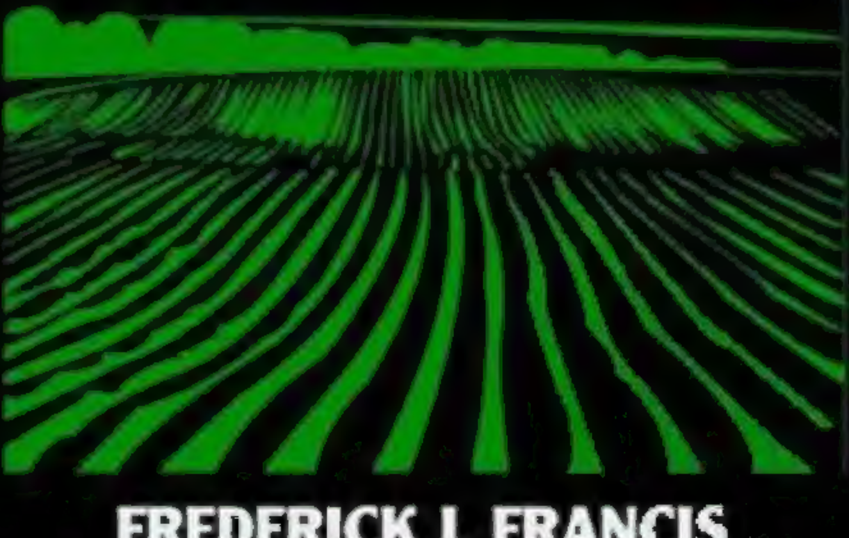
SECOND EDITION

WILEY ENCYCLOPEDIA OF FOOD SCIENCE AND TECHNOLOGY



FREDERICK J. FRANCIS

A

ACIDULANTS

Food acidulants are categorized either as general purpose acids or as specialty acids (1). General purpose acids are those that have a broad range of functions and can be used in most foods where acidity is desired or necessary. Specialty acids are those that are limited in their functionality and/or range of application.

Citric acid and malic acid are the predominant general purpose acidulants with tartaric and fumaric acids. Fumaric acid is included in this category even though its low solubility limits its potential range of application. However, it is used in popular and widely consumed food products.

All other acidulants fall into the specialty acid category. The most commonly used specialty acids are acetic acid (vinegar), cream of tartar (potassium acid tartrate), phosphoric acid, glucono-delta-lactone, acid phosphate salts, lactic acid, and adipic acid.

CITRIC ACID

Citric acid is the premier acid for the food and beverage industry because it offers a unique combination of desirable properties, ready availability in commercial quantities, and competitive pricing. It is estimated that citric acid worldwide accounts for more than 80% of general purpose acidulants used. It is found naturally in almost all living things, both plant and animal. It is the predominant acid, in substantial quantity, in citrus fruits (oranges, lemons, limes, etc), in berries (strawberries, raspberries, currants) and in pineapples. Citric acid is also the predominant acid in many vegetables, such as potatoes, tomatoes, asparagus, turnips, and peas, but in lower concentrations. The citrate ion occurs in all animal tissues and fluids. The total circulating citric acid in the serum of man is approximately 1 mg/kg of body weight (2).

Citric acid is manufactured by fermentation, a natural process using living organisms. The acid is recovered in pure crystalline form either as the anhydrous or monohydrate crystal depending on the temperature of crystallization. The transition temperature is 36.6°C. Crystallization above this temperature produces an anhydrous product while the monohydrate forms at lower temperatures. With an occasional exception for technological reasons, the anhydrous form is preferred for its physical stability and is the more widely available commercial form.

The salts of citric acid that are used in the food industry are sodium citrate dihydrate and anhydrous, monosodium citrate, potassium citrate monohydrate, calcium citrate tetrahydrate, and ferric ammonium citrate, in both brown and green powder. These salts are used either for functional purposes such as buffering or emulsification, as a source of cation for technological purposes, such as calcium to aid the gelation of low methoxyl pectin, or as a mineral source for food supplementation.

Citric acid is a hydroxy tribasic acid that is a white granule or powder. It is odorless with no characteristic taste other than tartness. Its physical and chemical properties are listed in Table 1. Citric acid and its salts are used across the broad spectrum of food and beverage products:

- Beverages
- Gelatin desserts
- Baked goods
- Jellies, jams, and preserves
- Candies
- Fruits and vegetables
- Dairy products
- Meats
- Seafood
- Fats and oils

MALIC ACID

This is the second most popular general purpose food acid although less than one-tenth the quantity of citric acid used. Malic acid is a white, odorless, crystalline powder or granule with a clean tart taste with no characterizing flavor of its own. The properties of malic acid are listed in Table 1.

Malic acid is made through chemical synthesis by the hydration of maleic acid. An inspection of its structure in Table 1 reveals that malic acid has an asymmetric carbon which provides for the existence of isomeric forms. The synthetic procedure for malic acid produces a mixture of the D and L isomers. The item of commerce, racemic D, L-malic acid does not occur in nature (3) although the acid is affirmed as GRAS for use in foods.

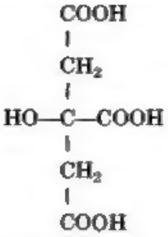
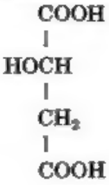
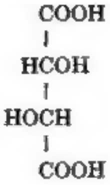
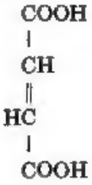
L-Malic acid is the isomeric form that is found in nature. It is the predominant acid in substantial quantity in apples and cherries and in lesser quantities in prunes, watermelon, squash, quince, plums, and mushrooms. Like citric acid, L-malic acid plays an essential part in carbohydrate metabolism in man and other animals.

There are no salts of malic acid that are items of commerce for the food and beverage industries. The application for malic and citric acid cover the same broad range of food categories.

TARTARIC ACID

While tartaric acid has the characteristics of a general purpose acid, fluctuating availability and price have caused users to reformulate where possible. Tartaric acid is a dibasic dihydroxy acid. The product is a white odorless granule or powder that has a tart taste and a slight characteristic flavor of its own. The properties of tartaric acid are listed in Table 1.

Table 1. Properties of General Purpose Food Acidulants

	Citric	Malic	Tartaric	Fumaric
Structure	 $\begin{array}{c}\mathrm{COOH}\\ \vert\\ \mathrm{CH_2}\\ \vert\\ \mathrm{HO{-}C{-}COOH}\\ \vert\\ \mathrm{CH_2}\\ \vert\\ \mathrm{COOH}\end{array}$	 $\begin{array}{c}\mathrm{COOH}\\ \vert\\ \mathrm{HOCH}\\ \vert\\ \mathrm{CH_2}\\ \vert\\ \mathrm{COOH}\end{array}$	 $\begin{array}{c}\mathrm{COOH}\\ \vert\\ \mathrm{HCOH}\\ \vert\\ \mathrm{HOCH}\\ \vert\\ \mathrm{COOH}\end{array}$	 $\begin{array}{c}\mathrm{COOH}\\ \vert\\ \mathrm{CH}\\ \Vert\\ \mathrm{HC}\\ \vert\\ \mathrm{COOH}\end{array}$
Formula	$C_6H_8O_7$	$C_4H_6O_5$	$C_4H_6O_6$	$C_4H_4O_4$
Molecular weight	192.12	134.09	150.09	116.07
Melting point, °C	153	131	169	286
Solubility at 25°C (g/100 mL of water)	181	144	147	0.63
Caloric value, kcal/g	2.47	2.39	1.84	2.76
Hygroscopicity (Resistance to moisture pick up)				
At 66% RH	Fair	Fair	Fair	Good
At 86% RH	Poor	Poor	Poor	Good
Ionization constants				
K_1	8.2×10^{-4}	4×10^{-4}	1.04×10^{-3}	1×10^{-3}
K_2	1.77×10^{-5}	9×10^{-6}	5.55×10^{-5}	3×10^{-5}
K_3	3.9×10^{-6}			

Tartaric acid has two asymmetric carbons which permit the formation of a dextro (+) rotatory form, a levo (−) rotatory form, and a meso form which is inactive due to internal compensation. A racemic mixture of dextro- and levo-rotatory forms is also a possibility.

Apart from very limited synthetic production in South Africa, tartaric acid is extracted from the residues of the wine industry. Therefore the natural form, L-(+)tartaric acid, is the article of commerce. The structure in Table 1 is L-(+)tartaric acid.

Potassium acid tartrate, also known as cream of tartar, is the major salt used in the food industry. It is occasionally used as the acid portion of a chemical leavening system for baked goods. It is also used as a doctor in candy making to prevent sugar crystallization by inverting a portion of the sucrose.

Tartaric acid can be used in most food categories and functions that require acidification but in practice it is limited to grape flavored products, particularly beverages and candies. It is also used where high tartness is desired in a highly soluble acid.

FUMARIC ACID

Fumaric acid is also a naturally occurring, organic, general purpose food acid. Although not found ubiquitously or in the concentrations of citric acid, fumaric nevertheless is found in all mammals as well as rice, sugar cane, wine, plant leaves, bean spouts, and edible mushrooms.

Fumaric acid is made synthetically by the isomerization of maleic acid. It is also produced by fermentation of glucose or molasses with *Rhizopus* spp. (4). It is a white crystalline powder that has the clean tartness necessary for a food acidulant. Table 1 shows that fumaric acid is the strongest of the food acids and is also the least soluble. The low solubility of fumaric acid limits its usefulness in foods. In processes where 50% stock solutions are mandatory such as carbonated beverages and jams and jellies, fumaric acid cannot be used.

Fumaric acid is extensively used in noncarbonated fruit juice drinks. Its greater acid strength allows lower use levels than citric acid and its solubility and rate of solution are sufficient for this process. Fumaric is also used in consumer packed gelatin desserts because of its low hygroscopicity. The only salt of fumaric acid that has had any application in the food industry is ferrous fumarate for iron fortification.

FUNCTIONS OF FOOD ACIDS

Food acidulants and their salts perform a variety of functions. These functions are as antioxidants, curing and pickling agents, flavor enhancers, flavoring agents and adjuvants, leavening agents, pH control agents, sequestrants, and synergists. The definitions for these functions are contained in the *U.S. Code of Federal Regulations* (5). Some of the functions overlap and in any given application an acidulant will often perform two or more functions.

Flavor Enhancer and Flavor Adjuvant

These functions are performed by a majority of the acidulants consumed by the food and beverage industries. Acids provide a tang or tartness that compliments and enhances many flavors but do not impart a characteristic flavor of their own. The acid itself should have a clean taste and be free of off notes that are foreign to foods. Some acids, such as succinic acid, have a distinctive taste which is incompatible with most food products; and hence, these acids have achieved very little use.

The need for tartness is obvious. Citrus and berry flavors would be flat and lifeless without at least a touch of acidity. However, not all fruit flavors require the same degree of tartness. Lemon candies and beverages are traditionally very sour, while orange and cherry are a little less tart. Flavors like strawberry, watermelon, and tropical fruits require only a trace of acidity for flavor enhancement.

In noncola carbonated beverages, beverage mixes, candies and confections, syrups and toppings, and any application where high solubility is required, citric and malic acids are used extensively. Fumaric acid is used in all ready constituted still beverages for economic reasons. Several acids are suitable for some flavors but not for others. For example, phosphoric acid is used in cola beverages but not in fruit flavored ones. Tartaric acid presents still another category. It has traditionally been used in grape flavored products even though it is suitable for other flavors.

The general purpose acids impart different degrees of tartness that are in part a result of their different acid strengths. Table 2 summarizes the tartness equivalence of the general purpose acids. The relationship shown is based only on tartness intensity and not character of flavor. This relationship can change depending on the formulation ingredients and the particular flavor system being studied. Malic acid, for example, has been claimed to be 10–15% more tart than citric acid in juice based, fruit flavored still beverages. In fruit and berry carbonated beverages, both acids have been perceived as being of equal tartness. Tartness is a difficult property to measure precisely and it must be determined by a trained and experienced taste panel.

Acids have also been used for their effects on masking undesired flavors in foods and food ingredients. Both citric and malic acids and citrate salts are known for their ability to mitigate the unpleasant aftertaste of saccharin. Gluconate salts and glucono-delta-lactone (GDL) have been patented for this function (6,7). Claims of enhanced benefits for malic acid over citric acid when used with the new intense sweeteners have been made but definitive advantages have not yet been demonstrated.

pH Control

Control of acidity in many food products is important for a variety of reasons. Precise pH control is important in the manufacture of jams, jellies, gelatin desserts, and pectin jellied candies in order to achieve optimum development of gel character and strength. Precise pH control is also important in the direct acidification of dairy products to achieve a smooth texture and proper curd formation. Increasing acidity enhances the activity of antimicrobial food preservatives, decreases the heat energy required for sterilization, inactivates enzymes, aids the development of cure color in processed meats, and aids the peelability of frankfurters.

Table 2. Tartness Equivalence

General-purpose acids	Weight equivalence
Citric	1.0
Fumaric	0.6–0.7
Tartaric	0.8–0.9
Malic	0.85–1.0

Gelatin desserts are generally adjusted to an average pH of 3.5 for proper flavor and good gel strength. However, the pH can range from 3.0–4.0. Adipic and fumaric acids are used in gelatin desserts that are packaged for retail sales. Their low hygroscopicity allows use of packaging materials that are less moisture resistance and less expensive.

In jams and jellies, the firmness of pectin gel is dependent on rigid pH control. Slow set pectin attains maximum firmness at pH 3.05–3.15 while rapid set pectin reaches maximum firmness at pH 3.35–3.45 (8). The addition of buffer salts such as sodium citrate and sodium phosphate assist in maintaining the pH within the critical pH range for the pectin type. These salts also delay the onset of gelation by lowering the gelation temperature. The acid should be added as late as possible in the process. Premature acid addition will result in some pectin hydrolysis and weakening of the gel in the finished product. The acid is added as a 50% stock solution and thus soluble acids are required. Citric is generally used in this application but malic and tartaric are also satisfactory.

The United States Federal Standards of Identity (9) provide for the direct acidification of cottage cheese by the addition of phosphoric, lactic, citric, or hydrochloric acid as an alternate procedure to production with lactic acid producing bacteria. Milk is acidified to a pH of 4.5–4.7 without coagulation, and then after mixing, is heated to a maximum of 120°F without agitation to form a curd. Glucono-delta-lactone is also permitted for this application. It is added in such amounts as to reach a final pH value of 4.5–4.8 and is held until it becomes coagulated. GDL is preferred for this application because it must undergo hydrolysis to gluconic acid before it can lower pH. Thus the rate at which the pH is lowered is slowed, avoiding local denaturation.

The activity of antimicrobial agents (benzoic acid, sorbic acid, propionic acid) is due primarily to the undissociated acid molecule (10). On the basis of undissociated acid concentration, Giannuzzi et al. (11) have shown that citric acid is more effective than ascorbic or lactic acids in inhibiting *Listeria monocytogenes* in a trypticase soya broth containing yeast extract. Under refrigerated temperatures, higher inhibition indices were obtained in the presence of lower concentrations of citric acid. Activity is therefore pH dependent and theoretical activity at any pH can be calculated. Table 3 shows the effect of pH on dissociation. It can be seen why acidification improves preservative perfor-

Table 3. Effect of pH on Dissociation[a]

pH	Sorbic	Benzoic	Propionic
3	98	94	99
4	86	60	88
5	37	13	42
6	6	1.5	6.7
7	0.6	0.15	0.7
(pK_a)	4.67	4.19	4.87

[a] Percentage of undissociated acid.

mance and why benzoates are not generally recommended above pH 4.5.

The use of acid to make heat preservation more effective, especially against spore-forming food spoilage organisms, is an established part of food technology. Under U.S. Federal Standards of Identity (12), the addition of a suitable organic acid or vinegar is required in the canning of artichokes (to reduce the pH to 4.5 or below) and is optional in the canning of the vegetables listed in Table 4. Vinegar is not permitted in mushrooms. Citric acid is specifically permitted as an optional ingredient in canned corn and canned field corn.

The advantage of acidification is especially well illustrated in the canning of whole tomatoes. When the pH of these is greater than 4.5, there is increased incidence of spoilage in the cans. When tomatoes of pH 3.9 are processed at 212°F, only 34 min are required to kill a normal or high spore load without decreases in color and flavor and deterioration of structure. In contrast, at pH 4.8 the cooking must be 110 min (13).

In the processing of fruits and vegetables, whether for canning, freezing, or dehydration, the prevention of discoloration in the fresh cut tissue is a major concern. Reactions in which polyphenolic compounds are changed by oxidation into colored materials play an important part in this discoloration which may be accompanied by undesirable flavors. The ascorbic acid naturally present in many fruits and vegetables offers some protection, but this is of relatively short duration because of destruction of ascorbic acid by natural enzymes and air. Heating, as applied in blanching, destroys the oxidative enzymes which cause discoloration but may alter flavor and texture if continued sufficiently to completely inactivate oxidative enzymes.

Lowering pH by addition of acid substantially decreases the activity of natural color producing enzymes in fruits. Citric acid also sequesters traces of metals which may accelerate oxidation. Even greater protection is obtained by using citric acid in conjunction with a reducing agent such as ascorbic or erythorbic acid. Some processors have found that a combination of sodium erythorbate and citric acid best serves their needs.

Leavening Agent

The basis for the formulation of effervescent beverage powders, effervescent compressed tablet products, and chemically leavened baked goods is the reaction of an acidulant with a carbonate or bicarbonate resulting in the generation of carbon dioxide. The physical state of some food acids as dry solids is a property appropriate for beverage mixes and chemical leavening systems. In the absence of water, there is essentially no interaction between such acids and sodium bicarbonate. Thus these dry mixes can be stored for long periods.

A desired property for an acidulant in a chemical leavening system is that it react smoothly with the sodium bicarbonate to assure desirable volume, texture, and taste. Leavening acids and acid salts vary quantitatively in their neutralizing capacity. This relationship is shown in Table 5. A new heat-activated leavening agent, dimagnesium phosphate, was recently reported for use in finished baked products (14).

The various acids differ in their rate of reaction in response to elevation of temperature. This must be taken into consideration in selecting an acidulant for a particular condition. Under some conditions, a mixture of acidulants may be most suitable to achieve desired reaction times. Table 6 compares the reaction times of GDL and cream of tartar.

Glucono-delta-lactone is an inner ester of gluconic acid that is produced commercially by fermentation involving *Aspergillus niger* or *A. suboxydans*. When it hydrolyzes, gluconic acid forms and this reacts with sodium bicarbonate. Although GDL is relatively expensive, there are certain specialized types of products such as pizza dough and cake doughnuts for which it is eminently suited as an acid component of the leavening system. Cream of tartar (potassium acid tartrate) has limited solubility at lower temperatures. There is a limited evolution of gas during the initial stages of mixing in reduced temperature bat-

Table 4. Canned Vegetables in which Use of Acids is Optional

Asparagus
Bean sprouts
Beans, butter
Beans, lima
Beans, shelled
Beet greens
Beets
Broccoli
Brussels sprouts
Cabbage
Carrots
Cauliflower
Celery
Greens, collard
Greens, dandelion
Greens, mustard
Kale
Mushrooms
Okra
Onions
Parsnips
Peas, black-eyed
Peas, field
Pimientos
Potatoes
Rutabagas
Salsify
Spinach
Sweet peppers, green
Sweet peppers, red
Sweet potatoes
Swiss chard
Tomatoes
Truffles
Turnip greens
Turnips

Table 5. Neutralizing Value of Various Acidulants Used in Chemical Leavening

Acid	Parts acid to neutralize one part sodium bicarbonate
Fumaric	0.69
Glucono-delta-lactone	2.12
Cream of tartar	2.24
Sodium acid pyrophosphate	1.39
Anhydrous monocalcium phosphate	1.20
Monocalcium phosphate monohydrate	1.25
Sodium aluminum sulfate	1.00
Sodium aluminum phosphate	1.00

Table 6. Comparison of Carbon Dioxide Evaluation in GDL and Cream of Tartar at Room Temperature

Time (min)	GDL (%)	Cream of tartar (%)
0.5	12.7	53.7
2	19.5	80.0
5	32.3	92.0
20	69.0	99.4
60	92.4	100.0

ters. At room temperature and above, the rate of reaction increases. Because of these characteristics, and its pleasant taste, cream of tartar is used in some baking powders and in the leavening systems of a number of baked goods and dry mixes.

Antioxidants, Sequestrants, and Synergists

Oxidation is promoted by the catalytic action of certain metallic ions present in many foods in trace quantities. If not naturally present in a food, minute quantities of these metals, particularly iron and copper, can be picked up from processing equipment. Oxidation is the cause of rancidity, an off flavor development in fat. It is also responsible for off color development that renders a food unappetizing in appearance. Hydroxy-polycarboxylic acids such as citric acid sequester these trace metals and render them unavailable for reaction. In this regard the acids function as antioxidants.

Hydroxy-polycarboxylic acids are often used in combination with antioxidants such as ascorbates or erythorbates to inhibit color and flavor deterioration caused by trace metal catalyzed oxidation. The ascorbates and erythorbates as well as BHA, BHT, and other approved antioxidants and reducing agents are oxygen scavengers and are effective when used alone. The effect of the combination of a sequestrant, such as a hydroxy-polycarboxylic acid, and an antioxidant is synergistically greater than the additive effect of either component used alone.

Citric acid is the most prominent antioxidant synergist although malic and tartaric acid have been used. In meat products, U.S. Department of Agriculture regulations permit citric acid in dry sausage (0.003%), fresh pork sausage (0.01%), and dried meats (0.01%). A short dip in a bath containing 0.25% citric and 0.25% erythorbic acid improves quality retention in frozen fish. This treatment is also applicable to shellfish to sequester iron and copper that catalyze complex blueing and darkening reactions.

Untreated fats and oils, both animal and vegetable, are likely to become rancid in storage. Oxidation is promoted by the catalytic action of certain metallic ions such as iron, nickel, manganese, cobalt, chromium, copper, and tin. Minute quantities of these metals are picked up from processing equipment. Adding citric acid to the oil sequesters these trace ions, thereby assisting antioxidants to prevent development of off flavors. Although the oil solubility of citric acid is limited, this can be overcome by first dissolving it in propylene glycol. The antioxidant can be dissolved in the same solvent so that the two can be added in combination.

Curing Accelerator

The acids approved by the U.S. Department of Agriculture for this function in meat products (1) must be used only in combination with curing agents. In addition to ascorbates and erythorbates, the approved acids are:

1. Fumaric acid to be used at a maximum of 0.065% (1 oz–100 lb) of the weight of the meat before processing.
2. GDL to be used at 8 oz to each 100 lb of meat in cured, comminuted meat products and at 16 oz per 100 lb of meat in Genoa salami.
3. Sodium acid pyrophosphate not to exceed, alone or in combination with other curing accelerators, 8 oz per 100 lb of meat nor 0.5% in the finished product.
4. Citric acid or sodium citrate to replace up to 50% of the ascorbate or erythorbates used or in 10% solution to spray surfaces of cured cuts.

In conjunction with sodium erythorbate or related reducing compounds, GDL accelerates the rate of development of cure color in frankfurters during smoking. This permits shortening smokehouse time by one half or more and products have less shrinkage and better shelf life.

The special property of GDL upon which these advantages depend is its lactone structure at room temperature. In this form there is no free acid group and the GDL can thus be safely added during the emulsifying stage of sausage making without fear of shorting out the emulsion. Under the influence of heat in the smoking process, the ester hydrolyzes rapidly and is converted in part to gluconic acid. This lowers the pH of the emulsion during smoking, providing conditions under which sodium erythorbate or other reducing compounds (erythorbic acid, ascorbic acid, and sodium ascorbate) react with greater speed to convert the nitrite of the cure mixture into nitric oxide. The nitric oxide, in turn, acts upon the meat pigment to form the desired red nitrosomyoglobin.

CONCLUSION

The many functions and broad range of applications of food acidulants makes the selection of the most suitable acid for a food product a matter of serious concern. The physical and chemical properties of food approved acidulants must be an essential part of the knowledge of those food technologists who make the decision.

BIBLIOGRAPHY

1. J. D. Dziezak, "Acidulants: Ingredients That Do More Than Meet the Acid Test," *Food Technol.* **44**, 75–83 (1990).
2. E. F. Bouchard and E. G. Merritt in M. Grayson, ed., Kirk-Othmer, *Encyclopedia of Chemical Technology*, Vol. 6, 3rd ed., John Wiley & Sons, Inc., New York, 1979, pp. 150–179.
3. *Code of Federal Regulations*, Title 21 §184.1069.
4. D. E. Blenford, "Food Acids," *Food Flavor Ingredients Processing and Packaging* **8**, 11 (1986).
5. *Code of Federal Regulations*, Title 21 §170.3(o).
6. U.S. Pat. 3,285,751 (Nov. 15, 1966) Artificial Sweetner Composition, P. Kracauer (to Cumberland Packing Company).
7. U.S. Pat. 3,684,529 (Aug. 15, 1972) Sweetening Compositions, J. J. Liggett (to William E. Hoerres).
8. M. Glicksman, *Gum Technology in the Food Industry*, Academic Press, Inc., Orlando, Fla., 1969, p. 179.
9. *Code of Federal Regulations*, Title 21 §133.129.
10. Davidson and Juneja, 1990.

11. L. Giannuzzi and N. E. Zaritsky, "Effect of Ascorbic Acid in Comparison to Citric Acid and Lactic Acid on *Listeria monocytogenes* Inhibition at Refrigerated Temperatures," *Lebensmittel Wissenschaft und Technologie* **29**, 278–285 (1996).
12. *Code of Federal Regulations*, Title 21 §155.200.
13. C. T. Townsend, cited in S. Leonard, R. M. Pangborn, and B. S. Luh, *Food Technology* **13**, 418 (1959).
14. D. R. Gard et al., "Evaluation of a New Leavening Acid, Dimagnesium Phosphate, With a Traditional Chemical Leavening System," *Proc. IFT Ann. Meet.*, 1996, p. 114.
15. *Code of Federal Regulations*, Title 9 §318.7(c)(4).

AFFLUENCE, FOOD EXCESS, AND NUTRITIONAL DISORDERS

Nutrition research in the first half of the present century focused mainly on identifying and assessing nutrients in foods and recognizing deficiency diseases. Since essential nutrients were unevenly distributed in individual foods, consumption of a variety of foods was recommended to provide a more nutritionally balanced diet (1). With undernutrition still a problem even in the richer countries, greater consumption of meat and dairy products was recommended. Increasing wealth in society allowed these recommendations to match producer and consumer wishes and acknowledged, at the same time, the perceived high nutritional values of these foods of animal origin. By the 1950s, however, evidence was accumulating that the high rates of premature deaths from some of the major chronic diseases could be related to diet. Originally this was thought to be a problem only in the industrialized countries but, as medicine conquered infectious diseases, the phenomenon became recognized as existing worldwide.

The major nutrition-related degenerative diseases include obesity, diabetes mellitus, cardiovascular diseases, and certain cancers. The still continuing Framingham Heart Study, which began in 1948, has been responsible for demonstrating many associations between these diseases and diet, notably excess intake of food energy and fat, especially saturated fat and cholesterol, as well as lifestyle factors such as smoking, emotional stress, and lack of physical exercise. Consequently, current diet/health recommendations differ dramatically from those made earlier.

OBESITY

Obesity affects many millions of Americans and is a major public health problem. It is characterized by excess body fat caused by an imbalance between energy intake and energy expenditure (2). The specific reasons behind such an imbalance, however, remain the subject of much debate.

Genetic, environmental and behavioral variables all influence the risk of becoming overweight (2,4), but the relative importance of each remains unclear. The prevalence of obesity for different age groups in the United States is shown in Table 1. Direct assessment of body fat can be used to evaluate obesity but the procedure is mainly limited to research. For public health studies and clinical practice, simple measures such as height and weight tables, body mass index (BMI), or skinfold measurements are used. The BMI (weight in kilograms divided by the square of height in meters) is an indicator that shows the best correlation with independent measures of body fat (2,5). The use of BMI was first proposed in 1871 and was long known as the Quetelet index. It is now widely used for assessing the degree of obesity or overweight (Table 2).

Obesity is more than a problem in its own right since it is closely linked to other diseases such as hypertension, diabetes, and cardiovascular disease. Many long-term studies have shown a greater risk of these diseases with increasing levels of obesity, even when other risk factors are present (5). Later studies have refined these conclusions and have demonstrated that the distribution of fat, especially in the abdominal area, is an additional factor (5). A waist circumference of over 40 in. (102 cm) in men and over 35 in. (89 cm) in women signifies increased risk in those who have a BMI of 25.0 to 34.9.

With increasing BMI, average blood pressure and total cholesterol levels rise while average high-density lipoprotein (HDL—a protective indicator) levels fall (6). Men in the highest obesity category have more than twice the risk of hypertension and elevated cholesterol when compared with men of normal weight. Women in the highest obesity category have four times the risk of either or both of these risk factors (6). Obese individuals are also at increased risk for several other problems, including lipid disorders, type II diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and certain cancers.

A number of efforts have been initiated to educate the public on altering behavioral risk factors such as improper dietary habits and lack of exercise. Examples include the Dietary Guidelines for Americans (Table 3), revised editions of which are periodically issued by the U.S. Department of Agriculture (USDA) (7). Recently the first federal guidelines on the identification, evaluation, and treatment of overweight and obesity in adults were released (6). These clinical practice guidelines are designed to help physicians in their care of overweight and obesity and include the scientific background for assessment as well as the principles of safe and effective weight loss. Three key indicators are recommended BMI, waist circumference, and the patient's risk factors for diseases and conditions associated with obesity. Overweight is defined as a BMI of 25.0 to 29.9 while obesity is a BMI greater than 30.0. These values are consistent with the definitions used in many other countries and support the Dietary Guidelines for Americans. A BMI of 30.0, for example, a weight of 221 lb for a 6-ft person (100 kg: 1.83 m) or 186 lb for a person of 5 ft, 6 in. (84 kg: 1.68 m) indicates about 30 lb (13.6 kg) overweight for both men and women. Highly muscular people may have a high BMI without increased health risks. For the majority, however, BMI is an excellent indicator of overall risk.

The most successful strategies for weight loss include reduction of food energy intake, increased physical activity,

Table 1. Prevalence of Overweight, Severe Overweight, and Morbid Obesity (NHANES II)

	Males		Females		Total
	Millions	Prevalence %	Millions	Prevalence %	Millions
Overweight	15.4	24.2	18.6	27.1	34.0
Severe overweight	5.1	8.0	7.4	10.6	12.5
Morbid obesity	0.327	0.6	1.7	2.5	2.0

Source: Ref. 3.

Table 2. Classification of Overweight and Obesity Based on Body Mass Index

Degree of Obesity	BMI[a]
Grade III (morbid obesity)	>40
Grade II (obese)	30–39.9
Grade I (overweight)	25–29.9
Grade 0 (normal)	20–24.9

[a] BMI = weight in kilograms divided by the square of the height in meters.
Source: Ref. 2.

Table 3. Dietary Guidelines for Americans

- Eat a variety of foods.
- Balance the food you eat with physical activity; maintain or improve your weight.
- Choose a diet with plenty of grain products, vegetables, and fruits
- Choose a diet low in fat, saturated fat, and cholesterol.
- Choose a diet moderate in sugars.
- Choose a diet moderate in salt and sodium.
- If you drink alcoholic beverages, do so in moderation.

Source: Ref. 7.

Table 4. Selected Recommendations from the First Federal Obesity Guidelines Panel

- Engage in moderate physical activity (30 min or more) on most/all days of the week.
- Reduce dietary fat and calories. Cutting back on dietary fat can help reduce calories and is heart-healthy.
- The initial goal of treatment should be to reduce body weight by about 10% from baseline, an amount that reduces obesity-related risk factors. With success, and if warranted, further weight loss can be attempted.
- A reasonable time line for a 10% reduction in body weight is six months of treatment (weight loss of 1 to 2 lb per week).
- Weight maintenance should be a priority after the first 6 months of weight-loss therapy.
- Overweight and obese patients who do not wish to lose weight, or are otherwise not candidates for weight-loss treatment, should be counseled on strategies to avoid further weight gain.
- Age alone should not preclude weight-loss treatment in older adults. A careful evaluation of potential risks and benefits in the individual patient should guide management.

Source: Ref. 6.

and behavior therapy designed to improve eating and physical activity habits. Other recommendations are listed in Table 4.

One in five children in the United States is now overweight. Care and treatment differ from adults, and therefore pediatric guidelines have been proposed by an expert committee (8). Clinicians who care for these children and their families are urged not to express blame but to show "sensitivity, compassion and a conviction that obesity is an important chronic medical condition that can be treated." Balanced nutritional intakes with avoidance of excess together with adequate physical activity are important goals for all family members.

HYPERTENSION

In the adult, hypertension (high blood pressure) is defined as a pressure greater than, or equal to, 140 mmHg systolic, or greater than or equal to, 90 mmHg diastolic pressure (9). In 90 to 95% of the cases of high blood pressure, the specific cause may be unknown (10). Hypertension is a risk factor for both coronary heart disease and stroke. Although it can occur in children and adolescents, it is more prevalent in the middle-aged and elderly, especially African Americans and the obese. Heavy drinkers and women who are taking oral contraceptives (11) are also at increased risk. Individuals with diabetes mellitus, gout, or kidney disease also have a higher frequency of hypertension. Salt consumption can increase blood pressure for some. High blood pressure is related to obesity and to increases in body weight over time (9,12). Factors increasing the risk of developing high blood pressure are listed in Table 5.

Weight loss, an active lifestyle, reduction in sodium intake, and moderation of alcohol consumption are recommended for prevention and management (9). For many, however, medical intervention with antihypertensive drugs is required to maintain acceptable blood pressure.

Table 5. Risk Factors for Hypertension (High Blood Pressure)

- Heredity
- Male gender
- Sodium or salt sensitivity
- Heavy alcohol consumption
- Sedentary and inactive lifestyle
- Race (African Americans are at greater risk)
- Age
- Obesity and overweight
- Use of oral contraceptives and some other medications

Source: Ref. 11.

DIABETES MELLITUS

What can now be recognized as diabetes was described in the ancient civilizations of Egypt, Greece, and India. The sweet taste of urine in those with the condition was noted in the 1600s and the term "mellitus" meaning honeylike was introduced (13). Diabetes mellitus is a major public health problem worldwide. It ranks sixth as a primary cause of death in the United States, but when its complications are included, it ranks third. These complications can be very serious and involve, in the United States, 50% of the amputations of all lower extremities in adults and 25% of all kidney failure and are also a leading cause of blindness.

Non-insulin-dependent diabetes (NIDDM, or type II) is the form of diabetes characterized as a chronic nutritionally related condition (Table 6) and is a disorder showing abnormalities in glucose, fat, and protein metabolism. The onset of type II diabetes can be triggered by dietary and lifestyle factors similar to those associated with cardiovascular diseases. Diabetes and heart disease in later life appear to be linked to weight at birth (13).

Table 6. Classification of Diabetes Mellitus

Spontaneous Diabetes Mellitus (DM)
Insulin-dependent (IDDM, or type I)
Non-insulin-dependent (NIDDM or type II)
  Nonobese NIDDM
  Obese NIDDM
  Maturity onset diabetes of young people
Secondary diabetes
Gestational diabetes

Source: Ref. 13.

CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) has been for many years the leading single cause of death in the United States (14): it includes both coronary heart disease (CHD) and stroke. CHD is the most common form of cardiovascular disease.

In 1987 nearly 1 million deaths in the United States, half of the total number, occurred due to some form of CVD (15). In 1998, CVD remains the leading cause of death in the United States (14), although there have been some reductions in the rate. Cardiovascular disease is often thought to affect mainly men and the elderly, but it is also a major killer of women and people in the prime of life (14). An estimated 58 million Americans live with some form of the disease, and almost 10 million Americans aged 65 years and older report disabilities caused by heart disease. Stroke is also a leading cause of disability in the United States, affecting more than 1 million people nationwide (14). The health burden of this condition is rivaled by the economic burden, which has a profound impact on the health care system.

Extensive clinical and epidemiological studies have identified several major and contributing risk factors of heart disease and stroke (16). The major risk factors are listed in Table 7. Some such as increasing age, male gender, and genetic background cannot be changed, treated, or modified (16). Others, for example, smoking, high serum cholesterol, high blood pressure, physical inactivity, obesity, and overweight, are under some control by the individual. Smokers have twice the risk of heart disease compared with nonsmokers. Nearly one-fifth of all deaths from cardiovascular diseases (180,000 deaths per year) are attributable to smoking (14). Surveillance data indicate that an estimated 1 million young people become regular smokers each year (14).

The risk of heart disease increases with a rise in cholesterol levels especially when other risk factors are present (17–19). Plasma total cholesterol was accepted as a causal factor (among multiple factors) by the World Health Organization (WHO) expert committee in 1982 and by the U.S. National Institute of Health Consensus Development Conference in 1985 (17). Diet and its effects on plasma cholesterol levels are discussed in the next section. Plasma triglyceride levels have also been correlated with increased risk of heart disease (17) and are associated with increased low-density lipoprotein (LDL) cholesterol levels. High blood pressure increases the risk of a stroke, heart attack, kidney failure, and congestive heart failure. When obesity, smoking, high blood cholesterol levels, or diabetes are also present, high blood pressure increases the risk of a heart attack or stroke severalfold.

Regular moderate-to-vigorous exercise plays a significant role in preventing heart and blood vessel disease (16). Exercise helps control blood cholesterol, diabetes, and obesity as well as maintaining blood pressure. However, surveys have shown that more than half of American adults do not practice the recommended level of physical activity. Obese people are at a greater risk of heart disease, high blood pressure, high cholesterol, and other chronic diseases and diabetes (14,16). Diabetes is also a serious risk factor for heart disease with more than 80% of diabetics succumbing to some form of heart or blood vessel disease (16).

ROLE OF DIET IN CARDIOVASCULAR DISEASE

Improper eating habits accompanied by the lack of exercise increase the risk of gaining excess weight, a major risk factor for heart disease, high blood pressure, and diabetes (14). Diet also affects plasma cholesterol levels. Cholesterol is carried in the blood associated with two major types of lipoproteins; LDL and HDL. LDL cholesterol has been

Table 7. Major Risk Factors for Cardiovascular Disease

- Increasing age
- Heredity
- High blood cholesterol levels
- Physical inactivity
- Diabetes mellitus
- Male gender
- Smoking
- High blood pressure
- Obesity and overweight

Source: Ref. 16.

correlated with increased risk of cardiovascular disease. For many years it has been recognized that dietary cholesterol has only a limited effect on plasma cholesterol levels (17). Absorption of ingested cholesterol is poor, and part of the cholesterol in plasma is synthesized in the liver.

Total lipid intake, and the type of fat consumed, have more effect in raising plasma cholesterol than does dietary cholesterol (17,20). Saturated fatty acids were found to raise cholesterol, polyunsaturated fatty acids lowered plasma cholesterol, and monounsaturated fatty acids had an intermediate effect. In the classic seven-country prospective study where lipid intake was correlated with CHD (21,22), the disease incidence was also related to the intake of saturated fat. Most dietary prevention trials have reduced total fat, saturated fat, and cholesterol intakes along with moderately increased polyunsaturated fat levels.

Similar recommendations are also inclusive in many of the guidelines for health in the United States. Recent research indicates that monounsaturated fatty acids such as oleic acid, which were thought to be neutral, have, in fact, a substantial cholesterol lowering effect (23). *Trans* fatty acids formed during hydrogenation of certain edible oils may, perhaps, increase LDL cholesterol and decrease HDL cholesterol (24). A high fish intake has been associated with beneficial effects on the prevention of CHD. Fish oils contain DHA (docosahexaenoic acid), which has a plasma triglyceride-lowering effect (25).

High levels of dietary carbohydrate, especially complex carbohydrate, are associated with a decreased risk of cardiovascular disease (17). A recent study found rice bran as well as oat bran to have a hypocholesterolemic effect (26). Increasing intakes of a number of vitamins have also been shown to be protective toward cardiovascular disease. These include vitamins B_6, C, E, and folate. Vitamin C and E are antioxidants and have been hypothesized as preventing damage to coronary arteries. Elevated serum vitamin C has also been correlated with increased HDL cholesterol levels in women (27). Increased consumption of folate and vitamin B_6 have also been shown to reduce risk of CHD in women (28). Current dietary recommendations (Table 3) incorporate many of these proven relationships between diet and health.

CANCER

Cancer is a disease condition characterized by excessive growth of cells due to abnormal multiplication and replication. The biological process shows several experimentally distinct phases following exposure to a carcinogen. These include: initiation, tumor promotion, and tumor progression (29). After CVD, cancer is the next highest cause of death in the United States. Many adverse effects that occur in patients are due, not only to the cancer itself, but also to the treatment. Loss of appetite (anorexia) is the most common side effect. When advanced and persistent, this along with other metabolic and physiologic changes can eventually lead to severe undernutrition termed cancer cachexia.

Anorexia is not unique to cancer; however, it is persistent and severe in certain cancers such as the carcinoma of the stomach, breast, and large bowel. Onset of anorexia is insidious and may not be accompanied by any obvious manifestations other than progressive weight loss. Energy expenditure is high in cancer patients (29) while glucose intolerance is found frequently and may be due to the increased insulin resistance or inadequate insulin release. Abnormalities also occur in fat metabolism and include excess body fat depletion, protein loss, increased lipolysis, changes in free fatty acid and glycerol turnover, as well as decreased lipogenesis. Abnormalities in protein metabolism include increased whole body protein turnover, increased hepatic protein synthesis, persistent muscle protein breakdown, and decreased levels of plasma branched-chain amino acids. Malabsorption as well as protein loss through the gastrointestinal tract may also occur.

DIET AND CANCER RISK

Diet may have either positive and negative influences on cancer risk (30). About 35% of cancer occurrence is related to dietary factors (31). The role of diet in cancer etiology is summarized in Table 8. Dietary fat intake was associated with breast cancer in animals as early as 1942 (32,33). More recently, higher intake levels of dietary fat have been related to increased risk of colon cancer (34). Positive correlations between per capita fat intakes and breast cancer rates have been described (35,36). It has been argued, however, that it may be the high food energy intake that is causative rather than the percentage of the food energy coming from dietary fat (37). Nevertheless, diets high in fat, particularly saturated fat, have also been associated with a higher incidence of cancer of the colon, prostate, and breast (17).

Increased consumption of fruits and vegetables has been recommended to reduce cancer risk as these foods contain protective factors. Many are also high in fiber, an increased consumption of which has been associated with decreased risk of cancer (especially colon cancer). Diets high in plant foods, starches, fiber, and various carotenes are commonly associated with a lower incidence of alimentary tract cancers. A number of dietary and nondietary factors have been found to decrease the incidence of various cancers. Lycopene found in tomatoes appears to be protective against colorectal cancer (38) as it can scavenge peroxyl radicals and quench singlet oxygen. Increased intakes of plants of the cabbage family appear to be protective against certain cancers (39). More recently, broccoli sprouts have been shown to contain high levels of the anticarcinogenic chemical sulforaphane. This compound has been known to help mobilize the body's natural cancer-fighting resources and reduces the risk of developing cancer (40).

ANOREXIA NERVOSA AND BULIMIA NERVOSA

Although not included within the category of nutritionally related chronic diseases, the eating disorders anorexia nervosa and bulimia nervosa are important. These diseases are primarily disorders of perception of body image and are characterized by an excessive concern over being fat. They

Table 8. Dietary Factors and Cancer Etiology

Carcinogenic dietary factors	Anticarcinogenic factors
Energy excess associated with increased cancer mortality in men and women	Energy deficit inhibits tumor growth
Amount and type of fat in the diet also related to increased cancer risk; high saturated fat, cholesterol, and low polyunsaturated fat are risk factors	High levels of monounsaturated fat in the diet show decreased incidence of certain cancers
High protein intake associated with increased risk of enhanced tumorigenesis	High levels of fiber from fruits and vegetables are associated with low levels of colon and rectal cancer.
Zinc deficiency associated with increased risk of tumors	Vitamin A and its analogues and precursor (carotenids) are possible inhibitors of carcinogenesis; β-carotene may be protective in a mechanism independent of its role as a vitamin A precursor
Excess alcohol intake	Vitamin C has antioxidant properties that may influence tumorigenesis
High intake of coffee is a possible risk factor	Vitamin E as an intracellular antioxidant may protect against carcinogens
Artificial sweeteners such as saccharin increase risk of bladder cancer	Calcium intake has a inverse association with colon cancer risk
Nitrates, nitrites, and nitrosamines may be causative factors of gastric cancer	Selenium intake has been associated with decreased tumor growth in animal models
Methods of food preparation, such as charcoal broiling, smoking food, and frying, may increase risk	

Source. Ref. 31.

are often regarded as modern disorders despite the fact that similar conditions have been recognized in medicine for more than a century.

Anorexia nervosa is a condition of self-engendered weight loss whose occurrence was originally thought to be restricted to young women. It also occurs in young men who are concerned with their body image such as dancers and models. The diseases appear to be largely confined to affluent societies that espouse Western cultural ideals.

Diagnostic criteria include: refusal to maintain minimally normal body weight for age and height; intense fear of gaining weight or becoming fat, even though already underweight; undue influence of body weight or shape on self-evaluation; and denial of the seriousness of the current low body weight with amennorhea often occurring in postmenarchal females (41). Associated symptoms include: depressed mood, irritability, social withdrawal, loss of sexual libido, preoccupation with food and rituals, as well as reduced alertness and concentration (42).

One form of the disease invokes restrictive feeding behavior commonly associated with normal dieting, such as undereating, refusal to take high-energy foods, and strenuous exercise. This behavior is abnormal only in the degree to which it is pursued. Restlessness is very common once emaciation sets in and continues until physical deterioration leads to weakness and lassitude. The "purging" form involves more dangerous behaviors, such as self-induced vomiting, and laxative or diuretic use.

Bulimia nervosa is a variant of anorexia nervosa and shares many of its clinical and demographic features. It is closely related to the purging form of anorexia nervosa. One of the major differences is that bulimic patients maintain normal weight. The condition generally involves persistent dietary restriction that is eventually interrupted by episodes of binge eating with compensatory behaviors such as vomiting and laxative abuse. Behavioral disturbances often become the focus of intense guilt feelings. In the early stages of the disease, all patients attempt to control their weight by dieting and abstaining from high-energy foods. They are constantly preoccupied by thoughts of food, but their pattern of eating alternates between fasting and gorging. Patients are extremely secretive about their bulimic episodes. It is this secrecy that makes the condition difficult to diagnose.

Both conditions occur predominantly in industrialized, developed countries and are rare elsewhere (43). Immigrants are more likely to develop eating disorders than their peers in their country of origin, probably indicating the importance of sociocultural factors in the etiology and distribution of these disorders (43).

CONCLUSION

The chronic, nutritionally related diseases just described are major causes of death and disability in rich industrialized countries. American and North European diets have tended to be high in animal foods (meat, dairy, fish, eggs) and low in foods of plant origin (grains, fruits, and vegetables). It is claimed by Garrow (44) that most of the chronic diseases in the Western society are the manifestation of the high availability and variety of foods leading to overconsumption. Only a small proportion of income is now required to be spent on food in the industrialized countries (45). Excessive intake of animal foods leads to a dietary pattern that is high in saturated fat and cholesterol and low in fiber.

In contrast, the southern European or the Mediterranean diet comprises fruits, vegetables, and grains with smaller amounts of meat, fish, eggs, and dairy products (46,47). Olive oil is often the major lipid, so that the diet is low in cholesterol and saturated fat and high in monounsaturated fatty acids. A comparative study between Italians and Americans was performed in the early 1950s. It was found that Italian diets were remarkably low in fat (20% of energy) or just half of the proportion observed in the diets of comparable American groups. The typical American diet, rich in meat and dairy fats was thus, together with higher concentrations of blood cholesterol, identified with increased risk of coronary heart disease (48). A seven-country study performed over 20 years confirmed these relationships (22). Recommendations for the "Mediterranean Diet" have become popular within the United States. This diet plan is indicated in Table 9 (49).

Ironically, while such diets are now being consumed by the affluent, recent dietary surveys carried out on the island of Crete have reported an increase in intake of meat, fish, and cheese and a decrease in intakes of bread, fruit, potatoes, and olive oil (50). Similar changes have been observed in Italy (51). An increased availability of animal foods throughout the Mediterranean area has also been documented (47). These dietary changes have been accompanied by increases in chronic disease risk factors such as higher concentrations of serum cholesterol, hypertension, and obesity as well as reduced levels of physical activity (50,52).

Chronic disease risk is increasing, not only in the Western society, but also in the more affluent classes of the developing countries (53). The rich in poor countries often have a similar pattern of food consumption to that observed in the affluent countries. They are also subject to many of the same lifestyle factors, including smoking and reduced physical activity.

Dietary Guidelines (Table 3) can help in reducing both heart disease and cancer risk. The guidelines now emphasize moderation in intake, especially of saturated fat, along with increased physical activity. Increasing intakes of fruits, vegetables, and complex carbohydrates are also recommended.

Paradoxically, these present recommendations for the affluent define diets and lifestyles closer to those common in the past for the less affluent. As a further paradox, these latter societies, as their wealth increases, are often attempting to emulate the diets and lifestyles of the West. Consequently, they are now increasingly subject to the same pattern of disease.

Table 9. The Mediterranean Diet Plan

Frequency of consumption	Foods
In significant amounts	
Daily	Whole grains and grain products (breads, pasta, rice, couscous, polenta, bulgur) and potatoes Fruits and vegetables Beans, other legumes, and nuts
In small or minimal amounts	
Daily	Cheese and yogurt
A few times a week	Fish, poultry, eggs, and sweets
A few times a month	Red meat (or in small amounts more often)

In addition, regular physical activity is important. Moderate wine consumption is optional.

Source. Ref. 49.

BIBLIOGRAPHY

1. P. James, "The Nature of Food: Essential Requirements," in B. Harriss and Sir R. Hoffenberg, eds., *Food: Multidisciplinary Perspectives*, Blackwell Publishers, Oxford, UK, 1994, pp. 27–40.
2. F. X. Pi-Sunyer, "Obesity," in M. E. Shils, J. A. Olson, and M Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed, no. 2, Lea & Febiger, Philadelphia, Pa., 1994, pp. 984–1006.
3. D. F. Williamson, "Prevalence and Demographics of Obesity," in K. D. Brownell and C G. Fairburn, eds., *Eating disorders and Obesity: A Comprehensive Handbook*, The Guilford Press, New York, 1995, pp. 391–395.
4. National Institutes of Health, "NIH Consensus Development Conference Statement: Health Implications of Obesity" (Feb. 11–13, 1985). URL: *http://text.nlm.nih.gov/nih/cdc/www/49txt.html* (last accessed Aug. 18, 1998).
5. National Institutes of Health and National Heart, Lung and Blood Institute, "First Federal Obesity Guidelines Released," URL: *http://www.nih.gov/news/pr/jun98/nhlbi-17.html* (last accessed June 17, 1998: 1–4).
6. Heart Information Network, "Most Americans Are Overweight," URL: *http://www.heartinfo.org/mosamfat197.html* (last accessed Aug. 18, 1998: 1–2)
7. U.S. Department of Agriculture, U.S. Department of Health and Human Services, *Nutrition and Your Health: Dietary Guidelines for Americans*, 4th ed., Home and Garden Bulletin No. 23, Hyattsville, Md.: U.S. Government Printing Office, Washington, D.C., 1995.
8. S. E Barlow and W. H. Dietz, "Obesity Evaluation and Treatment: Expert Committee Recommendations," *Pediatrics* **102**(3) (1998). URL: *http://www.pediatrics.org/cgi/content/full/102/3/e29* (last accessed Sept. 23, 1998: 1–11).
9. American Heart Association, "High Blood Pressure: AHA Recommendation," URL: *http://www/amhrt.org/Heart_and_Stroke_A_Z_Guide/hbp.html* (last accessed Aug. 18, 1998: 1–2).
10. American Heart Association, "High Blood Pressure Causes," URL: *http://www/amhrt.org/Heart_and_Stroke_A_Z_Guide/hbpc.html* (last accessed Aug. 18, 1998: 1).
11. American Heart Association, "High Blood Pressure, Factors That Contribute To," URL: *http://www/amhrt.org/Heart_and_Stroke_A_Z_Guide/hbpf.html* (last accessed Aug. 18, 1998: 1–2).
12. T. A. Kotchen and J. M. Kotchen, "Nutrition, Diet and Hypertension," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., no 2, Lea & Febiger, Philadelphia, Pa., 1994, pp. 1287–1297.
13. J. W. Anderson and P. B. Geil, "Nutritional Management of Diabetes Mellitus," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., no. 2, Lea & Febiger, Philadelphia, Pa., 1994, pp. 1259–1286.
14. U.S. Department of Health and Human Services: Public Health Service, Centers for Disease Control and Prevention, "Preventing Cardiovascular Disease," URL: *http://www.cdc.gov/nccdphp/cvd/cvdaag.html* (last accessed Aug. 18, 1998: 1–8).
15. D. J. McNamara, "Cardiovascular Disease," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., no. 2, Lea & Febiger, Philadelphia, Pa., 1994, pp. 1533–1544.
16. American Heart Association, "Risk Factors and Coronary Heart Disease," URL: *http://www/amhrt.org/Heart_and_Stroke_A_Z_Guide/riskfact.html* (last accessed Aug. 18, 1998: 1–3).

17. A. S. Truswell, "The Evolution of Diets for Western Diseases," in B. Harriss and Sir R. Hoffenberg, eds., *Food. Multidisciplinary Perspectives*, Blackwell Publishers, Oxford, UK, 1994, pp. 41–62.
18. A. Keys et al., "Lessons from Serum Cholesterol Studies in Japan, Hawaii and Los Angeles," *Ann. Intern. Med.* **48**, 83–94 (1958).
19. W B. Kannel et al., "Serum Cholesterol, Lipoproteins and the Risk of Coronary Heart Disease. The Framingham Study," *Ann. Intern. Med.* **74**, 1–12 (1971).
20. S. M. Mellinkoff, T. A. Marchella, and J. G. Reinhold, "The Effect of a Fat Free Diet in Causing Low Serum Cholesterol," *Amer. J. Med. Sci.* **220**, 203–207 (1950).
21. A. Keys, "Coronary Heart Disease in Seven Countries," *Circulation* **41** (Suppl. 1), 1–211 (1970).
22. A. Keys, *Seven Countries. A Multivariate Analysis of Death and Coronary Heart Disease*, Harvard Univ. Press, Cambridge, Mass., 1980.
23. R. P. Mensink and M B. Katan, "Effect of a Diet Enriched with Monounsaturated or Polyunsaturated Fatty Acids on the Levels of Low-Density and High-Density Lipoprotein Cholesterol in Healthy Men and Women," *New Engl. J. Med.* **321**, 436–441 (1989).
24. R. P. Mensink and M. B Katan, "Effect of Dietary Trans Fatty Acids on the High and Low Density Lipoprotein Cholesterol Levels in Healthy Subjects," *New Engl. J. Med.* **323**, 436–445 (1990).
25. H. Gerster, "Can Adults Adequately Convert Alpha-Linolenic Acid to Eicosapentaenoic Acid and Docosahexaenoic Acid," *Int J. Vitamin Nutr. Res.* **68**(3), 159–173 (1998).
26. A. L. Gerhardt and N. B. Gallo, "Full Fat Rice Bran and Oat Bran Similarly Reduce Hypercholsterolemia in Humans," *J. Nutr.* 5 **128**(5), 865–869 (1998).
27. J. A. Simon and E. S. Hudes, "Relation of Serum Ascorbic Acid to Serum Lipids and Lipoproteins in US Adults," *J. Amer. College Nutr.* **17**(3), 250–255 (1998).
28. E B. Rimm et al., "Folate and Vitamin B6 from Diet and Supplements in Relation to Risk of Coronary Heart Disease among Women," *J. Amer. Med. Assoc.* **279**(5), 359–364 (1998).
29. M. E Shils, "Nutrition and Diet in Cancer Management," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., no 2, Lea & Febiger, Philadelphia, Pa., 1994, pp. 1317–1348.
30. M. W. Pariza, "Diet, Cancer and Food Safety," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., no. 2, Lea & Febiger, Philadelphia, Pa., 1994, pp. 1545–1558.
31. C. L. Chenney and S. N. Aker, "Nutritional Care in Neoplastic Disease," in L. K. Mahan and M. Arlin, eds., *Krause's Food Nutrition and Diet Therapy*, 8th ed., W.B. Saunders Company, Harcourt Brace Jovanovich Inc., Philadelphia, Pa., 1992, pp. 625–642.
32. A. Tannenbaum, "The Genesis and Growth of Tumors. III Effects of a High Fat Diet," *Cancer Res.* **2**, 460 (1942).
33. T. L. Dao and R. Hilf, "Dietary Fat and Breast Cancer: A Search for Mechanisms," in Maryce M. Jacobs, ed., *Exercise, Calories, Fat and Cancer, Advances in Experimental Medicine and Biology 322*, Plenum Press, New York, 1992, pp. 223–238.
34. K. K. Carroll and H. T. Khor, "Dietary Fat in Relation to Tumorigenesis," *Prog. Biochem. Pharmacol.* **10**, 308 (1975).
35. S. Graham et al., "Diet in the Epidemiology of Breast Cancer," *Amer. J. Epidemiol.* **116**, 68–75 (1982).
36. G. R. Howe et al., "Dietary Factors and the Risk of Breast Cancer: Combined Analysis of 12 Case-Control Studies," *J. Nat. Cancer Inst.* **82**, 561–569 (1990).
37. R. K. Boutwell, "Caloric Intake, Dietary Fat Level and Experimental Carcinogenesis," in Maryce M. Jacobs, ed., *Exercise, Calories, Fat and Cancer, Advances in Experimental Medicine and Biology 322*, Plenum Press, New York, 1992, pp. 95–101.
38. C. La. Vecchia, "Mediterranean Epidemiological Evidence on Tomatoes and the Prevention of Digestive Tract Cancers," *Proc. Soc. Exp. Biol. Med.* **218**(2), 125–128 (1998).
39. U.S. Department of Health and Human Services, *The Surgeon General's Report on Nutrition and Health*, U.S. Government Printing Office, Washington, D.C., Publication No. 88-50210, 1988, 712 pp.
40. Health News, "Study Finds Broccoli Sprouts Have High Levels of Anticancer Chemical," URL: *http://www.canoe.ca/HealthNutrition/sep15-broccoli.html* (last accessed Sept. 15, 1997: 1–2).
41. P. E. Garfinkel, "Classification and Diagnosis of Eating Disorders," in K. D. Brownell and C. G. Fairburn, eds., *Eating and Obesity: A Comprehensive Handbook*, The Guilford Press, New York, 1995, pp. 125–134.
42. P. J V. Beumont, "The Clinical Presentation of Anorexia and Bulimia Nervosa," in K. D. Brownell and C. G. Fairburn, eds., *Eating Disorders and Obesity: A Comprehensive Handbook*, The Guilford Press, New York, 1995, pp. 151–158.
43. H. W. Hoek, "The Distribution of Eating Disorders," in K. D. Brownell and C. G. Fairburn, eds., *Eating Disorders and Obesity: A Comprehensive Handbook*, The Guilford Press, New York, 1995, pp. 207–211.
44. J. Garrow, "Diseases of Diet in Affluent Societies," in B. Harris and Sir R. Hoffenberg, eds., *Food: Multidisciplinary Perspectives*, Blackwell Publishers, Oxford, UK, 1994.
45. J. Waterlow, "Diet in the Classical Period of Greece and Rome," *Eur. J. Clin. Nutr.* **43** (Suppl. 2), 3–12 (1989).
46. A. Keys, "Mediterranean Diet and Public Health: Personal Reflections," *Amer. J. Clin. Nutr.* **61** (Suppl.), 1321S–1323S (1995).
47. M. Nestle, "Mediterranean Diets: Historical and Research Overview," *Amer. J. Clin. Nutr.* **61** (Suppl.), 1313S–1320S (1995).
48. A. Keys et al., "Studies on Serum Cholesterol and Other Characteristics on Clinically Healthy Men in Naples," *Arch. Intern. Med.* **93**, 328–335 (1954).
49. D. Schardt, B. Liebman, and S. Schmidt, "Going Mediterranean," *Nutr. Action Health Lett.* **21**, 1, 5–7 (1994).
50. A. Kafatos et al., "Coronary Heart Disease Risk Factor Status of the Cretan Urban Population in the 1980s," *Amer. J. Clin. Nutr.* **54**, 591–598 (1991).
51. A. Ferro-Luzzi and F. Branca, "The Mediterranean Diet, Italian Style: Prototype of a Healthy Diet," *Amer J. Clin. Nutr.* **61** (Suppl.), 1338S–1345S (1995).
52. G. A. Spiller, ed., *The Mediterranean Diets in Health and Disease*, AVI, Van Nostrand Reinhold, New York, 1991.
53. World Health Organization, "Diet, Nutrition, and the Prevention of Chronic Diseases," *Technical Report Series* No. 797, World Health Organization, Geneva, Switzerland, 1990.

S. Ghosh
P. L. Pellett
University of Massachusetts
Amherst, Massachusetts

AFLATOXINS. See MYCOTOXINS.

AGGLOMERATION AND AGGLOMERATOR SYSTEMS

GENERAL ASPECTS

Numerous food powders experience significant changes in their properties during storage, transportation, or processing, which are related to the particle size distribution. Attrition causes reduction in average particle size while aggregation increases it. Fines generated by attrition may either form clusters or coat larger particles (plating). Interparticle adhesion is decisively influenced by particle size, the ratio between adhesion and weight usually being inversely proportional to the square of the particle size (1). As a result, this ratio is two orders of magnitude higher for particles of 10 μm than for particles of 100 μm. Dry food powders with average sizes of 80 to 100 μm are usually free flowing, whereas powders having sizes below 20 to 30 μm become cohesive, form secondary particles (clusters) of larger size, and form lumps when rewetted.

Adhesion without formation of bridges between the adjacent particles occurs as a result of either van der Waals or electrostatic forces and causes the formation of comparatively weak agglomerates. Adhesion associated with the formation of bridges produces much stronger agglomerates. Free flowing and cohesive food powders may undergo segregation during their storage, transportation, and handling. Primarily because of the differences in particle size and also in density, shape, and resilience, fine particles migrate to the bottom while large particles find themselves at the top of the vessel. As a result, some minor components of beverage blends (colors, flavors, vitamins) may become unevenly distributed between packages.

The purpose of particle size enlargement by agglomeration is to improve powder properties like bulk density, flowability, meterability, dusting, powder mix homogeneity, storage stability, and optical appearance. Powdered foods, which are in most cases intended to be dispersed in liquid, should also have good wettability, sinkability, dispersibility, and (for soluble materials) solubility, that is good "instant properties." A powder layer spread on a liquid surface should imbibe the liquid, submerge, disperse, and dissolve within a few seconds with little mechanical aid and without forming lumps. A powder treated by a technical process to have such properties is called "instantized." Agglomeration is the predominant method for instantizing powdered foods, and an example of the dependency of the wetting time of a powder layer on the average agglomerate size is shown in Figure 1.

Another major quality factor for instant foods is the preservation of flavor components. Instant beverages containing, for example, coffee extract are particularly susceptible to flavor loss caused by high temperature or excessive contact with air, as, for example, in a fluidized bed.

Obviously, simultaneous improvement of all powder properties is impossible. Increasing agglomerate stability, for example, results in most cases in a decline of the instant properties. The way the agglomerates are formed in the production process determines their properties, and comprehension of the basic physical principles of particle adhesion and the mechanisms likely to predominate in a given agglomeration process is helpful.

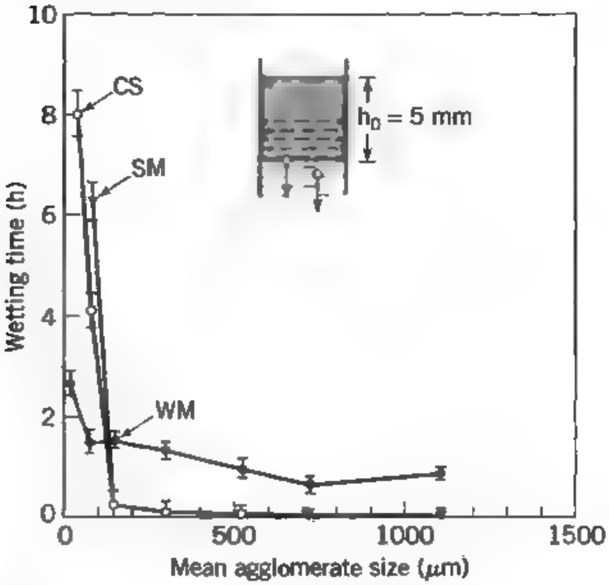


Figure 1. Wetting time (including standard deviations) of a powder layer of height h_0 = 5 mm for three commercial instantized food powders. CS, cocoa-sugar mix (21°C); SM, skim milk powder (21°C); WM, whole milk powder (50°C).

UNDESIRED AGGLOMERATION—CAKING

Agglomeration via caking may occur unintentionally since blends of particles are always exposed for some time to the ambient environmental conditions (temperature and/or humidity). For example, food powders that include lipids (soups, sauces, baking mixes) may undergo caking if the temperature exceeds the melting point of the lipids. As a result, sticky liquid bridges are formed. Once cooled, the lipids recrystallize, liquid bridges between particles become solid, and caking is reinforced. Although starchy and proteinaceous components are relatively insensitive to the environmental conditions, the soluble components of food powders (sugars, salts) absorb moisture and eventually change their state from solid to liquid.

The ability of sugars to soften depends on the conditions under which they were produced and stored. These conditions are responsible for the formation of areas of crystalline or amorphous structure. Amorphous sugars absorb much more moisture at a given water activity (relative humidity) and have lower glass-transition temperatures than crystalline sugars (2). Whereas a stable, crystalline structure is formed at equilibrium conditions, the amorphous one is created at nonequilibrium conditions. Relatively slow moisture withdrawal during carefully controlled crystallization (nuclei formation and crystal growth) leads to the development of a crystalline structure. Fast moisture withdrawal from a solution of carbohydrate via spray drying, roller drying, or freeze drying helps to produce mainly the amorphous form; even the mechanical impact of milling of sugar crystals produces an amorphous surface capable of recrystallization after absorbing water (3). Upon recrystallization, amorphous sucrose releases water, which facilitates formation of bridges between particles and initiates caking. Adding high molecular weight components (eg, maltodextrin) to a blend containing sugars may reduce caking (4).

Caking may be effectively suppressed by adding anticaking agents like tricalcium phosphate, magnesium oxide, calcium silicate, and so on, which absorb a portion of moisture from the blend and thus reduce the amount of available moisture. Although total moisture content of the blend with or without anticaking agent stays virtually unchanged, it is relative humidity generated by the blend in a sealed chamber that reflects the amount of available moisture: blend with added anticaking agent generates lower RH than blend without anticaking agent. The effectiveness of the anticaking agents depends largely on their water-holding capacity, so that with an unlimited source of humidity (open storage), their impact is lessened.

Even packaged food powders may undergo caking influenced by the environment inside their packages. Being relatively isolated, the headspace inside the package is affected not only by the surface moisture of the particles and temperature in the warehouse, but by the permeability and heat conductivity of the package film. Variations in the temperature and humidity outside of the packaged material often accelerate an exchange in surface moisture between the ingredients and initiate caking.

AGGLOMERATION METHODS

The first step in any agglomeration process (except drying methods starting with a slurry) is to make the primary particles contact each other, which is frequently achieved by external force. Powders for instant products usually consist of primary particles smaller than 200 μm to facilitate solubility. In a second step, permanent adhesion forces stronger than any possibly existing disruptive forces must be established between these particles. For food powders, this is usually achieved by wetting (which causes partial dissolution and the development of liquid bridges) and subsequent drying (which leaves solid bonds in place of the liquid bridges).

The duration and intensity of the forces acting among the particles during agglomerate formation and stabilization have an important influence on agglomerate porosity and stability. For example, an agglomeration process in which high forces act on the particles and agglomerates will turn out dense, smooth, and stable agglomerates that are easy to handle and dispense. However, instant properties would be poor owing to low agglomerate porosity and strong bonds between the primary particles. Such a process, like compaction, would be inappropriate for instantizing.

The final product should have the following properties:

- sufficient agglomerate porosity for fast liquid suction by capillary action, although a critical porosity must not be exceeded (5);
- particle size in the range of 0.2 to 2 mm; and
- sufficient agglomerate strength to withstand handling and transportation.

Agglomeration processes suitable for producing instantized food powders can be divided into three groups:

- moist agglomeration,
- agglomeration by drying, and
- combined methods.

Moist Agglomeration

Moist agglomeration, using capillary and liquid bridge forces to achieve sufficient interparticle adhesion during agglomeration, is the most important process for the production of instantized powders. This method starts from dry powder, which is moistened either by condensing vapor, atomized liquid, or a mixture of both. The material is then dried and solid bridges between the primary particles provide the necessary strength. A large variety of equipment is available for moist agglomeration. Schematics of some typical examples are shown in Figure 2.

Except for the static process Figure 2f, all methods are "dynamic"; that is, agglomerates are formed due to the collision and subsequent adhesion of the particles. Common features of all processes shown in Figure 2 are the moistening of the dry powder, the size enlargement of the wet particles, and subsequent drying and cooling, if required. The drying rate and temperature considerably influence the strength of the dry agglomerates because of different crystal structures and distributions of the interparticle solid bridges formed by crystallization of dissolved substances during drying (6).

Fluidized-bed agglomerators for batchwise or continuous production, Figure 2a and 2b, are provided by numerous manufacturers. Units for continuous operation (eg, APV Anhydro) utilize either a moisture product feed or a rewet system so that sufficient moisture is present to agglomerate the product. A typical unit has three fluidized zones. These include the entry or wetting zone, a drying zone, and a cooling zone.

Also, several mechanical agglomerators utilize mechanical mixers to provide the liquid addition, product interaction, and mixing to facilitate the agglomeration process.

The "SCHUGI" mixer (Bepex Corp.), shown in Figure 2d, has extremely short residence times (approximately 1 s) and has considerable flexibility in the types and amounts of feedstock. The system employs a flexible housing so that product buildup on the interior walls of the agglomerator is minimized, if not eliminated (7).

Jet agglomeration (Fig. 2e) has been used in the food industry for several years to produce agglomerates with favorable instant properties from fine powders. In a jet agglomeration plant, freely moving, wetted particles are made to collide with each other to form agglomerates. The solid material fed to the agglomerator consists of individ-

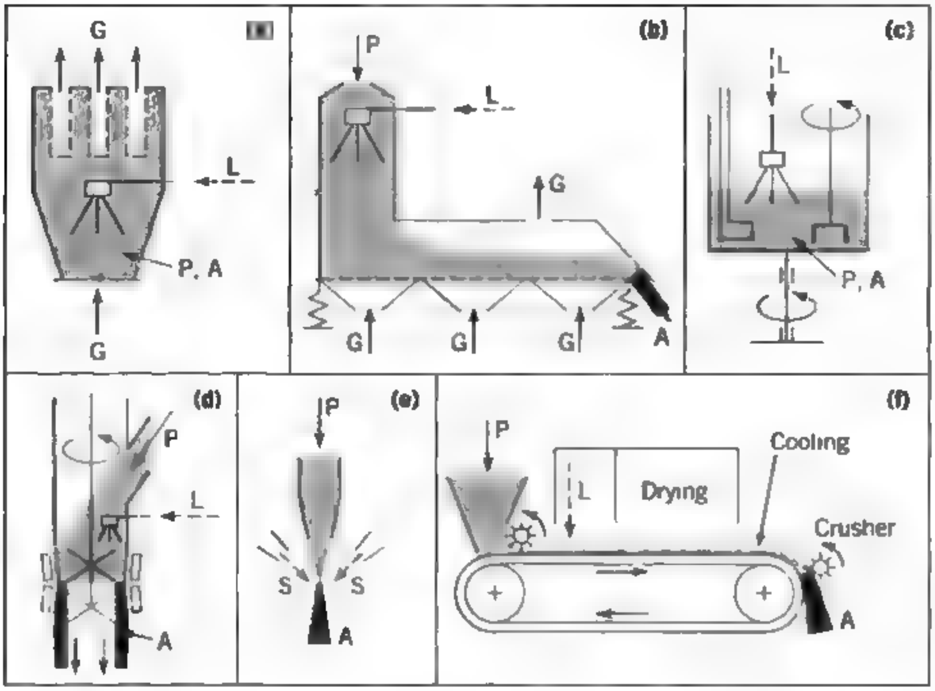


Figure 2. Principles of moist agglomeration processes for the production of instantized powdered foods. (**a**) Batch fluidized-bed agglomerator, (**b**) continuous mixing chamber with integrated fluidized-bed agglomeration, (**c**) batch mixer agglomerator, (**d**) continuous mixer agglomerator with a perpendicular balling region ("SCHUGI" mixer), (**e**) continuous steam fusion process ("jet agglomeration"), (**f**) continuous belt aggomerator. A, agglomerates; G, gas; L, liquid; P, powder; S, steam.

ual particles and dry preagglomerates bound mainly by van der Waals forces. The effectiveness of this method depends on a variety of process parameters influencing interparticle collision frequency, relative velocities of the particles, interparticle contact forces between wetted particles, and strain on the agglomerates (8).

Drying Processes

The second main group of processes for agglomerating food powders comprises special drying processes. Two examples are shown schematically in Figure 3, a spray drier with fluidized bed and a freeze drier.

Spray drying is one of the most widely used processes in food powder technology. The concentrate/slurry, either in the form of a suspension or a solution, is finely distributed using a nozzle or an atomizer disc, dried, and cooled in a connected fluidized bed from which the product is withdrawn in agglomerated form. The fluidized bed also serves the purpose of removing the fines, which are collected in a cyclone and recycled into the spray drier. Agglomeration occurs in the vicinity of the nozzle or atomizer, where the fine dry particles collide with the slurry droplets. In many cases the product is also after-dried in the fluidized bed, a process referred to as two-stage drying.

Freeze drying is relatively expensive but especially useful for products sensitive to high temperatures. Another advantage is the possibility to vary the porosity of the agglomerates over a wide range by foaming the concentrate before freezing (this can be achieved in a spray drier, too, by gassing the slurry immediately before atomization). As a novel technology, microwave drying in a vacuum chamber can be used instead of freeze drying.

Combined Methods

Two examples for combined agglomeration methods are presented in Figure 4. These are special spray driers, the "Filtermat" by GEA Niro AIs (Soeborg, Denmark), with integrated perforated belt drier (Fig. 4a), and spray drying with integrated fluidized bed and fines recycling (Fig. 4b).

In the "Filtermat" process, the product is partially dried in the spray-dry section of the machine to a moisture content of approximately 16 to 25%, whereupon it is deposited by gravity onto the perforated belt and further dried to approximately 5 to 10% moisture. Sufficient moisture is present in the intermediate dried product so that agglomerates are formed on the belt. These are then classified and/or size-reduced to the desired size (9).

These methods allow production of very loose, but still sufficiently strong agglomerates (5) with good instant properties and have been widely used in the food industry.

Further Methods

Pressure agglomeration is rarely applied to the production of redispersible products, because the resulting agglomerates are so compact that they show insufficient dispersibility. Substances dispersing readily—due to a bursting effect of special additives—can be agglomerated using die pressing or low pressure extrusion processes. If no great demands are set upon the redispersibility, roll pressing is a useful and inexpensive process. Mostly low-pressure ring-roller presses are used in which the moistened powder is pressed through holes and thereby shaped into agglomerates.

A special kind of agglomeration is coating, to improve the wetting behavior of the particles. Usually the particles are coated by pure liquids, solutions, or suspensions that harden on the surface of the solid material. A novel technology is coating with submicron particles by "mechanofusion" (10) to improve the wettability and for other applications (11).

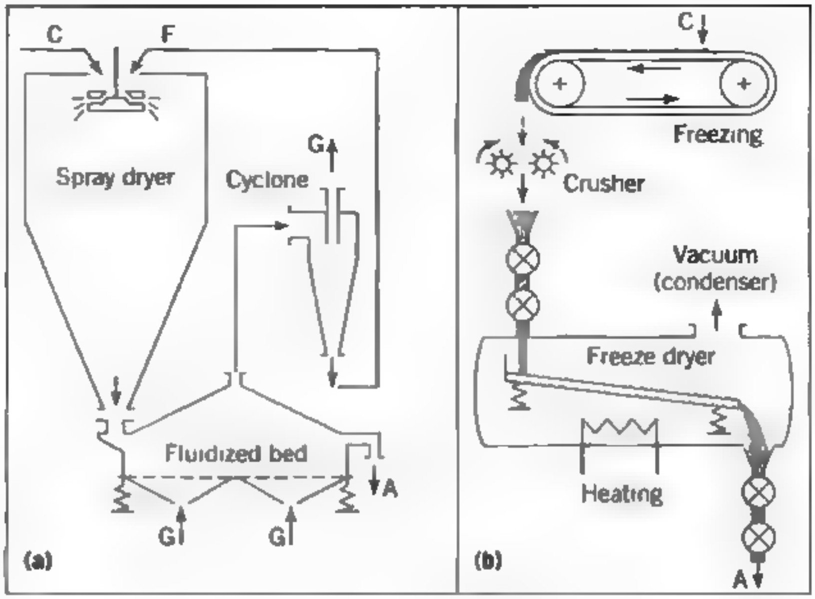


Figure 3. Principles of production of instantized powdered foods by spray drying (a) and freeze drying (b). A, agglomerates; C, concentrate; F, fines; G, gas.

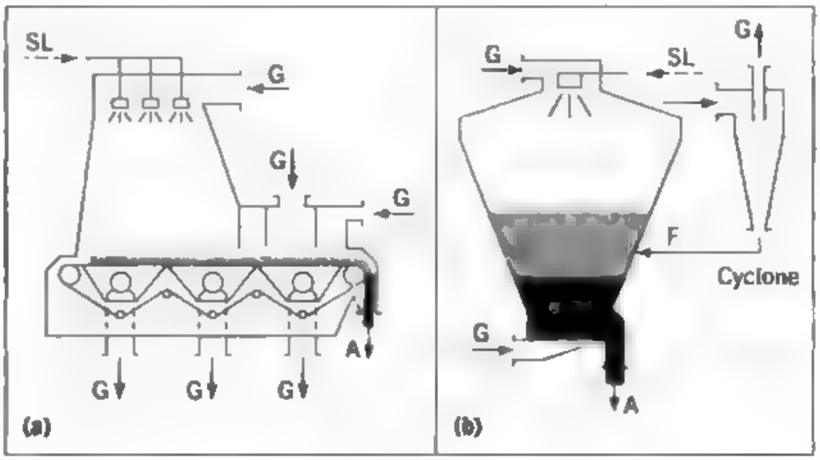


Figure 4. Principles of combined agglomeration processes for the production of instantized powdered foods: "Filtermat" spray drier with perforated belt drier (a) and spray drier with integrated fluidized bed and fines recycling (b). A, agglomerates; F, fines; G, gas; SL, slurry.

CHARACTERIZATION OF AGGLOMERATED POWDERS

Particle size distribution of agglomerated products should be measured using an adequately representative sample and with minimum disruption. Care should be taken to prevent swelling, dissolution, or disintegration. For sufficiently stable particles, sieving is still widely in use (dry for particles larger than 30 to 40 μm and wet for smaller particles), but over the past few years, laser diffraction has become a standard method due to the availability of a large number of different devices (Coulter, Malvern, Sympatec, etc). The last method is more versatile because it allows the measurement of larger particles with a dry feeder in a relatively nonviolent way and is also applicable for fines in a dispersing liquid. Automatic image analysis systems are a recent development employing charge-coupled device (CCD) camera imaging of particles falling out of a dry feeder.

Agglomerated particles may partially disintegrate when subjected to a more or less violent impact (vibration, shaking) during storage, transportation, and handling. In the case of agglomerated coffee, attrition is the primary cause in separation of fines from the outer surface of agglomerates (12). Formation of so-called secondary particles (shattering) from the disintegrated pieces is also taking place. The strength or resistance of agglomerated products to attrition may be measured by comparing its size distribution before and after rotation for a limited time inside a cylinder (friabilator). Other properties of agglomerates (bulk or free flow density, flowability, cohesion, angles of spatula and repose) are measured, for example, with the Hosokawa (MicroPul) tester.

For powdered foods, information about wetting and dispersing behavior is of special interest to the manufacturer. Wetting of a powder is easily tested by preparing a sample of defined height (5–8 mm) in a cylindrical testing vessel with a slide covering the liquid reservoir. When the slide is pulled out sideways, the powder sits on the liquid surface and is wetted. The wetting time measured in this way is useful for quality control purposes and for product property comparison.

Powder dispersion measurement is more difficult and depends on the ability of measuring the amount of material actually dispersed after the mixture/dispersion has been prepared in a defined way. For many products, this can be achieved by, for example, photometry (milk powder, cocoa beverages), conductometry (powdered extracts of coffee, tea) or refraction index measurement (sugars). The dispersed powder mass divided by the total powder mass in the sample is called degree of dispersibility (which varies between 0 and 1). Milk powder can reach a value of up to 0.8 (depending on fat content and age), instant cocoa beverages up to 0.98 and instant coffee up to 1.

PRODUCT EXAMPLES

Agglomerated food products are inseparable from some specific processes that were used to obtain these products. Steam fusion/steam jet agglomeration is used for agglomerating water-soluble instant beverage powders with high sugar content like cocoa drinks or products containing coffee extract (Fig. 5). After grinding, the particles with an average particle size of 25 to 75 μm are fed into an agglomeration chamber in the shape of dry clusters (average size up to the mm range). A uniformly distributed curtain of powder moves downward where it interacts with jets of steam. Steam wets the particles and fuses them into agglomerates. The wetted agglomerates pass from the top section of the agglomerating tower into a drying zone in the bottom portion of the tower, which is supplied with hot air. The agglomerates are dried and then cooled and screened. Critical parts of this agglomeration process are the feed port section, where initial cluster formation occurs, and the steam jet zone, where further agglomeration by collision of sticky clusters takes place (8).

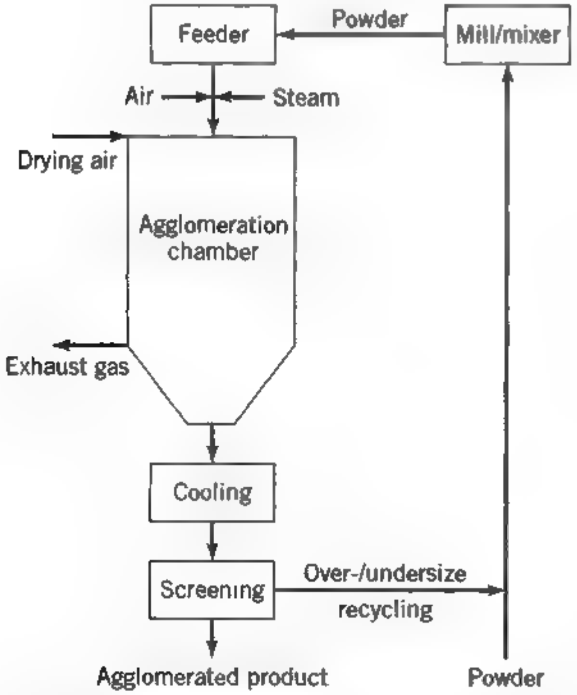


Figure 5. Principle flow diagram of a steam fusion/steam jet agglomeration process.

A method for making agglomerated bits containing aspartame includes preblending aspartame and a bulking agent (maltodextrin) to form a premix (13). The latter is then mixed with other dry ingredients (flavors, starch binders, dispersing agents, and vitamins) to form a dry mix. Liquid ingredients (vegetable oil and water) are blended into a dry mix with a ribbon blender or a paddle mixer to form moistened clumps. These granules must be dried in a forced-air convection oven and then screened to obtain a desirable particle size distribution. While starches (tapioca, corn, potato, modified wheat) and gums are used as the binders in forming agglomerates, baking soda and maltodextrin assist in the dispersion of the agglomerated product. These bits are suitable for use in home-cooked grain cereals and other foods.

Agglomerated potato granules can be prepared from a mixture of potato granules, egg white solids, and water (14). After the wet premix is formed, a gentle sieving, drying, and crushing are used to obtain agglomerates with the desired size and density.

Agglomerated bread crumbs may be produced from a starch containing raw material (flour, meal) and water in a continuous pellet mixer (15); subsequent baking in a humidified atmosphere (to control the desired gelatinization) and sizing (cutting) of the agglomerates are utilized. A controlled retrogradation (recrystallization of starch) occurs due to the controlled cooling process.

Agglomerated beverage blends having aspartame as a sweetener may be manufactured to prevent clumping of aspartame and to improve its water dispersibility (16). Agglomeration has been conducted in a jacketed blender so that a heating or cooling fluid may be passed through the jacket while the blender is rotated to provide adequate mixing. The blending time and temperature (but not, however, humidity) were controlled to obtain a desired agglomerate size distribution.

Aqueous, sugary syrups (honey, high fructose corn syrup, invert sugar, corn syrup, etc) were dehydrated using thin film drying in the presence of binders (soy protein and ungelatinized starch that was partially gelatinized *in situ*) (17). A spray of water was added during tumbling. The resultant agglomerates were dried and then slightly coated with a high melting point fat, apparently to prevent caking.

Agglomerates of garlic, onion, and their mixtures were produced in an upright chamber (18). Wetting of particles was provided by atomized water in the upper part of this chamber, followed by drying of the agglomerated product with air in the lower part of the chamber.

An agglomerated milk product was prepared by spraying a concentrate of milk into a stream of drying gas directed against the surface of a fluidized layer of already spray-dried particles (19). Adjustment of temperatures, flow rates of drying air, and residence times allowed better efficiency for skim milk, whole milk, and whey particles.

Agglomerates of meat analogues were prepared by extrusion cooking of soy concentrate, comminution of the extrudate, mixing it with a water slurry of binder, frying the mixture in edible fat or oil to produce an agglomerated mat, and sizing (20). Particles with the desired size distribution were used for meat-type sauces.

A porous and pelletized food product may be formed by premixing two or more ingredients, one of which is capable of forming sticky bonds after being moistened by an aqueous medium (21) This interaction occurs when the mixture of particles is tumbled and rolled on a pelletizing disc; the adhered particles form pellets or wet aggregates. Examples of a dry mix may include sugars, starches, dried milk products, proteinaceous materials, dehydrated juices, and powdered coffee concentrates. Some of these products (sugars, starches) may become self-adherent in contact with water and are used to form agglomerates. To provide pellets with controlled porosity, the particulate mixture also includes a chemical leavening system (sodium bicarbonate and leavening acid). Once moist agglomerates are in contact with hot air, two processes take place: drying and formation of gaseous carbon dioxide (due to a reaction between the bicarbonate and leavening acid). The resulting pellets have a porous, cellular structure with a crisp, crunchy, and friable texture.

The vast majority of gelatin dessert mixes require the use of hot water to dissolve the gelatin and an extended time (3–4 h) to prepare the meal. However, if gelatin-containing mix with a limited moisture content of 1 to 3% is agitated and slowly heated to 190 to 195°F, it forms agglomerates (22). Subsequent cooling helps to form so-called cold-water-soluble gelatin, which dissolves and disperses in water at 40 to 50°F. Agglomeration of gelatin mixes (ie, sucrose, gelatin, citric acid) was conducted in a jacketed rotating blender.

Other agglomerated (instantized) products include maltodextrin and dextrose, which may be used as carriers for flavors, colors, and nonnutritive sweeteners in instant beverages and desserts (23,24), soy protein isolates for high-protein beverage blends and for better dispersibility in meat emulsions, prejelled starches and gums as soup thickeners, whey protein concentrates and calcium caseinates for dairy blends, all developed by IFT, Inc. (25), and coated animal feed with improved nutritional value (26). Powders such as egg proteins, cocoa, or various fibers will have improved dispersibility after agglomeration in the presence of maltodextrin or surfactants (27).

BIBLIOGRAPHY

1. H. Schubert, "Food Particle Technology. Part 1: Properties of Particles and Particulate Food Systems," *J. Food Technol.* **6**, 1–32 (1987).
2. B. Makower and W. B. Dye, "Equilibrium Moisture Content and Crystallization of Amorphous Sucrose and Glucose," *Agric. Food Chem.* **4**, 72–77 (1984).
3. J. M. Flink, "Transition in Dried Carbohydrates," in M. Peleg and E B. Bagley, eds., *Physical Properties of Foods*, AVI Publishing, Westport, Conn., 1983.
4. E. C. To and J M. Flink, "Collapse," *J. Food Technol.* **13**, 551–594 (1978).
5. H. Schubert, "Instantization of powdered food products" *Int. Chem. Eng.* **33**, 28–45 (1993).
6. W. Pietsch, *Size Enlargement by Agglomeration*, Wiley, New York, 1991.
7. P. Koenig et al., *Bepex Corp. Product Literature*, Bepex Corp., Minneapolis, Minn.

[illegible] S. Hogekamp, "Über eine modifizierte Strahlagglomerationsanlage zur Herstellung schnell dispergierbarer Pulver," Ph.D. dissertation, Universität Karlsruhe, 1997

[illegible] S. Mignat, "Spray Drying and Instantization—A Survey," Dragoco Report, Factoring Information Service 4, Holzminden, Germany, 1988, pp. 105–116.

10. T. Yokoyama, "Mechanofusion," Micromeritics Laboratory, Hosokawa Micron Corp., Osaka, Japan, 1988
11. D. J. Harron, "Particle Fabrication: New Method of Agglomerating Heterogeneous Powders," *Proc. Fifth International Symposium on Agglomeration*, Brighton, UK, Inst. Chem. Engs., Rugby, UK, 1989, pp. 175–185.
12. J. Malave-Lopez and M. Peleg, "Patterns of Size Distribution Changes during the Attrition of Instant Coffees," *J. Food Sci.* **51**(3), 691–694 (1986).
13. U.S. Pat. 4,741,910 (May 3, 1988), J. Karwowski and A. M. Magliacano (to Nabisco Brands, Inc.).
14. U.S. Pat. 4,797,292 (Jan. 10, 1989), J. DeWit (to Nestec S.A.).
15. U.S. Pat. 4,344,975 (Aug. 17, 1982), W. Seiler (to Gebruder Buhler AG).
16. U.S. Pat. 4,554,167 (Nov. 19, 1985), R. M. Sorge et al. (to General Foods Corp.).
17. U.S. Pat. 3,941,893 (Mar. 2, 1976), E. F. Glabe et al. (to Food Technology, Inc.).
18. U.S. Pat. 4,394,394 (July 19, 1983), L. J. Nava and N. L. Ewing (to Foremost-McKesson, Inc.).
19. U.S. Pat. 4,490,403 (Dec. 25, 1984), J. Pisecky et al. (to A/S Niro Atomizer)
20. U.S. Pat. 4,441,461 (May 8, 1984), P. J. Loos et al. (to the Procter and Gamble Co.).
21. U.S. Pat. 4,310,560 (Jan. 12, 1982), R. C. Doster and S. L. Nelson (to Carnation Co.).
22. U.S. Pat. 4,571,346 (Feb. 18, 1986), D M. Lehmann et al. (to General Foods Corp.).
23. "New Ingredients," *Food Technol.* **43**(1), 169 (1989).
24. "A Sweetener for Specialized Applications," *Food Eng.* **59**(9), 56 (1986).
25. "Instantizing Ingredients," *Food Eng.* **57**(3), 58–59 (1984).
26. U.S. Pat. 5,786,008 (July 28, 1997), R. Humphry et al. (to Moorman Manufacturing Co.).
27. D. D. Duxbury, "Innovative Ingredient Properties Enhanced, Customized by Agglomeration, Coating Process," *Food Processing* **49**(5), 96–100 (May 1988).

STEFAN HOGEKAMP
HELMAR SCHUBERT
Universität Karlsruhe
Karlsruhe, Germany

ALCOHOL, POLYHYDRIC. See SUGAR: SUBSTITUTES, BULK, REDUCED CALORIE; SWEETENERS: NUTRITIVE.

ALCOHOLIC BEVERAGES AND HUMAN RESPONSES

Alcoholic beverages are, in essence, flavored solutions of ethanol. The flavors may come from grains, as in beer; or from grapes and other fruit, as in wine; or from any source of carbohydrates, grains, sugar, or grapes, as in whiskey, rum, and brandy. In addition, consumers may add their own flavors, as lime with some beers or fruits with some wine or carbonated sodas with distilled spirits. The spectrum of flavors is wide indeed. But the purpose of drinking any of these is to supply ethanol in measured doses to the user.

Ethanol is as unique as humanity itself. It is a food but requires no digestion. It acts on many organs in the body but has no cellular receptors as do all other drugs. It is stable in the atmosphere to any chemical change, whereas all other foods will undergo some kind of decomposition. It is the only food produced solely by microbial action. It enters any cell in the body, freely, without any transport mechanism. All other foods (and all other substances except water) require a transport mechanism to enter any cell. It provides energy more rapidly than any other food.

This article inquires more closely into these and other aspects of ethanol. Although alcohol is a generic term for a large group of related substances, so common is ethanol that the term alcohol has been usurped for it and will be used here from now on to mean ethanol. So common is the drinking of alcoholic beverages that the word drink or drinker implies the drinking of alcoholic beverages and not any others.

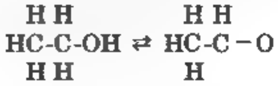
People have always needed a release from reality. From earliest recorded history this release has come quite effectively from alcohol. It must have been discovered by accident, and probably in more than one place. It is readily produced from any *saccharous* source, is pleasant tasting, and not prone to any pathogenic divergence.

Whether alcohol appeared first from grapes as wine or from grain as beer or from honey as mead is not known. The catalyst that converts any of these into alcohol is ubiquitous. A recipe for beer has been found on a clay tablet from Mesopotamia some 4000 yr old. It was probably known during the new Stone Age, some 6000 yr ago. All but three or four of the many cultures that have survived to modern times knew alcohol. It is absent from polar people and Australian aborigines.

Probably the nature of the alcohol in any culture depended on the prevalence of the source. In cool northern Europe it was likely to be beer or mead. In the Near East it may have been beer or wine. In the Far East it was probably beer. In early cultures the making of alcohol was so cherished that it fell under the domain of the priest and clergy. Vestiges of this still remain in many monasteries in Europe.

GENERAL METABOLISM OF ALCOHOL

The first step in the metabolism of alcohol is a dehydrogenation to acetaldehyde.



$$\begin{matrix} \mathrm{H\,H} & & \mathrm{H\,H} \\ \mathrm{HC\text{-}C\text{-}OH} & \rightleftarrows & \mathrm{HC\text{-}C-O} \\ \mathrm{H\,H} & & \mathrm{H} \end{matrix}$$

This is mediated by the enzyme alcohol dehydrogenase, with nicotine adenine dinucleotide (NAD^+) as hydrogen acceptor. The reaction is reversible, and the reverse reaction is the last step in the process by which alcohol is produced by yeast.

This reaction is followed by the oxidation of the aldehyde to acetate, brought about by another enzyme, aldehyde dehydrogenase, again with NAD^+. This reaction has never been reversed. The acetate in turn joins with coenzyme A to form the ever-present acetyl CoA. This can take part in the citric acid cycle and be oxidized to CO_2 and H_2O. This scheme is shown in Figure 1.

Alcohol dehydrogenase (ADH) exists in about 20 forms, each with differing activity toward ethanol and to other alcohols. These isozymes vary in concentration among diverse ethnic groups, no doubt accounting for different sensitivities to alcohol by different peoples. All forms have zinc as the core metallic element. ADH is found in all tissues, including red and white blood cells and the brain. Before 1970, it was thought that ADH existed only in the liver, but that is certainly not the case. That it is present in many isosteric forms is probably rooted in the many functions it performs and the many needs it satisfies in metabolism.

Aldehyde dehydrogenase (ALDH) also exists widely in humans. Cytoplasmic ALDH is the same in all people, whereas the mitochondrial ALDH does differ among people, with that found in Asians being less active than the form found in whites. But it is probable that mitochondrial ALDH is not nearly as important in oxidizing acetaldehyde as is cytoplasmic ALDH. Because very little acetaldehyde is found circulating in the blood even after high alcohol intake, it is assumed that the rate-limiting step in alcohol metabolism is the first step—its dehydrogenation to acetaldehyde.

HOW ALCOHOL IS CONSUMED

Wine

The fermentation of the juice of grapes produces a wine containing about 12% alcohol by volume (10% by weight). The stoichiometry of fermentation,

$$\begin{array}{ccccc} C_6H_{12}O_6 & \rightarrow & 2C_2H_5OH & + & 2CO_2 \\ 180 & & 92 & & 88 \end{array}$$

dictates that a 22°Brix grape juice will give an alcohol solution of somewhat more than 10% by weight. Perhaps by evolutionary coincidence, a 10% alcohol solution is close to the limit that most yeasts can produce.

Because most countries forbid the addition of water to grape juice before fermentation, wines worldwide are very similar in alcohol content. Champagnes, which are fermented twice, may be a little higher, say 14% by volume. Fortified wines, such as port and sherry, are wines to which brandy has been added at some stage. These may contain as much as 20% alcohol by volume.

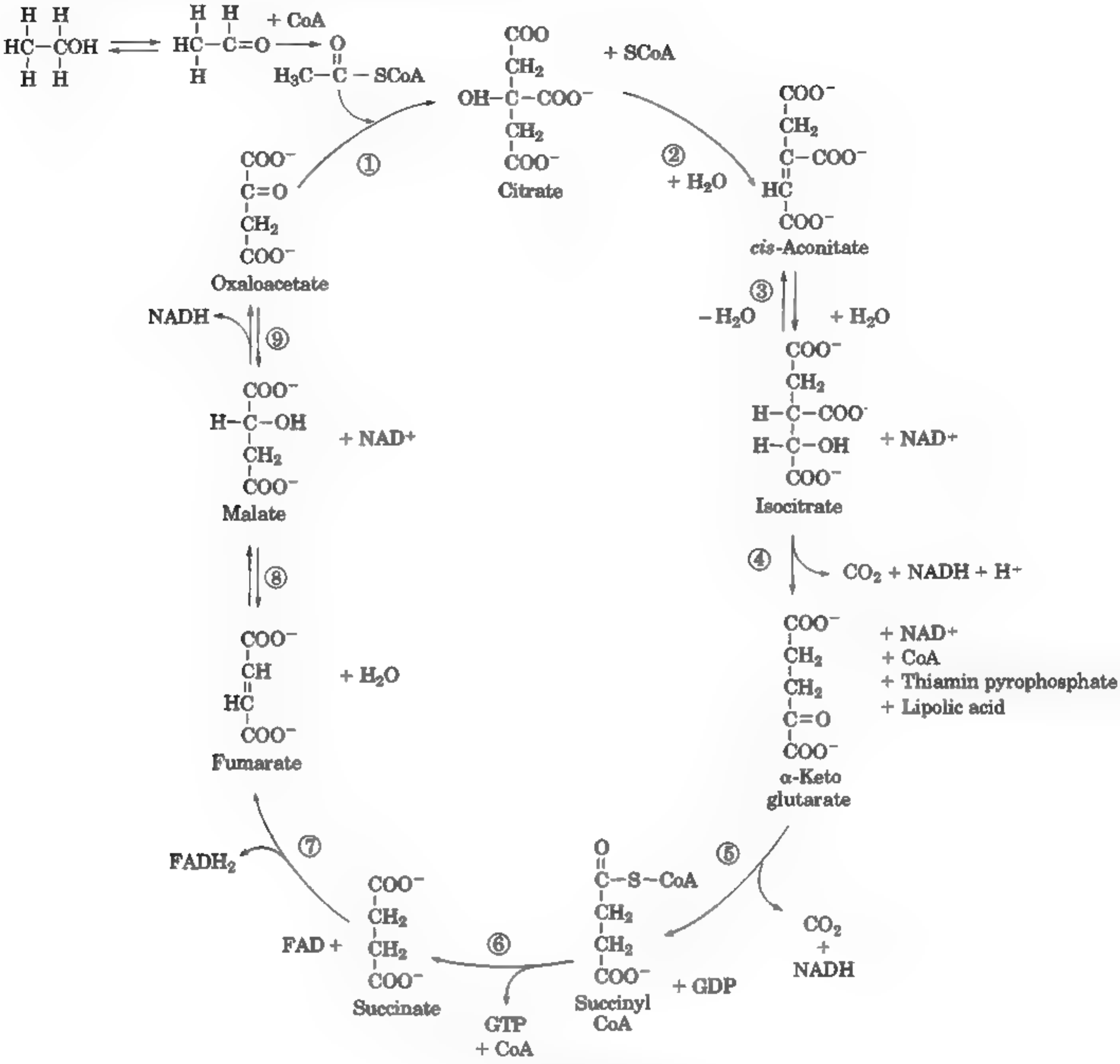

+ CoA
+ SCoA
Citrate
① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨
+ H₂O
cis-Aconitate
− H₂O
+ H₂O
Isocitrate
+ NAD+
CO₂ + NADH + H+
+ NAD+
+ CoA
+ Thiamin pyrophosphate
+ Lipolic acid
α-Keto glutarate
CO₂ + NADH
Succinyl CoA
+ GDP
GTP + CoA
Succinate
FAD +
FADH₂
Fumarate
+ H₂O
Malate
+ NAD+
NADH
Oxaloacetate

Figure 1. The citric acid cycle.

Other fruits may also be fermented to wine, but only apple juice, as cider, has found even limited acceptance.

Beer

The solution from which beer is made, called wort, is made at the brewery and not in nature. As such, its concentration and fermentability (see BEER) are designed for the beer being made and may vary widely. But the concentration of the wort only varies between 12 and 18°Brix, and the fermentability between about 60% and 90%. So beer is never as high in alcohol as wine. Typically, beer contains 5% alcohol by volume (4% by weight). So-called malt liquors contain about 6% alcohol by volume.

Mead

As honey is about 82% solids, it must be diluted to about 20°Brix and then fermented. The high price of honey and the rather weak flavor of its fermented solution has not permitted mead to be more than a historical curiosity.

Distilled Spirits

These spirits are distilled at a rather high proof, be they from grains (whiskey or vodka), molasses (rum), or wine (brandy), and are diluted to a marketable strength. The normal concentration is about 80 proof or 40% by volume or 32% by weight. Whiskeys are usually consumed after mixing with water, often carbonated, and also flavored. As purchased, wine is 2 stronger than beer, and whiskey is 3 or more times stronger than wine.

ABSORPTION OF ALCOHOL

Alcohol is absorbed into the bloodstream mainly from the upper small intestine. Although it enters the stomach, there is only a limited exit into the blood from that organ.

Emptying of the stomach's contents is controlled by the pyloric sphincter muscle at the base of the stomach. It opens when the pH of the contents falls below 3. The presence of proteins, which act as buffers, or fats, which delay access of the stomach's enzymes and acid to their substrates, delay the fall in pH and thus also the exit of food or drink from the stomach.

All the blood that nourishes the entire digestive tract, and that in turn carries all the digested foodstuffs from the tract, first enters the liver via the portal vein. Thus alcohol absorbed from the stomach or the small intestine goes first to the liver. This probably accounts for the old and incorrect belief that alcohol is oxidized to acetaldehyde only in the liver.

Alcohol consumed at high concentrations is absorbed more rapidly than when consumed in diluted forms. The presence of carbon dioxide also accelerates the absorption. Fatigue and exercise delay the absorption.

It has been recently found that a considerable amount of alcohol is metabolized in the stomach to acetaldehyde, and that the quantity oxidized is higher in men than in women and lower in alcoholic men and women (1). The blood alcohol curves for alcohol administered by mouth or intravenously for nonalcoholic and alcoholic men and women are shown in Figure 2. Of course, the alcohol metabolized in the stomach can have no effect elsewhere. This so-called first-pass metabolism accounts for the more ready effect that alcohol has on women than on men.

A comparison among whiskey, wine, and beer showing the maximum blood alcohol concentrations for the three beverages is shown in Table 1 (2). Wine and whiskey reach about the same maximum concentration, but whiskey is faster. Beer achieves a lower maximum, and it takes longer to get there.

At a lower alcohol level, the same relative relations are maintained, but the maximum concentrations attained are lower (Fig. 3) (3).

Table 2 shows the relationship between body weight, amount of beer and whiskey to reach certain blood alcohol levels, and the time at which the maximum concentration is reached. For example, an average person weighing 175 lb (79.5 kg) who consumes four 12-oz bottles of beer will have a maximum blood alcohol level of 0.08% in about 95 min (4).

Another set of experiments compared the sequential blood alcohol levels in a group of 50 men weighing between 145 and 175 lb who consumed 44 g of alcohol (four bottles of beer or four shots of whiskey) at once. The results in

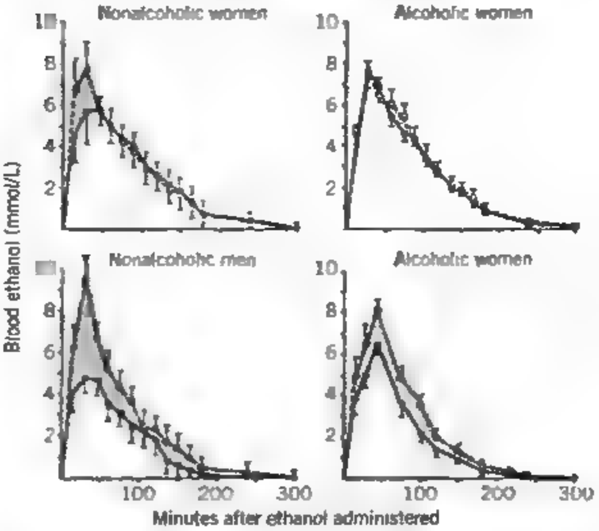


Figure 2. Effects of gender and chronic alcohol abuse on blood ethanol concentrations. Ethanol was administered orally (solid lines) or intravenously (dashed lines) in a dose of 0.3 g/body weight. The shaded area represents the difference between the curves for the two routes of administration (the first-pass metabolism).

Table 1. Effects of Alcoholic Beverages on the Maximum Blood Alcohol Levels and the Time at Which These Levels Are Reached

	c_{max}, %	t_{max}, min
Whiskey neat	0.108	57
Whiskey and water	0.107	71
Whiskey and soda water	0.104	51
Table wine	0.111	75
Beer	0.086	103

Note: Alcohol administered at a rate of 0.75 g/kg body weight. Equal to an all-at-once consumption of either four 12-oz. bottles of beer (for a 160-lb person) or two-thirds of a 750 mL bottle of 12% alcohol wine, or 3-1/2 shots (1-1/2 oz each) of 80-proof whiskey.

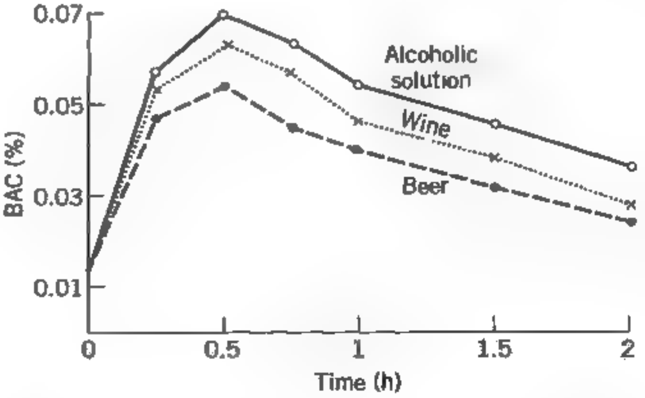


Figure 3. Blood alcohol curve after ingestion of 0.5 g alcohol per kg body weight in form of a diluted alcoholic solution (12.5%), of wine (11%), and of beer (5.5%) (mean value of 13 subjects).

Table 2.

Body weight, lb (kg)	g Alcohol per kg wt	To provide given amount of alcohol: No. of 12-oz bottles of beer (5%)	To provide given amount of alcohol: No. of 1-1/2-oz shots of whiskey (80 Proof)	Time to reach max. alc, min: Beer	Time to reach max. alc, min: Whiskey	Max. blood alcohol level, %: Beer	Max. blood alcohol level, %: Whiskey
120 (54.5)	0.25	<1	1	30	15	0.03	0.04
	0.50	2	2	50	35	0.06	0.075
	0.75	2-7/8	3	95	60	0.08	0.11
	1.0	3-3/8	4	150	95	0.10	0.14
	1.5	5-3/4	6	(240)	165	0.11	0.17
150 (68.2)	0.25	1-1/4	1-1/4	30	15	0.03	0.04
	0.50	2-1/2	1-1/2	50	35	0.06	0.075
	0.75	3-1/2	3-1/2	95	60	0.08	0.11
	1.0	4-3/4	5	150	95	0.10	0.14
	1.5	7-1/4	7-1/2	(240)	165	0.12	0.17
175 (79.5)	0.25	1-1/2	1-1/2	30	15	0.03	0.04
	0.50	2-4/5	3	50	35	0.06	0.075
	0.75	4	4-1/2	95	60	0.08	0.11
	1.0	5-1/2	6	150	95	0.10	0.14
	1.5	8-1/4	9	(240)	165	0.12	0.17
200 (90.9)	0.25	1-5/8	1-3/4	30	15	0.03	0.04
	0.50	3-1/4	3-1/2	50	35	0.06	0.75
	0.75	4-3/4	5	95	60	0.08	0.11
	1.0	6-1/2	6-3/4	150	95	0.10	0.14
	1.5	9-1/2	10-1/4	(240)	165	0.12	0.17

Figure 4 show that while whiskey produces a maximum blood alcohol level of 0.085, beer only gives a maximum of 0.045 (5).

Fig. 5 shows the maximum blood alcohol concentration, in percent of various alcoholic beverages. The effect of dilution on whiskey action is very marked (6).

PHYSIOLOGICAL ACTIONS OF ALCOHOL

Alcohol is one of only two substances to which human cells have never found a need to bar entrance. The only other freely moving substance is water. Because there is no barrier to entrance, there are no known cellular receptors to alcohol. This free movement indicates that the body does not consider alcohol toxic or in any way foreign. It is this facile movement that permits alcohol to be excreted in the urine and in breath and also permits it to be found and measured in the breath.

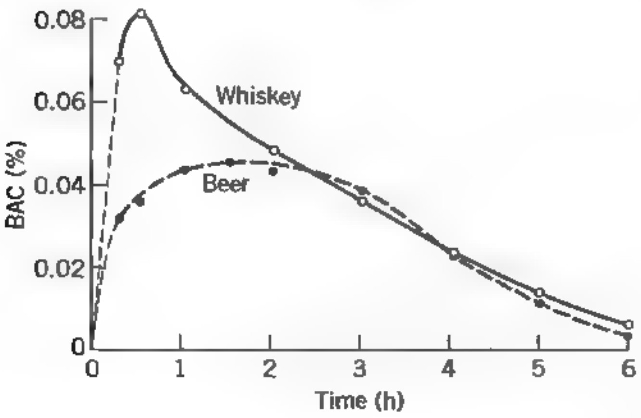


Figure 4. Blood alcohol curve after ingestion of 44 g alcohol in form of whiskey and of beer by 50 subjects, weighing between 65 and 79 kg.

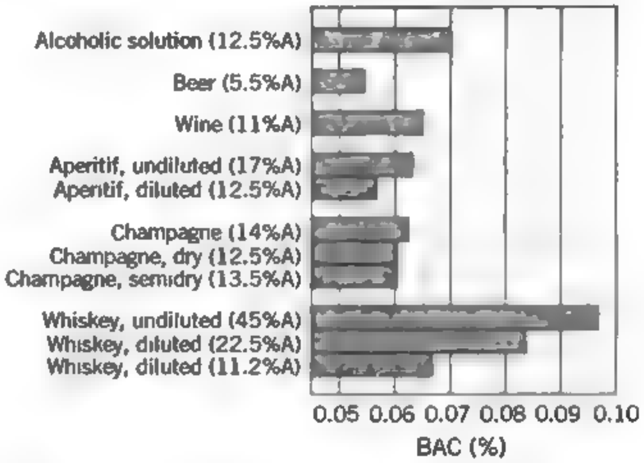


Figure 5. Blood alcohol maximum after ingestion of 0.5 g alcohol per kg body weight in form of several alcoholic beverages with different dilutions.

There is many-faceted evidence that alcohol is a product of normal metabolism in humans; it has even been found in teetotalers. The amount is low, about 0.001%.

Effect of Alcohol on Fluid Balance

The initial effect of alcohol is to produce a slight retention of water, followed by a more marked diuresis of water and sodium. This latter effect is due to an inhibition by alcohol of the pituitary antidiuretic hormone (vasopressin). This diuresis occurs only when the concentration of blood alcohol content is rising. The concerted drinking of wine or whiskey, but not beer, requires the imbibition of other fluids to maintain homeostasis (7).

Effect of Alcohol on Nutrition

Alcohol is readily metabolized and yields 6.9 kcal/g. The rate of metabolism varies from person to person and ranges from 50 to 100 mg/kg of body weight per hour. Alcohol does not affect the use of dietary protein and is as effective as carbohydrates in protecting the body from loss of protein.

Alcohol does not raise heat production or CO_2 production, so it has no thermogenic effect. There is no increase in alcohol metabolism with exercise, nor a decrease with rest.

Alcohol has only a minor effect on appetite. Distilled spirits consumed in concentrated form decrease gastric motility, whereas dilute alcohol increases gastric motion. These will appear to affect appetite.

In addition, during a meal, the palate-cleansing action of wine and beer will promote eating and lessen the feeling of satiety. It is probably the tannin in wine and the hops in beer that are responsible for this action. Yet heavy drinkers are far less likely to be obese than nondrinkers. Among a large group of low-income women in their 30s, 41% of nondrinkers were obese, whereas only 22% of heavy drinkers were so rated. Among men, the ratio is 31% obese for nondrinkers and 16% for drinkers.

Effects of Alcohol on the Central Nervous System

The primary effect of alcohol in the body is a sequential depressant action on the central nervous system. From the spinal cord rises the medulla oblongata, the pons, and the midbrain, the control centers for the autonomic nervous system, which regulates reflexes and those functions beyond control of the will. Above the midbrain lies the thalamus and hypothalamus, whose dominion includes functions not willfully regulated, such as body temperature, metabolism of fats, and blood sugar level.

Above all this, and more recent in the evolution of the species, lie the cerebellum and the cerebral cortex. The former has sway over voluntary muscular movements and manual skills, as well as balance and speech. The cerebral cortex, the corolla of the mind, is master of all that distinguishes humans and makes them distinguished.

Alcohol, which has ready access to all cells and crosses the almost impenetrable blood-brain barrier as if it were a sieve, exerts its earliest action on the cerebral cortex. Its action is not as a stimulant but as a tranquilizer or depressant. It lessens inhibitions and increases confidence. It replaces discontent with discovery, bashfulness with bravado, and cowardice with courage. Alcohol negates a no and affirms a yes.

These effects, which people had sought long before the tensions of modern life were imposed on them, occur at blood levels between 0.02 and 0.05%. Whatever pressures and problems were mankind's lot in Biblical days, they were surely different in scope and pervasiveness than those we confront today. But alcohol was used then and was considered of priestly stature. In many cultures, its production was limited to the clergy.

Apparently, the evolution of the cerebral cortex, with its awesome powers of the mind, brings with it a need to lessen its potent sway over our actions. This alcohol does with speed and thoroughness.

At blood alcohol levels between 0.05 and 0.10%, the sphere of its influence expands to the cerebellum. Balance and speech become less than normal. The gait becomes unsteady and speech uncertain. It is at the higher of these levels that ability to operate machinery is impaired.

At 0.2%, balance is more seriously affected and speech nearly incoherent. Neurotransmission in the cerebellum is disturbed. At increasing blood alcohol concentrations, the effects become deeper and parts of the brain developed earlier in evolution are also effected.

At 0.3–0.4%, most of the behavior that we call human is sharply curtailed if not lost. Anesthesia sets in. In fact, alcohol was the earliest anesthetic, before ether was discovered.

At 0.5–0.6% a remarkable shut-off valve operates and wakefulness is not maintained. This normally prevents any further action, because even the autonomic nervous system, controlling basic functions of respiration and heart beat, are denervated at a level above 0.6% and death ensues. But sleep normally prevents further inhibition, so fatal levels are not attained except with rapid drinking of strong whiskey. Fatal levels cannot be achieved with wine or beer.

Interaction of Alcohol and Other Nutrients

Alcohol and its primary metabolite, acetate, serve as excellent sources of energy. Alcohol and acetate penetrate the blood–brain barrier almost as efficiently as glucose. Everywhere, alcohol is used in preference to all other energy sources. Acetyl CoA, the entry point of all energy sources into the citric acid cycle, is most easily produced from alcohol.

Also, while all other energy sources—fatty acids, glucose, and amino acids—can be stored in polymolecular forms, alcohol and acetate cannot be stored. Neither do they act as substrates for conversion to glucose, amino acids, or lipids. Alcohol must thus be metabolized as it becomes available (8).

Effect on the Digestive Tract

Alcohol slightly increases gastric and intestinal mobility. It also has a slight positive but very transient effect on appetite. It has no effect on the absorption of fats or cholesterol from the intestinal tract. Some of the very slight appetite stimulation may not even be due to alcohol, but to other factors present in some alcoholic beverages—juniper in gin, bitters in some aperitifs, hops in beer.

Effect on Cardiovascular System

Alcohol slightly dilates peripheral capillaries, leading to blushing and a feeling of warmth. The effects are transitory and meager.

Moderate alcohol consumption reduces the risk of coronary heart disease. It also reduces the risk of cardiac mortality. Its action is probably mainly due to its effect on raising the HDL levels in the blood, as well as lowering the triglyceride levels. Physical activity has the same effect, and the effects are additive.

Moderate drinking is taken to be between 25 and 80 g of alcohol a day (two to six 12-oz. bottles of beer, 5% alcohol by volume; 250–750 ml of table wine [12% alcohol]; or 70–250 ml of whiskey [80 proof]).

The LDL in the blood (the "bad" cholesterol) is now believed to have its malign effect on atherosclerosis after it is oxidized. And so antioxidants have been shown to have a protective effect. This is the so-called French Paradox: the French consume diets high in cholesterol and saturated fats and yet have very low coronary artery disease. The explanation is their relatively high consumption of alcohol, mainly in the form of red wine. Many of the polyphenals, gallic acid, rutin, epicatechin, and quercitin present in red wine are potent antioxidants (9). Such phenolics are also present in oak-aged whiskey and in full-bodied and darker beers.

Alcoholic beverages therefore have a two-pronged effect on reducing coronary heart disease, raising the "good" HDL and keeping the LDL from becoming "bad."

Effect of Alcohol on Men versus Women

Alcohol per se has the same effects on either sex. However, a given amount of alcohol has a greater effect on women than on men for several reasons.

First, women are usually smaller than men, so a given volume of an alcoholic beverage will have a greater effect on the smaller person.

Second, women have a lower water content in their bodies than do men. Thus the alcohol, which is only soluble in water and not in fat, will reach a higher concentration in women. This difference in water content, on an equal weight basis, is about 12%.

And last, and probably most important, women have a lower gastric activity of alcohol dehydrogenase than do men. They therefore do not destroy as much alcohol in their stomach as do men, allowing more of it to be absorbed into the blood. This phenomenon, only recently discovered (1), is known as first-pass metabolism. It may differ by 20–30% between men and women. Thus the bioavailability of alcohol is considerably greater in women than in men. However, men over 50 years of age begin to lose the high alcohol dehydrogenase activity in their stomachs and become equal to women in their first-pass metabolism (10).

Alcohol as Medicine

Before the advent of the modern pharmacopoeia, alcohol was prescribed for many human ills. It dulls pain, summons sleep, soothes the spirit, and tames the teeming mind. Each of these functions can now be accomplished by a coterie of drugs, so alcohol is rarely recommended for these ills. But no one substance does all of what alcohol does, and people in all cultures have found alcohol to be the preferred, and possibly less noxious, means to tranquility. Furthermore, as a controlled way to peace and pleasure, alcohol has no peer.

Regulations in the United States prohibit any declaration of therapeutic value for any alcoholic beverage, so each generation must rediscover the value of alcohol. Many countries permit some therapeutic claim for alcohol, such as a bedtime relaxer or tension reducer.

As medicine, alcohol differs from drugs. All drugs are detoxified in the liver, ie, the liver so chemically modifies the drug as to render it inactive and able to be excreted, usually by the kidneys. This detoxification usually involves oxidation, hydrolysis, or sulfation. But alcohol is not detoxified, it is metabolized, just as is any foodstuff. No drug supplies energy; alcohol does. It may be more valid to consider alcohol as a food that has effects on the body rather than as a drug. As was pointed out earlier, alcohol has free entrance to every cell in the body; no drug has. All drugs operate by attaching themselves to a specific cellular receptor; alcohol needs none and has none.

Alcohol has at least one clinically proven and prescribed medicinal function. It is the only antidote to poisoning with ethylene glycol (antifreeze). Quick administration of alcohol, usually intravenously, makes all alcohol dehydrogenase sites act on the alcohol, harmlessly, instead of acting on the glycol, which produces very toxic oxalic acid. The glycol, not oxidized, is slowly excreted by the kidneys. The preferential reaction of alcohol dehydrogenase with alcohol permits this therapeutic action.

There are foods other than alcohol that affect the brain and human behavior. The amino acid tryptophane is a soporific and has been used as such. Meals high in carbohydrates produce high tryptophane blood levels. This they do by producing an influx of insulin into the blood, which in turn mobilizes some amino acids from plasma to muscle, leaving a higher concentration of tryptophane in the plasma. Histidine, threonine, tyrosine, and choline also are neuroactive, but their action is compromised by complex factors.

There are no confirmed allergies to alcohol per se. Its free entrance to all cells and ubiquitous presence mitigate against any allergic reaction.

Alcohol the Day After

The day after a serious session of drinking is often one of mental and physical fatigue and headache. In the United States, these symptoms are lumped together and called a hangover. In other languages the term is more descriptive, a wooden mouth in French, for example. The symptoms relate to the known constituents of alcoholic beverages and the known physiology of alcohol.

Alcohol masks fatigue, so a night of drinking will lead to excessive activity, which will be revealed by fatigue and malaise the next day. Rest always allows return to the normal state. The dry mouth or excessive thirst is the result of the diuretic effect of alcohol (and possibly the very salty nibbles that often accompany drinking). Drinking water or fruit juice, and a little time, alleviate this condition.

The headache may result from the fatigue and the dehydration but may also result from the congeners of distilled spirits and of wine. The congeners are simple compounds produced by yeast concomitant with alcohol. They range from ethyl acetate to amyl alcohol. Also, particularly in some wines, and in brandy, there may be measurable amounts of methyl alcohol. These levels of methyl alcohol are not toxic per se, but when metabolized to formaldehyde may produce headaches. The drinking of a small amount of some alcoholic beverage the next day, possibly beer or vodka, will induce the liver to return to oxidizing alcohol and allow the methanol to be eliminated by the kidneys.

Alcohol and Alcoholism

Alcohol, although it effects many organs and systems, is singularly responsible for only one disease, alcoholism. Those who have this disease are called alcoholics.

An alcoholic has been defined as a person who has a compulsion to drink and cannot stop drinking once begun, to the point where that person's social and economic life are adversely affected. There is no certain cause of this disease, although a genetic factor may well be involved. An alcoholic is a heavy drinker, but not all heavy drinkers are alcoholics. Drinking because they want to characterizes drinkers, heavy or light, but drinking because they have to is the hallmark of alcoholics. In an alcoholic drinking sets up a chain reaction, which terminates only with sleep. There is no clear evidence that an alcoholic metabolizes alcohol any differently than others, but there appears to be, in alcoholics, a strong predilection toward alcohol metabolism, rather than carbohydrate or fat metabolism, as the major energy source.

Alcoholism may lead to several other pathological conditions. It is important to understand that alcoholics may derive up to or even above 50% of their daily caloric requirements in the form of alcohol. The human system can only tolerate such univalent load with carbohydrates. Either fat or protein in such excess will lead to problems of a different nature, but none the less severe.

The major problem facing alcoholics is a series of metabolic and structural changes in the liver. The initial change is the accumulation of fats in the liver, resulting in a fatty liver. This is easily reversible. It occurs because, in the presence of alcohol, the liver uses it as a preferential source of energy and the fatty acids, which it would otherwise use, accumulate. This change is followed, after some years, by structural changes in the liver, including scarring or necrosis of the liver tissue, the mark of cirrhosis. About 20% of long-term alcoholics develop cirrhosis.

Alcohol consumption equivalent to about 50% of the daily caloric intake, for from 5 to 20 yr, is necessary to induce cirrhosis in susceptible persons. Cessation of drinking often leads to reversal of the cirrhotic condition.

The male to female ratio of alcoholics in the United States is now 4:1. Although alcohol is a necessary component in alcoholism, it is no more correct to say that alcohol is the cause of alcoholism than it would be to say that marriage is the cause of divorce. The roots of alcoholism are social, metabolic, and genetic. No medical treatment exists. The use of drugs that produce unpleasant symptoms if alcohol is consumed is not widespread because of toxic effects. Societal treatment, in groups of peers such as Alcoholics Anonymous, has proved most beneficial.

Prenatal alcohol abuse can lead to birth defects known as fetal alcohol syndrome. The most benign symptom is low birth weight, and the most serious is impaired brain development and mental retardation. The most severe effects are known to occur in chronic alcoholics, but because it is neither known what the safe lower limit of alcohol intake is nor when during the pregnancy alcohol is most likely to harm the fetus, it is generally recommended to avoid alcohol during pregnancy (11,12).

The Salubrity of Alcohol

The vast majority of consumers of alcohol—as beer, wine, or distilled spirits—do so because it makes them feel better. It relieves stress and smooths the way from work to winding down. Not for naught has the time in a tavern after work and before the trip home come to be called the happy hour.

In addition to general relief from stress and care, moderate drinkers have been found to show genuine improvement in health. Recall that the ill effects of alcohol are confined to those who consume, regularly and for many years, alcohol in excess of 80 g a day. This amounts to almost two cases (twenty-four 12 oz packages) of beer a week, or more than a 750 mL bottle of wine a day, or six 1-1/2 oz shots of whiskey a day.

The consumption of moderate amounts of alcohol has been found to decrease stepwise the risk of coronary heart disease. The lowest level of consumption produces the least salutary effect, and the highest levels, more than 31 g but less than 80 g a day, reduce the risk to only 20–75% of that in nondrinkers. These results have been found in many studies, in many lands, among men and women, and among various socioeconomic strata. The Framingham Study, the longest and largest research of its kind, has always found this correlation between alcohol consumption and coronary heart disease—moderate alcohol use lowers the risk of coronary heart disease.

The recent (1989) Physicians Health Study, in which 22,000 physicians were studied prospectively, to determine the effect of aspirin on cardiovascular mortality showed incidentally that daily alcohol consumption significantly reduced the overall mortality rate over that produced by aspirin alone.

Physicians using alcohol daily and taking aspirin had a myocardial infarction rate of 1% whereas those taking aspirin alone had a 1.4% rate. This 40% improvement was among the best of any other risk factor studied. The synergistic effect of moderate alcohol consumption was not expected.

Alcohol and Drugs

Alcohol has a potentiating effect on some drugs, particularly sedatives. This may be drug specific, but also in many cases is due to the fact that the liver, where all drugs are detoxified, may prefer to metabolize alcohol and the drugs may persist in the bloodstream longer than they would normally. Repeating the drug dosage, in, say, 4 or 6 h, may lead to an overdosage if the drug has not been removed by the liver.

Those taking drugs for any chronic ailments should find out if alcohol interferes with the action or metabolism of those drugs.

BIBLIOGRAPHY

1. M. Frezza et al., *New Eng. J. Med.* **322**, 95–99 (1990).
2. C. Leake and M. Silverman, *Alcoholic Beverages in Clinical Medicine*, World Publishing Co., Cleveland, 1966, p. 54.
3. A. Piendl, *How the Blood Alcohol Curve Develops After Drinking Beer*, Brauwelt International, 1993, pp. 154–157.
4. K. Crow and R. Batt, *Human Metabolism of Alcohol*, Vol. 1, CRC Press, Inc., Boca Raton, Fla., 1989, p. 53.
5. C. F. Gastineau et al., *Fermented Food Beverages in Nutrition*, Academic Press, New York, 1979.
6. B. Rouche, *The Netural Spirit*, Little, Brown, Boston, 1960.
7. L. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics*, Macmillan & Co., New York, 1970, p. 139.
8. L. Bisson et al., *Amer. J. Enol. Vit.* **46**, 449–462 (1995).
9. E. Frankel et al., *J. Agric. Food Chem.* **43**, 890–894 (1995).
10. H. Seitz et al., *New Eng J. Med.* **323**, 58 (1990).
11. *The Merck Manual*, 16th ed., 1992, p. 2009.
12. T. M. Roebuck et al., *Alcohol Clin. Exp. Res.* **22**, 339–344 (1998).

GENERAL REFERENCES

C. Leake and M. Silverman, *Alcoholic Beverages in Clinical Medicine*, World Publishing Co., Cleveland, 1966.

National Research Council (U.S.), *Diet and Health*, National Academy of Sciences, National Academy Press, Washington, D.C., 1989.

D. A. Roe, *Alcohol and the Diet*, AVI Publishing Co., Westport, Conn., 1979.

E. Rubin, "Alcohol and the Cell," *Annals of the New York Academy of Sciences* **492**, p. 1–7, 181–190 (1987).

F. A. Seixas, K. Williams and S. Eggleston, "Medical Consequences of Alcoholism," *Annals of the New York Academy of Sciences* **252**, p. 11–19, 63–78, 86–100 (1975).

F. A. Seixas, and S. Eggleston, "Alcohol and the Central Nervous System," *Annals of the New York Academy of Sciences* **215**, p. 10–36; 325–330 (1973).

JOSEPH L. OWADES
Consultant
Sonoma, California

ALKALOIDS

CLASSIFICATION AND PROPERTIES

Alkaloids are naturally occurring substances with a particularly wide range of structures and pharmacologic activities. They may be conveniently divided into three main categories: the true alkaloids, the protoalkaloids, and the pseudoalkaloids.

The true alkaloids have the following characteristics: they show a wide range of physiological activity, are usually basic, normally contain nitrogen in a heterocyclic ring, are biosynthesized from amino acids, are of limited taxonomic distribution, and occur in the plant as the salt of an organic acid. Exceptions are colchicine, aristolichic acid, and the quaternary alkaloids. The protoalkaloids, also known as the biological amines, include mescaline and *N,N*-dimethyltryptamine. They are simple amines synthesized from amino acids in which the nitrogen is not in a heterocyclic ring. The pseudoalkaloids, those not derived from amino acids, include two major series of compounds: the steroidal and terpenoid alkaloids (eg, cones-sine) and the purines (eg, caffeine). Most alkaloids occur in the Angiosperms, the flowering plants, but they are also found in animals, insects, marine organisms, microorganisms, and the lower plants. The only common characteristic of alkaloid names is that they end in "ine" (except camptothecin).

Alkaloids are preferably grouped by their biosynthetic origin from amino acids (eg, ornithine, lysine, phenylalanine, tryptophan, histidine, and anthranilic acid) rather than their heterocyclic nucleus. The pseudoalkaloids, which are not derived from amino acids and clearly cannot be classified this way, are best organized in terms of their parent terpenoid class, eg, diterpenoid and steroidal alkaloids, or as purines or ansamacrolides.

Physical Properties

Most alkaloids are colorless crystalline solids with a defined melting point or decomposition range (eg, vindoline and morphine). Some alkaloids are amorphous gums and some are liquids (eg, nicotine and conine) and some are colored (eg, berberine is yellow and betanidine is red).

The free base of the alkaloid is normally soluble in an organic solvent; however, the quaternary bases are only water soluble, and some of the pseudo- and protoalkaloids show substantial solubility in water. The salts of most alkaloids are soluble in water.

The solubility of alkaloids and their salts is of considerable significance in the pharmaceutical industry, both in the extraction of the alkaloid from plant or fungus and in the formulation of the final pharmaceutical preparation. Solubility is also of considerable significance in the clinical distribution of an alkaloidal drug.

Chemical Properties

Most alkaloids are basic, which makes them extremely susceptible to decomposition, particularly by heat and light.

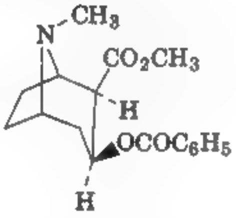
Ornithine-Derived Alkaloids

Ornithine-derived alkaloids include the tropanes (atropine, *l*-hyoscyamine, *l*-scopolamine, and cocaine), the Senecio alkaloids, and nicotine.

Tropane. Tropane alkaloids are derived from plants in the Solanaceae, Erythroxylaceae, and Convolvulaceae families. These alkaloids comprise two parts: an organic acid and an alcohol (normally a tropan-3α-ol). The pharmacologically active members of this group include atropine, the optically inactive form of *l*-hyoscyamine, which is isolated from deadly nightshade (*Atropa belladonna*); *l*-hyoscyamine and *l*-scopolamine, which are found in the leaves of *Duboisia metel* L., *D. meteloides* L., and *D. fastuosa* var. *alba*.

The tropane alkaloids are parasympathetic inhibitors. For example, atropine acts through antagonism of muscarinic receptors, the receptors responsible for the slowing of the heart, constriction of the eye pupil, vasodilation, and stimulation of secretions. Atropine prevents secretions (eg, sweat, saliva, tears, and pancreas) and dilates the pupil. Atropine is used to reduce pain of renal and intestinal cholic and other gastrointestinal tract disorders, to prolong mydriasis when necessary, and as an antidote to poisoning by cholinesterase inhibitors. Small doses produce respiratory and myocardial stimulation and decrease nasal secretion, and the drug has little local anesthetic action. Hyoscyamine and scopolamine have mydriatic effects. They are also used in combination as sedatives, in anti-motion-sickness drugs, and in antiperspirant preparations.

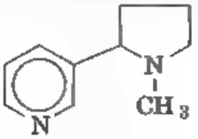
Cocaine. Cocaine is a potent central nervous system (CNS) stimulant and adrenergic blocking agent. It is extracted from South American cocoa leaves or prepared by converting ester alkaloids to exgonine, followed by methylation and benzoylation. It is too toxic to be used as an anesthetic by injection, but the hydrochloride is used as a topical anesthetic. It has served as a model for a tremendous synthetic effort to produce an anesthetic of increased stability and reduced toxicity.



Cocaine

Senecio Alkaloids. Senecio alkaloids possess a pyrrolizidine nucleus and occur in the genera Senecio (Compositae), Heliotropium and Trichodesma, and Crotalaria. They are biosynthetically derived from two units of ornithine in a manner similar to some of the lupin alkaloids. Certain of the alkaloids having an unsaturated nucleus are potent hepatotoxins.

Nicotine. Nicotine is toxic, soluble in water, and a constituent of tobacco. The lethal human dose is ca 40 mg/kg. Pharmacologically, there is an initial stimulation followed by depression and paralysis of the autonomic ganglia. The biosynthesis of nicotine is well established.



Nicotine

Lysine-Derived Alkaloids

Lysine-derived alkaloids contain the pyridine nucleus or its reduced form, piperidine. They include alkaloids derived from the Areca or Betel nut, Lobelia alkaloids, and those derived from pomegranate or club mosses.

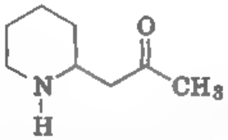
Arecoline, a colorless liquid alkaloid that has a pronounced stimulant action (in large doses, paralysis may occur) is found in the Areca or Betal nut. As the hydrobromide it is used as a diaphoretic and anthelmintic.

Lobelia inflata, known as Indian tobacco, contains lobeline, which is similar to, but less potent than, nicotine in pharmacologic action and is used as an emetic; the sulfate salt is used in antismoking tablets.

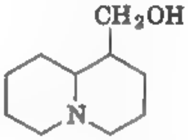
The root of *Punica granatum* contains alkaloids such as pelletierine and pseudopelletierine, which are formed from lysine and acetate. Pelletierine, is toxic to tapeworms and is used as an anthelmintic.

The club mosses, *Lycopodium* spp., produce polycyclic alkaloids such as lycopodine, whereas *Hydrangea* spp. yield febrifugine, an active antimalarial agent. Anabasine is found in *Haloxylon persicum* Bunge; this alkaloid has antismoking and respiratory muscle stimulation action similar to lobeline and is also used as a metal anticorrosive.

A host of complex alkaloids such as lupinine, sparteine, cytisine, and matrine are found in the lupins, a large plant family of the Leguminosae. Sparteine paralyzes motor nerve endings and sympathetic ganglia. The sulfate is used as an oxytoxic and the adenylate derivative is used to treat cardiac insufficiencies. Cytisine, found in the seeds of the highly toxic plant *Cytisus laburnum* L., is a strongly basic alkaloid that produces convulsions and death by respiratory failure.



Pelletierine



Lupinine

Anthranilic Acid-Derived Alkaloids

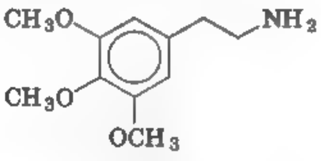
Anthranilic acid-derived alkaloids exhibit great structural diversity. This group includes dictamnine, platydesmine, vasicine, cusparine, and rutecarpine. Vasicine has oxytocic activity.

Phenylalanine- and Tyrosine-Derived Alkaloids

Phenylalanine- and tyrosine-derived alkaloids are by far the most numerous group of alkaloids, ranging from simple phenethylamines to the very complex dimeric benzylisoquinolines and the highly rearranged Cephalotaxus alkaloids.

Ephedrine. Ephedrine, from the Chinese drug Ma Huang, is soluble in water, alcohol, chloroform, and diethyl ether. It melts over the range of 33–42°C, depending on the water content. Little of the ephedrine of United States commerce is obtained from natural sources. Ephedrine is produced commercially through a biosynthetic process. Ephedrine has mydriatric effects and demonstrates adrenaline-like activity. It causes a rise in arterial blood pressure, increased secretions, and dilated pupils; it is a monoamine oxidase inhibitor and is used in nasal decongestants.

Peyote. Peyote, the small cactus of the Indians of north central Mexico, contain over 60 constituents. It is used as a hallucinogen in religious ceremonies and for medicinal purposes. A principal constituent is mescaline, a simple trimethoxyphenethylamine.



Mescaline

Ipecac. Ipecac, derived from *Cephaelis ipecacuanha* (native to Brazil), contains emetine and cephaeline, both of commercial significance. Emetine exhibits profound pharmacologic effects including clinical antiviral activity and is used in the treatment of amebic dysentery. Its side effects are cardiotoxicity, muscle weakness, and gastrointestinal problems including diarrhea, nausea, and vomiting. Two synthetic routes to emetine are of commercial importance: the Roche synthesis, which produces dehydroemetine, and the Burroughs-Wellcome synthesis. In handling emetine and its products, exposure should be limited as it can cause severe conjunctivitis, epidermal inflammation, and asthma attacks in susceptible individuals.

Isoquinoline and Related Alkaloids

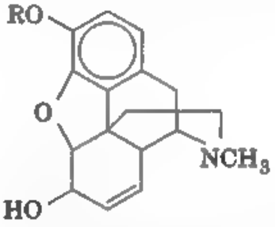
Isoquinoline and related alkaloids are the largest group of alkaloids derived from phenylalanine (or its hydroxylated derivatives) and corresponding β-phenylacetaldehydes. The alkaloids are prevalent in the plant families Fumariaceae, Papaveraceae, Ranunculaceae, Rutaceae, and Berberidaceae. There are many structural types, including the simple tetrahydroisoquinolines; the benzylisoquinolines; the bisbenzylisoquinolines, such as the *dl*- and *d*-isomers of tetrandrine, which exhibit anticancer activity; the proaporphines, such as glaziovine, which is an antidepressant; the aporphines, such as glaucine; the aporphine–benzylisoquinoline dimers, such as thalicarpine, which shows cytotoxic and antitumor activity; the oxoaporphines; the protoberberines, a group of over 70 alkaloids such as xylopinine, berberine, canadine, and corydaline, known for such diverse pharmaceutical uses as tranquilizers, CNS depressants, antibacterial and antiprotozoal agents, anticancer agents, and alpha adrenergic blockers; the benzophenanthridines, a group of 30 alkaloids such as fagaronine and nitidine, which exhibit antitumor properties; the protopines; the phthalideisoquinolines, a group that includes narcotine, which possesses antitussive activity, depresses smooth muscles, and is not narcotic, and hydrastine, which is used as an astringent in mucous membrane inflammation; and the homoaporphines.

The Opium Alkaloids

The opium alkaloids number over 25, some of which are of commercial importance and major significance. Opium is the air-dried milky exudate from incised, unripe capsules of *Papaver somniferum* L. or *P. albumen* Mill (Papaveraceae). Notable opium-derived alkaloids include morphine, codeine, thebaine, noscapine, and papaverine.

Morphine is the most important alkaloid. It is isolated from opium. Along with its salts, it is classified as a narcotic analgesic and is strongly hypnotic. Side effects include constipation, nausea, and vomiting in addition to habituation, reduced power of concentration, and reduction in fear and anxiety. Respiration is also deepened.

Codeine is the methyl ether of morphine, and thebaine is one of the methyl enol ethers of codeinone. Codeine pharmacologically resembles morphine but is weaker, less toxic, and exhibits less depressant action (it does not depress respiration in normal therapy). It is used in the treatment of minor pain and as an antitussive. Codeine is available as a free base or the sulfate or phosphate salt. Thebaine is a convulsant poison rather than a narcotic.



Morphine R = H
Codeine R = CH_3

Papaverine, a smooth-muscle relaxant that is neither narcotic nor additive, occurs in *P. somniferum* to the extent of 0.8–1.0%, commonly accompanied by noiscopine (narcotine). Most papaverine used is synthetically produced. The glyoxylate salt is used in treating arterial and venous disorders.

Amaryllidaceae

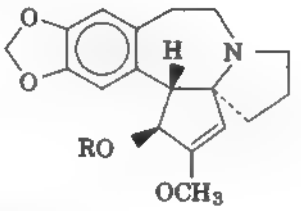
Amaryllidaceae alkaloids include galanthamine, margetine, and narciprimine. Galanthamine is a water-insoluble crystalline alkaloid that exhibits powerful cholinergic activity and analgesic activity comparable to morphine. It has been used to treat diseases of the nervous system. Its derivatives show anticholinesterase, antibacterial, and CNS depressant activity. Narciclasine, margetine, and narciprimine exhibit anticancer activity.

Colchicine

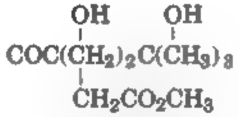
Colchicine, also known as hermodactyl, surinjan, and ephemeron, has some of the most unusual solubility characteristics of any alkaloid: it is soluble in water, alcohol, and chloroform, but only slightly soluble in ether or petroleum ether. Colchicine-type alkaloids are present in ten other genera of the Liliaceae and 19 species of Colchicum. Reviews of the chemistry of colchicine and related compounds and their history and pharmacology are available. Colchicine has the ability to artificially induce polyploidy or multiple chromosome groups. It is also used to suppress gout.

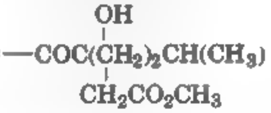
Cephalotaxus

Cephalotaxus alkaloids are found in the Japanese plum yews, *Cephalotoxus* spp. The esters, such as harringtonine and homoharringtonine, are potent antileukemic agents. The absolute configuration of the ester moiety has been determined. The α-hydroxy ester is essential for *in vivo* antileukemic activity.



Cephalotaxine R = H

Harringtonine R =

$$-\mathrm{COC}\overset{\mathrm{OH}}{\underset{\mathrm{CH_2CO_2CH_3}}{}}(\mathrm{CH_2})_2\overset{\mathrm{OH}}{\mathrm{C}}(\mathrm{CH_3})_3$$

Deoxyharringtonine R =

$$-\mathrm{COC}\overset{\mathrm{OH}}{\underset{\mathrm{CH_2CO_2CH_3}}{}}(\mathrm{CH_2})_2\mathrm{CH}(\mathrm{CH_3})$$

Securinine

Securinine, isolated from *Securinega suffruticosa* Rehd, is similar to strychnine in action, but exhibits lower toxicity, stimulates respiration, raises blood pressure, and increases cardiac output. The chemistry, pharmacology, and biosynthesis of securinine and related compounds have been reviewed.

Tryptophan-Derived Alkaloids

Tryptophan-derived alkaloids, which occur in the families Apocynaceae, Rubiaceae, and Loganiaceae, have recently become of great interest, particularly those derived from tryptamine and a monoterpene unit. There are many diverse structural types in this group. Attempts to determine the important details of the biosynthetic interconversions of these compounds have been reported.

The simplest indole alkaloids are derived from tryptamine itself. These include indole-3-acetic acid, a potent plant-growth stimulator; serotonin (5-hydroxytryptamine), a vital mammalian product that inhibits or stimulates smooth muscles and nerves; *N*-acetyl-5-methoxytryptamine (melotonin), a constituent of the pineal gland with melanophase-stimulating properties; 5-methoxy-*N*,*N*-dimethyltryptamine, a constituent of the hallucinogenic Virola snuffs; psilocybine, a hallucinogenic found in the mushroom *Psilocybe mexicana* Heim.

The harmala alkaloids, such as harmine and harmaline, are powerful monoamine oxidase inhibitors, previously used in the treatment of Parkinsonism. Harmine is the active ingredient of the narcotic drug yage.

Ellipticine and derivatives show anticancer activity.

Several alkaloids of *Calycanthus* spp. are the products of dimerization or trimerization of simple tryptamine residues, such as folicanthine.

The main indole alkaloid skeletons are derived from tryptamine and a C_{10} unit. Examples include corynanthine; yohimbine, which has hypotensive and cardiostimulant properties and is used to treat rheumatic disease; ajmaline, which has coronary dilating and antiarrhythmic properties; decarbomethoxydihydrovobasine, which shows vasodilating and hypotensive activities whereas related compounds exhibit antiviral activity; akuammicine; tabersonine; catharanthine; rhynchophylline; vindoline; dihydrovobasine; 10-methoxyibogamine, whose acyl derivatives exhibit analgesic and anti-inflammatory activity; and strychnine. This structural diversity has been the source of intense biosynthetic interest.

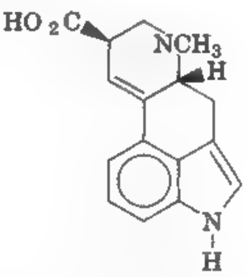
Physostigmine

Physostigmine, found in the perennial West African woody climber, *Physostigma venenosum* Balfour, is pharmacologically similar to pilocarpine and is a reversible cholinesterase inhibitor used to treat glaucoma.

The Ergot Alkaloids

The ergot alkaloids are obtained from ergot, the dried sclerotium of the fungus *Claviceps purpurea* (Fries) Tulsane (Hypocreaceae). Ergot alkaloids are produced by isolation from the crude drug grown in the field, by extraction from saprophytic cultures, and by partial and total synthesis.

The ergot alkaloids act pharmacologically to produce peripheral, neurohormonal, and adrenergic blockage, and to produce smooth-muscle contraction as well. The two medicinally important ergot alkaloids are ergotamine and ergonovine.



Lysergic acid

Three main groups of ergot alkaloids exist:

1. The clavine type, a group of over 20 alkaloids, which is water insoluble and does not give lysergic acid on hydrolysis. This group includes elymoclavine, agroclavine (a potent uterine stimulant), and chanoclavine-I.
2. The aqueous lysergic acid derivatives such as ergonovine, which in its maleate salt (or as methyl ergonovine maleate) is the drug of choice to treat postpartum hemorrhage.

3. The peptide ergot alkaloids, a group of water insoluble lysergic acid derivatives. This group includes ergotamine, ergocornine, and ergocryptine. Ergotoxine, a mixture of three peptide ergot alkaloids, possesses strong sympatholytic action and is used as a peripheral vasodilator and antihypertensive. Dihydroergotoxine is used for vascular disorders in the aged.

Some ergot alkaloids (eg, 2-bromo-α-ergocryptine) stimulate prolactin release and are being evaluated for treatment of breast cancer.

Catharanthus and Vinca Alkaloids

Catharanthus and Vinca alkaloids, usually discussed together, are quite distinct. The most important alkaloids of the Catharanthus genus are vincaleukoblastine, leurocristine, and leurosine, all antileukemic agents. Vincaleukoblastine and leurocristine are used clinically. The most important alkaloid of Vinca is vincamine, used to treat hypertension, angina, and migraine headaches. Alkaloids of this type produce marked hypotensive effects and curare-like action. The ethers of vincaminol are potent muscle relaxants.

Rauwolfia

Rauwolfia alkaloids include reserpine, the first tranquilizer, rescinnamine, and deserpidine. Reserpine is a sedative and tranquilizer useful in treating hypertension. It is also used as a rodenticide.

Strychnine

Strychnine, from the seeds of many *Strychnos* species, is a widely known poison (although, in fact, it is only moderately toxic). Pharmacologically, strychnine excites all portions of the CNS; it is a powerful convulsant and death results from asphyxia. It has no therapeutic uses in Western medicine, although its nitrate is used in treating chronic aplastic anemia.

Cinchona Alkaloids

Cinchona alkaloids, derived from the dried stem or root bark of various Cinchona species, include quinine and quinidine. These alkaloids are bitter tasting white crystalline solids, sparingly soluble in water. Quinine is toxic to many bacteria and other unicellular organisms and was the only specific antimalarial remedy until the Second World War. It is a local anesthetic of considerable duration. Quinine is commonly used as the sulfate and dihydrochloride. Quinidine, produced by the isomerization of quinine or found in Cuprea bark, is more effective on cardiac muscle than quinine and is used to prevent or abolish certain cardiac arrhythmias.

Camptothecine

Camptothecine, isolated from the Chinese tree *Camptotheca acuminata* Decsne, is used to treat cancer in the People's Republic of China.

Histidine-Derived Alkaloids

Histidine-derived alkaloids include pilocarpine and saxitoxin. Pilocarpine stimulates parasympathetic nerve endings and is used to treat glaucoma. The main commercial source of pilocarpine is *Pilocarpus microphyllus* Stapf., known as Marnham jaborandi. Saxitoxin is an extremely toxic neuromuscular blocking agent found in the so-called coastal red tides of North America.

Monoterpenoid Alkaloids

Monoterpenoid alkaloids include chaksine, a guanidine alkaloid from *Cassia lispikula* Vahl, which induces respiratory paralysis in mice; β-skytanthine, which is tremorigenic; cantleyine, derived from a monoterpene before loganin; and those derived from secologanin, such as gentianine, which exhibits hypotensive, anti-inflammatory, and muscle-relaxant actions, gentioflavine, gentiatibetine, pedicularine, and actinidine, a potent feline attractant.

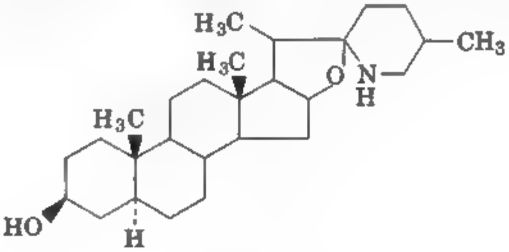
Diterpene Alkaloids

Diterpene alkaloids are not of commercial or therapeutic significance, but some have potent pharmacological activity, eg, aconitine and Erythrophleum alkaloids.

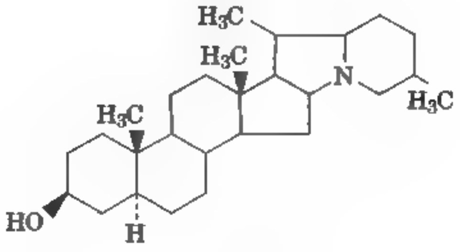
Steroidal and Triterpene Alkaloids

Steroidal and triterpene alkaloids are found in the plant families Solanaceae, Liliaceae, Apocynaceae, and Buxaceae. There are four main groups based on the botanical source: the Veratrum, Solanum, Holarrhena and Funtumia, and Buxus alkaloids.

The Veratrum alkaloids include jervine, protoveratrine A, and protoveratrine B; the latter two produce pronounced bradycardia and a fall of blood pressure by stimulation of vagal afferents. The Solanum alkaloids are of interest as potential sources of steroids. Examples of these alkaloids are tomatidine and solanidine. Some Solanum alkaloids exhibit fungistatic activity. Biosynthetically, the alkaloids are derived from acetate and mevalonate.



Tomatidine



Solanidine

Table 1. Effects of Natural Food Alkaloids on Humans and Animals

Alkaloid(s); identified or potential food sources	Clinical effects	Toxic symptoms
Amatoxins (α-, β-, γ-amanitins, amanin, amanullin); toxic mushrooms [Death Cup (*Amanita phalloides, A. verna, A. virosa*)]	Liver and kidney damage (man)	Delayed vomiting, abdominal pain and diarrhea, coma, death; LD^a (man, oral) = 0.11 mg/kg
Arecoline; Areca nut or Betal nut (*Areca catechu*)	Parasympathomimetic agent, cathartic (horses); cholinergic (man)	Flushing, perspiration, bronchial spasms, contraction of pupils, diarrhea, dyspnea, and collapse; LD^a (mouse, subcutaneous) = 102 mg/kg
Caffeine; coffee, tea, cola nuts, guarana	Cardiac, respiratory, and psychic stimulation, diuresis; vascular cephalalgesia (man)	Nausea, restlessness, vomiting, insomnia, tremors, tachycardia, cardiac arrhythmia (man); $\mathrm{LD}_{50}{}^b$ (rat, oral) = 19.6 mg/kg
Capsaicine; red peppers	Skin irritant	Sweating, salivation (mammals)
Carapine; papaya (*Carica papaya*)	CNS depression (mammals)	Bradycardia (mammals)
Caulerpicin; marine algae (*Caulerpa sertularioides C. racemosa* var. *clavifera*)	Anesthesia (mammals)	Numbness of lips and tongue (man)
Chavicine; black and white pepper (*Piper nigrum*)	Sharp peppery taste (man)	No information
Choline; brain, egg yolk, bivalve mollusks (*Callista brevisiphonata*), areca nut	Lipotrophic agent, antihypercholesterolemic agent	Nausea, vomiting, diarrhea (man); LD^a (rabbit, subcutaneous) = 450 mg/kg
Convicine; fava bean (*Vicia sativa* or *V. fava*)	Inhibition of glucose-6-phosphate dehydrogenase, decreased or reduced glutathione levels in red blood cells	Hemolysis, growth retardation (rat), hemoglobinemia (dog)
Curry alkaloids (murrayanine, mukoeic acid, girinimbine murrayacine, koenimbine, koenine, koeniginine, koenigine, mahanimbine, Cyclomahanimbine, curryanine); curry plant (*Murraya koenigii* Spreng)	For skin eruptions	No information
Cystisine; milk [contaminated from *Cystisus laburnum, Sophora secundiflora* (mountain laurel)	No information	Excitement, sweating, incoordination, convulsion, death (man, pig, cattle, horse); LD^a (as nitrate, dog, subcutaneous) = 4.3 mg/kg
3,4-Dihydroxy-L-phenylalanine (DOPA); fava bean (*Vicia fava*)	Decrease in reduced glutathione in red blood cells (man)	No information
Dopamine; banana, avocado, cephalopods	Hypertensive agent, increased cardiac output	No information
Dioscorine; wild yam (*Dioscorea hirsutus, D. hispida*)	CNS stimulation: analeptic, diuretic, expectorant	Sialorrhea, nausea, vomiting, diarrhea, confusion, cold sweat, pallor, clonic convulsion, paralysis, asphyxia (man), emetic hemolytic agent; LD^a (mouse, intraperitoneal) = 130 mg/kg
Eptatretin; Pacific hagfish (*Polistotrena stouti*)	Cardiac stimulant (frog, dog)	None, due to rapid detoxication
Ergot alkaloids (Eorgonovine, ergotamine, ergosine, ergocristine, ergocyptine, ergocornine, ergosinine, ergocristinine, etc); Barley, rye (contaminated from ergot produced by the fungi *Claviceps paspali* and *C. pupurea*)	Uterine stimulation, analgesic for migraine headache	Tachycardia, hypertension, vomiting, diarrhea, mental confusion, hallucinations, convulsions, gangrene, gastrointestinal (GI) disturbance; LD^a ergotamine, rat, intravenous) = 60 mg/kg
Gelsemine; Honey [contaminated from yellow jasmine] (*Gelsemium sempervirens*) nectar]	Uterine stimulation, CNS stimulant	Dizziness, dimness of vision, mydriasis, nausea, muscular debility, unusual prostration, weak pulse, dyspnea (man); minimum LD (rabbit, subcutaneous) = 0.15 mg/kg Glucobrassicin; broccoli, brussels sprouts, cabbage, cauliflower, kohlrabi, radish, rutabaga
	Goitrogenesis (rabbit, rat)	Hyperplasia of the thyroid; kidney and liver enlargement (rabbit, rat)

Table 1. Effects of Natural Food Alkaloids on Humans and Animals (*continued*)

Alkaloid(s); identified or potential food sources	Clinical effects	Toxic symptoms
Goitrin; cabbage, rape, rutabaga	Goitrogenesis (rat)	Hyperplasia of thyroid, liver and kidney enlargement (rat)
Gramine; barley (*Hordeum vulgare*)	Vasoactivity, stimulation of intestines and uterus (rabbit)	Hypertension, clonic convulsion, and excitation of respiratory center
Histamine; derived from beer, chocolate, fish, sauerkraut, wine, and yeasts	Vasoactivity (mammals)	Headache (man), hypotension (man, mammals); LD$_{50}^{b}$ (mouse, intraperitoneal) = 12.7 g/kg, LD^{a} (monkey, intravenous) = 52 mg/kg
Hordenine, germinated barley (*Hordeum vulgare*), sorghum (*Sorghum vulgare*), millet (*Panicum miliaceum*)	Sympathomimetic intestinal stimulation, respiratory stimulation (cat, dog); uterine stimulation (guinea pig)	Hypertension (man, dog), psychostimulation, convulsion, respiratory inhibition (man), LD^{a} (as sulfate, dog, oral) = 1.9 g/kg
Islanditoxin; rice (contaminated from *Penicillium islandicum*)	Carcinogenic hepatotoxin (mouse)	Liver damage, liver cancer (mouse, rat); LD$_{50}^{b}$ (mouse, oral) = 5.0 mg/10 g
Laminine; marine algae (*Laminaria* spp) and others	Hypotension (rabbit); depression of contraction of smooth muscles (mouse, guinea pig)	Hypotension (rabbit)
Methyl pyrazine (various derivatives); green peas (*Pisum sativum*)	No information	No information
Methyl pyrroline; black pepper (*Piper nigrum*)	No information	No information
Mimosine; *Leucaena glauca*	No information	Alopecia (mammals)
Murexine (urocanyl choline); shellfish (*Muricidae* spp.)	Excitation of respiratory center, neuromuscular blocking, muscle relaxation (vertebrates and invertebrates)	Muscular and respiratory paralysis (vertebrates and invertebrates); LD$_{100}$ (as oxalate, mouse, subcutaneous) = 310 mg/kg
Muscaridine; mushroom fly agaric (*Amanita muscaria*)	Uterine stimulation (rabbit, guinea pig)	Hashish or alcohol-like intoxication (man)
Muscarine; mushroom fly agaric (*Amanita muscaria* and other mushrooms)	Uterine stimulation (rabbit, guinea pig)	Sialorrhea, lacrimation, diaphoresis, nausea, vomiting, bradycardia, convulsions, coma, death; LD$_{100}$ (mouse) = 16 μg/day
Nicotine; tomato	Respiratory stimulation (man); uterine stimulation (cat, rabbit, pig); cerebral and visceral ganglial stimulation (man)	Hypotension (dog); respiratory depression and paralysis (cat); hyperglycemia, convulsion, dizziness, nausea (man), etc; LD (man, oral) = 40 mg/70 kg; LD$_{50}^{b}$ (rat, oral) = 60 mg/kg
Norepinephrine; banana (particularly the pulp), orange (trace), potato	Vasoactivity sympathomimetic, adrenergic	Hypertension
Pahutoxin; fishes [Hawaiian boxfish (Pahu), Ostraciontidae family]	Neuromuscular effects (mammals)	Hemolysis (mammals); ataxia, respiratory distress, coma (mouse); minimum LD^{a} (mouse, intraperitoneal) = 0.25 mg/g
Phallotoxins (phalloidin, phalloin, phallisin, phallicidin); toxic mushrooms [Death Cup, (*Amanita phalloides, A. verna, A virosa*)]	Liver and kidney damage (man)	Vomiting, abdominal pain, diarrhea, coma, and death (man); LD (man, oral) = 3 mg/kg
β-Phenylethylamine; Mushrooms, bitter almonds	Respiratory stimulation, intestinal relaxation, symphatomimetic (man)	Hyperventilation, hypertension, hypotension (cat); LD$_{50}$ (mouse, intraperitoneal) = 350 mg/kg
Piperine; black pepper (*Piper nigrum*)	No information	No information
Pyrrolizidine alkaloids; wheat and other cereals, legume (contaminated from *Crotalaria laburnifolia, C. striata*)	No information	Liver damage, carcinogenesis, venoocclusive disease (mammals); glaucoma (rat, mouse); LD$_{50}^{b}$ (mouse) = 20 mg/kg
Sanguinarine; mustard oil and cereal grains (contaminated from *Argemone mexicana*)	Expectorant; for chronic eczema and skin cancers	LD^{a} (as sulfate, intraperitoneal, rat) = 450 mg/kg

Table 1. Effects of Natural Food Alkaloids on Humans and Animals *(continued)*

Alkaloid(s); identified or potential food sources	Clinical effects	Toxic symptoms
Saxitoxin; shellfish, bivalve mollusks, and other pelecypods (contaminated from the dinoflagellates *Gonyaulax catenella* and *G. tamarensis*)	Neural stimulation (mammals); hypotension (cat and rabbit); myocardial and respiratory depression (mammals)	Peripheral paralysis, tingling and numbness of lips, respiratory failure (man and other mammals); $LD_{50}{}^{b}$ (man, oral) 10–20 μg/kg
Serotonine; avocado, banana, pineapple, octopus, papaya, plantain, passion fruit, red plum tomato	Respiratory stimulation (dog); respiratory inhibition (cat)	Hypertension (man); CNS depression (most animals); inhibition of ovulation (rabbit); teratogenesis (mouse)
Solanine (solanidine); Irish potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*)	Acetylcholine esterase inhibition (mammals)	Drowsiness, hyperesthesia, dyspnea, vomiting, diarrhea (man); LD^{a} (rabbit, intravenous) = 25 mg/kg
Tetrodotoxin; Pufferfish (Tetradontidae and Diodontidae families)	Neuromuscular blocking (mammals); hypotension (cat); respiratory inhibition, hypothermia (dog)	Weakness, dizziness, pallor and paresthesia, nausea, vomiting, sweating, salivation, muscular paralysis, cyanosis, respiratory paralysis, death (man); minimum LD (cat, subcutaneous) = 11 mg/kg; $LD_{50}{}^{b}$ (mouse, oral) = 325 mg/kg
Tetramine; shellfish (Buccinidae and Cymatidae families)	Curare-like effects, hypotension, bradycardia (mammals, frog)	Salivation, lacrimation, miosis, peristalsis (mouse); motor paralysis (mammals)
Threobromine; cacao bean; cola nuts; tea	Diuresis, myocardial stimulation, vasodilation, respiratory stimulation (cat)	GI distress; $LD_{50}{}^{b}$ (cat, oral) = 220 mg/kg
Theophylline; tea	Diuresis, hypertensive cephaloanalgesia (man)	Nausea and vomiting, vertigo, insomnia, flushing, convulsions, death; $LD_{50}{}^{b}$ (mouse, oral) = 550 mg/kg
Tomatine; tomato juice	No information	LD (rat, oral) 1 g/kg
Toxoflavin; Indonesian bongkrek (contaminated from *Pseudomonas cocovenenans*)	No information	$LD_{50}{}^{b}$ (mouse, oral) = 800 mg/kg $LD_{50}{}^{b}$ (mouse, intravenous) = 2.0 mg/kg
Trigonelline; green peas (*Pisum sativum*), coffee, soybeans, potatoes	No information	LD^{b} (rat, subcutaneous) = 4.9 g/kg
Tryptamine; plum (red and blue), orange, tomato	Vasoactivity, musculotropic (rabbit); intestinal and uterine contraction (rabbit, guinea pig)	Hypertension, headache (man)
Tyramine; avocado, banana, octopus, orange, spinach, potato, tomato	Respiratory stimulation (rat, cat); vasoconstriction (mammals)	Paroxysmal hypertension, intracerebral hemorrhage (man); mydriasis, bradycardia (dog); hypothermia (cat); LD (rabbit, intravenous) = 250 mg/kg
Vicine; fava bean (*Vicia sativa* or *V. fava*)	Inhibition of glucose-6-phosphate of dehydrogenase; decrease in reduced glutathione	Hemolysis, growth retardation (rat); hemoglobinemia (dog)

Source: Ref. 1, see also reference under S. W. Pettetier.

[a] LD = lethal dose.

[b] LD_{50} = median lethal dose.

Purine Alkaloids

Purine alkaloids are derivatives of the xanthine nucleus and include caffeine, theophylline, and theobromine, the principal constituents of plants used throughout the world as stimulating beverages.

Caffeine has the structure 1,3,7-trimethylxanthine. It is derived from cola, coffee (qv), tea (qv), guarana, and maté. Theophylline, 1,3-dimethylxanthine, is found in tea. Theobromine, 3,7-dimethylxanthine, is found in cocoa and tea. The xanthine derivatives have pharmacological properties in common: central nervous system (CNS) and respiratory stimulation; skeletal-muscle stimulation; diuresis; cardiac stimulation; and smooth-muscle relaxation. Caffeine is used to increase CNS activity; it acts on the cortex to produce clear thought and to reduce drowsiness and fatigue. Theophylline is used in smooth-muscle relaxants. Theophylline, as the ethylenediamine salt, is used in preference to caffeine in cardiac edema and in angina pectoris.

Miscellaneous Alkaloids

Coniine is an extremely toxic alkaloid that induces paralysis of the motor nerve endings and is the primary toxic constituent of poison hemlock. It was the first alkaloid to be synthesized. Carpaine, a crystalline macrocyclic alkaloid that induces bradycardia, depresses the CNS and is a potent amoebicide. Alkaloids are found in the poisonous Amanita species of mushrooms, such as α- and β-amanita toxins, ibotenic acid, muscimol, and muscazone. Maytansine and related ansamacrolides are potent antileukemic agents. Surugatoxin, found in the carnivorous gastropod *Babylonia japonica*, produces a pronounced mydriatic effect, sometimes resulting in death.

Ingestion and Human Health

The effects of natural alkaloids on humans and animals vary. This article is concerned mainly with food alkaloids and their clinical effects when ingested intentionally or accidentally. Table 1 summarizes the information.

BIBLIOGRAPHY

The section on classification and properties has been adapted from reference 1. Check original article for specific reference citations.

1. G. A. Cordell, "Alkaloids," in M. Grayson, ed., *Kirk-Othmer Concise Encyclopedia of Chemical Technology*, Wiley-Interscience, New York, 1985.

GENERAL REFERENCES

M. S. Abdel Kader et al., "DNA-Damaging Steroidal Alkaloids From *Eclipta alba* From the Suriname Rainforest," *Journal of Natural Products* **61**, 1202–1208 (1998).

Atta ur Rahman and M. I. Choudhary, "Diterpenoid and Steroidal Alkaloids," *Natural Products Report* **14**, 191–203 (1997).

J. M Betz et al., "Chiral Gas Chromatographic Determination of Ephedrine Alkaloids in Dietary Supplements Containing Ma Huang," *J. AOAC Int.* **80**, 303–315 (1997).

B. Beumann et al., "The Effect of Ephedrine Plus Caffeine on Plasma Lipids and Lipoproteins During a 4.2 MJ/day Diet," *International Journal of Obsesity Related Metabolism Disorders* **18**, 329–332 (1994).

[illegible]. F Domino, E. Hornbach and T. Demana, "The Nicotine Content of Common Vegetables," *N. Engl J. Med.* **329**, 437 (1993).

K. Frach and G. Blaschke, "Separation of Ergot Alkaloids and Their Epimers and Determination in Sclerotia by Capillary Chromatography," *J. Chromatogr.* **808**, 247–252 (1998).

J. R. Gallon, K. N. Chit and E. G. Brown, "Biosynthesis of the Tropane-Related Cyanobacterial Toxin—A Role of Ornithine Decarboxylase," *Phytochemistry* **29**, 1107–1111 (1990).

C. Giroud et al., "Thermospray Liquid Chromatography/Mass Spectrometry (TSP LC/MS) Analysis of the Alkaloids From Cinchona *in vitro* Cultures," *Planta Med.* 57 (2), 142–148 (1991).

B. Gutsche and M. Herderich, "HPLC-MS/MS Profiling of Tryptophan-Derived Alkaloids in Food. Identification of Tetrahydro-beta-carbolinedicarboxylic Acids," *J. Agric. Food Chem.* **45**, 2458–2462 (1997).

J. F. Hanock, "The Colchicine Story," *HortScience* **32**, 1011–1012 (1997).

X. J. Hao et al., "Dieterpene Alkaloids From Roots of *Spitaea japonica*," *Phytochemistry* **38**, 545–547 (1995).

J. N. Hathcock, ed., *Nutritional Toxicology*, Vols. 1 and 2, Academic Press, Orlando, Fla., 1987.

J Hong et al., "Enhancement of Catharanthine Production by the Addition of Paper Pulp Wastes Liquors to *Catharanthus roseus* in Chemostat Cultivation," *Biotechnol. Lett.* **19**, 967–969 (1997).

P. J. Houghton et al., "Two Securinega-type Alkaloids From *Phyllanthus amarus*," *Phytochemistry* **43**, 715–717 (1996).

A. Itoh, T. Tanahashi and N. Nakagura, "Six Tetrahydroisoquinoline-Monoterpene Glucosides From *Cephalis ipecacuanha*," *Phytochemistry* **30**, 3117–3123 (1991).

B. A. Kimball and C. A. Furcolow, "Ion-pair High Performance Liquid Chromatographic Determination of Strychnine Alkaloid in an Animal Tissue," *J. Agric. Food Chem.* **43**, 700–703 (1995).

Y. M. Liu and S. J. Sheu, "Determination of Ephedrine and Pseudoephedrine by Capillary Electrophoresis," *J. Chromatogr.* **637**, 219–223 (1993).

P. R. Martens and K. Vandevelde, "A Near Lethal Case of Combined Strychnine and Aconitine Poisoning," *J. Toxicol. Clin. Toxicol.* **31**, 133–138 (1993).

A. Montagnac et al., "Isoquinoline Alkaloids from *Ancistrocladus tectorius*," *Phytochemistry* **39**, 701–704 (1995).

P. M. Newberne, "Naturally Occurring Food-Borne Toxicants," in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Disease*, 7th ed., Lea & Febiger, Philadelphia, Pa., 1988.

A. J. Parr, "The Production of Secondary Metabolites by Plant Cell Cultures," *J. Biotechnol.* **10**, 1–26 (1989).

S. W. Pelletier, ed., *Alkaloids: Chemical and Biological Perspectives*, 6 Vols., Wiley-Interscience, New York, 1983–1988.

O. Poobrasert et al., "Cytotoxic Degradation Product of Physostigmine," *Journal of Natural Products* **59**, 1087–1089 (1996).

A. Rosso and S. Zuccaro, "Determination of Alkaloids From the Colchicine Family by Reversed-Phase High-Performance Liquid Chromatography," *J. Chromatogr. A* **825**, 96–101 (1998).

J. E. Saxton, "Recent Progress in the Chemistry of the Monoterpenoid Indole Alkaloids," *Natural Products Report* **14**, 559–590 (1997).

L. A. Skorupa and M. C. Assis, "Collecting and Preserving Ipecac (*Psychotria ipecacuanha, Rubiaceae*) Germplasm in Brazil," *Economic Botany* **52**, 209–210 (1998).

A. W. Smallwood, C. S. Tschee and R. D. Satzger, "Basic Drug Screen and Quantitation of Five Toxic Alkaloids in Milk, Chocolate Milk, Orange Juice, and Blended Vegetable Juice," *J. Agric. Food Chem.* **45**, 3976–3979 (1997).

L. Takano et al., "Alkaloids from *Cephalotaxus harringtonia*," *Phyochemistry* **43**, 299–303 (1996).

L. Takano et al., "Cephalotaxidine, a Novel Dimeric Alkaloid From *Cephalotaxus harringtonia* var. *Drupacea*," *Tetrahedron Lett.* **37**, 7053–7054 (1996).

S. L. Taylor and R. A. Scanlon, *Food Toxicity*, Marcel Dekker, New York, 1990.

A. Ulubelen et al., "Diterpene Alkaloids From *Delphinium peregrinum*," *Phytochemistry* **31**, 1019–1022 (1992).

S. J. P. van Belle et al., "Determination of Vinca Alkaloids in Mouse Tissues by High-Performance Liquid-Chromatography," *J. Chromatogr.* **578**, 223–229 (1992).

Y. H. Hui
American Food and Nutrition Center
Cutten, California

AMERICAN DIETETIC ASSOCIATION. See FOOD AND NUTRITION SCIENCE ALLIANCE (FANSA); FOOD AND NUTRITION SCIENCE ALLIANCE ORGANIZATIONS.

AMINO ACIDS. See PROTEINS: AMINO ACIDS.

ANIMAL BY-PRODUCT PROCESSING

Domesticated animals are grown and slaughtered for meat for human consumption. Normally, only 30 to 40% of the animal is utilized for human food (meat cuts, edible offals, processed meat products). In monetary terms this represents by far the most important product of meat processing plants. However, economics of operating the meat slaughter plant, together with the cost of pollution abatement and/or disposal of inedible material from the slaughter operation, demands that the rest of the material produced from the slaughter operation be used profitably. In most countries everything produced by or from the animal other than the dressed carcass is considered a by-product. Another name for by-products is coproducts. The term "coproduct" is coming into common acceptance, to indicate that these materials are contributing to process profitability. In some countries the terms "offal" and "by-product" are interchangeable. Hides and pelts are obvious by-products and are discussed elsewhere. A partial, but not all-encompassing, list of animal by-products is given in Refs. 1–4.

Many products can be made from the nonmeat parts of the animal (Fig. 1), and various by-products often contribute significantly to the meat plant's profitability. In some instances the commercial value of these by-products is often higher than the sum of the running expenses and the margin required for the meat plant to operate profitably. Also, because there is a worldwide shortage of animal protein, it is essential to maximize the use of these raw materials. This applies both to products used directly for human consumption and to protein-containing material that can be processed and fed back to animals.

By-products are usually classified as "edible" or "inedible." Edible by-products, such as heart, liver, tongue, oxtail, kidney, brain, sweetbreads, and tripe, that are segregated, chilled, and processed under sanitary conditions are called "variety meats" (or in some countries, offals). Chitterlings and natural casings (intestines) and fries (lamb or calf testicles) can also be eaten. In some countries, the blood and/or blood fractions from healthy animals, processed hygienically under conditions specified by the appropriate regulatory authority, can be an edible by-product. Some by-products must be processed or refined before they can be eaten. Examples include stomachs for tripe, bones and skin pieces for gelatin manufacture, and fatty tissue for edible fat. Most noncarcass material, if cleaned, handled, and processed in an appropriate manner, could be edible.

There is a sizable international trade in edible by-products because they are a very economical source of high-quality protein and fat. Custom, religion, palatability, and reputation of these products usually limit their use for human consumption. How a meat processor classifies a specific product depends both on the possible utilization of that product and the availability of a potential market. For example, many potentially edible by-products are downgraded to an inedible use because a profitable market does not exist. Many animal by-products are often underused. Their effective utilization depends on having a practical commercial process to convert the animal by-product into a usable commodity, actual or potential markets for the commodity produced, a large enough volume of economically priced raw material in one location for processing, a method for storing perishable material before and after

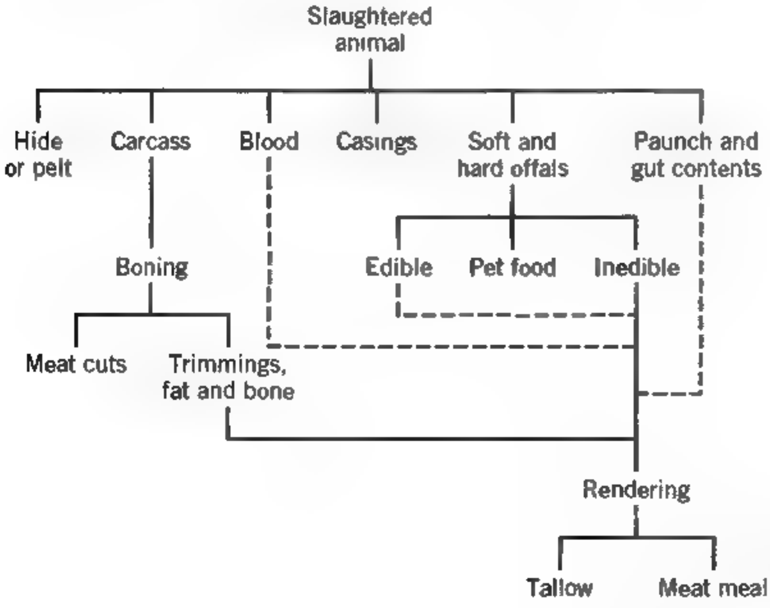


Figure 1. Flow diagram of material from a slaughtered animal.

processing, and, often, the availability of highly trained technical operators.

By-product yields (as a percentage of the animal's slaughter weight) depend on the species and category of animals slaughtered, the degree of animal processing, and on the end form of the by-product. Edible offals, material for pet food, and blood for edible use can represent up to 20% of carcass weight. When by-products are further processed (eg, rendered into meal and processed fat), one- to two-thirds of the original weight of raw material is lost as water.

BLOOD

Whole blood is a liquid with a dry matter content of 18 to 20%. Animal type and age may slightly influence blood composition. Blood undergoes complex biochemical reactions (clotting, coagulation, syneresis) once it has been released. Uncoagulated blood can be fractionated into plasma (a pale yellow fluid) and red blood cells (a fraction containing hemoglobin). Serum can be obtained when clotted blood synereses.

About 3 to 4% of the animal's live weight is blood, so large quantities are produced daily at slaughter facilities, even small ones. As 90 to 95% of the dry matter in blood is protein, blood is an obvious source of high-quality protein. Whole blood and the processed fractions (especially plasma) can be used as food ingredients. The dark red color of whole blood and red blood cells usually limits the use of these products in foods (5–7), although several processes exist for manufacturing decolorized blood fractions. However, the high costs and the logistics of collecting blood hygienically usually preclude extensive use of blood for edible products, so it is usually processed into an inedible blood meal. Although this processing into blood meal produces some income, it is done mainly to reduce pollution treatment costs: whole blood has a high biological oxygen demand (250,000 mg/L) and therefore can be a major source of pollution. Blood is usually not collected hygienically, in which case it may contain urine and ingesta. As well, it usually has been diluted with wash water. As a result, the dry matter content of the whole blood can be reduced to as low as 10%, especially if there is poor water management.

Whole blood can be stabilized chemically by adding urea, ammonia, metabisulfite or sulfuric acid, and the preserved blood can then be held without refrigeration and fed to animals (8). However, in most commercial processes, the blood is heated to coagulate the proteins, and these coagulated solids are then separated and dried to produce blood meal with a moisture content of 8 to 10%. This meal is a cheap source of animal protein high in lysine. The three most common methods of processing inedible blood are as follows:

1. Apply indirect heat to the whole blood to boil off most of the water. This process is very energy inefficient and produces a denatured product with very low solubility. This method is often used at smaller older plants that have not updated their equipment.
2. Inject live steam to bring the temperature to about 90°C to coagulate the blood, then remove most of the water in a decanter and dry the solids (eg, in a ring dryer with hot circulating gases, a rotating drum cooker, or a batch dryer). This method uses less energy than the previously mentioned technique because about half of the water is removed mechanically. A denatured protein powder is produced, the solubility of which depends on the type of dryer used: powders produced in ring dryers usually are more soluble. The high temperatures and/or long times used during processing lower the nutritional value of the blood meal.
3. Concentrate whole blood by ultrafiltration, then dry the concentrate in a spouted-bed drier (9). A very soluble powder is produced.

The yields of dried blood depend on the processing parameters (10). Aging the blood (leaving for 12 or more hours before processing) can increase yields. Any water added to the raw blood reduces yields. If the steam coagulator is not working efficiently, the losses of protein in the "blood water" from the centrifuge can increase.

Blood and its components (serum, albumin, red blood cells, hemoglobin) can be used in food, animal feeds, laboratory reagents, medical preparations, and industrial uses and as fertilizer. Blood albumin can be a substitute for egg albumen. Dried blood meal is used as a protein supplement in livestock feed. It is deficient in the amino acids tryptophan and isoleucine but is high in lysine, although lysine availability is affected by the drying method. Laboratory uses for blood include as a nutrient for tissue culture media and as a necessary ingredient in some agars for bacteriological use. Many blood components are isolated from whole blood and used in chemical and medical analyses or as nutritive supplements. Industrial uses of blood include as an adhesive and for its film-forming properties in the paper, lithographic, plywood, veneering, fiber, plastics, and glue industries. As a fertilizer, blood meal contributes nitrogen, aids humus formation, and improves soil structure.

Blood char, or blood charcoal, is the carbon component of whole blood or blood meal. Blood char is produced by treating 20% of the weight of whole blood or 50% of the weight of blood meal with activating agents and heating in airtight containers to 650 to 750°C for 6 to 8 h. Blood char contains 80% carbon and is used for absorption of gases, as an industrial decolorant, and as an antidote for chemical poisoning.

RENDERING

Introduction

Animal tissues are composed essentially of water, fat, and protein, with some minerals. The term "rendering" refers to a variety of processes that are used to separate the water, fat, and protein components, as far as is practicable, into commercial products. Until the 1850s, the highly perishable by-products from meat slaughtering were considered waste and were buried. The rendering industry developed to convert these materials into farm fertilizer and realize a profit from the operation. Proteinaceous by-products yielded nitrogen fertilizer, whereas bone produced phosphate fertilizer. Today, the rendering industry produces many useful products that can be broadly classified as edible and inedible fats, fine chemicals, meat meals, and bone meals.

Animal by-products used by renderers consist of fatty tissue, trimmings, bones, hooves, and soft offals (viscera). Processing carcasses into cuts and boneless meat increases the amount of material for rendering through the bones and trimmings that result. The material for rendering can represent 30 to 60 percent of the weight of a slaughtered animal. In developed countries, with centralized slaughter of large numbers of animals, a large volume of material is available for renderers. This has led to the development of sophisticated equipment and processes.

Basic Principles

The basic purpose of rendering is to produce stable products of commercial value, free of disease-bearing organisms, from raw material that is often unsuitable or unfit for human consumption. Most of the fat comes from fatty tissue, which can be located anywhere within connective tissue, and is made up of cells containing fat. This fat is deposited when animals have a surplus of dietary energy. The fat cells are surrounded by reticular fibers, and the fat cannot be released from animal tissue until the supporting structure has been broken. The two basic processes in rendering are separation of the fat and drying of the residue. The most common method used to rupture fat cells is heat, although enzymic and solvent-extraction rendering processes also exist.

A large proportion of the raw material for rendering is viscera (soft offal) and its associated contents. Because paunch and gut contents contain chemicals that can adversely affect fat quality, and solids that can downgrade meal quality, paunches are usually opened and emptied. The viscera are then cut and may be washed. Size reduction increases mass and heat transfer, although in some rendering processes it may be difficult to get even heat transfer in the processing vessel if the particles are too small. Both hard and soft offals may undergo size reduction. This size reduction is usually done in devices with rotating knives or anvils (termed hogors or prebreakers when producing large particles, grinders or mincers when producing fine-particle material) or with rotating hammer devices (hammer mills). Hooves and bones (hard offals) need to be reduced in size but usually do not require washing. The raw material is then processed to separate the fat from the nonfat phase.

Processes

A rendering process can generally be classified as wet or dry, depending on whether the fat is removed from the raw material before or after the drying operation. Processes can operate in a batch, semicontinuous, or continuous mode, and some of the newer rendering systems are classified as low-temperature-rendering (LTR) systems because of their milder heat treatment. Table 1 lists the best-known and most-used rendering systems, divided according to process type. Connected systems produce products of similar quality when processing similar raw material. There is no one "best" process for all applications; the most appropriate method will often depend on the application.

Profitability of any rendering system can be maximized by ensuring maximum yields and by obtaining the best possible product quality. Regular and planned maintenance of equipment will minimize repairs and maintenance costs. Processing additional water (from excessive washing and hosing) is wasteful, because more energy and a larger rendering capacity is required for a given throughput of solid material.

Wet Rendering. Wet rendering is an old processing method. In older systems, the preground raw material is cooked in a closed vertical tank (termed a digestor, autoclave, or cooker) under pressure by direct steam injection, usually to 380 to 500 kPa for 3 to 6 h (Fig. 2). Operators use past experience to gauge the end point. The pressure is then slowly released, and the liquid and solid phases are allowed to settle. The fat that has floated to the top is drawn off and can be "polished" in disc centrifuges to remove residual water and particles (fines). The water phase (liquor) in the cooker is drained off, then the solid material (greaves) is removed and can be pressed or centrifuged to remove additional liquid (stickwater) before being dried. The liquor and stickwater may be further processed to remove fines, which can be recycled to the greaves, and residual fat. Wet rendering produces good-quality fat (but only if the viscera are cut and washed). However, it requires very long cook times, is very labor intensive, and has significant losses (up to 25% of the solids may be lost in the stickwater). It is energy intensive, although heat can be recovered from the vent steam.

A more modern wet-rendering system is semicontinuous and involves cooking the raw material in a conventional dry-rendering cooker under pressure (to ensure sterilization) for a short time, then processing the cooked material in decanters to separate the liquor from the wet solids (Fig. 3). The meal is dried in continuous driers, and the fat is separated from the liquor in disc centrifuges. Process water is evaporated in multiple-effect evaporators and the concentrate added to the wet solids. The system produces high-quality fat and low-fat meal and uses less energy than conventional wet- or dry-rendering systems. However, capital costs, repair costs, and maintenance costs are high.

Dry Rendering. Both batch and continuous processes exist (Fig. 4). The material is heated in a horizontal, steam-jacketed vessel until most of the water has evaporated. The vessel has an agitator, which also may be steam heated. The evaporated water is usually condensed to recover heat and reduce atmospheric pollution. In batch systems, the raw material can be subjected to 200 to 500 kPa for some specified time to sterilize the material and/or hydrolyze wool and hair. It may take up to 3 h to produce a "dry" material, and the end-point temperature is often 120 to 140°C. The stage at which pressure is applied can influence the ease of further processing. Cooking times in continuous

Table 1. Best-Known and Most-Used Rendering Processes

			Cooker temperature, °C
Dry	Batch	Cookers	105–130
	Continuous	Keith	105–140
		Stord Bartz with centrifuges	110–140
		Duke	120–140
	Continuous with evaporator	Carver-Greenfield	90–105
		TM-1	120–140
		Retrofit	120–140
		Stord Barts with presses	120–140
Wet	Batch	Digesters	90–110
	Semicontinuous	Centrimeal	125–140
		Instant meal	125
	Continuous low temperature	Atlas	60–95
		Balfour	
		Centribone	
		MLTR	
		Pfaudler	
		Rendertech	
		Stord Bartz wet pressing	

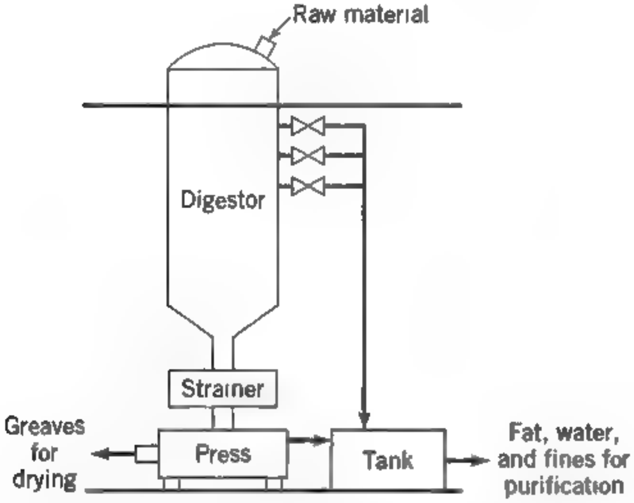

Raw material
Digestor
Strainer
Greaves for drying
Press
Tank
Fat, water, and fines for purification

Figure 2. Wet-rendering system.

systems depend on cooker volume, cooker heat-transfer capability, and the characteristics and feed-rate of the raw material. Cooker contents are discharged into a percolator to remove free-draining fat; then the solid material is pressed (a continuous operation) or centrifuged (a batch operation) to remove additional fat. The material is then ground into meat meal. The fat is polished in disc centrifuges to remove fines and moisture.

Batch dry rendering has the following advantages:

- Little material is lost.
- Cooking, pressurizing, and sterilizing can be carried out in the same vessel.
- Different cookers can be assigned for processing different raw material, and hence for producing different grades of fat.
- Heat can be recovered from the vent steam.

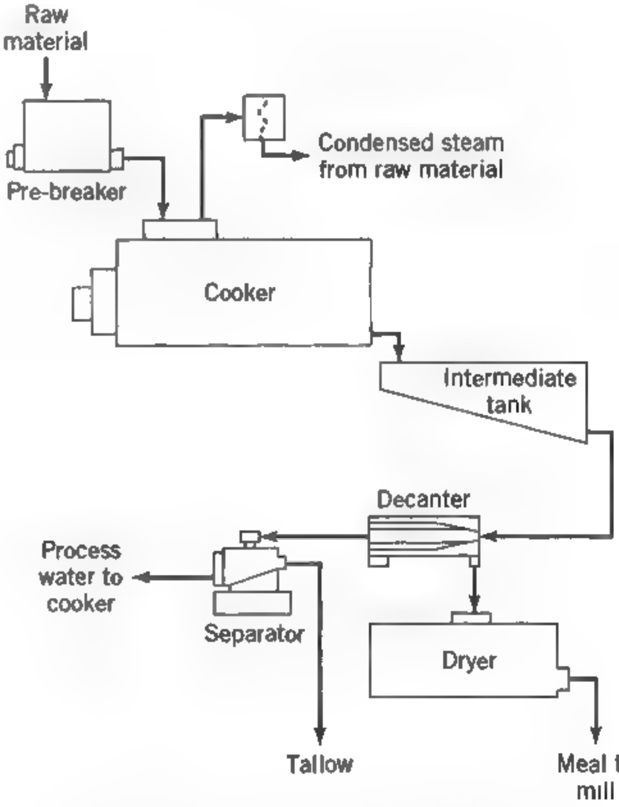

Raw material
Pre-breaker
Condensed steam from raw material
Cooker
Intermediate tank
Decanter
Process water to cooker
Separator
Dryer
Tallow
Meal to mill

Figure 3. Semicontinuous wet-rendering system.

The disadvantages are as follow:

- The fat is usually of poorer quality than that from wet or LTR systems.
- The high temperatures used produce fines, which can pass into the fat, degrading its quality, and which can be lost in the effluent from the polishing centrifuges.

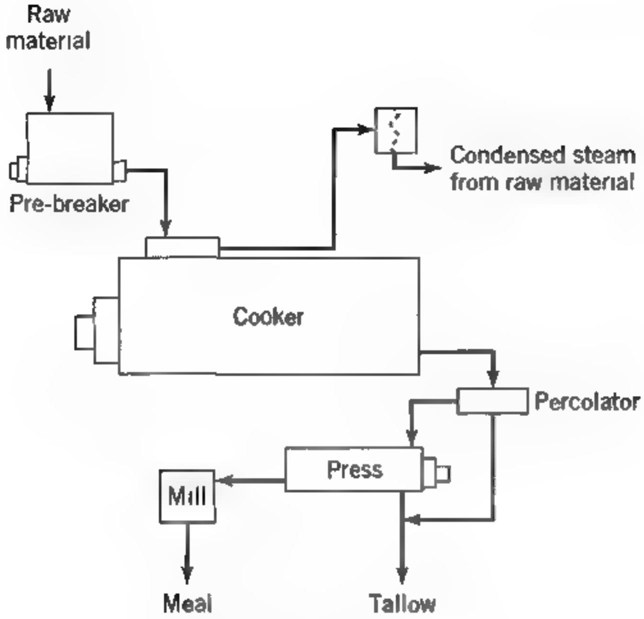


Figure 4. Dry-rendering system.

- The meal has a higher fat content than meal from wet and LTR systems.
- Raw material must be washed (producing effluent) to produce good-quality fat.
- Indiscriminate hosing and inadequate draining of washed material add extra water, increasing evaporation loadings and, hence, energy requirements.
- High-protein, low-fat material, such as slaughter wastes from calves and young lambs, is difficult to process.
- The end point of the cooks is difficult to control, so fat quality can be variable.
- It is difficult to keep the processing area clean and tidy; the process is not completely enclosed, so cooked products can be recontaminated.
- Energy usage is high, especially if vent steam is not recovered as hot water; dry-rendering cookers are not efficient dryers.
- The process is labor intensive.

Continuous dry rendering has most of the advantages of batch dry rendering, uses less labor, and requires less space. It has the disadvantages that the system cannot be pressurized, so sterilization and hydrolysis cannot be done, and tallow quality tends to be lower than with batch operations because end-point temperatures are often higher.

Low-Temperature Rendering (LTR). In the wet- and dry-rendering systems discussed so far, the raw material is subjected to high temperatures for long times. In dry rendering, near the end of the cooking process, when most of the free water has evaporated, the solids (cracklings) are essentially "frying" in the fat. With such high temperatures, the raw material must be washed to ensure that paunch contents and other "dirt" does not downgrade the color of the fat. Operators often overdry the cracklings to make pressing easier, so dry-rendered meal can be high in fat and low in moisture.

LTR systems were developed in the late 1970s to overcome some of the disadvantages of dry-rendering systems. Heat treatment is minimized and phase separation is carried out at low temperatures (70 to 100°C). Systems are usually continuous, so material flow through the size-reduction equipment, heating units, phase separating units, evaporators, and dryers must be balanced using surge bins and/or variable speed drives. Raw material, which can be unwashed, is minced, then heated to 70 to 95°C in a unit called a coagulator, preheater, melting section, or rendering vessel. The phases are then mechanically separated in decanters or presses.

In a MIRINZ low-temperature-rendering (MLTR) system (Fig. 5) processing 5 t of raw material per hour, the rendering vessel volume is only 1 m^3. Despite this, the residence time for the raw material, along with some recycled fat, to reach 80 to 90°C using indirect heat (in coils in the vessel) is just 6 to 8 min (11). The resulting liquor and solids are separated in a decanter, and the wet solids are dried, often in a direct-fired rotary drier, to give a low-fat meal. The liquor is separated into high-quality fat and an aqueous phase (stickwater). Product loss in the stickwater should be low.

In other low-temperature systems (eg, the Atlas or Stord Bartz wet pressing systems), raw material is heated indirectly to about 90 to 95°C over a 30- to 60-min period in a preheater, then pressed in a twin-screw press (Fig. 6). Drainings from the preheater and press are further heated to coagulate any soluble protein, then centrifuged to remove fines. The fines and pressed solids are dried, usually in contact driers.

The stickwater from LTR systems can be concentrated by ultrafiltration or in evaporators, then dried. The steam side of the evaporator is often supplied with waste vapors from the cooker/drier to save energy. Heat can be recovered from the stickwater and from the vapors from the drier and used to preheat incoming raw material, or to provide hot water.

The advantages of LTR systems are as follow:

- Energy requirements are usually about half those of dry-rendering systems.
- The raw material may not need to be washed.
- A low heat treatment is used, so high-quality fat is produced.
- The meal has a low fat content and has a high nutritional value because heat treatment is minimal.
- Labor requirements are low.
- The system can be easily automated.

However, the systems have high capital costs, may have high repair and maintenance costs, and usually require highly trained technical operators.

Other methods of treating waste by-products from the meat industry (and also the fish and other protein indus-

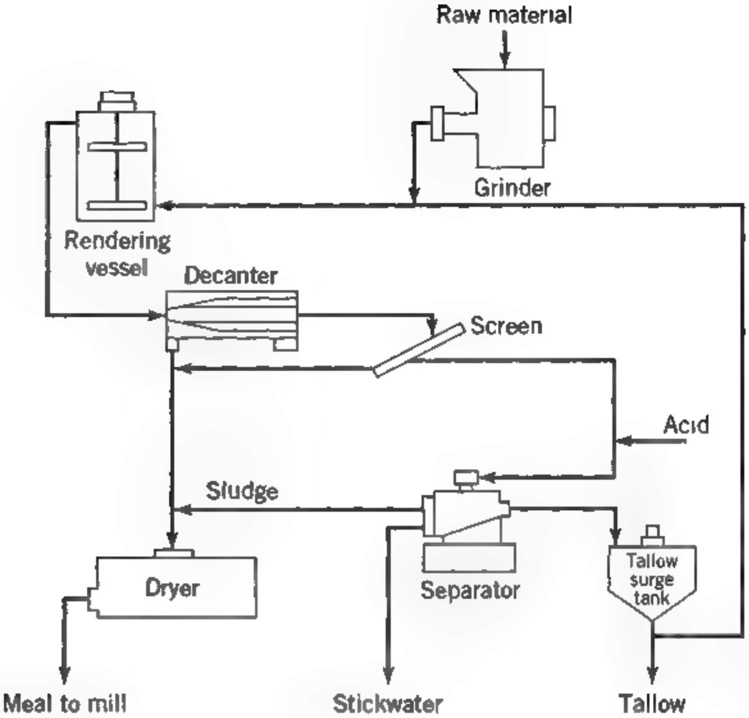


Figure 5. MIRINZ low-temperature rendering (MLTR) system.

tries) include hydrolysis using proteolytic enzymes or ensiling (12).

Uses of Fat

Animal fats are composed of triglycerides—three fatty acids esterified to glycerol. Fat quality is measured by titer; free fatty acid (FFA); FAC color (standard set by the Fat Analysis Committee of the American Oil Chemists Society); bleach color; and moisture, insoluble impurities, and unsaponifiable matter (MIU). Other tests that can be specified include saponification number, iodine value, peroxide value, and smoke point.

The fatty acid chain length and degree of saturation of the carbon bonds affect the fat's hardness or melting point (titer); the longer the chain length and the more saturated the fatty acid, the higher the titer. Fats of different species of animals and from different sites in the body have different titers. Type of feed can affect the titer, but rendering method does not.

When fat molecules break down, free fatty acids are released, so FFA content, which is usually expressed as percentage of free oleic acid, indicates the degree of spoilage that has occurred. To minimize FFA values, poor-quality raw material should be segregated from good-quality material, and material should be processed promptly. If material cannot be processed promptly, the raw material should be kept whole (unbroken) as long as practicable, to minimize microbial and enzymic activity, and preserved by cooling, or by adding acid. Processing equipment and storage tanks should be kept clean.

The factors affecting the color of fats include: animal breed, feed, age, and condition; source of the raw material; and presence of contaminants (feces, gut contents, etc). Fats can be almost white, yellow, or green (from contact with chlorophyll in digested plant material), or red or brown (from overheating or from contact with blood). Processing parameters during rendering (temperature, time) influence fat color. Some color components can be removed by bleaching with activated clay, and the color of the bleached sample read in a Lovibond tintometer. Fat bleachability indicates the temperature and handling conditions that the tallow has been subjected to; the cleaner the raw material and the lower the temperatures used, the lighter the bleach color.

The MIU value indicates fat purity. Moisture content should be as low as possible, because microbial and enzymic activity can hydrolyze fat at fat–water interfaces. Insoluble impurities such as protein fines, ground bone, manure, and so on that were not completely removed during processing may form colloidal fines that are not removed by settling or centrifuging. Trace amounts of copper, tin, and zinc can cause fat oxidation, and any polyethylene that has melted during rendering can adversely affect industrial processes that utilize fats. Unsaponifiable matter is fatty components such as cholesterol and gums that cannot be converted into soap by the use of alkali. Such matter reduces soap yields and affects catalyst efficiency. Unsaponifiable matter can also impart objectionable odors.

The saponification number indicates the average length of fatty acid chains, and the iodine number indicates the degree of unsaturation. These values can be used to identify types of fats and oils. The peroxide value indicates the degree of rancidity. Tallow that is not rancid and with good oxidative stability will have a low peroxide value. The smoke point is directly related to FFA and is the temperature to which the fat can be heated before it begins to smoke.

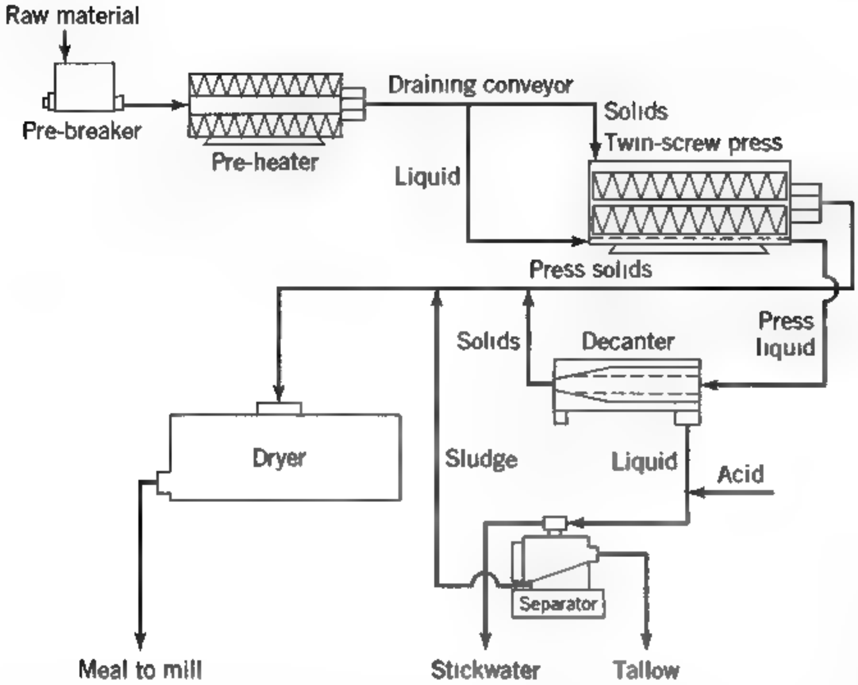


Figure 6. Wet pressing low-temperature-rendering system.

The two important fats of (land) animals used commercially are lard (fat from pigs) and tallow (fat from sheep and beef animals). Lard is made from specified, clean, sound tissues of healthy pigs, whereas rendered pork fat can be made from any fatty tissue. Edible tallow (dripping) is manufactured from specified edible fatty tissue. Oleo stock (premier jus) is a high-grade tallow prepared by low-temperature wet rendering of the fresh internal fat from beef. Inedible tallows (usually defined as fat with a melting point >40°C) and greases (melting point <40°C) are produced in many grades from inedible fatty tissue and dead stock.

Many different grades of tallow can be produced, and most countries have their own trading standards for tallow and grease. Tallow price reflects the prices obtained for other oils and fats, especially soy and palm oil. Vegetable oils are usually used for edible purposes and tend to be higher priced than tallow, which is used mainly for inedible processes such as soap manufacture, oleochemical production, and as an energy source in animal feed. Beef tallow is the largest component of bath bar soaps because of its economics and availability and its chemical composition. Fats are a useful component of animal feeds, as they have about twice the energy content of protein and carbohydrates. They also reduce dust, improve color and texture, enhance palatability, increase pelleting efficiency, and reduce machinery wear during feed production. Tallow can be used as an oleochemical feedstock. The resulting derivatives can be used to make abrasives, candles, cement additives, cleaners, cosmetics, paints, polishes, perfumes, detergents, plastics, synthetic rubber and water-repellent compounds. Tallow can be fractionated into its various components by crystallization, supercritical extraction, solvent extraction, or detergent fractionation. Tallow and its methyl esters can also be used as a fuel oil. Considerable amounts of tallow are still used for soap making. Glycerine, a coproduct of soap or fatty acid production, can be purified and used as a chemical in its own right.

Edible tallow and lard can be used in margarine, shortenings, and cooking fats. Tallow tends to give a better flavor to fried foods and is more stable during the cooking process than vegetable oils. However, because hard fats such as tallow can be associated with heart disease, the overall consumption of edible tallow has declined.

Uses of Meals

The material remaining after water and fat have been removed can have one of many names, depending on the rendering method and/or raw material used. These names include tankage, meat-meal tankage, digester tankage, wet-rendered tankage, or feeding tankage (finely ground, dried residue from wet-rendering material low in hair, hoof, horn, manure, and paunch contents), digester tankage with bone, meat and bone meal digester tankage, meat and bone meal tankage, or feeding tankage with bone (of higher phosphorus content than feeding tankage); meat meal (usually from dry rendering processes); and meat and bone meal or meat and bone scrap (of higher phosphorus content than meat meal). Feather meal is made from finely ground, wet-rendered feathers. This material is very digestible, but not well balanced nutritionally. Steamed bone meal (from wet rendering), or bone meal (from dry rendering), is defatted, dried, and ground bones suitable for animal feeding. Poultry meal has a similar composition, appearance, and nutritional value to meat meal but is made from poultry by-products. Fish meal is another high-quality meal similar to meat meal.

The type of raw material rendered and the rendering process used influence the composition of the meal produced. Meals made from proteinaceous material are high in nitrogen (the crude protein content is usually >50%) and also contain calcium, phosphorus, and fat. Bone meal and low-quality meat meals are made from material with a high bone content and have a relatively small market. One main use is as a fertilizer, but these meals are now being replaced by mineral fertilizers. Meals with a higher protein content are sold as meat and bone meal for use in animal feed formulations. They have a typical composition of 50% minimum crude protein, 4 to 10% moisture, 8 to 16% fat, and 20 to 30% ash. The price received for a given meal usually depends on its crude protein content, although some buyers may specify a minimum digestibility and availability of amino acids.

Bovine spongiform encephalopathy (BSE), commonly known as mad cow disease, was identified in the 1980s. Because of the belief that BSE transmission is via feeding-rendered ruminant protein to other ruminants, some countries introduced legislation banning the use of ruminant-derived animal proteins in animal feeds. This can have a great economic impact on the rendering industry. Researchers are developing economic sterilization processes and/or developing methods to monitor the sterilization effect of rendering processes (13,14).

FINE CHEMICALS AND PHARMACEUTICALS

Animals have ductless (endocrine) glands that secrete hormones, and ducted glands and organs that release enzymes and other biologically active molecules. Many biological chemicals can be recovered. A description of the animal glands and the medicinal and pharmaceutical uses of by-products is given in References 15 to 17.

The glands and tissues used for producing fine chemicals represent a small portion of the animal's live weight. Age, sex, and species of the animal determine the content of the active material. Most glands are very perishable and must be processed quickly to limit autolysis and microbial activity. The logistics of collecting sufficient raw material to operate a fine chemical plant or process usually limit operations either to larger meat plants or to centralized processing of material from many small plants.

SAUSAGE CASINGS

Intestines can be processed into natural casings used in sausage manufacture (18). Casing quality is affected by handling and cleaning procedures. Factors such as the age and species of the animal, breed, fodder consumed, and the conditions in which the animals are raised also affect casing quality and value. Animal casings naturally come in a wide variety of different shapes and sizes. The preference for a particular type of casing varies from country to country.

Reconstituted casings are manufactured from hide pieces (19), and artificial casings are often made from cellulose. Animal intestines are also used for the manufacture of surgical sutures, and strings for musical instruments and tennis rackets. Intestinal tract not used for these purposes is converted into pet food or is rendered to yield meat meal and tallow.

PET FOOD

Inedible animal tissue and rendered material can be used in both wet and dry pet foods. Pet foods require high-quality ingredients, which means that renderers may have to segregate raw material. Generally, pet-food manufacturers are using less fatty tissue and tallow and more protein-rich tissue and/or meat meal in their formulations.

BIBLIOGRAPHY

1. American Meat Institute Committee on Textbooks, *By-Products of the Meat Packing Industry*, Institute of Meat Packing, University of Chicago, Chicago, 1958.
2. A. Levie, *The Meat Handbook*, 2nd ed., AVI Publishing Co., Westport, Conn., 1967.
3. F. Gerrard, *Meat Technology*, Leonard Hill, London, 1971, pp. 65–77.
4. P. Filstrup, *Handbook for the Meat By-Products Industry*, Alfa-Laval Slaughterhouse By-products Department, Denmark, 1976.
5. C. W. Dill and W. A. Landmann, "Food Grade Proteins from Edible Blood," in A. M. Pearson and T. R. Dutson, eds., *Edible Meat By-Products*, Vol. 5, *Advances in Meat Research*, Elsevier Applied Science, New York, 1988, pp. 127–145.
6. C. L. Knipe, "Production and Use of Animal Blood and Blood Proteins for Human Food," A. M. Pearson and T. R. Dutson, eds., *Edible Meat By-Products*, Vol. 5, *Advances in Meat Research*, Elsevier Applied Science, New York, 1988, pp. 147–165.
7. V. M. Gorbatov, "Collection and Utilization of Blood and Blood Proteins for Edible Purposes in the USSR," A. M. Pearson and T. R. Dutson, eds., *Edible Meat By-Products*, Vol. 5, *Advances in Meat Research*, Elsevier Applied Science, New York, 1988, pp. 231–274.
8. J. Vrchlabsky and J Budig, "Animal Blood as Feed–Chemical Preservation," *Meat Focus Int.* **5**, 82–83 (1996).
9. Q. T. Pham, "Behaviour of a Conical Spouted-bed Dryer for Animal Blood," *Canadian J. Chem. Eng.* **61**, 426–434 (1983).
10. J. E. Swan, "*Maximising Yields from Conventional Blood Processing*," MIRINZ Food Technology and Research Ltd. Bulletin No. 10, Hamilton, New Zealand, 1985.
11. T. Fernando, "The MIRINZ Low-Temperature Rendering System," *Proc. of the 22nd Meat Industry Research Conference*, MIRINZ Food Technology and Research Ltd. Publ No 816, Hamilton, New Zealand, 1982, pp. 79–84.
12. B. Urlings, *Fermentation of Animal By-Products*, ADDIX, Wijk Bij Duurstede, The Netherlands, 1992.
13. H. Heubel, "The Continuous Sterilization Process for Slaughterhouse By-Products," in B. A. P. Urlings, ed., *Upgrading of Slaughter By-Products for Animal Nutrition*, ECCEAMST, Utrecht, The Netherlands, 1995, pp. 33–37.
14. D. M. Taylor, S. L. Woodgate, and M. J. Atkinson, "Inactivation of the Bovine Spongiform Encephalopathy Agent by Rendering Procedures," *Veterinary Record* **137**, 605–610 (1995).
15. H. W. Ockerman and C. L. Hansen, *Animal By-Product Processing*, Ellis Horwood, Chichester, England, 1988.

16. R. J. Banis, "Pharmaceutical and Diagnostic By-Products," in J. F. Price and B. S. Schweigert, eds., *The Science of Meat and Meat Products*, 3rd ed., Food and Nutrition Press, Inc., Westport, Conn., 1987.
17. A. M. Pearson and T. R. Dutson, eds., *Inedible Meat By-Products*, Vol. 8, *Advances in Meat Research*, Elsevier Applied Science, New York, 1992.
18. R. E. Rust, "Production of Edible Casings," A. M. Pearson and T. R. Dutson, eds., *Edible Meat By-Products*, Vol. 5, *Advances in Meat Research*, Elsevier Applied Science, New York, 1988, pp. 147–165
19. L. L. Hood, "Collagen in Sausage Casings," A. M. Pearson, T. R. Dutson, and A. J. Bailey, eds., *Collagen as a Food*, Vol. 4, *Advances in Meat Research*, AVI Publishing Co., New York, 1987, pp. 109–129

GENERAL REFERENCES

References 11 and 17 give a good overview.

T. Fernando, "Blood Meal, Meat and Bone Meal and Tallow," in A. M. Pearson and T. R. Dutson, eds., *Inedible Meat By-Products*, Vol. 8, *Advances in Meat Research*, Elsevier Applied Science, New York, 1992, pp. 81–112.
J. F. Price and B. S. Schweigert, eds., "Meat Animal By-Products and Their Utilization," *The Science of Meat and Meat Products*, 3rd ed., Food and Nutrition Press, Inc., Westport, Conn., 1987.
D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vols. 1–3, 4th ed., Wiley, New York, 1982.
I. A. Wolff, ed., "Red Meat and Meat Products," *CRC Handbook of Processing and Utilization in Agriculture*, Vol. 1, *Animal Products*, CRC Press, Boca Raton, Fla., 1982.

J. E. Swan
University of Waikato
Hamilton, New Zealand

ANIMAL SCIENCE AND LIVESTOCK PRODUCTION

The discipline of animal science is concerned with the application of the biological and physical sciences to the efficient production of livestock. Food and fiber are the primary products of livestock production in the United States. Examples of products derived from animal agriculture include meat, milk, eggs, wool, mohair, leathers, pharmaceuticals, and products used in research laboratories throughout the world.

BUSINESS ASPECTS OF LIVESTOCK PRODUCTION

Livestock are raised on a wide variety of terrains, climates, and management systems. However, concentrations of livestock raising often border on regions of grain raising (cattle and pigs are heavily concentrated in the Midwestern states of Iowa, Kansas, Nebraska, and Texas) or located near population centers (dairy cattle are concentrated in the Northeastern states, Wisconsin, Idaho, and California) (1). Fifty percent of the land area of the United States is classified as grazing or range area that cannot be used to cultivate crops (2). Ruminant livestock (cattle and sheep) are efficient utilizers of the millions of acres of land in the United States too rough or dry to grow crops.

Agriculture is the largest industry in the United States, accounting for 15% of the gross national product. About 2 million people are directly involved in production agriculture with ca 1.37 million agricultural operations involving beef or dairy cattle (2).

Beef Cattle

The beef cattle industry is the largest segment of American agriculture, accounting for almost 25% of all farm marketings (3). Indeed, nearly 26 billion lb of beef were produced in 1997 (4). Cattle are raised in every state, including Hawaii and Alaska. Commercial cattle operations tend to be large and diverse enterprises that involve many types and breeds of cattle. The beef cattle industry in the United States is distinctly segmented into three production schemes: cow-calf (both commercial and purebred), stocker (intensive grazing), and feedlot. The term feeders is sometimes used to describe cattle, pigs, and lambs going into intensive feeding programs. Feeder cattle production is the goal of commercial (nonpurebred) cow-calf and stocker operations. Feeder cattle may be either steers or heifers and are the animals of highest value in terms of beef production. The singular goal of purebred cattle production is the selective breeding and selection of superior offspring to attain distinguishable characteristics of the breed, such as carcass merit, production efficiency, or reproductive traits.

Stocker cattle are weaned calves grazed on grass for inexpensive growth. This period is characterized by growth of lean tissues and frame size. In the Midwest, stocker cattle may be grazed on small grain pastures, grain stubble, or legume pastures before being placed in feedlots for fattening. Stocker cattle are usually owned for less than 1 year.

Fattening steers and heifers in feedlots involves feeding nearly market-sized cattle moderately high to high energy rations (grain) until they have reached a sufficient finish (fattening) to produce a carcass that will grade USDA choice. Large feedlots predominate in the Western states and account for a large percentage of fed-beef marketings. Midwestern feedlots tend to be more plentiful, but are generally smaller and produce a smaller percentage of fed-beef marketings. Most fat steers and heifers are slaughtered at 15–24 months of age.

Bulls are sometimes fed for slaughter and are usually referred to as bullocks. Bulls generally gain weight more rapidly and are more feed efficient than steers or heifers. Bulls also tend to be leaner. Older cows culled from beef or dairy cow herds are also sometimes fed grain for short periods of time before slaughter to increase the palatability (juiciness and tenderness) characteristics of the meat.

Dairy Cattle

Dairy farms are highly specialized agricultural enterprises, with considerable financial and labor input and returns. Dairying as a secondary enterprise on farms and ranches is uncommon. The number of dairy farms and dairy cattle in the United States has steadily declined during the past 20 years. The number of farms with dairy cattle decreased about 92% from 2 million in 1950 to 164,000 in 1992 (1,5,6). However, the decrease has been offset by a significant increase in milk production (Fig. 1). Modern dairy cattle produce an average of 16,400 lb of milk per lactation (7).

The price dairy farmers receive for whole (fluid) milk has been, historically, on hundred weight basis according to the percentage of butterfat. However, as the value of butterfat has declined as the most valuable component of whole milk, the price differential has increased less than the price per pound. The decrease in demand for butterfat is directly attributable to the introduction of margarine (containing plant oils), increased consumption of cheeses (made from whole milk), and processed food containing nonfat dry milk. Also, there has been a strong trend for health-conscious people to eat and drink only low fat and non-fat dairy products such as low fat cottage cheese, low fat yogurt and skim milk.

Locations of dairy farms have traditionally been close to major markets. This is because of the high cost of transporting whole milk, which contains a high proportion of water, and because milk is relatively perishable. However, in recent years location of dairies near consumers has been a less important consideration than the high cost of real estate near major markets. Also, conflicts between large dairies and home owners over water quality, dust, flies, and odors have forced dairy farms to relocate to less populated areas. Improved processing techniques and more rapid transportation networks also have contributed to decreased costs in moving milk to markets.

Swine

Swine production units are located in every state of the United States, but are generally concentrated in or near feed-grain-growing regions of the central United States. The corn belt states of Iowa, Illinois, Minnesota, Indiana, and Nebraska are leaders in market hog production (1,9). Swine farrowing was traditionally a spring and fall operation, but with the development of improved housing and nutrition, production of swine is a year-round business. This has tended to equalize the monthly marketings of pigs and reduced the seasonal fluctuations in market price.

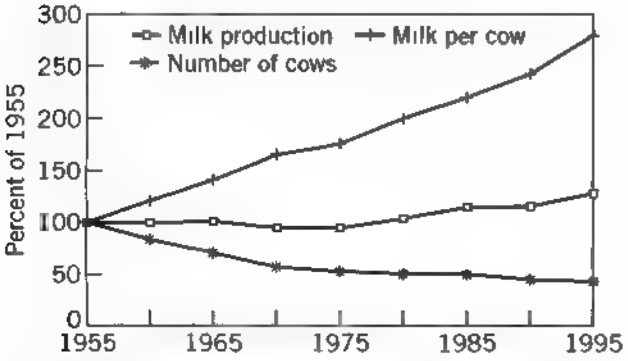


Figure 1. Index of milk production, milk per cow, and cow numbers in the United States *Source:* Refs. 5, 6, and 8.

The trend in pork production, as in other agricultural operations, has been toward larger and more specialized operations. There is also a trend toward more confinement units in the production of pigs. Swine production systems usually can be described by one of three basic systems: farrowing to finishing, feeder pig production, and finishing feeder pigs.

Finished pigs are usually sold directly to the processor by the producer. Selecting the market and timing the presentation of pigs for slaughter are two of the most important decisions made by the swine producer. Most pigs are sold on a live-weight basis, but some are sold based on carcass yield and grade. Computerized hog-marketing systems are also being used in some parts in the United States.

Sheep

The products of sheep production businesses are primarily lambs and mutton for meat and wool. Most of the sheep in the United States are raised in the Western states. Texas, California, Colorado, South Dakota, Wyoming, and Utah account for more than 50% of the sheep produced (10). Sheep production systems are generally classified as farm flocks or range flocks. Predators, such as coyotes and wild dogs, and disease are some of the common problems associated with raising sheep. Lamb mortality rates as high as 20% are not uncommon. Most feeder lambs are sold to feedlots weighing between 65–80 lb. Ewes culled from flocks are also marketed for slaughter. The seasonality of breeding for sheep unfortunately causes a shortage of fresh lamb during certain times of the year.

THE DISCIPLINE OF ANIMAL SCIENCE

The multiple and interdisciplinary needs of farm animal production that focus on growth, reproduction, and lactation are at the center of animal science programs in research, teaching, and extension. Animal science is primarily an applied science that incorporates advances in science and technology from all disciplines. Nearly all land-grant universities, as well as several state and private colleges with agricultural programs, offer a bachelor of science degree in animal science. Most animal science departments in land-grant universities also offer graduate programs leading to master of science (M.S.) and doctor of philosophy degrees (Ph.D.). The Ph.D. degree is a research-based science degree.

Curriculum at the undergraduate level is designed to prepare students for professional careers in animal agriculture. The student majoring in animal science usually takes specialized courses, which include nutrition, breeding and genetics, biochemistry, animal reproduction, agricultural economics, animal health, meat science, dairy science, and animal production and products courses.

The Journal of Animal Science. The Journal of Dairy Science, and *Meat Science* serve as the repository for advances in the science of livestock production as well as their products.

PRINCIPLES OF ANIMAL REPRODUCTION AND BREEDING

Productivity of livestock depends on reproductive efficiency and is usually measured by the number of offspring produced by an animal or herd per unit of time. Therefore, management of reproductive cycles is critical for obtaining maximum efficiency of reproducing animals.

Animal Reproduction

Dairy and beef cattle are derived from common ancestors and thus have common reproductive characteristics. The period of time from one ovulation to the next ovulation is called the estrous cycle. The estrous cycle in cattle is on average 21 days in length and is characterized as being one in which the cow does not permit mating to occur except at the time of ovulation. This period of time in which mating is permitted is relatively short, being in the order of 16–18 h. The estrous cycle in cattle is continuous (polyestrous) throughout the year and is not seasonal as is the case in some other ruminant-type animals such as sheep, deer, antelope, and elk. Cattle ovulate 10–14 h after the end of estrus, and normally one follicle ovulates per estrous cycle. The gestation period is 283 days, and twins occur only 1% of the time.

Sheep are generally seasonally polyestrous, with recurring estrous cycles during the fall of the year followed by a prolonged quiescent period. Some breeds of sheep that originated in equatorial regions and are subject to less variations in temperature and photoperiod have longer breeding seasons. The period of sexual receptivity in sheep lasts for 24–36 h, but may vary widely. The length of the estrous cycle is 14–19 days. Seasonal breeding in animals is associated with an increased frequency and size of episodic releases of luteinizing hormone (11). Ovulation normally occurs near the end of estrus. Ovulation rate is influenced by breed and nutrition, but twinning is extremely common in sheep. Gestation is 150 days in length.

The pig, like the cow, is polyestrous. The period of sexual receptivity in pigs lasts about 40–60 h and may be influenced by season, breed, and endocrine dysfunction (12). The length of the estrous cycle is 21 days. Ovulation rates are strongly influenced by weight of the gilt at breeding and the amount of inbreeding. Gestation in the pig is 114 days and average litter size is in the order of 9–12 pigs per litter.

The estrous cycle in all livestock is characterized by profound changes in behavioral patterns and blood hormone profiles. Cyclic changes during the estrous period reflect the secretory functions and interdependence of the ovary, uterus, hypothalamus, and pituitary gland. The estrous cycle is controlled by ovarian hormone secretions (estrogens and progesterone) and may be subdivided into a follicular phase, an ovulatory phase, and a luteal phase (Fig. 2). The ovary functions to provide fertilizable ova and a balanced ratio of steroid hormones to facilitate development of the reproductive tract for migration of the early embryo and successful implantation in the uterus.

The male reproductive system in livestock is comprised of two testes (producing both sperm and the male sex hormone testosterone), excretory ducts (epididymis, vas deferens, and ejaculatory duct), and accessory structures (prostate, seminal vesicles, bulbourethral glands, and penis). The scrotum, containing two testes, provides for efficient regulation of testicular temperature. Descent of both testes from the abdomen to their proper scrotal location is necessary for maximal fertility. Failure of one or both testes to descend is a common reproductive organ defect in livestock, especially in swine, where the condition is a hereditary defect transmitted by the male and is referred to as cryptorchidism (13).

Reproductive management of the male primarily involves maintenance of health and nutrition to optimize sperm production and libido. Methods for assessing individual male fertility, such as breeding soundness evaluations, are subjective and poorly correlated with pregnancy rates.

Breeds of Livestock

Decisions about breeding are some of the most important decisions a livestock producer must make. The livestock breeder must consider the heritability of a characteristic, such as prolificacy, feed conversion, milk yield, or carcass merit, for example. The livestock breeder also must consider the merits of inbreeding, outbreeding, crossbreeding, and the relative merits of different breeds of animals.

The development of modern breeds of livestock began in the 1700s. The origin of cattle and sheep breeds can be traced to Europe and the British Isles. For classification purposes there are four basic categories of modern beef and dairy cattle. The classification system for cattle is not consistent because two of the categories reflect geographical origin of the cattle and the other two reflect the purpose of the cattle.

1. British and continental breeds (beef): Includes Angus, Hereford, Shorthorn, Charolais, Limousin, Chianina, Gelbvieh
2. North American breeds (beef): Brahman, Brangus, Beefmaster, and Santa Gertrudis
3. Dual-purpose breeds (beef and dairy): Ayrshire, Milking Shorthorn, Red Poll, and Brown Swiss
4. Dairy breeds: Guernsey, Holstein, and Jersey

The foundation of modern-day pigs can be traced to European and Asiatic strains of pigs. Modern swine are raised to produce lean high-quality pork. However, not long ago, breeds of swine were classified as lard-type and bacon-type. Although the individual breeds of swine continue today, all swine producers strive for the same high-quality meat-type pig.

As in the case of cattle, the classification system for breeds of sheep is inconsistent. The Rambouillet and Merino breeds were developed for the production of the fine wool characteristic, but much lamb and mutton comes from these breeds. Likewise, income derived from wool represents a significant portion of the value of the meat-type breeds.

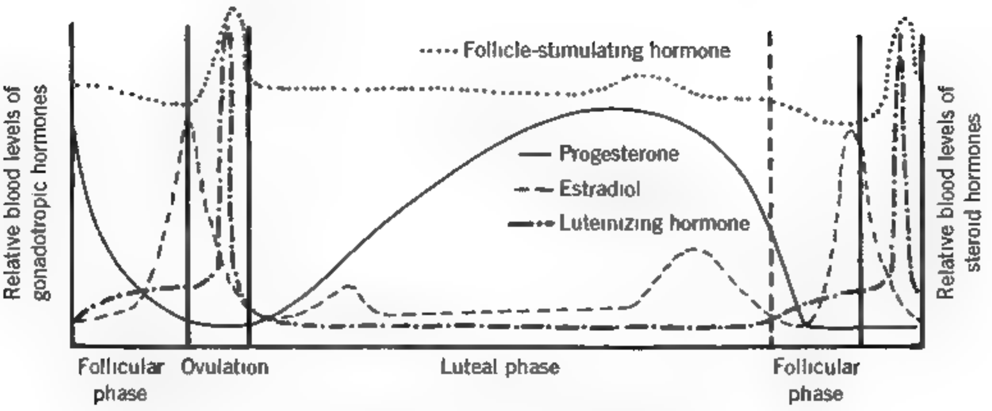


Figure 2. Cyclic changes in ovarian steroid and gonadotropic hormones in the ewe.

Animal Breeding

Crossbreeding is one of the quickest and most economical methods for increasing total beef or pork production. Heterosis, also sometimes commonly referred to as hybrid vigor, is the result of crossbreeding. Increased performance and vigor is not limited to animal breeding, but also results in hybrid plant production. Hybrid increases are seen in the first generation (F1) crossbred animals. F1 animals are the result of crossbreeding two purebred lines of livestock. Increased performance benefits may be as high as 25% for F1 calves. Hybrid chickens and crossbred pigs have contributed to the increased economics of poultry and pork production.

Three general crossbreeding systems are used to produce market animals: (1) Mating females of one breed with males of another to produce a F1 market animal; (2) three-breed terminal crossbreeding, where F1 females produced according to (1) are mated to bulls of a third breed to produce a three-breed market animal with a maximal level of heterosis (alternatively, the F1 female could be mated to one of the original parent breed to produce a backcross market animal); (3) rotational crossbreeding, where the breed of the sire is rotated among the three original breeds. Rotational breedings usually only use three breeds because the heterosis gained by the addition of a fourth breed is small.

PRINCIPLES OF ANIMAL NUTRITION AND FEEDING

The proper feeding of livestock is a matter of supplying them with the correct amount of nutrients essential for reproduction, growth, or lactation. Nutrients are chemical elements and compounds required by the cells of the animal's body in support of the three basic functions: (1) structural matter for building and maintaining the body; (2) a source of energy for work, thermogenesis (heat production), and fat deposition; and (3) regulating body processes or the synthesis of body regulators. An auxiliary function of nutrients would be their use in milk production.

The Nutrients

Nutrients required for livestock, just like for humans, can be categorized into six functional or chemical classes: carbohydrates, proteins, fats, minerals, vitamins, and water. All carbohydrates or saccharides are related structurally and chemically and contain the same amount of gross energy. Carbohydrates are comprised primarily of hexose ($C_6H_{12}O_6$) and pentose ($C_5H_{10}O_5$) molecules. Tetrose and triose molecules are present in small quantities but are generally not important in animal nutrition. Carbohydrates are usually categorized as monosaccharides, disaccharides, and polysaccharides based on how many hexose and pentose molecules are linked together. Common monosaccharides, also called simple sugars, consist of glucose, fructose, and galactose. Disaccharides consist of two monosaccharides linked together with hydroxyl groups of each sugar unit. The common monosaccharides include sucrose (table sugar), maltose, and lactose. Polysaccharides have the empirical formula $(C_6H_{10}O_5)_n$ and contain large polymers of monosaccharides. Polysaccharides important in livestock nutrition include starch, glycogen, and fiber (hemicellulose, cellulose, and lignin).

Carbohydrates are important energy sources in livestock feeds, comprising 65–80% of plant dry weight. Carbohydrate as a class of nutrients is usually divided into two groups: nitrogen free extract (NFE) and fiber. Fiber is what remains after a feed has been boiled in dilute acid or alkali and roughly approximates the amount of carbohydrate poorly digested in the animal's intestinal tract. NFE represents the soluble, readily digestible carbohydrate portion of a feedstuff. Corn grain, eg, contains only 2.2% fiber and 70% NFE. Good-quality alfalfa hay contains 26% fiber and 46% NFE. Cellulose is not an efficient energy source for nonruminant livestock (swine and poultry) but can be readily digested by the bacteria of the rumen. Because of this characteristic, ruminant livestock occupy an important niche in utilizing a potentially wasted feed resource.

Lipids are water-insoluble organic molecules that can be extracted from plant and animal tissues with nonpolar solvents such as benzene and ether. Lipids contain 2.25 times more energy per unit weight than either carbohydrate or protein. The term lipid is used in a general sense and used interchangeably with fat and oils. Livestock rations are generally low in fat, with most grains and roughages containing less than 5% lipid. Fats may sometimes be added to a ration, especially for swine and poultry, and high producing dairy cattle when higher energy rations are desired.

Lipids are composed of carbon, hydrogen, and oxygen, as are carbohydrates, but contain less oxygen. Lipids have several important biological functions, including storage and transport forms of energy and components of cell surface membranes. The fatty acid composition of body fat may be altered by the composition of dietary fats. This is especially true for swine and poultry.

Protein constitutes the most expensive portion of livestock feed. Protein primarily consists of 20 α amino acids linked together by peptide bonds. Both plant and animal tissues contain a diversity of proteins with variable amounts of amino acids. Ten amino acids cannot be synthesized by animal tissues or can be synthesized only to a limited extent. These 10 amino acids are referred to as dietary essential amino acids (Table 1). The primary function of dietary protein in livestock rations is to supply amino acids, the building blocks of proteinaceous body tissues. Ruminant livestock do not have dietary amino acid requirements as such, but depend on ruminal bacterial protein synthesis to supply these nutrients. Because of this, ruminant animals are able to utilize nonprotein nitrogen, such as urea, to replace part of the natural protein in the ration. The use of rumen bypass protein supplements, such as corn gluten meal and blood meal, has been shown to increase growth in beef cattle and milk production in dairy cattle (14,15). Swine rations are usually balanced for the first two or three most-limiting amino acids as well as for balancing the ration for crude protein.

Sixteen or more minerals are required by livestock. Of these, seven are classified as macrominerals (Ca, P, K, Na, S, Mg, Fe), with the remaining constituting microminerals (Co, Cu, F, I, Fe, Mn, Mo, Se, Zn). A description of the minerals' functions and deficiency characteristics in livestock nutrition has been reviewed in Ref. 16.

Vitamins are dietary-essential organic molecules that are required in minute quantities by livestock. Vitamins are usually classified into two groups: water soluble and fat soluble (Table 2). Approximately 15 vitamins are known to function in animal metabolism, but only several of these are needed in the ration of livestock because synthesis of certain vitamins occurs in the animal. Animal nutritionists formulating livestock rations are especially concerned about vitamins A and D for ruminants and certain B vitamins for nonruminants. Ruminants do not generally require B vitamin supplementation because the bacteria of the rumen synthesize sufficient quantities. Vitamin E is normally present in ample quantities in natural feeds, but may be supplemented to ruminants and nonruminant livestock receiving processed grain diets. Vitamin E supplementation of cattle diets also has been shown to stabilize the red color of fresh beef and help prevent oxidative rancidity (17). Livestock normally do not need ascorbic acid supplementation where adequate quantities are synthesized in the tissues. Ref. 16 outlines a review of vitamins in livestock nutrition.

Table 1. Dietary Essential Amino Acids Required by Swine and Poultry

Arginine	Lysine	Tryptophan
Histidine	Methionine	Valine
Isoleucine	Phenylalanine	Glycine (poultry)
Leucine	Threonine	Proline (poultry)

Table 2. Fat-Soluble and Water-Soluble Vitamins Required by Livestock

Fat-soluble	Water-soluble
Vitamin A	Thiamine
Vitamin D	Riboflavin
Vitamin E	Niacin
Vitamin K	Pyridoxine
	Biotin
	Pantothenic acid
	Vitamin B_{12}
	Choline
	Folic acid
	Inositol
	Para-aminobenzoic acid

Digestion

Digestion involves the physical and chemical preparation of feed in the gastrointestinal tract to absorption-ready nutrients. The digestive systems of livestock are anatomically and functionally similar, with the very important distinction of the ruminant having a large, four-compartment stomach (Fig. 3). The abomasum or the true stomach is functionally similar to the stomach of monogastrics. The rumen, the largest of the four compartments, with a capacity approaching 50 gal, functions as a fermentation vat and contains billions of bacteria and protozoa. The presence of the bacteria and the enzymes that they secrete enables the ruminant to efficiently use cellulose from fibrous plants and feedstuffs. The microorganisms also synthesize amino acids to make bacterial protein and supply the ruminant animal with essential amino acids.

RED MEAT PRODUCTION

The slaughtering procedure for beef and pork usually involves immobilization, bleeding, removing hair (pigs) or skin (cattle and sheep), and removing viscera—trachea, esophagus, lungs, heart, stomach, intestines, and reproductive organs. Immobilization of meat animals, except those killed for kosher meat, is accomplished according to humane, accepted methods (captive bolt or electric shock, eg) regulated by state and federal laws. Most commercial slaughtering plants use a movable rail where immobilized animals are individually hung by their shanks and moved along a deassembly line to dress the slaughtered animal. Electrical stimulation of fresh beef, pork, veal, and lamb carcasses is sometimes employed to improve tenderness

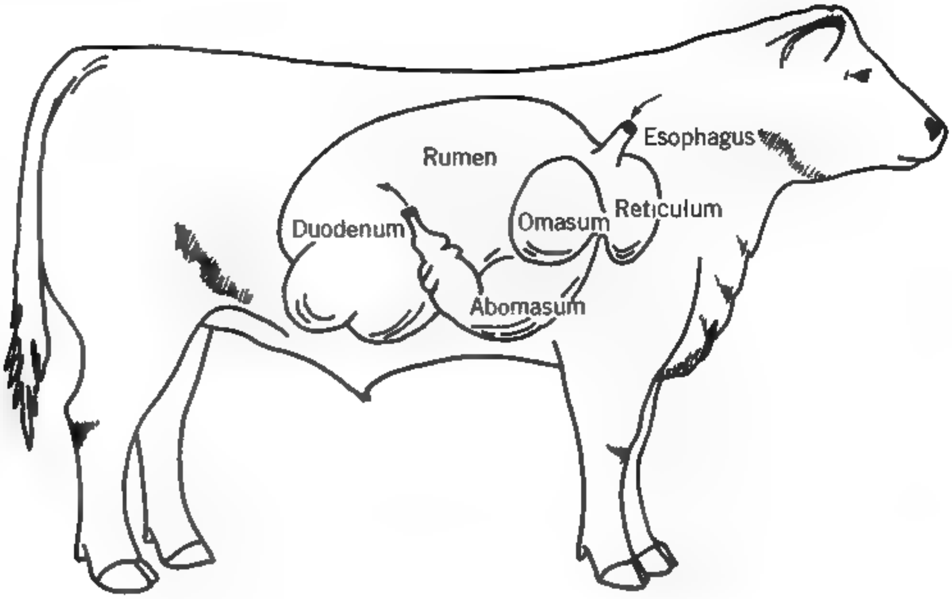


Figure 3. The digestive system of the polygastric ruminant.

(18). To facilitate rapid chilling of the carcass, beef carcasses are entirely split along the backbone, while pig carcasses are usually split to the neck.

The term carcass is what remains after dressing and usually refers to both sides of the same slaughtered animal. A side is one of the two halves of a split carcass. Beef quarters are the portions remaining after splitting a side of beef between the 12th and 13th rib. Wholesale cuts are divisions of the quarters including the round, full loin or short loin and sirloin, flank, rib, short plate, chuck, brisket, and fore shank. Primal cuts are any of the wholesale cuts except for short plates, briskets, flanks, and fore shanks.

The principal cuts of beef, pork, and lamb are shown in Figures 4, 5, and 6. Most beef is sold as carcass sides or quarter or wholesale cuts to retailers, wholesalers, chain-store breaking plants, and restaurants. However, an increasing portion of beef is sold as 60–80 lb saw- or knife-ready subprimal cuts shipped in boxes. Processors nearly always reduce pork carcasses to wholesale and retail cuts at the plant because hams and bellies, and sometimes other cuts, are smoked and cured. Most lamb is sold as intact carcasses, but some carcasses are reduced to wholesale cuts.

Significant quantities of beef are ground, tested for lean content, and blended to meet federal and state quality requirements for fat content. Low-quality carcasses, such as cutter and canner grades of beef, pale and soft pork carcasses, and cull grades of lamb and mutton, are not sold as wholesale or retail cuts but are instead boned-out at the plant. The edible meat from these carcasses may be sold as hamburger, or used in the preparation of a variety of meat products, such as canned meat products or sausage.

The USDA through the Food Safety and Inspection Service (FSIS) is responsible for ensuring the wholesomeness of the meat supply. FSIS assurance includes antemortem and postmortem inspection and compliance with sanitation and temperature, ingredients, and label requirements. Meat bearing the U.S. "inspected and passed" stamp informs the buyer the meat was processed in a federally approved plant and is wholesome and safe for human consumption. Additionally, the carcass may be graded for meat yield and quality. The yield grade is an estimate of the portion of the carcass that is edible meat. Quality grades are intended to identify meat on the basis of palatability and cooking attributes. Factors used to establish quality grades include kind or species of animal, class or physical attributes, maturity, marbling, firmness of flank, color and structure of lean, and conformation, fleshing, and finish of the carcass. USDA quality grades for various kinds of meat are given in Table 3.

The Food and Drug Administration (FDA) is responsible for ensuring the safety of the drugs used in livestock production and food additives. Both the FDA and the Federal Trade Commission have been involved with monitoring and regulating claims made during advertising of food products.

MILK PRODUCTION

Dairy cattle are normally milked two or three times per 24-h day. Following freshening (parturition), maximum milk production is normally reached after 45 days. A slow, but steady decline in milk produced by the cow occurs after peak yield until she is dried. Normal lactation periods for commercial dairy farms are 305 days lactation and 60 days dry (Fig. 7). The dry period allows for the cow to replenish her body reserves and rest her mammary tissue. Because a calf must be produced every 12.5 to 13.5 months in order to meet this idealized production schedule, cows are bred at approximately 80–90 days of lactation.

Feeding dairy cattle represents approximately 50% of the cost of milk production. The percentage of protein and fat may be influenced by feeding and management practices on dairy farms. Dairies may select feeding programs to produce fat and produce percentages that best suit milk

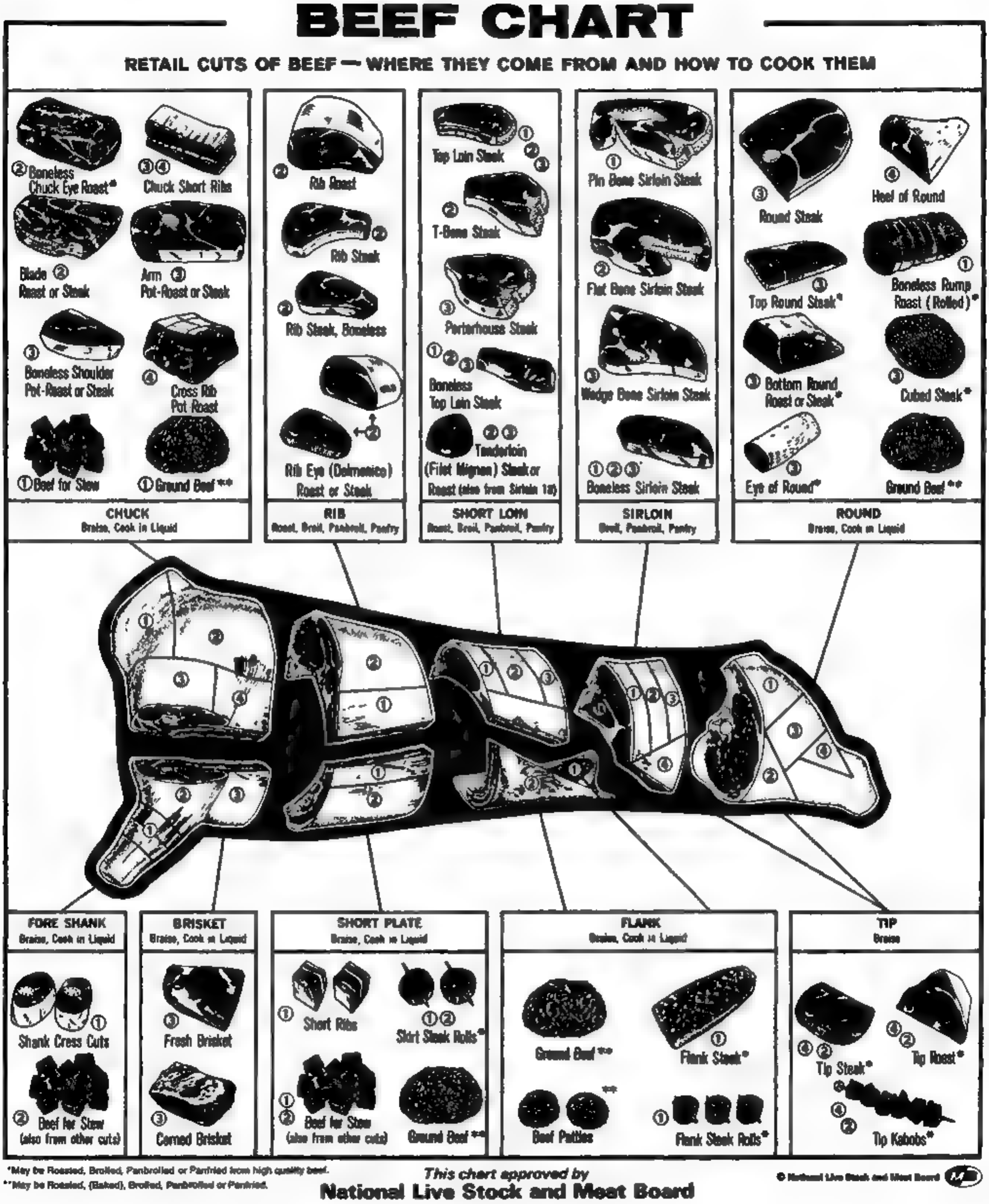

BEEF CHART
RETAIL CUTS OF BEEF — WHERE THEY COME FROM AND HOW TO COOK THEM
② Boneless Chuck Eye Roast*
③④ Chuck Short Ribs
Blade ② Roast or Steak
Arm ③ Pot-Roast or Steak
③ Boneless Shoulder Pot-Roast or Steak
④ Cross Rib Pot-Roast
① Beef for Stew
① Ground Beef**
CHUCK
Braise, Cook in Liquid
② Rib Roast
② Rib Steak
② Rib Steak, Boneless
② Rib Eye (Delmonico) Roast or Steak
RIB
Roast, Broil, Panbroil, Panfry
① ② ③ Top Loin Steak
② T-Bone Steak
③ Porterhouse Steak
① ② ③ Boneless Top Loin Steak
② ③ Tenderloin (Filet Mignon) Steak or Roast (also from Sirloin ①)
SHORT LOIN
Roast, Broil, Panbroil, Panfry
① Pin Bone Sirloin Steak
② Flat Bone Sirloin Steak
③ Wedge Bone Sirloin Steak
① ② ③ Boneless Sirloin Steak
SIRLOIN
Broil, Panbroil, Panfry
③ Round Steak
④ Heel of Round
③ Top Round Steak*
① Boneless Rump Roast (Rolled)*
③ Bottom Round Roast or Steak*
③ Cubed Steak*
③ Eye of Round*
Ground Beef**
ROUND
Braise, Cook in Liquid
①
②
③
④
FORE SHANK
Braise, Cook in Liquid
① Shank Cross Cuts
② Beef for Stew (also from other cuts)
BRISKET
Braise, Cook in Liquid
③ Fresh Brisket
③ Corned Brisket
SHORT PLATE
Braise, Cook in Liquid
① Short Ribs
① ② Skirt Steak Rolls*
① ② Beef for Stew (also from other cuts)
Ground Beef**
FLANK
Braise, Cook in Liquid
Ground Beef**
① Flank Steak*
Beef Patties**
① Flank Steak Rolls*
TIP
Braise
④ ② Tip Steak*
④ ② Tip Roast*
④ ② Tip Kabobs*
*May be Roasted, Broiled, Panbroiled or Panfried from high quality beef.
**May be Roasted, (Baked), Broiled, Panbroiled or Panfried.
This chart approved by
National Live Stock and Meat Board
© National Live Stock and Meat Board

Figure 4. Wholesale and retail cuts of beef.

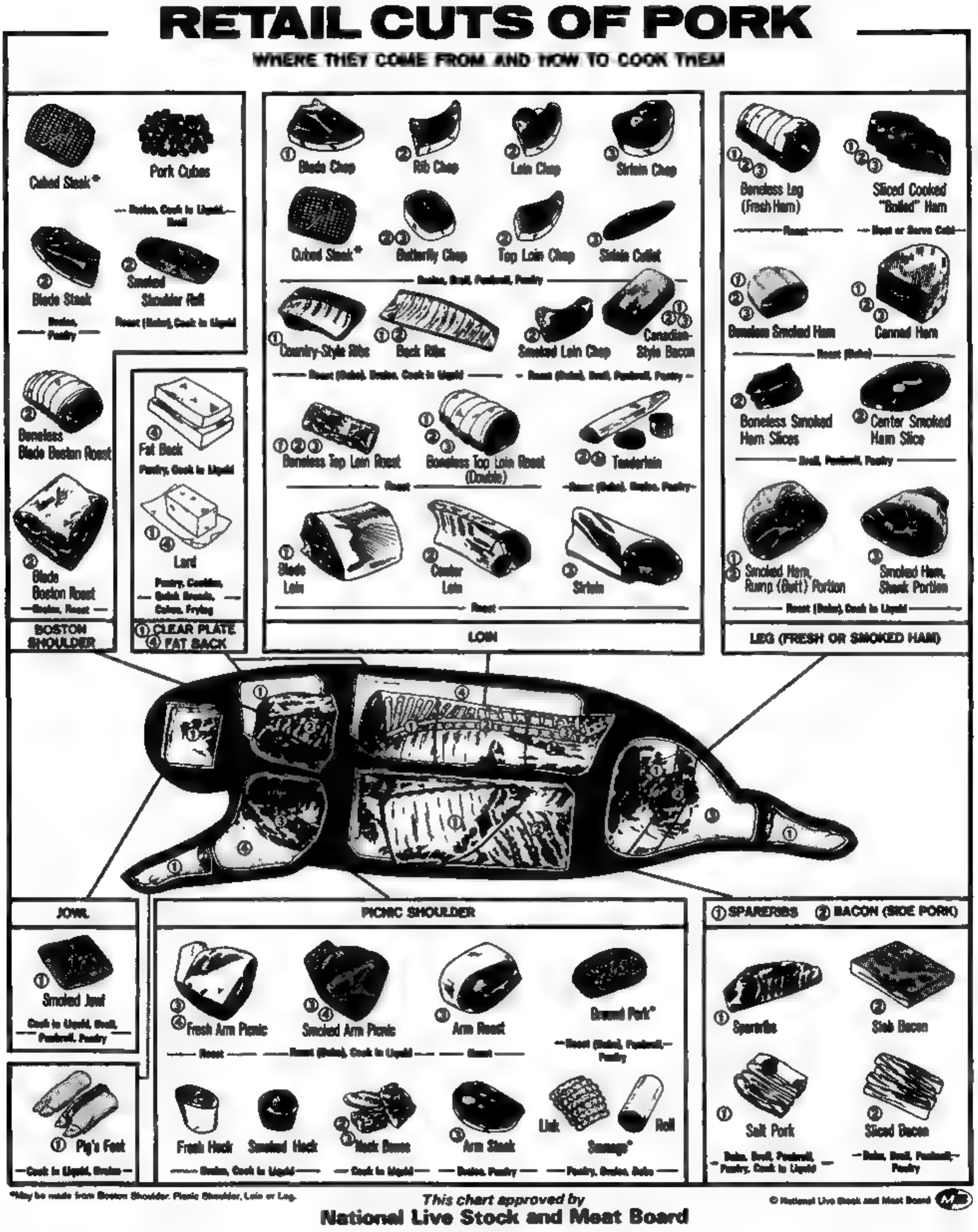

RETAIL CUTS OF PORK
WHERE THEY COME FROM AND HOW TO COOK THEM
Cubed Steak*
Pork Cubes
Braise, Cook in Liquid, Broil
② Blade Steak
Braise, Panfry
② Smoked Shoulder Roll
Roast (Bake), Cook in Liquid
② Boneless Blade Boston Roast
② Blade Boston Roast
Braise, Roast
BOSTON SHOULDER
④ Fat Back
Panfry, Cook in Liquid
①④ Lard
Pastry, Cookies, Quick Breads, Cakes, Frying
① CLEAR PLATE
④ FAT BACK
① Blade Chop
② Rib Chop
② Loin Chop
③ Sirloin Chop
Cubed Steak*
②③ Butterfly Chop
② Top Loin Chop
③ Sirloin Cutlet
Braise, Broil, Panbroil, Panfry
① Country-Style Ribs
①② Back Ribs
Roast (Bake), Braise, Cook in Liquid
② Smoked Loin Chop
①②③ Canadian-Style Bacon
Roast (Bake), Broil, Panbroil, Panfry
①②③ Boneless Top Loin Roast
①②③ Boneless Top Loin Roast (Double)
Roast
②③ Tenderloin
Roast (Bake), Braise, Panfry
① Blade Loin
② Center Loin
③ Sirloin
Roast
LOIN
①②③ Boneless Leg (Fresh Ham)
Roast
①②③ Sliced Cooked "Boiled" Ham
Heat or Serve Cold
①②③ Boneless Smoked Ham
①②③ Canned Ham
Roast (Bake)
② Boneless Smoked Ham Slices
② Center Smoked Ham Slice
Broil, Panbroil, Panfry
①② Smoked Ham, Rump (Butt) Portion
③ Smoked Ham, Shank Portion
Roast (Bake), Cook in Liquid
LEG (FRESH OR SMOKED HAM)
①
②
③
④
JOWL
① Smoked Jowl
Cook in Liquid, Broil, Panbroil, Panfry
① Pig's Feet
Cook in Liquid, Braise
PICNIC SHOULDER
③④ Fresh Arm Picnic
Roast
③④ Smoked Arm Picnic
Roast (Bake), Cook in Liquid
③ Arm Roast
Roast
Ground Pork*
Roast (Bake), Panbroil, Panfry
Fresh Hock
Smoked Hock
Braise, Cook in Liquid
②③ Neck Bones
Cook in Liquid
③ Arm Steak
Braise, Panfry
Link
Roll
Sausage*
Panfry, Braise, Bake
① SPARERIBS
② BACON (SIDE PORK)
① Spareribs
② Slab Bacon
① Salt Pork
Bake, Broil, Panbroil, Panfry, Cook in Liquid
② Sliced Bacon
Bake, Broil, Panbroil, Panfry
*May be made from Boston Shoulder, Picnic Shoulder, Loin or Leg.
This chart approved by
National Live Stock and Meat Board
© National Live Stock and Meat Board

Figure 5. Wholesale and retail cuts of pork.

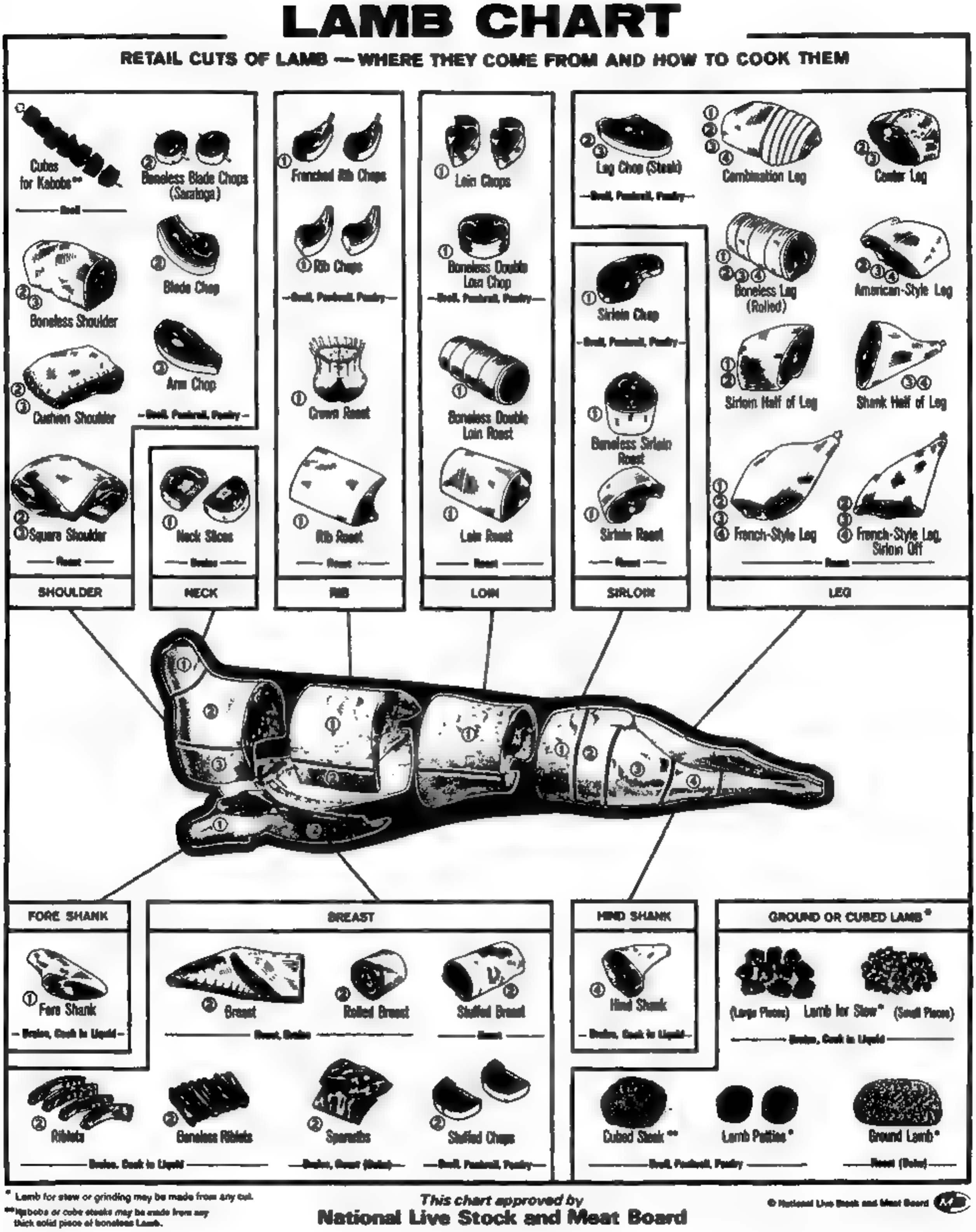

LAMB CHART
RETAIL CUTS OF LAMB — WHERE THEY COME FROM AND HOW TO COOK THEM
Cubes for Kabobs**
Broil
② Boneless Blade Chops (Saratoga)
② Blade Chop
②③ Boneless Shoulder
② Arm Chop
②③ Cushion Shoulder
Broil, Panbroil, Panfry
②③ Square Shoulder
Roast
SHOULDER
① Neck Slices
Braise
NECK
① Frenched Rib Chops
① Rib Chops
Broil, Panbroil, Panfry
① Crown Roast
① Rib Roast
Roast
RIB
① Loin Chops
① Boneless Double Loin Chop
Broil, Panbroil, Panfry
① Boneless Double Loin Roast
① Loin Roast
Roast
LOIN
②③ Leg Chop (Steak)
Broil, Panbroil, Panfry
① Sirloin Chop
Broil, Panbroil, Panfry
① Boneless Sirloin Roast
① Sirloin Roast
Roast
SIRLOIN
①②③④ Combination Leg
②③ Center Leg
①②③④ Boneless Leg (Rolled)
②③④ American-Style Leg
①② Sirloin Half of Leg
③④ Shank Half of Leg
①②③④ French-Style Leg
②③④ French-Style Leg, Sirloin Off
Roast
LEG
①
②
③
①
①
①
②
③
④
①
②
FORE SHANK
① Fore Shank
Braise, Cook in Liquid
BREAST
② Breast
② Rolled Breast
② Stuffed Breast
Roast, Braise
Roast
② Riblets
② Boneless Riblets
② Spareribs
② Stuffed Chops
Braise, Cook in Liquid
Braise, Roast (Bake)
Broil, Panbroil, Panfry
HIND SHANK
④ Hind Shank
Braise, Cook in Liquid
GROUND OR CUBED LAMB*
(Large Pieces) Lamb for Stew* (Small Pieces)
Braise, Cook in Liquid
Cubed Steak**
Lamb Patties*
Ground Lamb*
Broil, Panbroil, Panfry
Roast (Bake)
* Lamb for stew or grinding may be made from any cut.
** Kabobs or cube steaks may be made from any thick solid piece of boneless Lamb.
This chart approved by
National Live Stock and Meat Board
© National Live Stock and Meat Board

Figure 6. Wholesale and retail cuts of lamb.

Table 3. USDA Grades for Meat

Kind	Class	Grade names
Beef quality grades	Steer, heifer, cow	Prime, choice, good, standard, commercial, utiltiy, cutter, canner
	Bullock	Prime, choice, good, standard, utility
Beef yield grades	All classes	1, 2, 3, 4, 5
Calf quality grades		Prime, choice, good, standard, utility, cull
Veal quality grades		Same as calf
Lamb and mutton quality grades	Lamb, yearling lamb	Prime, choice, good, utility
Lamb yield grades	All classes	1, 2, 3, 4, 5
Pork carcasses	Barrow, gilt	U.S. No. 1, U.S. No. 2, U.S. No. 3, U.S. No. 4, U.S. utility
	Sows	U.S. No. 1, U.S. No. 2, U.S. No. 3, U.S. No. 4, U.S. utility, cull

Source. Ref. 19

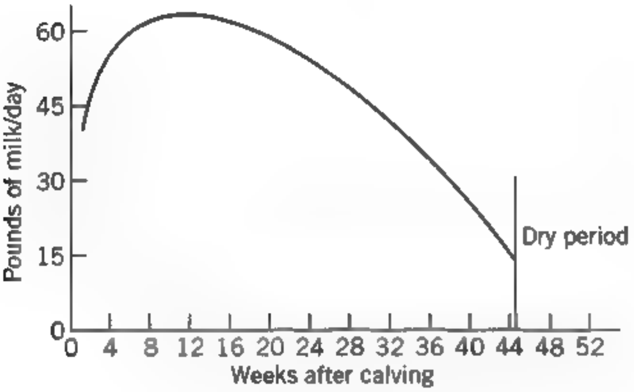


Figure 7. Typical lactation curve.

pricing schemes. However, because milk flavor is contained in the lipid component of milk, feed flavors are the most common causes of off-flavor in milk. Color of milk may also vary among breeds. Guernsey cattle milk, eg, has a more yellow color than milk from Holstein cattle.

The term for the facilities used for the milking of cows is the milking parlor. Modern milking parlors must meet rigid sanitation requirements, keep cows comfortable, and be labor efficient. In comparison to the Federal Meat Inspection Service, state and local laws regulate cow health, sanitation, and milk-handling equipment. Dairy cows are no longer milked by hand but instead by modern machines that rapidly move milk from the milking parlor to refrigerated storage tanks. Milking machines function to remove milk from the cow's udder by applying partial vacuum to the teat end. This vacuum is alternately applied by pulsation consisting of milk and rest cycle 45–60 times per minute.

Milk moves from the cow through a series of stainless-steel pipes to a bulk cooling-storage tank. Milk must be cooled rapidly to approximately 40°F to prevent bacterial growth. Some states allow for in-line rapid cooling coils. However, thorough cleaning of the coils must be ensured.

Most dairies sell their milk directly to processors or through cooperative milk marketing associations. Fluid milk is usually marketed and priced under federal milk marketing orders, in accordance with the Agricultural Marketing Act of 1937. The objective of the federal order is to promote and maintain orderly milk marketing conditions for dairy farmers and assure consumers of an adequate supply of milk.

Milk production is somewhat seasonal, with more milk being produced in the spring. The price of milk paid to dairy farmers is usually on the hundred-weight basis adjusted for percentage of components. Milk protein and fat are usually considered. The pricing scheme will consider local market factors and final use of the milk. For example, cheese plants usually will consider milk protein in establishing prices paid to producers. Milk purchased for the manufacture of cheese may be priced on a cheese yield formula that estimates the quantity of final product.

Almost 50% of the milk produced by farms is processed for consumption as whole, low-fat, or skim milk. All milk is pasteurized to destroy disease-producing microorganisms that may be present in unprocessed milk. The pasteurization process involves heating the milk to 161°F for 15 s in a continuous flow process.

Bovine somatotropin (bST) administration to dairy cattle to enhance milk production has been shown to be efficacious. Depending on the dosage of somatotropin administered, milk production will be enhanced by 10–40% without significant changes in the composition of the milk (20). Concern regarding the impact of this technology on the small dairy farmer has been shown to be unfounded. Since its 1994 approval and sale for use in dairy cattle, bST has been shown to have no deleterious effects on reproduction (21), hematological profiles (22), general health (23), or incidence of mastitis (24). This information confirms earlier work done in Europe (25). The success of bST in increasing milk production and the development of a slow-release product good for 14 days has made it one of the most popular products for use in dairy cattle in recent history.

FUTURE DEVELOPMENTS

The productivity of modern livestock production was made possible through basic and applied research. The application of the principles of genetics, discovery of vitamins and other nutrients necessary for health and productivity of livestock, and the control of reproduction have made large contributions to the economy of producing food. However, for livestock production and its products to remain economically competitive the emergence of new technologies must continue.

Several new technologies and discoveries with applications in livestock production hold promise for enhancing the competitiveness of United States livestock production. Recombinant DNA technology is largely responsible for the production of commercially abundant supplies of somatotropin, also commonly known as growth hormone, to enhance milk and meat production. Porcine somatotropin (PST) administration in pigs increases rate of gain, feed efficiency, and carcass muscling, while significantly reducing carcass fat deposition (26). Similar, though less dramatic, results are seen in beef cattle given bST (27). This technology, along with other methods to regulate lean tissue growth, such as β agonists, will help the pork and beef industry produce meat that is leaner and thus help to reduce fat consumption in the American diet.

Artificial insemination and embryo transplantation of superior lines of swine, sheep, and dairy and beef cattle will continue to increase in popularity among livestock breeders. The ability of scientists to develop improved embryo freezing techniques will greatly enhance the efficacy of this technology. The successful introduction of transgenic livestock with improved rates of growth, carcass characteristics, lactation, and disease resistance will influence the methods and efficiency of livestock production (28).

Transgenic animals provide opportunities to greatly decrease genetic lag in breeding schemes (29) and enhance transfer of superior genes (30). Transgenics may make it possible to alter milk composition in ways that enhance human health, such as decreasing lactose for lactose-intolerant individuals and increasing the resemblance to human milk to improve neonatal nutrition (31). There is even research into the use of the transgenic mammary gland as a mammalian bioreactor that could produce mammalian therapeutic proteins, thus avoiding the disadvantages of pharmaceutical proteins produced by microbial fermentors (lack of bioactivity, presence of allergenic prokaryotic proteins) (32). This process has been dubbed "pharming." Currently, transgenic technology is still inefficient, expensive, and socially controversial; however, it holds promise for enhancing the productivity of livestock and the quality of life for humans.

BIBLIOGRAPHY

1. *Change in U.S. Livestock Production, 1969–1992*, U.S. Department of Agriculture Economic Report, Economic Research Service, ERS-AER-754.
2. *Factsheet, "The Beef Industry,"* National Cattlemen's Association, Englewood, Colo., 1990.
3. C. Lambert, *Beef Industry Facts*, National Cattlemen's Association, Englewood, Colo., 1990.
4. *Livestock Slaughter 1997 Summary*. USDA National Agricultural Statistics Service, U.S. Government Printing Office, Washington, D.C. March, 1998.
5. *Dairy Producer Highlights*, National Milk Producers Federation, Arlington, Va., 1982.
6. U.S. Department of Agriculture, *Agricultural Statistics*, U.S. Government Printing Office, Washington, D.C., 1990.
7. U.S. Department of Agriculture Economics and Statistics System, *Dairy Outlook*, U.S. Government Printing Office, Washington, D.C., December, 1996.
8. *The Structure of Dairy Markets: Past, Present, Future*, U.S. Department of Agriculture Economic Report, Economic Research Service, ERS-AER-757
9. *Fact Book of Agriculture*, U.S. Department of Agriculture, Miscellaneous Publication No. 1063, 1989.
10. U.S. Department of Agriculture, *Agricultural Statistics*, U.S. Government Printing Office, Washington, D.C., 1989.
11. P Yuthasastrakosal et al., "Release of LH in Anoestrous and Cyclic Ewes," *Journal of Reproduction and Fertility* **50**, 319–321 (1977).
12. L. Anderson, "Pigs," in E. S. E. Hafez, ed., *Reproduction in Farm Animals*, 4th ed., Lea and Febiger, Philadelphia, Penn., 1980
13. R. Ashdown and J. Hancock, "Functional Anatomy of Male Reproduction," in E. S. E. Hafez, ed., *Reproduction in Farm Animals*, 4th ed., Lea and Febiger, Philadelphia, Penn., 1980.
14. National Research Council, *Ruminant Nitrogen Usage*, National Academy Press, Washington, D.C., 1985.
15. R. Preston and S. Bartle, "Quantification of Escape Protein and Amino Acid Needs for New Feedlot Cattle," *J. Animal Sci.* **68**, 531 (1990).
16. M. Jurgens, *Animal Feeding and Nutrition*, Kendall/Hunt, Dubuque, Iowa, 1982.
17. Q. Liu et al., "Titration of Fresh Meat Color Stability and Malondialdehyde Development with Holstein Steers Fed Vitamin E-Supplemented Diets," *J. Animal Sci.* **74**, 117–126 (1996).
18. Riley et al., "Palatability of Beef from Steer and Bull Carcasses as Influenced by Electrical Stimulation, Subcutaneous Fat, Thickness and Marbling," *J. Animal Sci.* **56**, 592–597 (1983).
19. M. Judge et al., *Principles of Meat Science*, 2nd ed., Kendall/Hunt, Dubuque, Iowa, 1989.
20. P. Eppard et al., "Effect of Dose of Bovine Growth Hormone on Lactation of Dairy Cows," *J. Dairy Sci.* **68**, 1109–1115 (1985).
21. C. Kirby et al., "Follicular Function in Lactating Dairy Cows Treated with Sustained-Release Bovine Somatotropin," *J. Dairy Sci.* **80**, 273–285 (1997).
22. P. Eppard et al., "Effect of Exogenous Somatotropin on Hematological Variables of Lactating Cows and Their Offspring," *J. Dairy Sci.* **80**, 1582–1591 (1997).
23. G. Hartnell et al., "Effect of Bovine Somatotropin Administered to Periparturient Dairy Cows on the Incidence of Metabolic Disease," *J. Dairy Sci.* **79**, 2170–2181 (1996).
24. L. Judge et al., "Recombinant Bovine Somatotropin and Clinical Mastitis: Incidence, Discarded Milk Following Therapy, and Culling," *J. Dairy Sci.* **80**, 3212–3218 (1997).
25. J. Skarda and H. Mader, "Impact of Bovine Somatotropin on Dairying in Eastern Europe," *J. Dairy Sci.* **74** (Suppl 2), 72–82 (1991).
26. E. Kanis et al., "Effect of Recombinant Porcine Somatotropin on Growth and Carcass Quality of Growing Pigs: Interaction With Genotype, Gender and Slaughter Weight," *J. Animal Sci.* **68**, 1193–1200 (1990).
27. B. Dalke et al., "Dose-Response Effects of Recombinant Bovine Somatotropin Implants on Feedlot Performance in Steers," *J. Animal Sci.* **70**, 2123–2137 (1992).

28. G. Seidel, "Characteristics of Future Agricultural Animals," in J. W. Evans and A. Hollaender, eds., *Genetic Engineering of Animals: An Agricultural Perspective*, Plenum Press, New York, 1986.
29. S. Bishop, "A Comparison of Bonus and Quota Mating Systems for Utilising the Sex-Determining Region Y Gene in Terminal Sire Beef Cattle Breeding," *Theoret. Appl. Genet.* **90**, 487–491 (1995).
30. L. Cundiff et al., "Challenges and Opportunities for Integrating Genetically Modified Animals Into Traditional Animal Breeding Plans," *J Animal Sci.* **71** (Suppl 3), 20–25 (1993).
31. C. Karatzas and J. Turner, "Toward Altering Milk Composition by Genetic Manipulation: Current Status and Challenges," *J. Dairy Sci.* **80**, 2225–2232 (1997).
32. R. Wall et al., "Transgenic Dairy Cattle: Genetic Engineering on a Large Scale," *J. Dairy Sci.* **80**, 2213–2224 (1997).

GENERAL REFERENCES

D. Acker, *Animal Science and Industry*, 3rd ed., Prentice-Hall, Englewood Cliffs, N.J., 1983.
J. Blakely and D. Bade, *The Science of Animal Husbandry*, 4th ed., Reston Publishing, Reston, Va, 1985.
M. Jurgens, *Animal Feeding and Nutrition*, 6th ed., Kendall Hunt, Dubuque, Iowa, 1988.
National Research Council, *Designing Foods: Animal Product Options in the Market Place*, National Academy Press, Washington, D.C., 1988.

RICHARD A. ROEDER
MIKELLE ROEDER
University of Idaho
Moscow, Idaho

ANTIBIOTICS IN FOODS OF ANIMAL ORIGIN

In 1877 Pasteur and Joubert discovered that growth of *Bacillus anthracis* could be inhibited by the presence of other microorganisms. This discovery led to the isolation of pyacyanase, the first antibiotic. In 1928 penicillin was discovered by Fleming when he observed that the outgrowth of an agar culture of *Staphylococcus aureus* was inhibited by mold. In 1932 Domagk discovered that the dye prontosil red, the first sulfonamide, demonstrated antimicrobial properties. Since these early discoveries the number of antimicrobials either isolated from natural sources or synthesized in the laboratory has grown tremendously. Antibiotics, in the narrowest sense, are products produced by living organisms that are not toxic to the producing organism but are capable of inhibiting the growth of or terminating other organisms. However, there are many synthetically manufactured compounds that inhibit or kill organisms as effectively as natural antibiotics. The chemistry and mode of action of these synthetic antimicrobial compounds are well documented. While there are virtually thousands of known antimicrobials, only a few are marketed for use in present-day food animal production.

Treatment of food animals with therapeutic and subtherapeutic dosage forms of antibiotic-antimicrobial drugs has increased in the last decade. This has been due to the advent of modern mass production operations that involve the maintenance of thousands of animals simultaneously and requires the utilization of carefully formulated and medicated diets that serve to maximize growth, minimize production costs, and provide an acceptable consumer product that is wholesome and affordable. Animal feeds that contain antibiotics and other antimicrobials as a prophylactic are now used routinely in beef, swine, chicken, and turkey production. In this way the antibiotics—antimicrobials help to maintain the optimal health of the animals so treated. Antibiotics may be coadministered with antimicrobial drugs resulting in a net increase in drug effectiveness compared to individual drug administrations. Strictly speaking, sulfonamides are not antibiotics. However, for purposes of this article sulfonamide antimicrobials will be collectively termed antibiotics.

The aminoglycoside, beta-lactam, ionophore, macrolide, sulfonamide, tetracycline, and other antibiotics are an integral part of food animal production. Regulations governing the use, dosage, and withdrawal times for many of the members of these antibiotic classes in animal production have been established in the United States by federal law, as outlined in the *Code of Federal Regulations*, Title 21, for each compound (1). The U.S. Food and Drug Administration (FDA) regulates the use of antibiotics in animal production while the U.S. Department of Agriculture (USDA) monitors residue levels by testing animal-derived products for antibiotics. Specific information regarding manufacturers, new animal drug application (NADA) codes, approved dosage forms and directions for proper use can be found in the *Code of Federal Regulations*, Title 21 and other published sources (2).

Antibiotics may manifest themselves as residues in animal-derived human foods if improperly used or if withdrawal times have not been observed for treated animals. Animal-derived human foods that contain violative antibiotic residues may pose a potential human health hazard. These potential health hazards can be broken down into three broad categories (3): toxicological, microbiological, and immunopathological. Toxicological concerns relate to the direct toxic effect of the compound on the consumer, resulting in physiological abnormalities, an example being sulfamethazine, which has recently been shown to produce cancer in laboratory animals (4). Microbiological concerns relate to the transmittance of antibiotic resistance. Antibiotics have the potential to act as a selective force that favors the emergence of resistant pathogenic bacteria in the natural flora of meat consumers. An example is a reported outbreak of salmonella poisoning in humans believed to be associated with a resistant strain in hamburger obtained from culled cattle that had been treated routinely with chloramphenicol (5). Finally, there are immunopathological mechanisms, whereby the drug serves as an antigen, demonstrates allergenic properties, and may result in hypersensitivity reactions to the drug that has sensitized some individuals. Penicillin is a prime example. Antibiotics are the principal compounds of concern in the federal residue control strategy (6). Such routine monitoring for antibiotic residues in animal-derived foods is intended to minimize the occurrence of violative antibi-

28. G. Seidel, "Characteristics of Future Agricultural Animals," in J. W. Evans and A. Hollaender, eds., *Genetic Engineering of Animals: An Agricultural Perspective*, Plenum Press, New York, 1986.
29. S. Bishop, "A Comparison of Bonus and Quota Mating Systems for Utilising the Sex-Determining Region Y Gene in Terminal Sire Beef Cattle Breeding," *Theoret. Appl. Genet.* **90**, 487–491 (1995).
30. L. Cundiff et al., "Challenges and Opportunities for Integrating Genetically Modified Animals Into Traditional Animal Breeding Plans," *J. Animal Sci.* **71** (Suppl 3), 20–25 (1993).
31. C. Karatzas and J. Turner, "Toward Altering Milk Composition by Genetic Manipulation: Current Status and Challenges," *J. Dairy Sci.* **80**, 2225–2232 (1997).
32. R. Wall et al., "Transgenic Dairy Cattle: Genetic Engineering on a Large Scale," *J. Dairy Sci.* **80**, 2213–2224 (1997).

GENERAL REFERENCES

D. Acker, *Animal Science and Industry*, 3rd ed., Prentice-Hall, Englewood Cliffs, N.J., 1983.

J. Blakely and D. Bade, *The Science of Animal Husbandry*, 4th ed., Reston Publishing, Reston, Va, 1985.

M. Jurgens, *Animal Feeding and Nutrition*, 6th ed., Kendall Hunt, Dubuque, Iowa, 1988.

National Research Council, *Designing Foods: Animal Product Options in the Market Place*, National Academy Press, Washington, D.C., 1988.

RICHARD A. ROEDER
MIKELLE ROEDER
University of Idaho
Moscow, Idaho

ANTIBIOTICS IN FOODS OF ANIMAL ORIGIN

In 1877 Pasteur and Joubert discovered that growth of *Bacillus anthracis* could be inhibited by the presence of other microorganisms. This discovery led to the isolation of pyacyanase, the first antibiotic. In 1928 penicillin was discovered by Fleming when he observed that the outgrowth of an agar culture of *Staphylococcus aureus* was inhibited by mold. In 1932 Domagk discovered that the dye prontosil red, the first sulfonamide, demonstrated antimicrobial properties. Since these early discoveries the number of antimicrobials either isolated from natural sources or synthesized in the laboratory has grown tremendously. Antibiotics, in the narrowest sense, are products produced by living organisms that are not toxic to the producing organism but are capable of inhibiting the growth of or terminating other organisms. However, there are many synthetically manufactured compounds that inhibit or kill organisms as effectively as natural antibiotics. The chemistry and mode of action of these synthetic antimicrobial compounds are well documented. While there are virtually thousands of known antimicrobials, only a few are marketed for use in present-day food animal production.

Treatment of food animals with therapeutic and subtherapeutic dosage forms of antibiotic-antimicrobial drugs has increased in the last decade. This has been due to the advent of modern mass production operations that involve the maintenance of thousands of animals simultaneously and requires the utilization of carefully formulated and medicated diets that serve to maximize growth, minimize production costs, and provide an acceptable consumer product that is wholesome and affordable. Animal feeds that contain antibiotics and other antimicrobials as a prophylactic are now used routinely in beef, swine, chicken, and turkey production. In this way the antibiotics—antimicrobials help to maintain the optimal health of the animals so treated. Antibiotics may be coadministered with antimicrobial drugs resulting in a net increase in drug effectiveness compared to individual drug administrations. Strictly speaking, sulfonamides are not antibiotics. However, for purposes of this article sulfonamide antimicrobials will be collectively termed antibiotics.

The aminoglycoside, beta-lactam, ionophore, macrolide, sulfonamide, tetracycline, and other antibiotics are an integral part of food animal production. Regulations governing the use, dosage, and withdrawal times for many of the members of these antibiotic classes in animal production have been established in the United States by federal law, as outlined in the *Code of Federal Regulations*, Title 21, for each compound (1). The U.S. Food and Drug Administration (FDA) regulates the use of antibiotics in animal production while the U.S. Department of Agriculture (USDA) monitors residue levels by testing animal-derived products for antibiotics. Specific information regarding manufacturers, new animal drug application (NADA) codes, approved dosage forms and directions for proper use can be found in the *Code of Federal Regulations*, Title 21 and other published sources (2).

Antibiotics may manifest themselves as residues in animal-derived human foods if improperly used or if withdrawal times have not been observed for treated animals. Animal-derived human foods that contain violative antibiotic residues may pose a potential human health hazard. These potential health hazards can be broken down into three broad categories (3): toxicological, microbiological, and immunopathological. Toxicological concerns relate to the direct toxic effect of the compound on the consumer, resulting in physiological abnormalities, an example being sulfamethazine, which has recently been shown to produce cancer in laboratory animals (4). Microbiological concerns relate to the transmittance of antibiotic resistance. Antibiotics have the potential to act as a selective force that favors the emergence of resistant pathogenic bacteria in the natural flora of meat consumers. An example is a reported outbreak of salmonella poisoning in humans believed to be associated with a resistant strain in hamburger obtained from culled cattle that had been treated routinely with chloramphenicol (5). Finally, there are immunopathological mechanisms, whereby the drug serves as an antigen, demonstrates allergenic properties, and may result in hypersensitivity reactions to the drug that has sensitized some individuals. Penicillin is a prime example. Antibiotics are the principal compounds of concern in the federal residue control strategy (6). Such routine monitoring for antibiotic residues in animal-derived foods is intended to minimize the occurrence of violative antibiotic levels in the food supply. Such a capability is dependent on the analytical methods utilized.

Methods for the determination of antibiotics can include but are not limited to bioassay (ba), thin layer chromatography (tlc), gas chromatography (gc), liquid chromatography (lc), and various immunoassay (ia) techniques. Each of these methods has found utility in antibiotic determinations and many have been shown to be precise, specific, or sensitive, depending on which analytical technique, detector, visualization method, and compound are used. In this regard, a method utilized to detect an antibiotic residue at low nanogram antibiotic per gram of sample (ng/g) or even picogram antibiotic per gram of sample (pg/g) levels may be confounded by the presence of interferences found in the sample matrix or in the sample extract. Thus analytical capability is governed ultimately by the sample preparation and extraction steps. The isolation of such residues from a complex biological matrix poses unique problems to the analyst and because of the number of antibiotics being utilized in animal production the need for multiresidue determinations exists. An emerging trend is the development of rapid multiresidue—multidrug class isolation techniques that result in clean, interference-free extracts and that minimize cost, time, and expendable material requirements, enabling the analyst to test for multiple drugs isolated from one sample. In conjunction with improved isolation methods the need to screen samples rapidly and accurately for antibiotic residues exists. Such antibiotic screening protocols and analytical capability can be enhanced by rapid, reproducible, efficient, and cost-effective residue isolation techniques.

SAMPLE PREPARATION AND RESIDUE ISOLATION

Sample preparation requirements are dependent on the particular analysis that is to be performed. For example, ba or ia procedures for antibiotics may require little or no sample preparation, whereas sample preparation for tlc, lc, and gc procedures can be a major limiting factor of the analyses.

Liquid samples such as milk, blood, urine, saliva, or other body fluids can be assayed directly for antibiotics by ba or ia techniques, or the samples may require only minor cleanup steps (centrifugation, pH adjustment, or a protein precipitation) prior to the assay. In some cases the antibiotic residues that may be present in the sample at low concentrations may require an analyte-concentration step prior to the analysis.

Residue enrichment methods that concentrate the antibiotic residue prior to an analysis may involve solvent extractions, column chromatography, or solid-phase extraction (spe) techniques. The utility of solvent-solvent extractions is limited because of the polar characteristics of many antibiotics such as beta-lactams, aminoglycosides, macrolides, polyether ionophores, and tetracyclines. Ion-exchange column chromatography techniques utilized to isolate polar ionizable antibiotics are only marginally effective. Solid-phase extraction appears to hold more promise as a routine residue enrichment approach. The application of this technique proved very effective in cleaning and concentrating penicillin residues in animal tissue prior to HPLC analysis (7). Methods that attempt to circumvent cleanup steps by assaying samples for antibiotics directly are only rarely efficient, except for ba or ia techniques of some liquid samples such as milk.

Supercritical fluid extraction (SFE), a relatively new extraction technique, is also superior to conventional sample preparation methods as no cleaning is required prior to analysis. SFE minimized sample manipulation and eliminated the use of organic solvents when used to extract sulfamethazine in chicken eggs (8). This advantage was also evident when supercritical CO_2 modified with acetonitrile was used to recover sulfonamide from chicken liver, beef liver, and egg yolk (9).

Sample-preparation steps may not be necessary in some cases for antibiotic determinations but their use can often facilitate more accurate analyses. Therefore, it is incumbent on the analyst to obtain the cleanest sample extract possible. Clean extracts from some liquids and most non-liquid matrices such as muscle or organ tissues are more difficult to obtain and generally require an extraction step and multiple manipulations to isolate the antibiotic residue from the sample with high percentage recoveries. Unfortunately, sample extracts obtained in this manner may contain naturally occurring inhibitors or interferences that could affect the analysis. Thus further cleanup of extracts is usually required prior to performing the analysis. Because of the polar nature of many antibiotics, buffered aqueous solutions are routinely employed for antibiotic extractions and it is difficult, if not impossible, to isolate antibiotic residues from these aqueous extracts by partitioning with organic solvents. Thus drying of the aqueous extract, a time-consuming process, or the use of other preparative steps such as column chromatography or spe techniques may be required before an analytical determination can be made. A direct assay of the aqueous extract may be possible if the concentration of the antibiotic in the extract is sufficiently high or if the analytical method is particularly sensitive. As the complexity of the sample and the number of sample cleanup steps increases, the utility of techniques such as ba, ia, and tlc for quick antibiotic screening purposes decreases. In addition, extracts suitable for ba or ia procedures may not be sufficiently clean for more sophisticated tlc, lc, or gc determinations, thereby requiring additional and different residue isolation procedures for confirmatory techniques.

Sophisticated techniques based on lc, gc, lc-mass spectrometry (ms), and gc-ms usually require more rigorous sample preparation to isolate the antibiotic free from interferences found in the sample extract. These classic isolation techniques can be laborious and time-consuming. Classic isolation techniques for aminoglycoside (10), β-lactam (11–13), chloramphenicol (14), ionophore (15), macrolide (13,16), sulfonamide (17–20), and tetracycline (21) antibiotics generally involve their extraction from biological matrices with large volumes of extracting solvents, chemical manipulations such as pH adjustments and protein precipitations, centrifugations, back washing, and the evaporation of large volumes of organic solvents. This approach limits the usefulness of many classic isolation techniques for multiresidue determinations by lc and gc.

Analytical capability is, therefore, limited to a large extent by interferences present in the sample extract. While state-of-the-art analytical techniques can detect picogram levels of pure antibiotic standard compounds, this same level of detection may not be achievable for antibiotics obtained from the extraction of biological samples. Specifically, coextracted interferences may hinder antibiotic determinations by tlc, lc, or gc because they may have similar detector and chromatographic characteristics or they may exist in such a large quantity that they overwhelm the detection method used. Interferences in ba or ia techniques can contribute to cross-reactions that may lead to false-positive or false-negative determinations. Thus a major limiting factor associated with antibiotic determinations is not the available analytical capability but the sample-preparation steps required to extract the antibiotics. The sample-preparation steps should ideally result in clean biological extracts that contain the antibiotic residue with high percentage recoveries and that have minimal interferences that might limit the choice of analysis.

The need to test for more antibiotic residues in more foods requires rapid, rugged, and multiresidue isolation techniques allowing the analyst to test for multiple drugs in the same sample. Classic residue isolation techniques have not been able to meet this challenge. Matrix solid-phase dispersion (mspd) techniques (22–28), recently developed for the isolation of drug residues from animal matrices, have the potential to greatly enhance many antibiotic residue isolation protocols. In mspd the sample (0.5 g) is dispersed onto octadecasilyl polymeric—derived silica beads [C-18 reversed-phase packing material (2 g), 1,000 m^2 surface area, theoretical]. The dispersion mechanism, utilizing a mortar and pestle, involves the disruption, unfolding, and rearrangement of matrix constituents by mechanical and hydrophobic forces onto the C-18 beads. Lipid and lipophilic materials associate with the lipophilic C-18, allowing the more hydrophilic components and protein regions to extend outward away from the nonpolar, inner C-18—lipid region. Water and more polar constituents preferentially associate with the hydrophilic ends. A column fashioned from the C-18—sample matrix blend can be eluted sequentially with solvents (8 mL) of different polarities to effectively remove interferences in one solvent and elute the target residue in a different solvent. The process can be envisioned as an exhaustive extraction process whereby a large volume of solvent is passed over a thin layer of sample. Mspd has been utilized for the isolation of beta-lactams from beef tissue (23); sulfonamides from milk (24), infant formula (25), and pork muscle tissue (26); as well as for chloramphenicol (27) and tetracycline (28) isolations from milk. The theoretical aspects describing the disruption, unfolding, and rearrangement of matrix constituents onto the C-18 have been published (22–28).

Advances in multiclass—multiresidue isolation procedures, which are rapid, rugged, generic in nature, and free from interferences and which facilitate the isolation of multiple antibiotic residues from one sample, will greatly enhance analytical determinations of antibiotics.

ANALYTICAL METHODOLOGY

Bioassays

Bioassays are used routinely to test for violative levels of antibiotics in milk, animal tissues, and feeds. Bioassays involve the inhibition of growth of specific bacterial spores or viable bacteria in the presence of a sample or sample extract that contains antibiotic residues. Bioassays may also utilize the measurement of labeled antibiotic analyte bound to receptors, a ligand assay, on vegetative bacterial cells. Bioassays have been utilized to detect aminoglycosides (29–32), β-lactams (30,32–34), chloramphenicol (32,35–37), ionophore (15), sulfonamide (29,30), and tetracycline (29,30,32,33,38) antibiotics in food.

For example, the swab test on premises (stop) procedure (30) is used by federal meat inspectors to test for chloramphenicol, tetracycline, aminoglycoside, penicillin G, and sulfonamide antibiotics. The stop method involves taking a sterile cotton swab and macerating the target organ or tissue with the noncotton end. The size of the macerated zone should be slightly larger in diameter and deeper than the cotton end of the swab The cotton end is then inserted into the disrupted area and allowed to absorb fluids (30 min). The fluid-soaked cotton swab is then placed onto a nutrient agar plate that has been inoculated with a lawn of specific bacterial spores and the plates are then incubated (16–22 h). Standards of known concentration are run in parallel. A zone of microbial growth inhibition on the sample plate is an indication of the presence of an antibiotic and the size of the zone of inhibition can be semicorrelated with the size of the zone of inhibition for a given concentration of pure antibiotic standard. The sample is positive if the size of the zone of inhibition is similar to that for a known pure standard. Unfortunately, the stop procedure detects only the presence of inhibitors, not their specific identity. Other antibiotics or naturally occurring inhibitors may contribute to the size of the zone of growth inhibition observed, indicating a positive sample although the amount of the individual antibiotic compounds present may be less than the violative level.

The classic disk assay procedure (34) is similar to the stop procedure for the same antibiotics except that a filter paper disk is placed on the inoculated nutrient agar, the liquid sample or suitable extract is added to the disk and then the plate is incubated for a minimum of 2.5 h or until a zone of inhibition can be observed. The zone of inhibition may be enhanced by dyeing techniques (39,40), which aid in its detection. Semiquantitative determinations by the disk assay can be accomplished, provided incubation times are increased (16–22 h). The disk assay procedure is the official method described in the Pasteurized Milk Ordinance (PMO) (41) for antibiotic testing in milk. The disk assay procedure suffers limitations similar to those of the stop procedure. However, the PMO allows for the use of any method that gives results equivalent to the disk assay method; therefore, many states utilize alternative techniques such as the color reaction test (crt) to test for antibiotics in milk. The Delvo test (39) is an example of a crt technique used to determine antibiotics, specifically beta-lactams, in milk. The test involves placing the milk sample onto agar containing a viable strain of *Bacillus*, nutrients, and pH indicators. If the color of the agar changes from purple (basic) to yellow (acid) after incubation (1.5 h) then no penicillin is present to prevent the outgrowth of the acid-producing bacteria. Color reaction tests can be rapid and simple to perform.

The microbial receptor assay (mra) (Charm test) technique (38) can be used to detect beta-lactam, macrolide, and aminoglycoside antibiotic classes. The mra method involves the use of C-14 or tritium isotopically labeled analyte to displace nonlabeled analyte from the bacterial receptors located on vegetative cells under a standard set of conditions. An equilibrium condition between unlabeled and isotopically labeled analyte results, allowing quantitative measurement of the amount of antibiotic present by an appropriate radiometric method. However, the identity of the compound is not known and must be determined by other methodology.

The thin layer chromatography—bioautography of tlb bioassay technique (15,32,40) uses traditional thin layer chromatography to separate sample constituents on silica or microcrystalline cellulose chromatography (mcc) plates. Different antibiotics will migrate on the plates according to their chemical characteristics and the developing solvent utilized. The developed plates are covered with a spore-inoculated nutrient agar and then incubated (16–24 h). Zones of growth inhibition observed for different locations on the plate after incubation indicate the presence of antibiotics. Aminoglycoside (32), β-lactam (32), chloramphenicol (32), ionophore (15,32), macrolide (32,40), and tetracycline (32) antibiotics have been assayed by tlb. The tlb method is more specific and sensitive (36) than either the stop or classical disk assay procedures because it takes advantage of the ability of tlc to separate 14 different antibiotics from each other as well as from other impurities that may be present in the sample. Therefore, zones of inhibition can be more closely correlated to different antibiotics. The tlc-developing solutions are designed to optimize separations between similar antibiotics. However, the separation of up to 14 different antibiotic residues can only be accomplished by utilizing three separate tlc plates. Furthermore, extensive sample-preparation and extraction steps are employed in the tlb procedure that are similar to those employed for more sophisticated analytical determinations. Because of the incubation time required the tlb procedure is not as rapid as the crt or the disk assay methods for screening purposes. However, the tlb procedure has an advantage over the stop or disk assay procedures for screening purposes because it can provide for more precise determinations between different antibiotics. Absolute confirmation of the antibiotics is not possible by tlb and time requirements are in excess of many sophisticated, more definitive, liquid and gas chromatographic techniques.

The stop, disk assay, crt, and tlb procedures are valuable screening tools. The first three techniques are relatively cheap, easy to perform, and require a minimal amount of equipment and technician training. However, they lack specificity and sensitivity and/or they may be subject to interpretive errors in the presence of naturally occurring inhibitors. Furthermore, each of these four methods requires confirmational testing of positives. The mra method using isotopically labeled antibiotics can be expensive and requires special handling and equipment; but it can provide for quantitative determinations. These bioassays, if used judiciously, can minimize the number of samples screened by more costly analytical methods, but they cannot replace such methods for absolute confirmations.

Immunoassay

Immunoassay techniques are based on classic antibody-antigen reactions whereby the antibody will bind with its corresponding antigen (antibiotic) and result in visible turbidity if reacted in solution or form visible immunoprecipitation in a gel at the location where the antigen and antibody meet. Visible end point immunoprecipitation that occurs at low antibiotic concentrations may be difficult to see and may require detection by more sensitive nonvisual means.

Immunoassay techniques such as enzyme immunoassay (eia), radio immunoassay (ria), enzyme-linked immunosorbent assay (elisa), fluorescence polarization immunoassay (fpia), particle-concentration immunoassay (pcia), particle-concentration fluorescence immunoassay (pcfia), quenching fluoroimmunoassay (fia), and latex-agglutination inhibition immunoassay (laia) require the measurement of by-products produced by linked enzyme systems or the measurement of radioactive, fluorescence, metal, or latex labels that have been attached to one of the reactants (42). The displacement between the unlabeled and labeled antigen or antibody allows for true measurement of the immunoprecipitate concentration, which corresponds to the concentration and type of analyte present in the sample extract. Ria determinations of chloramphenicol (43,44) as a residue in eggs, milk, and meat were comparable to values obtained by gc. The eia determination of monensin (45), a polyether antibiotic in urine, serum, and fecal extracts, may be applicable to food extracts as well. A review of ia techniques such as ria, fia, fpia, and laia for aminoglycoside determinations in body fluids has been published (46). The recent use of a monoclonal-antibody—based agglutination test (spot test) for beta-lactams in milk (47) and elisa for chloramphenicol determinations in swine tissue (48), milk (49), and sulfamethazine in swine tissue (50) has been adapted to other antibiotics including spectinomycin (51,52), enrofloxacin, (53) and gentamicin (54).

Immunoassays can be sensitive, class specific, accurate, and provide a means for rapidly screening samples for antibiotics; the future development of monoclonal antibodies will allow for more specificity in immunoassay determinations. The growth in the use of immunoassay-based detection has lead to the development of commercial kits allowing for on-site detection of chemical contaminants (55–57). Radioimmunoassay techniques that require radioisotopes may be subject to specific regulations, require expensive counting equipment, and pose disposal problems. Reagent kits can be relatively expensive and have a limited shelf life. In this regard, nonisotopic immunoassays such as elisa, fpia, pcia, or pcfia, and monoclonal-based ias will in all likelihood play an increasingly important role in antibiotic screening immunoassay determinations.

At present, ia techniques for antibiotics approved for use in animal production are limited and have minimal utility for aminoglycoside, β-lactam, chloramphenicol, and sulfonamide determinations, but they have the potential for use as screens for all the major antibiotic classes. Immunoassay techniques have been limited to liquid samples such as milk because of the difficulties associated with the isolation of antibiotic residues from matrices such as muscle or organ tissues. Increased use of ia techniques to screen for antibiotics in tissues is directly dependent on the development of rapid residue isolation techniques that isolate the antibiotic from the tissue and provide for an extract that is free from cross-reacting components and nonspecific binding (NSB) factors. The development of such isolation techniques will further the use of ia techniques for the screening of antibiotics in tissues.

Immunoassay techniques are more specific than microbiological based bioassays and would allow for the rapid screening of large numbers of samples. With the need to screen more samples for more drugs, the ia techniques will become more important in residue control protocols. Immunoassay techniques can greatly reduce the number of samples that are presently screened by more sophisticated analytical techniques and have the potential to replace many bioassay and tlc methods, provided rapid and efficient antibiotic extraction procedures can be developed. As a regulatory tool, ia techniques for antibiotic screening coupled to highly specific and accurate techniques, such as lc, gc, lc-ms, or gc-ms for confirmations of positives, mark the future with respect to a rapid and reliable residue control strategy.

Thin Layer Chromatography

Thin layer chromatography techniques have been used in chemical separations for decades. Thin layer chromatography is rapid and inexpensive, can be highly sensitive depending on the compound examined and the visualization technique employed, and is easy to use and versatile. It can be adapted to separations of all classes of antibiotics by utilizing different sorbents and solvent-developing systems. Aminoglycoside (31,32), β-lactam (32,48,58), chloramphenicol (32), ionophore (15,32), macrolide (16,32,40), sulfonamide (59,60), and tetracycline (38,61,62) antibiotics have been successfully assayed by tlc. However, the utility of tlc for the detection of picogram levels of antibiotics is limited and thus tlc techniques have come to play only a minor role in antibiotic analyses.

The sulfa on site (sos) tlc procedure for sulfonamide determinations in swine urine was developed by the USDA–FSIS and is presently in use in 100 of the largest swine-slaughtering facilities in the United States (63). This method was adopted in 1988 as the official method for in-plant testing of swine for sulfamethazine (64). Thin layer chromatography techniques can complement other antibiotic assay techniques such as tlc–bioautography bioassays (15,32,40). TLC/bioautography qualitative and semiquantitative methods are routinely used in Austria for the analysis of antibiotic residues in poultry, meat, and fish (65). This method is limited to a large degree by the cleanliness of the sample that is to be analyzed. Impurities in the sample can interfere with the chromatography of the antibiotic by altering the migration of the antibiotic on the tlc plates compared to pure standards, and these impurities can negate visualization techniques. Thin layer chromatography determinations also require confirmations of suspect residues isolated from the tlc plates to determine if the residue is indeed an antibiotic. In this regard bioassay and immunoassay techniques provide for a more precise determination of the presence of antibiotics in a sample extract. Thin layer chromatography will perhaps play a decreasing role in antibiotic residue control strategies where low-level detection is required for some antibiotics, but should not be eliminated from the analyst's tools utilized for the isolation and purification of compounds from animal-derived matrices.

Liquid Chromatography

The convenience and versatility of liquid chromatography has led to its adoption as the analytical method of choice for the determination of many drugs, especially antibiotics. Liquid chromatography, using selective detectors, can give reproducible results that are specific, sensitive, and precise. The polar characteristics of antibiotics make them well suited to lc procedures, in which mobile phase solvent systems and columns can be varied to facilitate specific antibiotic determinations.

Recent publications describing lc methods for the analysis of aminoglycoside (10,31,66), β-lactam (11–13,16,23,67–69), chloramphenicol (14,27,70,71), ionophore (15,16), macrolide (13,16,72), sulfonamide (17,19,24–26,73–75), and tetracycline (21,36,61,68,76–80) antibiotics underscore the utility of lc determinations for the analysis of these compounds as residues in foods and other biological matrices.

Food extracts containing residues of sulfonamide (15,18,24–27), β-lactam (11,16,23,67), chloramphenicol (14,27), tylosine (16) and spiramycin (13) macrolides, and tetracycline (21,36,61,68,78) antibiotics may be analyzed directly by ultraviolet (uv) detection at picogram—nanogram levels because they have characteristic uv absorbances and large extinction coefficients. Photodiode array uv detection of antibiotics can provide the analyst with uv spectra of suspect peaks (81) and thus serve as a preconfirmational screening tool. However, some beta-lactams and macrolides, which have a maximum absorbance in the low uv (210–240 nm) range or relatively small extinction coefficients, can be more difficult to analyze by lc—uv because of coextracted interferences that absorb readily in this range. Optimizing lc chromatographic conditions to separate a particular antibiotic residue from interferences that may be present in the extract severely limits lc techniques in terms of multiresidue antibiotic determinations.

Aminoglycoside and most macrolide antibiotics that have low or nonexistent uv-absorbing properties may require the formation of derivatives to aid in their detection or the use of alternative detection methods. Benzene sulfonyl chloride (82) and 1-fluoro-2,4-dinitrobenzene (83) have been used to prepare uv derivatives of aminoglycosides for analyses by lc. Detection methods for antibiotics that do not require making derivatives are ideal because reaction conditions for many derivatizations can be difficult to optimize. However, until improved or new detection methods are developed for antibiotics, derivatives will continue to be used to facilitate sensitive and selective detections, especially for aminoglycosides.

The analyses of aminoglycoside (10), β-lactam (12), macrolide (16), and tetracycline (38) antibiotics can also be facilitated by the formation of fluorescent derivatives. The ionophore lasalocid has native fluorescence due to its salicyclic acid-type aromatic moiety and, therefore, can be analyzed directly in food extracts at nanogram levels by fluorescence detection (15). Fluorescent derivatives of tetracyclines (38) can be made by simply complexing the tetracycline with different metal ions. The tetracycline—metal ion complexes have unique excitation and emission wavelengths that can be measured to quantitatively determine tetracycline concentrations in the food extract. In the case of aminoglycoside antibiotics, which do not have native fluorescence, fluorescent derivatives must be made to facilitate their detection. The preparation of fluorescent derivatives can require exacting reaction conditions, additional equipment in the form of reactors and delivery pumps, and in many cases the removal of reaction reagents before an analysis can be made. However, this cannot be avoided in the case of aminoglycosides because no other suitable chromatographic method presently exists for their detection at nanogram levels. Fluorescence detection of *o*-phthalaldehyde (OPA) or fluorescamine derivatives has been shown to give accurate and reliable values for aminoglycoside residues in animal tissue (10,31). The derivatization of the aminoglycoside amino group with a fluorogenic agent can be accomplished precolumn or postcolumn. The added cost of the postcolumn reactor and solvent pump needed to deliver the derivatization solution may be disadvantageous. Alternatively, precolumn derivatization reaction mixtures may require the removal of derivatizing reagents prior to the analyses. Specific applications may require either approach and are dependent on the type of food that is to be extracted and the inherent interferences present.

Fluorescent derivatives (12) of penicilloaldehyde products obtained by enzymatic hydrolysis of beta-lactam rings and reaction with dansyl hydrazine have been reported for eight neutral beta-lactams. This is a novel approach for beta-lactam determinations and might be applicable to other antibiotics that are inherently unstable and difficult to analyze. Techniques that serve to characterize unique enzymatic or degradative products of antibiotics may be a useful tool for many antibiotic determinations.

Liquid chromatography of antibiotics will continue to be the method of choice for most antibiotic determinations. Coupling of photodiode array uv, fluorescence, electrochemical and other yet to be developed detectors in tandem can provide information in terms of retention times, structures, and characteristic uv and fluorescent spectra. Information obtained by this approach may be sufficient to confirm the presence of specific antibiotics if coupled with positive results obtained by immunoassay techniques for the specific antibiotic in question. Liquid chromatography—mass spectrometry is not presently suitable for low-level detection of the major antibiotic classes, but advances in this area, the use of tandem detection methods, and specific monoclonal-based immunoassay screening techniques would contribute significantly to overall antibiotic residue control strategies needed to insure a safe and wholesome food supply.

Gas Chromatography

Gas chromatography coupled to specific detectors can provide valuable information about the retention time, structure, chemical characteristics, and identity of compounds. Gas chromatography has been utilized for antibiotic determinations but is limited to some extent by the molecular weight, high polarity and the relative lack of thermal stability of many antibiotics. Chemical derivatives can serve to impart greater stability and volatility to antibiotics. However, controlling reaction conditions to insure the formation of consistent derivative products with high yields can be difficult to accomplish. These difficulties have limited the usefulness of gc for routine antibiotic determinations.

Presently there are no suitable gc methods for the routine determination of aminoglycoside, beta-lactam, and most ionophore, macrolide, and tetracycline antibiotics. Gas chromatography of trimethylsilyl (TMS) derivatives of aminoglycoside (84), lasalocid ionophore (14), and tetracycline (85) antibiotics have been reported. However, the usefulness of this technique for low-level determinations of these antibiotics in food extracts is limited because of the need to control silylating reaction conditions carefully, the formation of multiple TMS antibiotic derivative products, the abundance of TMS interferences contributed by the sample, and the lack of stability of TMS derivatives.

Gas chromatography methods for chloramphenicol have been reviewed (14). Gas chromatography utilizing electron capture (ECD), flame ionization (FID) and thermionic (TID) detection of TMS and heptafluorobutyl derivatives of chloramphenicol isolated from muscle, liver, kidney, and milk resulted in picogram—nanogram detection giving results comparable to radioimmunoassay determinations (43). Gas chromatography of chloramphenicol residues in food extracts can provide for confirmations of suspect residues and complement lc determinations.

Gas chromatography methods for sulfonamides have also been reviewed (17). Sulfonamide determinations in animal tissues (20,86) by gc have been accomplished by analyzing volatile methylated or acylated derivatives. Methylation of sulfonamides at the *N*-1 position followed by acylation at the *N*-4 position can provide for stable volatile derivatives suitable for gc analysis. However, it can be difficult to control reaction conditions necessary to optimize this two-step derivative reaction. Low yields and the formation of multiple derivative products may result in nonrepresentative and misleading data. Although methods for the quantitative determination of sulfonamides (18,20,75) have been reported, it has been noted (18) that some sulfonamides determined in this manner resulted in low and variable recoveries. Because of this variability, it is necessary to be careful when quantitatively evaluating data obtained by this technique.

Advances in supercritical fluid chromatography (sfc) may eventually lead to applications involving antibiotic determinations. At present, sfc is limited as a routine analytical tool to nonpolar substances (87). Analysis of polar antibiotics by sfc will require advances in sfc hardware and columns. In addition, the solubility characteristics of different antibiotics in different supercritical media will require extensive laboratory investigation to define optimal conditions in terms of pressures and polar mobile phase modifiers that may be required:

Thus gas chromatography methods have minimal utility for antibiotic determinations. Gas chromatography will remain a supplemental analytical tool for antibiotic residue determinations until new derivatization schemes, reagents, and refined reaction conditions are developed. The development of such innovations can, however, advance present marginal gc antibiotic techniques to full-fledged quantitative antibiotic confirmatory methods, which will contribute significantly to an integrated antibiotic residue control strategy.

Lc–ms and Gc–ms

Mass detectors coupled to chromatographic techniques such as lc and gc can provide for the unequivocal identification of compounds. Sensitivity limitations associated with lc–mc precluded its use in antibiotic residue analyses when low-level determinations were required. However, in recent years lc–ms has been developed to permit such determinations. Keever, Voysker, and Tyczkowska (88) reported a quantitative procedure using lc–electrospray ms to detect ceftiofur in milk at levels down to 10 ppb. An interlaboratory study to monitor pirlimycin residues in bovine milk using lc–thermospray ms was able to detect as low as 0.4 ppm in milk and 0.5 ppm in liver tissue (89). Gc–ms techniques for antibiotics have been predominantly applied to colatile sulfonamide derivatives (17,20,75,86) but may provide for confirmation of suspect residues, although it may be inadequate as a regulatory tool. However, gc–ms procedures were reported suitable for identifying and quantifying chloramphenicol residues with detection limits of 0.1–1.0 ng/kg (90). This method compared well with results obtained using radioimmunoassay and enzyme immunoassay and was considered to be a good screening test. Nagota and Oka (91) reported a capillary gc–ms procedure capable of measuring antibiotic residues in yellowtail fish including chloramphenicol, florfenicol, and thamphenicol. Recovery of each antibiotic was greater than 65% with a detection limit of 5 ppb. Further refinement of lc–ms and gc–ms techniques, advances in ms sensitivity, more efficient lc–ms interfaces, and future developments may allow ms to become a routine confirmatory tool that will greatly enhance antibiotic residue determinations.

FUTURE TRENDS

Microbiological and bioassays have historically served to screen foods for the presence of antibiotics. While these assays have proven to be quite sensitive and inexpensive they are far too nonspecific and cause too lengthy a delay in obtaining data. This delay may allow antibiotic contaminated animal-derived foods to be marketed and consumed with drug detection being accomplished after the fact. This policy is inherently inadequate but has arisen from the limitations of the available analytical technologies. In this sense, bioassays and other methodologies that require lengthy waiting periods and prolonged and complex analytical procedures cannot meet the increasing need to perform rapid drug screening and confirmation on more samples for more drugs.

The advent of more specific, sensitive, and simple to perform immunoassays should overcome many of the problems of performing rapid screening and early detection of antibiotic contamination in animal-derived foods. In this regard, simple elisa, card, or test-strip assays that are suitable for use at production sites, slaughterhouses, and packing plants could dramatically reduce the occurrence of antibiotic violations and preclude the problems that can arise from antibiotic contamination of the food supply.

These and other more complex immunoassay methods could be performed on milk, urine, blood, or other body fluids. However, new methods for the rapid isolation of drugs from tissues may also make such immunoassays more directly applicable to the screening of this complex sample material. Matrix solid-phase dispersion (mspd) techniques have shown promise in providing a simple, rapid, and generic method for performing drug isolations from tissues and milk. Thus the combination of ia and mspd may prove to be a useful approach to the more rapid screening and analysis of tissues and animal derived products for antibiotics as well as other drugs. A further advantage of mspd is the capability to isolate a class of drugs or several classes of drugs from a single sample. In this regard the advances in immunoassay screening technology must be matched by advances in extraction procedures. Extraction methodology that is too narrow in extraction capability leads to a need to develop a specific method for each antibiotic drug. Extraction methods that are multidrug and multidrug class specific will have the greatest utility for performing screening analyses in the future, whether such screens are conducted by immunoassay, tlc, hplc, or gc.

Of these analytical techniques ia and tlc will be best utilized for rapid screening, whereas hplc and gc may be the best methods for subsequent confirmation and quantitation of antibiotic residues. However, hplc methods that are capable of separating and detecting several drug residues obtained, perhaps, from a single multidrug class extraction may also prove to be useful screening techniques. The usefulness of such screens is directly related to the efficiency of recovery of a multidrug extraction method, the elimination of background interferences, and the ability of various detectors to indicate the presence or absence of a drug with adequate sensitivity.

For the antibiotics, gc will remain of limited value but should always be considered and applied where feasible. It is expected that sfc will eventually play a major role in the extraction and analysis of compounds such as the antibiotics. However, current results are less than promising and the awaited applications may be long in development.

BIBLIOGRAPHY

1. *Code of Federal Regulations*, 21 CFR "Food and Drugs," U.S. Government Printing Office, Washington, D.C., 1989.
2. S. F. Sundlof, J. E. Riviere, and A. L. Craigmill. *Food Animal Residue Avoidance Databank. Trade Name File. A Compre-*

hensive Compendium of Food Animal Drugs, 3rd ed, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Fla., 1989.

3. Food and Agriculture Organization of the United Nations. *Residues of Veterinary Drugs in Foods*, FAO Food and Nutrition Paper **32**. Food and Agriculture Organization of the United Nations, Rome, Italy, 1985.
4. National Center for Toxicological Research, *Chronic Toxicity and Carcinogenicity of Sulfamethazine in B6C3F1 Mice*, technical report, National Center for Toxicological Research, Jefferson, Ark., 1988.
5. J. S. Spika et al., "Chloramphenicol-Resistant *Salmonella newport* Traced through Hamburgers to Dairy Farms," *New Eng. J. Med.* **316**, 565–570 (1987).
6. J. Brown, ed., U.S. Department of Agriculture/Food Safety and Inspection Service. *Compound Evaluation and Analytical National Residue Program Plan*, USDA, FSIS Science and Technology Program, Washington, D.C., 1988.
7. J. Boison and L. Keng, "Application of Solid Phase Technology for the Multiresidue Analysis of Penicillin Residues in Animal Tissues by High Performance Liquid Chromatography with Ultraviolet-Visible Detection," *Seminars in Food Analysis* **1**, 33–38 (1996).
8. J. W. Pensabene, W. Fiddler, and D. J. Donoghue, "Supercritical Fluid Extraction Compared with Solvent Method for Incurred Sulfamethazine in Chicken Eggs," *J. Food Sci.* **63**, 25–26 (1998).
9. M. T. Combs et al., "Quantitative Recovery of Sulfonamides from Chicken Liver, Beef Liver, and Egg Yolk Via Modified Supercritical Carbon Dioxide," *J. Agric. Food Chem.* **45**, 1779–1783 (1997).
10. V. K. Agarwal, "High Performance Liquid Chromatographic Determinations of Gentamicin in Animal Tissue," *J. Liquid Chromatography* **12**, 613–628 (1989)
11. J. O. Miners, "The Analysis of Penicillins in Biological Fluids and Pharmaceutical Preparations by High Performance Liquid Chromatography: A Review," *J. Liquid Chromatography* **8**, 2827–2843 (1985).
12. R. K. Munns et al., "Multiresidue Method for Determination of Eight Neutral β-Lactam Penicillins in Milk by Fluorescence-Liquid Chromatography," *Journal of the Association of Official Analytical Chemists* **68**, 968–971 (1985).
13. T. Nagata and M. Saeki, "Determination of Ampicillin Residues in Fish Tissues by Liquid Chromatography," *Journal of the Association of Official Analytical Chemists* **69**, 448–450 (1986).
14. E. H. Allen, "Review of Chromatographic Methods for Chloramphenicol Residues in Milk, Eggs and Tissues from Food Producing Animals," *Journal of the Association of Official Analytical Chemists* **68**, 990–999 (1985).
15. G Weiss and A. MacDonald, "Methods for Determination of Ionophore Type Antibiotic Residues in Animal Tissues," *Journal of the Association of Official Analytical Chemists* **68**, 971–980 (1985).
16. W. A. Moats, "Chromatographic Methods for Determination of Macrolide Antibiotic Residues in Tissues and Milk of Food-Producing Animals, *Journal of the Association of Official Analytical Chemists* **68**, 980–984 (1985).
17. W. Horwitz, "Analytical Methods for Sulfonamides in Foods and Feeds. II. Performance Characteristics of Sulfonamide Methods," *Journal of the Association of Official Analytical Chemists* **64**, 814–824 (1981).
18. A. J. Manuel and W. A. Steller, "Gas-Liquid Chromatographic Determination of Sulfamethazine in Swine and Cattle Tissues," *Journal of the Association of Official Analytical Chemists* **64**, 794–799 (1981).
19. B. L. Cox and A. Krzeminski, "High Pressure Liquid Chromatographic Determination of Sulfamethazine in Pork Tissue," *Journal of the Association of Official Analytical Chemists* **65**, 1311–1315 (1982).
20. R. M. Simpson, F. B. Suhre, and J. W. Shafer, "Quantitative Gas Chromatographic Mass Spectrometric Assay of Five Sulfonamide Residues in Animal Tissue," *Journal of the Association of Official Analytical Chemists* **68**, 23–26 (1985).
21. R. B. Ashworth, "Liquid Chromatographic Assay of Tetracyclines in Tissues of Food Producing Animals," *Journal of the Association of Official Analytical Chemists* **68**, 1013–1018 (1985).
22. S. A. Barker, A. R. Long, and C. R. Short, "A New Approach to the Isolation of Drug Residues From Animal Tissues," in W. Huber, ed., *Proceedings of the Sixth Symposium on Veterinary Pharmacology and Therapeutics*, Blacksburg, Va., 1988, pp. 55–56.
23. S. A. Barker, A. R. Long, and C. R. Short, "Isolation of Drug Residues from Tissues by Solid Phase Dispersion," *J. Chromatography* **475**, 353–361 (1989).
24. A. R. Long, C. R. Short, and S. A. Barker, "Multiresidue Method for the Isolation and Liquid Chromatographic Determination of Eight Sulfonamides in Milk," *J. Chromatography* **502**, 87–94 (1990).
25. A. R. Long et al., "A Multiresidue Method for the Isolation and Liquid Chromatographic Determination of Seven Sulfonamides in Infant Formula," *J. Liquid Chromatography* **12**, 1601–1612 (1989).
26. A. R. Long et al., "Multiresidue Method for the Determination of Sulfonamides in Pork Tissue," *J. Agric. Food Chem.* **38**, 423–426 (1990)
27. A. R. Long et al., "Method for the Isolation and Liquid Chromatographic Determination of Chloramphenicol in Milk," *J. Agric. Food Chem.* **38**, 427–429 (1990)
28. A. R. Long et al., "Matrix Solid Phase Dispersion (MSPD) Isolation and Liquid Chromatographic Determination of Oxytetracycline, Tetracycline and Chlortetracycline in Milk," *Journal of the Association of Official Analytical Chemists* **73**, 379–384 (1990).
29. R. B. Read, Jr. et al., "Detection of Sulfa Drugs and Antibiotics in Milk," *Applied Microbiology* **21**, 806–808 (1971).
30. R. W. Johnston et al., "A New Screening Method for the Detection of Antibiotic Residues in Meat and Poultry Tissues," *J. Food Protection* **44**, 828–831 (1981).
31. B. Shaikh and E. H. Allen, "Overview of Physical Chemical Methods for Determining Aminoglycoside Antibiotics in Tissues and Fluids of Food Producing Animals," *Journal of the Association of Official Analytical Chemists* **68**, 1007–1013 (1985).
32. E. Neidert, P. W. Saschenbrecker, and F. Tittiger, "Thin Layer Chromatographic/Bioautographic Method for Identification of Antibiotic Residues in Animal Tissues," *Journal of the Association of Official Analytical Chemists* **70**, 197–200 (1987).
33. J. R. Bishop et al., "Quantitative Assay for Antibiotics Used Commonly in Treatment of Bovine Infections," *J Dairy Sci.* **68**, 3031–3036 (1985).
34. American Public Health Association, *Standard Methods for the Examination of Dairy Products*, Port City Press Inc., Baltimore, Md., 1985.

35. C. J. Singer and S. E. Katz, "Microbiological Assay for Chloramphenicol Residues," *Journal of the Association of Official Analytical Chemists* **68**, 1037–1041 (1985).

36. G. O. Korsrud and J. D. MacNeil, "A Comparison of Three Bioassay Techniques and High Performance Liquid Chromatography for the Detection of Chlortetracycline Residues in Swine Tissues," *Food Additives and Contaminants* **5**, 149–153 (1987).

37 C. D. C. Salisbury et al., "Survey of Chloramphenicol Residues in Diseased Swine," *Can. J. Veterinary Res.* **52**, 15–17 (1988).

38. M. Riaz, "The Quantitative Analysis of Tetracyclines," *Journal of the Chemical Society of Pakistan* **8**, 571–583 (1986).

39. S. Williams, ed., *Official Methods of Analysis*, 14th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1984.

40. M. Petz et al., "Thin-Layer Chromatographic Determination of Erythromycin and Other Macrolide Antibiotics in Livestock Products," *Journal of the Association of Official Analytical Chemists* **70**, 691–697 (1987).

41. Food and Drug Administration, *PMO, Grade A Pasteurized Milk Ordinance*, Public Health Service Publication No. **229**, Washington, D.C., 1985.

42. B. A. Morris, M. N. Clifford, and R. Jackman, eds., *Immunoassays for Veterinary and Food Analysis—Part 1*, Elsevier Science Publishing Co., Inc., New York, 1988.

43. D Arnold et al., "Radioimmunological Determination of Chloramphenicol Residues in Musculature, Milk and Eggs," *Archiv für Lebensmittelhygiene* **35**, 131–139 (1984).

44. D. Arnold and A. Somogyi, "Trace Analysis of Chloramphenicol Residues in Eggs, Milk and Meat: Comparison of Gas Chromatography and Radioimmunoassay," *Journal of the Association of Official Analytical Chemists* **68**, 984–990 (1985).

45. M. E. Mount, D. L. Failla, and S. Wie, "Enzyme Immunoassay for the Feed Additive Monensin," *Journal of the Chemical Society of Pakistan* **8** (1986)

46. M. C. Rouan, "Antibiotic Monitoring in Body Fluids," *J. Chromatography* **340**, 361–400 (1985).

47. J. J. Ryan et al., "Detection of Penicillin, Cephapirin and Cloxacillin in Commingled Raw Milk by the Spot Test," *J. Dairy Sci.* **69**, 1510–1517 (1986).

48. C. Van de Water et al., "An Enzyme Linked Immunosorbent Assay for the Determination of Chloramphenicol Using Monoclonal Antibody," *Zeitschrift für Lebensmittel-Untersuchang und-Forschung* **185**, 202–207 (1987).

49. J. F. M. Nouws, J. Laurensen, and M. M. L. Aerts, "Monitoring Milk for Chloramphenicol Residues by an Immunoassay (Quick-Card)," *Veterinary Quarterly* **10**, 270–272 (1988).

50. D. E. Dixon-Holland and S. E. Katz, "Competitive Direct Enzyme-Linked Immunosorbent Assay for Detection of Sulfamethazine Residues in Swine Urine and Muscle Tissues," *Journal of the Association of Official Analytical Chemists* **71**, 1137–1140 (1988).

51. T. Tanaka et al., "Easy Enzyme-linked Immunosorbent Assay for Spectinomycin in Chicken Plasma," *J. AOAC Int.* **79**, 426–430 (1996).

52. J. D. Thacker, E. S Casale, and C. M. Tucker, "Immunoassays (ELISA) for Rapid, Quantitative Analysis in the Food Processing Industry," *J. Agric. Food Chem.* **44**, 2680–2685 (1996).

53. P. Hammer and W. Heeschen, "Antibody-Capture-Immunoassay for the Detection of Enrofloxacin in Raw Milk," *Milchwissenschaft* **50**, 513–514 (1995).

54. J. Ara et al., "Dot-ELISA for the Rapid Detection of Gentamicin in Milk," *J. Clin. Laboratory Analysis* **9**, 320–324 (1995).

55. B. M. Medina et al., "Evaluation of Commercial Immunochemical Assays for Determination of Sulfamethazine in Milk," *J. Food Protection* **55**, 330–332 (1992).

56. U. Samarajeewa et al., "Application of Immunoassays in the Food Industry," *CRC Reviews in Food Science and Nutrition* **29**, 403–434 (1991).

57. Y. Takahashi et al., "Enzyme Immunoassay of Sulfamethoxazole in Chicken Tissues: Interlaboratory Study," *J. Veterinary Medical Sci.* **56**, 1207–1208 (1994).

58. T. Saesmaa, "Identification and Purity Determination of Benzathine and Embonate Salts of Some Beta-Lactam Antibiotics by Thin Layer Chromatography, *J. Chromatography* **463**, 469–473 (1989).

59. J. Sherma et al., "Spectrometric and Thin-Layer Chromatographic Quantification of Sulfathiazole Residues in Honey," *J. Chromatography* **463**, 229–233 (1989).

60. B. Wyhowski de Bukanski, J. M. Degroodt, and H. Beernaert, "A Two-Dimensional High Performance Thin-Layer Chromatographic Screening Method for Sulphonamides in Animal Tissues," *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* **187**, 242–245 (1988).

61. H. Oka et al., "Improvement of Chemical Analysis of Antibiotics X. Determination of Eight Tetracyclines Using Thin Layer and High Performance Liquid Chromatography," *J. Chromatography* **393**, 285–296 (1987).

62. Y. Ikai et al., "Improvement of Chemical Analysis of Antibiotics. XIII. Systematic Simultaneous Analysis of Residual Tetracyclines in Animal Tissues Using Thin-Layer and High Performance Liquid Chromatography," *J. Chromatography* **411**, 313–323 (1987).

63. R. L. Ellis, "Less Expensive and Faster Than Laboratory Tests," *Association of Official Analytical Chemists Newsletter, The Referee* **13**, 9 (1989).

64. S. F. Sunderlof, "Drug and Chemical Residues in Livestock," *Veterinarian Clinical North American Food Animal Plant* **4**, 411–419 (1989).

65. I. Schwaiger, R. Schuch, and H. Hoertner, "TLC/Bioautographic Analysis of Antibiotic Residues in Poultry Meat and Milk," *Current Status and Future Trends, Proceedings of Euro Food Chem VIII*, Vol. 3, Vienna, 1995, pp. 727–731.

66 C. Stubbs and I. Kanfer. "High Performance Liquid Chromatography of Erythromycin Propionyl Ester and Erythromycin Base in Biological Fluids," *J. Chromatography* **427**, 93–101 (1988)

67. M. Margosis, "Quantitative Liquid Chromatography of Ampicillin: Collaborative Study," *Journal of the Association of Official Analytical Chemists* **70**, 206–212 (1987).

68 K. Tyczkowska and A. L. Aronson, "Ion-Pair Liquid Chromatographic Determination of Some Penicillins in Canine and Equine Sera," *Journal of the Association of Official Analytical Chemists* **71**, 773–775 (1988).

69. W. A. Moats and R. Harik-Khan, "Liquid Chromatographic Determination of β-Lactam Antibiotics in Milk: A Multiresidue Approach," *J. AOAC Int.* **78**, 49–54 (1995).

70. G. Knupp, G. Bugl-Kreickmann, and C. Commichaw, "A Method for the Verification of Chloramphenicol Residues in Animal Tissues and Eggs by High Performance Liquid Chromatography with Radioimmunological Detection (HPLC-RIA)," *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* **184**, 390–391 (1987).

71. M. F. Pochard, G. Burger, M. Chevalier, and E. Gleizes, "Determination of Chloramphenicol Residues with Reverse-Phase High Pressure Liquid Chromatography. Use of a Pharmacokinetic Study in Rainbow Trout with Confirmation by Mass Spectrometry," *J. Chromatography* **490**, 315–323 (1987).

72. B. Delepine, D. Hurtaud-Pessel, and P. Saunders, "Multiresidue Method for Confirmation of Macrolide Antibiotics in Bovine Muscle by Liquid Chromatography/Mass Spectrometry," *J. AOAC Int* **79**, 397–404 (1996).

73. M. M. Aerts, W. M Beek, and U. A. Brinkman, "Monitoring of Veterinary Drug Residues by a Combination of Continuous Flow Techniques and Column-Switching High Performance Liquid Chromatography. I. Sulfonamides in Egg, Meat and Milk Using Post-Column Derivatization with Dimethylaminobenzaldehyde," *J Chromatography* **435**, 97–112 (1988).

74. R. Malisch, "Multi-Method for the Determination of Residues of Chemotherapeutics, Antiparasitics and Growth Promoters in Foodstuffs of Animal Origin. Part I. General Procedure and Determination of Sulfonamides," *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* **182**, 385–399 (1986).

75. G. D. Paulson, A. D. Mitchell, and R. G. Zaylskie, "Identification and Quantitation of Sulfamethazine Metabolites by Liquid Chromatography and Gas Chromatography-Mass Spectrometry," *Journal of the Association of Official Analytical Chemists* **68**, 1000–1006 (1985).

76. W. A. Moats, "Determination of Tetracycline Antibiotics in Tissues and Blood Serum of Cattle and Swine by High Performance Liquid Chromatography," *J. Chromatography* **358**, 253–259 (1986).

77. F. Kondo, S. Morikawa, and S. Tateyama, "Simultaneous Determination of Six Tetracyclines in Bovine Tissue, Plasma and Urine by Reverse-Phase High Performance Liquid Chromatography," *J. Food Protection* **52**, 41–44 (1988).

78. I. Norlander, H. Johnsson, and B. Österdahl, "Oxytetracycline Residues in Rainbow Trout Analyses by a Rapid HPLC Method," *Food Additives and Contaminants* **4**, 291–295 (1987).

79. W. An-Bang, Y.-M. Chen, and C.-Y. Chen, "Simultaneous Determination of Sulfa Drugs and Tetracyclines in Feeds and Animal Tissues by High Performance Liquid Chromatography," *J. Food and Drug Analysis* **2**, 297–310 (1994).

80. S. Kawata et al., "Liquid Chromatographic Determination of Oxytetracycline in Swine Livers," *J. AOAC Int.* **79**, 1463–1465 (1997).

81. D. W. Hill and K. J. Langner, "HPLC Photodiode Array UV Detection for Toxicological Drug Analysis," *J. Liquid Chromatography* **10**, 377–409 (1987).

82. N. E. Larsen, K. Marinelli, and A. M. Heilesen, "Determination of Gentamicin in Serum Using Liquid Column Chromatography," *J. Chromatography* **221**, 182–187 (1980).

83. K. Tsuji, J. F. Goetz, W. Van Meter, and K. A. Gusciora, "Normal-Phase High Performance Liquid Chromatographic Determination of Neomycin Sulfate Derivatized with 1-Fluoro-2,4-dinitrobenzene," *J. Chromatography* **175**, 141–152 (1979).

84 K. Tsuji and J. H. Robertson, "Gas-Liquid Chromatographic Determination of Neomycins B and C," *Analytical Chem.* **41**, 1332–1335 (1969).

85. K. Tsuji and J. H. Robertson, "Formation of Trimethylsilyl Derivatives of Tetracyclines for Separation and Quantitation by Gas-Liquid Chromatography," *Analytical Chem.* **45**, 2136–2140 (1973).

86. S. J Stout, W. A. Stellar, A. J. Manuel, M. O. Poeppel, and A. R. DaCumha, "Confirmatory Method for Sulfamethazine Residues in Cattle and Swine Tissues Using Gas Chromatography-Chemical Ionization Mass Spectrometry," *Journal of the Association of Official Analytical Chemists* **67**, 142–144 (1984).

87. M. V. Novotny, "Recent Developments in Analytical Chromatography," *Science* **246**, 51–57 (1989).

88. J. Keever, R. D. Voykser, and K. L. Tyczkowska, "Quantitative Determination of Ceftiofur in Milk by Liquid Chromatography–Mass Spectrometry," *J. Chromatography* **794**, 57–62 (1998).

89. D. N. Heiller, "Determination of Pirlimycin Residue in Milk by Liquid Chromatographic Analysis of the 9-Fluorenylmethyl Chloroformate Derivative," *J. AOAC Int.* **80**, 975–981 (1997).

90. L. A. van Ginkel et al., "Development and Validation of a Gas Chromatographic–Mass Spectrometric Procedure for the Identification and Quantification of Residues of Chloramphenicol," *Analytica Chimica Acta* **237**, 61–69 (1990).

91. T. Nagata and H. Oka, "Detection of Residual Chloramphenicol, Florfenicol, and Thiamphenicol in Yellowtail Fish Muscles by Capillary Gas Chromatography–Mass Spectrometry," *J. Agric. and Food Chem.* **44**, 1280–1284 (1996).

AUSTIN R. LONG
STEVEN A. BARKER
Louisiana State University
Baton Rouge, Louisiana

ANTIMICROBIAL COMPOUNDS

Food antimicrobials are chemical compounds added to or present in foods for the purpose of retarding microbial growth or killing microorganisms. The major targets for antimicrobials are bacteria, molds, and yeasts that are either pathogenic or cause spoilage of foods. The effectiveness of food antimicrobials against viruses and parasites carried by foods is less well characterized. Food antimicrobials are sometimes referred to as food preservatives; however, the latter include food additives that are antimicrobials, antibrowning agents, and antioxidants. Under normal use conditions, food antimicrobials are bacteriostatic or fungistatic rather than bactericidal or fungicidal. The former indicates inhibition of growth of cells while the latter indicates killing of a population. Bacteriostasis is often reversible. Because food antimicrobials are generally static in nature, they will not preserve a food indefinitely. Depending on storage conditions, the food product eventually spoils or becomes hazardous. In addition, food antimicrobials are normally not capable of concealing spoilage of a food product. Rather, the food remains wholesome during its extended shelf life. Food antimicrobials are often used in combination with other food preservation procedures such as heat or refrigeration.

The effectiveness of food antimicrobials depends on many factors, including those related to the target microorganisms, characteristics of the food product, the storage environment, and processing of the food (1). Microbial factors that affect antimicrobial activity include inherent re-

71. M. F. Pochard, G. Burger, M. Chevalier, and E. Gleizes, "Determination of Chloramphenicol Residues with Reverse-Phase High Pressure Liquid Chromatography. Use of a Pharmacokinetic Study in Rainbow Trout with Confirmation by Mass Spectrometry," *J. Chromatography* **490**, 315–323 (1987).

72. B. Delepine, D. Hurtaud-Pessel, and P. Saunders, "Multiresidue Method for Confirmation of Macrolide Antibiotics in Bovine Muscle by Liquid Chromatography/Mass Spectrometry," *J. AOAC Int* **79**, 397–404 (1996).

73. M. M. Aerts, W. M Beek, and U. A. Brinkman, "Monitoring of Veterinary Drug Residues by a Combination of Continuous Flow Techniques and Column-Switching High Performance Liquid Chromatography. I. Sulfonamides in Egg, Meat and Milk Using Post-Column Derivatization with Dimethylaminobenzaldehyde," *J Chromatography* **435**, 97–112 (1988).

74. R. Malisch, "Multi-Method for the Determination of Residues of Chemotherapeutics, Antiparasitics and Growth Promoters in Foodstuffs of Animal Origin. Part I. General Procedure and Determination of Sulfonamides," *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* **182**, 385–399 (1986).

75. G. D. Paulson, A. D. Mitchell, and R. G. Zaylskie, "Identification and Quantitation of Sulfamethazine Metabolites by Liquid Chromatography and Gas Chromatography-Mass Spectrometry," *Journal of the Association of Official Analytical Chemists* **68**, 1000–1006 (1985).

76. W. A. Moats, "Determination of Tetracycline Antibiotics in Tissues and Blood Serum of Cattle and Swine by High Performance Liquid Chromatography," *J. Chromatography* **358**, 253–259 (1986).

77. F. Kondo, S. Morikawa, and S. Tateyama, "Simultaneous Determination of Six Tetracyclines in Bovine Tissue, Plasma and Urine by Reverse-Phase High Performance Liquid Chromatography," *J. Food Protection* **52**, 41–44 (1988).

78. I. Norlander, H. Johnsson, and B. Österdahl, "Oxytetracycline Residues in Rainbow Trout Analyses by a Rapid HPLC Method," *Food Additives and Contaminants* **4**, 291–295 (1987).

79. W. An-Bang, Y.-M. Chen, and C.-Y. Chen, "Simultaneous Determination of Sulfa Drugs and Tetracyclines in Feeds and Animal Tissues by High Performance Liquid Chromatography," *J. Food and Drug Analysis* **2**, 297–310 (1994).

80. S. Kawata et al., "Liquid Chromatographic Determination of Oxytetracycline in Swine Livers," *J. AOAC Int.* **79**, 1463–1465 (1997).

81. D. W. Hill and K. J. Langner, "HPLC Photodiode Array UV Detection for Toxicological Drug Analysis," *J. Liquid Chromatography* **10**, 377–409 (1987).

82. N. E. Larsen, K. Marinelli, and A. M. Heilesen, "Determination of Gentamicin in Serum Using Liquid Column Chromatography," *J. Chromatography* **221**, 182–187 (1980).

83. K. Tsuji, J. F. Goetz, W. Van Meter, and K. A. Gusciora, "Normal-Phase High Performance Liquid Chromatographic Determination of Neomycin Sulfate Derivatized with 1-Fluoro-2,4-dinitrobenzene," *J. Chromatography* **175**, 141–152 (1979).

84 K. Tsuji and J. H. Robertson, "Gas-Liquid Chromatographic Determination of Neomycins B and C," *Analytical Chem.* **41**, 1332–1335 (1969).

85. K. Tsuji and J. H. Robertson, "Formation of Trimethylsilyl Derivatives of Tetracyclines for Separation and Quantitation by Gas-Liquid Chromatography," *Analytical Chem.* **45**, 2136–2140 (1973).

86. S. J Stout, W. A. Stellar, A. J. Manuel, M. O. Poeppel, and A. R. DaCumha, "Confirmatory Method for Sulfamethazine Residues in Cattle and Swine Tissues Using Gas Chromatography-Chemical Ionization Mass Spectrometry," *Journal of the Association of Official Analytical Chemists* **67**, 142–144 (1984).

87. M. V. Novotny, "Recent Developments in Analytical Chromatography," *Science* **246**, 51–57 (1989).

88. J. Keever, R. D. Voykser, and K. L. Tyczkowska, "Quantitative Determination of Ceftiofur in Milk by Liquid Chromatography–Mass Spectrometry," *J. Chromatography* **794**, 57–62 (1998).

89. D. N. Heiller, "Determination of Pirlimycin Residue in Milk by Liquid Chromatographic Analysis of the 9-Fluorenylmethyl Chloroformate Derivative," *J. AOAC Int.* **80**, 975–981 (1997).

90. L. A. van Ginkel et al., "Development and Validation of a Gas Chromatographic–Mass Spectrometric Procedure for the Identification and Quantification of Residues of Chloramphenicol," *Analytica Chimica Acta* **237**, 61–69 (1990).

91. T. Nagata and H. Oka, "Detection of Residual Chloramphenicol, Florfenicol, and Thiamphenicol in Yellowtail Fish Muscles by Capillary Gas Chromatography–Mass Spectrometry," *J. Agric. and Food Chem.* **44**, 1280–1284 (1996).

Austin R. Long
Steven A. Barker
Louisiana State University
Baton Rouge, Louisiana

ANTIMICROBIAL COMPOUNDS

Food antimicrobials are chemical compounds added to or present in foods for the purpose of retarding microbial growth or killing microorganisms. The major targets for antimicrobials are bacteria, molds, and yeasts that are either pathogenic or cause spoilage of foods. The effectiveness of food antimicrobials against viruses and parasites carried by foods is less well characterized. Food antimicrobials are sometimes referred to as food preservatives; however, the latter include food additives that are antimicrobials, antibrowning agents, and antioxidants. Under normal use conditions, food antimicrobials are bacteriostatic or fungistatic rather than bactericidal or fungicidal. The former indicates inhibition of growth of cells while the latter indicates killing of a population. Bacteriostasis is often reversible. Because food antimicrobials are generally static in nature, they will not preserve a food indefinitely. Depending on storage conditions, the food product eventually spoils or becomes hazardous. In addition, food antimicrobials are normally not capable of concealing spoilage of a food product. Rather, the food remains wholesome during its extended shelf life. Food antimicrobials are often used in combination with other food preservation procedures such as heat or refrigeration.

The effectiveness of food antimicrobials depends on many factors, including those related to the target microorganisms, characteristics of the food product, the storage environment, and processing of the food (1). Microbial factors that affect antimicrobial activity include inherent resistance of a microorganism, initial number, growth stage, cellular composition (eg, gram reaction), previous exposure to stress, and injury. Important factors affecting activity associated with the food product include pH, oxidation reduction potential, and water activity. pH is the most important factor influencing the effectiveness of many food antimicrobials. Antimicrobials that are weak acids are most effective in their undissociated or protonated form because they are able to penetrate the cytoplasmic membrane of a microorganism more effectively in this form. Therefore, the pK_a value of these compounds is important in selecting a particular compound for an application. The lower the pH of a food product, the greater the proportion of acid in the undissociated form and the greater the antimicrobial activity. Storage factors affecting antimicrobial activity include time, temperature, and atmosphere. Processing of foods may lead to shifts in microflora and reduction in microbial numbers. Most of the preceding factors influence microbial inhibition or inactivation in an interactive manner.

Cellular targets of food antimicrobials may include the cell wall, cytoplasmic membrane, metabolic enzymes, protein synthesis, or genetic systems. The exact mechanisms for most food antimicrobials are not known or are not well defined. These compounds likely have multiple targets with concentration-dependent thresholds for inactivation or inhibition. A given target is important in an inhibitor's overall mechanism only when its sensitivity is within the range of the antimicrobial concentration that inhibits growth (2).

Following are discussions of compounds that are used traditionally in foods as antimicrobials including salt (sodium chloride), organic acids, nitrites, parahydroxybenzoic acid esters, sulfites, and dimethyl dicarbonate. In addition, selected compounds that are approved as food additives for other uses but have antimicrobial activity as well as compounds that occur naturally in animals, plants, and from microorganisms and have a potential for use as food antimicrobials are addressed.

SODIUM CHLORIDE

Sodium chloride (NaCl), or common salt, is probably the oldest known food preservative. Few present-day foods are preserved directly by high concentrations of NaCl. Rather salt is used primarily as an adjunct to other processing methods such as canning or curing (3). In general, foodborne pathogenic bacteria are inhibited by a water activity of 0.92 or less (equivalent to a NaCl concentration of 13% w/v). The exception is *Staphylococcus aureus*, which has a minimum water activity for growth of 0.83 to 0.86. Another relatively salt-tolerant foodborne pathogen is *Listeria monocytogenes*, which can survive in saturated salt solutions at low temperatures. Fungi are more tolerant to low water activity than bacteria. The minimum for growth of xerotolerant fungi is 0.61 to 0.62, but most are inhibited by 0.85 or lower (4).

The antimicrobial activity of sodium chloride is related to its ability to reduce water activity (a_w). As the water activity of the external medium is reduced, cells are subjected to osmotic shock and rapidly lose water through plasmolysis. During plasmolysis, a cell ceases to grow and either dies or remains dormant. To resume growth, the cell must reduce its intracellular water activity (5). In addition to osmotic influence on growth, other possible mechanisms of sodium chloride inhibition include limiting oxygen solubility, alteration of pH, toxicity of sodium and chloride ions, and loss of magnesium ions (6).

ORGANIC ACIDS AND ESTERS

Many organic acids are used as food additives, but not all have antimicrobial activity. The most effective antimicrobials are acetic, lactic, propionic, sorbic, and benzoic acids. The activity of organic acids is related to pH and the undissociated form of the acid is primarily responsible for antimicrobial activity (7). The use of organic acids is generally limited to foods with a pH less than 5.5, since most organic acids have pK_a's of pH 3.0 to 5.0 (7). Another factor affecting potential activity is polarity. This relates both to the ionization of the molecule and contribution of any alkyl side groups or hydrophobic parent molecules. Antimicrobials must be lipophilic to attach and pass through the cell membrane but also be soluble in the aqueous phase (8).

The mechanism of action of organic acids and their esters has some common elements. As already stated, in the undissociated form, organic acids can penetrate the cell membrane lipid bilayer more easily. Once inside the cell, the acid dissociates because the cell interior has a higher pH than the exterior (9). Bacteria maintain internal pH near neutrality to prevent conformational changes to the cell structural proteins, enzymes, nucleic acids, and phospholipids. Protons generated from intracellular dissociation of the organic acid acidify the cytoplasm and must be extruded to the exterior. This requires energy in the form of ATP, which will eventually deplete cellular energy.

Acetic Acid/Acetates

Acetic acid (pK_a = 4.75), the primary component of vinegar, and its sodium, potassium, and calcium salts are some of the oldest food antimicrobials. Derivatives of acetic acid including diacetate salts and dehydroacetic acid have also been used as food antimicrobials. In contrast to most organic acids, acetic acid is generally more effective against yeasts and bacteria than against molds (10). Bacteria inhibited include *Bacillus*, *Clostridium*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella*. Only *Acetobacter* species (microorganisms involved in vinegar production), lactic acid bacteria, and butyric acid bacteria are tolerant to acetic acid (7,11). Some yeasts and molds are sensitive to acetic acid and include species of *Aspergillus*, *Penicillium*, *Rhizopus*, and some strains of *Saccharomyces* (7).

Acetic acid and its salts have shown variable success as antimicrobials in food applications. Acetic acid can increase poultry shelf life and decreases the heat resistance of *Salmonella newport*, *Salmonella typhimurium*, and *Campylobacter jejuni* in poultry scald tank water (12,13). The compound has been used as a spray sanitizer at 1.5 to 2.5% on meat carcasses and as a dip for beef or lamb (14).

Two-percent acetic acid affected large-scale reductions in the number of viable *Escherichia coli* O157:H7 on beef after seven days at 5°C (15). Sodium acetate has been shown to increase the shelflife of meat and fish products and inhibits the molds, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. glaucus*, *Penicillium expansum*, and *Mucor pusillus* (7). Sodium diacetate inhibits mold growth in cheese spread and is an effective inhibitor of rope-forming bacteria (*Bacillus subtilis*) in baked goods (7). It is also inhibitory to *L. monocytogenes*, *E. coli*, *Pseudomonas fluorescens*, *S. enteritidis*, and *Shewanella putrefaciens*, but not *S. aureus*, *Yersinia enterocolitica*, *P. fragi*, *Enterococcus faecalis*, or *Lactobacillus* (7,16). Dehydroacetic acid has a high pK_a of 5.27 and is therefore active at higher pH values. It is inhibitory to bacteria at 0.1 to 0.4% and fungi at 0.005 to 0.1% (7).

Acetic acid is used commercially in baked goods, cheeses, condiments and relishes, dairy product analogues, fats and oils, gravies and sauces, and meats. The sodium and calcium salts are used in breakfast cereals, candy, cheeses, fats and oils, gelatin, jams and jellies, meats, snack foods, sauces, and soup mixes (7). Sodium diacetate is used in baked goods, candy, cheese spreads, gravies, meats, sauces and soup mixes (7).

Benzoic Acid/Benzoates

Benzoic acid and sodium benzoate were the first antimicrobial compounds permitted in foods by the U.S. Food and Drug Administration (FDA). Benzoic acid occurs naturally in cranberries, plums, prunes, apples, strawberries, cinnamon, cloves, and most berries. The undissociated form of benzoic acid (pK_a = 4.19) is the most effective antimicrobial agent; therefore, the most effective pH range is 2.5 to 4.5.

Benzoic acid and sodium benzoate are used primarily as antifungal agents. The inhibitory concentration of benzoic acid at pH less than 5.0 against most yeasts ranges from 20 to 700 μg/ml, whereas for molds it is 20 to 2000 μg/mL (11,17). Fungi including *Byssochlamys nivea*, *Pichia membranaefaciens*, *Talaromyces flavus*, and *Zygosaccharomyces bailii* are resistant to benzoic acid (18). While bacteria associated with food poisoning including *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* are inhibited by 1000 to 2000 μg/ml undissociated acid, the control of many spoilage bacteria requires much higher concentrations (11). Benzoic acid at 0.1% is effective in reducing viable *E. coli* O157:H7 in apple cider (pH 3.6–4.0) by 3 to 5 logs in seven days at 8°C (19).

Sodium benzoate is used as an antimicrobial at up to 0.1% in carbonated and still beverages, syrups, cider, margarine, olives, pickles, relishes, soy sauce, jams, jellies, preserves, pie and pastry fillings, fruit salads, and salad dressings and in the storage of vegetables (17).

Lactic Acid/Lactates

Lactic acid (pK_a = 3.79) is produced naturally during fermentation of foods by lactic acid bacteria including *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Carnobacterium*. While the acid and salts act as preservatives in food products, their primary uses are as pH control agents and flavorings. Lactic acid inhibits *Clostridium botulinum*, *C. perfringens*, *C. sporogenes*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, and *Yersinia enterocolitica* (7,20,21). Lactic acid at 1–2% reduces *Enterobacteriaceae* and aerobic mesophilic microorganisms on beef, veal, pork, and poultry and delays growth of spoilage microflora during long-term storage of products (7). Sodium lactate (2.5–5.0%) inhibits *C. botulinum*, *C. sporogenes*, *L. monocytogenes*, and spoilage bacteria in various meat products (7,22,23).

Propionic Acid/Propionates

Up to 1% propionic acid (pK_a = 4.87) is produced naturally in Swiss cheese by *Propionibacterium freudenreichii* ssp. *shermanii*. Propionic acid and sodium, potassium, and calcium propionates are used primarily against molds; however, some yeasts and bacteria are also inhibited. Propionates (0.1–5.0%) retard the growth of *E. coli*, *Staphylococcus aureus*, *Sarcina lutea*, *Salmonella*, *Proteus vulgaris*, *Lactobacillus plantarum*, and *Listeria monocytogenes*, and the yeasts *Candida* and *Saccharomyces cerevisiae* (7). Rope-forming bacteria in bread dough (*B. subtilis*) are inhibited by propionic acid at pH 5.6 to 6.0 (7).

Propionic acid and propionates are used as antimicrobials in baked goods and cheeses. Propionates may be added directly to bread dough because they have no effect on the activity of baker's yeast (7). There is no limit to the concentration of propionates allowed in foods, but amounts used are generally less than 0.4% due to sensory changes that might occur (7).

Sorbic Acid/Sorbates

Sorbic acid occurs naturally in the berries of the mountain ash tree (rowanberry) (24). As with other organic acids, the antimicrobial activity of sorbic acid is greatest when the compound is in the undissociated state. With a pK_a of 4.75, activity is greatest at a pH less than 6.0 to 6.5.

Sorbates are probably the most well characterized of all food antimicrobials as to their spectrum of action and inhibit bacteria, yeasts, and molds at concentrations of 0.05 to 0.3% (24). Food-related yeasts and molds inhibited by sorbates include *Byssochlamys*, *Candida*, *Saccharomyces*, and *Zygosaccharomyces* and *Aspergillus*, *Fusarium*, *Geotrichum*, and *Penicillium*, respectively (24). Sorbates inhibit the growth of yeasts and molds in microbiological media, cheeses, fruits, vegetables and vegetable fermentations, sauces, and meats. Sorbates inhibit growth and mycotoxin production by *Aspergillus flavus*, *A. parasiticus*, *Byssochlamys nivea*, *Penicillium expansum*, and *P. patulum* (24). Some of the genera of bacteria inhibited by sorbate include *Acinetobacter*, *Aeromonas*, *Bacillus*, *Campylobacter*, *Clostridium*, *Escherichia*, *Lactobacillus*, *Listeria*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, *Vibrio*, and *Yersinia* (24).

A number of *Penicillium*, *Saccharomyces*, and *Zygosaccharomyces* species can grow in the presence of and degrade potassium sorbate (24). Sorbates may be degraded through a decarboxylation reaction resulting in the formation of 1,3-pentadiene, a compound having a kerosene-like or hydrocarbon-like odor.

Sorbate inhibits the growth of many pathogenic bacteria in foods, including *Salmonella* and *S. aureus* in sausage; *S. aureus* in bacon; *Vibrio parahaemolyticus* in seafood; *Salmonella*, *S. aureus*, and *E. coli* in poultry; *Yersinia enterocolitica* in pork; and *Salmonella typhimurium* in milk and cheese (24,25). In addition, the compound inhibits growth of the spoilage bacteria, *Pseudomonas putrefaciens* and *P. fluorescens*, histamine production by *Proteus morgani* and *K. pneumoniae* and listeriolysin O production by *L. monocytogenes* (24,26). Sorbates are effective anticlostridial agents in cured meats and other meat and seafood products. The compound prevents spores of *C. botulinum* from germinating and forming toxin in beef, pork, poultry, and soy protein frankfurters and emulsions and bacon (24).

Sorbate is applied to foods by direct addition, dipping, spraying, dusting, or incorporation into packaging. Baked goods, icing, fruit, and cream fillings can be protected from yeast and molds through the use of 0.05 to 0.10% potassium sorbate applied either as a spray after baking or by direct addition (24). Sorbates may be used in or on beverages, jams, jellies, preserves, margarine, chocolate syrup, salads, dried fruits, dry sausages, salted and smoked fish, cheeses, and in various lactic acid fermentations (24).

Fumaric Acid/Fumarates

Fumaric acid has been used to prevent the malolactic fermentation in wines and as an antimicrobial agent in wines (7). The compound is lethal to ascospores of *Talaromyces flavus* and *Neosartorya fischeri* (7). Esters of fumaric acid (monomethyl, dimethyl, and ethyl) at 0.15 to 0.2% have been tested as a substitute or adjunct for nitrate in bacon. Fumaric acid esters inhibit *Clostridium botulinum* toxin formation in bacon, and the methyl, dimethyl, ethyl, and diethyl fumarates inhibit fungal growth in tomato juice and on bread (7).

Citric Acid/Citrates

Although citric acid is not used directly as an antimicrobial, it has activity against some molds and bacteria. Citric acid retards growth and toxin production by *Aspergillus parasiticus* and *A. versicolor*, but not *Penicillium expansum* (7). It is inhibitory to *Salmonella* in media and on poultry carcasses, to growth and toxin production by *C. botulinum* in shrimp and tomato products, to *S. aureus* in microbiological medium and to flat sour bacteria isolated from tomato juice (7). The mechanism of citrate inhibition has been theorized to be related to its ability to chelate metal ions (27). However, Buchanan and Golden (28) found that, while undissociated citric acid is inhibitory against *Listeria monocytogenes*, the dissociated molecule protects the microorganism. They theorized that this protection is due to chelation by the anion.

Fatty Acid Esters

Certain fatty acid esters have antimicrobial activity in foods. One of the most effective of the fatty acid esters is glyceryl monolaurate (monolaurin) (29,30). Monolaurin is effective as an antimicrobial against Gram-positive bacteria including *Bacillus*, *Listeria monocytogenes*, *Micrococcus*, and *Staphylococcus aureus* but generally ineffective against Gram-negative bacteria (31–33). Presence of ethylenediaminetetraacetic acid (EDTA) expands the activity spectrum of monolaurin to include Gram-negative bacteria such as *Salmonella*, *E. coli* O157:H7, and *Vibrio* and decreases the minimum inhibitory concentrations against Gram-positive strains (29,34). Monolaurin is inhibitory to molds and yeasts including *Aspergillus*, *Alternaria*, *Candida*, *Cladosporium*, *Penicillium*, and *Saccharomyces*. Monolaurin is effective against *L. monocytogenes* in cottage cheese, Camembert cheese, yogurt, and meat products (32,33).

NITRITES

Sodium ($NaNO_2$) and potassium (KNO_2) nitrite have a specialized use in cured meat products. Meat curing utilizes salt, sugar, spices, and ascorbate or erythorbate along with nitrite. In addition to serving as an antimicrobial, nitrite has many functions in cured meats, including formation of characteristic cured meat color, contribution to the flavor and texture, and as an antioxidant. The primary use for sodium nitrite as an antimicrobial is to inhibit *Clostridium botulinum* growth and toxin production in cured meats. In association with other components in the curing mix, such as salt and reduced pH, nitrite inhibits outgrowth of spores of *C. botulinum* and other clostridia. Nitrite inhibits bacterial spore formers by inhibiting outgrowth of the germinated spore (35). The effectiveness of nitrite depends on reduced pH, salt concentration, presence of ascorbate and isoascorbate, storage and processing temperatures, and initial inoculum size.

Meat products that may contain nitrites include bacon, bologna, corned beef, frankfurters, luncheon meats, ham, fermented sausages, shelf-stable canned cured meats, and perishable canned cured meat (eg, ham). Nitrite is also used in a variety of fish and poultry products. The concentration used in these products is specified by governmental regulations but is generally limited to 156 ppm (mg/kg) for most products and 100 to 120 ppm (mg/kg) in bacon. Sodium erythorbate or isoascorbate is required in products containing nitrites as a cure accelerator and as an inhibitor to the formation of nitrosamines, carcinogenic compounds formed by reactions of nitrite with secondary or tertiary amines. Sodium nitrate is used in certain European cheeses to prevent spoilage by *C. tyrobutyricum* or *C. butyricum* (35).

PARAHYDROXYBENZOIC ACID ESTERS (PARABENS)

Alkyl esters of *p*-hydroxybenzoic acid (parabens) have been known to possess antimicrobial activity since the 1920s. Esterification of the carboxyl group of benzoic acid allows the molecule to remain undissociated up to pH 8.5, giving the parabens an effective range of pH 3.0 to 8.0 (36). In most countries, the methyl, propyl, and heptyl parabens are allowed for direct addition to foods as antimicrobials while the ethyl and butyl esters are approved in a limited number of countries.

The antimicrobial activity of *p*-hydroxybenzoic acid esters is, in general, directly proportional to the chain length of the alkyl component (8,36). As the alkyl chain length of the parabens increases, inhibitory activity generally increases. Increasing activity with decreasing polarity is more evident against Gram-positive than against Gram-negative bacteria (8). Parabens are generally more active against molds and yeast than against bacteria (8).

To take advantage of their respective solubility and increased activity, methyl and propyl parabens are normally used in a combination of 2–3:1 (methyl.propyl). The compounds may be incorporated into foods by dissolving in water, ethanol, propylene glycol, or the food product itself. The *n*-heptyl ester is used in fermented malt beverages (beers) and noncarbonated soft drinks and fruit-based beverages. Parabens are used in a variety of foods including baked goods, beverages, fruit products, jams and jellies, fermented foods, syrups, salad dressings, and wine (8).

SULFITES

Sulfur dioxide (SO_2) and its salts (potassium sulfite [K_2SO_3], sodium sulfite [Na_2SO_3], potassium bisulfite [$KHSO_3$], sodium bisulfite [$NaHSO_3$], potassium metabisulfite [$K_2S_2O_5$], and sodium metabisulfite [NaS_2O_5]) have been used as disinfectants since the time of the ancient Greeks and Romans (37). Although sulfites now have multiple uses as food additives, their original purpose was as an antimicrobial. Sulfites are used primarily in fruit and vegetable products to control spoilage and fermentative yeasts, and molds on fruits and fruit products (eg, wine), acetic acid bacteria, and malolactic bacteria (37). In addition to use as antimicrobials, sulfites act as antioxidants and inhibit enzymatic and nonenzymatic browning in a variety of foods. Their primary application is in fruits and vegetable products, but they are also used to a limited extent in meats.

The most important factor impacting the antimicrobial activity of sulfites is pH. The inhibitory effect of sulfites is most pronounced when the acid or $SO_2 \cdot H_2O$ is in the undissociated form. As the pH decreases, the proportion of $SO_2 \cdot H_2O$ increases and the bisulfite (HSO_3^-) ion concentration decreases. Therefore, their most effective pH range is less than 4.0. Sulfites show increased effectiveness at low pH because the unionized sulfur dioxide can pass across the cell membrane in this form.

Sulfites, especially as the bisulfite ion, are very reactive. These reactions not only determine the mechanism of action of the compounds, they also influence antimicrobial activity. For example, sulfites form addition compounds (α-hydroxysulfonates) with aldehydes and ketones.

Sulfur dioxide is fungicidal even in low concentrations against yeasts and molds. The inhibitory concentration range of sulfur dioxide is 0.1 to 20.2 μg/ml for *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Hansenula*, and *Candida* species (37). Sulfur dioxide at 25 to 100 μg/ml inhibits *Byssochlamys nivea* growth and patulin production in grape and apple juices (38). Against bacteria, sulfur dioxide is more inhibitory to Gram-negative rods than to Gram-positive rods. Gram-negative bacteria susceptible to sulfites include *Salmonella*, *E. coli*, *Citrobacter*, *Yersinia enterocolitica*, *Enterobacter*, *Serratia marcescens*, and *Hafnia* (39).

Sulfur dioxide is used to control the growth of undesirable microorganisms in fruits, fruit juices, wines, sausages, fresh shrimp, and acid pickles and during extraction of starches (37). It is added at 50 to 100 mg/L to expressed grape juices used for making wines to inhibit molds, bacteria, and undesirable yeasts. At appropriate concentrations, sulfur dioxide does not interfere with wine yeasts or with the flavor of wine. The optimum level of sulfur dioxide (50–75 mg/L) is maintained to prevent postfermentation changes by microorganisms. In a few countries, sulfites may be used to inhibit the growth of microorganisms on fresh meat and meat products. Sulfite or metabisulfite added in sausages is effective in delaying the growth of molds, yeast, and salmonellae during storage at refrigerated or room temperature (39). Sulfur dioxide restores a bright color but may give a false impression of freshness.

DIMETHYL DICARBONATE

Dimethyl dicarbonate (DMDC) is a colorless liquid that is slightly soluble in water. The compound is very reactive with many substances, including water, ethanol, alkyl and aromatic amines, and sulfhydryl groups (40). The primary target microorganisms for DMDC are yeasts, including *Saccharomyces*, *Zygosaccharomyces*, *Rhodotorula*, *Candida*, *Pichia*, *Torulopsis*, *Torula*, *Endomyces*, *Kloeckera*, and *Hansenula*. The compound is also bactericidal at 30 to 400 mg/L to a number of species including *Acetobacter pasteurianus*, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, several *Lactobacillus* species, and *Pediococcus cerevisiae* (40). The compound has been shown to be bactericidal against *E. coli* O157:H7 in apple cider (41). Molds are generally more resistant to DMDC than yeasts or bacteria.

PHENOLIC ANTIOXIDANTS

Phenolic antioxidants including, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ), are used in foods primarily to delay autoxidation of unsaturated lipids. The first report on the antibacterial effectiveness of BHA was that of Chang and Branen (42) in which *E. coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* were inhibited in nutrient broth. Subsequent studies generally demonstrate that Gram-positive bacteria are more susceptible to BHA than Gram-negative bacteria. BHT is generally less effective than other phenolic antioxidants (43). TBHQ is an extremely effective inhibitor of Gram-positive bacteria including *S. aureus* and *Listeria monocytogenes* at concentrations generally less than 64 μg/mL (43).

BHA inhibits *Aspergillus flavus*, *A. parasiticus*, *Penicillium*, *Geotrichum*, *Byssochlamys*, and *Saccharomyces cerevisiae* in microbiological media (43). In addition to growth inhibition, BHA inhibits production of the mycotoxins (43).

A number of studies have been carried out to determine the antimicrobial effectiveness of phenolic antioxidants in foods (43). In nearly all studies, the concentration of phenolic antioxidants required for inhibition in a food, especially a meat product, is significantly higher than that needed for *in vitro* inhibition. This is probably because the presence of lipid or protein dramatically decreases the activity of phenolic antioxidants due to binding (44). Application studies in lower fat and protein products have shown more promise.

PHOSPHATES

Some phosphate compounds, including sodium acid pyrophosphate (SAPP), tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium tetrapolyphosphate, sodium hexametaphosphate (SHMP), and trisodium phosphate (TSP), have variable levels of antimicrobial activity in foods (45). Gram-positive bacteria are generally more susceptible to phosphates than Gram-negative bacteria. TSPP, SAPP, STPP, and SHMP have been shown to inhibit *Bacillus subtilis*, *Enterococcus faecalis*, *Clostridium sporogenes*, *C. bifermentans*, and *Staphylococcus aureus* (45,46). Sodium polyphosphates at 1% inhibited lag and generation times of *Listeria monocytogenes* in BHI broth, especially in the presence of NaCl (47). Wagner and Busta (48) found that SAPP has no effect on the growth of *C. botulinum* but delayed or prevented toxicity to mice. It was theorized that this was due to binding of the toxin molecule or inactivation of the protease responsible for protoxin activation.

Phosphate derivatives also have antimicrobial activity in food products (49). SAPP, SHMP, or polyphosphates enhance the effect of nitrite, pH, and salt against *C. botulinum* (45). Phosphates, sodium chloride, reduced water activity, water content, reduced pH, and lactic acid interact to prevent the outgrowth of *C. botulinum* in pasteurized process cheese (50). A 10% sodium tetrapolyphosphate dip preserved cherries against the fungal growth by *Penicillium*, *Rhizopus*, and *Botrytis* (45). Various phosphate salts have antimicrobial activity against rope-forming *Bacillus* in bread and *Salmonella* in pasteurized egg whites (51). Trisodium phosphate (TSP) at levels of 8 to 12% reduces pathogens, especially *Salmonella*, on poultry most likely due to a physical removal process or high pH (11–12) (52,53).

Several mechanisms have been suggested for bacterial inhibition by polyphosphates. The ability of polyphosphates to chelate metal ions, such as magnesium, appears to play an important role in their antimicrobial activity (45). Knabel et al. (54) stated that the chelating ability of polyphosphates is responsible for growth inhibition of *B. cereus*, *L. monocytogenes*, *S. aureus*, *Lactobacillus*, and *Aspergillus flavus*. In addition, inhibition is reduced at lower pH due to protonation of the chelating sites on the polyphosphates. It was concluded that polyphosphates inhibited Gram-positive bacteria and fungi by removal of essential cations from binding sites on the cell walls of these microorganisms (54).

NATURALLY OCCURRING COMPOUNDS AND SYSTEMS

Nisin

Nisin, a polypeptide produced by *Lactococcus lactis* spp. *lactis*, was isolated, characterized, and named by Mattick and Hirsh (55). The peptide has 34 amino acids and a molecular weight of 3,500 Da, however it usually occurs as a dimer. The solubility of the compound depends on the pH of the solution. At pH 2.2, the solubility of nisin is 56 mg/mL, at pH 5.0, 3 mg/mL, and it is less soluble at neutral and alkaline pH. Nisin solution in dilute HCl at pH 2.5 is stable to autoclaving (121°C) with no marked loss of antimicrobial activity. At pH 7.0, inactivation occurs even at room temperature. Nisin remains stable for years in the dry form, but activity is gradually lost in foods. The effectiveness of nisin increases as pH decreases. Nisinase from *Streptococcus thermophilus*, *Lactobacillus plantarum*, other lactic acid bacteria, and certain *Bacillus* species inactivate nisin (55). In addition, resistance to nisin may develop in cells exposed to the compound through alterations of the cell surface or cell membrane (18).

Nisin has a narrow spectrum affecting only Gram-positive bacteria, including lactic acid bacteria, streptococci, bacilli, and clostridia. By itself, it does not generally inhibit Gram-negative bacteria, yeasts, or molds. Nisin is inhibitory to the spore formers *Bacillus* and *Clostridium*, including *Clostridium botulinum*. Nisin concentrations necessary to inhibit *Clostridium botulinum* in brain heart infusion broth were 200, 80, and 20 μg/mL for types A, B, and E, respectively (56). In contrast, the concentration required to inhibit *C. botulinum* in cooked meat medium (CMM) was beyond the highest tested for types A (>200 μg/mL) and B (>80 μg/mL). It was theorized that the higher levels required in CMM were due to binding of the nisin by meat particles. Nisin reduces the heat resistance of spore formers. The sensitivity of vegetative bacteria to nisin varies. *Staphylococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus*, and *Listeria monocytogenes* have all been shown to be sensitive to nisin (57–60). Gram-negative bacteria are resistant to nisin activity, but they can be sensitized by disruption of the outer membrane either chemically with chelators, such as EDTA, or mechanically. This expands the spectrum of nisin to Gram-negative pathogens such as *Escherichia coli*, *Salmonella*, *Yersinia* (61).

The application of nisin as a food preservative has been studied extensively. Nisin-producing starter cultures were first used to prevent gas or "blowing" of Swiss-type cheese caused by *Clostridium tyrobutyricum* and *C. butyricum* (62). Nisin has been recommended for use in canned vegetable products to prevent the outgrowth of *Clostridium botulinum* when less severe sterilization conditions are desired or required (61). The compound has been shown to have potential benefit in some meat products, although, as already stated, binding to meat may be a problem. Nisin has been suggested as an adjunct to nitrite in cured meats to prevent the growth of clostridia (61). The compound has been tested as a preservative in seafood, dairy products, vegetables, soups, sauces, beer and ale (61). Nisin was less active against *L. monocytogenes* in milk and ice cream with increasing fat concentrations (63,64). This was probably due to binding of nisin to fat globules.

Nisin is permitted for use in foods in many countries including the U.S. It was approved by the U.S. FDA for use in pasteurized cheese spreads and pasteurized process cheese spread to inhibit the growth of *C. botulinum* at a maximum of 250 ppm in 1988.

Natamycin

Natamycin, a polyene macrolide antibiotic, was first isolated in 1955 from a culture of *Streptomyces natalensis*, a microorganism found in soil from Natal, South Africa. The name natamycin is synonymous with pimaricin, a name used in earlier literature. In the United States, natamycin was approved for use in cheese making as a mold spoilage inhibitor in 1982. Natamycin may be applied to the surface of cuts and slices of cheese by dipping or spraying an aqueous solution containing 200 to 300 ppm. Natamycin does not readily migrate into the cheese and does not adversely affect flavor or appearance.

Natamycin is active against nearly all molds and yeasts but has little or no effect on bacteria or viruses (65). Most molds are inhibited at concentrations of natamycin from 0.5 to 25 μg/mL. Most yeasts are inhibited at natamycin concentrations from 1.0 to 5.0 μg/mL. In addition to fungal growth inhibition, natamycin has been shown to inhibit aflatoxin B_1 production of *Aspergillus flavus* and penicillic acid production by *Penicillium cyclopium* as well as eliminate ochratoxin production by *A. ochraceus* and patulin production by *P. patulum* (66).

Several factors affect the stability and resulting antimycotic activity of natamycin. While pH has no apparent effect on antifungal activity, it does influence stability of the compound. In the pH range of most food products (pH 5–7), natamycin is very stable. Under normal storage conditions, temperature has little effect on natamycin activity when in neutral aqueous suspension. Sunlight, contact with certain oxidants (eg, organic peroxides and sulfhydryl groups), and heavy metals all adversely effect stability of natamycin solutions or suspensions (67).

Natamycin added in the wash water was effective in increasing the shelf life of cottage cheese up to 13.6 days at 4.4°C (68). Adding natamycin to the cottage cheese dressing was even more effective in extending shelf life. At 500 or 1000 μg/mL, natamycin delayed mold growth on cheese for up to six months but did not prevent it completely (69). Natamycin was shown to be effective in preserving Italian cheeses with no detrimental effect on ripening (70).

In addition to dairy products, early work on natamycin suggested it might be useful to inhibit fungal growth on fruit products and poultry. Natamycin was shown to be an effective antifungal agent on strawberries, raspberries, and cranberries and in orange juice (71,72). Natamycin, alone and in combination with chlortetracycline, inhibited yeast growth on chicken stored 12 to 15 days at 4.4°C (73).

Bacteriocins

It has long been known that lactic acid bacteria are antagonistic to other microorganisms through production of antimicrobial compounds such as hydrogen peroxide, acetic acid, lactic acid, and diacetyl. However, these and other food fermentation microorganisms also produce peptide antimicrobial compounds known as bacteriocins. Bacteriocins are bactericidal to susceptible genera or species and are often produced under plasmid control. These compounds are not considered to be antibiotics as they are not used for human disease control, are larger than medical antibiotics, and usually have a narrow spectrum. Potentially useful bacteriocins have been demonstrated from *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Propionibacterium*, *Bifidobacterium*, and *Carnobacterium*. Although none of the compounds are approved for use in foods, there is great potential for their use in the future. Most of the bacteria that produce bacteriocins being considered as food antimicrobials are used or associated with fermented dairy, meat, and vegetables products. For that reason, the antimicrobials they produce would be assumed to be safe from a toxicological standpoint. Many bacteriocins have been isolated and their effectiveness characterized in microbiological media, but much work remains to be done on determining their effectiveness in foods.

Excluding nisin, the most well characterized bacteriocins are those produced by *Pediococcus* species. *Pediococcus acidilactici* produces pediocin AcH and PA-1, and *P. pentosaceus* produces pediocin A. Pediocin AcH and PA-1 inhibit *Bacillus*, *Brochothrix*, *Clostridium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Listeria monocytogenes*, other *Pediococcus*, *Propionibacterium*, and *Staphylococcus aureus*. Pediocin A is inhibitory to other lactic acid bacteria and the pathogens *Bacillus cereus*, *Clostridium botulinum*, *C. perfringens*, *L. monocytogenes*, and *S. aureus*. Gram-negative bacteria can be made to be susceptible to pediocins following exposure to sublethal stress treatments such as freezing or heat (61). In foods, pediocin AcH inhibits spoilage bacteria in meats, dairy products, salads, and salad dressings, and it has a variable effect on growth of pathogens inoculated into foods (61). Limitations on the use of pediocins in foods include: (1) it may cause resistance development in some microorganisms; (2) it may select for resistant strains in foods; and (3) food components, especially lipids, could interfere with antimicrobial activity (61).

Several species of *Lactobacillus* have been shown to elucidate bacteriocins. These include *L. acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii* ssp. *bulgaricus*, *L. fermentum*, *L. helveticus*, *L. plantarum*, and *L. sake* (18,61). Bacteriocins produced by *Leuconostoc* include those produced by *L. carnosum*, *L. dextranicum*, *L. gelidum*, *L. mesenteroides*, and *L. paramesenteroides*. Various of these bacteriocins have shown activity against *C. botulinum*, *L. monocytogenes*, *S. aureus*, and *Yersinia enterocolitica* (61). *Carnobacterium piscicola* and *Bifidobacterium* have shown to produce bacteriocins with activity primarily against gram positive bacteria including *L. monocytogenes*. Milk fermented by *Propionibacterium freudenreichii* ssp. *shermanii* is effective against Gram-negative psychrotrophic bacteria in cottage cheese and spoilage microorganisms in a variety of other foods. Among the inhibitors in this product are propionic acid, lactic acid, and a low-molecular-weight proteinaceous compound (74).

Lysozyme

Lysozyme (1,4-β-*N*-acetylmuramidase) is a 14,600 Da enzyme present in avian eggs, mammalian milk, tears and other secretions, insects, and fish. It is most stable under acidic conditions. The enzyme catalyzes hydrolysis of the β-1,4 glycosidic bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan of bacterial cell walls. This causes cell wall degradation and eventual lysis.

Lysozyme is most active against Gram-positive bacteria, most likely because of the exposed peptidoglycan in the cell wall. The enzyme inhibits *Bacillus stearothermophilus*, *B. cereus*, *Clostridium botulinum*, *C. thermosaccharolyticum*, *C. tyrobutyricum*, *Listeria monocytogenes*, and *Micrococcus lysodeikticus* (18). Variation in susceptibility of Gram-positive bacteria is likely due to the presence of teichoic acids or other materials that bind the enzyme and that certain species have greater proportions of 1,6 or 1,3 glycosidic linkages in the peptidoglycan that are more resistant than the 1,4 linkage (75). For example, certain strains of *L. monocytogenes* are not inhibited by lysozyme unless EDTA is present (76). Lysozyme is less effective against Gram-negative bacteria due to a reduced peptidoglycan content and presence of outer membrane of lipopolysaccharide and lipoprotein. Gram-negative cell susceptibility can be increased by pretreatment with chelators (eg, EDTA) or if cells are subjected to shock (pH, heat, osmotic), drying or freeze–thaw cycling (75).

The most common use of lysozyme in foods is in hard cheeses such as Edam and Gouda to prevent a defect known as late blowing. This defect is characterized by formation of holes in the cheese and the production of unacceptable flavors caused by *C. tyrobutyricum*. In foods other than cheese, lysozyme has been evaluated as an antimicrobial in seafoods, sake, potato salad, sushi, Chinese noodles, creamed custard, and sausage. Lysozyme and lysozyme combined with EDTA may have potential for reducing *Salmonella* on poultry and spoilage microflora from shrimp. Lysozyme was bactericidal to *L. monocytogenes* in shredded cabbage and lettuce, fresh green beans, corn, and carrots, and this activity was enhanced by EDTA (76). The compound was less effective in refrigerated meats and soft cheese. In some studies, no inhibition was demonstrated with EDTA and lysozyme against *Salmonella typhimurium* or *Pseudomonas fluorescens* (77).

Lysozyme is one of the few naturally occurring antimicrobials approved by regulatory agencies for use in foods. In the United States and Europe, lysozyme is allowed to prevent gas formation in cheeses such as Edam and Gouda by *C. tyrobutyricum*. Lysozyme is used to a great extent in Japan to preserve seafood, vegetables, pasta, and salads.

Lactoperoxidase System

Lactoperoxidase (MW = 78,000 Da) is an enzyme that occurs in raw milk, colostrum, saliva and other biological secretions. Bovine milk naturally contains 10 to 60 mg/L of lactoperoxidase (78). This enzyme reacts with thiocyanate (SCN^-) in the presence of hydrogen peroxide and forms antimicrobial compound(s). The components are called the lactoperoxidase system (LPS). Fresh milk contains 1 to 10 mg/L of thiocyanate, which is not always sufficient to activate the LPS (79). Hydrogen peroxide, the third component of the LPS, is not present in fresh milk due to the action of natural catalase, peroxidase, or superoxide dismutase. Approximately 8 to 10 mg/L hydrogen peroxide are required to activate the LPS, which can be added directly, through the action of lactic acid bacteria or through the enzymatic action of xanthine oxidase, glucose oxidase, or sulfhydryl oxidase. In LPS reaction, thiocyanate is oxidized to the antimicrobial hypothiocyanate ($OSCN^-$), which also exists in equilibrium with hypothiocyanous acid (78).

The LPS is more effective against active against Gram-negative bacteria, including *Pseudomonas*, than Gram-positive bacteria. However, it does inhibit both Gram-positive and Gram-negative foodborne pathogens, including *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Campylobacter jejuni* (78). There is variable activity against catalase-negative microorganisms, including the lactic acid bacteria such as *Lactococcus*, *Lactobacillus*, and *Streptococcus*.

The LPS system can increase the shelf life of raw milk. This could be useful in countries that have poorly developed refrigerated storage systems (78). LPS has also been used as a preservation process in cream, cheese, liquid whole eggs, ice cream, and infant formula.

Lactoferrin and Other Iron-Binding Proteins

Lactoferrin is an iron-binding protein that occurs in milk and colostrum and has potential for use as a food antimicrobial (78). Lactoferrin is a glycoprotein with a molecular weight of around 76,500 Da. Lactoferrin has two iron-binding sites per molecule and, for each Fe^{3+} bound, requires one bicarbonate (HCO_3^-). Lactoferrin is potentially active in milk due to the low iron concentration and presence of bicarbonate (79). The exact biological role of lactoferrin is unknown; however, it may act as a barrier to infection of the nonlactating mammary gland and protect the gastrointestinal tract of the newborn against infection (78).

Lactoferrin is inhibitory to a number of microorganisms including *Bacillus subtilis*, *B. stearothermophilus*, *Listeria monocytogenes*, *Micrococcus*, *E. coli*, and *Klebsiella* (18). There is evidence that the most effective form of lactoferrin is the iron-unsaturated, or apolactoferrin, form (80). Some Gram-negative bacteria may be resistant because they adapt to low-iron environments by producing siderophores such as phenolates and hydroxamates. Microorganisms with a low iron requirement, such as lactic acid bacteria, would not be inhibited by lactoferrin (78).

Lactoferricin B is a small peptide (25 amino acids) produced by acid-pepsin hydrolysis of bovine lactoferrin (81). The compound is inhibitory to *Shigella*, *Salmonella*, *Yersinia enterocolitica*, *E. coli* O157:H7, *Staphylococcus aureus*, *L. monocytogenes*, and *Candida* species at concentrations ranging from 1.9 to 125 mg/mL (82). Another iron-binding molecule, ovotransferrin or conalbumin, occurs in egg albumen. Each ovotransferrin molecule has two iron-binding sites and, like lactoferrin, it binds an anions, such as bicarbonate or carbonate with each ferric iron bound. Ovotransferrin is inhibitory against both Gram-positive and Gram-negative bacteria, but the former are generally more sensitive, with *Bacillus* and *Micrococcus* species being particularly sensitive (75). Some yeasts are also sensitive.

Avidin

Avidin is a glycoprotein present in egg albumen. The concentration varies with hen's age, but the mean is 0.05% of the total egg albumen protein (75). The protein is 66,000 to 69,000 Da and has four identical subunits of 128 amino acids each. Avidin strongly binds the cofactor biotin at a ratio of four molecules of biotin per molecule of avidin. Biotin is a cofactor for enzymes in the tricarboxylic acid cycle and fatty acid biosynthesis. Avidin inhibits growth of some bacteria and yeasts that have a requirement for biotin with the primary mechanism being nutrient deprivation.

Spices and Their Essential Oils

Spices are roots, bark, seeds, buds, leaves, or fruit of aromatic plants added to foods as flavoring agents. It has been known since ancient times that spices and their essential oils have varying degrees of antimicrobial activity. Cloves, cinnamon, oregano, thyme, and, to a lesser extent, sage and rosemary have the strongest antimicrobial activity among spices.

The major antimicrobial components of clove and cinnamon are eugenol (2-methoxy-4-(2-propenyl)-phenol) and cinnamic aldehyde (3-phenyl-2-propenal), respectively. Cinnamon and clove extracts or their essential oils inhibit *Aeromonas hydrophila*, *Bacillus*, *Enterobacter aerogenes*, lactic acid bacteria, and *Staphylococcus aureus* in microbiological systems and in foods (8). Bullerman (83) observed that cinnamon in raisin bread inhibited growth and aflatoxin production by *Aspergillus parasiticus*. Cinnamon and clove were the most effective of 16 ground herbs and spices tested at 2% w/v against nine mycotoxin-producing *Aspergillus* and *Penicillium* species (84).

The antimicrobial activity of oregano and thyme has been attributed to their essential oils which contain the terpenes carvacrol (2-methyl-5-(1-methylethyl)phenol) and thymol (5-methyl-2-(1-methylethyl)phenol), respectively. These compounds have inhibitory activity against a number of bacterial species, molds, and yeasts including *Bacillus subtilis*, *E. coli*, *Lactobacillus plantarum*, *Pediococcus cerevisiae*, *Pseudomonas aeruginosa*, *Proteus*, *Salmonella enteritidis*, *S. aureus*, *Vibrio parahaemolyticus*, and *Aspergillus parasiticus* (8)

The active fraction of sage and rosemary has been suggested to be the terpene fractions of the essential oils. Rosemary contains primarily borneol (endo-1,7,7-trimethylbicyclo[2.2.1] heptan-2-ol) along with pinene, camphene, camphor while sage contains thujone (4-methyl-1-(1-methylethyl)bicyclo[3.1.0]-hexan-3-one). Sage and rosemary are more active against gram-positive than gram-negative bacterial strains (85). Sensitivity of *Bacillus cereus*, *S. aureus*, and *Pseudomonas* to sage is greatest in microbiological medium and significantly reduced in foods (86). It is theorized that loss of activity was due to solubilization of the antimicrobial fraction in the lipid of the foods.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a major constituent of vanilla beans, the fruit of an orchid (*Vanilla planifola*, *Vanilla pompona*, or *Vanilla tahitensis*). Vanillin is most active as against molds and nonlactic Gram-positive bacteria (87). Vanillin at 1500 μg/mL significantly inhibited strains of *Aspergillus* in fruit-based agars containing mango, papaya, pineapple, apple, and banana (88). The compound was least effective in banana and mango agars. This was attributed to binding of the vanillin by protein or lipid in these fruits, a phenomenon demonstrated for other antimicrobial phenolic compounds (44).

Many other spices have been tested and shown to have limited or no activity. They include allspice, anise, bay leaf, black pepper, cardamom, celery seed, chili powder, coriander, cumin, curry powder, dill, fenugreek, ginger, juniper oil, mace, marjoram, mustard, nutmeg, orris root, paprika, parsley, red pepper, sesame, spearmint, tarragon, and white pepper (89).

ONIONS AND GARLIC

Probably the most well characterized antimicrobial system in plants is that found in the juice and vapors of onions (*Allium cepa*) and garlic (*Allium sativum*). Growth and toxin production of many microorganisms have been shown to be inhibited by onion and garlic, including the bacteria *Bacillus cereus*, *Clostridium botulinum* type A, *E. coli*, *Lactobacillus plantarum*, *Salmonella*, *Shigella*, and *Staphylococcus aureus*, and the fungi *Aspergillus flavus*, *A. parasiticus*, *Candida albicans*, *Cryptococcus*, *Rhodotorula*, *Saccharomyces*, *Torulopsis*, and *Trichosporon* (18). The major antimicrobial component from garlic is allicin (diallyl thiosulfinate; thio-2-propene-1-sulfinic acid-5-allyl ester) (18), which is formed by the action of the enzyme allinase on the substrate alliin (*S*-(2-propenyl)-L-cysteine sulfoxide). The reaction only occurs when cells of the garlic are disrupted, releasing the enzyme to act on the substrate. A similar reaction occurs in onion except the substrate is *S*-(1-propenyl)-L-cysteine sulfoxide and one of the major products is thiopropanal-*S*-oxide. The products apparently responsible for antimicrobial activity are also responsible for the flavor of onions and garlic. In addition to antimicrobial sulfur compounds, onions contain the phenolic compounds protocatechuic acid and catechol, which could contribute to their antimicrobial activity (90). The mechanism of action of allicin is most likely inhibition of sulfhydryl-containing enzymes (18).

Other Plant Extracts

Isothiocyanates (R-N=C=S) are derivatives from glucosinolates in cells of plants of the Cruciferae or mustard family (cabbage, kohlrabi, Brussel sprouts, cauliflower, broccoli, kale, horseradish, mustard, turnips, rutabaga). They are potent antifungal and antimicrobial agents (91). These compounds are formed from the action of the enzyme myrosinase (thioglucoside glucohydrolase) on the glucosinolates when the plant tissue is injured or mechanically disrupted. Common isothiocyanate side groups include allyl (AIT), ethyl, methyl, benzyl, and phenyl. The compounds are inhibitory to fungi, yeasts, and bacteria in the range of 0.016 to 0.062 μg/mL in the vapor phase (92). Inhibition depends on the compound type against bacteria but generally Gram-positive bacteria are less sensitive to AIT than are Gram-negative bacteria. The mechanism by which isothiocyanates inhibit cells may involve enzymes by direct reaction with disulfide bonds or through thiocyanate (SCN^-) anion reaction to inactivate sulfhydryl enzymes (91). These compounds have very low sensory thresholds, but they may be useful as food antimicrobials due to their low inhibitory concentrations.

Phenolic Compounds

Phenolic compounds occurring in foods are classified as simple phenols and phenolic acids, hydroxycinnamic acid derivatives, and the flavonoids (93). The various classes of phenolics and individual compounds within each class have various antimicrobial activities.

Smoking of foods, such as meats, cheeses, fish, and poultry, has a preservative effect through drying and the deposition of chemicals. The major antimicrobial components of wood smoke are phenol and cresol. Smoke distillates and liquid smoke are inhibitory to *Staphylococcus aureus*, *E. coli*, *Listeria monocytogenes*, and *Saccharomyces cerevisiae* (43). Consistent with results using other phenolic compounds as antimicrobials, smoke is more effective against Gram-positive than Gram-negative bacteria. Isoeugenol is one of the most effective antimicrobial compounds in liquid smoke, followed by *m*-cresol and *p*-cresol (94).

The phenolic acids, including derivatives of *p*-hydroxybenzoic acid (protocatechuic, vanillic, gallic, syringic, ellagic) and *o*-hydroxybenzoic acid (salicylic) may be found in plants and foods. Esters of gallic acid including butyl, isobutyl, isoamyl, *p*-octyl, and *p*-dodecyl have been shown to be effective against *Clostridium botulinum* in microbiological media at 37°C (95). Another ester of gallic acid, propyl gallate, inhibited growth of *Alcaligenes faecalis*, *Enterobacter aerogenes*, *E. coli*, *Klebsiella pneumoniae*, *L. monocytogenes*, *Proteus vulgaris*, *Salmonella enteritidis*, *Salmonella paratyphi*, and *Shigella flexneri* (96). Tannic acid has antimicrobial activity against *Aeromonas hydrophila*, *E. coli*, *L. monocytogenes*, *S. enteritidis*, *S. aureus*, and *Streptococcus faecalis* (43). Stead (97) demonstrated stimulation of growth of the lactic acid spoilage bacteria, *Lactobacillus collinoides* and *Lactobacillus brevis*, by gallic, quinic, and chlorogenic acids. It was suggested that the compounds were being metabolized by the microorganisms and that presence of these compounds in beverages may potentially result in antagonistic reactions with other food-grade antimicrobials.

Resin from the flowers of the hop vine (*Humulus lupulus* L.) used in the brewing industry for imparting a desirable bitter flavor to beer contain α-bitter acids (humulone, cohumulone, adhumulone) and β-bitter acids (lupulone, colupulone, xanthohumol, adlupulone). Both types of acids possess antimicrobial activity primarily against Gram-positive bacteria and fungi at low water activity (98). A compound isolated from green olives and identified as the phenolic glycoside oleuropein, or its aglycone, inhibits *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Rhizopus* sp., and *Geotrichum candidum* (43).

Hydroxycinnamic acids, including caffeic, *p*-coumaric, ferulic, and sinapic acids, are found in plants and occur as esters and less often as glucosides (93). Hydroxycinnamic acids have been shown to inhibit bacteria, including *B. cereus*, *Erwinia carotovora*, *Lactobacillus collinoides*, *Lactobacillus brevis*, *S. aureus*, and fungi, including *Aspergillus flavus*, *A. parasiticus*, and *Saccharomyces cerevisiae* (43). Cinnamic acid was found to be inhibitory to *L. monocytogenes* Scott A growth and listeriolysin O activity at pH 5.5 (99). The degree of inhibition is generally inversely related to the polarity of the compounds.

The furocoumarins are related to the hydroxycinnamates. These compounds, including psoralen and its derivatives, are phytoalexins in citrus fruits, parsley, carrots, celery, and parsnips at levels of 2–6 μg/mL. They inhibit many Gram-positive bacteria including, *L. monocytogenes*, *E. coli* O157:H7 and *Micrococcus luteus*, following irradiation with long wave ultraviolet light (100). These compounds inhibit growth by interfering with DNA replication.

The flavonoids consist of catechins, proanthocyanidins, anthocyanidins and flavons, flavonols, and their glycosides (93). Proanthocyanidins or condensed tannins are polymers of favan-3-ol and are found in apples, grapes, strawberries, plums, sorghum, and barley (93). The tannins have been tested for their antimicrobial effectiveness against molds, yeasts, bacteria, and viruses. For example, proanthocyanidins and flavonols accounted for the majority of inhibition of *Saccharomyces bayanus* by cranberries (101). Tannins have been reported to be inhibitory to *Aeromonas*, *Bacillus*, *C. botulinum*, *C. perfringens*, *Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Shigella*, *S. aureus*, *Streptococcus*, and *Vibrio* (102). Extracts of blueberries, crabapples, strawberries, red wines, grape juice, apple juice, and tea inactivated poliovirus, coxsackievirus, echovirus, reovirus, and herpes simplex virus (103,104). It was concluded that the primary viral inhibitors in these products are condensed or hydrolyzable tannins.

Glucose Oxidase

Glucose oxidase (β-D-glucose:oxidoreductase) catalyzes the oxidation of glucose to gluconic acid with the production of hydrogen peroxide (89,105). Glucose oxidase acting on glucose in honey produces hydrogen peroxide as an inhibitory agent. Bacteria inhibited by glucose oxidase include *Acinetobacter*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Salmonella*, *Staphylococcus aureus*, and *Yersinia* (106,107). In most cases, it is hydrogen peroxide produced by the enzyme that is responsible for inhibition of these microorganisms. Glucose oxidase in the presence of added glucose was bactericidal against *Salmonella enteritidis*, *Micrococcus luteus*, and *B. cereus* and bacteriostatic against *P. fluorescens* in liquid whole egg stored at 7°C (105). In contrast, glucose oxidase had no effect on *Salmonella* or *Pseudomonas* on chicken breast meat (108).

Utilization of Naturally Occurring Antimicrobials in Foods

With the exception of nisin, natamycin, and lysozyme, no isolated naturally occurring antimicrobials are approved for use in foods by regulatory agencies. For acceptance by regulatory agencies, more information will be needed concerning the antimicrobial activity of the compounds in foods, their stability, and their toxicological effects on humans. In addition, prior to acceptance by the food industry, they must be shown to have no adverse effects on sensory qualities of foods and be cost-effective. These are huge barriers to the use of isolated compounds. It is more likely that the future of naturally occurring antimicrobials is in their use as crude or partially purified extracts of their component source.

BIBLIOGRAPHY

1. G. W. Gould, ed., *Mechanisms of Action of Food Preservation Procedures*, Elsevier Applied Science, London, 1989.
2. T. Eklund, "Organic Acids and Esters," in G. W. Gould, ed., *Mechanisms of Action of Food Preservation Procedures*, Elsevier Applied Science, London, 1989, pp. 161–200.
3. J. N. Sofos, "Antimicrobial Effects of Sodium and Other Ions in Foods," *J. Food Safety* **6**, 45–78 (1983).
4. J. E. L. Corry, "Relationships of Water Activity to Fungal Growth," in L. R. Beuchat, ed., *Food and Beverage Mycology*, 2nd ed., AVI/Van Nostrand Reinhold, New York, 1987, pp. 51–99.
5. W. H. Sperber, "Influence of Water Activity on Foodborne Bacteria—A Review," *J. Food Prot.* **46**, 142–150 (1983).
6. G. J. Banwart, *Basic Food Microbiology*, AVI Publishing, Westport, Conn., 1979.
7. S. Doores, "Organic Acids," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp 95–136.
8. P. M. Davidson, "Chemical Preservatives and Natural Antimicrobial Compounds," in M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds., *Food Microbiology: Fundamentals and Frontiers*, American Society for Microbiology, Washington, D.C., 1997, pp. 520–556.
9. D. Hunter and I. H. Segel, "Effect of Weak Acids on Amino Acid Transport by *Penicillium chrysogenum*. Evidence for a Proton or Charge Gradient as the Driving Force," *J. Bacteriol.* **113**, 1184–1192 (1973).
10. M. Ingram, F. J. H. Ottoway, and J. B. M. Coppock, "The Preservative Action of Acid Substances in Food," *Chem. Ind. (London)* **42**, 1154–1163 (1956).
11. A. C. Baird-Parker, "Organic Acids," International Commission on Microbiological Specifications for Foods, ed., *Microbial Ecology of Foods, Vol. I. Factors Affecting Life and Death of Microorganisms*, Academic Press, New York, 1980, pp. 135–141.
12. G. J. Mountney and J. O'Malley, "Acids as Poultry Meat Preservatives," *Poultry Sci.* **44**, 582 (1985).
13. A. J. Okrend, R. W. Johnston, and A. B. Moran, "Effect of Acetic Acid on the Death Rates at 52°C of *Salmonella newport*, *Salmonella typhimurium*, and *Campylobacter jejuni* in Poultry Scald Water," *J. Food Prot.* **49**, 500–503 (1986).
14. M. E. Anderson et al., "Counts of Six Types of Bacteria on Lamb Carcasses Dipped or Sprayed with Acetic Acid at 25°C or 55°C and Stored Vacuum Packaged at 0°C," *J. Food Prot.* **51**, 874–877 (1988).
15. G. R. Siragusa and J. S. Dickson, "Inhibition of *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7 on Beef Muscle Tissue by Lactic or Acetic Acid Contained in Calcium Alginate Gels," *J. Food Safety* **13**, 147–158 (1993).
16. L. A. Shelef, and L. Addala, "Inhibition of *Listeria monocytogenes* and Other Bacteria by Sodium Diacetate," *J. Food Safety* **14**, 103–115 (1994).
17. J. R. Chipley, "Sodium Benzoate and Benzoic Acid," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 11–48
18. J. N. Sofos et al., *Naturally Occurring Antimicrobials in Food*, Task Force Report No. 132, Council for Agricultural Science and Technology, Ames, Iowa, 1998.
19. T. Zhao, M. P. Doyle, and R. E. Besser, "Fate of Enterohemorrhagic *Escherichia coli* O157:H7 in Apple Cider With and Without Preservatives," *Appl. Env. Microbiol.* **59**, 2526–2530 (1993).
20. T. E. Minor and E. H. Marth, "Growth of *Staphylococcus aureus* in Acidified Pasteurized Milk," *J. Milk Food Technol.* **33**, 516–520 (1970).
21. R. E. Brackett, "Effect of Various Acids on Growth and Survival of *Yersinia enterocolitica*," *J. Food Prot.* **50**, 598–601 (1987).
22. N. Chen and L. A. Shelef, "Relationship Between Water Activity, Salts of Lactic Acid, and Growth of *Listeria monocytogenes* in a Meat Model System," *J. Food Prot.* **55**, 574–578 (1992).
23. G. A. Pelroy et al., "Inhibition of *Listeria monocytogenes* in Cold-Process (Smoked) Salmon by Sodium Lactate," *J. Food Prot.* **57**, 108–113 (1994).
24. J. N. Sofos and F. F. Busta, "Sorbic Acid and Sorbates," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 49–94.
25. M. C. Robach, "Use of preservatives to control microorganisms in food," *Food Technol.* **34**, 81–84 (1980).
26. R. C. McKellar, "Effect of Preservatives and Growth Factors on Secretion of Listeriolysin O by *Listeria monocytogenes*," *J. Food Prot.* **56**, 380–384 (1993).
27. A. L. Branen and T. W. Keenan, "Growth Stimulation of *Lactobacillus casei* by Sodium Citrate," *J. Dairy Sci.* **53**, 593 (1970).
28. R. L. Buchanan and M. H. Golden, "Interaction of Citric Acid Concentration and pH on the Kinetics of *Listeria monocytogenes* Inactivation," *J. Food Prot.* **57**, 567–570 (1994).
29. J. J. Kabara, "Medium-Chain Fatty Acids and Esters," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 307–342.
30. J. J. Kabara and T. Eklund, "Organic Acids and Esters," in N. J. Russell and G. W. Gould, eds., *Food Preservatives*, Blackie, Glasgow, United Kingdom, 1991, pp. 44–71.
31. S. M. Razavi-Rohani and M. W. Griffiths, "The Effect of Mono and Polyglycerol Laurate on Spoilage and Pathogenic Bacteria Associated With Foods," *J. Food Safety* **14**, 131–151 (1994).
32. D. H. Oh and D. L. Marshall, "Enhanced Inhibition of *Listeria monocytogenes* by Glycerol Monolaurate With Organic Acids," *J. Food Sci.* **59**, 1258–1261 (1994).
33. L.-L. Wang and E. A. Johnson, "Control of *Listeria monocytogenes* by Monoglycerides in Foods," *J. Food Prot.* **60**, 131–138 (1997).
34. L. R. Beuchat, "Comparison of Anti-Vibrio Activities of Potassium Sorbate, Sodium Benzoate and Glycerol and Sucrose Esters of Fatty Acids," *Appl. Environ. Microbiol.* **39**, 1178–1182 (1980).
35. R. B. Tompkin, "Nitrite," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 191–262.
36. T. R. Aalto, M. C. Firman, and N. E. Rigier, "P-hydroxybenzoic Acid Esters as Preservatives. I. Uses, Antibacterial and

Antifungal Studies, Properties and Determination," *J. Am. Pharm. Assoc. (Sci. Ed.)* **42**, 449–458 (1953).

37. C. S. Ough, "Sulfur Dioxide and Sulfites," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 137–190.
38. J. O. Roland et al., "Effects of Sorbate, Benzoate, Sulfur Dioxide and Temperature on Growth and Patulin Production by *Byssochlamys nivea* in Grape Juice," *J. Food Prot.* **47**, 237–241 (1984).
39. J. G. Banks and R. G. Board, "Sulfite-inhibition of *Enterobacteriaceae* Including *Salmonella* in British Fresh Sausage and in Culture Systems," *J. Food Prot.* **45**, 1292–1297 (1982).
40. C. S. Ough, "Dimethyl Dicarbonate and Diethyl Dicarbonate," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 343–368.
41. T. L. Fisher and D. A. Golden, "Survival of *Escherichia coli* O157:H7 in Apple Cider as Affected by Dimethyl Dicarbonate, Sodium Bisulfite, and Sodium Benzoate," *J. Food Sci.* **63**, 904–906 (1998).
42. H. C. Chang and A. L. Branen, "Antimicrobial Effects of Butylated Hydroxyanisole (BHA)," *J. Food Sci.* **40**, 349–351 (1975).
43. P. M. Davidson, "Parabens and Phenolic Compounds," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 263–306.
44. E. Rico-Muñoz and P. M. Davidson, "The Effect of Corn Oil and Casein on the Antimicrobial Activity of Phenolic Antioxidants," *J. Food Sci.* **48**, 1284–1288 (1983).
45. L. A. Shelef and J. A. Seiter, "Indirect Antimicrobials," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 325–328.
46. F. Kelch, "Effect of Commercial Phosphates on the Growth of Microorganisms," *Fleischwirtschaft* **10**, 325–328 (1958).
47. L. L. Zaika and A. H. Kim, "Effect of Sodium Polyphosphates on Growth of *Listeria monocytogenes*," *J. Food Prot.* **56**, 577–580 (1993).
48. M. K. Wagner and F. F. Busta, "Inhibition of *Clostridium botulinum* 52a Toxicity and Protease Activity by Sodium Acid Pyrophosphate in Media Systems," *Appl. Environ. Microbiol.* **50**, 16–20 (1985).
49. M. K. Wagner, "Phosphates as Antibotulinal Agents in Cured Meats. A Review," *J. Food Prot.* **49**, 482–487 (1986).
50. N. Tanaka, "Challenge of Pasteurized Process Cheese Spreads with *Clostridium botulinum* Using In-process and Post-process Inoculation," *J. Food Prot.* **45**, 1044–1050 (1982).
51. R. B. Tompkin, "Indirect Antimicrobial Effects in Foods: Phosphates," *J. Food Safety* **6**, 13–27 (1983)
52. J. Giese, "Experimental Process Reduces *Salmonella* on Poultry," *Food Technol.* **46**, 112 (1992).
53. H. S. Lillard, "Effect of Trisodium Phosphate on Salmonellae Attached to Chicken Skin," *J Food Prot.* **57**, 465–469 (1994).
54. S. J. Knabel, H. W. Walker, and P. A. Hartman, "Inhibition of *Aspergillus flavus* and Selected Gram-positive Bacteria by Chelation of Essential Metal Cations by Polyphosphates," *J. Food Prot.* **54**, 360–365 (1991).
55. A. Hurst and D. G. Hoover, "Nisin," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 369–394.
56. V. N. Scott and S. L. Taylor, "Effect of Nisin on the Outgrowth of *Clostridium botulinum* Spores," *J. Food Sci.* **46**, 117–120 (1981).
57. N. Benkerroum and W. E. Sandine, "Inhibitory Action of Nisin Against *Listeria monocytogenes*," *J. Dairy Sci.* **71**, 3237–3245 (1988).
58. A. Laukova, "Inhibition of Ruminal *Staphylococci* and *Enterococci* by Nisin *in Vitro*," *Lett. Appl. Microbiol.* **20**, 34–36 (1995).
59. F. Radler, "Possible Use of Nisin in Winemaking I. Action of Nisin Against Lactic Acid Bacteria and Wine Yeasts in Solid and Liquid Media," *Am. J. Enol. Vitic.* **41**, 1–6 (1990).
60. D. O. Ukuku and L. A. Shelef, "Sensitivity of Six Strains of *Listeria monocytogenes* to Nisin," *J. Food Prot.* **60**, 867–869 (1997).
61. B. Ray and M. Daeschel, *Food Biopreservatives of Microbial Origin*, CRC Press, Boca Raton, Fla., 1992.
62. A. Hirsch et al., "A Note on the Inhibition of an Anaerobic Sporeformer in Swiss-type Cheese by a Nisin-producing *Streptococcus*," *J. Dairy Res.* **18**, 205–206 (1951).
63. D. Jung, F. W. Bodyfelt, and M. A. Daeschel, "Influence of Fat and Emulsifiers on the Efficacy of Nisin in Inhibiting *Listeria monocytogenes* in Fluid Milk," *J. Dairy Sci.* **75**, 387–393 (1992).
64. J. P. Dean and E. A. Zottola, "Use of Nisin in Ice Cream and Effect on the Survival of *Listeria monocytogenes*," *J. Food Prot.* **59**, 476–480 (1996).
65. P. M. Davidson and C. H. Doan, "Natamycin," P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 395–408.
66. L. L. Ray and L. B. Bullerman. "Preventing Growth of Potentially Toxic Molds Using Antifungal Agents," *J. Food Prot.* **45**, 953–963 (1982).
67. H. Brik, "Natamycin," in K. Flory, ed., *Analytical Profiles of Drug Substances*, Academic Press, New York, 1981, pp. 513.
68. K. M. Nilson et al., "Pimaricin and Mycostatin for Retarding Cottage Cheese Spoilage," *J. Dairy Sci.* **58**, 668–671 (1975).
69. H. Lück and C. E. Cheeseman, "Mould Growth on Cheese as Influenced by Pimaricin or Sorbate Treatments," *S. Afr. J. Dairy Technol.* **10**, 143–146 (1978).
70. R. Lodi, R. Todesco, and V. Bozzetti, "New Applications of Natamycin with Different Types of Italian Cheese," *Microbiol. Aliments Nutr.* **7**, 81 (1989).
71. J. C. Ayres and E. L. Denisen. "Maintaining Freshness of Berries Using Selected Packaging Materials and Antifungal Agents," *Food Technol.* **12**, 562–567 (1958).
72. R. J. Shirk and W. L. Clark, "The Effect of Pimaricin in Retarding the Spoilage of Fresh Orange Juice," *Food Technol.* **17**, 1062–1066 (1963).
73. J. C. Ayres et al., "Use of Antibiotics in Prolonging Storage Life of Dressed Chicken," *Food Technol.* **10**, 563–568 (1956).
74. D. G. Hoover, "Bacteriocins with Potential for Use in Foods," in P. M. Davidson and A. L. Branen, eds., *Antimicrobials in Foods*, 2nd ed., Marcel Dekker, New York, 1993, pp. 409–440.
75. H. S. Tranter, "Lysozyme, Ovotransferrin and Avidin," in V. M. Dillon and R. G. Board, eds., *Natural Antimicrobial Systems and Food Preservation*, CAB International Wallingford, United Kingdom, 1994, pp. 65–98.
76. V. L. Hughey, R. A. Wilger, and E. A. Johnson, "Antibacterial Activity of Hen Egg White Lysozyme Against *Listeria monocytogenes* Scott A in Foods," *Appl. Env. Microbiol.* **55**, 631–638 (1989).
77. K. D. Payne, P. M. Davidson, and S. P. Oliver, "Comparison of EDTA and Apo-lactoferrin With Lysozyme on the Growth of Foodborne Pathogenic and Spoilage Bacteria," *J. Food Prot.* **57**, 62–65 (1994).

78. B. Ekstrand, "Lactoperoxidase and Lactoferrin," in V. M. Dillon and R. G. Board eds., *Natural Antimicrobial Systems and Food Preservation*, CAB International, Wallingford, United Kingdom, 1994, pp. 15–64.

79. K. M. Wilkins and R. G. Board, "Natural Antimicrobial Systems," in G. W. Gould, ed., *Mechanisms of Action of Food Preservation Procedures*, Elsevier Applied Science, London, 1989, pp. 285–362

80. K. D. Payne et al., "Influence of Bovine Lactoferrin on the Growth of *Listeria monocytogenes*," *J. Food Prot.* **53**, 468–472 (1990).

81. W. Bellamy et al., "Antibacterial Spectrum of Lactoferricin B, a Potent Bactericidal Peptide Derived from the N-terminal Region of Bovine Lactoferrin," *J. Appl. Bacteriol.* **73**, 472–479 (1992).

82. E. M. Jones et al., "Lactoferricin, a New Antimicrobial Peptide," *J. Appl. Bacteriol.* **77**, 208–214 (1994).

83. L. B. Bullerman, "Inhibition of Aflatoxin Production by Cinnamon," *J. Food Sci.* **39**, 1163–1165 (1974).

84. M. A. Azzouz and L. B. Bullerman, "Comparative Antimycotic Effects of Selected Herbs, Spices, Plant Components and Commercial Fungal Agents," *J. Food Sci.* **45**, 1298–1301 (1982).

85. L. A. Shelef, O. A. Naglik, and D. W. Bogen, "Sensitivity of Some Common Foodborne Bacteria to the Spices Sage, Rosemary, and Allspice," *J. Food Sci.* **45**, 1042–1044 (1980).

86. L. A. Shelef, E. K. Jyothi, and M. A. Bulgarelli, "Growth of Enteropathogenic and Spoilage Bacteria in Sage-containing Broth and Foods," *J. Food Sci.* **49**, 737–740 (1984).

87. J. M. Jay and G. M. Rivers, "Antimicrobial Activity of Some Food Flavoring Compounds," *J. Food Safety* **6**, 129–139 (1984).

88. A. López-Malo, S. M. Alzamora, and A. Argaiz, "Effect of Natural Vanillin on Germination Time and Radial Growth of Moulds in Fruit-based Agar Systems," *Food Microbiol.* **12**, 213–219 (1995).

89. E. H. Marth, "Antibiotics in Foods—Naturally Occurring, Developed and Added," *Residue Rev.* **12**, 65–161 (1966).

90. J. C. Walker and M. A. Stahmann, "Chemical Nature of Disease Resistance in Plants," *Ann. Rev. Plant Physiol.* **6**, 351–366 (1955).

91. P. J. Delaquis and G. Mazza, "Antimicrobial Properties of Isothiocyanates in Food Preservation," *Food Technol.* **49**, 73–84 (1995).

92. K. Isshiki et al., "Preliminary Examination of Allyl Isothiocyanate Vapor for Food Preservation," *Biosci. Biotechnol. Biochem.* **56**, 1476–1477 (1992).

93. C. T. Ho, "Phenolic Compounds in Food. An Overview," in C. T. Ho, C. Y. Lee, and M. T. Huang, eds., *Phenolic Compounds in Food and Their Effects on Health I. Analysis, Occurrence, and Chemistry*, ACS Symposium Series 506., American Chemical Society, Washington, D.C., 1992, pp. 2–7.

94. N. G. Faith, A. E. Yousef, and J. B. Luchansky, "Inhibition of *Listeria monocytogenes* by Liquid Smoke and Isoeugenol, a Phenolic Component Found in Smoke," *J. Food Safety* **12**, 303–314 (1992).

95. N. R. Reddy and M. D. Pierson, "Influence of pH and Phosphate Buffer on Inhibition of *Clostridium botulinum* by Antioxidants and Related Phenolic Compounds," *J. Food Prot.* **45**, 925–927 (1982).

96. K.-T. Chung et al., "Antimicrobial Properties of Tannic Acid, Propyl Gallate and Related Compounds," ASM General Meeting, New Orleans, La., 1992.

97. D. Stead, "The Effect of Hydroxycinnamic Acids on the Growth of Wine-spoilage Lactic Acid Bacteria," *J Appl. Bacteriol.* **75**, 135–141 (1993).

98. L. R. Beuchat, "Antimicrobial Properties of Spices and Their Essential Oils," in V. M. Dillon and R. G. Board eds., *Natural Antimicrobial Systems and Food Preservation*, CAB International, Wallingford, United Kingdom, 1994, pp. 167–180.

99. Y. Kouassi and L. A. Shelef, "Listeriolysin O Secretion by *Listeria monocytogenes* in the Presence of Cysteine and Sorbate," *Lett. Appl. Microbiol.* **20**, 295–299 (1995).

100. J. Ulate-Rodriguez et al., "Inhibition of *Listeria monocytogenes, Escherichia coli* O157:H7 and *Micrococcus luteus* by Linear Furocoumarins in Culture Media," *J. Food Prot.* **60**, 1046–1049 (1997).

101. A. G. Marwan and C. W. Nagel, "Microbial Inhibitors of Cranberries," *J. Food Sci.* **51**, 1009–1013 (1986).

102. K.-T. Chung, C.-I. Wei, and M. G. Johnson, "Are Tannins a Double-edged Sword in Biology and Health," *Trends Food Sci. Technol.* **9**, 168–175 (1998).

103. J. Konowalchuk and J. I. Speirs, "Antiviral Effect of Commercial Juices and Beverages," *Appl. Env. Microbiol.* **35**, 1219–1220 (1978).

104. J. Konowalchuk and J. I. Speirs, "Antiviral Effect of Apple Beverages," *Appl. Env. Microbiol.* **36**, 798–801 (1978).

105. D. Dobbenie, M. Uyttendaele, and J. Debevere, "Antibacterial Activity of the Glucose Oxidase/Glucose System in Liquid Whole Egg," *J. Food Prot.* **58**, 273–279 (1995).

106. C. A. Kantt and J. A. Torres, "Growth Inhibition by Glucose Oxidase of Selected Organisms Associated with the Microbial Spoilage of Shrimp (*Pandalus jordani*): In Vitro Model Studies," *J. Food Prot.* **56**, 147–152 (1993).

107. M. Tiina and M. Sandholm, "Antibacterial Effect of the Glucose Oxidase-Glucose System on Food-poisoning Organisms," *Int. J. Food Microbiol.* **8**, 165–174 (1989).

108. D. K. Jeong et al., "Trials on the Antibacterial Effect of Glucose Oxidase on Chicken Breast Skin and Muscle," *J. Food Safety* **13**, 43–50 (1992).

P. MICHAEL DAVIDSON
University of Tennessee
Knoxville, Tennessee

ANTIOXIDANTS

Oxidative reactions cause damage to lipids and proteins, thus influencing food quality. For example, oxidation of lipids results in the formation of volatile compounds that cause rancidity, oxidation of pigments (eg, carotenoids and myoglobin) leading to color changes, and oxidation of vitamins (eg, A, C, and E) leading to alterations in nutritional composition. The biological tissue from which we derive foods contains several distinctively different mechanisms to control oxidation catalysts, reactive oxygen species, and free radicals. In addition, many antioxidant additives are available to increase the oxidative stability of foods. Utilization of antioxidant additives and protection of endogenous antioxidants can be effective methods to increase the quality and shelf life of foods.

CHAIN-BREAKING ANTIOXIDANTS

Control of free radicals is an effective method to prevent both autoxidation and oxidation accelerated by prooxidants, since free radicals are a universal reaction intermediate for all lipid oxidation reactions. Chain-breaking antioxidants inhibit free-radical reactions by scavenging radicals resulting in inactivation of the original free radical and formation of a more stable antioxidant radical (1). The effectiveness of a chain-breaking antioxidant is a function of its chemical and physical properties. For a chain-breaking antioxidant to be effective, it must possess a hydrogen, which is weakly bound to the antioxidant so that it can be freely donated to the free radical. As the bond energy of the hydrogen on the chain-breaking antioxidant decreases, the transfer of the hydrogen to the free radical is more rapid (1,2). The effectiveness of a chain-breaking antioxidant is also related to its ability to decrease radical energy so that the antioxidant radical cannot promote further oxidation of unsaturated fatty acids. Effective antioxidants decrease free radical energy by resonance delocalization (2,3). Effective chain-breaking antioxidants also do not react rapidly with oxygen to form peroxides. Degradation of antioxidant peroxides can deplete the system of the antioxidant and can result in the formation of additional free radicals (1).

The most common functional groups that inactivate free radicals are hydroxyl (eg, phenolics), sulfhydryl (eg, cysteine and glutathione) and amino (eg, uric acid, spermine, and proteins) groups (4). Phenolic compounds are the most common forms of synthetic and natural chain-breaking antioxidants in foods. Examples of naturally occurring phenolics that inhibit lipid oxidation include α-tocopherol, epicatechin, ferulic acid, and carnosic acid (Fig. 1). Many of the natural antioxidants are used in foods as plant extracts such as rosemary and mixed tocopherol isomers. Synthetic phenolics approved as food additives include butylated hydroxytoluene, butylated hydroxyanisole, tertiary butylhydroquinone, and propyl gallate (Fig. 2). These phenolics are effective because of their ability to inactivate free radicals and form low-energy phenolic radicals. Natural phenolic extracts are typically added to foods at concentrations ≤500 ppm whereas the synthetic antioxidants are normally limited to concentrations ≤200 ppm of the fat content (5,6).

Effective utilization of phenolic antioxidants in foods often depends on the physical characteristics of the food and the solubility characteristics of the phenolics. Porter (7) originally described the "antioxidant paradox" as a phenomenon where the ability of a chain-breaking antioxidant to inhibit oxidation in bulk oils increased as the polarity of the antioxidant increased. Conversely in oil-in-water lipid emulsions, the effectiveness of lipid-soluble antioxidants increased as their polarity decreased. This phenomenon is due to the ability of polar, lipid-soluble chain-breaking antioxidants to concentrate at the oil–air interface of bulk oils (where oxidation was most prevalent) and the ability of nonpolar lipid-soluble chain-breaking antioxidants to be retained in the droplet of emulsified lipids. The structural diversity of naturally occurring phenolic antioxidants means that they vary greatly in polarity. Trolox (a water-soluble analog of tocopherol) and gallic acid inhibit lipid oxidation more effectively in bulk oils than their nonpolar counterparts, α-tocopherol and propyl gallate. In an oil-in-water emulsion, α-tocopherol is more effective at inhibiting lipid oxidation than Trolox, and methyl carnosate and carnosol are more effective than their more polar counterpart, carnosoic acid. In lipid emulsions, the phenolic antioxidants can partition into the continuous phase, the emulsifier and droplet interior. As the polarity of phenolic antioxidants decreases, the amount of the phenolic partitioning in the lipid droplet increases as does the ability of the phenolic to inhibit lipid oxidation (8–12).

Ascorbic acid and glutathione (Fig. 1) can also inactivate free radicals and act as chain-breaking antioxidants. Glutathione and ascorbate are endogenous food antioxidants, and ascorbate is often used as an antioxidant food additive. Since ascorbate and glutathione are highly water soluble, they would primarily interact with water-soluble free radicals and reactive oxygen species (4). However, ascorbate and glutathione are strong reducing compounds that can convert transition metals to their reduced state that, in turn, can promote the formation of free radicals from hydrogen and lipid peroxides (13). Therefore, ascorbate and glutathione can exhibit pro-oxidative activity if the activity of transition metals is not controlled.

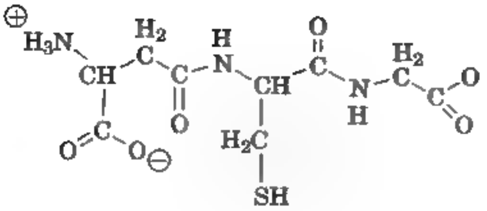
Chain-breaking antioxidants will go through a series of reactions with free radicals leading to their eventual inactivation (1). However, two or more chain-breaking antioxidants can interact synergistically, resulting in antioxidant regeneration. Synergistic antioxidant interactions can occur when one chain-breaking antioxidant preferentially reacts with free radicals due to its lower bond dissociation energies or physical location (eg, it is the same environment as the free radical) (3). Such a chain breaking is very effective at inhibiting oxidation; however, it is consumed rapidly during oxidation. However, it is possible that the highly reactive chain-breaking antioxidant can interact with another chain-breaking antioxidant resulting in antioxidant regeneration. An example of such a system is α-tocopherol and ascorbic acid (14). α-Tocopherol reacts more readily with lipid radicals due to its presence in the lipid phase. Ascorbic acid can then reduce the oxidized α-tocopherol back to its active form. In turn, the resulting dehydroascorbate is regenerated by enzymes that utilize reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) as reducing equivalents (1).

INHIBITING LIPID OXIDATION CATALYSTS

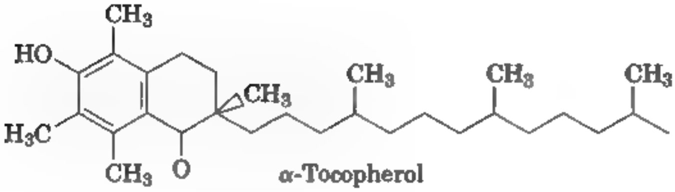
The presence and activity of lipid oxidation catalysts often determines the shelf life of foods. Therefore, control of lipid oxidation catalysts can be very important in preventing the oxidative deterioration of foods.

Transition Metals

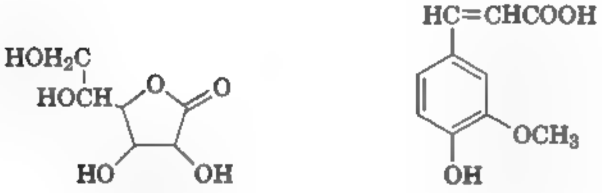
Transition metals promote the formation of free radicals by accelerating the decomposition of peroxides (15). The activity of pro-oxidant metals can be controlled by chelators that prevent metal redox cycling, occupy the reactive



Glutathione

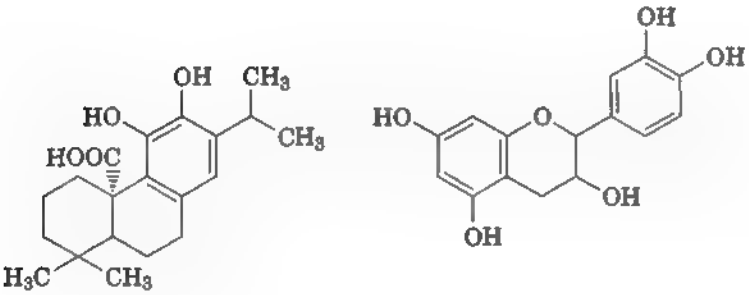


α-Tocopherol



Ascorbic acid

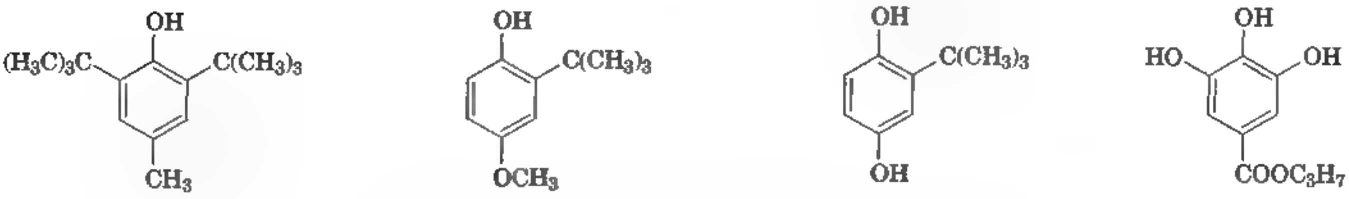
Ferulic acid



Carnosoic acid

Epicatechin

Figure 1. Examples of natural chain-breaking antioxidants in foods.



Butylated hydroxytoluene

2-Butylated hydroxyanisole

Tertiary butylhydroqunone

Propyl gallate

Figure 2. Synthetic chain-breaking antioxidants approved for food use.

metal coordination sites, form insoluble metal complexes, and stearically hinder interactions between metals and lipids (16). Common chelators added to foods include ethylenediaminetetraacetic acid (EDTA), polyphosphates, and citric acid (4). In addition, biological tissues from which we derive foods contain proteins and peptides that bind and inactivate metals such as serum albumin (Cu), transferrin (Fe), lactoferrin (Fe), ferritin (Fe), carnosine (Cu), and histidine (Cu) (4).

Combinations of chelators and chain-breaking antioxidants often result in increased inhibition of lipid oxidation (3). The ability of chelators to decrease metal-catalyzed free-radical generation decreases the rate at which chain-breaking antioxidants are consumed, thus making the concentration of the chain-breaking antioxidant greater at any given time.

Singlet Oxygen

Singlet oxygen is a high-energy form of oxygen that can directly interact with unsaturated fatty acids to form lipid peroxides (17). Singlet oxygen is most commonly formed from the interaction of oxygen with light-activated photosensitizers such as chlorophyll and riboflavin. Singlet oxygen can be inactivated by both chemical and physical mechanisms. Chemical inactivation occurs when singlet oxygen attacks the double bonds of compounds whose oxidation does not result in the development of rancidity (eg, carotenoids, tocopherols, amino acids, peptides, proteins, phenolics, and ascorbate) (17,18). Carotenoids can also inactivate singlet oxygen by physically quenching singlet oxygen through a transfer of energy from singlet oxygen to the carotenoid. The resulting excited state of the carotenoid then returns to its ground state by the slow dissipation of energy to the surrounding media (17). Similarly, carotenoids can absorb energy from photoactivated sensitizers, thereby decreasing their ability to promote the conversion of oxygen to singlet oxygen. For a carotenoid to be capable of the physical quenching of singlet oxygen it must possess a minimum of nine double bonds (19).

Lipoxygenases

Lipoxygenases catalyze the formation of lipid peroxides. Little is known about compounds that can directly inhibit lipoxygenase activity. Therefore lipoxygenases are most commonly inhibited by heat inactivation.

CONTROL OF REACTIVE OXYGEN SPECIES

The biological tissues from which we derive foods contain enzymes that control reactive oxygen species. The concentration and activity of the antioxidant enzymes can vary greatly in foods as a function of genetic and tissue variability and due to enzyme inactivation during food processing operations (4).

Superoxide Anion

Addition of an electron to molecular oxygen results in the formation of superoxide anion. Superoxide accelerates oxidative reactions by reducing transition metals to their more reactive states and by its conversion to the highly reactive perhydroxyl radical at low pH (15). Superoxide dismutase is an antioxidant enzyme found in foods that catalyzes the conversion of superoxide anion to hydrogen peroxide (4).

Peroxides

Both lipid peroxides and hydrogen peroxide will decompose into free radicals in the presence of transition metals, irradiation, and elevated temperatures (3). Catalase, glutathione peroxidase, and ascorbate peroxidase are antioxidant enzymes in food that convert peroxides into stable compounds. Catalase and ascorbate peroxidase are active against hydrogen peroxide, whereas glutathione peroxidase is active against both hydrogen and lipid peroxides (4,15). Hydrogen peroxide is converted to water, and lipid peroxides are converted to lipid alcohols (4).

EFFECTIVE UTILIZATION OF ANTIOXIDANTS IN FOODS

Utilization of antioxidant additives and preservation of endogenous antioxidants can be effective methods for improving food quality. Maximizing protection by endogenous antioxidants can be accomplished by minimizing antioxidant removal during the processing of raw materials, minimizing inactivation of antioxidants during processing (eg, inactivation of antioxidant enzymes during thermal processing operations) (20), selecting food sources that are naturally high in antioxidants, and including antioxidants in the diet of livestock (21). When antioxidant additives are used, several factors should be considered, including the antioxidant's mechanism of action in relation to the pro-oxidants in the food, the solubility characteristics of the antioxidant in relation to the physical location where oxidative reactions are most prevalent, how the antioxidant will interact with other food components (eg, prooxidative nature of ascorbate in the presence of transition metals), and how food processing operations will influence antioxidant stability.

BIBLIOGRAPHY

1. D. C. Liebler, "The Role of Metabolism in the Antioxidant Function of Vitamin E," *Crit. Rev. Toxicol.* **23**, 147–169 (1993).
2. F. Shahidi and J. P. K. Wanasundara, "Phenolic Antioxidants," *Crit. Rev. Food Sci. Nutr.* **32**, 67–103 (1992).
3. W. W. Nawar, "Lipids" in O. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, pp. 225–320.
4. E. A. Decker, "Antioxidant Mechanisms," in C. C. Akoh and D. B. Min, eds., *Lipid Chemistry*, Marcel Dekker, New York, 1997
5. J. T. R. Nickerson and L. J. Ronsivalli, *Elementary Food Science*, 2nd ed., AVI Publishing Company, Westport, Conn., 1980, pp. 62–63.
6. G. R. Schmidt, "Processing and Fabrication," in P. J. Bechtel, ed., *Muscle as Food*, Academic Press, Orlando, Fla., 1986
7. W. L. Porter, "Paradoxical Behavior of Antioxidants in Food and Biological Systems," in G. M. William, ed., *Antioxidants: Chemical Physiological, Nutritional and Toxicological Aspects*, Princeton Scientific, Princeton, N.J. 1993, pp. 93–122.

8. E. N. Frankel et al., "Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs. Emulsions," *J. Agric. Food Chem.* **42**, 1054–1059 (1994).
9. S.-W. Huang et al., "Antioxidant Activity of α-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs. Oil-in-Water Emulsions," *J. Agric. Food Chem.* **44**, 444–452 (1996).
10. S.-W. Huang et al., "Antioxidant Activity of Carnosic Acid and Methyl Carnosate in Bulk Oils and Oil-in-Water Emulsions," *J. Agric. Food Chem.* **44**, 2951–2956 (1996).
11. E. N. Frankel et al., "Antioxidant Activity of a Rosemary Extract and Its Constituents, Carnosic Acid, Carnosol, and Rosmarinic Acid in Bulk Oil and Oil-in-Water Emulsions," *J. Agric. Food Chem.* **44**, 131–135 (1996).
12. S.-W. Huang et al., "Partitioning of Selected Antioxidants in Corn Oil-in-Water Model Systems," *J. Agric. Food Chem.* **45**, 1991–1994 (1997).
13. E. A. Decker and H. O. Hultin, (1992) "Lipid Oxidation in Muscle Foods via Redox Iron," in A. J. St. Angelo, ed., *Lipid Oxidation in Foods*, American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C., 1992, pp. 33–54.
14. P. B. McCay, "Vitamin E: Interactions with Free Radicals and Ascorbate," *Ann. Rev. Nutr.* **5**, 323–340 (1985).
15. J. Kanner, J. B. German, and J. E. Kinsella, "Initiation of Lipid Peroxidation in Biological Systems," *Crit. Rev. Food Sci. Nutr.* **25**, 317–364 (1987).
16. E. Graf and J. W. Eaton, "Antioxidant Functions of Phytic Acid," in *Free Rad. Biol. Med.* **8**, 61–69 (1990).
17. D. G. Bradley and D. B. Min, "Singlet Oxygen Oxidation of Foods," *Crit. Rev. Food Sci. Nutr.* **31**, 211–236 (1992).
18. T. A. Dahl, W. R. Midden, and P. E. Hartman, "Some Prevalent Biomolecules as Defenses against Singlet Oxygen Damage," *Photochem. Photobiol.* **47**, 357–362 (1988)
19. P. DiMascio, P. S. Kaiser, and H. Sies, "Lycopene as the Most Efficient Biological Carotenoid Singlet Oxygen Quencher," *Arch. Biochem. Biophys.* **274**, 532–538 (1989).
20. L. Mei, A. D. Crum, and E. A. Decker, "Development of Lipid Oxidation and Inactivation of Antioxidant Enzymes in Cooked Beef and Pork," *J. Food Lipids* **1**, 273–283 (1994).
21. C. Faustman, "Food from Supplement-Fed Animals," in J. Smith, ed., *Technology for Reduced Additive Food*, Blackie Academic & Professional, New York, pp. 160–194, 1993.

ERIC A. DECKER
University of Massachusetts
Amherst, Massachusetts

AOAC INTERNATIONAL

AOAC INTERNATIONAL is a scientific association whose main purpose is to promote methods validation and quality measurements in the analytical sciences. AOAC also develops and validates chemical and microbiological analytical methods used in laboratories worldwide to analyze foods, feeds, agricultural and industrial chemicals, pharmaceuticals and cosmetics, water, soil, disinfectants, forensic materials, and many other substances.

HISTORY

AOAC INTERNATIONAL, originally known as the Association of Official Agricultural Chemists, was formed in 1884 to adopt uniform methods of analysis for fertilizers. Over the years, the number of areas of analytical sciences represented in AOAC increased steadily. A name change in 1965 to the Association of Official Analytical Chemists partially reflected the expansion of AOAC's analytical interests. By 1991 the Association had long ceased to be confined to regulatory (official) analytical chemists from North America—having a majority of members working in the private sector, a significant number involved in microbiology and other forms of analysis, and more than one-fourth living outside the United States. Consequently, in that year, the name was changed to AOAC INTERNATIONAL, thereby retaining the initials by which the Association had been known for more than 100 years, eliminating reference to a specific scientific discipline or profession, and reflecting the international membership and focus of AOAC.

MEMBERSHIP

Individual Membership

Individual members work in private industry, government, and academia in more than 85 countries worldwide. They include analytical chemists, microbiologists and other biologists, biochemists, toxicologists, forensic scientists, and other scientists in laboratory, administrative, and management positions. To qualify, they must have an interest in the purpose and goals of the Association, have a degree in science, and be engaged (directly or indirectly) in analysis or analytical science in fields relevant to the purpose of AOAC INTERNATIONAL.

Sustaining Membership

Sustaining member organizations are private firms, institutes, associations, and local, state, provincial, and national governmental agencies with an interest in AOAC INTERNATIONAL's mission. They are engaged, directly or indirectly, in analysis or research in the areas of interest to AOAC.

Sections of AOAC INTERNATIONAL

The AOAC INTERNATIONAL sections program was initiated in 1981. Geographically organized, it provides a forum in which members of AOAC and other analytical scientists can more easily and regularly share information. Most sections have an annual meeting and several produce newsletters and/or have their own websites.

Awards Programs

AOAC provides annual awards for scientific achievement, scientific potential, and service to the association. AOAC's most prestigious award, The Harvey W. Wiley Award, is presented each year to a scientist (or group of scientists) who has made an outstanding contribution to analytical methodology in an area of interest to the Association. AOAC's annual scholarship award is given to a worthy upper-level undergraduate or graduate student majoring in a field of study that supports the mission of the Associ-

8. E. N. Frankel et al., "Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs. Emulsions," *J. Agric. Food Chem.* **42**, 1054–1059 (1994).
9. S.-W. Huang et al., "Antioxidant Activity of α-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs. Oil-in-Water Emulsions," *J. Agric. Food Chem.* **44**, 444–452 (1996).
10. S.-W. Huang et al., "Antioxidant Activity of Carnosic Acid and Methyl Carnosate in Bulk Oils and Oil-in-Water Emulsions," *J. Agric. Food Chem.* **44**, 2951–2956 (1996).
11. E. N. Frankel et al., "Antioxidant Activity of a Rosemary Extract and Its Constituents, Carnosic Acid, Carnosol, and Rosmarinic Acid in Bulk Oil and Oil-in-Water Emulsions," *J. Agric. Food Chem.* **44**, 131–135 (1996).
12. S.-W. Huang et al., "Partitioning of Selected Antioxidants in Corn Oil-in-Water Model Systems," *J. Agric. Food Chem.* **45**, 1991–1994 (1997).
13. E. A. Decker and H. O. Hultin, (1992) "Lipid Oxidation in Muscle Foods via Redox Iron," in A. J. St. Angelo, ed., *Lipid Oxidation in Foods*, American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C., 1992, pp. 33–54.
14. P. B. McCay, "Vitamin E: Interactions with Free Radicals and Ascorbate," *Ann. Rev. Nutr.* **5**, 323–340 (1985).
15. J. Kanner, J. B. German, and J. E. Kinsella, "Initiation of Lipid Peroxidation in Biological Systems," *Crit. Rev. Food Sci. Nutr.* **25**, 317–364 (1987).
16. E. Graf and J. W. Eaton, "Antioxidant Functions of Phytic Acid," in *Free Rad. Biol. Med.* **8**, 61–69 (1990).
17. D. G. Bradley and D. B. Min, "Singlet Oxygen Oxidation of Foods," *Crit. Rev. Food Sci. Nutr.* **31**, 211–236 (1992).
18. T. A. Dahl, W. R. Midden, and P. E. Hartman, "Some Prevalent Biomolecules as Defenses against Singlet Oxygen Damage," *Photochem. Photobiol.* **47**, 357–362 (1988)
19. P. DiMascio, P. S. Kaiser, and H. Sies, "Lycopene as the Most Efficient Biological Carotenoid Singlet Oxygen Quencher," *Arch. Biochem. Biophys.* **274**, 532–538 (1989).
20. L. Mei, A. D. Crum, and E. A. Decker, "Development of Lipid Oxidation and Inactivation of Antioxidant Enzymes in Cooked Beef and Pork," *J. Food Lipids* **1**, 273–283 (1994).
21. C. Faustman, "Food from Supplement-Fed Animals," in J. Smith, ed., *Technology for Reduced Additive Food*, Blackie Academic & Professional, New York, pp. 160–194, 1993.

ERIC A. DECKER
University of Massachusetts
Amherst, Massachusetts

AOAC INTERNATIONAL

AOAC INTERNATIONAL is a scientific association whose main purpose is to promote methods validation and quality measurements in the analytical sciences. AOAC also develops and validates chemical and microbiological analytical methods used in laboratories worldwide to analyze foods, feeds, agricultural and industrial chemicals, pharmaceuticals and cosmetics, water, soil, disinfectants, forensic materials, and many other substances.

HISTORY

AOAC INTERNATIONAL, originally known as the Association of Official Agricultural Chemists, was formed in 1884 to adopt uniform methods of analysis for fertilizers. Over the years, the number of areas of analytical sciences represented in AOAC increased steadily. A name change in 1965 to the Association of Official Analytical Chemists partially reflected the expansion of AOAC's analytical interests. By 1991 the Association had long ceased to be confined to regulatory (official) analytical chemists from North America—having a majority of members working in the private sector, a significant number involved in microbiology and other forms of analysis, and more than one-fourth living outside the United States. Consequently, in that year, the name was changed to AOAC INTERNATIONAL, thereby retaining the initials by which the Association had been known for more than 100 years, eliminating reference to a specific scientific discipline or profession, and reflecting the international membership and focus of AOAC.

MEMBERSHIP

Individual Membership

Individual members work in private industry, government, and academia in more than 85 countries worldwide. They include analytical chemists, microbiologists and other biologists, biochemists, toxicologists, forensic scientists, and other scientists in laboratory, administrative, and management positions. To qualify, they must have an interest in the purpose and goals of the Association, have a degree in science, and be engaged (directly or indirectly) in analysis or analytical science in fields relevant to the purpose of AOAC INTERNATIONAL.

Sustaining Membership

Sustaining member organizations are private firms, institutes, associations, and local, state, provincial, and national governmental agencies with an interest in AOAC INTERNATIONAL's mission. They are engaged, directly or indirectly, in analysis or research in the areas of interest to AOAC.

Sections of AOAC INTERNATIONAL

The AOAC INTERNATIONAL sections program was initiated in 1981. Geographically organized, it provides a forum in which members of AOAC and other analytical scientists can more easily and regularly share information. Most sections have an annual meeting and several produce newsletters and/or have their own websites.

Awards Programs

AOAC provides annual awards for scientific achievement, scientific potential, and service to the association. AOAC's most prestigious award, The Harvey W. Wiley Award, is presented each year to a scientist (or group of scientists) who has made an outstanding contribution to analytical methodology in an area of interest to the Association. AOAC's annual scholarship award is given to a worthy upper-level undergraduate or graduate student majoring in a field of study that supports the mission of the Association. The Fellow of AOAC INTERNATIONAL Award recognizes the dedication of the volunteers who serve the Association.

TECHNICAL SERVICES

The AOAC® Official Methods℠ Program

The AOAC® *Official Methods*℠ program is designed to provide high-quality validated methods that can be used with confidence by regulatory agencies, regulated industry, product testing laboratories, academic institutions, and others. Proposed AOAC® *Official Methods*℠ are subjected to a multilaboratory collaborative study according to internationally recognized standards and receive rigorous scientific review of performance results. Adopted methods are published in the *Journal of AOAC INTERNATIONAL* and in *Official Methods of Analysis of AOAC INTERNATIONAL*.

The AOAC® Peer-Verified Methods℠ Program

This AOAC INTERNATIONAL method validation program, initiated in 1993, is the program of choice when speed of validation is essential and a lesser degree of confidence is acceptable. The performance characteristics of these methods are checked in one or more independent laboratories and reviewed against specific performance standards. Accepted *Peer-Verified Methods*℠ are listed in AOAC's magazine and journal, and copies are available for purchase.

The AOAC® Performance Tested Methods℠ Program

The AOAC® *Performance Tested Methods*℠ Program provides an independent third-party review for proprietary methods to confirm manufacturers' performance claims. This program is administered by the AOAC Research Institute, a subsidiary of AOAC INTERNATIONAL. Successfully validated test kits are licensed to use the AOAC Research Institute "Performance Tested" seal.

The AOAC® Laboratory Proficiency Testing Program

Developed in 1998, the AOAC® Laboratory Proficiency Testing Program provides a means for laboratories to test the quality and accuracy of their analytical results and benchmark them against those of other participating laboratories. The program supports laboratory accreditation and international standards through compliance with ISO guides.

TECHNICAL DIVISIONS

Technical Division on Reference Materials

The Technical Division on Reference Materials (TDRM) promotes the use of reference materials to verify the accuracy of methods. Liaisons from TDRM work closely with the AOAC methods committees to define needs for reference materials. TDRM plans and conducts educational and informational activities on the use of reference materials.

Technical Division on Laboratory Management

The Technical Division for Laboratory Management (TDLM) provides a forum for networking, information sharing, and problem solving on issues of mutual interest for managing an efficient, cost-effective, quality laboratory. It offers opportunities for leadership and mentoring as well as opportunities for learning and professional enhancement. TDLM maintains a listserv to discuss topics such as quality assurance, training, personnel management, safety, downsizing, budgeting, laboratory information management systems (LIMS), proficiency testing, and accreditation.

MEETINGS AND EDUCATION

Meetings

The annual meeting and exposition is the focal point of AOAC INTERNATIONAL's yearly work. The scientific/technical program includes symposia on a variety of topics of current interest as well as presentations of contributed papers, collaborative studies, and poster sessions organized to correspond with the topic areas of the methods committees. The program also includes workshops on methodology, forums on specialized subjects, and a panel presentation and round table discussion on regulatory topics. At its exposition, the annual meeting offers an opportunity for attendees to be introduced to and acquire information about new products and services offered by vendors of analytical equipment, services, and publications.

Training Courses

AOAC INTERNATIONAL has assumed a leadership role in providing continuing education opportunities for all members of the analytical community, including the scientists who use or develop methods, quality assurance personnel, and laboratory managers. In addition to courses scheduled by AOAC, the Association can also make arrangements with organizations to present AOAC training courses at their location.

PUBLICATIONS

Official Methods of Analysis of AOAC INTERNATIONAL

Considered to be the analytical methods "bible" by thousands of analysts who use and depend on it, *Official Methods of Analysis of AOAC INTERNATIONAL* (*OMA*) is AOAC's premiere publication. Methods validated and adopted by AOAC are published in *OMA*, and the book is updated annually via revisions available on a subscription basis to purchasers of the book.

Journal of AOAC INTERNATIONAL

The *Journal of AOAC INTERNATIONAL* publishes original research articles and reports on the development, validation, and interpretation of analytical methods for agricultural commodities, foods, drugs, cosmetics, and the environment. A limited number of invited reviews and feature articles on selected subjects are also published, as well as the proceedings from symposia. The *Journal of AOAC INTERNATIONAL* publishes fully refereed reports on developing, improving, and testing methods, including reports on the collaborative studies that precede and support adoption of AOAC® *Official Methods*^{SM}.

Inside Laboratory Management

Inside Laboratory Management (ILM) features articles on laboratory management, regulatory news, safety concerns, new instrumentation, and emerging technologies; publishes news of AOAC activities; disseminates information to and among methods volunteers; provides notice on stages of methods in progress in the AOAC® methods validation programs; offers a vehicle for communication among the AOAC constituencies; and generally promotes the purposes, activities, and accomplishments of the Association and its members.

MURI DUEPPEN
AOAC INTERNATIONAL
Gaithersburg, Maryland

APHRODISIACS AND STIMULANTS

When one thinks of aphrodisiacs, the image of oysters comes to mind. Yet there is no scientific evidence (short of possible pheromonal components of truffles [5α-androst-16-en-3α-ol] and celery [5α-androst-16-en-3α-one]) (1,2) that most of the traditional foods considered as aphrodisiacs have any aphrodisiac qualities at all. Alternatively, there is considerable evidence that foods can act to inhibit sexual arousal. In particular, alcohol has a marked inhibiting effect on sexual functions in both men and women (3,4). Shakespeare described, "It provokes the desire, but it takes away the performance" (5). Scientific research has just begun to investigate the aphrodisiacal effects of food on sexual arousal.

COMPONENTS OF FOOD

When one thinks of giving chocolates on Valentine's Day to a loved one, we hope this acts as an aphrodisiac . . . but does it? To understand this better, the sensory components of food need to be delineated. These include texture, visual appearance, viscosity, tactile properties, and gustatory and olfactory components.

A Twinkie, a cucumber, and a doughnut are particularly Freudian based on their shape and visual appearance. The texture and viscosity of oysters, likewise, may induce reminiscence of sexual organs. Alternatively, the sexual experience surrounding the ingestion of a food may also cause the food itself to become endowed with a sexual valence, which may be aphrodisiacal in nature. Another possible mechanism is not through achieving a direct sexual meaning, but rather, a food can become sexually charged by becoming indirectly associated with sexual function, for example, by being considered a "guilty pleasure." Since in our puritan society, sex and other hedonic pleasures are frowned upon and repressed, one pleasure can be unconsciously substituted for another, while retaining the same underlying meaning. Eating chocolate or strawberries and whipped cream may be viewed in a sinful way as a reward of "badness for good behavior" (as a dessert after having eaten the meal of brussels sprouts), or as a violation of an unwritten moral code that elevates these foods into a nonessential, potentially dangerous, risky (ie, exciting) food, and hence strongly desired. Thus, eating these foods is perceived on some level as a violation of social morals and revolt against hedonic repression, which may be manifested by sexual arousal. Another nonfood example of this repressed hedonic affective overflow is the Las Vegas effect of gambling, being perceived as a "naughty" thing to do, and hence becoming imbued with components of sexual arousal. Likewise, sinful foods may come to be endowed with other perceived sinful qualities, and this would include sexual arousal.

Alternatively, the taste of the food, that is, sweet, sour, bitter, or salty, if endowed with a high degree of sugar, may induce release of endorphins and norepinephrine, which may act to stimulate sexual arousal. Alternatively, sweet-induced reactive hypoglycemia may act to inhibit the associated sexual arousal. Sexual arousal in the male is manifested by erection and is predominantly parasympathetically mediated (6). Thus foods that induce parasympathetic activation (and inhibit sympathetic discharge) may have the greatest effect of inducing sexual arousal.

Last, the smell of the food can influence sexual arousal. Since approximately 90% of what is perceived as taste is really smell, the smell of food may have a far greater effect than is traditionally given credit in assessing food's overall effect on behavior (7). This can be demonstrated by holding one's nose while eating chocolates: it produces a taste of chalk. This is a manifestation of an olfactory synesthesia. The concept of synesthesia is when one sensation is misperceived as another sensation. For instance, pressing on the eyeballs induces an illusion of the presence of light, yet it is the pressure that is misperceived as light. In the olfactory realm, odors that are sniffed from outside the nose going up to the olfactory epithelium at the top of the nose are interpreted as smell. Alternatively, odors that do not go through the orthonasal route rather through the retronasal route, from the mouth up through the nasopharynx in the back of the throat to the top of the nose, are interpreted as taste.

SMELL AND SEXUAL AROUSAL

Throughout history aromas have been linked with sexual response. Perfume jars were preserved in the chambers designed for sexual relations in volcanic remnants of ancient Pompeii. Ancient Sumarians used perfumes to entice women. Traditional Chinese rituals highlight the relationship between smell and affection. Hieroglyphics also relate the two. Virtually all cultures use perfume in their marriage rites. In the modern world as well, the connection is pervasive. Use of perfumes and colognes as romantic enticements are a multibillion dollar business (8). The prominence of the historical connection between odors and sex among diverse cultures implies a high level of evolutionary importance. Could it be inbred within the brain of humans?

Freud, almost a hundred years ago, in *Civilization and Its Discontents* recognized the relationship between olfaction and sexual arousal. He suggested that to remain a civilized society we had to repress our olfactory instincts, otherwise humans would walk around sexually excited all the time (9). Freud, followed by Bieber and later analysts, delineated the connection between smell and sexual development (10). This linkage indicates a specific point in time in the development of Oedipal conflicts. When children recognize the smell of the same-sex parent and the scent of the opposite-sex parent, they learn to dislike the smell of the same sex and transfer positive feelings toward the smell of the opposite-sex parent (11). This establishes a psychodynamic interrelationship between smell and sexual identity. The transfer of emotions to a parent's odor imbues sexual meaning to otherwise inert odors (12). In those who are unable to work through this phase of Oedipal development, fetishes develop as in Freud's rat man (13).

Freud's analysis followed a millennia of pervasive cultural myth and practice that linked sexuality and olfaction. In Western culture the court jester wore a mask with a phalliclike nose (14). Native cultures practiced the Eskimo kiss (15). The Pacific Islanders engaged in a similar type of nasal kiss (16).

This cultural awareness may derive from the unique characteristics of human neuroanatomy. Humans have the largest limbic system compared with body size of any primate (17). The olfactory lobe directly connects with the limbic system (18). The relative size of the limbic system and the extensive olfactory/limbic interconnection implies olfaction has a significant effect on human behavior. The effect of external stimuli on the olfactory system and subsequent effect on behavior is well documented (19–23). Erectile tissue exists in both human genitals and within structures in the nose (24). With sexual arousal and excitation there is engorgement of tissue not only in the genitals but also in the nose (25). Other anatomical evidence is that common neurotransmitters facilitate both olfactory and sexual functioning (26,27).

Besides purely anatomical similarities, the linkage between olfactory function and sexual function is recognized in a clinical setting. More than 17% of individuals with chemosensory dysfunction develop impaired sexual desire or other sexual dysfunction (28). Genetic disorders can affect both systems; for example, those with Kallman's syndrome have both olfactory deficit and impaired sexual drive and functioning (29). Other diseases impair olfactory ability and sexual functioning concomitantly, including cerebral vascular disorders (18,30), Parkinson's disease (31,32), senile dementia of the Alzheimer's type (33), hypothyroidism (34,35), and vitamin deficiency states including B_{12} deficiency (36).

Research into the olfactory–sexual link has reached the laboratory as well as the physician's office. Olfactory-related sexual behavior has been well documented in laboratory animals. When the olfactory bulb is lesioned in hamsters, it causes an impaired sex drive (37). The lesion does not have to be in the brain itself; for example, lesion of the olfactory mucosa external to the brain, which prevents odorant molecule binding, similarly causes impaired sexual function and reduced sex drive (38). Homologous lesions in mice cause impaired copulatory ability (39,40). Alternatively, in the female rat, lesion of the olfactory bulb leads to an increase in mating behavior suggesting indiscriminate sexual activity (41). This olfactory–sexual connection is further highlighted in lab animals since castration of the animal leads to not only impaired sexual drive but also impaired olfactory functioning (42). Alternatively, ovariectomy leads to not only impaired sexual functioning but also reduced olfactory ability (43,44). Lesions of the olfactory bulb, or even of the nasal cartilage alone, cause both olfactory deficit (45,46) and malformation of the developing animal's sex organs. These studies suggest a strong link among olfaction, olfactory organs, and sexual functioning.

Following the strong evidence from laboratory animals, significant research has been accomplished in humans, especially regarding the sexual olfactory linkage in women. This could be because women's ability to smell is greater than that of men (47). There exists a fluctuation in olfactory ability concurrent with menstrual cycle (48), such that at the time of ovulation a woman's ability to smell a musk-like substance is about 100,000 times better than that of men (49). This increased olfactory ability at the time of ovulation is even prominent in women who are taking progesterone (50). A woman's superior ability to smell, as compared with man, does not occur until puberty, around the time when apocrine glands obtain their distinct "scents" smell (51). Unlike other sensory modalities, which do not increase at time of puberty (51), olfactory ability does, demonstrating the importance of interrelationship between sexual development and olfaction.

Many explanations exist to describe this olfactory–sexual connection; the first to be discussed here are pheromones. While pheromones have been well characterized extensively in animals, they have not been fully characterized in humans, although much evidence suggests that pheromones also exist in humans. For example, pheromones exist throughout the animal kingdom, including insects; mechanisms of such evolutionary importance that exist throughout so many species usually exist in humans as well (52).

Anatomic evidence suggesting pheromones may exist in humans is the presence of apocrine glands in the axilla and surrounding the genitals. Unlike eccrine, or sweat glands, apocrine glands secrete a high-density steroid substance (53). In nonhuman primates, pheromones are released from these glands (54). In these sites we find hair tufts, creating greater surface area, which would allow greater dispersion of any pheromone that may be present (55). Apocrine glands are larger in men than in women (56), which may act in harmony with women's greater olfactory ability; for example, it would do no good to secrete pheromones that cannot be detected. Further anatomical evidence is the existence of the vomeronasal organ (VNO). The VNO in some mammals is where pheromones act (57). In humans this organ is of unknown significance and until recently was thought to be only of vestigial origin (58,59).

It is now postulated to be the site where human pheromones act.

Physiologically one sees specific changes suggesting the presence of pheromones. For example, change in olfactory ability is associated with the ovulatory cycle as already described (50). Another change is the nasal engorgement at time of sexual excitation (60). This engorgement allows for greater olfactory ability, since the odorants form eddy currents in the nose, which do not go directly down to the lung, but rather reach the olfactory epithelium at the top of the nose (61). This mechanism would enhance an individual's ability to detect any potential pheromones that may be present.

Further evidence for the existence of pheromones is based on clinical observations. McClintock described the development of menstrual synchrony in an all-women dormitory (62). While entering as freshmen the women's menstrual cycles were asynchronous but became synchronized by midterm, suggesting that there was a pheromone present acting to synchronize the women. Likewise, increases in copulatory behavior occur in married couples associated with time of ovulation, suggesting that a pheromone may be present that stimulates the male to respond or self-stimulates arousal in the women and the men respond to this associated behavior (63). Michael et al. demonstrated that when a potential pheromone (copulant) was placed on the chest of women, as compared with a placebo odorant, there was an increase in copulation associated with the use of the copulant (64).

Pheromones that have received the most attention have been androstenol and androstenediol, both pig pheromones (1). Androstenol is reported to be less active than androstendiol, and there is an equilibrium whereby androstendiol is easily converted to androstenol. Kirk-Smith et al. placed androstendiol on surgical masks and had individuals rating slides, pictures of men and women (65). Both men and women tended to rate the figures of women as prettier, and so on, in the presence of the androstendiol. When androstendiol was placed on surgical masks in another experiment, and participants were asked to rate other individuals, they tended to rate the individuals in a more sexually oriented matter (66).

That women have a greater ability to detect potential pheromones is of significance since the propagation of the species is based on their sexual activity, and, hence, birth rates are maximized by women's appropriate response to pheromones. Existence of pheromones is also suggested based on territorial markings. Gustavson et al. placed androstenol and androstenediol underneath bathroom stalls in a men's room and watched where men sat (67). They tended to avoid those stalls with the androstenol, suggesting that it may act not only as a pheromone but also as a repellent or territorial marker for other males.

While pheromones are one mechanism used to explain the olfactory-sexual link, there are several others. For example, odors could act on sexual arousal through a learned conditioned response (68). If the odor is one that is associated in the past with being sexually aroused, it might induce a sexually arousing mechanism. This could be a primary conditioned response or through secondary effects, for instance by inducing a more positive mood state or relaxed state. Inducing a more positive or relaxed state might cause a reduction in inhibitions. Positive moods and relaxed states can be achieved either directly through a conditioned response, through a learned response, or through the phenomenon of olfactory-evoked nostalgia where an odor induces a positive mood state in an individual as a result of recalling the past (69).

Alternatively, odors may act to enhance sexual arousal by actually acting directly on areas of the brain that induce sexual arousal (eg, the septal nucleus), by acting directly, almost as a drug, influencing them through dopaminergic, cholinergic, or serotinergic mechanisms (70). Odors induce a change in dopamine in the olfactory bulb and its connections, and medications that have been said to induce sexual arousal include those that are known to affect dopamine (this side effect is seen in L-dopa treated Parkinson's patients) (71).

Alternatively odors could affect sexual arousal by inducing a state of risk taking or of generalized pleasure seeking, as in seeking food or other pleasure-oriented responses (72). Perhaps odors could inhibit associated cortical functioning, allowing a release of the id or the underlying limbic system functioning, thus manifesting more primitive responses (73). This release is seen in decorticate animals as in the Kluver-Bucy syndrome or in children with developmental cortical deficits, as in Down syndrome patients who become obese in response to their relatively uninhibited appetite. Similar responses are seen in individuals who became more tired, thus cortically suppressed, and hence more easily induced to sexual arousal or eating. Similarly, cortical suppression with alcohol leads to initially a lack of discrimination for sexual partners. Alternatively, this may be because of alcohol-induced inhibition of olfactory reception (74). This is seen in olfactory-deprived rats, which engage in indiscriminate mating.

Environmental stimuli that affect one sex in a species also usually affect the opposite sex. In a study by Hirsch and Gruss, odors were found to affect male sexual arousal (75). Each of the 30 odors produced an increase in penile blood flow. The combined odor of lavender and pumpkin pie had the greatest effect, increasing median penile blood flow by 40%.

Table 1 shows the median percentage increases in penile blood flow produced in the 31 participants by each of the odors. Second in effectiveness was the combination of black licorice and doughnut, which increased the median penile blood flow 31.5%, followed by the combination of pumpkin pie and doughnut, which produced a 20% increase. Least effective was cranberry, which increased penile blood flow by 2%. None of the odors tested were found to reduce penile blood flow.

Among individuals with normal olfactory ability, there were several significant correlations: higher brachial penile indices correlated with greater age and with greater responses to the odor of vanilla ($p = 0.05$); self-assessed level of sexual satisfaction correlated with greater responses to the odor of strawberry ($p - 0.05$); and frequency of sexual intercourse correlated with greater responses to the odors of lavender ($p = 0.03$), oriental spice ($p = 0.02$), and cola ($p = 0.03$).

Table 1. Increases in Penile Blood Flow Produced by Various Odors on 31 Individuals

Odor	Median % increase
Lavender and pumpkin pie	40.0
Doughnut and black licorice	31.5
Pumpkin pie and doughnut	20.0
Orange	19.5
Lavender and doughnut	18.0
Black licorice and cola	13.0
Black licorice	13.0
Doughnut and cola	12.5
Lily of the valley	11.0
Buttered popcorn	9.0
Vanilla	9.0
Pumpkin pie	8.5
Lavender	8.0
Musk	7.5
Cola	7.0
Doughnut	7.0
Peppermint	6.0
Cheese pizza	5.0
Roasting meat	5.0
Parsley	4.5
Cinnamon buns	4.0
Green apple	3.8
Rose	3.5
Strawberry	3.5
Oriental spice	3.5
Baby powder	3.3
Floral	3.0
Chocolate	2.8
Pink grapefruit	2.5
Cranberry	2.0

Table 2. Summary of Results in Study of the Effect of Odors on Female Sexual Arousal

Average change in vaginal blood flow (%)	Change in vaginal blood flow (%)
Good and Plenty and cucumber	+13
Baby powder	+13
Pumpkin pie and lavender	+11
Baby powder and chocolate	+4
Perfume	+0–1
Cologne	−1
Good and Plenty	−12
Charcoal barbeque meat	−14
Cherry	−18
Women who find masturbation extremely arousing	
Good and Plenty and banana nut bread	+28
Good and Plenty and cucumber	+22
Perfume	+18
Women who find masturbation not arousing or repulsive	
Baby powder	+16
Pumpkin pie and lavender	+10
Good and Plenty	−20
Women who are positively aroused by genital finger manipulation by a partner	
Good and Plenty and cucumber	+18
Pumpkin pie and lavender	+12
Women who are not aroused or are inhibited by finger manipulation by a partner	
Perfume	−14
Cologne	−14
Good and Plenty and cucumber	−13
Women who are not usually multiorgasmic in sexual encounters with partner	
Baby powder	+15
Women who are usually multiorgasmic in sexual encounters with partner	
Baby powder	−8

Odors found that consistently increased penile blood flow were those of food. If odors can increase penile blood flow, the ramifications extend beyond mere aphrodisiacs. Might odors be used to treat vasculogenic impotence, as in diabetics? Or if an odor could be found that decreases penile blood flow, might it be used to treat sexual deviants, such as pedophiles, as part of their aversion training?

In a homologous manner, odors' effect on women's sexual arousal were tested with results summarized in Table 2. Several questions remain as to why food odors enhance female sexual arousal. Possible mechanisms by which odors can induce female sexual arousal include Pavlovian conditioned response olfactory-evoked nostalgia, or physiological. Odors may act to induce the Pavlovian conditioned response (69) and may act to release sexual instincts or basic appetites for hedonic experiences including sexual arousal. Alternatively, there may be a conditioning response to the smell of food if this was something that frequently preceded enjoyable sexual experiences in the past, that induced arousal. Hence, in a conditioned response paradigm, reintroduction to food may induce the phenomena of sexual arousal. It's likely it is smell rather than the taste that elicits this response since approximately 90% of taste is actually smell (76). By eating food prior to sexual arousal, the women were primarily experiencing smell rather than taste, thus odors acted as the conditioning stimulus. Presentation of these odors may have acted to focus these women's attentions on the stimulus rather than on the external environment within the laboratory.

Another explanation is that the odors may have acted through a distractor effect. The women might think about the odor rather than think about all the negative aspects of being inside a laboratory. This would then remove associated negative emotional tone and hence remove inhibitions, allowing sexual arousal to occur.

Another potential mechanism is the phenomenon of olfactory-evoked nostalgia. Approximately 86% of women described a strong olfactory-evoked nostalgic response where odor induced a vivid memory of one's childhood (77). The odors might have induced an olfactory-evoked response of these women's childhood. Childhood memory is often associated with positive emotions. By inducing the women to be in a more positive mood state, odors may have reduced inhibitions, therefore allowing sexual arousal.

Memories of one's childhood might induce not only a positive mood state but also a sense of security and safety. These feelings would then be reintroduced to the women on exposure to the odor, allowing them to be less inhibited. By feeling safe and secure, urges would come forth as manifested by enhanced vaginal blood flow. Alternatively, the odors may have induced recall of sexual experiences in one's adolescence, which then would have a direct induction of sexual arousal.

Another possible mechanism by which odors could impact female sexual arousal is by directly acting in a physiological manner. The odors could have affected perception of another sensation, for example, touch or pain. Touch perception threshold may have been reduced by the odors. Hence, the women were able to feel the vaginal probe to a greater degree than they were without the odor present. This increased touch perception may have induced the vaginal probe to become a sexually arousing stimulus. Studies suggest that odors can enhance tactile perception (78), as well as affect perceptional thresholds in other forms, that is, perception of external space (79). Or the odors might not affect the touch threshold so much as the awareness of the touch threshold. Another possible physiological mechanism by which odors may enhance sexual arousal is by reducing pain. By reducing pain, odors may have acted to remove inhibitions. A low level of pain may have existed in these women as a result of the vaginal probe and odors may have reduced this pain. Evidence that odors can influence pain has been demonstrated in migraine headaches (21).

Another possible mechanism arises from the olfactory link to the limbic system. Odors, by inducing a strong emotional response associated with limbic system firing, may induce a neighborhood effect. Overflow limbic system discharges may project to all limbic system structures, including the septal nuclei, the sexual arousal center. Hence, the generalized emotional response to odors may have acted physiologically to induce arousal.

The effects of specific odors must also be considered. For example, an evolutionary hypothesis may be entertained in trying to understand why primarily food odors affect sexual arousal. Primitive humans congregated around food kills (80). Perhaps there they had the most opportunities to procreate. An increase in sexual arousal in response to food odors, therefore, may have conferred an evolutionary advantage for survival.

Humans can detect approximately 10,000 odors (81). Only 46 were examined. Studies have suggested that many odors affect behavior; for example, floral smells affect learning (82) and buying behavior (83), green apple affects claustrophobia, barbecue smoke induces agoraphobia (79), and food odors affect weight loss (84). Quite possibly other food odors would affect vaginal and penile blood flow more than those we examined.

BIBLIOGRAPHY

1. R. Claus, H. O. Hoppen, and H. Karg, "The Secret of Truffles: A Steroidal Pheromone?" *Experientia* **37**, 1178–1179 (1981).
2. R. Claus and H. O. Hoppen, "The Boar-Pheromone Steroid Identified in Vegetables," *Experientia* **35**, 1674–1675 (1979).
3. G. M. Farkas and R. C. Rosen, "Effect of Alcohol on Elicited Male Sexual Response," *J. Stud. Alcohol* **37**, 265–272 (1976).
4. G. T. Wilson and D. M. Lawson, "Effects of Alcohol on Sexual Arousal in Women," *J. Abnorm. Psychol.* **85**, 489–497 (1976).
5. W. Shakespeare, *Macbeth*, Act 2, Scene 3.
6. H. D. Weiss, "The Physiology of Human Penile Erection," *Ann. Intern. Med.* **76**, 792–799 (1972).
7. A. R. Hirsch, "Scentsation, Olfactory Demographics and Abnormalities," *Int. J. Aromatherapy* **4**(1), 16–17 (1992).
8. P. Dicther, "Second Hand Scents," *Drug and Cosmetic Industry* **156**, 72–75 (1995).
9. S. Freud, "Bemerkunger uber einen Fall von Zwangs Neurosa," *Ges. Schr.* **VIII**, 350 (1908).
10. I. Bieber, "Olfaction in Sexual Development and Adult Sexual Organization," *Am. J. Psychother.* **13**, 851–859 (1959).
11. I. Bieber, T. B. Bieber, and R. C. Friedman, "Olfaction and Human Sexuality: A Psychoanalytic Approach," in M. J. Serby and K. L. Chobor, eds., *Science of Olfaction*, Springer-Verlag, New York, 1992, pp. 396–409.
12. M. Kalogerakis, "The Role of Olfaction in Sexual Development," *Psychosom. Med.* **25**, 420–432 (1963).
13. S. Freud, "Notes Upon a Case of Obsessional Neurosis," (1909) in J. Strachey, ed. and trans., *The Standard Edition of the Complete Psychological Works of Sigmund Freud*, Vol. 10, Hogarth Press, London, United Kingdom, 1962, p. 247.
14. S. Freud, "Report on My Studies in Paris and Berlin 1886," (1892–1899), in J. Strachey, ed. and trans., *The Standard Edition of the Complete Psychological Works of Sigmund Freud*, Vol. 1, Hogarth Press, London, United Kingdom, 1962, pp. 173–280.
15. A. A. Brill, "The Sense of Smell in the Neuroses and Psychoses," *Psychoanal. Q.* **1**, 7–42 (1932).
16. I. Eibl-Eibesfeldt, *Human Ethology*, Aldine de Gruyter, New York, 1989.
17. E. Armstrong and M. Onge, "Number of Neurones in the Anterior Thalamic Complex of the Primate Limbic System," *Am. J. Phys. Anthropol.* **54**, 197 (Abstract) (1981).
18. R. B. Goodspeed, J. F. Gent, and F. A. Catalanotto, "Chemosensory Dysfunction: Clinical Evaluation Results from a Taste and Smell Clinic," *Postgrad. Med.* **81**, 251–260 (1987).
19. A. R. Hirsch and R. Gomez, "Weight Reduction Through Inhalation of Odorants," *J. Neurol. Orthop. Med. Surg.* **16**, 28–31 (1995).
20. A. R. Hirsch, "Effects of Ambient Odors on Slot Machine Usage in a Las Vegas Casino," *Psychology and Marketing* **12**, 585–594 (1995).
21. A. R. Hirsch and C. Kang, "The Effects of Green Apple Fragrance on Migraine Headache," *Headache* **37**, 312 (1997).
22. A. R. Hirsch and L. H. Johnson, "Odors and Learning," *J. Neurology Ortho Med Surg* **17**, 119–126 (1996).
23. A. R. Hirsch, "Negative Health Effects of Environmental Odor Pollution: A Review," in J. Lee and R. Phalen, eds., *The Proceedings of the Second Colloquium on Particulate Air Pollution and Human Health*, 1996, pp. 4-207 to 4-215.
24. H. E. Hamlin, "Working Mechanisms for the Liquid and Gaseous Intake and Output of the Jacobson's Organ," *Am. J. Physiol.* **91**, 201–205 (1929).

25. P. Cole, "*Respiratory Role of the Upper Airways*," Mosby-Yearbook, St. Louis, Mo., 1993, pp. 8–93.
26. N. Halasz and G. M. Shepherd, "Neurochemistry of the Vertebrae Olfactory Bulb," *Neurosci.* **10**, 579–619 (1983).
27. J. G. Herndon, Jr., "Effects of Midbrain Lesions on Female Sexual Behavior in the Rat," *Physiol. Behav.* **17**, 143–148 (1976).
28. A. R. Hirsch and T. J. Trannel, "Chemosensory Disorders and Psychiatric Diagnoses," *J Neurol Orthop Med Surg* **17**, 25–30 (1996).
29. F J. Kallann, W. A. Schoefeld, and S. E. Barrera, "The Genetic Aspects of Primary Eunuchoidism," *Am. J. Ment. Defic.* **48**, 203–236 (1943).
30. E. D. Goddess, N. N. Wagner, and D. R. Silverman, "Poststroke Sexual Activity of CVA Patients," *Med. Aspects Hum. Sex.* **13**, 16–30 (1979)
31 R. L. Doty, D. Deems, and S. Stellar, "Olfactory Dysfunction in Parkinson's Disease: A General Deficit Unrelated to Neurologic Signs, Disease Stage or Disease Duration," *Neurology* **38**, 1234–1237 (1988).
32. F. Boller and E. Frank, *Sexual Dysfunction in Neurological Disorders, Diagnosis, Management, and Rehabilitation*, Raven Press, New York, 1982, p. 50.
33. R. L. Doty, P. F. Reyes, and T. Gregor, "Presence of Both Odor Identification and Detection Deficits in Alzheimer's Disease," *Brain Res. Bull.* **18**, 597–600 (1987).
34. R. J. McConnell et al., "Defects of Taste and Smell in Patients with Hypothyroidism," *Am. J. Med.* **59**, 354–364 (1975).
35. R. C Kolodny, W. H. Masters, and V. E. Johnson, *Textbook of Sexual Medicine*, Little, Brown, Boston, 1979, pp. 142–143.
36. R. W. Rundles, "Prognosis in the Neurologic Manifestations of Pernicious Anemia," *Blood* **1**, 209–219 (1946).
37. M. Devor and M. Murphy, "The Effects of Peripheral Olfactory Blockade on the Social Behavior of the Male Golden Hamster," *Behav. Biol.* **9**, 31–42 (1973).
38. P. D. MacLean, *Triune Concept of the Brain and Behavior*, University of Toronto Press, Toronto, 1973
39. K. Larsson, "Impaired Mating Performances in Male Rats After Anosmia Induced Peripherally or Centrally," *Brain Behav. Evol.* **4**, 463–471 (1971).
40. D. A. Edwards, M. L. Thompson, and K. G. Burge, "Olfactory Bulb Removal vs. Peripherally Induced Anosmia: Differential Effects on the Aggressive Behavior of Male Mice," *Behav. Biol.* **7**, 823–828 (1972).
41. R. L. Moss, "Modification of Copulatory Behavior in the Female Rat Following Olfactory Bulb Removal," *J. Comp. Physiol. Psychol.* **74**, 374–382 (1971).
42. H. Mortimer, R. P. Wright, and J. B. Collip, "The Effect of Oestrogenic Hormones on the Nasal Mucosa; Their Role in the Nasosexual Relationship; and Their Significance in Clinical Rhinology," *Can. Med. Assoc. J.* **35**, 615–621 (1936).
43. R. P. Michael, J. Herbert, and J. Welegalla, "Ovarian Hormones and the Sexual Behaviour of the Male Rhesus Monkeys after Administration of Progesterone to Their Female Partners· Preliminary Communication," *Lancet* **1**, 1015–1016 (1966).
44. J. LeMagnen, "Physiologie des sensations: nouvelles donnees sur la phenomene de l'exaltolide," *Compt. Rend.* **230**, 1103 (1950) as referenced in M. G. Kalogerakis, "The Role of Olfaction in Sexual Development," *Psychosom. Med.* **25**, 420–432 (1963).
45. C. H. Best and N. B. Taylor, *The Physiological Basis of Medical Practice*, 6th ed., Williams & Wilkins, Baltimore, Md., 1955, p. 895, as referenced in M. G Kalogerakis, "The Role of Olfaction in Sexual Development," *Psychosom. Med.* **25**, 420–432 (1963).
46. W. K. Whitten, "The Effect of the Removal of the Olfactory Bulbs on the Gonads of Mice," *J. Endocrinol.* **14**, 160–163 (1956).
47. R. L. Doty et al., "Sex Differences in Odor Identification Ability: A Cross Cultural Analysis," *Neurophysiologia* **23**, 667–672 (1985).
48. R. A. Schneider and S. Wolf, "Olfactory Perception Thresholds for Citral Using a New Type of Olfactorium," *J. Appl. Physiol.* **8**, 337–342 (1955).
49. J. LeMagnen, "Physiologie des sensations-nouvelles données sur le phenomene de l'exaltolide," *C.R. Acad. Sci.* **230**, 1103–1105 (1950).
50. R. L. Doty et al., "Cyclical Changes in Olfactory and Auditory Sensitivity During the Menstrual Cycle: No Attenuation by Oral Contraceptives," in W. Briepohl, ed., *Olfaction and Endocrine Regulation*, IRL, London, 1982, pp. 35–42.
51. W. Velle, "Sex Differences in Sensory Functions," *Perspectives in Biology and Medicine* **30**, 491–522 (1987).
52. M. J. Rogel, "A Critical Evaluation of the Possibility of Higher Primate Reproductive and Sexual Pheromones," *Psychol. Bull.* **85**, 810–830 (1978).
53. B. W. L. Brooksbank, R. Brown, and R. Gustafson, "The Detection of 5α-androst-16-en-3α-ol in Human Male Axillary Sweat," *Experientia* **30**, 864–865 (1974).
54. B. H. Kingston, "The Chemistry and Olfactory Properties of Musk, Civet and Castoreum," Proceedings of the 2nd International Congress on Endocrinology, *Int. Congr. Ser.* **83**, 209–214 (1965).
55. A. M. Kligman and N. Shehadek, "*Pubic Apocrine Glands and Odour*," *Arch. Dermatol.* **89**, 461 (1964).
56. S. Bird and D. B. Gower, "The Validation and Use of a Radioimmunoassay for 5α-androst-16-en-3α-one in Human Axillary Collections," *J. Steroid Biochem.* **14**, 213–219 (1981).
57. M. Meredity et al., "Vomeronasal Pump; Significance for Male Hamster Sexual Behaviours," *Science* **207**, 1224–1226 (1980).
58. E. C. Crosby and T. Humphrey, "Studies of the Vertebrae Telencephalon," *J. Comp. Neurol.* **71**, 121 (1938).
59. J. Garcia-Velasco and M. Mondragon, "The Incidence of the Vomeronasal Organ in 1000 Human Subjects and Its Possible Clinical Significance," *J. Steroid Biochem. Mol. Biol.* **39**, 561–563 (1991).
60. N. D. Fabricant, "Sexual Functions and the Nose," *Am. J. Med. Sci.* **239**, 156–160 (1960).
61. M. Mozell et al., "Nasal Airflow," in T. V. Getchell et al., eds., *Smell and Taste in Health and Disease*, Raven Press, New York, 1991, pp. 481–492.
62. M. K. McClintock, "Menstrual Synchrony and Suppression," *Nature* **229**, 244–245 (1971).
63. D. B. Adams, A. R. Gold, and A. D. Burt, "Rise in Female Initiated Sexual Activity at Ovulation and Its Suppression by Oral Contraceptives," *N. Engl. J. Med.* **299**, 1145–1150 (1978).
64. R. P. Michael, R. W Bonsall, and P. Warner, "Human Vaginal Secretions: Volatile Fatty Acid Contents," *Science* **186**, 1217–1219 (1974).
65. M. Kirk-Smith et al., "Human Social Attitudes Affected by Androstenol," *Research Communications in Psychology, Psychiatry and Behavior* **3**, 379–381 (1978).

66. E. E. Filsinger, J. J. Braun, and W. C. Monte, "An Examination of the Effects of Putative Pheromones on Human Judgments," *Ethology and Sociobiology* **6**, 227–236 (1985).

67. R. Gustavson, E. Dawson, and G. Bonnett, "Androstenol, A Putative Human Pheromone, Affects Human (Homo Sapiens) Male Choice Performance," *J. Comp. Psychol.* **101**, 210–212 (1987).

68. I. P. Pavlov, *"Conditioned Reflexes,"* (G. V. Anrep, trans.) Oxford University Press, London, 1927.

69. A. R. Hirsch, "Nostalgia, The Odors of Childhood and Society," *Psychiatric Times* **9**(8), 29 (1992)

70. M. B Bowers, M. Van Woert, and L. Davis, "Sexual Behavior During L-dopa Treatment for Parkinsonism," *Am. J. Psychiatry* **127**, 6191–6193 (1971).

71. M. Hyyppa, U. K. Rinne, and V. Sonninen, "The Activating Effect of L-dopa Treatment on Sexual Functions and Its Experimental Background," *Acta Neurol. Scand.* **46**(Suppl. 43), 223–224 (1970).

72. H. R. Arkes, L. T. Herren, and A. M. Isen, "The Role of Potential Loss in the Influence of Affect on Risk-Taking Behavior," *Organizational Behavior and Human Decision Processes* **42**, 181–193 (1988).

73. H. Kluver and P. C. Bucy, "Psychic Blindness and Other Symptoms Following Bilateral Temporal Lobectomy in Rhesus Monkeys," *Am. J. Physiol.* **119**, 352–353 (1937).

74. R. L. Moss, "Modification of Copulatory Behaviour in the Female Rat Following Olfactory Bulb Removal," *J. Comp. Physiol. Psychol.* **74**, 374–382 (1971).

75. A. R. Hirsch and J. J. Gruss, "Olfactory Stimuli and Sexual Response in the Human Male," in J. Rose, ed., *The Proceedings of the World of Aromatherapy*, San Francisco, Calif., 1996, pp. 26–76.

76. T. Engen, *The Perception of Odors*, Academic Press, New York, 1982.

77. A. R. Hirsch, "Nostalgia: A Neuropsychiatric Understanding," *Advances in Consumer Research* **19**, 390–395 (1992).

78. J. Fox, "Improving Tactile Stimulation of the Blind," *Am. J. Ther.* **19**, 5 (1965).

79. A. R. Hirsch and J. J. Gruss, "Odors and Perception of Room Size," presented at the 148th Annual Meeting of the American Psychiatric Association, Miami, Fla. 1995.

80. J. Diamond, *Third Chimpanzee: Evolution and Future of the Human Animal*, Harper Collins, New York, 1992, p. 68.

81. D. Ackerman, *Natural History of the Senses*, Random House, New York, 1990, p. 26.

82. A. R. Hirsch and L. H. Johnston, "Odors and Learning," *J. Neurol. Orthop. Med. Surg.* **17**, 119–126 (1996).

83. A. R. Hirsch and S. Gay, "Effect of Ambient Olfactory Stimuli on the Evaluation of a Common Consumer Product," *Chem. Senses* **16**, 535 (1991).

84. A. R. Hirsch and R. Gomez, "Weight Reduction Through Inhalation of Odorants," *J. Neurol. Orthop. Med. Surg.* **16**, 28–31 (1995).

ALAN R. HIRSCH
Smell and Taste Treatment and Research Foundation
Chicago, Illinois

APPETITE

The term appetite does not have a single widely accepted meaning. Colloquially, it refers to a motivational state or desire, ordinarily for food but more generally for almost any reinforcer. Often the desire is not for food per se but for a specific food or nutrient (eg, salt). Hunger and appetite are often regarded as synonyms, although some investigators have distinguished between hunger as an objective depletion of energy or a specific nutrient and appetite as a subjective desire reflecting psychological factors more than physiological depletion. When people report a loss of appetite in conjunction with, say, an episode of clinical depression, the decline in the subjective desire for food is assumed to correspond to a suppression of the physiological correlates implicated in objective hunger processes; but it remains possible that subjective appetite and objective hunger might diverge under certain circumstances. Certainly the expression of an intense desire for an attractive dessert that appears unexpectedly at the end of a large meal cannot easily be reconciled with a simple depletion model of hunger.

People may report on appetite as a subjective state, but in accepting such reports at face value, one must proceed at one's own risk. First, people do not readily distinguish between their objective need for calories or nutrients (hunger) and their subjective desires (appetite). Second, few if any people can make sophisticated quantitative distinctions between different gradations of appetite or hunger. Indeed, it has been argued that people often infer the intensity of these internal states only retrospectively, by observing the amount of food they have consumed (1). Direct intro-(or viscero-)spection of hunger or appetite, except at the extremes of deprivation or satiation, may not be possible for the untrained subject. Thus, people may assume that they ate as much as they did because they were correspondingly hungry, rather than accept the less obvious or plausible explanation that they ate because of social influence or simply because the food was there (2). The possibility that intake might not be directly reflective of hunger or appetite also eludes many physiological researchers (behavioral neuroscientists), who accept as an axiom that all eating is driven by hunger and that the cessation is eating is necessarily a matter of the onset of physiological satiety.

To the extent that we rely on the amount consumed as an index of hunger or appetite, we may be misled by the fact that people often desire food but do not consume it even when it is available (as happens with dieters) and, obversely, people often consume food for which they have little or no reported desire (as occasionally happens under conditions of social pressure). Finally, much of the research on appetite is conducted on nonhuman animals, necessitating the substitution of physiological or consummatory assessments for verbal assessments. We readily assume the amount that an animal eats corresponds to its "subjective desire" for food, but of course we cannot be sure. Like humans, nonhuman animals often eat more in the presence of conspecifics (3), and we cannot simply infer that the presence of others increases hunger/appetite; it may well be that the presence of others increases intake directly, without affecting hunger/appetite.

Although we cannot complacently assume a direct correspondence between appetite and hunger, or between appetite and intake, the paucity of research on appetite (or hunger) as a phenomenon separate from intake demands that we suspend our concerns about the correspondence between desire and behavior and examine the factors that influence eating. Whether all of these factors operate through the mediator of subjective appetite is doubtful, but at this stage in the development of the research, we have little choice but to make that assumption. None of the research on nonhuman animals has connected appetite to intake, and even in humans, the majority of the research has focused on what is consumed, with a relative neglect of the corresponding subjective state. We are thus forced to shift our attention to the determinants of intake, on which a significant body of research has accumulated. These determinants may be classified as arising from the internal (physiological) and external environments. The role of appetite qua subjective state in this research is largely unexplored, but we may indulge ourselves with the probably only partly incorrect belief that eventually researchers will connect the subjective state of appetite to the more objective determinants of intake, both in humans and (somehow) even in nonverbal animals.

The factors that affect eating have not been systematically cataloged, let alone integrated into a complete systems approach to eating. We have elected to divide the influences on eating into internal and external categories, corresponding roughly to physiological versus environmental factors. Some factors, such as the palatability of the available food, depend jointly on the properties of food, the history of the organism, and the acute physiological state of the organism; such factors, obviously, do not fit neatly into the internal-external categorization. The internal and external distinction is also threatened by findings that demonstrate that through conditioning, external signals can stimulate hunger, or at least feeding (4). Likewise, the perception of attractive food triggers (cephalic) anticipatory digestive reflexes that in turn may affect feeding (5). By the same token, deprivation or other physiological manipulations can alter receptivity to external influences (6). Any comprehensive analysis of eating must contend with these mutual, interactive influences, but current research only hints at some of these complexities. In the absence of such a fully integrated view of eating, this article will be confined to a less satisfactory and somewhat arbitrary listing of influences.

PHYSIOLOGICAL INFLUENCES

As indicated, most laypeople and many researchers subscribe to an essentially physiological view of feeding. The feeding system is activated by various physiological events, most notably the progressive depletion over time of energy or more specific nutrients. This system is usually conceptualized as involving a central regulation of signals from the periphery, with eating as a tool in the service of homeostasis.

METABOLIC AND HORMONAL INFLUENCES

Metabolic factors governing the control of eating are generally separated into those associated with turning on eating (hunger) and those associated with turning off eating (satiety). Hunger (usually accompanied by eating) develops during the fasting phase of metabolism, after the ingestion and absorption of nutrients has been completed. Satiety (usually accompanied by cessation of eating) develops during the absorptive phase of metabolism, when a meal is being ingested and while nutrients are being absorbed from the intestine.

The principal sources of energy for all tissues are glucose and fatty acids, which may be drawn directly from the bloodstream. Energy reserves are stored in three different forms: protein, fat, and glycogen, which must be converted to glucose and fatty acids to be used. During the absorptive phase, metabolism is directed at the accumulation of these reserves; glucose and amino acids are converted to glycogen and fat, and amino acids are also stored as protein.

In the fasting phase, metabolic processes are aimed at utilizing energy. After a meal, blood glucose is used for energy needs, but as the supply of available glucose diminishes, energy reserves must be converted to usable forms. Glycogen is converted back to glucose and fat to fatty acid. These processes are associated with hunger. In the event of long-term deprivation or starvation, there is an increasing demand for energy derived from the conversion of amino acids to glucose, and fatty acids to ketones. These conversion processes provide the substrate for internal influences on eating.

Initiating Eating

Low levels of circulating glucose (and a corresponding higher rate of glycogen to glucose conversion) are important in the initiation of eating. Experimental manipulations that decrease glucose availability are a powerful means of stimulating eating (7). It is important to note, however, that a low level of circulating glucose is not an entirely accurate characterization of the metabolic trigger for eating. Consider untreated diabetics, who have a high level of circulating glucose but who are chronically hungry, because the glucose is not metabolically available owing to insufficient insulin, the hormone necessary for glucose uptake into cells. Thus, it is the unavailability of glucose that is the critical signal. Accordingly, because of its importance in clearing glucose from the bloodstream, insulin is considered an important hormone for the initiation of eating.

Recent research attempting to characterize more precisely the connection between blood glucose level and feeding indicates that meal onsets are preceded by small transient declines in blood glucose (8). If this decline in blood glucose is prevented, feeding will not occur. Feeding will not begin until a transient decline in blood glucose is experienced, supporting the notion that a drop in glucose availability is causally related to eating. Of special interest is the finding that the glucose-drop signal that initiates eating is of surprisingly short duration and small magnitude.

Whereas glucose appears to be important for regulating eating in the short term (ie, meal to meal), lipids appear to play a more important role in regulating food intake in the long term (9). During fasting, the body's fat reserves are converted to fatty acids for energy; this process probably provides an additional signal for turning on eating.

Terminating Eating

Physiological factors responsible for the cessation of eating can be divided into stomach and hormonal factors. Each of these contributes to what is typically referred to as satiety.

Stomach Factors. The stomach produces two types of satiety signal: one associated with the nutrient content of the meal and the other with the volume of the meal (10). It seems likely that these two meal-related signals are detected by separate mechanisms in the gut, because rats can regulate caloric intake independently from meal volume and meal volume can produce satiety cues that do not depend on caloric content.

Nutrient receptors in the stomach respond to the caloric density of a meal. A large but low-calorie meal would activate a large number of nutrient receptors but at a low rate, whereas a small but calorically dense meal would activate a small number of nutrient receptors at a high rate. In addition to this indirect effect on nutrient receptor activation rate, meal volume also induces satiety directly by activating stretch receptors in the stomach.

Current research indicates that stomach distension cues (which activate stretch receptors) and caloric cues (which activate nutrient receptors) are transmitted through separate channels. Stomach distension cues are transmitted to the brain by way of the vagus nerve, whereas stomach nutrient cues do not require the integrity of the vagus nerve and may therefore involve hormonal messengers (10).

Hormonal Factors. Although a number of different hormones have been implicated in satiety, the present discussion will briefly describe the influence of glucagon and cholecystokinin (CCK), the two hormones that have received the most attention as likely satiety agents. Glucagon is produced by islet cells of the pancreas as well as by cells in the gut. Animals treated with glucagon eat smaller than normal meals and animals treated with antibodies to glucagon eat larger than normal meals, suggesting that endogenous glucagon may contribute to meal termination. Because glucagon stimulates the conversion of glycogen (the stored form of glucose that cannot be directly utilized for energy) to glucose, the resultant increased levels of glucose released into the hepatic vein from the liver may stimulate glucose receptors and thereby decrease hunger signals.

Cholecystokinin has also received considerable attention as a signal associated with postprandial satiety (11). CCK is released from the duodenum (the upper portion of the small intestine) when food reaches the stomach. Animal and human studies indicate that systemic administration of CCK inhibits food intake through the normal behavioral sequence that characterizes satiety, without affecting water intake, implying that the behavioral effects of CCK are specific to feeding. Physiological studies indicate that CCK exerts its inhibitory effects on feeding through activation of vagus nerve input to the brain.

Brain Systems Controlling Eating

The Hypothalamus. Current notions regarding the organization of the brain's feeding system derive from early animal studies aimed at identifying brain regions responsible for turning on or turning off feeding. One of the original findings in this regard was that destruction of the medial hypothalamus, in particular the ventromedial hypothalamic nucleus (VMH), produced a behavioral syndrome characterized by severe overeating and ultimately obesity (12). Humans with VMH tumors display a similar behavioral profile, confirming across species the importance of the VMH for eating.

Initially, the VMH was viewed as the brain's satiety center, but it is now recognized that both the psychological and neural characteristics of satiety are far too complex to be accounted for solely by the VMH. However, it is generally agreed that the VMH and its connections are important for relaying satiety signals from the periphery to neural systems involved in turning off feeding. Recent evidence indicates that VMH lesions also destroy fibers projecting to the pituitary stalk and brain stem, both of which are important in the control of various hormones. Of relevance here are findings showing that VMH lesions increase insulin secretion and decrease glucagon secretion, a combination associated with overeating. Thus the overeating associated with VMH lesions may be secondary to the peripheral hormonal and metabolic effects of the lesions rather than a direct result of the missing VMH.

In contrast to the inhibitory role ascribed to the VMH, the lateral hypothalamus (LH) has become associated with stimulation of eating. Animal experiments reveal that lesions of the LH produce aphagia and adipsia, whereas stimulation of the LH produces eating responses (12). Although LH lesions produce aphagia and adipsia, artificially fed animals can eventually recover and maintain close to normal eating habits; this recovery indicates that the central nervous system is sufficiently plastic to enable other brain areas to take over, to some extent, the feeding functions of the LH. It should be noted, however, that although eating deficits can eventually recover, LH-lesioned rats seldom return to their prelesion weight. This finding suggests that the LH contributes to the establishment and maintenance of a set point for body weight, which may be altered permanently by the lesion.

Other Hypothalamic Structures Involved in Eating. Another important structure implicated in eating behavior is the paraventricular nucleus (PVN) of the hypothalamus. In fact, many of the effects associated with VMH manipulations have more recently been interpreted as PVN effects, owing to the discovery that VMH lesions destroy fibers originating from the PVN, some of which are associated with control of hormonal functions already described and others of which project to brain stem mechanisms involved in the control of eating. Moreover, it has been demonstrated that PVN lesions have effects on eating similar to those of VMH lesions. For these reasons, as well as for reasons to be discussed next, researchers have begun to view the PVN as a critical structure for the control of feeding.

Neurotransmitters and Eating. Any description of the neural basis of eating would be incomplete without a discussion of the neurotransmitter systems implicated in eating. Neurotransmitters are responsible for interneuronal communication. Thus, the activity of cells within particular brain regions is determined by the neurotransmitters that stimulate or inhibit them.

Norepinephrine, endorphins, galanin, and neuropeptide Y are transmitters that act in the PVN to facilitate food intake (13). Of special interest here is the finding that the stimulatory effects of norepinephrine in the PVN appear to be specific to carbohydrate intake (13). Elsewhere, growth hormone-releasing factor in the medial preoptic area of the hypothalamus stimulates eating (14). Neurotransmitter signals involved in inhibiting eating include dopamine and norepinephrine in the perifornical region of the hypothalamus as well as serotonin in the PVN (13). Interestingly, serotonin's inhibitory effect, like norepinephrine's stimulatory effect, is carbohydrate selective (13).

Although exactly how these transmitter signals are integrated during normal eating is still unclear, it is assumed that these chemical signals reflect food-relevant information derived from the periphery. For example, consider the regulation of carbohydrate intake. A high-carbohydrate meal increases tryptophan uptake in the brain and ultimately the synthesis of serotonin, for which tryptophan is the precursor. Increased serotonin activity, as discussed earlier, suppresses food intake, particularly carbohydrates. Thus increased carbohydrate intake eventually feeds back negatively on further carbohydrate intake (probably through serotonin's action in the PVN), providing a mechanism for the short-term regulation of carbohydrate intake.

Extrahypothalamic Influences on Feeding. Nonhypothalamic brain systems have also been implicated in the control of feeding. In particular, activation of the nigrostriatal and mesolimbic dopamine pathways, which use dopamine as a neurotransmitter, is associated with stimulatory effects on feeding. These pathways originate in the midbrain and project rostrally to striatal and limbic structures in the forebrain, with some of the axons involved coursing through the lateral hypothalamus en route. This anatomical fact has been taken to suggest that some of the feeding decrements associated with LH lesions may in fact be due to destruction of ascending dopamine neurons. Although the role of the nigrostriatal and mesolimbic dopamine pathways in feeding is not entirely understood, it is of interest to note that these pathways are crucial for reward and reinforcement functions in general (15), suggesting that activation of these systems may reinforce the sensory-motor associations involved in eating and also increase the rewarding properties of food or eating (16,17). Thus contrary to the conventional homeostatic view of eating regulation, these particular signals may be associated with nonregulatory influences on eating such as taste hedonics or learned feeding patterns.

CONDITIONED HUNGER

Ordinarily, the physiological substrate of appetite is thought to respond to energy considerations as previously outlined. However, these reactions are also subject to conditioning, such that particular environmental stimuli may, through repeated pairing, come to elicit the physiological states associated with the onset or offset of eating. For instance, it has been demonstrated that previously neutral stimuli repeatedly paired with the presentation of food can induce sated animals to continue eating (4). Visual, olfactory, or social cues associated with hunger may induce feeding in people who are not otherwise hungry; comparable cues that have been paired with satiety may likewise terminate eating in individuals who are not otherwise sated. It has been suggested that such external, conditioned cues do not control behavior directly, but rather operate on the physiological substrate of hunger and satiety; for example, the mere sight of attractive food cues (of the sort that have reliably predicted eating in the past) may induce physiological reactions (eg, insulin release) associated with hunger (18). Even temporal cues may stimulate appetite; people become hungry as mealtime approaches, but if for some reason the meal is skipped, hunger may subside (C. P. Herman, J. Polivy, and I. Biernacka, unpublished data, 1998).

SPECIFIC APPETITES AND NUTRIENT DEPLETIONS

The organism must concern itself not only with overall energy balance, but also with its inventory of the nutrients crucial to its well-being. Many well-known diseases (eg, scurvy and rickets) may appear in otherwise well-fed individuals who lack a particular vitamin or mineral. Specific appetites refer to hardwired cravings for certain substances the absence of which is detected and, wherever the food supply permits, rectified. It should be noted that whereas some deficits (eg, salt) produce clear cravings, not all deficiencies are detectable by the suffering organism.

Recently, speculation has arisen that the organism may regulate nutrients (especially macronutrients) not so much for their medicinal value as for their psychoactive effects. Most notably, a carbohydrate-craving hypothesis has been championed (19). It is argued that a deficiency of carbohydrates affects neurotransmitter levels to create a negative affective state; accordingly, individuals may learn to (over)eat carbohydrates in an attempt to bolster their mood, especially when they are feeling upset or depressed. This hypothesis has been offered to explain the overeating characteristic of bulimia nervosa and some forms of obesity.

SENSORY AND PERCEPTUAL FACTORS

Flavor

The term "flavor" is intended to encompass both gustatory (taste) and olfactory (smell) properties of food. Palatability is a matter of both aspects of flavor; when people say that a food tastes good, they tend to underestimate the contribution of smell. The palatability of food, which corresponds to how positively its flavor is rated and also includes certain secondary properties of the food such as its texture, is a major determinant of intake. Organisms are basically finicky, avoiding indiscriminate consumption in favor of selective consumption (where possible) of preferred foods. The finding that nonhuman animals consume more highly palatable food is compromised by the fact that palatability must often be inferred from intake itself; but humans also show a strong tendency to consume more of foods that they independently rate as more pleasant.

Palatability has a direct effect on intake, but whether palatability overrides other potentially competing considerations in controlling intake remains an active research question. It has been observed that the duration of the meal is largely a matter of the palatability of the available food but the likelihood of a rat initiating feeding is more closely tied to caloric deprivation (20). In short, palatability may affect some meal parameters more than others. In humans, extremes of food deprivation or satiety mute the effects of flavor; starving people tend to ignore the flavor of food and eat a great deal of unpalatable food if that is all that is available, whereas fully sated people eat very little of even quite palatable food. Interestingly, research indicates that at least under certain qualifying conditions hungry animals (including humans) do not display reduced finickiness; indeed, finickiness may be exaggerated both with respect to acceptance of good-tasting food and rejection of bad-tasting food (21,22). An apparent paradox has been identified in which "the animal eats . . . for taste when he needs calories [ie, when deprived]" (23). This paradox may be more apparent than real, in that the flavor of food is ordinarily confounded with its caloric density (ie, good-tasting foods are calorically denser, leading one humorist to describe the calorie as a term that scientists use to measure how good something tastes). Accordingly, the hungry animal may become more finicky as a way of maximizing energy intake in an energy deficiency crisis.

The potency of palatability as a determinant of eating may vary in different types of individuals. Obese people, for instance, have long been regarded as prone to overeat highly palatable foods (rather than food per se); this view was crystallized in the externality theory of obesity, which argues that obese people's eating is controlled virtually entirely by environmental cues, including taste, whereas normal-weight people's eating is more responsive to physiological cues of hunger and satiety (24). The observation that obese people are especially responsive to taste (24) may be meshed with the observation that hungry organisms are especially responsive to taste (23), if it is postulated that most obese people are chronically hungry as a result of their partially successful attempts to reduce their weight (25).

Other eating problems have likewise been associated with extreme partiality toward highly palatable foods. Although the evidence is conflictual (26), binge eating in normal-weight individuals is often interpreted as targeted specifically on highly palatable carbohydrates (27), which elevate serotonin levels and, ultimately, mood (19). Conceivably, this view might be extended in the direction of proposing that some flavors are deemed palatable precisely because of their central hedonic consequences.

Sensory Factors

If eating offset were associated solely with the accumulation of particular nutrient stores, then it would be difficult to account for the "dessert effect." That is, despite a satiating meal (fully repleting nutrient stores), people can still find room for dessert. One way of accounting for this effect is in terms of sensory specific satiety, which refers to an animal becoming satiated to a particular flavor without showing satiation to other normally preferred flavors (28). Thus the individual may become satiated to the sensory characteristics of the main course while not being satiated to the sensory characteristics of the dessert. (It should be noted that there may be other contributors to the "dessert effect," including social facilitation, as described later.)

Collateral support for the role of nongut sensory factors in satiety is found in studies of sham-feeding (esophagotomized) animals. In these animals, food is chewed and swallowed but does not reach the gut, and the nutrients are therefore not absorbed. Of special interest here is the fact that although these animals eat larger than normal meals (providing evidence for the involvement of postingestional factors in regulating eating offset), they do eventually stop eating. This has been interpreted to indicate that sensory aspects of the food (taste, olfaction, and texture) are sufficient to induce satiety-like effects on eating. A full explication of satiety will eventually have to incorporate and reconcile sensory and postingestional factors.

Perceptual Salience

Does the presence of food act merely to allow individuals to satisfy their preexisting hunger, or might the sight of food actually stimulate the appetitive drive, which would not have existed in the absence of food? One experiment found that normal-weight individuals ate the same amount (just under two sandwiches) regardless of whether they were initially presented with one sandwich (and had to obtain additional sandwiches from a refrigerator) or three sandwiches (29). This study suggests that the visual salience of food has a minimal impact on consumption. Obese subjects in this study, however, ate considerably more when presented with three sandwiches than when presented with only one, suggesting that visual cues have a strong effect on them. This finding was interpreted as support for the externality theory of obesity, with the visual salience of food representing an external cue par excellence. (One important implication of this study is that in the absence of excitatory external cues, obese people should eat less than do normal-weight people.) Another experiment found that normal-weight subjects ate the same amount of cashews regardless of whether the cashews were brightly illuminated or difficult to see (and regardless of whether they were instructed to think about the cashews or about something else), whereas obese subjects ate above-normal amounts of the visually salient cashews and below-normal amounts of the dimly illuminated cashews (30). Obese subjects also ate more when instructed to think about the cashews, reinforcing the point that it is awareness of food that matters and not simply optical effects. It should be noted that some researchers interpret these findings to mean that obese subjects eat in direct response to external cues and not because such cues stimulate hunger, which in turn drives eating. However, it has been suggested that exposure to food cues may trigger anticipatory (cephalic phase) reflexes at the physiological level, such that the sight or smell of palatable food may literally make the fat person hungry (18). Recently, we have shown that directing people's attention toward or away from food-related stimuli can increase or decrease their reported hunger (C. P. Herman, J. Ostovich, and J. Polivy, unpublished data, 1998); this effect was obtained in normal-weight nondieters, suggesting that the effects of food stimuli are not confined to the obese. Our study did not measure eating, however, so we cannot conclude that attentional or perceptual manipulations will affect intake.

Appetizer Effects

Does intake itself sometimes stimulate more intake? The term "appetizer" would suggest so, and common experience would seem to confirm that appetite can be stimulated by small delicacies. No compelling research has been conducted on this topic in humans, but if strong appetizer effects were confirmed, then the presumptive physiological mediation of such effects (ie, the appetizer triggers an anticipatory digestive reflex, which in turn demands satisfaction in the form of a complete meal) should be distinguished from alternative explanations (eg, the appetizer initiates a behavioral response sequence that is not hunger driven but that perseverates until satiety occurs). The role of behavioral momentum in feeding and other repetitive action patterns has not been adequately explored, although it has been argued that obese subjects overeat because of an inability to terminate repetitive behavior sequences (especially eating) once they have begun (31). This would suggest that appetizer effects might be stronger in the obese.

Preload studies, in which subjects are initially given a fixed amount of food and then permitted to continue ad lib, show a complex pattern. When subjects are classified as dieters (obese or normal weight) or nondieters, opposite reactions are observed, with nondieters eating ad lib in inverse proportion to preload size and dieters eating more following large preloads than following small preloads. The nondieter pattern would seem to contradict expectations based on an appetizer effect, and the data from dieters likewise do not fit in well, because a small preload (appetizer) would be expected to trigger the greatest amount of subsequent eating. Interpretations of these effects in dieters emphasize deliberate cognitive control of intake and its disruption, rather than the sort of analysis ordinarily invoked to explain the hypothetical appetizer effect (32).

EMOTIONAL FACTORS

It is readily agreed that emotional disturbance affects appetite and eating, but the precise nature of this effect is variable. In nonhuman animals, arousal tends to promote feeding. Nonspecific activation may render them more responsive to external, food-related stimuli, which direct behavior toward eating (33). (Such external responsiveness is presumably not specifically directed at food-related stimuli; rather, it focuses on whatever powerful behavior-relevant stimuli may dominate the animal's immediate perceptual environment, and might yield sexual, aggressive, or other stimulus-bound behavior depending on the environmental configuration that obtains when activation occurs.) It has been suggested that any sort of activation or drive induction might accentuate stimulus-bound eating in humans (C. P. Herman et al., unpublished data). Such stimulus-bound eating would not necessarily increase intake but rather make it more responsive to environmental conditions; if the food were bad-tasting, it might suppress eating. Such a mechanism might explain why hungry organisms sometimes eat less of a bad-tasting food than nondeprived organisms do (22,23).

Studies of arousal in humans tend to focus on particular valenced emotions (eg, anxiety, depression, and anger). In general, such negative emotions have developed a reputation for suppressing eating, even when palatable food is available. This effect is usually attributed to the sympathomimetic effects of emotional distress; these effects presumably act to suppress peripheral hunger signals. Clinical depression is indexed by a loss of appetite, among other criteria. Still, some depressed individuals tend to eat more and gain weight (34). Indeed, emotional agitation tends to increase eating among many people, to the point where emotional disturbance is often cited as a prime cause of weight gain and failure to maintain dietary restraint. How can these contradictory effects be reconciled?

It does appear to be the case that strong negative emotions produce physiological effects that oppose appetite. (It seems conceivable that minor agitation or disturbances might stimulate eating in humans much as seems to be the case in other animals, but such weak manipulations have not been explored.) Powerful distress (eg, intense clinical depression) probably suppresses appetite for virtually everyone, but short of overwhelming distress, emotional agitation appears to have opposite effects depending on the type of individual involved. Normal eaters, whose behavior is at least partially governed by the sort of hunger and satiety signals discussed previously, tend to respond to distress by eating less. Individuals who ordinarily attempt to suppress their eating (ie, dieters, including perhaps most of the obese), however, tend to eat more when they are upset (and possibly when elated, although studies of the effects of positive moods are so few as to defy conclusiveness). Dieters, because they must overcome normal physiological controls on eating if they are to succeed in reducing their intake, eventually dissociate their eating from such controls in favor of cognitive calculations of appropriate intake (see "Cognitive Factors"). Their intake is deliberately inhibited, but such inhibitions are vulnerable to disruption by (among other factors) emotional agitation. The emotionally distressed dieter, then, becomes disinhibited and overeats, unconstrained by the sort of normal satiety considerations that depend on physiological signals and conditioning, both of which are functionally atrophied in the dieter. The effects of distress may be exacerbated or attenuated depending on the type of distress involved; for example, physical threats tend to have a stronger suppressive effect, whereas ego threats tend to have a more facilitative effect (35; C. P. Herman et al., unpublished data, 1998). The exploration of overeating under conditions of distress remains a prominent concern of researchers studying obesity and especially bulimia nervosa.

SOCIAL FACTORS

Other people influence an individual's eating in a variety of ways. Social facilitation refers to increased intake in the presence of others; this usually occurs when these other people are eating substantial amounts as well (36). More typically, social inhibition of eating is encountered; for example, being observed by noneating others (D. A. Roth, et al., unpublished data, 1998) or eating along with others who eat very little (37) tends to reduce consumption. More generally, people seem to be highly vulnerable to social influences on eating, from indirect pressure (such as conformity to a model or experimental confederate) to more direct influences (such as requests or demands that a certain amount of food be eaten). These social effects are relatively powerful compared with other experimental manipulations of factors affecting eating and lend support to a nonhomeostatic view of eating, at least in the short term. Eating seems to be acutely responsive to social influences that bear little relation to an individual's physiological state or needs. Homeostatic considerations may eventually correct for such short-term deviations; for example, if a person undereats in a public spotlight, he or she may compensate by eating more later in a private setting. Another consideration is the likelihood that such social effects as copying others or not gorging when being watched serve a broader biological survival purpose. It is possible to benefit from others' experiences, and people may look to social guides when the appropriate amount or type of food to be consumed is ambiguous, as it often is. Furthermore, social norms may demand some sort of equity in the distribution of the food supply. Still it is clear that such social factors may have a profound effect on eating, and this effect may act in direct opposition to purely physiological needs and signals. Even 24-hour food-deprived experimental subjects, who ought to eat in response to powerful internal signals, are strongly influenced by the eating patterns of a model (38).

Another sort of social influence on eating occurs when people eat in a particular way to convey a certain impression. This impression-management view of eating focuses on eating as a self-presentational strategy (39). For example, women eat less when they are paired with a man than when paired with another woman, presumably because they wish to convey a more feminine impression to the man, and eating lightly is part of the feminine stereotype. Again, this sort of influence on eating seems to have little to do with the exigencies of internal state.

COGNITIVE FACTORS

The impact of both internal (eg, hunger) and external (eg, food salience) factors on eating is frequently mediated by cognition; people may decide to eat (or not eat) upon consideration of (or despite) the allure of such pressures. Because such internal and external factors have seemingly direct effects on eating (ie, because these factors can be interpreted as affecting eating directly, without the mediation of decisions that add little in the way of explanation) most analyses of eating do not bother to include deliberation or conscious choice as important elements. When we find that people eat in a manner opposed to both internal and external pressures, however, some sort of deliberative element seems necessary to account for the behavior. If someone were to continue eating an unpalatable food despite being sated, it would probably be necessary to have access to that individual's phenomenology to help provide an explanation. More commonly, people fail to eat despite their evident hunger and the availability of palatable food. Such dietary restraint demands an analysis that adds a set of mental factors to the control of eating in addition to physiological and environmental stimuli to which the person responds more or less reflexively (40). Such cognitive dietary calculations do not arise spontaneously, of course; they themselves are learned and may be reinforced or extinguished. But the prevalence (in humans, at least) of eating patterns that depart radically from the straightforward application of our knowledge of physiology and environmental stimulus control requires that attention be paid to willful opposition to such signals. One of the most serious problems arising from dieting is that the chronic overriding of normal controls on eating renders the dieter confused about whether or when she or he is feeling hunger or appetite. This problem is exacerbated in the recognized eating disorders (anorexia nervosa, bulimia nervosa, binge eating disorder).

INTERACTIONS AMONG RELEVANT FACTORS

A variety of examples have been presented of the nonadditive influence of factors affecting eating. For instance, hunger does not simply add to palatability as a determinant of eating, hungry people may eat more good-tasting food, as expected, but they may eat less bad-tasting food, depending on circumstances. Likewise, social influences may combine with, mask, or oppose hunger and satiety considerations in eating. An attempt has been made to reconcile the various influences on eating in a boundary model (41). The basic premise is that physiological influences will predominate when the person is particularly hungry or sated and that environmental and cognitive considerations will be most influential when the person is indifferent (neither hungry nor sated). This model has not been successful in predicting behavior, but its emphasis on the need to assess the influence of one factor (eg, social influence) in the context of other factors (eg, hunger-satiety) has proven fruitful experimentally. Research on the influence of one particular factor in isolation seems likely to misrepresent the importance of that influence; under certain conditions, its influence will be exaggerated, whereas under others it may be suppressed (42).

CONCLUSIONS

The scientific study of appetite has yielded a wide range of interesting phenomena. Still, the field is a painfully long way from achieving any sort of satisfying integration, in which the mutual and combined influences of various factors acting simultaneously might be predicted or explained. This conclusion is especially warranted in the case of human eating behavior, where social and cognitive factors are particularly evident. Such factors may apply as well to other animals. It can only be hoped that human and animal research, often seemingly at odds with each other, will eventually be synthesized into a comprehensive analysis of the determinants of feeding and appetite.

BIBLIOGRAPHY

1. D. J. Bem, "Self-perception Theory," in L. Berkowitz, ed., *Advances in Experimental Social Psychology*, Vol. 6, Academic Press, New York, 1972.
2. R. E. Nisbett and T. D. Wilson, "Telling More Than We Can Know: Verbal Reports on Mental Processes," *Psychol. Rev.* **84**, 231–259 (1977).
3. K. T. Hoyenga and S. Aeschleman, "Social Facilitation of Feeding in the Rat," *Psychonomic Sci.* **14**, 239–241 (1969).
4. H. P. Weingarten, "Conditioned Cues Elicit Feeding in Sated Rats: A Role for Learning in Meal Initiation," *Science* **220**, 431–433 (1983).
5. T. L. Powley, "The Ventromedial Hypothalamic Syndrome, Satiety, and a Cephalic Phase Hypothesis," *Psychol. Rev.* **84**, 89–126 (1977).
6. M. Cabanac, "Physiological Role of Pleasure," *Science* **173**, 1103–1107 (1971).
7. G. A. Bray and L. A. Campfield, "Metabolic Factors in the Regulation of Feeding and Body Energy Storage," *Metabolism* **24**, 99–117 (1975).
8. L. A. Campfield and F. J. Smith, "Functional Coupling between Transient Declines in Blood Glucose and Feeding Behavior: Temporal Relationships," *Brain Res. Bull.* **17**, 427–433 (1986).
9. N. Mrosovsky, "Body Fat: What Is Regulated?" *Physiol. Behav.* **38**, 407–414 (1986).
10. M. F. Gonzalez and J. A. Deutsch, "Vagotomy Abolishes Cues of Satiety Produced by Gastric Distention," *Science* **212**, 1283–1284 (1981).
11. G. P. Smith, "Gut Hormone Hypothesis of Postprandial Satiety," in A. J. Stunkard and E. Stellar, eds., *Eating and Its Disorders*, Raven, New York, 1984, pp. 67–76.
12. S. P. Grossman, "Contemporary Problems Concerning Our Understanding of Brain Mechanisms That Regulate Food Intake and Body Weight," in A. J. Stunkard and E Stellar, eds., *Eating and Its Disorders*, Raven, New York, 1984, pp. 5–14.
13. S. Liebowitz, "Brain Monoamines and Peptides: Role in the Control of Eating Behavior," *Federation Proc.* **45**, 1396–1403 (1986).
14. F J. Vaccarino and M. Hayward, "Microinjections of Growth Hormone-Releasing Factor into the Medial Preoptic Area/Suprachiasmatic Nucleus Region of the Hypothalamus Stimulate Food Intake in Rats," *Regulatory Peptides* **21**, 21–28 (1988).
15. F. J. Vaccarino, B. B Schiff, and S. E. Glickman, "Biological View of Reinforcement," in S. B Klein and R. R. Mowrer, eds., *Contemporary Learning Theories*, Erlbaum, Hillsdale, N.J., 1989, pp. 111–142.
16. K. R. Evans and F. J. Vaccarino, "Amphetamine and Morphine-Induced Feeding: Evidence for Involvement of Reward Mechanisms," *Neurosci. Biobehav. Rev.* **14**, 1–14 (1990).
17. N. M. White, "Control of Sensorimotor Function by Dopaminergic Nigroatriatal Neurons: Influence on Eating and Drinking," *Neurosci. Biobehav. Rev.* **10**, 15–36 (1989).
18. J. Rodin, "Has the Distinction between Internal Versus External Control of Feeding Outlived Its Usefulness?" in G. Bray, ed., *Recent Advances in Obesity Research*, Vol. II, Newman Publishing, London, 1978, pp. 75–85.
19. R. J. Wurtman, "Neurotransmitters, Control of Appetite, and Obesity," in M. Winick, ed., *Control of Appetite*, Wiley, New York, 1988, pp. 27–34.
20. J. Le Magnen, "Advances in Studies of the Physiological Control and Regulation of Food Intake," in E Stellar and J. M. Sprague, eds., *Progress in Physiological Psychology*, Vol 4, Academic Press, Orlando, Fla., 1971
21. P. Pliner, C. P. Herman, and J. Polivy, "Palatability as a Determinant of Eating, Finickiness as a Function of Taste, Hunger and the Prospect of Good Food," in E. D. Capaldi and T. L. Powley, eds., *Taste, Experience, and Feeding*, American Psychological Association, Washington, D.C., 1990, pp. 210–226.
22. N. Kauffman, C. P. Herman, and J. Polivy, "Hunger-Induced Finickiness in Humans," *Appetite* **24**, 203–218 (1995).
23. H. L. Jacobs and K. N. Sharma, "Taste Versus Calories: Sensory and Metabolic Signals in the Control of Food Intake," *Ann. New York Acad. Sci.* **157**, 1084–1125 (1969).
24. S. Schachter, "Some Extraordinary Facts about Obese Humans and Rats," *Amer. Psychol.* **26**, 129–145 (1971).
25. R. E. Nisbett, "Hunger, Obesity, and the Ventromedial Hypothalamus," *Psychol. Rev.* **79**, 433–453 (1972).
26. C. P. Herman and J. Polivy, "What Does Abnormal Eating Tell Us about Normal Eating?" in H. L. Meiselman and H. J. H. MacFie, eds., *Food Choice, Acceptance and Consumption*, Blackie Academic and Professional, London, 1996, pp. 207–238.
27. J. C. Rosen et al., "Binge Eating Episodes in Bulimia Nervosa: The Amount and Type of Food Consumed," *Int. J. Eating Disorders* **5**, 255–267 (1986).
28. B. J. Rolls, E. T. Rolls, and E. A. Rowe, "The Influence of Variety on Human Food Selection and Intake," in L. M. Barker, ed., *The Psychobiology of Human Food Selection*, AVT Publishing, Westport, Conn., 1982, pp. 101–122.
29. R. E. Nisbett, "Determinants of Food Intake in Obesity," *Science* **159**, 1254–1255 (1968).
30. L. Ross, "Effects of Manipulating Salience of Food upon Consumption by Obese and Normal Eaters," in S. Schachter and J. Rodin, eds., *Obese Humans and Rats*, Lawrence Erlbaum Assoc., Potomac, Md., 1974, pp. 43–52.
31. D. Singh, "Role of Response Habits and Cognitive Factors in Determination of Behavior of Obese Humans," *J. Personality Soc. Psychol.* **27**, 220–238 (1973).
32. C. P. Herman and J. Polivy, "Studies of Eating in Normal Dieters," in B. T. Walsh, ed., *Eating Behavior in Eating Disorders*, American Psychiatric Association Press, Washington, D.C., 1988, pp. 95–111
33. T. W. Robbins and P. J. Fray, "Stress-Induced Eating: Fact, Fiction or Misunderstanding?" *Appetite* **1**, 103–133 (1980).

34. J. Polivy and C. P. Herman, "Clinical Depression and Weight Change: A Complex Relation," *J. Abnormal Psychol.* **85**, 338–340 (1976).
35. T. F. Heatherton, C. P. Herman, and J. Polivy, "Effects of Physical Threat and Ego Threat on Eating Behavior," *J. Personality Soc. Psychol.* **60**, 138–143 (1991).
36. J. M. de Castro and E. S. de Castro, "Spontaneous Meal Patterns of Humans: Influence of the Presence of Other People," *Amer. J. Clin. Nutr.* **50**, 237–247 (1989).
37. J. Polivy et al., "Effects of a Model on Eating Behavior: The Induction of a Restrained Eating Style," *J. Personality* **47**, 100–112 (1979).
38. S. J. Goldman, C. P. Herman, and J. Polivy, "Is the Effect of a Social Model on Eating Attenuated by Hunger?" *Appetite* **17**, 129–140 (1991).
39. D. Mori and P. L. Pliner, "'Eating Lightly' and the Self-Presentation of Femininity," *J. Personality Soc. Psychol.* **53**, 693–702 (1987).
40. C. P. Herman and J. Polivy, "Restrained Eating," in A. J. Stunkard, ed., *Obesity*, Saunders, Philadelphia, 1980, 208–225.
41. C. P. Herman and J. Polivy, "A Boundary Model for the Regulation of Eating," in A. J. Stunkard and E. Stellar, eds., *Eating and Its Disorders*, Raven, New York, 1984, pp. 141–156.
42. C. P. Herman, "Human Eating: Diagnosis and Prognosis," *Neurosci. Biobehav. Rev.* **20**, 107–111 (1996).

C. P. Herman
F. J. Vaccarino
University of Toronto
Toronto, Ontario
Canada

APPLES. See Fruits, temperate.

APRICOTS. See Fruits, temperate.

AQUACULTURE

The Food and Agriculture Organization (FAO) of the United Nations has defined aquaculture as, "the farming of aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants" (1). Other definitions include "the rearing of aquatic organisms under controlled or semicontrolled conditions" and "underwater agriculture" (2). The list of organism groups mentioned in the FAO definition is not complete. Aquaculturists have also been involved in the production of echinoderms (sea urchins), cephalopods (octopus, squid, cuttlefish), reptiles (alligators, sea turtles, freshwater turtles), and amphibians (frogs). Aquaculture is an inclusive term that encompasses fresh, brackish, marine, and even hypersaline waters. The term mariculture is more restrictive in that it is usually defined as referring to aquaculture in saline environments.

Public sector aquaculture has historically involved production of aquatic animals to augment or establish recreational and commercial fisheries. More recently, it has also come to include attempts to recover threatened or endangered species by maintaining them in captivity and producing new generations that will ultimately be used to stock the waters where the species has become depleted. Public sector aquaculture is widely practiced in North America and to a lesser extent in other parts of the world. The FAO definition of aquaculture indicates that farming implies ownership of the organisms being cultured, which would seem to exclude public sector aquaculture.

Going hand in hand with attempts to recover endangered species are enhancement stocking programs aimed at releasing juvenile animals to build back stocks of aquatic animals that have been reduced due to overfishing. Examples of enhancement programs currently in existence include the stocking of cod in Norway, flounders in Japan, and red drum in the United States.

The bulk of global production from aquaculture is utilized directly as human food, with public aquaculture playing a minor role or being absent in many nations. Private aquaculture is not only about human food production, however. In some regions, well-developed private sector aquaculture is involved in the production of bait and ornamental fishes and invertebrates. The birth of aquaculture may have, in fact, been associated with attempts to produce ornamental fish to please royalty. Such might be the case with the development of colorful koi carp in China (koi are a colorful form of common carp, *Cyprinus carpio*).

Aquatic plants are cultured in many regions of the world. In fact, aquatic plants, primarily seaweeds, account for nearly 25% of the world's aquaculture production (3). Seaweed is widely used as food in the orient and elsewhere and can be found in many items that people use in their daily lives. Extracts from seaweed are important components of many pharmaceuticals, automobile tires, toothpaste, ice cream, and a wide variety of other products.

As implied, the origins of aquaculture may be rooted in China and could, in fact, go back some 4000 years. The ancient Egyptians may also have been early, if not the first aquaculturists. Pictographs in the tombs of the pharaohs can be interpreted to indicate that tilapia (fish native to North Africa and the Middle East) were being raised, possibly as food or display fish for the royal court. Regardless, Asia dominates the world in aquaculture production today (4). In North America, the culture of fish began in the nineteenth century and grew rapidly in the public sector after the establishment of the U.S. Fish and Fisheries Commission in 1871 (5). Private aquaculture existed as a minor industry for many decades, coming into prominence in the 1960s. Since then the United States has become one of the leaders in aquaculture research and development, although production, while significant at nearly 400,000 metric tons by 1996 (6), amounted to only about 2% of the world's total of 34 million metric tons.

The U.S. commercial aquaculture industry is dominated by channel catfish, trout, salmon, minnows, oysters, mussels, clams, and crawfish. A number of other fish and invertebrates are also being reared. Included are tilapia, striped bass and hybrid striped bass, red drum, goldfish,

34. J. Polivy and C. P. Herman, "Clinical Depression and Weight Change: A Complex Relation," *J. Abnormal Psychol.* **85**, 338–340 (1976).

35. T. F. Heatherton, C. P. Herman, and J. Polivy, "Effects of Physical Threat and Ego Threat on Eating Behavior," *J. Personality Soc. Psychol.* **60**, 138–143 (1991).

36. J. M. de Castro and E. S. de Castro, "Spontaneous Meal Patterns of Humans: Influence of the Presence of Other People," *Amer. J. Clin. Nutr.* **50**, 237–247 (1989).

37. J. Polivy et al., "Effects of a Model on Eating Behavior: The Induction of a Restrained Eating Style," *J. Personality* **47**, 100–112 (1979).

38. S. J. Goldman, C. P. Herman, and J. Polivy, "Is the Effect of a Social Model on Eating Attenuated by Hunger?" *Appetite* **17**, 129–140 (1991).

39. D. Mori and P. L. Pliner, "'Eating Lightly' and the Self-Presentation of Femininity," *J. Personality Soc. Psychol.* **53**, 693–702 (1987).

40. C. P. Herman and J. Polivy, "Restrained Eating," in A. J. Stunkard, ed., *Obesity*, Saunders, Philadelphia, 1980, 208–225.

41. C. P. Herman and J. Polivy, "A Boundary Model for the Regulation of Eating," in A. J. Stunkard and E. Stellar, eds., *Eating and Its Disorders*, Raven, New York, 1984, pp. 141–156.

42. C. P. Herman, "Human Eating: Diagnosis and Prognosis," *Neurosci. Biobehav. Rev.* **20**, 107–111 (1996).

C. P. Herman
F. J. Vaccarino
University of Toronto
Toronto, Ontario
Canada

APPLES. See Fruits, temperate.

APRICOTS. See Fruits, temperate.

AQUACULTURE

The Food and Agriculture Organization (FAO) of the United Nations has defined aquaculture as, "the farming of aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants" (1). Other definitions include "the rearing of aquatic organisms under controlled or semicontrolled conditions" and "underwater agriculture" (2). The list of organism groups mentioned in the FAO definition is not complete. Aquaculturists have also been involved in the production of echinoderms (sea urchins), cephalopods (octopus, squid, cuttlefish), reptiles (alligators, sea turtles, freshwater turtles), and amphibians (frogs). Aquaculture is an inclusive term that encompasses fresh, brackish, marine, and even hypersaline waters. The term mariculture is more restrictive in that it is usually defined as referring to aquaculture in saline environments.

Public sector aquaculture has historically involved production of aquatic animals to augment or establish recreational and commercial fisheries. More recently, it has also come to include attempts to recover threatened or endangered species by maintaining them in captivity and producing new generations that will ultimately be used to stock the waters where the species has become depleted. Public sector aquaculture is widely practiced in North America and to a lesser extent in other parts of the world. The FAO definition of aquaculture indicates that farming implies ownership of the organisms being cultured, which would seem to exclude public sector aquaculture.

Going hand in hand with attempts to recover endangered species are enhancement stocking programs aimed at releasing juvenile animals to build back stocks of aquatic animals that have been reduced due to overfishing. Examples of enhancement programs currently in existence include the stocking of cod in Norway, flounders in Japan, and red drum in the United States.

The bulk of global production from aquaculture is utilized directly as human food, with public aquaculture playing a minor role or being absent in many nations. Private aquaculture is not only about human food production, however. In some regions, well-developed private sector aquaculture is involved in the production of bait and ornamental fishes and invertebrates. The birth of aquaculture may have, in fact, been associated with attempts to produce ornamental fish to please royalty. Such might be the case with the development of colorful koi carp in China (koi are a colorful form of common carp, *Cyprinus carpio*).

Aquatic plants are cultured in many regions of the world. In fact, aquatic plants, primarily seaweeds, account for nearly 25% of the world's aquaculture production (3). Seaweed is widely used as food in the orient and elsewhere and can be found in many items that people use in their daily lives. Extracts from seaweed are important components of many pharmaceuticals, automobile tires, toothpaste, ice cream, and a wide variety of other products.

As implied, the origins of aquaculture may be rooted in China and could, in fact, go back some 4000 years. The ancient Egyptians may also have been early, if not the first aquaculturists. Pictographs in the tombs of the pharaohs can be interpreted to indicate that tilapia (fish native to North Africa and the Middle East) were being raised, possibly as food or display fish for the royal court. Regardless, Asia dominates the world in aquaculture production today (4). In North America, the culture of fish began in the nineteenth century and grew rapidly in the public sector after the establishment of the U.S. Fish and Fisheries Commission in 1871 (5). Private aquaculture existed as a minor industry for many decades, coming into prominence in the 1960s. Since then the United States has become one of the leaders in aquaculture research and development, although production, while significant at nearly 400,000 metric tons by 1996 (6), amounted to only about 2% of the world's total of 34 million metric tons.

The U.S. commercial aquaculture industry is dominated by channel catfish, trout, salmon, minnows, oysters, mussels, clams, and crawfish. A number of other fish and invertebrates are also being reared. Included are tilapia, striped bass and hybrid striped bass, red drum, goldfish, tropical fish, and shrimp. In the public sector, hatcheries produce large numbers of such species as salmon, trout, largemouth and smallmouth bass, sunfish, crappie, northern pike, muskellunge, walleye, and catfish for stocking or growout.

Global aquaculture production continues to grow annually, but increasing competition for suitable land and water, problems associated with wastewater from aquaculture facilities, disease outbreaks, and potential shortages of animal protein for aquatic animal feeds are becoming increasingly important. New technology, including the application of molecular genetic approaches to improving performance and disease resistance in aquaculture species, along with the development of water reuse (recirculating) systems, the establishment of offshore facilities, and the expansion of enhancement programs may provide the impetus for a resurgence in growth of the industry.

GLOBAL AQUACULTURE PRODUCERS AND PRODUCTION

Aquaculture production around the world grew at an average annual rate of 9.6% for the period 1984 through 1995 (3). In 1995, total production approached 28 million metric tons (3). Production jumped to 34.11 million metric tons in 1996 (6). A breakdown by type of organisms grown is presented in Table 1 for each year. The total value of the 1995 aquaculture crop exceeded $42 billion (3) and rose to $46.5 billion in 1996 (6).

The People's Republic of China is far and away the world's largest producer of aquaculture species. In 1995, production in China exceeded 17.5 million metric tons. India, in second place, produced slightly over 1.6 million metric tons, or less than 10% of China's production (3). In 1996, China's production exceeded 23 million metric tons, with India climbing modestly to 1.8 million metric tons (6). The top 20 aquaculture-producing nations during 1995 and 1996, in descending order, are shown in Table 2. The same countries are on both lists, but some changes in ranking are apparent. Most notable, perhaps, is the movement of Chile from ranking 16 to 12, reflecting the emphasis that country has been placing on aquaculture, particularly in the rearing of salmon.

U.S. aquaculture production actually dropped from 1995 to 1996 by 4.9%, and the United States dropped by one position in the top 20 from eighth to ninth (Table 2).

Table 1. Percentages of Total World Aquaculture Crop (by Weight) Associated with Different Types of Organisms during 1995 and 1996

Type of organism	1995 (%)	1996 (%)
Aquatic plants	24.5	22.7
Mollusks	18.3	24.9
Crustaceans	4.1	3.4
Finfish	52.8	48.8
Others	0.3	0.2
Total	*100.0*	*100.0*

Source. Refs. 3 and 6

Table 2. Top 20 Aquaculture-Producing Nations of the World in 1995 and 1996

Rank	1995	1996
1	People's Republic of China	People's Republic of China
2	India	India
3	Japan	Japan
4	South Korea	Philippines
5	Philippines	South Korea
6	Indonesia	Indonesia
7	Thailand	Thailand
8	United States of America	North Korea
9	Bangladesh	United States of America
10	Taiwan	Bangladesh
11	Norway	Norway
12	France	Chile
13	Italy	France
14	Viet Nam	Taiwan
15	North Korea	Spain
16	Chile	Italy
17	Spain	Viet Nam
18	Malaysia	United Kingdom
19	United Kingdom	Ecuador
20	Ecuador	Malaysia

Source. Refs. 3 and 6.

Of some importance is the fact that unlike most nations, where the bulk of the animals produced in aquaculture are used directly as human food, a considerable portion of U.S. aquaculture is associated with the production of bait minnows and ornamental fish. In 1997, aquaculture sales of tropical fish in the state of Florida were $57 million. Aquatic plant sales for species used in wetland restoration and aquaria netted an additional $13.2 million (7). The total value of U.S. aquaculture produce in 1996 was slightly more than $735 million (6).

SPECIES UNDER CULTIVATION

The emphasis here is on aquatic animal production, but as previously indicated, aquatic plants comprise nearly 25% of global aquaculture production by weight. Many hundreds of thousands of people are involved, worldwide, in aquatic plant production. The quantity of brown seaweeds, red seaweeds, green seaweeds, other types of algae, and miscellaneous aquatic plants such as watercress, water chestnuts, and ornamental plants produced in 1996 was estimated at more than 7.7 million metric tons (6). Several species of macroalgae are listed in Table 3. Microscopic algae and cyanobacteria are sometimes marketed as food or as a nutritional supplement (eg, *Spirulina* sp.). In addition, an undocumented quantity of algae (mostly of the single-celled variety) is produced for use as food for filter-feeding aquatic animals (primarily mollusks and zooplankton). Planktonic organisms such as rotifers are reared on algae and then used to feed the young of crustaceans and fish that do not accept prepared feeds.

Animal aquaculture is concentrated on finfish, mollusks, and crustaceans. Sponges, echinoderms, tunicates, turtles, frogs, and alligators are all being cultured, but production is insignificant in comparison with the three prin-

Table 3. Examples of Common and Scientific Names of Selected Aquaculture Species

Common name	Scientific name
Finfish	
African catfish	*Clarias gariepinus*
Atlantic halibut	*Hippoglossus hippoglossus*
Atlantic salmon	*Salmo salar*
Bighead carp	*Aristichthys nobilis*
Bigmouth buffalo	*Ictiobus bubalus*
Black crappie	*Pomoxis nigromaculatus*
Blue catfish	*Ictalurus furcatus*
Blue tilapia	*Oreochromis aureus*
Bluegill	*Lepomis macrochirus*
Brook trout	*Salvelinus fontinalis*
Brown trout	*Salmo trutta*
Catla	*Catla catla*
Channel catfish	*Ictalurus punctatus*
Chinook salmon	*Oncorhynchus tshawytscha*
Chum salmon	*Oncorhynchus keta*
Coho salmon	*Oncorhynchus kisutch*
Common carp	*Cyprinus carpio*
Fathead minnow	*Pimephales promelus*
Gilthead sea beam	*Sparus aurata*
Goldfish	*Carassius auratus*
Grass carp	*Ctenopharyngodon idella*
Largemouth bass	*Micropterus salmoides*
Milkfish	*Chanos chanos*
Mozambique tilapia	*Oreochromis mossambicus*
Mrigal	*Cirrhinus mrigala*
Mud carp	*Cirrhina molitorella*
Muskellunge	*Esox masquinongy*
Nile tilapia	*Oreochromis niloticus*
Northern pike	*Esox lucius*
Pacu	*Colossoma metrei*
Pink salmon	*Oncorhynchus gorbuscha*
Plaice	*Pleuronectes platessa*
Rabbitfish	*Siganus* spp.
Rainbow trout	*Oncorhynchus mykiss*
Red drum	*Sciaenops ocellatus*
Rohu	*Labeo rohita*
Sea bass	*Dicentrarchus labrax*
Shiners	*Notropis* spp.
Silver carp	*Hypophthalmichthys molitrix*
Smallmouth bass	*Micropterus dolomieui*
Sole	*Solea solea*
Steelhead	*Oncorhynchus mykiss*
Striped bass	*Morone saxatilis*
Walking catfish	*Clarias batrachus*
Walleye	*Stizostedion vitreum*
White crappie	*Pomoxis annularis*
Yellow perch	*Perca flavescens*
Yellowtail	*Seriola quinqueradiata*
Mollusks	
American oyster	*Crassostrea virginica*
Bay scallop	*Aequipecten irradians*
Blue mussel	*Mytilus edulis*
Northern quahog	*Mercenaria mercenaria*
Pacific oyster	*Crassostrea gigas*
Southern quahog	*Mercenaria campechiensis*
Crustaceans	
Blue shrimp	*Litopenaeus stylirostris*
Giant river prawn	*Macrobrachium rosenbergii*
Kuruma shrimp	*Marsupenaeus japonicus*
Marron crayfish	*Cherax tenuimanus*
Pacific white shrimp	*Litopenaeus vannamei*
Red claw crayfish	*Cherax quadricarinatus*
Red swamp crawfish	*Procambarus clarkii*
Tiger shrimp	*Penaeus monodon*
White river crawfish	*Procambarus acutus acutus*
White shrimp	*Penaeus setiferus*
Yabby crayfish	*Cherax destructor*
Algae (seaweeds)	
California giant kelp	*Macrocystis pyrifera*
Eucheuma	*Eucheuma cottoni*
False Irish moss	*Gigartina stellata*
Gracilaria	*Gracilaria* sp.
Irish moss	*Chondrus crispus*
Laminaria	*Laminaria* spp.
Nori or laver	*Porphyra* spp.
Wakame	*Undaria* spp.

cipal groups. Common and scientific names of many of the species of the finfish, mollusks, and crustaceans currently under culture are presented in Table 3. Included are examples of bait, recreational, and food animals.

Various species of carp and other members of the family Cyprinidae lead the world in terms of quantity of animals produced. In 1996, the total was more than 9.6 million metric tons (6). China is the leading carp-producing nation and is the world's leading aquaculture nation overall. Significant amounts of carp are also produced in India and parts of Europe. Species of carp where production exceeded 1 million metric tons in 1996 were silver carp, grass carp, common carp, and bighead carp.

Fish in the family Salmonidae (trout and salmon) are in high demand, with the interest in salmon being greatest in developed nations. Salmon, mostly Atlantic salmon, are produced in Canada, Chile, Norway, New Zealand, Scotland, and the United States. Fish in the family Cichlidae, which includes several cultured species of tilapia, are reared primarily in the tropics, but have been widely introduced throughout both the developed and developing world.

Catfish are not a major contributor to aquaculture production globally, but the channel catfish industry dominates U.S. aquaculture. Catfish production in the United States, primarily channel catfish, was 209,090 metric tons in 1992 (4) and 206,078 metric tons in 1995 (3). Total world catfish production in 1995, which includes walking catfish, was 312,000 metric tons.

Among the invertebrates, most of the world's production is associated with mussels, oysters, shrimp, scallops, and clams. Crawfish culture is of considerable importance in the United States but amounted to only 24,211 metric tons in 1992 (4), insignificant compared with some other invertebrate species. Globally, crawfish production was 29,350 tons, of which more than 28,000 tons was attributable to the red swamp crawfish (3).

Small amounts of crabs, lobsters, and abalone are being cultured in various nations. All three bring good prices in the marketplace but have drawbacks associated with their culture. Lobsters are highly cannibalistic. Rearing them separately to keep them from consuming one another during molting has precluded economic culture in most instances. Spiny lobster (*Panulirus* spp.) culturists produced a total of 69 metric tons in 1995 (3). Crab culture has also failed to develop in some parts of the world. In the United States, blue crab (*Callinectes sapidus*) has been attempted but has not been economically feasible, largely due to problems with cannibalism. Global production of crabs in the family Portunidae (of which *C. sapidus* is a member) during 1995 was only 20 metric tons. Chinese river crabs and various other species of marine crabs are being successfully cultured, with 1995 world production at more than 89,000 metric tons (3).

The dominant mollusks being cultured are oysters, clams, mussels, and to a lesser extent, scallops. Mollusk culture ranges from highly extensive to intensive. An example of one of those extremes is the placement of shell material (cultch) on the substrate (in public waters or a leased site) to provide additional substrate on which naturally produced oyster larvae (spat) can settle. That approach is highly extensive. At the other (intensive) extreme is producing and settling spat in a hatchery and rearing them on strings suspended from rafts or in trays.

Among the mollusks of aquaculture importance, abalones eat seaweeds and can only be reared in conjunction with a concurrent seaweed culture facility or in regions where large supplies of suitable seaweeds are available from nature. In some instances the value of the seaweed for direct human consumption may make the highest and best use of the plants.

Well over 100 species of aquatic animals are being cultured primarily for human food. That number expands to several hundred if ornamental, bait, and recreational species produced by aquaculturists are included. Many researchers have turned their attention to species for which there is demand by consumers, but for which the technology required for commercial production is marginal or not completely developed. Examples are dolphin, also known as mahimahi (*Coryphaena hippurus*), cobia (*Rachycentron canadum*), red snapper (*Lutjanus campechanus*), Pacific halibut (*Hippoglossus stenolepis*), southern flounder (*Paralichthys lethostigma*), winter flounder (*Pseudopleuronectes americanus*), American lobster (*Homarus americanus*), and blue crab (*Callinectes sapidus*). Each of the species mentioned is marine and has small eggs and larvae. Providing the first feeding stages with acceptable food has been a common problem, as has the fragility of the early life stages of many species, and the problem of cannibalism.

Fish with large eggs, such as trout, salmon, catfish, and tilapia, were among the first to be economically successful in modern times. Small eggs do not necessarily mean that sophisticated research is required to develop the technology required for successful culture. Carp, which have been cultured in China for millennia, have extremely small eggs. At the time the methodology for carp culture was developed, there were no research scientists, although there must have been dedicated farmers who used their common sense and trial-and-error methods to establish aquaculture.

In contrast, relatively large eggs do not necessarily mean that culture will be easy. Fish with very large eggs (eg, such marine catfish as the gaftopsail catfish, *Bagre marinus*) have eggs well over a centimeter in diameter but such low fecundity that the number of broodstock required to maintain a culture facility would doom the venture. Atlantic and Pacific halibut have eggs of about 3-mm diameter, much smaller than those of trout and salmon, but relatively large relative to many marine species, including other species of flatfish. Halibut eggs are relatively easy to hatch, but the larvae are extremely fragile so providing the nearly static conditions for development without causing water quality degradation is difficult. Nearly one month is required for egg incubation, followed by another approximately 35 days until first feeding, and then several additional weeks before metamorphosis. Following metamorphosis, halibut postlarvae become easy to maintain, but mortality rate from egg to metamorphosis is generally very high.

ECONOMICS

In nations where fish are produced for recreational fishing, funding for hatcheries is often raised from user fees such as fishing licenses. Such hatcheries are usually government facilities. For most private aquaculture companies to get started, outside funding is required. Funding may come through banks and other commercial lending sources or from venture capitalists. The high risks associated with aquaculture have made it difficult for many firms to obtain bank loans, although that situation is changing as bankers become more knowledgeable and comfortable with underwriting aquaculture ventures. Large corporations have also entered the aquaculture field. Some have abandoned aquaculture after a few years, but others continue to diversify into the arena. Highly profitable corporations may have an easier time obtaining the requisite funding for establishing aquaculture ventures than small entrepreneurs.

A key factor in obtaining funding support for aquaculture is development of a sound business plan. The plan needs to demonstrate that the prospective culturist has identified all costs associated with establishment of the facility and its day-to-day operation. One or more suitable sites should be identified and the species to be cultured selected before the business plan is submitted. Cost estimates should be verifiable. Having actual bids for specific tasks at specific locations, for example, pond construction, well drilling, building construction, and vehicle costs, in a particular geographic region will strengthen the business plan.

Land costs vary enormously both between and within countries. Compare, for example, the cost of coastal land in south Florida, where it might be possible to consider rearing shrimp, with that of Mississippi farmland suitable for catfish farming. The former might be thousands of dollars for every meter of oceanfront, while the latter may be obtained for one or two thousand dollars per hectare.

The amount of land required will also vary, not only as a function of the amount of anticipated production, but also on the type of culture system that is used. It may take several hectares of static culture ponds to produce the same biomass of animals as one modest size raceway through which large volumes of water are constantly flowing, for example. Construction costs vary from one location to another. Local labor and fuel costs must be factored into the equation. The experience of contractors in building aquaculture facilities is another factor to be considered.

Much of the world's aquaculture is conducted in tropical or subtropical regions that feature long growing seasons (often year-round). Many such areas lie within developing nations where labor and land are typically inexpensive and where governments sometimes offer tax incentives to prospective aquaculturists. Problems with obtaining good quality feed, parts, dependable energy supplies, and other infrastructure are common and represent a trade-off that needs to be considered. Skilled technical personnel, such as hatchery managers, may have to be brought into facilities sited in developing countries because individuals with the required credentials may not be available. Expatriate labor can be quite expensive but may be necessary.

The need for redundancy in the culture system needs to be assessed in conjunction with each facility. Failure of a well pump that brings up water to supply a static pond system may not be a serious problem in countries where new pumps can be purchased in a nearby town. However, it can be disastrous in developing countries where new pumps and pump parts are often not available but must be ordered from another country. Several weeks or months may pass before the situation can be remedied unless the culturist maintains a selection of spares. If the culture system requires constantly flowing water (eg, an open raceway system or a recirculating system), loss of flow due to pump failure for even a few minutes may result in disaster. A pump may break down, or there may be a power failure that results in pump failure. In either case, having a backup that will automatically come on to keep the system operational will help ensure against tragic losses. In the case of power failures, a backup diesel generator with an automatic switching mechanism is a popular choice.

Redundancy is not restricted to pumps but should be considered for all types of equipment where failure can lead to rapid loss of environmental control. The up-front costs of backup systems can be quite high, but loss of a crop may cause a business failure.

The business plan should provide projections of annual production. Based on those estimates and assumed food conversion ratios, an estimate of feed costs can be made. Food conversion ratio (FCR) is calculated by determining the amount of feed consumed for each unit of weight gain. For example, if a fish grows from 1 to 2 kg on 2 kg of feed, the FCR is 2 kg of feed consumed divided by 1 kg gain or 2.0. For many aquaculture ventures, between 40 and 50% of the variable costs involved in aquaculture can be attributed to feed.

Aquaculturists may elect to purchase animals for stocking or maintain their own broodstock and operate a hatchery. The decision may rest on such factors as the availability and cost of fry fish, postlarval fish, oyster spat, or other early life history stages in the location selected for the aquaculture venture. In regions where the species selected is being produced in large quantity (eg, channel catfish in Mississippi or penaeid shrimp in Thailand), sufficient local hatchery production may be available to supply a new venture. A catfish farmer in Nebraska or shrimp farmer in Alabama might find it more economical to establish a hatchery than to ship in young animals from long distances.

For aquaculturists working with banks and other types of lending institutions, money for land purchase, site preparation, and facility construction can be obtained through loans of 15 to 30 years. Equipment such as pumps and trucks are usually depreciated over a few years and are funded with shorter-term loans (often seven years). Operating expenses for such items as feed, chemicals, fuel, utilities, salaries, taxes, and insurance may require periodic short-term loans to keep the business solvent. The projected income should be based on a realistic estimate of farmgate value of the product and an accurate assessment of anticipated production. Each business plan should project income and expenses over the term of all loans to demonstrate to the lending agency or venture capitalist that there is a high probability the investment will be repaid. Realistic business plans often show losses for up to several years while the facility is being constructed and put into full production. Some aquaculturists even plan for an occasional crop loss when developing their long-range projections of profitability.

REGULATION

The extent to which governments regulate aquaculture varies greatly from one nation to another. In some parts of the world, particularly in developing nations, there has historically been little or no regulation. Inexpensive land and labor, low taxes, excellent climates, and a lack of government interference have drawn many aquaculturists to underdeveloped countries, most of which are in the tropics. Unregulated expansion of aquaculture in some countries has led to pollution problems and destruction of valuable habitats such as mangrove swamps and has enhanced the spread of disease from one farm to another. The need for imposing more restrictive regulations is now becoming evident around the world. Response to that need varies considerably from one nation to another.

Environmental problems associated with aquaculture have become a global phenomenon. In Japan, previously unrestricted development of net-pen culture of various kinds of marine fish in bays led to what was termed *self-pollution*. The waste feed and excretory products from the fish degraded water quality to the point that the aquacultured fish were unable to grow adequately or, in some cases, were unable to survive. Strict controls on the number of net-pens that could be established within each bay were established and the problem was adequately addressed. Similarly, strict regulations on aquaculture have been imposed throughout Europe in an attempt to prevent environmental deterioration. Inland aquaculture ventures on private lands have come under much less scrutiny and governmental control than those established in coastal regions in or adjacent to public waters.

The United States is an example of a mixture of local, state, and federal regulations. Permits from a county, state, or federal agency may be required for drilling wells, pumping water, releasing water, employing exotic species, constructing facilities, and so on. In the United States, most permits can be obtained at the local or state level. In some instances the federal government has devolved permitting authority to the states when state regulations are as rigorous or more so than national regulations. Federal agencies become involved when aquaculture projects are conducted in navigable waters (U.S. Army Corps of Engineers) or might impact threatened or endangered species (National Biological Service). For aquaculture ventures established in the marine environment outside of state waters, only federal permits are required. Once again, a U.S. Army Corps of Engineers permit will be required. With its mandate to regulate the nation's fisheries, the National Marine Fisheries Service will also become involved in the aquaculture permitting process in federal waters. Interest in establishing aquaculture operations in conjunction with operating or out-of-production oil and gas platforms has developed in recent years. For such a platform to be used for aquaculture, a permit from the Minerals Management Service will be required. During the period when the various permit applications are being reviewed, public hearings may be held and various other federal agencies may be provided with the opportunity to comment. The process can be long, arduous, and expensive whether in state or federal waters. It is not uncommon for the prospective aquaculturist to have to deal with more than a dozen agencies when seeking state permits.

In general, it is easier to establish an aquaculture facility on private land than in public waters such as a lake or coastal embayment. Prospective aquaculturists who want to establish facilities in public waters may be confronted at public hearings by outraged citizens who do not want to see an aquaculture facility in what they consider to be their water. The issue is highly contentious in some nations (eg, the United States). In other countries (eg, Japan), when properly conducted, aquaculture in public waters not only is seen as a good use of natural resources, but also can be considered an amenity.

Obtaining permits is often not simple. Few states have one office that can accommodate the prospective aquaculturist. In most cases it is necessary to contact a number of state agencies to apply for permits. As mentioned, public hearings may be required before permits are approved. The process can take months or even years to complete. The costs involved in going through the process may be prodigious. After the expenditure of considerable amounts of time and money, there is no guarantee that the permits will ultimately be granted.

Most states in the United States now have an aquaculture coordinator, usually housed in the state department of agriculture, who can assist prospective aquaculturists in finding a path through the permitting process. In other countries the process can vary from highly complicated to virtually unregulated. Anyone considering development of an aquaculture facility should become educated on the permitting process of the state or nation in which the facility will be developed. In cases where the process is involved, it should be initiated well in advance of the anticipated time of actual facility construction and, if possible, before land acquisition is completed.

CULTURE SYSTEMS

At one extreme, aquaculture can be conducted with a small amount of intervention from humans and the employment of little technology. At the other extreme, aquaculture involves total environmental control and the use of computers, molecular genetics, and complex modern technology. Many aquaculturists operate between the extremes. The range of culture approaches can be described as running from extensive to intensive, or even hyperintensive, with extensive systems being relatively simple and intensive systems being complex to very complex. In general, as the level of culture intensity increases, stocking density and, as a consequence, production per unit area of culture system or volume of water increase.

The most extensive types of aquaculture involve minimal human intervention to promote increases in natural productivity. One good example is the scattering of cultch material to provide substrates for oyster spat settlement as previously described. A second is the stocking of water bodies used in conjunction with recreational fishing.

The stocking of ponds, lakes, and reservoirs to increase the production of desirable fish that depend on natural productivity for their food supply and are ultimately captured by recreational anglers is practiced primarily in developed nations. In developing nations, community ponds are sometimes stocked to provide food for people living at the subsistence level. Some would consider the practice of stocking fish for recreation or subsistence as lying outside of the realm of aquaculture, but since it involves human intervention and often employs fish produced in hatcheries, such programs are certainly associated with, if not an actual part of, aquaculture. Since the stocked animals are expected to live off available natural food supplies in most instances (agricultural waste products are sometimes thrown into ponds by subsistence farmers to enhance productivity), production levels are often less than 100 kg/ha/yr.

Most of the aquaculture practiced around the world is conducted in ponds (Fig. 1). Ponds range in size, but production units are generally 0.1 to 10 ha in area. The intensity of aquaculture in ponds can range from a few kilograms per hectare to thousands of kilograms per hectare of annual production. Production levels in the U.S. channel catfish industry were only a few hundred kilograms per hectare in the early 1960s and rose to about 3000 kilograms per hectare by the end of that decade. A decade

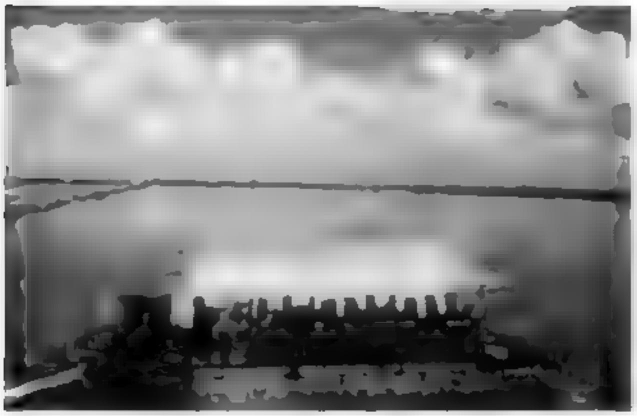


Figure 1. Catfish culture pond in Texas with paddlewheel aerator used to increase dissolved oxygen level, as necessary.

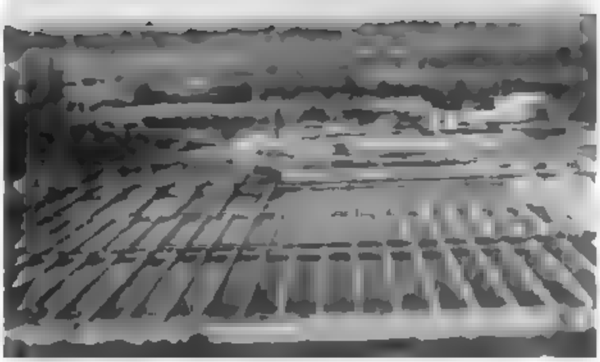


Figure 2. Concrete raceways at a trout farm in Idaho.

Figure 3. Circular tanks for the commercial production of tilapia in geothermal water in Idaho.

later, some farmers were able to produce 8000 to 10,000 kg/ha/yr in open ponds. Technological advances in feed quality, control of diseases, and water quality management were among the factors that allowed for the increased production rates.

The first step up from the recreational stocking or subsistence level of culture is to fertilize the water to increase natural productivity. Fertilization encourages algae blooms, which in turn stimulate the production of plankton and benthic organisms, all of which can provide a source of nutrients to the target culture species. Moving up in intensity from fertilization is the provision of supplemental feeds, which are those that provide some additional nutrition but cannot be depended upon to supply all the required nutrients. Provision of complete feeds, those that do provide all of the nutrients required by the culture species, translates to another increase in intensity. Associated with one or more of the stages described might be the application of techniques that lead to the maintenance of good water quality. Examples are continuous water exchange, mechanical aeration, and the use of various chemicals to adjust such factors as pH, alkalinity, and hardness.

With the application of increased technology and control over the culture system, intensity continues to increase. Utilization of specific pathogen-free animals, provision of nutritionally complete feeds, careful monitoring and control of water quality, and the use of animals bred for good performance can lead to impressive production levels.

Where water is plentiful and inexpensive, raceway culture is an attractive option and one that allows for production levels well in excess of what is possible in ponds. Trout are frequently reared in linear raceways from hatching to market size. Linear raceways are longer than they are wide and are usually no deeper than 1 to 2 in. (Fig. 2). High-density raceways used in production facilities are commonly constructed of poured concrete. Small raceways of the type used in hatcheries and research facilities may be constructed of fiberglass or other resilient materials. Water is introduced at one end and flows by gravity through the raceway to exit the other end. Circular raceways, called tanks (Fig. 3), are also used by aquaculturists. Tanks are usually no more than 2 in. deep and may be from less than 1 m to as much as 10 m in diameter. Concrete tanks can be found, but most are constructed of fiberglass, metal, or wood that is sealed and covered with epoxy or some other waterproof material. Plastic liners are commonly used in metal or wood tanks to prevent leakage, and in the case of metal, to avoid exposing the aquaculture animals to trace element toxicity.

Linear raceways are commonly used by trout and salmon culturists both for commercial production and for hatchery programs conducted by government agencies to produce fish for stocking. Large numbers of state and federal salmon hatcheries in Washington and Oregon, along with governmental and private hatcheries in Alaska, collect and fertilize eggs, hatch them, and rear the young fish to the smolt stage at which time they become physiologically adapted to enter seawater. It is following smoltification that the fish are transported to release sites. Migrating adults will have imprinted on the hatchery water as juveniles and can be counted upon to return to their hatcheries of origin with few exceptions.

Commercial salmon culturists can rear their fish to market size in freshwater raceways, although most salmon are grown from smolt to market size or adulthood in the marine environment, either as free roaming fish or in confinement. Releasing smolts into the open marine environment is called ocean ranching and is a technique that takes advantage of the homing instinct of salmon. When the fish that had been released as smolts return to spawn, sufficient numbers of adults are collected for use as broodfish to continue the cycle. The remainder may be harvested by the aquaculturist or by commercial fishers after which the fish are processed and marketed.

Salmon, steelhead trout, and a variety of marine fish are currently being reared in net-pens (Fig. 4). The typical salmon net-pen is several meters (sometimes as much as 20 m) on each side and may be 10 m or more deep (1). Smaller units, called cages, are sometimes used by freshwater culturists. Cages tend to have volumes of no more than a few cubic meters (Fig. 5).

Net-pen technology was developed in the 1960s but has only been widely employed commercially for salmon production since the 1980s, when the Norwegian salmon farming industry was developed. The Japanese began producing large numbers of sea bream and yellowtail in net-pens during the 1960s. Other nations have used the technology as well. Most of the net-pens currently in production are located in protected waters since they are easily damaged or destroyed by storms.

Competition by various user groups for space in protected coastal waters in much of the world has led to strict controls and, in some cases, prohibitions against the establishment of inshore net-pen facilities. As a result, there is growing interest in developing the technology to move offshore. Various designs for offshore net-pens have been developed and a few have been tested (Fig. 6). A number of different designs, including systems that are semisubmersible or totally submersible, have been able to withstand storm waves of at least 6 m, but the costs of those systems are very high compared with inshore net-pens, so commercial viability may be difficult to achieve unless the species being reared is of very high value. Tuna, for example, are being cultured to a limited extent and can bring very high prices, particularly if they are of high quality, for the Japanese sushi trade. Some success in spawning and larval rearing of tuna has been achieved, but in most instances juvenile fish are captured and placed in net-pens for growout to market size. Depending on the size at which the fish are stocked, the net-pen growout period may require only a few months.

The highest level of intensity that can be found in aquaculture is associated with reuse systems, often called recirculating systems. In these systems, the bulk of the wa-

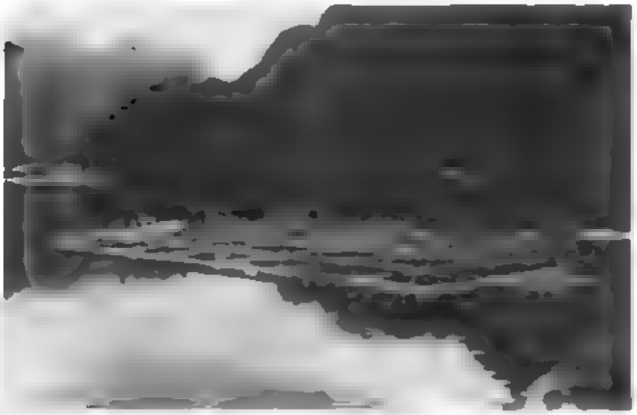


Figure 4. A marine net-pen facility in Norwegian fjord.

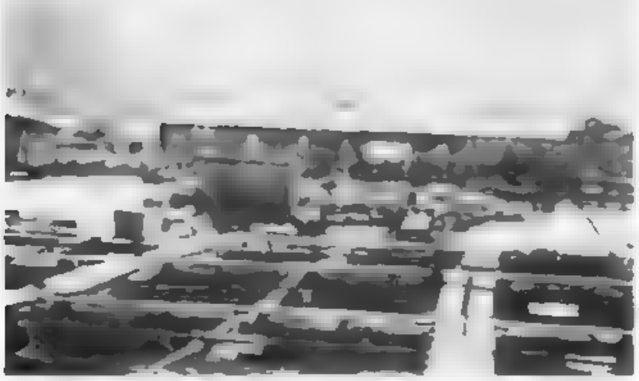


Figure 5. Marine cages in Malaysia.

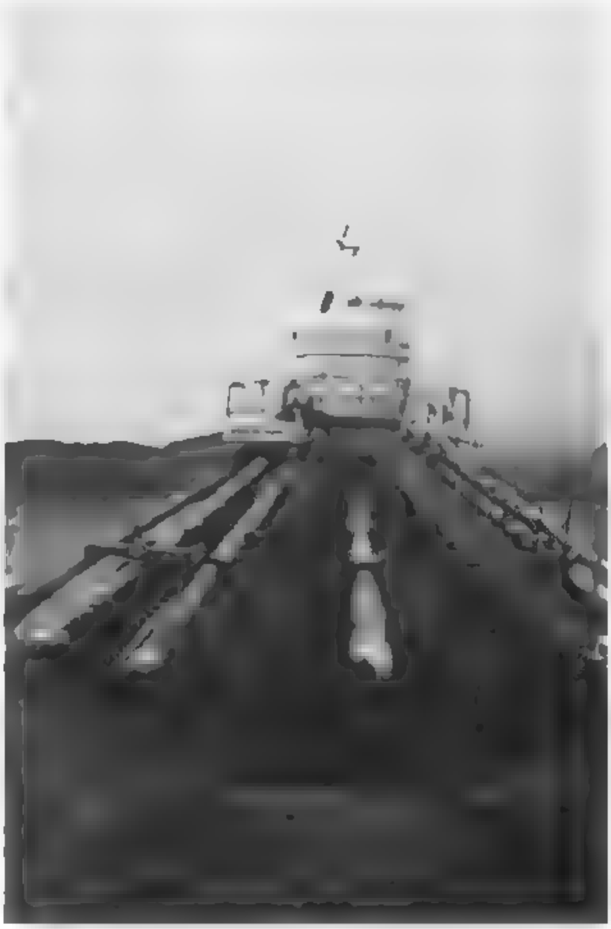


Figure 6. A salmon net-pen designed for use in areas where wave heights during storms may reach as high as 6 m.

ter passing through the chambers in which the finfish or shellfish are held is continuously treated and reused. Once filled initially, reuse systems can theoretically be operated for long periods of time without much water replacement. It is necessary to add some water to such systems to make up for that lost to evaporation and splashout and in conjunction with solids removal.

Recirculating systems can be used for all phases of culture, from broodstock maintenance through spawning, hatching, larval rearing, to growout. High energy requirements because of the requirement to run pumps and aerators 24 hours a day, and sometimes greatly increased if water must be heated or chilled, have rendered many recirculating systems uneconomical. Exceptions include systems used for the production of high-priced products (such as ornamental species), those where water and/or heat is basically free (such as systems that employ power plant cooling water), or when only part of the rearing cycle employs recirculating technology (eg, fingerling production in a reuse system followed by pond growout).

Many of the recirculating systems in use today are operated in a mode between entirely closed and completely open (open systems typically have sufficient flow rates to exchange the water in the culture chambers every hour or less). In most systems there is a significant percentage of replacement water added either continuously or intermittently on a daily basis. Such partial recirculating systems may exchange from a few percent to several hundred percent of system volume each day.

The heart of a recirculating water system is the biofilter, a device that contains a medium on which bacteria that help purify the water become established (Fig. 7). Fish and aquatic invertebrates produce ammonia as a primary metabolite. If not removed or converted to a less toxic chemical, ammonia can quickly reach lethal levels. Two genera of bacteria are responsible for ammonia removal in biofilters. The first, *Nitrosomonas*, converts ammonia (NH_3) to nitrite (NO_2^-). The second, *Nitrobacter*, converts nitrite to nitrate (NO_3^-). Nitrite is highly toxic to aquatic animals, although nitrate can be allowed to accumulate to relatively high levels. If both genera of bacteria are active, the conversion from ammonia through nitrite to nitrate is so rapid that nitrite levels remain within the safe range.

In addition to the biofilter and culture chambers, recirculating systems typically also employ one or more settling chambers or mechanical filters to remove solids such as unconsumed feed, feces, and mats of bacteria that slough from surfaces within the system. Each recirculating system requires a mechanical means of moving water from component to component. That usually means mechanical pumping, though air-lifts can also be used. Most systems incorporate one pump to lift water and rely on gravity to provide flow through the various system components and back to the pump (Fig. 8). That approach reduces the need to balance flow rates through two or more pumps and reduces operating costs.

Control of circulating bacteria and oxidation of organic matter can be obtained through ozonation of the water. Ozone (O_3) is highly toxic to aquatic organisms. Ozone must be allowed to dissipate prior to exposing the water to the aquaculture animals. With time, and with the assis-

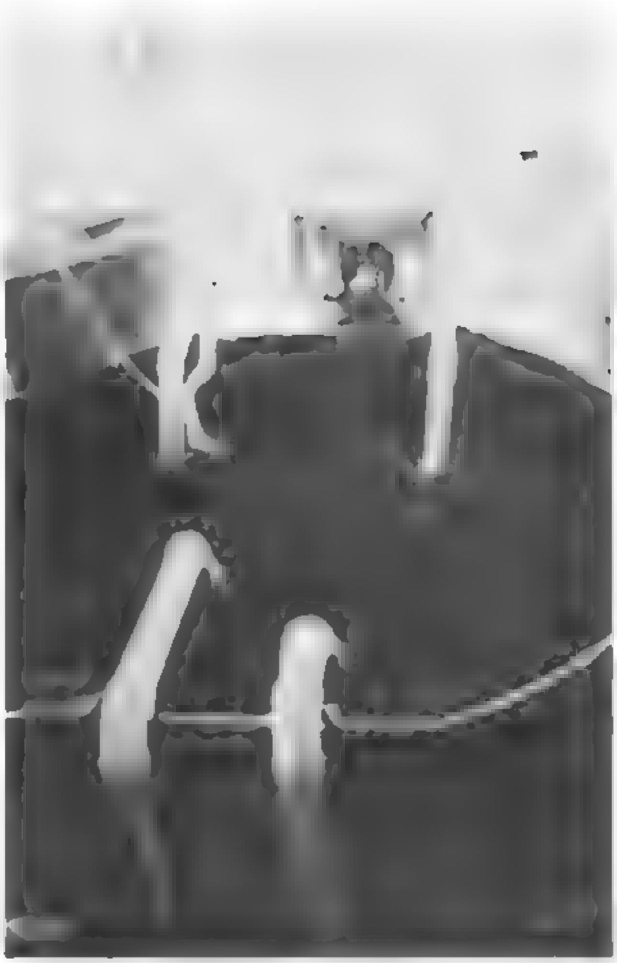


Figure 7. A circular tank filled with plastic balls to which bacteria attach is one type of biofilter that can be used by aquaculturists; in this case, the biofilter helps maintain water quality at a baitstand in Texas.

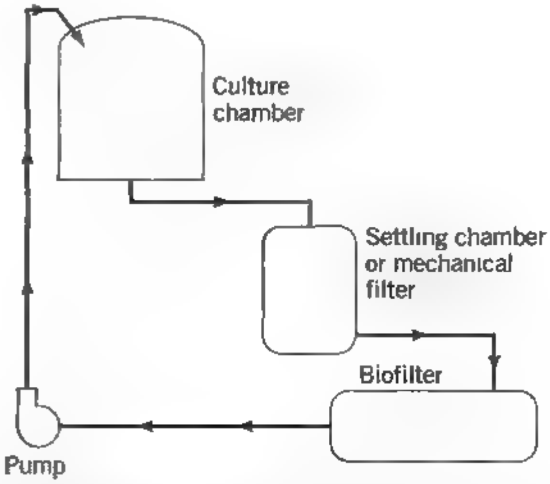


Figure 8. Schematic representation of a typical closed water system design.

tance of aeration, ozone can be driven off or converted to molecular oxygen. Various commercial firms market ozone generators and can assist aquaculturists in selecting the proper equipment to meet system needs.

Ultraviolet (uv) light has also been used to sterilize the water in aquaculture systems. The effectiveness of uv decreases with the thickness of the water column being treated, so the water is usually flowed past uv lights as a thin film (alternatively, the water may flow through a tube a few centimeters in diameter that is surrounded by uv lights). Ultraviolet light systems require more routine maintenance than ozone systems. The bulbs emit lower and lower quantities of uv light with time and need to be changed periodically. In addition, organic material exposed to uv light will foul the surface of the transparent quartz or plastic tubes separating the bulbs from the water, thereby creating a film between the water and the uv source that reduces the effectiveness of the uv light.

Recirculating systems often feature other types of apparatus, such as foam strippers and supplemental aeration. Devices for denitrifying nitrate to nitrogen gas have been developed to the point that they may find a place in commercial culture systems in the near future.

The technology associated with recirculating systems is expensive. Redundancy in the system, that is, providing backups for all critical components, and automation are important considerations. When a pump fails, for example, the failure must be instantly communicated to the culturist, who must have the ability to keep the system operating while the problem is being addressed. Loss of a critical component for even a few minutes can result in the loss of all animals within the system. Computerized water-quality monitoring systems that will sound alarms and dial emergency telephone numbers to report system failures are finding increased use among culturists who employ recirculating systems. The computer can also be used to put backup systems on line in the event of a primary system failure.

Recirculating systems can make aquaculture feasible in locations where conditions might otherwise not be conducive to successful operations. Tropical and subtropical animals such as tilapia and penaeid shrimp have, for example, been reared in recirculating systems in the northern tier of states in the United States. Recirculation systems can also be used to reduce transportation costs by making it possible to grow animals near markets. In areas where there are concerns about pollution from aquaculture system effluents or with the use of exotic species, recirculating systems provide an alternative approach to more extensive types of operations.

WATER SOURCES AND QUALITY

Sources of water for aquaculture include municipal supplies, wells, springs, streams, lakes, reservoirs, estuaries, and the ocean. The water may be used directly from the source or it may be treated in some fashion prior to use.

Most municipal water sources are chlorinated and contain sufficiently high levels of chlorine so as to be toxic to aquatic life. Chlorine can be removed by passing the water through activated charcoal filters or through the use of sodium thiosulfate metered into the incoming water at the proper rate. Municipal water is usually not used in aquaculture operations that require large quantities of water, either continuously or periodically, because of the initial high cost for the water and the cost of pretreatment to remove chlorine.

Most aquaculturists, if asked, would probably indicate a preference for well water over other sources. Both freshwater and saline wells are common sources of water for aquaculture. The most commonly used pretreatments for well water include temperature alteration (either heating or cooling); aeration to add oxygen or to drive off or oxidize such substances as carbon dioxide, hydrogen sulfide, and iron; and modification of salinity (in mariculture systems). Pretreatment may also include adjusting hardness and alkalinity through the application of appropriate chemicals. For example, limestone ($CaCO_3$) is commonly used to increase both hardness and alkalinity.

To heat or cool water requires large amounts of energy. A major consideration in locating an aquaculture facility is to have not only a sufficient supply of water but also water at or near the optimum temperature for growing the species that has been selected. The vast supply of spring water of almost perfect temperature in the Hagerman Valley of Idaho supports the majority of the rainbow trout production in the United States. Where geothermal water is available, tropical species can be grown in locations where ambient winter temperatures would otherwise not allow them to survive. Tilapia farmers have been successful in parts of Idaho and other states where geothermal water of suitable quality is available. Not all geothermal wells produce water of suitable quality, however. High levels of sulfur and other toxic chemicals are commonly found in geothermal water, so thorough testing should precede any attempt to use such water sources for aquaculture.

Another large cost associated with incoming water is associated with its movement. Many aquaculture facilities that utilize surface waters and those that obtain their water from wells other than artesian wells are required to pump the water into their facilities. Pumping costs can be a major expense, particularly when the facility requires continuous inflow or high volumes of makeup water to replace evaporative losses from ponds.

Surface water can sometimes be obtained through gravity flow by locating aquaculture facilities at elevations below those of adjacent springs, streams, lakes, or reservoirs. Coastal facilities may be able to obtain water through tidal flow.

The most common treatment of incoming surface water is removal of particulate matter. This can be effected through the use of settling basins or mechanical filters. Particle removal may involve the reduction or elimination of suspended inorganic material such as clay, silt, and sand. It may also involve removal of organic material, including living organisms. Organisms that will enter aquaculture facilities if not filtered from the incoming water include phytoplankton and zooplankton, plants and plant parts, macroinvertebrates, and fish. Some of the organisms, if not removed, can be expected to survive and grow to become predators on, or competitors with, the target aquaculture species. Very small organisms, such as bacteria, can be removed mechanically though filtering particles of a few microns in size; such removal becomes inordinately expensive if there are large volumes involved. In most cases, filtration of incoming water involves passing the water through screens of various sizes, the smallest of which is often no smaller than a window screen. If a situation calls for elimination of microorganisms, other forms of water treatment, such as ozonation and the use of uv radiation, are more efficient and effective than filtration.

For many freshwater species that can be characterized as warmwater (such as channel catfish and tilapia with an optimum of about 30°C) or coldwater (such as trout with an optimum of about 20°C), the conditions outlined in Table 4 should provide an acceptable environment. So-called midrange species are those with an optimum temperature for growth of about 25°C (examples are walleye, northern pike, muskellunge, and yellow perch). Typically, midrange species do well under the conditions, other than temperature, specified in Table 4 for coldwater species. Some species have higher or lower tolerances than others. For example, tilapia can tolerate temperatures in excess of 34°C but have poor tolerance for low temperature. Most tilapia species die when the temperature falls below about 12°C. Tilapia have a remarkably high tolerance for ammonia compared with such species as trout and salmon, which have a high tolerance for cold water but cannot tolerate water temperatures much above 20°C. Marine fish may be able to tolerate a wide range of salinity (such euryhaline species include many species of flounder, red drum, salmon, and some species of shrimp), or they may have a narrow tolerance range (they are called stenohaline species, examples of which are dolphin, halibut, and lobsters). Recommended water quality conditions for marine fish production systems are presented in Table 5.

The water quality criteria for each species should be determined from the literature or through experimentation when literature information is unavailable. Synergistic effects that occur among water quality variables can have an influence on the tolerance a species has under any given set of circumstances. Ammonia is a good example. Ionized ammonia (NH^+) is not particularly lethal to aquatic animals, but unionized ammonia (NH^3) can be toxic even when present at a fraction of a part per million (depending on species). The percentage of unionized ammonia in the water at any given total ammonia concentration changes in relation to such factors as temperature and pH. As either temperature or pH increases so does the percentage of unionized ammonia relative to the level of total ammonia. Thus, in warmwater and in seawater (which generally has a higher pH than freshwater), ammonia toxicity will occur at a lower total ammonia concentration than in cool or lower pH water.

Table 4. General Water Quality Requirements for Trout and Warmwater Aquatic Animals in Fresh Water

Variable	Acceptable level or range	
	Coldwater	Warmwater
Temperature, °C	<20	26–30
Alkalinity, mg/L	10–400	50–400
Dissolved oxygen, mg/L	>5	~5
Hardness, mg/L	10–400	50–400
pH	6.5–8.5	6.5–8.5
Total ammonia, mg/L	<0.1	<1.0
Ferrous iron, mg/L	0	0
Ferric iron, mg/L	0.5	0–0.5
Carbon dioxide, mg/L	0–10	0–15
Hydrogen sulfide, mg/L	0	0
Cadmium, μg/L	<10	<10
Chromium, μg/L	<100	<100
Copper, μg/L	<25	<25
Lead, μg/L	<100	<100
Mercury, μg/L	<0.1	<0.1
Zinc, μg/L	<100	<100

Source: Refs. 2, 8, and 9.

Table 5. Suggested Water Quality Conditions for Marine Fish Production Facilities

Variable	Acceptable level or range
Temperature, °C	1–40 (depends on species)
Salinity, g/kg	1–40 (depends on species)
Dissolved oxygen, mg/L	>6
pH	<7.9–8.2
Total ammonia, μg/L as NH_3	<10
Iron, μg/L	100
Carbon dioxide, mg/L	<10
Hydrogen sulfide, μg/L	<1
Cadmium, μg/L	<3
Chromium, μg/L	<25
Copper, μg/L	<3
Mercury, μg/L	<0.1
Nickel, μg/L	<5
Lead, μg/L	<4
Zinc, μg/L	<25

Source. Ref. 10.

Another example is dissolved oxygen (DO). The amount of DO that water can hold at saturation is affected by both temperature and salinity. The warmer and/or saline the water, the lower the saturation DO level. Atmospheric pressure also affects oxygen saturation. The saturation oxygen level decreases as elevation increases.

Biocides should not be present in water used for aquaculture. Sources of herbicides and pesticides include runoff from agricultural land, contamination of the water table, and spray drift from crop-dusting activity. Excessive levels of phosphorus and nitrogen may occur where runoff from fertilized land enters an aquaculture facility either from surface runoff or groundwater contamination. Trace metal levels should be low as indicated in Tables 4 and 5.

Most aquaculture facilities release water constantly or periodically into the environment without passing it through a municipal sewage treatment plant. The effects of aquaculture effluents on natural systems have become a subject of intense scrutiny in recent years and have, in some instances, resulted in opposition to further development of aquaculture facilities in some locales, particularly in public waters. There have even been demands that some existing operations should be shut down.

Regulation of aquaculture varies greatly both between and within nations. Some governmental agencies with jurisdiction over aquaculture have placed severe restrictions on the levels of such nutrients as phosphorus and nitrogen that can be released into receiving waters. Regulations on suspended solids levels in effluent water are also common. The installation of settling ponds or created wetlands, exposure of the water to filter feeding animals that will remove solids, and mechanical filtration have been used to treat effluents. Reduction or removal of dissolved nutrients through tertiary treatment is possible but is generally not economically feasible with current technology. Research is currently under way to develop feeds containing reduced levels of nutrients or to provide nutrients in forms that the culture animals can better utilize. The goal in both approaches is to reduce losses of nutrients to the environment through excretion.

NUTRITION AND FEEDING

Problems associated with excessive levels of nutrients and unwanted nuisance species have already been mentioned. In some cases, aquaculturists use intentional fertilization to produce desirable types of natural food for the species under culture. Examples of this approach include inorganic fertilizer applications in ponds to promote phytoplankton and zooplankton blooms that provide food for young fish such as channel catfish in the United States, the development of algal mats (called lab-lab) through fertilization of milkfish ponds in Asia, and the use of organic fertilizers (from livestock and human excrement) in Chinese carp ponds to encourage the growth of phytoplankton, macrophytes, and benthic invertebrates. In the latter instance, various species of carp with different food habits are stocked to ensure that all the types of natural foods produced as a result of fertilization are consumed.

Provision of live foods is currently necessary for survival of the early stages of many aquaculture species because acceptable prepared feeds have yet to be developed. Algae is routinely cultured for the early stages of mollusks produced in hatcheries. Once the mollusks are placed in growout areas, natural productivity is depended on to provide the algae upon which the shellfish feed.

In cases where zooplankton are reared as food for predatory larvae or fry, it may be necessary to maintain three cultures (algae, zooplankton, and the desired aquaculture species). Though wild zooplankton have been used successfully in some instances (eg, in Norway, wild zooplankton have been collected and fed to larval Pacific halibut), it is much more common practice to provide rotifers or brine shrimp nauplii as zooplanktonic food for first-feeding stages of animals being reared for marketing. After being fed rotifers and/or brine shrimp nauplii for periods ranging from several days to several weeks, depending on the species being reared, the aquaculture animals can usually be trained to accept pelleted feeds, though a weaning process during which both natural and prepared feeds are offered is sometimes required. Problems associated with utilizing prepared feeds from first feeding include difficulty in providing very small particles that contain all the required nutrients, loss of soluble nutrients into the water from small particles before the animals consume the feed, and in some cases, the fact that prepared feeds do not behave the same as live foods when placed in the water. For species that are sight feeders, behavior of the food is often an important factor. Feed color may also be a factor, as is texture, in whether a food item is accepted. Some fish, for example, reject hard pellets but will accept pellets that have a more spongy texture.

Some of the most popular aquaculture species accept prepared feeds from first feeding. Included are catfish, tilapia, salmon, and trout. All of the fish listed have relatively large eggs (several millimeters in diameter) that develop into fry that have concomitantly large yolk sacs. The nutrients in the yolk sac lead to production of first-feeding fry with well-developed digestive tracts that produce the enzymes required to efficiently digest diets that contain the same types of ingredients used for larger animals. First-feeding fry of catfish and so on have mouth gapes of sufficient size that they can engulf particles that do contain all essential nutrients.

Over the last few decades, fish nutritionists have successfully determined the nutritional requirements of many aquaculture species and have developed practical feed formulations based on those requirements. For species such as Atlantic salmon, various species of Pacific salmon and trout, common carp, channel catfish, and tilapia, sufficient information exists to design diets precisely suited to each species. Aquaculturists are always interested in the development of new species. In each instance, the nutritional requirements of the new species must be investigated. Although there are many similarities among aquatic animals with respect to nutritional requirements, diets that produce the best growth at the least cost will vary significantly from species to species and can only be formulated when precise nutritional requirements are known. Determination of those requirements may require several years of research, although diets based on existing formulations may be at least adequate to produce reasonable growth and survival and can be employed while the research is being conducted.

Requirements for energy, protein, carbohydrates, lipids, vitamins, and minerals have been determined for the species commonly cultured (11). As a rule of thumb, trout and salmon diets will, if accepted, support growth and survival in virtually any aquaculture species. Such diets often serve as the control against which experimental diets are compared.

Since feeds contain substances other than those required by the animals of interest, studies have also been conducted on antinutritional factors in feedstuffs and on the use of additives. Certain feed ingredients contain chemicals that retard growth or may actually be toxic Examples are gossypol in cottonseed meal and trypsin inhibitor in soybean meal. Restricting the amounts of the feedstuffs used in a particular diet is one way to avoid problems. In some cases, as is true of trypsin inhibitor, proper processing can destroy the antinutritional factor. In this case, heating of soybean meal is effective.

Animals that do not readily accept pelleted feeds may be enticed to do so if the feed carries an odor that induces ingestion. Color development is an important consideration in aquarium species and some animals produced for human food. External coloration is desired in aquarium species. Pink flesh in cultured salmon is desired by much of the consuming public. Coloration, whether external or of the flesh, can be achieved by incorporating ingredients that contain pigments or by adding extracts or synthetic compounds. One class of additives that impart color is the carotenoids.

Prepared feeds are marketed in various forms from very small crumbles to flakes and pellets of various sizes. Pelleted rations may be hard, semimoist, or moist. Hard pellets typically contain less than 10% water and can be stored under cool, dry conditions for at least 90 days without deterioration of quality. Dry pellets should, however, always be stored in a cool, dry place. Semimoist pellets are chemically stabilized to protect them from degradation and mold if they are properly stored (either refrigerated or in a cool, dry place), while moist pellets that contain high percentages of water must be frozen if they are not used immediately after manufacture.

Hard pellets are the type preferred if the species under culture will accept them. Semimoist feeds are most commonly used in conjunction with feeding young fish and species that find hard pellets unpalatable. Semimoist diets tend to be very expensive and can often only be used economically in conjunction with early life history stages where only small amounts of feed are required. Moist feeds, which contain high percentages of fresh fish, are usually available only in the vicinity of fish-processing plants.

Moist and semimoist feed pellets are produced in machines similar to sausage grinders. The most widely used types of prepared feeds, hard pellets, are produced by pressure pelleting or extrusion (crumbles are produced by grinding larger pellets). Pressure pelleting involves pushing the ground and mixed feed ingredients through holes in a die that is a few centimeters thick to produce spaghetti-like strands of the desired diameter. The strands are cut to length as they exit the die. Steam is often injected into the pellet mill in a location that exposes the feed mixture to moist heat just before the mix enters the die. Exposure to steam improves binding and extends pellet water stability. In general, a pelleted diet should remain stable in water for at least 10 minutes so the fish have sufficient opportunity to consume the feed before it dissolves.

Extruded pellets are produced by exposing the ground and mixed ingredients to much higher heat and pressure and for a longer time than is the case with pressure pellets. In the extrusion process the ingredients undergo some cooking that can be beneficial in reducing the levels of certain antinutritional factors, such as trypsin inhibitor. There may be concomitant losses of heat labile nutrients such as vitamin C, so overfortification to obtain the desired level in the final product may be required. In some cases, a heat labile nutrient is dissolved in oil and sprayed on the pellets after they have exited the extruder.

Specialty feeds such as flakes can be made by running newly manufactured pellets through a press or through use of a double drum dryer. The latter type of flakes begin as a slurry of feed ingredients and water. When the slurry is pressed between the hot rollers of the double drum dryer, wafer thin sheets of dry feed are produced that are then broken into smaller pieces. The different colors observed in some tropical fish foods represent a mixture of flakes, with each of the different colored flakes containing one or more different additives that impart color.

Pressure pellets sink when placed in water, whereas under the proper conditions, floating pellets can be produced through the extrusion process. That is accomplished when the feed mixture contains high levels of starch. When heated, starch expands and the pellets produced will trap air as they leave the barrel of the extruder. The trapped air gives the pellets a density of less than 1.0. Floating pellets are desirable for species that come to the surface to feed, because the aquaculturist can visually determine that the fish are actively feeding and can control daily feeding rates based on observed consumption.

Sinking extruded pellets are used for shrimp and other species that will not surface to obtain food and require pellets with high water stability. Shrimp consume very small particles, so they will nibble pieces from a pellet over an extended period of time. Unless heavily fortified with binders, pressure pellets will dissolve before shrimp have adequate time to fully consume them. Extruded feeds, whether sinking or floating, may remain intact for up to 24 hours after being placed in the water. Pressure pellets begin to disintegrate after a few minutes, unless, as mentioned, supplemental binders are incorporated into the feed mixture.

Nearly all aquaculture feeds contain at least some animal protein since the amino acid levels in plant proteins cannot meet the requirements of most aquatic animals. Fish meal is the most commonly used source of animal protein in aquaculture feeds, though blood meal, poultry by-product meal, and meat and bone meal have also been successfully used. In 1995 more than 15,500,000 tons of prepared feeds were manufactured for use by the world's aquaculture producers. To make that amount of feed, 1,728,000 tons of fish meal were used. That amounted to 25.6% of the world's fish meal production (12).

Commonly used plant proteins include cornmeal, cottonseed meal, peanut meal, rice, soybean meal, and wheat. A number of other ingredients have also been used, many of which are only locally available. Most formulations contain a small percentage of added fat from such sources as fish oil, beef tallow, or more commonly, oilseed oils such as corn oil and soybean oil.

Complete rations contain added vitamins and minerals. Purified amino acids, binders, carotenoids, and antioxidants are other components found in many feeds. Growth hormone and antibiotics are sometimes used. Regulations on the incorporation of hormones along with other chemicals and drugs into aquatic animal feeds are in place in the United States and some other countries (Table 6). Few such regulations have been promulgated in developing nations.

Feeding practices vary from species to species. It is important not to overfeed since waste feed not only means wasted money, it can also lead to degradation of water quality. Most species require only 3 to 4% of body weight

Table 6. Therapeutants and Disinfecting Agents Approved for Use in U.S. Aquaculture

Name of compound	Use of compound
Therapeutants	
Copper	Antibacterial for shrimp
Formalin	Parasiticide for various species
Furanace (Nifurpyrinol)	Antibiotic for aquarium fishes
Oxytetracycline (Terramycin)	Antibiotic for fish and lobsters
Sodium chloride	Osmoregulatory enhancer for fish
Sulfadimethoxine (Romet)	Antibacterial for salmonids and catfish
Trichlorofon (Masoten)	Parasiticide for baitfish and goldfish
Disinfectants	
Calcium hypochlorite (HTH)	Used in raceways and on equipment
Didecyl dimethyl ammonium chloride (Sanaqua)	Used in aquaria and on equipment
Povidone-iodine compounds (Argentyne, Betadine, Wescodyne)	Disinfection of fish eggs

Source: Ref. 13.

in dry feed daily for optimum growth. Very young and adult animals are exceptions. Young animals are fed at higher rates because they are growing rapidly and consume a greater percentage of body weight daily than older animals. In addition, it is important to have food readily available to them. Food should be spread evenly over the culture chamber area so the young animals do not have to expend a great deal of energy searching for a meal. That is easily possible in tanks and small raceways, but large culture units do not lend themselves to having feed spread across the entire water surface. In those instances the feed can be spread along the pond or raceway's sides. Feeding rates as high as 50% of body weight daily are not uncommon for young animals. Since total biomass is small, even in intensively stocked units such as raceways, the economic cost is not high. Water quality in raceways can be maintained by siphoning out waste feed periodically. In ponds, any unconsumed feed acts as fertilizer, and the quantities used are not high enough to affect water quality adversely. Over time as the animals become increasingly mobile, the feeding rate can be reduced to satiation, which will often be about 10% of body weight daily for early juveniles and will fall to the previously mentioned 3 to 4% as the animals grow. Broodfish and other adult animals, such as ornamental species, being held in captivity are often fed at a maintenance level, which is usually about 1% of body weight daily.

Young animals may be fed several times daily. Examples include the standard practices of feeding fry channel catfish every three hours and young northern pike as frequently as every few minutes. Keeping carnivorous species such as northern pike satiated helps reduce the incidence of cannibalism. Animals stocked in growout culture chambers may be fed several times a day, but it is common practice to feed only once or twice to satiation. In warm weather, feed should be provided early in the day (but after making certain that the dissolved oxygen level is within the optimum range) and late in the afternoon. As the water cools in the fall in temperate climates, feeding rate is often reduced. Channel catfish farmers often feed during winter on warm days or every three days (unless there is ice cover) at a rate of no more than 1% of body weight at each feeding. The fish will overwinter without being fed but will lose weight. Weight loss can be averted by providing a modest amount of feed during winter.

REPRODUCTION AND GENETICS

Species such as carp, salmon, trout, channel catfish, and tilapia have been bred for many generations in captivity, though they usually differ little in appearance or genetically from their wild counterparts. A few exceptions exist, such as the leather carp, a common carp strain selectively bred to produce only one row of scales, and the Donaldson trout, a strain of rainbow trout developed over numerous generations to grow more rapidly to larger size and with a stouter body than its wild cousins.

Selective breeding has been long practiced as a means of improving aquaculture stocks. In some instances it has not been possible or is at least quite difficult and expensive to produce broodstock and spawn them in captivity, so culturists continue to rear animals obtained from nature. Most of the species that are being reared in significant quantities around the world are produced in hatcheries using either captured or cultured broodstock. Milkfish is a notable exception. That species has been spawned in captivity, but most of the fish reared in confinement are collected as juveniles in seines and sold to fish culturists. Wild shrimp postlarvae continue to be used to stock ponds in some parts of the world, though hatcheries may also be available in the event sufficient numbers of wild postlarvae are unavailable in a given year. In the United States, where shrimp culture involves the use of exotic species, all the animals reared come from hatcheries, both in the United States and in Latin America.

Spawning techniques vary widely from one species to another. Tilapia and catfish are typically allowed to spawn in ponds. Artificial nests are provided for catfish while tilapia dig their own nests in the pond bottom. Fertilized eggs can be collected from the mouths of female tilapia, but it is common practice to collect schools of fry after they are released from the mother's mouth to forage on their own. Catfish lay eggs in adhesive masses. Spawning chambers such as milk cans and grease cans are placed in ponds and may be examined every few days for the presence of egg masses. Some catfish farmers allow the eggs to hatch in the pond, though most farmers collect eggs and incubate them in a hatchery.

Adult Pacific salmon die after spawning. Females are usually sacrificed by cutting open the abdomen to release the eggs. Milt is obtained by squeezing the belly of males. Trout and Atlantic salmon can be reconditioned to spawn annually. Eggs are usually obtained from those species in the same fashion as from male Pacific salmon. Banks of egg-hatching trays, called *Heath trays*, through which water is flowed are typically used to hatch trout and salmon eggs (Fig. 9).

Unlike catfish, tilapia, trout, and salmon that produce from several hundred to several thousand relatively large eggs per female, many marine species produce large numbers of very small eggs. Hundreds of thousands to millions of eggs are produced by such species as halibut, flounder, red drum, striped bass, and shrimp. Catfish, salmon, and trout spawn once a year, while tilapia and some marine species spawn repeatedly at intervals of a few days to a few weeks for as long as several months if the proper environmental conditions are maintained (2).

Fish breeders have worked with varying degrees of success to improve growth and disease resistance in several species. As genetic engineering techniques are adapted to aquatic animals, dramatic and rapid changes in the genetic makeup of aquaculture species may be possible. However, since it is virtually impossible to prevent the escape of animals into the natural environment from aquaculture facilities, potential negative impacts of such organisms on wild populations cannot be ignored. Maintaining genetic diversity like that of the wild population in cultured stocks makes good sense, particularly in conjunction with enhancement and when escape from confinement systems would produce a high likelihood of escapees intermingling with wild counterparts.

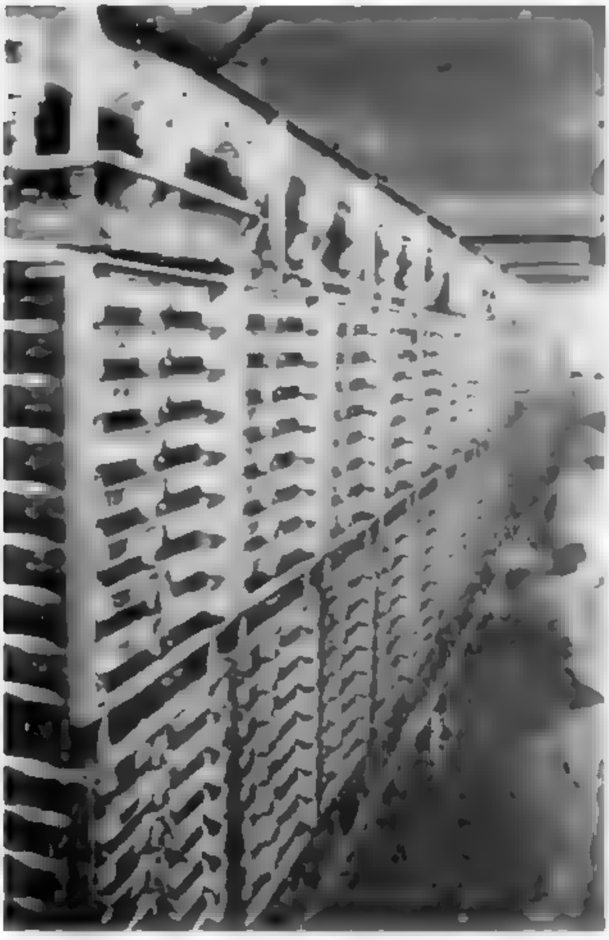


Figure 9. A bank of Heath trays in a salmon hatchery in Washington State.

For some species, one sex may grow more rapidly than the other. A prime example is tilapia, which mature at an early age (often within six months of hatching). At maturity, submarketable females divert large amounts of food energy to egg production. Also, since they are mouth brooders (holding the eggs and fry within their mouths for about two weeks) and repeat spawners (spawning about once a month if the water temperature is suitable), the females grow very slowly once they mature. Males, on the other hand, continue to grow rapidly and can become marketable within a few months after reaching maturity. All-male, or predominantly male, populations of tilapia can be produced by feeding androgens to fry, which are undifferentiated sexually. Various forms of testosterone have been used effectively in sex reversing tilapia and other fishes.

In species such as flatfish, females may grow more rapidly than males and ultimately reach much larger sizes. For them, producing all-female populations for growout might be beneficial.

DISEASES AND THEIR CONTROL

Aquatic animals are susceptible to a variety of diseases, including those caused by viruses, bacteria, fungi, and parasites. A range of chemicals and vaccines has been developed for treating the known diseases, although some conditions have resisted all control attempts to date, and severe restrictions on the use of therapeutants in some nations has impaired the ability of aquaculturists to control disease outbreaks. The United States is a good example of a nation in which the variety of treatment chemicals is limited (Table 6). In many instances when a drug is cleared for use on aquatic animals in the United States, the species on which the drug can be used is limited. Clearing a drug for catfish, for example, does not necessarily mean it can be used on trout. The cost of obtaining clearance for drug and chemical use can be in the millions of dollars, and it is often uneconomical to attempt gaining clearance for species that are not major contributors to overall aquaculture production.

Maintenance of conditions in the culture environment that minimize stress is one of the best methods of avoiding diseases. Vaccines have been developed against several diseases and more are under development. Selective breeding of animals with disease resistance has met with only limited success. Good sanitation and disinfection of contaminated facilities are important avoidance and control measures. Some disinfectants are listed in Table 6. Pond soils can be sterilized with burnt lime (CaO), hydrated lime ($Ca[OH]_2$), or chlorine compounds (14).

When treatment chemicals have to be used, they may be incorporated in the food; dissolved in water for use in dips, flushes, and baths that allow relatively short exposure periods; or allowed to remain in the water until the chemical breaks down. Higher dosages can be used in dips, flushes, and baths than with chronic exposure. Since one of the first responses of aquatic animals to disease is reduction or cessation of feeding, treatments with medicated feeds must be initiated as soon as development of an outbreak is suspected and before the animals quit feeding. Antibiotics, such as oxytetracycline, can be dissolved in the water but may be less effective than when given orally.

Vaccines can be administered through injections, orally, or by immersion. Injection is the most effective means of vaccinating aquatic animals, but it is stressful, time-consuming, and expensive. The time and expense may be acceptable for use in conjunction with broodfish and other valuable animals. Oral administration of vaccines may be ineffective, because many vaccines are deactivated in the digestive tract of the animals the vaccines are intended to protect. Dip treatment by which the vaccines enter the animals through diffusion from the water are not generally as effective as injection but can be used to vaccinate large numbers of animals in short periods of time. Vaccines have been developed for a number of fish diseases, but scientists have not achieved much success with invertebrates, which have a primitive immune system.

HARVESTING, PROCESSING, AND MARKETING

Harvesting techniques vary depending on the type of culture system involved. Seines are often used to capture fish from ponds, or the majority of the animals can be collected by draining the pond through netting. Fish pumps are available that can physically remove aquatic animals directly onto hauling trucks from ponds, raceways, cages, and net-pens without causing damage to the animals.

Aquaculturists may harvest and even process their own crops, although custom harvesting and hauling companies are often available in areas where the aquaculture industry is sufficiently developed to support them. Some processing plants also provide harvesting and live-hauling services.

Some species, with channel catfish being a good example, can develop off-flavors. A characteristic off-flavor in catfish is often described as an earthy-musty, or muddy, flavor. The problem is associated with the chemical geosmin and related compounds that are produced by certain types of algae (2). Processors often require that a sample fish from each pond scheduled for harvest be brought to the plant about two weeks prior to harvest for a testing. Subsequent samples are taken to the processor three days before and during the day of processing. A portion of the sample fish is cooked and tasted. If off-flavor is detected during any of the tests, the plant will reject the fish from that pond until the problem is resolved. Once the source of geosmin is no longer present, the fish will metabolize the compound. Purging fish of geosmin may involve moving them into clean well water or merely waiting until the algae bloom dissipates, after which the geosmin will be rapidly metabolized. Within a few days after the source of the problem has been eliminated, the fish can be retested and, if no off-flavor is detected, harvested and processed.

Centralized processing plants specifically designed to handle regional aquaculture crops are established in areas where production is sufficiently high. In coastal regions, aquacultured animals are often processed in plants that also serve capture fisheries.

Marketing can be done by aquaculturists who operate their own processing facilities. Most aquaculture operations depend on a regional processing plant to market the final product. In all cases aquaculturists should remember that their job is not complete until the product reaches the consumer in prime condition.

BIBLIOGRAPHY

1. FAO, *Agriculture Production Statistics, 1974–1993*, Food and Agriculture Organization of the United Nations, 1995.
2. R. R. Stickney, *Principles of Aquaculture*, John Wiley & Sons, New York, 1994.
3. FAO, *Aquaculture Production Statistics 1986–1995*, Food and Agriculture Organization of the United Nations, Circular 815, Rev. 9, Rome (1997).
4. *Aquaculture Buyer's Guide '95 and Industry Directory, Vol. 8*, 1995, Aquaculture Magazine, Asheville, N. Car.
5. R. R. Stickney, *Aquaculture in the United States: A Historical Review*, John Wiley & Sons, New York, 1996.
6. A. Tacon, *International AquaFeed* **2**, 13–16 (1998).
7. Anonymous, *Aquaculture News* **6**, 1, 12 (1998).
8. R. G. Piper, I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard, *Fish Hatchery Management*, U.S. Fish and Wildlife Service, Washington, D.C. 1982.
9. C. E. Boyd, *Water Quality in Ponds for Aquaculture*, Alabama Agricultural Experiment State, Auburn University, 1990.
10. J. Huegenin and J. Colt, *Design and Operating Guide for Aquaculture Seawater Systems*, Elsevier, New York, 1989.
11. National Research Council, *Nutrient Requirements of Fish*, National Academy Press, Washington, D.C., 1993.
12. A. Tacon, *International Aquafeed Directory and Buyers' Guide 1997/98* (1998).
13. F. P. Meyer and R. A. Schnick, "A Review of Chemicals Used for the Control of Fish Diseases," *Rev. Aquatic Sci.* **1**, 693 (1989).
14. C. E. Boyd, *Bottom Soils, Sediment, and Pond Aquaculture*, Chapman and Hall, New York, 1995.

ROBERT R. STICKNEY
Texas Sea Grant College Program
Bryan, Texas

AQUACULTURE: ENGINEERING AND CONSTRUCTION

BACKGROUND

Aquaculture's origins are ancient, but the explicit application of engineering to aquaculture is very recent. Only during the last 20 to 30 years has there been visible research activity in this field. This activity has accelerated recently fueled in part by the newly formed Aquacultural Engineering Society (AES, c/o The Freshwater Institute, P.O. Box 1746, Shepherdstown, WV 25443). The AES is an international organization bringing together individuals interested in the engineering applications to aquaculture. Although the number of aquacultural engineering practiof the first responses of aquatic animals to disease is reduction or cessation of feeding, treatments with medicated feeds must be initiated as soon as development of an outbreak is suspected and before the animals quit feeding. Antibiotics, such as oxytetracycline, can be dissolved in the water but may be less effective than when given orally.

Vaccines can be administered through injections, orally, or by immersion. Injection is the most effective means of vaccinating aquatic animals, but it is stressful, time-consuming, and expensive. The time and expense may be acceptable for use in conjunction with broodfish and other valuable animals. Oral administration of vaccines may be ineffective, because many vaccines are deactivated in the digestive tract of the animals the vaccines are intended to protect. Dip treatment by which the vaccines enter the animals through diffusion from the water are not generally as effective as injection but can be used to vaccinate large numbers of animals in short periods of time. Vaccines have been developed for a number of fish diseases, but scientists have not achieved much success with invertebrates, which have a primitive immune system.

HARVESTING, PROCESSING, AND MARKETING

Harvesting techniques vary depending on the type of culture system involved. Seines are often used to capture fish from ponds, or the majority of the animals can be collected by draining the pond through netting. Fish pumps are available that can physically remove aquatic animals directly onto hauling trucks from ponds, raceways, cages, and net-pens without causing damage to the animals.

Aquaculturists may harvest and even process their own crops, although custom harvesting and hauling companies are often available in areas where the aquaculture industry is sufficiently developed to support them. Some processing plants also provide harvesting and live-hauling services.

Some species, with channel catfish being a good example, can develop off-flavors. A characteristic off-flavor in catfish is often described as an earthy-musty, or muddy, flavor. The problem is associated with the chemical geosmin and related compounds that are produced by certain types of algae (2). Processors often require that a sample fish from each pond scheduled for harvest be brought to the plant about two weeks prior to harvest for a testing. Subsequent samples are taken to the processor three days before and during the day of processing. A portion of the sample fish is cooked and tasted. If off-flavor is detected during any of the tests, the plant will reject the fish from that pond until the problem is resolved. Once the source of geosmin is no longer present, the fish will metabolize the compound. Purging fish of geosmin may involve moving them into clean well water or merely waiting until the algae bloom dissipates, after which the geosmin will be rapidly metabolized. Within a few days after the source of the problem has been eliminated, the fish can be retested and, if no off-flavor is detected, harvested and processed.

Centralized processing plants specifically designed to handle regional aquaculture crops are established in areas where production is sufficiently high. In coastal regions, aquacultured animals are often processed in plants that also serve capture fisheries.

Marketing can be done by aquaculturists who operate their own processing facilities. Most aquaculture operations depend on a regional processing plant to market the final product. In all cases aquaculturists should remember that their job is not complete until the product reaches the consumer in prime condition.

BIBLIOGRAPHY

1. FAO, *Agriculture Production Statistics, 1974–1993*, Food and Agriculture Organization of the United Nations, 1995.
2. R. R. Stickney, *Principles of Aquaculture*, John Wiley & Sons, New York, 1994.
3. FAO, *Aquaculture Production Statistics 1986–1995*, Food and Agriculture Organization of the United Nations, Circular 815, Rev. 9, Rome (1997).
4. *Aquaculture Buyer's Guide '95 and Industry Directory, Vol. 8*, 1995, Aquaculture Magazine, Asheville, N. Car.
5. R. R. Stickney, *Aquaculture in the United States: A Historical Review*, John Wiley & Sons, New York, 1996.
6. A. Tacon, *International AquaFeed* **2**, 13–16 (1998).
7. Anonymous, *Aquaculture News* **6**, 1, 12 (1998).
8. R. G. Piper, I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard, *Fish Hatchery Management*, U.S. Fish and Wildlife Service, Washington, D.C. 1982.
9. C. E. Boyd, *Water Quality in Ponds for Aquaculture*, Alabama Agricultural Experiment State, Auburn University, 1990.
10. J. Huegenin and J. Colt, *Design and Operating Guide for Aquaculture Seawater Systems*, Elsevier, New York, 1989.
11. National Research Council, *Nutrient Requirements of Fish*, National Academy Press, Washington, D.C., 1993.
12. A. Tacon, *International Aquafeed Directory and Buyers' Guide 1997/98* (1998).
13. F. P. Meyer and R. A. Schnick, "A Review of Chemicals Used for the Control of Fish Diseases," *Rev. Aquatic Sci.* **1**, 693 (1989).
14. C. E. Boyd, *Bottom Soils, Sediment, and Pond Aquaculture*, Chapman and Hall, New York, 1995.

ROBERT R. STICKNEY
Texas Sea Grant College Program
Bryan, Texas

AQUACULTURE: ENGINEERING AND CONSTRUCTION

BACKGROUND

Aquaculture's origins are ancient, but the explicit application of engineering to aquaculture is very recent. Only during the last 20 to 30 years has there been visible research activity in this field. This activity has accelerated recently fueled in part by the newly formed Aquacultural Engineering Society (AES, c/o The Freshwater Institute, P.O. Box 1746, Shepherdstown, WV 25443). The AES is an international organization bringing together individuals interested in the engineering applications to aquaculture. Although the number of aquacultural engineering practitioners is still small, interest in the field is very high, and engineering sessions are typically some of the best attended at meetings of aquaculture societies.

The development of aquacultural engineering has followed a path analogous to that followed by agricultural engineering 50 to 75 years earlier. Typically, engineers trained in related disciplines have acquired enough understanding of the biological components of aquaculture production systems to be able to design, evaluate, and manage these systems. These early activities have led to the training of students, graduate and undergraduate, in the combination of engineering and biological principles that would allow them to function effectively as aquacultural engineers. However, the number of educational programs that offer aquacultural engineering courses and degree programs continues to be small, and there continue to be all too frequent cases of engineers and other designers working in aquaculture without adequate training in the discipline, pointing to the continuing need to expand educational offerings in aquacultural engineering.

Aquaculture encompasses a wide range of activities, culture techniques, and species. The application of engineering principles to the different forms of aquaculture varies widely from the sophisticated, explicit engineering needed for an intensive system that incorporates water reuse, to the simple, nonformal engineering used for extensive, subsistence-type aquacultural operations. Some of the processes described here, then, will be applicable only to certain types of production systems.

DEFINITION

Aquacultural engineering is the application of engineering principles to the design, construction, and management of systems for the production of aquatic animals or plants. Specific areas covered include the overall system design and management; the physical system used to hold the animals or plants; the supporting structures; processes, monitoring equipment, and techniques needed to ensure an adequate environmental quality in the production system; and the facilities and equipment needed to handle fish* and other materials such as feed. As a research discipline, aquacultural engineering also deals with the evaluation of the effects of water quality and other environmental conditions on fish, as well as the effect that fish have on the water. Effects on the fish that are of concern include growth rates, susceptibility to disease, and reproductive success. An area of increasing concern is the potential environmental impact of aquaculture operations, and aquacultural engineers are being called upon to help address this topic. In addition, traditional engineering disciplines ranging from environmental to mechanical to electrical engineering are used in aquaculture.

SYSTEM DESIGN

An aquaculture system must be designed to provide a healthy environment for the target fish or plant, and this must be achieved within constraints inherent in the choice of location where the facility is to be constructed. Other considerations that need to be taken into account include: marketing, economics, and regulatory restrictions. The procedure for the development of an aquaculture system is summarized in Figure 1 and follows stages of data collection, evaluation, design, and construction (1,2). Background information needed includes data on the biological (or bioengineering as they are sometimes referred to) characteristics of the target species, as well as data on the site, possible markets for the product, and regulations affecting aquaculture at the chosen site. The biological data needed to quantify the relationship between the target organism and its environment include information on tolerances and optimum levels for various water quality parameters, the effect of target animal activity on water quality, space and water velocity requirements, reproductive characteristics, feeding behavior, and others. Site data are primarily related to water availability and to the quality of the water, but other factors are important, such as soil characteristics (soil permeability, physical properties affecting possible construction, and fertility), topography, climate, other land uses, transportation, and other infrastructure. Site characteristics may be considered at the level of a specific aquaculture site, or at the regional planning level, where attempts have been made at incorporating satellite imaging and geographic information system (GIS) techniques into the site characterization process (3). Desirable marketing information includes not only the type of species that is salable but also the particular requirements imposed by the market on product size, level of processing, seasonal fluctuations in demand, and so on. Last, regulatory aspects of aquaculture must not be overlooked. Restrictions on land and water use are augmented by considerations of species approvals, and in some cases by the lack of familiarity of regulators with aquaculture, and their unwillingness to consider it as a form of agriculture, rather than as an industrial enterprise. The Food and Agriculture Organization (FAO) of the United Nations has recently released a "code of practice" for aquaculturists (4). The intent of the code is to promote the safe and environmentally sound development of aquaculture by encouraging aquaculture practitioners to consider the full scope of possible impacts of aquaculture installations.

A last topic on which background information should be gathered is an overall review of the methods and procedures used for the culture of the species of interest. Wherever possible this should focus on conditions that are similar to those of the planned facility.

From this point on, the design process follows fairly well established overall guidelines for engineering design. A preliminary evaluation is carried out where the background information is reviewed and an initial decision is made about the possible viability of the operation, or of the need to make changes and collect additional background information.

Once a decision has been made to proceed with the planning of a facility, detailed review of the practices used by others in the culture of the target species is undertaken. This review should be based on published information as well as on personal contacts with other producers, with

---

*In the context of this article, fish may be a finfish such as trout or catfish, or a crustacean such as shrimp or lobsters, or a mollusk such as oysters or clams.

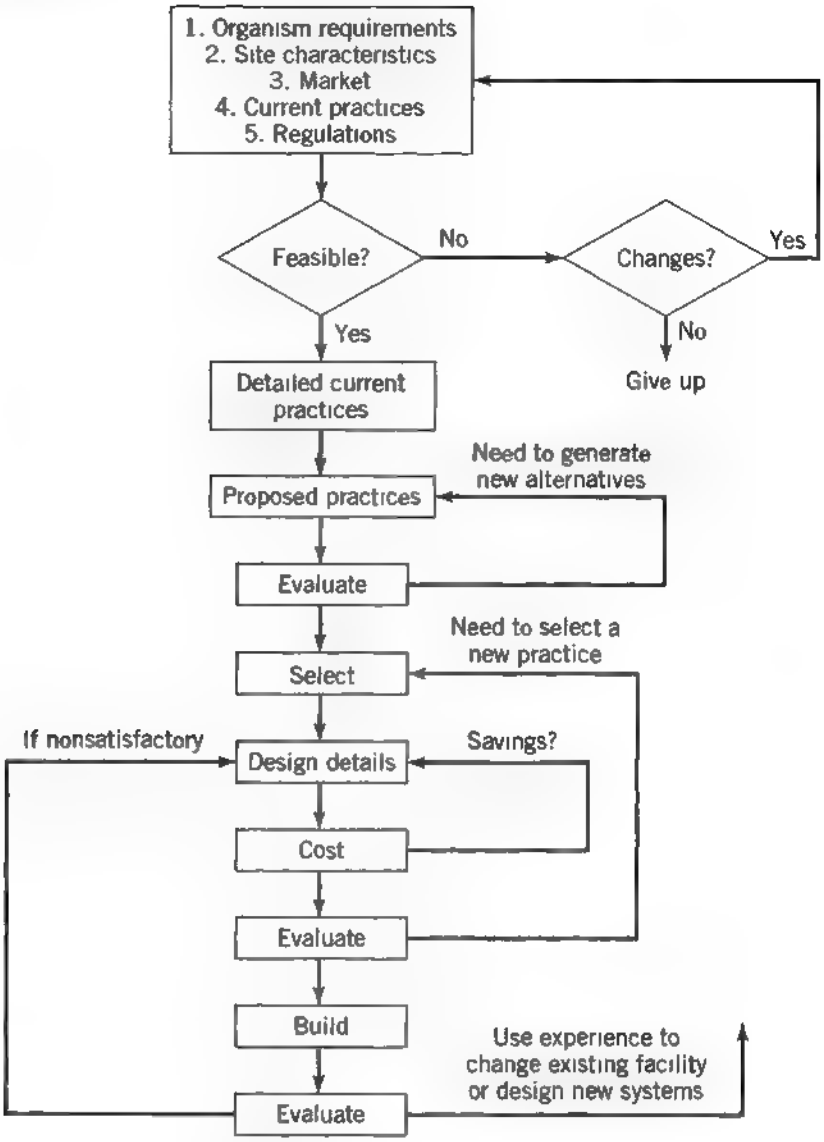

1. Organism requirements
2. Site characteristics
3. Market
4. Current practices
5. Regulations
Feasible?
No
Changes?
Yes
Yes
No
Give up
Detailed current practices
Need to generate new alternatives
Proposed practices
Evaluate
Need to select a new practice
Select
If nonsatisfactory
Design details
Savings?
Cost
Evaluate
Build
Use experience to change existing facility or design new systems
Evaluate

Figure 1. Flow diagram of the design process followed for aquaculture operations.

extension agents, and with researchers. As a first step, several alternative designs may be generated and evaluated to select the most promising one for further development. After evaluation and selection, the details of the design are completed, including estimated costs and preparation of construction documents. Evaluation of the plans and facilities should be carried out at the various stages of the process to minimize the probability of errors, as well as to ensure maximum functionality and operational efficiency for the investment. Evaluation of the completed installation will serve in the preparation of future designs or in the possible expansion of the facility.

HOLDING SYSTEMS

The types of impoundments or holding systems used in aquaculture are determined by the requirements of the particular species and by the production and management practices being employed. Typical systems include ponds, raceways, tanks, and cages. Although construction materials for the various types of holding systems differ depending on specific requirements, they should have general characteristics, including being corrosion resistant, nontoxic, with smooth and nonabrasive surfaces, strong and lightweight, resistant to ultraviolet radiation, and low in cost (5,6). Typically, no material satisfies all criteria optimally, and selection is based on the particular constraints of a given project.

Ponds

Pond size, shape, and construction technique varies widely (7,8). Ponds tend to be shallow, less than 1.5 m deep, and vary in area between less than 100 m^2 to more than 10 ha (Fig. 2). The pond bottom is sometimes covered with concrete or plastic liners to prevent water losses to infiltration. The ponds may be built by excavation, by construction of dikes, or more commonly by a combination of the two techniques. In all cases, pond design should incorporate struc-

Figure 2. Pond being used for the culture of shrimp with supplemental mechanical aeration.

tures for water inflow for filling and maintenance flows, and for effluents including flows during pond drainage and emptying.

Raceways

Raceways are relatively narrow, long channels with water constantly entering through one end of the channel and leaving through the opposite end (Fig. 3). Raceways are often built of concrete, and the normal dimensions are in a ratio of approximately 30:3:1 (length:width:depth). Raceways are the most common holding system for the production of trout and are usually approximately 30 m long. Flow in a raceway approaches plug flow characteristics, creating a gradient of water quality along the raceway; water quality is best near the head of the raceway where the water enters and degrades toward the drain side of the raceway. Raceways are often constructed in series of up to six raceways with reaeration between raceways to replenish some of the oxygen used by the fish.

Tanks

Aquaculture tanks are normally designed to optimize the movement of water around the center of the tank. As such, common shapes are round, square with rounded corners, hexagonal, and octagonal (5,9,10) (see Fig. 4). The choice is often made on the basis of space availability and construction materials, as there aren't large differences in the hydraulic properties of the various shapes. Tank size ranges from an equivalent diameter of less than 1 m to more than 10 m. Water depth is normally maintained between 0.5 m for the smaller tanks and 1 to 2 m for the larger tanks. The two most common materials used for tanks are fiberglass and concrete.

The size and shape of tanks used in aquaculture vary greatly, but the common characteristic is that flow approaches the ideal continuously stirred tank reactor (CSTR) model in which water quality within the tank is approximately uniform. To achieve this uniformity, water is introduced along the periphery of the tank through one or more inlets that normally inject the water tangentially, and the water effluent is collected from the center of the tank (10,11). Contrary to raceways, it is possible to achieve a certain degree of independence between water flow rate and water velocity in a tank by manipulating the inlet and outlet characteristics and location. An important feature is that by adjusting the circulation pattern and water velocity to maintain particulate matter in suspension, tanks may be made to be "self-cleaning" (10,11).

A recent development in tank culture is the introduction of "double drains" in which two water streams are created in the drain, one constituting a large percentage of the flow containing a very small fraction of the solids released to the tank water (fish feces and uneaten feed), and the second stream conveying no more than 5 to 10% of the total flow from the tank, but containing up to 90 or 95% of the tank solids (10,11). These new double drains offer opportunities for improved water utilization efficiency and water treatment systems, with the high flow–low solids stream being relatively easy to treat for reuse, and the low flow–high solids stream being suitable for treatment prior to disposal. Flow rates in tanks are normally selected to result in residence times ranging from 10 to 15 min to several hours, and the value used depends on the level of water treatment (normally aeration) taking place within the tank and on the fish biomass being held.

Figure 3. Farm that uses raceways for the culture of trout. The raceways are laid out in series and in parallel to optimize land and water use.

Figure 4. Round concrete tanks used for the culture of striped bass. Water in the tanks is reoxygenated using pure oxygen injection systems and aerated for carbon dioxide removal.

Cages

Although cages have been used in aquaculture for many years, they have received a great deal of attention recently, especially with the development of the salmon industry in Norway and other countries (Fig. 5). Conventional cage technology is based on a rigid frame that is either floating or fixed, and to which a cage made of some type of netting material is attached. Traditionally, cages had been rectangular and relatively small, with total volumes of less than 10 m^3. The recent growth of the industry, the availability of new materials and technologies, and the presence of competing users for coastal resources have resulted in the development of large cage systems suitable for open sea deployment (6,12). Some of these cages may be more than 50 m in diameter, 30 m deep, with production volumes of more than 50,000 m^3, and are designed to hold up to 1,000,000 kg of fish (6,12). Engineering contributions into the design, construction, and anchoring of cages becomes very specialized as the cage size increases and as cages are placed in more exposed locations. Cages are subjected to static and dynamic loads. Static loads are relatively easy to quantify and are caused by the weight of the fish and the structure itself, but transient loads caused by wind, waves, and currents may be orders of magnitude greater than static loads and are very difficult to determine accurately (6). Frequent cage maintenance is required to prevent fouling of the net from restricting the flow of water. Exchange of water through the net must be sufficient to maintain adequate water quality for the fish.

QUANTIFYING PROCESSES

As was mentioned in the definition of aquacultural engineering, many engineering disciplines can be applied to the design of aquaculture systems. In this section, some of the critical processes that are unique in an aquaculture system and that define design parameters will be described. The key processes in the design of aquatic systems have to do with the primary water quality factors that affect the cultured organism and with how the organism affects water quality.

Figure 5. Cages used for the culture of Atlantic salmon in Norway.

As a starting point in the description of important processes in an aquaculture operation, one can consider how a fish relates to its environment. Fish consume oxygen from the water and release water products from their metabolism back into the water. The main waste products of concern are ammonia, carbon dioxide, and particulate and dissolved organics. Depletion of dissolved oxygen below a certain level, which is species and size dependent, results in increased stress to the fish and may ultimately result in suffocation. Similarly, the accumulation of waste products may reach toxic levels, as in the case of ammonia and carbon dioxide; it may alter the pH of the water, again as a result of carbon dioxide accumulation; it may affect growth and reproductive behavior as a result of the accumulation of dissolved organics such as hormones; or it may affect the overall oxygen balance of the system by the oxygen demand created by the decomposition of particulate and dissolved organics.

A major difference between aquaculture and land-based food production systems is that in an aquaculture system the animal or plant takes its nutrients and oxygen and releases its waste products into the same environment: the water. Maintaining water quality in an aquaculture system is based on providing sufficient oxygen to satisfy the needs of the fish, and in removing metabolites to prevent their buildup to toxic levels. Design of systems to achieve those goals requires the quantification of processes that take place within the system, especially those related directly to the target species. The relative importance of the various consumption and production processes depends on the type of aquatic production system being considered. In general, the shorter the residence time of the water in the production system, the more important are the fish-related processes with respect to other biological processes taking place. If, on the other hand, the residence time is long (more than one day and as long as one year), as is the case in most ponds, biological and chemical processes that are not directly associated with the target product's metabolism become very important in determining water quality. As an example, dissolved oxygen in a tank with a short residence time will be determined by the concentration in the influent water, the fish biomass being held in the tank, the amount of oxygen being added to the system through aeration mechanisms, and the oxygen consumption by the fish. In a pond, on the other hand, many processes can have significant effects on dissolved oxygen concentration. These processes include photosynthetic oxygen production, respiration by all the organisms in the ponds (phytoplankton, zooplankton, fish, bacteria, and sediment decomposition processes), and gas exchange between the water and the atmosphere.

Quantification, design, and management of aquaculture production systems are most predictable in those systems with short residence times, where the majority of water quality changes are associated with fish activity. Pond systems, often with long residence times, are difficult to quantify and their management remains very much an art rather than a science.

Design of systems is ultimately based on mass balances on the critical water quality factors already mentioned. The general form of the mass balance equation for a particular substance (eg, dissolved oxygen or ammonia) in a control volume can be written as

$$\text{ACCUMULATION} = \text{INFLOW} + \text{PRODUCTION} - \text{OUTFLOW} - \text{CONSUMPTION} \quad (1)$$

For simplicity in calculations and because of incomplete information on the time dependence of production and consumption rates, the mass balance is often simplified to consider steady-state conditions in which ACCUMULATION becomes zero, and equation 1 can be written as:

$$\text{INFLOW} + \text{PRODUCTION} = \text{OUTFLOW} + \text{CONSUMPTION} \quad (2)$$

And the INFLOW and OUTFLOW terms may be expressed as the product of the concentration of the substance being observed and the flow rate:

$$\text{INFLOW} = Q \times C_i \quad (3)$$

$$\text{OUTFLOW} = Q \times C_o \quad (4)$$

where Q is the flow rate ($m^3\ d^{-1}$), C_i is the influent concentration ($g\ m^{-3}$), and C_o is the effluent concentration ($g\ m^{-3}$).

In a fully mixed rearing impoundment, the effluent concentration is approximately equal to the concentration at any point within the impoundment and is the concentration to which the target species is exposed. The production and consumption functions depend on the species, size, and condition of the fish and on the environmental conditions to which they are exposed. In addition, these rates fluctuate through diel cycles in response to activity levels associated primarily with feeding and digestion. In general, there is little quantitative information on how the various factors affect the rates of oxygen consumption and metabolite production, and for purposes of design, it is common to relate these rates to feeding levels (13,14):

$$\text{DO}_{\text{cons}} = \text{FEED} \times \text{DO}_{\text{rc}} \quad (5)$$

$$\text{CO}_{2\text{prod}} = \text{FEED} \times \text{CO}_{2\text{rc}} \quad (6)$$

$$\text{TAN}_{\text{prod}} = \text{FEED} \times \text{TAN}_{\text{rc}} \quad (7)$$

$$\text{SOLIDS}_{\text{prod}} = \text{FEED} \times \text{SOLIDS}_{\text{rc}} \quad (8)$$

where DO_{cons} is the rate of oxygen consumption by fish within the impoundment ($g\ d^{-1}$), FEED is the amount of feed applied to the impoundment ($g_{\text{feed}}\ d^{-1}$), DO_{rc} is the oxygen consumption per unit of feed applied ($g\ g_{\text{feed}}^{-1}$), and similarly for carbon dioxide (CO_2), total ammonia nitrogen (TAN), and solids productions. Feeding rate is often expressed as a proportion of the fish biomass applied per day, and tables are available from feed manufacturers for various fish species and ages. Approximate values used for the consumption of oxygen and production of carbon dioxide, ammonia, and solids per unit mass of feed ($g\ \text{gfeed}^{-1}$) are 0.20, 0.28, 0.03, and 0.30, respectively (13,14). These values can be useful as a first approximation, but detailed calculations must be based on values specifically developed for the species of interest and for the feed and culture system being used.

WATER CONDITIONING

Water conditioning operations may be used to treat the water and increase the fish biomass that can be held for a given water flow rate. The rates of oxygen consumption and metabolite production and the tolerances of fish to concentrations of these substances are such that oxygen is the first limiting factor encountered. If aeration is used to replenish oxygen, then the second limiting factor reached may be either carbon dioxide or ammonia accumulation, depending on characteristics of the water supply (in particular alkalinity and pH of the water) and on fish tolerances.

Oxygenation

Oxygen sources differ between pond and other types of holding systems. Possible sources of oxygen in a pond are the production by aquatic plants, including phytoplankton, mechanical reaeration, and reaeration across the water surface with minimum contributions by the water exchange, with the relative importance of the sources depending on the type of production system being used. In raceways and tanks, oxygen sources are limited to the water supply and some form of mechanical reaeration.

Oxygen Production by Phytoplankton. Phytoplankton produce oxygen through photosynthesis. The rate of oxygen production by phytoplankton is very difficult to quantify but conceptually may be expressed as

$$\text{DOPHYTO} = \text{DO}_{\text{equi}} \times \text{PHYTO} \times \text{MAX} \times \text{NUTRI} \times \text{LIGHT} \times \text{TEMP} \quad (9)$$

where DOPHYTO is the oxygen production rate by phytoplankton ($g\ m^{-3}\ h^{-1}$), DO_{equi} is the oxygen production per unit of phytoplankton biomass growth ($g\ g^{-1}$), PHYTO is the phytoplankton concentration ($g\ m^{-3}$), MAX is the maximum phytoplankton growth rate under optimum conditions (h^{-1}), NUTRI is the nutrient limitation on growth (0–1), LIGHT is the light limitation on growth (0–1), and TEMP is the temperature limitation on growth (0–1).

A multitude of expressions have been proposed for the various limitation terms (eg, Ref. 15).

The overall effect of phytoplankton on oxygen concentration in a pond is further complicated by their respiration at night. This combination of oxygen production by phytoplankton during the day and consumption at night by phytoplankton, fish, and other organisms results in wide variations in dissolved oxygen over diurnal cycles (8). Maintaining a balance between the phytoplankton production and consumption terms in ponds becomes the primary goal of water quality management in most pond production systems.

A new production system has been developed recently that makes use of the water-treating and oxygen-producing capabilities of ponds. The system has been called the Partitioned Aquaculture System (PAS) (16) and consists of a shallow pond used for water treatment and oxygen production, and a small raceway in which fish are held. Water is continuously recirculated between the raceway and the pond. A secondary fish crop is raised in a section of the raceway downstream from the primary fish crop. The species to be raised as the secondary crop is selected such that it has the ability to filter the water and remove particulates such as algae and uneaten feed from the primary crop. These new systems are still in the experimental stage but offer great promise given their high production rates per unit area and the fact that the water is recycled and no effluent is produced.

Oxygen Production by Mechanical Aeration. Oxygen transfer into the water takes place as a result of a driving force equal to the difference between the saturation concentration and the actual concentration found in solution, a mass-transfer coefficient, and the area of contact between the liquid and gas phases. The mass-transfer coefficient and the area of contact are normally combined into an overall gas-transfer coefficient, K_{La}, that may be determined experimentally. The equation describing the rate of oxygen transfer can be written as

$$\mathrm{DO}_{\mathrm{Trans}} = (\mathrm{DO}_{\mathrm{Sat}} - \mathrm{DO})K_{La}V \qquad (10)$$

where $\mathrm{DO}_{\mathrm{Trans}}$ is the dissolved oxygen transfer rate (g h^{-1}), $\mathrm{DO}_{\mathrm{Sat}}$ is the saturation concentration of dissolved oxygen (g m^{-3}), DO is the dissolved oxygen concentration in solution (g m^{-3}), K_{La} is the overall gas-transfer coefficient (h^{-1}), and V is the volume over which the aeration is taking place (m^3).

Saturation concentration is determined by the composition of the gas phase, by temperature, by atmospheric pressure, and by the presence of dissolved substances in the water (eg, salinity) (17). Although more complete estimation procedures are available that consider the factors just mentioned (17), saturation concentration for fresh water as a function of temperature may be expressed as a regression equation (18):

$$\begin{aligned}\mathrm{DO}_{\mathrm{Sat}} = {} & 14.652 - 0.41022 \times \mathrm{TEMP} \\ & + 0.007991 \times \mathrm{TEMP}^2 \\ & - 0.000077774 \times \mathrm{TEMP}^3 \qquad (11)\end{aligned}$$

where TEMP is the water temperature (°C).

Equation 12 and tables of dissolved oxygen saturation are normally prepared for an atmosphere of 20.9% oxygen. Oxygen enrichment of the atmosphere will result in a corresponding increase in saturation dissolved oxygen concentration. Enrichment may be achieved by aerating with "pure" oxygen.

An important difference between aeration systems used for aquaculture and those used in water and wastewater treatment is that the "driving force" ($\mathrm{DO}_{\mathrm{Sat}} - \mathrm{DO}$) in aquaculture tends to be lower, reducing the rate of oxygen transfer for a given aerator. The lower driving force is caused by the requirement of most fish for dissolved oxygen concentrations above 3 to 4 g m^{-3}, compared with the usual limit of just above zero in wastewater treatment.

Common types of aerators used in aquaculture may be classified as surface, gravity, and diffuser types (5,9,19). Surface aerators spray water into the air or beat the water, increasing the turbulence and area of contact between the water and air. Gravity aerators rely on the fall of water for aeration. Different types of structures such as plates, baffles, and so on may be used in gravity aerators to increase transfer rates. A particularly useful and common gravity aerator is the packed column aerator (PCA), where water is introduced at the top, and air or another gas is introduced at the bottom of a column filled with a medium designed to maximize turbulence and transfer area. Design equations for the use of PCAs in aquaculture have been proposed by Hackney and Colt (20). In diffuse aeration, gas bubbles are introduced into the water, and transfer takes place between the bubble and the water. The simplest type of diffuse aerator is an airstone where bubbles are released at the bottom of a tank or pond. This type of aerator tends to be inefficient in conventional aquaculture systems due to the shallow water depths used and the resulting short contact times between the bubble and the water. Variations on the basic concept of diffuse aeration have been developed, including some systems designed to be used as part of a pipeline and that pressurize and trap bubbles allowing for longer contact times and, in some cases, complete dissolution of the bubbles into the liquid (5,9).

The use of pure oxygen in aquaculture operations has become commonplace over the last few years, especially for intensive systems that include some form of water reuse. Pure oxygen systems rely on either liquid bottled oxygen or on the on-site production of oxygen. The use of pure oxygen makes possible the addition of large amounts of oxygen to the water, increasing fish biomass held in a given water supply.

Oxygen Production by Surface Reaeration. The transfer of oxygen by reaeration is important in ponds where it can result in substantial net gains or losses of oxygen depending on whether the dissolved oxygen concentration is below or above saturation. Estimates of reaeration rates may be obtained from the following (21):

$$\mathrm{DO}_{\mathrm{Reaer}} = (\mathrm{DO}_{\mathrm{Sat}} - \mathrm{DO})K_{\mathrm{Reaer}}/D \qquad (12)$$

where $\mathrm{DO}_{\mathrm{Reaer}}$ is the reaeration rate (g m^{-3} h^{-1}),

$$\begin{aligned}K_{\mathrm{Reaer}} = {} & 0.03 \times v_w^{0.5} - 0.0132 \times v_w + 0.0015 \\ & \times v_w^2 (\mathrm{m\ h^{-1}}) \qquad (13)\end{aligned}$$

v_w is the wind velocity (m s^{-1}), and D is the pond depth (m).

An implicit assumption in equation 13 is that conditions in the pond are uniform and there is a minimum of stratification.

Ammonia Removal

Ammonia is toxic to aquatic animals and is also the waste product of their protein metabolism. The sensitivity of aquatic animals to ammonia concentration depends on species, live stage, level of stress, and on other environmental conditions. Ammonia exists in water as the equilibrium product of ammonium ion and unionized ammonia:

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad pK_a = 9.25 \text{ at } 25°C \qquad (14)$$

Unionized ammonia (NH_3) is the toxic form of ammonia (5,14). Approximate estimates of the concentration of unionized ammonia in fresh water can be obtained form measures of total ammonia (NH_3 plus NH_4^+ concentrations) and pH as (5):

$$[NH_3] = [TA] \times (1/10^{(pK_a - pH)}) \qquad (15)$$

where $[NH_3]$ is the unionized ammonia concentration (mol/L) and [TA] is the total ammonia concentration (mol/L).

Removal of ammonia from aquaculture systems may be achieved by ion exchange or by biological filtration. Ion exchange is carried out with a natural zeolite (clinoptilolite) that has a high affinity for ammonium ions. Removal capacity of the resin varies widely, but a common design value is around 1 mg NH_4^+ $(\text{g clino})^{-1}$ (22). Clinoptilolite resin may be regenerated with brackish or salt water, making the use of the resin suitable for fresh water applications only. Ion exchange columns need to be designed to incorporate the downtime involved with the recharging and reconditioning. In addition, unless properly maintained, these columns have a tendency to be colonized by bacteria and become biological filters.

The biological removal of ammonia by the process of nitrification is carried out by two groups of bacteria: nitrosomonas, which take ammonia to nitrite; and nitrobacter, which complete the reaction to nitrate. Nitrate is toxic to fish only at very high concentration and therefore is a suitable end product for ammonia and nitrite, forms of nitrogen toxic to fish. The nitrification process is relatively slow and sensitive to temperature, pH, and ammonia concentration. To achieve practical nitrification rates, high bacterial biomass must be maintained, which is normally achieved with some form of attached growth filter (23,24). Many types of filters are used for ammonia removal. downflow, upflow, rotating disks, submerged, trickling, fluidized beds, and soon (23,24). Removal rates are highly variable, and this is an area of active research at the present time.

Solids Removal

Removal of solids produced by aquaculture may be attempted for making the water suitable for reuse, or to meet discharge guidelines. Solids account for a high percentage of the biochemical oxygen demand (BOD) in aquaculture effluents; they tend to be highly variable in size and break up easily if subjected to mechanical forces. Solids are formed from uneaten feed and fecal material. Their density tends to be very close to that of water.

Removal is achieved by filtration with particulate (eg, sand) or microscreen (eg, stainless steel) filters, or by sedimentation (25). The high organic content of the particulates make them highly biodegradable, and filters need to be backwashed frequently to prevent clogging by a mat of biological slime. Screen filters are common in aquaculture and a number of filters have been developed that incorporate some form of automated cleaning (eg, drum or disk filters). Simple settling tanks or ponds with overflow rates between 40 and 80 m d^{-1} can result in total suspended solids removal rates of 65 to 85% (25). Aquaculture settling ponds traditionally have been operated with infrequent sludge removal, relying on biological decomposition to prevent excessive accumulation of sludge.

Recent concerns with the possible release of nutrients, especially phosphorus, from aquaculture operations to natural waters have caused a reevaluation of techniques available for solids removal. Given the technical difficulties and expense associated with treating aquaculture effluents, a combination of nutritional and engineering approaches are being studied to reduce the solids (and nutrient) content in effluents.

FUTURE DEVELOPMENTS

The aquaculture farm of the future will take many forms. The difference with today's farms will be in the level of intensity of water use, in the degree of control over the culture environment exerted by the operator, and in the predictability of growth and overall production rates. The application of engineering will become more and more important as the aquaculture industry continues to grow and diversify. Developments will be in new culture systems, materials, equipment, and water quality monitoring, treatment, and control. The efficient use of natural resources, especially water, will be a primary constraint on new aquaculture operations. To make more efficient use of water, higher fish biomass will be maintained per unit of water volume and per unit of water use by the implementation of water treatment and recirculation systems. The safe rearing of these large biomasses will require continuous monitoring of water quality, and the use of alarms and backup systems. In most cases these will be all computer controlled.

Improvements in water treatment are also needed to make possible reductions in water use and the commercial viability of culture systems based on water reuse. New, more efficient and reliable filtration systems for the removal of particulate and dissolved organics and of ammonia will be developed.

Systems for inventorying and handling fish are needed. Obtaining accurate estimates of number and size of fish in a culture system are practically impossible today as they are based on sampling or on the use of growth functions and initial stocking values. Equipment and techniques that can be used to count animals and estimate their size with a minimum of disruption and stress to the fish will have to be developed.

BIBLIOGRAPHY

1. R. D. Mayo, "A Format for Planning a Commercial Model Aquaculture Facility," paper presented at Northeast Fish and Wildlife Conference, February 25–28, 1974, Grate Gorge, N.J. Technical Reprint No. 30. Kramer, Chin and Mayo, Inc., Consulting Engineers, Seattle, Wash., 1974.

2. C. M. Brown and C. E. Nash, "Planning an Aquaculture Facility—Guidelines for Bioprogramming and Design," Aquaculture Development and Coordination Programme Report ADCP/REP/87/24, United Nations Development Programme, Food and Agriculture Organization of the United Nations, Rome, 1988.
3. S. S. Nath et al., "Applications of Heat Balance and Fish Growth Models for Continental-Scale Assessment of Aquaculture Potential in Latin America," in D. Burke et al., eds., *Pond Dynamics / Aquaculture Collaborative Research Support Program, Fourteenth Annual Technical Report*, PD/A CRSP Office of International Research and Development, Oregon State University, Corvallis, Oreg., 1996.
4. Food and Agriculture Organization of the United Nations (FAO) Fisheries Department, Aquaculture Development, FAO Technical Guidelines for Responsible Fisheries, No. 5, Rome, 1997.
5. T. B. Lawson, *Fundamentals of Aquacultural Engineering*, Chapman & Hall, New York, 1995.
6. M. C. M. Beveridge, *Cage Aquaculture*, 2nd ed. Fishing News Books Ltd., Surrey, England, 1996.
7. C. R. dela Cruz, "Fishpond Engineering: A Technical Manual for Small- and Medium-Scale Coastal Fish Farms in Southeast Asia," *South China Sea Fisheries Development and Coordinating Programme Manual No. 5*, United Nations Development Programme, Food and Agriculture Organization of the United Nations. Manila, Philippines, 1983.
8. H. S. Egna, and C. E. Boyd, eds. *Dynamics of Pond Aquaculture*, CRC Press, Boca Raton, Fla., 1997.
9. F. W. Wheaton, *Aquacultural Engineering*, Wiley, New York, 1977.
10. S. Skybakmoen, "Fish Rearing Tanks," *Aquaculture Series Brochure*, AGA AB, Lidingö, Sweden, 1993
11. M. B. Timmons and S. T. Summerfelt, "Advances in Circular Culture Tank Engineering to Enhance Hydraulics, Solids Removal, and Fish Management," in M. B. Timmons and T. M. Losordo, eds., *Advances in Aquacultural Engineering*, Aquacultural Engineering Society Proceedings III. Northeast Regional Agricultural Engineering Service, NRAES-105, Ithaca, New York, 1997, pp. 66–84.
12. J. Gunnarsson, "Bridgestone HI-SEAS Fish Cage: Design and Documentation," in H. Reinertsen et al., eds., *Fish Farming Technology, Proc. of the First International Conference on Fish Farming Technology*, Trondheim, Norway, August 9–12, 1993, A. A. Balkema, Rotterdam, 1993, pp. 199–201.
13. J. Colt and K. Orwicz, "Modeling Production Capacity of Aquatic Culture Systems under Freshwater Conditions," *Aquacultural Eng.* **10**, 1–29 (1991).
14. T. M. Losordo and H. Westers, "System Carrying Capacity," in M. B. Timmons and T. M. Losordo, eds., *Aquaculture Water Reuse Systems: Engineering Design and Management*, Elsevier, Amsterdam, 1994, pp. 9–60.
15. S. E. Jørgensen, *Fundamentals of Ecological Modelling*, Elsevier, Amsterdam, 1986.
16. D. E. Brune and J. K. Wang, "Recirculation in Photosynthetic Aquaculture Systems," *Aquaculture Magazine* **24**(3), 63–71 (1998).
17. J. Colt, "Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity, and Pressure," American Fisheries Society Special Publication No. 14, Bethesda, Md., 1984.
18. H. L. Elmore and T. W. Hayes, "Solubility of Atmospheric Oxygen in Water," *J. Sanitary Eng. Div. ASCE* **86**(SA4), 41 (1960).
19. B. J. Watten, "Aeration and Oxygenation," in M. B. Timmons and T. M. Losordo, eds., *Aquaculture Water Reuse Systems: Engineering Design and Management*, Elsevier, Amsterdam, 1994, pp. 173–208.
20. G. Hackney and J. Colt, "The Performance and Design of Packed Column Aeration Systems for Aquaculture," *Aquacultural Eng.* **1**, 275–295 (1982).
21. R. B. Banks and F. F. Herrera, "Effects of Wind and Rain on Surface Reaeration," *J. Environ. Eng Div. ASCE* **103**(EE3), 489–504 (1977).
22. W. Bruin, J. W. Nightingale, and L. Mumaw, "Preliminary Results and Design Criteria from and On-line Zeolite Ammonia Removal Filter in a Semi-closed Recirculating System," in L. J. Allen and E. C. Kinney, eds., *Proc. of the Bio-Engineering Symposium for Fish Culture*, American Fisheries Society, Bethesda, Md., 1981, pp. 92–96.
23. F. W. Wheaton, et al., "Nitrification Filter Principles," in M. B. Timmons and T. M. Losordo, eds., *Aquaculture Water Reuse Systems: Engineering Design and Management*, Elsevier, Amsterdam, 1994, pp. 101–126.
24. F. W. Wheaton, et al., "Nitrification Filter Design Methods," in M. B. Timmons and T. M. Losordo, eds., *Aquaculture Water Reuse Systems: Engineering Design and Management*, Elsevier, Amsterdam, 1994, pp. 127–171.
25. S. Chen, D. Stechey, and R. F. Malone, "Suspended Solids Control in Recirculating Aquaculture Systems," in M. B. Timmons and T. M. Losordo, eds., *Aquaculture Water Reuse Systems: Engineering Design and Management*, Elsevier, Amsterdam, 1994, pp. 61–100.

RAUL H. PIEDRAHITA
University of California
Davis, California

AROMATHERAPY

Aromatherapy is a branch of the alternate medicine approach that uses herbal remedies to improve an individual's health and appearance and to alter one's mood. The alleged benefits from aromatherapy range from stress relief to enhancement of immunity and the unlocking of emotions from past experience. The concept has ancient roots but is primarily used today by the cosmetics, fragrance, and alternative-medicine industries.

The proponents of aromatherapy claim that the tools of the trade, such as wood-resin distillates and flower, leaf, stalk, root, grass, and fruit extracts, contain antibiotics, antiseptics, hormones, and vitamins. Some proponents go even further and have characterized the essential oils, which are volatile, aromatic, and flammable, as the soul or spirit of plants. One of aromatherapy's promises is that the essential oils have a "spiritual dimension" and can restore "balance" and "harmony" to one's body and to one's life. One of its principles is the "doctrine of signatures," which claims that a plant's visible and olfactory characteristics reveal its secret qualities. For example, the violet suggests shyness so the proponents conclude that the scent of violets engenders shyness and modesty. Aromatherapy encompasses topical application of essential oils, bathing in water containing essential oils, sniffing them, or actually ingesting them. Products supporting this concept include shaving gels, aftershaves, facial cleansers, bath salts, shower gels, shampoos, hair conditioners, body masks, moisturizers, sunscreen preparations, lipsticks, deodorants, candles, lamps, diffusers, pottery, massage oils, massage devices, and jewelry such as lockets and pendants for carrying essential oils.

The most common aromatherapy applications are aesthetic, where a sense of well-being is derived from enjoying perfumes, scented candles and baths, and other fragrances. At the opposite end of the spectrum is medical aromatherapy, also known as aromatic medicine, which includes massage therapists, naturopaths, some nurses, and some medical doctors. The alleged beneficial effects are numerous: essential oil from bergamot normalizes emotions; essential oil from roses or sandalwood increases confidence; essential oil from eucalyptus alleviates sorrow, and oil from patchouli creates a desire for peace. Eucalyptus oil and peppermint oil have been used to treat respiratory diseases. But it is conceded that some oils can be harmful. Concentrated oils from cloves, cinnamon, nutmeg, and ginger can burn the skin and ingestion of oil from pennyroyal can cause miscarriages. One of the obvious applications of aromatherapy involves the sense of smell, and one researcher believes that the lack of ability to smell is correlated with a gain in weight. Others believe that the ability to smell or even the ability of the body to produce certain odors indicates certain diseases or impairments. If this proves to be true, it would be an interesting diagnostic tool.

The concept behind aromatherapy is accepted in parts of Europe. For example, in France, medical students are taught how to prescribe essential oils, and in Britain, hospital nurses use aromatherapy to treat patients suffering anxiety and depression and to make terminal-care patients more comfortable. Dorene Peterson, principal of the Australasian College of Herbal Studies, commented, "There is a philosophical difference between hard-core science and the approach that believes there's vibrational energy that's part of the healing process. Alternative medicine is offered now in quite a number of medical schools. I think a lot of hard-core scientists and doctors who have been trained in the data-oriented scientific approach are realizing there's more to heaven and earth than we really know about." However, she admits that empirical evidence is necessary for widespread acceptance. In the United States, there are no legal standards concerning the education in aromatherapy, or the certification or occupational practice of aromatherapy. Several nonaccredited organizations offer short courses or correspondence courses, but no accredited educational institutions offer majors in aromatherapy. It is safe to say that aromatherapy remains outside the mainstream of medical therapy, but it is well ensconced in the cosmetic area.

GENERAL REFERENCE

C. A. Sweet, "Scents and Nonsense," *Priorities* **9**, 30–33 (1997).

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amherst, Massachusetts

ARTIFICIAL INTELLIGENCE

BACKGROUND

Artificial intelligence is defined as the application for computer technology to resemble human thought and action. It is also the branch of computer science concerned with the development of machines having this ability. Artificial intelligence was widely acclaimed in the 1980s as revolutionary technology. However, when expectations went unrealized, its popularity faded. More recently it is reemerging with revamped development tools and languages and advanced flexibility. The restrictions of expensive, large machines and obscure languages have been overcome. Many of the lofty early claims have been dismissed and replaced with more pragmatic and profitable applications. The use of artificial intelligence is subtly in place for a variety of industrial, business, and consumer applications.

Areas of artificial intelligence include robotics, machine vision, voice recognition, natural language processing, expert systems, neural networks, and fuzzy logic. The term artificial intelligence was first used in 1956 at a conference held at Dartmouth College in Hanover, New Hampshire. The basis of the conference was the conjecture that every feature of intelligence can be so precisely described that a machine can be made to simulate it (1). Since that time the field has grown, evolved, fragmented, developed, and in many ways become incorporated into mainstream software.

Robotics

Robotics, along with computer vision, have been applied in food-processing operations for sorting and evaluation. Robots are used in manufacturing, performing such functions as welding and painting. Labor-intensive, repetitive tasks such as those found in packaging have been prime applications for robots. Even routine lab testing has incorporated robotics when the volume of samples justifies the expense. Through artificial intelligence techniques robots can function in unstructured situations, performing tasks such as identifying objects and selecting and assembling them into a unit.

Machine Vision

Machine vision is the ability of a machine to look at or see objects and to differentiate between those objects intelligently. A vision system can observe defects even at microscopic levels. Because of its success, the implementation of machine vision is extensive. In the food industry, machine vision is used for sorting purposes in quality control applications. Machine vision, coupled with neural network-based products, is being used to recognize patterns, optical characters, and bar codes.

Voice Recognition

Voice recognition systems respond to the human voice instead of a keyboard or other input. In certain industrial settings such as package routing, voice recognition has been successful. Initially, because of the wide variety in human voice patterns, voice recognition was constrained to a narrow range of applications or subjects. The technology has since advanced to common usage in telecommunications for automated information exchange.

Natural Language Processing

Natural language processing allows computer software to understand normal conversational commands. Natural language is gaining strength in foreign language translation. Although syntax often suffers, at least approximate translations can be commercially provided and are readily available by means of the Internet. In database applications, natural language programs translate queries from natural language to some database query language. Natural language processing is steadily developing in order to facilitate the user/computer interface.

Expert Systems

Expert systems are computer programs designed to solve problems requiring experts. In the field of artificial intelligence, knowledge-based expert systems received most of the early attention. An expert system includes a knowledge base and an inference engine. The knowledge base is the accumulation and representation of knowledge specific to a particular task. It is often constructed in the form of rules, but methods such as frames and semantic nets are also used. Rule-based expert systems rule statements normally include a premise and a conclusion, using If and Then statements. The inference engine is the system's machinery for selecting and applying knowledge from the knowledge base to the specific problem. An inference engine uses the knowledge base along with responses from the user to solve problems. The inference engine takes information and proceeds forward through the knowledge base looking for a valid path. The engine may either take information and proceed forward through the knowledge base looking for a valid path, or start with goals and work backward until a clear path is found. In conventional programs the knowledge base is part of the program, but with an expert system it is separate. This distinction makes it possible to substitute a new knowledge base for a new task in place of the existing knowledge base. Expert systems can also be updated readily as new knowledge is discovered. Expert systems take advantage of an expert's heuristics or knowledge from experience. The combination of heuristics and learned principles helps account for the value of an expert. Usually an expert system is developed through an iterative process where an initial program is prepared and then developed and improved with additional items of knowledge. Expert systems cannot exactly model human problem-solving processes, but rather they attempt to interact with expert thought concerning specific areas of knowledge. Most expert systems allow confidence factors in order to express uncertainty. Uncertainty may arise from either the stored knowledge base or user responses.

Although an expert system is useful for arriving at a solution from numerous options, its range of focus needs to be narrow to avoid an excessively complex system. The economic advantages of going to the time and expense of developing an expert system need to be established. If the problem is very simple or if ample resources are already available to solve the problem within the company, then the investment may not be worthwhile. Some problems are too broad or general to be solved by an expert system. To be solved by an expert system, the problem would have to be solvable to start with by a human expert in the field. Expert systems work well with programs where symbolic logic and rules are required.

Expert systems can benefit problem-solving situations in which humans suffer from cognitive overload or fail to monitor all available information. It can be difficult to simultaneously manipulate all relevant information to obtain the optimal solution. An expert system may be more consistent in certain situations in which there is little time to think. Expert systems can often deliver solutions more quickly than their human counterparts. They are also readily available at any time. Human experts are then able to better use their skills on more difficult problems beyond the ability of the expert system. The process of building a knowledge base can help to clarify and organize an expert's logic and thinking process. A disadvantage of an expert system is that it can be costly to develop. It also needs to be maintained and updated to remain current.

Neural Networks

Neural networks attempt to figuratively model the neurological processes of the brain, in a stimulus/response structure. Neural networks essentially recognize patterns in data leading from initial events to results. These programs are well suited to systems in which traditional statistics are not applicable. The technology's power lies in its ability to analyze large combinations of variables very quickly. The neural network is trained by observing a multitude of patterns. For many processes indications of product quality parameters, such as moisture, color, texture, concentration, or taste, cannot be measured at the point of control. Quality labs check samples from the end of the line, which is too late for process control, resulting in considerable scrap or costly rework. Neural networks can be trained to predict quality parameters from on-line process data at the point of control. This information can then be used either manually or automatically for controlling the process. Whenever product quality needs to be controlled based on measurements taken after the process, neural networks are useful.

Fuzzy Systems

Fuzzy systems are distinguished primarily by their support for a gradual and continuous transition rather than a sharp and abrupt change between binary values. Information obtained in food process control may be noisy, incomplete, scrambled, erroneous, or generally uncertain (2). Fuzzy systems are most suitable for uncertain or approximate reasoning, particularly systems with a mathematical model that is difficult to derive. Designers are able to represent descriptive or qualitative expressions such as "slow" or "moderately fast" and incorporate them with symbolic statements. Integrated circuit manufacturers have fabricated chips with built-in fuzzy logic capabilities.

EXAMPLES

The addition of an expert system can advise a process controller when to make adjustments. These devices use pattern recognition algorithms, calculate control loop performance on-line, and readjust operator intervention. Expert systems can help monitor a process and notify operators of an out-of-specification situation. Self-tuning controllers can also use model-based references where the difference in response between the process and the model is used to tune the controller and also update the model. A classic expert system for process control was designed for advisory control of thermal processing in a major soup company (3). The expertise of a retiring staff member for troubleshooting, start-up, and shutdown of hydrostatic and rotary sterilizers was captured in an expert system program, saving the company up to US$3 million per year. An expert system can respond to user questions, query the user for additional information, and provide a report of the interaction along with a process and instrumentation diagram. Fault analyzers respond to alarms and other process inputs to diagnose abnormal conditions. They can find a previously determined pattern in a set of alarm messages. Rather than receiving a number of alarms, the operator will receive a simplified message stating the recommendation. Corrective action can then be taken.

A widely used rule-based expert system from Gensym Corp (Cambridge, Mass.), among other applications, has been useful in controlling wastewater treatment. The arrangement of equipment and controls is a complex, time-consuming assignment and many factors need to be considered. With control-system configuration, operating conditions and parameters, objectives, and constraints such as equipment limitations can be entered into a process model. The configuration best satisfying all requirements is then presented to the user.

Neural networks can predict patterns in process data, which in turn can be used to control the process as desired. With neural network pattern recognition software, responses are observed and necessary control adjustments are made. Applications have been reported across a wide variety of industries. Pattern recognition methods have been used for the identification of flours (4). BrainMaker (Nevada City, Calif.) has reported a wide variety of applications for its neural network product including investment advising, heart attack diagnosis, beverage quality testing, chemical structure recognition, and protein sequence pattern recognition. The application of neural networks in the dairy industry was recently discussed in an Australian seminar (5). Reports included neural networks in ultrahigh temperature (UHT) operations, natural language computer control of crucial steps in cheese making, and fermentation processes. The effects of transmembrane pressure and cross-flow velocity on cross-flow filtration were modeled using a neural network approach. Modeling was obtained after only five experimental trials for either raw cane sugar remelt or natural gum solution (6). The use of neural networks for modeling instrumental-sensory relationships has been investigated. The advantages and disadvantages of using neural networks were compared with multivariate linear methods of principal components regression (PCR) and partial least-squares (PLS) regression. It was concluded that neural networks cannot replace PCR and PLS for linear relationships but do offer potential for modeling nonlinear relationships (7).

Questions regarding expansion of neural networks when necessary have prompted research on network configuration. Learning rates for neural networks, which were expanded by coupling modules, rather than simply directly expanding the existing network were improved. Coupled neural networks were found to improve food starch identification (8).

The relationships between process parameters and flow rates through ultrafiltration membranes have been examined using fuzzy mathematics (9). Main parameters in the permeate flow were identified. Fuzzy logic has been used in many familiar applications including, home appliances, video cameras, automobiles, robots, and aircraft. One company has developed a hybrid product combining neural networks, fuzzy logic, genetic algorithms, and chaos theory (Neuristics, Baltimore, Md.). The company maintains this design overcomes limitations of neural networks, explaining the logic behind its forecasts.

Regarding business applications, neural networks have been successfully used for bank loan applications. A program called Countrywide Loan Underwriting Expert System, or CLUES (Brightware, Inc, Novato, CA), evaluates a loan file's strengths and weaknesses in human terms. Loans can be approved in less than 2 min compared with 50 min for human underwriters. Benefits include personnel cost savings and better quality and consistency in underwriting. Neural networks can be used to set up intelligent agents for information services. These agents are able to automatically locate and retrieve competitive intelligence information. Consumer electronics industries have developed software programs with the ability to operate autonomously, performing operations ranging from electronic shopping to getting data over the Internet. The growth of the Internet may create new artificial intelligence applications.

ADVANTAGES/FUTURE DEVELOPMENT

The many facets of artificial intelligence are able to reduce labor costs, improve productivity and quality, and remove human bias. Expert systems can act as training tools, both during the process of development, as well as during implementation. Advanced computer-aided systems can improve food safety, reduce processing times, and achieve higher quality and yields at minimal costs if applied correctly to the food processing system (10). Human talents and abilities can be better utilized and even assisted as these programs are made available for process control and analysis.

The large number of computing techniques within the very broad terms of artificial intelligence will continue to drive many of the advanced applications in science, industry, and consumer products. Products will continue to improve in data assimilation and communication, replacing an increasing number of tasks previously requiring human intervention.

Artificial intelligence will continue in importance as it becomes embedded in mainstream systems. The different components of artificial intelligence are each finding their own particular embedded applications.

BIBLIOGRAPHY

1. E. Charniak and D. McDermott, *Introduction to Artificial Intelligence*, Addison-Wesley, Reading, Mass., 1985.
2. T. Eerikainen et al., "Fuzzy Logic and Neural Network Applications in Food Science and Technology," *Trends Food Sci. Technol.* **4**, 237–242 (1993)
3. R. K. Tyson and R. A. Herrod, "Capturing 32 Years on Computer," *Chilton's Food Eng.* **57**, 69–71 (Dec. 1985).
4. P. Zagrodzki et al., "Characterisation of Flour by Means of Pattern Recognition Methods," *Food Chem.* **53**, 295 (1995).
5. J. A. Hourigan, "Artificial Neural Networks in the Dairy Industry," *Aust. J. Dairy Technol.* **49**, 110–121 (1994).
6. M. Dornier et al., "Interest of Neural Networks for the Optimization of the Crossflow Filtration Process," *Lebensmittel-Wissenschaft Technol.* **28**, 300–309 (1995).
7. C. Wilkenson and D. Yuksel, "Using Artificial Neural Networks to Develop Prediction Models for Sensory-Instrumental Relationships: An Overview," *Food Quality Preference* **8**, 439–445 (1997).
8. Y. W. Huang et al., "Modular Neural Networks for Identification of Starches in Manufacturing Food Products," *Biotechnol. Progr.* **9**, 401–410 (1993).
9. P. Vaija et al., "Prediction of the Flux through an Ultrafiltration Membrane Using Fuzzy Mathematics," *J. Membrane Sci.* **83**, 173–179 (1993).
10. S. Linko, "Expert Systems: What Can They Do for the Food Industry?" *Trends Food Sci. Technol.* **9**, 3–12 (1998).

ROBERT OLSEN
Schreiber Foods, Inc.
Tempe, Arizona

ASEPTIC PROCESSING AND PACKAGING SYSTEMS

In aseptic processing, packages and food product are sterilized in separate systems. The sterile package is then filled with sterile product, closed, and sealed in a sterile chamber. Because aseptic processing is a continuous operation, the behavior of one part of the system can affect the overall performance of the entire system. As a result, there are numerous critical factors associated with aseptic processing and packaging often requiring automated control systems. Process establishment for aseptic systems must consider not only the sterilization of the product, the processing equipment, and downstream piping, but also the sterilization of the packaging material and the packaging equipment and the maintenance of sterile conditions throughout the aseptic system.

DEFINITIONS

To assist in the discussion of aseptic processing and packaging systems, some definitions are presented here.

Aseptic describes a condition in which there is an absence of microorganisms, including viable spores. In the food industry, the terms aseptic, sterile, and commercially sterile are often used interchangeably.

Aseptic system refers to the entire system necessary to produce a commercially sterile product contained in a hermetically sealed container. This term includes the product processing system and the packaging system.

Aseptic processing system refers only to the system that processes the product and delivers it to a packaging system.

Aseptic packaging system refers to any piece of equipment that fills a sterile package or container with sterile product and hermetically seals it under aseptic conditions. These units or systems may also form and sterilize the package.

BASIC ASEPTIC SYSTEM

Figure 1 is a diagram of a simplified aseptic system. Raw or unprocessed product is heated, sterilized by holding at high temperature for a predetermined amount of time, then cooled and delivered to a packaging unit for packaging. Commercial sterility is maintained throughout the system, from product heating to the discharge of hermetically sealed containers.

DESCRIPTION OF THE ASEPTIC PROCESSING SYSTEM

Although the equipment for aseptic processing systems varies, all systems have certain common features:

1. A pumpable product
2. A means to control and document the flow rate of product through the system
3. A method of heating the product to sterilizing temperatures
4. A method of holding product at an elevated temperature for a time sufficient for sterilization
5. A method of cooling product to filling temperature
6. A means to sterilize the system before production and to maintain sterility during production
7. Adequate safeguards to protect sterility and prevent nonsterile product from reaching the packaging equipment

Preproduction Sterilization

Producing a commercially sterile product cannot be assured unless the processing system and filler have been adequately sterilized before starting production. It is important that the system be thoroughly cleaned before sterilization; otherwise the process may not be effective. Some systems, or portions thereof, use saturated steam for sterilization. However, for most systems, equipment sterilization is accomplished by circulation of hot water through the system for a sufficient length of time to render it commercially sterile. When water is used, it is heated in the

Artificial intelligence will continue in importance as it becomes embedded in mainstream systems. The different components of artificial intelligence are each finding their own particular embedded applications.

BIBLIOGRAPHY

1. E. Charniak and D. McDermott, *Introduction to Artificial Intelligence*, Addison-Wesley, Reading, Mass., 1985.
2. T. Eerikainen et al., "Fuzzy Logic and Neural Network Applications in Food Science and Technology," *Trends Food Sci. Technol.* **4**, 237–242 (1993)
3. R. K. Tyson and R. A. Herrod, "Capturing 32 Years on Computer," *Chilton's Food Eng.* **57**, 69–71 (Dec. 1985).
4. P. Zagrodzki et al., "Characterisation of Flour by Means of Pattern Recognition Methods," *Food Chem.* **53**, 295 (1995).
5. J. A. Hourigan, "Artificial Neural Networks in the Dairy Industry," *Aust. J. Dairy Technol.* **49**, 110–121 (1994).
6. M. Dornier et al., "Interest of Neural Networks for the Optimization of the Crossflow Filtration Process," *Lebensmittel-Wissenschaft Technol.* **28**, 300–309 (1995).
7. C. Wilkenson and D. Yuksel, "Using Artificial Neural Networks to Develop Prediction Models for Sensory-Instrumental Relationships: An Overview," *Food Quality Preference* **8**, 439–445 (1997).
8. Y. W. Huang et al., "Modular Neural Networks for Identification of Starches in Manufacturing Food Products," *Biotechnol. Progr.* **9**, 401–410 (1993).
9. P. Vaija et al., "Prediction of the Flux through an Ultrafiltration Membrane Using Fuzzy Mathematics," *J. Membrane Sci.* **83**, 173–179 (1993).
10. S. Linko, "Expert Systems: What Can They Do for the Food Industry?" *Trends Food Sci. Technol.* **9**, 3–12 (1998).

Robert Olsen
Schreiber Foods, Inc.
Tempe, Arizona

ASEPTIC PROCESSING AND PACKAGING SYSTEMS

In aseptic processing, packages and food product are sterilized in separate systems. The sterile package is then filled with sterile product, closed, and sealed in a sterile chamber. Because aseptic processing is a continuous operation, the behavior of one part of the system can affect the overall performance of the entire system. As a result, there are numerous critical factors associated with aseptic processing and packaging often requiring automated control systems. Process establishment for aseptic systems must consider not only the sterilization of the product, the processing equipment, and downstream piping, but also the sterilization of the packaging material and the packaging equipment and the maintenance of sterile conditions throughout the aseptic system.

DEFINITIONS

To assist in the discussion of aseptic processing and packaging systems, some definitions are presented here.

Aseptic describes a condition in which there is an absence of microorganisms, including viable spores. In the food industry, the terms aseptic, sterile, and commercially sterile are often used interchangeably.

Aseptic system refers to the entire system necessary to produce a commercially sterile product contained in a hermetically sealed container. This term includes the product processing system and the packaging system.

Aseptic processing system refers only to the system that processes the product and delivers it to a packaging system.

Aseptic packaging system refers to any piece of equipment that fills a sterile package or container with sterile product and hermetically seals it under aseptic conditions. These units or systems may also form and sterilize the package.

BASIC ASEPTIC SYSTEM

Figure 1 is a diagram of a simplified aseptic system. Raw or unprocessed product is heated, sterilized by holding at high temperature for a predetermined amount of time, then cooled and delivered to a packaging unit for packaging. Commercial sterility is maintained throughout the system, from product heating to the discharge of hermetically sealed containers.

DESCRIPTION OF THE ASEPTIC PROCESSING SYSTEM

Although the equipment for aseptic processing systems varies, all systems have certain common features:

1. A pumpable product
2. A means to control and document the flow rate of product through the system
3. A method of heating the product to sterilizing temperatures
4. A method of holding product at an elevated temperature for a time sufficient for sterilization
5. A method of cooling product to filling temperature
6. A means to sterilize the system before production and to maintain sterility during production
7. Adequate safeguards to protect sterility and prevent nonsterile product from reaching the packaging equipment

Preproduction Sterilization

Producing a commercially sterile product cannot be assured unless the processing system and filler have been adequately sterilized before starting production. It is important that the system be thoroughly cleaned before sterilization; otherwise the process may not be effective. Some systems, or portions thereof, use saturated steam for sterilization. However, for most systems, equipment sterilization is accomplished by circulation of hot water through the system for a sufficient length of time to render it commercially sterile. When water is used, it is heated in the

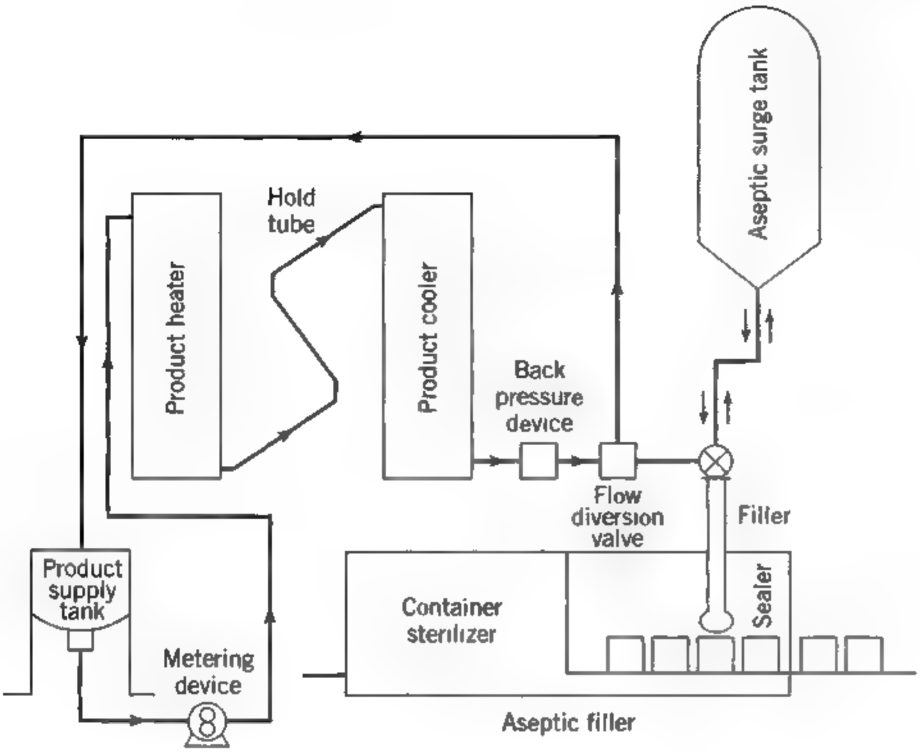


Figure 1. Simplified diagram of an aseptic processing system.

product heater and then pumped through all downstream piping and equipment up to (and generally past) the filler valve on the packaging unit. All product contact surfaces downstream from the product heater must be maintained at or above a specified temperature by continuously circulating the hot water for a required period of time.

Surge tanks are generally sterilized with saturated steam rather than hot water owing to their large capacity. Although sterilization of surge tanks may occur separately, it is usually conducted simultaneously with hot water sterilization of the other equipment.

To control aseptic system sterilization properly, it is necessary that a thermometer or thermocouple be located at the coldest point(s) in the system to ensure that the proper temperature is maintained throughout. Thus, the temperature-measuring device is generally located at the most distant point from the heat exchangers. Timing of the sterilization cycle begins when the proper temperature is obtained at this remote location. If this temperature should fall below the minimum, the cycle should be restarted after the sterilization temperature is reestablished.

Flow Control

Sterilization time or residence time, as indicated in the scheduled process, is directly related to the rate of flow of the fastest-moving particle through the system. The fastest-moving particle is a function of the flow characteristics of the food. Consequently, a process must be designed to ensure that product flows through the system at a uniform and constant rate so that the fastest-moving particle of food receives at least the minimum amount of heat for the minimum time specified by the scheduled process. This constant flow rate is generally achieved with a pump, called a timing or metering pump.

Timing pumps may be variable speed or fixed rate. The pumping rate of the fixed-rate pump cannot be changed without dismantling the pump. Variable-speed pumps are designed to provide flexibility and allow for easy rate changes. When a variable-speed pump is used, it must be protected against unauthorized changes in the pump speed that could affect the rate of product flow through the system.

Product Heating

A product heater brings the product to sterilizing temperature. There are two major categories of product heaters in aseptic food processing: direct and indirect.

Direct heating, as the name implies, involves direct contact between the heating medium (steam) and the product. Direct heating systems can be one of two types: steam injection and steam infusion.

Steam injection introduces steam into the product in an injection chamber as product is pumped through the chamber (Figure 2). Steam infusion introduces product through a steam-filled infusion chamber (Fig. 3). These systems are currently limited to homogeneous, low-viscosity products.

Direct heating has the advantage of very rapid heating, which minimizes organoleptic changes in the product. The problems of fouling or burn-on of product in the system may also be reduced in direct heating systems compared with indirect systems.

There are also some disadvantages. The addition of water (from the condensation of steam in the product) in-

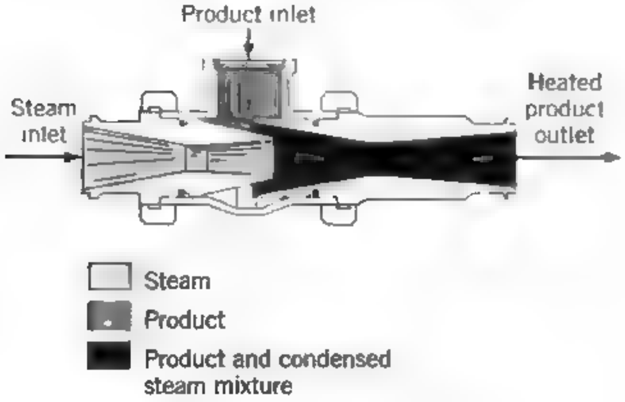


Figure 2. Steam injection.

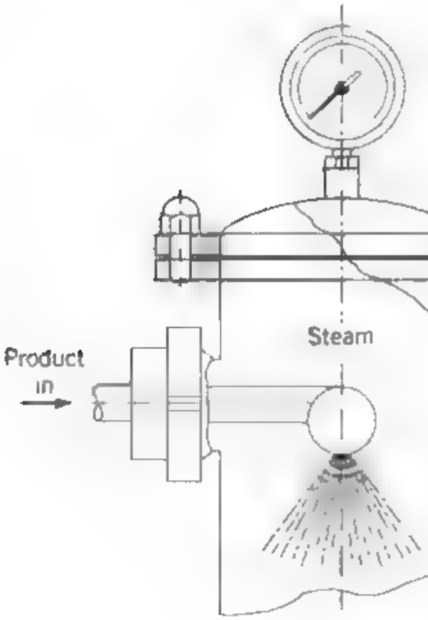


Figure 3. Steam infusion.

creases product volume. Because this change in volume increases product flow rate through the hold tube, it must be accounted for when establishing the scheduled process. Depending on the product being produced, water that was added as steam may need to be removed. Water removal is discussed under product cooling. Steam used for direct heating must be of culinary quality and must be free of noncondensable gases. Thus, strict controls on boiler feed water additives must be followed.

The other major category of product heaters is indirect heating units. *Indirect heating* units have a physical separation between the product and the heating medium. There are three major types of indirect heating units: plate, tubular, and swept-surface heat exchangers.

Plate heat exchangers are used for homogeneous liquids of relatively low viscosity. The plates serve as both a barrier and a heat-transfer surface with product on one side and the heating medium on the other. Each plate is gasketed, and a series of plates are held together in a press. The number of plates can be adjusted to meet specific needs.

Tubular heat exchangers employ either two or three concentric tubes instead of plates as heat-transfer surfaces. Product flows through the inner tube of the double-tube style and through the middle tube of the triple-tube style, with the heating medium in the other tube(s) flowing in the opposite direction to the product. In a shell and tube heat exchanger (considered a type of tubular exchanger), the tube is coiled inside a shell. Product flows through the tube while the heating medium flows in the opposite direction through the shell. As with plate heat exchangers, tubular heat exchangers are used for homogeneous products of low viscosity.

Scraped-surface heat exchangers are normally used for processing more viscous products (Fig. 4). The scraped-surface heat exchanger consists of a mutator shaft with scraper blades concentrically located within a jacketed, insulated heat exchange tube. The rotating blades continuously scrape the product off the wall. This scraping reduces buildup of product and burn-on. The heating medium flowing on the opposite side of the wall is circulating water or steam.

Some systems incorporate the use of product-to-product heat exchangers. These devices are either plate or tubular heat exchangers with product flowing on both sides of the plates or through both sets of tubes. This process allows the heat from the hot, sterile product to be transferred to the cool incoming, nonsterile product. Energy and cost savings can be significant by recycling the heat from sterile product.

When a product-to-product regenerator is used, the regenerator must be designed, operated, and controlled so that the pressure of the sterilized product in the regenerator is at least 1 psi greater than the pressure of any nonsterilized product in the regenerator. This pressure differential helps ensure that any leakage in the regenerator will be from sterilized product into nonsterilized product.

Hold Tube

Once the product has been brought to sterilizing temperature in the heater, it flows to a hold tube. The time required for the fastest product particle to flow through the hold tube is referred to as the residence time. The residence time must be equivalent to or greater than the time necessary at a specific temperature to sterilize the product and is specified in the scheduled process. Hold-tube volume, which is determined by hold-tube diameter and length, combined with the flow rate and flow characteristics of the product, determines the actual residence time of the product in the hold tube. Because the hold tube is essential for ensuring that the product is held at sterilization temperatures for the proper time, certain precautions must be followed:

1. The hold tube must have an upward slope in the direction of product flow at least 0.25 in./ft to assist in eliminating air pockets and prevent self-draining.

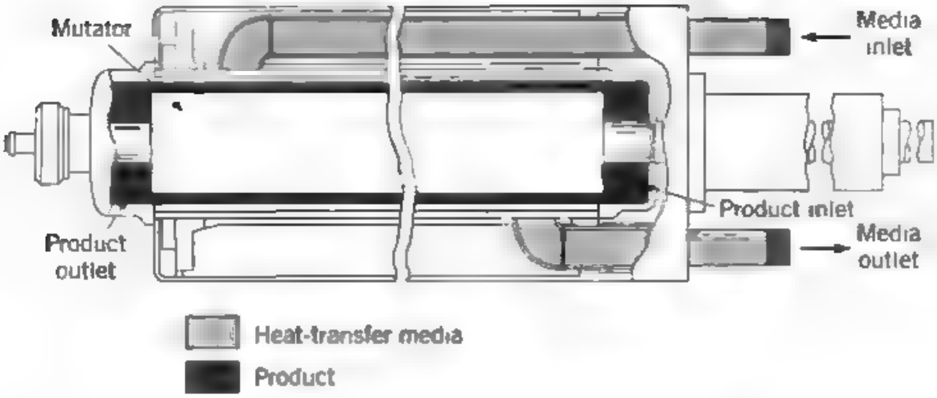


Figure 4. Scraped-surface heat exchanger.

2. If the hold tube can be taken apart, care should be taken that all parts are replaced and that no parts are removed or interchanged to make the tube shorter or different in diameter. Such accidental alterations could shorten the time the product remains in the tube.
3. If the hold tube can be taken apart, care should be exercised when reassembling to ensure that the gaskets do not protrude into the inner surface. The tube interior should be smooth and easily cleanable.
4. There must be no condensate drip on the tube, and the tube should not be subjected to drafts or cold air, which could affect the product temperature in the hold tube.
5. Heat must not be applied at any point along the hold tube.
6. The product in the hold tube must be maintained under a pressure sufficiently above the vapor pressure of the product at the process temperature to prevent flashing or boiling because flashing can decrease the product residence time in the hold tube. The prevention of flashing is usually accomplished by use of a back-pressure device.

The temperature of the food in the hold tube must be monitored at the inlet and outlet of the tube. The temperature at the inlet of the tube is monitored with a temperature recorder-controller sensor that must be located at the final heater outlet and must be capable of maintaining process temperature in the hold tube. A mercury-in-glass thermometer or other acceptable temperature-measuring device (such as an accurate thermocouple recorder) must be installed in the product sterilizer hold tube outlet between the hold tube and the cooler inlet. An automatic recording thermometer sensor must also be located in the product at the hold tube outlet between the hold tube and cooler to indicate the product temperature. The temperature-sensing device chart graduations must not exceed 2°F (1°C) within a range of 10°F (6°C) of the required product sterilization temperature.

Product Cooling

Product flows from the hold tube into a product cooler that reduces product temperature before filling. In systems that use indirect heating, the cooler will be a heat exchanger that may be heating raw product while cooling sterile product. Those systems that use direct heating will typically include a flash chamber or vacuum chamber. The hot product is exposed to a reduced pressure atmosphere within the chamber, resulting in product boiling or flashing. The product temperature is lowered, and a portion or all of the water that was added to the product during heating is removed by evaporation. On discharge from the flash chamber, product may be further cooled in some type of heat exchanger.

Maintaining Sterility

After the product leaves the hold tube, it is sterile and subject to contamination if microorganisms are permitted to enter the system. One of the simplest and best ways to prevent contamination is to keep the sterile product flowing and pressurized. A back-pressure device is used to prevent product from boiling or flashing and maintains the entire product system under elevated pressure.

Effective barriers against microorganisms must be provided at all potential contamination points, such as rotating or reciprocating shafts and the stems of aseptic valves. Steam seals at these locations can provide an effective barrier, but they must be monitored visually to ensure proper functioning. If other types of barriers are used, there must be a means provided to permit the operator to monitor the proper functioning of the barrier.

Aseptic Surge Tanks

Aseptic surge tanks have been used in aseptic systems to hold sterile product before packaging. These vessels, which range in capacity from about 100 gal to several thousand gallons, provide flexibility, especially for systems in which the flow rate of a product sterilization system is not compatible with the filling rate of a given packaging unit. If the valving that connects a surge tank to the rest of the system is designed to allow maximum flexibility, the packaging and processing functions can be carried out independently, with the surge tank acting as a buffer between the two systems. A disadvantage of the surge tank is that all sterile product is held together, and if there is a contamination problem, all product is lost. A sterile air or other sterile gas supply system is needed to maintain a protective positive pressure within the tank and to displace the contents. This positive pressure must be monitored and controlled to protect the tank from contamination.

Automatic Flow Diversion

An automatic flow-diversion device may be used in an aseptic processing system to prevent the possibility of potentially unsterile product from reaching the sterile packaging equipment. The flow-diversion device must be designed so that it can be sterilized and operated reliably. Past experience has shown that flow-diversion valves of the gravity drain type should not be used in aseptic systems owing to the possibility of recontamination of sterile product. Because the design and operation of a flow-diversion system is critical, it should be done in accordance with the recommendations of an aseptic processing authority.

NONTHERMAL PROCESSING

The major disadvantage of an aseptic processing (and any thermal processing system) is that heat treatment results in destruction of nutrients and organoleptic properties of foods. An alternative to aseptic processing is nonthermal preservation of food. Methods of nonthermal preservation include Pulsed Electric Field (PEF), High-Pressure Processing (HPP), and Ultraviolet Processing (UV). PEF treatment and UV are inherently adaptable to a continuous processing system, whereas HPP was developed as a batch system, although there is one continuous HPP system.

PEF is a process that uses very short duration (~2μs) of high electric field pulses (~30–50 kV) to destroy microorganisms in liquid and other pumpable foods. Research has shown that PEF is very effective against vegetative microorganisms; however, its effect against spore-forming microorganisms is still under study. PEF causes an electroporation of the semipermeable microbial cell envelope causing cell injury and eventual death.

HPP involves the application of high pressures (up to 140,000 psi) to food. The high pressure disrupts microbial cells and deactivates certain enzymes. Up until recently HPP has been a batch operation. However, one company, Flow International, has developed a semicontinuous high-pressure system that is currently undergoing testing. HPP has also been shown to be quite effective on vegetative microorganisms; however, for HPP to be effective against spore formers, moderate heat (60–70°C) is necessary in combination with the high pressure.

UV processing, as the name implies, uses ultraviolet electromagnetic rays to affect microbial destruction. Currently, UV has been shown to be effective only against vegetative microorganisms. UV has also been used effectively for water treatment.

ASEPTIC PACKAGING SYSTEMS

Basic Requirements

Aseptic packaging units are designed to combine sterile product with a sterile package, resulting in a hermetically sealed shelf-stable product. As with aseptic processing systems, certain features are common to all aseptic packaging systems. The packaging units must do the following:

1. Create and maintain a sterile environment in which the package and product can be brought together
2. Sterilize the product contact surface of the package
3. Aseptically fill the sterile product into the sterilized package
4. Produce hermetically sealed containers
5. Monitor and control critical factors

Sterilization Agents

Sterilization agents are used in aseptic packaging units to sterilize the packaging material and the internal equipment surfaces to create a sterile packaging environment. In general, these agents involve heat, chemicals, high-energy radiation, or a combination of these. For aseptic packaging equipment the sterilization agents used must effectively provide the same degree of protection in terms of microbiological safety that traditional sterilization systems provide for canned foods. This requirement applies to both the food contact surface of the packaging material and the internal machine surfaces that constitute the aseptic or sterile zone within the machine. The safety and effectiveness of these agents must be proven and accepted or approved by regulatory agencies for packaging commercially sterile low-acid or acidified foods in hermetically sealed containers. Food processors considering use of an aseptic packaging unit should request written assurances that the equipment has passed such testing and that the equipment and sterilizing agents are acceptable to the regulatory agencies for their intended use.

Heat is the most widely used method of sterilization. Steam or hot water is commonly used and referred to as moist heat. Superheated steam or hot air may also be used in certain situations and is referred to as dry heat. Dry heat is a much less effective sterilization agent than moist heat at the same temperature. Systems that use moist heat operate at elevated pressures compared with dry heat systems, which operate at atmospheric pressures. Other methods may be used to generate heat, such as microwave radiation or infrared light. As new methods are developed, they will have to be evaluated by aseptic processing authorities.

Chemical agents, primarily hydrogen peroxide, are often used in combination with heat as sterilization agents. The Food and Drug Administration (FDA) regulations specify that a maximum concentration of 35% hydrogen peroxide may be used for food contact surfaces. If hydrogen peroxide is used as a sterilant, the packaging equipment must be capable of producing finished packages that also meet FDA requirements for residuals. Not more than 0.5 ppm hydrogen peroxide may be present in tests done with distilled water packaged under production conditions. These regulations apply also to products regulated by the U.S. Department of Agriculture (USDA).

Other sterilants, such as high-energy radiation (UV-light, γ radiation, or electron-beam radiation), could be used alone or in combination with existing methods. Completely new alternative sterilants may be developed in the future. Whatever methods are developed, they will have to be proven effective in order to protect the public health and will be compared with existing methods.

Aseptic Zones

The aseptic zone is the area within the aseptic packaging machine that is sterilized and maintained sterile during production. This is the area in which the sterile product is filled and sealed in the sterile container. The aseptic zone begins at the point where the package material is sterilized or where presterilized package material is introduced into the machine. The area ends after the seal is placed on the package and the finished package leaves the sterile area. All areas between these two points are considered as part of the aseptic zone.

Before production, the aseptic zone must be brought to a condition of commercial sterility analogous to that achieved on the packaging material or other sterile product contact surfaces. This area may contain a variety of surfaces, including moving parts composed of different materials. The sterilant(s) must be uniformly effective and their application controllable throughout the entire aseptic zone.

Once the aseptic zone has been sterilized, sterility must be maintained during production. The area should be constructed in a manner that provides sterilizable physical barriers between sterile and nonsterile areas. Mechanisms must be provided to allow sterile packaging materials and hermetically sealed finished packages to enter and leave the aseptic zone without compromising the sterility of the zone.

The sterility of the aseptic zone can be protected from contamination by maintaining the aseptic zone under positive pressure of sterile air or other gas. As finished containers leave the sterile area, sterile air flows outward, preventing contaminants from entering the aseptic area. The sterile air pressure within the aseptic zone must be kept at a level proven to maintain sterility of the zone. Air or gases can be sterilized using various sterilization agents, but the most common methods are incineration (dry heat) and/or ultrafiltration.

Production of Aseptic Packages

A wide variety of aseptic packaging systems are in use today. These are easily categorized by package type:

1. Preformed rigid and semirigid containers, including
   a. Metal cans
   b. Composite cans
   c. Plastic cups
   d. Glass containers
   e. Drums
2. Web-fed paperboard laminates and plastic containers
3. Partially formed laminated paper containers
4. Thermoform-fill-seal containers
5. Preformed bags or pouches
6. Blow-molded containers

A number of different packaging systems are represented in these categories. Not all of these systems, however, are being used in the United States for aseptic applications.

Containers in these categories may be sterilized by a variety of means. For example, one system utilizing metal cans uses superheated steam to sterilize the containers. In other systems, preformed plastic cups may be sterilized by hydrogen peroxide and heat or by saturated steam. Systems using containers formed from paperboard laminates also utilize hydrogen peroxide and heat or hydrogen peroxide and ultraviolet irradiation to sterilize packages. Thermoform-fill-seal containers may be sterilized by the heat of extrusion (dry heat) or by hydrogen peroxide and heat. Plastic pouches or bags may be sterilized by gamma irradiation, by the heat of extrusion, or by chemical means such as hydrogen peroxide.

Research is now being conducted to explore alternative sterilization methods for most categories of packaging. Thus, it can be said with some certainty that currently familiar equipment may not be state of the art tomorrow. Nevertheless, whatever equipment or sterilant or packaging material is used, the monitoring and control of critical factors will be vital to successful operation.

SUMMARY

1. Aseptic is a term that describes the absence of microorganisms and may be used interchangeably with commercial sterility.
2. An aseptic processing system consists of a timing pump, a means to heat the product, a hold tube, and a means to cool the product.
3. The processing system from the hold tube past the fillers is brought to a condition of commercial sterility before the introduction of product into the system.
4. The thermal process occurs in the hold tube where flow rate, or residence time, and temperature are critical factors.
5. In the event of a process deviation, flow-diversion devices are used to prevent potentially inadequately processed product from reaching the filler.
6. Aseptic packaging machines create and maintain an aseptic zone (sterile environment) in which sterilized containers are filled and sealed.
7. Sterilization agents such as heat, chemicals, high-energy radiation, or a combination of these, can be used to sterilize packages or machine surfaces.

GENERAL REFERENCES

Adapted from *Canned Foods—Principles of Thermal Process Control, Acidification and Container Closure Evaluation*, 6th ed., The Food Processors Institute, Washington, D.C., 1995.

K. Stevenson
D. Chandarana
National Food Processors Association
Dublin, California

ASEPTIC PROCESSING: OHMIC HEATING

The recent development of the ohmic heater represents a major advance in the continuous processing of particulate food products. The ohmic heater uses resistance heating within the flow of electrically conducting liquid and particulates to provide heat and is capable of handling food products containing particulates of up to 25 mm.

When combined with an aseptic container or bag-in-box filling system, this new process offers the food processor with the possibility of producing high-quality, ready-prepared meals containing large quantities of undamaged chunks of meat and vegetables, which can be stored at ambient temperatures for long periods of time. The process has also been successfully applied to the sterilization of diced and sliced fruit at solids concentrations of up to 80% drained weight.

PRINCIPLE OF OHMIC HEATING

The ohmic heating effect occurs when an electric current is passed through a conducting product (Fig. 1). In practice, low-frequency alternating current (50 or 60 Hz) from the public main supply is used to eliminate the possibility of adverse electrochemical reactions and minimize power supply complexity and cost.

In common with microwave heating, electrical energy is transformed into thermal energy. However, unlike microwave heating, the depth of penetration is virtually unlimited and the extent of heating is governed by the spatial uniformity of electrical conductivity throughout the product and its residence time in the heater. For most practical purposes the product does not experience a large temperature gradient within itself as it heats, and liquid and particulate are heated virtually simultaneously. The requirement to overprocess the liquid to ensure sterility at the center of a large particulate as with scraped surface and tubular heat exchangers is therefore reduced. This results in less heat damage to the liquid phase and prevents overcooking of the outside of the particulates.

Another major advantage is that there are no heat transfer surfaces, which reduces the possibility of deposit formation and the transfer of burnt particles to the finished product. Nor is there a need for mechanical agitation of the product, which can cause loss of true particulate identity.

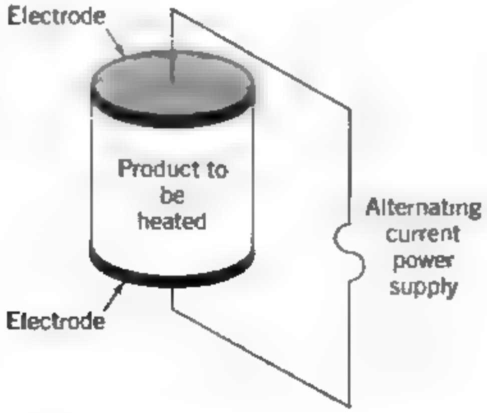


Figure 1. Principle of ohmic heating.

The applicability of ohmic heating depends on product electrical conductivity. Most food preparations contain a moderate percentage of free water with dissolved ionic salts and hence conduct sufficiently well for the ohmic effect to be applied. The system will not directly heat fats, oils, alcohol, bone, or crystalline structures such as ice.

DESIGN OF THE OHMIC HEATER

The ohmic heater column typically consists of four or more electrode housings machined from a solid block of poly(tetrafluoroethylene) (PTFE) and encased in stainless steel, each containing a single cantilever electrode (Fig. 2). The electrode housing are connected using stainless steel interconnecting tubes lined with an electrically insulating plastics liner. Suitable lining materials include poly(vinylidene difluoride) (PVDF) and glass. These flanged tube sections are bolted together and sealed with flat food-grade rubber gaskets.

The column is mounted in a vertical or near vertical position with the flow of product in an upward direction. A vent valve positioned at the top of the heater ensures the column is always full. The column is configured such that each heating section has the same electrical impedance; hence, the interconnecting tubes generally increase in length toward the outlet. This is because the electrical

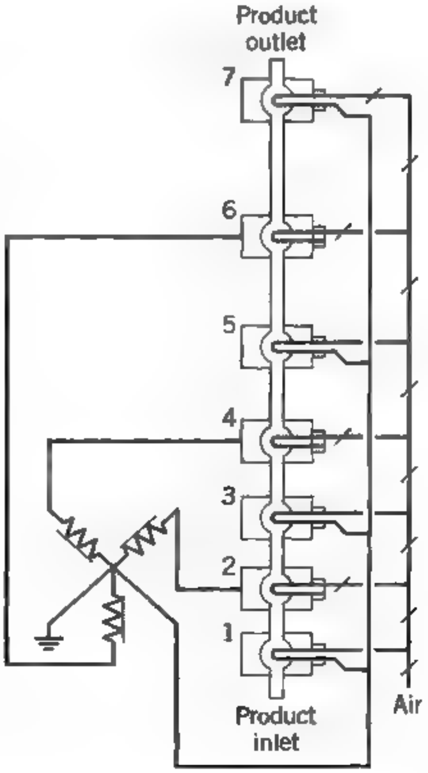


Figure 2. Design of an ohmic heater column.

conductivity of food products usually increases with increase in temperature; indeed, for aqueous solutions of ionized salts there is a linear relationship between temperature and electrical conductivity. This phenomenon is attributed to increased ionic mobility with increase in temperature and also applies to most food products. Exceptions could be products in which viscosity increases markedly at higher temperatures such as those containing ungelled starches

MEASUREMENT OF ELECTRICAL CONDUCTIVITY OF PARTICULATE FOOD PRODUCTS

Conductivity measurements of products with large particles are often impossible using commercially available cells because of their small internal clearances.

As consistent results can only be obtained using a cell having a cross-sectional area considerably greater than that of the largest particle, APV has developed a special cell to overcome this problem, and can now accurately measure the conductivity of products containing large particulates.

TEMPERATURE CONTROL OF THE OHMIC HEATER

Ohmic heating plants are supplied with a fully automatic feed-forward temperature control system. Pure feedback is not satisfactory due to the time constant in the heater column.

Inlet changes, which will affect the final product outlet temperature, are changes in inlet temperature, mass flow rate and product specific heat capacity. In the feed-forward control system, a microprocessor scans these variables including the latter, which is an operator-entered or computer-generated variable. It continuously computes the electrical power required to heat the product and compares this value with the signal from a power transducer on the output side of the transformer. Feedback monitoring is used to prevent any long-term drift in outlet temperature.

ASEPTIC PROCESSING USING THE OHMIC HEATER

The ohmic heater assembly can be visualized in the context of a complete product sterilization or cooking process. A typical line diagram of such a plant is shown in Figure 3. In common with other types of continuous systems, careful design of the ancillary process equipment is necessary, and once heated, the product must be cooled by more conventional means such as scraped surface or tubular heat exchangers. The latter is generally preferred for particulate processing to maximize the greatest advantage of ohmic heating, that of a minimal structural damage to the particulates.

Presterilization of the ohmic heater assembly, holding tube and coolers, is carried out by recirculation of a solution of sodium sulfate at a concentration that approximates the electrical conductivity of the food material that will subsequently be processed. Sterilization temperatures are achieved through the passage of electrical current and backpressure is controlled using a backpressure valve. The aseptic storage reservoir, interface catch tank, and connecting pipework to the filler are sterilized by traditional steam methods.

The use of sterilizing solution of similar product electrical conductivity minimizes adjustment of electrical power during subsequent change to product, thus ensuring a smooth and efficient changeover period with little temperature fluctuation.

Once the plant has been sterilized, the recycled solution is cooled using a heat exchanger in the recirculation line. When steady-state conditions are reached, sterilizing solution is run to drain and product introduced into the hopper of a positive displacement feed pump. Typically, this might be an auger-fed mono or rotary or a Marlen reciprocating piston pump. Product is usually prepared in premix vessels, which can incorporate preheating, or blanching operations.

Backpressure during the changeover period is controlled by regulation of top pressure in a catch tank using sterile compressed air or nitrogen. This tank serves to collect the sodium sulfate/product interface. Once the interface has been collected, product is diverted to the main aseptic storage vessel where top pressure is similarly used to control backpressure in the system.

Backpressure is maintained at a constant 1 bar when sterilizing high-acid food products at temperatures of 90 to 95°C. A 4-bar backpressure is used for low-acid food products where sterilization temperatures of 120 to 140°C are necessary. Safety features are incorporated to ensure power is automatically switched off should there be any loss of pressure.

The use of pressurized storage vessels has proven to be an extremely effective method of controlling backpressure in pilot-plant heaters where throughputs are typically less than 750 kg/h. In larger systems operating in excess of 2 t/h, aseptic positive displacement pump downstream of the cooler can be used as an alternative depending on the composition of the food product. This alternative overcomes the requirement for two aseptic storage vessels in order for the system to continuously feed product to the filling machine.

Product is progressively heated to the required sterilization temperature as it rises through the ohmic heater assembly. It then enters an air-insulated holding tube before being cooled in a series of tubular heat exchangers. More rapid cooling can be achieved with some products in which the final particulate level will be less than 40%. This system involves a combination of ohmic and traditional heat treatment and takes advantage of the capability of the ohmic heater in being able to process products containing up to 80% particulates. During preparation, the product is formulated into a high concentration particulate stream and a separate liquid stream. The liquid stream is conventionally sterilized and cooled in a plate or tubular heat exchanger system before being injected into the particulate stream leaving the holding tube of the ohmic heater. Such operation has the advantage that it reduces the capital and operating cost for a given throughput. It also allows the electrical conductivity of the carrier fluid in the ohmic heater to be more closely matched to that of

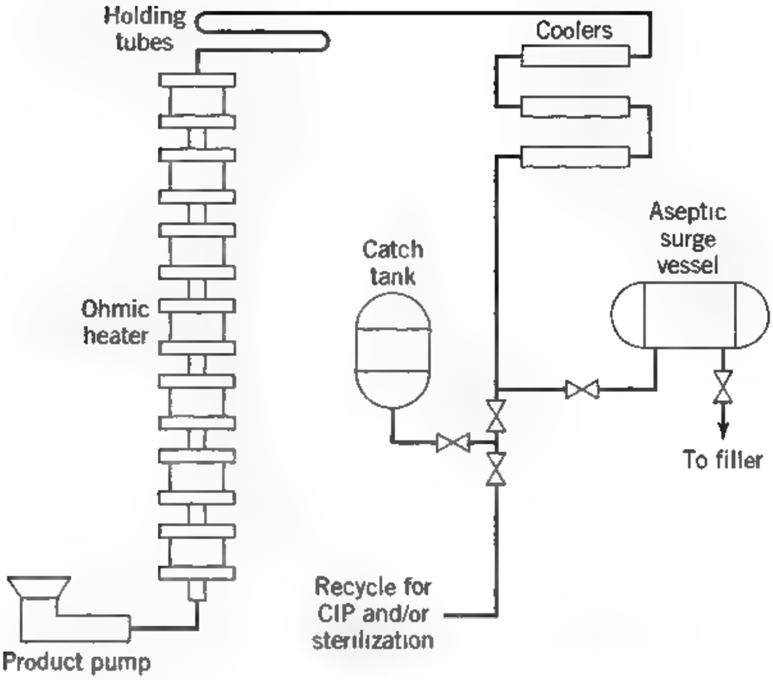


Figure 3. Ohmic plant line diagram.

the particulate, should they be markedly different, by manipulation of the dissolved ionic contents of the two processing streams. This assists in ensuring that both particulates and carrier fluid are heated at the same rate.

After cooling, product enters the main storage reservoir prior to aseptic filling. It is not usually necessary to carry out intermediate cleaning and/or resterilization of the plant when several different products are processed. This is due to the almost complete absence of fouling with most food materials. After one product is processed, the plant is flushed with a food-compatible liquid or base sauce before introducing the next product. The catch tank is used to collect product/sauce interfaces.

When food processing is complete, the power is turned off and the plant rinsed with water. This is followed by cleaning with a 2% (w/v) solution of caustic soda recirculated at 60 to 70°C for 30 min. Heating of the cleaning solution is by standard methods and not using the ohmic principle. This is because the electrical conductivity of the cleaning solution is too high for the ohmic principle to be applied.

PRODUCT QUALITY

Included in the list of perceived advantages of continuous aseptic processing (Fig. 4) is that of improved product quality, with the basic standard being set by products sterilized by traditional canning methods.

These quality standards include microbiological safety (ie, process lethality), cooking effects, and nutrient/vitamin retention. Unlike other processes requiring heat penetration, either from the outside to the center of a can (in-can sterilization) or from the outside of the particulate to the center (tubular or swept surface), ohmic has the ability to provide exceptionally fast heating of all of the product virtually simultaneously; this provides a high degree of microbiological security, as there is a very narrow range of lethality from the surface to the center of the particulates.

- Fresher tasting, more nutritional products can be produced containing large particulates.
- There is ability to heat food product in continuous flow without the need for any hot heat transfer surfaces.
- Process is ideal for shear-sensitive products.
- Risk of fouling is considerably reduced.
- Heat can be generated in the product solids without reliance on thermal conductivity through liquid.
- System is quiet in operation.
- Maintenance costs are lower.
- Process is easy to control and can be started or shut down instantly.
- There are potential savings in processing and packaging costs.

Figure 4. Advantages of electrical resistance heating.

In a 1996 publication by Kim et al. (2), a team involving the U.S. Army Soldier and Biological Chemical Command and APV UK Ltd., had demonstrated that not only the center temperature of a particulate rises faster than the surface, but also particulates could heat faster than fluid in ohmic heating. A higher temperature of the particulates provided additional heat to the surrounding fluid as both phases exited the ohmic column and entered the holding tube. Therefore a higher temperature reading was registered at the outlet than that at the inlet of holding tube (Fig. 5). They also suggested that lethality estimation of microorganisms based on fluid temperature at the ohmic column outlet would be very conservative. This is a very

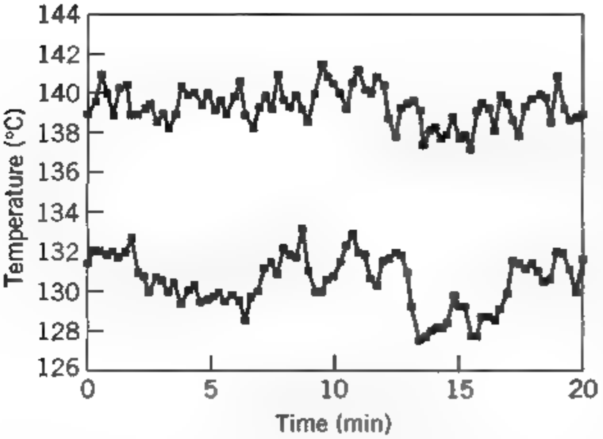


Figure 5. Fluid temperature increases in the holding tube in ohmic heating. Bottom: ohmic column outlet; top: holding tube exit.

important information for the application of regulatory approval of such novel technology.

Challenge tests carried out (on now commercially available products) by the Campden Food and Drink Research Association have involved particulates of meat and vegetable up to 19 mm cube. An average of 10 million spores of *Bacillus stearothermophilus* per particle embedded in a food/alginate mixture were included in batches of mince and mushrooms in bolognese sauce, and in kidney, peas, and gravy, in order to determine the lethality at the center and throughout particles of various sizes from 3 mm to 19 mm.

The results obtained (Table 1) demonstrate the high level of microbiological security of the process and the very narrow range of lethality from the surface to the center of the particulates.

The cook value (C_0) of a process is also a function of time and temperature (dependent upon the viscosity of the product). For small cans, F_0 values for viscous products can typically range from 5 to 300 resulting in high cook values. Larger cans will show even greater variations in F_0 values and higher degrees of overcook to achieve satisfactory F_0 values at the center.

Continuous systems using heat penetration will also have wide variations in F_0 values from the center of the particulate to the surface, the variation depending greatly on the size of the particulate, the ratio of solids to liquid (as the carrier fluid must provide the excess heat required to penetrate the particulate), and the sterilization temperature selected.

Fig. 6 and Fig. 7 show the F_0 value given to the carrier fluid to achieve a minimal F_0 value of 5 in the center of the particulate, and the processing times required at various temperatures. It can be seen that for particulates larger than 10 mm, a considerable wide range of F_0 values result

Table 1. Lethality Tests of APV Ohmic System on *Bacillus stearothermophilus*

Particle type	Number of particles	F_z range, min	Mean	Standard deviation
		Run 1		
Mince (5 mm)	41	23.6–34.3	27.9	2.2
Mushroom (19 mm)	16	34.9–40.3	37.9	1.4
Mushroom (3 mm)	6	31.9–41.8	36.0	4.1
		Run 2		
Pea (5 mm)	28	33.7–46.4	38.5	2.8
Kidney (19 mm)	30	47.–62.8	54.8	3.8
Kidney (3 mm)	20	45.6–56.2	50.2	3.2

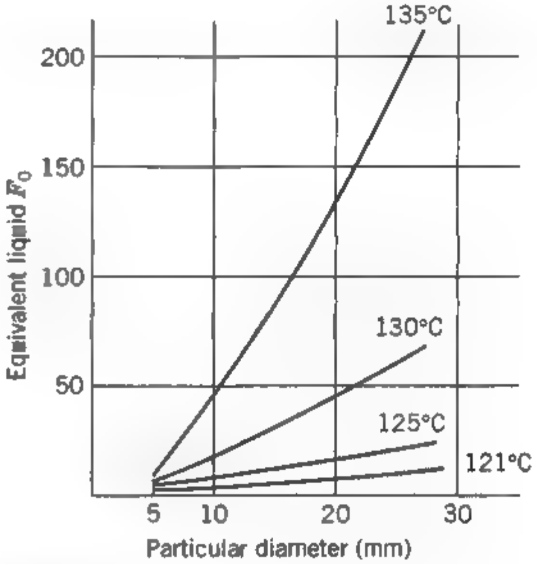


Figure 6. Liquid F_0 necessary to obtain F_0 = 5 in center of particulate.

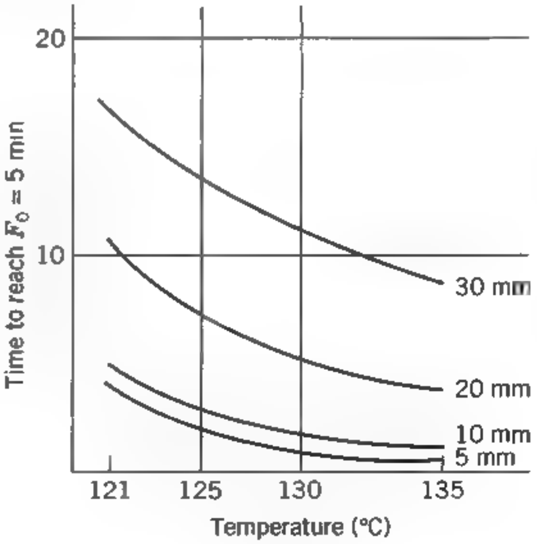


Figure 7. Time taken to reach F_0 = 5 in center of particulate.

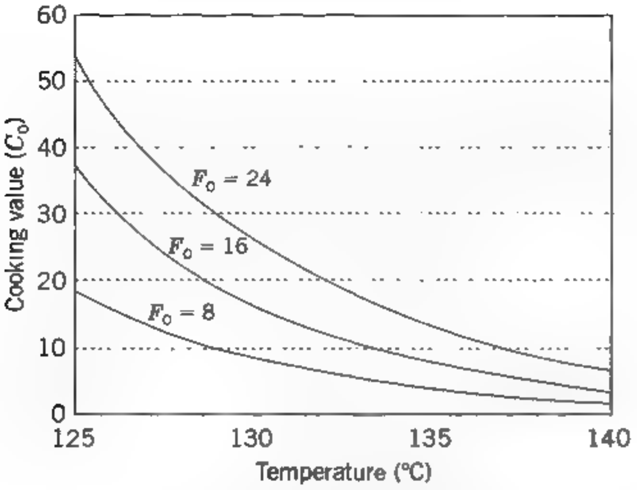


Figure 8. Cooking values versus temperature

irrespective of the sterilization temperature. This results in undesirable overcooking of the carrier fluid and the outside surface of the particulates. For example, processing a 19-mm particle to a minimum F_0 value of 5 at a sterilization temperature of 135°C will require the carrier fluid to have had an equivalent F_0 treatment of 108, as the heat penetration time will have been the order of 4 min. In contrast, ohmic heating can achieve a high F_0 level with a low value of cook and nutrient/vitamin destruction. Figure 8 shows the effect of processing temperature on F_0 and C_0. The C_0 value is based on a Z value of 33 and is calculated as

$$10^y[T]$$

where y is the [(°C − 100)/Z] and T is the time, min. Sterilization at 140°C to a virtually uniform F_0 of 24 equates to a likewise uniform C_0 value of less than 5.

This relationship allows such products as diced potato to be subjected to an F_0 value greater than 24 and still not be fully cooked; thus, the particle retains its sharp edges throughout the process. Indeed such products processed through the ohmic system therefore require further cooking (as part of the reheat process) by the end user.

The ability to heat particulates uniformly, without mechanical damage, with lower nutrient and vitamin loss, and with no fouling of heat transfer surfaces leaves no doubt that ohmic heating will play a major role in the growing requirement for aseptic food products containing large particulates, as it provides the food processor with an opportunity to produce new high-quality, long-life products previously unseen with alternative sterilization techniques.

Typical of such high-quality added-value products are those already fully tested that contain meat chunks, shrimps, baked beans, mushrooms, diced or sliced vegetables, pasta, whole strawberries, black currants, blackberries and sliced kiwi fruit.

BIBLIOGRAPHY

1. D. F. Dinnage, "Continuous Aseptic Processing Using the Ohmic Heating Process," *dfi News* 11(3), 6–13 (1989).
2. H.-J. Kim et al., "Validation of Ohmic Heating for Quality Enhancement of Food Products," *Food Technol.* **50**(5), 253–261 (1996).

T. C. S. Yang
U.S. Army Soldier and Biological Chemical Command
Natick, Massachusetts

B

BAKERY LEAVENING AGENTS

All baked foods are leavened. Even unleavened bread such as matzoh is raised by the steam generated during baking. Normally, leavening is thought of as being due to the products of fermentation (carbon dioxide, alcohol) or to CO_2 generated by chemical reactions. However, the expansion of air incorporated into a dough during mixing and the formation of steam, from water in the dough, also cause the baking piece to expand. If this does not happen, the product is dense and unappetizing. This article discusses leavening obtained by chemical reactions as well as from yeast fermentation.

CHEMICAL LEAVENING

The common form of chemical leavening involves a food acid and soda (sodium bicarbonate). The soda dissolves readily in the aqueous phase of the dough or batter. When the acid dissolves, the hydrogen ion reacts with bicarbonate ion, releasing carbon dioxide (CO_2), which expands the baking piece. Water-vapor pressure increases as the internal temperature of the bakery item increases. Air incorporated during dough mixing also expands. Finally, certain materials, notably ammonium bicarbonate, decompose on heating and generate gases that leaven the product.

Air

During dough mixing air is incorporated as numerous small bubbles (1,2). The actual amount varies, but with an ordinary horizontal mixer a bread dough contains about 15 to 20% air by volume. If yeast fermentation is occurring, this volume shrinks by 20% owing to oxygen depletion (3), and during baking, the nitrogen remaining expands by 17% owing to temperature rise, so the net leavening contribution due to air is nil. In the absence of yeast, thermal expansion is still only 17%, so the overall effect is small.

In cookie doughs and cake batters the amount of air retained is variable. In a standard sugar cookie dough, for example, the dough contains about 10% air by volume (4). Cake batter specific gravity may vary from 0.60 to 1.10 g/mL, depending on the formulation and emulsifier system used. The density of air-free batter is about 1.30 g/mL (5), so the amount of air in the batter may range from 15 to 55% by volume. Even at the highest level, however, the thermal expansion of this air contributes only slightly to the final volume of the cake.

Nevertheless, it is important to get good air incorporation in the dough or batter during mixing, because the air bubbles serve as nuclei for other leavening gases. The internal pressure produced by the surface tension of a gas bubble is inversely related to bubble diameter. If a small bubble and a large bubble are near each other, the gas diffuses from the small one to the large one, and the small bubble disappears. If no gas bubbles are already present in, say, a cake batter when baking is begun, then the CO_2 that is formed by the chemical reactions in the batter has no place to go, because a new bubble cannot form (with a zero radius the surface tension is infinite). If the cake batter contains relatively few air bubbles, the CO_2 diffuses to these, and the resulting cake has a coarse, open grain, often accompanied by tunnels. With many small air nuclei, the grain is fine and close.

Surfactants aid incorporation and subdivision of air into dough (2). Emulsifiers help incorporate air into shortening during the preparation of cookie doughs and cake batters. In all cases the presence of many air bubbles for nucleation is an important part of any leavening action.

Steam

The formation of steam by evaporation of water would seem to be important only when the temperature in the baking piece exceeds 100°C in items such as cookies, crackers, and snacks such as tortilla chips. Also, it has been suggested that the water present in roll-in margarine, used in making puff pastry, evaporates and helps to give the characteristic open structure of this product. Extruded goods, of course, depend almost entirely on steam for their expansion.

The vapor pressure of water increases with increasing temperature, and this increased pressure can expand the gas bubbles in cake batter and in bread. In calculations of the expansion of sponge-cake batter (which contained no soda for chemical leavening), it was found that the theoretical curves (which took into account the thermal expansion of the air) fitted the experimental measurements within a few percent (5). When theoretical expansion for bread dough in the oven (see "Oven Spring") was calculated, it was concluded that water evaporation accounted for 60% of the total volume increase (6).

Ammonium Bicarbonate

Ammonium bicarbonate is often used as a supplementary leavening agent for cookies and snack crackers. At room temperature, dissolved in the dough water, it is stable, but when the temperature reaches 40°C (104°F) in the early stages of the oven, it decomposes:

$$NH_4HCO_3 \rightarrow NH_3 + CO_2 + H_2O$$

One mole of ammonium bicarbonate (79 g, or 2.8 oz) gives 2 mol of gas (44.8 L, or ca 1.6 ft^3).

This potential for extensive gas formation means that ammonium bicarbonate must be uniformly distributed throughout the dough; the presence of small undissolved pieces would give rather large blowouts in the product. Ammonium bicarbonate is usually dissolved in a few quarts of warm water and then added to the mixer along with the other water.

Because ammonia is water soluble, this leavener is applicable only in low-moisture products. If the finished moisture is above 5% (say, in a soft cookie), the water will retain some of the NH_3 and the product has a characteristic ammonia taste. In contrast to this general rule, ammonium bicarbonate is sometimes added to eclair and popover doughs (*pâté de choux*). In this case the combination of high baking temperatures, thin walls, and a large internal cavity allows enough space for ammonia to diffuse out of the baked good.

Sodium Bicarbonate

Baking soda (sodium bicarbonate) has been the workhorse chemical leavener of baked goods for well over a century. In most cases it is combined with a leavening acid to form CO_2, but soda itself undergoes thermal decomposition:

$$2NaHCO_3 + \text{heat} \rightarrow Na_2CO_3 + CO_2 + H_2O$$

This reaction usually requires rather high temperatures (>120°C) to happen at an appreciable rate, so using soda as a sole leavening agent is restricted mainly to cookies and snack crackers in which the internal temperatures can approach this range.

The more usual reaction is with hydrogen ions from leavening acids:

$$NaHCO_3 + H^+ \rightarrow Na^+ + CO_2 + H_2O$$

Soda is readily soluble in water (saturation solubility of 6.5% at 0°C, 14.7% at 60°C) and dissolves in the dough or batter water during mixing. The rate of reaction is governed by the rate of dissolution of the leavening acid (see "Leavening Acids"); no H^+ is present until the acid dissolves and ionizes. Of course, the soda can react with acidic ingredients in the formula such as buttermilk and even flour itself. Chlorinated cake flour, which is more acidic than ordinary pastry flour, will neutralize about 0.27 g of soda per 100g of flour. Soda is a mild alkali; the pH of an aqueous solution is about 8.2. Increasing the amount of soda in a formula raises the pH of the dough, for example, in raising the dough pH of saltine crackers to 7.4. Excess soda (more than that neutralized by available leavening acid) is added to devil's food cake batter to raise the pH of the finished cake crumb to about 7.8 and generate the dark chocolate color desired (cocoa is lighter colored in mild acid, darker colored in mild alkali).

Sodium bicarbonate is available in various granulations (Table 1); it is important to choose the correct one for the application. If the ingredient is added at the mixer, and the dough or batter will be processed fairly quickly, then rapid dissolution is wanted and No. 3 (fine powdered) or No. 1 (powdered) would be appropriate. At the other extreme is the inclusion of soda in a dry mix (eg, cake, cake doughnut) that is packaged and stored for a period of time before use. In this case thermal decomposition of the dry powder becomes a factor. Grade No. 1 (powdered) decomposes 50% faster than grade No. 5 (coarse granular) in this situation. A loss of 2 to 4% per week at 50°C (122°F) and 0.5 to 1% at 30°C has been reported. A common practice is to add 5 to 10% more than needed when the dry mix is blended, in the hope that when the mix is used (with uncontrolled variables of age and storage temperatures), the amount of soda will be adequate to perform as expected. It would be preferable to use grade No. 4 (granular) or No. 5 (coarse granular) for this application and reduce the amount of overage in the dry mix.

Potassium Bicarbonate

Recently this material has become commercially available as a substitute for sodium bicarbonate; it is functionally equivalent but lowers the sodium content of the finished product. As shown in Table 1 the granulation grade currently offered is intermediate between No. 4 (granular) and No. 5 (coarse granular) sodium bicarbonate. The effect on pH and the reactions to CO_2 are the same. Because the molecular weight is greater (100.11 for $KHCO_3$ vs 84.01 for $NaHCO_3$), 19% more is required to get the same effect; 10 lb of sodium bicarbonate is replaced with 11.9 lb (11 lb 14-1/4 oz) of potassium bicarbonate.

A review of bicarbonates for leavening has been published (7).

Leavening Acids

The traditional acidulants for baking were vinegar (acetic acid), lemon juice (citric acid), cream of tartar (potassium acid tartrate), or sour milk (lactic and acetic acids). In all cases the acid reacted with the soda as soon as they were mixed and the baker relied on batter viscosity to retain the CO_2 until the cake or cookie was baked in the oven. In 1864 monocalcium phosphate hydrate was patented for use in making a commercial baking powder. Later (ca 1885) sodium aluminum sulfate began to be used; this compound has a low solubility in water at room temperature and essentially none in the aqueous phase of a batter, so it does not release CO_2 until late in the baking cycle. The combi-

Table 1. Sodium Bicarbonate Granulation

Grade number	Cumulative % retained, minimum-maximum							
USS sieve:	60	70	80	100	140	170	200	325
Microns:	250	210	177	149	105	88	74	44
1. Powdered				0–2			20–45	60–100
2. Fine granular			0–Tr	0–2			70–100	90–100
3. Fine powdered				0–Tr	0–5		0–20	20–50
4. Granular			0–Tr	0–2			80–100	93–100
5. Coarse granular	0–8	0–35		65–100		95–100		
6. Potassium bicarbonate	0–5			40–60			80–100	

nation of the two leavening acids gave rise to double-acting baking powder. During the first third of the twentieth century a number of phosphate compounds were studied, and the range of leavening acids available today to formulators was developed. These acids have the advantage of controlled and predictable rates of CO_2 release and account for essentially all commercial leavening acids sold today. Several good reviews on the properties and uses of leavening acids have been published (8–12) that give more details about various specific applications.

Table 2 lists the leavening acids and the chemical reactions that occur when they dissolve in water to liberate H^+. Of use to the formulator are the neutralizing value (NV) and the equivalence value (EV). Suppliers characterize their products by NV, which is defined as the pounds of sodium bicarbonate that are neutralized by 100 lb of the acid. When developing product formulas, it is more convenient to use EV; for example, if 2% soda is included in the formula, 2.78% sodium acid pyrophosphate (SAPP) is added to achieve exact equivalence.

Neutralizing value is determined by experimental titration (9,10). The yield of H^+ shown in Table 2 corresponds to NV, although there are some discrepancies. For instance, dicalcium phosphate dihydrate (DCP.Di) should yield 2.15 H^+ to have an NV of 35. One that is even more puzzling is the reaction of SAPP. The pyrophosphate ion has four ionization constants, the first two of which are neutralized during manufacture of disodium pyrophosphate. The additional ionization constants for the dihydrogen dianion formed on solution in water are 5.77 for pK_1 and 8.22 for pK_2. In a cake batter at pH 7 the dianion should yield only 1.1 H^+, equivalent to an NV of 42, but in actual fact NV is 72 as shown in the table. Obviously other, unrecognized, reactions occur that increase the acidifying potential of SAPP. Neutralizing value (and EV) are for practical guidance; the reactions are for interest and edification.

Reaction Rates. Two kinds of reaction rates are of interest to bakers: (*1*) immediate, at room temperature; and (*2*) during baking. Both are functions of the solubility of the leavening acid. The first rate, called the dough reaction rate (DRR), is measured by mixing all the dry ingredients (cake flour, nonfat dry milk, shortening, salt, soda, and leavening acid), adding water, stirring briefly, and then measuring the evolution of gas at 27°C for 10 min (13). In the absence of leavening acid there is a blank reaction of about 20% of the soda due to acidity of the flour and dry milk. Monocalcium phosphate monohydrate (MCP.H_2O) reacts about as quickly as cream of tartar; that is, it is a very fast acting acid. Anhydrous MCP is about 80% as reactive. Dicalcium phosphate dihydrate (DCP.Di) is essentially unreactive under these conditions, as are sodium aluminum phosphate (SAlP) and sodium acid pyrophosphate for refrigerated dough (SAPP-RD). The various grades of SAPP (see the following discussion) show increasing extents of reaction, with the reaction rate for sodium aluminum sulfate (SAS) being somewhere in the middle of the SAPPs (9) and dimagnesium phosphate (DMP) slightly slower than SAlP.

Baking (temperature-dependent) reaction rates measure the rate of CO_2 release from a dough at various temperatures. The rate is actually governed by the solubility of the acid. A slow acid such as SAlP is nearly insoluble at room temperature, so it cannot ionize to give H^+ for reaction with soda; as the temperature rises, the material dissolves and starts to perform its function. Figure 1 shows the rates for several leavening acids (8,9,14). The ranking of acids is the same as that given by the DRR test, but Figure 1 is useful in choosing acids for giving lift at various points in the baking cycle. For instance, MCP hydrate generates CO_2 in a cake batter during mixing, whereas SAlP starts to leaven the cake only midway through the baking cycle. DCP.Di is effectively unreactive (insoluble) until the temperature reaches about 80°C, near the end of the bake cycle. However, it has value in triggering a late release of CO_2, which helps prevent dipped centers or fallen layer cakes.

Sodium acid pyrophosphate is provided in various grades ranging from fast to slow baking reaction rates. Al-

Table 2. Reactions of Leavening Agents

Leavening acid	NV[a]	EV[b]	Reaction
Monobasic calcium phosphate (MCP)	80[c]	1.25[d]	$3Ca(H_2PO_4)_2 \rightarrow Ca_3(PO_4)_2 + 3HPO_4^{2-} + H_2PO_4^- + 7H^+$
Sodium acid pyrophosphate (SAPP)	72	1.39	$Na_2H_2P_2O_7 \rightarrow 2Na^+ + P_2O_7^{2-} + 2H^+$
Sodium aluminum phosphate (SAlP)	100	1.00	$NaH_{14}Al_3(PO_4)_8 \cdot 4H_2O + 5H_2O \rightarrow 3Al(OH)_3 + Na^+ + 4H_2PO_4 + 4HPO_4^{2-} + 11H^+$
Sodium aluminum sulfate (SAS)	104	0.96	$NaAl(SO_4)_2 + 3H_2O \rightarrow Al(OH)_3 + Na^+ + 2SO_4^{2-} + 3H^+$
Dimagnesium phosphate (DMP)	40	2.50	$MgHPO_4 \cdot 3H_2O \rightarrow Mg(OH)_2 + H_2PO_4 + H^+ + H_2O$
Dicalcium phosphate · $2H_2O$ (DCP.Di)	35	2.86	$3CaHPO_4 \cdot 2H_2O \rightarrow Ca_3(PO_4)_2 + HPO_4^{2-} + 6H_2O + 2H^+$

[a] Neutralizing value, grams of $NAHCO_3$ neutralized by 100 g of the leavening acid.

[b] Equivalence value = 100/NV, grams of leavening acid to neutralize 1 g of $NaHCO_3$.

[c] NV is 83 for anhydrous MCP, 80 for the monohydrate form.

[d] EV is 1 20 for anhydrous MCP, 1.25 for the monohydrate form.

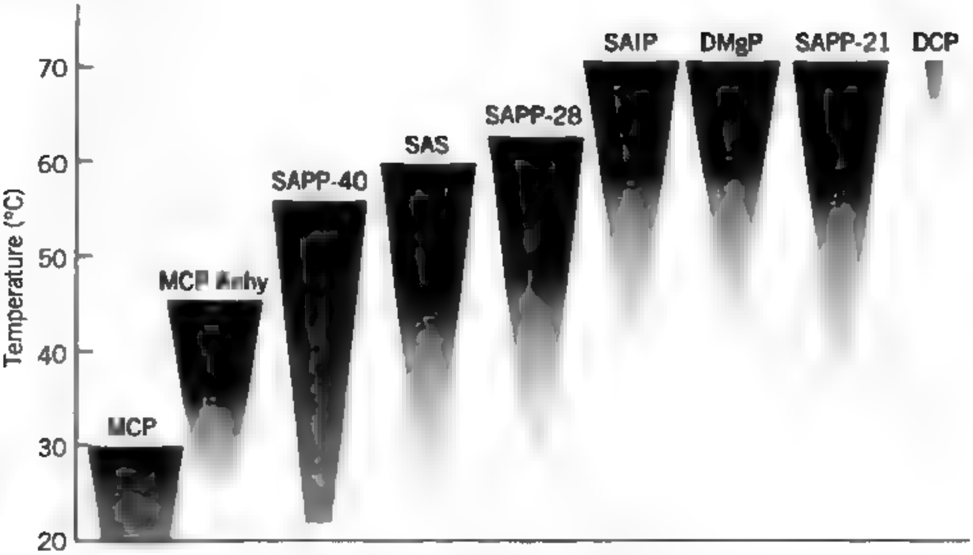


Figure 1. The rate of reaction of various leavening acids as a function of temperature. The width of the triangles indicates the rate of reaction. MCP is the fastest-acting acid, while DCP.Di is the slowest leavening acid. *Source:* Ref. 9 (data for MGP from B. Heidolph, personal communication).

though the basic molecule is the same in all cases, Ca^{++} slows the dissolution rate of SAPP granules. Thus the inclusion of milk in a SAPP-leavened formulation (eg, a pancake batter) delays the time of leavening slightly. The various reaction grades are made by intentionally adding a certain amount of Ca^{++} to the disodium salt during manufacture (adjustments in granule size also control dissolution rate). The grade designations used by Monsanto Chemical Co. range from SAPP-43 (the fastest) through 40, 37, and 28 to 21 (formerly called SAPP RD-1, the slowest). The corresponding grades marketed by Stauffer Chemical Co. (now a division of Rhone-Poulenc) are named Perfection, Donut Pyro, Victor Cream, BP Pyro, and SAPP #4. The slowest grade is used in making refrigerated biscuits and doughs, whereas for other uses (cakes, cake doughnuts, biscuits, and pancakes) a combination of SAPP acids is used to get continuous leavening throughout the baking cycle.

The high reaction rate of MCP may be moderated by coating it with a somewhat insoluble material. This coating dissolves slowly with time, allowing the MCP to then dissolve and form H^+. Instead of 70% of the reaction occurring during the first 10 min after mixing, coated MCP typically gives 20% reaction immediately and then the other 50% over the next 30 min.

The slow-reacting SAIP and DMP leavening acids are useful in pancake and waffle batter for restaurant use, where little or no evolution of CO_2 is desired during the holding period between uses.

Formula Balance. Some of the ions found in leavening acids may influence other properties of certain baked goods. The pyrophosphate ion gives a certain taste that is detectable by some people. Aluminum ion plays a role in developing optimum layer cake structure (15), and SAIP and/or SAS is useful as part of the leavening system. Sodium aluminum sulfate is reported to contribute to rancidity in dry cake mixes stored for a long time. Calcium ion also helps set protein structure and contributes to fine grain in cakes and cake doughnuts. One leavening acid may be selected over another with an equivalent reaction rate just to take advantage of these ion effects.

Developing a formulation with a properly balanced chemical leavening system is partly science and partly art—preplanning followed by trial and adjustment. The selection of timing of the leavening acids, the ratio between the various ones that might be used, the total amount of CO_2 required, and the amount of excess of sodium bicarbonate desired are all factors that interplay with other ingredients and structural functionalities in the baked goods. Achieving the correct leavening balance in a particular formula requires a certain amount of trial and error.

Baking Powder

In the latter part of the nineteenth century, baking powders were developed that combined both parts of the chemical leavening system—baking soda and a leavening acid. The first baking powder contained only one leavening acid, anhydrous MCP, and reacted fairly quickly in cake batters, biscuits, and so on. Soon afterward, sodium aluminum sulfate was incorporated; this gives leavening action during the middle of the baking process, in addition to the early action due to the MCP. This double-acting baking powder was a great success. In Table 3 some typical compositions of baking powders are shown. In addition to the soda and acid, various fillers and calcium salts are also included. The level of potential leavening gas is nearly constant: it is the amount of CO_2 equivalent to 28% sodium bicarbonate in the single-acting powder, and carbon dioxide equivalent to 30% soda in all the double-acting types. Thus these powders are interchangeable from the standpoint of the amount of leavening achieved during baking.

Table 3. Baking Powder Compositions

		Double-acting types					
	Single-acting	Household			Commercial		
Sodium bicarbonate, granular	28.0	30.0	30.0	30.0	30.0	30.0	30.0
Monocalcium phosphate, hydrate		8.7	12.0	5.0	5.0		5.0
Monocalcium phosphate, anhydrous	34.0						
Sodium aluminum sulfate		21.0	21.0	26.0			
Sodium acid pyrophosphate					38.0	44.0	38.0
Cornstarch, redried	38.0	26.6	37.0	19.0	24.5	26.0	27.0
Calcium sulfate		13.7					
Calcium carbonate				20.0			
Calcium lactate					2.5		

Note. Composition given in percentage of each ingredient in the baking powder

Household double-acting baking powder is based on MCP.H_2O and SAS, whereas the commercial types contain MCP.H_2O and SAPP as the leavening acids. The use of a combination of different grades of SAPP in place of SAS allows better control of the timing of CO_2 release in the oven, which is sometimes an advantage in commercial bakery production.

BIOLOGICAL LEAVENING

The use of yeasts in food processing is prehistoric in origin. Baking, brewing, and wine-making all depend on the ability of yeasts to carry out anaerobic fermentation of sugars, yielding CO_2 and ethanol. In brewing and wine-making, alcohol is the prime product of interest; in baking, the leavening effect of CO_2 and ethanol is more important. In this article only the leavening action of yeast in baked goods is discussed.

Oven Spring

The use of yeast in bread production gives a product containing many small gas cells—leavened bread. By the end of the proofing period the aqueous phase of the bread is saturated with CO_2 and the volume has roughly doubled owing to the pressure of CO_2 that has diffused to air cells or bubble nuclei. During the first part of the bake cycle the loaf expands; then at some temperature the matrix sets, expansion stops, and the rest of the baking time is for the purpose of starch gelatinization, crust coloring, flavor development, and so on. The increase in loaf volume during baking is called oven spring (16). The magnitude of oven spring depends on two factors: (*1*) generation and expansion of gases; and (*2*) the amount of time available for loaf expansion before the structure sets. The first factor is primarily a function of yeast fermentation; the second one is affected by dough components such as shortening, surfactants, gluten protein, and flour lipids.

The influence of several dough components on finished volume has been explored (17,18). A key observation is shown in Figure 2 (18,19). Dough with or without shortening expands at the same rate initially, but at 55°C the structure of the fat-free dough begins to set, slowing the expansion rate. In the presence of 3% shortening, dough continues to expand up to 80°C; the longer time at the initial expansion rate results in a larger final volume. Similar results were found in the presence of 0.6% monoglyceride or 0.5% sodium stearoyl lactylate (17).

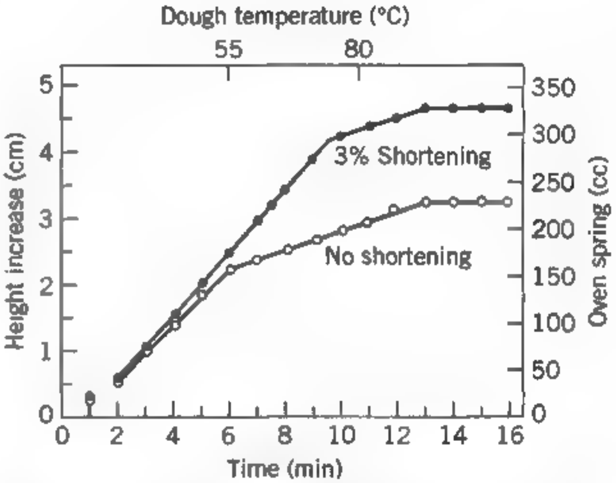


Figure 2. The increase in volume of bread dough in a resistance-heating oven. Doughs contained no shortening or 3% (flour basis) shortening. Initial rate of expansion is the same for both doughs. In the absence of shortening some (as yet unknown) event takes place at 55°C that results in a decreased rate of expansion. This slowdown occurs at about 75°C in the presence of shortening. Both loaves cease all expansion at about 90°C. *Source:* Ref. 19.

Much of the CO_2 formed during fermentation is lost to the atmosphere (20). If the internal gases in a bread were at equilibrium with the atmosphere, there would be no excess CO_2 pressure inside the gas cells. The fact that there is such a pressure (as shown by increased dough volume) is due to inhibition of diffusion of CO_2 from the gas cells to the surrounding air. The causes of this inhibition are not known, but increased viscosity due to water-soluble pentosans and/or the developed gluten phase has been suggested (21). At the end of proof, about 35% of the CO_2 formed during fermentation is present in gas cells, about 5% is dissolved in the aqueous phase, and 60% has been lost to the atmosphere (22). When the solubility of CO_2 in bread dough was measured experimentally, a value of 0.81

mL (equivalent) dissolved per gram of dough was found (21). Others calculated 0.32 mL (equivalent) dissolved per gram of dough, based on the known solubility of CO_2 in water (20).

A mass balance calculation for a pup loaf based on 100 g of flour (173.5 g of dough) found that CO_2 and air occluded during mixing accounted for 370 mL of loaf volume at the end of proofing, and in the oven this gas phase expanded by 62 mL (20). Dissolved CO_2, driven out of the aqueous phase owing to rising temperature, contributed 43 mL to oven expansion, and CO_2 generated by increased yeast activity during early stages of baking amounted to 4 mL. The total volume expansion in the oven from CO_2 and air was 109 mL, but the actual increase in loaf volume was 360 mL. (If the solubility data of Ref. 22 are used, the volume of CO_2 expelled from the aqueous phase would be 109 mL, and the total volume expansion due to CO_2 and air would be 175 mL.)

The difference, 251 mL (or 185 mL, if Ref. 23 data are used), must be supplied by the evaporation of ethanol and water. The amount of dough expansion that could be expected owing to evaporation of water, based on the vapor pressure of water as internal dough temperature rises was calculated (6). This is a slight overestimate, as the aqueous phase of the dough is roughly 0.5 M in salt and 0.3 M in sugar, as well as containing water-soluble proteins, flour pentosans, and fermentation acids. These solutes lower the vapor pressure of the water by roughly 3%.

The amount of ethanol formed during fermentation and proofing usually amounts to 1% of total dough weight (24), or about 2.5% by weight of the liquid phase. About half this ethanol is vaporized during baking. The boiling point of the ethanol azeotrope (95% ethanol, 5% water) is 78°C, so all of it would be expected to vaporize by the end of the bake (although half is apparently trapped and does not escape to the surrounding atmosphere). At 1% of the weight of the pup loaf, 1.7 g of ethanol vaporizes to 830 mL. Vaporization of only half the ethanol is more than sufficient to supply the volume of oven spring over and above that from CO_2 and air.

To summarize, CO_2 contributes about 25% of the gas pressure responsible for oven spring in the standard white loaf considered; expansion of air accounts for about 15%; and ethanol vaporization makes up the rest. The actual magnitude of oven spring depends on other dough components that govern the temperature at which rapid expansion ceases (the foam structure of leavened dough converts to a porous sponge, allowing leavening gases to escape).

Proofing Temperature

Another factor that influences the amount of oven spring is the temperature of proofing. In a study using a sponge and dough variety bread (containing wheat bran) it was found that lower proofing temperatures produced larger final loaf volumes (25). The 1-lb loaves were proofed to a height of 0.5 in. above the pan rim in 50 to 55 min. Additional yeast was added on the dough side to maintain this proof time at each experimental temperature (75–125°F, in 10-degree steps). The bread proofed at 75° had a loaf volume of 3058 cc, while that proofed at 125° had a final volume of 2365 cc. Further, the crumb was progressively more open and overall bread quality was poorer as proofing temperatures increased.

The probable mechanism for this enhanced oven spring was analyzed in some detail (26). Expansion of CO_2 and air accounted for only a small part of the difference. The main contributor to better bread quality is probably twofold: (*1*) improved gluten strength (less weakening effect from yeast cells dying at the higher temperature), and (2) a longer loaf expansion time until the foam (dough) converts to a sponge (bread) structure.

BIBLIOGRAPHY

1. J. C. Baker and M. D. Mize, "The Origin of the Gas Cell in Bread Dough," *Cereal Chemistry* **18**, 19–34 (1941).
2. R. C. Junge, R. C. Hoseney, and E. Variano-Marston, "Effect of Surfactants on Air Incorporation in Dough and the Crumb Grain of Bread," *Cereal Chemistry* **58**, 338–342 (1981).
3. N. Chamberlain, "The Use of Ascorbic Acid in Breadmaking," in J. N. Councell and D. H. Homig, eds., *Vitamin C*, Applied Science, London, 1981, pp. 87–104.
4. J. L. Vetter et al., "Effect of Shortening Type and Level on Cookie Spread," *Technical Bulletin of the American Institute of Baking* **6**(10), 1–5 (1984).
5. M. Mizukoshi, H. Maeda, and H. Amano, "Model Studies of Cake Baking. II. Expansion and Heat Set of Cake Batter During Baking," *Cereal Chemistry* **57**, 352–355 (1980).
6. A. H. Bloksma, "Rheology of the Breadmaking Process," *Cereal Foods World* **35**, 228–236 (1990).
7. M. S. Lajoie and M. C. Thomas, "Versatility of Bicarbonate Leavening Bases," *Cereal Foods World* **36**, 420–424 (1991).
8. T. P. Kichline and J. F. Conn, "Some Fundamental Aspects of Leavening Agents," *Bakers Digest* **44**(4), 36–40 (1970).
9. J. F. Conn, "Chemical Leavening Systems in Flour Products," *Cereal Foods World* **26**, 119–123 (1981).
10. H M Reiman, "Chemical Leavening Systems," *Bakers Digest* **51**(4), 33–34, 36, 42 (1977).
11. D. K. Dubois, "Chemical Leavening," *Technical Bulletin of the American Institute of Baking* **3**(9), 1–6 (1981).
12. B. B. Heidolph, "Designing Chemical Leavening Systems," *Cereal Foods World* **41**, 118–126 (1996).
13. J. R. Parkes et al., "Method for Measuring Reactivity of Chemical Leavening Systems," *Cereal Chemistry* **37**, 503–518 (1960).
14. C. E. Stauffer, "Precise Leavening," *Baking & Snack* **19**(1), 46, 48, 50, 52, 54 (1997).
15. N. B. Howard, "The Role of Some Essential Ingredients in the Formation of Layer Cake Structures," *Bakers Digest* **46**(5), 28–30, 32, 34, 36–37, 64 (1972).
16. J. C. Baker, "Function of Yeast in Baked Products. Effects of Yeast on Bread Flavor," in C. S. McWilliams and M S. Peterson, eds., *Yeast—Its Characteristics, Growth, and Function in Baked Products*, National Academy of Science, National Research Council, Washington, D.C., 1957.
17. W R. Moore and R. C. Hoseney, "Influence of Shortening and Surfactants on Retention of Carbon Dioxide in Bread Dough," *Cereal Chemistry* **63**, 67–70 (1986).
18. W. R. Moore and R. C. Hoseney, "The Effects of Flour Lipids on the Expansion Rate and Volume of Bread Baked in a Resistance Oven," *Cereal Chemistry* **63**, 172–174 (1986).

19. R. C. Junge and R. C. Hoseney, "A Mechanism by Which Shortening and Certain Surfactants Improve Loaf Volume in Bread," *Cereal Chemistry* **58**, 408–412 (1981).
20. W. R. Moore and R. C. Hoseney, "The Leavening of Bread Dough," *Cereal Foods World* **30**, 791–792 (1985).
21. C. E. Stauffer, "Principles of Dough Formation," in S. Cauvain and L. Young, eds., *Technology of Breadmaking*, Chapman & Hall, London, 1998
22. R. C. Hoseney, "Gas Retention in Bread Doughs," *Cereal Foods World* **29**, 306–308 (1984).
23. G. E. Hibberd and N. S. Parker, "Gas Pressure–Volume–Time Relationships in Fermenting Doughs. I. Rate of Production and Solubility of Carbon Dioxide in Dough," *Cereal Chemistry* **53**, 338–346 (1976).
24. J. W Stitley et al., "Bakery Oven Ethanol Emissions. Experimental and Plant Survey Results," *Technical Bulletin of the American Institute of Baking* **9**(12), 1–11 (1987).
25. K. Siffring and B. L. Bruinsma, "Effects of Proof Temperature on the Quality of Pan Bread," *Cereal Chemistry* **70**, 351–353 (1993).
26. C. E Stauffer, "Low-Temperature Proofing," *Baking & Snack* **19**(9), 50, 52–55 (1997).

Clyde E. Stauffer
Technical Food Consultants
Cincinnati, Ohio

BAKERY SPECIALTY PRODUCTS

The main items produced by commercial bakeries, in terms of total tonnage, are bread and rolls, cakes, cookies, and crackers, with bread (either pan bread or hearth-baked types) representing the largest part of this production. Over many centuries many other types of food products, using wheat flour as their main ingredient, have been developed. This article discusses several of these products that are available to consumers today.

NATIVE FLAT BREADS

The earliest form of wheat bread is made by grinding cereal grain(s), adding water, mixing to a coherent mass, and allowing this mass to rest and ferment for a period of time. Then small portions are taken, flattened into a sheet, and baked quickly on a hot surface, usually either the floor or the wall of a heated oven. The process is usually a sourdough type; that is, a portion of the fermented dough is retained at the end of the day's baking and mixed in with the next day's dough, thus inoculating it with the yeast and other fermentative microorganisms that produce ethanol, carbon dioxide, and organic acids as they metabolize and grow.

These breads are produced widely throughout the Middle East and the Indian subcontinent. Depending on details of formulation and production, they are called chappati, nan, roti, tamouri, tamees, korsan, shamuf, and several other names. The simplest formula consists of whole wheat meal (frequently stone-ground by hand in the kitchen), salt, water, and sourdough starter. At the other extreme, the dough also contains ground pulses, sesame seeds, shortening (either ghee or sesame seed paste), honey or other sweetener, and spices (anise, cardamom, curry powder, dill, etc). Although wheat is the major cereal grain used, ground rice, barley, maize, or sorghum are also sometimes included at up to 20% of the weight of wheat meal or flour.

Production may be either in the home or in small commercial establishments. Home production is done strictly by hand. Commercial bakeries have a mechanical slow-speed mixer and perhaps a set of motorized sheeting rollers. The oven is usually heated by building a wood fire inside it, raking out the coals when it is hot, and baking on the floor (hearth) of the oven. In the home, ovens have been seen in the shape of an urn, about 3 ft high with an 18-in. opening in the top and a small opening in the bottom for feeding the fire. When the sides of the oven are hot, the sheeted dough is inserted through the top and plastered (by hand) against the oven wall. Dexterity and practice is required for this, because the oven temperature approaches 500°F. The baked bread is removed with wooden tongs. In the commercial hearth oven, insertion and removal is performed using a wooden peel.

As is common practice in most parts of the world, the bread is produced, purchased, and consumed on a daily basis, so staling and development of mold is not a problem. The meat and vegetable dishes, in the countries where flat breads are common, tend to be stews and purées, and freshly baked flat bread is an ideal and flavorful accompaniment to such a meal.

A similar sort of bread called tortilla, based on maize, is common in Latin American countries and recently in the United States. The maize (corn, in U.S. terminology) is soaked in dilute lime (calcium hydroxide) for up to 24 h to remove the hull. The endosperm is ground, while wet, to make masa (dough), then shaped into balls, flattened, and baked as described above. These differ from the flat breads described previously in the way in which the grain is processed and in having no fermentation step. The leavening of the tortilla, such as it is, is by steam. Tortillas can also be made using wheat flour. Commercial flour tortillas are usually leavened by the addition of a small amount of sodium bicarbonate. Commercial production of tortillas, to accompany Mexican food specialties, incorporates a number of refinements; space does not allow a full discussion of these factors in this article.

PITA BREAD

Another type of Middle Eastern bread, related to the flat breads, is pita (pocket, balady, burr) bread. A wheat flour is used, with an extraction rate of 75 to 82%, depending on the specific type of bread being made. The strength of this flour is greater than that of the flour (or whole wheat meal) used in flat breads. Usually the bread is made by a sourdough process, but with a rather large (20%) portion of sourdough, so that fermentation is more rapid than with the usual flat bread. (Pita doughs can be made adding yeast to each dough, as for regular bread production.) The absorption is somewhat greater than for regular bread dough. The dough is allowed to ferment for about 1 h after mixing, then it is divided into pieces weighing about 3 to 4 oz each. These receive a short intermediate proof, are flattened into circles about 1/2 in. thick and 6 to 9 in. in diameter, and allowed to proof for 30 to 45 min.

The proofed pieces are baked for a short time (1.5–2 min) in an extremely hot oven (700–900°F). The high oven heat causes formation of a top and bottom crust almost instantaneously after the piece is inserted. As the heat penetrates the dough the ethanol and water rapidly evaporate and the vapors, trapped by the crust, cause the bread to balloon. As the bread cools, after removal from the oven, the internal pressure dissipates and it collapses to the familiar round, flat shape, but with the internal "pocket" still present.

PIZZA DOUGH

Pizza is basically an open-faced sandwich made on a leavened flat bread, in which the topping and the bread are baked together. It has become a popular baked food worldwide, and although the familiar tomato sauce/sausage/cheese topping is the most popular, fish, ground lamb, puréed legumes, and a wide variety of chopped vegetables are also used. The possible variety of pizzas is limited only by the availability of ingredients and the ingenuity of the chef.

Pizza crusts are of two types—thin cracker-type crusts and thick bread-type crusts—and are mixed shortly before use or are mixed and retarded in a commissary for later use. These variations lead to differences in dough formulas and handling. The flour used for pizza dough should be a good grade of bread flour with 12.0 to 12.5% protein. The thin-crust dough for immediate use requires even more protein, so rather than carry a 14% protein flour for this dough and a 12% flour for thick-crust doughs, it is more convenient to add 2 to 3% of vital wheat gluten to the 12% flour. Gluten generally is not used in commissary doughs unless flour of an adequate strength is not available. The gluten protein forms a seal at the crust/topping interface, minimizing formation of a soggy layer during baking.

Dough for immediate use receives little fermentation time beyond a 10 to 15-min floor time. It should be somewhat undermixed (just beyond the "cleanup" stage) and quite warm (33–38°C, 90–100°F). These doughs often resist sheeting and tend to shrink back in the oven. To counteract this effect, a reductant, either L-cyteine or sodium bisulfite, is used. Cornmeal also reduces dough elasticity. Adding it does away with the need for reductants and improves crust color, flavor, and eating properties. Sodium stearoyl lactylate improves the crust volume of thick-crust pizzas, giving closer grain and a more tender crust.

The variations in water absorption between the two types of crusts are consonant with the descriptions of cracker-type crust and bread-type crust. If absorption is too high, the dough is sticky, whereas if it is too low, the dough tears when it is sheeted.

Commissary pizza doughs are mixed, divided, rounded, and immediately placed in the retarder at 5 to 8°C (40–45°F). The dough ball should double its volume in 24 h at this temperature. If the volume increase is greater than this, the dough temperature (usually around 25°C, 78°F) should be reduced; if the volume increase is less than double, mix the doughs to a warmer temperature. Slight adjustments in yeast levels may be made to get the amount of fermentation to the desired point, but too much yeast in the dough may result in large blisters on the crust when it is sheeted and baked in the pizza shop.

Frozen pizza is a common supermarket food item. The consumer does not thaw the pizza and allow it to proof; rather, the piece is simply placed directly in the oven and baked. For this application, chemical leavening rather than yeast is used to give the desired texture to the crust. The leavening is of the double-acting baking powder type.

BAGELS

Bagels supposedly originated in Vienna, during the period (late seventeenth century) when the city was fighting off the Turks. Jan Sobieski, the king of Poland and a famous horseman, was a major factor in the defeat of the Turks, and a hard roll in the shape of a stirrup (bügel in German), immersed briefly in a boiling-water bath before being baked to preserve the shape, was developed in his honor. In later times the shape was simplified and the name corrupted to bagel, but the same production procedures are still followed.

Bagels traditionally are made from a lean, low-absorption straight dough, using a high-protein (ca 13.5–14%), clear, hard-red spring-wheat flour. In recent years numerous adjustments have been made to the basic formula, in the direction of making the bagel richer (eg, adding whole eggs), softer (by the addition of shortening or enzymes for longer shelf life), or sweeter (by adding honey and/or raisins). The main feature that separates bagels from other bakery items (besides the short boiling-water treatment) is the type of fermentation schedule used.

A combination of proofing (35–40°C, 95–105°F) and retarding (5–10°C, 40–50°F) is used to obtain the desired product volume as well as the internal and external characteristics. If the bagels are overproofed, they collapse in the oven; if they are underretarded, they tend to ball, that is, expand and close the center hole. Also, tiny blisters on the surface of the finished bagel are considered desirable. These blisters are due to the presence of lactic acid formed during retarding, and if they are absent (but the bagels are otherwise acceptable), 0.1 to 0.5% lactic acid may be added to the dough.

The sequence and timing of the fermentation steps is open to experimentation. Proofing may be done before retarding, after retarding, or both. Retarding times may be as short as 8 h or as long as 24 h. Specific process parameters must be worked out by the baker, depending on the formula and equipment being used, to make the product that is most acceptable to customers.

Just before baking, the proofed bagels are placed in a boiling-water bath; they float on the top of the bath and are boiled for about 1 min, being flipped halfway through. This treatment gelatinizes the starch on the surface of the bagel and causes the formation of a hard, shiny crust when the bagel is baked. The usual baking procedure is directly in a hearth oven, although they also may be baked on sheet pans. Various toppings (poppy seed, sesame seed) are applied to the boiled (and still moist) bagel; other flavorings such as dried onion are mixed in with the dough.

PRETZELS

Considered to be the world's oldest snack foods, pretzels reputedly originated around the year 600 in a monastery in the Italian Alps. Supposedly, the monk in charge of the bakery formed strips of bread dough into a shape intended to resemble arms folded across the breast in prayer. When baked, these pretiola (little reward in Italian) were given to children who learned their prayers properly. Later, these baked bread snacks became popular north of the Alps, where the name was germanicized to bretzels, a spelling still used by some pretzel bakers.

Soft pretzels, presumably identical with those made by the Italian monk, are still popular today. They have a moisture content similar to that of bread and a short shelf life. Hard pretzels were supposedly discovered when a baker's apprentice forgot to remove the last trays of pretzels from the oven at the end of the day, and the overnight residence in the cooling oven dried them, giving them a hard, crisp texture. The rest of this discussion concerns itself with the low-moisture (2–3%) hard pretzel.

Pretzel dough is extremely stiff, made with a low-protein flour (that from soft white winter wheat is preferred), and only 38 to 42% water. The only other ingredients are yeast (ca 0.25%) and roughly 1% each of salt, shortening, and dry malt. This dough is mixed in a horizontal sigma-arm mixer, allowed to ferment for up to 4 h, then processed.

The dough is divided into small pieces, elongated into a thin strip, and twisted into the typical pretzel shape by a special machine. The formed pretzels receive a short intermediate proof, then are carried through a caustic bath, which contains 1.25 ± 0.25% sodium hydroxide, held at about 190°F. After exiting this bath, salt is sprinkled on the tops and the pretzels enter the oven. Here they are baked at about 450°F for 4 to 5 min. The caustic bath gelatinizes the starch on the surface of the pretzel, giving the crisp, shiny crust. In the oven all residual caustic is converted to sodium bicarbonate. The baked pretzels are then piled onto slower-moving conveyors that carry them through a drying stage, where they remain for 30 to 90 min (depending on the size of the piece) at a temperature of approximately 250°F. This treatment slowly reduces the moisture to about 2% and also allows equilibration so that the finished pretzel does not check (form small cracks on its surface).

Variations on this production scheme are many. A machine is available that extrudes the dough through a die already shaped in the twisted form, so tying is not necessary. By changing the die the machine will also make straight rods, which are a popular form for this snack. Also, the exact dough formulation and extent of fermentation varies among manufacturers, the details being closely held as proprietary information, each company sure that its particular formula makes the best possible pretzel. As with any low-moisture snack, the shelf life is governed by the quality of the packaging; the loss in consumer acceptance is directly related to moisture uptake, with about 3.5% moisture marking the upper limit of acceptability.

ENGLISH MUFFINS

The nomenclature of this item is anomalous, as it was developed in the United States; the term *English* applied to them is a highly successful marketing ploy. They are a disc-shaped baked product, usually around 3 to 4 in. diameter and about three-quarters in. high. A good general description is: "Good eating muffins are relatively tough, chewy, and honeycombed with medium to large size holes (1/8–1/4 in. diameter). Flavor is bland and somewhat sour. Side walls are straight and light colored. The edge between the sides and the flat, dark brown top crust is gently rounded, not sharp." They are baked in a covered griddle cup on a highly automated line. The main features of importance are that the dough must be soft so it flows to fill the cup, and the formulation must lead to the formation of the large internal holes.

A typical English muffin dough contains 83 to 87% water (flour basis equals 100%), 2% sugar, 1.5% salt, 5 to 8% yeast, and 0.5 to 0.7% calcium propionate. Vital wheat gluten at 1 to 2% is sometimes added to increase the chewiness of the finished muffin. At this absorption level the dough is very soft, and it must be cold (20°C, 68°F) when taken from the mixer so that it does not stick when it is divided, rounded, and deposited from the intermediate proofer into the griddle cups. Dusting material, usually a blend of corn flour and cornmeal, is used rather liberally (3–4% of the dough weight) to facilitate the various transfers.

Fermentation of the dough piece after it is divided and rounded is rather short, usually about 30 min. Three factors—short time, cold dough, and high calcium propionate levels—all tend to retard fermentation, so a rather high yeast level is required. Leavening in the griddle cups is due to CO_2, ethanol, and steam; in addition, some bakers add 0.5% baking powder to get an early kick in the cups so that the mold is quickly filled by the dough. The finished English muffin has a rather high moisture content (about 45%) and is prone to mold formation. Calcium propionate is used at the elevated level mentioned to help overcome this problem, and it is also added to the dusting flour. Another method is to spray the muffin with a potassium sorbate solution as it leaves the oven. If this is done, the propionate may be omitted from the dough, which in turn allows a decrease of about 1% in the amount of yeast.

In some instances the internal grain porosity is still less than desired, and the addition of protease enhances the openness of the crumb. This additive also increases pan flow, so absorption might be reduced by 1 to 2%, which might or might not be desirable. Sometimes a tangy, acidic taste is wanted (sourdough muffins), and a dry sour base or vinegar (acetic acid) is added to the dough, The increased acidity (lower pH) of the finished product enhances the antimold action of calcium propionate. Another popular variety contains raisins, added in amounts equal to 25 to 50% of the flour weight.

DOUGHNUTS

Doughnuts are fried in deep fat rather than baked in an oven as are most other bakery products. They are related to another product, fritters, which are made with a soft, rich dough containing pieces of fruit (apples) or vegetables (corn), dropped by spoonfuls into hot fat. An apocryphal story relates that a nineteenth-century Yankee ship captain loved apple fritters but had trouble holding onto them when at the ship's wheel during high seas. His cook, anxious to please, got the idea of making the fritters with a hole in the center so the captain could stick them on the handspokes of the wheel during busy moments. Although there is a certain implausibility about this story, the fact remains that doughnuts, in one form or another, are extremely popular sweet snacks or desserts.

There are three basic types of doughnuts: cake doughnuts, yeast-raised doughnuts, and French crullers. Cake doughnuts are chemically leavened, yeast-raised doughnuts use a sweet yeasted dough, and French crullers are steam leavened. These will be discussed separately.

Cake Doughnuts

Batters for this product are a leaner, lower-sugar version of layer cake batters. A typical regular cake doughnut mix contains (on 100% flour basis) about 40% sugar and 13% nonemulsified shortening. Other ingredients frequently found in doughnut mixes are defatted soy flour, nonfat dry milk, potato starch, and dried egg yolk. These ingredients give a tender eating quality to the finished product, as well as limiting fat absorption during frying. The protein ingredients decrease fat absorption, while potato starch helps retain moisture in the finished doughnut and gives a longer shelf life. Lecithin is sometimes used to enhance the wetting of the dry mix during batter preparation, and occasionally monoglyceride is added to make a more tender final product. Other emulsifiers are not used, as they increase fat absorption without conferring any quality enhancement.

Most cake doughnuts are produced by extruding (or depositing) the batter from a reservoir directly into the hot fat fryer. Bench-cut doughnuts are made using the same recipe, but with a reduced amount of water. This gives a dough (rather than a batter) that can be rolled out on the workbench and cut into rings using a doughnut cutter. This process takes much more hand labor than depositing and is usually used only in home baking or in small, traditional doughnut shops.

In commercial bakeries and most doughnut shops, doughnut batter is deposited through a cutter into the frying fat. Proper batter viscosity is extremely important to attain uniformity of flow through the cutter and uniform spread in the fryer. Batter viscosity is governed by the amount of water used in mixing (usually about 70% based on flour, or 38–44% based on total dry mix weight) and the way that water is partitioned between flour, sugar, soy flour, and the other ingredients present. Sometimes high-viscosity gums (guar, locust bean, sodium carboxymethylcellulose) are added to the dry mix at levels of 0.1 to 0.25%. The gum helps give uniform viscosity from batch to batch and also acts as a water binder that reduces fat absorption and lengthens shelf life.

Leavening in cake doughnuts is by 1.5% soda (flour basis) and 2.1% sodium acid pyrophosphate (SAPP). The fast-reacting grades of SAPP are required, from SAPP-37 to SAPP-43 (the fastest grade). When the doughnut is first deposited in the hot (190°C, 375°F) frying fat, the ring of dough sinks and is supported on a bottom plate. Leavening begins, and after 3 to 7 s the doughnut rises to the surface and frying of the first side commences. As heat penetrates the dough piece it slowly expands; after the first side is fried, the doughnut is flipped and the second side is fried. During this time expansion continues, but the diameter of the doughnut has been fixed because the first side has set (the starch has gelatinized and proteins have denatured). Thus, second-stage expansion tends to be into the center hole and the typical star is formed (or the doughnut balls and fills the center hole if conditions are incorrect).

When the finished doughnut is cut crosswise, three regions are readily seen. The central core is surrounded by a more porous leavened ring, but this porosity tends to be greater on the second side than the first. If the core is inadequately leavened, it will be dense and gummy, and the doughnut will not have a good eating quality. Part of the art of the doughnut formulator is in choosing leavening acids that strike the best compromise to achieve moderate porosity of the two sides along with at least some opening up of the core.

Yeast-Raised Doughnuts

These doughnuts are made using a rather lean, sweet dough. After full development of the dough, it is sheeted, cut into rings, and the doughnuts are proofed for 30 to 45 min. The raised doughnuts are then introduced into the fryer, where they are fried for about 1 min on each side. (Because they are already leavened, they float on the fat throughout the frying cycle.) They are then removed, the surface fat is drained, and the fried doughnuts are (most often) glazed with a simple sugar/water glaze. If they are to be iced or otherwise coated, they are usually cooled before these operations are carried out.

Yeast-raised doughnuts can be made in many different shapes. In addition to rings, the dough is also frequently cut into strips and twisted into various fanciful shapes before proofing and frying. In the form of rectangles about 2 by 4 in., they are iced and topped with chopped nuts or fruit to make Long Johns. In the form of discs, the fried piece can be filled with jelly or custard. This product, filled with raspberry-flavored jelly, is known as a Berliner in Austria and Germany. (This explains why the Germans roared with laughter when, in 1963, President Kennedy proclaimed, "Ich bin ein Berliner." He was telling them that he was a jelly-filled doughnut.)

French Crullers

These doughnuts are made using eclair dough (see the next section). The dough is deposited in a ring shape onto a sheet of parchment paper, and after a few moments to allow it to set, the parchment is dipped into the frying fat and the rings of dough are loosened from the paper. The dough can also be deposited using a specially shaped cutter that produces a fluted surface on the ring of dough. The dough piece expands to a much greater extent than does cake doughnut batter, and the finished piece is much less dense than other doughnuts. It is frequently glazed with a butter/rum flavored glaze.

ECLAIRS

The dough for eclairs and French crullers (*paté de choux*) is a paste made in a rather unusual procedure. Water (two parts) and shortening (one part) are placed in a kettle and brought to a rolling boil. After the fat is melted, a pinch of salt is added, flour (1–1.5 parts) is added, and the mixture is stirred briskly. The gelatinized flour paste is cooled to about 150°F, and two parts whole egg are added in several increments, with continuous stirring. A small amount of ammonium bicarbonate, dissolved in one-half part milk, is stirred in. The final paste should be soft enough to deposit from a pastry bag, yet firm enough to hold its shape once deposited. If it is too stiff, it may be softened by the addition of a small amount of milk or water.

The finished dough is deposited on sheet pans lined with parchment paper, then baked in a hot (425°F) oven until the eclairs are crispy. Leavening action is due to the formation of steam in the interior of the piece. If inadequate leavening occurs, a small amount of ammonium bicarbonate (no more than 1% of the weight of the water used) may be dissolved in a little water and mixed in at the end of the dough-making procedure. The finished piece should be crisp on the outside, hollow on the inside, and with no soggy layer on the interior wall.

Eclairs are usually filled with a chilled custard, either plain or a French custard (butter-flavored). They are then iced, with chocolate being the most popular flavor, or dusted with confectioners' (very finely powdered) sugar. To prevent bacterial growth, custard-filled eclairs should be refrigerated until served.

LAYERED DOUGH PRODUCTS

Numerous bakery products have a unique texture, in that the baked dough is present in many thin layers rather than one continuous matrix. This effect is obtained by placing a layer of fat between two layers of dough, rolling out this sandwich to a thin sheet, folding the sheet over onto itself into three or four layers, and repeating this rolling and folding process two or three more times. If done properly, the final sheet consists of 50 to 100 layers of dough interspersed with an equal number of layers of fat. When baked, the dough layers set up more or less independently, giving the flaky texture of the finished product.

Puff Pastry

Puff pastry dough is used for a large number of culinary items, from basic pastries such as fruit turnovers and napoleons, to patty shells for holding a variety of fillings for appetizers, to dough for enclosing fish or meat, such as beef Wellington. It needs no fermentation but does require some care in the roll-in process to obtain a flaky final product.

The dough for this product is a flour/water dough (no yeast), containing about 6% shortening and 6% whole eggs. After it is fully developed it is sheeted out, fat is spread over two-thirds of the dough, which is then folded to give a sandwich having three layers of dough separated by two layers of fat. This is sheeted out and folded as already described. Usually the dough is refrigerated after the second sheeting and folding, to keep the fat in the plastic state. If the dough becomes too warm, the fat melts and gets incorporated into the dough itself, and the layering effect is lost. The fat usually used is a special puff-paste margarine, having a broad plastic range, and containing about 15% water. In the oven the water (which is finely emulsified in the fat) forms steam, which helps give the puff to puff pastry. Thus, puff pastry is a steam-leavened product.

Croissants

Croissants are another legacy of the siege of Vienna. The story is that the Turks were busy digging a tunnel under the city walls but were overheard by a baker in the early hours of the morning, as they were digging under his shop. He alerted the authorities, and a countertunnel foiled the plot. After the siege was lifted, the baker made rolls in the shape of a crescent (the emblem on the Turkish flag), which were sold to celebrate the victory, and which became a Viennese tradition. Marie Antoinette introduced these Kipfeln to the French court, and they became popular under the French name croissant.

A typical croissant dough is basically a straight dough, containing 10% sugar, 1% salt, and 4% margarine (flour basis), with a fairly high (9%) yeast level and slightly below normal absorption. Croissant dough is mixed cold (18–20°C, 64–68°F). This practice, together with the slight underabsorption, retards fermentation. Proofing temperatures are also relatively low, not exceeding the melting point of the fat (ca 37°C, 98°F). For these reasons a rather high level of yeast is used in the dough. Numerous variations on the basic mixing, sheeting, and roll-in process are used, most of which seem to be based more on tradition than on actually yielding superior product. After mixing, the dough is allowed to ferment for up to 1 hr, then the fat is rolled in, essentially as described for puff pastry. The traditional fat for croissants is unsalted butter, although many commercial companies today use a bakers' margarine that does not get as hard as butter at retarder temperatures and so spreads more uniformly during the sheeting and folding process. The usual fat-to-dough ratio is about 25%, that is, 1 lb of butter to 4 lb of dough.

The rolled-in dough is sheeted out, then cut into triangles having a height about twice the width of the base This triangle is rolled up, starting from the base, the shaped dough piece is placed on a sheet pan, and the ends are pulled down to give the typical crescent shape. After proofing for 1 to 3 h, it is baked and cooled. Care must be taken that the piece is not underproofed, otherwise the finished product loses its flakiness and is tough, chewy, and unpalatable. Croissants are frequently sliced horizontally and used to make sandwiches, with a wide variety of fillings being offered by various specialty shops.

Danish Pastry

Danish dough is a rich, sweet, yeasted dough made with a higher than usual absorption. The dough is quite soft, mixed cold (62–64°F), and not fully developed in the mixer. Gluten development takes place during the roll-in process. The dough is divided into pieces of a suitable size (eg, 16 lb) and then retarded for several hours. Some fermentation takes place during this time. The cold dough is then sheeted out and fat is rolled in, as already described. Specialty shops use butter or bakers' margarine for the roll-in fat, but all-purpose shortening works equally well. The dough sheet is usually given the first two sheetings and folds, then returned to the retarder for 6 to 12 h. It is then given one more sheeting and three-fold, and returned to the retarder for 24 to 48 h for further fermentation.

Danish pastry is almost invariably made by sheeting out the rolled-in dough, cutting into various shapes, then adding some sort of sweet filling (fruit, nuts, cinnamon, etc) and folding or rolling up the dough to retain this filling during proofing and baking. The variety of shapes and fillings is limited only by the ingenuity of the baker. The proofed pieces are sometimes washed lightly with diluted egg just before baking, to give a shine to the finished piece. Alternatively, the piece may be sprayed with a light sugar syrup as it exits the oven, to give the same effect. If a Danish coffee cake, for example, is to receive an icing, this is usually a rather thin icing that is drizzled over the top of the coffee cake in a random pattern, rather than applied in a solid layer (as on sweet rolls). A good Danish pastry is one of the foundation stones of any small retail bakery today.

PIES

The term *pie* (like biscuit) means something rather different in the United States than it does on the other side of the Atlantic Ocean. In Europe a pie usually contains meat, potatoes, and other vegetables and may have a crust of puff pastry, of mashed potatoes, or of a flour dumpling. It is normally the main dish of a meal. In the United States, on the other hand, a pie is for dessert, or is a sweet snack. It need not be a baked food; for example, ice cream pies are now popular. In at least one instance (Boston cream pie), pie is more properly a filled, layered sponge cake. Having said that, this section discusses pies primarily in terms of the traditional one-crust or two-crust baked American dessert pie, and, briefly, the fried pie, a related, traditional American dessert.

A pie crust is one of the simplest of baked doughs, comprising only flour, shortening, salt, and water. The ability to combine these few ingredients properly to produce a flaky, tender pie crust, however, is rare, as attested by the number of leathery, soggy, nearly inedible pie crusts that are served to diners in U.S. restaurants every day. The first step in making a pie crust is to cut the shortening into the flour; that is, mix the two using a fork or similar mixing tool, reducing the particle size of the shortening to small lumps, perhaps one-quarter the size of a pea. Then a measured amount of cold water (even ice water, in hot weather), with the salt dissolved in it, is added, and the dough is gently mixed until the flour is wetted and the dough forms a lumpy, slightly sticky ball. The dough should then be allowed to rest for a time, preferably in a refrigerator, to allow the water to equilibrate with the flour protein.

An amount of this dough just sufficient to form the crust (either top or bottom) is then rolled out into a circle. An absolute minimum of dusting flour is used, and it is best to use cloth sleeve on the rolling pin and a flat cloth on the bench top, both of which have been rubbed with flour and the excess flour removed. Once the desired diameter of crust is achieved, the circle of dough is loosely rolled up on the rolling pin and transferred to the pie pan, where it is gently shaped and the edges sealed (if it is the top crust, and the filling is in place).

The main mistake in making a flaky, tender pie crust lies in excessive working of the dough and consequent development of the gluten in the flour. This can happen if too much water (or water that is warm) is added to the dough, and it is mixed until the water disappears. Also, excessive dusting flour, and rolling the dough more than once, will contribute to gluten formation. In large-scale pie production (either in a commercial bakery, or in an institutional setting) the dough just described may be too tender for the kind of handling it receives, and a certain amount of gluten development will be done on purpose, in the interests of production efficiency. Although this speeds up the operating line, it produces the leathery crusts that are abandoned on dessert plates across the country.

Fillings for pies are usually either some kind of fruit in a starch-stabilized juice matrix or a custard. The formulation of a good pie filling deserves an entire article to itself and will not be explored here. However, the stabilizing system should be such that during baking the filling will not boil or foam excessively, to the point where it overflows the side of the pie. If the pie crust at the filling/crust interface does not bake rapidly enough, it will soak up moisture from the filling, and the final crust will be soggy. This may be due to using a cold filling in the pie or having too low an oven temperature.

One type of pie avoids this pitfall, by adding the filling after the bottom crust is baked. An example is strawberry pie; a single crust is baked in the pie pan, then the fruit is placed in the cooled crust and a strawberry-flavored starch glaze is poured over the top. The pie is refrigerated until served.

Fried Pies

These desserts are traditional in the southeastern part of the United States, although they are now produced commercially and sold throughout the country. A pie crust with a slightly developed gluten is used. A circle is rolled out, fruit filling is placed on one half the circle, the other half of the crust is folded over, and the edges are sealed. This is then fried in a half inch of hot fat, in a heavy skillet. In the commercial version, the shaped and filled piece is fried in a deep fat (doughnut) fryer. A top conveyor belt keeps the pie submerged throughout its journey through the hot fat. The crust absorbs some of the frying fat, tenderizing it so that it is not leathery as it would be if baked. The commercial fried pie is given a sugar glaze before being cooled, wrapped, and dispatched to the consumer. The traditional fried pie, on the other hand, is usually eaten while still warm, fresh from the kitchen, and any glaze or other topping would be superfluous.

GENERAL REFERENCES

Technical Bulletins of the American Institute of Baking: *Bagels*, **8**(11) 1986; **10**(4), 1988; *Croissants*, **8**(10), 1986; *Donuts*, **1**(6,7) 1979; **2**(7), 1980; **4**(9), 1982; *English Muffins*, **1**(1), 1979; *Pizza Dough*, **1**(11), 1979; **8**(12), 1986; *Chemically Leavened Pizza Dough*, **19**(11), 1997.

A. Matz, *Bakery Technology and Engineering*, AVI Publishing, Westport, Conn., 1972.

E. J. Pyler, *Baking Science & Technology*, 3rd ed., Sosland Publishing, Merriam, Kans. 1988.

W. J. Sultan, *Practical Baking*, 5th ed., Van Nostrand Reinhold, New York, 1990.

CLYDE E. STAUFFER
Technical Food Consultants
Cincinnati, Ohio

BAMBOO SHOOTS

FAMILY AND RELATED SPECIES

Bamboo belongs to the family Gramineae. The emerging shoots of several species, of the genera *Dendrocalamus, Bambusa, Phyllostachys*, and others are used as a vegetable (1).

PRACTICAL DESCRIPTION

The members of the family Gramineae are tall woody shoots of perennial grasses. The reproductive organs resemble the grasses, particularly the oat (*Avena sativa*). The anthers are borne on long filaments resembling those of corn (*Zea mais*) (2).

ORIGIN, DISTRIBUTION, AND PRODUCTION

The majority of the edible bamboos originated in China, Japan, and Southeast Asia. Some are wild and others are cultivated. The use of bamboo shoots outside of this area is restricted (1). The important edible species include the tropical clump bamboos, *Bambusa oldhami* Nakai and *Dendrocalamus latiflorus* Munro (Fig. 1), and the spreading bamboos. *Phyllostachys edulis* (Fig. 2), *P. pubescens*, and *P. makinoi*, which are not confined to the tropics.

The planted area of edible bamboos in Taiwan is ca 30,000 ha with an average yield of 12 t/ha. The annual production of bamboo shoots in 1989 was 401,152 t (3).

CULTURE

The cultivation of bamboo has been practiced in the Orient for thousands of years. Bamboo is propagated by the transfer of clumps or rhizomes as shown in Figure 3 (4). Some bamboos can be propagated from culm cuttings. In the life cycle of a clone, the bamboo flowers once, and the top dies. Parts of the same clone, even when transferred to different areas and conditions, will flower at the same time (2). Very few bamboo flowers produce viable seeds, but the collection of seeds is important for the breeding of an entirely new seedling, which can start a new life cycle of 50–60 yr (5).

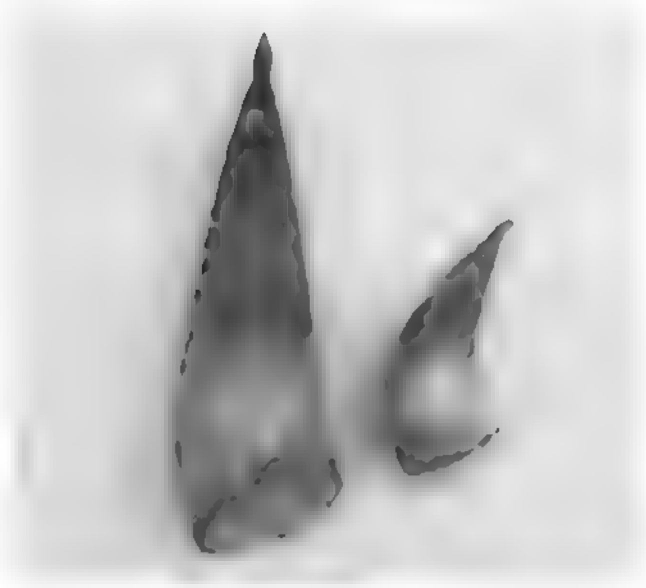


Figure 1. Shoots of *Bambusa oldhami* (right) and *Dendrocalamus latiflorus* (left).

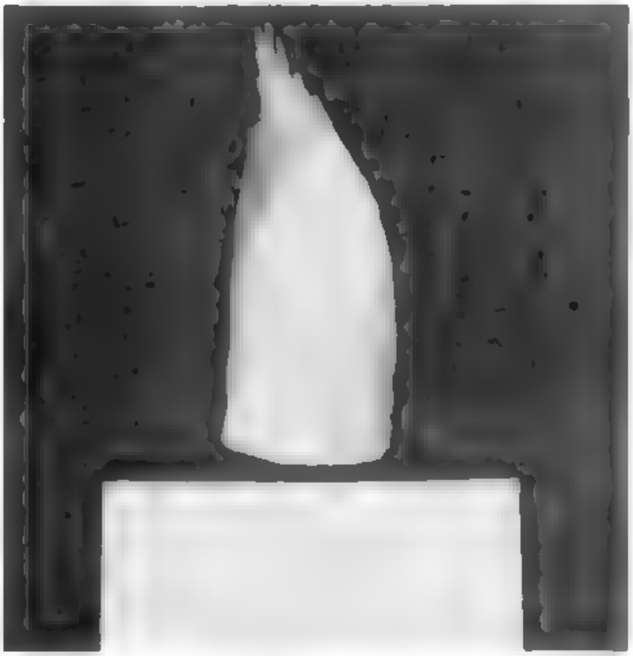


Figure 2. Shoot of *Phyllostachys edulis*.

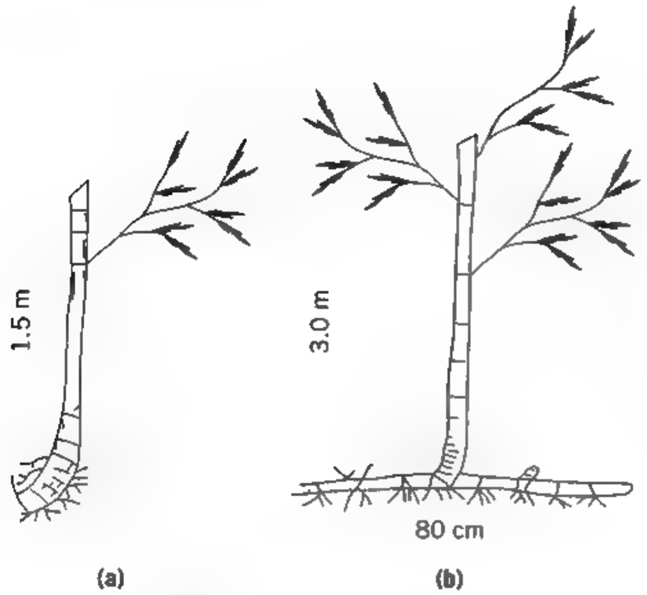


Figure 3. Rhizomes transferred for propagation of bamboos (**a**) *Bambusa* sp, or *Dendrocalamus* sp; (**b**) *Phyllostachys* sp.

HARVEST

The harvest of bamboo for its edible shoots is an ancient and highly specialized act (6). The grower must know what proportion of the young shoots may be cut and when to cut without endangering the vigor of the parent plant. The usual practice is to cover and mound up the clumps with soil, rice hull, rice straw, bamboo leaves, etc so that the emerging shoots are in the dark. Polyethylene (PE) or poly(vinyl chloride) (PVC) sheets have recently been used on top of the mounds (7). Exposure of shoots to light causes greening and bitterness and these shoots are mainly used for processing. The shoots are ready for cutting when the tips are just emerging from the surface of the soil in the mound, if left much later they will become woody. First the mud is removed by hand and then the shoot is cut off with a sharp knife or spade designed for the purpose (Fig. 4) (5).

In Japan, bamboo shoots are forced by growing under mulch heated with electric cables placed 6–8 cm below the soil. About 2–3 cm of rice straw are placed on the soil surface, an additional 4–5 cm layer of soil is put on top, and finally plastic sheeting is laid on top of all. The soil temperature is kept between 13 and 15°C. By the mulching procedure, bamboo shoots can be harvested almost a month earlier than those not mulched (2).

STORAGE

Harvested bamboo shoots are highly perishable with a respiration rate over 100 mg CO_2/kg·h at 20°C (8). The fiber content increases quickly from the cut end toward the tip. The quality and yield of canned bamboo shoots depend greatly on the holding condition of raw materials before processing (9,10). Rapid precooling and storage at low temperature and high humidity conditions can prevent quality deterioration and extend the shelf life of the bamboo shoot from 1–2 days to 7–10 days (11). In Taiwan, harvested bamboo shoots are washed and packed in plastic or bamboo baskets for nearby markets or in PE-lined cartons, which are shipped to the wholesale fresh markets in Taipei.

PRODUCTION SEASON

In Taiwan markets shoots of *B. oldhami* Nakai are available from May to early October. They are called green bamboo shoots and are sweet and tender. Green bamboo shoots

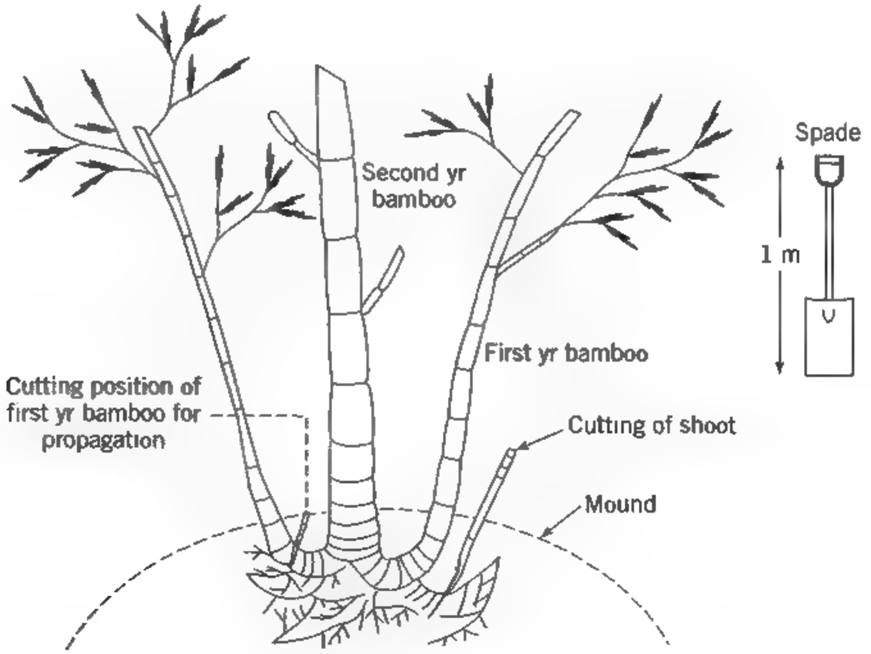


Figure 4. Shooting and harvesting position of *Bambusa oldhami*.

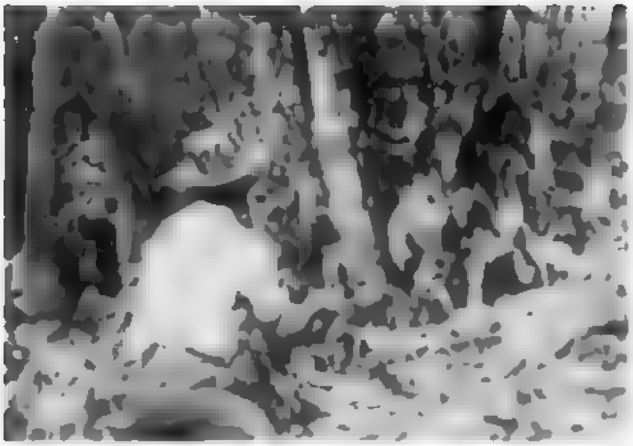


Figure 5. Harvest of *Dendrocalamus latiflorus* shoot.

Figure 6. Sun drying of fermented shoots of *D. latiflorus*.

are popular in summer when other vegetables are in shortage. *Dendrocalamus latiflorus* Munro has a longer production season from late March to early November. The average weight of each shoot is 2 kg. Green bamboo shoots weigh 0.65 kg. The shoots of *D. latiflorus* are cut when 50–100 cm above the ground (Fig. 5) and are mainly used for processing because they are relatively bitter and tough.

Phyllostachys edulis yields winter shoots, available from November to February, which are smaller, sweeter, and of better quality than spring shoot, available from March to May. Winter shoots of *P. edulis* are considered the best flavored shoots and are precious among winter vegetables due to small production.

FOOD USES

In the preparation of shoots for use, the sheaths and tough basal portions are removed, and the tender shoots are then sliced or cut lengthwise and boiled in water. The still crispy slices can be used in many dishes. Upon tasting, if the shoot is bitter, it is boiled again to remove the bitterness. Raw bamboo shoot is acrid, and boiling removes the acridity. The bitterness is from cyanogenic glucosides, which, when ingested, is poisonous because cyanide is released. Parboiling in water leaches the compound to render the shoots nontoxic (2). Green bamboo shoots and the winter shoots of *P. edulis* are not bitter because they are harvested before they emerge from the soil surface. Both shoots can be directly cooked with other food without precooking.

In Taiwan, shoots of *D. latiflorus* Munro, which are much larger in size, are used for canning or fermentation, fewer go to fresh market. Canned bamboo shoots are mainly for export and the amount is decreased year on year due to high labor cost. Dried fermented shoots are prepared from fresh bamboo shoots of ca 1 m long, the green portion is removed before processing. After blanching and fermenting for about one month the shoots are sun dried and sliced (Fig. 6). The final product has a crispy, tender texture and good flavor from the lactic acid fermentation (12).

Table 1. Approximate Composition of *Bambusa oldhami* and *Phyllostachys edulis* in a 100-g Edible Portion

	Composition	
Constituent	*B. oldhami*	*P. edulis*
Energy (cal/100 g)	60	22
Macroconstituent, 100%		
Water	83	92
Protein	3.9	2.4
Fat	1.1	0.3
Ash	0.7	1.0
Fiber	1.3	0.9
N-free extract	10.3	3.2.
Minerals, (mg %)		
Ca	44	43
Mg	21	45
P	39	38
Fe	3.4	2.2
Na	98	26
K	330	435
Vitamins, (mg %)		
A (IU)	30	20
B_1	0.02	0.08
B_2	0.03	0.07
Niacin	2.23	1.58
C	3	3

NUTRITIONAL VALUE

Bambusa oldhami and *Phyllostachys edulis* are two varieties sold in Taiwan fresh markets. The approximate nutritional composition in a 100-g edible portion is given in Table 1 (13).

BIBLIOGRAPHY

1. R. M. Ruberté, "Leaf and Miscellaneous Vegetables," in F. W. Martin, ed., *Handbook of Tropical Food Crops*, CRC Press, Inc., Boca Raton, Fl., 1984.
2. M. Yamaguchi, "Bamboo Shoot," *World Vegetables*, AVI Publishing Co., Westport, Conn., 1983.
3. Department of Agriculture and Forestry, "Crop Production," in *Taiwan Agricultural Yearbook*, Taiwan Provincial Government, Taichun, Taiwan, 1990.

4. B. N. Lee, "Bamboo Shoot," *Farmers Guide. Harvest Farm Magazine* Ltd., Taipei, Taiwan, 1980 pp. 885–888.
5. G. M Leu, "Disappearing of Bamboos" *Harvest* **35**, 16–17 (June 28, 1985).
6. G A. C. Herklots, *Vegetables in South-East Asia*, George Allen & Unwin Ltd., London, 1972.
7. M. T. Chang, "Cultivation of Bamboo Shoot," *Harvest* **36**, 42–45 (Aug. 1, 1986).
8. M. L. Liao et al., "Respiration Measurement of Some Fruits and Vegetables of Taiwan," *FIRDI Technical Report* **E-66**, Hsinchu, Taiwan, 1982.
9. T. R. Yen et al., "Quality Improvement of Canned Bamboo Shoot," *FIRDI Technical Report* **237**, Hsinchu, Taiwan, 1982.
10. R. Y. Chen et al., "Postharvest Handling and Storage of Bamboo Shoots," *Acta Horticulture* 258.309–316, 1989.
11. R. Y. Chen et al , "Control of Postharvest Quality of Bamboo Shoot," *FIRDI Technical Report* **423**, Hsinchu, Taiwan, 1986.
12. G. T. Huang et al., "Improvement on the Quality of Dried Fermented Bamboo Shoots," *FIRDI Technical Report* E-121, Hsinchu, Taiwan, 1985.
13. FIRDI, "Table of Taiwan Food Composition," *FIRDI Technical Report* **24**, Hsinchu, Taiwan, 1971.

Ming-Sai Liu
Food Industry Research and Development Institute
Hsinchu, Taiwan

BANANAS. See Fruits, tropical.

BARLEY. See Cereals science and technology.

BEEKEEPING

Honey bees represent less than 12 of the thousands of bee species that exist around the world. Honey bees are unique in that they live in perennial social societies, and they produce a number of "hive products" that are used by humans in various ways. However, their greatest value to humans is their role as pollinators of cultivated crops. Approximately one-third of the diet consumed by individuals living in advanced societies is the product of honey bee pollination.

HISTORY

Honey bees (genus *Apis*) are believed to have originated in Southeast Asia (1). New species of *Apis* are still being described from that region today. Four species that naturally inhabit large areas of the world include the Western honey bee (*Apis mellifera*) from Europe and Africa, and the Eastern honey bee (*Apis cerana*), the giant honey bee (*Apis dorsata*), and the dwarf honey bee (*Apis florea*) from Southeast Asia. Only *A. mellifera* and *A. cerana* are cavity nesting species and thus have been collected by humans and successfully transferred and managed in artificial domiciles or hives.

It is likely that the first beekeepers were honey hunters or honey gathers, as depicted in a Paleolithic rock painting found in eastern Spain dating from around 7000 B.C. (2). There, gatherers collected honey by robbing wild bee nests. Honey hunting is still widely practiced in Asia to obtain honey from *A. dorsata* and to a lesser extent from *A. florea* nests. It can be postulated that an early stage in the development of beekeeping involved the cutting of a log section enclosing a bee colony and transporting the log to the dwelling of the gatherer. There the log and bees would provide a convenient source of honey and brood for periodic robbing by the new owner. The use of log sections as beehives persists in some African regions. The first hives made by humans probably were composed of clay, straw, or tree bark. In the Middle East, honey bees are still kept in clay pottery, mud, and straw hives. Some of these hive materials were used some 6000 years ago.

The advent of what is considered the "modern era" of beekeeping came about with the discovery of movable-frame beehives. The person generally credited with publicizing the significance of the "bee space" was Lorenzo L. Langstroth who, in 1851, discovered that bees would refrain from building wax connections between neighboring combs, or combs and the side of the beehive, if the space separating them was approximately 3/8 in. (3). This seemingly modest discovery led directly to the evolution of hives that had fully removable frames that enclose and support the beeswax combs. These frames could be manipulated easily by the beekeeper and removed for the purpose of examining the brood or extracting honey. The latter part of the nineteenth century saw the development of an amazing array of inventions based on this technology, including machines for removing wax (uncapping) and extracting honey from the new removable frames. Eventually, the Langstroth hive became established as the standard-size beehive that remains largely unchanged to the present day.

BIOLOGY OF HONEY BEES

All members of the genus *Apis* are eusocial, which means truly social. To entomologists this term indicates that there is cooperative care of the young by individuals of the same species, an overlap in generations, and production of a sterile or nonreproducing worker caste. This type of social development is uncommon considering that there are over 1 million named insect species. Living as a colony, eusocial species like honey bees provide more food and protection for themselves and their young than can any solitary individual. It is because honey bees store food for future needs that humans have been able to exploit them.

Scientists subdivide the wide-ranging species *A. mellifera* and *A. cerana* into a number of races (4). These races differ in behavioral and physical characteristics that are the result of adaptations of the species over time, following isolation in geographical regions. In fact, such races are sometimes called geographic races. In *A. mellifera* some races can be physically distinguished only through the use of computer-assisted morphometric (body measurement) analysis. To describe honey bee races, specialists use a trinomial system of nomenclature as opposed to the traditional binomial. For example, the Italian honey bee race's

4. B. N. Lee, "Bamboo Shoot," *Farmers Guide. Harvest Farm Magazine* Ltd., Taipei, Taiwan, 1980 pp. 885–888.
5. G. M Leu, "Disappearing of Bamboos" *Harvest* **35**, 16–17 (June 28, 1985).
6. G A. C. Herklots, *Vegetables in South-East Asia*, George Allen & Unwin Ltd., London, 1972.
7. M. T. Chang, "Cultivation of Bamboo Shoot," *Harvest* **36**, 42–45 (Aug. 1, 1986).
8. M. L. Liao et al., "Respiration Measurement of Some Fruits and Vegetables of Taiwan," *FIRDI Technical Report* **E-66**, Hsinchu, Taiwan, 1982.
9. T. R. Yen et al., "Quality Improvement of Canned Bamboo Shoot," *FIRDI Technical Report* **237**, Hsinchu, Taiwan, 1982.
10. R. Y. Chen et al., "Postharvest Handling and Storage of Bamboo Shoots," *Acta Horticulture* 258.309–316, 1989.
11. R. Y. Chen et al , "Control of Postharvest Quality of Bamboo Shoot," *FIRDI Technical Report* **423**, Hsinchu, Taiwan, 1986.
12. G. T. Huang et al., "Improvement on the Quality of Dried Fermented Bamboo Shoots," *FIRDI Technical Report* E-121, Hsinchu, Taiwan, 1985.
13. FIRDI, "Table of Taiwan Food Composition," *FIRDI Technical Report* **24**, Hsinchu, Taiwan, 1971.

MING-SAI LIU
Food Industry Research and Development Institute
Hsinchu, Taiwan

BANANAS. See FRUITS, TROPICAL.

BARLEY. See CEREALS SCIENCE AND TECHNOLOGY.

BEEKEEPING

Honey bees represent less than 12 of the thousands of bee species that exist around the world. Honey bees are unique in that they live in perennial social societies, and they produce a number of "hive products" that are used by humans in various ways. However, their greatest value to humans is their role as pollinators of cultivated crops. Approximately one-third of the diet consumed by individuals living in advanced societies is the product of honey bee pollination.

HISTORY

Honey bees (genus *Apis*) are believed to have originated in Southeast Asia (1). New species of *Apis* are still being described from that region today. Four species that naturally inhabit large areas of the world include the Western honey bee (*Apis mellifera*) from Europe and Africa, and the Eastern honey bee (*Apis cerana*), the giant honey bee (*Apis dorsata*), and the dwarf honey bee (*Apis florea*) from Southeast Asia. Only *A. mellifera* and *A. cerana* are cavity nesting species and thus have been collected by humans and successfully transferred and managed in artificial domiciles or hives.

It is likely that the first beekeepers were honey hunters or honey gathers, as depicted in a Paleolithic rock painting found in eastern Spain dating from around 7000 B.C. (2). There, gatherers collected honey by robbing wild bee nests. Honey hunting is still widely practiced in Asia to obtain honey from *A. dorsata* and to a lesser extent from *A. florea* nests. It can be postulated that an early stage in the development of beekeeping involved the cutting of a log section enclosing a bee colony and transporting the log to the dwelling of the gatherer. There the log and bees would provide a convenient source of honey and brood for periodic robbing by the new owner. The use of log sections as beehives persists in some African regions. The first hives made by humans probably were composed of clay, straw, or tree bark. In the Middle East, honey bees are still kept in clay pottery, mud, and straw hives. Some of these hive materials were used some 6000 years ago.

The advent of what is considered the "modern era" of beekeeping came about with the discovery of movable-frame beehives. The person generally credited with publicizing the significance of the "bee space" was Lorenzo L. Langstroth who, in 1851, discovered that bees would refrain from building wax connections between neighboring combs, or combs and the side of the beehive, if the space separating them was approximately 3/8 in. (3). This seemingly modest discovery led directly to the evolution of hives that had fully removable frames that enclose and support the beeswax combs. These frames could be manipulated easily by the beekeeper and removed for the purpose of examining the brood or extracting honey. The latter part of the nineteenth century saw the development of an amazing array of inventions based on this technology, including machines for removing wax (uncapping) and extracting honey from the new removable frames. Eventually, the Langstroth hive became established as the standard-size beehive that remains largely unchanged to the present day.

BIOLOGY OF HONEY BEES

All members of the genus *Apis* are eusocial, which means truly social. To entomologists this term indicates that there is cooperative care of the young by individuals of the same species, an overlap in generations, and production of a sterile or nonreproducing worker caste. This type of social development is uncommon considering that there are over 1 million named insect species. Living as a colony, eusocial species like honey bees provide more food and protection for themselves and their young than can any solitary individual. It is because honey bees store food for future needs that humans have been able to exploit them.

Scientists subdivide the wide-ranging species *A. mellifera* and *A. cerana* into a number of races (4). These races differ in behavioral and physical characteristics that are the result of adaptations of the species over time, following isolation in geographical regions. In fact, such races are sometimes called geographic races. In *A. mellifera* some races can be physically distinguished only through the use of computer-assisted morphometric (body measurement) analysis. To describe honey bee races, specialists use a trinomial system of nomenclature as opposed to the traditional binomial. For example, the Italian honey bee race's trinomial is *A. mellifera ligustica*. Other examples are *A. mellifera mellifera*, a race from northern Europe and the first honey bee race known to have been introduced into the New World, and *A. mellifera carnica*, the Carniolan honey bee from southern Austria and Yugoslavia.

A colony of honey bees may contain more than 45,000 adult individuals during the summer when the population peaks. Four life stages present in the colony are the eggs, larvae, pupae, and adults. These stages can be further subdivided by the two sexes, females and males (drones). The females may be divided further into two castes, workers and queens.

WORKERS

Workers, as their name implies, do the work of the colony. The worker caste builds the combs used to house developing eggs, larvae, and pupae (collectively called brood) and to store honey and pollen. They forage outside the colony for nectar (carbohydrate) and pollen (proteins, vitamins, minerals, and fats) to provide food for the colony. Workers also feed the brood, queen, and drones and defend the colony against intruders. When a worker bee stings, the barbed stinger usually pulls out of her abdomen and remains attached to her victim, where its odor attracts more bees to sting. Bees that have stung die within a day.

Sexually, workers are females with 32 chromosomes and usually lack functional ovaries. However, in certain situations, such as the absence of a queen, it is possible for workers to lay eggs. Laying workers are incapable of laying fertile eggs since they do not mate with drones to obtain sperm. Consequently, they lay eggs that only produce haploid drones. This leads to eventual death of the colony. In rare situations, laying workers can produce females impaternate (without fathers). This parthenogenic means of reproduction is found in the highest frequency in the race *A. mellifera capensis*.

During the busy foraging season, the life span of a worker is about six weeks. Workers literally work themselves to death. However, workers reared late in the fall often live through the winter into the following spring.

QUEENS

Queens are also females. They have the capacity to mate and lay fertilized eggs. Usually each colony has a single queen. However, under certain conditions colonies may have more than one queen. A few commercial beekeepers intentionally manipulate beehives so they can maintain a two-queen colony that is sometimes more productive than a single-queen colony. Queens live for as long as five years but most commercial beekeepers replace them every two years. The queen is the egg-laying female of the colony. The maximum number of eggs a queen lays varies throughout the season. It is estimated that a good queen can lay 1500 to 2000 eggs a day. A colony will produce new queens if its queen becomes a drone layer (depletes her stored sperm) or is injured or if the colony is about to swarm. New queens have to be raised in specially constructed cells. The workers feed the developing larva in such a way that a queen, rather than a worker, is produced.

DRONES

The drones are males and their primary function is to mate with virgin queens. They are raised in specially constructed cells that are larger than the worker cells. Genetically, drones are haploid, having a chromosome number of 16, in contrast to the diploid status of workers and queens, which have a chromosome number of 32. In temperate areas, drones are produced in large numbers, usually beginning in March, and are maintained until the first killing frost. A typical colony in summer will have 1000 to 2000 drones. Populous colonies sometimes will keep their drones into late November. Some scientists suspect that drones have functions other than mating, but no evidence has been forthcoming to support this viewpoint.

MATING

In nature, mating of drones and queens occurs in flight. The queen mates only at one early period of her life, during the so-called nuptial flight(s). About seven days after she emerges from her cell as an adult, she is considered sexually mature and will leave the hive to mate. A queen mates with about 17 drones (5). Queens are believed to fly to a location known as a drone congregation area (DCA) to mate. To this day, it is not possible to predict with certainty what geographical condition or other attributes are needed to establish a DCA. The results of recent work done with radar suggest that DCAs may be busy intersections in a complex network of drone pathways. Drones injure themselves mortally during mating, after which they fall to the ground and die.

SWARMING

The process of swarming represents the natural method of colony reproduction. Under certain conditions, often associated with the seasonal growth cycle of the colony, workers begin to raise additional queens in the colony. At some point before the virgin queens emerge from their cells, the old queen will depart from the nest with approximately half of the workers and drones and attempt to establish a colony at a new home site. The propensity for swarming and the conditions leading up to this behavior vary within and among races of honey bees. Sometimes one or more of the newly emerged virgin queens will also depart with a group of workers and drones in an "after swarm," also attempting to establish a new colony.

DISEASES AND PESTS

Larval Diseases

Like all living animals, the honey bee is subject to the ravages of diseases and pests. The diseases range from viral to fungal in origin. One troublesome brood disease is called

American foulbrood disease. This disease brought about the establishment of many state bee inspection programs (6). American foulbrood disease is caused by *Paenibacillus larvae larvae* (formerly *Bacillus larvae*). Only the spore stage of this bacterium is infective and only in honey bee larvae less than three days of age. Another important brood disease is European foulbrood disease, caused by *Melissococcus pluton*. In both cases, beekeepers sometimes use an antibiotic (oxytetracycline) for disease prevention and treatment. Another bacterial disease of minor importance is powdery scale disease, which is caused by *Paenibacillus larvae pulvifaciens*.

All the known fungal diseases that affect the brood are of minor significance except for chalkbrood disease, which is caused by *Ascosphaera apis*. Until 1968, no chalkbrood disease had been found in the United States. Currently, chalkbrood is reported present in most of the states, and many beekeepers report reductions in their honey crop due to this disease. There is no effective chemical control for chalkbrood.

A number of viruses affect honey bees. Most virus diseases of bees are considered of minor importance and no treatment is necessary. Two brood diseases of viral origin are sacbrood and filamentous virus disease (formerly believed to be of rickettsial origin).

Adult Diseases

Nosema disease is caused by the microsporidian *Nosema apis*. This protozoan shortens the life span of adult bees and reduces their production of royal jelly. Consequently, brood and honey production are reduced by 30 to 40%. The subtle effects of this disease are difficult to recognize and most beekeepers do not realize that their honey bees are infected. Fumagillin is used by some beekeepers to treat this disease.

Chronic bee paralysis is a virus-induced disease that is occasionally seen in honey bee colonies. This disease mimics the effects of pesticide kills, and many beekeepers may misdiagnose the condition. Fortunately, the disease is not serious. It is generally believed that requeening can abate the disease.

Other minor diseases affect the health of honey bees but are of little economic significance. Noninfectious conditions caused by pesticides and poisonous plants, such as California buckeye (*Aesculus californica*), cornlily (*Veratrum califonicum*), death camas (*Zygadenus venenosus*), and some locoweeds (*Astragalus* spp) can be serious problems in some years. A more detailed discussion of this subject has been published (7).

Parasitic Mites

The first of two problematic, then "exotic," parasitic mites of honey bees was detected in the United States in 1984 (8,9). *Acarapis woodi*, the tracheal mite, lives and reproduces in the thoracic tracheae (breathing tubes) of adult honey bees. During warmer times of the year, mites remain in young worker hosts only during the three weeks that they perform chores in the hive. When the workers begin to forage, the mites leave their original hosts at night, when the bees are relatively motionless, and seek newly emerged bees to infest. All castes of bees are susceptible, and the mites tend to remain in drones and queens. Tracheal mites feed by pushing their mouthparts through membranous areas of the tracheal tubes and sucking hemolymph (bee blood). Mite feeding causes hemolymph chemistry changes, destruction of flight muscles, darkening (healing?) of tracheal tubes, transmission of diseases, and reduced life expectancy. Colony losses are common in areas of colder climates, where new bees are not reared for months during the winter.

Menthol fumigation inside the beehive currently is the only registered and legal means of reducing the mite population. Treatments are temperature sensitive, with high heat driving bees from the boxes resulting in some queen losses. Once mite populations are reduced, they often can be prohibited from increasing by placing thin patties, made of two parts sugar to one part vegetable shortening, in contact with the bees in the hive.

The second important mite to be detected in the United States was the varroa mite, *Varroa jacobsoni*, found in 1987 (10,11). *Varroa* is an ectoparasite that feeds through membranous portions of the exoskeleton of adult and larval bees. Reproduction takes place only in capped cells containing worker or drone pupae. All developmental stages of the mite feed on the pupa. Physiologically immature male offspring mate with their sisters, delivering sperm that complete maturation in their sisters. When the bee emerges from its cell, the mites are liberated.

As with tracheal mites, *Varroa* feeding reduces protein content in the host's hemolymph and reduces its life span. As mite populations increase, two or more *Varroa* infest the same cell. The host pupa is apt to shrink and have malformed wings and legs. Such individuals are carried out of their cells by worker bees and discarded outside the hive. Without replacement workers, the colony perishes. *Varroa* also are disease vectors. In 1995 and 1996 there were so many *Varroa* in the country that we lost nearly 90% of our feral (unmanaged) colonies. Gardeners and commercial growers realized these losses when they noticed a total lack of honey bees on crops requiring pollination.

Introduction of plastic strips containing a time-release acaricide is the only currently registered, legal chemical control for *Varroa* mites. The selective acaricide kills the mites on contact without killing the bees. In a number of locations around the world, scientists are searching for new and different chemistries to control the mite Other researchers are screening U.S. and foreign bee stocks for populations demonstrating tolerance or resistance to *Varroa* and the tracheal mite. Results look much better for the tracheal mite than for *Varroa*, which is an interspecific transplant from its original bee host, *A. cerana* (12).

AFRICANIZED HONEY BEES

An especially defensive, man-made, hybrid race of honey bees reached southern Texas in 1990. The result of intentional crossing of European and African stocks of honey bees in Brazil, the Africanized honey bees (AHBs) expanded their population and the territory in which they live at a phenomenal rate. The leading edge of the population expansion progressed about 250 to 300 miles per year as the bees moved from Brazil to Mexico. Fortunately they slowed down considerably as they entered more temperate climates. Population expansion to the east seems to have stopped west of Houston, Texas, but is continuing to the west in New Mexico, Arizona, Nevada, and California. Various opinions exist, but it appears that AHBs may not move, on their own, too much further north than their current locations (13).

Away from their hives, AHBs forage similarly to European honey bees (EHBs), can be used for pollinating crops, and produce good honey yields if managed carefully. However, their propensity to defend their nests extremely quickly, with very large number of stinging bees, makes them inappropriate for the type of beekeeping that is practiced in the United States. Fortunately, there is a segment of the commercial beekeeping industry that produces new queens, mated outside of areas "colonized" by AHBs. These new queens may be substituted for old queens or for queens of unsuitable stocks, but only after the old queen has been found and removed from the colony. The worker bee population will be converted fully to offspring of the new queen in about six weeks.

POLLINATION

The contribution of honey bees to agriculture far exceeds the value of honey production. Whereas honey bees produce an average of 200 million lb of honey a year valued at $150 million, the annual value of crops pollinated by honey bees exceeds $10 billion (14,15).

PRODUCTS OF THE HIVE

Most people think only of honey when honey bees are mentioned. Other useful products of the hive, on a worldwide basis, include bee venom, propolis, royal jelly, pollen, and beeswax (16). The larvae and pupae also can be eaten. However, in most cases, bee brood is a novelty food or is crushed in the process of extracting honey and consumed with the honey.

Bee Venom

Bee venom is used in the United States primarily for desensitizing individuals allergic to honey bee stings. In some countries bee venom is also used for treating arthritis. Interest is increasing in the United States in using bee venom therapy for other autoimmune conditions, such as multiple sclerosis (17). Specific devices are used by beekeepers for the collection of bee venom. One device generates a mild electric current to irritate the honey bees enough to sting a plastic surface. The surface material must allow withdrawal of the barbed stinger after venom is deposited. After air drying, the bee venom is scraped off the underside of the plastic material, diluted to known concentrations, sterilized, and packaged for medical use.

Propolis

Propolis or "bee glue" is a resinous material the bees collect from plants. This material is used by honey bees to seal small openings or cracks and for mummification of foreign objects that are too large for the bees to remove from the hive (such as a dead mouse). Because the bees also use propolis to cement together wooden parts of man-made beehives, most beekeepers consider propolis a nuisance and maintain bees that use less of the substance. Propolis has been shown to possess antimicrobial properties and is used in some European and Asian countries as the active ingredient in tinctures and throat lozenges. Propolis can be scraped from wooden hive parts and can also be harvested from a perforated plastic mat that is placed between the supers (boxes that make up a beehive). After the bees deposit propolis on it, the mat is removed and frozen previous to breaking off the substance. Periodically, bee journals carry advertising for propolis, but the demand for this material is quite limited.

Royal Jelly

Royal jelly (bee milk) is fed to young honey bee larvae, and it is this food that differentiates queens from workers. Only female larvae selected to be queens are fed royal jelly voluminously throughout their developmental period. Royal jelly is used in some cosmetic products and food supplements. In some countries it is believed that royal jelly possesses medicinal properties. Collection of royal jelly is labor intensive. In commercial production, larvae are grafted (moved into a queen cell cup) as if to produce queens. Nurse bees are induced to feed the larvae liberal amounts of royal jelly. Three days after grafting, the larvae are removed from the cells and the royal jelly harvested.

Pollen

Pollen is consumed by humans primarily as a dietary supplement. Its protein, vitamin, mineral, and fat content is variable, depending on the season and plant source. In a survey of a mixed sample of bee-collected pollen from seven states, the average protein content of pollen on a dry weight basis was 24.1%. However, when individual plant sources were analyzed, the range was 7.02 to 29.87% protein.

No precise data are available on the amount of pollen needed by one colony over an entire year. This would depend on the population and brood rearing activities. Nevertheless, a good estimate is 44 lb. Because most colonies collect pollen in excess of their immediate needs, beekeepers can supplement their income by collecting pollen.

Pollen is collected with a pollen trap, a device with a screen that literally scrapes the pollen off the hind legs of returning foragers. Pollen traps must be emptied frequently, because pollen is an attractive food source for many insects and mites, and it will absorb moisture and become moldy. Some of the pollen collected by beekeepers is used to feed honey bee colonies during dearth periods. Processing of pollen is generally a simple matter after its removal from the hive. The first step is to dry the pollen, usually by air drying. Then foreign debris, such as insect parts and the remains of diseased bees, is removed. In some cases, pollen pellets are also separated by color.

The market for pollen is not especially lucrative. Some foreign pollen is imported into the United States for sale in health food stores. There are no reliable figures on the size of this trade.

Beeswax

One of the most useful products of the hive is beeswax. Honey bees produce beeswax from honey or sugar syrup through internal body chemical processes. It is estimated that it takes 8 lb of honey to produce 2 lb of beeswax. In terms of costs of both products, it is economically more profitable for a beekeeper to produce honey. Because beekeepers must remove the beeswax cappings from honeycombs prior to extracting honey, beeswax is usually considered to be a by-product of honey production. Cappings and old combs can be liquefied by heat to extract the beeswax, which floats on the surface and hardens when the mixture is allowed to cool. Beeswax ranges in color from almost white through light green or tan to golden yellow. Like honey, the color of beeswax from cappings varies according to the floral source, while beeswax from old combs is usually darkest.

The majority of beeswax produced in the United States is used in candle making and cosmetics. Other uses include dental wax, furniture polish, lubricants, jewelry making ("lost-wax casting"), batik, egg coloring, sewing, and honeycomb foundation (used by beekeepers).

Honey

Honey can be defined simply as a plant exudate of floral or extrafloral origin that is modified by honey bees and is stored in honeycombs. Nectars collected by honey bees are processed into honey. Principally, this ripening procedure involves enzymatic inversion of sucrose into fructose and glucose and reduction in the water content of nectar. Other changes occur in the process, but not much is known about them. The color, odor, and taste of honey are strongly influenced by floral source (18). Depending on the floral source and location, honey is traditionally harvested whenever the combs are filled and capped with wax. Both the stage of the ripening process, achieved before the honey is harvested, and the ambient relative humidity affect the moisture content of the final product. Honey contains spores of yeast that will germinate and ferment the honey if the moisture content is much above 18%.

Honey yields are extremely variable, and two colonies placed side by side seldom produce identical honey crops. Honey depends on a number of factors, in addition to differences in nectar production among plant species. Honey production also is influenced by ground moisture, air temperature (day and night), wind conditions, and rainfall, because they affect both nectar production and bee flight. Honey bees exhibit preferences among plant species and may collect nectar from many sources. Consequently, honey from only one plant source is extremely rare. For instance, honey that is advertised as tulip poplar frequently contains honey from a number of floral sources, though the predominant floral flavor is that of tulip poplar. As a result, many beekeepers label their honey as mountain honey, wildflower honey, and so forth.

Honey is available in many forms, although the most popular and well known is extracted honey in the familiar queen-line jars. Extracted honey is harvested from the combs by first removing the beeswax cappings and then extracting the cell contents by centrifugal force. Some honey packers heat liquid honey (72°C for 4–5 min) to destroy yeasts and to delay granulation. When honey granulates, the remaining liquid portion of the honey increases in moisture content and the likelihood of fermentation also increases. Other beekeepers only heat their honey enough (34–38°C) to aid in the process of filtration, and still others do not heat their honey at all. The filtration process is somewhat variable, ranging from merely straining out dead bees, comb, and other large debris, to filtration that removes bits of beeswax and even pollen.

The U.S. Department of Agriculture (USDA) has established a system of grading honey based on percentage of sugars, clarity, flavor, aroma, and absence of defects (beeswax, propolis, etc). In addition to grades, honey is also classified by color: water white, extra white, white, extra light amber, light amber, amber, and dark amber. Honey color varies according to floral source. There have been attempts to develop worldwide honey standards. Meanwhile, in the United States, the USDA Standards for Grades of Extracted Honey and Comb Honey are currently being used.

In addition to liquid, extracted honey, many products using honey are available on grocery store shelves. These include honey-roasted nuts, cereals, baked goods, ice cream, and candy. Honey is widely used in baked goods because of its hygroscopic properties. The fructose in honey attracts moisture and prolongs freshness in baked products. To the purist, however, comb honey is the ultimate form for eating. Within this category of packaging there are several subdivisions, such as whole combs, cut combs, chunk honey, and comb honey sections (square and round). Honey sold in combs is unheated, unfiltered, and unlikely to be adulterated.

The typical beekeeper removes only the honey that is surplus to the colony's needs. In the United States, the commercial beekeeper's colonies produce an average of 80 lb of honey annually. In good years, beekeepers report honey yields of 200 lb or more in some areas of the United States and Canada.

FUTURE DEVELOPMENT

In recent years the honey bee industry in the United States has faced many difficult problems. Foreign honey imports and lower honey prices, coupled with increased cost of production, have created a considerable financial challenge. Because of the recent establishment of the two parasitic mites and Africanized honey bees in the United States, Canada and Mexico have banned imports of U.S. package bees and queens, an action severely damaging to the queen-producing segment of the industry. The reliance of U.S. agriculture on honey bees, however, makes it imperative that these problems be solved. Many crops cannot be effectively pollinated without the placement of honey bee colonies in the orchards or fields. Fortunately, the demand for one of the direct products of the insects, honey, shows signs of increasing. The formation of the National Honey Board, with its stated goal of expanding domestic and foreign markets for honey and to develop and improve markets, bodes well for the future of commercial and hobby beekeeping in the United States.

BIBLIOGRAPHY

1. E. Crane, "The World's Beekeeping—Past and Present," in J. M. Graham, ed., *The Hive and the Honey Bee*, revised ed., Dadant & Sons, Hamilton, Ill., 1992.
2. E Crane, *The Archaeology of Beekeeping*, Duckworth, London, 1983.
3. L. L. Langstroth, *Langstroth on the Hive and the Honey-bee, A Bee Keeper's Manual*, Hopkins, Bridgman & Co., Northampton, Mass., 1853.
4. A. Dietz, "Honey Bees of the World," in J. M. Graham, ed., *The Hive and the Honey Bee*, revised ed., Dadant & Sons, Hamilton, Ill., 1992.
5. R. Page, "Sperm Utilization in Social Insects," *Ann. Rev. Entomol.* **31**, 297–320 (1986).
6. A. S. Michael, D. A. Knox, and H. Shimanuki, "U.S. Federal and State Laws Relating to Honey Bees," in R. A. Morse and R. Nowogrodzki, eds , *Honey Bee Pests, Predators, and Diseases*, 2nd ed., Comstock Publishing, Cornell University Press, Ithaca, New York, 1990.
7. E. L. Atkins, "Injury to Honey Bees by Poisoning," in J. M. Graham, ed., *The Hive and the Honey Bee*, revised ed., Dadant & Sons, Hamilton, Ill., 1992.
8. M. Bruner, "Acarine Mites Found in Texas," *Gleanings in Bee Culture* **112**, 402 (1984).
9. M. Delfinado-Baker, "*Acarapis woodi* in the United States," *Amer. Bee J.* **124**, 805–806 (1984).
10. J. M. Graham, "Varroa Mites Found in the United States," *Amer. Bee J.* **127**, 745–746 (1987).
11. K. Flottom, "Of Mites and Men (Again!)," *Gleanings in Bee Culture* **115**, 613 (1987).
12. M. Delfinado-Baker and C. Y. S. Peng, "*Varroa jacobsoni* and *Tropilaelaps clareae*—A Perspective of Life History and Why Asian Bee-mites Preferred European Honey Bees," *Amer. Bee J.* **135**, 415–420 (1995).
13. O. R. Taylor, "African Bees: Potential Impact in the United States," *Bull. Entoml. Soc. of Amer.* **31**, 14–24 (1985).
14. M. Levin, "Value of Bee Pollination to United States Agriculture," *Amer Bee J* **124**, 184–186 (1984).
15. W. S. Robinson, R. Nowogrodzki, and R. A. Morse, "The Value of Honey Bees as Pollinators of U.S. Crops, (Two part series)," *Amer. Bee J.* **129**, 411–423 and 478–487 (1989).
16. A. Mizrahi and Y. Lensky, *Bee Products—Properties, Applications, and Apitherapy*, Plenum Press, New York, 1997.
17. M. Simics, *Bee Venom: Exploring the Healing Power*, Apitronic Publishing, Calgary, Alberta, Canada, 1994.
18. E. Crane, *Honey: A Comprehensive Survey*, Heinemann, London, 1976.

ERIC C. MUSSEN
University of California Entomology Extension
Davis, California

BEER

Beer is traditionally, and in many countries, legally, defined as an alcoholic beverage derived from barley malt, with or without other cereal grains, and flavored with hops.

Barley malt is barley that has been purposely wetted, allowed to partly germinate, and then dried or kilned. This three-step process is called malting. Because barley is virtually the only cereal that is malted, it is often just referred to as malt. The drying stabilizes the malt and allows beer to be produced year round and in places far removed from where barley is grown. It thus differs from wine, which is only produced seasonally and only near grape-growing areas.

Other cereal grains that are used to make beer are rice, corn, sorghum, and wheat. The choice, depends on the individual brewer and often on the availability of the different cereals.

HISTORY

Beer was likely a casual discovery when a bowl of grain that got wet, and then very wet, accidentally fermented with airborne yeast. It has been made intentionally for more than 6,000 years and is indigenous to most temperate and tropical cultures.

The oldest known written recipe was found on a 4,000-year-old Mesopotamian clay tablet, and it was for beer (Fig. 1). The Babylonians made 16 kinds of beer, using barley, wheat, and honey. The Egyptians described a "beer of truth" and a "beer of eternity".

Beer making was an important trade in ancient Mesopotamia, Egypt, Greece, and Rome. In less-settled cultures in Europe, Africa, China, India, and South America some kind of fermented grain beverages were made on a small scale for home consumption. It may well have been discovered that brewing beer makes any water safe for human consumption.

Large-scale brewing began in medieval Europe as a result of increased travel and trade and the attendant increase in inns and taverns offering food and lodging. Monasteries began brewing beer, as well as wine and liqueurs, and accepted voyagers as guests. This helped spread knowledge about beer.

In North America, beer was introduced with the early English and Dutch settlers. The Pilgrims landed in Plymouth, instead of going farther south, because they had run out of beer. Many of the founders of the United States were active brewers or maltsters.

All the beer of the early settlers was actually ale, fermented by strains of yeast, *Saccharomyces cerevisiae*, that are airborne and are also used in making bread and wine. Ales are produced at ambient temperatures.

In the 1840s German brewers came to the United States and began brewing lager beer, fermented by strains of a yeast *S. carlsbergensis* that exists only in breweries and is used only for making beer. A characteristic of this yeast is that it can only function at temperatures below 70°F. For this reason, the early lager breweries were built near riveffectively pollinated without the placement of honey bee colonies in the orchards or fields. Fortunately, the demand for one of the direct products of the insects, honey, shows signs of increasing. The formation of the National Honey Board, with its stated goal of expanding domestic and foreign markets for honey and to develop and improve markets, bodes well for the future of commercial and hobby beekeeping in the United States.

BIBLIOGRAPHY

1. E. Crane, "The World's Beekeeping—Past and Present," in J. M. Graham, ed., *The Hive and the Honey Bee*, revised ed., Dadant & Sons, Hamilton, Ill., 1992.
2. E. Crane, *The Archaeology of Beekeeping*, Duckworth, London, 1983.
3. L. L. Langstroth, *Langstroth on the Hive and the Honey-bee, A Bee Keeper's Manual*, Hopkins, Bridgman & Co., Northampton, Mass., 1853.
4. A. Dietz, "Honey Bees of the World," in J. M. Graham, ed., *The Hive and the Honey Bee*, revised ed., Dadant & Sons, Hamilton, Ill., 1992.
5. R. Page, "Sperm Utilization in Social Insects," *Ann. Rev. Entomol.* **31**, 297–320 (1986).
6. A. S. Michael, D. A. Knox, and H. Shimanuki, "U.S. Federal and State Laws Relating to Honey Bees," in R. A. Morse and R. Nowogrodzki, eds., *Honey Bee Pests, Predators, and Diseases*, 2nd ed., Comstock Publishing, Cornell University Press, Ithaca, New York, 1990.
7. E. L. Atkins, "Injury to Honey Bees by Poisoning," in J. M. Graham, ed., *The Hive and the Honey Bee*, revised ed., Dadant & Sons, Hamilton, Ill., 1992.
8. M. Bruner, "Acarine Mites Found in Texas," *Gleanings in Bee Culture* **112**, 402 (1984).
9. M. Delfinado-Baker, "*Acarapis woodi* in the United States," *Amer. Bee J.* **124**, 805–806 (1984).
10. J. M. Graham, "Varroa Mites Found in the United States," *Amer. Bee J.* **127**, 745–746 (1987).
11. K. Flottom, "Of Mites and Men (Again!)," *Gleanings in Bee Culture* **115**, 613 (1987).
12. M. Delfinado-Baker and C. Y. S. Peng, "*Varroa jacobsoni* and *Tropilaelaps clareae*—A Perspective of Life History and Why Asian Bee-mites Preferred European Honey Bees," *Amer. Bee J.* **135**, 415–420 (1995).
13. O. R. Taylor, "African Bees: Potential Impact in the United States," *Bull. Entoml. Soc. of Amer.* **31**, 14–24 (1985).
14. M. Levin, "Value of Bee Pollination to United States Agriculture," *Amer. Bee J.* **124**, 184–186 (1984).
15. W. S. Robinson, R. Nowogrodzki, and R. A. Morse, "The Value of Honey Bees as Pollinators of U.S. Crops, (Two part series)," *Amer. Bee J.* **129**, 411–423 and 478–487 (1989).
16. A. Mizrahi and Y. Lensky, *Bee Products—Properties, Applications, and Apitherapy*, Plenum Press, New York, 1997.
17. M. Simics, *Bee Venom: Exploring the Healing Power*, Apitronic Publishing, Calgary, Alberta, Canada, 1994.
18. E. Crane, *Honey: A Comprehensive Survey*, Heinemann, London, 1976.

ERIC C. MUSSEN
University of California Entomology Extension
Davis, California

BEER

Beer is traditionally, and in many countries, legally, defined as an alcoholic beverage derived from barley malt, with or without other cereal grains, and flavored with hops.

Barley malt is barley that has been purposely wetted, allowed to partly germinate, and then dried or kilned. This three-step process is called malting. Because barley is virtually the only cereal that is malted, it is often just referred to as malt. The drying stabilizes the malt and allows beer to be produced year round and in places far removed from where barley is grown. It thus differs from wine, which is only produced seasonally and only near grape-growing areas.

Other cereal grains that are used to make beer are rice, corn, sorghum, and wheat. The choice, depends on the individual brewer and often on the availability of the different cereals.

HISTORY

Beer was likely a casual discovery when a bowl of grain that got wet, and then very wet, accidentally fermented with airborne yeast. It has been made intentionally for more than 6,000 years and is indigenous to most temperate and tropical cultures.

The oldest known written recipe was found on a 4,000-year-old Mesopotamian clay tablet, and it was for beer (Fig. 1). The Babylonians made 16 kinds of beer, using barley, wheat, and honey. The Egyptians described a "beer of truth" and a "beer of eternity".

Beer making was an important trade in ancient Mesopotamia, Egypt, Greece, and Rome. In less-settled cultures in Europe, Africa, China, India, and South America some kind of fermented grain beverages were made on a small scale for home consumption. It may well have been discovered that brewing beer makes any water safe for human consumption.

Large-scale brewing began in medieval Europe as a result of increased travel and trade and the attendant increase in inns and taverns offering food and lodging. Monasteries began brewing beer, as well as wine and liqueurs, and accepted voyagers as guests. This helped spread knowledge about beer.

In North America, beer was introduced with the early English and Dutch settlers. The Pilgrims landed in Plymouth, instead of going farther south, because they had run out of beer. Many of the founders of the United States were active brewers or maltsters.

All the beer of the early settlers was actually ale, fermented by strains of yeast, *Saccharomyces cerevisiae*, that are airborne and are also used in making bread and wine. Ales are produced at ambient temperatures.

In the 1840s German brewers came to the United States and began brewing lager beer, fermented by strains of a yeast *S. carlsbergensis* that exists only in breweries and is used only for making beer. A characteristic of this yeast is that it can only function at temperatures below 70°F. For this reason, the early lager breweries were built near riv-

Figure 1. Mesopotamian clay tablet. *Source:* Courtesy of the Beer Institute.

ers or lakes that froze in the winter. Ice was cut from these and stored in cellars for refrigeration during the summer. Thus New York, St. Louis, and Philadelphia became centers of lager brewing. By the 1870s mechanical refrigeration was invented and used first in a brewery in Brooklyn.

Lager beer has almost completely replaced ale as the beverage of choice, and today ale is only produced in the United Kingdom and parts of Canada, with only token amounts elsewhere. In the United Kingdom ale is referred to as beer and what the rest of the world calls beer is called lager. In what follows, beer will be used to refer to both ale and beer.

The Making of Beer

Beer production involves three distinct but interrelated stages. First is the preparation of an extract of the malt and the grains selected. This extract is called wort (pronounced to rhyme with "hurt"). This step takes place in the brewhouse and is often referred to as brewing. It takes about 4–10 h. A photograph of a typical brewhouse is shown in Figure 2.

The next stage is fermentation, the conversion of this liquid by yeast into beer. This is a temperature-controlled process that takes 3–10 d.

Fermentation is usually conducted in large stainless steel vessels that hold the volume of an entire brew, or several brews. Their size may be between 7,000 and 375,000 gal, and they may be horizontal rectangular in shape, or vertical cylindrical, with or without a conical bottom. The conical bottom allows simple gravity expulsion of yeast. A set of cylindroconical tanks are shown in Figure 3. The rectangular tanks require manual raking of yeast for reuse in subsequent brews.

Figure 2. Brewhouse, showing lauter tubs above and kettles below. *Source:* Courtesy of Anheuser-Busch, Inc.

The final stage is finishing or the refining of this liquid into salable beer by the brewery. It may take 2–25 d. It takes place in tanks equal in size to the fermentation tanks

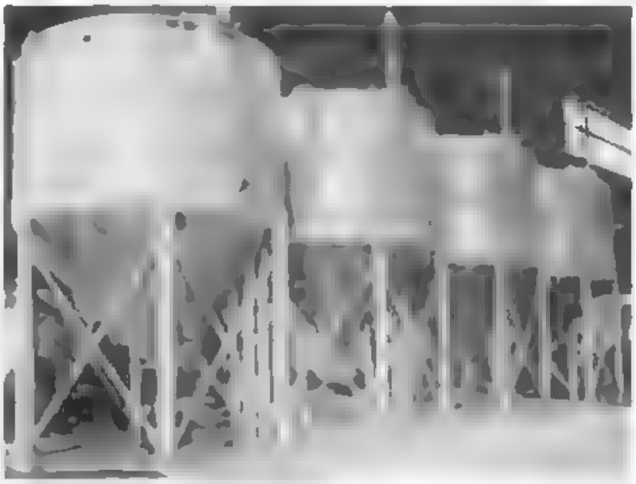


Figure 3. Cylindroconical tanks. *Source:* Courtesy of Paul Mueller Company.

or multiples of the fermenter size. Aging tanks, preassembled on site, are shown in Figure 4.

Each stage offers the brewery many choices. It is for these reasons that there are not many beers, perhaps none, that are exactly alike. The finished beer may be packed in large containers, holding 2–31 gal, or into small ones, holding 7–40 oz.

RAW MATERIALS OF BREWING

Water

Water is, only quantitatively, the major component of beer. Its requirements for brewing are fairly simple. First, it must meet local or international standards for potable water. The World Health Organization's standards for drinking water are shown in Table 1.

The second requirement is that it not be too alkaline. A maximum alkalinity of 50 ppm (as calcium carbonate) is acceptable.

A third requirement is that it be hard and contain calcium. The level of calcium considered most desirable is 100 ppm (as calcium), but lesser amounts may also be used. The water in Pilsner, Czechoslovakia, had these attributes, neutrality and hardness, and soon became the standard by which all beer is made.

All water supplies are either surface water or groundwater. Surface water is derived from rainfall or snow and may be quite pure. If it is collected in reservoirs near the source of rain or snow, it is usually soft and neutral. If it is collected in rivers, its purity will depend on the distance between the collection and distribution points and the uses it suffers in between. Such water may need purification with sand and charcoal.

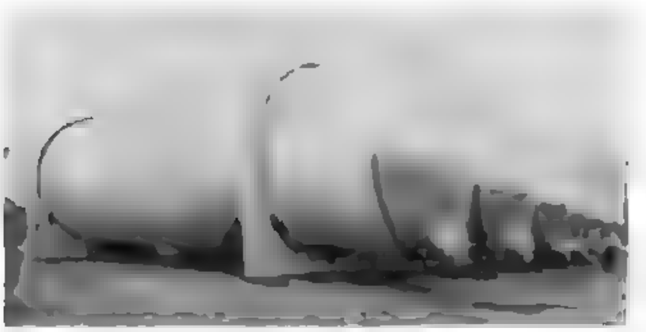


Figure 4. Aging tanks assembled on site. *Source:* Courtesy of Paul Mueller Company.

Table 1. World Health Organization Standards for Drinking Water

	Maximum permissible concentrations (mg/L)
Chlorides	60
Sulfates	400
Calcium	200
Magnesium	150
Total dissolved solids	1,500

Groundwater comes from wells or springs and is usually tasteless but may be quite alkaline or hard, or both. Such water may need acidification to remove alkalinity and, less often, softening if it is too hard. Such water rarely needs treatment with activated charcoal.

Barley Malt

Malted barley is the principal ingredient of beer. It furnishes almost all the required elements for making beer. Malt provides starch and sugars, which will, partly or completely, become alcohol during fermentation; and protein and amino acids, which will supply nutrition for the yeast, as well as color, foam, and flavor in the finished beer.

Barley used to make malt is almost always selected from special strains that have been developed and grown specifically for this purpose. There are two major types of barley, distinguished by the number of rows on the stalk. Six-rowed barley is grown mainly in the midwest of the United States and Canada, but now also in Europe and Australia. Two-rowed barely is grown in the far west, and also in Europe and other barley growing regions. The two-rowed barleys are considered by some to give a smoother beer. A drawing of a barley kernel is shown in Figure 5.

The husk, which protects the seed and is composed of the palea and lemma, is, in barley, alone among the cereals, fused to the testa and the pericarp. It is this fusion that permits barley to be malted efficiently and to be the universal basis for making beer. Grains that lose their husks at germination tend to become moldy when malted in bulk.

The malting of barley begins with steeping, in which the barley, in tanks, is soaked in aerated water. The steeped water is changed several times. The steeping is complete when the barley reaches a moisture content of 45%. It is then transferred to long, horizontal compartments, through which moist air is blown. In 4–6 d, the barley germinates and starts to grow as if it were to become a barley plant. During this time the barley produces starch-splitting enzymes, notably α-amylase and β-amylase, and also proteinases and cellulases. The amylases break down some of the starch in the barley; the proteinases degrade some of the proteins to amino acids, and the cellulases soften the cell walls. All these enzymes are secreted by the aleurone layer and migrate into the endosperm. These changes, which collectively are called modification, prepare the barley for its use in brewing.

At the time when the modification is considered complete, the malt is transferred to a kiln and dried. The temperature at which the malt is dried determines the color and, partly, the flavor of the beer from which it will be made. Kilning stabilizes the malt and allows it to be shipped widely and to be stored for a year or more.

The analysis of a typical malt is shown in Table 2.

UNMALTED CEREALS (ADJUNCTS)

Cereals such as rice, corn, and sorghum are often used to complement or attenuate the malt. These grains do not

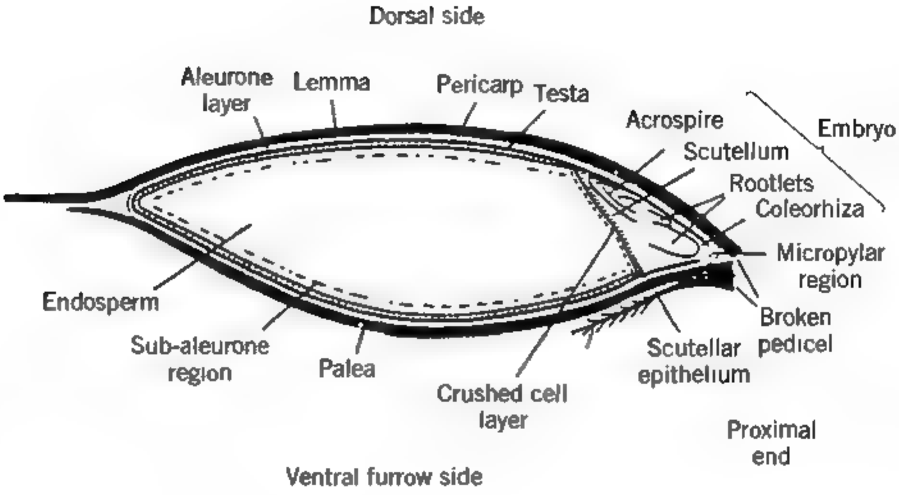


Figure 5. Schematic longitudinal section of a barley grain, taken to one side of the furrow, showing the disposition of the parts.

Table 2. Analysis of Barley Malt (%)

Moisture	4.0
Starch and dextrins	52.5
Simple sugars	9.5
Total protein	13.0
Soluble protein	5.4
Cellulose	6.0
Other fiber	10.0
Fats	2.5
Minerals	2.5

contribute to flavor, color, or foam but serve only to supply carbohydrates to the wort. Their use probably arose when malt was in short supply. They are widely used throughout the world. Only in parts of Germany, in Switzerland, and in Greece is their use prohibited by law. This prohibition, which dates to 1516 in Bavaria, as the *Reinheitsgebot*, originally was intended as a means to police the taxation of beer because malt could only be made in a malthouse. The prohibition on the use of other sources of carbohydrates precluded the surreptitious increase in beer production. It remains to this day as a vestigial reminder of days past that has served to prevent the importation into those three countries of beers made with unmalted cereals. However, the European Common Market has overruled this law and such beers may now be imported into the all-malt countries.

The quantity of unmalted cereals may vary from 10 to 50% of the total mash, depending on the beer to be produced. Increased use gives a lighter, less bodied beer. If rice or sorghum is used, it must be ground before mashing. If corn is chosen, then a physically separated fraction of the ground corn kernel that contains mainly starch and protein and very little corn oil is used.

Starch is the major component of both malt and the other cereals. It is a complex polysaccharide made up of about 35% of a straight-chain polymer of glucose known as amylose and about 65% of a branched polymer, amylopectin. The links in all of amylose are 1,4′ bonds between neighboring glucose units. The bonds in amylopectin are both 1,4′ and 1,6′,—the latter at the branch points. Schematic diagrams of amylose and amylopectin are shown in Figure 6 and 7, respectively.

SPECIAL MALTS

Some malts are made with special properties, mainly differing in color and flavor. A caramel, or crystal, malt is made by heating a wet malt with steam under pressure. The resulting malt is used with regular malt to produce dark beers. A so-called chocolate malt is a variant of crystal malt. These malts provide color and special flavor to the beer. Malt may also be darkened by kilning at higher temperatures (200°F or higher) or for longer times. Such malt only imparts more color to the beer.

Wheat may be malted, with difficulty, but it produces an ordinary beer unless a yeast is used that by itself produces a spicy flavor. This has found some favor in parts of Germany and Belgium.

HOPS

Hops, *Humulus lupulus*, is a truly remarkable plant. Although it originally grew wild, and still does, it is intensively cultivated in a few countries for use only in beer. The major hop-growing countries are Germany, the United States, the Soviet Union, Czechoslovakia, Yugoslavia, and the United Kingdom. Figure 8 shows hops growing in Germany. Hop picking by hand in Czechoslovakia is shown in Figure 9.

The use of hops in beer only goes back some 500 years. Many plants had been used to flavor beer, until it was discovered that hops not only makes beer pleasantly flavored but also controls the growth of spoilage bacteria. The use of hops is now universal in beer.

Hops contain a group of compounds, humulones, which are very insoluble in water, but which undergo a chemical rearrangement during the brewing process to form an isomeric group of compounds called isohumulones. The isohumulones are soluble in water and impart to beer a

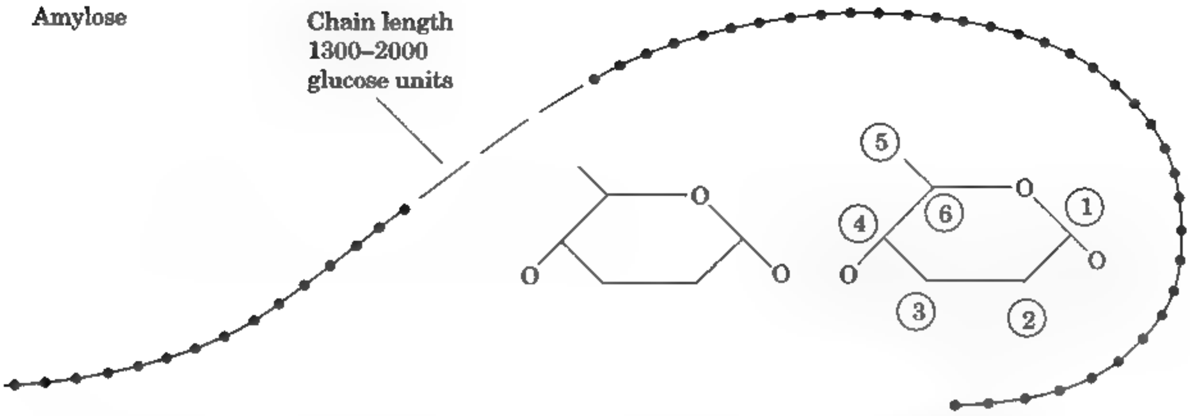


Figure 6. Structure of the linear starch fraction. Each black circle represents a glucose unit, and the aldehydic terminus of the chain is indicated by an asterisk. Interglucose bonding is α-1,4, as shown in the inset, with carbon atoms in the glucose unit numbered in conventional fashion.

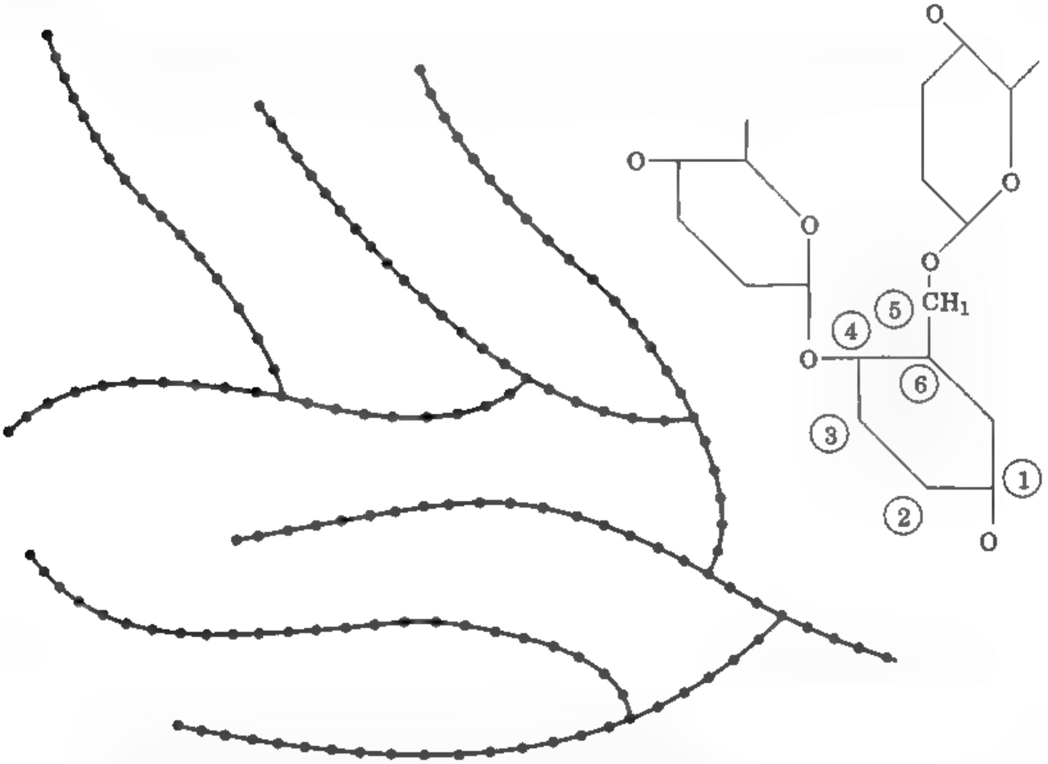


Figure 7. Structure of a small section of the branched starch fraction. Branching is effected through an α-1,6 linkage, as shown in the inset.

palate-cleansing bitterness that provides beer with its unusual property of drinkability. The isohumulones in beer can be readily analyzed, permitting a quantitative measure of their presence and thus of the beer's bitterness. Not many flavor compounds in other foods are so easily measured. A diagram of humulone and its conversion product isohumulone is shown in Figure 10. The analogues of humulone (and isohumulone)—cohumulone, adhumulone, and prehumulone—differ only in the number of carbon atoms in the side chains.

In addition, hops contain a volatile oil containing many odoriferous compounds, some of which survive into the finished beer. Some varieties of hops, such as Clusters and Galena, are used primarily for bitterness, whereas others, such as Hallertau and Cascade, are used for their aroma.

The essential oil of hops is, as in all plant materials, of terpenic origin. The major terpenes are myrcene (1), a diterpene; humulene (2), and caryophyllene (3) sesquiterpenes (Fig. 11). Oxidation products of these hydrocarbons, such as humaladienone and caryophyllene epoxide have

Figure 8. Hops growing in Germany. *Source:* Courtesy of S. S. Steiner.

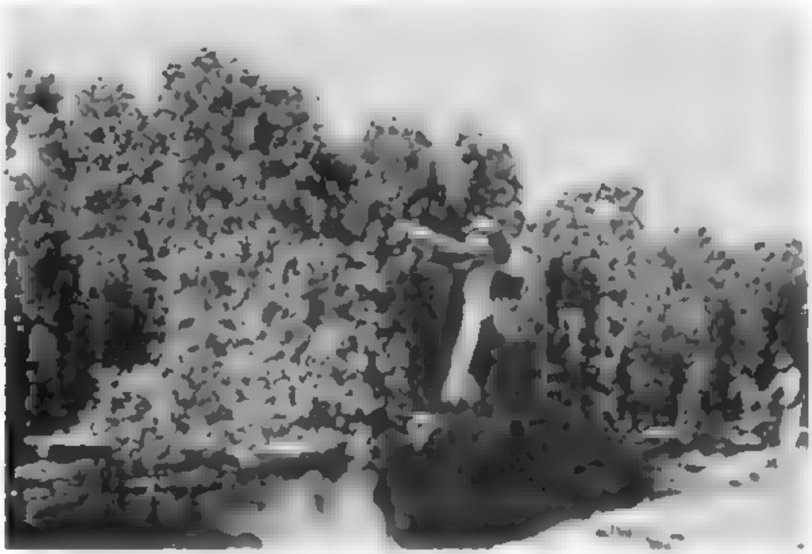


Figure 9. Hand picking of hops. *Source:* Courtesy of S. S. Steiner.

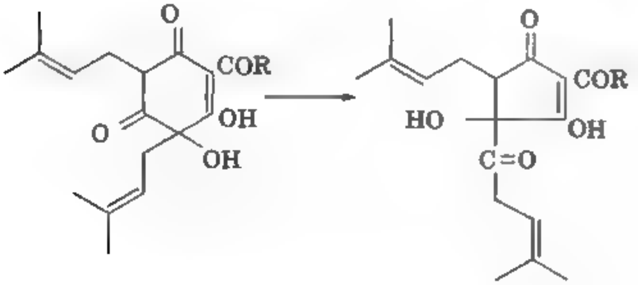


Figure 10. Humulone and isohumulone.

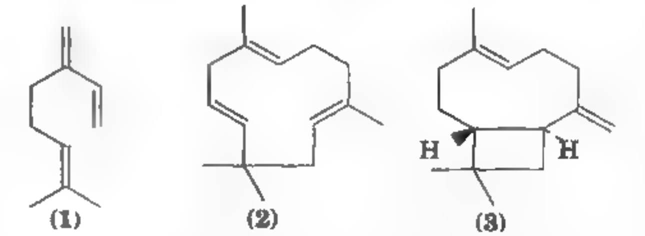


Figure 11. The terpenes (1) myrcene, (2) humulene, and (3) caryophyllene.

been found and are probably more important to the flavor of beer.

Also present in hop oil are alcohols, such as linalool and geraniol; ketones, such as undecanone-2; and esters, such as geranyl butyrate. These survive into beer and are important components of the hoppy aroma of some beers.

Hops is a perennial vine that grows to more than 20 ft in one season. They are trained to high wires held by long poles. The flowers are mechanically picked and dried in a kiln. Hops are used in several forms. The dried cones, compressed into bales weighing 200 lb, are used by many brewers. In this state, hops must be kept refrigerated. Alternatively, the hop cones can be milled to a powder and then recompressed into pellets. These are easier to use at a brewery, but also need refrigeration. Lastly, the hops may be extracted, with an organic solvent such as hexane or alcohol, or with liquid carbon dioxide, and the extract used. These do not need refrigeration and are popular in tropical countries. A cluster of Fuggle hops is shown in Figure 12. Some varieties of hops grown commercially are listed in Table 3, with the country producing the major quantities indicated.

A typical analysis of hops is shown in Table 4.

YEAST

Yeast is a unicellular microscopic organism that is fairly distinctive in being able to metabolize sugars either to carbon dioxide and water, in the presence of air; or to alcohol and carbon dioxide, in the absence of air. It is this latter trait that is used in all alcoholic fermentations.

One genus of yeast, Saccharomyces is used in brewing. But there are two species. One is *S. cerevisiae*, used in producing ales, and also for making bread, wine, and whiskey. It is a fairly hardy yeast and survives in the atmosphere.

The other is *S. carlsbergensis* (uvarum) used only for lager beers and found no where else but in breweries. It is temperature-sensitive and does not survive in the atmosphere.

Yeast reproduces asexually and multiplies severalfold during a normal fermentation. Because yeast enters a commercially sterile liquid, wort, it may be reused many times in successive brews without danger of contamination. Beer is the only fermented product that starts with a sterile medium. A photograph through an electron microscope of a dividing yeast cell is shown in Figure 13.

The two species of yeast differ in many biochemical characteristics. Table 5 gives some of these differences.

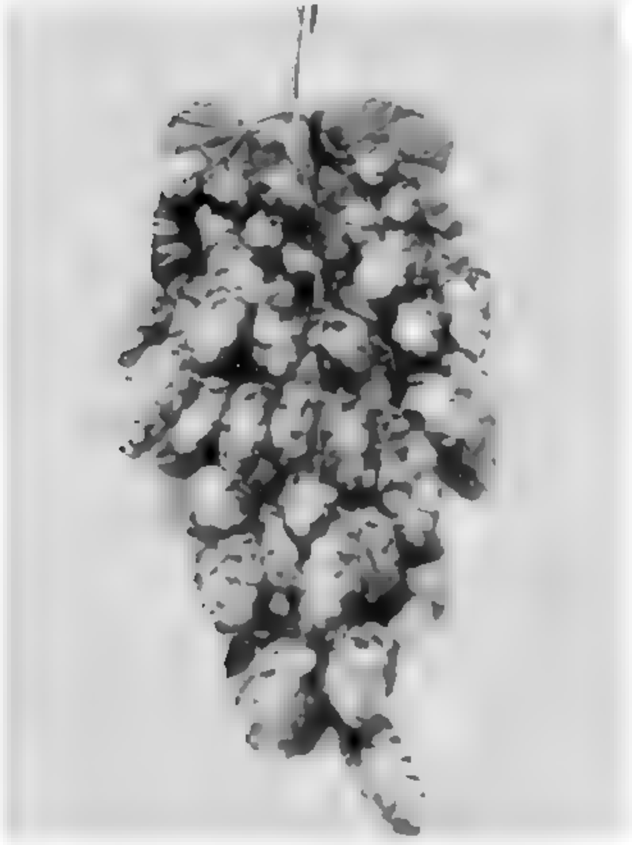


Figure 12. A cluster of Fuggle hops. *Source:* Courtesy of S. S. Steiner.

Table 3. Hop varieties (In Order of Acreage Devoted to Each Variety)

United States	Europe	Australia
Washington	Perle (G)	Pride of Ringwood
Galena	Hallertau Magnum (G)	
Nugget	Hershrucker (G)	
Columbus	Hallertau Tradition (G)	
Willamette	Spalter Select (G)	
Cluster	Hallertauer (G)	
Chinook	Nugget (G)	
Cascade	Tettnanger (G)	
Mt. Hood	Northern Brewer (UK & G)	
Perle	Fuggle (UK)	
Tettnanger	Golding (UK & SU)	
Horizon	Brewers Gold (UK & G)	
Olympic	Styrian (Y & SU)	
Golding	Saaz (Cz)	
	Hallertau Tarus (G)	
Idaho	Spalter (G)	
	Target (G)	
Galena	Huller (G)	
Cluster	Orion (G)	
Chinook	Record (G)	
Willamette	Bullion (G)	
Nugget		
Mt. Hood		
Oregon		
Willamette		
Nugget		
Perle		
Golding		
Mt. Hood		
Fuggle		
Tettnanger		

Note: G, Germany; Y, Yugoslavia; SU, Soviet Union; Cz, Czechoslovakia; and UK, United Kingdom.

Table 4. Chemical Composition of HopS

Moisture	10%
Total resins	17–20%
Volatile oils	0.3–1.2%
Polyphenols	2–5%
Waxes and lipids	3%
Ash	7%
Cellulose	55%

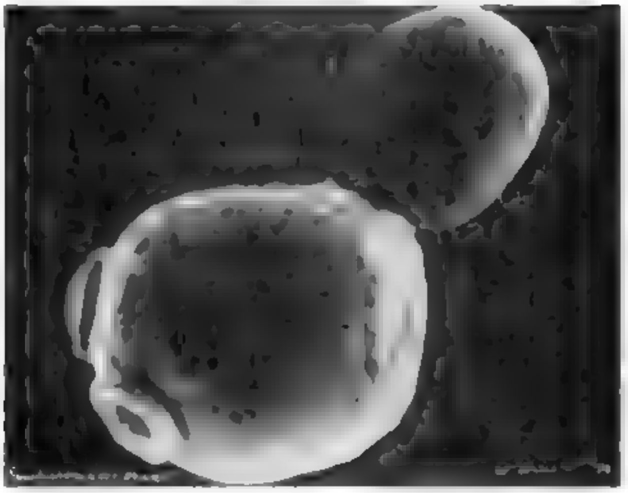


Figure 13. Dividing yeast cell.

Table 5. Biochemical Characteristaics of Yeast Used in Beer Making

	S. carlsbergensis	*S. cerevisiae*
Ferment melibiose	+	−
Ferment raffinose	+ + +	+
Utilize ethanol aerobically	−	+
Produce hydrogen sulfide	+ + +	+
Attitude after fermentation	Settles to bottom	Rises to top

Most breweries have their own strains of yeast and continue to use them indefinitely. Very often a pure yeast culture is maintained and propagated in each plant, or, more often, in the parent plant if the brewery has more than one plant.

THE BREWING PROCESS

The Brewhouse

Milling. Grains are normally received in breweries in bulk and transferred to silos. Malt needs to be crushed before it is used. This is done just before use. The aim in milling is to crush the endosperm of the barley, but leave the husk as intact as possible. These husks act as the filtration medium during later processing. A six-roller malt mill is shown in Figure 14.

Corn as received in a brewery does not need any treatment before use. It has already been crushed by a corn miller and separated pneumatically. The lighter germ of the corn is conveyed in a different airstream from the heavier, starchy endosperm. It is this latter fraction that is used by breweries.

Rice needs merely to be crushed before use.

Mashing. This step determines the ultimate structure of the finished beer. A typical mashing cycle requires the use of four vessels, a cooker, mash tub (tun in Europe), lauter tub, and kettle. Examples of some brewhouse control panels are shown in Figure 15.

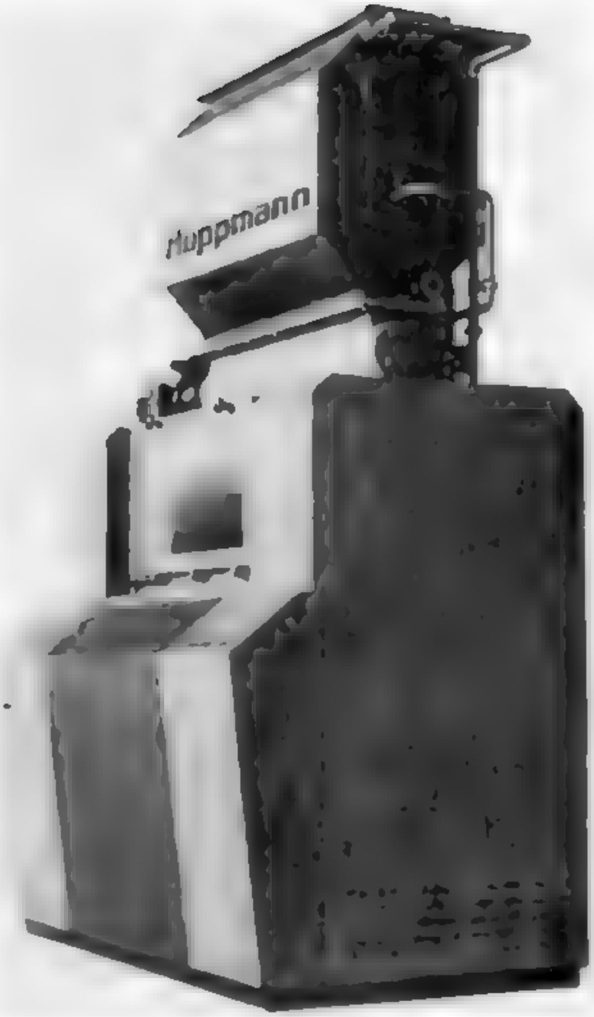


Figure 14. Six-roller malt mill. *Source:* Courtesy of Wittemann Hasselberg.

The cooker is a simple vessel, with an agitator and some means of being heated. The lauter tub is a complex vessel with a specially perforated false bottom through which the mash is strained or filtered. It has variable speed rakes, with adjustable flights that can be raised and lowered. The husks of the malt serve as the filtration medium in the lauter tub. A cut-out diagram of a lauter tub is shown in Figure 16. The kettle is also complex. It is heated internally with steam passing through coils and a percolator, or directly with a flame; or externally in a steam boiler. Steam-heated kettles are shown in Figure 17

There are several systems of mashing, each with certain advantages and each setting the requirements for the brewhouse equipment. The most common system in the United States and most other countries is a double-mash system. The corn or rice is boiled in the cooker, with a small amount of malt to lower the viscosity. The main portion of malt is admitted to the mash tub, and, after a stand at about 50°C for proteolysis, the temperature is raised by

Figure 15. Brewhouse control panels. *Source:* Courtesy of Wittemann Hasselberg.

adding the contents of the boiling cooker. The combined mash is allowed to rest at the temperature that results from this mixing for a variable time, and then the temperature is raised selectively until the final mashing off temperature, usually 75°C, is reached.

In the United Kingdom and its brewing satellites the most common mashing system is a simple infusion method. In this system, the mashing process takes place at a single temperature. This system developed with mash tubs that could not be heated. It is not nearly as versatile as the double-mash system. Mashing, with or without corn or rice, boiled separately in a small vessel, takes place at about 65°C.

In Germany, and its brewing followers, still a third system, called decoction brewing, is common. Here too the mash tub cannot be heated, but a smaller decoction vessel can. The malt is transferred into the mash tub at a low temperature. A small portion of this mash is pumped to the decoction vessel and boiled. The boiled mash is pumped back into the main mash vessel. This serves to raise its temperature to a level that depends on the relative volumes in both vessels. This transfer is repeated once or twice (double decoction or triple decoction), leading to a series of rising temperatures in the mash tub. This process is rather lengthy and has not found wide use.

RMDG
Lauter
Tuns

Figure 16. Cut-out diagram of lauter tub. *Source:* Courtesy of Robert Morton.

The reactions that occur in the mash tub are very complex. At a low temperature, about 50°C, proteolytic and cytolytic enzymes in the malt are operative. The corn or rice are not present at this stage and don't need to be. Proteolysis is necessary to supply amino acids for the growth of yeast and also to break down large, insoluble proteins to simple soluble peptides for foam enhancement. Cytolysis is required to reduce the viscosity of the barley gums (soluble fiber) to accelerate the later filtration. After some 10 to 60 min, the temperature is raised by the addition of the boiled cooker (or decoction vessel). The resulting temperature may be held for 10–60 min, and the temperature raised again, finally ending at 75°C. During these elevated temperatures, two amylolytic enzymes, α-amylase and β-amylase, cooperate to hydrolyze most of the starch to fermentable sugars. Beta-amylase, which operates best at about 63°C, attacks starch from its nonreducing end and splits off the disaccharide, maltose. It cannot proceed past a 1,6′ linkage, and so depends on the concurrent action of α-amylase. Alpha-amylase, which operates best at 70°C, splits any 1,4′ linkage at random, and so provides a supply of nonreducing ends for the β-amylase to operate on. Cooperatively, the action of these two enzymes can result in the splitting of about 65–75% of the starch into mono-, di-, and trisaccharides.

The mash temperature is raised to 75–77°C when the desired action of the amylolytic enzymes has been achieved. This arrests any further enzymatic action and prepares the mash for the next step, lautering.

Lautering (Straining or Filtration). The converted mash is transferred to a vessel that will permit separation of the liquid (wort) from the insoluble solids (husk, fiber) of the malt and grains. Three different types of vessels accomplish this separation. The one most widely used is a lauter tub, a large cylindrical vessel with a slotted false bottom and a set of movable and adjustable flights. The mash is pumped into this tub, allowed to settle for a few minutes, and then liquid is allowed to flow to the next stage, the kettle. The first runnings are always cloudy and are run back onto the mash. When it runs clear, it is run to the kettle. After the clear liquid, called first wort, stops run-

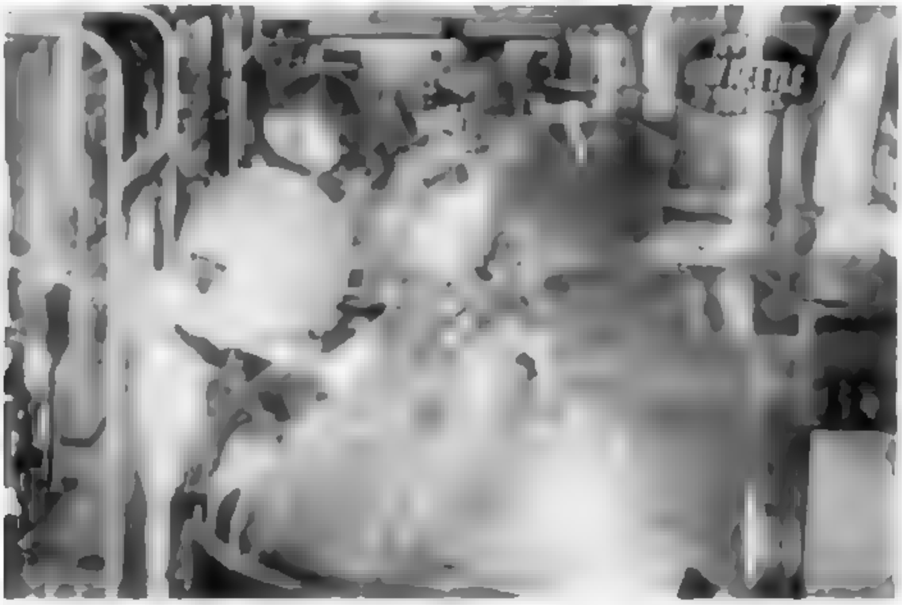


Figure 17. A row of brew kettles. *Source:* Courtesy of Miller Brewing Co.

ning, fresh water (sparge water) is sprayed onto the grain bed to elute the wort adhering to the grains.

Sparging continues until the next vessel is full, the concentration of the wort has reached the desired value, or the solids in the effluent are too dilute to be worthwhile. In some breweries, these last runnings are recovered and used for a succeeding brew. At the end of sparging, the rakes are lowered, their angle changed, and the spent grains are pushed into now-open large holes at the bottom of the lauter tub. These grains have a ready market as a valuable animal feed.

Another device used to separate wort from grain is a mash filter, a plate-and-frame press with plastic cloth as the barrier. These are faster than lauter tubs, but lack flexibility to grain depth. They have found some use in the United States, but wider use outside that country.

A third device, patented by Anheuser-Busch, is a Strainmaster, a modified lauter tub with perforated tubes projecting into the grain bed that serve to collect the wort. It has fallen out of favor and is being replaced by the lauter tub. This also is more rapid than a lauter tub. They are used almost exclusively in the United States.

The Kettle. The clear wort running from any of the grain-separation vessels is collected in a large vessel, the kettle, and boiled. Heat is normally supplied by steam in a set of coils and a center-mounted percolator. Alternatively, the vessel may be heated by direct fire or by an external collandria heated by steam.

During the heating period hops are added, in one or several portions, and at varying times. The varieties, the quantity, and the duration of their boiling time, all affect the flavor of the finished beer.

The boiling of the wort serves many vital functions:

1. It extracts the resin from the hops.
2. It isomerizes the humulones to soluble isohumulones.
3. It volatilizes most, but not all of the volatile oils in the hops.
4. It stops all enzymatic action.
5. It sterilizes the wort.
6. It concentrates the wort.
7. It removes grainy odors from the malt and other cereals.
8. It darkens the wort and produces flavor-inducing melanoidin reactions between the amino acids and simple sugars.

At the end of the boil, the wort is passed through a strainer to remove hop leaves, if whole hops were used, or directly to a tank in which the wort is allowed to settle. Recently, tangential entrance to this tank was found to produce a rapid settling of the precipitate that always forms in the kettle. This precipitate, called trub, contains hop bitter resins, proteins, and tannins and needs to be eliminated from the beer.

After settling some 20–30 min the wort is passed through a heat exchanger and cooled to the temperature desired for fermentation. Immediately after cooling, the wort is aerated to provide oxygen for the yeast.

Yeast, from a preceding brew, is added (pitched) in measured amounts to the aerated wort. The usual quantity is 1 lb of liquid yeast per barrel of wort. This will give a count of about 12 million yeast cells per milliliter. The second stage of the brewing process now begins.

Fermentation

Yeast enters a cool solution containing oxygen, fermentable sugars, and various nutrients. The yeast quickly absorbs the oxygen as well as minor nutrients that it requires, such as phosphate, potassium, magnesium, and zinc. It then begins to metabolize sugars and amino acids. The glycolytic pathway that yeast uses is common to many higher organisms, and only the ability to split pyruvic acid into acetaldehyde and carbon dioxide makes yeast distinctive. The scheme for the fermentation of glucose is shown in Figure 18.

The temperature during fermentation is very rigidly controlled. Because fermentation itself is exothermic, the fermentation vessels in all beer fermentations are cooled by a refrigerated fluid circulating through coils in the tanks or through jackets surrounding the tanks. The process usually takes 5–9 d.

During this time the yeast also metabolizes amino acids and makes three to four times as much yeast as was pitched. The excess yeast, over that used for succeeding brews, is collected and sold for use in dietary supplements and flavors for soups and snack foods.

The metabolism of amino acids is orderly and proceeds in a specific sequence. Certain amino acids are metabolized first, followed by the others, as shown in Table 6.

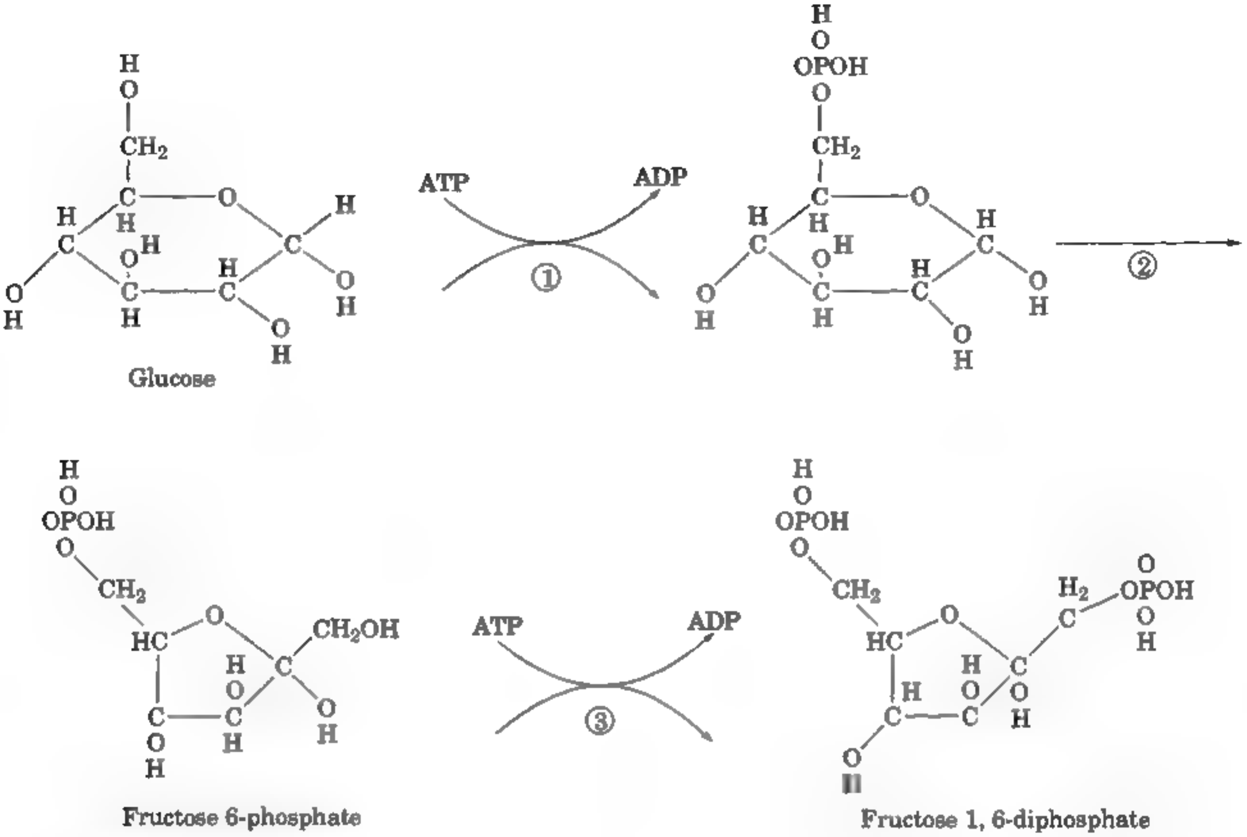
S. carlsbergensis has a marked ability to flocculate when it completes fermentation. This tendency allows the yeast to settle quickly when fermentable sugars are exhausted and makes collection and reuse of the yeast fairly simple. Some brewers hasten this settling by using centrifuges to collect their yeast.

S. cerevisiae, on the other hand, first rises to the top of its fermenting tank, where it may be collected by skimming, but then settles to the bottom and may also be collected there. Certain strains of *S. cerevisiae* settle more quickly and have been chosen by many brewers of ales and stouts.

Finishing

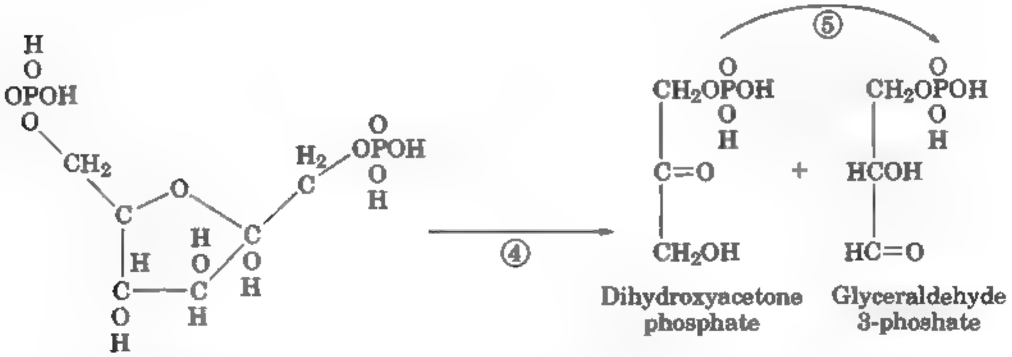
The fermented beer may now be finished in one of several ways. The simplest and most widely used is merely to transfer the beer to another tank, chilling it en route. This stage, called ruh (rest), allows much of the still suspended yeast to settle and also removes some harsh, sulfury notes. Furthermore, the yeast removes some undesirable flavor compounds, notably diacetyl, which were produced during the earlier fermentation. Ruh normally takes 7–14 d.

An alternative way of finishing beer is to move it to another tank sometime before it is completely fermented. Again, it is chilled en route, and the so-called secondary fermentation is allowed to proceed at a much lower tem-


Glucose
ATP
ADP
①
②
ATP
ADP
③
Fructose 6-phosphate
Fructose 1, 6-diphosphate

(1) Needs hexokinase, ATP, Mg^{2+} (or Mn^{2+})
(2) Needs glucosephosphate isomerase
(3) Needs phosphofructokinase—rate limiting

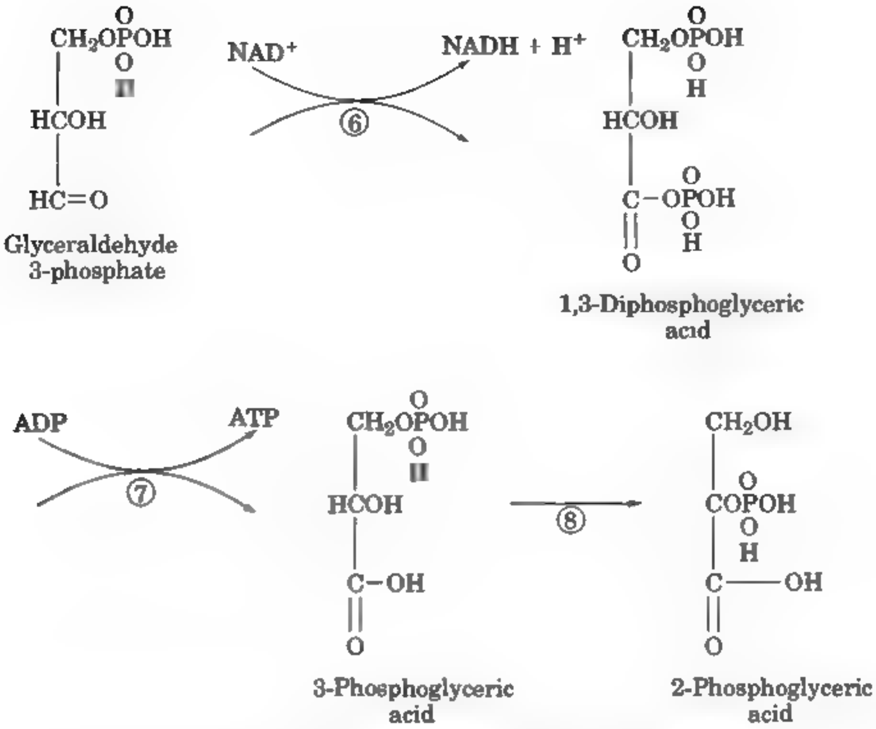
(a) Phosphorylation


⑤
④
Dihydroxyacetone phosphate
Glyceraldehyde 3-phoshate

(4) Needs aldolase, Zn^{2+}, is a reverse aldol condensation
(5) Needs triose isomerase

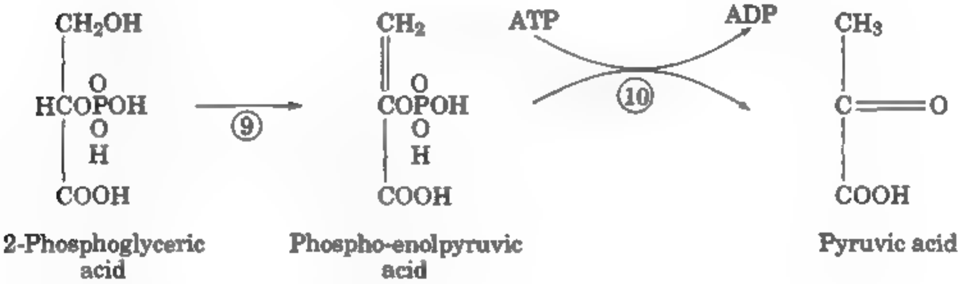
(b) Splitting of hexose

Figure 18. Chemistry of alcoholic fermentation.


NAD⁺
NADH + H⁺
⑥
Glyceraldehyde 3-phosphate
1,3-Diphosphoglyceric acid
ADP
ATP
⑦
⑧
3-Phosphoglyceric acid
2-Phosphoglyceric acid

(6) Needs glyceraldehyde phosphate dehydrogenase and NAD^+
(7) Needs phosphoglyceric kinase
(8) Needs phosphoglyceromutase

(c) Oxidation without oxygen


⑨
ATP
ADP
⑩
2-Phosphoglyceric acid
Phospho-enolpyruvic acid
Pyruvic acid

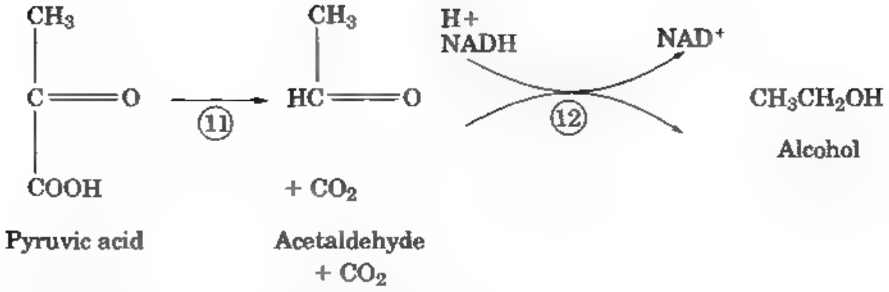
(9) Needs enolase and Mg^{2+}
(10) Needs pyruvate kinase

(d) Formation of pyruvic acid

Figure 18. Chemistry of alcoholic fermentation (*continued*).

perature, 0–3°C. Much the same cleansing action occurs, but the early transfer ensures the presence of sufficient yeast to cleanse the beer more efficiently. It takes 2–4 w. It has not found wide favor, probably because of the need to transfer the beer at times that could be inconvenient.

A third, and most elegant way, is to transfer the fermented beer to another tank, chilling it less, and treating it with a small quantity of beer that just started to ferment a day earlier. This subjects the fermented beer not just to a specific quantity of yeast, but to very active yeast that very effectively cleanses the fermented beer. This process, called krauesening, is used by very fastidious brewers. It normally takes 3–5 w.



(11) Needs carboxylase, Mg^{2+} and thiamin
(12) Needs alcohol dehydrogenase and Zn^{2+}

(e) Production of alcohol and carbon dioxide

Figure 18. Chemistry of alcoholic fermentation (*continued*).

Table 6. Metabolism of Amino Acids

Extent of absorption	Acid
Group A	
Almost completely absorbed within 24 hours	Glutamic acid Glutamine Aspartic acid Asparagine Serine Threonine Lysine Arginine
Group B	
Absorbed gradually during fermentation	Valine Methionine Leucine Isoleucine Histidine
Group C	
Absorbed only after Group A amino acids are gone	Glycine Phenylalanine Tyrosine Tyrptophane Alanine Ammonia
Group D	
Absorbed only slightly	Proline

After any of these finishing processes, the beer is filtered, always in the cold, to remove insoluble particles and yeast. This filtration uses diatomaceous earth as the filter medium. The beer may be filtered again, and this filtration may be a sterile filtration to remove all yeast and lactobacilli or another diatomaceous earth filtration. A sterile filtration may involve cotton fibers, a porous plastic sheet, or a ceramic filter as the retaining barrier.

Beer Packaging

Beer is packaged in either cans or bottles or in large containers or kegs for use in restaurants and taverns. The packaging machinery for beer has become very sophisticated and fast. Because oxygen changes the flavor of beer (see later), modern fillers evacuate the bottle before it is filled and replace the air with CO_2. Then the beer enters against a counter pressure of CO_2. Bottles are being filled at more than 20/s, and cans at more than 30/s. A high-speed can filler is shown Figure 19.

If such packaged beer has not been sterile-filtered, it must be pasteurized, as beer is a fertile medium for many microbes. The pasteurization may be done just before filling, a so-called bulk pasteurization, or after filling in long tunnels with hot-water sprays. Bulk pasteurization takes about a minute, tunnel pasteurization about an hour.

Kegs may be filled with diatomaceous earth-filtered beer, in which case they are kept refrigerated; or they may be filled with sterile filtered or bulk pasteurized beer, in which case they do not need refrigeration.

Problems of Beer

The complexity of beer makes it prone to several problems. First and foremost is that the delicate flavor of beer is adversely affected by oxygen, so great pains are taken in the brewery and in the packaging operation to minimize the pick up of oxygen. When yeast is present, it will scavenge oxygen and keep the beer oxygen-free. But after filtration beer is very prone to pick up oxygen. At every transfer, steps are taken to avoid oxygen pickup, which is monitored closely. The filling operation has been mentioned earlier.

Correctly packaged beer has a flavor shelf life of about 4 m: if kept cold, it will last longer. The change in flavor with oxidation is difficult to describe because it is unique to beer. It has been described as papery, bready, or cardboard. But mainly it loses the fine appeal of fresh beer.

Another problem of beer is that it develops a peculiar, skunky aroma if packaged in clear or green bottles and is exposed to light. The problem has been eliminated with the

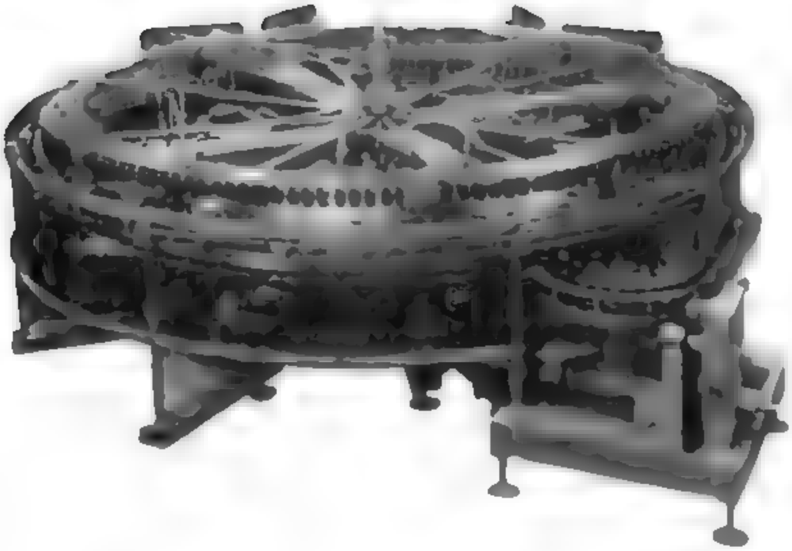


Figure 19. High-speed can filler. *Source:* Courtesy of H&R Inc.

discovery that brown bottles filter the offending wavelengths of light. The aroma is caused by the action of light on isohumulone, which splits off an unsaturated hydrocarbon, which in turn reacts with a trace of hydrogen sulfide in the beer to give a mercaptan.

A third problem is that beer is a good medium for some nonpathogenic microbes and so must be sterile-filtered or pasteurized. Either of these treatments effectively eliminate the problem.

Another shortcoming that has largely been eliminated is the tendency for beer to become cloudy when it is chilled. This haze is caused by a reversibly insoluble protein-polyphenol complex. It is removed in one of several ways. The protein may be solubilized by the enzyme papain, or the polyphenols may be removed by absorption onto silica gel. These treatments are effective and widely used.

Light Beer

The enzymes present in barley malt cannot completely degrade starch to fermentable sugars. So all regular beers contain dextrins, fragments of starch held by 1,6′ linkages and too large to be fermented by yeast. There is an enzyme, however, that can break up all the starch—amyloglucosidase.

A true light beer is one in which all the starch has been made fermentable. This is accomplished by the use of amyloglucosidase, either in the brewhouse to work with the α-amylase and β-amylase or during fermentation to work after the α-amylase and β-amylase.

Some light beers are made by merely extending the action of the α-amylase and β-amylase. This produces a beer reduced in dextrins but not free of them. The legal definition of a light beer in this country permits this.

Analysis of Beer

Typical analysis of some American beers are shown in Table 7.

Table 7. Analysis of American Beers

	Standard beer	All malt beer	Light beer
Water	92	91	6
Alcohol, wt%	3.8	3.9	3.3
Carbohydrates, %	3.4	3.8	<1.0
Protein, %	0.3	0.6	0.2
Acids, as lactic, %	0.1	0.1	0.1
Ash, %	0.1	0.2	0.1
Isohumulones, ppm	15	25	15

Table 8. Vitamins in Beer

Vitamin	Vitamin content of beer (mg/L)	% Minimum daily requirements supplied by 1 L
Thiamin	0.02	<2
Riboflavin	0.3	20%
Pantotenic acid	1.0	25%
Pyridoxine	0.5	20%
Biotin	0.007	7%
Cyanocobalamin	0.1	3%

Types of Beer

Beer may be classified in many ways:

- By type yeast
  1. Lager yeast, S. carlsbergensis—By color: Light to amber color—lager beer; Dark color—porter; Dark and strong—bock beer. Or by process: Ruh beer; Krauesened beer.
  2. Ale yeast, S. cerevisiae—By color: Light to amber color—ale; Dark colored—stout.
- By properties of the water
  1. Alkaline, hard water—Munich type

    2. Neutral, hard water—Pilsen type (All beers made now are Pilsen type)
- By alcohol content
    1. No-alcohol beer
    2. Low-alcohol beer (shank beer)
    3. Beer, ale
    4. Malt liquor
    5. Barley wine
- By market category
    1. Super premium
    2. Premium
    3. Standard, regional
    4. Generic

Dietary Aspects

Beer, being made from grains, has considerable nutritional value. Table 8 shows the vitamin content of a typical beer.

Beer also contains the trace minerals zinc, copper, and manganese. It is also very low in sodium, 12–18 mg sodium per liter, and high in potassium, 110–125 mg potassium per liter. This ratio of sodium to potassium has made for the inclusion of beer in the diets of patients with fluid retention problems. The well-known diuretic effect of beer is also a factor in many diets.

PLANT OPERATIONS

Breweries are operated most efficiently on round-the-clock schedules. The limiting factor in production is almost always capacity of the brewhouse, mainly the lauter tub and kettle. Some breweries will have one malt mill, one cereal cooker, one mash tub, two lauter tubs, and two kettles. With a mash filter or Strainmaster, only one may be required. Large breweries will have more than one brewing line. The maximum volume of beer that can be produced from the largest practical kettle (31,000 gal) is about 3–4 million barrels (90–120,000,000 gal). Any plant making more than this has more than one brewing line.

Round-the-clock brewhouse operation also demands 24-h manning in the fermentation cellars. Although fermenting and aging tanks are always above street level, the rooms they are in are referred to as cellars, from the days when the absence of mechanical refrigeration made underground storage necessary to keep the fermenting beer cool.

The speed of packaging equipment allows the so-called bottleshop section of a brewery to run on less than round-the-clock operation, even if the rest of the brewery is on a 24-h schedule.

The many choices involved in the brewing process, and the actual day-to-day running of the brewery (but not the bottleshop) are under the domain of the brewmaster. All else in a brewery, including the bottleshop, are the domain of the plant manager.

QUALITY CONTROL

Quality are those attributes of a product that makes it desirable to a consumer. In the production of beer there are many opportunities for undesirable properties—in aroma, in taste, and in appearance—to develop. Brewers have learned to be very careful about their products.

Quality control begins with the raw materials used in brewing. The water used is monitored for taste, alkalinity, hardness, and trace metals.

Barley malt is controlled very closely. The variety of barley is always specified, and sometimes the region in which it is grown. The malting process is controlled indirectly, by the analysis of the finished malt. The kilning cycle is specified, and the temperatures on the kiln are controlled.

Analyses exist for malt that reveal the size and vitality of the barley used, the extent to which the barley is germinated, how well it was modified or malted, and how well it will perform in the brewery. A typical analysis of a two-row and six-row malt is shown in Table 9.

The assortment indicates the size and grade of the barley used to make this malt. Uniformity in size is as important as absolute values. The growth shows how many dead kernels there were in the barley and how extensively and how uniformly the barley germinated.

The extract values, which give the amount of soluble matter in the malt, is an important economic factor. The difference in extract between a coarsely ground and a finely ground malt indicates how well the barley was modified; the smaller the difference the better the modification.

The diastatic power and α-amylase value measure the β-amylase and α-amylase, respectively. The ratio of soluble protein over total protein indicates how well the barley grew during malting and can predict yeast behavior in the brewery.

Hops are specified by variety and country or state in which they are grown. Samples from lots that could be purchased are examined visually by the brewer. Acceptance

Table 9. Analyses of Two-Row and Six-Row Malts

	Two-row malt	Six-row malt
Assortment		
On 7/64 in., %	61.4	47.5
6/64	30.7	43.3
5/64	7.0	9.2
Through 5/64	0.9	1.0
Growth		
0–1/4	0	0
1/4–1/2	1	2
1/2–3/4	5	6
3/4–1	84	83
Over 1	10	9
Moisture, %	4	4
Extract, fine grind, as is, %	77.3	75.2
Extract, coarse grind, as is, %	76.0	73.8
Diastatic power	133	142
α-Amylase	51	55
Total protein, %	13.7	13.8
Soluble protein, %	5.8	5.6

depends on aroma and appearance. Analysis for humulones accompanies the sample and may be a criterion also.

Corn and rice are judged by extract values and low oil content. In the brewery, control begins with the wort produced in the kettle. This is judged for solids content, color, bitterness, pH, degree of fermentability, and sterility.

The fermentation process is monitored for rate of fermentation (decrease in specific gravity) and temperature and biologically for the presence of lactobacilli. The aging process is checked for gravity and microbiologic state.

After filtration, the oxygen content is monitored, as is the carbon dioxide level. Just before packaging, and possibly again in the package, the beer is analyzed completely to maintain standards and uniformity.

Tasting is an integral part of quality control. In many breweries beer is tasted just before it is transferred from one tank to another. It is again tasted just before packaging, and again, critically, in the package. Taste panels are selected from brewery workers and are trained to detect certain flavors in beer and to describe them. These panels operate daily, or twice daily, with rotating membership. A view of a taste panel is shown in Figure 20.

WORLD BEER PRODUCTION

Beer is made in almost every country in the world. It probably ranks next to bread in the ubiquity of its production. Table 10 lists the production, in 1,000 hectoliters, in these countries.

GLOSSARY

Apparent extract. The density, usually measured with a hydrometer or a densitometer of a fermenting or fermented liquid. In beer it measures the extent to which the wort has fermented. The value reflects the residual solids in the beer, modified by the alcohol present (which lowers the reading).

Figure 20. A view of a taste panel. *Source:* Courtesy of Miller Brewing Co.

Table 10. World Beer Production in 1997 (in 1,000 hL)

The Americas	
United States	236,430
Brazil	88,200
Mexico	51,949
Canada	22,355
Colombia	20,000
Venezuela	17,232
Argentina	12,063
Peru	8,500
Chile	3,900
Dominican Republic	2,400
Ecuador	2,270
Bolivia	1,800
Paraguay	1,600
Cuba	1,500
Panama	1,350
Costa Rica	1,200
Honduras	1,184
Guatemala	1,000
Uruguay	800
El Salvador	700
Jamaica	574
Nicaragua	400
Trinidad	320
Puerto Rico	314
Guyana	180
Haiti	140
Bahamas	139
Netherlands Antilles	135
Barbados	112
Surinam	89
Belize	70
Martinique	70
Santa Lucia	49
St. Vincent	35
Grenada	34
Antigua	22
St. Kitts	18
Dominica	12
Total	479,146
Far East	
China	170,000
Japan	67,695
South Korea	16,740
Philippines	13,475
Thailand	8,360
Vietnam	5,638
India	4,250
Taiwan	3,900
Indonesian	1,722
Malaysia	1,477
Hong Kong	890
Singapore	804
Nepal	350
Laos	286
Sri Lanka	266
Cambodia	175
Lebanon	126
Mongolia	100
Burma	60
Pakistan	20
Total	296,334

Table 10. World Beer Production in 1997 (in 1,000 hL) *(continued)*

Near East	
Israel	800
Syria	102
Jordan	56
Iraq	50
Total	1,008
South Pacific	
Australia	17,349
New Zealand	3,214
Papua-New Guinea	390
Figi	161
Tahiti	146
New Caledonia	125
Samoa	45
Solomon Islands	19
Total	22,457
Europe	
Germany	114,800
UK	58,139
USSR	25,249
Spain	24,879
Netherlands	24,701
France	19,483
Poland	18,800
Czech Republic	18,649
Belgium	14,168
Italy	11,455
Austria	9,366
Denmark	9,180
Ireland	8,152
Romania	7,506
Turkey	7,448
Hungary	7,168
Portugal	6,623
Yugoslavia	6,106
Ukraine	6,090
Sweden	4,810
Finland	4,793
Slovak Republic	4,394
Greece	3,945
Croatia	3,607
Switzerland	3,563
Bulgaria	3,004
Norway	2,298
Slovenia	2,123
Lithuania	1,356
Kazakhstan	730
Latvia	687
Bosnia-Herzegovina	620
Macedonia	600
Estonia	585
Uzbekistan	500
Luxembourg	481
Georgia	450
Cyprus	320
Albania	152
Malta	132
Armenia	52
Iceland	51
Azerbaijan	15
Total	437,230
Africa	
South Africa	25,000
Nigeria	4,300
Kenya	3,000
Cameroon	3,253
Zimbabwe	1,649
Tanzania	1,615
Zaire	1,525
Ivory Coast	1,240
Burundi	1,161
Angola	980
Ethiopia	856
Namibia	888
Uganda	830
Rwanda	808
Gabon	801
Marocco	800
Tunisia	780
Guinea	773
Malawi	760
Mozambique	698
Zanbua	558
Upper Volta	458
Botswana	419
Egypt	400
Lesotho	398
Benin	358
Algeria	350
Madagascar	350
Congo	347
Mauritius	347
Swaziland	294
Togo	292
Reunion	239
Eritrea	218
Central African Republic	207
Senegal	162
Chad	157
Guinea	131
Sierra Leone	100
Niger	72
Seychelles	70
Mali	65
Liberia	60
Cape Verde	54
Gambia	20
Total	57,868

Barley malt. A legally required ingredient in beer; it is barley that has been wetted and induced to germinate and then heated to stop the germination. The germination partly digests the starch and protein in the barley and produces some enzymes. The temperature at which the terminal drying is done determines the color of the resulting beer. Barley may be either two-rowed or six-rowed and may be either blue or white. Malt provides carbohydrates, protein, and yeast nutrients to the brewing process.

Bitterness unit or isohumulones. A quantitative measure of the amount of isohumulones—the major bitter compound from hops—in beer, in milligrams per liter.

Corn grits, corn flakes, corn starch, corn syrup, rice, milo, and sorghum. Ingredients used as adjuncts to dilute the barley malt; they are only a source of carbohydrates.

Enzyme. A protein, made by a living cell or organism, that catalyzes or promotes a chemical reaction. Most of the processes of life, and most of those in brewing, are mediated by enzymes.

Hops. The flower of a perennial vine Humulus lupulus grown in the United States only in Washington, Oregon, Idaho, and California. Many varieties exist, with differing aromas and bittering qualities. The United States grows the varieties called Clusters, Northern Brewer, Fuggles, Bullion, and Cascades.

Krauesen. A stage after primary fermentation in which about 15% by volume of freshly fermenting beer is added to beer that has completed its fermentation. The term, as a noun, is also used to describe the foamy head that appears on top of a fermenting tank at the height of fermentation.

Original gravity. The concentration of solids in the wort from which beer is made. In the United States and most other countries, the concentration is expressed in percent solids, or °Plato or °Balling (after the workers who laboriously determined these values). Thus a wort with 12% solids would be said to have a gravity of 12°P (or 12°B), and the beer made from it would be said to have an original gravity of 12°P. An analysis of beer permits you to calculate the original gravity of the wort from which it was made, without reference to the wort. In the United Kingdom, the concentration of solids in the wort is expressed as the actual specific gravity. A wort with 12% solids would have a specific gravity of 1.048 and would be said to have a gravity of ten forty-eight.

Real extract. The actual percentage of solids in a beer, determined after removing the alcohol present.

Ruh. The period of rest between fermentation and filtration during which the beer clarifies itself and loses some harsh notes.

Wort. The liquid from which beer is made; a warm-water extract of malt and other grains.

Yeast. The microorganism that converts simple sugars to alcohol and carbon dioxide. Two types of yeast are used in brewing—Saccharomyces carlsbergensis for lager beers and S. cerevisiae for ales. Most brewers have their own strain of these yeasts. During a normal brewers fermentation, yeast multiplies three- to fourfold, yielding more than enough yeast for successive brews. Yeast also produces many compounds that have a marked influence on the flavor of the beer.

GENERAL REFERENCES

Briggs, D. E., J. S. Hough, R. Stevens, and T. W. Young, *Malting and Brewing Science*, Vol. 1, *Malt and Sweet Wort*; Vol. 2, *Hopped Wort and Beer*, Chapman and Hall, New York, 1981.

Broderick, H. M., *The Practical Beer*, Master Brewers Association of the Americas, Madison, Wis., 1977.

Broderick, H. M., *Beer Packaging*, Master Brewers Association of the Americas, Madison, Wis., 1982.

Burgess, A. H., *Hops*, Interscience Publishers, New York, 1964

European Brewery Convention, Fermentation and Storage Symposium, *E.B.C. Monograph V*, European Brewery Convention, Amsterdam, The Netherlands, 1978.

European Brewery Convention, Symposium on the Relationship Between Malt and Beer, Helsinki, *E.B.C. Monograph VI*, European Brewery Convention, Amsterdam, The Netherlands, 1980.

European Brewery Convention, Flavour Symposium, Copenhagen November 1981, *E.B.C. Monograph VII*, European Brewery Convention, Amsterdam, The Netherlands, 1981.

Findlay, W. P. K., *Modern Brewing Technology*, Macmillan, London, 1971

Pollock, J. R. A., *Brewing Science*, Vol 1, 1979; Vol. 2, 1981; Vol. 3, 1987, Academic Press, Orlando, Fla.

Preece, I. A., *The Biochemistry of Brewing*, Oliver & Boyd, London, 1954.

Rose, A. H., and J. S. Harrison, *The Yeasts*, Vol 2, Academic Press, Orlando Fla., 1987

Strausz, D A., *The Hop Industry of Eastern Europe and the Soviet Union*, Washington State University Press, Pullman, Wash., 1969.

JOSEPH L. OWADES
Consultant
Sonoma, California

BEVERAGES: CARBONATED

Carbonated beverages, or soft drinks, are the most popular beverages in North America and a growing category worldwide. Per capita consumption in the United States reached 54 gallons per year in 1997. Worldwide consumption is estimated at almost 7.5 gallons per year for every man, woman, and child alive. This has created an industry with estimated annual U.S. sales of over $54 billion, employing more than 136,000 workers, producing hundreds of different flavors.

Few products are as closely associated with American ingenuity, enterprise, and culture as soft drinks. The trademark of the market leader, the Coca-Cola Company, is the most recognized trademark in the world. That one company served an estimated 1 billion customers in 1997. Although a few large corporations dominate sales, there has always been a market for hundreds of small businesses offering unique alternatives to the mass-marketed brand names.

The emergence and growth of this category resulted from the ideas of individuals in the United States and Europe, who found better flavors, better marketing, or better ways of producing their beverages. Although descriptions of naturally carbonated mineral waters date back 2,000 years, the production and distribution of flavored soft drinks began little more than 200 years ago. The development of soft drinks follows (see "History of Carbonated Beverages"). What is a carbonated beverage and how is it different from other beverages?

DESCRIPTION

The favored term for carbonated beverages is now carbonated soft drink, or simply soft drink, to differentiate them

Corn grits, corn flakes, corn starch, corn syrup, rice, milo, and sorghum. Ingredients used as adjuncts to dilute the barley malt; they are only a source of carbohydrates.

Enzyme. A protein, made by a living cell or organism, that catalyzes or promotes a chemical reaction. Most of the processes of life, and most of those in brewing, are mediated by enzymes.

Hops. The flower of a perennial vine Humulus lupulus grown in the United States only in Washington, Oregon, Idaho, and California. Many varieties exist, with differing aromas and bittering qualities. The United States grows the varieties called Clusters, Northern Brewer, Fuggles, Bullion, and Cascades.

Krauesen. A stage after primary fermentation in which about 15% by volume of freshly fermenting beer is added to beer that has completed its fermentation. The term, as a noun, is also used to describe the foamy head that appears on top of a fermenting tank at the height of fermentation.

Original gravity. The concentration of solids in the wort from which beer is made. In the United States and most other countries, the concentration is expressed in percent solids, or °Plato or °Balling (after the workers who laboriously determined these values). Thus a wort with 12% solids would be said to have a gravity of 12°P (or 12°B), and the beer made from it would be said to have an original gravity of 12°P. An analysis of beer permits you to calculate the original gravity of the wort from which it was made, without reference to the wort. In the United Kingdom, the concentration of solids in the wort is expressed as the actual specific gravity. A wort with 12% solids would have a specific gravity of 1.048 and would be said to have a gravity of ten forty-eight.

Real extract. The actual percentage of solids in a beer, determined after removing the alcohol present.

Ruh. The period of rest between fermentation and filtration during which the beer clarifies itself and loses some harsh notes.

Wort. The liquid from which beer is made; a warm-water extract of malt and other grains.

Yeast. The microorganism that converts simple sugars to alcohol and carbon dioxide. Two types of yeast are used in brewing—Saccharomyces carlsbergensis for lager beers and S. cerevisiae for ales. Most brewers have their own strain of these yeasts. During a normal brewers fermentation, yeast multiplies three- to fourfold, yielding more than enough yeast for successive brews. Yeast also produces many compounds that have a marked influence on the flavor of the beer.

GENERAL REFERENCES

Briggs, D. E., J. S. Hough, R. Stevens, and T. W. Young, *Malting and Brewing Science*, Vol. 1, *Malt and Sweet Wort*; Vol. 2, *Hopped Wort and Beer*, Chapman and Hall, New York, 1981.

Broderick, H. M., *The Practical Beer*, Master Brewers Association of the Americas, Madison, Wis., 1977.

Broderick, H. M., *Beer Packaging*, Master Brewers Association of the Americas, Madison, Wis., 1982.

Burgess, A. H., *Hops*, Interscience Publishers, New York, 1964

European Brewery Convention, Fermentation and Storage Symposium, *E.B.C. Monograph V*, European Brewery Convention, Amsterdam, The Netherlands, 1978.

European Brewery Convention, Symposium on the Relationship Between Malt and Beer, Helsinki, *E.B.C. Monograph VI*, European Brewery Convention, Amsterdam, The Netherlands, 1980.

European Brewery Convention, Flavour Symposium, Copenhagen November 1981, *E.B.C. Monograph VII*, European Brewery Convention, Amsterdam, The Netherlands, 1981.

Findlay, W. P. K., *Modern Brewing Technology*, Macmillan, London, 1971

Pollock, J. R. A., *Brewing Science*, Vol 1, 1979; Vol. 2, 1981; Vol. 3, 1987, Academic Press, Orlando, Fla.

Preece, I. A., *The Biochemistry of Brewing*, Oliver & Boyd, London, 1954.

Rose, A. H., and J. S. Harrison, *The Yeasts*, Vol 2, Academic Press, Orlando Fla., 1987

Strausz, D A., *The Hop Industry of Eastern Europe and the Soviet Union*, Washington State University Press, Pullman, Wash., 1969.

JOSEPH L. OWADES
Consultant
Sonoma, California

BEVERAGES: CARBONATED

Carbonated beverages, or soft drinks, are the most popular beverages in North America and a growing category worldwide. Per capita consumption in the United States reached 54 gallons per year in 1997. Worldwide consumption is estimated at almost 7.5 gallons per year for every man, woman, and child alive. This has created an industry with estimated annual U.S. sales of over $54 billion, employing more than 136,000 workers, producing hundreds of different flavors.

Few products are as closely associated with American ingenuity, enterprise, and culture as soft drinks. The trademark of the market leader, the Coca-Cola Company, is the most recognized trademark in the world. That one company served an estimated 1 billion customers in 1997. Although a few large corporations dominate sales, there has always been a market for hundreds of small businesses offering unique alternatives to the mass-marketed brand names.

The emergence and growth of this category resulted from the ideas of individuals in the United States and Europe, who found better flavors, better marketing, or better ways of producing their beverages. Although descriptions of naturally carbonated mineral waters date back 2,000 years, the production and distribution of flavored soft drinks began little more than 200 years ago. The development of soft drinks follows (see "History of Carbonated Beverages"). What is a carbonated beverage and how is it different from other beverages?

DESCRIPTION

The favored term for carbonated beverages is now carbonated soft drink, or simply soft drink, to differentiate them from beverages containing alcohol. They are characterized by containing carbon dioxide (CO_2) gas, which effervesces (bubbles) when pressure is released, as when opening a bottle Soft drinks have some added flavor and usually a sweetener to distinguish them from seltzer water. The primary ingredient of course is water, comprising from 85 to 99% of the formula.

INGREDIENTS

The water used in soft drinks must be free of objectionable tastes, odors, or colors. It must be safe to drink and low in organic and mineral content. Most soft drink bottlers employ extensive water treatment to assure this primary ingredient is pure and suitable for their products. Although the water must meet federal drinking water standards, most bottlers go well beyond those specifications to assure stability in a complex beverage system that must be shelf stable for six months or more.

Carbon dioxide is delivered to the bottling plant as a liquid compressed under high pressure. As the gas is needed, the liquid CO_2 is metered through a regulator where pressure is decreased and the liquid is vaporized to a gas. The gas is then dissolved in water in direct proportion to the pressure and temperature, following the universal gas laws. The amount of CO_2 in the product is measured in gas volumes, where *one gas volume equals the volume of gas that will dissolve in water under standard conditions*. The treated water is chilled to absorb more CO_2 and carbonated by exposing it to a countercurrent flow of CO_2 gas under pressure. Different flavors specify different levels of carbonation, up to a practical maximum of 5.0 gas volumes.

Most soft drinks are sweetened with sugars, predominantly in recent years by a liquid corn sweetener known as high fructose corn syrup (HFCS). Corn syrup is refined from cornstarch, which is a polymer of the simple sugar dextrose (*d*-glucose). Starch is hydrolyzed to dextrose and then a portion of the dextrose is enzymatically converted to fructose to enhance sweetness. HFCS is transported across North America by rail tank car or by tank truck and delivered in bulk. Prior to the 1950s, granulated sugar (sucrose) was most common, delivered in bags or bulk, and this sweetener still predominates in most areas of the world.

Sugar, HFCS, and honey are nutritive sweeteners, meaning they supply some food energy or calories. A significant portion of the U.S. market, about 15%, prefers diet or sugar-free soft drinks made with nonnutritive sweeteners. These are typically intense sweeteners, hundreds of times sweeter than sugar, and are therefore used in small amounts measured in parts per million (ppm or μg/L).

Where sugars may constitute 10 to 12% by weight of the beverage, an intense sweetener makes up a fraction of 1%. Sugars also contribute to the mouth-feel of the beverage and help carry and smooth flavors across the tongue. This presents a challenge to the flavor chemist formulating sugar-free products. The balance of flavors found in a sugar-sweetened beverage is difficult to duplicate in the diet version. Examples of nonnutritive sweeteners include aspartame, saccharin, sucralose acesulfame potassium, and alitame. Table 1 lists several of the sweeteners used in the beverage industry with relative sweetness compared with sucrose, solubility at 20°C, and approximate usage levels in beverages.

Another important ingredient is an acidulent or acid, typically citric acid or phosphoric acid, which serves several purposes. The primary function is to modify the flavor by contributing a bite or tang sensation while balancing the sweetness in a sweet–sour relationship The acid also lowers the beverage pH (a measure of acidity or hydrogen ion concentration) increasing the solubility of some ingredients and helping to prevent spoilage. Carbon dioxide gas forms carbonic acid in solution and therefore is itself a minor acidulent. Other common acid ingredients include malic acid, found in apples and cherries; tartaric acid, primarily found in grapes; and acetic acid, found in vinegar.

The defining ingredient of soft drinks is the flavor system, which differentiates one product from another. Flavors can be simple or very complex combinations of ingredients blended together to achieve a desired balance. Some soft drink flavors have hundreds of ingredients, some of which may be added just to prevent analysis and unauthorized duplication. The secrecy of such formulas is legendary. Fruit juices or juice extractives are common in fruit flavors. Extracts of spices, nuts, and herbs characterize colas, ginger ales, and root beers.

Flavors are categorized by law as "natural" or "artificial" depending on their source and means of manufacture. The U.S. Code of Federal Regulations (21 CFR 101.22) states:

> Natural flavors include: essential oils, oleoresins, essence or extractive. . . . or product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf. . . . meat, seafood or dairy product . . . whose significant function is flavoring rather than nutritional.

Artificial flavors are not derived from this list of foods, but typically are synthesized chemicals that may or may not be identical to natural flavor constituents. A great amount of research has been done and is continuing to find new flavoring materials in nature and in the laboratory. All in-

Table 1. Beverage Sweeteners

Sweetener	Relative sweetness	20°C (%) solubility	Approx. (mg/L) usage level
Acesulfame potassium	200	27	500
Alitame	2,000	14	50
Aspartame	180	1	600
Crystalline fructose	1.5	80	90,000
Cyclamate	30	13	(Blend)
HFCS 42	0.9		111,000 (solids)
HFCS 55	1		100,000 (solids)
Saccharin	300		350
Sorbitol	0.6	230	(Blend)
Sucralose	600	28	180
Sucrose	1	60	100,000

gredients used in foods must be approved by the U.S. Food and Drug Administration (FDA). Most common soft drink flavoring materials are on the generally recognized as safe (GRAS) list.

It is well established that our perception and memory for flavor is primarily based on our sense of smell. Taste receptors on the tongue play a role but are not as sensitive as the olfactory receptors found in our nasal passages. This is why the volatile essences or "top notes" are so important to successful flavors.

Colors are also important ingredients in beverages. Consumer acceptance and even flavor perception is dependent on the appearance of the product. Until recently, the choices of stable color ingredients have been limited to about a dozen useful in soft drinks. As our scientific understanding of chemistry advances, more choices are becoming available, allowing a rainbow of colors to be offered. The regulation of colors can present some contradictions: the natural color component of red cranberries can be used in a cranberry drink and labeled "natural"; however, the same natural color ingredient used in cherry flavor must be labeled "artificial color."

Preservatives are found in most soft drinks to prevent spoilage, protect flavors, and extend shelf life. Soft drinks are generally packaged cold to maintain carbonation, and they cannot be heat processed to eliminate spoilage yeast and mold spores. Although conditions in modern bottling plants are sanitary, the total elimination of microorganisms is nearly impossible. Agents that prevent microbial growth in soft drinks include CO_2, acids, and some flavoring materials. Beyond these, many formulas include a preservative to prevent spoilage and assure product safety. The most common are sodium or potassium benzoate, salts of the common benzoic acid found in fruits and berries. Another type of preservative is an antioxidant (such as vitamin C), which protects sensitive flavor ingredients from oxidation and the resulting development of off-flavors. Sequestrants (such as citric acid) prevent chemical reactions by binding with the metals that act as catalysts.

Other functional ingredients include foaming agents, emulsifiers, clouding agents, and gums. Foaming agents stabilize the foam "head" on a glass of root beer or sarsaparilla. Emulsifiers stabilize oil-in-water emulsions critical to oil-based flavors, such as citrus flavors. Clouding agents make beverages cloudy or opaque to appear more natural, like orange juice. Gums can have a variety of functions including improving the mouth-feel of diet drinks.

Table 2 compares the ingredients in popular soft drink flavors.

Table 2. Typical Soft Drink Ingredients

Flavor	Brix (% sugar)	Acid	pH	Co$_2$ gas volumes	Calories/8 oz.
Cola	10	Phosphoric	2.3	3.0	93
Cream	11–13	Citric/none	5.5	2.9	115
Grape	14	Tartaric	3.0	2.8	125
Lemon-lime	11	Citric	2.7	3.5	108
Orange	12–14	Citric	3.5	2.5	120
Ginger ale	8	Citric	2.6	4.0	80
Root beer	11–12	Varies	5.0	3.2	113

NUTRITIONAL VALUE

Soft drinks are usually consumed for refreshment and enjoyment, not for nutritional value, but they do have value. They are a significant source of essential water in the diet. Many people who drink soft drinks on the job or while travelling may have limited choices of safe or appealing liquid alternatives. Soft drinks supply quick energy in the form of readily absorbable carbohydrates (sugars), which can be valuable to athletes, workers, and those with calorie deficient diets. Soft drinks have often provided much needed relief to victims of floods and natural disasters, who have no clean source of water for drinking or preparing meals. Recent lifting of government restrictions has allowed fortification of soft drinks with vitamins and nutritive additives. Now we have "value added" products enriched with vitamin C or calcium to increase nutritional benefits.

AVAILABILITY

One important reason for the increasing consumption of soft drinks is availability. Historically, soft drinks were made at home, or close to home, and by the early 1800s, many cities had choices of several purveyors. Even before the advent of bottled product, soda fountains were common in drugstores, candy shops, and saloons. As the industry grew in the later nineteenth and early twentieth centuries, mobility increased, bottling "pop" became common, and bottlers did their best to make it available in grocery stores, gas stations, and corner stands. Now you can buy soft drinks from convenience stores, fast food counters, and the ubiquitous vending machines. Of course they are available on the Internet! Increasing availability is a corporate marketing priority, and the competition is fierce.

TERMINOLOGY

Carbonated beverage. Preferred term adopted by manufacturers in 1919 to favorably disassociate the packaged effervescent beverages from other types of soft drink.

Soft drink. Commonly adopted early in the twentieth century to disassociate flavored refreshment waters from alcoholic beverages. Includes all varieties of carbonated and still beverages in bottles and cans and also those prepared at the fountain.

Soda. Originally a specific type of carbonated water, which included sodium bicarbonate for its medicinal properties. Term became generic in America for the broad category of flavored carbonated soft drinks. Because soda is not an ingredient in modern beverages, the term "soda water" is disfavored by the industry, although "soda" persists as a popular designation for carbonated beverages, particularly in the northeastern United States.

Pop. Popular term in the late nineteenth century for bottled flavored soda waters and still widely used by consumers in the central parts of the United States. Derived from popping noise made when the cork or other closure was removed from the bottle.

Tonic. A New England generic name for packaged carbonated beverages derived from the health values attributed to early carbonated waters. Also an English term now used in the United States to designate carbonated quinine water.

HISTORY OF CARBONATED BEVERAGES

Ancient Greeks and Romans knew the virtues of bathing in naturally occurring carbonated mineral waters. By the sixteenth century, extensive efforts were made by physicians and scientists to characterize and reproduce these physical benefits. The Swiss alchemist Phillipus Aureolus Paracelsus, recorded the earliest written observations. A century later, the Belgian physician Jean Baptiste Van Helmont (1577–1644) applied the name "gas" to mineral spring vapors. In 1757, Joseph Black, a Scottish physician, established that "fixed air" (CO_2 gas) could be extracted from limestone with oil of vitriol (sulfuric acid). In the 1780s Thomas and William Henry discovered that increasing pressure increased solubility of gases (Henry's law). By 1823, Sir Humphry Davy and Michael Faraday were liquifying CO_2 gas in England.

Simultaneously in America, scientists were studying mineral springs near Saratoga, New York, and developing ways to duplicate their properties. In 1798 the term "soda water" was first introduced. In 1807, Dr. Benjamin Silliman began selling carbonated mineral waters in New Haven, Connecticut. The first U.S. patent for the manufacture of imitation mineral waters was issued to Joseph Hawkins of Philadelphia. Developments followed rapidly on both sides of the Atlantic Ocean. Bottled soda water was first sold in the United States in 1835. Flavors were added soon after that in Germany and the United States, the earliest being fruit juices. Artificial flavors appeared in the 1850s along with ginger ale, root beer, and vanilla flavors sold through pharmacies. These early "tonics" were compounded and dispensed by chemists in apothecaries for consumption on the premises. The health benefits of mineral waters were expanded with the addition of herbs and sodium bicarbonate to soothe the stomach. By the late 1800s, the availability of liquid CO_2 in steel cylinders allowed the transition from soda fountains to bottling shops, and the industry took off. Some of the earliest products that grew into businesses include:

- Cantrell & Cochrane (C&C) ginger ale was exported from Belfast, Ireland, in the 1850s.
- Vernors ginger ale was developed in Detroit, Michigan, by James Vernor when he returned from the Civil War in the late 1860s.
- Hires root beer was introduced at the Philadelphia Centennial in 1876 by Charles Hires.
- In 1881, Henry Millis started selling Clicquot Club ginger ale in Boston.
- In 1884, Dr. Augustin Thompson developed Moxie in Lowell, Massachusetts.
- Dr Pepper formula developed by young Charles Alderton, was sold to Robert S. Lazenby, proprieter of the Circle "A" Ginger Ale Company in Waco, Texas, in 1885.
- In 1886, Dr. John Pemberton, an Atlanta druggist, produced the first 25 gallons of what became Coca-Cola, in a three-legged pot in his backyard. By 1904, 123 franchised bottlers sold one million gallons of Coke.
- Caleb Bradham of New Bern, North Carolina, named his new beverage Pepsi-Cola in 1898. By 1910 he had sold franchises to 280 bottlers.
- In 1890, John McLaughlin opened a small plant near Toronto to sell carbonated water to local drug stores. He introduced Canada Dry pale dry ginger ale in 1904.
- The Royal Crown name was trademarked by Hatcher Grocery Company in Columbus, Georgia, in 1905.

By the turn of the twentieth century the soft drink industry was well on its way to success. In 1900, 2,800 bottling plants produced over 1 billion 6 oz. bottles. Coca-Cola's first newspaper advertisement appeared in 1902. By 1921 there were over 6,000 bottlers helped in part by Prohibition, which closed many beer breweries. The number of bottlers peaked in 1929 at 7,920 and then tapered down during the Great Depression. After World II, the number of plants increased to 6,900 in 1949, but has been decreasing ever since, to less than 500 at the end of the twentieth century. But throughout the century, except for a few periods of war rationing restriction, the volume of soft drinks has increased as the plants became more efficient, more sanitary, and extended their reach through better packaging, transportation, and marketing.

SOFT DRINK INDUSTRY

An important factor in the sales growth of soft drinks has been innovation. The earliest successful purveyors had better ideas, better products, and better locations. To expand their businesses, they needed to develop better packaging, better production methods, and improved distribution. The important milestones on the road to success are acknowledged as (*1*) addition of flavors to mineral waters; (*2*) development of the fluted crown closure (bottle cap), capable of withstanding the pressures exerted by carbonated beverages; and (*3*) mass-produced glass bottles of uniform size, allowing development of faster bottling machinery. With a uniform package, the next innovation was the sale of franchise agreements, which allowed a franchisee to bottle and sell product exclusively within a prescribed geographic territory. Franchising the product quickly expanded its sales area with little capital cost to the franchisor. The Coca-Cola Company (Coke) and the Pepsi-Cola Company (Pepsi) quickly had large networks of hundreds of bottling plants each, few of which were owned by the parent company. Bottlers were independent businessmen operating within their territories as they wished with some minor constraints by the franchise contract. The most significant restriction was they must buy the flavor syrup from the parent company and follow the formula specifications.

Bottlers, being independent businessmen, became very competitive with bottlers of rival brands. Early "turf" battles for space in neighborhood stores and gas stations gradually evolved into the corporate marketing competition known as the cola wars. The advertising and distribution rivalry between Coke and Pepsi is legendary for its competition for priority in shelf space, sales outlets, stadium rights, and the bragging rights of being the "Official Soft Drink" of any and every event.

Bottlers also came to recognize the value of cooperation with their rivals on certain issues that adversely affected their business. The issues that finally brought them together were taxes and bottle "misappropriation," the stealing of empty bottles by a competitor to be refilled with the rival brand. The second issue led to the development of distinctive bottle designs for each brand and the controversial imposition of bottle return deposits. Bottlers formed regional and statewide organizations to discuss and resolve common issues.

SOFT DRINK BOTTLER ASSOCIATIONS

The first attempt at national organization was the U.S. Bottlers Protective Association in 1882. This group dissolved within five years and was replaced by the American Bottlers Protective Association (ABPA) in 1889. The ABPA held meetings to oppose taxes and resolve differences between members, but was largely ineffective. Finally, in 1919, the American Bottlers of Carbonated Beverages (ABCB) was formed under the strong leadership of James Vernor of Detroit. The group raised money to fight tax proposals that unfairly burdened the industry. They held regular meetings and formed committees to address industry issues. Soon, the annual meetings included equipment displays and the presentation of technical papers for the education of the membership.

The 1920s were boom years for soft drinks with amazing growth in sales and production technology. The ABCB hastened the spread of modern technology and equipment. One of the first accomplishments was the establishment of a scientific research program at Iowa State University to study industry needs and develop better testing methods. In 1923 they adopted the slogan "Quality—Purity—Service" to focus the image of the industry as a safe and reliable source of refreshment for consumers. The group introduced the Sanitary Code for Bottling Plants in 1929, establishing benchmarks for sanitary operations. In 1933 ABCB opened a beverage testing lab in Washington, D.C., to work with bottlers on product and ingredient specifications. This was supplemented by a mobile laboratory, which traveled the nation visiting bottling plants, upgrading local understanding and technical standards. This mobile lab continued for 27 years until supplanted by the growth of corporate field programs.

The ABCB also published important guidelines on the operations and financial controls of bottling plants. The industry faced many challenges from state and federal regulations and taxes that held the organization together, despite the competitive interests of the membership. The Great Depression of the 1930s drove many businesses out of existence, and bottling plants closed, but the five cent price of a bottle of cold soda made it an affordable treat. The advent of the rationing of sugar, trucks, and equipment in World War II also affected many bottlers, but the industry grew rapidly after the war. Some companies benefited from the war as American GIs spread their preference for soft drinks across Europe and Africa. Major brands were becoming international businesses.

ABCB was instrumental in the formation of the scientific association Society of Soft Drink Technologists in 1953. This group, now known as the International Society of Beverage Technologists, has greatly advanced the technical understanding of beverage chemistry and production. Annual technical meetings are attended by bottler, ingredient, and equipment members from around the world with the presentation and publishing of peer-reviewed technical papers.

In 1967, the ABCB changed its name to the National Soft Drink Association (NSDA), headquartered in Washington, D.C. A permanent staff of lawyers and technical managers coordinates activities of various volunteer committees to promote the industry and protect it from excessive legislation and taxes. The original reasons bottlers came together over 100 years ago, taxes and bottle return deposits, are still hot issues today.

SOFT DRINK PRODUCTION

The reason more beverage units are produced each year while the number of producing plants decreases is the advancement of beverage manufacturing technology. The industry has modernized in all aspects as it has developed larger and more efficient factories. Many related advances have combined to improve water purification, material handling, refrigeration, packaging speeds, warehousing, and transportation. The bottler of 1950 mixed a few bags of sugar to make 100 cases of 7 oz. bottles with a 32-station bottle filler filling about 150 bottles per minute. In 1998, the bottler mixes a tank truck of liquid sweetener to make 30,000 cases of 12 oz. cans with a 120-station can filler running at 2,000 cans per minute. He has automatic equipment to unload truckloads of empty cans to feed the conveyors and automatic equipment to load the finished product onto pallets, keeping several forklifts busy loading 15 semitrailer trucks to carry the product to market. Thanks to the interstate highway system, these trucks can now travel a couple hundred miles in a few hours to a distribution warehouse for local delivery. Table 3 summarizes the steps in the production of the modern soft drink can.

The modern beverage plant uses the latest in management tools and techniques to increase efficiencies, productivity, and product quality. Inventories are kept low by scheduling incoming materials to arrive hours before needed with "just-in-time" deliveries from suppliers. Production runs are scheduled to fill weekly orders from customers, assuring rapid warehouse turnover to keep fresh

Table 3. Steps in Soft Drink Can Production

Step	Description
Water treatment	Coagulation, sand filtration, carbon filtration, and polishing
Ingredients	Flavors, acidulent, colors, and preservative measured
Liquid sweetener	Filtered and metered into syrup tank
Syrup blending	Syrup compounded from ingredients, sweetener, and water
Water	Chilled and carbonated
Beverage proportioning	Beverage = 1 part syrup + 5 parts carbonated water
Can receiving	Empty cans depalletized, inspected, and cleaned
Can filling	Cans gravity filled through open end on revolving filler
Can seaming	Filled cans have lid applied and sealed with double seam.
Date coding	Each can has a date code printed on the side or bottom.
Fill check	Net contents measured, low fill cans rejected to scrap
Can warmer	Cans warmed above dew point to prevent condensation in cases
Multipacker	Cans grouped and wrapped in 6, 12, 18, or 24 packs
Case packer	Six or 12 packs combined into cases of 24
Palletizer	One hundred cases oriented into alternating stack patterns on wood pallets
Stretch wrap	Pallets wrapped in clear plastic film for protection and stability
Warehousing	Product stored in warehouse until needed to fill orders
Bulk delivery	Semitrailer loads transported to customer warehouses
Local delivery	Smaller trucks deliver to stores, restaurants, vending machines, etc.

product on the store shelves. Heating and cooling energy is recycled to minimize waste. Packaging materials are recycled, solid waste is minimized, and liquid wastes are pretreated before release to municipal treatment facilities.

Many plants run 16- to 20-hour daily production schedules with a sanitation shift during downtime. Quality improvement and worker safety programs are constantly reinforced by management. Strict quality programs are monitored by parent company representatives, who provide materials, training, and technical support to each plant in their system. Every facility has a modern testing laboratory with calibrated instrumentation to assure accurate quality test results. Many now have on-line instrumentation providing constant data logging of critical measurements.

MARKETING

The soft drink story begins with marketing and ends with consumer satisfaction. Carbonated beverage marketing is big business and possibly the most visible product promotion campaign existing. Direct advertising on signs, motor vehicles, store displays, print ads, television, radio, clothing, and anywhere else a logo or jingle can be found constantly reminds us we are thirsty. From subtle placements in movies to giant skywriting over sports events, soft drink advertising is ubiquitous. From the early patent medicine purveyors to the latest extreme-sport computer-graphic commercials, the point is the same, sell more soft drinks. These are fun refreshing products, so the ads tend to be more attractive and enjoyable than most. Not many products have more appealing packaging graphics or vending machine decoration to be sure.

Although good quality advertising and packaging increase desire to purchase, the product must also consistently deliver the promised refreshment at a reasonable price. Competitive promotion pressures have kept the price of soft drinks quite reasonable. There are times during holiday promotions when products are sold at or below cost to increase market share and meet volume targets. This discounting has contributed to the demise of the small, inefficient bottler and the growth of the larger franchisors. Another factor in that evolution is the consolidation of supermarket chains, giving stores more control over which products get shelf space and end-of-aisle displays.

Soft drinks are marketed primarily to the young, from teenage years through the twenties, because early preferences often continue as people age. The brands want to be associated with the "in crowd" and good times. The brand confers status to those that are seen with a can. As the population demographics are aging, more ads will be seen with older people enjoying soft drinks, perhaps with nostalgia.

Not only do the advertising campaigns adjust to changing times, but new product offerings will be available for changing tastes and lifestyles. The recent proliferation of sports drinks, flavored waters, and good-for-you beverages are keeping up with the trends. The future is unpredictable, but you can be sure soft drinks will evolve to fill the niche for liquid refreshment.

GENERAL REFERENCES

American Bottlers of Carbonated Beverages, *Plant Operation Manual*, Washington, D C., 1940.

American Bottlers of Carbonated Beverages, *Bottlers Tentative Sugar Standard*, Washington, D.C., 1953.

American Bottlers of Carbonated Beverages, *Bottlers Tentative Liquid Sugar Standard*, Washington, D.C., 1956.

American Can Company, *Carbonated Beverages in the United States—Historical Review*, Greenwich, Conn., 1972.

Beverage Digest Online home page, URL: *www.beverage-digest.com* (last accessed April 19, 1999).

BEVnet, The Beverage Network home page, URL: *www.bevnet.com* (last accessed April 19, 1999).

Beverage World home page, URL: *www.beverageworld.com* (last accessed June 25, 1999).

Cadbury Schweppes plc. home page, URL: *www.cadburyschweppes.com* (last accessed April 19, 1999).

Coca-Cola Company, *Portrait of a Business*, Atlanta, Ga., 1971.

Dr. Pepper Museum and Free Enterprise Institute home page, URL: *www.drpeppermuseum.com* (last accessed April 19, 1999).

M. B. Jacobs, *Manufacture and Analysis of Carbonated Beverages*, Chemical Publishing, New York, 1959

C. H. Lofland, *The National Soft Drink Association—A Tradition of Service*, National Soft Drink Association Washington, D.C., 1986.

National Soft Drink Association home page, URL: *www.nsda.org* (last accessed April 19, 1999).

T Oliver, *The Real Coke, The Real Story*, Random House, New York, 1986.

J. J. Riley and the American Bottlers of Carbonated Beverages, *Organization in the Soft Drink Industry*, Washington, D.C., 1946.

J. G. Woodroof and G. F. Phillips, *Beverages: Carbonated and Noncarbonated*, revised edition, AVI Publishing, Westport, Conn., 1981.

SCOTT M. GRIFFITH
Admix, Inc.
Manchester, New Hampshire

BEVERAGES: NONCARBONATED

How many types of beverages can you name? There are thousands of different flavors, categories, brands, and packages of beverages available in the United States today. They all have one thing in common: They are all predominantly water. Our bodies are composed primarily of water and water-soluble compounds. We must drink liquids to maintain a water balance and replenish the water our bodies expel constantly. The amount of liquid required will vary with our physical condition, exertion level, and environment. We can only survive for a week or two without some water intake. Fortunately, most of us consume plenty of beverages to meet our biological need for water.

Our water balance controls the natural desire for a drink, called *thirst*. When our bodies sense an excess of salts, called *electrolytes*, in our blood, we feel the sensation of thirst. We can satisfy our thirst by restoring the water balance through beverage consumption, but we have many other reasons to consume beverages beyond our physical need.

Natural water was undoubtedly the first beverage consumed by prehistoric people, and it remains the most popular beverage in areas where it is safe and available to drink. Unfortunately, as the world becomes more developed and crowded, those areas are shrinking fast. Throughout history, we have developed beverage alternatives with desirable flavors, physical sensations, and nutritional value. We have learned to identify and purify unsafe water and change it into the rainbow of beverages available today.

Several other beverages are found in nature, the most obvious and important to our development is milk. As mammalian babies, mother's milk is our first beverage and source of nutrition following birth. Until the relatively recent development of nutritional baby formula, milk was essential to a baby's survival. As we grow, we can experience other beverages and eventually solid food. Frequently the next beverage after milk and water, is fruit juice, another naturally occurring drink derived by squeezing ripe fruit. Of course, the fruit juice we enjoy today is much improved over what was available from fruit growing in the wild. Other naturally occurring beverages would also be liquids derived from plants such as nectars, saps, and vegetable juices.

BEVERAGE TYPES

Webster's defines a beverage simply as a drinkable liquid. We drink beverages to satisfy our thirst, for enjoyment, for psychological reward, for ceremony or celebration, and for nutrition. The proliferation of beverage choices was most likely driven by curiosity at first, but now seems to be more profit driven. We have beverages for all occasions: to stimulate us in the morning, to calm us down at night, to sooth our physical maladies, to celebrate holidays and special occasions, and to perform religious ceremonies. There are ethnic drinks identified with specific cultures, sports drinks for strenuous exercise, health drinks for improved nutrition, and even astronaut drinks developed for space travel. See Table 1 for a list of the popular categories available in the U.S. market. There are two common measures of beverage sales and consumption: liquid volume and retail sales in dollars. These measures can rank beverages quite differently because some beverages are very expensive per unit volume. For instance, alcoholic beverages including beer, wine, and distilled spirits, account for almost half of the beverage dollars spent by consumers, but only comprise about 15% of the volume sold.

HISTORY

Early beverages of commerce include milk, mineral waters, tea, beer, and wine. Chapters could be written on the histories of each of these, with roots in early civilizations. Explorers constantly expanded the list with discoveries of herbs, spices, and fruits from distant lands. Beverage purveyors developed preservation methods, processing techniques, and improved packaging to expand their markets. New machines such as grinders, presses, distilleries, and dehydrators improved yields and quality. Pasteurization and natural preservatives, along with better packaging to reduce spoilage, greatly extended shelf life and distribution range. As availability of supplies increased, marketing

Table 1. Beverage Categories

Water—tap, purified, spring, sparkling, flavored, caffeinated
Carbonated soft drinks
Alcoholic—beer, cider, wine, distilled spirits, low-calorie beer
Milk and dairy-based beverages
Coffee and tea—hot and cold, cappucino, latte
Fruit juices and juice drinks (less than 100% juice)
Fruit flavored (no juice)
Powdered drink mixes
Vegetable juices
Sports drinks—isotonics
Frozen drinks—shakes, slushes
Health drinks—nutrition fortified, herbal, nutraceuticals
Diet drinks—low-calorie, low-fat, weight control aids
Chocolate based—hot cocoa, cold sweetened
New Age—Innovative new blends that cross categories

and promotion of beverages accelerated the trial and proliferation of new drinks. The beverage market grew to become huge. Beverages make up the largest portion of our daily food intake. Proliferation of products and packages has made drinks conveniently available almost everywhere we go in the Western world. Packages range from little tea bags and powder packets up to 5-gal bulk dispensers and kegs.

U.S. BEVERAGE MARKET

What are the most popular beverages available in the United States in the late 1990s? By far the volume leader is carbonated soft drinks, with a per capita consumption of more than 55 gal per year and growing. They are also the largest single category in retail dollars, estimated at more than $50 billion. Tap water may be second in volume, but declining and contributing negligible dollar sales. Beer is in third place, with steady annual volume of about 22 gal per person, followed by coffee, milk, bottled water, juice, and tea, in that order. The fastest growing segment is bottled water.

RECENT DEVELOPMENTS

The decades of the 1980s and 1990s saw a tremendous proliferation of new products in ready-to-drink teas, juice blends, New Age, and nutraceuticals. Ready-to-drink teas (bottles or cans) were pioneered by the big dry-tea producers such as Lipton, Nestlé, Red Rose, and Tetley. These were cold-filled 12-oz cans with preservatives, offered as an alternative to carbonated soft drinks. Drinking cold or iced tea was an American innovation very popular in the South. The packaged product did not really catch on until a small New York company introduced Snapple, all natural flavored teas. These were hot-filled products in 16-oz bottles with fruit flavors. With some clever marketing, Snapple grew at an amazing rate for several years. It spawned many imitators and created a new market segment. Snapple sales plummeted when a large food conglomerate bought the company and tried to change its successful distribution system.

At about the same time, imported bottled waters became popular in small, fancy bottles appealing to upscale consumers. Perrier and Clearly Canadian expanded their offerings with flavored waters and created a new category of innovative products, dubbed New Age, that could not be defined by the existing beverage categories. New Age products were attractively packaged, very expensive, and very profitable when consumers made them a trend. But as more products tried to ride the coattails of their success, the market was diluted, and many new products failed to last long. Many industry watchers felt too many new products were being introduced; sales outlets and distributors had no room to take on so many products. An industry shakeout was inevitable as supermarkets raised the cost of new product introductions by charging for shelf space and quickly dropping products that did not sell well. Many smaller companies failed or were sold to larger companies, but innovation continued at the more successful firms.

Sports drinks are a recent innovation that has grown steadily but is still dominated by the pioneer, Gatorade. Sports drinks are designed to replace the water, energy, and electrolytes lost during strenuous exercise. Gatorade was developed in the 1960s by researchers led by Dr. Robert Cade to help the University of Florida football team (Gators) maintain stamina while playing in the hot and humid Florida climate. It was successful with the athletes and soon was used by other teams. Clever marketing got it placed on professional football team benches with large logos on coolers and cups plainly visible on national television. This rapid brand identification made it popular with athletes and sports fans across the country. Soon, Gatorade was sponsoring youth sports events with associated promotion of branded accessories like water bottles, hats, and headbands. Its marketing clout kept other sports drinks from making any inroads until Coke and Pepsi developed products and used their distribution power to challenge the market leader. Their market shares are slowly gaining on Gatorade.

Juice companies have been livening up their product lines with exotic juice blends, featuring tropical fruits like guava, papaya, mango, and kiwi. Juices are expensive, so juice drinks with 5 or 10% juice were introduced to compete at a lower price. Lately the trend has swung back toward 100% juice blends, which allow some cost savings with lower-priced juices predominating in the blend. Juice sales are not growing overall, but certain segments such as orange juice and the new blends are doing well.

Coffee sales have been reinvigorated by specialty stores like Starbucks and Dunkin Donuts and upscale specialty products like cappuccino and latte. New cold and frozen products from these stores may help even out the seasonal sales cycles of hot products.

The latest trend in beverages is the healthy or "good for you" segment. This seemed to start with herbal teas making claims to have physiological affects. There has been a sharp increase in fortification of products with calcium, beginning with orange juice. This was based on the health benefits of calcium in preventing Osteoporosis, a degenerative bone condition common in older women.

Now a new class of nutraceutical beverages has emerged claiming health benefits derived from herbs, trace minerals, and amino acids. A list of some of these esoteric ingredients follows in Table 2. The term nutraceutical comes from combining nutrition and pharmaceutical, as

Table 2. Some Interesting New Beverage Ingredients

Ginseng root	Gotu kola
Guarana	Echinacea
Ginko biloba	Kava kava
Dong quai	Bee pollen
Fo-ti	Carnitine
Damiana	Proline
Agavé nectar	Taurine
Yerba maté	Yohimbe

these products are targeted toward better health through diets rich in these ingredients with curative properties.

OTHER TRENDS AFFECTING BEVERAGES

Consumer trends that affect beverage developments include the healthier lifestyle movement, desire for more "natural" products with fewer additives, the increase in meals eaten away from home, and the desire for convenience. Natural products and products with fewer additives have become more popular. Improvements in processing have made these products possible while consumer demand has allowed producers to raise prices to cover increased costs. Changes in packaging technology have also had an impact. Aseptic packaging allows less heat processing and resulting flavor loss for shelf-stable juice products. Better oxygen and light barrier materials have improved shelf life and reduced vitamin losses in dairy products. Easy-opening spouts on water and sports drink bottles have become popular. Convenience trends have hurt the sales of powder mix beverages and frozen juice concentrates prepared at home. At the same time, convenience store and vending machine packaged product sales have grown. Bottled water is a good example of convenience packaging increasing sales and margins for a commodity product. The convenience trend is making beverages of all kinds more readily available where we work, shop, and recreate.

GENERAL REFERENCES

Beverage Digest LLC home page, URL: *www.beverage-digest.com* (last accessed June 25, 1999).

Bill Communications home page, URL: *www.beverageworld.com* (last accessed June 25, 1999).

Hoovers, Inc. home page, URL: *www.hoovers.com* (last accessed June 25, 1999).

Kraft Foods, Inc. home page, URL: *www.kraftfoods.com* (last accessed June 25, 1999)

Quaker Oats Company home page, URL: *www.quakeroats.com* (last accessed June 25, 1999).

J. G. Woodroof and G F. Philips, *Beverages: Carbonated and Noncarbonated*, rev. ed., AVI Publishing, Westport, Conn., 1981.

SCOTT M. GRIFFITH
Admix, Inc.
Manchester, New Hampshire

BIOLOGICALLY STABLE INTERMEDIATES

The ability to preserve food from one harvest to another has been vital to the development of civilization and has played a very important role in discovery and survival. Methods of food preservation, in one form or another, have been employed for thousands of years and are usually defined as a procedure that delays spoilage and makes food items available for consumption at a later date. Often the food item can be eaten without further preparation as, for example, when the inhabitants of the hot dry desert areas used to bury dates, figs, and grapes in the hot dry sands to dehydrate them. With partial removal of water, the sugar content of the fruit was high enough to prevent the growth of microorganisms, and the fruit would keep for long periods of time. The fruit could be consumed at a later date without the need for further processing. A number of other technologies were also developed to accomplish this. These include simple dehydration, chemical preservation, pickling, fermentation, canning, freezing, and, more recently, a number of more-sophisticated approaches such as irradiation, hypobaric applications, pulsed light, and so on. All of these were concerned with processing food in a manner that would make it edible at a later date. But with the increasing importance of food fabrication, another concept emerged. Food commodities were essential as ingredients in many formulated foods, and a need arose to have food ingredients available in a "biologically stable form" for future formulation. With the limited harvesting schedules available for many fruits and vegetables, it was economically prohibitive to install sufficient formulation equipment to make all the desired product at time of harvest. The manufactures wanted stable ingredients such that they could run their formulation lines on a year-round basis. The following passages describe some food commodities available in biologically active form that are not usually consumed in that form.

Nature has provided many foods in a biologically stable form. The most obvious example is cereal grains. It is no surprise that cereal grains became the main source of food for humans from the dawn of civilization. Cereal grains, when properly matured, dried, and stored under good conditions, will last almost forever. They are subject to loss from insects, birds, and animals but seldom from microorganisms. Cereal grains are the ideal biologically stable food, but they do require processing such as grinding, soaking, and cooking prior to consumption. Many vegetables are normally biologically stable. Root crops such as potatoes, carrots, parsnips, turnips, rutabagas, and beets can be stored throughout the winter season. The early settlers in New England depended on storage of root crops in "root cellars," which were simply excavations in the side of a hill, to store root vegetables and cabbage over the winter. The natural humidity of the earthen surroundings in the cave provided sufficient moisture to prevent dehydration and also sufficient insulation to maintain a cool storage temperature without freezing. The availability of these vegetables in the winter months led to the development of the well-known "New England boiled dinner" (1), which was composed of potatoes, carrots, onions, cabbage, turnips, parsnips, and chunks of meat, usually corned beef, but sometimes salt pork or codfish. Very few fruits are biologically stable, with the possible exception of the banana. Bananas are the world's largest fresh fruit crop. Its popularity, aside from its delicious flavor, may be partially due to the fact that it requires a three-week ripening period after harvesting, which was long enough to allow bananas to be shipped by boat from the tropical producing areas to nearly all parts of the world. The central Asian nomads had their own biologically stable food. They simply opened a vein in their horse's neck and drank the blood (2). In the following examples, biologically stable foods will be confined to those that are processed to form an intermediate commodity that is not normally consumed as such and requires further processing into a consumer item.

DRIED CODFISH

Probably the oldest, and certainly the most successful, biologically stable product is dried codfish (3). The earliest record is attributed to the Vikings who traveled from Norway to Iceland to Greenland to Canada, which coincidentally is the range of the Atlantic cod (*Gadus morhua*). In the tenth century, Thorwald and his son Erik the Red were expelled from Norway for murder and traveled to Iceland where they were again expelled for murder and in the year 965 traveled to Greenland. The Vikings were able to travel such long distances because they dried cod by splitting the fish and exposing the carcasses to the dry freezing air. The low temperatures prevented the frozen fish from decaying and the dry air was effective in removing moisture. The dried product resembled a wooden plank, but it did not decay. This was the same process developed by the natives in the high mountains of South America to produce "chofa" from potatoes. The Vikings had a thriving trade in dried cod with the Romans many centuries earlier, but it really flourished in the trade with Europe in the Middle Ages. Erik the Red colonized Greenland and his son Leif Eriksson sailed on to what he called Stoneland but was probably the coast of Labrador. The Norsemen made five expeditions to Canada between 985 and 1011 and all were possible because they had rations of dried cod.

In the Middle Ages in Europe, dried cod was an important food commodity, and the supply was coming from Scandinavia and Iceland. The dried cod being sold in Europe was a superior product to that being used in the Norse exploratory journeys because the Europeans had salt, and dried salt cod lasted longer and simply tasted better. The Basques in northern Spain entered the European market with large quantities of dried salt cod and for centuries no one knew where it was coming from. Actually, the Basques had discovered the limitless cod stocks of the eastern shore of America. Europe, in the Middle Ages, was a big market for whale meat, and the Basques were providing a large portion of it. In their whaling expeditions, they discovered the cod fishing grounds. When John Cabot landed in Newfoundland in 1497, he claimed the land for England. Jacques Cartier arrived in 1534 and reported seeing 1000 Basque ships fishing for cod. But the Basques, in the interests of secrecy, never claimed the land, and it remained for Jacques Cartier to claim the land for Canada. The Pilgrims landed in 1621 and the wealth of the New England cod fishery began to unfold.

The eighteenth century saw a race to exploit the New England cod fishery, and the European countries of Spain, Portugal, France, England, and Scandinavia rushed to supply the huge European demand for dried cod. The Americans soon learned that this was a lucrative world trade and developed the fishing industry. The French colonies on Haiti, Martinique, and Guadalupe, and the Dutch Republic of Suriname (then Dutch Guiana) became good customers because salt cod provided a nutritious and cheap source of food for slaves. Ships sailed for Africa loaded with salt cod and used half the cargo to purchase slaves and then set sail for the Carribbean where they exchanged the slaves and the rest of the cargo for molasses. The molasses was used to produce rum, which had become a naval necessity. A tot of rum was issued each day to each sailor, and we are led to believe that it was appreciated. Rum became a generic term for alcoholic beverages. The trade in dried cod became intertwined with the slave and molasses trade, and the Boston "Cod Aristocracy" was born. Today a model of a codfish hangs from the ceiling of the state house in Boston.

Codfish was an ideal candidate for biologically stable products for several reasons. First, it was very low in fat, which reduces the tendency to become rancid when stored, as opposed to salmon, which the early New Englanders also had in abundance. Salmon has a high oil content and cannot be preserved by drying with the same efficiency as codfish. Second, codfish was available in limitless quantities and could be easily caught. Third, abundant rocky shorelines were available to allow for drying in the open air. The Vikings reported that the dried cod without salt could be eaten directly by chipping off pieces, but this must have been very difficult. They probably soaked the dry slabs in water to soften the product. Dried salt cod cannot be eaten directly but must be soaked to lower the salt content. A number of changes of water were necessary to lower the salt content, and some ingenious methods were developed. The French reported that the journey by boat from Bordeaux to Aveyron took about two days, which was approximately the soaking time required for salt cod, so they towed it in a mesh bag behind the boat. Pollution of the Lot River discouraged this practice. In the meantime, flush toilets were developed and the old "water closet" had a tank of water elevated above the toilet. Storing cod in the tank provided an efficient way to remove the salt.

It is ironic that the "limitless" harvest of cod that was responsible for so much international trade turned out to be anything but limitless. Ships became bigger and more powerful for dragging trawls and more efficient in finding fish with electronic gear, and the capacity to process fish at sea was developed. The development of large factory ships made it possible for any country to exploit the cod fishing grounds, and soon ships from Europe, Russia, Korea, China, and many others were appearing in the Atlantic off the New England coast. The fisheries could not sustain the fishing effort, and cod became commercially extinct. In 1994 Canada closed the fisheries off Newfoundland, and in 1999 the United States closed a large portion of the cod fishing grounds. Fishery biologists say that the fisheries will need a number of old spawners to replenish the cod population. This means fish up to 15 years of age, so the fisheries will be closed for a long time.

SULFITED FRUITS

The use of sulfur dioxide and its salts to preserve fruits and some vegetables is a very old process. Most fruits were harvested over a very short period and had to be preserved in bulk until they could be processed into jams and jellies. Sulfur dioxide served this role very well. Fruit could be cleaned, placed into barrels, and covered with a solution of sulfur dioxide in water, or a solution of sodium bisulfite or one of its salts. After mixing in the barrels, the fruit could be kept for years. The fruit could be removed from the barrels, placed in a kettle, and boiled to remove the sulfur dioxide. After addition of pectin and sugars and boiling to produce the required moisture level, the product could be filled into consumer-sized packages. Strawberries were an important sulfited product because of the large market for strawberry jam but the sulfite treatment bleached out the red color. The chemical reaction was a simple addition reaction between the red anthocyanin pigment and the sulfite ion that was easily broken such that the boiling process reduced the sulfite content and restored the desirable red color.

The sulfite process is important today in the manufacture of maraschino cherries. Nearly all the cherries used for maraschino cherries are mechanically harvested and suffer some mechanical damage in the process of being shaken off the tree. Since cherries discolor very quickly, they are conveyed in a matter of seconds into a brine tank on the mechanical harvester. The simplest of the sulfite brines is a solution of sodium bisulfite in water, but a number of other formulations are used. The purpose of the sulfite brine is to preserve the fruit by inhibiting the enzymes that produce discoloration and to bleach out the red color. Sometimes a secondary bleach in sodium chlorite is used. A source of calcium ions is usually added to the brine to firm the cherries. The brined cherries are leached to remove the sulfite ions and any other soluble material and conveyed to a tank containing a red colorant dissolved in a sugar syrup. The cherries readily absorb the red colorant and are then drained and packaged. Maraschino, candied, and glaceéd cherries are made the same way except that the sugar content is different.

Around the beginning of the twentieth century, sulfiting was the major way to preserve fruits in bulk for further processing in Europe, particularly in England because of the English people's liking for strawberry jam. The introduction of freezing provided a method of preservation that produced a superior final product from both an appearance and a flavor point of view and sulfiting decreased in importance. Sulfiting is still used in some parts of the world but the technology has been essentially displaced except for maraschino cherries.

TOMATO PRODUCTS

Tomato juice and tomato concentrate are more recent examples of biologically stable products and are ideal candidates for preserving. The tomato harvesting season is relatively short, the crop is huge in size, the demand for tomato products in formulated foods was growing rapidly, and nearly all the tomatoes for processing are mechanically harvested. Mechanical harvesting is an economic necessity, but it does result in more mechanical damage to the crop than the old-fashioned hand harvesting. This means that the crop must be processed as soon after harvesting as possible. Minimal processing to produce a biologically stable intermediate is a virtual necessity to minimize the costs of production and ensure a high-quality product.

Biogically stable intermediate products became feasible with the concept of aseptic packaging developed by W. M. Martin in 1935 (4). He developed the concept of sterilizing a product, sterilizing the container, and bringing the two together, with a sterile cap, in a sterile atmosphere. Conventional canning at that time consisted of filling and capping a container and heat treating the closed container to ensure sterility. This means that the center point of the container needed to receive sufficient heat treatment to ensure sterility; however, the outer portions of the container would be overheated and the quality of the food would be lowered. This effectively limited the size of the container to a No. 10 can, approximately 1 gal. If the product could be sterilized in a thin film outside the container, and packaged aseptically, the size of the container was limitless. I remember visiting a large food processor in the 1940s. They required a large quantity of tomato paste to formulate into products like pork and beans, soup, pizza sauce, sardines in tomato sauce, barbecue sauce, and many others. During the tomato season they would pack their year's quota of tomato juice, tomato ketchup, and tomato soup and make the remainder into tomato concentrate or paste. The concentrate would be filled into No. 10 cans, capped, and stored in a huge warehouse. The concentration process would make the puree essentially sterile, and the hot fill would sterilize the cans and lids. This process worked very well, but it was labor intensive and it was not perfect. Some of the cans leaked, and one leaky can makes quite a mess.

Bulk storage of tomato juice in the United States started essentially with the work of Nelson at Purdue University (5–7). He experimented with aseptic storage of tomato juice in tanks up to 100,000 gal, and even larger, and now numerous large storage systems are in place worldwide. The concept is simple, but the implementation is more complicated. Tomatoes can be mechanically harvested into large containers and brought immediately to the factory for processing. After washing, the tomatoes are chopped and screened to remove skins and seeds and pumped to a heat exchanger that sterilizes the juice. A number of systems are available to sterilize the juice, but nearly all employ highly efficient heat exchangers that use the high temperature/short time (HTST) or the ultrahigh temperature (UHT) approach to deliver a quantity of heat lethal to microorganisms. The short heating time ensures maximum retention of nutrients and flavor. A deaeration system is necessary to remove dissolved air in order to minimize loss of quality during subsequent storage. Large tanks are sterilized with hot water and chlorine, and the juice is pumped into them. The whole system, including the pumps, pressure reduction valves, heat exchangers, and the valve system, is closed and operated aseptically, which ensures that sterile air enters the tank to replace the product that is removed on demand for later formulation. The tanks can be refrigerated with interior coils or simply stored in a refrigerated building, if desired, depending on the quality demands of the final product. The tanks can also be mounted on a rail car or truck for shipment to any area. The same system can be operated with tomato concentrate, depending on the requirements of the formulator.

GRAPE JUICE

The development of bulk storage of grape juice paralleled the experience with tomato juice, but the products are different primarily due to the higher sugar content and flavor lability of grape juice. The aims are similar. The "Concord" grape juice industry primarily in the eastern United States is used for juice, jams, jellies, and fruit juice drinks. The western U.S. grape industry is about 10 times as big and is used for wines, brandies, raisins, juices, and fruit juices. Both have a need for bulk storage of grape juice. Friedman (8) attributed the development of the Concord grape juice industry in the last 70 years to three major technological developments: (*1*) bulk storage of juice, (*2*) continuous processing, and (*3*) mechanical harvesting. Continuous processing was introduced about 1955 to replace the batch process being used by the apple and grape industry prior to that time. The batch process consisted of crushing the heated grapes and building a pad of grapes on a filter cloth on a wooden rack. The racks and layers of grapes were alternated to build a large pile and the whole stack was placed in a filter press to remove the juice. A number of types of continuous presses were developed that removed the juice in one pass. The batch process was labor intensive and slow, whereas the new presses enabled some plants to process 2000 tons of grapes per day. The introduction of mechanical harvesters, at approximately the same time as continous pressing, enabled the operators to harvest a large quantity of fruit in a short time. Most harvesters consist of a machine that straddles the row of grapes, and beater blades separate the grapes from the vine. The grapes are collected and transferred to a tank in the vineyards for transport to the processing factory. Today, nearly all grapes for processing are mechanically harvested.

A typical bulk storage operation would be as follows. Grapes are crushed and destemmed, heated to about 60°C, and treated for 30 to 40 min with a depectinizing enzyme to disintegrate the pulp and release the juice. A filter aid, usually a cellulose derivative, is added, and the skins and seeds are separated in a continuous press. The juice is filtered, flash-pasteurized, cooled, and pumped directly to the sterile storage tanks with the same sterility precautions as described for tomato juice. The storage tanks may be made of stainless steel or, more usually, of mild steel lined with a phenolic (epoxy) resin. Tanks holding 320,000 gal were standard in the industry for years, but modern installations usually hold 714,000 gal. Apparently grape juice can be held at 30°F for 12 months or more with little loss in quality. The large tanks are usually freestanding with refrigerated coils inside the tank, but smaller tanks could be stored in a refrigerated room. Bulk storage of juice has several important advantages: (*1*) The "argols," crystals of potassium bitartrate, precipitate out, which prevents the accumulation of tartrates as unsightly sediment in consumer packages; (*2*) a substantial amount of pooling takes place with a resulting beneficial standardization and uniformity; and (*3*) the juice can be withdrawn, as desired, to maintain year-round production lines. The same procedure may be used to produce and store juice concentrates. Juice can be pumped to the concentrators at 15 to 20° Brix and stored at 60° Brix. The concentrate is more stable, microbiologically, because of the high sugar content. Alternatively, single strength juice can be stored for later concentration, but this is only done with white juices because two heat treatments would cause too much color degradation for colored grape juice.

Many of the fruit drinks on the market use grape juice as the main ingredient for several reasons. It is inexpensive, available in large quantities, provides sweetness, and has a desirable fruity flavor that is compatible with other added flavors. Some grape concentrates have another advantage in that they can provide a very attractive color in addition to the sugar and flavor.

FROZEN FOODS

Frozen foods do not qualify under the definition of biologically stable foods that are not normally consumed in that form since they obviously can be consumed, but the packages are much bigger. Frozen foods are one of the best biologically stable foods from a quality standpoint and play a very important role in the reformulation of convenience foods. Products such as peas, green beans, lima beans, corn, sliced carrots, and almost any vegetable can be frozen individually and packed in containers up to 2000 lb for future reformulation into entrees, pies, and so on. Many fruits can be prepared, frozen individually, and stored in large containers for reformulation into fruit cocktail, specialty desserts, and so on.

BIBLIOGRAPHY

1. *American Cooking* Time-Life Series on Foods of the World, New York, 1968, p. 90.
2. R. Tannahill, Food in History Stein and Day, New York, 1973.
3. M. Kurlanski, *Cod*. Penguin Books, New York, 1997.
4. American Can Co., *The Story of Canco Research*, American Can Co., Chicago, 1955.
5. P. E. Nelson, "Technical Developments in Bulk Storage Processing," *Hortscience* **6**(3), 222–223 (1971).
6. U.S. Pat. 3,714,956 (1973), P. E. Nelson (to Nelson).
7. P. E. Nelson, "Aseptic Bulk Processing of Fruits and Vegetables," *Food Technol.* **44**(2), 96–97 (1990).
8. I. E. Friedman, "Industrial Application of Bulk Storage in the "Concord" Grape Industry," *Hortscience* **6**(3), 228–229 (1971).

F. J. Francis
Editor-in-Chief
University of Massachusetts
Amherst, Massachusetts

BISCUIT AND CRACKER TECHNOLOGY

If a definition of a biscuit were to encompass the wide range of products as understood by consumers and the baking industry, that definition would be different from the one contained in most standard dictionaries. In the United States, the common biscuit is a chemically leavened bread/roll that is generally circular in outline and flat in profile; it is rather similar in composition to some British scones. This product is sometimes called a baking powder biscuit.

In the United Kingdom, and most of the rest of the English-speaking world, biscuits are the two types of products called crackers and cookies. Crackers are nonsweet products used like bread. Cookie items include a vast array of dessert foods, characterized mainly by being baked in small pieces and having a texture or consistency that is drier, chewier (or crisper), and denser than most cakes, and are usually sweet. An important characteristic of crackers and cookies is that they usually have a much longer shelf life than baked products such as bread and cake. However, intermediate moisture cookies (8–12% moisture) are being sold that confound this distinction (eg, soft-filled cookies, brownies, and fruit-filled bars).

Except for their lower moisture content, crackers and cookies are similar in composition to breads and cakes. The range of ingredient percentages in cracker and cookie formulas will be found to overlap with formulas for breads and cakes. Manufacturing processes, procedures, and equipment are also similar, yet different, for many parts of the process (ingredient handling, mixing, and baking), although the forming, depositing, and packaging equipment may be highly specialized.

INGREDIENTS

Flour

The principal structure-forming ingredient used in biscuit doughs is wheat flour. Very few cookies or crackers are made without any wheat flour, and those are usually quite atypical in organoleptic characteristics. To provide for flour strength in weaker flours, vital wheat gluten can replace wheat flour to a limited extent.

There are many different kinds of wheat flour, and the specifications of this ingredient must be carefully chosen, if it is to impart satisfactory machining properties to the dough and have a desirable appearance with good eating qualities. Cracker doughs typically use soft wheat flour fortified with relatively high protein content flour made from hard red winter wheat or spring wheat. Some cracker doughs and most cookies will be based on soft wheat flour, sometimes with a small proportion of flour from hard wheat.

Soft wheat flours suitable for biscuits may vary in protein content (mostly gluten) from 7.0 to 9.5% (for cookies) to 10% or more (for crackers). The flour may be unbleached or heavily bleached to a pH range of 4.4 to 4.8 for certain specialty items, such as soft cookies that contain higher levels of sugar and shortening. Cookies using bleached flour will rise higher with little spread; in contrast, unbleached flour will typically spread more than it rises. Cracker sponge flours may contain added malt (wheat or barley). Malt flours are made by wetting whole grains and allowing the enzymes to become active. The malted grains are then dried at low heating temperatures to remove moisture but not deactivate the enzymes. Such malted flour is then added to the cracker sponge for its enzymatic properties (1).

Ingredient specifications for cookie flour will usually include protein content, moisture content, ash content, particle size, starch damage, pH, odor, and flavor. Microbiological tests will be required to reveal contamination by insects, fungi, pesticides, and other unwanted materials. It is also common to specify certain rheological tests (eg, Alveograph), which are expected to correlate with dough response to processing conditions. Finally, the flour must yield cookies of specified characteristics (spread/height), when it is used in dough that is prepared and baked under standardized conditions. One relevant test that gives an indication of flour function is the bake-spread test. Written procedures for this test can be found in AACC Method 10-53 (2). The diameter and height of the finished test cookie are measured. Analyzing the results indicates the amount of rise or spread performance of a flour. Sometimes this measurement is expressed as a ratio of the height to diameter, but that approach does not fully characterize flour performance. It is better to analyze and compare both results for trends.

Flours other than wheat can be used in crackers and cookies for color, texture, and flavor. Prime examples of these ingredients would be rye and corn flour. Sorghum flour, cottonseed meal, soybean meal, triticale flour, barley flour, and other cereal and noncereal powders have been suggested as additives for enhancing nutritional or other properties. These nonwheat ingredients have structure-forming properties that do not function as well as typical wheat varieties and must be supplemented with a strong wheat flour or vital wheat gluten for most purposes. Rice flour has been used to make cookies suitable for persons who are allergic to wheat gluten; it is incapable of forming typical dough. Rice flour has been used successfully for the production of rice crackers, but the process and procedures are very different from standard baking practices. A modified form of wheat flour starch, called resistant starch, passes easily through the digestive system, thus reducing its caloric contribution to the body.

Shortenings

Shortenings are essential components of most crackers and cookies. The type and amount of shortenings and emulsifiers in a formula affect both the machining response of a dough or batter and the quality of a finished product. Coatings (such as chocolate) and fillings (such as sandwich cremes) depend on specific fats and oils to furnish the structural part of such components.

Natural fats and oils suitable for shortenings include butter, lard, beef fats, and vegetable oils. The latter material includes refined and modified soybean, cottonseed, coconut, palm, and corn oils. The processing of shortenings may include some or all of the steps of refining (to remove contaminants), deodorizing, winterizing (to remove high-melting-point fats), bleaching, hydrogenation, fractionation, and blending. The purpose of these operations is to yield a shortening that is bland in flavor, essentially colorless, and free of aromas; has a melting point and solid fat content within the desired ranges for the application for which it is destined; and has other characteristics desired by the purchaser. Butter, and to a lesser extent lard and beef fats, have natural flavors and physical qualities that are considered desirable in some applications.

Recently, new fat systems that are non- or poorly digestible have been developed. One of these fat systems is a combination of sugar esters that is nondigestible in the body. Another fat system, which contributes a reduced calorie load, modifies the triglyceride composition through esterification with nondigestible fatty acids.

The function of shortenings in baked goods is primarily to modify the physical properties or texture of the finished product, making it more tender or flakier and, in some cases, giving it a glossier, more appealing appearance. Shortenings may also affect the rheological characteristics of doughs. Most crackers have a topping or spray oil applied after baking, to add flavors or solids (cheese, spices and/or herbs) that improve the eating qualities.

Emulsifiers are specialized fats or fat systems that act as surfactants between various systems (eg, oil and water). Emulsifiers are specialized fats that can be composed of mono-, di-, or triglycerides or their combinations. Chemically, an emulsifier is a molecule composed of a water-soluble or hydrophilic portion and a water-insoluble or hydrophobic portion. Different solubility tendencies thus exist within the same molecule. This leads to the phenomenon of incomplete solubility in both water and oils. Emulsifiers partition, or orient, themselves at the interface between oil and water phases (3). Naturally occurring emulsifiers include egg yolk or fluid lecithin. Fluid lecithin is derived from either soybeans or corn during the milling process.

Sweeteners

All cookies and many crackers contain some form of sweetener. The quantity of sweetener is usually such that it has significant effects on the texture and appearance of the product, as well as on its flavor. Machining properties and response of the dough piece to oven conditions are also related to the type and quantity of sweetener employed. In fermented goods, sugars serve as substrates from which yeast and other microorganisms form carbon dioxide and the flavoring substances characteristic of these products. Commercial sucrose (cane or beet sugar) functions not only as a nutritive sweetener but also as a texturizer, coloring agent, and as a means of controlling spread during baking. However, differences in the crystallization properties between cane and beet sugars can be quite significant. In marshmallow, jellies, and fruit jams with relatively high moisture content, sucrose has the valuable property of delaying microbiological spoilage, when it is present in a high-enough concentration.

Sucrose can be obtained in various particle sizes or in the form of syrups. When added to a dough as granulated or powdered sugar, its particle size influences the dimensions of the finished cookie, by controlling the extent to which the dough piece spreads as it is baking.

Syrups are easier to handle, being adaptable to fluid-transfer systems of pumps, pipes, valves, and meters. Syrups can crystallize in handling systems, causing problems. Sucrose will not form a stable aqueous solution of greater than 67% concentration at room temperature, and this is not high enough to ensure resistance to all forms of microbiological growth. Treating a solution of sucrose with an acid or an enzyme known as invertase hydrolyzes the sucrose into fructose and dextrose (3). Invert sugar solutions are sweeter than pure sugar solutions of the same concentration. Invert syrups are less likely to crystallize and are more resistant to spoilage.

Corn syrups are sweeteners prepared by hydrolyzing cornstarch with acids or enzymes. The standard types contain glucose and varying amounts of maltose, larger oligomers, polymers, and other carbohydrates, plus minor amounts of impurities. In preparing the high-fructose corn syrup (HFCS) varieties, manufacturers isomerize part of the glucose to fructose. Because fructose is sweeter than glucose, a substantial increase in sweetening power results. HFCS can be substituted for invert sugar syrups in many applications, but they are not identical.

Regular corn syrup is categorized by type, according to dextrose equivalent (DE), which is a measure of reducing sugar content. A second parameter is total solids content of a syrup. Cookies made with syrups with higher-reducing sugar contents have a greater sweetness that those made with a similar content of low-DE syrup. In addition, the browning of the crust and, for that matter, the interior of the cookie is affected by high contents of reducing sugar from any source. Low-DE corn syrups can increase the apparent viscosity of doughs and batters and make a finished product chewier.

Molasses is less-refined, concentrated sugar syrup containing some of the impurities, flavoring and coloring materials from the sugar-refining process. Molasses is available in various degrees of darkness; the darker the color, the more bitter and less sweet the syrup is. True blackstrap, the final residue of the refining process, is not suitable for human consumption and is used primarily as an animal feed additive.

Commercial brown sugar is granulated sugar that has been coated with a small percentage of molasses. No commercial brown sugar is produced by removing partially refined granulated sugar from the refining process. There are, however, less-refined sugars available from refiners (eg, Demarara) that occasionally provide some economic advantages over pure cane or beet sugar.

Honey is primarily invert syrup with various impurities that give typical flavors and color. Various concentrated fruit juices are used, as alternatives to sucrose and corn syrups, as natural sweeteners. These fruit juices are principally from grape, pear, or apple sources that have been treated to have low characterizing flavor. These fruit syrups are usually more expensive than sucrose or corn syrup.

Caramel color is a widely used brown pigment made from acid- or alkali-treated corn syrup (or sugar syrup). Caramel color is used in many types of cookies, in liquid or powder form, for its colorant properties and its low cost. It contributes no flavor to a finished product.

Synthetic sweeteners, such as saccharin and aspartame, can be used in certain dietetic cookies. However, sugar serves as a primary bulking agent in cookies. When significant reductions in sugar content are made, texture and other physical characteristics are affected. Most synthetic sweeteners are not heat stable, but recent developments are changing that limitation. New synthetic sugars are under development, which have up to 2000 times the sweetening power of sugar. Of course, most of these synthetic sweeteners are regulated by law and must pass stringent food and drug tests. Nonnutritive and reduced-calorie sweeteners are playing a more important role, as consumer demand requires such specialized products.

Leavening Agents

A leavening agent is a substance or system that expands or lightens a dough or batter at some stage of its processing. Leavening can be achieved in three ways: mechanically, biologically, or chemically. The leavening effect is absolutely essential to the formation of a finished product having the appearance and eating qualities that are required by consumers. Leaveners familiar to every baker are baking powder and yeast. Air and water vapor (steam) are also leaveners. They cause expansion of the product during baking, providing the dough or batter has a structure capable of retaining the gas. Ammonium bicarbonate is a chemical additive that has leavening and other effects on cookie and cracker doughs.

Yeast alters the physical properties of dough and its handling characteristics during mixing and fermentation. Yeast acts on certain sugars to form alcohol and carbon dioxide. Gluten from the flour absorbs water and forms extensible membranes that trap carbon dioxide and expand to decrease the density of the dough mass. The diffusion and accumulation of carbon dioxide throughout the dough mass generates powerful stretching action. These changes in gluten are described as the "mellowing," maturing, or conditioning of the dough (3).

An egg white meringue, which is the basis of angel food cake, relies on egg albumen to initially entrap the air that has been beaten into the mixture. The addition of flour strengthens the dough mass in the latter stages of mixing and baking.

Few cookies depend on yeast leavening. Crackers made by the sponge-and-dough method (eg, soda crackers, saltines, and certain snack crackers) require a fermentation period to develop their textures and characterizing flavors. Typical sponges contain flour, water, and yeast mixtures; the doughs usually contain the remaining ingredients, including sodium bicarbonate. The acids produced during sponge fermentation will react with sodium bicarbonate to neutralize the acid and yield additional leavening.

The great majority of cookies contain some sort of chemical leavening system such as baking powder, which is a mixture of sodium bicarbonate and a reactive acidic compound such as cream of tartar (potassium acid tartrate). In addition, fillers such as starch and other additives will be included in commercial baking powders to standardize their strength and delay storage deterioration.

When formulating cookie doughs, most food technologists add sodium bicarbonate in combination with a reactive acidic material, in proportions appropriate to the pH of the dough, rather than rely on premixed baking powder. Other types of chemical leaveners (eg, monocalcium phosphate, sodium acid pyrophosphate) have specific characteristics that can be tailored to the requirements of a given dough formula. The goal is usually to obtain a finished product with a pH close to neutrality, although this generality may not apply to certain products (eg, chocolate doughs containing Dutched cocoa, with a resulting higher pH).

Ammonium bicarbonate is a self-reacting leavener that decomposes as the dough is heated during the early stages of baking, giving off ammonia, carbon dioxide, and water. It is acceptable only for products baked to lower moistures (3–9%). In high-moisture products, the ammonia dissolves into the water, producing an unpleasant aroma and/or taste that most people find offensive. However, ammonium bicarbonate can be very effective in altering finished product characteristics (height, spread, and texture) for both cookies and crackers.

Many cookies undergo an increase in volume (decrease in density due to leavening) that is quite small compared with that of bread. For example, traditional shortbread cookies may contain no added soda and do not seem to increase in size during baking. An examination of their interior shows, however, that some expansion has taken place, as a result of air and water (steam) that have expanded during the baking process.

Other Ingredients

Ingredient water can have a significant effect on dough properties, but most production plants are able to develop formula modifications that offset any undesirable effects that might result from use of the water from their particular supply system. The water from any potable source, such as the municipal pipelines or a well, can be regarded as legally suitable for incorporation in a biscuit formula. However, it should not be necessary to say that any water that has an undesirable odor or flavor should be subjected to a purification process before it is mixed into a dough. The pH of the ingredient water supply, especially from municipal sources, can in some cases vary widely throughout the week or day, and this can cause the physical properties of the dough to change, adversely affecting the machining of this material.

Fresh milk and eggs are not common ingredients in most commercial cookies partly because they are relatively costly. When present, they are likely to constitute only a small percentage of the product. Eggs can have a substantial effect on the physical properties of a dough or batter, acting as a structure former, leavener (due to entrapment of air and water vapor), emulsifier, and lubricants. Egg whites are better as a structure former and leavener; yolks are more effective as an emulsifier and lubricant. Eggs do affect flavor, not always positively, and yolks add color, usually a desirable result. Nonfat milk has ambivalent results on structure while whole milk generally has a weakening effect due to the fat. Both milk and eggs add good-quality protein to a biscuit and are sometimes used as a wash on the surface to color or glaze the finished piece, but for some consumers, cholesterol concerns may be a negative attribute.

Salt is an important ingredient. Not only is it an essential flavor-enhancing ingredient in any baked product, but it has effects on the physical characteristics of doughs. Some of these effects may be favorable, such as the slight toughening effect it has on some doughs, and others are considered unfavorable, such as its retarding effect on yeast-leavened systems, when incorporated at high levels.

Crackers and especially cookies are susceptible to rancidity, because of their high fat content and their fairly long shelf life. Antioxidants can counteract this tendency to a considerable degree. Common antioxidants are butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ). Amounts that can be added are restricted by federal regulations, and antioxidants must be labeled as cancer-causing agents in some states and may be banned outright in certain countries. Citric acid and phosphoric acid are sometimes useful for chelating metal ions that would otherwise accelerate rancidity. Research has shown that some natural tocopherols (vitamin E) and other antioxidants (rosemary extract) can prevent oxidation.

FORMULAS AND PROCEDURES

In general, formulas can be divided into different groups. Saltines and other fermented products use a sponge-and-dough system that is normally formulated with yeast. In contrast, straight cracker doughs and the like will have small amounts of sugar, fat, and other characterizing additives and are chemically leavened. Cookie formulas will contain moderate to high amounts of sugar and shortening and are primarily leavened with sodium bicarbonate and ammonium bicarbonate in combination with a leavening acid to produce a baked good with high volume. Cookies may also contain other ingredients such as icings, fillings, fruits, nut pastes or pieces, flavors, and chocolate to give them distinctive value.

Cookie formulas are generally classified according to the kind of equipment used to form the individual pieces. Stamping machines, rotary cutters, rotary molders, wire-cut machines and depositors are used for more than 90% of the cookies commercially produced in the United States. The type of equipment being used for a product sets limits on the dough rheological properties or the final composition of the dough.

Deposit cookies are the machine-made counterparts of hand-dropped cookies, and many published (1) formulas for the latter can be easily adapted to factory requirements. General rules are that deposit cookies should contain (on the basis of flour as 100%), about 35 to 40% sugar, 65 to 75% shortening, and 15 to 25% whole eggs. The flour should be milled from soft wheat, and it should be unbleached.

Although wire-cut cookie equipment is rather tolerant as far as dough consistency is concerned, there are still some rheological requirements that must be observed. The dough should be sufficiently cohesive enough to hold together as it is extruded through orifices. Yet it must be relatively nonsticky and short enough, so that it separates cleanly when cut by the wire. Such formulas may contain several times as much sugar as flour, shortening up to 100% of the flour, and are generally the type of doughs to which particulates (nuts, chocolate chips, or candy pieces) are added. Doughs may be almost as soft as some cake batters or too stiff to be easily molded by hand. The softest wire-cut doughs overlap deposit doughs in consistency, while the other extreme is close to the consistency of some rotary-molded doughs.

Whatever the mixing procedure, it must be sufficient to produce a uniform distribution of ingredients. Many cookie doughs are rather tolerant in the amount and type of mixing that will yield satisfactory performance and products. Saltines and the like must be developed (or brought to a stage of near-maximum dough strength) by the mixing process. For high-sugar-fat formulas, a preliminary creaming stage, in which the sugar and shortening are mixed together for a time before other ingredients are added, is said to ensure uniformity and air incorporation. This creaming step also produces a finer texture in the finished cookie. Most particulates (eg, chocolate chips or nuts) are best added at a late stage of mixing, either with or after the addition of flour.

Brownies are one of the few kinds of cookies that can be baked in a continuous sheet on an oven band, then cut into pieces during or after cooling. Brownies are made with high proportions of sugar, invert syrup and other hygroscopic ingredients, so that they are soft and chewy when fresh, and retain this texture fairly well over a period of weeks or months when properly packaged. The batter, which is often quite soft, is extruded directly onto the oven band as a sheet of uniform thickness, and no other forming operations are performed.

The manufacturing process for sponge-and-dough (eg, saltines) consists of the following steps: weighing the sponge ingredients, mixing the sponge, fermenting the sponge in troughs, mixing the sponge with the remainder of the dough ingredients, fermenting the dough, laminating the dough, sheeting the dough to a required thickness, cutting and embossing the dough sheet, removing the scrap, sprinkling salt on the dough sheet, baking, breaking the baked dough sheet into pieces, and packaging. The manufacturing steps for yeast-leavened or chemically leavened snack crackers resemble the foregoing sequence to a considerable extent, with variations depending on the need for special shapes or content of additional ingredients.

Reciprocating cutters (stamping type) and rotary-cutting machines must be fed, by conveyor belts, with a continuous sheet of dough. An important requirement of these machines is that the scrap dough must be removed in one piece and reincorporated, if the operation is to be efficient. The scrap is returned to a prelamination stage in an operation that will uniformly reincorporate the scrap back into the fresh dough. Care must be taken, in that excessive scrap or rework may affect machining and finished product attributes. Also, the sheet thickness must be maintained within a narrow range, so that the weight of the dough pieces will not vary significantly. The dough must have some elasticity and be cohesive enough to bear its weight, if it is to form a sheet that will retain its continuity and not tear. Excessive elasticity creates problems with shrinkage of the pieces and difficulties in maintaining uniform piece weight. To obtain the desired characteristics, the dough must contain a substantial amount of wheat flour, to provide the gluten that will give the dough strength and elasticity. In processing, the dough must be developed by a mixing operation that orients the gluten molecules, or it must be repeatedly sheeted and layered. The content of ingredients that weaken or "shorten" the dough, such as sweeteners and shortenings, must be kept relatively low. Moisture content, for all practical purposes, is the amount of ingredient water added and should be sufficient enough to allow full hydration of the gluten, without weakening the dough excessively. Nearly all cookies and crackers are baked after forming. A few recipes for fried cookies and crackers can be found in the literature, but these do not appear to have been commercialized to an appreciable extent.

EQUIPMENT

The type of equipment that can be used to process a given biscuit dough is highly dependent on the consistency of the dough. The types of dough that can be processed satisfactorily with one or more of the kinds of available equipment include everything from soft, fluffy batters and wet, flowable doughs to very stiff, claylike masses and tough but elastic doughs resembling bread doughs in many respects.

Mixers

Cookie and cracker doughs are mixed in two main types of mixers: upright and spindle mixers. The upright mixer is used mainly for cookie and some cracker doughs. Various arrangements of the mixing arms can be used, including sigma, z-type, and double-armed. Depending on the product to be made, the characteristics of the formula will determine what mixing arm arrangement is used. Spindle mixers are much more aggressive and are used for saltine and like doughs or other high-strength cracker doughs.

Other specialized mixers, called aerators, are used to produce batters for most sugar wafer production and some drop cookies (eg, vanilla wafers). These machines take a liquid to semipaste batter and incorporate pressurized air into the mixture. Through formulation and mechanical agitation, the air is incorporated into the batter, thus reducing the specific gravity of the resultant batter.

Laminators

Many cracker doughs, processed through cutting machines, first undergo multiple sheeting and folding steps. The basic principle is to form a thin, endless dough sheet by passing the dough between two rollers (or multiple pairs of rollers may be used in sequence), folding this thin dough sheet into a stack of multiple layers, then incorporating together the multiple layers. This laminating step gives the dough internal structure, which enhances its performance in the cutting machine, and gives the final product desired characteristics. If a saltine is examined closely, traces of this layering process can be found.

Forming Equipment

Each type of forming equipment has been designed to process a dough or batter with a particular range of physical characteristics. The action or method required for shaping the dough necessarily sets certain limits on the appearance of the product. It is convenient to separate the following discussion into cookie equipment and cracker equipment, because the machines used in forming these two types of product are quite different.

Cookie Equipment

The following three broad types of forming or shaping devices, as described afterward, are used to manufacture the great majority of commercial cookies:

1. Extruders push the dough or batter through a constricting orifice; they are exemplified by deposit machines, bar presses, and wire-cut equipment.
2. Rotary molders shape the dough in die cavities cut into the surface of a metal cylinder.
3. Stamping machines or rotating cutters cut shaped pieces from a continuous sheet of dough.

Extruders. Extruders vary widely in complexity, from simple equipment consisting of a hopper fitted with feed rollers that press the dough through adjustable slits, to complicated devices that extrude deposit-cookie batters through orifices moving in predetermined patterns. The most common type of machine consists of a hopper and one or more feed rollers that force the dough through an array of tubes called die cups. The dies may have orifices of different shapes: square, round, oval, scalloped, and so on. On wire-cut machines, disks are sliced from a continuously extruded cylinder of dough and allowed to drop onto the oven band or transfer belt. Other, more complicated extruder-type machines can extrude multiple doughs or doughs with fillers. Such machines are sometimes called encrusters. A dough casing and filler are coextruded and either sliced by a wire device or pinched closed via a mechanical device. Sometimes, the dough and filler combination is applied directly to the oven band, forming a continuous strip (eg, fruit bar).

Rotary Molders. A simple rotary-molding machine consists of a hopper, a feed roll, a cylindrical die, a knife or scraper, a cloth or woven-plastic belt (also called a web or apron), and a rubber-covered compression roller. There will also be a frame, motors, controls, and so on. Such machines may be permanently affixed to the oven frame or they may be constructed as movable attachments for cutting machine lines.

The curved surface of the metal cylinder is covered with engraved cavities having the shape desired for the dough piece. Alternatively, plastic die cavities may be fastened to the surface. Pressure is exerted on the dough in the hopper. This causes the dough to be forced into the die cavities, as the die roller rotates beneath a slit at the bottom of the hopper. The cylinder rotates past a knife or scraper (which forms the flat bottom on the dough piece), until it contacts the cloth belt passing beneath it. The belt is forced against the die by a rubber-covered roller, which can be adjusted to vary the pressure. As the cylinder lifts off the belt in its continuing rotation, the dough piece adheres to the belt more strongly than it does to the cavity and is, therefore, drawn out of the die. The belt carries the dough piece to the oven band, where transfer is completed by various kinds of simple mechanisms. There are limits to the adjustments that can be made on the machine to achieve these operational essentials, so the physical characteristics of the dough can vary only slightly.

Stamping Machines. In deposit machines, the batter is extruded intermittently through shaped nozzles. Separation into cookie-size portions is achieved by lowering the oven band during the time the extrusion is stopped. This causes the batter on the band to be pulled away and separated from the batter still in the extrusion orifice. Then the band is moved up again toward the nozzle, and extrusion begins again. Such machines are relatively simple, but they are also quite demanding with regard to dough characteristics. In more advanced machines, the nozzles can be moved to form various patterns such as curves, wavy figures, swirls, and circles. A second depositor can be synchronized with the first to put jelly or some other filling on top of the cookie.

A bar-press machine (sometimes called rout presses) extrudes continuous strings or strips of dough directly onto the oven band. Separation of these strips into individual cookie pieces can be made, either before or after baking, by a cutting device. The die plate may be inclined in the direction of the extrusion, so that the dough ribbon is supported for a longer period of time, an arrangement that reduces breaking or thinning of the dough strand caused by gravitational pull. The die orifices are usually slots with a straight lower edge to give a flat bottom to the cookie, and a grooved top edge, to give a ribbed upper surface. Height of the strip can be varied by moving one of the slot edges.

Wire-cut machines represent an advance in complexity over bar-press and deposit machines in that they include a device that cuts off pieces of the extruded dough as it emerges from the die orifice. The cutoff device consists of a wire or blade that is quickly drawn through the dough by a reciprocating harp. The harp is simply a frame or support attached to a mechanism that moves it back and forth beneath the die cups. The dough emerges from the cups in approximately cylindrical form, if round cookies are being made. However, changing the shape of the orifice can modify the cross section of the dough strand. Several of the die cups are held on a bar that fits snugly into a channel at the bottom of the hopper. Wire-cut machines can probably handle more types of dough than any other cookie-forming apparatus, but they do not operate well on elastic, extensible doughs.

Cracker Equipment

Vertically reciprocating cutters, or stamping machines, are used mostly for crackers, although such devices are also used to make embossed cookies. Rotary cutters employ metal cylinders with cutting dies attached to the curved surface. Two stamping machines may be placed in tandem to perform two different operations on the same piece of dough (eg, cutting the outline and docking the piece).

Ovens

It is frequently said that the biscuit factory must be designed around the oven, because the limitations of this device affect nearly every other operation in the bakery. The bakery equipment found in nearly all large biscuit factories include some form of band oven, a type of oven that is ideally suited for rapid and uniform processing of small pieces of dough. Small operations may use reel ovens, deck ovens, rotary ovens, or just about any other kind of baking equipment. The following discussion will concentrate on the band oven.

The band oven consists of an endless steel belt passing through a baking chamber that can be heated directly or indirectly. The band may be a solid strip of steel or a belt of woven steel wire. Perforated steel or wire-mesh bands allow steam to escape from the bottom of the dough pieces. This helps to prevent gas pockets and retard spread and other distortions in the finished baked good.

The baking chamber consists of a series of modular units (eg, 10 ft long, 7 ft wide, and 5 ft high). Heating means are supplied above and below the band. Thick insulation covers all surfaces that are not occupied by air input and output ducts, ports, and other fixtures. In direct-heated ovens, heat is often provided by ribbon burners above and below the band. Open-flame or surface combustion (ceramic) elements have been used, but the former type is more common. Gas (methane or propane), oil, or even diesel fuel is burned, depending on the limitations of the heating system and the market price of fuel. Electrically heated ovens function satisfactorily but are economically impractical in most locations.

Indirect-heated ovens burn the fuel in a chamber separate from the baking tunnel, and hot air is transferred to the band by fans and convection. The length of the oven is divided into zones, which permits different temperatures to be applied to the product during different stages of baking.

Electronic ovens, of both the dielectric and microwave varieties, have been used to bake cookies and crackers. Microwave heating has demonstrated considerable success in finishing or drying, after baking in a conventional oven. This form of energy application enables the manufacturer to reduce moisture in a cracker, without undue browning of the cracker's surface. New designs in oven technology have led to the development of hybrid ovens, which combine conventional hot air and microwave technology.

Other Equipment

Sugar wafers are made from wafer sheets that are baked in a specialized wafer oven. The oven consists of an enclosed chamber through which pairs of plates, which can be brought together and separated, as required, are transported. At the beginning of the process, a fluid batter is deposited on the bottom plate, and then the other plate in the pair is immediately brought near the first to form a closed chamber within which the batter rapidly expands under the influence of heat. The plates are patterned on their contiguous surfaces so the baked wafer emerges with a sort of waffle pattern or any other desired pattern. In fact, there are many points where the process resembles home baking of waffles. The baked wafers are removed from the griddle, cooled, spread with a filling, topped with another wafer, cut into desired shapes, and packed.

Sandwiching machines assemble two cookie wafers (usually of the molded type, but sometimes wire-cut) and a layer of filling to form a sandwich cookie. Many variations in shapes and flavors are possible; in fact, crackers can be sandwiched with cheese or peanut butter filling to make a nonsweet snack. The cookies, called basecakes, arrive at the sandwiching machines from cooling conveyors leading from the oven or from chutes. Vibrating conveyors align the basecakes into a stacked-on-edge orientation. Basecakes are fed by the conveyor into magazines or chutes in the proper orientation for sandwiching; they are removed one at a time by means of double pins on double chains. As the bottom cakes travel through the machine with their embossed side downward, they receive a deposit of soft creme extruded through a rotating sleeve having shaped orifices. As the bottom basecake with its creme deposit reaches the second set of magazines, the top cakes are dropped onto the creme. Then the sandwich is gently pressed together to ensure adherence of the components, and to establish uniform thickness of the finished cookies. A much more complete discussion of biscuit equipment has been published elsewhere (4).

PACKAGING, STORAGE, AND DISTRIBUTION

Most biscuits will have moisture contents of less than 5%, with some of the crisper varieties, such as saltines, averaging around 2%. Exceptions are the chewy cookies such as brownies and cakelike cookies, which, when fresh, will have moisture contents of more than 10%. Within the range of normal ambient relative humidities experienced in the United States, drier cookies and crackers will tend to absorb water vapor. Chewier cookies will lose moisture, so it is highly desirable to package all biscuits in containers having low rates of moisture vapor transfer, if extended shelf lives are to be achieved. A more detailed discussion of packaging technology has been published elsewhere (5).

Deterioration of cookies and crackers generally takes the form of loss of flavor or acquisition of stale flavors, due to chemical changes (such as oxidation and loss of volatiles by evaporation), and undesirable texture changes. These changes are caused by loss or uptake of moisture, depending on whether a given biscuit is expected to be chewy or crisp, respectively. Sometimes, the appearance also changes because the surface reflects and refracts light differently, due to physical changes such as crystallization of sugars, solidification or liquefaction of fat fractions, or formation of empty vacuoles as moisture evaporates.

Microbiological changes are usually not particularly important causes of storage deterioration of cookies and crackers, because the low "water activity" (actually, relative humidity) of the substrate strongly inhibits the growth of yeasts, bacteria, and molds. The water activity (denoted as A_w) should be less than 0.70, which is the point below which most yeast, mold, and bacteria cannot survive. Sometimes, mold inhibitors such as potassium sorbate or calcium propionate are used to control spoilage, but these additives are regulated and are pH dependent. Occasionally, fermentation by osmophilic yeasts will take place in marshmallow and jellies, but formula adjustments to decrease the water activity of these materials, plus improvements in sanitation, can often remedy such problems. An extensive discussion of packaging and preservation methods for biscuits and other baked goods has been published elsewhere (5).

CURRENT AND FUTURE TRENDS

New product introductions in the United States in recent years have emphasized health and nutritional claims (not always the same things) and high-priced gourmet variations. Health claims are faddish in nature and vary according to the enthusiasm of the moment. High-fiber cookies and crackers can be produced through heavy additions of wheat, oat, or corn bran or more exotic ingredients such as psyllium seed husks, purified cellulose (from wood, sugar beet pulp, etc), and pectin-based materials. No-fat and reduced-fat cookies and crackers have also been popular. Consumers realize there are health benefits in such products, but it is difficult for manufacturers to predict such product trends.

It can be expected that future trends will follow new dietary fads, as they become entrenched in the popular imagination. The exact direction cannot be predicted, but products of reduced caloric content or those meeting health requirements of an aging population will probably be popular.

Gourmet cookies are achieved by formula variations that are heavy on chocolate, fruit preserves, nuts, butter, and other highly indulgent ingredients. Pricing structure usually restricts such products to select market niches. Other gourmet cookies can be made by the addition of perishable components such as whipped cream, butter-cream fillings, and other adjuncts that are practical only for freshly prepared items or for cookies that are distributed in frozen form

BIBLIOGRAPHY

1. P. Ellis, *Biscuit and Cracker Technology and Manufacturing*, Crompton Press, Morris Plains, New Jersey, 1988.
2. American Association of Cereal Chemists (AACC), 1995, *Approved Methods of the AmericanAmerican Association of Cereal Chemists*, Method 10-53 (approved October 15, 1997).
3. P. Ellis, *Cookie and Cracker Manufacturing*, Vols 1 and 2, The Biscuit and Cracker Manufacturers Association, Washington, D.C., 1990.
4. S. A. Matz, *Ingredients for Bakers*, Pan-Tech International, Inc., McAllen, Tex., 1987.
5. M. Bakker, *Encyclopedia of Packaging Technology*, John Wiley and Sons, New York, 1986.

GENERAL REFERENCES

H. Faridi, *The Science of Cookie and Cracker Production*, Chapman & Hall, New York, 1994.

D. J. R. Manley, *Technology of Biscuits, Crackers and Cookies*, Ellies Heorrwood Ltd., Chichester, UK, 1983.

S. A. Matz, *Formulas and Process for Bakers*, Pan-Tech International, Inc., McAllen, Tex., 1988.

S. A. Matz, *Equipment for Bakers*, Pan-Tech International, Inc., McAllen, Tex., 1988

S. A. Matz, *Bakery Technology*, Pan-Tech International, Inc., McAllen, Tex., 1989.

S. A. Matz and T. D. Matz, *Cookie and Cracker Technology*, 2nd ed., AVI Publishing Co., Inc. Westport, Conn., 1978.

W. H. Smith, *Biscuits, Crackers, and Cookies*, Magazines for Industry, Inc., New York, 1972

P. R. Whiteley, *Biscuit Manufacture*, Elsevier Publishing Co., London, UK, 1971.

RICHARD R. MCFEATERS
DOMENICO R. CASSONE
Nabisco, Inc.
East Hanover, New Jersey

BLANCHING

The blanching process typically utilizes temperatures around 75–95°C for times of about 1–10 min, depending on the product requirements. It is a necessary pretreatment for many vegetables in order to achieve satisfactory quality in dehydrated, canned, and frozen products. A blanching process may be needed if there is likely to be a delay in reaching enzyme inactivation temperatures or, as in freezing preservation, if such temperatures are never achieved. The process should ensure the required reduction of enzyme activity that otherwise might cause undesirable changes in odor, flavor, color, texture, and nutritive value during frozen storage. Another major effect is the removal of intercellular gases. This reduces the potential for oxidative changes in the food and allows the achievement of suitable headspace vacua within cans. As a heat process, blanching may result in some reduction in microbial load, and texture may be improved. Vegetable matter tends to shrink because of loss of turgor, which can aid the achievement of the required fill weight. Undesirable losses of heat sensitive nutrients may be caused, and, in water blanching, soluble constituents may be leached, resulting in large volumes of effluent (1).

BLANCHING METHODS

Water Blanching

Water blanching is the most widely used method. There are four basic designs of water blancher.

1. The tubular blancher in which particulate vegetable matter is transported by pumped water through a system of tubes for the required residence time. Direct steam injection is used to heat the water to blanch temperature, while tube length and product flow rate govern the blanch time.

2. The rotary screw blancher in which a central screw rotates, at a speed that moves the product forward to give the required blanch time, within a static drum containing the blanch water.

3. The rotary blancher, which comprises a drum with a scroll attached to the inner wall, rotating on trunnions.

4. The thermascrew blancher in which the rotating central screw is hollow and contains the heating medium.

Other designs have been developed for various products, such as the countercurrent blanching tower in which product and hot water are moved in opposite directions to promote rapid heat transfer. Product characteristics vary considerably and types (b) and (c) have been used widely. For rotary water blanchers, by a relatively simple scale-up of the basic design, ie, increasing blancher length, blancher capacity is increased for a range of blanch times. Estimates of product outputs can be predicted from data based on trials, as shown by the example in Figure 1 and Table 1.

Capital and running costs are relatively low for water blanchers, and thermal efficiency has been reported to be as high as 60% (2). Improvements in design have been directed at reducing energy and water consumption to minimize leaching and effluent. Mean heat utilization for a blancher with an average spinach capacity of 58,000 kg/h has been estimated at 39%, assuming a value of 758 kJ/kg for raw spinach leaves free from adhering water (3). Energy consumptions of a tubular water blancher and a water blancher with a screw conveyor have been measured as a basis for suggesting energy conserving modifications. The former required 0.54 MJ/kg and the latter 0.91 MJ/kg, indicating the importance of complete steam condensation.

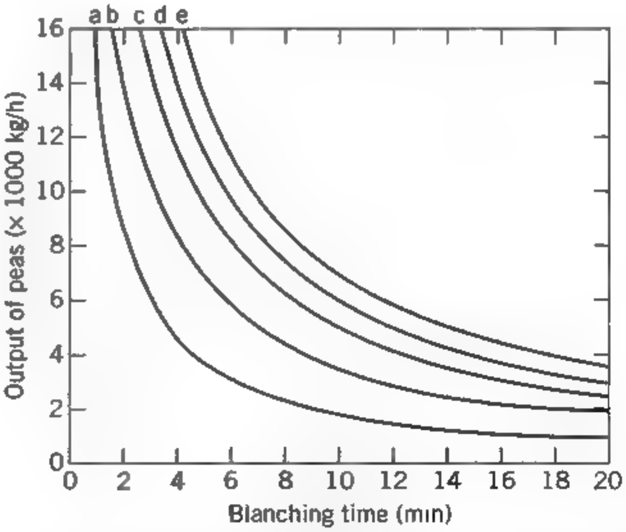


Figure 1. Typical example of relation between product output and blanch time for various products blanched in various sizes of rotary water blancher. For a blancher diameter = 2 m, blancher length is approximately a = 3.1 m, b = 4.5 m, c 5.9 m, d 7.4 m, and e = 8.8 m.

Table 1. Multiply Graph Outputs by Appropriate Factor for These Products

Product	Factor
Potatoes	
whole	1.15
french fried	1.00
Carrots, whole	1.10
Beans, soaked	1.08
Peas	
soaked	1.08
fresh	1.00
Green beans	
cross cut	0.70
whole	0.60
Cauliflower, florets	0.50
Spinach, leaf	0.30
Brussels sprouts	0.30
Cabbage, leaf	0.30

Note. See Figure 1.

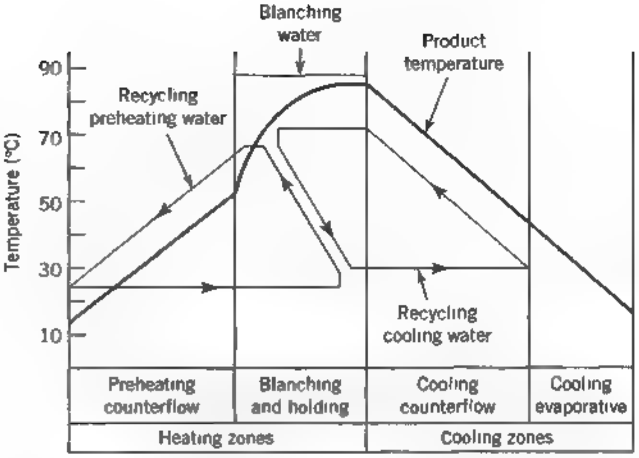


Figure 2. Representation of flow of water and temperatures in a Cabinplant integrated blancher–cooler with heat exchanger.

A study of water heating by heat exchange (68% efficient) and by steam injection (17% efficient) confirmed the energy savings potential of heating by using a heat exchanger to minimize the escape of steam rather than by steam injection (4). Energy consumption can be reduced by minimizing the flow rate of water to the blancher (5), and a comparison of energy consumptions and effluent volumes of various water blanchers is shown in Table 2.

Loss of solubles may be reduced by maximizing product to water ratio, by recycling the blanch water, and by minimizing fresh water addition. This allows the solute concentration in the blanch water to rise toward that of the vegetable cell sap, the isotonic condition thus minimizing net solute loss (6). Tissue damage may promote more rapid loss of solutes and oxidative changes, for example, in peas (7), and hence mechanically induced product movement may be significant both to nutrient quality and product break-up of the more delicate vegetables such as broccoli. The Cabinplant integrated blancher–cooker (Denmark) utilizes minimal water volumes, heat exchangers, and a design to optimize many of the above points, and is the most sophisticated water blancher available (Fig. 2) (8).

One advantage of water blanching is that processing aids such as sodium chloride and sodium bicarbonate may be added to the blanch water to obtain the preferred quality of, for example, cabbage (9). However, blanch waters can also provide a good microbial growth medium. Total microbial loads may be reduced on heating by 10^4–10^5 per gram of product, but numbers of thermoduric organisms may not be reduced. It is therefore important that blanchers be easily accessible for cleaning, be constructed of stainless steel, and that dead spaces where food splashes may collect be designed out wherever possible.

Steam Blanching

As a means of reducing leaching losses, much attention has been given to the development of steam blanchers. The first major development of steam blanching was that of the Individual Quick Blanch (IQB) system (10). This consisted of a 25-s exposure to steam of a single layer of diced carrot, followed by 50 s in a deep bed to allow equilibriation of temperature throughout the carrot pieces. A venturi thermocycle blancher was described, which was claimed to reduce steam consumption by 50%, maintain a uniform temperature throughout the blanching area, and, because of

Table 2. Comparison of Water Blanchers

Type of blancher	Product	Energy efficiency (%)	Energy (MJ/kg product)	Effluent volume or mass/ton product
Ordinary water blancher, calculated values	Peas	60		240–384 L
Ordinary water blancher	Peas		4.8	1,080–3,600 kg
Ordinary water blancher			4.5–6.8	400–1,800 kg
Tank water blancher	Spinach	30.7	1.5	
Ordinary screw blancher	Cauliflower	31.3	0.9	
Tubular blancher	Beans	34.7	0.54	
Pilot screw blancher with steam injection	Cauliflower	16.8	2.09	
Pilot screw blancher with heat exchanger	Cauliflower	67.6	0.51	
Screw blancher	Peas		2.09	4.000 L
Cabinplant blancher and cooling section	Peas		0.27	240 L

Source. Ref. 13.

the water steam traps, provide a vapor-free plant environment (11). Further development of the IQB concept involved an integrated blanching–cooling system comprising vertical helical vibratory conveyors and condensate spray cooling, although problems were encountered when handling nonparticulate material such as leafy green vegetables (12).

In spite of the improved performance of such blancher designs regarding energy and leaching, capital costs and other variable costs have been so high that the conventional water blancher has remained the most competitive system. For many large-scale operations this may no longer be true, and Table 3 compares the performance of various steam blanchers (13). The most recently developed energy efficient steam blancher is that manufactured by the Atlantic Bridge Company (ABCO), Canada (14). The principle of operation of the ABCO K2 was developed from the earlier IQB concept.

Hot-Gas Blanching

Hot-gas blanching has been studied because of the potential for reducing leaching and, in particular, for reducing effluent. One report indicated that for spinach, green beans, corn on the cob, and leeks the retention of water-soluble vitamins was much the same as for other commercial blanching operations. Excessive surface drying in some cases and the presence of atmospheric oxygen, which could promote oxidative changes, were disadvantages. Also, it was shown that operational costs could be much higher than water blanching for some vegetables (15).

Microwave and Electroconductive Blanching

A number of studies of microwave blanching have been carried out to explore the potential for reduced leaching and rapid heating, particularly for vegetables of large cross section such as potatoes, corn on the cob, and brussels sprouts (16). On an industrial scale, processing advantages have been identified, but in the past it was concluded that these were outweighed by the high electricity costs (17). Until recently microwave generators were limited to about 30 kW, with the microwave blancher incorporating steam preheating and humidity control for optimum efficiency. However, with the recent development of 60-kW generators, which also permit more uniform heating, the cost effectiveness for blanching operations needs reviewing (18). There has been limited interest to date in electroconductive blanching (19).

OBJECTIVES FOR BLANCHER CONTROL

Close control of blanch time and temperature influences uniformity of product quality as well as energy consumption. For example, where a vegetable does require blanching prior to freezing, it is now being established that the blanch treatment required depends largely on the heat stability of those enzymes directly responsible for the main deteriorative changes in a given product during frozen storage. Hence energy may be wasted if the blanching conditions are loosely controlled to inactivate peroxidase enzymes, whereas the less heat stable lipoxygenases are the relevant enzymes to inactivate (20).

Accepting that there will always be variations in the vegetable raw material in terms of the physical and thermal properties, consistently uniform blanching conditions will result in consistent blanching effects. This may be less easy to achieve where one blancher is to be used to meet the requirements for a range of vegetable products. Consistently controlled blanching also permits the prediction of the degree of enzyme inactivation (21) and of leaching losses of various solutes using suitable models for water blanching (22).

EFFECTS OF BLANCHING

Water blanchers have been shown to be the main operation contributing to the solids content of cannery wastes, and some recent studies have considered the treatment and concentration of waste blanch water. The use of water as the blanch medium also allows the addition of certain chemicals as processing aids, such as citric acid, and this is reviewed elsewhere (1).

Table 3. Comparison of Steam Blanchers

Type of blancher	Product	Energy efficiency (%)	Energy (MJ/kg product)	Effluent volume or mass/ton product
Ordinary steam blancher, calculated values	Peas	5		191–313 L
Ordinary steam blancher	Peas		6.7–9.0	200 kg
Ordinary steam blancher	Spinach	13	2.12	
Ordinary steam blancher with water cooling	Peas			5.151 L
Steam blancher with end seals	Spinach	27	0.95	
Steam blancher with water curtains	Spinach	19	1.56	
Vibratory spiral blancher	Beans	85		28 L
Individ. quick blancher	Peas			225 L
Steam blancher with end seals and steam recycling	Spinach	31	0.91	
Steam blancher with thermocompression			0.32–0.49	
IQB-prototype K1 & evap. cooling	Peas		0.27–0.32	130–150 kg
IQB-prototype K2 & evap. cooling			0.32–0.56	75–81 kg

Source Ref. 13.

Weight

In some instances weight losses can be excessive; for example, when blanching mushrooms, weight losses in excess of 19% and volume losses between 11 and 15% have been recorded (23). It has been shown that weight loss from vegetable tissue during water blanching occurs by two main mechanisms. In the typical temperature range of 50 to 55°C the cytoplasmic membranes that enclose the cell contents become disorganized, and on loss of turgor the cells contract and express some cell solution. Simultaneously, the damaged cell membranes allow free diffusion of solutes out of the cells. Because of continued diffusion of solutes out of the tissue during the blanch time, the net tissue weight loss also increases. The example of whole peas blanched in the laboratory at 85°C is shown in Figure 3. The kinetics of mushroom shrinkage have been described by three apparently first-order reactions (24). Artificially altering the water content of the vegetable prior to blanching will also influence subsequent weight loss, although the effect on the diffusional loss of solutes is small (25).

The blanching of dried vegetables has been studied since it was found that the standard rehydration step of around 18 h in cold water could be avoided by an extended blanch of 10–70 min at 71–85°C, depending on the vegetable. During this time sufficient water was taken up to compensate for lack of soaking, with resulting improved overall quality (26).

Nutrients

Some loss of nutrients occurs in all cases of blanching, but water blanching may promote excessive losses, typically up to 40% for minerals and certain vitamins, 35% for sugars, and 20% for protein (1). The most commonly measured nutrient is ascorbic acid because of its high water solubility, susceptibility to oxidation, and ease of analysis. Loss of vitamin C from spinach has been reported as being greater than 70% after 3–5-min blanching (27). Spinach blanched for a range of times at different temperatures was found to lose vitamin C and thiamin following first-order reaction kinetics (28). The decimal reduction times decreased as temperature increased, and Z values (temperature range over which the decimal reduction time changes tenfold) were about 65°C for both these vitamins.

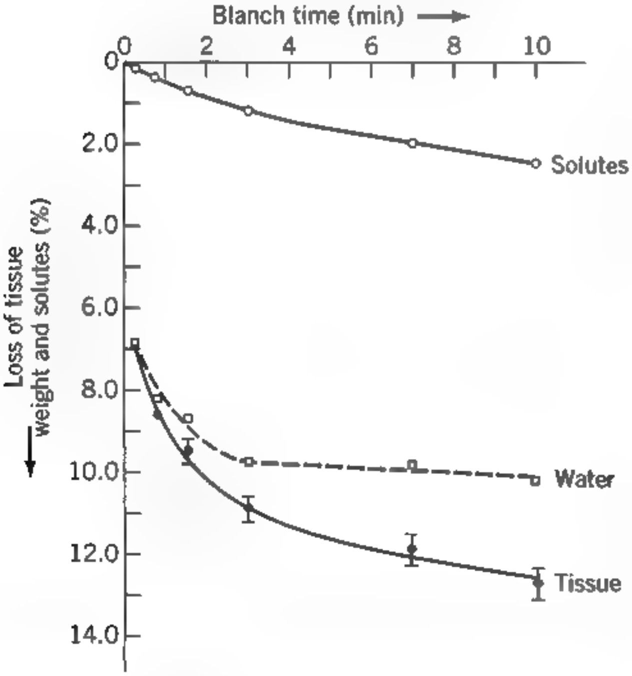


Figure 3. Percentage loss of tissue weight, solutes, and water (by difference) from whole pea samples after the given blanch time at 85°C (means of two duplicated replicates). *Source:* Ref. 25.

Losses of vitamin C from peas during water blanching have been studied extensively. Temperatures of 35–97°C for times of 15 s to 25 min were used, and the changes in ascorbic acid and dehydroascorbic acid levels were measured (7,29). It was also found that vitamin C concentration was higher in the seed coat (30). A typical 1-min blanch at 97°C resulted in 28% loss of ascorbic acid from Dark Skinned Perfection peas. A broader study showed that at 94–99°C the K1 blanch–cool system (see also Table 3) had a profound effect on ascorbic acid content of some vegetables but not others. For example, large losses were observed for peas and broccoli but not green beans. An analysis of the beans showed that ascorbic acid was concentrated in the seeds (38 mg/100 g fresh wt), which are protected by the pod (8.4 mg/100 g fresh wt) (31). This highlights the need to study the location and distribution of nutrients within various tissues and to study how the concentrations vary with maturity and variety.

Toxic Constituents

In addition to the leaching of nutrients, toxic constituents naturally present in the vegetable may also be leached. The level of nitrates in foods has caused concern because of potential toxicity, and high levels of nitrites may cause methaemoglobinaemia in infants. Nitrate levels in spinach petioles have been shown to be as much as 5 to 10 times greater than in leaves, but water blanching subsequently reduced levels by more than 50% (32). Any nitrite formed by reduction was eliminated, and it was concluded that a combination of leaf selection and blanching could reduce nitrate levels to 25% of the original content. Nitrate levels in carrots were reported for raw carrot, 339 mg nitrate/kg; water washed at 17°C, 226 mg/kg; blanched at 65°C, 187 mg/kg; blanched at 80°C, 181 mg/kg; and blanched at 95°C, 165 mg/kg (33).

The calcium uptake from blanch water influences the ratio of soluble and insoluble oxalates (32). Although excess calcium chloride in the blanch water reduced soluble oxalate in spinach, it also adversely affected color. The influence of washing and blanching on the cadmium content of several vegetables has been studied. Contents initially were 0.121–0.379 mg % for spinach, 0.029 mg % for green beans, and 0.038 mg % for peas. Although 0.1 ppm cadmium was recommended as the maximum in the fresh weight, some spinach still contained higher levels after blanching (34).

Contaminants

Blanching can significantly reduce the level of contaminating microorganisms, which is especially important prior to freezing or dehydration. A 3-min blanch of soy sprouts in boiling water reduced the total bacteria to ≤14,000/g, coliforms to <10/g, and salmonella to zero (35). However, the numbers of thermoduric organisms may not be reduced, and *Bacillus stearothermophilus* was isolated from blanch water used at 90°C for 5-min treatments prior to canning peas in an investigation of the cause of spoiled packs (36). Because blanching may render the vegetable more easily infected by microorganisms, hygienic handling is important.

The content of pesticide residues in vegetables has been studied recently. Commercial blanching was shown to remove 50% of DDT (dichlorodiphenyltrichloroethane) and 68–73% of carbonyl residue from green beans (37). The fate of di-syston has been studied during the processing of potatoes (38), and a 15-min blanch at 100°C reduced the levels of Aldrin, heptachlor epoxide, and Endrin in Irish and sweet potatoes (39). Blanching for 3 min at 100°C reduced levels of DDT isomers in turnip greens (40). The effects of water cooking on the pesticide residues in spinach have been studied (41).

Laboratory canning operations were highly effective in reducing strontium and cesium concentrations in beans and kale. However, blanching of sweet potatoes appeared to result in a transfer of radioactivity from the peel to the core, suggesting that skins of contaminated potatoes should be removed prior to thermal treatment (42).

Enzymes

Peroxidase has often been used as a blanching efficiency indicator enzyme because it is the most heat resistant enzyme and is easy to measure (43). Recent work has shown that a significant but not well-defined proportion of active peroxidase can be left in many vegetables after blanching and a long storage life can still be achieved (44). Less than 5% residual peroxidase activity did not affect quality during storage of carrot, cauliflower, french bean, onion, leek, and swede stored at −20 or −30°C for 15 months (20). Good quality was retained in carrots after storing at −20°C when palmitoyl-CoA hydrolase was inactivated by blanching, even though catalase and peroxidase activity was present. Although this hydrolase was a better indicator enzyme, there was no cheap, easy method of measurement. The complete inactivation of peroxidase does correlate well with the achievement of best quality in peas, but the best sensory quality of green beans was achieved prior to complete peroxidase inactivation.

A study on brussels sprouts found that the activities of polyphenoloxidase and peroxidase were related to the size of the sprouts, and increased from the center to the outer layers. Reducing activity to about 1% of the initial level required 7 min at 98°C for large sprouts, 5 min for medium, and 4 min for small. Residual activity increased toward the center of the sprout (45). The inactivation of lipid-degrading enzymes has also been shown to be very important to final sensory quality (46).

Color, Flavor, and Odor

The blanching operation promotes the thermal degradation of the blue-green chlorophyll pigments to the yellow-green pheophytins (47,48). This has been demonstrated in peas, and it has been shown that the addition of sodium carbonate or calcium oxide or alkalinization with sodium hydroxide during a 3-min blanch at 90°C increased chlorophyll stability. Addition of calcium chloride had the opposite effect. Iron and tin ions promoted, and copper and tin inhibited, chlorophyll decomposition; chlorophyll *b* was more resistant to heat than chlorophyll *a* (49).

Steam blanching of ground red peppers gave a major improvement in color in the first 10 min. This effect was greatest in material that had not been stored, in which the ripening process was not terminated. Because of changes in the conjugated system of carotenes, the red proportion increased and the yellow decreased (50). The effect of different types of blanch on the color of sliced carrots using hot water, in-can steam, direct steam, and microwaves resulted in similar color changes. These arose from changes in ultrastructure, especially in chromoplasts, from which liberated carotenes dissolved into lipids, and some were lost into the water (51).

The use of sodium acid pyrophosphate has been reported in blanch solutions, especially for potato and cauliflower to prevent discoloration during storage of the processed products. A cause of the discoloration may be the reaction between *ortho*-dihydroxyphenols with ferrous ions, forming a pigment in the ferric form on oxidation. Pyrophosphate is unique among condensed phosphates because of its strong ferric ion binding ability.

When peaches were microwaved a uniform browning of the skin resulted because the high levels of polyphenoloxidase in the outer fruit parts received the least heating effect. To prevent this a combination of microwave heating to blanch the inner parts and lye peeling to scald the outer parts was proposed (52).

Soluble or volatile flavor components may be lost during blanching. However, the resulting enzyme inactivation and oxygen removal may aid greater flavor retention during subsequent frozen storage. Unblanched carrot, cauliflower, and french bean developed off-odor after 9, 3, and 6 months, respectively, at −30°C (53). Unblanched onion, leek, and swede did not develop detectable off-flavor or off-odor, and no changes in total lipid content were found. In onion, no lipoxygenase or peroxidase has been found.

Structure and Texture

Blanching may induce both physical and biochemical changes in the structure and textural properties, depending on blanch time and temperature, and type and state of the vegetable. Blanching green beans at 90°C for 30 to 240 s did not seem to influence the degree of cell damage (54), and no tissue disruption in carrots was observed after blanching for 3 min at 100°C or cooking for 10 min at 100°C. This suggested that physiological and chemical rather than physical changes may have occurred to cause softness of the tissues. Subsequent freezing did cause cell disruption (55).

It has been observed that the effects of moisture and heating during blanching lead to a swelling of the cell walls, for example, of green bean pods (150–200%), and to a beginning of separation of the individual cells. This is attributed to the extraction of protopectin from the middle lamella. It was found that not only short blanching but also boiling to doneness preserved the histological structure well. No evidence of any cell wall rupture was observed during cooking, and it was concluded that earlier suggestions that cell wall rupture did occur probably arose through poor methodology in preparing sections (56).

The effects of boiling, steaming, and pressure cooking on the loss of pectic substances, and also the effect of water hardness on potato structure, have been reported (57). Steaming and pressure cooking gave similar results, but boiling resulted in significantly greater losses followed by a characteristic leveling off with time. Steaming probably caused less cell wall damage than boiling because of the lack of water to wash away degraded pectic substances. Boiled potatoes retained significantly more pectic substances when cooked with hard water. Surface to volume ratio was increased by slicing, thus increasing the rate of calcium diffusion into the tissue and of degraded pectic substance out of the tissue.

French fries have been preblanched in disodium dihydrogen pyrophosphate to sequester ions, such as calcium, and prevent discoloration, and this treatment did not reduce protopectin stability or cause deterioration of French fry texture. Low-specific-gravity potatoes were also studied because these gave a soft texture after frying. After 15 min at 70°C in 0.5% calcium chloride the best firming results were obtained, although still not equal to the firmness of high-specific-gravity potatoes (58). It was shown that blanching increased shear value and springiness but decreased hardness of asparagus. The optimum blanch times at 100°C were 135 s for stalk diameters of 16–25 mm, 120 s for 12–15 mm, 105 s for 9–11 mm, and 90 s for 6–8 mm (59).

The texture and color of beans were improved by a stepwise blanch treatment involving high and low temperatures (60). Others found that low-temperature blanching at 74°C for 20–30 min resulted in firmer carrots. Their studies confirmed the conclusion that the increase in firmness was caused by the effects of pectin methyl esterase, which was activated at low temperature and inactivated at a higher temperature (61). The rate of thermal softening of these vegetables follows first-order kinetics (62). Mung bean shoota were blanched at 75°C for 30 s to activate pectinesterase and then held at 55°C for 30 min to maximize deesterification. Such firming has been applied to tomato, potato, and cauliflower. Subsequent addition of calcium salts to the canning liquor caused insoluble calcium pectate gels in the cell walls, thus firming the cell walls (63). The noncooking effects of salts added after cooking on the texture of canned snap beans has been studied (64). Blanching may also affect the dietary fiber content of vegetables (65).

Intercellular Gases

Blanching induces the expansion and removal of intercellular gases from within vegetable tissues. This reduces the potential for subsequent oxidative changes and permits the attainment of adequate headspace vacua in cans. Air removal from the surfaces of a vegetable may change the apparent color shade. When peas of various sizes were blanched between 66 and 95°C for a few seconds up to 10 min it was found that removal of internal gases had largely occurred within the first minute. Gas volume varied from 0.47 mL/100 g grade 2 peas to 1.21 mL/100 g grade 7 peas (66).

The gas and volume changes in sliced green peppers and beans were measured during blanching in steam at 1.5 atm and in water at 100, 90, and 80°C. Initially the peppers contained 14% gas, which was reduced to 2.8–3.8% by steam or water at 100°C, but only to 3.2–5.8% at the lower temperatures. Beans initially contained 19.3%, which was reduced to 1.1–4.1% by steam or water at 100°C, and to 4.2–6.4% at the lower temperatures. Maximum gas reduction was achieved after 2 min with beans and after 1 min with peppers (67). Further data are available for red cabbage and carrots (68).

Several early blanching studies reported that steam blanching was slower than water blanching. Since the heat transfer coefficient of condensing steam is at least an order of magnitude greater than that of hot water to the same surface, it was postulated that the noncondensable gases mixed with steam accumulated at the surfaces being heated, thus interfering with the heat transfer from the steam. Blancher designs where the noncondensable gases are not swept away continually and removed from the system might be prone to a stagnant layer of gas accumulating at the surfaces, thus reducing heat transfer rate. Studies using pure steam revealed that the most important source of noncondensable gases was from the interior of the vegetables being blanched. Foaming was observed, demonstrating that heat transfer had to occur through an insulating layer of foam from the steam to the vegetable. A surface heat transfer coefficient for carrot in steam at atmospheric pressure was given as 1136 $W/m^2{\cdot}K$ (69). The work showed that the rate of heat transfer was increased by vacuum pretreatment and decreased by pressure pretreatment. Results indicated that some means of degassing vegetable particles prior to steam blanching would reduce the heating time much more effectively than increases in steam velocity.

Heat and Mass Transfer

The calculation of heat transfer rates during blanching requires a knowledge of the geometry of the vegetable piece, the blanching conditions, including the initial food temperature and blanch medium temperature, and the thermal properties of the vegetable. For unsteady-state heat conduction, numerical solutions to Fourier's general law are available for the central temperature history of the three elementary shapes; an infinite slab, an infinite cylinder, and a sphere (70). Thermokinetic models for enzyme inactivation have also been studied using unsteady-state heat conduction procedures (71).

The two contributions to the total resistance to mass transfer in the blanching of vegetables are the surface resistance due to convection and the internal resistance due to mass diffusion. These may be represented by Fick's first and second laws, together with a mass balance at the interface. When there is sufficient agitation of the blanching liquid, the surface resistance becomes small, and it can be assumed that the total resistance is due to only the internal resistance. Then only the solution to Fick's second law is required, and this is available for the infinite slab, infinite cylinder, and sphere, with the mean concentration obtained after integration with respect to position as a function of time, again in nondimensionalized form (72).

These solutions were originally developed for drying applications over a wide range of concentrations; however, in the case of blanching vegetables, the concentrations are much smaller and near to zero. The solutions have been recalculated for smaller increments in this range of concentrations using the first ten terms for the three series (6). Where the apparent diffusion coefficient for a solute is known at various temperatures it is possible to predict the changes in concentration that will occur under defined conditions of blanching (73,74).

BIBLIOGRAPHY

1. J. D. Selman, "The Blanching Process," in S. Thorne, ed., *Developments in Food Preservation*, Vol. 4, Elsevier Applied Science, London, 1986.
2. J. L. Bomben, "Effluent Generation, Energy Use and Cost of Blanching," *Journal of Food Process Engineering* **1**, 329–341 (1977).
3. A. Schaller, "Heat Utilisation in Water Blanching of Spinach Leaves," *Confructa* **17**, 264–273 (1973).
4. T. R. Rumsey, E. P. Scott, and P. A. Carroad, "Energy Consumption in Water Blanching," *Journal of Food Science* **47**, 295–298 (1982).
5. M. A. Rao, H. J. Cooley, and A. A. Vitali, "Thermal Energy Consumption for Blanching and Sterilisation of Snap Beans," *Journal of Food Science* **51**, 378–380 (1986).
6. J. D. Selman, P. Rice, and R. K. Abdul-Rezzak, "A Study of the Apparent Diffusion Coefficients for Solute Losses from Carrot Tissue during Blanching in Water," *Journal of Food Technology* **18**, 427–440 (1983).
7. J. D. Selman and E. J. Rolfe, "Effects of Water Blanching on Pea Seeds, II: Changes in Vitamin C Content," *Journal of Food Technology* **17**, 219–234 (1982).
8. M. Togeby, N. Hansen, E. Mosekilde, and K. P. Poulsen, "Modelling Energy Consumption, Loss of Firmness and Enzyme Inactivation in an Industrial Blanching Process," *Journal of Food Engineering* **5**, 251–267 (1986).
9. C. Srisangnam, D. K. Salunkhe, N. R. Reddy, and G. G. Dull, "Quality of Cabbage, I: Effects of Blanching, Freezing and Freeze-Dehydration on the Acceptability and Nutrient Retention of Cabbage," *Journal of Food Quality* **3**, 217–231 (1980).
10. M. E. Lazar, D. B. Lund, and W. C. Dietrich, "A New Concept in Blanching," *Food Technology* **25**, 684 (1971).
11. C. R. Havighorst, "Venturi Tubes Recycle Heat in Blancher," *Food Engineering* **45**, 89–90 (1973).
12. J L. Bomben, "Vibratory Blancher-Cooler Saves Water, Heat and Waste," *Food Engineering International* **1**, 37–38 (1976).
13. K. P. Poulsen, "Optimisation of Vegetable Blanching," *Food Technology* **40**, 122–129 (1986).
14. D. Atherton and J. B. Adams, "New Blanching Systems," *Technical Memorandum No. 319*, Campden Food and Drink Research Association, Chipping Campden, England, 1983
15. J. W Ralls, H. J. Maagdenberg, N. L. Yacoub, M. E. Zinnecker, J. M. Reiman, H. D Karnath, D. M Homnick, and W. A. Mercer, "In-Plant Hot-Gas Blanching of Vegetables," *Proceedings of the 4th National Symposium of Food Processing Wastes*, Environmental Protection Agency, Syracuse, N Y., pp. 178–222, 1973.
16. W. C. Dietrich, C. C. Huxsoll, and D. C. Guadagni, "Comparison of Microwave, Conventional and Combination Blanching of Brussels Sprouts for Frozen Storage," *Food Technology* **24**, 613–617 (1970).
17. A. C. Cross and D. Y. C Fung, "The Effect of Microwaves on Nutrient Value of Foods," *Critical Reviews in Food Science and Nutrition* **16**, 355–381 (1982).
18. W. Stolp, D. J. van Zuilichem, and F. Westbrook, "Microwave Blanching of Fresh Mushrooms," *Vaedingsmiddelentechnologie* **22**, 45–47 (1989)
19. R. L. Garrote, E. R. Silva, and R. A. Bertone, "Blanching of Small Whole Potatoes in Boiling Water and by Electroconductive Heating," *Lebensmittel-Wissenschaft und Tecnologie* **21**, 41–45 (1988).
20. P. Baardseth, "Quality Changes in Frozen Vegetables," *Food Chemistry* **3**, 271–282 (1978).
21. R. P. Singh and G. Chen, "Lethality-Fourier Method to Predict Blanching," in P. Linko, Y. Malkki, J. Olkku, and J. Larinkari, eds., *Food Process Engineering*, Vol. 1, Applied Science, London, 1980, pp. 70–74.
22. S. M. Alzamora, G. Hough, and J. Chirife, "Mathematical Prediction of Leaching Losses of Water Soluble Vitamins during Blanching of Peas," *Journal of Food Technology* **20**, 251–262 (1985).
23. T. R. Gormley and C. MacCanna, "Canning Tests on Mushroom Strains," *Irish Journal of Food Science and Technology* **4**, 57–64 (1980).
24. M. Konanayakam and S. K. Sastry, "Kinetics of Shrinkage of Mushrooms during Blanching," *Journal of Food Science* **53**, 1406–1411 (1988).
25. J. D. Selman and E. J. Rolfe, "Effects of Water Blanching on Pea Seeds, I: Fresh Weight Changes and Solute Loss," *Journal of Food Technology* **14**, 493–507 (1979).
26. UK Patent Application GB2088695 A (1982), I. J. Farrow, Cleary & Co. Ltd, "Blanching Dried Vegetables."
27. H. Koaze, T. Okamura, K. Ishibashi, K. Hironaka, and S. Kato, "Studies on Frozen Vegetables, IV: Effect of Blanching on Retention of Reduced Ascorbic Acid, Chlorophyll and Peroxidase Activity of Frozen Spinach," *Refrigeration (Reito)* **55**, 771–776 (1980).
28. K. Paulus, R. Duden, A. Fricker, K. Heintze, and H. Zohm, "Influence of Heat Treatment of Spinach at Temperatures up to 100°C, II: Changes of Drained Weight and Contents of Dry Matter, Vitamin C, Vitamin B1 and Oxalic Acid," *Lebensmittel Wissenschaft Technologie* **8**, 11–16 (1975).
29. J. D. Selman, "Review—Vitamin C Losses from Peas during Blanching in Water," *Food Chemistry* **3**, 189–197 (1978).
30. J. D. Selman and E. J. Rolfe, "Studies on the Vitamin C Content of Developing Pea Seeds," *Journal of Food Technology* **14**, 157–171 (1979).
31. D. B. Cumming, R. Stark, and K. A. Sanford, "The Effect of an Individual Quick Blanching Method on Ascorbic Acid Retention in Selected Vegetables," *Journal of Food Processing and Preservation* **5**, 31–37 (1981).
32. B. L. Bengtsson, "Effect of Blanching on Mineral and Oxalate Content of Spinach," *Journal of Food Technology* **4**, 141–145 (1969).
33. T. Mari and I. Binder, "Reduction of the Nitrate Content of Carrots by Various Blanching Methods," *Hütoipar* **25**, 7–9 (1978).

34. H. J. Beilig and H. Treptow, "Influence of Processing on Cadmium Content of Spinach, Green Beans and Peas," *Berichte über Landwirtschaft* **55**, 809–816 (1977).

35. G Marcy and W. Adam, "Soy Sprout Salad as a Health Hazard," *Archiv für Lebensmittelhygiene* **28**, 197–198 (1977).

36. A. C. Georgescu and M. Bugulescu, "Thermophilic Organisms in Insufficiently Sterilized Canned Peas," *Industria Alimentara* **20**, 251–253 (1969)

37. E. R. Elkins, F. C Lamb, R. P. Farrow, R. W. Cook, M. Kawai, and J. R. Kimball, "Removal of DDT, Malathion and Carbaryl from Green Beans by Commercial and Home Preparation Procedures," *Journal of Agricultural and Food Chemistry* **16**, 962–966 (1968)

38. M. G. Kleinschmidt, "Fate of Di-syston-in Potatoes during Processing," *Journal of Agricultural and Food Chemistry* **19**, 1196–1197 (1971).

39. J M. Solar, J. A. Liuzzo, and A. F Novak, "Removal of Aldrin, Heptachlor Epoxide and Endrin from Potatoes during Processing," *Journal of Agricultural and Food Chemistry* **19**, 1008–1010 (1971).

40. J. G. Fair, J. L. Collins, M. R. Johnston, and D. L. Coffey, "Levels of DDT Isomers in Turnip Greens after Blanching and Thermal Processing," *Journal of Food Science* **38**, 189–191 (1973).

41. G. Melkebeke, M. van Assche, W. Dejonckheere, W. Steurbaut, and R. H. Kips, "Effect of Some Culinary Treatments on Pesticide Residue Contents of Spinach," *Revue de l'Agriculture* **36**, 369–378 (1983).

42. C. M. Weaver and N. D. Harris, "Removal of Radioactive Strontium and Caesium from Vegetables during Laboratory Scale Processing," *Journal of Food Science* **44**, 1491–1493 (1979).

43. D. C. Williams, M. H. Lim, A. O. Chen, R. M Pangborn, and J. R. Whitaker, "Blanching of Vegetables for Freezing—Which Indicator Enzyme to Choose," *Food Technology* **40**, 130–140 (1986).

44. H. Böttcher, "The Enzyme Content and the Quality of Frozen Vegetables, II: Effects on the Quality of Frozen Vegetables," *Nahrung* **19**, 245–253 (1975).

45. E. Pogorzelski, J. Rotsztejn, and J. Berdowski, "Effect of Blanching Conditions on Polyphenoloxidase and Peroxidase Activities of Brussels Sprouts," *Przemysl Fermentauyjny i Owocowo-Warszawny* **25**, 38–39 (1981).

46. P. Baardseth and E. Naesset, "The Effect of Lipid-Degrading Enzyme Activities on Quality of Blanched and Unblanched Frozen Stored Cauliflower, Estimated by Sensory and Instrumental Analysis," *Food Chemistry* **32**, 39–46 (1989).

47. J. Abbas, M. A. Rouet-Mayer, and J. Philippon, "Comparison of the Kinetics of Two Pathways of Chlorophyll Degradation in Blanched or Unblanched Frozen Green Beans," *Lebensmittel Wissenschaft und Technologie* **22**, 68–72 (1989).

48. N. Muftugil, "Effect of Different Types of Blanching on the Color and the Ascorbic Acid and Chlorophyll Contents of Green Beans," *Journal of Food Processing and Preservation* **10**, 69–76 (1986).

49. B. Segal, "Chlorophyll Decomposition in Green Peas during Processing," *Industria Alimentara* **21**, 493–497 (1970).

50. K. Klyamov, "Effect of Blanching on Colour of Ground Red Peppers," *Bilgarski Plodove Zelenchutsi i, Konservi* **8**, 25–27 (1975).

51. S. Mirza and I. D. Morton, "Blanching of Carrots and Nutrient Loss," *Journal of the Science of Food and Agriculture* **25**, 1043 (1974).

52. C. Avisse and P. Varoquaux, "Microwave Blanching of Peaches," *Journal of Microwave Power* **12**, 73–77 (1977).

53. P. Baardseth, "Meat Products and Vegetables (Thermal Processing), in *Physical, Chemical and Biological Changes in Food Caused by Thermal Processing*, Applied Science Publishers, London, 1977, pp. 280–289.

54. C. Buonocore and G. Crivelli, "Structural Changes in Vegetable Products as a Function of Freezing Time, V: Observations on Green Beans," *Industrie Agrarie* **8**, 225–230 (1970).

55. A. R. Rahman, W. L. Henning, and D. E. Westcott, "Histological and Physical Changes in Carrots as Affected by Blanching, Cooking, Freezing, Freeze-drying and Compression," *Journal of Food Science* **36**, 500–502 (1971).

56. M. Grote and H. G. Fromm, "Fine Structural Analysis of the Morphological Changes Involved in the Blanching, Cooking, Dehydration and Rehydration of Green Bean Pod Tissue," *Zeitschrift für Lebensmittel Untersuchung und Forschung* **166**, 203–207 (1978).

57. D. E. Johnston, D. Kelly, and P. P. Dornan, "Losses of Pectic Substances during Cooking and the Effect of Water Hardness," *Journal of the Science of Food and Agriculture* **34**, 733–736 (1983).

58. A. S. Jaswal, "Effects of Various Chemical Blanchings on the Texture of French Fries," *American Potato Journal* **47**, 13–18 (1970).

59. T. Motohiro and N. Inoue, "Manufacture of Canned Asparagus, IV: Changes in Shear Value, Springiness and Hardness of Asparagus on Heat Treatment," *Journal of Food Science and Technology (Japan)* **20**, 1–4 (1973).

60. E. Steinbuch, "Technical Note: Improvement of Texture of Frozen Vegetables by Stepwise Blanching Treatments II," *Journal of Food Technology* **12**, 435–436 (1977).

61. C. Y. Lee, M. C. Bourne, and J. P. van Buren, "Effect of Blanching Treatments on the Firmness of Carrots," *Journal of Food Science* **44**, 615–616 (1979)

62. M. C. Bourne, "Effect of Blanch Temperature on Kinetics of Thermal Softening of Carrots and Green Beans," *Journal of Food Science* **52**, 667–668, 690 (1987).

63. A. J. Taylor, J. M. Brown, and L. M. Downie, "The Effect of Processing on the Texture of Canned Mung Bean Shoots," *Journal of the Science of Food and Agriculture* **32**, 134–138 (1981).

64. J. P. van Buren, "Effects of Salts Added after Cooking on the Texture of Canned Snap Beans," *Journal of Food Science* **49**, 910–912 (1984).

65. M. Nyman, K. E. Palsson, and N. G. Asp, "Effects of Processing on Dietary Fibre in Vegetables," *Lebensmittel Wissenschaft und Technologie* **20**, 29–36 (1987).

66. R. S. Mitchell, D. J. Casimir, and L. J. Lynch, "Blanching of Green Peas, II: Puncturing to Improve Efficiency of Gas Removal," *Food Technology (Champaign)* **23**, 819–822 (1969).

67. S. I. Jankov, "Physical Changes in Sliced Green Peppers, Beans and Peas during Blanching, *Confructa* **15**, 88–92 (1970).

68. C. Kluge, K. Melhardt, and L. Linke, "Studies on Gas Escape during the Thermal Treatment of Vegetables," *Lebensmittelindustrie* **33**, 234–237 (1986).

69. C. C. A. Ling, J. L. Bomben, D. F. Farkas, and C. J. King, "Heat Transfer from Condensing Steam to Vegetable Pieces," *Journal of Food Science* **39**, 692–695 (1974).

70. P. J. Schneider, *Conduction Heat Transfer*, 6th ed., Addison-Wesley, Reading, Mass., 1974.

71. J. A. Luna, R. L. Garrote, and J. A. Bressan, "Thermo-kinetic Modeling of Peroxidase Inactivation during Blanching–Cooling of Corn on the Cob," *Journal of Food Science* **51**, 141–145 (1986).
72. A. B. Newman, "The Drying of Porous Solids: Diffusion Calculations," *Transactions of the AIChE* **27**, 310 (1931).
73. G. Hough, S. M. Alzamora, and J. Chirife, "Effect of Piece Shape and Size on Leaching of Vitamin C during Water Blanching of Potato," *Journal of Food Engineering* **8**, 303–310 (1988).
74. P. Rice, J. D. Selman, and R. K. Abdul-Rezzak, "Nutrient Loss in the Hot Water Blanching of Potatoes," *International Journal of Food Science and Technology* **25**, 61–65 (1990).

GENERAL REFERENCES

"Steaming into the Future," *Potato Business World* **6**, 19–25 (1998).
S. Boyes et al., "Microwave and Water Blanching of Corn Kernels: Control of Uniformity of Heating During Microwave Blanching," *Journal of Food Processing and Preservation* **21**, 461–484 (1997).
J. I. Mate et al., "Effect of Blanching on Structural Quality of Dried Potato Slices," *J. Agric. Food Chem.* **46**, 676–681 (1998).
S. Meenakshi, B. Raghavan, and K. O. Abraham, "Processing of Marjoram (*Marjorana hortensis Moensch.*) and Rosemary (*Rosmarinus officinalis L.*). Effect of Blanching Methods on Quality," *Nahrung* **40**, 264–266 (1996).
G. Prestemo, C. Fuster, and M. C. Risueno, "Effects of Blanching and Freezing on the Structure of Carrot Cells and Their Implications for Food Processing," *J. Sci. Food Agric.* **77**, 223–229 (1998).
A. R. Quinero et al., "Optimization of Low Temperature Blanching of Frozen Jalapeno Pepper (*Capsicum annuum*) Using Response Surface Methodology," *J. Food Sci.* **63**, 519–522 (1998).
H. S. Raswamy and M. O. Fakhouri, "Microwave Blanching: Effect on Peroxidase Activity, Texture and Quality of Frozen Vegetables," *Journal of Food Science and Technology India* **35**, 216–222 (1998).
U.S. Patents 752,431,730,717 (1998), D. R. Zittel.

JEREMY D. SELMAN
Campden Food & Drink Research Association
Campden, Gloucestershire
United Kingdom

BLOOD. See ANIMAL BY-PRODUCT PROCESSING; KOSHER FOODS AND FOOD PROCESSING.

BLUEBERRIES. See FRUITS, TEMPERATE.

BOTULISM. See FOODBORNE DISEASES.

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Bovine spongiform encephalopathy (BSE, mad cow disease), a progressive degenerative disorder of the central nervous system of cattle, was first recognized in the United Kingdom (UK) in November 1986 (1). Believed to be caused by a newly classified proteinaceous infectious particle, a prion, BSE is one of several recognized fatal transmissible spongiform encephalopathies (TSEs) found in various species. The epizootic of BSE in the UK affected more than 170,000 cattle, 70 domestic cats, and some zoo animals and is believed to have resulted in 40 human cases of variant Creutzfeldt-Jakob disease (v-CJD, also known as new variant or nv-CJD). The human cases resulted from the ingestion of beef contaminated with the BSE agent.

OVERVIEW

TSEs disrupt the function of nerve cells. In TSEs there are characteristic vacuolar degeneration of neurons in the brain and spinal cord, giving the tissue a spongelike appearance (Fig. 1). TSEs evoke no immune response and are inevitably fatal (2–5).

The incubation period (ie, the time between exposure and the onset of clinical signs) in cattle is very long, rang-

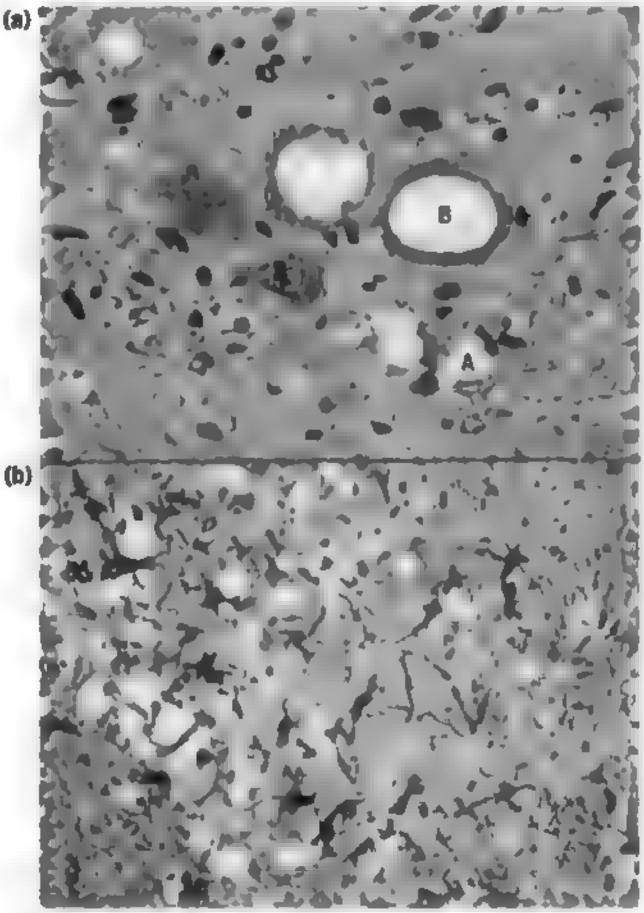


Figure 1. Lesions in the gray matter of the brain of a sheep with scrapie: (A) typical spongiform change in neurons; (B) spongiform change and astrocytic hypertrophy and hyperplasia. (**a**) Hematoxylin and eosin stain; (**b**), glial fibrillar acid protein (GFAP) stain. Magnification ×500. *Source:* Courtesy of Dr. Robert Higgins, School of Veterinary Medicine, University of California, Davis.

71. J. A. Luna, R. L. Garrote, and J. A. Bressan, "Thermo-kinetic Modeling of Peroxidase Inactivation during Blanching–Cooling of Corn on the Cob," *Journal of Food Science* **51**, 141–145 (1986).
72. A. B. Newman, "The Drying of Porous Solids: Diffusion Calculations," *Transactions of the AIChE* **27**, 310 (1931).
73. G. Hough, S. M. Alzamora, and J. Chirife, "Effect of Piece Shape and Size on Leaching of Vitamin C during Water Blanching of Potato," *Journal of Food Engineering* **8**, 303–310 (1988).
74. P. Rice, J. D. Selman, and R. K. Abdul-Rezzak, "Nutrient Loss in the Hot Water Blanching of Potatoes," *International Journal of Food Science and Technology* **25**, 61–65 (1990).

GENERAL REFERENCES

"Steaming into the Future," *Potato Business World* **6**, 19–25 (1998).

S. Boyes et al., "Microwave and Water Blanching of Corn Kernels: Control of Uniformity of Heating During Microwave Blanching," *Journal of Food Processing and Preservation* **21**, 461–484 (1997).

J. I. Mate et al., "Effect of Blanching on Structural Quality of Dried Potato Slices," *J. Agric. Food Chem.* **46**, 676–681 (1998).

S. Meenakshi, B. Raghavan, and K. O. Abraham, "Processing of Marjoram (*Marjorana hortensis Moensch.*) and Rosemary (*Rosmarinus officinalis L.*). Effect of Blanching Methods on Quality," *Nahrung* **40**, 264–266 (1996).

G. Prestemo, C. Fuster, and M. C. Risueno, "Effects of Blanching and Freezing on the Structure of Carrot Cells and Their Implications for Food Processing," *J. Sci. Food Agric.* **77**, 223–229 (1998).

A. R. Quinero et al., "Optimization of Low Temperature Blanching of Frozen Jalapeno Pepper (*Capsicum annuum*) Using Response Surface Methodology," *J. Food Sci.* **63**, 519–522 (1998).

H. S. Raswamy and M. O. Fakhouri, "Microwave Blanching: Effect on Peroxidase Activity, Texture and Quality of Frozen Vegetables," *Journal of Food Science and Technology India* **35**, 216–222 (1998).

U.S. Patents 752,431,730,717 (1998), D. R. Zittel.

JEREMY D. SELMAN
Campden Food & Drink Research Association
Campden, Gloucestershire
United Kingdom

BLOOD. See ANIMAL BY-PRODUCT PROCESSING; KOSHER FOODS AND FOOD PROCESSING.

BLUEBERRIES. See FRUITS, TEMPERATE.

BOTULISM. See FOODBORNE DISEASES.

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Bovine spongiform encephalopathy (BSE, mad cow disease), a progressive degenerative disorder of the central nervous system of cattle, was first recognized in the United Kingdom (UK) in November 1986 (1). Believed to be caused by a newly classified proteinaceous infectious particle, a prion, BSE is one of several recognized fatal transmissible spongiform encephalopathies (TSEs) found in various species. The epizootic of BSE in the UK affected more than 170,000 cattle, 70 domestic cats, and some zoo animals and is believed to have resulted in 40 human cases of variant Creutzfeldt-Jakob disease (v-CJD, also known as new variant or nv-CJD). The human cases resulted from the ingestion of beef contaminated with the BSE agent.

OVERVIEW

TSEs disrupt the function of nerve cells. In TSEs there are characteristic vacuolar degeneration of neurons in the brain and spinal cord, giving the tissue a spongelike appearance (Fig. 1). TSEs evoke no immune response and are inevitably fatal (2–5).

The incubation period (ie, the time between exposure and the onset of clinical signs) in cattle is very long, rang-

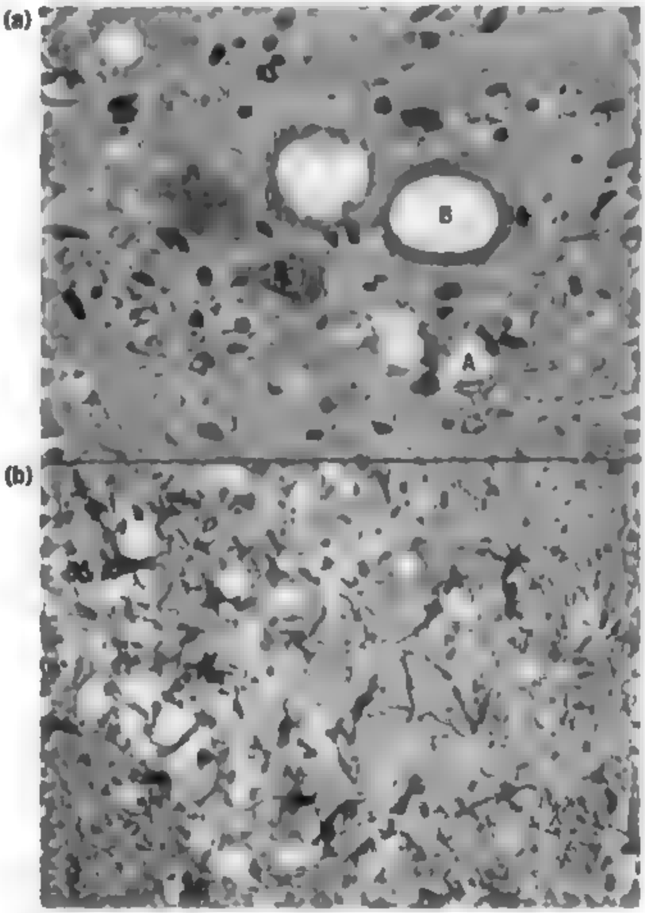


Figure 1. Lesions in the gray matter of the brain of a sheep with scrapie: (A) typical spongiform change in neurons; (B) spongiform change and astrocytic hypertrophy and hyperplasia. (**a**) Hematoxylin and eosin stain; (**b**), glial fibrillar acid protein (GFAP) stain. Magnification ×500. *Source:* Courtesy of Dr. Robert Higgins, School of Veterinary Medicine, University of California, Davis.

ing from two to eight years. Most animals show clinical signs between three and five years of age and demonstrate a slowly progressive disease with nonspecific neurological signs, including incoordination, weakness, tremors, and anxiety. Once signs develop, animals usually die within two weeks to six months.

Epidemiologically, BSE has been considered an "expanded point source epizootic"; that is, it resulted from the consumption of contaminated protein feed supplements (6). Meat and bone meal (MBM) produced from rendered carcasses and offal from dead and butchered livestock has been a common protein supplement used for decades in livestock production. Changes in rendering practices in the late 1970s and early 1980s were considered a risk factor in amplifying the disease through the resulting manufacture of infectious MBM feed supplements (6–10).

About 2000 cattle have been diagnosed with the disease in other countries, some as the result of exportation from the UK of live cattle incubating the disease, and the majority from exportation of contaminated MBM subsequently fed to native cattle (7–9).

More than 170,000 cattle from more than 33,500 British herds (67% of all dairy herds, 16% of all beef herds) have been confirmed with the disease. The low average within-herd incidence, with 35% of the farms experiencing only one case, and 69% with four or fewer cases, is attributed to uneven exposure to the agent in widely spaced batches of feed (6).

In 1996 a possible link was made between BSE in cattle and 10 cases of Creutzfeldt-Jakob disease (CJD) in humans (11). To date 29 humans have been confirmed with v-CJD (12), shown to share identical lesion patterns and chemical characteristics with BSE (13–15). Evidence strongly supports the hypothesis that v-CJD in people and BSE in cattle are the same disease, with transmission to people occurring through the ingestion of infected beef products (16,17).

Perishable foods are especially vulnerable to acute crises in consumer confidence. Following the March 1996 announcement of the possible link between BSE and human disease, British beef consumption fell for more than a month to 25% of its preannouncement level, and the European Union (EU) banned British beef imports. Global markets, including those in countries without BSE, have been impacted by this epizootic.

Beginning in July 1988 the UK responded to the cattle epizootic with a series of control measures. Mandatory programs were enacted requiring euthanasia of affected cattle, prohibiting the use of affected cattle carcasses or specified offal from sheep or cattle in feeds, and eventually banning MBM from all agricultural uses. A selective cull program was instituted targeting herds that had BSE cases and removing nonaffected cattle that might have been shared exposure to the agent. Current projections are for BSE eradication between 2001 and 2005.

Measures to protect human health began in 1989 with the Specified Offal Ban (SBO), prohibiting the use of brain, spinal cord, tonsil, thymus, spleen, and intestinal tissues of cattle origin in foods intended for human consumption. Additional measures, requiring meat from animals over 6 months of age to be deboned prior to sale, and banning most cattle over 30 months of age from use as human food, were enacted.

By-products from slaughtering cattle are used in horticultural applications, pet foods, cosmetics, and pharmaceuticals. Those tissues not meeting specified standards are prohibited from use in manufacturing these products and are now incinerated.

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

TSEs are caused by similar, but not identical, agents and produce specific and characteristic lesions in the brain of affected hosts. The apparent resistance/susceptibility factors in animals and humans are not yet fully understood, and multiple "strains" have been identified in some TSEs, including scrapie (2,3,13,14,17). All TSEs have long incubation periods, evoke no immune response, and are inevitably fatal.

TSEs in animals include cattle BSE, scrapie of sheep and goats, transmissible mink encephalopathy, and chronic wasting disease of mule deer and elk (6,9,18). Confirmed cases in kudus, a gemsbok, an eland, a nyala, an oryx, an Ankole cow, cheetahs, pumas, a tiger, ocelots, and more than 70 domestic cats are also believed to be BSE caused by the feeding of contaminated protein supplements (19).

Human TSEs include kuru, Creutzfeldt-Jakob disease, Gerstmann–Straussler–Scheinker syndrome, and fatal familial insomnia. Human TSEs are heritable in about 15% of the cases and are considered sporadic in about 85% of the cases. Worldwide a few hundred cases have been associated with the use of tissues from infected donors (corneal transplants, cadaveric dura mater, and human pituitary extracts used to prepare growth and gonadotropin hormones), or contaminated intracranial electrodes and neurosurgical instruments. Kuru, a TSE associated with the ritual handling and cannibalistic consumption of the brain of deceased tribal members in a region of New Guinea, with a known incubation period up to 30 years, has all but disappeared due to changes in local customs (2,3).

BSE LINK TO HUMAN DISEASE

In 1990 the UK established the Spongiform Encephalopathy Advisory Committee to advise on the potential implications TSEs have on advised the government that it had become concerned about 10 cases of Creutzfeldt-Jakob disease with onset of symptoms in 1994 and 1995. These cases were unusual because they included patients younger than the expected (average age 27 vs 63 for sporadic CJD cases). In addition, the neuropathology and clinical course in these patients was distinguishable from that in other CJD patients. A review of medical histories, genetic analysis, and consideration of other possible causes of CJD "failed to explain these cases adequately" (4,5,11).

As many as 900,000 cattle might have been infected between 1984 and 1995, with 450,000 entering the human food chain prior to 1989 and 283,000 between 1989 and 1995 (6). Cattle are slaughtered at young ages and BSE's long incubation period would preclude most infected animals from showing any clinical signs. Cattle in the earlier stages of incubation are expected to have had much lower accumulations of infectious agent in their brain and spinal cord.

There have been no confirmed cases of v-CJD in people with occupations expected to have the highest risk of exposure, including farmers, abattoir workers, butchers, and veterinarians. The number of new cases of all types of CJD in the UK remains stable, at about one new case per million people per year. This rate is about the same in all countries, including Australia and New Zealand, where neither scrapie nor BSE have been identified. Currently 29 people, one in France and the remainder in the UK, are believed to have v-CJD (13). The proposed link between BSE and v-CJD was eventually substantiated (13,14). There are no predictions of the number of human victims that might eventually result.

THE CAUSATIVE AGENT

Prions and the "Protein Only" Hypothesis

Prions, the name coined by Stanley Prusiner for proteinaceous infectious particles, have changed our understanding of infectious agents. All previously recognized pathogenic agents use nucleic acid, either DNA or RNA, to reproduce. Prions are abnormal structural variants of normal cellular proteins found in the membranes of neurons and other tissues (2,3).

Although scientific opinion generally supports Prusiner's 1982 hypothesis that TSEs are caused by abnormal prion proteins, other research groups continue looking for an unrecognized organism with nucleic acid (3), and one group continues to promote *Spiroplasma* sp. as a potential causative agent (20).

Prions are single molecules of about 250 amino acids and contain no nucleic acid. Abnormal prion protein is formed within the host by genetic mutation (eg, familial CJD), or by unknown means (eg, sporadic CJD), or enters the body via oral or other routes (eg, BSE, kuru, scrapie, etc), and proceeds to convert by contact normal protein into structurally aberrant forms. The conversion of normal cellular prion protein to the distorted prion conformation is characterized by a structural change from predominantly alpha helices to beta sheets (alpha helix:beta sheet 42:3 in normal prion protein, 30:43 in abnormal) (3,21). The distorted form has increased resistance to natural proteases and, as a result, accumulates in tissues, contributing to neuronal death and spongiform changes in the brain. Amyloid plaques are formed from remnants of prion protein in some, but not all, forms of TSE disease.

TSEs such as scrapie have been shown to exist as different "strains," evidenced by different incubation periods, lesion patterns, and chemical characteristics. Historically, strain differences in pathogenic organisms have always been associated with genetic differences represented by varying nucleic acid sequences (3). Strain differences in prion diseases appear to be from conformational differences of the same protein molecule. Functional behavior of the molecule is linked to the conformation and may influence susceptibility/resistance, infectious dose, virulence, disease pattern, and incubation period (13,14,17).

Transmission

Variant CJD is believed to have been transmitted by consuming infected beef products such as sausages, franks, and hamburgers that contained portions of brain, spinal cord, dorsal root ganglia, retina, intestines, or bone marrow as components. A list of Specified Risk Materials (SRM) has been adopted by the European Union (EU) Scientific Steering Committee as a result of calf assay experiments using various tissues from known infected animals (Table 1; 22).

Prions are extremely resistant to heat, ultraviolet light, ionizing radiation, and common disinfectants that normally inactivate viruses and bacteria, and destroy nucleic acid (2,3,23–25). Contaminated surgical instruments have been shown to retain infectivity following standard hospital sterilization techniques. Transplanted tissues from humans with CJD, including dura mater grafts, extracts from pituitary glands, and corneas, have been demonstrated vehicles of transmission. Strategies to protect the blood supply, including provisions to deplete blood products of leukocytes because these white blood cells may play a role in the transport of ingested abnormal prions to the nervous system, are currently under consideration in the UK (26).

Kuru was transmitted by ingestion and through open cuts or abrasions. Scrapie in sheep has been transmitted through open wounds and new infections have been associated with lambing of normal animals with infected animals. Although the majority of cattle infections resulted from ingestion of contaminated protein feed supplements, studies on potential maternal transmission report a small risk of infection for calves born shortly before or after the onset of clinical signs of the disease in infected dams. Experts indicate this rate is insufficient to perpetuate the epizootic in cattle but will delay the eradication effort (6,27).

Table 1. Levels of TSE Infectivity Associated with Various Ruminant Tissues

Category	Organs
High infectivity	Bovine brain, eyes, spinal cord and dorsal root ganglia; *dura mater,* pituitary, skull and bovine vertebral column, lungs ovine/caprine brain, eyes, and spinal cord, dorsal root ganglia and vertebral columns; ovine and caprine spleens, lungs
Medium infectivity	Total intestine from duodenum to rectum, tonsils Bovine and caprine spleen, placenta, uterus, fetal tissue, adrenal, cerebrospinal fluid, lymph nodes
Low infectivity	Liver, pancreas, thymus, bone marrow, other bones, nasal mucosa, peripheral nerves
No detected infectivity	Skeletal muscle, heart, kidney, colostrum, milk, discrete adipose tissues, salivary gland, saliva, thyroid, mammary gland, ovary, testis, seminal testes, cartilaginous tissue, skin, hair, blood clot, serum, urine, bile, feces

Source: Ref. 22.

The persistence of chronic wasting disease of mule deer and elk raises questions about possible methods of lateral transmission between unrelated animals. There is a small risk of transmission to uninfected lambs if they are born in the immediate physical vicinity of infected ewes also giving birth.

Susceptibility

The amino acid sequence in the prion protein of cattle differs from that of sheep at 7 positions, and that of humans at 30 positions. It is thought that greater differences provide a more formidable "species barrier" to transmission. Scrapie strains have not been shown to transmit to cattle; however, BSE has been experimentally transmitted to sheep and several other species. Prions from cattle and humans, but not sheep, share specific amino acid sequences in two areas of the protein, possibly accounting for the transmission in spite of the amino acid sequence differences (2,3,21,28).

All cases of v-CJD have been recorded in people homozygous for methionine at prion protein codon 129. This genotype is found in 38% of the UK population. Genotypes that are heterozygous for methionine/valine (UK frequency 51%), or homozygous valine (UK frequency 11%), may have different incubation periods or increased resistance to infection (15,29).

BSE has a characteristic pattern of lesions in all species examined when changes are mapped by region of the brain. Also, protein digestion patterns of BSE prion are identical between species and differ from other TSEs. Other TSEs, however, demonstrate "strain" differences using these tests and in clinical progression of the disease. Strain differences are believed to represent differing molecular conformations of identical proteins (13,14).

Prevention and Treatment

Both normal and distorted prion proteins fail to stimulate any detectable host immune response, and no inflammatory reaction is associated with accumulations of abnormal prion protein in neurons. The agent presents special challenges to those attempting to develop effective drugs for treatment and vaccines for prevention.

A reliable antemortem test is an area of special need, and a number of promising methods are under evaluation. The long incubation period contributes to the difficulty of validating the various tests being developed. Ultimately the determination of the risk from medical products incorporating organs, cells, sera, and bovine by-products, and that from various food products, depends on sensitive and specific antemortem testing methods.

THE BSE EPIZOOTIC

First recognized in November of 1986, with retrospective histological evidence of the first case dating from November 1985, the BSE epizootic peaked in 1992 with more than 1000 cattle cases confirmed per week (see Fig. 2). Reviews of archived tissue samples from cattle with undiagnosed disease have failed to identify any unrecognized cases prior to 1985 (1,6).

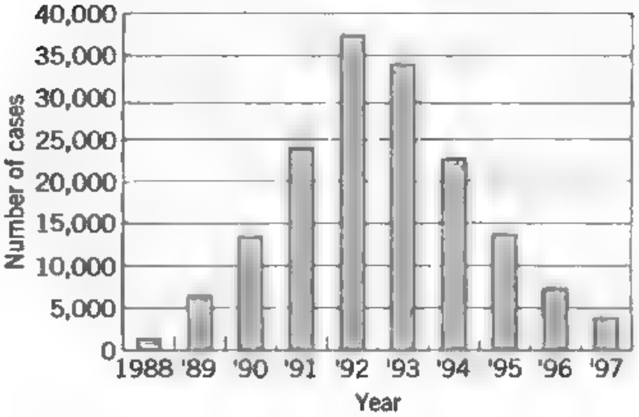


Figure 2. Confirmed cases of BSE in UK cattle by year.

Epidemiological studies suggest that UK cattle became infected through exposure to contaminated MBM feed supplements in 1981 to 1982 (6). Although the original source of the contamination is unknown, the most accepted theory proposes that sheep scrapie prions completed a species jump and induced normal cattle prion protein to become distorted (6–9). This distorted cattle prion protein distorts others, eventually resulting in clinical disease. Affected animals were slaughtered or died, rendered, and the resulting MBM containing abnormal cattle prion protein was fed to other cattle. The UK sheep population has a high enzootic rate of scrapie, and a high ratio of sheep to cattle (45 million to 12 million). This resulted in sheep representing 14% of rendered products, compared with 0.6% in the United States. The higher level of MBM supplements fed to young calves in the UK (2–4% MBM in rations) may explain the high incidence of BSE as compared with other EU countries (30).

A second theory suggests that cattle have always had a low incidence of BSE, perhaps at a rate similar to the 1:1,000,000/year incidence of CJD found in humans, which was unrecognized (31). Changes in rendering practices in the late 1970s and early 1980s no longer destroyed infectivity sufficiently to prevent accumulation of the necessary infectious dose.

A third hypothesis, offered in an effort to explain the rapid appearance and spread of the epizootic, theorizes importation of the agent to the UK in MBM containing abnormal proteins from an unknown ruminant other than cattle (32). The original source would have to be a country or countries lacking the infrastructure necessary to identify clinical cases and with rendering systems inadequate to destroy the agent. The imported material would need to be in sufficient quantity, or commingled with domestic MBM, to result in the volumes of contaminated product with infectious dose levels necessary to account for the epizootic.

Whatever the original source, changes in British rendering methods in the late 1970s and early 1980s are proposed to have played a role in the widespread amplification of the disease (6–8). The solvent extraction step with steam recovery was eliminated, and a liquid slurry process was introduced (33). Both changes may have resulted in decreased exposure of materials to heat during rendering. Critics note that TSEs such as scrapie have been demonstrated to be resistant to solvents, and the temperatures used (about 100°C) are too low to inactivate the agent (23,24,32). The impacts from these changes in the rendering system on the epizootic are unknown. The use of MBM in cattle rations as a source of supplemental protein had been a common practice for decades prior to the emergence of BSE, and scrapie is known to have been endemic in the UK for more than 250 years.

BSE has been confirmed in native cattle in Ireland, France, Portugal, the Netherlands, and Switzerland. Exported British cattle have been found with the disease in Oman, the Falkland Islands, Germany, Denmark, Canada, and Italy (1). The export of rendered animal feed products from the UK is considered the source of the disease outbreaks in native cattle in other countries. There are concerns that the number of cattle, and the amount of feed, exported should have resulted in significantly more disease than reported (30). There is confusion about whether the expected cases failed to occur for unknown reasons, went unrecognized within the surveillance systems in place, or were unreported.

ACTIVITIES TO PROTECT THE FOOD SUPPLY

In the UK

In July 1988, with about 2000 cattle cases confirmed, and in response to a just completed epidemiological investigation implicating MBM (6), the UK made BSE a notifiable disease. Euthanasia of affected cattle became mandatory, and their carcasses were banned from use in feed supplements. A few months later the carcass ban was expanded to include offal from sheep and cattle, although strict enforcement was not achieved. In 1989 the Specified Bovine Offal (SBO) ban prohibited the use of brain, spinal cord, tonsil, thymus, spleen, and intestinal tissues of cattle origin in foods intended for human consumption and fertilizer manufacturing.

Beginning in 1991 controls on MBM went through a number of iterations, starting with prohibitions from use in ruminant feeds, and eventually resulting in a ban from all agricultural use. In August 1996 mammalian MBM was made illegal to possess or use in any part of the food chain including on-farm, in transit, or at feed mills. A surveillance system was imposed including statistical sampling of protein supplements for the presence of ruminant and porcine proteins, and sheep and goat heads, spinal cords, and spleens were banned from rendered use.

Special programs for calf processing were initiated to prevent male calves less than 20 days old from being used for human food. Data showing that cattle did not harbor significant amounts of abnormal prion protein until later in life led to the "Over Thirty Month scheme" (OTM, OTMS), which prohibited the slaughter of cattle over 30 months of age for human food. Beef cattle from BSE-free herds, and reared on grass, are considered at very low risk of the disease and can be exempted and slaughtered for human consumption up to 42 months of age.

In 1997, the selective cull program was initiated, requiring all cattle born on farms six months before and after a confirmed case of BSE to be removed from the herd, euthanized, and handled as if they are infected. No replacements are allowed onto the farm until all such animals have been removed. Additional restrictions required all meat from cattle more than 6 months of age to be deboned as a precaution to prevent any nervous tissue or bone marrow from being consumed.

By-products from slaughtering cattle approved for use as human food are considered "clean" and approved for use in horticultural applications, pet food, cosmetics, and pharmaceuticals, or they can be buried in landfills. All other by-products are considered "dirty" and are stained to prevent accidental use, incinerated at 1000°C for sufficient time to result in a protein-free ash, and then disposed of in landfills. No rendered ruminant products can be used for agricultural purposes.

The number of new cases of BSE has fallen from its peak in 1992 of 900 to 1000 confirmed diagnoses per week, to less than 200 per week in 1998. The UK expects to eradicate the disease between the years 2001 and 2005 (1,9,27).

In the European Union

On March 27, 1996, the EU imposed a ban on exports from the UK of beef, live bovine animals, semen, embryos, and bovine products (including MBM) that might enter the human or animal food chains or be used in pharmaceuticals or cosmetics. In December of 1996, the UK published the Export Certified Herds Scheme (ECHS) to enable documentation that an animal was from a BSE-free herd. In March of 1998, EU farm ministers approved the ECHS, permitting legal exports of beef from Northern Ireland from June 1, 1998. The European Commission then proposed to lift the export ban for the rest of the UK, as applied to animals born after August 1, 1996 (the date the MBM ban became fully effective).

In the United States

The United States has not imported processed beef, live cattle, semen, embryos, MBM, or other beef products from the UK since 1989. In 1990 the U.S. Department of Agriculture (USDA) began tracing the 496 UK cattle imported to the United States between 1981 and 1989 and found no traces of BSE among those they were able to locate. A surveillance program was begun that has examined thousands of brain tissue samples from slaughtered animals across 43 states without finding any evidence of BSE.

In 1991 the USDA began enforcing stringent restrictions on importations from other countries either known to have had cases of BSE or considered to have inadequate surveillance systems to detect the disease. The rendering of sheep for use in animal feed was discouraged, and risk analysis activities were pursued. In 1993 the slaughter surveillance program was expanded to include "downer" cattle in response to reports suggesting disabled cattle, those that are unable to stand without assistance, represent unrecognized cases of BSE. Downer cattle cannot stand for many different reasons including metabolic diseases, injured musculoskeletal systems, or systemic illness resulting in general weakness. BSE-affected cattle may be downers, but downers are not necessarily related to BSE.

On March 29, 1996, livestock and professional animal health organizations in the United States requested an American ban on the use of ruminant protein in ruminant feeds. In addition, these groups requested increased national surveillance, enhanced BSE educational activities, and expanded cooperative research efforts to provide extra measures to prevent the introduction of BSE into the United States. Replacing animal origin proteins for feeds in the United States will require about 3 million additional acres of soybean production (25).

In 1997 USDA banned the importation of live ruminants and most ruminant products from European countries, and the Food and Drug Administration announced a permanent prohibition of specified substances for use in ruminant feed.

GLOSSARY

Amino acids. Organic compounds serving as the building blocks of proteins.

BSE. Bovine spongiform encephalopathy.

Chronic wasting disease. A TSE of mule deer and elk.

CJD. Creutzfeld-Jakob disease of humans.

Codon. The sequence of three chemical units in nucleic acid that determines the specific amino acid formed in protein synthesis.

Dura matter. The membranous covering of the brain.

Enzootic. Diseases continuously prevalent in animals.

Epizootic. Diseases spreading widely among animals.

Fatal familial insomnia. A TSE of humans.

Genotype. The genetic makeup of an organism, as distinguished from its appearance.

GSS. Gerstmann–Strausser–Sheinkers syndrome, a TSE of humans.

Heterozygous. Differing genes for one characteristic.

Homozygous. Identical genes for one characteristic.

Kuru. A TSE of humans.

Leukocytes White blood cells.

Maternal transmission. Spread of a disease from dam to offspring before birth.

MBM. Meat and bone meal.

Nucleic acid. DNA or RNA.

Offal. Waste products of animals slaughtered for food.

OTM. Over thirty months.

OTMS. Over thirty months scheme.

Prions. Proteinaceous infectious particles.

Rendering. To melt fat or other animal matter.

Scrapie. A TSE of sheep and goats.

SBO. Specified bovine offal.

Species barrier. A hypothetical barrier delaying a TSE of one species from inducing disease in another species.

Species jump. A TSE of one species inducing disease in another species.

SRM. Specified risk material.

SEAC. Spongiform Encephalopathy Advisory Committee.

Transmissable mink encephalopathy. A TSE of mink.

TSE. Transmissible spongiform encephalopathy.

BIBLIOGRAPHY

1. British Ministry of Agriculture, Fisheries and Food, *A Progress Report on Bovine Spongiform Encephalopathy in Great Britain*, 1996, 25 pp.
2. S. B. Prusiner, "Prions," *Sci. Am.* **251**, 50–59 (1984).
3. S. B. Prusiner, "The Prion Diseases," *Sci. Am.* **272**, 47–57 (1995).
4. J. G. Collee and R. Bradley, "BSE—A Decade on—Part 1," *Lancet* **349**, 636–641 (1997).
5. J. G. Collee and R. Bradley, "BSE—A Decade on—Part 2," *Lancet* **349**, 715–721 (1997).
6. R. M. Anderson et al., "Transmission Dynamics and Epidemiology of BSE in British Cattle," *Nature* **382**, 779–788 (1996).
7. J. W. Wilesmith et al., "Bovine Spongiform Encephalopathy: Epidemiological Studies," *Vet. Rec.* **123**, 638–644 (1988).
8. J. W. Wilesmith et al., "BSE: Epidemiological Studies on the Origin," *Vet. Rec.* **128**, 199–203 (1991).
9. J. W. Wilesmith et al., "Bovine Spongiform Encephalopathy: Epidemiological Features 1985 to 1990," *Vet. Rec.* **130**, 90–94 (1992).
10. I. H. Pattison, "Origins of BSE," *Vet. Rec.* **128**, 262–263 (1991).
11. R. G. Will et al., "A New Variant of Creutzfeldt-Jacob Disease in the UK," *Lancet* **347**, 921–925 (1996).
12. National CJD Surveillance Unit, Creutzfeldt-Jakob Disease Surveillance in the UK, Seventh Annual Report," URL: *www.CJD.ed.ac.uk/rep98.html* (last accessed April 19, 1999).
13. J. Collinge et al., "Molecular Analysis of Prion Strain Variation and the Aetiology of 'New Variant' CJD," *Nature* **383**, 685–690 (1996).
14. M. Bruce et al., "Transmissions to Mice Indicate That 'New Variant' CJD Is Caused by the BSE Agent," *Nature* **389**, 498–501 (1997).
15. J. Almond and J. Pattison, "Human BSE," *Nature* **389**, 437–438 (1997).
16. World Health Organization, *Report of a WHO Consultation on Public Health Issues Related to Human and Animal TSE With the Participation of FAO and OIE*, World Health Organization, Geneva, Switzerland, 1996.
17. G. J. Raymond et al., "Molecular Assessment of the Potential Transmissibility of BSE and Scrapie to Humans," *Nature* **388**, 285–288 (1997).
18. T. R. Spraker et al., "Spongiform Encephalopathy in Free-Ranging Mule Deer (*Odocoileus hemionus*), White-Tailed Deer (*Odocoileus virginianus*), and Rocky Mountain Elk (*Cervus elaphus nelsoni*) in North Central Colorado," *Journal of Wildlife Diseases* **33**, 1–6 (1997).
19. J. K. Kirkwood and A. A. Cunningham, "Epidemiological Observations on Spongiform Encephalopathies in Captive Wild Animals in the British Isles," *Vet. Rec.* **135**, 296–303 (1994).

20. F. O. Bastian, "Bovine Spongiform Encephalopathy: Relationship to Human Disease and Nature of the Agent," *ASM News* **59**, 235–240 (1993).

21. S. B. Prusiner and M. R. Scott, "Genetics of Prions," *Annu. Rev. Genet.* **31**, 139–175 (1997).

22. EU Scientific Steering Committee, "Listing of Specified Risk Materials: A Scheme for Assessing Relative Risks to Man. Opinion of the Scientific Steering Committee," adopted Dec. 9, 1997, URL: *http://www.easynet.co.uk/ifst/hottop5c.htm.*

23. D. M. Taylor, "Survival of Mouse-Passaged Bovine Spongiform Encephalopathy Agent After Exposure to Paraformaldehyde-Lysine-Periodate and Formic Acid," *Vet. Microbiol.* **44**, 111–112 (1995).

24. D. M. Taylor, "Deactivation of BSE and Scrapie Agents: Rendering and Other UK Studies," in Editor(s) name(s), *Transmissible Spongiform Encephalopathies*, Scientific Veterinary Committee of European Communities, Brussels, Belgium, 1995, pp. 205–223

25. D. Harlan, "Risk Reduction—An Ounce of Prevention," *International Symposium on Spongiform Encephalopathies: Generating Rational Policy in the Face of Public Fears*, The Ceres Forum, Georgetown University, December 12–13, 1996.

26. World Health Organization, *Report of an International Consultation of Experts Convened in Geneva from 24 to 26 March 1997 on the Issue of Human and Animal Spongiform Encephalopathies and Medical Products*, World Health Organization, Geneva, Switzerland, 1997.

27. D. J. Stekel et al., "Prediction of Future BSE Spread," *Nature* **381**, 119 (1996).

28. D. C. Krakauer et al., "Phylogenesis of Prion Protein," *Nature* **380**, 675 (1996).

29. R. C. Moore et al., "Mice With Gene Targeted Prion Protein Alterations Show That *Prnp, Sinc* and *Prni* Are Congruent," *Nature Genet.* **18**, 118–125 (1998).

30. D. Butler, "Creutzfeldt-Jakob Researchers Seek Greater Access to Data on Cases," *Nature* **381**, 453 (1996).

31. C. J. Gibbs, "Is BSE Endemic?" *International Symposium on Spongiform Encephalopathies: Generating Rational Policy in the Face of Public Fears*, The Ceres Forum, Georgetown University, December 12–13, 1996.

32. O. Hotz de Baar, "BSE: A Hypothetical Genesis," *PROMED* communication, 12 December 1996.

33. "Origins of BSE [comment]," *Vet. Rec.* **128**, 310 (1991).

Donald Klingborg
Dean Cliver
University of California
Davis, California

BREAKFAST CEREALS

Breakfast cereals may be classified as hot cereals, requiring some preparation such as the addition of hot water, or ready-to-eat (RTE) cereals, usually consumed with cold milk. RTE cereals are so-called because they are precooked using one of a number of processes. Corn, wheat, rice, oats, or other grains may be puffed, flaked, shredded, or extruded; coated with sugars; enriched with micronutrients; or enhanced with fruits to provide a convenient, nutritious, and readily digestible breakfast food.

The history of breakfast cereals parallels the growth of the Kellogg Company and the Postum Cereal Company, the latter becoming the nucleus of General Foods (1,2). Health and nutrition were the driving forces in the development of breakfast cereals. At the turn of the century, people were looking for lighter, more digestible foods as they moved into less physically demanding work environments. The heavy fried and starchy foods of the pioneer days became a major cause of stomach disorders among the sedentary segment of the population (2).

About this time Dr. J. H. Kellogg was superintendent of the Battle Creek sanitarium. Kellogg, with his brother W. K. Kellogg, developed the first flaked cereal as a health food to feed the sanitarium patients. C. W. Post was a patient at the sanitarium and was most intrigued at the commercial possibilities of the health foods being served there. When he regained his health, Post went on his own to develop the cereal products that are still known today as Grape Nuts and Post Toasties.

From humble beginnings, breakfast cereals became a large industry in the United States. In 1987 it was a $5.3 billion business, with 90% coming from the RTE segment and 10% from the hot cereal category. The per capita consumption of breakfast cereals in 1987 was 11.7 lb for RTE and 3.6 lb for hot cereals (3).

READY-TO-EAT BREAKFAST CEREALS

Ready-to-eat breakfast cereals can be described by grouping them into a few basic cereal types: puffed, flaked, shredded, baked, and agglomerated.

Puffed Cereals

Puffed cereals are whole grains, grain parts, or a grain dough that has been formed into a specific shape and then expanded by subjecting it to heat and pressure to produce a very light and airy grain product. The two types of process that are normally used for puffed cereals are gun puffing and extrusion.

Gun Puffing. Gun puffing consists of a chamber (gun) that is charged with a quantity of grain material. The chamber is subjected to high-pressure steam (100–250 psig) for 5–45 s. The pressure is then released very rapidly giving a sound reminiscent of a gun. The shot of grain is propelled into an expansion vessel. The change from the high-pressure steam to atmospheric conditions causes the grain piece to expand to about 3–10 times its original volume. The puff is then dried to a final moisture content of 1–3% (4,5).

Extrusion. Extrusion employs either a single- or twin-screw extruder. A single flour or a mixture of grain flours is fed into the extruder. These flours may have been preconditioned with water or steam before extrusion. Other ingredients may be added to the extruder such as water or sugar syrups. The design of the screw, barrel elements, and die of the extruder impart desired characteristics to the finished product. Heating or cooling is accomplished through mechanical energy from the screw (6) and through jacketing the extruder barrel. Pressures in the extruder range from 500 to 2500 psig and temperatures range from

250 to 400°F. As the material exists from the end of the extruder it expands to release steam from the product. The produce is then dried to 1–4% moisture.

Puffed cereals are often coated with a sugar solution that may be flavored and dried to give a presweetened cereal. This coating is normally done in a rotating drum or screw auger where the puffed cereal is fed at a controlled rate. Sugar syrup is sprayed onto the cereal in the desired amount. The coated cereal is dried to remove excess moisture.

Flaked Cereals

Flaked cereals are cooked whole grains, parts of grains, or flours that have been pelletized or agglomerated, flattened into a flake, and dried or toasted. A normal process involves blending the grain with a flavoring syrup that may contain sugar, water, malt, or salt as well as other ingredients or combinations thereof. The blend is cooked in a steam atmosphere in a batch or continuous cooker. Cooking conditions involve moistures between 25 and 40%, time between 30 min and 3 h, and steam pressures of 15–40 psig. The cooked material is dried (pelletized and sized if required) to the desired flaking moisture, normally between 12 and 22% moisture. The material is tempered for 1–24 h to equilibrate moisture throughout the grain piece. After tempering, the pieces are fed through flaking rolls to get the desired flake thickness, normally between 0.010 and 0.035 in. After flaking, the wet flakes go to toasting ovens where the product moistured is quickly brought down to 2–3%. At times, grain flakes (especially rice) are oven puffed. This requires that the wet flakes be subjected to a very high temperature (up to 600°F) for a short period of time, often 1 min or less (7). This is done effectively in a fluidized bed dryer.

Shredded Products

Shredded products are whole grains or mixtures of grains that have been shaped into thin strands or a crosshatch shape, then layered, cut into biscuits or geometric shapes, and finally dried or toasted. For a whole grain such as wheat, the grain is cooked either in atmospheric conditions with an excess of water or in a pressurized steam vessel. Moisture content at this point is approximately 40–43% (8). After cooking, the grain is allowed to temper for several hours to equilibrate; it is then fed into shredding rolls consisting of one smooth roll and one grooved roll. The smooth roll forces the cooked grain into the grooved roll. A comb scrapes the grain material out of the grooves and deposits the strands onto a conveyer belt. Some 18–20 layers of shreds are deposited one on top of another from a bank of shredding rolls, and then cut into bite-size pieces. The pieces go through an oven to bake and dry (4,6).

Crosshatched types of products (chex) are normally made with rice or corn plus a flavoring syrup. Cooking is done in a batch or extrusion cooker and the shredding rolls are basically the same except for the addition of a horizontal groove on the grooved roll which is slightly less deep than the vertical groove. The layered web is then cut into bite-size pieces and puffed in a fluidized bed dryer to develop the characteristic light texture.

Baked Cereals

Baked cereals are the nugget type or rotary mold type. They are normally made in a typical bakery process. The nugget type involves blending grain flours, yeast, salt, and water together in a dough mixer, allowing the dough to proof, forming the dough into loaves or sheets, baking in an oven, then breaking the baked grain material into chunks, and drying to a final moisture of approximately 2%. The chunks are ground into finished food size (8). The rotary mold type utilizes a cookie baking line where the ingredients—grain, water, sugar, and shortening—are made into a dough. Pieces are formed by a cookie mold with a shape and size appropriate for RTE cereal. These are baked and dried in a typical baking oven.

Agglomerated Cereals

Agglomerated cereals are blends of grain products such as rolled oats, crisp rice, and toasted rolled wheat that have been glued together using a sugar or maltodextrin syrup as a binding agent. Vegetable oil is added to prevent sugar absorption into the agglomerated piece and to improve crispness retention in milk. The process involves blending the ingredients together in a ribbon blender or continuously in a mixer auger. The product is spread onto the belt of a traveling screen dryer (4) and moisture is reduced to approximately 2–3%. The sheets of agglomerated grain are sized off the dryer through the use of a grinder and then readied for packaging. Ingredient addition and sequencing are important to insure good agglomeration of the product without causing sugar lumps or bricking of the product in the package.

HOT BREAKFAST CEREALS

Oats

Hot breakfast cereals are usually made of oat flakes. The two principal factors that make oats different from other cereals are its retention of its whole grain identity and its nutlike flavor. Products vary from rolled oats (whole oat flakes), which require five or more minutes to prepare, to instant products, which can be prepared in a bowl with hot water.

In making oat flakes, the oat grain is first heated with dry steam, which reduces its moisture content to approximately 6%. Enzymes that produce rancid flavors during storage are deactivated during steaming. A high-speed impact operation separates the dry friable hull from the groat (oat berries). Even a small percentage of whole oats remaining is unacceptable because the hull fraction in oat flakes is unpalatable. Whole rolled oats are produced by flaking the whole groat.

Steel cutting reduces the groats for further processing. Another steaming step softens the cut groats for the rolling step. The size of the flake is determined by the type of rollers used to flatten the groats. Instant cook-in-the-bowl oats are produced from highly polished rollers with a narrow gap.

Oat flakes vary in sizes from 0.02 to 0.03 in. for rolled oats to 0.011 to 0.013 in. for instant oat flakes. Thicker flakes require longer cooking and retain more flake integrity (6,9).

Wheat

Hot wheat breakfast cereal is made with farina, which is a fraction of middlings from hard wheat. Soft wheat is not suitable because it produces a product that loses particle integrity too rapidly during cooking. Particle size is critical for quality. United States standards require that 100% of the product pass through a 20-mesh sieve and not more than 10% pass through a 45-mesh sieve. Farina must be cooked in boiling water for several minutes to insure complete starch gelatinization.

Instant farina cooks in about 1 min with boiling water. Proteolytic enzymes are used to instantize farina by opening avenues for easier penetration of water into particles to facilitate hydration during cooking (6).

Corn

In the southern portion of the United States a breakfast cereal made from corn called grits or hominy grits is popular. Grits are produced by dry milling white corn and are essentially small pieces of endosperm (6).

NUTRITIONAL CONSIDERATIONS

Breakfast Cereals and Dietary Recommendations

Dietary recommendations from the government and major health organizations suggest that the intake of dietary fat and cholesterol should decrease and the caloric intake from complex carbohydrates should increase (10). One way this can be accomplished is to increase the amount of cereals and grains in the diet. A RTE breakfast cereal is right in line with these dietary recommendations.

A recent General Foods study showed that adults who consumed breakfast, especially one that incorporated RTE cereal, had a better overall diet quality than those who skipped breakfast. In addition, RTE cereal users usually have lower daily intake of fat and cholesterol (11).

Another survey showed that 95% of the survey population consumes some kind of food item in the morning. Eighty-nine percent of these people eat breakfast. RTE cereals were the second most frequently consumed meal, behind a food group that included rolls, muffins, and toast (12). A third survey showed that 88% of the survey population consumed breakfast, and approximately 32% consumed cereal, primarily RTE cereal (13).

Breakfast cereals are, therefore, an excellent vehicle for delivery of calories from complex carbohydrates and, at the same time, reduce fat calories. Figure 1 shows the distribution of calories in a complete cereal breakfast (1 oz cereal, 12 fl oz lowfat milk, two slices toast with spread, and 6 fl oz orange juice) compared with a bacon and egg breakfast (one egg, two slices bacon, one slice toast with spread, 6 fl oz orange juice, and 8 fl oz lowfat milk). The cereal breakfast is lower in fat and higher in carbohydrates than the bacon and egg breakfast.

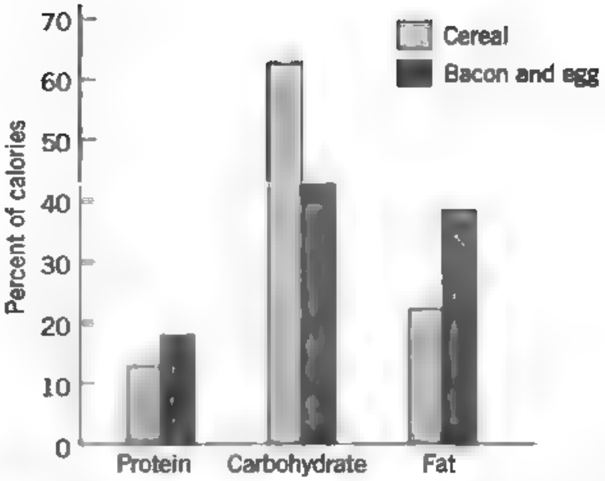


Figure 1. Percent calories in a cereal breakfast versus a bacon and egg breakfast.

Fortification

Cereal fortification has been practiced in the industry for decades and most RTE cereals today are fortified with vitamins and minerals. In 1974 the Food and Drug Administration (FDA) proposed guidelines on cereal fortification that were later incorporated into a general fortification regulation (14). This regulation, along with other guidelines (15), serves as the cornerstone for cereal fortification. Cereal manufacturers today use the following guidelines for specific nutrient fortification.

1. The intake of the nutrient is below the desirable level in the diets of a significant number of people.
2. The food used to supply the nutrient is likely to be consumed in quantities that will make a significant contribution to the diet of the population in need.
3. The addition of the nutrient is not likely to create an imbalance of essential nutrients.
4. The nutrient added is stable under conditions of storage.
5. The nutrient is physiologically available from the food.
6. There is reasonable assurance against excessive intake to a level of toxicity.

Other factors considered when fortifying include the estimated frequency of consumption of the food, the target consumer group, current health issues, and the dietary role of the product.

Breakfast cereals therefore make a significant contribution to the overall diet quality of an individual through their ingredient and fortification profiles. They provide a logical way to translate dietary recommendations to increase carbohydrate intake and decrease fat consumption; at the same time they provide a means for enhancement of vitamins and minerals in the diet.

STABILITY OF BREAKFAST CEREALS

Breakfast cereals should remain stable for up to one year when stored under reasonably cool and dry conditions. RTE cereals should remain crisp without developing off-flavors in storage. They should stay crisp in milk for at least 3–5 min.

Texture Stability

Loss of crispness (staling) in RTE cereals is associated with moisture pickup. Fresh RTE cereals have a moisture content of 2–3% and are very crisp. A good indicator of sensory crispness is water activity (A_w), which is the ratio of the vapor pressure of water over the food material to the vapor pressure of pure water, both measured at the same temperature. Fresh cereals generally have an A_w of about 0.20. As the moisture content goes up, the A_w increases until it reaches a critical value; for most cereals, this is around 0.45 (16). Beyond the critical A_w, the cereal becomes stale and unacceptable.

In the case of fruited cereals such as raisin bran, the raisin, which contains up to 18% moisture, will transfer its moisture during storage to the cereal, which has about 2–3% moisture. As long as the critical A_w of the cereal is not exceeded, the cereal will remain crisp. But the raisin could become unacceptably hard.

Some remedies to keep fruit soft while retaining cereal crispness include controlled addition of moisture to the cereal to modulate moisture migration from the fruit. In some cases this could make the cereal lose crispness. Infusion of fruits with edible humectants such as glycerol will keep the fruits soft while preventing the cereal from becoming stale (16,17). Coating RTE cereals with hydrophobic ingredients such as fats and oils, or incorporation of ingredients like magnesium stearate in the formulation, can significantly improve the bowl life of the cereal (18,19).

Flavor Stability

Breakfast cereals can develop off-flavors during storage. Unsaturated fatty acids and other compounds containing unsaturated linkages, such as vitamins, may undergo autooxidation, resulting in oxidative rancidity. Other reactions that can cause off-flavors are fat hydrolysis and reversion (8,20). Susceptibility to oxidation depends on the type of cereal grain used. Breakfast cereals made from oats are more susceptible to oxidative rancidity because oats contain higher levels of oil, about 7%. Other breakfast cereals, such as those made from rice, wheat, and corn, contain much less oil and thus are more stable in storage.

Antioxidants are used to retard autooxidation in breakfast cereals. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are the most common phenolic antioxidants used. Federal regulations allow BHT and BHA to be added directly to breakfast cereals up to 50 ppm. The more common practice is to add antioxidants in the wax liner of the cereal box. These phenolic antioxidants, volatile at room temperature, diffuse from the liner to the product, thus providing protection from oxidation (21).

Nutrient Stability

Breakfast cereals are usually fortified with vitamins and minerals up to 25% of the U.S. recommended daily allowance or higher. These micronutrients, especially vitamins A, D, and C, are not stable during cereal processing. Hence, micronutrient fortification is done after processing, just before packaging (8,22). Properly maintained temperature and humidity will protect micronutrients during storage.

BREAKFAST CEREAL PACKAGING

The forerunner of today's RTE cereal packaging was introduced about 1900 by Nabisco in packaging the Uneeda Biscuit. For the consumer, a folding carton with an internal liner became a convenient alternative to cracker-barrel–type bulk packaging. Although today's packaging resembles that of the early 1900s, technological changes have occurred in packaging materials and equipment as a result of more demanding product specifications, consumer needs, distribution, merchandising, and regulatory and production requirements.

The major functions of the RTE cereal package are containment, preservation, identification, and integrity. Most RTE cereal packaging systems comprise three components: primary (liner), secondary (folding carton), and tertiary (corrugated shipping container).

Primary Package

The cereal liner's principal function is preservation. The liner evolved over time from an uncoated, bleached sulfate paper to a more protective waxed glassine paper. Plastic films were introduced in the mid-1970s. These coextruded films provide superior product protection via improved moisture and grease barriers and seal integrity. In 1985 films were made with bidirectional barrier properties to retain product aroma while preventing external contamination (23). Cereal liners can be modified to preserve other product attributes. Phenolic antioxidants can be incorporated into both waxed paper liners and plastic film coextrusions to minimize oxidative rancidity, which may impart off-flavors to the product (21). Titanium dioxide is generally added to plastic films to provide varying degrees of opacity where transparency is not desired.

Secondary Package

The principal functions of the folding carton are liner containment and communication (graphics). Most cereal cartons are produced from a machine clay-coated newboard comprising multiple layers of recycled paper fiber and are printed by either offset lithography or rotogravure. A second method prints clay-coated sulfite paper before lamination to an uncoated, special-bending newsboard using adhesives or waxes. Other less-economical, higher quality constructions using virgin fiberboard include coated, solid, bleached sulfate and coated, solid, unbleached sulfate.

Tertiary Package

Maintenance of the integrity of the retail package through the distribution cycle is the main function of the corrugated shipping case. Shipping cases are generally regular slotted containers able to withstand a burst test ranging from 150–275 lb. Top-to-bottom compression strength of the case is important to prevent crushing during handling and storage. Minimum standards for corrugated containers have been established for rail and truck shipments and are covered under specific uniform classification codes (24–26).

Cereal Packaging Line

The bag-in-the-box (BNB) packaging system is replacing the double package liner packing lines. BNB packaging uses vertical form–fill–seal baggers coupled with horizontal cartoners. Product is deposited in the bag (liner) during bag formation and is sealed prior to carton insertion. In contrast, traditional packing lines form a liner and carton around a mandrel prior to product fill. BNB equipment is more efficient and flexible, operates with wider liner material selection, and produces a higher quality cereal liner.

BIBLIOGRAPHY

1. G. Carson, *The Cornflake Crusade*, Rinehart & Co., New York, 1957, pp. 147–175.
2. J. Berry, *Inside Battle Creek*, rev. ed., Battle Creek Public Schools, Brochure No. 6, Battle Creek, Michigan, 1989.
3. The Staff, "State of the Food Industry, Breakfast Cereals," *Food Engineering* **60**, 86 (1988).
4. R. Fast, "Breakfast Cereals: Processed Grains for Human Consumption," *Cereal Foods World* **32**, 241–244 (1987).
5. R. Daniels, *Breakfast Cereal Technology*, Noyes Data Corp., Park Ridge, N.J., 1974
6. R. Hosney, *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986, pp. 293–304.
7. D. Tressler and W. Sultan, *Food Products Formulary*, Vol. 2, AVI Publishing Co., Westport, Conn., 1975.
8. N. Kent, *Technology of Cereals*, Pergamon Press, Elmsford, NY, 1983, pp. 144–153.
9. F. H. Webster, "Oat Utilization: Past, Present, and Future," in F. H. Webster, ed., *Oats: Chemistry and Technology*, American Association of Cereal Chemists, Inc., St. Paul, Minn., 1986, pp. 413–426.
10. Committee on Diet and Health, *Diet & Health: Implications for Reducing Chronic Disease Risk*, National Academy Press, Washington, D.C., 1989.
11. K. Morgan, M. Zabik, and G. Stampley, "The Role of Breakfast in Diet Adequacy of the U.S. Adult Population," *Journal of the American College of Nutrition* **5**, 551–563 (1986).
12. J. Markle, F. Vellucci, and G. Coccodrilli, *Food Selection Patterns of Individuals Who Consume a Food Item in the Morning*, Internal Research Report, General Foods USA, White Plains, N.Y., 1985.
13. J Heyback and F. Vellucci, *Clustering Patterns of Food Intake at Breakfast*, Internal Research Report, General Foods USA, White Plains, N.Y., 1985.
14. 21 *CFR* Part 104, *Federal Register* **45**, 6314–6324 (1980).
15. AMA Council on Foods and Nutrition and the Food and Nutrition Board of the National Academy of Science—National Research Council "Improvement of Nutritive Quality of Foods," *Journal of the American Medical Association* **205**, 160 (1968).
16. U.S. Pat. 4,256,772 (Mar. 17, 1981), A. Shanbhag and A. Szczesniak (to General Foods Corp.)
17. U.S. Pat. 4,103,035 (July 25, 1978), C. Fulger and T. Morfee (to Kellogg Co.).
18. U.S. Pat. 3,484,250 (Dec. 16, 1969), W. Vollink and M. Steeg (to General Foods Corp.).
19. U.S. Pat. 4,588,596 (May 13, 1986), D. Bone and co-workers (to the Quaker Oats Co.).
20. R. B. Coulter, "Extending Shelf Life by Using Traditional Phenolic Antioxidants," *Cereal Foods World* **33**, 207–210 (1988).
21. R. Sims and J. Fioriti, "Antioxidants as Stabilizers for Fats, Oils, and Lipid Containing Foods," in T. E. Furia, ed., *CRC Handbook of Food Additives*, Vol. 11, 2nd ed., CRC Press, Inc., Boca Raton, Fl., 1980, pp. 13–56.
22. R. H. Anderson and co-workers, "Effects of Processing and Storage on Micronutrients in Breakfast Cereals," *Food Technology* **30**, 110–114 (1976).
23. W. Drennan, "Barrier Resins Key New Package Development," *Plastics Packaging*, 17–21 (July–Aug. 1988).
24. *Uniform Freight Classification*, Uniform Freight Classification Committee, Chicago, Ill., annual.
25. *National Motor Freight Classification*, American Trucking Associations, Washington, D.C., annual.
26. *Coordinated Freight Classification*, The New England Motor Rate Bureau, Inc., Burlington, Mass., annual.

GENERAL REFERENCES

A. Brody and K. Marsh, *The Wiley Encyclopedia of Packaging Technology*, 2nd ed., John Wiley & Sons, Inc., New York, 1997.

S. A. Matz, *The Chemistry and Technology of Cereals as Food & Feed*, AVI Publishing Co., Westport, Conn., 1959.

S. A. Matz, *Cereal Technology*, AVI Publishing Co., Westport, Conn., 1970.

DICK FIZZELL
G. COCCODRILLI
CHARLES J. CANTE
General Foods USA
White Plains, New York

BROWNING REACTION, ENZYMATIC

The change in color following mechanical or physiological injury of fruits and vegetables is due to oxidative reactions of phenolic compounds by polyphenol oxidase (PPO) and the reaction products, o-quinones, to various polymerized products. In food processing, such color change is commonplace during preparation for canning, dehydration, freezing, or storage. Formation of the dark brown color causes the product to become unattractive and is accompanied by undesirable changes in flavor and a reduction in nutritive value. However, in some other cases such as the manufacture of tea, coffee, or cocoa, this enzymatic browning reaction is an essential part of the processing. Enzyme-catalyzed oxidative browning has been recognized since 1895, when the change in color of fresh apple cider was gated shipping case. Shipping cases are generally regular slotted containers able to withstand a burst test ranging from 150–275 lb. Top-to-bottom compression strength of the case is important to prevent crushing during handling and storage. Minimum standards for corrugated containers have been established for rail and truck shipments and are covered under specific uniform classification codes (24–26).

Cereal Packaging Line

The bag-in-the-box (BNB) packaging system is replacing the double package liner packing lines. BNB packaging uses vertical form–fill–seal baggers coupled with horizontal cartoners. Product is deposited in the bag (liner) during bag formation and is sealed prior to carton insertion. In contrast, traditional packing lines form a liner and carton around a mandrel prior to product fill. BNB equipment is more efficient and flexible, operates with wider liner material selection, and produces a higher quality cereal liner.

BIBLIOGRAPHY

1. G. Carson, *The Cornflake Crusade*, Rinehart & Co., New York, 1957, pp. 147–175.
2. J. Berry, *Inside Battle Creek*, rev. ed., Battle Creek Public Schools, Brochure No. 6, Battle Creek, Michigan, 1989.
3. The Staff, "State of the Food Industry, Breakfast Cereals," *Food Engineering* **60**, 86 (1988).
4. R. Fast, "Breakfast Cereals: Processed Grains for Human Consumption," *Cereal Foods World* **32**, 241–244 (1987).
5. R. Daniels, *Breakfast Cereal Technology*, Noyes Data Corp., Park Ridge, N.J., 1974
6. R. Hosney, *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986, pp. 293–304.
7. D. Tressler and W. Sultan, *Food Products Formulary*, Vol. 2, AVI Publishing Co., Westport, Conn., 1975.
8. N. Kent, *Technology of Cereals*, Pergamon Press, Elmsford, NY, 1983, pp. 144–153.
9. F. H. Webster, "Oat Utilization: Past, Present, and Future," in F. H. Webster, ed., *Oats: Chemistry and Technology*, American Association of Cereal Chemists, Inc., St. Paul, Minn., 1986, pp. 413–426.
10. Committee on Diet and Health, *Diet & Health: Implications for Reducing Chronic Disease Risk*, National Academy Press, Washington, D.C., 1989.
11. K. Morgan, M. Zabik, and G. Stampley, "The Role of Breakfast in Diet Adequacy of the U.S. Adult Population," *Journal of the American College of Nutrition* **5**, 551–563 (1986).
12. J. Markle, F. Vellucci, and G. Coccodrilli, *Food Selection Patterns of Individuals Who Consume a Food Item in the Morning*, Internal Research Report, General Foods USA, White Plains, N.Y., 1985.
13. J Heyback and F. Vellucci, *Clustering Patterns of Food Intake at Breakfast*, Internal Research Report, General Foods USA, White Plains, N.Y., 1985.
14. 21 *CFR* Part 104, *Federal Register* **45**, 6314–6324 (1980).
15. AMA Council on Foods and Nutrition and the Food and Nutrition Board of the National Academy of Science—National Research Council "Improvement of Nutritive Quality of Foods," *Journal of the American Medical Association* **205**, 160 (1968).
16. U.S. Pat. 4,256,772 (Mar. 17, 1981), A. Shanbhag and A. Szczesniak (to General Foods Corp.)
17. U.S. Pat. 4,103,035 (July 25, 1978), C. Fulger and T. Morfee (to Kellogg Co.).
18. U.S. Pat. 3,484,250 (Dec. 16, 1969), W. Vollink and M. Steeg (to General Foods Corp.).
19. U.S. Pat. 4,588,596 (May 13, 1986), D. Bone and co-workers (to the Quaker Oats Co.).
20. R. B. Coulter, "Extending Shelf Life by Using Traditional Phenolic Antioxidants," *Cereal Foods World* **33**, 207–210 (1988).
21. R. Sims and J. Fioriti, "Antioxidants as Stabilizers for Fats, Oils, and Lipid Containing Foods," in T. E. Furia, ed., *CRC Handbook of Food Additives*, Vol. 11, 2nd ed., CRC Press, Inc., Boca Raton, Fl., 1980, pp. 13–56.
22. R. H. Anderson and co-workers, "Effects of Processing and Storage on Micronutrients in Breakfast Cereals," *Food Technology* **30**, 110–114 (1976).
23. W. Drennan, "Barrier Resins Key New Package Development," *Plastics Packaging*, 17–21 (July–Aug. 1988).
24. *Uniform Freight Classification*, Uniform Freight Classification Committee, Chicago, Ill., annual.
25. *National Motor Freight Classification*, American Trucking Associations, Washington, D.C., annual.
26. *Coordinated Freight Classification*, The New England Motor Rate Bureau, Inc., Burlington, Mass., annual.

GENERAL REFERENCES

A. Brody and K. Marsh, *The Wiley Encyclopedia of Packaging Technology*, 2nd ed., John Wiley & Sons, Inc., New York, 1997.

S. A. Matz, *The Chemistry and Technology of Cereals as Food & Feed*, AVI Publishing Co., Westport, Conn., 1959.

S. A. Matz, *Cereal Technology*, AVI Publishing Co., Westport, Conn., 1970.

DICK FIZZELL
G. COCCODRILLI
CHARLES J. CANTE
General Foods USA
White Plains, New York

BROWNING REACTION, ENZYMATIC

The change in color following mechanical or physiological injury of fruits and vegetables is due to oxidative reactions of phenolic compounds by polyphenol oxidase (PPO) and the reaction products, o-quinones, to various polymerized products. In food processing, such color change is commonplace during preparation for canning, dehydration, freezing, or storage. Formation of the dark brown color causes the product to become unattractive and is accompanied by undesirable changes in flavor and a reduction in nutritive value. However, in some other cases such as the manufacture of tea, coffee, or cocoa, this enzymatic browning reaction is an essential part of the processing. Enzyme-catalyzed oxidative browning has been recognized since 1895, when the change in color of fresh apple cider was recognized first as a result of oxidation of tannin by the oxidase present in apple tissue (1).

POLYPHENOL OXIDASE

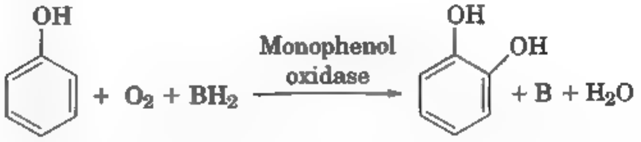
PPO (phenolase or catechol oxidase) is a ubiquitous group of copper metalloproteins and it is found in microorganisms (bacteria and fungi), plants, and animals, where it is involved in the biosynthesis of melanins and other polyphenolic compounds. PPO is of particular importance in the processing and marketing of horticultural products because of the brown discoloration of bruised fruits and vegetables resulting from the action of this enzyme. Not only does an undesirable color form, but browning is accompanied by a loss of nutrient quality and the development of undesirable flavors. In some tropical fruits, the action of PPO leads to major economic losses (up to 50%). Black spots in shrimp are caused by PPO-catalyzed browning; the browned shrimp are not acceptable to the consumer and they are downgraded in quality. Millions of dollars are spent each year on attempts to control PPO oxidation; to date none of the control methods are entirely successful. On the other hand, browning by PPO is a desired reaction in certain foods such as tea, coffee, cocoa, prunes, and dates.

PPO was first discovered in mid 1800s in mushrooms (2). PPO is a generic term for the group of enzymes that catalyze the oxidation of phenolic compounds at the expense of oxygen to quinones. Quinones are highly reactive, electrophilic molecules, which covalently modify and crosslink a variety of cellular constituents including nucleophiles of proteins such as sulfhydryl, amine, amide, indol, and imidazole substituents. These quinone adducts produce brown or black color on cut surfaces of fruits and vegetables, and they are the major detrimental effect of the enzyme in postharvest physiology and food processing.

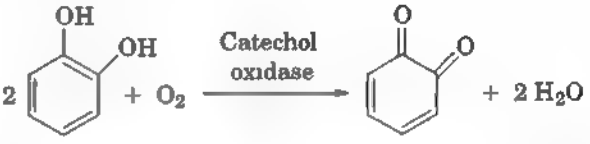
PPO is a copper-containing enzyme that belongs to a group of oxidoreductases. Although the function of PPO in plant metabolism is not well understood yet, the recent cloning of PPO presents opportunities to explore its function in plants as well as to explore the extent to which PPO expression can be manipulated. Various aspects of PPO have been reviewed recently (3–8).

Depending on the substrate specificity, International Enzyme Nomenclature has designated monophenol monooxygenase or tyrosinase as EC 1.14.18.1, which hydroxylates monophenols to *o*-diphenols; diphenol oxidase or catechol oxidase or diphenol oxygen oxidoreductase as 1.10.3.2, which dehydrogenates *o*-dihydroxyphenols to *o*-quinones; and laccase or *p*-diphenol oxygen oxidoreductase as 1.10.3.1. The first two enzymes, tyrosinase and catechol oxidase, which many authors refer to as PPOs, occur in practically all plants.

PPO was first described by G. Bertrand in 1895 who demonstrated that the darkening of mushroom was due to the enzymatic oxidation of tyrosine (9). In addition to its general occurrence in plants, PPO is also found in some bacteria, fungi, algae, bryophytes, and gymnosperms (10–13). Laccase was first observed by H. Yoshida in the latex of the Japanese lacquer tree (14). PPO catalyzes two distinct reactions: (*1*) hydroxylation of monophenols to give *o*-diphenols. This monophenol oxidase activity often requires priming with reducing agents or trace amounts of *o*-diphenol indicated as BH_2,

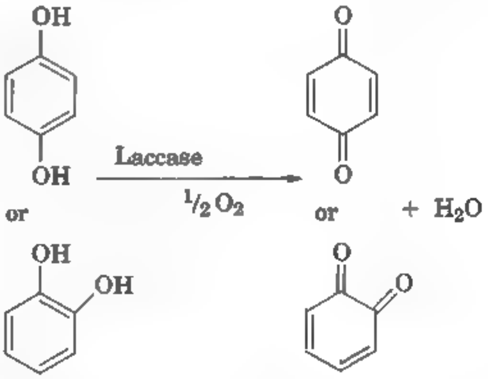


and (*2*) the removal of hydrogens from *o*-diphenol to give *o*-quinone.



It was reported in 1944 that PPO preparations from mushrooms had the ability to catalyze oxidation of monophenols in addition to catalyzing oxidation of polyphenols (15). However, the ratio of rate of monophenol oxidation to that of polyphenol oxidation varied considerably from different mushroom sources. Since then many reports have shown a wide range of PPO activities in many different fruits and vegetables in different ratios. Today, it is known that enzyme preparations from many other sources possess these two activities in different ratios and that the ratios may change during isolation and purification. Most PPO preparations from potato, apple, mushroom, and bean possess both activities, whereas those from tea leaf, tobacco, mango, banana, pear, clingstone peach, and sweet cherry have been reported not to act on monohydroxy phenols (16).

Laccase is less frequently encountered as a cause of browning in fruits and vegetables than is catechol oxidase. Laccase oxidizes *o*- and *p*-diphenols,



In addition, it oxidizes various other *p*-diphenols, *m*-diphenols and *p*-phenylenediamine, but laccase does not oxidize tyrosine.

PPO appears in almost all tissues of plants; however, significant differences in both the level of the activity and in the concentration of its substrates have been observed among different cultivars of fruits and vegetables. The enzyme has been found in a variety of cell fractions, both in organelles and in the soluble fraction of the cell (17,18). The level of PPO often changes markedly during the development of the plant (19–21) and may be significantly affected by growing conditions.

Recent emerging new scientific development in the field of molecular biology offers not only the possibility of understanding the essential function of PPO in plants, which has remained a puzzle for years, but it also promises to control PPO and its substrates to prevent browning in foods.

PPO ANALYSIS

Qualitative and quantitative colorimetric tests and manometric determinations have been used to detect and measure enzyme activity. Because PPOs use molecular oxygen to oxidize phenolic substrates, their activity can be determined by measuring the rate of substrate disappearance or the rate of product formation. When based on the rate of substrate disappearance, oxygen absorption is usually measured either manometrically in a Warburg respirometer or polarographically with an oxygen electrode. The most popular method of assay is to follow the initial rate of formation of the quinone spectrophotometrically by measuring the optical density. Care must be taken to restrict measurement to the initial phase of the oxidation, because the reaction soon slows down. Some researchers object to the use of spectrophotometric analysis because it measures the secondary reaction products of PPO, and the secondary reactions are influenced by many factors difficult to control. Thus the presence of ascorbic acid creates a lag phase and lowers the values obtained, whereas autooxidation products of polyphenols may increase the levels of enzyme activity. It is important to note that the reaction changes with time and temperature and depends on the substrate type and concentration, on the pH of the reaction mixture, and on the buffer used (22,23). A wide variety of substrates can be used with spectrophotometric methods: catechol (24), pyrogallol (25), or chlorogenic acid (26). It has to be taken into account that the reaction products formed from the oxidation of various phenols have absorption maxima at different wavelengths, that the substrate may undergo autooxidation, and that an excess of some substrates causes strong inhibition of the enzyme (26). Other methods for PPO activity are based on disappearance of ascorbic acid, a reaction that is directly proportional to enzyme activity (27) and spectrophotometric methods using Besthorn's hydrazone (28), or 2-nitro-5-thiobenzoic acid (29).

It is important to differentiate between catechol oxidase and laccase on the one hand and peroxidase on the other. Because peroxidative oxidation of phenols is often mistaken for PPO or laccase, peroxides should be removed from the reaction mixture by addition of catalase and alcohol to prevent any oxidation of phenols (30). For the determination of laccase, syringaldehyde (31) and 2,6-dimethoxyphenol (32) have been used as substrates of laccase. The PPO and laccase activities can be differentiated by the use of cinnamic acid derivatives to inhibit PPO and cationic detergents to inhibit laccase (33).

PPO LOCATION IN PLANT

The vascular elements of many fruits and vegetables darken rapidly when they are cut and exposed to air. The relative PPO activity of different parts of apricots, apples, or potato plants vary. PPO is considered to be an intracellular enzyme in plants except in a few cases where it is found in the cell wall fraction. The enzyme is located in a variety of cell fractions, both in organelles where it may be tightly bound to membranes and in the soluble fraction (17,18). Histochemical studies using the electron microscope and the density gradient centrifugation support the idea that PPO is bound within chloroplast lamellae and grana and in mitochondria (34). Recent study suggested more specifically that PPO is located in the grana lamellae loosely associated with photosynthetic photo system II (35). Many studies indicated that PPO is membrane-bound in plastides of nonsenescing tissues (3,5). It was suggested that PPO is located exclusively in plastids and is only released to the cytosol upon wounding, senescence, or deterioration of the organelle (36). So far, no PPO genes encoding a nonplastidic enzyme have been isolated (8). Conversion of particulate forms of the enzyme to soluble forms occurs in fruits when they are exposed to stress conditions or during ripening and storage. Browning reactions that take place after the disruption of tissues rich in PPO may cause binding of soluble PPO to a particulate fraction. The microbial extracellular laccase has been studied extensively but the location of laccase in higher plants is not well known. Although it is believed that the enzyme is found in a cytoplasmic soluble form, more information is needed on its location and distribution in plant tissues.

EXTRACTION AND PURIFICATION

Due to extremely low concentrations of PPO and laccase in plants and their instability, preparation and purification of PPO and laccase are not easy. PPO has been isolated from a variety of sources, but pigment contamination and the occurrence of multiple forms have frequently hampered its characterization. Oxidation reactions taking place during the isolation of the enzyme, due to natural substrates within the plant, result in changes in enzyme properties as well as in apparent multiplicity. Such reactions can be partially prevented by isolation under nitrogen gas, by using reducing agents such as ascorbic acid or cysteine, or by using phenol-adsorbing agents, such as poly(vinylpyrrolidone) or poly(ethylene glycol) (37,38). The binding of PPO to membranes in many tissues further complicates its isolation. Solubilization of acetone powder preparations or extracting with detergents also result in modification in structure and properties of the enzyme. To minimize these changes, all extraction steps should be carried out at temperature below 0°C, preferably at −20 to −30°C. Communition and homogenization are often carried out in liquid nitrogen or under a nitrogen atmosphere (39). Acetone precipitation followed by buffer extraction is one of the methods most often applied (16).

For the purification of the enzyme, several methods have been used that vary according to the enzyme source and the degree of purity to be attained. Most often, precipitation with ammonium sulfate of different saturation, gel chromatography on Sephadex columns, ion exchange chromatography, DEAE-cellulose or DEAE-Sephadex are applied. Recently, hydrophobic gel chromatography using Phenyl-Sepharose CL-4B has been used effectively (40,41). The separation of enzymes by electrophoresis is now a standard procedure. Sodium dodecylsulfate (SDS) techniques and electroblotting of catechol oxidase also have been successfully used (42,43).

PHYSIOCHEMICAL PROPERTIES

Molecular Weight

Most reports on the molecular weight of PPO are based on estimates on partially purified preparations using gel filtration or acrylamide gel electrophoresis. Therefore, a wide range of values are reported. Often crude or partially purified preparations show a multiplicity of forms, which may have resulted from association–dissociation reactions. Depending on the source of the enzyme, the molecular weights vary between 29,000 and 200,000 with subunit molecular weight from 29,000 to 67,000. Until recently, the enzyme from the bacterium *Streptomyces glaucescens* was found to have the smallest functional unit with one copper pair per polypeptide chain of 29,000 (44). The molecular weight of mushroom PPO has been reported to be between 116,000 and 128,000 daltons (37). The enzyme consisted of four identical subunits of M_W ca 30,000, each containing one copper atom (45). Two types of polypeptide chains, heavy (M_W 43,000) and light (M_W 13,400) were reported in mushroom (46). Avocado, banana, tea leaf, and other sources also showed multiple molecular weights in a wide range. The predominant form of PPO in grape had a molecular weight of 55,000 to 59,000 but upon storage it dissociated into 31,000–33,000 and 20,000–21,000 subunits (47). Based on recent knowledge regarding multiplicity of PPO, it is believed that many previous observations on multiplicity were due to secondary reactions, such as tanning, during enzyme preparation.

With the advent of recombinant DNA technology in recent years, numerous amino acid sequences have become available. These include PPO from the bacterium *Streptomyces glaucescens* (48) and *Streptomyces antibioticus* (49), tomato (50,51), broad bean (52), and potato (53). All PPO molecules sequenced are single-chain proteins with calculated M_W of 30,900 (273 amino acid residues) for *S. glaucescens* and *S. antibioticus*, 46,000 (407 amino acid residues) for *N. crassa*, 68,000 (606 amino acid residues) for the broad bean *Vicia faba*, and 66,300 (587 amino acid residues) for tomato.

The molecular weight of laccase was also reported to vary according to their sources. *Rhus vernicifera* laccase has been reported to vary between 101,000 and 140,000 depending on the species and variety. The M_W of the fungal laccase varies between 56,000 and 390,000; peach laccase was reported to have a M_W of 73,500. These data suggest that laccases are a heterogeneous group of glycoproteins, having a basic structural unit of between 50,000 and 70,000 M_W, which can undergo aggregation to give larger units.

pH and Temperature Optima

The optimum pH for the activity of an enzyme preparation from any one source usually varies with different substrates and is characteristic of the substrate as well as the enzyme preparation. The optimum pH of PPO activity has a relative wide range, pH 5.0 and 7.0. Some preparations were reported to be inactive below 4.0. The type of buffer and the purity of the enzyme affect the pH optimum; also, isoenzymes have different pH optima. Enzyme preparations obtained from the same fruit or vegetable at various stages of maturity have been reported to differ in optimum pH of activity as well. The pH optimum of laccase activity depends on the enzyme source and on the substrate. The stability of the enzyme is reported to be dependent upon buffer and other factors in the medium. Optima between pH 4.5 and 7.5 have been reported for enzymes from various fungi and peaches (54).

The optimum temperature of PPO depends essentially on the same factors as the pH optimum. Generally, the activity of PPO increases as the temperature rises from cold to temperatures of maximum activity (25–35°C); activity declines rapidly at temperatures of 35 to 45°C, depending on the enzyme source and substrate (16). Like most enzymes, the application of heat (70–90°C) to plant tissues or enzyme solutions for a short period causes rapid inactivation. Exposures to temperatures below zero may also affect activity. Concentrated solutions of the enzyme in dilute phosphate buffer at a pH near neutrality can be held without detectable loss of activity for several months at 1°C or when frozen at −25°C. However, the enzyme slowly losses activity even in the frozen state. Thermotolerance of PPO also depends on the source of the enzyme. It is difficult to compare heat stability data of purified enzyme preparations with that of the enzyme when in tissues or juice. Different molecular forms of PPO from the same source may have different thermostabilities. Laccase is usually more sensitive to inactivation by heat than is catechol oxidase.

Activators and Inhibitors

The activation mechanism of PPO from different sources is not well understood, but it has been suggested that conformational changes of protein and perhaps protein association or dissociation are involved in the process. Anionic detergents, such as sodium dodecyl sulfate, were found to reactivate PPO in crude and partially purified preparations from different avocado cultivars (55). Short exposures to acid pH or urea also resulted in a severalfold reversible activation of grape and bean PPO.

Many compounds inhibit PPO activity but only a limited number of PPO inhibitors are considered acceptable on grounds of safety and expense for use to control enzymatic browning during food processing. Practical aspects of browning inhibition are discussed later. There are two principal types of PPO inhibitor: compounds that interact with the copper in the enzyme and compounds that affect the site for the phenolic substrate. The metal-chelating agents such as cyanide, diethyldithiocarbamate, carbon monoxide, dimercaptopropanol, sodium azid, phenylthiourea, and potassium methyl xanthate are all inhibitory. In general, there are five major types of PPO inhibitors based on their chemical characters (56,57): (*1*) *reducing agents*, such as ascorbic acid and sulfur dioxide; (*2*) *copper-chelating agents*, such as carbon monoxide, tropolone, and diethyl dithiocarbamate; (*3*) *quinone couplers*, such as cysteine and glutathione; (*4*) *substrate analogues*, such as cinnamic acid, *p*-coumaric acid, and benzoic acid and some substituted cinnamic acids; and (*5*) *miscellaneous compounds*, such as 4-hexylresorcinol and poly(vinylpyrrolidone). Some other natural inhibitors of PPO from various sources have been reported (57). The activity of laccase is not inhibited by carbon monoxide or phenylhydrazine, both of which inhibit PPO. This response permits an easy differentiation between laccase and PPO.

Substrate Specificity

Whereas PPOs from animal tissues are relatively specific for tyrosine and dopa, those from fungi and higher plants act on a wide range of monophenol and *o*-diphenols. There are individual differences among PPOs from different sources. The rate of oxidation of *o*-diphenols by PPO increases with increasing electron withdrawing power of substituents in the *para* position. *o*-Diphenol substitution (-CH_3) at one of the positions adjacent to the -OH groups prevents oxidation. These positions should remain free for oxidation to take place. As mentioned earlier, some PPO preparations from some plants lack cresolase activity, which may be due to changes in the structure of the protein during preparation. Some arguments regarding the physical relationships between the cresolase and catechol oxidase functions suggested that both functions are catalyzed by a single site, whereas others imply the participation of two sites, either on the same enzyme molecule or different ones. Although PPO could oxidize a wide range of phenolics, the individual enzymes tend to prefer a particular substrate or certain type of phenolic substrates. The affinity of plant PPOs of the phenolic substrates is relatively low. The K_m is high, usually around 1 mM, which is higher than most of fungi and bacteria, 0.1 mM. The affinity to oxygen is also relatively low, ranging from 0.1 to 0.5 mM. Monophenols and o-diphenols have been considered as the exclusive substrates of PPO for a long time, but a recent study showed that aromatic amines and *o*-aminophenols also undergo the same catalytic reactions (ortho hydroxylation and oxidation), as documented by product analysis and kinetic studies (44).

Cloning of PPO Genes

Scientific interest in molecular biology of PPO is that the molecular weight and the form of PPO have long been a matter of speculation because of the various forms of the PPO as described earlier. Identification of PPO genes offers a great potential to produce transgenic plants having regulated levels of PPO. Because PPO is responsible for significant decreases in postharvest quality of many crops due to enzymatic browning associated with injury or senescence, downregulation of PPO using antisense could significantly increase quality attributes of most fruits and vegetables. However, overexpression of PPO may potentially minimize pest damage to crop plants. This plant defense system is one of many theories of PPO function in plant. Enzymatic browning generated by PPO in a multitude of plant–pest interactions and its wound inducibility have led some scientists to the view that the primary role of PPO is in plant defense (58). Therefore, the challenge here is to selectively modify PPO expression in specific tissue to minimize its effects on browning of fruits and vegetables, while maximizing PPO expression in other parts of the plant to maintain pathogen or herbivore resistance. Much progress has been made recent years. PPO genes have been characterized from broad bean, tomato, potato, and grape, and all PPOs cloned have been found to encode mature peptides of 57,000 to 62,000 Da with 8,000 to 11,000 Da putative transit peptides, which posttranslationally directs the protein to the chloroplast envelope (7,35,51–53). The catalytic centers of the copper binding regions for *V. faba* and tomato PPOs have been elucidated. The further characterization of PPO in various plants is being made, and there is a great potential in near future to manipulate the levels of PPO in plant foods to overcome the adverse effects of PPO.

Mechanism of Oxidation

In spite of extensive studies on PPOs, its reaction mechanism is still unclear. One of the reasons is that the mechanism of PPOs on phenolic compounds is complicated. As stated earlier, the cresolase reaction apparently involves a hydroxylation reaction, which differs from the oxidation of the o-diphenols by the catecholase reaction. For a long time there was a difference of opinions as to whether hydroxylation of monophenols and oxidation of o-dihydroxy phenols were catalyzed by the same enzyme. The oxidation mechanism of PPO from *N. crassa* has been extensively investigated (44), and a plausible theory of its catalytic activation is shown in Figure 1.

The proposed mechanisms for hydroxylation and dehydrogenation reactions with phenols probably occur by separate pathways but are linked by a common *deoxy* PPO intermediate (middle in Fig. 1). In dehydrogenation of catechol oxidase (cycle A in Fig. 1), oxygen is bound first to the Cu(I) groups of *deoxy* PPO to give *oxy* PPO in which a Cu-Cu distance of the two Cu(II) groups is about 3.4 to 3.6 Å. This would allow two Cu(II) groups of *oxy* PPO to bind to the oxygen atom of the two hydroxyl groups of catechol (an efficient 2e-transfer from catechol to the binuclear site) and from the O_2-PPO-catechol complex (at about the 1 o'clock position on the A cycle of Fig. 1). The catechol is then oxidized to *o*-benzoquinone (at about 11 o'clock position), and the enzyme is reduced to *met* PPO. Another molecule of catechol binds to *met* PPO, is oxidized to *o*-benzoquinone (and water) and the enzyme reduced to *deoxy* PPO, completing the A cycle.

The mechanism of *o*-hydroxylation of a monophenol by PPO is shown in B cycle in Figure 1. In vitro, the reaction begins with *met* PPO of A cycle. *Met* PPO must be reduced by a reducing compound BH_2 (shown previously) if a lag period is to be avoided, to give *deoxy* PPO. *Deoxy* PPO binds O_2 to give *oxy* PPO, the monophenol is bound to one of the Cu(II) groups via the oxygen atom of the hydroxyl group to give the O_2-PPO-monophenol complex (at about 5 o'clock

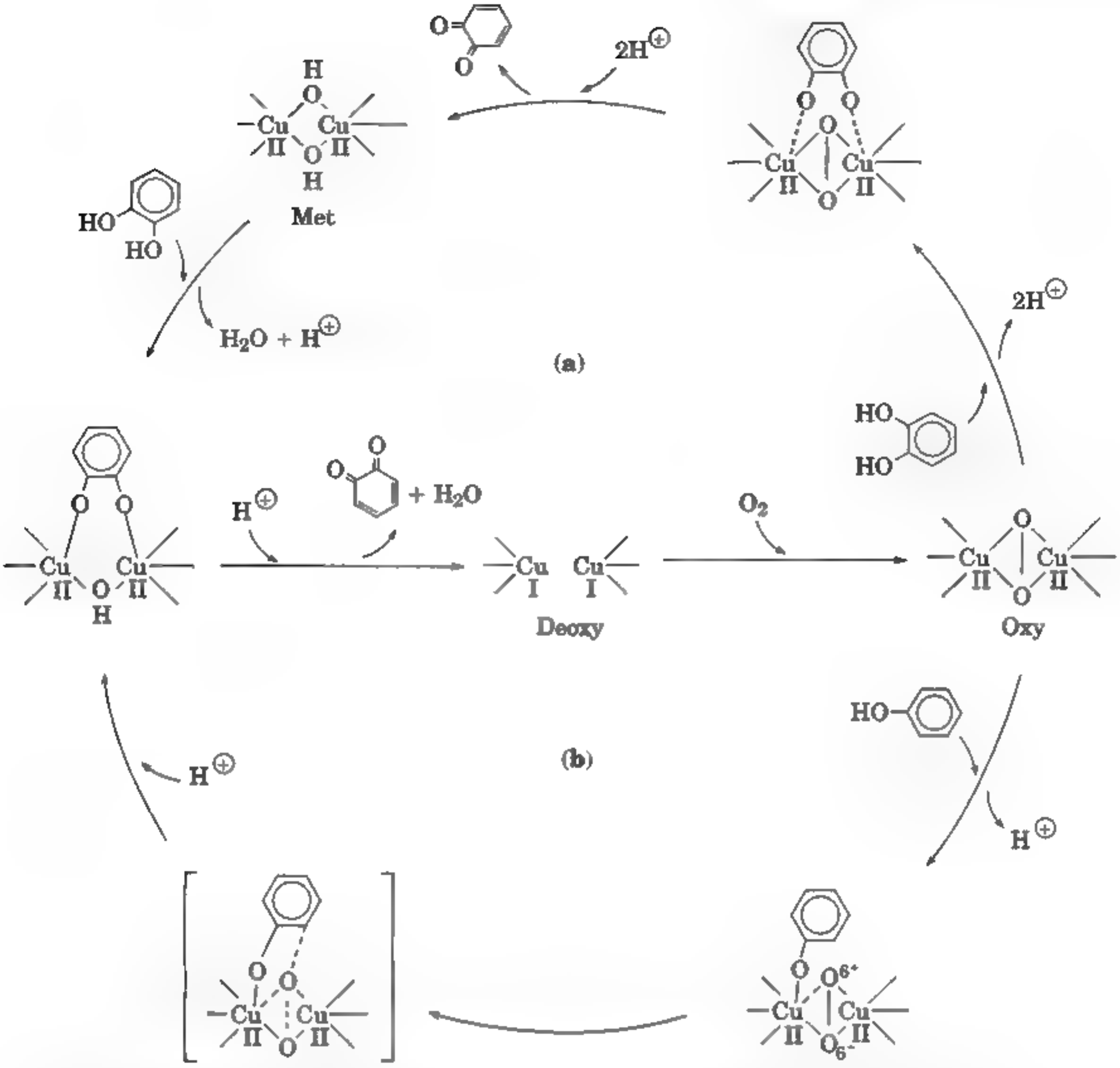


Figure 1. Proposed mechanisms of oxidation of *o*-diphenol (cycle A, for catechol) and monophenol (cycle B) for *Neurospora crassa* PPO. *Source:* Adapted from Refs. 2 and 44.

on the B cycle). Subsequently, the *o*-position of the monophenol is hydroxylated by an oxygen atom of the O_2 of the O_2-PPO-monophenol complex to give catechol, which then dissociates to give *deoxy* PPO, to complete the cycle. Only the first cycle of hydroxylation on a monophenol requires starting at the *met* PPO; all subsequent cycles begin with *deoxy* PPO (2).

Because copper is the prosthetic group, the catalytic activity of PPOs is based on the cupric-cuprous valency change. On isolation of the enzyme in its natural state, the copper is in the cuprous form, but in the presence of *o*-dihydroxy phenols, the copper would be oxidized to cupric form. The substrate is oxidized by losing two electrons and two protons. The two electrons are taken up by the copper of the enzyme, which then passes into the cuprous state. The cuprous enzyme rapidly transfers the two electrons to oxygen, which immediately forms water with the two protons that were liberated, and the enzyme returns to the cupric state. This two-state reaction can be represented by

$$4\,\mathrm{Cu}^{2+}(\text{enzyme}) + 2\text{ catechol} \rightarrow 4\,\mathrm{Cu}^{+}(\text{enzyme}) + 2\ o\text{-quinone} + 4\,\mathrm{H}$$

$$4\,\mathrm{Cu}^{+}(\text{enzyme}) + 4\,\mathrm{H}^{+} + \mathrm{O}_2 \rightarrow 4\,\mathrm{Cu}^{2+}(\text{enzyme}) + 2\,\mathrm{H_2O}$$

Recent understanding of the complete amino acid sequence of fungal PPO and the site of interaction with the phenolic substrate made it possible to create models that include a binuclear center. Histidine residues appear to lignand at least one of the two active sites of the copper atoms. Monophenols bind to one of the Cu^{2+} atoms, whereas diphenols bind to both of them, as shown in Figure 1 (59,60). Catechol oxidase can undergo conformational changes induced by the substrate O_2 and by pH. The changes in conformation are accompanied by changes in the K_m of the enzyme for both its substrates, oxygen and diphenol.

A two-step reaction mechanism by laccase has been reported (61). The first step in the oxidation of quinol by laccase was the formation of the semiquinone, with transfer of an electron from substrate to the copper in the enzyme. The second step was a nonenzymatic disproportionation reaction between two semiquinone molecules to give one molecule of quinone and one of quinol. The function of copper and electron transfer in the reaction mechanism has been studied by using inorganic ions, electron paramagnetic resonance, and spectrophotometric methods (3,62).

The Brown Pigment

Although the mechanism of pigment formation from phenolic substrates is not understood completely, the general course of pigmentation is known to involve enzymatic oxidation, nonenzymatic oxidation, nonoxidative transformations, and polymerizations. The *o*-quinones formed from phenolic compounds by PPOs are the precursors of the brown color. The *o*-quinones themselves possess little color, but they are among the most reactive intermediates occurring in plants. They take part in a secondary reaction, bringing about the formation of more intensely colored secondary products. The most important secondary reactions are coupled oxidation of substrates oxidized, complexing with amino compounds and proteins, and condensation and polymerization. The principal reaction of *o*-quinones in browning reaction is the one leading to the formation of the unstable hydroxyquinones. The hydroxyquinones polymerize readily and are easily further oxidized nonenzymatically to a dark brown pigment.

As a typical oxidation reaction, tyrosine is oxidized to dihydroxyphenylalanine (dopa) quinone by PPO and then proceeds to melanin (Scheme 1). Tyrosine is first converted into dopa, which is then oxidized to the corresponding dopa quinone. The dopa quinone, on intramolecular rearrangement, is converted into 5,6-dihydroxy indole-2-carboxylic acid, which on further oxidation is converted into red 5,6-quinone indole carboxylic acid; then finally the black melanins are formed. Quinones also react readily with simple amines, such as

$$\text{o-Benzoquinone} + \text{glycine} \rightarrow \text{4-}N\text{-glycyl-}o\text{-benzoquinone}$$

This reaction product is the intermediate responsible for the deamination of glycine with the concomitant formation of deeply colored pigments. *o*-Quinones also react with proteins, sulfhydryl compounds, such as cysteine, and produce dark-colored, insoluble products.

Dimerization or polymerization of *o*-quinones that lead to the colored products is common. The formation of dimers or oligomers of *o*-quinone by condensation of a hydroxy quinone with a quinone was recently demonstrated. The enzymatically generated caffeoyl tartaric acid o-quinones were shown to oxidize other phenols, such as 2-*S*-glutathionyl caffeoyl tartaric acid and flavans, by coupled oxidation mechanisms, with reduction of the cafeoyl tartaric acid quinones back to caffeoyl tartaric acid (63). The *o*-quinones formed by enzymatic or coupled oxidation can also react with a hydroquinones to yield a condensation product (64–66). It is possible to regenerate the original phenolic from an intermediate by reduction provided that oxidation and subsequent transformation had not gone too far. In later stages the browning is no longer reversible.

CONTROL OF BROWNING IN FOODS

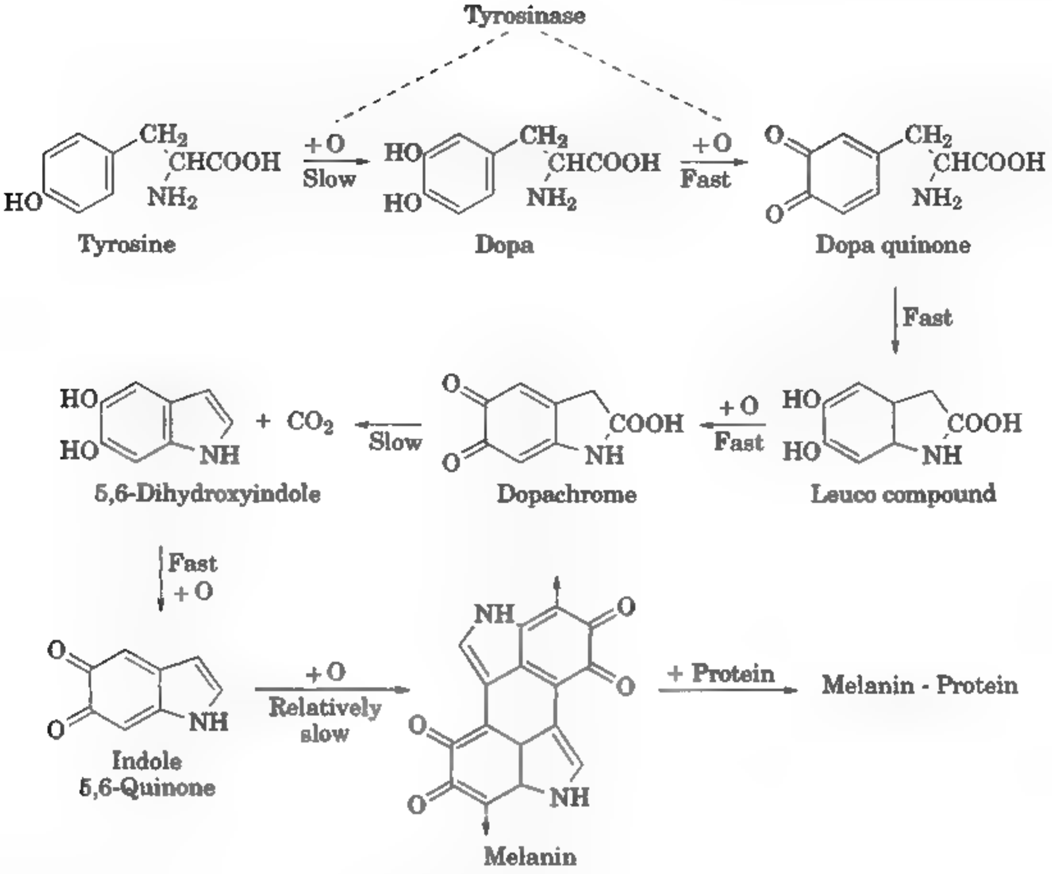
Extensive studies have been carried out to control enzymatic browning ever since Lindet (1) recognized in 1895 that the change in color occurring in freshly pressed cider was enzymatic. The practical control of enzymatic browning in foods has been carried out by several methods. The method of choice is dependent upon the food product and the intended use. The enzymatic browning in fruits and vegetables can be controlled at the beginning by selecting cultivars that are least susceptible to discoloration either because of the absence of the certain phenolic substrate or because the substrate or enzyme is present at low concentration. It is also possible to select fruits and vegetables at stages of maturity where discoloration is at minimum. Other methods include the removal of oxygen from fruit and vegetable tissues as well as from the atmosphere surrounding the food; the addition of acids to reduce the pH and thus reduce PPO activity; the addition of antioxidants or reducing substances; the addition or treatment with permissible inhibitors; and heat inactivation of the enzyme. The use of antioxidants during processing and heat inactivation of the enzyme are widely used practices that have been moderately successful.

All fruits susceptible to browning should be processed as quickly as possible. Heating destroys the enzymes responsible for the reaction; thus when fruit is canned or made into jams or jellies, browning stops as soon as the fruit is heated sufficiently to denature the enzyme. The exact temperature necessary varies with enzyme, rate of heating, pH, and other factors such as size of the fruits. Unfortunately some undesirable effects on quality often result from an adequate heat process. In general, cherry, peach, and apricot take 2 to 3 minutes in boiling water and apple, pear, and plum take 4 to 5 minutes to inactivate enzymes responsible for browning on surface. Vegetables such as beans and peas also take 3 to 5 minutes. The optimum blanching conditions for strawberry, black currant, sour cherry, and prune range from 1.5 to 3 minutes at 85°C.

In the preparation of fruit for freezing, sugars and sugar solution have been used to sweeten and to exclude direct contact of the fruit tissue with molecular oxygen. The sugar solutions inhibit discoloration by reducing the concentration of dissolved oxygen and by retarding the rate of diffusion of the oxygen from the air into the fruit tissues. Concentrated sugar solutions also exert an inhibiting effect on fruit PPO. The pH also affects the rate of the browning reaction: acid dips are sometimes used to lower the pH and by this method delay or retard browning. The effect of many salts and compounds such as sulfur dioxide, hydrogen sulfide, hydrocyanic acid, and thiourea on PPO has been studied extensively.

In the home preparation of fruits, pineapple juice and lemon juice have long been used to prevent browning. Pineapple juice has a relatively high concentration of sulfhydryl compounds, which are active antioxidants, whereas lemon juice contains relatively high amounts of both citric acid and ascorbic acid.

Heat treatments and the application of sulfur dioxide or sulfites are common commercial methods for inactivating PPO. PPO activity can be inhibited by the addition of



Scheme 1.

sufficient amounts of acidulants, such as citric, malic, or phosphoric acids to yield a pH of 3 or lower. Oxygen can be eliminated by vacuum or by immersing the plant tissues in a brine or syrup. Phenolic substrates can be protected from oxidation by reaction with borate salts, but these are not approved for food use.

For products such as vegetables, which ultimately end up cooked, heat inactivation by steam or hot water blanching is the most practical method of inactivating PPOs. Overblanching should be avoided due to loss of firmness and nutrients. Most commercial blanching of vegetables has been based on the residual activity of the peroxidase because peroxidase is one of the most stable enzymes in plants. It has been generally accepted that if peroxidase is destroyed it is quite unlikely that other enzymes including PPOs will have survived. There are, however, problems associated with the use of peroxidase as an indicator of an adequate blanch in that several enzymes other than peroxidase have been shown to be largely responsible for quality deterioration during frozen storage of different vegetables (67,68). Therefore, optimization of blanching of individual products has to be established in order to tailor the process to each type of raw material and product desired. Heat inactivation of PPO in fruit products has been applied to fruit juices and purees and to fruit intended for such products. Because the enzyme is very labile to heat at 85°C and above, the higher the temperature, the shorter the time required for inactivation. To minimize the undesirable changes due to excess heating, optimum temperature–time requirements for PPO must be established. Rapid cooling after enzyme inactivation is also necessity for best quality retention.

Sulfur dioxide is a very effective inhibitor of PPO and has been used for many years. It readily reacts with compounds such as aldehyde and other carbonyl-containing molecules. These reaction products are ineffective against PPO, and therefore, a sufficient amount of free SO_2 must be maintained. In order to be effective in preventing PPO activity, SO_2 must penetrate throughout the tissues. Sulfurous acid penetrates better than the bisulfite form. The use of excess SO_2 produces both undesirable flavors and an excessively soft product. In recent years the safety of sulfites in foods has been questioned because of their hazard to certain asthmatics. Since August 9, 1986, the U.S FDA has banned the use of sulfur dioxide in fresh fruits and vegetables and has required a label declaration on those food products (such as dehydrated fruits, frozen potato products, wine) containing more than 10 ppm of sulfiting agent. Because of these restrictions, food processors have turned to a number of sulfite alternatives, mostly formulations effective against enzymatic browning, with varying success. The demand for more effective browning inhibitors has stimulated considerable research activity in this area during the last decade.

Ascorbic acid and its isomer, erythorbic acid, and derivatives such as ascorbic acid-2-phosphate, ascorbic acid-triphosphate, ascorbic acid-6-fatty acid esters are reported to control browning. Ascorbic acid is a very effective reducing agent, as it reduces the *o*-quinones formed by PPO to the original o-dihydroxy phenolic compounds. Ascorbic acid alone or in combination with citric acid have been used widely by the food industry. Its prevention of browning lasts as long as any residual ascorbic acid remains, and, therefore, stabilized forms of ascorbic acid should be effective sulfite substitutes. One should keep in mind that the excess amounts of oxidized ascorbic acid can produce brown pigments by nonenzymatic browning reactions. Browning inhibitor penetration can be enhanced by vacuum infiltration, which also removes air from the products' void spaces.

Cinnamic acid and benzoic acid were found to be effective for apple products, and carbon monoxide has been applied to control browning in mushrooms. 4-Hexylresorcinol (Everfresh®) is known to be effective on shrimp. Kojic acid and salicylhydroxamic acid were also known as PPO inhibitors. Compounds that bind or complex PPO substrates also may be potential inhibitors. Poly(vinylpolypyrollidone) and cyclodextrins can bind polyphenols and prevent their participation in enzymatic browning. Glutathione and *N*-acetylcystein were also reported to be effective in controlling browning in apple, potato, and fresh juices. Protease enzymes as well as low molecular weight peptides were claimed to be effective browning inhibitors (69,70). Selecting raw materials for processing that have a low tendency to brown is another way to control browning. Empire and Granny Smith apples and Atlantic potato are examples of cultivars that brown slowly (21,71).

Another approach involves enzymes that transform the substrates of PPOs by methylation or oxidative cleavage of the benzene nucleus. *o*-Methyl transferase methylates the 3-position of 3,4-dihydroxy aromatic compounds in the presence of a methyl donor, converting PPO substrates into inhibitors of PPOs such as caffeic acid to ferulic acid. The exclusion of oxygen as a means of controlling enzymatic browning is generally used in combination with other methods. For example, retail packs of frozen peach slices maintain their high quality when packaged in ascorbic acid-containing syrup combined with hermetically sealed containers wherein the oxygen has been removed from the head space. Oxygen concentrations in the atmosphere of packaged produce can be controlled by modified atmosphere packaging. Although this method can delay browning, low oxygen may induce an anaerobic condition that entails a risk of undesirable quality change and anaerobic microbial growth. Edible coating materials such as xanthan were reported to prevent enzymatic browning and extend the shelf life of fruits and vegetables used in salad bars and prepared salads. Ultrafiltration removes PPO and prevents browning. Osmotic dehydration using sugar or syrup also inhibits enzymatic browning and protect flavors.

Most of chemicals mentioned here are not fail-safe, not acceptable to some consumers, and cannot be used to prevent browning in intact fruits and vegetables. Through better understanding of the mechanism of action of PPO and its essential metabolic roles in plants, it is expected that genetic engineering techniques will be the most important tools in preventing unwanted enzymatic browning. A molecular biology approach will provide a more precise method of decreasing PPO expression, while retaining the desirable genetic traits of plants.

BIBLIOGRAPHY

1. M. Lindet, "Sur l'oxydation du tannin de la pomme a cidre," *Comptes Rendus des Seances de l'Academie des Sciences* **120**, 370–372 (1895).
2. J. R. Whitaker and C. Y. Lee, "Recent Advances in Chemistry of Enzymatic Browning," in C. Y. Lee and J. R. Whitaker, eds., *Enzymatic Browning and Its Prevention*, ACS Symposium Series 600, Washington, D.C., 1995, pp. 2–7.
3. A. M. Mayer, "Polyphenol Oxidases in Plants—Recent Progress," *Phytochemistry* **26**, 11–20 (1987).
4. J. Macheix, J. Sapis, and A. Fleuriet, "Phenolic Compounds and Polyphenol Oxidase in Relation to Browning in Grapes and Wines," *Crit. Rev. Food Sci. Nutr.* **30**, 441–486 (1991).
5. A. M. Mayer and E. Harel, "Phenoloxidases and Their Significance in Fruit and Vegetables," in P. F. Fox, ed., *Food Enzymology*, Elsevier, New York, 1991, pp. 373–398.
6. J. J. Nicolas et al., "Enzymatic Browning Reactions in Apple and Apple Products," *Crit. Rev. Food Sci. Nutr.* **34**, 109–157 (1994).
7. J. C. Steffen, E. Harel, and M. D. Hunt, "Polyphenol Oxidase" B. E. Ellis, ed., in *Genetic Engineering of Secondary Metabolism*, Plenum Press, New York, 1994, pp. 275–312.
8. J. C. Steffen et al., "Polyphenol Oxidase," in *Proceedings of 18th International Conference on Polyphenols*, INRA, Paris, 1996, pp. 223–250.
9. G. Bertrand, "Sur la recherche et la presence de la laccase dans les vegetaux," *Comptes Rendus Hebdomadaires des Sciences de l'Academie des Sciences* **121**, 166–168 (1895).
10. K. Prabhakaran, "Properties of Phenoloxidase in *Mycobacterium leprae*," *Nature* **218**, 973–974 (1968).
11. R. D. Tochner and B. J. D. Meeuse, "Enzymes of Marine Algae. 1. Studies on Phenolase in the Green Algae, *Monostroma fuscum*," *Can. J. Botany* **44**, 551–561 (1966).
12. M. von Poucke, "Copper Oxidase in Thalli *Marchantia pollumorpha*," *Physiological Plantarum* **20**, 932–945 (1967).
13. R. C. Cambie and S. M. Bocks, "A *p*-Diphenol Oxidase from Gymnosperms," *Phytochemistry* **5**, 391–396 (1966).
14. H. Yoshida, "Chemistry of Lacquer (Urushi). Part I," *J. Chem. Soc.* **43**, 472–486 (1883).
15. J. M. Nelson and C. R. Dawson, "Tyrosinase," *Advances in Enzymology* **4**, 99–152 (1944).
16. L. Vamos-Vigyazo, "Polyphenol Oxidase and Peroxidase in Fruits and Vegetables," *Crit. Rev. Food Sci. Nutr.* **15**, 49–127 (1981).
17. E. Harel, A. M. Mayer, and Y. Shain, "Catechol Oxidases from Apples; Their Properties, Subcellular Location and Inhibition," *Physiological Plantarum* **17**, 921–930 (1964).
18. A. M. Mayer, "Nature and Location of Phenolase in Germinating Lettuce," *Physiological Plantarum* **14**, 322–331 (1961).
19. E. Harel, A. M. Mayer, and Y. Shain, "Catechol Oxidases, Endogenous Substrates and Browning in Developing Apples," *J. Sci. Food Agric.* **17**, 389–392 (1966).

20. K. W. Wissemann and C. Y. Lee, "Polyphenoloxidase Activity During Grape Maturation and Wine Production," *Am. J. Enology Viticulture* **31**, 206–211 (1980).

21. Y. CoSeteng and C. Y. Lee, "Changes in Apple Polyphenoloxidase and Polyphenol Concentration in Relation to Degree of Browning," *J. Food Science* **52**, 985–989 (1987).

22. H. S. Mason and C. I. Wright, "The Chemistry of Melanin: V. Oxidation of Dihydroxyphenylalanine by Tyrosinase," *J. Biol. Chem.* **180**, 235–247 (1949).

23. W. S. Pierpoint, "The Enzymatic Oxidation of Chlorogenic Acid and Some Reactions of the Quinone Produced," *Biochem. J.* **98**, 567–580 (1966).

24. V. Khan, "Some Biochemical Properties of Polyphenol Oxidase from Two Avocado Varieties Differing in Their Browning Rates," *J. Food Sci.* **42**, 38–43 (1977).

25. L. Vamos-Vigyazo, K. Vas, and N. Kiss-Kutz, "Studies into the *o*-Diphenol Oxidase Activity of Potatoes: I. Development of an Enzyme Activity Assay Method," *Acta Alimentaria Academiae Scientarum Hungaricae* **2**, 413–429 (1973).

26. K. Mihalyi and L. Vamos-Vigyazo, "A Method for Determining Polyphenol Oxidase Activity in Fruits and Vegetables Applying a Natural Substrate," *Acta Alimentaria Academiae Scientarum Hungaricae* **5**, 69–85 (1976).

27. M. El-Bayoumi and E. Frieden, "A Spectrophotometric Method for the Determination of the Catecholase Activity of Tyrosinase and Some of Its Applications," *J. Am. Chem. Soc.* **79**, 4854–4859 (1957).

28. F. Mazzocco and P. G. Pifferi, "An Improvement of the Spectrophotometric Method for the Determination of Tyrosinase Catecholase Activity by Besthorn's Hydrozone," *Anal. Biochem.* **72**, 643–647 (1976).

29. H. Esterbauer, E. Schwarzl, and M. Hayn, "A Rapid Assay for Catechol Oxidase and Laccase Using 2-Nitro-5-thiobenzoic Acid," *Anal. Biochem.* **77**, 486–494 (1977).

30. A. M. Mayer, E. Harel, and Y. Shain, "2,3-Naphthalenediol, a Specific Competitive Inhibitor of Phenolase," *Phytochemistry* **3**, 447–451 (1964).

31. R. J. Petroski, W. Peczynska-Czoch, and J. P. Rosazza, "Analysis, Production, and Isolation of an Extracellular Laccase from *Polyporus anceps*," *Appl. Environ. Microbiol.* **40**, 1003–1006 (1980).

32. V. Kovac, "Effet des moisissures du genre penicillium sur l'activite laccase du *Botrytis cinerea*," *Annales de Technologie Agricole* **28**, 345–355 (1979).

33. J. R. L. Walker and R. F. McCallion, "The Selective Inhibition of ortho- and para-Diphenol Oxidases," *Phytochemistry* **19**, 373–377 (1980).

34. A. M. Mayer and E. Harel, "Polyphenol Oxidases in Plants," *Phytochemistry* **18**, 193–215 (1979).

35. A. R. Lax and J. W. Cary, "Biology and Molecular Biology of Polyphenol Oxidase," in C. Y. Lee and J. R. Whitaker, eds., *Enzymatic Browning and Its Prevention*, ACS Symposium Series 600, Washington, D.C., 1995, pp. 120–128.

36. K. C. Vaughn, A. R. Lax, and S. O. Duke, "Polyphenol Oxidase: The Chloroplast Oxidase With No Established Function," *Physiology Plantarum* **72**, 659–665 (1988).

37. D. Kertesz and R. Zito, "Mushroom Polyphenol Oxidase. I. Purification and General Properties," *Biochim. Biophys. Acta* **96**, 447–462 (1965).

38. W. D. Loomis and J. Battaile, "Plant Phenolic Compounds and the Isolation of Plant Enzymes," *Phytochemistry* **5**, 423–438 (1966).

39. N. O. Benjamin and M. W. Montgomery, "Polyphenol Oxidase of Royal Ann Cherries: Purification and Characterization," *J. Food Sci.* **38**, 799–806 (1973).

40. K. W. Wisseman and M. W. Montgomery, "Purification of d'Anjou Pear (*Pyrus communis L.*) Polyphenol Oxidase," *Plant Physiology* **78**, 256–262 (1985).

41. K. W. Wisseman and C. Y. Lee, "Purification of Grape Polyphenoloxidase With Hydrophobic Chromatography," *J. Chromatgr.* **192**, 232–235 (1980).

42. E. L. Angleton and W. H. Flurkey, "Activation and Alteration of Plant and Fungal Polyphenoloxidase Isoenzymes in Sodium Dodecylsulfate Electrophoresis," *Phytochemistry* **23**, 2723–2725 (1984).

43. M. Hruskocy and W. H. Flurkey, "Detection of Polyphenoloxidase Isoenzymes by Electroblotting and Photography," *Phytochemistry* **25**, 329–332 (1986).

44. K. Lerch, "Tyrosinase: Molecular and Active-Site Structure," in C. Y. Lee and J. R. Whitaker, eds., *Enzymatic Browning and Its Prevention*, ACS Symposium Series 600, Washington, D.C., 1995, pp. 64–80.

45. S. Bouchilloux, P. McMahill, and H. S. Mason, "The Multiple Forms of Mushroom Tyrosinase. Purification and Molecular Properties of the Enzymes," *J. Biol. Chem.* **238**, 1699–1707 (1963).

46. K. G. Strothkamp, R. L. Jolley, and H. S. Mason, "Quaternary Structure of Mushroom Tyrosinase," *Biochem. Biophys. Res. Commun.* **70**, 519–524 (1976).

47. E. Harel, A. M. Mayer, and E. Lehman, "Multiple Forms of *Vitis vinifera* Catechol Oxidase," *Phytochemistry* **12**, 2649–2654 (1973).

48. M. Huber, G. Hintermann, and K. Lerch, "Primary Structure of Tyrosinase from *Streptomyces glaucescens*," *Biochemistry* **24**, 6038–6044 (1985).

49. V. Bernan et al., "The Nucleotide Sequence of the Tyrosinase Gene from *Streptomyces antibioticus* and Characterization of the Gene Product," *Gene* **37**, 101–110 (1985).

50. T. Shahar et al., "The Tomato 66.3-kD Polyphenoloxidase Gene: Molecular Identification and Development Expression," *Plant Cell* **4**, 135–147 (1992).

51. S. M. Newman et al., "Organization of the Tomato Polyphenol Oxidase Gene Family," *Plant Mol. Biol.* **21**, 1035–1051 (1993).

52. J. W. Cary, A. R. Lax, and W. H. Flurkey, "Cloning and Characterization of cDNA Coding for *Vicia faba* Polyphenol Oxidase," *Plant Mol. Biol.* **20**, 245–253 (1992).

53. M. D. Hunt et al., "cDNA Cloning and Expression of Potato Polyphenol Oxidase," *Plant Mol. Biol.* **21**, 59–68 (1993).

54. A. M. Mayer and E. Harel, "Laccase-like Enzyme in Peaches," *Phytochemistry* **7**, 1253–1256 (1968).

55. V. Kahn, "Latency Properties of Polyphenol Oxidase in Two Avocado Cultivars Differing in Their Rate of Browning," *J. Sci. Food Agric.* **28**, 233–239 (1977).

56. J. R. L. Walker, "Enzymatic Browning in Fruits," in C. Y. Lee and J. R. Whitaker, eds., *Enzymatic Browning and Its Prevention*, Symposium Series 600, Washington, D.C., 1995, pp. 8–22.

57. C. Y. Lee and J. R. Whitaker, *Enzymatic Browning and Its Prevention*, ACS Symposium Series 600, Washington, D.C., 1995.

58. S. Duffy and G. Felton, "Enzymatic Antinutritive Defenses of the Tomato Plant Against Insects," in P. Hedin, ed., *Naturally Occurring Pest Bioregulators*, American Chemical Society, Washington, D.C., 1991, pp. 166–197.

59. M. E. Winkler, K. Lerch, and E. I. Solomon, "Competitive Inhibitor Binding to the Binuclear Copper Active Site in Tyrosinase," *J. Am. Chem. Soc.* **103**, 7001–7003 (1981).
60. J. R. Whitaker, *Principles of Enzymology for the Food Sciences*, 2nd ed., Marcel Dekker, New York, 1994.
61. T. Nakamura, "On the Process of Enzymatic Oxidation of Hydroquinone," *Biochem. Biophys. Res. Commun.* **2**, 111–113 (1960).
62. R. A. Holwerda, S. Wherland, and H. B. Gray, "Electron Transfer Reactions of Copper Protein," *Ann. Rev. Biophys. Bioeng* **5**, 363–396 (1976).
63. V. Cheynier, C. Osse, and J. Rigaud, "Oxidation of Grape Juice Compounds in Model Solutions," *J. Food Sci.* **53**, 1729–1732 (1988).
64. V. L. Singleton, "Oxygen with Phenols and Related Reactions in Musts, Wines, and Model Systems: Observations and Practical Implications," *Am. J. Enology Viticulture* **38**, 69–77 (1987).
65. V. Chenyier, N. Basire, and J. Rigaud, "Mechanism of trans-Caffeoyltartaric acid and Catechin Oxidation in Model Solutions Containing Grape Polyphenoloxidase," *J. Agric. Food Chem.* **37**, 1069–1071 (1989).
66. J. Oszmianski and C. Y. Lee, "Enzymatic Oxidation Reaction of Catechin and Chlorogenic Acid in a Model System," *J. Agric. Food Chem.* **38**, 1202–1204 (1990).
67. M. H. Lim et al., "Enzyme Indicators of Adequate Blanching," in *Proceedings International Conference on Technical Innovations in Freezing and Refrigeration of Fruits and Vegetables*, University of California–Davis, Calif., July 9–12, 1989, pp. 67–72.
68. C. Y. Lee, N. L. Smith, and D. E. Hawbecker, "An Improved Blanching Technique for Frozen Sweet Corn-on-the-cob," in *Proceedings International Conference on Technical Innovations in Freezing and Refrigeration of Fruits and Vegetables*, University of California–Davis, Calif., July 9–12, 1989, pp. 85–90.
69. T. P. Labuza, J. H. Lillemo, and P. S. Taoukis, "Inhibition of Polyphenoloxidases by Proteolytic Enzymes," *Flussiges Obst.* **59**, 15–20 (1992).
70. J. Oszmianski and C. Y. Lee, "Inhibition of Polyphenol Oxidase Activity and Browning by Honey," *J. Agric. Food Chem.* **38**, 1892–1895 (1990).
71. G. M. Sapers and F. W. Douglas, "Measurement of Enzymatic Browning in Cut Surfaces and in Juice of Raw Apple and Pear Fruits," *J. Food Sci.* **52**, 1258–1262 (1987).

CHANG Y. LEE
Cornell University
Geneva, New York

BULKING AGENTS. See SUGAR: SUBSTITUTES, BULK, REDUCED CALORIE.

BUTTER AND BUTTER PRODUCTS

The art of butter making dates back to times immemorial. Reference to the use of butter for sacrificial worship, for medicinal and cosmetic purposes, and as a human food may be found long before the Christian era. Documents indicate that, at least in the Old World, the taming and domestication of animals constituted the earliest beginnings of human civilization and culture. There is good reason to believe, therefore, that the milking of animals and the origin of butter making aforedate the beginning of organized and permanent recording of human activities.

The evolution of the art of butter making has been intimately associated with the development and use of equipment. With the close of the eighteenth century the construction and use of creaming and butter-making equipment, other than that made of wood, began to receive consideration, and the barrel churn made its appearance.

By the middle of the nineteenth century attention was given to improvement in methods of creaming. These efforts gave birth to the deep-setting system. Up to that time, creaming was done by a method called shallow pan. The deep-setting system shortened the time for creaming and produced a better-quality cream. An inventive Bavarian brewer in 1864 conceived the idea of adapting the principle of the laboratory centrifuge. In 1877 a German engineer succeeded in designing a machine that, although primitive, was usable as a batch-type apparatus. In 1879 engineers in Sweden, Denmark, and Germany succeeded in the construction of cream separators for fully continuous operation (1).

In 1870, the last year before introduction of factory butter making, butter production in the United States was 514 million lb, practically all farm made. Authentic records concerning the beginning of factory butter making are meager. It appears that the first butter factory was built in Iowa in 1871. This beginning also introduced the pooling system of creamery operation (1).

Other inventions that assisted the development of the butter industry included the Babcock test (1890), which accurately determines the percentage of fat in milk and cream, the use of pasteurization to maintain milk and cream quality; and the use of pure cultures of lactic acid bacteria and refrigeration to help preserve cream quality.

BUTTER CONSUMPTION

Butter production and consumption has recently increased from a long decline Table 1–4. A dramatic shift occurred, starting in 1985, to the table spreads category of products (less than 80% fat) from full-fat butter, margarine, and blends (2, Land O'Lakes, unpublished data, 1985). The spreads category encompasses all non-Standard-of-Identity table spreads (ie, from 0 to 79.9% fat). Major market forces have changed this production consumption turnaround. As noted in a 1984 survey, the most important

Table 1. Market Shares for Butter, Margarine, Blends, and Spreads for 1980 to 1995

Year	Butter	Light butter	Margarine	Blends	Spreads
1980	25	—	74	1	—
1985	22	—	73	3	2
1990	17	—	48	2	33
1995	21	1	23	11	44

Source: Ref. 2 and Land O'Lakes unpublished data, 1996.

59. M. E. Winkler, K. Lerch, and E. I. Solomon, "Competitive Inhibitor Binding to the Binuclear Copper Active Site in Tyrosinase," *J. Am. Chem. Soc.* **103**, 7001–7003 (1981).
60. J. R. Whitaker, *Principles of Enzymology for the Food Sciences*, 2nd ed., Marcel Dekker, New York, 1994.
61. T. Nakamura, "On the Process of Enzymatic Oxidation of Hydroquinone," *Biochem. Biophys. Res. Commun.* **2**, 111–113 (1960).
62. R. A. Holwerda, S. Wherland, and H. B. Gray, "Electron Transfer Reactions of Copper Protein," *Ann. Rev. Biophys. Bioeng* **5**, 363–396 (1976).
63. V. Cheymer, C. Osse, and J. Rigaud, "Oxidation of Grape Juice Compounds in Model Solutions," *J. Food Sci.* **53**, 1729–1732 (1988).
64. V. L. Singleton, "Oxygen with Phenols and Related Reactions in Musts, Wines, and Model Systems: Observations and Practical Implications," *Am. J. Enology Viticulture* **38**, 69–77 (1987).
65. V. Chenyier, N. Basire, and J. Rigaud, "Mechanism of trans-Caffeoyltartaric acid and Catechin Oxidation in Model Solutions Containing Grape Polyphenoloxidase," *J. Agric. Food Chem.* **37**, 1069–1071 (1989).
66. J. Oszmianski and C. Y. Lee, "Enzymatic Oxidation Reaction of Catechin and Chlorogenic Acid in a Model System," *J. Agric. Food Chem.* **38**, 1202–1204 (1990).
67. M. H. Lim et al., "Enzyme Indicators of Adequate Blanching," in *Proceedings International Conference on Technical Innovations in Freezing and Refrigeration of Fruits and Vegetables*, University of California–Davis, Calif., July 9–12, 1989, pp. 67–72.
68. C. Y. Lee, N. L. Smith, and D. E. Hawbecker, "An Improved Blanching Technique for Frozen Sweet Corn-on-the-cob," in *Proceedings International Conference on Technical Innovations in Freezing and Refrigeration of Fruits and Vegetables*, University of California–Davis, Calif., July 9–12, 1989, pp. 85–90.
69. T. P. Labuza, J. H. Lillemo, and P. S. Taoukis, "Inhibition of Polyphenoloxidases by Proteolytic Enzymes," *Flussiges Obst.* **59**, 15–20 (1992).
70. J. Oszmianski and C. Y. Lee, "Inhibition of Polyphenol Oxidase Activity and Browning by Honey," *J. Agric. Food Chem.* **38**, 1892–1895 (1990).
71. G. M. Sapers and F. W. Douglas, "Measurement of Enzymatic Browning in Cut Surfaces and in Juice of Raw Apple and Pear Fruits," *J. Food Sci.* **52**, 1258–1262 (1987).

CHANG Y. LEE
Cornell University
Geneva, New York

BULKING AGENTS. See SUGAR: SUBSTITUTES, BULK, REDUCED CALORIE.

BUTTER AND BUTTER PRODUCTS

The art of butter making dates back to times immemorial. Reference to the use of butter for sacrificial worship, for medicinal and cosmetic purposes, and as a human food may be found long before the Christian era. Documents indicate that, at least in the Old World, the taming and domestication of animals constituted the earliest beginnings of human civilization and culture. There is good reason to believe, therefore, that the milking of animals and the origin of butter making aforedate the beginning of organized and permanent recording of human activities.

The evolution of the art of butter making has been intimately associated with the development and use of equipment. With the close of the eighteenth century the construction and use of creaming and butter-making equipment, other than that made of wood, began to receive consideration, and the barrel churn made its appearance.

By the middle of the nineteenth century attention was given to improvement in methods of creaming. These efforts gave birth to the deep-setting system. Up to that time, creaming was done by a method called shallow pan. The deep-setting system shortened the time for creaming and produced a better-quality cream. An inventive Bavarian brewer in 1864 conceived the idea of adapting the principle of the laboratory centrifuge. In 1877 a German engineer succeeded in designing a machine that, although primitive, was usable as a batch-type apparatus. In 1879 engineers in Sweden, Denmark, and Germany succeeded in the construction of cream separators for fully continuous operation (1).

In 1870, the last year before introduction of factory butter making, butter production in the United States was 514 million lb, practically all farm made. Authentic records concerning the beginning of factory butter making are meager. It appears that the first butter factory was built in Iowa in 1871. This beginning also introduced the pooling system of creamery operation (1).

Other inventions that assisted the development of the butter industry included the Babcock test (1890), which accurately determines the percentage of fat in milk and cream, the use of pasteurization to maintain milk and cream quality; and the use of pure cultures of lactic acid bacteria and refrigeration to help preserve cream quality.

BUTTER CONSUMPTION

Butter production and consumption has recently increased from a long decline Table 1–4. A dramatic shift occurred, starting in 1985, to the table spreads category of products (less than 80% fat) from full-fat butter, margarine, and blends (2, Land O'Lakes, unpublished data, 1985). The spreads category encompasses all non-Standard-of-Identity table spreads (ie, from 0 to 79.9% fat). Major market forces have changed this production consumption turnaround. As noted in a 1984 survey, the most important

Table 1. Market Shares for Butter, Margarine, Blends, and Spreads for 1980 to 1995

Year	Butter	Light butter	Margarine	Blends	Spreads
1980	25	—	74	1	—
1985	22	—	73	3	2
1990	17	—	48	2	33
1995	21	1	23	11	44

Source: Ref. 2 and Land O'Lakes unpublished data, 1996.

Table 2. Composition of Lipids in Whole Bovine Milk

Lipid	Weight percent
Hydrocarbons	Trace
Sterol esters	Trace
Triglycerides	97–98
Diglycerides	0.28–0.59
Monoglycerides	0.016–0.038
Free fatty acids	0.10–0.44
Free sterols	0.22–0.41
Phospholipids	0.2–1.0

Source. Refs. 7 and 8.

Table 3. Composition of Lipids from Milk Fat Globule Membrane

Lipid component	Percentage of membrane lipids
Carotenoids (pigment)	0.45
Squalene	0.61
Cholesterol esters	0.79
Triglycerides	53.4
Free fatty acids	6.3[a]
Cholesterol	5.2
Diglycerides	8.1
Monoglycerides	4.7
Phospholipids	20.4

Source: Refs. 9 and 10.

[a] Contained some triglycerides.

Table 4. Per Capita Consumption of Butter for the Years 1950 to 1995

Year	Pounds per capita
1950	10.7
1960	7.7
1970	5.4
1980	4.5
1990	4.0
1995	4.5

Source: Refs. 3, 4, and Land O'Lakes unpublished data, 1996.

barriers to increased butter sales were listed in the following order (2):

1. Price (opinion of an overwhelming majority when butter is compared to margarine)
2. Health (negative consumer attitudes toward cholesterol and saturated fats are increasing)
3. Poor spreadability
4. Inadequate promotional spending
5. Product innovation in margarine and spreads
6. Legislation and regulatory restrictions

The forces that have had a positive impact on butter are as follows:

1. The U.S. Department of Agriculture (USDA) has eliminated all price supports for butter. Prices follow demand/consumption economics with the world market price (which has traditionally been significantly below the U.S. support price) operating as a base floor price. When price dips to world market price, butter becomes very price competitive to margarine/blends/spreads.
2. Health issues associated with butter have been greatly negated, for the public has become apathetic to negative cholesterol messages. In addition, the issue of trans fatty acids in fairly high concentration in margarines, and the negative health impacts of these trans fats has given butter a positive health message.

Butter manufacture continues to serve as the safety valve for the dairy industry. It absorbs surplus milk supply above market requirements for other dairy products. Milk not required by the demand for these products overflows into the creamery, is skimmed, and the cream is converted to butter. When the milk supply for other products runs short of their demand, milk normally intended for butter making is diverted into the channels where needed. Even though consumption patterns have dramatically changed over the years, the butter industry thus provides a never-failing balance wheel that takes up the slack in the relationship of supply and demand for all other dairy products.

DEFINITIONS AND GRADING OF BUTTER

Standards for butter in the United States were established by an Act of Congress and are supported by the USDA standards for grades of butter. In the revised standards the following definitions apply. Butter refers to the food product usually known as butter, which is made exclusively from milk, cream, or both, with or without common salt; and with or without additional coloring matter. The milk fat content of butter is not less than 80% by weight, allowing for all tolerances. Cream refers to the cream separated from milk produced by healthy cows. Cream is pasteurized at a temperature of not less than 73.9°C for not less than 30 min; or it can be pasteurized at a temperature of not less than 89°C for not less than 15 s. Other approved methods of pasteurization give equivalent results (5).

The cream may be cultured by the addition of harmless lactic acid bacteria to enhance flavor, natural flavors obtained by distilling a fermented milk, or cream may be added to the finished butter. In addition, color, derived from a Food and Drug Administration (FDA) approved source, may be used.

There are three U.S. grades of butter: AA, A, and B. Butter is graded by first classifying its flavor organoleptically. In addition to the overall quality of the butter flavor itself, the standards list 17 flavor defects and the degree to which they may be present for each grade. If more than one off-flavor is noted, assignment is made on the basis of the flavor resulting in the lowest grade. This grade is then lowered by defects in the workmanship and the degree to which they are apparent. Disratings are characterized by negative body, flavor, or salt attributes, which are fully described in the standards. Butter that does not meet the requirements for U.S. Grade B is not graded. To bear the USDA seal, the finished product must fall within the following microbiological specifications: proteolytic count not more than 100 per gram, yeast and mold not more than 20 per gram, and coliform not more than 10 per gram. Butter should be stored at 4.4°C or lower or at less than −17.8°C, if it is to be held for more than 30 days (6).

Legal requirements for butter vary considerably in different countries. For example, in Europe butter must contain 82% fat and in France it may contain a maximum of 16% moisture (6). In some tropical parts of the world, milk fat is used in nearly anhydrous form because it is less susceptible to bacterial spoilage. This product is known as ghee. In the Middle East and India ghee is prepared from heated cow or buffalo milk.

COMPOSITION

The composition of milk fat is somewhat complex. Although dominated by triglycerides, which constitute some 98% of milk fat (with small amounts of diglycerides, monoglycerides, and free fatty acids), various other lipid classes are also present in measurable amounts. It is estimated that about 500 separate fatty acids have been detected in milk lipids; it is probable that additional fatty acids remain to be identified. Of these, about 20 are major components; the remainder are minor and occur in small or trace quantities (7,8). The other components include phospholipids, cerebrosides, and sterols (cholesterol and cholesterol esters). Small amounts of fat-soluble vitamins (mainly A, D, and E), antioxidants (tocopherol), pigments (carotene), and flavor components (lactones, aldehydes, and ketones) are also present.

The composition of the lipids of whole bovine milk is given in Table 2 (7,8). The structure and composition of the typical milk fat globule is exceeding complex. The globule is probably 2 to 3 μ in diameter with a 90-Å-thick membrane surrounding a 98 to 99% triglyceride core. The composition of the milk fat membrane is quite different from milk fat itself in that approximately 60% triglycerides are present, much less than in the parent milk fat (Table 3) (9,10).

It has been generally recognized that butterfat consists of about 15 major fatty acids with perhaps 12 or so minor (trace quantity) acids. Triglycerides are normally defined with respect to their carbon number (CN), that is, the number of fatty acid carbon atoms present in the molecule; the three carbon atoms of the glycerol moiety are ignored. Because the fatty acid spectrum of milk fat is dominated by acids containing an even number of carbon atoms, so is the triglyceride spectrum. However, the proportion of triglycerides with an odd carbon number is about three times greater than the proportion of odd-numbered fatty acids.

Although obvious correlations exist between fatty acid composition and triglyceride distribution, detailed information is lacking that would enable the triglyceride distribution to be predicted from the fatty acid composition. Much more needs to be understood of the strategy used in the bovine mammary gland in assembling a complex array of fatty acids into triglycerides. This is not an arcane study; it is necessary if processes such as fractionation are to yield products with consistent qualities throughout the year. In effect, the detailed structure of milk fat is not yet understood. Perhaps this is not surprising if we consider only the 15 major fatty acids; there are 15^3 (3375) possible triglyceride structures using a purely random model.

The concentration of milk fat results in a dairy product called butter. The concentration is a two-stage process with the first stage being effected in a mechanical separator, which causes a 10-fold concentration of the fat into an intermediate product, cream. The second stage is the churning process, which results in a further twofold concentration of the fat.

In butter manufacture, the preparation of the cream is effected quite independently of the butter-manufacturing stage. Today most butter plants receive their cream from other operations rather than directly from the farm, as was the case in the past. Because of general improvement in sanitation practices, the receipt of fresher milk and cream, and advances in the knowledge and understanding of deleterious handling conditions, the quality of cream is constantly improving.

The composition of milk fat is the most important factor affecting the firmness of butter and, therefore, its spreadability. The composition of milk fat changes primarily according to the feed; therefore, the entire problem is connected to the animal's diet. Today, the fatty acid composition of milk fat produced in various countries is rather accurately known, along with its seasonal variations. In Europe the amount of saturated fatty acids is generally highest in winter and lowest in summer or fall (Table 5) (11). Green fodder decreases the amount of saturated fatty acids and correspondingly increases the amount of unsaturated fatty acids (12). The differences between the maximum and minimum values can be fairly large. For palmitic and oleic acids, the quantitatively most important fatty acids, a difference of more than 10% between the maximum and minimum values was found in some cases. This makes it understandable that there are also significant differences in the physical characteristics of the butter. The structure of the triglycerides in the milk fat, along with the fatty acid composition, is important in determining the physical characteristics of the fat, because the softening point of fat has been found to rise as the result of interesterification (13).

Protected oils are hydrolyzed in the abomasum, and the fatty acids are absorbed in the small intestine, thereby avoiding hydrogenation. The 18:2 content in the milk fat was increased about fivefold, and the 14:0, 16:0, and 18:0 were decreased accordingly. Plasma and depot fats were also increased in 18:2 content by this program (14).

Results at the USDA are similar: cow's milk can be increased in 18:2 acid from 3 to 35% by feeding protected safflower oil (15,16). However, at high 18:2 levels, milk develops an oxidized off-flavor, usually after about 24 h, and creams require a longer aging time for satisfactory churning. As expected, butter that contains more than 16% linoleic acid is soft and sticky (8).

Table 5. Compositional Characteristics of Summer and Winter Milk Fat, 1970

	Fatty acids				
Samples investigated ($N = 140$)	Volatile	Saturated	Monounsaturated	Polyunsaturated	Iodine number
Total	10.98	56.50	29.81	2.50	32 2
Summer	9.49	58.82	33.53	3.14	36.8
Winter	12.45	59.15	26.15	1.86	27.7

Source Ref 11.

There are also differences in the butter fat of different cows on identical rations, and the age of the animal and duration of lactation have some influence on butter fat composition. Much of the dairy literature provides information relating dairy animal species and the composition of the butter fat from them.

Textural characteristics of butter are significantly dependent on milk fat composition and method of manufacture. It is possible, therefore, knowing the chemical composition of milk fat, to select the appropriate technological parameters of butter making to improve the texture. To obtain butter with constant rheological characteristics and to control the parameters of the butter-making process it is necessary to consider the difference in the chemical compositions and the properties of the milk fat in various seasons.

From the standpoint of nutritional value, the vitamin A content of butter is important. Because the source of vitamin A in butter is β-carotene or other carotenoid pigments in the feed of the cows, the content of this vitamin varies considerably, being highest in the summer when the dairy herds are in pasture and lowest in winter when there are no green feedstuffs in the rations. A portion of the carotene in the feed that is transferred by the cow into the butter fat varies with the feeding regimen parallel to variations in the production of vitamin A, so that the intensity of the yellow color of butter to some extent serves to indicate its vitamin A content.

The vitamin A potency of butter is in part due to vitamin A as such and in part to carotene, which is partially converted to the vitamin in the human body. The vitamin A content of butter is usually within the range of 6 to 12 mg/g, and the carotene content is in the range of 2 to 10 mg/g (17); 1 IU of vitamin A is defined as the amount possessing the biological activity of 0.6 μg of pure β-carotene.

The vitamin D content of butter is much less significant than that of vitamin A, but it is nevertheless appreciable. It varies from about 0.1 to 1.0 IU/g, being highest in the summer and lowest in the winter (17).

MODIFICATION OF MILK FAT

Melting and Crystallization of Milk Fat Triglycerides

The complex fatty acid composition of milk fat is reflected in its melting behavior. Melting begins at −30°C and is complete only at 37°C. At any intermediate temperature, milk fat is a mixture of solid and liquid. To a large extent, the solid: liquid ration determines the rheological properties of the fat. For example, at refrigeration temperature, butter has a higher solids content than does a tub margarine. Hence the latter product is more easily spread (18).

As crystallization proceeds, the growing crystals impinge to form aggregates. A network results, in which both the solid and liquid phases may be regarded as continuous. Formation of the network greatly increases the firmness of the fat.

As a liquid fat is cooled, crystallization begins. There are two parts to the crystallization: (*1*) nucleation and (*2*) growth. In a bulk fat, nucleation occurs at the surfaces of impurities, a phenomenon described as heterogeneous nucleation. A considerable degree of supercooling is necessary to initiate nucleation. Subsequent growth of the nuclei tends to be slow in natural fats because of competitive inhibition. In materials of low molecular weight, impurities are rejected at the face of growing crystal. In fats, however, the various triglyceride species are so closely related that the term *impurity* tends to lose its meaning (19).

Hydrogenation

Hydrogenation of various fats and oils is used extensively in industry but is not generally applied to butter fat (the high cost of the raw material argues against its use as a feedstock). The process reduces the degree of unsaturation of the fat and increases its melting point.

Given the criticism directed at milk fat because of its saturated nature, there appears to be little future in increasing the degree of saturation by means of hydrogenation. The reverse procedure, desaturation or dehydrogenation, offers more attractive prospects.

Cream Quality

Raw cream should be processed without delay to minimize deterioration of quality. If the cream is to be held for more than two hours between separation and processing, it should be cooled below 5°C. For holding periods exceeding one day it may be advantageous to heat, treat, or pasteurize the cream.

To make high-quality butter, the cream must be of high quality. The flavor of butter is the most important organoleptic property, and it depends in no small part on the flavor quality of the cream from which it is made. There is no real alternative to tasting in assessing flavor and this simple but important procedure should be conducted routinely as part of the overall quality-control program.

The application of hazard analysis of critical control points (HACCP) to butter production is one of the newer techniques to ensure improved butter safety and quality (20).

Fat Content

The percentage of fat in the cream must be known and controlled. It influences fat losses in churning. Knowledge of the fat content assists in yield estimations for operational conditions in continuous manufacture. A number of satisfactory procedures are available, with the Babcock test being the most common.

The chemical composition of the triglycerides, which comprise milk fat, varies throughout the year depending on the stage of lactation and the cow's diet. This causes a cyclic change in the melting properties of the fat. In the control of the butter-making process and the physical properties of the finished butter this factor must be monitored. The term melting property is used rather than softness or hardness because these more correctly refer to an altogether different attribute of solids. A number of procedures have been used to follow the seasonal change in the melting properties. The iodine number, refractive index and differential scanning calorimetry, or pulsed nmr spectroscopy can be used to prepare a melting curve. However, the expense and complexity of these melting curve techniques precludes the approach in most quality-control situations (21). The traditional chemical determinations for fat, saponification value and Polenske value, are of limited value. They are scarcely relevant for quality control, and the information they represent can be more usefully quantified by the determination of the fatty acid profile using gas chromatography.

Today the use of stainless steel has essentially eliminated the exposure of the fat to copper and iron. The presence of copper and, to a lesser extent, iron can catalyze oxidative deterioration of butter during storage, particularly in the presence of salt and a low pH.

Many procedures have been used or proposed to assess the microbiological quality of the milk or cream. Generally, microbiological tests are performed to determine the hygiene of production and storage conditions or for safety reasons. Tests include total counts and counts for specific classes of microorganisms such as yeasts and molds, coliforms, psychotrophs, and pathogens such as *Salmonella*. Rapid-screening tests based on dye reduction or direct observation using a microscope or automatic total counters are also in use.

Lipase Activity

Lipolysis in butter after manufacturing caused by thermoresistant lipase enzymes created in milk or cream by psychotrophic bacteria is an increasing problem. Based on a termination of the lipase activity in cream, the keeping quality of manufactured butter in regard to lipolysis can be predicted with reasonable accuracy. A similar prediction for sweet cream butter can be based on activity determination in the serum phase (22).

Oxidation

The flavor of dairy products is largely determined by the fat component. Consequently, it is particularly important to restrict the development of oxidized off-flavors in the fat source before use. Oxidation is the chief mode of deterioration of fats and a major factor in determining the shelf life of fat-containing foods (23). Unsaturated fatty acid esters react with oxygen to form peroxides. Although flavorless themselves, peroxides are unstable and readily decompose to yield flavorful carbonyl compounds. The latter are the source of the characteristic oxidized flavors that are detectable at low concentrations. The rate of oxidation depends on the concentration of dissolved oxygen, the temperature, the presence of prooxidants such as copper and iron, the degree of unsaturation of the fat, and the presence of antioxidants that may retard the onset of oxidation. Compared with many fats, milk fat has a good oxidative stability, because it is high in total saturates, low in polyunsaturates, and contains natural antioxidants, principally α-tocopherol.

The development of oxidative rancidity in milk fat is the major determinant of the stability of the fat on storage. Dissolved air in the milk fat can give dissolved oxygen levels of up to 40 ppm at 30°C. In practice, the dissolved oxygen level in the freshly processed milk fat would be about 5 ppm at 45°C, a level sufficient to permit the development of oxidative rancidity, but if the milk fat were allowed to equilibrate with the air, then this level could increase to 33 ppm with a consequent increase in the rate of development of oxidative rancidity.

The oxygen level in the milk fat may be limited by either active or passive actions. For passive control, processing procedures and plant design are established to minimize oxygen (air) exposure. Deaeration devices (24), the use of antioxidants, effective destruction of lipases, and nitrogen spanning of container headspace are examples of active control of product quality.

Consistency

Consistency has been defined as "that property of the material by which it resists permanent change of shape and is defined by the complete force flow relation" (8). This implies that the concept of consistency includes many aspects and cannot be expressed by one parameter. Today more importance is attached to spreadability than anything else in the evaluation of the consistency of butter. The general consensus is that butter should be spreadable at refrigerator temperatures. There is no suitable method available to measure such a subjective criteria as the spreadability of butter. For this reason, the firmness of butter, which should correlate well with spreadability, was selected as the parameter to be measured. It was recommended that the use of the cone penetrometer, along with other methods, could give good results (25).

Flavor

One of the most important consumer attributes of butter is the pleasing flavor. Butter flavor is made up of many volatile and nonvolatile compounds. Researchers have identified more than 40 neutral volatiles, of which the most prominent are lactones, ethyl esters, ketones, aldehydes, and free fatty acids (26). The nonvolatiles, of which salt (sodium chloride) is the most prominent, contribute to a balanced flavor profile. Diacetyl and dimethyl sulfide also contribute, especially in cultured butter flavor (27).

Ripened or cultured creams use lactic acid bacterial cultures selected for their ability to produce volatile acid and diactyl flavors. The NIZO (Dutch Research Institute for Dairy) process was developed to incorporate concentrated lactic starters or to directly inject starter distillates to produce the flavor and characteristics of cultured butter (25). In the United Kingdom, Canada, Ireland, and the United States, sweet cream, salted butter is primarily used, but in France, Federal Republic of Germany, and Scandinavia, the taste of cultured butter is preferred (6).

Body and Texture

The physical properties of butter that are noted by the senses are described by butter graders by means of appropriate qualifications of the terms body and texture. The exact meaning of these terms has not been clearly defined. Frequently they are used as if they had the same meaning. Certain properties such as hardness and softness refer to the body of butter, whereas properties such as openness refer to texture. But some of the properties, such as leakiness or crumbliness, lead to confusion. Usually, most body and texture terms are used to describe a defect such as gritty, gummy, sticky, and the like.

Good butter should be of fine and close texture; have a firm, waxy body; and be sufficiently plastic to be spreadable at cold temperatures.

Color

The color of butter may vary from a light creamy white to a dark creamy yellow or orange yellow. Differences in butter color are due to variations in the color of the butter fat, which is affected by the cows' feed and season of the year; variations in the size of the fat globule; presence or absence of salt; conditions of working the butter; and the type and amount of natural coloring added.

Butter colorings are oil soluble and most often are natural annatto, an extraction of the seeds of the tropical tree *Bixa orellana*, or natural carotenes, extractions from various carotene-rich plants. Because they are oil soluble, colorings are added to the cream to obtain the most uniform dispersion.

PROCESSING

Milk and Cream Separation

The most basic and oldest processing method is cream separation. Ancient people are known to have used milk freely. It is probable that they used the cream that rose to the top of milk that had been held for some time in containers, although there is little in ancient literature to suggest that such use was common. It is well established that in early times butter was produced by churning milk.

The principal questions concerning separation in a butter-manufacturing facility are the choice of cream fat content and the choice of the separation technique, milk separation before or after pasteurization, temperature of separation, and regulation of the fat content. Separation of cream from milk is possible because of a difference in specific gravity between the fat and the liquid portion, or serum. Whether separation is accomplished by gravity or centrifugal methods, the result depends on this difference (28).

Crystallization

The crystal structure of fat and the resulting physical properties of butter made by both conventional and alternative processes have received considerable study. When conventionally churned or Fritz butter is made, most of the milk fat is contained within the fat globule in cream during the cooling and crystallization process. The fat globule provides a natural limit to the growth of fat crystals. Cooling and holding of cream is normally carried out overnight, and thus sufficient time exists for the crystallization process to approach equilibrium (29).

The principles of crystallization of plastic fats in the type of equipment used for margarine manufacture have been described (30). It is important to develop small fat crystals that remain substantially discrete and do not form a strong interlocking structure. Small crystals (eg, 5-μm diameter) have a greater total surface area than large crystals and will bind water and free liquid fat by absorption more effectively (29). Large crystals impart a gritty texture to the product. When fats are cooled rapidly in a scraped surface heat exchanger, fat crystallization commences, but the fat is substantially supercooled on exiting. If crystallization is then permitted to continue under quiescent conditions, crystals will grow together and form a lattice structure. The product will thus be hard and brittle and may tend to leak moisture. If, however, crystallization is permitted to occur under agitated conditions (eg, for 1–3 min in a pin worker), the formation of small, independent crystals will be favored and the product will have a fine, smooth texture. If crystallization under agitated conditions is permitted to continue for too long, the product will be too soft for most patting or bulk filling operations, and it is likely to be too soft and greasy at warm room temperatures.

Neutralization

When lactic acid has developed in the raw, unpasteurized cream by microbial activity to a degree considered excessive, neutralizer may be added to return the cream acidity to a desirable level. Sodium carbonates have been found suitable in practice for batch neutralization. For continuous neutralization by pH control, sodium hydroxide is more suitable. These chemicals should be of food grade.

Fractionation

Fractionation by thermal crystallization, steam stripping, short-path distillation, supercritical fluid extraction, or crystallization using solvents can achieve fat alterations of significance to the dairy industry. Milk fat is a mixture of triglycerides with a range of molecular weights, degrees of unsaturation, melting points, and other physical properties. Milk fat is an important component of most dairy products, but it has been consumed traditionally for the most part as butter.

Fractionation of butter fat for the selective removal of cholesterol has been recently reported using supercritical fluid extraction (31), steam stripping or deodorization (32), short-path molecular distillation (33), and absorption processes (28). Various enzymatic investigations are under way to isolate cholesterol reductase (34) and to insert the gene for cholesterol reductase into lactic and acid bacteria biogenetically (35), resulting in the reduction of the dairy fat cholesterol to its major by-product, coprostanol.

Heat Treatment

The heat treatment of cream plays a decisive role in the butter-manufacturing process and the eventual quality of the butter. It is important that milk and cream be handled in the gentlest possible way to avoid mechanical damage to the fat, a serious problem in continuous manufacture (Fritz process) of butter (36). Cream is pasteurized or heat treated for the following reasons: to destroy pathogenic microorganisms and reduce the number of bacteria; to deactivate enzymes, to liquefy the fat for subsequent control of crystallization, and to provide partial elimination of undesirable volatile flavors.

Batch Butter Manufacture

Today batch processing is not used to any extent for the production of large quantities of butter. Batch systems are still encountered in small butter plants, primarily in less industrially developed countries. Continuous systems are more efficient and cost effective for large outputs; batch systems have low capital intensity.

The processing of cream by a batch churn requires filling to approximately 30 to 50% capacity at a cream temperature of 4.4 to 12.8°C. Cream temperature will vary depending on seasonably of the cream, the butter characteristics desired, and the desired rate of fat inversion. Churning is accomplished by rotation of the churn at approximately 35 rpm until small butter granules appear. The process usually requires about 45 min for coalescence of the fat globules and clean separation of the buttermilk so that it can be drained (37). The granules may be washed with cold water to remove surface buttermilk. Salt is then added with water if the fat content standardization is required. The butter is worked to ensure uniformity and desirable body and texture characteristics.

Continuous Butter Manufacture

Between 1930 and 1960 a number of continuous processes were developed. In the Alfa, Alfa-Laval, New Way, and Meleshin processes phase inversion takes place by cooling and mechanical treatment of the concentrated cream. In the Cherry-Burrel Gold'n Flow and Creamery Package processes, phase inversion takes place during or immediately after concentration, producing a liquid identical to melted butter, prior to cooling and working. The Alfa, Alfa-Laval, and New Way processes were unsuccessful commercially. The Meleshin process, however, was adopted successfully in the Republic of Russia. The Cherry-Burrel Gold'n Flow process appears to have been the more successful of the two American processes (29).

The Fritz continuous butter-making process, which is based on the same principles as traditional batch churning, is now the predominant process for butter manufacture in most butter-producing countries. In the churning process crystallization of milk fat is carried out in the cream, with phase inversion and milk fat concentration taking place during the churning and draining steps. However, because of the discovery that cream could be concentrated to a fat content equal to or greater than that of butter, methods have been sought for converting the concentrated or plastic cream directly into butter. Such methods would carry out the principal butter-making steps essentially in reverse order, with concentration of cream in a centrifugal separator, followed by a phase inversion, cooling, and crystallizing of the milk fat (37).

Increased demands on the keeping qualities of butter require that, in addition to careful construction, operation, and cleaning of the milk and cream processing equipment, research to develop machines that will ensure butter production and packing under conditions eliminating contamination and air admixture must be carried out. It has been demonstrated that butter produced under closed conditions has a better keeping quality than butter produced in open systems (38).

There are two classes of continuous processes in use: one using 40% cream, such as the Fritz process, and the other using 80% cream, such as the Cherry-Burrell Gold'n Flow. As much as 85% of the butter in France is made by the Fritz process. In this process 40% fat cream is churned as it passes through a cylindrical beater in a matter of seconds. The butter granules are fed through an auger where the buttermilk is drained and the product is squeeze dried to a low moisture content. It then passes through a second working stage where brine and water are injected to standardize the moisture and salt contents. As a result of the efficient draining of the buttermilk, this process is suitable for the addition of lactic acid bacteria cultures at this point. The process then becomes known as the NIZO method when the lactic starter is injected (Land O'Lakes, unpublished data, 1985). Advantages of the NIZO method over traditional culturing are improved flavor development; acid values as a result of lower pH; more flexible temperature treatment of the cream, because culturing and tempering often are accomplished concurrently; and, most important, sweet cream buttermilk is produced.

The latest in developments in butter making have been outlined in the *European Dairy Magazine* (39), with the article centering on dosing systems, and for the addition of salt and culture. In addition, a number of patents have been issued for improved butter-making processes (40), making butter directly from milk (41), and for flexible integration of added ingredients, and molding and demolding (42).

The Cherry-Burrell Gold'n Flow process is similar to margarine manufacture (43). The process starts with 18.3°C cream that is pumped through a high-speed destabilizing unit and then to a cream separator from which a 90% fat plastic cream is discharged. It is then vacuum pasteurized and held in agitated tanks to which color, flavor, salt, and milk are added. Then this 80% fat–water emulsion, which is maintained at 48.9°C, is cooled by use of scraped surface heat exchangers to 4.4°C. It then passes through a crystallizing tube, followed by a perforated plate that works the butter. Prior to chilling, 5% nitrogen gas is injected into the emulsion.

Although the Meleshin process continues to be in widespread use in the former USSR, the use of alternative continuous butter-making processes based on high-fat cream has declined in Western countries during the past 20 years (29). The principal reasons for this decline appear to be the economics and butter quality, particularly when compared with the Fritz process. A Fritz manufacturing process can be installed in existing batch churn factories with almost no modification to cream-handling or butter-packing equipment. The churns could be retained in case the Fritz breaks down. However, very little batch plant equipment could be reused in the alternative systems (ie, Gold'n Flow). When a completely new plant is being bought, the alternative systems still tend to be more expensive, and operational advantages over the Fritz are not significant. Butter from the Fritz process is nearly identical in its physical and flavor characteristics to batch-churned butter, whereas butter produced by the alternative processes tends to be different. These differences may be perceived as defects by the consumer, and manufacturers have been reluctant to alter a traditional product.

The alternative systems have a number of advantages compared with a modern Fritz line: (*1*) The most attractive advantage is the flexibility to produce a wide range of products with fat contents ranging from 30 to 95% butter–vegetable oil blends and the incorporation of fractionated fats; (*2*) these processes also present the possibility of a number of operational advantages by use of an efficient centrifuge during the cream concentration stage; fat losses in the buttermilk can be substantially reduced; and (*3*) the composition of the butter can be more accurately controlled, either by including a batch standardization step or by the use of accurate continuous metering systems.

Reworking Butter

Since significant amounts of butter are made during high milk production periods of the year (spring) and highest consumption tends to be during the late fall, butter by necessity needs to be stored under conditions to ensure continuing quality. These conditions are usually in bulk frozen form. Thawing and the subsequent reworking have resulted in development of new and superior processes and equipment. The equipment is in a closed system with clean-in-place, and the process includes butter blocks at temperatures > -5°C that are transported to a shredder, from which the butter chips pass via large-diameter screw conveyors to the silo; from here they are pumped through the reworker where optimal moisture distribution is achieved by two counterrotating kneading rollers, and reworked butter emerges at 6 to 14°C. Equipment for reworking of butter blocks that have a temperature of > -25°C incorporates a butter tempering system after the shredder, and temperature of the emerging butter can be regulated (44).

BUTTER PRODUCTS

Cultured Butter Manufacture

There are several ways of making cultured butter from sweet cream. Pasilac-Danish Turnkey Dairies, Ltd. developed the IBC method (45). The main principles of the IBC method are as follows. After sweet cream churning and buttermilk drainage, a starter culture mixture is worked into the butter, which produces both the required lowering of butter pH and, because of the diacetyl content of the starter culture mixture, the required aroma. The starter mixture consists of two types of starter culture. (*1*) *Lactococcus lactis* and (*2*) *L. cremoris* and *L. lactis* ssp. *diacetylactis*. With respect to production costs, the experience with this method shows that for the manufacture of mildly cultured butter, the direct costs are only about one-third of the costs of other methods (45).

Butter-Vegetable Oil Blends

In the early 1980s blends of butter and vegetable oil products appeared in the U.S. market. These blends generally were 40% butter and 60% vegetable oil for a total fat content of 80%, within the margarine Standard of Identity and designation. With the increasing popularity of reduced-fat (less than 80%), spreads, starting in the mid-1980s, other blends with butter fat contents of 2 to 25% were introduced. As noted in Table 1, the full 80% fat blends have declined in preference to the lower-fat spreads; the blend spreads have taken a portion of this market share (Land O'Lakes, unpublished data, 1996).

A number of processes were developed using continuous churns (46) and alternative systems similar to the Cherry-Burrell Gold'n Flow process (47). The major disadvantage to churning, either batch or continuous, was that the resultant buttermilk would be adulterated with some vegetable fat and would be less valuable than standard buttermilk. An advantage of alternative processing systems is their ability to easily accommodate the manufacture of reduced-fat spread blends.

With the popularity of blends in the United States, several products were introduced in Europe and Australia and have subsequently expanded the market for butter fat usage (Land O'Lakes, unpublished data, 1996). An even earlier entry into the blend category was Swedish Bregott in Europe.

Whipped Butter

Whipped butter is a product produced to improve spreadability; air or nitrogen is incorporated. With the incorporation of gas, the butter's volume increases by approximately 33%. Generally, whipped butter is sold in tub rather than stick form.

Spreadable Butter

In 1937 a process known as the Alnarp method was developed to increase the spreadability of butter made from winter milk fat. The apparent reason for its effectiveness is that it increases the liquid fat content of the milk fat (Fig. 1) (48).

The consistency of butter is determined by the percentage of solid fat present, which is directly influenced by the fat composition, the thermal treatment given to cream prior to churning, the mechanical treatment given to butter after manufacture, and the temperature at which the

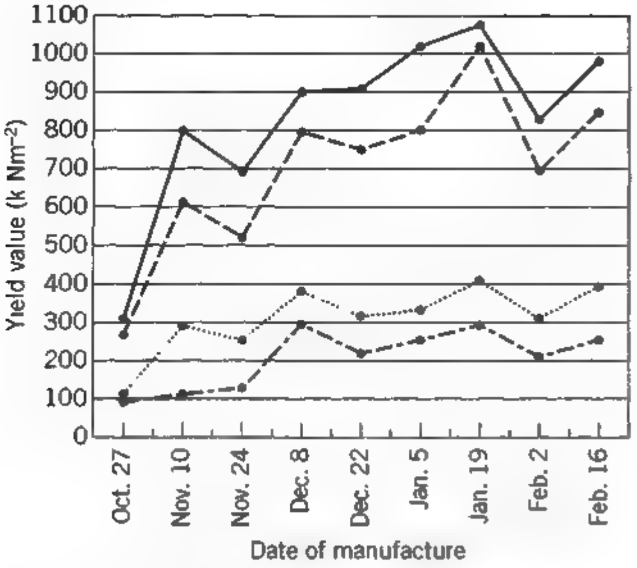


Figure 1. Effect of Alnarp-type treatment on firmness of winter butter. —●— Control 5°C; – –●– – Alnarp; -●- control 15°C; – · ● · – Alnarp; —- upper limit of spreadability. *Source:* Ref. 49.

butter is held (50). The European butter market demands that butter be softer and more spreadable in winter and harder in summer. With information on the changes in fat composition from gas–liquid chromatography analysis and the use of nuclear magnetic resonance to estimate solid fat, suitable tempering procedures can be selected to modify the fat composition and produce the most acceptable product for the consumer. A spreadable consistency of butter can be achieved by either varying the fatty acid composition or varying heat-step cream-ripening times and temperatures (51).

In efforts to improve the spreading properties of butter in relation to hard butter fat, one alternative put forward is the use of soft fat fractions obtained in the fractionation of anhydrous milk fat. Although several practical methods of fractionation have been presented, the use of soft fat fractions in butter making has not become general practice. This is evidently because fractionation in all cases significantly raises the cost of the butter produced. In addition, a common problem has been to find suitable uses for the hard fat fractions. Furthermore, in fractionation methods that use solvents or additives, fractionation should be linked to fat refining; and in this process butter also loses its natural food classification. In studies that have used soft butter fat fractions, a substantial softening of the butter has been obtained; however, this butter, like normal butter, hardens as the temperature increases and again decreases. One of the new ways to improve butter spreadability is to add vegetable oil during manufacturing. The best known of these preparations, which cannot be called butter, is the Swedish Bregott, in which 20% of the fat used is vegetable oil. A similar product is made in many other countries, Finland among them, where it has been well received, possibly just because of its better spreadability compared with normal butter.

Two industrial processes in practice for the fractionation of milk fat are the Tirtiaux system, and the DeSmet, which is a semicontinuous bulk crystallization process. They are dry fractionation processes enabling one- or two-step fractionation of butter oil at any temperature from 50 to 2°C. The milk fat fractions thus obtained can be used as such or they can be blended in different proportions for use as ingredients in various food fat formulations or in preparing spreadable butter (52).

Figure 2 depicts the solid fat content profiles of the control and anhydrous milk fat: the low melting, high melting, and 20% very high melting milk fat; the milk fat fractions used in the low melting, high melting, and 20% very high melting butter: the 30S fraction; the 13L fraction; and the 15S fraction. The fraction number includes the fractionation temperature (°C) and its physical form (solid or liquid) (53).

The major shortcoming inherent in this system is the long residence time (8–12 h) for nucleation and crystal growth.

Butter samples made from low melting liquid fractions and from a combination of primarily low melting liquid fractions and a small amount of high melting solid fractions exhibited good spreadability at refrigerator temperature (4°C) but were almost melted at room temperature (21°C). Butters made with a high proportion of low melting liquid fraction, a small proportion of high melting solid, and a small proportion of very high melting solid fractions were spreadable at refrigerator temperature and maintained their physical form at room temperature (53).

Some newer modifications and improvements to processes for the enhancement of spreadability have recently been patented (54,55). They include the use of a hydropho-

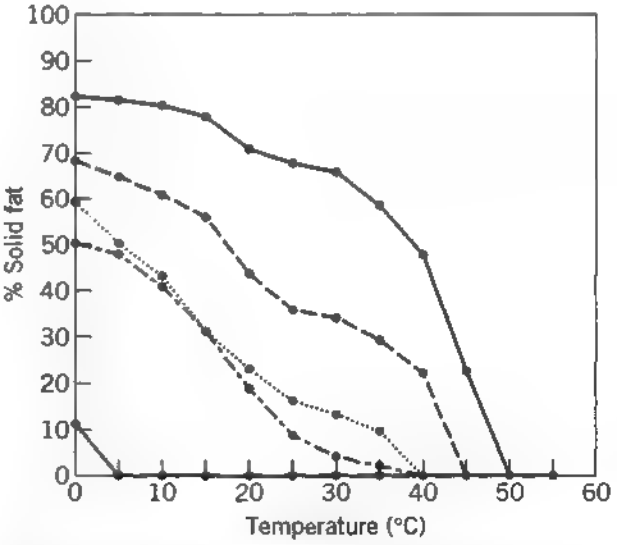


Figure 2. The fraction number includes the fractionation temperature (°C) and its physical form (solid or liquid). —— 15S Fraction (top line); ––––– and the milk fat fractions used in the low melting, high melting, and 20% very high melting butter: 30S fraction; ···· the low melting, high melting, and 20% very high melting milk fat; –·–·–· solid fat content profiles of the control and anhydrous milk fat; —— 13L fraction (bottom line).

bic membrane to separate high and low melting point fractions and improved blending techniques.

Reduced-Fat Butter

The first reduced-fat butter (50% fat), called Light Butter, was introduced in the United States by the Lipton Co. in the mid 1980s. The product was withdrawn due to FDA objections of not meeting Standards of Identity for nomenclature. Also, the product contained stabilizers not allowed in the Standard of Identity for butter. In the late 1980s, Ault, Inc. introduced a reduced-fat butter (39% fat) called Pure and Simple, which contained no unusual additives (56). Unfortunately, this all-natural product had severe negatives: it had a short shelf life, experienced moisture seepage, and lacked the highly desirable butter notes. In 1990, Land O'Lakes, Inc. launched its Light Butter (52% fat), which contained emulsifiers, added vitamin A, and preservatives. The FDA was in the process of establishing standards for reduced-fat products at this time and no objection was registered. The new standards were established in 1993 (57), which automatically required Land O'Lakes to reformulate to a 40% butterfat content; it did so and relaunched. The product was a success and has established dominance in the U.S. market.

Manufacturers have experienced many problems with the production of low-fat butter (58). Low-fat butter cannot be manufactured in conventional continuous butter makers. The technology of producing low-fat butter and margarine products is similar to that of ordinary margarine production, and it has nothing in common with modern butter production. These low-calorie water-in-fat emulsions have such a dense package of water droplets that unwanted phase inversion during processing and/or structural weak points in the product can occur, which may, for example, severely limit the microbiological shelf life. The scraped-surface heat exchanger type of machine is preferred for production of low-fat products.

Butterlike products with reduced-fat content are manufactured in several countries. Stabilizers, milk and soy proteins, sodium albumin or caseinate, fatty acids, and other additives are used. A product is now available on a commercial scale in Russia that has the following composition: 45% milk fat, 10% nonfat solids, and 45% moisture. It has a shelf life of 10 days at 5°C (59).

Decreasing the energy content of a diet has clearly been the motive behind those milk fat products in which the fat content is approximately half that of normal butter. Although these products can no longer be called butter according to international standards, they are nonetheless often called low-calorie butter, half-butter, or similar names. A large number of patents have been obtained for these products, because raising the water content to nearly 50% in the manufacture of butterlike spreads requires considerably higher emulsifying properties than the manufacture of normal butter. In addition, the emulsion must often be stabilized with additives. In some countries, a low-fat butter (40% total fat) containing vegetable oil has been designated as Minarine, but Minarine can also be prepared using only butter fat (6).

Anhydrous Milk Fat Manufacture

The quality assurance program for manufacture of butter oil, or anhydrous milk fat (AMF), also focuses on the quality of the raw materials. Naturally, many of the same considerations apply to handling raw cream for AMF manufacture that apply to butter, except that vacreation is not used. Because it is stored under ambient conditions, care against oxidation is essential. Oxidation is perhaps the most important mechanism by which milk fat deteriorates in quality. Because the oxidation reaction is autocatalytic (ie, the products of the reaction act as catalysts to promote further reaction), the normal quality control tests, peroxide value and free fat acidity, could give misleading results when applied to stored butter. Methods of deaeration have been developed that could reduce potential oxidation (24).

Milk fat is present in milk or cream as part of a stable oil-in-water emulsion. The emulsion is stabilized as a result of the protein and phospholipid-rich milk fat globule membrane (MFGM) surrounding the milk fat. During AMF manufacture the aim is to break the emulsion and to separate out all of the nonfat solids and water. To achieve this, the MFGM must first be disrupted mechanically or chemically. Homogenization, an example of mechanical treatment, disrupts the membrane, destroying the membrane layer. For chemical destruction of the membrane layer, an acid such as citric acid can be added to lower the pH of milk or cream to about 4.5 (60). The protein will precipitate, removing a component to maintain an intact fat globule. Direct-from-cream AMF plants usually has three separators. The first concentrates cream from 40% to about 75% fat before phase inversion in a homogenizing device. The oil separator then separates the liberated butter oil to about 99% purity. The oil is washed with water before the third (polishing) separator and the final traces of moisture are removed in a dehydrator at 95°C and under a vacuum of 35 to 50 torr. The dehydrator is usually a simple vessel, and the butter oil is introduced either as a thin film on to the walls or as a spray, to maximize the surface area exposed to the vacuum. Such a device will not remove significant off-flavors, because the vapor flows, temperatures, and pressures are inappropriate for flavor stripping. When producing AMF from butter, fresh or block butter is softened just to a pumpable stage (approximately 50°C) and transferred to a plate heat exchanger to increase the temperature (70–80°C). The oil phase is concentrated through separators and dried under vacuum. Some washing is possible before the final separator removes the last traces of nonfat components (60).

In terms of the preparation of products and their appearance and texture, AMF has several advantages over traditional butter. The latter is in fact subject to seasonal variations, which affect its physical properties. The advantages of AMF are linked to the possibility of standardizing its physical properties (by the selection and mixture of the raw materials used in production) and the possibility of adapting its properties using the fractionation technique.

Ghee

By definition, ghee is a product obtained exclusively from milk and/or fat-enriched milk products of various animal species by means of processes that result in the near total removal of water and nonfat solids (similar to anhydrous milk fat) and in the development of a characteristic flavor and texture. Even so, most ghee contains some nonfat solids to enhance the flavor.

Typically, ghee is manufactured by heating butter to temperatures well above those used during AMF manufacture. The high temperature treatment of the nonfat milk solids and milk fat leads to the development of a strong buttery flavor. However, traditional ghee, as produced in the Middle East and Asia, has a more rancid taste due to less-sophisticated methods of preparation and storage. Manufacturers in the European community are also producing ghee by adding ethyl butyrate to AMF (60,61). Alternate synthetic flavors have been developed to add ghee flavor notes to butter oil.

Technologies continue to advance and processes have been developed to create or enhance AMF flavor. For instance, the continuous production of lipolyzed butter oil or AMF has been developed utilizing pregastric esterases immobilized in a hollow fiber reactor (62).

Butter Flavors

Technologies for the hydrolysis of butterfat to produce and concentrate the free fatty acids to enhance the butter flavor of products have been available for decades. More recently, biotechnologists have developed methods for producing a variety of fairly pure enzymes, economically and in large quantities. The increased availability of lipases (glycerol ester hydrolases) from microbial sources has made it possible for researchers to employ the catalytic properties of these enzymes in innovative ways. One application in which the use of lipases has become well established is the production of lipolyzed flavor from feedstocks of natural origin. Immobilization of lipases on hydrophobic supports has the potential to (*1*) preserve, and in some cases enhance, the activity of lipases over their free counterparts; (*2*) increase their thermal stability; (*3*) avoid contamination of the lipase-modified product with residual activity; (*4*) increase system productivity per unit of lipase employed; and (*5*) permit the development of continuous butter flavor.

Decholesterolized Butter

Fractionation by crystallization, supercritical fluid extraction, and other technology are methods being applied commercially to milk fat to create desirable new products, such as decholesterolized butter. The essential purpose of milk fat application development is to adapt products to fit user demands.

In the 1980s, there was significant research and market activity in developing decholesterolized milk fat. All this activity was for naught, for the hypothesis of creating a "healthier" fat (for butter or milk or other dairy product) was not sound. The nutrition community had long recognized that the link between dietary cholesterol and serum cholesterol was weak and that the ratio of total fat–saturated fat had a greater impact on health. In addition, the FDA issued new standards in 1993 (57) that effectively negated the value of decholestering milk fat. The new law required that to be called low cholesterol, the fat must contain no more than 2 g of saturation fat per serving. Butterfat is approximately 65% saturated. Since the technology to desaturate milk fat is not cost-effective, decholesterization has no economic value.

Desaturated Milk Fat

In addition to chemical and enzymatic means of desaturation, there have been extensive studies on feeding cows specific diets to change butterfat saturation as well as increasing the ratio of potentially desirable fatty acids (19,63,64). In general, good progress in the understanding of rumen physiology, digestion, and function has occurred, but economic potential remains unacceptable. The most promising technologies are the use of protected fats in a feeding regimen. These fats are protected in a way that they pass through the rumen (point of fat hyrogenation) into the remaining digestive system for absorption and subsequently into the mammary glands. Unsaturated fats that are fed to cows have a great opportunity to remain unsaturated as they are synthesized into milk fat. Biotechnology may offer alternatives in the modification of edible fats and milk fat. Research has led to new methods of lipolysis and esterification, but the developments are still at the laboratory level. Nevertheless, commercial application may emerge from these interesting areas of research.

Part of the reason for consumer popularity of the butter–vegetable oil mixtures may be seen in the emphasis on saturated animal fats in current nutritional debates as well as the alleged cause-and-effect relation between butter and heart disease. For these reasons, people interested in good nutrition willingly change over to products that contain a certain amount of vegetable oil but also have the natural butter aroma.

The rise in concern for fat and cholesterol in the U.S. market overshadowed the concern for chemicals and preservatives in the 1980s. By 1990, the concern for cholesterol started to significantly decline, whereas concern for fat, particularly saturated fat, remained high (Fig. 3) (65). The U.S. Nutritional Labeling and Education Act of 1990 has provided the consumer with product label information that has influenced dietary decisions and has educated the consumer, particularly for fat and saturated fat contents.

Because the physical properties of milk fat influence the rheological properties of dairy products, especially butter,

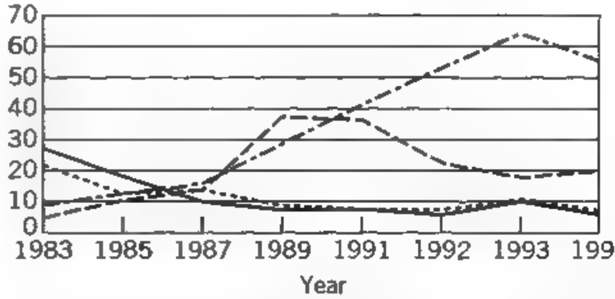


Figure 3. Rise in concern over fats, cholesterols. — - — Fat content; — — cholesterol; —— chemicals/additives; ---- preservatives.

there has been considerable interest in the modification of milk fat by physical and chemical means. Economical fractionation of milk fat into oil and plastic fat fractions will facilitate an increased utilization of milk fat in many food applications, such as chocolate, confectionery and bakery products, and in developing new convenient (spreadable) and dietetic (reduced cholesterol, variable fatty acid composition) butter or butter fat–containing products.

BIBLIOGRAPHY

1. O. F. Hunziker, *The Butter Industry*, 3rd ed., Printing Products Corp., Chicago, Ill , 1940.
2. "The World Market for Butter," *International Dairy Federation Bulletin*, Document **170**, 1984.
3. *Statistical Abstract of the United States*, 117th ed., U.S. Department of Commerce, Bureau of the Census, Washington, D.C., 1997.
4. R. Rizek, N. Raper, and K. Tippett, "Trends in U.S. Fat and Oil Consumption," *J. Am. Oil Chem. Soc.* **65**(5), 723 (1988).
5. *U.S. Code of Federal Regulations*, Title 7, Part. 58, Subpart P, U.S. Government Printing Office, Washington, D.C., 1983.
6. M. M. Chrysam, "Table Spreads and Shortenings," in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, John Wiley & Sons, New York, 1985, pp. 65–106.
7. W. R. Morrison, "Milk Lipids," in F. D. Gunstone, ed., *Topics in Liquid Chemistry*, Logos Press, London, United Kingdom, 1970, pp. 51–106
8. R. G. Jensen, "Symposium: Milk Lipids," *J. Am. Oil Chem. Soc.* **50**, 186–192 (1973).
9. J. R. Brunner, "Milk Lipoproteins," in E. Tria and A. M. Scann, eds., *Structural and Functional Aspects of Lipoproteins in Living Systems*, Academic Press, London, United Kingdom, 1969, pp. 545–578.
10. U. Bracco, J. Hidalgo, and H. Bohren, "Lipid Consumption of the Fat Globule Membrane of Human and Bovine Milk," *J. Dairy Sci.* **55**, 165 (1972).
11. V. Antila, "The Consistency of Butter and the Possibilities to Manipulate It," *Proceedings of the XXI International Dairy Congress, Moscow* **2**, 159 (1982).
12. J. Kiska et al., "Butter Texture and Consistency as a Function of Milk Fat Composition and Method Manufacture," *Brief Communication* **1**(1), 352 (1982).
13. P. W. Parodi, "Relationship Between Triglyceride Structure and Softening Point of Milk Fat," *J. Dairy Res.* **48**, 131 (1981).
14. T. W. Scott, L. J. Cook, and S. C. Mills, "Protection of Dietary Polyunsaturated Fatty Acids Against Microbial Hydrogenation in Ruminants," *J. Am. Oil Chem. Soc.* **48**, 358 (1972).
15. L. F. Edmondson et al., "Feeding Encapsulated Oils to Increase the Polyunsaturation in Milk and Meat Fat," *J. Am. Oil Chem. Soc.* **51**, 72 (1974).
16. R. D Plowman et al., "Milk Fat with Increase Polyunsaturated Fatty Acids," *J. Dairy Sci.* **55**, 204 (1972).
17. C. R. Brewington, E. A. Caress, and D. P. Schwartz, "Isolation and Identification of New Constituents in Milk Fat," *J. Lipid Res.* **11**, 355 (1970)
18. A. Boudreau and J. Aurl, "Utilizations of Milkfat: Physical and Chemical Modification of Milkfat," *International Dairy Federation Bulletin* **260**, 7–10, 13–16 (1991).
19. W. Banks, "Utilizations of Milkfat: Milk Lipids," *International Dairy Federation Bulletin* **260**, 3–6, 13, 22 (1991).
20. A. Albertini et al., "Applications of the Hazard Analysis Critical Control Point System to Butter Production," *Mondo del Lattle* **50**(2), 95 (1996).
21. B. D. Dixon, "Continuous Butter Manufacture: Raw Materials," *International Dairy Federation Bulletin* **204**, 3–5 (1986).
22. B. Schaffer and S. Szakaly, "Heat-Step Cream Ripening for Producing Spreadable Butter in Hungary," *Brief Communication* **1**(1), 337 (1982).
23. R. Norris, "Utilizations of Milkfat: Uses of Milkfat," *International Dairy Federation Bulletin* **260**(5), 23–25 (1991).
24. German Patent DF 36 23 313 Al (1988), M. Schroder (to Schroeder).
25. G. Van den Berg, "Developments in Buttermaking," *Proceedings of the XXI International Dairy Congress, Moscow* **2**, 153 (1982).
26. T. Siek and J. Lindsay, "Semiquantitative Analysis of Fresh Sweet-Cream Butter Volatiles," *J. Dairy Sci.* **53**, 700 (1970).
27. J. E. Kinsella, "Butter Flavor," *Food Technol.* **29**(5), 82–98 (1975).
28. D. H. Hettinga, *Altering Fat Composition of Dairy Products*, CAST Publication, Ames, Iowa, 1991.
29. M. Kimenai, "Continuous Butter Manufacture," *International Dairy Federation Bulletin* **204**, 16 (1986).
30. L. H Wiedermann, "Margarine and Margarine Oil, Formulation and Control," *J. Am. Oil Chem. Soc.* **55**, 823 (1978).
31. D. H. Hettinga, "Processing Technologies for Improving the Nutritional Value of Dairy Products," in Board of Agriculture, ed., *Designing Foods*, National Academy Press, Washington, D.C., 1988, p. 292.
32. R&D Applications, "Cholesterol Reduced Fat: They're Here," Prepared Foods, Chicago, Ill., 1989.
33. "Utilizations of Milk Fat," IDF Report B-Doc **164**, in *International Dairy Federation Annual Sessions*, Copenhagen, Denmark, 1989.
34. B. J. Spalding, "Combating Cholesterol via Enzyme Research," *Chemish Weekblad*, June 1, 1988, p. 42.
35. S. Harlander, "Biotechnology: Applications in the Dairy Industry," *J. Am. Oil Chem. Soc* **65**, 1727 (1988).
36. M. Schweizer, "Continuous Butter Manufacture: Heat Treatment," *International Dairy Federation Bulletin* **204**, 10 (1986).
37. F. H. McDowall, *The Butter Maker's Manual*, New Zealand University Press, Wellington, 1953.
38. P. Friis, "Advantages of Butter Production in Closed Systems," *Brief Communication* **1**(1), 326 (1982).
39. "New Generation of Butter Making Equipment," *European Dairy Magazine* **4**, 39 (1996).
40. PCT International Patent Application WO95/27392 A1 (1995), W. Vennewald (to Westfalia Separator).
41. PCT International Patent Application WO94/26123 A1 (1994), N. Shtukarin (to U.L. Karbysheva).
42. French Patent Application FR 2 743 311 A1 (1997), J. Peneveyre and P. Y. A. R. Guyard (to Peneveyre and Guyard).
43. *Gold'n Flow Continuous Buttermaking Method*, Bulletin **G-493**, Cherry-Burrell Corp., Chicago, Ill., 1954.
44. R. Egli, "Butter Reworking," *Food Tech Europe* **2**(3), 110 (1995).
45. P. Bjerre, in *Milk Fat*, Society of Dairy Technology, Huntingdon, U.K., 1991, pp. 63–73.
46. U.S. Pat. 4,425,370 (Jan. 10, 1984), F. Graves (to Land O'Lakes, Inc.).
47. U.S. Pat 4,447,463 (May 8, 1984), D. Antenore, D. Schmadeke, and R. Stewart (to Land O'Lakes, Inc.).

48. B. Schaffer and S. Szakaly, "The Effect of Temperature Treatment of Cream on the Liquid Fat Ratio," *Brief Communication* **1**(1), 363 (1982).
49. A. M. Fearon and D. E. Johnston, "Improving Butter Spreadability—the Scientific Approach," *Dairy Industry International* **53**(10), 25–29 (1988).
50. N Cullinane and D. Eason, "Variation in Percentage Solid Fat of Irish Butter," *Brief Communication* **1**(1), 349 (1982).
51 B. K. Mortensen and K. Jansen, "Lipase Activity in Cream and Butter," *Brief Communication* **1**(1), 334 (1982).
52. U.S. Pat. 4,839,190 (June 13, 1989), J. Bumbalough (to Wisconsin Milk Marketing Board, Inc.).
53. K. E. Kaylegian and R. C. Lindsey, "Performance of Selected Milk Fat Fractions in Cold-Spreadable Butter," *J. Dairy Sci.* **75**, 3307–3317 (1992).
54. PCT International Patent Application WO 97/10719 A1 (1997), K. Glugovsky (to Glugovsky and Lassnig Vertriebsges).
55. French Patent Application FR 2 713 656 A1 (1995), M. Parmentier, S. Bomaz, and B. Journet (to Union Beurriere SA).
56. I. Macnab, "Ault Achieves Technological Breakthrough with World's First Pure Light Butter," *Modern Dairy* **68**(3), 23 (1989).
57. R. Wood et al., "Effect of Butter Mono and Polyunsaturated-Enriched Butter Trans Fatty Acid Margarine and Zero Trans Fatty Acid Margarine on Serum Lipids and Lipoproteins in Healthy Men," *J. Lipid Res.* **34**, 1–11 (1993).
58. O. Gerstenberg, "Pitfalls in Low Butter Production," *Dairy Industry International* **53**(11), 28–29 (1988).
59. S. Gulyayev-Zaitsev et al., "Low Calorie Butter," *Brief Communication* **1**, 328 (1982).
60. K. K. Rajah and K. J. Burgess, in *Milk Fat*, Society of Dairy Technology, Huntingdon, U.K., 1991, pp. 37–43.
61. B. K. Wadhwa and M K. Jain, "Chemistry of Ghee Flavour—a Review," *Indian J Dairy Sci.* **44**, 372–374 (1992).
62. H. S. Garcia and C. G. Hill, Jr., "Improving the Continuous Production of Lipolyzed Butter Oil With Pregastric Esterases Immobilized in a Hollow Fiber Reactor," *Biotechnol. Tech.* **9**, 467 (1995).
63. W. Banks and W. W. Christie, "Feeding Cows for the Production of Butter With Good Spreadability at Refrigeration Temperatures," *Outlook Agriculture* **19**, 43–47 (1990).
64. R. P. Middaugh et al., "Characteristics of Milk and Butter from Cows Fed Sunflower Seeds," *J. Dairy Sci.* **71**, 3179–3187 (1988).
65. *Trends in the U.S. Consumer Attitudes and the Supermarket*, Food Marketing Institute, Washington, D.C., 1997.

DAVID HETTINGA
Land O' Lakes
Arden Hills, Minnesota

C

CACAO BEAN. See CHOCOLATE AND COCOA.

CAFFEINE

Caffeine (1,3,7-trimethylxanthine) was isolated from coffee in 1820 (1). Thein was isolated from tea in 1827 and later shown to be identical with the coffee isolate. It was subsequently identified in cocoa, maté, kola nuts, and other plants; structure was determined in 1875. Caffeine is one of several methylxanthines that occur naturally, primarily in plant matter that is used to prepare beverages. Other methylxanthines that usually accompany it include theobromine and theophylline. The methylxanthines, as dioxypurines, are related to two nucleic acids: adenine and guanine. This is relevant to their biosynthesis and physiological effects. Their relationship to uric acids accounts for their metabolic fate. The structures of caffeine and related compounds are shown in Figure 1.

Total synthesis was first achieved in 1895 starting with dimethylurea. Dimethyluric acid, chlorotheophylline, and chlorocaffeine were intermediates (2).

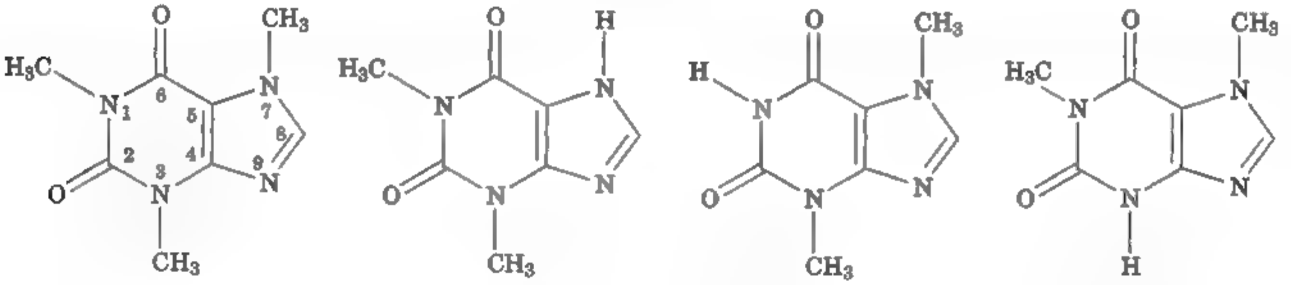
PROPERTIES

Physical Properties

Caffeine is a white, odorless powder with a slightly bitter taste. The anhydrous form obtained by crystallization from nonaqueous solvents is a crystalline solid that melts at 235 to 237°C. At atmospheric pressure it begins to sublime without decomposition at 120°C and at 80°C under high vacuum. Its crystals are hexagonal and form parallel plates. When crystallized from water, the monohydrate forms long, silky, white needles. It becomes anhydrous at 80°C. Caffeine is soluble to the extent of 0.6% in water at 0°C, 2.13% at 25°C and 66.7% at 100°C. The pH of dilute solutions is 6.9. In aqueous solution, caffeine forms dimers and higher polymers by base stacking (3). It is fairly stable in dilute bases and acids, forming salts with the latter. Caffeine is considerably more soluble in chlorinated solvents: 8.67% in methylene chloride and 12.20% in chloroform at 25°C. Its ultraviolet absorption spectrum shows a maximum at 274 nm with no variation over the pH range 2 to 14.

Chemical Properties

Complexation occurs with many acid radicals, notably chlorogenate, salicylate, citrate, benzoate, and cinnamate. Chlorogenic acid forms a tightly bound 1:1 complex. It is soluble in chloroform and can be recrystallized from aqueous alcohol, attesting to its stability. In plant matter much of the caffeine exists in this form (4). Complexes with phenolic molecules are only stable below pH 6. Caffeine is a weak base; its acid salts hydrolyze readily. It is converted

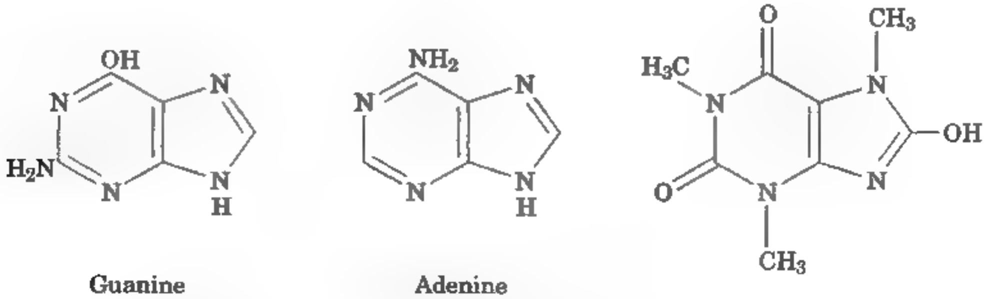


Caffeine
1,3,7-Trimethylxanthine

Theophylline
1,3-Dimethylxanthine

Theobromine
3,7-Dimethylxanthine

Paraxanthine
1,7-Dimethylxanthine



Guanine
2-Aminohypoxanthine

Adenine
6-Aminopurine

1,3,7-Trimethyluric acid

Figure 1. Methylxanthines and related compounds.

to caffeidine by treatment with concentrated NaOH (5). Alloxans are formed by oxidation with chlorine and other oxidants (6). Reaction products are shown in Figure 2.

ANALYSIS

Concern with the physiological effects of caffeine has stimulated research on its analysis in food and biological systems. High-performance liquid chromatographic (hplc) analysis of caffeine in food products requires only the filtration of aqueous extracts and injection onto a suitable column. Detection is frequently based on the measurement of µv absorption at 280 nm (7). Determination of caffeine at the low concentrations present in body fluids is accomplished by direct injection onto reversed-phase hplc columns (8). Gas chromatographic determination of caffeine in beverages using a flame ionization detector has also been described (9). Capillary electrophoresis has also been used for caffeine determination (10).

OCCURRENCE

Caffeine is present in various tissues of about 60 plant species, several of which are used to prepare beverages (11). The most important are the following:

Coffee beans (*Coffea arabica* and *Coffea canephora* var. *robusta*)
Tea leaves (*Camellia sinensis* vars. *assamica* and *sinensis*)
Cocoa beans (*Theobroma cacao*)
Maté stems and leaves (*Ilex paraguariensis*)
Guarana seeds (*Paullinia cupana*)
Kola nuts (*Cola acuminata* and *Cola verticillata*)

BIOGENESIS

The biogenesis of caffeine is probably similar in all plants, but with differences in the rates of various steps. It has its origin in the cell purine pool. Precursors include 7-methylxanthosine, 7-methylxanthine, and theobromine. Synthesis of caffeine from adenine occurs readily in young tea leaf. 7-Methylguanilic acid and 7-methylguanasine are also on the biosynthetic path. A 7-methyl-*N*-nucleoside hydrolase mediates the removal of ribose from 7-methylxanthosine. *S*-adenosylmethionine is the methyl donor for the methylation of the xanthines in reactions catalyzed by *N*-methyltransferase (12,13). A possible reaction path is shown in Figure 3. Caffeine biosyntheses in the coffee plant (12–14) and in the tea plant (14–16) have been studied in detail.

CAFFEINE IN THE HUMAN DIET

Natural Products

A high proportion of the human population has consumed caffeine-containing beverages for many centuries. Authenticated usage of tea in China dates from A.D. 350 The beverage reached Europe in 1600. Coffee came into use as a beverage in Arabia around A.D. 1000 and was brought to Europe in the seventeenth century. Cocoa was consumed by the Aztecs in Mexico. In many parts of South America maté has been a source of substantial caffeine intake. Caffeine content of several contemporary food products is shown in Table 1.

Variations in caffeine content in specific plant species result from varietal diversity, climatic changes in the growing areas, and horticultural techniques. In tea, youngest leaf has the highest concentration (17). Processing conditions also affect caffeine content. High coffee roasting temperatures result in caffeine loss by sublimation (18). There is a higher level of caffeine in tea than in coffee beans, but 200 cups of tea beverage are obtained per pound of tea leaves, whereas only about 40 to 60 cups of coffee are usually prepared per pound of coffee beans.

By far, the greatest sources of dietary caffeine are coffee and tea, but cola beverages provide an increasing portion of the intake. The total per capita intake of caffeine by adults in the United States is about 3 mg/kg of body weight with about 2 mg/kg coming from coffee and most of the rest from tea. For caffeine users, average intake is approximately 4 mg/kg (19). The consumption by heavy users is of interest because of concern with possible health effects. Mean daily intake (mg/kg body weight) in the United States by consumers in the 90th to 100th percentiles of consumption varies with age level as follows:

1–5 yr	4.7	12–17 yr	2.9
6–11 yr	3.2	18 yr +	7.0

Tea and soft drinks are the major caffeine sources for users under the age of 18. Coffee becomes the major source for those over that age (20).

Caffeine as a Food Additive

In addition to its natural occurrence in plants, caffeine is added to the very widely consumed cola drinks and other

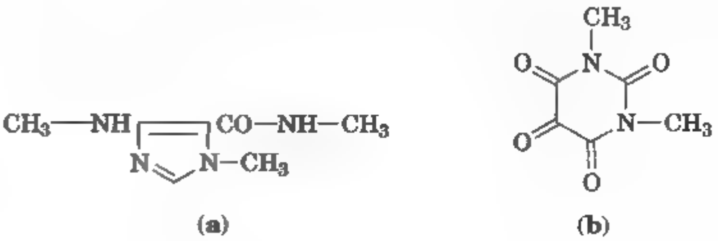


Figure 2. Reaction products of caffeine: (a) caffeidine and (b) dimethylalloxan.

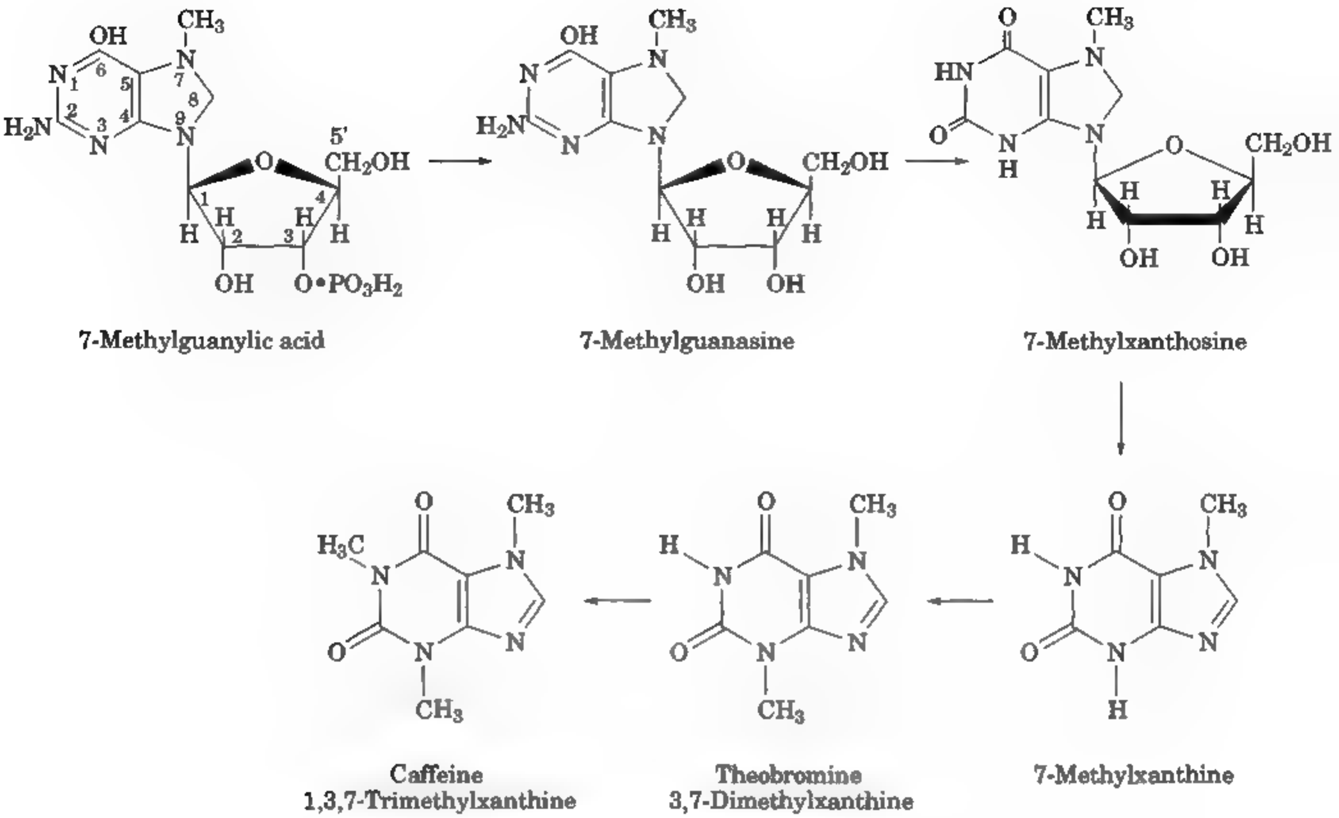


Figure 3. Biosynthesis of caffeine.

Table 1. Caffeine in Food Products

	Caffeine content (%)		Caffeine per serving[a] (mg)	
Food product	Range	Mean	Range	Mean
Coffee, arabica	0.58–1.7	1.2	35–80	60
Coffee, robusta	1.2–3.3	2.2	60–140	120
Coffee, instant	2.2–5.0	3.3	45–105	65
Coffee, decaf.	0.03–0.09	0.06	2–6	3
Tea, black	1.2–4.6	3.0	20–48	35
Tea, green	1.0–2.4	1.9	17–40	24
Tea, instant	4.0–5.0	4.8	20–30	22
Cocoa, powder	0.08–0.35	0.2	1–8	4
Chocolate milk	0.01–0 025	0.02	2–7	5
Milk chocolate	0.005–0.04	0.02	2–14	6
Dark chocolate	0.02–0.10	0.08	6–28	23
Maté	0.9–2.2	2.0	36–42	40
Guarana paste	3.9–5.8	4.7	—	—
Kola nut	0.8–2.2	1.5	—	—
Cola drinks	0.08–0.16	0.09	20–30	28

[a] Serving size is assumed to be 6 oz for hot beverages, 8 oz for cold beverages, and 1 oz for chocolate products.

soda products. This usage is based on its stimulatory properties and the slight degree of bitterness that it imparts. In 1978 the U.S. Select Committee on GRAS Substances reaffirmed the safety of caffeine as a beverage additive at levels current at that time 10 to 12 mg/100 mL). A very few soft drinks have a higher level. No-caffeine colas have become widely consumed.

Prescriptions and Over-the-Counter Medications

In the United States prescriptions and over-the-counter (OTC) medications use a total of about 50 metric tons of caffeine annually. Caffeine-containing medications are used for certain types of pain relief, treatment of infant apnea, alertness increase, and weight loss.

CAFFEINE PRODUCTION

Approximately 450 tons of caffeine are required annually in the United States for addition to beverages and for pharmaceutical use. This need is partially met by the decaffeination of coffee (21). The methylation of theobromine obtained by extraction of cocoa hulls provides part of the remainder of the requirements. Dimethyl sulfate, diazomethane, and methyl iodide can be used to prepare caffeine from xanthine or any of the related monomethyl- or dimethylxanthines (22). Patented synthetic processes are also based on classical organochemical ring closure techniques and do not require the availability of xanthines as starting materials.

In 1995 the United States imported about 1000 tons of caffeine. About 500 tons came from China. Domestic synthetic caffeine was list-priced at $7.25/lb. The product from China was approximately $4.50/lb (23).

METABOLISM OF CAFFEINE

The caffeine molecule is sufficiently hydrophobic to pass freely through biological membranes (24). It is completely absorbed from the gastrointestinal tract and rapidly attains peak plasma levels. Caffeine is equally well absorbed from each of its beverage sources. There are no effective barriers to penetration of any tissue, including placenta and fetus. This characteristic prevents efficient excretion of caffeine by the human kidney since it is readily reabsorbed from the renal tubules.

The half-life of caffeine in plasma varies not only with specie differences but also with age and condition of the individual. The half-life in rodent plasma is 1 to 2 h but it is 6 h in that of the healthy adult human. There is variation depending on smoking habits and the use of some medications. During pregnancy the half-life is increased to 18 h. The immature liver of the newborn human is limited in its ability to metabolize caffeine so that its half-life is three to four days, similar to that of adults with severe liver damage. These differences are significant in extrapolating safety data from animal studies and also in considering diet during pregnancy (24).

The metabolic pathways of caffeine in those mammalian species that have been studied exhibit many similarities (24). The same major reactions take place: demethylation, oxidation at the 8-position on the xanthine ring to form uric acids, and ring cleavage between the 8- and 9-positions to form diaminouricils. *N*-acetylation also occurs. The three dimethyl- and the three monomethylxanthines that can be formed are all found in human urine after caffeine ingestion along with all of the corresponding methylated uric acids. Uracils derived from caffeine, theobromine, and paraxanthine as well as large amounts of acetylated products are also present. No xanthine is formed. The human metabolic pathway is shown in Figure 4.

Specie variations in the metabolic pathways of caffeine were first observed in 1900 (1). In humans, about 70% of ingested caffeine is initially converted to paraxanthine, 25% to a mixture of theobromine and theophylline, and about 5% is oxidized without demethylation to form the corresponding uric acid and uracil compounds. Some primates produce theophylline as the predominant initial metabolite. In the human the final product mix is the result of competing reactions, the rates of which vary with gender, dosage, medications in the diet, and individual differences. Minor amounts of uracils derived from caffeine, theobromine, and paraxanthine also occur. Averaged values for caffeine metabolites found in human urine are shown in Table 2.

The acetylated product (A) in Table 2 is believed to be a true metabolite. Product (B) is probably an artifact of analytical procedures.

Reaction mechanisms are not well known. The biochemical changes take place in the liver. Xanthine oxidase mediates the formation of the uric acid derivatives. It is interesting to note that in the human infant, theophylline, often used for treatment of apnea, is methylated to caffeine as the first step in its metabolism (1).

PHARMACOLOGY

Effects on Cardiovascular System

Caffeine produces minor, transitory increases in blood pressure. Habitual users are less prone to exhibit this effect. Its significance is not known (26). Caffeine has also been reported to cause cardiac arrhythmias, but there is conflicting evidence (26). There appears to be a caffeine-intolerant population that is susceptible to this effect. Caffeine may exacerbate an existing tendency toward arrhythmias. Low dosages may decrease heart rate slightly; high dosages may cause tachycardia in sensitive subjects (27). Cerebral blood flow is decreased, and this effect is the basis for its inclusion in drug preparations for the treatment of migraine headaches (28).

Some studies indicate a positive correlation between caffeine intake and the development of hypercholesterolemia, but many very large efforts to confirm this effect, such as the Framingham study, show no correlation between atherosclerotic cardiovascular disease and coffee intake. Other large studies have shown no correlation between coffee drinking and any form of coronary heart disease (27). The disparate results concerning the effects of caffeine on the cardiovascular system probably relate to the size of the studies, lack of control of unrecognized factors and, very importantly, to the acceptance of "cups of coffee" as a quantitative measure of caffeine content. Coffee and tea contain many physiologically active compounds other than caffeine. Coffee oils, especially kahweol, have been shown to be cholesterogenic (29).

Effects on the Central Nervous System

Although caffeine is the stimulatory drug most widely used throughout the world, it is difficult to quantify its behavioral effects. This is primarily due to the differences in the form of its ingestion, that is, coffee, tea, or the pure substance. Another confounding factor is the varying degrees of tolerance that develop among its users.

Studies of the effects of caffeine on information processing (30), vigilance performance (31), ease of distraction (32), reaction time performance (30), decision making (33), and cognitive functioning (34) have been reported. Results are difficult to assess, especially with regard to cognitive functioning, but in general, low or intermediate dosages of caffeine are beneficial while high dosages impair performance. Gender, age, and task difficulty have notable effects on results.

The effects of caffeine on mood have also been studied. Again, improvements are experienced until dosage levels become high (30). At a dosage of 2 mg/kg, which generates a peak plasma concentration of 5 to 10 μM, individuals generally feel more alert and better able to carry out routine tasks after having become bored or fatigued (35). Caffeine may even counteract mood deficits caused by profound sleep deprivation (36). Conversely, omission of a habitual morning dosage of caffeine often results in nervousness, irritability, and poor work performance. The most widely noticed effect of caffeine is the prolongation of the sleep latency period. Its effect on the quality of sleep shows wide individual variation and is dose, time, and age

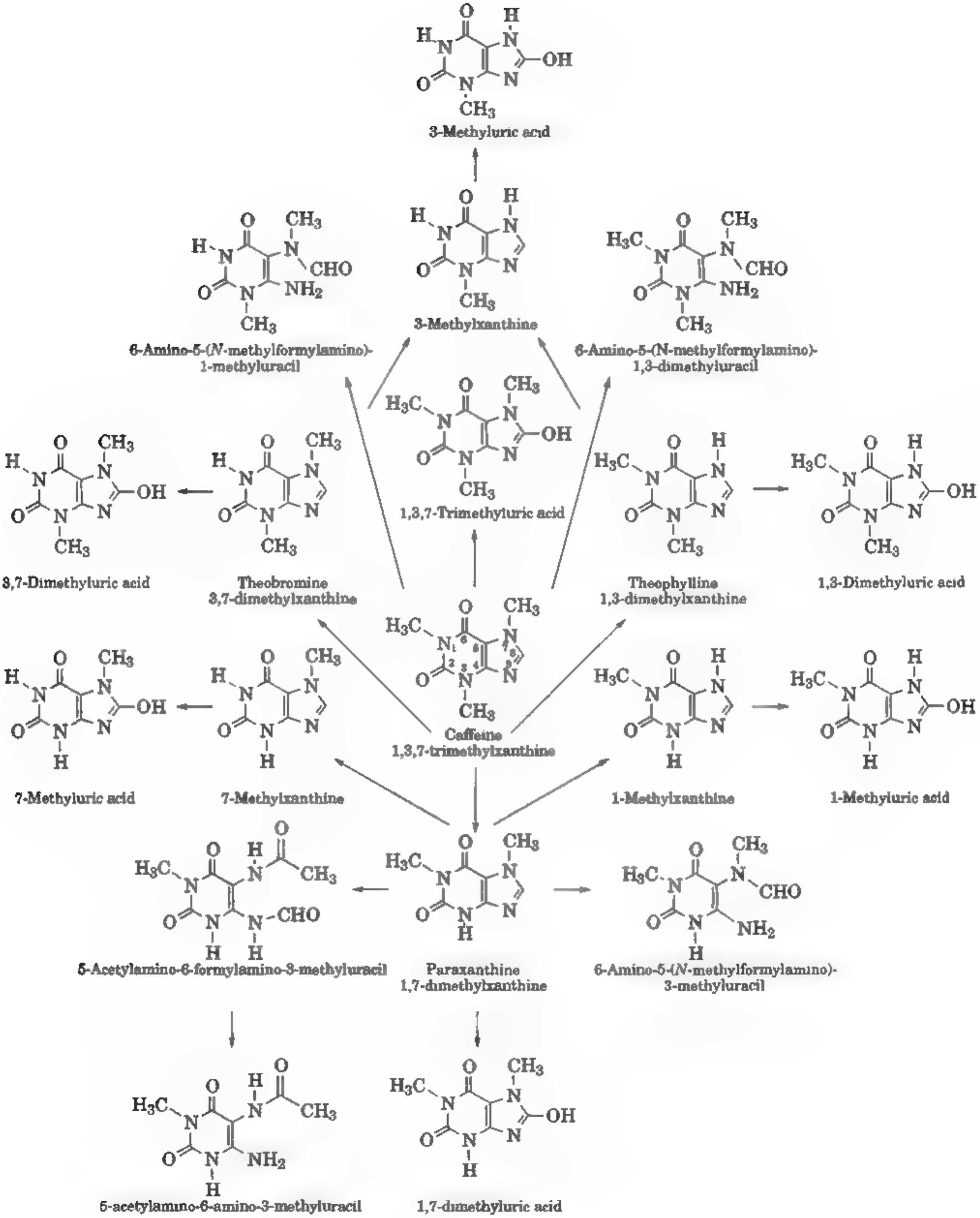

3-Methyluric acid
6-Amino-5-(N-methylformylamino)-1-methyluracil
3-Methylxanthine
6-Amino-5-(N-methylformylamino)-1,3-dimethyluracil
1,3,7-Trimethyluric acid
3,7-Dimethyluric acid
Theobromine
3,7-dimethylxanthine
Theophylline
1,3-dimethylxanthine
1,3-Dimethyluric acid
Caffeine
1,3,7-trimethylxanthine
7-Methyluric acid
7-Methylxanthine
1-Methylxanthine
1-Methyluric acid
5-Acetylamino-6-formylamino-3-methyluracil
Paraxanthine
1,7-dimethylxanthine
6-Amino-5-(N-methylformylamino)-3-methyluracil
5-acetylamino-6-amino-3-methyluracil
1,7-dimethyluric acid

Figure 4. Human metabolism of caffeine.

Table 2. Caffeine Metabolites in Human Urine as % of a 5 mg/kg Dosage

Caffeine	1.2	1,3,7-trimethyluric acid	1.2
Paraxanthine	5.9	1,7-dimethyluric acid	5.4
Theobromine	1.9	3,7-dimethyluric acid[1]	
Theophylline	0.9	1,3-dimethyluric acid	1.8
1-methylxanthine	18.1	1-methyluric acid	14.8
7-methylxanthine	7.7	7-methyluric acid	1.0
3-methylxanthine	4.1	3-methyluric acid	
5-acetylamino-6-amino-3-methyluracil (A)	3.2		
5-acetylamino-6-formylamino-3-methyluracil (B)	14.7		

Source: Ref. 26.

dependent. It is markedly subject to the development of tolerance.

The arousal model as an explanation for the foregoing effects has been suggested (37). Positive response to the arousal effect of caffeine plus those of any other biological and background impacts, increases with an increase in stimuli but is reversed as the total arousal level exceeds the tolerance level.

Other Effects

Caffeine has been reported to increase work output and endurance in long-term exercise regimens. Ergogenic properties of caffeine have been observed in many studies of athletic performance but are subject to confounding factors: nature of activity, degree of training of athletes, level of caffeine tolerance, and habitual caffeine usage. There are also many "no effect" reports. An interesting interpretation of these varying results is that the mood-elevating properties of caffeine may be responsible for some of the positive effects reported (38).

Caffeine produces a mild diuresis in humans and increases the excretion of sodium, potassium, and chloride ions (28). The thermogenic properties of caffeine cause an increase in the resting metabolic rate. Consideration has been given to its use in managing obesity (39). Respiratory rate is increased by caffeine at a plasma level of 5 mg/L. The mechanisms for this stimulation are complex and involve many separate effects. They include increased pulmonary blood flow through vasodilation, increased sensitivity of the respiratory centers to carbon dioxide, and improved skeletal muscle contraction (28). This multiple response is utilized in the treatment of infantile apnea.

Mechanism of Physiological Activities

The effects of caffeine on the cardiovascular, neurological, and renal systems and on lipolysis are primarily triggered by the blockage of adenosine receptors through competitive antagonism (40). The structural relationship between caffeine and adenine (and therefore adenosine) was shown previously. Other suggested mechanisms such as the inhibition of phosphodiesterases and modification of calcium metabolism may also be operative but are inadequate to explain these effects at reasonable caffeine concentrations. Adenosine and caffeine show contrasting effects on blood pressure, activity of the central nervous system, urine output, and lipolysis. The well-known induction of tolerance to caffeine also becomes explicable by this mechanism since caffeine is known to induce the formation of additional adenosine receptors in the rat (40).

Physical Dependence

Addiction to caffeine may develop even from short-term (seven days) administration of high dosages. Headache and fatigue are the most common withdrawal symptoms but depression, irritability, anxiety, and vomiting may also occur. Severity of symptoms that may last for up to one week range from mild to incapacitating. Many caffeine consumers exhibit no withdrawal symptoms. The physiology of caffeine withdrawal is not well understood (41).

Toxicity

The physiological effects of caffeine become greatly intensified at high dosages. Caffeine affects the cortex, the medulla, and eventually the spinal cord as dosages are increased (28). The LD50 of caffeine is similar for many mammals—about 200 mg/kg (42). This would suggest an LD50 of about 3.5 g for humans based on normal metabolic weight corrections. It is more generally accepted that the lethal oral dose is about 10 g. Lethal plasma concentration is in the range of 0.5 to 1.0 mM. To achieve this concentration would require the consumption of about 75 cups of coffee over a very short period of time (40). Lethality resulting from beverage consumption is most unlikely. The few recorded cases of death from caffeine consumption have involved the pure substance or medications containing it.

Caffeine as a Drug

The primary uses of caffeine as a drug are based on its effects on the respiratory, cardiovascular, and central nervous systems. Premature infants, for example, are subject to apnea, a transient but potentially dangerous cessation of breathing. Caffeine has been used to control this syndrome. It decreases apneic episodes and regularizes breathing patterns (28). It is also used for the treatment of bronchiospastic disease in asthmatic patients. Caffeine is used widely in drug mixtures designed for relief from migraine-type headaches because of its vasoconstrictor effects on the cerebral circulation. Many OTC preparations of this type are available. They contain 30 to 200 mg of caffeine per tablet. A common application of caffeine's stimulatory effects on the central nervous system is the use of 100- to 200-mg tablets to prevent drowsiness when driving or whenever this condition is undesirable. There are about 2000 nonprescription and about 1000 prescription drugs containing caffeine.

MUTAGENICITY

Caffeine attains the same concentration in gonadal tissue as in the circulating plasma and may thus reach levels of up to 0.05 mM for very heavy consumers (43). *In vitro* mutagenicity studies in which positive results are obtained generally involve concentrations at least 100 times greater and are therefore at or above the lethal concentration for humans. At these high concentrations, DNA repair is inhibited, an effect attributable to its purine analog structure. Caffeine may thus be considered a weak mutagen with no significance for humans.

TERATOGENICITY

Caffeine is teratogenic to rodents at high dosage levels (44). Effects such as cleft palates and ectrodactyly result from administration of 100mg/kg/d or more, but only when given in a single dose by gavage or injection. Increased fetal absorption and decreased fetal weight also occur at these levels, which approach lethality. True teratogenic responses have usually been associated with dosages that result in maternal toxicity. Delayed bone ossification is observed at much lower levels, but this effect is rapidly compensated postnatally.

The role of caffeine in the outcome of human pregnancy is generally determined by retrospective questioning. Studies have been based on beverage consumption recall and are therefore inexact with regard to the amount of caffeine ingested or the effects of other beverage components. There is no clear evidence of teratogenic effects due to caffeine (45). Conflicting data exist concerning the relationship between maternal caffeine consumption and infant birth weight (46). Since caffeine crosses the placental barrier, it is possible for high concentrations to occur in the neonate (47). Most medical advice cautions against high caffeine intake during pregnancy.

CARCINOGENICITY

The carcinogenetic potential of caffeine has been studied intensively in rodents (48). The results of about a dozen well-controlled studies indicate no carcinogenic effects attributable to caffeine. Epidemiological studies regarding the risk of cancers of the bladder, colon, rectum, pancreas, breast, ovaries, and liver to human coffee drinkers have been carried out in different parts of the world (49). Conflicting results have been obtained. In almost all instances, dose-response relationships have not been observed, casting doubt on any causal relationships between the effect and the consumption of coffee. The fact that tea consumption often produces different effects, sometimes converse, makes it unlikely that caffeine itself is a causative agent of increased cancer risk. An extensive survey of human studies, based primarily on coffee and tea consumption, show little correlation between consumption of the beverages and cancer incidence (50).

Theoretical considerations suggest a lack of carcinogenic potential of caffeine based on the facts that it does not alkylate or combine covalently with DNA, it does not undergo metabolic activation, and it lacks some of the structural features of known purine carcinogens (47).

Determination of the effect of caffeine on fibrocystic disease of the breast has also been pursued. Results of the studies are mixed. There may be some correlation between caffeine consumption (based on dietary coffee) and fibrocystic breast disease (51).

DECAFFEINATION

Concern with the physiological effects of coffee prompted early consideration of ways to decaffeinate the product. The first commercial plant was established by the firm Kaffee HAG in Germany in 1906 and in the United States a few years later. The U.S. plant was expropriated by the government during World War I, terminating production of decaffeinated coffee in the United States until 1932. Only one brand was available until the mid-1950s and solely as the instant product. Consumption later increased rapidly and in 1997 accounted for 35% of the instant product and 12 to 14% of ground roasted coffee. Decaffeinated tea appeared on the market in 1978. In 1997 it accounted for approximately 15% of leaf tea consumed in the United States and 20% of pure instant tea.

Coffee

Chlorinated compounds were the first caffeine extractants used with coffee. To expedite removal of caffeine, coffee beans are steam treated to bring about swelling for greater solvent permeability and to break down caffeine complexes. In all extraction processes it is necessary to wet the coffee for efficient caffeine removal. Green beans are decaffeinated to prevent extraction and loss of the aromatic components that are generated only during roasting. After solvent removal by steam distillation, the beans are roasted.

Trichlorethylene was commonly used as the solvent until it was eliminated in 1977 because of its suspected carcinogenicity. Methylene chloride then became the decaffeinating solvent of choice (36). A residue level of 10 ppm is approved by the U.S. Food and Drug Administration. An unofficial standard providing for the removal of 97% of the original caffeine has come into use for decaffeinated coffee. The beverage therefore contains 2 to 4 mg of caffeine per cup. To eliminate residues of chlorinated compounds, other decaffeinating solvents are now used. Ethyl acetate is approved in the United States. The process has some disadvantages: 5 to 6% solids loss and more difficult desolventization.

Decaffeination using water as the only solvent to contact the coffee is also carried out. A concentrated coffee extract freed of caffeine contacts the green beans. Noncaffeine solids are not removed under these conditions. The extract is then regenerated for use by adsorbing the caffeine on activated carbon (52). Coffee oil expressed from beans is used as a decaffeinating solvent along with other vegetable oils in another operating process. Caffeine is removed from the oils by a liquid–liquid extraction system with water as the second solvent (53).

Supercritical CO_2 extraction is now widely used for decaffeination (54). The process is carried out at 300 bars and at 60 to 90°C. Caffeine solubility increases as temperature and pressure are raised. The extracted caffeine may be removed from the supercritical fluid by reducing the pressure, thereby decreasing its solubility or by passing the mixture over activated carbon. Techniques that avoid the necessity for repressurizing the gas are preferable. Supercritical processes are capital intensive but result in products of superior quality with no solvent residues. In 1997 supercritical extraction was used to prepare about 50% of the decaffeinated coffee in the United States.

Tea

The commercial procedures for the decaffeination of tea are, in principle, similar to those used for coffee. For practical reasons it is necessary to utilize manufactured black tea rather than fresh green leaf. Conservation of aroma during processing is desirable. Decaffeinated tea appeared on the market in 1978. Similar process evolution took place as with coffee. Decaffeination with supercritical CO_2 became a commercial process in 1984 (55). Aroma may be previously stripped with dry supercritical CO_2 or with the moist gas at atmospheric pressure for later addition to the product (56).

BIBLIOGRAPHY

1. M. J. Arnaud, "Products of Metabolism of Caffeine," in P. B. Dews, ed., *Caffeine*, Springer-Verlag, Berlin, 1984, pp. 3–38.
2. E. Fischer and L. Ach, "Synthese des Caffeins," *Chem. Ber.* **28**, 3135–3142 (1895).
3. A. L. Thakkar et al., "Self-association of Caffeine in Aqueous Solution: A Nuclear Magnetic Resonance Study," *J. Chem. Soc. Chem. Commun.* **9**, 524–525 (1970).
4. I. Horman and R. Viani, "The Caffeine-Chlorogenate Complex of Coffee, an NMR Study," ASIC, 5th Colloque, Lisbon, 1971, pp. 102–111.
5. H. Biltz and H. Rakett, "Kaffeiden und Kaffeiden-carbonsaure," *Chem. Ber.* **61**, 1409–1422 (1928).
6. A. C. Cope et al., "1,3-Dimethyl-5-alkyl Barbituric Acids," *J. Am. Chem. Soc.* **63**, 356–358 (1941).
7. W. J. Hurst, R. A. Martin, Jr., and S. M. Tarka, Jr., "Analytical Methods for the Quantitation of Methylxanthines," in G. A. Spiller, ed., *Caffeine*, CRC Press, New York, 1998, pp. 13–31.
8. R. Dorizzi and F. Tagliaro, "Direct HPLC Determination of Theophylline, Theophylline Analogs and Caffeine in Plasma with an ISRP (Pinkerton) Column," *Clin. Chem.* **36**, 566 (1989).
9. E. D. Conte and E. F. Barry, "Gas Chromatographic Determination of Caffeine in Beverages by Alkali Aerosol Flame Ionization Detection," *Microchem. J.* **48**, 372–376 (1993).
10. W. J. Hurst and R. A. Martin, Jr., "The Quantitative Determination of Caffeine in Beverages Using Capillary Electrophoresis Analysis," *Analysis* **21**, 389–391 (1993).
11. B. A. Kihlman, in *Caffeine and Chromosomes*, Elsevier, Amsterdam, 1977, pp. 11–13.
12. E. Looser, T. W. Bauman, and H. Warner, "The Biosynthesis of Caffeine in the Coffee Plant," *Phytochemistry* **13**, 2515–2517 (1974).
13. P. Mazzafera et al., "*S*-Adenosyl-L-Methionine:Theobromine I-*N*-Methyltransferase, an Enzyme Catalyzing the Synthesis of Caffeine in Coffee," *Phytochemistry* **37**, 1577–1584 (1994).
14. T. Suzuki, H. Ashihara, and G. R. Wallace, "Purine and Purine Alkaloid Metabolism in Camellia and Coffea Plants," *Phytochemistry* **31**, 2575–2582 (1992).
15. H. Ashihara and H. Kubota, "Biosynthesis of Purine Alkaloids in Camellia Plants," *Plant Cell Physiol.* **28**, 535–539 (1987).
16. O. Negishi, T. Ozzawa, and H. Imagawa, "Biosynthesis of Caffeine from Purine Nucleotides in Tea Plant," *Biosci. Biotechnol. Biochem.* **56**, 499–503 (1992).
17. M. A. Bokuchava and N. I. Skobeleva, "The Biochemistry and Technology of Tea Manufacture," *Crit. Rev. Food Sci. Nutr.* **12**, 348 (1980).
18. M. Dong et al., "The Occurrence of Caffeine in the Air of New York City," *Atmos. Environ.* **11**, 651–653 (1977).
19. J. J. Barone and H. R. Roberts, "Caffeine Consumption," *Food Chem. Toxicol.* **34**, 119–129 (1996).
20. H. R. Roberts and J. J. Barone, "Biological Effects of Caffeine; History and Use," *Food Technol.* **37**, 32–39 (1983).
21. U.S. Pat. 4,818,552 (Apr. 4, 1989), L. Kaper (to Douwe Egberts).
22. U.S. Pat. 2,534,331 (Dec. 19, 1950), D. W. Woodward (to E.I. du Pont de Nemours & Co.)
23. Anonymous communication, *Chemical Marketing Reporter* **247**, no. 8 Feb. 20 (1995).
24. R. W. Von Borstel, "Biological Effects of Caffeine; Metabolism," *Food Technol.* **37**, 40–45 (1983).
25. D. W. Yesair, A. R. Branfman, and M. M. Callahan, "Human Disposition and Some Biochemical Aspects of Methylxanthines," *Phytochemistry* **13**, 215–233 (1974).
26. M. G. Meyers, "Effects of Caffeine on Blood Pressure," *Arch. Int. Med.* **148**, 1189–1193 (1988).
27. T. K. Leonard, R. R. Watson, and M. E. Mohs, "The Effects of Caffeine on Various Body Systems: A Review," *J. Am. Diet. Assoc.* **87**, 1048–1053 (1987).
28. M. J. Arnaud, "The Pharmacology of Caffeine," *Progress in Drug Research* **31**, 273–313 (1987).
29. R. Urgert et al., "Separate Effects of the Coffee Diterpenes Cafestol and Kahweol on Serum Lipids and Liver Aminotransferases," *Am. J. Clin. Nutr.* **65**, 519–524 (1997).
30. M. Hasenfratz and K. Battig, "Acute Dose–Effect Relationships of Caffeine and Mental Performance, EEG, Cardiological and Subjective Parameters," *Psychopharmacology (Berlin)* **114**, 281–287 (1994).
31. D. Fagan, C. G. Swift, and B. Tiplady, "Effects Of Caffeine on Vigilance and Other Performance Tests in Normal Subjects," *J. Pharmopsychol.* **2**, 19–25 (1988).
32. L. M. Giambra et al., "The Influence of Caffeine Arousal on the Frequency of Task-Unrelated Image and Thought Intrusions," *Imagination, Cognition and Personality* **13**, 215–223 (1994).
33. R. Lubit and B. Russett, "The Effects of Drugs on Decision Making," *Conflict Resolution* **28**, 85–102 (1984).
34. B. D. Smith and K. Tola, "Caffeine: Effects on Psychological Functioning and Performance," in G. A. Spiller ed., *Caffeine*, CRC Press, New York, 1998, pp. 263–266.

35. A. Leviton, "Biological Effects of Caffeine; Behavioral Effects," *Food Technol.* **37**, 44–46 (1983).
36. D. Penetar et al., "Caffeine Reversal of Sleep Deprivation Effects on Alertness and Mood," *Psychopharmacology (Berlin)* **112**, 359–365 (1993).
37. B. Smith, "Effects of Acute and Habitual Caffeine Ingestion in Physiology and Behavior; Tests of a Biobehavioral Arousal Theory," *Pharmopsychoecologia* **7**, 151–167 (1994).
38. R. J. Lamarine, "Caffeine as an Ergogenic Aid," in G. A. Spiller, ed., *Caffeine*, CRC Press, New York, 1998, pp. 244–245.
39. A. G. Dulloo et al., "Normal Caffeine Consumption: Influence on Thermogenesis and Daily Energy Expenditure in Lean and Postobese Human Volunteers," *Am. J Clin. Nutr.* **49**, 44–50 (1989).
40. R. Von Borstel and R. J. Wurtman, "Caffeine and the Cardiovascular Effects of Physiological Levels of Adenosine," in P. B. Dews, ed., *Caffeine*, Springer-Verlag, Berlin, 1984, pp. 142–150.
41. R. R. Griffiths and P. P. Woodson, "Caffeine Physical Dependence: A Review of Human and Laboratory Animal Studies," *Psychopharmacology (Berlin)* **94**, 437–451 (1988).
42. B. Stavric, "Toxicity to Humans. 2. Caffeine," *Food Chem. Toxicol.* **26**, 645–662 (1988).
43. R. H. Haynes and J. D. B. Collins, "The Mutagenic Potential of Caffeine" in P. B. Dews, ed., *Caffeine*, Springer-Verlag, Berlin, 1984, pp. 231–238.
44. J. G. Wilson and W. J. Scott, Jr., "The Teratogenic Potential of Caffeine in Laboratory Animals," in P. B. Dews, ed., *Caffeine*, Springer-Verlag, Berlin, 1984, pp. 165–187.
45. A. Berger, "Effects of Caffeine Consumption on Pregnancy Outcome," *J. Reproduct. Med.* **33**, 945–956 (1988).
46. J. Nash and T. V. N. Persaud, "Reproductive and Teratological Risks of Caffeine," *Anatomischer Anzeiger* **167**, 265–270 (1988).
47. W. D. Parsons and A. H. Niems. "Prolonged Half-Life of Caffeine in Healthy Term Newborn Infants," *J. Pediatrics* **98**, 640–641 (1981).
48. H. C. Grice, "The Carcinogenic Potential of Caffeine," in P. B. Dews, ed., *Caffeine*, Springer-Verlag, Berlin, 1984, pp. 153–164.
49. Committee on Diet and Health, National Research Council, *Diet and Health*, National Academy Press, Washington, D.C., 1989.
50. G. A. Spiller and B. Bruce, "Coffee, Tea, Methyl Xanthines, Human Cancer, and Fibrocystic Breast Disease," in G.A. Spiller, ed., *Cancer*, CRC Press, New York, 1998, pp. 326–336.
51. U.S. Pat. 3,671,263 (June 20, 1972), J. M. Patel and A. B. Wolfson (to Proctor and Gamble Co.).
52. Anonymous, "Coffex Avails Swift Water Process to N. America," *Tea and Coffee Trade Journal* **160**, 45–47 (1988).
53. U.S. Pat. 4,837,038 (June 6, 1989), J. C. Proudley (to Nestea SA).
54. U.S. Pat. 4,820,537 (Apr. 11, 1989), S. N. Katz (to General Foods Corp.).
55. W. Ger. Pat. 3,515,740 (May 2, 1985), H. Klima, E. Schütz, and H. Vollbrecht (to SKW Trostberg AG).
56. Eur. Pat. 269,970 (June 8, 1988), E. Schütz and H. Vollbrecht (to SKW Trostberg AG).

HAROLD GRAHAM
Consultant
New York, New York

CANCER RISK AND DIET

Cancer can be regarded as a mass of cells that are no longer under normal regulatory growth control. Cancer develops in several stages, namely, initiation, promotion, and progression. The initiation stage is rapid and irreversible and begins with attack of a cancer-inducing agent on a target site in the DNA of the cell. In this process a normal cell is converted into a cancer cell. The transformation is irreversible. During the promotion stage the transformed cell may grow into a tumor; not all cells do. Progression is the stage wherein a benign tumor may become malignant.

Nutritional studies of the influence of diet on cancer risk can be carried out in animals by direct experimentation and in humans by using epidemiological methods. Both methods provide useful data that can then be examined further to determine influences of diet on causation and, possibly, inhibition of tumor growth.

Animal studies are relatively easy to carry out. The animal of choice (almost always a mouse or rat) is subject to treatment with a carcinogenic agent and observed for a specified length of time before being subjected to necropsy. The advantages of this system are an experimental consistency and total control over diet and life span. But even here different strains of mice or rats do not always give the same result. Additionally, the experimental animal is caged, which may produce a certain amount of stress; a lifetime effect of treatment is never seen; and there are virtually no studies in which the diet is varied. Still, animal studies provide useful data concerning mechanisms of carcinogenesis and of effects of dietary and drug treatment. The results of animal studies are not directly applicable to humans.

Epidemiological studies of cancer in humans use varied approaches. Ecological or correlation studies are studies in which differences between populations are examined. These may be examinations of specific nutrients or of patterns of food intake. Generally investigation of food disappearance data is not as useful as studies of individual dietary components. Studies in humans may include migrant studies (cancer incidence in the new location vs cancer incidence in the old location); case-control studies, in which cases are compared with age-matched controls; and follow-up studies, in which appearance of cancer is compared between exposed or unexposed individuals. Studies involving assessment of past dietary intake are subject to errors in recall. Many foods are important sources of more than one nutrient—meat, for instance, is a source of both protein and fat—which adds to the complexity of diet assessment.

A good example of the complexity of epidemiological studies may be found in two studies of diet effects on colon cancer carried out in Australian cities that are about 400 miles apart. In Adelaide, Potter and McMichael (1) found colon cancer risk to be associated with protein and energy intake, but fiber intake was, if anything, a risk for colon cancer. Kune et al. (2) in Melbourne found dietary fiber to be protective.

Armstrong and Doll (3) reviewed the effects of diet on cancer worldwide and suggested that the correlations found in their survey be regarded as guides to further research rather than as evidence of causation. In 1987 a review by Doll and Peto (4) raised interest in diet and cancer to a new level. They suggested that 30 to 70% of cancer in the United States might be related to diet and stated that this value was "highly speculative and chiefly refers to dietary factors which are not yet reliably identified."

In 1982 a committee of the National Academy of Sciences (U.S.) (5) reviewed influences of diet on cancer. Their conclusions were (in part):

1. Epidemiological and experimental data suggest an association between a high-fat diet and cancers of the breast, large bowel, and prostate. The data are not unanimous, however.
2. Dietary restriction reduces tumor incidence in experimental animals.
3. Foods rich in vitamin A and beta-carotene reduce risk of lung and bladder cancer.
4. Selenium may reduce cancer risk, but the results are inconclusive.
5. Diets high in vitamin C may reduce the risk of esophageal or gastric cancer.
6. Studies relating cancer incidence to carbohydrate, fiber, vitamin E, and the B vitamins are inconclusive.
7. Alcohol acts synergistically with smoking to increase risk of cancers of the upper GI and respiratory tracts.

Few new revelations have occurred in the 17 years since that report, except that the role of fat in breast cancer has been downgraded and importance of fruits and vegetables somewhat increased.

One can review dietary data related to cancer by nutrient or by type of cancer. The former model will be used in this review.

MACRONUTRIENTS

Protein

In general there is virtually no relationship between protein intake and either colorectal or breast cancer (6). The data relating to meat intake and the risk of colorectal cancer overall suggest little association if any. Recently, an American multicenter case-control study has concluded, "This study provides little support for an association between meat consumption and colon cancer risk" (7). The finding that cooking meat resulted in formation of heterocyclic amines that are carcinogenic for mice (8) was used as a point of departure for many inquiries into the effects of meat, but a recent report by Augustsson et al. (9) finds that heterocyclic amine intake within the usual dietary range is not likely to increase risk of cancer of the colon, rectum, kidney, or bladder in humans.

CARBOHYDRATE

There is little evidence to support a role for carbohydrates per se in carcinogenesis. Drasar and Irving (10) found a correlation between incidence of breast cancer and intake of simple sugars as did Hems (11), but an inverse correlation was seen with starch intake (12). Longtime feeding of sucrose to rats (13) or mice (14) did not result in increased tumorigenesis. The available evidence is scanty, and it is not possible to form any conclusion except for the safe one; namely, more research is needed. Most dietary fiber is carbohydrate in nature, but that represents a special case that is discussed later.

FAT AND ENERGY

Overweight has been associated with increased risk of a number of cancers (15). Since overweight is often linked to fat intake, the search for dietary correlations with cancer incidence has led to investigation of fat intake and cancer. There is some positive correlation between fat intake and colon cancer risk, but it is not very strong (16). Studies in Great Britain (17) and Sweden (18) found no relation between fat intake and either colon or breast cancer. One of the earliest epidemiologic studies of diet and colon cancer was carried out in 1933 by Stocks and Karn (19) who found negative correlations between colon cancer and intake of bread, vegetables, and dairy food. Similarly, a study comparing diets of Danes (high colon cancer) and Finns (low colon cancer) found positive correlations only with alcohol intake and fecal bile and concentration; among the negative correlations was intake of saturated fat (20).

Data from the National Health and Nutrition Examination Study (NHANES) show no correlation between fat intake and breast cancer (21). The same finding has emerged from broad reviews of the literature (22,23). A study by Willett et al. (24) of more than 89,000 American nurses has revealed no link between fat intake and risk of breast cancer. As with virtually all diet-cancer data the findings are not unanimous.

A new nutritional entity in the diet-cancer arena is conjugated linoleic acid (CLA). Linoleic acid contains double bonds that are separated by a methylene group. A microorganism that occurs in the rumen of ruminants such as the cow or sheep moves the double bonds into conjugation, meaning they are contiguous and not methylene separated. What is now termed CLA is a mixture of positional and geometric isomers of octadecadienoic acid. CLA has been shown to inhibit formation of chemically induced skin and forestomach tumors in mice and mammary tumors in rats (25). CLA is present in dairy foods and meat of ruminant animals. Efforts are being made to increase its content in the common sources to determine if increased dietary intake can deter human carcinogenesis. In rats CLA is effective at levels of 0.25% of the diet. This extrapolates to more than the current intake in humans.

Berg (26) suggested in 1975 that cancers prevalent in developed countries could be due to overnutrition. Almost a century ago Moreschi (27) found that transplanted tumors grew poorly in underfed mice. The effects of caloric (energy) restriction on growth of spontaneous, chemically induced or transplanted tumors has confirmed Moreschi's observation almost unanimously. Studies of chemically induced mammary tumors in rats have shown that at 25% caloric restriction, rats fed 25% fat have fewer tumors than freely fed rats ingesting only 5% fat (28). This area of investigation is very active at the present time, and emerging data show that energy restriction affects enzyme activity and gene expression.

A number of studies have shown that breast cancer risk is related positively to age at menarche, body weight or body mass index, and height (29). These factors can be influenced by nutrition. A role of caloric intake on human cancer has been suggested by several groups of investigators (1,30,31). Energy utilization may also play an important part in carcinogenesis Women who engaged in organized athletics while in college (32) or men who have spent most of their working life at energy-intensive jobs (33) have less breast or colon cancer, respectively. In 1945 Potter (34) recommended exercise and reduction of caloric intake as means of preventing cancer. These activities are cheap and effective but unpopular.

Alcohol consumption has been suggested to be a risk for breast cancer. The studies that have been reported indicate a bias in that direction, but it is not overly strong (23). Alcohol, as a source of calories, lies between carbohydrate (4 cal/g) and fat (9 cal/g). Alcohol provides about 7 cal/g and can be an important contributor to daily caloric intake.

FIBER

Dietary fiber is a generic term that describes dietary components that are not broken down during the digestive process. Except for lignin, fiber is carbohydrate in nature and is often classified as soluble (pectin, guar gum) or insoluble (cellulose, wheat bran). The many substances that are included under the designation "dietary fiber" are unique chemical compounds and exhibit individual physiological effects.

Interest in fiber was stimulated by the work of Burkitt (35) who attributed the freedom from colon cancer of native African populations to their high fiber intake. Earlier, Higginson and Oettlé (36) made a similar observation, but they did not pursue it with Burkitt's missionary fervor. Armstrong and Doll (3) had observed a negative correlation between cereal intake and cancer risk. In a comprehensive review of the literature up to 1986 (37), data from 18 ecological and 22 case-control studies were considered. Protection from colon cancer by a high-fiber diet was adduced from 66.7% of the ecological studies and 36 4% of the case-control studies; no effect was seen in 27.8% of the ecological studies and 40.9% of the case-control studies; and enhancement was found in the rest (5.6% of the ecological studies and 22.7% of the case control studies). Later reviews also find a preponderance of studies that show a protective effect of fiber but without unanimity.

Fiber fulfills a number of functions that may contribute to its protective effects. It increases fecal bulk, which has been suggested as a protective factor (38); it dilutes colonic contents, which may reduce contact between carcinogens and colonic mucosa; and it reduces fecal pH (39), levels of fecal mutagens (40), and concentration of fecal bile acids (20). Degradation of fiber by colonic microflora also produces water, methane, carbon dioxide, and short-chain fatty acids (SCFA). The major SCFA are acetate, propionate, and butyrate and, of these, butyrate increases differentiation of colonic cells. Butyrate exerts many actions at the cellular and molecular level, all of which work toward protection against carcinogenesis (41).

A summary of epidemiological trials that support a preventive role for dietary fiber has been published recently (41). Reviews by Hill (42) and Jacobs et al. (43) have shown that cereals and cereal fiber do indeed protect against colon cancer. The comparison of colon cancer in Danes and Finns (20) found negative correlations with carbohydrate, cereals, protein, saturated fat, starch, and total dietary fiber. A recent study by Fuchs et al. (44) found that dietary fiber did not protect against colorectal cancer or adenomas. The median intake of cereal fiber in the 88,757 women studied was rather low, ranging from 1.0 to 4.5 g/day going from the first to fifth quintile of intake. An intake of 4.5 g of cereal fiber is on the low side. In their review Rogers and Longnecker (6) state, "most epidemiologic studies of fiber or fiber-containing food intake in relation to the risk of colorectal cancer are consistent with a very small inverse association or no association." However, specific fiber sources such as cereals may offer protection.

A problem in interpretation of data for review purposes is that all fiber is viewed to be the same. In fact dietary fiber from different sources may vary in fiber content and structure and in the accompanying nutrients, which may also have an effect. The epidemiologic data are derived from populations ingesting a fiber-rich diet, not a single source of fiber. A high-fiber diet will also contain carotenoids and other substances unique to specific plant sources, plant sterols, n-3 and n-6 polyunsaturated fatty acids, and possibly trace minerals. The reductionist approach to the data tends to single out a particular substance and assume that to be the protective substance. The substance chosen may be one currently in vogue or may be a specific interest of the investigator. One case in point—many studies have suggested that diets rich in beta-carotene are protective against lung cancer. But adding beta-carotene to the diets of heavy smokers actually increased the incidence of lung cancer (45). In a similar vein total dietary fiber did not lower the risk of colorectal adenomas in women, but fiber from fruits and vegetables did (46).

In assessing the risk of colon cancer in 47,949 (205 cases) as a function of fiber source, fruit fiber ingestion was most strongly correlated with negative risk (47). In an earlier study of a cohort of 7284 men (170 cases) the same authors found that all sources of fiber reduced significantly the risk of colorectal adenoma (48). The data beg the question of why most sources of fiber no longer protect when the adenoma progress to carcinoma. Is it due to the presence of other factors in fruit? Fiber from fruits and vegetables implies other components of fruits and vegetables were present and may have played a role. Several large prospective studies of the effect of wheat bran on colon cancer will be reported in 1999 and 2000.

FRUITS AND VEGETABLES

Substances found in fruits and vegetables may exert anticarcinogenic action by inhibiting formation of carcinogens, by inducing detoxifying enzymes, or by blocking the carcinogen from reaching target tissue. Tumor promotion may be inhibited by retinol and beta-carotene (green/yellow vegetables and fruits), tocopherol (nuts, wheat germ), vitamin C (vegetables, fruits), organosulfur compounds (garlic, onions), curcumin (tumeric, curry), and capsaicin (chili peppers). Covalent DNA binding is inhibited by phenylisothiocyanate (broccoli, cabbage), ellagic acid (fruits, nuts, berries), and flavonoids (fruits, vegetables). Biotransformation of potential carcinogens is inhibited by indole-3-carbinol (cruciferous vegetables). Thus, citrus fruit contains carotenoids, but it also contains flavonoids, glucarates, terpenes, and phenolic acids. Does protection require one or more of these plus carotenoids? Studies along this line of reasoning are virtually unknown. Perhaps studies of combinations of protectants will yield an answer where studies of single substances haven't. Block et al. (49) compiled an exhaustive review of the roles of fruit and vegetables in cancer prevention. A statistically significant protective was found in 128 of 156 dietary studies (82%). Significant protection against lung cancer was found in 24 of 25 studies and against cancers of the oral cavity, larynx, or esophagus in 28 of 29 studies. They also found significant protection against pancreatic or stomach cancer (26 of 30 studies), colorectal and bladder cancer (23 of 38 studies), and cancers of the ovary, cervix, and endometrium in 11 of 13 studies. Protection against breast cancer was adduced from metanalysis. Which particular components of fruit and vegetables or which combination of components is active remains to be elucidated.

MINERALS

Tumorigenesis due to deficiencies of various minerals has been reported, among them selenium (50), iron (51), zinc (52), and molybdenum (53). Data regarding others are sparse but available (5). Antitumor effects of selenium in experimental carcinogenesis have also been reported (54,55). A recently reported clinical trial designed to test the efficacy of dietary selenium treatment for prevention of skin cancer found no effect with regard to the stated objective (56). However, selenium treatment proved efficacious against other forms of cancer. A total of 1312 patients with basal or squamous cell carcinomas were treated with a selenium-containing preparation. Cancer incidence in the selenium-treated group was 77 cases compared with 119 cases in the controls, a highly significant difference. All-cause mortality was lower in the selenium-treated individuals (108 vs 129 in controls). Total cancer deaths numbered 29 in the treated group compared with 57 in the placebo group. Over the total observation period of treatment (4.5 ± 2.8 years) and follow-up (6.4 ± 2.0 years), mortality from colorectal or prostate cancer was significantly lower in the treatment group. Total cancer incidence and mortality were reduced significantly. Considering the original aims of the study, the results were serendipitous and most welcome but illustrate some of the vagaries of nutrition-cancer research. A difficulty with studies of trace minerals is that there is a fine line between necessity and toxicity. Efforts to synthesize mineral-containing compounds with lower toxicity are under way.

SUMMARY

Most dietary guidelines, whether general or aimed at a particular disease state, recommend eating a variety of foods and maintaining desirable weight. The foregoing discussion shows that these suggestions might apply to cancer as well. The risk of overweight has been alluded to. The presence of anticancer substances in fruits and vegetables is well documented. A trend toward reductionism in assessing data has led us to choose a particular component of a food when that same food contains many substances that may act synergistically. The interaction of foods or components of foods is addressed rarely. A study of Japanese men in Hawaii showed a significant association between fiber intake and risk in men who ingested less than 61 g/day of fat, but no association if daily fat intake exceeded 61 (57). Lee et al. (58) found the dietary ratio of meat to vegetables influenced risk of colon cancer in Singapore. More studies of food or food component interactions are needed to clarify the relationship of diet to cancer risk. In the meantime, the dietary suggestion of balance, variety, and moderation may be the most useful.

BIBLIOGRAPHY

1. J. D. Potter and A. J. McMichael, *J. Natl. Cancer Inst.* **76**, 557–569 (1986).
2. S. Kune, G. A. Kune, and L. F. Watson, *Nutr Cancer* **9**, 21–42 (1987).
3. B. Armstrong and R. Doll, *Int. J. Cancer* **15**, 617–631 (1975).
4. R. Doll and R. Peto, *J. Natl. Cancer Inst.* **66**, 1191–1308 (1981).
5. Committee on Diet, Nutrition, and Cancer, *Diet, Nutrition and Cancer*, National Academy Press, Washington, D.C., 1982
6. A. E. Rogers and M. P. Longnecker, *Lab. Invest.* **59**, 729–759 (1988).
7. E. Kampman et al., *Cancer Epidemiol. Biomarkers, Prev.* **8**, 15–24 (1999).
8. T. Sugimura, *Mutat. Res.* **376**, 211–219 (1997).
9. K. Augustsson et al., *Lancet* **353**, 703–707 (1999).
10. B. S. Drasar and D. Irving, *Br. J. Cancer* **27**, 167–172 (1973).
11. G. Hems, *Br. J. Cancer* **37**, 974–982 (1978).
12. G. Hems and A. Stuart, *Br. J. Cancer* **31**, 118–123 (1975).
13. L. Friedman et al., *J. Natl. Cancer Inst.* **49**, 751–764 (1972).
14. F. J. C. Roe, L. S. Levy, and R. L. Carter, *Food Cosmet. Toxicol.* **8**, 135–145 (1970).
15. E. A. Lew and L. Garfinkel, *J. Chronic Dis.* **32**, 563–576 (1979).
16. C. LaVecchia et al., *Int. J. Cancer* **41**, 492–498 (1988).
17. L. J. Kinlen, *Lancet* **1**, 946–949 (1982).
18. M. Rosen, L. Nystrom, and S. Wall, *Am. J. Epidemiol.* **127**, 42–49 (1988).
19. P. Stocks and M. K. Karn, *Ann. Eugen. (London)* **5**, 237–280 (1933).
20. O. M. Jensen, R. MacLennon, and J. Wahrendorf, *Nutr. Cancer* **4**, 5–19 (1982).
21. D. Y. Jones et al., *J. Natl. Cancer Inst.* **79**, 465–471 (1987).
22. P. J. Goodwin and N. F. Boyd, *J. Natl. Cancer Inst.* **79**, 473–485 (1987).

23. T. Byers, *Cancer* **62**, 1713–1724 (1988).
24. W. C. Willett et al., *N. Engl. J. Med.* **316**, 22–28 (1987).
25. M. A. Belury, *Nutr. Rev.* **53**, 83–89 (1995).
26. J. W. Berg, *Cancer Res.* **35**, 3345–3350 (1975).
27. C. Moreschi, *Z. Immunitätsforsch* **2**, 651–675 (1909).
28. D. M. Klurfeld et al., *Int. J. Cancer* **43**, 922–925 (1989).
29. D. Kritchevsky, *Cancer* **66**, 1321–1325 (1990).
30. J. B. Bristol et al., *Br. Med. J.* **291**, 1467–1470 (1985).
31. J. L. Lyon et al., *J. Natl. Cancer Inst.* **78**, 853–861 (1987).
32. R. E. Frisch et al., *Br. J. Cancer* **52**, 885–891 (1985).
33. D. H. Garabrant et al., *Am. J. Epidemiol.* **119**, 1005–1014 (1984).
34. V. R. Potter, *Science* **101**, 105–109 (1945).
35. D. P. Burkitt, *Cancer* **28**, 3–13 (1971).
36. J. Higginson and A. G. Oettlé, *J. Natl. Cancer Inst.* **24**, 589–671 (1960).
37. S. M. Pilch, ed., *Physiological Effects and Health Consequences of Dietary Fiber*, FASEB, Bethesda, Md., 1987.
38. J. H. Cummings et al., *Gastroenterology* **103**, 1783–1789 (1992).
39. A. R. P. Walker, B. F. Walker, and A. J. Walker, *Br. J. Cancer* **53**, 489–495 (1986).
40. B. S. Reddy et al., *Cancer Res.* **47**, 644–648 (1987).
41. D. Kritchevsky, *Eur. J. Cancer Prev.* **7**(Suppl. 2), S33–S39 (1998).
42. M. J. Hill, *Eur. J. Cancer Prev.* **6**, 219–225 (1997).
43. D. R. Jacobs, Jr., et al., *Nutr. Cancer* **30**, 85–96 (1998).
44. C. S. Fuchs et al., *N. Engl. J. Med.* **340**, 169–176 (1999).
45. The Alpha Tocopherol, Beta Carotene, Cancer Prevention Study Group, *N. Engl. J. Med.* **330**, 1029–1035 (1994).
46. R. S. Sandler et al., *J. Natl. Cancer Inst.* **85**, 884–891 (1993).
47. E. Giovannucci et al., *Cancer Res.* **54**, 2390–2397 (1994).
48. E. Giovannucci et al., *J. Natl. Cancer Inst.* **84**, 91–98 (1992).
49. G. Block, B. Patterson, and A. Subar, *Nutr. Cancer* **18**, 1–29 (1992).
50. R. J. Shamberger, *J. Natl. Cancer Inst.* **44**, 931–936 (1970).
51. L. G. Larsson, A. Sandström, and P. Westling, *Cancer Res.* **35**, 3308–3316 (1975).
52. H. J. Lin et al., *Nutr. Rep. Int.* **15**, 635–643 (1977).
53. C. S. Yang, *Cancer Res.* **40**, 2633–2644 (1980).
54. J. R. Harr et al., *Clin. Toxicol.* **5**, 187–194 (1972).
55. C. Ip and D. K. Sinha, *Cancer Res.* **41**, 31–34 (1981).
56. L. C. Clark et al., *JAMA, J. Am. Med. Assoc.* **276**, 1957–1963 (1996).
57. L. K. Heilbrun et al., *Int. J. Cancer* **44**, 1–6 (1989).
58. H. P. Lee et al., *Int. J. Cancer* **43**, 1007–1016 (1989).

GENERAL REFERENCES

R. B. Alfin-Slater and D. Kritchevsky, eds., *Human Nutrition. A Comprehensive Treatment*, Vol. 7, Plenum Press, New York 1991.

C. Ip et al., eds., *Dietary Fat and Cancer*, Alan R. Liss, New York, 1986

M. J. Hill, A. Giacosa, and C. P. J. Caygill, eds., *Epidemiology of Diet and Cancer*, Ellis Horwood Ltd., Chichester, W. Sussex, England, 1994.

F. L. Meyskins, Jr., and K. N. Prasad, eds., *Vitamins and Cancer*, Humana Press, Clifton, N. J., 1986.

I. R. Rowland, ed., *Nutrition, Toxicity, and Cancer*, CRC Press, Boca Raton, Fla., 1991.

David Kritchevsky
The Wistar Institute
Philadelphia, Pennsylvania

CANNING: REGULATORY AND SAFETY CONSIDERATIONS

Food safety and spoilage have been the major issues of the canning industry since its beginnings in the early 1900s. Preventing food from spoilage during storage was indeed a major reason that the canning industry came to be. However, at the turn of the twentieth century there was little or no scientific knowledge, as we know it today, about canning processes. In the late 1700s, Nicolas Appert in France found a "new" way to preserve food for the armed forces that involved corking bottles filled with food and heating them in boiling water. Not until Pasteur's work in 1864 was the relationship between food spoilage and microorganisms discovered. Several other developments in following decades then brought necessary components together to make use of this new knowledge. The science of bacteriology in the late 1800s to early 1900s provided information about specific bacteria and their growth characteristics. Inventions related to thermocouple technology by 1920 and subsequent determination of mathematical methods for calculating sterilization processes laid the foundation for today's safety standards and regulations in the canning industry.

REGULATORY CONTROLS

Regulations are in place to ensure the control of critical parameters in the canning of foods. The U.S. Federal Food, Drug and Cosmetic Act prohibits the distribution and sale of foods that carry disease-causing contaminants as well as a list of undesirable elements categorized as "filth." Two episodes with botulism in the canning industry in 1971 led to a petition from the National Canners Association, now the National Food Processors Association (NFPA), to the U.S. FDA proposing a statement of policy that was handled as a proposal for regulation. After receiving comments, the FDA published minimum good manufacturing practice (GMP) regulations. After various revisions and comment periods since that time, the FDA now has published several GMP regulations that pertain to canned foods in the *Code of Federal Regulations* (CFR).

"Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food" (21 CFR 110) sets forth general requirements for maintaining sanitary conditions in food establishments, such as design and maintenance of facilities and equipment, and pest control. More specific requirements are set forth in an additional series of regulations in Title 21 of the CFR. "Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers" (Part 113) defines low-acid foods, requires design of a scheduled process by a process authority, defines commercial sterility for low-acid canned foods, and specifies design, control, and instrumentation for retorting systems. This regulation also specifies record keeping requirements, procedures for process deviations, and container closure inspection procedures. "Acidified Foods" (Part 114) defines acidified foods and procedures for acidification; this part describes requirements for process determination, corrections for process deviations, and record keeping. "Emergency Permit Control" (Part 108) describes procedures for exemptions from and compliance with registering an establishment when requirements for operation are not being met.

The U.S. Department of Agriculture (USDA) has published regulations for canning of meat (21 CFR 318.300) and poultry (21 CFR 381.300) products, "Canning and Canned Products." The U.S. Meat Inspection Act and the Poultry Products Inspection Act authorize regulation of canned products by the USDA Food Safety and Inspection Service. The canning regulations for the two products are essentially the same and contain requirements similar to those for low-acid canned foods regulated by the FDA.

Personnel and Training

Canning regulations recognize the importance of people in maintaining the integrity of the canning process, in addition to food, process design, and equipment concerns. All retort (canner), container closure, and inspection operations must be performed under the supervision of trained supervisors. Supervisors of low-acid and acidified foods operations are required by regulations in Parts 108, 113, and 114 to satisfactorily complete instruction in a school or training approved by the FDA. These courses have become known as Better Process Control Schools, developed in cooperation with the NFPA's educational arm, the Food Processors Institute (FPI). Instructors for these schools are selected from the FDA, selected universities, the FPI, the NFPA, and industry. Regulations for canned meat and poultry products (Parts 318 and 381) specify that the supervisor of canning operations must attend a school generally recognized as adequate for properly training supervisors of canning operations.

Imported Canned Foods

Food regulations also allow for protection from unsafe imported thermally processed foods. Foreign food processors of low-acid canned foods and acidified foods must register their establishments with the FDA before exporting products to the United States. The FDA assigns each establishment a number that helps track the registration and processing records. Imported low-acid canned foods and acidified foods are subject to all the requirements under the U.S. Federal Food, Drug, and Cosmetic Act and the Fair Packaging and Labeling Act. The processing of low-acid and acidified foods must also comply with GMP regulations in 21 CFR Parts 113 and 114, respectively. Canning processes must be determined by a thermal process authority and filed with the FDA before being exported.

SAFETY CONCERNS

Microbiology of the Canning Process

The microbiology of the can environment during storage is an extremely important consideration in the safety of canned foods. The objective in food canning is to produce a food and container interior that are commercially sterile. This is accomplished through the application of heat alone or in combination with adjusted parameters of water activity, chemicals, or acidity (usually measured as pH). Commercial sterility results from destruction of all viable microorganisms of public health significance as well as those capable of reproduction at normal nonrefrigerated temperatures during storage and distribution.

Generally, the residual oxygen content of canned foods is minimized. Microorganisms that require an environment with oxygen (obligate aerobes) would not be expected to present spoilage or health hazards. (Special consideration would have to be given to products where oxygen is not completely removed and mild heat treatments are used in conjunction with other preservation means, such as curing salts in canned, cured meat products.) Of greatest concern, then, in the anaerobic environment of canned foods is the pH of the food, as acidity does affect the ability of microorganisms to reproduce and survive destruction by heat.

The foremost health concern with canned foods is survival of the pathogenic *Clostridium botulinum* bacteria. *C. botulinum* is a spore-forming anaerobe. Destruction of the spores in canned low-acid foods is essential to prevent germination and toxin formation during nonrefrigerated storage. It is an accepted generalization that spores of *C. botulinum* can germinate into vegetative cells that multiply, with the ultimate production of toxin if the pH of the canned food product is higher than 4.6. Therefore, the pH value of 4.6 is one delineating factor between low-acid and acid or acidified categories of canned foods. A low-acid food means any food, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity greater than 0.85.

There are some extremely heat-resistant spore-forming bacteria that may survive a process for commercial sterility. These bacteria do not present a health hazard, however, and do not present a spoilage problem under normal, recommended conditions of storage. These are thermophilic, or heat-loving, bacteria that require high temperatures to germinate. If this happens, they may produce acid with little or no gas, or gases that swell cans. It is generally recommended that canned foods be quickly cooled below 105°F in thermal processing and then stored below 95°F to prevent problems from these surviving thermophiles.

Process Design

Critical factors influencing the process needed to obtain commercial sterility include:

1. The composition and nature of the food (eg, pH, sauce viscosity, starch content, particle tendency to mat or clump)

2. Initial number of microorganisms on the food, also called bacterial load
3. The microorganism(s) of concern with the particular food and the heat resistance of those organisms
4. Pack style, or preparation of the food as it affects particle size, viscosity, solids-to-liquid ratio, acid-to-low-acid ratio (for acidified foods) and others depending on the product
5. Jar size and conformation
6. Fill weight
7. Initial (or fill) temperature of the food
8. Retort temperature and style (eg, rotating or still)

Designing a thermal process (canning schedule) that takes into account all the critical factors that can influence safety of the finished product requires the use of specific scientific methods. It requires knowledge and data about the microorganisms of concern in that food and the rate at which they are destroyed by heat in the product and container. Process design also requires and involves collecting data showing heat penetration into the food product in its container, under the conditions used during production. These pieces of information are used to calculate a theoretical process that is then tested by inoculated pack studies, to test that the calculated process does provide commercial sterility.

Regulations for all canned foods state that the food processor must obtain scheduled processes established by a competent processing authority. The regulations do not specifically define acceptable process authorities; there are generally recognized organizations, such as the NFPA, members of the Institute for Thermal Processing Specialists, faculty at certain universities, and a number of consultants recognized as having this expertise. The regulations also do not specify actual procedures for thermal process design. Again, the NFPA provides guidance in how to conduct heat penetration tests as well as performing the tests itself. The Institute for Thermal Processing Specialists has also developed protocols for thermal process design.

Process Filing

Critical details of the process used to can a low-acid or acidified food product must be filed with the FDA. The process time, temperature, and other factors critical to adequacy of the process must be filed for each product and product style in each container type and size. Critical factors that influence the integrity of the process in addition to processing time and temperature include: type of processing equipment used; minimum initial temperature of the food; sterilizing value of the process (F_0) or other evidence of process adequacy; pH; water activity; and factors affecting heat penetration such as formulation, fill weight, drained weight, and headspace. The filing of a scheduled process with all necessary parameters delineated shall be done not later than 60 days after registration of the establishment and before any new product is packed. The FDA then edits all processing information filed for questionable, incomplete, or incorrectly filed information, and returns forms needing clarification or completion. The FDA does not certify or approve processes; it is the responsibility of the processor and thermal processing authority to produce a safe process.

Equipment and Procedures

Scheduled processes determined through testing must be applied under equivalent conditions in commercial practice. The regulations contain specifications for equipment design and management to ensure that critical controls important to a thermal process are in place. The regulations regarding equipment and procedures are detailed, and for actual requirements, the reader should consult the CFR. However, the following is a summary of the controls required to ensure the safety of canned foods.

Equipment design features are prescribed for various types of retorts (equipment used for the canning process). They include type and placement of temperature- and pressure-indicating devices and of temperature-recording devices. Because the steam supply affects maintenance of the temperature in the retort, requirements for steam controllers, the steam inlet to the retort, and steam spreaders are also described. Size and positioning of steam bleeders are specified for certain retorts. Air and water supply and drainage are also critical components discussed. Much attention is given to the placement of retort vents and venting processes; removal of air from the retort is critical to the temperature obtained for steam pressure processing.

Raw Materials

All raw materials and ingredients that are susceptible to microbiological contamination must be suitable for use in processing. Specifications for suppliers should be in place to ensure their wholesomeness and quality. Receiving procedures should ensure adequate examination, especially so as to determine their microbiological condition. If stored before use, they should be examined again immediately prior to processing.

Raw agricultural products should be cleaned to remove excess soil from the surfaces. All raw materials should be stored or held in a manner that prevents contamination, increases in microbiological loads, and loss of quality. Physical characteristics of the product may also affect the thermal process. Product size, texture, and condition can affect heat penetration. Raw product controls important to maintain are sorting and grading for the purposes of size and quality.

The water supply must be of sufficient quality for the intended use and from an acceptable source. Any water that contacts food or food-contact surfaces shall be safe and sanitary. Water must be able to be delivered at appropriate temperatures suitable for its use at different locations in the establishment. The residual chlorine content of water needs to be checked and adjusted at various steps in the process and plant operations.

Product Preparation

Blanching is often done. It helps inhibit enzymatic action, which may later alter the characteristics of the goods.

Blanching also expels respiratory gases and oxygen, a step that assists in control of headspace in the container and allows a greater vacuum to be achieved. Softening of food to enable easier packing, setting of flavors and colors, and cleaning are some additional benefits from blanching. Finally, blanching may allow peeling, dicing, cutting, and other steps to be done more easily.

Blanchers must be operated in such a way as to minimize thermophilic bacterial growth and contamination. Rapid heating is preferred. Blanchers should be operated at temperatures in the 190 to 200°F range to control thermophilic growth. Lower temperatures may be used, but the procedures must be evaluated for the growth of organisms. If the product is not to be immediately packed and processed after blanching, it should be rapidly cooled after heating. Potable water must be used on blanched foods washed before filling.

Product formulation must be controlled; starches, gums, sugars, and other thickening agents are particularly critical factors in heat penetration. Inspectors should determine if changes have been made, and if so, if the scheduled process was tested and changed accordingly if needed. Rehydration of dry ingredients, such as beans, rice, peas, and potatoes also affects thickening and heat penetration. Rehydration should be controlled. Viscosity of packing mediums must also be controlled, and inspectors should check for deviations from the original formulations.

Filling Operations

Containers must be appropriate to the product being processed. They must be appropriately cleaned and even sanitized, if required. The filling operation should be inspected to ensure that no contamination occurs during the filling, such as might occur from a ventilator used to prevent trapped air in the product.

Filling of the container prior to the canning process may also be a critical control. Filling requirements specified in the process must be followed. Fill weights and ratio of solids and liquids must be controlled. Headspace may be critical, especially for agitated products that need a gas bubble to achieve movement of the product. Headspace is normally controlled by the fill weight or volume. Too little headspace may result in underprocessing of the product as well as a low vacuum. After filling, exhausting of containers to remove air from the headspace shall be controlled to meet conditions specified in the scheduled process. This is usually accomplished by hot filling, mechanical methods, steam injection, or gas flushing.

Container closing operations are another area requiring safety considerations. Intact, properly formed seams and seals are essential to successful canning and food safety. They are so essential, in fact, that careful examination of can seaming operations and frequent inspection of seals at the end of retorting and after cooling are required by regulations (see "Container Evaluations"). To avoid container leaks, can seaming equipment or other sealing equipment must be properly maintained and operated. Unacceptable practices at this stage are overfilling and using cans with defective flanges or jars with damaged lugs or threads. Food must not overhang or rest in seal areas.

Holding filled containers in the growth range of microorganisms for long periods of time before processing can lead to spoilage before canning takes place. This is known as incipient spoilage. It may increase the microbiological load before processing and reduce the vacuum obtained. Steps to eliminate incipient spoilage include controlling microbial growth on the raw material, during production, and in the container prior to processing. Holding time before processing should be minimized or carefully controlled to prevent growth. Incipient spoilage does not always result in a health hazard, but may occur, particularly with contamination from staphylococci which produces an enterotoxin.

Processing Operations

Operations in the thermal processing area, often called the "cook room," are vital to safety of the canned food and are heavily regulated. Retort operating procedures and venting procedures to be used for each food and container size must be readily available to the retort operator and the inspector. As discussed earlier, the design of the retorts and their critical operating components are described in detail in regulations for low-acid foods. The operator should be sure all aspects are operating properly before processing food. A system must be established and used that ensures that no product moves through the processing room without being retorted. Labels or other visual indicators should be used to provide indication that the containers have been exposed to the retort process.

All sealed containers must be marked with a code that is permanently visible. The code must identify the establishment; the product; and the year, day, and period when packed. Coding is critical to having a means for isolating and/or recalling questionable product.

The initial temperature of the container contents is a critical safety factor. These temperatures should be measured and recorded with sufficient frequency to be sure that the minimum initial temperature specified in the scheduled process is obtained. This is another reason why lag time between filling and processing should be minimized. Initial temperature is normally determined using a handheld thermometer. (Glass stem thermometers are not used due to potential breakage.) For batch retort loads, a container may be removed from the load going into a retort and set aside where it is not subject to extreme temperature change. When the retort lid is closed and steam turned on, the initial temperature is determined. When retort systems use water to cushion or hold containers, the temperature of that water is a critical concern so the initial food temperature is not lowered. The initial temperature is determined by shaking or stirring the container contents prior to measuring; it is the average temperature of the contents.

Other critical factors during processing are measurements, meters, and devices. Equipment must be accurate, and pressure gauges and thermometers must be located for ease in reading. Timing devices that record process information are to be in place and accurate. Venting of the steam retort is extremely important to achieving the desired processing temperature. Placement of containers in the retort must be consistent with conditions of the scheduled process and must not block venting of the retort.

Deviations from the intended process may occur. A line breakdown may occur at several locations and for many reasons. Procedures should be in place to provide direction for continued use or disposition of the product. Full records must be kept of any reprocessing. Product may be set aside for evaluation by a competent processing authority as to whether it may be used or should be destroyed. Records of evaluation procedures, reasons for the deviation, and actions taken must all be recorded and filed.

During inspection, all these factors of processing operations must be determined, and at least one complete cycle of retort operation should be observed.

Cooling and Storage Operations

Cooling of containers after the retort processing must be controlled to prevent recontamination and damage to seams or seals. It also should be done quickly to prevent growth of thermophilic organisms, as well as overcooking, although the latter is usually detrimental only to quality and not to safety.

Containers may be air or water cooled. Water used for cooling must be of good sanitary quality. Cooling water must be chlorinated or otherwise sanitized. The level of free chlorine in the water must be checked and care given to where the samples are taken for this testing. After processing, the sealing compound used in containers is softened, and as it cools, small amounts of cooling water may be drawn into the container. The sealing compound may then set and seal the leak without loss of vacuum. If contaminated cooling water is used, spoilage and possible danger to the consumer may result.

The time required and the temperature to which retorted foods are cooled must be checked. Cans are generally cooled to 95 to 105°F to leave enough heat in the cans to dry them (when water cooled) but not enough to permit the growth of thermophilic organisms. At this temperature the cook is also stopped, preventing overcooking of the product.

Some cooling procedures may result in damaged seams. Precautions should be taken to prevent buckling of metal containers or damage to seals in glass and other packages. If pressure is reduced too quickly during cooling, buckling may occur from the inability of seams to withstand the change. Buckling is a problem due to the potential for the can seams to be pulled apart enough to leak, and contamination of the contents may occur.

Steps to maintain the integrity of container seals must be continued into postcooling container handling. Bacterial contamination can occur in seam and seal areas if handling lines are not sanitary. Moisture present on containers or the can handling equipment can suspend the organisms and allow them to grow. Rough handling can also damage seals. Crates and baskets used to transfer containers should not have sharp edges or protrusions that damage packages. Leaks from dents or defective seams can result in postprocessing contamination of the commercially sterile product. Cleaning and sanitizing procedures should be in place for all crates, racks, conveyors, belts, and machinery.

Before product is stored prior to distribution, it must be sufficiently cooled to prevent growth of thermophilic organisms (105°F). The storage area must be clean with no product stored directly on the floor. Storage temperatures should not exceed 95°F.

Rust should be prevented or it may lead to damaged seams and seals. If the temperature of the warehouse is too high, the cans may sweat and the moisture will lead to rusting. Rust on the can exteriors may be due to other reasons, including improper operation of retort vents, too long come-up time in retort, or steam containing moisture. Failure to remove the surface water or chemical composition of the cooling water may cause rust.

Container Evaluations

Regular observations and examinations are made to determine that container closures are not defective. The establishment's personnel responsible for these procedures and the inspector need equipment that is used to conduct "tear-down" examinations of the can and its seams. Teardown of cans to determine seam integrity must be done by a trained individual. Samples shall be taken from each seaming station not more than every 4 hours. During teardown, measurements are made on cover and body "hooks" of the double seam. Thickness, tightness, and width are measured or observed. Can seam terminology and procedures are the subject of specialized training in the canning industry, due to the importance of producing and maintaining hermetic seals for food safety.

Sampling of containers for closure evaluation is also done during production runs. At this time, gross closure defects can be observed and corrected. Any defects and corrective actions must be recorded. Measurements and recordings should be made no more than every 30 minutes. Additional inspections of closures are made immediately following a jam in a closing machine, after any adjustment to closing machines, or following a prolonged shutdown before a machine is started up.

SAFEGUARDS

Inspections

One of the protections provided for by canning regulations is inspection of a canning establishment. The regulations recognize that there are certain *critical* elements or steps common to every canning process, and low-acid canning process in particular, that must be controlled to prevent a health hazard. It is the responsibility of the inspection authority (eg, FDA for low acid canned foods) to monitor a firm's compliance with these preventive principles.

The FDA and the USDA publish inspection guidance documents that explain procedures to be followed. These documents may be consulted for detailed information about standards. Changes are being made as needed to keep these procedures updated; for example, they must be consistent with Hazard Analysis Critical Control Points (HACCP) regulatory systems being implemented in various segments of the food processing industry in the 1990s. Although the HACCP system has its origin in the industry's quality control functions of the 1960s, its emphasis is food safety and prevention of health hazards. The use of HACCP systems for regulatory purposes is growing in the 1990s; however, the current GMPs covering low-acid canned foods and acidified foods already have their origins in the HACCP principles presented at the 1971 Conference on Food Protection.

Before an inspection, the investigators should become familiar with the firm's scheduled processes and all critical control factors. A *critical factor* is defined in 21 CFR 113.3(f) as any property, characteristic, condition, aspect, or other parameter, variation of which may affect the scheduled process and the attainment of commercial sterility. Regulations state that critical factors specified in the scheduled process *must* be measured and recorded in processing records. The inspector must review documentation at the firm to determine all critical factors are being controlled.

Recalls

Another safeguard in regulation of the canning industry is the provision for recalls. The FDA and USDA have the authority and responsibility to issue recalls for potentially harmful foods. A company may discover defective product on its own and issue a recall. In other instances, the FDA or USDA finds a problem, informs the company, and suggests or requests a recall. Recalls may then be done voluntarily by the processor in cooperation with the governing agency. However, it the company does not comply with a requested recall, the agency can seek a court order authorizing seizure of the product.

The FDA and USDA have guidelines on how to conduct a recall for companies to follow. There are three classes of recalls according to the level of hazard involved. The strictest procedures are in place to ensure appropriate follow-through for recalls presenting the greatest hazard.

GENERAL REFERENCES

D. L. Downing, ed., *A Complete Course in Canning and Related Processes*, 13th ed., Vols. 1–3, CTI Publications, Baltimore, Md., 1996.

A. Gavin and L. M Weddig, *Canned Foods. Principles of Thermal Process Control, Acidification and Container Closure Evaluation*, 6th ed., The Food Processors Institute, Washington, D.C., 1995.

Y. H. Hui, "Canning: Regulatory and Safety Considerations," in Y. H. Hui *Encyclopedia of Food Science and Technology*, 1st ed., John Wiley & Sons, New York, 1992, pp. 260–264.

Protocol for Carrying Out Heat Penetration Studies, Institute for Thermal Processing Specialists, Fairfax, Va., 1995.

Thermal Processes for Low-Acid Foods in Metal Containers, Bulletin 26-L, 13th ed., National Food Processors Association, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 9, Part 318, Subpart G (.300–.311), Canning and Canned Products (Meat), U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 9, Part 318, Subpart X (.300–.311), Canning and Canned Products (Poultry), U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 114, Acidified Foods, U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 110, Current Good Manufacturing Practice in Manufacturing, Packing or Holding Human Food, U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 108, Emergency Permit Control, U.S Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 113, Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers, U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Food and Drug Administration, *Guide to Inspections of Acidified Food Manufacturers*, 1998.

U.S. Food and Drug Administration, *Guide to Inspections of Low Acid Canned Food Manufacturers*, Part 1 (PB97-196141), 1996; Part 2 (PB97-196158), 1997; Part 3, 1998.

ELIZABETH ANDRESS
University of Georgia
Athens, Georgia

CANOLA OIL

BACKGROUND

The term *canola* refers to cultivars of an oilseed crop, known in many countries as rapeseed, that is a major source of food and feed throughout the world. The crop has become the world's third most important edible oil source after soybeans and palm (1). The development by plant breeders of low erucic acid and low glucosinolate (double-low) varieties of rapeseed has proved pivotal to the rapid expansion in production and use of rapeseed worldwide. The rapeseed industry in Canada adopted the name canola in 1979 to distinguish those cultivars of *Brassica napus* and *Brassica rapa* (formerly, *B. campestris*) that are genetically low in both erucic acid and glucosinolates. Since the oil and meal from the double-low cultivars are nutritionally superior to those of earlier-grown varieties, the generic term *canola* also was used to identify the products from these varieties. The definition of canola was last updated in 1997 to take account of the development of mustard seed, *Brassica juncea*, varieties with low levels of erucic acid and glucosinolates. The definition adopted by the Canola Council of Canada in 1997 reads: "The oilseeds shall be the seed of the genus *Brassica* which shall contain less than 18 micromoles of total glucosinolates per gram of whole seed at a moisture content of 8.5%; and the oil component of which the seed shall contain less than one percent of all fatty acids as erucic acid" (2). Canola is not an exclusive Canadian trademark; the oilseed industry in other countries, such as Australia, Japan, the United States, and the United Kingdom, also have accepted canola to describe double-low rapeseed.

Canola has become Canada's second most valuable crop after wheat. Its rapid rise in economic importance is due to some positive agronomic advantages and the marketing alternatives it offers producers. Canada is the leading exporter of seed in the world, exporting an average volume of 2.8 million tons annually over the period 1992–1997 (3); Japan is the major importer of Canadian rapeseed/canola. In 1996–1997, the domestic crush of seed in Canada ex-industry's quality control functions of the 1960s, its emphasis is food safety and prevention of health hazards. The use of HACCP systems for regulatory purposes is growing in the 1990s; however, the current GMPs covering low-acid canned foods and acidified foods already have their origins in the HACCP principles presented at the 1971 Conference on Food Protection.

Before an inspection, the investigators should become familiar with the firm's scheduled processes and all critical control factors. A *critical factor* is defined in 21 CFR 113.3(f) as any property, characteristic, condition, aspect, or other parameter, variation of which may affect the scheduled process and the attainment of commercial sterility. Regulations state that critical factors specified in the scheduled process *must* be measured and recorded in processing records. The inspector must review documentation at the firm to determine all critical factors are being controlled.

Recalls

Another safeguard in regulation of the canning industry is the provision for recalls. The FDA and USDA have the authority and responsibility to issue recalls for potentially harmful foods. A company may discover defective product on its own and issue a recall. In other instances, the FDA or USDA finds a problem, informs the company, and suggests or requests a recall. Recalls may then be done voluntarily by the processor in cooperation with the governing agency. However, it the company does not comply with a requested recall, the agency can seek a court order authorizing seizure of the product.

The FDA and USDA have guidelines on how to conduct a recall for companies to follow. There are three classes of recalls according to the level of hazard involved. The strictest procedures are in place to ensure appropriate follow-through for recalls presenting the greatest hazard.

GENERAL REFERENCES

D. L. Downing, ed., *A Complete Course in Canning and Related Processes*, 13th ed., Vols. 1–3, CTI Publications, Baltimore, Md., 1996.

A. Gavin and L. M Weddig, *Canned Foods. Principles of Thermal Process Control, Acidification and Container Closure Evaluation*, 6th ed., The Food Processors Institute, Washington, D.C., 1995.

Y. H. Hui, "Canning: Regulatory and Safety Considerations," in Y. H. Hui *Encyclopedia of Food Science and Technology*, 1st ed., John Wiley & Sons, New York, 1992, pp. 260–264.

Protocol for Carrying Out Heat Penetration Studies, Institute for Thermal Processing Specialists, Fairfax, Va., 1995.

Thermal Processes for Low-Acid Foods in Metal Containers, Bulletin 26-L, 13th ed., National Food Processors Association, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 9, Part 318, Subpart G (.300–.311), Canning and Canned Products (Meat), U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 9, Part 318, Subpart X (.300–.311), Canning and Canned Products (Poultry), U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 114, Acidified Foods, U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 110, Current Good Manufacturing Practice in Manufacturing, Packing or Holding Human Food, U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 108, Emergency Permit Control, U.S Government Printing Office, Washington, D.C., 1996.

U.S. Code of Federal Regulations, Title 21, Part 113, Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers, U.S. Government Printing Office, Washington, D.C., 1996.

U.S. Food and Drug Administration, *Guide to Inspections of Acidified Food Manufacturers*, 1998.

U.S. Food and Drug Administration, *Guide to Inspections of Low Acid Canned Food Manufacturers*, Part 1 (PB97-196141), 1996; Part 2 (PB97-196158), 1997; Part 3, 1998.

ELIZABETH ANDRESS
University of Georgia
Athens, Georgia

CANOLA OIL

BACKGROUND

The term *canola* refers to cultivars of an oilseed crop, known in many countries as rapeseed, that is a major source of food and feed throughout the world. The crop has become the world's third most important edible oil source after soybeans and palm (1). The development by plant breeders of low erucic acid and low glucosinolate (double-low) varieties of rapeseed has proved pivotal to the rapid expansion in production and use of rapeseed worldwide. The rapeseed industry in Canada adopted the name canola in 1979 to distinguish those cultivars of *Brassica napus* and *Brassica rapa* (formerly, *B. campestris*) that are genetically low in both erucic acid and glucosinolates. Since the oil and meal from the double-low cultivars are nutritionally superior to those of earlier-grown varieties, the generic term *canola* also was used to identify the products from these varieties. The definition of canola was last updated in 1997 to take account of the development of mustard seed, *Brassica juncea*, varieties with low levels of erucic acid and glucosinolates. The definition adopted by the Canola Council of Canada in 1997 reads: "The oilseeds shall be the seed of the genus *Brassica* which shall contain less than 18 micromoles of total glucosinolates per gram of whole seed at a moisture content of 8.5%; and the oil component of which the seed shall contain less than one percent of all fatty acids as erucic acid" (2). Canola is not an exclusive Canadian trademark; the oilseed industry in other countries, such as Australia, Japan, the United States, and the United Kingdom, also have accepted canola to describe double-low rapeseed.

Canola has become Canada's second most valuable crop after wheat. Its rapid rise in economic importance is due to some positive agronomic advantages and the marketing alternatives it offers producers. Canada is the leading exporter of seed in the world, exporting an average volume of 2.8 million tons annually over the period 1992–1997 (3); Japan is the major importer of Canadian rapeseed/canola. In 1996–1997, the domestic crush of seed in Canada exceeded seed export for the first time (2.71 vs 2.52 million tons). Domestic utilization of canola oil in 1996–1997 was 6.82×10^5 t, up from 4.33×10^5 t in 1992–1993, while oil exports increased from 3.67×10^5 to 5.07×10^5 over the same period (4). Among the processed oils and fats, canola has made substantial gains into the shortening and salad and cooking oil segments of the Canadian market during the past decade. Canola accounted for 70.4% of all deodorized fats and oils, 72.8% of the vegetable oils, and more than 80% of the salad oils produced in Canada in the calendar year 1997 (4). Although current canola production in the United States is relatively small, there is a growing demand for canola products; for 1996–1997 alone, Canada exported 4.24×10^5 t of oil and 8.49×10^5 t of meal to the United States, which accounted for more than 80% of the total oil and meal exports (5).

Canola oil is characterized by a low level of saturated fatty acids and a relatively high content of oleic acid; canola oil is second to olive oil in oleic acid content among common vegetable oils. The blood cholesterol-lowering effect observed in humans, when canola oil made up a major portion of the total dietary fat (6), has been an important factor in the increased use of canola products in the world's markets. Rapeseed/canola now ranks third, behind soybean oil and palm oil, in the global disappearance of fats and oils.

Botanical Origin

Although rapeseed production in Canada commenced after World War II, the cultivation of *Brassica* oilseeds has a long history in Europe and Asia (7). Unlike other oilseed crops, rapeseed is not a product of a single species, but comes from two species of the genus *Brassica: B. napus* and *B. rapa*. The cytogenic relationships between rapeseed and its close relatives have been discussed extensively in several reports and are important in understanding the origin, evolution, and plant-breeding strategies of the *Brassica* species (8–11). The two rapeseed species along with *Brassica juncea* (mustard), commonly referred to as oilseed *Brassicas*, collectively provide more than 13.2% of the world's edible oil supply (11). The small, round *Brassica* seeds contain 40 to 44% oil (dry weight basis) and produce a high-protein content (38–41%) oil-free meal. Within the rapeseed species, both spring and winter cultivars exist; the latter are higher yielding than the spring varieties but are less winter hardy than cereals. Winter cultivars of *B. napus* predominate in Europe, whereas spring cultivars of *B. napus* and *B. rapa* are grown in Western Canada. *B. napus* varieties have a generally higher seed-yielding potential (15–20%) than those of *B. rapa*, but they require an additional 8 to 15 frost-free days to mature; in Western Canada, cultivars of *B. napus* require 95 to 105 days for maturity (9,12).

Development of Double-Low Cultivars

Rapeseed production in Canada began with the introduction of seed of *B. napus* from Argentina and *B. rapa* from Poland; these materials, highly heterogenous, constituted the seed stock for the establishment of breeding programs. Although improvements in seed yield and oil content were the first objectives, particular emphasis was also given to oil and meal quality. The elimination of nutritionally undesirable components—erucic acid, found to cause cardiac lipidosis in several animal species (13), from the oil and sulfur-containing glucosinolates from the meal—became a target at the early stages of the breeding programs. Changes in fatty acid composition were made by introducing the inherited traits of low erucic acid into adapted lines of *B. napus* and *B. rapa*. Canadian breeders also accomplished the transfer of genes responsible for the low glucosinolate characteristic into rapeseed varieties. These sulfur-containing constituents are hydrolyzed by an endogenous enzyme, called *myrosinase* (thioglucosidase, EC 3.2.3.1), into thiocyanates, isothiocyanates, and under some conditions nitriles (Fig. 1) when seed cells are ruptured (eg, during seed crushing). The characteristic odor and flavor of *Brassica* vegetables and condiments (mustard) are largely due to the presence of these compounds. Glucosinolate breakdown products also reduce the palatability and, in nonruminant animals, adversely affect the iodine uptake by the thyroid gland (14,15). Breeding for lower glucosinolate content of the seed has resulted in nutritional upgrading of rapeseed meal for all classes of livestock and poultry. Further reduction in glucosinolates content is required, particularly of the indoyl glucosinolates (16), if proteins from canola meal are to be used in human food formulations. This has been partly accomplished by extraction processes employed in preparation of protein concentrates and isolates (17). The ultimate solution would be to breed varieties free of both alkenyl and indolyl glucosinolates. Total elimination of glucosinolates in canola is a future possibility since a strain of *B. rapa* essentially free of alkenyl glucosinolates has been produced (16).

HORTICULTURE-POSTHARVEST HANDLING

Cultivation-Agronomic Considerations

Canola is a cool season crop that requires more available moisture than wheat, as well as cool night temperatures to recover from hot and dry weather. Hence, canola is usually grown at the wetter and cooler margins of the cereal-growing regions of Canada and other countries.

Because *B. juncea* (mustard) is more heat and drought tolerant than the rapeseed cultivars *B. napus* and *B. rapa*, a double-low germplasm of *B. juncea* has been developed in Canada and is being tested for its potential as an oilseed crop in the dry prairie region of western Canada. This approach is seen as the most promising means of increasing the production area for "canola-type" oilseeds in Canada.

Although rapeseed grows well on a variety of soils, it does best on loamy soils that do not crust and impede seedling emergence (18). Good yields are also obtained on light and heavy soils if rainfall, fertilization, and drainage are adequate (12). Canola is moderately tolerant to saline soils and has greater moisture requirements than cereals. Canola also requires more intensive management practices; for a good crop, the need for nitrogen is 20% higher than for a comparable cereal crop. Certified seed is usually used for sowing to ensure absence of mustard or weeds and the quality characteristics of the cultivar.

Canola is grown in Canada on summer fallow or cereal stubble land. From an agronomic viewpoint, the crop fits

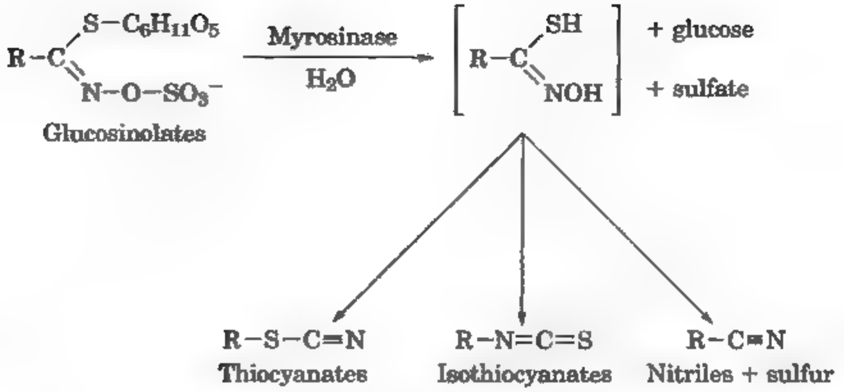


Figure 1. General structure of glucosinolates and their enzymic hydrolysis products.

well as a crop rotation with cereals; wheat or barley following canola is the preferred sequence. Most fields are not cropped with canola more than once every four years. This practice permits a break in disease and insect pest cycles, while preserving the soil structure. Canola is susceptible to several fungal diseases such as blackleg (*Leptosphaeria maculans*), stem rot (*Sclerotinia sclerotiorum*), damping-off (*Fusarium* spp.), root rot (*Rhizoctonia* spp.), and black spot or leaf spot (*Alternaria* spp.); disease severity varies with year and location (19). A number of different insects can also cause serious damage to canola (eg, flea beetle) (20). Crop rotation is effective in controlling fungi and insects. Several herbicides are also available for a variety of weed species (12,21).

Harvest and Storage Practices

The date and method of canola harvesting are important determinants of yield and seed quality (12,18,22). Immature seeds have higher chlorophyll and free fatty acids contents. On the other hand, if the harvest is delayed, canola is susceptible to shattering of the pods, particularly in windy areas. To avoid this, swathing (leaving cut plants to dry in the field) is applied when the majority of the seeds are at a firm doughlike stage (moisture content 35–40%). Swathing facilitates uniform maturation and yields seeds of good color and high in oil and protein. Premature swathing (seed moisture >45%) results in reduced yields, whereas late swathing (moisture <20%) increases shattering losses and the possibility of frost damage. Canola is ready to combine when the moisture content is below 15%. Freshly harvested seeds normally require up to six weeks to become dormant; storage in dry and cool environments minimizes respiration losses. Canola is usually transported after harvest to dryer-stocker companies and therefrom to oil-processing plants. For storage, silos of various sizes are used, while on the farms the seed is stored in bins. Immature and field-germinated seeds should not be stored for long periods because they deteriorate rapidly. A high percentage of damaged or fragmented seeds also gives rise to a rapid increase in free fatty acids (22,23).

The moisture content of the seed, because of its importance to enzyme activity and the growth of microflora, is the main criterion for safe storage. In the case of oilseeds, including canola, moisture is held by the nonfatty components, thus bringing the critical level of overall seed moisture content much below that required for safe storage of cereal grains (15%). The maximum moisture content for storage of clean canola seeds in a cool environment (<15°C) is 10% (24); others sources (22,25) even suggest lower optimum levels of moisture (8–9%) for storage over periods of several months. Above this level, the seeds must be air dried; air temperatures must be kept below 38°C to prevent heat damage. Aeration during storage, although at much lower airflow rates than drying, is also required to prevent moisture gradients and to cool the stored grain. Figure 2 shows how storage is affected by both temperature and moisture content. Canola is particularly prone to microbial spoilage, with severe losses in seed and oil quality. The rate of spoilage rapidly increases if nonuniform distributions of moisture and temperature exist in the stored grain. An important consideration regarding moisture content of canola is that before solvent extraction of the oil, the seed must be cooked rapidly at temperatures between 80 and 100°C and at water contents between 6 and 10% to inactivate myrosinase; above 10% moisture, hydrolysis of glucosinolates would occur; below 6%, heat inactivation of the enzyme is difficult.

Chlorophyll is another important quality parameter of canola. Besides imparting an undesirable color to the oil, chlorophyll promotes oxidative rancidity in salad oils. Several environmental factors and agronomic practices, including length of growing season, temperature during seed

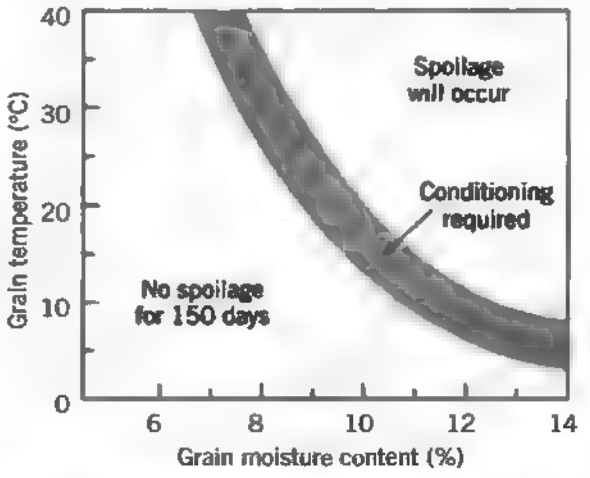


Figure 2. Effects of moisture and temperature on storage of canola. *Source:* Ref. 24.

ripening, cultivar and seeding rate, have been shown to affect the chlorophyll level (26–28). Swathing at 35 to 37% seed moisture followed by drying is effective in reducing substantially the chlorophyll content of canola seeds (29).

BREEDING CANOLA FOR IMPROVED YIELD AND QUALITY—ROLE OF BIOTECHNOLOGY

Important objectives in canola breeding are both agronomic (seed yield, winter and frost hardiness, disease resistance, early maturity, herbicide tolerance, and resistance to shattering) and quality (oil and protein content and composition) traits (9–11,30,31). Although seed yield is one of the most desirable attributes, improvement through selection is a difficult task because of the strong influence from environmental factors.

Oil, being the most valuable component of the seed, is deposited in the form of small lipid droplets in the cytoplasm of embryonal cells. Protein bodies (storage proteins) are also stored in the cytoplasm of these cells (32). In *Brassica* spp., yellow-seeded cultivars have thinner seed coats and thus higher oil and protein content than seeds of darker color (31). It is, therefore, of great interest to producers and crushers to develop yellow-seeded forms or cultivars with increased seed size.

Oil quality is determined by the fatty acid composition. Fatty acid biosynthesis in oilseeds is carried out by assemblies of enzymes located in specific cell compartments (viz., plastids and endoplasmic reticulum of the cytoplasm) (33). Fatty acid synthesis via the fatty acid synthetase (FAS) complex in the plastids produce saturated aliphatic fatty acids (lauric, C12:0; palmitic, C16:0; stearic, C18:0) (Fig. 3). Production of the various saturated fatty acids depends on the presence and activity of specific thioesterases. In most oilseed plants, C18:0 is the primary product of the FAS pathway and it, in turn, is largely desaturated to oleic acid (C18:1n-9) by a specific (stearoyl-ACP) desaturase. Further desaturation of C18:1n-9 to linoleic acid (C18:2n-6) and linolenic acid (18:3n-3) and chain elongation to eicosaenoic acid (C20:1n-9) and erucic acid (C22:1n-9) occurs in the cytoplasm (8,33). Hence, changes in the fatty acid profile in *Brassica* can be achieved by modification of the biosynthetic pathways (Fig. 3). Changes in single genes can have profound effects on the fatty acid composition of the oil. For example, the amount of erucic acid depends on a series of alleles (at two gene loci in *B. napus* and a single locus in *B. rapa*) that influence the elongation of C18:1n-9; by varying the alleles present, it is possible to have erucic acid levels between 0 and over 50%. Interest in erucic acid for use as a feedstock in the oleochemical industry has stimulated interest in producing rapeseed cultivars with very high levels of erucic acid. In general, *Brassica* spp. do not incorporate erucic acid into the *sn*-2 position of the triacylglycerol molecule. However, identification of a genotype of *B. oleracea*, which can accumulate erucic acid in the *sn*-2 position, provides a possible route to *B. napus* lines with enhanced erucic acid levels (34). Another approach being investigated is the cloning of genes for *sn*-2 acyltransferases that recognize erucic acid, for example, from *Limnanthes* spp., and then inserting these genes into rapeseed (34).

Another oil quality breeding objective of canola has been to reduce the level of linolenic acid (C18:3n-3) from 8 to 10% to less than 2% for the purpose of improving oxidative stability of the oil. The first low-linolenic acid cultivar (Stellar), which was introduced in 1987, contained less than 3% linolenic acid (9,35). The oil from this cultivar exhibited improved stability at 60°C and showed negligible changes in sensory and chemical indices of rancidity, compared with regular canola oil (36); improved odor scores from frying tests were also reported for the low-linolenic canola oil (37). Cultivars with even lower levels of linolenic acid (eg, Apollo, avg. 1.7% C18:3n-3) have recently been released (9). Other modifications also have been made to the fatty acid composition of *Brassica* spp., for example, the development of cultivars with 86% oleic acid (C18:1n-9). These modified canola oils were produced in response to the demand for a frying oil with a low level of saturated and *trans* fatty acids and a high stability to oxidative changes without having to be hydrogenated. Another example is the development of cultivars with 10 to 12% palmitic (C16:0) plus palmitoleic (C16:1n-7) acids (15) in order to make margarines exclusively from low erucic acid rapeseed oils. For such products, the tendency to develop large crystals on storage would be diminished.

The application of biotechnology techniques (genetic engineering) to *Brassica* spp. has resulted in some novel oils. Scientists at Calgene Inc. have produced both a high stearic acid and a high lauric acid cultivar of rapeseed/canola using transgenic techniques. The high-lauric canola (>40% C12:0), which is intended for use in the manufacture of detergents and surfactants, was produced by adding the lauroyl-ACP thioesterase gene from the California Bay laurel plant into canola (38,39). The high-stearic canola was produced by inserting the antisense gene construct for stearoyl-ACP desaturase into canola (40). This high-stearic canola could be used in the production of margarine or the manufacture of cocoa butter substitutes (39). Hence, it is reasonable to expect the development of a broad range of rapeseed/canola oils having fatty acid composition appropriate for specific applications (Table 1).

In contrast to oil, relatively little attention has been paid to the protein quality of the meal. Indeed, the reduction of glucosinolate levels in the meal has been the primary objective in the breeding programs. Recessive alleles, responsible for the low-glucosinolate characteristics, have been introduced from *B. napus* var. Bronowski into cultivars of *B. napus* and *B. rapa* by backcrossing (43); several gene loci are involved in the inheritance of glucosinolate content (30,31). Additional improvement in the nutritional value and palatability of canola meal would also result if the 1.0 to 1.5% sinapine and phytic acids present in *Brassica* oilseeds are reduced or eliminated by breeding.

There is great interest among breeders and producers in developing hybrid cultivars of canola for increased yield. Cytoplasmic male sterility (CMS), self-incompatability (SI), and transgenic techniques are among the systems being investigated as means of producing hybrid seeds (10,11,31,39,44). For the CMS system, three inbred lines are used: (*1*) the female parent (male-sterile line owing to cytoplasmic components—incompatibility of "foreign" mitochondria and the nuclear material), (*2*) a maintainer line

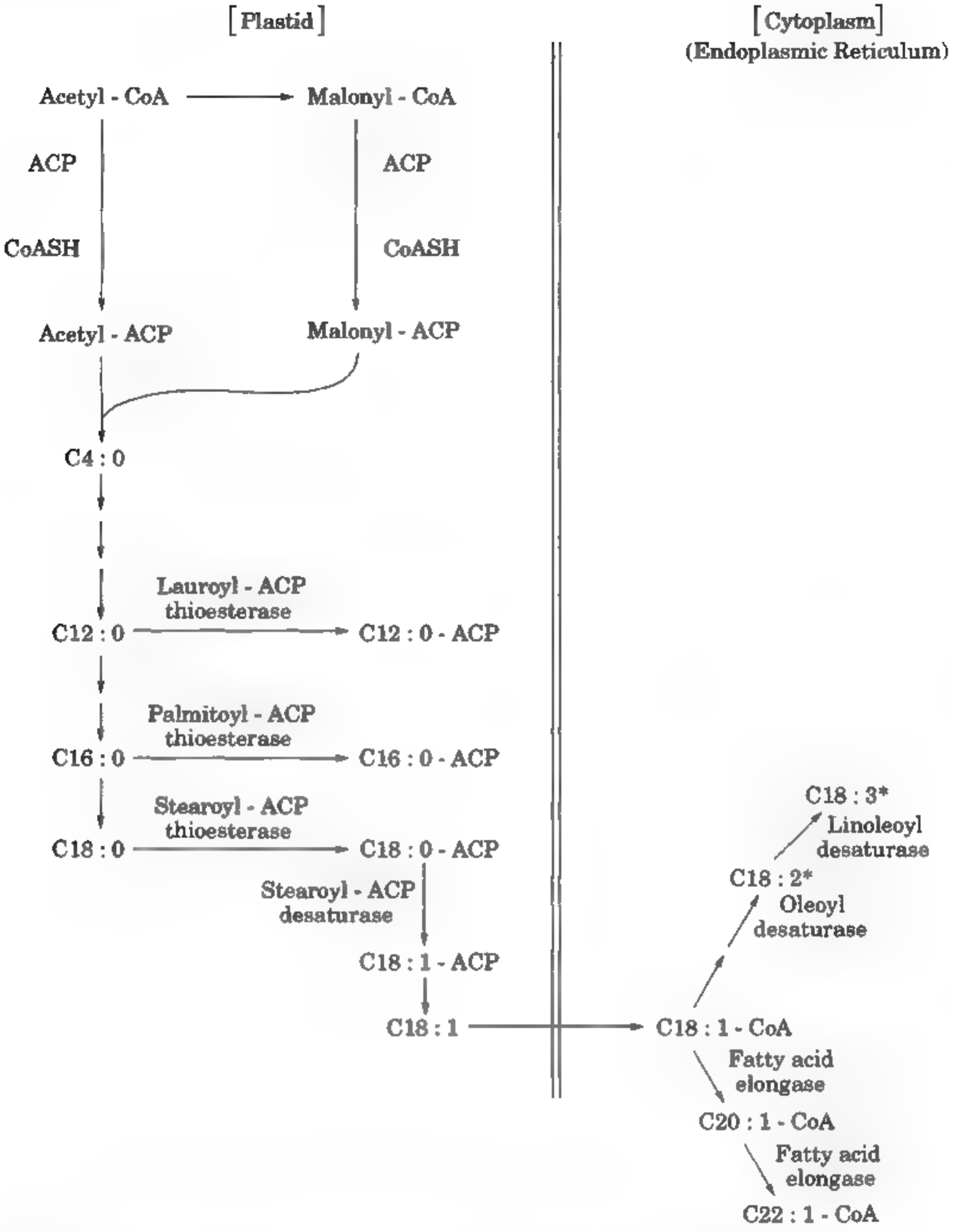

[Plastid]
[Cytoplasm]
(Endoplasmic Reticulum)
Acetyl - CoA
Malonyl - CoA
ACP
CoASH
ACP
CoASH
Acetyl - ACP
Malonyl - ACP
C4 : 0
Lauroyl - ACP thioesterase
C12 : 0
C12 : 0 - ACP
Palmitoyl - ACP thioesterase
C16 : 0
C16 : 0 - ACP
Stearoyl - ACP thioesterase
C18 : 0
C18 : 0 - ACP
Stearoyl - ACP desaturase
C18 : 1 - ACP
C18 : 1
C18 : 1 - CoA
C18 : 3*
Linoleoyl desaturase
C18 : 2*
Oleoyl desaturase
Fatty acid elongase
C20 : 1 - CoA
Fatty acid elongase
C22 : 1 - CoA

Figure 3. A simplified scheme for the synthesis of fatty acids in plants seeds. Abbr.: ACP—acyl carrier protein; CoASH—coenzyme A. * The oleoyl and linoleoyl desaturases act on C18:1 and C18:2 moieties of phosphatidylcholine to produce C18:2 and C18:3, respectively.

(possess cytoplasm that allows normal production of pollen), and (*3*) a restorer or male parent line that carries nuclear genes that will restore normal fertility to the hybrid cultivars. The expected level of heterosis (up to 40% seed yield increase) is sufficient to offset the cost associated with the development of an effective cytoplasmic male-sterile, genetic restorer system (10,31). Although SI hybrids have been released in Canada, a limitation of the self-incompatibility system for field crops, even though it is used for vegetable crops, is the relatively high cost.

The *Brassica* oilseeds have responded to genetic manipulation better than any other crop. The rapidly growing interest for crop improvement and modification by nonconventional breeding approaches as a result of advances in molecular biology and tissue culture techniques is currently focused on several areas of research (39,45,46).

1. Production of haploids by culture of reproductive organs to reduce the time required for new cultivar development.

Table 1. Fatty Acid Composition of Normal and Modified Rapeseed Oil Resulting from Selection

	Fatty acid							
Oil Type	16:0	16:1	18:0	18:1	18:2	18:3	20:1	22:1
				High erucic				
Normal	3	<1	1	11	13	8	8	50
Selected	2	<1	1	11	12	9	9	57
				Canola				
Low erucic	4	<1	1.5	61	27	9.5	21	<1
High-oleic	3.5	<1	2.5	77	8	2.5	1.5	<1
Low-linolenic	4	<1	1	61	27	2	1	<1
High-lauric[a]	3	<1	1.5	33	11	6.5	1	<1

[a] C12:0, 39%; C14:0, 4%.
Source: Refs. 41 and 42.

2. Protoplast fusion to transfer desirable genetic traits, particularly those carried by cytoplasmic organelles (chloroplast, mitochondria). Transfer of cytoplasmic traits such as male sterility and herbicide resistance was made possible via protoplast fusion and microinjection techniques (39).
3. Selection of cell variants with desirable agronomic characteristics (eg, herbicide resistance, drought tolerance, winter hardiness) after treatment with mutagenic agents and selection pressure of treated cells.
4. Gene transfer using suitable vectors (*Agrobacterium tumefaciens*) or by microinjection techniques.

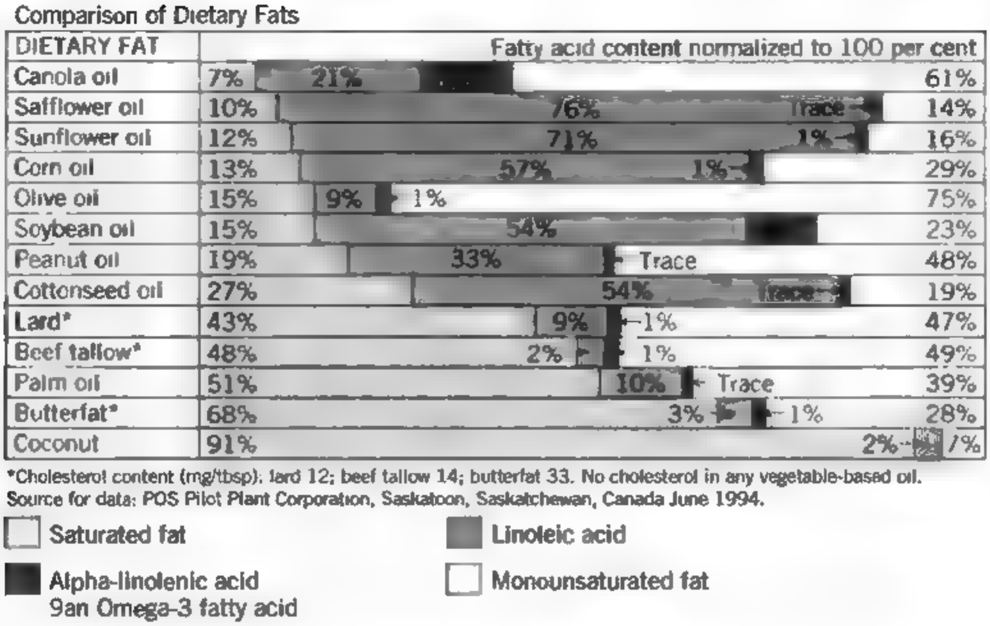
Consideration is primarily given to genes responsible for herbicide and fungal disease resistance, osmotolerance, manipulation of triglyceride composition, and production of novel high value chemicals (eg, jojoba-type waxes having as precursors eicosenoic and erucic acids).

Most prevalent among the novel-trait canola cultivars are herbicide-tolerant cultivars. Several *B. napus* varieties that are tolerant to the herbicides glyphosate (Round-up Ready), glufosinolate-ammonium (Liberty Link), and imidozolonone (Smart) are presently being grown in Canada. In fact, herbicide-tolerant varieties were estimated to account for 55 to 60% of the total acreage devoted to canola production in 1998 (47). By contrast, it was estimated that only 25 to 30% of the total acreage was devoted to transgenic herbicide-tolerant varieties in 1997. In addition to the development and testing of *B. napus* lines, several lines of herbicide-tolerant *B. rapa* also have been developed and are being tested for agronomic properties. The rapid development of transgenic canola varieties in Canada has been the result of effective collaboration among industry, government, and university researchers and the fact that canola breeding in Canada is conducted primarily by private industry, companies such as Pioneer Hi-Bred, Zeneca Seeds, Plant Genetic Systems (Canada) Lyd., Saskatchewan Wheat Pool, AgrEvo, Limagrain Canada, and Svalof AB (48). The impact of genetic engineering and conventional breeding is expected to result in even more valuable and versatile *Brassica* oilseeds in the future Specialty oils and by-products for both food and industrial uses, which are within reach of today's breeders, are expected to influence the production and marketing of rapeseed/canola in the years ahead.

COMPARISON OF CANOLA WITH OTHER OILSEEDS

The reduction of erucic acid in rapeseed oil resulted in a marked increase in oleic acid along with smaller increases in linoleic and linolenic acids. Canola oil has a fatty acid composition similar to peanut and olive oil with the exception of the lower palmitic and higher linolenic acid contents. Canola oil contains the lowest level of saturated fatty acids (mainly palmitic and stearic acids) and the highest content of unsaturated fat (mainly monounsaturated) of the common edible vegetable oil sources (Fig. 4). The total quantity of polyunsaturated fatty acids (C18:2n-6 and C18:3n-3) in canola oil is intermediate among the vegetable oils, although it contains an excellent balance between linoleic acid and linolenic acid compared with other widely used vegetable oils. The relatively high linolenic acid content of regular canola oil (10%) makes it susceptible to oxidative rancidity. This problem has been alleviated in the newly developed low linolenic acid cultivars (35,36), although the presence of lipoxygenase in canola seeds has been reported (48). In most respects, there is no obvious physical property distinguishing canola oil from other common vegetable oils. Rapeseed oils (both high and low erucic acid), however, contain a C_{28} brassicasterol, which is not found in other edible vegetable oils (49); brassicasterol is thus a suitable marker to identify *Brassica* oils in vegetable oil blends.

The protein content of canola meal varies with the variety from which it is produced; it ranges between 36 and 42%. Canola meal is higher in fiber than soybean meal (Table 2). The amino acid composition compares well with that of soybean meal (50,51). Although the latter has a greater lysine content, canola meal contains more of the sulfur-containing amino acids (methionine and cystine). Consequently, the two meals tend to complement each other when used as feedstuffs in rations for livestock and poultry. Canola meal is a richer source of minerals than soybean meal (50,51) (Table 3). However, the presence of higher amounts of phytic acid and fiber in canola meal reduces the availability of calcium, magnesium, manganese, phosphorus, zinc, and copper for nonruminant animals (viz., poultry and swine) (51,52). Nevertheless, canola meal is a better source of available calcium, iron, manganese, phosphorus, selenium, and most vitamins than is soybean meal. Canola meal has been shown to have less metabolizable energy than soybean meal (50); on a nutrient content basis alone, canola meal is equivalent to 70 to 75% of 44% protein-content soybean meal for feeding poultry and to about 75 to 80% of the same for feeding swine and ruminants. A reduction in fiber content of the meal would improve the metabolizable energy value. Seeds with yellow color have a lower hull content and, in turn, fiber content than dark seeds, and the hull is more digestible (50). Nonetheless, the available energy in canola meal is a critical factor in its use in animal feeds.



*Cholesterol content (mg/tbsp): lard 12; beef tallow 14; butterfat 33. No cholesterol in any vegetable-based oil.
Source for data: POS Pilot Plant Corporation, Saskatoon, Saskatchewan, Canada June 1994.

Figure 4. Fatty acid composition of common dietary fats. *Source:* Canola Council of Canada.

Table 2. Proximate Analysis and Amino Acid Composition of Canola and Soybean Meals

	Canola meal		Soybean meal	
	As fed, %	In protein, %	As fed, %	In protein, %
	Proximate composition			
Moisture	8.0		11.0	
Crude fiber	12.6		7.3	
Ether extract	3.4		0.8	
Protein (N × 6.25)	39.0		45.2	
	Amino acid composition			
Alanine	1.73	4.65	1.95	4.31
Arginine	2.32	6.15	3.03	6.71
Aspartic acid	3.03	8.16	5.27	11.66
Cystine	1.05	2.80	0.71	1.61
Glutamic acid	6.46	17.38	8.43	18.65
Glycine	1.92	5.18	2.06	4.55
Histidine	1.26	3.34	1.12	2.48
Isoleucine	1.66	4.38	1.82	4.03
Leucine	2.68	7.07	3.36	7.44
Lysine	2.21	5.95	2.82	6.24
Methionine	0.76	2.03	0.70	1.59
Phenylalanine	1.52	4.04	2.13	4.72
Proline	2.48	6.67	2.27	5.03
Serine	1.69	4.55	2.38	5.27
Threonine	1.68	4.52	1.74	3.85
Tryptophan	0.44	1.19	0.52	1.15
Tyrosine	1.14	3.02	1.33	2.95
Valine	2.14	5.68	1.89	4.18

Source: Refs. 50 and 51.

PROCESSING

Processing of edible oils, including canola oil, is designed to produce the purest extract of triacylglycerols possible. This necessitates removing minor components, naturally present in the oil, that are detrimental to the quality of the finished product (52).

Cleaning Seeds

Before processing, canola seeds undergo cleaning to reduce the presence of any foreign materials. This material, referred to as dockage, was shown to consist mainly of damaged canola seed together with weed seeds (53). A number of operations are involved, including fanning and sieving mills to remove pods and weed seeds; indent machines to remove any large noncanola seeds; destoners to remove dirt and small stones; and finally gravity tables to eliminate anything not removed in the previous processes. Removal of foreign material is designed to ensure less than 2.5% remains in the seed. The presence of damaged canola seed has been shown to be detrimental to the quality of the extracted oil and should be reduced as much as possible before oil extraction (54).

Oil Extraction

Extracting oil from canola seeds is carried out with as little chemical alteration to the oil or meal as possible (55). Once cleaned, canola seeds are rolled or flaked to fracture the seed coat and rupture the oil cells. The production of thin flakes, 0.2- to 0.3-mm thick, is extremely important as a high surface-to-volume ratio is critical during oilseed processing. To facilitate good oil release, the flakes are cooked at 77 to 100°C for 15 to 20 min, to rupture any intact oil cells remaining (56). Flaked and cooked canola seeds gen-

Table 3. Mineral and Vitamin Content of Canola and Soybean Meals

	Canola meals as fed	Soybean meal as fed
Minerals		
Calcium, %	0.66	0.29
Magnesium, %	0.58	0.27
Phosphorus, %	1.10	0.65
Potassium, %	1.29	2.00
Copper, mg/kg	5.8	23.0
Iron, mg/kg	159	120
Manganese, mg/kg	52	30
Selenium, mg/kg	1.0	0.1
Zinc, mg/kg	70	52
Vitamins		
Choline, %	0.67	0.28
Biotin, mg/kg	1.0	0.32
Folic acid, mg/kg	2.3	0.6
Niacin, mg/kg	160	29
Pantothenic acid, mg/kg	5.9	16 0
Riboflavin, mg/kg	5.8	2.9
Thiamin, mg/kg	5.2	6.0

Source: Refs. 50 and 51.

erally undergo mild pressing or prepress to reduce oil content from 42 to 16–20%, while compressing the thin flakes into large cake fragments. Canola cake fragments are solvent extracted with normal hexane to remove the remaining oil. This is achieved by countercurrent movement of the cells of pressed canola cake and hexane, thus interfacing the oil in the flake or cake with a rich solvent-oil solution (57). The solvent is recovered from the oil-hexane solution by the conventional distillation system that ensures the oil and meal are solvent free.

The solvent-extracted oil (43–47% of the total oil) is combined with the prepressed oil (53–56% of the total oil) to form the crude oil fraction, as outlined in Figure 5. The crude oil contains a variety of minor constituents (Table 4) detrimental to oil quality that are removed by a series of unit processing steps including degumming, alkali refining, bleaching, hydrogenation, and deodorization.

Degumming

Conventional degumming is carried out in most plants by treating the crude oil with either hot water (85–90°C) or steam (2–5%) while mixing intensively from 1 to 30 min (59). This precipitates the water-hydrated phospholipids, which are then removed by centrifugation. The major drawback to this type of degumming process is that it only removes hydratable phospholipids and still leaves 100 to 200 ppm of phosphorus (0.25–0.5% phosphatides) in the oil. To achieve better results, most Canadian processing plants now carry out acid degumming using citric, phosphoric, or malic acid. This is followed by steam, which results in an oil with phosphorus levels of 50 ppm or less. This product is referred to as acid degummed or super degummed oil.

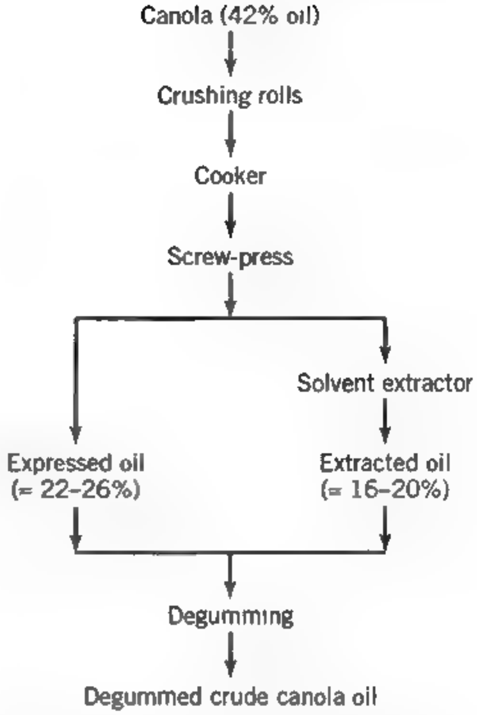


Figure 5. Outline of the primary processing of canola.

Table 4. Minor Constituents in Crude, Degummed Canola Oil

Constituents	Amount
Free fatty acids, %	0.5–0.8
Phospholipids (gums), %	0.5–0.8
Unsaponifiables (sterols and sterol esters), %	0.1–0.8
Color bodies, ppm	20–30
Sulfur compounds, ppm	2–10
Iron, ppm	2
Copper, ppm	5

Source Ref. 58.

Refining

The crude degummed oil is then subjected to refining, which removes free fatty acids, phospholipids, color bodies, iron and copper, as well as some sulfur compounds. The major type of refining in Canada is alkali refining, although there is a shift towards physical refining due to the fewer environmental problems associated with the latter process.

Five major steps are involved in alkali refining.

1. Initial pretreatment of the crude degummed oil with phosphoric acid (0.2–0.5% of 85% phosphoric acid) at 40°C for a minimum of 30 min. This conditions or acidifies the nonhydratable phospholipids (gums) and permits their separation during the refining process. In addition to precipitating nonhydratable phosphatides, chlorophyll as well as pro-oxidant

metals such as iron and copper also are removed (60,61).

2. The second and major step in alkali refining, neutralization with sodium hydroxide (8–12%), depends on the free fatty acid content of the oil and the amount of phosphoric acid used during pretreatment. A slight excess is normally added to ensure complete saponification of these fatty acids.
3. The addition of sodium hydroxide in step 2 produces a soap-stock phase that contains precipitated nonhydratable phospholipids as well as free fatty acids and other insoluble impurities. This soap stock is separated from the oil by centrifugation.
4. Any residual soap stock in the oil is further reduced to 50 ppm or less by washing with water at 86 to 95°C. Citric or phosphoric acid may be added to the washed oil to remove remaining traces of soap stock.
5. During this stage the oil is heated at 105°C with agitation until dry.

Most oil processors use a continuous alkali refining process, although batch refining is still used by some processors. The overall result of alkali refining is a marked reduction in free fatty acids, phosphorus, chlorophyll, and sulfur, as summarized in Table 5.

An alternative method to alkali refining is physical refining, which removes free fatty acids from canola oil by steam distillation. This avoids the production of a soap stock and attendant disposal problems. The oil is first acid degummed with citric or phosphoric acid and then bleached to remove phosphatides and trace metals. This method reduces the phosphorus content to less than 5 ppm, as higher levels cause a darkening of the oil during steam distillation. The latter process (distillative deacidification) is carried out in a specially designed deodorizer. An alternative method to alkali refining is physical or steam refining. This method is used widely in Europe and has been only recently introduced into North America.

Bleaching

Before hydrogenation or deodorization, canola oil is bleached with acid-activated bleaching clay (at 0.125–2.0%) under vacuum at 100 to 125°C for 15 to 30 min. This is an adsorptive process attracting polar substances to active sites of clay surface particles. These include pigments such as chlorophylls and carotenes, residual soaps, phospholipids, and trace metals, as well as primary and secondary oxidation products in the oil. Of particular importance is the presence of very high levels of chlorophylls, which are extremely detrimental to oil quality and must be removed before deodorization. Unlike carotenes, which are heat bleachable in the deodorizer, chlorophylls are not and must be removed by bleaching (63). Most modern oil-processing plants use a continuous bleaching system.

Table 5. Effect of Alkali Refining on the Quality of Canola Oil

Constituents	Crude degummed	Alkali refined
Free fatty acids, %	0.4–1.0	0.05
Phosphorus, ppm	150–250	0–5
Chlorophyll, ppm	5–25	0–25
Sulfur, ppm	3–10	2–7

Source: Ref. 62.

Hydrogenation

Canola oil, like other edible oils, is hydrogenated to improve oxidative stability and to modify the physical properties of the fat or oil. The basic principle of this method is to add hydrogen at the double bonds of the polyunsaturated fatty acids (those containing two or more double bonds), thus eliminating these sites from reaction with oxygen. Only a small portion of these fatty acids may be affected in partially hydrogenated oil stocks, depending on the type of hydrogenation system used. This process requires a catalyst (usually nickel), which may be poisoned by the presence of low levels of sulfur compounds (3–5 ppm) originating from breakdown products of glucosinolates (64). Once hydrogenated, the oil becomes physically harder and more resistant to oxidation. In Canada, canola oil is selectively hydrogenated for the production of margarines and shortenings.

Selective hydrogenation of canola oil is carried under conditions of high temperature, low pressure (200°C, 6 psig), and agitation and affects the more unsaturated fatty acids first. This is illustrated by the fatty acid profiles during the course of hydrogenation of soybean oil shown in Figure 6. All the unsaturated fatty acids are hydrogenated at the same time but at different rates, as indicated by the reaction rate constants. Linolenic acid is hydrogenated 2.3 times faster than linoleic acid, where the latter is hydrogenated 12 times faster than oleic acid (65). This method results in the formation of higher levels of *trans* fatty acids, steeper solid fat index curves, and higher melting points at lower iodine values. The formation of *trans* fatty acids changes the physical properties of the oil as a *trans* double bond is equivalent in physical properties to a saturated

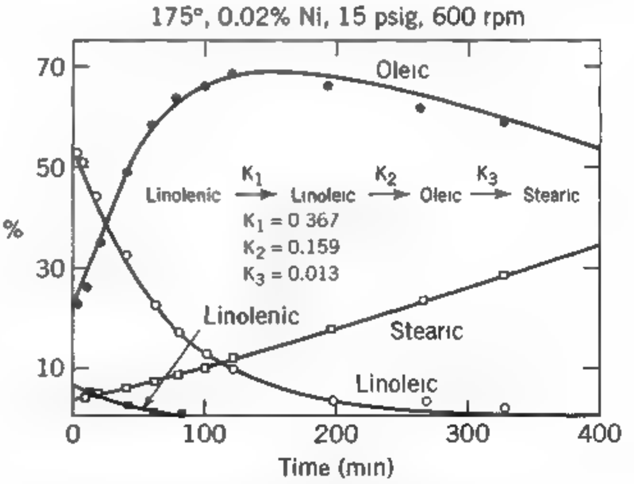


Figure 6. Time-dependent changes in fatty acid composition during hydrogenation of soybean oil. *Source:* Ref. 65.

single bond fatty acid; for example, *cis, trans*-linoleyic acid is equivalent in physical properties to oleic acid. Hardness of margarine oils is due to the higher levels of *trans* fatty acids present rather than to a marked decrease in unsaturation of the fatty acids (66). Because different degrees of hydrogenation are often required by the industry, hydrogenation is a batch process.

Deodorization

Free fatty acids or odiferous or flavor degradation products remaining in the oil are removed by deodorization. This involves steam distillation carried out at very high temperatures (240–270°C) under vacuum (3–8 mmHg). Most plants use a semicontinuous or continuous deodorizing system that is comprised of a large cylindrical tank or shell through which oil is pumped in and passed through a series of trays where it is deaerated and successively deodorized with sparging steam. In the case of canola oil, it must be essentially free of chlorophylls, phosphatidic material, and heavy metals prior to deodorization. Following deodorization, the oil is cooled, passed through a polishing filter, and sparged with nitrogen. The final product is a bland oil that is treated with citric acid (0.005–0.01%) to sequester any trace metals still remaining and other antioxidants to prevent oxidation.

EDIBLE BY-PRODUCTS

Two major edible by-products obtained by processing canola seeds are the oil and the meal. Although canola oil remains the primary edible product, the meal provides an important source of animal feed.

Canola Oil

The major salad oil in Canada is canola, which accounts for more than 80% of the total market. It remains clear and liquid under refrigerated conditions and has acceptable shelf life if protected from direct light. In recent years canola oil has been recognized in the United States as a premium quality oil because of the health implications afforded by its unique fatty acid composition (6,9). This is attributed to the low saturated fatty acid content, the hypocholesterolemic effect of oleic acid (C18:1n-9), a monounsaturated fatty acid present at high levels (ca 60%) in canola oil (Fig. 4), and the favorable balance between linoleic acid and linolenic acid. Oleic acid has been shown equivalent to polyunsaturated fatty acids in lowering low-density lipoprotein (LDL) cholesterol levels (67,68). Studies in Canada (69,70), the United States (71), and Finland (72) have shown canola equally as effective as sunflower oil, soybean oil, and safflower oil in lowering plasma total and LDL cholesterol levels. Because of its low level of saturated fatty acids (<6%) and its relatively high oleic acid content, canola oil has become a premium salad oil in North America. Canola oil, because of its relatively high linolenic acid content and the favorable linolenic acid to linoleic acid balance (viz., C18:3/C18:2 approx. 1:2.5), also may have a favorable effect on thrombosis (clot formation); like atherosclerosis, another major event in coronary heart disease (6,9). Several groups have found canola oil resulted in higher levels of very long chain n-3 polyunsaturated fatty acids (viz., C20:5n-3 and C22:5n-3) in plasma and platelet phospholipids than sunflower oil, soybean oil, or customary mixed fat diets (6,9). The very long chain n-3 polyunsaturated fatty acids in fish oils have been shown to inhibit platelet aggregation (73).

The stability of canola oil is comparable to other edible oils, although its high linolenic acid content (8–10%), like that of soybean oil, makes it susceptible to oxidative rancidity (9). Among eight different vegetable oils stored at 60°C for 8 to 16 days, canola oil had lower peroxide values and volatiles than corn, cottonseed, olive, peanut, safflower, soybean, and sunflower oils (74). A recent study showed a low linolenic acid canola oil product (<2.0%) to be remarkably stable to both heat-accelerated and photochemical oxidation (36,75).

In addition to use as a salad oil, canola oil is used in the production of margarines and shortenings. In North America, margarine is a strictly defined alternative to butter with 80% fat. Although some margarines are made from 100% canola oil, it is generally blended with soybean oil or palm oil. This is carried out to ensure the development of small crystals (β') essential for smooth creaming properties. The unacceptable sandy mouth-feel (due to large fat crystals) for margarines originates from $\beta' \rightarrow \beta$ polymorphic transitions; one way to alleviate this problem is by increasing the heterogeneity of acyl chain lengths of the constituent fatty acids in the oil. Canola oil is selectively hydrogenated and the fat and aqueous phases are mixed together in a crystallizer system or votator to form an emulsion. A variety of different soft and stick margarines are available on the North American market. Shortenings are made in a similar manner to margarines, although in this case, the fat is aerated with air or nitrogen (12%) to improve whiteness and opacity.

High Erucic Acid Oil

As discussed earlier, rapeseed oil has been systematically modified by plant breeders from high erucic acid rapeseed (HEAR) (60%) to a low erucic acid oil (<2%). Nevertheless, HEAR is still permitted in the United States as a stabilizer and thickener component in peanut butter at a maximum level of 2% (76). Superglycerinated, 100% hydrogenated HEAR has been allowed in cake-mix formulations since 1957 in the United States as an emulsifier in the shortening (76).

Canola Meal

After extraction of the oil and removal of hexane, the meal contains approximately 1.5% residual oil and 8 to 10% moisture. It may be granulated to uniform consistency and pelletized or sent directly to storage for marketing (77). Gums removed during degumming are usually added back to the meal to a level of 1.5%; some of the acidulated soap stock is added back as well. The final meal produced is used primarily for the animal feed industry. Canola meal has found much wider acceptance in North America over the past 20 years due primarily to the substantial reduction in glucosinolates accomplished by plant breeding.

Canola meal has, as yet, not found wide acceptance in human nutrition despite the high quality of its protein. This is due to a combination of high levels of phytate and polyphenols as well as the presence of some glucosinolates. A dual solvent process has successfully removed all glucosinolates as well as phytate and hulls (17); the resulting meal was reported to be bland, free-flowing, free from glucosinolates, low in polyphenols, and light in color, with a protein content of 50%. Protein isolates containing 90% protein produced from this meal could have considerable potential for use in human foods.

NONEDIBLE BY-PRODUCTS

The majority of rapeseed currently grown in Canada is canola, although there still remains some acreage devoted to HEAR. This is due to the industrial applications of erucic acid and its cleavage products.

Canola Oil

Canola oil–based printing ink has been found to be superior to petroleum-based ink by reducing the rub problem. Switching to canola-based ink also reduces environmental problems, as 75% of the ink is biodegradable (78). The superior rub properties of canola oil permit both black and color inks to be made with this oil. A number of major Canadian newspapers are now printed with canola oil–based ink.

High Erucic Acid Oil

Erucic acid, a long chain fatty acid ($C_{22:1}$) containing a single double bond, exhibits high fire and smoke points (271°C). These properties allow erucic acid to withstand high temperatures and still remain a liquid at room temperature (79). Consequently, oils containing high levels of erucic acid are used as lubricants or in lubricant formulations (80). Because of their lubricant properties, HEAR oils have found applications as spinning lubricants in the textile, steel, and shipping industries, as drilling oils, and as marine lubricants (81). High erucic acid oil has also been used for the clinical treatment of adrenoleukodistrophy, a rare children's disease. Hydrogenated HEAR oils have industrial applications by producing hard, glossy waxes (82).

Erucic Acid Derivatives

Erucic acid and its hydrogenated derivative, behenic acid, and erucyl and behenyl alcohols as well as other derivatives including esters, amides, amines, and their salts are used extensively in industry as slip, softening, antifoaming, and release agents; emulsifiers; processing aids; conditioners; antistatic agents; stabilizers; and corrosion inhibitors (80).

$$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$$

erucic acid

$$CH_3(CH_2)_7CH_2CH_2(CH_2)_{11}COOH$$

behenic acid

Erucamide, the amide derivative of erucic acid, has been used for many years as a processing aid and antiblock agent in plastic films. It facilitates the production of plastic parts by acting as a lubricant and forming a thin layer on the surface of the plastic, thus preventing the sheets from sticking to each other.

$$CH_3(CH_2)CH{=}CH(CH_2)_{11}CONH_2$$

erucamide

Behenic acid and its esters are used to enhance the performance of a wide range of pharmaceutical, cosmetic, fabric softener, and hair conditioner products. As antifriction coatings they soften and improve the texture and sewability of cotton and synthetic fabrics (80). In addition to the compounds discussed, there are a large number of HEAR oil-derived alcohols and esters that are used in the cosmetic and pharmaceutical industries.

Erucic acid cleavage products have considerable potential in the production of plastics, resins, and nylons. For example, the oxidative cleavage of erucic acid yields brassylic acid, a 13-carbon dicarboxylic acid, and pelargonic acid, a 9-carbon monocarboxylic acid. A number of long chain nylons prepared from brassylic acid are important in automotive parts and products (79, 83,84).

$$CH_3(CH_2)_6CH{=}CH(CH_2)_{11}COOH$$

oxidative cleavage

$$\rightarrow \underset{\text{pelargonic acid}}{CH_3(CH_2)_7COOH} + \underset{\text{brassylic acid}}{HOOC(CH_2)_{11}COOH}$$

Alkyl esters of brassylic acid are excellent plasticizers. The corresponding allyl and vinyl esters form polymers and copolymers that may be used in molding compounds, reinforced plastics, laminates, sealants, and coatings (85–88).

STANDARDS AND SAFETY

The standards for crude and refined, bleached, deodorized canola oil, as outlined in Table 6, were established in 1987 by the Canadian General Standard Board (CGSB). Such standards are used in purchasing, consumer protection, health and safety, international trade, and regulatory reference (90). In addition, there is also the Canada Agricultural Products Standard Act, which defines the quality of products, including canola oil (Table 4), for regulating the grading, packing, and marketing of processed canola oil in Canada. These come under the jurisdiction of Agriculture Canada (90).

Canola oil has been approved by Health and Welfare Canada under the Food and Drugs Act and Regulations as safe for human consumption (91). The Canadian legislation favors consumption of canola oil over that of rapeseed oil by limiting the amount of C_{22} monoenoic fatty acids such as erucic acid ($C_{22:1}$) permitted in dietary fats. The three sections of this act, outlined in Table 7, refer to this particular restriction. For example, Section B.09.022 refers to a restriction of less than 5% C_{22} monoenoic fatty acids in fats and oils, in shortenings, salad and cooking oils, as well as table spreads. A similar restriction is cited in Section B.07.043 for salad dressings, while Section

Table 6. Canadian General Standards Board Requirements for Canola Oil

A. Crude canola oil

Characteristics	Super degummed	Degummed
Free fatty acids (as oleic acid), % by mass, maximum	1.0	1.0
Moisture and impurities, combined % by mass, maximum	0.3	0.3
Phosphorus, ppm, maximum	50	200
Chlorophyll, ppm, maximum	30	30
Sulfur, ppm, maximum	10	10
Refined, bleached color, Lovibond (133.4 mm cell), maximum	1.5 red	1.5 red
Erucic acid, % by mass, maximum	2.0	2.0

B. Refined, bleached, and deodorized canola oil

Characteristics	Minimum	Maximum
Free fatty acids (as oleic acid), % by mass	—	0.05
Moisture and impurities, combined % by mass	—	0.05
Lovibond color (133.4 mm cell)	—	1.5 red, 15 yellow
Peroxide value, mEq/kg	—	2.0
Cold test, h	12	—
Smoke, point, °C	232	—
Unsaponifiable matter, g/kg	—	15
Saponification value, milligrams potassium hydroxide per gram oil	182	193
Refractive index (n_D 40°C)	1.465	1.467
Iodine value (Wija)	110	126
Crismer value, °C	67	70
Relative density (20°C/water 20°C)	0.914	0.920
Erucic acid, % by mass	—	2.0

Source. Ref 89.

Table 7. Regulations in Canada's Food and Drugs Act Relating to Canola and Rapeseed Oils

B.09.001 (S)	Vegetable fats and oils shall be fats and oils obtained entirely from the botanical source after which they are named, shall be dry and sweet in flavour and odour and, with the exception of olive oil, may contain Class IV preservatives, an antifoaming agent, and β-carotene in a quantity sufficient to replace that lost during processing, if such addition is shown on the label.
B09.022	No person shall sell cooking oil, margarine, salad oil, simulated dairy products, shortening or food that resembles margarine or shortening, if the product contains more than 5 percent. C_{22} Monoenoic Fatty Acids calculated as a proportion of the total fatty acids contained in the product.
B07.043	No person shall sell a dressing that contains more than 5 percent C_{22} Monoenoic Fatty Acids calculated as a proportion of the total fatty acids contained in the dressing.
B25.054 (1)	Except as otherwise provided in this Division, no person shall sell or advertise for sale of human milk substitute unless it contains, when prepared according to directions for use. (a) per 100 available kilocalories (i) not less than 3.3 and not more than 6.0 grams of fat. (ii) not less than 500 milligrams of linoleic acid in the form of a glyceride. (iii) not more than 1 kilocalories from C_{22} Monoenoic Fatty Acids.

Source: Ref. 92.

B.25.054(a)(iii) limits C_{22} monoenoic acids in infant formula to a maximum of 1% of the calories. In Canada, only 2% erucic acid is now permitted in canola oil, which is much lower than the 5% indicated in the Codex standard for low erucic acid oils by Codex Alimentarious Commission FAO/WHO 1982 (89). Erucic acid oil with a maximum of 2% was subsequently granted GRAS (generally regarded as safe) status in the United States (1985) by the FDA (92). This oil, now identified as canola oil, has since been recognized by the American Health Foundation as a healthful product in the consumer market. A comprehensive review on the safety and health aspects of canola oil (93) concluded that, because of its fatty acid composition, canola oil can be a good substitute for saturated fat in diets intended to reduce the risk of hypercholesterolemic and coronary heart disease. Current nutritional recommendations by many health organizations suggest a reduction of the calories derived from fat in the diet to about 30% and no more than 10% of the caloric intake as saturated fat.

FUTURE OF CANOLA

The discovery of cytoplasmic male sterility (CMS) in *Brassica* spp. a decade ago led to considerable research in developing new hybrids. Because *B. napus* spp. of canola are mainly self-pollinated, the development of hybrids requires cross-pollination. The CMS system has been perfected and is now used for the production of commercial hybrids. Future research should result in improved hybrids for commercial production. The application of biotechnology (and genetic engineering) will assist in the development of improved canola lines that mature early, are frost resistant, are resistant to pesticides, are blackleg tolerant, as well as higher yielding. *B. napus* varieties require long days to flower, so future research will develop lines that mature earlier without any loss in yield (8,31,35,94,95). New high protein lines will also be developed for *B. napus* varieties, whereas higher yields and oil content and modified fatty acid composition are the long-term goals for *B. rapa* breeding research programs. Biotechnology should also result in new lines with cold temperature hardiness and frost resistance that can germinate at much lower soil temperatures and permit early seeding. As breeders and genetic engineers manage to tailor rapeseed/canola, segregating the crop on the basis of specific characteristics will evolve. Processors may develop contracts with farmers to plant varieties that provide products with the desired end-use properties. In addition to the breeding programs, modifications in canola processing technologies will be sought to improve the finished products while being environmentally safe.

BIBLIOGRAPHY

1. "Fats and Oils Industry Changes," *J. Am. Oil Chem. Soc.* **65**, 702–713 (1988).
2. J. Daun and D. Adolphe, "A Revision of the Canola Definition," *Bulletin of Groupe Consultatif International de Recherche sur la Colza* **14**, 134–141 (1997).
3. Canola Council of Canada, URL: *http://www.canola-council.org/Stats/seedsupplydemand.htm*
4. Canola Council of Canada, URL: *http://www.canola-council.org/Stats/oilmealsupplydemand.htm*.
5. Canola Council of Canada, URL: *http://www.canola-council.org/Stats/oilmealexports.htm*.
6. B. E. McDonald, "Oil Properties of Importance in Human Nutrition," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 291–299.
7. L. A. Appelqvist, "Historical Background," in L. A. Appelqvist and R. Ohlson, eds., *Rapeseed: Cultivation, Composition, Processing and Utilization*, Elsevier, Amsterdam, 1972, pp. 1–8.
8. D. S. Kimber and D. I. McGregor, "The Species and Their Origin, Cultivation and World Production," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp 1–7.
9. N. A. M. Eskin et al. "Canola Oil," in Y. H. Hui ed., *Bailey's Industrial Oil & Fat Products Edible Oil and Fat Products*, 5th ed., Vol. 2, *Edible Oil and Fat Products: Oils and Oil Seeds*, John Wiley & Sons, New York, 1995, pp. 1–95.
10. R. K. Downey and G. F. K. Rakow, "Rapeseed and Mustard," in W. R. Fehr, ed., *Principles of Cultivar Development*, Vol. 2, Macmillan, London, 1987, pp. 437–486.
11. R. K. Downey and G. Robbelen, "Brassica Species," in G. Robbelen, R. K. Downey, and A. Ashri, eds., *Oil Crops of the World: Their Breeding and Utilization*, McGraw-Hill, New York, 1989, pp. 339–362.
12. *Rapeseed-Canola*, Canola Council of Canada, Winnipeg, Canada, 1981, pp. 12–14.
13. F. D. Sauer and J. K. G. Kramer, "The Problem Associated with the Feeding of High Erucic Acid Rapeseed Oils and Some Fish Oils to Experimental Animals," in J. K. G. Kramer, F. D. Sauer, and W. J. Pigden, eds., *High and Low Erucic Acid Rapeseed Oils: Production, Usage, Chemistry and Toxicological Evaluation*, Academic Press, New York, 1983, pp. 253–292.
14. G. R. Fenwick, R. K. Heaney, and W. J. Mullin, "Glucosinolates and Their Breakdown Products in Food and Food Plants," *Crit. Rev. Food Sci. Nutr.* **18**, 123–201 (1983).
15. B. Uppstrom, "Seed Chemistry," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 217–242.
16. D. S. Hutcheson, "Development of Improved *B. campestris* Cultivars Essentially Free from Both Alkenyl and Indolyl Glucosinolates," *Canola Research Summary 1985–1989*, Canola Council of Canada, Winnipeg, Manitoba, 1989, p. 54.
17. L. L. Diosady, L. J. Rubin, C. R., Phillips, and M. Naczk, "Effect of Alkanol-Ammonia-Water Treatment on the Glucosinolate Content of Rapeseed Meal," *Can. Inst. Food Sci. Technol. J.* **18**, 311–315 (1985).
18. B. Loof, "Cultivation of Rapeseed," L. A. Appelqvist and R. Ohlson, eds., *Rapeseed: Cultivation, Composition, Processing and Utilization*, Elsevier, Amsterdam, 1972, pp. 49–59.
19. S. R. Rimmer and L. Buchwaldt, "Diseases," in D. S. Kimber and D. I. McGregor eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 111–140.
20. B. Ekbom, "Insect Pests," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 141–152.
21. J. Orson, "Weeds and Their Control," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 93–109.
22. A. Pouzet, "Agronomy," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 65–92
23. L. A. Appelqvist and B. Loof, "Postharvest Handling and Storage of Rapeseed," in L. A. Appelqvist and R. Ohlson, eds., *Rapeseed: Cultivation, Composition, Processing and Utilization*, Elsevier, Amsterdam, 1972, pp. 60–100.
24. *Guide to Farm Practice in Saskatchewan*, University of Saskatchewan, Saskatoon, 1987, pp. 60–61.
25. H. B. W. Patterson, *Handling and Storage of Oilseeds, Oils, Fats and Meal*, Elsevier Applied Science, London, 1989, pp. 133–140.
26. K. M. Clear and J. K. Daun, "Chlorophyll in Canola Seed and Oil," in *8th Progress Report on Canola Seed, Oil and Meal Fractions*, Canola Council of Canada, Winnipeg, Manitoba, 1987, pp. 277–279
27. K. Ward, et al., "Genotypic and Environmental Effects on Seed Chlorophyll Levels in Canola (*Brassica napus*)," in D. I. McGregor, ed., *Proceedings of the 8th International Rapeseed Congress*, Saskatoon, SK, 1991, pp. 1241–1245.
28. K. Ward et al., "Effects of Genotype and Environment on Seed Chlorophyll Degradation During Ripening in Four Cultivars of Oilseed Rape (*Brassica napus*)," *Can. J. Plant Sci.* **72**, 643–649 (1992).
29. S. Cenkowski, S. Sokhansanj, and F. W. Sosulski, "The Effect of Drying Temperature on Green Color and Chlorophyll Content of Canola Seeds," *Can. Inst. Food Sci. Technol. J.* **22**, 383–386 (1989).
30. B. R. Stefansson, "The Development of Improved Rapeseed Cultivars," *Bulletin of Groupe Consultatif International de Recherche sur la Colza* **14**, 143–159 (1997).
31. G. C. Buzza, "Plant Breeding," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseeds: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 153–175.
32. L. Bengtsson, A. Von Hofsten, and B. Loof, "Botany of Rapeseed," in L. A. Appelqvist and R. Ohlson, eds., *Rapeseed: Cul-*

tivation, Composition, Processing and Utilization, Elsevier, Amsterdam, 1972, pp. 36–48.

33. C. H. Foyer and W. P. Quick, *A Molecular Approach to Primary Metabolism in Higher Plants*, Taylor and Francis, London, 1997, pp. 311–326
34. D. C. Taylor et al., "Stereospecific Analysis of Seed Triacylglycerols from High-Erucic Acid Brassicaceae: Detection of Erucic Acid at the *sn*-2 Position in *Brassica oleracea* L. Genotype," *J. Am. Oil Chem. Soc.* **71**, 163–167 (1994).
35. R. Scarth, et al., "Stellar Low Linolenic-High Linoleic Acid Summer Rape," *Can. J. Plant Sci.* **68**, 509–511 (1988).
36. N. A. M. Eskin et al., "Stability of Low Linolenic Acid Canola Oil to Frying Temperature," *J. Am. Oil Chem. Soc.* **66**, 1081–1084 (1989).
37. A. Prevot et al., "A New Variety of Low-Linolenic Rapeseed Oil: Characteristics and Room-Odor Tests," *J. Am. Oil Chem. Soc.* **67**, 161–164 (1990).
38. Anonymous, "Calgene Seeks Approval for Modified Canola," *INFORM* **5**, 716 (1994).
39. D. J. Murphy and R. Mithen, "Biotechnology," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseed: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 177–193.
40. D. S. Knutzon et al., "Modification of Brassica Seed Oil by Antisense Expression of a Stearoyl-Acyl Carrier Protein Desaturase Gene," *Proc. Natl. Acad. Sci.* **89**, 2624–2628 (1992).
41. B. E. McDonald and K. Fitzpatrick, "Designer Vegetable Oils," in G. Mazza, ed. *Functional Foods: Biochemical and Processing Aspects*, Technomic Publishing, Lancaster, Pa., U.S.A., 1998.
42. R. K. Downey, "It's All in the Breeding," *Proceedings of the 19th Annual Convention of the Canola Council of Canada Meeting*, San Francisco, Calif., March 24–26, 1986, pp. 25–36.
43. R. K. Downey, "The Origin and Description of the Brassica Oilseed Crops," in J. K. G. Kramer, F. D. Sauer, and W. J. Pigden, eds., *High and Low Erucic Acid Rapeseed Oils: Production, Usage, Chemistry and Toxicological Evaluation*, Academic Press, New York, 1983, pp. 1–20.
44. K. F. Thompson and W. G. Hughes, "Breeding and Varieties," in D. H. Scarisbrick and R. W. Daniels, eds., *Oilseed Rape*, Collins, London, 1986, pp. 32–82.
45. W. A. Keller, "The Application of Biotechnology to Canola Improvement," *Crit. Rev. Food Sci. Nutr.* **18**, 56 (1983).
46. Anonymous, "Breeding Canola for Success," *J. Am. Oil Chem. Soc.* **65**, 1567–1568 (1988).
47. Canola Council of Canada, URL: *http://www.canola-council.org/board/biotech/31.html* (last accessed Feb. 17, 1998).
48. A. Khalyfa, S. Kermasha, and I. Alli, "Partial Purification and Characterization of Lipoxygenase of Canola Seed (*Brassica napus* var. Westar)," *J. Agric. Food Chem.* **38**, 2003–2008 (1990)
49. R. G. Ackman, "Chemical Composition of Rapeseed Oil," Bulletin of *Groupe Consultatif International de Recherche sur la Colza* **14**, 85–129 (1997).
50. D. R. Clandinin et al., "Composition of Canola Meal," in R. Salmon and D. R. Clandinin, eds., *Canola Meal for Livestock and Poultry*, Canola Council of Canada, Winnipeg, Manitoba, 1989, pp. 5–7.
51. J. M. Bell, "Meal and By-product Utilization in Animal Nutrition," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseed: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 301–337.
52. N. A. M. Eskin and R. Bacchus, "Processing of Canola Oil," in M. Vaisey-Genser and N. A. M. Eskin, eds., *Canola Oil: Properties and Performance*, Canola Council of Canada, Winnipeg, Canada, 1987.
53. F. W. Hougen, J. K. Daun, and D. C. Durnin. "The Composition and Quality of Canola Dockage," in D. S. Kimber and D. I. McGregor, eds., *Brassica Oilseed: Production and Utilization*, CAB International, Wallingford, UK, 1995, pp. 287–297
54. F. Ismail, N. A. M. Eskin, and M. Vaisey-Genser, "The Effect of Dockage on the Stability of Canola Oil," in *6th Progress Report on Canola Seed, Oil, Meal and Meal Fractions*, Canola Council of Canada, Winnipeg, Manitoba, 1980, pp. 234–239.
55. R. Simmons, "The Effect of Rapeseed Quality on Processing Procedures," in *Proceedings of the International Conference on Science, Technology and Marketing of Rapeseed and Rapeseed Products*, Rapeseed Association of Canada, St. Adele, Quebec, 1970, p. 121–126.
56. D. L. Beach, "Primary Processing of Vegetable Oils," in J. T. Harapiak, ed., *Oilseed and Pulse Crops in Western Canada*, Western Cooperative Fertilizers Ltd., Calgary, Alberta, 1975, pp. 541–550.
57. D. L. Beach, "Rapeseed Crushing and Extraction," *Bulletin of Groupe Consultatif International de Recherche sur la Colza* **14**, 181–195 (1997).
58. B. F. Teasedale, "Processing of Vegetable Oils," in D. H. Scarisbrick and R. W. Daniels, eds., *Oilseed Rape*, Collins, London, 1986, pp. 551–585.
59. T. K. Mag, "Canola Processing in Canada," *J. Am. Oil Chem. Soc.* **60**, 332A–336A (1983).
60. R. Ohlson and C. Scensson, "Comparison of Oxalix Acid and Phosphoric Acid as Degumming Agents for Vegetable Oils," *J. Am. Oil Chem. Soc.* **53**, 8–11 (1976).
61. G. R. List et al., "Steam-refined Soybean Oil 1. Effect of Refining and Degumming Methods on Oil Quality," *J. Am. Oil Chem. Soc.* **55**, 277–279 (1978).
62. B. F. Teasedale and T. K. Mag, "The Commercial Processing of Low and High Erucic Acid Rapeseed Oils," *Bulletin of Groupe Consultatif International de Recherche sur la Colza* **14**, 199–228 (1997).
63. H. Niewiadomski, "Progress in the Technology of Rapeseed Oil for Edible Purposes," *Chem. Ind.*, 883–888 (1970).
64. J. K. Daun and F. W. Hougen, "Sulfur Content of Rapeseed Oils," *J. Am. Oil Chem. Soc.* **53**, 169–171 (1976).
65. R. R. Allen, "Hydrogenation," *J. Am. Oil Chem. Soc.* **58**, 166–169 (1981).
66. T. J. Weiss, *Foods, Oils and Their Uses*, AVI Publishing, Westport, Conn., 1970.
67. R. S. Mattson and S. M. Grundy, "Comparison of Effects of Dietary Saturated, Monounsaturated and Polyunsaturated Fatty Acids on Plasma Lipids and Lipoproteins in Man," *J. Lipid Res.* **26**, 194–202 (1985).
68. R. P. Mensink and M. B. Katan, "Effect of a Diet Enriched with Monounsaturated or Polyunsaturated Fatty Acids on Level of Low-Density Lipoprotein Cholesterol in Healthy Women and Men," *N. Engl. J. Med.* **321**, 436–441 (1989).
69. B. E. McDonald et al., "Comparison of the Effect of Canola Oil and Sunflower Oil on Plasma Lipids and Lipoproteins and on in vivo Thromboxane A_2 and Prostacyclin Production in Healthy Young Men," *Am. J. Clin. Nutr.* **50**, 1382–1388 (1989).
70. J. K. Chan, V. M. Bruce, and B. E. McDonald, "Dietary α-Linolenic Acid Is as Effective as Oleic Acid and Linoleic Acid in Lowering Blood Cholesterol in Normolipidemic Men," *Am. J. Clin. Nutr.* **53**, 1230–1240 (1991).
71. G. M. Wardlaw et al., "Serum Lipid and Apolipoprotein Concentration in Healthy Men on Diets Enriched in Either Canola Oil or Safflower Oil," *Am. J. Clin. Nutr.* **54**, 104–110 (1991).

72. L. M. Valsta et al., "Effects of a Monounsaturated Rapeseed Oil and a Polyunsaturated Sunflower Oil on Lipoprotein Levels in Humans," *Arter. Thromb.* **12**, 50–57 (1992).
73. P. M. Herold and J. E. Kinsella, "Fish Oil Consumption and Decreased Risk of Cardiovascular Disease: a Comparison of Findings from Animal and Human Feeding Trials," *Am. J. Clin. Nutr.* **43**, 556–598 (1986).
74. J. M. Snyder, E. N. Frankel, and E. Selke, "Capillary Gas Chromatographic Analyses of Headspace Volatiles from Vegetable Oils," *J. Am. Oil Chem. Soc.* **62**, 1675–1679 (1985).
75. R. Przybylski et al., Stability of low linolenic acid canola oil to accelerated storage at 60°C, *Lebensm.-Wiss. Technol.* **26**, 205–209 (1993).
76. Federal Register, **42**, 44335–48336 (1977).
77. M. D. Pickard et al., "Processing of Canola Seed for Quality Meal," in R. Salman and D. R. Clandinin, eds., *Canola Meal for Livestock and Poultry*, Canola Council of Canada, Winnipeg, Canada, 1989, pp. 3–4.
78. Anonymous, "What's Black and White and Read All Over?" *Canola Digest* **22**, 1 (1988).
79. H. J. Nieschlag et al., "Synthetic Wax Esters and Diesters from Crambe and Limnanthes Seed Oils," *Ind. Eng. Chem. Prod. Res. Dev.* **16**, 202–207 (1977).
80. D. L. van Dyne, M. G. Blase, and K. D. Carlson, *Industrial Feedstocks and Products from High Erucic Acid Oil: Crambe and Industrial Rapeseed*, University of Missouri, Columbia, 1990.
81. H. J. Nieschlag and I. A. Wolff, "Industrial Uses of High Erucic Oils," *J. Am. Oil Chem. Soc.* **41**, 1–5 (1971).
82. T. K. Miwa and I. A. Wolff, "Fatty Acids, Fatty Alcohols, Wax Esters and Methyl Esters from *Crambe abyssinica* and *Lunaria annua* Seed Oils," *J. Am. Oil Chem. Soc.* **40**, 742–744 (1963).
83. J. L. Greene, Jr. R. Perkins, Jr., and I. A. Wolff, "Aminotridecanoic Acid from Erucic Acid," *Ind. Eng. Chem. Prod. Res. Dev.* **8**, 171–176 (1969).
84. R. B. Perkins, Jr. et al., "Nylons from Vegetable Oils: − 13, − 13/13 and − 6/13," *Mod. Plastics* **46**, 136–139 (1969).
85. S. P. Chang, T. K. Miwa, and I. A. Wolff, "Alkyl Vinyl Esters of Brassylic Acid," *J. Polym. Sci.*, Part A-1, **5**, 2457–2556 (1967).
86. S. P. Chang, T. K. Miwa, and W. H Tallent, "Allylic Prepolymers from Bassylic and Azelaic Acids," *J. Appl. Polym. Sci.* **18**, 319–334 (1974).
87. H. J. Nieschlag et al., "Brassylic Acid Esters as Plasticizers for Polyvinylchloride," *Ind. Eng. Chem. Prod. Res. Dev.* **3**, 146–149 (1964).
88. H. J. Nieschlag et al., "Diester Plasticizers from Mixed Crambe Dibasic Acids," *Ind. Eng. Chem. Prod. Res. Dev.* **6**, 201–204 (1967).
89. FAO/WHO, *Codex Standards for Edible Low Erucic Acid Rapeseed Oil* Codex Standard 123-1981. Codex Alimentarious Commission, Joint Food and Agriculture Organization/World Health Organization Food Standards Programme, April 1982.
90. M. Vaisey-Genser, "Regulations Related to Canola Oil," in M. Vaisey-Genser and N. A. M. Eskin, eds., *Canola Oil: Properties and Performance*, Canola Council of Canada, Winnipeg, Canada, 1987, pp. 10–15.
91. Health and Welfare Canada, *The Food and Drugs Act and Regulations*, Supply and Services Canada, Ottawa, 1953, amended to September 1986.
92. Food and Drug Administration, *Direct Food Substances Affirmed as Generally Regarded as Safe; Low Erucic Acid Rapeseed Oil*. Federal Food, Drug and Cosmetic Acit 21 CRF Part 184, 1555 (c), amended 9 Jan. Department of Health and Human Services, Food and Drug Administration, Washington, D.C., 1985.
93. J. Dupont et al., "Food Safety and Health Effects of Canola Oil," *J. Am. College Nutr.* **8**, 360–375 (1989).
94. Anonymous, "Contiseed Banks on Hybrids," *Canola Digest* **22**, 8 (1988).
95. Anonymous, "Improving Oil/Protein Important at U. of A.," *Canola Digest* **22**, 4 (1988).

GENERAL REFERENCES

References 9, 25, 78, and 89 are also good general references.

L. A. Appelqvist and R. Ohlson, *Rapeseed Cultivation, Composition, Processing and Utilization*, Elsevier, Amsterdam, 1972.

D. S. Kimber and D. I. McGregor, *Brassica Oilseeds: Production and Utilization*, CAB, International, Wellingford, UK, 1995.

J. K. G. Kramer, F. D. Sauer, and W. J. Pigden, eds., *High and Low Erucic Rapeseed Oils—Production, Usage, Chemistry and Toxicological Evaluation*, Academic Press, New York, 1983.

G. Robbelen, R. K. Downey, and A. Ashri, *Oil Crops of the World*, McGraw-Hill, New York, 1989.

R. E. Salmon and D. R. Clandinin, *Canola Meal for Livestock and Poultry*, Canola Council of Canada, Winnipeg, Manitoba.

D. H. Scarisbrick and R. W. Daniels, *Oilseed Rape*, Collins, London, 1986.

D. Swern, *Bailey's Industrial Oil and Fat Products*, 4th ed., John Wiley & Sons, New York, 1985.

M. Vaisey-Genser and N. A. M. Eskin, *Canola Oil: Properties and Performance*, Canola Council of Canada, Winnipeg, Manitoba 1987.

N. A. M. ESKIN
B. E. MCDONALD
University of Manitoba
Winnipeg, Manitoba, Canada

CARAMEL. See COLORANTS: CARAMEL.

CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, AND LABELING

Carbohydrates can be, and are, classified in several different ways. They are often classified according to functionality in the minds of food scientists, for example, as sweeteners, nonsweet (or only slightly sweet) bulking agents, texturizing materials, suspending agents, and so on. While tables generally have not been constructed in this way, that is the approach taken later in this article. First the more traditional method will be presented. Both types of classification are included in Table 1.

MONOSACCHARIDES

Monosaccharides, often called simple sugars, are individual carbohydrate molecules that cannot be hydrolyzed to smaller, simpler carbohydrate units. The only ones found in food, beverage, and confectionery products to any appreciable extent are D-glucose and D-fructose. Most monosaccharides present in prepared food, beverage, and con-

Table 1. Major Carbohydrate Ingredients

Natural	Derived
Monosaccharides	
Glucose/dextrose[a]	Sorbitol[a]
Fructose[a]	Xylitol[a]
	Mannitol
	Glucono-δ-lactone (glucono-delta-lactone)
Oligosaccharides	
Sucrose[b]	Hydrogenated glucose syrup (maltitol syrup)
Lactose[c]	
Maltodextrins[a]	
Carbohydrate sweeteners	
Sugar[b]	
Glucose (corn) syrups[a]	
High-fructose (corn) syrups[a]	
Honey[a]	
Molasses[a]	
Sorbitol[a]	
Sugar products[b]	
Granulated sugar	
Coarse	
Extrafine	
Fine	
Baker's special, etc	
Brown sugar/soft sugar (light yellow → dark brown)	
Powdered sugar	
Fondant sugar	
Transformed sugar/agglomerated sugar	
Liquid sugar	
Molasses	
Polysaccharides	
Starches[d] (cookup, pregelatinized, and cold-water-swelling)	
Food gums[e]	
Dietary fiber	
Modified food starches[d]	
Crosslinked	cookup, pregelatinized, and cold water swelling
Stabilized	
Crosslinked and stabilized	
Dextrins	
Bulking agents	
Starches[c] and modified food starches[d]	
Food fiber[f]	
Polydextrose	
Fat replacers	
Microcrystalline cellulose	
Dextrins[d]	
Maltodextrins[a]	
Pectin microgel particles	
Inulin oligosaccharides	
β-glucan products	
Sources of fiber	
Cellulose (powdered, microcrystalline, microfibrillated, microreticulated)	
Brans (wheat, rice, barley, corn, oat)	
β-glucan preparations	
Ordinary food gums[e]	
Psyllium gum	
Resistant starch	
Polydextrose	
Sugar beet fiber	
Fruit fiber preparations	

[a] See SWEETENERS: NUTRITIVE.
[b] See SUGAR: SUCROSE.
[c] See LACTOSE.
[d] See STARCH.
[e] See GUMS.
[f] See FIBER, DIETARY.

fectionery products have been added as ingredients, primarily as high-fructose syrups or invert sugar.

D-Glucose is a six-carbon sugar (a hexose) whose carbonyl function is an aldehydo group. Aldehyde-based sugars are known as aldoses. Therefore, D-glucose is an aldohexose (Fig. 1). D-Glucose may appear on labels as glucose or dextrose.

The chemistry of D-glucose is that of aldehydo groups and hydroxyl groups (1,2). The aldehydo group reacts with the hydroxyl group on carbon atom 5 to form a cyclic, hemiacetal structure (a pyranose ring), which is by far the predominate form of D-glucose in solution. Ring formation involves formation of a new chiral carbon atom (carbon atom 1 [C-1], the one on the far right in the structure of β-D-glucopyranose (Fig. 1), so two configurations are formed at that position, the one with the hydroxyl group on C-1 down being the α form and the one with the hydroxyl group up being the β form.

Oxidation of the aldehydo or hemiacetal carbon atom (C-1) produces, respectively, the corresponding acid, D-gluconic acid (or its salt, D-gluconate), or the cyclic ester (lactone) (Fig. 1), the latter being produced directly in commercial production, which employs the enzyme glucose oxidase. This food acidulant is identified on ingredient labels as gluconolactone or glucono-delta-lactone. The product of reduction, D-glucitol (Fig. 2), is commonly known as sorbitol, which is how it most often appears on labels. Glucitol (sorbitol) belongs to the class of carbohydrates known as polyols, polyhydroxy alcohols or, scientifically, alditols. Other monosaccharidic polyols used as food ingredients are mannitol, xylitol, and erythritol (Fig. 3), which are labeled as such. A classification of these and other nutritive sweeteners can be found in the article SWEETENERS: NUTRITIVE.

D-Fructose is a six-carbon sugar (hexose) (Fig. 4) whose carbonyl function is a keto group, making it a ketose, more specifically a ketohexose. D-Fructose may appear on labels as fructose, levulose, or fruit sugar.

The keto group of D-fructose is also involved in ring formation. In solution, such as in honey and high-fructose

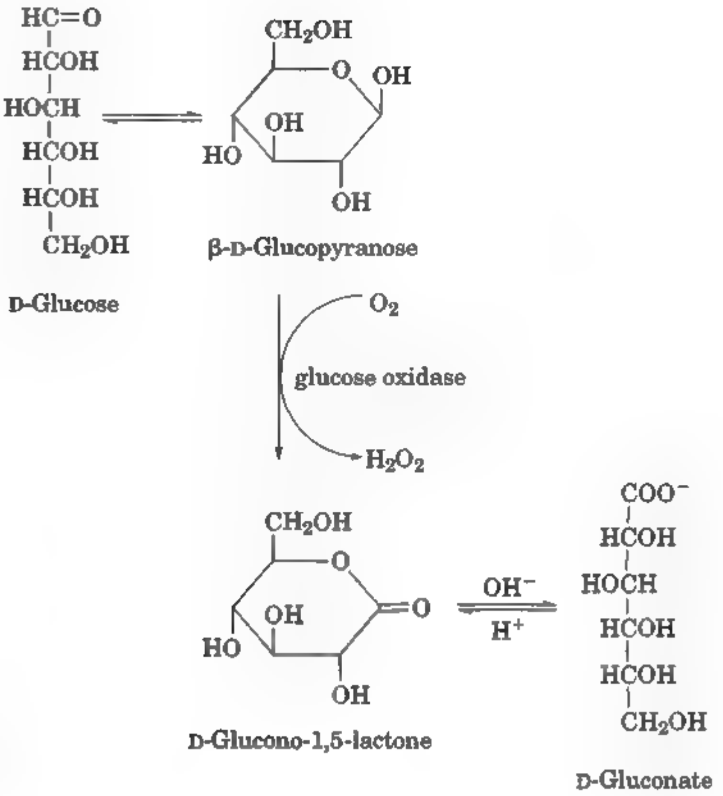

D-Glucose
β-D-Glucopyranose
O2
glucose oxidase
H2O2
D-Glucono-1,5-lactone
OH−
H+
D-Gluconate

Figure 1. The open-chain (acyclic) and one of the two six-membered (pyranose) ring forms of D-glucose, its enzyme-catalyzed oxidation, and the open-chain (salt) and lactone ring forms of D-gluconic acid.

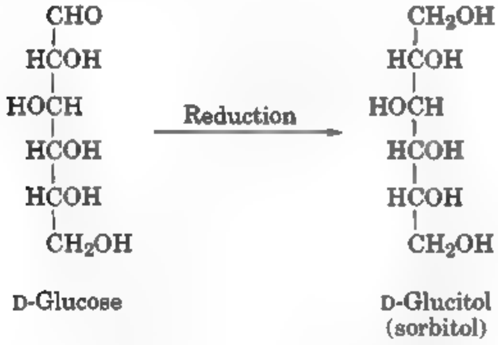

D-Glucose
Reduction
D-Glucitol
(sorbitol)

Figure 2. Reduction of D-glucose to sorbitol.

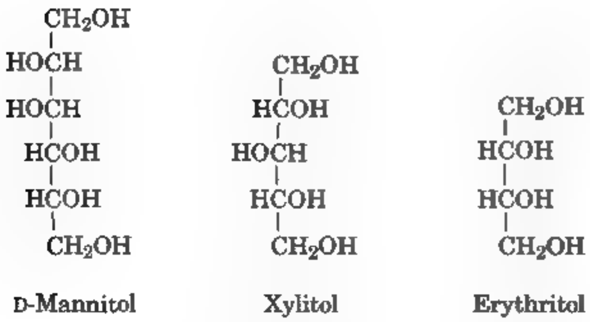

D-Mannitol
Xylitol
Erythritol

Figure 3. Structures of other food alditols.

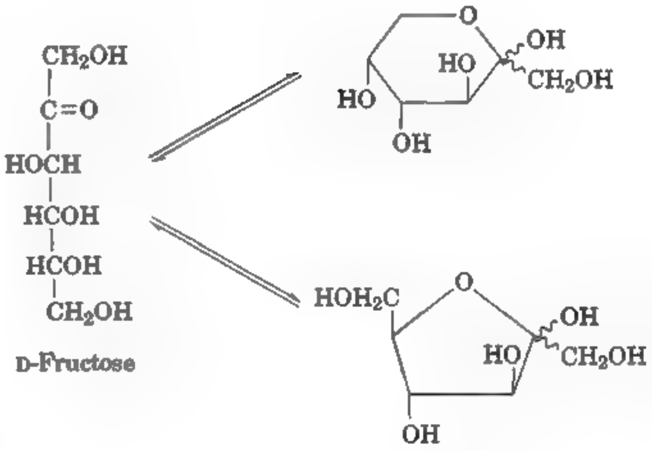


Figure 4. Cyclization of D-fructose to pyranose and furanose ring forms.

syrups, D-fructose occurs as the more stable six-membered (hemiacetal/hemiketal, pyranose) ring (Fig. 4); in the disaccharide sucrose (described in the next section), D-fructose occurs in its five-membered (furanose) ring form (lower ring structure in Fig. 4). (The squiggly lines on carbon 2 in this figure and at the reducing end of Figure 5 indicate a mixture of two configurations—the α and β configurations.) Reduction of D-fructose produces a mixture of D-glucitol (sorbitol) and D-mannitol. Fructose cannot be oxidized to an acid or lactone.

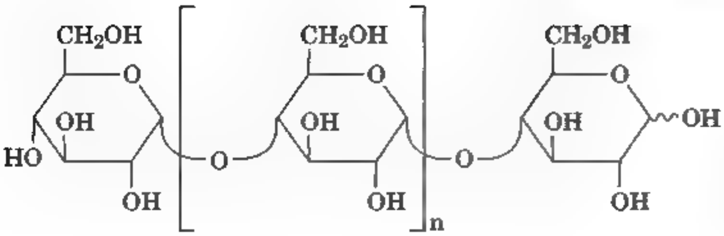


Figure 5. Structures of molecules related to the two starch polymers, amylose and amylopectin: $n = 0$, maltose: $n = 1$, maltotriose; $n = 2$, maltotetraose; $n = 3$, maltopentaose; and so on. In amylose, $n = \sim1000\text{–}15{,}000$.

Glucose is isomerized to fructose by the enzyme glucose oxidase, which is the basis for the production of high-fructose syrups (high-fructose corn syrups, HFCS). First starch is completely enzyme-converted to D-glucose, which is then isomerized to D-fructose. Generally, two fructose syrups are made, a 42% fructose and a 55% fructose syrup. Crystalline fructose is also available from this process.

In addition to starch being completely converted to glucose (crystalline and solution/syrup form), various glucose syrups (corn syrups) are also produced from it. Such syrups are described by their dextrose (glucose) equivalence (DE), which is defined as the percent reducing sugar calculated as D-glucose on a dry-weight basis. Thus, the DE is a measure of the degree of hydrolysis. D-Glucose has a DE value of 100 and starch a DE value of 0. Glucose syrups normally have DE values ranging from ~25% to >95%. A DE 25 syrup contains ~5% glucose, ~6% maltose, ~11% maltotriose, and ~78% higher oligosaccharides. A DE 80 syrup contains about 80% glucose.

Reactions of hydroxyl groups are of little significance in monosaccharides of foods, except as they are involved in hemiacetal and lactone ring formation. However, an important process that can occur with any carbohydrate containing a free, or potentially free, aldehydo or keto group also involves the remainder of molecule. Nonenzymic browning begins with a reaction known as the Maillard reaction, which is a chemical reaction between an amino group of an amino acid or protein molecule and reducing sugars, which are any carbohydrate molecules with a carbonyl group (free or in the form of a hemiacetal). The end products of a series of steps in several related pathways are flavors, aromas, and dark-colored soluble and insoluble polymers.

Heating sugars in the absence of compounds containing amino groups to a sufficient temperature effects a complex series of pyrolytic reactions resulting in the production of caramel colors and flavors.

OLIGOSACCHARIDES

Oligosaccharides are short chains of a few sugar units joined in acetal linkages; that is, the hemiacetal groups of a ring form (most often the pyranose ring form) of monosaccharides have been joined during biosynthesis to a hydroxyl group of another monosaccharide unit to create an acetal. In carbohydrate chemistry, the half of the acetal bond joining two sugar units is called a glycosidic linkage, the other half being that which forms the pyranose or furanose ring (1,2).

The upper limit to the number of saccharide (glycosyl) units in an oligosaccharide is undefined. It is often stated to be 10, sometimes 20. There are 2 monosaccharide units in a disaccharide, 3 in a trisaccharide, 4 in a tetrasaccharide, 10 in a decasaccharide, and so on. Chains containing more than 20 units are generally considered to be polymer molecules, that is, polysaccharides. With the exception of only a few naturally occurring oligosaccharides, the glycosyl (generally pyranosyl, occasionally furanosyl) units in oligo- and polysaccharides are joined together by glycosidic linkages in a head-to-tail fashion. As a result, each oligosaccharide has one, and only one, reducing end, that is, an end with a carbonyl or hemiacetal group (Fig. 5), but because most oligosaccharides are produced by depolymerization of polysaccharides, and because polysaccharides are often branched and may have many nonreducing ends, oligosaccharides may have more than one nonreducing end.

Depolymerization of starch molecules produces a series of branched (see "Polysaccharides") and unbranched oligosaccharides known chemically as maltooligosaccharides (Fig. 5) and, perhaps, larger fragments, depending on the extent of depolymerization. Commercially, these mixtures are known as, and labeled, maltodextrins. A range of maltodextrin products with different properties are produced. Mixtures of starch depolymerization products with smaller average degrees of polymerization, but large enough to allow conversion to a dry powder form (rather than a syrup) are known as, and labeled, corn syrup solids, glucose syrup solids, dried glucose syrup, or glucose syrup, dried.

The primary exception to the head-to-tail linkage arrangement is found in the structure of the disaccharide sucrose (1,2) (see the article SUGAR: SUCROSE), in which the linkage is from hemiacetal group to hemiacetal (hemiketal) group (Fig. 6), leaving the molecule with no free or potentially free carbonyl group. Therefore, sucrose is classified as a nonreducing sugar. Tri- and tetrasaccharides related to sucrose (raffinose, and stachyose, respectively)

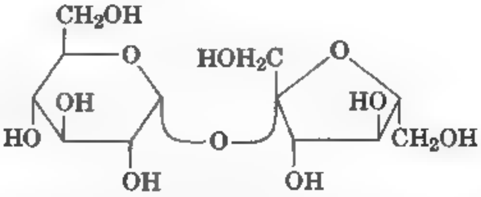


Figure 6. Sucrose. The left-hand portion is an α-D-glucopyranosyl unit. The right-hand portion is a β-D-fructofuranosyl unit.

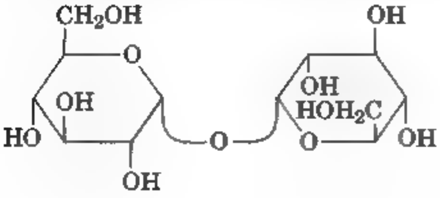


Figure 7. α,α-Trehalose.

Table 2. Classification of Selected Food Polysaccharides by Source

Source	Examples
	Algal (seaweed extracts)
	Agars, algins, carrageenans, furcellaran
	Higher plants
Insoluble	Cellulose
Extract	Pectins
Seeds	Corn starches, rice starches, wheat starch, guar gum, locust bean gum, psyllium seed gum
Tubers and roots	Potato starch, tapioca starch, konjac mannan
Exudates	Gum arabics, gum tragacanth
	Microorganisms (fermentation gums)
	Xanthans, gellan, curdlan, dextrans
	Derived
From cellulose	Carboxymethylcelluloses (CMC), hydroxypropylcelluloses (HPC), hydroxypropylmethylcelluloses (HPMC), methylcelluloses (MC)
From starch	Starch acetates, starch 1-octenylsuccinates, starch phosphates, starch adipates, starch succinates, hydroxypropylstarches, dextrins
	Synthetic
	Polydextrose

Table 3. Classification of Selected Food Polysaccharides by Structure

Classification schemes	Examples
	By shape
Linear	Algins, starch amyloses, carrageenans, cellulose, konjac mannan, pectins
	Branched
Short branches on a linear backbone	Guar gum, locust bean gum, xanthan
Branch-on-branch structures	Starch amylopectins, gum arabics, gum tragacanth (tragacanthin)
	By different kinds of monosaccharide units
One	Starch amylopectins, starch amylose, cellulose
Two	Algins, carrageenans, galactomannans, konjac mannan, pectins
Three	Gellan, xanthan
Four	Gum arabics
Five	Gum tragacanth (tragacanthin)
	By charge
Neutral	Starch amylopectins, starch amyloses, cellulose, galactomannans, konjac mannan
Anionic (acidic)	Algins, carrageenans, gellan, gum arabics, gum tragacanth (tragacanthin), pectins, xanthan

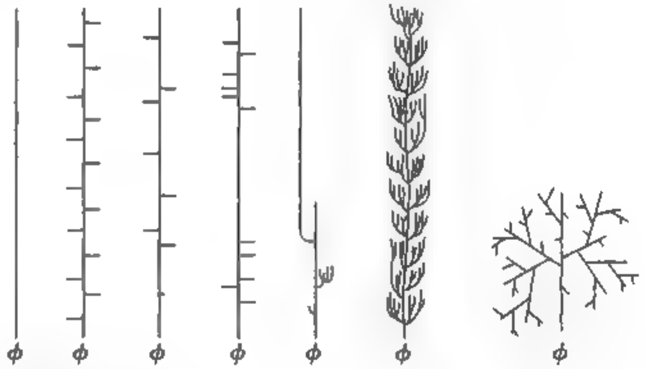


Figure 8. General structural features of polysaccharides. ϕ, Reducing end unit.

are found primarily in beans. Added sucrose in a food product most often is indicated on the ingredient label as sugar.

The other exception to head-to-tail linkages is that of α,α-trehalose (Fig. 7). Also in this disaccharide, the two monosaccharide (α-D-glucopyranosyl) units are linked reducing end to reducing end.

All glycosidic linkages are susceptible to hydrolysis in the presence of acid and heat. That of sucrose is especially so. This hydrolysis to produce an equimolar mixture of D-glucose and D-fructose is called inversion and the product invert sugar. Invert sugar and invert sugar syrup may be used as label designations.

Unique structures that do involve head-to-tail linkages are those of the cyclodextrins (cycloamyloses), which as their name implies, are cyclic structures with neither a reducing or nonreducing end. Also, as their name implies, they are formed from starch. The commercial one is β-cyclodextrin, which is a ring composed of seven α-D-glucopyranosyl units linked (1 → 4); that is, a structure like that of Figure 5 in which $n = 5$, and the ends are joined in the same type of linkage joining the other units.

Reactions of hydroxyl groups of oligosaccharides are generally not practiced. There are a few exceptions. Derivatives of β-cyclodextrins have been made to improve

Table 4. Label Designations of Food Polysaccharides

Chemical or generic name of food gum	Approved label designations (in addition to chemical or generic name)
Agar	Agar–agar
Alginic acid	
Sodium or potassium alginate	Algin
Propylene glycol alginate	Algin derivative
Carboxymethylcellulose	Sodium carboxymethylcellulose Sodium carboxymethyl cellulose Carboxymethylcellulose Carboxymethyl cellulose CMC Sodium CMC Cellulose gum
κ-carrageenan	Carrageenan Chondrus extract Irish moss extract
λ,ι-carrageenan	Carrageenan
Cellulose, powdered	
Furcellaran	Danish agar
Gellan	Gellan gum
Guar gum	
Gum arabic	Arabic gum Gum acacia Acacia gum
Gum ghatti	Ghatti gum
Gum karaya	Karaya gum
Gum tragacanth	Tragacanth gum
Hydroxypropylcellulose	Hydroxypropyl cellulose Modified cellulose
Hydroxypropylmethylcellulose	Hydroxypropyl methylcellulose Hydroxypropyl methyl cellulose HPMC Modified cellulose
Konjac mannan	Konjac gum Konjac flour Yam Yam flour
Locust bean gum	Carob gum
Methylcellulose	Methyl cellulose MC Modified cellulose
Microcrystalline cellulose	Cellulose microcrystalline Cellulose gel
Pectin	
Polydextrose	
Psyllium gum	Gum psyllium
Corn, waxy maize, waxy corn, high-amylose corn, potato, tapioca, cassava, wheat, arrowroot, rice, waxy rice starch	Starch
Starch acetate, starch phosphate, starch octenylsuccinate, crosslinked starch, oxidized starch, hydroxypropylstarch, thin-boiling starch	Food starch-modified Modified food starch
Dextrins	
Xanthan	Xanthan gum

Note Approved for the United States.

its solubility. Sucrose is monoesterified with fatty acids to produce food-grade emulsifiers/surfactants and more completely esterified (6–8 fatty acyl groups) to produce the fat substitute olestra. The modified carbohydrate, sorbitan, is also fatty acyl esterified and/or etherified with poly(ethylene glycol)/poly(oxyethylene) chains to produce food-approved emulsifiers/surfactants.

POLYSACCHARIDES

Polysaccharides are polymers of monosaccharide units, which may be substituted with other groups such as ester, ether, and/or cyclic acetal moieties (1). Polysaccharides may be attached to proteins, in which case the substance is referred to as a protein-polysaccharide (when they originate from plants) or a proteoglycan (when they originate from animals), or to a lipid, in which case the substance is referred to as a lipopolysaccharide. Certain polysaccharides of plants, namely, hemicelluloses, may be covalently bound to lignin.

Polysaccharides of foods, either naturally occurring or added as ingredients, are classified in several different ways (3,4), by source (Table 2) and by structure (Table 3) being the most common ways. They may also be classified by composition (3) and by imparted functionalities (4). Water-soluble polysaccharides of foods, especially those added as ingredients, are known as food gums and hydrocolloids (see the article GUMS).

One feature to note from Table 3 is that polysaccharides, unlike proteins, can be branched. Because each monosaccharide unit of a polysaccharide has several hydroxyl groups that can be involved in glycosidic linkages, each unit can potentially be involved in multiple linkages. Therefore, branching can, and does, occur. Branched polysaccharide structures can have several shapes, including essentially linear structures with a very few, very long branches; linear structures decorated with short branches, either regularly spaced, irregularly spaced, or in clusters; branch-on-branch structures with branches in clusters (as in starch amylopectin) (1) (see the article STARCH); and bushlike, branch-on-branch structures, with and without decoration with short branches (Fig. 8). Each polysaccharide has one and only one reducing end unit but may have many nonreducing end units.

The chemistry of polysaccharides, like that of oligosaccharides, is primarily the chemistry of hydroxyl groups and the chemistry of glycosidic linkages; in the case of polysaccharides containing uronic acid units, the chemistry of carboxyl groups may also be involved (3). Hydroxyl groups may be reacted to form ethers (practiced with cellulose [1] and starches [1], primarily corn, waxy maize, and potato starches). Hydroxyl groups may also be reacted to form esters (practiced with starch) (1). Some naturally occurring ester groups are removed by saponification during processing of the food gums konjac mannan (acetate ester groups) and gellan (acetate and glycolate ester groups). Oxidants will convert hydroxyl groups into carbonyl functions; carboxyl groups may also be introduced during oxidation.

Oxidation is usually effected at alkaline pH values. Under these conditions, β-eliminations may result in depolymerization (1). Glycosidic bonds are also cleaved by acid-catalyzed hydrolysis (practiced with starches and gums in general). Starches are also depolymerized by enzyme-catalyzed hydrolyses in the formation of corn syrups and other hydrolytic products. Under low-moisture conditions, heating in the presence of an acid results in both glycosidic bond hydrolysis and transfer, the latter reaction resulting in a more highly branched product (production of dextrins from a starch).

Some of the uronic acid carboxyl groups of algins are esterified in the production of propylene glycol alginate (1). Some naturally occurring methyl ester groups of a high-methoxyl pectin are saponified during its conversion into a low-methoxyl pectin. In other low-methoxyl pectins, the methyl carboxylate groups are converted into carboxamide groups by treatment with ammonia (1).

Allowed label designations of food gums are given in Table 4.

BIBLIOGRAPHY

1. R. L. Whistler and J. N. BeMiller, *Carbohydrate Chemistry for Food Scientists*, Eagen Press, St. Paul, Minn., 1997.
2. J. N. BeMiller and R. L. Whistler, "Carbohydrates," in O. R. Fennema, ed., *Food Chemistry*, Marcel Dekker, New York, 1996.
3. J. N. BeMiller, "Structure–Property Correlations of Non-starch Food Polysaccharides," in H. N. Cheng, G. Cote, and I. C. Baianu, eds., *Application of Polymers in Foods*, Marcel Dekker, New York, 1999.
4. J. N. BeMiller, "Classification, Structure, and Chemistry of Polysaccharides in Foods," in S. S. Cho and M. Dreher, eds., *Handbook of Dietary Fiber and Functional Foods*, Marcel Dekker, New York, 1999.

J. N. BEMILLER
Purdue University
West Lafayette, Indiana

CARBOHYDRATES: FUNCTIONALITY AND PHYSIOLOGICAL SIGNIFICANCE

Carbohydrates are the most important source of food energy in the world. They provide between 40 to 80% of total food energy intake, depending on location, cultural considerations, or economic status. Despite the importance of dietary carbohydrates, the human body only comprises about 1%. Following digestion and absorption, available carbohydrates may be (*1*) used to meet immediate energy needs of tissue cells, (*2*) converted to glycogen and stored in the liver and muscle for later energy needs, and (*3*) converted to fat as a larger reserve of energy.

In addition to the role that carbohydrates play as an energy source, they have a number of other roles as well. Sugars are used as sweeteners to make food more palatable and to assist in preservation. Starches and gums provide important textural properties to processed foods such as baked goods and desserts. Diets high in carbohydrates may reduce individual propensity to obesity, and there is growing evidence that such diets may also provide some protection against various noncommunicable human diseases and conditions.

DESCRIPTION

The major class of dietary carbohydrates (degree of polymerization) are sugars (1–2), oligosaccharides (3–9), and polysaccharides (>9) (1). Sugars comprise monosaccharides, disaccharides, and polyols (sugar alcohols). Oligosaccharides include malto-oligosaccharides, mainly from the hydrolysis of starch, and other oligosaccharides such as fructo-oligosaccharides. Polysaccharides may be divided into starch (α-glucans) and nonstarch polysaccharides, of originate from plants) or a proteoglycan (when they originate from animals), or to a lipid, in which case the substance is referred to as a lipopolysaccharide. Certain polysaccharides of plants, namely, hemicelluloses, may be covalently bound to lignin.

Polysaccharides of foods, either naturally occurring or added as ingredients, are classified in several different ways (3,4), by source (Table 2) and by structure (Table 3) being the most common ways. They may also be classified by composition (3) and by imparted functionalities (4). Water-soluble polysaccharides of foods, especially those added as ingredients, are known as food gums and hydrocolloids (see the article GUMS).

One feature to note from Table 3 is that polysaccharides, unlike proteins, can be branched. Because each monosaccharide unit of a polysaccharide has several hydroxyl groups that can be involved in glycosidic linkages, each unit can potentially be involved in multiple linkages. Therefore, branching can, and does, occur. Branched polysaccharide structures can have several shapes, including essentially linear structures with a very few, very long branches; linear structures decorated with short branches, either regularly spaced, irregularly spaced, or in clusters; branch-on-branch structures with branches in clusters (as in starch amylopectin) (1) (see the article STARCH); and bushlike, branch-on-branch structures, with and without decoration with short branches (Fig. 8). Each polysaccharide has one and only one reducing end unit but may have many nonreducing end units.

The chemistry of polysaccharides, like that of oligosaccharides, is primarily the chemistry of hydroxyl groups and the chemistry of glycosidic linkages; in the case of polysaccharides containing uronic acid units, the chemistry of carboxyl groups may also be involved (3). Hydroxyl groups may be reacted to form ethers (practiced with cellulose [1] and starches [1], primarily corn, waxy maize, and potato starches). Hydroxyl groups may also be reacted to form esters (practiced with starch) (1). Some naturally occurring ester groups are removed by saponification during processing of the food gums konjac mannan (acetate ester groups) and gellan (acetate and glycolate ester groups). Oxidants will convert hydroxyl groups into carbonyl functions; carboxyl groups may also be introduced during oxidation.

Oxidation is usually effected at alkaline pH values. Under these conditions, β-eliminations may result in depolymerization (1). Glycosidic bonds are also cleaved by acid-catalyzed hydrolysis (practiced with starches and gums in general). Starches are also depolymerized by enzyme-catalyzed hydrolyses in the formation of corn syrups and other hydrolytic products. Under low-moisture conditions, heating in the presence of an acid results in both glycosidic bond hydrolysis and transfer, the latter reaction resulting in a more highly branched product (production of dextrins from a starch).

Some of the uronic acid carboxyl groups of algins are esterified in the production of propylene glycol alginate (1). Some naturally occurring methyl ester groups of a high-methoxyl pectin are saponified during its conversion into a low-methoxyl pectin. In other low-methoxyl pectins, the methyl carboxylate groups are converted into carboxamide groups by treatment with ammonia (1).

Allowed label designations of food gums are given in Table 4.

BIBLIOGRAPHY

1. R. L. Whistler and J. N. BeMiller, *Carbohydrate Chemistry for Food Scientists*, Eagen Press, St. Paul, Minn., 1997.
2. J. N. BeMiller and R. L. Whistler, "Carbohydrates," in O. R. Fennema, ed., *Food Chemistry*, Marcel Dekker, New York, 1996.
3. J. N. BeMiller, "Structure–Property Correlations of Non-starch Food Polysaccharides," in H. N. Cheng, G. Cote, and I. C. Baianu, eds., *Application of Polymers in Foods*, Marcel Dekker, New York, 1999.
4. J. N. BeMiller, "Classification, Structure, and Chemistry of Polysaccharides in Foods," in S. S. Cho and M. Dreher, eds., *Handbook of Dietary Fiber and Functional Foods*, Marcel Dekker, New York, 1999.

J. N. BEMILLER
Purdue University
West Lafayette, Indiana

CARBOHYDRATES: FUNCTIONALITY AND PHYSIOLOGICAL SIGNIFICANCE

Carbohydrates are the most important source of food energy in the world. They provide between 40 to 80% of total food energy intake, depending on location, cultural considerations, or economic status. Despite the importance of dietary carbohydrates, the human body only comprises about 1%. Following digestion and absorption, available carbohydrates may be (*1*) used to meet immediate energy needs of tissue cells, (*2*) converted to glycogen and stored in the liver and muscle for later energy needs, and (*3*) converted to fat as a larger reserve of energy.

In addition to the role that carbohydrates play as an energy source, they have a number of other roles as well. Sugars are used as sweeteners to make food more palatable and to assist in preservation. Starches and gums provide important textural properties to processed foods such as baked goods and desserts. Diets high in carbohydrates may reduce individual propensity to obesity, and there is growing evidence that such diets may also provide some protection against various noncommunicable human diseases and conditions.

DESCRIPTION

The major class of dietary carbohydrates (degree of polymerization) are sugars (1–2), oligosaccharides (3–9), and polysaccharides (>9) (1). Sugars comprise monosaccharides, disaccharides, and polyols (sugar alcohols). Oligosaccharides include malto-oligosaccharides, mainly from the hydrolysis of starch, and other oligosaccharides such as fructo-oligosaccharides. Polysaccharides may be divided into starch (α-glucans) and nonstarch polysaccharides, of which the major components are the polysaccharides of plant cell walls (eg, cellulose, hemicellulose, and pectin). The term *complex carbohydrates* is often used in the United States to describe starch, dietary fiber, and nondigestible oligosaccharides (2). One of the major developments in the understanding of the importance of carbohydrates in health over the last 20 years has been the discovery of resistant starch, which is defined as "starch and starch degradation products not absorbed in the small intestine of healthy humans" (1).

FOOD FUNCTIONALITY

Carbohydrates have a number of important roles in food functionality including effects on texture, taste, and stability.

Sugars

Sugars contribute primarily to the sweetness of foods (3). Other important properties of sugars include preservation, fermentation, browning, and viscosity. Concentrated sugar solutions act as a preservative by controlling water activity. Yeast cells in fermented bread and in alcoholic beverages metabolize sugars, under anaerobic conditions, to produce carbon dioxide or ethanol. Carmelization reactions occur when sugars are heated. Sugars are able to increase boiling point and depress the freezing point of a solution. Polyols impart bulking or humectant properties and lower energy content than sugars.

Oligosaccharides

Oligosaccharides provide unique health benefits (4,5). More than half of the "functional foods" on the Japanese market contain prebiotic oligosaccharides as an active component, with the aim of promoting favorable gut microflora. Oligosaccharides such as polydextrose can provide a 1 kcal/g bulking agent providing the body and texture of sugar for energy controlled foods.

Starches

Starch is the major component in flours and commodities such as rice, corn, wheat, and beans (6). One of the major advantages of granular starches is that, being insoluble in cold water, they can be easily dispersed and then rendered functional by heating, which causes the granules to swell, absorb water, and affect viscosity. The behavior of starch depends on the temperature and the concentration. The gelatinization of starch occurs at a critical temperature of between 56 and 68°C depending on the starch type. The rheological properties of starch paste have important food applications. Pregelatinized starches can be used in food products for reduced preparation and processing requirements. Starch retrogradation accompanies the aging of cooked foods such as breads, cereals, puddings, or gravies. With bread, the staling process involves hardening of the starch by retrogradation and the redistribution of the water from the crumb to the crust.

Polysaccharide Gums

Polysaccharide gums are widely used in food processing as thickeners and gelling agents (3). These functions allow the development of a wide variety of unique processed food products such as dietetic jams, jellies, salad dressings, and sauces. There are a variety of gum types derived from (*1*) plant materials such as seaweed, seeds, roots, or tree exudates; (*2*) microbial biosynthesis; and (*3*) chemical modification of natural polysaccharides. Thickening and gelling of foods by gums is a common practice in processed foods to achieve a certain texture, mouth-feel, and body. Most gums have the ability to increase viscosity and many gums can form gels that result from intermolecular associations. Gums tend to be effective at low levels, such as less than 1% of formulation weight.

BASIC PHYSIOLOGICAL EFFECTS

Energy Source

Dietary carbohydrates are generally given an energy value of 4 kcal/g (17 kJ/g), although where carbohydrates are expressed as monosaccharides, the value of 3.75 kcal/g (15.7 kJ/g) is used. However, a number of carbohydrates are only partially or not digested in the small intestine and are fermented in the large bowel to short-chain organic acids. These include the nondigestible oligosaccharides, resistant starch, and nonstarch polysaccharides. This process of fermentation is less efficient than absorption in the small intestine. Although energy values vary for different carbohydrates, a value of 2 kcal/g (8 kJ/g) is a reasonable average figure for carbohydrates that reach the colon (1,4).

Glucose and Insulin

The digestion of carbohydrates starts in the mouth, where salivary α-amylase initiates starch hydrolysis (7). The starch fragments formed are maltose, some glucose, and dextrins. The α-amylase hydrolysis of starch is completed by the pancreatic amylase active in the small intestine. Disaccharides also need to be broken down to monosaccharides to be absorbed. The final hydrolysis is accomplished by "disaccharidases" attached to the intestinal brush border membrane. Disaccharidase deficiencies occur in some populations, causing malabsorption and intolerance, such as in the case of lactose. Lactose, a disaccharide of glucose and galactose, is the principal sugar in milk. At birth, lactase activity is high in the brush border of the small intestine of infants, but declines after weaning, so that most non-Caucasian populations of the world have low activity in adult life (1,8).

Glucose and galactose are transported actively against a concentration gradient into the intestinal mucosal cells by a sodium-dependent transporter (1,7). Fructose undergoes facilitated transport by another mechanism. When absorbed into circulation, carbohydrates cause an elevation of blood glucose concentration. Fructose and galactose have to be converted to glucose mainly in the liver and therefore produce a slower rate of glucose elevation. Insulin, necessary for glucose uptake by body cells, is secreted as a response to blood glucose elevation but is modified by many factors, such as neural and endocrine stimuli.

Plasma Lipids

There is concern that very high carbohydrate diets at the expense of fat might result in a decrease in high density lipoprotein and a corresponding increase in plasma triglycerides. However, this does not appear to be an issue when the increased carbohydrates are in the form of vegetables, fruits, and processed cereal over prolonged periods.

Polysaccharides like oat β-glucan and those of psyllium have been repeatedly shown to lower plasma cholesterol levels in individuals with elevated levels; slight to moderate change of plasma levels are normal (1,9). Proposed mechanisms include impaired bile acid and cholesterol reabsorption through physical entrapment in the small intestine, or inhibitory effects on cholesterol synthesis by products of lower bowel fermentation.

Bowel Function

It has long been recognized that the nonstarch polysaccharide portion of dietary fiber is the principal dietary component affecting bowel regularity (10). This occurs through increases in bowel content bulk and speeding up of intestinal transit time. The degree of effect depends on the chemical and physical properties of the polysaccharides and the extent of fermentation. One important development in recent years has been the demonstration that specific dietary carbohydrates selectively stimulate the growth of individual groups or species of bacteria. For example, fructo-oligosaccharides specifically stimulate growth of bifidobacteria that may protect the host from invasion by pathogenic species and have other health benefits. Foods used to selectively stimulate the growth of gut bacteria are known as prebiotics.

HEALTH MAINTENANCE AND DISEASE PREVENTION

Although the amount of carbohydrate required to avoid ketosis is relatively small (about 50 g/day), carbohydrate provides the majority of the energy in the diets of most people (1). This is desirable not only for providing easily available energy for oxidative metabolism, but also carbohydrate-containing foods are good vehicles for micronutrients and phytochemicals. Carbohydrates are important in maintaining glycemic homeostasis and for gastrointestinal integrity and function. Diets high in carbohydrates, as compared with diets high in fat, reduce the likelihood of developing obesity and related conditions. An optimum diet should consist of at least 55% of the total energy coming from carbohydrates obtained from a variety of food sources. However, carbohydrates intakes at or above 75% of total energy could have significant adverse effects on nutritional status by the exclusion of adequate quantities of protein, fat, and other essential nutrients.

Energy Balance and Weight Maintenance

In adults, it is very important that the energy ingested be matched to the amount of energy expended (1,11). Maintenance of energy balance is necessary to avoid obesity and associated chronic diseases such as diabetes and cardiovascular disease. Positive energy balance (weight gain) occurs when total energy intake exceeds total energy expenditure, regardless of the composition of the excess energy. However, composition of the diet may affect to what extent positive energy balance occurs.

The composition of the diet may affect the body's ability to maintain energy (1,11). Especially, diets containing at least 55% of energy from a variety of carbohydrate sources, as compared with high-fat diets, reduce the probability of body fat accumulation. Substantial data suggest those diets high in fat tend to promote consumption of more total energy than diets high in carbohydrates. This effect may be due to the low energy density of high-carbohydrate diets, because total volume of food consumed appears to provide an important satiety cue. Although there are no data to suggest that different types of carbohydrates affect total energy differently, the composition of the diet may affect the proportion of excess energy stored as body fat. The body has a large fat storage capacity, and excess dietary fat is stored very efficiently in adipose tissue. Alternatively the body's capacity to store carbohydrates is very limited, and excess dietary carbohydrates are not efficiently stored as body fat. Instead, excess carbohydrate tends to be oxidized readily, resulting in indirect fat accumulation by reductions in fat oxidation. Although the overall contribution of de novo lipogenesis from carbohydrates is small, it may increase with appreciable carbohydrates overfeeding, insulin resistance, and with extremely high intakes of sucrose or fructose.

There is considerable controversy surrounding the extent to which sugars and starch promote obesity (1,11). There is no direct evidence to implicate either form of carbohydrate in the etiology of obesity, based on data derived from studies in Western countries. In spite of that, it is important to state that excess energy in any form promotes body fat accumulation. Excess consumption of low-fat foods, although not as obesity producing as excess consumption of high-fat foods, leads to obesity if energy expenditure (such as physical activity) is not increased.

Physical Activity

Maintaining regular physical activity reduces the likelihood of creating positive energy balance or weight gain, regardless of diet composition (1,12). There is general agreement that the combination of high-carbohydrate diets and regular physical activity is the best way to avoid positive energy balance. The increased energy needs of people with high physical activity can be efficiently met by dietary carbohydrates. In many developing countries, the major challenge is to meet the daily energy needs of high physical labor. The importance of carbohydrates in the diet becomes more critical as the amount of and the intensity of physical activity increases.

There is substantial evidence that supplemental carbohydrates can improve performance for elite, endurance-trained athletes (1,12,13). A high-carbohydrate diet including meals and snacks such as energy bars have been shown to enhance performance during long-distance cycling and running. There is, however, no clear evidence that carbohydrate supplements and snacks would improve the performance for the majority of people who engage in recreational physical activity of lower intensity and duration. On the other hand, carbohydrate intake after exercise can help to quickly replenish depleted glycogen stores.

Dental Caries

The incidence of dental caries is influenced by a number of factors (1). Foods containing sugars and starch may be easily hydrolyzed by α-amylase and bacteria in the mouth, which can increase the risk of caries as a result of excess production of organic acids. Starches with a high glycemic index produce more pronounced changes in plaque pH than low glycemic index starch, especially when combined with sugars. However, the impact of carbohydrates on caries is dependent on the food, frequency of consumption, degree of oral hygiene performed, availability of fluoride, salivary function, and genetic factors.

Behavior

There is emerging evidence that food intake may have important effects on behavior (1,14). Although providing breakfast to children who do not typically eat breakfast can increase cognitive performance, it is less clear that overall composition of the diet can affect behavior. Although it is often suggested that sugar consumption may lead to hyperactivity in children, there is no evidence to support the claim that refined sugars have a significant influence on child hyperactivity and related behaviors. Because glucose is an essential energy source for the central nervous system, carbohydrate has been suggested to play a role in memory and cognitive function. Although there appears to be a relationship between glucose levels and memory processing, the clinical significance of this effect has yet to be determined.

Blood Glucose and Diabetes Management

The rise of blood glucose in normal and diabetic people after meals varies markedly and depends on many factors, including the source of the carbohydrate, its method of preparation, and composition of the total meal (1,15). Classification of carbohydrates as simple or complex does not predict their effects on blood glucose or insulin. Rapidly absorbed carbohydrates, which promote large blood glucose responses, may be in the form of both sugars and starches. Sugars added to foods have no different effects on blood glucose from those of sugars alone. The natural sugars from fruits and fruit juices raise blood glucose approximately as much as does sucrose and less than do most refined starchy carbohydrate foods.

Consuming a wide range of carbohydrate foods is now regarded as acceptable in the nutrition management of people who already have non-insulin-dependent diabetes (1). It is suggested that between 60 and 70% of total energy should be derived from a mix of carbohydrates and monounsaturated fats. Carbohydrates should mainly come from a wide range of appropriately processed cereals, vegetables, and fruit. Sucrose and other sugars have not been directly implicated in the etiology of diabetes, and recommendations concerning intake relate primarily to the avoidance of all energy-dense foods in order to reduce obesity. Most recommendations for the management of diabetes permit modest (30–50 g/day) intakes of sucrose and other added sugars in the diabetic dietary plan.

Blood Lipids and Cardiovascular Disease Risk

The cornerstone of dietary advice aimed at reducing coronary heart disease risk is to increase the intake of carbohydrate-rich foods, especially cereals, vegetables, and fruits rich in nonstarch polysaccharides, at the expense of fat (1). There is increasing evidence that antioxidants have a protective effect against coronary heart disease, and complex carbohydrates such as fruits and vegetables tend to be rich sources of antioxidant nutrients and food components. Cereal foods rich in nonstarch polysaccharides tend to have a protective effect, and there is not evidence that sucrose consumption increases risk. Certain nonstarch polysaccharides such as β-glucan have been shown to have an appreciable effect on lowering plasma cholesterol when consumed in naturally occurring foods, enriched foods, or dietary supplements. Many carbohydrate-rich plant foods are rich in potassium, which may help to reduce the risk of hypertension.

Gastrointestinal Illnesses and Cancer Risk Management

Minimally refined carbohydrate staple foods may be a good source of dietary fiber or oligosaccharides and phytochemicals, which might mitigate the risk of colorectal and other cancers (1). The process of complex carbohydrate (eg, dietary fiber, resistant starch, oligosaccharides) fermentation in the large intestine may protect the colorectal area against the genetic damage that may lead to cancer through a range of mechanisms that include (*1*) the dilution of potential carcinogens; (*2*) the reduction of products of protein fermentation through the stimulation of bacterial growth; (*3*) pH effects through the production of butyric acid; (*4*) maintenance of the gut mucosal barrier; and (*5*) effects of bile degradation. High intake of prebiotics such as fructo-oligosaccharides may facilitate the colonization of bifidobacteria and lactobacilli in the large gut, this may reduce the risk of acute infective gastrointestinal illnesses.

Inherited Conditions

There are a number of inherited conditions having significant implications for restricting dietary intake in infants and children (1). These include rare conditions such as fructose intolerance, galactosemia, and sucrase deficiencies. Though rare diseases, their early detection and careful dietary management is important in avoiding severe handicap or pathology.

BIBLIOGRAPHY

1. Food and Agricultural Organization, *Carbohydrates in Human Nutrition*, FAO Food and Nutrition Paper 66, Rome, Italy, 1998.

2. International Life Sciences Committee, "Complex Carbohydrates. The Science and the Label," *Nutr. Rev.* **53**, 186–193 (1995)
3. P. Chinachoti, "Carbohydrates: Functionality in Foods," *Am. J. Clin. Nutr.* **61**, 922S–929S (1995).
4. F. R. J. Bornet, "Undigestible Sugars in Food Products," *Am. J. Clin. Nutr* **59**, 763S–769S (1994)
5. S. K. Figdor and H. H. Rennhard, "Caloric Utilization and Disposition of Polydextrose in the Rat," *J. Agric. Food Chem.* **29**, 1181 (1981)
6. D. J. Mauro, "An Update on Starch," *Cereal Foods World* **41**, 776–780 (1996).
7. B. Szepesi, "Carbohydrates," in *Present Knowledge in Nutrition*, ILSI Press, Washington, D.C., 1996.
8. L. V. Muir, K. D. Sanders, and W. P. Bishop, "Gastrointestinal Disease," in *Present Knowledge in Nutrition*, ILSI Press, Washington, D.C., 1996.
9. A. S Truswell and A. C. Beynen, "Dietary Fibre and Plasma Lipids: Potential for Prevention and Treatment of Hyperlipidemias," in *Dietary Fibre—A Component of Food*, Springer-Verlag, New York, 1992.
10. N. W. Read and M. A. Eastwood, "Gastrointestinal Physiology and Function," in *Dietary Fibre—A Component of Food*, Springer-Verlag, New York, 1992.
11. J. O. Hill and B. J. Rolls, *Carbohydrates and Weight Management*, ILSI NA Monograph, ILSI Press, Washington, D.C., 1998.
12. E. F. Coyle, "Substrate Utilization During Exercise in Active People," *Am. J. Clin. Nutr.* **61**, 968S–979S, (1995).
13. W. M. Sherman, "Metabolism of Sugars and Physical Activity," *Am. J. Clin. Nutr.* **62**, 228S–241S (1995).
14. J. W. White and M Wolraich, "Effect of Sugar on Behavior and Mental Performance," *Am. J. Clin. Nutr.* **62**, 242S–249S (1995).
15. T. M. S. Wolever and J B. Miller, "Sugars and Blood Glucose Control," *Am. J. Clin. Nutr.* **62**, 212S–227S (1995).

Mark L. Dreher
Mead Johnson Nutritionals, Bristol-Myers Squibb
Evansville, Indiana

CAROTENOID PIGMENTS

Carotenoids as natural pigments are unique in that they are found in both plants and animals. Animals cannot make the basic 40 carbon structure but are capable of making changes in the hydrocarbon rings and chain. Table 1 shows the distribution of the various plant pigments in food. The carotenoid pigments are important from a food science point of view because of the color they impart to various food products and the fact that a number of the pigments have vitamin A activity.

The carotenoids are chemically and biochemically related to the more general class of compounds known collectively as terpenes and terpenoids. These are compounds composed of repeating isoprenoid like units. Mevalonic aid has been shown to be a precursor for the biosynthesis of the terponoids.

The carotenoids have traditionally been classified as carotenes (hydrocarbons) and xanthophylls (containing oxygen in some form). Generally, the hydrocarbons (lycopene, β-carotene, etc) are produced first and the xanthophylls are produced at a later stage. However, it has recently been shown that some fish can reverse the process and make retinol from oxygenated carotenoids.

There are four periods of carotenoid research (1). During the first period (1800s) pigments were characterized by measurements of light absorption. During the second period (1900–1927) attempts were made to define the empirical formulas of the isolated carotenoids. The third period (1928–1949) was a time in which some carotenes with provitamin A activity were discovered. Vitamin A and some of the carotenes, including β-carotene, were synthesized. The fourth, the most recent, period witnessed a virtual explosion in the number of known carotenoids. The instrumentation that has made this possible was developed during this time (2). There were about 80 known carotenoids, and of these perhaps only 35 had established structures. Today there are greater than 600 known carotenoids.

Today there is much interest in the carotenoids because they are natural pigments and also because of their proposed role in medical applications.

Every three years a formal group meets in Europe, the United States, and Japan, and plenary and session lectures are published (2–9). A book on the technical aspects of carotenoids (10) and a book on vitamin A deficiency (11) has also been published.

The carotenoids are the ultimate sources of vitamin A in the diet, and the lack of retinol or provitamin A has led to nutritional blindness. A number of countries have been listed as having a potentially serious vitamin A deficiency problem (12). In some well-studied Southeast Asian countries it has been estimated that half a million children will go blind each year because of a lack of vitamin A. When one extrapolates to other countries in Asia, Africa, and Latin America, the mortality and morbidity rates are staggering.

A body of evidence suggests that some of the carotenoids themselves have medical applications (13). These include the deposition of carotenoid pigments in the skin of people who do not develop protective pigmentation and protection against certain cancers. Although we have known of the protection of green plants from photodynamic destruction by the carotenoids, the protection of animals from neoplastic transformation is a recent discovery.

CAROTENOID NOMENCLATURE

Trivial names were used because the structures were not established and the number was small. With more than 600 carotenoids of proven structure now known there is still a need for trivial names, but there is also a need for more precise names that follow a chemical convention.

Under trivial names the carotenoids are generally grouped as carotenes (hydrocarbons) and the oxygen-containing compounds as xanthophylls. Some of the carotenes' names are preceded by a Greek letter (eg, β, ϵ), and others have names denoting the source (lycopene tomato). Some are a combination such as β-zeacarotene and α-zeacarotene. Here the ring double-bond position and the source (maize) are indicated.

Table 1. Distribution of Natural Coloring Matter

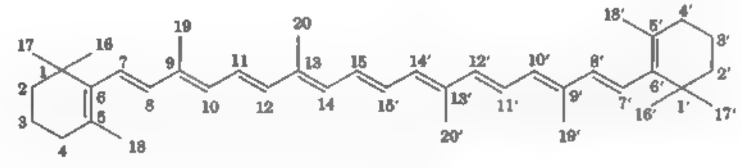
Food	Heme	Carotenoids	Chlorophyll	Betalains	Anthocyanins	Flavonoids	Caramel	Melanina
Red meat	X	—	—	—	—	—	—	—
Fish	X	X	—	—	—	—	—	X
Eggs	—	X	—	—	—	—	—	—
Crustaceans	—	X	—	—	—	—	—	—
Dairy products	—	X	—	—	—	—	—	—
Green vegetables	—	X	X	—	X	X	—	—
Root vegetables	—	X	X	X	X	X	—	—
Fruits	—	X	X	—	X	X	—	—
Cereals	—	X	X	—	—	—	X	—

Xanthophylls are pigments that contain hydroxyl, carbonyl, epoxide, ester, and carboxylic acid groups. The convention today is to indicate the source and add the suffix xanthin (eg, tunaxanthin). Some of the older trivial names such as lutein denoted their yellow color. Structural features are indicated by the prefix iso, as in isozeaxanthin; the hydroxyl group in zeaxanthin is in the three position, and it is in the four position in isozeaxanthin *cis*-Lycopene would indicate a departure from the expected all-trans structure.

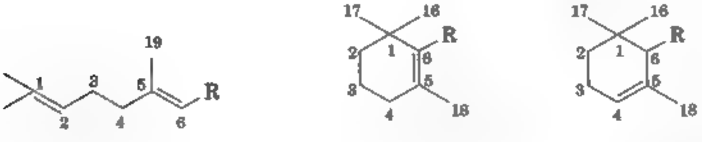
In recent years an attempt has been made to limit the number of trivial names by building on a known name such as β-carotene. Thus astaxanthin might be 3,3′dihydroxy-4,4′-diketo-β-carotene.

The systematic method of carotenoid nomenclature uses the IUPAC convention (14–15) (Fig. 1). The advantage is that any carotenoid can be exactly named, including chiral centers. The disadvantage is that the name for a complicated compound can take two or three lines, whereas the trivial name is a single work (eg, fucoxanthin). In the IUPAC system the central chain is assumed to be unsaturated and all-trans. Any departure from this structure is specified. Thus β-carotene is β, β-carotene. The term nor indicates that a methyl is missing, and seco indicates a break in the ring structure such as 5,6, SECO, β, β-carotene-5,6-dione (semi-β-carotenone). The change in the hydrogenation level to form saturated positions as well as acetylenes and allenes can be designated by hydro and dehydro.

Oxygenated derivatives of carotenoid hydrocarbons are named by use of suffixes and prefixes according to the rules of general chemical nomenclature. The principal group is chosen and is cited by use of a suffix, and the other groups are cited as prefixes. The sequence is as follows: carboxylic acid, ester of carotenoid acid, aldehyde, ketone, alcohol, ester of carotenoid alcohol, and epoxide. Apo-carotenoids are found naturally as well as occurring synthetically. The apo designates where the parent compound has been



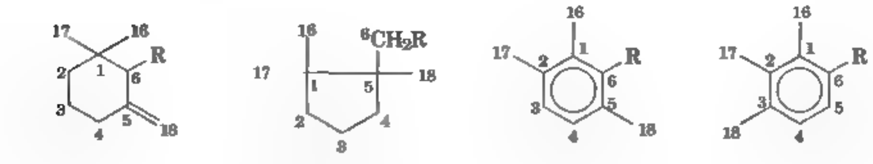
β-Carotene



Acylic
ψ (psi)

Cyclohexene
β (beta)

Cyclohexene
ε (epsilon)



Methylene cyclohexane
γ (gamma)

Cyclopentane
κ (kappa)

Aryl
ϕ (phi)

Aryl
χ (chi)

Figure 1. IUPAC rings.

cleaved. Thus β-apo-8′-carotenal should be designated 8′-apo-β-carotene-8′-al.

There are rules for carotenoids with more than 40 carbons, retro double-bond structures, designated chiral centers, and the use of the primes. This area is covered in more detail elsewhere (14). Pfander gives the structure and reference for more than 600 carotenoids (16).

BIOSYNTHESIS

In 1950 a biosynthetic pathway for the conversion of phytoene to lycopene to β-carotene was proposed (17). While the structures of several of these compounds were not proven at that time, it nevertheless was the first sequential pathway proposal.

The 1950s and 1960s were intense years in the polyene biosynthetic field because steroid and carotenoid biogenesis followed similar pathways. Thus when mevalonic acid (MVA) was discovered it was a breakthrough for both groups. The use of stereospecific [14C]-and [3H] mevalonic acids incorporated into cell-free systems provided a valuable tool for studying biosynthetic conversions. Acetyl-CoA may be considered the starting point in terpene biosynthesis. As can be seen in Figure 2, two acetyl-CoA molecules condense to form acetoacetyl-CoA. This in turn condenses to form the branched six-carbon acid β-hydroxy-β-methylglutaryl CoA (HMG-CoA). Through a series of reactions, HMG-CoA can also be formed from leucine (18). HMG-CoA is reduced from the dicarboxylic acid to a monoacidic-monohydroxyl compound, MVA. For most systems, MVA once formed cannot be converted back to HMG-CoA. MVA in the presence of ATP is converted to MVAP and then to MVAPP. With a third ATP, MVAPP is converted to isopentenyl pyrophosphate (IPP). This step involves a phosphorylation and a decarboxylation. IPP is the key intermediate to the terpenoids, which include rubber, sterols, bile acids, squalene, some sex hormones, ubiquinones, essential oils, the phytol side chain of chlorophyll, and vitamins E and K. It is isomerized to form dimethylallylpyrophosphate (DMAPP). IPP and DMAPP condense to form the C_{10} compound geranyl PP (GPP). The 10-carbon unit is further reacted with IPP to form the C_{15} farnesyl (FPP) and the C_{20} geranylgeranyl (GGPP). FPP can be dimerized to form squalene and the C20 GGPP, to form phytoene. The formation of phytoene follows a rather complicated pathway. By analogy to squalene, lycopersene with a saturated 15-15′ position was proposed as the first carotenoid and the precursor to phytoene. More recent studies have shown that the unlikely reduction to form lycopersene from GGPP and the oxidation to form phytoene from lycopersene does not occur; see Figure 2. Plants are able to form phytoene and the higher carotenoids, but animals can only make changes to preformed carotenoids.

More recently a new pathway to IPP has been reported (18). This pathway does not involve mevalonic acid, but follows five carbon intermediates. Β-carotene has been shown to be formed by this pathway. It remains to be seen how significant these findings are.

There is a branch of neurosporene leading into either lycopene or α- or β-zeacarotenes. The data on the cyclization of neurosporene or lycopene seem to favor lycopene as the major pathway. In the red tomato, synthesis of β-zeacarotene rules out lycopene as the sole precursor to β-carotene because it is difficult to explain the higher temperature-selective inhibition of lycopene (19, Fig. 2). When tomatoes were treated with DMSO at various stages of maturity (20,21), it was found that the acyclic but not the cyclic carotenoids were inhibited. It was proposed that parallel pathways exist for phytoene to lycopene and phytoene to β-carotene. By the use of a mutant tomato it was found that the DMSO inhibited the formation of lycopene at one stage and both pathways at an earlier stage of maturity. Also, the β-carotene is found in the chloroplast, whereas the lycopene is found crystallized in the chromoplasts.

Research has been reported on the use of cell-free homogenates. However, the interpretation of the results of these cell-free systems was difficult because the cell was no longer intact. A number of inhibitors were used. These included mainly nitrogen-containing compounds. One compound, 2-(4-chlorophenyl)triethylamine (CPTA), inhibits the cyclization of lycopene and thus lycopene accumulates. When washing out the CPTa, β-carotene is formed at the expense of lycopene. A number of other inhibitors cause an increase in β-zeacarotene and a decrease in β-carotene. Still other compounds have been found to stimulate β-carotene and β-zeacarotene synthesis. Clearly both pathways are operating and it is possible to "prove" a pathway by the choice of inhibitor, stimulant, or bioregulator (22).

The great deal of information on the biosynthesis of the carotenoids has come from the use of $^{14}C/^{3}H$ dual-labeled MVA. By use of MVA with ^{3}H in the two and four positions and ^{14}C in the two position, it was found that the α- and β-rings are formed independently. Eight carbons and a number of 3H would be found in the entire molecule. Two ^{3}H atoms are found in position four of the β-ring and one in α-carotene. However, if the ϵ-ring is converted to the β-ring, one 3H would be lost. This is not found experimentally. Likewise, the 4-^{3}H from MVA is in the six position; 3H must be lost in the formation of β-carotene but not in the direct formation of α-carotene. Because the 6′-^{3}H is retained in α-carotene, the β-ring is not converted to the ε-ring (23). The nonrandomized dimethyl groups in torularhodin were proved with double labeled MVA. This result suggested that the interconversion of IPP and DMAPP did not result in the randomization of the C-2 hydrogen from MVA (24).

The ring formation in the red pepper to form capsanthin and capsorubin has been suggested to follow a pathway through a postulated epoxide intermediate. It is generally thought that the insertion of oxygen occurs at a later stage in the biosynthetic pathway, and that the epoxide, hydroxyl, and one-carboxyl oxygen came from molecular oxygen.

The view had long been held that the direction of biosynthesis was from saturated to unsaturated, from hydrocarbon to the xanthyphylls. Recently, however, it has been shown that some fish can convert astaxanthin to retinol. Bacteria have also been shown to form a series of 45- and 50-carbon carotenoids. Lycopene, leutein, and cellulose

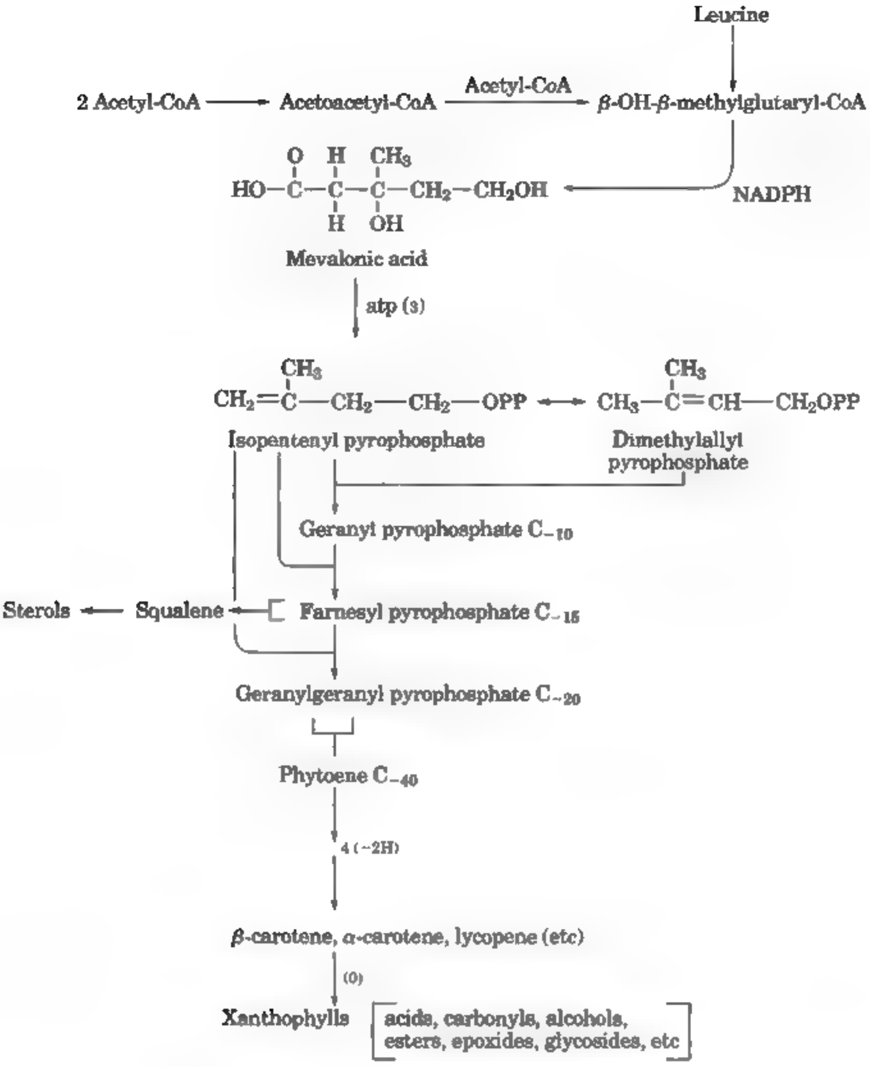

Leucine
2 Acetyl-CoA
Acetoacetyl-CoA
Acetyl-CoA
β-OH-β-methylglutaryl-CoA
NADPH
Mevalonic acid
atp (3)
Isopentenyl pyrophosphate
Dimethylallyl pyrophosphate
Geranyl pyrophosphate C₋₁₀
Sterols
Squalene
Farnesyl pyrophosphate C₋₁₅
Geranylgeranyl pyrophosphate C₋₂₀
Phytoene C₋₄₀
4 (−2H)
β-carotene, α-carotene, lycopene (etc)
(O)
Xanthophylls
acids, carbonyls, alcohols, esters, epoxides, glycosides, etc

Figure 2. Pathway for terpene biosynthesis.

were fed to rats, and it was found that the conversion of β-carotene to vitamin A was inhibited (25).

OCCURRENCE IN NATURE

As indicated in Table 1 the carotenoids are widely distributed in foods and are the most widespread group of pigments in nature. They are present without exception in photosynthetic tissue; occur with no definite pattern in nonphotosynthetic tissues such as roots, flower petals, seeds, fruits, vegetables; and are found sporadically in the Kingdom Protista, including the (fungi-yeast mushrooms and bacteria). The red, yellow, and orange pigments in the skin, flesh, shell, or exoskeleton of some animal species are due to these pigments. These would include the lobster, shrimp, salmon, goldfish, and flamingo. People who consume large amounts of tomato juice may turn orange or red because of an intake of the pigments. The carotenoids, which cannot be made by animals, are the precursors to vitamin A, but unlike vitamin A, are not toxic in large doses (26).

Generally the concentration of the carotenoids in various tissues is low but can vary widely. The cornea of the eye narcissus contains 18% β-carotene (dry), whereas the concentration in foods is much lower. It has been estimated that the annual production of carotenoids is in on the order of 10^8 tons. The major pigment is fucoxanthin, the pigment in marine brown algae. Other major pigments include those found in green leaves, mainly lutein, violaxanthin, and neoxanthin. By contrast, β-carotene occurs widely but in smaller amounts. It is of interest that (note: zea is major pigment in some algae) 3,3′-dihydroxy-α-carotene (lutein) is a major pigment, whereas 3,3′-dihydroxy-β-carotene (zeaxanthin) is a minor pigment in nature. In contrast, the major hydroxarbon carotenoid found in nature is β-carotene, whereas α-carotene is a minor pigment. A typical percentage profile of the xanthophylls of a green plant could be lutein, 40%; violaxanthin, 34%; neoxanthin, 19%; cryptoxanthin, 4%; and zeaxanthin, 2%. β-carotene, lycopene, and capsanthin can be high in some tissues such as the sweet potato, tomato, and red pepper. By contrast, the carrot is a major course of α-carotene where the concentration can be 30 to 40%.

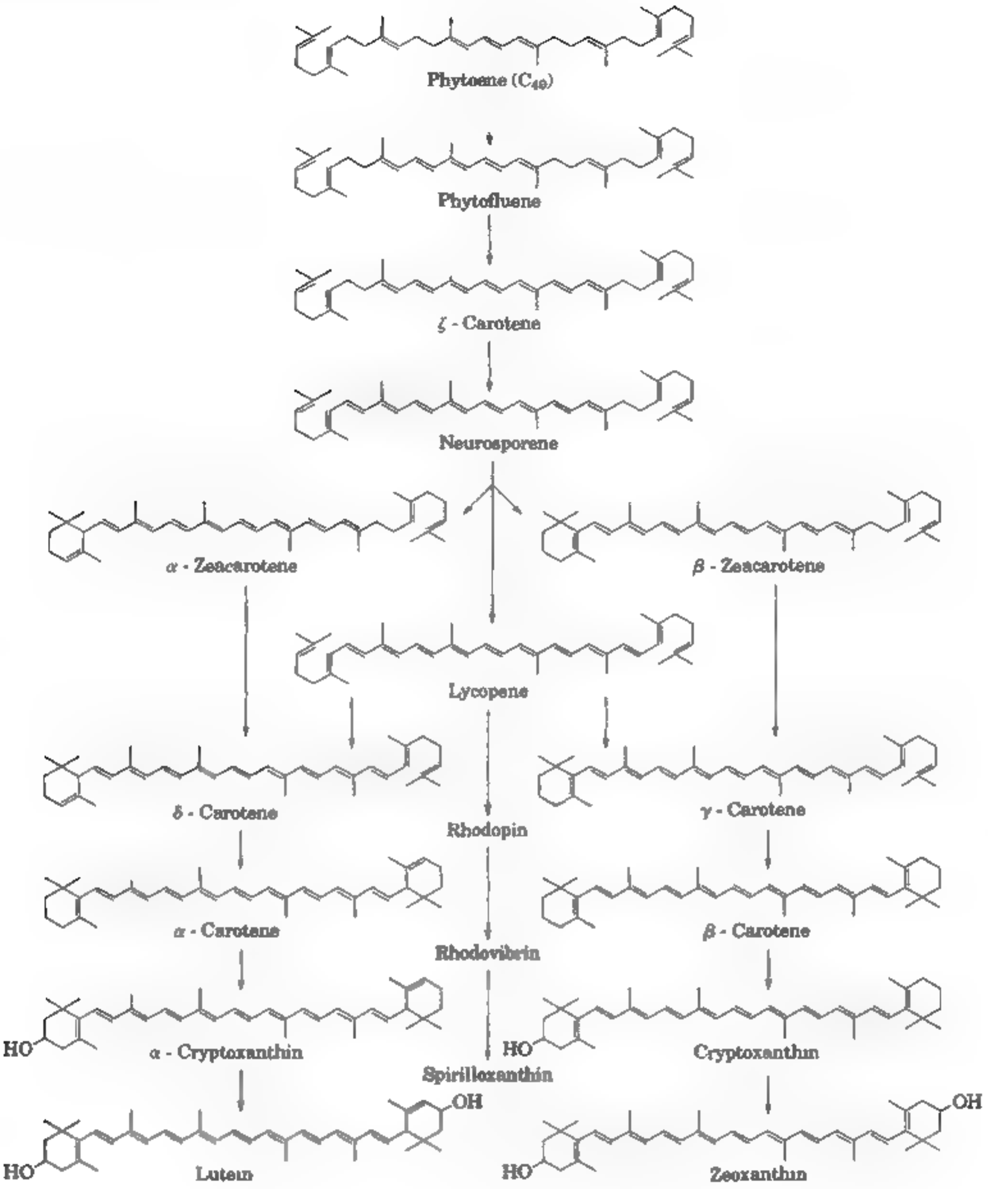

Phytoene (C_{40})
Phytofluene
ζ - Carotene
Neurosporene
α - Zeacarotene
β - Zeacarotene
Lycopene
δ - Carotene
Rhodopin
γ - Carotene
α - Carotene
Rhodovibrin
β - Carotene
HO
α - Cryptoxanthin
Spirilloxanthin
HO
Cryptoxanthin
OH
HO
Lutein
OH
HO
Zeoxanthin

Figure 3. Common carotenoids.

The level of carotenoids is often directly related to the quality of food. The lack of pigments as in white butter or salmon, for example, is a quality defect (27). However, flour is bleached to destroy the carotenoids, and β-carotene is often removed from red palm oil to make it colorless.

The acyclic pigments phytoene, phytofluene, zeta-carotene, neurosporene, and lycopene are often found in carotenoid-producing systems such as higher and lower plants. They are not often found in animal tissue. Of these, lycopene is the most common and may occur in large amounts in the tomato, watermelon, orange, pink grapefruit, and some apricots. Further reactions in products of lycopene lead to the closing of one or both rings. However in some oranges (Valencia) 3,4-didehydrolycopene is found. There have been a number of acyclic xanthophylls isolated mainly from the purple photosynthetic bacteria. The 1,2-epoxide has been isolated from tomatoes as a minor pigment. Because phytofluene is fluorescent, it must be accounted for in the fluorometric analysis of vitamin A (25).

The alicyclic carotenoids (eg, β-carotene) are very common in higher plants, bacteria, fungi, algae, and animals. It has been estimated, on the basis of structure, that some 50 to 60 alicyclic compounds have potential vitamin A activity. Apricots are an excellent source of β-carotene (60%), whereas the level in tomatoes (10%) and peaches (10%) is much lower. Papaya, if it is yellow/orange, is a good source, whereas the red flesh papaya contains mainly lycopene. Where the flesh is green, some plastid pigments would be present and thus some β-carotene would be present. Red grapes, pears, figs, red apples, and beet root, although brightly colored, are not good sources of β-carotene.

δ-Carotene has been isolated from certain tomato varieties where apo-polycis-isomers of β, α, and ϵ-carotene, and lycopene have been found.

Vegetables may be excellent sources of the provitamin A carotenoids. Spinach may have up to 4.0 mg/100 g β-carotene and carrots may have as high as 6.0 mg/100 g of β-carotene. Yellow corn (maize) contains small amounts of β-carotene and β-zeacarotene. Sweet potatoes may be purple, white, or bright orange. The two former potatoes are low in carotenoids and the latter may be greater than 9 mg/100 g. Squash, broccoli, peas, and pumpkin contain relatively large amounts of carotenes. Most vegetable oils are slightly yellow indicating a small amount of carotenoids. The red palm oil produced in Brazil, Southeast Asia, and parts of Africa may contain large amounts of β- and α-carotene (50 mg/100 g total carotenoids). Egg yolks are a rich source of carotenoids (lutein and zeaxanthin) but relatively poor sources of β-carotene. Green peppers are a better source of carotene than are the red peppers. Wheat flour is low in carotene and this amount is further lowered in the bleaching process.

Hydroxylated carotenoids are very common in plants, where lutein and zeaxanthin are to a lesser extent, β-cryptoxanthin are major pigments. Lutein is a common pigment in freshwater fish, whereas tunaxanthin (3,3′-dihydroxy ϵ-carotene) is common in marine fish. Peaches are a good source of β-cryptoxanthin. Many fruits contain xanthophyll esters. In the persimmon the carotenoid alcohol is β-cryptoxanthin. Corn varieties are selected for their high content of zeaxanthin and lutein. These pigments are fed in order to pigment the skin and egg yolks.

The xanthophylls with hydroxyl or keto substitution in the four position are common in various animal tissues. Echinenone (4-keto-β-carotene) is widely found in marine invertebrates and algae and canthaxanthin (4,4′-keto-β-carotene). The usual epoxides are in the five, six position and these are easily converted to the 5,8-epoxide with acid. Where a keto group is in the four position, in-chain epoxides are formed (eg, 9,10-epoxides of canthaxanthin). The 1,2-epoxides of acylic pigments have been reported in tomato leaves. The allenic seaweed pigment fucoxanthin is thin. The cyclopentyl ketones are found in red peppers and are probably formed by a pinacolic rearrangement of the epoxides of zeaxanthin.

The acetylenic carotenoids are found in diatoms, *Euglena*, giant scallops, mussels, and starfish. A number of apo-carotenoids are formed in some fruit, bacteria, orange peel, and fungi. The most well known apo-compound is bixin, the pigment in annatto seeds. Crocin is an apo-compound occurring in saffron. In a sense, retinol can be considered a degraded β-carotene. The carotenoid acid, torularhodin, is a common pigment in Rhodotorula yeast. Canthaxanthin is found in edible mushrooms, blue-green algae, trout, brine shrimp, and flamingos.

Astaxanthin is the pigment of the salmon and the invertebrates, annelida and crustacea. Recently it has been isolated from red yeast and Adonis flowers (28). Shrimp raised in intensive culture are often blue because of a protein complex with astaxanthin. This complex extends the chromophore resulting in a blue color. This has been mistakenly described as a disease, the condition, however, results in a lower price for the shrimp, particularly on the Japanese market. When cooked, the complex is broken and the red color is seen. The lobster can be green, black, red, or blue because of various protein-astaxanthin complexes all of which are broken with heat, yielding a red pigment. When treated with alkali, astaxanthin is converted to astacene.

Aromatic carotenoids have recently been isolated from photosynthetic bacteria and some sea sponges. Carotenoid epoxides are fairly common in nature. The so-called violaxanthin cycle is an example of the natural occurrence of epoxidation.

$$\text{zeaxanthin} \rightleftharpoons \text{antheraxanthin} \rightleftharpoons \text{violaxanthin}$$

EFFECT OF AGRICULTURAL PRACTICES

A number of agricultural practices, particularly those with plants, directly affect the carotenoid content. Thus the nutrient content of edible plants can vary severalfold to as much as 20 times (29). The major changes are caused by genetic manipulation. However, a number of factors such as sunlight, rainfall, topography, soils, location, season, fertilization of soils, and maturity may affect carotene content. Thus the nutritional tables give an average value with an understood large variation. A number of countries have developed nutritional tables that, when compared, show a great variation for the same fruit or vegetable. In the past, genetic manipulation has been concerned with the color, flavor, texture, and yield. Today there is more of an effort being made to produce foods that are nutritionally superior. Thus, interest is being shown in developing not only the color but also the quality of the color (eg, more β-carotene). No agricultural practice has been as effective as genetic manipulation. High β-carotene tomatoes have been developed but because of the yellow color were not popular. Sweet potatoes can vary from 0 to 7 mg/100 g of carotene and the carotene from carrots can be increased five times. Generally light has a positive effect on carotene synthesis. Fruit grown in the dark, however, will develop some color (30). Temperature has an effect, but this depends on the product. The most pronounced effect is on the ripening of the tomato. In temperatures below 27°C the synthesis of lycopene is favored, whereas a yellow tomato results at a higher temperature. Gamma irradiation also affects the formation of carotene (31).

Most vegetables and fruits increase in carotene during the ripening stage. The carotene content of the carrot increases until a level in maturity is reached. The β-carotene level falls off upon further growth. In the case of the red pepper, the conversion from green to red lowers the carotene content. Because of cultural practices and shipping constraints, fruits may be picked green and thus the full color may not develop.

ANALYSIS

A universal extraction and analysis scheme for the carotenoids is not possible due to the wide variation in carotenoid structure. Thus, numerous methods are suggested for the separation of carotenoids from such things as foodstuffs and blood plasma. The classic methods used by most investigators rely on some method of extraction and a chromatographic separation of the carotenoids (32,33). The methods require some sort of size reduction to increase surface area followed by extraction with a suitable solvent or solvent mixture. Often a bipolar solvent is used to extract nonpolar carotenoids from polar tissue. Ethyl alcohol and acetone are examples of bipolar solvents, and $CHCl_3$/MeoH is an example of a bipolar solvent mixture. The extraction may be followed by saponification, removal of alkali, transfer to a developing solvent, and chromatography under vacuum or pressure. The column may be developed with a gradient of solvents, and the pigments are separated by elution or are cut from the column and extracted. Often rechromatography is required of some bands, followed by verifying chemical reactions. The quantity of each purified fraction is determined by spectroscopy. The method requires several hours, and in some cases artifacts (*cis-trans* isomers, epoxides) are formed due to the long exposure to solvents, absorbents, light, and oxygen. Although the method does give a separation of provitamin A precursors from inactive carotenoids, it is time-consuming and requires skilled technical operators. Thus, the method may not be suitable for analysis of the large number of food items for a table of food compositions. A much simpler method has been developed that chromatographically separates the carotenes from the oxygenated compounds, but it may not separate individual carotenes, their *cis* isomers or carotenoid esters (34). Therefore, this method tends to overestimate provitamin A activity, especially if β-carotene represents only a small part of the total. High-performance liquid chromatography (HPLC) is clearly the method of choice, and the more recent values in the nutritional tables are generally determined by this method. A number of reports have appeared that show the general overestimation of the Association of Official Analytical Chemists (AOAC) method. A modified AOAC method and chromatographic procedures that separated the individual components were used to estimate the provitamin A content of Clingstone peaches (35).

The U.S. RDA for 100 g was much higher by the AOAC method than by the complete separation (60% vs 11% of the RDA for raw, 12% vs 6% for canned).

The comparison of the AOAC method with a stepwise gradient, a saponification step, and HPLC has been published. The AOAC method and HPLC were comparable for green vegetables. For the carrot and pumpkin, the AOAC method was higher for both vegetables than the gradient elution and HPLC method. Where fruit xanthophylls were esterified, these compounds were chromatographed with β-carotene, and thus the estimation was higher in the AOAC method when the fruit extract was not saponified (36).

A number of papers (37) have shown that the open column packet with 40 to 50 μm gives good separation of vitamin A compounds as well as carotenoid compounds. The procedure used a pipette for the injector. Nitrogen gas or vacuum is used in place of a pump.

EFFECT OF FOOD PROCESSING

The carotenoids are altered or partly destroyed by acids, usually but not always stable in inorganic bases, can be destroyed by some enzymes (eg, lipoxygenase), and are usually bleached by light. Most carotenoids are fat soluble and thus are not subject to leaching losses. Generally they are fairly stable to the heat involved in canning but are rapidly lost on dehydration because of oxidation

Although the most stable form of the carotenoids is the all-*trans* form, the *cis*-geometrical isomer can form upon heating, especially in the presence of H^+. The *cis*-isomer carotenoids are naturally present in the tangerine tomato. It has been shown that if β-carotene activity was set at 100%, the neo-β-carotene vitamin A activity was 38%, and the neo-α-carotene activity was 13%. The transformation from *trans* to *cis* results in a loss of extinction and a shift in absorption maxima to shorter (2–5 mm). Cooking causes the formation of *cis* isomers from all-*trans* β-carotene (38). Broccoli lost 13%, spinach lost 7%, and the sweet potato lost 32% of the all-*trans* isomer. A 15% loss of vitamin A was reported, mainly due to a *cis-trans* isomerization of α- and β-carotene during canning. High-temperature short-time (HTST) processing was shown to have less effect on the carotene in the sweet potato than conventional cooking (35).

Heat processing produced enough acid in pineapple to cause the formation of *cis* isomers. This could result in a lighter yellow color.

The formation of 5,6-and 5,8-epoxides of β-carotene in model systems has been studied. Diphenylamine, a free-radical inhibitor stopped the loss of β-carotene. It is generally thought that epoxide formation is the initial step in the degradation of the carotenoid. Several reports have been published that show the complexity of the reactions. Stored hydrogenated oils and enzymatic-coupled oxidation of carotenoids result in the formation of epoxides and other products.

A number of studies have reported an apparent increase in the level of carotenes in various canned products over the fresh product. There are two reasons for this surprising result. Where soluble solids are leached out of the products during cooking and storage, the fat-soluble carotenes appear to increase in amount. It has also been observed that the fresh control may have an active lipoxygenase-like enzyme that bleaches the carotenoids. When antioxidants were added to the fresh sample before extraction, the vitamin A value in the fresh product increased.

Dehydration and size reduction of fruits and vegetables increase surface area and, if products are not protected from light and oxygen, result in poor stability of the carotenoid pigments. The shelf life of the carotenoids in the dry state is shorter than in the wet state. Different dehydration methods result in varying losses of carotenes. Retention of carotene in fresh carrots varies; fresh carrots retain more than (100%), blanched (95%), freeze-dried (89%), air-dried (80%), and puff-dried (12%). Carotenoid oxidation in foods is generally associated with unsaturated fatty acids and is usually autocatalytic. The oxidation may or may not be enzymatic in nature and is directly related to the available water (A_w), oxygen, heat, and certain metals. Generally antioxidents are effective in slowing the reaction. The decomposition products from a simulated deoderization of red palm oil were isolated. Beta 15^1- and $\beta^{14'}$-apo-carotenals and 13 apo-carotenone were isolated from the reaction mixture and a pathway for the reaction was suggested.

CAROTENOIDS AS FOOD COLORS

It was the color of the carrot that first interested researchers 160 years ago to start work on carotenoids. Many years later it was again the color that was the means of identifying compounds separated by chromatography. It is the same today as it has always been; color is one of the means by which we identify the quality of food. People often reject the unfamiliar. Most pigment changes such as the changes in chlorophyll on thermal processing, the bleaching of anthocyanins, the browning of fresh meat, or the off-coloring of flavonoids do not in themselves affect the product. In case of the destruction of carotene, loss or change in color would result in poorer quality, but also may result in loss of vitamin A activity. β-Carotene has 11 double bonds in conjugation, which is the reason for its yellow-orange color. Any disruption in the chromophore, such as that which is depicted in Figure 2 would result in bleaching.

CONVERSION OF CAROTENOIDS TO VITAMIN A

The carotenoids of fruits, vegetables, and animal products are generally fat soluble and are associated with the lipid fractions. In some cases they may be in the crystalline state, as lycopene is in the tomato. During the digestion process, esterases, lipases, and proteases act on the carotenoids, which are solubilized by the bile salts. Within the mucosal cell β-carotene and the other provitamin A, carotenoids are split by carotenoid dioxygenase(s) resulting in an oxidative cleavage. In theory a molecule of β-carotene should yield two molecules of retinal. Retinol is formed by reduction of retinal. It is esterified and transported to the liver for storage. There is some difference of opinion as to whether there is a central cleavage enzyme or whether there is also a random-splitting enzyme that acts on various places on the chain. The carotenes may also be absorbed, carried by low-density lipoproteins and deposited in the skin, fat, and various organs. The extent of accumulation, rate of accumulation, time to reach saturation, and turnover time were different for each tissue. Liver adrenal and ovary have high concentrations of β-carotene.

Animals differ in the efficiency of the splitting reaction. The rat is generally regarded as very efficient. The mink and the fox are relatively inefficient in this reaction. Man is average with a ratio of about 6:1. The cat does not contain the enzymes necessary to utilize provitamin A carotenes.

Generally, for a compound to be active it must have an unsaturated central chain and an unsubstituted β-ring. On the basis of structure out of the greater than 600 carotenoids listed, the provitamin A compounds would be between 50 and 60. Vitamin A conversion factors include:

$$\mathrm{RE} = \mu\mathrm{g\ retinol} + \frac{\mu\mathrm{g}\beta\text{-carotene}}{6} + \frac{\mu\mathrm{g\ other\ provitamin\ A\ compounds}}{12}$$

$$\begin{aligned} 1\ \mathrm{IU} = {} & 0.3\ \mu\mathrm{g\ retinol} \\ & 0.6\ \mu\mathrm{g}\ \beta\text{-carotene} \\ & 1.2\ \mu\mathrm{g\ other\ provitamin\ A\ carotenoids.} \end{aligned}$$

α-Carotene on the preceding basis would be half as active as β-carotene. The metabolism of α-carotene has been shown to result in the liver storage of retinol and α-vitamin A. The latter compound does not combine with retinol binding protein (RBP). It can induce hypervitaminosis A because it is not delivered to the tissues. There is some confusion in regard to the capacity of the diepoxide to serve as a vitamin A source. The compound is listed as active *in vivo* but not active *in vitro*. Recently, radioactive β-carotene-5,6,5′,6′-diepoxide and luteochrome (5,6,5′,8′-β-carotene-diepoxide) were fed to rats and C^{14} retinol was not detected in the liver. The absorption or conversion to retinal is enhanced by bile salts, proteins, lipids, and zinc. It is not clear what the effect of various nonprovitamin A compounds have on the bioavailability of the provitamin A compounds. Lycopene is reported to enhance carotene uptake. It was found that nonprovitamin A compounds may decrease the utilization of provitamin A pigments, whereas β-cryptoxanthin and α-carotene promote utilization.

Because of the uncertainty of some of the artificial pigments and because of the delisting of several (eg, Red No. 2 in the United States, Red No. 40 in the United Kingdom), interest has increased for using carotenoids as food colorants.

We can consider the use of carotenoids as following a direct or an indirect route. As a case of the former, margarine might be colored with β-carotene. Examples of the latter might be the coloring of poultry skin, or eggs, or salmon flesh with the appropriate pigment. Some confusion exists regarding natural and artificial pigments. The infrared fingerprints for synthetic and natural β-carotene are identical. Many people prefer the term nature-identical to describe the synthetic carotenes.

One of the most common natural coloring preparations is obtained by extracting the seeds of the *Bixa orellana* tree, which grows in the tropics. The apo-compound bixin is the main component of the oil-soluble annatto preparations, and nor-bixin is the 6,6′-diapo-dicarboxylic acid (bixin has one methyl ester). Bixin is most stable at pH 8 and shows a diminished stability in the 4 to 8 pH range. In general, annatto extracts have a good shelf life. Annatto oil applications include the coloring of butter, bakery products, and salad oil. Combined with paprika, annatto oils are used to color cheese. Water-soluble preparations may be used in ice cream.

Saffron consists of the dried stigmas of *Crocus sativus*. It contains crocin, the digentiobioside of crocetin. The preparation is yellow in color. Saffron is also used as a spice and is widely accepted in such foods as soups, meat products, and cheese. Formerly, it was used in cakes and other bakery products.

Tomato extracts are red because of the pigment lycopene. The color of the product would be red to orange depending on the medium. Lycopene is not stable, and its use is limited.

Carrot extracts, carrot oil, and red palm oil contain large amounts of α- and β-carotene. These were used in fat-based products. They have the advantage of being natural but have limited use because of the lower price of synthetic β-carotene. Recently, hypersaline algae have been cultured for their β-carotene content.

Oleoresin paprika is an oil extract of paprika. The main carotenoids are capsanthin and capsorubin. The color of products colored by paprika vary from red to pinkish yellow. Oleoresin paprika can be used in salad dressings, sauces, meat products, and processed cheeses. The indirect coloring of trout with oleoresin paprika resulted in a yellow rather than a red hue of the flesh.

The synthetic pigments have a number of advantages over the natural product extracts. Although the principal advantage is price, it should not be overlooked that the large variation in quality is not a problem with the synthetic FDC dyes. The major synthetic carotenoids that are marketed include β-carotene, β-apo-8′-carotenal, and canthaxanthin. All occur naturally, and only canthaxanthin does not have vitamin A activity.

β-Carotene is widely encountered in nature. In foods it has been used in butter, margarine, salad oil, popcorn, baked goods, confections and candy, eggnog, coffee whiteners, juices, and soups to name a few. β-apo-8′-carotenal has been isolated from oranges and several natural sources. However the main source is the chemical synthesis. It is also yellow, and its uses are similar to those for β-carotene.

Canthaxanin (rosanthin) (4,4′-diketo-β-carotene) is used where a red color is desired. It was first isolated from the mushroom, but it is also a widely distributed animal pigment. Many crustaceans, especially brine shrimp, and birds, notably the flamingo, contain canthaxanthin. One of its main uses is as a pigmenting agent for salmon. Although a red color is obtained, it has been observed that the pigment tends to cook out. Recently canthaxanthin has been used in simulated meats, shrimp, crab, and lobster products.

Synthetic astaxanthin is now available and is approved for use in Europe and the United States. This is the major pigment in a number of fish and shellfish species. The color tends to be more stable than canthaxanthin. When shrimp are raised in intensive culture, often the blue carotenoprotein is found. This can be corrected by feeding more green algae or astaxanthin. Marigold flowers contain mainly lutein and are fed to chickens to supply yellow-orange pigments for the skin and egg yolks.

The crystalline carotenoids are not absorbed or metabolized by animals. This problem has been solved by the development of special water-soluble forms marketed to satisfy various product needs. Two approaches have been primarily employed: the production of oil suspensions of micropulverized crystals and the development of emulsions or beadlet forms containing the carotenoid in supersaturated solution or finely colloidal forms in liquid or dry products.

CAROTENOIDS BY CHEMICAL SYNTHESIS

The total synthesis of β-carotene was reported in 1950. Since that date β-carotene, retinol, *cis*-retinoic acid, canthaxanthin, β-apo-8′-carotenal, and more recently astaxanthin are produced by commercial chemical synthesis. A great many other carotenoids have been synthesized that at the moment have no commercial significance.

The Roche synthesis of β-carotene starts from β-ionone and is related to the vitamin A synthesis. The β-ionone may be prepared from citral, a constituent of lemongrass oil, or prepared synthetically from acetylene as a starting material. In the β-carotene procedure, β-ionone is converted to C-14 aldehyde, which, through further chain-lengthening steps, is converted to C-19 aldehydes. These are joined through a Grignard reaction to form the C-40 diol, which, through allylic rearrangement and dehydration, is converted to 15,15-dehydro-β-carotene. A partial hydrogenation and rearrangement yields *trans*-β-carotene.

Crystalline carotenoids produced by chemical synthesis are of high purity and uniform color. The vast majority of carotenoids, when pure, can be obtained in crystalline form. In general, the melting points of crystalline carotenoids are fairly high and quite sharp for the pure compounds. The synthesis of the carotenoid series of compounds employs a variety of organic synthetic techniques.

The literature on the synthesis of the various carotenoids is extensive, and the details are certainly beyond this chapter.

FUNCTION OF CAROTENOIDS

It is tempting to propose a single universal function for the carotenoids since they are found in such diverse tissues. Failing this, it is tempting to ascribe some function, wherever a carotenoid is found. Although the function of the carotenoids has been proven in some cases, their universal function, if any, remains to be determined. Where the function has been proven, it generally is with some aspect of the light-absorbing property of the carotenoid pigment. The critical role of these pigments in photosynthesis has been the best documented. Phototropism and phototaxis responses of plants seem to be related to the light absorption spectra of the carotenoids. Functions in animals are related to the antioxidant and to the light-absorbing property of these pigments. The function in vitamin A formation has been discussed. Because these pigments are found in many reproductive tissues, it has been suggested that they have a role in reproduction. The results in this area are less convincing than those in other areas.

In the photosynthetic process the carotenoids have been shown to be active in the light-gathering process (eg, they absorb light at wavelengths not absorbed by chlorophyll). In *Chlorella*, other green algae, and higher plants, the light absorbed by the carotenoids was used at low efficiency. In the diatom and brown algae the energy transfer is comparable to chlorophyll where the main pigment in fucoxanthin. In the photosynthetic process two photo systems are involved. In general, more carotenes are found in photosystem I and xanthophylls in photosystem II.

The role of the carotenoids in photoprotection of photosynthetic bacteria has been well documented. A mutant, which only produced the colorless polyenes phytoene and phytofloene, was compared with the wild type *Rhodopseu-*

dowonas sphaeroides. The wild type showed good growth under all conditions of light and oxygen. However, the mutant could only grow under anaerobic-light or aerobic-dark conditions.

Under light with O_2, massive killing resulted at room temperature and bacterial chlorophyll was destroyed. If the mutant was grown in the dark with air, chlorophyll disappeared. Where chlorophyll was missing, light and O_2 have no effect.

Diphenylamine (DPA) inhibits the formation of colored carotenoids. When the wild type blue-green bacteria is treated with DPA, it is destroyed like the mutant. At low temperatures, killing proceeds in the mutant but bacterial chlorophyll is not destroyed.

Corynebacterium poinsettiae is not photosynthetic, and both the wild type and the carotenoid-less mutant can tolerate high intensities of light in the presence of air. When the exogenous dye, toluidine blue, is added as a photosensitizing pigment, the carotenoid-less mutant is killed. The mechanism involves the simultaneous interaction of visible light, a photosensitizing dye, and O_2. Generally, the carotenoids are effective for visible light but have no effect in ultraviolet, gamma, or X radiation.

The reactions are listed as follows:

$$\text{CHL} + h_v \rightarrow {}^*\text{CHL excited state}$$

$$^*\text{CHL} + \rightarrow \text{photosynthesis or } {}^3\text{CHL triplet-excited state intersystem crossing}$$

$$^3\text{CHL} + {}^3O_2 \rightarrow {}^1\text{CHL} + {}^1O_2 \text{ or } {}^3\text{CHL} + {}^1\text{CAR} \rightarrow {}^1\text{CHL} + {}^3\text{CAR}$$

$$^3\text{CAR}\ {}^1\text{CAR} \rightarrow \text{harmless decay}$$

$$^1O_2 + \text{CAR CAR } O_2 \text{ CAR can be regenerated or } {}^1O_2 + \text{A A } O_2 \text{ photodynamic action}$$

$$^1O_2 + {}^1\text{CAR}\ {}^3O_2 + {}^3\text{CAR}$$

$$^3\text{CAR} \rightarrow {}^1\text{CAR harmless decay}$$

As can be seen in these reactions, carotenoids may protect photosynthetic bacteria at various levels by quenching the singlet-excited state of O_2 or the triplet-excited state of chlorophyll. The ground states of oxygen would be $^3O_2$ and for CHL the triplet state. The carotenoids may be the preferred substrate for oxidation or may act in quenching reactive species.

Phototropism is a response of higher plants and some fungi that results in the plant turning to the light. Rival claims for either β-carotene or riboflavin as the active compound have been made based on the action spectra. More recent reports seem to rule out β-carotene as the mediator in the response.

Phototaxis is a response in which an organism such as *Euglena* can move toward the light. The action spectra does not match the spectra of the carotenoids.

The involvement of the carotenoids in reproduction appear to be coincidental to the process of sexual reproduction and of no known significance to the process. The major pigment of the brine shrimp is canthaxanthin; however, the female *Artemia* converts the all-*trans* canthaxanthin to *cis* canthaxanthin during the time of sexual activity.

In animals the major function of carotenoids is as a precursor to the formation of vitamin A. It is assumed that in order to have vitamin A activity a molecule must have one-half of the structure similar to that of β-carotene. Recently it has been shown that astaxanthin can be converted to zeaxanthin in trout where the fish is sufficient in vitamin A. Tritiated astaxanthin was converted to retinol in strips of duodenum or inverted sacks of trout intestines. Astaxanthin, canthaxanthin, and zeaxanthin can be converted to vitamin A and A_2 in guppies.

We have become increasingly aware of a mounting body of evidence that suggests that the carotenoids can function in medical applications apart from their role as vitamin A precursors. The symptoms of erythropoietic *Protoporphyria* can be relieved by large doses of β-carotene (38). This pigment was deposited in the skin of patients who lacked skin pigmentation.

As of late some carotenoids have been claimed to be effective in the treatment of cancer, particularly cancer of epithelial origin. Other carotenoids such as canthaxanthin and phytoene with no vitamin A activity were, in cases, found to be as effective as β-carotene.

Some of the first studies were based on epidemiological studies. In one such study it was shown that the participants who consumed diets rich in carotenoids developed fewer lung cancers whether or not they smoked. This study assumed that the active ingredients are the carotenoids contained in fruits and vegetables.

More recently a number of animal studies have shown that β-carotene or other carotenoids can prevent or slow down the growth of skin cancer and other cancerous tumors. Review have been published on the role of the carotenoids in relation to cancer. Human studies are presently being conducted (39,40).

BIBLIOGRAPHY

1. O. Isler, "Introduction," in O. Isler, ed., *Carotenoids*, Birkhauser Verlag, Basel, 1971, pp. 11–27.
2. P. Karrer and E. Jucker, *Carotenoids*, Birkhauser Verlag, Basel, 1948.
3. *Plenary and Session Lectures, First International Symposium on Carotenoids Other Than Vitamin A-1*, Butterworth, London, 1967, p. 278.
4. *Plenary and Session Lectures, Second International Symposium on Carotenoids Other Than Vitamin A*, Butterworth, Kent, United Kingdom, 1969.
5. *Plenary and Session Lectures, Third International Symposium on Carotenoids Other Than Vitamin A*, Butterworth, Kent, United Kingdom, 1973, p. 130.
6. B. L. C. Weeden, ed., *Plenary and Session Lectures, Fourth International Symposium on Carotenoids—4*, Pergamon Press, Oxford, United Kingdom, 1976, p. 243.
7. T. W. Goodwin, ed., *Plenary and Session Lectures, Fifth International Symposium on Carotenoids—5*, Pergamon Press, Oxford, United Kingdom, 1979, p. 886.
8. G. Britton and T. W. Goodwin, eds., *Plenary and Session Lectures, Sixth International Symposium on Carotenoids—6*, Pergamon Press, Oxford, United Kingdom, 1982.
9. G. Britton, ed., *Plenary and Session Lectures, Seventh International Symposium on Carotenoids—7*. Pergamon Press, Oxford, United Kingdom, 1985.

10. N. I. Krinsky, M. M. Mathews-Roth, and R. F. Taylor, *Carotenoids*, Plenum, New York, 1989
11. J. C. Bauernfeind, ed., *Carotenoids as Colorants and Vitamin A Precursors. Technological and Nutritional Applications*, Academic Press, Orlando, Fla., 1981, p. 938.
12. J. C. Bauernfiend, ed., *Vitamin A Deficiency and Its Control*, Academic Press, Orlando, Fla., 1986, p. 530.
13. H. Gerster, "Anticarcinogenic Effect of Common Carotenoids," *Int. J. Vitam. Nutr. Res.* **63**, 93–121 (1993).
14. Anonymous, "Tentative Rules for the Nomenclature of Carotenoids," in O. Isler, ed., *Carotenoids*, Birkhauser Verlag, Basel, 1971, pp. 851–864.
15. "Nomenclature of Carotenoids (Rules Approved 1974)," *Journal of Pure and Applied Chemistry* **41**, 406–431 (1975).
16. H. Pfander, *Key to Carotenoids*, Birkhauser, Boston, Mass., 1987.
17. J. W. Porter and R. E. Lincoln, "I. Lycopersicon Selections Containing a High Content of Carotenes and Colorless Polyenes: II. The Mechanism of Carotene Biosynthesis," *Arch. Biochem. Biophys.* **27**, 390–403 (1950).
18. H. K. Lichtenthaler et al., "Biosynthesis of Isoprenoids in Higher Plant Chloroplasts Proceeds via a Mevalonate-Independent Pathway," *FEBS Lett.* **400**, 271–274 (1997).
19. G. Britton, "Biosynthesis of Carotenoids," in T. W. Goodwin, ed., *Chemistry and Biochemistry of Plant Pigments*, Vol. 1, 2nd ed., Academic Press, Orlando, Fla., 1976, pp. 202–327.
20. L. C. Raymundo, A. E. Griffiths, and K. L. Simpson, "Effect of Dimethyl Sulfoxide (DMSO) on the Biosynthesis of Carotenoids in Detached Tomatoes," *Phytochemistry* **6**, 1527–1532 (1967).
21. L. C. Raymundo, A. E. Griffiths, and K. L. Simpson, "Biosynthesis of Carotenoids in the Tomato Fruit," *Phytochemistry* **9**, 1239–1245 (1970).
22. M. Elahi et al., "Effect of CPTA Analogs and Other Nitrogenous Compounds on the Biosynthesis of the Carotenoids in *Phycomyces blakesleeanus* Mutants," *Phytochemistry* **14**, 133–381 (1975).
23. R. J. H. Williams, G. Britton, and T. W. Goodwin, "Biosynthesis of Cyclic Carotenes," *Biochem. J.* **105**, 99–105 (1967).
24. R. E. Tefft, T. W. Goodwin, and K. L. Simpson, "Aspects of the Stereospecificity of Torularhodin Biosynthesis," *Biochem. J.* **117**, 921–927 (1970).
25. S.-I. Huang, Ph.D. Thesis, "Inhibitory Effects of Cellulose and Nonprovitamin A Carotenoids on Beta-Carotene Utilization in Rats," University of Rhode Island, 1995.
26. T. W. Goodwin, *The Biochemistry of the Carotenoids*, Vol. 1, 2nd. ed., Academic Press, Orlando, Fla., 1980, p. 377.
27. J. Ostrander et al., "Sensory Testing of Pen Reared Salmon and Trout," *J. Food Sci.* **41**, 386–390 (1975).
28. T. Kamata and K. L. Simpson, "Study of Astaxanthin Diester Extracted from *Adonis aestivalis*," *Comp. Biochem. Physiol. B* **86**, 587–596 (1987).
29. R. S. Harris, "Effects of Agricultural Practices on the Composition of Foods," in R. S. Harris and E. Karmas, eds., *Nutritional Evaluation of Food Processing*, 2nd ed., AVI Publishing, Westport, Conn., 1957, pp 33–57.
30. L. C. Raymundo, C. O. Chichester, and K. L. Simpson, "Light Dependent Carotenoid Synthesis in the Tomato Fruit," *J. Agric. Food Chem.* **24**, 59–64 (1976).
31. C. N. Villegas et al., "The Effect of Gamma Irradiation on the Biosynthesis of Carotenoids in the Tomato Fruit," *Plant Physiol.* **50**, 694–697 (1972).
32. B H. Davies, "Analytical Methods—Carotenoids," in T. W. Goodwin, ed., *Chemistry and Biochemistry of Plant Pigments*, Vol. 2, 2nd ed., Academic Press, Orlando, Fla., 1976, pp. 38–165.
33. E. DeRitter and A. E. Purcell, "Carotenoid Analytical Methods," in J. C. Bauernfiend, ed., *Carotenoids as Colorants and Vitamin A Precursors Technological and Nutritional Applications*, Academic Press, Orlando, Fla., 1981, pp. 915–823.
34. S. E. Gebhardt, E. R. Elkins, and J. Humphrey, "Comparison of Two Methods of Determining the Vitamin A of Clingstone Peaches," *J. Agric. Food Chem.* **25**, 628–631 (1977).
35. D. B. Rodriguez-Amaya, "Assessment of Provitamin A Contents of Foods—The Brazilian Experience," *J. Food Composition and Analysis* **9**, 96–230 (1998).
36. K. L. Simpson, "Chemical Changes in Natural Food Pigments," in T. Richardson and J. W. Finley, eds., *Chemical Changes in Food During Processing*, AVI Publishing, Westport, Conn., 1985, pp 409–441
37. L. Wenhong et al., "Rapid Determination of Blood Serum Retinol by Reverse Phase Open Column Chromatography," *Int. J. Vitam. Nutr. Res.* **63**, 82–86 (1993).
38. M. M. Mathews-Roth et al., "Beta-Carotene as a Photoprotective Agent in Erythropoietic Protoporphyria," *N. Engl. J. Med.* **282**, 1231–1234 (1970).
39. R. Peto et al., "Can Dietary B-Carotene Materially Reduce Human Cancer Rates?" *Nature (London)* **290**, 201–208 (1981).
40. M M. Mathews-Roth, "Carotenoids and Cancer Prevention—Experimental and Epidemiological Studies," *Pure Appl. Chem.* **51**, 717–722 (1985).

KENNETH L. SIMPSON
University of Rhode Island
Kingston, Rhode Island

See also COLORANTS: CAROTENOIDS.

CENTRIFUGES: PRINCIPLES AND APPLICATIONS

Centrifuges are used widely in food processing to separate liquids from solids, liquids from liquids, and even to separate two immiscible liquids from the accompanying solids. Centrifuges can be blanketed with inert gas and operate under high temperatures and pressures, as well as discharge centrate under pressure to retard foam and oxidation. They rinse the mother liquor from solids. They can be cleaned in place, and some can be steam sterilized. Centrifuges may also be used to separate large particles from similar small particles when they are operated as classifiers.

Centrifuges were first used in the 1880s to separate milk from cream. They use rotational acceleration to separate heavier phases from lighter phases, similar to a gravity-settling basin. The separating force is measured in units called *g*'s, which are units of acceleration; 1 g = 980 cm/s.^{2}

Centrifuges usually generate at least 1,200 g for low-speed machines and up to 63,000 g for small tubular centrifuges. From a process standpoint, a higher g force results in a higher capacity for a given centrifuge; capacity is measured by volume throughput, degree of solids concentration, clarity of the liquid, and entrainment of one phase in another. Also, because the length of time the fluid is held under high g affects the separation, the volume of

Best ●
Not recommended ○
Not applicable ⊗

	Decanter centrifuge	High speed decanter	Disc separator	Disc nozzle	Solids ejecting	Tubular separator	Imperforate basket	Perforate basket
G Force	◑	◕	●	●	●	●	◔	◔
High flow rate	●	◑	●	●	●	◔	◑	◑
Solids dryness	●	●	⊗	○	○	◑	◑	●
High solids rate	●	◑	⊗	◑	◑	○	◔	◔
Low solids rate	◕	◕	○	◑	●	●	◑	◕
Liquid capacity	●	◑	●	●	●	◔	◑	◑
Thickening	●	●	◑	●	●	○	○	⊗
Abrasion resistance	●	◑	⊗	○	○	●	●	●
Rinse efficiency	◑	◑	◑	⊗	⊗	⊗	⊗	●
Antifoam	●	●	●	●	●	●	○	○
Pressure/sealing	●	○	◔	◔	◔	◔	○	○
Continuous	●	●	●	●	●	○	○	○
Operator attention	●	●	●	◕	◕	○	◕	◕

Figure 1. Properties of different centrifuge types.

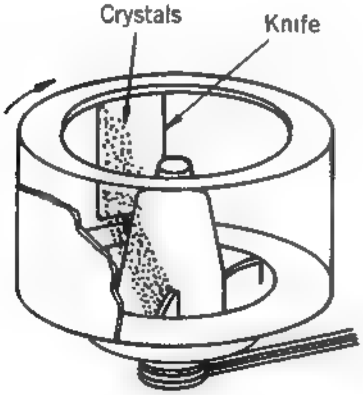


Figure 2. Basket centrifuge: imperforate bowl. Low *g* force (500–1,000 *g*) and intermittent feed limit these to applications where moderate solids dryness is needed. Capacity: 100–200 L/min.

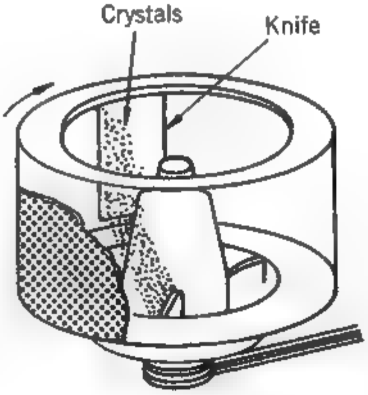


Figure 3. Basket centrifuge: perforate bowl. Same as Figure 2 except that the liquid filters through the solids. Excellent rinsing capability. Very dry cakes if the particle size is large enough to filter well.

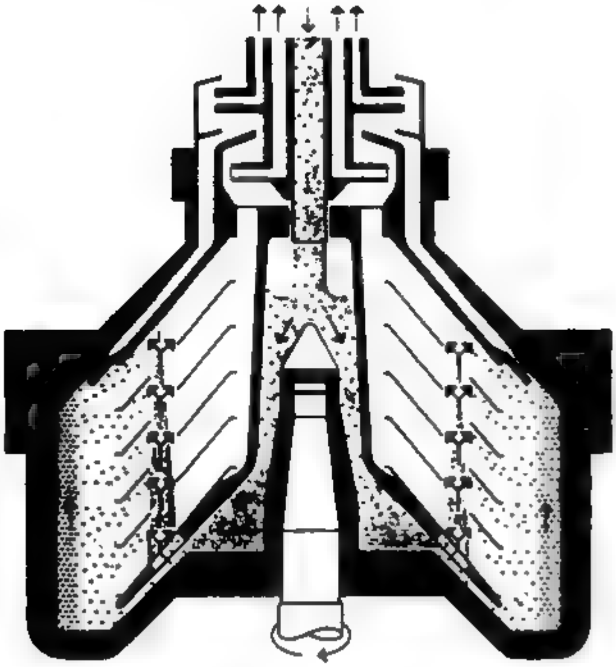


Figure 4. Disc stack centrifuge. The solid bowl disc centrifuge is a solid bowl version liquid-liquid separator operating at 5,000–10,000 *g* with some models as high as 15,000 *g*. They can handle a wide range of rates up to 2,400 L/min.

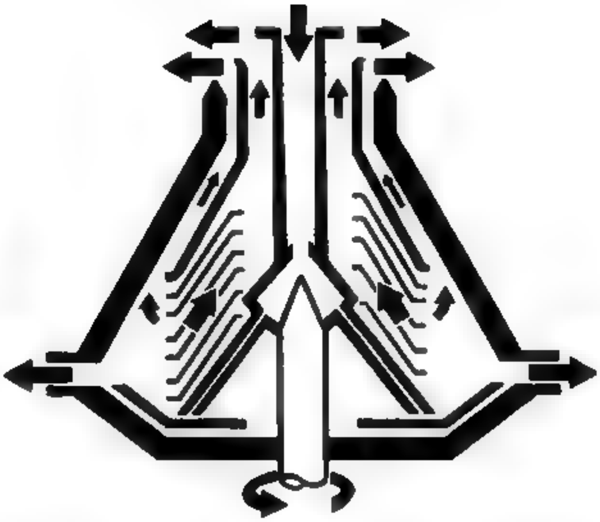


Figure 5. Disc stack centrifuge: nozzle version. Same as Figure 4 except that the solids are continuously discharged as a pumpable slurry. Rates as high as 5,000 L/min are possible.

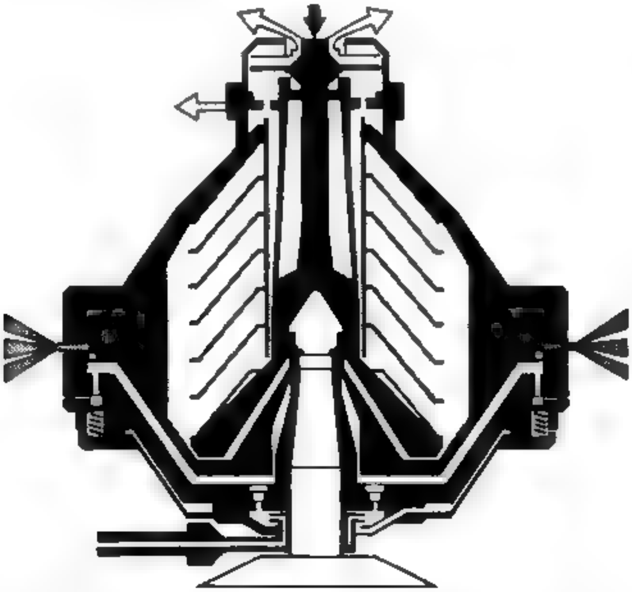


Figure 6. Disc stack centrifuge: solids ejecting version. Used where the solids volume is low, the bowl opens up intermittently to dump the solids as pumpable slurry. Maximum rates 40 L/min solids, 2,500 L/min liquid; *g* force 5,000–10,000 *g*.

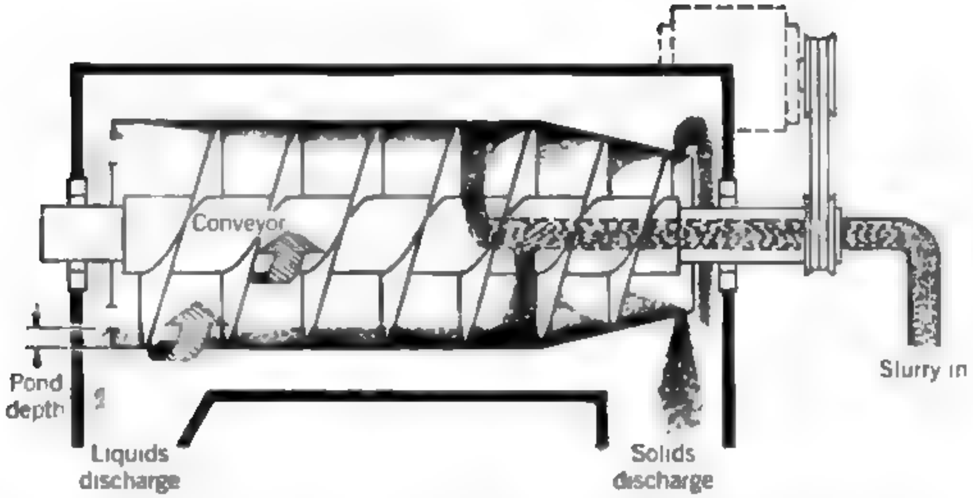

Conveyor
Pond depth
Liquids discharge
Solids discharge
Slurry in

Figure 7. Decanter centrifuges. Solid bowl decanter centrifuges develop 1,000–3,000 *g*. They are used where large volumes of solids are present and the solid phase must be as dry as possible. Rates vary from 8 to 4,000 L/min. Very high speed decanters from 5–10,000 *g* are available but rates are lower, 10–200 L/min.

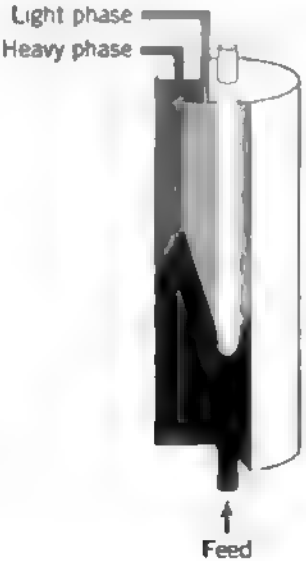

Light phase
Heavy phase
Feed

Figure 8. Tubular centrifuges. Manual disassembly to remove solids limits these to applications with little or no solids in the feed. As liquid-liquid separators, they develop much higher *g* force than most other centrifuges; maximum feed rates are 40–80 L/min.

fluid in the centrifuge directly affects its capacity. Physically, the separation follows Stokes' law for the settling velocity of a particle in a fluid, except that the acceleration of the rotational field is substituted for the earth's gravitational field.

STOKES' LAW

$$v_g = \frac{\Delta\varphi d w^2 r}{18\mu}$$

where V_g = settling velocity, w = rotational speed, r = radial position of the particle, d = diameter of particle, $\Delta\varphi$ = difference in the specific gravity of the liquid and solid, and μ = viscosity of fluid. Stokes' law is used to design separation systems because the faster the settling velocity of a particle, the greater the chance of capture will be. Generally the temperature and the particle size are, to some extent, variables in a system.

For example, in rendering the fat from animal tissues, the amount of fat separated from the tissue increases with increasing temperature. Above 45°C, the residual tissue changes character, and its value as a meat additive drops. For edible rendering, the separation is made at 45°C, for the maximum fat yield with high-value solids. Inedible rendering is done at 90–95°C where the fat yield is at a maximum, because the value of the inedible solids is quite low.

Stokes' law can be generalized as follows. Separation is improved if

1. The difference in specific gravity between the phases is large.
2. The particle size is large.
3. The viscosity is low.
4. The centrifuge speed is high.
5. The feed rate to the centrifuge is low.

Scale-up to estimate between different types of centrifuges is difficult, but within a class (eg, all disk centrifuges) a reasonably accurate scale-up can be developed. Centrifuges are usually scaled up based on *g* times volume or *g* times area. More detailed scale-up is usually left to centrifuge specialists.

SCREENING THE VARIOUS CENTRIFUGE TYPES

The goals of the separation must be defined. Centrifuges in general can:

Separate liquids from liquids.
Separate liquids from solids.
Separate solids from immiscible liquids.
Classify solids by size or density.
Rinse mother liquid from solids.

In brewing, the spent grains must be as dry as possible, the solids rate is high, and the solids are somewhat abrasive. Based on Figure 1, the following relationships can be seen.

	Solids dryness	High flow rate	Abrasion resistance
Decanter centrifuge	●	●	●
High-speed decanter	●	◑	◑
Perforate basket	●	◑	●

The decanter centrifuge is the clear choice followed by the perforate basket and, a distant third, the high speed decanter. An inquiry of decanter manufacturers would provide much data and reference accounts. The perforate basket centrifuge, in fact, is a filtration device and is not suitable for the application. Most manufacturers have laboratories where tests of new applications can be run at nominal cost. Some manufacturers also rent portable equipment to test on location.

CENTRIFUGE TYPES AND CONCLUSION

The centrifuge has wide and growing applications in the food industry for both liquid-liquid and solid-liquid separations. Advances in both speed and capacity have been made in the last few years, which increase yields and capacities. There is extensive literature on both theory and applications to aid the design engineer in equipment selection (Figs. 2–8).

Peter L. Montagne
Sharples, Inc.
Warminster, Pennsylvania

CEREALS, NUTRIENTS, AND AGRICULTURAL PRACTICES

CEREAL GRAIN PRODUCTION AND COMPOSITION

Wheat, rice, and corn were the three largest cereal grains produced in the world in 1995/1996 and 1996/1997 (Table 1). Average chemical compositions of these and other commonly consumed grains are listed in Table 2. All cereal grains contain starch as the principal component. Vitamin, mineral, fatty acids, and amino acid contents of cereal grains have been published (3). Content and nutritive value of cereal proteins depend both on seed heredity and environment during cultivation and harvest.

Table 1. Production of Cereal Grains, Million Metric Tons

	World		United States	
Grain	1995/1996	1996/1997	1995/1996	1996/1997
Wheat	536	583	59.4	62.1
Corn	514	591	187	236
Rice	551	565	7.9	7.8
Barley	142	153	7.8	8.7
Sorghum	55.2	68.2	11.7	20.4
Oats	28.7	30.6	2.4	2.2
Rye	21.9	22.2	0.26	0.23

Source: Refs. 1 and 2. Each year such as 1995/1996 covered a 12-month period.

fluid in the centrifuge directly affects its capacity. Physically, the separation follows Stokes' law for the settling velocity of a particle in a fluid, except that the acceleration of the rotational field is substituted for the earth's gravitational field.

STOKES' LAW

$$v_g = \frac{\Delta\varphi d w^2 r}{18\mu}$$

where V_g = settling velocity, w = rotational speed, r = radial position of the particle, d = diameter of particle, $\Delta\varphi$ = difference in the specific gravity of the liquid and solid, and μ = viscosity of fluid. Stokes' law is used to design separation systems because the faster the settling velocity of a particle, the greater the chance of capture will be. Generally the temperature and the particle size are, to some extent, variables in a system.

For example, in rendering the fat from animal tissues, the amount of fat separated from the tissue increases with increasing temperature. Above 45°C, the residual tissue changes character, and its value as a meat additive drops. For edible rendering, the separation is made at 45°C, for the maximum fat yield with high-value solids. Inedible rendering is done at 90–95°C where the fat yield is at a maximum, because the value of the inedible solids is quite low.

Stokes' law can be generalized as follows. Separation is improved if

1. The difference in specific gravity between the phases is large.
2. The particle size is large.
3. The viscosity is low.
4. The centrifuge speed is high.
5. The feed rate to the centrifuge is low.

Scale-up to estimate between different types of centrifuges is difficult, but within a class (eg, all disk centrifuges) a reasonably accurate scale-up can be developed. Centrifuges are usually scaled up based on *g* times volume or *g* times area. More detailed scale-up is usually left to centrifuge specialists.

SCREENING THE VARIOUS CENTRIFUGE TYPES

The goals of the separation must be defined. Centrifuges in general can:

Separate liquids from liquids.
Separate liquids from solids.
Separate solids from immiscible liquids.
Classify solids by size or density.
Rinse mother liquid from solids.

In brewing, the spent grains must be as dry as possible, the solids rate is high, and the solids are somewhat abrasive. Based on Figure 1, the following relationships can be seen.

	Solids dryness	High flow rate	Abrasion resistance
Decanter centrifuge	●	●	●
High-speed decanter	●	◑	◑
Perforate basket	●	◑	●

The decanter centrifuge is the clear choice followed by the perforate basket and, a distant third, the high speed decanter. An inquiry of decanter manufacturers would provide much data and reference accounts. The perforate basket centrifuge, in fact, is a filtration device and is not suitable for the application. Most manufacturers have laboratories where tests of new applications can be run at nominal cost. Some manufacturers also rent portable equipment to test on location.

CENTRIFUGE TYPES AND CONCLUSION

The centrifuge has wide and growing applications in the food industry for both liquid-liquid and solid-liquid separations. Advances in both speed and capacity have been made in the last few years, which increase yields and capacities. There is extensive literature on both theory and applications to aid the design engineer in equipment selection (Figs. 2–8).

Peter L. Montagne
Sharples, Inc.
Warminster, Pennsylvania

CEREALS, NUTRIENTS, AND AGRICULTURAL PRACTICES

CEREAL GRAIN PRODUCTION AND COMPOSITION

Wheat, rice, and corn were the three largest cereal grains produced in the world in 1995/1996 and 1996/1997 (Table 1). Average chemical compositions of these and other commonly consumed grains are listed in Table 2. All cereal grains contain starch as the principal component. Vitamin, mineral, fatty acids, and amino acid contents of cereal grains have been published (3). Content and nutritive value of cereal proteins depend both on seed heredity and environment during cultivation and harvest.

Table 1. Production of Cereal Grains, Million Metric Tons

	World		United States	
Grain	1995/1996	1996/1997	1995/1996	1996/1997
Wheat	536	583	59.4	62.1
Corn	514	591	187	236
Rice	551	565	7.9	7.8
Barley	142	153	7.8	8.7
Sorghum	55.2	68.2	11.7	20.4
Oats	28.7	30.6	2.4	2.2
Rye	21.9	22.2	0.26	0.23

Source: Refs. 1 and 2. Each year such as 1995/1996 covered a 12-month period.

Table 2. Average Composition of Cereal Grains (As-is)

	Barley (pearled)	Corn (field)	Oats (oatmeal)	Rice (brown)	Rye	Sorghum	Wheat[a]
Constituents (%)							
Water	10.1	10.4	8.8	10.4	11.0	9.2	13.1
Protein	9.9	9.4	16.0	7.9	14.8	11.3	12.6
Fat	1.2	4.7	6.3	2.9	2.5	3.3	1.5
Starch	57.5	65.0	50.3	69.2	57.1	65.1	55.7
Total dietary fiber	15.6	7.3	10.6	3.5	14.6	7.6	12.2
Ash	1.1	1.2	1.9	1.5	2.0	1.6	1.6
Energy value, Kcal/100 g	352	365	384	370	335	339	327

Source: Refs. 3–6.

[a] Hard red winter.

WHEAT

Dry and Wet Milling

Wheat (*Triticum vulgare*) products from dry milling hard wheat are farina, straight grade flour, patent flour, first clear flour, second clear flour, germ, bran, and shorts. Similar flour milling processes for soft and durum wheats provide products for many foods that differ from those incorporating hard wheat products, although durum farina is called semolina. A small amount of the world's wheat is processed to starch and gluten (about 80% of the total wheat flour protein) by wet milling. The baking industry is the largest user of gluten. Alkaline extraction procedures can also yield protein and starch from whole wheat (7) and mill feeds (8). Total food uses for wheat was 24 million metric tons in the United States for 1996/1997.

Nutrient Composition of Wheat Products

White wheat flour, the principal refined product of wheat milling, is the major ingredient in many foods: hard wheat flour for breads and noodles; soft wheat flour for cakes, pastries, quick breads, crackers, and snack foods; and durum semolina for spaghetti, macaroni, and other pasta products. The nutritional value of wheat foods depends mainly on the chemical composition of the flours used in their preparation. The average percentage composition of hard wheat milled products is given in Table 3. Wheat averages nearly 13% protein (the range is 7–22%). The lysine content of wheat protein varies between 2.2 and 4.2%, with a mean value of approximately 3.0%. Successful breeding of wheat for better protein quality and quantity could have an important impact on the nutrient content of wheat food products. Celiac disease, a rare allergic reaction, is an ingestion intolerance to wheat gluten (the major protein of wheat) that interferes with the absorption of nutrients.

CORN

Dry and Wet Milling

Corn (*Zea mays* L.) is processed to provide food ingredients, industrial products, feeds, alcoholic beverages, and fuel ethanol. Wet millers produce starch; modified starch products, including dextrose and syrups (by starch hydrolysis); feed products; and oil (10). Dry millers produce hull, germ, and endosperm fractions that vary in particle size and fat content. Endosperm products—grits, meal, and flour—are the primary products used by the food processors and in consumer markets (11).

Corn germ flour prepared from a commercial dry-milled fraction appears to be a promising fortifying ingredient for the food industry (12). Protein concentrates from normal and high-lysine corns (13) and from defatted dry-milled corn germ (14) are obtained by alkaline extraction of corn or corn germ and then precipitating the extracted proteins with acid.

Nutrient Composition of Corn Products

Typical yields and analyses of dry-milled products from corn have been published (11,15). About 95% of the corn produced in the United States in 1996 was yellow dent. Specialty corns such as white, waxy, hard endosperm/food grade, high-oil, high-lysine, and high-amylose are also commercially available. High-oil corn, which has about 7% oil compared with about 4.5% oil for dent corn, is the fastest growing market product among all corns in the United

Table 3. Average Percentage Composition of Dry-Milled Hard Wheat Products (As-is)

Constituent, %	Wheat	Farina	Patent flour	First clear flour	Second clear flour	Germ	Shorts	Bran
Moisture	13.1	14.2	13.9	13.4	12.4	10.5	13.5	14.1
Ash	1.6	0.4	0.4	0.7	1.2	4.0	4.1	6.0
Protein	12.6	10.3	11.0	12.7	13.5	30.0	16.0	14.5
Total dietary fiber	12.2	2.6	2.7	—[a]	—[a]	15.1	—[a]	40.5
Fat	1.5	0.8	0.9	1.3	1.3	10.0	4.5	3.3

Source. Refs. 3, 4, and 9.

[a] Not determined.

States from 1993 to 1997. High-oil corn also has higher protein and higher essential amino acids compared with regular dent corn.

RICE

Rice (*Oryza sativa* L.) consists of 18 to 28% hull and the edible portion (brown rice). After the hull is removed, the resulting brown rice is further processed by abrasive milling, which removes 6 to 10% of the weight as bran polish. The remainder is known as white or milled rice. Conventional and Japanese rice milling systems consist of precleaning, dehulling, rough rice separation, and whitening (16); the chemical composition of rice and its fractions have been listed (17). Brown rice and white rice are usually consumed as food after cooking. Rice bran, because of rancidity problems, was used exclusively as animal feed until recently. Stabilized rice bran is now commercially available in the United States, and perhaps 1% of rice bran in the United States, is used as human food.

Rice is a staple diet item in half of the world's population, but only a relatively small proportion of adverse food reactions are from rice proteins. The main allergen of rice is the 16-kDa globulin, and rice mutants containing low levels of the 16-kDa allergenic protein were obtained by γ-ray irradiation or ethyl methanesulfonate treatment (18). Another method to make hypoallergenic rice is to digest the allergenic protein with a proteolytic enzyme, Actinase (19).

OATS

Processing

Oat (*Avena sativa* L.) processing includes cleaning, dehulling, steaming, and flaking (20). Typical products from milling oats are rolled oats, oat hulls, feed oats, mixed grains and seeds, and fines. Oats are dry milled into break flour, reduction flour, shorts flours, shorts, bran, and hulls (21). Weight distribution and protein contents of dry-milled oat fractions, sequential solvent extraction of proteins from milled fractions, and amino acid compositions of milled fractions and oat extracts were also determined (21). Several commercial high-protein oat cultivars are available. Protein concentrates have been prepared from oat groats (dehulled oats) by wet-milling procedures (22). Air classification (separation of particles by size in a stream of air) of oat groats can concentrate protein in the fine fraction and concentrate β-glucan, a polysaccharide that can lower elevated serum cholesterol in humans, in the coarse fraction (23). Amylodextrins with soluble β-glucan contents as high as 10% were prepared from milled oat flours and brans by α-amylases treatment (24). These amylodextrins are used as fat substitutes in bakery goods, dairy products such as cheese, and beverages. Estimated production of oat amylodextrins was around 5 million lb in 1998.

Nutrient Composition of Oat Products

The nutritional quality of oat protein is good compared with other cereals; rolled oats have a protein efficiency ratio (PER) of 2.2 compared with the milk protein casein with a PER of 2.5. Oat groats used for food contain 11 to 15% protein. However, some dehulled oats from the Near East have protein contents of up to 25%.

BARLEY

Conventional roller milling of dehulled barley (*Hordeum vulgare* L.) gives four principal products: flour, tailings flour, shorts, and bran (25). The yields, protein contents, and amino acid composition of roller-milled barley fractions have been reported (26). Barley protein concentrates from normal and high-protein, high-lysine varieties were prepared by an alkaline extraction method (27). Air classification of flour from high-protein, high β-glucan barleys can concentrate protein in the fine fraction and concentrate β-glucan in the coarse fraction (28).

A hulless high β-glucan barley with 17% β-glucan on dry basis in flour has been reported (28). A high-lysine and high-protein barley variety, Hiproly, has been described (29). Many other high-lysine barleys have also been developed since 1970 (30). Food uses of barley include parched grain, pearled grain, flour, and ground grain. About half of the total U.S. barley crop is used for malting and the remainder is used for feed.

SORGHUM

Sorghum is a major feed grain in the United States. Most of the sorghum that is consumed throughout the world as food is prepared directly from the whole grain. Dry milling of sorghum (*Sorghum bicolor* [L.] Moench) grain ranges from cracking to debranning and degermination, which yield refined bran, germ, meal, and grit fractions (31).

Normal sorghum has the lowest lysine content per 100 g protein among the cereal grains. However, two floury lines of Ethiopian origin were exceptionally high in lysine at relatively high levels of protein (32). Another high-lysine sorghum, *P721 opaque*, was produced by treating normal grain with a chemical mutagen (33). An alkaline extraction process gives protein concentrates and starch from ground normal and high-lysine sorghum (34). Air classification of flour and hard endosperm from high-lysine sorghum yielded fractions with higher protein contents than the starting flour or hard endosperm (35).

[illegible]

Rye (*Secale cereale* L.) can be milled into flours and meals for bread, crackers, and snack foods. Rye milling is similar to wheat dry milling in general. Ground meat products may contain rye flours as fillers. However, labels for meat with grains must indicate the inclusion of these grains.

DISCLOSURE STATEMENT

Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. All programs and services of the USDA are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

BIBLIOGRAPHY

1. U.S. Department of Agriculture, *Agricultural Statistics 1997*, U.S. Government Printing Office, Washington, D.C., 1997.
2. U S. Department of Agriculture, *Agricultural Statistics 1998*, U.S. Government Printing Office, Washington, D.C., 1998
3. D. L. Drake, S. E. Gebhardt, and R. H. Matthews, "Composition of Foods: Cereal Grains and Pasta, Raw, Processed, Prepared," in *Agricultural Handbook*, Number 8-20, U.S. Department of Agriculture, Washington, D.C., 1989.
4. R. H. Matthews and P. R. Pehrsson, *Provisional Table on the Dietary Fiber Content of Selected Foods*, HNIS/PT-106, U.S. Department of Agriculture, Human Nutrition Information Service, Washington, D C., 1988.
5. R. A. Olson and K. J. Frey, eds., *Nutritional Qualities of Cereal Grains*, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WS., 1987.
6. K. E. Bach Knudsen and L. Munck, "Dietary Fibre Contents and Compositions of Sorghum and Sorghum-Based Foods," *J. Cereal Sci.* **3**, 153–164 (1985).
7. Y. V. Wu and K. R. Sexson, "Preparation of Protein Concentrate from Normal and High-Protein Wheats," *J. Agric. Food Chem.* **23**, 903–905 (1975).
8. R. M. Saunders et al., "Preparation of Protein Concentrates from Wheat Shorts and Wheat Mill Run by Wet Alkaline Process," *Cereal Chem.* **52**, 93–101 (1975).
9. Y. V. Wu and G. E. Inglett, "Effects of Agricultural Practices, Handling, Processing, and Storage on Cereals," in E. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., AVI/van Nostrand Reinhold, New York, 1988, pp. 101–118.
10. J. B. May, "Wet Milling: Process and Products," in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987, pp. 377–397.
11. R. J. Alexander, "Corn Dry Milling: Processes, Products, and Applications," in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St Paul, Minn., 1987, pp. 351–376.
12. C. W. Blessin et al., "Composition of Three Food Products Containing Defatted Corn Germ Flour," *J. Food Sci.* **38**, 602–606 (1973)
13. Y. V. Wu and K. R. Sexson, "Protein Concentrates from Normal and High-Lysine Corns by Alkaline Extraction: Preparation," *J. Food Sci.* **41**, 509–511 (1976).
14. H. C. Nielsen et al., "Corn Germ Protein Isolate—Preliminary Studies on Preparation and Properties," *Cereal Chem.* **50**, 435–443 (1973).
15. S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
16. H. T. L. van Ruiten, "Rice Milling: An Overview," in B. O. Juliano, ed., *Rice: Chemistry and Technology*, 2nd ed., American Association of Cereal Chemists, St. Paul, Minn., 1985, pp. 349–388.
17. B. O. Juliano and D. B. Bechtel, "The Rice Grain and its Gross Composition," in B. O Juliano, ed., *Rice: Chemistry and Technology*, 2nd ed., American Association of Cereal Chemists, St. Paul, Minn., 1985, pp. 17–57.
18. T. Nishio and S. Iida, "Mutants Having a Low Content of 16-kDa Allergenic Protein in Rice (*Oryza sativa* L.)," *Theoret. Appl. Genet.* **86**, 317–321 (1993).
19. M. Watanabe et al., "Production of Hypoallergenic Rice by Enzymatic Decomposition of Constituent Proteins," *J. Food Sci.* **55**, 781–783 (1990).
20. D. Deane and E. Commers, "Oat Cleaning and Processing," in F. H. Webster, ed., *Oats: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986, pp. 371–412.
21. Y. V. Wu et al., "Oats and Their Dry-Milled Fractions. Protein Isolation and Properties of Four Varieties," *J. Agric. Food Chem.* **20**, 757–761 (1972).
22. J. E. Cluskey et al., "Oat Protein Concentrates from a Wet-Milling Process: Preparation," *Cereal Chem.* **50**, 475–481 (1973).
23. Y. V. Wu and A. C. Stringfellow, "Enriched Protein- and β-Glucan Fractions from High-Protein Oats by Air Classification," *Cereal Chem.* **72**, 132–134 (1995).
24. G. E. Inglett, "Amylodextrins Containing β-Glucan from Oat Flours and Bran," *Food Chem.* **47**, 133–136 (1993).
25. Y. Pomeranz, H. Ke, and A. B. Ward, "Composition and Utilization of Milled Barley Products. I. Gross Composition of Roller-Milled and Air-Separated Fractions," *Cereal Chem.* **48**, 47–58 (1971).
26. G. S. Robbins and Y. Pomeranz, "Composition and Utilization of Milled Barley Products. III. Amino Acid Composition," *Cereal Chem.* **49**, 240–246 (1972).
27. Y. V. Wu, K. R. Sexson, and J. E. Sanderson, "Barley Protein Concentrate from High-Protein, High-Lysine Varieties," *J. Food Sci.* **44**, 1580–1583 (1979).
28. Y. V. Wu, A. C. Stringfellow, and G. E. Inglett, "Protein- and β-Glucan Enriched Fractions from High-Protein, High β-Glucan Barleys by Sieving and Air Classification," *Cereal Chem.* **71**, 220–223 (1994).
29. A. Hagberg and K. E. Karlsson, "Breeding for High Protein Content and Quality in Barley," in *Symposium: New Approaches to Breeding for Improved Plant Protein*, edited by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, International Atomic Energy Agency, Vienna, 1968, pp. 17–21.
30. R. S. Bhatty, "Nonmalting Uses of Barley," in A. W. MacGregor and R. S. Bhatty, eds., *Barley: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1993, pp. 355–417.
31. D. A. V. Dendy, *Sorghum and Millets, Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1995.
32. R. Singh and J. D. Axtell, "High Lysine Mutant Gene HL That Improves Protein Quality and Biological Value of Grain Sorghum," *Crop Sci.* **13**, 535–539 (1973).
33. D. P. Mohan and J. D. Axtell, "Diethyl Sulfate Induced High Lysine Mutants in Sorghum," *Proceedings of the Ninth Biennial Grain Sorghum Research and Utilization Conference*, Lubbock, Tex., March 4–6, 1975.
34. Y. V. Wu, "Protein Concentrate from Normal and High-Lysine Sorghums: Preparation, Composition, and Properties," *J. Agric. Food Chem.* **26**, 305–309 (1978).
35. Y. V. Wu and A. C. Stringfellow, "Protein Concentrate from Air Classification of Flour and Horny Endosperm from High-Lysine Sorghum," *J. Food Sci.* **46**, 304–305 (1981).

GENERAL REFERENCES

W. Bushuk, ed., *Rye: Production, Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1976

R. C. Hoseney, *Principles of Cereal Science and Technology*, 2nd ed., American Association of Cereal Chemists, St. Paul, Minn., 1994.

Y. Pomeranz, ed., *Wheat: Chemistry and Technology*, 3rd ed., American Association of Cereal Chemists, St. Paul, Minn., 1988.

Y. VICTOR WU
U.S. Department of Agriculture, Agricultural Research Service
Peoria, Illinois

CEREALS SCIENCE AND TECHNOLOGY

Cereal grains supply the most calories per acre than do other food sources, can be stored safely for a long time, and can be processed into many acceptable products. They are adapted to a variety of soil and climatic conditions, and can be cultivated both on a large scale mechanically with a small amount of labor, and on a garden scale almost entirely by human labor. They are excellent sources of energy and relatively good sources of inexpensive protein, certain minerals, and vitamins. More than two-thirds of the world's cultivated area is planted with grain crops. Most developing countries rely on cereals as food sources. Cereals provide more than half of the calories consumed for human energy, and in many developing countries they provide more than two-thirds of the total diet. As feeds, cereals also contribute greatly to the production of animal proteins (1–9). Total world production of cereal grains (except for sorghum where data were not available) and their production in various parts of the world are shown in Table 1.

GRAINS

Wheat

Wheat grows on almost every kind of arable land, from sea level to elevations of 3000 m, in regions where water is sufficient or areas that are relatively arid; it also thrives in well-drained loams and clays. Wheat is a basic food throughout the world. In developing countries, wheat consumption is increasing and generally accompanies rising living standards. The increase in wheat consumption comes at the expense of the more costly rice or the less costly barley or sorghum. Wheat is the most widely cultivated grain crop in temperate areas. Winter wheat may be multicropped prior to soybeans while spring wheat is often grown in rotation with barley and oats to reduce loss of soil fertility (5,10–13).

Wheat provides about one-fifth of all calories consumed by humans. It accounts for nearly 30% of worldwide grain production and for over 50% of the world trade in grains. It is harvested somewhere in the world nearly every month of the year. The United States, Canada, Australia, Argentina, and France are the main wheat exporters. Wheat production in North America and Western Europe has declined relative to that of the rest of the world since 1950. These two areas accounted for 41% of world production in 1950 but only 36% in 1951. Eastern Europe and Asia accounted for 48% of the total world production in 1950 and 56% in 1981.

The main wheat-producing areas in Asia are China, India, Turkey, and Pakistan. China produces more wheat than any other nation. In fact, wheat is second only to rice as a source of human food in China. Japan is a large wheat importer; Argentina and Australia are major wheat exporters. The United States is the fourth largest wheat-producing country behind China, the former USSR, and India.

Hexaploid wheats are the most widely grown around the world. They can be classified according to:

1. Growth habitat: spring wheats are sown in the spring and harvested in late summer. Winter wheats are sown in the fall and harvested early the next summer. For winter wheats to produce seed it must be vernalized (be subjected to freezing temperature for several days). Winter wheats are preferred by growers because of a generally higher yield potential.
2. Bran color: white (colorless), red, purple or black.
3. Kernel hardness: hard or soft; and vitreousness: glassy-vitreous or mealy–starchy–floury. In many parts of the world all bread wheats (hard and soft)

Table 1. Production of Cereal Grains, in 10^3 Metric Tons, During 1996–1997 in Various Production Areas

Production area	Wheat	Rye	Rice	Corn	Oats	Barley
North America	95,367	538	6930	266,214	6714	24,578
South America	22,276	60	11,337	56,550	865	1,285
W. Europe	99,970	5,765	1,600	34,969	7,313	52,937
E. Europe	26,300	6,151	42	25,315	2,526	9,708
F. Soviet Union	64,309	9,456	839	4,824	10,322	29,765
Africa	21,881	—	9,704	40,513	164	8,101
Asia	229,106	250	347,667	161,190	902	20,350
Oceania	23,786	20	1,006	516	1,747	7,017
Total World	*583,007*	*22,233*	*379,125*	*590,091*	*30,553*	*153,743*

Source: USDA-NASS Agricultural Statistics 1998. Data for sorghum were not available.

are called soft, and the term hard is reserved for the very hard durum wheats. In the United States the term hard is used for bread wheats; soft is reserved for wheats used to produce cookies, cakes, and so on; and durum for those tetraploid wheats used to produce alimentary pastes.

4. Geographic region of growth.
5. End use properties: protein content, physical dough properties, bread-making quality.
6. Variety.
7. Composite designations (14–17).

Corn

Corn (called maize in much of the world) is now the most popular grain for use as animal feed in temperate regions. In many tropical areas, it is a basic human food. There are five major types of corn: flint, dent, floury, pop, and sweet. Dent varieties, grown most widely in the United States, provide high yields and are used to a large extent as feed grain. Flint corn, with a somewhat higher food value, is common in Europe and South America. It is valued for its physical properties and as a raw material in the production of certain Central American foods (tortilla and arepa). Pop, sweet, and floury corn are relatively minor food crops (18–21).

In corn, the starch contains about 25% amylose and 75% amylopectin. Mutants of corn are grown in which the starch is almost entirely amylopectin (called waxy) or 70 to 80% amylose (amylomaize types). These starches vary widely in their physical properties (gelatinization characteristics, water-binding capacity, gel properties, etc) and usefulness in various foods and industrial applications.

Maize is generally deficient in the basic amino acids. However, high-lysine and high-lysine-tryptophan genetic mutants have improved the nutritional properties of corn and are now available as dent or flint corn. In the future those mutants are likely to be acceptable for the production of maize-based foods.

The United States accounts for more than half of the total world corn production and about 80% of the annual world corn exports. In the United States, most corn is used as feed or seed or in the production of alcoholic beverages. Only about 10% is used as food. This includes the production of starch, corn syrups, breakfast cereals, and various foods. Livestock feed accounts for almost 85% of the total U.S. domestic use. The main importers of U.S. corn are Western Europe and Japan, primarily for livestock feed.

Rice

Rice is the staple food of about half of the human race, providing more than one-fifth of the total food calories consumed by the people of the world. Most rice is produced in the Far East and is primarily consumed within the borders of the country of origin. Asia, which has nearly 60% of the world population, produces and consumes about 90% of the world rice production. The United States produces less than 2% of the world crop, but accounts for about 30% of the world rice trade (22,23).

Rice varieties vary in their kernel shape and properties. They are classified as long, medium, and short grain. Long-grain rice accounts for about 50%, medium-grain rice for 40%, and short-grain rice for about 10% of the U.S. rice production. California is practically the exclusive producer of short-grain rice. California and Louisiana lead in medium-grain rice production. Growers in Arkansas, Texas, and Mississippi produce mostly long-grain rice.

Barley

Barley is a winter-hardy and drought-resistant grain. It matures more rapidly than do wheat, oats, or rye and is used mainly as feed for livestock and in malting and distilling industries. The two main types of barley, two-row and six-row, differ in the arrangement of grains in the ear. The former predominates in Europe and parts of Australia; the latter is more resistant to extreme temperatures and is grown in North America, India, and the Middle East (24,25).

Although barley is grown throughout the world, production is concentrated in the northern latitudes. Since 1950 the world production of barley tripled with most of its increase in Europe. The former USSR is the largest barley producer. Barley is adaptable to a variety of conditions and is produced commercially in 36 states in the United States. In the United States, the use of barley as a livestock feed is declining, while the use for processing into malt is increasing.

Oats

Oats are grown most successfully in cool, humid climates and on neutral to slightly acidic soil. The bulk of the crop is consumed as animal feed (primarily for horses). A covered cereal, the oat hull must first be removed before further processing. The resulting product is called a groat. Heavy oats (with high groat to hull ratios) are processed into rolled oats, breakfast foods, and oatmeal (26).

The world production of oats is about one-tenth of the world production of wheat and slightly over one-fourth of the world production of barley. The chief oat-producing states in the United States are in the north-central Corn Belt and farther northward. About 90% of the oat is left on the farm as a feed, and only a small proportion is processed as food or used for industrial products. The use of oats in food processing is increasing, especially in breakfast cereals.

Sorghum

Sorghum is the fifth most widely grown grain in the world. However, sorghum production is less than 5% of the total grain production. There has been a small increase in the area planted and harvested with sorghum, but the increase in production has been substantial because of increases in yield. This has resulted from the development of high-yielding hybrids. The primary sorghum-producing regions are Asia, Africa, and North America. The primary sorghum-producing countries are the United States, China, India, Argentina, Nigeria, and Mexico. These countries produce more than 75% of the world total, with the United States producing about 30%.

More than 50% of the worldwide production of sorghum is destined for human consumption. However, in the United States, sorghum is grown almost entirely as a feed grain for local use or for export. Sorghum is produced under a wide range of climatic conditions. It tolerates limited moisture conditions and adapts to high temperature conditions (27,28).

The nucellar tissue of some varieties of sorghum contains pigments that complicate production of acceptable and white sorghum starch. Varieties with no nucellar tissue persisting to maturity could be used for starch production, but the ready availability of corn and other problems with the wet-milling of sorghum has discouraged such attempts. Waxy (starch) sorghums are potentially promising for special food uses. Some sorghums are rich in condensed tannins. This reduces both their palatability and feed value.

Rye

Rye is characterized by its good resistance to cold, pests, and diseases, but it cannot compete with wheat or barley on good soils and under improved cultivation practices. Approximately 90% of the world's rye production is in Europe (mainly in Poland and Germany) and in the former USSR. Rye is considered a grain for use in bread in Europe, but its proportion in mixed wheat-rye bread is decreasing. Much of the rye is used as a feed grain and a small proportion in the distilling industry (29).

PHYSICAL PROPERTIES AND STRUCTURE

Optimal utilization of cereal grains requires knowledge of their structure and composition. The practical implications of kernel structure and composition are numerous (30–32). They relate to the various stages of grain production, harvest, storage, marketing, and use.

Some physical properties of cereal grains are listed in Table 2 (33). Table 3 summarizes approximate grain size and the proportions of the principal parts comprising the mature kernels of different cereals (34).

Kernel Structure

The cereal grain is a one-seeded fruit (called a caryopsis), in which the fruit coat adheres to the seed at maturity. As the fruit ripens, the pericarp (fruit wall) becomes firmly attached to the wall of the seed proper. The pericarp, seed coats, nucellus, and aleurone cells form the bran. The embryo comprises only a small part of the seed. The bulk of the seed is taken up by the endosperm, which is a food reservoir for the germinating plant.

The floral envelopes (modified leaves known as lemma and palea), or chaffy parts, within which the caryopsis develops, persist to maturity in the grass family If the chaffy structures envelop the caryopsis so closely that they remain attached to it when the grain is threshed (as with rice and most varieties of oats and barley), the grain is considered to be covered. However, if the caryopsis readily separates from the floral envelopes when the grain is threshed, as with common wheats, rye, hull-less barleys, and the common varieties of corn, these grains are considered to be naked.

Wheat. The structure of the wheat kernel is shown in Figure 1 (35). The dorsal or back side of the wheat grain is rounded, while the ventral side has a deep groove or crease along the entire longitudinal axis. At the apex or small end (stigniatic end) of the grain is a cluster of short, fine hairs known as brush hairs. The pericarp, or dry fruit coat, consists of four layers: the epidermis, hypodermis, cross cells, and tube cells. The remaining tissues of the grain are the inner bran (seed coat and nucellar tissue), endosperm, and embryo (germ). The aleurone layer consists of large rectangular, thick-walled, starch-free cells. Botanically, the aleurone is the outer layer of the endosperm, but as it tends to remain attached to the outer layers during wheat milling. It is shown in the diagram as the innermost bran layer.

The embryo (germ) consists of the plumule and radical, which are connected by the mesocotyl. The outer layer of the scutellum, the epithelium, may function as both a secretory or an absorptive organ. In a well-filled wheat kernel, the germ comprises about 2 to 3% of the kernel, the bran 13 to 17%, and the endosperm the remainder. The inner bran layers (aleurone) are high in protein, whereas

Table 2. Some Physical Properties of Cereal Grains

Name	Length (mm)	Width (mm)	Grain mass (mg)	Bulk density (kg/m³)	Unit density (kg/m³)
Rye	4.5–10	1.5–3.5	21	695	—
Sorghum	3–5	2.5–4.5	23	1360	—
Paddy rice	5–10	1.5–5	27	575–600	1370–1400
Oats	6–13	1–4.5	22	356–520	1360–1390
Wheat	5–8	2.5–4.5	37	790–825	1400–1435
Barley	8–14	1–4.5	37	580–660	1390–1400
Corn	8–17	5–15	285	745	1310
Bulirush millet	3–4	2–3	11	760	1322
Wild rice	8–20	0.5–2	22	388–775	—
Tef	1–1.5	0.5–1	0.3	880	—
Findi	1	1–1.5	0.4	790	—
Finger millet	1.5	1.5	—	—	—

Source. Ref. 31.

Table 3. Approximate Grain Size and Proportions of the Principal Parts Comprising the Mature Kernel of Different Cereals

Cereal	Grain mass (mg)	Embryo (%)	Scutellum (%)	Pericarp (%)	Aleurone (%)	Endosperm (%)
Barley	36–45	1.85	1.53	18.3	79.0	
Bread wheat	30–45	1.2	1.54	7.9	6.7–7.0	81–84
Durum wheat	34–46	1.6		12.0	86.4	
Maize	150–600	1.15	7.25	5.5		82
Oats	15–23	1.6	2.13	28.7–41.4		55.8–68.3
Rice	23–27	2–3	1.5	1.5	4–6	89–94
Rye	15–40	1.8	1.73	12.0	85.1	
Sorghum	8–50	7.8–12.1		7.3–9.3		80–85
Triticale	38–53	3.7		14.4	81.9	

Source: Ref. 32

the outer bran (pericarp, seed coats, and nucellus) is high in cellulose, hemicelluloses, and minerals. The germ is high in proteins, lipids, sugars, and minerals; the endosperm consists largely of starch granules embedded in a protein matrix. The structure of rice, barley, oats, rye, and triticale are similar in structure to wheat, except that rice has no crease.

Rice. Rice is a covered cereal. In the threshed grain (termed rough or paddy rice), the kernel is enclosed in a tough siliceous hull that renders it unsuitable for human consumption. Once this hull is removed during processing, the kernel (or caryopsis), comprised of the pericarp (outer bran) and the seed proper (inner bran, endosperm, and germ), is known as brown rice or sometimes as unpolished rice. Brown rice is in little demand as a food. Unless stored under favorable conditions, it tends to become rancid and is more subject to insect infestation than are the various forms of milled rice. When brown rice is milled further, the bran and germ are removed to produce the purified endosperm marketed as white rice or polished rice. Milled rice is classified according to size as head rice (whole endosperm) and various classes of broken rice, known as second head, screenings, and brewer's rice, in order of decreasing size.

Corn. The corn grain is the largest of all cereals (Fig. 2) (35). The kernel is flattened, wedge shaped, and broader at the apex than at its attachment to the cob. The aleurone cells contains relatively high levels of protein and oil. They also contain the pigments that make certain varieties appear blue, black, or purple. Each corn kernel contains two types of starchy endosperms, horny (vitreous) and floury (opaque). The horny endosperm is tightly packed. The starch granules in this region are polyhedral in shape, In dent corn varieties, horny endosperm is located on the sides and back of the kernel and bulges toward the center at the sides. The floury endosperm fills the crown (upper part) of the kernel and extends downward to surround the germ. The starch granules in the floury endosperm are ellipsoidal in shape. As dent corn matures, the endoperm shrinks causing an indentation at the top of the kernel. In a typical dent corn, the pericarp comprises about 6%, the germ, 11%, and the endosperm, 83% of the kernel by weight. Flint corn varieties contain a higher ratio of horny to floury endosperm than do dent corn.

Barley. The husks of barley are cemented to the kernel and remain attached after threshing. The husks protect the kernel from mechanical injury during commercial malting, strengthen the texture of steeped barley, and contribute to more uniform germination of the kernels. The husks are also important as a filtration bed in the separation of extract components during mashing and contribute to the flavor and astringency of beer. The main types of cultivated covered barley, differ in the arrangement of grains in the ear, two-rowed and six-rowed ear. The axis of the barley ear has nodes throughout its length. The nodes alternate from side to side. In the six-rowed types of barley, three kernels develop on each node, one central kernel and two lateral kernels. In the two-rowed barley, the lateral kernels are sterile and only the central kernels develop.

The kernel of covered barley consists of the caryopsis and the flowering glumes (or husks). The husks consist of two membranous sheaths that completely enclose the caryopsis. During development of the growing barley, a cementing substance causing adherence is secreted by the caryopsis within the first two weeks after pollination. The husk in cultivated malting barley amounts to about 8 to 15% of the grain. The proportion varies according to type, variety, grain size, and climatic conditions. Large kernels have less husk than small kernels. The husk in two-rowed barley is generally lower than that in six-rowed barley. As in wheat, the caryopsis of barley is a one-seeded fruit in which the outer pericarp layers enclose the aleurone, the starchy endosperm, and the germ. The aleurone layer in barley is at least two cell layers thick; in other cereal grains (rice excepted), it is one cell layer thick.

Oats. The common varieties of oats have the fruit (caryopsis) enveloped by a hull composed of the floral envelopes. Naked or hull-less oat varieties are known but not grown extensively. In light thin oats, hulls may comprise as much as 45% of the grain. In very heavy or plump oats, the hull may represent only 20%. The hull normally makes up about 30% of the grain. Oat kernels, obtained by removing hulls, are called groats.

Sorghum. Sorghum kernels are generally spherical to flattened spheres, have a kernel mass of 20 to 30 mg, and may be white, yellow, brown, or red. Sorghum has both vitreous and opaque regions within the kernel similar to that of corn. Sorghum grains contain polyphenolic com-

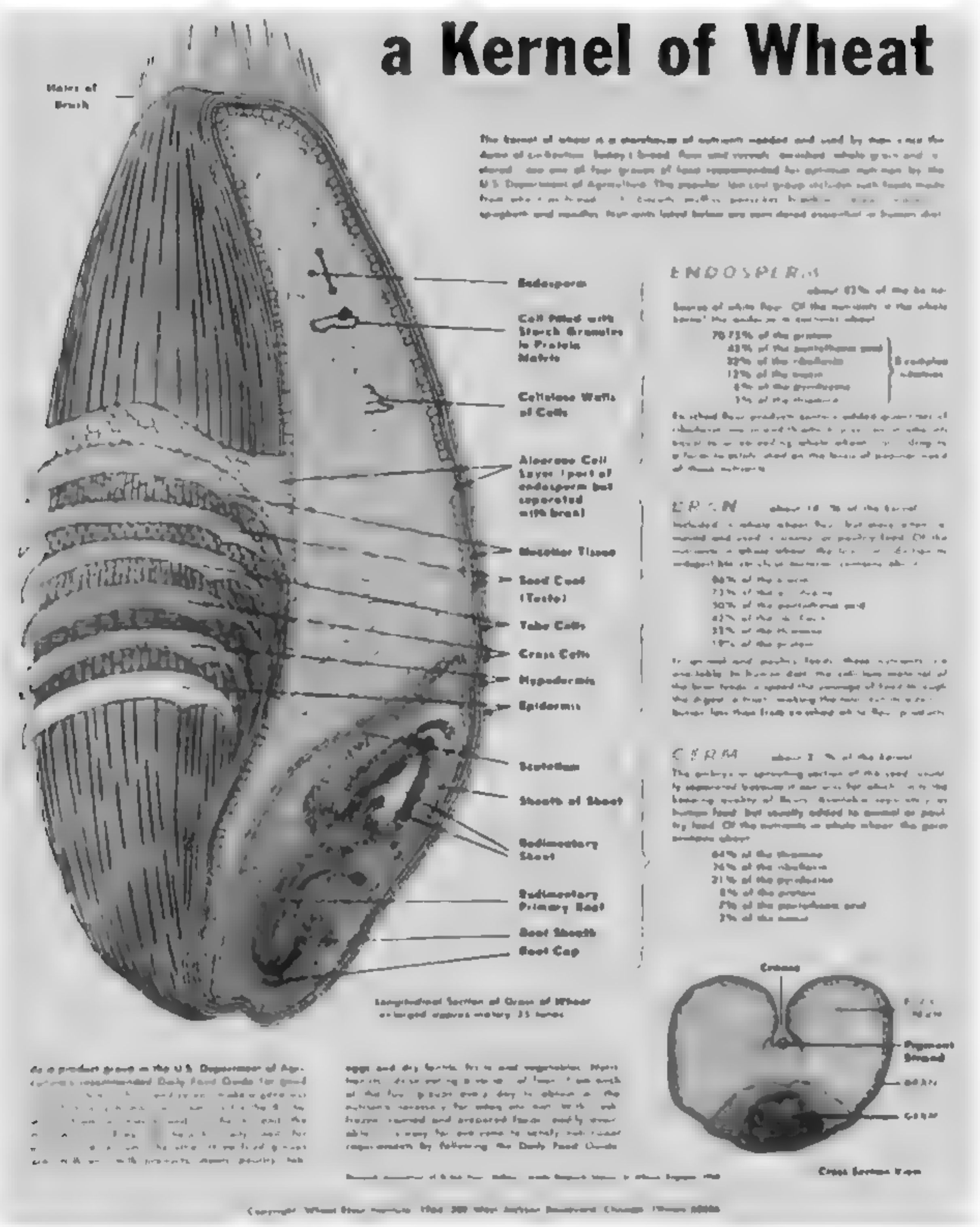

a Kernel of Wheat
Hairs of Brush
ENDOSPERM
BRAN
GERM
Endosperm
Cell Filled with Starch Granules in Protein Matrix
Cellulose Walls of Cells
Aleurone Cell Layer (part of endosperm but separated with bran)
Nucellar Tissue
Seed Coat (Testa)
Tube Cells
Cross Cells
Hypodermis
Epidermis
Scutellum
Sheath of Shoot
Rudimentary Shoot
Rudimentary Primary Root
Root Sheath
Root Cap
Longitudinal Section of Grain of Wheat enlarged approximately 35 times
Crease
Pigment Strand
BRAN
GERM
Cross Section View

Figure 1. A wheat kernel. Longitudinal section (enlarged ca 12×). *Source:* Ref. 35.

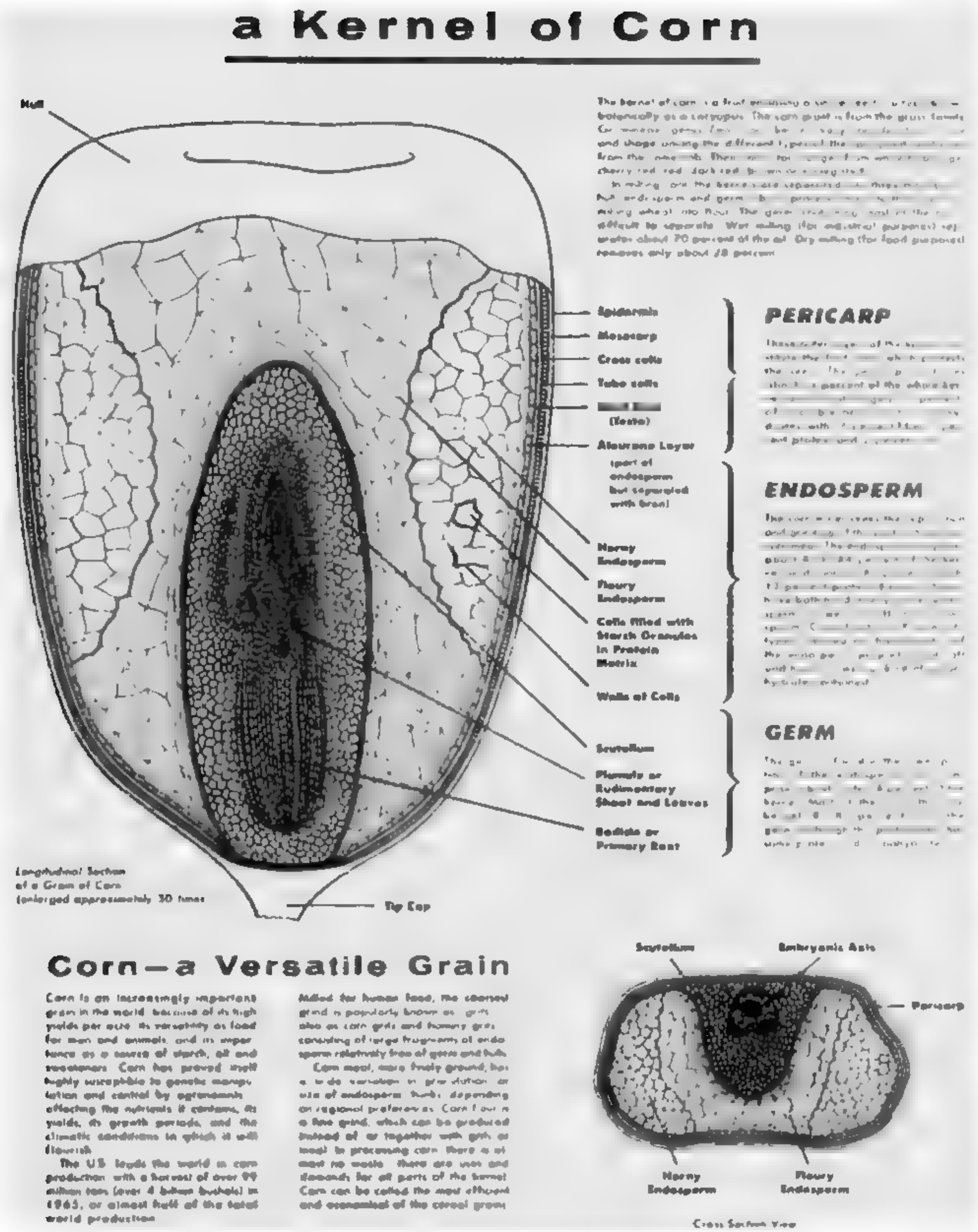

a Kernel of Corn
Hull
Epidermis
Mesocarp
Cross cells
Tube cells
(Testa)
Aleurone Layer (part of endosperm but separated with bran)
Horny Endosperm
Floury Endosperm
Cells filled with Starch Granules in Protein Matrix
Walls of Cells
Scutellum
Plumule or Rudimentary Shoot and Leaves
Radicle or Primary Root
Tip Cap
Longitudinal Section of a Grain of Corn (enlarged approximately 30 times)
PERICARP
ENDOSPERM
GERM
Corn—a Versatile Grain
Scutellum
Embryonic Axis
Pericarp
Horny Endosperm
Floury Endosperm
Cross Section View

Figure 2. A corn kernel. Longitudinal section (enlarged ca 8×). *Source:* Ref. 35.

pounds, primarily in the outer kernel layers and to some degree in the endosperm. These compounds are colored and impart various colors to certain foods from sorghums. Condensed tannins, present only in some sorghum lines, are polyphenolic compounds that interact with and precipitate protein. Those tannins impart a bird resistance to the sorghum. This may be important if the sorghum is grown in small plots in a forest (such as parts of Africa). However, the tannins also reduce nutritional value and germinability.

Composition

The chemical composition of the dry matter of different cereal grains, as with other foods of plant origin, varies widely (36). A proximate analysis of the principal cereal grains is summarized in Table 4. Variations are encountered in the relative amounts of proteins, lipids, carbohydrates, pigments, vitamins, and ash. Mineral elements that are present and the quantities of them also vary widely. As a food group, cereals are characterized by relatively low protein and high carbohydrate contents. The carbohydrates consist of starch (90% or more of the total), pentosans, and simple sugars (monosaccharides).

The various components are not distributed uniformly through the different kernel structures. The hulls and pericarp are high in cellulose, pentosans, and ash; the germ is high in lipid and rich in proteins, sugars, and ash components. The endosperm contains the starch and is lower in protein content than the germ and, in some cereals, than bran. It is also low in crude fat and ash. As a group, cereals are low in nutritionally important calcium. Its concentration and that of other ash components is greatly reduced by the milling processes used to prepare refined flours and grits. In these processes, hulls, germ, and bran, which are the structures rich in minerals and vitamins, are in large part removed.

All cereal grains contain vitamins of the B group, but all are completely lacking in vitamin C (unless the grain is sprouted) and vitamin D. Yellow corn differs from white corn and the other cereal grains in containing carotenoid pigments (principally cryptoxanthin, with smaller quantities of carotenes). These are convertible by the body to vitamin A. Wheat also contains yellow pigments, but they are almost entirely xanthophylls, which are not precursors of vitamin A. The oils of the embryos of cereal grains are rich sources of vitamin E. The relative distribution of vitamins in the kernel is not uniform, although the endosperm invariably contains the lowest concentration.

Table 4. Approximate Analysis of Important Cereal Grains, Percentage of Dry Mass

Cereal	Nitrogen	Protein	Fat	Fiber	Ash	NFE
Barley (grain)	1.2–2.2	11	2.1	6.0	3.1	—
(kernel)	1.2–2.5	9	2.1	2.1	2.3	78.8
Maize	1.4–1.9	10	4.7	2.4	1.5	72.2
Millet	1.7–2.0	11	3.3	8.1	3.4	72.9
Oats (grain)	1.5–2.5	14	5.5	11.8	3.7	—
(kernel)	1.7–3.9	16	7.7	1.6	2.0	68.2
Rice (brown)	1.4–1.7	8	2.4	1.8	1.5	77.4
(milled)	—	—	0.8	0.4	0.8	—
Rye	1.2–2.4	10	1.8	2.6	2.1	73.4
Sorghum	1.5–2.3	10	3.6	2.2	1.6	73.0
Triticale	2.0–2.8	14	1.5	3.1	2.0	71.0
Wheat (bread)	1.4–2.6	12	1.9	2.5	1.4	71.7
(durum)	2.1–2.4	13	—	—	1.5	70.0
Wild rice	2.3–2.5	14	0.7	1.5	1.2	74.4

Note. Typical or average figure. NFE = nitrogen free extract (an approximate measure of the starch content).
Source. Ref. 32.

The protein contents of wheat and barley are important quality indices for the manufacture of various foods. For example, the quantity and quality of wheat protein is critical in bread making. Cereal grains contain water-soluble proteins (albumins), salt-soluble proteins (globulins), aqueous alcohol-soluble proteins (prolamins), and acid- and alkali-soluble proteins (glutelins). The prolamins are characteristic of the grass family, and together with the glutelins, comprise the bulk of the proteins of cereal grains. The following are names given to prolamins in proteins of the cereal grains: gliadins in wheat, hordeins in barley, zeins in maize, avenins in oats, kaffirins in grain sorghum, and secalins in rye.

The various proteins are not distributed uniformly in the kernel. Thus, the proteins isolated from the inner endosperm of wheat consist chiefly of prolamins (gliadin) and glutelins (glutenin). The embryo proteins consist of nucleoprotein, an albumin (leucosin), and globulins, whereas in wheat bran a prolamin predominates with smaller quantities of albumins and globulins. In the presence of water, the wheat endosperm proteins, gliadin, and glutenin form a tenacious colloidal complex known as gluten. Gluten is mostly responsible for the superiority of wheat over the other cereals in the manufacture of leavened products because it makes possible the formation of a dough that retains the carbon dioxide produced by yeast or chemical leavening agents. The gluten proteins collectively contain 17.55% nitrogen. Consequently in estimating the crude protein content of wheat and wheat products from the determination of total nitrogen, the factor 5.7 is normally employed rather than the customary value of 6.25. The latter value is based on the assumption that proteins contain an average of 16% nitrogen.

In general, cereal proteins are not as high in biological value as are those of certain legumes, nuts, or animal products. Most cereals are low in lysine and tryptophan. Oats are a notable exception to this generalization. The biological value of whole cereal grains is greater than that of refined milled products, which consist chiefly of the endosperm. Studies have shown that adults, if satisfying their calorie needs, will not be protein deficient on nearly 100% cereal diets. This may not be true for young children or pregnant women. In general, American and Western European diets normally include animal products as well as cereals and, thus, satisfy protein requirements many times over. Under these conditions, the different proteins tend to supplement each other.

The predominant form of carbohydrate in cereals is starch, which is the primary source of calories provided by the grains. Most of the carbohydrates are in the starchy endosperm.

Table 5. FDA Cereal Enrichment Standards

Product	Thiamine (mg/lb)	Riboflavin (mg/lb)	Niacin (mg/lb)	Iron (mg/lb)	Calcium (mg/lb)	Vitamin D (1U/lb)
Flour[a]	2.9	1.8	24	20	(960)	
Self-rising flour	2.9	1.8	24	20	960	
Corn grits	2.0–3.0	1.2–1.8	16–24	13–26	(500–750)	(250–1000)
Corn meal	2.0–3.0	1.2–1.8	16–24	13–26	(500–750)	(250–1000)
Rice	2.0–4.0	1.2–2.4	16–32	13–26	(500–1000)	(250–1000)
Macaroni products	4.0–5.0	1.7–2.2	27–34	13–16.5	(500–625)	(250–1000)
Noodle products	4.0–5.0	1.7–2.2	27–34	13–16.5	(500–625)	(250–1000)
Bread[a]	1.8	1.1	15	12.5	(600)	
U.S. RDA (mg or IU/day)	1.5	1.7	20	18	1000	400

[a] In addition, flour is to contain 0.7 mg/lb and bread 0.43 mg/lb of folic acid. The figures in this table are the nutrient levels to be present in the final products as set forth by the Food and Drug Administration (21 CFR 101.9, 137 and 139). Figures in parentheses are optional. When figures are shown as a range, these indicate minimum/maximum values. When one figure is shown, it is the minimum required. Calcium is added by the milling company. It cannot be included in the enrichment product. Enriched pasta products are normally made from flours enriched to these levels. Macaroni products with fortified protein must have the maximum figures but no Vitamin D. If enriched bread is manufactured using at least 62.5% of enriched flour, the bread will meet standards for enriched bread.

U.S. RDA (recommended daily allowances) as published in the 1986 21 CFR 104.20.

The lipids in cereals are relatively rich in the essential fatty acid linoleic acid. Saturated fatty acids (mainly palmitic) represent less than 25% of the total fatty acids of most grains.

In summary, cereal grains are a diverse yet primary source of nutrients. Their high starch contents make them major sources of calories. They also contribute to human needs for proteins, lipids, vitamins, and minerals. Vitamins and minerals lost during milling to produce refined food products can be, and in many countries are, replaced by nutrient fortification (Table 5). The composition of cereal grains and their milled products makes them uniquely suited for producing wholesome, nutritional, and consumer-acceptable foods.

STANDARDS AND CLASSIFICATION

At the turn of the century, many organizations in the United States and in several other grain-producing countries tried to develop uniform grain standards. The demand for uniform grades and application of the standards resulted in the introduction during 1903 to 1916 of 26 bills calling for the same in the U.S. Congress.

Those bills and the extensive hearings that accompanied them culminated in the U.S. Grain Standard Act, which was passed August 11, 1916, and amended in 1940 to include soybeans. This act provides in part for the establishment of official grain standards, the federal licensing and supervision of the work of grain inspectors, and the mechanisms for filing appeals concerning grades assigned by licensed inspectors. The official U.S. standards for grain cover wheat, corn, barley, oats, rye, sorghum, flaxseed, soybeans, mixed grain, and triticale.

According to U.S. grain standards, wheat is divided into seven classes on the basis of type and color: hard red spring, durum, red durum, hard red winter, soft red winter, white, and mixed. The division of wheat into classes and subclasses is according to suitability of different wheats for specific uses. Hard red spring, hard red winter, and hard white wheats are valued for the production of flours used in making yeast-leavened breads. Soft red winter, soft white, and white club wheats are especially suited for making chemically leavened baked goods (cookies, cakes). Amber durum wheat is prized for the manufacture of semolina used to produce alimentary pastes (macaroni, vermicelli, etc). Red durum is used primarily as a poultry and livestock feed.

The wheat in each subclass is sorted into a number of grades on the basis of quality and condition. The tests generally used to determine the grade of grain and its conformity to the official U.S. grain standards include plumpness, soundness, cleanliness, dryness, purity of type, and the general condition of the grain (37,38).

Other cereals are graded in a similar manner. Corn is divided into three classes: yellow, white, and mixed. These class designations apply to dent-type corn only. If the corn consists of at least 95% flint varieties, the word flint is used. If the corn contains more than 5% but less than 95% flint varieties, the corn is designated as flint and dent. Waxy corn is corn of any class consisting of at least 95% waxy corn. For many years moisture content was a grading factor for corn and sorghum. Especially at the beginning of the harvest, moisture content is the single most important quality factor of corn (high being problematic). Broken corn and foreign material (BCFM) is determined by sieving through a 12/64 in. (4.76 mm) sieve.

Rough rice is divided into three classes: long, medium, and short. There are separate standards for rough, brown, and milled rice. All rough rice is graded on the basis of milled rice yields.

Barley is divided into three classes (with several subclasses): six-rowed (malting, blue malting, and barley), two-rowed (two-rowed malting and two-rowed), and barley. Plumpness is determined by sieving. The designation malting requires inclusion of only varieties that have been approved as suitable for malting purposes. Special grades and designations of oats are: heavy or extra heavy (test weights of 48.9–51.5 kg/hL and >51.5 kg/hL, respectively), thin, bleached, bright, ergoty, garlicky, smutty, tough (moisture between 14 and 16%), and weevily.

BIBLIOGRAPHY

1. Canadian International Grains Institute, *Grain and Oilseeds, Handling, Marketing, Processing*, 2nd ed., Canadian International Grains Institute, Winnipeg, Canada, 1975.
2. R. C. Hoseney, *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986.
3. D. W. Kent-Jones and A. J. Amos, *Modern Cereal Chemists*, 6th ed., Food Trade Press, London, United Kingdom, 1967.
4. R. A. Olson and K. J. Frey, eds., *Nutritional Quality of Cereal Grains*, American Society of Agronomy, Madison, Wis., 1987.
5. Y. Pomeranz, *Modern Cereal Science and Technology*, VCH Publishers, New York, 1987.
6. Y. Pomeranz, ed., *Industrial Uses of Cereals*, American Association of Cereal Chemists, St. Paul, Minn., 1973.
7. Y. Pomeranz, ed., *Advanced Cereal Science and Technology*, Vols. I–X, American Association of Cereal Chemists, St. Paul, Minn., 1976–1990.
8. Y. Pomeranz, ed., *Cereals '78: Better Nutrition for the World's Millions*, American Association of Cereal Chemists, St. Paul, Minn., 1978.
9. Y. Pomeranz and L. Munck, eds., *Cereals: A Renewable Resource, Theory and Practice*, American Association of Cereal Chemists, St. Paul, Minn., 1981.
10. H. Hanson, N. E. Bourlaug, and R. G. Anderson, *Wheat in the Third World*, Westview Press, Boulder, Colo., 1982.
11. E. G. Heyne, ed., *Wheat and Wheat Improvement*, 2nd ed., American Society of Agronomy, Madison, Wis., 1987.
12. Y. Pomeranz, ed., *Wheat is Unique*, American Association of Cereal Chemists, St. Paul, Minn., 1989.
13. Y. Pomeranz, ed., *Wheat Chemistry and Technology*, 3rd ed., American Association of Cereal Chemists, St Paul, Minn., 1988.
14. N. L. Kent, *Technology of Cereals With Special Reference to Wheat*, Pergamon, Oxford, United Kingdom, 1960.
15. I. D. Morton, ed., *Cereals in a European Context*, VCH Publishers, New York, 1987.
16. W. R. Akroyd and J. Doughty, *Wheat in Human Nutrition*, FAO Nutritional Studies No. 23, FAO, Rome, Italy, 1970.
17. J. Holas and J Kratochvil, eds., *Progress in Cereal Chemistry and Technology*, 2 Elsevier Science, New York, 1983.
18. R. W. Jugenheimer, *Corn: Improvement, Seed Production and Uses*, John Wiley & Sons, New York, 1976.
19. P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press, Orlando, Fla., 1983.
20. M N Leath, L. H. Meyer, and L. D. Hill, *U.S. Corn Industry*, Report No. 479, U.S. Department of Agriculture, Economic Research Service, Washington, D.C., 1982.
21. S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
22. B. O. Juliano, ed., *Rice, Chemistry and Technology*, 2nd ed., American Association of Cereal Chemists, St. Paul, Minn., 1987
23. B. S. Luh, ed., *Rice: Production and Utilization*, AVI Publishing, Westport, Conn., 1980.
24. W. G. Heid, *U.S. Barley Industry*, Agr. Econ. Report No. 395, U.S. Department of Agricultural Economics, Statistics and Cooperative Service, Washington, D.C., 1978.
25. D. C. Rasmusson, ed., *Barley*, American Society of Agronomy Madison, Wis., 1985.
26. F. H. Webster, ed., *Oats, Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986.
27. H. Doggett, *Sorghum*, Longmans, Green, and Co., London, United Kingdom, 1970.
28. L. W. Rooney, D. S. Murthy, and J. V. Mertui, eds., *International Symposium on Sorghum Grain Quality*, Proceedings, INCRASAT Patancheru, A. P. India, 1982.
29. W. Bushuk, ed., *Rye: Production, Chemistry, and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
30. *Yearbook of Agriculture*, U.S. Government Printing Office, Washington, D.C., 1985.
31. Y. Pomeranz, *Functional Properties of Food Components*, Academic Press, Orlando, Fla., 1985.
32. J. E. Kruger, D. R. Lineback, and C. E. Stauffer, eds., *Enzymes and Their Role in Cereal Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
33. H. G. Muller, "Some Physical Properties of Cereals and Their Products as Related to Potential Industrial Utilization," in Y. Pomeranz, ed., *Industrial Uses of Cereals*, American Association of Cereal Chemists, St. Paul, Minn., 1973, pp. 20–50.
34. D. H. Simmonds, "Structure, Composition, and Biochemistry of Cereal Grains," in Y. Pomeranz, ed., *Cereals '78 Better Nutrition for the World's Millions*, American Association of Cereal Chemists, St. Paul, Minn., 1978, pp. 105–137.
35. *From Wheat to Flour*, Wheat Flour Institute, Chicago, Ill., 1965.
36. B. Holland, I. D. Unwin, and D. H. Buss, *Cereals and Cereal Products*, Royal Society of Chemistry, Ministry of Agriculture, Fisheries, and Food, London, United Kingdom, 1988.
37. C. M Christensen, ed., *Storage of Cereal Grains and Their Products*, 3rd ed., American Association of Cereal Chemists, St. Paul, Minn., 1982
38. J. L. Multon, ed., *Preservation and Storage of Grains, Seeds, and Their By Products*, Lavoisier Publishing, New York, 1988.

R. CARL HOSENEY
R & R Research Services
Manhattan, Kansas

See also WHEAT SCIENCE AND TECHNOLOGY.

CHEESE

ORIGIN

The first use of cheese as food is not known. References to cheeses throughout history are widespread. "Cheese is an art that predates the biblical era" (1). The origin of cheese has been dated to 6000–7000 B.C. and the worldwide number of varieties has been estimated at 500, with an annual production of more than 12 million tons growing at a rate of about 4% (2). This food served as a source of vital nourishment in huts and castles and on journeys; armies also used cheese. It also was a way of preserving food during periods of shortages. A plausible tracing of the origin and development of cheese, as a food, around the world has been published (1).

BIBLIOGRAPHY

1. Canadian International Grains Institute, *Grain and Oilseeds, Handling, Marketing, Processing*, 2nd ed., Canadian International Grains Institute, Winnipeg, Canada, 1975.
2. R. C. Hoseney, *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986.
3. D. W. Kent-Jones and A. J. Amos, *Modern Cereal Chemists*, 6th ed., Food Trade Press, London, United Kingdom, 1967.
4. R. A. Olson and K. J. Frey, eds., *Nutritional Quality of Cereal Grains*, American Society of Agronomy, Madison, Wis., 1987.
5. Y. Pomeranz, *Modern Cereal Science and Technology*, VCH Publishers, New York, 1987.
6. Y. Pomeranz, ed., *Industrial Uses of Cereals*, American Association of Cereal Chemists, St. Paul, Minn., 1973.
7. Y. Pomeranz, ed., *Advanced Cereal Science and Technology*, Vols. I–X, American Association of Cereal Chemists, St. Paul, Minn., 1976–1990.
8. Y. Pomeranz, ed., *Cereals '78: Better Nutrition for the World's Millions*, American Association of Cereal Chemists, St. Paul, Minn., 1978.
9. Y. Pomeranz and L. Munck, eds., *Cereals: A Renewable Resource, Theory and Practice*, American Association of Cereal Chemists, St. Paul, Minn., 1981.
10. H. Hanson, N. E. Bourlaug, and R. G. Anderson, *Wheat in the Third World*, Westview Press, Boulder, Colo., 1982.
11. E. G. Heyne, ed., *Wheat and Wheat Improvement*, 2nd ed., American Society of Agronomy, Madison, Wis., 1987.
12. Y. Pomeranz, ed., *Wheat is Unique*, American Association of Cereal Chemists, St. Paul, Minn., 1989.
13. Y. Pomeranz, ed., *Wheat Chemistry and Technology*, 3rd ed., American Association of Cereal Chemists, St Paul, Minn., 1988.
14. N. L. Kent, *Technology of Cereals With Special Reference to Wheat*, Pergamon, Oxford, United Kingdom, 1960.
15. I. D. Morton, ed., *Cereals in a European Context*, VCH Publishers, New York, 1987.
16. W. R. Akroyd and J. Doughty, *Wheat in Human Nutrition*, FAO Nutritional Studies No. 23, FAO, Rome, Italy, 1970.
17. J. Holas and J Kratochvil, eds., *Progress in Cereal Chemistry and Technology*, 2 Elsevier Science, New York, 1983.
18. R. W. Jugenheimer, *Corn: Improvement, Seed Production and Uses*, John Wiley & Sons, New York, 1976.
19. P. J. Barnes, ed., *Lipids in Cereal Technology*, Academic Press, Orlando, Fla., 1983.
20. M N Leath, L. H. Meyer, and L. D. Hill, *U.S. Corn Industry*, Report No. 479, U.S. Department of Agriculture, Economic Research Service, Washington, D.C., 1982.
21. S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
22. B. O. Juliano, ed., *Rice, Chemistry and Technology*, 2nd ed., American Association of Cereal Chemists, St. Paul, Minn., 1987
23. B. S. Luh, ed., *Rice: Production and Utilization*, AVI Publishing, Westport, Conn., 1980.
24. W. G. Heid, *U.S. Barley Industry*, Agr. Econ. Report No. 395, U.S. Department of Agricultural Economics, Statistics and Cooperative Service, Washington, D.C., 1978.
25. D. C. Rasmusson, ed., *Barley*, American Society of Agronomy Madison, Wis., 1985.
26. F. H. Webster, ed., *Oats, Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986.
27. H. Doggett, *Sorghum*, Longmans, Green, and Co., London, United Kingdom, 1970.
28. L. W. Rooney, D. S. Murthy, and J. V. Mertui, eds., *International Symposium on Sorghum Grain Quality*, Proceedings, INCRASAT Patancheru, A. P. India, 1982.
29. W. Bushuk, ed., *Rye: Production, Chemistry, and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
30. *Yearbook of Agriculture*, U.S. Government Printing Office, Washington, D.C., 1985.
31. Y. Pomeranz, *Functional Properties of Food Components*, Academic Press, Orlando, Fla., 1985.
32. J. E. Kruger, D. R. Lineback, and C. E. Stauffer, eds., *Enzymes and Their Role in Cereal Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
33. H. G. Muller, "Some Physical Properties of Cereals and Their Products as Related to Potential Industrial Utilization," in Y. Pomeranz, ed., *Industrial Uses of Cereals*, American Association of Cereal Chemists, St. Paul, Minn., 1973, pp. 20–50.
34. D. H. Simmonds, "Structure, Composition, and Biochemistry of Cereal Grains," in Y. Pomeranz, ed., *Cereals '78 Better Nutrition for the World's Millions*, American Association of Cereal Chemists, St. Paul, Minn., 1978, pp. 105–137.
35. *From Wheat to Flour*, Wheat Flour Institute, Chicago, Ill., 1965.
36. B. Holland, I. D. Unwin, and D. H. Buss, *Cereals and Cereal Products*, Royal Society of Chemistry, Ministry of Agriculture, Fisheries, and Food, London, United Kingdom, 1988.
37. C. M Christensen, ed., *Storage of Cereal Grains and Their Products*, 3rd ed., American Association of Cereal Chemists, St. Paul, Minn., 1982
38. J. L. Multon, ed., *Preservation and Storage of Grains, Seeds, and Their By Products*, Lavoisier Publishing, New York, 1988.

R. CARL HOSENEY
R & R Research Services
Manhattan, Kansas

See also WHEAT SCIENCE AND TECHNOLOGY.

CHEESE

ORIGIN

The first use of cheese as food is not known. References to cheeses throughout history are widespread. "Cheese is an art that predates the biblical era" (1). The origin of cheese has been dated to 6000–7000 B.C. and the worldwide number of varieties has been estimated at 500, with an annual production of more than 12 million tons growing at a rate of about 4% (2). This food served as a source of vital nourishment in huts and castles and on journeys; armies also used cheese. It also was a way of preserving food during periods of shortages. A plausible tracing of the origin and development of cheese, as a food, around the world has been published (1).

CLASSIFICATION

Cheeses have been classified in several ways. Several attempts to classify the varieties of cheese have been made. One suggestion consists of scheme that divides cheeses into the following superfamilies based on the coagulating agent.

1. *Rennet Cheeses*. Cheddar, brick, Muenster
2. *Acid Cheeses*. Cottage, Quarg, cream
3. *Heat–Acid*. Ricotta, sapsago
4. *Concentration—Crystallization*. Mysost

A more simple but incomplete scheme, would be to classify cheeses as follows.

1. *Very Hard*. Parmesan, Romano
2. *Hard*. Cheddar, Swiss
3. *Semisoft*. Brick, Muenster, blue, Havarti
4. *Soft*. Bel Paese, Brie, Camembert, feta
5. *Acid*. Cottage, baker's, cream, ricotta

A broad look at cheeses might divide them into two large categories, ripened and fresh.

Ripened

Cheeses can be ripened by adding selected enzymes or microorganisms (bacteria or molds) to the starting milk, to the newly made cheese curds, or to the surface of a finish cheese. The cheese is then ripened (cured) under conditions controlled by one or more of the following elements: temperature, humidity, salt, and time.

Depending on the style of cheese, the ripening can be principally carried out on the cheese surface or the interior. The selection of organisms, the appropriate enzymes, and ripening regime determine the texture and flavor of each cheese type.

Fresh

These cheeses do not undergo curing and are generally the result of acid coagulation of the milk. The composition, as well as processing steps, provides the specific product texture, while the bacteria used to provide the acid usually generate the characteristic flavor of the cheese.

CHEESEMAKING—GENERAL STEPS

1. Milk is clarified by filtration or centrifugation.

2. Depending on the composition of the final cheese, the fat content is standardized in a special centrifuge (separator).

3. Depending on the variety, the milk is pasteurized (generally at about 72°C (161°F) for 16 s) or undergoes any equivalent heat treatment that renders the milk phosphatase negative. Phosphatase is inactivated in the same range as the tuberculin bacilli. The phosphatase test insures that the milk is free of tuberculin bacilli and other pathogens.

4. Before pumping the milk to the cheese vat, the milk fat can be homogenized, if desired. The extent of this homogenization will depend on the cheese variety being made (Fig. 1).

5. A bacterial starter culture is added to the milk, which is at 30–36°C, depending on the cheese being made. This inoculated milk is generally ripened (held at this temperature) for 30–60 min to allow the lactic organisms to multiply sufficiently for their enzyme systems to convert some of the milk sugar (lactose) to lactic acid.

6. After ripening, a milk-coagulating enzyme is added with stirring. The milk in the vat is then allowed to set in a quiescent state.

7. The resulting coagulum is usually ready to be cut 20–30 min after the enzyme addition. For most fresh cheeses little or no coagulating enzyme is added. Coagulation is allowed to occur by the development of lactic acid alone.

8. When the coagulum is firm enough to be cut, a combination of vertical and horizontal stainless steel knives (wires) are pulled lengthwise and crosswise through the coagulum in a rectangular vat. If an automatic enclosed circular vat is used, the cutting is programmed to insure a curd-size range by the speed and timing of the automatic knives.

9. For most varieties a heal time is allowed where no stirring is done for 5–15 min after cutting. This allows the newly cut curd particles to firm up slightly and begin syneresis (excluding whey from the particle during the shrinking process).

10. Following the heal period, heat is applied to the jacket of the vat and gentle stirring begins. For most ripened cheeses the curds are cooked in the whey until the temperature of the mixture reaches 37–41°C, depending on the variety. For some cheeses, for example, Swiss and Parmesan, this temperature can be as high as 53°C. The cheese is generally cooked for 30 min. Fresh cheeses, such as cottage, cream, and Neufchâtel, are cooked at temperatures as high as 51.5–60°C to promote syneresis and provide product stability.

11. Generally, when the cook temperature is reached, a 45–60 min period is allowed to promote curd firming and the releasing of whey. During this stir-out time the contents of the vat are agitated somewhat vigorously.

12. From this point the whey is drained and the treatment of the new curd depends on the nature of the final product.

For cheeses such as Cheddar, Monterey Jack, Colby, brick, and mozzarella the curd can be handled in a number of ways. Cheddar can be made by allowing the curd to settle in a rectangular vat, then draining the whey from the curd. When the whey is removed, the curd is trenched down the middle of the vat, lengthwise. The curd is hand cut, turned over, and then piled, at intervals, one slab on another. During this time acid continues to develop and whey continues to be exuded from the curd. When the proper pH is reached (about 5.1–5.3) the curd is milled by machine into finger-size pieces and slowly dry-salted to

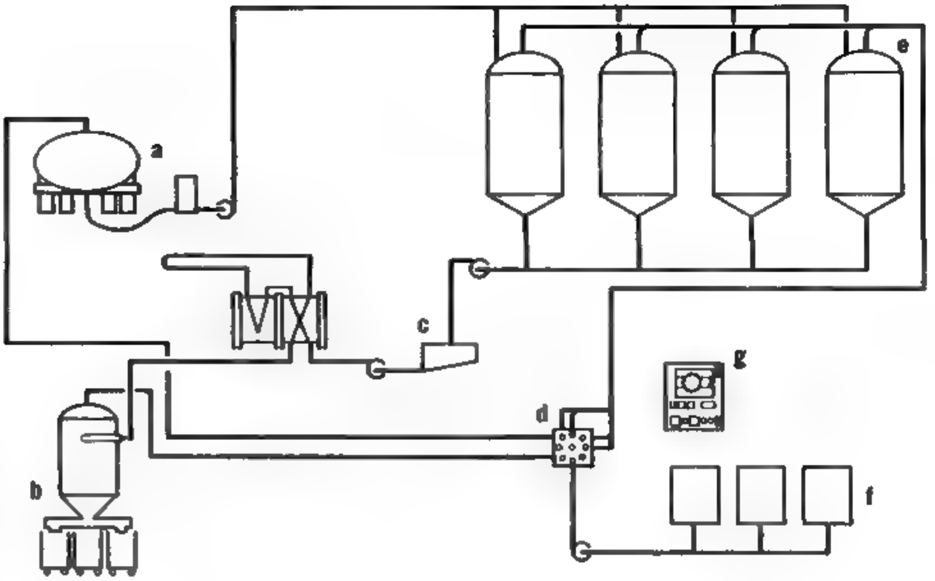


Figure 1. Natural cheese system flow key. a, Receiving pump/air eliminator; b, cyclone filter; c, balance tank; d, flow direction control; e, cheese vat; f, CIP system; g, CIP control panel. *Source:* Courtesy of Sherping Systems, Winsted, Minn.

provide about 1.6–1.8% salt in the final cheese. The salted curds are then packed into 40-lb stainless-steel hoops with porous netting lining the hoops or into 600–700-lb boxes. These containers are generally pressed at 15–20 psi to remove more whey over several hours and then placed into a vacuum chamber while under pressure to fuse the curd particles into a solid mass.

Several methods are used to arrive at a solid cheese block. One process provides for pumping the curds into a tower that allows whey drainage and compacting of the curds by their own weight. At the bottom of the tower a portion of the curd is cut off to fit into a 40-lb hoop, which travels to a press station and, finally, to a packaging station.

In another method, curd and whey are pumped from the vat to a draining-and-matting conveyer (DMC) under controlled temperature and curd depth. When the curd has reached the proper pH, the mat is cut and is automatically salted and transferred onto another conveyer where it is finally carried to a boxing operation (Fig. 2).

There is also a stirred-curd procedure used for mild- and medium-flavored Cheddar as well as Colby, brick, mozzarella, etc. In this method the curd and whey are pumped to a drain table or automatic-finishing vat (AFV) where the whey is drained and the curds are continually stirred to prevent matting. When the proper pH is achieved the cubes are salted and then boxed as previously described (Fig. 3).

If stirred curd Cheddar is intended for use in process cheese it can be packed into 500-lb drums and used after a short curing period. It should be noted that cheeses such as Monterey Jack and Colby, should not be placed under vacuum to preserve a slight open texture.

Blue cheese is made in a similar manner up to this point, but the mold (*Penicillium* sp.) can be added to the starting milk or to the drained curds. This cheese is usually formed in 5-lb hoops to promote an open texture. When the cheese is removed from the hoops it is dry-salted several times over a number of days and then punctured to create air channels in which the mold grows; this develops the characteristic blue veining. This cheese may be salt brined instead to obtain a 4% salt content in the final cheese. The cheese is cured for 60–120 days at 13°C and 95% humidity to achieve full veining and flavor.

Swiss, Parmesan, and other varieties are handled somewhat differently at the whey drainage step. For years these cheeses were manufactured in Europe in wheel-shaped hoops; 20-lb wheels were used for Parmesan and 175–225-lb hoops, for Swiss. The principles are the same so the description here will pertain to most U.S. products. For Swiss cheese the curds and whey are pumped to a large stainless-steel universal vat, which has been equipped with porous side plates, where the curd is allowed to settle evenly at the bottom. Large stainless-steel plates are used to press the curd at a precise depth of the curd mass. This pressing under whey results in a tightly fused cheese, required later for eye development. The whey is drained and the huge curd block is kept under pressure for about 16 h while it continues to develop acidity. Then the 3,000–3,500-lb block is cut into sections of about 180 lb and immersed into 2°C saturated brine for 24 h. The surface of the blocks are then dried. The blocks are packaged and boxed. These boxes are stacked and banded to help keep the block shape as the cheese cures in a hot room at 20–25°C. When the eyes have formed (about 18–28 days) the blocks are transferred to a room at about 2°C to prevent further eye development and held for at least 60 days before cutting into retail sizes. The cheese can be held for longer periods if stronger Swiss flavor is desired.

For Parmesan cheese, the curd is stirred while draining off the whey and then placed into round 20-lb hoops. After pressing, the round cheeses are allowed to surface dry before brining. If salt has not been added during the stirring of the curds, additional brining time is required. The cheeses are held at 13–16°C until the proper weight is reached (moisture reduced) and then vacuum packed in

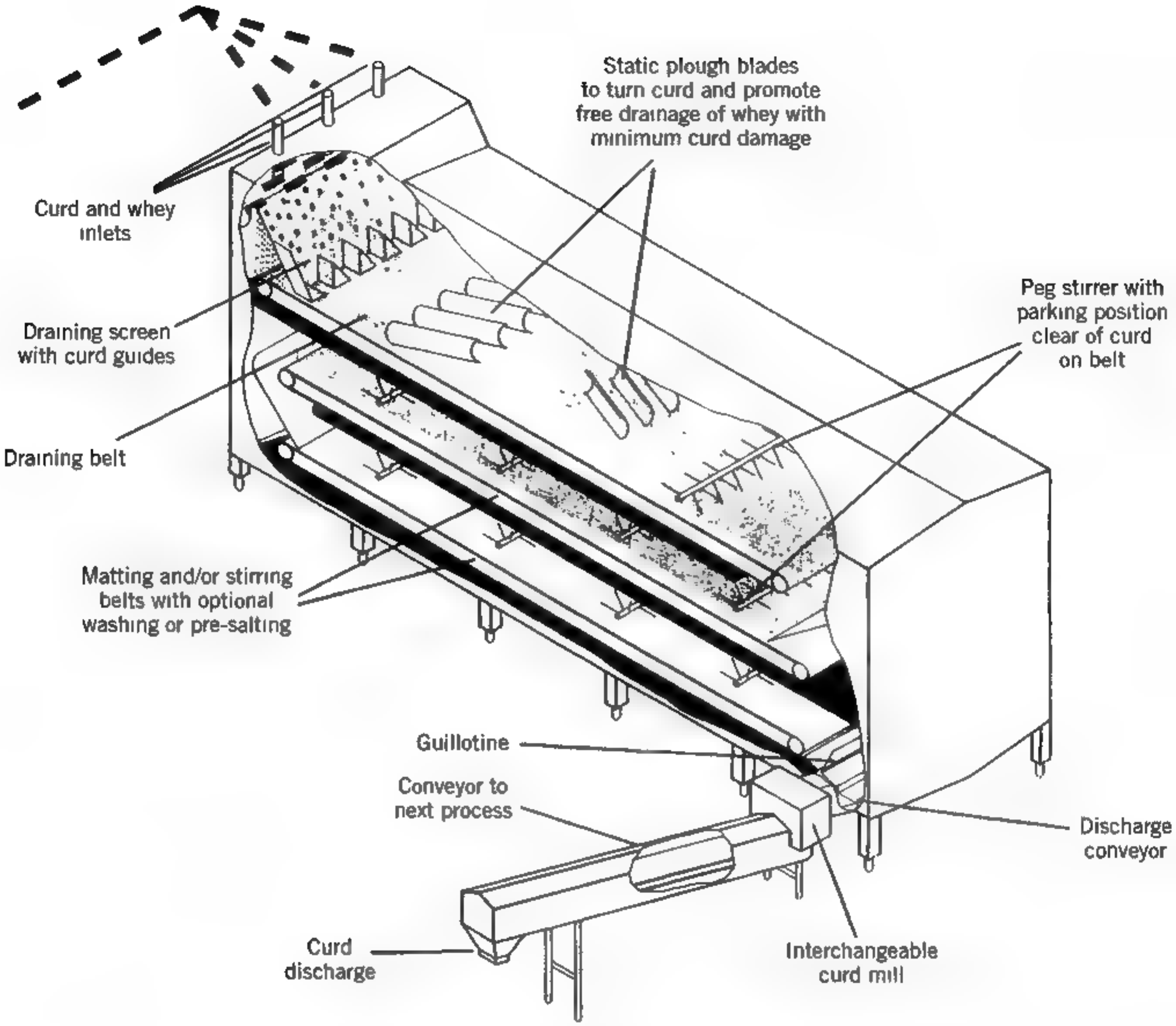

Static plough blades to turn curd and promote free drainage of whey with minimum curd damage
Curd and whey inlets
Peg stirrer with parking position clear of curd on belt
Draining screen with curd guides
Draining belt
Matting and/or stirring belts with optional washing or pre-salting
Guillotine
Conveyor to next process
Discharge conveyor
Curd discharge
Interchangeable curd mill

Figure 2. Cheese curd draining conveyor. *Source:* Courtesy of Sherping Systems, Winsted, Minn.

plastic bags with a tight seal. Some factories wax the cheese. This cheese requires a minimum of a 10-mo cure time by federal regulation. Most of the U.S. Parmesan is grated for use as a retail product.

Mozzarella and provolone are among those cheeses referred to as pasta filata varieties. Provolone has some lipase enzymes added to produce its characteristic flavor and sometimes is sold naturally smoked or with smoke flavor added. These cheeses have traditionally been further cooked, after whey drainage, in hot water and stretched by hand with subsequent shaping into the familiar forms. Today, however, most mozzarella and provolone are mechanically cooked under water and further stretched to provide texture and then shaped in molding machines. The cheeses are then brined to the proper salt level.

Cheeses such as Camembert, Brie, Muenster, and Limburger are made in smaller equipment and formed into smaller shapes to allow surface salting or brining and treatment of the cheese surface with selected bacteria or molds to provide the characteristic flavors, and textures.

Fresh cheeses such as cottage, queso fresco, and cream are mostly acid in nature and, following cooking of the curds, are treated differently. Cottage curds are washed with filtered water and then combined with a cream dressing. Queso fresco is pressed into loaves after salting and eaten fresh after slicing. Cream cheese curd is separated centrifugally and then standardized in regard to fat, salt, and stabilizers. Stabilizers are added to prevent moisture release. It can then be packaged in small loaves, hot or chilled.

RECENT DEVELOPMENTS

A number of cheese varieties have been manufactured from milk concentrated by ultrafiltration (3,4). Patents

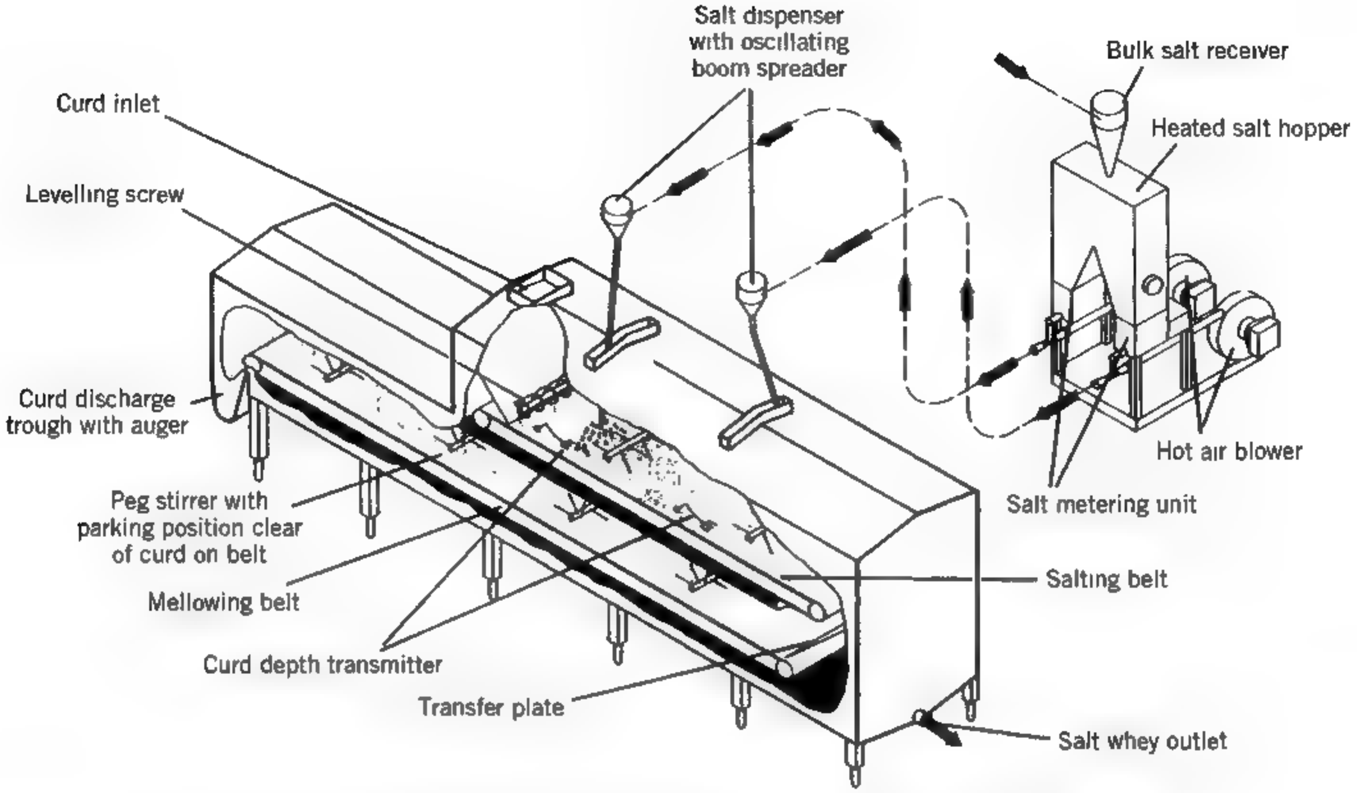


Figure 3. Dual salt applicating and cheese curd mellowing system. Two stage continuous salting and mellowing of milled or granular curd. *Source:* Courtesy of Sherping Systems, Winsted, Minn.

have been issued (5) and some procedures have been reduced to practice, but after more than 20 years of research in this area only a minimal amount of the total world production of cheese is made with ultrafiltered milk.

Bactofugation used to remove microbes and spores from cheese milk has sparked some interest, especially in Europe (6). Microfiltration may find significant application in special milk treatment prior to, or during, product manufacture. Significant developments in recombinant biotechnology and fermentation technology may provide specificity previously not available for milk coagulation and cheese flavor development. New cultures, which are resistant to phage, may be on the horizon. New cheese-making technology may allow previously unattainable processes and product control to become reality.

Cheese Starter Cultures

Starter cultures are organisms that ferment lactose to lactic acid and other products. These include streptococci, leuconostocs, lactobacilli, and streptococcus thermophilus. Starter cultures also include propionibacteria, brevibacteria, and mold species of Penicillium. The latter organisms are used in conjunction with lactic acid bacteria for a particular characteristic of cheese, eg, the holes in Swiss cheese are due to propionibacteria, and the yellowish color and typical flavor of brick cheese is due to brevibacterium linens. Blue cheese and Brie derive their characteristics from the added blue and white molds, respectively. Lactic acid-producing bacteria have several functions:

1. Acid production and coagulation of milk.
2. Acid gives firmness to the coagulum, which influences cheese yield.
3. Developed acidity determines the residual amount of animal rennet influencing cheese ripening; more acid curd binds more rennet.
4. The rate of acid development influences dissociation of colloidal calcium phosphate, which then influences proteolysis during manufacture and the rheological properties of cheese.
5. Acid development and production of other antimicrobials control the growth of certain nonstarter bacteria and pathogens in cheese.
6. Acid development contributes to proteolysis and flavor production in cheese.

Types of Culture

There are some impending changes in the nomenclature of starter culture organisms; the nomenclature used here follows present standards (7). Mesophilic cultures are used for cheese types that do not exceed 40°C during cheese making (8). These starters are propagated at 21–23°C and include *Streptococcus lactis* ssp. *lactis, S. lactis* ssp. *cremoris, S. lactis* ssp. *diacetylactis* and *Leuconostoc cremoris*. These are used for Cheddar, Gouda, brick, Muenster, cream, cottage, and Quarg cheese types, leuconstoc and *S. lactis* ssp. *diacetylactis* ferment citrate to produce carbon dioxide and diacetyl to characterize Edam, Gouda, and cream cheese.

Thermophilic cultures are used in cheese types where curd is cooked to 45–56°C. These starters are propagated at 40–45°C and include *S. thermophilus*, sometimes fecal streptococci, *Lactobacillus helveticus, L. delbrueckii* ssp. *lactis*, and *L. delbrueckii* ssp. *bulgaricus*. These are used for Swiss, Emmentaler, Gruyère, Parmesan, Romano, mozzarella, and Gorgonzola cheeses (9).

Propagation

Cultures are propagated in milk or a medium containing milk components and other nutrients and are heat treated (85°–90°C for 45 min) to render milk free of contaminants. The medium is then cooled to incubation temperature before inoculation. This processing is accomplished in a jacketed stainless-steel vessel provided with agitation and supplied with sterile air pressure. Culture inoculum is available from suppliers in frozen or freeze-dried form. It consists of appropriate mixture of culture strains and is free of contamination. After inoculation, culture is allowed to grow in quiescent state for 12–18 h. At the end of growth the medium is acidic with a pH of 4.5 or lower, depending on the culture. Ripened culture cell population is about 10^9 cfu/g. The ripened culture is cooled to at least 5°C. This form of propagation is called bulk-starter propagation. The culture may be used immediately or held for several days. The growth medium may contain buffering salts or the acid produced may be neutralized and controlled by the addition of ammonia, potassium hydroxide, or sodium hydroxide under controlled conditions. The latter is called pH-controlled propagation.

Starter cultures are susceptible to bacteriophages (viruses), which destroy the cultures and hamper cheese production. Cultures are also sensitive to antibiotics and bacteriocins (proteinaceus bacterial inhibitors). Propagated cultures are used at 1–5% of cheese milk. Highly concentrated (10^{10} cfu/g) frozen cultures are available for direct addition to cheese milk (350 g/5,000 lb). These are called direct vat set (DVS). This form is convenient, but expensive.

Coagulation

Coagulation of milk is essential to cheese making. The coagulation entraps fat and other components of milk. Most proteolytic enzymes can cause milk to coagulate. Rennet (chymosin, EC 3.4.23.4) is widely used for milk coagulation in cheese making (10). It is extracted from the fourth stomach of young calves. Commercial rennets may include blends of chymosin and pepsin (bovine or other animals). Microbial rennets with similar functionality prepared from *M. miehei* are known as Marzyme, Hannilase, Rennilase, and Fromase. Preparations from *M. pusillus* and *Endothia parasiticus* are called Emporase and Sure Curd, respectively. Proteases from plants are known to coagulate milk but are not used in commercial cheese making. Coagulation of milk is also effected by lowering pH to about 4.6 in quiescent state either by fermentation of lactose to lactic acid or by hydrolysis of gamma—delta lactones to produce cottage, Quarg, ricotta, and cream cheese.

Animal and microbial rennets are aspartic proteases with optimum activity under acidic conditions. Their molecular weights range from 30,000 to 38,000. The primary stage of rennet coagulation involves partial hydrolysis of *K*-casein, the principal stabilizing factor of milk protein, at Phe-105 to Met-106 bond. Destabilization of the residual protein (*para*-casein) occurs in the presence of Ca^{2+} at temperatures >18°C in the secondary, nonenzymatic stage of rennet coagulation. About 30% of calf rennet is retained in the curd before pressing and only 5–8% after pressing (11). The amount of rennet retained is governed by the pH of the curd. Microbial rennets are retained to a lesser extent (3–5%).

During coagulation, linkages between casein micelles are formed and many micelles are joined by bridges, but later these appear to contract, bringing the micelles into contact and causing partial fusion. The firmness of curd is due to an increase in both the number and strength of linkages between micelles. It is suggested that phosphoryl side chains of casein specially *β*-casein are involved. These may be linked by Ca^{2+} bridges. In cheese, *αs*-1-casein plays a structural role (12).

Casein aggregation and fusion continues after coagulum cutting and throughout cheese making and early stages of cheese ripening. This process of rennet coagulation of milk is fundamental to the conversion of milk to cheese curd and its manipulation is essential for the control of cheese manufacture (Table 1). Casein aggregation leads to curd formation and syneresis. Increase in acidity and temperature and the addition of calcium chloride accelerates the rate of curd formation and its syneresis. On the other hand, cold storage of milk, homogenization, higher pasteurization temperature, and increased fat content impede the two processes. Acid coagulation of milk, as in cottage-cheese making results in less aggregation of casein than is seen with rennet. During cottage-cheese making the pH of milk casein drops to its isoelectric point resulting in a soft, fragile gel, firm enough to be cut. For large-curd cottage cheese, a very small amount of rennet is added. Essentially all added rennet is inactivated at the cooking temperature of curd (57–60°C).

MICROBIOLOGICAL AND BIOCHEMICAL CHANGES DURING CHEESE MAKING AND CHEESE RIPENING

During cheese manufacture, milk is dehydrated to a given composition, characteristic of a family of cheeses. The distinguishing features of a cheese within a family are a function of smaller but significant deviations from set practices. The following pairs of cheeses represent good examples of the effect of manufacturing variations: Edam and Gouda, brick and Limburger, mozzarella and provolone, and Cheddar and Colby. Rennet curds have unique properties that can be handled to produce high- and low-moisture cheeses without hardening. Limited, but essential, proteolysis of milk protein by rennet enzymes augments the shift of microbial populations that, in turn, are pressed into summoning those metabolic activities that must transform the milk component simply to survive. In doing so, the chemical entities generated interact among

Table 1. Milk Coagulants for Cheese Making

	Supplier/designation			
Coagulant	Hansen's	Marschall's	Pfizer	SANOFI
Calf	Standard	Calf rennet Cheese rennet	Calf rennet	American rennet[a]
Bovine	Bovine			Beef rennet
Porcine			Maria Set	Pepsin
Calf-porcine	50-50	Chymoset	Econozyme	Quick Set[b]
Bovine-porcine	B-P			
Calf-bovine-porcine				Trizyme
Mucor miehei (modified thermolabile equivalent to Novo Rennilase XL)	Hannilase (HL)		Mor Curd	
Mucor miehie (modified extra thermolabile equivalent to Novo Rennilase XL)	Superlase	Marzyme Supreme	Mor Curd Plus	
Endothia parasiticus			Sure Curd	
Mucor pusillus				Emporase[c]
Microbial, animal	C and P (Chees-zyme and Pep-zyme blend)	M-P (50-50 Hog and *M. miehei*)		Regalase (porcine and Emporase) Beemase (bovine and Emporase)

[a] DFL sells three grades of calf rennet: PD300 (highest chymosin), SF100 (intermediate chymosin), and EL400 (lowest chymosin).
[b] DFL sells three grades of Quick Set.
[c] DFL sells three grades of Emporane.

themselves and with the microbial population to result in a more flavorful and preserved milk. Both casein and milk fat hydrolysis is needed for cheese flavor development, but the rate and extent of such hydrolysis must be controlled to maintain cheese identity. Different cheese types are discussed below with these comments in mind.

Lactic starter cultures are added to vat milk to give about 10^6–10^7 cfu/mL. The amount and type of starter may vary significantly depending on the type of cheese and characteristics desired. In most cheese types an overnight pH range is 4.95–5.3.

Cheddar-Type Cheese

In Cheddar, Caciocavallo, and Colby types of cheese, about 30–40% of the added culture cells are lost in whey. The cells trapped in the rennet coagulum rapidly multiply and ferment lactose to lactic acid. The population of starter organisms may reach in excess of 5×10^8 cfu/g in curd before salting. These bacterial numbers include a 10-fold increase due to milk solids concentration. The cultures multiply only slightly during coagulation and cooking, but growth and acid production accelerate after whey is removed and continues through cheddaring as the starter cells are concentrated in the curd. Acid production will continue until the lactose is depleted. At the time of milling the curd may have a pH of 5.3 and titratable acidity of whey at 0.57% or higher.

The milling operation involves cutting the curd into small pieces to facilitate uniform salt distribution and whey drainage. Enough salt is applied to the curd in two or three applications to yield a salt-in-moisture phase ($S/S + M$) of the cheese of about 4–5%. Due to steady acid production throughout cheese making the casein curd is demineralized with respect to calcium and phosphorus. The rate and extent of acid production governs the residual chymosin, minerals, moisture, and the cheese texture and structure. Higher acid in cheese reflects higher starter populations, possibly resulting in bitter cheese due to total proteinase activity from starter cells.

Cheddar-type curd is dry salted. Addition of salt to the curd is the last step in the manufacture of cheese. It controls microbial growth and activity and the final pH of hooped cheese. At hooping Cheddar cheese may contain 0.6% lactose. Its fermentation to lactic acid depends on $S/S + M$. Culture activity is inhibited by $S/S + M$ of >5% (13). Salt also helps to expel moisture. Proteolytic activity of chymosin and pepsin on αs-1-casein is stimulated by salt, whereas β-casein proteolysis is inhibited by $S/S + M$ of 5%. Salt appears to affect physical changes in cheese protein, which influence cheese texture, protein solubility and probably protein conformation. Cheddar-type cheese is internally ripened by chymosin in concert with starter proteinases and adventitious lactobacilli. For normal ripening a high starter population must lyse to release proteinases and peptidases affecting cheese flavor, body, and texture development. In most cheese types there is a shift in the population of starter organisms to lactobacilli and in some cases pediococci. It has been demonstrated that in young cheese there are inhibitory and stimulatory factors for lactobacilli. As the cheese ripens, most of the inhibition vanishes. These stimulatory factors for lactobacilli appear to originate primarily in αs-casein (14). In Cheddar cheese, β-casein is not extensively degraded (15). Studies with starters lacking proteinase indicate the importance of starter proteinases (16). Proteolysis and increasing concentration of free amino acids add to the savory taste and provide substrate for sulfur compounds. Many flavor compounds are chemical interactions of microbially derived substrates under conditions of low pH and low oxidation–reduction potential. Numerous compounds, such as hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones, and sulfurs are important in cheese flavor. Hydrogen sulfide and methanethiol along with phenylacetaldehyde, phenylacetic acid, and phenethanol are considered key compounds of good Cheddar flavor (17). Lactic acid bacteria are not lipolytic but small increases in butyric and other fatty acids appear desirable.

Swiss, Emmentaler, and Gruyère

These cheese types are made with thermophilic streptococci and lactobacilli to which propionibacteria are added for eye formation. During the early phases of cheese making *S. thermophilus* multiplies rapidly and utilizes the glucose moiety of lactose to produce *L*-lactate, leaving behind galactose. Lactobacilli start vigorous acid production after whey drainage when the curd temperature drops to 46–49°C. Acid production and starter bacteria number are greater toward the periphery where the cheese has cooled down. Lactic acid fermentation in cheese is complete within 24 h with no detectable sugars.

Changes During Ripening. Increase in starter organisms population and acidity are halted when the cheese is brined. This is due to exhaustion of fermentable sugar and the low brine temperature. Cheese is cured in a hot room (20°–25°C) for 18–40 days. During this period the added starter organisms disappear and the niche is occupied by propionibacteria, adventitious lactobacilli, and fecal streptococci. Propionibacteria ferment lactate to propionic acid and other flavor compounds according to the reaction: $3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$.

Eye Formation. The quality of Swiss cheese is judged by the size and distribution of eyes. Swiss cheese eyes are essentially due to carbon dioxide production, diffusion, and accumulation in the cheese body (18). The number and size of eyes depend on carbon dioxide pressure, diffusion rate, body texture, and temperature of cheese. The bulk of protein breakdown products are derived from *αs*-casein. *β*-Casein is not greatly proteolysed in Swiss cheese (15). Bacterial proteinases and plasmin (milk proteinase) are considered important in Swiss cheese ripening.

Flavor. Cheese flavor is derived in part from cheese milk. However, the characteristic flavor of Swiss-type cheese comes from the microbial transformation of milk components. These contain water-soluble volatiles (acetic acid, propionic acid, butyric acid, and diacetyl), which give the basic sharpness and general cheesy notes (17). Water-soluble nonvolatile amino acids (especially proline), peptides, lactic acid, and salts provide mainly sweet notes. Oil-soluble fractions (short-chain fatty acids) other than the water-soluble volatiles are important to flavor. Nutty flavor is attributed to alkylpyrazines. Several compounds, eg, ketones, aldehydes, esters, lactones, and sulfur-containing compounds are important. Due to the activity of certain strains of lactobacilli and fecal streptococci, biogenic amines are sometimes found in Swiss cheese.

Camembert Cheese

This is a soft cheese with 48–52% moisture. Milk inoculated with 1–5% mesophilic lactic starter is renneted at about 30°C. When the acidity reaches 0 20–0.23% the curd is dipped, molded, and held at 21°C in rooms with 85–90% humidity until it reaches pH 4.5–4.6. During storage, yeast and *Geotrichum candidum* appear on the surface. After about a week at about 10°C *Penicillium camemberti* covers the surface and deacidifies the cheese in 15–20 days. This is followed by colonization of the cheese surface by *B. linens* and micrococci. During ripening, the surface pH rises to about 7.0. *β*-Casein is not extensively degraded. Appearance of some *γ*-casein suggests plasmin activity. There is *αs*-casein degradation but less compared to Cheddar cheese. In Camembert, due to extensive deamination, ammonia constitutes 7–9% of the total *N*. Free fatty acids found in large quantities contribute to the basic flavor of cheese and serve as the precursors of methylketones and secondary alcohols. Aldehydes, methylketones, 1-alkanols, 2-alkanols, esters (C_2, 4, 6, 8, 10-ethyl 2-phenylethylacetate), phenol, *p*-cresol, lactones, hydrogen sulfide, methanethiol, methyldisulfide and other sulfur compounds along with anisoles, amines, and other compounds constitute the volatile compounds.

Blue Cheese

Blue cheese and its relatives, Roquefort, Stilton, and Gorgonzola are characterized by peppery, piquant flavors produced by the mold *Penicillium roqueforti*. Milk for cheese making may be homogenized to promote lipolysis in cheese. An acid curd with pH 4.5–4.6 is produced with mesophilic lactic starter and sometimes include *S. lactics* ssp. *diacetylactis*. Gorgonzola is made with *S. thermophilus* and *Lactobacillus bulgaricus*. The blue mold is added to the milk or curd. The molded curd is dry salted, and salt is rubbed on cheese to attain 4–5% salt. After about a week the cheese heads are pierced with needles to aerate the cheese and let carbon dioxide escape and air enter. This promotes mold growth.

Due to high acid and salt, starter population declines rapidly and the blue mold grows throughout the cheese. Salt-tolerant *B. linens* and micrococci appear on the cheese surface in two to three weeks. Due to deacidification of the cheese and extensive proteolysis, the cheese pH rises from 4.5 to 4.7 at 24 h to a maximum of 6.0–6.25 in two to three months.

Molds are both proteolytic and lipolytic, resulting in extensive proteolysis and lipolysis of cheese. In blue cheese both *αs*- and *β*-casein are degraded. There is a large accumulation of free amino acids due to extracellular acidic and alkaline endopeptidases (19). *B. linens* and micrococci are also proteolytic and contribute to the overall proteolysis. Occurrence of citrulline, ornithine, *γ*-aminobutylic acid, and biogenic amines reflect amino acid break-down products. Other volatile compounds such as ammonia, aldehydes, acids, alcohols, and methanethiol are also produced from amino acid metabolism.

Mold-ripened cheeses have a high free fatty acid content, 27–45 meq of acid per 100 g of fat. *Penicillium roqueforti* produces two lipases, one active at pH 7.5–8.0 or higher and the acid lipase active at pH 6.0–6.5. The fatty acids produced are converted into methylketones via ketoacyl–coenzyme A and the β-keto acids (20). Thiohydrolases are also present in cheese, resulting in the accumulation of 2-heptanone. The rate of fatty acid release governs the rate of methylketone formation (21).

Brick Cheese

Brick cheese is representative of a large group of cheeses (Limburger, Muenster, Tilsiter, Bel Paese, and Trappist) ripened with the aid of surface flora made of yeast, micrococci, and coryneform bacteria (*B. linens*). A culture of *S. lactis* ssp. *lactis*, *S. lactis* ssp. *cremoris*, and sometimes in combination with *S. thermophilus* is used to cause a cheese pH of 5.0–5.2. After brining for one or more days cheese is held at about 15°C (60°F) in rooms with a 90–95% RH where yeast (*Mycoderma*) appear on the surface in two or three days followed by micrococci and then *B. linens*. Organisms from the genus *Arthrobacter* have also been isolated from the cheese surface. Cheese ripens in four to eight weeks depending on the moisture and intensity of cheese flavor desired. Film-wrapped cheese has more rounded flavor than waxed cheese.

Growth of yeast lowers the acidity on the surface, making it suitable for micrococci and *B. linens* colonization (22). Sometimes *G. candidum* may also be present. α_s-Casein is always hydrolyzed but β-casein disappearance was seen in Muenster and not in brick. Yeasts found on Limburger cheese synthesize considerable amounts of pantothenic acid, niacin, and riboflavin (23). Pantothenic acid and *para*-aminobenzoic acid are required by *B. linens* Thus explaining the population sequence. Liberated free amino acids are much higher on the surface than in the interior of cheese. Activities of micrococci appear essential to the development of typical flavor of brick or Limburger cheese. These proteolytic organisms are able to convert methionine to methanethiol. Acetyl methyl disulfide is characteristic of Limburger flavor.

Edam and Gouda

These Dutch cheese varieties are made with *S. lactis* ssp. *lactis*, *S. lactis* ssp. *cremoris*, *Leuconstoc cremoris* and/or *L. lactis*. *Leuconstoc* and *S. lactis* ssp. *diacetylactis* ferment citrate to produce carbon dioxide, diacetyl, and other compounds. The starter organisms reach $\sim 10^9$ cfu/g in curd with a pH of about 5.7. The remaining lactose in curd is fermented in an uncoupled state to lower cheese pH to 5.2. Molded cheese forms are placed in 14% brine at 14°C for three to seven days. After brief drying, the cheese is coated with two to three coats of plastic emulsion, which permits slow moisture evaporation. Carbon dioxide production is essential to eye formation. Ripening changes involve proteolysis by rennet enzymes, enzymes of starter bacteria, and, to a small extent, native milk proteinase. Rennet is responsible for extensive degradation of α_s-casein to large molecular weight water-soluble peptides. This proteolysis is significant in determining the body characteristics of cheese. Starter organism production of low molecular weight peptide fractions and amino acids are stimulated by rennet action.

Lipolysis occurs to a small degree mainly due to enzymes of starter bacteria liberating free fatty acids from monoglycerides and diglycerides formed by milk or microbial lipases. In well-made Edam and Gouda lactobacilli do not exceed 10^5–10^6/g or certain defects in the cheese are noticed. Lactic acid, carbon dioxide, diacetyl, aldehydes, ketones, alcohols, esters, and organic acids in proper balance are important to flavor. Anethole and bismethylthiomethane are important odor compounds of Gouda cheese (24).

Mozzarella and Provolone

These cheese types may contain 45–60% moisture. *S. thermophilus*, thermophilic *Lactobacillus* sp. or mesophilic lactics with *S. thermophilus* combination starters are used. Sometimes citric acid or other acids are used in place of lactic starters.

The cultures used generally cleave the galactose moiety of lactose in cheese, which, if not utilized, result in dark brown cheese color on baking. In fresh brined cheese there may be 10^8 cfu/g of starter organisms in 1:1 ratio of streptococci and lactobacilli. α_s-Casein is proteolysed to a lesser degree by rennet enzymes compared to other cheese types, while β-casein is largely intact. This level of rennet proteolysis of milk protein is sufficient to give the melt and stretch characteristics to cheese during hot water kneading at about 57°C. The molded cheese is brined. The pH of cheese should be 5.1–5.4. There is little lipolysis and fatty acids liberation in traditional mozzarella cheese (25). Cheese made with mesophilic starter and *S. thermophilus* tends to have greater protein and fat hydrolysis during storage. Such cheese is difficult to shred and has atypical flavor when aged.

Mozzarella and provolone are manufactured in a similar manner. The former is consumed fresh while the latter may be ripened at 12.5°C for three to four weeks and then stored at 4.5°C for 6–12 months for grating. The ripened cheese have mainly *Lactobacillus casei* and its subspecies. Sometimes leuconstocs are detected in cheese, particularly if no starter was used.

Parmesan and Romano

Parmesan and Romano cheese are made with *S. thermophilus*, *Lactobacillus bulgaricus*, or other species of thermophilic lactobacilli. In addition to rennet, pregastric estrases, or rennet paste may be added to cheese milk for their lipolytic activity. The curd is cooked to 51–54°C, when the whey acidity reaches about 0.2% it is hooped (packed) in round forms. Sometimes salt is added to the curd, which slows the starter and regulates moisture. The cheese at pH 5.1–5.3 is placed in 24% brine for several days.

Compared to other cheese types, the starter populations in the fresh cheese is low. Throughout cheese ripening, 12–24 months, cheese flora seldom exceeds 10^5 cfu/g. Fecal streptococci and salt-tolerant lactobacilli predominate. In these hard grating cheeses α_s- and β-caseins are not overly proteolysed compared to other cheese varieties. This is attributed to high cooking temperatures of curd, which inactivates the coagulant, and the high salt in the moisture, which discourages growth of adventitious flora. In ripened cheeses quality varies from location to location. Volatile free fatty acid and nonvolatile free fatty acid (C_4 through C_{18}) concentrations are high in these cheeses, particularly in Romano (25). Butyric acid and minor branched chain fatty acids that occur in milk appear to contribute to the piquant flavor of Parmesan. For a more balanced flavor a concomitant increase in free amino acids (glutamic acid, aspartic acid, valine, and alanine) has been noted. Too high a free fatty acid level in cheese gives a strong, soapy, undesirable flavor.

Cottage Cheese and Cream Cheese

These are cultured to the isoelectric point of casein (pH ~4.6) followed by heating to separate the cheese solids. Cottage cheese curd is blended with a cultured or uncultured cream to no more than 4% fat. Cream cheese is cultured with lactics and citrate-fermenting cultures. Diacetyl, ethanol, acetone, lactic acid, acetic acid, and other less-characterized compounds are responsible for the flavor of these products.

ACCELERATED CHEESE RIPENING

One of the major costs of cheese is the expense of curing time before desired flavor develops. While some maturation time is inevitable, there are systems available where ripening time is shortened by speeding up proteolysis and lipolysis to generate flavor and modify texture. Elevated temperature (13°C or higher) curing offers the simplest approach to speed up ripening of otherwise normal cheese. Cheese intended for this type of curing must not contain measurable levels of heterofermentative lactobacilli or leuconostocs, because an open-texture defect and off-flavors will develop (26).

Microbial proteinases and gastric esterases have been used with little success to achieve acceptable cheese with uniformity. Activity of these exogenous enzymes is unregulated and may contribute to the detriment of cheese quality. Several unproven systems are available from culture houses.

Additions of partially inactivated starter organisms have been used with mixed results. Presently, this is not economical. Most of the proprietary systems investigated cause a minor to major deviation from characteristic flavor, body, and texture of cheese.

CHEESE DEFECTS

Cheese varieties exist because manufacturing and curing are carried out to preserve characteristic flavor, body, and texture of a given cheese. Attributes of one cheese may prove to be defects in another.

Even though certain molds are used for manufacturing and curing of cheese, their growth on most cheeses is undesirable (27). Gas production with putrid, unclean odors due to nonstarter bacteria constitute a defect in cheese. Moisture accumulation on the surface of hard cheeses permits rind rot due to yeasts, molds, and proteolytic bacteria growth, sometimes accompanied by discoloration. Milk itself can at times contribute weedy, feedy, cowy, barny, and related flavors coming from the cow. Psychrotrophic organism growth in milk can generate rancidity due to lipolysis and bitterness due to proteases from these organisms. Specific defects are discussed below

Swiss Cheese

Swiss is graded by the size, number, and distribution of eyes; there are many defects of the eyes (18). *Blind* or cheese with no eyes may be due to inhibition of carbon dioxide production of proprionibacteria by pH of less than 5.0, prolonged brining (high salt), and antibiotics or other inhibitors in milk or cheese.

Overset (pinny or too many eyes) is generally caused by improper acidity, high moisture, entrapment of air in curd, and other factors that prevent knitting of curd before gas production. Early gas is caused by *Enterobacter aerogenes*. Flavor defects, such as stink spots, white spots (28), and excessive amine production, are caused by *Clostridium tyrobutyricum*, *S. faecalis* ssp. *liquefaciens*, and strains of *Lactobacillus buchenerii*, respectively.

Cheddar Cheese

Excessive acidity, below pH 5.0 at three or more days, causes short, crumbly body and a sour, often bitter flavor. Normal body and flavor development may not occur in this cheese. On the other hand, too little acid or pH above 5.3 at three or four days may lead to corky, pasty body with off-flavor development. Open-texture defect may be caused by lactobacillus fermentum, L. brevis, and leuconostocs. Raw milk and rennet preparations have been linked to these defects.

Edam and Gouda

Edam and Gouda are subject to spoilage similar to Cheddar. In these cheeses, brine has been known to contaminate the cheese surface with gas-forming lactobacilli. These may cause the occurrence of phenols, putrid odor, and sulfidelike flavors and excessive production of carbon dioxide. Butyric acid fermentation and carbon dioxide production are due to *C. tyrobutyricum*. Growth of propionibacteria may cause a sweet taste and an open texture due to carbon dioxide.

Brick Cheese

Excessive acidity is a major defect. Coliform bacteria can grow during draining and salting, causing early gas, which if excessive can yield a spongy condition. Sometimes, due to lack of surface smear growth, typical flavor does not develop.

Blue Cheese

Blue cheese may not have sufficient mold growth, leading to lack of flavor and general development of cheese attributes. Excessive growth of mold may cause mustiness and loss of flavor. Mold-ripened cheeses are sensitive to bitterness development due to excessive acidity in the curd followed by abundant mycelium growth and proteases. Surface discoloration by other molds is not uncommon. Excessive slime and undesirable flavors occur due to excessive humidity in curing rooms.

Mozzarella

Mozzarella cheese is generally shredded before use. Softening of this cheese, due to *Lactobacillus casei* strain, is a problem during curing. It is also prone to softening and gas production by thermoresistant strains of *Leuconostoc*.

Cottage Cheese

Cottage cheese has a short shelf life generally due to *Pseudomonas*, yeasts, and molds. Sometimes bitterness and excessive acid development due to surviving starter culture is noticed during refrigerated storage (Tables 2, 3).

For total dollar annual sales and a breakout by cheese type see Tables 4 and 5.

PROCESS CHEESE

Process cheese is the food produced by grinding and blending various cheese types into a uniform, pliable mass with the aid of heat. Water, emulsifying salts, color, etc are usually added and the hot plastic mass is cooled into the desired form.

Process cheeses were prepared as early as 1895 in Europe, but the use of emulsifying salts was not widely practiced until 1911 when Gerber and Co. of Switzerland invented process cheese. A patent issued to J. L. Kraft in 1916 marked the origin of the process cheese industry in America and describes the method of heating natural cheese and its emulsification with alkaline salts.

Table 2. General Cheese Composition Ranges

Variety	Moisture (%)	Fat (dry basis, %)	Salt (%)
Blue cheese	43.0–45.0	51.0–54.0	3.0–4.0
Brick	40.0–42.0	51.0–54.0	1.5–1.7
Cheddar	36.5–38.0	51.0–55.0	1.5–1.8
Colby	38.0–39.5	51.5–55.0	1.5–1.8
Cream cheese	53.0–54.5	34.0–36.0	1.0–1.2
Edam	35.0–38.0	41.0–47.0	1.6–2.0
Gorgonzola	35.0–38.0	51.0–53.0	3.0–4.0
Granular-stirred curd (CM)[a]	33.0–35.0	51.0–54.0	1.8–2.0
Limburger	46.0–48.0	51.0–54.0	2.0–2.1
Monterey Jack	40.5–42.5	51.0–54.0	1.5–1.7
Mozzarella	54.0–58.0	46.0–50.0	1.8–2.4
LM mozzarella[b]	45.0–48.0	46.0–50.0	1.7–2.2
PS mozzarella[c]	54 0–58.0	34.0–39.0	1.7–2.2
LMPS mozzarella[d]	46.0–50.0	34.0–39.0	1.6–1.9
Muenster	41.5–43.5	51.0–54.0	1.5–1.7
Neufchâtel	61.0–63.0	21.0–26.0	0.8–1.0
Parmesan	29.5–31.5	36.0–39.0	3.5–4.0
Provolone	41.0–44.0	46.0–48.0	1.8–2.2
Romano	32.5–33.5	39.0–41.0	3.5–4.0
Skim curd	47.0–50.0	11.0–15.0	0.9–1.1
Swiss	37.0–39.5	46.0–48.5	0.2–0.6

[a] Cheese for manufacturing.

[b] Low moisture.

[c] Part skim.

[d] Low moisture, part skim.

Process cheeses in the United States generally fall into one of the following categories (29).

1. Pasteurized blended cheese. Must conform to the standard of identity and is subject to the requirements prescribed by pasteurized process cheese except
   - A mixture of two or more cheeses may include cream or Neufchâtel.
   - None of the ingredients prescribed or permitted for pasteurized process cheese is used.
   - The moisture content is not more than the arithmetic average of the maximum moisture prescribed by the definitions of the standards of identity for the varieties of cheeses blended.
   - The word process is replaced by the word blended.
2. Pasteurized process cheese.
   - Must be heated at no less than 65.5°C for no less than 30 s. If a single variety is used the moisture content can be no more than 1% greater than that prescribed by the definition of that variety, but in no case greater than 43%, except for special provisions for Swiss, Gruyère, or Limburger.
   - The fat content must not be less than that prescribed for the variety used or in no case less than 47% except for special provisions for Swiss or Gruyère.
   - Further requirements refer to minimum percentages of the cheeses used.
3. Pasteurized process cheese food.
   - Required heat treatment minimum is the same as pasteurized process cheese.
   - Moisture maximum is 44%; fat minimum is 23%.
   - A variety of percentages are prescribed.
   - Optional dairy ingredients may be used, such as cream, milk, skim milk, buttermilk, and cheese whey.
   - May contain any approved emulsifying agent.
   - The weight of the cheese ingredient is not less than 51% of the weight of the finished product.
4. Pasteurized process cheese spread.
   - Moisture is more than 44% but less than 60%.
   - Fat minimum is 20%.
   - Is a blend of cheeses and optional dairy ingredients and is spreadable at 21°C.
   - Has the same heat treatment minimum as pasteurized process cheese.
   - Cheese ingredients must constitute at least 51%.
   - A variety of percentages are prescribed.

Advantages of Process Cheese

- Compact, dense body.
- Smooth texture.

Table 3. Code of Federal Regulations Cheese Composition Standards

Cheese type	Legal maximum moisture (%)	Legal minimum fat (dry basis, %)	Legal minimum age
Asiago, fresh	45	50	60 days
Asiago, soft	45	50	60 days
Asiago, medium	35	45	6 months
Asiago, old	32	42	1 year
Blue cheese	46	50	60 days
Brick cheese	44	50	—
Caciocavello Siciliano	40	42	90 days
Cheddar	39	50	—
Low-sodium Cheddar	(Same as cheddar but less than 96 mg of sodium per pound of cheese)		
Colby	40	50	—
Low-sodium Colby	(Same as colby but less than 96 mg of sodium per pound of cheese)		
Cottage cheese (curd)	80	0.5	—
Cream cheese	55	33	—
Washed curd	42	50	—
Edam	45	40	—
Gammelost	52	(skim milk)	—
Gorganzola	42	50	90 days
Gouda	45	46	—
Granular-stirred curd	39	50	—
Hard grating	34	32	6 months
Hard cheese	39	50	—
Gruyère	39	45	90 days
Limburger	50	50	—
Monterey Jack	44	50	—
High-moisture Monterey Jack	44–50	50	—
Mozzarella and scamorza	52–60	45	—
Low-moisture mozzarella and scamorza	45–52	45	—
Part-skim mozzarella and scamorza	52–60	30–45	—
Low-moisture, part-skim mozzarella	45–52	30–45	—
Muenster	46	50	—
Neufchâtel	65	20–33	—
Nuworld	46	50	60 days
Parmesan and reggiamo	32	32	10 months
Provolone	45	45	—
Soft-ripened cheese	–	50	—
Romano	34	38	5 months
Roquefort (sheep's milk)	45	50	60 days
Samsoe	41	45	60 days
Sapsago	38	(skim milk)	5 months
Semisoft cheese	39–50	50	—
Semisoft, part-skim cheese	50	45–50	—
Skim-milk cheese for manufacturing	50	(skim milk)	—
Swiss and Emmentaler	41	43	60 days

- Improved slicing properties without crumbling.
- Smooth melting without phase separation.
- Flavor ranges depending on the cheese used.
- Can control organoleptic and physical properties.
- Versatile in use attributes (cooking cheese, dips, sauces, snacks, etc).
- Shelf stability (nonrefrigeration, fixed properties).
- Can take on various shapes and forms.

Basic Emulsification Systems for Cheese Processing

Citrates

- Trisodium citrate (most common, used for slices).
- Tripotassium citrate (used in reduced-sodium formulations, promotes bitterness).
- Calcium citrate (poor emulsification).

Orthophosphates

- Disodium phosphate and trisodium phosphate (most common, used for loaf and slices).
- Sodium aluminum phosphate (used in slices).
- Dicalcium phosphate and tricalcium phosphate (poor emulsification, used for calcium ion fortification).
- Monosodium phosphate (acid taste, open texture).

Table 4. Manufacturers' Sales of Cheese

Year	Total ($, millions)	Annual percent change
1967	1,751.8	—
1972	3,094.6	12.1%[a]
1973	3,644.4	17.8
1974	4,504.7	23.6
1975	4,900.5	8.8
1976	5,764.1	17.6
1977	6,073.6	5.4
1978	6,688.5	10.1
1979	7,903.6	18.2
1980	9,415.9	19.1
1981	10,188.0	8.2
1982	10,170.0	−0.2
1983	10,561.7	3.9
1984	10,492.1	−0.7
1985	10,707.5	2.1
1986	11,378.3	6.3
1987	11,232.5	−1.3
1988[b]	11,388.8	1.4
1997[b]	17,644.8	4.6[a]

Source: Ref 31.

[a] Average annual growth.

[b] Estimate.

Condensed Phosphates

- Sodium tripolyphosphate (nonmelting).
- Sodium hexametaphosphate (used to restrict melts).
- Tetrasodium pyrophosphate and sodium acid pyrophosphate (minimal usage).

General Rules for Emulsifier Selection

Citrates. Used where firm-textured, close-knit products are desired, such as slices, and when products are refrigerated.

Phosphates. Used where shelf stability is required, pH is to be controlled for melting, and other physical properties.

Optional Dairy Ingredients

- Milk fat.
- Milk and skim milk.
- Nonfat dry milk (NFDM).
- Whey (lots of lactose, some sweetness).
- Whey protein.
- Buttermilk.
- Skim-milk cheese.

Other Optional Ingredients

- Acidifying agents.
- Salt.

Table 5. Manufacturers' Sales of Cheese by Type

	Natural cheese		Process cheese and related products		Cottage cheese		Other cheese[a]	
	Sales ($, millions)	Percent change	Sales ($, millions)	Percent change	Sales ($, millions)	Percent change	Sales ($, millions)	Percent change
1967	829.2	—	662.5	—	218.0	—	142.1	—
1972	1,400.0	11.0[c]	1,134.1	15.1[a]	340.9	9.4[c]	219.6	9.1[b]
1973	1,705.9	21.9	1,363.5	20.2	405.6	19.0	169.4	−22.9
1974	2,458.7	44.1	1,496.6	9.8	456.0	12.4	93.4	−44.9
1975	2,668.7	8.5	1,654.4	10.5	506.7	11.6	68.7	−26.4
1976	3,267.9	22.5	1,859.7	12.4	530.7	4.3	105.8	54.0
1977	2,727.2	−16.5	2,518.5	35.4	545.6	2.8	282.3	66.8
1978	3,104.1	13.8	2,681.4	6.5	588.5	7.9	314.5	11.4
1979	3,949.3	27.2	2,822.0	5.2	729.3	23.9	403.0	28.1
1980	4,821.1	22.1	3,303.4	17.1	840.9	15.3	450.5	11.8
1981	5,225.6	8.4	3,567.9	8.0	856.5	1.9	538.0	19.4
1982	5,625.6	7.7	3,194.3	−10.5	683.2	−20.2	666.9	24.0
1983	5,824.0	3.5	3,325.4	4.1	693.8	1.6	719.5	7.9
1984	5,617.3	−3.5	3,390.1	1.9	748.3	7.9	736.4	2.3
1985	5,664.6	0.8	3,552.6	4.8	738.3	−1.3	752.0	2.1
1986	6,289.8	11.0	3,548.9	−0.1	725.1	−1.8	814.5	8.3
1987	6,208.0	−1.3	3,463.7	−2.4	731.6	0.9	829.2	1.8
1988[c]	6,294.9	1.4	3,529.5	1.9	722.8	−1.2	841.6	1.5
1997[c]	9,826.9	4.7[b]	5,482.8	4.7[b]	1,083.9	3.9[b]	1,251.2	4.2[b]

Source: Ref 31.

[a] Includes cheese substitution.

[b] Average annual growth.

[c] Estimate.

- Artificial coloring.
- Spices or flavorings (other than cheese flavors).
- Mold inhibitors.
- Enzyme-modified cheese (EMC).
- Gums.
- Sweetening agents.

Processing Equipment

- Grinders.
- Transfer of cheese from grinder to blender.
- Blenders.
- Scale hoppers and auger cart.
- Batch cooking.
- Continuous cooking.
- Aseptic processing.
- Surge tanks.
- Flash tanks, scraped surface heat exchange (SSHE).
- Line screens.
- Shear pumps and silverson mixers.
- Specialty equipment.

Forms of Packaging

- Can and cup.
- Jar.
- Pouch.
- Loaf.
- Slice.

Methods of Cooling

- Chill roll.
- Hot pack.
- Fast cooling.
  Blast coolers.
  Water spray tunnels.
- Slow cooling.
  Cased product.
  Restricted melt properties.

CHEESE ANALOGUES: HISTORY

Cheese analogues are products that resemble natural or process cheeses. They are intended to have the appearance, taste, texture, and nutrition of their counterpart cheeses, but are made without butterfat. The procedures by which they are made are also quite similar to those used for traditional cheeses.

Throughout history, substitute food products have been developed as the result of shortages or the opportunity to reduce costs. With cheese analogues, the impetus was the opportunity to replace valuable butterfat with less-expensive fats made from vegetable oils. Early natural cheese analogues were made by skimming butterfat from whole milk, replacing it with some other fat, then following traditional cheese-making procedures. These products, called filled cheeses, were first made around the turn of the century (30).

In the early 1970s technology was developed in which process cheese analogues could be made by combining dried milk protein, hydrogenated vegetable oil in place of butterfat, emulsifying salts, and other ingredients, then cooking the mixture. These products simulated process American and mozzarella cheeses.

DEFINITIONS

Filled Cheese

A product that simulates a natural cheese, made by substituting the butterfat in fluid whole milk with some other fat, then following traditional natural cheese-making procedures. Typical products are similar to mild Cheddar, Colby, or similar cheeses. A filled cream cheese can also be made by replacing butterfat with vegetable fat following conventional procedures.

Process Cheese Analogue

A product that simulates a process cheese, made by substituting the butterfat in a formulation, combining it with a protein source, emulsifying salts, and other ingredients and cooking the mixture. Typical products are simulations of pasteurized process cheese, pasteurized process cheese food, or pasteurized process cheese spread. Imitation mozzarella cheese is also made with this method.

Cheese analogues are classified as imitation or substitutes depending on their functional and compositional characteristics. An imitation cheese need only resemble the cheese it is designed to replace. Thus the choice of ingredients that can be used is quite extensive. However, a product can generally be called a substitute cheese only if it is nutritionally equivalent to the cheese it is replacing and meets the minimum compositional requirements for that cheese. Thus substitute cheeses will often have higher protein levels than imitation cheeses and be fortified with vitamins and minerals.

COMMERCIAL USE

Since the introduction of these products in the 1970s, cheese analogue consumption in the United States grew to about 208 million lb in 1988, or about 2.2% of the total cheese markets (31). The main incentive was a lower price than natural cheeses. In 1980, for example, the average price of cheese analogues was $0.47/lb less than natural cheese (5). This was due to the use of vegetable oil in analogues, rather than butterfat, as well as imported casein products as the source of dairy protein.

The principal use of cheese analogues has been in sandwich slices or as an ingredient in food products manufactured by food-processing companies or food service operators. In these applications, the analogues can be used as extenders of natural cheeses. Common uses are in frozen pizzas and in food service cheese shreds and cheese sauces. The United States retail market has not grown substantially, because cheese analogues have a lower quality than natural cheeses. Nonetheless, analogues of pasteurized process American cheese and mozzarella shreds are being sold in the grocery stores.

INGREDIENTS IN CHEESE ANALOGUES

Formulations

Cheese analogues all contain a protein source, a fat component, and a variety of minor components chosen to provide the desired flavor, texture, color, nutrition, and keeping quality. Table 6 lists ingredient statements provided by these types of analogue product: a filled Cheddar type, an imitation process cheese, and an imitation cream cheese. The formulations are considerably more complex than for natural cheeses.

PROTEIN SOURCES

Casein Products

The most critical component in cheese analogues is the protein. The cheese analogues that have had commercial success are all based on casein, the protein in milk. In some cases a soluble casein salt, or caseinate, is used. One of three forms of casein is generally used. For each, the starting material is skim milk (Table 7).

Vegetable Proteins

Several patents have been issued for, and papers written about, the use of vegetable proteins such as soy or cottonseed protein in place of caseins (16,32). However, these products have not been commercially successful to date because of many technical problems. These include such important characteristics as flavor, melting properties, gel strength, color, translucence, and mouth feel (such as creaminess or chewiness). The vegetable proteins simply do not function as milk proteins without further modifications, and these developments remain in the laboratory. However, some soybean protein isolates have been used as an extender in cheese analogues to replace up to 30% of the casein.

Table 7. Composition of Casein(ates)

	Acid casein (lactic)	Sodium caseinate	Rennet casein
Protein ($N \times 6.35$)			
Dry basis, %	96.7	94.7	90.6
As is, %	87.3	90.4	80.6
Ash, %	1.8	3.8	7.8
Moisture, %	9.7	4.5	11.0
Fat, %	1.2	1.1	0.5
Lactose, %	0.1	0.1	0.1
pH (5% at 20°C)	4.8	6.7	7.1

Vegetable Fats

Fats are essential ingredients in cheese analogues, for they are replacing butterfat in the products. The fats utilized become emulsified and intermixed with protein and water and, therefore, contribute to melting and textural characteristics. The fats also must behave in the mouth something like butterfat, which does melt in a characteristic way and influences creaminess, flavor, and other factors. Consequently, fats most often utilized in cheese analogues are partially hydrogenated or mixtures of hydrogenated and unhydrogenated oils that stimulate butterfat. These fats are similar to vegetable shortenings and can be made from soybean, corn, cottonseed, palm, or coconut oils or blends of these oils. These blends often have a melting point of about 35–47°C (33).

Table 6. Ingredients in Cheese Analogues

Kraft Golden Image Imitation Cheddar Cheese	Kraft Lunchwagon Imitation Pasteurized Process Cheese	American Whipped Products King Smoothee Imitation Cream Cheese
Pasteurized part-skim milk	Whey	Water
Liquid and partially hydrogenated corn oil	Partially hydrogenated soybean oil	Partially hydrogenated vegetable oil (may contain one or more of the following: coconut oil, soybean oil, corn oil)
Cheese culture	Sodium caseinate	Corn syrup solids
Salt	Granular cheese	Sodium caseinate
Enzymes	Food starch, modified	Tapioca flour
Apocarotenal (color)	Sodium citrate	Monodiglycerides
Vitamin A palmitate	Monodiglycerides	Salt
	Potassium chloride	Lactic acid
	Sorbic acid as a preservative	Guar gum
	Magnesium oxide	Monopotassium phosphate
	Zinc oxide	Artificial flavor
	Vitamin B_{12}	Citric acid
	Apocarotenal (color)	Carageenan
	Vitamin A palmitate	Artificial color
	Calcium pantothenate	Sorbic acid (as a preservative)
	Riboflavin	

Table 8. Cheese Analogues Characteristics

Advantages	Disadvantages
Cost of raw materials	Flavor
Keeping quality	No U.S. large sources of casein
Nutritional equivalency or superiority	Unnatural image
Varying functional properties	

Other Ingredients

The principal ingredients in cheese analogues are protein, fat, and water. Thereafter, ingredients are added to solubilize the protein, add flavor and color, and provide nutritional supplements. A typical formula for an imitation pasteurized process American cheese is presented below (34).

Water	45.20
Acid casein	25.00
Hydrogenated soybean oil	20.60
Adipic acid	1.30
Na–Ca hydroxide	0.80
Color–flavor blend	0.45
Dry ingredients	
Salt	1.50
Tapioca flour	1.50
Tricalcium phosphate	1.10
Disodium phosphate	1.00
Vitamin mix	0.50
Cheese flavor	0.50
Amino acids	0.31
Dipotassium phosphate	0.14
Sorbic acid	0.10
	100.00%

As can be seen, the formulation of cheese analogues is quite complex.

CHARACTERISTICS

Cheese analogues have both advantages and disadvantages compared to their natural counterparts. To date, the biggest problem is flavor. Casein products have a characteristic off-flavor that needs to be masked, in some manner. Likewise, vegetable proteins do not have characteristic, clean dairy flavors. But there are some offsetting advantages (Table 8).

Analogues generally have better keeping quality than natural cheeses because hydrogenated vegetable oil is less likely to develop rancidity than butterfat. The absence of microbial cultures and their enzyme systems creates stable flavor and texture. Analogues made with vegetable oil also have less cholesterol than natural cheeses and can be formulated to have less saturated fat and fewer calories. The varying functional properties can be incorporated by the utilization of different ingredients.

BIBLIOGRAPHY

1. A. Carlson, G. Hill, and N. F. Olson, *Biotechnology and Bioengineering*, Vol. 29, John Wiley & Sons, Inc., New York, 1987, p. 582.
2. P. F. Fox, *Cheese: Chemistry, Physics and Microbiology*, Vol. 2, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1987.
3. M. L. Green, "Effect of Milk Pretreatment and Making Conditions on the Properties of Cheddar Cheese from Milk Concentrated by Ultrafiltration," *Journal of Dairy Research* **52**, 555–564 (1985).
4. U.S. Pat. 3,914,435 (Oct. 21, 1975), J. L. Maubois and G. Mocquot (to Institut National de la Recherche Agronomique-France).
5. G. Sillen, "Modern Bactofuges in the Dairy Industry," *Dairy Industries International* **52**, 27–29 (1987).
6. D. D. Duxbury, "Breakthrough for Cheese—100% Chymosin Rennet," *Food Processing* **50**, 19–22 (1989).
7. P. H. A. Sneath, ed., *Bergey's Manual of Systematic Bacteriology*, Vol. 2, Williams and Wilkins, Baltimore, Md., 1984.
8. C. Daly, "The Use of Mesophilic Cultures in the Dairy Industry," *Antonie Van Leeuwenhock* **49**, 297–312 (1983).
9. J. Auclair and J. P. Accolas, "Use of Thermophilic Lactic Starters in the Dairy Industry," *Antonie Van Leeuwenhock* **49**, 313–326 (1983).
10. M. L. Green, "Review of the Progress of Dairy Science: Milk Coagulants," *Journal of Dairy Research* **44**, 159–188 (1977).
11. C. A. Ernstrom, "Enzyme Survival during Cheesemaking," *Dairy & Ice Cream Field* **159**, 43–46 (1976).
12. D. J. McMahon and R. J. Brown, "Enzymic Coagulation of Casein Micelles: A Review," *Journal of Dairy Science* **67**, 919–929 (1984).
13. R. C. Lawrence and J. Gilles, "Factors That Determine the pH of Young Cheddar Cheese," *New Zealand Journal of Dairy Science Technology* **17**, 1–14 (1982).
14. K. R. Nath and R. A. Ledford, "Growth Response of *Lactobacillus casei* var *casei* to Proteolysis Products Occurring in Cheese During Ripening," *Journal of Dairy Science* **56**, 710–715 (1973).
15. R. A. Ledford, A. C. O'Sullivan, and K. R. Nath, "Residual Casein Fractions in Ripened Cheese Determined by Polyacrylamide-Gel Electrophoresis," *Journal of Dairy Science* **49**, 1098–1101 (1966).
16. J. Stadhouders, L. Toepoal, and T. M. Wouters, "Cheesemaking With prt$^-$ and prt$^+$ Variants of *N*-Streptococci and Their Mixtures. Phage Sensitivity, Proteolysis and Flavor Development during Ripening," *Netherlands Milk Dairy Journal* **42**, 183–193 (1988).
17. D. J. Manning and H. E. Nursten, "Flavor of Milk and Milk Products," in P. F. Fox, ed., *Developments in Dairy Chemistry*, Vol. 3, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1985.
18. G. W. Reinbold, *Swiss Cheese Varieties, Pfizer Cheese Monographs*, Vol. 5, Pfizer, Inc. New York, 1972.
19. P. Trieu-cuot and J. C. Gripon, "A Study of Proteolysis during Camembert Cheese Ripening Using Isoelectric Focusing and Two Dimensional Electrophoresis," *Journal of Dairy Research* **49**, 501–510 (1982).
20. J. E. Kinsella and D. Hwang, "Biosynthesis of Flavors by *Penicillium roqueforti*," *Biotechnology and Bioengineering* **18**, 927–938 (1976).
21. R. D. King and G. H. Clegg, "The Metabolism of Fatty Acids, Methyl Ketones and Secondary Alcohols by *Penicillium roqueforti* in Blue Cheese Slurries," *Journal of the Science of Food and Agriculture* **30**, 197–202 (1979).
22. W. L. Langhus, W. Y. Price, H. H. Sommer, and W. C. Frazier, "The "Smear" of Brick Cheese and Its Relation to Flavor Development," *Journal of Dairy Science* **28**, 827–838 (1945).

23. M. Purko, W. O. Nelson, and W. A. Wood, "The Associative Action between Certain Yeasts and *Bacterium linens*," *Journal of Dairy Science* **34**, 699–705 (1951).
24. D. Sloot and P. D. Harkor, "Volatile Trace Components in Gouda Cheese," *Journal of Agriculture and Food Chemistry* **23**, 356–357 (1975).
25. A. H. Woo and R. C. Lindsay, "Concentration of Major Free Fatty Acids and Flavor Development in Italian Cheese Varieties," *Journal of Dairy Science* **67**, 960–968 (1984).
26. T. Conner, "Advances in Accelerated Ripening of Cheese," *Cultured Dairy Products Journal* **23**, 21–25 (1988).
27. E. M. Foster, F. E. Nelson, R. N. Doetsch, and J. C. Olson, Jr., *Dairy Microbiology*, Prentice Hall, Englewood Cliffs, N.J., 1957.
28. K. R. Nath and B. J. Kostak, "Etiology of White Spot Defect in Swiss Cheese Made from Pasteurized Milk," *Journal of Food Protection* **49**, 718–723 (1986).
29. *Code of Federal Regulations*, Title 21 §100 120.
30. S. K. Gupta, G. R. Patil, and A. A. Patel, "Fabricated Dairy Products," *Indian Dairyman* **39**, 199–208 (1987).
31. U.S. Department of Commerce, Bureau of the Census, International Trade Administration, reprinted in *U.S. Industrial Outlook 1989—Food, Beverages, and Tobacco*, U.S. Government Printing Office, Washington, D.C., Jan. 1989.
32. U.S. Pat. 4,303,691 (Dec. 1, 1981), R. E. Sand and R. E. Johnson (to Anderson, Clayton & Co.).
33. U.S. Pat. 4,822,623 (Apr. 18, 1989), J. L. Middleton (to Universal Foods Corp.).
34. U.S. Pat. 3,922,374 (Nov. 25, 1975), R. J. Bell, J. D. Whynn, G. T. Denton, R. E. Sand, and D. L. Cornelius (to Anderson Clayton & Co.).

GENERAL REFERENCES

V. K. Babayan and J. R. Rosenau, "Medium Chain Triglyceride Cheese," *Food Technol.* **45**, 111–114 (1991).

A. Baer et al., "Microplate Assay of Free Amino Acids in Swiss Cheeses," *Lebensm.-Wiss. Technol.* **29**, 58–62 (1996).

P. Bican and A. Spahni, "Proteolysis in Swiss-type Cheeses: A High Performance Liquid Chromatography Study," *International Dairy Journal* **3**, 73–84 (1993).

P. Bican, A. Spahni, and J. Schaller, "Partial Characterization of Peptides From Emmentaler Cheese," *Food Biotechnology* **8**, 229–241 (1994).

J. O. Bosset et al., "Occurrence of Terpenes and Aliphatic Hydrocarbons in Swiss Gruyere and Etivaz Alpine Cheese Using Dynamic Headspace GC-MS Analysis of Their Volatile Compounds," *Schweizerische Milchwirtschafliche Forschung* **23**, 37–42 (1994).

J. O. Bosset, M. Cullomb, and R. Sieber, "The Aroma Composition of Swiss Gruyere Cheese IV. The Acidic Volatile Components and Their Changes in Content During Ripening," *Lebensm.-Wiss. Technol.* **26**, 581–592 (1993).

S. E. Brummel and K. Lee, "Soluble Hydrocolloids Enable Fat Reduction in Process Cheese Spreads," *J. Food Sci.* **55**, 1290–1292, 1307 (1990).

L. Cabezas, P. Alvarez Martin, and M. D. Cabezudo, "Proteolysis in Gruyere de Comte Cheese Accentuating the Effect of Traditional Salting," *Revista Espanola de Ciencia y Tecnologia de Alimentos* **33**, 501–516 (1993).

S. L. Caldwell et al., "Development of Lactose-Positive *Pediococcus* Species Cheese Starter Cultures," *Journal of Dairy Science* **78**(Suppl. 1), 108 (1995).

E. Carrera et al., "The Effect of Milk Coagulant on the Formation of Hydrophobic Peptides in Cheese Manufactured From Cow, Ewe or Goat Milk," *Journal of Dairy Science* **78**(Suppl. 1), 143 (1995).

F. L. Davies and B. A. Law, eds., *Advances in Microbiology and Biochemistry of Cheese and Fermented Milk*, Elsevier Applied Science Publishers, Ltd., Barking, United Kingdom, 1986.

K. Elsborg, "Cheese Cultures Adapted to Specific Technologies," *European Dairy Magazine* No. 4, 44–45 (1997).

P. F. Fox, *Cheese, Chemistry, Physics and Microbiology*, Vol. 1, Chapman and Hall, London, United Kingdom, 1993.

P. F. Fox, *Cheese: Chemistry, Physics and Microbiology*, Vol. 2, Elsevier Applied Science Publishers, Ltd., Barking, United Kingdom, 1987.

S. K. Garg and B. N. Johri, "Rennet: Current Trends and Future Research," *Food Rev. Int.* **10**, 313–355 (1994).

M. Grand et al., "Development of Ripening Flora on the Surface of Gruyere Cheese," *Schweizerische Milchwirtschaftliche Forschung* **21**, 3–5 (1992).

R. Grappin and J. L. Berdague, "Ripening and Quality of Gruyere de Comte Cheese, VIII. Synthesis and Conclusions," *Lait* **69**, 183–196 (1989).

Y. H. Hong, "Influences of Ingredients and Melting Temperatures on the Physicochemical Properties of Process Cheese," *Korean Journal of Food Science and Technology* **21**, 710–713 (1989).

S. Issanchou, I. Lesschaeve, and E. P. Koester, "Screening Individual Ability to Perform Descriptive Analysis of Food Products: Basic Statements and Application to a Camembert Descriptive Panel," *Journal of Sensory Studies* **10**, 349–368 (1995).

M. Kalab et al., "Structure, Meltability, and Firmness of Process Cheese Containing White Cheese," *Food Structure* **10**, 193–201 (1991).

T. Khieokhachee, "Kinetic Studies of Cheese Starter Cultures," *Dissertation Abstracts International B* **56**, 3522–3523 (1996).

F. A. M. Klaver and M. Twigt, "Improvement of Eye Formation in Cheese," *Voedingsmiddelentechnologie* **26**, 11–14 (1993).

F. Kosikowski, *Cheese and Fermented Milk Foods*, 2d ed. rev., F. V. Kosikowski & Associates Publishers, Brooktondale, New York, 1982.

J. Kubickova and W. Grosch, "Quantification of Potent Odorants in Camembert Cheese and Calculation of Their Odour Activity Values," *International Dairy Journal* **8**, 17–23 (1998).

J. Kubickova and W. Grosch, "Evaluation of Flavour Compounds of Camembert Cheese," *International Dairy Journal* **8**, 11–16 (1998).

B. Kupiec and B. Revell, "Specialty and Artisanal Cheese Today: The Product and the Consumer," *British Food Journal* **100**, 236–243 (1998).

S. Lubbers, N. Cayot, and C. Taisant, "Blue Cheese Taste Intensification in Process Cheese Products," *Science des Ailments* **17**, 393–402 (1997).

S. Marchesseau et al., "Influence of pH on Protein Interactions and Microstructure of Process Cheese," *Journal of Dairy Science* **80**, 1483–1489 (1997).

F. G. Martlery and V. L. Crow, "Open Texture in Cheese: The Contributions of Gas Production by Microorganisms and Cheese Manufacturing Practices," *Journal of Dairy Research* **63**, 489–507 (1996).

P. Palich, W. Derengiewicz, and J. Switka, "Studies on the Shelf Life of Modified Camembert Cheese," *Acta Alimentaria* **19**, 321–329 (1990).

D. B. Perry, J. MacMahon, and C. J. Oberg, "Manufacture of Low Fat Mozzarella Cheese Using Exopolysaccharide-Producing Starter Cultures," *Journal of Dairy Science* **81**, 536–566 (1998).

L. M. Popplewell and J. R. Rosenau, "Incorporation of Milk Proteins Harvested by Direct Acidification Into Process Cheese Products," *Journal of Food Process Engineering* **11**, 203–220 (1989).

M. Preininger and W. Grosch, "Evaluation of Key Odorants of the Neutral Volatiles of Emmentaler Cheese by Calculation of Odour Activity Values," *Lebensm.-Wiss. Technol.* **27**, 237–244 (1994).

S. Rapposch, "Occurrence and Significance of Nonstarter Lactic Acid Bacteria in Cheese," *Deutsche Milchwirtschaft* **48**, 838–841 (1997).

M. Rosenberg, M. McCarthy, and R. Kauten, "Evaluation of Eye Formation and Structural Quality of Swiss-Type Cheese by Magnetic Resonance Imaging," *Journal of Dairy Science* **75**, 2083–2091 (1992)

J. E. Schlesser, S. J. Schmidt, and R. Speckman, "Characterization of Chemical and Physical Changes in Camembert Cheese During Ripening," *Journal of Dairy Science* **75**, 1753–1760 (1992).

E. K. Shumaker and W. L. Wendorff, "Factors Affecting Pink Discoloration in Annato-Colored Pasteurized Process Cheese," *J. Food Sci.* **63**, 828–831 (1998).

R. Smeets, "Enzyme-Coagulation," *Dairy Technology-Paper* **5**, 14–18 (1995).

M. J. Sousa and F. X. Malcata, "Comparison of Plant and Animal Rennets in Terms of Microbiological, Chemical, and Proteolysis Characteristics of Ovine Cheese," *J. Agric. Food Chem.* **45**, 74–81 (1997).

M. Sutheerawattananonda et al., "Fluorescence Image Analysis of Process Cheese Manufactured With Trisodium Citrate and Sodium Chloride," *Journal of Dairy Science* **80**, 620–627 (1997).

F. Vance et al., "Autolysis of *Lactobacillus helveticus* and *Propionbacterium freudenreichii* in Swiss Cheeses: First Evidence by Using Species-Specific Lysis Markers," *Journal of Dairy Research* **65**, 609–620 (1998).

R. Warme, H. D. Belitz, and W. Grosch, "Evaluation of Taste Compounds of Swiss Cheese (Emmentaler)," *Z. Lebensm.-Unters. Forsch.* **203**, 230–235 (1996).

B. H. Webb, A. H. Johnson, and J. A. Alford, *Fundamentals of Dairy Chemistry*, 2d ed., AVI Publishing Co., Westport, Conn., 1974.

G. H. Wilster, *Practical Cheesemaking*, 11th ed., O.S.C. Cooperative Association, Corvalis, Ore., 1959.

P. Zanerl, "Detection of Clostridia Responsible for Cheese Defects in Raw Milk and Cheese," *Deutsche Milkchwirtschaft* **44**, 936, 938, 940 (1993).

K. RAJINDER NUATH
JOHN T. HINES
RONALD D. HARRIS
Kraft, General Foods, Inc.
Glenview, Illinois

CHEESE RHEOLOGY

The word rheology in the most literal sense means the study of flow. At first sight it appears an unlikely pursuit to apply to the study of cheese. However, one may extend the concept of the flow of a material to include the idea of any change in its shape, under the action of an external agency, which is not instantaneous, and which is not entirely recoverable. These are characteristics of flow (1), and the application to cheese becomes immediately obvious. In fact, in the history of rheology applied to food materials, many of the pioneers found dairy products, and particularly cheese, very suitable materials for their experiments. This followed naturally from the fact that cheese-making was originally a craft industry. Long before rheology developed as a science, the skilled cheesemaker used various tactile tests to estimate the progress of the development of body in the curd and the firmness of the final cheese, using memory as a guide to the consistency of the results. The rheologist has, in more recent years, attempted to apply instrumental techniques and universally adopted physical units to these measurements.

At the same time as the science of rheology was developing, and often in parallel with it, the methods of testing using the human senses have been developed into a separate field of study now known as texture studies (2–4). It is necessary for the complete food rheologist to keep abreast of these developments since food is judged, in the final analysis, by its deformation in the mouth during mastication, a purely sensory judgment. Food scientists devote much effort to correlating the physical and sensory observations. It may well be that in endeavoring to seek parity between instrumental rheological measurements and properties judged subjectively one is asking the wrong question and a question to which there can be no final and complete answer. The real questions which can be asked are: What can instrumental measurements tell us about the properties and in particular the structure of the material on which those properties depend? How can this information be useful to the cheese-maker? How may the instrumental measurements be used as a guide to the consumer's assessment?

FIRST PRINCIPLES

Before proceeding to the detailed study of the application of rheology to cheese, it is pertinent to consider a few basic principles. These will appear obvious to the reader educated in a physical science, but may be less obvious to those from other walks of life. One is taught that matter exists in one of three distinct states: gas, liquid, and solid. This context is mainly concerned with only the latter two. Both are characterized by the fact that any sample has a well-defined volume. Solids have a definite shape which can be altered only by the application of an external agency. Liquids, on the other hand, have no characteristic shape: they take up the shape of the vessel containing them.

The property, then, which distinguishes a solid is its rigidity and this term has been formally adopted by physicists as a measure of the effort required to change the sample's shape. The precise definition will be given later. If the material is a true solid, it will follow that this rigidity is invariant for the material. Furthermore, time does not enter into this, so it is understood that the change is instantaneous and once the effort is removed, the sample will recover its original shape spontaneously. This is, in plain language, the physicist's concept of an elastic solid.

In contrast with the solid, the characteristic by which a layman distinguishes a liquid is its fluidity. This, again, has a precise definition, but in practice it is more convenient to use the inverse concept, which is known as the vis-

L. M. Popplewell and J. R. Rosenau, "Incorporation of Milk Proteins Harvested by Direct Acidification Into Process Cheese Products," *Journal of Food Process Engineering* **11**, 203–220 (1989).

M. Preininger and W. Grosch, "Evaluation of Key Odorants of the Neutral Volatiles of Emmentaler Cheese by Calculation of Odour Activity Values," *Lebensm.-Wiss. Technol.* **27**, 237–244 (1994).

S. Rapposch, "Occurrence and Significance of Nonstarter Lactic Acid Bacteria in Cheese," *Deutsche Milchwirtschaft* **48**, 838–841 (1997).

M. Rosenberg, M. McCarthy, and R. Kauten, "Evaluation of Eye Formation and Structural Quality of Swiss-Type Cheese by Magnetic Resonance Imaging," *Journal of Dairy Science* **75**, 2083–2091 (1992)

J. E. Schlesser, S. J. Schmidt, and R Speckman, "Characterization of Chemical and Physical Changes in Camembert Cheese During Ripening," *Journal of Dairy Science* **75**, 1753–1760 (1992).

E. K. Shumaker and W. L. Wendorff, "Factors Affecting Pink Discoloration in Annato-Colored Pasteurized Process Cheese," *J. Food Sci.* **63**, 828–831 (1998).

R. Smeets, "Enzyme-Coagulation," *Dairy Technology-Paper* **5**, 14–18 (1995).

M. J. Sousa and F. X. Malcata, "Comparison of Plant and Animal Rennets in Terms of Microbiological, Chemical, and Proteolysis Characteristics of Ovine Cheese," *J. Agric. Food Chem.* **45**, 74–81 (1997).

M. Sutheerawattananonda et al., "Fluorescence Image Analysis of Process Cheese Manufactured With Trisodium Citrate and Sodium Chloride," *Journal of Dairy Science* **80**, 620–627 (1997).

F. Vance et al., "Autolysis of *Lactobacillus helveticus* and *Propionbacterium freudenreichii* in Swiss Cheeses: First Evidence by Using Species-Specific Lysis Markers," *Journal of Dairy Research* **65**, 609–620 (1998).

R. Warme, H. D. Belitz, and W. Grosch, "Evaluation of Taste Compounds of Swiss Cheese (Emmentaler)," *Z. Lebensm.-Unters. Forsch.* **203**, 230–235 (1996).

B. H. Webb, A. H. Johnson, and J. A. Alford, *Fundamentals of Dairy Chemistry*, 2d ed., AVI Publishing Co., Westport, Conn., 1974.

G. H. Wilster, *Practical Cheesemaking*, 11th ed., O.S.C. Cooperative Association, Corvalis, Ore., 1959.

P. Zanerl, "Detection of Clostridia Responsible for Cheese Defects in Raw Milk and Cheese," *Deutsche Milkchwirtschaft* **44**, 936, 938, 940 (1993).

K. RAJINDER NUATH
JOHN T. HINES
RONALD D. HARRIS
Kraft, General Foods, Inc.
Glenview, Illinois

CHEESE RHEOLOGY

The word rheology in the most literal sense means the study of flow. At first sight it appears an unlikely pursuit to apply to the study of cheese. However, one may extend the concept of the flow of a material to include the idea of any change in its shape, under the action of an external agency, which is not instantaneous, and which is not entirely recoverable. These are characteristics of flow (1), and the application to cheese becomes immediately obvious. In fact, in the history of rheology applied to food materials, many of the pioneers found dairy products, and particularly cheese, very suitable materials for their experiments. This followed naturally from the fact that cheese-making was originally a craft industry. Long before rheology developed as a science, the skilled cheesemaker used various tactile tests to estimate the progress of the development of body in the curd and the firmness of the final cheese, using memory as a guide to the consistency of the results. The rheologist has, in more recent years, attempted to apply instrumental techniques and universally adopted physical units to these measurements.

At the same time as the science of rheology was developing, and often in parallel with it, the methods of testing using the human senses have been developed into a separate field of study now known as texture studies (2–4). It is necessary for the complete food rheologist to keep abreast of these developments since food is judged, in the final analysis, by its deformation in the mouth during mastication, a purely sensory judgment. Food scientists devote much effort to correlating the physical and sensory observations. It may well be that in endeavoring to seek parity between instrumental rheological measurements and properties judged subjectively one is asking the wrong question and a question to which there can be no final and complete answer. The real questions which can be asked are: What can instrumental measurements tell us about the properties and in particular the structure of the material on which those properties depend? How can this information be useful to the cheese-maker? How may the instrumental measurements be used as a guide to the consumer's assessment?

FIRST PRINCIPLES

Before proceeding to the detailed study of the application of rheology to cheese, it is pertinent to consider a few basic principles. These will appear obvious to the reader educated in a physical science, but may be less obvious to those from other walks of life. One is taught that matter exists in one of three distinct states: gas, liquid, and solid. This context is mainly concerned with only the latter two. Both are characterized by the fact that any sample has a well-defined volume. Solids have a definite shape which can be altered only by the application of an external agency. Liquids, on the other hand, have no characteristic shape: they take up the shape of the vessel containing them.

The property, then, which distinguishes a solid is its rigidity and this term has been formally adopted by physicists as a measure of the effort required to change the sample's shape. The precise definition will be given later. If the material is a true solid, it will follow that this rigidity is invariant for the material. Furthermore, time does not enter into this, so it is understood that the change is instantaneous and once the effort is removed, the sample will recover its original shape spontaneously. This is, in plain language, the physicist's concept of an elastic solid.

In contrast with the solid, the characteristic by which a layman distinguishes a liquid is its fluidity. This, again, has a precise definition, but in practice it is more convenient to use the inverse concept, which is known as the viscosity. This is a measure of the relation between the effort applied to the liquid and the rate at which it flows. As before, once the effort has been withdrawn, the flow ceases, but the liquid remains where it is—there is no recovery. It is the rate of flow which spontaneously reverts to its original value. This describes the physicist's concept of a viscous liquid.

It is important to keep clearly in mind that the fundamental difference in behavior between solids and liquids involves the dimension of time. Time does not enter at all into the description of the behavior of an elastic solid—only spatial dimensions are involved—whereas time is equally as important as the spatial dimensions in the description of fluid behavior.

So far, only ideal materials have been described. These are seldom encountered in practice and are only of interest to rheologists as reference points, the simplest extremes of material behavior, one may say the "black and white" of classical physics. It is that extensive "gray" area between that is the rheologist's domain. Everyone knows that a piece of cheese, once squeezed, may recover its original shape only slowly and probably not completely. Indeed, some of the softer cheeses may, if sufficient effort be applied, be spread and only recover very little. This leads to a definition of a third category of materials. Any material falling within this gray area, possessing at the same time some of the characteristics of elastic and of viscous behavior, is known as a viscoelastic material.

Yet another class of materials may be mentioned in passing, since they may be encountered in everyday parlance. This is the plastic material. Plasticity may be defined as that property of a material whereby it remains rigid until a certain minimum force is applied, whereupon it deforms. Once this force is removed or falls below the critical value, the material again becomes rigid but remains in the deformed state. There is a simple mathematical model for this type of behavior (5) and the rheologist may be particularly interested in the transition from solid to fluid behavior, known as the yield point; however, the ideal plastic is probably as rare as the ideal solid or liquid.

DEFINITIONS

Before the rheological measurements which have been made on cheese can be discussed it is necessary to define a few of the terms used by rheologists. In the preceding paragraphs the words effort and force have been used in their everyday connotation. The term used by the rheologist is stress, denoted by the Greek letter σ. This is formally defined as the force measured in newtons (N) divided by the area in square meters (m^2) over which it is applied, and is measured in pascals (Pa). A convenient aide-mémoire for the size of these units is that a newton is approximately the weight, under the action of gravity, of a medium-sized apple. The stress may be applied normal to the surface, as in Figure 1a, or tangentially, as in Figure 1b. In either case it is equal to the force divided by the area over which it is applied.

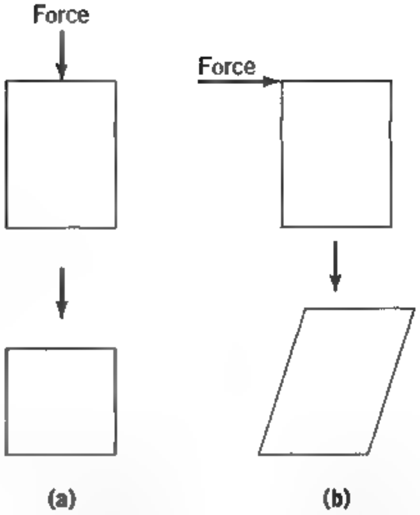


Figure 1. Application of stress to a sample: (a) compressive strain; and (b) shear.

In the case of Figure 1a, which depicts a weight resting on the upper surface of a sample, the deformation which occurs is a change (Δh) in the height (h) of the sample. The fractional change in height, $\Delta h/h$, defines the strain, which is denoted by the Greek letter ϵ. There is a practical difficulty here: the height is continuously changing during the deformation, so there is some uncertainty over what value should be used for h (6). For small deformations this is relatively unimportant, but when large deformations occur, such as is usual when examining cheese, it is necessary to be clear on this point. For the sake of clarity, the ratio of the change in height to the original height will be referred to as the fractional compression and expressed as a percentage. When the change is related to the varying height, the formula for the strain becomes

$$\epsilon = \ln(h_0/h_1) \tag{1}$$

This is sometimes called the true strain and will be given in decimal form.

If the stress is applied tangentially, as in Figure 1b, the resulting strain is described as a shear. It is defined as the distance through which one plane has traveled relative to another divided by the distance between them and is given the symbol γ. Both the strain and the shear are the quotients of two lengths and are therefore dimensionless numbers.

With these definitions of the physical quantities stress, strain, and shear, it is now possible to define the properties of some ideal materials. The rigidity of a solid, hitherto used loosely, can now be defined formally as the ratio of the tangential stress to the shear produced and is given the symbol n:

$$n = \sigma/\gamma \tag{2}$$

The dimensions of n are evidently the same as the dimensions of the stress, but it is usual to express the modulus of rigidity in newtons per square meter and to reserve the use of pascals for stresses only, thereby preserving an easily recognizable distinction between them.

The second property of an elastic solid which may be defined can be seen by referring again to Figure 1a. This is the modulus of elasticity and is the ratio of the stress to the strain. If the material is ideal, it will be isotropic; that is, the properties will be the same in all directions. During compression the volume will remain constant, so that while the height of the sample is changing in the direction of the applied stress, the dimensions in the two directions at right angles will change simultaneously. At any point within the sample only one-third of the stress will be used in causing the change in height. Another third will be used in each of the two other directions normal to this, causing the sample to expand laterally. Hence, for any ideal elastic material the modulus of elasticity will be three times the modulus of rigidity. However, if the material is compressible, some of the stress will be used up in compressing it and the factor 3 relating the modulus of elasticity to that of rigidity falls toward 2. The importance of this is that some authors express their results in terms of elasticity and some in terms of rigidity. An accurate comparison is possible only when some information is available concerning the compressibility of the material. In practice the ideal incompressible material is as unlikely to exist as any other ideal material. Almost all cheese is somewhat compressible, but in general not so compressible that any serious error is introduced by using the factor 3 to convert from one unit to the other.

In the case of liquids there is only one material constant that is of interest. The rate of flow or deformation is now given by the quotient of the strain and the time taken to reach it if the motion is constant, or in the unsteady state by the first derivative with respect to time of the strain, $\dot{\epsilon}$. The ratio of the applied stress to the rate of flow is called the viscosity and this is denoted by the Greek letter η, so that

$$\eta = \sigma/\dot{\epsilon} \tag{3}$$

The unit of viscosity is the pascal-second. A rough guide to its magnitude is that the viscosity of water at room temperature is very nearly 1/1000 Pa-s.

MODELS OF RHEOLOGICAL BEHAVIOR

In order to provide a theoretical framework within which to discuss the determination of the physical quantities just described it is necessary to consider some models for a viscoelastic body. There are two ways in which this may be visualized. Viscoelasticity may be considered either to be a property intermediate between viscosity and elasticity, or the result of a combination of viscous and elastic behavior occurring simultaneously in the material. In the first case, recalling that the elastic modulus is a function of spatial dimensions only, whereas viscosity is a function also of the first derivative of the strain with respect to time, the suggestion is that viscoelasticity is a function of a fractional derivative, the fraction lying between zero and unity. Although the concept is simple to describe, the mathematical equipment required to convert measurements which are always made in a conventional three-dimensional space and real time to this hypothetical continuum where space and time are no longer independent is formidable. The concept is now of historical interest only, but readers may find some early writers used it to discuss the rheology of cheese.

The alternative hypothesis, that both elastic and viscous behavior occur simultaneously, leads to a number of simple models (7), which require only elementary mathematics. It should be emphasized at this stage that these are only mathematical models, whose purpose is to codify the experimental observations; they cannot be taken to be real physical structures. It will be shown later that it is sometimes possible to relate elements in a model to the existence of particular structural elements within a material. The simplest model for an elastic solid is a spring whose strain or compression ϵ_s is always proportional to the stress. Writing G for the constant of proportionality to distinguish it from the rigidity or elasticity of any real material,

$$\epsilon_s = \sigma/G \tag{4}$$

In a similar manner a viscous liquid may be modeled by an infinitely long dashpot whose displacement is always inversely proportional to the viscosity

$$\dot{\epsilon} = \sigma/\eta \tag{5}$$

There are two ways in which one spring and one dashpot can be combined to give a compound structure. If they are placed in tandem as shown in Figure 2a, the stress is continuous throughout the model and the total displacement is given by the sum of those of the elements:

$$\epsilon = \sigma/G + \sigma t/\eta \tag{6}$$

The first term of the right-hand side expresses the elastic component and indicates that immediately on applying the stress there will be some deformation; thereafter (second term) the deformation will increase with time t. On removal of the stress, the elastic component recovers immediately while the strain due to the dashpot remains.

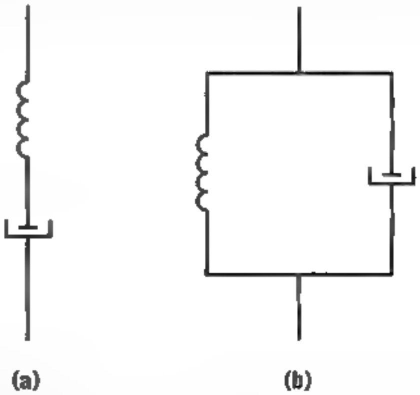


Figure 2. Viscoelastic models. (a) Maxwell body, and (b) Voigt body.

This model is called a Maxwell body. A complete creep and recovery curve of this model is given in Figure 3a. It is already apparent that a two-component model represents the rheological behavior of a sample of cheese better than the assumption that it is either a solid or a fluid.

If the two components are combined in the other possible way, so that they share the stress but the strain is common to both, we arrive at a formula for a Voigt body, sometimes known as a Kelvin body:

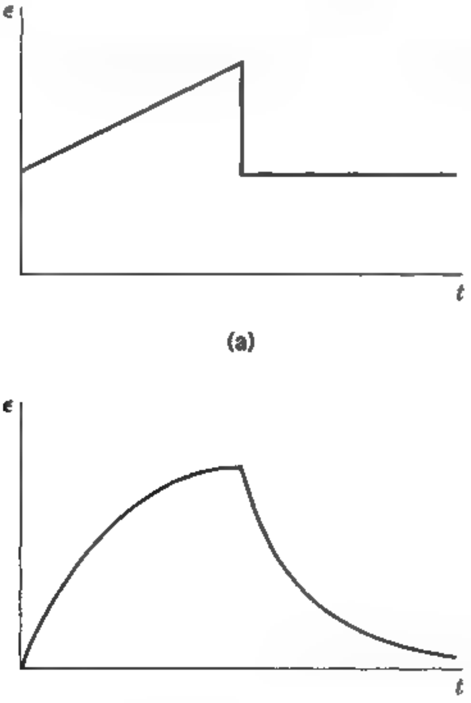
$$\sigma = G\epsilon + \frac{\eta\dot{\epsilon}}{t} \qquad (7)$$

Transforming this equation so that it may be compared with equation 6 gives

$$\epsilon = \frac{\sigma}{\eta}\left[1 - \exp\left(-\frac{G}{\eta}\,t\right)\right] \qquad (8)$$

The creep and recovery curve of this model is given in Figure 3b. Again, there is some resemblance between this curve and the behavior observed with cheese. The deformation and the recovery are not instantaneous in this case and this is more in line with practical experience, but the model predicts a complete recovery, which is not always observed.

The student of electric circuit theory will be aware of some familiarity with the Voigt and Maxwell models. If one replaces the spring with a capacitor and the dashpot with a resistor, the two models become the two alternative representations of a leaky dielectric. Just as in electrical network theory these binary combinations can be built up into more complicated networks by adding more elements, so the Voigt and Maxwell bodies can be extended. One popular combination is of a Voigt body placed end-to-end with a Maxwell body. This is known as a Burgers body and it does indeed give a creep and recovery curve in which both the deformation and the recovery are retarded to different degrees and in which the recovery is incomplete.



(a)

(b)

Figure 3. Creep recovery curve: (**a**) Maxwell body; and (**b**) Voigt body.

Although the process of building up more complicated models may be continued indefinitely, the expressions for the behavior rapidly become cumbersome. As the number of elements increases it becomes disproportionately difficult to analyze the data, and the accuracy of the parameters extracted decreases (8). Indeed, the precision of any experimental measurements made on cheese may well be insufficient for more than two or three parameters to be identified with any pretensions of accuracy. Only if the rheological behavior can be linked unequivocally to the structure of the sample may the use of complex models be justified. Otherwise, the model should be kept as simple as possible.

EMPIRICAL INSTRUMENTAL TESTS

The earliest applications of rheology to cheese, even before rheology was conceived as a scientific discipline, consisted of making subjective estimations of some of the more obvious properties and trying to describe these in plain language. Often the cheese-maker or the grader, though skillful and competent in judgment, would find it difficult to define precisely the terms being used. One can see that confusion can arise when one tries to distinguish between terms such as consistency and body, firmness and hardness, chewiness and meatiness. The grader's mind may be clear about what is understood by each, but communicating the precise meaning to the outside world is less easy. The difficulty is further compounded by the fact that these words do not translate precisely into other languages, making international communication more difficult. To some extent the development of texture studies, particularly in the United States, has clarified the semantic problem (9), but subjective assessment can never match the precision which can be attained with sophisticated instrumentation. Much of the earlier instrumental rheology sought to replace, or at least to reinforce, subjective judgment with numerical quantities which could be measured reproducibly by any competent technical assistant (10).

The earliest instruments were empirical (3) and, in general, intended to give some indication of the firmness or similar qualities. To this end, they were designed to simulate what the scientist perceived to be the mechanical action of the hand or fingers of the expert during examination of the cheese. They were generally unsophisticated, not too expensive, and made no pretence of measuring any precisely defined physical property. Because of this, their measurements cannot be analyzed and expressed in terms of fundamental rheological parameters as defined above. Nevertheless, some of them may still perform a useful function if their limitations are understood, because they possess the undoubted merit that their measurements are easily reproducible between different laboratories and different times.

One such instrument became known as the Ball Compressor (11). In the course of examining a cheese, one of the actions of any grader is to press either a thumb or a finger into the surface and, from the reaction which is felt, gain an impression of the firmness or springiness of the cheese. The Ball Compressor might in fact be described as a mechanical thumb. It consists of a metal hemisphere which is initially placed on the surface of a whole cheese and allowed to sink into the cheese under the action of a load; the depth of indentation can be measured and recorded after a fixed time. The load can then be removed and the recovery of the cheese observed. The instrument is shown diagrammatically in Figure 4.

Making a number of simplifying assumptions, it is possible to convert a reading obtained with the Ball Compressor into a modulus G (12,13), which may be considered to be the equivalent of the modulus of rigidity, using the formula

$$G = 3M/16(RD^3)^{1/2}. \tag{9}$$

where M is the applied load, R the radius, and D the depth of the indentation. This formula has been arrived at assuming that the sample is a homogeneous isotropic elastic solid, the load is static, and there is no friction between the sample and the surface of the hemisphere. It is clear that every one of these conditions is violated. Cheese is far from homogeneous, particularly if it has a rind or has been salted by immersion in brine, causing the properties to vary from the surface to the center. It is not isotropic: the method of manufacture of some varieties, such as Cheddar, on which many of the earlier experiments were carried out, or Mozzarella, is intended to produce an oriented structure. Some of the objections, however, would appear to be much less serious in the case of other cheeses where uniformity of texture is the aim of the manufacturer. Cheese is not an elastic solid; it is viscoelastic. The indentation is not instantaneous and may not be completed within the duration of the experiment. Finally, if the load is removed, some indentation remains, showing that some of the energy has been dissipated in causing flow and not stored up as in an elastic solid. In spite of all these limitations, if the test proceeds at least until the indentation becomes so slow that it has virtually ceased, the use of equation 9 gives an indication of the magnitude of the firmness in readily understandable physical units. This enables the reader to compare measurements given in the early literature with those made more recently by more sophisticated means.

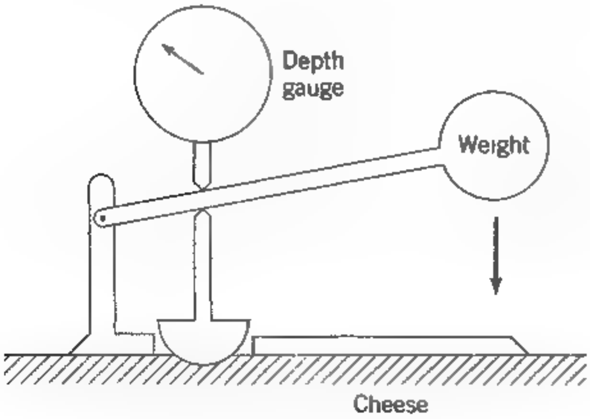


Figure 4. The Ball Compressor (diagrammatic).

The Ball Compressor has been discussed at some length, not only because of its historical importance, but because it has the merit of being a nondestructive means of testing and does not require an operator to undergo any specialized training. Also, it is used on the whole cheese, whereas almost every other rheological test requires that a sample be cut from the whole. Using the Ball Compressor, it has been shown that in a whole cheese such as a Cheddar or a Cheshire weighing some 25 kg there are considerable variations in the firmness over the surface of the cheese (14) and that the firmness differs on the upper and lower sides. This difference is influenced by the frequency with which the cheese is turned in the store during its maturing period, and the time which has elapsed since it was last turned. The implication of this is that it is not possible, whatever instrumental measurement is made, to assign a single number to any property of the cheese, nor is it possible to assess the properties of the whole cheese by means of a measurement made at one local point in that cheese.

Clearly there is a need for some form of nondestructive test and for many purposes there is no reason why it should not be empirical, provided that the implications are understood. The Ball Compressor has the merits of cheapness and simplicity, but the time taken to obtain a representative reading limits its use to the research laboratory. The problem of devising a suitable test is as yet unsolved. It is unfortunate that the application of ultrasonic techniques, which have proved so useful in the nondestructive testing of many engineering materials, have proved unrewarding with cheese (15,16). This is because the dimensions of the cracks and other inhomogeneities in cheese are commensurate with, or sometimes even larger than, the wavelength of the ultrasound and large-scale scattering takes place. It is difficult for a pulse to penetrate the body of the cheese and both the velocity of propagation and the attenuation are more influenced by the scattering than by the properties of the bulk.

One other empirical test deserves to be mentioned on account of its simplicity. This is the penetrometer (17–19). It is not quite nondestructive, but very nearly so, since it only requires that a needle be driven into the body of the cheese; no separate sampling is required. A penetrometer test may take one of several forms. For example, a needle may be allowed to penetrate under the action of a fixed load (17,18), or it may be forced into the cheese at a predetermined rate (19) and the required force measured.

Whichever method is used, let the specific actions be considered. As the needle penetrates the cheese, that part of the cheese immediately ahead of it is ruptured and forced apart. If the needle is thin, the deformation normal to its axis is small, so that the force required to accomplish this may be ignored. On the other hand, the progress of the needle is retarded by the adhesion of its surface to the cheese through which it passes. This may be expected to increase with the progress of the penetration until a point is reached when the restraining force matches the applied load and further penetration ceases. If a suitable diameter for the needle and a suitable weight have been chosen, this test may be completed in a few seconds. The test will be more useful for cheese whose body is reasonably homogeneous on the macroscopic scale, as, for example, the Dutch cheeses. With cheeses such as Cheddar or the blue cheeses the heterogeneities are generally on too large a scale and the penetration becomes irregular. The needle may pass through weaknesses in the structure or even cracks and so give rise to the impression that the cheese is less firm than it really is, or the point of the needle may attempt to follow a line of weakness, not necessarily vertical, and as a result there will be additional lateral forces acting on the needle and its penetration will be arrested prematurely.

Measurements made with a penetrometer cannot be converted theoretically to any well-defined physical unit. Both the cohesive forces within the cheese and the adhesive forces between the cheese and the surface of the needle are a consequence of the forces binding the structure together. One may infer that these are related to its viscoelastic properties but there is no simple theory which attempts to establish these relations. It has been shown experimentally (20) that there is a statistically significant correlation between firmness as measured by the Ball Compressor and by penetration, but this differs among different types of cheese (21). It has also been shown experimentally that a curvilinear relation exists between the resistance to penetration and an elastic modulus of some Swiss cheeses, calculated from the results of a compression experiment (Fig. 5). While this gives some idea of the magnitude of the forces involved, it has little practical application since it refers only to one series of experiments on only one type of cheese. In the absence of a similar experimentally determined relation for the cheese in which one is particularly interested, penetrometer measurements can only be regarded as empirical.

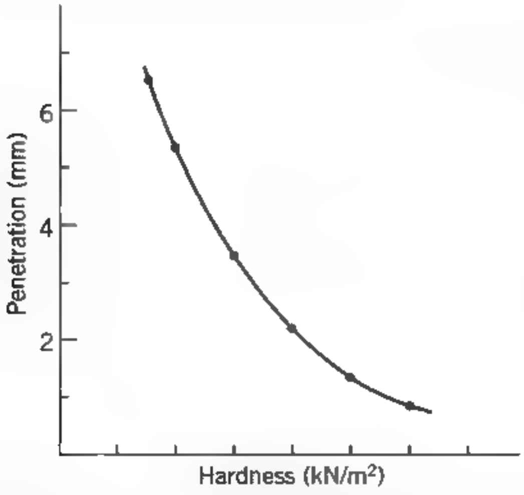


Figure 5. Penetrometer readings compared with compression modulus.

Another test of a similar nature is the use of a cone to penetrate the sample (22). This test was originally developed for use with high-viscosity lubricants. A loaded cone with its apex pointed vertically downward and initially just making contact with the upper surface of the sample is allowed to penetrate the sample until equilibrium is reached. If a suitable combination of the apical angle of the cone and the load is used, the equilibrium is usually reached within a few seconds and the penetration is deep enough to be measured comfortably. The test assumes that the sample behaves as a plastic or Bingham body; that is, when acted upon by small stresses it behaves as a solid, but once a critical stress, the yield stress, is exceeded, it flows as a viscous liquid.

In fact, the test works in reverse. At the commencement the stress is infinite, since the area of application, which is the tip of the cone, is zero. As the cone penetrates, the material is caused to flow laterally and the rate of penetration is controlled by this lateral flow. As the penetration proceeds, the cross-sectional area increases proportionally with the square of the depth of penetration and the stress decreases correspondingly. When the stress no longer exceeds the critical or yield point, motion ceases and an equilibrium is established. It is a matter of contention among rheologists whether a yield value really exists. Some maintain that there is always some flow, albeit minimal, however low the stress. For practical purposes, however, the cone soon appears to be stationary and the depth of penetration can be recorded. This is a simple, quick test and, while it is not entirely nondestructive, it does only a little damage near the surface of the sample. The vertical stress Y may be calculated from the penetration and the angle of the cone:

$$Y = (Mg/\pi h^2)\cot^2\alpha/2 \tag{10}$$

where α is the apical angle of the cone, h the penetration depth, M the applied load, and g the gravitational constant. This is greater than the yield stress since it does not take into account the stresses involved in causing the sample to flow laterally. An estimate of the yield stress may be obtained by multiplying the equilibrium vertical stress by the factor 1/2 sin α (23). It is only an estimate, though, because it takes no account of any friction between the surface of the cone and the cheese.

Another form of penetrometer-type test is that sometimes referred to as the puncture test (24). In place of a needle or cone, a rod is driven into the sample and the resistance to its motion measured. The mechanism of the deformation of the sample is more complicated in this case. At least four principal factors are involved. In the first place, the sample ahead of the rod is compressed. Second, the rod must cut through the sample at its leading edge: hence the name puncture, and this requires a force. Third, there is the frictional resistance between the surface of the rod and the cheese, and finally, there is the force required to set up the lateral flow within the sample. If the sample is large enough compared with the dimensions of the rod so that any compression in directions perpendicular to the motion may be ignored, once a dynamic equilibrium has been set up the first two factors will be constant and the third will increase linearly with the penetration. By using rods of different cross-sectional areas and keeping the perimeter the same it is possible to separate these effects (25,26).

Instead of performing this test on an extensive sample, such as a whole cheese, it is more often carried out on a small sample contained within a rigid box (27–29). When done in this manner, additional forces are called into play. The lateral forces on the rod as the sample is compressed between the rod and the walls of the container now become important. In addition, the compressive force on the leading face of the rod is no longer constant as the distance between this face and the bottom of the container decreases. If the sample is shallow, it is arguable (27,28,30) that this may indeed be the largest single contributory force, and, by ignoring the others in comparison with it, one can calculate a modulus from the ratio of the measured stress to the compression. It is obvious that this will be an overestimate of any true modulus which could have been derived in a simple unrestrained test and that the excess will be arbitrary and unknown. In spite of this the test is useful because it gives an indication of the magnitude of the rigidity of the sample and allows comparisons to be made between similar types of cheese.

PHYSICAL MEASUREMENTS

It has already been pointed out that rheological phenomena take place in real time. The basic rheological experiment consists in applying a known stress for a known time and observing the strain which ensues (31). In this context it is immaterial whether one refers to normal stresses, which result in strains, or to tangential stresses, which result in shears. For the purpose of the argument strain and shear are interchangeable. The generic mathematical equation which connects the variables is

$$\sigma = f(\epsilon, t)$$

This is the equation of a surface in a three-dimensional diagram. The material is said to be linearly viscoelastic if, for any value of t, the line representing the variation of strain with stress is a straight one. For many substances this is approximately true, at least while the strains and stresses are small. Cheeses vary in this respect. For a linear viscoelastic material the two-dimensional graph of strain versus time will contain all the required rheological information.

The basic graph is the creep curve. If the experiment is prolonged indefinitely, some equilibrium will ultimately be established. If the material is essentially a solid, but one whose deformation is retarded, the strain will eventually become constant. There are obviously two parameters which can be used to denote the material behavior, one based on the ultimate deformation and the other on the rate at which it is (asymptotically) established. On the other hand, if the material is more akin to a liquid, the final equilibrium will be a dynamic one in which a constant rate of strain is established. As before, two parameters are needed to describe the behavior: one, the ultimate flow; the other, the rate of attaining it. The experiment may also be conducted rather differently. Instead of allowing it to proceed ad infinitum, the stress may be removed at a predetermined point. Should the material possess any elastic characteristics, some of the energy used in the deformation will be stored within it; on removal of the stress the strain will decrease again and once more eventually reach an equilibrium. This part of the graph is known as the recovery curve. Again there are two points of particular interest—the amount of recovery and the rate

So far the description refers to what may be called an ideal viscoelastic material and is an oversimplification of real-life experience. If the material is not linearly viscoelastic, the creep and recovery curve can still be obtained, but a single curve will not include all the rheological information about the sample. A series of such curves is necessary, giving the full three-dimensional representation. It has also been assumed so far that the properties of the material are not altered in any way by the action of the strain itself. In the case of cheese it is very evident that at large strains the whole structure breaks down and cannot recover. Indeed, the stress and strain at the point of onset of this breakdown may be a useful quantity when describing the properties of any cheese, as will be seen later. Even at lower strains there may well be unobserved changes in the structure. These could occur during the early part of a cycle of measurement, in which case they may be revealed by the failure of the recovery curve to follow the expected pattern. However, they may occur during handling of the cheese at any stage between manufacture and sampling. A complete rheological description of a sample really needs not only the experimental curve or curves but a statement of the history of the straining of the sample up to the moment of measurement. This is most likely to be unavailable and it is necessary to presume that the sample is in good condition and to allow that variations in its history are included within the natural variation among samples.

It is often more convenient from the point of view of practical instrumentation to carry out the compression experiment in a different way. Instead of applying a fixed stress and observing the reaction, the sample is subjected to a known rate of strain and the build-up of the stress observed. Again, this may be prolonged indefinitely, if the operation of the instrument will allow it. If the straining is stopped at any point, it is not usually practicable to apply a reverse rate of strain. In this case, the strain is usually held constant and the relaxation of the stress observed. Again, a complete cycle of deformation and relaxation contains all the available information about the rheological properties of the sample. Quite sophisticated instruments have been developed which enable this type of test to be carried out with precision and then record the results, making this now the most widely used test. In principle, the operation of these instruments is simple. The sample is compressed between parallel plates at a known fixed rate and the force required is monitored.

However, before discussing the results of any measurements made on these instruments it is important to consider the exact mode of operation. As the measurement is usually carried out, the sample is in the form of a small cylinder, although other right prisms may be used. This is placed with its axis vertical on one plate and is then compressed along that axis. Since the compression is linear, the height of the sample at any instant after the compression has begun is given by

$$h = h_0(1 - \alpha t)$$

where h_0 is the original height of the sample and α denotes the rate at which the two places approach each other, ie,

$$\alpha = -dh/dt$$

Using equation 1 for the strain leads to

$$\epsilon = -\ln(1 - \alpha t) \qquad (11)$$

If the instrument's recorder treats time (or its equivalent linear distance traveled) as one axis, then this is not a true strain axis. As the compression increases from 0 to 100% the true strain increases from zero to infinity. In fact, whatever definition of strain is used (6), other than the linear compression, the time axis is a distortion of the strain axis. Second, as a sample is compressed its volume remains constant, so that the cross-sectional area increases as the height decreases. Assuming that the plates are perfectly smooth, so that there is no lateral friction between them and the end surfaces of the sample, a purely viscous, or equally a perfectly elastic solid, sample would deform uniformly and the cross-sectional area A at any height would vary precisely inversely with the height, ie,

$$A = A_0(h_0/h) \qquad (12)$$

A viscoelastic material does not deform so simply, but in such a way that the lateral movement is greatest near the ends, so that a concave shape results (32), as shown in Figure 6. In this case the stress is not uniform throughout the sample. When there is some friction between the plates and the sample, the lateral movement of the end layers is restricted and a rotational couple is set up within the sample, using the perimeter of the end surfaces as fulcrum. If the sample is homogeneous or at least cohesive, the effect of this is that the middle of the sample will spread outward, giving rise to barrel-shaped distortion (Fig. 6c). When the rotational forces which develop are sufficient to cause fractures to develop, even though they may be undetected by eye, then with the weakened structure the sample may spread out near the ends, and what is known as mushroom distortion results (33) (Fig. 6). Both barrel and mushroom distortion may occur simultaneously, and a shape such as Figure 6d may be the outcome.

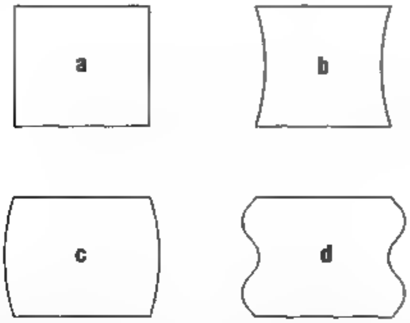


Figure 6. Compression of a cylinder: (**a**) ideal; (**b**) concave; (**c**) barrel; and (**d**) complex.

Also, during the compression some liquid, which may be free fat or water, or both, may be squeezed out. This might be thought to lubricate the sample ends to some extent; but it might also ensure closer contact between what is inevitably the slightly rough cut surface, the degree of roughness depending largely upon the type of cheese, and the smooth plates of the instrument. In practice it has been found (34) that the ends of the cheese do not expand as much as would be predicted by the use of equation 12; yet on the other hand it would be quite erroneous to assume that the cross section remained at its original value. The instrument does not measure stress, but the total force transmitted by the end surface to the measuring transducer. The stress within the sample is obtained by dividing the force F by the cross-sectional area. In the case of a perfectly lubricated sample this is given by

$$\sigma = F/A = (F/A_0)(1 - \alpha t) \qquad (13)$$

When barrel distortion occurs, the calculation of the true stress is less simple. If one assumes that the material behaves as a perfect elastic solid and that the ends of the sample are firmly bonded to the instrument plates so that they cannot expand laterally, the barreling can be shown to assume a parabolic profile. The actual correction to be applied depends on the height and radius of the uncompressed sample as well as the degree of compression. It is somewhat greater than the correction for a lubricated sample. A force–compression curve is thus not a true representation of a stress–strain curve. Near the origin it is close enough, but the distortion increases as the compression progresses.

If the sample behaves as one of the simple models, it is possible to predict the shape of the stress–strain curve. In the case of the Voigt body the solution is straightforward. The stress is the sum of the elastic and the viscous contributions (eq. 7). From equations 1 and 11

$$\epsilon = \alpha e^{\epsilon}$$

so that

$$\sigma = G\epsilon + \alpha\eta e^{\epsilon} \qquad (14)$$

This is the equation of a curve in which the intercept on the σ axis is proportional to the viscous component and the rate of compression, and the initial slope is proportional to the elastic component. The curve is concave upward throughout. On substituting for σ and ϵ, equation 14 becomes

$$F = [A_0/(1 - \alpha t)][\alpha\eta/(1 - \alpha t) - G\ln(1 - \alpha t)] \qquad (15)$$

This is not so convenient to analyze but there is still an intercept on the F axis proportional to the viscous component and the rate of compression. The solution for the Maxwell body is more complicated. Making the same substitutions as before leads to an infinite series

$$\sigma = Ge^{(G/\alpha\eta)\epsilon - \epsilon}\left[\epsilon + \sum_{1}^{\infty} \frac{(-G/\alpha\eta)^{i}(1 - e^{-\epsilon i})}{i.i!}\right] \quad (16)$$

At the commencement of the compression the stress is zero and the initial slope is proportional to the elastic component. Initially the curve is convex upward, but as the compression proceeds a point of inflexion is reached, depending on the ratio of the viscous to the elastic components and the rate of compression. Finally, the curve becomes asymptotic to a line through the origin, with a slope given by the elastic constant.

If the stress–strain curve obtained for a particular sample can be recognized as being similar to one of these two patterns, the appropriate model can be identified and the material constants evaluated. It is seldom that such a simple fit occurs. A material as heterogeneous as cheese is unlikely to conform to the behavior of a two-element model. More sophisticated models may give a better representation, but adding further elements only makes the theory more complex and the analysis that much more difficult. One word of caution must be entered here. The Voigt body is characterized by the finite intercept on the stress axis. The converse is not necessarily true. A finite intercept also arises in the case of any material possessing a yield value, since this is the stress which must be overcome before any deformation takes place.

In the usual way in which compression tests are carried out, the compression is allowed to proceed to a point far beyond that at which any simple theory may be expected to apply. Often it reaches 80%, a true strain of 1.609. Long before this is reached, any structure which may have been present in the original sample of cheese can be seen to have broken down. If it is carried to this extent, only the early part of the test may be considered as measuring the rheological properties of the original sample, while the latter part becomes a test of the mechanical strength of the structure. A typical compression curve is shown in Figure 7. It is often difficult to decide whether there is an intercept on the stress axis, ie, whether there is an instantaneous build-up of stress at the moment the compression begins or a rapid development of stress rising from zero but in a finite though brief time. Nevertheless, it is essential that the distinction be made if the correct model is to be assigned. The instrument itself necessarily has a finite, even if very rapid, acceleration from rest, and the response of the recording mechanism also takes a finite time, so that even if the stress were instantaneous, it would appear as a very steep rise (35).

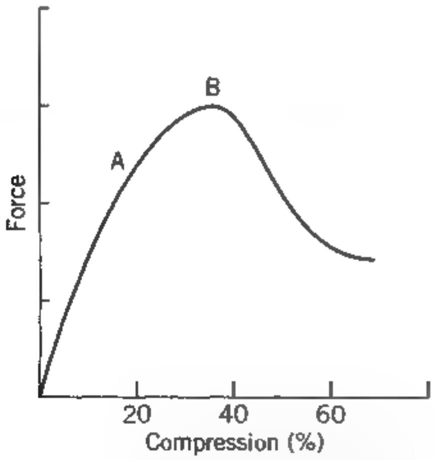


Figure 7. Typical force–compression curve.

However, much more serious than any limitations in the instrument is the problem of attaining perfection in the shape of the sample. Usually the dimensions of the sample and the rate of compression are chosen so that the strain rate at the commencement is of the order of 0.01 s^{-1}. In a sample, say 20 mm in height, a lack of parallelism of the two end surfaces of only 0.2 mm means that for the whole of the first second of compression only part of the sample is being compressed. This will inevitably appear on the record as a flow, whatever the real properties of the cheese.

Assuming that any doubts about the shortcomings of either sample or instrumentation may be satisfactorily allayed, it is pertinent to consider the principal features of the compression curve. In the early stages, after the initial rise there is a smooth increase of stress with the strain. Should the compression be halted at this stage, the cheese would recover either completely or in part and a repeat of the curve could then be obtained, the new curve having the same general shape as the original, showing that the internal structure of the cheese had remained more or less intact, although there may have been some rearrangement. Eventually a point is reached, the point A in Figure 7, where the structure begins to break down and the slope becomes noticeably less. The conventional view is that this is the point at which cracks in the structure appear and the cracks then spread spontaneously (18,36). In the case of a hard cheese these cracks may be evident to the eye, but they may at first be so small and so localized that they do not become visible until the compression has proceeded somewhat further. If the cheese has a very homogeneous structure, it is to be expected that these cracks will develop throughout the structure at about the same time and the change of slope will be clearly defined. In a more heterogeneous cheese they may appear over a range of strains and the change of slope will be much more diffuse. Once this region has been passed, the cracks continue to develop at an increasing rate and become more evident, until at point B the rate at which the structure breaks down overtakes the rate of build-up of stress through further compression and a peak is reached. This value is obviously dependent on a balance between the spontaneous failure of the structure and the effect of increasing deformation applied by the instrument to any residual structure. It is a convenient parameter to determine and may be used as a measure of firmness or hardness of the cheese (18). Beyond this point the stress may continue to fall if the failure of the structure becomes catastrophic, until the fragments become compacted into a new arrangement which can take up the stress and this now rises again.

If the compression test is carried on beyond the point at which it is reasonable to attempt to interpret it in terms of an acceptable model, and hence to evaluate any of the customary rheological parameters, it becomes purely empirical. One of the points on the curve most frequently used is the peak (18,37) (point B). This is a kind of yield point and one can obtain from it two useful parameters, the stress required to cause catastrophic breakdown of the structure and the amount of deformation that the cheese will stand before this breakdown occurs. The two are not entirely independent. In a dynamic measurement such as this on an essentially viscoelastic material, the rate of strain as well as the strain itself influences the moment at which the breakdown occurs. For reliable comparisons, all measurements should be made at the same rate of straining. Fortunately, many workers have found it convenient to use similar sizes and rates of compression, so that at least approximate comparisons can be made between their results. The stress measured at point B is in fact sometimes called a yield value, but it should not be confused with the yield stress of a plastic material determined by other methods such as a cone penetrometer. The yield at point B is not a material constant defining the strength of the sample; breakdown has already been occurring at least since point A, and maybe earlier. The peak value at B only indicates the maximum to which the stress rises before the collapse of structure overtakes the build-up of stress in what remains of that structure.

The other point frequently used is the stress at 80% compression. The damage to the structure when the compression reaches this extent is generally so complete that it is unrealistic to regard the stress as applying to the original sample. This is particularly true in the case of hard cheeses such as the typical English and Grana varieties, which crumble long before this degree of compression is reached. Nevertheless, compressions of this order and much greater arise during mastication (38). The stress at 80% can therefore give some indication of the consumer's response to the firmness of the cheese, although it must be said that in mastication the rate of compression is also much greater.

Measurements have been made on four types of hard cheese, Cheddar, Cheshire, Double Gloucester (36), and Leicester (39), which confirm that the value of the stress at the peak point B is dependent on the rate at which the compression is carried out. It was shown that over at least a twentyfold range of the factor a in equation 11, the rate at which the plates approached each other, the peak stress was linearly related to the fourth root of a. This is shown in Figure 8, where straight lines have been drawn through the points for each cheese type. If one bears in mind that this is a destructive test, so that each measurement had to be made on a different sample, the fit of the lines to the experimental points is quite acceptable. This result is, however, purely empirical; as far as can be seen there is no theoretical justification for it and it is limited to those types of cheese which crumble on breakdown. But there is a practical benefit. Using this finding, it is reasonable to reduce measurements made at any practical rate of compression to a standard rate so that the results of workers in different laboratories may be compared.

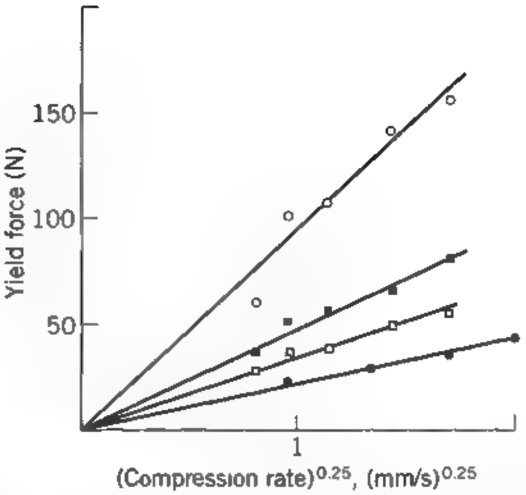


Figure 8. Effect of rate of compression on stress at yield point ●, Double Gloucester; □, Cheshire; ■, Leicester; and ○, Cheddar.

The investigation was carried one step further on Double Gloucester cheese (36). Not only were the peak values found to obey this fourth-root law but so were the stresses at other degrees of compression; the results are shown in Figure 9. The fact that the relation between stress and rate of deformation is more or less constant before, at, and beyond the yield point may be considered to lend support to the hypothesis that the processes taking place within the cheese are similar throughout the compression. The stress at any point is the result of a balance between the rate of collapse of structure, ie, the spread of cracks, and the build-up of stress in that structure which remains. If it is assumed that the basic framework within the cheese has at least some structural strength, there will be some build-up of stress before any cracks appear, and the hypothesis predicts that the stress—strain curve will show an initial instantaneous rise due to viscoelastic deformation before

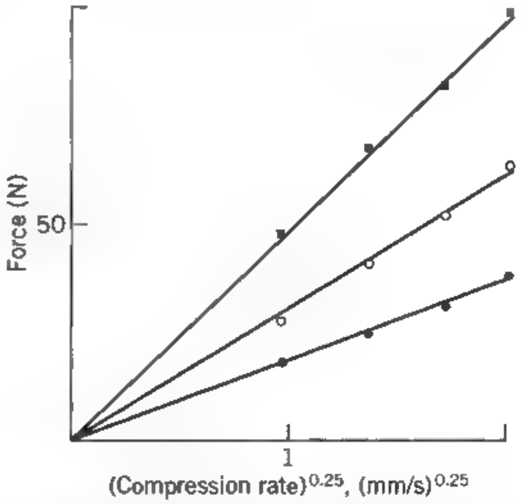


Figure 9. Effect of rate of compression on stress at various compressions: ●, 10%; ○, 40%; and ■, 70%.

any cracks develop, followed by a rising curve, convex upward, as the discrete minute cracks increase, with a pronounced change of slope as they begin to coalesce.

There are alternative explanations which may be advanced for the shape of the curves. In the early stages the curve rises from the origin and is often convex upward. This is characteristic of a Maxwell body. The interpretation could be that cheese behaves as an elastic fluid with a very high viscosity term. Neither the elasticity or the viscosity are readily obtained from the curves, but it is possible to calculate an apparent elasticity from the slope. Sometimes the slope at the origin is taken; more usually, the slope over the middle portion of the rise.

On the other hand, it is arguable that the structure of cheese is basically a solid one, but that even under very small stresses minute cracks begin to appear in that structure (40,41), even though these are far too small to be observed by the naked eye and may be disguised to some extent by the fact that some liquid component from the fat could flow into some of the opening interstices. Experiments on cheese analogs (42) at very low strains have confirmed that the structure does indeed break down well before the compression reaches 1%. It is possible to analyze the curves further on the basis of this hypothesis. Suppose that the cheese has an initial rigid structure giving rise to an elasticity E_0. Then at the instant at which the compression commences the slope of the stress—strain curve $d\sigma/d\epsilon$ is equal to E_0. If the cheese breaks down continuously by the appearance of cracks, infinitessimal at first but becoming gradually more widespread and larger, the strength of the cheese is progressively reduced, so that at any subsequent instant the elasticity $E = d\sigma/d\epsilon$ is less than E_0. If the breakdown of the structure is consequent upon the extent of the strain, the equation may be written

$$E = d\sigma/d\epsilon = E_0[1 - f(\epsilon)] \qquad (17)$$

This is the equation of a curve through the origin with an initial slope E_0 and subsequently convex upward, as is usually observed.

Pursuing this a little further, the function $f(\epsilon)$ is a distribution function of the breaking strains of the interparticulate junctions within the cheese. As a trial hypothesis, one may postulate that the distribution function is linear up to the point at which all the junctions are broken. Replacing $f(\epsilon)$ by a constant c and integrating equation 17 one gets

$$\sigma = E_0\epsilon - c\epsilon^2(\epsilon < \epsilon_{\text{critical}}) \qquad (18)$$

This is the equation of a parabola with its apex upward and a maximum stress of $\sigma = E_0^2/4c$ at a strain of $\epsilon = E_0/2c$. Eventually the situation is reached, at $\epsilon_{\text{critical}}$, where all the structure is more or less completely destroyed and individual crumbs may move more or less independently as in the flow of a powder. If the cheese is spreadable, the stress required to maintain this flow may be expected to be constant. On the other hand, some further compaction may take place and the stress begin to rise again. In general, the distribution would not be expected to be a linear one. While the argument remains the same, leading to a convex upward curve with a peak and a subsequent trough, algebraic analysis is more involved. However, if the constant c is replaced by a general expression, such as a series expansion in ϵ, the slope at the origin is still given by E_0 but the parabolic form becomes distorted.

When studying individual curves it is not always possible to determine the slope at the origin with any confidence, particularly since this is the region where the response time of the recorder is most likely to introduce its own distortion. However, rewriting equation 18 as

$$\sigma/\epsilon = E_0 - c\epsilon$$

it is possible to construct fresh curves of σ/ϵ versus ϵ and these may be easier to extrapolate to zero strain and thereby estimate the value of E_0. The (negative) slope of this curve is then the distribution function of c. In a few cheeses c has been found to be more or less constant up to strains approaching unity (ie, about 60% compression) but with most cheeses the value of c decreases as the strain increases, indicating that the rate of breakdown of structure is actually greatest at the lowest strains. Figure 10 shows a few typical derived curves.

RELAXATION

If the compression of the sample is halted before the sample is completely broken up and then held, the stress which has built up during that compression will decay. The two binary models show characteristically different types of decay behavior. In the Voigt body the stress at any instant is due partly to the strain and partly to the rate of straining. At the instant of arresting the motion the strain rate falls abruptly to zero, so that its contribution to the stress disappears, leaving only the stress due to the amount of strain, which then remains constant. In the Maxwell body the stress decays progressively, ie, relaxes, falling exponentially to zero if followed indefinitely according to the equation

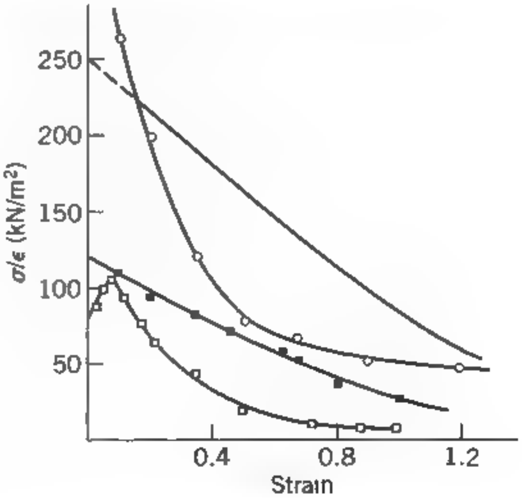


Figure 10. Derived curves of apparent modulus versus strain.

any cracks develop, followed by a rising curve, convex upward, as the discrete minute cracks increase, with a pronounced change of slope as they begin to coalesce.

There are alternative explanations which may be advanced for the shape of the curves. In the early stages the curve rises from the origin and is often convex upward. This is characteristic of a Maxwell body. The interpretation could be that cheese behaves as an elastic fluid with a very high viscosity term. Neither the elasticity or the viscosity are readily obtained from the curves, but it is possible to calculate an apparent elasticity from the slope. Sometimes the slope at the origin is taken; more usually, the slope over the middle portion of the rise.

On the other hand, it is arguable that the structure of cheese is basically a solid one, but that even under very small stresses minute cracks begin to appear in that structure (40,41), even though these are far too small to be observed by the naked eye and may be disguised to some extent by the fact that some liquid component from the fat could flow into some of the opening interstices. Experiments on cheese analogs (42) at very low strains have confirmed that the structure does indeed break down well before the compression reaches 1%. It is possible to analyze the curves further on the basis of this hypothesis. Suppose that the cheese has an initial rigid structure giving rise to an elasticity E_0. Then at the instant at which the compression commences the slope of the stress—strain curve $d\sigma/d\epsilon$ is equal to E_0. If the cheese breaks down continuously by the appearance of cracks, infinitessimal at first but becoming gradually more widespread and larger, the strength of the cheese is progressively reduced, so that at any subsequent instant the elasticity $E = d\sigma/d\epsilon$ is less than E_0. If the breakdown of the structure is consequent upon the extent of the strain, the equation may be written

$$E = d\sigma/d\epsilon = E_0[1 - f(\epsilon)] \qquad (17)$$

This is the equation of a curve through the origin with an initial slope E_0 and subsequently convex upward, as is usually observed.

Pursuing this a little further, the function $f(\epsilon)$ is a distribution function of the breaking strains of the interparticulate junctions within the cheese. As a trial hypothesis, one may postulate that the distribution function is linear up to the point at which all the junctions are broken. Replacing $f(\epsilon)$ by a constant c and integrating equation 17 one gets

$$\sigma = E_0\epsilon - c\epsilon^2(\epsilon < \epsilon_{\text{critical}}) \qquad (18)$$

This is the equation of a parabola with its apex upward and a maximum stress of $\sigma = E_0^2/4c$ at a strain of $\epsilon = E_0/2c$. Eventually the situation is reached, at $\epsilon_{\text{critical}}$, where all the structure is more or less completely destroyed and individual crumbs may move more or less independently as in the flow of a powder. If the cheese is spreadable, the stress required to maintain this flow may be expected to be constant. On the other hand, some further compaction may take place and the stress begin to rise again. In general, the distribution would not be expected to be a linear one. While the argument remains the same, leading to a convex upward curve with a peak and a subsequent trough, algebraic analysis is more involved. However, if the constant c is replaced by a general expression, such as a series expansion in ϵ, the slope at the origin is still given by E_0 but the parabolic form becomes distorted.

When studying individual curves it is not always possible to determine the slope at the origin with any confidence, particularly since this is the region where the response time of the recorder is most likely to introduce its own distortion. However, rewriting equation 18 as

$$\sigma/\epsilon = E_0 - c\epsilon$$

it is possible to construct fresh curves of σ/ϵ versus ϵ and these may be easier to extrapolate to zero strain and thereby estimate the value of E_0. The (negative) slope of this curve is then the distribution function of c. In a few cheeses c has been found to be more or less constant up to strains approaching unity (ie, about 60% compression) but with most cheeses the value of c decreases as the strain increases, indicating that the rate of breakdown of structure is actually greatest at the lowest strains. Figure 10 shows a few typical derived curves.

RELAXATION

If the compression of the sample is halted before the sample is completely broken up and then held, the stress which has built up during that compression will decay. The two binary models show characteristically different types of decay behavior. In the Voigt body the stress at any instant is due partly to the strain and partly to the rate of straining. At the instant of arresting the motion the strain rate falls abruptly to zero, so that its contribution to the stress disappears, leaving only the stress due to the amount of strain, which then remains constant. In the Maxwell body the stress decays progressively, ie, relaxes, falling exponentially to zero if followed indefinitely according to the equation

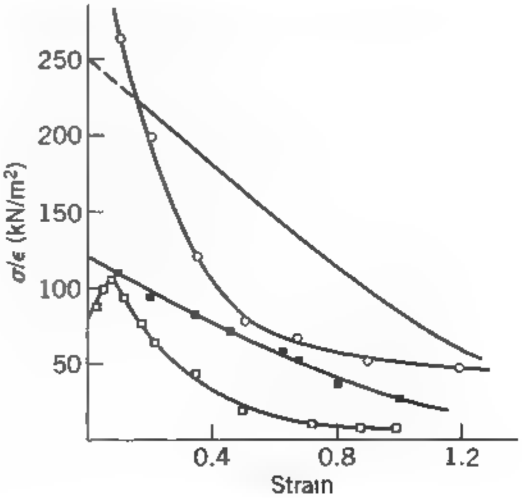


Figure 10. Derived curves of apparent modulus versus strain.

$$\sigma = \sigma_0 \exp(-Gt/\eta) \tag{19}$$

where σ_0 is the stress at the instant of halting. The ratio ν/G has the dimensions of time and is known as the relaxation time. This is readily determined from the experimental curve, either by redrawing it in logarithmic form or as the time for the stress to fall to $1/e$ of its starting value. The relaxation curve by itself does not allow the elastic and viscous components to be obtained separately. When the simple Maxwell model is evidently insufficient to describe the relaxation behavior, an analysis of the curve may become more difficult. The sample may be described by a more complex model created by combining several simple binary models. There are computational procedures which enable the various constants of the model to be evaluated in such a case, but the precision falls off rapidly as the number of constants increases. The rheologist may sometimes be interested in determining several relaxation times and moduli for a particular sample, especially if these can be assigned to recognizable structural elements in the material, but more often the proliferation of constants does little to clarify the behavior of cheese.

There is an empirical treatment which is sometimes useful in analyzing relaxation data (43,44), particularly in cases where the precision of the data may be in some doubt. If one takes Y_t as the fraction of the stress which has decayed in time t, ie,

$$Y_t = (\sigma_0 - \sigma_t)/\sigma_0$$

where σ_0 is the stress at the commencement of the relaxation and σ_t the stress after time t, it has been found that many complex viscoelastic materials relax in such a way that the relation

$$1/Y_t = k_1 + k_2/t \tag{20}$$

holds to a fair degree of approximation; k_1 and k_2 may easily be found. Then $1/k_2$ is the extent to which the stress eventually will decay, while the ratio k_2/k_1 is a measure of the rate of decay. For a perfectly elastic body or a Voigt body $1/k_2$ is zero; there is no relaxation. For a liquid or a Maxwell body it is unity. For more complex models it lies somewhere in between. This is a useful device for deciding whether a simple model will suffice. For a Cheddar cheese $1/k_2$ was found to be in the region of 0.8, so a simple model is not adequate for describing the relaxation behavior of this cheese (43). An alternative treatment has been suggested (45), which, it is claimed, sometimes fits experimental data more closely. This may be derived by considering a binary model of the Maxwell type, but one in which the viscosity associated with the dashpot is not constant but follows a power-law variation with stress. The resulting expression is

$$(\sigma_0/\sigma)^{n-1} = 1 + k(n - 1)t \tag{21}$$

where n is the exponent of the power law. This is an interesting suggestion: One of the consequences of the power-law variation of viscosity is that it becomes infinite at zero stress, and a Maxwell body with an infinitely viscous dashpot is in fact a solid. Thus this is a model for a material which is solid while at rest but becomes progressively more fluid as the stress increases. This is not too different from the description of cheese given above: a solid material whose structure breaks down progressively when subject to strain.

RHEOLOGY AND STRUCTURE

From the foregoing considerations it will be evident that the rheological properties of any material are consequent upon its structure. The three major constituents of cheese, casein, fat, and water, each contribute to the structure and therefore to the rheological properties in their own specific way. At normal room temperatures the casein is solid, the fat is an intimate mixture of solid and liquid fractions, giving it what may be described as plastic properties, while the water is liquid. The casein forms an open meshlike structure (46–48). Within this mesh is entrapped the fat, which had its origin as the fat globules of the milk. The water is more precisely the aqueous phase, since dissolved in it are the soluble constituents of the milk serum together with any salt which has been added during the cheese-making. Some of the water is bound to the protein and therefore largely immobilized; the remainder is free and fills the interstices between the casein matrix and the fat. This structure is common to all types of cheese. The differences among various types of cheese are brought about by the influence of different manufacturing regimes on that structure. In addition to the characteristic differences arising from different procedures, adventitious variations may also occur within the same type of cheese. These reflect differences in the original milk or in the conditions during the manufacturing or maturing processes.

It is the casein within the cheese which is responsible for its solid nature. The primary structure is a three-dimensional cage whose sides consist of chains of casein molecules (49,50). This provides a structure of considerable inherent rigidity. The chains are not linear (51), but have an irregular, somewhat gnarled structure. This may be deformed elastically under the action of external forces and this elasticity modifies the rigidity of the cage.

During the clotting process these chains have been formed by the joining together of individual casein particles in the serum. This serum surrounds the fat globules so that each cage may be expected to encase at least one globule or cluster of globules (52). The distribution of sizes of these cells will be controlled by the distribution and size of the fat globules. For instance, when the milk is first homogenized, the cheese made from it will have a more uniform distribution of cells than cheese made from fresh milk (53). The complete cheese curd at this stage consists of an aggregate of these cells of casein plus fat and the whole is pervaded by the aqueous phase (52).

If a force is applied to such a structure, the deformation will be primarily controlled by the rigidity of the cage, modified by any elasticity in its structural members. In the absence of the fat and water this would behave simply as any other open-type structure and its deformation would be characterized by a single modulus of rigidity or elasticity. However, the deformation of any of the cells is limited by the fat within it. At very low temperatures the fat would be solid and this would only add to the rigidity. At the normal temperatures at which cheese is matured and used the fat has both solid and liquid constituents and has its own peculiar rheological properties. Any deformation of the casein matrix would also require the fat to deform. At the same time, the water between the fat and the casein acts as a lubricant. As a result, the rigidity of the fat is added to that of the casein in a complex manner and it is this which gives rise to the peculiar viscoelastic properties of the cheese.

Even this is not the whole picture. The final cheese is not merely a continuous aggregate of cells as just described. During manufacture the curd is cut into small pieces at least once to allow any excess serum to drain away. As the serum drains away, the casein matrix shrinks on to the fat globules, making a more compact whole. The granules so formed may then be further distorted, as in the cheddaring process, or may be allowed to take up a random distribution, as in a Cheshire cheese. The final cheese mass is an aggregation of these granules which forms a secondary structure having its own set of rheological properties. Even this may be further modified by subsequent processes such as milling, which gives rise to a tertiary structure, and by pressing, which distorts the whole (54).

This rudimentary account of the factors contributing to the rheological properties applies to any cheese. During the course of manufacture, and subsequently during ripening, the basic structure may be modified by mechanical or thermal treatment, or the casein itself may be acted upon by bacteria and any residual enzymes. These agencies may change the organization of the structure or they may cause contiguous fat globules to coalesce. Finally, water may be lost by evaporation from the surface.

Before discussing the effect of the structure of the cheese on its rheological properties it is appropriate to consider differences which may occur within a single cheese or between cheeses from the same batch which might be expected to be alike. A cheese which matures in contact with the air will develop a pronounced rind and may show considerable variation throughout its body. The surface layers lose moisture more rapidly than the inner portions. This results in a difference in composition, which may be reflected in the rheological properties. It may also affect the progress of the maturation process itself. Another source of variation in some cheeses, particularly some of the larger varieties with long maturation periods, is that they are turned at intervals: The top and bottom layers are subjected to alternate low and high compressive forces due to their own weight, while the middle will have a more uniform treatment. This again may be reflected in the nature of the maturation. An otherwise uniform cheese will show a distribution of firmness as in Figure 11. A practical consequence of this is that measurements made on or near the surface of a large cheese will not be characteristic of the main body of that cheese.

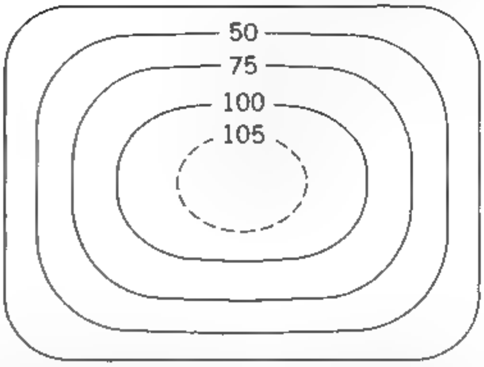


Figure 11. Typical distribution of firmness throughout a mature cheese.

Differences between cheeses may be expected to be greater and the magnitude of the variations will depend to some extent on the method of measurement. Penetration methods, where the instrument only acts locally on a limited quantity of the sample, give greater differences than compression testing in which a larger volume of the sample is involved. Variations of the order of 10% of the mean value of a parameter have been found on different samples from the same batch in a compression test (37); this probably indicates the limit of reproducibility which one may reasonably expect when making measurements on cheese. Differences between batches may be considerably larger. As an extreme example, one experimenter measuring eight different lots of cream cheese obtained readings which ranged from 54 to 251 units (55). Before leaving the topic of variation, one other source which must always be borne in mind is that between laboratories nominally making similar measurements. Because this is not a problem unique to either rheology or cheese, it will be disregarded in what follows.

Although it is the interaction of the properties of the principal constituents which gives cheese its viscoelasticity, it is profitable to consider some of the features associated with each of the constituents. First, consider the casein, which, it has been pointed out, gives the cheese its solid appearance. Because the casein forms chains within the spaces around the fat globules, there must necessarily be a minimum amount of casein below which any continuous structure cannot exist. This will depend on the number, size, and size distribution of the fat globules and on the size and size distribution of the casein micelles themselves. Once the quantity of casein required for this minimum structure has been exceeded, any additional casein will serve to strengthen the branches and the junctions. It is to be expected that, irrespective of the type of cheese, there will be a general relationship between the amount of casein present in the cheese and its firmness. Figure 12 shows this relation for some ten different types of hard cheese (29). Naturally, since the data refer to cheeses of very different provenance, there is considerable scatter, but it is possible to draw a regression line through the points and this indicates that about 25% of the weight of the cheese must be casein in order to provide a rigid framework and that above this limit more protein only strengthens it.

The requirement that there be sufficient casein to build a structure around the fat has been clearly shown in mea-

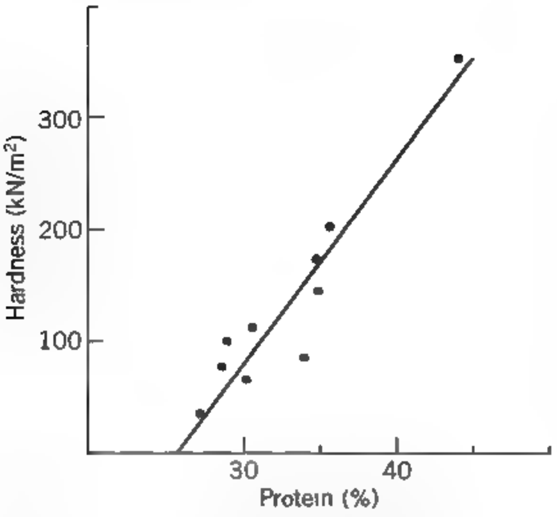


Figure 12. Relation between firmness of cheeses and total protein content.

surements on two other varieties of cheese. In some Meshanger cheese (56) it was found that, unless the casein occupied more than about 37% of the volume of the cheese not occupied by the fat, it had virtually no rigidity and behaved like a soft paste. In a similar way, it has been shown quite dramatically (Fig. 13) that in Mozzarella cheese (57) the protein must exceed about 42% of the nonaqueous part of the cheese for any rigidity to exist. Again, this corresponds to a minimum casein content of about 25% for a rigid structure.

Although it has been claimed that the casein matrix gives rigidity to the cheese, there is still a theoretical question which has not been answered. Is the matrix continuous throughout the whole cheese, so that it may be treated as a solid body, or do the dislocations in the structure which have been introduced by cutting allow it to flow, albeit imperceptibly, however small the stress? The evidence is inconclusive. In theory, an examination of the stress–strain curves at very low strains should decide the issue. If there is a continuous structure, there may be elastic deformation but no flow until a finite stress is reached which is sufficient to rupture some of that structure. Force–compression curves should indicate whether the cheese is of the Maxwell body or the Voigt body type. Commercially available instruments are generally not precise enough in this region, for they are not designed for this purpose. More precise measurements on cheese analogs have already been mentioned as showing that these began to break down at very small strains (42). Measurements of the recovery after compression of a few real cheeses (58) showed that even the smallest strains applied broke down some structure in a Mozzarella cheese, but a Cheddar cheese and a Muenster were able to recover completely (Fig. 14).

Cheddar cheese has also been studied by means of a relaxation experiment (44). Samples were compressed under different stresses, and hence at different rates, to the same ultimate deformation and the subsequent relaxation of the stress was observed. Using Peleg's treatment (43) it was found that the value of $1/k_2$ in equation 20 was dependent on the stress applied. The greater the stress, the more the structure broke down, although only the same strain was reached; the relation was curvilinear, particularly at the lowest stresses (Fig. 15). Extrapolating this curve back to zero stress should indicate whether any breakdown occurred. A zero value of $1/k_2$ would indicate a solid structure. Unfortunately, any extrapolation of this curve would be speculative. It is possible that it would lead to a positive intercept on the vertical axis. If this were so, it would lead to the conclusion that there was no continuous structure throughout that cheese, but to establish this with confidence would require measurements at much lower stresses.

Another series of experiments which could throw light on this question has been carried out (59). A small sinusoidal vibration was applied to one surface of the cheese sample and the stress transmitted through it observed. If

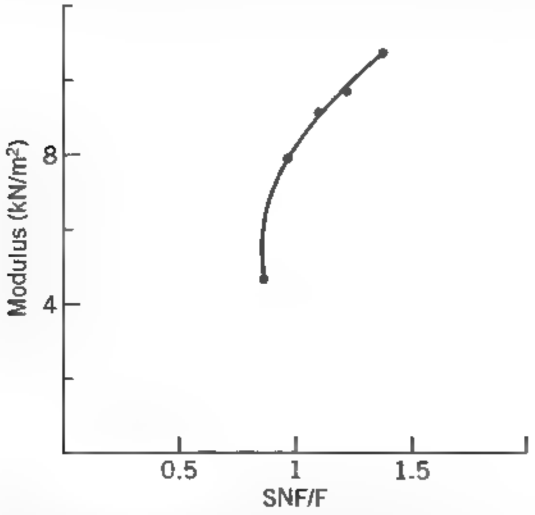


Figure 13. Relation between firmness and protein content in a single cheese. SNF/F ratio of nonfat to fat solids.

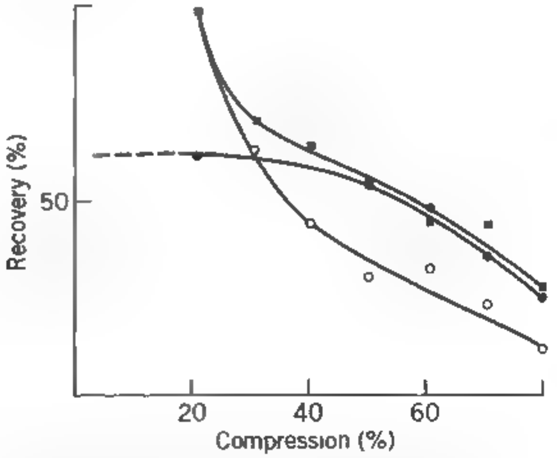


Figure 14. Relation between recovery of cheese and previous compression: ○, Cheddar; ●, Mozzarella; and ■, Muenster.

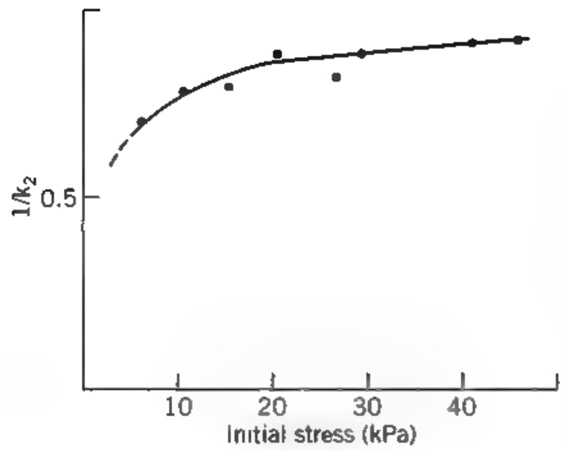


Figure 15. Amount of relaxation after previous stress.

one makes the assumption that the cheese behaves as a Voigt body, a modulus of rigidity can be calculated from the inphase component of the signal received and a viscosity from the out-of-phase component. For a true Voigt body both of these should be independent of the frequency. In fact, neither was constant with either Cheddar or Gouda cheese, even when the strain was as low as 0.04. The inference is that even at this strain some failure in the rigid structure had taken place. Either internal cracks or slip planes had developed within the cheese (42). However, everyday experience suggests that cheese should have some rigid structure. The stress on the lower layers of a large cheese, such as a Parmigiano or a Cheddar, is of the order of 3 to 4 kPa; yet such a cheese retains its shape more or less indefinitely.

Summarizing the previous paragraphs, it appears that the rheological role of the casein is to provide a continuous elastic framework within the individual granules. It is likely that where the casein chains lie in the granule surface and are contiguous with chains in neighboring granules they may be held together by physicochemical bonds which develop during maturation and give rise to some rigidity in the aggregation (54). However, these bonds will be sparser than those within the original network, since they form only where chance contacts occur. This results in a weaker secondary structure. These bonds may be broken when a positive strain is applied, giving a Maxwell body type of response, but are strong enough to preserve rigidity under the cheese's own weight. Some further support is given to this view by the fact that individual granules have a much higher modulus than the whole cheese (60).

The role of the other major constituents is more clearly documented than is that of the casein. The fat derived from the original milk is very roughly one third of the total mass. Its rheological properties are very sensitive to temperature changes and, as is to be expected, this sensitivity is imparted to the whole cheese. At 5°C (around normal refrigeration temperatures) many of the glycerides in the milk fat are solid. The proportion of solid fat decreases with rise of temperature, particularly sharply in the 12–15°C region, which is close to the ripening temperature for many varieties. Above this region the proportion of solid decreases further until at around 35°C almost all is liquid. This is near the temperature that a small portion will rapidly attain if it is placed in the mouth and chewed. The ratio of the solid to liquid fat components is the principal factor influencing the rheological properties of the fat (61). Although factors such as breed and species of animal, pasture, and herd management have some effect on this ratio, temperature is by far the most important. Fat also supercools readily, so that the thermal history is almost as important as the absolute value of the temperature itself. During maturation and storage cheese is usually kept at a lower temperature than that at which it was made. Originally most of the glycerides will have been liquid and at the lower temperature these will slowly solidify. Most of the change will take place in the first day or two, but solidification will continue progressively, maybe for several weeks before final equilibrium is reached.

Measurements made on Cheddar, Cheshire (39), Emmentaler (62), Gouda (63), and Russian (64) cheeses at a range of temperatures, using different instruments, all show the same general trend (Fig. 16). It may be mentioned that different instrumental methods give rise to somewhat different results. In general, penetrometers appear to indicate a rather steeper dependence of firmness on temperature than compression tests. Nevertheless, bearing in mind the difference in origin of the cheeses and the limitations in accuracy of the individual measurements, already discussed, this curve shows clearly the influence of the (test) temperature on the firmness of the cheeses.

Differences in the glyceride composition of the original milk fat are also reflected in the final cheese. Using the iodine number as an indication of the ratio of solid to liquid fat at any one temperature, the firmness of Swiss cheese was shown to vary seasonally with the solidity of the fat (65) (Fig. 17). The lower the iodine number, the greater the degree of saturation of the glycerides and the higher the

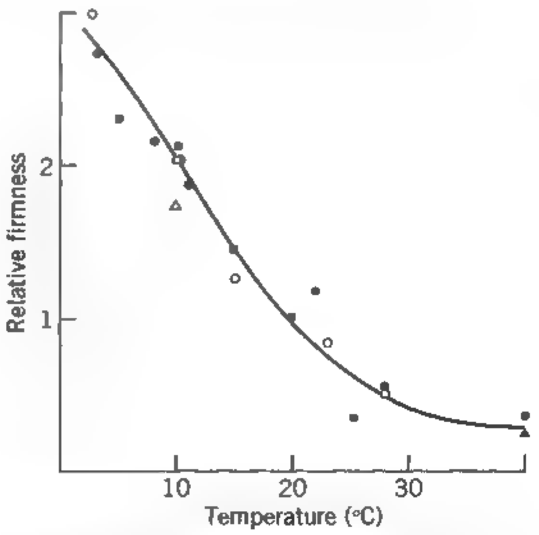


Figure 16. Variation of firmness with temperature: ●, Cheddar; ○, Cheshire; ■, Emmental; □, Gouda; and ▲, Russian.

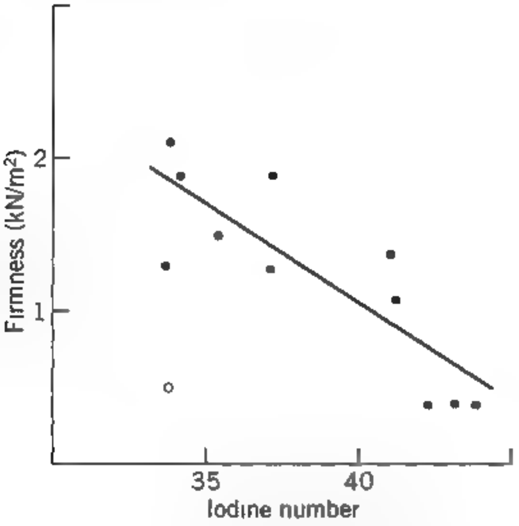


Figure 17. Variation of firmness with saturation of glycerides.

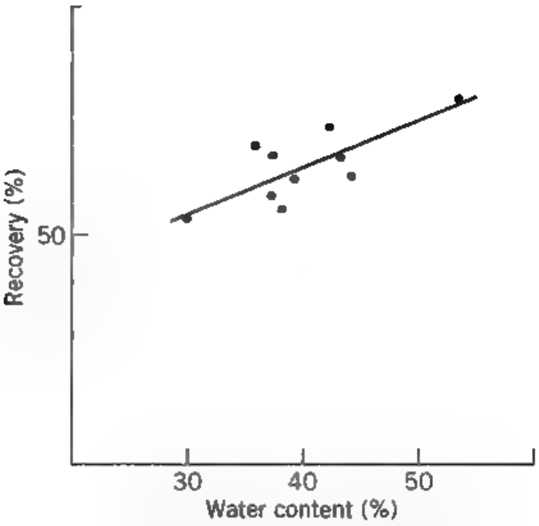


Figure 18. Relation between elastic recovery and moisture content.

proportion of solid fat. In these particular experiments the cheese produced in December appeared to be anomalous. However, even including the December result, the relation was statistically significant: excluding it, it was highly so and this regression has been drawn on the figure.

The water serves two functions, one purely physical, in that it is a low viscosity liquid, the other in its action as a carrier for the substances dissolved in it which may undergo chemical reactions with the fat or casein. It has already been stated that the water occupies the space not taken up by the casein matrix and the fat. Provided that there is a sufficient quantity, it takes up all of that space. Only near the outer surface, for instance, in the rind, is there likely to be any deficiency. In the case of Swiss-type cheeses, which contain eyes filled with gas, the main body of the cheese is still replete with water. The rheological consequence of this arrangement is that the water acts as a low-viscosity lubricant between the surfaces of the fat and the casein. The greater the space between these, which is another way of saying the higher the water content of the cheese, the easier the flow of water within these spaces. Hence there should be less restraint on the movement of the casein mesh around the enclosed fat. This freer movement should manifest itself as a lesser resistance to the deformation of the whole cheese and as a greater recovery after deformation.

Taking the latter point first, a study was made of the elastic recovery of samples of some ten varieties of cheese after they had been compressed (29), but only up to a point at which no visible damage to the structure had occurred (Fig. 18). The cheese with the lowest water content, Parmigiano, showed the least recovery, while a Mozzarella with over 50% water showed the most. From the figure it is seen that while there was considerable scatter about the regression line, as was to be expected with cheeses of different origins, a general trend was well established.

The lubrication effect can be more clearly demonstrated with a single variety and within a single cheese. In a freshly made cheese the water is distributed more or less evenly throughout the whole mass. During the maturation period of those cheeses which are allowed to ripen in air, water evaporates from the surface and is only slowly replaced by more water migrating from the interior. A moisture gradient is set up within the cheese. Measurements made on Emmentaler cheese (62) (Fig. 19) showed a very close relation between the water content of successive layers and the firmness as determined by a penetrometer. Similar results have been obtained with Edam cheese (66). In that series of experiments a close relation was observed between the water content of the cheeses and the firmness, although they were made in two batches at different temperatures (Fig. 20).

In its other role as carrier of active ingredients the presence of water is principally seen in the effect on the

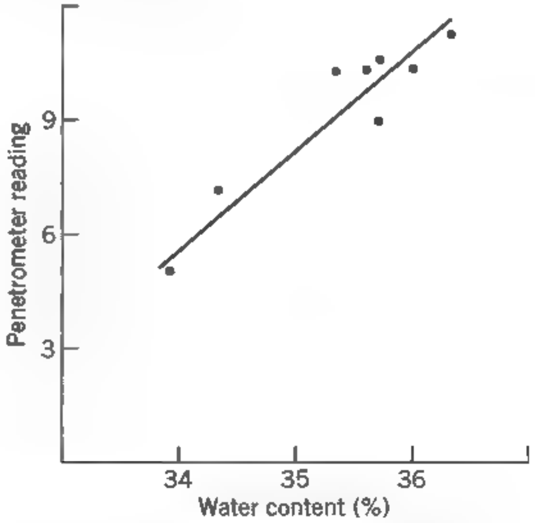


Figure 19. Relation between firmness and moisture content in a single cheese.

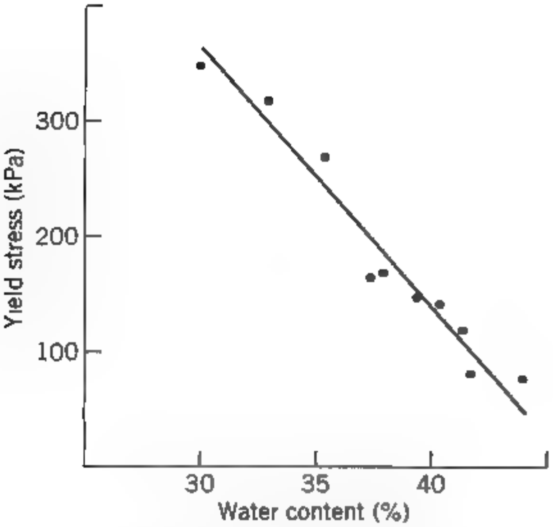


Figure 20. Relation between quasi-modulus and moisture content in a single cheese variety.

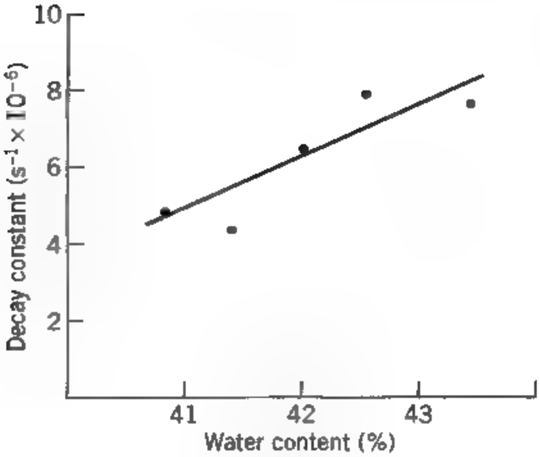


Figure 22. Relation between rate of casein breakdown and moisture content.

changes which take place in the casein network. An illustration of this is found in Meshanger cheese (56). This cheese has the usual well-organized casein structure when freshly made. As the ripening proceeds, the structure breaks down until it becomes a more or less amorphous loose mass of casein submicelles. Figure 21 shows the relation found between an arbitrary measurement of the firmness and the water content of cheeses from some ten batches during ripening. In the same series of experiments the rate of degradation of the casein was determined for some of the batches over the first few weeks of life. The rate of degradation, calculated as a first-order reaction, showed good agreement with the water content (Fig. 22).

Although protein, fat, and water constitute by far the greatest part of any cheese mass, other constituents cannot be ignored. Salt, when added dry before the cheese is pressed, may sometimes appear as crystals embedded in the fat–protein mass. Then it is not usually present in large enough quantities to make any sensible contribution to the rheological properties of the whole. Almost all of the salt is present only in solution in the water. There its effect on the properties of the whole cheese is minimal. Any serious contribution of the salt to the rheological properties of the whole cheese is by indirect action (67). A high concentration of salt increases the osmotic pressure, diverting a significant quantity of water from the structural bonds of the casein network (68,69). This may be seen in the effect on the ripening of some samples of Mozzarella cheese (70). When the salt content was low (0.27%) the modulus decreased from 120 to 45 N/m^2 in five weeks, but when salt was present in excess of 1% no change was observed within the same period.

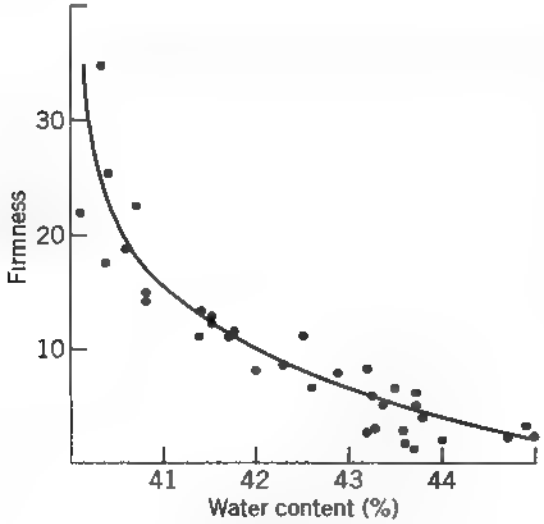


Figure 21. Relation between firmness and moisture content.

In cheese where the salt is added by immersion in brine there is a further consideration. The inward diffusion of the brine results in a concentration gradient being set up within the cheese. This has a twofold effect. First, the presence of the salt simply excludes some of the water; this in turn reinforces the moisture gradient simultaneously arising from the evaporation of water from the surface layers. Probably more important is the fact that the diffusion of the salt into the cheese is a slow process (71). In the early stages of ripening the salt concentration in the inner regions is unlikely to be sufficient to limit the protein degradation described above. The presence of more water and an enhanced proteolytic activity affect the rheological properties in the same sense, giving rise to a weaker structure and therefore a less firm cheese. It is therefore probable that the variation in firmness within a single cheese, which, as described earlier, is closely related to the moisture distribution, is accentuated by bringing.

Some cheeses also contain a significant quantity of gas. In the Swiss-type cheeses this is concentrated in the eyes; the remainder of the cheese has a close waxy texture and is virtually free of any entrapped gas. In hard and crumbly cheeses the gas may simply be air which has infiltrated into cracks which have developed in the mass. The significance of either eyes or cracks is that measurements made on the whole cheese or on its surface are not a fair representation of the properties of the main body of the cheese. Hidden cracks or eyes will give rise to irregularities when measurements are made by any form of penetrometer and probably account for a substantial amount of the observed scatter. In compression measurements they will give rise to premature breakdown. They also account for the fact that difficulties are encountered when any attempt is made to assess the rheological properties of cheese by studying the propagation of ultrasound through it. The velocity of propagation is more influenced by the scattering at the discontinuities than by the properties of the cheese mass.

SOME EXPERIMENTAL RESULTS

Most of the rheological methods described earlier have been used at one time or another for the study of cheese. Problems associated with penetrometers and tests on the surface have already been indicated. The most satisfactory tests are made on samples cut out specifically from the cheese mass. In this case the sample can be inspected to insure that there are at least no major cracks or other inhomogeneities in it and the dimensions can be precisely determined. The force–compression test using small prepared samples has been widely used for routine measurements. However, in spite of its widespread use, there has been no consensus of opinion on the most suitable operating conditions. Sample size and shape, rate of compression, and even temperature have all varied. A most interesting feature is that, notwithstanding these differences and the number of cheeses which have been examined, the force–compression curves show a remarkably common pattern. This underlines its general usefulness.

There are several important considerations to be taken into account when deciding on the operating conditions. In the first place, the sample should be large enough to be representative of the whole (72); the more heterogeneous the cheese, the larger the sample. On the other hand, it should not be so large that it may contain hidden cracks or irregularities. These two requirements are almost mutually exclusive. As a compromise most workers have taken samples with linear dimensions of 10 to 25 mm. The preferred shape has usually been a cylinder. It is easier to prepare a rectangular sample with precise dimensions but the symmetry of the cylindrical shape helps to minimize the development of irregular cracks during compression (63). A wide range of compression rates has been used: from 2 mm/min up to 100 mm/min (3.33×10^{-5} to 1.66×10^{-3} m/s). The faster rates tend to obscure the true behavior at the onset of compression unless a recorder of very short response time is available. The strain rate at any instant depends on both the rate of compression and the height of the sample at that instant.

Table 1. Principal Measurements of Hard Cheeses Using the Force–Compression Test

Cheese	Modulus, E_c (kN/m^2)	Yield point σ_y (kPa)	 ϵ_y	80% Stress, σ_{80} (kPa)	Ref.
American loaf	70	25	0.72	46	58
Bel Paese	300	44	1.1		37
Caerphilly	1100	92	0.16	67	36
Cheddar	280	41	0.34	50	22
Cheddar		53	0.25		39
Cheddar		63	0.24		47
Cheddar (mild)	160	40	0.60	78	58
Cheddar (strong)	400	58	0.22	65	58
Cheddar	320	45	0.21	64	73
Cheddar (green)	75	54	0.80	37	47
Cheddar (mature)	170	23	0.20	22	47
Double Gloucester	1600 +	139	0.24	75	36
Edam	660	214	0.60		36
German loaf	300	18	ca 1.1		33
Gouda	240	98	0.72	52	36
Lancashire	2000 +	105	0.23		36
Leicester	400	56	0.31	38	32
Montasio	40	85	0.80		37
Mozzarella	300	(no peak)			34
Mozzarella	80	14	0.57		58
Muenster	40	16	0.72	59	58
Parmigiano	700	132	0.28		37
Provolone	120	37	0.60		37
Silano	230	26	ca 0 30		57
Processed	196	57	0.39	30	36
Processed	440	71	0.27		37
Processed	60	9	0.21		37

Table 1 summarizes the principal measurements that have been reported on hard cheeses using the force–compression test. In order to produce this table and make the results from different workers comparable, the original curves from the references cited have been transformed into stress—strain curves. As previously mentioned, the instrument in its usual commercially available form produces curves of force versus linear travel. While linear travel is easily converted to true strain (eq. 11), the stress correction requires a knowledge of the actual shape at any instant during compression. Often this is unknown, or at least unreported. Unless special precautions have been taken, the behavior of the sample will be intermediate between that obtaining in the lubricated and in the bonded situations. For the present purposes the simpler correction applying to the lubricated condition has been used throughout, although it is realized that it will not quite compensate for the barreling which occurs. The values were then read from the transformed curves. The modulus given in the column headed E_0 is from the slope at the origin, ie, it refers to the undeformed sample. The entries in the columns headed yield refer to the stress and strain at the peak of the curve.

Table 2 summarizes a number of results which have been obtained by other methods where it has been possible to calculate a modulus from the data. In order to compare the two tables, it will be recalled that Table 1 lists the values of an elastic modulus. It must be most nearly comparable with any quasi-modulus determined by the puncture test or by simple compression. The yield stress measured by a cone penetrometer is more likely to be comparable with the yield value in the force–compression

Table 2. Modulus Measurements Using Various Methods

Cheese	Modulus (kN/m^2)	Method	Ref.
Brie	1.3	Extruder	74
Sbrinz	41	Extruder	74
Chanakh	58	Cone	69
Emmental	40	Cone	69
Emmental	3.5	Cone	22
Gouda	44	Cone	69
Kostroma	54	Cone	69
Lori	40	Cone	69
Cheddar	195	Punch	29
Edam	340	Punch	29
Emmental	220	Punch	29
Gouda	270	Punch	29
Kachkaval	120	Punch	75
Mozzarella	170	Punch	29
Mozzarella	22	Punch	74
Muenster	120	Punch	29
Parmigiano	550	Punch	29
Provolone	130	Punch	29
Cheddar	270–400	Compression	76
Mozzarella	80	Compression	70
Kachkaval	60–100	Ball Compressor	77

test, since both are influenced by partially broken-down structure. It must be pointed out, however, that lack of adequate documentation makes it difficult to make meaningful comparisons between the results from different laboratories.

VARIATION WITH AGE

The development of firmness during the ripening and subsequent storage of cheese has always been of particular interest to both the practical cheese-maker and the rheologist. During this period all three principal constituents undergo change. Moisture evaporates from the surface of the cheese if this is exposed to the air; additional moisture migrates from the interior to replace that lost and some of this also evaporates. Eventually a moisture gradient is set up, but even the center will be drier than it was originally. The protein in the matrix undergoes progressive change as the available water is reduced and the residual enzymes and bacteria continue to act on it (46,47,75). Furthermore, on the purely physical plane, as the water content decreases, the matrix may shrink or collapse under pressure so that voids do not form and the protein becomes denser. Some of the glycerides in the fat slowly crystallize, resulting in a more solid mass of fat.

These changes which take place in the structural components of the cheese are reflected in force–compression curves. Figure 23 shows curves for two cheeses, Cheddar (47) and Gouda (63), when green and when mature. During aging the strain sustainable by the cheese before breaking down at the yield point (point B in Fig. 7) decreased more or less exponentially with time in both cases. Figure 24 shows this for one batch of Cheddar cheese. At the same time the stress at the yield point diminished. The strength of the matrix had clearly been reduced through aging. In the Gouda cheese the behavior was rather different. Although the yield strain decreased, the stress at this point, ie, the ultimate strength of the matrix, had increased. It is only to be expected that as the actual balance between the different mechanisms of aging of the components varies from one cheese to another, so the paths of change in the whole cheese will vary. Figure 25 shows some of the results that have been reported (47,78,79).

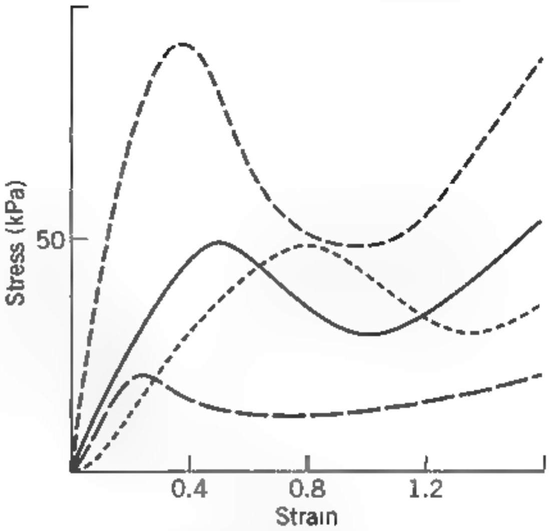


Figure 23. Force–compression curves for young and mature cheeses: ——, Cheddar (mature); - - -, Cheddar (young); —, Gouda (young); and – –, Gouda (mature).

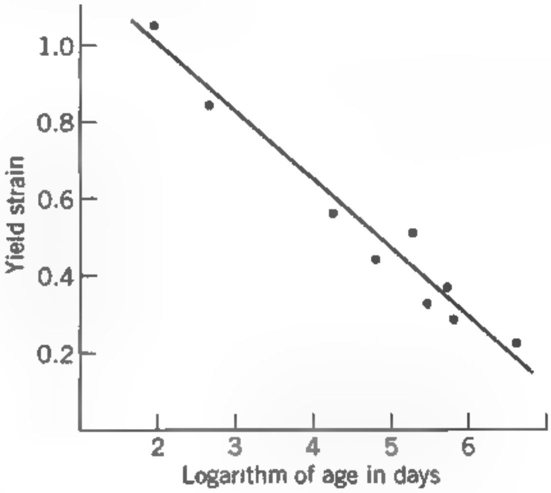


Figure 24. Relation between age and strain at yield point for Cheddar cheese.

Not only does the firmness of cheese change with age, but also its springiness. The degradation of the protein, the solidification of the fat, and the reduction of the water available to lubricate the relative motion all tend to reduce the springiness. Springiness is not a simple rheological concept. It implies not only the ability to recover from compression or indentation but also that this will be immedi-

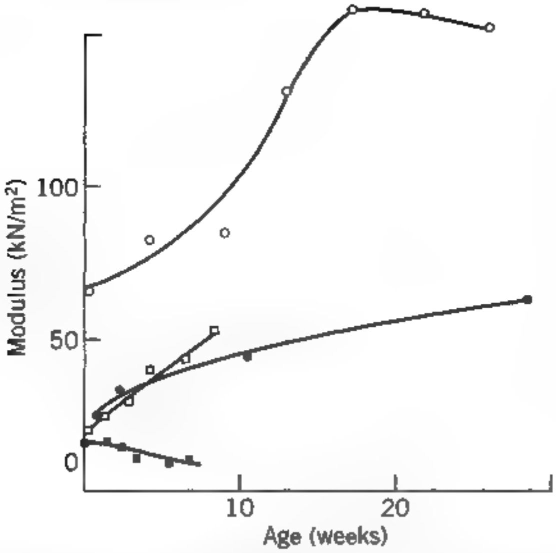


Figure 25. Variation of firmness with age: ●, Cheddar; ○, Kachkaval; □, Tamismi and Russian; and ■, unspecified French cheese.

ately evident. It does not show itself directly in a force–compression curve; a recovery curve is more appropriate for this purpose. Nevertheless, this decrease of springiness has been remarked upon consistently using subjective observations on a number of cheeses (27,29,47,49,80).

SUBJECTIVE ASSESSMENTS

How the rheological properties measured by instruments, as just described, relate to the grader's or the consumer's assessments of the cheese remains to be considered. The emphasis in this article has been on the measurement of the firmness of cheese which arises from its structure. It should be stressed that meaningful comparisons can only be made between instrumental and subjective methods if the subjective terminology is unambiguous and the instrumental measurements precise. Early experiments on single varieties of cheese showed that the subjective assessment of firmness, which is a fairly simple concept, correlates very highly with measurements made with simple apparatus, such as the Ball Compressor (81). But a simple correlation by itself does not indicate to what extent the instrumental measurement should be used to predict a typical user's appraisal. Nor can the results of experiments on a single variety be extrapolated to other types of cheese without verification. Some authors have arbitrarily assigned a specific consumer quality to a particular instrumental reading. For instance, the quasi-modulus calculated from a simple compression test (27), the yield value given by a cone penetrometer (22), and the stress at 80% compression have all been proposed as measures of firmness. The recovery after a limited compression (29,82), arbitrarily decided upon, has been used as an indication of springiness. These intuitive opinions may help visualize the significance of an instrumental measurement and are probably adequate for internal comparisons within a single investigation, but they lack scientific rigor.

The force–compression test, giving as it does a characteristic curve with several readily identifiable features, provides a number of potentially useful parameters. In Figure 26 the values of hardness as assessed by a panel, of a number of different cheeses, versus the stress measured at 80% compression are plotted (73). A logarithmic regression line has been drawn through the points. The standard deviation about this regression line was no greater than that due to the differences among the panel. In this case, then, using the regression line, the instrumental reading can be safely used to predict that panel's assessments. In the same series of experiments the instrumental measurements were carried further. As soon as the compression had reached 80% the compressing plates were withdrawn and the recovered height of the sample was measured. At the same time the springiness was assessed subjectively. As has already been pointed out, springiness involves not only the extent of recovery but also its speed; possibly all individuals will have, subconsciously, their own ideas of the relative contribution of the two to the final judgment. As was to be expected, there was poorer agreement among the panel members in this part of the experiment. Even so, there was a highly significant correlation between the measured recovery and the average panel assessment of springiness.

The relation between user acceptance and instrumental rheological measurement is an ongoing study (82). The few examples cited above show that, if the proper parameter is chosen and the terms clearly understood, rheological measurements can successfully predict users' assessments.

CONCLUSION

Although the study of cheese rheology is as old as rheology itself, only a few cheese varieties have been investigated. These have been drawn almost exclusively from among the

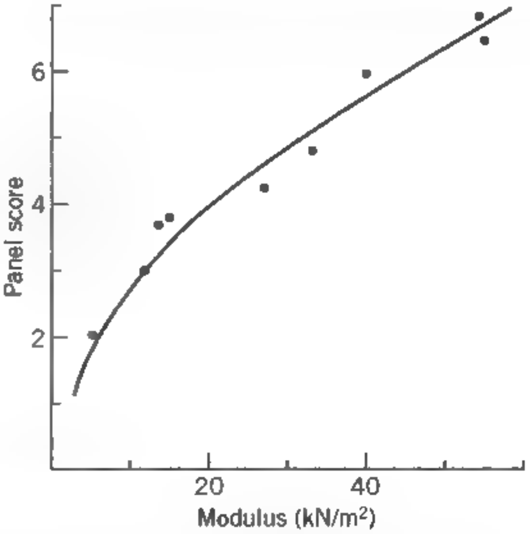


Figure 26. Comparison of subjective and objective measurements of firmness.

firmer varieties. The softer cheeses, of which there are many, have received scant attention. They pose rather different rheological problems because they are less like solids and more akin to stiff pastes or creams; they should, however, be nonetheless interesting to rheologists.

It has been shown, by bringing together diverse observations on the firmer cheeses from a number of workers, that it is possible to build up plausible theories to relate the behavior of the finished cheese to the structure developed during its manufacture. Mathematical models may be posited for the rheological behavior, but with a material as heterogeneous as cheese, these, if they are to be simple enough to be easily handled, are unlikely to give more than an approximate representation of the observed pattern. Using these, or directly from experimental measurements, material constants may be extracted to characterize the cheese. A knowledge of these may be helpful to the cheesemaker, both as a means of controlling the production regime and as a guide to the acceptability of the product.

DISCLOSURE STATEMENT

This article previously appeared in "*Cheese: Chemistry, Physics and Microbiology*," R. F. Fox, ed., Elsevier Applied Science, New York, 1987. It is printed here with some revision by permission of the publishers.

BIBLIOGRAPHY

1. M. Reiner, *Twelve Lectures in Theoretical Rheology*, North Holland Publishing Co., Amsterdam, 1949, p. 19.
2. A. S. Szczesniak, *Journal of Food Science* **28**, 385 (1963).
3. M. Baron, *The Mechanical Properties of Cheese and Butter*, United Trade Press, London, 1952, p. 29.
4. R. Jowitt, *Journal of Texture Studies* **5**, 351 (1974).
5. R. Houwink, *Elasticity, Plasticity and Structure of Matter*, Dover Publications, New York, 1958, p. 5.
6. M. Peleg, *Journal of Texture Studies* **15**, 317 (1984).
7. J. H. Prentice, *Measurements in the Rheology of Foodstuffs*, Elsevier Applied Science Publishers, London, 1984, p. 32.
8. R. B. Bird, *Annual Review of Fluid Mechanics* **8**, 13 (1976).
9. A. S. Szczesniak and M. C. Bourne, *Journal of Texture Studies* 1, 52 (1969).
10. J. E. Caffyn, *Dairy Industries* **10**, 257 (1946).
11. J. E. Caffyn and M. Baron, *The Dairyman* **64**, 345 (1947).
12. N. N. Mohsenin, *Physical Properties of Plant and Animal Materials*, Gordon & Breach Scientific Publishers, New York, 1970, p. 288.
13. N. N. Mohsenin, C. T. Morrow, and Y. M. Young, *Proceedings of the 5th International Congress on Rheology*, Vol. 2, 1970, p. 647.
14. C. P. Cox and M. Baron, *Journal of Dairy Research* **22**, 386 (1955).
15. A. D. Konoplev, P. E. Krashenin, and V. P. Tabachnikov, *Trudy Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta masl. i. syr. Prom.* **17**, 40 (1947).
16. S. Poulard, J. Roucou, G. Durrange, and J. Manry, *Rheologica Acta* **13**, 761 (1974).
17. M. Baron, *Dairy Industries* **14**, 146 (1954).
18. P. Eberhard and E. Flückiger, *Schweizerische Milchzeitung* **104**, 24 (1978).
19. V. P. Tabachnikov, V. Ya. Borkov, V. B. Ilyushkin, and I. I. Tetereva, *Trudy Vsesoyuznogo Nauchno-issledovatel'skogo Instituta masl. i. syr. Prom.* **27**, 61 (1979).
20. R. Harper and M. Baron, *British Journal of Applied Physics* **2**, 35 (1951).
21. W. G. Wearmouth, *Dairy Industries* **19**, 213 (1954).
22. E. Flückiger and E. Siegenthaler, *Schweizerische Milchzeitung Wissenschaftliche Beilage* **89**, 707 (1963).
23. F. J. Mottram, *Laboratory Practice* **10**, 767 (1961).
24. M. C. Bourne, in P. Sherman, ed., *Food Texture and Rheology*, Academic Press, London, 1979, p. 95.
25. J. M. deMan, *Journal of Texture Studies* **1**, 114 (1969).
26. B. S. Kamel and J. M. deMan, *Lebensmittel-Wissenschaft und Technologie* **8**, 123 (1975).
27. R. Davidov and N. Barabanshchikov, *Molochnaya Promyshlennost* **9**, 27 (1950).
28. V. P. Tabachnikov, *Trudy Vsesoyuznogo Nauchno-issledovatel'skogo Instituta masl. i. syr. Prom.* **17**, 84 (1974).
29. A. H. Chen, J. W. Larkin, C. J. Clarke, and W. E. Irvine, *Journal of Dairy Science* **62**, 901 (1979).
30. R. Ramanauskas, S. Urbene, L. Galginaitye, and P. Matsulis, *Trudy Litovskii Filial Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta Maslodel'noi Syrodel'noi Promyshlennosti* **13**, 64 (1979).
31. Ref. 7, p. 9.
32. E. J. V. Carter and P. Sherman, *Journal of Texture Studies* **9**, 311 (1978).
33. P. Sherman and G. Atkin, *Advances in Rheology* **4**, 133 (1976).
34. E. M. Casirighi, E. B. Bagley, and D. D. Christianson, *Journal of Texture Studies* **16**, 281 (1985).
35. P. W. Voisey and M. Kloek, *Journal of Texture Studies* **6**, 489 (1975).
36. P. Shama and P. Sherman, *Journal of Texture Studies* **4**, 344 (1973).
37. P. Masi, in *Physical Properties of Foods*, Vol. 2, Elsevier Applied Science Publishers, London, 1987, p. 383.
38. M. M. Bryan and D. Kilcast, *Journal of Texture Studies* **17**, 221 (1986).
39. E. Dickinson and I. C. Goulding, *Journal of Texture Studies* **11**, 51 (1980).
40. N. V. Polak, *Dissertation Abstracts International B* **42**, 3178 (1982).
41. R. Jowitt, in P. Sherman, ed., *Food Texture and Rheology*, Academic Press, London, 1979, p. 146.
42. R. J. Marshall, *Journal of the Science of Food and Agriculture* **50**, 237 (1990).
43. M. Peleg, *Journal of Food Science* **44**, 277 (1979).
44. M. Peleg, *Journal of Rheology* **24**, 451 (1980).
45. B. Launay and S. Buré, *DECHEMA Monographien* **77**, 137 (1974).
46. A. M. Kimber, B. E. Brooker, D. G. Hobbs, and J. H. Prentice, *Journal of Dairy Research* **41**, 389 (1974).
47. H. K. Creamer and N. P. Olson, *Journal of Food Science* **47**, 631 (1982).
48. M. V. Taranto, P. J. Wan, S. L. Chen, and K. C. Rhee, *Scanning Electron Microscopy*, 273 (1979).
49. M. Green, A. Turvey, and D. G. Hobbs, *Journal of Dairy Research* **48**, 343 (1981).
50. T. van Vliet and P. Walstra, *Netherlands Milk and Dairy Journal* **39**, 115 (1985).
51. J. H. Prentice, *Measurements in the Rheology of Foodstuffs*, Elsevier Applied Science Publishers, London, 1984, p. 157.

52. M. L. Green and A. I. Grandison, in P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology*, Vol. 1, Elsevier Applied Science Publishers, London, 1987, p. 97.
53. D. B. Emmons, M. Kalab, E. Larmond, and K. J. Lorne, *Journal of Texture Studies* **11**, 15 (1980).
54. M. Kalab, *Milchwissenschaft* **32**, 449 (1977).
55. Z. D. Roundy and W. V. Price, *Journal of Dairy Science* **24**, 135 (1941).
56. L. de Jong, *Netherlands Milk and Dairy Journal* **32**, 1 (1978).
57. P. Masi and F. Addeo, *Advances in Rheology* 4, 161 (1976).
58. E. M. Imoto, C-H. Lee, and C. K. Rha, *Journal of Food Science* **44**, 343 (1979).
59. S. Taneya, T. Izutsu, and T. Sone, in P. Sherman, ed., *Food Texture and Rheology*, Academic Press, London, 1979, p. 367.
60. E. P. Suchkovae, A. M. Maslov, and N. G. Alekseev, *Izvestiya Vysshikh Uchebnykh Zavedenii Pishchevaya Tekhnologiya* **4**, 113 (1985).
61. H. Jonsson and K. Anderson, *Milchwissenschaft* **31**, 593 (1976).
62. C. Steffen, *Schweizerische Milchwirtschaftliche Forschung* **5**, 43 (1976).
63. J. Culioli and P. Sherman, *Journal of Texture Studies* **7**, 353 (1976).
64. N. Ya. Dykalo and V. P. Tabachnikov, *Trudy Vsesoyoznogo Nauchno-Issledovatel'shoko Instituta masl. i. syr. Prom.* **77**, 85 (1979).
65. E. Flückiger, P. Walser, and H. Hanni, *Deutsche Molkerei Zeitung* **96**, 1524 (1975).
66. C. W. Raadsveld and H. Mulder, *Netherlands Milk and Dairy Journal* **3**, 117 (1949).
67. T. P. Gunee and P. Fox, in P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology*, Elsevier Applied Science Publishers, London, 1987, p. 251
68. R. Ramanauskas, *Proceedings of the 20th International Dairy Congress* 1978, p. E 265.
69. G. G. Khachatryan, K. Zh. Dilanyan, V. P. Tabachnikov, and I. I. Tetereva, *Proceedings of the 19th International Dairy Congress* 1974, 1E, p. 717.
70. M. A. Cervantes, D. B. Lund, and N. F. Olson, *Journal of Dairy Science* **66**, 204 (1983).
71. T. J. Guerts, P. Walstra, and H. Mulder, *Netherlands Milk and Dairy Journal* **34**, 229 (1980).
72. M. Peleg, *Journal of Food Science* **42**, 649 (1977).
73. C-H. Lee, E M. Imoto, and C. K. Rha, *Journal of Food Science* **43**, 579 (1978).
74. C. S. T. Yang and M. V. Taranto, *Journal of Food Science* **42**, 906 (1982).
75. R. Stefanovic, *DECHEMA Monographien* **77**, 211 (1973).
76. J. C. Weaver and M. Kroger, *Journal of Food Science* **43**, 579 (1978).
77. G. Szabo, *Proceedings of the 19th International Dairy Congress* 1974, 1E, p. 505.
78. S. D. Sakharov, V. P. Tabachnikov, V. K. Nebert, and P. F. Krasheninin, *Trudy Vsesoyuznogo Nauchno-Issledovalel'skogo Instituta masl. i. syr Prom.* **18**, 29 (1975).
79. M. Ostojic, D. Miocinovic, and G. Niketic, *Mljekarstvo* **32**, 139 (1982).
80. B. A. Nikolaev and R. M. Abdullina, *Trudy Vologogradskaya* moloch. Inst. **30**, 27 (1969).
81. N Baron and R. Harper, *Dairy Industries* **15**, 407 (1950).
82. A. S. Szczesniak, *Journal of Texture Studies* **18**, 1 (1987).

GENERAL REFERENCES

G. F. Amantea, B. J. Skura and S. Nakai, "Culture Effects on Ripening Characteristics and Rheological Behavior of Cheddar Cheese," *J. Food Sci.* **51**, 912–918 (1986).

E. B. Bagley and D. D. Christianson, "Measurement and Interpretation of Rheological Properties of Foods," *Food Technol.* **41**, 96–99 (1987).

O. H. Campanella et al., "Elongational Viscosity Measurements of Melting Process Cheese," *J. Food Sci.* **52**, 1249–1251 (1987).

S. Cavella, S. Chemin, and P. Masi, "Objective Measurement of the Stretchability of Mozzarella Cheese," *J. Texture Stud.* **23**, 185–194 (1992).

Y. S. Chang et al., "Viscoelasticity of Cheese," *J. Chem. Educ.* **63**, 1077–1078 (1986).

N. Y. Farke and P. F. Fox, "Objective Indices of Cheese Ripening," *Trends Food Sci. Technol.* **1**, 37–40 (1990).

P. Fox, *Cheese Chemistry, Physics and Microbiology*, Vol. 1, Chapman and Hall, London, United Kingdom, 1994, pp. 303–340.

H. C. Goh and P. Sherman, "Influence of Surface Friction on the Stress Relaxation of Gouda Cheese," *J. Texture Stud.* **18**, 389–404 (1987).

T. Izutsu et al., "Rheological Properties of Mozzarella Cheese Curds," *Nippon Kagaku Kaishi* **1990**, 621–627 (1990).

F. R. Jack and A. Patterson, "Texture of Hard Cheeses," *Trends Food Sci. Technol.* **3**, 160–164 (1992).

J. Korolczuk, "Rheological Properties of Fresh Cheeses. I. Stress Evolution During Compression," *Milchwissenschaft* **50**, 674–678 (1995).

J. Koroluczuk and M. Mahaut, "Viscometric Studies on Acid Type Cheese Texture," *J. Texture Stud.* **20**, 169–178 (1989).

J. Korolczuk and M. Mahaut, "Relaxation Studies of Acid Type Cheese Texture by a Constant Speed Cone Penetrometric Method," *J. Texture Stud.* **21**, 107–122 (1990).

E. Kucukoner, "Effect of Various Commercial Fat Replacers on the Physicochemical Properties and Rheology of Low Fat Cheddar Cheese," *Dissertation Abstracts International B* **57**, 4113–4114 (1997).

R. J. Marshall, "Composition, Structure, Rheological Properties, and Sensory Texture of Processed Cheese Analogues," *J. Sci. Food Agric.* **50**, 237–252 (1990).

P. Masi, "Characteristics of History-Dependent Stress-Relaxation Behavior of Cheeses," *J. Texture Stud.* **19**, 373–388 (1989).

E. J. Nolan, V. H. Helsinger, and J. J. Shieh, "Dynamic Rheological Properties of Natural and Imitation Mozzarella Cheese," *J. Texture Stud.* **20**, 179–189 (1989).

A. A. Omar, A. A. Baky Abdel and S. A. Metwally, "Rheological Properties and Microstructure of Ripened Camembert Cheese," *Egyptian Journal of Dairy Science* **26**, 175–192 (1998).

M. Sutheerawattananonda and E. D. Bastian, "Monitoring Process Cheese Meltability Using Dynamic Stress Rheometry," *Journal of Texture Studies* **29**, 169–183 (1998).

M. H. Tunick et al., "Cheddar and Cheshire Cheese Rheology," *J. Dairy Sci.* **73**, 1671–1675 (1990).

M. H. Tunick et al., "Effects of Composition and Storage on the Texture of Mozarella Cheese," *Netherland Milk Dairy Journal* **45**, 117–125 (1991).

M. H. Tunick et al., "Rheology and Microstructure of Low-Fat Mozzarella Cheese," *International Dairy Journal* **3**, 649–662 (1993).

J. H. PRENTICE
Axminster
Devon, United Kingdom

CHERRIES. See FRUITS, TEMPERATE.

CHILLED FOODS

The chilled-food market has expanded since the advent of freezing technology in the 1940s. Although foods were sold chilled at that time, these were generally raw product such as fish and meats. The current needs of society for time-saving and easily prepared meals has led to a wider range of products being offered to the consumer, including many ethnic dishes. Much of this chilled product development has coincided with expansion in the technology of chilling, modified atmosphere packaging, and an understanding of the microbiological safety issues during storage. The addition of microbiological hurdles has allowed shelf-life extension to many products, which has helped to meet the needs for an economic distribution system. Most supermarkets now offer chilled prepared foods that are fully cooked and ready to eat. The development of a food service industry both in vending and restaurants has allowed large companies to quickly exploit the refrigerated product market and to make the most of such opportunities. This article summarizes the issues and developments in the area of chilled foods related to production, safety, and market trends from different perspectives.

MARKET FOR CHILLED FOODS

The global market for chilled foods was estimated at more than $7 billion in 1996. Although other regions such as Canada and Southeast Asia have significant chilled-food markets, the global market is dominated by sales in the United States ($3.4 billion), Europe ($2.0 billion), and Japan ($590 million) (1). The market share in these regions could be attributed to a modern lifestyle in which less time is set aside for cooking at home as well as a good infrastructure for chilled-food distribution. As the types of available products, distribution of the products, and trends in each of the three largest chilled-food markets differ significantly from the others, these markets will be addressed separately.

UNITED STATES

Available Products

The chilled-food market in the United States can be divided into seven main product categories: precut vegetables and salads, lunches and snacks, fully cooked poultry, pasta, pizza, sauces, and fully cooked entrees and dinners (1). A breakdown of the major product types in the United States chilled-food market can be found in Figure 1.

Although many food companies may distribute precut vegetables and salads, these items are also prepared, packaged, and stored at the retail level in supermarkets or convenience stores. Prepared salads may also include dressings and/or seasonings, croutons, or breadsticks. Lunches and snacks include small kits for making a sandwich or snack for one person. These kits may include cold cuts, tuna or chicken salads, or cheese and crackers; some kits targeted specifically for children's lunches may include chips or dessert items and a beverage. Fully cooked poultry includes parts (legs, breasts, etc) as well as quarter, half, and whole birds. The majority of chilled poultry marketed in the United States is chicken, with turkey receiving an increasing market share. Most fully cooked, chilled poultry is roasted or fried and may be breaded, seasoned (barbecue, cajun, spicy hot, etc), or marinated. Chilled pasta is not typically fully cooked and usually requires some preparation. The main selling points to this particular category are freshness, variety, and reduced preparation time. Some chilled pasta products are filled with cheese or meat, some may be made with nonstandard flours (corn, bran, or pumpkin), and some may be flavored (1). Chilled pizza products fall into two main segments: kits and completely assembled uncooked or partially cooked pies (1). A pizza kit usually includes the uncooked crust, sauce, cheese, spices, and possibly other toppings. Completely assembled pizzas may be uncooked or partially cooked, but the consumer is usually required to finish the cooking. Chilled sauces tend to be upscale products positioned on gourmet recipes. Italian pasta sauces are the most popular of this category, followed by ethnic and traditional sauces. Examples may include alfredo, pesto, spicy marinara with clams, Thai with lemongrass, Mexican mole, barbecue, and seafood sauces. Fully cooked entrees and dinners are mostly cooked and meant to be heated and served. Examples may include lasagna, meatloaf, and fajitas as well as complete turkey dinners with side dishes for the holiday season (1).

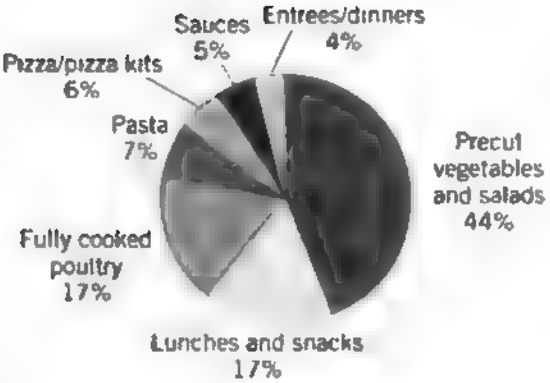


Figure 1. Available product types and market share of chilled foods in the United States, 1996.

Current and Future Market Trends

The retail market for chilled prepared foods in the United States advanced from $1.8 billion to $3.4 billion during the years 1992 to 1996, an increase of 87.6% (1). Most of the growth has been earned by precut vegetables/salads and lunches/snacks, with fully cooked poultry, pasta, and pizza earning small advances and chilled sauces and entrees/dinners declining slightly. The total chilled-food market is expected to increase to more than $5 billion by the year 2001; this is demonstrated in Figure 2.

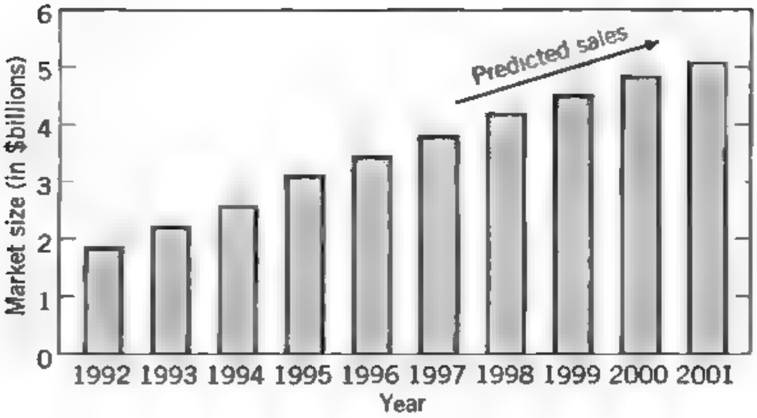


Figure 2. Actual and predicted market for chilled foods in the United States, 1992–2001.

Most growth in the U.S. chilled-food market is predicted to occur in the precut vegetables/salads and lunches/snacks areas. Other chilled categories are expected to remain stable or decline somewhat. Despite the large market growth between 1992 and 1996, most chilled foods are marketed as line extensions of successful frozen or shelf-stable company brands. Although food companies are aware of the consumer demand for fresh, convenient foods, most do not provide proper market support or distribution channels for chilled products. Refrigerated food marketing strategies in the past have included trying to capitalize on already successful frozen or shelf-stable brands rather than pushing the products on their own merits. Most consumers purchase chilled prepared foods because they are perceived as being fresher and more convenient than similar frozen or shelf-stable products. Future trends in the chilled-food area include achieving fresher products through improved distribution practices, improving the market by focusing on the convenience of chilled foods, and developing a greater variety of value-added products to offset premium price levels of chilled foods (1).

EUROPEAN AND JAPANESE MARKETS

Available Products

The European chilled-food market can be divided into seven main product categories: salad lunches, meat dishes, seafood dishes, pasta-based dishes, pizza and pizza toppings, ethnic foods, and other foods such as cheese and dairy products (1). The types of products in each of the preceding categories will vary greatly throughout Europe due to the breadth of cultural diversity. Nonseafood salad lunches account for 21% of the total chilled prepared-food sales, closely followed by meat dishes, seafood dishes, pasta-based dishes, and pizza and pizza toppings. Figure 3 illustrates the major product types and market share of each product category in the European chilled-food market.

The chilled-food market in Japan is comprised of three main product categories: Japanese-style foods, other Asian dishes, and Western dishes (1). Chilled foods in Japan tend to be marketed as snacks or luncheon replacements rather than main meal replacements.

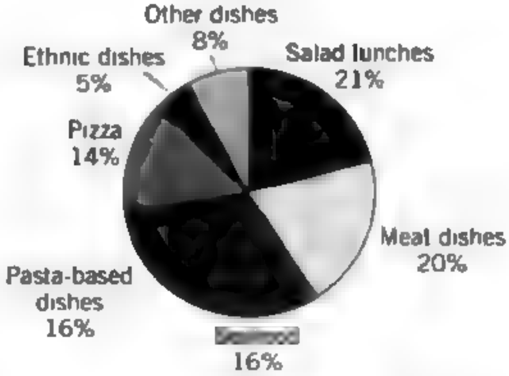


Figure 3. Available product types and market share of chilled foods in the European Community, 1996.

Current and Future Market Trends

Sales of chilled prepared foods in Europe increased from $1.5 billion to more than $2 billion during the period 1992 to 1996 (1); actual and forecasted sales figures for the European chilled-food market can be seen in Figure 4.

The growth in the chilled-food market in Europe is not as dramatic as that in the United States over the same time period. It should be noted, however, that chilled-food marketers experienced a drop in unit prices and profit margins due to the marketing of larger product portions in order to gain or expand market share (1). Growth of the European chilled-food market during the period 1997 to 2001 is also expected to be relatively slow; sales of chilled foods in Europe are expected to increase to only $2.4 billion by the year 2001. Nevertheless, chilled foods account for 19% of the total prepared-foods market in Europe, and this position is expected to increase to 22% by 2001. Scandinavia and the United Kingdom account for more than 50% of the total chilled-food market in Europe, with Germany and France accounting for another 27%. These markets are seen as relatively mature, but greater growth is expected in countries such as Spain, Italy, and Eastern Europe, where chilled-food sales are still relatively small. The chilled prepared-food market in Japan increased from $433 million to $587 million during the period 1992 to 1996. Chilled-food sales in Japan are expected to increase

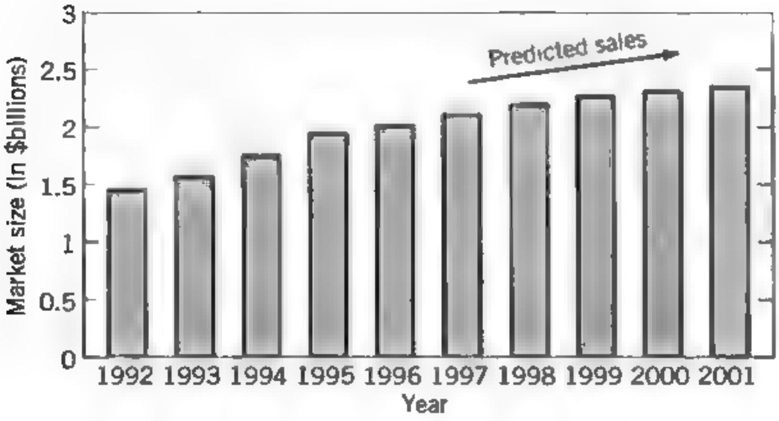


Figure 4. Actual and predicted market for chilled foods in Europe, 1992–2001.

to more than $840 million by 2001 (1); the current and future chilled-food market trend for Japan can be seen in Figure 5.

Most of this market growth is due to the expanding presence of supermarkets, although much of the available chilled foods is distributed through neighborhood convenience stores. The chilled-food market in Japan is seen as relatively new and expanding by market research companies, as chilled-food distribution systems have only recently been opened to Western companies (1). Home delivery of food to the elderly and the expanding presence of automatic vending machines are expected to contribute significantly to the Japanese chilled-food market growth.

Consumers in both Europe and Japan view the nutritional value and health benefits of chilled foods as being superior to similar frozen or shelf-stable products. A demand for superior quality is also evident in both Europe and Japan, as consumers are willing to pay a premium for chilled foods with authentic home-cooked or restaurant-quality taste. Convenient and functional packaging of chilled foods is given as much accord by Japanese customers as the nutritional quality of the food. Consumers in both Europe and Japan are also becoming more price conscious in light of the 1990 European recession and the recent economic troubles in Asia (1).

PROCESSING OF CHILLED FOODS

Chilled-food processing lines are designed to produce quality finished products as well as to control the presence and growth of microorganisms in finished products. A particular production method may be used in concert with another to produce a product of superior organoleptic and microbiological quality. The following procedures are common throughout the industry in the production of chilled foods.

Controlled Product Handling During Processing

Product handling during all phases of chilled-food production is crucial from a microbiological safety and quality standpoint. A typical processing diagram for chilled foods indicating separation of production zones can be found in Figure 6.

Separate zones for raw receiving, preprocess preparation, product assembly and cooking, pasteurization, chilling, and packaging are usually identified and maintained in chilled-food operations (2). Physical separation of different production zones is necessary to prevent cross-contamination between raw and cooked or processed product. Other aspects of hygienic process design, such as clean rooms and air handling systems, may be incorporated into chilled-food processing to reduce the risk of microbial contamination. For example, clean rooms may be maintained in the assembly and packaging of already-processed constituents, and air handling systems may be used to filter particles 0.5 μm or smaller from product assembly areas to minimize postprocess microbial contamination. A rigorous cleaning protocol for machines and the factory environment is also to be included as part of a good manufacturing practice for the manufacture of chilled-food products (3).

Pasteurization

Pasteurization involves heating food products to eliminate specific types of microorganisms. Chilled products can be pasteurized before packaging and clean-filled (hot fill hold) or filled and pasteurized in-pack (IPP). Cooking processes may be considered as part of the pasteurization process, but care must be taken to prevent contamination during latter processing steps. For chilled products having an expected shelf life of 10 to 14 days, a minimal heat process to eliminate infectious pathogens is recommended, especially if the product is ready to eat. An example of a typical process for these product types is 70°C per 2-min hold, which will eliminate 10^6 *Listeria monocytogenes* per gram of product. Chilled products having an expected shelf life of more than two weeks generally require a more severe process to eliminate spore-forming psychrotrophic pathogens. Typical processing conditions for these extended shelf-life products are at least 90°C per 10-min hold to eliminate 10^6 nonproteolytic, psychrotrophic *Clostridium botulinum* per gram of product.

Pasteurization processes are defined in terms of *P*-value, which is the time a product is held at a certain processing temperature (typical value for nonproteolytic *C. botulinum* would be written as $P_{90^\circ C} = 10$). Conventional

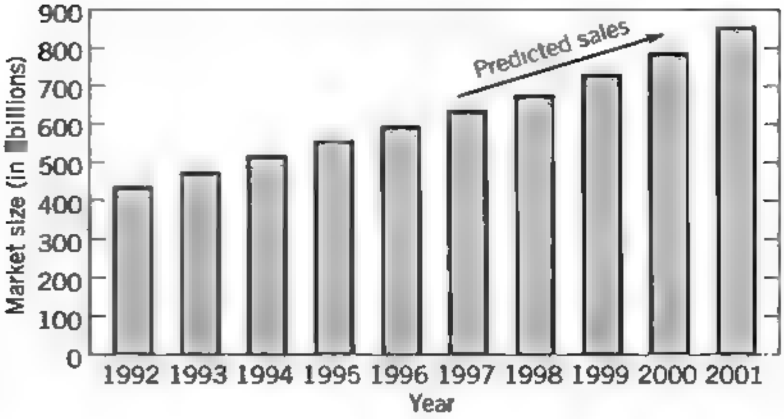


Figure 5. Actual and predicted market for chilled foods in Japan, 1992–2001.

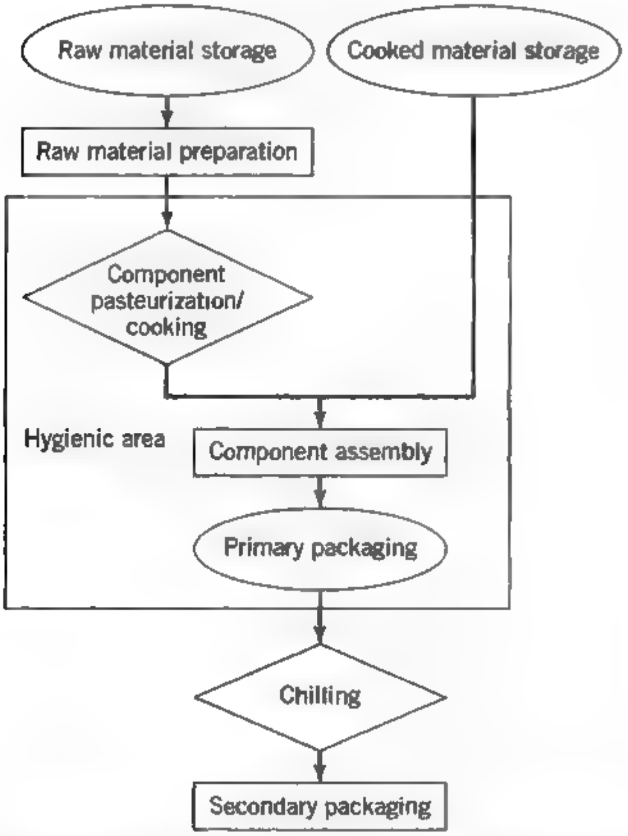


Figure 6. Typical process diagram for the manufacture of chilled foods.

thermal processing *z*-value, which is used to calculate the increase or decrease in temperature necessary for a 10-fold difference in the reduction of microorganisms, may also be used to describe pasteurization processes. *P*-values are usually used, however, because unlike commercial sterilization processes, both *z*-value and heat resistance of the organism may increase during pasteurization (4). The heat resistance of microorganisms generally increases in food products having a lower water activity and decreases in food products having a lower pH.

Examples of pasteurization methods include heating mixes and particles suspended in sauces in jacketed vessels, heating liquid or pumpable ingredients via heat exchangers, heating solid particles in atmospheric ovens, frying, and heating packaged products in water baths or retorts for in-pack pasteurization. Any method used must provide a critical minimum heat treatment, which must be defined by heat penetration analyses and thermal death time experiments. Factors affecting the pasteurization process include the initial temperature of the ingredients, amount of solid particulates, equipment heating rate, and rate of circulation/agitation. These parameters, as well as temperature profiles of the finished product during processing, are usually Hazard Analysis Critical Control Points (HACCPs) and must be accurately and reliably controlled and maintained.

INTRINSIC PRODUCT FACTORS

Chilled-product formulation may have a significant effect on the process applied to a particular chilled food. Several factors may be incorporated into the formulation of chilled foods that may increase the overall keeping quality of the product and subsequently decrease the amount of processing necessary to achieve a certain shelf life. One factor that reduces the amount of heat necessary to provide a particular shelf life is product pH. Microorganisms tend to be more heat sensitive in products with a lower pH, and lower pH may also enhance the keeping quality in combination with chilled storage temperatures, as the optimum pH range for the growth of most foodborne microorganisms is between 5 and 8. An example of utilizing the synergistic effect between storage temperature and product pH is in the prevention of *C. botulinum* type E outgrowth. A product pH of 5.5 is necessary to prevent the outgrowth of *C. botulinum* type E spores at 15.5°C, but if the storage temperature is maintained at 5°C, the optimum pH for control of outgrowth rises to 6.4. Product pH may be lowered using acidic ingredients like tomatoes or by the addition of organic acids such as lactic, citric, or acetic acid. The type of acid selected depends both on sensory and shelf-life requirements. In general, acetic and lactic acids have greater antimicrobial activity than citric acid.

Another factor that may be used to control the possible growth of microorganisms in chilled products, especially nonproteolytic *C. botulinum*, is water activity (a_w). Water activity values below 0.85 may prevent or delay the outgrowth of microorganisms during shelf storage of chilled products, although products with a lower a_w may require a more severe initial heat process. Most chilled products have an a_w above 0.90. Preservatives such as potassium sorbate or sodium benzoate may also be added to chilled foods to control the growth of certain microorganisms during shelf storage. Use of these preservatives is primarily to control the growth of yeasts and molds, and the activity of these substances may depend on product pH. Potassium sorbate may also delay the outgrowth of some spore formers and lactic acid bacteria. Nitrites and sodium chloride have been used in chilled meats, poultry, and fish to protect against *C. botulinum*. The factors just listed may be used in concert for chilled-product safety and quality while improving the overall product quality and appearance. They assist by reducing the pasteurization process necessary for the control of microbial growth. The development of products with these intrinsic factors is known as "Hurdle Technology" and is widely practiced in the food industry.

CHILLING TECHNIQUES

Products distributed in the chilled state must be sufficiently cooled in a reasonable time after cooking and processing. European Economic Community (EEC) Directive 77/99 requires that foods for chilled distribution must be cooled to 10°C or less within two hours of processing and maintained at or below that temperature for the duration of product shelf storage. The U.S. Food and Drug Administration (FDA) requires similar cooling conditions (cooled to less than 7.2°C in less than two hours after processing and maintained at or below that temperature during product shelf storage) for chilled foods.

Several techniques are currently used in the industry that allow for the processed products to be chilled according to the preceding requirements. One of these is the use of air-blast coolers, which circulate chilled air at high velocities (up to 16 m/s for smaller product sizes) to effect a rapid cooling rate. This rapid rate is important for sensitive foods that have been heat processed prior to cooling. The design of these units provides rapid and even chilling, and coolers may also be designed for humidity control in unwrapped product applications. Safety issues such as *L. monocytogenes* contamination can arise in systems for chilling unwrapped products if conditions such as temperature and humidity are not controlled. Chilling tunnels can also provide good product chilling characteristics by altering the airflow methods in different tunnel sections. Products cooled by this method are usually carried through the tunnel on trays or pallets. Direct-contact refrigerants such as liquid nitrogen and dry ice (carbon dioxide) shavings may also be used to rapidly chill heat-processed products. This equipment is usually designed to prevent products from freezing. Immersion hydrocooling and ice bank chilling are two other common methods of rapidly cooling chilled products; these methods provide more control over the chilling process, as they do not allow for the product to freeze. Hydrocooling is carried out by spraying or immersion of the product and can be included as part of a continuous process. Ice bank chilling is primarily used with vegetables, and systems may be designed to maintain a high humidity to prevent moisture loss. After rapid chilling, products may be held in lower-velocity air system cold rooms before packaging or distribution.

Some products may be prepared frozen and held until they reach the retail or food service market. At the retail outlet, the products are thawed at 4 to 6°C and maintained at this temperature. This approach is referred to as "freeze-thaw" and allows greater flexibility in the distribution chain as well as longer product shelf life and improved safety. Sale of such products requires that the consumer be informed that the product was "previously frozen." Another approach, often referred to as Latent Zone chilling, uses temperatures of −2 to 0°C for the preparation and storage of products. Such temperatures have been shown to extend the shelf life of a number of products for up to six weeks. The concept was developed to provide more efficient production of prepared foods.

PACKAGING OF CHILLED FOODS

There are a number of methods for packaging chilled foods, and the diversity is evident when the chilled-product areas in supermarkets are viewed. A series of requirements for chilled-food packaging from both the consumer and manufacturer point of view are listed in Table 1.

Foods undergo numerous changes including chemical, physical, and microbiological changes during storage. Food packaging acts as a protective unit against such changes by providing a barrier against contamination from outside. These properties have been described as active, intelligent, or interactive and may delay or control many of the undesirable changes to the product during the storage period.

Packaging materials are chosen based on the properties necessary to maintain product safety or quality as well as package integrity. The most common materials are paper, variable types of polymers or films, aluminum, or combinations of each as laminates. Lamination of these materials provides strength, improved heat resistance for the package (eg, from direct microwaving), better sealing properties, and more efficient barriers against moisture loss.

Table 1. Principal Package Requirements for Chilled Foods

Manufacturer basic requirements	Consumers' requirements
Nontoxic	Easy opening
Withstand factory process conditions	Resealable
Maintain integrity during transit	Shelf life/nutritional/ingredient information
Control moisture/gas losses	Contents clearly visible
Maintain seal integrity	Minimal handling required during preparation

Rigid polymer materials have become a popular choice for chilled products; these include polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polystyrene (PS). The need for lower-cost packaging materials such as the flexible polymers or packaging films consists of multiple layers of laminates. Ethylene vinyl alcohol (EVOH), for example, is the film of choice for improving the oxygen barrier properties. A good overview of chilled food packaging can be found in Reference 5.

Modified Atmosphere Packaging

Chilled products may be packaged under a modified atmosphere to allow extended shelf storage (6). This type of approach allows products to be shipped longer distances and enables a reduction in economic losses due to spoilage. Modified atmosphere packaging (MAP) depends on replacing air in a package with different gas mixtures to regulate microbial activity within the food. The composition of gases in the pack does not remain constant and will change during storage as a result of chemical, enzymatic, and microbial activity of the product. There are three parameters to consider for the successful use of MAP in chilled foods: the packaging film/container, the gas or gas mixtures being used, and the storage temperature. The film must have high barrier properties against moisture loss and low rates of transmission for the gas mixture being used. The role of the gases is quite distinct; carbon dioxide is inhibitory to both bacteria and yeast and molds and acts by extending the lag phase and, therefore, the generation time. The concentration of the gas and the age, type, and load of the initial bacterial population influence this effect. Low temperatures increase the inhibitory action of carbon dioxide. Use of high levels of this gas can affect sensory properties of a food, producing off-odors and pigment, taste, and texture changes. It may also cause package "collapse" as a result of the carbon dioxide being absorbed by the food. Nitrogen has little or no effect on bacterial growth unless the residual oxygen levels are extremely low (0.2%) and usually acts as a filler for the package. The presence of oxygen in MAP retards anaerobic growth and can improve or stabilize color within the pack. The application of MAP alone for chilled foods may not always provide shelf-life extension but should be considered as a part of a hurdle system. Some commonly used gas mixtures for MAP are shown in Table 2.

Advantages and limitations of MAP in chilled foods are listed in Table 3. Further discussions on MAP technology can be found in References 7 and 8. The desired atmospheres within a package can also be achieved by use of sachet technology, an alternative to gas flushing. This approach referred to as active packaging involves placing a sachet of a gas generator or absorber directly into the package before sealing. Currently there are two kinds of MAP sachets available—oxygen absorbers and carbon dioxide generators. The most common oxygen absorbers work on the principle of iron being converted to ferric hydroxide by oxygen and water vapor. This technology is easily applied during the manufacturing stage, and the atmosphere generated can be maintained during storage. Another application of sachet technology is the use of an ethanol pack. A sachet containing ethanol is placed in a package, and the released vapor acts as an antimicrobial agent.

DISTRIBUTION OF CHILLED FOODS

Chilled products must be distributed under temperature-controlled conditions matching the previously described EEC or FDA guidelines. Once the chilled products have been prepared, a temperature-controlled means of transport must be used to ensure that critical product handling temperatures are maintained. Road transports and intermodal freight containers are generally used to transport chilled food products from factory to warehouse to retail outlet. Road transports may include temperature-controlled trailers that may be run independently of the tractor units or trucks with temperature-controlled storage areas. Intermodal freight containers have self-contained temperature control units and may be transported via train or on the bed of a trailer. Temperature-controlled road transports or intermodal freight containers must be capable of not only maintaining a chilled environment in warmer months but also of maintaining chilled products in a refrigerated state during colder seasons.

Chilled food products are generally maintained in exclusive temperature-controlled warehouses or in temperature-controlled sections of other warehouses via the use of cold room techniques previously described. Once the chilled products have been distributed to the retail outlet, refrigerated display units are generally used to maintain product temperature for the remainder of product shelf

Table 2. Commonly used Gas Mixtures in MAP for Microbial Stability in Chilled Products

Chilled product	Target organisms	CO_2 (%)	Nitrogen (%)	Oxygen (%)
Pasta	Molds, *S. aureus*	>35	>20	—
Meats	Spoilage organisms	—	100	—
Salads	Spoilage organisms	20	—	—
Fish	*C. botulinum*	50–60	—	25
Sandwiches	Spoilage organisms	—	100	—
Pizza	Molds, lactics	50	50	0–10
Poultry	Spoilage organisms	>40	60	—
Cottage cheese	*Listeria*	35	—	—
Soft Cheeses	*Listeria*, molds	—	100	—
Hard Cheeses	*Listeria*, molds	—	100	—

Table 3. Advantages and Limitations of MAP in Chilled Foods

Advantages	Limitations
Shelf-life extension	Does not inactivate pathogens or prevent growth
Restrict growth of aerobic organisms	Shelf-life extension depends on initial population of microorganisms
Delays growth of some facultative organisms	Requires more costly packaging
May improve flavor	Can lead to lower line speeds
Adjunct to other processes	Does not control the growth of lactic acid bacteria

storage. Refrigerated display units may be configured in a variety of ways, and the most common types are freestanding bins without doors and upright shelf units with protective glass doors. Several sources have noted that retail display is the aspect of distribution in which a control of storage conditions is most lacking (9). Proposed regulations in the United States are more stringent in that the temperature requirement has been lowered from 6 to 4°C for refrigerated storage of chilled products. The goal of this regulation if to provide a greater margin of safety and quality to chilled products, which should benefit both the consumer and the processing company.

TEMPERATURE CONTROL AND MONITORING

In the production of chilled foods the focus of any production strategy is either to eliminate or minimize the growth of pathogens or spoilage organisms. However, this focus must be balanced with quality issues such as color, flavor, and the desired shelf life. A number of options will allow such a balance, but all of these depend on temperature control.

A key factor for successful production of chilled foods is the constant awareness of controlling the temperature from the production and manufacturing site to the retail or food service markets and the consumer. The rate of quality loss for chilled products, for example, can increase up to eight times for each 10°C increase in temperature, and the penalty for mishandling of products can be severe both in terms of human illness and product loss (10).

A number of temperature-measuring devices are available for monitoring products during production or transporting or in the retail marketplace. An excellent overview of this area has been published in Reference 11. The most common types consist of single readout units, sensors such as semiconductors or thermocouples, recording charts, data loggers, and chemical indicators. The single-unit devices such as the dial and stick thermometer, often seen in supermarket chill cabinets, lack accuracy in determining the air temperature. Changes to electronic readout versions of these bimetal units have not added significantly to their accuracy. A much more accurate unit is the "point and shoot" infrared thermometers. These handheld guns measure radiation temperatures of a product surface, which is translated to a digital readout. Since the units measure surface temperature, the actual temperature of a food product may need to be confirmed by other means. Similarly the liquid crystal devices seen on the front surfaces of chill cabinets suffer the same problem since they measure the surface temperature of the cabinet.

These single-unit devices are now being replaced with more accurate remote systems. These systems can be linked to a computer for data storage and temperature control. Such systems can give temperature displays from a number of sites at once and may be linked to an alarm device. The temperature-measuring devices in these units may be either thermocouples or thermistors. The accuracy of these sensors ranges between 0.3 and 1.5°C. The thermistor, a device that measures changes in resistance with temperature, is preferable to a thermocouple because of its accuracy.

Data loggers may also be used to monitor refrigerated transport and storage of chilled foods. These units can be programmed to measure and record temperatures at regular intervals over a period of time. When linked to a computer, these data can then be retrieved, and the temperature history during storage is revealed. Current developments with these units have reduced the size of these loggers so that they can easily be placed inside small packages. Temperature monitoring and control is an important issue, especially for chilled food where it remains the primary hurdle against spoilage and foodborne disease. An indication of this is that chilled-product mishandling and abuse due to incorrect storage temperatures contributed to 43% of foodborne disease outbreaks in the United States in 1996. A temperature-monitoring approach that has been successful in the pharmaceutical industry is the use of time-temperature indicators (TTI). These indicators measure temperature or temperature changes based on chemical or biological reactions. The results of these reactions are displayed in the form of color changes. In chilled foods the best type of TTI is the one that integrates both time and temperature. Such a product is available on the market, but current costs for these units prevent many companies from using them on individual packs. Consumer preference with TTI, especially with chilled products, is a "go/no-go" indicator. Systems indicating how much shelf life is remaining in a product are also preferred by consumers (10). Future developments should be able to overcome cost and ease-of-use issues.

Distribution and Storage Temperature Studies

Several studies on refrigeration temperatures maintained at retail outlets and homes of consumers revealed several interesting facts (12). Studies carried out in the U.S. market have shown that the temperature of storage of chilled products varies within a store and by product type. Supermarket surveys have shown that storage temperatures and fluctuations within the raw meat cabinets are better controlled than in the delicatessen areas. A summary of this information can be found in Figure 7.

Comprehensive studies have observed that many refrigerated foods both at the retail and consumer level exceeded 10°C (13). This was particularly true for delicatessen items, where 13% of these items were above 12°C.

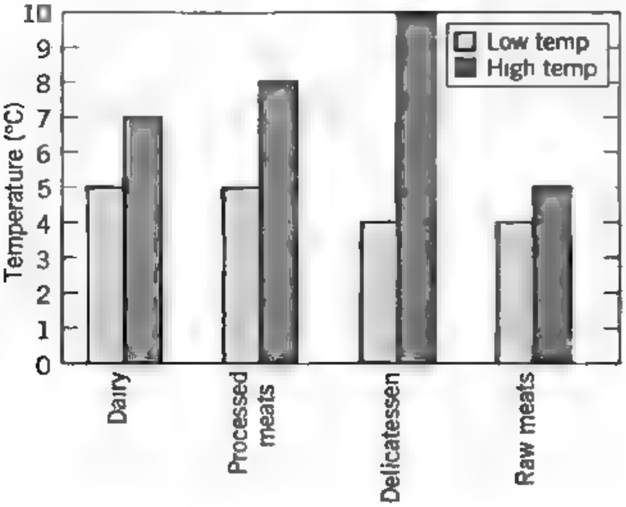


Figure 7. Temperature distribution of chilled products at the retail market.

Twenty percent of home refrigerators surveyed were also operating above 10°C (14). Factors such as shopping time and chilled-product temperature change from store to home have also been studied. In the United States, the variation in shopping time was found to be from 30 to 45 min to over 1 h and 30 min. Product temperatures during transit by consumers were shown to rise from 5°C to 15°C in some instances.

MICROBIOLOGY OF CHILLED FOODS

Other factors apart from temperature control influence microbial growth. These are moisture/water activity (a_w), acidity (pH), packaging, the use of preservatives, and type of initial processing. The most commonly used combinations are pH, a mild heat process, modified atmosphere packaging, and preservatives. These are combined with the use of chilled temperatures for better safety and shelf life of the product.

Microbiological Safety

Significant foodborne pathogens in chilled foods include both infectious and toxigenic organisms. An assessment of the ability of these organisms to grow in such products is critical for an evaluation of hazards. Organisms such as *Staphylococcus aureus*, *Bacillus cereus*, and proteolytic *C. botulinum* are unable to grow below 7°C, but under temperature abuse conditions they should be considered potential hazards. Spore formers such a *B. cereus* and *C. botulinum* are also likely to survive mild pasteurization treatments often given to chilled products, so the outgrowth of these pathogens is controlled by temperature alone. Organisms important to the safety of chilled foods, as well as the parameters for their growth, are listed in Table 4.

The major microbiological concern in chilled foods is pathogens such as *Listeria* and nonproteolytic *C. botulinum*. Recent outbreaks in the United States of *Listeria* in chilled meats emphasize the importance of controlling this pathogen in a chilled-food processing environment. Ready-to-eat deli meats produced by one large company were associated with at least 62 listeriosis cases caused by serotype 4b strain. At the same time, reports of recalls due to contamination by *Listeria* from other ready-to-eat meat producers were also published. The organism is ubiquitous and can be found in raw products such as vegetables, meat, and dairy products. It typically can be isolated from several locations in a factory environment and can contaminate chilled foods as a postprocess contaminant (15). Because the organism cannot be eliminated from food processing plants, strict management of the processing environment is necessary to minimize the probability of cross-contamination. This includes monitoring of ingredients, surfaces, and the environment. This infectious pathogen has the capacity to grow at refrigerated temperatures, with a generation or doubling time at 4°C of about 24 h. Thus chilled foods that do not receive a terminal heat process prior to consumption by the consumer can contain levels of *Listeria* capable of causing listeriosis. Listeriosis is characterized by a range of symptoms from flulike illness to miscarriages, septicemia, and death, depending on the sensitivity of the person. The infectious dose has yet to be determined but the current literature suggests <100 organisms per gram. This organism has limited heat resistance, and a process of 70°C per 2-min hold will ensure the elimination of this organism from the food.

Most serotypes of *C. botulinum* are of little significance in chilled foods with the exception of type E and nonproteolytic type B strains. Such serotypes are capable of growing at chilled temperatures and produce a neurotoxin. Incubation periods under anaerobic conditions of up to one month are required for toxin production at 4°C in meat/fish-containing products. The spores are capable of being destroyed with milder heat treatments (eg, 85°C for 15–30 min) than their proteolytic counterpart strains, which require temperatures above 100°C for any measurable death. Other public heath–related organisms that are capable of growing at refrigerated temperatures include *Yersinia enterocolitica* and *Aeromonas hydrophila*. The more important of these two is *Y. enterocolitica*, as this has been clearly implicated in foodborne outbreaks with chilled products (16). Like *Listeria*, this organism has been isolated from foods such as meats, dairy products, and vegetables. Species of *A. hydrophila* have been associated with chilled salads and other uncooked foods.

B. cereus is a spore-forming organism that functions both as a spoilage organism and a foodborne pathogen. While it is reported that psychrotrophic strains of *B. cereus* have been isolated from refrigerated products, most strains are unable to grow below 8°C. The spores are capable of surviving pasteurization temperatures associated with milk and can germinate and produce toxins under the right conditions. Sources of *B. cereus* include spices, herbs, dairy products, and meats.

Infectious agents such as *Salmonella*, *Campylobacter*, and *Escherichia coli* 0157:H7, although unable to grow below 7°C, are potential hazards in chilled foods due to their low infectious doses. Such organisms must be eliminated

Table 4. Microorganisms Important in Chilled Foods with Respect to Safety

Microorganism	Minimum growth temperature (°C)	Minimum a_w for growth	Minimum pH for growth
Listeria monocytogenes	0	0.92	4.4
Yersinia enterocolitica	1	0.93	4.2
Clostridium botulinum (nonproteolytic)	3.3	0.96	4.6
Salmonella	7	0.94	3.8
Escherichia coli 0157:H7	7	0.95	4.4
Bacillus cereus	8	0.93	4.4
Clostridium botulinum (proteolytic)	10	0.93	4.6
Staphylococcus aureus	7	0.86	4.5

in ready-to-eat products. Some viruses such as the Norwalk virus have been associated with oysters and have caused foodborne outbreaks when the product has been eaten raw.

Microbiological Spoilage

Many of the spoilage organisms associated with chilled foods are adapted to their environment (17). At 0°C, for example, the lag time for many organisms is usually about two to three days before initiating growth. Once this occurs, the generation time for these psychrotrophic bacteria at 4°C is in the region of 10 to 12 h. For a chilled food product to achieve maximum shelf life with optimum quality, the initial level of these psychrotrophic spoilage microorganisms must be minimized. The principal groups of spoilage organisms associated with chilled foods are *Pseudmonas* spp., lactic acid bacteria, members of the coliform group, yeast, and molds. These groups are widely distributed in nature and therefore present both in raw ingredients and the factory environment. They are good competitors and capable of producing off-flavors or odors, especially when bacterial populations reach about 1 million cells per gram of food.

Each of the microbial spoilage groups contributes certain attributes to a food product during growth. *Pseudomonas* species cause slime formation, color changes within the pack, and, as a result of their proteolytic metabolism, strong amine odors. These changes occur in an aerobic environment and can be controlled by removing the oxygen from the food. Other Gram-negative groups contribute less drastic changes but will contribute to off-odors and acid flavors. The lactic acid group has a unique role in spoilage since lactic acid is produced as a result of their metabolism. In addition to lactic acid, other antimicrobial agents that are capable of controlling the growth of certain pathogens and competitive microflora may be produced. Lactic acid bacteria are also one of the more persistent groups in chilled foods because they tend to be resistant to most of the preservatives used and can grow under anaerobic conditions. Yeasts and molds are also a problem in chilled foods, and this type of spoilage is easily visible to the consumer. Yeasts cause spoilage problems in chilled products because they are facultative with respect to oxygen requirements, but they can be inhibited by a preservative such as sorbic acid or growth delayed by high carbon dioxide levels in the pack. Molds require aerobic conditions for growth and can be controlled by use of oxygen scavengers in the pack or modified atmosphere packaging.

Nonmicrobial Issues Pertaining to Chilled-Product Safety and Quality

Several toxicological issues relate to the safety of chilled foods. Natural toxicants exist usually at low levels in several types of raw products. These include biogenic amines, glycoalkaloids, phytotoxins, nutritional inhibitors, antivitamins, and antigens that can stimulate anaphylactic shock in sensitive people. Fortunately, many such agents are modified during processing as a result of culling, washing, and heating. Potatoes, for example, can produce glycoalkaloids such as solamine, which is a potent cholinesterase that acts on the central nervous system and interferes with nerve impulses. These amines are produced by stress conditions in the potato and accumulate close to the skin. The process of culling and peeling reduces human exposure to this alkaloid. Lectins associated with legumes such as kidney beans have been associated with toxicosis when eaten without prior treatment. When such products are soaked overnight and subsequently cooked, the toxic lectins are inactivated. An important toxin associated with fish products is caused by algal growth. The algal toxins gain access to the food chain through ingestion by shellfish. These toxins can accumulate in marine life at high levels without adverse effects. It is particularly dangerous to consume such shellfish during these periods of high algal concentration in coastal waters when the nutrient levels and temperature reach an optimum level. Depending on the type of toxin present, consumption by humans of contaminated shellfish causes a number of symptoms including vomiting, disorientation, numbness, and paralysis.

Other chemicals arising from the environment can occur at low levels in foods and should be accounted for when considering safety of a product. The most common and closely monitored contaminants are heavy metals, pesticides, antibiotic residues, and incidental additives from packaging. Toxic metals are of a particular concern, as problems arise as a result of using sludge from sewage farms and waste from settling pools for fertilizing crops. The dissemination of antibiotics from livestock into the food chain has been demonstrated in milk and meats. Although the levels are low, there is a concern with respect to the development of resistant strains of bacteria entering the food chain. See Reference 18 for further information.

Quality Control Plans

Control plans for chilled food include shelf-life testing and the use of predictive microbiology, HACCP plans, and risk assessment to ensure that all products meet local and national regulations.

Shelf-Life Testing. The shelf life of a refrigerated product is, in a sense, a record of its production and applied processes. Products with a long shelf life have to be prepared with more stringent control over ingredients and environment as well as the use of more severe heat treatment or the application of multihurdle technology. Product shelf life can be defined as the period in which the product, when stored under proper chilled conditions, is safe and wholesome to eat. In determining shelf life at chilled temperatures, both chemical and microbiological parameters have to be considered. Likewise, there should be a balance between spoilage and safety with respect to microbiological issues. Chemical issues usually involve texture changes, color degradation, syneresis or separation of water from the product, and flavor changes. Texture changes usually occur as a result of migration of water to or from an ingredient or as a result of enzymatic activity. Color degradation of pigments such as chlorophyll or carotenes, for example, can occur from exposure to high temperatures or pH changes. Sensory testing is an important part of shelf-life evaluation for determining the optimum product quality and how long it can be maintained during storage.

Microbiological shelf life testing usually consists of testing products at both standard chilled temperatures (4–5°C) and at abuse temperatures (10–15°C) over the claimed shelf life. The abuse temperatures are used to determine the margin of safety built into the product by allowing for the growth of certain pathogens if present. Several sampling periods and storage trials are necessary to get a true picture of the microbiological profile of the product. Shelf life is then designated based on data from these studies. In some instances, challenge studies are incorporated in shelf-life studies, especially if new formulations or technology is used. The food is challenged with the pertinent pathogens over the shelf-life standard and abuse temperatures (19). The data give some idea as to the ability of the growth characteristics of the pathogen in that food. Mathematical modeling of data can be used for such studies as a basis for experimental design. These models may aid in predicting growth conditions of selected pathogens under a given set of condition at different temperatures. Using such data can be timesaving and limit the number of tests that need to be performed. Current predictive models also include a number of variables associated with the intrinsic properties of the food such as a_w, pH, and preservative systems.

Needless to say, most of the data used in the establishment of predictive models has been derived from both studies and cannot completely replace standard challenge experiments. Both shelf-life and challenge experiments require technical expertise and adequate resources to carry out the task successfully (20).

Hazard Analysis. Data from shelf-life and challenge studies focus on the safety and quality issues of the product during storage. This information can be tied into the HACCP plan during the manufacturing of the chilled products (21). In essence, this approach examines the safety issues likely to arise during the complete food chain (production to consumer) and lists hazards that are deemed critical. Such hazards are recognized as critical control points (CCP), and control measures are put in place to control the identified hazard during the production, distribution, and sale of the product. The system is science based, applying a systematic approach to the issue of safety (and sometimes quality) for food production. Briefly, the approach uses seven steps that include hazard identification, setting of standards for CCPs, corrective action to be taken, and auditing. The application of a HACCP program requires a team approach that draws on expertise in disciplines such as engineering, product development, microbiology, chemistry, and public health. The HACCP system can also provide other benefits such as reducing end product testing, aid inspection programs and promote international trade.

Risk Assessment. The decision on what is a hazard or whether it is critical is sometimes unclear, and risk assessment can help link relevant steps in the HACCP plan to public health concerns (20). Risk assessment is the scientific basis for the estimation of risks, either qualitative or quantitative and has been used successfully for environmental issues for many years. When a person is exposed to a hazard, it is possible to estimate the severity and likelihood of harm resulting from that exposure. One of the steps in a HACCP study is the establishment of critical limits for the CCPs. These critical limits would more meaningful if they were established by quantitative means. The steps in risk assessment would allow this by determining (*1*) hazard identification, (*2*) assessment of the exposure to the hazard, (*3*) dose response or negative effects of such exposure, and (*4*) estimation of the magnitude of the public health problem.

Thus, the overall objective is to generate and obtain quantitative information of the microbial risks associated with the manufacturing and distribution of the product. This is achieved by assessing the frequency of pathogens in raw materials, the survival or growth patterns during the process, storage, and distribution. This data, along with the dose response for the pathogen, will provide information for a risk assessment to be determined. For further reading see Reference 22.

Legislation

Many countries have standards or guidelines that cover relevant legislation for chilled foods. The FDA in the United States has requirements under Good Manufacturing Practices for the production of chilled foods (CFR Part 110). Included in this law is the requirement that raw materials and ingredients be free from pathogenic microorganisms or be treated in such a way "so that they no longer contain levels that would cause the product to be adulterated." This regulation also has provisions for maintaining the food at 7.2°C or below "as appropriate for the food." It also requires that chilled products be handled in such a way as to protect the product from recontamination. The FDA has also developed the Food Code as a model to assist other state agencies in the area of food service, retail, and vending. It provides guidance for thawing, cold storage, and reheating of potentially hazardous foods. The Code also provides information on labeling requirements and shelf life at specific temperatures for chilled foods. Although this code was intended as a guideline, several states have adopted it into legislation. The U.S. Department of Agriculture (USDA) provides general processing guidelines for meat and poultry products as well as requirements related to the sanitation of the plant. It also requires any manufacturer of chilled foods under its jurisdiction have a sanitation standard operating procedures (9CFR 416) and a HACCP plan for all processed products.

The Food Act of 1990 provides the main provisions in the United Kingdom for the safety and hygiene controls of chilled foods (23). It introduces the concept of "due diligence" by stating that all reasonable precautions and control measures (eg, HACCP plans) must be in place in food preparation. As with other European countries the European Community Directives on Food Hygiene (93/43/EEC) are implemented as part of the regulations on hygiene.

The Food Sanitation Law governs food regulations in Japan and covers a full range of topics including additives and labeling and matters pertaining to chilled foods.

Codes of practice or industrial guides for chilled foods also exist in many countries and these lay out guidelines for the manufacturing of refrigerated products (24). Typical is the Chilled Foods Association (25) chilled-foods guidelines, which covers hygiene requirements, HACCP considerations, lethal rates for selected pathogens, and other relevant codes of practice. The outstanding feature of any legislation for chilled products is the temperature requirements for storage, and this often forms the only basis for local or provincial legislation.

BIBLIOGRAPHY

1. *The International Market for Chilled Foods*, Packaged Facts, New York, 1996.
2. M. H. Brown and G. W. Gould, "Processing," in C. Dennis and M. Stringer, eds., *Chilled Foods, A Comprehensive Guide*, Ellis Horwood Limited, Chichester, United Kingdom, 1992, pp. 111–146.
3. M. Waite-Wright, "Paper 1 Chilled Foods—The Manufacturer's Responsibility," *Food Science and Technology Today* 4, 223–227 (1990).
4. B. M. McKay and C. M. Derrick, "Changes in Heat Resistance of *Salmonella typhimurium* During Heating at Rising Temperatures," *Lett. Appl. Microbiol* **3**, 1316 (1987).
5. B. P. F. Day, "Chilled Food Packaging," in C. Dennis and M. Stringer, eds., *Chilled Foods, A Comprehensive Guide*, Ellis Horwood Limited, Chichester, United Kingdom, 1992, pp. 147–163.
6. J. M. Farber, "Microbiological Aspects of Modified Atmosphere Packaging Technology—A Review," *Journal of Food Protection* **54**, 58–70 (1991).
7. C. D. Gill and G. Molin, "Modified Atmospheres and Vacuum Packaging," in N. J. Russel and G. W. Gould, eds., *Food Preservatives*, Blackie and Sons, London, United Kingdom, 1991, pp. 172–199.
8. T. P. Labuza, B. Fu, and P. S. Taokis, "Prediction of Shelf Life and Safety of Minimally Processed CAP/MAP Chilled Foods: A Review," *Journal of Food Protection* **55**, 741–750 (1992).
9. A. L. Brody, "Chilled Foods Distribution Needs Improvement," *Food Technol.* **51**, 120 (1997).
10. M. Sherlock and T. P. Labuza, "Consumer Perceptions of Consumer Time–Temperature Indicators for Use on Refrigerated Products," *J. Dairy Sci.* **75**, 3167–3176 (1992).
11. M. L. Woolfe, "Temperature Monitoring and Measurement," in C. Dennis and M. Stringer, eds., *Chilled Foods, A Comprehensive Guide*, Ellis Horwood, Chichester, United Kingdom, 1992, pp. 77–109.
12. G. E. Skinner and J. W. Larkin, "Conservative Predictions of Time to *Clostridium botulinum* Toxin Formation for Use With Time–Temperature Indicators to Ensure the Safety of Foods," *Journal of Food Protection* **61**, 1154–1160 (1998).
13. R. W. Daniels, "Applying HACCP to New Generation Refrigerated Foods at Retail and Beyond," *Food Technol.* **45**, 122–124 (1991).
14. S. J. Van Garde and M. J. Woodburn, "Food Discard Practices of Households," *Journal of American Dieticians Association* **87**, 322–327 (1987).
15. L. J. Cox et al., "Listeria species in Food Processing, Non-food Processing and Domestic Environments," *Food Microbiol.* **6**, 49–61 (1989).
16. S. A. Rose, "Chilled Foods," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, Vol. 1., John Wiley and Sons, New York, 1992, pp. xx–xx.
17. V. N. Scott, "Interaction of Factors to Control Microbial Spoilage of Refrigerated Foods," *Journal of Food Protection* **52**, 431–435 (1989).
18. H. M. Brown, "Non-Microbiological Factors Affecting Quality and Safety," in C. Dennis and M. Stringer, eds., *Chilled Foods, A Comprehensive Guide*, Ellis Horwood, Chichester, United Kingdom, 1992, pp. 261–288.
19. S. Notermans et al., "A User's Guide to Microbial Challenge Testing for Ensuring the Safety and Stability of Food Products," *Food Microbiol.* **10**, 145–157 (1993).
20. W. B. McNab, "A Literature Review Linking Microbial Risk Assessment, Predictive Microbiology and Dose-Response Modeling," *Dairy, Food and Environmental Sanitation* **17**, 405–416 (1997).
21. A. C. Baird-Parker, "Use of HACCP by the Chilled Food Industry," *Food Control* **5**, 167–170 (1994).
22. L. Jaykus, "The Application of Quantitative Risk Assessment to Microbial Food Safety Risks," *Crit. Rev. Microbiol.* **22**, 279–293 (1996).
23. *Guidelines for the Handling of Chilled Food*, 2nd ed., Institute of Food Science and Technology, London, United Kingdom, 1990.
24. *Guidelines for the Development, Production, Distribution and Handling of Refrigerated Foods*, National Food Processors Association, Washington, D.C., 1989.
25. *Technical Handbook for the Chilled Food Industry*, Chilled Foods Association, Atlanta, Ga., 1990.

DAVID COLLINS-THOMPSON
JOHN M. SIDDLE
Nestlé R&D Center, Inc.
New Milford, Connecticut

CHLOROPHYLL. See COLORANTS: CHLOROPHYLLS.

CHOCOLATE AND COCOA

In the United States, chocolate and coca are standardized by the U.S. Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act. The current definitions and standards resulted from prolonged discussions between the U.S. chocolate industry and the Food and Drug Administration (FDA). The definitions and standards originally published in the *Federal Register* of December 6, 1944, have been revised only slightly.

The FDA announced in the *Federal Register* of January 25, 1989 a proposal to amend the U.S. chocolate and cocoa standards of identity. The proposed amendments respond principally to a citizen petition submitted by the Chocolate Manufacturers Association (CMA) and, to the extent practicable, will achieve consistency with the Codex standards. The proposed amendments would allow for the use of nutritive carbohydrate sweeteners, neutralizing agents, and emulsifiers; reduce slightly the minimum milkfat content and eliminate the nonfat milk solids-to-milkfat ratios in certain cocoa products including milk chocolate; update the language and format of the standards; and provide for optional ingredient labeling requirements. FDA has also received a proposal to establish a new standard of identity for white chocolate. Comments regarding the proposal amendments are under review by FDA, and a final ruling is expected to be issued in the near future.

WHITE CHOCOLATE

There is at present no standard of identity in the United States for white chocolate.

White chocolate has been defined by the European Economic Community (EEC) Directive 75/155/EEC as free of coloring matter and consisting of cocoa butter (not less than 20%); sucrose (not more than 55%); milk or solids obtained by partially or totally dehydrated whole milk, skimmed milk, or cream (not less than 14%); and butter or butter fat (not less than 3.5%).

COCOA BEANS

The cocoa bean is the basic raw ingredient in the manufacture of all cocoa products. The beans are converted to chocolate liquor, the primary ingredient from which all chocolate and cocoa products are made. Figure 1 depicts the conversion of cocoa beans to chocolate liquor, and in turn to the chief chocolate and cocoa products manufactured in the United States, ie, cocoa powder, cocoa butter, and sweet and milk chocolate.

Significant amounts of cocoa beans are produced in about 30 different localities. These areas are confined to latitudes 20° north or south of the equator. Although cocoa trees thrive in this very hot climate, young trees require the shade of larger trees such as banana, coconut, and palm for protection.

Fermentation (Curing)

Prior to shipment from producing countries, most cocoa beans undergo a process known as curing, fermenting, or sweating. These terms are used rather loosely to describe a procedure in which seeds are removed from the pods, fermented, and dried. Unfermented beans, particularly from Haiti and the Dominican Republic, are used in the United States.

Commercial Grades

Most cocoa beans imported into the United States are one of about a dozen commercial varieties that can be generally classified as Criollo or Forastero. Criollo beans have a light color, a mild, nutty flavor, and an odor somewhat like sour wine. Forastero beans have a strong, somewhat bitter flavor and various degrees of astringency. The Forastero varieties are more abundant and provide the basis for most chocolate and cocoa for formulations. The main varieties of cocoa beans imported into the United States, usually named for the country or port of origin, are Ivory Coast, Accra (Ghana), Lagos, Nigeria, Fernando Po, and Sierra Leone (from Africa); Bahia (Brazil), Arriba (Ecuador), and Venezuelan (from South America); Malaysia, New Guinea, Indonesia, and Samoa (in the Pacific); and Sanchez (Dominican Republic), Grenada, and Trinidad (in the West Indies).

Blending

Most chocolate and cocoa products consist of blends of beans chosen for flavor and color characteristics.

Production

Worldwide cocoa bean production ranged from 2.3–2.5-million t and cocoa bean production was stagnant between 1988 and 1993. Indonesia was the only country significantly expanding production. Production in Brazil and Malaysia actually dropped.

Consumption

Worldwide cocoa bean consumption increased by 14% between 1988 and 1993 from approximately 2.1 million t in the 1988–1989 crop year to almost 2.4 million t today. North America and Western Europe increased grind by approximately 26% over this time period, whereas in Russia and Eastern Europe grind dropped by 46%.

Marketing

Most of the cocoa beans and products imported into the United States are done so by New York and London trade houses. The New York Sugar, Coffee, and Cocoa Exchange provides a mechanism by which both chocolate manufacturers and trade houses can hedge their cocoa bean transactions.

CHOCOLATE LIQUOR

Chocolate liquor is the solid or semisolid food prepared by finely grinding the kernel or nib of the cocoa bean. It is also

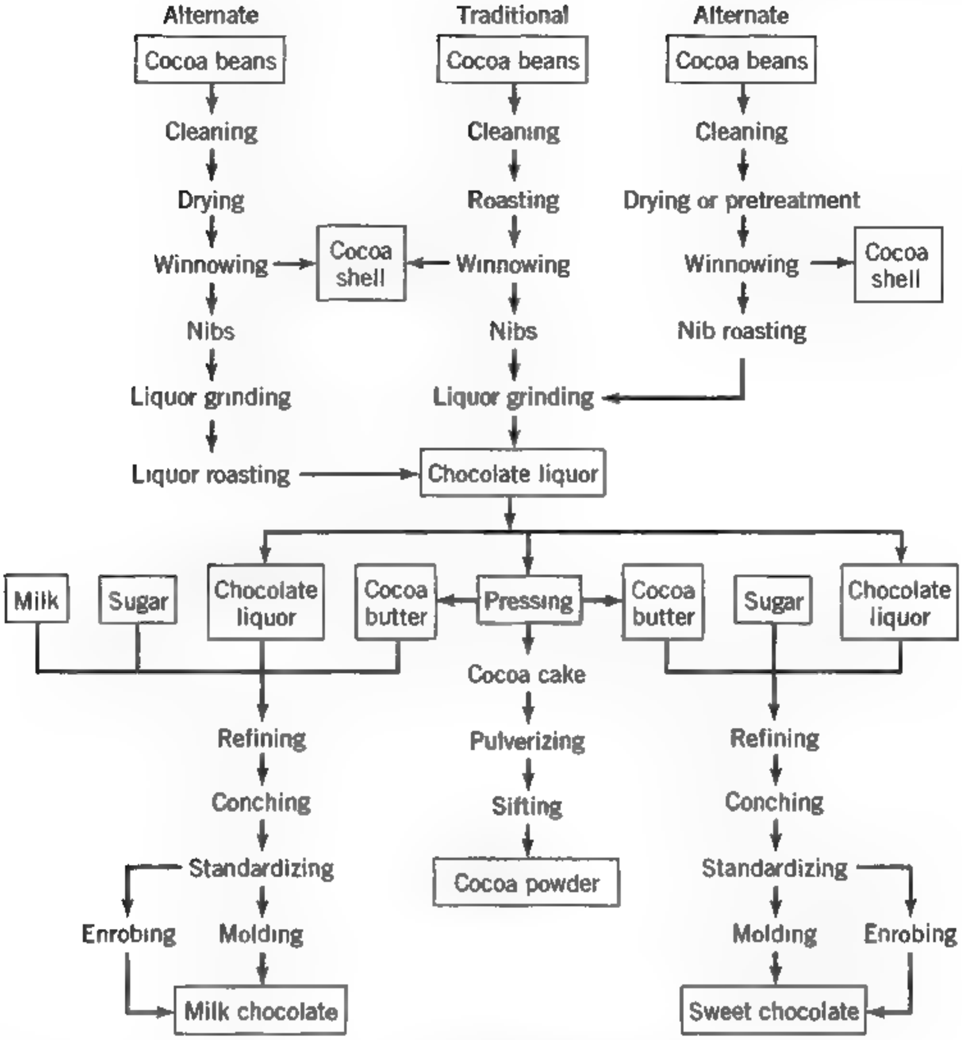

Alternate
Traditional
Alternate
Cocoa beans
Cocoa beans
Cocoa beans
Cleaning
Cleaning
Cleaning
Drying
Roasting
Drying or pretreatment
Winnowing
Cocoa shell
Winnowing
Winnowing
Cocoa shell
Nibs
Nibs
Nib roasting
Liquor grinding
Liquor grinding
Liquor roasting
Chocolate liquor
Milk
Sugar
Chocolate liquor
Cocoa butter
Pressing
Cocoa butter
Sugar
Chocolate liquor
Cocoa cake
Refining
Pulverizing
Refining
Conching
Sifting
Conching
Standardizing
Cocoa powder
Standardizing
Enrobing
Molding
Molding
Enrobing
Milk chocolate
Sweet chocolate

Figure 1. Flow diagram of chocolate and cocoa production.

commonly called chocolate, unsweetened chocolate, baking chocolate, or cooking chocolate. In Europe chocolate liquor is often called chocolate mass or cocoa mass.

COCOA POWDER

Cocoa powder (cocoa) is prepared by pulverizing the remaining material after part of the fat (cocoa butter) is removed from chocolate liquor. The U.S. chocolate standards define three types of cocoas based on their fat content. These are breakfast, or high fat cocoa, containing not less than 22% fat; cocoa, or medium fat cocoa, containing less than 22% fat but more than 10%; and low fat cocoa, containing less than 10% fat.

Cocoa powder production today is an important part of the cocoa and chocolate industry because of increased consumption of chocolate-flavored products. Cocoa powder is the basic flavoring ingredient in most chocolate-flavored cookies, biscuits, syrups, cakes, and ice cream. It is also used extensively in the production of confectionery coatings for candy bars.

COCOA BUTTER

Cocoa butter is the common name given to the fat obtained by subjecting chocolate liquor to hydraulic pressure. It is the main carrier and suspending medium for cocoa particles in chocolate liquor and for sugar and other ingredients in sweet and milk chocolate.

The FDA has not legally defined cocoa butter, and no standard exists for this product under the U.S. Chocolate Standards. For the purpose of enforcement, the FDA defines cocoa butter as the edible fat obtained from cocoa beans either before or after roasting. Cocoa butter as defined in the *U.S. Pharmacopeia* is the fat obtained from the roasted seed of *Theobroma cacao Linne*.

Composition and Properties

Cocoa butter is a unique fat with specific melting characteristics. It is a solid at room temperature (20°C), starts to soften around 30°C, and melts completely just below body temperature. Its distinct melting characteristic makes cocoa butter the preferred fat for chocolate products.

Cocoa butter is composed mainly of glycerides of stearic, palmitic, and oleic fatty acids. The triglyceride structure of cocoa butter has been determined as tri-saturated, 3%; mono-unsaturated (oleo-distearin), 22%; oleo-palmitostearin, 57%; oleo-dipalmitin, 4%; di-unsaturated (stearodiolein), 6%; palmitodiolein, 7%; and tri-unsaturated, triolein, 1%.

Although there are actually six crystalline forms of cocoa butter, four basic forms are generally recognized as alpha, beta, beta prime, and gamma.

Substitutes and Equivalents.

In the past 25 years, many fats have been developed to replace part or all of the added cocoa butter in chocolate-flavored products. These fats fall into two basic categories commonly known as cocoa butter substitutes and cocoa butter equivalents.

Cocoa butter substitutes and equivalents differ greatly with respect to their method of manufacture, source of fats, and functionality; they are produced by several physical and chemical processes. Cocoa butter substitutes are produced from lauric acid fats such as coconut, palm, and palm kernel oils by fractionation and hydrogenation; from domestic fats such as soy, corn, and cotton seed oils by selective hydrogenation; or from palm kernel stearines by fractionation. Cocoa butter equivalents can be produced from palm kernel oil and other specialty fats such as shea and illipe by fractional crystallization; from glycerol and selected fatty acids by direct chemical synthesis; or from edible beef tallow by acetone crystallization.

In the early 1990s, the most frequently used cocoa butter equivalent in the United States was derived from palm kernel oil but a synthesized product was expected to be available in the near future.

SWEET AND MILK CHOCOLATE

Most chocolate consumed in the United States is consumed in the form of milk chocolate and sweet chocolate. Sweet chocolate is chocolate liquor to which sugar and cocoa butter have been added. Milk chocolate contains these same ingredients and milk or milk solids.

U.S. definitions and standards for chocolate are quite specific. Sweet chocolate must contain at least 15% chocolate liquor by weight and must be sweetened with sucrose or mixtures of sucrose, dextrose, and corn syrup solids in specific ratios. Semisweet chocolate and bittersweet chocolate, though often referred to as sweet chocolate, must contain a minimum of 35% chocolate liquor. The three products, sweet chocolate, semisweet chocolate, and bittersweet chocolate, are often simply called chocolate or dark chocolate to distinguish them from milk chocolate.

Sweet chocolate can contain milk or milk solids (up to 12% max), nuts, coffee, honey, malt, salt, vanillin, and other spices and flavors as well as a number of specified emulsifiers. Many different kinds of chocolate can be produced by careful selection of bean blends, controlled roasting temperatures, and varying amounts of ingredients and flavors.

The most popular chocolate in the United States is milk chocolate. The U.S. Chocolate Standards state that milk chocolate shall contain no less than 3.66 wt % of milk fat and not less than 12 wt % of milk solids. In addition, the ratio of nonfat milk solids to milk fat must not exceed 2.43:1 and the chocolate liquor content must not be less than 10% by weight.

Production

The main difference in the production of sweet and milk chocolate is that in the production of milk chocolate, water must be removed from the milk. Many milk chocolate producers in the United States use spray-dried milk powder. Others condense fresh whole milk with sugar, and either dry it, producing milk crumb, or blend it with chocolate liquor and then dry it, producing milk chocolate crumb. These crumbs are mixed with additional chocolate liquor, sugar, and cocoa butter later in the process. Milk chocolates made from crumb typically have a more caramelized milk flavor than those made from spray-dried milk powder.

THEOBROMINE AND CAFFEINE

Chocolate and cocoa products, like coffee, tea, and cola beverages, contain alkaloids. The predominant alkaloid in cocoa and chocolate products is theobromine, though caffeine is also present in smaller amounts. Concentrations of both alkaloids vary depending on the origin of the beans. Published values for the theobromine and caffeine content of chocolate vary widely because of natural differences in cocoa beans and differences in analytical methodology.

NUTRITIONAL PROPERTIES OF CHOCOLATE PRODUCTS

Chocolate and cocoa products supply proteins, fats, carbohydrates, vitamins, and minerals. The Chocolate Manufacturers' Association of the United States (McLean, Virginia) completed a nutritional analysis from 1973 to 1976 of a wide variety of chocolate and cocoa products representative of those generally consumed in the United States.

ECONOMIC ASPECTS

Chocolate consumption (wholesale Dollar value) on a global basis was approximately $23 billion in 1992. In the United States, Hershey, Mars, and Nestlé control about 70% of the market.

The leading chocolate companies continue to pursue a global confectionery business strategy with an increase in the early 1990s of confectionery business activity in the Eastern Bloc countries, Russia, China, and South America. Generally, as per capita income increases, chocolate consumption increases and sugar consumption decreases. Consumer demographics, the declining child population, and the increase in consumer awareness of health issues play important roles in the economics of chocolate consumption. Chocolate confectionery business trends during the early 1990s include product down-sizing leading to snack size finger foods, increased emphasis on specialty chocolates with concentration on dessert chocolates, and chocolate brand equity spread into beverages, baked goods, frozen novelties, and even sugar confections.

GENERAL REFERENCES

W. T. Clarke, *The Literature of Cacao*, ACS, Washington, D.C., 1954.

L. R. Cook, *Chocolate Production and Use*, Magazines for Industry, Inc., New York, 1972.

B. W. Minifie, *Chocolate, Cocoa, and Confectionery: Science and Technology*, AVI, Westport, Conn., 1970.

E. M. Chatt, in Z. J. Kertesz, ed., *Economic Crops*, Vol. 3, Interscience Publishers, Inc., New York, 1953, p. 185

B. L. ZOUMAS
J. F. SMULLEN
Hershey Foods Corporation
University Park, Pennsylvania

CITRUS. See FRUITS, SEMI-TROPICAL; FRUITS, TROPICAL.

CLEANING-IN-PLACE (CIP)

From the time of its early use in the 1950s, the practice of cleaning-in-place (CIP) for cleaning of plants processing potable liquids and other products such as ice cream and butter has become widespread and is now considered an established cleaning technique (1,2). The technique of CIP stands for cleaning of the tanks, pipelines, processing equipment, and process lines by circulation of water and chemical solutions (hereafter referred to as solution) through them. The term CIP or cleaning-in-place emphasizes that the technique does not require dismantling of pipelines or equipment, which was the case with manual cleaning. Manual cleaning was extremely time-consuming and expensive, and often the level of hygiene (bacteriologic cleanliness) achieved through it was low and inconsistent (2). Introduction of CIP, which became inevitable in the face of economic pressures to increase throughput, increasing cost of labor, scarcity of labor, and technical developments by equipment manufacturers and detergent chemists, alleviated the problems associated with manual cleaning (2,3). Three forms of energy—chemical, kinetic, and thermal—are generally needed for any cleaning operation. In comparison to manual cleaning, higher temperatures and stronger chemical (detergent) concentrations can be used in CIP. Solution temperatures up to 88°C and detergent pH up to 13 can be used in CIP (4). The equipment design and the properties of the material to be cleaned impose limits on temperature and strength of solution. Lower temperatures may have to be used if product residue (soil on product contact surface) becomes harder to clean at higher temperatures. Manual brushing, which contributes a great deal in manual cleaning, is totally eliminated in CIP and, in some sense, is replaced by the kinetic energy of turbulent flow in pipelines or impingement of jets in vessels (1,4). The circulation time is also a factor in cleaning, and an increase in the time, within a limit, improves the cleaning achieved. Circulation time from 5 min to 1 h is used in practice (5). One of the most important though less noticeable advantages of CIP over manual cleaning is the fact that CIP provides freedom in plant design from the severe limitation of keeping the plant manually cleanable and hence helps in development of new processes and ideas (3). The use of CIP has also made it possible and convenient to automate the cleaning operations in a plant where it was impossible in the case of manual cleaning. The sequence or cycle of operations in any cleaning are the same regardless of cleaning technique (5). A normal cleaning sequence consists of prerinsing with water, washing with detergent solution, and postrinsing with clean water. In addition, there may be a disinfection (sanitizing) stage followed by a final water rinse. Prerinsing is an important stage. It should be started as soon as possible and should continue until the discharging water is free from product residue (soil).

REQUIREMENTS FOR CIP

General Requirements

Only a plant that is properly designed to be cleaned by CIP can be cleaned by CIP efficiently (4). The design of a plant that is to be cleaned in place and the design of the equipment and system that is to apply the CIP technique should be compatible. The CIP system should be designed as an integral part of the processing plant; modifying the plant later for CIP may pose problems (4). All product contact surfaces must be accessible to the pre- and post-rinse water and solution. The material that comes in contact with cleaning solution must be able to withstand the solution at its concentration and temperature. For this reason, most of the construction material is stainless steel.

Requirements for Pipelines

There should be no crevice condition, especially in the pipe joints. Crevice condition can lead to bacterial trap, which may be impossible to clean by CIP (6). As far as possible, pipelines should have welded joints. Proper drainage of prerinse water is important in CIP system to avoid any dilution or cooling of solution. To provide the drainage, all piping should have a minimum fall (pitch or slope) of 1:100. Pipe work should also have good support to prevent the pipes from sagging. Any sags in the pipeline would prevent complete drainage and put strain on the pipe joints. Uncleanable dead pockets must be avoided inside the pipeline. This may not be achieved, if CIP is added to the plant as an afterthought instead of being an integral part (2). A mean flow rate of 1.5 m/s is normally recommended, but a mean solution velocity of 1.0 m/s may be sufficient in some cases. In practice, there is not much gain in exceeding the mean velocity beyond 2.0 m/s (4). Volume flow rate would depend on the diameter of the pipe. Higher flow rates create a higher hydraulic pressure drop, hence power requirements of circulating pumps could be considerably larger. Abrupt changes in the diameter of pipes that disturb the flow and reduce the cleaning efficiency should be avoided (6).

Requirements for the Vessels and Tanks

The cleaning-in-place of storage tanks or vessels is performed by spraying the cleaning solution onto the surface of the vessel through pressure spray devices located inside the vessel. The spray devices may be rotating, oscillating, or fixed. The fixed device, which is in the form of a perforated ball, is used most commonly. The fixed or static spray ball device does not have any moving parts and therefore gives trouble-free operation with minimal maintenance. Rotating or oscillating devices may wear and give a distorted spray pattern. They may also jam or stick in one place with the consequence of incomplete cleaning of the vessel. A single rotating or oscillating jet, however, can clean a larger-sized vessel than can a single static spray ball. The installation of the spray device should result in a spray pattern that must always cover all parts of the vessel, including probes, agitators, and areas shadowed by them. If required, more than one static spray ball should be used for total coverage in the vessel. Suitable filters should be used to prevent blockage of the spray device. Spray devices should be run at designed pressure and throughput. Too high a pressure can cause atomization of the solution and too low a pressure will reduce the force of jet impingement—both resulting in unsatisfactory cleaning. Permanently installed spray devices are commonly used, but removable spray devices may be preferred in certain special circumstances. Adequate venting of vessels is extremely important to avoid the collapse of the vessel due to a vacuum created during in-place-cleaning when a cold-water rinse immediately follows a wash period with hot detergent. The vessel and the supply and return CIP lines to it should have adequate drainage, otherwise undrained prerinse water can dilute and cool the solution, hence resulting in unsatisfactory cleaning.

Cleaning Circuits

Factory installations as a whole are generally divided into a number of circuits that can be cleaned at different times by CIP technique. It is a usual practice to group pipelines, vessels, and special equipment such as heat exchangers and evaporators into different cleaning circuits because of their different cleaning requirements with respect to flow rates, pressures, and chemicals (1). The product residue deposit (soil) should be of the same kind in a circuit, and all components of the circuit must be available for cleaning at the same time. Hot and cold lines of the plant may be placed in different circuits. After the introduction of automation, it has become practical to clean some parts of the plant while production continues in the adjacent areas. In such circumstances, it is necessary to prevent contamination of product by the cleaning solution. In recent years, double seat valves with the internal leakage drains being used to safeguard against such contamination (2).

TYPES OF CIP SETS/SYSTEMS

CIP sets can be categorized as centralized, local, and satellite or decentralized. In a centralized system, the various CIP circuits are connected by a network of pipes to one or two central CIP stations or units, which consist of all necessary equipment for storage and monitoring of cleaning fluids (water for rinse and solution). Large capacity water and detergent tanks (13,600 to 27,700 L) are used in centralized CIP (4). The system may work well except in the case of large processing plants where long pipe runs require large pump capacities and excessive energy use owing to heat losses. Also, the longer pipelines may contain some water after the prerinse operation that can dilute the detergent solution (7). Overcoming this problem increases chemical consumption. Another disadvantage of a centralized system is the fact that total reliance for cleaning the plant is placed on one or two central stations or units (4). In case of failure or malfunctioning of the central units, the whole plant may have to be shut down. In contrast to the centralized system, the local system requires a greater number of smaller tanks and pumps and shorter pipe runs. The system is more reliable. If one local unit fails or malfunctions, the cleaning of the plant not served by that local unit can still be achieved. The local system would require a greater number of heating units and detergent-strength (concentration) controllers (4). The satellite system is a combination of central and local systems. In the satellite system, there are central solution tanks and the local units draw the required volume of solution using properly sized pipes from the central tanks. The heating of the solution is arranged locally (4). Large modern food-processing plants generally employ a satellite system (7) because of the advantages of saving energy, water, and detergent.

The CIP systems are also classified as single-use, reuse, or multiuse systems, depending on whether the same cleaning solution is used for one, many, or few cycles of cleaning (2). The single-use system is most suited for cleaning heavy soil loads (such as in thermal processing equipment) or cleaning of small plants (8). The system uses the minimum amount of detergent needed for cleaning a circuit and discharges the solution to the sewer after one cleaning cycle is over. The system may be wasteful of detergent and energy and can cause effluent problems (4). However, it is simple in installation and operation. By contrast, the reuse system is complex in installation and operation. The reuse system provides for the reclamation and reuse of the cleaning solution and final rinse water—the latter is used as prerinse for the next cleaning cycle. More detergent may be added to the solution between the cleaning cycles to counteract the loss of detergency due to expending chemical energy to remove the soil. The solution is discharged to the sewer when it becomes very dirty. The reuse systems require greater capital expenditure but offer savings on volume of water and detergent solutions and energy used (4). Greater benefits can be derived from the reuse systems in plants that have light soil loads and use large-diameter pipe circuits (especially in the brewing industry) (8). The multiuse system is a compromise between the single-use and reuse systems. The final rinse water and solution are used for a few cleaning cycles before discharging to the sewer. In terms of capital and operating costs and complexity of the installation and operation, it is between single-use and reuse systems. Many factors: type and size of plant to be cleaned, soil loading, range and type of chemicals available, pressure drop in pipeline circuits, and pressure and throughput required for vessel spray device, should be considered before selecting the type and size of the CIP system.

CONTROL SYSTEM

Control systems for operating CIP must try to perform CIP operations in a precise sequence. In the early CIP control systems, pumps and valves were controlled manually and time was kept using a stopwatch. Overdependence on the human element prevented the precise sequencing in early control systems (4,9). As the CIP control system evolved with time, the use of electromechanical relays became common. The relays were used to perform the automatic sequencing, but the system had an inherent ability to fail at the wrong time (9). The present-day CIP control systems are microprocessor-based. The development of inexpensive and reliable microprocessors has accelerated the use of microprocessor-based control systems in CIP. Control systems for CIP have improved a great deal in the past 40 years from the unreliable to a very reliable and flexible microprocessor-based controls where cycle time, chemical concentration, and temperature are fully adjustable to suit individual cleaning needs (10).

The center of any microprocessor-based control system is a process controller containing a central processing unit (CPU), memory, and an input/output (I/O) interface. Input—output devices provide the operator access to the control system. The CPU accesses the operator-oriented equipment (such as pushbuttons, pilot lights, rotary switches, thumbwheel switches, LED readouts, CRT screens, and printers) and process-oriented equipment (such as pump motors, valve solenoids, pressure sensors, level probes, flow meters, and temperature switches) through the I/O devices. An operating logic, which may originate as a verbal description or an algorithm, describes the interaction and coordination among the operator-oriented equipment, the process-oriented equipment, and the control system. The operating logic or computer program can be prepared from the algorithm. The CPU executes the operating logic or the program. Because the controller's actions are determined by the computer program, any configuration of equipment and/or program sequence can be implemented and controlled by such a control system. The application of microprocessor-based control systems has helped in making the modern food-processing plants and associated CIP systems completely automatic (7,9,10). With precisely controlled operations, a more sophisticated CIP unit design has become possible, resulting in increased savings on energy, water, and cleaning chemicals (10). The microprocessor-based control systems have provided new opportunities for modifying and improving CIP operations and concepts.

Even very reliable modern control systems can malfunction; therefore, regular routine checks and maintenance are necessary (1,4).

ENERGY AND COST CONSIDERATIONS

Heat energy required for CIP operations constitutes 99% of the energy requirement (11). Heat energy needed is related to cleaning temperature and hence energy savings can be achieved by reducing the cleaning temperature. However, this would necessitate an increase in the detergency requirement of the solution and/or cleaning time. The most energy efficient method, therefore, may not be the most cost effective method. Cost and energy calculations are required in several plants for combinations of time, temperature, and strength of detergent to achieve a standard level of bacteriologic cleanliness before any meaningful conclusion can be reached (11).

Type of plant to be cleaned, CIP system used, mechanical components, control system, building construction, and plant installation would all influence the capital cost associated with CIP (12). However, it is the operating cost that becomes the major factor in making decisions about the CIP system to be used (12). Cleaning chemicals, water supply, required heat energy, operating labor, effluent discharge, maintenance, and electric power would all contribute to the operating cost. The satellite or decentralized system may be the best option in economic terms (12).

BIBLIOGRAPHY

1. D. A. Timperley, "Cleaning in Place (CIP)," *Journal of the Society of Dairy Technology* **42**, 32–33 (1989).
2. N. P. B. Sharp, "CIP System Design and Philosophy," *Journal of the Society of Dairy Technology* **38**, 17–21 (1985).
3. J. R. Franklin, "The Concept of CIP," in proceedings of a seminar in Melbourne, Australia, June 3–4, 1980.
4. W. Barron, "A Practical Look at CIP," *Dairy Industries International* **49**, 34–34 (1984).
5. R. K. Guthrie, *Food Sanitation*, AVI Publishing Co., Inc., Westport, Conn., 1980.
6. J. Farmer, "Engineering Design for CIP," in proceedings of a seminar in Melbourne, Australia, June 3–4, 1980.
7. "Cleaning of Dairy Equipment," *Dairy Handbook*, Alfa-Laval, S-221 03 Lund, Sweden, pp. 307–318.
8. P. F. Davis, "Single Use, Re-Use and Multi-Use CIP systems," in proceedings of a seminar in Melbourne, Australia, June 3–4, 1980.
9. A. L. Foreshew, "Control Systems for Operating CIP," in proceedings of a seminar in Melbourne, Australia, June 3–4, 1980.
10. J. Hyde, "State-of-the-Art CIP/Sanitation Systems," *Dairy Record* **84**, 101–105 (1983).
11. C. C. Sillett, "Energy Aspects of Cleaning-in-Place," *Journal of the Society of Dairy Technology* **35**, 87–91 (1982).
12. G. F. Taylor, "The Economics of CIP," in proceedings of a seminar in Melbourne, Australia, June 3–4, 1980.

D. S. Jayas
P. Shatadal
S. Cenkowski
University of Manitoba
Winnipeg, Manitoba, Canada

COCONUTS. See Nuts in the Supplement section.

COFFEE

Coffee, originally a wild plant from East Africa, was presumably first cultivated by the Arabians in about 575 A.D. (1). By the sixteenth century, it had become a popular drink in Egypt, Syria, and Turkey. The name coffee derives from the Turkish pronunciation *kahveh* of the Arabian word *gahweh*, signifying an infusion of the bean. Coffee was introduced as a beverage in Europe early in the seventeenth century and its use spread quickly. In 1725 the first coffee plant in the Western Hemisphere was planted on Martinique in the West Indies. Its cultivation expanded rapidly, and its consumption soon gained the wide acceptance it enjoys today.

MODERN COFFEE PRODUCTION

Commercial coffees are grown in tropical and subtropical climates at altitudes up to roughly 1800 m; the best grades are grown at high elevations. Most individual coffees from different producing areas possess characteristic flavors. Commercial roasters obtain preferred flavors by blending varieties before or after roasting. Colombian and washed Central American coffees are generally characterized as mild, winey-acid, and aromatic; Brazilian coffees as heavy body, moderately acid, and aromatic; and African robusta coffees as heavy body, neutral, slightly acid, and slightly aromatic. Premium coffee blends contain higher percentages of Colombian and Central American coffees.

ECONOMIC IMPORTANCE OF COFFEE

Coffee, a significant factor in international trade for about 185 years, is among the leading agricultural products in international trade along with wheat, corn, and soybeans. The total world exportable production of green coffee in the 1997–1998 growing season is approximately 81.7 million bags (Table 1). This compares with a total production of about 107.5 million bags, the difference being internal consumption. Table 2 shows imports and consumption data.

Table 1. Green Coffee Production and Exportable Product 1997–1998

	Million of 60-kg bags[a]	
Principal countries	Production	Exportable product
Brazil	35.8	23.3
Colombia	11.0	9.4
Indonesia	7.0	4.6
Vietnam	5.8	5.5
Mexico	5.6	4.6
Ivory Coast	4.1	4.0
Uganda	3.8	3.7
Ethiopia	3.7	2.1
India[b]	3.5	2.6
Guatemala	3.1	2.8
Honduras	2.6	2.4
Costa Rica	2.2	1.9
El Salvador	2.0	1.8
Total	*107.5*	*81.7*

[a] 1996–1997 figures used because 1997–1998 data were not yet available.
Source Ref. 2.

Table 2. Imports 1997 and Consumption 1995–1996 of Green Coffee

	Million bags[a]	
Principal countries	Imports	Consumption
United States	18.1[b]	18.1
Germany	13.6	9.7
France	6.7	5.6
Japan	5.6	6.4
Italy	5.6	4.9
Spain	3.6	3.0
Netherlands	3.3	2.4
UK	3.1	2.4
Total	*82.8*	

[a] 60-kg bags.
[b] Total 1997 U.S. coffee imports including green, roasted, and soluble coffee were equivalent to 20.2 million bags of green coffee.
Source: Ref. 4.

The International Coffee Organization and Association of Coffee Producing Companies assign export quotas to their members to help stabilize coffee pricing. Composite U.S. prices for green coffees in 1997 and 1998 ranged between $2.20/kg and $3.97/kg. For Colombians, the range was $3.22/kg to $5.82/kg, whereas for robustas it was $1.49/kg to $2.11/kg. These compare with a peak of about $6.60/kg in April 1977 following a disastrous frost in Brazil in 1975.

PROCESSING AND PACKAGING

Green Coffee Processing

The coffee plant is a relatively small tree or shrub, often controlled to a height of 2 to 3 m, belonging to the family Rubiaceae. *Coffea arabica* accounts for 70%, *Coffea robusta*, 29%, and *Coffea liberica* and others, 1% of world production. Arabustas, hybrid coffees that combine many disease-resistant characteristics of robustas with many favorable flavor qualities of arabicas, are also produced. Each species includes several varieties. After the spring rains, the plant produces white flowers. About six months later, the flowers are replaced by fruit, approximately the size of a small cherry. The ripe fruit is red or purple. The outer portion of the fruit is removed by curing; yellowish or light green seeds, the coffee beans, remain. They are covered with a tough parchment and a silvery skin known as the spermoderm. Each cherry normally contains two coffee beans.

Curing is effected by either the dry or wet method. The dry method produces so-called natural coffees; the wet method, washed coffees. The latter coffees are usually more uniform and of higher quality.

Dry curing is used in most of Brazil and in other countries where water is scarce in the harvesting season. The ripe cherries are spread on open drying ground and turned frequently to permit thorough drying by the sun and wind.

Sun drying usually takes two to three weeks depending on weather conditions. Some producing areas use, in addition to the sun, hot air, indirect steam, and other machine-drying devices. When the coffee cherries are thoroughly dry, they are transferred to hulling machines that remove the skin, pulp, parchment shell, and silver skin in a single operation.

In wet curing, freshly picked coffee cherries are fed into a tank for initial washing. Stones and other foreign material are removed. The cherries are then transferred to depulping machines that remove the outer skin and most of the pulp. However, some pulp mucilage clings to the parchment shells that encase the coffee beans. Fermentation tanks, usually containing water, remove the last portions of this pulp. Fermentation may last from 12 h to several days. Because prolonged fermentation may cause development of undesirable flavors and odors in the beans, some operators use enzymes to accelerate the process.

The beans are subsequently dried either in the sun, in mechanical dryers, or in combination. Machine drying continues to gain popularity, in spite of higher costs, because it is faster and independent of weather conditions. When the coffee is thoroughly dried, the parchment is broken by rollers and removed. Further rubbing removes the silver skin to produce ordinary green unroasted coffee containing about 12 to 14% moisture.

Coffee prepared by either the wet or dry method is machine graded into large, medium, and small beans by sieves, oscillating tables, and airveyors. Damaged beans and foreign matter are removed by handpicking, machine separators, electronic color sorters, or a combination of these techniques. Commercial coffee is graded according to the number of imperfections present—black beans, damaged beans, stones, pieces of hull, or other foreign matter. Processors also grade coffee by color, roasting characteristics, and cup quality of the beverage.

Chemical Composition of Green Coffee

Coffee varies in composition according to the type of plant, region from which it comes, altitude, soil, and method of handling the beans. As shown in Table 3, differences are greater between species, for example, arabica versus robusta (African), than within the same species grown in different regions, for example, Colombian versus Brazilian arabicas.

Lower oil, trigonelline, and sucrose contents are typical of robusta beans, as is a higher caffeine content. Green coffee contains little reducing sugar but a considerable quantity of carbohydrate polymers. The polymers are mainly mannose with varying percentages of glucose, arabinose, and galactose. Table 4 summarizes the polysaccharide composition of different sources of green coffee (4).

Effects of Roasting on Major Components

Green coffee has no desirable taste or aroma; these are developed by roasting. Many complex physical and chemical changes occur during roasting, including the obvious change in color from green to brown, and a large increase in bean volume. As the roast nears completion, strong exothermic reactions produce a rapid rise in temperature, usually accompanied by a sudden expansion, or puffing, of the beans, with a volume increase of 50 to 100%. However, this behavior varies widely among coffee varieties because of differences in composition and physical structure.

Table 5 shows the most significant and well-established chemical changes that occur in green coffee as a result of roasting. The principal water-soluble constituents of green coffee are protein, sucrose, chlorogenic acid, and ash, which together account for 70 to 80% of the water-soluble solids. Most sucrose disappears early in the roast. Reducing sugars are apparently formed first and then react rapidly so that the total amount of sugar decreases as the roast nears completion. The sugar reactions, dehydration and polymerization, form high molecular weight water-soluble and water-insoluble materials. Formation of carbon dioxide and other volatile substances as well as the loss of reaction-formed water account for most of the dry-weight roasting loss. These losses range from 2 to 5% of green bean weight for light-roast coffees used in the United States and northern Europe to up to 8 to 11% for dark roasts used in southern Europe.

Roasting essentially insolubilizes the proteins, which constitute 10 to 12% of green coffee, and 20 to 25% of the fraction soluble in cold water. The flavor and aroma of roasted coffee are probably due in large part to breakdown and interaction of the amino acids derived from these proteins. Analyses of the amino acids present after acid hydrolysis in both green and the corresponding roasted coffee show marked decreases in arginine, cysteine, lysine, serine, and threonine in Colombian and Angola robusta types after roasting. The amounts of glutamic acid and leucine for both coffee types, and in the robusta, phenylalanine, proline, and valine increase with roasting (5). Cysteine is the probable source of the many sulfur compounds found in the coffee aroma.

About 15 to 40% of the trigonelline is decomposed during roasting. Trigonelline is a probable source of niacin, which reportedly increases during roasting, and of potent

Table 3. Typical Analyses of Green Coffee Types

Variety	H_2O	Oil	Total nitrogen	Ash	Caffeine	Chlorogenic acid[a]	Trigonelline	Protein	Reducing sugar	Sucrose	Total carbohydrate
African robusta	11.5	7.0	2.5	3.8	2.06	4.7	0.76	11.4	0.40	4.2	35.0
Colombian arabica	13.0	13.7	2.1	3.4	1.10	4.1	0.94	10.5	0.17	7.2	34.1
Brazilian arabica	11.0	14.3	2.2	4.1	1.01	4.1	1.24	11.1	0.27	7.1	32.0

[a] Chlorogenic acid values vary somewhat with the method of analysis used.

Source Ref. 3.

Table 4. Polysaccharide Analysis of Green Coffee Beans Polysaccharide Content (wt%)

Variety	Arabinose	Mannose	Glucose	Galactose	Total
India robusta	4.1	21.9	7.8	14.0	48.2
Ivory coast robusta	4.0	22.4	8.7	12.4	48.3
Sierra Leone robusta	3.8	21.7	8.0	12.9	46.9
El Salvador arabica	3.6	22.5	6.7	10.7	43.5
Colombia arabica	3.4	22.2	7.0	10.4	43.0
Ethiopia arabica	4.0	21.3	7.8	11.9	45.0

Note. Polysaccharide content is expressed as anhydro-sugars. Wt% is on dry basis

Table 5. Average Composition of Green and Roasted Coffee

Constituents	Green, percent dry basis	Roasted, percent dry basis[a]
Hemicelluloses	23.0	24.0
Cellulose	12.7	13.2
Lignin	5.6	5.8
Fat	11.4	11.9
Ash	3.8	4.0
Caffeine	1.2	1.3
Sucrose	7.3	0.3
Chlorogenic acid	7.6	3.5
Protein (based on nonalkaloid nitrogen)	11.6	3.1
Trigonelline	1.1	0.7
Reducing sugars	0.7	0.5
Unknown	14.0	31.7
Total	*100.0*	*100.0*

[a] Not corrected for dry-weight roasting loss.

aromatic nitrogen ring compounds, such as pyridine, found in roasted coffee aroma. However, pyrazines, oxazoles, and thiazoles, also components of the coffee aroma, are probably products of protein breakdown. Caffeine is relatively stable, and only small amounts are lost by sublimation during roasting.

The chlorogenic acids—3-caffeoylquinic acid (cholorogenic acid), 4-caffeoylquinic acid (cryptochlorogenic acid), and 5-caffeoylquinic acid (neochlorogenic acid)—occur at least in part on potassium-caffeine-chlorogenate complexes. They decompose in direct relationship to the degree of roast. Table 6 shows the changes that occur in these acids during roasting. Apparently, chlorogenic acids modify and control reactions that occur during the roast and are particularly important to the decomposition of sucrose.

Glycerides of linoleic and palmitic acids, along with some glycerides of stearic and oleic acids, make up the 7 to 16% fat content of coffee. Some cleavage of glycerides and some loss of unsaponifiables occur during roasting. Table 7 details these losses (7).

Aroma

Combinations of advanced chromatography and mass spectra analytical techniques have advanced the identification of volatile flavor components in roasted coffee to more than 800. Although present in minute quantities, they are extremely significant to the balance of flavor in a cup of coffee. A historical account of this work has been published (8). A partial summary of the volatile components by chemical class is in Table 8.

Freshly roasted ground coffee rapidly loses its fresh character when exposed to air, and within a few weeks develops a noticeable stale flavor. The mechanism for the development of staling is not known but is believed to be caused by an oxidation reaction that can be catalyzed by increasing levels of moisture (9).

Table 6. Changes in Chlorogenic Acids during Roasting by Percentage

	Santos				Colombians			
Acid	Green	Light roast	Medium roast	Dark roast	Green	Light roast	Medium roast	Dark roast
Chlorogenic acid	5.56	2.90	1.96	1.11	3.77	2.74	2.16	0.93
Neochlorogenic acid	0.88	1.59	1.02	0.63	0.60	1.53	1.16	0.49
Isochlorogenic acid	0.41		0.24					

Source: Ref. 6.

Table 7. Characteristics of Oil from Green and Roasted Coffee by Percentage

	Green			Roasted		
Variety	Oil	FFA[a]	Unsaponifiables	Oil	FFA[a]	Unsaponifiables
Santos	12.77	0.78	6.56	16.05	2.70	6.10
Robusta (Indonesia)	9.07	1.00	6.40	11.27	1.99	5.65

[a] As oleic; FFA = free fatty acid.

Table 8. Aromatic Components of Roasted Coffee

Hydrocarbons	72
Alcohols	20
Aldehydes	29
Ketones	70
Acids	22
Esters	30
Lactones	9
Amines	22
Sulfur thiols	13
Phenols	44
Furans[a]	115
Thiophenes	30
Pyrroles[a]	74
Oxazoles[a]	29
Thiazoles	27
Pyridines[a]	16
Pyrazines[a]	82
Others	11

[a] Includes benzoderivatives.

ROASTING TECHNOLOGY

The main processing steps in the manufacture of roasted coffee are blending, roasting, grinding, and packaging.

Green coffee is shipped in bags weighing from 60 to 70 kg. Prior to processing, the green coffee is dumped and cleaned of string, lint, dust, hulls, and other foreign matter. Coffees from different varieties or sources are usually blended before or after roasting.

Roasting is usually carried out batchwise by contacting beans with hot combustion gases in rotating cylinders. Continuous roasting using long cylinders fitted with helical conveyor flights is also employed. The beans absorb heat at a fairly uniform rate, and most moisture is removed during the first two-thirds of this period. As the temperature of the coffee increases rapidly during the last few minutes, the beans swell and unfold with a noticeable cracking sound, like that of popping corn, indicating a reaction change from endothermic to exothermic. This stage is known as development of the roast. The final bean temperature, 200 to 230°C, is determined by the blend, variety, or flavor development desired. A water or air quench terminates the roasting reaction. Most, but not all, of any added water is evaporated from the heat of the beans.

Theoretically, about 700 kJ of heat are needed to roast a kilogram of coffee beans. However, the hot gas-recirculation rate determines the thermal efficiency achieved. Older roasters that do not use recirculation may have an efficiency rate as low as 25%, requiring as much as 2800 kJ. Roasters with high levels of gas recirculation have an efficiency rate of 75% or more, requiring about 930 kJ. Volatile organic compounds in roaster discharge gases and smoke have to be reduced to tolerable levels. Some reduction can be effected by partial recycling of roaster gas, but to achieve adequate reduction, discharged roaster gas must be passed through an afterburner.

Most roasters are equipped with controls that regulate the temperature of the hot gas used and automatically quench the roast when a chosen end-of-roast bean temperature is reached. The chosen end-of-roast bean temperature correlates well with the desired color of ground roast coffee as measured by photometric reflectance. Roasts are stopped by diverting the heating gas, injecting a controlled amount of cold water, and blowing room-temperature air through the beans. Control systems introduced in the 1990s provide improved control of roasting reactions and reproducible development of coffee flavor by controlling bean temperature versus time behavior during roasting (10).

Conventional roasting by hot combustion gases in rotating cylinders requires 8 to 15 min. Fast roasting technologies patented in the 1980s develop roast flavor in under 5 min, sometimes in 1 to 3 min (11,12).

Fast roasting, whether carried out in a batch or continuous roaster, is achieved by increasing rates of heat transfer into beans. This may be done by using much more heated gas than used conventionally. The heated gas may flow upward through a bed of beans, fluidizing it. The bed may also be fluidized by hot gas reflected upward from a solid surface after issuing from downward-pointed jets. Rapidly rotating paddles and centrifugal devices that throw beans through heated gas are also used to improve heat transfer to beans. Recirculative lifting of beans by heated gas in a spouted bed is also used. When much larger quantities of gas are used to transfer heat to beans, the gas temperature can be lower, that is, 250 to 310°C compared with 425 to 550°C.

Fast-roasted coffees are less dense, retain more chlorogenic acid, free acid, moisture, and carbon dioxide than conventionally roasted coffees and release 20% more soluble solids during home brewing. It is further claimed that the lower roasting temperature results in higher aroma retention.

Air must be circulated through the beans to remove excess heat before the finished and quenched roasted coffee is conveyed to storage bins. Residual foreign matter, such as stones and tramp iron, which may have passed through the initial green coffee cleaning operation, must be removed before grinding. This is accomplished by an air-lift adjusted to such a high velocity that the roasted coffee beans are carried over into bins above the grinders, and heavier impurities are left behind. The coffee beans flow by airveying or by gravity to mills where they are ground to the desired particle size.

Small and intermediate-size coffee roasters, after virtually disappearing from the United States, have staged a marked comeback. Now, some 1200 to 1500 small, custom roasters roast roughly 12% of the coffee consumed in the United States.

Grinding

Roasted coffee beans are ground to improve extraction efficiency in the preparation of the beverage. Particle size distributions ranging from about 1100 μm average (very coarse) to about 500 μm average (very fine) are tailored by the manufacturer to the various kinds of coffee makers used in households, hotels, restaurants, and institutions.

Finer grinds are provided for espresso coffee (300 to 400 μm), and Turkish coffee ($<100\ \mu$m).

Most coffee is ground in mills that use multiple steel cutting rolls to produce the most desirable uniform particle size distribution. After passing through cracking rolls, the broken beans are fed between two more rolls, one of which is cut or scored longitudinally, the other, circumferentially. The paired rolls operate at controlled speeds to cut, rather than crush, the coffee particles. A second pair of more finely scored rolls, installed below the main grinding rolls and running at higher speeds, is used for finer grinds. A normalizer section is then used to distribute particles uniformly

Packaging

Most roasted and ground coffee sold directly to consumers in the United States is vacuum packed in metal cans; 0.45, 0.9, or 1.35 kg are optional, although other sizes have been used to a limited extent. After roasting and grinding, the coffee is conveyed, usually by gravity, to weighing-and-filling machines that achieve the proper fill by tapping or vibrating. A loosely set cover is partially crimped. The can then passes into the vacuum chamber, maintained at about 3.3 kPa (25 mmHg) absolute pressure, or less. The cover is clinched to the can cylinder wall, and the can passes through an exit valve or chamber. This process removes 95% or more of the oxygen from the can. Polyethylene snap caps for reclosure are placed on the cans before they are stacked in cardboard cartons for shipping. A case usually contains 10.9 kg of coffee, and a production packing line usually operates at a rate of 250 to 350 0.45-kg cans per minute.

Though slight losses in fresh roasted character occur due mostly to chemical reactions with the residual oxygen in the can and previous exposure to oxygen prior to packing (9), vacuum-packed coffee retains high quality for at least one year.

Coffee vacuum packed in flexible, bag-in-box packages has gained wide acceptance in Europe and the United States. The inner liner, usually a preformed pouch of plastic-laminated foil, is placed in a paperboard carton that helps shape the bag into a hard brick form when vacuum is applied (13). The carton also protects the package from physical damage during handling and shipping. This type of package provides a barrier to moisture and oxygen as good as that of a metal can.

Inert gas flush packing in plastic-laminated pouches, although less effective than vacuum packing, can remove or displace 80 to 90% of the oxygen in the package. These packages offer satisfactory shelf life and are sold primarily to institutions.

Some coffee in the United States, and an appreciable amount in Europe, is distributed as whole beans, which are ground in the stores or by consumers in their homes. Whole-bean roasted coffee remains fresh longer than unprotected ground coffee and retains its fresh roasted flavor for several days longer than ground.

Roasted whole beans are often packed in bags that contain a one-way valve to allow escape of carbon dioxide gas produced during roasting and prevent air from entering package.

Modified Coffees

Coffee substitutes, which include roasted chicory, chickpeas, cereal, fruit, and vegetable products, have been used in all coffee-consuming countries. Although consumers in some locations prefer the noncoffee beverages, they are generally used as lower-cost beverage sources, rather than as coffee.

Chicory is harvested as fleshy roots, which are dried, cut to a uniform size, and roasted. Chicory contains no caffeine and, on roasting, develops an aroma compatible with that of coffee. It gives a high yield, about 70%, of water-soluble solids with boiling water and can also be extracted and dried in an instant form. Chicory extract has a darker color than does normal coffee brew (14). The growing technology for the processing and use of roasted cereals and chicory is evidenced by the introduction on the market of coffees extended with these materials.

Roughly 2% of the coffee sold in the United States contains added flavors, for example, vanilla, chocolate, and nut flavors. These coffees are manufactured by spraying flavors dissolved in alcohol on roasted beans and drying the beans.

BREWING

Roast and ground coffee is brewed by a wide variety of methods. In most cases, hot water is caused to percolate though a shallow bed of ground coffee supported by a porous surface that retains grounds. Steam-bubble-induced flow may be used to repeatedly apply hot water or hot partly brewed coffee to a bed of relatively coarse grounds, or near-boiling water may be poured on a bed of finer grounds.

Near-boiling water is forced through beds of very fine grounds by a pump when espresso coffee is brewed in modern machines. Steam may be passed through the brew or mixtures of brewed coffee and milk to prepare foamed beverages such as cappucino or latté. Very finely ground, darkly roasted coffee, low water:coffee ratios, and demitasse cups are used when espresso is brewed in Italy. Higher water:coffee ratios and larger-sized cups are often used for espresso made in the United States.

Ground coffee is also cooked in or mixed with boiling water and separated from the resulting brew by decantation, filtration, or sedimentation. Coarse grounds may be used, as sometimes done in Scandinavia, where the practice is decreasing. Superfine grounds are used when Turkish coffee is brewed. Compared with percolation, brewing by mixing grounds with boiling water releases much more caffestol, which elevates serum cholesterol (15).

Roasted coffee contains colloidal material that does not pass through intact cell walls. Very fine and superfine coffees contain many ruptured cells and consequently release colloidal matter during brewing, producing brewed coffee with a mouth-feel different from that produced by more coarsely ground coffee.

Gourmet coffee bars that sell brewed coffee prepared by both standard and espresso methods from custom-roasted, high-quality beans have increased in number in the United

States from 500 in 1991 to roughly 12,000 to 15,000 in 1998.

INSTANT COFFEE

Instant coffee is the dried water-extract of roasted, ground coffee. Although used in army rations during the Civil War, instant coffee did not become a popular consumer item until after World War II. Improvements in manufacturing methods and product quality as well as a demand for convenience foods accounted for its rise in popularity. Some of the soluble coffee consumed in the United States is now manufactured in coffee-growing countries. This may be part of the reason why only 41% as much instant coffee was made in the United States in 1997 as in 1977.

Most soluble coffee blends contain Brazilian, Central American, Colombian, and African robusta coffees. Beans for instant coffee are blended and roasted as for regular coffee but are ground more coarsely to avoid excessive pressure drop and flow maldistribution during subsequent extraction (16). The roasted, ground coffee is charged into columns called percolators through which hot water is pumped to produce a concentrated coffee extract. The extracted solubles are dried, usually by spray or freeze drying, and the final powder is packaged in glass jars at rates of up to 200 jars per minute.

Extraction

Commercial extraction equipment and conditions have been designed to obtain the maximum soluble yield and acceptable flavor. In most processes, the water-soluble components in roasted coffee are first extracted at temperatures slightly lower than 100°C and atmospheric pressure. Additional solubles are then created by hydrolytically converting hemicelluloses and other insoluble components of the roasted coffee into water-soluble materials by pressure extraction at higher temperatures.

The factors influencing extraction efficiency and product quality are (*1*) grind of coffee, (*2*) temperature of water fed to the extractors and temperature profile through the system, (*3*) percolation time, (*4*) ratio of coffee to water, (*5*) premoistening or wetting of the ground coffee, (*6*) design of extraction equipment, and (*7*) flow rate of extract through the percolation columns (16).

Cylindrical percolators with height-to-diameter ratios ranging from 7:1 to 4:1 are common. They are usually operated in series as semicontinuous units of 5 to 10 percolators, with the water flowing countercurrent to the coffee. The ground coffee may be steamed or wetted with water or coffee extract; this supposedly improves extraction (17). Feed water temperatures range from 154 to 182°C, and unless the columns are heated, the temperature drops so that the extract effluent will have cooled to 60 to 82°C. The effluent extract temperature is further reduced by water cooling in plate heat exchangers to minimize flavor and aroma loss prior to drying.

The extract is removed from the percolators and stored in insulated tanks until dried. The extract solubles yield is calculated from the extract weight and soluble solids concentration as measured by specific gravity or refractive index. Yield is controlled directly by adjusting the weight of soluble solids removed and depends primarily on the properties of the coffee, operating temperatures, and percolation time. Soluble yields of 24 to 48% on a roasted coffee basis are possible. Robusta coffees give yields about 10% higher than arabica coffees (16).

High solubles concentrations are desirable to reduce evaporative load in drying and provide good flavor retention during drying. Water inflows that provide extract concentrations in the 20 to 30% soluble solids range are frequently used. More dilute extracts are obtained if greater water inflow is used, but lipophilic flavors and aromas that reside largely in coffee oil in the beans are extracted more efficiently. Some processors concentrate solubles by vacuum evaporation prior to drying. The concentrated percolate may also be clarified by centrifuging prior to drying so that the dry product will be completely free of insoluble fine particles.

The flavor of instant coffee can be enhanced by recovering and returning some of the natural aroma lost during processing. Aroma constituents are liberated and can be collected subsequently by condensation when roasted coffee is ground, when roasted coffee is steamed in a vented extraction column, or when percolate is concentrated. Many patents have been issued dealing with the separation, collection, and transfer of aroma from roasted coffee to instant coffee.

Drying

The following factors are important criteria for good instant coffee drying processes: (*1*) minimum loss or degradation of flavor and aroma, (*2*) free-flowing particles of desired uniform size and shape, (*3*) suitable bulk density for packaging requirements, (*4*) desirable product color, and (*5*) moisture content below 4.5%. Operating costs, product losses, capital investment, and other economic aspects must be considered in selecting the drying process.

Spray Drying

Most instant coffee is spray dried. Concentrated coffee extract is atomized, usually by pumping it through pressure nozzles. Nozzles and nozzle combinations are used that provide the desired particle size, product bulk density, and flow capacity based on the properties of the extract. The resulting stream of drops, joined by concurrently flowing heated air, flows downward into a tall cylindrical drying chamber. The air transfers heat to the drops, causing water evaporation and progressive drying of the drops. The air progressively cools and becomes more and more humid as drying progresses. Most processors prefer to use low inlet-air temperatures (200–260°C) for best flavor quality, and use enough air to provide outlet-air temperatures between 107 and 121°C. Spray dryers are usually constructed of stainless steel and must be provided with adequate dust collection systems, such as cyclones or bag filters (16).

The particles of dried instant coffee are collected from the conical bottom of the spray dryer and from cyclones through rotary valves and are conveyed to bulk storage or packaging bins. Processors may screen the dry product to obtain a uniform particle-size distribution.

Agglomeration

At one time most instant coffee was marketed as small, spherically shaped particles. Now it is marketed mostly in a granular form. Instant coffee granules are produced by moistening small spray-dried particles with steam, thereby fusing clusters of particles together. These clusters are then dried in a tower similar to a spray-drying tower using a low level of heat. Particle fusion and drying may also be carried out on a continuous belt.

Freeze Drying

Freeze-dried instant coffee is made by freezing coffee extract, grinding the frozen extract into granules and then converting the ice and bound-water content of the granules into water vapor by sublimation under high vacuum. Absolute pressures of 67 Pa (500 μmHg), or lower, are used. The heat used to induce sublimation is radiantly transferred from hot plates to the outer surface of beds of granules and then conductively transferred in the bed and granules to sublimation sites. Freeze drying occurs at much lower temperatures than spray drying. Heat input is controlled to give maximum end-point temperatures between 38 and 49°C (18). Drying times are much longer than for spray drying (18). Freeze-dried coffees use high-quality coffee and have better retention of volatile aromatics than do spray-dried coffees and are thus considered quality instant coffees.

Packaging

In the United States, instant coffee for the consumer market is usually packaged in glass jars containing from 56 to 340 g of coffee. Larger units for institutional, hotel, restaurant, and vending-machine use are packaged in bags and pouches of plastic or paper. In Europe, instant coffee is packaged in glass jars and, frequently, plug-closure metal containers with foil liners.

Protective packaging is primarily required to prevent moisture pickup. The flavor quality of regular instant coffee changes very little during storage. However, the powder is hygroscopic, and moisture pickup can cause caking and flavor impairment. Moisture content should be kept below 5%.

Many instant coffee producers in the United States incorporate natural coffee aroma fixed in expelled coffee oil in the powder. These highly volatile and chemically unstable flavor components necessitate inert-gas packing to prevent aroma deterioration and staling from exposure to oxygen.

DECAFFEINATED COFFEE

Decaffeinated coffee was first developed on a commercial basis in Europe about 1900. The basic process is described in a 1908 patent (19). Green coffee beans are moisturized by steam or water to a moisture content of at least 20%. The added water and heat separate the caffeine from its natural complexes and aid its transport through the cell wall to the surface of the beans. Solvents are then used to remove the caffeine from the wet beans.

Up to the 1980s man-made organic solvents were commonly used. The caffeine is removed either by direct contact of solvent with the beans or by contact with a secondary water system that has previously removed the caffeine from the beans (13). In either case additional steaming or stripping is used to remove solvent from the beans. The beans are dried to their original moisture content of about 10 to 12% prior to roasting.

In the 1980s decaffeination processes were commercialized making use of solvents that occur in nature or can be made from substances that occur in nature. The use of these processes are the basis of positioning a coffee product as naturally decaffeinated.

In a 1970 patent, Studiengesellschaft Kohle of Mulheim, Germany, showed that dense supercritical carbon dioxide is a very specific solvent for caffeine. Subsequent patents (20) describe use of this technology to decaffeinate coffee in a semicontinuous commercial process. Caffeine selectively transfers from wetted green coffee beans to supercritical carbon dioxide and then transfers from the carbon dioxide to water, thereby regenerating the carbon dioxide for reuse and recovering the caffeine.

Fats and oils, including oil from roasted coffee, (21) and edible esters, including ethyl acetate, which is present in coffee, are also used to selectively extract caffeine from coffee. Direct water contact with green beans is used in another process. This contact removes caffeine as well as some noncaffeine solids. The caffeine is adsorbed on activated carbon or an ion exchange resin, and the noncaffeine solids, containing flavor precursors, are reabsorbed on green beans prior to drying and roasting.

In all of the preceding decaffeination processes, prewetting of the green beans is necessary, and drying afterward is needed prior to roasting. These steps, in addition to caffeine removal, cause changes in the beans that affect roast flavor development.

The degree of decaffeination as claimed on the product is based on the caffeine content of the starting material and the time-temperature process conditions used by the manufacturer to achieve a desired end point.

Roasted decaffeinated coffee is vacuum packed as ground coffee or whole beans for consumer use. Roasted and ground decaffeinated coffee is made into instant coffee by methods previously described. Decaffeinated coffee represents about 9% of the coffee consumed in 1997 in the United States, declining from a peak of 18% in 1987 (22).

BIBLIOGRAPHY

This article was adapted and updated from the article "Coffee" by A. Stefanucci, W. P. Clinton, and M. Hamell, in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 6, 3rd ed., John Wiley & Sons, New York, 1979, pp. 511–522 and from an earlier version of this article by W. Clinton in *The Wiley Encyclopedia of Food Science and Technology*.

1. W. A. Ukers, All About Coffee, 2nd ed., *Tea & Coffee Trade Journal*, New York, 1935, pp. 1–3.
2. NTIS, U.S. Department of Commerce, *Tropical Products (Coffee, Cocoa, Spices, Essential Oils)*, June 1998.

3. A. Stefanucci and K. Sloman, *Internal Report*, Technical Center, General Foods Corp.
4. A. G. W. Bradbury and D. J. Halliday, "Polysaccharides in Green Coffee," paper presented at the Association Scientifique Internationale du Cafe, 12th colloquium, Montreux, Switzerland, 1987, p. 266
5. H. Thaler and R. Gaigal, *Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung* **120**, 449–454 (1963).
6. J. R. Feldman, W. S. Ryder, and T. J. Kung, *J. Agric. Food Chem.* **17**(4), 733–739 (1969)
7. J. Carisano and L. Gariboldi, *J Sci. Food Agric.* **15**, 619–622 (1964).
8. I. Flament, "Research on the Aroma of Coffee," paper presented at the A.S.I.C. 12th Colloquium, Montreux, Switzerland, 1987, p. 146.
9. W. Clinton, "Evaluation of Stored Coffee Products," paper presented at the A.S.I.C. 9th Colloquium, London, 1980, p. 273.
10. Praxis Werke Inc., *Logofile*, 1995.
11. U.S. Patent 4,169,164 (Sept. 25, 1979), M. Hubbard (to Hills Bros. Coffee Co.).
12. U.S. Patent 4,737,376 (Apr. 12, 1988), L. Brandlein et al. (to General Foods Corp.).
13. U.S. Patent 2,309,092 (Jan. 26, 1943), N. E. Berry and R.H. Walters (to General Foods Corp.).
14. R. J. Clarke and R. Macrae, eds., *Coffee*, Vol. 5, *Related Beverages*, Elsevier Applied Science, Barking, UK.
15. M B. Katan and R. Ungert, "The Cholesterol Elevating Factor from Coffee Beans," 16th A.S.I.C. Colloquium, Kyoto, 1995, p. 49.
16. M. Sivetz and N. W. Desrosier, *Coffee Processing Technology*, Avi Publishing, Westport, Conn., 1979.
17. U.S. Patent 3,549,380 (Dec. 22, 1970), J.M. Patel et al. (to Procter & Gamble).
18. U.S. Patent 3,438,784 (Apr. 15, 1969), W.P. Clinton and coworkers (to General Foods Corp.).
19. U.S. Patent 897,763 (Sept. 1, 1908), J. F. Meyer and coworkers (to Kaffee-Handels-Aktien-Gesellschaft).
20. U.S. Patent 4,820,537 (Apr. 8, 1989), S. Katz (to General Foods Corp.).
21. U.S. Patent 4,465,699 (Aug. 14, 1984), F.A. Pagliaro and coworkers (to Nestlé, SA).
22. National Coffee Organization, "Coffee Drinking Study, U.S.A.," Winter, 1997.

GENERAL REFERENCES

R. J. Clarke and R. Macrae, eds., *Coffee*, Vols. 1–6, Elsevier Applied Science Publishers, Barking, UK, 1985–1988.

M. N Clifford and K. C Wilson, eds., *Coffee: Botany, Biochemistry and Production of Beans and Beverages*, Croom Helm, London, 1985

B. Rothfos, *Coffee Consumption*, Gordian-Max Rieck GmbH, Hamburg, 1986

M. Sivetz and N. W. Desrosier, *Coffee Processing Technology*, Avi Publishing, Westport, Conn., 1979.

Proceedings of International Scientific Colloquium on Coffee (held every two or three years since 1963), Assoc. Scientifique Internationale du Cafe, Paris, France.

HENRY G. SCHWARTZBERG
University of Massachusetts
Amherst, Massachusetts

COLLOID MILLS

A colloid mill is a device used in the preparation of emulsions and dispersions. The name implies that it can generate colloidal size droplets (1–1,000 nm) for the disperse phase, but in reality the colloid mill produces emulsions with droplets in the size range of 1 to 25 μm. It is sometimes used for dispersing solid particulates throughout the continuous phase, but in most cases it does not grind particles, rather it deagglomerates and disperses the solids. In the early years of the colloid mill, one author suggested that it be called a dispersion mill to reflect its function better (1). However, the term colloid mill is still used to describe this type of device.

HISTORY

The first mention of a colloid mill came from a Russian colloid chemist, Von Weimarn, who in 1910 proposed using a mechanical device to generate shearing action to produce colloidal dispersions (1–5). In Hamburg, another Russian engineer named Plauson developed and patented in 1921 a colloid mill. Plauson modified a hammer mill " . . . so that the beater arms impinged on a liquid surface. Further, he operated the rotor at very high peripheral speed to increase the number of blows in a given time" (2). The Plauson mill used excessive horsepower, was intermittent in its operation, and was not very successful on a plant scale (1).

After the introduction of the Plauson mill, many different designs of colloid mill were introduced. In 1928, Clayton, in his book *The Theory of Emulsions and Their Technical Treatment*, described 22 different colloid mill designs, many protected by patents (2). The colloid mill became popular because of its high volume output and low maintenance costs.

GENERAL DESCRIPTION OF DESIGN

There are many different colloid mills commercially available, but each share some common elements. Colloid mills usually consist of a rotor and a stator (Fig. 1). The rotor is a disk that can have many different forms and surfaces, but it is most commonly conical. This disk rotates at a high speed, usually between 1,500 and 20,000 rpm depending on the particular design. The speed of the rotor is more commonly in the intermediate range of 3,000 to 10,000 rpm for smaller machines and roughly half that range for larger machines. The rotor may be directly connected to the motor, or it may be driven by a gear or drive belt system. The stator is a fixed surface that is close to the rotor. However, colloid mills have been designed with many variations of these elements, such as two rotors spinning in opposite directions close to each other; and rotors and stators with slots, channels, flutes, teeth, projections, corrugations, steps, and intercalating ridges forming a sinuous passage (2). The diameter of the rotor may range from 1 to 21 inches (2.54–53.3 cm). In operation some colloid mills require a feed pump, whereas others are self-pumping.

Flowing between the rotor and stator is a thin film of liquid that is subjected to a very high shear field. The thick-

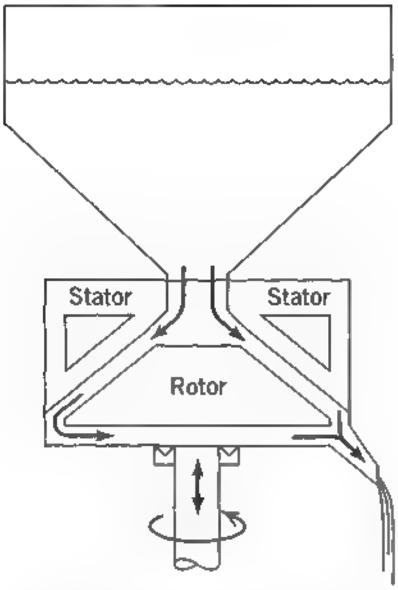


Figure 1. Basic components of a colloid mill: a rotor attached to a rotating shaft and a stator close to the rotor with a small gap between them.

ness of this layer of liquid is typically between 0.001 and 0.050 inches (0.0254–1.27 mm). It is characteristic of a colloid mill that the intensity of the shear field can be adjusted so as to achieve the desired process result. This adjustment is most often made by varying the thickness of the gap between the rotor and the stator.

THEORY OF OPERATION

The prevailing theory of the action in a colloid mill is shear. Fluid shear occurs in a layer of liquid between two planes, one stationary and the other mobile. There exists a gradient of velocity between these two planes. At the mobile plane the velocity is at its maximum, and at the stationary plane it is at its minimum (zero velocity). This means there are parallel layers of liquid moving past each other at different velocities subjecting dispersed particles to a disrupting force. The rate of change in velocity over the distance between these two planes is the shear rate. A common method of estimating the shear rate in a colloid mill is given as (8)

$$\gamma = n\pi D_r/h$$

where n is the number of revolutions per second, Dr is the rotor diameter, and h is the gap width. By this equation, a colloid mill with a rotor diameter of 2 inches (50.8 mm), a speed of 20,000 rpm, and a gap of 0.010 inches (0.254 mm) produces a shear rate of 2.09×10^5 sec ^{1}. At a gap of 0.002 inches (0.0508 mm) the shear rate is 1.05×10^6 sec^{-1}. A rotor diameter of 5 inches (127 mm) at 3,600 rpm with a gap of 0.005 inches (0.127 mm) has a shear rate of 1.88×10^5 sec. ^{1}. These examples illustrate the high shear rate generated in a colloid mill, and how this shear rate varies with operating conditions.

Some authors have suggested that other mechanisms besides shear are important in a colloid mill. Turbulence and cavitation may occur in mills with corrugated rotors and stators (6). Some researchers believe that the dispersion is caused by the collision of particles against each other at the surface of the rotor. Particles are set into rotation by the high shear field in the gap between the rotor and stator. A lift force pushes the rotating particles against the rotor. The comminution or emulsification occurs by "autogenous grinding empowered by the rotational kinetic energy of particles in suspension" (7). The rotational energy spectrum varies with "particle size, fluid viscosity, gap size, mill speed, and other parameters in the system" (7).

A recent paper describing the mechanism of emulsification in a colloid mill emphasizes the importance of residence time in the working area (8). Two models for droplet breakup are presented. One model is capillary breakup, where a droplet is stretched into an elongated form that is then broken into many droplets. The other model is a binary breakup where a cascade of events is needed to complete the reduction of the droplet. There was good agreement between experimental observation and the capillary model. One significant parameter in these models is residence time in the gap. Because these models require a certain time for breakup of the droplets, the residence time is an important factor in understanding scale-up and variations in colloid mill designs.

Residence time in a colloid mill is important because the flow rate in a mill is dependent on several factors. The flow rate depends on the rheological properties of the product, the clearance between the rotor and stator, the in-feed pressure to the mill, the shape of the rotor and stator, the amount of restriction to flow at the discharge from the mill, and the speed of the rotor. Some factors that reduce the flow rate of the mill are high viscosity, reduced clearance between the rotor and stator, restriction to flow at the discharge, and a flow path that is axial or tends radially inward. Factors that increase flow through the mill are increased in-feed pressure, large clearance between the rotor and stator, increased speed of the rotor, low viscosity fluid, no restriction to flow at the discharge, and a flow path that tends radially outward.

The temperature rise in a product flowing through a mill depends on the residence time in the working area. The longer the fluid is in the working area, the greater the temperature rise. Therefore, all the factors that increase residence time most likely increase temperature of the product.

Figure 2 shows the consequences of varying these factors for mayonnaise. Mayonnaise was processed in a laboratory-sized colloid mill. The flow rate, the product viscosity, and the in-feed pressure were measured during the test. When the in-feed pressure is increased, the flow rate through the mill is also increased. However, as the in-feed pressure is increased, the product viscosity is decreased because the residence time for the product is reduced and less work is done on the product.

PRODUCTS

The most common food applications of the colloid mill are the processing of mayonnaise and salad dressing.

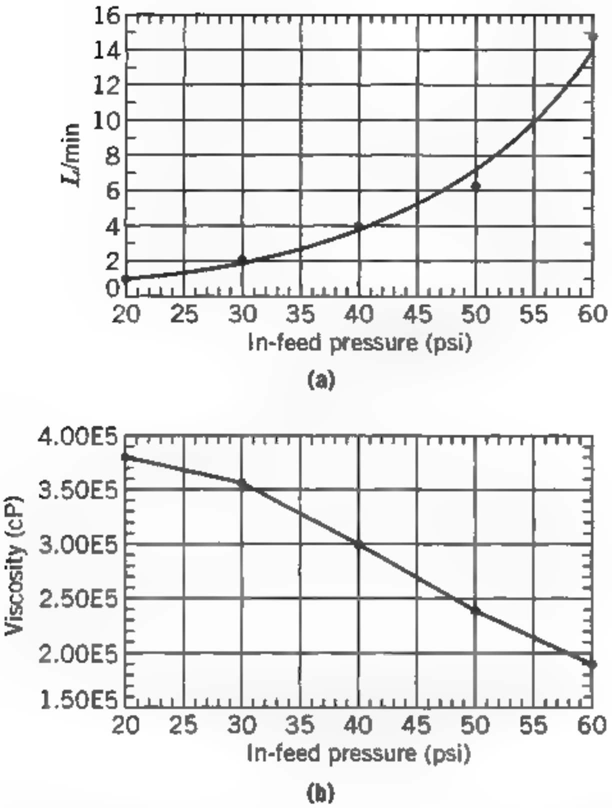


Figure 2. (**a**) Flow rate of a mayonnaise through a colloid mill increases with in-feed pressure. The gap between the rotor and the stator was 0.010 inches (0.254 mm). Density was 920 g/L. (**b**) Viscosity is reduced when the in-feed pressure is increased, causing an increase in flow rate and reduction in residence time. *Source:* From the research laboratory APV Americas-Homogenizers.

Mayonnaise contains 65% or more vegetable oil. The colloid mill produces the best combination of fluid processing conditions to make a good emulsion at this oil level. If this high oil level emulsion is processed on a homogenizer, then the results are poor. The homogenizer makes too small a droplet size, which leads to instability and separation of the emulsion because the natural emulsifier in mayonnaise cannot stabilize the large interfacial area generated. The colloid mill produces a droplet size range that makes a stable, viscous product. On the other hand, a salad dressing at 30% oil can be made on a homogenizer or a colloid mill. Because of the high viscosity usually associated with a salad dressing, a small oil droplet size is not necessary, and the energy level of a colloid mill is adequate to make this type of emulsion.

There are many other products made on the colloid mill. In the cosmetic industry colloid mills are used for making creams and lotions. In the chemical industry they are used for preparing bituminous emulsions, silicone oil emulsions, grease, and pigment dispersions. In many of these applications, the colloid mill works well because of high dispersed phase concentration in the product, high viscosity components, and moderate droplet size requirements.

BIBLIOGRAPHY

1. P. M. Travis, *Mechanochemistry and the Colloid Mill*, Chemical Catalog Co., New York, 1928.
2. W. Clayton, *The Theory of Emulsions and Their Technical Treatment*, 3rd ed., P. Blakiston's Son & Co., Philadelphia, Penn. 1935.
3. E. A. Hauser, *Colloidal Phenomena*, MIT Press, Cambridge, Mass., 1954.
4. E. K. Fischer, *Colloidal Dispersions*, John Wiley & Sons, New York, 1950.
5. J. W. McBain, *Colloid Science*, D. C. Heath, Boston, 1950.
6. P. Walstra, "Formation of Emulsions," in P. Becher, ed., *Encyclopedia of Emulsion Technology*, Vol. 1, Marcel Dekker, New York, 1983, pp. 57–127.
7. A. G. King and S. T. Keswani, "Colloid Mills: Theory and Experiment," *J. Am. Ceram. Soc.* 77, 769–777 (1994).
8. J. A. Wieringa et al., "Droplet Breakup Mechanisms During Emulsification in Colloid Mills at High Dispersed Phase Volume Fraction," *Trans. Inst. Chem. Eng.* **74**, 554–562 (1996).

WILLIAM D. PANDOLFE
APV Americas-Homogenizers
Wilmington, Massachusetts

COLOR AND FOOD

IMPORTANCE OF FOOD COLOR

The role that color plays in the reaction to food is so automatic that it may be taken for granted. This does not make that role any less important it merely decreases awareness. The color of a food has important considerations for both the consumer and the technologist. These considerations are quite different yet they are interrelated as shown in Figure 1.

The psychological effects of color have long been recognized. A room decorated in red exudes warmth and may increase pulse and respiration rates and even increase vivacity. Whereas a room decorated in blue or green is cool and peaceful encouraging concentration and relaxation. But darkening that blue can turn it into a subdued and even depressing atmosphere. The color of food also has its psychological aspects but these are less of mood swings than they are of learned associations. The color of a food not only sends a message of expectation but can also provide clues as to the condition of that food; a yellow peach is ripe, brown strawberry jam is old. Food of an unnatural color raises a barrier that most people have difficulty overcoming. Witness the red-fleshed potato that cooks up blue. Most people given the chance will avoid tasting it even when assured it tastes similar to the familiar white varieties. In contrast a recently introduced yellow-fleshed variety, reminiscent of buttery mashed potatoes, is gaining in popularity. In a very real sense the appearance of a food acts as a gate keeper and the old saying "We eat with our eyes" is not far off the mark. Experience and memory play important roles in food assessment. This is easily demonstrated by mismatching gelatin samples for color and flavor. Even a familiar flavor such as orange is difficult to

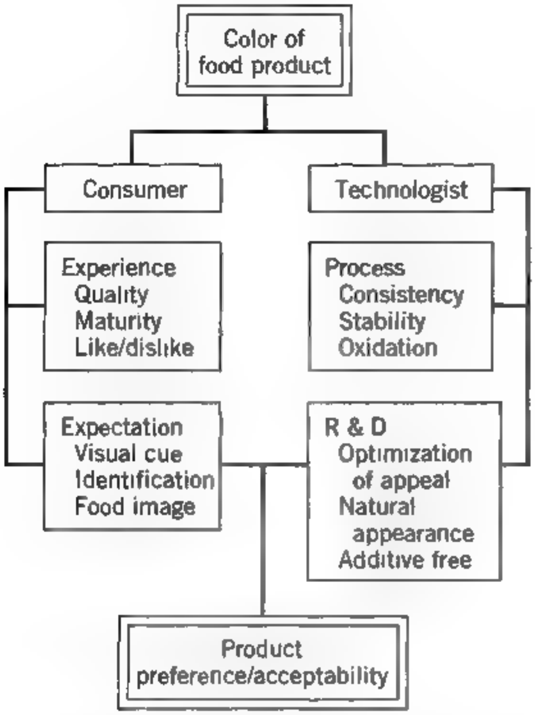


Figure 1. Food color considerations for the consumer and for the food technologist.

recognize when colored red. This is because red is associated with red fruits, thus a red-colored orange gelatin sends the wrong visual cue. This is of such proportion that it is difficult to rely on the messages sent by the taste buds and olfactory receptor.

In practice consumers do not isolate their sensory perceptions of a food but combine them in obtaining a total assessment for a food. This association is sufficiently strong that when asked to separate them there may still be a strong influence of one sensory perception on another. Yet the visual sense is both sophisticated and sensitive, it is only that experience has confirmed what the eye sees, which has caused the interaction of the senses to become unconscious.

The technologist has an interest in the color of food for several reasons. One is the need to maintain a uniformity of color over production runs. A second is the avoidance of color changes brought about by chemical reactions occurring during processing or storage life of the product. A third is the optimizing of color and appearance in relation to consumer preference. A fourth is the maintenance of a color in accordance with consumer experience and expectations. The need to maintain color uniformity over production runs varies with the food and especially with the packaging used. For example, the need is greater for fruit juices packaged in clear containers than for those packaged in individual drinking boxes where very little of the juice is actually seen. The color problems of processed green vegetables are examples of color changes arising from unstable pigment reactions. The retailing practices of fresh meat, particularly beef, have been limited for many years by the need to maintain meat pigments in the bright cherry red of oxymyoglobin because of the strong effect on consumer acceptability.

For many new products the product developer must consider the eye appeal of the product, and this should be given serious consideration in consumer acceptability ratings in any optimization procedure undertaken. The need to be aware of dynamic consumer reactions in regard to color and appearance is paramount. What is preferred in the color of a product is influenced by experience and availability and changes over time. The replacement of nitrites for curing meat products is complicated by the fact that the nitrites contribute to the characteristic color of cured meat. A satisfactory replacement has yet to be found. To support the deletion of nitrites from the process, consumers must exhibit sufficient concern about their diets that they would be willing to accept brown cured meat products or at least products that appeared different from those to which they are accustomed.

COLOR THEORY

A thorough review of color theory is beyond the scope of this article; however, several references have been published (1–5). The portion of the electromagnetic spectrum that comprises visible light falls between 380 and 750 nm, bordered by ultraviolet light on the low end and infrared light on the high end. The visible spectrum is represented by the colors seen in the rainbow with blue existing at wavelengths less than 480 nm; green roughly 480–560 nm; yellow, 560–590; orange 590–630; and red, at wavelengths longer than 630 nm (1). Purple is achieved by mixing blue and red and is considered to be a nonspectral color. If white light is dispersed by a prism a spectrum is obtained representing all the visible colors at appropriate wavelengths. The relative power or energy (power multiplied by time) emitted at these wavelengths can be plotted to produce the spectral power distribution curve of the light source. A group of light sources, black bodies, change from black through red to white when heated, the color produced being dependent on the temperature reached. This is referred to as color temperature. Tungsten filaments are close approximations of black bodies, although their color temperature is not exactly equal to their actual temperatures. Real daylight and fluorescent lights do not approximate black bodies.

When light falls on an object it may be reflected, absorbed, or transmitted or a combination of these may occur. When light passes through a material essentially unchanged it is transmitted. Wherever light is slowed down, as occurs at the boundary of two materials, the light changes speed and the direction of the light beam changes slightly (refractive index). The change in direction is dependant on the wavelength and accounts for the dispersion of light into a spectrum by a prism. The change in refractive index results in some light being reflected from the object. Light that is absorbed by the material is lost as visible light. If light is absorbed completely the object appears black and opaque. If some of the light is not absorbed but transmitted the object appears colored and transparent. Lambert's law, which states that the fraction of light that is absorbed by a substance is independent of the intensity of the incident light, is always true in the absence of light scattering. Beer's law states that the light absorption is directly proportional to the number of molecules through which the light passes. In practice, Beer's law is often not exactly observed, possibly due to chemical changes in some of the absorbing molecules.

When light interacts with matter it may travel in many directions. The sky is blue because of light scattered by molecules of air. Scattering caused by larger particles produces the white of clouds, smoke, etc. Scattering results in light being diffusely reflected from an object. Translucent materials transmit part of the light and scatter part of the light, if no transmittance occurs, the object is said to be opaque. The color of the object is dependent on the amount and kind of scattering and the absorption occurring. The amount of light that is scattered is dependent on the difference between the refractive indexes of the two materials. The boundary between two materials having the same refractive index cannot be seen because no light scattering occurs. Large particles scatter more light than small particles until the particle size approaches the size of the wavelength, at which point scattering decreases. Many foods are not completely reflecting or transmitting materials, which introduces an element of empiricism into an attempt to measure the color (6). With these foods absorption and scattering are factors affecting visual judgments. The absorption coefficient K and the scatter coefficient S in the Kubelka-Munk equations have been used in attempts to deal with this (7,8).

The chemist tends to think of color rather simplistically as being determined by the amount of light absorbed at a specified wavelength. But that is not how color is seen, for it is the light that is reflected from an object that determines color. The appearance of an object may vary over the entire object because of the angle of viewing and the light falling on the object. Appearance attributes have been divided into two categories: color, and geometric or spatial attributes (3). Color refers to the light reflected from the object to provide a portion of the spectrum as well as white, gray, black, or any intermediate. Geometric attributes result from the spatial distribution of the light from the object and are responsible for the variation in perceived light over a surface of uniform color, such as gloss or texture. The mode in which the eye is operating affects the visual evaluation. There are three modes: the illuminant mode, in which the stimulus is seen as a source of light; the object mode, in which the stimulus is an illuminated object; and the aperture mode, in which the stimulus is seen as light. The aperture mode may be thought of as a lighted window where first the light is seen, but at a closer range the object mode takes over and the contents of the room can be seen. The aperture mode views an object through a smallish aperture removing the effects of spatial distribution of light. This method is usually used for visual color-matching experiments. However, it is the object mode that is of practical importance in assessing the appearance of a food. The everyday evaluations of color, haze, clarity, gloss, and opacity are made by observing what the object does to the light falling on it.

The human eye is an incredibly sensitive and discriminating sensor; it can detect up to 10,000,000 different colors (6). The basic units of sight are the eye, the nervous system, and the brain. The cone light receptors located in the fovea of the retina of the eye are responsible for the ability to see color. It is generally agreed that there are three types of cone receptor responding to red, green, or blue and that these responses are converted in nerve-signal-switching areas within the eye and the optic nerve to opponent-color signals as proposed by Muller. Three opponent-color systems, black-white, red-green, and yellow-blue, were first proposed by Hering (3). The other type of light receptor, the rods, are also located in the retina; they increase in density as the distance from the fovea increases. The rods are responsible for black-and-white vision, the ability to see in dim light. Rods do not contribute to color vision. Approximately 8% of the population, predominately men, have abnormal color vision. For all humans the mode of presentation including viewing angle, source of illumination, and background, affect the color perceived by the viewer.

COLOR MEASUREMENT

Tristimulus Colorimetry

It is possible to match colors with a simple laboratory setup. Three projectors with a red, green, and blue filter, respectively, can be focused with the image superimposed on a screen. A color to be matched is projected on the screen by a fourth projector. An operator can match the desired color by determining the amounts of red, green, and blue required for a match. Unfortunately, not all colors can be matched by the red, green, and blue primary colors, so the researchers were given free rein to choose other primaries. They chose X (roughly corresponding to red), Y (roughly corresponding to green), and Z (roughly corresponding to blue). X Y and Z are unreal in that they cannot be produced directly in the laboratory, but they are very useful mathematically. This concept was accepted by the Commission Internationale de Eclairage (CIE) and became known as the CIE system. These tristimulus (three-stimuli) values for the equal-energy spectrum were used to define the 2° 1931 standard observer and were designated as $\bar{x}$, $\bar{y}$, and $\bar{z}$, which are special cases of X, Y, and Z; the 2° refers to the angle of observation. Later systems of measurement were developed with a 10° angle of viewing and are thus called 10° 1964 standard observers. The definition of the XYZ system offered a number of advantages. First, all colors lie within the primary coordinates. Second, the Y value contained all the lightness, and third, many of the coordinates for orange and red colors lie along a straight line, which is a great advantage in color formulation.

The XYZ values, sometimes presented as Y,x,y or xyz as described below, provided a measure of the color of a sample when viewed under a standard source of illumination by a standard observer. The CIE organization defined a number of standard sources of illumination. CIE Source A represented incandescent light and a color temperature of 2,854 K; CIE Source B simulated noon sunlight; and CIE Source C simulated overcast-sky daylight. These light sources were supplemented by the CIE in 1965, and illuminant D_{65} with a color temperature of 6,500 K is now widely used. In 1931 the CIE adopted the standard observer as having color vision representative of the average of that of the population having normal color vision. These data were used to obtain CIE standard-observer curves for the visible spectrum for the tristimulus values r, g, and b, for a set of red, green, and blue primaries. The red, green, and blue data were transformed into XYZ values and provided the basis for the standard observer curves shown in Figure 2 (1). The definition of the standard observer curves made it possible to design a simple colorimeter. Figure 3 shows white light falling on a sample to be measured. The reflected light is passed through a glass filter to determine the X component, the Y component, and the Z component, respectively. The signal from each filter is measured electronically and constitutes a tristimulus reading for the sample. The accuracy of the reading depends on the ability of the manufacturer to produce a filter/photocell combination that duplicates the response of the human eye, that is, the standard observer curves. They have been very successful in this aspect, and several commercial colorimeters available today are based on this principle.

Spectrophotometry

The CIE values of a color can be calculated by multiplying the energy spectrum of the illuminant by the reflectance, or transmission, spectrum of the sample and the standard observer curves. They can be mathematically represented by the following integral equations:

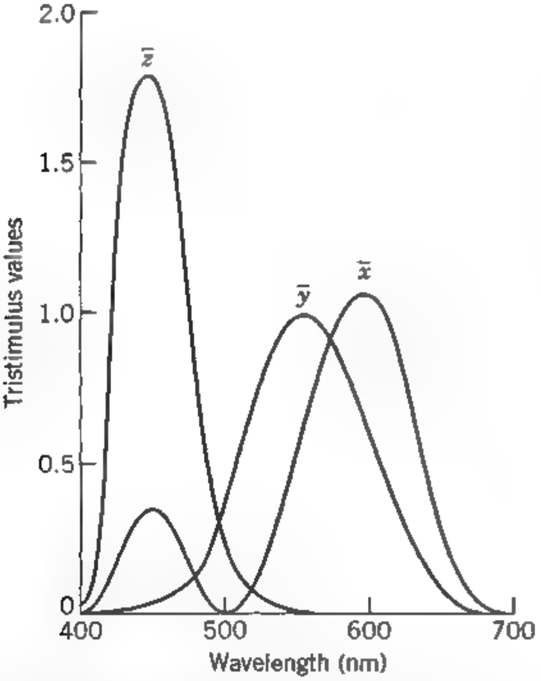


Figure 2. Curves for 2° standard observer expressed as tristimulus values $\bar{X}$, $\bar{Y}$, and $\bar{Z}$.

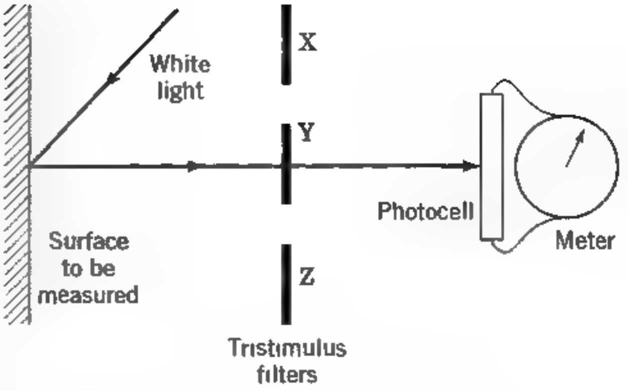


Figure 3. A simple tristimulus colorimeter. The light reflected from the sample passes through the three tristimulus filters, X, Y, and Z, and the signals are recorded by the photocell. The combined outputs from the filter/photocell combination duplicate the standard observer curves x,y, and z and represent the color of the sample.

$$X = \int_{380}^{750} \mathrm{RE}\bar{x}dx$$

$$Y = \int_{380}^{750} \mathrm{RE}\bar{y}dy$$

$$Z = \int_{380}^{750} \mathrm{RE}\bar{z}dz$$

where R is the sample spectrum, E is the source light spectrum, and $\bar{x}$, $\bar{y}$, $\bar{z}$ are the standard observer curves.

Many of the more expensive colorimeters available today record a spectrum from the sample as the initial signal and calculate CIE values from it. This approach allows considerable flexibility because the signal can be programmed to produce data for any illuminant, several types of sample presentation, a number of mathematical color systems, special color scales, and many types of color difference calculations. Interestingly, the early spectrophotometers designed for color measurement 70 years ago all used this approach, but the calculations for CIE values were tedious. Mechanical integrators helped, but they were expensive. This led to the development of the much simpler tristimulus colorimeters about fifty years ago. Today with the development of electronic calculation, the cost of calculation is simply not a significant consideration, and spectrophotometers are coming back into fashion.

Interestingly, a tristimulus colorimeter can also be used as a chemical absorptimeter for analytical purposes (9,10).

Data Presentation

Spectrophotometers usually present data in XYZ units but there are other conventional ways. Chromaticity coordinates (xyz) calculated by the following equations are often used to plot a chromaticity diagram (Fig. 4). The convention Yxy is also used, where Y represents CIE Y expressed as a percent of $X + Y + Z$:

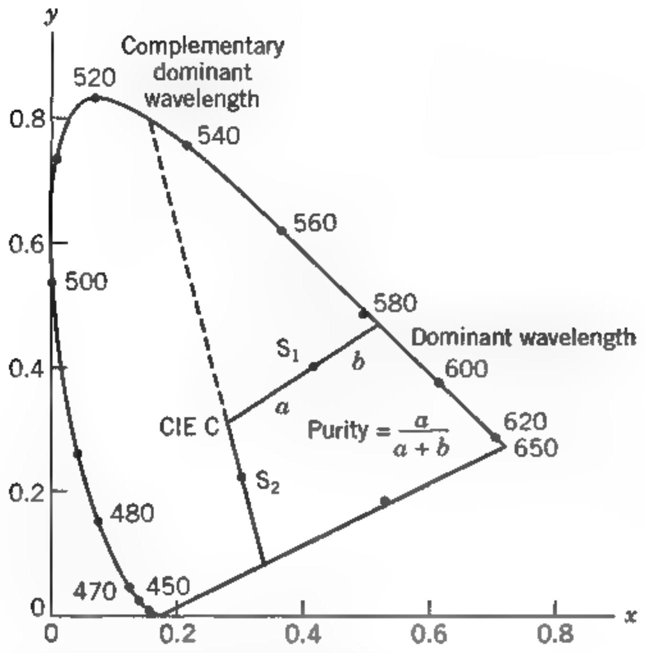


Figure 4. CIE chromaticity diagram showing dominant wavelength and purity.

$$x = \frac{X}{X + Y + Z}$$

$$y = \frac{Y}{X + Y + Z}$$

$$z = \frac{Z}{X + Y + Z}$$

By convention x and y are plotted on the diagram that features the horseshoe-shaped spectrum locus. Dominant wavelength is obtained by drawing a line from the illuminant through the sample to the spectrum locus as shown in Figure 4 and represents visual hue. Purity is obtained by the equation $a/(a + b)$ and represents visual chroma or saturation. Samples located nearer the spectrum locus are more saturated. Colors placing below the illuminant at the horseshoe base are considered nonspectral colors, and a complimentary dominant wavelength is calculated by drawing a line from the sample through the illuminant to the spectrum locus. Purity is determined as for spectral colors. The horseshoe spectrum locus represents two dimensions of color and the third is represented by Y as shown in Figure 5. The plane of the horseshoe rises as the color becomes lighter. Spectrum colors on the spectrum locus have a low lightness factor.

The Hunter L, a, and b color scale is an opponent-type system and has the advantage of being more uniform than the CIE system. It has proved popular for measuring food products. This scale was developed in 1958, allowing computation and direct readout from the dials of the colorimeter (3). Readout is as L_L, a_L, and b_L, and the functions are related to the CIE X, Y, and Z as

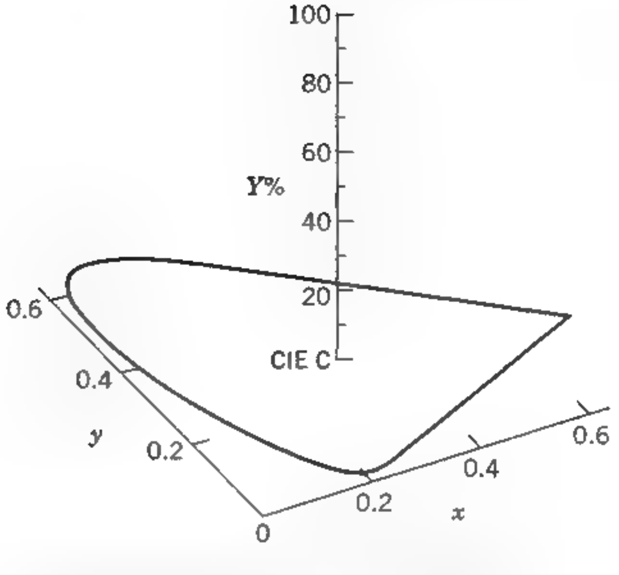


Figure 5. Third dimension of color represented by Y function rising from the plane of the chromaticity diagram.

$$L_L = 10(Y)^{1/2}$$

$$a_L = \frac{17.5(X - Y)}{(Y)^{1/2}}$$

$$b_L = \frac{7.0(Y - Z)}{(Y)^{1/2}}$$

The a and b chromaticity dimensions are expanded so that intervals on the scale for a and b approximate the visual correspondences to those of the 100-unit lightness scale L_L. This color scale is based on Hering's theory that the cone receptors in the eye are coded for light–dark, red–green, and yellow–blue signals. It is argued that a color cannot be both red and green at the same time; therefore, a is used to represent these; $-a$ represents greenness and $+a$ represents redness. Similarly, $-b$ represents blueness and $+b$ represents yellowness. Lightness is represented by L_L (Fig. 6). Hue is calculated as the angle going counterclockwise from $+a$ and hue angle is calculated as $\tan^{-1} b/a$. Chroma is calculated as $(a^2 + b^2)^{1/2}$. Color difference

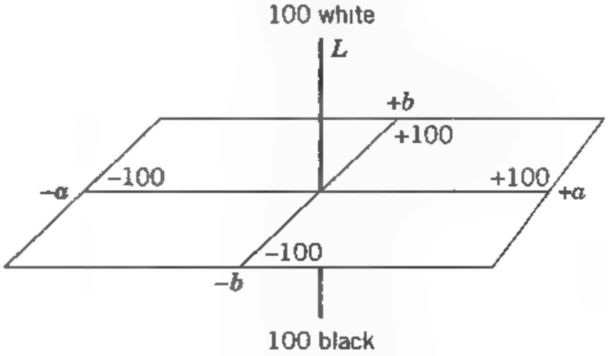


Figure 6. Hunter L a b color scale.

is calculated as $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 b_2)^2]^{1/2}$, but this value gives no indication in which dimension the difference lies. The Hunter color scale was first developed for use with reflectance and later adapted for transmission measurements. Confusion arises with luminosity of dark liquids such as dark fruit juices. This prompted adaptation and revision of scales for use at low luminosity levels (6). These scales are useful when the ratio of absorbance to transmittance is high.

The three-dimensionality of color is an important concept for measuring the color of a food. Examination of the wavelength distribution along the spectrum locus makes it clear that color is not equally visually spaced with this system. The elliptical nature of the color spaces prove troublesome when trying to match or establish specifications for color. Much attention has been devoted to providing more uniform color spaces and the CIELAB (1) system with parameters L*a*b* was designed to do this. Another color system was called CIELCH with parameters L*C*H* (9). The CIELAB system seems to be gaining more prominance. Color-matching computers are now available and are much used in textile and other industries. It may be important to determine whether a color difference exceeds the just-noticeable difference (JND). Computers have removed the computational labor of the more accurate calculations of differences.

SAMPLE PRESENTATION

Most instruments treat foods subjected to measurement as having opaque, matte, and uniform surfaces. In practice, foods seldom meet these requirements but deviate from ideal conditions of flatness, uniformity, opaqueness, diffusion, and specularity in ways that affect the measurement. This introduces an element of empiricism into any attempt to instrumentally assess the color of a food and sample preparation, and presentation becomes important. Color measurements are made to obtain repeatable numbers that correspond to visual assessments of the color of the food. Repeatability requires standardized procedures for sample preparation (9).

Many foods require a container of optical material thus introducing an element of gloss in contrast to the ideal matte surface. A uniform sample works best but grinding, mixing, milling, and blending are all processes that affect the light scattering properties of the sample. This may be to the extent that the sample no longer represents visual evaluation of the product, ie, crushed potato chips. The presence of water or other liquids in the mixture decreases the light scattering of the sample. Foods that consist of pieces that do not fit tightly together result in light from the source becoming trapped and thus reducing the light scattering. These foods are best measured with an aperture larger than the incident light beam to maximize collection of the light that has been trapped and diffused beyond the normal-size aperture. Liquids that depend on the transmission of light require optical cells of a thickness that will maximize the color difference. Translucent samples measured by reflection also pose the problem of light being trapped because it is reflected from within the food not from the surface. Light so trapped is not collected to be included in the measurement and a large aperture is useful.

Because most foods do not represent ideal conditions it is recommended that a second measurement be made after turning the sample 90° and the two readings be averaged. In devising a presentation technique that works well for the measurement it is well to remember to check that it retains a true relationship with the visual assessment of the product in its original form.

DATA INTERPRETATION

For quick assessments the simplest evaluation that will do the job is desired. Three-dimensional data may be considered too complex and difficult to deal with. It is important that any color measurement used to assess a food relates to what the eye sees and this should go further to determining those aspects of color that are important to consumers' assessment of quality. Any reduction of data needs to take these factors into account. Consumer assessments are generally obtained in less-controlled conditions, which are useful in determining the breadth of the problem. An intermediate stage under more—controlled conditions may be necessary in translating consumer results into instrumental measurements. However, using company personnel, closely involved with the technical side of the operation, as a substitute for consumers is risky because they are not representative of the consuming population.

Generally it is advisable to go beyond the basic CIE X, Y, and Z or Hunter L, a, and b instrument readouts to get a true picture of the color of the food. Lightness, hue, and chroma or saturation dimensions should be examined to obtain an indication of how the color of the samples measured differ. If the samples differ in only one dimension that dimension may be enough to provide a useful assessment of the product. However it should be remembered that lightness varies with both hue and chroma.

Some caution is required in using color difference calculations. The general ΔE calculation is affected by the equation selected for the calculation and by the geometry of the instrument on which the data were collected. Therefore, a suitable equation should be selected and used consistently. In addition, the ΔE calculation does not provide information about the nature of the difference. Calculating differences for the components as either CIELAB ΔL^*, Δa^*, and Δb^* or ΔH for hue, ΔL, or ΔC for chroma, is likely to be more informative. If these are to be used for establishing tolerances they must be checked against visual judgments. It is important to note that while numbers may be convenient, color acceptions or rejections in practice are not number based but made on visual assessments.

The availability of computer-equipped instruments has removed much of the tedium from calculating color-measurement results and made readily available data in different forms. This should not be allowed to lull researchers into a false sense of security. It is still important to use well-prepared, representative samples for measurement. It still takes time to think about the problem, to evaluate, and to understand the results obtained.

COLOR MEASUREMENT OF SPECIFIC FOODS

An extensive review of the color measurement of various foods has been published (10,11). This discussion highlights treatments of color measurement that represent newer developments or deal with conditions that present real problems in color measurement in attempting to relate measured color to what the eye sees.

Orange juice is a food for which color is considered important enough to represent a large proportion of the grading system yet it is difficult to measure because of translucency that may be compounded by the presence of particles. One approach to color measurement of this type of product has been the use of Kubelka-Munk equations developed for reflectance at a single wavelength (7), use with colorimeters is strictly an empirical adaptation. It has been observed that the effect of different rates of transmittance change relative to concentrations; <1.0 produced large changes in measured lightness, hue, and chroma (12). The falloff in lightness and chroma observed at higher concentrations resulted from smaller change in transmittance at concentrations >2.0. It was concluded that for strongly colored scattering materials in dilute suspension, instrumental measurement was inadequate because it did not measure what the human vision perceived as appearance (12). This is because the instrument measures intensity of back-scattered light over a limited angle whereas human vision is stimulated by internally scattered light emerging multidirectionally from the suspension in addition to that which is reflected directly. For purposes of grading, the color of orange juice is measured using the Citrus Colorimeter (3). This instrument has two scales: citrus redness (CR); and a subsidiary citrus yellow (CY), which are used to calculate a color-score equivalent to a visual color score.

The color of fresh meat is of interest because of the importance of the degree of oxygenation of the surface to consumer acceptability and also because of the incidence of dark cutting beef and pale, soft exudate pork. More traditional approaches have been to determine relative concentrations of the myoglobin, oxymyoglobin, and metmyoglobin pigments spectrophotometrically at appropriate wavelengths (13). The problem of measuring meat color is made difficult because of the variability in concentration of the heme pigment myoglobin, the condition of the cut surface that may have undergone a degree of desiccation, the chemical state of the myoglobin, and the light-scattering properties of the muscle pigments (12). The muscle of a freshly slaughtered animal is dark and translucent in appearance becoming lighter and more opaque as the pH falls and the glycogen is converted to lactic acid. Because of the degree of translucency inherent in fresh meat, the Kubelka-Munk equations have been explored as a means of measuring meat color (12,13). The scatter coefficient has been used to measure color as a condition of fresh meat; however, the range of scatter is too large to estimate pigment concentration accurately (12). Scattering data were supplemented with data for L, hue angle, and chroma to produce typical values for conditions of fresh meat. It has been pointed out that derived formulas and multiple regression equations for expressing meat color suffer in accuracy because most people tend to think of how the viewed samples differ from a mental image of ideal meat color (14).

A mathematical approach rather than a visual approach has been used for estimating physical color parameters L^*, a^*, and b^* for red and tawny ports based on consumer data (15). Consumers ratio-scaled 15 blends of port for redness, brownness, and intensity of color and then provided ratings for their ideal port. Assessors were separated on the basis of those who preferred tawny and those who preferred ruby port. Deviations of the samples from the ideal were regressed against L^*, a^*, b^*, hue angle, and chroma. Three simultaneous equations including port preference, hue angle, and chroma were established for relating sensory information with physical information. These data were further treated by the method of inverse simultaneous estimation to obtain estimates for color parameters for ideal ports. However, as with all ideal assessments, caution must be exercised because of the large degree of error. In this case the ideal fell outside the range of blends, a common occurrence with ideal data. Although the study does demonstrate that appropriate use of mathematics and data collection can increase efficiency in relating sensory and physical color data.

ROLE OF COLORANTS

Because the color of a food is used by consumers to aid in forming a judgment about that food, it is not surprising that food processors and manufacturers consider colorants important adjuncts. The use of colorants to enhance the appearance of a food is not a recent development. Spices and condiments have been used since early civilization. However, the use of colorants has not been without its more unscrupulous aspects such as the propensity to use them to mask inferior products in the eighteenth and nineteenth centuries. The use of copper sulfate or copper fittings in making pickles to obtain an appetizing green appearance has lingered to more recent times. Candies, wines, liquors, and even flours have all been subjected to harmful coloring practices (16). As a result the use of colorants in foods came under scrutiny in the late nineteenth century, and the use of food colors has been controlled at the federal level in the United States since an act of Congress in 1886 allowed butter to be colored. This act recognized the need to provide the marketplace with products having both an attractive and a consistent appearance. By 1900 a wide range of foods such as jellies, catsup, alcoholic beverages, milks, ice creams, candy, sausages, pastries, etc were allowed to be colored. Both the substances allowed and the amounts specific to a food have been regulated where coloring has been permitted.

Colorants allowed in foods include those from both natural and synthetic sources. Natural colorants as a rule are more expensive, less stable, and possess lower tinctorial power. In addition, they are frequently present as mixtures in the host materials and vary with season, region, variety, etc. Disadvantages associated with natural colorants have been identified as low yields; color instability resulting from effects of pH, light, heat, and freezing; and possible association with other properties that may be undesirable (17). However, they do present the advantage of being perceived as safer than synthetic colors. Pigments to be used as colorants must be extracted from the host materials and prepared in some form, such as a dried powder or beadlets, which increases the ease of use in a food product (16). A breakthrough in the use of natural pigments occurred with the synthesization of the carotenoids β-carotene, β-apo-8′-carotenal, and canthaxanthin. But even here considerable confusion exists in the interpretations by regulatory bodies. In the United States these synthetic duplications of natural extracts must be identified as an artificial color, whereas in Canada they must be labeled a natural color.

The safety of synthetic colorants continues to be questioned. In 1938 the FDA evaluated synthetic colorants and determined that 15 colorants met the criteria for use in food, drugs, and cosmetics, and these were identified as FD&C colors. Since then the list of FD&C colors has been reduced to seven. The most controversial deletion was FD&C Red No. 2, or amaranth, which was banned in the United States based on concerns regarding teratogenic and reproduction effects raised by USSR studies in 1970 and later confirmed by an in-house FDA study. This colorant was also under fire at the time as contributing to behavioral problems in children. However the Canadian Health Protection Branch, having assessed the same data, retained amaranth as a colorant on the basis of lack of carcinogenic evidence and inadequate experimental control used in the FDA study (16). Sweden, Denmark, the FRG, Japan, and nine European Economic Community countries also retained it. In contrast the FDA gave approval in 1974 for FD&C Red No. 40, and it is now the only general-purpose red color certified in the United States. Canada's Health Protection Branch did not allow FD&C Red Dye No. 40 known as Allura Red, for use in that country. A result of these discrepancies in regulations between countries is an added complexity between trading partners and to global trade.

A quick assessment of the commodity use of synthetic colors indicates that a large proportion are used in fruits and fruit juices and nonalcoholic beverages. These are foods where water solubility and stability are requirements and suitable natural colorants have yet to be developed.

EFFECT OF COLOR ON SENSORY PERCEPTIONS

The effect of color on sensory perceptions has been well demonstrated. Color can influence people's perception of the basic tastes and affect their ability to distinguish threshold levels (18). Presenting the basic tastes in colored solutions raised the thresholds of the test subjects. Thresholds for sour presented in green or yellow solutions were higher than those presented in red solutions, which in turn were higher than those presented in colorless solutions. This is not entirely accounted for by color association with certain foods, for example, both green and yellow sour and nonsour foods are common. In the case of bitter taste, green and yellow presentations resulted in higher concentrations being required for detection than for colorless or red presentations. It was concluded that red color was not associated with bitter flavor (18). In contrast to sour and bitter, sweet thresholds were lower for green-colored solutions. Thresholds for colored salt solutions were no different than those for colorless solutions. This was attributed to the wide variety of foods associated with a salty taste.

The levels of basic tastes in most foods are well above threshold levels and taste-color associations vary with specific foods. Color was shown to be a distraction in correctly identifying the sweeter sample in a paired comparison test of pear nectar, but it was not confirmed that the color green lowers the perception of sweetness, a result obtained in an earlier study (19). It was concluded that the effect of color varied considerably between individuals. The intensity of red color has been shown to affect a subject's perception of sweetness in beverages and solutions (20). A red color in fruit-flavored beverages was associated with an increase in perceived sweetness although the sucrose level was constant. Sweetness of darker red solutions was reported to be perceived as being 2–10% greater than the uncolored reference when actual sucrose concentrations were 1% less. There is an interrelationship between color and flavor, for example, the introduction of blue color to cherry- and strawberry-flavored drinks markedly decreased perception of tartness and flavor (21). The addition of yellow color decreased perception of sweetness to a lesser extent, and the addition of red increased sweetness. This study introduces the possibility of using color to reduce the concentration of sucrose required in a beverage. The perception of sweetness seems to be more affected by color than the perception of salt. It has been observed that color had no influence on salt perception of chicken broth colored red or yellow (22). Neither was flavor preference affected by color.

An investigation into the effect of color on aroma, flavor, and texture of foods, showed that appropriately colored foods were perceived to contain greater intensity of aroma and flavor and as having a higher quality, with aroma being the most affected (23). Perception of texture was not affected by color. Another study found that fruit-flavored beverages were correctly identified when their color was visible but when their color could not be seen dramatic reductions in correct identification occurred (24). Only 70% of the tasters correctly identified grape; 50%, lemon-lime; 30%, cherry; and 20%, orange. When the study was expanded to include combinations of color, flavor, colorless, and flavorless in beverages, flavor identification was color cued, and in the case of the colorless beverages a spread of flavors was identified. Flavorless beverage colored red was identified as strawberry by 22% of the tasters; orange-colored, as orange by 22%; and green-colored, as lime by 26%. Identification as having no flavor ranged from 41% for colored beverages to 48% for the colorless beverage. These same researchers observed that when white cake was varied in lemon flavor and yellow color that flavor intensity was most affected by color when no lemon flavoring was added, but the effect was nonlinear. Intensity of flavor was most affected between no added color and the lowest level. Color has been shown to have a strong influence on the assessment of aroma and flavor for port wines (25). These ratings were shown to be influenced by visual cues when these were made available. Examination of assessments by individual assessors showed assessors differed in the degree of this influence. These researchers reported that trained panelists were able to remove color bias from evaluation of Bordeaux wines when instructed to do so.

That color plays a role in the acceptability of many foods cannot be denied. One study varied the color of cherry- and orange-flavored beverages above and below normal levels. It was observed that the overall liking peaked around the normal color (24). It was also noted that overall acceptability was more closely related to flavor than to color acceptability, suggesting that color cannot substitute for flavor. This same study demonstrated a significant color-flavor interaction for overall acceptability of lemon-flavored cakes. More intense flavors were liked less at higher color concentrations. As flavor levels increased, overall acceptability peaked at lower color levels. These findings suggest that intensities reach a point where they exceed the accustomed expectation and are, therefore, liked less. The interrelationships between pleasantness, color, and sucrose concentration have been observed (16). The interaction was evident in the lower pleasantness rating given to the darker red intensity beverage. This may have resulted because the beverage was perceived as sweeter and, therefore, as less pleasant or the red color may not have been associated with the tasters' expectations of a desirable red color. One study examined the perceived pleasantness of beverages varying in sweetness and color and concluded that the most intense color or sweetest flavor did not always produce the optimum product formulation (21). Color and appearance have been shown to have important influences on overall acceptability of wines but the degree varies with the individual assessor and the study (25).

MARKETING IMPLICATIONS

Any research and development technologist should be aware of the marketing implications related to the color of a food as they affect product development and quality control. The technologist must bear in mind that it is what is perceived by the consuming public that controls sales not necessarily the truth of the perception.

A 1985 survey conducted by *Good Housekeeping* magazine, asked respondents to indicate whether nutrition or appetite appeal was the more-important selection criterion for food purchases; 45% stated appetite appeal (26). Color certainly plays a considerable role in appetite appeal and this is one reason that foods are colored. Yet some consumers consider the addition of color as perpetrating deception and in Europe there have been attempts to ban the use of colorants (17). It has been reported that the lack of artificial colorings and the 3presence of natural color rank high as positive food attributes, whereas the use of artificial color was ranked as negative (26). However, 35–40% of respondents considered artificial color as somewhat acceptable. More than 50% of the respondents considered coal tar color derivatives to be unacceptable. Apparently, the use of colorants for cosmetic reasons, once considered to enhance acceptability, may now be a detractor because of the pressure to reduce the use of additives in processed foods as a result of prevailing consumer attitudes and safety concerns. It should also be remembered that experiences change over time and that this influences acceptability. The combination of experience and prevailing consumer concerns can combine to bring about changes in acceptability. The Spy variety of apple was once prized as a cooking apple but it has gradually been superseded by other varieties for processing and retail sales. When cooked, the Spy apple produces a very intense yellow color. This bright yellow color is less preferred by today's consumers than the duller browner color of the cooked product of more familiar varieties. Part of this is undoubtedly that the intense color is unexpected and unfamiliar but the lower preference may also result from the fact that the intense yellow, although natural, has become suspect.

Although within the marketing profession there has been much exploration of the effects of color, particularly in the packaging, on the sales of food products, little research of this nature has been published. Package design and color are important factors in attracting a buyer at the point of purchase. That packaging becomes part of a familiar product has been witnessed by any shopper who has had to search for an often-purchased product because the color of the package has been changed. Marketers are concerned about product image and thus both the product and the package must contribute to this image. Both can become dated and in an industry that experiences a short life for many of its products this can be of concern for the long-lived products. Black has not generally been considered a desirable color for food packages but with the popularity of art deco design, sophisticated black packages and labels are beginning to appear. Marketers are quick to take advantage of color association with consumer concerns such as that of green with environmental concerns. One Canadian food retailer has capitalized on this to develop an array of G.R.E.E.N. products, which have been promoted as being environmental friendly. This sort of use of color with food is usually beyond the purvey of the technologist, but such marketing decisions may result in formulation requests to research and development.

It can be concluded that color has an important association with food, which extends far beyond the technologist's concern with measurement. An examination of all the facets as they affect any product is becoming increasingly important in coping with the greater degree of sophistication and competitiveness occurring in the food industry.

BIBLIOGRAPHY

1. F. W. Billmeyer, Jr., and M. Saltzman, *Principles of Color Technology*, 2nd ed., John Wiley & Sons, Inc., New York, 1981
2. D. B. Judd and G. Wyszecki, *Color in Business, Science, and Industry* John Wiley & Sons, Inc., New York, 1963.
3. R. S. Hunter, *The Measurement of Appearance*, John Wiley & Sons, Inc., New York, 1975, pp. 44, 81, 167.
4. J. B. Hutchings, *Food Colour and Appearance*, Blackie Academic & Professional, Glasgow, Scotland, 1994.
5. R. G. Kuehni, *Color: An Introduction to Practice and Principles*, John Wiley & Sons, New York, 1997.

6. F. J. Francis, "Colour and Appearances as Dominating Sensory Properties of Foods," in, G. G. Birch, J. G. Brennan, and K. J. Parker, eds., *Sensory Properties of Foods*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1977, p. 27.
7. E. A. Gullett, F. J. Francis, and F. M. Clydesdale, "Colorimetry of Foods: Orange Juice," *Journal of Food Science* **37**, 389 (1972).
8. D. B. MacDougall, "Colour in Meat" in G. G. Birch, J. G. Brennan, and K. J. Parker, eds., *Sensory Properties of Foods*, Applied Science Publishers Ltd., 1977, p. 59.
9. F. J. Francis, "Color Analysis," in S. S. Nielson, ed., *Food Analysis*, 2nd ed., Aspen Publishers, Gaithersburg, Md., 1998, pp. 599–612.
10. F. J. Francis, "Color Measurement and Interpretation," in D. Y. C. Fung and R. F. Matthews, eds., *Instrumental Methods for Quality Assurance in Foods*, Marcel Dekker, Inc. New York, 1991, pp. 189–210.
11. F. J. Francis and F. M. Clydesdale, *Food Colorimetry: Theory and Applications*, AVI Publishing Co., Inc., Westport, Conn., 1975.
12. D. B. MacDougall, "Instrumental Assessment of the Appearance of Foods," in A. A. Williams and R. K. Atkin, eds., *Sensory Quality in Foods and Beverages*, Ellis Horwood Ltd., 1983, p. 121.
13. F. M. Clydesdale and F. J. Francis, "Color Measurement of Foods. 28. The Measurement of Meat Color," *Food Product Development* **5**, 87 (1971).
14. B. A. Eagerman, F. M. Clydesdale, and F. J. Francis, "Determination of Fresh Meat Color by Objective Methods," *Journal of Food Science* **42**, 707 (1977).
15. A. A. Williams, C. R. Baines, and M. S. Finnie, "Optimization of Colour in Commercial Port Blends," *Journal of Food Technology* **21**, 451 (1986).
16. Institute of Food Technologists' Expert Panel on Food Safety & Nutrition and the Committee on Public Information, *Food Colors. Scientific Status Summary, Institute of Food Technologists*, Chicago, Ill., 1980.
17. D. Blenford, "Natural Food Colours," *Food Flavourings, Ingredients and Processing* **7**, 19 (1985).
18. J. A. Maga, "Influence of Color on Taste Thresholds," *Chemical Senses and Flavor* **1**, 115 (1974).
19. R. M. Pangborn and B. Hansen, "The Influence of Color on Discrimination of Sweetness and Sourness in Pear-Nectar," *American Journal of Psychology* **76**, 315 (1963).
20. J. Johnson and F. M. Clydesdale, "Perceived Sweetness and Redness in Colored Sucrose Solutions," *Journal of Food Science* **47**, 747 (1982).
21. J. L. Johnson, E. Dzendolet, R. Damon, M. Sawyer, and F. M. Clydesdale, "Psychophysical Relationships Between Perceived Sweetness and Color in Cherry-Flavored Beverages," *J. Food Protect.* **45**, 601 (1982).
22. S. R. Gifford and F. M. Clydesdale, "The Psychophysical Relationship Between Color and Sodium Chloride Concentrations in Model Systems," *Journal of Food Protection* **49**, 977 (1986).
23. C. M. Christensen, "Effects of Color on Aroma, Flavor and Texture Judgements of Foods," *Journal of Food Science* **48**, 787 (1983).
24. C. N. DuBose, A. V. Cardello, and O. Maller, "Effects of Colorants and Flavorants on Identification, Perceived Flavor Intensity, and Hedonic Quality of Fruit-Flavored Beverages and Cake," *Journal of Food Science* **45**, 1393 (1980).
25. A. A. Williams, S. P. Langron, C. F. Timberlake, and J. Bakker, "Effect of Colour on the Assessment of Ports," *Journal of Food Technology* **19**, 659 (1984).
26. K. W. McNutt, M. E. Powers, and A. E. Sloan, "Food Colors, Flavors and Safety: A Consumer Viewpoint," *Food Technology* **40**, 72 (1986).

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amhert, Massachusetts

See also COLORANTS section.

COLORANTS

Colorants: Introduction 372
Colorants: Anthocyanin 375
Colorants: Anthraquinones 378
Colorants: Betalains 380
Colorants: Caramel 383
Colorants: Carotenoids 385
Colorants: Carthamin 390
Colorants: Chlorophylls 391
Colorants: Food, Drug, and Cosmetic Colorants 393
Colorants: Haems 396
Colorants: Iridoids 397
Colorants: Monascus 398
Colorants: Phycobilins 399
Colorants: Polyphenols 400
Colorants: Turmeric 401
Colorants: Miscellaneous Colorants 402

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amhert, Massachusetts

COLORANTS: INTRODUCTION

The appreciation of color dates back to antiquity, and recorded history is replete with descriptions of the use of colorants in everyday life (1). The art of making colored candy is depicted in paintings in Egyptian tombs as far back as 1500 B.C. Pliny the Elder described the artificial coloration of wines in 400 B.C. Spices and condiments were colored at least 500 years ago. Colorants for use in cosmetics were probably more widespread than those in foods or at least were better documented. Archaeologists have pointed out that Egyptian women used green copper ores as eye shadow as early as 5000 B.C. Henna was used to dye hair, carmine to redden lips, and kohl, an arsenic compound, to blacken eyebrows. It was common practice in India thousands of years ago to color faces yellow with saffron or to dye feet red with henna. The Romans used white lead and chalk on their faces and blue dyes on their hair and beards. More recently, in Britain, sugar was imported in the

6. F. J. Francis, "Colour and Appearances as Dominating Sensory Properties of Foods," in, G. G. Birch, J. G. Brennan, and K. J. Parker, eds., *Sensory Properties of Foods*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1977, p. 27.
7. E. A. Gullett, F. J. Francis, and F. M. Clydesdale, "Colorimetry of Foods: Orange Juice," *Journal of Food Science* **37**, 389 (1972).
8. D. B. MacDougall, "Colour in Meat" in G. G. Birch, J. G. Brennan, and K. J. Parker, eds., *Sensory Properties of Foods*, Applied Science Publishers Ltd., 1977, p. 59.
9. F. J. Francis, "Color Analysis," in S. S. Nielson, ed., *Food Analysis*, 2nd ed., Aspen Publishers, Gaithersburg, Md., 1998, pp. 599–612.
10. F. J. Francis, "Color Measurement and Interpretation," in D. Y. C. Fung and R. F. Matthews, eds., *Instrumental Methods for Quality Assurance in Foods*, Marcel Dekker, Inc. New York, 1991, pp. 189–210
11. F. J. Francis and F. M. Clydesdale, *Food Colorimetry: Theory and Applications*, AVI Publishing Co., Inc., Westport, Conn., 1975.
12. D. B. MacDougall, "Instrumental Assessment of the Appearance of Foods," in A. A. Williams and R. K. Atkin, eds., *Sensory Quality in Foods and Beverages*, Ellis Horwood Ltd., 1983, p. 121.
13. F. M. Clydesdale and F. J. Francis, "Color Measurement of Foods. 28. The Measurement of Meat Color," *Food Product Development* **5**, 87 (1971).
14. B. A. Eagerman, F. M. Clydesdale, and F. J. Francis, "Determination of Fresh Meat Color by Objective Methods," *Journal of Food Science* **42**, 707 (1977).
15. A. A. Williams, C. R. Baines, and M. S. Finnie, "Optimization of Colour in Commercial Port Blends," *Journal of Food Technology* **21**, 451 (1986).
16. Institute of Food Technologists' Expert Panel on Food Safety & Nutrition and the Committee on Public Information, *Food Colors. Scientific Status Summary, Institute of Food Technologists*, Chicago, Ill., 1980.
17. D. Blenford, "Natural Food Colours," *Food Flavourings, Ingredients and Processing* **7**, 19 (1985).
18. J. A. Maga, "Influence of Color on Taste Thresholds," *Chemical Senses and Flavor* **1**, 115 (1974).
19. R. M. Pangborn and B. Hansen, "The Influence of Color on Discrimination of Sweetness and Sourness in Pear-Nectar," *American Journal of Psychology* **76**, 315 (1963).
20. J. Johnson and F. M. Clydesdale, "Perceived Sweetness and Redness in Colored Sucrose Solutions," *Journal of Food Science* **47**, 747 (1982).
21. J. L. Johnson, E. Dzendolet, R. Damon, M. Sawyer, and F. M. Clydesdale, "Psychophysical Relationships Between Perceived Sweetness and Color in Cherry-Flavored Beverages," *J. Food Protect.* **45**, 601 (1982).
22. S. R. Gifford and F. M. Clydesdale, "The Psychophysical Relationship Between Color and Sodium Chloride Concentrations in Model Systems," *Journal of Food Protection* **49**, 977 (1986)
23. C. M. Christensen, "Effects of Color on Aroma, Flavor and Texture Judgements of Foods," *Journal of Food Science* **48**, 787 (1983).
24. C. N. DuBose, A. V. Cardello, and O. Maller, "Effects of Colorants and Flavorants on Identification, Perceived Flavor Intensity, and Hedonic Quality of Fruit-Flavored Beverages and Cake," *Journal of Food Science* **45**, 1393 (1980).
25. A. A. Williams, S. P. Langron, C. F. Timberlake, and J. Bakker, "Effect of Colour on the Assessment of Ports," *Journal of Food Technology* **19**, 659 (1984).
26. K. W. McNutt, M. E. Powers, and A. E. Sloan, "Food Colors, Flavors and Safety: A Consumer Viewpoint," *Food Technology* **40**, 72 (1986).

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amhert, Massachusetts

See also COLORANTS section.

COLORANTS

Colorants: Introduction 372
Colorants: Anthocyanin 375
Colorants: Anthraquinones 378
Colorants: Betalains 380
Colorants: Caramel 383
Colorants: Carotenoids 385
Colorants: Carthamin 390
Colorants: Chlorophylls 391
Colorants: Food, Drug, and Cosmetic Colorants 393
Colorants: Haems 396
Colorants: Iridoids 397
Colorants: Monascus 398
Colorants: Phycobilins 399
Colorants: Polyphenols 400
Colorants: Turmeric 401
Colorants: Miscellaneous Colorants 402

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amhert, Massachusetts

COLORANTS: INTRODUCTION

The appreciation of color dates back to antiquity, and recorded history is replete with descriptions of the use of colorants in everyday life (1). The art of making colored candy is depicted in paintings in Egyptian tombs as far back as 1500 B.C. Pliny the Elder described the artificial coloration of wines in 400 B.C. Spices and condiments were colored at least 500 years ago. Colorants for use in cosmetics were probably more widespread than those in foods or at least were better documented. Archaeologists have pointed out that Egyptian women used green copper ores as eye shadow as early as 5000 B.C. Henna was used to dye hair, carmine to redden lips, and kohl, an arsenic compound, to blacken eyebrows. It was common practice in India thousands of years ago to color faces yellow with saffron or to dye feet red with henna. The Romans used white lead and chalk on their faces and blue dyes on their hair and beards. More recently, in Britain, sugar was imported in the twelfth century in attractive red and violet colors, probably from the colorants Madder and Kermes for the reds, and Tyrian purple for the violet colors.

Early applications of colorants were usually associated with religious festivals and public celebrations. Obviously they could be added to foods, but in the Middle Ages, the trade guilds policed their members and protected the quality of their products. However, when the industrial revolution evolved with its massive social and population changes and much wider food distribution quality safeguards were weakened and adulteration became rampant. Wines and confectionery were particularly suspect. Ancient history contains many examples of the concern about food adulteration and its regulation. We can go back to the laws of Moses, the Roman statutes, and down through the Middle Ages. England seems to stand out in the laws enacted by Parliament in the Middle Ages. For example, in 1266 Parliament prohibited a number of important food staples if they were so adulterated as to be "not wholesome for man's body." This unambiguous statement could form the basis of food regulation today.

In 1920 Accum published a book describing practices such as the coloring of tea leaves with verdigris (copper acetate), Gloucester cheese contaminated with red lead, pickles boiled with a penny to make them green, confectionery with added red lead and copper, and many other appalling practices (2). Hassell in 1857 published a book on adulteration of foods that really raised the public conscience, and things began to change (3). Nearly every country passed laws dealing with the adulteration of foods, and colorants were a major concern since they could be used to conceal inferior food and in some cases were actually dangerous. Until the middle of the nineteenth century, the colorants used in cosmetics, drugs, and foods were those of natural origin such as those from animals, plants, and minerals. In 1856 when Sir William Henry Perkins discovered the first synthetic organic dyestuff, mauve, the supply situation changed drastically. The German dyestuff industry synthesized a number of "coal tar" dyes, which apparently were utilized very quickly. For example, French wines were colored with fuchsine as early as 1860. In the United States, colorants were allowed to be added to butter in 1886 and to cheeses in 1896. By 1900 Americans were eating a wide variety of colored foods such as jellies, cordials, ketchup, butter, cheese, candy, ice cream, wine, noodles, sausage, confectionery, and baked goods. Many of these practices constituted economic fraud but were not actually dangerous. But some were Marmion described a classic horror story in 1860 in which a druggist gave a caterer copper arsenite to use in making a green pudding for a public dinner and two people died (4). At the turn of the century, there were nearly 700 synthetic colorants available, and obviously very little control was exercised over the type and purity of the colorants offered for use in foods. Because of public concerns over these practices, the American food manufacturers decided to police their own industry. One example was a list, published in 1899 by the National Confectioners Association, of 21 colorants that they considered unfit for addition to foods. However, it became apparent that some form of government control was needed, and, in 1900, the Bureau of Chemistry in the U.S. Department of Agriculture (USDA) was given funds to study the problem. A series of Food Inspection Decisions (FID) resulted. One FID in 1904 declared a food to be adulterated "if it be colored, powdered or polished with intent to deceive or to make the article appear to be of better quality than it really is." Another FID in 1905 required the labeling of such products as artificially colored imitation chocolate. Another FID in 1906 concerned a coal tar dye considered to be unsafe for use in food. In effect, it stopped the importation of macaroni colored with Martius Yellow. At this time, the (USDA) launched a monumental task to determine which colorants were safe for use in food and what restrictions should be placed on their use. This effort led to the regulations for the use of synthetic colorants in the United States today.

The development of the regulatory aspects of food colorants is one small portion of the much bigger picture concerning food additives and food quality in general. One of the driving forces for food regulation down through the years has been food safety. This aspect has been brought to public attention, sometimes in rather stormy fashion, by a series of books such as *The Jungle* by Upton Sinclair in 1906. Public laws to minimize obviously unpalatable practices concerned with food sources, processing, handling, ingredients, and so on date back seriously only about 150 years. Much of the criticism has been directed toward the artificial colorants and very little toward the naturally occurring colorants. Perhaps this is partially due to the naive belief that humans have somehow become conditioned to tolerate certain compounds after a long history of use. But it is unlikely that 5000 years is enough to create significant genetic changes in humans. It is true, however, that history has allowed us to identify acute hazards and eliminate them from our diet, but long-term or lifetime exposures are another story. Regardless, much of the regulatory activity has centered around food safety.

The development of food laws in the United States followed in concept those in Europe (5) and are governed by the Food and Drug Acts. The first one of importance was in 1906, and it has been modified several times. The 1938 modification was one of the more important developments, and the 1938 principles are still in effect. The 1938 Act established the term FD& C colors with the initials standing for Food, Drug, and Cosmetic. There is also a D&C classification for colorants allowed in drugs and cosmetics. The Act specified a list of colorants allowed in foods, and presumably they would be held to a higher standard of purity than those available for use in industry. Colorants used in food would have to be identified by their FD&C name, but colorants used for other purposes in industry could still be referred to by their common name. The Society of Dyers and Colorists in England and the American Association of Textile Chemists and Colorists in the United States developed a Color Index (CI) system. Each colorant was given a CI number and name. Colorants are also identified by their Chemical Abstract Service (CAS) Registry code number. The U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) uses a CAS-like code for those substances without a number assigned by CAS.

The 1960 Color Additive Amendment to the Act specified two groups of colorants: certified color additives and color additives exempt from certification. The first group listed seven synthetic FD&C colorants that were certified to comply with the purity specifications required by the FDA. The second group has 26 colorants (6,7) for which the FDA does not require that each batch be analyzed and certified (Table 2 in COLORANTS: FOOD, DRUG AND COSMETIC COLORANTS). Many of the colorants that would be called "natural" in other countries are on the list of 26. The FDA does not accept the concept of "natural" versus "synthetic" and requires that compounds in each group be subjected to the same standards of safety. Actually, in practice, the safety studies required for some of the natural colorants were considerably less than those required for the synthetics. This approach is understandable if one accepts the concept of the decision tree approach for determining safety as suggested by the Food Safety Council (8). The Nutritional Labeling and Education Act of 1990 mandated that certified color additives be specifically declared by their individual names, but the requirements for exempt colorants were left unchanged. Exempt colorants can still be declared generically as "artificial color" or any other specific or generic name for the colorant. But the term "natural" is prohibited because it may lead the consumer to believe that the color is derived from the food itself. There is no such thing as a natural colorant in the United States. Detailed accounts of the safety of the noncertified colorants have recently been published (6,9).

The emphasis on food safety possibly has had another influence, namely the worldwide trend toward the use of colorants derived from natural sources. In one survey of food colorant patents in the 15-year period from 1969 to 1984, there were 356 patents on colorants from natural sources and 71 on synthetics (10). The ratio is even more lopsided when one considers that 21 of the 71 were on colorants linked to a polymer background (the Dynapol concept), and this approach is no longer being considered. There is little effort worldwide being devoted to the development of novel synthetic colorants, but considerable effort is being devoted to the development of colorants from natural sources.

Food colorants are one small portion of the much larger concept of food additives. The FDA has made it easy to determine the status of a food additive by publishing a book entitled *Everything Added to Food in the United States* (11). The information in the book was derived from the files of the FDA and contains 2922 total food additives of which 1755 are regulated food additives, including direct, secondary direct, color, and Generally Recognized as Safe (GRAS) additives. The book also contains administrative and chemical information on 1167 additional substances. The book is arranged alphabetically, and each compound is identified by its CAS number. The appropriate section in Title 21 of the U.S. Code of Federal Regulations for each substance is also listed. For example, Paragraph (e) of CFR 21-178.3297 provides a description and a long list of compounds that "may be safely used as colorants in the manufacture of articles or components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting or holding food, subject to the provisions and definitions set forth in this section. . . . The term colorant means a dye, pigment, or other substance that is used to impart color or to alter the color of a food-contact material, but that does not migrate into the food in amounts that will contribute to that food any color apparent to the naked eye. For the purposes of this section, the term 'colorant' includes substances such as optical brighteners and fluorescent whiteners, which may not themselves be colored, but whose use is intended to affect the color of a food-contact material." The preceding section is very general, but the sections on individual colorants are more specific. For example, talc has only one entry (21-182.90) and FD&C Red No. 40 has three (21-74.340, 21-74.1340, and 21-74.2340). The data in *Everything Added to Food in the United States* are also listed in a much broader publication entitled *Food Additives Toxicology, Regulation, and Properties*, edited by F. M. Clydesdale (12). This publication is a CD-ROM playable on Windows software.

Food colorants are an important aspect of the formulation of foods. Walford summarized the reasons for adding colorants to food today:

> 1. To give an attractive appearance by replacing the natural colour destroyed during processing or anticipated storage conditions.
> 2. To give colour to those processed foods such as soft drinks, confectionery and ice creams, which otherwise would have little or no colour.
> 3. To supplement the intensity of the natural colour where this is perceived to be weak.
> 4. To ensure batch to batch uniformity where raw materials of different source and varying colour intensity have been used. (1)

With the increased emphasis on increasing food production for an ever-increasing population, and the necessity to make food appealing and acceptable, food colorants will remain a major concern. They already are an important part of the economy since Pszczola estimated that the worldwide market for colorants in 1998 was about 939 million U.S. dollars (13). Of this 65% was represented by natural colorants.

BIBLIOGRAPHY

1. J. Walford, "Historical Development of Food Coloration," in J. Walford, ed., *Development of Food Colours*-1, Applied Science, London, 1980, pp. 1–25
2. F. Accum, *A Treatise on the Adulteration of Food and Culinary Poisons*, London, 1820.
3. A. H. Hassell, *Food and Its Adulterants: Comprising the Reports of the Analytical Sanitary Commission of the Lancet 1857*, London, 1857.
4. D. M. Marmion, *Handbook of U.S. Colorants for Foods, Drugs and Cosmetics*, 2nd ed., Wiley-Interscience, New York, 1984.
5. L. E. Parker, "Regulatory Approaches to Food Coloration," in J. Walford, ed., *Developments in Food Colours-2*, Applied Science, London, 1984, pp. 1–22.

6. J. B. Hallagan, D. C. Allen, and J. F. Borzellaca, "The Safety and Regulatory Status of Food, Drug and Cosmetic Color Additives Exempt from Certification," *Food Chem. Toxicol.* **33**, 515–528 (1995).
7. F. J. Francis, *Handbook of Food Colorants*, Eagen Press, St. Paul, Minn., 1999.
8. *A Proposed Food Safety Evaluation Process. Food Safety Council. Final Report*, Nutrition Foundation, Washington D.C., 1982
9. F. J. Francis, "Safety of Natural Food Colorants," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie, Glasgow, Scotland, 1996, pp. 112–130.
10. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.
11. U.S. Food and Drug Administration, *Everything Added to Food in the United States*, CRC Press, Boca Raton, Fla., 1993.
12. F. M. Clydesdale, *Food Additives: Toxicology, Regulation, and Properties* [CD ROM], CRC Press, Boca Raton, Fla. 1997.
13. D. E. Pszczola, D. E. "Natural Colors Pigments of Imagination," *Food Technol.* **52**, 70–72 (1998).

COLORANTS: ANTHOCYANINS

The anthocyanins are probably the best known of the natural pigments. They are ubiquitous in the plant kingdom and are responsible for many of the orange, red, blue, violet, and magenta colors in plants. Their very visibility, combined with their role as taxonomic markers, has attracted the efforts of many research workers in the past 75 years. Their use as a colorant goes back to antiquity. The Romans used highly colored berries to augment the color of wine. The American Indians added cranberries to a "trail-stable" product called pemmican. Pemmican is a high-calorie mixture of dried meat strips, fat, and berries. The cranberries did provide an attractive color and flavor, and probably the highly acid cranberries improved storage stability. Certainly the acid would remove the possibility of botulism. Cranberries also contain the well-known preservative benzoic acid. Pemmican may be one of the earliest "fast foods" in the United States.

In view of the ubiquity of the anthocyanins and their high tinctorial power it comes as no surprise that many sources have been suggested as colorants. Francis listed more than 40 plants as potential sources with their pigment profiles and methods of extraction (1,2). Francis also listed 49 patents on anthocyanin sources (3). However, despite the large number of potential sources, only two have had commercial success, grapes and, more recently, red cabbage. Colorants from grapes have been available for nearly 120 years primarily from press cake as a by-product of the wine industry. Grapes are the world's largest fruit crop for processing. The Food and Agriculture Organization (FAO) 1989 estimate of annual production was 60,000,000 metric tons, of which about 80% was used for wine making. This ensures a limitless source of inexpensive raw material for colorant production.

The anthocyanins are chemically similar to the much larger group of flavonoids and are usually included in the general classification of flavonoids. Anthocyanins are usually orange-red in color, whereas the other flavonoids are usually colorless to yellow.

CHEMICAL COMPOSITION

The composition of any colorant containing anthocyanins will reflect the composition of the raw material. At present it is grapes and red cabbage, but other sources may become available in the future. About 275 anthocyanins are known currently and are part of about 5000 flavonoid compounds of similar chemical structure (4). The anthocyanins are composed of an aglycone (anthocyanidin), sugar, and perhaps organic acids. Twenty-two aglycones are known, of which 18 occur naturally. Only 6 are important in foods, and the structure of these is shown in Figure 1. Free aglycones occur very rarely in plants and are nearly always combined with sugars. One reason for this is that the sugars stabilize the molecule. In order of relative abundance, the sugars are glucose, rhamnose, galactose, xylose, arabinose, and glucuronic acid. Anthocyanins may also be acylated, which adds a third component to the molecule One or more molecules of *p*-coumaric, ferulic, caffeic, malonic, or acetic acids may be esterified to the sugar molecule. The aglycones in grapes are cyanidin, peonidin, malvidin, petunidin, and delphinidin with the organic acids acetic, coumaric, and caffeic (5). The only sugar present is glucose. Grapes usually have a very complex anthocyanin profile with the Concord variety having 31, the greatest number in any single variety. The 15 anthocyanins in red cabbage contain only cyanidin and glucose with the acids ferulic and coumaric.

Plants that contain anthocyanins also invariably contain flavonoids, and the distribution of flavonoids is more widespread than the anthocyanins. In view of the large number of flavonoids (5000), the pigment profile is usually characteristic of the plant family. A number of other polyphenolic compounds are also present in grapes (6). The polyphenolic compounds in grapes, in view of their importance in wines, have received much more research attention than those in red cabbage but the latter can be assumed to also have a complex polyphenol profile. A typical compound from each group of compounds is shown in Figure 2. The composition of grape extracts is further complicated by polymers produced by association with catechins and flavonoids with or without the interaction with acetaldehyde. Actually most of the tinctorial power in enocyanin preparations is due to the complexed anthocyanins. The overall conclusion is that it is impossible to specify accurately the composition of enocyanin preparations.

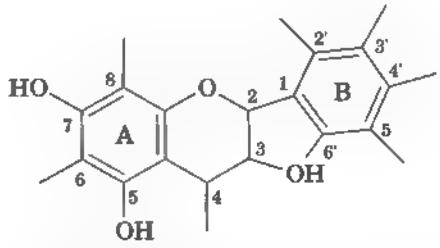


Figure 1. The anthocyanidin nucleus. Pelargonidin (4′ OH); cyanidin (3′,4′ = OH); delphinidin (3′,4′,5′ OH); peonidin (4′ = OH, 3′ = OMe); petunidin (4′,5′ OH, 3′ = OMe); malvidin (4′ = OH, 3′,5′ = OMe); all other substitution positions = H.

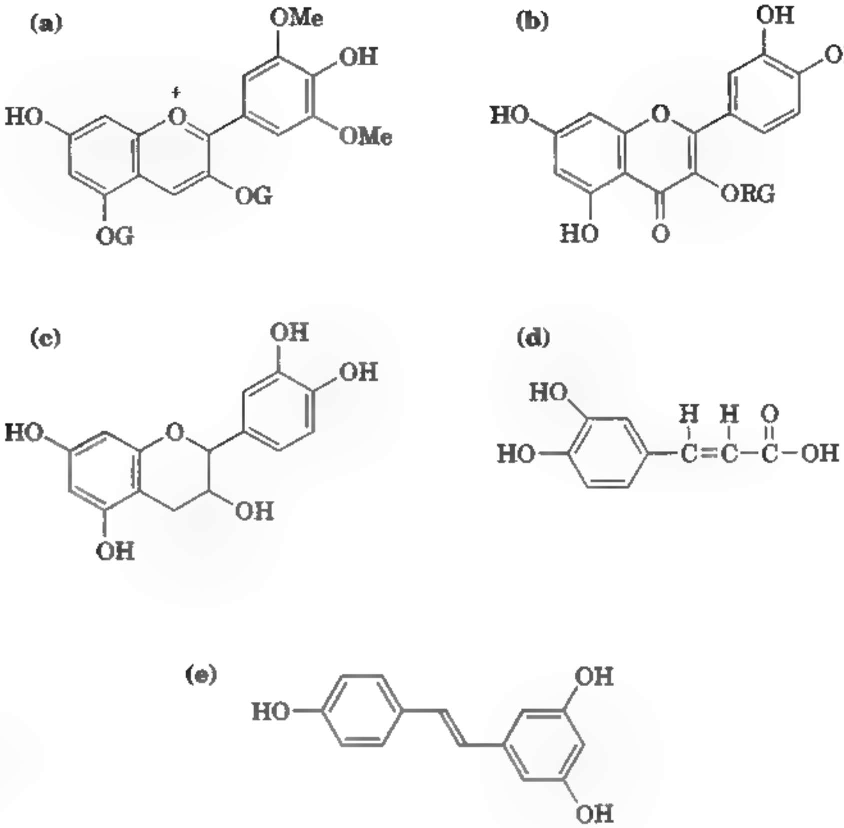


Figure 2. Typical components of grape colorants extracts. (**a**) A red anthocyanin, malvidin-3,5-diglucoside; (**b**) a yellow flavonoid, quercetin-3-rhamnoglucoside, (rutin); (**c**) a component of tannin catechin; (**d**) a phenolic acid, caffeic acid; (**e**) a stilbene phytoalexin, resveritol.

Commercial specifications are usually confined to tinctorial power, total acidity, %solids, %ash, heavy metals, sulfur dioxide, tannins, and alcohol. The tinctorial strength of grape extracts is often expressed as the absorbance of a 1% solution in a 1-cm cell at 520 nm in citrate buffer at pH 3. The tinctorial power of red cabbage extracts is sometimes expressed as the absorbance of a 10% solution in a 1-cm cell measured in citrate buffer at pH 3 (eg, E 10%, 1 cm = 190).

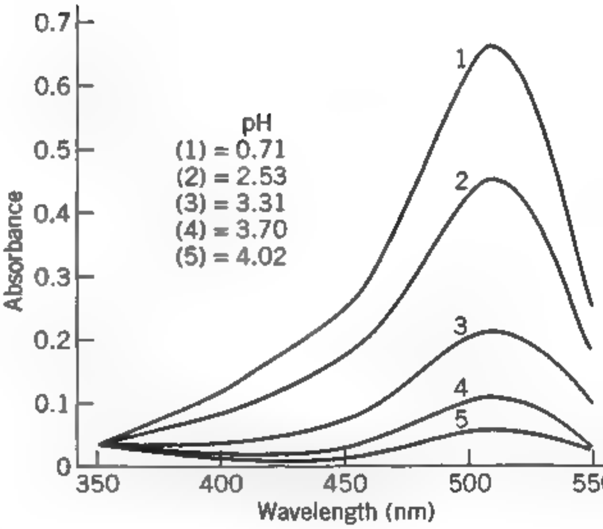


Figure 3. Absorption spectra of cyanidin-3- rhamnoside in buffer solutions at pH values of 0.71–4.02. The concentration of the pigment is 1.6×10^2 g/L.

COMMERCIAL PREPARATION

The most common commercial method involves treatment of the skins with water containing up to 3000 ppm of sulfur dioxide or its equivalent in bisulfite or metabisulfite. After 48 to 72 h, the liquid is removed from the skins, filtered, desulfured, and concentrated. Sometimes a fermentation step is allowed prior to the extraction, in which case the alcohol is removed during subsequent processing. The final product is a particle-free liquid of high tinctorial power. The concentrated liquid may also be dried to produce a soluble dry powder. The presence of sulfur dioxide in the extracting medium results in increased extraction of the pigments as well as increased stability of the final product. The sulfite-anthocyanin complex is colorless, so the sulfite must be removed prior to the final concentration and filtration step. Addition of acid makes the extraction step more efficient, but addition of mineral acid requires neutralization at some point. The addition of tartaric acid can be removed by addition of potassium hydroxide since the resulting precipitate of potassium hydrogen tartrate can be easily removed by filtration. Purification of the crude extract can be accomplished by passing the extract through an ion-exchange resin bed with the added advantage that the colorant eluate can be fractionated. The first portion of

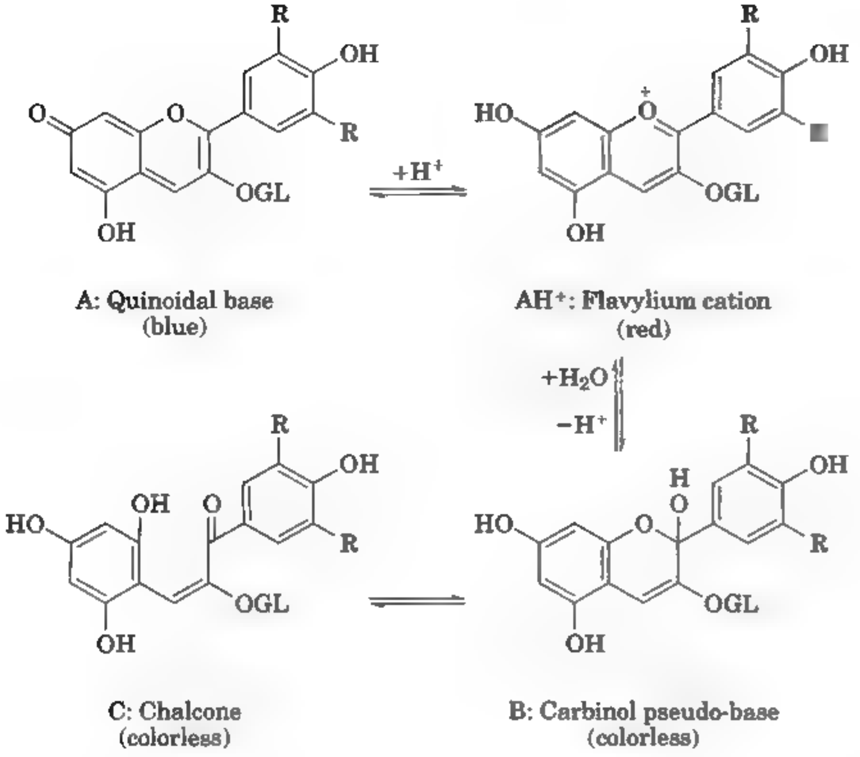


Figure 4. Structural changes in anthocyanins with pH. Malvidin-3-glucoside at 25°C.

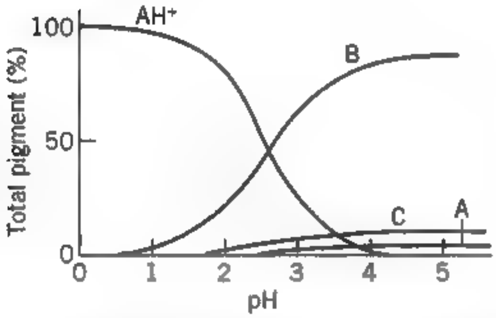


Figure 5. Effect of pH on the distribution of anthocyanin structures of malvidin-3-glucoside. A, B, C, and AH⁺ refer to the forms in Figure 4.

the eluate will be redder than the bluer trailing portion, because the higher molecular weight compounds will go through the resin bed at a slower rate. Treatment with resins produces a purer product but at a higher price. The FDA-approved procedures for extraction are specified in the CFR Section 73.170 for Grape Skin Extract.

Extraction of grape skins is much more efficient with acidified methanol or ethanol, but this requires another step to remove the alcohol. Wineries are usually familiar with handling alcohol, but other potential colorant producers seem reluctant to put an alcohol recovery unit in a food plant. Regardless, alcohol extraction does not seem to have commercial appeal.

Current emphasis is on attempts to make colorants containing anthocyanins more stable. Considerable research emphasis has been on the copigmentation or association of anthocyanins with themselves or with a series of other compounds such as flavonoids, polysaccharides, proteins, tannins, and other polyphenolic compounds.

SYNTHETIC COMPOUNDS

Studies on synthetic compounds related to the anthocyanins have shown that methyl or phenyl moieties substituted at the C-4 position in the molecule are virtually resistant to nucleophilic attack at the C-2 position. This observation led to the suggestion that a number of synthetic analogues would prove to be very stable (7). The first natural C-4 substituted anthocyanin reported was purpurinidin fructo-glucoside isolated from willow bark, but shortage of raw material has precluded its commercial development (8). A second group of naturally occurring C-4 substituted anthocyanins was reported by Cameira dos Santos et al. in red wine (9). The new pigments contain a vinylphenol group attached to positions 4 and 5 of the grape anthocyanins and were named anthocyanin-3-glucoside adducts. Sarni-Manchado et al. prepared the vinylphenol adducts of five of the major anthocyanins of wine, namely, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside and tested their stability at 55°C in a model beverage at pH 3 and 5 (10). The adduct pigments were much more stable than the original pigments, particularly at pH 5. The adducts also were unaffected by SO_2. The color of the adducts was slightly more orange than the corresponding anthocyanins. The authors did not state the concentration of the adducts found in red wine. Chemical synthesis of a number of C-4 substituted analogues of anthocyanins is feasible but obviously would require testing for safety and legislative approval.

COMMERCIAL APPLICATIONS

In view of the large body of research on the appearance, stability, analysis, and plant breeding of anthocyanins, that occur naturally in fruit and vegetable products, it is not surprising that anthocyanin colorants would be used to enhance the aesthetic appeal of existing plant products or formulated substitutes. Actually, one of the first applications of enocyanin, a generic term for colorants from grapes, was to improve the color of wine. In the United States, fruit drinks are the biggest market. Colorants containing anthocyanins have been suggested for beverages, jellies, jams, ice cream, yogurt, gelatin desserts, canned fruits, fruit sauces, candy and confections, bakery fillings, toppings, drink-mix crystals, pastries, cosmetics, and pharmaceuticals.

Anthocyanin colorants have their greatest color intensity at pH values between 2 and 4. Figure 3 shows the changes in absorption of cyanidin-3-rhamnoglucoside as the pH is changed from 0.71 to 4.02. Figure 4 shows the chemical changes that accompany a pH change Figure 5 shows the amounts of each pigment present at each pH value. It is possible to use an anthocyanin colorant to color a product with a pH value above 4, but the color will be bluish and considerably more colorant will be required. The reduction in color intensity as the pH is raised, known as the pH effect, is much more obvious with preparations that contain a high proportion of monomeric anthocyanins and less in preparations with a higher content of polymerized or degraded pigments, but all preparations show the pH effect to some degree. The pigments are also susceptible to degradation by light, oxygen, iron, copper, tin, ascorbic acid, and sulfur dioxide. Applications are also restricted to those with minimal heat treatment, but despite these apparent problems with stability, this group of colorants has met with good commercial success.

BIBLIOGRAPHY

1. F. J. Francis, "Miscellaneous Colorants," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie, Glasgow, Scotland, 1992, pp 242–272.
2. F. J. Francis, "Polyphenols as Natural Food Colorants," in A. Scalbert, ed., *Polyphenolic Phenomena*, INRA, Versailles, France 1993, pp. 209–220.
3. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986
4. J. B. Harborne, *The Flavonoids: Advances in Research Since 1980*, Chapman and Hall, London, 1988.
5. G. Mazza, "Anthocyanins in Grapes and Grape Products," Crit. Rev Food Sci. Nutr. **35**, 341–371 (1995).
6. F. J. Francis, *Handbook of Food Colorants*, Eagen Press, St. Paul, Minn., 1998
7. D. Amic, J. Baranac, and V. Vukadinovic, "Reactivity of Some Flavylium Cations and Corresponding Anhydrobases," *J. Agric. Food Chem.* **38**, 936–940 (1990).
8. P. Bridle, K. G. Scott, and C. F. Timberlake, "Antocyanins in *Salix* Species. New Anthocyanin in *Salix purpurea* Bark," *Phytochemistry* **12**, 1103–1106 (1973).
9. H. Fulcrand et al., "A New Class of Wine Pigments Generated by Reaction Between Pyruvic Acid and Grape Anthocyanins," *Phytochem.* **47**, 141–147 (1998).
10. P. Sarni-Manchado et al., "Stability and Color of Unreported Wine Anthocyanin-Derived Pigments," *J. Food Sci.* **61**, 938–941 (1996).

COLORANTS: ANTHRAQUINONES

COCHINEAL AND CARMINE

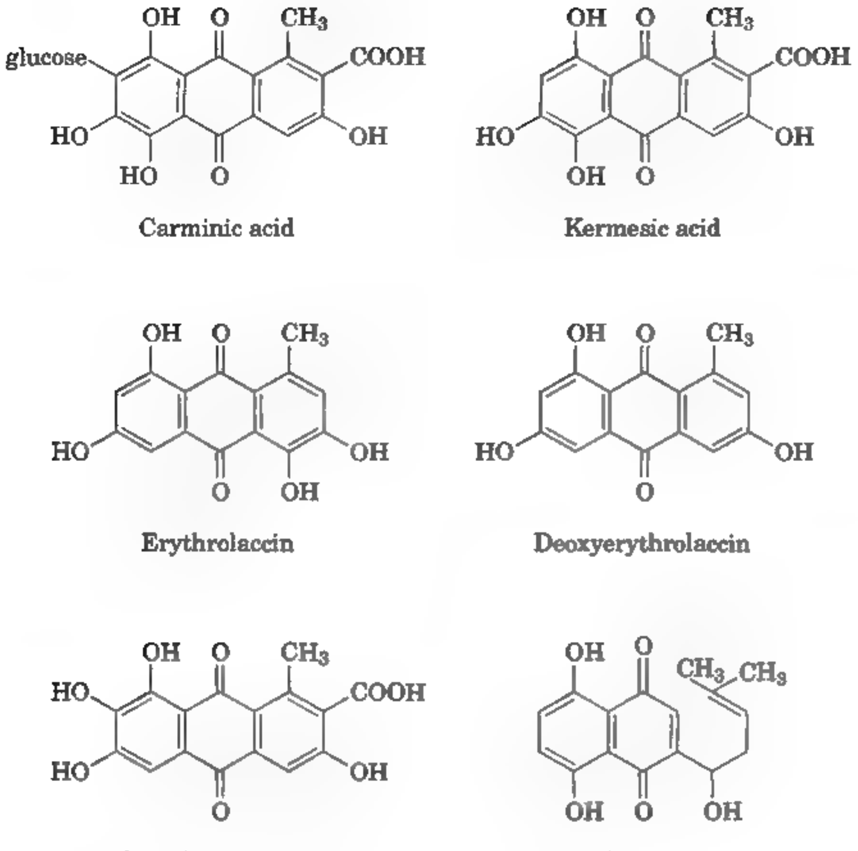
Cochineal extract is a very old colorant with references as far back as 5000 B.C. when the Egyptian women used it to color their lips. It was introduced to the Western world by Cortez when he found it in Mexico in 1518. The Aztecs had been using it for many years, and the native Mexicans were cultivating the cochineal insect on the aerial parts of cactus, *Opuntia* and *Nopalea* species, particularly *N. coccinelliferna*. The Spaniards guarded the secret of cochineal jealously, and by 1700 as much as 500,000 pounds of cochineal were being shipped to Spain from Mexico each year. This is impressive when one considers that it takes 50,000 to 70,000 insects to produce 1 lb of colorant. Other areas, including the East and West Indies, Central and South America, Palestine, India, Persia, Europe, and Africa, developed the ability to produce cochineal. The cochineal trade peaked in 1870 and then declined rapidly due to the introduction of the synthetic colorants in 1856. Peru today is the major supplier with an annual production of about 400 tons. This constitutes 85% of the world production with Mexico and the Canary Islands sharing the remaining 15%.

American cochineal is the most significant form today, but there are other sources. Similar pigments are produced by insects from the families *Coccoidea* and *Aphidoidea* and have various historical names. Armenian Red is obtained from the insect *Porphyrophera hameli*, which grows on the roots and stems of several grasses in Ajerbaizan and Armenia. Polish cochineal is obtained from the insects *Margaroides polonicus* or *P. pomonica* found on the roots of *Scleranthus perennis*, a grass found in Central and Eastern Europe.

Cochineal and its derivatives are staging a comeback today as a food colorant because of their superior technological properties and the influence of the "natural" trend.

Chemistry

Cochineal extract (CI Natural Red 4, CI No. 75470, EEC No. **E** 120) is extracted from the bodies of female insects, particularly *Dactylopius coccus* Costa, just prior to egg laying, at which time the insects may contain as much as 22% of their dry weight as pigment. One may wonder at the biological significance of this, but presumably it is a protection from predators. Historically the insects were extracted with hot water, and the colorants were known as "simple" extracts of cochineal. Later methods involved extraction with aqueous alcohol. After removal of the alcohol, the preparation, called cochineal extract, contains approximately 2 to 4% carminic acid (Fig. 1) as the main colorant



Carminic acid

Kermesic acid

Erythrolaccin

Deoxyerythrolaccin

Ceroalbolinic acid

Alkannet

Figure 1. Structures of some anthraquinones.

compound. More recently, purification methods including proteinase enzyme treatments have produced colorants sometimes known as the "carmines of cochineal." The word *carmine* has been used a general term for this class of anthraquinones, but the more usual meaning of the term carmine (CAS Reg. No. 1390-65-4) denotes a magnesium or aluminum lake of carminic acid. Carminic acid (Fig. 1) is the pigment in cochineal, and the lakes usually contain about 50% of carminic acid. The content of carminic acid is usually the way of specifying the strength, but Schul (1) suggested that the price of carmine should be based on its color strength, not on the carminic acid content, and provided details of a method. The FDA-approved procedures are specified in the CFR Section 73.100 for Carmine.

Solutions of carminic acid at pH 4 show a pale yellow color and actually have little intrinsic color at pH values below 7, but they do complex with metals to produce stable brilliant red hues. Complexes with tin and aluminum produce the most desirable hues, and nearly all commercial preparations contain aluminum. A range of hues from "strawberry" to "black currant" can be produced by adjusting the ratio of carminic acid to aluminum. The color is essentially independent of the pH value, being red at pH 4 and bluish-red at pH 10. Aluminum lakes are soluble in alkaline media and insoluble in acids. Carmine is very stable to heat and light, resistant to oxidation, and not affected by sulfur dioxide. The presence of other metal ions may shift the color slightly toward the blue.

Carminic acid is usually available as an aqueous solution with a colorant content about 2 to 5%. It may also be spray-dried. Formulations may also contain propylene glycol citric acid, and sodium citrate. Carmine is usually supplied as an alkaline solution with a carminic acid content of 2 to 7%. Traditionally, ammonia was used as an alkanizing agent, but recently formulations with potassium hydroxide, spray-dried with maltodextrin as a carrier, have become available. They may also contain sodium hydroxide, ammonium hydroxide, and glycerol. The intensity of carmine lakes is almost twice that of carminic acid; thus, they are more cost-effective as colorants.

Applications

Carmine is considered to be technologically a very good food colorant. Its only significant limitation is with products at low pH values. It is ideally suited for comminuted meat products such a sausages, processed poultry products, surimi, and red marinades. Other important uses are in jams and preserves, gelatin desserts, baked goods, confections, icings, toppings, dairy products and soft drinks. The level of usage varies with the product and is usually 0.05 to 1.0%. Cochineal and carmine are permitted as food colorants in the United States.

KERMES

Kermes is a well-known red colorant in eastern Europe. It is derived from the insects *Kermes ilicis* or *Kermococcus* *vermilis*, which are found in the aboveground portions of several species of oak, particularly *Quercus coccifera*, the "Kermes Oak." The pigment in kermes is kermisic acid, the aglycone of carminic acid (Fig. 1). It also occurs as an isomer ceroalbolinic acid (Fig. 1). The pigment is obviously closely related to carminic acid, and the properties are very similar. Colorants from kermes are not permitted in the United States.

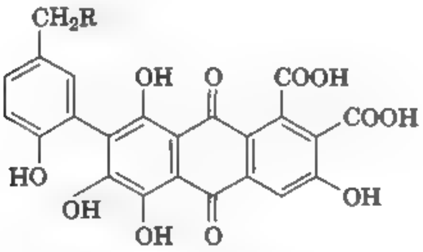
LAC

Lac is a red colorant obtained from the insect *Laccifera lacca* found on the trees *Schleichera oleosa*, *Ziziphus mauritiana*, and *Butea monosperma*, which grow in India and Malaysia. The lac insects are better known as a source of shellac. The lac pigments are a complex mixture of anthraquinones (Fig. 2). The mixture also contains the closely related pigments erythrolaccin and deoxyerythrolaccin (Fig. 2). The chemistry and applications of lac are similar to cochineal. Colorants from lac are not permitted in the United States.

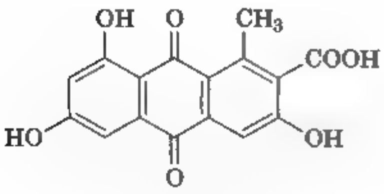
ALKANNET

Alkannet is a red pigment (Fig. 1) extracted from the roots of *Alkanna tinctoria* Taush and *Alchusa tinctoria* Lom from southern Europe. The pigment is almost insoluble in water but readily soluble in organic solvents. It has been used in Europe to color confectionery, ice cream, and wines, but it is not permitted in the United States.

The anthraquinones and napthoquinones have been the subject of considerable research interest lately. Twelve patents were issued in the 1963 to 1984 era (2), and a large number of new compounds in this class have been reported (3). Interest as food colorants stems mainly from their stability and high tinctorial strength. They were the chromophores of choice in the "linked polymer" concept of colorants pioneered by the Dynapol Company, but this concept is no longer being commercialized (2).



Laccaic acid A: R = $CH_2.NH.COCH_3$
Laccaic acid B: R = CH_2OH
Laccaic acid C: R = $CH.NH_2.COOH$
Laccaic acid E: R = $CH_2.NH_2$



Laccaic acid D

Figure 2. Structures of the anthraquinone pigments in lac.

BIBLIOGRAPHY

1. I. J. Schul, *Proceedings of the First International Symposium on Natural Colorants*, Amherst, Mass., November 7–10, 1993, The Hereld Co, Norwalk, Conn.
2. F. J. Francis, *Handbook of Food Colorant Patents*, Food ands Nutrition Press, Westport, Conn., 1986.
3. F. J. Francis, *Lesser Known Food Colorants*, in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Publishing, Glasgow, Scotland, 1996, pp. 310–341.

COLORANTS: BETALAINS

The red roots of beetroot, *Beta vulgaris*, have been known for centuries as an attractive food and as a means of imparting a desirable red color to other foods. Extracts of red beets as colorants are a relatively recent development, but the concept dates back well over 100 years. The berries of pokeweed, *Phytolacca americana*, are intensely colored with betalains and provided some insurance for the wine industry against years with poorly colored grapes. The addition of pokeberry juice to wine was forbidden in France in 1892, primarily because the juice also contains a purgatory and emetic saponin called phytolaccatoxin. The red pigment was formerly called phytolaccanin until it was established that the pigment was identical with betanin in beets (1,2). A method to remove the phytolaccatoxin from pokeberry juice was also published. There was considerable interest in the United States in the development of colorants from beets in the 1980s, but, unfortunately, it coincided with the unfolding of the role of nitrites in the formation of the toxic nitrosamines. Beets are notorious accumulators of nitrates and nitrites in the growing period, and the necessity of reducing the nitrite level was one more hurdle.

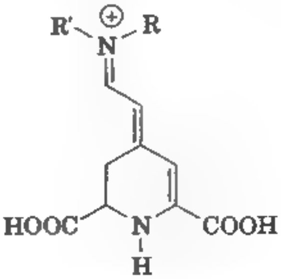


Figure 1. The diazoheptamethin structure.

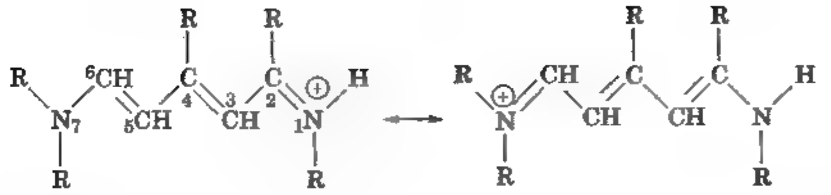


Figure 2. Betacyanin resonating structures.

CHEMISTRY

The betalain group contains about 50 red pigments called betacyanins and 20 yellow pigments termed betaxanthins. The betacyanins are all derivatives of the diazoheptamethin structure shown in Figure 1. The color is due to the resonating structures in Figure 2. If the R or R′ group extends the resonance, the pigment is red as in betanin (Fig. 3). If the resonance is not extended, the pigment is yellow as in vulgaxanthin (Fig. 4). The betacyanins contain the aglycones, betanidin, isobetanidin, and the rarer forms neobetanidin and 2-decarboxybetanidin (Fig. 5). The aglycones are combined usually with glucose and much less frequently with the disaccharide sugars sophorose and rhamnose. The betacyanin molecule may also be acylated with sulfuric, malonic, 3-hydroxy-3-methylglutaric, citric, *p*-coumaric, ferulic, caffeic, and synapic acids. A number of other betalains have been reported, and it is likely that more will be isolated, but those that are most important from a food colorant point of view are betanin for the red colors and vulgaxanthin-1 and vulgaxanthin-2 for the yellow colors.

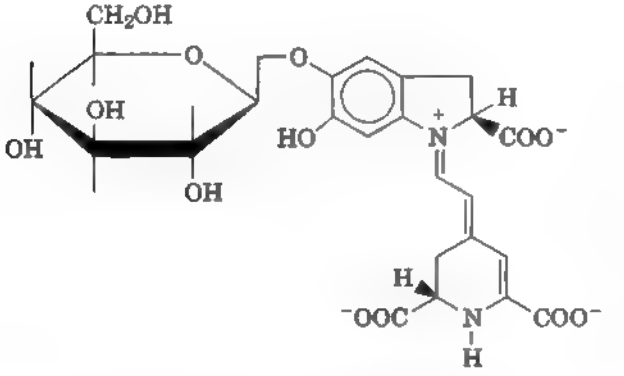


Figure 3. The structure of betanin.

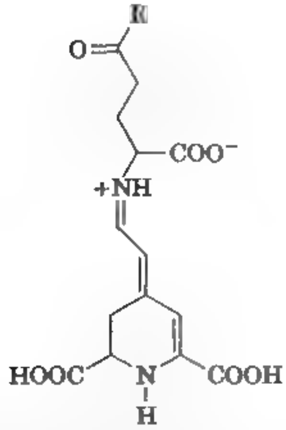


Figure 4. Structures of vulgaxanthin-1 ($R = NH_2$) and vulgaxanthin-2 ($R = OH$).

OCCURRENCE

The betalains are confined to 10 closely related families of the order *Caryophyllales*. The only foods containing betacyanins are the red beet, *B. vulgaris*; chard, *B. vulgaris*; cactus fruit, *Opuntia ficus-indica*; and pokeberries, *P. americana*. Pokeberries are not a normal food source, but the leaves are eaten as a green vegetable. Betalains are also found in a number of flowers and the poisonous mushroom, *Amanita muscaria*, but again these are not normal food sources. Their importance as a food colorant derives solely from extracts of red beets. Red beets contain 75 to 95% betanin with the remainder isobetanin, prebetanin, and isoprebetanin. The last two are the sulfate monoesters of betanin and isoprebetanin (Fig. 5), respectively. Beets contain both the red betacyanins and the yellow betaxanthins, but cultivars are available with different ratios of the red and yellow pigments. Cultivars also are available that contain only the yellow betaxanthins. Cultivars of red beets with a wide ratio of red to yellow pigments have been developed. Thus it is possible by selecting appropriate cultivars of red beets and/or blending with extracts of yellow beets to provide a range of red to yellow colorants.

The anthocyanin/flavonoid and betacyanin/betaxanthin groups are mutually exclusive in the plant kingdom. Anthocyanins and betacyanins have never been reported in the same plant.

PREPARATION

Commercial preparations from red beets are currently restricted in most countries to two products. Liquid concentrates can be prepared by pressing blanched ground beets, filtering, and concentrating under vacuum to 60 to 65% total solids. Powders can be prepared by spray-drying the liquid concentrate. The Food and Drug Administration (FDA) approved extraction procedures are described in the 21 CFR Section 73.400 for Beet Powder. Beet juice contains considerable sugar, so a yeast fermentation is sometimes included to reduce the sugar content. The alcohol produced in the fermentation step would have to be removed. Beet juice usually exhibits a beetlike taste and odor due to the

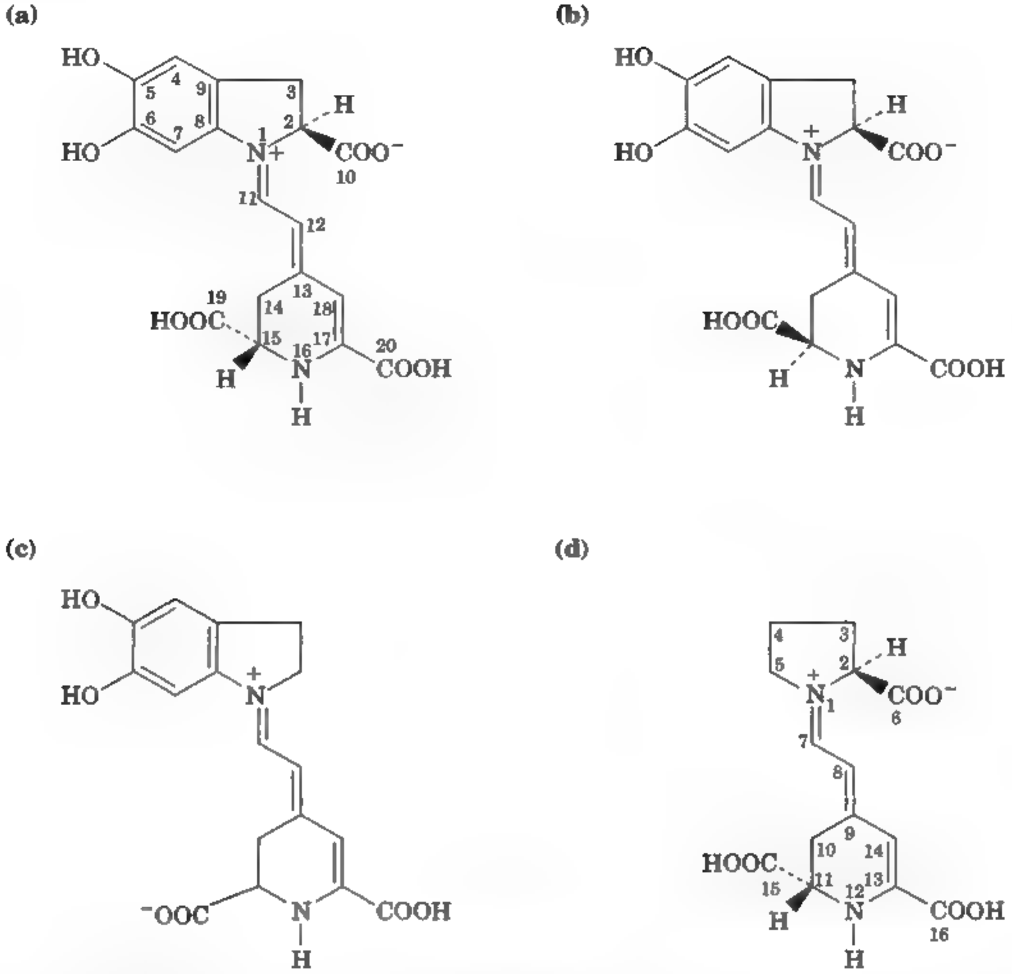


Figure 5. Structures of betanidin (**a**), isobetanidin (**b**), 2-decarboxybetanidin (**c**), and indicaxanthin (**d**).

presence of geosmin. Fermentation with *Aspergillus niger*, *Candida utilis*, and *Saccharomyces oviformis* has been reported to produce a product with improved stability and also to remove the geosmin. Traditionally, hydraulic or rotary presses have been used, but they recover only about 50% of the pigment. Recoveries as high as 90% can be accomplished by continuous diffusion processes. A number of resin and other absorption processes such as Dowex 50 W followed by polyamide column chromatography with methanol as the eluent, polyacrylamide (Bio-Gel P-6), and numerous others (3) are available for purification of the pigments, but they are not usually permitted by legislation. *In vitro* cell suspensions have been reported to yield high levels of pigment production, and they have a number of advantages. The high growth rates of cultures makes pigment production efficient. The characteristic odor of geosmin is not produced. A number of pigments can be produced selectively as desired. Economic commercialization of cell culture production systems has yet to be demonstrated. But there is a need for more highly purified and concentrated colorants.

Commercial beet powders usually contain 0.4 to 1.0% pigment expressed as betanin, 80% sugar, 8% ash, and 10% protein together with citric and/or ascorbic acid as a preservative. The tinctorial power is usually expressed as % betanin. Betanin is identified as EEC No. E 162 or CAS Reg. No. 7659-95-2.

APPLICATIONS

Betanin preparations are water soluble with high tinctorial strength. They are relatively unchanged in color from pH 3 to 7 but are violet at pH values below 3 and bluer at pH values above 7. The absorption spectra of betanin at three pH values are shown in Figure 6 (4). A shift in dominant wavelength toward the left would indicate a yellower color and to the right a bluer color. The spectrum at pH 9 does show a small shift in color toward the bluish-red. Both the red and yellow pigments are thermolabile with and without the presence of oxygen and are also degraded by light. Metal cations such as iron, copper, tin, and aluminum accelerate the degradation. With these limitations, beetroot preparations may be used ideally to color products with short shelf life, which are packaged to reduce exposure to light, oxygen, and high humidity; do not receive

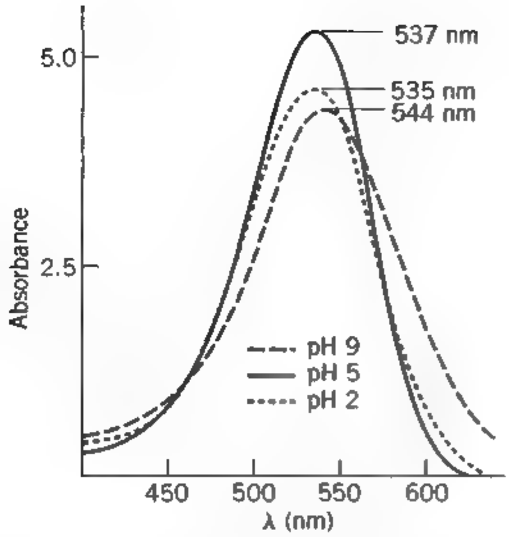


Figure 6. Spectra of aqueous betanin solutions at pH 2, 5, and 9.

extended or high heat treatment; and are marketed in the dry state (3). Beet extracts have been suggested for dairy products such as ice cream and yogurt, salad dressings, frostings, cake mixes, gelatin desserts, meat substitutes, poultry meat sausages, gravy mixes, soft drinks, powdered drink mixes, marshmallow candies, hard candies, and fruit chews. Betanin colorants can also be blended with other colorants to provide desired color matches. Extracts from beets are the only betalain colorants permitted in the United States today.

BIBLIOGRAPHY

1. M. W. Driver and F. J. Francis, "Stability of Phytolaccanin, Betanin, and FD & C Red No. 2 in Dessert Gels," *J. Food Sci.* **44**, 518–520 (1979).
2. M. W. Driver and F. J Francis, "Purification of Phytolaccanin by Removal of Phytolaccatoxin from *Phytolaccca americana*," *J. Food Sci.* **44**, 52–523 (1979).
3. R. L. Jackman and J. L. Smith, "Anthocyanins and Betalains," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Publishers, Glasgow, Scotland, 1992.
4. J. H. von Elbe, "Stability of Betalains as Food Colors," *Food Technol.* **29**, 42–46 (1975).

COLORANTS: CARAMEL

The heating of sugar preparations to create brown, flavorful, and pleasant-smelling products has been practiced in home cooking for centuries. The sauces or candies are known as caramels, but commercial practices to prepare caramel colorants began in Europe about 1850. The first caramel colorants were prepared by heating sugars in an open pan, but in view of their popularity, modifications were soon introduced. Caramel colorants that were compatible with various food products were desired, and essentially compatibility consists of the absence of flocculation and precipitation in the food. These undesirable effects result from colloidal interactions of charged macromolecular components of caramel with a particular food. Hence, the net ionic charge of the caramel color macromolecules at the pH of the product in which it is used is a prime determinant of compatibility (1).

Caramel (CI Natural Brown 10, EEC No. E 150) is a brown colorant obtained by heating sugars. The official FDA definition is "The color additive caramel is the dark brown liquid or solid resulting from carefully controlled heat treatment of the following food-grade carbohydrates: dextrose, invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions thereof, or sucrose."

COMMERCIAL PREPARATION

An appropriate amount of carbohydrate is added to a reaction vessel at 50 to 70°C, the temperature is raised to 100°C, and the reactants are added. After appropriate heating the mass is cooled and filtered, and the pH and specific gravity are adjusted with acids, alkalies, or water to meet customer specifications. The carbohydrate hydrolysates may be obtained from sucrose, corn, wheat, and tapioca. High glucose content is desirable because caramelization only occurs through the monosaccharide. The type and concentration of reactant determines the properties of the caramel colorant. Caramels can be prepared by using carbonates, hydroxides, ammonium compounds, sulfites, and both sulfite and ammonium compounds. There is a direct relationship between reactant concentration and color intensity. Too little produces an underreacted product, and too much produces a product that is too viscous or even solid.

CHEMISTRY

In 1980 the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO)/World Health Organization (WHO) recommended that further information on the chemical properties of caramel be obtained to establish a suitable classification and specification system. This reaction is common to a number of natural extracts and also to some manufactured colorants. For example, with the colorants from grapes, no manufacturer has attempted to provide a chemical profile of the components of the extract. This is understandable in view of the complexity of the chemical profile. A somewhat similar situation occurs with a synthesized product such as FD&C Yellow No. 5. When it was approved for food use in 1916, the manufacturers wanted to produce as pure a product as possible. The impurities they had to cope with were unreacted ingredients and about three compounds produced by side reactions. In the usual process of chemical synthesis, the desired reaction does not normally go to 100% completion with no side reactions, and it is the job of the manufacturer to remove the unwanted by-products. But the increasing sophistication of analytical procedures that can routinely detect one part per billion, or even as low as one molecule, has allowed researchers to detect more impurities. Today, FD&C Yellow No. 5 has been shown to have as many as 17 components present, some in exceedingly low concentrations (2). The situation with other FD&C colorants is probably similar. The same situation exists with synthetic β-carotene. Modern analytical instrumentation has made it possible to identify the impurities in chemicals produced with rigid process control as illustrated with FD&C Yellow No. 5 and β-carotene. The situation with caramel and colorants produced from grapes is different in that it is not possible to specify in detail the structures of all the compounds present.

The question that arises with knowledge of the impurities is, What do they mean from a safety point of view? It also emphasizes that the chemical profile of a given additive in routine manufacture be the same as that in the batches used for toxicology studies. It is economically unfeasible to remove all impurities in food additives; thus, some form of risk assessment is unavoidable. Fortunately all four products mentioned earlier have been given a clean bill of health.

The JECFA wanted data on caramel for the reasons just described. The International Technical Caramel Committee (ITCA) undertook an extensive research program to provide this information. This resulted in the grouping of the caramel formulations into four classes depending on the net ionic charge and the presence of reactants.

Class	Charge	Reactants
I	−	No ammonium or sulfite compounds
II	−	Sulfite compounds
III	+	Ammonium compounds
IV	+	Both sulfite and and ammonium compounds

It is obvious that heating carbohydrates in the presence of a reactant would produce a wide range of chemical compounds. Complete characterization is not feasible; thus, a series of profiles were developed using 157 samples from 11 manufacturers in seven countries. Since Caramel Color IV accounts for 70% of all caramel colors manufactured, it was chosen as a prototype for this ambitious undertaking. The profiles developed were HPLC screening, size fractionation by ultrafiltration to provide low molecular weight, intermediate molecular weight and high molecular weight groupings, and subfractionation by cellulose chromatography. Each fraction was examined by a series of sophisticated analytical approaches. These data combined with 11 physical characteristics confirmed that the four groupings provided real and reproducible classifications (3).

Color is a physical parameter of interest to the formulator. It could, of course, be specified in any of the conventional tristimulus color scales, but it is possible to specify the color accurately by a simpler method (4). The color of caramel is due to a large number of chemical chromophores; thus, different formulations do not show markedly different spectral curves and do not change shape with concentration. Curves of different concentrations can be superimposed on each other by plotting log absorbency against concentration. This is the "optical signature" of a colorant. When log absorbency is plotted against wavelength for caramel solutions, the result is a straight line that moves up or down depending on the concentration. The slope of the line will change according to the hue of the solution. This makes it possible to determine two colorimetric indices, the Hue Index and the Tinctorial Power, from two simple absorbency measurements

The Hue Index is:

$$10 \log(A_{0.51}/A_{0.61})$$

where 0.51 is the absorbency at 0.51 microns, and 0.61 is the absorbency at 0.51 microns.

The Tinctorial Power is:

$$K^{0.56} = \frac{A_{0.56}}{cb}$$

where 0.56 is the absorbency at 0.565 microns, c is the concentration (g/L), and b is the cell thickness (cm). Both the Hue Index and the Tinctorial Power should reflect the visual appearance over the whole visual spectrum. The preceding equations are reproduced as they appeared in the original publication, but today the term nanometers would be used instead of microns.

One of the major considerations in this research was the safety aspect of the caramel colorants. This program resulted in 11 papers being published in the same 1992 Vol. 30 issue of the journal *Food Chemical Toxicology*, and 7 of them were on toxicology. Caramel was given a clean bill of health and the JECFA assigned an Acceptable Daily Intake (ADI) of 0 to 200 mg/kg/day.

APPLICATIONS

Caramel color is freely soluble in water but insoluble in most organic solvents. In concentrated form the colorant has a characteristic burnt taste, which is not detectable at the levels normally used in foods and beverages. Commercial preparations vary from 50 to 70% total solids with a range of pH values depending on the type of caramel. More than 80% of the caramel produced in the United States is used to color soft drinks, particularly colas and root beers. Type I is designed for use with distilled spirits, desserts, and spice blends. Type II is used with liqueurs. Type III is suitable for baked goods, beer, and gravies. Type IV is used in soft drinks, pet foods, and soups.

BIBLIOGRAPHY

1. D. V. Myers and J. C. Howell, "Characterization and Specifications of Caramel Colours: An Overview," *Food Chem. Toxicol.* **30**, 359–363 (1992).
2. J. E. Kassner, "Modern Technologies in the Manufacturer of Certified Food Colors," *Food Technol.* **41**, 74–76 (1987).
3. B. H. Licht, J. Orr, and D. V. Myer, "Characterization of Caramel Color IV," *Food Chem. Toxicol.* **30**, 365–373 (1992).
4. R. T. Linner, "Caramel Coloring: A New Method of Determining its Color Hue and Tinctorial Power," *Amer. Soft Drink J.*, 26–30 (1971).

COLORANTS: CAROTENOIDS

The carotenoids are one of the largest group of pigments produced in nature, second only to the chlorophylls. They are very widespread with more than 100,000,000 tons produced annually in nature. Most of this amount is produced in the form of fucoxanthin in algae in the ocean and the three main carotenoids of green leaves: lutein, violaxanthin, and neoxanthin (Fig. 1). Other pigments predominate in certain plants, such as lycopene in tomatoes (Fig. 1), capsanthin and capsorubin (Fig. 2) in red peppers, and bixin in annatto (Fig. 2) Colorant preparations have been made from all of these (1), and obviously the composition of the colorant extracts reflects the profile of the starting material. The chemistry and occurrence of the carotenoids is well described in the article entitled COLORANTS: CAROTENOIDS. Carotenoids are probably the best known of the food colorants derived from natural sources (2).

ANNATTO

Annatto (CI Natural Orange 4, CI No. 75120, EEC No. E 160b) is found in the outer layers of the seeds of the shrub *Bixa orellana*, a tropical plant grown in South America, India, East Africa, the Philippines, and the Carribbean. Peru and Brazil are the dominant sources of supply. Annatto is one of the oldest colorants and has been used in antiquity for coloring foods, cosmetics, and textiles. It has been used for more than a hundred years in the United States and Europe primarily as a colorant for dairy products. The colorant is prepared by leaching, with gentle mechanical friction, of the seeds with various solvents, including vegetable oil, fats, and alkali aqueous and alcoholic solutions. Depending on the application, the crude extract may be refined by precipitation with acids and/or recrystallization. Spray-dried powders are also available in both water-soluble and oil-soluble forms. The Food and Drug Administration (FDA) approved procedures for extraction are specified in the 21 CFR section 73.30 for Annatto Extract.

The pigments in annatto are a mixture of bixin, the monomethyl ester of a dicarboxylic carotenoid, and norbixin, the dicarboxylic derivative of the same carotenoid (Fig. 2). Both bixin and norbixin normally occur in the *cis* form, but small amounts of the more stable *trans* form are formed on heating. A yellow degradation product termed C_{17} yellow pigment (Fig. 2) is also produced on heating. The *cis* forms are redder than the *trans* forms or the C_{17} compound; thus, a source of red and yellow pigments are available. The carboxylic portion of the molecule contributes to the solubility in water, and the ester form contributes to oil solubility. This has provided flexibility in applications, and annatto has been used in a wide variety of applications.

Annatto is available in both water-soluble and oil-soluble liquids and powders. The oil-soluble form is somewhat unstable under oxidative conditions, and degradation is increased by exposure to light and is catalyzed by metals. Addition of antioxidants, such as ascorbic acid, tocopherols, and polyphenols, helps to minimize oxidative degradation. Annatto shows more stability to exposure to air than other carotenoids and is moderately stable to heat. Little change in color occurs with pH changes, but products with a low pH may show a pinkish tinge due to isomerization of the pigments.

LYCOPENE

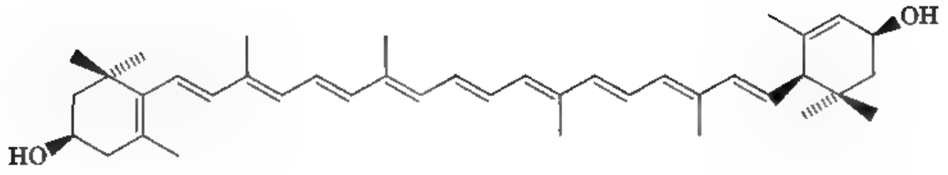
Lycopene occurs as the major (85–90%) pigment (Fig. 1) in red tomatoes, *Lycopersicum esculentum*. The other pigments are β-carotene (10–15%) with small quantities of about 10 other carotenoids. In spite of the fact that large quantities of lycopene are available in the waste from the tomato-processing industry, colorants containing lycopene were not commercially available. This was probably due to the belief that lycopene was susceptible to degradation by oxidation and light. But recently a combination of better manufacturing practices, and the development of a tomato cultivar particularly high in lycopene led to the commercialization of lycopene as a food colorant. Preparation of lycopene extracts from tomatoes is relatively simple, involving an alkali saponification and extraction with a mixture of solvents such as acetone and hexane. The acetone can be removed by washing with water and the hexane by vacuum treatment, leaving a relatively pure lycopene extract. The extract would, of course, contain the other carotenoids in the tomato. Commercialization probably was also helped by the health aspects because it may be an efficient *in vivo* radical scavenger. Lycopene preparations are being marketed as a neutraceutical and as a food colorant. Colorant preparations containing lycopene are currently not allowed in the United States.

PAPRIKA

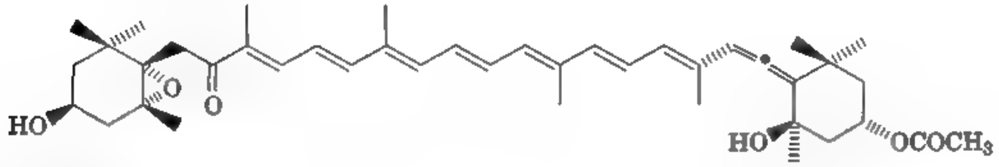
Paprika is a deep red pungent powder prepared from the ground dried pods of the sweet pepper *Capsicum annum*. Paprika is produced in many warm countries around the world, and several areas have developed products with specific characteristics such as the Hungarian paprika and the Spanish paprika. The same peppers are used in salads and as a source of pimento. The other principal type of red pepper (also *C. annum*), typically called cayenne pepper or cayenne, is usually much more pungent in flavor. Both types are highly pigmented. The red pepper *C. frutescens* is the source of the highly colored and very pungent Tabasco sauce.

Paprika contains capsanthin and capsorubin (Fig. 2) occurring mainly as the lauric acid esters. Smaller quantities of about 20 other carotenoids are also present. Specifications do not attempt to describe the pigment profile but usually specify color strength by the American Spice Trade Association (ASTA) Color Value. This is essentially the absorption at 460 nm in acetone measured against a cobalt solution or a glass standard used as a reference (3).

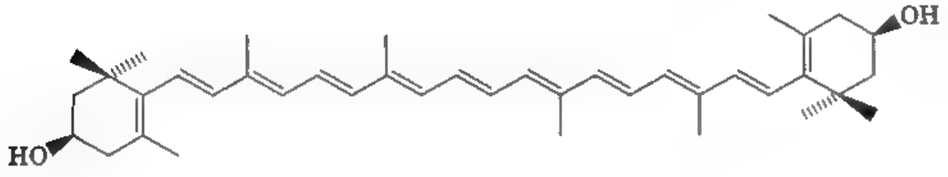
Paprika oleoresin (EEC No. E 160c) is an orange-red oil-soluble extract from *C. annum*. The FDA-approved procedures are specified in 21 CFR Section 73.345 for Paprika Oleoresin. The dried and ground red peppers are extracted with a volatile solvent, usually a chlorinated hydrocarbon



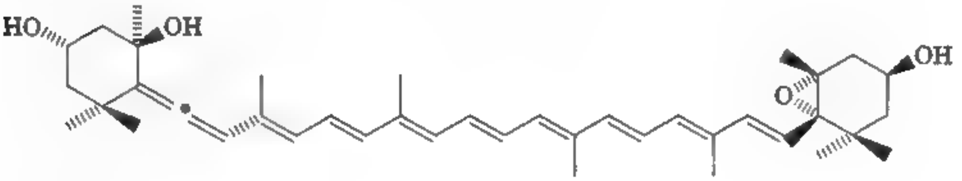
Lutein


O
O
HO
HO
OCOCH3

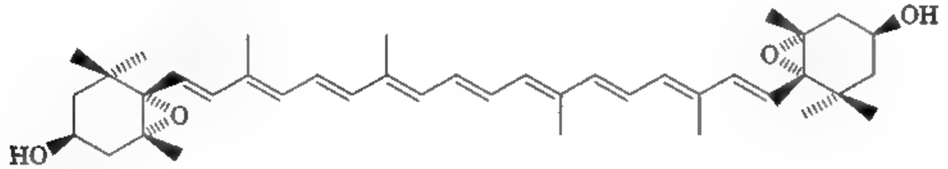
Fucoxanthin



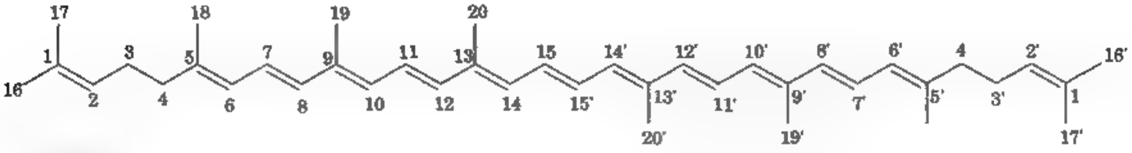
Zeaxanthin



Neoxanthin

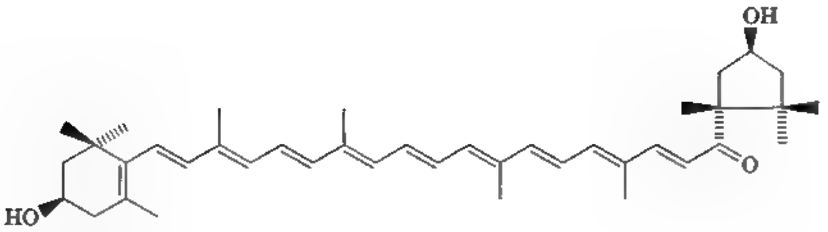


Violaxanthin

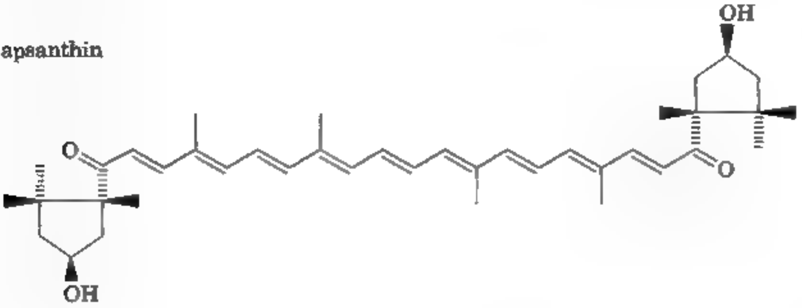

17
18
19
20
1
3
5
7
9
11
13
15
14'
12'
10'
8'
6'
4
2'
16'
16
2
4
6
8
10
12
14
15'
13'
11'
9'
7'
5'
3'
1
20'
19'
17'

Lycopene

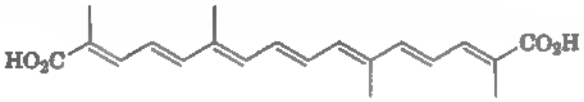
Figure 1. Selected structures for the carotenoids in plants.



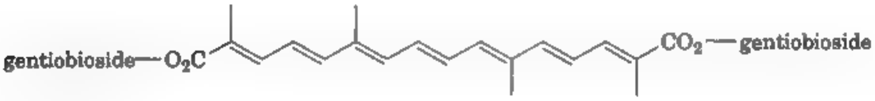
Capsanthin



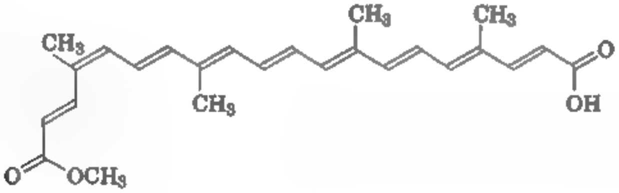
Capsorubin



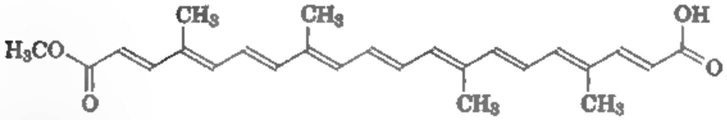
Crocetin


gentiobioside—O$_2$C
CO$_2$—gentiobioside

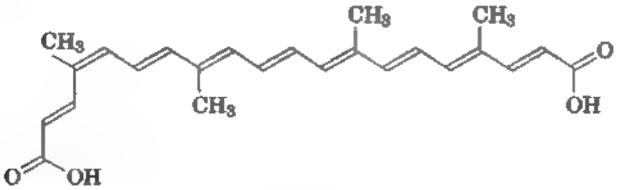
Crocin


CH$_3$
CH$_3$
CH$_3$
CH$_3$
O
OH
O
OCH$_3$

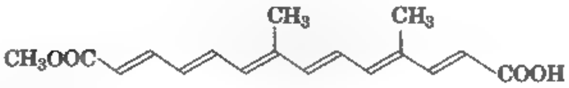
Bixin


H$_3$CO
O
CH$_3$
CH$_3$
OH
O
CH$_3$
CH$_3$

Trans-bixin


CH$_3$
CH$_3$
CH$_3$
CH$_3$
O
OH
O
OH

Norbixin


CH$_3$OOC
CH$_3$
CH$_3$
COOH

C_{17} Yellow Pigment

Figure 2. The carotenoids of paprika (capsanthin and capsorubin), saffron (crocetin and crocin), and annatto (bixin, transbixin, norbixin, and C_{17} yellow pigment).

followed by removal of the solvent. Preparations can also be made by extraction with a vegetable oil to provide an oil-soluble colorant. Paprika and paprika oleoresin add both color and flavor to a product; thus, the applications are usually limited to products in which both characteristics are desirable. For example, the recent rise in demand for tomato products in the form of pizza, salsa, and so on has increased the demand for paprika. Most of the spice extract used in Europe is used to flavor meat products, soups, and sauces. Smaller quantities are used in salad dressings, snacks, processed cheese, confectionery, and baked goods.

SAFFRON

Saffron (CI Natural Yellow 6, CI No. 75100) is a very old colorant dating back to the twenty-third century B.C. It has come to be known as the "gourmet spice" because of its high price, but it also provides both spice and colorant. Saffron consists of the dried stigmas of the flowers of the crocus bulb, *Crocus sativus*, grown primarily in North Africa, Spain, Switzerland, Austria, Greece, and France. The high price is due to the fact that it takes about 150,000 flowers to produce 1 kg of colorant.

The pigments in saffron are chemically similar to those in annatto (Fig. 2). They are crocetin, a dicarboxylic carotenoid, together with its gentiobioside ester. Gentiobioside is a diglucoside with a β 1–6 linkage. The sugar portion confers solubility in water and makes the colorant very flexible in its applications to a variety of food and pharmaceutical products. The same pigments occur in a number of other plants but *C. sativus* is the only commercial source. The fruits of the Cape Jasmine, *Gardenia jasminoides*, produce the same pigments and have been suggested as an alternate source. But the gardenia fruits supply only the colorant, not the spice flavor, so the sources are quite different. Saffron extracts also contain β-carotene, zeaxanthin, and traces of several other carotenoids.

Saffron preparations are fairly stable to light, oxidation, microbiological attack, and changes in pH. Overall, technically it is a good colorant with high tinctorial strength. Its strength is usually judged by its carotenoid content as measured by the absorption of an aqueous extract at 440 nm, but Alonso et al. (4) suggested that tristimulus methods were more appropriate for classifying samples of saffron.

TAGETES

Tagetes erecta L. (Aztec marigold) is an annual herb that grows from 3 to 4 tall in temperate climates. Colorants are available in three forms: dried ground flower petals, oleoresin extracts, and purified oleoresin extracts. The first two are used primarily in the poultry industry and the third in the food industry. The principal producers are Mexico, Peru, the United States, Spain, and India (5).

Tagetes petals contain up to 80% lutein with smaller amounts of zeaxanthin, cryptoxanthin, β-carotene, and about 14 other carotenoids. The lutein compounds exist as dipalmitate, dimyristate, myristate-palmitate, palmitate-stearate, and distearate esters. Because of the seasonality of the crop, the mature marigold flowers are collected and the petals separated prior to storage in large bins containing up to 80 tons. The petals are removed as needed, pressed, and then dried to less than 10% moisture. They can then be ground and sold as tagetes meal. The ground petals can also be extracted with a number of solvents, but hexane seems to be preferred. After removal of the solvent, a brown oleoresin is obtained, which can be incorporated directly into poultry feed. The resin can be further purified by saponification and sold as Saponified Marigold Extract. This product is suitable for incorporation into poultry feed. The product can be further purified to produce a dry powder suitable for use as a food colorant.

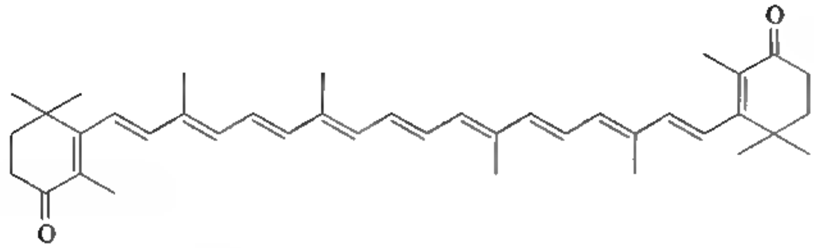
Colorants from tagetes are available in a variety of forms. Formulations for animal feeds are usually ground dried petals, oleoresins, or saponified oleoresins. Food colorants are available in a number of forms such as purified lutein esters in oil-soluble or water-soluble dispersible systems, spray-dried emulsions, gum-based emulsions, and emulsifier-based emulsions. They show good stability to heat, light, pH changes, and sulfur dioxide. They are susceptible to oxidation, which can be minimized through the addition of antioxidants such as ethoxyquin, ascorbic acid, tocopherols, BHA and BHT, and encapsulation. The strength of tagetes extracts is usually measured as the absorption of a 1%/1 cm solution in an appropriate solvent and is often quantified by calculations using the specific extinction coefficient of lutein or lutein esters.

Tagetes colorants provide yellow to orange colors suitable for use in pastas, vegetable oils, margarine, mayonnaise, salad dressings, baked goods, confectionery, dairy products, ice cream, yogurts, citrus juices, and mustard. Tagetes meal and its extracts are approved only as colorants in poultry feed in the United States.

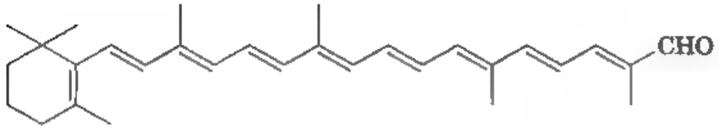
SYNTHETIC CAROTENOIDS

Plant extracts of carotenoids have been used for centuries as food and cosmetic colorants, so it was only natural that synthetic carotenoids would become available as colorants. The original synthesis of β-carotene was reported in 1950, and commercial production followed in 1954. It was followed by β-apo-8′-carotenal in 1962 and canthaxanthin in 1964. The methyl and ethyl esters of β-apo-8′-carotenoic acid, citranaxanthin, and astaxanthin followed (Fig. 3). All carotenoids by virtue of their structure are susceptible to degradation by oxidation, light, and heat. In addition, they are insoluble in water and almost insoluble in oil. But appropriate commercial preparations are currently available for almost any type of food in the yellow to red range.

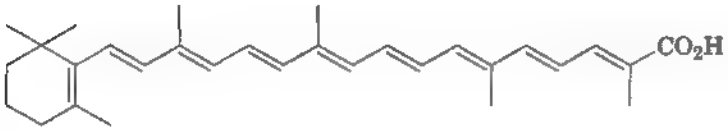
A variety of approaches are used to prepare appropriate formulations. These involve reducing particle size for suspensions in oil and stabilization with antioxidants. Three approaches are used to develop water-soluble products. These are formulation of colloidal suspensions, emulsification of oily solutions, and dispersion in suitable colloids. They can be stabilized by a wide variety of additives in the protein, carbohydrate, and lipid categories and stabilized



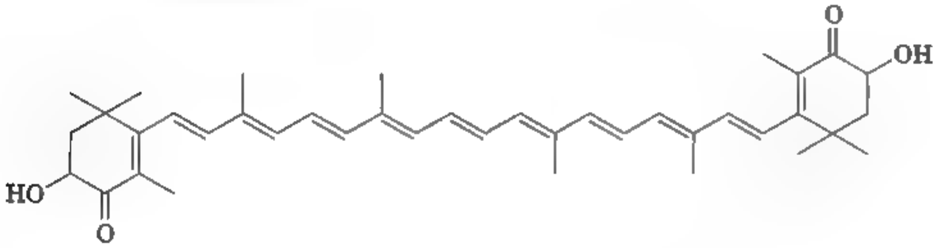
Canthaxanthin



β-apo-8'-carotenal



β-apo-8'-carotenoic acid



Astaxanthin

Figure 3. Carotenoids synthesized for commercial colorant applications. *β*-Apo-8′-carotenoic acid is usually in the form of the methyl or ethyl esters. *β*-Carotene was also synthesized.

with antioxidants (6). Carotenoid colorants are appropriate for margarines, oils, fats, shortenings, fruit juices, beverages, dry soups, canned soups, dairy products, milk substitutes, coffee whiteners, dessert mixes, preserves, syrups, confectionery, salad dressings, meat products, pasta, egg products, baked goods, and many others.

MISCELLANEOUS CAROTENOID EXTRACTS

The red oil obtained from the fruit of the palm tree *Elaeis guineensis* is very highly pigmented with about 500 mg carotenoids per kilogram of oil. The carotenoid mixture is very complex, containing mainly *β*-carotene and about 20 other carotenoids. The oil can be used as an ingredient to color margarine and other oil-based products, but its chief use is as a cooking oil due to its distinctive flavor or, after refining, as a general purpose edible oil. Xanthophyll pastes are well known in Europe and consist of extracts of alfalfa (lucerne), nettles, or broccoli. Unless they are saponified they will be green due to their high content of chlorophyll. Many concentrated xanthophyll pastes contain as much as 30% carotene with the major pigments as lutein (45%), *β*-carotene (25%), violaxanthin (15%), neoxanthin (15%), and a number of minor pigments. Highly concentrated extracts from alfalfa became available some years ago as by-products of the Pro-Xan process for preparing protein concentrates from alfalfa. The carotenoid extracts were widely promoted as colorant additives for poultry feed, but the Pro-Xan process was never commercially successful. Extracts from citrus peels have been suggested as a means of augmenting the natural color of orange juice since the more highly colored juices command a premium price, but addition of colorants from citrus is not permitted in the United States at the present time. Citrus peel extracts contain *β*-carotene, cryptoxanthin, antheroxanthin, violaxanthin, lycopene and β-apo-8′-carotenol together with a number of others. About 115 carotenoid pigments have been reported in citrus. Colorants from carrots usually contain about 80% β-carotene and up to 20% α-carotene plus small amounts of several others. Some of the high pigment strains of carrots used for colorant extracts also contain lycopene. Astaxanthin is a desirable addition to the diet of salmon and trout in aquaculture because of its ability to impart a desirable red color to the flesh. The usual sources of astaxanthin are the by-products of the lobster and shrimp processing industry, but the demand exceeds the supply. This has led to an interest in growing the red yeast *Phaffia rhodozyma* as a raw material for a concentrated extract. But unfortunately *Phaffia* produces the wrong optical isomer of astaxanthin for optimal accumulation in the flesh of salmon. Hinostroza et al. (7) reported that carotenoids from three sources, synthetic astaxanthin, crabs (*Pleurocodes planiples*), and *P. rhodozyma*, were deposited in trout muscle at the rate of 7.8%, 5.0%, and 4.0%, respectively. The FDA has approved the addition of *Phaffia* products to fish food. There is an ongoing interest to develop other plant sources to compete with the nature-like synthetic carotenoids. Several mutants of the carotogenic molds *Blakeslea trispora* and *Phycomyces blakesleeanus* produce high concentrations of β-carotene, but recent interest has shifted to the microalgae. Species of *Dunaliella* can accumulate up to 10% dry weight of β-carotene. Ponds in Australia originally developed to produce salt by evaporation of salt water are now being used to grow *Dunaliella*. Other installations are in Hawaii. Regardless, both sources command a premium because of their "natural" association. Interestingly, the same ponds can be used to grow *Haematococcus* species, which accumulate astaxanthin. A gene from *H. pluvialis* has been transferred to tobacco plants to provide a plant source of astaxanthin. In view of the widespread occurrence of the carotenoid pigments, it is not surprising that other plants and animals would be suggested as potential sources of colorants. These include krill, chlorella, shrimp, algae, bacteria, molds, and a variety of plant sources (8).

BIBLIOGRAPHY

1. J. C. Bauernfeind, *Carotenoids as Colorants and Vitamin A Precursors*, Academic Press, New York, 1981.
2. F. J. Francis, *Handbook of Food Colorants*, Eagen Press. St. Paul, Minn., 1999.
3. D. M. Marmion, *Handbook of US Colorants*, John Wiley & Sons, New York, 1991.
4. G. L. Alonzo et al., "Color Analysis of Saffron," *Proc. 2nd Int. Symp. on Natural Colorants*, Puerto de Acapulco, Mexico, January 23–26, 1996.
5. J. Verghese, "Focus on Xanthophylls from *Tagetes erecta*, L.—The Giant Natural Colour Complex," *Proc. 2nd International Symposium on Natural Colorants*, Puerto de Acapulco, Mexico, January 23–26, 1996.
6. F. J. Francis, "Carotenoids as Colorants," *World of Ingredients*, 34–38 (1995).
7. G. C. Hinostoza et al., "Pigmentation of Rainbow Trout *Oncorhynchus mykiss* by Astaxanthin from Red Crab *Pleuroncodes planipes* in Comparison with Synthetic Astaxanthin and *Phaffia rhodozyma* Yeast," *Proc. 2nd Int. Symp. on Natural Colorants*, Puerto de Acapulco, Mexico, January 23–26, 1996.
8. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.

See also CAROTENOID PIGMENTS.

COLORANTS: CARTHAMIN

Carthamin (CI Natural Red 26; CI 75140) is a very old colorant. Its use as a textile colorant dates back to antiquity under a variety of names such as Spanish saffron, African saffron, American saffron, thistle saffron, false saffron, bastard saffron, Dyer's saffron, and so on. More modern names are carthemone, carthamic acid, and safflor red. Carthamin is a yellow to red preparation from safflower flowers, *Carthemus tinctorius*, of the family Compositae, which is cultivated extensively in Europe and America.

Carthamin is classified biochemically as a chalcone in the yellow flavonoid group of pigments. The flavonoids are a very large group of plant pigments with more than 4000 compounds, but only carthamin has been suggested as a colorant. Carthamin contains three chalcones: the red carthamin, safflor Yellow A, and safflor Yellow B (Fig. 1). Fresh yellow flower petals contain precarthamin, which oxidizes to form the red carthamin. Carthamin, under acid conditions, equilibrates to two isomers: red carthamin and yellow isocarthamin (1). The earlier patents on carthamin involved simple aqueous extraction of the petals or crushing the petals prior to extraction to allow for oxidation (2). The three main pigments can be purified by passage through a styrene resin bed. Purification and stabilization can be greatly enhanced by absorption of the pigments on cellulose powder. Apparently cellulose has a great affinity for carthamin and this is known as the "Saito effect." "The effect is so strong that the carthamin may be retained for more than a thousand years without appreciable change to the coloration" (3). No storage data were provided to substantiate this claim. The cellulose absorption method was adapted to large-scale production using a methanolic extract and a cellulose column in acid media. Another method using a cellulose derivative diethylaminoethylcellulose yielded pure precarthamin, carthamin, safflor Yellow A and safflor Yellow B. Tissue culture approaches have been successful in producing the safflower pigments as well as some novel closely related pigments (4,5).

Carthamin has been suggested as a colorant for pineapple juice, yogurt, butter, liqueurs, confectionery and so on. The yellow to red range of colors gives it some flexibility. Currently it is not approved for use in foods in the United States.

violaxanthin, lycopene and β-apo-8′-carotenol together with a number of others. About 115 carotenoid pigments have been reported in citrus. Colorants from carrots usually contain about 80% β-carotene and up to 20% α-carotene plus small amounts of several others. Some of the high pigment strains of carrots used for colorant extracts also contain lycopene. Astaxanthin is a desirable addition to the diet of salmon and trout in aquaculture because of its ability to impart a desirable red color to the flesh. The usual sources of astaxanthin are the by-products of the lobster and shrimp processing industry, but the demand exceeds the supply. This has led to an interest in growing the red yeast *Phaffia rhodozyma* as a raw material for a concentrated extract. But unfortunately *Phaffia* produces the wrong optical isomer of astaxanthin for optimal accumulation in the flesh of salmon. Hinostroza et al. (7) reported that carotenoids from three sources, synthetic astaxanthin, crabs (*Pleurocodes planiples*), and *P. rhodozyma*, were deposited in trout muscle at the rate of 7.8%, 5.0%, and 4.0%, respectively. The FDA has approved the addition of *Phaffia* products to fish food. There is an ongoing interest to develop other plant sources to compete with the nature-like synthetic carotenoids. Several mutants of the carotogenic molds *Blakeslea trispora* and *Phycomyces blakesleeanus* produce high concentrations of β-carotene, but recent interest has shifted to the microalgae. Species of *Dunaliella* can accumulate up to 10% dry weight of β-carotene. Ponds in Australia originally developed to produce salt by evaporation of salt water are now being used to grow *Dunaliella*. Other installations are in Hawaii. Regardless, both sources command a premium because of their "natural" association. Interestingly, the same ponds can be used to grow *Haematococcus* species, which accumulate astaxanthin. A gene from *H. pluvialis* has been transferred to tobacco plants to provide a plant source of astaxanthin. In view of the widespread occurrence of the carotenoid pigments, it is not surprising that other plants and animals would be suggested as potential sources of colorants. These include krill, chlorella, shrimp, algae, bacteria, molds, and a variety of plant sources (8).

BIBLIOGRAPHY

1. J. C. Bauernfeind, *Carotenoids as Colorants and Vitamin A Precursors*, Academic Press, New York, 1981.
2. F. J. Francis, *Handbook of Food Colorants*, Eagen Press. St. Paul, Minn., 1999.
3. D. M. Marmion, *Handbook of US Colorants*, John Wiley & Sons, New York, 1991.
4. G. L. Alonzo et al., "Color Analysis of Saffron," *Proc. 2nd Int. Symp. on Natural Colorants*, Puerto de Acapulco, Mexico, January 23–26, 1996.
5. J. Verghese, "Focus on Xanthophylls from *Tagetes erecta*, L.—The Giant Natural Colour Complex," *Proc. 2nd International Symposium on Natural Colorants*, Puerto de Acapulco, Mexico, January 23–26, 1996.
6. F. J. Francis, "Carotenoids as Colorants," *World of Ingredients*, 34–38 (1995).
7. G. C. Hinostoza et al., "Pigmentation of Rainbow Trout *Oncorhynchus mykiss* by Astaxanthin from Red Crab *Pleuroncodes planipes* in Comparison with Synthetic Astaxanthin and *Phaffia rhodozyma* Yeast," *Proc. 2nd Int. Symp. on Natural Colorants*, Puerto de Acapulco, Mexico, January 23–26, 1996.
8. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.

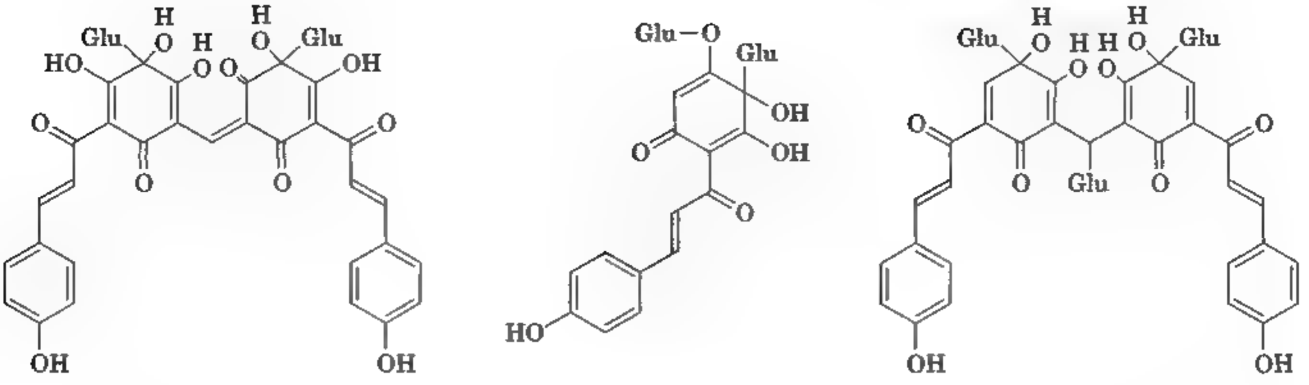
See also CAROTENOID PIGMENTS.

COLORANTS: CARTHAMIN

Carthamin (CI Natural Red 26; CI 75140) is a very old colorant. Its use as a textile colorant dates back to antiquity under a variety of names such as Spanish saffron, African saffron, American saffron, thistle saffron, false saffron, bastard saffron, Dyer's saffron, and so on. More modern names are carthemone, carthamic acid, and safflor red. Carthamin is a yellow to red preparation from safflower flowers, *Carthemus tinctorius*, of the family Compositae, which is cultivated extensively in Europe and America.

Carthamin is classified biochemically as a chalcone in the yellow flavonoid group of pigments. The flavonoids are a very large group of plant pigments with more than 4000 compounds, but only carthamin has been suggested as a colorant. Carthamin contains three chalcones: the red carthamin, safflor Yellow A, and safflor Yellow B (Fig. 1). Fresh yellow flower petals contain precarthamin, which oxidizes to form the red carthamin. Carthamin, under acid conditions, equilibrates to two isomers: red carthamin and yellow isocarthamin (1). The earlier patents on carthamin involved simple aqueous extraction of the petals or crushing the petals prior to extraction to allow for oxidation (2). The three main pigments can be purified by passage through a styrene resin bed. Purification and stabilization can be greatly enhanced by absorption of the pigments on cellulose powder. Apparently cellulose has a great affinity for carthamin and this is known as the "Saito effect." "The effect is so strong that the carthamin may be retained for more than a thousand years without appreciable change to the coloration" (3). No storage data were provided to substantiate this claim. The cellulose absorption method was adapted to large-scale production using a methanolic extract and a cellulose column in acid media. Another method using a cellulose derivative diethylaminoethylcellulose yielded pure precarthamin, carthamin, safflor Yellow A and safflor Yellow B. Tissue culture approaches have been successful in producing the safflower pigments as well as some novel closely related pigments (4,5).

Carthamin has been suggested as a colorant for pineapple juice, yogurt, butter, liqueurs, confectionery and so on. The yellow to red range of colors gives it some flexibility. Currently it is not approved for use in foods in the United States.



(a) (b) (c)

Figure 1. Pigments of carthamin (a) carthamin; (b) Safflor Yellow A; (c) Safflor Yellow B.

BIBLIOGRAPHY

1. K. Saito and A. Fukushima, "Effect of External Conditions on the Stability of Enzymatically Synthesized Carthamin," *Acta. Soc. Bot. Pol.* **55**, 639–651 (1986).
2. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.
3. K. Saito and A. Fukushima, "On the Mechanism of the Stable Red Colors of Cellulose-bound Carthamin," *Food Chem.* **29**, 161–175 (1988).
4. F. J. Francis, "Less Common Natural Colorants," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, 2nd ed., Blackie Academic and Professional, Glasgow, Scotland, 1996, pp. 310–342.
5. F. J. Francis, "Miscellaneous Colorants," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Academic and Professional, Glasgow, Scotland, 1992, pp. 242–272.

COLORANTS: CHLOROPHYLLS

The chlorophylls are a group of naturally occurring pigments present in all photosynthetic plants including the algae and some bacteria. They are in greater abundance than any other organic pigment produced in nature. Hendry estimated annual production at about 1,100,000,000 tons as compared with carotenoids at 100,000,000 tons, with about 75% being produced in aquatic, primarily marine, environments (1). Obviously, as a source of raw material for food colorants, supply is no problem.

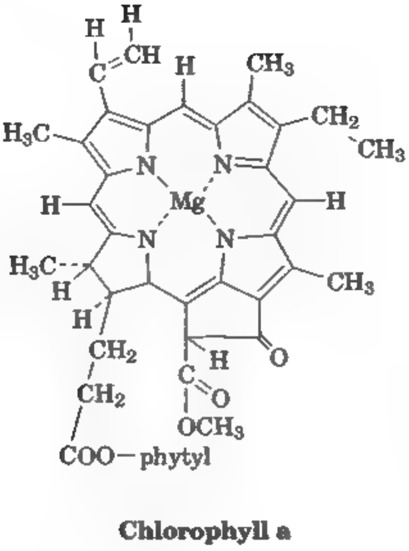
CHEMISTRY

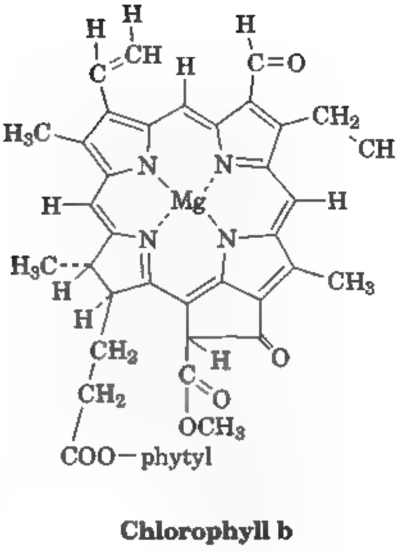
Five chlorophylls and five bacteriochlorophylls are known in nature. Only two, chlorophylls a and b (Fig. 1) are important as source material for food colorants. Chlorophylls a and b have a complex structure, but they differ only by a -CH_3 and a -CHO group on carbon 7. The numbering system for chlorophylls is shown in Figure 2 (1). The chlorophyll a molecule is easily changed in the presence of acids to remove the magnesium ion, resulting in pheophytin a. The phytyl group is easily removed to produce chlorophyllide a. If both magnesium and phytyl groups are removed, the compound is called pheophorbide a. A similar series of reactions occurs with chlorophyll b. Several other degradative reactions also occur, but the change to pheophytin is the major change that occurs when products containing chlorophyll are heated. The trivial names chlorophyll, chlorophyllide, pheophytin, and pheophorbide were developed historically and have been retained even though the chemical nomenclature has been changed by the IUB-IUPAC Joint Commission on Nomenclature in 1980. A good description of the new nomenclature and the chemistry of chlorophylls has been provided by Hendry (1).

The chlorophylls have a bright green color, whereas the pheophytins are olive green; thus, major efforts to maintain the attractive green color in vegetables during processing have been to retain the magnesium in the molecule. Pretreatment and treatment during processing in alkaline solutions containing magnesium (the Blair Process) were successful in retaining the green color for a short time after thermal processing. Enzymatic treatments to convert the chlorophyll to chlorophyllide were attempted because the chlorophyllides have a color similar to the chlorophylls and are more stable, and they did help to maintain the color. A combination of the two processes with high-temperature short-time (HTST) processing also maintained the color a little longer. But the overall effect was that the retention of attractive color was only a few weeks and not long enough to provide an economic incentive, so the attempts were abandoned. The same problems with stability were experienced with attempts to make colorants containing chlorophyll.

PREPARATION OF COLORANTS

The land plants that contain chlorophylls a and b have been the source of all chlorophyll-containing colorants. The order of stability of the chlorophylls is c > b > a, but chlorophyll c has been ignored as a source of green colorants. It does occur in the brown seaweeds used as a commercial source of alginates and also in single-celled phytoplankton. Algae are already being grown as a source of carotenoids and conceivably could also be grown for chlorophyll content if the increased stability was sufficient to provide an economic motivation. The plants currently being used include





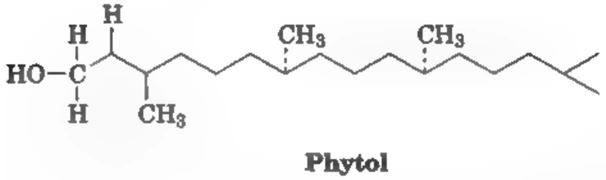


Figure 1. Formulas for chlorophylls a and b and the phytol moiety.

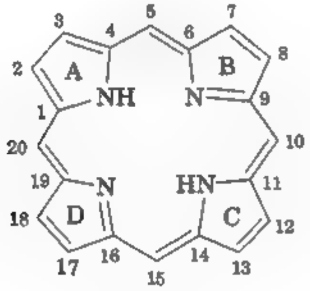


Figure 2. Numbering system for the chlorophylls.

alfalfa (lucerne), *Medicago sativa*; nettles, *Urtica dioica*; and a series of pasture grasses such as fescue. In a living cell, within the chloroplasts, the chlorophylls are complexed with but not covalently bound to one of a series of polypeptides. The chlorophyll–polypeptide or chlorophyll–protein complexes are closely associated with carotenoids and tocopherols (vitamin E) and are actively involved in the processes of photosynthesis. Preparation of a colorant involves recovery and purification of an appropriate form of chlorophyll.

Land plants are usually available for only a few weeks of the year; thus, the chlorophyll colorants are usually made from bulk harvested and dried plants. The dried plants are ground and extracted with acetone or a chlorinated hydrocarbon solvent and washed, and the solvent is removed. A yield of 20% of a mixture of chlorophylls, pheophytins, and other degraded chlorophyll compounds is usually obtained. The dry residue after removal of the solvent can be further purified by treatment with a water-immiscible solvent to obtain an oil-soluble preparation known as metal-free pheophytin. The dry residue can also be acidified in the presence of copper salts to produce an oil-soluble copper pheophytin. It is not commercially practical to produce a colorant containing chlorophyll itself because of the instability of the magnesium compound. The dry residue can also be saponified to replace the phytyl group with sodium or potassium to form water-soluble compounds. After further purification, the product may be marketed as a water-soluble, metal-free, gray-green product. Acidification in the presence of copper salts converts the pigments to more-stable green pigments known as copper chlorophyllins.

APPLICATIONS

The copper-substituted derivatives of pheophytin are relatively stable to light and mineral acids and are more stable than the metal-free pheophytins and pheophorbides. The major portion of the estimated $15 million (in 1994 U.S. dollars) in annual sales of colorants derived from chlorophyll is in the water-soluble forms (1). The major portion of both oil-soluble and water-soluble forms is used in dairy products, edible oils, soups, chewing gum, sugar confections, and drinks. A minor proportion is used in cosmetics and toiletries.

Chlorophyll colorants are widely used in Europe and other parts of the world but are not permitted in the United States.

BIBLIOGRAPHY

1. G. A. F. Hendry, "Chlorophylls and Chlorophyll Derivatives," in G. A. F Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Publishers, Glasgow, Scotland, 1996, pp. 131–156.

COLORANTS: FOOD, DRUG, AND COSMETIC COLORANTS

The synthetic colorants are the most obvious of the long list of colorants and probably the most important in terms of scope of applications. They are also the most controversial. The history of synthetic colorants dates back to the discovery of the first synthetic dye, mauve, by Sir William Henry Perkin in 1856. Since then more than 700 colorants have been available to the paint, plastic, textile, and food industry. In 1907 about 80 colorants were offered for use in foods (1), and obviously few, if any, had been tested for safety. At that time, Dr. Bernard Hesse, a German dye expert employed by the U.S. Department of Agriculture (USDA), was asked to study the colorants available for use in foods. He concluded that only 16 of the 80 colorants were probably more or less harmless. Most of Hesse's information was embodied into the Food and Drug Act of 1906. This Act together with FID No. 76 in July 1907 put an end to the indiscriminate use of colorants in food. The new legislation required that only colorants of known chemical structure and that had been tested for safety could be used. The Act also set up a system for certification of synthetic organic food colorants designed for use in foods. The certification of each batch included proof of identity and the levels of impurities. During the next three decades, 10 more colorants were added to the list. In 1938 the federal Food, Drug, and Cosmetic Act of 1938 was enacted. It stated that the use of any uncertified coal-tar color in any food, drug, or cosmetic shipped in interstate commerce was forbidden. The Act also created three categories of coal-tar colors:

1. *FD&C colors*. Those certifiable for use in coloring foods, drugs, and cosmetics.
2. *D&C colors*. Dyes and pigments considered safe in drugs and cosmetics when in contact with mucous membranes or when ingested.
3. *Ext. D&C colors*. Those colorants that, because of their oral toxicity, were not certifiable for use in products intended for ingestion but were considered safe for use in products externally applied.

Each colorant had to be identified by its specific FD&C, D&C, or Ext. D&C name.

SAFETY OF COLORANTS

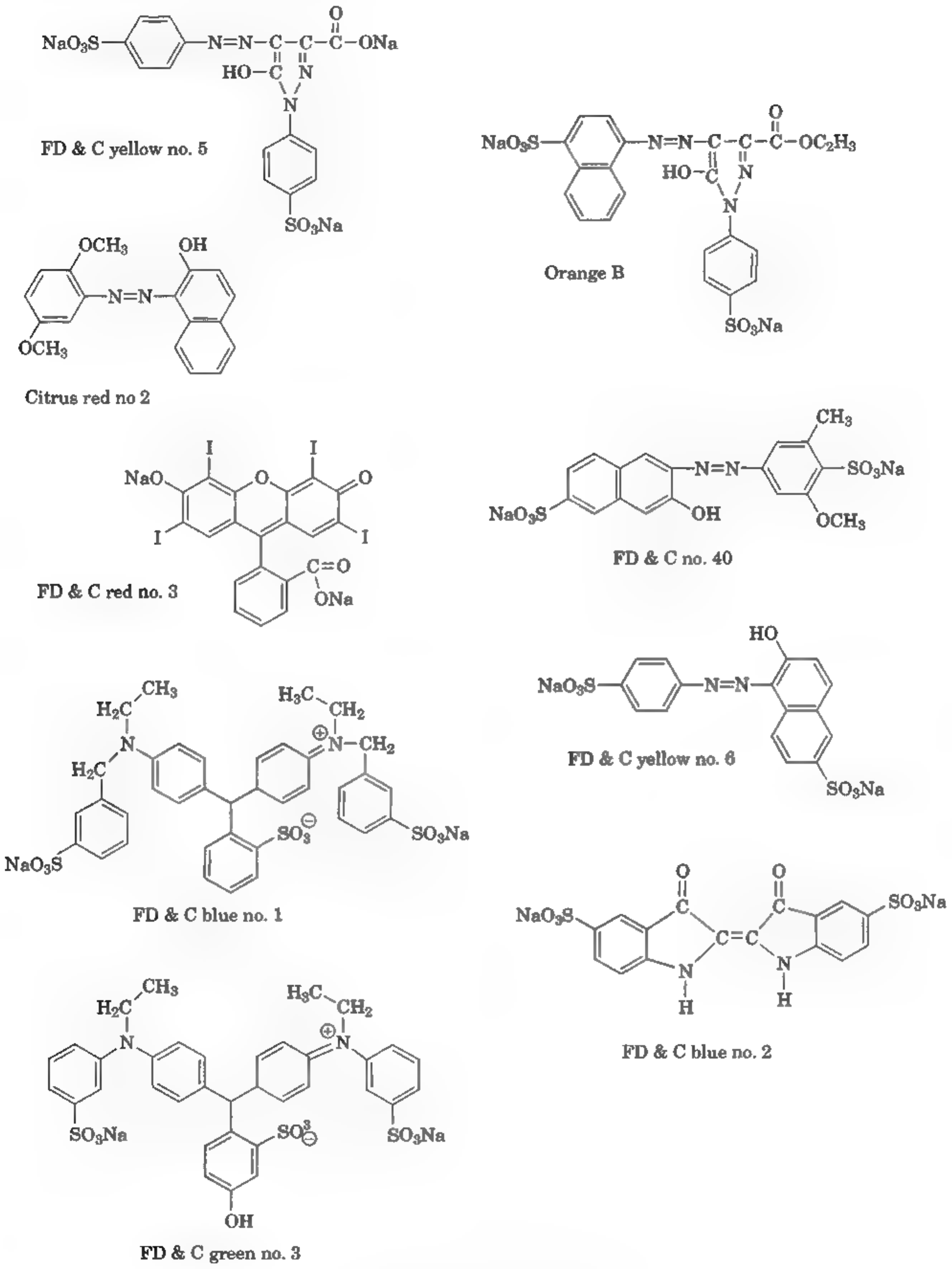
The passage of the 1938 Act drew public attention to the question of safety of colorants and led to the publication of the Service and Regulatory Announcement, Food, Drugs, and Cosmetics No. 3 in 1940. This document listed specific colorants that could be used together with purity specifications and regulations pertaining to manufacture and sale. In 1950 a new dilemma developed, which was precipitated by two developments. First, a number of children became sick after reportedly eating popcorn and candy colored with excessive amounts of colorant. Second, the Food and Drug Administration (FDA) launched a new round of toxicity tests with animals fed much higher levels of colorants and for a longer time than any tests previously conducted. The results were unfavorable for FD&C Orange No. 1, FD&C Orange No. 2, and FD&C Red No. 32. The FDA based its conclusions on the interpretation of the 1938 Act, which states: "The Secretary shall promulgate regulations providing for the listing of coal-tar colors which are harmless and suitable for food." The FDA interpreted "harmless" to mean harmless at any level. The manufacturers argued that this interpretation was too strict and the FDA should set safe limits. The Supreme Court held that the FDA did not have the authority to set limits, and several more colorants were delisted including FD&C Yellows Nos. 1 to 4. It was obvious that the law was unworkable and, through the efforts of the Certified Color Industry and FDA, a new law was developed called the Color Additive Amendment of 1960. The Amendment allowed for current use of colorants pending completion of testing, and, equally important, it authorized the secretary of health, education, and welfare to establish limits of use, thereby eliminating the "harmless per se" interpretation. Another

Table 1. FD&C Colorants Approved for Use in the United States

FDA name	Common name	CI number	Year listed	Chemical class	Hue
FD&C Blue No. 1	Brilliant Blue	42090	1929	Triphenylmethane	Greenish blue
FD&C Blue No 2	Indigotine	73015	1907	Indigoid	Deep blue
FD&C Green No. 3	Fast Green	42053	1927	Triphenylmethane	Bluish green
FD&C Yellow No. 5	Tartrazine	19140	1916	Pyrazolone	Lemon yellow
FD&C Yellow No. 6	Sunset Yellow	15985	1929	Monoazo	Reddish yellow
FD&C Red No. 3	Erythrosine	43430	1907	Xanthine	Bluish pink
FD&C Red No. 40	Allura Red	16035	1971	Monoazo	Yellowish red
Orange B[a]	Orange B		1966	Monoazo	
Citrus Red No 2[b]	Citrus Red No. 2		1959	Monoazo	

[a] Allowed only on the surfaces of sausages and frankfurters at concentrations up to 150 ppm by weight.

[b] Allowed only on the skins of oranges, not intended for processing, at concentrations up to 2 ppm by weight of the whole fruit.



FD & C yellow no. 5

Orange B

Citrus red no 2

FD & C red no. 3

FD & C no. 40

FD & C yellow no. 6

FD & C blue no. 1

FD & C blue no. 2

FD & C green no. 3

Figure 1. Structure of 9 FD&C colorants

feature, known as the "Delany Clause," was also inserted. Briefly, the clause states that no additive shall be added to food "if it is found, after tests which are appropriate for the evaluation of safety of food additives, to induce cancer in man or animals." Unfortunately Congressman Delany did not appreciate the ingenuity of modern chemists to push back the limits of analytical sensitivity. Today, chemists can find almost anything in anything. The clause is clearly unworkable and is currently being replaced by some form of "de minimis," which comes from the term "de minimis curat lex." Freely translated, this means that the law does not concern itself with trifles. Another feature of the 1960 Amendment allowed the secretary of health, education, and welfare to determine which colorants had to be certified and which would be exempt from certification. In view of the expense and time required for adequate testing, some of the colorants had "provisional" listing for a number of years, but this category for straight FD&C colorants has expired at the present time. Because of the expense involved, only commercial colorants of significant economic value were tested, so a number of colorants were delisted by default. Actually this should not be a hardship, because the seven currently allowed colorants (Table 1) should be sufficient in view of the opportunities for blending. Interestingly, Hesse, around 1900, recommended seven colorants for general use in food. Of the seven, only two (FD&C Red No. 3 and FD&C Blue No. 2) remain on the current list of approved colorants. Table 1 shows a list of seven colorants approved for general use, and two approved for restricted use, together with the FD&C name, common name, CI number, year listed, and chemical class. FD&C Blue No. 2 was provisionally listed until final approval in 1987. Similarly, FD&C Yellow No. 6 was finally approved in 1986. Formulae for the nine colorants are shown in Figure 1. The regulatory status of 26 colorants exempt from certification is shown in Table 2.

APPLICATIONS IN FOODS

The seven colorants listed in Table 1 should be sufficient to enable a formulator to create any desired color by mixing

Table 2. Regulatory Status of Colorants Exempt from Certification in the United States

Name of color additive	U.S. food use limit	E. C. status	JECFA ADI[a] (mg/kg/bw)
Annatto	GMP[b]	E 160b	0–0.065 (calculated as bixin)
Dehydrated beets	GMP	E 162	Not evaluated
Ultramarine blue	Salt for animal feed up to 0.5% by weight	Not listed	None
Canthaxanthin	Not to exceed 30 mg/pd of solid/semisolid food or pint of liquid food or 4.41 mg/kg of chicken feed	E 161g	None
Caramel	GMP	E 150	0–200
β-apo-8′-carotenal	Not to exceed 15 mg/pd of solid or semisolid food or 15 mg/pt of liquid food	E 160a	0–5
β-Carotene	GMP	E 160a	0–5
Cochineal extract or carmine	GMP	E 120	0–5
Toasted partially defatted cooked cottonseed flour	GMP	Not listed	Not evaluated
Ferrous gluconate	GMP for ripe olives only	Not listed	Not evaluated
Grape color extract	GMP for nonbeverage foods	E 163	Not evaluated
Grape skin extract (Enocianina)	GMP for beverages	E 163	0–2.5
Synthetic iron oxide	Pet food up to 0.25%	E 172	0–2.5
Fruit juice	GMP	Not listed	Not evaluated
Vegetable juice	GMP	Not listed	Not evaluated
Dried algal meal	GMP for chicken feed	Not listed	Not evaluated
Tagetes (Aztec marigold) meal and extract	GMP for chicken feed	Not listed	Not evaluated
Carrot oil	GMP	Not listed	Not evaluated
Corn endosperm oil	GMP for chicken feed	Not listed	Not evaluated
Paprika	GMP	E 160c	None allocated
Paprika oleoresin	GMP	E 160c	Self-limiting as spice
Riboflavin	GMP	E 101	0–0.5
Saffron	GMP	Not listed	Food ingredient
Titanium dioxide	Not to exceed 1% by weight of food	E 171	Not specified
Turmeric	GMP	E 100 (curcumin)	Temporary ADI not extended
Turmeric oleoresin	GMP	E 100 (curcumin)	Temporary ADI not extended

---

[a] JECFA refers to the Joint Expert Committee on Food Additives set up by the World Health Organization and the Food and Agriculture Organization of the United Nations. The term ADI refers to Acceptable Daily Intake as usually determined by the JECFA.

[b] GMP = Good manufacturing practice.

colorants as discussed in COLOR AND FOOD. But each colorant probably will behave differently in each food system; therefore prediction from a knowledge of each colorant is difficult. The blending will have to be done under actual food conditions. The stability of the FD&C colorants under acid, alkaline, light, heat, and oxidation/reduction conditions, and the presence of sugars, acids, diluents, solubility, and so on discussed by Francis (2). The FD&C colorants are appropriate for a wide variety of foods, particularly those requiring a water-soluble colorant.

LAKES

Lakes are colorants prepared by precipitating a soluble colorant onto an insoluble base or substratum. A variety of bases such as titanium dioxide, zinc oxide, talc, calcium carbonate, and aluminum benzoate are approved for D&C lakes, but only aluminum, magnesium, and calcium salts are approved in the United States for manufacturing FD&C lakes. The process of manufacture is fairly simple. First, a substratum is prepared by adding sodium carbonate to a solution of aluminum sulfate. Next a certified colorant is added to the slurry, then aluminum sulfate is added to convert the colorant to an aluminum salt, which is absorbed onto the surface of the alumina. The slurry is then washed, dried, and ground to a fine powder. The colored powder can be marketed as is or mixed with a diluent such as hydrogenated vegetable oil, coconut oil, propylene glycol, glycerol, sugar syrup, or other media appropriate for food consumption or for printing food wrappers. Diluents for both pure colorants or lakes have to be either on the Generally Recognized As Safe (GRAS) list or on the list approved by the FDA Code of Federal Regulations (1–3).

Lake colorants have many advantages, primarily because they are insoluble in most solvents including water. They have a high opacity, are easily incorporated into dry media, and have superior stability to light and heat. They are effective colorants for candy and pill coatings since they do not require removal of water prior to processing. They are particularly effective for coloring hydrophobic foods such as fats and oils, confectionery products, bakery products, salad dressings, chocolate substitutes, and other products in which the presence of water is undesirable. Lakes made from some water-soluble FD&C colorants may show some bleeding when formulated in foods outside the pH range 3.5 to 9, but few foods are in this pH range. Lakes have been used to color food packaging materials including lacquers, plastic films, and inks from which straight water-soluble colorants would be soon leached.

BIBLIOGRAPHY

1. D. C. Marmion, *Handbook of U.S. Colorants for Foods, Drugs, and Cosmetics*, 2nd ed., John Wiley and Sons, New York, 1984.
2. D. C. Marmion, *Handbook of U.S. Colorants for Foods, Drugs, and Cosmetics*, 3rd ed., John Wiley and Sons, New York, 1991.
3. F. J. Francis, *Handbook of Food Colorants*, Eagen Press, St. Paul, Minn., 1999

COLORANTS: HAEMS

Haemoglobin from blood is the only commercially important colorant in the haem group. The term haem, or heme, is used to describe iron derivatives of the cyclic tetrapyrrole protoporphin with the structure shown in Figure 1. The numbering system is similar to that for chlorophyll (see COLORANTS: CHLOROPHYLLS, Fig. 2). This structure is basic to a number of compounds such as chlorophyll, important in photosynthesis; haemoglobin, in oxygen transport; cytochrome, in energy transport; and a number of other enzymes. Haemoglobin is probably the best known, and it is composed of a haem group associated with a protein. Haemoglobin forms a loose association with oxygen as the basis for oxygen transport and carbon dioxide removal and is remarkably stable *in vivo* but not *in vitro*. This introduces some difficulty in using haems as colorants.

Free haem can be easily extracted from blood by treatment with acidified organic solvents. Preparation of haem from blood has been known for centuries and can be accomplished by the addition of whole blood to glacial acetic acid saturated with salt at 100°C. Crystalline haem precipitates out, but it is not stable enough to function as a food colorant. More stable compounds can be produced by allowing other ligands such as carbon monoxide, nitrous oxide, hydroxides, and cyanide to displace the oxygen from the central iron atom. A number of patents exist (1), and they fall into three broad areas: (*1*) treatment of whole blood with a variety of reagents, (*2*) stabilization of the color with a variety of ligands, and (*3*) purification of haem derivatives. Treatment of whole blood to remove the haemoglobin has considerable appeal because the iron is in a very nutritionally available form as a dietary supplement and the protein has a high biological value. It would be still another advantage if the preparation could be made stable enough to act as a food colorant. A number of the patents claim

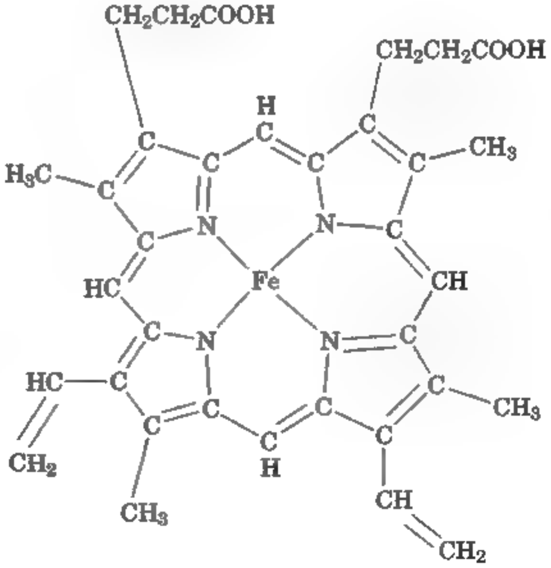


Figure 1. Structure of haem.

that the preparations are suitable for coloring products that normally contain meat, meat analogues, sausages, and so on.

The use of whole blood as a food ingredient is a very old custom. The Europeans are famous for "blood pudding," which may be a delectable food item but is black in color not red. Haemoglobin is denatured in the cooking process. Houghton reported an interesting application of naturally occurring haem pigments, namely, the bile pigments bilirubin and biliverdin (2). They occur in gallstones and hair balls and are in demand in Chinese medicine as an aphrodisiac.

Blood is permitted as a food ingredient if it has been collected and processed in an appropriate sanitary manner, but colorants derived from blood are not permitted in the United States.

BIBLIOGRAPHY

1. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.
2. J. D. Houghton, "Haems and Bilins," in G. A. F Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Publishers, Glasgow, Scotland, 1996, pp. 157–196.

COLORANTS: IRIDOIDS

Colorants from saffron have enjoyed good technological success and gustatory appeal, but their high price has led to searches for other sources with the same pigments. It became apparent that the same pigments, but not the flavor, could be obtained in much larger quantities from the fruits of the gardenia or Cape Jasmine plant. The fruits of gardenia, *Gardenia jasminoides*, contain three major groups of pigments: crocins (see COLORANTS: CAROTENOIDS, Fig. 1), iridoids, and flavonoids. The structures of nine iridoid pigments are shown in Figure 1. This group also comprises a series of flavonoid compounds. The structures of five flavonoids isolated from *G. fosbergii* are shown

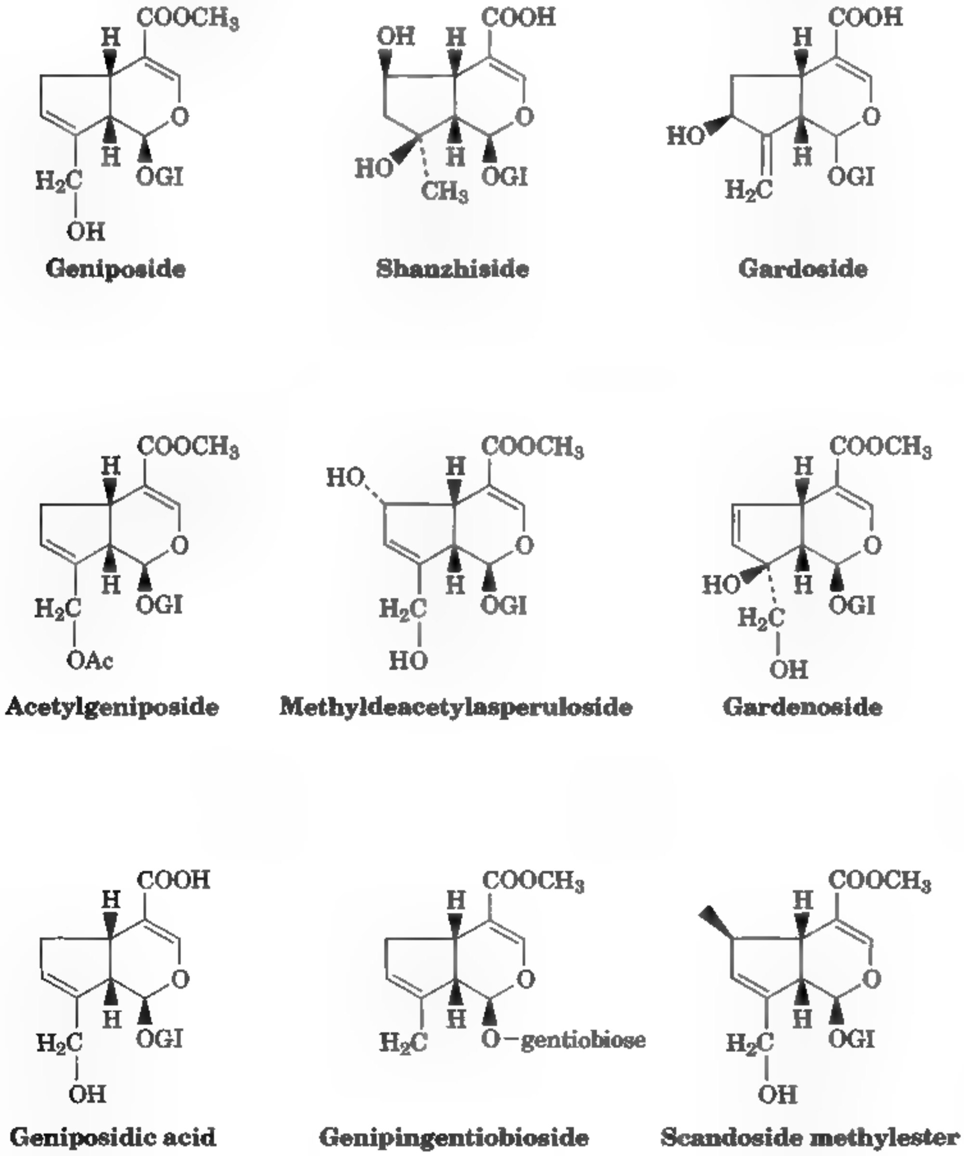
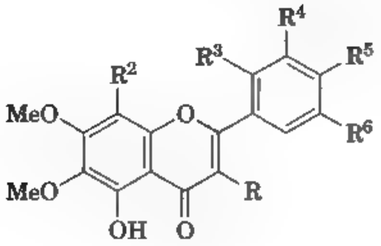

COOCH3
H
O
H
OGI
H2C
OH
Geniposide
OH
H
COOH
HO
CH3
OGI
Shanzhiside
HO
H2C
Gardoside
OAc
Acetylgeniposide
Methyldeacetylasperuloside
Gardenoside
Geniposidic acid
O−gentiobiose
Genipingentiobioside
Scandoside methylester

Figure 1. Structures of nine iridoid pigments in *G. jasminoides*. Gl = glucose.



Flavonoid compounds

Compound	R^1	R^2	R^3	R^4	R^5	R^6
1	H	H	H	OMe	OMe	OMe
2	OMe	H	H	OH	OMe	OH
3	H	H	OMe	OMe	H	OH
4	OMe	H	H	OMe	OMe	OH
5	OMe	OMe	H	H	OH	H

Figure 2. Structures of five flavonoid pigments in *G. fosbergii*.

in Figure 2. This is a different species from *G. jasminoides*, but the flavonoid compounds in closely related species tend to be similar. The flavonoids would contribute a pale yellow color and the carotenoids an orange color.

The iridoid pigments constitute an interesting situation as a food colorant because a range of colors from green to yellow, red and blue, can be produced (1). The patent literature suggests extraction of the fruit with water, treatment with enzymes having β-glycosidic or proteolytic (bromelain) activity, followed by reaction with primary amines from amino acids or a protein such as soy. The reaction conditions such as time, pH, temperature, oxygen content, degree of polymerization, and conjugation of the primary amino groups allow a range of colorants to be produced. Several patents involve culturing extracts of gardenia with microorganisms such as *Bacillus subtilis, Aspergillus japonicus*, or a species of *Rhizopus*. Several patents involve hydrolysis of the iridoid glycoside geniposide by the action of β-glucosidase to produce genipin. The genipin can be reacted with taurine to produce a blue colorant. Other amino acids such as glycine, alanine, leucine, phenylalanine, and tyrosine can be reacted to produce brilliant blue colorants. They are claimed to be stable for two weeks at 40°C in 40% ethanol. Four greens, two blues, and one red have been commercialized in Japan.

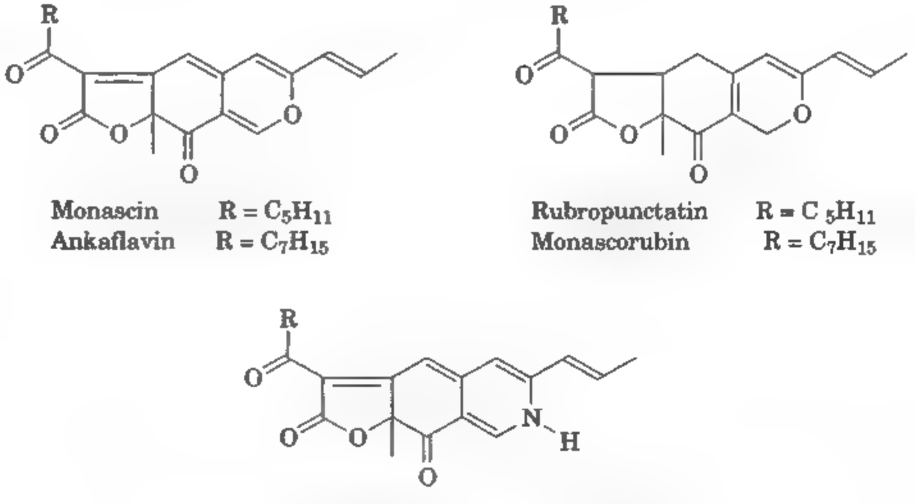
Colorant preparations from gardenia have been suggested for use with candies, sweets, colored ices, noodles, imitation crab, fish eggs, glazed chestnuts, beans, dried fish substitutes, liqueurs, baked goods, and so on. In view of the wide range of colors and apparent stability, colorants from gardenia appear to have good potential. They are not approved for use in the United States.

BIBLIOGRAPHY

1. F. J. Francis, "Less Common Food Colorants," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Publishers, Glasgow, Scotland, 1996, pp. 310–342.

COLORANTS: MONASCUS

Monascus products have been well known in south and east oriental countries for centuries. Wong and Koehler listed their use as a Chinese medicine as far back as 1590 (1). The genus *Monascus* includes several fungal species that will grow on a number of carbohydrate substrates, especially steamed rice. Traditionally, *Monascus* species were grown on rice, and the whole mass was eaten as an attractive red food. It could also be dried, ground, and incorporated into other foods.



Monascin $R = C_5H_{11}$
Ankaflavin $R = C_7H_{15}$

Rubropunctatin $R = C_5H_{11}$
Monascorubin $R = C_7H_{15}$

Rubropunctamine $R = C_5H_{11}$
Monascorubramine $R = C_7H_{15}$

Figure 1. Pigments of *Monascus*.

CHEMISTRY

Monascus species produce six pigments with the structures shown in Figure 1. Monascin and ankaflavin are yellow, rubropunctatin and monascorubin are red, and rubropunctamine and monascorubramine are purple. The red and yellow pigments are considered to be normal secondary metabolites of fungal growth, and the purple pigments may be formed by enzymatic modification of the red and yellow pigments. The red pigments are very reactive and readily react with amino groups via a ring opening and a Schiff rearrangement to form water-soluble compounds such as that in Figure 2 (2). The *Monascus* pigments have been reacted with amino groups, polyamino acids, amino alcohols, chitin amines or hexamines, proteins, peptides and amino acids, sugar amino acids, browning reaction products, aminoacetic acid, and amino benzoic acid (3). Obviously a number of derivatives are possible, and they have been shown to have greater water solubility, thermostability, and photostability than the parent compounds.

The *Monascus* pigments are readily soluble in ethanol and only slightly soluble in water. Ethanolic solutions are orange at pH 3 to 4, red at 5 to 6 and purplish red at 7 to 9. The pigments fade under prolonged exposure to light, but the pigments in 70% alcohol are more stable than in water. The pigments are very stable to temperatures up to 100°C in neutral or alkaline conditions.

COMMERCIAL PRODUCTION

Monacus colorants are currently being produced in Japan, China and Taiwon. Traditionally, *Monascus* has been grown on solid cereal substrates such as rice and wheat and the entire mass used as a food or a food ingredient. But it became obvious that the fungus could be grown in liquid or fluid solid state media and optimized for pigment production. Much research has been devoted to the determination of the optimal conditions for pigment production on a wide variety of cereal substrates. It is possible to maximize the production of either the red or yellow pigments to produce a range of red to yellow colors. For example, Tadao et al. reported that *M. anka* grown on bread produced only yellow pigments (4). Criticism was directed at the earlier production methods because the fungus produced an antibiotic, and this is considered to be an undesirable food ingredient. Recent strain selections and control of growth conditions have eliminated the antibiotic problem. Han and Mudgett (1992) concluded that *M. purpureus* ATCC 16365 was the most appropriate organism for solid-state fermentation and confirmed that it had no antibiotic activity (5). *Monascus* produces a range of compounds normal to growth metabolism such as alcohol, enzymes, coenzymes, monascolins that modify fat metabolism, antihypertensive agents, and flocculents. This is a strain selection problem, growth optimization, and finally a removal problem for a food colorant.

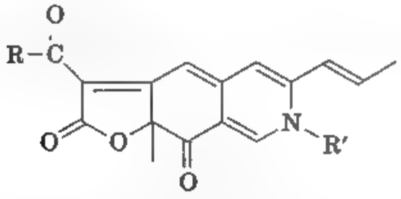


Figure 2. A red derivative of the monascin pigment. R represents an aliphatic radical and R′ represents a compound of the formula HN-R, which represents an amino sugar, a polymer of an amino sugar, or an amino alcohol. *Source:* Ref. 2.

APPLICATIONS

Monascus colorants offer considerable advantages since they can be produced in any quantity on inexpensive substrates. Their range of colors from yellow to red and their stability in neutral media is a real advantage. Sweeny et al. suggested *Monascus* colorants for processed meats, marine products, jam, ice cream, and tomato ketchup (6). They should be appropriate for alcoholic beverages such as saki and also for koji, soy sauce, and kamboko. There is considerable interest in the *Monascus* group, with 38 patents issued in the years 1969 to 1985 (3). Currently *Monascus* colorants are not permitted in the United States.

BIBLIOGRAPHY

1. H. C. Wong and P. E. Koehler, "Mutants for Monascus-pigment Production," *J. Food Sci.* **46**, 956–957 (1981).
2. U.S. Pat. 3,993,789, H. R. Moll and D. R. Farr.
3. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.
4. Jap. Pat. 81,006,263 (1981), H. Tadao et al.
5. O. H. Han and R. E. Mudgett, "Effects of Oxygen and Carbon Dioxide on Monascus Growth and Pigment Production in Solid State Fermentation," *Biotechnol. Prog.* **8**, 5–12 (1992).
6. J. G. Sweeny et al., "Photoprotection of the Red Pigments of *Monascus anka* in Aqueous Media by 1.4,6-Trihydroxynaphthalene," *Agric. Food Chem.* **29**, 1189–1193 (1981).

COLORANTS: PHYCOBILINS

Phycobilins are found in abundance as components of the blue-green, red, and cryptomonad algae. They are colored, fluorescent, water-soluble pigment-protein complexes classified into three major groups according to color: the phycoerythrins, which are red in color with a bright orange fluorescence, and the phycocyanins and allophycocyanins, which are both blue and fluorescent red. The range of color and the apparent stability of the pigments appear to make this group attractive as food colorants.

The structures for the two chromophores of the cyanobilins, termed phycocyanobilin and phycoerythrobilin, are shown in Figure 1. Phycocyanins and allophycocyanins share the same chromophore, and the differences in color are due to the different protein groups. Figure 1 shows a bilin written in the conventional linear form and also in acyclic, possibly truer, form, showing its relationship to other pigments such as chlorophyll and haemoglobin. The

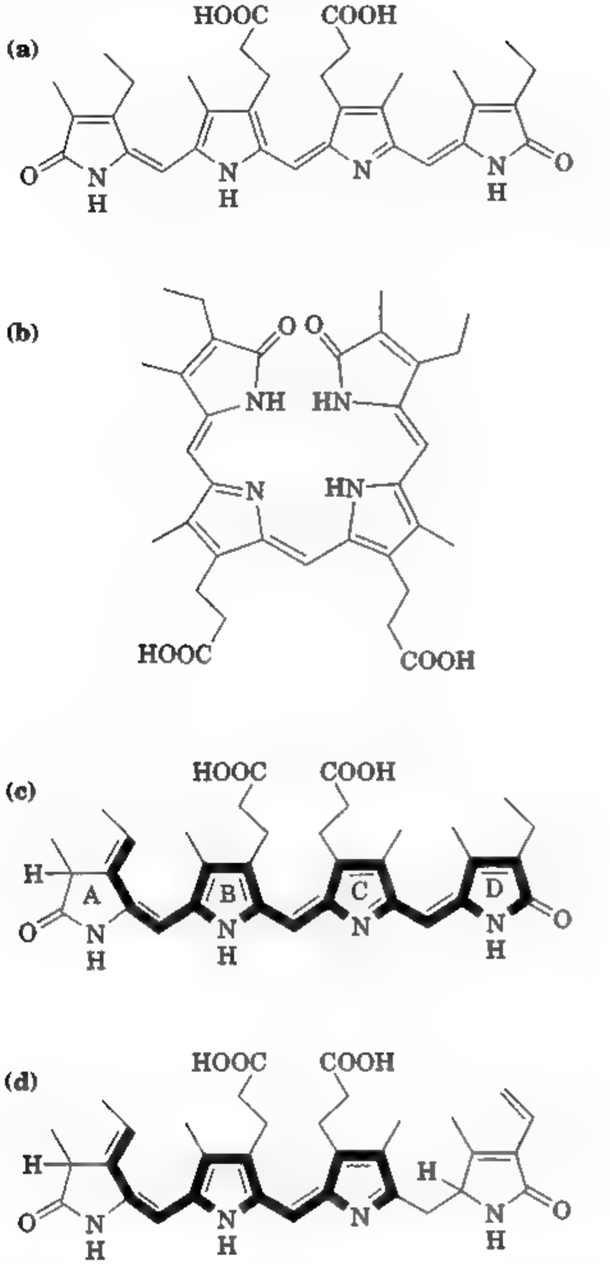


Figure 1. Basic structure of the phycobilins. (**a**) is a bilin in the conventional linear form. (**b**) is the same structure in a cyclic form to show its relationship with the porphyrins. (**c**) is a blue phycocyanobilin pigment and (**d**) is a red phycoerythrobilin pigment.

attachment of the bilin chromophore to its protein is very stable due to covalent bonds between the ethylidene group on position 3 (see COLORANTS: CHLOROPHYLLS, Fig. 2) of the bilin with a cysteine group in the protein. A second covalent bond may be on position 18. The major function of the bilin pigments is to act as light absorbers in the energy transport system.

Phycobilin preparations can be obtained by simply freeze-drying an algal cell suspension, producing a highly colored powder. The chlorophyll and carotenoid components can be removed by centrifuging after breaking the algal cells. The centrifugate is a brightly colored solution of water-soluble protein of which 40% may be phycobilin. Further purification by precipitation with ammonium sulphate and ion-exchange yields almost pure phycobilin. The free chromophore can be obtained by extended refluxing in methanol. An alternative to extensive purification would be to grow an organism such as *Cyanidium caldarium*, which, on addition of 5-aminolevulinic acid to the growth medium, yields pure phycocyanobilin (1). Another approach is to use the existing technology for growth of *Spirulina platensis* in open ponds. This organism has been used for eons in Africa and Mexico, probably because of its high protein content and digestibility. It is important in health food stores as a dietary supplement. The cells are simply harvested and dried. Another unicellular organism, the red algae *Porphyridium cruentum* can also be grown in open ponds or in tubular reactors for production of phycobilin and phycoerythrin. A number of patents (2) exist for the extraction, stabilization, purification, and use of phycocyanins from *Spirulina* and *Aphanotheca nidulans*, and three firms are marketing the product.

The presence of a protein in the pigment suggests that the colorant would be used for products requiring a minimum of heat treatment. But the fact that it takes 16 hours in boiling methanol to separate the protein from the chromophore suggests that applications could include products with mild heat treatment. Suggested applications for phycocyanin colorants include chewing gums, frozen confections, soft drinks, dairy products, sweets, and ice cream. No patents have been filed for the use of phycoerythrin, but it seems like a good candidate for a red colorant.

There are several industrial applications for phycobilins such as fluorescent tracers in biochemical research, fluorescence-activated cell sorting, fluorescence microscopy, and so on (1). Existence of other markets will certainly help to obtain the critical volume necessary for production of food colorants. Colorants containing phycobilins are currently not permitted in the United States.

BIBLIOGRAPHY

1. J. D. Houghton, "Haems and Bilins," in G. A. F. Hendry and J. D. Houghton, eds., *Natural Food Colorants*, Blackie Publishers, Glasgow, Scotland, 1996, pp. 157–196.
2. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.

COLORANTS: POLYPHENOLS

The major brown food colorant is obviously caramel, but other sources are available. A brown colorant can be prepared from an appropriate mixture of FD&C colorants or from extracts of cacao or tea.

CACAO

Cacao, *Theobroma cacao*, has been suggested as a potential source of brown colorants. The cocao, pods, beans, shells, cotyledons, husks, and stems all contain a complex mixture of polyphenols such as cyanidin glycosides, leucoanthocyanins, (-)-epicatechins, quercitin glycosides, and the acyl acids *p*-coumaric and gentisic. Colorants prepared from cacao are likely to contain a complex mixture of leucoanthocyanins, flavonoid polymers, and catechin-type polymers.

The suggested extraction methods involve hot acid or alkaline water or ethanol followed by filtration and concentration. A more reddish extract can be obtained by prior roasting of the beans followed by extraction with hot aqueous alkaline solutions. The colorant is suitable for most foods where a brown color is desired. Cacao products are permitted as food ingredients in the United States but not as a source of colorants.

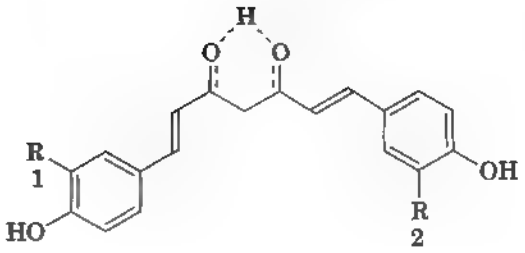
TEA

Extracts of tea, *Thea sinensis*, have been used as a brown colorant for centuries. The polyphenols in tea comprise a very complex mixture including the glucosides and rhamnoglucosides of myricetin, quercetin and kaempferol, di-*C*-glycosylapigenins, 7-*O*-glucosylisovitexin, epicatechin, epigallocatechins, epicatechin gallate, gallic acid, chlorogenic acid, ellagic acid, coumarylquinic acid, and many related compounds. In black tea, they may act as precursors to the poorly defined pigments thearubin and theaflavin. Black tea also contains theaflavin gallate, digalloylbisepigallocatechin, triacetinidin, flavanotropolone, and flavanotropolone gallate.

Preparation of extracts of tea is usually a simple extraction of the leaves and stems with warm water or ethanol, followed by filtration and concentration. Extracts of tea are legal food ingredients in the United States but specific colorants prepared from tea products are not. This may appear to be a fine distinction, but it depends on whether the colorant is classified as an ingredient or an additive.

COLORANTS: TURMERIC

Turmeric has been used as a spice and colorant for thousands of years and is today still one of the principal ingredients of curry powder. Turmeric is produced in many tropical countries, including India, China, Pakistan, Haiti, Peru, and the East Indies. Turmeric (CI Natural Yellow 3, CI No. 75300, EEC No. E 100), also called tumeric or curcuma, is the dried ground rhizomes of several species of *Curcuma longa*, a perennial herb of the Zingiberaceae family native to northern Asia.



Curcumin	$R_1 = R_2 = OCH_3$
Demethoxycurcumin	$R_1 = H$ $R_2 = OCH_3$
Bisdemethoxycurcumin	$R_1 = R_2 = H$

Figure 1. Pigments of turmeric.

Turmeric is a bright yellow powder with a characteristic odor and taste and contains three major pigments: curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Fig. 1). These pigments together with the flavor compounds turmerone, cineol, zingeroni, and phellandrene constitute the turmeric and turmeric oleoresin of commerce. The oleoresin is prepared by extraction of dried rhizomes with one or a combination of solvents, including methanol, ethanol, isopropanol, ethyl acetate, hexane, methylene chloride, ethylene dichloride, and trichloroethylene, and removal of the solvent. Other methods include extraction with ether, removal of the ether, and suspension of the residue in vegetable oil. Powdered turmeric preparations consisting of the powdered rhizome or the oleoresin standardized with maltodextrin usually contain 8 to 95% cucurmin. Liquid concentrates of oleoresin suspended in ethanol and/or propylene glycol with a polysorbate emulsifier usually contain 0.5 to 25% curcumin. A variety of other carriers such as edible oils and fats, and monoglycerides are also available (1). The FDA-approved extraction procedures are specified in the CFR Section 73.615 for Turmeric.

Turmeric and turmeric oleoresins are both unstable to light and alkaline conditions; thus, several patents involve formulation with citric, gentisic, gallic, and tannic acids. Polyphosphate, sodium citrate, waxy maize, and an emulsifier are sometimes added. Both powder and liquids are susceptible to degradation by oxidation. Both show good tinctorial strength with turmeric usually used in the 0.2 to 60 ppm and the oleoresin in the 2 to 640 ppm range. The tinctorial strength is usually expressed as % turmeric even though preparations from different geographical regions differ in the relative content of curcumin and demethoxycurcumin. Both turmeric and the oleoresin produce bright yellow to greenish yellow shades and are sometimes used as a replacement for FD&C Yellow No. 5. Curcumin is insoluble in water, but water-soluble complexes can be made by complexing with heavy metals such as stannous chloride and zinc chloride to produce an intense orange colorant. Curcumin colorants can also be prepared by absorbing the pigments on finely divided celllose.

The major applications of turmeric are to color cauliflower in pickles and as an ingredient in mustard and curry powder. It is also used alone or in combination with other colorants such as annatto in spices, ice cream, cheeses, baked goods, confectionery, cooking oils, and salad dressings.

BIBLIOGRAPHY

1. F. J. Francis, *Handbook of Food Colorant Patents*, Food and Nutrition Press, Westport, Conn., 1986.

COLORANTS: MISCELLANEOUS COLORANTS

INORGANIC COLORANTS

A number of miscellaneous preparations may be added to foods for various reasons. Some, such as titanium dioxide and carbon black, are obvious colorants, but others, such as zinc oxide and calcium carbonate, are nutritional supplements. Calcium carbonate is a pH adjuster. In both cases their contribution to color is minimal. Still others, such as talc, are indirect additives used in processing or packaging where they do contribute incidentally to appearance but it is not their primary function. There is a long list of compounds in this category as described in CFR 21-178.3297.

Titanium Dioxide

Titanium dioxide (CI Pigment White, CI No. 77891, CAS Reg. No. 1 3463-67-7, EEC No. E 171, Titanic Earth) is a large industrial commodity with current world production estimated at nearly 4 million tons, but only a small percentage is used as a food colorant. Commercial TiO_2 is obtained from the naturally occurring mineral ilmenite ($FeTiO_2$) and occurs in three crystalline forms, anatase, brookite, and rutile, with anatase as the most common form in industry. The only form allowed in food is synthetically produced anatase.

TiO_2 is the whitest pigment known today with a hiding power five times greater than its closest rival zinc oxide. This is the reason for its importance in paint formulations. TiO_2 is a very stable compound with excellent stability toward light, oxidation, pH changes, and microbiological attack. Water-dispersible and oil-dispersible forms are available usually in water, glycerol, propylene glycol, sugar syrups, vegetable oils, or polyglycerol esters of fatty acids. Xanthan gum may be added as a stabilizer and potassium sorbate, citric acid, and methyl and ethyl parabens as preservatives. It is used in confectionery, baked goods, cheeses, icings, and numerous pharmaceuticals and cosmetics up to 1% of the weight of the final product.

Carbon Black

Carbon black is a large volume industrial commodity, but food usage is very small. Food-grade carbon black is derived from vegetable material, usually peat, by complete combustion to residual carbon. The powder colorant has a very small particle size, usually less than 5 nm, and consequently is very difficult to handle. Therefore, it is usual for carbon black to be sold to the food industry in the form of a viscous paste with the colorant suspended in glucose syrup. Little safety data are available, and in the United States in the 1970s when the GRAS list was being reviewed, toxicological data were requested in view of the theoretical possibility of contamination with heterocyclic amines. Apparently the cost of obtaining the data was higher than the entire annual sales of food-grade carbon black so the tests were never done. Carbon black is not currently permitted in the United States.

Carbon black is a very stable and technologically a very effective colorant. It is widely used in Europe and other countries in sugar confectionery.

Ultramarine Blue

Ultramarine blue (CI No. 77007, E 180) is a synthetic blue pigment of rather indefinite composition. The group of ultramarine pigments are aluminosulphosilicates with empirical formulas approximated as $Na_2Al_6Si_6O_{24}S_3$. Ultramarine blue is well known as a cosmetic and is intended to resemble the colorants produced from the naturally occurring semiprecious gem lapis lazuli.

The basic ingredients for the production of the ultramarines are kaolin (China clay), silica, sulfur, soda ash, and sodium sulfate plus a reducing agent such as rosin or charcoal pitch. Depending on the processing conditions, ultramarine green, ultramarine blue, ultramarine violet, and ultramarine red can be produced. All four are important as cosmetic colorants, but the only food use is in salt intended for animal use at the 0.5% w/w level.

Iron Oxides

The iron oxides represent a group of synthetic colorants. The iron oxides are known under a variety of names such as CI Pigment Black 11 and CI Pigment Browns 6 and 7 (CI No. 77499), CI Pigment Yellows 42 and 43 (CI No. 77492), and CI Pigment Reds 101 and 102 (CI No. 77491). The chemical composition varies with the method of manufacture but can be represented by the empirical formula $FeO.xH_2O,Fe_2O_3.xH_2O$ or some combination. Most are produced from ferrous sulfate $FeSO_4.7H_2O$, and the most common forms are yellow hydrated oxides (ochre) and the brown, red, and black oxides.

Iron oxides are very stable compounds insoluble in most solvents but usually soluble in acids. Their main uses are in cosmetics and drugs, but they are allowed (CI Nos. 77491, 77492, 77499) in dog and cat food at levels up to 0.25% by weight of the finished food.

Talc

Talc (CI Pigment White, CI No. 77018, CAS Reg. No. 14807-96-6) is a naturally occurring magnesium silicate, $3MgO.4SiO_2H_2O$, sometimes containing a small amount of aluminum silicate. It is a large industrial commodity produced in many countries, particularly France, Italy, India, and the United States. The lumps are known as soapstone or steatite and the fine powders as talc, tateum, or French chalk. Its major uses are as a dusting powder in medicine; as a white filler in paints, varnishes, and rubber; and as a lubricant in molds for manufacturing. It is used as a release agent in the pharmaceutical and baking industries as well as in oatings for rice grains.

Zinc Oxide

Zinc oxide (CI Pigment White, CI No. 77947, CAS Reg. No. 1314-13-2) is a white or yellowish-white odorless amorphous powder. It is insoluble in water but soluble in most mineral acids and alkali hydroxides. Zinc oxide is the most important white powder used in the cosmetic industry. It has the advantages of brightness, ability to provide opacity without blue undertones, and it has antiseptic and healing effects. It does not have the hiding power of titanium dioxide but sometimes is used as a whitener in wrappers for food. Zinc oxide is also added as a nutrient dietary supplement.

Calcium Carbonate

Calcium carbonate (CI Pigment White 18, CI No. 77220, EEC No. E 170, CAS Reg. No. 471-34-1) is a fine white powder prepared by precipitating $CaCO_3$ from solution. Calcium carbonate occurs naturally as limestone and marble, but the impurities make it unacceptable as a food ingredient. Calcium carbonate has a minor role in foods as a whitener. Its major roles are as a pH adjuster, dietary nutrient supplement primarily for cereals, dough conditioner, firming agent, and yeast food. There are no limitations on use other than Good Manufacturing Practices.

Silver

Silver (EEC No. E 174) is a fine crystalline powder prepared by the reaction of silver nitrate with ferrous sulfate in the presence of nitric, phosphoric, or sulfuric acid. Polyvinyl alcohol is sometimes added to prevent the agglomeration of crystals and the formation of amorphous silver. Its primary use is as a fingernail polish colorant. Colloidal silver is sometimes used in the food industry as a bactericidal aid in purifying solutions.

Silver dragees are small silver-coated candy balls used as an ornament and ingredient in confectionery and baked goods.

Silicon Dioxide

Silicon dioxide is an amorphous material produced synthetically by either a vapor-phase hydrolysis process yielding fumed (colloidal) silica, or by a wet process that yields precipitated silica, silica gel, or hydrous silica. Fumed silica occurs as a hygroscopic, white, fluffy, nongritty powder of very fine particle size. The wet-process silicas occur as hygroscopic, white, fluffy powders or white microcellular beads or granules.

The colorant uses of silicon dioxide are minimal, but it is used widely in foods as an anticaking agent, defoaming agent, conditioning agent, and chillproofing agent in malt beverages.

Gold

Gold is allowed in some alcoholic beverages, usually in the form of flakes.

ORGANIC COLORANTS

Riboflavin

Riboflavin (EEC No. E 101) is a yellow pigment, in the vitamin B group, found in plant and animal cells. Riboflavin is usually added to food as an essential nutrient, but it is also an effective colorant. Together with riboflavin-5′-phosphate, it is a well-known colorant in Europe, but only riboflavin is allowed in the United States. In pure form it is a yellow orange crystalline powder, soluble in water and alcohol and insoluble in ether and chloroform. Riboflavin is somewhat unstable to light and oxidation and is unstable in alkaline media. It is stable to heat. For colorant purposes, riboflavin is confined mainly to cereals, dairy products, and sugar-coated tablets. Riboflavin produces an attractive greenish-yellow color with an intense green fluorescence.

Corn Endosperm Oil

Corn endosperm oil (CAS No 977010 506) is obtained by extraction of corn gluten with isopropyl alcohol and hexane. It is a reddish brown liquid containing fats, fatty acids, sitosterols, carotenoid pigments, and their degradation products. It is added as a nutrient, but it also contributes a yellow/orange color to feedstuffs.

Dried Algal Meal

Dried algal meal is a dry mixture of algal cells produced by the growth of *Spongiococcum* spp. on molasses or cornsteep liquor. It contains a maximum of 0.3% ethoxyquin.

Algae, Brown Extract

Brown extract algae (CAS No. 977026 928) are produced by extraction of the seaweeds *Macrocystis* and *Laminaria* spp.

Algae, Red Extract

Red extract algae (CAS No. 977090 042) are produced by extraction of the seaweeds *Porphyra* spp. *Gloiopeltis furcata*, and *Rhodomenia palmata*. All three algal products are added primarily for nutrient reasons, but they do add to the color.

Cottonseed Products

The FDA recognizes four products from cottonseed kernels:

Cottonseed flour (CAS No 977050 546). It is partially defatted and cooked.

Cottonseed flour (CAS No. 977043 778). It is partially defatted, cooked, and roasted.

Cottonseed kernels (CAS No. 997043 778). Glandless raw kernels.

Cottonseed kernels (CAS No. 997043 78. Glandless roasted kernels.

All four are nutrients, but they do contribute to the color.

Shellac

Shellac is obtained from the resinous secretions of the insect *Lassifer lacca*. Shellac is used primarily in foods as a coating agent, surface-finishing agent, and glaze in baked goods and some fruits, vegetables, and nuts. Shellac is not primarily a colorant, but it does affect the appearance of a product.

Octopus Ink

The secretions of the octopus and squid are a complex mixture of melanoidin polymers. It is an effective black colorant for pasta for special occasions in some ethnic groups, particularly the Portuguese.

COMPUTER APPLICATIONS IN THE FOOD INDUSTRY

BACKGROUND AND DEFINITIONS

The use of computers has become so pervasive in our professional and personal lives that a basic description of computer terms and functions is generally unnecessary. From laptop computers to mainframes the speed, power, and memory of personal computers continues to increase. Computer manufacturers and software developers compete fiercely for market share. New products and systems are constantly emerging or are being revised. Hundreds of books are being printed on computer-related topics every year. Rapidly changing dynamics of the computer industry requires constant updating to stay informed.

Current computer developments must be considered within the context of the entire information marketplace. The information marketplace includes the collection of computers, communications, software, and services engaged in all business and personal transactions. Today's computers successfully support many applications such as spreadsheets and databases. However, the larger problem involves the exchange of information between computers. A lack of standardization of data forms at different sites and with different machines and software packages makes communication difficult. Some degree of standardization may occur as large companies try to impose their standards on the marketplace in order to achieve dominance. The development of tools to encode and decode information and transactions is necessary to take advantage of widespread information exchange. A current area of great interest and growth is information exchange via the Internet. Although the information available through the Internet is extensive, Web browsers can require a great deal of time for searches, and often with uncertain results. The Web has great potential for information exchange, but it is still quite chaotic. Search engines will need to be improved. Security for confidential and financial electronic transactions is uncertain. Many companies provide general information about their operation on a Web page. Most suppliers and trade organizations use Web pages to advertise their products or to report on their activities. Web pages can offer attractive graphics and provide access to company literature and personal contacts for further information or sample requests.

Many computer applications have incorporated some manifestation of artificial intelligence. This term includes devices and applications that exhibit human intelligence and behavior, including robots, expert systems, voice recognition, natural and foreign language processing (1). Expert systems use a knowledge base of human expertise to solve problems. The knowledge base is commonly in the form of if-then-else rule statements, which interact with the user through an inference engine. Expert systems are often used for diagnostic or troubleshooting applications. Knowledge-based expert systems are often embedded as tools within larger programs, somewhat losing their identity. Additional applications widely used include object-oriented programs and neural networks. Object-oriented programs use modules of software to allow their reuse and interchange between programs. Neural networks somewhat mimic biological neurons in that loosely related data can be trained to reflect patterns in a process. Once trained the pattern can predict a particular output for specified input variables, just as a nerve stimulus generates a specific response. Neural networks are being used for more sophisticated process control schemes.

FOOD INDUSTRY APPLICATIONS

Production

Computers have long been a part of agriculture. Much early statistical work began with agricultural experimentation, generating an early need for mathematical computational power. Computers continue to play an important role in agriculture, both in research as well as for farm management. Models of crop growth and yield have been developed to predict the effects of land use and other management practices on yields, profitability, and the environment (2). Dairy cattle nutrition regimens and culling practices are adjusted according to computer records of growth and milk production. Computer-based tools are available to help farmers predict profitability investments (3) and to simulate production and population growth.

Product/Process Development

One of the earliest applications for computers in food processing was for linear programming to obtain optimal use of resources. When a variety of ingredients at varying costs is available to choose from, least-cost formulation programs are widely used. Given certain constraints such as moisture, salt, and fat content, the lowest cost ingredients are selected. More sophisticated programs can consider additional factors such as raw materials, equipment and time requirements, scheduling, and finished goods inventory. A modular neural network system has been developed to optimize the selection of starches for use in processed foods (4). Types of starch along with variables such as source, cost, flavor impact, function, and pH were incorporated into the program. The modular arrangement consisted of two networks trained independently on half of the starches, and then coupled in the final network. A faster learning period resulted. A program based on matrices has been developed that combines food component data with information on process control, inventory management, costing, and quality (5). The information is used to track inventories and is presented in a form ready for label printing. Random centroid optimization has been used to develop a process foods formulation program (6). Compared with other formula optimization techniques it was found to work more efficiently. Accommodating up to 20 factors it was found to be more useful when formulating with constraints.

Product development can be expensive in terms of personnel, ingredients, and plant time. Modeling or simulation programs that predict behaviors given certain conditions can reduce the time and expense of actual trials. Commercial tools are available for developing process models (The MathWorks, Inc., Natick, MA). Numerous reports of food process models have been prepared. Computer-based mathematical models were developed to predict the quality of apple juice during storage (7). Models were based on mass transfer of product through the package and accompanying ascorbic acid oxidation. Computer simulation was used to develop mathematical models for moisture transfer in different packaging systems (8). The models were successfully used in two component bakery mixes. A computer program for calculation of temperature during continuous heating of a particulate food product has been developed (9). The program is useful in predicting the effect of processing on product quality in an aseptic system comparing surface heat transfer and heat conduction inside the particles. Specialized computer packages have been developed for modeling sugar processing (10). Modeling activities include simulation, prediction, optimization, design, evaluation, and control. Optimal conditions for thermal processing have been determined using neural networks (11). Input variables included can size, thermal diffusion, and sterilization temperatures.

Statistical programs such as principal components analysis (PCA) and analysis of variance (ANOVA) have been integrated into programs specific for food product development (12). Statistical and experimental design tools are commonly integrated into packages for product development work. Computer-aided design/computer-aided manufacture (CAD/CAM), along with Latin squares experimental design have been used for label design (13). Consumer response to label changes was identified. Computerized systems have the potential to improve the accuracy of sensory evaluation as it relates to consumer preference and competitor analysis (14). Continual improvements in dietary consumption databases can be helpful in a better understanding of consumer behavior (15).

Quality Assurance

Laboratory automation systems (LAS) and laboratory information management systems (LIMS) have been developed to reduce errors and increase efficiency of laboratory analyses. Rapid access to previous analyses, data manipulation, and report preparation are valuable features of these systems. LIMS continue to evolve as with all computer software. Vendors range from dedicated, smaller companies to business units of larger instrument manufacturers such as Hewlett-Packard (Wilmington, DE) and Beckman Instruments (Fullerton, CA). Continuing improvements include user interfaces, sample scheduling, data storage and retrieval, instrument interfacing, and Web accessibility. Trade journals abound with descriptions of LIMS. Additional information is available through the Internet.

Statistical Quality Control (SQC) charts are widely used to monitor process control and product quality. Important factors to consider when selecting SQC software include the ability to handle process and lab data and the ability to exchange data with corporate or plantwide information systems. Computer programs have been developed for designing hazard analysis and critical control points (HACCP) systems. HACCP software can quickly access critical control points along a process.

Sophisticated computer-based tools for product analysis and identification are available. Computer-controlled automated sampling procedures have been developed to predict noodle discoloration over time (16). Computer vision technology can be used as a quality control process for gauging, verification, flaw determination, recognition, and locating (17). Machine vision and image processing have been used to identify corn kernel shape (18). Grain kernels, nuts, and crackers have also been examined using computerized image analysis and classified with a neural network program (19). Snack and chips with varying morphological and texture features as input and sensory attributes as output were used to train a neural network for quality analysis. The program was validated with predicted output compared with a sensory evaluation panel (20). Computer-controlled shape and size grading and filleting in the seafood industry have been developed (21). Computer-simulated models of flavor loss due to volatilization during heating or baking have been used to improve quality (22). Flavor was related to product-to-air partition coefficients of the flavor components and texture of the foods.

An artificial neural network has been developed to relate residual chlorine in stored water to various water quality and operational parameters (23). A fault analysis program has been developed to diagnose problems at all levels of cake production in order to optimize quality (24). Hybrid object-oriented and rule-based systems have been used to design safe food processes (25). The system relates food composition and process parameters with microbiological safety and food quality.

Processing

Process control systems monitor signals from various sensors, perform analyses on the information, and provide the proper control. Control is commonly accomplished with a programmable logic controller (PLC). Data can be stored in and accessed from the PLC by other hardware for report preparation. The typical function of a PLC is to keep certain parameters constant. More advanced process control systems adjust continuously to provide the best possible settings for the existing process and materials. Most food processing operations have at least some and many a large amount of computer-controlled automation. For example, a milk processing plant has a number of steps in a process. Each step contains a series of logic statements that may control valves, pumps, or thermal devices. To start up the pasteurizer, valves allowing water into the balance tank open, a pump turns on, steam or hot water are applied to the heating section. Local control devices maintain water and temperature levels. A flow diversion valve recycles water until the pasteurization temperature is reached. Once the temperature is reached the operator is cued to switch to milk. The operation is stepped up to another set of valve openings and pump activations, allowing milk into the balance tank. During pasteurization, if the holding tube temperature falls below the legal minimum, milk flow is diverted back to the balance tank. Additional sets of instructions are given for shutdown and cleaning. Neural networks have been used to control process parameters, inspect systems, and monitor machine performance. Incorporation of automation and computers has been credited with increased productivity, reduced waste, more effective cleaning, and improved quality assurance.

Integrated computer systems have been developed in which the food processing operation acts as part of a supply chain. In these systems materials flow from suppliers, through the processor, and on to distribution. Information flows in both directions. Computer integrated manufacturing (CIM) has incorporated business plans and information strategies with business applications software. Electronic data interchange, use of bar codes, automated data collection and retrieval, scheduling, and process control are brought together in a CIM environment. Statistical process control (SPC) programs are commonplace in the food processing industry. Automated production lines require sensors such as thermocouples, flowmeters, and colorimeters to determine when adjustments are required. Data collected from the sensors is used by SPC programs to display the immediate and historical state of the process. Feedback can be sent directly to controllers for changing process variables when required.

Computer automation in which a machine is made to duplicate some labor intensive human operation has involved the implementation of robots. Robots are able to withstand harsh working and cleaning conditions. Labor savings is another robotics benefit. Robots have been extensively used in packaging and palletizing, relieving humans of much physically demanding work. From robotics to control charts, there has been a proliferation of software for the process industries. Mathematical models have been used to determine optimal locations and the method of producing and transporting products. Effective use is made of machine capacity, manpower, raw materials, and warehouse space. Mathematical models and rule-based systems are useful for planning and are able to show the effects of minute by minute changes. For example, given characteristics such as vessel size the program indicates processing time, sequence of products, selection of equipment, and costs. Fuzzy logic programs have been useful for monitoring complex food processes (26). Fuzzy logic control offers a combination of objective process data with subjective human operator input to achieve performance output such as reduced quality defects. In the past raw process data would be modified and reduced before entering into an operational database. With low cost disk space and inexpensive database servers, more often raw data is being saved for further analysis. New techniques are then used to extract information from this data to improve process control and other process optimization. Data can be used with an expert diagnostic system for troubleshooting problems. Neural network technology can also be used to recognize patterns in data unsuitable for conventional statistical techniques.

Manufacturing execution systems (MES) claim to merge process control systems and management information systems (MIS). They integrate plant floor operations with production planning (27). Using current data MES guides, these systems initiate, respond to, and report on plant activities as they occur. Rapid responses can then be made to changing conditions. MES provides tools to integrate other programs such as SPC and supervisory control and data acquisition (SCADA) and then integrate them with planning systems such as manufacturing resource planning (MRP II). As these various computer-based control systems often overlap. Promoters claim MES as an information hub that links to all these other manufacturing systems. Enterprise resource planning (ERP) systems, which already integrate material resource planning with other upper-level business systems (such as financial, sales, and marketing information; cost management; human resources data; and supply-chain management) requires close integration with MES. Object-oriented program technology will help in this integration. Object-oriented programs are based on modular programs that can be modified without affecting each other, reducing costs to customize programs for new business needs.

Warehouse and Distribution

Computer programs have been developed to monitor suppliers and supplies. These systems provide enhanced communication within the plant and between the plant and suppliers. Simulation software has been developed to predict temperature changes under various conditions during storage or transit (28). The predictions can be useful in developing HACCP programs. It is becoming more common for suppliers to be automatically linked to customer inventory databases so that they can keep the customer supplied automatically. This offers advantages of stable, long-term business for the supplier and a never-failing supply of raw materials, parts, and other resources for the customer.

Administrative Operations

Business applications for computers such as word processing, databases, spreadsheets, and presentation graphics are commonly used in the food industry. Human resource management, including health and benefits, accounting, scheduling, training, strategic planning, and all business-related operations in a company, is very dependent upon standard computer programs. Graphic presentation and word processing programs have become very sophisticated with features such as powerful computational functions and data linking operations. Plants and administrative facilities can be closely linked for communications regardless of location. Data from remote and varied locations can be combined for analysis and report generation. International locations and contacts have become more accessible. Plant security systems, which often use key cards for entry and tracking of employee arrivals and departures, are becoming more sophisticated. Biometric recognition has used fingerprints, hand geometry, and eye scans for identification (29).

Food Safety

Implementation of HACCP programs has been greatly facilitated with the use of computer programs. Systems for recall in the event of a product failure require the speed and power of electronic coding and tracking in order to be effective. Microbial modeling programs have created databases and calculated growth, survival, thermal-death-time, and time-to-growth model for various pathogens. Microbial behavior in food is largely determined by factors such as pH, temperature, water activity, and atmosphere. Although limitations of models must be considered, they have been useful in estimating microbial behavior, developing HACCP programs, assisting in planning laboratory experimentation, and educating nonmicrobiologists. In addition, changes in composition or processing can be quickly evaluated, assisting in development of safer products. A limitation of predictive models occurs with outliers. Samples with high contamination, the first pathogenic cells to adjust to a new environment, or the organism with greatest thermal resistance may elude the model.

FUTURE DEVELOPMENTS

In the highly competitive and often saturated markets in which food companies operate, growth is not likely to occur through price increase or geographical expansion. Rather, growth depends on being the low-cost supplier and having access to competitive information. Integration of computer-based tools for sales and marketing management, manufacturing, maintenance, logistics, supply and inventory data, and financial management will be the goal of computer systems suppliers. Use of the Internet and other forms of network communication will continue to have a major impact on information exchange. Competitive intelligence is becoming a critical business strategy. Increased surveillance will cause security of electronic data to become increasingly important. With the proliferation of software, control systems, and networking tools, food processing industries are able to operate more efficiently and competitively. Proper implementation, monitoring, and continual training are key factors for success.

BIBLIOGRAPHY

1. A. Freedman, *The Computer Desktop Encyclopedia*, AMACOM, New York, 1996.
2. P. K. Thornton, H. W. G. Booltink, and J. J. Stoorvogel, "A Computer Program for Geostatistical and Spatial Analysis of Crop Model," *Agronomy Journal* **89**, 620–627 (1997).
3. J. A. A. M. Verstegen et al., "Economic Value of Management Information Systems in Agriculture: A Review of Evaluation Approaches," *Computers and Electronics in Agriculture* **13**, 273–288 (1995).
4. Y. W. Huang et al., "Modular Neural Networks for Identification of Starches in Manufacturing Food Products," *Biotechnol. Prog.* **9**, 401–410 (1993).
5. C. Barcenas and J. P. Norback, "A System for Tracking Food Components for Labeling and Other Purposes," *Food Technol.* **47**, 97–100, 102 (1993).
6. J. Dou, S. Toma, and S. Nakai, "Random-centroid Optimization for Food Formulation," *Food Research International* **26**, 27–37 (1993).
7. J. N. Kim et al., "Computer Simulation Model for Diffusion of Oxygen into a Packaged Liquid Food System with Simultaneous Oxidation of Ascorbic Acid," *IFT Annual Meeting: Book of Abstracts* **129**, (1996).
8. G. C. P. Rangarao, U. V. Chetana, and P. Veerraju, "Mathematical Model for Computer Simulation of Moisture Transfer in Multiple Package Systems," *Lebensmittel-Wissenschaft und Technologie* **28**, 38–42 (1995).
9. C. Skjoldebrand and T. Ohlsson, "A Computer Simulation Program for Evaluation of the Continuous Heat Treatment of Particulate Food Products: II," *Journal of Food Engineering* **20**, 167–187 (1993).
10. P. Thompson, "The Engineers' Toolbox. A Review of Modeling and Analysis Tools and their Application," *International Sugar Journal* **99**, 427–432 (1997).
11. S. S. Sablani, H. S. Ramaswamy, and S. O. Prasher, "A Neural Network Approach for Thermal Processing Applications," *Journal of Food Processing and Preservation* **19**, 283–301, (1995).
12. S. Schonkopf et al., "Computer-aided Product Development in the Food Industry," *Food Technol.* **50**, 69–70 (1996).
13. R. P. Hamlin and K. J. Leith, "The Use of CADCAM and the Latin Square to Isolate and Investigate Individual Consumer Cues in Food Products and Their Packaging," *Food Technologist* **25**, 32–38 (1995).
14. H. McIlveen and G. Armstrong, "Sensory Analysis and the Food Industry: Can Computers Improve Credibility?" *Nutr. Food Sci.* **1**, 36–40 (1996).
15. G. M. Price et al., "Measurement of Diet in a Large National Survey: Comparison of Computerized and Manual Coding of Records in Household Measures," *Journal of Human Nutrition and Dietetics* **8**, 417–428 (1995).
16. H. Corke, L. Au-Yeung, and X. Chen, "An Automated System for the Continuous Measurement of Time-dependent Changes in Noodle Color," *Cereal Chem.* **74**, 356–358 (1997).
17. S. Gunasekaran, "Computer Vision Technology for Food Quality Assurance," *Trends Food Sci. Technol.* **7**, 245–256 (1996).
18. B. Ni, M. R. Paulsen, and J. F. Reid, "Corn Kernel Crown Shape Identification Using Image Processing," *Transactions of the ASAE* **40**, 833–838 (1997).
19. K. Ding, "Food Shape and Microstructure Evaluation with Computer Vision," *Dissertations Abstracts International B* **56**, 3872–3873 (1996).
20. M. S. Sayeed, A. D. Whittaker, and N. D. Kehtarnavaz, "Snack Quality Evaluation Method Based on Image Features and Neural Network Prediction," *Transactions of the ASAE* **38**, 1239–1245 (1995).
21. B. Scudder, "Computers Working for Fish," *Seafood International* **10**, 37–39 (1995).
22. K. B. Roos and E. Graf, "Nonequilibrium Partition Model for Predicting Flavor Retention in Microwave and Convection Heated Foods," *J. Agric. Food Chem.* **43**, 2204–2211 (1995).
23. J. B. Serodes and M. J. Rodriguez, "Predicting Residual Chlorine Evolution in Storage Tanks Within Distribution Systems: Application of a Neural Network Approach," *Aqua* **45**, 57–66 (1996).
24. L. S. Young, "Getting the Product Right," *Food Manufacture* **70**, 43 (1995).
25. M. Schellekens et al., "Computer Aided Microbial Safety Design of Food Processes," *Int. J. Food Microbiol.* **24**, 1–9 (1994).

26. Q. Zhang, J B Litchfield, and J. Bentsman, "Fuzzy Logic Control for Food Processes," *AIChE Symposium Series* **89**, 90–97 (1993).
27. C. E. Morris, "MES Boosts Manufacturing Flexibility," *Food Engineering* **69**, 59–66 (1997).
28. C. Alasalvar et al., "Prediction of Time-Temperature Profiles of Atlantic Salmon (*Salmo salar*) During Chilled Transport Using the MAILPROF Computer Program," *IFT Annual Meeting: Book of Abstracts* 80 (1996).
29. R. W. Hardin, "Biometric Recognition: Photonics Ushers in a New Age of Security," *Photonics Spectra* **31**, 88–100 (1997).

ROBERT L. OLSEN
Schreiber Foods, Inc.
Tempe, Arizona

CONTROLLED ATMOSPHERES FOR FRESH FRUITS AND VEGETABLES

Controlled-atmosphere (CA) storage is a technique for maintaining the quality of fresh fruits and vegetables in an atmosphere that differs from normal air with respect to the concentrations of oxygen (O_2), carbon dioxide (CO_2), and/or nitrogen (N_2). The desired compositions of the atmosphere for storing commodities are usually obtained by initially increasing CO_2 or decreasing O_2 levels in a gas-tight storage room or container. Sometimes, the addition of carbon monoxide (CO) or removal of ethylene (C_2H_4) may also be beneficial.

Modified atmosphere (MA) is a condition similar to CA, but with less or no active control of the gas concentrations. In MA, the O_2 level is reduced and the CO_2 level is increased at a rate determined by the respiration rate of the commodity, the storage temperature, and the permeability of the container and film wrap to the gases. Judicious selection of the commodity, the package dimensions, and the package material will insure establishment and maintenance of the desired atmosphere under specified storage temperatures.

TYPES OF CA STORAGE

During the past 60 years, tremendous progress has been made in the technology of CA storage. The commercial application of CA during transit and storage has received considerable attention since 1960, resulting in the development of different methods of establishing and maintaining CA. These include regular CA; short-term, high-CO_2 treatment; rapid CA; low-oxygen CA; low-ethylene CA; and low-pressure, or hypobaric, storage.

Short-Term High-CO_2 Treatment

Short-term, high-CO_2 treatment was originally developed to maintain the firmness of Golden Delicious apples (1). Subsequently, it was found that pears and other fruits and vegetables also benefit from this treatment (2,3). This treatment involves the exposure of fruit to 10–20% CO_2 for four to seven days prior to adjustment of the atmosphere to regular CA concentrations. Carbon dioxide injury of the fruit skin may occur if moisture has condensed on the surface of the fruit. This high-CO_2 treatment gives excellent results in maintaining the quality of Golden Delicious apples and Anjou pears (1,2,4).

Rapid CA

Rapid CA is a strategy that shortens the time between harvest and establishment of the desirable CA conditions (5). The faster the CA conditions are attained after harvest, the better the fruit quality can be maintained, providing that the cooling rate in storage is not adversely affected by the rapid loading of the room. To achieve the objectives of rapid CA, the storage room should be filled and sealed within three days or less of harvest.

Low-Oxygen CA

Low-oxygen CA has recently received increased attention; not only because it markedly retards fruit softening, but also because it greatly reduces the development of storage scald and breakdown of apples and pears (6). In regular CA, the recommended O_2 concentrations are usually 2% or higher. It has been found that O_2 levels between 1 and 1.5% are even more effective in extending the storage life of some fruits and vegetables (7). Careful monitoring to maintain the precise O_2 level is essential to avoid damage due to anaerobic respiration.

Low-Ethylene CA

In low-ethylene CA, ethylene is scrubbed from the CA room to improve storage quality of the fruit. Removal of ethylene from the storage atmosphere results in retardation of ripening, retention of flesh firmness, and reduction of the incidence of superficial scald of apples (8). It is generally recognized that the concentration of ethylene should be maintained below 1 ppm to obtain the beneficial effect of low-ethylene CA. Storage life of several apple varieties such as Empire and Bramley's Seedling can be extended by this technique (9,10).

Low-Pressure Hypobaric, Storage

Low-pressure, or hypobaric, storage consists of storing fruits and vegetables at below-normal atmospheric pressure. Enhanced diffusion of gases under reduced-pressure facilitates the loss of CO_2 and ethylene from the commodity and reduces the O_2 gradient between the inside and outside of the commodity. The partial pressures of O_2 is directly related to the absolute pressure of air (11). Thus the O_2 concentration is equivalent to 0.55% at 20 mm Hg. Ethylene inside the fruit is also reduced proportionally. Therefore, this storage technique combines the advantages of low O_2 storage and low-ethylene storage. Ripening can be

26. Q. Zhang, J B Litchfield, and J. Bentsman, "Fuzzy Logic Control for Food Processes," *AIChE Symposium Series* **89**, 90–97 (1993).
27. C. E. Morris, "MES Boosts Manufacturing Flexibility," *Food Engineering* **69**, 59–66 (1997).
28. C. Alasalvar et al., "Prediction of Time-Temperature Profiles of Atlantic Salmon (*Salmo salar*) During Chilled Transport Using the MAILPROF Computer Program," *IFT Annual Meeting: Book of Abstracts* 80 (1996).
29. R. W. Hardin, "Biometric Recognition: Photonics Ushers in a New Age of Security," *Photonics Spectra* **31**, 88–100 (1997).

ROBERT L. OLSEN
Schreiber Foods, Inc.
Tempe, Arizona

CONTROLLED ATMOSPHERES FOR FRESH FRUITS AND VEGETABLES

Controlled-atmosphere (CA) storage is a technique for maintaining the quality of fresh fruits and vegetables in an atmosphere that differs from normal air with respect to the concentrations of oxygen (O_2), carbon dioxide (CO_2), and/or nitrogen (N_2). The desired compositions of the atmosphere for storing commodities are usually obtained by initially increasing CO_2 or decreasing O_2 levels in a gas-tight storage room or container. Sometimes, the addition of carbon monoxide (CO) or removal of ethylene (C_2H_4) may also be beneficial.

Modified atmosphere (MA) is a condition similar to CA, but with less or no active control of the gas concentrations. In MA, the O_2 level is reduced and the CO_2 level is increased at a rate determined by the respiration rate of the commodity, the storage temperature, and the permeability of the container and film wrap to the gases. Judicious selection of the commodity, the package dimensions, and the package material will insure establishment and maintenance of the desired atmosphere under specified storage temperatures.

TYPES OF CA STORAGE

During the past 60 years, tremendous progress has been made in the technology of CA storage. The commercial application of CA during transit and storage has received considerable attention since 1960, resulting in the development of different methods of establishing and maintaining CA. These include regular CA; short-term, high-CO_2 treatment; rapid CA; low-oxygen CA; low-ethylene CA; and low-pressure, or hypobaric, storage.

Short-Term High-CO_2 Treatment

Short-term, high-CO_2 treatment was originally developed to maintain the firmness of Golden Delicious apples (1). Subsequently, it was found that pears and other fruits and vegetables also benefit from this treatment (2,3). This treatment involves the exposure of fruit to 10–20% CO_2 for four to seven days prior to adjustment of the atmosphere to regular CA concentrations. Carbon dioxide injury of the fruit skin may occur if moisture has condensed on the surface of the fruit. This high-CO_2 treatment gives excellent results in maintaining the quality of Golden Delicious apples and Anjou pears (1,2,4).

Rapid CA

Rapid CA is a strategy that shortens the time between harvest and establishment of the desirable CA conditions (5). The faster the CA conditions are attained after harvest, the better the fruit quality can be maintained, providing that the cooling rate in storage is not adversely affected by the rapid loading of the room. To achieve the objectives of rapid CA, the storage room should be filled and sealed within three days or less of harvest.

Low-Oxygen CA

Low-oxygen CA has recently received increased attention; not only because it markedly retards fruit softening, but also because it greatly reduces the development of storage scald and breakdown of apples and pears (6). In regular CA, the recommended O_2 concentrations are usually 2% or higher. It has been found that O_2 levels between 1 and 1.5% are even more effective in extending the storage life of some fruits and vegetables (7). Careful monitoring to maintain the precise O_2 level is essential to avoid damage due to anaerobic respiration.

Low-Ethylene CA

In low-ethylene CA, ethylene is scrubbed from the CA room to improve storage quality of the fruit. Removal of ethylene from the storage atmosphere results in retardation of ripening, retention of flesh firmness, and reduction of the incidence of superficial scald of apples (8). It is generally recognized that the concentration of ethylene should be maintained below 1 ppm to obtain the beneficial effect of low-ethylene CA. Storage life of several apple varieties such as Empire and Bramley's Seedling can be extended by this technique (9,10).

Low-Pressure Hypobaric, Storage

Low-pressure, or hypobaric, storage consists of storing fruits and vegetables at below-normal atmospheric pressure. Enhanced diffusion of gases under reduced-pressure facilitates the loss of CO_2 and ethylene from the commodity and reduces the O_2 gradient between the inside and outside of the commodity. The partial pressures of O_2 is directly related to the absolute pressure of air (11). Thus the O_2 concentration is equivalent to 0.55% at 20 mm Hg. Ethylene inside the fruit is also reduced proportionally. Therefore, this storage technique combines the advantages of low O_2 storage and low-ethylene storage. Ripening can be inhibited and storage life prolonged when fruit are stored under hypobaric conditions

BENEFICIAL EFFECTS OF CA STORAGE

Beneficial effects of CA storage include reduction of respiration, scald, decay, discoloration, and internal breakdown, inhibition of ethylene production and ripening, and retention of firmness, flavor, and nutritional quality.

The rate of respiration of fresh fruits and vegetables has been shown to be reduced by low O_2 or high CO_2 (12). The lower respiration rate indicates that CA has an inhibitory effect on the overall metabolic activities of stored commodities. A slower rate of utilization of carbohydrates, organic acids, and other reserves usually leads to prolonging the life of the produce.

Ethylene production of fresh fruits and vegetables is suppressed by low O_2 and/or elevated concentrations of CO_2. The biosynthesis of ethylene in plant tissues requires the presence of O_2 (13,14). When O_2 is absent or when plant tissue is under low O_2 atmosphere, ethylene biosynthesis is inhibited. The time of onset of ethylene production in apples is inversely related to the O_2 concentration in storage, and the maximum rate of production is directly related to the O_2 level (15). High CO_2 inhibits ethylene action on ripening (16), which in turn inhibits the autocatalytic production of enzymes involved in ethylene biosynthesis, including 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ethylene-forming enzymes (17). These inhibitory effects on ethylene action and production by CA consequently lead to the delay of the ripening process.

The loss of organic acids in apples and pears during storage is reduced by CA (18,19). This reduced loss of organic acids in CA fruits is probably due to an increase in CO_2 fixation, an inhibition of respiratory metabolism, and a lower consumption of acids under CA (19,20).

A slower decline in carbohydrates under CA has been reported in sugar beet, Chinese cabbage, apricots, and peaches (21,22,23). CA storage is also beneficial in the retention of ascorbic acid and amino acids in several fresh fruits and vegetables (3,22,24,25).

EFFECTS ON PHYSIOLOGICAL DISORDERS

Scald is one of the most serious physiological disorders in apples, pears, and many other fruits during and after storage. Storage of fruits in CA, particularly in low O_2 atmosphere, has been shown to reduce the susceptibility of fruits to scald (6,26). Russet spotting of lettuce can be caused by exposure to ethylene or warm shipping or storage temperatures (27). This physiological disorder can be substantially reduced by low O_2 atmosphere (28,29). The development and spread of necrotic spots on the outer leaves of cabbage was largely prevented by low O_2 atmosphere but not by high-CO_2 treatment (30). The incidence and severity of vein streaking of cabbage leaves was also reported to be reduced in CA storage (31).

Controlled atmosphere reduces chilling injury in certain sensitive crops, while it aggravates or has no effect in other crops. Holding zucchini squash in low O_2 alleviated chilling injury during storage at 2.5°C (32,33). Conditioning grapefruit with short-term prestorage treatment of high CO_2 (40%) at 21°C reduced subsequent brown staining and rind pitting, two symptoms of chilling injury, at 1°C (34). Intermittent exposure of unripe avocados to 20% CO_2 reduced chilling injury at 4°C (35). Addition of CO_2 to the storage atmospheres was also effective in reducing chilling-induced internal breakdown and retaining ripening ability of peaches (36,37). Controlled atmosphere has also been reported to reduce the severity of chilling injury symptoms in okra (38). However, increasing the level of CO_2 or reducing O_2 concentration can accentuate symptoms of chilling injury in cucumbers, bell peppers, and tomatoes (39,40).

Some other physiological disorders have also been reported to be aggravated by CA conditions. The development of a brown discoloration in and around the core and adjacent cortex tissue of apples and pears has been associated with high CO_2 (41,42). White core inclusions in kiwifruit can also be induced by elevated CO_2 in combination with ethylene (43). The severity of brown stain on lettuce has also been found to increase with increasing CO_2 levels (44).

CONCLUSION

Controlled atmosphere storage has withstood the test of time and has been proven to be effective for maintaining quality and extending the storage life of a number of horticultural crops. The rate of deterioration of fresh produce is often greatly retarded by CA storage. Each type of fruit or vegetable has its own specific requirement and tolerance for atmosphere modification. The optimum CA condition for each commodity is continually being revised and improved. Table 1 presents the most recent recommendations for O_2 and CO_2 levels, the most suitable storage temperatures, feasible storage periods, and pertinent remarks for some commodities.

The maintenance of CA storage requires continual monitoring of the gases and temperature to prevent any deviation from the recommended conditions. Injuries induced by low O_2 or high CO_2 often lead to tissue discoloration or off-flavor and loss of market value. Proper maturity, the internal condition of the fruit at harvest, and the speed at which storage atmospheres are established are some of the key factors affecting the success of CA storage.

It should be emphasized that proper CA storage should always be accompanied by good temperature control. CA storage is considered to be a supplement—not a substitute—for proper refrigeration and careful handling.

Table 1. Summary of Controlled-Atmosphere Storage Requirements for Fruits and Vegetables

Commodity Cultivar	Beneficial concentration (%)		Suitable temperature		Approximate storage period	Remarks	Ref.
	O_2	CO_2	°C	°F			
			Fruits of temperate zone				
Apple (*Malus domestica*)							
Boskoop	1.5–2	<1.5	4	39	5–7 mo	Sensitive to low temperature breakdown	45
Bramley's Seedling	2	6	3–4	37–39	7 mo	CA reduces bitter pit	45
Cortland	2–3	5	2	36	4–6 mo		46
Cox Orange Pippin	1–3	<1	3	37	4–6 mo	2% O_2 first week, then 1.00–1.25% O_2	45
Empire	1.5–3	1–5	2	36	5–7 mo		45
Fuji	2–2.5	1–2	0	32	7–8 mo	CA reduces scald	45
Gala	1–2	1–5	0	32	5 mo		45
Golden Delicious	1–3	1–5	0	32	7–11 mo	Rapid CA is beneficial	45
Granny Smith	1–2	1–3	0	32	7–9 mo	CA reduces scald	45
Jonathan	1–3	1–6	0–3	32–37	4–7 mo	CA reduces Jonathan spot	45
McIntosh	1.5–3	1–5	3	37	7–9 mo		45
Newtown	3	5–8	2–4	36–39	8 mo	Susceptible to low temperature injury	
Northern Spy	2–3	2–3	0	32	8 mo		46
Red Delicious	1–3	1–3	0	32	8–10 mo	Susceptible to scald	45
Rome Beauty	2–3	2–5	0	32	6–8 mo		45
Spartan	1.5–2.5	1–2	0	32	6–8 mo		45
Stayman	2–3	2–5	0	32	7–8 mo		46
Worcester Pearmain	3	5	1	34	6 mo		45
Apricot (*Prunus armeniaca*)	2–3	2–3	−0.5–0	31–32	7 wk	CA delays ripening	47
Blackberry (*Rubus* sp.)	5–10	15–20	−0.5–0	31–32	1 wk	Prompt cooling is important	47
Black currant (*Ribes nigrum*)	—	25–50	2	36	4 wk	50% first week, then 25% CO_2	48
Blueberry (*Vaccinium* sp.)	5–10	15–20	−0.5–0	31–32	2–3 wk	Prompt cooling is important	47
Cherry, sweet (*Prunus avium*)	3–10	10–15	−1–0	30–32	4 wk	High CO_2 reduces decay	47
Cranberry (*Vaccinium macrocarpon*)	1–2	0–5	3	37	2–4 mo		47
Fig (*Ficus carica*)	5–10	15–20	−1–0	30–32	2 wk		47
Grape (*Vitis vinifera*)	2–5	1–3	−1–0	30–32	1–6 mo		47
Kiwifruit (*Actinidia chinensis*)	1–2	3–5	0	32	3–5 mo	CA delays ripening	47
Nectarine (*Prunus persica*)	1–2	3–5	0.5–0	31–32	6–9 wk	CA reduce internal breakdown	47
Peach (*Prunus persica*)	1–2	3–5	−0.5–0	31–32	6–9 wk	Cultivars differ in response	47
Pear, European (*Pyrus communis*)							
Anjou	0.5–2	0.5–2	−1	30	7–9 mo	CA reduce scald	49
Bartlett	1–2	1–2	−1	30	3–5 mo	Rapid cooling recommended	49
Bosc	1–3	0.5–1	−1	30	4–6 mo	Optimum maturity is critical	49
Comice	2	2–3	−1	30	5–7 mo		49
Conference	2	2	−1	30	4–6 mo		49
Packham's Triumph	2–3	1–2	−0.5	31	8 mo	Optimum maturity is critical	49
Passe Crassane	3–4	5–7	−1	30	6–7 mo	Tolerant to high CO_2	49

Pear, Asian (*Pyrus serotina* and *Pyrus bretschneideri*)							
Nijiseiki (20th Century)	3	1	0	32	9–12 mo		49
Tsu Li	1–2	3	0	32	6–8 mo		49
Ya Li	3–4	2	0–3	32–37	6–8 mo		49
Persimmon (*Diospyros kaki*)	3–5	5–8	−1–0	30–32	4 mo		47
Plum (*Prunus domestica*)	1–2	0–5	−0.5–0	31–32	4–5 mo	CA delays ripening	47
Rasberry (*Rubus idaeus*)	5–10	15–20	−0.5–0	31–32	1 wk	Prompt cooling is important	47
Strawberry (*Fragaria* sp.)	5–10	15–20	−0.5–0	31–32	1 wk	Commercial use during transport	47
			Subtropical and tropical fruits				
Avocado (*Persea americana*)	2–5	3–10	10	50	3–6 wk	CA reduces chilling injury	47
Banana (*Musa* spp)	2–5	2–5	14	58	1–6 mo	CA delays ripening	47
Grapefruit (*Citrus paradisi*)	3–10	5–10	13	55	6–8 wk	CA reduces pitting	47
Lemon (*Citrus limon*)	5–10	0–10	13	55	1–6 mo	CA reduces decay	47
Lime (*Citrus aurantifolia*)	5–10	0–10	13	55	6–8 wk	CA retards degreening	47
Mango (*Mangifera indica*)	3–5	5–10	13	55	5 wk	CA delays ripening	47
Olive (*Olea europaea*)	2–3	0–1	7	45	2 mo		47
Orange (*Citrus sinensis*)	5–10	0–5	7	45	8–12 wk	Storage life varys with cultivars	47
Papaya (*Carica papaya*)	3–5	5–10	12	54	2–3 wk		47
Passion fruit (*Passiflora edulis*)	5	5	7–10	45–50	6 wk		50
Pineapple (*Ananas comosus*)	2–5	5–10	10	50	4 wk		47
			Vegetables				
Artichoke (*Cynara scolymus*)	2–3	2–3	0	32	1 mo	CA decreases discoloration	51
Asparagus (*Asparagus officinalis*)	Air	10–14	2	36	3 wk	High CO_2 is beneficial	51
Bean, lima (*Phaseolus limensis*)	>5	10–35	5–7	40–45	7–10 d	Shelled only	52
Bean, snap (*Phaseolus vulgaris*)	2–3	4–7	8	46	2 wk	CA reduces color loss	51
Broccoli (*Brassica oleracea italica*)	1–2	5–10	0	32	1 mo	CA maintain green color	51
Brussels sprouts (*Brassica oleracea gemmifera*)	1–2	5–7	0	32	3–5 wk	CA reduces yellowing	51
Cabbage (*Brassica oleracea, capitata*)	2–3	3–6	0	32	6–8 mo	Large scale commercial use	51
Cantaloupe (*Cucumis melo*)	3–5	10–20	8	46	2–3 mo	CA reduces ripening	51
Cauliflower (*Brassica oleracea botrytis*)	2–3	3–4	0	32	1 mo		51
Celery (*Apium graveolens*)	1–4	3–5	0	32	3 mo		51
Chinese cabbage (*Brassica campestris*)	1–2	0–5	0	32	4–5 mo	CA reduces leaf abscission	51
Corn, sweet (*Zea mays*)	2–4	5–10	0	32	2 wk	CA reduces sugar loss	51
Cucumber (*Cucumis sativus*)	1–4	0	12	54	3 wk	CA reduces yellowing	51
Leek (*Allium porrum*)	1–6	5–10	0	32	4 mo		51
Lettuce, head (*Lactuca sativa*)	1–3	0	0	32	3–4 wk		51
Mushroom (*Agaricus bisporus*)	Air	10–15	0	32	1–2 wk	CO_2 retards cap opening	51
Onion, dry (*Allium cepa*)	0–1	0–5	0	32	8 mo	Tolerant to low O_2	51
Onion, green (*Allium cepa*)	1	5	0	32	2 mo		53
Parsley (*Petroselinum crispum*)	8–10	8–10	0	32	3 mo		51
Pepper, sweet (*Capsicum annuum*)	2–5	0	12	54	3 wk		51
Radish (*Raphanus sativus*)	1–2	2–3	0	32	4 mo		51
Spinach (*Spinacia oleracea*)	7–10	5–10	0	32	3 wk		51
Tomato (*Lycopersicon esculentum*)	3–5	2–3	12	54	4–6 wk		

Note: The approximate storage periods are compiled by the authors based on all the information available.

BIBLIOGRAPHY

1. H. M. Couey and K. L. Olsen, "Storage Response of Golden Delicious Apples Following High Carbon Dioxide Treatment," *Journal of the American Society of Horticulture Science* **100**, 148–150 (1975)
2. C. Y. Wang and W. M. Mellenthin, "Effect of Short Term High CO_2 treatment on Storage of d'Anjou Pear," *Journal of the American Society of Horticulture Science* **100**, 492–495 (1975).
3. C. Y. Wang, "Effect of Short Term High CO_2 Treatment on the Market Quality of Stored Broccoli," *Journal of Food Science* **44**, 1478–1482 (1979).
4. H. M. Couey and T. R. Wright, "Effect of a Prestorage CO_2 Treatment on the Quality of d'Anjou Pears After Regular or controlled Atmosphere Storage," *HortScience* **12**, 244, 245 (1977).
5. O. L. Lau, "Storage Responses of Four Apple Cultivars to a 'Rapid CA' Procedure in Commercial Controlled Atmosphere Facilities," *Journal of the American Society of Horticulture Science* **108**, 530–533 (1983).
6. W. M. Mellenthin, P. M. Chen, and S. B. Kelly, "Low Oxygen Effects on Dessert Quality, Scald Prevention, and Nitrogen Metabolism of d'Anjou Pear Fruit During Long Term Storage," *Journal of the American Society of Horticulture Science* **105**, 522–527 (1980).
7. D. G. Richardson and M. Meheriuk, eds., *Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities*, Timber Press, Beaverton, Oreg., 1982.
8. F. W. Liu, "Conditions for Low Ethylene CA Storage of Apple: A Review," in S. M. Blankenship, ed., *Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities*, Horticulture Report 126, North Carolina State University, Raleigh, 1985.
9. G. D. Blanpied, "Low Ethylene CA Storage for Empire Apples," in Ref. 8.
10. C. J. Dover, "Effects of Ethylene Removal during Storage of Bramley's Seedling," in Ref 8.
11. D. R. Dilley, P. L. Irwin, and M. W. McKee, "Low Oxygen, Hypobaric Storage and Ethylene Scrubbing," in Ref. 7.
12. J. B. Biale, "Respiration of Fruits," in W. Ruhland, ed., *Encyclopedia of Plant Physiology*, Vol. 12, Springer-Verlag, New York, 1960.
13. E. Hansen, "Quantitative Study of Ethylene Production in Relation to Respiration of Pears," *Botanical Gazette* **103**, 543–558 (1942).
14. D. O. Adams, and S. F. Yang, "Ethylene Biosynthesis: Identification of 1-Aminocyclopropane-1-Carboxylic Acid as an Intermediate in the Conversion of Methionine to Ethylene," *Proceedings of the National Academy of Sciences of the United States of America* **76**, 170–174 (1979).
15. M. Knee, "Physiological Responses of Apple Fruits to Oxygen Concentrations," *Annals of Applied Biology* **96**, 243–253 (1980).
16. S. P. Burg and E. A. Burg, "Molecular Requirements for the Biological Activity of Ethylene," *Plant Physiology* **42**, 144–152 (1967).
17. S. F. Yang, "Biosynthesis and Action of Ethylene," *HortScience* **20**, 41–45 (1985).
18. P. H. Li and E. Hansen, "Effects of Modified Atmosphere Storage on Organic Acid and Protein Metabolism of Pears," *Proceedings of the American Society for Horticultural Science* **85**, 100–111 (1964).
19. D. A. Kollas, "Preliminary Investigation of the Influence of Controlled Atmosphere Storage on the Organic Acid of Apples," *Nature (London)* **204**, 758–759 (1964).
20. N. Allentoff, W. R. Philips, and F. B. Johnston, "A ^{14}C Study of Carbon Dioxide Fixation in the Apple. II. Rates of Carbon Dioxide Fixation in the Detached McIntosh Apple," *Journal of the Science of Food and Agriculture* **5**, 234–238 (1954).
21. M. T. Wu, B. Singh, J. C. Theurer, L. E. Olson, and D. K. Salunkhe, "Control of Sucrose Loss in Sugarbeet During Storage by Chemicals and Modified Atmosphere and Certain Associated Physiological Changes," *Journal of the American Society of Sugar Beet Technology* **16**, 117–127 (1970).
22. C. Y. Wang, "Postharvest Responses of Chinese Cabbage to High CO_2 Treatment and Low O_2 Storage," *Journal of the American Society for Horticultural Science* **108**, 125–129 (1983)
23. B. N. Wankier, D. K. Salunkhe, and W. F. Campbell, "Effects of CA Storage on Biochemical Changes in Apricot and Peach Fruits," *Journal of the American Society for Horticultural Science* **95**, 604–609 (1970).
24. J. N. McGill, A. I. Nelson, and M. P. Steinberg, "Effects of Modified Storage Atmospheres on Ascorbic Acid and Other Quality Characteristics of Spinach," *Journal of Food Science* **31**, 510–517 (1966).
25. T. Suzuki, N. Kubo, S. Haginuma, S. Tamura, and H. Yamamoto, "Changes in Free Amino Acid Content During Controlled Atmosphere Storage of Lettuce, Chinese Cabbage and Cucumber," *Report of National Food Research Institute (Japan)* **38**, 46–55 (1981).
26. C. R. Little, H. J. Taylor, and F. McFarlane, "Postharvest and Storage Factors Affecting Superficial Scald and Core Flush of Granny Smith Apples," *HortScience* **20**, 1080–1082 (1985).
27. W. J. Lipton and J. K. Stewart, *An Illustrated Guide to the Identification of Some Market Disorders of Head Lettuce*, U.S. Department of Agriculture, Marketing Research Report, U.S. Government Printing Office, Washington, D.C., **950** (1972).
28. W. J. Lipton, *Market Quality and Rate of Respiration of Head Lettuce Held in Low Oxygen Atmospheres*, U.S. Department of Agriculture, Marketing Research Report, U.S. Government Printing Office, Washington, D.C., **777** (1967).
29. C. S. Parsons, J. E. Gates, and D. H. Spalding, "Quality of Some Fruits and Vegetables after Holding in Nitrogen Atmospheres," *Proceedings of the American Society for Horticultural Science* **84**, 549–556 (1964).
30. H. Bohling and H. Hansen, "Storage of White Cabbage (*Brassica oleracea* var. Capitata) in Controlled Atmospheres," *Acta Horticulturae* **62**, 49–54 (1977).
31. L. S. Beard, "Effect of CA on Several Storage Disorders of White Cabbage," in Ref. 8.
32. F. Mencarelli, W. J. Lipton, and S. J. Peterson, "Responses of Zucchini Squash to Storage in Low O_2 Atmospheres at Chilling and Nonchilling Temperatures," *Journal of the American Society for Horticultural Science* **108**, 884–890 (1983).
33. C. Y. Wang and Z. L. Ji, "Effect of Low Oxygen Storage on Chilling Injury and Polyamines in Zucchini Squash," *Scientia Horticulture* **39**, 1–7 (1989).
34. T. T. Hatton and R. H. Cubbedge, "Conditioning Florida Grapefruit to Reduce Chilling Injury During Low Temperature Storage," *Journal of the American Society for Horticultural Science* **107**, 57–60 (1982).
35. P. Marcellin and A. Chaves, "Effects of Intermittent High CO_2 Treatment on Storage Life of Avocado Fruits in Relation to Respiration and Ethylene Production," *Acta Horticulture* **138**, 155–162 (1983).

36. R. E. Anderson, C. S. Parsons, and W. L. Smith, *Controlled Atmosphere Storage of Eastern-Grown Peaches and Nectarines*, U.S. Department of Agriculture, Marketing Research Report, U.S. Government Printing Office, Washington, D.C.
37. N. L. Wade, "Physiology of Cold Storage Disorders of Fruits and Vegetables," in J. M. Lyons, D. Graham, and J. K. Raison, eds., *Low Temperature Stress in Crop Plants*, Academic Press, Orlando, Fla., 1979.
38. Y. Ilker and L. L. Morris, "Alleviation of Chilling Injury of Okra," *HortScience* **10**, 324 (1975).
39. I. L. Eaks, "Effect of Modified Atmosphere on Cucumbers at Chilling and Nonchilling Temperatures," *Proceedings of the American Society for Horticultural Science* **67**, 473–478 (1956).
40. A. A. Kader, "Biochemical and Physiological Basis for Effects of Controlled and Modified Atmospheres on Fruits and Vegetables," *Food Technology (Chicago)* **40**, 99–104 (1986).
41. R. M. Smock and A. Van Doren, "Controlled Atmosphere Storage of Apples," *Cornell University Agricultural Experimental Station Bulletin* **762** (1941).
42. E. Hansen, "Reactions of Anjou Pears to Carbon Dioxide and Oxygen Content of the Storage Atmosphere," *Proceedings of the American Society for Horticultural Science* **69**, 110–115 (1957).
43. M. L. Arpaia, F. G. Mitchell, A. A. Kader, and G. Mayer, "Effects of 2% O_2 and Varying Concentrations of CO_2 with or without C_2H_4 on the Storage Performance of Kiwifruit," *Journal of the American Society for Horticultural Science* **110**, 200–203 (1985).
44. P. E. Brecht, A. A. Kader, and L. L. Morris, "The Effect of Composition of the Atmosphere and Duration of Exposure on Brown Stain of Lettuce," *Journal of the American Society for Horticultural Science* **98**, 536–538 (1973).
45. M. Meheriuk, "Controlled Atmosphere Recommendations for Apples," in J. Fellman, ed., *Proceedings of the Fifth International CA Research Conference*, Washington State University, Wenatchee, Washington, 1989.
46. R. E. Hardenburg, A. E. Watada, and C. Y. Wang, *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*, U.S. Department of Agriculture, Agricultural Handbook **66** (1986).
47. A. A. Kader, "A Summary of CA Requirements and Recommendations for Fruits Other than Pome Fruits," in Ref. 45.
48. W. H. Smith, "The Use of Carbon Dioxide in the Transport and Storage of Fruits and Vegetables," *Advances in Food Research* **12**, 95–146 (1963).
49. D. G. Richardson and M. Meheriuk, "Controlled Atmosphere Recommendations for Pears," in Ref. 45.
50. J. S. Pruthi, "Physiology, Chemistry, and Technology of Passion Fruit," *Advances in Food Research* **12**, 203–282 (1963).
51. M. E. Saltveit, "A Summary of Requirements and Recommendations for the Controlled and Modified Atmosphere Storage of Harvested Vegetables," in Ref. 45.
52. A. L. Ryall and W. J. Lipton, *Handling, Transportation, and Storage of Fruits and Vegetables*, Vol. 1, AVI Publishing Co., Westport, Conn., 1979.
53. H. W. Hruschka, *Storage and Shelf Life of Packaged Green Onions*, U.S. Department of Agriculture, Marketing Research Report, U.S. Government Printing Office, Washington, D.C. **1015** (1974).

GENERAL REFERENCES

D. Batram, "CA Storage Key to Growth of State's Apple Industry," *Good Fruit Grower* **47**, 44–46 (1996).

P. Bertolini et al., "Effects of Controlled Atmosphere Storage on the Physiological Disorders and Quality of Conference Pears," *Italian Journal of Food Science* **9**, 303–312 (1997).

G. Briones Lopez et al., "Storage of Common Mushroom Under Controlled Atmospheres," *International Journal of Food Science and Technology* **27**, 493–505 (1992).

D. M. Burmeister and D. R. Dilley, "A Scald-like Controlled Atmosphere Storage Disorder of Empire Apples—A Chilling Injury Induced by CO2," *Postharvest Biology and Technology* **6**, 1–7 (1995).

S. R. Drake, "Elevated Carbon Dioxide Storage of Anjou Pears Using Ourge-Controlled Atmosphere," *HortScience* **29**, 299–301 (1994).

S. R. Drake and T. A. Eisele, "Quality of Gala Apples as Influenced by Harvest Maturity, Storage Atmosphere and Concomitant Storage With Bartlett Pears," *Journal of Food Quality* **20**, 41–51 (1997).

I. U. Elgar, D. M. Burmeister and C. B. Watkins, "Storage and Handling Effects on a CO2-related Internal Browning Disorder of Braeburn Apples," *HortScience* **33**, 719–722 (1998).

Y. Gariepy, G. S. V. Raghavan, and J. A. Munroe, "Long-Term Storage of Leek Stalks Under Regular and Controlled Atmospheres," *International Journal of Refrigeration* **17**, 140–147 (1994).

G. A. Gonzalez et al., "Low Oxygen Treatment Before Storage in Normal or Modified Atmosphere Packaging of Mangoes to Extend Shelf Life," *Journal of Food Science and Technology India* **34**, 399–404 (1997).

M. Hansen, "New Process Extends Storage Life of Peaches," *Good Fruit Grower* **48**, 27 (1997).

P. L. Hurst et al., "Biochemical Responses of Asparagus to Controlled Atmosphere Storage at 20 Degree," *J. Food Biochem.* **20**, 463–472 (1997).

H. Izumi, A. E. Watada and W. Douglas, "Optimum O2 or CO2 Atmosphere for Storing Broccoli Florets at Various Temperatures," *J. Am. Soc. Hortic. Sci.* **121**, 127–131 (1996).

A. King and Y. Joon Yong, "Effects of Low Temperature and CA on Quality Changes and Physiological Characteristics of Chilling During Storage of Squash (*Curcubitai imoschatai*)," *Journal of the Korean Society for Horticultural Sciences* **39**, 402–407 (1998).

R. E. Lill and V. K. Corrigan, "Asparagus Responds to Controlled Atmospheres in Warm Conditions," *International Journal of Food Science and Technology* **31**, 117–121 (1996).

S. Meir et al., "Further Studies on the Controlled Atmosphere Storage of Avocadoes," *Postharvest Biology and Technology* **5**, 323–330 (1995).

A. Noomhorn and N. Tiasuwan, "Controlled Atmosphere Storage of Mango Fruit, *Mangifera indica L. cv. Rad*," *Journal of Food Processing and Preservation* **19**, 271–281 (1995).

T. Palma et al., "Respiratory Behavior of Cherimoya (*Annona cherimola Mill.*) Under Controlled Atmospheres," *HortScience* **28**, 647–649 (1993).

B. Ratanchinakorn, A. Klieber, and D. H. Simons, "Effect of Short-Term Controlled Atmospheres and Maturity on Ripening and Eating Quality of Tomatoes," *Postharvest Biology and Technology* **11**, 149–154 (1997).

S. Y. Rogers and N. R. Knowles, "Effects of Storage Temperature and Atmosphere on Saskatoon (*Amelancher alnifolia Nutt.*) Fruit Quality, Respiration and Ethylene Production," *Postharvest Biology and Technology* **13**, 183–190 (1998).

T. Saray, "Controlled Atmosphere Storage of Vegetable: The Possibilities," *Food Technology International Europe*, 69–73 (1994).

J. Stow, "The Effects of Storage Atmosphere on the Keeping Quality of Idared Apples," *Journal of Horticultural Science* **70**, 587–595 (1995).

T. K. Thomas and E. M. Sfakiotakis, "Effect of Low-Oxygen Atmosphere on Storage Behaviour of Kiwifruit," *Advances in Horticultural Science* **11**, 137-141 (1997).

A. B. Truter, J. C. Combrink, and S. A. Burger, "Control of Superficial Scald in Granny Smith Apples by Ultra-Low Levels of Oxygen as an Alternative to Diphenylamine," *Journal of Horticultural Science* **69**, 581–587 (1994).

R. K. Voltz et al., "Prediction of Controlled Atmosphere-Induced Flesh Browning in Fuji Fruit," *Postharvest Biology and Technology* **13**, 97-102 (1998).

H. S. Yong and L. A. Kyung, "Physiological Characteristics of Chilling Injury and CA Effect on its Reduction During Cold Storage of Pepper Fruit," *Journal of the Korean Society for Horticultural Sciences* **38**, 478–482 (1997).

H. S. Yong, I. I. Kim, and L. C. Jae, "Effects of Harvest Maturity and Storage Environments on the Incidence of Watercore, Flesh Browning, and Quality in Fuji Apples," *Journal of the Korean Society for Horticultural Sciences* **39**, 569–573 (1998).

P. Younmoon et al., "Preharvest Factors Affecting the Incidence of Physiological Disorders During CA Storage of Fuji Apples," *Journal of the Korean Society for Horticultural Sciences* **38**, 725–729 (1997).

CHIEN YI WANG
ALLEY E. WATADA
USDA/ARS
Beltsville, Maryland

See also PACKAGING: PART IV—CONTROLLED/MODIFIED ATMOSPHERE/VACUUM FOOD PACKAGING.

CORN. See CEREALS SCIENCE AND TECHNOLOGY.

CORROSION AND FOOD PROCESSING

MATERIALS OF CONSTRUCTION

The "Stainless Steels"

Many people refer to this range of alloys as austenitic stainless steels, 18/8s, 18/10s, etc, without a full appreciation of what is meant by the terminology. It is worth devoting a few paragraphs to explain the basic metallurgy of stainless steels.

It was in 1913 that Harry Brearley discovered that the addition of 11% chromium to carbon steel would impart a good level of corrosion and oxidation resistance, and by 1914 these corrosion resisting steels had become commercially available. It was Brearley who pioneered the first commercial use of these steels for cutlery, and it was also he who coined the name "stainless steels." For metallurgical reasons, which are outside the scope of this article, these were known as ferritic steels because of their crystallographic structure. Unfortunately, they lacked the ductility to undergo extensive fabrication and furthermore, they could not be welded. Numerous workers tried to overcome these deficiencies by the addition of other alloying elements and to produce a material where the ferrite was transformed to austenite (another metallurgical phase) that was stable at room temperature. Soft stainless steels that were ductile both before and after welding were developed in Sheffield, England (then the heart of the British steel industry) exploiting scientific work undertaken in Germany. This new group of steels was based on an 18% chromium steel to which nickel was added as a second alloying element. These were termed the austenitic stainless steels. The general relationship between chromium and nickel necessary to maintain a fully austenitic structure is shown diagrammatically in Figure 1. It will be seen that the optimum combination is 18% chromium, 8% nickel—hence the terminology 18/8s.

Probably the next major advance in the development of stainless steels was the discovery that relatively small additions of molybdenum had a pronounced effect on the corrosion resistance, greatly enhancing the ability to withstand the effects of mineral acids and other corrodents such as chloride solutions. Needless to say, from these early developments, there has been tremendous growth in production facilities and the number of grades of stainless steel available. Table 1 lists some of the more commonly available grades, while Figure 2 illustrates how the basic 18/8 composition is modified to enhance specific physical or chemical properties.

In spite of the plethora of stainless steels available, grades 304 and 316 have, and continue to be, the workhorses for fabrication of dairy and food processing equipment.

Although 316 stainless steel offers excellent resistance to a wide range of chemical and nonchemical environments, it does not offer immunity to all. In the case of the food industry, these are notably anything containing salt, especially low-pH products. There was, therefore, a demand by industry to develop more corrosion-resistant materials and these are finding increasing use in the food industry for certain specific processing operations.

Super Stainless Steels and Nickel Alloys

The super stainless steels are a group of alloys that have enhanced levels of chromium, nickel, and molybdenum, compared to the conventional 18/8s. The major constituent is still iron; hence the classification under the "steel" title.

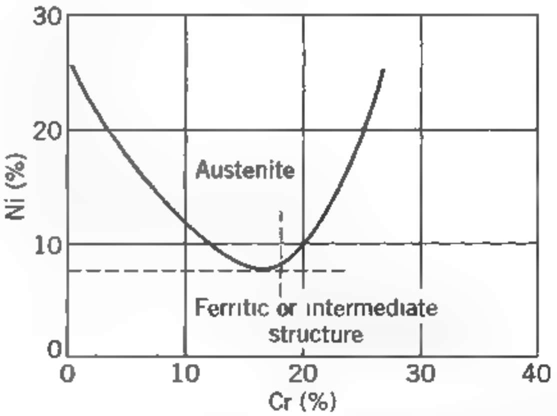


Figure 1. Graph showing the various combinations of chromium and nickel that form austenitic stainless steels. *Source:* Ref. 1.

T. K. Thomas and E. M. Sfakiotakis, "Effect of Low-Oxygen Atmosphere on Storage Behaviour of Kiwifruit," *Advances in Horticultural Science* **11**, 137-141 (1997).

A. B. Truter, J. C. Combrink, and S. A. Burger, "Control of Superficial Scald in Granny Smith Apples by Ultra-Low Levels of Oxygen as an Alternative to Diphenylamine," *Journal of Horticultural Science* **69**, 581–587 (1994).

R. K. Voltz et al., "Prediction of Controlled Atmosphere-Induced Flesh Browning in Fuji Fruit," *Postharvest Biology and Technology* **13**, 97-102 (1998).

H. S. Yong and L. A. Kyung, "Physiological Characteristics of Chilling Injury and CA Effect on its Reduction During Cold Storage of Pepper Fruit," *Journal of the Korean Society for Horticultural Sciences* **38**, 478–482 (1997).

H. S. Yong, I. I. Kim, and L. C. Jae, "Effects of Harvest Maturity and Storage Environments on the Incidence of Watercore, Flesh Browning, and Quality in Fuji Apples," *Journal of the Korean Society for Horticultural Sciences* **39**, 569–573 (1998).

P. Younmoon et al., "Preharvest Factors Affecting the Incidence of Physiological Disorders During CA Storage of Fuji Apples," *Journal of the Korean Society for Horticultural Sciences* **38**, 725–729 (1997).

CHIEN YI WANG
ALLEY E. WATADA
USDA/ARS
Beltsville, Maryland

See also PACKAGING: PART IV—CONTROLLED/MODIFIED ATMOSPHERE/VACUUM FOOD PACKAGING.

CORN. See CEREALS SCIENCE AND TECHNOLOGY.

CORROSION AND FOOD PROCESSING

MATERIALS OF CONSTRUCTION

The "Stainless Steels"

Many people refer to this range of alloys as austenitic stainless steels, 18/8s, 18/10s, etc, without a full appreciation of what is meant by the terminology. It is worth devoting a few paragraphs to explain the basic metallurgy of stainless steels.

It was in 1913 that Harry Brearley discovered that the addition of 11% chromium to carbon steel would impart a good level of corrosion and oxidation resistance, and by 1914 these corrosion resisting steels had become commercially available. It was Brearley who pioneered the first commercial use of these steels for cutlery, and it was also he who coined the name "stainless steels." For metallurgical reasons, which are outside the scope of this article, these were known as ferritic steels because of their crystallographic structure. Unfortunately, they lacked the ductility to undergo extensive fabrication and furthermore, they could not be welded. Numerous workers tried to overcome these deficiencies by the addition of other alloying elements and to produce a material where the ferrite was transformed to austenite (another metallurgical phase) that was stable at room temperature. Soft stainless steels that were ductile both before and after welding were developed in Sheffield, England (then the heart of the British steel industry) exploiting scientific work undertaken in Germany. This new group of steels was based on an 18% chromium steel to which nickel was added as a second alloying element. These were termed the austenitic stainless steels. The general relationship between chromium and nickel necessary to maintain a fully austenitic structure is shown diagrammatically in Figure 1. It will be seen that the optimum combination is 18% chromium, 8% nickel—hence the terminology 18/8s.

Probably the next major advance in the development of stainless steels was the discovery that relatively small additions of molybdenum had a pronounced effect on the corrosion resistance, greatly enhancing the ability to withstand the effects of mineral acids and other corrodents such as chloride solutions. Needless to say, from these early developments, there has been tremendous growth in production facilities and the number of grades of stainless steel available. Table 1 lists some of the more commonly available grades, while Figure 2 illustrates how the basic 18/8 composition is modified to enhance specific physical or chemical properties.

In spite of the plethora of stainless steels available, grades 304 and 316 have, and continue to be, the workhorses for fabrication of dairy and food processing equipment.

Although 316 stainless steel offers excellent resistance to a wide range of chemical and nonchemical environments, it does not offer immunity to all. In the case of the food industry, these are notably anything containing salt, especially low-pH products. There was, therefore, a demand by industry to develop more corrosion-resistant materials and these are finding increasing use in the food industry for certain specific processing operations.

Super Stainless Steels and Nickel Alloys

The super stainless steels are a group of alloys that have enhanced levels of chromium, nickel, and molybdenum, compared to the conventional 18/8s. The major constituent is still iron; hence the classification under the "steel" title.

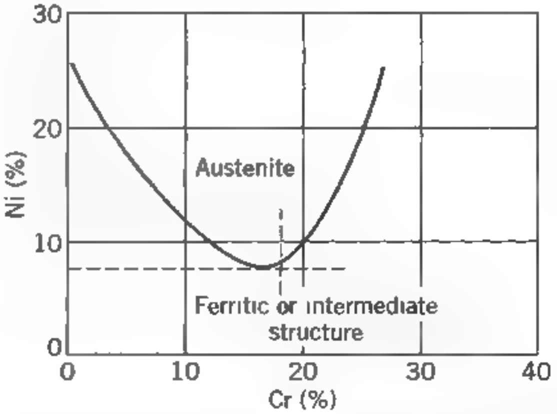


Figure 1. Graph showing the various combinations of chromium and nickel that form austenitic stainless steels. *Source:* Ref. 1.

Table 1. Composition of Some of the More Commonly Used Austenitic Stainless Steels

		Composition (%)						
Alloy	UNS no.	Carbon	Manganese	Silicon	Chromium	Nickel	Molybdenum	Others
302	S30200	0.15	2.00	1.00	16.0–18.0	6.0–8.0		Sulfur 0.030 Phosphorus 0.045
304	S30400	0.08	2.00	1.00	18.0–20.0	8.0–10.5		Sulfur 0.030 Phosphorus 0.045
304L	S30403	0.03	2.00	1.00	18.0–20.0	8.0–12.0		Sulfur 0.030 Phosphorus 0.045
316	S31600	0.08	2.00	1.00	16.0–18.0	10.0–14.0	2.0–3.0	Sulfur 0.030 Phosphorus 0.045
316L	S31603	0.03	2.00	1.00	16.0–18.0	10.0–14.0	2.0–3.0	Sulfur 0.030 Phosphorus 0.045
317	S31700	0.08	2.00	1.00	18.0–20.0	11.0–15.0	3.0–4.0	Sulfur 0.030 Phosphorus 0.045
317L	S31703	0.03	2.00	1.00	18.0–20.0	11.0–15.0	3.0–4.0	Sulfur 0.030 Phosphorus 0.045
321	S32100	0.08	2.00	1.00	17.0–19.0	9.0–12.0		Sulfur 0.030 Ti $\nless$ 5 × Carbon
347	S34700	0.08	2.00	1.00	17.0–19.0	9.0–13.0		Sulfur 0.030 Cb + Ta $\nless$ 10 × Carbon

Note: Unless indicated otherwise, all values are maxima.

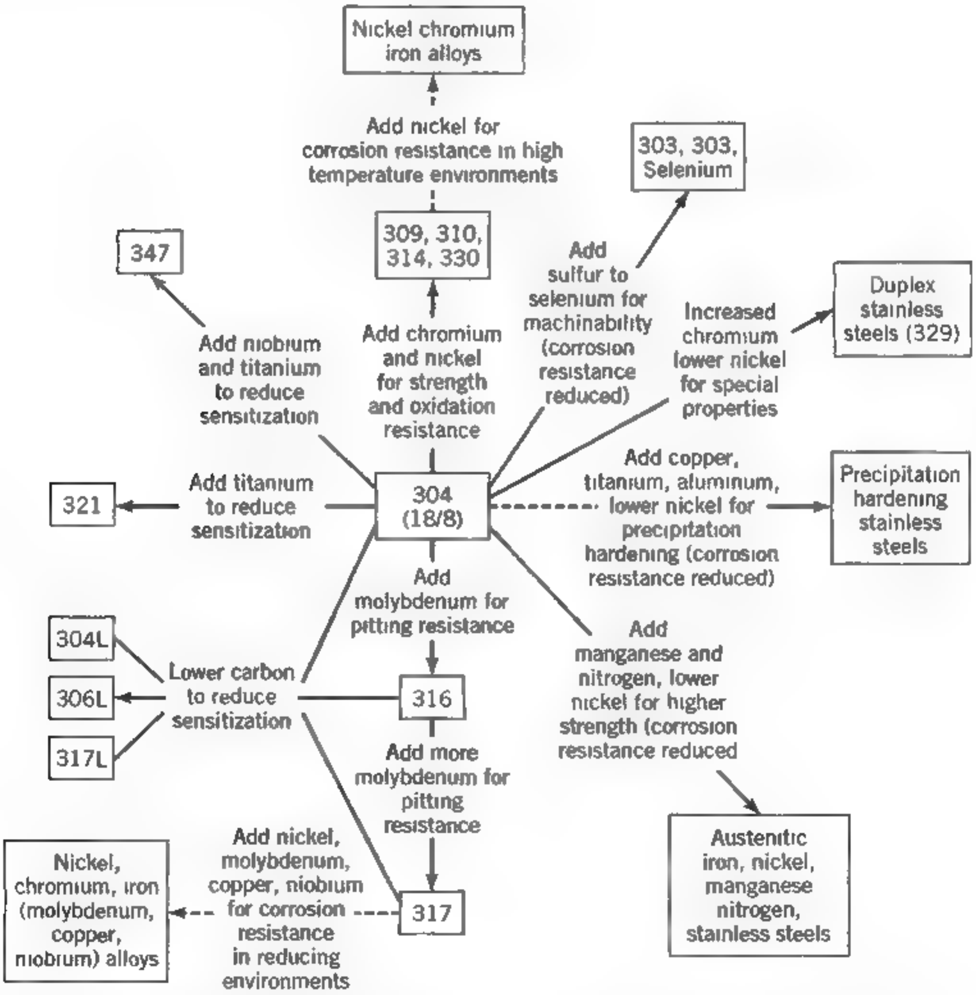

Nickel chromium iron alloys
Add nickel for corrosion resistance in high temperature environments
303, 303, Selenium
309, 310, 314, 330
347
Add sulfur to selenium for machinability (corrosion resistance reduced)
Duplex stainless steels (329)
Add niobium and titanium to reduce sensitization
Add chromium and nickel for strength and oxidation resistance
Increased chromium lower nickel for special properties
Add copper, titanium, aluminum, lower nickel for precipitation hardening (corrosion resistance reduced)
Precipitation hardening stainless steels
321
Add titanium to reduce sensitization
304 (18/8)
Add molybdenum for pitting resistance
304L
306L
317L
Lower carbon to reduce sensitization
316
Add manganese and nitrogen, lower nickel for higher strength (corrosion resistance reduced
Add more molybdenum for pitting resistance
Austenitic iron, nickel, manganese nitrogen, stainless steels
Nickel, chromium, iron (molybdenum, copper, niobium) alloys
Add nickel, molybdenum, copper, niobium for corrosion resistance in reducing environments
317

Figure 2. Outline of some compositional modifications of 18/8 austenitic stainless steel to produce special properties. *Source:* Ref. 2.

Still further increases in the three aforementioned alloying elements result in the nickel alloys. (The classification of an alloy is generally under the heading of the major constituent.)

There are a large number of these alloys but those of primary interest to the food industry are shown in Table 2 together with their composition. In general terms, it will be noted that the increase in nickel content is accompanied by an increase in chromium and molybdenum. As stated previously, this element is particularly effective in promoting corrosion resistance.

Just like insurance, you get only what you pay for, and generally speaking, the higher the corrosion resistance, the more expensive the material. In fact, the differential between type 304 stainless steel and a high-nickel alloy may be as much as 20 times, depending on the market prices for the various alloying elements that fluctuate widely with the supply and demand position.

Aluminum

High-purity grades of aluminum (±99.5%) and its alloys still are preferred for some food and pharmaceutical applications because of the reasonable corrosion resistance of the metal. This resistance is attributable to the easy and rapid formation of a thin, continuous, adherent oxide film on exposed surfaces. This oxide film, in turn, exhibits a good corrosion resistance to many foodstuffs, and it is reported that fats, oils, sugar, and some colloids have an inhibitory or sealing effect on these films (3).

As aluminum salts formed by corrosion are colorless, tasteless, and claimed to be nontoxic, the metal is easy to clean, inexpensive, and light and has a high thermal conductivity. It still is used quite extensively in certain areas of food manufacture and distribution. However, in recent years, the claim of nontoxicity is being questioned as a high dietary incidence has been implicated in Alzheimer's disease (senile dementia) with compounds of aluminum (aluminosilicates) being found in the brain tissue of sufferers (4). However, the case is far from proven, and it is not clear if the increased levels of aluminosilicates are due to a high intake of aluminum per se or other factors such as a dietary deficiency of calcium.

For many years, aluminum was used extensively for containment vessels in the diary and brewing industry, and it was Richard Seligman who founded the then Aluminium Plant and Vessel Company (now APV plc) to exploit the technique of welding this material for the fabrication of fermenting vessels in the brewing industry (5). Many of these original vessels are still in use in some of the smaller, privately owned breweries in the United Kingdom.

Although large fermenting vessels and storage tanks now tend to be fabricated from stainless steels, there is still widespread use of aluminum for beer kegs, beer cans, and a miscellany of small-scale equipment where the resistance of aluminum is such that it imparts no change or modification of flavor, even after prolonged storage.

While still used for holding vessels and some equipment when processing cider, wines, and perry, prolonged contact is inadvisable because of the acidity of the sulfites employed as preservatives for these products—inadvisable, that is, unless the surface of the metal has been modified by anodizing or has been protected with a lacquer.

In the manufacture of preserves, aluminum is still employed for boiling pans, the presence of sugar appearing to inhibit any corrosion. In the field of apiculture, it has even been used for making prefabricated honeycombs, which the bees readily accepted.

Extensive use is made of aluminum and the alloys in the baking industry for baking tins, kneading troughs, handling equipment, etc.

In other areas of food manufacture and preparation, the use of aluminum extends virtually over the whole field of activity—butter, margarine, table oils, and edible fats, meat and meat products, fish and shellfish, certain sorts of vinegar, mustards, spices; the list is almost endless.

No mention has so far been made of the application of this metal in the dairy industry, and indeed it still has limited application mostly in the field of packaging, eg, bottle caps, wrapping for cheese, butter, carton caps for yogurt, cream.

It will be appreciated that the uses of aluminum in the food industry so far mentioned have tended to be for equipment used in batch operation, hand utensils, and packaging. There are probably three major factors that have mitigated against its more widespread use, not only in the dairy industry but in brewing and many other branches of food processing.

1. Modern, highly automated plants operating on a continuous or semicontinuous basis employ a wide va-

Table 2. Composition of Some of the More Commonly Used Wrought Super Stainless Steels and Nickel Alloys

		Composition (%)						
Alloy	UNS no.	Carbon	Silicon	Manganese	Chromium	Nickel	Molybdenum	Others
904L	N08904	0.02	0.70	2.0	19.0–21.0	24.0–26.0	4.2–4.7	Cu 1.2–1.7
Avesta 254 SMO	S31254	0.02	0.80	2.0	19.5–20.5	17.5–18.5	6.0–6.5	Cu 0.5–1.0, N 0.18–0.22
Incoloy 825	N08825	0.05	0.50	1.0	19.5–23.5	38.0–46.0	2.5–3.5	Al 0.2, Ti 0.6–1.2
Hastelloy G-30	N06030	0.03	0.08	1.5	28.0–31.5	Bal.	4.0–6.0	Co 5.0, Cu 1.0–2.4, Cb + Ta 0.3–1.5, Fe 13.0–17.0
Inconel 625	N06625	0.10	0.50	0.50	23.0–28.0	Bal.	8.0–10.0	Co 1.0, Fe 5.0, Al 0.4, Ti 0.4
Hastelloy C-276	N10276	0.02	0.08	1.0	14.5–16.5		15.0–17.0	W 3.0–4.5, V 0.35

Note Unless indicated otherwise, all values are maxima.

riety of materials of construction. Because of the position in the electrochemical series (to be discussed later), aluminum and its alloys are susceptible to galvanic corrosion when coupled with other metals.

2. The commercial availability of stainless steels and their ease of fabrication, strength, ease of maintenance, appearance, and proven track record of reliability.
3. The fact that since modern plants operate on a semicontinuous basis with much higher levels of fouling, cleaning regimes require strongly alkaline detergents to which aluminum has virtually zero corrosion resistance.

Copper and Tinned Copper

Copper and tinned copper were used extensively in former times because of their excellent thermal conductivity (8 times that of stainless steel), ductility, ease of fabrication, and reasonable level of corrosion resistance. However, the demise of copper as a material of construction is largely attributable to the toxic nature of the metal and its catalytic activity in the development of oxidative rancidity in fats and oils. Even at the sub-part-per-million level, copper in vegetable oils and animal fats rapidly causes the development of off-flavors. In equipment where high levels of liquid turbulence are encountered (eg, plate heat exchanger or high-velocity pipe lines) copper is subject to erosion. Nevertheless, there is an area of the beverage industry where copper is still the only acceptable material of construction, ie, pot stills for Scotch and Irish whiskey production. It is also used in the distillation of the spirits such as rum and brandy. Much old copper brewing equipment such as fermenting vessels and wort boilers is still in use throughout the world, and an interesting observation is that even though the wort boilers in modern breweries are fabricated from stainless steel, they are still known as "coppers" and UK craftsmen fabricating stainless steel are still known as coppersmiths.

Titanium

There are certain areas of the food industry, especially in equipment involving heat transfer, where stainless steels are just not capable of withstanding the corrosive effects of salty, low-pH environments. Food processors are increasingly accepting the use of titanium as an alternative, in the full knowledge that it offers corrosion immunity to the more aggressive foodstuffs and provides a long-term solution to what was an on-going problem with stainless steels. Titanium is a light metal, the density of which is almost half that of stainless steels. Although relatively expensive (6–7 times the cost of stainless steel), being a low-density material offsets this price differential for the raw material by almost half. It is ductile and fabricable using normal techniques, although welding it does require a high degree of expertise.

Other Metals

Tin, in the form of tin plate, is used extensively in the canning industry, where its long-term corrosion resistance to a wide range of food acids makes it a material par excellence for this purpose.

Cadmium, used as a protective coating for carbon steel nuts and bolts, was favored at one time. However, the high toxicity of the cadmium compounds has come under increasing scrutiny from many health regulatory bodies and now cadmium-plated bolting is not permitted in food factories. Indeed, Denmark and Sweden have totally banned the import of cadmium-plated components into their countries, and many other countries are likely to follow suit.

Lead and lead-containing products are generally not acceptable for food contact surfaces, although some codes of practice permit the use of lead-containing solder for capillary pipeline joints on water supplies and service lines.

SELECTING MATERIALS OF CONSTRUCTION

Designing equipment is a multidiscipline exercise involving mechanical engineers, materials–corrosion engineers, stressing experts, draftsmen, etc. The corrosion engineer has an important role in this team effort, namely, to ensure the materials specified will offer a corrosion resistance that is just adequate for all the environmental conditions likely to be encountered during normal operation of the equipment. A piece of equipment that prematurely fails by corrosion is as badly designed as one in which the materials have been overspecified. Unfortunately, all too often the functional requirements for a piece of equipment are analyzed in a somewhat arbitrary manner and all too often, the basic cost of the material tends to outweigh other equally important considerations (6).

Figure 3 shows the primary criteria that must be considered in the initial selection process.

- *Corrosion Resistance*. For any processing operation, there will be a range of materials that will offer a corrosion resistance that is adequate (or more than adequate) for a particular job. When considering corrosion resistance, the operational environment is the obvious one, but the other point must be whether the material will also offer corrosion resistance to the chemicals used for cleaning and sanitizing.
- *Cost*. Many of the materials originally considered will be eliminated on the grounds of their high cost. For example, there is no point in considering a high-nickel alloy when a standard 300 series stainless steel at a lower cost will be perfectly satisfactory.

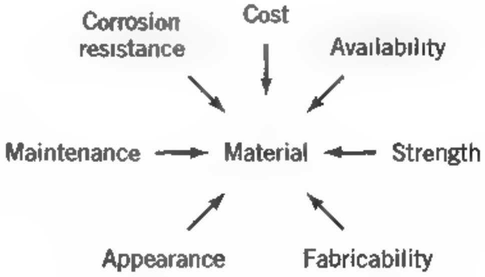


Figure 3. Materials selection criteria.

- *Availability.* Availability is a less obvious feature of the material selection process. Many steel producers will require a minimum order of, say, 3 tons for a nonstandard material. Clearly, the equipment manufacturer is not going to buy this large quantity when the job that is to be done may only require the use of one ton of material.
- *Strength.* Strength is a factor that is taken into account at the design stage but, as with all the others, cannot be considered in isolation. For example, many of the new stronger stainless steels, although more expensive on a ton-for-ton basis than conventional stainless steels, are less expensive when considered on a strength/cost ratio.
- *Fabricability.* There is little point in considering materials that are either unweldable (or unfabricable) or can be welded only under conditions more akin to a surgical operating theater than a general engineering fabrication shop.
- *Appearance.* Appearance may or may not be an important requirement. Equipment located outside must be resistant to environmental weathering and therefore may require the application of protective sheathing, which could double the basic material cost.
- *Maintenance.* Is the equipment to be essentially maintenance-free or is some maintenance, such as periodic repainting, tolerable? How long will the equipment operate without the need for major servicing?

When all these interrelated criteria have been considered, the long list of possible starters will have been reduced to maybe one or two. Also, somewhere through the selection process some of those materials initially rejected because, for example, of their high cost, may have to be reconsidered because of other factors.

TYPES OF CORROSION

Defining Corrosion

Before embarking on a discussion of the various forms of corrosion, it is worthwhile considering exactly what corrosion is. There are several definitions of corrosion. For example, Fontant (7) defines it as extractive metallurgy in reverse using the diagram, shown in Figure 4, to illustrate the point.

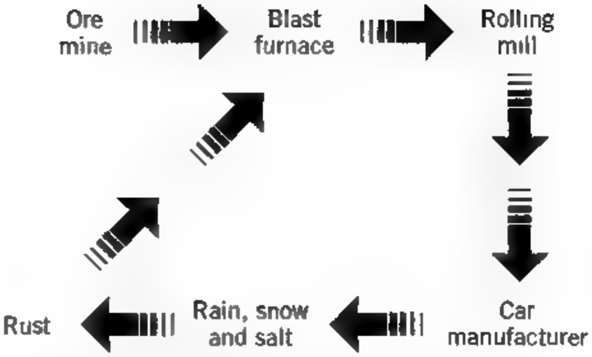


Figure 4. Extractive metallurgy in reverse.

A more general and descriptive definition is "it is the deterioration or destruction of a material through interaction with its environment." This covers all materials of construction including rubber and plastics as well as metal. However, the primary object of this article is to deal with corrosion of metals, in particular stainless steels, and how this corrosion can be classified.

There are two basic forms of corrosion—wet corrosion and dry corrosion. Dry corrosion is concerned with the oxidation of metals at high temperature and clearly outside the scope of this text. Wet corrosion occurs in aqueous solutions or in the presence of electrolytes and is an electrochemical process. It should be noted that the "aqueous" component of the system may be present in only trace quantities (eg, present as moisture); the classical example is the corrosion of steel by chlorine gas. In fact, steel is not corroded by chlorine since steel is the material used for storing liquid chlorine. However, in the presence of even trace quantities of moisture, chlorine rapidly attacks steel and, for that matter, most metals.

The corrosion of metals involves a whole range of factors. These may be chemical, electrochemical, biological, metallurgical, or mechanical, acting singly or conjointly. Nevertheless, the main parameter governing corrosion of metals is related to electrochemistry. Electrochemical principles therefore are the basis for a theoretical understanding of the subject. In fact, electrochemical techniques are now the standard method for investigating corrosion although the standard "weight loss" approach still provides invaluable data. It is not proposed to discuss in depth the electrochemical nature of corrosion but should further information be required, several excellent texts are available (7,8).

Forms of Corrosion

Wet corrosion can be classified under any of eight headings, namely:

- Galvanic or bimetallic corrosion
- Uniform or general attack
- Crevice corrosion
- Pitting corrosion
- Intergranular corrosion
- Stress corrosion cracking
- Corrosion fatigue
- Selective corrosion (castings and free-matching stainless steels)

Galvanic Corrosion. When two dissimilar metals (or alloys) are immersed in a corrosive or conductive solution, an electrical potential or potential difference usually exists between them. If the two metals are electrically connected, then, because of this potential difference, a flow of current occurs. As the corrosion process is an electrochemical phenomenon and dissolution of a metal involves electron flow, the corrosion rates for the two metals is affected. Generally, the corrosion rate for the least corrosion resistant is enhanced while that of the more corrosion resistant is diminished. In simple electrochemical terms, the least resistant metal has become anodic and the more resistant cathodic. This, then, is galvanic or dissimilar metal corrosion.

The magnitude of the changes in corrosion rates depends on the so-called electrode potentials of the two metals; the greater the difference, the greater the enhancement or diminution of the corrosion rates. It is possible to draw up a table of some commercial alloys that ranks them in order of their electrochemical potential. Such a table is known as the galvanic series. A typical one as shown in Table 3 is based on work undertaken by the International Nickel Company (now INCO Ltd.) at their Harbor Island, NC, test facility. This galvanic series relates to tests in unpolluted seawater, although different environments could produce different results and rankings. When coupled, individual metals and alloys from the same group are unlikely to show galvanic effects that will cause any change in their corrosion rates.

The problem of dissimilar metal corrosion, being relatively well understood and appreciated by engineers, is usually avoided in plant construction and in the author's experience, few cases have been encountered. Probably the most common form of unintentional galvanic corrosion is on service lines where brass fittings are used on steel pipelines—the steel suffering an increase in corrosion rate at the bimetallic junction.

One of the worst bimetallic combinations is aluminum and copper. An example of this is in relation to aluminum milk churns used to transport whey from Gruyere cheese manufacture (in Switzerland), where copper is used for the cheesemaking vats and the whey picks up traces of this metal. The effect on the aluminum churns which are internally protected with lacquer that gets worn away through mechanical damage is pretty catastrophic.

Another, somewhat unique, example of galvanic corrosion is related to a weld repair on a 304 stainless-steel storage vessel. Welding consumables containing molybdenum had been employed to effect the repair, and although it is most unusual for the potential difference between molybdenum and nonmolybdenum containing stainless steels to be sufficient to initiate galvanic corrosion, the environmental factors in this particular case were obviously such that corrosion was initiated (Fig. 5). As stated, this is somewhat unique and it is not uncommon for 316 welding consumables to be used for welding 304 stainless steel with no adverse effects. As a practice, however, it is to be deprecated and the correct welding consumables should always be employed.

Not all galvanic corrosion is bad; indeed, galvanic corrosion is used extensively to protect metal and structures by the use of a sacrificial metal coating. A classic example is the galvanizing of sheet steel and fittings, the zinc coating being applied not so much because it does not corrode, but because it does. When the galvanizing film is damaged, the zinc galvanically protects the exposed steel and inhibits rusting. Similarly, sacrificial anodes are fitted to domestic hot water storage tanks to protect the tank.

Table 3. The Galvanic Series of Some Commercial Metals and Alloys in Clean Seawater

↑	Platinum
	Gold
	Graphite
	Titanium
Noble or cathodic	Silver
	Chlorimet 3 (62 Ni, 18 Cr, 18 Mo)
	Hastelloy C (62 Ni, 17 Cr, 15 Mo)
	18/8 Mo stainless steel (passive)
	18/8 stainless steel (passive)
	Chromium stainless steel 11–30% Cr (passive)
	Inconel (passive) (80 Ni, 13 Cr, 7 Fe)
	Nickel (passive)
	Silver solder
	Monel (70 Ni, 30 Cu)
	Cupronickels (60–90 Cu, 40–10 Ni)
	Bronzes (Cu-Sn)
	Copper
	Brasses (Cu-Zn)
	Chlorimet 2 (66 Ni, 32 Mo, 1 Fe)
	Hastelloy B (60 Ni, 30 Mo, 6 Fe, 1 Mn)
	Inconel (active)
	Nickel (active)
	Tin
	Lead
	Lead-tin solders
	18/8 Mo stainless steel (active)
	18/stainless steel (active)
	Ni-resist (high-Ni cast iron)
	Chromium stainless steel, 13% Cr (active)
	Cast iron
Active or anodic	Steel or iron
	2024 aluminum (4.5 Cu, 1.5 Mg, 0.6 Mn)
	Cadmium
	Commercially pure aluminum (1100)
	Zinc
↓	Magnesium and magnesium alloys

Uniform or General Attack. As the name implies, this form of corrosion occurs more or less uniformly over the whole surface of the metal exposed to the corrosive envi-

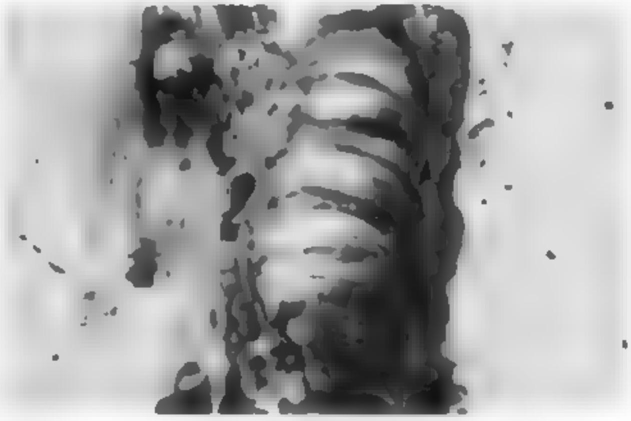


Figure 5. Galvanic corrosion of 304 stainless steel initiated by a 316 weld deposit. Note the large pit associated with the weld splatter.

ronment. It is the most common form of corrosion encountered with the majority of metals, a classic example being the rusting of carbon steel. Insofar as the corrosion occurs uniformly, corrosion rates are predictable and the necessary corrosion allowances built into any equipment. In the case of stainless steels, this form of corrosion is rarely encountered. Corrodents likely to produce general attack of stainless steel are certain mineral acids, some organic acids, and high-strength caustic soda at concentrations and temperatures well in excess of those ever likely to be found in the food industry. The same remark applies to cleaning acids such as nitric, phosphoric, and citric acids, but not for sulfuric or hydrochloric acids, both of which can cause rapid, general corrosion of stainless steels. Hence, they are not recommended for use, especially where corrosion would result in a deterioration of the surface finish of process equipment.

The behavior of both 304 and 316 stainless steels when subjected to some of the more common acids that are encountered in the food industry is graphically illustrated in Figure 6. These isocorrosion graphs, ie, lines that define the conditions of temperature and acid concentration that will produce a constant corrosion rate expressed in mils (0.001 in.) or mm loss of metal thickness per year, are used extensively by corrosion engineers in the material selection process when the form of corrosion is general attack. *They are of no value whatsoever when the corrosion mode is one of the other forms that will be defined, such as pitting or crevice corrosion.*

Crevice Corrosion. This form of corrosion is an intense local attack within crevices or shielded areas on metal surfaces exposed to corrosive solutions. It is characteristically encountered with metals and alloys that rely on a surface oxide film for corrosion protection, eg, stainless steels, titanium, aluminum.

The crevices can be inherent in the design of the equipment (eg, plate heat exchangers) or inadvertently created by bad design. Although crevice corrosion can be initiated at metal-to-metal surfaces (see Fig. 7), it is frequently encountered at metal to nonmetallic sealing faces. Any nonmetallic material that is porous and used, for example,as a gasket, is particularly good (or bad!) for initiating this form of attack. Fibrous materials that have a strong wicking action are notorious in their ability to initiate crevice attack. Similarly, materials that have poor stress relaxation characteristics, ie, have little or no ability to recover their original shape after being deformed, are also crevice creators, as are materials that tend to creep under the influence of applied loads and/or at elevated temperatures. Although used for gasketing, PTFE suffers both these deficiencies. On the other hand, elastomeric materials are particularly good insofar as they exhibit elastic recovery and have the ability to form a crevice-free seal. However, at elevated temperatures, many rubbers harden and in this condition, suffer the deficiencies of many nonelastomeric gasketing materials.

Artificial crevices can also be created by the deposition of scale from one of the process streams to which the metal is exposed. It is necessary, therefore, to maintain food processing equipment in a scale-free condition, especially on surfaces exposed to service fluids such as services side hot/cold water, cooling brines, which tend to be overlooked during plant cleaning operations.

Much research work has been done on the geometry of crevices and the influence of this on the propensity for the initiation of crevice corrosion (9). However, in practical terms, crevice corrosion usually occurs in openings a few tenths of a millimeter or less and rarely is encountered where the crevice is greater than 2 mm (0.08 in.).

Until the 1950s, crevice corrosion was thought to be due to differences in metal ion or oxygen concentration within the crevice and its surroundings. While these are factors in the initiation and propagation of crevice corrosion, they are not the primary cause. Current theory supports the view that through a series of electrochemical reactions and the geometrically restricted access into the crevice, migration of cations, chloride ions in particular, occurs. This alters the environment within, with a large reduction in pH and an increase in the cations by a factor of as much as 10. The pH value can fall from a value of, say, 7 in the surrounding solution to as low as pH 2 within the crevice. As corrosion is initiated, it proceeds in an autocatalytic manner with all the damage and metal dissolution occurring within the crevice and little or no metal loss outside. The confined and autocatalytic nature of crevice corrosion results in significant loss of metal under the surface of site of initiation. As a result, deep and severe undercutting of the metal occurs (see Fig. 8). The time scale for initiation of crevice corrosion can vary from a few hours to several months and, once initiated, can progress very rapidly. Stopping the corrosion process can be extremely difficult as it is necessary to remove all the trapped reactants and completely modify the occluded environment. The difficulty of attaining this will be appreciated by reference to Figure 8, where the entrance to the corroded region is only 0.5 mm (0.020 in.).

While methods for combating the onset of crevice corrosion can be deduced from the foregoing text, a reiteration of some of the more important precautions is not out of place, viz:

- Good-quality, crevice-free welded joints are always preferable to bolted joints
- Good equipment design (well-designed gasket sealing faces) that avoids unintentional crevices and does not permit the development of stagnant regions
- Frequent inspection of equipment and removal of surface deposits
- Use of good-quality rubber gaskets rather than absorbent packings
- Good gasket maintenance; replacement when hardened or damaged

However, certain pieces of equipment are by virtue of their design highly creviced. In such cases, it is necessary to recognize the potential corrosion risk and select the materials of construction that will resist the initiation of crevice corrosion by the environment. Similarly, cleaning and sanitizing regimes must be developed to avoid the onset of attack.

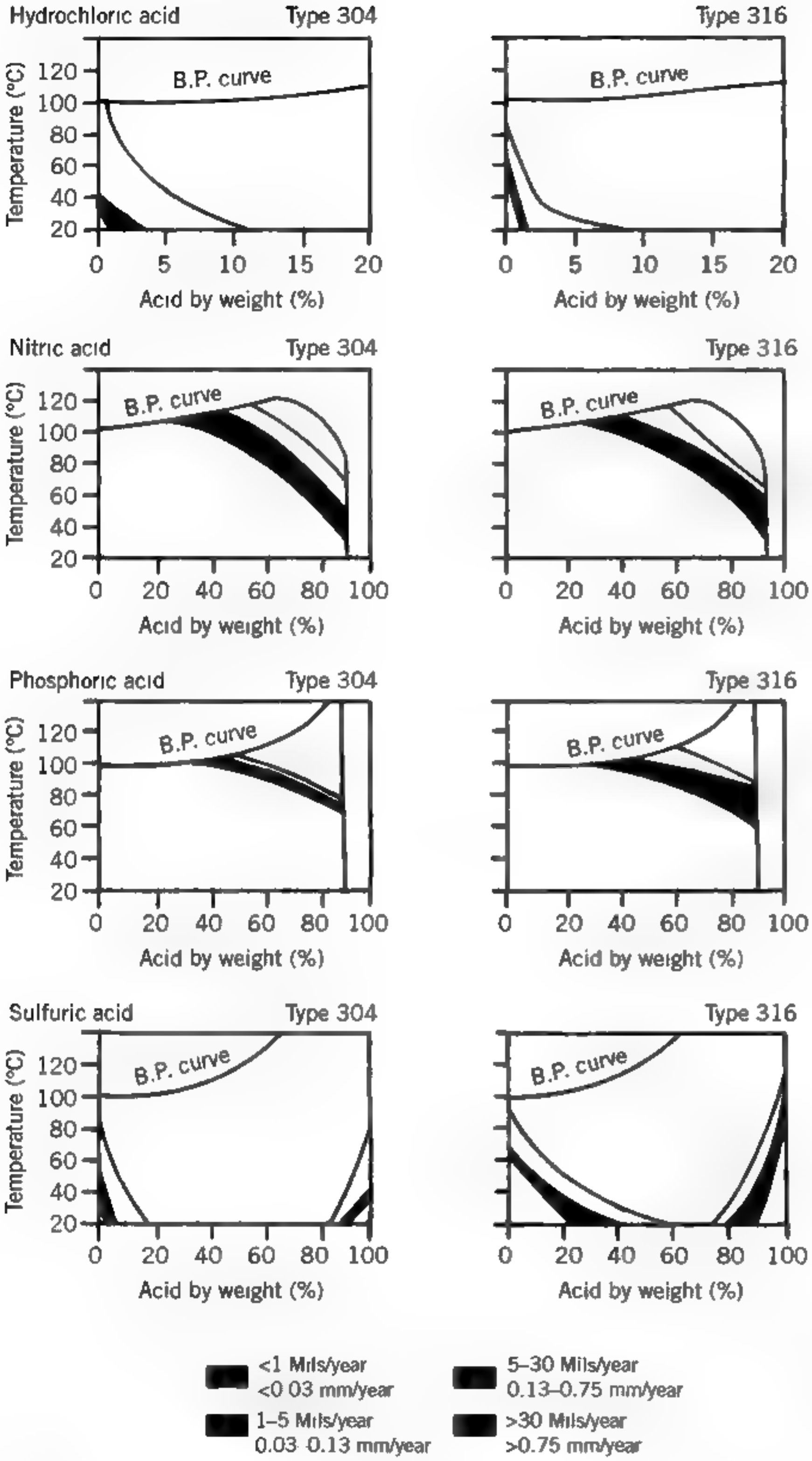


Figure 6. Corrosion resistance of 304 and 316 stainless steels to mineral acids. *Source:* Reproduced by permission of British Steel Plc.

In the case of stainless steels, although there are several ionic species that will initiate the attack, by far the most common are solutions containing chloride. The presence of salt in virtually all foodstuffs highlights the problem. Low pH values also enhance the propensity for initiation of attack.

Other environmental factors such as temperature and the oxygen or dissolved air content of the process stream all play a role in the corrosion process.

Because the presence of oxygen is a prerequisite for the onset of crevice corrosion (and many other forms of attack), in theoretical terms complete removal of oxygen from a

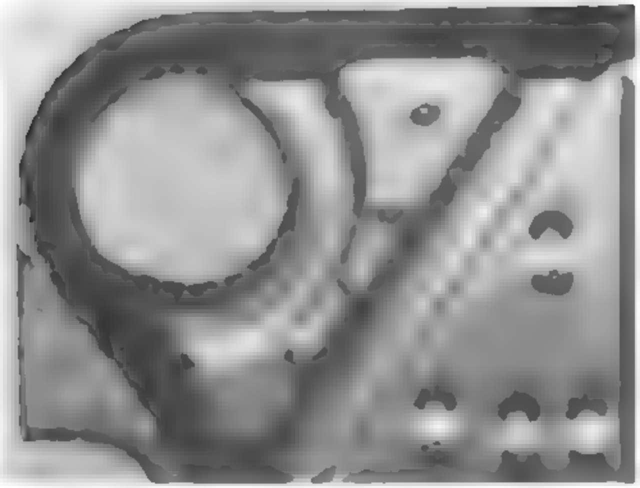


Figure 7. Crevice corrosion at the interplate contact points of a heat-exchanger plate.

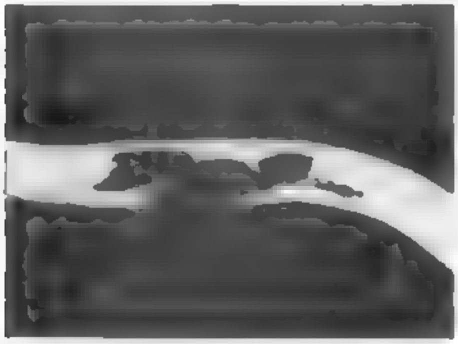


Figure 8. Photomicrograph of a section through a site of crevice corrosion. Note the deep undercutting which is typical of chloride-induced attack on stainless steel.

process stream will inhibit corrosion. In practice, however, this is difficult to achieve. Only in equipment where complete and effective deaeration occurs, such as a multiple effect evaporator operating under reduced pressure, will the beneficial effect of oxygen removal be achieved.

Stainless steels containing molybdenum (316, 317) have a much higher resistance to crevice corrosion than do alloys without this element (304, 321, 347). The higher the molybdenum content, the greater the corrosion resistance. For particularly aggressive process streams, titanium is often the only economically viable material to offer adequate corrosion resistance.

Pitting Corrosion. As the name implies, pitting is a form of corrosion that leads to the development of pits on a metal surface. It is a form of extremely localized but intense attack, insidious insofar as the actual loss of metal is negligible in relation to the total mass of metal that may be affected. Nevertheless, equipment failure by perforation is the usual outcome of pitting corrosion. The pits can be small and sporadically distributed over the metal surface (Fig. 9) or extremely close together, close enough, in fact, to give the appearance of the metal having suffered from general attack.

In the case of stainless steels, environments that will initiate crevice corrosion will also induce pitting. As far as the food industry is concerned, it is almost exclusively caused by chloride containing media, particularly at low pH values.

Many theories have been developed to explain the cause of initiation of pitting corrosion (10), and the one feature they have in common is that there is a breakdown in the passive oxide film. This results in ionic migration and the development of an electrochemical cell. There is, however, no unified theory that explains the reason for the film breakdown. Evans (11) for example, suggests that metal dissolution at the onset of pitting may be due to a surface scratch, an emerging dislocation or other defects, or random variations in solution composition. However, propagation of the pit proceeds by a mechanism similar to that occurring with crevice corrosion. Like crevice corrosion, the pits are often undercut and on vertical surfaces may assume an elongated morphology due to gravitational effects (Fig. 10).

The onset of pitting corrosion can occur in a matter of days but frequently requires several months for the development of recognizable pits. This makes the assessment of the pitting propensity of a particular environment very difficult to determine, and there are no short-cut laboratory testing techniques available. Methods and test solutions are available to rank alloys; the best known and most frequently quoted is ASTM Standard G48 (12), which employs 6% ferric chloride solution. Another chemical method involving ferric chloride determines the temperature at

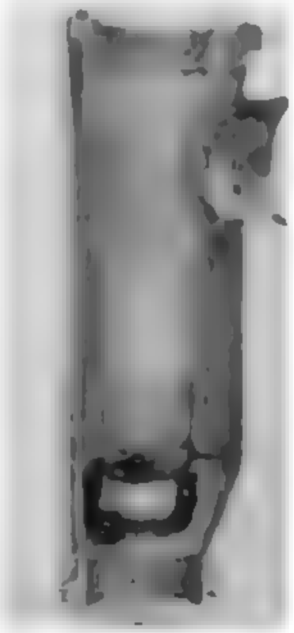


Figure 9. Pitting corrosion of a stainless-steel injector caused by the presence of hydrochloric acid in the steam supply.

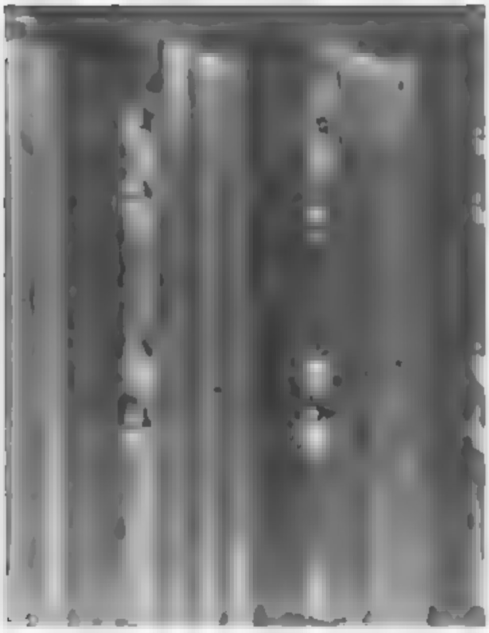


Figure 10. Elongated pitting attack on a 316 stainless-steel heat exchanger plate.

which the solution will cause pitting within a 24-h test period, the results being expressed as the critical pitting temperature or CPT (13). However, as stated, both of these methods are used to rank the susceptibility of a range of alloys rather than define the performance of a material in a service environment. Electrochemical methods have also been used.

As with crevice corrosion, alloy composition has a profound effect on the resistance of a material to pitting attack. Greene and Fontana (14) summarized the effect of various elements as shown in Table 4.

Intergranular Corrosion. A fact not often appreciated is that metals and alloys have a crystalline structure. However, unlike crystalline solids such as sugar or salt, metallic crystals can be deformed or bent without fracturing; in other words, they are ductile. In the molten state, the atoms in a metal are randomly distributed but on cooling and solidification, they become arranged in crystalline form. Because crystallization occurs at many points in the solidification process, these crystals or grains are randomly orientated and the region where they meet are grain boundaries. In thermodynamic terms, the grain boundaries are more susceptible to corrosion attack because of their higher free energy, although in practice the difference in free energy of the grain boundaries and the main crystals or grains in a homogeneous alloy are too small to be significant. However, when the metal or alloy has a heterogeneous structure, preferential attack at or adjacent to the grain boundaries can occur. This is intergranular corrosion as shown in Figure 11.

When austenitic stainless steels are heated to and held in the temperature range of 600–900°C (1100–1650°F), the material becomes sensitized and susceptible to grain boundary corrosion. It is generally agreed that this is due to chromium combining with carbon to form chromium carbide, which is precipitated at the grain boundaries. The net effect is that the metal immediately adjacent to the grain boundaries is denuded of chromium and instead of having a composition of, say, 18% chromium and 8% nickel, it may assume an alloy composition where the chromium content is reduced to 9% or even lower. As such, this zone depleted in chromium bears little similarity to the main metal matrix and has lost one of the major alloying elements on which it relied for its original corrosion resistance. Indeed, the lowering of corrosion resistance in this zone is so great that sensitized materials are subject to attack by even mildly corrosive environments.

As supplied from the steel mills, stainless steels are in the so-called solution annealed condition, ie, the carbon is in solution and does not exist as grain boundary chromium carbide precipitates. During fabrication where welding is involved, the metal adjacent to the weld is subjected to temperatures in the critical range (600–900°C/1100–1650°F) where sensitization can occur. As such, therefore, this zone may be susceptible to the development of intergranular carbide precipitates. Because the formation of chromium carbides is a function of time, the longer the dwell time in the critical temperature zone, the greater the propensity for carbide formation. Hence, the problem is greatest with thicker metal sections due to the thermal mass and slow cooling rate.

By heating a sensitized stainless steel to a temperature of 1050°C (1950°F), the carbide precipitates are taken into solution and by rapidly cooling or quenching the steel from this temperature, the original homogeneous structure is reestablished and the original corrosion resistance restored.

The first stainless steels were produced with carbon contents of up to 0.2% and as such, were extremely susceptible to sensitization and in-service failure after welding. In consequence, the carbon levels were reduced to 0.08%, which represented the lower limit attainable with steel making technology then available. Although this

Table 4. The Effect of Alloying on Pitting Resistance of Stainless-Steel Alloys

Element	Effect on pitting resistance	Element	Effect on pitting resistance
Chromium	Increases	Molybdenum	Increases
Nickel	Increases	Nitrogen	Increases
Titanium/Niobium	No effect in media other than ferric chloride	Sulfur (and selenium)	Decreases
Silicon	Decrease or increase depending on the absence or presence of molybdenum	Carbon	Decreases if present as grain boundary precipitates

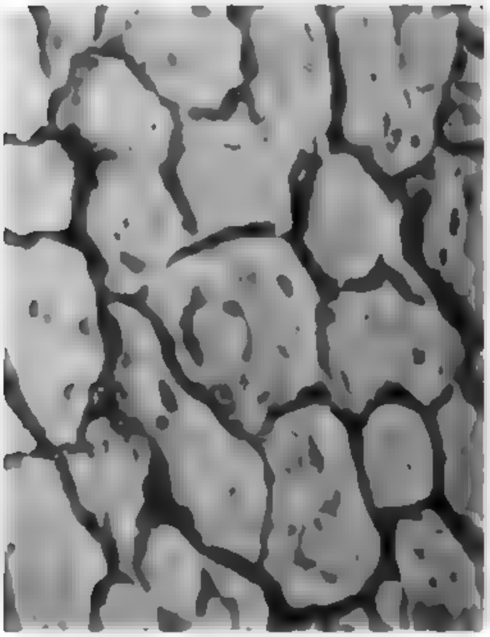


Figure 11. Scanning electron micrograph of the surface of sensitized stainless steel showing preferential attack along the grain boundaries.

move alleviated the problem, it was not wholly successful, particularly when welding thicker sections of the metal. Solution annealing of the fabricated items was rarely a practical proposition and there was a need for a long-term solution. It was shown that titanium or niobium (columbium) had a much greater affinity for the carbon than chromium and by additions of either of these elements, the problem was largely overcome. The titanium or niobium carbides that are formed remain dispersed throughout the metal structure rather than accumulating at the grain boundaries.

Grade 321 is a type 304 (18 Cr, 8 Ni) with titanium added as a stabilizing element, while grade 347 contains niobium. By far, the most commonly used is 321, grade 347 being specified for certain chemical applications.

Modern steelmaking techniques such as AOD (air-oxygen decarburization) were developed to reach even lower levels of carbon, typically less than 0.03%, to produce the "L" grades of stainless steel. These are commercially available and routinely specified where no sensitization can be permitted. With these advances in steel making technology, even the standard grades of stainless steels have typically carbon levels of 0.04–0.05% and, generally speaking, are weldable without risk of chromium carbide precipitates at metal thicknesses of up to 6 mm (1/4 in.). Above this figure or where multipass welding is to be employed, the use of a stabilized or "L" grade is always advisable.

Stress Corrosion Cracking. One of the most insidious forms of corrosion encountered with the austenitic stainless steels is stress corrosion cracking (SCC). The morphology of this type of failure is invariably a fine filamentous crack that propagates through the metal in a transgranular mode. Frequently, the crack is highly branched as shown in Figure 12, although sometimes it can assume a single-crack form. Factors such as metal structure, environment, and stress level have an effect on crack morphology. The disturbing feature of SCC is that there is virtually no loss of metal and frequently, it is not visible by casual inspection and is only apparent after perforation occurs. Some claim that as much as 50% of the failures of stainless steel are attributable to this cause.

Another characteristic of SCC in stainless steels is that once detected, repair by welding is extremely difficult. Crack propagation frequently occurs below the surface of the metal, and any attempt to weld repair results in the crack opening up and running ahead of the welding torch. The only practical method of achieving a satisfactory repair is to completely remove the affected area with a 15–25 cm (6–9 in.) allowance all around the area of visible damage and replace the section. Even then, there is no guarantee that the damaged zone has been entirely removed. In most cases, there are three prerequisites for the initiation of SCC.

- *Tensile Stress.* This may be either residual stress from fabricating operations or applied through the normal operating conditions of the equipment. Furthermore, it has been observed that a corrosion pit can act both as a stress raiser and a nucleation site for SCC.

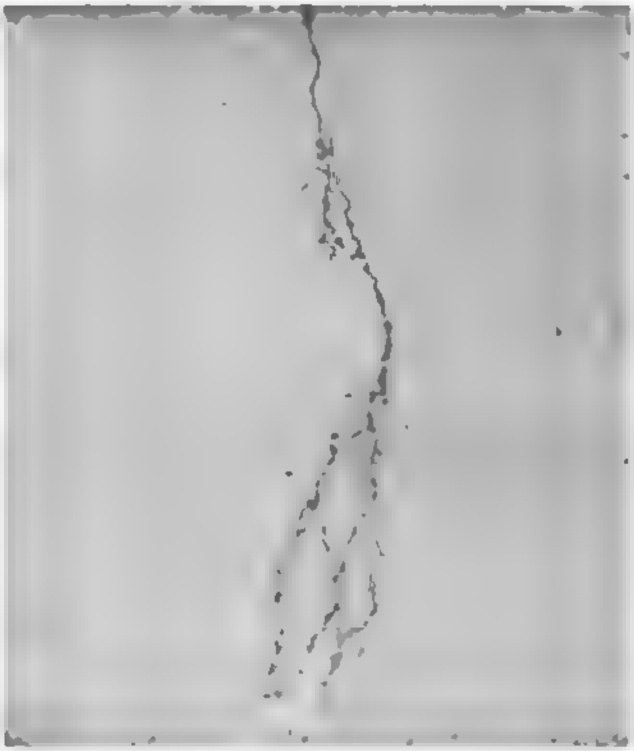


Figure 12. Photomicrograph of a typical stress corrosion crack showing its highly branched morphology and transgranular propagation.

- *Corrosive Species*. Although there are a number of ionic compounds that will act as the corrodent, in the food industry this invariably is the chloride ion. High-strength caustic soda at elevated temperatures will also induce SCC, but the concentrations and temperatures required are well in excess of those ever likely to be encountered. Furthermore, the crack morphology is inter- rather than transgranular. pH also plays a role and generally speaking, the lower the pH the greater the propensity for SCC.
- *Temperature*. It generally is regarded by many that a temperature in excess of 60°C (140°F) is required for this type of failure, although the author has seen examples occurring at 50°C (122°F) in liquid glucose storage vessels.

In the absence of any one of these prerequisites, the initiation of SCC is eliminated. Therefore, it is worth considering the practical approach to its elimination from equipment.

Figure 13 is a diagrammatic representation of the effect of stress on "time-to-failure." As will be seen, by reducing the stress level below a certain critical point, the "time-to-failure" can be increased by several orders of magnitude. On small pieces of equipment, residual stress from manufacturing operations can be removed by stress-relief annealing, which, in the case of stainless steels, is the same as solution annealing. For large pieces of equipment such as storage vessels this approach is clearly impractical. Applied stress is very much a function of the operational conditions of the equipment and only by reducing the stress level by increasing the thickness of the metal can this be reduced. However, this too is a somewhat impractical and uneconomic approach. Some (15) claim that by placing the surface of the metal under compressive rather than tensile stress, by shot peening with glass beads, the problem of SCC can be minimized or eliminated. This too is not a practical proposition for many items of food processing equipment.

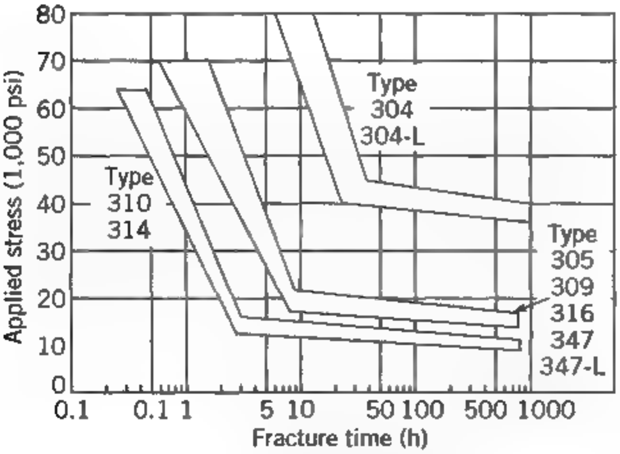


Figure 13. Composite curves illustrating the relative resistance to stress corrosion cracking of some commercial stainless steel in a specific test solution.

As for the matter of corrosive species, it is questionable if anything can be done about elimination. With foodstuffs, for example, this invariably will be the chloride ion, a naturally occurring or essential additive.

Similarly, little can be done in respect to the temperature as this is going to be essential to the processing operation.

As shown by Copson (16), the tendency for iron-chromium-nickel alloys to fail by SCC in a specific test medium (boiling 42% magnesium chloride solution) is related to the nickel content of the alloy. Figure 14 shows this effect, and it is unfortunate that stainless steels with a nominal 10% nickel have the highest susceptibility to failure. Increasing the nickel content of the alloy results in a significant increase in the time-to-failure, but of course, this approach incurs not only the increased cost of the nickel but also the added penalty of having to increase the chromium to maintain a balanced metallurgical structure. The more effective approach is by reducing the nickel content of the alloy.

A group of stainless steels have been developed that exploit this feature, and although their composition varies from producer to producer, they have a nominal composition of 20/22% chromium, 5% nickel. Molybdenum may or may not be present, depending on the environment for which the alloy has been designed. These alloys differ from the austenitic stainless steels insofar as they contain approximately 50% ferrite; hence their designation, austenitic-ferritic, or more commonly, duplex stainless steels. It is only with the advent of modern steel making technology, particularly in relation to the lower carbon levels that can be achieved, that these alloys have become a

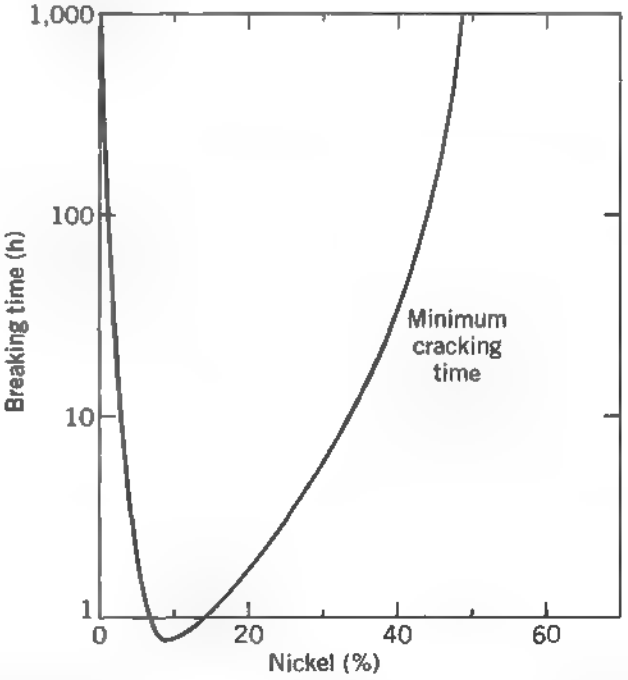


Figure 14. Stress corrosion cracking of iron, chromium, nickel alloys—the Copson curve. Data points have been omitted for clarity.

commercially viable proposition. Because of their low carbon content, typically 0.01–0.02%, the original problems associated with welding ferritic stainless steels and chromium carbide precipitation have been overcome. The alloys are almost twice as strong as the austenitic stainless steels and are ductile and weldable. From a general corrosion standpoint, they are comparable with, or marginally superior to, their 300 series equivalent; but from an SCC standpoint in test work and from field experience, they offer a resistance orders of magnitude better.

Also now available are fully ferritic stainless steels such as grade 444, which contains 18% chromium and 2% molybdenum. This alloy contains carbon at the 0.001% level and therefore does not suffer the problems of welding that were encountered with the original ferritic steels. Furthermore, stabilizing elements such as titanium and niobium are also alloying additions that minimize the tendency for intergranular chromium carbide formation. The main disadvantage of these materials is their susceptibility to grain growth during welding (Fig. 15), which makes them extremely sensitive to fracture even at room temperature. Welding sections thicker than 3 mm (1/8 in.) are not regarded as a practical proposition, and, therefore, their use tends to be limited to tubing.

Corrosion Fatigue. Fatigue is not a form of corrosion in the accepted sense as there is no loss of metal, but can be associated with other forms of localized attack. Because pure fatigue is an *in vacuo* phenomenon, a more correct term is corrosion fatigue or environmental cracking, which is the modern expression and takes into account cracking where the corrosive factor has played a major role on the crack morphology.

The primary cause of corrosion fatigue is the application of fluctuating pressure loads to components that, while of adequate design to withstand normal operating pressures eventually fail under the influence of cyclic loading. The components can be of extremely rigid construction such as a homogenizer block or of relatively light construction such as pipework. There are many potential sources of the fluctuating pressure, the most common of which are positive-displacement pumps (eg, homogenizer or metering pumps), rapid-acting on-off valves that will produce transient pressure peaks, frequent stop-start operations, dead-ending of equipment linked to a filling machine, etc.

Generally speaking, fatigue cracks are straight, without branching and without ductile metal distortion of the material adjacent to the crack. The one characteristic of fatigue cracks is that the crack face frequently has a series of conchoidal markings that represent the stepwise advance of the crack front (Fig. 16). Although, as stated, corrosion fatigue cracks are generally straight and unbranched, where fatiguing conditions pertain in a potentially corrosive environment, the influence of the corrosive component may be superimposed on the cracks. This can lead to branching of the cracks, and in the extreme case, the crack may assume a highly branched morphology which is almost indistinguishable from stress corrosion cracking. It is only when the crack face is examined under high-power magnification that it is possible to categorize the failure mode. An example of this is shown in Figure 17, which has all the features of stress corrosion cracking but the scanning electron micrograph (Fig. 18) clearly shows the stepwise progression of the crack front.

The site for initiation of corrosion fatigue is frequently a discontinuity in the metal section of the component. This may be, for example, a sharp change in diameter of a shaft where inadequate radiusing of the diametric change results in a high cycle stress level through shaft rotation and thus an initiation point for fatigue. A corrosion pit, which under cyclic loading will have a high stress-level association, can also act as a nucleation site (epicenter) for fatigue failure. Because of the corrosive element, it is sometimes difficult to establish whether the pit and associated corrosion were the initiating mechanism for the cracking or the result of corrosion superimposed on a crack, the frac-

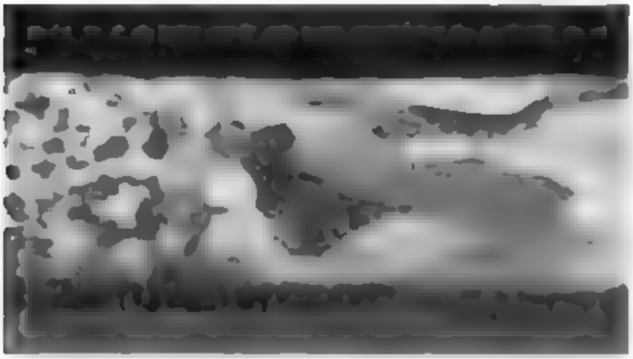


Figure 15. Photomicrograph of a weld deposit on a ferritic stainless steel. Compare the size of grains in the weld with those in the parent material on the left.

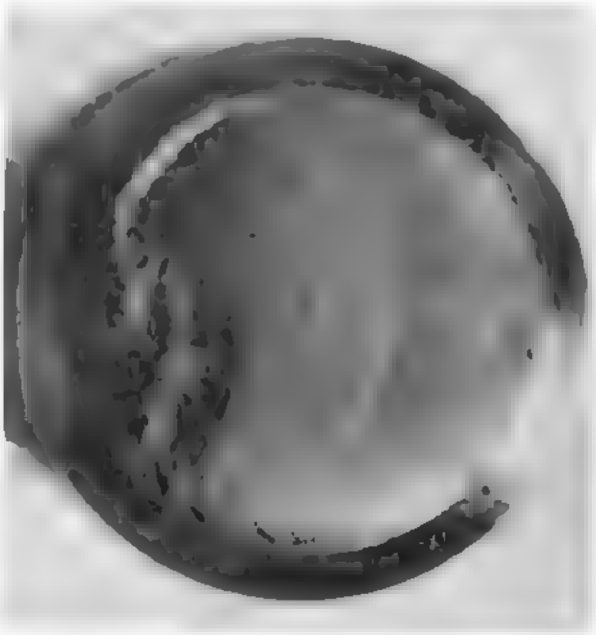


Figure 16. Fatigue cracking of a 1-in.-diameter stainless steel bolt showing the characteristic conchoidal striations on the crack face.

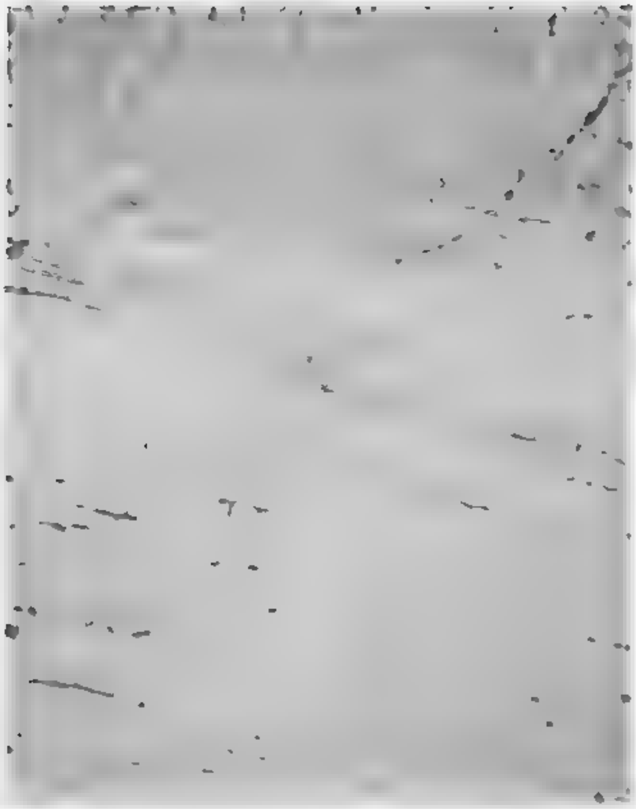


Figure 17. Photomicrograph of a fatigue crack showing all the features of stress corrosion cracking. Compare this with Figure 12.

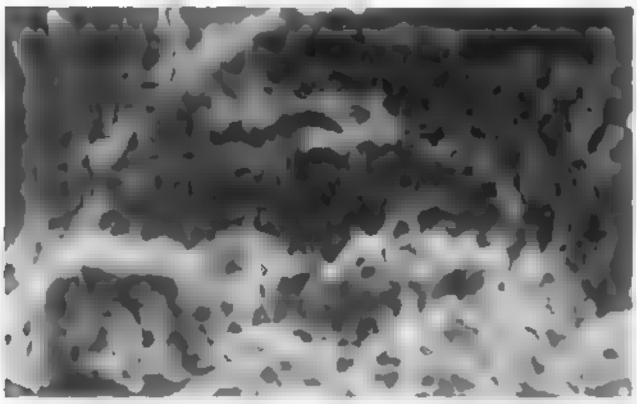


Figure 18. Scanning electron micrograph of the fracture face of the crack shown in Figure 17. Note the stepwise progression of the crack.

ture face of which will be in an "active" state and therefore more susceptible to corrosion processes.

The whole subject of fatigue and corrosion fatigue is complex. However, as far as food processing equipment is concerned, avoidance of fatigue failure is best achieved by avoiding pulsing and pressure peaks. This requires the use of well-engineered valving systems and avoiding the use of positive-displacement pumps. Where this is impractical, provision should be made to incorporate pulsation dampers to smooth out the pressure peaks and minimize the risk of fatigue failures.

Selective Corrosion *Corrosion of Castings.* There are a number of stainless-steel components found in food processing plants such as pipeline fittings and pump impellers that are produced as castings rather than fabricated from wrought material—notably, the cast equivalents of grade 304 (CF8) and 316 (CF8M). Although the cast and wrought materials have similar, but not identical, compositions with regard to their chromium and nickel contents, metallurgically they have different structures. Whereas the wrought materials are fully austenitic, castings will contain some ferrite or more terminologically correct, δ (delta) ferrite in the basic austenitic matrix. The ferrite is necessary to permit welding to the castings, to avoid shrinkage cracking during cooling from the casting temperature, and to act as nucleation sites for the precipitation of chromium carbides, which will invariably be present as it will not always be possible to solution anneal the cast components.

The nominal ferrite level is usually 5–12%. Below 5%, cracking problems may be experienced while above 12%, the ferrite tends to form a continuous network rather than remain as isolated pools.

Because the crystallographic structures of the ferrite and austenite differ (austenite is a face-center cubic and the ferrite, body-center cubic), the ferrite has, thermodynamically speaking, a higher free energy, which renders it more susceptible to attack, particularly in low-pH chloride-containing environments such as tomato ketchup and glucose syrups. Although the problem is not so severe when the ferrite occurs as isolated pools, when present as a continuous network, propagation of the corrosion occurs along the ferrite, with the austenite phase being relatively unaffected (Fig. 19). Because the products of corrosion are not leached out from the corrosion site and are more voluminous than the metal, corroded castings frequently assume a blistered or pockmarked appearance. The common environments encountered in the industry that produce preferential ferrite attack are the same as those causing

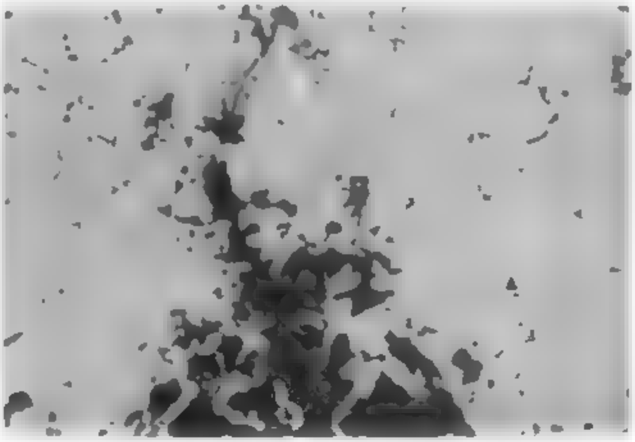


Figure 19. Photomicrograph of a section of cast 316 (CF8M) showing preferential attack of the ferrite phase.

stress corrosion cracking. Therefore, this form of damage is frequently also present (Fig. 20).

Depending on the method used to make the casting, the surface of the castings can be chemically modified, which reduces the corrosion resistance. Small components are usually cast by either the shell molding process or produced as investment castings. In the shell molding process, the sand forming the mold is bonded together with an organic resin that carbonizes when the hot metal is poured. This results in the metal adjacent to the mold having an enhanced carbon level with the formation of intergranular carbide precipitates and hence, a susceptibility to intergranular attack and other forms such as crevice, pitting, and stress corrosion cracking. Methods of overcoming this include solution annealing or machining off the carburized skin of metal.

With investment castings, the mold is made of zircon sand (zirconium silicate), and fired at a high temperature to remove all traces of organic material and wax that is used as a core in the moldmaking process. They do not, therefore, have this carburized layer and offer a much better resistance to surface corrosion.

Free-Machining Stainless Steels. Stainless steels are notoriously difficult to machine, especially turning, not so much because they are hard, but because the swarf tends to form as continuous lengths that clog the machine and to weld to the tip of the machine tools. One method of overcoming this is to incorporate a small amount, typically 0.2%, of sulfur or selenium in the alloy. These elements react with the manganese to form manganese sulfide or selenide, which, being insoluble in the steel, forms as discrete pools in castings or as elongated, continuous stringers in, say, wrought bar. The effect of the sulfide inclusions is to cause the material to form chips rather than long strings of swarf when being machined. Both manganese sulfide and selenide have virtually zero corrosion resistance to dilute mineral acids or other corrosive media. Thus, the free-cutting variants have a much lower corrosion resistance than their designation would imply. Indeed, some believe that the addition of sulfur to a type 316 material will offset the beneficial effect of the alloying addition of molybdenum. As stated, the sulfide inclusions will occur in castings as discrete pools, and therefore there will not be a continuous corrosion path. However, one of the products of corrosion in acidic media will be hydrogen sulfide, which has a profound effect on the corrosivity of even dilute mineral acids, causing attack of the austenitic matrix.

In the case of wrought materials, in particular bar stock, the sulfide inclusions are present as semicontinuous stringers and can suffer so-called end-grain attack in mildly corrosive media.

Stainless-steel nuts and bolts, which are produced on automatic thread-cutting machines, are invariably made from free-cutting materials. Figure 21 illustrates the difference in corrosion resistance of a bolt made from this and a nonfree machining 316. Both bolts were exposed to the same mildly acidic environment.

When specifying materials of construction, quite clearly due cognizance of this difference must be recognized. Any components turned from bar stock which are likely to come into contact with potentially corrosive environments should always be specified in 316 and not in the free machining, sulfur-containing variant.

CORROSION OF SPECIFIC ENVIRONMENTS

From a corrosion standpoint, the environments likely to be encountered in the food industry that may cause premature equipment failure may be classified under main headings:

Noncorrosive
Mildly corrosive
Highly corrosive
Service fluids
Alkaline detergents
Acidic detergents
Sanitizing agents

Noncorrosive Foodstuffs

In general terms, natural foodstuffs such as milk, cream, natural fruit juices, and whole egg do not cause corrosion

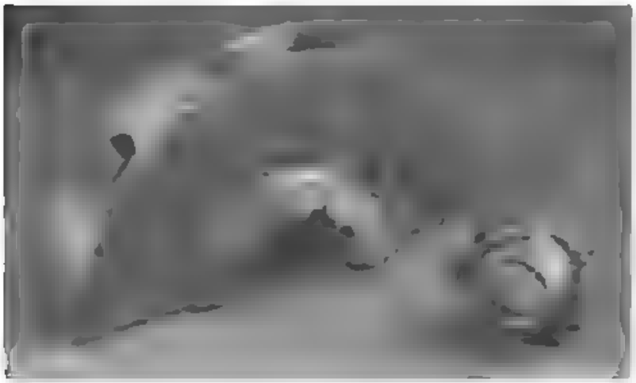


Figure 20. Corroded pump impeller (above) and valve body (below) caused by tomato ketchup. Note the pocklike corrosion sites.

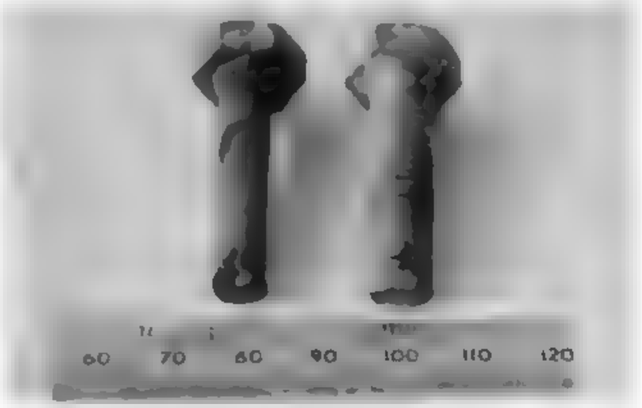


Figure 21. Free machining and nonfree machining bolts after immersion in a mildly acidic environment.

stress corrosion cracking. Therefore, this form of damage is frequently also present (Fig. 20).

Depending on the method used to make the casting, the surface of the castings can be chemically modified, which reduces the corrosion resistance. Small components are usually cast by either the shell molding process or produced as investment castings. In the shell molding process, the sand forming the mold is bonded together with an organic resin that carbonizes when the hot metal is poured. This results in the metal adjacent to the mold having an enhanced carbon level with the formation of intergranular carbide precipitates and hence, a susceptibility to intergranular attack and other forms such as crevice, pitting, and stress corrosion cracking. Methods of overcoming this include solution annealing or machining off the carburized skin of metal.

With investment castings, the mold is made of zircon sand (zirconium silicate), and fired at a high temperature to remove all traces of organic material and wax that is used as a core in the moldmaking process. They do not, therefore, have this carburized layer and offer a much better resistance to surface corrosion.

Free-Machining Stainless Steels. Stainless steels are notoriously difficult to machine, especially turning, not so much because they are hard, but because the swarf tends to form as continuous lengths that clog the machine and to weld to the tip of the machine tools. One method of overcoming this is to incorporate a small amount, typically 0.2%, of sulfur or selenium in the alloy. These elements react with the manganese to form manganese sulfide or selenide, which, being insoluble in the steel, forms as discrete pools in castings or as elongated, continuous stringers in, say, wrought bar. The effect of the sulfide inclusions is to cause the material to form chips rather than long strings of swarf when being machined. Both manganese sulfide and selenide have virtually zero corrosion resistance to dilute mineral acids or other corrosive media. Thus, the free-cutting variants have a much lower corrosion resistance than their designation would imply. Indeed, some believe that the addition of sulfur to a type 316 material will offset the beneficial effect of the alloying addition of molybdenum. As stated, the sulfide inclusions will occur in castings as discrete pools, and therefore there will not be a continuous corrosion path. However, one of the products of corrosion in acidic media will be hydrogen sulfide, which has a profound effect on the corrosivity of even dilute mineral acids, causing attack of the austenitic matrix.

In the case of wrought materials, in particular bar stock, the sulfide inclusions are present as semicontinuous stringers and can suffer so-called end-grain attack in mildly corrosive media.

Stainless-steel nuts and bolts, which are produced on automatic thread-cutting machines, are invariably made from free-cutting materials. Figure 21 illustrates the difference in corrosion resistance of a bolt made from this and a nonfree machining 316. Both bolts were exposed to the same mildly acidic environment.

When specifying materials of construction, quite clearly due cognizance of this difference must be recognized. Any components turned from bar stock which are likely to come into contact with potentially corrosive environments should always be specified in 316 and not in the free machining, sulfur-containing variant.

CORROSION OF SPECIFIC ENVIRONMENTS

From a corrosion standpoint, the environments likely to be encountered in the food industry that may cause premature equipment failure may be classified under main headings:

Noncorrosive
Mildly corrosive
Highly corrosive
Service fluids
Alkaline detergents
Acidic detergents
Sanitizing agents

Noncorrosive Foodstuffs

In general terms, natural foodstuffs such as milk, cream, natural fruit juices, and whole egg do not cause corrosion

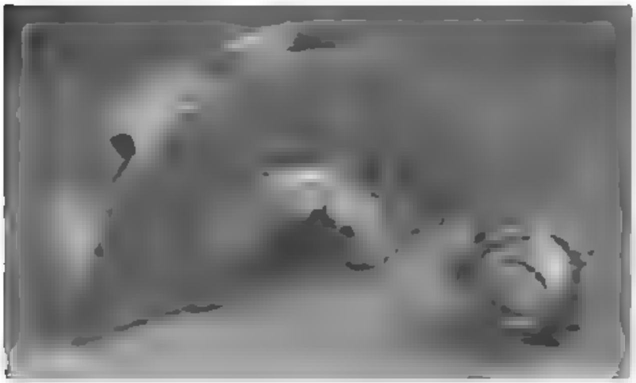


Figure 20. Corroded pump impeller (above) and valve body (below) caused by tomato ketchup. Note the pocklike corrosion sites.

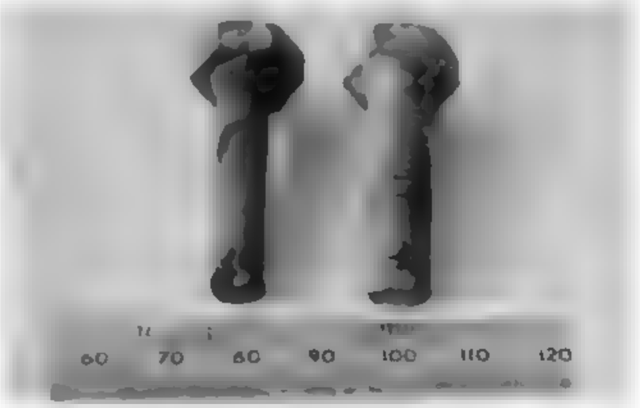


Figure 21. Free machining and nonfree machining bolts after immersion in a mildly acidic environment.

problems with 304 or 316 stainless steels. Prepared foodstuffs to which there is no added salt such as yogurt, beer, ice cream, wines, spirits, and coffee also fall within this classification. For general storage vessels, pipelines, pumps, fittings or valves, grade 304 is perfectly satisfactory. However, for plate heat exchangers that are highly creviced and therefore prone to crevice corrosion, grade 316 frequently is employed. This offers a higher degree of protection against some of the more acidic products such as lemon juice, which may contain small quantities of salt and also provides a higher level of integrity against corrosion by service liquids and sanitizing agents.

It is quite common to use sulfur dioxide or sodium bisulfite for the preservation of fruit juices and gelatin solutions and in such cases, storage vessels always should be constructed from 316. Although the sulfur dioxide is noncorrosive at ambient temperature in the liquid phase, as a gas contained in significant quantities within the head space in a storage tank it tends to dissolve in water droplets on the tank wall. In the presence of air, the sulfurous acid that forms is oxidized to sulfuric acid at a concentration high enough to cause corrosion of 304 but not of 316.

Mildly Corrosive Foodstuffs

This category of foodstuffs covers products containing relatively low levels of salt and where pH values are below 7. Examples include glucose/fructose syrups and gelatin, the production of which may involve the use of hydrochloric acid. For storage vessels, pipelines, fittings, and pumps, grade 316 has established a good track record, and boiling pans in this grade of steel are perfect for long and satisfactory service. The corrosion hazards increase however in processing operations involving high temperatures and where the configuration of the equipment is such as to contain crevices, especially when the product contains dissolved oxygen. For example, multistage evaporators operating on glucose syrup will usually have the first stage, where temperatures may approach 100°C (212°F), constructed in a super stainless steel such as 904L. Subsequent effects where temperatures are lower and where the product has been deoxygenated may be fabricated in grade 316 stainless steel.

As previously indicated, it is common practice to use sulfur dioxide as a preservative in dilute gelatin solutions during storage prior to evaporation. In some cases, excess hydrogen peroxide will be added to neutralize the sulfur dioxide immediately before concentration. This can have a catastrophic effect on the 300 series stainless steels and indeed on even more highly alloyed metals such as 904L, because of the combined effect of the chlorides present with the excess hydrogen peroxide. Because of this, it is a more acceptable practice to make the peroxide addition after rather than before evaporation.

Gelatin for pharmaceutical end use is subject to UHT treatment to ensure sterility. This will involve heating the gelatin solutions to 135°C (285°F) and holding at that temperature for a short period of time. Plate heat exchangers are used extensively for this duty. Although plates made from 316 stainless steel give a reasonable life of typically 2–3 years, a corrosion resistant alloy with an enhanced-level molybdenum is to be preferred.

Highly Corrosive Foodstuffs

The list of foodstuffs falling in this category is almost endless—gravies, ketchups, pickles, salad dressings, butter, and margarine—in fact, anything to which salt has been added at the 1–3% level or even higher. Also within this category must be included cheese salting brine and other brines used in the preservation of foodstuffs that undergo pasteurization to minimize bacterial growth on food residues remaining in the brine. Although these brines are usually too strong to support the growth of common organisms, salt resistant strains (halophiles) are the major problem.

Low-pH products containing acetic acid are particularly aggressive from a corrosion standpoint, but selection of materials for handling these products depends to a great extent on the duty involved.

When trying to define the corrosion risk to a piece of equipment handling potential corrodents, several factors come into play. While temperature, oxygen content, chloride content, and pH are the obvious ones, less obvious and equally important is contact time. All three main forms of corrosion induced in stainless steels (crevice, pitting, and stress corrosion cracking) have an induction period before the onset of corrosion. This can vary from a few hours to several months depending on the other operative factors. In a hypothetical situation where stainless steel is exposed to a potentially corrosive environment, removal of the steel and removal of the corrodents will stop the induction and the status quo is established. On repeating the exposure, the induction period is the same. In other words, the individual periods the steel spends in contact with the corrodent are not cumulative and each period must be taken in isolation.

When the contact period is short, temperatures are low and a rigorous cleaning regime is implemented at the end of each processing period, 316 stainless steel will give excellent service. However, where temperatures are high and contact periods are long, the corrosion process may be initiated. This is especially so in crevices such as the interplate contact points on a plate heat exchanger where, albeit at a microscopic level, corrodents and corrosion products are trapped in pits or cracks. Geometric factors may prevent the complete removal of this debris during cleaning and under such circumstances, the corrosion process will be ongoing.

Because of the perishable nature of foodstuffs, storage is rarely for prolonged periods or at high temperatures, and regular, thorough cleaning tends to be the norm. The one exception to this is buffer storage vessels for holding "self-preserving" ketchup and sauces. For such duties, an alloy such as 904L, Avesta 254 SMO, or even Inconel 625 may be required.

While all the foregoing applies to general equipment, the one exception is plate heat exchangers. Their highly creviced configuration and the high temperatures employed render them particularly susceptible. Plates made from grade 316 have a poor track record on these types of duties. Even the more highly alloyed materials do not offer complete immunity. The only reasonably priced material that is finding increased usage in certain areas of food processing is titanium.

The fact that butter and margarine have been included in this group of corrodents requires comment. Both these foodstuffs are emulsions containing typically 16% water and 2% salt A fact not often appreciated is that the salt is dissolved in the water phase, being insoluble in the oil. From a corrosion standpoint, therefore, the margarine or butter may be regarded as a suspension of 12% salt solution and as such is very corrosive to 316 stainless steel at the higher processing temperatures. The only mitigating feature that partly offsets their corrosivity is the fact that the aqueous salty phase is dispersed in an oil rather than the reverse and that the oil does tend to preferentially wet the steel surface and provide some degree of protection. However, the pasteurizing heat exchanger in margarine rework systems invariably has titanium plates as the life of 316 stainless steel is limited and has been known to be as little as 6 weeks

Corrosion by Service Fluids

Steam. Because it is a vapor and free from dissolved salts, steam is not corrosive to stainless steels. Although sometimes contaminated with traces of rust from carbon steel steam lines, in the author's experience no case of corrosion due to industrial boiler steam ever has been encountered.

Water. The quality and dissolved solids content of water supplies varies tremendously, with the aggressive ionic species, chloride ions, being present at levels varying between zero, as found in the lakeland area of England, to several hundred parts per million, as encountered in coastal regions of Holland. It is also normal practice to chlorinate potable water supplies to kill pathogenic bacteria with the amount added dependent on other factors such as the amount of organic matter present. However, most water supply authorities aim to provide water with a residual chlorine content of 0.2 ppm at the point of use. Well waters also vary in composition depending on the geographical location, especially in coastal regions where the chloride content can fluctuate with the rise and fall of the tide.

What constitutes a "good" water? From a general user viewpoint, the important factor is hardness, either temporary hardness caused by calcium and magnesium bicarbonates that can be removed by boiling, or permanent hardness caused by calcium sulfate that can be removed by chemical treatment. While hardness is a factor, chloride content and pH probably are the most important from a corrosion standpoint.

What can be classed as a noncorrosive water supply from a stainless-steel equipment user? Unfortunately, there are no hard-and-fast rules that will determine whether corrosion of equipment will occur. As has been repeatedly stated throughout this article, so many factors come into play. The type of equipment, temperatures, contact times, etc all play a role in the overall corrosion process. Again, as has been stated before, the most critical items of equipment are those with inherent crevices—evaporators and plate heat exchangers among others. Defining conditions of use for this type of equipment will be the regulatory factor. Even then, it is virtually impossible to define the composition of a "safe" water, but as a general guideline, water with less than 100 ppm chloride is unlikely to initiate crevice corrosion of type 316 stainless steel while a maximum level of 50 ppm should be used with type 304.

Cooling tower water systems are frequently overlooked as a potential source of corrodents. It must be appreciated that a cooling tower is an evaporator, and although the supply of makeup water may contain only 25 ppm chloride, over a period of operation this can increase by a factor of 10 unless there is a routine bleed on the pond

Water scale deposits formed on heat-transfer surfaces should always be removed as part of the routine maintenance schedule. Water scale deposits can accumulate chloride and other soluble salts that tend to concentrate, producing higher levels in contact with the metal than indicated by the water composition. Furthermore, water scale formed on a stainless-steel surface provides an ideal base for the onset of crevice corrosion.

As stated previously, potable water supplies usually have a residual free chlorine content of 0.2 ppm. Where installations have their own private wells, chlorination is undertaken on site. In general terms, the levels employed by the local water authorities should be followed and overchlorination avoided. Levels in excess of 2 ppm could initiate crevice corrosion.

Cooling Brines. Depending on the industry, these can be anything from glycol solutions, sodium nitrate/carbonate, or calcium chloride. It is the latter which are used as a 25% solution that can give rise to corrosion of stainless steel unless maintained in the ideal condition, especially when employed in the final chilling section of plate heat exchangers for milk and beer processing. However, by observing certain precautions, damage can be avoided.

The corrosion of stainless steels by brine can best be represented as shown in Figure 22. It will be noted that an exponential rise in corrosion rate with reducing pH occurs in the range pH 12-7. The diminution in number of pits occurring in the range pH 6-4 corresponds with the change in mode of attack, ie, from pitting to general corrosion. It will be seen that ideally the pH of the solution should be maintained in the region 14-11. However, calcium chloride brine undergoes decomposition at pH values higher than 10.6:

$$CaCl_2 + 2NaOH \rightarrow Ca(OH)_2\downarrow + 2NaCl$$

Scale deposition occurs and heat-transfer surfaces become fouled with calcium hydroxide scale. Furthermore, the scale that forms traps quantities of chloride salts that cannot be effectively removed and remain in contact with the equipment during shutdown periods. This is particularly important in equipment such as plate heat exchangers that are subjected to cleaning and possibly hot-water sterilization cycles at temperatures of 80°C (176°F) or higher.

The other aspect, nonaeration, is equally important. Air contains small quantities of carbon dioxide that form a slightly acidic solution when dissolved in water. This has

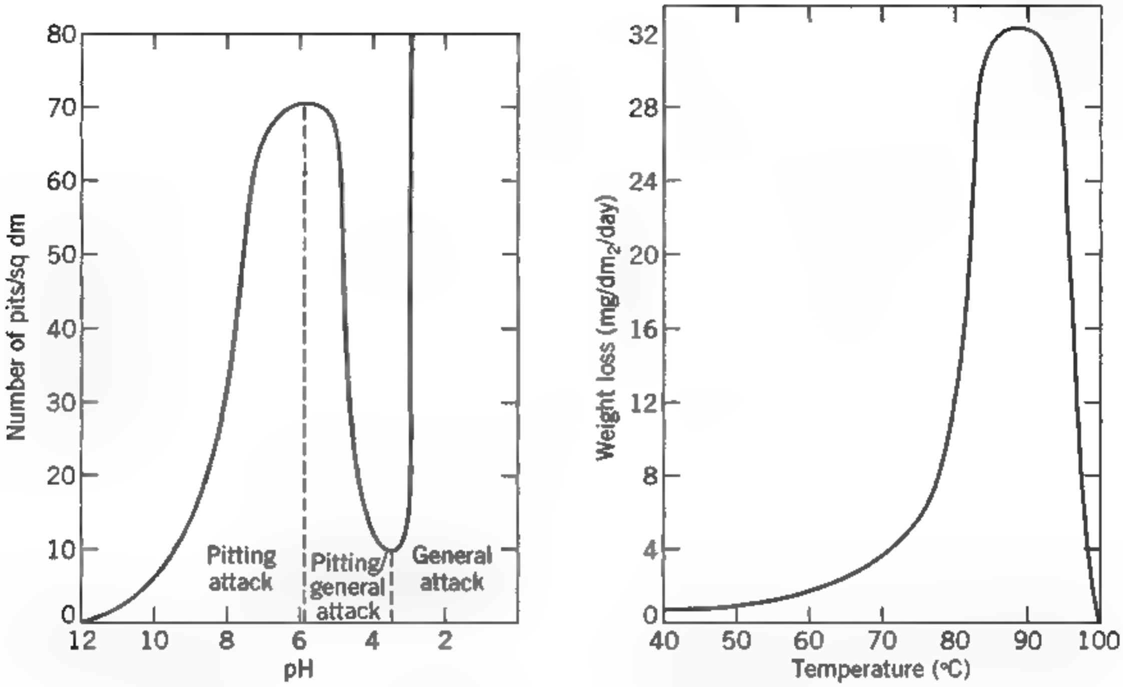


Figure 22. The effect of pH and temperature on the pitting of stainless steel by brine.

the effect of neutralizing the buffering action of any alkaline components in the brine:

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$
$$Na_2CO_3 + CaCl_2 \rightarrow 2NaCl + CaCO_3\downarrow$$

or

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3\downarrow + H_2O$$

Therefore, the pH of the brine decreases and assumes a value of about pH 6.5, which is the region where pitting incidence is highest. Furthermore, scale deposits of calcium carbonate are laid down on heat-transfer surfaces, creating the problems referred to above.

The precautions to be observed when using brine circuits are

- Ensure correct pH control and maintain in the range pH 9.5–10.
- Eliminate aeration. In particular, make certain that the brine return discharge line is below the surface in the storage tank during running and that the method of feeding brine from the tank does not cause vortexing with resultant air entrainment. Baudelot type evaporators cause aeration of the chilling liquor and should never be used on brine circuits.
- When cleaning and sterilizing the brine section of a pasteurizer, flush out all brine residues until the rinse water is free of chloride. As an added precaution, it is advisable to form a closed circuit and circulate a 1/4–1/2% caustic soda or sodium metasilicate solution to ensure that any brine residues are rendered alkaline.
- In plate heat exchangers and similar equipment, make sure that stainless-steel brine section components remain free of scale.
- When shutting down the plant after a cleaning run, it is advisable to leave the section full with a dilute (1/4%) caustic solution. Before startup, this should be drained and residues flushed out prior to reintroducing brine.

When operating conditions prevailing in a plant do not permit such a disciplined cleaning, operating, and shutdown procedure, only two materials can be considered for the brine section of a plate heat exchanger. These are Hastelloy C-276 or titanium. The corrosion resistance of both of these materials is such that cleaning and sanitizing of the product side of the heat exchanger can be carried out without removing the brine. Although both are more expensive than stainless steels, especially Hastelloy C-276, the flexibility of plant operation which their use permits could offset their premium price.

Alkaline Detergents

Supplied to food processing plants either as bulk shipments of separate chemicals or as carefully preformulated mixtures, the composition of alkaline detergent formulations can vary widely in accordance with individual preference or the cleaning job to be done. The detergents do, however, generally include some or all of the following compounds:

Sodium hydroxide
Sodium polyphosphate
Sodium metasilicate
Sodium carbonate

Additionally, it is not uncommon to find that a selection of sequestering agents such as EDTA and any of the many available wetting agents also may be present in the formulations

None of these compounds are corrosive to stainless steels at the concentrations and temperatures used by the food industry for cleaning. Indeed, 316 stainless steel is unaffected by concentrations of sodium hydroxide as high as 20% at temperatures up to 160°C (320°F). They can, therefore, be used with impunity at their usual maximum concentration of 5%, even in ultra-high-temperature (UHT) operation where temperatures can rise to 140°C (284°F).

Companies have reported that some of these preformulated alkaline detergents cause discoloration of the equipment.

The discoloration starts as a golden yellow, darkening to blue through mauve and eventually black. It has been established that this discoloration is caused by the EDTA sequestering agent, which complexes with traces of iron in the water. It then decomposes under certain conditions of pH and temperature to form an extremely fine film of hydrated iron oxide, the coloration being interference colors that darken as the film thickness increases. Although the film is not aesthetically pleasing, it is in no way deleterious and removing it by conventional cleaning agents is virtually impossible.

Some alkaline detergents are compounded with chlorine release agents such as sodium hypochlorite, salts of di- or trichlorocyanuric acid that form a solution containing 200–300 ppm available chlorine at their usage strength. Although the high alkalinity reduces the corrosivity of these additives, generally speaking they should not be employed on a regular basis at temperatures exceeding 70°C (160°F).

Acidic Detergents

Alkaline detergents will not remove the inorganic salts such as milkstone and beer-stone deposits frequently found in pasteurizers. For this, an acidic detergent is required and selection must be made with regard to their interaction with the metal. As was shown earlier, sulfuric and hydrochloric acids will cause general corrosion of stainless steels. Although it could be argued that sulfuric acid can be employed under strictly controlled conditions because stainless steels, especially grade 316, have a very low corrosion rate, its use could result in a deterioration of surface finish. This, in corrosion terms, is an extremely low rate but from an aesthetic viewpoint, is undesirable.

Acids such as phosphoric, nitric, and citric, when used at any concentration likely to be employed in a plant cleaning operation, have no effect on stainless steels and can be used with impunity. Three cautionary notes are worthy of mention:

- It is always preferable to use alkaline cleaning before the acid cycle to minimize the risk of interacting the acid with any chloride salts and, therefore, minimize the formation of hydrochloric acid.
- It is inadvisable to introduce an acid into a UHT sterilizing plant when it is at full operating temperature (140°C/285°F) as part of a "clean-on-the-run" regime.
- Nitric acid, which is a strong oxidizing agent, will attack certain types of rubber used as gaskets and seals. As a general guideline, concentrations should not exceed 1% and temperature 65°C (150°F), although at lower concentrations, the upper recommended temperature is 90°C (195°F).

Another acid that is finding increasing use in the food industry for removing water scale and other acid-soluble scales is sulfamic acid. Freshly prepared solutions of up to 5% concentration are relatively innocuous to stainless steels but problems may arise when CIP systems incorporating recovery of detergents and acids are employed. Sulfamic acid will undergo hydrolysis at elevated temperatures to produce ammonium hydrogen sulfate

$$NH_2SO_2OH + H_2O \rightarrow NH_4HSO_4$$

which behaves in much the same way as sulfuric acid. In situations where the use of this acid is contemplated, prolonged storage of dilute solutions at elevated temperature is inadvisable, although at room temperature the hydrolysis is at a low rate.

Sanitizing Agents

Terminology for the process of killing pathogenic bacteria varies from country to country. In Europe, disinfection is preferred; in America, sanitizing. Regardless, the term should not be confused with sterilization, which is the process of rendering equipment free from all live food spoilage organisms including yeasts, mold, thermophilic bacteria, and most importantly, spores. Sterilization with chemicals is not considered to be feasible and the only recommended procedure involves the circulation of pressurized hot water at a temperature of not less than 140°C (285°F).

For sanitization, while hot water (or steam) is preferred, chemical sanitizers are extensively used. These include noncorrosive compounds such as quaternary ammonium salts, anionic compounds, aldehydes, amphoterics, and potentially corrosive groups of compounds that rely on the release of halogens for their efficacy. By far, the most popular sanitizer is sodium hypochlorite (chloros), and this is probably the one material that has caused more corrosion in food plants than any other cleaning agent. For a detailed explanation of the corrosion mechanism, the reader is referred to an article by Boulton and Sorenson (17) that describes a study of the corrosion of 304 and 316 stainless steels by sodium hypochlorite solutions. It is important, therefore, if corrosion is to be avoided, that the conditions under which it is used are strictly controlled. For equipment manufactured from grade 316 stainless steel, the recommended conditions are.

- Maximum concentration—150 ppm available chlorine
- Maximum contact time—20 min
- Maximum temperature—room temperature that is well in excess of the minimum conditions established by Tastayre and Holley to kill *Pseudomonas aeruginosa* (18).

In addition, several other precautions must be observed:

- Before introducing hypochlorite, equipment should be thoroughly clean and free of scale deposits. Organic residues reduce the bactericidal efficiency of the disinfectant and offer an artificial crevice in which stagnant pools of hypochlorite can accumulate.
- It is imperative that acidic residues be removed by adequate rinsing before introducing hypochlorite solutions. Acid solutions will react with hypochlorite to release elementary chlorine, which is extremely corrosive to all stainless steels.
- The equipment must be cooled to room temperature before introducing hypochlorite. In detergent cleaning runs, equipment temperature is raised to 80–85°C (176–185°F), and unless it is cooled during the rinsing cycle, a substantial increase in temperature of the disinfectant can occur. An important point, frequently overlooked, is that a leaking steam valve can cause a rise in the temperature of equipment even though it theoretically is shut off.
- After sanitizing, the solution should be drained and the system flushed with water of an acceptable bacteriological standard. This normally is done by using a high rinse rate, preferably greater than that used in the processing run.

While these comments relate specifically to the sanitizing of plate heat exchangers, similar precautions must be taken with other creviced equipment. Examples include manually operated valves that should be slackened and the plug raised to permit flushing of the seating surface. Pipeline gaskets also should be checked frequently to make sure that they are in good condition and not excessively hardened. Otherwise they will fail to form a crevice-free seal over their entire diameter. Where it is not possible to completely remove hypochlorite residues such as in absorbent gland packing materials, hot water is preferred.

All the foregoing relate specifically to sodium hypochlorite solutions but other sanitizing agents that rely on halogen release, such as di- and trichlorocyanuric acid, should also be used under strictly controlled conditions, including such factors as pH.

Iodophors also are used for sanitizing equipment. These are solutions of iodine in nonionic detergents and contain an acid such as phosphoric to adjust the pH into the range at which they exhibit bactericidal efficacy. This group of sanitizers is employed where hot cleaning is not necessary or on lightly soiled surfaces such as milk road tankers, and farm tanks. Extreme caution should be exercised with this group for, although used at low concentrations (50 ppm), prolonged contact with stainless steel can cause pitting and crevice corrosion. Furthermore, in storage vessels that have been partially filled with iodophor solutions and allowed to stand overnight, pitting corrosion in the head space has been observed as a result of iodine vaporizing from the solution and condensing as pure crystals on the tank wall above the liquid line. Another factor is that iodine can be absorbed by some rubbers. During subsequent processing operations at elevated temperatures, the iodine is released in the form of organic iodine compounds, especially into fatty foods, which can cause an antiseptic taint. The author knows of one dairy that used an iodophor solution to sanitize a plate pasteurizer to kill an infection of a heat resistant spore-forming organism. The following day, there were over 2000 complaints of tainted milk. CIP cleaning cycles did not remove the antiseptic smell from the rubber seals, and complete replacement with new seals was the only method of resolving the problem.

Another sanitizing agent that is assuming increasing popularity, especially in the brewing industry because of its efficacy against yeasts, is peracetic acid. As such, peracetic acid will not cause corrosion of 304 or 316 stainless steels, and the only precautionary measure to be taken is to use a good quality water containing less than 50 ppm of chloride ions for making up the solutions to their usage concentration. Because of the strongly oxidizing nature of some types of peracetic acid solutions, deterioration of some types of rubber may occur. A recent survey undertaken by the IDF for the use of peracetic acid in the dairy industry (19) found few corrosion problems reported. The general consensus of opinion was that it permitted greater flexibility in the conditions of use, compared with sodium hypochlorite, without running the risk of damage to equipment.

For comprehensive information on the cleaning of food processing equipment, albeit primarily written for the dairy industry, the reader is referred to the British Standards Institute publication BS 5305 (20).

CORROSION BY INSULATING MATERIALS

Energy conservation now is widely practiced by all branches of industry, and the food industry is no exception. For example, in the brewing industry, wort from the wort boilers is cooled to fermentation temperature, and the hot water generated in the process is stored in insulated vessels (hot-liquor tanks) for making up the next batch of wort. An area of corrosion science that is receiving increased attention is the subject of corrosion initiated in stainless steels by insulating materials. At temperatures in excess of 60°C (140°F) these can act as a source of chlorides that will induce stress corrosion cracking and pitting corrosion of austenitic stainless steels.

Among the insulating materials that have been used for tanks and pipework are

Foamed plastics—polyurethane, polyisocyanurate, phenolic resins, etc
Cellular and foamed glass
Mineral fiber—glass wool, rock wool
Calcium silicate

Magnesia
Cork

All insulating materials contain chlorides to a lesser (10 ppm) or greater (1.5%) extent. The mineral based insulants such as asbestos may contain them as naturally occurring water soluble salts such as sodium or calcium chloride. The organic foams, on the other hand, may contain hydrolysable organochlorine materials used as blowing agents (to form the foam), fire retardants such as chlorinated organophosphates, or chlorine containing materials present as impurities. Insulation manufacturers are becoming increasingly aware of the potential risk of chlorides in contact with stainless steels and are making serious efforts to market a range of materials that are essentially chloride-free.

A point not frequently appreciated is that even though many of the insulating materials contain high levels of chloride, in isolation they are not corrosive.

Corrosion, which is an electrochemical process, involves ionic species, and in the absence of a solvent (water) the chloride or salts present in the insulation cannot undergo ionization to give chloride ions. Therefore, they are essentially noncorrosive. Similarly, where organochlorine compounds are present, water is necessary for hydrolysis to occur with the formation of hydrochloric acid or other ionisable chloride compounds.

The main problem, therefore, is not so much the insulant but the interaction of the insulant with potential contaminants to release corrosive species. Under ideal conditions, that is, if the insulating material could be maintained perfectly dry, the chloride content would not be a critical factor in the material specification process. Unfortunately, it must be acknowledged that even in the best regulated installation, this ideal is rarely (if ever) achieved and therefore, thought must be given to recommended guidelines.

Any material that is capable of absorbing water must be regarded as a potential source of chloride. Although the chloride content of the insulant may be extremely low (25 ppm), under extractive conditions when the insulation becomes wet and concentration effects come into play, even this material may cause the initiation of stress corrosion cracking. The chloride content of the contaminating water cannot be ignored. Even a "good-quality" water with a low chloride content (30 ppm), if being continuously absorbed by the insulant, forms a potential source of chlorides that, through evaporation, can reach very significant levels and initiate the corrosion mechanism.

The more absorptive the insulant, the greater the risk, and materials such as calcium silicate and certain types of foams must be regarded as least desirable. It is interesting to note that one of the least absorptive insulants from those listed is cork, and in the experience of the author, there have been no reported cases of this insulant causing problems with stress corrosion cracking of stainless steels. Of course, it could be argued that the bitumen used as an adhesive to stick the cork to the vessel walls has to be applied so thickly that the bitumen forms an impermeable barrier preventing contact of the contaminated water with the stainless-steel substrate. Irrespective of the protection mechanism, the net effect is that cork has an extremely good "track record." Unfortunately, due to the cost, cork is now rarely used.

No hard-and-fast rules can be laid down for specifying the acceptable maximum tolerable chloride content of an insulating material. Any specification must take into account what is commercially available as well as all the other factors such as price, flammability, and ease of application. To specify zero chloride is obviously impractical and even a figure of 10 ppm may be difficult to achieve in commercially available products. As a general compromise, 25 ppm maximum is considered to be technically and commercially feasible while minimizing the potential source level of chloride corrodent.

The primary function of the insulant is to provide a thermal barrier between the outside of the vessel and the environment. It does not provide a vapor or moisture barrier, and provision of such protection must be regarded as equally important in the insulating process. As mentioned previously, the use of bitumen as an adhesive for cork insulation must provide an extremely good water barrier, although there are a wide range of products marketed specifically for this purpose. These include specially developed paints of undefined composition and zinc free silicone alkyd paints as well as silicone lacquer. These silicone based products are particularly appropriate because of their inherent low water permeability and also their stability at elevated temperatures. Aluminum foil has also been successfully used as a water-vapor barrier between the stainless steel and the insulant. There is little doubt that the foil provides an extremely good barrier but at laps between the sheets of foil, ingression of water can occur unless sealing is complete, the achievement of which is most unlikely. Furthermore, it is likely that there will be tears in the foil, providing yet another ingression path for water. However, it is believed that aluminum foil has a role additional to that of a barrier. As it is, from an electrochemical aspect, "anodic" to stainless steel, it will provide galvanic protection of the steel in areas where there is a "holiday" in the foil, thus inhibiting corrosion mechanisms.

When insulation material becomes wet, the insulating efficiency shows a dramatic fall-off. In fact, the thermal conductivity of wet insulation will approach that of the wetting medium, and it is ironic that the thermal conductivity of water is among the highest known for liquids. It is imperative that from the standpoint of preventing moisture ingression to minimize corrosion risks and maintain insulation efficiency, the insulation is externally protected from rain and water. There is a variety of materials available for this such as aluminum sheeting, plastic-coated mild steel, and spray applied polyurethane coatings. Much of the value of the protection will be lost unless particular attention is taken to maintaining a weather-tight seal at the overlaps in individuals sheets of cladding. There are many semiflexible caulking agents that can be employed for this. One that is particularly effective is the RTV silicon rubber, which exhibits extremely good weather resistance and long-term reliability. Nevertheless, maintenance is required, especially around areas of discontinuity such as flanged connections and manway doors. Operators are beginning to realize that routine maintenance work on in-

Table 5. Some Synthetic Rubbers

Rubber	Common trade names	Basic structure
Polychloroprene	Neoprene Perbunan C Butachlor Nairit	Poly (2-chlor-1,8 butadiene)
SBR (styrene butadiene rubber)	Buna S Plioflex Intol Krylene	Copolymer of styrene and 1,4 butadiene
Nitrile	Buna N Chemigum N Paracril Perbunan Hycar	Copolymer of acrylonitrile and 1,4 butadiene
EPDM (ethylene propylene diene methylene)	Nordel Royalene Vistalon Dutral Keltan Intolan	Copolymer of ethylene and propylene with a third monomer such as ethylidene norbonene, cyclopentadiene
Butyl	GR-1 Bucar Socabutyl	Copolymer of isobutylene and isoprene
Silicone	Silastic Silastomer Sil-O-Flex	Poly(dimethyl siloxane), poly(methyl vinyl siloxane), etc.
Fluoroelastomer	Viton Technoflon Fluorel	Copolymer of hexafluoropropylene and vinylidene fluoride

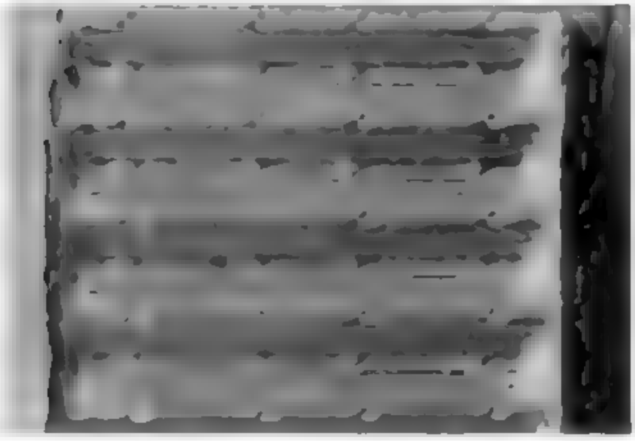


Figure 23. Catastrophic failure of the gasket groove of a heat-exchanger plate by stress corrosion cracking caused by the use of a polychloroprene based adhesive.

sulation is as important as that on all other items of plant and equipment.

It will be appreciated from all the foregoing that insulation of a vessel or pipeline is a composite activity with many interactive parameters. It is impossible to lay down specification rules, as each case much be viewed in the light of requirements. These notes must, therefore, be regarded as guidelines rather than dogma, and for further information, an excellent publication by the American Society for Testing Materials (21) is essential reading.

CORROSION OF RUBBERS

Many involved in the field of corrosion would not regard the deterioration of rubber as a corrosion process. The author's opinion differs from this viewpoint as "rubber undergoes deterioration by interaction with its environment."

General

Rubber and rubber components form an essential part of food processing equipment—joint rings on pipelines, gaskets on heat exchangers, and plate evaporators. Although natural rubber was the first material to be used for manufacturing these components, nowadays they are made almost exclusively from one of the synthetic rubbers listed in Table 5.

Unlike metals and alloys, which have a strictly defined composition, the constituents used in the formulation of rubbers are rarely stipulated. More often, they will reflect the views and idiosyncrasies of the formulator on how to achieve the desired end product. The important constituents of a rubber are

- *Basic Polymer.* Largely determines the general chemical properties of the finished product.
- *Reinforcing Fillers.* These are added to improve the mechanical properties and will invariably be one of the grades of carbon black—or if a white rubber is required, mineral fillers such as clays or calcium silicate.
- *Vulcanizing Agents.* These cross-link the basic polymer and impart rubberlike properties that are maintained at elevated temperatures.
- *Antioxidants.* To stabilize the rubber against oxidative degradation, hardening or softening, after prolonged operating periods at elevated temperatures.
- *Processing Aids.* Which facilitate the molding of the rubber.
- *Plasticizers.* To modify the mechanical properties.

A complicating factor to be considered when formulating rubber for food-contact surfaces is the acceptability (or nonacceptability) of the compounding ingredients. Some countries, notably Germany and the USA, have drawn up lists of permitted ingredients (22,23), while other countries regulate the amount of material that can be extracted from the finished article by various test media.

Invoking these regulations may impose limits on the in-service performance of a rubber component, which could be a compromise, exhibiting desirable properties inferior to those achievable were it for a nonfood application. For example, the resistance to high pressure steam of some rubbers can be enhanced by using lead oxide as an ingredient. Obviously, such materials could not be contemplated for any food contact application.

Table 6. Performance of Rubbers in Some Environments Found in the Food and Beverage Industries

	SBR	Medium nitrile	Natural rubber	Polychloroprene	Butyl	EPDM	Silicone[a]
			Products				
Whole milk	E	E	E	E	E	E	E
Beer, wines, spirits	G-F	E	F	E	E	E	E
Fats, oils, cream	F	E	F	G	P	G	E
Sauces	E	E	F[b]	E	E[b]	E	E
Salad dressings	F	E	F	G	P	G	E
Fruit drinks and juices[d]	E	E	G	E	E	E	E
			Cleaning Agents				
Sodium hydroxide[c]	G	E	G	G	E	E	E
Sodium carbonate[f]	E	E	E	E	E	E	E
Sodium hypochlorite[g]	G	G	G	G	E	E	E
Nitric acid[h]	F	F	P	F	G	G	G
Phosphoric acid[i]	E	E	E	E	E	E	E
Quaternary ammonium compounds[e]	E	E	E	E	G	G	G

[a] Depending on the type of basic polymer.

[b] Depending on the fat/oil content.

[c] Used as an aqueous 1% solution.

[d] The performance of a rubber will be affected by the presence of essential oils.

[e] All strengths up to 5% at maximum operating temperatures of the rubber

[f] Sodium carbonate and other detergent/additives, eg, sodium phosphate, silicate.

[g] Sodium hypochlorite as used at normal sterilizing concentration—150 mg/L.

[h] Nitric acid as used at normal cleaning strength of 1/2-1%.

[i] Up to 5% strength.

Table 7. Suggested Limits of Concentration and Temperature for Peracetic Acid in Contact with Rubber

Peracetic acid concentration (active ingredient)	Temp. (°C)	Medium nitrile	Butyl (resin-cured)	EPDM	Silicon	Fluoro-elastomer
0.05% (500 mg/L)	20	R[a]	R	R	R	R
	60	R	R	R	R	R
	85	(?)	R	R	R	(?)[b]
0.10% (1 g/L)	20	R	R	R	R	R
	60	NR[c]	R	R	NR	R
	85	NR	(?)	R	NR	NR
0.25% (2.5 g/L)	20	R	R	R	(?)	R
	60	NR	R	R	NR	R
	85	NR	NR	R	NR	NR
0.5% (5 g/L)	20		R	R	NR	R
	60	NR	R	R	NR	R
	85	NR	NR	R	NR	NR

[a] R—Little effect on the rubber

[b] (?)—Possibly some degradation.

[c] NR—Not recommended as significant degradation may occur.

Corrosion by Rubber

The majority of rubbers and formulating ingredients have no effect on stainless steels even under conditions of high temperature and moisture. There are two notable exceptions, namely, polychloroprene and chlorosulfonated polyethylene. Both of these contain chlorine, which under the influence of temperature and moisture, undergo hydrolysis to produce small quantities of hydrochloric acid. In contact with stainless steel, this represents a serious corrosion hazard causing the three main forms of attack.

When specifying a rubber component, it is easy to avoid these two polymers but a fact not often appreciated is that many of the rubber adhesives are produced from one of these polymers. That is the reason why manufacturers of heat exchangers and other equipment, which necessitates sticking the rubber onto metal, specify what type of adhesive should be used. Many DIY adhesives and contact adhesives are formulated from polychloroprene, and it is not unknown for a maintenance engineer having to stick gaskets in a heat exchanger to get the supply of rubber cement from the local hardware shop. The results can be catastrophic, as shown in Figure 23. Similarly, many self adhesive tapes used as a polychloroprene-based adhesive and direct contact of these with stainless steels should be avoided.

Corrosion of Rubber by Environments

From the standpoint of food processing, the environments likely to interact with rubber are classified under the following headings:

- Foodstuffs containing no fat or a low level of fat, eg, milk
- Fatty products: butter, cream, cooking oils, shortenings
- Alkaline detergents
- Acid detergents
- Sanitizing agents

Unlike the corrosion of metals, which is associated with oxidation and loss of metal, rubber deterioration usually takes other forms. When a rubber is immersed in a liquid, it absorbs that liquid or substances present in it to a greater or lesser degree. The amount of absorption determines whether the rubber is compatible with the environment. The absorption will be accompanied by changes in mass, volume, hardness, and tensile strength. For example, immersing and oil resistant rubber in vegetable oil may produce a change in volume of only 2–3%, whereas a non-oil-resistant rubber may swell by 150% or more. Such a volumetric change will be accompanied by a large reduction in the tensile strength and a high degree of softening.

Broadly, speaking, a rubber should not exhibit a volumetric or weight change greater than 10% nor a hardness change of more than 10 degrees (International Rubber Hardness Degrees—IRHD or Shore A) to be classed as compatible. For general guidance, data presented in Tables 6 and 7 (19) indicate the compatibility of rubbers with some food industry environments. But for more information, the reader should refer to one of the national or international test procedures (24–26).

BIBLIOGRAPHY

Adapted from: C. T. Cowan, "Materials Science and Corrosion Prevention for Food Processing Equipment," *APV Corrosion Handbook*, March 1990, 44 pp.
Copyrighted APV Crepaco, Inc. Used with permission.

1. N Warren, *Metal Corrosion in Boats*, London Stanford Maritime, 1980.
2. A. J. Sedricks, *Corrosion of Stainless Steels*, Wiley, New York, 1979.
3. The British Aluminium Co., Ltd., *Aluminium in the Chemical and Food Industries*, 1951 (London: The British Aluminium Co., Ltd.)
4. G. Ferry, *New Scientist*, 27 Feb. 1986, 23.
5. G. A. Dummett, *From Little Acorns, A History of the APV Company*, Hutchison Benham, London, 1981.
6. C. T. Cowan, "Corrosion Engineering with New Materials," *The Brewer* 163–168 (1985).
7. M. G. Fontana, *Corrosion Engineering*, McGraw-Hill, New York, 1986.
8. L. L. Shrier, *Corrosion*, Newnes-Butterworth, London, 1976.
9. J. W. Oldfield and B. Todd, Transactions of the Institute of Marine Engineering (C), 1984, Conference 1, 139.
10. J. W. Oldfield, *Test Techniques for Pitting and Crevice Corrosion of Stainless Steels and Nickel Based Alloys in Chloride-Containing Environments*, NiDi Technical Series 10 016, 1987.
11. U. R. Evans, *Corrosion* 7, 238 (1981).
12. ASTM G48-76, *Standard Methods for Pitting and Crevice Corrosion Resistance of Stainless Steels and Related Alloys by Use of Ferric Chloride Solution*, American Society for Testing Materials, Philadelphia.
13. R. J. Brigham, *Materials Performance* 13, 29 (Nov. 1974).
14. N. D Greene and M G. Fontana, *Corrosion* 15, 25t (1959).
15. M. Woelful and R. Mulhall, *Metal Progress*, 57–59 Sept. 1982.
16. M. R. Copson, "Effect of Composition on SCC of Some Alloys Containing Nickel," in T Rhodin, ed., *Physical Metallurgy of Stress Corrosion Fracture*, Interscience Publishers, New York, 1989.
17. L. H. Boulton and M. M Sorensen, *New Zealand Journal of Dairy Science & Technology* 23, 37–49 (1988).
18. G. M. Tastayre and R. A. Holley, Publication 1806/B. Agriculture Canada, Ottawa, 1986.
19. IDF Bulletin 236, *Corrosion by Peracetic Acid Solutions*, International Dairy Federation, 41 Square Vergote, 1040 Brussels, Belgium.
20. BS 5305 Code of practice for cleaning and disinfecting of plant and equipment used in the dairying industry, British Standards Institute, London.
21. ASTM Special Technical Publication 880. Corrosion of Metals under Thermal Insulation. American Society for Testing Materials, Philadelphia.
22. Sonderdruck aus Bundesgesundheitsblatt, 22.1,79, West Germany.
23. USA *Code of Federal Regulations*. Title 21. Food and Drugs Section 177.2600 Rubber Articles Intended for Repeated Use.
24. BS 903 Part A16, Resistance of Vulcanized Rubbers to Liquids, British Standards Institution, London.
25. ASTM D. 471, Testing of Rubbers and Elastomers. Determination of Resistance to Liquids, Vapors, and Gases, American Society for Testing Materials, Philadelphia.
26. ISO 1817, Vulcanized Rubbers—Resistance to Liquids—Methods of Test.

APV Crepaco, Inc.
Lake Mills, Wisconsin

CRABS AND CRAB PROCESSING

Crabs play an important part in the U.S. fishing industry. Ex-vessel landings and values usually rank low in quantity (Fig. 1) and high in value (Fig. 2). The quantity and value of the major crab fishes in the United States in 1996 are shown in Table 1 (1). Variations in processing methods for crabs are related to the species and the seasonal characteristics that affect yield, ease of meat removal, and color. Speed in handling the product from time of harvesting to freezing or canning is a most important process requirement (2,3). Quality control procedures generally emphasize sanitation, food regulatory requirements, and compliance with end-product specifications of the processor (4).

Corrosion of Rubber by Environments

From the standpoint of food processing, the environments likely to interact with rubber are classified under the following headings:

- Foodstuffs containing no fat or a low level of fat, eg, milk
- Fatty products: butter, cream, cooking oils, shortenings
- Alkaline detergents
- Acid detergents
- Sanitizing agents

Unlike the corrosion of metals, which is associated with oxidation and loss of metal, rubber deterioration usually takes other forms. When a rubber is immersed in a liquid, it absorbs that liquid or substances present in it to a greater or lesser degree. The amount of absorption determines whether the rubber is compatible with the environment. The absorption will be accompanied by changes in mass, volume, hardness, and tensile strength. For example, immersing and oil resistant rubber in vegetable oil may produce a change in volume of only 2–3%, whereas a non-oil-resistant rubber may swell by 150% or more. Such a volumetric change will be accompanied by a large reduction in the tensile strength and a high degree of softening.

Broadly, speaking, a rubber should not exhibit a volumetric or weight change greater than 10% nor a hardness change of more than 10 degrees (International Rubber Hardness Degrees—IRHD or Shore A) to be classed as compatible. For general guidance, data presented in Tables 6 and 7 (19) indicate the compatibility of rubbers with some food industry environments. But for more information, the reader should refer to one of the national or international test procedures (24–26).

BIBLIOGRAPHY

Adapted from: C. T. Cowan, "Materials Science and Corrosion Prevention for Food Processing Equipment," *APV Corrosion Handbook*, March 1990, 44 pp.
Copyrighted APV Crepaco, Inc. Used with permission.

1. N Warren, *Metal Corrosion in Boats*, London Stanford Maritime, 1980.
2. A. J. Sedricks, *Corrosion of Stainless Steels*, Wiley, New York, 1979.
3. The British Aluminium Co., Ltd., *Aluminium in the Chemical and Food Industries*, 1951 (London: The British Aluminium Co., Ltd.)
4. G. Ferry, *New Scientist*, 27 Feb. 1986, 23.
5. G. A. Dummett, *From Little Acorns, A History of the APV Company*, Hutchison Benham, London, 1981.
6. C. T. Cowan, "Corrosion Engineering with New Materials," *The Brewer* 163–168 (1985).
7. M. G. Fontana, *Corrosion Engineering*, McGraw-Hill, New York, 1986.
8. L. L. Shrier, *Corrosion*, Newnes-Butterworth, London, 1976.
9. J. W. Oldfield and B. Todd, Transactions of the Institute of Marine Engineering (C), 1984, Conference 1, 139.
10. J. W. Oldfield, *Test Techniques for Pitting and Crevice Corrosion of Stainless Steels and Nickel Based Alloys in Chloride-Containing Environments*, NiDi Technical Series 10 016, 1987.
11. U. R. Evans, *Corrosion* 7, 238 (1981).
12. ASTM G48-76, *Standard Methods for Pitting and Crevice Corrosion Resistance of Stainless Steels and Related Alloys by Use of Ferric Chloride Solution*, American Society for Testing Materials, Philadelphia.
13. R. J. Brigham, *Materials Performance* 13, 29 (Nov. 1974).
14. N. D Greene and M G. Fontana, *Corrosion* 15, 25t (1959).
15. M. Woelful and R. Mulhall, *Metal Progress*, 57–59 Sept. 1982.
16. M. R. Copson, "Effect of Composition on SCC of Some Alloys Containing Nickel," in T Rhodin, ed., *Physical Metallurgy of Stress Corrosion Fracture*, Interscience Publishers, New York, 1989.
17. L. H. Boulton and M. M Sorensen, *New Zealand Journal of Dairy Science & Technology* 23, 37–49 (1988).
18. G. M. Tastayre and R. A. Holley, Publication 1806/B. Agriculture Canada, Ottawa, 1986.
19. IDF Bulletin 236, *Corrosion by Peracetic Acid Solutions*, International Dairy Federation, 41 Square Vergote, 1040 Brussels, Belgium.
20. BS 5305 Code of practice for cleaning and disinfecting of plant and equipment used in the dairying industry, British Standards Institute, London.
21. ASTM Special Technical Publication 880. Corrosion of Metals under Thermal Insulation. American Society for Testing Materials, Philadelphia.
22. Sonderdruck aus Bundesgesundheitsblatt, 22.1,79, West Germany.
23. USA *Code of Federal Regulations*. Title 21. Food and Drugs Section 177.2600 Rubber Articles Intended for Repeated Use.
24. BS 903 Part A16, Resistance of Vulcanized Rubbers to Liquids, British Standards Institution, London.
25. ASTM D. 471, Testing of Rubbers and Elastomers. Determination of Resistance to Liquids, Vapors, and Gases, American Society for Testing Materials, Philadelphia.
26. ISO 1817, Vulcanized Rubbers—Resistance to Liquids—Methods of Test.

APV Crepaco, Inc.
Lake Mills, Wisconsin

CRABS AND CRAB PROCESSING

Crabs play an important part in the U.S. fishing industry. Ex-vessel landings and values usually rank low in quantity (Fig. 1) and high in value (Fig. 2). The quantity and value of the major crab fishes in the United States in 1996 are shown in Table 1 (1). Variations in processing methods for crabs are related to the species and the seasonal characteristics that affect yield, ease of meat removal, and color. Speed in handling the product from time of harvesting to freezing or canning is a most important process requirement (2,3). Quality control procedures generally emphasize sanitation, food regulatory requirements, and compliance with end-product specifications of the processor (4).

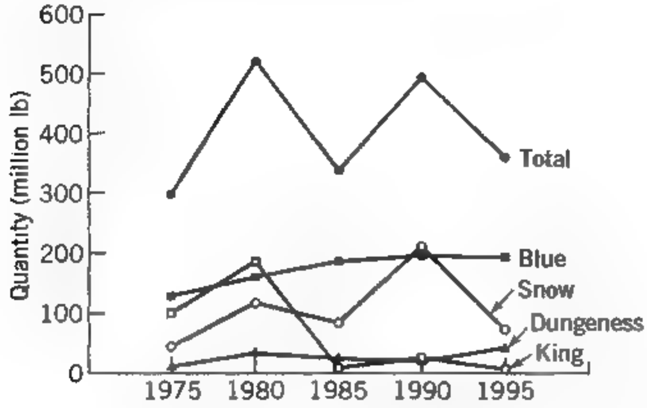


Figure 1. Landings of U.S. crab species.

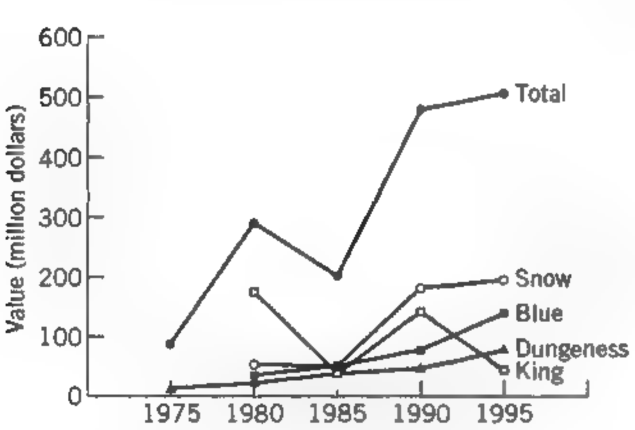


Figure 2. Value of U.S. crab species.

Table 1. Landings and Values of U.S. Crab Species in 1996

	Quantity (million lb)	Value (million $)	Average ex-vessel price ($/lb)
Blue	219.0	147.1	0.67
Chesapeake	68.3		
South Atlantic	86.6		
Gulf	56.9		
Middle Atlantic	7.2		
Dungeness	65.0	87.9	1.35
Washington	27.5		
Alaska	5.9		
Oregon	19.3		
California	12.3		
King			
Alaska	21.0	62.6	2.98
Snow			
Alaska	67.9	93.2	1.37

KING CRAB

Three species of king crab harvested in the North Pacific and Bering Sea are of commercial importance. The most important species is red king crab (*Paralithodes camtschatica*). Blue king crab (*P. platypus*) is caught in commercial quantities primarily near the Pribilof Islands. Brown or golden king crab (*Lithodes aequispina*) are found in deeper waters at the edge of the continental shelf.

King crabs are actually not "true crabs" like dungeness, tanner, or blue crab, but are more closely related to an order of hermit crabs. The legs of true crabs are jointed forward, whereas the legs of king crabs are jointed to fold behind its body (5).

Harvesting

The world's most extensive king crab populations dwell in Alaskan waters, where 15-year-old male king crabs may grow to a weight of 24 lb and a leg span of 6 ft. Most of the commercial catch consists of 7- or 8-year-old crabs that weigh about 6 lb. They are captured by means of large pots consisting of iron frames about 6 ft square and 3 ft high covered with synthetic webbing. These pots may weigh between 300 and 700 lb apiece. The pots are baited with fresh fish (herring is the most preferred) and then dropped to the bottom at 60 to 300 ft depth with a heavy line and marker buoy. The pots are lifted every few days and the male crabs of legal size are kept. The crabs are held alive on the vessel in a well with circulating seawater. At the docks or on the deck of a processor vessel, the live crabs are transferred to seawater tanks to await processing. Weak crabs may be sorted out and processed immediately, but generally all dead and injured crabs are discarded.

Processing

The crab is butchered by use of a stationary iron blade. The back, or carapace, is removed and the crab is split into halves, each with legs attached, called sections. A quick shake will jar out viscera that cling to the body cavities. Revolving nylon brushes are generally used to clean off gills and viscera.

Batch cooking is common, although a continuous cooker requires less labor, and cooking is done without the delay of filling a basket. It also has automatic steam controls and a fixed rate of travel through the tank, ensuring identical cooking. For crab planned for frozen sales, the sections are cooked for 22 to 28 min in either boiling fresh water or seawater. Cook times will vary slightly, but cooking should be continued to ensure that the internal temperature of the crab meat reaches at least 88°C (190 °F).

For canned or retorted products, crab need only be cooked to make meat removal easy. A two-stage cook of 20 min at 60°C (140 °F), rinsing the sections well with a spray of water followed by cooking in boiling water for 12 min, has been proposed (6). This prevents a blue discoloration in canned Kegani crab since the crabmeat is coagulated during the precook while the blood (hemocyanin) can be washed free of the meat. Regardless of cooking method, cooked crab meat should be picked and thermally processed as rapidly as possible to ensure an excellent canned product (7). Normally, canned crabmeat is packed in 307 by 113 C-enamel cans, sealed in a vacuum, retorted for 50 min at 116°C (240°F), then water cooled (8).

For freezing, the sections are cooled in cold running water after cooking. Ideally, chilled water is used to cool the sections to at least 4°C (40°F). Usually, sections are then frozen either in brine or air-blast freezers. The majority of Alaskan king crab production is brine-frozen. The sections are packed with the legs stacked in the center of baskets, shoulders out, and held in liquid brine (22–23%) at a temperature of −18 to −20°C (0 to −5°F) for 30 to 60 min. Internal temperature should reach at least 15°F. The sections are then removed from the brine and dipped in cold fresh water to rinse the salt from the section and to form a protective glaze on the surface of the crab. Blast freezing freezes the product in air by blowing refrigerated air (less than −30°C or −20°F) over it. Proponents of blast freezing contend the method results in a product with a lower sodium content. Brine freezing, if carefully controlled, will be similar to blast freezing, and some feel it results in a moister product.

Product Forms

Almost all of Alaska's king crab is sold frozen. Frozen king crab is packed in a variety of product forms, including both in-shell products and extracted crab meat. The most common king crab products include the following.

Bulk Clusters (Sections). These are packs of shoulders, legs, and claws, commonly packed in 80-lb random weight cartons. The pack should average about 75% legs and 25% shoulder-body meat by weight.

Legs and Claws. These packages are available in a number of forms, including both whole legs and claws or split legs and claws. King crab have six walking legs, two of which have claws. Packs of legs and claws should be in equivalent proportion to the live crab and should include the whole leg from shoulder to walking tip or claw. As a general rule, the larger the leg the higher the price.

King Crab Meat. The body, leg, and claw meats are removed by shaking or by blowing with water under pressure. After the shoulder-body meats are removed, each leg is broken just below the uppermost joint and the two connective tendons that run through the leg meat are removed by pulling the remaining leg sections free. This step is repeated for each leg segment, allowing the leg meats to be removed intact. For larger industrial processes, the legs are divided at the joints, in some cases, by use of a band saw, and fed into two large rubber rollers. The rollers are adjusted for proper clearance and are continuously rotated so that the shell is squeezed as it passes through and the meat is forced out. Meat yield averages about 20% but may be 26% or more by weight of the live crabs (9,10).

The meats are washed, sorted, inspected for shell and debris, and packed into cartons or trays for freezing. King crab meat is available in a number of forms including:

Merus meat. The red-colored merus meat is the largest segment of a king crab's leg and the most expensive. It is available in both blocks and individually quick frozen (IFQ) in poly bags.

Fancy meat. The most common pack of king crab meat and consists of a mixture of merus meat (25–35%), shoulder-body meat (40–50%), and red leg meat (30–35 %). The blocks of meat are packed in triple layers beginning with the merus meat, then white meat, finally red leg meat, and then frozen. The higher the percentage of leg meat in the block, the higher the price.

Salad meat. Salad meat is a cheaper frozen pack of small chunks of both red and white meat.

Rice meat. The cheapest form of king crab meat is rice meat, which consists of uniformly shredded body and leg meats.

King crab that is adequately protected from dehydration can be held for six months or more at −18 to −23°C (0 to −10°F).

SNOW CRAB

Commonly referred to as "tanner crab," the two crab species, *Chionoecetes bairdi* and *C. opilio*, were relatively unknown in the United States until the king crab stocks began to decline. Today, these crab species are marketed as snow crab and have emerged as the most valued of the crabs harvested in the United States (1). *C. bairdi*, the larger and thus more desirable of the two snow crab species, is caught only in the North Pacific and Bering Sea. The smaller species, *C. opilio*, is caught in the North Atlantic as well. Pacific *C. opilio* is caught primarily by Alaskan fishermen while Atlantic *C. opilio*, which is slightly smaller, is caught by Canadians. Canadian snow crab may be marketed as queen crab (5).

Snow crabs are members of the spider crab family. In the North Pacific, they occur broadly from the southeast coast of Alaska westward along the coast throughout the Aleutian Islands and the Bering Sea. Snow crabs are caught in the winter and spring by the same boats that fish for king crab in the fall. Although only half the size of king crabs, snow crabs are still larger than most other species of crab. The largest, *C. bairdi*, occasionally exceed 3 lb and have a leg span of 2 ft.

Alaskan snow crab are harvested and processed in approximately the same manner as king crab and by the same fishers and processors. Snow crab is almost always marketed frozen and the product forms are similar to king crab; that is, clusters, claws, legs, and meats (fancy, merus, salad, and shredded). The yield of picked meats from snow crab is slightly lower than for king crab and averages 17%, ranging from 12 to 25% (9,11). Like fancy meat from king crab, frozen blocks of fancy snow crab meat consist of three layers: red meat (45%), white meat (35–45%), and shreds (10–20%). Snow crab has the same handling characteristics as king crab. Like king crab, care should be taken to ensure the product is well protected by proper glazing and packaging in order to prevent dehydration and subsequent quality loss during frozen storage.

A snow crab product that is popular in the Japanese market is called green crab, which is raw frozen snow crab sections. It is very important to handle, process, and freeze the snow crab quickly and carefully because of the tendency for a bluish to black discoloration to develop in the sections. Although there is no general agreement on the cause(s) for the discoloration (12), it may be the result of the enzymatically mediated oxidation and polymerization of phenolic compounds naturally present in crab (2). Thus antioxidants, sulfites, and chelating agents have been used to block the bluing discoloration. However, speed or quick processing is the best assurance in preventing the bluing, which will still occur and even develop at a faster rate after the frozen raw crab section is thawed. If crab are not cooked properly to destroy these enzymes, then the crab should be further processed quickly or consumed soon after thawing.

DUNGENESS CRAB

Dungeness (*Cancer magister*) crabs inhabit sandy and grassy bottoms along the Pacific Coast from California to the Aleutian Islands in Alaska. They are found at various depths below the intertidal zone but are usually harvested commercially offshore in depths from 12 to 120 ft. Dungeness crabs can grow to a weight of more than 3 lb; however, the commercial catch usually ranges between 1.25 and 2.25 lb. They are harvested with circular Iron pots covered with wire mesh, 3 ft in diameter and constructed with two entrance tunnels on the sides. Pots are hauled up every day or two, the legal-size male crabs are placed in seawater wells in the boat either dry or flooded, and the crabs are delivered alive at the plant. Only live, vigorous crabs are processed (13).

Product Forms

Dungeness crabs are marketed in a variety of forms, although the current trend is more toward whole crabs, both live and cooked.

Live Crab. Live Dungeness are commonly air freighted in 50-lb wetlock boxes (14). As with snow crab, the live crab may be packed in sawdust that has been saturated with water.

Whole-Cooked Crab. For Dungeness crab planned for fresh sales, whole crab are cooked for 20 to 25 min in salted (4–5%) boiling water in stainless steel batch or continuous cookers. Adding citric acid (0.1–0.2%) to the saltwater may enhance the color of the cooked crab by bleaching the surface of the shell, creating a whiter color. The hot crabs are cooled in cold water and, if the crab are to be sold fresh, packed in ice immediately. Properly handled, the shelf life should be up to seven days. If not sold fresh, the crab is frozen as soon after cooking as possible. Brine freezing has become very popular, but any rapid freezing system can be as good if care is taken to avoid any loss of quality from dehydration.

Meat. Dungeness crab sections intended for picking are prepared in the same manner as king crab and generally cooked in fresh (unsalted) boiling water for 10 to 12 min in either stainless steel batch or continuous cookers. After cooling the sections in cold water, the meat is removed from the crab section by hand. The steps in meat extraction have been well documented (15). Generally, while holding the legs of a section with one hand, the body is pressed with the free hand against a stainless steel table and then the key bone is removed. This step frees the body meat, which can be dislodged from the shell by striking the section and hand vigorously against the side of an aluminum or stainless steel pan. Then, the shell associated with the body is broken free of the legs just below the joint of each leg. Using a small metal mallet and anvil the top section of each leg is hit to crack the shell. The leg meat is then removed by striking the leg against the pan. This step is repeated at each joint to remove the leg meat Body and leg meat are usually kept separate. The meat is placed in a strong salt brine (25% sodium chloride solution) to separate the bits of shell from the meat. The meat floats to the top of the tank and is conveyed out of the tank, where it is immediately inspected under ultraviolet light (shell pieces will fluoresce), then washed with cold water sprays to remove excess salt.

The body and leg meats are weighed separately and packed in about equal proportions in #10 C-enamel cans holding 5 lb net weight of drained crab meat or in #2 cans holding 1 lb of meat. The cans are sealed under a low vacuum, frozen in a blast freezer, and stored at −18 to −23°C (0 to −10°F) or colder. Frozen Dungeness crab meat stores well only if protected by suitable packaging against dehydration and oxidation and is stored at −23°C (−10°F) or lower. Under these conditions it stores well for six months.

BLUE CRAB

Blue crabs (*Callinectes sapidus*) are found along the Atlantic and Gulf Coasts from Massachusetts to Texas. Essentially a shallow water crab, blue crabs live in bays, sounds, and channels near the mouths of coastal rivers. Blue crab, when fully grown, average 5 to 7 in. across the carapace. The top of the shell is generally green and the bottom is a whitish shade. The name blue crab comes from the coloring of the legs, of which there are five pairs. The hind legs, flattened at the outer ends, are used for swimming. At times, using their hind swimming legs, blue crab can swim beautifully through the water with great speed and ease.

Harvesting

Catching methods depend on season, regional preferences, and state regulations. Crabs are taken by crab pots, trotlines, dredges, scrapes, and dip nets. The trotline is a long length of rope with pieces of bait attached at intervals. It is laid on the bottom, ends anchored and marked with buoys. When the fisherman collects his catch, he runs his boat along the line, forcing it to pass over a roller attached to the boat. As the boat moves forward, the crabs cling to the bait until they reach the surface where they are caught with a dip net and placed in a basket or barrel. Fishing boats are usually small. Fishing is usually a one- or two-person operation, employing inexpensive gear. The pots used for blue crab are cubical in shape, 2 ft on each side, and covered with chicken wire. The pot consist of two parts: the crab enter the lower or bait compartment and then pass into the upper or trap compartment through a slit in the partition. The pots are lifted daily, and the crabs are removed from the top compartment.

Processing

At the plant, the crabs are weighed and dumped into baskets for cooking in vertical retorts. Cooking conditions vary based on type of equipment used, but most plants use steam at 121°C (250°F) for 9 to 20 minutes (4,16). A few plants use boiling water for 15 to 20 min. After cooling, many processors deback the cooked crabs, and then wash and refrigerate the crabs overnight before picking the meat.

The meat is picked primarily by hand and divided into three categories: the lump meat, which is the large muscle controlling the swimming legs; the regular or flake meat, which is the remainder of the meat from the body; and claw meat. Total meat yields consist of 50%, flake meat; 25%, lump meat; and 25%, claw meat and varies from 12 to 17% of the weight of the whole crab.

Blue crab meat does not freeze and store well; therefore, only a small volume of frozen meat is produced. Most of the meat is packed in hermetically sealed cans or plastic bags and sold in the fresh chilled form. To extend the marketing period for the chilled product from several days to several weeks, a heat pasteurization process was developed and has been used successfully for 1-lb sealed cans of meat, which must be stored and distributed at 0°C (32°F) or as near to that as practicable (16).

Also, a popular and important product is the soft-shell crab, a product common only in the blue crab industry. Soft-shelled crabs are crabs that have just molted. They are considered a delicacy and bring higher prices. The entire body of a soft-shelled crab may be eaten after cooking. Because the optimal period for shipping soft crabs is so short, crab fishermen of the Atlantic and Gulf Coasts are by necessity close observers of the molting cycle. As the molt approaches, the peelers move from their normal foraging areas to shallow beds of marine grasses near the shoreline. The peelers are collected in pots or with trawl-like devices called scrapes and held in peeler floats, large floating containers. Immediately after molting, the now soft crab is removed, graded for size, and shipped in a refrigerated container to market. Occasionally, soft-shelled crabs are frozen. For freezing, the crabs may be held in refrigerated storage for two to three days, then are killed, eviscerated, washed, wrapped individually with parchment, packed in a carton, and frozen (4).

Handling Tips for Crab

A common problem with processed crab is a discoloration called bluing, although the actual color may range from light blue to blue-gray to black. Despite almost a century of study, general agreement on the causes of or cures for bluing in crab has not been reached (12). However, a scheme for bluing relating the discoloration to the enzymatically mediated oxidation and polymerization of phenolic compounds naturally present in crab has been proposed and can be used to illustrate the importance of quick and proper handling of crab (2).

Only crabs that are alive and in good condition should be processed. Crabs that have been held in a cooler (1–3°C) even for up to four days are edible if properly cooked. However, the chances of bluing occurring in the cooked crab are greatly increased even if proper cooking times and temperatures are used because the compounds causing the bluing have already been formed (2). Certainly, care must be taken to properly cook the crab, since underprocessing will not destroy the enzymes responsible for the bluing. Molting is a factor in the bluing reaction, since the concentration of phenolic compounds responsible for bluing is very high as they are involved in the formation of the new shell (sclerotization). So, during times of molting, extra care must be used in handling and processing crab to prevent bluing.

If the crab meat is to be thermal processed, the cooking time and temperatures of the raw crab are not as critical. However, as soon as the crab is cooked, the meat from the shell must be removed and thermal processed. Regardless of how long the live crab are cooked, any delay in thermal processing the crab meat will result in a higher incidence of bluing. Using frozen crab sections to produce canned crab meat will also increase the risk of bluing. Whole crab or crab sections should be thoroughly cooked before freezing to reduce the risk of bluing. Finally, avoid any contact of copper or iron with the crab since these metals can greatly intensify the bluing discoloration.

BIBLIOGRAPHY

1. M. C. Holliday and B. K. O'Bannon, eds., *Fisheries of the United States, 1988*, Current Fishery Statistics No. 8800, U.S. Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Washington, D.C., 1990.
2. J. K. Babbitt, "Blueing Discoloration of Dungeness Crabmeat," in R. E. Martin et al., eds., *Chemistry and Biochemistry of Marine Food Products*, AVI Publishing, Westport, Conn., 1982, p. 423
3. E. B. Dewberry, "Speed is the Essence," *Food Processing Industry* **39**, 50–53 (1970).
4. J. A. Dassow, "Preparation for Freezing and Freezing of Shellfish," in D. K. Tressler and C. F. Evers, eds., *The Freezing Preservation of Foods*, 4th ed., Vol. 3. AVI Publishing, Westport, Conn., 1968, p. 266.
5. P. Redmayne, "Crab: King, Snow, and Dungeness," *Seafood Leader* **2**, 72 (1982).
6. I. Osakabe, "Low Cook Produces High Quality in Kegani Crab," *Pacific Fisherman* **55**, 48 (1957).
7. J. K. Babbitt, D. K. Law, and D. L. Crawford, "Effect of Precooking on Copper Content, Phenolic Content and Blueing of Canned Dungeness Crabmeat," *J. Food Sci.* **40**, 649 (1975).
8. National Food Processors Association, *Processes for Low-Acid Canned Foods in Metal Containers*, Bulletin 26-L, NFPA, Washington, D.C., 1976.
9. C. Crapo, B. Paust, and J. K. Babbitt, *Recoveries and Yields from Pacific Fish and Shellfish*, Marine Advisory Bulletin No. 37, Alaska Sea Grant College Program, Fairbanks, Alaska, 1988.
10. R. A. Krzeczkowski, R. D. Tenney, and C. Kelley, "Alaska King Crab: Fatty Acid Composition, Carotenoid Index and Proximate Analysis," *J. Food Sci.* **36**, 604 (1974).

11 R. A. Krzeczkowski and F. E. Stone, "Amino Acid, Fatty Acid and Proximate Composition of Snow Crab. (*Chionoecetes bairdi*)," *J. Food Sci.* **39**, 386 (1974).

12. D. D. Boon, "Discoloration in Processed Crabmeat. A Review," *J. Food Sci.* **40**, 756 (1975).

13 J. K. Babbitt, *Improving the Quality of Commercially Processed Dungeness Crab*, Oregon State University Extension Marine Advisory Program SG65, Corvallis, Oreg., 1981.

14 H. J Barnett, R. W. Nelson, and P. J. Hunter, "Shipping Live Dungeness Crabs by Air to Retail Market," *Commercial Fisheries Review* **31**, 21 (1969).

15. B. Engesser, *Crab Meat Extraction* [training film], College of Engineering, Oregon State University, Corvallis, Oreg., 1972.

16. T. M. Miller, N. B. Webb, and F. B. Thomas, *Technical Operations Manual for the Blue Crab Industry*, University of North Carolina Sea Grant Publication UNC-SG-74-12, Special Scientific Report No. 28, Raleigh, N.C., 1974.

Jerry K. Babbitt
National Marine Fisheries Service
Kodiak, Alaska

CRANBERRIES. See Fruits, temperate.

CULTURAL NUTRITION

A LITERAL MELTING POT

Most of us can easily answer the question: What are your favorite foods? Generally, we respond with tastes developed during childhood. Basic food habits usually prove resistant to change.

Despite their food habits, Americans have a wide variety of food tastes. This variety is due partly to the significant mobility of Americans, partly to a wide variety of choices, and partly to such trends as eating at restaurants and eating ethnic. It is considered stylish to sample the cuisines of other countries.

At home, though, we usually prefer less variation. Our food patterns tend to follow family history—and especially what our parents liked and disliked. Where an ethnic community is established, food habits can become readily apparent. Especially in major cities, it is not uncommon to find neighborhood grocery stores that have shelves of food catering to ethnic tastes of a significant segment of the surrounding community.

Personal food patterns become especially important when we are old and when we are ill. Success in encouraging such patients to eat often depends on including their special preferences in their diets. This article addresses food patterns of people in the United States of different regions, ethnic origins, cultural backgrounds, religious backgrounds, and life-styles.

REGIONAL DIFFERENCES

For many years, obvious regional food patterns could be found in the United States. Those pronounced differences have faded considerably as techniques for processing, storing, and transporting foods have advanced. National advertising has had its effects, as has the mobility of our population.

In certain parts of the country, though, remnants of regional food preference persist. Such preferences often reflect availability that has spawned popularity. For example, seafood enjoys popularity in coastal areas because of ready availability, lower cost that reflects lower transportation expense, and local industry advertising. Preference is thus influenced by climate, geography, and economics.

In California, the state's agricultural richness affects preference, as do ethnic demographics—characteristics of human populations. California's large quantities of fruits and vegetables, the popularization of the salad as a part of the meal, and the large numbers of Mexican-Americans, Orientals, and Italians all combine to give California its distinct cuisine.

In the southern states, corn, fish, and rice are often used. Hot breads, such as biscuits or corn bread, accompany meals. Green leafy vegetables are cooked with a piece of fatback (pork back fat), a regional practice that yields a consumable liquid known as pot liquor. Before mechanical refrigeration was developed, milk and cheese were consumed only in small quantities; storage and shipping were not possible. The national consumption of dairy products has shown an increase since the 1940s and 1950s.

ETHNIC, CULTURAL, AND RELIGIOUS INFLUENCES

Each ethnic, cultural, and religious group has certain characteristic food patterns or preferences. Sometimes these characteristics remain unique to the group, but more often they become a part of our nation. In that regard the United States can be described as a melting pot, both figuratively and literally. The remaining part of this article explores such influences.

Europeans

Many of the foods we have come to think of as typically American were in fact brought to us from European countries. Norwegian, Swedish, and Danish immigrants brought a greater use of milk, numerous cheeses, cream, and butter. In their native countries, Western Europeans relied on fish, shellfish, and vegetables. Especially popular were potatoes, dark breads, eggs, cheese, beef, pork, and poultry.

Central Europeans favored potatoes, rye flour, wheat, pork, sausage, and cabbage. Cabbage was especially favored and was eaten raw, cooked, or salted as sauerkraut. Common vegetables included turnips, carrots, squash, onion, beans, and greens.

Italian in the United States have perpetuated many favorite foods of their ancestors and have concurrently made them basics of the American diet. The huge sale of pizza and pasta products and the number of Italian restaurants confirm this fact. There are nearly as many pizza stands in the United States as there are hamburger and fried chicken businesses. Daily, the typical Italian diet features pasta made from hard-wheat dough. Its innumerable forms are seasoned with sauces, onions, tomatoes, cheese, peppers, and meat. Bread usually accompanies the meal.

Differences exist between northern and southern Italian foods. The northern area of Italy is more industrialized, and meat, root vegetables, and dairy products are more popular there. Southern Italy's preference tends toward fish, spices, and olive oil. The southern Italian diet can benefit from more green vegetables, eggs, fruits, meat, and milk. The last two food items can especially benefit the nutrient intake of children.

Experienced dietitians and nurses know that hospitalization can be especially difficult for Italians who have a strong sense of family and a strong preference for traditional foods. Institutional diets that cater to Italian patients can both reduce feelings of isolation and increase nutritional intake.

Native Americans

It may come as a surprise that more than half the plant foods you eat—including corn, potatoes, squash, pumpkin, tomatoes, peppers, beans, wild rice, cranberries, and cocoa—come from North, Central, and South American Indians. The Native American diet also used acorns, wild fruit, fish, wild fowl, small and large game, and seafood. The food preservation methods that produce jerky and pemmican were introduced to the frontier Americans by the Native Americans. Corn, which has become a world staple, is a significant example of their culture's contribution.

Diet varied from tribe to tribe depending on geographic location. Historically, the natural resources of the tribe's home area determined such occupations as hunting, fishing, agriculture, and herding. Native Americans who lived near the coast used large amounts of seafood. Those in the Northwest were and still are great fishermen, and from Alaska to the Columbia River one of the most prized items is salmon. A favorite method of preserving fish and meat is smoking; racks of drying fish can be seen along the river banks where Native Americans live. Along the New England coast, shellfish is a favorite, especially clams. A favored method of cooking is open-air baking of clams and fish over hot coals. Sometimes seafood is baked in the sand. The item is wrapped in seaweed or similar materials and placed in sand kept hot by a fire.

In the past, the Plains Indians depended more on game, fowl, and freshwater fish. Native vegetables and fruits were generously used and cultivated if the group was a stationary, agricultural one. Many vegetables that originated in North and South America have become part of the American and world diet. Because little food storage was possible, nutritional life was characterized by the extremes of bounty and scarcity. Despite such fluctuations in availability, the historical Native American diet was undoubtedly more nutritional than is their diet today.

With the introduction of trading-post products such as lard, sugar, coffee, and canned meat and canned milk, the American Indian diet began to assimilate European food customs, leaving little today that can be distinguished as a Native American food pattern.

Native American food specialties that can be found today include clambakes, fry bread (biscuit dough fried in fat), and wajupi, a pudding made from berries, sugars, and cornstarch. Northern California Indians have revived the use of acorns in preparing soup and flour, methods of preparing eels, and stews combining meat, vegetables, and berries. Often these traditional foods play a part in tribal ceremonies. As cultural renewal has taken place among Native Americans, and as the Native American heritage is becoming better understood, interest in traditional Native American foods is being revived.

The nutritional weakness of the Native American diet is a low intake of milk by children after weaning. Vitamin C intake is also low, and the greater use of fruits, vegetables, and lean meat will improve the diet.

The present generation of Native Americans may be consuming too much sugar and soft drinks. Sound nutritional education is needed. Extensive alcohol abuse among some adult males has also hurt the nutritional status of these people.

Black Americans

Among middle- and upper-income blacks, food selections are similar to those of their white counterparts. Among poor families, many of whom have come from or still live in the southern states, the traditional eating pattern is determined to a large extent by the cost and availability of foods. However, there are many food items preferred by black families, rich or poor.

The popularization of the term *soul food* indicates the effort to recognize the uniqueness of black American food habits. One characteristic black food habit is the use of hominy grits, especially for breakfast with some form of pork. Corn bread, muffins, or biscuits are preferred to yeast bread. There are differences between the dietary habits of southern blacks and northern blacks, and one of these differences is in the customary use of greens.

Among southern blacks, fresh greens such as mustard, turnip, and collard are popular and are usually served with pork such as fatback or salt pork. In the north, fresh greens are less available, and a much wider variety of frozen greens are used. Other vegetable favorites include fresh corn, lima beans, cabbage, sweet potatoes, and squash. The use of fresh fruit emphasizes oranges, watermelon, and peaches (Table 1).

Black-eyed peas and corn are well liked, as indicated earlier; a mixture of black-eyed peas and rice called Hoppin John is a favorite southern dish. A favorite method of preparing green vegetables is to boil them with a small amount of fat pork; the resultant juice (pot liquor) is also used in the meal. Potatoes (white and sweet) and yams are popular, and sweet potatoes are often made into pies.

Carbohydrate is generally obtained from grits, potatoes, and rice, though black-eyed peas and dried beans are also used. The last-named provide some protein, as does fried fish, especially when locally caught. Other meats frequently used include poultry, pork (both cured and fresh), and wild game. All parts of an animal are used, especially among poor families. Dishes made from a slaughtered pig, for instance, include chitlings (the small intestines, cleaned and fried crisp); hog maws (the stomach lining); boiled pig's feet; and neckbone, jaws, snout, and head,

Table 1. Characteristic Black Americans' Food Choices

Protein foods	Milk and milk products	Grain products	Vegetables	Fruits	Other
Meat	Milk	Rice	Broccoli	Apples	Salt pork
Beef	Fluid	Corn bread	Cabbage	Bananas	Carbonated beverages
Pork and ham	Evaporated, in coffee	Hominy grits	Carrots	Grapefruit	Fruit drinks
Sausage	Buttermilk	Biscuits	Corn	Grapes	Gravies
Pig's feet, ears, etc.	Cheese	Muffins	Green beans	Nectarines	
Bacon	Cheddar	White bread	Greens	Oranges	
Luncheon meat	Cottage	Dry cereal	Mustard	Plums	
Organ meats	Ice cream	Cooked cereal	Collard	Tangerines	
Poultry		Macaroni	Kale	Watermelons	
Chicken		Spaghetti	Spinach		
Turkey		Crackers	Turnips, etc.		
Fish			Lima beans		
Catfish			Okra		
Perch			Peas		
Red snapper			Potatoes		
Tuna			Pumpkins		
Salmon			Sweet potatoes		
Sardines			Tomatoes		
Shrimp			Yams		
Eggs					
Legumes					
Kidney beans					
Red beans					
Pinto beans					
Black-eyed peas					
Nuts					
Peanuts					
Peanut butter					

Source: Ref. 1.

which are cooked and made into scrapple. Frying, stewing, and barbecuing are the favored methods of preparation. Sweets such as molasses, pastries, cakes, and candy are especially popular.

Milk consumption, which is often low, should be encouraged, especially among children. Buttermilk and ice cream are well liked by black Americans, although there is little use of cheeses. The use of more citrus fruits should be encouraged.

As the economic status of black people improves, they eat more meat and their diet improves generally. Because their typical diet is high in fat and carbohydrate and low in protein, iron, and vitamin C, an increase in consumption of lean meat, milk, and citrus fruits will help balance their nutrient intake.

It is in preparation that the essence of the term soul food can be found. It is not necessarily the unique foods but rather the care in preparation that is emphasized. Food preparation offers the opportunity to minister to the physical health and well-being of those who will consume the food. The opportunity to bring happiness, health, and love through food preparation lies at the figurative and literal heart of the customary black dietary habits.

Jews

Orthodox Jews follow Old Testament and rabbinical dietary laws. Conservative Jews distinguish between meals served in the home and those outside it. Reformed Jews do not observe dietary regulations. The country of origin can further influence a Jewish family's dietary practices.

Foods used according to strict Jewish laws are referred to as Kosher. Those who follow those dietary laws eat only animals that are designated as clean and are killed in a ritualistic manner. The method of slaughter minimizes pain and maximizes blood drainage. Blood, the symbol of life, is strictly avoided by soaking the meat in cold water, draining it, salting it, and rinsing it three times. Permitted meats include poultry, fish with fins and scales, and quadruped animals that chew the cud and have divided hooves. (Thus, pork cannot be eaten.) The hindquarter of quadruped animals must have the hip sinew or the thigh vein removed.

Kosher laws additionally require separation of meat and milk. Milk and its products must be excluded from meals involving meat but can be consumed before the meat meal. If a milk meal is to be consumed, meat and its products are excluded from the milk meal and for 6 hours thereafter. Usually two milk meals and one meat meal are eaten each day. A Kosher household will maintain two sets of dishes, utensils, and cooking supplies, one for meat meals and one for milk meals.

Certain foods, viewed as neutral, are referred to as pareve. Fruits, uncooked vegetables, eggs, and clean fish, because they are recognized as neutral, can be consumed with either meat or milk meals.

No food can be cooked or heated on Saturday, the Sabbath, so the evening meal preceding the Sabbath tends to the most substantial of the week. Both chicken and fish are served at that meal. Any foods eaten on the Sabbath must be cooked on a preceding day.

On Jewish holidays, symbolic foods are eaten. For example, Passover, a spring festival, commemorates the flight of the Jews from Egypt. Passover celebration lasts 8 days, and only unleavened bread, called matzo, is permitted. An Orthodox household will, therefore, use separate utensils for preparing unleavened bread to avoid any contact with leavening. Other holidays include Rosh Hashanah (Jewish New Year), Sukkoth (fall harvest), Chanukkah (the festival of lights), Purim (the arrival of spring), and Yom Kippur (the day of atonement) when fasting occurs.

Mexican-Americans

People of Mexican descent make up a large part of the population of the Southwest. They therefore reside close to their native country and maintain a close tie to traditional Mexican foods. A lack of refrigeration in such warmer climates creates a scarcity of meat, milk, eggs, vegetables, and fruits. For lower-income families, the expense of these nutrient-rich foods can be prohibitive.

Items basic to the Mexican diet include dry beans, chili peppers, tomatoes, and corn. The corn is ground, soaked in lime water, and baked on a griddle to make tortillas, the popular flat breads that can be rolled and filled with ground beef and vegetables to make tacos. Tacos provide nutrients from all four food groups, although the user of wheat tortillas reduces the calcium obtained from lime-soaked corn tortillas. Many foods are fried, and beans are even refried until they absorb all the fat.

Nutritionists encourage retention of the basic Mexican diet with only minor modifications. Because of iron, vitamin A, and calcium deficiencies, use of lean meat is recommended in taco fillings, dark green and yellow vegetables, fruits, and milk—dried if fresh milk is too expensive—are also recommended. The milk is especially important for children. One Mexican dietary practice that is particularly encouraged is the practice of limiting the amount of sweets consumed (Table 2).

Puerto Ricans

The Puerto Rican diet has similarities to the dietary patterns of other Caribbean Islands but is noteworthy because so many Puerto Ricans emigrate to the United States.

Traditional emphasis is on beans, rice, and starchy root vegetables (and plantains) known as viandas. Rice and beans eaten together are a high-quality, complementary protein combination. The beans are often boiled and then cooked with sofrito, a mixture made from tomatoes, green peppers, onions, garlic, salt pork, lard, and herbs. Viandas are good sources of B vitamins, iron calories, and, in some cases, vitamin C. Salt codfish is more popular than fresh fish. Chicken, beef, and pork are favored. Coffee with 2 to 5 oz of milk per cup—cafe con leche—is drunk several times a day, contributing to an otherwise low consumption of milk. Fruits prove an especially popular food owing to availability, with such native fruits as papaya, mango, and acerola (the West Indian cherry, which is extremely high in vitamin C) being joined by bananas, oranges, and pineapple.

The mainstays of the Puerto Rican diet may be hard to find or expensive in the United States. And because Puerto Ricans come from an agrarian background and thus often lack the job skills for a highly industrialized society, they often find themselves in the lowest socioeconomic classes in the United States, living in crowded urban conditions with poor cooking and refrigeration facilities and unable to feed their families as well as they did in Puerto Rico.

Table 2. Characteristic Mexican-American Food Choices

Protein foods	Milk and milk products	Grain products	Vegetables	Fruits	Other
Meat	Milk	Rice	Avocados	Apples	Salsa
Beef	Fluid	Tortillas	Cabbage	Apricots	(tomato-pepper-onion
Pork	Flavored	Corn	Carrots	Bananas	relish)
Lamb	Evaporated	Flour	Chilies	Guavas	Chili sauce
Tripe	Condensed	Oatmeal	Corn	Lemons	Guacamole
Sausage (chorizo)	Cheese	Dry cereals	Green beans	Mangos	Lard (manteca)
Bologna	American	Cornflakes	Lettuce	Melons	Pork cracklings
Bacon	Monterey jack	Sugar coated	Onions	Oranges	Fruit drinks
Poultry	Hoop	Noodles	Peas	Peaches	Kool-aid
Chicken	Ice cream	Spaghetti	Potatoes	Pears	Carbonated
Eggs		White bread	Prickly pear	Prickly pear	beverages
Legumes		Sweet bread	cactus leaf	cactus fruit	Beer
Pinto beans		(pan dulce)	(nopales)	(tuna)	Coffee
Pink beans			Spinach	Zapote	
Garbanzo beans			Sweet potatoes	(or sapote)	
Lentils			Tomatoes		
Nuts			Zucchini		
Peanuts					
Peanut butter					

Source: Ref. 1.

Consequently, malnutrition is not uncommon among their children. Also, Puerto Rican children born in the United States may have adopted favorite mainland foods such as hamburgers, hot dogs, canned spaghetti, and cold cereals instead of the traditional Puerto Rican food prepared by their mothers. If these children have picked up the mainland habit of snacking on nutrient-light foods, they may be more poorly nourished than people adhering to the relatively inexpensive but nutritionally adequate traditional diet of Puerto Rico.

Nutritionists encourage Puerto Ricans living in northern mainland cities to become familiar with different fruits, which are inexpensive when they are in season or canned; to use more milk, cheese, and inexpensive cuts of meat; and to substitute canned tomatoes for fresh tomatoes, which are expensive when not in season.

Middle Eastern People

Middle East people—Greeks, Iranians, Arabs, Turks, Armenians, and Lebanese—tend to be farmers. Dietary emphasis is on crops and animals raised—cattle, sheep, goats, chickens, ducks, geese, grains, fruits, and vegetables. Lamb holds the position of the favored meat, and wheat products, grains, and rice are the major energy sources. Popular dairy foods, derived from sheep, goats, and camels, are sour milk, including yogurt, fermented milk, and sour cream.

Lentils and beans may be boiled or stewed with tomatoes, onions, and olive oil; they may be eaten alone or mixed with other foods. A favorite combination is seasoned chick-peas mixed with bulgur (wheat that has been steamed, cracked, and fried) and spices and then fried in fat.

Traditional vegetables include okra, squash, tomatoes, onions, leeks, peppers, spinach, brussel sprouts, cabbage, peas, green beans, dandelion greens, eggplant, artichokes, and olives. Grape leaves, used either fresh or canned, are stuffed with rice, bulgur, meat, and seasoning.

Fruits grown in these warm climates are used extensively. Some common ones are dates, figs, melons, cherries, oranges, apricots, and raisins.

Common Middle Eastern cereals include rice, wheat, and barley. Typical breads are flat, thin, and round and may be baked outdoors. Olive oil and butter from sheep's and goat's milk are used generously. Turkish coffee, a favorite beverage in the Middle East, is a strong, dark drink containing the crushed coffee bean that is served with a

Table 3. Characteristic Chinese Food Choices

Protein foods	Milk and milk products	Grain products	Vegetables	Fruits	Other
Meat	Flavored milk	Rice	Bamboo shoots	Apples	Soy sauce
Pork	Milk (cooking)	Noodles	Beans	Bananas	Sweet and sour sauce
Beef	Ice cream	White bread	Green	Figs	
Organ meats		Barley	Yellow	Grapes	Mustard sauce
Poultry		Millet	Bean sprouts	Kumquats	Ginger
Chicken			Bok choy	Loquats	Plum sauce
Duck			Broccoli	Mangos	Red bean paste
Fish			Cabbage	Melons	Tea
White fish			Carrots	Oranges	Coffee
Shrimp			Celery	Peaches	
Lobster			Chinese cabbage	Pears	
Oyster			Corn	Persimmons	
Sardines			Cucumbers	Pineapples	
Eggs			Eggplant	Plums	
Legumes			Greens	Tangerines	
Soybeans			Collard		
Soybean curd (tofu)			Chinese broccoli		
Black beans			Mustard		
Nuts			Kale		
Peanuts			Spinach		
Almonds			Leeks		
Cashews			Lettuce		
			Mushrooms		
			Peppers		
			Potatoes		
			Scallions		
			Snow peas		
			Sweet potatoes		
			Taro		
			Tomatoes		
			Water chestnuts		
			White radishes		
			White turnips		
			Winter melons		

Source. Ref. 1

generous amount of sugar. The Middle Eastern diet is a good one, contributing adequate nutrients. But Middle Eastern people in the United States may have difficulty obtaining the foods if they cannot afford them, as they are usually expensive.

Asians

The United States is also home to many people of Asian heritage. The diets of the Chinese, Japanese, and Filipino are discussed below.

Chinese. China is a vast country with many different regional foods. In the United States, most of the Chinese on the West Coast come from southern China and adhere to a Cantonese diet. This type of cookery uses little fat and subtle seasonings. Beef, pork, poultry, and all kinds of seafoods are well liked. In Chinese cooking, all parts of the animal are utilized, including organs, blood, and skin. Rice is the predominant cereal used by the Cantonese, both at home and in the United States. Northern Chinese use more bread, noodles, and dumplings, which are prepared from wheat, corn, and millet (Table 3).

The Chinese diet is varied, containing eggs, fish, meat, soybeans, and a great variety of vegetables. Many green, leafy vegetables unfamiliar to Americans, such as leaves of the radish and shephard's purse, are enjoyed. Sprouts of bamboo and beans are incorporated into some dishes, giving a distinctive flavor and texture. Many Chinese recipes call for mushrooms and nuts. Eggs from ducks, hens, and pigeons are widely used—fresh, preserved, and pickled. Soy sauce is used both in preparation and serving. The high salt content of this flavorable condiment is a problem for Chinese patients whose salt intake may be restricted by the physician.

Chinese cookery is unique in many ways. Food is quickly cooked over the heat source, usually cut into small pieces so that the short cooking period is adequate. Vegetables retain their crispness, flavor, and practically all their nutrients; this method of cookery, which has been widely adopted, is quite beneficial. When vegetables are cooked in water, the liquid is also consumed.

Probably the greatest weakness, in the Chinese diet is a low intake of milk and milk products, a consequence of lactose intolerance. However, a higher consumption of meat and the frequent use of soybeans prevent calcium and protein deficiency. In addition, for undefined reasons, Chinese children born in the United States have a higher tolerance for milk.

Japanese. Japanese people who have emigrated to the United States, especially the older people, tend to follow

Table 4. Characteristic Japanese Food Choices

Protein foods	Milk and milk products	Grain products	Vegetables	Fruits	Other
Meat	Milk	Rice	Bamboo shoots	Apples	Soy sauce
Beef	Ice cream	Rice crackers	Bok choy	Apricots	Nori paste (used to season rice)
Pork	Cheese	Noodles (whole wheat noodle called soba)	Broccoli	Bananas	Bean thread (konyaku)
Poultry		Spaghetti	Burdock root	Cherries	Ginger (shoga; dried form called denishoga)
Chicken		White bread	Cabbage	Grapefruits	Tea
Turkey		Oatmeal	Carrots	Grapes	Coffee
Fish		Dry cereals[a]	Cauliflower	Lemons	
Tuna			Celery	Limes	
Mackerel			Cucumbers	Melons	
Sardines (dried form called mezashi)			Eggplants	Oranges	
Sea bass			Green beans	Peaches	
Shrimp			Gourd (kampyo)	Pears	
Abalone			Mushrooms	Persimmons	
Squid			Mustard greens	Pineapples	
Octopus			Napa cabbage	Pomegranates	
Eggs			Peas	Plums (dried pickled plums called umeboshi)	
Legumes			Peppers	Strawberries	
Soybean curd (tofu)			Radishes (white radish called daikon; pickled white radish called takawan)	Tangerines	
Soybean paste (miso)			Snow peas		
Soybeans			Spinach		
Red beans (azuki)			Squash		
Lima beans			Sweet potatoes		
Nuts			Taro (Japanese sweet potato)		
Chestnuts (kuri)			Tomatoes		
			Turnips		
			Water chestnuts		
			Yams		

Source: Ref. 1.

[a] Nisei only.

the food habits of their homeland rather closely. Typical of the Japanese food pattern is much use of fish, both fresh and saltwater. Methods of preparation vary. Fish may be eaten raw or deep-fat fried, dried, or salted. Many kinds of vegetables may be prepared with meat or eaten separately. Protein intake comes from meat, fish, eggs, legumes, nuts, and, most popular of all, soybean curd (tofu), which is sold in many American grocery stores. Eggs are prepared in many ways and are well liked.

The basic cereal is rice, although since World War II wheat has been consumed. Milk and cheese are limited in the traditional Japanese diet, mainly because of lactose intolerance. Japanese born in the United States have a greater tolerance for dairy products such as milk and cheese, and they also eat more fruits. It is important that the Japanese in this country eat enriched, converted, or whole-grain rice, which contains more nutrients than unenriched rice with the husks removed. To avoid excessive nutrients loss, they should also be told to refrain from washing the rice repeatedly before preparing it (Table 4).

Filipinos. Filipino food patterns are similar to those of the Chinese and Japanese. The basic cereal is rice, and the principal sources of protein are fish, meat, eggs, legumes, and nuts. Meat is prepared by roasting, frying, or boiling; the fish used may be dried.

A large variety of vegetables and fruits is found in the diet. Vegetables are usually boiled or pan-fried, and some are used as salad dishes. Most fruits are eaten raw, but they are sometimes used in cooking (Table 5).

Again, because of lactose intolerance, Filipinos have a low intake of milk and milk products. The consumption of good calcium and protein sources should be encouraged, especially among the young. Growing children may also have a problem with low caloric intake. Larger servings of food should be encouraged, especially more meat; food preparers should also be instructed about the use of enriched rice without prewashing.

The Eskimos: Meeting of Two Cultures

For years, the Eskimos of the far north have maintained their culture on a diet consisting almost entirely of meat and fish. This culture has fascinated nutritionists, as it runs counter to all that is known about the necessity of a balanced diet.

Caribou, whale, and seals from the staples of the traditional diet, with walrus, fish, and birds being of minor importance. Edible plants and berries are scarce. Although it had been believed that Eskimos consumed the stomach contents of the animals, that assumption is debatable. There is agreement the Eskimo diet is very high in protein and fat and very low in carbohydrate.

Table 5. Characteristic Filipino Food Choices

Protein foods	Milk and milk products	Grain products	Vegetables	Fruits	Other
Meat	Milk	Rice	Bamboo shoots	Apples	Soy sauce
Pork	Flavored	Cooked cereals	Beets	Bananas	Coffee
Beef	Evaporated	Farina	Cabbage	Grapes	Tea
Goat	Cheese	Oatmeal	Carrots	Guavas	
Deer	Gouda	Dry cereals	Cauliflower	Lemons	
Rabbit	Cheddar	Pastas	Celery	Limes	
Variety meats		Rice noodles	Chinese celery	Mangos	
Poultry		Wheat noodles	Eggplants	Melons	
Chicken		Macaroni	Endive	Oranges	
Fish		Spaghetti	Green beans	Papayas	
Sole			Leeks	Pears	
Bonito			Lettuce	Pineapples	
Herring			Mushrooms	Plums	
Tuna			Okra	Pomegranates	
Mackerel			Onions	Rhubarb	
Crab			Peppers	Strawberries	
Mussels			Potatoes	Tangerines	
Shrimp			Pumpkins		
Squid			Radishes		
Eggs			Snow peas		
Legumes			Spinach		
Black beans			Sweet potatoes		
Chick peas			Tomatoes		
Black-eyed peas			Water chestnuts		
Lentils			Watercress		
Mung beans			Yams		
Lima beans					
White kidney beans					
Nuts					
Peanuts					
Pili nuts					

Source: Ref. 1.

The high protein content provides calories essential to an active life and additionally contributes to blood glucose, an essential metabolic fuel for the nervous system. What carbohydrate has been available has been obtained through synthesis of glycogen from muscles. A by-product of the traditional diet has been the virtual nonexistence of diabetes. This disease has, however, been on the increase since the introduction of sugar to the culture.

A unique characteristic of Eskimo nutrition is the utilization of fatty acids from oils as energy. Tissues can adapt to the utilization of ketone bodies instead of glucose as energy source. The ketone bodies form in the liver and are characteristic of high-fat, low-carbohydrate diets. Eskimos thus provide a human example of a unique metabolic phenomenon.

As we saw in the discussion of vitamins, we should expect scurvy among Eskimos. But their culture has escaped scurvy because they eat fish and meat either raw or only slightly cooked. What little vitamin C is available is not destroyed by oxidation in the cooking process.

Vitamins A, D, E, and K are obtained in sufficient quantities from the traditional Eskimo diet, as are B vitamins. The diet is low in calcium, a problem that surfaces in bone fragility among Eskimos. Children are somewhat protected by a traditionally long nursing period.

As nontraditional dietary habits have gained popularity in the Eskimo culture, our problems have become theirs. Heart disease, diabetes, tooth decay, and hypertension are now on the increase.

PEOPLE AND THEIR LAND

As you have probably determined from these discussions of culture and nutrition, our food habits are very much influenced by our geographic location. Relationships between people and their land can be profound, and they have given rise to fields of knowledge ranging from cultural geography to literary analysis focusing on agrarian mythology. Although it has been said that we are what we eat, perhaps we should also say that we eat where we are.

BIBLIOGRAPHY

This article has been adapted from Y. H. Hui, *Principles and Issues in Nutrition*, Jones and Bartlett Publishers, Inc., Boston, 1985. Used with permission.

1. *Nutrition During Pregnancy and Health*, California Department of Health, Sacramento, Calif., 1975.

GENERAL REFERENCES

L. Bakery, ed., *The Psychobiology of Human Food Selection*, Van Nostrand Reinhold, New York, 1982.
L. K. Brown and K. Mussell, eds., *Ethnic and Regional Foodways in the United States The Performance of Group Identity*, University of Tennessee Press, Knoxville.
C. A. Bryant et al., 1985, *The Cultural Feast: An Introduction to Food and Society*, West Publishing Co., St. Paul, Minn., 1985
P. Farb and G. Armelagos, *Consuming Passions: The Anthropology of Eating*, Houghton Mifflin, Boston, 1980.
P. Fieldhouse, *Food and Nutrition: Customs and Culture*, Methuen, New York, 1985
Food and Nutrition Board, *What is America Eating?* National Academy Press, Washington, D C., 1986.
Food and Nutrition Board, National Research Council, National Academy of Sciences, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
N Jerome et al., eds., *Nutritional Anthropology*, Redgrave Publishing, Pleasantville, N.Y., 1980.
F. E. Johnston, ed., *Nutritional Anthropology*, Alan R. Liss, New York, 1987.
P. G. Kittler and K. Sucher, *Food and Culture in America*, Van Nostrand Reinhold, New York, 1989
A. W. Logue, *The Psychology of Eating and Drinking*, W. H. Freeman, New York, 1986.
J. M. Newman, *Melting Pot: An Annotated Bibliography and Guide to Food and Nutrition Information for Ethnic Groups in America*, Garland, New York, 1986.
W. Root and R. DeRochement, *Eating in America: A History*, Eco Press, New York, 1976
D. Sanjur, *Social and Cultural Perspectives in Nutrition*, Prentice-Hall, Englewood Cliffs, N.J., 1982.

Y. H. Hui
American Food and Nutrition Center
Cutten, California

CULTURED MILK PRODUCTS

Cultured milk products are produced by the lactic fermentation of milk using various bacteria cultures. Some products may also have other fermentations taking place, for example, alcohol. These fermentations lead to the coagulation of milk and the production of typical cultured milk product flavor.

Fermented milk products originated in the Near East and then spread to parts of southern and eastern Europe. The earliest forms of fermented products were developed accidentally by nomadic tribes who carried milk from cows, sheep, camels, or goats. Under warm storage conditions, milk coagulated or clabbered due to the production of acid end products by lactic bacteria. Fortunately the predominant bacteria were lactic types and, therefore, helped to preserve the product by suppressing spoilage and pathogenic bacteria. Humans evidently enjoyed the refreshing tart taste of their discovery and began to handle milk so that this preserving action would be encouraged.

Milk and curdled milk products are mentioned throughout history dating back as far as 4000 B.C.: "He asked for water, and she gave him milk; in a bowl fit for nobles she brought him curdled milk" (Old Testament, Judges 5:25). There is also remarkable pictorial evidence that the custom of keeping milk in containers for later consumption was already a craft systematically practiced by the Sumerians around 2900 B.C. (1). Through applied scientific principles and advances in manufacturing technology, these early products have developed into a highly diversified group of foods that are popular throughout the world.

Actual figures for world consumption of fermented milk products are not known. According to the International Dairy Federation (IDF), an estimated 1 trillion lb of cow's milk is produced per year that is manufactured into cultured milk products. The northern European countries consume the greatest amount of fermented milk products on a per capita basis with Asian countries and the United States showing rapid increases. Worldwide increases in consumption are leading to advances in the fermented product manufacturing industry. One reason for the continued increase in product consumption is the successful development of functional foods/neutraceuticals that contain health-promoting probiotic cultures.

Throughout the world there are great differences in cultured products. These differences are due to variations in the cultures used and manufacturing principles. Products are most often classified as traditional or nontraditional; however, products can be classified according to culture medium (milk and cream), manufacturing procedure, further processing (packaging and addition of fruits, vegetables, meat, fish, or grains), end use (baking and consumption), and microbial action (type of bacteria or yeast and temperature). Traditional fermented milk products have a long history and are known and made all over the world. Their manufacture is crude and relies on ill-defined procedures and nonstandard cultures that lead to inconsistencies in product characteristics (1). The production of nontraditional products is based on sound scientific principles and leads to the creation of products with consistent characteristics. Cultures and manufacturing methods have been standardized to produce the highest quality possible. Despite some differences, most modern cultured products use the following basic manufacturing steps: (*1*) culture or starter selection and preparation; (*2*) milk processing and treatment (eg, pasteurization to kill undesirable bacteria, standardization of composition by separation of fat and addition of ingredients, and homogenization); (*3*) inoculation with bacterial culture; (*4*) incubation for culture growth and acid production; (*5*) agitation to break the coagulum; (*6*) cooling to stop bacterial growth and acid production; and (*7*) packaging. Table 1 lists the world's principal cultured milk products including type, location, and bacterial culture used.

MICROORGANISMS

A culture (starter) is a controlled bacterial population that is added to milk or milk products to produce acid and flavorful substances that characterize cultured milk products. Cultures may be a single strain of bacteria or a combination of cultures (mixed strain). Mixed strain cultures may be used to enhance the production of specific flavors or characteristics and, therefore, must be compatible and balanced. Some cultures will be antagonistic to each other while others will act in a symbiotic relationship.

The proper production of lactic acid is critical in fermented product manufacture. Lactic acid is not only re-

Table 1. Fermented Foods and Geographical Location of Production

Name	Type	Location	Bacteria
Acidophilus	Dairy	Europe, North America	*Lactobacillus acidophilus* *Bifidobacterium bifidum*
Bulgarian buttermilk	Dairy	Europe	*Lactobacillus bulgaricus*
Buttermilk	Dairy	North America, Europe, Middle East, North Africa, Indian subcontinent, Oceania	*Lactococcus cremoris, Lc. Lacticus, Lc. Lacticus* ssp *diacetylactis Leuconostoc cremoris*
Filmjolk	Dairy	Europe	*Lc. Cremoris, Lc. Lacticus, Lc. Lacticus diacetylactis, Lc. Cremoris, Alcaligenes viscosus, Geatrichus candidum*
Flummery	Cereal and dairy	Europe, South Africa	Naturally present lactic bacteria
Ghee	Dairy and miscellaneous	Indian Subcontinent, Middle East, South Africa, Southeast Asia	*Streptococcus, Lactobacillus, and Leuconostoc* spp
Junket	Dairy	Europe	*Streptococcus and Lactobacillus* spp.
Kefir	Dairy	Middle East, Europe, North Africa	*Streptococcus, Lactobacillus, and Leuconostoc* spp. *Candida kefyr, Kluyveromyces fragilis*
Kishk	Dairy and cereal	North Africa, Middle East, Europe, Indian subcontinent, East Asia,	*Streptococcus, Lactobacillus, and Leuconostoc* spp
Kolatchen	Cereal and dairy	Middle East, Europe	*Lc. Lacticus, Lc. Lacticus* ssp. *diacetylactis, Lc. Cremoris Saccharomyces cerevisiae*
Koumiss	Dairy	Europe, Middle East, East Asia	*Lc. Lacticus, Lb. bulgaricus, Candida Kefyr, Torulopsis*
Kurut	Dairy	North Africa, Middle East, Indian subcontinent, East Asia	*Lactobacillus and Streptococcus* spp. *Saccharomyces lactis, Penicillium*
Lassi	Dairy	Indian subcontinent, East Asia, Middle East, North Africa, South Africa, Europe	*Str. thermophilus, Lb. bulgaricus,* sometimes yeast
Prokllada	Dairy	Europe	*Streptococcus and Lactobacillus* spp
Sour cream	Dairy	Europe, North America, Indian subcontinent, Middle East	*Lc. cremoris, Lc. lacticus, Lc. lacticus* ssp. *diacetylactis*
Yakult	Dairy	East Asia	*Lactobacillus casei*
Yogurt	Dairy	Worldwide	*Str. thermophilus, Lb. bulgaricus*

Source: Ref. 2.

sponsible for the refreshing tart flavor of cultured products but is responsible for the destabilization of the milk protein structure (casein micelle) that allows the milk protein to coagulate, thus contributing to the product's body and texture characteristics. The culture may also contribute other flavorful compounds (Table 2). The major flavor compounds, other than lactic acid, encountered in fermented milk products are acetaldehyde, diacetyl, ethanol, carbon dioxide, and acetic acid. These flavor compounds are responsible for the unique flavor characteristics of various products and great care is taken to promote their production in certain products. For example, proper yogurt flavor depends on the production of acetaldehyde while proper cultured buttermilk flavor depends on the production of diacetyl. Products such as kefir and koumiss also undergo alcoholic fermentation with final levels of ethanol reaching 1 to 2.5%.

NUTRITION

The primary nutritional benefits of cultured milk products are due to their compositional makeup. All milk products are excellent sources of high-quality proteins (casein and whey), calcium, and vitamins (especially riboflavin and other B vitamins). Early in the twentieth century Eli Metchnikoff (1908) proposed in his book, *Prolongation of Life Optimistic Studies*, that the consumption of cultured milk products such as yogurt result in the prolongation of life. Metchnikoff based his claims on the ability of lactic bacteria to prevent putrefactive processes in the digestive tract (4). Although lactic cultures do produce antimicrobial effects from the production of acids, hydrogen peroxide, and antibiotics such as nisin, scientific evidence does not support Metchnikoff's claims of prolonged life due to the consumption of cultured products. However, there is growing scientific evidence that the addition of alternative organisms to cultured products such as *Lactobacillus acidophilus* and *Bifidobacterium* sp. may have therapeutic effects in the lower digestive system (5). Many of these organisms are believed to be able to pass through the upper digestive system and then produce beneficial effects in the lower intestine. Recent research suggest that the consumption of these probiotic cultures in cultured milk products could aid in the prevention of intestinal infections, diarrheal diseases, hypercholesterolaemia, lactose digestion, and cancer.

Table 2. Starter Cultures and Their Principal Metabolic Products

Starter organisms	Important metabolic products
Mesophilic bacteria (optimum growth temperature of 20–35°C)	
Lc. cremoris	Lactic acid
Lc. lactis	Lactic acid
Lc. lactis ssp. diacetylactis	Lactic acid, diacetyl
Leuc. cremoris	Lactic acid, diacetyl
Lb. acidophilus	Lactic acid
Lb. breves	CO_2, acetic acid, lactic acid
Lb. casei	Lactic acid
Thermmophilic bacteria (optimum growth temperature of >35°C)	
Str thermophilus	Lactic acid, acetaldehyde
Lb. bulgaricus	Lactic acid, acetaldehyde
Lb. lactis	Lactic acid
Bifidobacteria	Lactic acid, acetic acid
Yeast	
Saccharomyces cerevisiae	Ethanol, CO_2
Candida (Torula) kefir	Ethanol, CO_2
Kluveromyces fragilis	Acetaldehyde, CO_2

Note. Lc. = Lactococcus, Leuc. = Leuconostoc, Lb. = Lactobacillus, Str. = Streptococcus.
Source: Ref. 3.

BIBLIOGRAPHY

1. M. Kroger, J. A. Kurmann, and J. L. Rasic, "Fermented Milks—Past, Present, and Future," *Food Technol.* **43**, 92–99 (1989).
2. G. Campbell-Platt, *Fermented Foods of the World*, Butterworth, Kent, United Kingdom, 1987.
3. B. A. Law, *Microbiology and Biochemistry of Cheese and Fermented Milk*, Blackie Academic and Professional, London, United Kingdom, 1997.
4. E. Metchnikoff, *The Prolongation of Life Optimistic Studies*, G. P. Putnam's Sons, New York, 1908.
5. K. Kailasapathy and S. Rybka, "*L. acidophilus* and *Bifidobacterium spp.*—Their Therapeutic Potential and Survival in Yogurt," *Aust. J. Dairy Tech.* **52**, 28–35 (1997).

JOHN U. MCGREGOR
Louisiana State University Agricultural Center
Baton Rouge, Louisiana

CYCLOSPORA. See FOODBORNE DISEASES.

D

DAIRY FLAVORS

Dairy products are very ancient foods and are one of the most important classes of foods in our diet. The basic dairy product, milk, is not a very stable food. Today's practice allows us to heat process it to keep it stable from microbiological attack for various periods of time depending on the nature of the processing conditions to which the milk is subjected. The commercial practice of heat processing (pasteurization) has only been in place for the last 60 or so years. In ancient times milk was allowed to spoil in a controlled way so that it would remain eatable.

The development of ghee (butter) from buffalo milk, an early practice of controlling the spoilage of milk, has been traced to India of perhaps about 4,000 years ago. The method of butter making was to appear in Europe at a much later date. The first commercial creamery in the United States was built by Alanson Slaughter in 1881 in Orange County, New York.

Cheese from milk also has a prehistoric origin. Cheese was an excellent way to preserve the important nutrients of milk. Because of the many ways of stabilizing milk into cheese, many forms of cheese with a variety of flavor and taste profiles have been developed. Recently, a USDA bulletin indicated over 800 varieties and the (United States Food and Drug Administration) U.S. FDA has established standards of identity for 30 different type of cheeses. Typically, cheeses are named after the immediate region where they were originally produced.

At first the manufacture of dairy products, including milk, milk products, butter, and cheese, was based on historical practice and the art of the producers. It took the great scientific revelation of Louis Pasteur working in France between 1857 and 1876 to relate the manufacturing process to the new world of the microorganism and fermentation. The use of pure culture techniques began around 1870, and the dairy and cheese industry was transformed from an industry of inconsistent product quality and quantity. The various organisms responsible for the development of the flavor, taste, and texture of dairy and cheese products were discovered and used in day-to-day operation of the manufacturing plants. More recent scientific advances in the area of organic, analytic, and biochemistry have allowed for the identification of those chemical components that develop during the manufacturing steps and contribute to the flavor profiles that we associate with these products.

Besides the well-known nutritional properties of these products, they are very popular owing to their unique flavor, taste, and textual attributes.

OVERVIEW OF DAIRY PRODUCTS

Dairy products may be defined as food products based on milk developed through a variety of manufacturing methods and fluid milk itself. There are several animal sources of milk, including the cow, water buffalo, goat, and sheep, that are commonly used sources.

In the 10 year period from 1987 to 1997, the production of milk has increased by approximately 15 billion lb to 155 billion lb, with 60% of the milk used to create other dairy products. The percent use of milk products to make other dairy products increased by 2% during that period. Total cheese production, excluding cottage cheese, was 7.33 billion lb in 1997, an annual increase of 2%. Wisconsin was the leading state for cheese product, with 29% of the total production. By far the most popular types of cheese are the American varieties, which include mainly cheddar. Cottage cheese is produced by far more plants in the United States (332) and represents production of 1.2 billion lb. Butter production has been declining; in 1997 production was recorded at 1.2 billion lb, a decrease of 2% from 1996. California, at 27% of butter production, was slightly ahead of Wisconsin, at 26%. Table 1 represents the statistics for production of various dairy products in the United States during 1997.

In discussing the nature of the flavors of these products it is natural to organize the discussion based on the types of products manufactured. Based on the major groups of products, there are four distinct areas that will be discussed. They are fluid milk and milk products (dried and canned), milk-derived products (yogurt and similar types of fermented milk products and ice cream), butter, and cheeses.

Milk and Milk Products

These products are minimally processed. Typically they may be reduced in fat, have the fat suspended (homogenized), or be heat treated. The heat-processing step may vary in its time/temperature relationship to assure a safe

Table 1. Dairy Product Production in the United States for 1997

Products	Plants	Total volume in 1997 (in 1,000 pounds)	Growth rate (%)
Butter	91	1,151,250	2.0
Cheeses			2.0
American	215	3,285,203	
Swiss	42	207,583	
Muenster	42	100,191	
Brick	21	8,535	
Limburger	1	746	
Italian types	156	2,880,404	
Cream & Neufchatel	32	614,921	
Cottage Cheese	332	1,164,172	
Canned milk	9	580,965	
Skim milk	51	997,802	
Dry Milk (nonfat)	47	1,217,562	15.0
Yogurt	104	1,574,050	

Source: Ref. 1.

product. Although the heat treatment can have some influence on the taste and flavor of fresh milk, there is a much greater relationship to the time of year, nature of the animals' feed, and the species of animal from which the milk is derived.

Milk is a very complex substance based on fats, sugars (mainly lactose), proteins (such as lactalbumin), and salts (such as calcium phosphate). The taste of milk is mainly affected by the change in this mixture and the degree to which the fat is emulsified (homogenized) in a continuous phase with the aqueous phase. The sensory perception determines the physical nature of the emulsion, with a slightly salty/sweet taste from the salts and lactose. Although some slight flavor changes can be detected in even the mildest of heat-treated milk, the flavor defects are generally considered to be notes associated with good quality, pasteurized milk.

Of the various constituents of the milk, the lipid (fat) fraction has the greatest effect on milk's flavor. It is also the precursor of many chemical components considered important in all types of dairy flavors. The fatty acid composition of the milk lipids is also very complex and unique among food products. More than 60 fatty acids have been reported in cow's milk (2). Quantities of butyric acid and caproic, caprilic, decanoic (fatty acids with 6, 8, and 10 carbon atoms) acids are unique to milk and of great importance in the development of flavors in products based on milk. Oxidation of these lipids gives rise to some key flavor components. For example, the oxidation of unsaturated octadecadienoic acid leads to the formation of 4-*cis*-heptenal, which has been identified as the cream-like flavor component in milk and butter (3). The free fatty acid distribution of milk and butter is shown in Table 2.

The heat treatment of milk is divided into two types of processing: pasteurization in which the milk is heated for 15 s at 72°C and ultra high-temperature treatment (uht), where it is heated at 135–150°C for a few seconds. The flavor changes that occur during the heat treatment may be formed from three distinct chemical mechanisms:

- Degradation of thermally labile precursor found in milk.
- Reaction between the sugars and proteins (Maillard reactions).
- The release or formation of sulfur-containing components.

Table 2. Free Fatty Acids in Milk

Fatty acid (carbon no : unsaturation)	Fat (%)		
	Fresh milk	Rancid milk	Butter
4:0	7.23	6.03	1.32
6:0	3.40	3.42	0.65
8:0	1.37	2.07	0.83
10:0	5.16	3.98	1.41
12:0	4.89	3.98	4.22
14:0	10.02	9.58	11.02
16:0	23.61	26.09	22.69
18:0	9.98	13.65	12.04
18:1	29.78	27.05	38.40
18:2	4.55	4.14	4.66
18:3	—	—	2.69

Source: Ref. 4.

Degradation of Precursors. The triglycerides (lipids) in milk fat are formed from complex as well as simple fatty acids of many types, including substituted acids such β-keto acids and hydroxy acids. These acids originate in the diet of the animal and also may be the result of normal metabolism. Methyl ketones are formed from the β-keto acids (5). Although they are formed in fresh milk, maximum yield occurs at prolonged elevated heating (140°C for 3 h). The liberation of methyl ketones is generally below their individual odor thresholds, yet they contribute to the aroma of milk because of their synergistic interaction due to their combined concentration (6). Methyl ketones are formed from the decarboxylation of the β-keto acids, whereas ring closure of δ- and τ-hydroxy acids form the corresponding lactones.

The Maillard Reaction. Reaction between the lactose and the milk protein can lead to many different compounds of flavor significance. These reactions are referred to as Maillard reactions. Discussion of the Maillard reaction is complex and outside the scope of this article, but its basic chemical pathway is the reaction between aldose and ketone sugars and α-amino acids resulting in the formation of aldosylamines or ketosylamines. These materials then undergo further rearrangement, resulting in the creation of many key flavoring materials, such as acetyls, furans, pyrroles, aldols, and pyrones. The normal heat treatment of milk does not appear to produce enough of the Maillard reaction product to be a flavor contributor. However, the cooked flavor of milk is noted when it is heated to above 75°C (7). At that point many Maillard reaction products start to contribute to the flavor of the milk. Further heating above the cooked-flavor range produces a caramelization flavor, due to the intense amount of Maillard reaction products being formed and the liberation of many sulfur-containing chemicals.

Formation of Sulfur-Containing Compounds. Dimethyl sulfide is produced from the *S*-methyl methionine sulfonium salt (from the vegetable matter in the animal's diet) that is decomposed on heating (8). At high concentrations this chemical produces a malty or cowy flavor defect in the milk (9). The flavor from the caramelization of milk has found great use in various culinary schools of fine food preparation.

Flavor Defects in Milk. Caramelization is a wanted defect in many food applications; yet, there are some defects that are not wanted. Extended storage of dry milk causes the stale off-flavor where the release to the fatty acid contributes to the further development of methyl ketones above their threshold value, but below the quantity seen in certain cheeses; for example, the family of blue cheeses (10).

The oxidation of milk causes the cardboardy or cappy defect that is evaluated as the milk being metallic, tallowy, oily, or fishy. The oxidation of the unsaturated fatty acids leads to the methyl ketones and series of aldehydes. Very low levels of *cis*-4-heptenal, hexenal, and 1,3 octenone contribute to the oxidized flavor defect (11).

The lipolysis of the milk by lipases remaining active after the heat treatment can cause rancidity of the milk. This is due to the increase of both the short-chain fatty acids and the increased acidity of the milk. In normal milk the total free acid content is about 360 mg/kg, but in rancid milk it rises to 500–1500 mg/kg (4).

One final major flavor defect is known as the sunlight defect. It is believed to be produced from the degradation of methional (a common amino acid found in milk) to several sulfur-containing compounds, including mathanethiol, dimethyl sulfide, and dimethyl disulfide. These contribute to a flavor characterized as burnt or cabbagey. The extent of the flavor defect is related to the length and strength of exposure to light (12).

Milk's Basic Flavor. Milk's basic flavor, then, is due mainly to the taste components found in the milk as noted in Table 3. The fats, sugars, salts, and protein all contribute to give milk a subtle, but enjoyable, flavor and taste. Unless the milk has been mistreated or subjected to high temperature for prolonged time, no significantly predominate flavor is noted. In contrast, products made from milk have characteristic and, in some cases, strongly predominate aroma and tastes.

Cultured Dairy Products

Among the first products of milk to be developed were cultured products. Cultured products are produced throughout the world, as seen in Table 4. Originally, fermentation occurred naturally to milk set aside for later use. The spoilage organisms in the milk produced the proper conditions for the preservation of the cultured product. The lowering of the pH of the milk by the organisms allows for the preservation of the product and contributes to its characteristic aroma and taste.

The preparation of these cultured products became an art that survives today. The consumption of these products varies around the world, with the northern European countries consuming the greatest amount (~20 kg/y/person); and the United States consuming the least (~1.2 kg/y/person) (14). However, the United States is a 300-fold increase over the 1966 figure. This, no doubt, reflects the healthy eating trend in the United States prompted by television commercials and magazine ads listing the health benefits of cultured milk products.

Table 3. Fat Content of Cow's Milk

Type of fats	Range of occurrence (%)
Triglycerides	97.0–98.0
Diglycerides	0.25–0.48
Monoglycerides	0.015–0.038
Keto acid glycerides	0.85–1.28
Aldehydo glycerides	0.011–0.015
Glyceryl ethers	0.011–0.023
Free Fatty Acids	0.10–0.44
Phospholipids	0.2–1.0
Cerebrosides	0.013–0.066
Sterols	0.12–0.4

Source: Ref. 13.

Table 4. Cultured Milk Products

Product type	Fermentation	Organism
Yogurt (U.S.)	Moderate acid	*Streptococcus thermophilus* *Lactobacillus bulgaricus*
Buttermilk (U.S.)	Moderate acid	*Streptococcus cremoris* *Streptococcus lactis*
Acidophilus milk (U.S.)	High acid	*Lactobacillus acidophilus*
Bulgarican milk (EUR)	High acid	*Lactobacillus bulgaricus*
Yakult (Japan)	Moderate acid	
Dahi (India)	Moderate acid	*Streptococcus diacetyllactic*
Leben (Egypt)	High acid	*Streptococci, Lactobacilli, yeast*
Kifur (USSR)	High alcohol	*Lactobacillus caucascus* *Leuconostoc* spp

The basic fermentation is due to lactic acid bacteria. Historically, this was fermentation done by a mixed or unknown culture or back-slop, but modern technology has developed the pure culture with its predictable performance potentials.

The major flavor and taste component in these products is the lactic acid produced by the fermentation of the sugars by the bacteria. Although this flavor is considered acceptable in many societies, it is rejected completely in others. To increase the commercialization of these products, manufacturers have introduced products that contain added sugars, fruit preserves, and flavors. These innovations have also contributed to increased consumption, particularly in the United States.

During the fermentation the lactose in the milk is transformed via pyruvic acid to lactic acid. The small amount of lactose or its hydrolysis products galactose and glucose is below the threshold for detecting sweetness, but the lactic acid may account for more than 2% of the mass of the product and, by that, contribute a significant flavor and taste effect. The fermentation pathway may favor both enantimer formation (L(+) and D(−) lactic acid), as depicted in Figure 1. The preferred metabolite, in flavor terms, is the L(+) or a harsh off-flavor will be observed (15).

The basic microorganisms used are the lactobacilli, streptococci, and leuconostocs. The major difference in their flavor is the amount of lactic acid produced and the perception of the acid taste. One exception is the product from the USSR called Kifur, which undergoes a secondary fermentation to create ethyl alcohol. This product is also known as the champagne of milk (16).

Yogurt Flavor. Yogurt flavor is also influenced by acetaldehyde, another product of lactose from the fermentation of pyruvic acid by *Lactobacillus bulgaricus*. In yogurt the acetaldehyde level differs greatly with the type of microorganism(s) used (17). There is no agreement on the acetaldehyde values that give an optimum flavor effect (18).

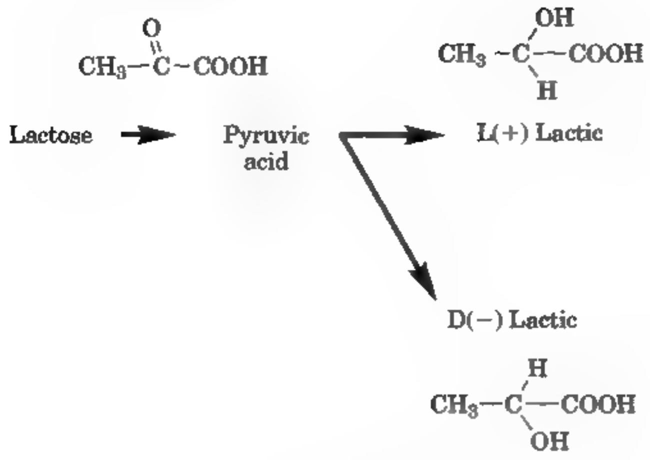


Figure 1. Lactic acid fermentation.

The value of 23–41 ppm is given in some reports, whereas the value of 13–16 ppm is considered by others to be the optimum level (19,20).

One further compound of flavor interest in cultured milk products is diacetyl. It is formed from the citrate present in milk by the action of Streptococci, Leuconostocs, and other organisms that can metabolize citrate. (21) This component strongly contributes to the acid cream butter flavor usually at level of 1–2 ppm (22). It is diacetyl that is a significant contributor to butter's flavor.

Butter and Cream

We have discussed the nature of milk flavor and the cultured products derived from milk. The production of butter from milk involves the concentration of the milk fat in the milk by mechanical separation. The resultant product is a water in oil emulsion that has a mild characteristic flavor. During this process the exogenous enzymes (those enzymes existing outside the cell) can break down the triglycerides to free fatty acids; with improper handling this may occur at a high level, leading to a flavor effect known as rancidity. It is the short-chain free fatty acids (eg, butyric acid) that are responsible for the development of rancidity in butter. The role of other free fatty acids directly on flavor seems small, except the deca- and dodecanoic acids, which appear in sweet cream butter at levels exceeding their flavor threshold (23). Because the lipases responsible for the hydrolysis of the triglycerides are very heat stable, their activity continues even at low temperature during refrigerated or frozen storage.

Rancidity. Rancidity is the major flavor defect in butter. The most useful index of rancidity is related to the amount of butyric acid found in the butter. A rancidity score developed by the University of Wisconsin correlates sensory data with individual free fatty acids concentration by stepwise regression and discriminatory analysis to index the relative rancid flavor intensities in butter (24). This is a very useful flavor index for the evaluation of butter.

Lactone Formation. Lactones have also been considered a major flavor attribute in butter and cream. They are generated from the small amount of δ-hydroxy acids occurring in milk fat. On hydrolysis these compounds may spontaneously lactonize to form the corresponding δ-lactones. The τ-lactones also may be formed from unsaturated C18 fatty acids via hydration and β-oxidation. The amount of lactones formed is related to the type of feed, the season, the type of breed, and the stage of lactation of the animal (25). Estimates of the amount of lactone that can create a flavor effect is reported at 7–85 ppm (26,27).

Heated-Milk Defects. Heated milk fat increases the level of lactones found in butter (28) Although this is considered a defect in butter or cream, it is a desired effect when cooking. The flavor developed is characterized as a coconut-type note, which has been observed in heated butter used to flavor popcorn. Several patents have been issued to commercial companies for the use of lactones or lactone precursors for the flavoring of butter analogues, including margarine.

Imitation Products. Although margarine is not a dairy product, it bears mention here as it is produced in great quantity throughout the world as a substitute for butter. The process goes back to the Napoleonic times as a commercial product. The flavors used in these products generally are composed of diacetyl and lactones or lactone precursors for the butter flavor impression. To various degrees of sophistication, all commercially available margarine has these materials added to the vegetable fat/water emulsion.

Ice Cream. One other product produced from milk and butter is ice cream. Ice cream represents a congealed product produced from cream, milk, skim milk, condensed milk, butter, or a combination of these ingredients, plus the addition of sugar, flavorings, fruits, stabilizers and colors. Again, the basic contribution of the dairy ingredients to the flavor is the fat and lactose, with those aromatic components mentioned in the foregoing discussion and associated with butter or cream flavor. Typically, the aromatic portion of the flavor is greatly modified by the addition of flavors and extracts.

Most of these basic dairy products, like butter, have flavor profiles that are simple. The real flavor masterpieces of the dairy industry are the many varieties of cheese, with their extremes in flavor types and intensities.

Cheese

The many varieties of cheese represent many centuries of the cultivation of the art of cheese-making. Many different, probably accidentally developed, flavors, tastes, and textures have been developed. The products of cheese-making are the result of many types of microorganisms (yeast, bacteria, and mold) working together with the basic milk source (typically cow's, goat's or sheep's milk) and the knowledge of the control of fermentation. The variety of methods is great and, so too, as has been noted, are the varieties of cheese produced throughout the world. We will not attempt to discuss all the types known, but focus our flavor discussion on those well-known cheeses and the basic science of their formation.

Chemistry. The basic chemistry or biochemistry of cheese-making is the slow degradation of the macromolecules (protein and lipid material) to small components of flavor (aroma and taste) value. Proteolysis (breakdown of the protein by enzymes) during cheese ripening and aging has a major influence on peptide formation. Peptides have various taste characteristics based on their polarity. The basic taste sensations of sour/sweet and salt/bitter and the flavor-enhancing property known as umami (the flavor-enhancer taste associated with monosodium glutamate, MSG) are all taste attributes of peptides and amino acids.

It has been observed that the acid- (sour), hydrophobic- (bitter), and glutamic/aspartic-rich (umami) peptides are the key taste peptides and amino acids in aged cheese (29). The hydrophilic (sweet) and salt-based peptides contribute very little, as they are overwhelmed by the basic taste attributes of the cheese. The natural sweetness from lactose and the saltiness due to the salt (sodium chloride) added to all cheese to produce a selective environment and the chemistry needed to develop the specific cheese character play a more significant flavor role than do the peptides that have salty or sweet taste character.

The salt content of selected cheeses is listed in Table 5. Note that the Roquefort or blue-cheese types have the highest salt content and those of the Swiss types have the lowest. This is reflected in the basic salt taste of these cheeses. As we will see, the volatile chemicals formed from the amino acid, peptides, lipids, and sugar are the backbone of the characterizing flavors for the various cheese.

Types of Cheeses. The family of cheeses generally recognized are

- Fresh soft low-fat cheese
- Cream/Neufchâtel
- Gouda/Muenster
- Port du Salut
- Brie/Camembert
- Blue cheeses
- Goat cheeses
- Emmentaler
- Cheddar
- Parmesan/Romano
- Whey/ricotta

Processing. The basic processing involves the progressive dehydration and working of the coagulated and separated curds of milk to form a homogeneous compact mass. The initial coagulation is achieved by using an added enzyme, rennin, or by lactic acid fermentation, or both, depending on the desired character of the final product. The green cheese is then ripened via the addition of certain microorganisms that will accomplish the breakdown of the protein, lipids, and sugars to the small molecular weight components that contribute to the cheesey flavors found in the final product.

Table 5. Salt Content of Certain Cheeses

Cheese variety	Salt (NaCl) (% w/w)
Roquefort	4.1–5.0
Feta	2.4–4.4
Parmesan	2.1–3.5
Edam	1.7–1.8
Cheddar	1.6–1.7
Emmentaler	0.9–1.0

Flavor Attributes of Cheese by Families. The less cheesey flavored cheeses are those with the least amount of ripening: fresh soft low-fat, higher fat cream/Neufchâtel, and the whey/ricotta type. Their flavors are primarily due to the components contributing to milk, cream, and butter flavor. They should be low in flavor intensity or they would be considered to have a flavor defect.

The next group in terms of flavor strength consist of the Swiss (Emmentaler), German (Muenster), and Dutch (Gouda) cheeses. The importance of peptides was recognized in studies with Swiss cheese (30). Results indicated that the small peptide that interacted with calcium and magnesium gives the cheese a sweet flavor, with the small free peptides and amino acid contributing a slight brothy-nutty flavor. An important reaction in Emmentaler is the conversion of pyruvic acid to propionic acid by propionibacterium. Propionic acid is found in Emmentaler in levels of up to 1%, and it is considered the basis of the sweet taste of that cheese (31). Gouda has a defect called catty taint that is due to the formation of 4-mercato-4-methylpentaone-2 (32).

Cheddar cheese is by far the most consumed cheese in the United States. It is produced by a unique process, cheddaring, introduced in 1857 to repress the growth of spoilage organisms during cheese-making. Cheddaring involves the piling and repiling of blocks of warm cheese curd in the cheese vat for ca 2 h. During this period the lactic acid increases rapidly to a point where coliform bacteria are destroyed.

The basic taste of cheddar is due to the peptide fraction and, in particular, to the umami and bitter components. The development of acetic acid during ripening contributes to the sharpness of aged cheddar. The release of the acetic acid and amino acids continue during the ripening process. The medium chain fatty acids (C6 to C18) that are released during the ripening process have also been shown to be important in cheddar cheese flavor character (33).

Many other studies over a 10-year period indicated that it is necessary to produce the right level of acidity to provide a reducing environment in the finished cheese so that there may be a release of sulfur-containing compounds. The lost of these compounds, in particular, methyl mercaptan, results in a cheese with little cheddar character (34–36). Methanethiol has also been shown to be a significant factor in aged cheddar (37–41). Methanethiol has a very fecal-like aroma with a threshold of detection reported at 0.02 ppb (42).

It has been suggested that acetylpyrazine and 2-methoxy-3-ethylpyrazine are important in aged cheddar (43). The possible microbial origins of pyrazines became evident when it was reported that dimethylpyrazine and 2-methoxy-isopropylpyrazine were isolated from milk con-

Table 6. Summary of the Flavor Chemistry of Dairy Products

Product	Process	Chemistry	Major flavor contributors
Milk	Homogenization and mild heat	Natural components	Balance of fats, salts, and lactose (milk sugar)
Defects	Cooked >57°C	Maillard reactions	Aldehydes and pyrazines
	Prolonged cooking	Caramelization	Sulfur-containing components
	Oxidation	Oxidation	Aldehydes
	Sunlight exposure	Sulfur reactions	Methanethiol
	Rancidity	Lipolysis	Butyric acid
Cream	Fat separation	Enzymolysis	Methyl ketones (low level) diacetyl and lactones
Butter	Further fat separation	Enzymolysis	Diacetyl and butyric acid
Cultured products	Fermentation	Lactic acid formation	L(+) Lactic acid
Yogurt	Fermentation		Acetaldehyde
Cheeses	Salting		NaCl
	Aging	Proteolysis	Peptide formation
Swiss types	Fermentation	Propionibacteria	Propionic acid
Cheddar types	Cheddaring	Lactic acid fermentation	Lactic and acetic acid
		Maillard reactions	Acetyl pyrazine and other pyrazines
Blue types	Fermentation	Penicillium	Methyl ketones (high amounts)

taminated by *Pseudomonas taetrolens* (44). Further alkylpyrazines have been isolated from mold ripened cheese using *Penicillium caseicolum* cultures (45). The overall flavor of good cheddar is a balance between the free fatty acids and the other flavor components noted above.

The blue cheese family has been found to contain from 13 to 37 times the amount of free fatty acids found in cheddar. This is due to the lipolysis of the fat by the lipases generated from the use of the mold *Penicillium roqueforti* (46). Roquefort is made from sheep milk and does not have the high levels of free fatty acids seen in the blue cheese made from cow's milk. Part of the flavor difference between the two is due to the lower concentration of propionic acid (4:0) in Roquefort and the relative larger amount of C8:0 and C10:0 free fatty acids.

The major aroma character of the blue cheeses is due to the high levels of methyl ketones found in the cheese. These components have been isolated in milk and are assumed to be the product of the free fatty acid. The importance of the methyl ketone in creating an imitation blue cheese flavor has been well reported (47–52).

Secondary alcohol may be produced from the methyl ketones when they are generated in high amounts driving the equilibrium from the methyl ketones to the secondary alcohols. Although the secondary alcohols have a similar flavor profile to the methyl ketones, their intensity and quantity in the cheese is less, therefore, making only a small contribution to the complete flavor (52).

CONCLUSION

The flavors of dairy products are extremely varied and represent a great range of chemistry, biochemistry, and microbiology. A summary of the major dairy products and their associated significant flavor values is given in Table 6. It summarizes the nature of the components contributing to the flavor and taste characters of the particular material. Science has yet to identify completely the components responsible for the overall subtle aromas and taste found in dairy products, but Table 6 represents a good basic start to an understanding of the complexities of dairy flavors.

BIBLIOGRAPHY

1. United States Department of Agriculture, *Dairy Industry Report*, National Agricultural Statistics Service, April 1998.
2. S. F. Herb, P. Magidma, F. E. Lubby, and R. W. Riemenscheider, "Fatty Acids of Cow Milk (II). Compounds by Gas-Liquid Chromatography Aided by Fractionation," *Journal of the American Oil Chemists' Society* **39**, 142–149 (1962).
3. P. H. Haverkamp-Begemann and J. C. Koster, "4 Cis-heptenal—cream flavor component of butter," *Nature* **202**, 552–523 (1964).
4. J. A. Kintner and E. A. Day, "Major Free Fatty Acids in Milk," *Journal of Dairy Science* **48**, 1557–1581 (1965).
5. S. Patton and G. W. Kurtz, "A Note on The Thiobarbituric Acid Test for Milk Lipid Oxidation," *Journal of Dairy Science* **38**, 901 (1955).
6. J. E. Langler and E. A. Day, "Development and Flavor Properties of Methyl Ketone in Milk Fat," *Journal of Dairy Science* **47**, 1291–1296 (1964).
7. D. V. Josephson and F. J. Doan, "Cooked Flavor in Milk, Its Sources and Significance," *Milk Dealer* **29**, 35–40 (1939).
8. T. W. Keenan and R. C. Lindsay, "Evidence for a Dimethyl Sulfide Precursor in Milk," *Journal of Dairy Science* **51**, 122–128 (1968).
9. S. Patton, D. A. Forss and E. A. Day, "Methyl Sulfide and the Flavor of Milk," *Journal of Dairy Science* **39**, 1469–1473 (1956).
10. O. N. Parks and S. Patton, "Volatile Carbonyl Compounds in Stored Dry Whole Milk," *Journal of Dairy Science* **44**, 1–9 (1961).
11. H. T. Badings and R. Neeter, "Recent Advances in the Study of Aroma of Milk and Dairy Products," *Netherlands Milk and Dairy Journal* **34**, 9–30 (1980).
12. R. L. Bradley, Jr., "Effect of Light on Alteration of Nutritional Value and Flavor of Milk—A Review," *Journal of Food Protection* **43**, 314–320 (1980).
13. F. E. Kurtz, *Fundamentals of Dairy Chemistry*, The AVI Publishing Co., Inc., Westport, Conn. 1965, pp. 135–180.

14. International Dairy Foundation, *Bulletin 119*, International Dairy Foundation, Brussels, Belgium, 1980.

15. G. Kielwein and U. Dawn, "Occurrence and Significance of D(−)-Lactic Acid in Fermented Milk Products with Special Consideration of Yogurt Based Mixed Products," *Deutsche Molkerei Zeitung* **100**, 290–293 (1979).

16. K. M. Shahani, B. A. Friend, and R. C. Chandan, "Natural Antibiotic Activity of Lactobacillus acidophilus and bulgaricus," *Cult. Dairy Prod. J.* **18**, 1519 (1983).

17. R. K. Robinson, A. Y. Tamine, and L. W. Chubb, "Yogurt-Perceived Regulatory Trends in Europe," *Dairy Ind. Int.* **41**, 449–451 (1976).

18. M. Groux, "Component of Yogurt Flavor," *Le Lait* **53**, 146–153 (1973).

19. H. T. Badings and R. Neeter, "Recent Advances in the Study of Aroma Compounds of Milk and Dairy Products," *Netherlands Milk and Dairy Journal* **34**, 9–30 (1980).

20. M. L. Green and D. J. Manning, "Development of Texture and Flavor in Cheese and Other Fermented Products, *Journal of Dairy Research* **49**, 737–748 (1982).

21. E. B. Collins, "Biosynthesis of Flavor Compounds by Microorganisms," *Journal of Dairy Science* **55**, 1022–1028 (1972)

22. V. K. Steinholt, A. Svensen, and G. Tufto, "The Formation of Citrate in Milk," *Dairy Research Institute* **155**, 1–9 (1971).

23. E. H. Ramshaw, "Volatile Components of Butter and their Relevance to Its Desired Flavor," *Australian Journal of Dairy Technology* **29**, 110–115 (1974).

24. A. H. Woo and R. C. Lindsay, "Statistical Correlation of Quantitative Flavor Intensity Assessments of Free Fatty Acid Measurements for Routine Detection and Prediction of Hydrolytic Rancidity of Flavor in Butter," *Journal of Food Science* **48**, 1761–1766 (1983).

25. P. S. Dimick, N. J. Walker, and S. Patton, "Occurrence and Biochemical Origin of Aliphatic Lactones in Milk Fat—A review," *Journal of Agricultural Food Chemistry* **17**, 649–655 (1969).

26. J. E. Kinsella, S. Patton, and P. S. Dimick, "The Flavor Potential of Milk Fat. A Review of Its Chemical Nature and Biochemical Origins," *Journal of the American Oil Chemistry Society* **44**, 449–454 (1967).

27. R. Ellis and N. P. Wong, "Lactones in Butter, Butter Oil, and Margarine," *Journal of the American Oil Chemistry Society* **52**, 252–255 (1975).

28. G. Urbach, "The Effect of Different Feeds on the Lactone and Methyl Ketone Precursors of Milk Fat," *Lebensmittel Wissenschaft Technologie* **15**, 62–69 (1982)

29. K. H. Ney, "Recent Advances in Cheese Flavor Research," in G. Charalambous and G. Inglett, eds., *The Quality of Foods and Beverages*, Vol. 1, Academic Press Inc., New York, 1981, pp. 389–435.

30. S. L. Biede, A Study of the Chemical and Flavor Profiles of Swiss Cheese, Doctoral Dissertation, Department of Food Science, Iowa State University, Ames, Iowa, 1977.

31. K. H. Ney, "Bitterness and Gel Permeation Chromatography of Enzymatic Protein Hydrolysates," *Fette Seifen Anstrichmittel* **80**, 323–325 (1978).

32. H. T. Badings, "Causes of Ribes Flavor in Cheese," *Journal of Dairy Science* **50**, 1347–1351 (1967).

33. D. D. Bill and E. A. Day, "Determination of the Major Free Fatty Acids of Cheddar Cheese," *Journal of Dairy Science* **47**, 733–738 (1964).

34. T. J. Kristoffersen and I. A. Gould, "Cheddar Cheese Flavor, II., Changes in Flavor Quality and Ripening Products of Commercial Cheddar Cheese During Controlled Curing," *Journal of Dairy Science* **43**, 1202–1209 (1960).

35. B. A. Law, "New Methods for the Controlling Spoilage of Milk and Milk Products," *Perfumer & Flavorist* **7**, 9–13 (1983).

36. T. J. Kristoffersen, I. A. Gould, and G. A. Puruis, "Cheddar Cheese Flavor. III. Active Sulfhydryl Group Production During Ripening," *Journal of Dairy Science* **47**, 599–603 (1964).

37. L. M. Libbey and E. A. Day, "Methyl Mercaptan as a component of Cheddar Cheese," *Journal of Dairy Science* **46**, 859 (1963).

38. D. J. Manning, "Cheddar Cheese Micro Flavor Studies II. Relative Flavor Contribution of Individual Volatile Components," *Journal of Dairy Research* **46**, 531–539 (1979).

39. D. J. Manning, "Headspace Analysis of Hard Cheese," *Journal of Dairy Research* **46**, 539–545 (1979).

40. D. J. Manning, "Cheddar Cheese Aroma—The Effects of Selectively Removing Specific Classes of Compound from Cheese Headspace," *Journal of Dairy Research* **44**, 357–361 (1977).

41. D. J. Manning, "Sulfur Compounds in Relation to Cheddar Cheese Flavor," *Journal of Dairy Research* **41**, 81–87 (1974).

42. R. G. Buttery, D. G. Guadaghi, L. C. Ling, R. M. Seifert, and W. Lipton, "Additional Volatile Components of Cabbage, Broccoli and Cauliflower," *Journal of Agricultural Food Chemistry* **24**, 829–832 (1976).

43. W. A. McGugan, "Cheddar Cheese Flavor, A Review of Current Progress," *Journal of Agricultural Food Chemistry* **23**, 1047–1050 (1975).

44. M. E. Morgan, "The Chemistry of Some Microbially Included Flavor Detects in Milk and Dairy Foods," *Biotechnology and Bioengineering* **18**, 953–965 (1976).

45. T. O. Bosset and R. Liardon, "The Aroma of Swiss Gruyere Cheese, III. Relative Changes in the Content of Alkaline and Neutral Volatile Components During Ripening," *Lebensmittel Wissenchaft Technologie* 18–85, 178 (1985).

46. D. Anderson and E. A. Day, "Quantitative Analysis of the Major Free Fatty Acids in Blue Cheese," *Journal of Dairy Science* **48**, 248–249 (1965).

47. B. K. Dwivedi and J. E. Kinsella, "Continuous Production of Blue-Type Cheese Flavor by Submerged Fermentation of Penicillium Roquefort," *Journal of Food Science* **39**, 620–622 (1974).

48. R. C. Jolly and F. V. Kosikowski, "Flavor Development in Pasteurized Milk Blue Cheese by Animal and Microbial Lipase Preparations," *Journal of Dairy Science* **58**, 846–852 (1975).

49. M. Godinho and P. F. Fox, "Ripening of Blue Cheese. Influence of Salting Rate on Lipolysis and Carbonyl Formation," *Milchwissenschaft* **36**, 476–478 (1981).

50. T. Kanisawa and H. Itoh, "Production of Methyl Ketone Mixture from Lipolyzed Milk Fat by Fungi Isolated from Blue Cheese," *Nippon Shokuhin Kogyo* **31**, 483–487 (1984).

51. J. H. Nelson, "Production of Blue Cheese Flavor via Submerged Fermentation by Penicillium Roqueforti," *Journal of Agricultural Food Chemistry* **18**, 567–569 (1970).

52. E. A. Day and D. F. Anderson, "Gas Chromatographic and Mass Spectral Identification of Natural Components of the Aroma Fraction of Blue Cheese," *Journal of Agricultural Food Chemistry* 13–14, 2 (1965).

Charles H. Manley
Takasago International Corporation, USA, Inc.
Teterboro, New Jersey

DAIRY INGREDIENTS: APPLICATIONS IN MEAT, POULTRY, AND SEAFOODS

Meat and dairy products have several things in common. Both are appreciated from organoleptic and nutritional points of view. They have been traditionally regarded as two of the main protein sources for humans. In a modern diet, meat contributes about 35% of the protein intake, and milk about 25% (1).

Meat and dairy industries, furthermore, have a common interest in maintaining a positive image for protein of animal origin. However, in recent years there has been criticism about the fat content of meat and dairy products, and in some countries nutritional councils have recommended substituting vegetable protein for animal protein in the diet. In response to this criticism, meat and dairy manufacturers have succeeded in introducing low-fat products. A whole new range of meat and dairy foods is available, enabling the consumer to decide on the fat level desired in the diet. In addition to providing protein and fat, meat and dairy products contribute to a great extent to the intake of micronutrients. Meat is very important for the intake of iron and certain vitamins such as thiamin and riboflavin, while dairy foods are critical for the intake of calcium and vitamins.

Liquid or solid dairy products are widely used in the meat industry. They are mostly used with the objective to improve taste or eye appeal, eg, cheese topping on burgers, cordon bleu entrees, and cheese franks. Fresh dairy cream is often used in pâtés or liver products and in many seafood products. These applications are not within the scope of this article on functionality. The two most important ingredients from milk that find application in meat products are milk sugar (lactose) and predominantly milk protein. Milk protein causes functional advantages, while lactose is mostly used for taste improvement.

This article reviews and updates the application of these milk derivates in meat, poultry, and fish products. Scientific backgrounds will be highlighted.

PROCESSED MEAT PRODUCTS

Processed meat products are all meat-based products that need more than just cutting from the carcass. It is estimated that ca 25% of the worldwide meat production is being processed (Table 1). In these processed meats dairy ingredients can be used as functional ingredients.

Table 1. Production of Meat in Different Areas of the World (in 1,000 tons) 1987

Area	Total meat[a]
European community	29,985
United States	26,787
USSR	18,997
Total world	159,669

Source: Ref. 2

[a] Beef, veal, pork, poultry, and sheep expressed as gross carcass weight.

Meat products can be classified in many different ways. Usually they are categorized as noncomminuted, coarsely comminuted, and finely comminuted. The degree of comminution determines to a large extent the way of applying dairy ingredients (see Table 2).

DAIRY INGREDIENTS IN MEAT PRODUCTS

The two most important ingredients from milk that find application in meat are predominantly milk protein and to some extent the milk sugar or lactose.

Lactose

Water binding in coarse and noncomminuted meats such as pumped ham is improved by extraction and swelling of meat proteins. For optimum extraction of protein from meat, usually phosphate and salt are used. The concentration of these ingredients is limited for organoleptic reasons. The effective salt and phosphate concentration on water and so the ionic strength can be increased by increasing the total solids of the brine mixture. For this purpose lactose is often used. Lactose has advantageous effects on

Taste. Lactose has the capacity of masking the bitter aftertaste of salts and phosphates.

Stability. Lactose improves water binding and sliceability and enhances the cured color.

Yield. Lactose is therefore used in liver products, cooked hams, and cooked sausages. In sterilized products the addition of lactose is less desirable because of the browning reaction.

Lactose as an integrated part of whey powder or skim milk powder (smp) is being used in many meat products.

Table 2. Classification of Meat Product

Degree of comminution	Examples
None	Hams (cooked, smoked, dry), bacon, cooked loins, shoulders, reformed hams
Coarse	(Semi)dry sausages (salami, cervelat), fresh sausages (bratwurst), hamburgers, meat patties, meatballs, chicken nuggets
Finely	Frankfurters, hot dogs, polony, pariser, bologna, mortadella, liver sausages/spreads, meat loaves

Table 3. Ingredients in Final Product

Ingredient	Percent of final product
Phosphates	0.3
Nitrited salt	1.8
Lactose	1.2
Powdered egg white	0.3
Milk protein	Varying from 0–15
Sodium ascorbate	0.05

The protein from smp also contributes to some extent to the stability, but as this is present in micellar form, it is not optimum for emulsifying properties. Also, it is known that calcium may negatively influence the binding properties of meat proteins (3). Therefore Ca-reduced smp has found quite some application in meat products.

Milk Protein

The application of milk protein in the form of sodium caseinate has found broad acceptance in the meat industry (4). The functional properties of milk protein can be explained from its molecular structure (5) (Fig. 1).

Due to the high proline and the low sulfuric amino acids content, caseinates will have a random coil structure with a low percent helix. As a consequence, caseinates will show no heat gelation and denaturation and will have a high viscosity in solution.

Caseinates have a high electrical charge and have several very hydrophobic groups. This makes them perfect emulsifiers, with a strong preference for interfaces fat/water or air/water. The high charge makes them perfectly soluble in water.

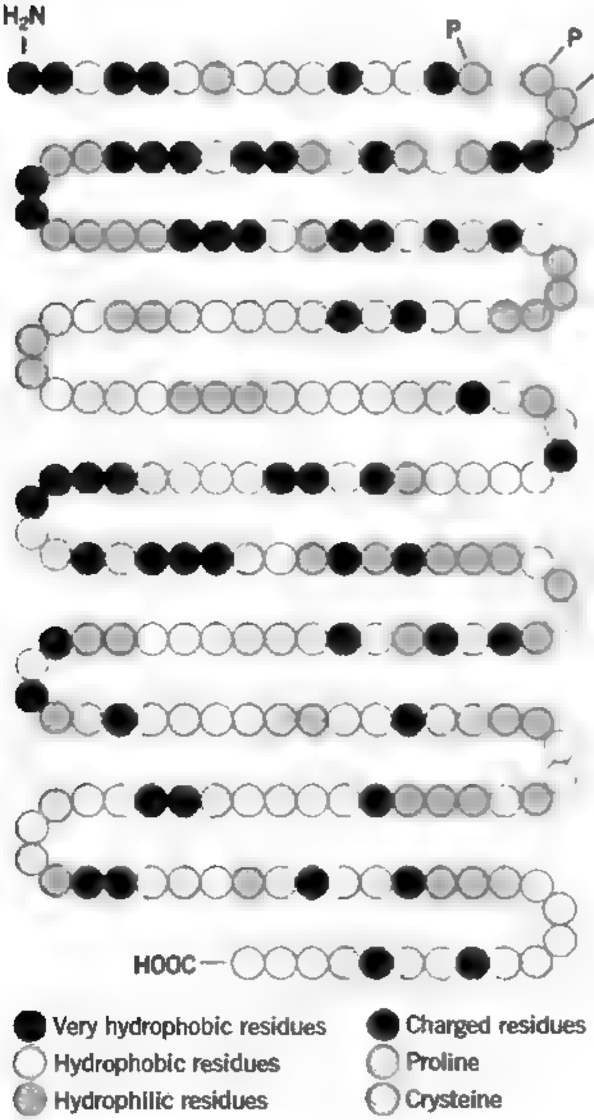


Figure 1. Amino acid sequence of β-casein A.

Caseinates build strong, flexible membranes that will hardly be influenced by heat. Whey proteins show a different pattern (Fig. 2). They are low in proline and have many S-S bonds, leading to a globular, strongly folded and organized structure. Whey proteins are sensitive to heat. During heating they unfold and, depending on pH and concentration, they will build intermolecular disulfide bonds resulting in gelation. The globular form is also the reason for the low viscosity in solution.

Whey proteins have a rather high hydrophobicity, which is more evenly distributed than in caseinate. Because of this structure, the application possibilities of whey proteins in meat products are very limited and will not be discussed in this article.

FINELY COMMINUTED MEAT PRODUCTS

The various finely comminuted meat systems, such as frankfurters, Vienna sausages, mortadellas, luncheon meat, chicken sausages, liver sausages, and pâtés, are commonly indicated as meat emulsions (6).

These products are generally prepared by chopping or grinding meat with water or ice, salt, and frequently phos-

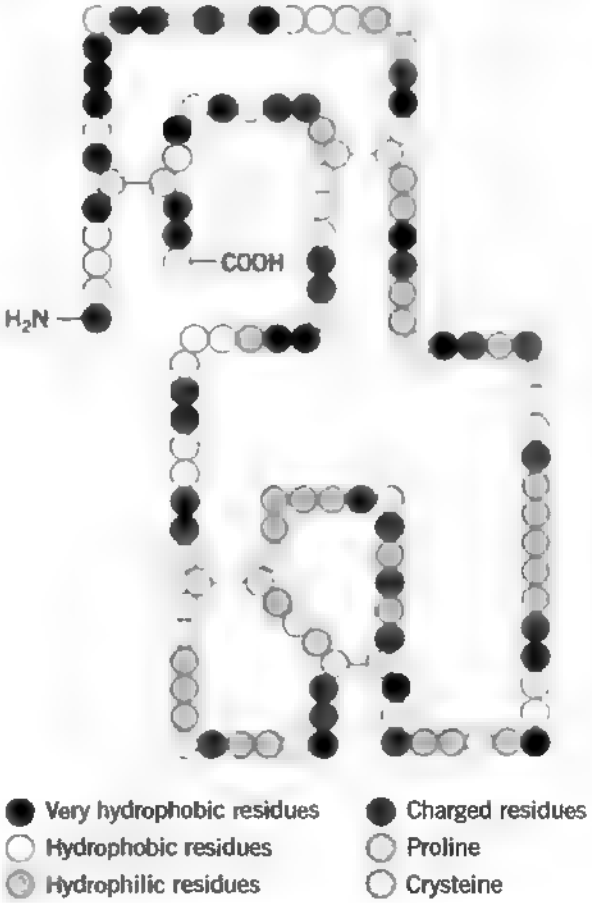


Figure 2. Amino acid sequence of α-lactalbumin B.

phates to a kind of cold meat slurry, then forming the matrix in which the animal fatty tissue and possibly connective tissue material is dispersed.

In general, the final temperature during processing does not exceed 15–18°C. Often flavorings, binders, or other additives are mixed in. Having this in mind, it will be clear that hardly any of the abovementioned finely comminuted products will show structures that are equal to true and simple emulsions as defined in physical chemistry.

The stability of finely comminuted meat products is—apart from production technology, formulation, the use of binders, etc—mainly determined by the quality of the meat cuts used (6,7).

Structure of Lean Meat

Lean meat generally contains about 20% protein, which can be divided into:

30–35% Sarcoplasmic proteins.
50–55% Myofibrillar or structural proteins (myosin, actin).
15–20% Stromal proteins (collagen, elastin).

The essential unit of all muscle is the fiber (8) (Fig. 3). Each fiber is surrounded by a membrane, the sarcolemma, and consists of a great number of parallel ordered myofibrils (8). In turn, each myofibril represents a similarly parallel-ordered structure of very thin protein threads, the actin- and the myosin filaments (Fig. 4).

Within the sarcolemma, surrounding the myofibrils, is the meat juice or sarcoplasma. This meat juice contains the water soluble meat proteins (wsp), consisting of enzymes, the red meat color myoglobin, and structured bodies. It is easily extracted on pressing, freezing/thawing, or chopping the meat. The myofibrillar proteins in warm slaughtered meat are present as free actin- and myosin filaments and are soluble in brine. Therefore, they are called the salt soluble proteins (ssp). In chilled and matured meat, however, the actin and myosin have gone into a reaction to form the complex actomyosin (7,8). This actomyosin is only partly soluble in brine, depending on the condition of the meat, age of the animal, (pre)slaughter conditions, anatomic location, mechanical treatment, etc.

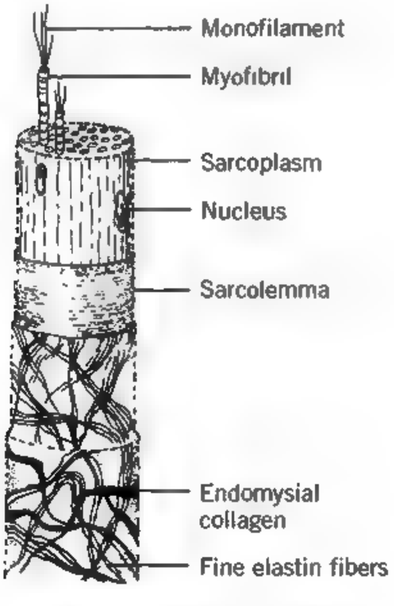


Figure 3. Sketch of a muscle fiber.

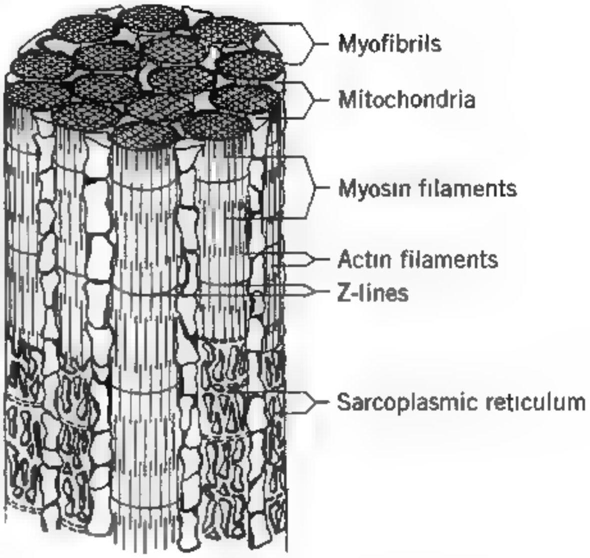


Figure 4. Section of a muscle fiber.

The nonsoluble part of the myofibrillar proteins is able to swell (take up water). In this postrigor condition the water- and fat-holding capacity of the meat are considerably lower than in warm slaughtered meat.

Meat Emulsions

As indicated in the introduction, hardly any of the abovementioned finely comminuted meat products is a true emulsion. In meat emulsion there are at least four different phase systems at the same time:

1. A true aqueous solution of salt, phosphates, nitrite, sugars, etc and a colloidal solution (sol) of sarcoplasmic (water-soluble) proteins and salt-solubilized myofibrillar proteins. This solution is the continuous phase of three different dispersed systems at a time.
2. A suspension of undissolved proteins and fatty tissue.
3. An emulsion containing emulsified fat droplets
4. A foam; during the comminution process some air is corporated.

This system has to be stabilized against heat treatments such as pasteurization or sterilization, against smoking, cooling, frying, reheating, vacuum treatments, etc—whatever physical stress is put upon the products during conserving, storing, and commercializing.

These stabilization properties are important, not only from the standpoint of production practices, but also from the standpoint of cost and quality characteristics, such as texture, consistency, bite, tenderness, juiciness, appearance, and palatability of the finished sausages.

The mechanism for the binding and stabilization phenomenon still is not completely understood, and there are several conflicting viewpoints expressed in the literature (8–15). It is, however, generally agreed on that the myofibrillar proteins are mainly responsible for this stability (8–10,16,17).

Stability of the Aqueous Matrix

The stability of the matrix is, apart from the effect of binders, determined by the water holding and gelling capacity of the meat proteins, and, more in particular, of the myofibrillar part. In relation to the stability of fresh sausage batters, the water-binding capacity of the nonsolubilized myofibrillar proteins is decisive. Changes in the net-charge of the proteins, resulting in either attraction or repulsion of the filaments, are responsible for shrinkage and swelling of the protein network and run parallel with the decrease or increase, respectively, in water holding (18).

This mechanism of water binding may be compared to that of a sponge. The more salt-soluble proteins are solubilized, the better the water binding will be.

Stability of the Suspended and Emulsified Fat

Animal fatty tissue consists of a cellular network in which the fat is enclosed. The network is built up of connective tissue and water. As long as these cell walls are intact, only minor fat separation will take place. However, on chopping the fatty tissue an increasing number of fat cells is damaged and more and more fat is set free. This free fat should be stabilized to prevent fat separation from the product. The origin of the fat (pork, beef, mutton, poultry, etc) and the anatomic location determine the amount of free fat during comminution and as a consequence the application of the fatty tissue.

Most beef, mutton, and chicken fat, as well as pork flare fat are hard to stabilize in finely comminuted meat products, unless they are pre-emulsified with a nonmeat protein such as sodium caseinate (13,19,20).

Pork fats other than flare fat (back fat, shoulder fat) are softer and consequently can be used directly in the production of the meat emulsion, because there is more fat in a liquid state.

Particularly in finely comminuted meat products, the interaction between the free fat and the meat proteins plays an important role in the stabilization of both fat and water (14).

The salt-soluble myofibrillar meat proteins have excellent emulsifying properties and are quickly preferentially absorbed in the fat/water interface (21). The sarcoplasmic proteins are relatively unimportant in this respect. In addition to their gelling capacity and their role in water binding, this emulsification is another reason to aim at a sufficient extraction of these myofibrillar proteins during first stage of chopping.

However, when the myofibrillar meat protein enters the fat/water interface, the protein structure is altered and, as a result, the myofibrillar protein is no longer capable of gel formation and water binding (8). This means that the consumption of ssp for the emulsification of free fat goes at the expense of the water binding

Milk Proteins in Meat Emulsions

Milk protein, type sodium caseinate, is a perfect emulsifying protein, which is very strongly attracted by the fat/water interface (12). If this type of milk protein gets the opportunity to surround the free-fat particles during sausage manufacture and before the myofibrillar (ssp) proteins do this, the latter are saved for denaturation in the interface.

Research has proved that milk protein, type sodium caseinate, indeed is better and more quickly absorbed in the oil/water interface when emulsification takes place when both milk proteins and meat proteins are present in the continuous phase (Fig. 5).

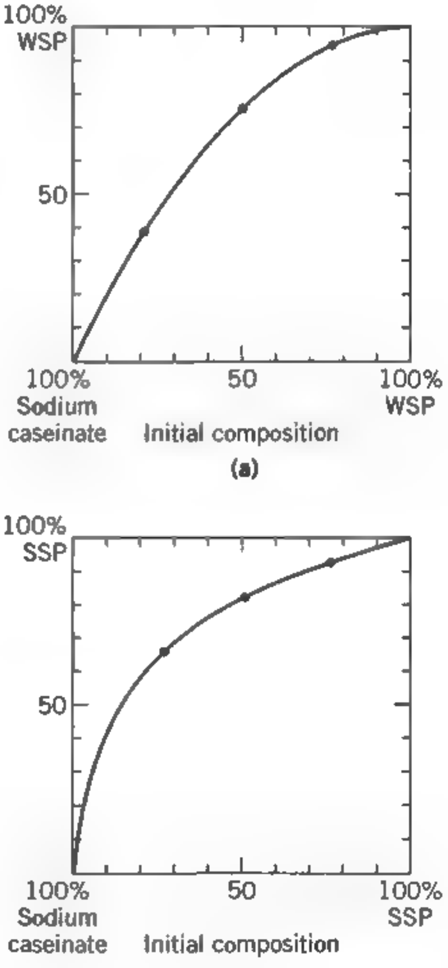


Figure 5. The preferential adsorption of milk protein, sodium caseinate over water soluble protein (WSP) and salt soluble protein (SSP), in a fat/water/protein emulsion; (**a**) the initial composition of the protein soluble mixtures is shown on the abscissae; (**b**) the composition of the protein solution after removal of the emulsified fat is shown on the ordinates.

In this way the abovementioned loss in gelling capacity is avoided. As milk protein is doing the fat binding, the meat proteins can use their full power in water binding and texture formation. In this way sodium caseinate contributes directly to a better fat binding and indirectly to an improved water binding and texture formation (20–22).

Milk proteins can be applied in three ways:

1. Addition of the powder at the beginning of the comminution process. When milk protein is used in this way, it is important that the addition takes place just before the addition of water/ice. In that way the protein is optimally hydrated; this hydration is very important for its functionality.
2. Addition as a jelly. The milk protein is predissolved in water in a bowl chopper or colloid mill. A common ratio is 1 part milk protein dissolved in 6 parts water.
3. In the form of a pre-emulsion. These pre-emulsions consist of milk protein, fat, and water. Technologically difficult to stabilize fats can be used perfectly by this pre-emulsifying technique (eg, beef suet and flare fat). A common ratio of milk protein:fat:water is 1:5:5. Addition levels of these emulsions to meat emulsions can vary from 10 to 25% maximum.

Temperature control during the production of finely comminuted meat products is very important; when temperatures at the end of comminution exceed 15–18°C, the stability of the final product is affected very negatively. This means that there is little tolerance in processing.

A unique feature of milk proteins is their capacity to widen the processing tolerance as far as temperatures are concerned. This is illustrated in Figure 6.

SECTIONED AND FORMED MEAT PRODUCTS

Principles of Production

Sectioned and formed products are made by mechanically working meat pieces to disrupt the normal muscle cell structure (16). This produces a creamy, tacky exudate on the surface of the meat pieces. When the product is heated during thermal processing, this exudate binds the meat pieces together; during this heating process the solubilized salt-soluble proteins (mainly myosin) are denaturated and form a gel (23–27). The mechanical action of tumbling and massaging primarily affects external tissues to produce the surface exudate. Some internal tissue disruption also occurs, which explains the enhanced tenderness, brine penetration and distribution, and improved water-holding capacity. The protein exudate is not only produced by the mechanical action during massaging and tumbling, but also by the synergistic effect of addition of salt combined with alkaline phosphates, which improves yields and maximizes myofibrillar protein solubilization.

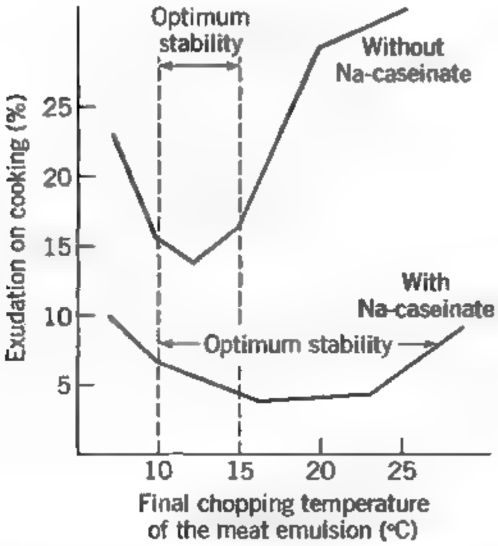


Figure 6. Influence of Na-caseinate on the stability of a meat emulsion in dependence of the final chopping temperature.

Optimum product quality is achieved with the addition of brine, which produces a final product containing 2 to 3% salt and 0.3 to 0.5% phosphate.

Dairy Ingredients

In the production of reformed hams both lactose and milk proteins (sodium caseinate) are very useful ingredients. Lactose can be used in levels varying from 0.5 to 2%. When lactose is used, it serves as a water binder, thereby increasing the yield. It has the capacity of masking the bitter aftertaste of phosphates, giving the final product a more delicate flavor. Milk protein or milk protein hydrolysates can be used in levels varying from 0.8 to 1.6% by dissolving them in the brines used for injection of the meat pieces. Milk protein strongly affects the binding between the meat pieces, thereby an improved sliceability is obtained and they have a positive effect on yield. The effects on yield after cooking as function of milk protein percentage in the final product is presented in Figure 7.

INTERACTION OF MILK PROTEINS WITH MEAT PROTEINS

The results of application research indicate that there is a specific interaction between milk protein and meat proteins. To study this effect we have to make a classification of proteins present in meat. Meat protein can be divided

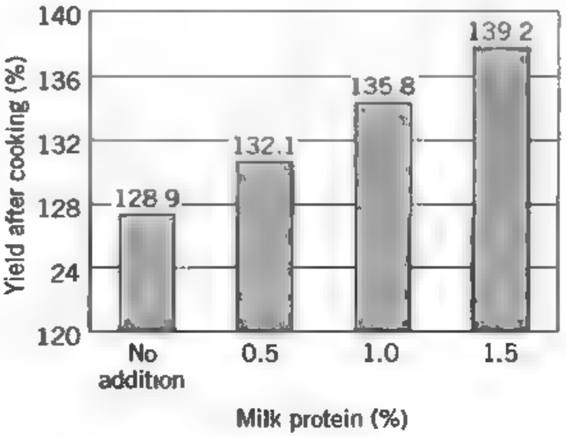


Figure 7. Milk proteins in reformed cooked ham (60% injection).

into different groups according to their specific function and solubility in different solvents (Table 4).

To study the effect of interaction between milk protein and salt-soluble meat proteins, lean meat was extracted according to the extraction procedure shown in Figure 8. Sixty-three percent lean meat (*M. semimembranosis*) is extracted with 2% salt and 35% ice/water in the bowl chopper. After dilution with 200% brine (2% salt), the lean meat slurry is centrifuged.

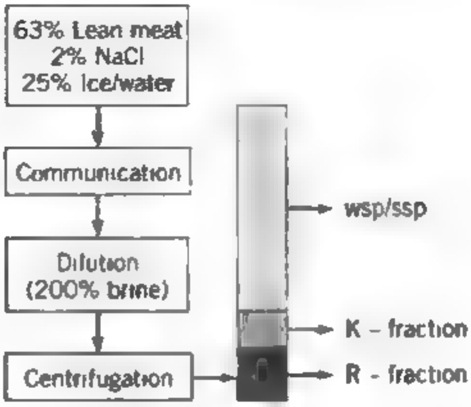


Figure 8. Fractionation of meat proteins.

After centrifugation there are three distinct layers in the centrifuge tube:

Upper layer: contains wsp (sarcoplasmic proteins) and the salt-soluble myofibrillar proteins.

K-fraction: contains nonsolubilized and swollen myofibrillar proteins, mainly actomyosin.

R-fraction: contains connective tissue materials.

The gellification of wsp/ssp proteins was tested in the following solution:

3% ssp/wsp proteins.

3% NaCl.

2% milk protein.

The equipment for these tests is the gelograph (Fig. 9).

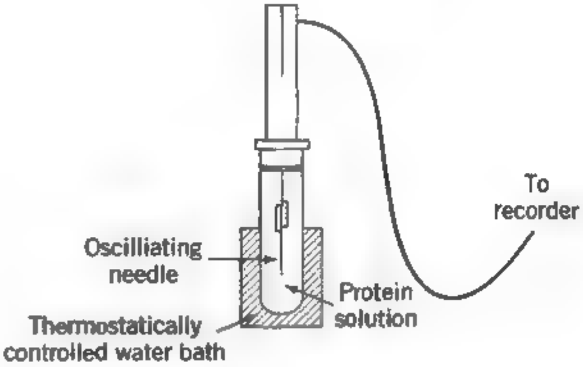


Figure 9. The gelograph.

The principle of this gelograph is as follows: A protein solution that is placed in a waterbath is slowly heated (1°C/min). During this heat treatment the viscosity/gel strength of the solution is continuously measured by means of an oscillating needle; this equipment operates in a nondestructive way; this means the gel structure that is formed during the heating of the solution is not destroyed because of minimal oscillating of the needle. In this way it is possible to imitate the gelling of meat proteins in pasteurized meat products (eg, a cooked ham).

An example is a product containing 60% lean meat, which contains about 12% total meat protein. Roughly 50% of this protein is myofibrillar proteins. This is about 6%. Suppose that 50% of total myofibrillar proteins are extracted during massaging/tumbling. This means that the concentration of ssp will be around 3%. This is the same concentration as used in the model experiments. Results of gelograph experiments are presented in Figures 10 and 11.

Figure 10 shows the gel strength of a 3% wsp/ssp as a function of temperature. The temperature starts at 20°C (68°F) and is increased at a rate of 1°C/min; during this temperature increase the gel strength is measured as miligels. At 44°C (110°F) there is a sudden increase in gel strength; this is caused by the aggregation of myosin molecules (24). At higher temperatures the viscosity decreases again. The reason for this phenomenon is not yet exactly known (23,26,27).

At a temperature of 57°C there is again an increase in gel strength. With further heating of the solution to 75°C the gel strength reaches a constant value of ±150 m gels. Cooling down the solution results in a final gel strength of ±200 m gels. The same experiments are done in the presence of 2% milk proteins and 2% soy protein isolate. The results are presented in Figure 12. A conclusion from this figure—addition of 2% milk protein (which has no gellifying capacity itself) results in a much higher gel strength of a heated meat protein solution.

This result supports the theory that there is a synergistic effect of milk proteins on the gellifying capacity of salt-soluble meat proteins, either milk proteins play a role in the cross-linking of myosin molecules or they absorb a significant amount of water, which results in a higher net concentration of myosin molecules. Other nonmeat proteins

Table 4. Classification of Meat Proteins

Protein	Percent	Role	Solubility	Gellifying properties	Emulsifying properties
Sarcoplasmic	30–35	Metabolic	Water	Weak	Weak
Stromal	15–20	Connective	Insoluble	No	Weak
Myofibrillar	50–55	Contractile	Salt soluble	Strong	Strong

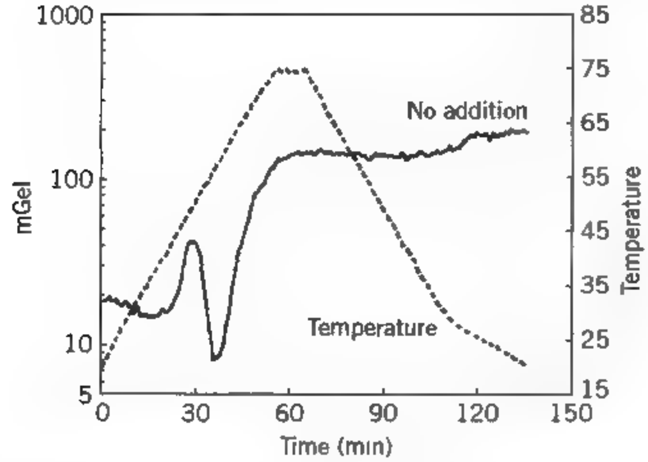


Figure 10. Results of an experiment on gelograph meat protein (no addition).

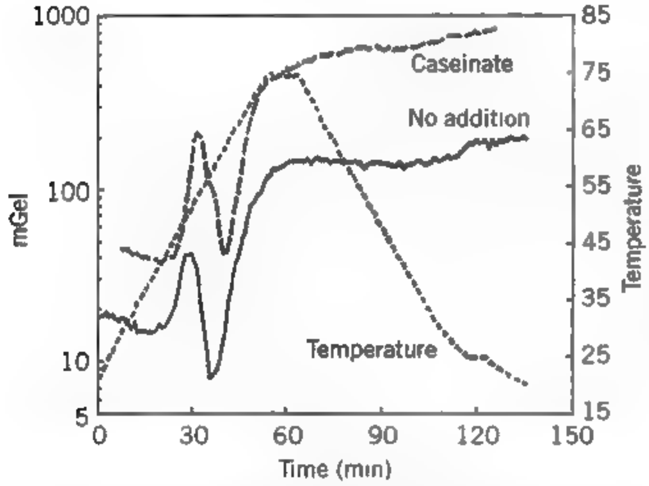


Figure 11. Results of an experiment on gelograph meat protein (no addition, caseinate).

only partially have this specific function (eg, soy protein isolate) or do not have it at all.

POULTRY

Poultry Consumption

Whereas the consumption of red meat is stable or even decreasing, poultry consumption has grown impressively over the last decade. The main reasons for the increase of consumption of poultry are a healthy image (lean, low in fat and cholesterol), increase in the amount of further-processed poultry meat (convenience), and relatively cheap source of animal protein.

The consumption of poultry per capita in different areas of the world is given in Table 5.

Poultry Rolls and Poultry Bolognas

Poultry rolls and poultry bolognas are in appearance very similar to their red meat counterparts. They can be manufactured from materials such as trimmings, skin, fat, and mechanically deboned poultry (mdp). The basic difference between a poultry roll and a poultry bologna is that the bologna sausage contains curing salt and is finely emulsified (20). Poultry rolls normally have a certain ratio of fine meat emulsion to meat chunks.

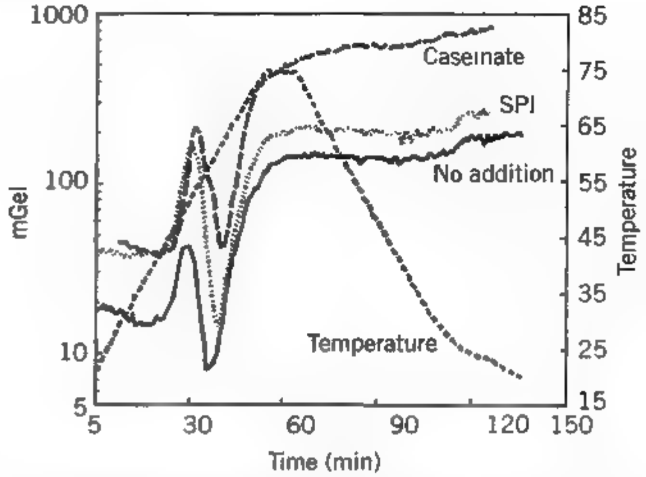


Figure 12. Results of an experiment on gelograph meat protein (no addition, caseinate, SPI).

Table 5. Per Capita Consumption of Poultry, 1982–1987 (kg)

Area	1982	1983	1984	1985	1986	1987
United States	29.3	29.9	30.5	32.0	33.1	35.6
European community	15.5	15.6	15.6	15.9	16.2	16.9
USSR	9.6	9.4	9.5	10.3	10.7	11.2
Japan	11.1	11.4	11.8	12.2	12.9	13.6

Source. Ref. 2.

It is important to create an even color appearance within the roll, ie, the color of the surrounding fine emulsion must match the color of the meat chunks. To obtain this even and white color, it is recommended that milk proteins be used in both basic components: part in the fine emulsion and part mixed into the meat chunks.

Some chicken or turkey bolognas are made from 100% mechanically deboned poultry. From a technologic point of view the poultry meat emulsion is not very different from the red meat emulsion. Sometimes poultry bolognas can have a dry mouthfeel. This can be corrected by the addition of approximately 10% chicken skin/fat emulsion. Such a pre-emulsion is made of 1 part milk protein (sodium caseinate), 5 parts chicken skin, 5 parts chicken fat, 6 parts water, and 2 parts ice.

Without the assistance of such a prestabilized fat/skin emulsion, fatting out often occurs when the total fat content reaches 20% or higher. The addition of milk protein as a dry powder to such products (addition level generally between 1.0 and 1.5%) improves the consistency of the final product as well; generally firmer and better sliceable products are obtained.

Chicken Nuggets and Patties

Since their introduction in the early 1980s, these poultry items have become very popular. Initially, a chicken nugget was a solid piece of slightly marinated breast meat that was battered and breaded (20).

Increased demand has forced the poultry processor to use such parts as thigh meat, trimming, mechanically deboned meat, and chicken skin. The target in combining all these ingredients in a nugget or a patty is to reach the appearance, flavor, and bite of the original product as closely as possible. Important in the stabilization of the final product is the binding of the meat pieces, the water holding capacity, and the stability of the fat.

Again, some basic rules in meat technology are very important: for optimal binding of the meat pieces, it is necessary to grind the meat before use; this creates relatively small meat pieces with a large surface area. During the mixing stage, when salt and phosphates are added, it is important that sufficient meat proteins are extracted and become hydrated in the continuous phase (28–30). On frying, this continuous phase will gellify and cement the meat pieces together. When milk protein is being used in these systems, they boost the meat protein gelation, resulting in higher yields and better consistency of the final products.

Products based on lean meat exclusively tend to be dry and tough after frying. By incorporation of prestabilized chicken fat/skin emulsion (usually 10%), flavor, consistency, and juiciness can be improved.

Whole Muscle Poultry Products

One of the most popular food items in this group of poultry products is breast of turkey. Essentially the manufacturing process comes very close to the production process of reformed hams. Important differences are: lower injection levels are applied (up to 25%); lower salt concentrations are being used; no curing ingredients are being used, because a white color is desired; and the tumbling process is generally much shorter and less intense than that practiced in ham manufacture.

For the production of breast of turkey both milk protein (type sodium caseinate) and milk protein concentrates are used frequently. Usage levels vary from 0.8 to 1.5% (calculated on the final product). Yield increases from 3.0 to 6.0% can be reached. At the same time the consistency, binding, and sliceability of the product are improved. One of the most important characteristics of milk proteins in comparison to, eg, carbohydrate-based stabilizers is the fact that milk proteins do not affect the original flavor and palatability of the final product.

SEAFOOD

Since the growing interest in food and health in general, seafood items have become very popular over the last 10 years. The major reasons for the impressive increase in seafood consumption are sea foods are low in fat, high in protein, contain high percentages of polyunsaturated fatty acids, and most sea foods are relatively cheap.

Although fish and meat are two completely different sources of protein, there is a striking similarity from a technologic point of view. Muscle fibers in seafood are almost identical to fibers in red meat (31). A remarkable difference is the very low content of collagen protein in fishmeat. This is the main reason for its softer texture and bite compared to red meats.

Because of the similarity between red meat and fishmeat structure a lot of processing technologies from the red meat industry can be transferred to processed seafood. This is especially true for fish sausages, fish nuggets, and fish burgers.

Table 6. Water-Packed Tuna

Component	Percent of total
Steam-cooked tuna	75
Brine	25
Brine formula	
Water	86
Salt	4
Milk protein hydrolysate	10

Canned Tuna

Canned tuna, but eg, also canned salmon, mackerel, and sardines, has been on the market for years as preserved fish with a long shelf life. For a good heat transfer and maintenance of the natural appearance and taste during sterilization, it is absolutely necessary that the fish is canned with a liquid as heat transfer medium. Until recently the liquid used was usually oil, but as a result of changes in the food consumption pattern and health consciousness of people, an aqueous solution has been applied more frequently in recent times. The switch from oil to an aqueous solution is naturally associated with some changes in product characteristics, and it is apparent in practice that milk protein hydrolysate is a perfect ingredient for stabilizing the canned product in respect to flavor, juiciness, and yield.

In the preparation of canned tuna and other species of fish the starting point is usually precooked fish. After steam-cooking the fish is cooled and bones, skin, and the dark fish flesh are removed carefully and the fish is suitable for canning. A formula for water-packed tuna is given in Table 6.

Milk Protein Functionality

A light color, mild flavor, juicy texture, and good water penetration during sterilization are extremely important characteristics of premium quality canned tuna. Milk protein hydrolysate promotes the juciness of the fish structure, gives a mild taste, and has an excellent effect on the water retention of the tuna during sterilization. Adding 10% milk hydrolysate, as indicated in the brine formula, improves the drain weight of the tuna by 6 to 8%. This means that milk proteins not only effect the quality of the final product, but have important economic advantages as well (30).

BIBLIOGRAPHY

1. R. Hermus and H. Albers, "Meat and Meat Products in Nutrition," *Proceedings of 32nd European Meeting of Meat Research Workers*, Gent, Belgium, 1986.

2. ZMP, Bilanz 1987, Zentrale Markt- und Preisberichtstelle für Erzeugnisse der Land-, Forst- und Ernährungswirtschaft GmbH, Bonn, FRG, 1988.
3. F. M. W Visser, "Dairy Ingredients in Meat Products", *Proceedings of the International Dairy Federation Seminar: Dairy Ingredients in the Food Industry*, Luxembourg, May 19–21, 1981.
4. S. S. Gulayev-Zaitsev, "The Use of Caseinates in Foods," in Organizing Committee of the XXII International Dairy Congress, ed., *Milk the Vital Force*, D. Reidel Publishing Company, Dordrecht, Holland, 1986.
5. P. Walstra and R. Jenness, *Dairy Chemistry and Physics*, John Wiley & Sons, Inc. New York, 1984.
6. P. V. Farrant, "Muscle Proteins in Meat Technology", in P. F. Fox and J. J. Cordon, eds., *Food Proteins*, Applied Science Publishers Ltd , New York, 1982
7. R. Hamm, *Kolloidchemie des Fleisches*, Paul Parey, Berlin and Hamburg, 1972.
8. J. Schut, "Meat Emulsions" in S. Friberg, ed., *Food Emulsions*, Marcel Dekker Inc., New York, 1976.
9. P. M. Smith, "Meat Proteins: Functional Properties in Comminuted Meat Products," *Food Technology* 4, 116–121 (1988).
10. N. L. King and J. J. MacFarlane, "Muscle Proteins," in A. M. Pearson and T. R. Dutson, eds., *Advances in Meat Research, Restructured Meat and Poultry Products*, Vol. 3, Van Nostrand Reinhold Co., New York, 1987.
11. J. Schut, F. Visser, and F. Brouwer, "Microscopical Observations During Heating of Meat Protein Fractions and Emulsions Stabilized by These," *Proceedings of 25th European Meeting of Meat Research Workers*, Budapest, Hungary, Aug. 27–31, 1979, Paper 6.12.
12. J. Schut, "Zur Emulgierbarkeit von Schlachtfetten bei der Herstellung von Brühwurst," *Fleischwirtschaft* **48**, 1201–1204 (1968).
13. J. Schut and F. Brouwer, "The Influence of Milk Proteins on the Stability of Cooked Sausages," *Proceedings of 21st European Meeting of Meat Research Workers*, Aug. 31–Sept. 5, Bern, Switzerland, 1975, pp. 80–82
14. J. Schut, F. Visser, and F. Brouwer. "Fat Emulsification in Finely Comminuted Sausage. A Model System," *Proceedings of 24th European Meeting of Meat Research Workers*, Kulmbach, FRG, 1978, paper W 12.
15. S. A. Ackerman, C. Swift, R. Carroll, and W Townsend, "Effects of Types of Fat and of Rates and Temperatures of Comminution on Dispersion of Lipids in Frankfurters," *Journal of Food Science* **36**, 266–269 (1971).
16. L. Knipe, "Sectioned and Formed Meat Products," *Proceedings Annual Sausage and Processed Meat Short Course*, Ames, Iowa, 1982, Res. Paper, 2113 E-7.
17. P. A. Morrisey, D. M. Mulvihill, and E. O'Neill, "Functional Properties of Muscle Proteins", in B. J. F. Hudson, ed., *Development in Food Proteins*, Vol. 5, Elsevier Applied Science, London, 1987.
18. J. E. Kinsella, "Relationships between Structure and Functional Properties of Food Proteins," in P. F. Fox and J. J. Gordon, eds., *Food Proteins*, Applied Science Publishers Ltd., New York, 1982.
19. R. Hamm and J. Grambowska, "Proteinlöslichkeit und Wasserbindung unter den in Brühwurstbräten gegebenen Bedingungen, Part II," *Fleischwirtschaft* **58**, 1345–1348 (1978).
20. H. Hoogenkamp, *Milk Protein. The Complete Guide to Meat, Poultry, and Sea Food*, DMV Campina bv., Veghel, Holland, 1989.
21. J. Schut and F. Brouwer, "Preferential Adsorption of Meat Proteins during Emulsification," *Proceedings of 17th European Meeting of Meat Research Workers*, Bristol, UK, 1971
22. A. S. Bawa, H. Z. Orr, and W. R. Usborne, "Evaluation of Synergistic Effects Obtained in Emulsion Systems for the Production of Wieners, *Journal of Food Science Technology* **5**, 285–290 (1988).
23. A. Asghar, K. Samejima, and T. Yasui, "Functionality of Muscle Protein in Gelation Mechanisms of Structured Meat Products," *CRC, Critical Reviews in Food Science and Nutrition* **22**, 27–106 (1985).
24. E. A. Foegeding and T. C. Lanier, "The Contribution of Non Muscle Proteins to Texture fo Gelled Muscle Protein Foods," in R. D. Philips and J. W. Fixley, eds., *Protein Quality and the Effects of Processing* Marcel Dekker Inc., New York, 1989.
25. B. Egelandsdal, K. Fretheim, and O. Honbitz, "Dynamic Rheological Measurements on Heat Induced Myosin Gels. An Evaluation of the Methods Suitable for the Filamentous Gels," *Journal of the Science of Food and Agriculture* **37**, 944–954, 1986.
26. M. K. Knight, "Interaction of Proteins in Meat Products: Part III, Functions of Salt Soluble Protein, Insoluble Myofibrillar Protein and Connective Tissue Protein in Meat Products," Research report no. 630, Leatherhead Food R. A., Leatherhead, UK, 1988.
27. G. R. Ziegler and J. C. Acton, "Mechanisms of Gel Formation by Proteins of Muscle Tissue," *Food Technology* **5**, 77–82 (1984).
28. K. Samejima, M. Ishioroshi, and T. Yasui, "Relative Roles of the Head and Tail Portions of the Molecule in Heat Induced Gelation of Myosin," *Journal of Food Science* **46**, 1412–1418 (1981).
29. J. A. Dudziak, E. A. Foegeding, and J. A. Knopp, "Gelation and Thermal Transitions in Post Rigor Turkey Myosin/Actomyosin Suspensions," *Journal of Food Science* **53**, 1278–1281 (1988).
30. D. G. Huber and J. H. Regenstein, "Emulsion Stability Studies of Myosin and Exhaustively Washed Muscle from Adult Chicken Breast Muscle," *Journal of Food Science* **53**, 1282–1293 (1988).
31. J. Jongsma, *Milk Proteins in Processed Sea Food*, DMV Campina bv., Veghel, Holland, 1987.

GENERAL REFERENCES

M. Anese and R. Gormley, "Retaining Quality in Frozen Fish Mince," *Farm & Foods* **5**, 13–15 (1995).

M. Anese and R. Gormley, "Effects of Dairy Ingredients on Some Chemical, Physico-chemical and Functional Properties of Minced Fish During Freezing and Frozen Storage," *Lebensm.-Wiss. Technol.* **29**, 151–157 (1996).

P. Baardseth et al., "Dairy Ingredients Effects on Sausage Sensory Properties Studies by Principal Component Analysis," *J. Food Sci.* **57**, 822–828 (1992).

B. C. Beuschel, J. A. Partridge, and D. M Smith, "Insolubilized Whey Protein Concentrate and/or Chicken Salt-soluble Protein Gel Properties," *J. Food Sci.* **57**, 852–855 (1992).

A. M. Bhoyar et al., "Effect of Extending Restructured Chicken Steaks With Milk Co-precipitates," *Journal of Food Science and Technology India* **35**, 90–92 (1998).

H. K. Cheng, E. Kolbe, and M English, "A Nonlinear Programming Technique to Develop Least Cost Formulations of Surimi Products," *Journal of Food Process Engineering* **20**, 179–186 (1997).

S. Cunningham, "Marketing of Dairy Ingredients. Part 1," *World of Ingredients* Jan/Feb., 22–23, 25, 27 (1995).

S. Cunningham, "Marketing of Dairy Ingredients. Part II," *World of Ingredients* March/April, 38–41 (1995).

B. N Dobson and D. P. Cornforth, "Nonfat Dry Milk Inhibits Pink Discoloration in Turkey Rolls," *Poultry Science* **71**, 1943–1946 (1992)

B. N. Dobson et al., "Instrument for Measuring Bind Strength of Restructured and Emulsion-Type Meat Products," *J. Texture Stud.* **24**, 303–310 (1993).

M. R. Ellekjaer, T Naes, and P. Baardseth, "Milk Proteins Affect Yield and Sensory Quality of Cooked Sausages," *J. Food Sci.* **61**, 660–666 (1996).

S. B El-Magoli, S Larola, and P. M. T. Hansen, "Ultrastructure of Low-Fat Ground Beef Patties With Added Whey Protein Concentrate," *Food Hydrocolloids* **9**, 291–306 (1995).

S. B. El-Magoli, S. Larola, and P. M. T. Hansen, "Flavor and Texture Characteristics of Low Fat Ground Beef Patties Formulated With Whey Protein Concentrate," *Meat Science* **42**, 179–193 (1996).

J. B. German and C. J. Dillard, "Fractionated Milk Fat: Composition, Structure, and Functional Properties," *Food Technol* **52**, 33–34, 36–38 (1998).

T. P. Guinee and M. O. Corcoran, "Expanded Use of Cheese in Processed Meat Products," *Farm & Food* 4, 25–28 (1994).

A. Hendrickx, "New Protein Additive for Meat and Poultry Products: Milk-Based Functional Protein Product," *Fleischerei* **43**, 880, 883–884 (1992).

C. K. Hsu and E. Kolbe, "The Market Potential of Whey Concentrate as a Functional Ingredient in Surimi Seafoods," *Journal of Dairy Science* **79**, 2146–2151 (1996).

L. King, "Whey Protein Concentrates as Ingredients," *Food Technology Europe* **3**, 88–89 (1996).

A. Konno and M. Kimura, "Effects of Addition of Whey Protein Concentrates on the Water-Retention Properties and Texture of Ham," *Journal of Japanese Society of Food Science and Technology* **40**, 427–432 (1993).

H. A. M. Lemmers, "The Use of Dairy Ingredients in Further Processed Poultry Products," *Fleischerei* **42**, III–VI (1991).

P. H. Lyons et al., "The Influence of Whey Protein/Carrageenan Gels and Tapioca Starch on the Textural Properties of Low Fat Pork," *Meat Science* **51**, 43–52 (1999).

L. Mancini, "Dairy Ingredients, The natural Choice for Versatility," *Food Engineering* **64**, 73–75 (1992).

A. McCord, A. B. Smith and E. E. O'Neill, "Heat-Induced Gelation Properties of Salt-Soluble Muscle Proteins as Affected by Nonmeat Proteins," *J. Food Sci.* **63**, 580–583 (1998).

L. G. Phillips, M. J. Davis and J. E. Kinsella, "The Effects of Various Milk Proteins on the Foaming Properties of Egg White," *Food Hydrocolloids* **3**, 163–174 (1989).

E. Pitts, "The European Market for Dairy Ingredients," *Journal of the Society for Dairy Technology* **48**, 79–85 (1995).

K. Piyachomkwan and M. H. Penner, "Inhibition of Pacific Whiting Surimi-Associated Protease by Whey Protein Concentrate," *J. Food Prot.* **18**, 341–353 (1995).

C. Ramesh, *Dairy-Based Ingredients*, Eagan Press, Minn., 1997.

D. M. Smith and A. J. Rose, "Properties of Chicken Salt-Soluble Protein and Whey Protein Concentrate Gels as Influenced by Sodium Tripolyphosphate," *Poultry Science* **74**, 169–175 (1995).

S. Solberg, "Effective Product Development for Sausages," *Livsmedelsteknik* **38**, 42–43 (1996).

H. Y. Tan and D. M. Smith, "Dynamic Rheological Properties and Microstructure of Partially Insolubilized Whey Protein Concentrate and Chicken Breast Salt-Soluble Protein Gels," *J. Agric. Food Chem.* **41**, 1372–1378 (1993).

L. Thorsen and T. Bergersen, "Quantitation of Milk Proteins in Heat-Treated Comminuted Meat Products," *InformMAT* **2**, 62–64 (1989).

P. Walkenstrom and A. M. Hermansson, "Fine-Stranded Mixed Gels of Whey Proteins and Gelatin," *Food Hydrocolloids* **10**, 51–62 (1996).

P. Walkenstrom and A. M. Hermansson, "High-Pressure Treated Mixed Gels of Gelatin and Whey Proteins," *Food Hydrocolloids* **11**, 195–208 (1997).

P. Walkenstrom and A. M. Hermansson, "Mixed Gels of Gelatin and Whey Proteins, Formed by Combining Temperature and High Pressure," *Food Hydrocolloids* **11**, 457–470 (1997).

V. C. Weerasinghe et al., "Whey Protein Concentrate as a Proteinase Inhibitor in Pacific Whiting Surimi," *J. Food Sci.* **61**, 367–371 (1996).

J. N. de Wit, "Nutritional and Functional Characteristics of Whey Proteins in Food Products," *Journal of Dairy Science* **81**, 597–608 (1998).

O. Zorba et al., "The Possibility of Using Fluid Whey in Comminuted Meat Products: Capacity and Viscosity of the Model Emulsions Prepared Using Whey and Muscle Protein," *Z. Lebensm.-Unters. Forsch.* **200**, 425–427 (1995).

MARTIEN VAN DEN HOVEN
Creamy Creations
Rijkevoort, The Netherlands

See also DAIRY INGREDIENTS FOR FOODS; WHEY: COMPOSITION, PROPERTIES, PROCESSING, AND USES.

DAIRY INGREDIENTS FOR FOODS

Milk and its products have been consumed by many generations of people of Western, Middle Eastern, Indian, and some African cultures. The nutritional value of dairy materials, properly handled, has been proven by widespread use, and their safety is assured by processing procedures that have been introduced and refined since the time of Pasteur. The great bulk of dairy products today recognize traditions of simple processes and physical separations, avoiding chemicals and thereby preserving the natural properties and the very desirable connotations of traditional products.

The more recently developed food-processing industries have generated requirements for sophisticated performance of dairy materials as ingredients for both familiar and fabricated foods. Milk production does not require the use of pesticides and hormones. Well-managed herds can produce milk with contaminant levels far below the requirements of regulatory authorities. Active components that directly inhibit the processes of digestion, metabolism, and assimilation of nutrients are not present in milk.

The nature of milk and its components is probably better characterized than the properties of any other basic food. Consequently, the ability to transform milk and its individual components into appropriate food ingredients is extensive, as demonstrated by Figure 1.

S. Cunningham, "Marketing of Dairy Ingredients. Part 1," *World of Ingredients* Jan/Feb., 22–23, 25, 27 (1995).

S. Cunningham, "Marketing of Dairy Ingredients. Part II," *World of Ingredients* March/April, 38–41 (1995).

B. N Dobson and D. P. Cornforth, "Nonfat Dry Milk Inhibits Pink Discoloration in Turkey Rolls," *Poultry Science* **71**, 1943–1946 (1992)

B. N. Dobson et al., "Instrument for Measuring Bind Strength of Restructured and Emulsion-Type Meat Products," *J. Texture Stud.* **24**, 303–310 (1993).

M. R. Ellekjaer, T Naes, and P. Baardseth, "Milk Proteins Affect Yield and Sensory Quality of Cooked Sausages," *J. Food Sci.* **61**, 660–666 (1996).

S. B El-Magoli, S Larola, and P. M. T. Hansen, "Ultrastructure of Low-Fat Ground Beef Patties With Added Whey Protein Concentrate," *Food Hydrocolloids* **9**, 291–306 (1995).

S. B. El-Magoli, S. Larola, and P. M. T. Hansen, "Flavor and Texture Characteristics of Low Fat Ground Beef Patties Formulated With Whey Protein Concentrate," *Meat Science* **42**, 179–193 (1996).

J. B. German and C. J. Dillard, "Fractionated Milk Fat: Composition, Structure, and Functional Properties," *Food Technol* **52**, 33–34, 36–38 (1998).

T. P. Guinee and M. O. Corcoran, "Expanded Use of Cheese in Processed Meat Products," *Farm & Food* **4**, 25–28 (1994).

A. Hendrickx, "New Protein Additive for Meat and Poultry Products: Milk-Based Functional Protein Product," *Fleischerei* **43**, 880, 883–884 (1992).

C. K. Hsu and E. Kolbe, "The Market Potential of Whey Concentrate as a Functional Ingredient in Surimi Seafoods," *Journal of Dairy Science* **79**, 2146–2151 (1996).

L. King, "Whey Protein Concentrates as Ingredients," *Food Technology Europe* **3**, 88–89 (1996).

A. Konno and M. Kimura, "Effects of Addition of Whey Protein Concentrates on the Water-Retention Properties and Texture of Ham," *Journal of Japanese Society of Food Science and Technology* **40**, 427–432 (1993).

H. A. M. Lemmers, "The Use of Dairy Ingredients in Further Processed Poultry Products," *Fleischerei* **42**, III–VI (1991).

P. H. Lyons et al., "The Influence of Whey Protein/Carrageenan Gels and Tapioca Starch on the Textural Properties of Low Fat Pork," *Meat Science* **51**, 43–52 (1999).

L. Mancini, "Dairy Ingredients, The natural Choice for Versatility," *Food Engineering* **64**, 73–75 (1992).

A. McCord, A. B. Smith and E. E. O'Neill, "Heat-Induced Gelation Properties of Salt-Soluble Muscle Proteins as Affected by Nonmeat Proteins," *J. Food Sci.* **63**, 580–583 (1998).

L. G. Phillips, M. J. Davis and J. E. Kinsella, "The Effects of Various Milk Proteins on the Foaming Properties of Egg White," *Food Hydrocolloids* **3**, 163–174 (1989).

E. Pitts, "The European Market for Dairy Ingredients," *Journal of the Society for Dairy Technology* **48**, 79–85 (1995).

K. Piyachomkwan and M. H. Penner, "Inhibition of Pacific Whiting Surimi-Associated Protease by Whey Protein Concentrate," *J. Food Prot.* **18**, 341–353 (1995).

C. Ramesh, *Dairy-Based Ingredients*, Eagan Press, Minn., 1997.

D. M. Smith and A. J. Rose, "Properties of Chicken Salt-Soluble Protein and Whey Protein Concentrate Gels as Influenced by Sodium Tripolyphosphate," *Poultry Science* **74**, 169–175 (1995).

S. Solberg, "Effective Product Development for Sausages," *Livsmedelsteknik* **38**, 42–43 (1996).

H. Y. Tan and D. M. Smith, "Dynamic Rheological Properties and Microstructure of Partially Insolubilized Whey Protein Concentrate and Chicken Breast Salt-Soluble Protein Gels," *J. Agric. Food Chem.* **41**, 1372–1378 (1993).

L. Thorsen and T. Bergersen, "Quantitation of Milk Proteins in Heat-Treated Comminuted Meat Products," *InformMAT* **2**, 62–64 (1989).

P. Walkenstrom and A. M. Hermansson, "Fine-Stranded Mixed Gels of Whey Proteins and Gelatin," *Food Hydrocolloids* **10**, 51–62 (1996).

P. Walkenstrom and A. M. Hermansson, "High-Pressure Treated Mixed Gels of Gelatin and Whey Proteins," *Food Hydrocolloids* **11**, 195–208 (1997).

P. Walkenstrom and A. M. Hermansson, "Mixed Gels of Gelatin and Whey Proteins, Formed by Combining Temperature and High Pressure," *Food Hydrocolloids* **11**, 457–470 (1997).

V. C. Weerasinghe et al., "Whey Protein Concentrate as a Proteinase Inhibitor in Pacific Whiting Surimi," *J. Food Sci.* **61**, 367–371 (1996).

J. N. de Wit, "Nutritional and Functional Characteristics of Whey Proteins in Food Products," *Journal of Dairy Science* **81**, 597–608 (1998).

O. Zorba et al., "The Possibility of Using Fluid Whey in Comminuted Meat Products: Capacity and Viscosity of the Model Emulsions Prepared Using Whey and Muscle Protein," *Z. Lebensm.-Unters. Forsch.* **200**, 425–427 (1995).

MARTIEN VAN DEN HOVEN
Creamy Creations
Rijkevoort, The Netherlands

See also DAIRY INGREDIENTS FOR FOODS; WHEY: COMPOSITION, PROPERTIES, PROCESSING, AND USES.

DAIRY INGREDIENTS FOR FOODS

Milk and its products have been consumed by many generations of people of Western, Middle Eastern, Indian, and some African cultures. The nutritional value of dairy materials, properly handled, has been proven by widespread use, and their safety is assured by processing procedures that have been introduced and refined since the time of Pasteur. The great bulk of dairy products today recognize traditions of simple processes and physical separations, avoiding chemicals and thereby preserving the natural properties and the very desirable connotations of traditional products.

The more recently developed food-processing industries have generated requirements for sophisticated performance of dairy materials as ingredients for both familiar and fabricated foods. Milk production does not require the use of pesticides and hormones. Well-managed herds can produce milk with contaminant levels far below the requirements of regulatory authorities. Active components that directly inhibit the processes of digestion, metabolism, and assimilation of nutrients are not present in milk.

The nature of milk and its components is probably better characterized than the properties of any other basic food. Consequently, the ability to transform milk and its individual components into appropriate food ingredients is extensive, as demonstrated by Figure 1.

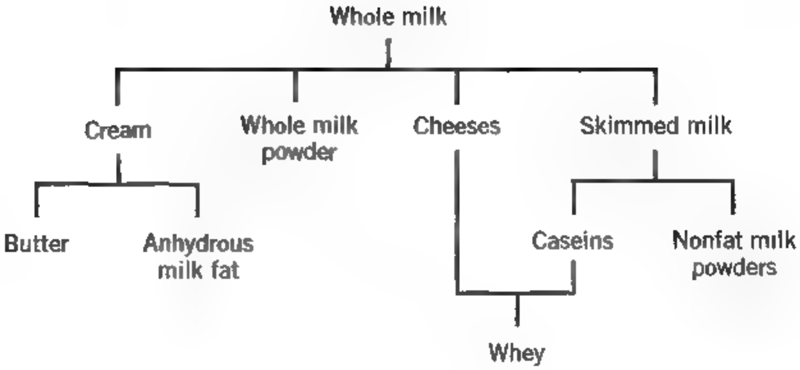


Figure 1. The cascade of dairy materials.

FORMS OF DAIRY INGREDIENTS

Dairy ingredients are provided in many different forms, which can be grouped according to the effect of the process on the products.

1. Compositionally complete or only partially rearranged ingredients (e.g., removal of water and/or fat).

Liquid milk and cream are available, but it is normally more convenient for the food processors to use products from which the water has been removed (as in the manufacture of whole milk powder and butter). The centrifugal removal of cream results in the manufacture of nonfat milk powder. These processes have been industrialized on a very large scale in order to minimize costs. Although they are inherently simple, sophisticated knowledge and process control are required to achieve consistent product performance as may be specified.

2. Products in which a desired component of milk is prepared (e.g., milkfat, protein).

Complete removal of water and nonfat solids from cream will result in anhydrous milkfat that is usable in the same manner as other oils but that is prepared without requiring a complex series of extractive procedures in its manufacture. The major protein of milk, casein, is obtained by simple isoelectric or enzymic precipitation followed by subsequent washing with water and drying.

3. Products obtained by the reassembly, or rearrangement, of the individual components.

At its simplest level the compositional adjustment of milk ingredients is readily achieved. For example, the control of fat levels in cheese products and milk powders is widely practiced; increased levels of whey solids in infant-food products are provided because they are nutritionally desirable; the recombination of powdered ingredients at locations that are far distant from the point of milk production has provided international availability of milk and its products.

4. Products in which the ingredients have been modified for specific purposes.

Judicious application of heat modifies the properties of milk components and can result in a range of milk powders tailored to satisfy specific industrial requirements (1). Addition of food-grade alkali to casein results in its solubilization (2). Physical modification by processes such as homogenization, to provide homogeneity, and agglomeration of powder particles to provide improved dispersibility, is of considerable value. More extensive modifications of dairy materials have been intensively researched. New processes are becoming available, and products that result from these newer technologies hold considerable promise in meeting the increasingly sophisticated requirements of food formulators. Transformations of milk components are being achieved. Lactose can be converted to lactulose (3), protein is being transformed into its hydrolysates (4), and milkfat can be deodorized and decolorized.

Extensively modified materials have been prepared, but the market has not yet taken up all the possibilities; the great bulk of commercial usage in the food industry lies with products that are well known and are based on simple processes.

THE INGREDIENT RANGE

The composition of dairy ingredients varies both naturally (because of their biological origin) and by design (through the manufacturing procedures) but can, in general terms, be set out as in Table 1.

Control of Component Ratios and Performance

A wide variety of performance requirements are imposed by the needs of food formulation. Products that contain various combinations of milk components are available in traditional, widely used forms and also in forms that have been recently modified for food-industry use. The composition of some available product groups is provided in Table 2.

Table 2 reviews the products that have been traditionally available and that are widely recognized. More recently, the specific needs of the food industry have been met by the development of milk powders for milk recombination plants. Recombiners require particular properties such as heat stability for the manufacture of recombined evaporated milks. Similarly, nonfat milk powders that provide manageable viscosity characteristics in the produc-

Table 1. Typical Composition of Dairy Products

	Content, % dry basis				
Product	Fat	Protein	Lactose	Mineral	Moisture content (%)
Milk	32	27	35	5	87
Cheese (cheddar)	53	38	2[a]	6[b]	33
Products with concentrated fat levels					
Cream	87	5	7	1	55
Butter	98	1	1	<0.5	16
Anhydrous milkfat (AMF)	100	<0.1	<0.1	<0.1	<0.1
Products with concentrated protein levels					
Nonfat dry milk	1	40	50	9	3.5
Casein (acid)	1	96	<0.1	2	11
Caseinate	1	95	<0.1	4	4
Whey powder	1	15	76	8	3.4
Whey protein concentrate (WPC)[a]	3	37	54	6	4
Whey protein concentrate[b]	5	79	12	4	4
Whey protein isolate	1	96		3	4
Isolated products					
Lactose			100		5
Minerals	1	8	1	83	10

[a] As lactate.
[b] Includes salt.

Table 2. Combinations of Milk Components

Product class	Performance required by food formulator	Process of manufacture	Reference
Whole milk	A natural liquid	Pasteurized for safety	5
	A stable liquid	Sterilized for microbial stability	5
	A stable liquid with dairy flavor	UHT[a] sterilized and asceptically packaged to protect from oxygen and light	6
Compositionally altered milks	A powder for dry-blend purposes	Dehydrate by roller or spray drier	1
	Fat reduced or zero fat contribution	Remove milk fat centrifugally	7
	Protein enrichment	Protein added before drying	
	Sweetened and stable	Sugar addition, appropriate heat, crystallization concentration	8
	Cocoa crumb as chocolate ingredient	Codried cocoa and sweetened condensed milk	9
Modified milk	Reduced mineral levels (especially for infant foods) or mineral profile adjustment	Ion exchange and/or electrodialysis (of whey generally)	10
	Heat-stable powder	Mineral adjustment and appropriate heat control	11
	A powder for nutritional improvement in bread without loaf volume depression	Appropriate heat control	12
	Sweet but without sugar addition (especially for dietetic uses)	Hydrolysis of lactose by lactase	13
	A powder with high dispersibility	Agglomeration plus surface-coating with hydrophilic materials	14

[a] UHT, ultra-high temperature.

tion of sweetened condensed milks are also required. Manufacture by the appropriate use of heat and by control of the mineral balance of the milks during processing are the essential manufacturing processes. An improvement in the dispersibility of powdered products has been achieved by progressively improved drier design so that powder particles can be agglomerated without damage to the flavor or nutritional properties of the product. More recently, effective coating of the particles with natural materials that impart hydrophillic properties has been achieved. The reduction of mineral levels by the processes of ion exchange and electrodialysis are widely practiced (15,16). This has allowed, in particular, the development of infant foods with low osmotic pressure and with managed mineral profiles that appropriately meet nutritional needs for nutrient balance.

Powders that contain milk fat are manufactured by means that ensure that stale flavors, due to oxidation developed during storage, do not arise in less than 12 months. Fat-containing powders that are intended for dispersion in water require particular care in manufacture (17).

High-fat powders, such as cream and butter powders which are particularly useful in the bakery industry, require appropriate formulation to ensure that free-flow properties are retained without loss of functional performance (18).

PRODUCTS IN WHICH MILKFAT IS THE DOMINANT COMPONENT

The use of cream, butter, and anhydrous milkfat (AMF), represent around 30% of the milkfat that is produced on dairy farms worldwide. The 6.8 million tons used annually means that milkfat is the third most widely used fat in worldwide commerce. The nature of fat on presentation to the manufacturing process is pure and wholesome so that the use of extensive clean-up processes and chemical preservative procedures are unnecessary. Milkfat has a desirable and prestigious flavor, so the maintenance of that flavor is of special importance. For this reason, refrigerated storage of the products of milkfat is common. Because of a significant level of naturally occurring materials that have antioxidant activity, and the widespread use of stainless steel, which prevents contamination of milk with prooxidants, extended shelf life can be achieved without recourse to added antioxidents.

The manufacturing processes that are used are inherently simple physical procedures such as churning and dehydration. The product range is shown in Figure 2.

Rearrangement of the textural properties of milkfat is achieved in a number of ways (19):

1. Mechanical and thermal procedures are often used simultaneously in scraped surface heat exchangers and pin-working machines and result in plasticization. Butter-type products with controlled physical properties are the result.
2. Processes known as dry fractionation consist of crystallization from melted fat after controlled cooling followed by separation of the liquid from solid phase by vacuum filtration or centrifugation without coming into contact with any contaminating process aid. These processes yield products that contain increased solid fat levels that are particularly useful for croissants and puff pastry (20).
3. Compounding of milkfat with vegetable oil to modify spreadability properties of spreadable products results in a number of products that are successful at the retail level and are attractive to the catering industry. Commonly, these products employ 50% milkfat, 40% liquid vegetable oil to increase the proportion of fat liquid at 5°, and 10% hard stock to provide standup properties.

The intensity of the yellow color of milkfat is, to some extent, controllable. The natural color of milk fat is carotene, a precurser of vitamin A, and is not implicated in any concern for health. Selection of the milkfat origin according to breed of cow, feed source to the herd, and season of production will provide a range of color properties. More extensive color modification can be achieved by use of steam deodorization processes that are commonly employed in the oils industry. It yields a white milkfat that is finding extensive use in the manufacture of white cheeses and coffee whiteners and holds promise in other foods.

Experimental investigation into the extensive modification of milkfat by complex processes such as interesterification and hydrogenation has not, so far, yielded products having adequately improved benefits to justify the cost of the processes. Such approaches would simultaneously harm both the distinctive flavor and the naturalness of milkfat (Table 3).

MILK PROTEINS

The products that contain milk proteins are derived from skimmed (nonfat) milk (Fig. 3). The proteins of milk have a well-balanced amino acid profile, having been designed by nature for the rapid early phase of growth that is necessary for infant mammals. By comparison with a reference protein, which has been described by a FAO/WHO

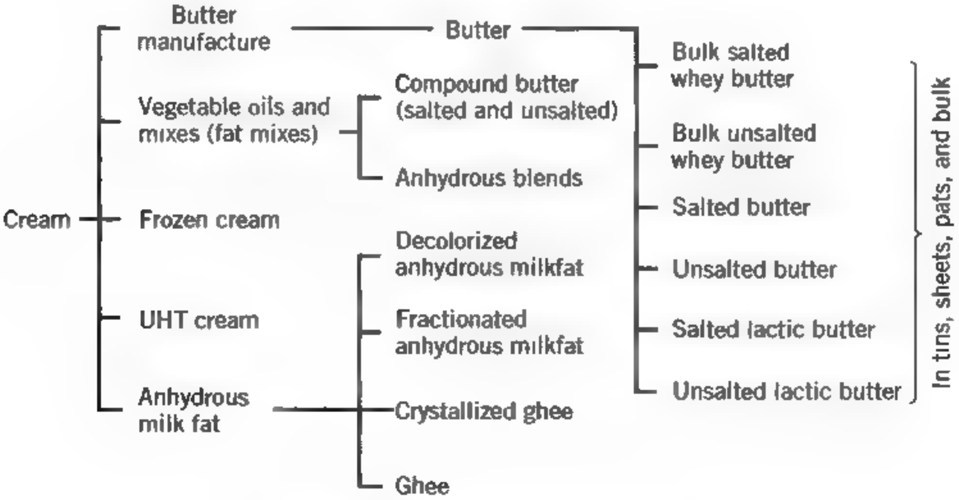


Figure 2. The products derived from cream.

Table 3. Milk Fat Ingredients

Product class	Performance requirement by food formulator	Process of manufacture	Reference
Whole milk			
Cream	Natural flavor and liquid, whippable	Centrifugal separation	7
Butter	Solid or spreadable form	Churning	21
	Spreadability	Ammix process	22
Component. Anhydrous milk fat	Strong dairy flavor with no interfering components	Dehydrate and wash	23
Rearrangement of composition	Control of melting characteristics while retaining natural flavor performance	Selection of natural properties	24
		Fractional crystallization and separation	25
		Inclusion of nondairy oils into churning process (compound butter)	
	Greater spreadability or increased levels of polyunsaturated fatty acids	Inclusion of polyunsaturated oils before churning or by recombining	26
		Introduce protected polyunsaturated oils to feed of the cows	27
Modifications	Light color	Destruction of carotene by steam deodorization	
	Deep color	Select milkfat by origin	28
	Melting-point control	Fractional crystallization and separation	29

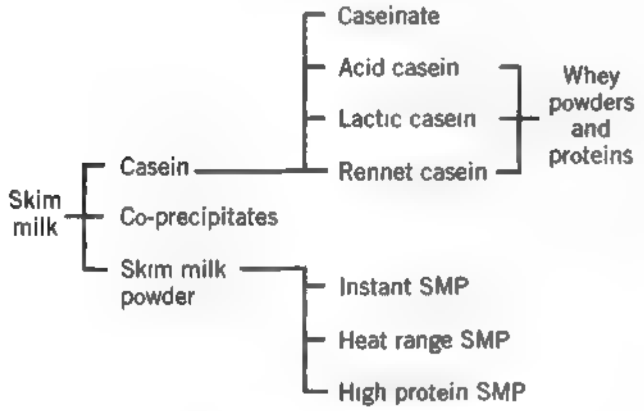


Figure 3. The products derived from skim milk.

group of experts, milk proteins meet all the requirements for essential amino acids. Whey proteins are particularly rich in the sulfur-containing amino acids (methionine and cystine), so they serve a valuable role complementing the amino acid profile of proteins of vegetable origin in food formulations that require high nutritional quality (30).

A physiological role for milk proteins in human nutrition is based on the activity of the immunoglobulins, lactoferrin, and other proteins that are present in small amounts (31). The particular amino acid profile of whey proteins has been proposed as a fundamental property that improves immunocompetence of humans (32).

The proteins in milk are recoverable by long-practiced separation procedures such as by very specific enzymatic precipitation (as in the case of rennet casein), heat precipitation (as in lactalbumin), and by isoelectric precipitation (as in acid caseins). The most widely used of these is the last-named (33).

Recent technological advances have resulted in the manufacture of soluble whey protein concentrates that are produced by the membrane process of ultrafiltration of whey. Functional whey protein concentrates that contain up to about 80% protein are commercially available.

Products that provide the combined benefits of casein and whey protein have been available in the form of coprecipitates, which are manufactured by control of temperature, acidity level, and calcium level (33). More recently developed technology using shifts in pH has resulted in new products that combine strong functionality with high PER levels (34,35).

Specific functional performance has been engineered into caseinates, which have been solubilized from acid casein by reaction with alkali (2). Arising from a precise understanding of the influence of minerals, a growing comprehension of the modifications achievable by controlled management of enzymatic reaction a number of highly specific protein products have been created. Also of considerable importance to the food formulator is the fact that the protein component of a food should not contribute unwelcome flavors. Adsorption technology has been used to enhance the already bland flavor of casein products. The use of casein (and caseinate) as an ingredient in foods is very extensive, as it provides strong functional performance, high levels of protein relative to milk, competitive cost, and strong nutritive quality (36)

Similar product development has been applied to whey protein concentrates (WPCs) with the result that enhanced gelation performance and products with unusual heat stability have been achieved. The selection of an appropriately functional WPC for a particular use in food formulation can offer substantial benefits (37).

Very high purity levels of whey protein have been achieved on the industrial scale by ion exchange processes that produce isolates, but the cost penalty that is incurred means that only the very highest-priced market applications can be considered for such products.

The enzymatic hydrolysis of proteins may be used to produce products of low or zero allergenicity. This technique is receiving intensive scientific attention and is likely to result in products that will have considerable impact in improving the health of infants, and possibly others, who have specific nutritional requirements (Table 4).

CHEESE AND CULTURED PRODUCTS

Cheese and cultured milk products generally are consumed in their native form, but they also can provide a nutritionally valuable ingredient resource with especially useful flavor and compositional characteristics that are well regarded by consumers (48). The cheesemaker can provide a graduated series of flavor, texture, and compositional properties to suit a variety of needs. Consequently cheese types vary widely, as shown in Figure 4.

Natural cheeses are manufactured by controlled fermentation, which influences both acidity and flavor, and which in combination with controlled composition, influences texture (49). Texture control extends from gels, through spreadable pastes, to firmly structured semisolids. Processed cheese, which is made by melting the natural cheese and then dissolving the protein so that a new emulsion is established, extends the range of textural properties and forms of presentation that can be offered.

High-melt properties that can provide intact cheese pieces in, eg, heated sausages have been achieved. Stretch properties as may be required for pizza, and melt charac-

Table 4. Milk Protein

Product class	Performance requirement of food formulation	Process of manufacture	Reference
	Whole proteins		
Coprecipitate	Balanced amino acid profile	Precipitated under managed conditions of heat, acid, and calcium concentration	38
Isolates	Strong functional performance	pH shifts and isoelectric precipitation	34,35
	Components		
Casein-acid	Broad functional performance, bland, nutritionally strong	Isoelectric precipitation, multiple washing procedure	33,36
Casein-rennet	Nutritionally strong, including bioavailable calcium, stable bland flavor, stretch properties for analogue cheese	Enzymic precipitation and multiple washing	39
Whey protein			
Lactalbumin	Amino acid balance complementary to vegetable proteins No interfering function	Heat precipitation and spray dried or ring dried	40
WPC[a] low protein level	Milk powder alternative at economical price	Ultrafiltration to around 35% protein content	
WPC[a], high protein level	Soluble, bland, heat setting	Ultrafilter and diafilter to around 75% protein content	41
Isolates	Pure proteins	Ionic adsorption	42
	Compounding		
	Specific functional performance	Introduce vegetable proteins in blends or by interreaction	43
	Modification		
Caseinates			
Sodium	Solubility, natural emulsification, foam, fat binding, water holding Bland flavor	Treat with sodium alkali and spray dry Carbon asorption	36,44
Calcium	Colloidal protein, viscosity control	Treat with calcium alkali and spray dry	45
Potassium	Soluble and functional with low sodium	Treat with potassium alkali	
Various	Special performance (e.g., high foam, low viscosity, gelation)	Various proprietary procedures	46
Whey proteins, WPC[a]	Special performance (e.g., strong gels, heat stability)	Various proprietary procedures	47
Hydrolysates	Nutritional performance, reduced allergenicity	Enzymic modification	4

[a] WPC, whey protein concentrate.

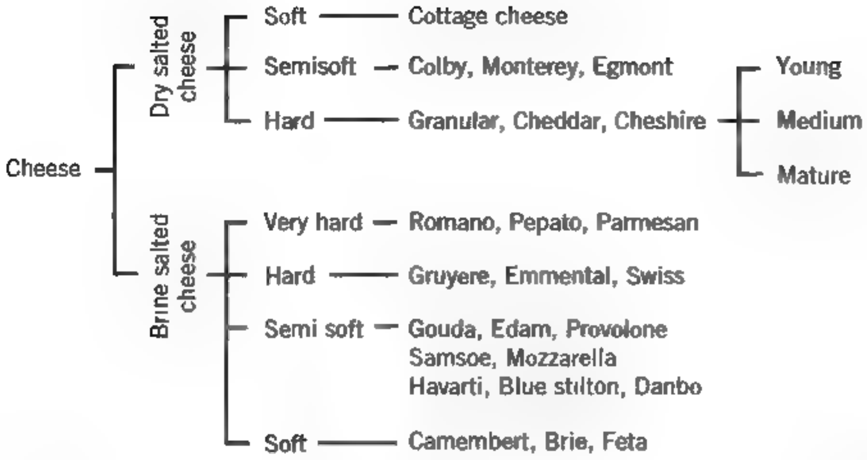


Figure 4. Cheese types.

teristics as may be required for crust appearance, can be built-in during the manufacturing process.

Cheese flavors are often desired characteristics, and they range widely. They include fresh and carbohydrate-derived flavor as in lactic cottage cheese and in creamy harvati; protein-derived flavor as in matured cheddar and camembert; and fat-derived flavors as in parmesan and blue cheeses. Further flavor extension by inclusion of natural materials such as spices and herbs can be used with both natural and processed cheeses. Flavor contributions are particularly significant to cheese sauces, dressings, and parmesan toppings for European-style foods.

Particulate forms of cheese, prepared by grating, dicing, or drying, provide convenience for incorporation into food formulations. Varied flavor intensity (such as by enzyme treatment) can also be built into powdered cheeses. Powdered yogurts are available, for coating and as formulation ingredients. These can be used, along with cream cheese, cottage cheese, and quarg for a range of cheese-cakes and similar products.

New technologies, such as the use of ultrafiltration of milk before cheesemaking, have already yielded commercially successful forms of feta cheese with increased shelf life and promise to further extend the range of product composition that will be created (Table 5).

LACTOSE

The sugar of milk is lactose, a disaccharide that has one of the lowest levels of sweetness contributed by a carbohydrate. Because it is approximately 25% as sweet as sucrose, it can be used in food formulations without dominating the natural flavors of other food components. Its solubility and sweetness vary with temperature and concentration, and these properties may require control in some forms of use (55).

Lactose is prepared by the processes of concentration and multiple crystallization followed by management of crystal size and properties. Lactose is available both at high levels of purity (including pharmaceutical quality) and, at lower cost, as the major component of dry whey powder or dry whey permeates (55).

When used in the baking industry, it acts as a reducing sugar to promote the Maillard reaction, which increases the browning of the crust. At higher temperatures it will caramelize and so contribute to flavor.

In formulated powdered products lactose crystals are slow to take up moisture and consequently minimize the likelihood of caking and lumping. This same property is also a valuable aid to tableting.

Nutritionally, lactose has been shown to promote calcium and phosphorous absorption, which is especially useful in infant feeding preparations (56). It protects against destabilization of the caseinate complex during the drying process. Its presence can also maintain biological activity in the preparation of enzyme products. In brewing and baking applications, it is not fermented by conventional strains of yeast so its contribution to sweetness and color is not destroyed by the biological processes of those industries.

The hydrolysis of lactose yields a sweet syrup that contains glucose and galactose, which may have nutritional advantages in some dietary applications. Lactose-hydrolyzed syrups from permeates and wheys are commercially available and are being used in confectionery and ice cream (Table 6).

THE NUTRITIONALLY VALUABLE MINOR COMPONENTS OF MILK

The new technologies that have arisen from the biotechnology revolution are permitting the extraction of a number of biologically active materials. Some of these biologically active components are present in large proportions in the colostrum milk but decline to low levels during the lactational period. The extraction of lactoferrin, which is an iron-binding protein, has been achieved commercially by sophisticated procedures of chromatography. This protein has been shown to be inhibitory to a number of pathogenic microorganisms, plays a role in the immunological process, and also provides a highly available source of iron. The enzyme lactoperoxidase has also been extracted from dairy sources and is capable of acting to destroy microbes in a

Table 5. Cheeses

Product Class	Performance requirement of food formulation	Process of manufacture	Reference
	Whole		
Natural	Flavor and texture selection, strong nutritional contribution Stretch and melt control	Fermentation, controlled acidity and heat, controlled maturation	50
Powders	Flavor enhancement	Selection of type and maturity, emulsify and spray dry	51
Particulate	Sprinkling (onto pizza)	Shredding or dicing	
	Compositionally modified		
Skim milk cheese	Protein source	Fat-reduced milk	
Cream cheese	Fat source flavor contribution and texture	Cream addition, culturing	
	Modified cheese		
	Flavor enhanced	Enzymic modification	53
	Stretch control	Acidity management	
	Processed cheese		
	Specific texture requirements, flavor modification	Emulsification. Use of nondairy ingredients	53
	Specific shape	e.g., Slices	54

Table 6. Lactose

Product class	Performance requirement	Process	Reference
Component	Low sweetness, reduction of water activity, enhanced browning. Promotion of calcium and phosphorus absorption (especially in infant feeding), dispersing agent	Concentration crystallization and particle size control	55,56,57,59
Modified	Sweet syrup	Hydrolysis by lactase, concentration	13,60
Transformed	New materials	Various	55,61

completely safe manner that would provide an alternative to the use of antimicrobial agents.

Natural antibodies are also present in very low concentration in unprocessed milk, but traditional procedures have resulted in their inactivation. Procedures are now available that allow for preparation of protein products having biological activity, and there is evidence that disease control is achievable (62). Currently these products are targeted toward the problems of animal health. Scientific enquiry is targeted toward the improvement of human health. Wide-ranging implications for a new range of foods that have physiological functions exist and may provide a new wave of the future (63,64).

The recognition of the value of calcium naturally present in milk and cheese in meeting the nutritional needs of aging people, particularly women, has resulted in a demand for pure mineral ingredients of natural origin, fine particle size, and clean flavor. The natural calcium content of milk is being recovered by precipitation processes for direct incorporation into foods (Table 7).

PREPARED INGREDIENTS FOR FOODS

Sophisticated food ingredients have been specifically designed to provide physical functional performance for particular food systems. Strong functional properties are provided by dairy-based ingredients.

Casein protein has an open structure and has separate areas of hydrophobic and hydrophyllic nature along the protein molecule. Consequently, it diffuses readily to interfaces and has powerful emulsion-forming end stabilizing properties. Whey proteins unfold on heating and so, similarly, expose hydrophillic areas of the polypeptide chains. They also gel and can provide natural thickening and stabilizing function. The properties of caseinates and coprecipitates can be tailored by control of ionic content to provide a range of viscosities, solubilities, foaming and whipping (67), and surface activity (68). Measurements of the function of milk proteins have proved difficult to relate to the performance of these proteins in the complex environment of food systems (69), but considerable progress has been made (70,71). Manufacturing techniques that control the function of proteins are being practiced (72).

The functional properties of milkfat vary because of the effects on the secretory process in the mammary glands of cows by the breed of the cow and by the type of feed that she receives and that changes seasonally. Levels of technological control by temperature control during processing, texturizing by physical means, and fractionation by controlled crystallization minimize these variations (19).

Table 7. Nutritionally Valuable Components in Minor Concentrations

Product class	Performance requirement of food formulations	Process of manufacture	Reference
Minerals	Natural, origin, low flavor, bioavailable	Precipitation	
Biologically active			
Lactoferrin	Microbiological inhibition by iron binding. Nutritionally available form of iron	Sophisticated chromatography	65
Lactoperoxidase	Natural antimicrobial system	Sophisticated chromatography	65
Immunoglobulins	Natural antibody active against a wide range of diseases	Specialized ultrafiltration	66

Milkfats tailor-made for specific purposes are available (73). The primary role of milkfat, when incorporated into a food, is the contribution that it makes to flavor, texture, and consumer acceptability (74).

The benefits that dairy ingredients provide to food formulators differ according to the particular needs of each industry. For confectionery products, milkfat is compatible with cocoa butter in that it becomes part of the continuous fat phase and it contributes to smooth flavor and texture of milk chocolate. Caramel flavor is best developed from sweetened condensed milk by utilizing the browning properties of lactose and protein. Proteins, especially caseins, enhance moisture retention by candy and control the quantity of free and bound water (75). Hydrolyzed milk proteins act as whipping agents for frappés and marshmallows (76).

For bakery products, milk powders improve crust color, resilience, and structural strength of cakes. Whey powders improve tenderness and shortness. Whey powder and skim milk powder, when used in dough for cookies and biscuits, reduces the tendency of the dough to tear and produces smooth, even browning. Milk proteins at levels up to 20% improve the nutritional value of cookies end biscuits because they contain high levels of the essential amino acids that are deficient in soy and wheat flour. Butter imparts distinctive flavor to butter cookies and croissants (77,78).

For meat products, milk proteins offer improved appearance, increased yield, and economy. The powerful emulsion capacity of sodium caseinate is particularly valuable in comminuted meat products. Whey protein concentrates of high gel strength are important in reformed ham. Lactose will mask salts, phosphates, and bitter aftertaste while providing a low sweetness profile in liver products, cooked hams, and cooked sausages (79,80).

A very wide variety of proprietary dairy ingredients are manufactured for convenience of use in the food industry. Specific attention is paid to the physical form of powders to ensure dispersibility, mixibility, and low dust levels. Butter is available as flakes and in sheet form. Cheese may be shredded, diced, or sliced. Flavor enhancement, particularly for cheese and milk powder, is offered (81).

CONCLUSION

The remarkably wide range of products that has been obtained from milk demonstrates the intensity of technological attention that has been devoted to dairy materials over a long period of time. The increasing interest in progressively more sophisticated materials that will be required by the food industry is opening up an increasing range of possibilities for the future. While the opportunities for sophisticated materials are exciting, the great bulk of dairy ingredient purchases are based on the recognition of value for money and a clear understanding of the increasing range of uses for products that can be produced by relatively simple processes from a pure and natural raw material.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the valuable assistance of the staff of the New Zealand Dairy Research Institute during the preparation of this paper.

BIBLIOGRAPHY

This article has been adapted and used with permission from K. J. Kirkpatrick and R. M. Fedwick, "Manufacture and General Properties of Dairy Ingredients," *Food Technology* **41**, 58–65 (1987).

1. M. E. Knipschildt, "Drying of Milk and Milk Products." In R. K. Robinson, ed., *Modern Dairy Technology*, vol. 1, Elsevier Applied Science Publishers, London, 1986, p. 131.
2. C. Towler, "Conversion of Casein Curd to Sodium Caseinate," *New Zealand Journal of Dairy Sciences and Technology* **11**, (1976).
3. U.S. Pat. 4,273,922 (1981) K. B. Hicks.
4. R. J. Knights, "Processing and Evaluation of the Antigenicity of Protein Hydrolysates," in F. Lifshitz, ed., *Nutrition for Special Needs in Infancy*, Marcel Dekker, New York, 1985.
5. M. J. Lewis, "Advances in the Heat Treatment of Milk," in Ref. 1, p. 131.
6. International Dairy Federation, *New Monograph on UHT Milk*, Document No. 133, International Dairy Federation, Brussels, Belgium, 1981.
7. C. Towler, "Developments in Cream Separation and Processing," in Ref. 1.
8. B. H. Webb, "Condensed Products," in B. H. Webb and E. O. Whittier, eds., *By-products from Milk*, 2nd ed., AVI Publishing Co., Westport, Conn., 1970, p. 83.
9. B. H. Webb, 1970b. "Miscellaneous Products," in Ref. 8, p. 285.
10. J. G. Zadow, "Utilization of Milk Components: Whey," in Ref. 1, p. 93.
11. K. J. Kirkpatrick, "Raw Material Selection for Recombined Evaporated Milk Products." In *Proceedings of IDF Seminar on Recombination of Milk and Milk Products*, Document No. 142. International Dairy Federation, Brussels, 1982, p. 91.
12. E. J. Guy, "Bakery Products," in Ref. 8, p. 197.
13. R. R. Mahoney, "Modification of Lactose and Lactose-containing Dairy Products with Beta-Galactosidase," *Developments in Dairy Chemistry* **3**, 69 (1985).

14. H. G. Kessler, "Drying-Instantizing," in *Food Engineering and Dairy Technology*, Verlag A. Kessler, Germany, 1981.

15 B. T. Batchelder, "Electrodialysis Applications in Whey Processing," in *Proceedings of International Whey Conferences*. Whey Products Institute and International Dairy Federation, 1986.

16. H. Jonsson and S-O. Arph, "Ion Exchange for Demineralization of Cheese Whey," in Ref 15.

17. U.S. Pat. 4,737,369 (1988) I. A. Suzuka, and K. Mori.

18. F G. Kieseker, J. G. Zadow, and B. Aitkin, "Further Developments in the Manufacture of Powdered Whipping Creams," *Australian Journal of Dairy Technology* **34**, 112 (1979).

19 E Frede, "Technological and Analytical Aspects of Milk Fat Modification," in *Conference Proceedings Food Ingredients Europe 1989*, Expoconsult Publishers, Maarsen, The Netherlands, 1989, pp. 55–61.

20. L. Eyres, "Milkfat Product Development," *Lipid Technology* **1**, 12 (1989).

21. R. A. Wilbey, "Production of Butter and Dairy-based Spreads," Ref. 1, p. 93.

22. H. T. Truong and D. S. Munroe, "The Quality of Butter Produced by the "Ammix" Process," *Brief Communications*, 21st International Dairy Congress, vol. 1, p. 341.

23. A. Fjaervoll, "Anhydrous Milkfat, Manufacturing Techniques and Future Applications," *Dairy Industries* **35**, 424 (1970).

24. M. W. Taylor and R. Norris, "The Physical Properties of Dairy Spreads," *New Zealand Journal of Dairy Science and Technology* **12**, 166 (1977).

25. Austr. Pat. 431,955 (1968), I. T. H. Olsson.

26. M. M. Chrysam, "Table Spreads and Shortenings" in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, vol. 3, 1985, p. 1.

27. A. D. Fogerty and A. R. Johnson, "Influence of Nutritional Factors on the Yield and Content of Milkfat. Protected Polyunsaturated Fat in the Diet," Document No. 125, International Dairy Federation, Brussels, 1980, p. 96.

28. A. R. Keen, "Seasonal Variation in the Colour of Milkfat from Selected Herds and Two Dairy Plants," *New Zealand Journal of Dairy Science and Technology* **19**, 263 (1984).

29. A. E. Thomas, "Fractionation and Winterization: Processes and Products," in Ref. 26, p. 1.

30. E. Renner, "Milk Proteins," in *Milk and Dairy Products in Human Nutrition*, W-GmbH, Volkswirtschaftlicher Verlag. Munich, Germany, 1983, pp. 90–115.

31 L. Hambraeus, "Importance of Milk Proteins in Human Nutrition: Physiological Aspects," in T. E. Galesloot and B J. Tinvbergen, eds. *Milk Proteins 1984*, Pudoc, Wageningen, Germany, 1985, p. 63.

32. G. Bounous and P. A. L. Kongshavn, "Influence of Protein Type in Nutritionally Adequate Diets on the Development of Immunity," in M. Friedman, ed., *Absorption and Utilization of Amino Acids*, vol. 2, CRC Press, Inc., Boca Raton, Fla., 19, pp. 219–233.

33 C. R. Southward and N. J. Walker, "Casein Caseinates and Milk Protein Coprecipitates," in *CRC Handbook of Processing and Utilization in Agriculture*, vol. 1, 1982, p. 445.

34. U.S. Pat. 4,376,072 (1982), P. B. Connolly.

35. U.S. Pat. 4,519,945 (1985), H. A. W. E. M. Ottenhof.

36. C. R. Southward, "Uses of Casein and Caseinates," in P. F. Fox, ed., *Developments in Dairy Chemistry*, vol. 4, *Functional Milk Proteins*, Applied Science Publishers, London, 1989, pp. 173–244.

37. M. J. Rockell, "Selecting the Correct WPC for Your Food Applications via a Knowledge of the Functional Properties of WPC's," in *Food Ingredients Europe, Conference Proceedings*, Expaconsult Publishers, Maarssen, The Netherlands, 1989, p 51.

38. L. L. Muller, "Manufacture of Casein, Caseinates and Coprecipitates," in Ref. 36, vol. 1, 1982, p. 315.

39. C. R. Southward and N. J. Walker, "The Manufacture and Industrial Use of Casein," *New Zealand Journal of Dairy Technology*, (1980).

40. B. P. Robinson, J. L. Short, and K. R. Marshall, "Traditional Lactalbumin—Manufacture, Properties, and Uses," *New Zealand Journal of Dairy Science and Technology* **11**, 114 (1976).

41. K. R. Marshall, "Proteins, Industrial Isolation of Milk Proteins: Whey Proteins" in Ref. 36, vol. 1, 1982, p. 339.

42. D. E. Palmer, "Recovery of Protein from Food Factory Wastes by Ion Exchange," in P. E. Fox and J. J. Congson, eds., *Food Proteins*, Applied Science Publishers, New York, 1981, p. 341.

43. U.S. Pat. 4,486,343 (1984), N. J. Walker and P. B. Connolly.

44. A. Bergman, "Continuous Production of Spray Dried Sodium Caseinate," *Journal of the Society of Dairy Technology* **25**, 89 (1972).

45. J. Roeper, "Preparation of Calcium Caseinate from Casein Curd," *New Zealand Journal of Dairy Science and Technology* **12**, 182 (1977).

46. U.S. Pat. 4,126,607 (1978), W. C. Easton.

47. J. E. Kinsella, "Proteins from Whey: Factors Affecting Functional Behaviour and Uses," *Proceedings of IDF Seminar, Atlanta, Ga.*, International Dairy Federation, Brussels, Belgium, 1986, p. 87.

48. J. C Dillon, "Cheese in the Diet," in Ref. 50, pp. 499–511.

49. M E. Johnson, "Cheese Chemistry," in *Fundamentals of Dairy Chemistry*, 3rd ed., by Van Nostrand Reinhold Co., New York, 1988, pp 634–654

50. A. Eck, *Cheesemaking, Science and Technology*, Lavoisier Publishing, Inc., New York, 1986.

51. T. I. Hedrick, "Spray Drying of Cheese," in *Proceedings of Second Marschall International Cheese Conference*, 1981, p. 76.

52. L. Talbott, "The Use of Enzyme Modified Cheeses for Flavouring Processed Cheese Products," in Ref. 51, p. 81.

53. A. Meyer, *Processed Cheese Manufacture, Benckiser-Knapsack GMBH, 1970.*

54. J. H. Carne, "Sliced Process Cheese Production," in *Proceedings from the First Marschall International Cheese Conference*, 1979, p. 305.

55. V. H. Holsinger, "Lactose," in Ref. 49, pp. 279–342.

56. S. G. Coton, T. R. Poynton, and D. Ryder, "Utilisation of Lactose in the Food Industry," *Bulletin of International Dairy Federation*, Document 147, p. 23 (1982).

57. E. Renner, "Lactose," in Ref 30, pp. 154–171.

58. T. A. Nickerson, "Lactose" in Ref. 8, p 356.

59. P. A. Morrissey, "Lactose: Chemical and Physico-Chemical Properties," *Developments in Dairy Chemistry* **3**, 1 (1985).

60. J. Rothwell, "Uses for Dairy Ingredients in Ice Cream and Other Frozen Desserts," *Journal of the Society of Dairy Technology* **37**, 119 (1984).

61. L. A. W. Thelwall, "Developments in the Chemistry and Chemical Modification of Lactose," *Developments in Dairy Chemistry* **3**, 35 (1985).

62. C. O. Tacket, G. Losonosky, H. Link, Y. Hoang, P. Guesry, H. Hilpert, and M. M. Levine, "Protection by Milk Immunoglobulin Concentrate Against Oral Challenge with Enteroxgenic

Escherichia coli," *New England Journal of Medicine* **318**, 1240 (1988).

63. S. Dosako, "Application of Milk Proteins to Functional Food," *Japan Food Science* **27**, 25–34 (1988).
64. A. S. Goldman, "Immunologic Supplementation of Cow's Milk Formulations," in *Bulletin No 244*, International Dairy Federation, Brussels, Belgium, 1990.
65. B. Reiter, "The Biological Significance of the Non-immunoglobulin Protective Proteins in Milk," *Developments in Dairy Chemistry* **3**, 281 (1985).
66. Jap. Pat. 60-75433 (1985) Y. Minami.
67. H. W. Modler, "Properties of Non-fat Dairy Ingredients—A Review," *Journal of Dairy Science* **68**, 2195–2205 (1985).
68. J. Leman and J. E. Kinsella, "Surface Activity, Film Formulation, and Emulsifying Properties of Milk Proteins," *Critical Reviews in Food Science and Nutrition* **28**, 115–138 (1989).
69. P. D. Patel and J. C. Fry, *The Search for Standard Methods for Assessing Protein Functionality*.
70. C. V. Morr, "Utilization of Milk Proteins as Starting Materials for Other Foodstuffs," *Journal of Dairy Research* **46**, 369–376 (1979).
71. C. V. Morr, "Functional Properties of Milk Proteins and Their Use as Food Ingredients," Chapter 12, p. 375, in Ref. 36, vol. 1, Proteins, p. 375.
72. H. W. Modler and J. D. Jones, "Selected Processes to Improve the Functionality of Dairy Ingredients," *Food Technology* **41**, 114–117 (1987).
73. D. Illingworth, J. C. Lloyd, and R. Norris, "Tailor-made Fats from Milkfat—the New Zealand Experience," in *Fats for the Future II, International Conference of New Zealand Institute of Chemistry*, IUPAC, 1989.
74. M. I. Gurr and P. Walstra, "Fat Content of Dairy Foods in Relation to Sensory Properties and Consumer Acceptability," *International Dairy Federation Bulletin*, No. 244, 44–46 (1990).
75. L. B. Campbell and S. J. Pavlasek, "Dairy Products as Ingredients in Chocolates and Confections," *Food Technology* **41**, 78–85 (1987).
76. L. Munksgaard and R. Ipsen, "Confectionery Products," in *Dairy Ingredients for the Food Industry 3*, International Dairy Federation, Brussels, Belgium, 1989.
77. W. B. Sanderson, "Cakes, Cookies, & Biscuits," in *Dairy Ingredients for the Food Industry*, International Dairy Federation, Brussels, Belgium, 1985.
78. R. O. Cocup and W. B. Sanderson, "Functionality of Dairy Ingredients," *Bakery Products Food Technology* **41**, 86–90 (1987).
79. J. M. G. Lankveld, "Meat and Meat Products," in *Dairy Ingredients for the Food Industry 2*, International Dairy Federation, Brussels, Belgium, 1987.
80. van deu Hoven, "Functionality of Dairy Ingredients in Meat Products," *Food Technology* **41**, 72–77 (1987).
81. "Product Update—Ingredients from and for Dairy Products," *Food Technology* **43**, 108–122 (1989).

GENERAL REFERENCES

"Research Efforts Aim To Find New Uses for Milkfat Fractions in Food Ingredients," *Canadian Dairy* **75**, 13 (1996).

N. Dhanapati et al., "Effects of Mechanical Agitation, Heating and pH on the Structure of Bovine Alpha Lactalbumin," *Animal Science and Technology* **68**, 545–554 (1997).

D. A. Dionysius and J. M. Milne, "Antibacterial Peptides of Bovine Lactoferrin: Purification and Characterization," *Journal of Dairy Science* **80**, 667–674 (1997).

M. A. Drake et al., "Rheological Characteristics of Milkfat and Milkfat-Blend Sucrose Polyesters," *Food Research International* **27**, 477–481 (1994).

M. A. Drake et al., "Milkfat Sucrose Polyesters as Fat Substitutes in Cheddar-Type Cheeses," *J. Food Sci.* **59**, 326–327, 365 (1994).

D. D. Drohan et al., "Milk Protein–Carrageenan Interactions," *Food Hydrocolloids* **11**, 101–107 (1997).

S. El Shibiny, N. M. Shahein, and M. El Shiekh, "Preparation and Functional Properties of UF Whole Milk Protein Isolates," *Bulletin of the International Dairy Federation* No. 311, 28–30 (1996).

M. J. Facon, "Antibacterial Factors in Cow's Milk and Colostrum: Immunoglobulins and Lactoferrin," *Dissertation Abstracts International B* **56**, 4077 (1996).

L. O. Figura, "Lactose With Tailor-Made Properties," *Lebensmitteltechnik* **25**, 43–45 (1993).

J. B. German and C. J. Dillard, "Fractionated Milk Fat: Composition, Structure and Functional Properties," *Food Technol.* **52**, 33–34, 36–38 (1998).

J. Getler, A. Nielsen, and J. Sprogo, "Powdered Milk Protein Concentrate," *Scandinavian Dairy Information* **10**, 42–43 (1996).

J. Getler, A. Nielsen, and J. Sprogo, "Functional Process for Milk Protein Concentrate (MPC)," *Dairy Industries International* **62**, 25, 27 (1997).

M. S. Haddadin, S. A. Ibrahim, and R. K. Robinson, "Preservation of Raw Milk by Activation of the Natural Lactoperoxidase Systems," *Food Control* **7**, 149–152 (1996).

L. Hambraeus, "Lactoferrin—A Milk Protein in the News," *Livsmedelsteknik* **36**, 42 (1994).

W. R. Harmer and C. Wijesundera, "Heat Stability of Milkfat in Relation to Vegetable Oils," *Australian Journal of Dairy Technology* **51**, 108–111 (1996).

A. G. Khramtsov et al., "Application of Lactulose in Food for Children, Dietetic and Clinical Nutrition," *Voprosy Pitaniya* No. 2, 25–26 (1997).

M. Kosempel, A. McAloon, and L. Roth, "Simulated Scale-Up and Cost Estimate of a Process for Alkaline Isomerization of Lactose to Lactulose Using Boric Acid as Complexation Agent," *J. Chem. Technol. Biotechnol.* **68**, 229–235 (1997).

P. Lee and H. E. Swaisgood, "Modification of Milkfat Physical Properties by Immobilized Fluorescens Lipase," *J. Agric. Food Chem.* **45**, 3343–3349 (1998).

V. V. Mistry and J. B. Pulgar, "Physical and Storage Properties of High Milk Protein Powder," *International Dairy Journal* **6**, 195–203 (1996).

D. L. Moreau and M. Rosenberg, "Storage Stability of Anhydrous Milkfat Microencapsulated in Whey Proteins," *Journal of Dairy Science* **78** (Suppl. 1), 114 (1995).

R. L. Motion, "Hydrolysates of Milk Protein," *Proceedings of the Nutrition Society of New Zealand* **17**, 56–63 (1992).

M. S. Nam et al., "Lactoferrin: A Review," *Korean Journal of Dairy Science* **18**, 289–298 (1996).

I. Nor-Aini et al., "Chemical and Physical Properties of Shortenings Based on Palm Oil and Milkfat," *ASEAN Food Journal* **9**, 141–146 (1994).

I. Nor-Aini et al., "Physical Characteristics of Shortenings Based on Modified Palm Oil, Milkfat and Low Melting Milkfat Fraction," *Fett Wissenschaft Technologie* **97**, 253–260 (1995).

C. I. Onwulata, R. P. Konstance, and V. H. Holsinger, "Flow Properties of Encapsulated Milkfat Powders as Affected by Flow Agent," *J. Food Sci.* **61**, 1211–1215 (1996).

C. I. Onwulata, P. W. Smith, and V. H. Holsinger, "Flow and Compaction of Spray-Dried Powders of Anhydrous Butteroil and High Melting Milkfat Encapsulated in Disaccharides," *J. Food Sci.* **60**, 836–840 (1995).

C. I. Onwulata et al., "Particle Structures of Encapsulated Milkfat Powders," *Lebensm. Wiss. Technol.* **29**, 163–172 (1996).

M. Papalois et al., "Australian Milkfat Survey—Physical Properties," *Australian Journal of Dairy Technology* **51**, 114–117 (1996).

D Rousseau, A. R. Hill, and A. G. Marangoni, "Rheological Behaviour of Modified Milkfat," *Journal of Dairy Science* **78** (Suppl 1), 103 (1995)

V. K. S. Shukla, "Milkfat and Its Applications," *World of Ingredients*, 30–31, 33 (Jan./Feb. 1995).

W. Strohmaier, "Lactulose, an Innovative Food Ingredient—Physiological Aspects," *Food Ingredients Europe Conference Proceedings*, 69–72 (1997).

C. Versteeg et al., "New Fractionated Milkfat Products," *Australian Journal of Dairy Technology* **49**, 57–61 (1994).

X. Ye, "Studies on Minor Proteins in Bovine Milk," *Journal of the Faculty of Applied Biological Science Hiroshima University* **34**, 194–195 (1995).

ROBIN M. FENWICK
K. J. KIRKPATRICK
New Zealand Dairy Board
Wellington, New Zealand

See also DAIRY INGREDIENTS: APPLICATIONS IN MEAT, POULTRY, AND SEAFOODS; WHEY: COMPOSITION, PROPERTIES, PROCESSING, AND USES.

DEHYDRATION

Dehydration (or drying) is defined as the application of heat under controlled conditions to remove the majority of the water normally present in a food by evaporation (or in the case of freeze-drying by sublimation). This definition excludes other unit operations that remove water from foods (eg, mechanical separations, membrane concentration, evaporation, and baking) as these normally remove much less water than dehydration. The main purpose of dehydration is to extend the shelf life of foods by a reduction in water activity. This inhibits microbial growth and enzyme activity, but the product temperature is usually insufficient to cause inactivation. The reduction in weight and bulk of food reduces transport and storage costs and, for some types of food, provides greater variety and convenience for the consumer. Drying causes deterioration of both the eating quality and the nutritive value of the food. The design and operation of dehydration equipment aim to minimize these changes by selection of appropriate drying conditions for individual foods. Examples of commercially important dried foods are sugar, coffee, milk, potato, flour (including bakery mixes), beans, pulses, nuts, breakfast cereals, tea, and spices.

THEORY

Dehydration involves the simultaneous application of heat and removal of moisture from foods. Factors that control the rates of heat and mass transfer are described elsewhere in the encyclopedia. Dehydration by heated air or heated surfaces is described in this article. Microwave, dielectric, radiant, and freeze-drying are described in other entries.

Psychrometrics

The capacity of air to remove moisture from a food depends on the temperature and the amount of water vapor already carried by the air. The content of water vapor in air is expressed as either absolute humidity—the mass of water vapor per unit mass of dry air (in kilograms per kilogram), termed moisture content in Fig. 1)—or relative humidity (RH) (in percent)—the ratio of the partial pressure of water vapor in the air to the pressure of saturated water vapor at the same temperature, multiplied by 100. Psychrometry is the study of the interrelationships of the temperature and humidity of air. These properties are most conveniently represented on a phychrometric chart (Fig. 1).

The temperature of the air, measured by a thermometer bulb, is termed the dry-bulb temperature. If the thermometer bulb is surrounded by a wet cloth, heat is removed by evaporation of the water from the cloth and the temperature falls. This lower temperature is called the wet-bulb temperature. The difference between the two temperatures is used to find the relative humidity of air on the psychrometric chart. An increase in air temperature, or reduction in RH, causes water to evaporate from a wet surface more rapidly and therefore produces a greater fall in temperature. The dew point is the temperature at which air becomes saturated with moisture (100% RH). Adiabatic cooling lines are the parallel straight lines sloping across the chart, which show how absolute humidity decreases as the air temperature increases.

Sample Problems 1. Using the psychrometric chart (Fig. 1), calculate the following.

1. The absolute humidity of air that has 50% RH and a dry-bulb temperature of 60°C.
2. The wet-bulb temperature under these conditions.
3. The RH of air having a wet-bulb temperature of 45°C and a dry-bulb temperature of 75°C.
4. The dew point of air cooled adiabatically from a dry-bulb temperature of 55°C and 30% RH.
5. The change in RH of air with a wet-bulb temperature of 39°C, heated from a dry-bulb temperature of 50°C to a dry-bulb temperature of 86°C.
6. The change in RH of air with a wet-bulb temperature of 35°C, cooled adiabatically from a dry-bulb temperature of 70°C to 40°C.

Solution to Sample Problems 1.

1. 0.068 kg/kg of dry air. Find the intersection of the 60°C and 50% RH lines and then follow the chart horizontally right to read the absolute humidity.
2. 47.5°C. From the intersection of the 60°C and 50% RH lines, extrapolate left parallel to the wet-bulb lines to read the wet-bulb temperature.

C. I. Onwulata, P. W. Smith, and V. H. Holsinger, "Flow and Compaction of Spray-Dried Powders of Anhydrous Butteroil and High Melting Milkfat Encapsulated in Disaccharides," *J. Food Sci.* **60**, 836–840 (1995).

C. I. Onwulata et al., "Particle Structures of Encapsulated Milkfat Powders," *Lebensm. Wiss Technol.* **29**, 163–172 (1996).

M. Papalois et al., "Australian Milkfat Survey—Physical Properties," *Australian Journal of Dairy Technology* **51**, 114–117 (1996).

D Rousseau, A. R. Hill, and A. G. Marangoni, "Rheological Behaviour of Modified Milkfat," *Journal of Dairy Science* **78** (Suppl 1), 103 (1995).

V. K. S. Shukla, "Milkfat and Its Applications," *World of Ingredients*, 30–31, 33 (Jan./Feb. 1995).

W. Strohmaier, "Lactulose, an Innovative Food Ingredient—Physiological Aspects," *Food Ingredients Europe Conference Proceedings*, 69–72 (1997).

C. Versteeg et al., "New Fractionated Milkfat Products," *Australian Journal of Dairy Technology* **49**, 57–61 (1994).

X. Ye, "Studies on Minor Proteins in Bovine Milk," *Journal of the Faculty of Applied Biological Science Hiroshima University* **34**, 194–195 (1995).

ROBIN M. FENWICK
K. J. KIRKPATRICK
New Zealand Dairy Board
Wellington, New Zealand

See also DAIRY INGREDIENTS: APPLICATIONS IN MEAT, POULTRY, AND SEAFOODS; WHEY: COMPOSITION, PROPERTIES, PROCESSING, AND USES.

DEHYDRATION

Dehydration (or drying) is defined as the application of heat under controlled conditions to remove the majority of the water normally present in a food by evaporation (or in the case of freeze-drying by sublimation). This definition excludes other unit operations that remove water from foods (eg, mechanical separations, membrane concentration, evaporation, and baking) as these normally remove much less water than dehydration. The main purpose of dehydration is to extend the shelf life of foods by a reduction in water activity. This inhibits microbial growth and enzyme activity, but the product temperature is usually insufficient to cause inactivation. The reduction in weight and bulk of food reduces transport and storage costs and, for some types of food, provides greater variety and convenience for the consumer. Drying causes deterioration of both the eating quality and the nutritive value of the food. The design and operation of dehydration equipment aim to minimize these changes by selection of appropriate drying conditions for individual foods. Examples of commercially important dried foods are sugar, coffee, milk, potato, flour (including bakery mixes), beans, pulses, nuts, breakfast cereals, tea, and spices.

THEORY

Dehydration involves the simultaneous application of heat and removal of moisture from foods. Factors that control the rates of heat and mass transfer are described elsewhere in the encyclopedia. Dehydration by heated air or heated surfaces is described in this article. Microwave, dielectric, radiant, and freeze-drying are described in other entries.

Psychrometrics

The capacity of air to remove moisture from a food depends on the temperature and the amount of water vapor already carried by the air. The content of water vapor in air is expressed as either absolute humidity—the mass of water vapor per unit mass of dry air (in kilograms per kilogram), termed moisture content in Fig. 1)—or relative humidity (RH) (in percent)—the ratio of the partial pressure of water vapor in the air to the pressure of saturated water vapor at the same temperature, multiplied by 100. Psychrometry is the study of the interrelationships of the temperature and humidity of air. These properties are most conveniently represented on a phychrometric chart (Fig. 1).

The temperature of the air, measured by a thermometer bulb, is termed the dry-bulb temperature. If the thermometer bulb is surrounded by a wet cloth, heat is removed by evaporation of the water from the cloth and the temperature falls. This lower temperature is called the wet-bulb temperature. The difference between the two temperatures is used to find the relative humidity of air on the psychrometric chart. An increase in air temperature, or reduction in RH, causes water to evaporate from a wet surface more rapidly and therefore produces a greater fall in temperature. The dew point is the temperature at which air becomes saturated with moisture (100% RH). Adiabatic cooling lines are the parallel straight lines sloping across the chart, which show how absolute humidity decreases as the air temperature increases.

Sample Problems 1. Using the psychrometric chart (Fig. 1), calculate the following.

1. The absolute humidity of air that has 50% RH and a dry-bulb temperature of 60°C.
2. The wet-bulb temperature under these conditions.
3. The RH of air having a wet-bulb temperature of 45°C and a dry-bulb temperature of 75°C.
4. The dew point of air cooled adiabatically from a dry-bulb temperature of 55°C and 30% RH.
5. The change in RH of air with a wet-bulb temperature of 39°C, heated from a dry-bulb temperature of 50°C to a dry-bulb temperature of 86°C.
6. The change in RH of air with a wet-bulb temperature of 35°C, cooled adiabatically from a dry-bulb temperature of 70°C to 40°C.

Solution to Sample Problems 1.

1. 0.068 kg/kg of dry air. Find the intersection of the 60°C and 50% RH lines and then follow the chart horizontally right to read the absolute humidity.
2. 47.5°C. From the intersection of the 60°C and 50% RH lines, extrapolate left parallel to the wet-bulb lines to read the wet-bulb temperature.

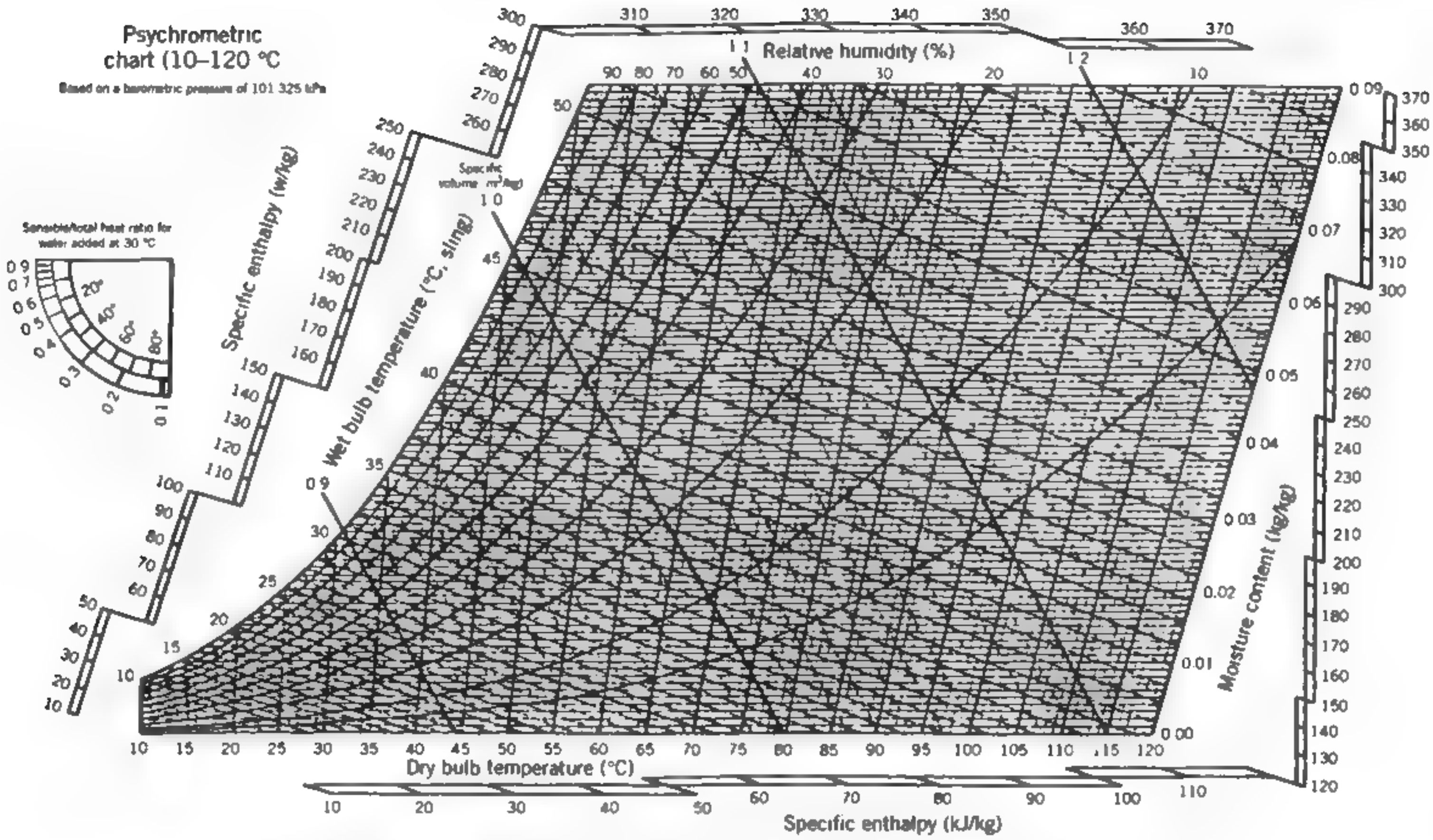


Figure 1. Psychometric chart (10–120°C based on barometric pressure of 101.325 kPa. *Source:* Courtesy of Chartered Institution of Building Services Engineers.

3. 20%. Find the intersection of the 45°C and 75%C lines and follow the sloping RH line upward to read the % RH.
4. 36°C. Find the intersection of the 55°C and 30% RH lines and follow the wet-bulb line left until the RH reaches 100%.
5. 50–100%. Find the intersection of the 39°C wet-bulb and the 50°C dry-bulb temperatures and follow the horizontal line to the intersection with the 86°C dry-bulb line; read the sloping RH line at each intersection (this represents the changes that take place when air is heated before being blown over food.
6. 10–70%. Find the intersection of the 35°C wet-bulb and 70°C dry-bulb temperatures and follow the wet-bulb line left until the intersection with the 40°C dry-bulb line; read sloping RH line at each intersection (this represents the changes taking place as the air is used to dry food; the air is cooled and becomes more humid as it picks up moisture from the food).

Water Activity

Deterioration of foods by microorganisms can take place rapidly, whereas enzymatic and chemical reactions take place more slowly during storage. In either case, water is the single most important factor controlling the rate of deterioration. The moisture content of foods can be expressed either on a wet-weight basis:

$$m = \frac{\text{mass of water}}{\text{mass of sample}} \times 100 \tag{1}$$

$$m = \frac{\text{mass of water}}{\text{mass of water} + \text{solids}} \times 100 \tag{2}$$

or a dry-weight basis (1):

$$M = \frac{\text{mass of water}}{\text{mass of solids}} \tag{3}$$

The dry-weight basis is more commonly used for processing calculations, whereas the wet-weight basis is frequently quoted in food composition tables. It is important, however, to note which system is used when expressing a result. Dry-weight basis is used throughout this article unless otherwise stated.

A knowledge of the moisture content alone is not sufficient to predict the stability of foods. Some foods are unstable at a low moisture content (eg, peanut oil deteriorates if the moisture content exceeds 0.6%), whereas other foods are stable at relatively high moisture contents (eg, potato starch is stable at 20% moisture) (2). It is the availability of water for microbial, enzymatic, or chemical activity that determines the shelf life of a food, and this is measured by the water activity (A_w) of a food. Examples of unit operations that reduce the availability of water in foods include those that physically remove water (dehydration, evaporation, and freeze-drying or freeze concentration) and those that immobilize water in the food (eg, by the use of humectants in intermediate-moisture foods and by formation of ice crystals in freezing). Examples of the moisture content and A_w of foods are shown in Table 1. The effect of reduced A_w on food stability is shown in Table 2.

Water in food exerts a vapor pressure. The size of the vapor pressure depends on

1. The amount of water present.
2. The temperature.
3. The concentration of dissolved solutes (particularly salts and sugars) in the water.

Water activity is defined as the ratio of the vapor pressure of water in a food to the saturated vapor pressure of water at the same temperature:

$$A_W = \frac{P}{P_0} \tag{4}$$

where P (Pa) is vapor pressure of the food and P_0 (Pa) the vapor pressure of pure water at the same temperature. A_w is related to the moisture content by the Brunauer-Emmett-Teller (BET) equation

$$\frac{A_W}{M(1 - A_W)} = \frac{1}{M_1C} + \frac{C - 1}{M_1C} A_W \tag{5}$$

where A_W is the water activity, M the moisture as percentage dry weight, M_1 the moisture (dryweight basis) of a monomolecular layer, and C a constant (2).

A proportion of the total water in a food is strongly bound to specific sites (eg, hydroxyl groups of polysaccharides, carbonyl and amino groups of proteins, the hydrogen bonding). When all sites are (statistically) occupied by adsorbed water the moisture content is termed the BET monolayer value. Typical examples include gelatin (11%), starch (11%), amorphous lactose (6%), and whole spray-dried milk (3%). The BET monolayer value therefore represents the moisture content at which the food is most stable. At moisture contents below this level, there is a higher rate of lipid oxidation and, at higher moisture contents, Maillard browning and then enzymatic and microbiological activities are promoted.

The movement of water vapor from a food to the surrounding air depends on both the moisture content and composition of the food and the temperature and humidity of the air. At a constant temperature the moisture content of food changes until it comes into equilibrium with water vapor in the surrounding air. The food then neither gains nor loses weight on storage under those conditions. This is called the equilibrium moisture content of the food, and the relative humidity of the storage atmosphere is known as the equilibrium relative humidity. When different values of relative humidity versus equilibrium moisture content are plotted, a curve known as a water sorption isotherm is obtained (Fig. 2).

Each food has a unique set of sorption isotherms at different temperatures. The precise shape of the sorption isotherm is caused by differences in the physical structure, chemical composition, and extent of water binding within the food, but all sorption isotherms have a characteristic shape, similar to that shown in Figure 2. The first part of

Table 1. Moisture Content and Water Activity of Foods

Food	Moisture content (%)	Water activity	Degree of protection required
Ice (0°C)	100	1.00[a]	Package to prevent moisture loss
Fresh meat	70	0.985	
Bread	40	0.96	
Ice (−10°C)	100	0.91[a]	
Marmalade	35	0.86	
Ice (−20°C)	100	0.82[a]	
Wheat flour	14.5	0.72	Mininum protection or no packaging required
Ice (−50°C)	100	0.62[a]	
Raisins	27	0.60	
Macaroni	10	0.45	
Cocoa powder		0.40	
Boiled sweets	3.0	0.30	Package to prevent moisture uptake
Biscuits	5.0	0.20	
Dried milk	3.5	0.11	
Potato crisps	1.5	0.08	

Source. Refs. 2–4.

[a]

Table 2. The Importance of Water Activity in Foods

A_W	Phenomenon	Examples
1.00		Highly perishable fresh foods
0.95	Pseudomonads, bacillus, *Clostridium perfringens*, and some yeasts inhibited	Foods with 40% sucrose or 7% salt; cooked sausages, bread
0.90	Lower limit for bacterial growth (general), salmonella, *Vibrio parahemolyticus, Clostridium botulinum,* lactobacillus, and some yeasts and fungi inhibited	Foods with 55% sucrose, 12% salt; cured ham, medium-age cheese. Intermediate-moisture foods (A_W = 0.90 − 0.55)
0.85	Many yeasts inhibited	Foods with 65% sucrose, 15% salt; salami, mature cheese, margarine
0.80	Lower limit for enzyme activity and growth of most fungi; *Staphlococcus aureus* inhibited	Flour, rice (15–17% water) fruit cake, sweetened condensed milk, fruit syrups, fondant
0.75	Lower limit for halophilic bacteria	Marzipan (15–17% water), jams
0.70	Lower limit for growth of most xerophilic fungi	
0.65	Maximum velocity of Maillard reactions	Rolled oats (10% water), fudge, molasses, nuts
0.60	Lower limit for growth of osmophilic or xerophilic yeasts and fungi	Dried fruits (15–20% water), toffees, caramels (8% water), honey
0.55	Deoxyribonucleic acid becomes disordered (lower limit for life to continue)	
0.50		Dried foods, spices, noodles
0.40	Minimum oxidation velocity	Whole egg powder (5% water)
0.30		Crackers, bread crusts (3–5% water)
0.25	Maximum heat resistance of bacterial spores	
0.20		Whole milk powder (2–3% water), dried vegetables (5% water), cornflakes (5% water)

the curve, to point *A*, represents monolayer water, which is very stable, unfreezable, and not removed by drying. The second, relatively straight part of the curve (*AB*) represents water absorbed in multilayers within the food and solutions of soluble components. The third portion (above point *B*) is free water condensed within the capillary structure or in the cells of a food. It is mechanically trapped within the food and held only by weak forces. It is easily removed by drying and easily frozen, as indicated by the steepness of the curve. Free water is available for microbial growth and enzyme activity, and a food that has a moisture content above point *B* on the curve is likely to be susceptible to spoilage.

The sorption isotherm indicates the A_w at which a food is stable and allows predictions of the effect of changes in moisture content on A_w and hence on storage stability. It is used to determine the rate and extent of drying, the optimum frozen storage temperatures, and the moisture-barrier properties required in packaging materials.

The rate of change in A_w on a sorption isotherm differs according to whether moisture is removed from a food (desorption) or it is added to dry food (adsorption) (Fig. 2). This is termed a hysteresis loop. The difference is large in some foods, eg, rice, and is important for example in determining the protection required against moisture uptake.

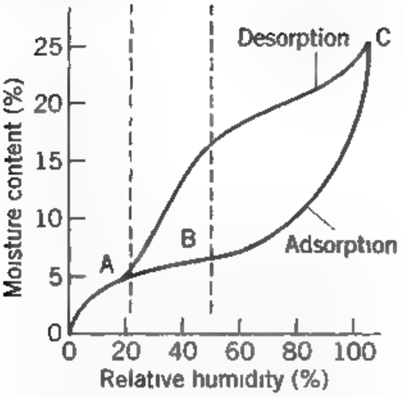


Figure 2. Water sorption isotherm.

Mechanism of Drying

When hot air is blown over a wet food, heat is transferred to the surface, and latent heat of vaporization causes water to evaporate. Water vapor diffuses through a boundary film of air and is carried away by the moving air (Fig. 3). This creates a region of lower water vapor pressure at the surface of the food, and a water vapor pressure gradient is established from the moist interior of the food to the dry air. This gradient provides the driving force for water removal from the food.

Water moves to the surface by the following mechanisms:

1. Liquid movement by capillary forces.
2. Diffusion of liquids, caused by differences in the concentration of solutes in different regions of the food.
3. Diffusion of liquids, which are adsorbed in layers at the surfaces of solid components of the food.
4. Water vapor diffusion in air spaces within the food caused by vapor pressure gradients.

Foods are characterized as hygroscopic and nonhygroscopic. Hygroscopic foods are those in which the partial pressure of water vapor varies with the moisture content. Nonhygroscopic foods have a constant water vapor pressure at different moisture contents. The difference is found by using sorption isotherms.

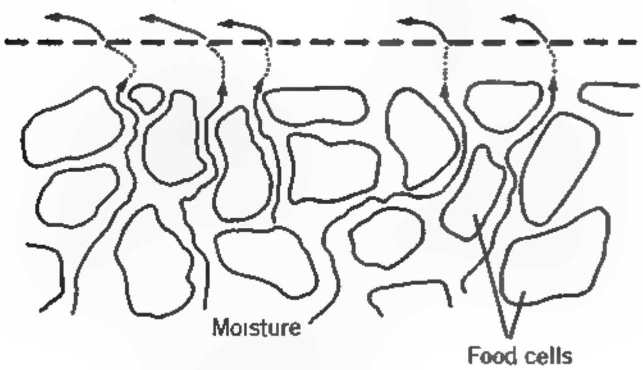


Figure 3. Movement of moisture during drying.

When food is placed in a dryer, there is a short initial settling down period as the surface heats up to the wet-bulb temperature (*AB* in Fig. 4a). Drying then commences and, provided that water moves from the interior of the food at the same rate as it evaporates from the surface, the surface remains wet. This is known as the constant-rate period and continues until a certain critical moisture content is reached (*BC* in Figs. 4a and b). In practice, however, different areas of the surface of the food dry out at different rates and, overall, the rate of drying declines gradually during the constant-rate period. Thus the critical point is not fixed for a given food and depends on the amount of food in the dryer and the rate of drying.

The three characteristics of air that are necessary for successful drying in the constant rate period are

1. A moderately high dry-bulb temperature.
2. A low RH.
3. A high air velocity.

The boundary film of air surrounding the food acts as a barrier to the transfer of both heat and water vapor during

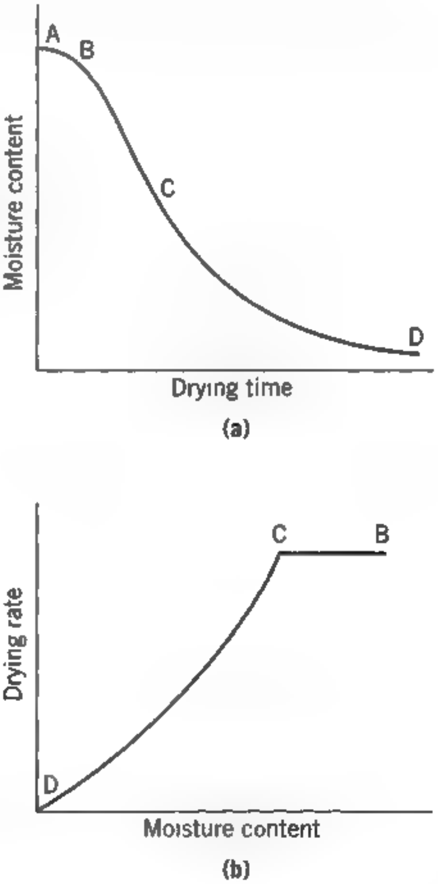


Figure 4. (**a**) and (**b**) Drying curves. The temperature and humidity of the drying air are constant and all heat is supplied to the surface by convection.

drying. The thickness of the film is determined primarily by the air velocity. If this is too low, water vapor leaves the surface of the food and increases the humidity of the surrounding air, to cause a reduction in the water vapor pressure gradient and the rate of drying (Similarly, if the temperature of the drying air falls or the humidity rises, the rate of evaporation falls and drying slows).

When the moisture content of the food falls below the critical moisture content, the rate of drying slowly decreases until it approaches zero at the equilibrium moisture content (ie, the food comes into equilibrium with the drying air). This is known as the falling-rate period. Nonhygroscopic foods have a single falling-rate period (*CD* in Fig. 4a and b), whereas hygroscopic foods have two periods. In the first period, the plane of evaporation moves inside the food, and water diffuses through the dry solids to the drying air. It ends when the plane of evaporation reaches the center of the food and the partial pressure of water falls below the saturated water vapor pressure. The second period occurs when the partial pressure of water is below the saturated vapor pressure and drying is by desorption.

During the falling-rate period, the rate of water movement from the interior of the food to the surface falls below the rate at which water evaporates to the surrounding air. The surface therefore dries out. This is usually the longest period of a drying operation and, in some foods, eg, grain drying, where the initial moisture content is below the critical moisture content, the falling-rate period is the only part of the drying curve to be observed. During the falling-rate period, the factors that control the rate of drying change. Initially, the important factors are similar to those in the constant-rate period, but gradually the rate of mass transfer becomes the controlling factor. This depends mostly on the temperature of the air and the thickness of the food. It is unaffected by both the RH of the air (except in determining the equilibrium moisture content) and the velocity of the air. The air temperature is therefore controlled during the falling rate period, whereas the air velocity and temperature are more important during the constant-rate period. In practice, foods may differ from these idealized drying curves owing to shrinkage, changes in the temperature and rate of moisture diffusion in different parts of the food, and changes in the temperature and humidity of the drying air.

The surface temperature of the food remains close to the wet-bulb temperature of the drying air until the end of the constant-rate period, owing to the cooling effect of the evaporating water. During the falling-rate period the amount of water evaporating from the surface gradually decreases but, as the same amount of heat is being supplied by the air, the surface temperature rises until it reaches the dry-bulb temperature of the drying air. Most heat damage to food therefore occurs in the falling rate period.

Calculation of Drying Rate

The rate of drying depends on the properties of the dryer (the dry-bulb temperature, RH and velocity of the air, and the surface heat transfer coefficient) and the properties of the food (the moisture content, surface-to-volume ratio, and the surface temperature and rate of moisture loss). The size of food pieces has an important effect on the drying rate in both the constant- and the falling-rate periods. In the constant-rate period, smaller pieces have a larger surface area available for evaporation, whereas, in the falling-rate period, smaller pieces have a shorter distance for moisture to travel through the food. Other factors that influence the rate of drying include.

1. The fat content of the food (higher fat contents generally result in slower drying, as water is trapped within the food).
2. The method of preparation of the food (cut surfaces lose moisture more quickly than losses through skin.
3. The amount of food placed into a dryer in relation to its size (in a given dryer, faster drying is achieved with smaller quantities of food).

The rate of heat transfer is found using

$$Q = h_s A(\theta_a - \theta_s) \tag{6}$$

The rate of mass transfer is found using

$$-m_c = K_g A(H_s - H_a) \tag{7}$$

Because during the constant-rate period, an equilibrium exists between the rate of heat transfer to the food and the rate of mass transfer in the form of moisture loss from the food, these rates are related by

$$-m_c = \frac{h_c A}{\lambda}(\theta_a - \theta_s) \tag{8}$$

where Q (J/s) is the rate of heat transfer, h_c W/m^2/°K the surface heat transfer coefficient for convective heating, A (m^2) the surface area available for drying, θ_a (°C) the average dry-bulb temperature of drying air, θ_s (°C) the average wet-bulb temperature of drying air, m_c (kg/s) the change in mass with time (drying rate), K_g (kg/m^2/s) the mass transfer coefficient, H_s (kilograms of moisture per kilogram dry air) the humidity at the surface of the food (saturation humidity), H_a (kilograms of moisture per kilogram dry air) the humidity of air, and λ (J/kg) the latent heat of evaporation at the wet-bulb temperature.

The surface heat transfer coefficient (h_c) is related to the mass flow rate of air using the following equations: for parallel airflow,

$$h_c = 14.3G^{0.8} \tag{9}$$

and for perpendicular airflow,

$$h_c = 24.2G^{0.37} \tag{10}$$

Where G (kg/m^2/s) is the mass flow rate of air.

For a tray of food in which water evaporates only from the upper surface, the drying time is found using

$$m_c = \frac{h_c}{\rho\lambda x}(\theta_a - \theta_s) \tag{11}$$

where ρ (kg/m^3) is the bulk density of food and x (m) the thickness of the bed of food.

The drying time in the constant-rate period is found using

$$t = \frac{\rho\lambda x(M_i - M_c)}{h_c(\theta_a - \theta_s)} \tag{12}$$

where t (s) is the drying time, M_i (kg/kg of dry solids) the initial moisture content, and M_c (kg/kg of dry solids) the critical moisture content.

For water evaporating from a spherical droplet in a spray dryer, the drying time is found using

$$t = \frac{r^2\rho_l\lambda}{3h_c(\theta_a - \theta_s)}\,\frac{M_i - M_f}{1 + M_i} \tag{13}$$

where ρ_l (kg/m^3) is the density of the liquid, r (m) the radius of the droplet, M_f (kg/kg of dry solids) the final moisture content.

In the falling-rate period, moisture gradients change throughout the food and the temperature slowly increases from the wet-bulb temperature to the dry-bulb temperature as the food dries. The following equation is used to calculate the drying time from the start of the falling-rate period to the equilibrium moisture content using a number of assumptions concerning, eg, the nature of moisture movement and the absence of shrinkage of the food:

$$t = \frac{\rho x(M_c - M_e)}{K_g(P_s - P_a)}\ln\left(\frac{M_c - M_e}{M - M_e}\right) \tag{14}$$

where M_e (kg/kg of dry solids) is the equilibrium moisture content, M (kg/kg of dry solids) the moisture content at time t from the start of the falling-rate period, P_s (mmHg) the saturated water vapor pressure at the wet-bulb temperature, and P_a (mmHg) the partial water vapor pressure.

The velocity of the air needed to achieve fluidization of spherical particles is calculated using

$$v_f = \frac{(\rho_s - \rho)g}{\mu}\,\frac{d^2\varepsilon^3}{180(1 - \varepsilon)} \tag{15}$$

where v_f(m/s) is the fluidization velocity, ρ_s (kg/m^3 the density of the solid particles, ρ (kg/m^3) the density of the fluid, g (m/s^2) the acceleration due to gravity, μ (N · s/m^2) the viscosity of the fluid, d (m) the diameter of the particles, ϵ the voidage of the bed.

Formulas for foods of other shapes are described in Ref. 5. The minimum air velocity needed to convey particles is found using:

$$v_e = \sqrt{\left[\frac{4d(\rho_s - \rho)}{3C_d\rho}\right]} \tag{16}$$

where v_e (m/s) is the minimum air velocity and C_d (= 0.44 for Re = 500–200,000) the drag coefficient.

Sample Problem 2. Peas with an average diameter of 6 mm and a density of 880 kg/m^3 are dried in a fluidized-bed dryer. The minimum voidage is 0.4 and the cross-sectional area of the bed is 0.25 m^2. Calculate the minimum air velocity needed to fluidize the bed if the air density is 0.96 kg/m^3 and the air viscosity is 2.15×10^{-5} N · s/m^2.

Solution of Sample Problem 2. From equation 15

$$v_F = \frac{(880 - 0.96)9.81}{2.15 \times 10^{-5}}\,\frac{(0.006)^2(0.4)^3}{180(1 - 0.4)} = 8.5 \text{ m/s}^1$$

Derivations of these equations are described in Refs. 6–8.

Sample Problem 3. A conveyor dryer is required to dry peas from an initial moisture content of 78% to 16% moisture (wet-weight basis) in a bed 10 cm deep with a voidage of 0.4. Air at 85°C with a relative humidity of 10% is blown perpendicularly through the bed at 0.9 m/s. The dryer belt measures 0.75 m wide and 4 m long. Assuming that drying takes place from the entire surface of the peas and there is no shrinkage, calculate the drying time and energy consumption in both the constant and the falling-rate periods. (Additional data: The equilibrium moisture content of the peas is 9%, the critical moisture content 300% (dry-weight basis), the average diameter 6 mm, the bulk density 610 kg/m^3, the latent heat of evaporation 2300 kJ/kg, the saturated water vapor pressure at wet-bulb temperature 61.5 mmHg, and the mass transfer coefficient 0.015 kg/m^2/s).

Solution to Sample Problem 3. In the constant-rate period, from equation 10:

$$h_c = 24.2(0.9)^{0.37} = 23.3 \text{ W/m}^2/^\circ\text{K}$$

From Fig. 1 for θ_a = 85°C and RH = 10%,

$$\theta_s = 42^\circ\text{C}$$

To find the area of the peas,

$$\text{Volume of a sphere} = \frac{4}{3}\pi r^3 = 4 \times 3.142(0.003)^3$$
$$= 339 \times 10^{-9} \text{ m}^3$$

$$\text{Volume of the bed} = 0.75 \times 4 \times 0.1 = 0.3 \text{ m}^3$$

$$\text{Volume of peas in the bed} = 0.3(1 - 0.4) = 0.18 \text{ m}^3$$

$$\text{Number of peas} = \frac{\text{volume of peas in bed}}{\text{volume each pea}}$$
$$= \frac{0.18}{339 \times 10^{-9}} = 5.31 \times 10^5$$

$$\text{Area of a sphere} = 4\pi r^2 = 4 \times 3.142(0.003)^2$$
$$= 113 \times 10^{-6} \text{ m}^2$$

and

$$\text{Total area of peas} = 5.31 \times 10^5 \times 113 \times 10^{-6}$$
$$= 60 \text{ m}^2$$

From equation 8:

$$\text{Drying rate} = \frac{23.3 \times 60}{2.3 \times 10^6}(85 \quad 42) = 0.026 \text{ kg/s}$$

From a mass balance

$$\text{Volume of peas} = 0.18 \text{ m}^3$$
$$\text{Bulk density} = 610 \text{ kg/m}^{-3}$$

Therefore,

$$\text{Mass of peas} = 0.18 \times 610 = 109.8 \text{ kg}$$
$$\text{Initial solids content} = 109.8 \times 0.22 = 24.15 \text{ kg}$$

Therefore,

$$\text{Initial mass water} = 109.8 - 24.15 = 85.6 \text{ kg}$$

After constant-rate period, solids remain constant and

$$\text{Mass of water} = 96.6 - 24.15 = 72.45 \text{ kg}$$

Therefore,

$$(85.6 - 72.45) = 13.15 \text{ kg water lost}$$

at a rate of 0.026 kg/s

$$\text{Drying time} = \frac{13.15}{0.026} = 505 \text{ s} = 8.4 \text{ min}$$

Therefore,

$$\begin{aligned}\text{Energy required} &= 0.026 \times 2.3 \times 10^5 = 6 \times 10^4 \text{ J/s} \\ &= 60 \text{ kW}\end{aligned}$$

In the falling-rate period,

$$\text{RH} = \frac{P_A}{P_o} \times 100$$
$$10 = \frac{P}{61.5} \times 100$$

Therefore,

$$P = 6.15 \text{ mmHg}$$

The moisture values are

$$M_c = \frac{75}{25} = 3$$
$$M_f = \frac{16}{84} = 0.19$$
$$M_e - \frac{9}{91} - 0.099$$

From Equation 14,

$$\begin{aligned}t &= \frac{(3 - 0.099)610 \times 0.1}{0.015(61.5 - 6.15)} \ln\left(\frac{3 - 0.099}{0.19 - 0.099}\right) = 737.7 \text{ s} \\ &= 12.3 \text{ min}\end{aligned}$$

From a mass balance, at the critical moisture content, 96.6 kg contains 25% solids = 24.16 kg. After drying in the falling-rate period, 84% solids = 24.16 kg. Therefore,

$$\text{Total mass} = \frac{100}{84} \times 24.16 = 28.8 \text{ kg}$$

and

$$\text{Mass loss} = 96.6 - 28.8 = 67.8 \text{ kg}$$

Thus,

$$\text{Average drying rate} = \frac{67.8}{737.7} = 0.092 \text{ kg/s}$$

$$\begin{aligned}\text{average energy required} &= 0.092 \times 2.3 \times 10^6 \\ &= 2.1 \times 10^5 \text{ J/s} = 210 \text{ kW}\end{aligned}$$

Drying Using Heated Surfaces

Heat is conducted from a hot surface, through a thin layer of food, and moisture is evaporated from the exposed surface. The main resistance to heat transfer is the thermal conductivity of the food. Knowledge of the rheological properties of the food is necessary to determine the thickness of the layer of food and the way in which it should be applied to the heated surface. Additional resistances to heat transfer arise if the partly dried food lifts off the hot surface. Equation 17 is used in the calculation of drying rates.

$$Q = UA(\theta_a - \theta_b) \qquad (17)$$

U is the overall heat transfer coefficient (W/m^2/K), θ_a is the temperature of hot surface (°C), and θ_b is the temperature of food (°C).

Sample Problem 4. A single-drum dryer 0.7 m in diameter and 0.85 m long operates at 150°C and is fitted with a doctor blade to remove food after three-fourths of a revolution. It is used to dry a 0.6-mm layer of 20% w/w solution of gelatin, preheated to 100°C, at atmospheric pressure. Calculate the speed of the drum required to produce a product with a moisture content of 4 kg of solids per kilogram of water. (Additional data: The density of gelatin feed is 1020 kg/m^3, and the overall heat transfer coefficient is 1200 W/m^2/K; assume that the critical moisture content of the gelatin is 450%, dry weight basis.)

Solution to Sample Problem 4. First,

$$\text{Drum area} = \pi dl = 3.142 \times 0.7 \times 0.85 = 1.87 \text{ m}^2$$

Therefore,

$$\text{Mass of food on the drum} = (1.87 \times 0.75)0.0006 \times 1020 = 0.86 \text{ kg}$$

From a mass balance (initially the food contains 80% moisture and 20% solids),

$$\text{Mass of solids} = 0.86 \times 0.2 = 0.172 \text{ kg}$$

After drying, 80% solids = 0.172 kg. Therefore,

$$\text{Mass of dried food} = \frac{100}{80} \times 0.172 = 0.215 \text{ kg}$$

$$\text{Mass loss} = 0.86 - 0.215 = 0.645 \text{ kg}$$

From equation 17,

$$Q = 1200 \times 1.87\ (150 - 100) = 1.12 \times 10^5 \text{ J/s}$$

$$\text{Drying rate} = \frac{1.12 \times 10^5}{2.257 \times 10^6} \text{ kg/s} = 0.05 \text{ kg/s}$$

and

$$\text{Residence time required} = \frac{0.645}{0.05} = 13 \text{ s}$$

As only three-quarters of the drum surface is used, 1 rev should take (100/75) × 13 = 17.3 s. Therefore, speed = 3.5 rev/min.

EQUIPMENT

Most commercial dryers are insulated to reduce heat losses, and they recirculate hot air to save energy. Many designs have energy-saving devices that recover heat from the exhaust air or automatically control the air humidity (9,10). Computer control of dryers is increasingly sophisticated (11) and also results in important savings in energy. The criteria for selection of drying equipment and potential applications are described in Table 3. The relative costs of different drying methods are reported as follows (12): forced-air drying, 198; fluidized-bed drying, 315; drum drying, 327; continuous vacuum drying, 1840; freeze-drying, 3528. Relative energy consumption (in kWh/kg of water removed) are as follows: roller drying, 1.25; pneumatic drying, 1.8; spray drying, 2.5; fluidized-bed drying, 3.5 (13).

Hot-Air Dryers

Bin Dryers (Deep-Bed Dryers). Bin dryers are cylindrical or rectangular containers fitted with a mesh base. Hot air passes up through a bed of food at relatively low speeds (eg, 0.5 $m^3/s/m^2$ of bin area). These dryers have a high capacity and low capital and running costs. They are mainly used for finishing (to 3–6% moisture content) after initial drying in other types of equipment. Bin dryers improve the operating capacity of initial dryers by taking the food when it is in the falling-rate period, when moisture removal is most time consuming. The deep bed of food permits variations in moisture content to be equalized and acts as a store to smooth out fluctuations in the product flow between drying stages and packaging. However, the dryers may be several meters high, and it is therefore important that foods are sufficiently strong to withstand compression at the base and to retain an open structure to permit the passage of hot air through the bed.

Cabinet Dryers (Tray Dryers). These dryers consist of an insulated cabinet fitted with shallow mesh or perforated trays, each of which contains a thin (2–6 cm deep) layer of food. Hot air is circulated through the cabinet at 0.5–5 m/s per square meter tray area. A system of ducts and baffles is used to direct air over and/or through each tray, to promote uniform air distribution. Additional heaters may be placed above or alongside the trays to increase the rate of drying. Tray dryers are used for small-scale production (1–20 tons/day) or for pilot-scale work. They have low capital and maintenance costs, but compared to dryers that have more sophisticated control they produce more variable product quality.

In developing countries the high capital investment for sophisticated dryers often cannot be justified, but there is a need for better-quality products than those produced by sun or solar drying. A small dryer consisting of a 60 kW gas or kerosine heater/blower unit and a cabinet fitted with 15 mesh trays has been developed to meet this need (14). In operation, air is passed across the food in each tray. Trays are loaded at the top of the dryer and unloaded at the base using metal fingers to move the tray stack. The dryer therefore operates semicontinuously and with counter-current airflow. Typical tray loadings are 5 kg, and the tray change cycle is 15–20 minutes.

Conveyor Dryers (Belt Dryers). Continuous conveyor dryers are up to 20 m long and 3 m wide. Food is dried on a mesh belt in beds 5–15 cm deep. The airflow is initially directed upward through the bed of food and then downward in later stages to prevent dried food from blowing out of the bed. Two- or three-stage dryers (Fig. 5) mix and repile the partly dried shrunken food into deeper beds (to 15–25 cm and 250–900 cm in three-stage dryers). This improves uniformity of drying and saves floor space. Foods are dried to 10–15% moisture content and then transferred to bin dryers for finishing. This equipment has good control over drying conditions and high production rates. It is used for large-scale drying of foods (eg, fruits and vegetables are dried in 2–3.5 h at up to 5.5 tons/h). It has independently controlled drying zones and is automatically loaded and unloaded, which reduces labor costs. As a result, it has largely replaced the tunnel dryer in many applications.

A second application of conveyor dryers is foam-mat drying in which liquid foods (eg, fruit juices) are formed into a stable foam by the addition of a stabilizer and aeration with nitrogen or air. The foam is spread on a perforated belt to a depth of 2–3 mm and dried rapidly in two stages by parallel and then counter-current airflows (Table 4). Foam drying is approximately three times faster than drying a similar thickness of liquid. The thin, porous mat

Table 3. Characteristics of Dryers

	Characteristics of the food											
			Initial moisture content		Size of pieces				Drying rate required		Final moisture content required	
Type of dryer	Solid	Liquid	Moderate to high	Low	Heat-sensitive	Small	Intermediate to large	Mechanically strong	Moderate to fast	Slow	Moderate	Low
Bin	*			*			*	*		*		*
Cabinet	*		*				*		*	*	*	
Conveyor	*		*				*		*	*	*	
Drum		*	—	—		—	—	—	*			*
Foam mat		*	—	—	*	—	—	—	*			*
Fluid bed	*		*			*		*	*		*	
Kiln	*		*				*			*	*	
Pneumatic	*			*		*		*	*			*
Rotary	*		*			*		*	*			*
Spray		*	—	—	*	—	—	—	*		*	*
Trough	*		*		*	*		*	*		*	
Tunnel	*		*				*		*	*	*	*
Vacuum band		*	—	—	*	—	—	—	*		*	*
Vacuum shelf	*	*	*		*	*			*	*	*	*
Radiant	*			*		*			*			*
Microwave or dielectric	*			*		*			*	*		*
Solar (sun)	*		*				*				*	

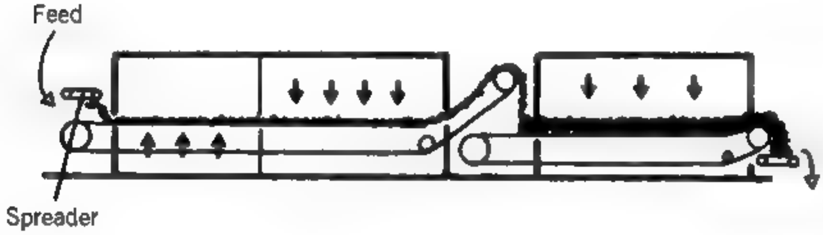


Figure 5. Two-stage conveyor dryer. *Source:* Courtesy of Proctor and Schwartz Inc.

Table 4. Advantages and Limitations of Parallel Flow, Counter-Current Flow, Center-Exhaust, and Cross-Flow Drying

Type of air flow	Advantages	Limitations
Parallel or cocurrent type: Food → Airflow →	Rapid initial drying. Little shrinkage of food. Low bulk density. Less heat damage to food. No risk of spoilage	Low moisture content difficult to achieve as cool, moist air passes over dry food
Counter-current type: Food → Airflow ←	More economical use of energy. Low final moisture content as hot air passes over dry food	Food shrinkage and possible heat damage. Risk of spoilage from warm moist air meeting wet food
Center-exhaust type: Food → Airflow → ↑ ←	Combined benefits of parallel and counter-current dryers but less than cross-flow dryers	More complex and expensive then single-direction air flow
Cross-flow type: Food → Airflow ↑ ↓	Flexible control of drying conditions by separately controlled heating zones, giving uniform drying and high drying rates	More complex and expensive to buy, operate, and maintain

of dried food is ground to a free-flowing powder that has good rehydration properties. The rapid drying and low product temperatures result in high-quality product. However, a large surface area is required for high production rates, so capital costs are therefore high.

Fluidized-Bed Dryers. Metal trays with mesh or perforated bases contain a bed of particulate foods up to 15 cm deep. Hot air is blown through the bed (Fig. 6), causing the food to become suspended and vigorously agitated (fluidized). The air thus acts as both the drying and the fluidiz-

Figure 6. Fluidized-bed drying. *Source:* Courtesy of Petrie and McNaught Ltd.

ing medium and the maximum surface area of food is made available for drying. A sample calculation of the air speed needed for fluidization is described in sample problem 2. Dryers may be batch or continuous in operation; the latter are often fitted with a vibrating base to help move the product. Continuous cascade systems, in which food is discharged under gravity from one tray to the next, employ up to six dryers for high production rates.

Fluidized-bed dryers are compact and have good control over drying conditions, relatively high thermal efficiencies, and high drying rates. In batch operation, products are mixed by fluidization, this leads to uniform drying. In continuous dryers, there is a greater range of moisture content in the dried product; bin dryers are therefore used for finishing. Fluidized-bed dryers are limited to small particulate foods that are capable of being fluidized without excessive mechanical damage (eg, peas, diced or sliced vegetables, grains, powders, or extruded foods). These considerations also apply to fluidized-bed freeze-dryers and freezers.

A development of the fluidized-bed dryer, named the Torbed dryer, has potential applications for drying particulate foods. A fluidized bed of particles is made to rotate around a torus-shaped chamber, by hot air blown directly from a burner (Fig. 7). The dryer has very high rates of heat and mass transfer and substantially reduced drying times. It is likely that some products (eg, vegetable pieces) would require a period of equilibration to allow moisture redistribution before final drying. The dryer operates semicontinuously under microprocessor control and is suitable for agglomeration and puff drying in addition to roasting, cooking, and coating applications.

Kiln Dryers. These dryers are two-story buildings in which a drying room with a slatted floor is located above a furnace. Hot air and the products of combustion from the furnace pass through a bed of food up to 20 cm deep. These dryers have been used traditionally for drying apple rings or slices in the United States, and hops or malt in Europe. There is limited control over drying conditions, and drying times are relatively long. High labor costs are incurred by the need to turn the product regularly, and by manual loading and unloading. However, the dryers have a large capacity and are easily constructed and maintained at low cost.

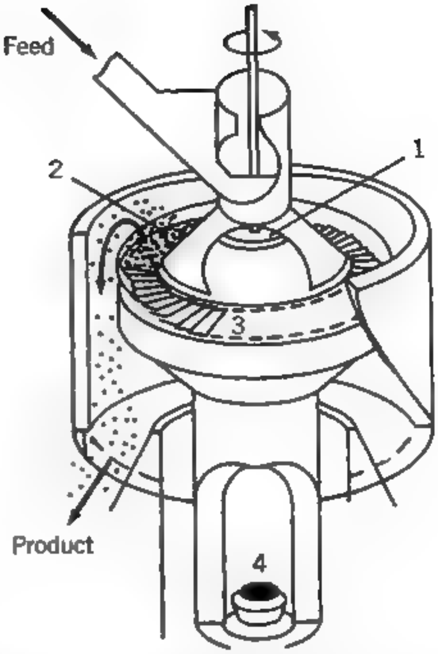


Figure 7. Torbed dryer: 1, rotating disc distributor to deliver raw material evenly into processing chamber; 2, rotating bed of particles; 3, fixed blades with hot gas passing through at high velocity; 4, burner assembly. *Source:* Courtesy of Torftech Ltd.

Pneumatic Dryers. In pneumatic dryers, powders or particulate foods are continuously dried in vertical or horizontal metal ducts. A cyclone separator is used to remove the dried product. The moist food (usually less than 40% moisture) is metered into the ducting and suspended in hot air. In vertical dryers the airflow is adjusted to classify the particles; lighter and smaller particles, which dry more rapidly, are carried to a cyclone more rapidly than are heavier and wetter particles that remain suspended to receive the additional drying required. For longer residence times the ducting is formed into a continuous loop (pneumatic ring dryers) and the product is recirculated until it is adequately dried. High-temperature short-time ring dryers are used to expand the starch-cell structure in potatoes or carrots to give a rigid, porous structure, which enhances subsequent conventional drying and rehydration rates. Calculation of air velocities needed for pneumatic drying is described in equation 16.

Pneumatic dryers have relatively low capital costs, high drying rates and thermal efficiencies, and close control over drying conditions. They are often used after spray drying to produce foods that have a lower moisture content than normal (eg, special milk or egg powders and potato granules). In some applications the simultaneous transportation and drying of the food may be a useful method of materials handling.

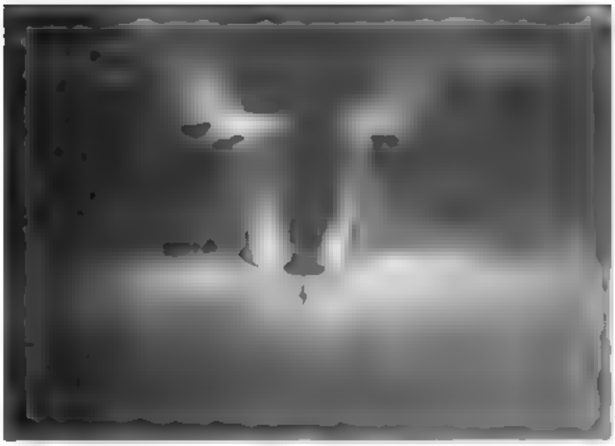
Rotary Dryers. A slightly inclined rotating metal cylinder is fitted internally with flights to cause the food to cascade through a stream of hot air as it moves through the dryer. Airflow may be parallel or counter-current (Table 4). The agitation of the food and the large area of food exposed to the air produce high drying rates and a uniformly dried product. The method is especially suitable for foods that tend to mat or stick together in belt or tray dryers. However, the damage caused by impact and abrasion in the dryer restrict this method to relatively few foods (eg, sugar crystals and cocoa beans).

Spray Dryers. A fine dispersion of preconcentrated foods is first atomized to form droplets (10–200 μm in diameter) and sprayed into a current of heated air at 150–300°C in a large drying chamber. The feed rate is controlled to produce an outlet air temperature of 90–100°C, which corresponds to a wet-bulb temperature (and product temperature) of 40–50°C. Complete and uniform atomization is necessary for successful drying, and one of the following types of atomizer is used.

1. *Centrifugal Atomizer*. Liquid is fed to the center of a rotating bowl (with a peripheral velocity of 90–200 m/s). Droplets, 50–60 μm in diameter, are flung from the edge of the bowl to form a uniform spray (Fig. 8a).
2. *Pressure Nozzle Atomizer*. Liquid is forced at a high pressure (700–2000 kPa) through a small aperture. Droplet sizes are 180–250 μm. Grooves on the inside of the nozzle cause the spray to form into a cone shape and therefore to use the full volume of the drying chamber.
3. *Two-Fluid Nozzle Atomizer*. Compressed air creates turbulence, which atomizes the liquid (Fig. 8b). The operating pressure is lower than the pressure nozzle, but a wider range of droplet sizes is produced.

Both types of nozzle atomizer are susceptible to blockage by particulate foods, and abrasive foods gradually widen the apertures and increase the average droplet size. Studies of droplet drying, including methods for calculating changes in size, density, and trajectory of the droplets are reported in Refs. 10, 16, and 17.

Rapid drying takes place (1–10 s) because of the very large surface area of the droplets. The temperature of the product remains at the wet-bulb temperature of the drying air, and there is minimum heat damage to the food. Airflow may be co- or counter-current (Table 4). The dry powder is collected at the base of the dryer and removed by a screw conveyor or a pneumatic system with a cyclone separator. There are a large number of designs of atomizer, drying chamber, air heating, and powder collecting systems (10,18). The variations in design arise from the different requirements of the very large variety of food materials that are spray dried—eg, milk, egg, coffee, cocoa, tea, potato, ground chicken, ice cream mix, butter, cream, yogurt and cheese powder, coffee whitener, fruit juices, meat and yeast extracts, encapsulated flavors (19), and wheat and corn starch products. Spray dryers may also be fitted with fluidized bed facilities to finish powders taken from the drying chamber.


(a)

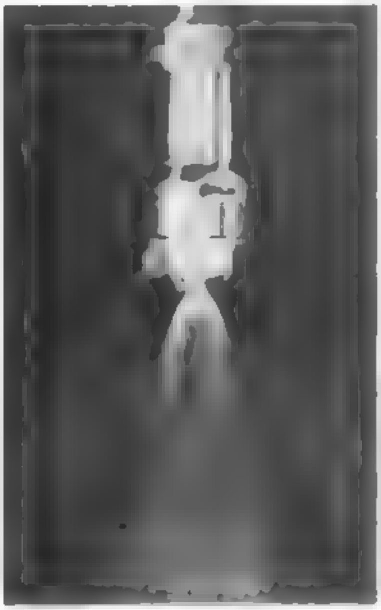

(b)

Figure 8. Atomizers: (a) centrifugal atomizer; (b) two-fluid nozzle atomizer (15). *Source:* Courtesy of Elsevier Applied Science.

Spray dryers vary in size from small pilot-scale models for low-volume high-value products (eg, enzymes and flavors) to large commercial models capable of producing 80,000 kg of dried milk per day (20) (Fig. 9). The main advantages are rapid drying, large-scale continuous production, low labor costs, and simple operation and maintenance. The major limitations are high capital costs and the requirement for a relatively high feed moisture content to ensure that the food can be pumped to the atomizer. This results in higher energy costs (to remove the moisture) and higher volatile losses. Conveyor band dryers and fluidized bed dryers are beginning to replace spray dryers, as they are more compact and energy efficient (21).

The bulk density of powders depends on the size of the dried particles and on whether they are hollow or solid.

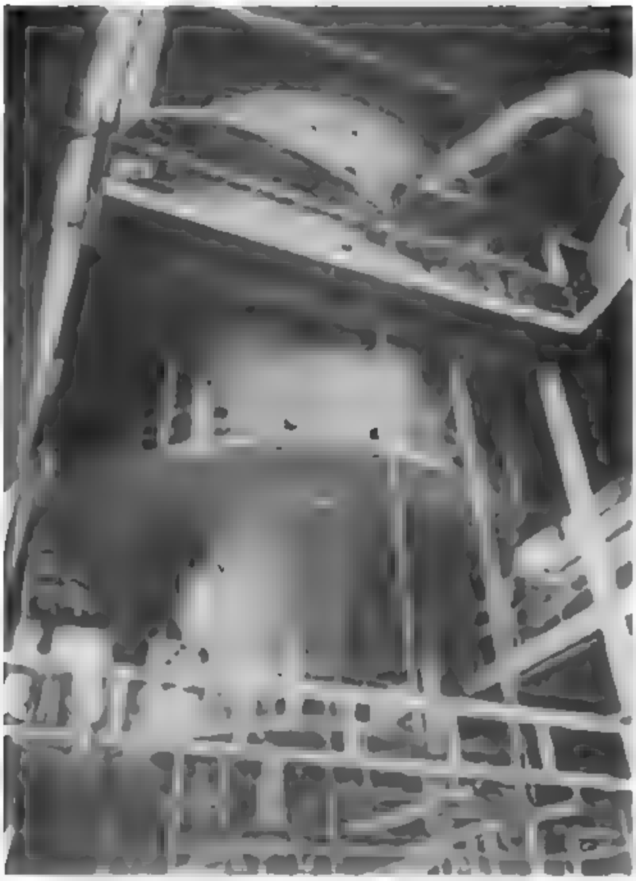


Figure 9. Spray dryer. *Source:* Courtesy of De Melkindustrie Veghel.

This is determined by the nature of the food and the drying conditions (eg, the uniformity of droplet size, temperature, solids content, and degree of aeration of the feed liquid). Instant powders are produced by either agglomeration or non-agglomeration methods. Agglomeration is achieved by remoistening particles in low-pressure steam in an agglomerator, and then redrying. Fluidized-bed, jet, disc, cone, or belt agglomerators are described in Ref. 22. Alternatively, straight-through agglomeration is achieved directly during spray drying. A relatively moist powder is agglomerated and dried in an attached fluidized bed dryer. Nonagglomeration methods employ a binding agent, (eg, lecithin), to bind particles. This method was previously used for foods with a relatively high fat content, (eg, whole milk powder) but agglomeration procedures have now largely replaced this method (23).

Trough Dryers (Belt-Trough Dryers). Small, uniform pieces of food, (eg, peas or diced vegetables) are dried in a mesh conveyor belt that hangs freely between rollers to form the shape of a trough. Hot air is blown through the bed of food, and the movement of the conveyor mixes and turns it to bring new surfaces continually into contact with the drying air. The mixing action moves food away from the drying air, and this allows time for moisture to move from the interior of the pieces to the dry surface. The moisture is then rapidly evaporated when the food again contacts the hot air. The dryer operates in two stages, to 50–60% moisture and then to 15–20% moisture. Foods are finished in bin dryers. These dryers have high drying rates (eg, 55 min for diced vegetables, compared with 5 h in a tunnel dryer), high energy efficiencies, good control, and minimal heat damage to the product. However, they are not suitable for sticky foods.

Tunnel Dryers. Thin layers of food are dried on trays, which are stacked on trucks programmed to move semicontinuously through an insulated tunnel. Different designs use one of the types of air flow described in Table 4. Food is finished in bin dryers. Typically, a 20-m tunnel contains 12–15 trucks with a total capacity of 5000 kg of food. This ability to dry large quantities of food in a relatively short time (5–16 h) made tunnel drying widely used, especially in the United States. However, the method has now been largely superseded by conveyor drying and fluidized-bed drying as a result of their higher energy efficiency, reduced labor costs, and better product quality.

Sun and Solar Drying. Sun drying (without drying equipment) is the most widely practiced agricultural processing operation in the world; more than 250,000,000 tons of fruits and grains are dried by solar energy per annum. In some countries foods are simply laid out on roofs or other flat surfaces and turned regularly until dry. More sophisticated methods (solar drying) collect solar energy and heat air, which in turn is used for drying. Solar dryers are classified into three categories (4):

1. Direct natural-circulation dryers (a combined collector and drying chamber).
2. Direct dryers with a separate collector.
3. Indirect forced-convection dryers (separate collector and drying chamber).

Both solar and sun drying are simple, inexpensive technologies, in terms of both capital input and operating costs. Energy inputs and skilled labor are not required. Sun drying is therefore the preferred option in developing countries that have a suitable dry season after harvesting food crops such as paddy or maize. The major disadvantages of sun drying are poor control over drying conditions; lower drying rates than in artificial dryers; dependence on sunlight, which causes cessation of drying operations at night or during rain; and contamination of the dried product by dust, etc. Each of these factors contributes to a more variable and generally lower quality product than that produced by artificial dryers.

Solar dryers aim to improve product quality by providing greater control over drying conditions, protection from rain or dust, and higher drying rates. However, they have a relatively small capacity for drying the large bulk of crops at harvest time when compared to sun drying. In addition, the higher capital investment may not result in a higher income from improved quality of the dried crop. More valuable crops such as herbs and spices offer better potential for solar drying owing to the smaller quantities involved and the increased income from improved quality. However, the dependence of solar dryers on sunlight and the better control over drying conditions achieved in artificial (fuel-fired) dryers again limit the potential for solar drying.

Heated-Surface Dryers. Dryers in which heat is supplied to the food by conduction have two main advantages over hot-air drying: (*1*) It is not necessary to heat large volumes of air before drying commences, and the thermal efficiency is therefore high; (*2*) Drying may be carried out in the absence of oxygen to protect components of foods that are easily oxidized.

Typically, heat consumption is 2000–3000 kJ/kg of water evaporated compared with 4000–10,000 kJ/kg of water evaporated for hot-air dryers. However, foods have low thermal conductivities that become lower as the food dries. There should therefore be a thin layer of food to conduct heat rapidly, without causing heat damage. Foods may shrink during drying and lift off the hot surface, therefore introducing an additional barrier to heat transfer. Careful control is necessary over the rheological properties of the feed slurry to minimize shrinkage and to determine the thickness of the feed layer.

Drum Dryers (Roller Dryers). Slowly rotating hollow steel drums are heated internally by pressurized steam to 120–170°C. A thin layer of food is spread uniformly over the outer surface by dipping, spraying, spreading, or auxiliary feed rollers. Before the drum has completed 1 rev (within 20 s/3 min), the dried food is scraped off by a doctor blade that contacts the drum surface uniformly along its length. Dryers may have a single drum (Fig. 10a) or double drums (Fig. 10b) or twin drums. The single drum is widely used as it has greater flexibility, a larger proportion of the drum area available for drying, easier access for maintenance, and no risk of damage caused by metal objects falling between the drums.

Drum dryers have high drying rates and high energy efficiencies. They are suitable for slurries in which the particles are too large for spray drying. However, the high capital cost of the machined drums and heat damage to sensitive foods from high drum temperatures have caused a move to spray drying for many bulk dried foods. Drum drying is used to produce potato flakes, precooked cereals, molasses, some dried soups, and fruit purees, and whey or distillers' solubles for animal feed formulations.

Developments in drum design to improve the sensory and nutritional qualities of dried food include the use of auxiliary rolls to remove and reapply food during drying, the use of high-velocity air to increase the drying rate, and the use of chilled air to cool the product. Drums may be enclosed in a vacuum chamber to dry food at lower temperatures, but the high capital cost of this system restricts its use to high-value heat-sensitive foods.

Vacuum Band and Vacuum Shelf Dryers. A food slurry is spread or sprayed onto a steel belt (or band) that passes over two hollow drums within a vacuum chamber at 1–70 mmHg. The food is dried by the first steam-heated drum, and then by steam-heated coils or radiant heaters located over the band. The dried food is cooled by the second water-cooled drum and removed by a doctor blade. Vacuum shelf dryers consist of hollow shelves in a vacuum chamber. Food is placed in thin layers on flat metal trays that are carefully made to ensure good contact with the shelves. A particular vacuum of 1–70 mmHg is drawn in the chamber and steam or hot water is passed through the shelves to dry the food.

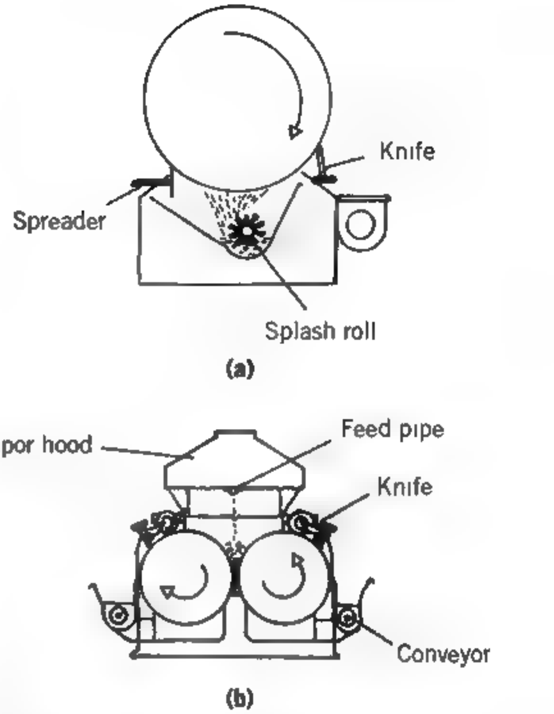


Figure 10. Drum dryers: (**a**) single drum; (**b**) double drum. *Source:* Courtesy of APV Mitchell Ltd

Rapid drying and limited heat damage to the food make both methods suitable for drying heat-sensitive foods. However, care is necessary to prevent the dried food from burning onto trays in vacuum shelf dryers, and shrinkage reduces the contact between the food and heated surfaces of both types of equipment. Both have relatively high capital and operating costs and low production rates.

Vacuum-band shelf dryers are used to produce puff-dried foods. Explosion puff drying involves partially drying food to a moderate moisture content and then sealing it into a pressure chamber. The pressure and temperature in the chamber are increased and then instantly released. The rapid loss of pressure causes the food to expand and develop a fine porous structure. This permits faster final drying and rapid rehydration. Sensory and nutritional qualities are well retained. The technique was first applied commercially to breakfast cereals and now includes a range of fruit and vegetable products.

EFFECTS ON FOODS

The effect of A_w on microbiological and selected biochemical reactions is shown in Fig. 11 and Table 2. Almost all microbial activity is inhibited below $A_w - 0.6$; most fungi

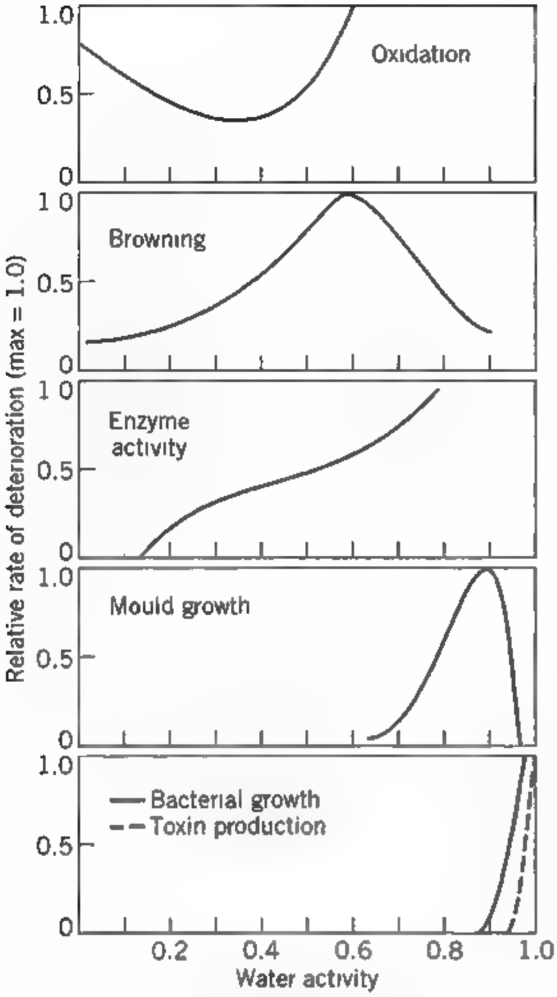


Figure 11. Effect of water activity on microbial, enzymic and chemical changes to foods (7). *Source:* Courtesy of Marcel Dekker.

are inhibited below $A_w = 0.7$; most yeasts are inhibited below $A_w = 0.8$; and most bacteria below $A_w = 0.9$. The interaction of A_w with temperature, pH, oxygen, and carbon dioxide, or chemical preservative has an important effect on the inhibition of microbial growth. When any one of the other environmental conditions is suboptimal for a given microorganism, the effect of reduced A_w is enhanced (Fig. 11). This permits the combination of several mild control mechanisms that result in the preservation of food without substantial loss of nutritional properties or sensory properties (Table 5).

Enzymatic activity virtually ceases at A_w values below the BET monolayer value. This is due to the low substrate mobility and its inability to diffuse to the reactive site on the enzyme. Chemical changes are more complex. The two most important things that occur in foods that have a low A_w are Maillard browning and oxidation of lipids. The A_w that causes the maximum rate of browning varies with different foods. However, in general, a low A_w restricts the mobility of reactants and browning is reduced. At a higher A_w, browning reaches maximum. Water is a product of the condensation reaction in browning, and at higher moisture levels, browning is inhibited by end-product inhibition. At high moisture contents, water dilutes the reactants and the rate of browning falls (Fig. 11).

Oxidation of lipids occurs at low A_w values owing to the action of free radicals. Above the BET monolayer value, antioxidants and chelating agents (which sequester trace metal catalysts) become soluble and reduce the rate of oxidation. At higher A_w values the catalytic activity of metals is reduced by hydration and the formation of insoluble hydroxides but, at high A_w values, metal catalysts become soluble and the structure of the food swells to expose more reactive sites (Fig. 11).

Texture

Changes to the texture of solid foods are an important cause of quality deterioration. The nature and extent of pretreatments (eg, the addition of calcium chloride to blancher water), the type and extent of size reduction, and peeling each affect the texture of rehydrated fruits and vegetables. In foods that are adequately blanched, loss of texture is caused by gelatinization of starch, crystallization of cellulose, and localized variations in the moisture content during dehydration, which set up internal stresses. These rupture, compress, and permanently distort the relatively rigid cells, to give the food a shrunken, shrivelled appearance. On rehydration the product absorbs water more slowly and does not regain the firm texture associated with the fresh material. There are substantial variations in the degree of shrinkage with different foods.

Drying is not commonly applied to meats in many countries owing to the severe changes in texture compared with other methods of preservation. These are caused by aggregation and denaturation of proteins and a loss of water-holding capacity, which leads to toughening of muscle tissue.

The rate and temperature of drying have a substantial effect on the texture of foods. In general, rapid drying and high temperatures cause greater changes than do moderate rates of drying and lower temperatures. As water is removed during dehydration, solutes move from the interior of the food to the surface. The mechanism and rate of movement are specific for each solute and depend on the type of food and the drying conditions used. Evaporation of water causes concentration of solutes at the surface. High air temperatures (particularly with fruits, fish, and meats) cause complex chemical and physical changes to the surface and the formation of a hard, impermeable skin. This is termed case hardening. It reduces the rate of drying and produces a food with a dry surface and a moist interior. It is minimized by controlling the drying conditions to prevent excessively high moisture gradients between the interior and the surface of the food.

In powders, the textural characteristics are related to bulk density and the ease with which they are rehydrated. These properties are determined by the composition of the food, the method of drying, and the particle size of the product. Low-fat foods (eg, fruit juices, potatoes, and coffee) are more easily formed into free-flowing powders than are whole milk or meat extracts. Powders are instantized by

Table 5. Interaction of A_W, pH, and Temperature in Selected Foods

Food	pH	A_W	Shelf life	Notes
Fresh meat	>4 5	>0.95	Days	Preserve by chilling
Cooked meat	>4.5	0.95	Weeks	Ambient storage when packaged
Dry sausage	>4.5	<0.90	Months	Preserved by salt and low A_W
Fresh vegetables	>4.5	>0.95	Weeks	Stable while respiring
Pickles	<4.5	0.90	Months	Low pH maintained by packaging
Bread	>4.5	>0.95	Days	Preserved by heat and low A_W in crust
Fruitcake	>4.5	<0.90	Weeks	Preserved by heat and low A_W
Milk	>4.5	>0.95	Days	Preserved by chilling
Yogurt	<4.5	<0 95	Weeks	Preserved by low pH and chilling
Dried milk	>4.5	<0.90	Months	Preserved by low A_W

treating individual particles so that they form free-flowing agglomerates of aggregates, in which there are relatively few points on contact (Fig. 12). The surface of each particle is easily wetted when the powder is rehydrated, and particles sink below the surface to disperse rapidly through the liquid. These characteristics are respectively termed wettability, sinkability, dispersibility, and solubility. For a powder to be considered instant, it should complete these four stages within a few seconds.

The convenience of instantized powders outweighs the additional expense of production, packaging, and transport for retail products. However, many powdered foods are used as ingredients in other processes, and these are required to possess a high bulk of density and a wider range of particle sizes. Small particles fill the spaces between larger ones and thus exclude air to promote a longer storage life. The characteristics of some powdered foods are described in Table 6.

Table 6. Bulk Density and Moisture Content of Selected Powdered Foods

Food	Bulk density (kg m^3)	Moisture content (%)
Cocoa	480	3–5
Coffee (ground)	330	7
Coffee (instant)	330	2.5
Coffee creamer	470	3
Cornstarch	560	12
Egg, whole	340	2–4
Milk, powdered, skimmed	640	2–4
Milk, instant, skimmed	550	2–4
Salt, granulated	960	0.2
Sugar, granulated	800	0.5
Wheat flour	450	12

Source: Refs. 24 and 25.

Flavor and Aroma

Heat not only vaporizes water during drying but also causes loss of volatile components from the food. The extent of volatile loss depends on the temperature and solids concentration of the food and on the vapor pressure of the volatiles and their solubility in water vapor. Volatiles that have a high relative volatility and diffusivity are lost at an early stage in drying. Fewer volatile components are lost at later stages. Control of drying conditions during each stage of drying minimizes losses. Foods that have a high economic value due to their characteristic flavors (eg, herbs and spices) are dried at lower temperatures.

A second important cause of aroma loss is oxidation of pigments, vitamins, and lipids during storage. The open, porous structure of dried food allows access of oxygen. The rate of deterioration is determined by the storage temperature and the water activity of the food.

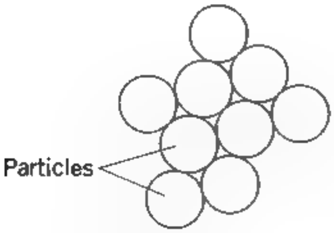


Figure 12. Agglomerated powder.

In dried milk the oxidation of lipids produces rancid flavors owing to the formation of secondary products, including δ-lactones. Most fruits and vegetables contain only small quantities of lipid, but oxidation of unsaturated fatty acids to produce hydroperoxides, which react further by polymerization, dehydration, or oxidation to produce aldehydes, ketones, and acids, causes rancid and objectionable odors. Some foods, (eg, carrots), may develop an odor of violets produced by the oxidation of carotenes to β-ionone. These changes are reduced by vacuum- or gas-packing, low storage temperatures, exclusion of ultraviolet or visible light, maintenance of low moisture contents, addition of synthetic antioxidants, or preservation of natural antioxidants.

The technical enzyme glucose oxidase is also used to protect dried foods from oxidation. A package that is permeable to oxygen but not to moisture and that contains glucose and the enzyme is placed on the dried food inside a container. Oxygen is removed from the headspace during storage. Milk powders are stored under an atmosphere of nitrogen with 10% carbon dioxide. The carbon dioxide is absorbed into the milk and creates a small partial vacuum in the headspace. Air diffuses out of the dried particles and is removed by regassing after 24 h. Flavor changes, due to oxidative or hydrolytic enzymes, are prevented in fruits by the use of sulfur dioxide, ascorbic acid, or citric acid, by pasteurization of milk or fruit juices, and by blanching of vegetables.

Other methods used to retain flavors in dried foods include

1. Recovery of volatiles and their return to the product during drying.
2. Mixing recovered volatiles with flavor-fixing compounds, which are then granulated and added back to the dried product, eg, dried meat powders.
3. Addition of enzymes, or activation of naturally occurring enzymes, to produce flavors from flavor precursors in the food (eg, onion and garlic are dried under conditions that protect the enzymes that release characteristic flavors). Maltose or maltodextrin are used as a carrier material when drying flavor compounds.

Color

Drying changes the surface characteristics of food and hence alters the reflectivity and color. Chemical changes to carotenoid and chlorophyll pigments are caused by heat and oxidation during drying. In general, longer drying times and higher drying temperatures produce greater pigment losses. Oxidation and residual enzyme activity cause browning during storage. This is prevented by improved blanching methods and treatment of fruits with ascorbic acid or sulfur dioxide. For moderately sulfured fruits and vegetables the rate of darkening during storage is inversely proportional to the residual sulfur dioxide content. However, sulfur dioxide bleaches anthocyanins, and residual sulfur dioxide is an important cause of color deterioration in stored dried fruits and vegetables.

The rate of Maillard browning in stored milk and fruit products depends on the water activity of the food and the temperature of storage. The rate of darkening increases markedly at high drying temperatures, when the moisture content of the product exceeds 4–5%, and at storage temperatures above 38°C (26).

Nutritive Value

Large differences in reported data on the nutritive value of dried foods are due to wide variations in the preparation procedures, the drying temperature and time, and the storage conditions. In fruits and vegetables, losses during preparation usually exceed those caused by the drying operation. For example, losses of vitamin C during preparation of apple flakes are reported to be 8% during slicing, 62% from blanching, 10% from pureeing, and 5% from drum drying (27).

Vitamins have different solubilities in water, and, as drying proceeds, some (eg, riboflavin) become supersaturated and precipitate from solution. Losses are therefore small (Table 7). Others, (eg, ascorbic acid) are soluble until the moisture content of the food falls to very low levels and react with solutes at higher rates as drying proceeds. Vitamin C is also sensitive to heat and oxidation. Short drying times, low temperatures, and low moisture and oxygen levels during storage are necessary to avoid large losses. Thiamin is also heat sensitive, but other water-soluble vitamins are more stable to heat and oxidation, and losses during drying rarely exceed 5–10% (excluding blanching losses).

Oil-soluble nutrients (eg, essential fatty acids and vitamins A, D, E, and K) are mostly contained within the dry matter of the food, and they are not therefore concentrated during drying. However, water is a solvent for heavy-metal catalysts that promote oxidation of unsaturated nutrients. As water is removed, the catalysts become more reactive, and the rate of oxidation accelerates (Fig. 12). Fat-soluble vitamins are lost by interaction with the peroxides produced by fat oxidation. Losses during storage are reduced by low oxygen concentrations and storage temperatures and by exclusion of light.

The biological value and digestibility of proteins in most foods does not change substantially. However, milk proteins are partially denatured during drum drying, and this results in a reduction in solubility of the milk powder, aggregation, and loss of clotting ability. A reduction in biological value of 8–30% is reported, depending on the temperature and residence time (30). Spray drying does not affect the biological value of milk proteins. At high storage temperatures and at moisture contents above approximately 5%, the biological value of milk protein is decreased by Maillard reactions between lysine and lactose. Lysine is heat sensitive, and losses in whole milk range from 3–10% in spray drying and 5–40% in drum drying (31).

The importance of nutrient losses during processing depends on the nutritional value of a particular food in the diet. Some foods, eg, bread and milk, are an important source of nutrients for large numbers of people. Vitamin losses are therefore more significant in these foods than in those that either are eaten in small quantities or have low concentrations of nutrients.

In industrialized countries, the majority of the population achieve an adequate supply of nutrients from the mixture of foods that is eaten. Losses due to processing of one component of the diet are therefore insignificant to the long-term health of an individual. In one example, complete meals that initially contained 16.5 μg of vitamin A lost 50% on canning and 100% after storage for 18 months. Although the losses appear to be significant, the original meal contained only 2% of the recommended daily allowance (RDA), and the extent of loss is therefore of minor importance. The same meal contained 9 mg of thiamin and lost 75% after 18 months' storage. The thiamin content is 10 times the RDA, so adequate quantities therefore remained. Possible exceptions are the special dietary needs of preterm infants, pregnant women, and the elderly. In these groups there may be either a special need for certain nutrients or a more restricted diet than normal. These special cases are discussed in detail in Refs. 32, 33 and 34.

Reported vitamin losses during processing give an indication of the severity of each unit operation. However, such data should be treated with caution. Variation in nutrient losses between cultivars or varieties can exceed differences caused by alternative methods of processing. Growth conditions, or handling and preparation procedures before processing, also cause substantial variation in nutrient loss. Data on nutritional changes cannot be di-

Table 7. Vitamin Losses in Selected Dried Foods

	Loss (%)						
Food	Vitamin A	Thiamin	Vitamin B_2	Niacin	Vitamin C	Folic acid	Biotin
Fruits[a]	6	55	0	10	56		
Fig (sun-dried)	—	48	42	37	—	—	—
Whole milk (spray-dried)	—	—	—	—	15	10	10
Whole milk (drum-dried)	—	—	—	—	30	10	10
Pork		50–70					
Vegetables[b]	5	<10	<10				

Source Refs. 28 and 29.

[a] Fruits. mean loss from fresh apple, apricot, peach, and prune.

[b] Vegetables: mean loss from peas, corn, cabbage, and beans (drying stage only).

rectly applied to individual commercial operations, because of differences in ingredients, processing conditions, and equipment used by different manufacturers.

REHYDRATION

Rehydration is not the reverse of drying. Texture changes, solute migration, and volatile losses are each irreversible. Heat reduces the degree of hydration of starch and the elasticity of cell walls and coagulates proteins to reduce their water-holding capacity. The rate and extent of rehydration may be used as an indicator of food quality; those foods that are dried under optimum conditions suffer less damage and rehydrate more rapidly and completely than poorly dried foods.

NOMENCLATURE

A	Area
A_w	Water activity
C	Constant, drag coefficient
d	Diameter
G	Mass flow rate of air
g	Acceleration due to gravity (9.81 m/s^2)
H	Humidity
h	Surface heat transfer coefficient
K	Mass transfer coefficient
L	Length
M	Moisture content (dry-weight basis)
m	Mass, mass flow rate, moisture content (wet-weight basis)
P	Pressure, vapor pressure
Q	Rate of heat transfer
r	Radius
Re	Reynolds Number
t	Time
U	Overall heat transfer coefficient
v_f	Velocity, air velocity needed for fluidization
x	Thickness, depth
ϵ	Voidage of fluidized bed
θ	Temperature
λ	Latent heat
μ	Viscosity
τ	Constant = 3.142
ρ	Density

ACKNOWLEDGMENTS

Grateful acknowledgment is made for information supplied by the following: APV Mitchell Dryers Ltd, Carlisle, Cumbria, CA5 5DU, UK; Petrie and McNaught Ltd, Rochdale, UK; Zschokke Wartmann Ltd, Stahlrain, CH-5200 Brugg, Switzerland, Proctor and Schwartz Inc., Glasgow, Scotland; Torftech Ltd, Mortimer, Reading, Berkshire, UK; Unilever, London EC4P 4BQ, UK; Chartered Institute of Building Services Engineers, 222 Balham High Road London, SW12 985, UK; De Melkindustrie Veghel.

BIBLIOGRAPHY

Reproduced with permission from P. Fellows, *Food Processing Technology*, Ellis Horwood Limited, Chichester, UK, 1988.

1. M. J. Lewis, *Physical Properties of Foods and Food Processing Systems*, Ellis Horwood, Chichester, West Sussex, UK, 1987.
2. C. Van den Berg, "Water Activity," in D. MacCarthy, ed., *Concentration and Drying of Foods*, Elsevier Applied Science, Barking, Essex, UK, 1986, pp. 11–36.
3. J. A. Troller and J. H. B. Christian, *Water Activity and Food*, Academic Press, London, 1978.
4. B. Brenndorfer, L. Kennedy, C. O. Oswin-Bateman, and D. S. Trim. *Solar Dryers*, Commonwealth Science Council, Commonwealth Secretariat, Pall Mall, London, 1985.
5. D. Kunii and O. Leverspiel, *Fluidisation Engineering*, John Wiley & Sons, New York, 1969.
6. M. Loncin and R. L. Merson, *Food Engineering—Principles and Selected Applications*, Academic Press, New York, 1979.
7. M. Karel, "Dehydration of Foods," in O. R. Fennema, ed., *Principles of Food Science. Part 2. Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975, pp. 309–357.
8. C. W. Hall, *Dictionary of Drying*, Marcell Dekker, New York, 1979.
9. P. E. Zagorzycki, "Automatic Humidity Control of Dryers," *Chemical Engineering Progress*, 66–70 (April 1983).
10. K. Masters, *Spray Drying*. Leonard Hill, London, 1972, pp. 15, 144, 160, 230, 545–586.
11. K. Grikitis, "Dryer Spearheads Dairy Initiative," *Food Process* (1986).

12. S. F. Sapakie and T. A. Renshaw, "Economics of Drying and Concentration of Foods," in B. M. McKenna, ed., *Engineering and Food*, Vol. 2, Elsevier Applied Science, London, 1984, pp. 927–938.
13. C. Tragardh, "Energy and Energy Analysis in Some Food Processing Industries," *Lebensmittel Wissenschaft Technologie* **14**, 213–217 (1981).
14. A. Axtell, A. B. Bush, and M. Molina, *Try Drying It. A Case Study of Small Industry Dryers*, IT Publications, London, 1991.
15. K. Masters, "Recent Developments in Spray Drying," in S. Thorne, ed., *Developments in Food Preservation*, Vol. 2, Applied Science, London, 1983, pp. 95–121.
16. S. E. Charm, *Fundamentals of Food Engineering*, 3rd ed., AVI Publishing Co., Westport, Conn., 1978, pp. 298–408.
17. P. J. A. M. Kerkhof and W. J. A. H. Schoeber, "Theoretical Modelling of the Drying Behaviour of Droplets in Spray Dryers," in A. Spicer, ed., *Advances in Preconcentration and Dehydration of Foods*, Applied Science, London, 1974, pp. 349–397.
18. O. G. Kjaergaard, "Effects of Latest Developments on Design and Practice of Spray Drying. In Ref. 17, pp. 321–348.
19. H. B. Heath, "The Flavour Trap," *Food Flavour Ingredients* **7**, 21, 23, 25 (1985).
20. M. Byrne, "The £8M Dryer," *Food Manufacture*, 67, 69 (September 1986).
21. J. C. Ashworth, "Developments in Dehydration," *Food Manufacture*, 25–27, 29 (December 1981).
22. H. Schubert, (1980) "Processing and Properties of Instant Powdered Foods," in P. Linko, Y. Malkki, J. Olkku, and J. Larinkari, eds., *Food Process Engineering*, Vol. 1, *Food Processing Systems*, Applied Science, London, 1980, pp. 675–684.
23. U.S. Pat. 4,490,403 (1983) J. Pisecky, J. Krag, and Ib. H. Sor[illegible]
24. B. K. Watt and A. L. Merrill, *Composition of Foods*, Agriculture Handbook 8, U.S. Department of Agriculture, Washington D.C., 1975.
25. M. Peleg, "Physical Characteristics of Food Powders," in M. Peleg and E. B. Bagley, eds., *Physical Properties of Foods*, AVI Publishing Co., Westport, Conn., 1983, pp. 293–323.
26. C. H. Lea, "Chemical Changes in the Preparation and Storage of Dehydrated Foods," in *Proceedings of Fundamental Aspects of Thermal Dehydration of Foodstuffs*, Aberdeen, March 25–27, 1958, Society of Chemical Industry, London pp. 178–194.
27. F Escher and H. Neukom, "Studies on Drum-drying Apple Flakes," *Trav. Chim. Aliment. Hyg.* **61**, 339–348 (1970) (in German).
28. B A. Rolls, "Effects of Processing on Nutritive Value of Food: Milk and Milk Products, in M Rechcigl, ed., *Handbook of the Nutritive Value of Processed Foods*, Vol. 1, CRC Press, Boca Raton, Fla., 1982, pp 383–389.
29. D. H. Calloway, "Dehydrated Foods," *Nutrition Reviews* **20**, 257–260 (1962).
30. W. Fairbanks and H. H. Mitchell, "The Nutritive Value of Skim-milk Powders with Special Reference to the Sensitivity of Milk Proteins to Heat," *Journal of Agricultural Research* **51**, 1107–1121 (1935).
31. B. A. Rolls and J. W G. Porter, "Some Effects of Processing and Storage on the Nutritive Value of Milk and Milk Products," *Proceedings of the Nutrition Society* **32**, 9–15 (1973).
32. A. E. Bender, "The Nutritional Aspects of Food Processing," in A. Turner, ed., *Food Technology International Europe*, Sterling, London, 1987, pp. 273–275.
33. R. R. Watson, *Nutrition for the Aged*. CRC Press, Boca Raton, Fla., 1986.
34. D. Francis, *Nutrition for Children*, Blackwell, Oxford, UK, 1986.

GENERAL REFERENCES

References 7, 10, and 12 are good general references.

C. W. Hall, *Dictionary of Drying*, Marcel Dekker, New York, 1979

O. G. Spicer Kjaergaard, "Effects of Latest Developments on Design and Practice of Spray Drying," in A. Spicer, ed., *Advances in Preconcentration and Dehydration of Foods*. Applied Science, London, 1974, pp. 321–348.

P. Fellows
Intermediate Technology Development Group
Rugby, United Kingdom

DETERGENTS

A detergent is any material that cleanses or provides surface active properties. Detergents assist in the removal of unwanted soils from surfaces. The use of detergents in food-processing facilities is an important component of their sanitation program. Food-processing plants perform regular sanitation within their operations for two primary reasons: to provide a clean and aesthetically appealing environment and to eliminate microbial organisms that cause food spoilage and health problems for the consumer. Detergents selected for use in food-processing areas should be products that are free rinsing, leaving no residual toxins that might contaminate the food. Detergents used in food-processing areas should also leave no residual odor, after rinsing, that might serve to conceal malodors caused by microbial activity in those areas.

The Food and Drug Administration (FDA) publishes a listing of materials that are acceptable for use in the food environment (1). These include ingredients for food and substances present on food contact surfaces, along with parameters for their use. The U.S. Department of Agriculture (USDA) follows the FDA guidelines when approving detergents and other products acceptable for use in meat- and poultry-processing plants.

TYPES OF DETERGENT

Detergents may be either solvent-based or water-soluble products. Water-soluble detergents may be acidic, neutral, or alkaline. Solvent-based detergents are not widely used in food-processing areas. Many solvents have toxic hazards associated with them, possess strong persistent odors and are not readily biodegradable. The few solvent detergents that are acceptable in food-processing plants tend to be either readily biodegradable or are volatile. Most volatile solvents are also flammable. Because of all the liabilities that are associated with solvent-based detergents, their use is limited to specialty applications where other detergents do not provide adequate cleaning. Acid detergents are generally used to remove mineral deposits such as

12. S. F. Sapakie and T. A. Renshaw, "Economics of Drying and Concentration of Foods," in B. M. McKenna, ed., *Engineering and Food*, Vol. 2, Elsevier Applied Science, London, 1984, pp. 927–938.
13. C. Tragardh, "Energy and Energy Analysis in Some Food Processing Industries," *Lebensmittel Wissenschaft Technologie* **14**, 213–217 (1981).
14. A. Axtell, A. B. Bush, and M. Molina, *Try Drying It. A Case Study of Small Industry Dryers*, IT Publications, London, 1991.
15. K. Masters, "Recent Developments in Spray Drying," in S. Thorne, ed., *Developments in Food Preservation*, Vol. 2, Applied Science, London, 1983, pp. 95–121.
16. S. E. Charm, *Fundamentals of Food Engineering*, 3rd ed., AVI Publishing Co., Westport, Conn., 1978, pp. 298–408.
17. P. J. A. M. Kerkhof and W. J. A. H. Schoeber, "Theoretical Modelling of the Drying Behaviour of Droplets in Spray Dryers," in A. Spicer, ed., *Advances in Preconcentration and Dehydration of Foods*, Applied Science, London, 1974, pp. 349–397.
18. O. G. Kjaergaard, "Effects of Latest Developments on Design and Practice of Spray Drying. In Ref. 17, pp. 321–348.
19. H. B. Heath, "The Flavour Trap," *Food Flavour Ingredients* **7**, 21, 23, 25 (1985).
20. M. Byrne, "The £8M Dryer," *Food Manufacture*, 67, 69 (September 1986).
21. J. C. Ashworth, "Developments in Dehydration," *Food Manufacture*, 25–27, 29 (December 1981).
22. H. Schubert, (1980) "Processing and Properties of Instant Powdered Foods," in P. Linko, Y. Malkki, J. Olkku, and J. Larinkari, eds., *Food Process Engineering*, Vol. 1, *Food Processing Systems*, Applied Science, London, 1980, pp. 675–684.
23. U.S. Pat. 4,490,403 (1983) J. Pisecky, J. Krag, and Ib. H. Sor-[illegible]
24. B. K. Watt and A. L. Merrill, *Composition of Foods*, Agriculture Handbook 8, U.S. Department of Agriculture, Washington D.C., 1975.
25. M. Peleg, "Physical Characteristics of Food Powders," in M. Peleg and E. B. Bagley, eds., *Physical Properties of Foods*, AVI Publishing Co., Westport, Conn., 1983, pp. 293–323.
26. C. H. Lea, "Chemical Changes in the Preparation and Storage of Dehydrated Foods," in *Proceedings of Fundamental Aspects of Thermal Dehydration of Foodstuffs*, Aberdeen, March 25–27, 1958, Society of Chemical Industry, London pp. 178–194.
27. F Escher and H. Neukom, "Studies on Drum-drying Apple Flakes," *Trav. Chim. Aliment. Hyg.* **61**, 339–348 (1970) (in German).
28. B A. Rolls, "Effects of Processing on Nutritive Value of Food: Milk and Milk Products, in M Rechcigl, ed., *Handbook of the Nutritive Value of Processed Foods*, Vol. 1, CRC Press, Boca Raton, Fla., 1982, pp 383–389.
29. D. H. Calloway, "Dehydrated Foods," *Nutrition Reviews* **20**, 257–260 (1962).
30. W. Fairbanks and H. H. Mitchell, "The Nutritive Value of Skim-milk Powders with Special Reference to the Sensitivity of Milk Proteins to Heat," *Journal of Agricultural Research* **51**, 1107–1121 (1935).
31. B. A. Rolls and J. W G. Porter, "Some Effects of Processing and Storage on the Nutritive Value of Milk and Milk Products," *Proceedings of the Nutrition Society* **32**, 9–15 (1973).
32. A. E. Bender, "The Nutritional Aspects of Food Processing," in A. Turner, ed., *Food Technology International Europe*, Sterling, London, 1987, pp. 273–275.
33. R. R. Watson, *Nutrition for the Aged*. CRC Press, Boca Raton, Fla., 1986.
34. D. Francis, *Nutrition for Children*, Blackwell, Oxford, UK, 1986.

GENERAL REFERENCES

References 7, 10, and 12 are good general references.

C. W. Hall, *Dictionary of Drying*, Marcel Dekker, New York, 1979

O. G. Spicer Kjaergaard, "Effects of Latest Developments on Design and Practice of Spray Drying," in A. Spicer, ed., *Advances in Preconcentration and Dehydration of Foods*. Applied Science, London, 1974, pp. 321–348.

P. Fellows
Intermediate Technology Development Group
Rugby, United Kingdom

DETERGENTS

A detergent is any material that cleanses or provides surface active properties. Detergents assist in the removal of unwanted soils from surfaces. The use of detergents in food-processing facilities is an important component of their sanitation program. Food-processing plants perform regular sanitation within their operations for two primary reasons: to provide a clean and aesthetically appealing environment and to eliminate microbial organisms that cause food spoilage and health problems for the consumer. Detergents selected for use in food-processing areas should be products that are free rinsing, leaving no residual toxins that might contaminate the food. Detergents used in food-processing areas should also leave no residual odor, after rinsing, that might serve to conceal malodors caused by microbial activity in those areas.

The Food and Drug Administration (FDA) publishes a listing of materials that are acceptable for use in the food environment (1). These include ingredients for food and substances present on food contact surfaces, along with parameters for their use. The U.S. Department of Agriculture (USDA) follows the FDA guidelines when approving detergents and other products acceptable for use in meat- and poultry-processing plants.

TYPES OF DETERGENT

Detergents may be either solvent-based or water-soluble products. Water-soluble detergents may be acidic, neutral, or alkaline. Solvent-based detergents are not widely used in food-processing areas. Many solvents have toxic hazards associated with them, possess strong persistent odors and are not readily biodegradable. The few solvent detergents that are acceptable in food-processing plants tend to be either readily biodegradable or are volatile. Most volatile solvents are also flammable. Because of all the liabilities that are associated with solvent-based detergents, their use is limited to specialty applications where other detergents do not provide adequate cleaning. Acid detergents are generally used to remove mineral deposits such as hard-water scale. These products are also effective in removing coagulated protein films. Specialized applications use acid detergents for microbial control. A product formulation that combines an acid with an anionic surfactant makes an effective sanitizer; as do iodophors, which are acid detergents that release iodine in solution. Neutral detergents (pH between 5.0 and 9.0) are generally synergistic surfactant blends. Some neutral products also contain solvents to complement the cleaning action of the surfactants. Because of their pH neutrality these detergents are widely used for hard scrubbing. Neutral detergents tend to be most effective on oils, greases, and fats because of their excellent ability to emulsify and disperse these types of soil. A specialized application in this category is the use of quaternary ammonium chloride formulations as sanitizers. Alkaline detergents are effective on organic soils including fats, proteins, and carbohydrates. These detergents are used extensively to clean food-processing facilities. They work by solubilizing, saponifying, peptizing, or hydrolyzing the soils to be removed.

DETERGENT PROPERTIES

Detergents assist the cleaning process by performing one or more of the following: wetting or penetration, dispersing or deflocculating, suspension, emulsification, peptizing, dissolving, chelating or sequestering, rinsing, and sanitizing (2,3). Wetting refers to the detergent's ability to penetrate through and come in contact with all soils and equipment surfaces, thus loosening the soil and facilitating its removal. A dispersant promotes the breakdown of large soil particles into smaller particles and inhibits the formation of larger soil particles by holding them uniformly distributed in the detergent bath. When soil particles are uniformly distributed throughout the detergent bath, without any precipitation to the bottom or flotation to the surface, they are in suspension. An emulsifier breaks down fat and oil into tiny particles and disburses them uniformly throughout the water phase where they are held in suspension. (This is an oil-in-water emulsion.) Peptizing is the partial solubilization and colloidal dispersion of protein by an alkaline detergent. When a soil is converted into a form that is completely soluble in water it will dissolve. Chelation is the process of chemically removing minerals such as calcium, magnesium, or iron from water. This is done by forming a soluble complex with the mineral thus preventing their precipitation from the detergent bath. This enhances the rinsability of the detergent solution and prevents interference of those minerals with the detergent's cleaning ability. Rinsing is the ability to flush the detergent solution and soil from the surfaces being cleaned without leaving any residue. Sanitizing reduces the total microbial population present on the cleaned surfaces. Sanitizers work most efficiently on cleaned surfaces free of any soil. The ideal detergent would possess the following characteristics: rapid and complete solubility in water and complete removal of all soil from the surfaces being cleaned. It would rinse free of all residue and provide germicidal activity. Finally it would be inexpensive to use and safe to handle. To date no universal product possessing all these characteristics exists; hence the plethora of products available in the marketplace.

DETERGENT COMPONENTS

Most detergents are blends of surfactants and builders, although they may be strictly surfactant blends or blends of builders. Surfactants are classed as anionic, nonionic, or cationic. Anionic surfactants are negatively charged molecules and are the most widely used in cleaning. Nonionic surfactants have no charge and are excellent oil and grease solubilizers and emulsifiers. Cationic surfactants are positively charged molecules and are not used to any significant degree as cleaners. Their use is primarily in specialty applications such as fabric softeners and sanitizers.

In aqueous solution, most solid soils are negatively charged minerals (4). If cationic surfactants are present, an electrostatic attraction occurs that is not favorable to soil removal. The negative charge of the anionic surfactants, on the other hand, creates an electrostatic repulsion that facilitates removal of these soils and prevents their redeposition. Uncharged oily soils are most readily removed by solubilization in micelles or by emulsification with nonionic surfactants (4). The solubilization process is most effective when the nonionic surfactant concentration is above the critical micelle concentration (CMC). Also the effectiveness can be further optimized by maintaining a temperature just below the cloud point of the surfactant. Builders are all the other ingredients that are formulated into a detergent to enhance the performance of the surfactants. Detergents that contain no surfactants rely on saponification to produce surfactants *in situ*. Some builders found in detergents are alkalies, acids, phosphates, silicates, chelants, chlorine, defoamers, antiredeposition agents, abrasives, and corrosion inhibitors (2,3,5). By a judicious selection of builders, detergents can be formulated to enhance one or more of the following properties: emulsification, saponification, hydrolysis, removal of mineral deposits, wetting and penetration, dispersing, antiredeposition, peptizing, water softening and antiscaling, defoaming, and sanitizing.

CLEANING

Regular cleaning is essential to maintaining sanitary conditions in food-processing facilities. A good cleaning program should schedule regular cleaning of all interior building surfaces (floors, walls, and ceilings), all processing equipment, removal of garbage, trash, and other wastes, leaving clean rinsed surfaces that are free of any chemical residues that might contaminate the food (5). The basic steps of the cleaning process are the physical removal of the gross soil load, detergent cleaning, rinsing, and sanitizing. The physical removal of gross soil is accomplished by such methods as squeegeeing, shoveling, scraping, rinsing, or any other mechanical means appropriate to the soil and the surface being cleaned. By reducing the soil load the detergents can work more efficiently. The detergent cleaning process loosens the remaining soil. Rinsing flushes the soil from the surface and prevents its redeposition. Sanitizing inhibits microbiological growth on the cleaned surfaces. Three classes of soil have been characterized: soils bound to the surface by oils or greases, soils absorbed onto the surface, and scale deposits. These soils are separated from surfaces by three possible mechanisms: mechanical action, chemical alteration of the nature of the soil, or reduction of the energy binding the soil to the surface by reduction of the surface tension through wetting and penetration (3). The procedure used for cleaning and the type of detergent selected depend on a number of factors: the type and condition of the soil, the type of surface being cleaned, the condition and temperature of the water supply, the cleaning equipment available, the time alloted for cleanup, and the cost of the sanitation program. The ideal water used in sanitation would be free of microorganisms, clear, colorless, noncorrosive, and free of minerals. Other factors to effective cleaning are time, temperature, concentration of detergent, and mechanical action. In general, cleaning is enhanced with longer time, higher temperature, higher concentrations of detergent, and increased mechanical action. However, this generalization needs to be tempered by practical considerations and limitations of the clean-up project. For example, protein starts to coagulate above 55°C (3). In general the following criteria are used to select an appropriate detergent for cleaning:

Heavy soils, cooked on soils	High alkaline cleaner
Fats and proteins	Chlorinated cleaner
Hand scrubbing, soft metals	Light duty or neutral cleaner
Remove mineral deposits, brighten equipment	Acid cleaner

There are four primary procedures used to apply the detergent cleaners with an infinite variety of variations on these methods. Cleaning in place (CIP) is used primarily on enclosed equipment and uses pumps to circulate the detergent solution through the equipment. This process normally entails the use of an alkaline cleaner followed by an acid cleaner. These cleaners typically are low-foaming detergents, because foam hinders the cleaning process when the solutions are pumped through pipes and spray balls to clean large tanks and other parts of the system. Foaming and high-pressure spraying techniques are the most common methods for cleaning the exposed surfaces of equipment, conveyors, walls, and floors. Foaming is a modification of the high-pressure application where compressed air is injected into the detergent stream to produce foam. Foam is popular for use on vertical surfaces because it holds the detergent onto those surfaces for several minutes before draining off. Soak tank cleaning is used on trays, molds and other portable equipment that can be readily removed to one location for cleaning by soaking in a detergent bath. Boil out is a special soak tank procedure where the equipment to be cleaned is filled with a detergent solution and heat is applied until cleaning is effected. Hand scrubbing is used where none of the other procedures are appropriate, or to supplement inadequate cleaning by those methods. This technique combines the chemical action of the detergent with the mechanical action of wiping or scouring the soil from the surface.

DISPOSAL

Disposal of detergents, as with all chemicals, comes under the purview of federal, state, and local laws and regulations. Food processors should maintain close contact with their waste-disposal facilities to keep abreast of current requirements for disposal in their respective locales. Generally, spent detergents can be flushed down the drain. However, care does need to be taken to ensure that this waste solution does comply with the requirements of the local wastewater treatment plant. Usually their primary concerns are the pH and the phosphate levels in the discharged waste. Avoid using detergents containing phosphates where stringent phosphate disposal levels exist. The pH can be readily neutralized, if necessary, before sending the solution out to the wastewater treatment plant. In the event of a detergent spill, several options exist. One would be to neutralize and dilute the detergent with water and dispose as wastewater. Another would be to contain the spill, absorb onto an appropriate substrate, if a liquid, and dispose in a landfill in accordance with all applicable laws and regulations. Certain solvent cleaners present special disposal problems unique to them, and special care must be taken to comply with the disposal regulations for those products.

FOOD SAFETY

In general, food products must be segregated from the clean-up area to avoid contamination. There are a few specialized cases where detergents are used to process edible food products. These special applications are stringently regulated to ensure the safety and wholesomeness of the resulting food product. However, where this is not the case, the food either must be removed from the clean-up area or otherwise covered or segregated to protect it from any possible contamination during the clean-up process. Also, the containers of detergents should be stored in an area segregated from the food-processing and storage areas, again to avoid the possibility of contamination.

WORKER SAFETY

Many detergents used to clean food processing plants are strongly alkaline, strongly acid, release chlorine, or present other safety hazards to the clean-up personnel. These personnel should familiarize themselves with the products they are using and the safety hazards associated with them by studying the material safety data sheet (MSDS), the product label, and any other information the detergent supplier has made available. Pay special attention to the first aid measures that have been recommended. Acids and alkalies can cause both chemical and thermal burns. Chlorine causes respiratory distress. Many chemicals, such as quaternary ammonium chloride compounds, can be sensitizing agents or allergens. Even mild detergents, because of their ability to solubilize oils and fats, can cause dryness, chapping, and cracking of the skin. Appropriate, safety measures need to be taken by clean-up personnel to avoid injury. The purpose of safety equipment is to ensure a safe air supply for breathing and to protect the skin from direct contact with any hazardous materials being used. Generally the first aid step to take, after eye or skin exposure, is to rinse with copious quantities of water; for breathing difficulties, remove to fresh air. See a doctor if distress persists. Furthermore, in the area of eye safety, contact lenses should never be worn when working with potentially hazardous chemical products. In the event of any chemical splattering in the eye, the contact lens makes it very difficult to adequately flush the eye. When mixing chemical products such as detergents, they should always be added to water. Furthermore, use cold water where the chemical addition produces heat of reaction as, for example, adding a strong alkaline detergent to water, which can generate sufficient heat to boil water. A healthy, clean, and safe working environment should be the goal of every food-processing plant.

BIBLIOGRAPHY

1. *Code of Federal Regulations*, Title 21 §172, U.S. Government Printing Office, Washington, D.C., 1986.
2. M. E. Parker and J. H. Litchfield, *Food Plant Sanitation*, Reinhold Publishing Corp., New York, 1962.
3. N. G. Marriot, *Principles of Food Sanitation*, 2nd ed., Van Nostrand Reinhold, Co., Inc., New York, 1989.
4. D. Myers, *Surfactant Science and Technology*, VCH Publishers, Inc., New York, 1988, pp. 315–316.
5. R. K. Guthrie, *Food Sanitation*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1980.

GENERAL REFERENCES

M. Agosti et al., "In-Field Comparative Evaluation of the Antimicrobial Effects of Plain Detergents and Biocides-Supplemented Detergents," *Industrie Alimentari* **33**, 631–635 (1994).

E. Andueza, "Contributions of Detergents to Sustainable Development in the Dairy Industry," *Alimentacion Equipos y Tecnologia* **17**, 57–59 (1998).

"Developing a Total Cleaning/Sanitation Program," *Food Processing* **48**, 112–113 (1987).

M. J. Banner, "Keeping it Clean," *Beverage World* **106**, 41–49 (1987).

W. G. Cutler and E. Kissa, eds., *Detergency Theory and Technology*, Marcel Dekker, New York, 1987.

E. A. Erten and R. F. Ellis, "Efficient Cleaning Practices Safeguard Product Quality," *Food Processing* **49**, 104–106 (1988).

C. Grimmett, "Developments in Detergents and Disinfectants," *Brewing & Distilling International* **23**, 18–20 (1992).

P. Helisto and T. Korpela, "Effects of Detergents on Activity of Microbial Lipases as Measured by the Nitrophenyl Alkanoate Esters Method," *Enzyme Microb. Technol.* **23**, 113–117 (1998).

D. R. Karsa, *Industrial Applications of Surfactants*, The Royal Society of Chemistry, Burlington House, London, 1987.

F. J. C. Lacroix et al., "*Salmonella typhirium* TnphoA Mutants With Increased Sensitivity to Biological and Chemical Detergents," *Research in Microbiology* **146**, 659–670 (1995).

M. Lucchese, "Ecological Aspects of the Use of Detergents and Sanitizers in the Wine and Food Industries. Effects on Effluents and Purification Aspects," *Vini d'Italia* **34**, 53–60 (1992).

M. H. C. R. Passos and A. Y. Kuaye, "Cleaning of Milk Deposit on Heat Exchange Surface by Alkaline and Acid Detergents," *Cienca e Tecnologia de Alimentos* **14**, 105–112 (1994).

Sanitation Operations Manual, National Restaurant Association, 1979.

A. M. Sjoeberg, G. Wirtanen, and T. S. Mattila, "Biofilm and Residue Investigations of Detergents on Surfaces of Food Processing Equipment," *Food and Bioproducts Processing* **73**(C1), 17–21 (1995).

State Water Quality Board, *Detergent Report, A Study of Detergents in California*, California Department of Water Resources, California Department of Public Health, 1965.

G. K. York, "A Guide to Food Plant Sanitizers," *Food Processing* **48**, 118–120 (1987).

TERRY L. MCANINCH
Birko Corporation
Westminster, Colorado

DISINFECTANTS

DEFINITIONS

Disinfection originally referred to the destruction of the germs of disease. In modern use, a disinfectant is an agent capable of destroying a wide range of microorganisms, but not necessarily bacterial spores. Sterilization is the process of destroying all forms of life, including bacterial spores. Sanitation is a more general term referring to those factors that improve the general cleanliness of our living environment and aid in the preservation of health. A sanitizer is a chemical substance that reduces numbers of microorganisms to an acceptable level. This term is widely used in the United States and it is virtually synonymous with the term disinfectant. In the food industry, sanitation and sanitizers are used to refer to processes and agents, respectively, for cleaning inanimate (work) surfaces. The term hygiene (from *Hygeia*, the Greek goddess of health) is frequently used to refer to personal cleanliness, hence hand hygiene. A soil is an organic or inorganic residue on equipment and other work surfaces. A soiled surface is cleaned by washing with a detergent. Detergency does not imply anything more than assisting in cleaning a surface; microorganisms are reduced only to the extent that they are physically removed from the surface (1).

Disinfectants may be characterized on the basis of their mode and range of antimicrobial action. A biocide is a disinfectant that kills all forms of life, whereas a biostat is a disinfectant that inhibits the growth of all forms of life. Many biocides are biostats at low concentrations. Bactericides and bacteriostats are agents active against bacteria. The prefixes fungi-, spori-, and viru- indicate action against fungi, spores, and viruses, respectively. The term germicide is sometimes used to describe disinfectants that destroy disease-causing organisms.

A sanitation program refers to the schedule and procedures for cleaning and sanitizing a food-processing plant or food-handling facility, as well as requirements for personal hygiene. Procedures for sanitation of inanimate surfaces and equipment involve a two- or three-step process. In each case the initial step is removal of gross soil. In a three-step process, the initial rinse is followed by a detergent wash, rinse, and sanitizing. In a two-step process a combined detergent–sanitizing agent is used, followed by a rinse.

CHEMICAL DISINFECTING AGENTS

Lister used phenol (carbolic acid) as a germicide in 1867. Although it is the parent compound of chemical disinfection, its use today is limited to substituted phenols, eg, the *bis*-phenols used in germicidal soaps. Both chlorine (in the form of hypochlorite) and phenol were used to deodorize waste materials in the early 1800s, before Pasteur established the germ theory of infection and putrefaction. The use of disinfectant chemicals began in clinical surroundings in the late nineteenth century. In 1908 the first large-scale use of chlorine (chloride of lime) in water purification started in Chicago, and its use for this purpose spread rapidly. Yet disinfectants were not readily accepted in food production until the 1940s, when hypochlorite treatment was permitted in the dairy industry as an alternative to steam sterilization. The range of antimicrobial chemical agents has been comprehensively reviewed (2).

Heavy metal ions such as Hg^{2+}, Cu^{2+} and Ag^+ are universal biocides, but they are generally too toxic to use on food equipment. Alcohols such as ethanol and isopropanol and others with chain length up to 8–10 carbon atoms are biocidal except for bacterial spores. Water is essential for the antimicrobial action of alcohols, and 60–70% (v/v) is the most effective concentration. The alkylating agents formaldehyde and ethylene oxide are active gaseous disinfectants with a broad spectrum of biocidal activity, including bacterial spores. The sporicidal activity is probably due to their ability to penetrate into the spores and the fact that they do not require water for their action. Formaldehyde is too toxic for use with food equipment because it is absorbed onto surfaces as a reversible polymer (paraformaldehyde) that slowly depolymerizes to give free formaldehyde as a food contaminant. Ethylene oxide is the most reliable substance for gaseous disinfection of dry surfaces, especially heat-sensitive materials such as plastics (3).

Halogens

The halogens include fluorine, chlorine, bromine, and iodine, all of which are extremely active oxidizing agents. Fluorine is the most reactive, but it is too toxic, irritant and corrosive to be used as a disinfectant (2). Bromine is also too toxic and irritant for widespread use, but it has some applications in mixed halogen compounds (4). Chlorine is an effective disinfectant (Table 1) through the action of hypochlorous acid (HOCl). Hypochlorous acid is unstable and it is normally stabilized by adding caustic soda to form sodium hypochlorite (NaOCl). HOCl can be formed by any of the following reactions:

Chlorine

$$Cl_2 + H_2O \underset{OH}{\overset{H^+}{\rightleftharpoons}} HOCl + H^+ + Cl^-$$

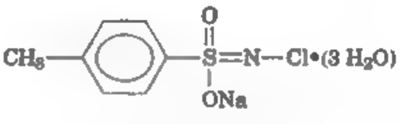
Hypochlorite

$$NaOCl + H_2O \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} HOCl + Na^+ + OH$$

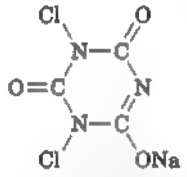
Chloramine

$$NH_2Cl + 2H_2O \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} HOCl + NH_4^+ + OH$$

At acid pH, solutions have greater germicidal activity because greater amounts of HOCl are formed; however, for product stability a pH > 8 is used. Sodium hypochlorite (NaOCl) in commercial liquid form contains 10–14% available chlorine. Calcium hypochlorite [$Ca(OCl)_2$] is a powder with about 30% available chlorine. Chlorine-based sanitizers are normally used at concentrations that yield 100–200 ppm available chlorine with contact times of 3–30 min. Organic chlorines such as chloramine-T and isocyanuric acid derivatives are more stable than hypochlorites in the presence of organic matter, they are less irritant and less toxic, and they release chlorine more slowly.



Chloramine-T



Isocyanuric acid derivatives

Chloramines have 25–30% available chlorine, but they are weaker bactericides than hypochlorites except that they are more active at pH > 10. Isocyanuric acid products are active over the pH range 6–10. Both of these organic chlorines can be formulated as detergent–sanitizers, but they are relatively expensive (1).

Chlorine dioxide is another form of chlorine that has antimicrobial properties. It is considered to have the same antimicrobial activity as hypochlorite. Principal advantages are that it is not as readily inactivated by organics as hypochlorite, and it retains activity at higher pH values than does hypochlorite. Additionally, chlorine dioxide does not react with ammonia, it can break down phenolics to remove phenolic tastes and odors from water, and it does not form trihalomethanes with nitrogenous organic components of water. Because chlorine dioxide is highly reactive, it cannot be produced and shipped in bulk. Therefore, it is generated at the site of use, usually by combining separate solutions of chlorine and sodium chlorite at carefully controlled reaction conditions. Chlorine dioxide decahydrate can be prepared as a commercial product, but it must be refrigerated because it decomposes with rising temperatures. Under certain conditions it can be explosive. Chlorine dioxide, although it is more expensive than hypochlorite, has been used for treatment of water supplies, in plant chlorination of wastewater treatment, and chlorination of recycled or recirculated waters where slime buildup may occur (5).

Iodine is too insoluble in water to be used as a disinfectant; however, if it is complexed with a nonionic surface

Table 1. Advantages and Disadvantages of Different Classes of Sanitizers

	Hypochlorite	Iodophor	QAC	Acid–Anionics
Antimicrobial spectrum				
Gram-positive bacteria	+	+	+	+
Gram-negative bacteria	+	+	−	+
Spores	+	−	−	−
Viruses	+	−		+
Low toxicity	+	+	+	
Nonirritating to skin	−	+	+	
Noncorrosive	−[a]	−	+	
Nonstaining	+	−[b]	+	+
Stability during storage	−	+	+	+
in hard water	+	+		+
with organic matter	−	±	+	+
Use at temperatures	+	−	+	+
Mineral–protein films removed	−	+	−	+
Leaves no residues	+	±	−	−
Leaves no flavors or odors	−	−	+	+
Detergent–sanitizer	−	±	+	+
Self-indicating	±[c]	+	−	−
Low cost	+	±	−	

Note: Advantages attribute = −, disadvantageous attribute = −.

[a] Only a problem if misused.

[b] Only some plastic materials and painted walls; staining of skin is temporary.

[c] By odor of available chlorine.

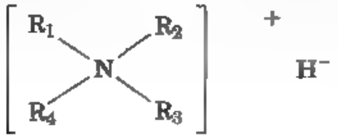
active agent, the resulting iodophor can be used as a detergent–sanitizer (Table 1). Diatomic iodine (I_2) is highly bactericidal; hypoiodous acid (HOI) and the hypoiodite ion (IO^-) are less bactericidal; the iodate ion (IO_3^-) is inactive.

$$I_2 + H_2O \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} HOI + H^+ + I^- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} I^- + IO_3^-$$

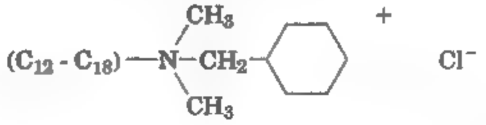
Iodophors are generally used at 12.5–25.0 ppm; at these concentrations they are self-indicating, ie, the presence of available iodine is indicated by the amber color of the solution. They must be used at < 50°C to avoid the release of toxic iodine vapor. Iodophors are formulated with an acid, usually phosphoric acid, because they are more active at pH 3–5. They also solubilize and remove mineral and protein films from food-processing equipment (4).

Surface Active Agents (Surfactants)

These compounds are classified in three categories: (*1*) cationic, (*2*) anionic, and (*3*) nonionic or amphoteric. All three categories have antimicrobial activity to varying degrees, depending on the specifics of molecular moiety and formulation with other ingredients to produce disinfectant or sanitizing compounds. Quaternary ammonium compounds (QACs), or quats, are cationic wetting agents or surfactants. The general chemical configuration of a QAC is



where R_1, R_2, R_3, and R_4 are covalently bound organic groups such as alkyl, methyl, benzyl, and cetylbenzyl. The H^- is a halogen, most often chlorine or occasionally bromide. These are synthesized compounds that are formed by reacting tertiary amines with quarterizing agents such as alkyl or cetyl halides. A wide array of groups may be substituted, and this may result in the formation of quaternaries with widely variable properties. The cation or positively charged proton of the molecule is microbiologically active and lyophobic. The negatively charged halide is lyophilic. Because QACs are actively cationic, they are not compatible with detergents or other compounds that are anionic in solution. The chemical configuration for alkyldimethylbenzylammonium chloride, one of the frequently employed quaternaries, is



Because of the great diversity of chemical composition with QACs, great variation in the antimicrobial activity may occur. Generalization is difficult with these compounds. QACs principally are antibacterial in activity. Often they are more effective against gram-positive bacteria than gram-negative bacteria, especially at alkaline pH. The antifungal activity of quaternaries is variable and depends on the moiety of the molecule of the specific QAC employed and the specific species of fungal organism(s) of concern. Some quaternaries are very effective fungicides, while others may be mediocre at best. Proper selection of these products for antifungal activity is necessary. The QACs are not considered to be effective agents for controlling either bacterial spores or fungal spores. They are variable as viricides, being moderately active against lipophilic viruses but not effective against hydrophilic viruses. In addition to molecular configuration, other factors have an influence on the antimicrobial activity of QACs. As stated previously, quaternaries at alkaline pH generally are more effective against gram-positive than gram-negative bacteria. However, there are complications, conflicts, and resistances related to the pH of solutions. Some gram-negative bacteria, such as *Pseuomonas* and *Escherichia*, show resistance to QACs at alkaline pH but may be much less resistant to neutral or slightly acid quaternary solutions. The effect of organic residues on antimicrobial effectiveness of QACs can be variable. To a large extent, the degree depends on the specific quaternary compound and the type of organic soil that may be present. In general, QACs are more stable to the presence of organic residues than hypochlorite and about equal to that of the iodophors. The activity of quaternary ammonium compounds may be affected by the presence of calcium and magnesium water-hardness salts in solutions. This effect varies with the quaternary compound employed. The QACs that are formulated with chelating agents, such as EDTA and/or sequesterants, have good tolerance to high levels of water-hardness salts. There is good agreement that the effectiveness of QACs increases as the solution temperature increases and that these compounds are more stable than both hypochlorite and iodophors at high temperatures (6).

The quaternaries have found use as sanitizing solutions for dairy and food equipment, skin antiseptics, and hospital sanitizing applications. Quaternary compounds may be combined with nonionic wetting agents and other detergent enhancers into detergent–sanitizer formulations for specific applications. If QACs are to be on sanitizing food-contact surfaces, they must be of a type and in a form that has FDA approval according to provisions of 21 CFR (7). Because of the great variability inherent to these products, it is imperative that they be selected after consultation with QAC manufacturers or suppliers who have been advised of the prevailing conditions under which the quaternary will be expected to perform. After this has been done, the selected product(s) should be used only under the explicit instructions that have been given.

The acid–anionic products are mixtures of anionic surfactant and an acid. Most frequently phosphoric acid is the acid employed. The chemical structure of the anionic surfactants usually is in the form of an alkyl aryl sulfonate. Some examples of these are dodecylbenzene sulfonic acid, sodium lauryl sulfate, sodium dioctylsulfosuccinate, and 1-octane sulfonate. For a more comprehensive list of anionics that have been approved for use in sanitizer solutions for application to food contact surfaces, refer to 21 CFR (7).

The antimicrobial activity of the acid–anionic sanitizers is most pronounced at low pH levels, with the pH range of 3.0 to 1.5 being the most optimal. At the recommended-use concentrations, for specific applications, they provide excellent antimicrobial activity against vegetative bacterial cells and most yeasts. Both bacterial spores and fungal spores show resistance to these products. At pH values above 3.5, the effectiveness of the acid–anionic sanitizers against gram-negative bacteria is reduced, and this becomes more pronounced with increasing pH. Water hardness at up to 1,000 ppm as $CaCO_3$ has not been shown to affect the antimicrobial activity of acid–anionics, but if the water-hardness level should be high enough to cause a rise in pH of a sanitizing solution above 3.5, then reduced sanitizing effectiveness could be anticipated. Because of their acidic reaction in solution, the acid–anionic surfactant sanitizers have found important applications for sanitizing both circulation cleaned-in-place (CIP) equipment and spray-cleaned tanks and vessels in dairy and beverage processing plants where mineral residuals may affect the sanitizing efficiency of the other types of cleaners. The acid–anionic sanitizers are more costly than hypochlorite, but they are less expensive than either the iodophors or the quaternary ammonium sanitizers (8).

Amphoteric surfactants are disinfectants that have been used in European and some other countries for more than 40 years. They have not received FDA approval in the United States for use as sanitizers on food contact surfaces. Most of the commercial amphoteric products are sold under the trade name Tego. They are termed amphoteric or ampholytic because in water solution they yield anions, cations, and zwitterions (dual-charged molecules). The chemical structure of amphoteric surfactants is a molecule composed of an amino acid, most frequently glycine, substituted with a long-chain alkyl amine group. Examples of the most frequently employed amphoterics include dodecylglycine, dodecylaminoethylglycine, and dodecyldiaminoethylglycine. The amphoteric surfactants have been purported to be bactericidal, fungicidal, and viricidal and effective in the presence of soil, lipids, and proteins. The contact times required for effective microbial inactivation are longer than that for other more frequently used sanitizers. Because of their surfactant properties, the amphoteric sanitizers may adsorb on surfaces to form a film that is resistant to rinsing by water. This is an advantageous property when residual antimicrobial activity is desired but may be a disadvantage from the standpoint of possible sanitizer residuals in foods. The effect of organic residues on the antimicrobial of amphoteric surfactant sanitizers are contradictory. This has created a problem for their gaining acceptance and approval as sanitizers. The amphoteric sanitizers have been used as hand-wash disinfectants, floor-wash disinfectants, and food plant equipment sanitizers in European and some other countries (9).

The Peroxides

The two peroxides that are most frequently used for disinfection and sanitizing are hydrogen peroxide and peroxyacetic acid (peracetic acid). Hydrogen peroxide at 3% concentration has been employed as a topical antiseptic and wound irrigation agent for many years. More recently, processes have been developed that allow the production of stable, highly concentrated solutions that may contain up to 90% hydrogen peroxide. This has resulted in the availability of hydrogen peroxide, of various grades and purity, that could be used for microbial control in the food, cosmetic and electronic industries. Hydrogen peroxide has shown effective antimicrobial activity against both non-spore-bearing and spore-bearing bacteria, yeasts, molds, and viruses. It is very effective against the anaerobes because they lack catalase enzyme, which hydrolyses hydrogen peroxide. Also, gram-negative bacteria are more easily inactivated by hydrogen peroxide than are gram-positive bacteria. Among the factors known to affect the antimicrobial activity of hydrogen peroxide are the concentration of chemical, pH, temperature, and presence of metallic salts. Hydrogen peroxide follows the classical relationship that the higher the concentration of chemical antimicrobial in solution, the more rapid the rate of microbial inactivation. Hydrogen peroxide is most active at acidic conditions; its activity is slower at neutrality and becomes progressively less active as the alkalinity of the solution increases. Temperature affects the action of hydrogen peroxide. In general, higher solution temperatures can result in lower concentrations and shorter contact times to obtain microbial inactivation. The presence of certain metallic salts or ions increases the antimicrobial effectiveness of hydrogen peroxide. These include copper, chromium, iron, and molybdenum. A good review on the antimicrobial activity of hydrogen peroxide has been presented by Turner (10).

In industrial applications, hydrogen peroxide has FDA approval for use as a sterilant of containers and equipment used in aseptic packaging of foods (7). For treatment of packaging rooms, 30 to 40% hydrogen peroxide for up to 30 minutes or more contact time at room temperature may be required; whereas at a temperature of 60 to 71°C, the sterilization contact time for containers may be reduced to seconds. Because of the potential hazard of hydrogen peroxide, it is important to insure that all necessary safety practices are in effect for handling and using this material to prevent serious harm to personnel. Hydrogen peroxide has shown promise as an agent for use in vapor-phase sterilization applications (11).

Peroxyacetic acid (peracetic acid) is an organic peroxide that has been used for practical microbial control. Peroxyacetic acid is a strong oxidizing agent that is soluble in water and has a characteristic pungent, vinegarlike odor. Concentrated solutions of peroxyacetic acid are highly unstable, particularly at high temperatures and in the presence of heavy metallic ions. Current commercially available products contain stabilizing ingredients, and they are safe to use. Typically these contain from 4 to 40% peroxyacetic acid. As with all chemicals, they must be handled, stored, and used according to specific label declarations and product safety data sheets for the product. Peroxyacetic acid is superior in action to hydrogen peroxide as an antimicrobial and has a broad spectrum of activity against both gram-positive and gram-negative bacteria, spore-bearing bacteria, and microbial spores. It has both fungicidal and viricidal activity at recommended conditions of use. The FDA allows the use of peroxyacetic acid sanitizing solutions on food-contact surfaces at a concentration of 100 to 200 mg/L (ppm) (7). The antimicrobial activity of peroxyacetic acid is affected by such factors as concentration, pH, and contact time. As is true with other chemical antimicrobials, higher concentrations of peroxyacetic acid in solution are more cidal than are lower concentrations, provided that all other factors are constant. With regard to pH, acid conditions are most optimal to inactivation of microbes, and as the pH increases above pH 7 to 8, there is decreasing cidal activity for a constant concentration of peroxyacetic acid in solution. As might be expected, the temperature of the solution affects the killing power of peroxyacetic acid at a given concentration. Increasing temperature improves the germicidal effect, but peroxyacetic acid retains some antibacterial activity at a temperature as low as 7°C. Because of this, peroxyacetic acid has an advantage over most other commonly used chemical sanitizers for certain applications. Water-hardness minerals do not have a great effect on peroxyacetic acid, and it is not affected as adversely by organic residuals as are other oxidative sanitizers (12,13). As with all hazardous chemicals, necessary precautions are required when using this product. It is essential that personnel be made aware of these and that labeling directions and product safety instructions be followed precisely.

Phenols

Many phenolic compounds are strong germicides, but their potential for use in the food industry is limited by their odor and the possibility of causing off-flavors in foods. Their action involves cell lysis. Depending on concentration, phenols are either bactericidal or bacteriostatic. They have only limited activity against viruses, and they are not sporicidal. Halogenation of phenols increases their activity 3–30 times. Substitution in the *para* position is more effective than in the *ortho* position, substitution with two halogen atoms is more effective than one, and bromophenols are more active than chlorophenols (14). Chloroxylenol or *para*-chloro-*meta*-xylenol (PCMX), the active ingredient of Dettol, is a phenolic compound that can be used as a skin germicide.

Table 2 provides information on comparative information on some of the characteristics of the most common chemical sanitizers that have FDA (7) approval for no-rinse application on food-contact surfaces. Because of the wide variations in the formulation of commercial products, there may be anomalies from that normally expected. In all instances, the information and recommendations given by the manufacturers or supplies of commercial proprietary sanitizer products should be sought and followed explicitly for any application.

Many halogenated *bis*-phenols (or phenylphenols) have considerable activity against bacteria and fungi, but they have low activity against pseudomonads (2). They are used as clinical disinfectants and in germicidal soaps, eg, hexachlorophene.

Hexachlorophene is not very volatile and lacks the unpleasant odor of phenols. It is more active against Gram-positive than Gram-negative bacteria. It is bacteriostatic for *Staphylococcus aureus* at extremely low concentrations (0.05 μg/mL) and requires a suitable quenching agent to inactivate residues of the disinfectant so that its bactericidal activity can be accurately assessed (2). It was widely used as a skin antiseptic marketed as pHisoHex and in a wide range of over-the-counter (OTC) pharmaceutical and personal hygiene products. It can be absorbed through in-

Table 2. Comparative Characteristics of Different Sanitizers

	Hypoclorite	Iodophors	Quarternary ammoniums	Acid anionics	Peroxyacetic acid
Biocidal spectrum					
Gram-positive bacteria	+ +	+ +	+ +	+ +	+ +
Gram-negative bacteria	+ +	+ +	+	+	+ +
Bacterial spores	+ +	+	±	±	+
Yeasts	+	+ +	+	+	+
Molds	+ +	+ +	+	+	+ +
Viruses	+ +	+	±	±	+
pH effectiveness					
Acid	± (unstable)	+ +	±	+ +(<3.5)	+ +
Neutral	+ +	±	+	−	+ +
Alkaline	+	−	+	−	+
Stability to					
Organic residues	−	±	+	+	±
Hard water	+ +	+	±	+	+
Noncorrosive	−	±	+ +	±	±
Skin irritant	+ +	−	−	±	±
Residual activity	−	+	+ +	+	−
High temperature stability	+ +	−	+	+	+ +
Use solution stability	−	+	+ +	+ +	+
Maximum use concentration by FDA[a]	200 mg/l[b]	25 mg/l[b]	200 mg/l[b]	200–430 mg/l[b] (depends on type)	100–200 mg/l[b]

Note. Relative properties: + +, definite; + moderate; ±, variable, −, very low to none

[a] According to 21CFR, 178:1010; no rinsing after application.

[b] mg/L = milligrams per liter or ppm.

flamed and infant skin with the possibility of serious systemic toxicity (15). As a result, OTC products have generally been limited to 0.75% hexachlorophene. Higher concentrations are sold with medical prescription.

Restrictions on the use of hexachlorophene resulted in the development of a range of other disinfectants for use in germicidal soaps. For example, Irgasan DP 300, also known as triclosan, 2-4-4′trichlor-2′-hydroxy diphenyl ether is active against Gram-positive and Gram-negative organisms.

Other Antimicrobial Agents

Chlorhexidine is one of a family of N^1,N^5-substituted biguanides (2). Its use was licensed in the UK and Canada, but not in the United States.

Chlorhexidine is active against Gram-positive and Gram-negative bacteria; it has limited antifungal activity, but it is not active against acid-fast bacilli, bacterial spores, or viruses. However, some resistant pseudomonads can contaminate aqueous solutions of this compound. It is not compatible with anionic compounds. The digluconate salt is freely soluble in water; however, the diacetate and dihydrochloride salts are only poorly soluble. Alcoholic chlorhexidine or a 4% chlorhexidine detergent (Hibiscrub) are highly effective skin germicides (16).

The salicylanilides and carbanilides are families of antimicrobial chemicals (17). One of the more popular disinfectants among these chemicals is 3,4,4′-trichlorocarbanilide. (TTC). It has been incorporated into soaps to give an antimicrobial product.

PHYSICAL DISINFECTING AGENTS

In addition to chemical compounds, heat and ultraviolet (uv) light can be used to disinfect food contact surfaces. Heat, particularly moist heat, is the most reliable and most widely used method of destroying all forms of microbes (2). The efficacy of heat treatment with steam or hot water (80°C or above) depends on the temperature achieved and the time of exposure. Temperatures above 60°C progressively kill microbial cells. Vegetative cells may be killed or injured. Injured cells can recover in an appropriate environment, for example, in foods. Heat injury has been described in two ways: the extension of the lag phase of growth (18) and the inability to grow on selective media, for example, on salt-containing media for *Staphylococcus aureus* (19) or violet red bile agar for coliform bacteria (20). Because heat cannot be universally applied it is necessary to rely on chemical agents for sanitation programs.

Radiation is an alternative to gaseous disinfection. Gamma (cobalt-60) and electron-accelerated β-ray (<10 MeV) irradiation have good penetrating ability and, therefore, have the potential for use not only in disinfection, but also in food processing. Ultraviolet radiation has a wavelength between 210 and 328 nm, with maximum antimicrobial activity between 240 and 280 nm. Ultraviolet radiation is low energy, so it does not penetrate foods. It is absorbed by glass and plastics, but it can be used for surface disinfection (2). *Micrococcus radiodurans* and bacterial and mold spores are highly resistant to ionizing and uv irradiation. The primary effect of radiation on living cells is by action on DNA. Radiation resistance is genetically determined. Sublethally damaged cells can recover by photoreactivation or by excision and recombination events, which give rise to mutations (2).

EVALUATION OF DISINFECTANTS

A number of laboratory tests have been developed to evaluate the efficacy of disinfectants (2). The Rideal–Walker and Chick–Martin methods of determining the phenol coefficient with *Salmonella typhi* were developed in the early 1900s. Although the Chick–Martin test includes an organic soil, the phenol coefficient tests are artificial in concept, have poor reproducibility and phenol is an unreliable control disinfectant (1). The improved Kelsey–Sykes capacity test attempted to resolve the deficiencies of the phenol tests by using at least four organisms in preliminary screening tests, the most resistant organism being selected for further testing. The disinfectants are prepared in a standardized hard water, with or without a standard soil. Recovery broths are prepared with a neutralizer against the disinfectant. This test also has its shortcomings, but it is used as the official test for disinfectants in the UK (21).

Several tests have been designed to simulate in-use conditions by preparing an air-dried film of microorganisms on an appropriate surface, such as stainless steel, with or without an organic soil. The Lisboa test was developed for testing sanitizers for dairy equipment. Stainless steel tubes are contaminated and disinfected using standardized procedures and neutralized to inactivate any residual disinfectant; the number of surviving organisms is then determined (1). Various modifications of this in-use testing have been developed (2,22). Methods of the Association of Official Analytical Chemists (AOAC) include use-dilution carrier techniques with *Salmonella choleraesuis, Staphylococcus aureus*, or *Pseudomonas aeruginosa* contaminated onto polished stainless-steel cylinders. Tests for fungicidal and sporicidal activity are also specified (23).

Tests for evaluating skin germicides under in-use conditions have been even more difficult to standardize (24), and there are no official methods comparable to the AOAC use-dilution technique for evaluating disinfectants for use on inanimate surfaces. Multiple-basin techniques measure the rate of mechanical removal of microorganisms from the skin. Testing of skin disinfectants must take into account the transient and residual microflora of the skin, the time of exposure, methods of sampling the skin after washing, and methods of contaminating skin with a transient microflora appropriate to the use environment (25). Depending on the germicidal agent used, it may be necessary to neutralize the rinse solution. With skin disinfectants it is appropriate to test for a residual (or substantive) effect, ie, improved results with repetitive use of the same agent compared with nongermicidal soaps. Occlusion and expanded flora tests (in which agents are applied topically to the skin, covered with a plastic film, and sealed with impermeable plastic tape) represent alternative approaches to testing skin disinfectants (26).

Comparisons of antimicrobial soaps by various standardized techniques have given results that question the in-use efficacy of most products tested. Studies have indicated that under the specific test conditions, only an iodophor product (0.75%) and 4% chlorhexidine gluconate significantly reduced the number of microorganisms released from hands (25,27,28). Other products, including intermediate strength iodophor products, 2% chlorhexidine, Irgasan DP 300, *para*-chloro-*meta*-xylenol (PCMX), trichlorocarbanilide or tribromosalicylanilide, under in-use conditions of testing were no better than a nongermicidal soap.

MODULAR SYSTEMS OF DISINFECTION

Food industries that process liquid products, such as milk, beer, and soft drinks, use CIP systems for disinfection. The principles are similar to those for manual cleaning, but mechanical force generated by the velocity of liquid flow through the system is relied on to remove food soils. CIP offers many advantages over manual sanitizing programs, including lower labor costs, more economic operation, better sanitation, faster cleanup and reuse of equipment, less dismantling and reassembly, and greater safety of use and operation (1). The CIP systems can be based on single or multiple use of cleaning solutions. Multiple-use systems are usually automatic, and solutions are recovered according to a preset program and stored in holding tanks for reuse. A typical CIP program is as follows (1):

1. Prerinse (5 min) with cold water.
2. Alkali detergent wash (15 min at 80°C).
3. Intermediate rinse (3 min) with cold water from mains.
4. Cold hypochlorite solution (10 min).
5. Final rinse (3 min) with cold water from mains.

For large tanks that would be uneconomical to fill with cleaning fluids, a permanent or portable spray system is fitted to the vessel. The spraying devices should allow every part of the inside of the vessel to come in contact with the cleaning solutions. Good drainage must be insured to avoid accumulation of fluids and residues on equipment surfaces.

Mechanical aids for plant sanitation include pressurized steam, high-pressure water jets, compressed air, and ultrasound. All of these cleaning aids require specialized equipment and have specific uses. High-pressure steam can remove debris and sterilize. High-pressure water jets also remove debris from inaccessible parts of machinery; however, such inaccessibility should be avoided in the design of food equipment and machinery. Compressed air removes dry powder, dust, and soil, but it spreads, rather than eliminates, the soil. Vacuum cleaners are preferable to compressed air for removing dry solids and dust. Ultrasound is used to clean small or sensitive items of equipment that are otherwise difficult to clean. It requires immersion of the objects in an ultrasound tank for exposure to ultrasonic vibrations, which remove soils by cavitation.

Foam sanitation is an efficient system for cleaning walls, floors, equipment with large contact surfaces, and immovable food-handling equipment. A foaming agent is added to the detergent formulation to produce a thick, long-lasting foam. This gives the cleaning agent a long contact time with the soiled surfaces. This form of cleaning requires a special pressure-generating system. The foam must be removed and the bactericidal agent applied. Foam systems give a good visible awareness of the sanitation process.

LEGISLATION

The food legislation of most countries requires that food is handled in a sanitary manner. The details and standards differ between countries. In the United States the principal federal legislation governing the handling of foods is the Food, Drug and Cosmetic Act of 1938, as amended. This covers all foods except meats and poultry, which are covered by various food inspection acts. Regulations promulgated through provisions of these acts are published in the *Code of Federal Regulations* (CFR), which is revised annually as needed. Emphasis is on good manufacturing practices and hazard analysis and critical control point (HACCP) standards for the handling, preparation, processing, and storage of food. Each state may enact its own laws and establish its own regulations for food sanitation control that apply to its territorial jurisdiction. In most cases, the state laws and regulations are in substantive agreement with the federal counterpart. The U.S. Department of Agriculture has responsibility for meat, poultry, rabbit, and egg products inspection programs in the United States. Under the Federal Insecticide, Fungicide, and Rodenticide Act of 1978, sanitizers for use in food establishments are defined as insecticides and are regulated by the U.S. Environmental Protection Agency (EPA). Antimicrobial agents for applications on humans are regulated by the Food and Drug Administration.

In Canada food legislation is enacted through the federal Food and Drugs Act of 1953, as amended. The Health Protection Branch (HPB) of Health and Welfare Canada is responsible for administering the act. Other agencies also have responsibility for food inspection, including Agriculture Canada, and Fisheries and Oceans Canada. Disinfectants for use in food premises are deemed to be drugs and require a Drug Identification Number (DIN) issued by HPB.

In the UK, food legislation is based on the Food and Drugs Act of 1955, which is the enabling legislation for regulations governing food hygiene and is controlled by the Department of Health and Social Security or the Ministry of Agriculture, Fisheries and Food (MAFF). Enforcement of the regulations is the responsibility of Environmental Health Officers employed by elected local authorities.

In Europe, the EEC has mandatory standards that automatically become law in member countries. In a broader sense, the Codex Alimentarius program of the Food and Agriculture and World Health Organizations (FAO/WHO) has the intention of establishing international agreements on food standards that would safeguard health and encourage good handling practices for foods.

BIBLIOGRAPHY

1. P. R. Hayes, *Food Microbiology and Hygiene*, Elsevier Applied Science Publishers, Ltd., Barbing, UK, 1985.
2. A. D. Russell, W. B. Hugo, and E. A. J. Ayliffe, eds., *Principles and Practice of Disinfection, and Sterilization*, Blackwell Scientific Publications, London, 1982.
3. C. R. Phillips, "Gaseous Sterilization," in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 2nd. ed., Lea and Febiger, Philadelphia, 1977.
4. J. R. Trueman, "The Halogens" in W. B. Hugo, ed., *Inhibition and Destruction of the Microbial Cell*, Academic Press, Orlando, Fla., 1971, pp. 137-183
5. M. A. Bernarde et al., "Efficiency of Chlorine Dioxide as a Bactericide," *Appl. Microbiol.* **13**, 776–780 (1965).
6. J. J. Merianos, "Quaternary Ammonium Antimicrobial Compounds," in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 4th ed., Lea and Febiger, Philadelphia, 1991, pp. 225–255.
7. CFR, *Code of Federal Regulations*, Title 21, Parts 178.1005-1010, Office of Federal Register, U.S. Government Printing Office, Washington, DC, April 1998.
8. G. R. Dychdala and J. A. Lopes, "Surface Active Agents: Acid-Anionic Compounds," in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 4th ed., Lea and Febiger, Philadelphia, 1991, pp. 256–262.
9. S. S. Block, "Peroxygen Compounds," in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 4th ed., Lea and Febiger, Philadelphia, 1991, pp. 167–181.
10. F. J. Turner, "Hydrogen Peroxide and Other Oxidant Disinfectants" in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 3rd ed., Lea and Febiger, Philadelphia, 1983. Penn., 1983, pp. 240–250.
11. N. A. Klapes and D. Vesley, "Vapor-phase Hydrogen Peroxide as a Surface Decontaminant and Sterilant," *Appl. Environ. Microbiol.* **56**, 503–506 (1990).
12. M. G. C. Baldry, "The Bactericidal, Fungicidal and Sporocidal Properties of Hydrogen Peroxide and Peracetic Acid," *J. Appl. Bact.* **54**, 417–423 (1983).
13. M. G. C. Baldry and J. A. L. Fraser, "Disinfection with Peroxygens," in K. R. Payne, ed., *Industrial Biocides*, John Wiley and Sons, New York, 1988, pp. 91–116.
14. R. F. Prindle, "Phenolic Compounds," in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 3rd ed., Lea and Febiger, Philadelphia, 1983, pp. 197–224.
15. R. A. Chilcote et al., "Hexachlorophene Storage in a Burn Patient Associated with Encephalopathy," *Pediatrics* **59**, 457–459 (1977).
16. E. J. L. Lowbury and H. A. Lilly, "Use of 4% Chlorohexidine Detergent Solution (Hibiscrub) and Other Methods of Skin Disinfection," *Br Med. J.* **1**, 510–515 (1973).
17. H. C. Stecker, "The Salicylanides and Carbanilides" in S. S. Block, ed., *Disinfection, Sterilization and Preservation*, 2nd ed., Lea and Febiger, Philadelphia, 1977, pp. 282–300.
18. H. Jackson and M. Woodbine, "The Effect of Sublethal Heat Treatment on the Growth of *Staphylococcus aureus*," *J. Appl. Bact.* **26**, 152–158 (1963).
19. M. E. Stiles and L. D. Witter, "Thermal Inactivation, Heat Injury and Recovery of *Staphylcoccus aureus*," *J. Dairy Sci.* **48**, 677–681 (1965).
20. L. A. Roth, M. E. Stiles, and L. F. L. Clegg, "Reliability of Selective Media for the Enumeration and Estimation of *Escherichia coli*," *Can. Inst. Food Sci. Tech. J.* **6**, 230–234 (1973).
21. B. Croshaw, "Disinfectant Testing—With Particular Reference to the Rideal-Walker and Kelsey-Sykes Tests," in C. H. Collins et al., ed. *Disinfectants: Their Use and Evaluation of Effectiveness*, Academic Press, Orlando, Fla., 1981, pp. 1–15.
22. R. M. Blood, J. S. Abbiss, and B. Jarvis, "Assessment of Two Methods for Testing Disinfectants and Sanitizers for Use in the Meat Processing Industry," in C. H. Collins et al., eds., *Disinfectants: Their Use and Evaluation of Effectiveness*, Academic Press, Orlando, Fla., 1981, pp. 17–31.
23. Association of Official Analytical Chemists, *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., AOAC, Arlington, Va., Vol. 1, 1990, pp. 135–142.

24. G. A. J. Ayliffe, J. R. Babb, and Ha. A. Lilly, "Tests for Hand Disinfection," in C. H. Collins et al., eds., *Disinfectants: Their Use and Evaluation of Effectiveness*, Academic Press, Orlando, Fla., 1981, pp. 37–44.
25. A. Z. Sheena and M. E. Stiles, "Efficacy of Germicidal Hand Wash Agents in Hygienic Hand Disinfection," *J. Food Protection* **45**, 713–720 (1982).
26. R. R. Marples and A. M. Kligmen, "Methods for Evaluation Topical Antibacterial Agents on Human Skin," *Antimicrob. Agents and Chemotherapy* **2**, 8–15 (1974).
27. A. Z. Sheena and M. E. Stiles, "Efficacy of Germicidal Hand Wash Agents Against Transient Bacteria Inoculated Onto Hands," *J. Food Protection* **46**, 722–727 (1983).
28. A. Z. Sheena and M. E. Stiles, "Low Concentration Iodophor for Hand Hygiene," *J. Hygiene* **94**, 269–277 (1985).

David A. Evans
University of Massachusetts
Amherst, Massachusetts

DISTILLATION: TECHNOLOGY AND ENGINEERING

Although the use of distillation dates back in recorded history to about 50 BC the first truly industrial exploitation of this separation process did not occur until the 12th century, when it was used in the production of alcoholic beverages. By the 16th century, distillation also was being used in the manufacture of vinegar, perfumes, oils, and other such products.

As recently as 200 years ago, distillation stills were small, of the batch type, and usually operated with little or no reflux. With experience, however, came new developments. Tray columns appeared on the scene in the 1820s, along with feed preheating and the use of internal reflux. By the latter part of that century, considerable progress had been made by Hausbrand in Germany and Sorel in France, who developed mathematical relations that turned distillation from an art into a well-defined technology.

Distillation today is a widely used operation in the petroleum, chemical petrochemical, beverage, and pharmaceutical industries. It is important not only for the development of new products but also in many instances for the recovery and reuse of volatile liquids. Pharmaceutical manufacturers, for example, use large quantities of solvents, most of which can be recovered by distillation with a substantial savings in cost and a reduction in pollution.

Although one of the most important unit operations, distillation unfortunately is also one of the most energy-intensive operations. It easily is the largest consumer of energy in petroleum and petrochemical processing and as such, must be approached with conservation in mind. It is a specialized technology in which the correct design of equipment is not always a simple task.

DISTILLATION TERMINOLOGY

To provide a better understanding of the distillation process, the following briefly explains the terminology most often encountered.

Solvent Recovery

The term solvent recovery often has been a somewhat vague label applied to the many and very different ways in which solvents can be reclaimed by industry

One approach when an impure solvent contains both soluble and insoluble particles is to evaporate the solvent from the solids. This requires the use of a small forced-circulation-type evaporator that combines a heat exchanger, external separator, and vacuum system with a special orifice that causes back pressure in the exchanger and arrests vaporization until the liquid flashes into the separator. Although this will recover a solvent, it will not separate solvents if two or more are present.

A further technique is available to handle an airstream that carries solvents. By chilling the air by means of vent condensers or refrigeration equipment, the solvents can be removed from the condenser. Solvents also can be recovered by using extraction, adsorption, absorption, and distillation methods.

Solvent Extraction

Essentially a liquid/liquid process where one liquid is used to extract another from a secondary stream, solvent extraction generally is performed in a column somewhat similar to a normal distillation column. The primary difference is that the process involves two liquids instead of liquid and vapor.

During the process, the lighter (ie, less dense) liquid is charged to the base of the column and rises through packing or trays while the more dense liquid descends. Mass transfer occurs, and a component is extracted from one stream and passed to the other. Liquid/liquid extraction sometimes is used when the breaking of an azeotrope is difficult or impossible by distillation techniques.

Carbon Adsorption

The carbon adsorption technique is used primarily to recover solvents from dilute air or gas streams. In principle, a solvent-ladened airstream is passed over activated carbon and the solvent is adsorbed into the carbon bed. When the bed becomes saturated, steam is used to desorb the solvent and carry it to a condenser. In such cases as toluene, for example, recovery of the solvent can be achieved simply by decanting the water/solvent two-phase mixture that forms in the condensate. Carbon adsorption beds normally are used in pairs so that the airflow can be diverted to the secondary bed when required.

On occasion, the condensate is in the form of a moderately dilute miscible mixture. The solvent then has to be recovered by distillation. This would apply especially to ethyl alcohol, acetone-type solvents.

Absorption

When carbon adsorption cannot be used because certain solvents either poison the activated carbon bed or create so much heat that the bed can ignite, absorption is an alternative technique. Solvent is recovered by pumping the solvent-ladened airstream through a column countercurrently to a water stream that absorbs the solvent. The

24. G. A. J. Ayliffe, J. R. Babb, and Ha. A. Lilly, "Tests for Hand Disinfection," in C. H. Collins et al., eds., *Disinfectants: Their Use and Evaluation of Effectiveness*, Academic Press, Orlando, Fla., 1981, pp. 37–44.
25. A. Z. Sheena and M. E. Stiles, "Efficacy of Germicidal Hand Wash Agents in Hygienic Hand Disinfection," *J. Food Protection* **45**, 713–720 (1982).
26. R. R. Marples and A. M. Kligmen, "Methods for Evaluation Topical Antibacterial Agents on Human Skin," *Antimicrob. Agents and Chemotherapy* **2**, 8–15 (1974).
27. A. Z. Sheena and M. E. Stiles, "Efficacy of Germicidal Hand Wash Agents Against Transient Bacteria Inoculated Onto Hands," *J. Food Protection* **46**, 722–727 (1983).
28. A. Z. Sheena and M. E. Stiles, "Low Concentration Iodophor for Hand Hygiene," *J. Hygiene* **94**, 269–277 (1985).

DAVID A. EVANS
University of Massachusetts
Amherst, Massachusetts

DISTILLATION: TECHNOLOGY AND ENGINEERING

Although the use of distillation dates back in recorded history to about 50 BC the first truly industrial exploitation of this separation process did not occur until the 12th century, when it was used in the production of alcoholic beverages. By the 16th century, distillation also was being used in the manufacture of vinegar, perfumes, oils, and other such products.

As recently as 200 years ago, distillation stills were small, of the batch type, and usually operated with little or no reflux. With experience, however, came new developments. Tray columns appeared on the scene in the 1820s, along with feed preheating and the use of internal reflux. By the latter part of that century, considerable progress had been made by Hausbrand in Germany and Sorel in France, who developed mathematical relations that turned distillation from an art into a well-defined technology.

Distillation today is a widely used operation in the petroleum, chemical petrochemical, beverage, and pharmaceutical industries. It is important not only for the development of new products but also in many instances for the recovery and reuse of volatile liquids. Pharmaceutical manufacturers, for example, use large quantities of solvents, most of which can be recovered by distillation with a substantial savings in cost and a reduction in pollution.

Although one of the most important unit operations, distillation unfortunately is also one of the most energy-intensive operations. It easily is the largest consumer of energy in petroleum and petrochemical processing and as such, must be approached with conservation in mind. It is a specialized technology in which the correct design of equipment is not always a simple task.

DISTILLATION TERMINOLOGY

To provide a better understanding of the distillation process, the following briefly explains the terminology most often encountered.

Solvent Recovery

The term solvent recovery often has been a somewhat vague label applied to the many and very different ways in which solvents can be reclaimed by industry

One approach when an impure solvent contains both soluble and insoluble particles is to evaporate the solvent from the solids. This requires the use of a small forced-circulation-type evaporator that combines a heat exchanger, external separator, and vacuum system with a special orifice that causes back pressure in the exchanger and arrests vaporization until the liquid flashes into the separator. Although this will recover a solvent, it will not separate solvents if two or more are present.

A further technique is available to handle an airstream that carries solvents. By chilling the air by means of vent condensers or refrigeration equipment, the solvents can be removed from the condenser. Solvents also can be recovered by using extraction, adsorption, absorption, and distillation methods.

Solvent Extraction

Essentially a liquid/liquid process where one liquid is used to extract another from a secondary stream, solvent extraction generally is performed in a column somewhat similar to a normal distillation column. The primary difference is that the process involves two liquids instead of liquid and vapor.

During the process, the lighter (ie, less dense) liquid is charged to the base of the column and rises through packing or trays while the more dense liquid descends. Mass transfer occurs, and a component is extracted from one stream and passed to the other. Liquid/liquid extraction sometimes is used when the breaking of an azeotrope is difficult or impossible by distillation techniques.

Carbon Adsorption

The carbon adsorption technique is used primarily to recover solvents from dilute air or gas streams. In principle, a solvent-ladened airstream is passed over activated carbon and the solvent is adsorbed into the carbon bed. When the bed becomes saturated, steam is used to desorb the solvent and carry it to a condenser. In such cases as toluene, for example, recovery of the solvent can be achieved simply by decanting the water/solvent two-phase mixture that forms in the condensate. Carbon adsorption beds normally are used in pairs so that the airflow can be diverted to the secondary bed when required.

On occasion, the condensate is in the form of a moderately dilute miscible mixture. The solvent then has to be recovered by distillation. This would apply especially to ethyl alcohol, acetone-type solvents.

Absorption

When carbon adsorption cannot be used because certain solvents either poison the activated carbon bed or create so much heat that the bed can ignite, absorption is an alternative technique. Solvent is recovered by pumping the solvent-ladened airstream through a column countercurrently to a water stream that absorbs the solvent. The air from the top of the column essentially is solvent-free whereas the dilute water/solvent stream discharged from the column bottom usually is concentrated in a distillation column. Absorption also can be applied in cases where an oil rather than water is used to absorb certain organic solvents from an airstream.

Azeotropes

During distillation, some components form an azeotrope at a certain stage of the fractionation and require a third component to break the azeotrope and achieve a higher percentage of concentration. In the case of ethyl alcohol and water, for example, a boiling mixture containing less than 96% by weight ethyl alcohol produces a vapor richer in alcohol than in water and is readily distilled. At the 96% by weight point, however, the ethyl alcohol composition in the vapor remains constant, ie, the same composition as the boiling liquid. This is known as the azeotrope composition. Further concentration requires use of a process known as azeotropic distillation. Other common fluid mixtures that form azeotropes are formic acid/water, isopropyl alcohol/water, and isobutanol/water.

Azeotropic Distillation

In a typical azeotropic distillation procedure, a third component such as benzene, isopropyl ether, or cyclohexane is added to an azeotropic mixture such as ethyl alcohol/water to form a ternary azeotrope. Because the ternary azeotrope is richer in water than the binary ethyl alcohol/water azeotrope, water is carried over the top of the column. The azeotrope, when condensed, forms two phases. The organic phase is refluxed to the column whereas the aqueous phase is discharged to a third column for recovery of the entraining agent.

Certain azeotropes such as the *n*-butanol/water mixture can be separated in a two-column system without the use of a third component. When condensed and decanted, this type of azeotrope forms two phases. The organic phase is fed back to the primary column, and the butanol is recovered from the bottom of the still. The aqueous phase, meanwhile, is charged to the second column, with the water being taken from the column bottom. The vapor from top of both columns is condensed, and the condensate is run to a common decanter (Fig. 1).

Extractive Distillation

This technique is somewhat similar to azeotropic distillation in that it is designed to perform the same type of task. In azeotropic distillation, the azeotrope is broken by carrying over a ternary azeotrope at the top of the column. In extractive distillation, a very high boiling compound is added and the solvent is removed at the base of the column.

Stripping

In distillation terminology, stripping refers to the recovery of a volatile component from a less volatile substance. Again, referring to the ethyl alcohol/water system, stripping is done in the first column below the feed point where the alcohol enters at about 10% by weight and the resulting liquid from the column base contains less than 0.02% alcohol by weight. This is known as the stripping section of the column. This technique does not increase the con-

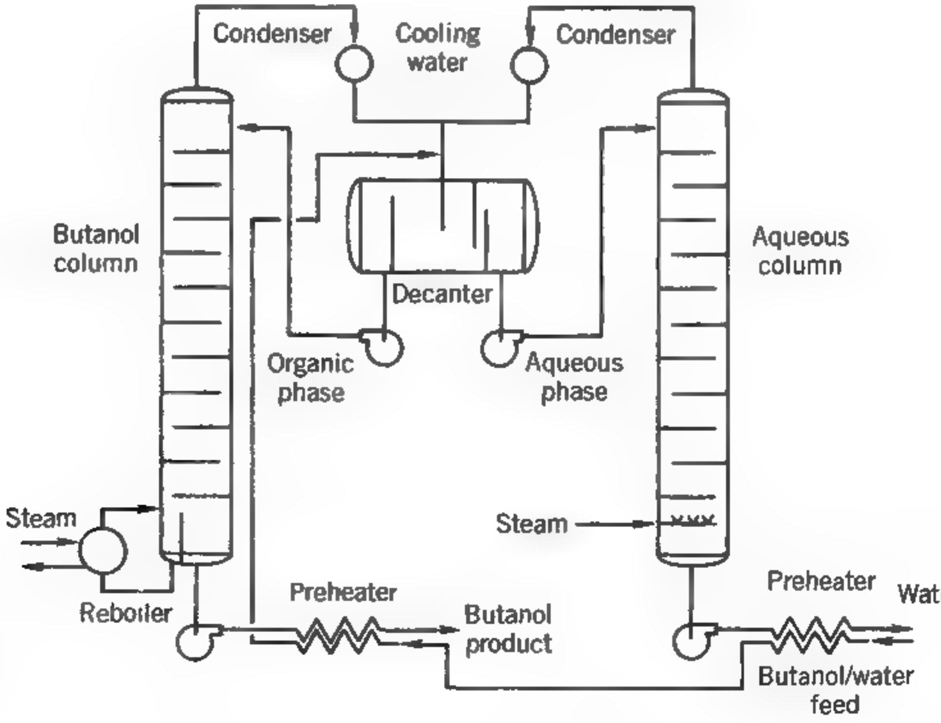


Figure 1. System for recovering butanol from butanol/water mixture.

centration of the more volatile component but rather, decreases its concentration in the less volatile component.

A stripping column also can be used when a liquid such as water contaminated by toluene cannot be discharged to a sewer. For this pure stripping duty, the toluene is removed within the column while vapor from the top is decanted for residual toluene recovery and refluxing of the aqueous phase.

Rectification

For rectification or concentration of the more volatile component, a top section of column above the feed point is required. By means of a series of trays and reflux back to the top of the column, a solvent such as ethyl alcohol can be concentrated to more than 95% by weight.

Batch Distillation

When particularly complex or small operations require recovery of the more volatile component, there are batch-distillation systems of various capacities. Essentially a rectification-type process, batch distillation involves pumping a batch of liquid feed into a tank where boiling occurs. Vapor rising through a column above the tank combines with reflux coming down the column to effect concentration. This approach is not very effective for purifying the less-volatile component.

For many applications, batch distillation requires considerable operator intervention or, alternatively, a significant amount of control instrumentation. Although it is more energy intensive than a continuous system, steam costs generally are less significant on a small operation. Furthermore, it is highly flexible and a single batch column can be used to recover many different solvents.

Continuous Distillation

The most common form of distillation used by the chemical, petroleum, and petrochemical industries is the continuous-mode system. In continuous distillation, feed constantly is charged to the column at a point between the top and bottom trays. The section above the feed point rectifies the more volatile component while the column section below the feed point strips out the more volatile component from the less volatile liquid. In order to separate N components with continuous distillation, a minimum of $N - 1$ distillation columns is required.

Turndown

The turndown ratio of a column is an indication of the operating flexibility. If a column, for example, has a turndown ratio of 3, it means that the column can be operated efficiently at 33% of the design maximum throughput.

SYSTEM COMPONENTS: COLUMNS, INTERNALS, INSTRUMENTATION, AND AUXILIARY EQUIPMENT

The following briefly defines the many components required for a distillation system and the many variations in components that are available to meet different process conditions.

Column Shells

A distillation column shell can be designed for use as a free-standing module or for installation within a supporting steel structure. Generally speaking, unless a column is of very small diameter, a self-supporting column is more economical. This holds true even under extreme seismic 3 conditions.

There are distillation columns built of carbon steel, 304 stainless steel, 316 stainless steel, Monel, titanium, and Incoloy 825. Usually, it is more economical to fabricate columns in a single piece without shell flanges. This technique not only simplifies installation but also eliminates danger of leakage during operation. Columns more than 80 feet long have been shipped by road without transit problems.

Although columns of more than 3-ft diameter normally have been transported without trays to prevent dislodgement and possible damage, recent and more economical techniques have been devised for factory installation of trays with the tray manways omitted. After the column has been erected, manways are added and, at the same time, the fitter inspects each tray.

With packed columns of 20-in. diameter or less that use high-efficiency metal mesh packing, the packing can be installed before shipment. Job-site packing, however, is the norm for larger columns. This prevents packing from bedding down during transit and leaving voids that would reduce operating efficiency. Random packing always is installed after delivery except for those rare occasions when a column can be shipped in a vertical position. Access platforms and interconnecting ladders designed to Occupational Safety and Health Administration (OSHA) standards also are supplied for on-site attachment to free-standing columns.

Installation usually is simple because columns are fitted with lifting lugs and, at the fabrication stage, a template is drilled to match support holes in the column base ring. With these exact template dimensions, supporting bolts can be preset for quick and accurate coupling as the column is lowered into place.

Column Internals

During recent years, the development of sophisticated computer programs and new materials has led to many innovations in the design of trays and packings for more efficient operation of distillation columns. In designing systems for chemical, petroleum, and petrochemical use, full advantage can be taken of available internals to assure optimum distillation performance.

Tray Devices

Although there are perhaps five basic distillation trays suitable for industrial use, there are many design variations of differing degrees of importance and a confusing array of trade names applied to their products by tray manufacturers. The most modern and commonly used devices are sieve, dual-flow, valve, bubble-cap, and baffle trays—each with its advantages and preferred usage. Of these, the sieve- and valve-type trays currently are most often specified.

For a better understanding of tray design, Figure 2 defines and locates typical tray components. The material of construction usually is 14 gauge, with modern trays adopting the integral truss design, which simplifies fabrication. A typical truss tray is shown in Figure 3. For columns less than 3 ft in diameter, it is not possible to assemble the truss trays in the column. Trays therefore must be preassembled on rods into a cartridge section for loading into the column. Figure 4 shows this arrangement in scale-model size.

The hydraulic design of a tray is a most important factor. The upper operating limit generally is governed by the flood point, although, in some cases, entrainment also can restrict performance. By forcing some liquid to flow back up the column, entrainment reduces concentration gradients and lowers efficiency. A column also can flood by downcomer backup when tray design provides insufficient down-comer area or when the pressure drop across the tray is high. When the down-comer is unable to handle all the liquid involved, the trays start to fill and pressures increase. This also can occur when a highly foaming liquid is involved. Flooding associated with high tray pressure drops and small tray spacing takes place when the required liquid seal is higher than the tray spacing. Downcomer design also is particularly important at high operating pressures due to a reduction in the difference between vapor and liquid densities.

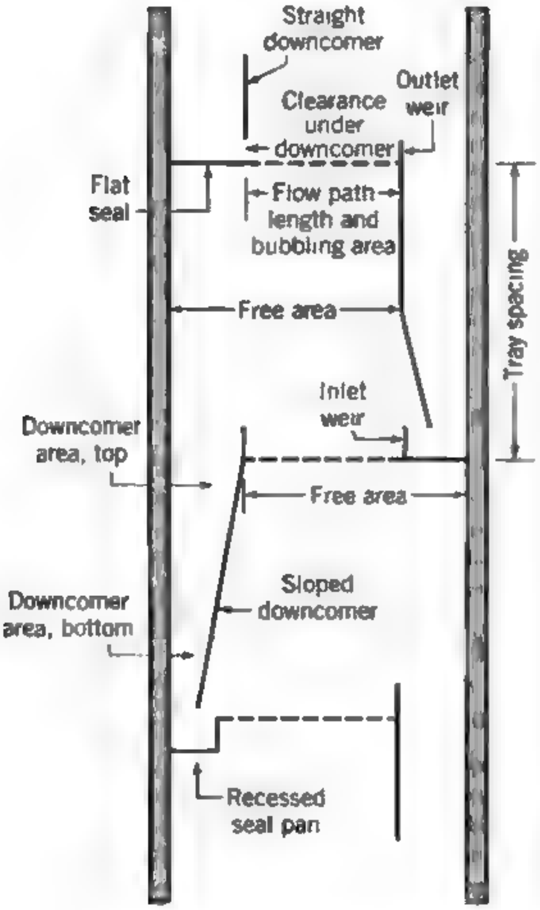


Figure 2. Tray component terminology.

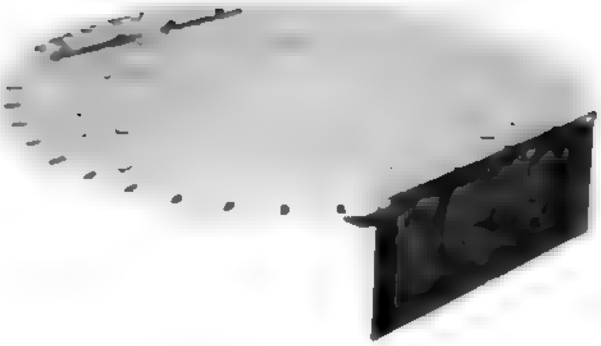


Figure 3. Typical tray of integral truss design.

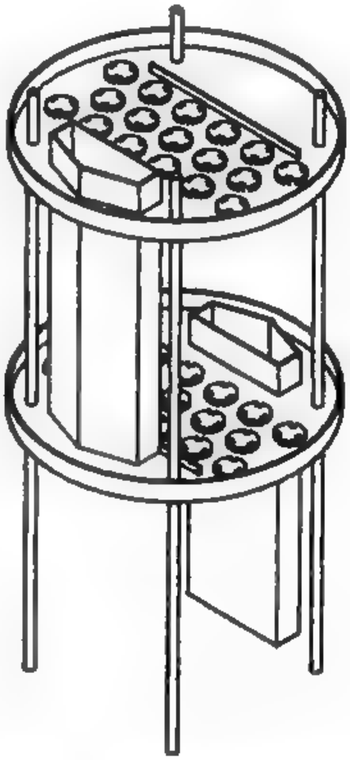


Figure 4. Cartridge tray assembly.

The lower limit of tray operation, meanwhile, is influenced by the amount of liquid weeping from one tray to the next. Unlike the upward force of entrainment, weeping liquid flows in the normal direction and considerable amounts can be tolerated before column efficiency is significantly affected. As the vapor rate decreases, however, a point eventually is reached when all the liquid is weeping and there is no liquid seal on the tray. This is known as the dump point, below which there is a severe drop in efficiency.

Sieve Tray. The sieve tray is a low-cost device that consists of a perforated plate that usually has holes of 1/16 in. to 1 in. diameter, a down-comer, and an outlet weir. Although inexpensive, a correctly designed sieve tray can be comparable to other styles in vapor and liquid capacities, pressure drop, and efficiency. For flexibility, however, it is inferior to valve and bubble-cap trays and sometimes is unacceptable for low liquid loads when weeping has to be minimized.

Depending on process conditions and allowable pressure drop, the turndown ratio of a sieve tray can vary from 1.5 to 3 and occasionally higher. Ratios of 5, as sometimes claimed, can be achieved only when the tray spacing is large, available pressure drop is very high, liquid loadings are high, and the system is nonfoaming. For many applications, a turndown of 1.5 is acceptable.

It also is possible to increase the flexibility of a sieve tray for occasional low throughput operation by maintaining a high reboil and increasing the reflux ratio. This may be economically desirable when the low throughput occurs for a small fraction of the operating time. Flexibility, likewise, can be increased by the use of blanking plates to reduce the hole area. This is particularly useful for initial operation when it is proposed to increase the plant capacity after a few years. There is no evidence to suggest that blanked-off plates have inferior performance to unblanked plates of similar hole area.

Dual-Flow Tray. The dual-flow tray is a high hole areas sieve tray without a down-comer. The liquid passes down through the same holes through which the vapor rises. Because no down-comer is used, the cost of the tray is lower than that of a conventional sieve tray.

In recent years, use of the dual-flow tray has declined somewhat because of difficulties experienced with partial liquid/vapor bypassing of the two phases, particularly in larger diameter columns. The dual-flow column also has a very restricted operating range and a reduced efficiency because there is no cross flow of liquid.

Valve Tray. Although the valve tray dates back to the rivet type first used in 1922, many design improvements and innumerable valve types have been introduced in recent years. A selection of modern valves as illustrated provide the following advantages (Fig. 5):

1. Throughputs and efficiencies can be as high as sieve or bubble-cap trays.
2. Very high flexibility can be achieved and turndown ratios of 4 to 1 are easily obtained without having to resort to large pressure drops at the high end of the operating range.
3. Special valve designs with venturi-shaped orifices are available for duties involving low pressure drops.
4. Although slightly more expensive than sieve trays, the valve tray is very economical in view of its numerous advantages.
5. Because an operating valve is continuously in movement, the valve tray can be used for light-to-moderate fouling duties. Valve trays can be successfully used on brewery effluent containing waste beer, yeast, and other materials with fouling tendencies.

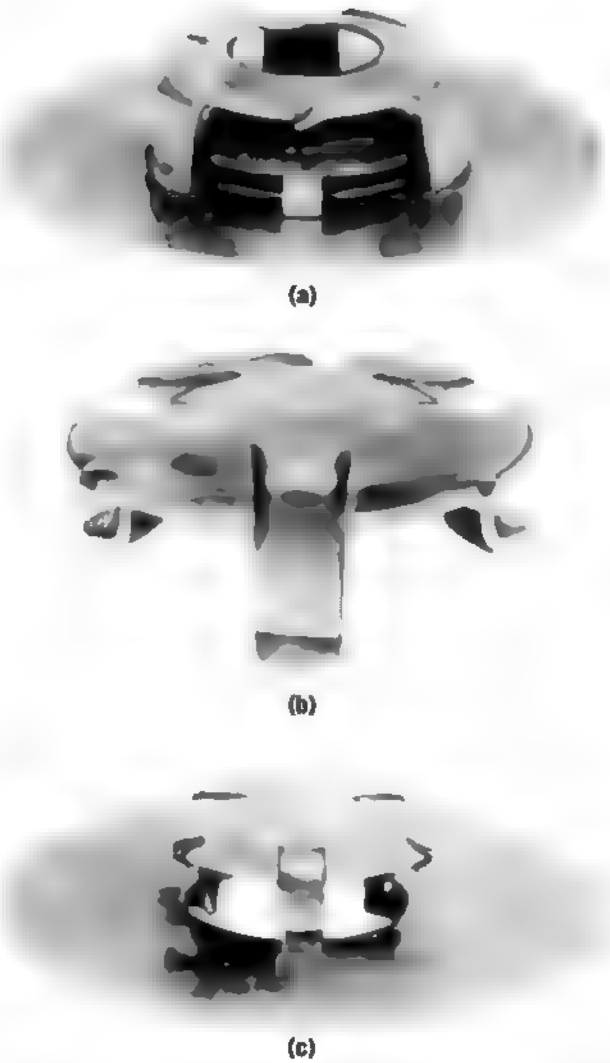


Figure 5. (a) Special two-stage valve with lightweight orifice cover for complete closing; (b) and (c) two typical general purpose valves which may be used in all types of services.

Bubble-Cap Tray. Although many bubble-cap columns still are in operation, bubble-cap trays rarely are specified today because of high cost factors and the excellent performance of the modern valve-type tray. The bubble cap, however, does have a good turndown ratio and is good for low liquid loads.

Baffle Tray. Baffle trays are arranged in a tower in such a manner that the liquid flows down the column by splashing from one baffle to the next lower baffle. The ascending gas or vapor, meanwhile, passes through this curtain of liquid spray.

Although the baffle-type tray has a low efficiency, it can be useful in applications where the liquid contains a high fraction of solids.

Packings

For many types of duties, particularly those involving small-diameter columns, packing is the most economical tower internal. One advantage is that most packing can be purchased from stock on a cubic-foot basis. In addition, the mechanical design and fabrication of a packed column is quite simple. Disadvantages of packing include its unsuitability for fouling duties, breakage of ceramic packing, and, according to some reports, less predictive performance, particularly at low liquid loads or high column diameters.

The most widely used packing is the random packing, usually Rashig rings, Pall rings, and ceramic saddles. These are available in various plastics, a number of different metals and, with the exception of Pall rings, in ceramic materials. Although packings in plastic have the advantage of corrosion resistance, the self-wetting ability of some plastic packing, such as fluorocarbon polymers, sometimes is poor, particularly in aqueous systems. This considerably increases the HETP when compared with equivalent ceramic rings.

High-efficiency metal mesh packing as shown in Figure 6 has found increasing favor in industry during recent years. One type uses a woven wire mesh that becomes self-wetting because of capillary forces. This helps establish good liquid distribution as the liquid flows through the packing in a zig-zag pattern.

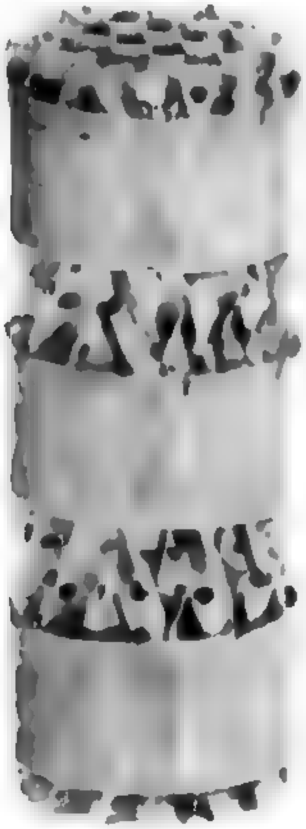


Figure 6. Segment of high-efficiency metal mesh packing.

If properly used, high efficiency packings can provide HETP values in the range 6–12 in. This can reduce column heights, especially when a large number of trays is required. Such packings, however, are very expensive, and each application must be studied in great detail.

With random and, in particular, high-efficiency packing, considerable attention must be given to correct liquid distribution. Certain types of high-efficiency packing are extremely sensitive to liquid distribution and should not be used in columns more than 2 ft in diameter. Positioning of these devices and the design of liquid distribution and redistribution are important factors that should be determined only by experts.

Instrumentation

One of the most important aspects of any distillation system is the ability to maintain the correct compositions from the columns by means of proper controls and instrumentation. Although manual controls can be supplied, this approach rarely is used today in the United States. Manual control involves the extensive use of rotameters and thermometers, which, in turn, involves high labor costs, possible energy wastage, and, at times, poor quality control. Far better control is obtained through the use of pneumatic or electronic control systems.

Pneumatic Control Systems. The most common form of distillation column instrumentation is the pneumatic-type analogue control system. Pneumatic instruments have the advantage of being less expensive than other types and, because there are no electrical signals required, there is no risk of an electrical spark. One disadvantage is the need to ensure that the air supply has a very low dew point (usually $-40°F$) to prevent condensation in the loops.

Electronic Control Systems. Essentially, there are three types of electronic control systems:

1. Conventional electronic instruments.
2. Electronic systems with all field devices explosion proof.
3. Intrinsically safe electronic systems.

The need to have a clear understanding of the differences is important. Most distillation duties involve at least one flammable liquid that is being processed in both the vapor and liquid phases. Because there always is the possibility of a leak of liquid or vapor, particularly from pump seals, it is essential for complete safety that there be no source of ignition in the vicinity of the equipment. Although many instruments, such as controllers and alarms, can be located in a control room removed from the process, all local electronic instruments must be either explosion proof or intrinsically safe.

With explosion-proof equipment, electrical devices and wiring are protected by boxes or conduit that will contain any explosion that may occur. With intrinsically safe equipment, barriers limit the transmission of electrical energy to such a low level that it is not possible to generate a spark. As explosion-proof boxes and conduits are not required, wiring costs are reduced.

For any intrinsically safe system to be accepted for insurance purposes, FM (Factory Mutual) or CSA (Canadian Standards Association) approval usually must be obtained. This approval applies to a combination of barriers and field devices. Therefore, when a loop incorporates such instruments from different manufacturers, it is essential to ensure that approval has been obtained for the combination of instruments.

Auxiliary Equipment

In any distillation system, the design of auxiliary equipment such as the reboiler, condenser, preheaters, and product coolers is as important as the design of the column itself.

Reboiler. Although there are many types of reboilers, the shell-and-tube thermosyphon reboiler is used most frequently. Boiling within the vertical tubes of the exchanger produces liquid circulation and eliminates the need for a pump. A typical arrangement is shown in Figure 7.

For certain duties, particularly when the bottoms liquid has a tendency to foul heat transfer surfaces, it is desirable to pump the liquid around the heat exchanger. Because boiling can be suppressed by use of an orifice plate at the outlet of the unit, fouling is reduced. The liquid being pumped is heated under pressure and then is flashed into the base of the column where vapor is generated.

An alternative approach is the use of a plate heat exchanger as a forced-circulation reboiler. With this technique, the very high liquid turbulent flow that is induced within the heat exchanger through the use of multiple corrugated plates holds fouling to a minimum. Meanwhile, the superior rates of heat transfer that are achieved reduce the surface area required for the reboiler.

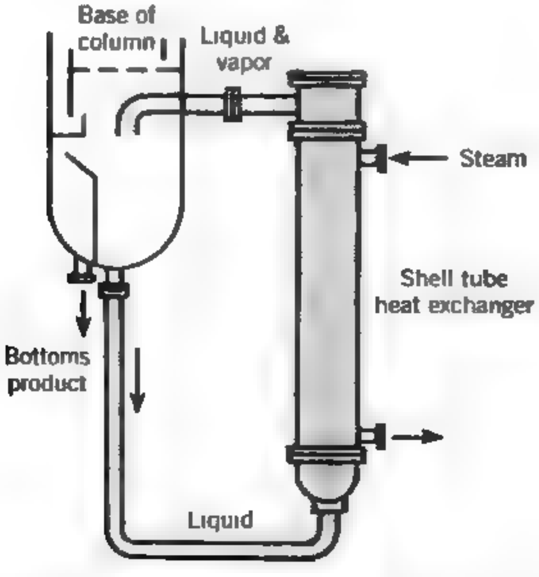


Figure 7. Typical shell and tube thermosyphon reboiler arrangement.

Condensers. Because most distillation column condensers are of shell-and-tube design, the processor has the option of condensing on either the shell or tube side. From the process point of view, condensation on the shell side is preferred because there is less subcooling of condensate and a lower pressure drop is required. These are important factors in vacuum duties. Furthermore, with cooling water on the tube side, any fouling can be removed more easily.

Tube-side condensation, on the other hand, can be more advantageous whenever process fluid characteristics dictate the use of more expensive, exotic materials. Capital cost of the unit then may be cut by using a carbon steel shell.

Preheaters/Coolers. The degree to which fluids are aggressive to metals and gasketing materials generally determines the selection of plate or shell-and-tube preheaters and product coolers. If fluids are not overly aggressive toward gasket materials, a plate heat exchanger is an extremely efficient preheater because a very close temperature approach may be achieved. Added economy is realized by using heat from the top and bottoms product for all necessary preheating.

Very aggressive duties normally are handled in a number of tubular exchangers arranged in series to generate a good mean temperature difference. The use of multiple tubular units obviously is more expensive than a single plate heat exchanger but is unavoidable for certain solutions such as aromatic compounds.

Vent Condenser. It is normal practice on distillation systems to use a vent condenser after the main condenser to minimize the amount of volatiles being driven off into the atmosphere. Usually of the shell-and-tube type, the vent condenser will have about one-tenth the area of the main unit and will use a chilled water supply to cool the noncondensible gases to about 45–50°F.

Pumps. Because most distillation duties involve fluids that are highly flammable and have a low flash point, it is essential that explosion-proof (Class 1, Group D, Division 1) pump motors be supplied. Centrifugal pumps generally are specified as they are reliable and can provide the necessary head and volumetric capacity at moderate costs.

PACKAGED DISTILLATION SYSTEMS

For distillation systems of moderate size, it often can be economical to fabricate and supply columns, heat exchangers, tanks, pumps, and other elements as a fully preassembled package. This technique was used for the distillation unit as pictured in Figure 8 during fabrication as a complete, ready to transport system. For this particular project, which was designed to separate both ethyl alcohol and isopropyl alcohol from water, three columns of relatively small diameter were positioned with associated components and instrumentation on a prefabricated structure. Although insulation normally would not be supplied in order to preclude the possibility of damage during shipment, major elements of this project were insulated at the cus-

Figure 8. Packaged distillation system during fabrication.

tomer's request and were trucked 600 miles to the job site without problems. Final size of the package was 65′ × 12′ × 8′.

The packaged approach proved to be beneficial in a number of ways. Factory fabrication and installation of piping was far more economical than field finishing, while erection time for the system was reduced considerably with significant savings in local labor costs.

An alternative approach for larger systems is the fabrication of equipment modules. For one large batch-distillation unit, the column itself can be supplied as one module, the batch tank and reboiler as the second, and the heat exchangers, decanter, pumps, and auxiliary items as the third. This modular construction before shipment was successful despite the fact that the batch tank was more than 8 ft in diameter.

For systems involving columns in excess of 6-ft diameter, prepackaging generally is not feasible. Components of such systems normally have to be installed and piped at the job site.

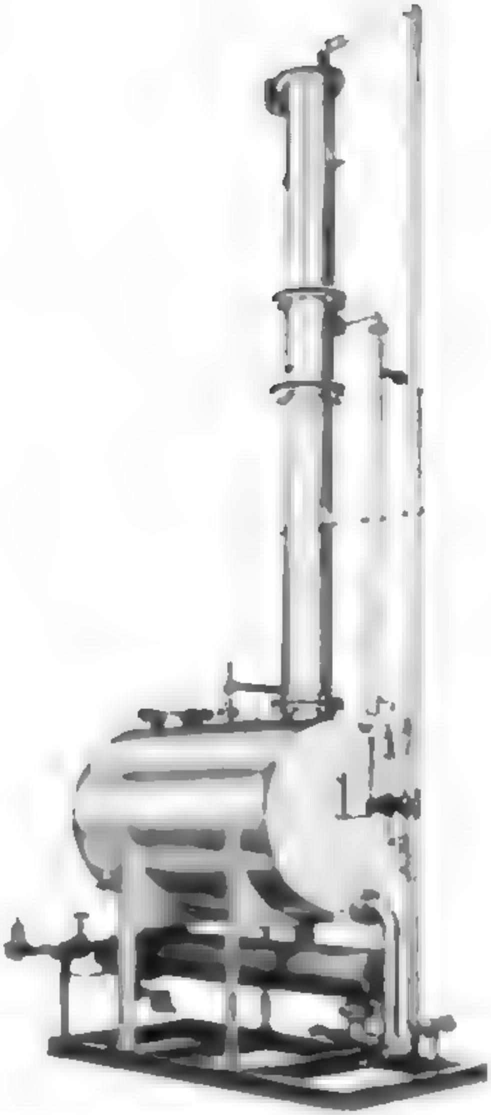


Figure 9. Batch distillation/solvent recovery silhoutte mask.

BATCH DISTILLATION

With process plants becoming increasingly larger, there has been a tendency for the chemical and petroleum industries to focus attention on the use of continuous distillation because this approach becomes progressively more economical as the scale of operation grows. As a result, batch distillation has become a somewhat neglected unit operation. There are, however, many areas where batch distillation can be used more economically than continuous. Batch sizes are available from about 300 to more than 5000 gal. A 300 gal unit is shown in Figure 9.

Advantages

The main advantage of batch distillation is its flexibility. A single unit can, by changing reflux ratios and boil-up rates, be used for many different systems. It also is possible to separate more than two components in the same column, whereas with continuous distillation, at least $N - 1$ separate columns are required to separate N components.

A batch distillation process is very simple to control, and there is no need to balance the feed and draw-off, as is the case with the continuous distillation approach.

Batch distillation is particularly useful when applied to feeds containing residues that have a tendency to foul surfaces as these residues remain in the still and cannot contaminate the rectification column internals. For aqueous systems-handling materials that leave very heavy fouling, it is possible to heat with direct steam injection into the still and thus alleviate problems of buildup on the heat transfer surfaces.

COLUMN DESIGN

Before designing a batch column, it obviously is desirable to have as much detailed information on the system as is possible. Data on vapor liquid equilibria (VLE), vapor and liquid densities, liquid viscosity, and the boiling temperatures of the components are essential if a column is to be properly designed. Failure to have accurate VLE data means that it is necessary to run a small-scale experiment in order to characterize the system. In addition, the customer must identify the product feed composition, the required composition of the residue and distillate, the batch size, and the batch time. Having acquired this data, the vendor finally can commence design work.

To illustrate the design principles, it is useful to study the case of binary mixtures. A typical system is shown in Figure 10. Once the VLE data have been established, it becomes a straightforward task to calculate the number of theoretical stages and reflux ratios.

There are two main techniques for operating a batch column. One is to work with constant reflux ratio during the complete run. The effect of this method is charted in Figure 11. As the composition of the more volatile component (MVC) in the still, x_w, decreases, the fraction of MVC in the top product decreases. For example, to obtain a set composition of 90% in the total amount of top product collected, it will always be necessary to collect initially at a higher composition of about 95% to compensate for a composition below specification at the end of the run. The advantage of constant reflux is that control and operation are very simple.

The second method is to increase the reflux ratio during the run in order to maintain a steady top-product composition. This is shown in Figure 12, where the increase in the slope of the operating line is obtained by increasing the reflux ratio. The gradient of the operating line (L/V) is obtained from the enrichment equation given below.

$$Y_n = \frac{L}{V} x_{n+1} + \frac{D}{V_n} x_d$$

This equation assumes constant molal overflow. Y_n is the mole fraction of the MVC in the vapor leaving the nth stage, x_{n+1} is the mole fraction of the MVC in the liquid arriving at the nth stage, x_d is the mole fraction of the MVC in the top product, D is the molal flow rate of top product, V is the molal vapor flow rate in the column, and L is the molal liquid flow rate in the column The reflux ratio R is given by

$$R = \frac{L/V}{1 - (L/V)}$$

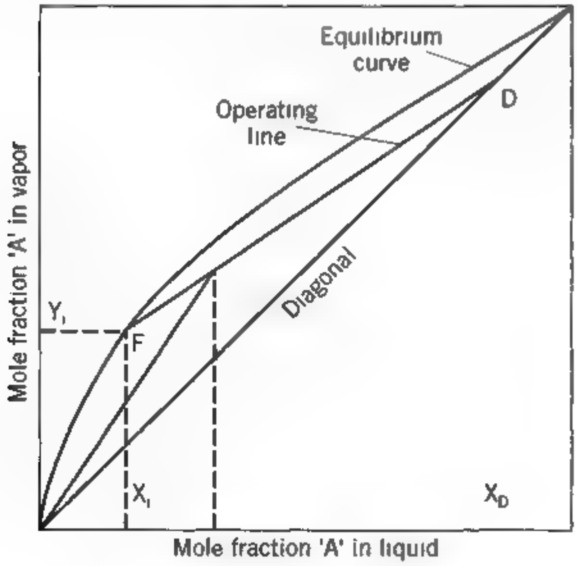


Figure 10. A typical system illustrating the design principles.

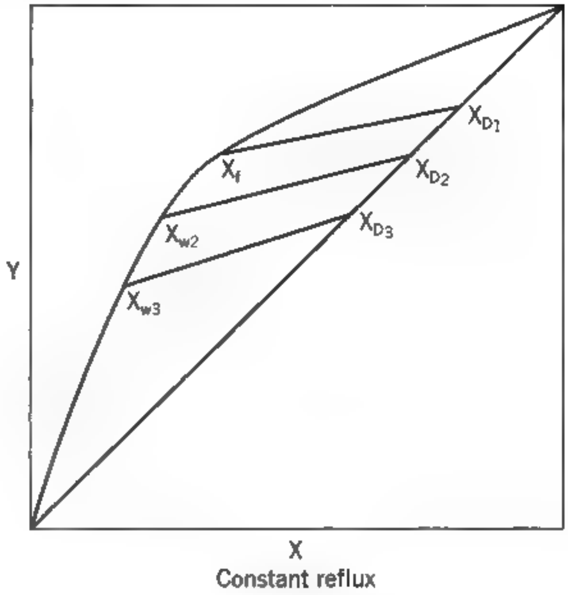


Figure 11. A chart illustrates the effect of the method of working with constant reflux ratio during the complete run. One of two

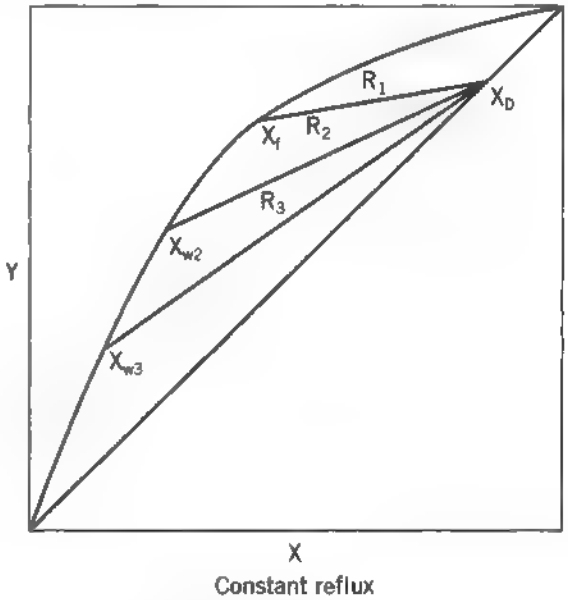


Figure 12. The second method is to increase the reflux ratio during the run in order to maintain a steady top product composition.

With the VLE data and the top and bottom compositions, it then is possible to calculate graphically by the

Table 1. Computer Program Modes

Mode	Specified	Program calculation
1	Top-product and still composition	Minimum number of theoretical stages Minimum reflux ratio
2	Top-product still composition, reflux ratio	Number of theoretical stages
3	Top-product and still composition Number of theoretical stages	Reflux ratio
4	Top-product composition required Initial and final still composition	Reflux ratio at stat and end of batch
	Still charge Batch time Number of theoretical stages	Boiling rate Product quality
5	Average top-product composition Initial and final still composition Still charge	Reflux ratio required constant throughout batch
	Batch time Number of theoretical stages	Boil-up rate Product quality

McCabe and Thiele procedure the minimum reflux ratio, minimum number of theoretical stages, and other such parameters. In batch distillation in particular, these procedures are tedious and time-consuming because, of course, the composition of the liquid in the still changes with time and it is necessary to repeat the calculations many times. Obviously, the procedures become even more time-consuming with multicomponent systems.

For this type of design work, computer programs can be used to enable the engineer to produce an efficient design very quickly. One program is extremely flexible and operates in a different series of modes, as is charted in Table 1. Naturally, the VLE data have to be specified for all modes. The program further assists in the determination of the number of theoretical stages. Other modes also are available to provide different permutations of operation.

To help determine the sizing of the column diameter, various programs can be used to incorporate different proprietary methods, including those of Fractionation Research Inc. (FRI) of California.

Column Internals

The choice of internals for the column depends mainly on the product being processed and the size of the column to be used. To meet virtually every parameter, packed columns as well as sieve, bubble, and ballast trays are available. As a general rule, sieve trays are not used frequently in batch columns because the turndown ratio of most trays of this type is only about 1.5 to 1. This reduces one of the main advantages of batch columns, mainly flexibility. Usually, small batch columns are packed because the efficiency of trays of less than 2-ft diameter often decreases rapidly. Ballast trays, although expensive, are often used for larger columns because they are efficient and have turndown ratios of up to 9 to 1.

Control

The control of batch columns is very simple, and, therefore, required instrumentation usually is quite inexpensive. Constant reflux operation can be handled by a ratio controller or by using a timed deflection of condensate. For operation with variable reflux, the control of the reflux ratio must be tied to some property of the top product that undergoes a sufficiently large change in value for change in composition. Manual control of this type of reflux operation is not feasible.

Numerous batch distillation systems have been supplied to customers who require the separation of many different components. To simplify the operation of these systems, there are proprietary computers specifically designed for process control. For example, use of certain microprocessors enables the operator to switch from one type of separation to another without having to make many manual adjustments on the control panel. In addition, the computer can be programmed to automatically take care of all necessary tails cuts as well as to clean the system during interims between the various separations.

Batch Systems/Continuous Distillation

In a number of duties, it is essential that the base product contain only very small amounts of one component. For example, in the recovery of solvents from water, it is mandated that the water may retain only trace quantities of solvents before being pumped to waste. Because one disadvantage of a batch system is that there is no stripping section, it is necessary to boil the batch pot for relatively long periods of time in order to reduce the residual solvent to trace quantities.

To resolve this problem, a number of batch distillation systems with the capability of being operated in the continuous mode have been supplied. By incorporating one, two, or three feed points on the column, the feed can be pumped directly to the column instead of to the batch tank. Furthermore, a separate small holdup/reboiler is furnished in certain cases so that the batch tank and its associated reboiler need not be used for the continuous operation.

BIBLIOGRAPHY

This article was adapted from and is used with permission of A. Cooper, ***APV Distillation Handbook***, 3rd ed., Lake Mills, Wisc., 1987. Copyrighted APV Crepaco, Inc.

APV Crepaco, Inc.
Lake Mills, Wisconsin

DISTILLED BEVERAGE SPIRITS

HISTORY

The history of distilled spirits goes back into antiquity. Scientists have unearthed pottery in Mesopotamia depicting fermentation scenes dating back to 4200 B.C., a small wooden model of a brewery from about 2000 B.C. is on display at the Metropolitan Museum of Art in New York City, and Aristotle mentions a wine that produces a spirit. The first real distiller was probably a Greek-Egyptian alchemist who in the first or second century A.D., in an attempt to transmute base metal into gold, boiled some wine in a crude still. The discovery of ardent spirits that resulted from this effort was looked on with awe. It was kept a secret for centuries.

The technique of distillation probably came from the Egyptians who had been interested in alchemy since the pre-Christian era. At a later time the Arabians gained this knowledge from the Egyptians. Distillation was introduced into Western Europe either through Spain about A.D. 1150, or by the crusaders who learned about it from the Moslems in the 12th and 13th centuries. Distilled spirits were probably known in Ireland and Scotland before the 12th century, but actually it was not until then that there is a recorded history of distilled spirits in Europe. The first written evidence is in the description by Master Salernus who died in A.D. 1167. However, for another three centuries distilled spirits were regarded only as a rare and costly medicine called *aqua vitae*, "the water of life."

The first treatise on distillation was written by a French chemist, Arnold de Villeneuve, sometime before 1311, and was printed in 1478 in Venice. A Spaniard, Raymond Lully, was also instrumental in spreading the knowledge of distillation through Europe in the late 13th century. *Liber de Arte Distillandi*, by Hieronimus Brunswick, a well-known author of medical works, was printed in 1500 in Strasbourg. A more comprehensive book by Ryff, another medical author, appeared in 1556 in Frankfurt am Main. Both works contain elaborate chapters on herbs and their distillates with indications of their uses as medicines.

Monks had been producing fermented liquors on a substantial scale since A.D. 800, and were the first to practice the new art of distillation because churchmen were the only one capable of reading and understanding the treatises on distillation. Gradually the knowledge of *aqua vitae* spread widely, and during the 16th and 17th centuries the production of distilled spirits became a full-scale industry. The popularity of distilled spirits in Europe grew during this era and may best be traced through court records.

The word *whisky* comes from the Gaelic word *uisgebeatha*, or *usquebaugh*, as the Irish called it, meaning "the water of life." *Usquebaugh*, supposedly the Celtic form of whisky, was actually a cordial made with aniseed, cloves, nutmeg, ginger, caraway seeds, raisins, licorice, sugar, and saffron. The real whiskey of the Irish was called potheen, reputedly a formidable drink, full of heavy-bodied flavors resulting from simple distillations.

The early methods of distillation used the alembic, a simple closed container to which heat was applied. The vapors were transferred through a tube to a cooling chamber in which they were condensed. Alembics made of copper, iron, or tin were preferred. Other metals were said to have an adverse effect on the distillate. Occasionally, glass and potter's earth were used. From this developed stills consisting of clay or brick fireboxes into which the copper pots of the still were fitted. Direct heat was applied to the body or cucurbit containing the fermented mixture, and the vapors rose into the head, passed through a pipe (the crane neck) to the worm tub, where a copper coil was immersed in a barrel of cool water. Variations and improvements of this technique resulted in pot stills, some of which are still in use in the production of malt whiskies, notably in Scotland, and brandies, in France.

During the latter part of the 18th century, great strides were made in the development of distillation equipment, mostly in France. Because brandy (from fruit) was the principal distillate in France, the French had an advantage over the British and Germans, who were both hampered by the thick mashes obtained from grains. Argand invented the preheater, and in 1801, Edouard Adam designed the prototype of a charge still. He used egg-shaped vessels to hold the alcoholic liquid through which vapors from the kettle passed to the condenser. In the early 19th century several stills were patented in France and England, and in 1830 Aeness Coffey, in Dublin, developed his continuous still. The Coffey still (Fig. 1) is composed of two columns: in one, the fermented mash is stripped of its alcohol and, in the other, the vapors are rectified to a high proof (94–96%). Almost all of the fundamentals of distillation had been recognized and incorporated into the Coffey still. Later developments do not differ fundamentally from the stills of Cellier-Blumenthal and Coffey.

The early history of distillation in the United States is as poorly documented as it is elsewhere. No one knows who made the first rum, colonial New England's most important product; no one knows when or where the first rye whisky was made, or with any certainty who made the first bourbon in Kentucky. The Indians may possibly have known distillation. They had fermented drinks from maple syrup, corn, ground acorns, chestnuts, and chinquapins. Columbus reported an Indian drink made from the marrow of maguey. The fermented sap of maguey makes pulque, and distilled pulque becomes mezcal. Whether or not Columbus tasted mezcal is conjectural. However, the Spanish and Portugese brought the still.

The first recorded beverage spirits made from grains (corn and rye) were distilled on Staten Island in 1640 by William Kieft, the director general of the Dutch Colony of New Netherland. During the early colonial days, fermented beverages made from sugar-bearing fruits and vegetables were very popular. Pumpkins, maple sugar, parsnips, peaches, pears, apples, currants, grapes, and elderberries provided a ready source. The more aristocratic colonists preferred imported wines. However, in the 18th century colonial drinking customs changed to distilled drinks, especially rum. Rum was made in Barbados as early as 1650. In colonial America, the earliest reference is in the records of the General Court of Massachusetts in May 1657. By 1750, there were 63 distilleries. One of the first acts of the Continental Congress was to establish a

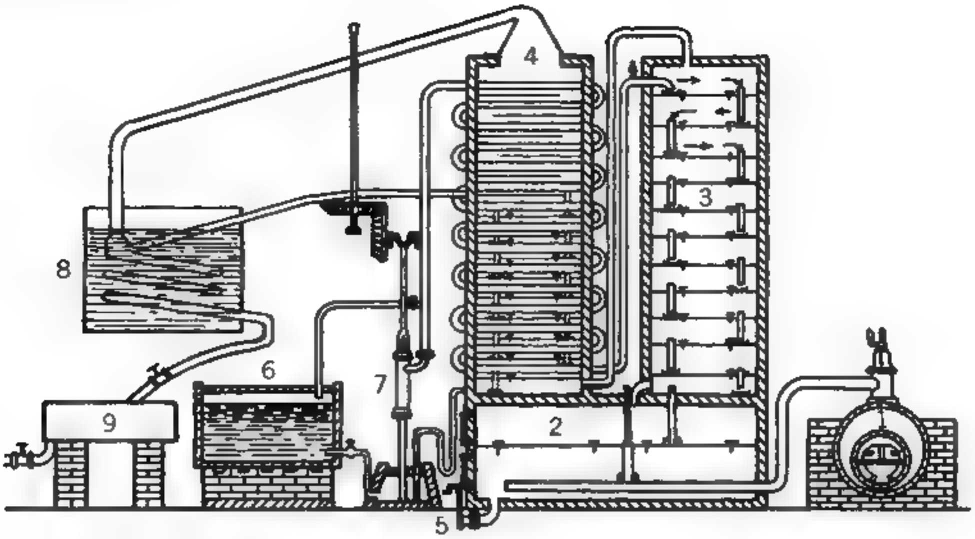


Figure 1. Coffey still 1, Boiler; 2, spent mash chamber; 3, stripping column (analyzer); 4, rectifying column; 5, residual mash (stillage) outlet; 6, fermented mash (feed); 7, feed pump; 8, condenser; 9, product tank.

ration of rum for soldiers and sailors. Brandy, which had been made in the colonies as early as 1650, and gin, which was popular with the poorer classes in England, never achieved the popularity of rum in this country. Nevertheless, rum was not a native drink, because the raw materials had to be imported. But rum starts with an inexpensive by-product of the sugar industry, molasses, and so could be the least-expensive distilled spirit. There are sufficient sugars left after most of the cane sugar has been removed to serve as the source of alcohol for rum.

With the decline of the three-cornered trade, the revolution, and the movement of settlers from the coastal areas inland, the rum industry slowly declined. Americans turned to rye, a successful crop in Pennsylvania and Maryland, and to corn, which grew in the South and West. George Washington is reported to have made rye whisky. His distillery had an excellent reputation for making fine liquor under the supervision of James Anderson, a Scotsman. In 1798, Washington's net profit was £83 with an inventory of 587 L (155 gal) still unsold.

Canada's first distillery produced rum from molasses in Quebec in 1769, but whisky did not develop until the 19th century. By 1850, there were some 200 distilleries operating in Ontario alone. Today there are only 27 of which 2, Joseph E. Seagram & Sons, Ltd., and Hiram Walker & Sons, Ltd., have been in business continuously since 1857 and 1858, respectively.

The Whisky Rebellion in 1791 resulted in the rise of Kentucky as the greatest whisky-producing state in the union. In 1810, Kentucky which had achieved statehood in 1792 with over 70,000 inhabitants, many of them Scotch-Irish and German farmer immigrants with distiller experience, had 2,000 stills out of 14,000 in the United States producing well over 7,500,000 L (2 million gal) of whisky annually. Many counties of Kentucky claim the first production of bourbon. However, it is generally agreed that the first bourbon whisky (genuine, old-fashioned, handmade, sour-mash bourbon) was made by a Baptist minister, the Reverend Elijah Craig, in 1789 at Georgetown in Scott County which was part of Bourbon County, one of the Virginia counties that made up the Kentucky territory, from which Craig's corn whisky got its name. In order to distinguish this corn whisky from Pennsylvania rye, it was called Kentucky bourbon, with a mashing formula of at least 51% corn grains. Until 1865, few Kentucky distilleries produced more than 160,000 L (1,000 bbl) per year. Most of them were small, with an annual production of only 8,000–80,000 L (50–500 bbl).

The method used in Kentucky of making whisky, with the exception of malt preparation, was in most respects similar to that used in Scotland and Ireland for hundreds of years. Ground corn and rye meal were scalded in tubs somewhat larger than barrels, stirred with paddles, and allowed to cool and sour overnight. Then malt made from rye, corn, or barley was added for conversion of the starch to a fermentable grain sugar. Yeast was added and the mash allowed to ferment for 72–96 h. A simple, single-chambered copper still was used to separate the spirits from the mash. When redistilled, the product was called double distilled. In 1819, New Orleans received some 750,000 L (200,000 gal) per month of this product. By the time it had floated down the Ohio and Mississippi on flatboats, the hot sun and boat movement had aged it.

Before the Civil War, not much attention was paid to aging, even though it was recognized that whisky left in charred oak barrels took on a golden color and some mellowness. Originally, whisky was sold in its natural white state or artificially colored to resemble the amber glint of brandy. No one bothered with brand names, whisky was whisky, as everyone knew, and not too much was made of the wide variation in palatability. The hunter or riverman who drank raw white whisky was not particular about quality.

After the Civil War, the rise in taxes made storage in bond desirable. Many family names, always important in Kentucky lore, now became associated with distinctive whiskies. As the industry grew, it engaged in bitter controversy over what was whisky. As a result, in 1909 during President Taft's administration, whisky was finally defined as any volatile liquor distilled from grain, and standards of identity based on current manufacturing processes for various whisky types, such as rye and bourbon, were established (1). In 1906, the Pure Food and Drug Act was passed requiring a statement of manufacturing process and materials on the label. By 1920 when prohibition began, blended whiskies comprised 70% of the whisky market.

Prohibition brought with it evils that were greater than those it was designed to prevent. During Prohibition, consumption of spirits increased from 530 to 750 million L (140 to 200 million gal) annually. In 1930, the Prohibition Enforcement Bureau estimated that the production of moonshine was more than 3 billion L (800 million gal) per year. In December 1933, Prohibition was repealed and the distilling industry began to catch up with developments in bacteriology, chemistry, engineering, and sanitation. Many of the small producers were absorbed by the major organizations, and capital was provided for new equipment and inventory.

Per capita consumption continued to decline; it was 10.26 L (2.71 gal) in 1864, 6.47 L (1.71 gal) in 1922–1930, and only 4.96 L (1.31 gal) in 1960. However, the growth in population accounted for an increase in total consumption from 550 million L (145 million wine gal) in 1940 to over 1.6 billion L (420 million gal) in 1976. In World War II the industry offered its facilities to the government for war production, even before the United States entered the war. From Pearl Harbor to V-J Day, almost 3 billion L (750 million gal) of 190° proof (95%) alcohol was produced for the war effort in 127 distilling plants.

TAXATION, GOVERNMENT REGULATIONS

The distilling industry has always been taxed heavily, not only in the United States, but throughout the world. On the one hand, governments have laid an unduly heavy burden on the legal producers of distilled beverage spirits. On the other hand, dishonest men have evaded legal obligations and brought the industry into disrepute. Moreover, in many instances, dry interests have attempted to further their cause by advocating high taxes. For example, in England, by 1730, the laws were so complicated and onerous that they all but destroyed the industry, and in 1743 Parliament completely revised the regulations. Whenever taxes were too high, illicit distilling flourished.

Alcoholic beverages were subject to regulations as early as the Babylonian Code of Hammurabi, ca 2000 B.C., which contained provisions for the quality, sale, and use of fermented liquors. In the Magna Carta, a clause provided a standard of measurement for the sale of ale and wine. With the increase in consumption of distilled spirits in Europe, governments became increasingly interested in the tax revenues, and in 1643, Parliament imposed the first tax on distilled spirits.

The first liquor tax in the United States (2 guilders on each half-vat of beer) was imposed in 1640 by William Kieft, director general of New Netherland. The Molasses Act of 1733 and the Sugar Act of 1763, imposing a heavy tax on French and Dutch rum and molasses and leaving the more expensive British products free, provoked protest and action against its enforcement.

In 1791, Alexander Hamilton, in testing the strength of the new federal government, imposed an excise tax to be collected by revenue officers assigned to each district. This tax was fiercely contested and was repealed in 1800 during Jefferson's administration and, except for the years between 1812 and 1817, as a war measure, there was no further excise tax on domestic beverage spirits until 1862.

In 1975, the combined federal excise tax and the average state tax (32 licensed states) amounted to $13.13 per proof gal; federal taxes alone, $10.50 per proof gal. Table 1 lists the federal excise tax rates for various years. The federal excise tax was raised in 1991 to $13.50 per proof gal, ie, a gallon of liquid containing 50% by volume of ethyl alcohol (100° proof). Prior to that, and since 1985, it had been $12.50. per proof gal.

Even though the metric system for containers has been adopted, determination of the excise tax remains on a tax-gallon basis. The Bureau of Alcohol, Tobacco, and Firearms has established an official conversion factor: 1 L = 0.26417 U.S. gal (Treasury Decision A.F.F. 39, Jan. 21, 1977).

In the United States, the revenue from excise taxes on distilled spirits has become a substantial part of income realized by the three levels of government: federal, state, and local. In 1863, with a $0.20 rate, the federal government collected over $5 million; in 1960, $3,090 million; and in 1975 the total revenue amounted to $6,277 million, of which 60% represented the federal government's share, collected at a rate of $10.50 per tax gallon. In 1997, the federal government collected $3.6 billion from distilled spirits and $3.4 billion from beer and $0.6 billion from wine. In addition, the states collected $1.9 billion from beer alone. State and local governments collected $7.5 billion in 1988, of which $3.1 billion came from distilled spirits and $4.4 billion from wine and beer.

Supervision over the production of distilled beverage spirits is maintained by the Bureau of Alcohol, Tobacco,

Table 1. Federal Excise Tax Rates on Distilled Spirits

Year	Rate[a] (dollars)	Year	Rate[a] (dollars)
1812–1817	0.02 (0.09)	1934 (Jan.)	0.53 (2.00)
1862	0.05 (0.20)	1938 (July)	0.59 (2.25)
1846 (July)	0.16 (0.60)	1940 (July)	0.79 (3.00)
1864 (Dec.)	0.53 (2.00)	1941 (Oct.)	1.06 (4.00)
1894	0.29 (1.10)	1942 (Nov.)	1.59 (6.00)
World War I	0.61 (2.30)	1944 (Apr.)	2.38 (9.00)
1919	1.69 (6.40)	1951 (Nov.)	2.77 (10.50)
1933 (Dec.)	1.29 (1.10)	1985 (Oct.)	3.30 (12.50)
		1991 (Jan.)	3.56 (13.50)

[a] Per liter (gal).

and Firearms, Department of the Treasury, which succeeded the Alcohol and Tobacco Tax Division (ATTD) of the Internal Revenue Service in July 1972. (The ATTD was called the Alcohol Tax Unit from the repeal of prohibition to 1952.) This organization's enforcement agents are charged with the responsibility of eliminating illicit operations.

Prior to Prohibition, revenue agents and deputy collectors investigated illegal liquor operations, made arrests, seizures, etc, along with income tax and other miscellaneous work. On January 16, 1920, the effective date of Prohibition, federal prohibition agents took over the duties. An act of Congress created the Bureau of Prohibition in the Treasury Department on April 1, 1927. The Prohibition Reorganization Act, effective July 1, 1930, terminated the Bureau of Prohibition and created the Bureau of Industrial Alcohol in the Treasury Department, responsible for the permissive provisions of the act, and the Bureau of Prohibition in the Department of Justice, responsible for the enforcement of the penal provisions of the National Prohibition Act. These two were merged on December 6, 1933, as the Alcohol Tax Unit, Internal Revenue Department, and on March 15, 1952 it was named the Alcohol and Tobacco Tax Division of the Internal Revenue Service.

PRODUCTION AND CONSUMPTION

In 1810, according to government records, Pennsylvania produced 24.5 million L (6.5 million gal); Indiana 83,000 L (22,000 gal); and Kentucky more than 7.5 million L (2 million gal). In 1917, apparently in anticipation of Prohibition, over 1.1 billion L (300 million gal) of beverage spirits were produced, of which 225 million L (60 million gal) were whisky. In the year 1930, the Prohibition Enforcement Bureau estimated that the illicit production amounted to 3 billion L (800 million gal). Table 2 shows distilled spirits entering trade channels from 1955 to 1995.

STANDARDS OF IDENTITY

Distilled alcoholic beverages are usually characterized by their geographical origin, type of material used in production, and standard of quality, as evaluated by organoleptic analysis (taste and bouquet). Secretiveness was a way of life for ancient distillers; producers today operate according to their own methods. As it is not possible to correlate taste and bouquet with chemical composition, evaluation becomes a matter of organoleptic analysis.

Over the centuries legends, traditions, and to some extent political influences were important factors in the production and identification of potable distilled liquors. Since most governments had an economic interest because of the great source of revenue realized, they established standards for distilled alcoholic beverages, generally in keeping with the customs prevailing in their country. As a result, geographical identification has become accepted, and each country respects the identity and exclusiveness of the other's products.

Within each category of product wide variations in flavor can be caused by (*1*) types of material and their proportions, (*2*) methods of preparation, (*3*) selection of yeast types, (*4*) fermentation conditions, (*5*) distillation processes, (*6*) maturation techniques, and (*7*) blending. Since the alcoholic and water components are insignificant factors in flavor intensity or palatability, distillers are primarily interested in the more flavorful constituents, the so-called congeners, substances that are generated with the alcohol during the fermentation process and in the course of maturation. To produce a palatable product, it is, therefore, necessary to select the proper configuration of these constituents (congeners). In consideration of items 1–7 above, this cannot be accomplished by production techniques alone; therefore, the majority of alcoholic beverages are blended to provide uniformity, balanced bouquet, and palatability. To illustrate the variations in flavor constituents an analysis of some types is given in Table 3.

Whiskies

Although brandy, rum, and gin are substantial items in world markets, whiskies are by far the leading distilled alcoholic beverages, with those from Canada, Scotland, and the United States accounting for most of the sales. Irish whiskey, a distinctive product of Ireland, although not enjoying a large volume of sales, does have good distribution as a specialty item. The Irish use the spelling whiskey, the Scotch and Canadians use whisky, and U.S. citizens use both, although U.S. regulations use the spelling whisky.

Canadian. Canadian whisky is manufactured in compliance with the laws of Canada regulating the manufacture of whisky for consumption in Canada and containing no distilled spirits less than three years old. Canadian whiskies are premium products usually bottled at six years of age or more, and because they are blended, they are not designated as straight whiskies. They are light bodied and, although delicate in flavor, they nevertheless retain a distinct positive character. The Canadian government exercises the customary rigid controls in matters pertaining to labeling and in collection of the excise tax. However, it sets no limitations as to grain formulas, distilling proofs, or special types of cooperage for the maturation of whisky.

The major cereal grains (corn, rye, and barley malt) are used, and their proportions in the mashing formula remains a distiller's trade secret; otherwise, the process is substantially the same as is found in the distilleries of the United States. Because Canadian distillers are not faced with artificial proof restrictions in their distillation procedures, they are able to operate batch and continuous distillation systems under conditions that are optimum for the separation and selection of desirable congeners.

White oak casks (189 L, 50 U.S. gal) are used in the maturation process. A substantial amount of Canadian whiskies are stored in preused cooperage from other whiskies, which should lower the cost. Again, the proportions of new and matured cooperage used for maturation are each distiller's trade secret.

Scotch. Although Scotch whisky has enjoyed a worldwide reputation for its unique smoky flavor and high stan-

Table 2. U.S. distilled spirits entering trade channels, including exports and imports in 1000 wine gallons

	in 1000 wine gallons								
	1955	1960	1965	1970	1975	1981	1985	1990	1995
Domestic whiskey									
Bonded	12,868	9,394	7,482	6,004	3,993	2,276	1,128	721	327
Straight	44,838	58,939	69,417	77,895	69,531	63,288	48,933	44,193	39,143
Blend of straights	1,249	792	577	751	1,855	1,789	952	3,131	3,154
Blend of netural spirits	81,493	74,074	74,054	—	674	35,398	26,571	18,640	13,869
Other	(−448)	(−118)	—	73,936	58,484	4,153	3,747	878	1,322
Imported whiskey									
Scotch	12,284	20,584	32,523	51,911	55,275	47,656	40,271	27,889	20,902
Canadian	9,157	12,551	19,636	33,535	48,425	54,639	49,788	54,607	36,240
Irish and others	13	78	104	231	271	821	632	787	535
Belgian	—	—	141	—	—	—	—	—	—
Other	—	72	2	—	—	—	—	—	—
Gin									
Domestic	20,446	22,000	29,627	35,122	38,916	38,295	31,494	26,836	24,153
Imported	291	1,149	2,498	3,772	4,817	5,872	6,139	5,326	5,045
Rum									
Puerto Rican	1,856	2,611	4,650	9,049	13,601	26,881	27,077	29,962	18,564
Virgin Island	16	112	136	248	1,314	3,122	3,084	2,739	3,457
Other domestic	662	804	1,625	2,909	1,936	1,218	2,342	2,580	5,902
Imported	181	219	108	141	706	1,357	1,526	2,572	1,784
Brandy									
Domestic	3,726	5,300	8,263	11,795	12,388	14,541	14,470	13,353	11,642
Imported	893	1,163	1,142	1,764	3,035	6,769	7,862	6,391	6,332
Vodka									
	6,968	19,405	31,157	50,172	86,775	100,617	98,672	91,995	91,996
Cordials									
Domestic	6,173	9,156	14,755	20,505	26,323	26,059	32,039	26,881	26,952
Imported	455	812	1,247	2,405	4,769	13,704	16,176	16,274	18,107
Totals	*203,306*	*239,373*	*300,116*	*384,453*	*448,550*	*465,511*	*431,447*	*403,358*	*364,238*

Table 3. Congeneric Content of Various Types of Distilled Alcoholic Beverages, mg/L (ppm) at 80° proof (40%)

	Whisky					
				Bourbon		
Component	U.S.	Canadian	Scotch	Straight	Blended	Cognac brandy
Fusel oil[a]	664	464	1,144	1,624	1,560	1,544
Total acids[b]	240	160	120	552	540	288
Esters[c]	136	112	136	448	344	328
Aldehydes[d]	21.6	23.2	36	54.4	43.2	60.8
Furfural	2.6	0.88	0.88	3.6	7.2	5.4
Total solids	896	776	1,016	1,440	1,272	5,584
Tannins	168	144	64	416	384	200
Total congeners, wt/vol %	0.116	0.085	0.160	0.292	0.309	0.239

Note. Determinations were made according to the official methods of analysis of the Association of Office Agricultural Chemists.

[a] Determined by the Komarowsky colorimetric method.

[b] As acetic acid.

[c] As ethyl acetate

[d] As acetaldehyde.

dard of quality, consumers know very little about it. Not much information is revealed by government regulations, which only specify the use of cereal grains and a minimum requirement for aging in oak casks; in Britain,

> spirits described as Scotch Whisky shall not be deemed to correspond to that description unless they have been obtained by distillation in Scotland from a mash of cereal grain saccharified by the diastase of malt and have been matured in warehouse in cask for a period of at least three years.

In the United States,

> Scotch Whisky is a distinctive product of Scotland, manufactured in Scotland in compliance with the laws of Great Britain regulating the manufacture of Scotch Whisky for consumption in Great Britain, and containing no distilled spirits less than three years old.

This minimum age requirement is greatly exceeded by the Scotch distillers. For example, nothing under 4 years of age is included in their exports to the United States, and for the most part, 6-, 8-, 10-, and 12-yr minimum ages are featured in their brands. Most Scotch brands are blends of grain whiskies and numerous distinctive malt whiskies are produced by more than 100 distilleries in four major areas of Scotland. Malt whiskies are characterized by their location.

Even though there are many distilleries and, no doubt, slight variations in their production methods (that is how single whiskies acquire the characteristics attributable to each specific plant) there are definite processes generally used. A basic knowledge of these traditional methods, still in use today, is needed to fully appreciate the quality concept inherent in Scotch whisky.

As in Canada, no government limitations are placed on production and maturation techniques. The Scotch distillers are guided by their production experience developed over many centuries and by consumer reactions.

The outstanding taste characteristic of Scotch, its subtle smoky flavor, is due to the techniques used in the production of malt whiskies. Malted barley, dried over peat fires, is the only grain ingredient in the mash. The kind and the amount of peat used in the fires determines the intensity of flavor in the final product. The aroma of the burning peat, known as peat reek, is absorbed by the barley malt. This smoky flavor is carried through to the final distillate and becomes a characteristic of single malt whisky. Peat is heather, fern, and evergreen that have been subjected to aging and compression processes over the centuries.

The dried malted barley is ground to a grist and allowed to hydrolyze in a mash tub. After conversion is completed, the liquid portion, or wort, is drained off, cooled, and placed in a fermenter. After fermentation, the separation of malt whisky from the fermented wort takes place in a batch distillation system, a copper kettle with a "worm," or spiral tube, leading from its head. The size and shape of these pot stills exert a definite influence on the character of the whiskies. Another critical factor is the selection of the product during that portion of the distillation cycle that will produce the desired flavors. The first portion in the cycle is referred to as foreshots and the last as feints (heads and tails in the United States). The middle portion, after further distillation, becomes high wines and is subsequently reduced to maturation proof for storage in oak casks. The final distillation proof is in the 140–160° (70–80%) range.

The grain whiskies used in Scotch brands are produced in a manner similar to the production techniques used in Canada and in the United States. Corn (referred to in the UK as maize), rye, and barley malt are the ingredients. The proportions again depend on the individual distiller. Because delicate flavors are desired, the distillation proof is around 180–186° (90–93%). The distillation system is basically a Coffey still composed of two columns.

The grain whiskies are generally aged in matured oak casks of 190-L capacities not unlike U.S. and Canadian barrels. Some malt whiskies acquire other distinctive qualities by being matured in oak casks that were previously used for sherry.

Whereas the materials, geographic location, and production processes are responsible for the uniqueness of Scotch whisky, it is the skill of the blender that achieves the quality of the final product. As many as 20 and sometimes more, different malt and grain whiskies are married to produce a brand. Of course, the formulae are well-guarded secrets.

Irish. Irish whiskey is manufactured either in the Republic of Ireland or in Northern Ireland in compliances with their laws regulating the manufacture of Irish whiskey for home consumption and containing no distilled spirits less than three years old. Like Scotch, Irish whiskeys are blends of grain and malt whiskeys. Unlike Scotch, Irish whiskey does not have the unique smoky taste because the barley malt is not dried over peat fires. Because whiskey has been known in Ireland as long as in Scotland, it is not surprising that their production techniques are closely related. One variation, however, is the use of some small grain, mostly barley, in addition to barley malt in the production of malt whiskey. The production process involves four basic steps: brewing (mashing), fermentation, distillation, and maturation. Mashing takes place in a Kieve (mash tun), a circular metal vessel with two bottoms; the upper is perforated, which permits the wort (converted grain starch or maltose) to be filtered to the underback and sent on to the washbacks (fermenters) where the inoculation with and action by yeast produces the whiskey. The wash (fermented mash) is then distilled in a pot still (batch), producing a low wine of about 100° proof (50%), which in turn is redistilled to produce first shots (fore shots) from which the final product or whiskey of about 140–150° proof (70–75%) is distilled. Irish whiskey brands are generally considered to be more flavorful and heavier bodied than Scotch blended whiskies.

United States. Distilled spirits for beverage purposes in the United States are characterized specifically as to type, materials, composition, distillation proofs, maturation proofs, storage containers, and the extent of the aging period. The federal government also requires that a detailed statement of the production process be filed for each type and any subsequent improvements or changes must be filed and approved before being placed into operation. In addition, a generalized application of the regulations is made to establish the identity of products where the intensity of flavor may not conform to an arbitrary organoleptic evaluation based on chemical analysis. As a result, in spite of extensive manufacturing facilities and know-how available for the production of a wide range of whiskies, the distiller is restrained within narrow limits and does not enjoy the degree of latitude available to the Canadian and Scotch distillers. U.S. regulations, by the Bureau of Alcohol, Tobacco, and Firearms of the Treasury Department, are very specific and more limiting than those of Canada or the UK. Title 27, *Code of Federal Regulations*, Subpart C, paragraph 5.22 *et seq.* sets the standards of identity for all distilled spirits. Those that are a factor in the U.S. market are given here.

> *Whisky* is an alcoholic distillate from a fermented mash of grain produced at less than 190° proof in such manner that the distillate possesses the taste, aroma, and characteristics generally attributed to whisky, stored in oak containers, and bottled at not less than 80° proof.
>
> *Neutral Spirits* or *alcohol* are distilled spirits produced from any material at or above 190° proof.

The requirement of distilling above 190° proof (95%) gives a distiller an opportunity to take advantage of the physical relationship that exists between water, alcohol, and congeners that are produced during fermentation. The sophisticated equipment utilized by progressive distillers permits a technique known as selective distillation, whereby the distiller can remove all congeners or retain those that are deemed desirable.

> *Grain spirits* are neutral spirits distilled from a fermented mash of grain and stored in oak containers.

Because grain spirits have delicate flavors, they must be stored in oak barrels previously seasoned through the storage of whisky or grain neutral spirits. It is important that the barrel is compatible with the flavor intensity of the grain neutral spirits; otherwise, the woody character of the barrel would overwhelm the light flavor of the grain neutral spirits and thus prevent proper development during aging. Grain neutral spirits are produced on continuous and batch distillation systems. Each produces a number of distillates having a low flavor intensity that, when stored in barrels, develop in flavor in the same manner as whisky (4).

The batch system, related to the pot still, is simpler in concept and offers an opportunity to use the heart-of-the-run principle for the production of grain neutral spirits. Sometimes referred to as a time-cycle distillation system, it involves three stages: (*1*) the heads (aldehydes) are removed, (*2*) the product is removed, and (*3*) the residual distillate (tails) remaining in the kettle is removed for subsequent redistillation in the continuous system. The batch system is composed of a large kettle with a capacity of up to 190,000 L (50,000 wine gal) with a vapor pipe leading to a product-concentrating column having as many as 55 bubble-cap plates. The large capacity of the kettle is important in maintaining product uniformity. Straight whisky, produced in the normal manner in the whisky column, is pumped into the kettle, indirect steam heat is applied through a coil within the kettle, and the alcoholic vapors rise into the product column where they are refined. The grain neutral spirits thus produced are reduced in proof with deionized water to between 110 and 160° proof (55 and 80%), put in oak barrels, and placed in government bonded warehouses for storage.

> *Vodka* is neutral spirits so distilled, or so treated after distillation with charcoal or other materials, as to be without distinctive character, aroma, taste, or color

This definition has come under fire recently because some vodkas on the market claim to have some flavor. Since the 1950s drinking patterns in the United States have become more diversified. Vodka has moved up from negligible sales in 1949 to 36% in 1996. The fact that it can be mixed with any flavored substance seems to be the reason for its wide acceptance.

> *Rye whisky, bourbon whisky, or malt whisky* is whisky produced at not more than 160° proof from a fermented mash of not less than 51% rye, corn, or malted barley respectively, and stored at not more than 125° proof in charred new oak containers.
>
> *Corn whisky* is whisky produced at not more than 160° proof from a fermented mash of not less than 80% corn It may or may not be stored in oak containers, but only in used or uncharred ones.

In producing bourbon whisky, eg, the mashing formula must contain at least 51% corn and the remaining ingredients (49%) generally are proportioned between rye grains and barley malt. Each distiller selects a preferred formula. Very little bourbon with 49% small grains is produced. The most popular proportions are 60% corn, 28% rye, 12% barley malt (referred to as 40% small grains), 70-18-12 (30% small grains), and 75-13-12 (25% small grains). Although some bourbons use as much as 15% barley malt, the general practice in the industry is to use 12% barley malt for all bourbon production.

Because rye and barley malt produce more intensive flavors than corn, the formula with the greater small-grains proportion will produce a bourbon with more body, provided, of course, that the same distillation techniques are used.

> *Straight whisky* may be any of the whiskies in the preceding two paragraphs that have been stored in the prescribed oak containers for two years or more. A straight whisky may further be identified as *bottled in bond*, provided it is at least four years of age, bottled at 100° proof (50%), and distilled at one plant by the same proprietor. A bottled-in-bond whisky may contain homogeneous mixtures of whiskies, provided they represent one season, or if consolidated with other seasons, the mixture shall be the distilling season of the youngest spirits contained therein, and shall consist of not less than 10% of spirits of each such season.

> *Light whisky* is whisky produced in the United States at more than 160° proof, and stored in used or uncharred new oak containers; and also includes mixtures of such whiskies. If light whisky is mixed with less than 20% by volume of 100° proof (50%) straight whisky the mixture shall be designated Blended Light Whisky.
>
> *Blended whisky* is a mixture which contains straight whisky or a blend of straight whiskies at not less than 20 percent on a proof gallon basis, and, separately, or in combination, whisky or neutral spirits. A blended whisky containing not less than 51 percent on a proof gallon basis of one of the types of straight whisky shall be further designated by that specific type of straight whisky; for example, "blended rye whisky."
>
> *Scotch whisky* is whisky which is a distinctive product of Scotland, manufactured in Scotland in compliance with the laws of the United Kingdom regulating the manufacture of Scotch whisky for consumption in the United Kingdom: such product is a mixture of whiskies, such mixture is "blended Scotch whisky."
>
> *Irish whisky* is whisky which is a distinctive product of Ireland, manufactured either in the Republic of Ireland or in Northern Ireland, in compliance with their laws regulating the manufacture of Irish whisky for home consumption: if such product is a mixture of whiskies, such mixture is "blended Irish whiskey."
>
> *Canadian whisky* is whisky which is a distinctive product of Canada, manufactured in Canada in compliance with the laws of Canada regulating the manufacture of Canadian whisky for consumption in Canada: if such product is a mixture of whiskies, such mixture is "blended Canadian whisky."

Gins

> *Gin* is a product obtained by original distillation from mash, or by redistillation of distilled spirits, or by mixing neutral spirits, with or over juniper berries and other aromatics, or with or over extracts derived from infusions, percolations, or maceration of such materials, and includes mixtures of gin and neutral spirits. It shall derive its main characteristic flavor from juniper berries and be bottled at not less than 80° proof.

France de La Boe, a 17th-century professor of medicine at Leyden University, The Netherlands, is credited with being the originator of the botanical-flavored spirits known as gin. Because his product's primary flavor was due to the essential oils extracted from juniper berries, he gave it the French name *jenièvre*, which appeared later as the Dutch *geneva* and finally as the English *gin*. Gin produced exclusively by original distillation or by redistillation may be further designated as distilled. Gin derives its main characteristic flavor from juniper berries. In addition to juniper berries, other botanicals may be used, including angelica root; anise; coriander; caraway seeds; lime, lemon and orange peel; licorice; calamus; cardamom; cassia bark; orris root; and bitter almonds. The use and proportion of any of these botanicals in the gin formula is left to the producer, and the character and quality of the gin depends to a great extent on the skill of the craftsman in formulating the recipe. The more skilled producers formulate their aromatic ingredients on the basis of the essential oil content in the raw materials to assure a greater degree of product uniformity.

To expose the essential oils, the ingredients are reduced to a granular form and then immersed directly into the kettle (pot), which is filled with grain neutral spirits at approximately 100° proof (50%). A vapor-phase extraction may also be used. In this case, the botanical mixture is placed on trays or in baskets in the head of the kettle where the alcoholic vapors passing by extract the essential oils and rise to the condenser.

It is important that the grain spirits be as neutral as possible (devoid of congeners) to avoid undesirable flavors. In addition to the kettle, some gin stills have a refinement section (as many as six plates) above the kettle for flavor stability and enrichment. Indirect steam heat is applied and the various essential oils are distilled over during the entire distillation cycle. The first (heads) and last (tails) portions of the cycle are not included in the product. Only the heart of the run is used, representing approximately an 85% recovery of the original alcohol concentration and varying with the type of product desired. Some distillers, to avoid thermal decomposition of the delicate flavors and to acquire a degree of softness, conduct the distillation under reduced pressure at a temperature of about 57°C. London Dry Gin, for example, is produced in this manner.

British and Canadian regulations permit and recognize the use of maturation techniques for gin. Gins stored in special oak casks acquire a pale golden hue and a unique dryness of flavor. Although distillers are permitted to store gins in the United States for further flavor development, the federal government does not permit any reference to aging to appear on the label.

Holland Gin, characterized by its high flavor intensity derived mostly from juniper berries and cereal grains (corn, rye, and barley malt), is produced by immersing the botanical mixture directly into the grain mash before distillation or by extracting the essential oils from the botanical mixture with the heavy distillate (high wines) from a fermented mash of grain, consisting of corn, rye, and barley malt. Consequently, the flavors produced during fermentation become flavor components of the final product. Compound gin is a mixture of grain spirits and essential oil extracts from botanicals. It does not undergo any distillation procedure.

Brandies

> *Brandy* is an alcoholic distillate from the fermented juice, mash, or wine of fruit, or from the residue thereof, produced at less than 190° proof in such manner that the distillate possesses the taste, aroma, and characteristics generally attributed to the product, and bottled at not less than 80° proof.

The most important category of brandy is fruit brandy, distilled solely from the juice or mash of whole, sound, ripe fruit or from standard grape, citrus, or other fruit wine. Brandy derived exclusively from one variety of fruit is so designated. However, a fruit brandy derived exclusively from grapes may be designated as brandy without further qualification, and unless the product is specifically identified, the term brandy always means grape brandy. Brandy is subject to a distillation limitation of 170° proof (85%). If distilled over 170° proof (85%), it must be further identified as neutral brandy. A minimum of two years of maturation in oak casks is required, otherwise the term immature must be included in the designation. Although the age is not indicated on the label, brandies are normally aged from three to eight years.

Brandies are produced in batch or continuous distillation systems. The pot still or its variation is universally used in France, whereas in the United States both systems are employed. The batch system produces a more flavorful product, the continuous system a lighter, more delicate flavor.

The history of brandy can be said to be the history of distillation, because in the distant past it was the distillation of wine in crude stills that produced *aqua vitae*. In the ensuing evolution, many areas of Europe and of the United States became renowned for their brandies. Perhaps the most popular brandy comes from the Cognac region of France, in the Department of Charente and Charente Inférieure. As such, it enjoys an exclusive identity, Cognac, under which no other brandy may be labeled. Cognac is produced in the traditional pot stills by small farmers and sold to the bottlers who age the brandies in limousin oak casks. When the brandies reach maturity, they are skillfully blended for marketing under their own brand name.

Cognac is a blend of some Grande Champagne, Petite Champagne, Borderies, and Fins Bois, the proportions of each being a well-kept secret. To further characterize Cognac, the bottle is labeled: E, especial; F, fine; V, very; O, old; S, superior; P, pale; X, extra; C, Cognac; eg, VSOP means very superior old pale, and is considered to be a better-quality product.

Another well-known brandy of Frances is Armagnac, produced in southern France. Armagnac is distilled from wines in a continuous system using two pot stills in series. Armagnac is considered to be more heavy-bodied and drier than Cognac. Brandies are distilled in almost every wine region of France; they are called *eau de vie*, exported as French Brandy, never as Cognac.

In the United States, California produces almost all of the grape brandy. Generally, it is a well-integrated operation, the cultivation of the grapes, the making of the wine, the distilling, aging, bottling, and the marketing of the brandy being done by the same firm. Usually a continuous multicolumn distillation system is employed. Of the total U.S. brandy consumption of approximately 56 million L (15 million gal) California brandies account for over 80%.

Blended applejack is accorded a special classification as a mixture that contains at least 20% of apple brandy (applejack) on a proof basis, stored in oak containers for not less than two years, and not more than 80% of neutral spirits on a proof gallon basis and bottled at not less than 80° proof (40%). Another class of beverage spirits, flavored brandy, is a brandy to which natural flavoring materials have been added with or without the addition of sugar; it is bottled at no less than 70° proof (35%). The name of the predominant flavor appears as part of the designation, ie, blackberry flavored brandy; cherry flavored brandy, etc. Such a flavored brandy may contain up to 12.5% of wine derived from the particular fruit corresponding to the labeled flavor.

Certain areas in Europe and in South America are well known for their specialty brandies, such as Spanish brandies, distilled from Jerez sherry wine; the fragrant, fruity Portuguese brandies, distilled from port wine; the pleasant and flowery muscat bouquet of Pisco brandy from Peru; Kirschwasser brandy, with its almond undertone flavor, distilled from a fermented mash of small black cherries, which grow along the Rhine Valley in Germany and Switzerland; and Slivovitz, the plum brandy, which is produced in Hungary and in the Balkan countries.

> *Cognac or Cognac (grape) brandy*, is grape brandy distilled in the Cognac region of France, which is entitled to be so designated by the laws and regulations of the French Government.

Rums

> *Rum* is an alcoholic distillate from the fermented juice of sugar cane, sugar cane syrup, sugar cane molasses, or other sugar cane by-products, produced at less than 190° proof in such manner that the distillate possesses the taste, aroma and characteristics generally attributed to rum, and bottled at not less than 80° proof.

Blackstrap molasses is the most common raw material for the manufacture of rum. Otherwise, the same basic factors that produce different whiskies are responsible for the flavor varieties of rums. The type of yeast, fermentation environment, distillation techniques and systems, the maturation conditions, and not least the blending skill all contribute to the final character and quality of rum. Blackstrap molasses varies in composition according to origin owing to the environment and to some extent on the processing of cane (a greater recovery of sugar is usually reflected in a lower concentration of residual sugar in the molasses). A typical composition of Puerto Rican blackstrap molasses is as follows:

pH	4.3
Density (% solids)	89°Brix
Nonfermentable solids	28 7%
Sucrose	33.8%
Reducing sugars	26.9%
Total sugars	60.7%

Rums are characterized as light bodied, of which the Puerto Ricans are the best known, and full bodied, which come from Jamaica and certain other islands of the West Indies. Light rums are distilled on multicolumn continuous distillation systems over a proof range of 160–180° (80–90%). They are matured in oak casks that are reused for rum storage. Age may, but need not, be stated on the labels.

Rum has been produced in Jamaica for more than 200 yr as some plantation crop records indicate. A typical small plantation would produce a ton of sugar and 41,000 L (9,000 imperial gal) of rum. Today, only a few large facilities exist and the law requires that a distillery may only be operated in conjunction with a sugar refinery. As in every area where distilled spirits are produced, terminology and local practice play a major role in determining the identity and the flavor characteristics of the product; eg, molasses acid results from a natural fermentation that produces alcohol and subsequently an organic acid mixture. Some of this is used in the wash (fermenting mash) for the production of certain flavorful rums. Dunder is the term used for stillage, the material left after the beverage spirits are removed by distillation. Dunder is also used as an ingredient in the fermenting mash, as much as 40 vol %, to provide buffering action, and flavor development.

Jamaican and other full-bodied rums are distilled between 140 and 160° proof (70 and 80%) in pot stills. They are matured in large casks of 422.4 L (111.6 gal) called puncheons. Unlike the light-bodied rums, which use cultured yeast for inoculation, the Jamaican rums rely on natural fermentation, sometimes referred to as wild fermentation. In this method the mash is inoculated by the yeast that is present in the air and in the raw material. Time of fermentation may vary from 2 to as much as 11 days, depending on the desired flavor characteristics.

Puerto Rican rums are generally labeled as white or gold label. The latter is a little more amber in color and has a more pronounced flavor. Although the rums produced in various areas are not considered distinctive types, they do retain their local characteristics and their names may not be applied to rum produced in any other place than the particular region indicated in the name.

Venezuela and to some extent Mexico are major producers of rum. The former requires a minimum two-year aging period and in the latter the amount of production is closely regulated by the government and keyed to the availability of cane and the need for industrial alcohol. In all other respects, the light-bodied rums are similar to those produced in Puerto Rico. A limited amount of more flavorful rums for use in blending is produced in both countries. Continuous and batch distillation systems are used and thus offer a capability of producing rums with varying degrees of flavor intensities and individual characteristics.

Tequila

Tequila, a distinctive product of Mexico, is an alcoholic distillate produced in Mexico from the fermented juice of the heads of *Agave Tequilana Weber* (blue variety), with or without additional fermentable substances, distilled in such a manner that it possess the taste, aroma, and characteristics generally attributed to tequila. This is Mexico's most popular distilled spirits drink.

The mezcal azul (blue mezcal), the primary source for tequila, is cultivated and usually propagated from 2-yr-old sprouts obtained from 7-yr-old mezcal plants. After 8–12 yr the plants are matured; the trimmed heads, referred to as pine apples because of their appearance and weighing 36–59 kg (80–130 lb) each, are transported to the distillery. In the State of Jalisco, up to 20,000 t of mezcal heads are harvested annually. The heads contain (unsteamed): moisture, 62%; total solids, 38%; fiber, 11%; inulin, 20%; and ash, 2.5%, and they have a pH of 5.5.

The juice from the mezcal heads is first extracted in masonry ovens with a capacity of approximately 40 t for 9–24 h at approximately 93°C. The length of the steaming period is critical for the acid hydrolysis of the inulin to monosaccharides. During a 12-h cooling period, some additional juice (*mieles de escurrido*, drained molasses) is recovered. The mezcal heads are now dark brown, soft in texture, with a taste similar to maple syrup. Residual juice is removed by shredding the steamed heads, compressing the strips between roller mills, and finally washing the strips (bagasse) to recover all of the sugary syrup.

The mezcal juice from the steaming ovens, the roller mills, and the bagasse washes is pumped into fermenters of 3,800–7,500 L (1,000–2,000 gal) capacity made of masonry or of local pine wood. Nitrogen nutrients are added to facilitate fermentation. The Mexican government also permits the addition of piloncillo, a brown sugar, up to 30 wt % of the fermentable sugar in the mezcal heads after steaming. After a fermentation period of about 42 h, the alcoholic concentration is 4.5 vol% of the fermenter mash. When piloncillo is not used, the alcoholic yield is lower and the fermentation takes longer.

The fermented mash is pumped to a copper pot still of about 1,100 L (300 gal) capacity provided with a steam coil within the kettle for heating and a condenser for cooling the vapors. The intermediate of the first distillation called ordinario is collected at 28° proof (14%) and redistilled in a slightly larger pot still. This distillation cycle can be controlled to yield a product of approximately 106° proof (53%). The residual distillate from this process is combined with the fermented mash, starting a new cycle in the primary distillation system.

Tequila, as consumed in Mexico, is unaged and usually bottled at 80–86° proof (40–43%). However, some producers do age tequila in seasoned 190-L (50-gal) white oak casks imported from the United States. In aging, tequila becomes golden in color and acquires a pleasant mellowness without altering its inherent taste. Tequila aged one year is identified as Anejo; aged as much as two to four years, it is identified as Muy Anejo. The annual production of tequila in Mexico is around 20 million L (5.2 million U.S. gal), the major portion of which is produced by 28 plants located in the county of Tequila.

Cordials and Liqueurs

Cordials and liqueurs are the same, with the former term being American and the latter European. They are obtained by mixing or redistilling neutral spirits, brandy, gin, or other distilled spirits with or over fruits, flowers, plants, or pure juices therefrom, or other natural flavoring materials, or with extracts derived from infusions, percolations, or maceration of such materials. Cordials must contain a minimum of 2.5 wt% of sugar or dextrose, or a combination of both. If the added sugar and dextrose are less than 10 wt% then it may be designated as dry. Synthetic or imitation flavoring materials cannot be included in United States cordials, nor can they be designated as distilled or compound.

Cordials were known in ancient Egypt and Athens, but commercial production was started in the Middle Ages when alchemists, physicians, and monks, among others, were searching for an elixir of life. From this activity many well-known cordials were developed, such as Benedictine and Chartreuse, both derived from aromatic plant flavors and bearing the names of the monasteries where they were first prepared.

A great variety of cordials are available encompassing a wide spectrum of flavors from fruits, peels, leaves, roots, herbs, and seeds. Organoleptic attainment, however, becomes a matter of experience and skill in the selection of botanicals and in the extraction and formulation of flavors. Although these elements are carefully guarded secrets, the producer must rely on three basic processes, namely maceration, percolation, and distillation, or any combination thereof. Maceration involves the steeping of the raw materials in the spirits, usually in a vat, to impart the desired aroma, flavor, and color. The liquid is then drawn off and provides the base for further processing. Percolation is accomplished by recirculating the spirits through a percolator containing the raw materials. As the spirits seep down through the raw material, the desired constituents are extracted, which will give the proper aroma, flavor intensity, and color. The distillation method is similar to that used in gin production. The ingredients are either immersed in the beverage spirits or placed in trays or pans in the head of the still. The rising vapors extract the essential flavors, which are then condensed and discharged as a colorless liquid. This distillate contains the basic flavor that is used for further processing.

Cordials are characterized and marketed according to their generic names; eg, anisette (aniseed), crème de menthe (peppermint), triple sec (citrus fruit peel), slow gin (sloe berries), and by their trade names (proprietary brands) of which Benedictine and Chartreuse are well-known examples.

THE MANUFACTURING PROCESS

Any material rich in carbohydrates is a potential source of ethyl alcohol, which for industrial purposes is obtained by the fermentation of materials containing sugar (molasses), or a substance convertible into sugar, such as the starches. In the production of distilled spirits for beverage purposes, however, cereal grains are the principal types of raw material used. Any reference to alcohol in beverages is always to ethyl alcohol (C_2H_5OH). Although other alcohols may be present, they are referred to as higher alcohols, fusel oils, or by their specific name.

The chemical composition of grain varies considerably and depends to a large extent on environmental factors such as climatic conditions and the nature of the soil. Another variable is the malt (sprouted or germinated grain) used. Malt is generally understood to be germinated barley, unless it is further qualified as rye malt, wheat malt, etc. The purpose of malting is the development of the amylases, the active ingredients in malt. Amylases are enzymes of organic origin, which change grain starch into the sugar, maltose. Besides providing the means of converting the grain starch into sugar, the malt also contributes to the final flavor and aroma of the distillate. Malting techniques may produce a malt of such unusual character that it may indeed furnish the outstanding characteristics of the final product, as in Scotch whisky. Figure 2 shows the process flow sheet of a modern beverage spirits plant.

Grain Handling and Milling

The beverage distilling industry utilizes premium cereal grains. Each distiller supplements government grain standards with personal specifications, especially in regard to the elimination of grain with objectionable odors, which may have developed during storage or kiln drying at the elevators. Hybrid corn, usually of the readily available dent variety and plump rye, developed from Polish strains (Rosen), are used for beverage alcohol production. Modern distilleries use Airveyor unloading systems, others the traditional power shovel in conjunction with screw conveyors and bucket elevators. Even though the grain has been subjected to a cleaning process at the elevator, it is passed over receiving separators, a series of vibrating screens which sift out the foreign materials. Air jets and dust collectors remove any light material and magnetic separators remove metallic substances.

Milling breaks the outer cellulose protective wall around the kernel and exposes the starch to the cooking and conversion processes. Distillers require an even, coarse meal without flour. Milling is accomplished by three methods: (*1*) in roller mills, using pairs of corrugated rolls (breaks), run sharp to sharp (projections facing projections); (*2*) in hammer mills, where a series of revolving hammers within a close-fitting casing and rotating at 1,800–3,600 rpm shear the grain to a meal, which is removed by suction through a screen, different for various types of grain; and (*3*) in attrition mills (not widely used) where the grain is ground by two counterrotating disks (1,200–2,000 rpm). This mill is not entirely satisfactory because of excessive flour produced. A three-break roller mill (three pairs of corrugated rolls, arranged vertically) with rolls 23 cm in diameter × 76 cm long has a capacity of 3.5 m^3 (100 bu) of corn per hour; a hammer mill 61 cm in width, 10.6 m^3 (300 bu) per hour; and an attrition mill with 41 cm grinding plates, 3.5 m^3 (100 bu) per hour, on the basis that 25% of the ground corn remains above the no. 12 mesh (1.68 mm) screens.

Mashing

The mashing process, consists of cooking, ie, gelatinization of starch, and conversion (saccharification), ie, changing starch to grain sugar (maltose). Cooking can be carried out at atmospheric or higher pressure in a batch or a continuous system. For whisky production, batch cooking at atmospheric pressure is widely used although some batch pressure cooking is practiced. For grain neutral spirits production, both batch and continuous systems are used under pressure. After cooling conversion is accomplished in the cooking vessel by the addition of barley malt meal to the cooked grain. Some distillers pump the mash immediately to a converter for the necessary holding time and thus make the cooking vessel available for the next cook. The converted mash is cooled and pumped to the fermenters.

Distillers vary mashing procedures, but generally conform to basic principles, especially in the maintenance of sanitary conditions. The cooking and conversion equipment is provided with direct or indirect steam, propeller or rake-type agitation, and cooling coils or a barometric condenser. Mashing procedures for rye, corn, and malt grains are described below.

Rye. In the preparation of a bourbon mash, rye is generally subjected to the corn-cooking process. However, rye comes a matter of experience and skill in the selection of botanicals and in the extraction and formulation of flavors. Although these elements are carefully guarded secrets, the producer must rely on three basic processes, namely maceration, percolation, and distillation, or any combination thereof. Maceration involves the steeping of the raw materials in the spirits, usually in a vat, to impart the desired aroma, flavor, and color. The liquid is then drawn off and provides the base for further processing. Percolation is accomplished by recirculating the spirits through a percolator containing the raw materials. As the spirits seep down through the raw material, the desired constituents are extracted, which will give the proper aroma, flavor intensity, and color. The distillation method is similar to that used in gin production. The ingredients are either immersed in the beverage spirits or placed in trays or pans in the head of the still. The rising vapors extract the essential flavors, which are then condensed and discharged as a colorless liquid. This distillate contains the basic flavor that is used for further processing.

Cordials are characterized and marketed according to their generic names; eg, anisette (aniseed), crème de menthe (peppermint), triple sec (citrus fruit peel), slow gin (sloe berries), and by their trade names (proprietary brands) of which Benedictine and Chartreuse are well-known examples.

THE MANUFACTURING PROCESS

Any material rich in carbohydrates is a potential source of ethyl alcohol, which for industrial purposes is obtained by the fermentation of materials containing sugar (molasses), or a substance convertible into sugar, such as the starches. In the production of distilled spirits for beverage purposes, however, cereal grains are the principal types of raw material used. Any reference to alcohol in beverages is always to ethyl alcohol (C_2H_5OH). Although other alcohols may be present, they are referred to as higher alcohols, fusel oils, or by their specific name.

The chemical composition of grain varies considerably and depends to a large extent on environmental factors such as climatic conditions and the nature of the soil. Another variable is the malt (sprouted or germinated grain) used. Malt is generally understood to be germinated barley, unless it is further qualified as rye malt, wheat malt, etc. The purpose of malting is the development of the amylases, the active ingredients in malt. Amylases are enzymes of organic origin, which change grain starch into the sugar, maltose. Besides providing the means of converting the grain starch into sugar, the malt also contributes to the final flavor and aroma of the distillate. Malting techniques may produce a malt of such unusual character that it may indeed furnish the outstanding characteristics of the final product, as in Scotch whisky. Figure 2 shows the process flow sheet of a modern beverage spirits plant.

Grain Handling and Milling

The beverage distilling industry utilizes premium cereal grains. Each distiller supplements government grain standards with personal specifications, especially in regard to the elimination of grain with objectionable odors, which may have developed during storage or kiln drying at the elevators. Hybrid corn, usually of the readily available dent variety and plump rye, developed from Polish strains (Rosen), are used for beverage alcohol production Modern distilleries use Airveyor unloading systems, others the traditional power shovel in conjunction with screw conveyors and bucket elevators. Even though the grain has been subjected to a cleaning process at the elevator, it is passed over receiving separators, a series of vibrating screens which sift out the foreign materials. Air jets and dust collectors remove any light material and magnetic separators remove metallic substances.

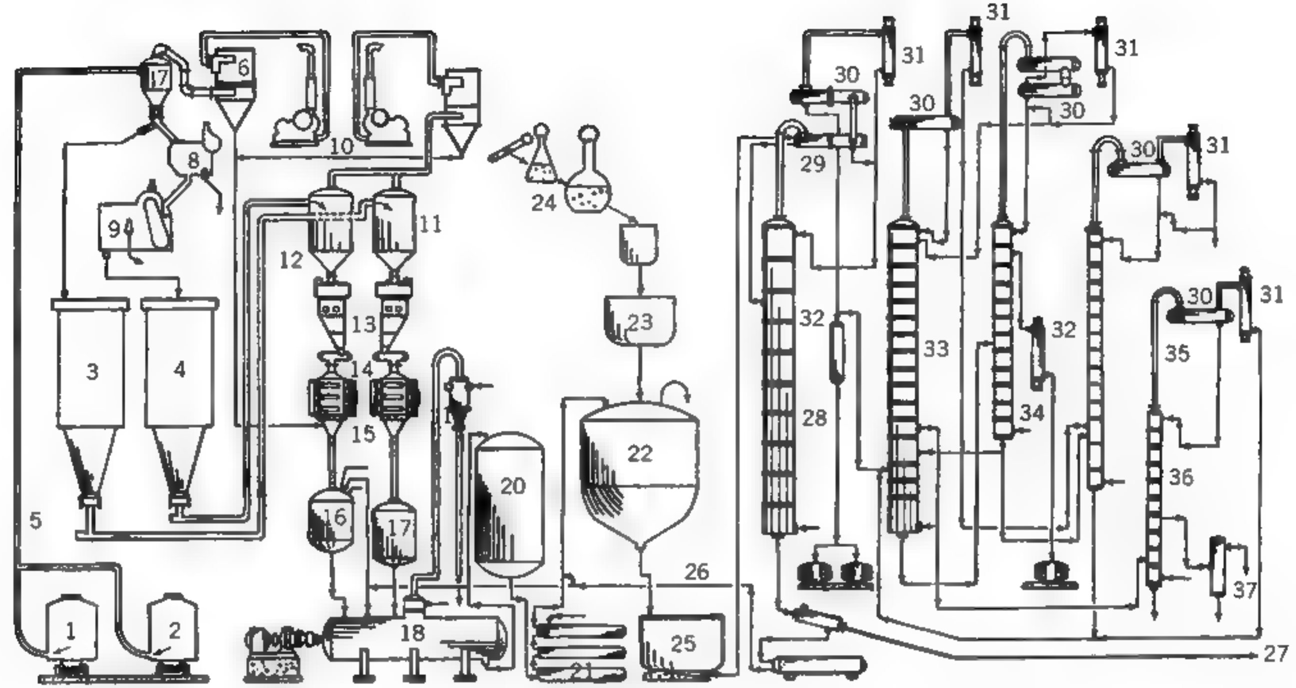
Milling breaks the outer cellulose protective wall around the kernel and exposes the starch to the cooking and conversion processes. Distillers require an even, coarse meal without flour. Milling is accomplished by three methods: (*1*) in roller mills, using pairs of corrugated rolls (breaks), run sharp to sharp (projections facing projections); (*2*) in hammer mills, where a series of revolving hammers within a close-fitting casing and rotating at 1,800–3,600 rpm shear the grain to a meal, which is removed by suction through a screen, different for various types of grain; and (*3*) in attrition mills (not widely used) where the grain is ground by two counterrotating disks (1,200–2,000 rpm). This mill is not entirely satisfactory because of excessive flour produced. A three-break roller mill (three pairs of corrugated rolls, arranged vertically) with rolls 23 cm in diameter × 76 cm long has a capacity of 3.5 m^3 (100 bu) of corn per hour; a hammer mill 61 cm in width, 10.6 m^3 (300 bu) per hour; and an attrition mill with 41 cm grinding plates, 3.5 m^3 (100 bu) per hour, on the basis that 25% of the ground corn remains above the no. 12 mesh (1.68 mm) screens.

Mashing

The mashing process, consists of cooking, ie, gelatinization of starch, and conversion (saccharification), ie, changing starch to grain sugar (maltose). Cooking can be carried out at atmospheric or higher pressure in a batch or a continuous system. For whisky production, batch cooking at atmospheric pressure is widely used although some batch pressure cooking is practiced. For grain neutral spirits production, both batch and continuous systems are used under pressure. After cooling conversion is accomplished in the cooking vessel by the addition of barley malt meal to the cooked grain. Some distillers pump the mash immediately to a converter for the necessary holding time and thus make the cooking vessel available for the next cook. The converted mash is cooled and pumped to the fermenters.

Distillers vary mashing procedures, but generally conform to basic principles, especially in the maintenance of sanitary conditions. The cooking and conversion equipment is provided with direct or indirect steam, propeller or rake-type agitation, and cooling coils or a barometric condenser. Mashing procedures for rye, corn, and malt grains are described below.

Rye. In the preparation of a bourbon mash, rye is generally subjected to the corn-cooking process. However, rye



1. Cereal grains
2. Malt
3. Malt bin
4. Cereal grain bin
5 Unloading elevator
6. Dust filter
7. Collector
8. Scalperator
9. Millerator
10. Reclaiming exhauster
11. Malt receiver
12. Cereal grain receiver
13. Automatic scale
14. Mill feeder
15. Roller mills
16. Precooker
17. Malt infusion
18. Cooker
19. Barometric condenser
20 Converter
21 Mash coolers
22. Fermenter
23. Final yeast propagator
24. Yeast culture and intermediate yeast propagator
25. Fermented-mash holding vessel
26. Stillage return system
27. Stillage flow to recovery system
28. Whisky separating column
29. Heat exchanger
30. Dephlegamtor
31. Vent condenser
32. Product cooler
33. Selective distillation column
34. Product concentrating column
35. Aldehyde concentrating column
36. Fusel oil concentrating column
37. Fusel oil decanter

Figure 2. Material process flow, modern beverage spirits plant.

undergoes liquefication at a much lower temperature than corn, which avoids thermal decomposition of critical grain constituents adversely affecting the final flavor of the distillate. For that reason, many distillers mash rye separately.

Water is drawn at the rate of 35 L/m^3 (28 gal/bu) and rye and malt meal are added. The mash is slowly heated to 54°C and held for approximately 30 min. Proteolytic enzymes, active at 43–46°C, aid in reducing the viscosity, and the optimum temperature for β-amylase is 54°C. The mash is then heated to 63–67°C and held for 30–45 min to ensure maximum conversion. The mash (pH 6.0) is then cooled to the fermenting temperature 20–22°C. This process of converting small grains is called infusion mashing.

Corn. Although the starch in corn grains converts rather easily, higher cooking temperatures are necessary to make the starch available. Usually malt is not added at the beginning, but to reduce viscosity, premalt of 0.5% may be added before cooking, preferably at around 66°C. Thin stillage (the residual dealcoholized fermented mash from the whisky distillation process) is added by some producers to adjust pH to 5.2–5.4. For cookers operating at atmospheric pressure, a mashing ratio of 95–115 L (25–30 gal) of slurry (grain, water, and stillage mixture) per 0.03 m^3 (1 bu) and a holding time of 30 min at 100°C are preferable. The mash is cooled to 67°C and malt is added. Primary conversion, the saccharification taking place during conversion, is in the order of 70–80% of the available starches. The remainder of the conversion to fermentable sugar takes place during the fermentation process and is referred to as secondary conversion. For batch cooking under pressure only 65–83 L (17–22 gal) of water are drawn, and the maximum temperature is 120–152°C. In continuous pressure cooking, water is drawn at a ratio of 30 L/m^3 (24 gal/bu) of meal and sufficient thin stillage is added to adjust the pH to 5.2–5.4. The mash is pumped through the continuous pressure cooker, where it is exposed to temperatures of 170–177°C for 2–6 min, and then into a flash chamber where it is cooled immediately by vacuum to the malting (conversion) temperature of 63°C. A malt slurry is continuously introduced and the mixture proceeds through the water cooling system to the fermenters.

Fermentation

In fermentation the grain sugars (largely maltose), produced by the action of malt enzymes (amylases) on gelatinized starch, are converted into nearly equal parts of ethyl alcohol and carbon dioxide. This is accomplished by the enzymes in yeast. Yeast multiples by budding, and a new cell is produced about every 70 min. Although yeasts of several genera are capable of some degree of fermentation, *Saccharomyces cerevisiae* is almost exclusively used by the distilling industry. It has the ability to reproduce prolifically under normal growth conditions found in distilleries, has a high fermentation rate and efficiency, and can tolerate relatively high alcohol concentrations (up to 15–16 vol %). A great variety of strains exist and the characteristics of each strain are evidenced by the type and amount of congeners the yeast is capable of producing.

Alcoholic fermentation is represented by the following reactions:

$$\underset{\text{maltose}}{C_{12}H_{22}O_{11}} \xrightarrow{\text{maltase}} \underset{\text{dextrose}}{C_6H_{12}O_6} \rightarrow \underset{\substack{\text{ethyl}\\ \text{alcohol}}}{2C_2H_5OH} + \underset{\substack{\text{carbon}\\ \text{dioxide}}}{2CO_2}$$

A fermentation efficiency of 95% is obtained based on the sugar available. Of the starch converted to grain sugar and subsequently subjected to fermentation, 5–6% is consumed in side reactions. The extent and type of these reactions depend on: (1) yeast strain characteristics, (2) the composition of the wort, and (3) fermentation conditions such as the oxidation—reduction potential, temperature, and degree of interference by bacterial contaminants.

Secondary products formed by these side reactions largely determine the characteristics and organoleptic qualities of the final product. In the production of whisky, the secondary products (known as congeners) formed and retained during the subsequent operations include a number of aldehydes, esters, higher alcohols (fusel oils), some fatty acids, phenolics or aromatics, and a great many unidentified trace substances (Table 3). In the production of grain neutral spirits, the congeners are removed from the distillate in a complex multicolumn distillation system. Some distillers, however, retain a small portion of the low-boiling esters in the distillate when the grain neutral spirits are to be matured. Fermentation of grain mashes is initiated by the inoculation of the set mash with 2–3 vol % of ripe yeast prepared separately (see below) and followed by three distinct phases.

1. Prefermentation involves rapid multiplication of yeast from an initial 4–8 million/mL to a maximum of 125–130 million/mL of the liquid and an increasing rate of fermentation.
2. Primary fermentation is a rapid rate of fermentation, as indicated by the vigorous "boiling" of the fermenting mash, caused by escape of carbon dioxide. During this phase secondary conversion takes place, ie, the changing of dextrins to fermentable substances.
3. Secondary fermentation is a slow and decreasing rate of fermentation. Conversion of the remaining dextrins, which are difficult to hydrolyze, takes place.

The degree of conversion, agitation of the mash, and temperature directly affect the fermentation rate. Fermenter mash set at a concentration of 144 L (38 gal) of mash per bushel of grain 25.4 kg (56 lb) will be fermented to completion in two to five days, depending on the set and control temperatures. The set temperature (temperature of the mash at the time of inoculation) is largely determined by the available facilities for cooling the fermenting mash. If cooling facilities are adequate, temperatures of 27–30°C may be employed; otherwise, the set temperature must be low enough to ensure that the temperature will not exceed 32°C during fermentation. When no cooling facilities are provided, the inoculation temperature must be below 21°C. Excessive temperatures during the prefermentation phase retard yeast growth and stimulate the development of bacterial contaminants which are likely to produce undesirable flavors.

In the production of sour mash whisky U.S. federal regulations require that a minimum of 25 vol % of the fermenting mash must be stillage (cooled, screened liquid recovered from the base discharge of the whisky separating column, pH 3.8–4.1). In addition to producing a heavier-bodied whisky, this procedure provides the distiller with an economical means of adjusting the setting pH (4.8–5.2) to inhibit bacterial development. It also provides buffering action during the fermentation cycle, which is important because secondary conversion does not take place if the fermenting pH drops below 4.1 in the immediate stages. Thin stillage also provides a means of diluting the cooker mash to the proper fermenter mash concentration, 3,220–3,870 L/m^3 (30–36 gal/bu) of grain for the production of spirits, and 4,080–4,850 L/m^3 (38–45 gal/bu) for making whisky. This concentration gives about a 12–16% soluble solids in the fermenting liquid, within the range used in beer fermentations but lower than that in wine fermentation.

Preparation of the yeast involves a stepwise propagation, first on a laboratory scale and then on a plant scale to produce a sufficient quantity of yeast for stocking the main mash in the fermenters. A strain of yeast is usually carried in a test tube containing a solid medium (agar slant). A series of daily transfers, beginning with the removal of some yeast from the solid medium, are made into successively larger flasks containing liquid media—diamalt (commercial malt extract) diluted to 15–20° Balling, malt extract, or strained sour yeast mash—until the required amount of inoculum is available for the starter yeast mash, called a dona. After one day's fermentation, the dona is added to a yeast mash normally composed of barley malt and rye grains, and representing approximately 2–3.5 wt % of the total grain mashed for each fermenter.

The yeast mash is generally prepared by the infusion mashing method and then soured (acidified) to a pH of 3.9–4.1 by a 4–8-h fermentation at 41–54°C with *Lactobacillus delbrucki*, which ferments carbohydrates to lactic acid. Satisfactory souring can be induced with an inoculum of approximately 0.25% of culture per volume of mash. The water-to-meal ratio of 2,580–3,000 L/m^3 (24–28 gal/bu) attains a yeast mash balling of approximately 21°. Before inoculation with yeast, the soured mash is pasteurized to 71–87°C to curtail bacterial activity, then cooled to the setting temperature of 20–22°C. The sour mash medium offers an optimum condition for yeast growth and also has an inhibitory effect on bacterial contamination. In 16 h the yeast cell count reaches 150–250 million/mL. Some distillers use the sweet yeast method for yeast development. In this instance the lactic acid souring is not included and the inoculation temperature is usually above 26.6°C to insure rapid yeast growth.

Distillation

Distillation separates, selects, and concentrates the alcoholic products of yeast fermentation from the fermented grain mash, sometimes referred to as fermented wort or distillers beer. In addition to the alcohol and the desirable secondary products (congeners), the fermented mash contains solid grain particles, yeast cells, water-soluble proteins, mineral salts, lactic acid, fatty acids, and traces of glycerol and succinic acid. Although a great number of different distillation processes are available, the most common systems used in the United States are (1) the continuous whisky separating column, with or without an auxiliary doubler unit for the production of straight whiskies; (2) the continuous multicolumn, system used for the production of grain neutral spirits; and (3) the batch rectifying column and kettle unit, used primarily in the production of grain neutral spirits that are subsequently stored in barrels for maturation purposes. In the batch system, the heads and tails fractions are separated from the product resulting from the middle portion of the distillation cycle.

Although most modern plants have various capacity whisky stills available, a whisky separating column is usually incorporated into the multicolumn system, thus acquiring a greater range of distillation selectivity, ie, the removal or retention of certain congeners. For example, absorptive distillation involving the addition of water to the upper section of a column in the whisky distillation system is a method of controlling the level of heavier components in a product. In the beverage distillation industry, stills and auxiliary piping are generally fabricated of copper, although stainless steel is also used. All piping that conveys finished products is tin-lined copper, stainless steel, or glass.

The whisky column, a cylindrical shell that is divided into sections and may contain from 14 to 21 perforated plates, spaced 56–61 cm apart. The perforations are usually 1–1.25 cm in diameter and take up about 7–10% of the plate area. The vapors from the bottom of the still pass through the perforations with a velocity of 6–12 m/s. The fermented mash is introduced near the top of the still, and passes from plate to plate through down pipes until it reaches the base where the residual mash is discharged. The vapor leaving the top of the still is condensed and forms the product. Some whisky stills are fitted with entrainment removal chambers and also with bubble-cap plate sections (wine plates) at the top to permit operation at higher distillation proofs. Because whisky stills made of copper, especially the refinement section, supply a superior product, additional copper surface in the upper section of the column may be provided by a demister, a flat disk of copper mesh. The average whisky still uses approximately 1.44–1.80 kg/L (12–15 lb of steam/proof gal) of beverage spirits distilled. Steam is introduced at the base of the column through a sparger. Where economy is an important factor, a calandria is employed as the source of indirect heat. The diameter of the still, number of perforated and bubble-cap plates, capacity of the doubler, and proof of distillation are the critical factors that largely determine the characteristics of a whisky.

The basic continuous distillation system for the production of grain neutral spirits usually consists of a whisky separating column, an aldehyde column (selective distillation column), a product concentrating column (sometimes referred to as an alcohol or rectifying column, from which the product is drawn), and a fusel oil concentrating column. In addition, some distillers, to secure a greater degree of refinement and flexibility, may include an aldehyde concentrating column (heads concentrating column) or a fusel oil stripping column. Bubble-cap plates are used throughout the system (except in the whisky column, which may have some bubble-cap plates).

This distillation system offers a wide range of flexibility for the refinement of distilled beverage spirits. Figure 3 shows a five-column, continuous distillation system for the production of grain neutral spirits. A fermented mash (generally 90% corn and 10% barley malt) with an alcohol concentration of approximately 7 vol % is pumped into the whisky column somewhere between the 13th and 19th perforated plate for stripping. The residual mash is discharged at the base and pumped to the feed recovery plant; the overhead distillate [ranging in proof from 105 to 135° (52.5 to 67.5%)] is fed to the selective distillation column (also called the aldehyde column), which has over 75 bubble-cap plates. The main stream [10–20° proof (5–10%)] from the selective distillation column is pumped to the product concentrating column. A heads draw (aldehydes and esters) from the condenser is pumped to the heads concentrating column (also called the aldehyde concentrating column), and a fusel oil and ester draw is pumped to the fusel oil concentrating column. The product is withdrawn from the product concentrating column.

Some accumulation of heads, at the top of the product concentrating column, are removed at the condenser, and transferred to the aldehyde concentrating column, where the heads from the system are removed for disposal. The fusel oil concentrating column removes the fusel oil from the system. Figures 3, 4, and 5 show the distribution and concentration of alcohols and congeners through the selective distillation column, the product concentrating column, and the heads concentrating column.

By-Products. The discharge from the base of the whisky column is called stillage and contains in solution and in suspension substances derived from grain (except the starch, which has been fermented), and from the mashing and fermentation processes. The suspended solids are recovered by screening and then subjected to a pressing and a drying operation (dehydrating), usually in rotary, steam-tube dryers. The liquid portion, called thin stillage, is concentrated by a multieffect evaporator to a syrup with a solid content of 30–35%. This concentrate can be mixed and dried with the previously screened-out solids, or it can be dried separately, on rotary-drum dryers. These by-products are known as distillers' dried grains and distillers' solubles and are used by the feed industry to fortify

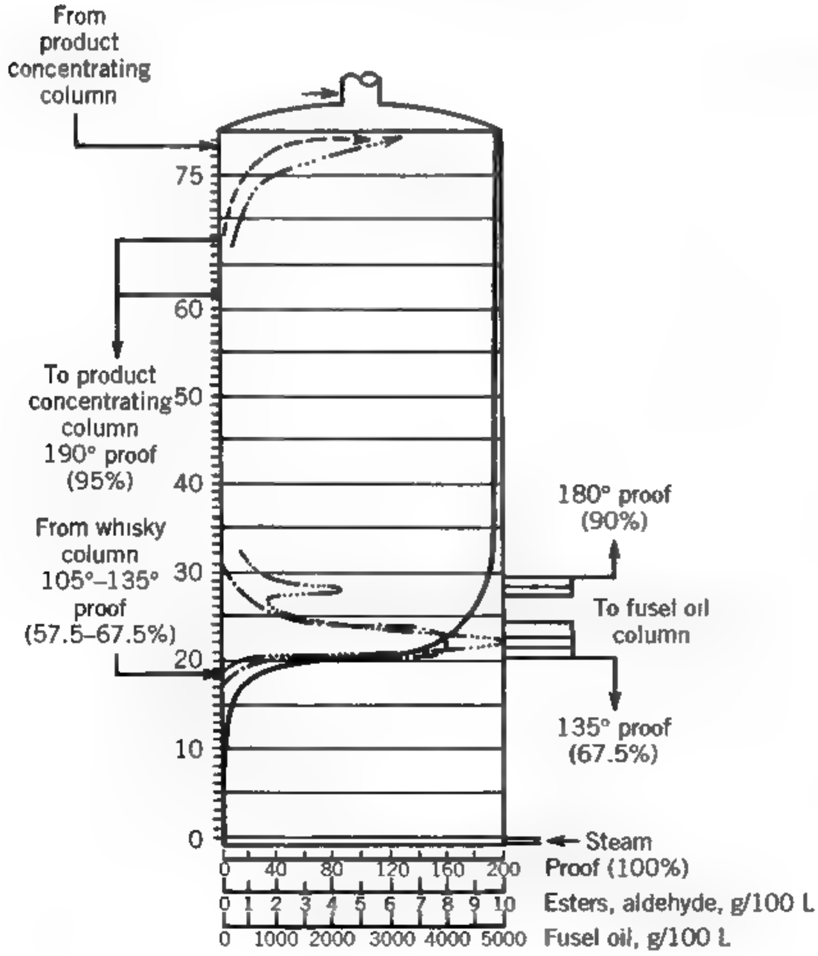

From product concentrating column
75
60
To product concentrating column 190° proof (95%)
50
40
From whisky column 105°–135° proof (57.5–67.5%)
30
20
10
0
180° proof (90%)
To fusel oil column
135° proof (67.5%)
Steam
0 40 80 120 160 200 Proof (100%)
0 1 2 3 4 5 6 7 8 9 10 Esters, aldehyde, g/100 L
0 1000 2000 3000 4000 5000 Fusel oil, g/100 L

Figure 3. Selective distillation column.

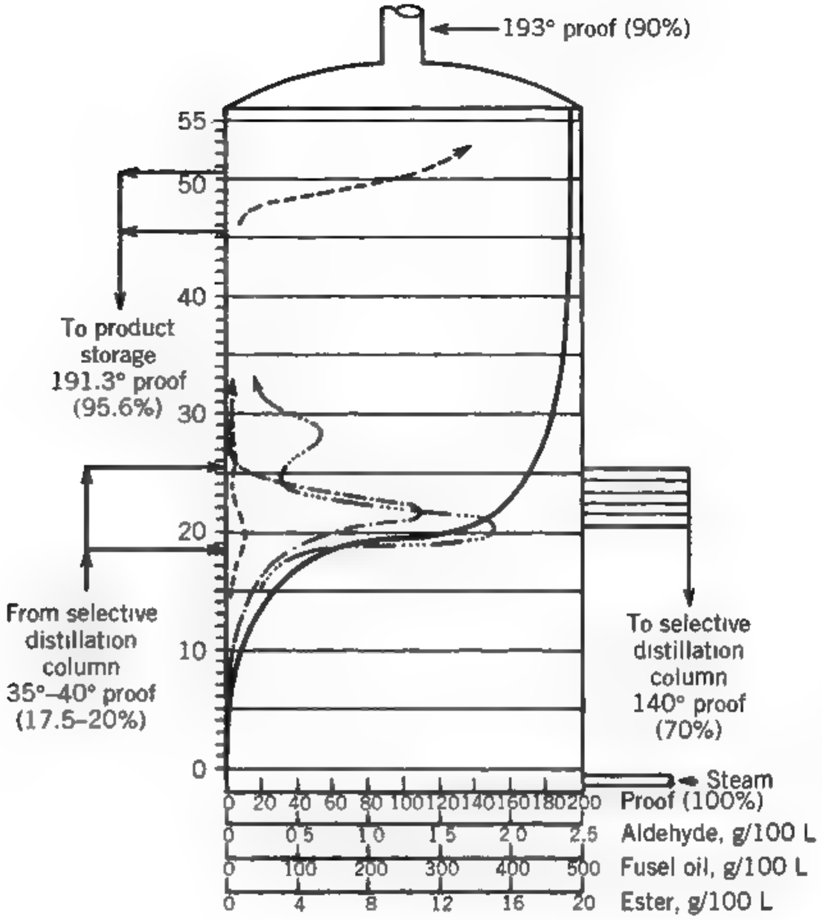

193° proof (90%)
55
50
40
30
20
10
0
To product storage 191.3° proof (95.6%)
From selective distillation column 35°–40° proof (17.5–20%)
To selective distillation column 140° proof (70%)
Steam
0 20 40 60 80 100 120 140 160 180 200 Proof (100%)
0 0.5 1.0 1.5 2.0 2.5 Aldehyde, g/100 L
0 100 200 300 400 500 Fusel oil, g/100 L
0 4 8 12 16 20 Ester, g/100 L

Figure 4. Product concentrating column.

dairy, poultry, and swine formula feeds. Distillers' feeds are rich in proteins (24–35%), fat (8%), choline, niacin, and other B-complex vitamins and contain vital fermentation growth factors that produce good growth responses in livestock and poultry.

Maturation

In the United States, the final phase of the whisky production process, called maturation, is the storage of beverage spirits (which at this point are colorless and rather pungent in taste) in new, white oak barrels, whose staves and headings are charred. The duration (years) of storage in the barrel depends on the time it takes a particular whisky to attain the desirable ripeness or maturity. The staves of the barrel may vary in thickness from 2 to 2.85 m. The outside dimensions of a 190-L (50-gal) barrel are approximately: height, 0.88 m (34.5 in.) and diameter at the head, 0.54 m. Whisky storage warehouses vary in construction from brick and mortar types, single and multiple floors (as many as six) having capacities up to 100,000 barrels of 190-L (50-gal) capacity, to wooden sheet-metal-covered buildings (called iron clads), generally not exceeding a capacity of 30,000 barrels of 190-L (50-gal) capacity. It is customary to provide natural ventilation. The whisky in storage is subject to critical factors that determine the character of the final product. The thickness of the stave, depth of the char (controlled by regulating the duration of the firing, 30–50 s), temperature and humidity, entry proof, and, finally, the length of storage impart definite and intended changes in the aromatic and taste characteristics of a whisky. These changes are caused by three types of reaction occurring simultaneously and continually in the barrel: (*1*) extraction of complex wood constituents by the liquid, (*2*) oxidation of components originally in the liquid and of material extracted from the wood, and (*3*) reaction between the various organic substances present in the liquid, leading to the formation of new congeners.

Comprehensive studies of changes occurring during the maturation of whisky have been published by a number of investigators (4–11). Although these reports contain detailed information on the concentration of various congeners throughout the course of the maturation period, little is revealed of the interrelationships between various congeners except for the nonvolatile groups, such as solids, nonvolatile acids, tannin, and color. Notable among the observed changes are increases in the concentrations of acids, esters, and solids. These studies reveal that throughout the maturation period a linear relationship exists between the increase in acids and esters and the increase in dissolved solids; this suggests that acid and ester formation is dependent on some precursor that is extracted from the barrel at the same rate as the bulk of the solid fraction (9).

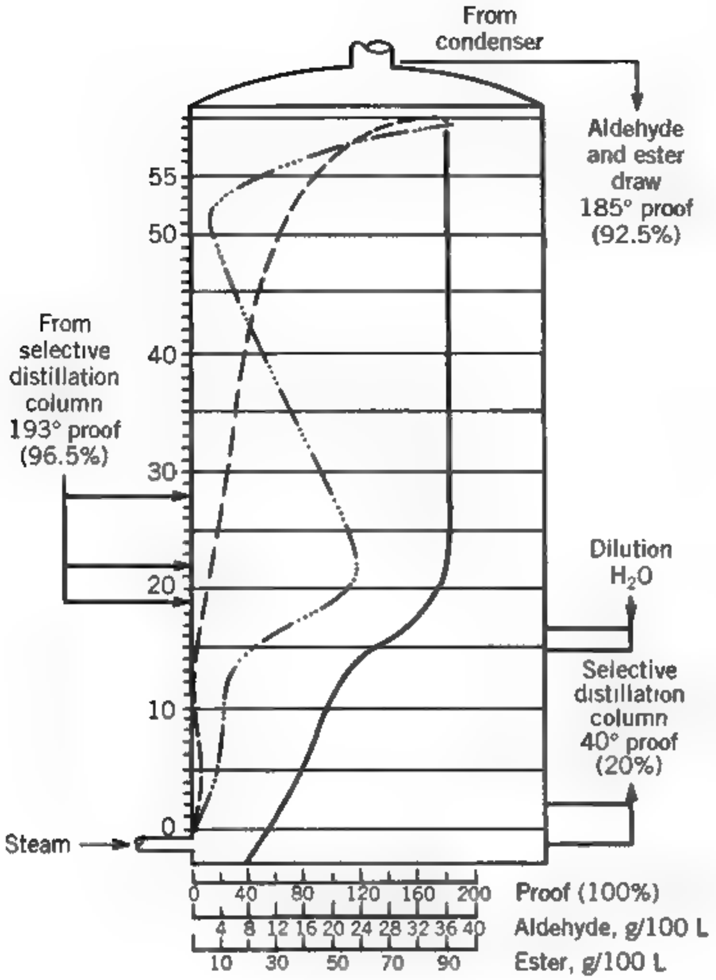

From condenser
Aldehyde and ester draw 185° proof (92.5%)
From selective distillation column 193° proof (96.5%)
Dilution H₂O
Selective distillation column 40° proof (20%)
Steam
55
50
40
30
20
10
0
0 40 80 120 160 200 Proof (100%)
4 8 12 16 20 24 28 32 36 40 Aldehyde, g/100 L
10 30 50 70 90 Ester, g/100 L

Figure 5. Aldehyde (heads) concentrating column.

In 1962 the U.S. Treasury Department increased the maximum allowable entry proof (storage in barrels) for straight whisky from 110 to 125° (55 to 62.5%). The industry engaged in a study in December 1985 to determine the flavor development, chemical composition, and evaporation loss rates of distillates at six different proofs from 109 to 155° (54.5 to 77.5%) over a planned 8-yr period, and the study was continued an additional 4 yr. Evaporation losses averaged approximately 3.0% a year over the 12-year period.

In addition to chemical analysis, they applied regressive analysis for most congeners listed in Table 4. Regression analysis illustrates the functional relationship between congeners. Except for fusel oil, there was an appreciable continuing development of congeners through 12 yr. Esters, the most important flavor constituent in whisky, developed linearly over this time period. Later studies, using radioactive carbon for direct monitoring, determined the congeners derived from ethanol during whisky maturation. Esters (ethyl acetate) are formed in a system that is affected by changes in concentration of acetic acid, ethanol, and water, ie, a loss of water by evaporation; an increase in acetic acid results in an increase of ethyl acetate.

The maturing progress of a type of bourbon as measured by the principal ingredients is illustrated in Tables 4 and 5. It is evident that the congeners amount to only about 0.5–0.75% of the total weight. Yet it is this small fraction that determines the quality of the final product. Thus matured whiskies vary widely in taste and aroma because of the wide variation in congener concentration. For example, esters may vary from 8.0 to 18.0 g/100 L, and aldehydes from 0.7 to 7.6 g/100 L. Nevertheless, there is no correlation between chemical analysis and quality, ie, taste and aroma. Only the consumer can detect the fine variations and thus evaluate the quality of whiskies.

ANALYSIS

Analytical results are expressed in terms of one component for each chemical class; ie, acetic acid for acids, acetaldehyde for aldehydes, etc (Tables 4 and 5). Individual constituents can, of course, be determined by more refined techniques. Until the advent of gas chromatography, tedious chemical procedures were generally required for separation of the congeners. Distillation, countercurrent distribution, and other physical methods affected concen-

Table 4. Chemical Analysis^a of Bourbon Whiskies Matured at Six Proofs from 109 to 155° (54.5 to 77.5%), g/100 L at 100° Proof (50%)

Age (yr)	Proof	Color^a	pH^b	Solids	Fixed acids	Volatile acids	Esters	Fusel oil	Aldehydes	Furfural	Tannins
0	109	nd^c	nd	0	0	1.6	4.8	125	0.3	0.06	0
1	106	0.666	4.2	94	6	29	11	134	3.1	1.02	31
	112	0.678	4.2	99	7	27	12	134	3.1	1.03	31
	122	0.686	4.3	90	7	23	11	132	3.1	0.95	29
	132	0.652	4.3	70	6	21	11	128	2.9	0.84	26
	143	0.688	4.2	71	5	21	11	130	2.6	0.78	25
	155	0.652	4.2	63	5	18	11	129	2.6	0.75	23
2	107	0.793	4.1	124	7	31	16	147	3.5	1.16	37
	113	0.810	4.2	105	7	32	18	141	3.5	1.10	37
	123	0.848	4.3	107	7	27	16	143	3.2	1.12	36
	133	0.785	4.1	103	6	28	17	136	2.9	0.96	32
	143	0.807	4.1	92	6	23	16	145	2.5	0.82	30
	152	0.764	4.2	85	5	22	15	142	2.3	0.80	29
3	106	1.036	4.1	149	9	36	21	148	4.5	1.12	45
	112	1.013	4.2	145	9	36	21	146	4.2	1.14	43
	123	1.031	4.1	130	8	32	22	151	3.8	1.09	41
	132	0.928	4.2	118	7	29	19	153	3.5	0.89	36
	142	0.917	4.1	107	7	27	21	142	3.2	0.78	35
	151	0.845	4.1	88	6	25	21	149	2.8	0.71	31
4	106	1.086	4.1	177	12	42	23	148	5.2	1.16	52
	112	1.081	4.1	162	11	40	22	145	5.3	1.16	49
	122	1.081	4.0	152	10	33	21	152	4.9	1.11	45
	131	1.000	4.0	137	9	32	22	148	5.1	0.96	42
	140	0.977	4.1	124	8	26	20	146	4.2	0.88	40
	150	0.910	4.1	110	8	26	20	148	4.0	0.84	36
5	106	1.229	4.1	194	12	43	30	157	6.1	1.20	49
	112	1.260	4.1	182	12	41	31	157	6.0	1.19	50
	122	1.180	4.0	164	10	34	26	155	5.7	1.14	45
	131	1.086	4.1	144	9	30	27	157	5.4	1.02	40
	140	1.102	4.0	131	8	30	28	155	5.3	0.96	38
	149	1.004	4.1	114	7	27	28	159	4.9	0.94	34
6	107	1.215	4.1	186	12	40	33	159	5.6	1.25	49
	113	1.284	4.1	185	12	40	33	163	5.5	1.15	49
	123	1.284	4.0	176	11	34	32	159	5.1	1.30	47
	132	1.155	4.0	153	10	33	32	163	5.1	1.05	42
	141	1.155	4.0	138	10	28	31	163	4.8	1.00	40
	150	1.056	4.0	122	8	27	29	166	4.7	1.00	36
7	107	1.456	4.1	214	13	51	37	157	6.1	1.34	57
	113	1.409	4.1	209	13	49	35	160	6.1	1.34	54
	123	1.409	4.1	190	12	46	36	155	5.7	1.29	51
	132	1.284	4.0	169	10	37	35	164	5.8	1.15	45
	141	1.215	4.0	152	10	32	33	162	5.2	1.08	42
	149	1.137	4.0	135	8	29	35	162	5.3	1.03	40
8	107	1.509	4.1	223	13	50	42	148	6.8	1.38	56
	113	1.523	4.1	218	13	49	43	159	6.8	1.35	57
	122	1.409	4.1	196	12	38	38	155	6.6	1.29	52
	131	1.276	4.0	175	10	34	37	153	5.9	1.13	60
	140	1.284	4.0	57	10	32	40	151	6.1	1.06	60
	149	1.168	4.0	147	9	27	39	152	5.4	1.08	58
9	108	1.509	4.0	225	14	50	48	164	8.7	1.45	57
	113	1.538	4.1	226	13	50	50	160	7.9	1.40	58
	122	1.432	4.0	204	12	38	43	171	7.8	1.27	53
	131	1.284	4.1	179	9	37	42	155	6.2	1.14	45
	140	1.301	4.0	164	9	33	45	159	6.7	1.06	44
	148	1.168	4.1	143	8	28	41	160	6.4	1.06	40
10	108	1.509	4.1	240	15	57	57	182	8.3	1.37	58
	113	1.482	4.1	231	13	52	54	173	8.4	1.33	55
	124	1.523	4.0	206	12	41	54	168	8.2	1.27	53
	132	1.367	4.0	183	11	41	41	175	8.1	1.15	47
	140	1.377	4.0	171	10	37	37	176	7.1	1.03	43
	147	1.215	4.0	152	9	32	32	169	7.1	1.00	41

Table 4. Chemical Analysis[a] of Bourbon Whiskies Matured at Six Proofs from 109 to 155° (54.5 to 77.5%), g/100 L at 100° Proof (50%) *(continued)*

Age (yr)	Proof	Color[a]	pH[b]	Solids	Fixed acids	Volatile acids	Esters	Fusel oil	Aldehydes	Furfural	Tannins
11	108	1.699	4.0	252	14	60	60	166	8.2	1.37	64
	113	1.721	4.0	242	13	56	56	168	7.7	1.37	62
	123	1.658	3.9	219	12	49	57	179	7.8	1.34	59
	131	1.509	3.9	192	12	42	54	171	7.7	1.21	53
	139	1.398	3.9	181	10	38	51	178	6.7	1.08	50
	146	1.319	4.0	162	9	34	51	166	6.7	1.05	47
12	107	1.770	4.0	270	21	68	65	189	9.1	1.41	67
	112	1.745	4.0	258	19	52	62	187	9.3	1.37	65
	123	1.699	3.9	231	17	46	59	187	8.5	1.30	61
	130	1.538	3.9	211	15	41	62	187	9.4	1.20	55
	138	1.409	3.9	190	14	36	58	184	8.0	1.09	52
	145	1.319	3.9	168	10	33	54	185	7.8	1.07	47

Note: Analyses performed annually on samples reduced to 100° proof (50%). Acids as acetic acid, esters are ethyl acetate, aldehydes as acetaldehyde, and tannins as tannic acid (11).

[a] Absorbance at 430 nm at 100° proof (50%).

[b] At 100° proof (50%).

[c] Not determined.

Table 5. Changes Taking Place in Bourbon Whisky Stored in Wood

Age[a] (yr)	Range	Proof	Extract[a]	Acids[a]	Esters[a]	Aldehydes[a]	Furfural[a]	Fusel oil[a]	Color
New	Average	101.0	26.5	10.0	18.4	3.2	0.7	100.9	0.0
	Maximum	104.0	161.0	29.1	53.2	7.9	2.0	171.3	0.0
	Minimum	100.0	4.0	1.2	13.0	1.0	Trace	71.3	0.0
								42.0	
1	Average	101.8	99.4	41.1	28.6	5.8	1.6	110.1	7.1
	Maximum	103.0	193.0	55.3	55.9	8.6	7.9	173.4	10.9
	Minimum	100.0	61.0	24.7	17.2	2.7	Trace	58.0	5.4
			54.0	10.4	10.4			42.8	4.6
2	Average	102.2	126.8	45.6	40.0	8.4	1.6	110.1	8.6
	Maximum	104.0	214.0	61.7	59.8	12.0	9.1	197.1	11.8
	Minimum	100.0	81.0	25.5	24.4	5.9	0.4	86.2	6.9
			78.0	23.5	11.2			42.8	5.7
			78.0	23.5	11.2			42.8	5.7
4	Average	104.3	151.9	58.4	53.5	11.0	1.9	123.9	10.8
	Maximum	108.0	249.0	73.0	80.6	22.0	9.6	237.1	14.8
	Minimum	100.0	101.0	40.0	28.2	6.9	0.8	95.0	8.6
			92.0	40.0	13.8			43.5	7.4
6	Average	107.9	185.1	67.1	64.0	/9	1.8	135.3	13.1
	Maximum	116.0	287.0	81.0	83.9	23.3	9.5	240.0	17.5
	Minimum	102.0	132.0	53.6	36.4	7.7	0.9	98.1	12.0
			127.0	45.0	17.9				9.8
8	Average	111.1	210.3	76.4	65.6	12.9	2.1	143.5	14.2
	Maximum	124.0	326.0	91.4	93.6	28.8	10.0	241.8	20.9
	Minimum	102.0	152.0	64.1	37.7	8.7	1.0	110.0	12.3
			141.0	53.7	22.1			47.6	10.5

Source. Ref. 5.

[a] Grams per 100 L of 100° proof (50%) spirit.

tration of various congener groups. These concentrates reacted to form chemical derivatives that were then subjected to further separation. Paper and column chromatography have been used extensively for this work. After separation of the components, identification is possible by infrared and mass spectrometry.

With the high sensitivity (flame and β-ray ionization detection) and efficiency offered by gas chromatographic techniques, a qualitative profile of alcoholic distillates can be obtained without prior treatment of the sample. Flame ionization uses a hydrogen flame for the combustion of organic substances to produce electrons and negative ions, which are collected on an anode. The resulting electrical current is proportional to the amount of material burned. β-Ray ionization uses beta particles emitted from a source such as strontium-90 to ionize the carrier gas and its components. The measure of the electrical current resulting from the collection of electrons on the anode is used in the determination or detection of the substance. This may augment sensory evaluations. A positive identification of the separated congeners can be made by infrared and mass spectrometers. Nmr may offer possibilities in the identification of congeners present in alcoholic beverages.

PACKAGING

Because distilled spirits are packaged under federal government supervision and the product is then distributed to the various states in compliance with laws and regulations, many factors must be considered normally not involved in glass packaging. First, the product represents a high value, because it includes the federal tax at the time of bottling. In the United States, a bottle of whisky retailing at $5.44 is taxed at $3.11. Obviously, care must be taken to avoid losses. Second, in addition to dealing with cases, bottles, cartons, closures, and labels, the distiller must apply to each bottle a federal strip stamp indicating that the excise tax has been paid and also apply bottle stamps of the decal type to indicate identification or tax payment in the seven states that require these in their system of control. With the heavy investment in product taxes and the necessity of applying state stamps, the distiller's ability to build up a substantial case goods inventory to provide immediate service to his customers in those states having this requirement is limited. Third, because this is a licensed industry, in addition to the normal record keeping necessary for efficient operations and control, federal and state records are required.

Federal regulations prescribe and limit the standards of fill (size of containers). On October 1, 1976, the adoption of the metric system permitted distillers to incorporate the new sizes into their operations as circumstances allow. Once the metric size is adopted for a brand, the distiller may not revert to the former standard.

Now, only the metric size will be permitted, such as 1.75 L (59.2 fl oz); 1.00 L (33.8 fl oz); 750 mL (25.4 fl oz); 500 mL (16.9 fl oz); 200 mL (6.8 fl oz); and 50 mL (1.7 fl oz). Individual states likewise limit the number of sizes that can be distributed within their borders and need not adopt all the sizes made available by the federal government.

The most common packaging operation utilized in the industry is the straight-line system with a line speed from 120 to 200 bottles/min. Along with the general progress in packaging, the distilling industry is moving in the direction of automatic-line operation with variable frequency control systems keyed to the fill and labeling operations. This is only possible, however, when volume on certain sizes is substantial. Most distillers are faced with mechanical equipment changes, requiring approximately four hours per line, to handle various sizes of bottles. In addition to material specifications for quality control on all supplies, built-in quality-control inspection systems are included on the bottling lines. Likewise, the quality-control department, independent of bottling operations, takes random samples for evaluation purposes.

Table 6. U.S. Commercial Exports of Liquor by Class 1996 (in wine gal.)

Class and type	
Domestic whiskey	
Bonded	0
Straight	8,293,519
Blended straights	34,814
Distilled over 160/p	2,355
Blended whiskey	158,576
Subtotal	8,489,264
Imported whiskey	
Scotland and N. Ireland	31,515
Bottled in U.K.	0
Bottled in U.S.	31,616
Canadian	110,562
Bottled in Canada	0
Bottled in U.S.	110,562
Ireland and others	0
Subtotal	142,077
Total all whiskey	*8,631,341*
Gin	
Domestic	553,942
Imported	0
Subtotal	553,942
Vodka	
Domestic	6,008,700
Imported	0
Subtotal	6,008,700
Rum	
Puerto Rico	1,034,908
Virgin Islands	89,834
Other domestic	244,789
Subtotal dom. rum	1,369,531
Subtotal imp. rum	0
Subtotal all run	1,369,531
Brandy	
Domestic	260,337
Imported	0
Subtotal	260,337
Cordials and Specs.	
Domestic	578,408
Imported	0
Subtotal	578,408
Cocktails and mixed drinks	
Domestic	110,167
Imported	0
Subtotal	110,167
Tequila	
Subtotal	308,068
Non-whiskey	
Domestic	8,881,075
Imported	308,058
Total	*9,183,133*

Table 6. U.S. Commercial Exports of Liquor by Class 1996 (in wine gal.) *(continued)*

Class and type	
N.E.S.	
Domestic	14,630,239
Imported	0
Subtotal	14,630,239
Total Dom. Distilled Spirits	*32,000,574*
Total Imp. Distilled Spirits	*450,135*
Grand Total	*32,450,713*

EXPORTS OF DISTILLED SPIRITS

The United States is well known throughout the world for its world-class whiskies and these are exported to many lands. Tastes differ widely in other countries. In some, like Germany and Japan the major export by far is bourbon whisky. In others, like Belgium, it is vodka. In all, bourbon, the prototypical U.S. whisky, is the largest distilled spirit export. Table 6 shows exports of distilled spirits in 1996.

GLOSSARY

Balling. A measure of the sugar concentration in a grain mash, expressed in degrees and approximating percent by weight of the sugar in solution.

Bushel. A distillers bushel of any cereal gain is 25.4 kg (56 lb).

Congeners. The flavor constituents in beverage spirits that are responsible for its flavor and aroma and that result from the fermentation, distillation, and maturation processes.

Feints. The third fraction of the distillation cycle derived from the distillation of low wines in a pot still. This term is also used to describe the undesirable constituents of the wash which are removed during the distillation of grain whisky in a continuous patent still (Coffey). These are mostly aldehydes and fusel oils.

Foreshots. The first fraction of the distillation cycle derived from the distillation of low wines in a pot still.

Fusel oil. An inclusive term for heavier, pungent-tasting alcohols produced during fermentation. Fusel oil is composed of approximately 80% amyl alcohols, 15% butyl alcohols, and 5% other alcohols.

Grain whisky. An alcoholic distillate from a fermented wort derived from malted and unmalted barley and maize (corn), in varying proportions, and distilled in a continuous patent still (Coffey).

Heads. A distillate containing a high percentage of low-boiling components such as aldehydes.

High wines. An all-inclusive term for beverage spirit distillates that have undergone complete distillation.

Low wines. The term for the initial product obtained by separating (in a pot still) the beverage spirits and congeners from the wash. Low wines are subjected to at least one more pot still distillation to attain a greater degree of refinement in the malt whisky.

Malt whisky. An alcoholic distillate made from a fermented wort derived from malted barley only, and distilled in pot stills. It is the second fraction (heart of the run) of the distillation process.

Proof. In Canada, the UK, and the United States, the alcoholic concentration of beverage spirits is expressed in terms of proof. The United States statutes define this standard as follows: proof spirit is held to be that alcoholic liquor that contains one-half its volume of alcohol of a specific gravity of 0.7939 at 15.6°C; ie, the figure for proof is always twice the alcoholic content by volume. For example, 100° proof means 50% alcohol by volume. In the UK as well as Canada, proof spirit is such that at 10.6°C weighs exactly twelve-thirteenths of the weight of an equal bulk of distilled water. A proof of 87.7° would indicate an alcohol concentration of 50%. A conversion factor of 1.142 can be used to change British proof to U.S. proof.

Proof gallon. A U.S. gallon of proof spirits or the alcoholic equivalent thereof; ie, a U.S. gallon of 231 in.^{3} (3,785 cm^3) containing 50% of ethyl alcohol by volume. Thus a gallon of liquor at 120° proof is 1.2 proof gal; a gallon at 86° proof is 0.86 proof gal. A British and Canadian proof gallon is an imperial gallon of 277.4 in.^{3} (4,546 cm^3) at 100° proof (57.1% of ethyl alcohol by volume). An imperial gallon is equivalent to 1.2 U.S. gal. To convert British proof gallons to U.S. proof gallons, multiply by the factor 1.37. Since excise taxes are paid on the basis of proof gallons, this term is synonymous with tax gallons.

Single whisky. The whisky, either grain or malt, produced by one particular distillery. Blended Scotch whisky is not a single whisky.

Spirits. Distilled spirits including all whiskies, gin, brandy, rum, cordials, and others made by a distillation process for nonindustrial use.

Tails. A residual alcoholic distillate.

Wash. The liquid obtained by fermenting wort with yeast. It contains the beverage spirits and congeners developed during fermentation.

Wine gallon. Measure of actual volume; U.S. gallon (3.7845118 L) contains 231 in.^{3} (3,785 cm^3); British (Imperial) gallon contains 277.4 in.^{3} (4,546 cm^3).

Wort. The liquid drained off the mash tun and containing the soluble sugars and amino acids derived from the grains.

BIBLIOGRAPHY

1. *Proceedings Before and By Direction of the President Concerning the Meaning of the Term Whisky,* U.S. Government Printing Office, Washington, D.C., 1909.
2. *Business Week* (Feb. 17, 1962).
3. *Business Week* (Mar. 21, 1977).
4. S. Baldwin et al., *Journal of Agricultural and Food Chemistry* **15**, 381 (1967).
5. C. A. Crampton and L. M. Tolman, *Journal of the American Chemical Society* **30**, 97 (1908).
6. A. J. Liebmann and M. Rosenblatt, *Industrial and Engineering Chemistry* **35**, 994 (1943)

7. A. J. Liebmann and B. Scherl, *Industrial and Engineering Chemistry* **41**, 534 (1949).
8. P. Valaer and W. H. Frazier, *Industrial and Engineering Chemistry* **28**, 92 (1936).
9. M. C. Brockmann, *Journal of the Association of Official Agricultural Chemists* **33**, 127 (1950).
10. G. H. Reazin et al., *Journal of the Association of Official Agricultural Chemists* **59**, 770 (1976).
11. S. Baldwin and A. A. Andreasen, *Journal of the Association of Official Agricultural Chemists* **53**, 940 (1974).
12. Bureau of the Census, U.S. Department of Commerce, *Distilled Spirits Council of the U.S.*, Aug. 1989.

GENERAL REFERENCES

Annual Statistical Revue (1976), Distilled Spirits Council of the US, Inc., Washington, D.C., 1977.

A. Barnard, *The Whiskey Distilleries of the United Kingdom*, London, 1887.

H. Barron, *Distillation of Alcohol*, Joseph E. Seagram & Sons, Inc., New York, 1944.

C. S. Boruff and L. A. Rittschol, *Agric. Food Chem.* **7**, 630 (1959).

Canada, Its History, Products, and Natural Resources, Department of Agriculture of Canada, Ottawa, 1906.

A. Cooper, *The Complete Distiller*, London, 1760.

H. G. Crowgey, *Kentucky Bourbon, The Early Years of Whiskey Making*, The University Press of Kentucky, Lexington, 1971.

Distillers Feed Research, Distillers Feed Research Council, Cincinnati, Ohio, 1971.

G. A. DeBecze, "Alcoholic Beverages, Distilled," *Encyclopedia of Industrial Chemical Analysis*, Vol. 4, Interscience Division of John Wiley & Sons, Inc., New York, 1967, pp. 462–494.

The Excise Act, 1934, Department of National Revenue, Ottawa, Canada, 1947, Chap. 52, pp. 24–25

G. Foth, *Handbuch der Spiritusfabrikation*, Verlag Paul Parey, Hamburg, Germany, 1929.

G. Foth, *Die Praxis des Brennereibetriebs*, Verlag Paul Parey, Hamburg, Germany, 1935.

A. Herman, E. M. Stallings, and H. F. Willkie, "Chemical Engineering Developments in Grain Distillery," *Trans. Am. Inst. Chem. Eng* **31**, 1942.

A. J. Liebmann, "Alcoholic Beverages," in R. E. Kirk and D. F. Othmer, eds., *Encyclopedia of Chemical Technology*, Vol. 1, Interscience Publishers, New York, 1947, pp. 228–303.

A. McDonald, *Whisky*, Glasgow, UK, 1934.

Methods of Analysis, 12th ed., Association of Official Agricultural Chemists, New York, 1975.

G. W. Packowski, "Alcoholic Beverages, Distilled" in A. Standen, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 1, 2nd ed., Interscience Division of John Wiley & Sons, Inc., New York, 1963, pp. 501–531.

G. W. Packowski, "Distilled Beverage Spirits," in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol 3, 3rd ed., Wiley Interscience, New York, 1978, pp. 830–869.

D. R. Peryam, *Ind. Qual. Control* **11**, 17 (1950).

Regulations, Distilleries and Their Products, Circular ED 203, Department of National Revenue, Ottawa, Canada, Mar. 30, 1961.

Regulations under the Federal Alcohol Administration Act, Alcohol, Tobacco Products and Firearms (27 CFR) ATF P 5100.8 (12/77), U.S. Government Printing Office, Washington, D.C.

J. Samuelson, *The History of Drink*, London, 1880.

E. D. Unger, H. F. Willkie, and H. C. Blankmyer, "The Development and Design of a Continuous Cooking and Mashing System for Cereal Grains," *Trans. Am. Inst. Chem. Eng.* **40**, 1944.

H. F. Willkie, *Beverage Spirits in America—A Brief History*, The Newcomen Society in North America, Downington, Pa., 1949

H. F. Willkie and J. A. Proschaska, *Fundamentals of Distillery Practice*, Joseph E. Seagram & Sons, Inc., New York, 1943.

F. B. Wright, *Distillation of Alcohol*, London, 1918.

H. Wustenfeld, *Trinkbranntweine und Liquöre*, Berlin, Germany, 1931.

C. L. Yaws, J. R. Hopper, "Methanol, Ethanol, Propanol and Butanol, Physical and Thermodynamic Properties," *Chemical Engineering* 119 (June 7, 1976).

Joseph L. Owades
Consultant
Sonoma, California

DRY MILK

HISTORY

The development of the dry milk industry apparently stems from the days of Marco Polo in the thirteenth century. Reports are that Marco Polo encountered "sun dried milk" on his journeys through Mongolia and that from this beginning dry milk products evolved.

Through early pioneering scientists, such as Nicholas Appert and Gail Borden, basic processing methods were developed for drying milk products. Martin Ekenberg and Lewis Merrill have been acknowledged as developers of the first commercial roller- and spray-process drying systems, respectively, in the United States.

Since the initial development of commercial drying systems, significant technological advances have been made resulting in such widely recognized and used dry milk products as nonfat dry milk, dry whole milk, and dry buttermilk, which may be manufactured by roller- or spray-dryers (now mostly by the latter) or by more unique processes such as foam or freeze drying. These same dry milk products also may be processed in such a manner as to make them readily soluble and thus "instantized."

PROCESSING

The steps in a typical dry milk processing operation are as follows:

- Receipt of fresh, high-quality milk from modern dairy farms, delivered in refrigerated, stainless steel bulk tankers.
- Separation of the milk (if nonfat dry milk is to be manufactured) to remove milkfat, which commonly is churned into butter. If dry whole milk is to be manufactured, the separation step is omitted but may be replaced by clarification.
- Pasteurization by a continuous high-temperature short-time (HTST) process whereby every particle of milk is subjected to a heat treatment of at least 71.7°C for 15 sec.
- Holding the pasteurized milk at an elevated temperature for an extended period of time (76.7°C for

7. A. J. Liebmann and B. Scherl, *Industrial and Engineering Chemistry* **41**, 534 (1949).
8. P. Valaer and W. H. Frazier, *Industrial and Engineering Chemistry* **28**, 92 (1936).
9. M. C. Brockmann, *Journal of the Association of Official Agricultural Chemists* **33**, 127 (1950).
10. G. H. Reazin et al., *Journal of the Association of Official Agricultural Chemists* **59**, 770 (1976).
11. S. Baldwin and A. A. Andreasen, *Journal of the Association of Official Agricultural Chemists* **53**, 940 (1974).
12. Bureau of the Census, U.S. Department of Commerce, *Distilled Spirits Council of the U.S.*, Aug. 1989.

GENERAL REFERENCES

Annual Statistical Review (1976), Distilled Spirits Council of the US, Inc., Washington, D.C., 1977.

A. Barnard, *The Whiskey Distilleries of the United Kingdom*, London, 1887.

H. Barron, *Distillation of Alcohol*, Joseph E. Seagram & Sons, Inc., New York, 1944.

C. S. Boruff and L. A. Rittschol, *Agric. Food Chem.* **7**, 630 (1959).

Canada, Its History, Products, and Natural Resources, Department of Agriculture of Canada, Ottawa, 1906.

A. Cooper, *The Complete Distiller*, London, 1760.

H. G. Crowgey, *Kentucky Bourbon, The Early Years of Whiskey Making*, The University Press of Kentucky, Lexington, 1971.

Distillers Feed Research, Distillers Feed Research Council, Cincinnati, Ohio, 1971.

G. A. DeBecze, "Alcoholic Beverages, Distilled," *Encyclopedia of Industrial Chemical Analysis*, Vol. 4, Interscience Division of John Wiley & Sons, Inc., New York, 1967, pp. 462–494.

The Excise Act, 1934, Department of National Revenue, Ottawa, Canada, 1947, Chap. 52, pp. 24–25

G. Foth, *Handbuch der Spiritusfabrikation*, Verlag Paul Parey, Hamburg, Germany, 1929.

G. Foth, *Die Praxis des Brennereibetriebs*, Verlag Paul Parey, Hamburg, Germany, 1935.

A. Herman, E. M. Stallings, and H. F. Willkie, "Chemical Engineering Developments in Grain Distillery," *Trans. Am. Inst. Chem. Eng* **31**, 1942.

A. J. Liebmann, "Alcoholic Beverages," in R. E. Kirk and D. F. Othmer, eds., *Encyclopedia of Chemical Technology*, Vol. 1, Interscience Publishers, New York, 1947, pp. 228–303.

A. McDonald, *Whisky*, Glasgow, UK, 1934.

Methods of Analysis, 12th ed., Association of Official Agricultural Chemists, New York, 1975.

G. W. Packowski, "Alcoholic Beverages, Distilled" in A. Standen, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 1, 2nd ed., Interscience Division of John Wiley & Sons, Inc., New York, 1963, pp. 501–531.

G W. Packowski, "Distilled Beverage Spirits," in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol 3, 3rd ed., Wiley Interscience, New York, 1978, pp. 830–869.

D. R. Peryam, *Ind. Qual. Control* **11**, 17 (1950).

Regulations, Distilleries and Their Products, Circular ED 203, Department of National Revenue, Ottawa, Canada, Mar. 30, 1961.

Regulations under the Federal Alcohol Administration Act, Alcohol, Tobacco Products and Firearms (27 CFR) ATF P 5100.8 (12/77), U.S. Government Printing Office, Washington, D.C.

J. Samuelson, *The History of Drink*, London, 1880.

E. D. Unger, H. F. Willkie, and H C. Blankmyer, "The Development and Design of a Continuous Cooking and Mashing System for Cereal Grains," *Trans. Am. Inst. Chem. Eng.* **40**, 1944.

H. F. Willkie, *Beverage Spirits in America—A Brief History*, The Newcomen Society in North America, Downington, Pa., 1949

H. F. Willkie and J. A. Proschaska, *Fundamentals of Distillery Practice*, Joseph E. Seagram & Sons, Inc., New York, 1943.

F. B. Wright, *Distillation of Alcohol*, London, 1918.

H. Wustenfeld, *Trinkbranntweine und Liquöre*, Berlin, Germany, 1931.

C. L. Yaws, J. R. Hopper, "Methanol, Ethanol, Propanol and Butanol, Physical and Thermodynamic Properties," *Chemical Engineering* 119 (June 7, 1976).

JOSEPH L. OWADES
Consultant
Sonoma, California

DRY MILK

HISTORY

The development of the dry milk industry apparently stems from the days of Marco Polo in the thirteenth century. Reports are that Marco Polo encountered "sun dried milk" on his journeys through Mongolia and that from this beginning dry milk products evolved.

Through early pioneering scientists, such as Nicholas Appert and Gail Borden, basic processing methods were developed for drying milk products. Martin Ekenberg and Lewis Merrill have been acknowledged as developers of the first commercial roller- and spray-process drying systems, respectively, in the United States.

Since the initial development of commercial drying systems, significant technological advances have been made resulting in such widely recognized and used dry milk products as nonfat dry milk, dry whole milk, and dry buttermilk, which may be manufactured by roller- or spray-dryers (now mostly by the latter) or by more unique processes such as foam or freeze drying. These same dry milk products also may be processed in such a manner as to make them readily soluble and thus "instantized."

PROCESSING

The steps in a typical dry milk processing operation are as follows:

- Receipt of fresh, high-quality milk from modern dairy farms, delivered in refrigerated, stainless steel bulk tankers.
- Separation of the milk (if nonfat dry milk is to be manufactured) to remove milkfat, which commonly is churned into butter. If dry whole milk is to be manufactured, the separation step is omitted but may be replaced by clarification.
- Pasteurization by a continuous high-temperature short-time (HTST) process whereby every particle of milk is subjected to a heat treatment of at least 71.7°C for 15 sec.
- Holding the pasteurized milk at an elevated temperature for an extended period of time (76.7°C for

25–30 min.)—a step used only in the manufacture of high-heat nonfat dry milk, which commonly is used as an ingredient in yeast-raised bakery products and meat products.

- Condensing the milk by removing water in an evaporator or vacuum pan until a milk solids content of 40% is reached.
- Delivery of the condensed product to the dryer.

Commercial U.S. drying processes are of two types—roller (drum) and spray. The former currently is used only to a limited extent. In this process, two large rollers, usually steam-heated internally and located adjacent and parallel to each other, revolve in opposite directions contacting a reservoir of either pasteurized fluid or condensed milk. During rotation, the fluid milk product dries on the hot roller surface. After approximately three-quarters of a revolution, a carefully positioned, sharp stationary knife detaches the milk product, now in the form of a thin dry sheet. The dry milk next is conveyed by an auger to a hammermill where it undergoes a physical treatment to convert it into uniformly fine particles which then are packaged, usually in 25-kg polyethylene-lined multiwall bags.

Two basic configurations of spray dryers presently are in use, these being horizontal (box) and vertical (tower) dryers. In both, pasteurized fluid milk, which has been condensed to a total solids of 40% or greater, is fed under pressure to a spray nozzle, or an atomizer, where the dispersed liquid then comes into contact with a current of filtered, heated air. The droplets of condensed milk are dried almost immediately and fall to the bottom of the fully enclosed, stainless steel drying chamber. The dry milk product continuously is removed from the drying chamber, transported through a cooling and collecting system, and finally conveyed into a hopper for packaging—usually into 25-kg bags, or in tote bins.

Table 1. Yearly U.S. Production of Dry Milks[a]

Year	Nonfat dry milk	Dry whole milk	Dry buttermilk
1960	1,818.6	98.0	86.4
1961	2,019.8	81.7	89.0
1962	2,230.3	86.1	86.4
1963	2,106.1	91.0	87.5
1964	2,177.2	87.6	92.0
1965	1,988.5	88.6	87.4
1966	1,579.8	94.4	76.2
1967	1,678.7	74.3	72.6
1968	1,594.4	79.8	70.4
1969	1,452.3	70.2	66.5
1970	1,444.4	68.9	59.5
1971	1,417.6	72.2	51.7
1972	1,223.5	75.2	49.5
1973	916.6	78 0	43.3
1974	1,020.0	67.7	45.3
1975	1,001.5	63.1	42.8
1976	926.2	78.1	46.3
1977	1,106.6	69 4	53.2
1978	920.4	74 6	47.6
1979	908.7	85.3	44.7
1980	1,160.7	82.7	43.9
1981	1,314.3	92.7	43.8
1982	1,400.5	102.2	38.6
1983	1,499.9	111.2	46.5
1984	1,160.8	119.6	43.5
1985	1,390.0	118.9	51.5
1986	1,284.1	122.4	65.7
1987	1,056.8	145.9	55.6
1988	978.5	172.3	58.7
1989	874.7	175.8	60.5
1990	879.2	175.1	55.9
1991	877.5	106.8	60 0
1992	872.1	168.3	61.1
1993	954.5	153.8	51.0
1994	1,215.6	166.8	52.3
1995	1,233.0	171.3	54.8
1996	1,061.6	134.4	48.8
1997	1,217.6	122.1	49.3
1998	1,121.3	138.7	49.3

[a] In millions of pounds.

THE PRODUCT

The primary dehydrated dairy products manufactured domestically are nonfat dry milk, dry whole milk, and dry buttermilk.

Nonfat dry milk is the product resulting from the removal of fat and water from milk. It contains lactose, milk proteins, and milk minerals in the same relative proportions as in the fresh milk from which it was made. Nonfat dry milk contains not over 5% by weight of moisture. The fat content is not over 1.5% by weight unless otherwise indicated.

Dry whole milk is the product resulting from the removal of water from milk and contains not less than 26% milkfat and not more than 4% moisture. Dry whole milk with milkfat contents of 26 and 28.5% commonly are produced.

Table 2. Approximate Composition and Food Value of Dry Milks

Constituents	Nonfat dry milk	Dry whole milk	Dry buttermilk
Protein ($N \times 6.38$) (%)	36.0	26.0	34.0
Lactose (milk sugar) (%)	51.0	38.0	48.0
Fat (%)	0.7	26.75	5.0
Moisture (%)	3.0	2.25	3.0
Minerals (ash) (%)	8.2	6.0	7 9
Calcium (%)	(1.31)	(0.97)	(1.3)
Phosphorus (%)	(1 02)	(0 75)	(1.0)
Vitamin A (IU/lb)	165.0	4,950.0	2,300.0
Riboflavin (mg/lb)	9.2	6.7	14.0
Thiamin (mg/lb)	1.6	1.2	1.2
Niacin (mg/lb)	4.2	3.1	4.5
Niacin equivalents[a] (mg/lb)	42.2	30.6	40.6
Pantothenic acid (mg/lb)	15.0	13.0	14.0
Pyridoxine (mg/lb)	2.0	1.5	2.0
Biotin (mg/lb)	0.2	0.185	0.2
Choline (mg/lb)	500.0	400.0	500.0
Energy (cal/lb)	1,630.0	2,260.0	1,700.0

[a] Includes contribution of tryptophan.

Dry buttermilk is the product resulting from the removal of water from liquid buttermilk derived from the manufacture of butter. It contains not less than 4.5% milkfat and not more than 5% moisture.

All of these products may be processed in such a way that their dispersing and reliquefaction properties are substantially improved. When this is accomplished, the products are called instantized. "Single-pass" and "agglomerating" processes are used to make instant dry milk products, with the primarily method being the latter.

Table 1 reflects the annual domestic production of nonfat dry milk, dry whole milk, and dry buttermilk during the period 1960 to 1998. Standards for dry milk products initially were developed by the dairy industry in 1929. Since that time, additional product standards have been developed—and revised—both by industry and government agencies. These standards are based on various general and specific product characteristics, which serve as a basis for determining overall quality of the dry milk products. Table 2 shows the approximate composition and food value of the three most commonly manufactured dry milk products.

UTILIZATION

An industry-wide survey of end uses for dry milk products is conducted annually by the American Dairy Products Institute. Data compiled in the survey are published under the title *Dry Milk Products—Utilization and Production Trends*. Included in the census data are end-use markets for nonfat dry milk, dry whole milk, and dry buttermilk. For the past several years the largest markets for nonfat dry milk have been dairy, prepared dry mixes, confectionery, and bakery. These market areas accounted for 781 million pounds in 1998—approximately 89% of the domestic sales. The primary market for dry whole milk is confectionery. In 1998, this market area accounted for 88 million pounds of dry whole milk—over 66% of the domestic sales for this product. Dry buttermilk is used in three primary markets—dairy, bakery, and prepared dry mixes. In 1998, these markets used 39.6 million pounds of dry buttermilk—representing 89% of domestic sales.

GENERAL REFERENCES

American Dairy Products Institute, *Standards for Grades of Dry Milks Including Methods of Analysis*, Bulletin 916 (Rev.), American Dairy Products Institute, Chicago, Ill., 1990.

American Dairy Products Institute, *Dry Milk Products—Utilization and Production Trends, 1997*, Bulletin 1000, American Dairy Products Institute, Chicago, Ill., 1999.

C. E Beardslee, *Dry Milks—The Story of an Industry*, American Dry Milk Institute, Chicago, Ill., 1948.

C. W. Hall and T. I. Hedrick, *Drying of Milk and Milk Products*, 2nd ed., AVI Publishing, Westport, Conn., 1971.

WARREN S. CLARK, JR.
American Dairy Products Institute
Chicago, Illinois

DRYERS: TECHNOLOGY AND ENGINEERING

Throughout the food processing industries, there are many and varied requirements for thermal drying. Some involve the removal of moisture or volatiles from various food ingredients or products that differ in both chemical and physical characteristics. Others involve the drying of solutions or liquid suspensions and different approaches to the problem. To assist manufacturers in arriving at a reasonably accurate first assessment of the type, size, and cost of element for a particular duty, this article describes the most widely used types of both batch and continuous dryers in the food industries and gives an indication of approximate sizes and capital costs for typical installations.

Three basic methods of heat transfer are used in industrial dryers in varying degrees of prominence and combinations, specifically, convection, conduction, and radiation.

In the chemical processing industry, the majority of dryers employ forced convection and continuous operation. With the exception of the indirectly heated rotary dryer and the film drum dryer, units in which heat is transferred by conduction are suitable only for batch use. This limitation effectively restricts them to applications involving somewhat modest production runs.

Radiant, or "infrared," heating is rarely used in drying materials such as fine chemicals or pigments. Its main application is in such operations as the drying of surface coatings on large plane surfaces since for efficient utilization, it generally is true that the material being irradiated must have a sight of the heat source or emitter. There is, however, in all the dryers considered here a radiant component in the heat-transfer mechanism.

Direct heating is used extensively in industrial drying equipment where much higher thermal efficiencies are exhibited than with indirectly heated dryers. This is because there are no heat exchanger losses and the maximum heat release from the fuel is available for the process. However, this method is not always acceptable, especially where product contamination cannot be tolerated. In such cases, indirect heating must be used.

With forced-convection equipment, indirect heating employs a condensing vapor such as steam in an extended surface tubular heat exchanger or in a steam jacket where conduction is the method of heat transfer. Alternative systems that employ proprietary heat-transfer fluids also can be used. These enjoy the advantage of obtaining elevated temperatures without the need for high-pressure operation as may be required with conventional steam heating. This may be reflected in the design and manufacturing cost of the dryer. Furthermore, in addition to the methods listed above, oil- or gas-fired indirect heat exchangers also can be used.

In general, dryers are either suitable for batch or continuous operation. A number of the more common types are listed in Table 1, where an application rating based on practical considerations is given. In the following review, some of the factors likely to influence selection of the various types are discussed for particular applications.

BATCH DRYERS

It will be apparent that batch operated equipment usually is related to small production runs or to operations requir-

Dry buttermilk is the product resulting from the removal of water from liquid buttermilk derived from the manufacture of butter. It contains not less than 4.5% milkfat and not more than 5% moisture.

All of these products may be processed in such a way that their dispersing and reliquefaction properties are substantially improved. When this is accomplished, the products are called instantized. "Single-pass" and "agglomerating" processes are used to make instant dry milk products, with the primarily method being the latter.

Table 1 reflects the annual domestic production of nonfat dry milk, dry whole milk, and dry buttermilk during the period 1960 to 1998. Standards for dry milk products initially were developed by the dairy industry in 1929. Since that time, additional product standards have been developed—and revised—both by industry and government agencies. These standards are based on various general and specific product characteristics, which serve as a basis for determining overall quality of the dry milk products. Table 2 shows the approximate composition and food value of the three most commonly manufactured dry milk products.

UTILIZATION

An industry-wide survey of end uses for dry milk products is conducted annually by the American Dairy Products Institute. Data compiled in the survey are published under the title *Dry Milk Products—Utilization and Production Trends*. Included in the census data are end-use markets for nonfat dry milk, dry whole milk, and dry buttermilk. For the past several years the largest markets for nonfat dry milk have been dairy, prepared dry mixes, confectionery, and bakery. These market areas accounted for 781 million pounds in 1998—approximately 89% of the domestic sales. The primary market for dry whole milk is confectionery. In 1998, this market area accounted for 88 million pounds of dry whole milk—over 66% of the domestic sales for this product. Dry buttermilk is used in three primary markets—dairy, bakery, and prepared dry mixes. In 1998, these markets used 39.6 million pounds of dry buttermilk—representing 89% of domestic sales.

GENERAL REFERENCES

American Dairy Products Institute, *Standards for Grades of Dry Milks Including Methods of Analysis*, Bulletin 916 (Rev.), American Dairy Products Institute, Chicago, Ill., 1990.

American Dairy Products Institute, *Dry Milk Products—Utilization and Production Trends, 1997*, Bulletin 1000, American Dairy Products Institute, Chicago, Ill., 1999.

C. E Beardslee, *Dry Milks—The Story of an Industry*, American Dry Milk Institute, Chicago, Ill., 1948.

C. W. Hall and T. I. Hedrick, *Drying of Milk and Milk Products*, 2nd ed., AVI Publishing, Westport, Conn., 1971.

WARREN S. CLARK, JR.
American Dairy Products Institute
Chicago, Illinois

DRYERS: TECHNOLOGY AND ENGINEERING

Throughout the food processing industries, there are many and varied requirements for thermal drying. Some involve the removal of moisture or volatiles from various food ingredients or products that differ in both chemical and physical characteristics. Others involve the drying of solutions or liquid suspensions and different approaches to the problem. To assist manufacturers in arriving at a reasonably accurate first assessment of the type, size, and cost of element for a particular duty, this article describes the most widely used types of both batch and continuous dryers in the food industries and gives an indication of approximate sizes and capital costs for typical installations.

Three basic methods of heat transfer are used in industrial dryers in varying degrees of prominence and combinations, specifically, convection, conduction, and radiation.

In the chemical processing industry, the majority of dryers employ forced convection and continuous operation. With the exception of the indirectly heated rotary dryer and the film drum dryer, units in which heat is transferred by conduction are suitable only for batch use. This limitation effectively restricts them to applications involving somewhat modest production runs.

Radiant, or "infrared," heating is rarely used in drying materials such as fine chemicals or pigments. Its main application is in such operations as the drying of surface coatings on large plane surfaces since for efficient utilization, it generally is true that the material being irradiated must have a sight of the heat source or emitter. There is, however, in all the dryers considered here a radiant component in the heat-transfer mechanism.

Direct heating is used extensively in industrial drying equipment where much higher thermal efficiencies are exhibited than with indirectly heated dryers. This is because there are no heat exchanger losses and the maximum heat release from the fuel is available for the process. However, this method is not always acceptable, especially where product contamination cannot be tolerated. In such cases, indirect heating must be used.

With forced-convection equipment, indirect heating employs a condensing vapor such as steam in an extended surface tubular heat exchanger or in a steam jacket where conduction is the method of heat transfer. Alternative systems that employ proprietary heat-transfer fluids also can be used. These enjoy the advantage of obtaining elevated temperatures without the need for high-pressure operation as may be required with conventional steam heating. This may be reflected in the design and manufacturing cost of the dryer. Furthermore, in addition to the methods listed above, oil- or gas-fired indirect heat exchangers also can be used.

In general, dryers are either suitable for batch or continuous operation. A number of the more common types are listed in Table 1, where an application rating based on practical considerations is given. In the following review, some of the factors likely to influence selection of the various types are discussed for particular applications.

BATCH DRYERS

It will be apparent that batch operated equipment usually is related to small production runs or to operations requir-

Table 1. Product Classification and Dryer Types as an Aid to Selection

	Evaporation rate, lb/ft^2 · h mean rate = E_{av}	Fluids, liquid suspension	Pastes, dewatered cake	Powders	Granules, pellets, extrudates	Operation
Forced convection (cross-airflow)	0.15–0.25 E_{av} = 0.2	Poor	Fair	Fair	Good	Batch
Forced convection (throughflow)	1.0–2.0 E_{av} = 1.5	—	—	—	Good	Batch
Agitated pan (sub-atmospheric)	1.0–5.0 E_{av} = 3.0	Fair	Fair	Fair	Poor	Batch
Agitated pan (atmospheric)	1.0–5.0 E_{av} = 3.0	Fair	Fair	Fair	Poor	Batch
Double-cone tumbler (sub atmospheric)	1.0–3.0 E_{av} = 2.0	—	Poor	Fair	Poor	Batch
Fluidized bed (throughflow)	2–50 E_{av} = 26	—	—	Good	Good	Continuous
Conveyor band (throughflow)	2.0–10 0 E_{av} = 6.0	—	Fair	—	Good	Continuous
Rotary (indirect)	1.0–3.0^a E_{av} = 2.0	—	Poor	Good	Fair	Continuous
Rotary (direct)	2.0–6.0^a E_{av} = 4.0	—	Fair	Fair	Good	Continuous
Film drum (atmospheric)	3.0–6.0 E_{av} = 4.5	Good	Fair	—	—	Continuous
Pulumatic or flash	50–250 E_{av} = 150	—	Fair	Good	Fair	Continuous
Spray	7.0–33.0 E_{av} = 20.0	Good	—	—	—	Continuous

[a] Evaporation rates for rotary dryers are expressed in lb/ft^3 · h.

ing great flexibility. As a result, the batch-type forced-convection unit certainly finds the widest possible application of any dryer used today.

The majority of designs employ recirculatory air systems incorporating large-volume, low-pressure fans that, with the use of properly insulated enclosures, usually provide thermal efficiencies in the region of 50–60%. However, in special applications of this type of dryer that call for total air rejection, this figure is somewhat lower and is largely related to the volume and temperature of the exhaust air. Capital investment is relatively low, as are installation costs. Furthermore, by using the fan systems, both power requirements and operating costs also are minimal. Against these advantages, labor costs can be high.

In such a plant, the drying cycles are extended, with 24–45 hours being quite common in certain cases. This is a direct result of the low evaporative rate, which normally is in the region of 0.15–0.25 lb/ft^2 h.

Following the recent trend and interest shown in preforming feedstock and in particular with regard to the design of extruding and tray-filling equipment for dewatered cakes, it is now possible to obtain the maximum benefit of enhanced evaporative rates by using through-air circulation dryers when handling preformed materials. Figure 1 shows how a high-performance dryer can product 1950 pounds of dried material in a 24-h period at a terminal figure of 0.5% moisture when handling a preformed filter cake having an initial moisture content of 58%. The very great improvement in performance can readily be seen from the curve, in which it is clear that the corresponding number of conventional two-truck recirculatory units would be between seven and eight for the same duty. The advantage is more apparent when it is seen that respective floor areas occupied are 55 ft^2 for the Thruflo dryer pictured in Figure 2 and 245 ft^2 in the case of conventional units using transverse airflow.

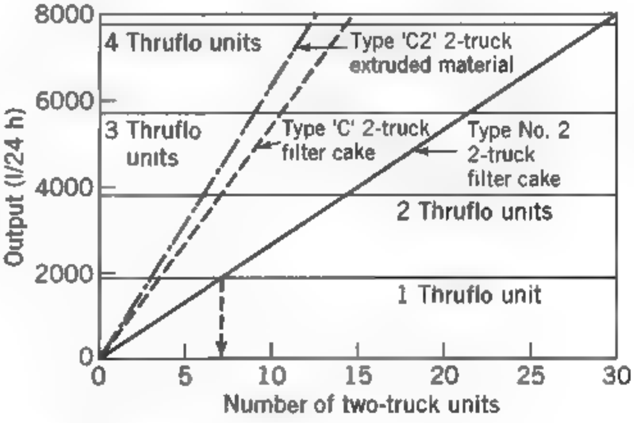


Figure 1. Comparative performance curves for Thruflo and conventional units.

Reference to the drying curves for the processing of materials in solid or filter cake form or, in fact, in the case of wet powders, clearly indicates that the ultimate rate-governing factor is the rate of diffusion of moisture from the wet mass. This becomes increasingly so during the falling rate period of drying. This situation, however, can be

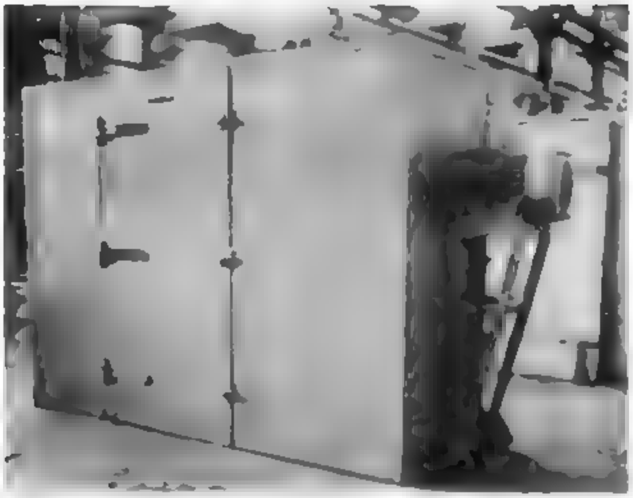


Figure 2. Thruflo dryer.

improved by preforming the product in order to increase the effective surface area presented to heat and mass transfer. The logical extension of this technique is to total dispersion drying (flash or pneumatic dryers, fluid beds, etc) where discrete particles can be brought into contact with the hot gas. This produces rapid heat transfer with correspondingly short drying times.

Batch-type fluidized-bed dryers have, therefore, superseded forced-convection units in many cases, notably in the drying of pharmaceuticals. These machines generally are available in a range of standard sizes with batch capacities of 50–200 lb, although much larger units are made for special applications.

When considering this type of dryer, it is important to ensure that the feed material can be fluidized in both initial and final conditions. It also should be remembered that standard fan arrangements are not equally suitable for a variety of materials of different densities. Therefore, it is necessary to determine accurately the minimum fluidizing velocity for each product.

If the feedback is at an acceptable level of moisture content for fluidization, this type of dryer provides many advantages over a batch-type unit. Simplified loading and unloading results in lower labor costs, high thermal efficiencies are common, and the drying time is reduced to minutes as opposed to hours in conventional units. Current developments of this type of equipment now include techniques for the simultaneous evaporation of water and the granulation of solids. This makes the units ideal for use in the pharmaceutical field.

The various batch dryers referred to operate by means of forced convection, the transfer of thermal energy being designed to increase the vapor pressure of the absorbed moisture while the circulated air scavenges the overlying vapor. Good conditions thus are maintained for continued effective drying. Alternatively and where the material is thermosensitive—implying low temperatures with consequently low evaporative rates—some improvement can be effected by the use of subatmospheric dryers, ie, by reducing the vapor pressure. Several different configurations are in use and all fall into the category of conduction-type dryers. The most usual type of heating is by steam, although hot water or one of the proprietary heat-transfer fluids can be used.

Two particular types are the double-cone dryer (Fig. 3) with capacities of ≤400 ft^3 and the agitated-pan dryer not normally larger than 8 ft diameter, where average evaporative rates per unit wetted area usually are in the region of 4 lb/ft^2 h. These units are comparatively simple to operate and, when adequately insulated, are thermally quite efficient, although drying times can be extended. They are especially suitable for applications involving solvent recovery and will handle powders and granules moderately well. They do, however, suffer from the disadvantage with some materials that the tumbling action in double-cone dryers and the action of the agitator in agitated-pan machines can produce a degree of attrition in the dried product that may prove unacceptable.

Similarly, quite large rotary vacuum dryers are used for pigment pastes and other such materials, especially where organic solvents present in the feedstock have to be recovered. These units normally are jacketed and equipped with an internal agitator that constantly lifts and turns the material. Heat transfer here is entirely by conduction from the wall of the dryer and from the agitator. Owing to the nature of their construction, initial cost is high relative to capacity. Installation costs also are considerable. In general, these dryers find only limited application.

CONTINUOUS DRYERS

For the drying of liquids of liquid suspensions, two types of dryers can be used: film drum dryers for duties in the region of 600 lb/h for a larger dryer of about 4 ft diameter × 10 ft face length or large spray dryers (as in Fig. 4) with drying rates of approximately 22,000 lb/h. Where tonnage production is required, the drum dryer is at a disadvan-

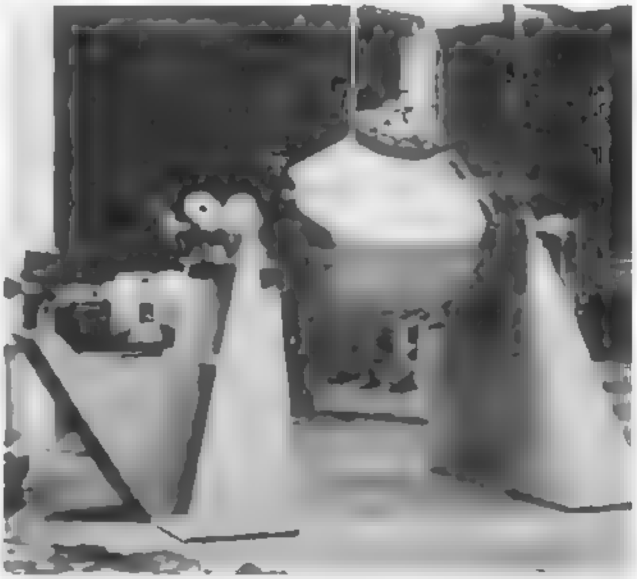


Figure 3. Double-cone vacuum dryer.

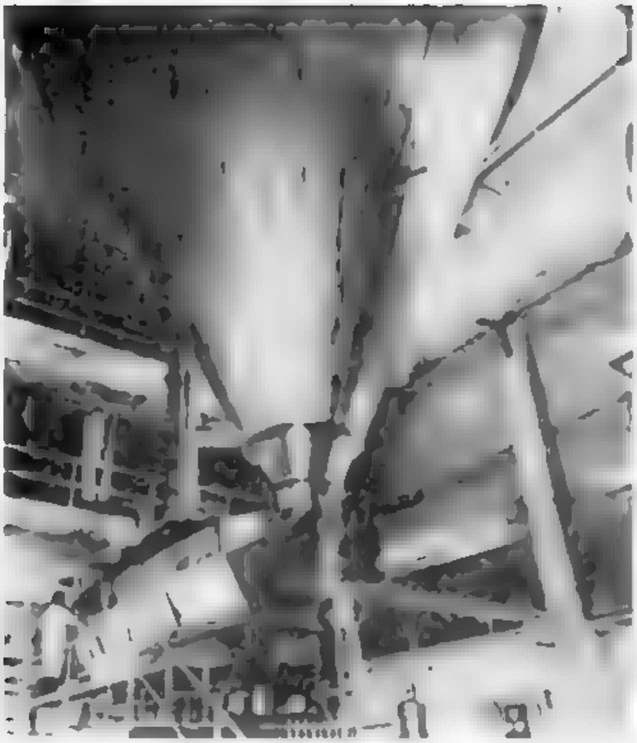


Figure 4. Conical section of a large spray dryer.

tage. However, the thermal efficiency of the drum dryer is high in the region of 1.3–1.5 lb steam/lb of water evaporated and for small to medium production runs, it does have many applications.

Drum dryers usually are steam-heated, although work has been done involving the development of units for direct gas or oil heating. Completely packaged and capable of independent operation, these dryers can be divided into two broad classifications, ie, single-drum and double-drum.

Double-drum machines normally employ a "nip" feed device with the space between the drums capable of being adjusted to provide a means of controlling the film thickness. Alternatively, and in the case of the single-drum types, a variety of feeding methods can be used to apply material to the drum. The most usual is the simple "dip" feed. With this arrangement, good liquor circulation in the trough is desirable in order to avoid increasing the concentration of the feed by evaporation. Again, for special applications, single-drum dryers use top roller feed. While the number of rolls is related to the particular application and the material being handled, in general this method of feeding is used for pasty materials such as starches. Where the feed is very mobile, rotating devices such as spray feeds are used.

It will be seen from the Figure 5 drawings that there are a number of different feeding arrangements for drum dryers, all of which have a particular use. In practice, these variants are necessary owing to the differing characteristics of the materials to be dried and to the fact that no universally satisfactory feeding device has yet been developed. This again illustrates the need for testing, not only in support of theoretical calculations for the determination of the best dryer size but also to establish where a satisfactory film can be formed.

It must be emphasized that the method of feeding the product to the dryer is of paramount importance to selection or design. There are, of course, certain materials that are temperature-sensitive to such a degree that their handling would preclude the use of an atmospheric drum dryer. In such cases, special subatmospheric equipment may provide the answer, although the capital cost in relation to output generally would restrict its use to premium grade products.

As an alternative, the spray dryer offers an excellent solution to many drying problems. Many materials that would suffer from thermal degradation if dried by other methods often can be handled by spray drying owing to the rapid flash evaporation and its accompanying cooling effect. The continuous method of operation also lends itself to large outputs and with the correct application of control equipment, to low labor cost as well.

SPRAY DRYERS

Fundamentally, the spray-drying process is a simple one. However, the design of an efficient spray-drying plant requires considerable expertise along with access to large-scale test facilities, particularly where particle size and bulk density requirements in the dried product are critical. The sizing of spray dryers on a purely thermal basis is a comparatively simple matter since the evaporation is entirely a function of the Δt across the dryer. Tests on pilot-scale equipment are not sufficient in the face of such imponderables as possible wall buildup, bulk density, and particle size predictions. Atomization of the feed is of prime importance to efficient drying and three basic feed devices are used extensively:

1. Single-fluid nozzle or pressure type
2. Two-fluid nozzle or pneumatic type
3. Centrifugal (spinning disk)

The single-fluid nozzle produces a narrow spray of fine particles. While a multiplicity of nozzles of this type are used in tonnage plants to obtain the desired feedrate, because of the high pressures employed (up to 7000 psig), excessive wear can result, particularly with abrasive products, as an alternative, the two-fluid nozzle with external mixing is used for a variety of abrasive materials. This system generally is limited to small-capacity installations. Normally, the feed is pumped at about 25 psig merely to induce mobility while the secondary fluid is introduced at 50–100 psig, thus producing the required atomization.

Centrifugal atomization achieves dispersion by centrifugal force; the feed liquor is pumped to a spinning disk. This system is suitable for and generally used on large productions. When stacked or multiple disks are employed, feed rates of 40,000–60,000 lb/h are not uncommon.

Many spray dryer configurations are in current use along with a variety of airflow patterns. The nature of the chamber geometry selected is strictly related to the system

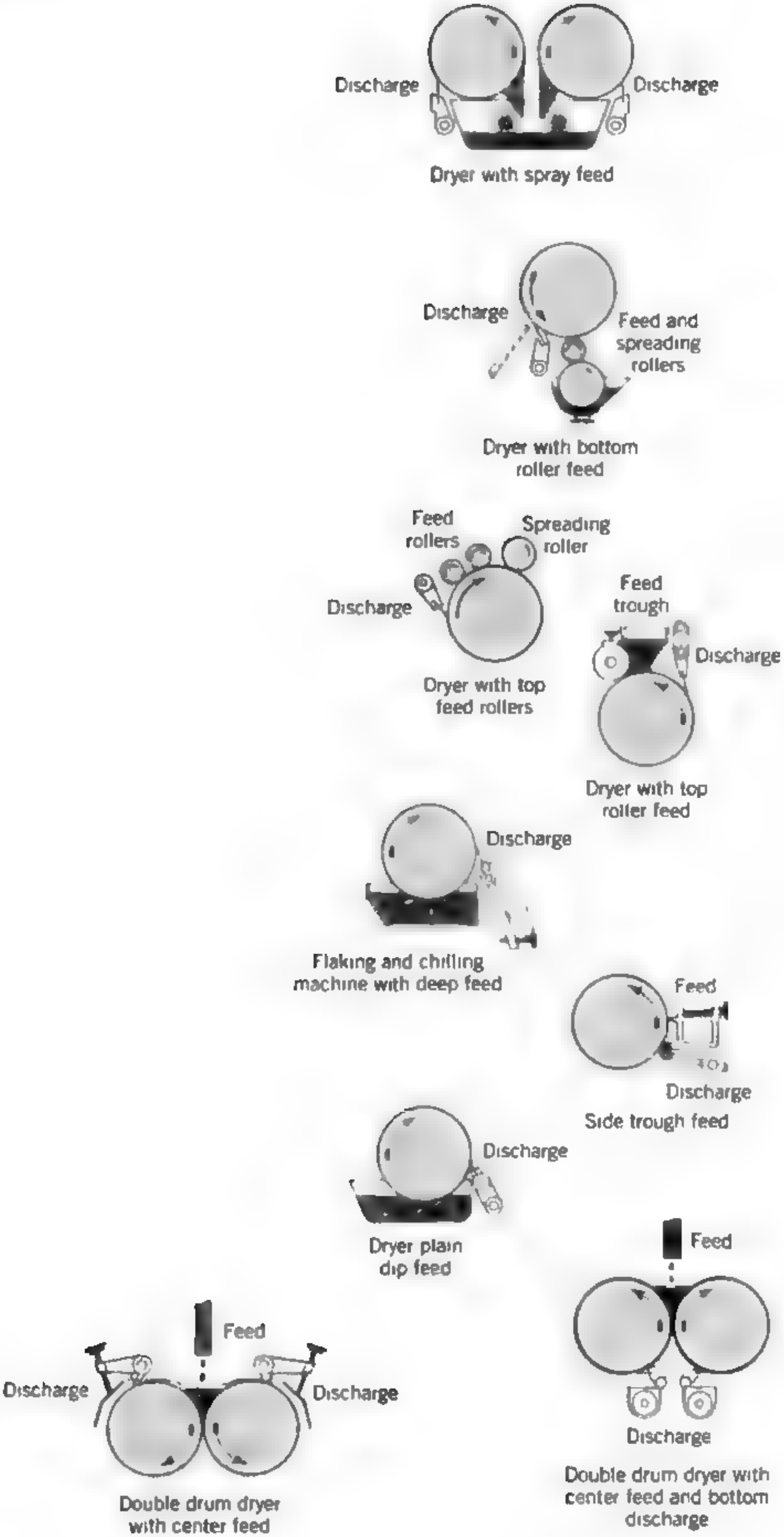

Discharge
Discharge
Dryer with spray feed
Discharge
Feed and spreading rollers
Dryer with bottom roller feed
Feed rollers
Spreading roller
Discharge
Dryer with top feed rollers
Feed trough
Discharge
Dryer with top roller feed
Discharge
Flaking and chilling machine with deep feed
Feed
Discharge
Side trough feed
Discharge
Dryer plain dip feed
Feed
Discharge
Double drum dryer with center feed and bottom discharge
Feed
Discharge
Discharge
Double drum dryer with center feed

Figure 5. Feeding arrangements for drum dryers.

of atomization used. An example of this is the tower configuration designed to accommodate the inverted jet of the two-fluid nozzle whereas the cylinder and cone of the more usual configuration are designed for the spray pattern produced by a disk-type atomizer (Fig. 6).

The product collection systems incorporated in spray-drying installations are many and varied and can constitute a substantial proportion of the total capital investment. In some cases, this can be as high as 20–25% of the installed plant cost. It also must be remembered that to be suitable for spray drying, the feed must be in a pumpable condition. Therefore, consideration must be given to the upstream process, ie, whether there is any need to reslurry in order to make the feed suitable for spray drying.

It generally is accepted that mechanical dewatering is less costly than thermal methods and while the spray dryer exhibits quite high thermal efficiencies, it often is at a disadvantage relative to other drying systems because of the greater absolute weight of water to be evaporated owing to the nature of the feed. It is, however, interesting to consider this point further. A classic case for comparison is provided by a thixotropic material that may be handled either in a spray dryer using a mechanical disperser doing work on a filtered cake to make it amenable to spray drying or alternatively, drying the same feedstock on a continuous-band dryer. In the latter instance, the cake is fed to an extruder and suitably preformed prior to being deposited on the conveyor band.

The operating costs presented in Table 2 are based on requirements for thermal and electrical energy only. No consideration is given to labor costs for either type of plant since these are likely to be approximately the same. Probably the most obvious figure emerging from this comparison is the 20% price differential in favor of the continuous-band dryer. Furthermore, while energy costs favor spray drying, they are not significantly lower.

It is, of course, impossible to generalize since the economic viability of a drying process ultimately depends on the cost per pound of the dried product and, as mentioned previously, the spray dryer usually has a greater amount of water to remove by thermal methods than other types. In the particular case illustrated, the spray dryer would have an approximate diameter of 21 ft for the evaporation of 6000 lb/h. If, however, the feed solids were reduced to 30% by dilution, the hourly evaporation rate would increase to 14,000 lb and the chamber diameter would be about 30 ft with corresponding increases in thermal input and air volume. The system would, as a result, also require larger fans and product collection systems. The overall thermal efficiency would remain substantially constant at 76% with reducing feed solids. Installed plant costs, however, increase proportionally with increasing dryer size necessary for the higher evaporation involved in producing a dried output equivalent to that shown in Table 2.

Spray drying does have many advantages, particularly with regard to the final product form. This is especially so where pressing grade materials are required, ie, in the production of ceramics and dust-free products such as dyestuffs. It is certain that with the introduction of new ge-

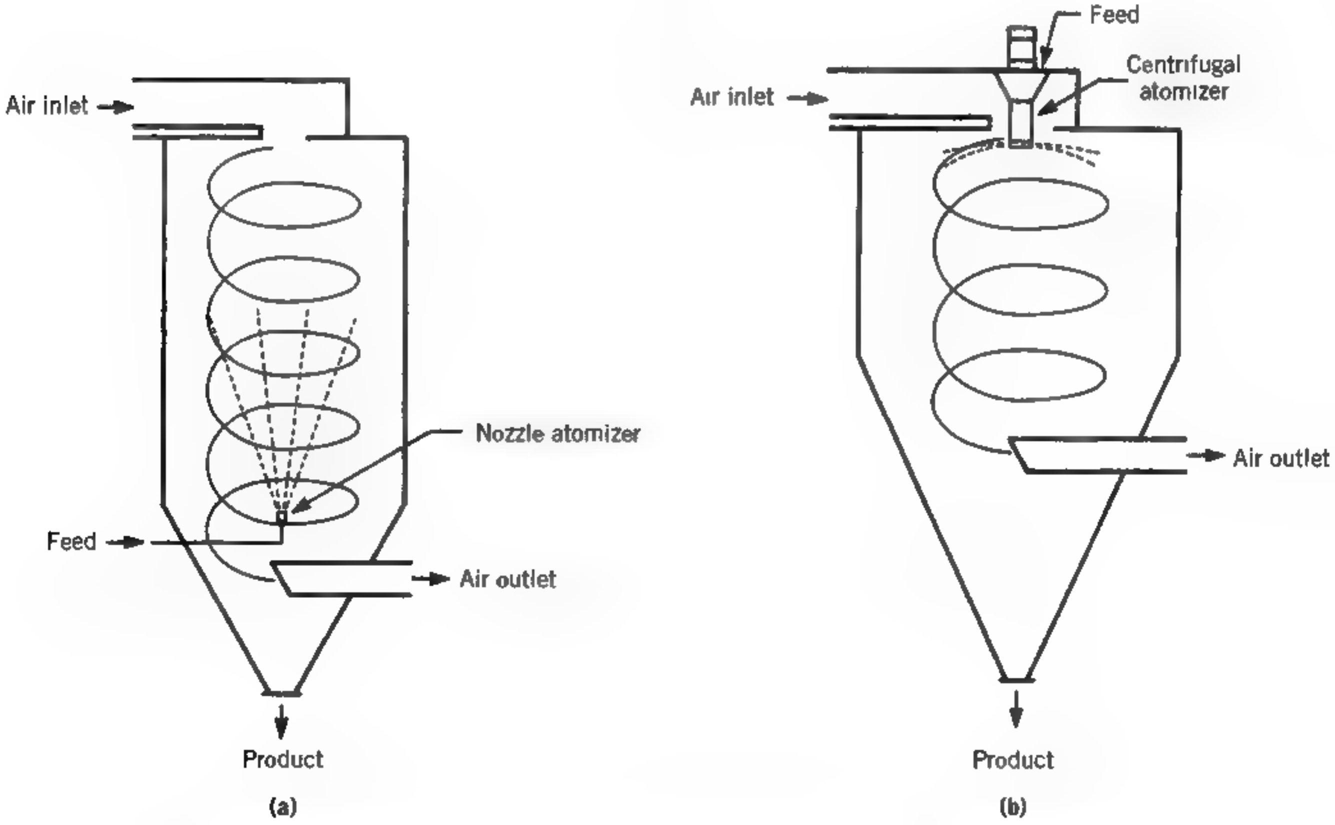


Figure 6. Alternative configurations of spray dryers showing (a) tall form type and (b) conical.

Table 2. Comparison between Direct-Fired Band Dryer and Spray-Dryer Costs in the Processing of Titanium Dioxide

	Spray dryer	Band dryer
Initial moisture content, W/W	50%	50%
Final moisture content, W/W	0.5%	0.5%
Feed, lb/h	12,000	12,000
Evaporation lb/h	5970	5970
Output, lb/h	6030	6030
Inlet air temperature, °F	1150	393
Exhaust air temperature, °F	250	300
Direct gas firing	Gas $CV = 1000$ Btu/ft^2	Gas $CV = 1000$ Btu/ft^2
Hourly operating cost gas and electricity	$39.00	$42.26
Thermal efficiency	76.0%	69.5%
Installed horsepower	149	154
Floor area occupied, ft^2	650	2100
Installed cost	$465,000	$330,000
Energy cost per pound of dried product	$0.065	$0 070

ometries and techniques there will be further development into areas such as foods and in the production of powders that may be easily reconstituted.

ROTARY DRYERS

Another type of dryer that is very much in evidence in the chemical and process industries is the continuous rotary dryer. This machine generally is associated with tonnage production and as a result of its ability to handle products having a considerable size variation, can be used to dry a wide range of materials. The principal sources of thermal energy are oil, gas, and coal. While typical inlet temperatures for direct-fired dryers using these fuels is in the order of 1200°F, in certain instances they may be as high as 1500–1600°F depending largely on the nature of the product handled. Where feed materials are thermosensitive, steam heating from an indirect heat exchanger also is used extensively. These dryers are available in a variety of designs but in general, can be divided into two main types: those arranged for direct heating and those designed for indirect heating. As seen from Figure 7, certain variants do exist. For example, the direct—indirect dryer uses both systems simultaneously.

Where direct heating is used, the product of combustion are in intimate contact with the material to be dried while in the case of the indirect system, the hot gases are arranged to circulate around the dryer shell. Heat transfer then is by conduction and radiation through the shell.

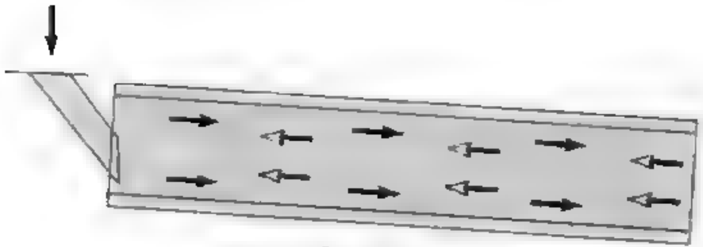
With the indirect—direct system, hot gases first pass down a central tube coaxial with the dryer shell and return through the annular space between the tube and the shell. The material being cascaded in this annulus picks up heat from the gases and also by conduction from direct contact with the central tube. This design is thermally very efficient. Again, while there are a number of proprietary designs employing different systems of airflow, in the main these dryers are of two types: parallel and countercurrent flow. With parallel flow, only high-moisture-content material comes into contact with the hot gases, and, as a result, higher evaporative rates can be achieved than when using countercurrent flow.

In addition, many thermosensitive materials can be dried successfully by this method. Such an arrangement lends itself to the handling of pasty materials since the rapid flashing off of moisture and consequent surface drying limits the possibility of wall buildup or agglomeration within the dryer. On the other hand, countercurrent operation normally is used where a low terminal moisture content is required. In this arrangement, the high-temperature gases are brought into contact with the product immediately prior to discharge where the final traces of moisture in the product must be driven off.

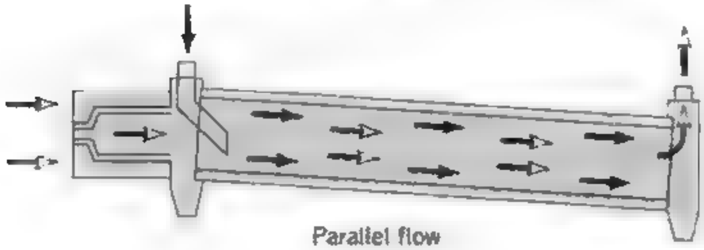
In both these types, however, gas velocities can be sufficiently high to produce product entrainment. They therefore would be unsuitable for low-density or fine-particle materials such as carbon black. In such cases, the indirect-fired function-type dryer is more suitable since the dryer shell usually is enclosed in a brick housing or outer steel jacket into which the hot gases are introduced. As heat transfer is entirely by conduction, conventional flighting and cascading of the material is not used. Rather, the inside of the shell is fitted with small lifters designed to gently turn the product while at the same time maintaining maximum contact with the heated shell.

Another type of indirectly heated dryer that is particularly useful for fine particles or heat-sensitive materials is the steam-tube unit. This dryer can be of the fixed-tube variety equipped with conventional lifting flights designed to cascade the product through a nest of square section tubes, or alternatively, a central rotating tube nest can be used. Figure 8 shows a fixed-tube rotary dryer, which normally has an electrical vibrator fitted to the tube nest in order to eliminate the possibility of bridging of the product with consequent loss of heat-transfer surface. Since the heat exchanger is positioned within the insulated shell in this type of dryer, the air rejection rate is extremely low and thermal efficiencies are high. In general, this design is suitable only for free-flowing materials.

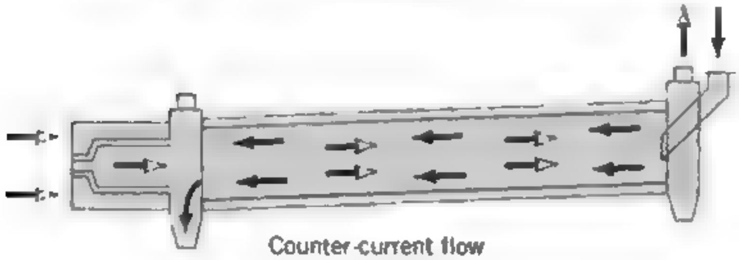
A considerable amount of work has been done on the development of various types of lifting flights, all designed to produce a continuous curtain of material over the cross section of the dryer shell. Other special configurations involve cruciform arrangements to produce a labyrinth path. The object is to give longer residence times where this is



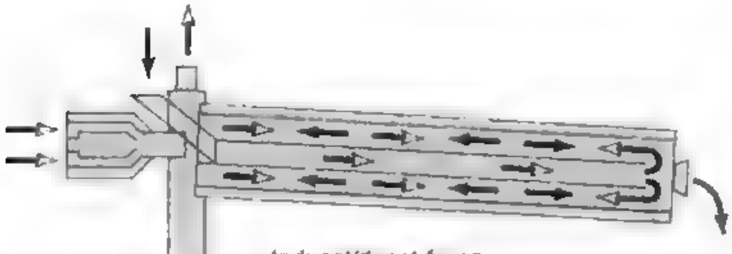
Direct firing



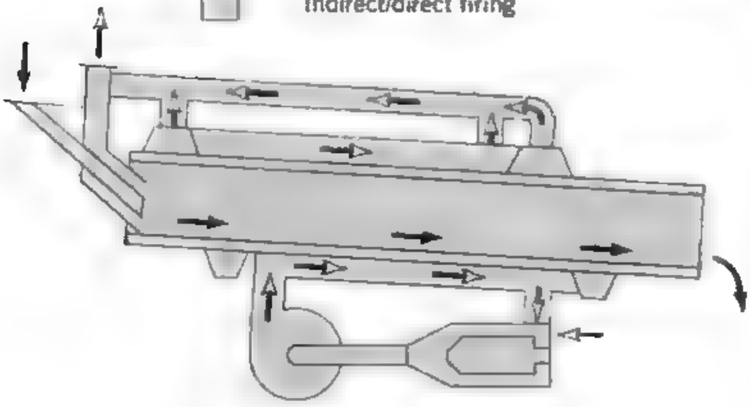
Parallel flow



Counter-current flow



Indirect/direct firing



Indirect firing conduction type

Materials →   Heat flow —▷

Figure 7. Typical rotary dryer arrangements.

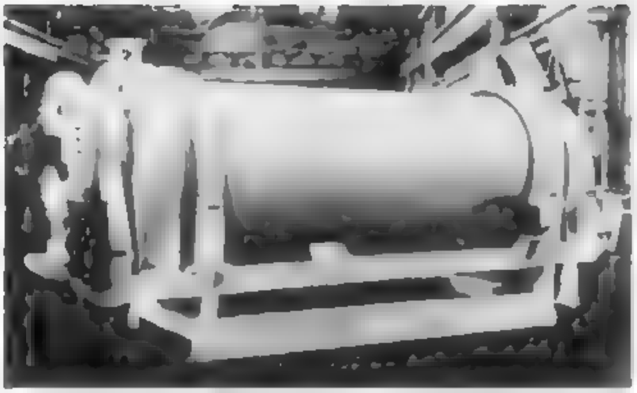


Figure 8. Fixed-tube rotary dryer.

necessary. When the diffusional characteristics of the material or other process considerations call for extended residence times, these machines no doubt will continue to find application.

PNEUMATIC DRYERS

Where total dispersion of the product in a heated gas stream can be achieved with a significant increase in evaporative rates, pneumatic or continuous fluid-bed dryers are preferred. The capital cost of these alternatives generally is lower and maintenance is limited to such components as circulating fans and rotary valves. When considering these two types of dryers, it is convenient to examine them together since both share similar characteristics. Both employ forced convection with dispersion of the feedstock, and as a result of the intimate contact between the drying medium and the wet solids, both exhibit much higher drying rates than do any of the other dryers mentioned previously.

In a fluidized-bed dryer, the degree of dispersion and agitation of the wet solids is limited, whereas in a pneumatic dryer, the degree of dispersion is total and the material is completely entrained in the gas stream. This often is turned to advantage as the drying medium is used as a vehicle for the partially dried product. Other operations such as product classification also can be carried out where required. A further feature of fluid-bed and flash dryers is that the method of operation allows many temperature-sensitive materials to be dried without thermal degradation due to the rapid absorption of the latent heat of vaporization. This generally permits high-rate drying, whereas in other types of dryers lower temperatures would be necessary and correspondingly larger and more costly equipment would be required.

A very good degree of temperature control can be achieved in fluid-bed dryers and the residence time of the material can be varied either by the adjustment of the discharge weir or by the use of multistage units. Similarly, the residence time in the flash dryer can be adjusted by the use of variable cross-sectional area and, therefore, variable velocity. In addition, multiple-effect columns can be incorporated to give an extended path length or continuous recirculatory systems employing both air and product recycle can be used as illustrated in Figure 9.

Generally speaking, the residence time in fluidized-bed dryers is measured in minutes and in the pneumatic dryer in seconds. Both dryers feature high thermal efficiencies, particularly where the moisture content of the wet feed is sufficiently high to produce a significant drop between inlet and outlet temperatures. While the condition of the feed in the pneumatic dryer is somewhat less critical than that in the fluid-bed dryer because it is completely entrained, it still is necessary to use backmixing techniques on occasion in order to produce a suitable feed. A variety of feeding devices are used with these machines.

In fluid-bed dryers, special attention must be paid to the nature of the proposed feed since one condition can militate against another. To some extent, this is reflected in the range of variation in the figures given in Table 1 for evaporative rates. If a large or heavy particle is to be dried, the fluidizing velocities required may be considerable and involve high power usage. In such circumstances, if the moisture content is low and the surface/mass ratio also is low, the thermal efficiency and evaporation would be low. This would make selection of a fluid-bed dryer completely unrealistic and probably would suggest a conventional rotary dryer for the application.

Another case is that in which the minimum fluidizing velocity is so low that a dryer of very large surface dimensions is necessary to obtain the required thermal input. This also occurs in problems of fluid-bed cooling and usually is overcome by the introduction or removal of thermal energy by additional heating or cooling media through an extended-surface heat exchanger immersed in the bed.

With both types of dispersion dryer many configurations are available. While the power requirements of each usually is well in excess of other dryers because of the use of high-efficiency product recovery systems, the smaller size of the fluid-bed dryer compared with conventional rotaries and the fact that the flash dryer can be arranged to fit in limited floor space makes them very attractive.

BAND DRYERS

When selecting a dryer, it always is necessary to consider the final product form. When the degree of product attrition common to pneumatic and fluidized-bed dryer operation is unacceptable, continuous-band or "apron" dryers can provide an effective solution. These are widely used where moderately high rates of throughput on a continuous basis are called for. The most commonly used continuous-band dryer is the single-pass machine employing through-air circulation. Alternatively, and where there is limited floor space or a possible need for long residence time, multi-pass units are used with the conveyors mounted one above the other. In similar circumstances, another special type of multi-deck dryer can be used that employs a system of tilting trays so that the product is supported on both the normal working and the inside of the return run of the conveyor band. This arrangement considerably increases the residence time within the dryer and is particularly useful where the product has poor diffusional characteristics.

The method of airflow employed in these dryers is either vertically downward through the material and the sup-

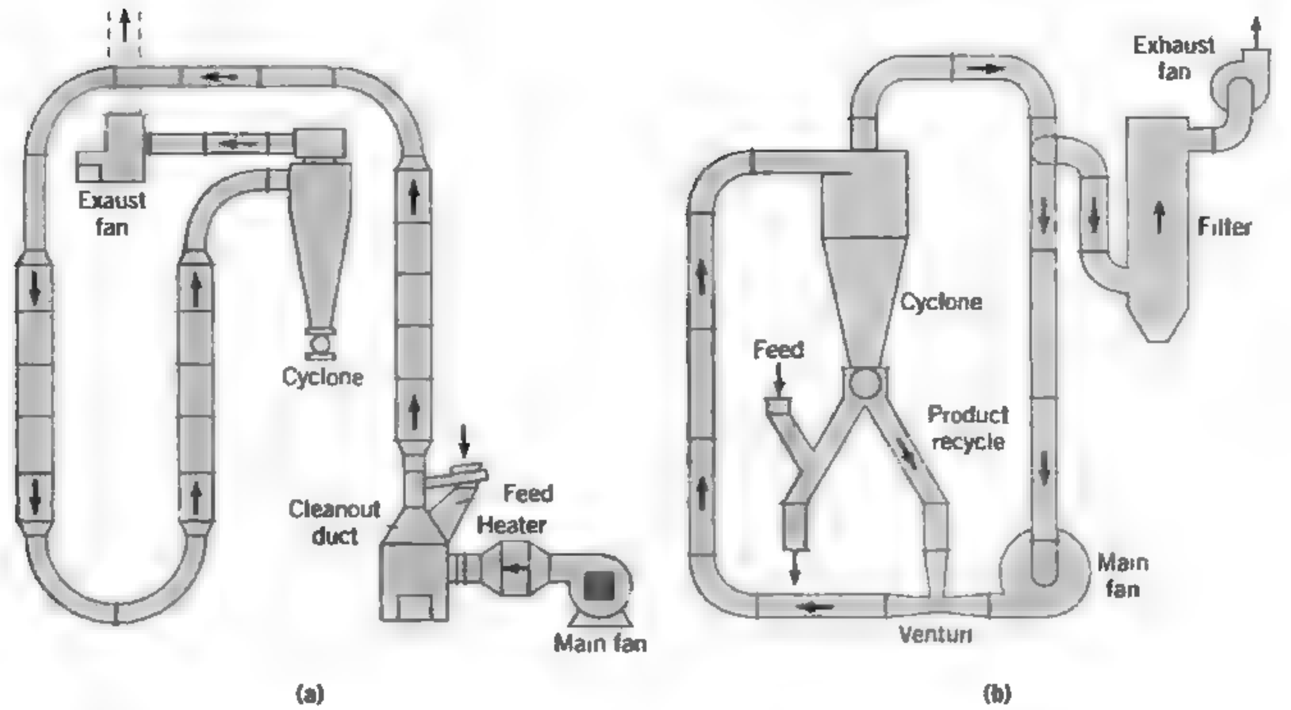


Figure 9. Multipass (a) and air recycle (b) arrangements in flash dryers.

porting band or alternatively upward. Sometimes a combination of the two may be dictated by the nature of the wet feed. It occasionally happens that extruded materials have a tendency to coalesce when deposited on the band, in which case one or more sections at the wet end of the dryer may be arranged for upward airflow to reduce the effect. Wherever possible, through-air circulation is used as opposed to transverse airflow. This results in greatly increased evaporative rates as may be seen from Table 1.

An illustration of the relatively high performance of a band dryer operating on this system as compared with a unit having transverse airflow can be cited in a case involving the processing of a 70% moisture content filter cake. When this material is dried in a conventional unit, the cycle time is in the region of 28 h. This is reduced to 55 min in the through-circulation band dryer largely as a result of using an extruder–performer designed to produce a dimensionally stable bed of sufficient porosity to permit air circulation through the feed.

In view of this, transverse airflow usually is used only where the type of conveyor necessary to support the product does not allow through-flow or where the product form is not suitable for this method of airflow. The most usual method of heating is by steam through heat exchangers mounted in the side plenums or above the band, although direct oil and gas firing sometimes is used. In such cases, the products of combustion normally are introduced to a hot well or duct at an elevated temperature from where they are drawn off and mixed with circulating air in each zone or section of the dryer.

Another alternative with direct firing is to use a series of small individual burners positioned so that each serves one or more zones of the dryer. Typical single-pass dryers of modular construction are illustrated in Figures 10, 11, and 12.

With this type and size of dryer, the average product throughput is about 5600 lb/h and involves an evaporation of 1600 lb/h moisture. It is not unusual, however, to find equipment with evaporative capacities of 3000 lb/h. Such outputs involve quite a large band area with correspondingly large floor area requirements. Various types of feeding arrangements are available to spread or distribute the wet product over the width of the band. Here again, the nature of the feed is an important prerequisite for efficient drying. Steam-heated, finned drums have been used as a means of producing a partially dried, preformed feed. While the amount of predrying achieved is reflected in increased output for a given dryer size or, alternatively, enables a smaller dryer to be used, these items are usually much more costly than many of the mechanical extruders that are available.

Generally, these extruders operate with rubber covered rollers moving over a perforated die plate with feed in the form of pressed cakes or more usually, as the discharge from a rotary vacuum filter. Others of the pressure type employ a gear pump arrangement, with extrusion taking place through a series of individual nozzles, while some use screw feeds that usually are set up to oscillate in order to obtain effective coverage of the band. Alternative designs include rotating cam blades or conventional bar-type granulators, although the latter often produce a high proportion of fines because of the pronounced shearing effect. This makes the product rather unsuitable because of the entrainment problems that can occur.

Each of the types available is designed to produce continuous–discontinuous extrudates or granules, with

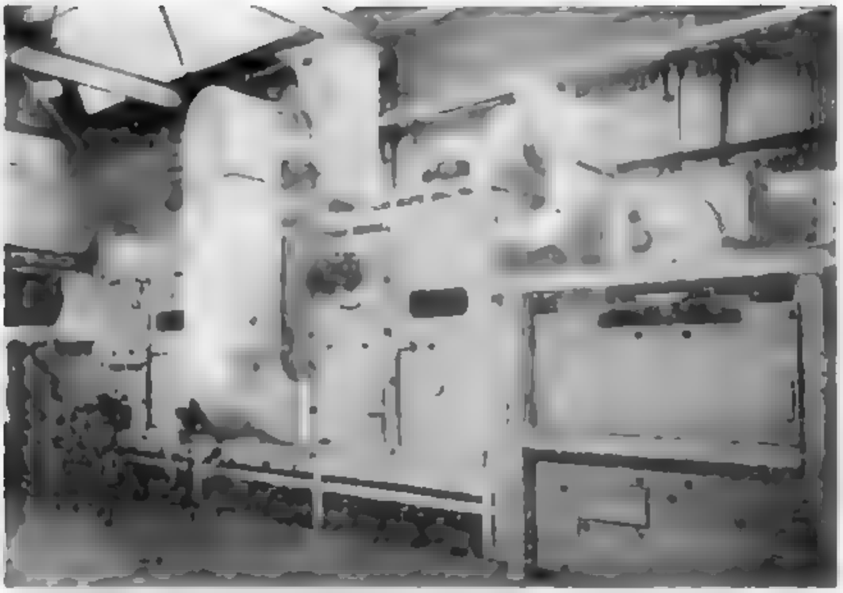


Figure 10. Continuous conveyor band dryer arrangement for direct gas firing.

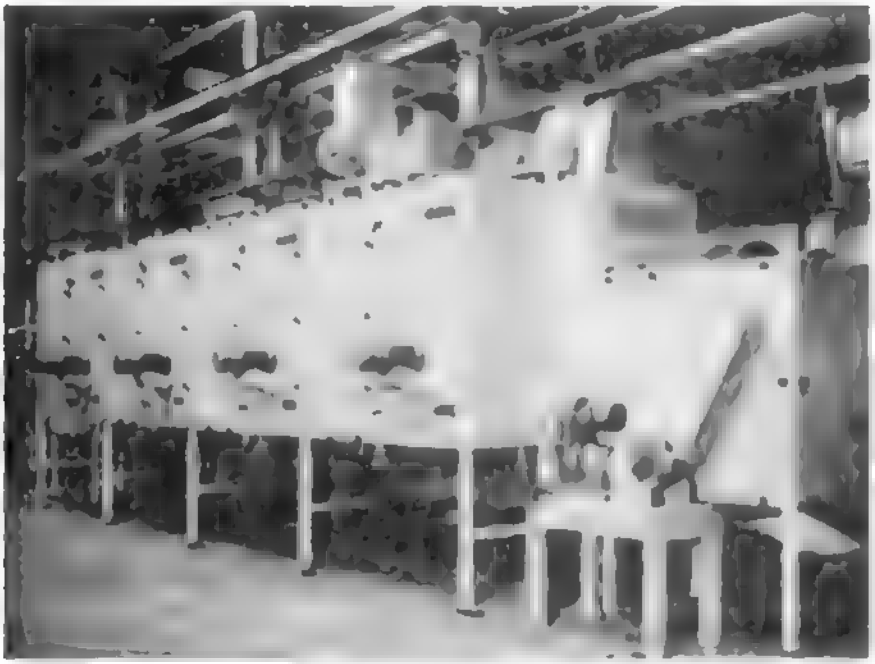


Figure 11. Multistage band dryer.

the grid perforations spaced to meet product characteristics. In selecting the proper type of extruder, it is essential to carry out tests on semiscale equipment as no other valid assessment of suitability can be made.

As a further illustration of the desirability of using a preforming technique, tests on a designated material exhibited a mean evaporative rate of 1.9 lb $ft^2 \cdot h$ when processed in filter-cake form without preforming. When extruded, however, the same material being dried under identical conditions gave a mean evaporative rate of 3.8 lb $ft^2 \cdot h$. This indicates, of course, that the effective band area required when working on extruded material would be only 50% of that required in the initial test. Unfortunately, the capital cost is not halved as might be expected since the feed and delivery ends of the machine housing the drive and terminals remain the same and form an increased proportion of the cost of the smaller dryer. While the cost of the extruder also must be taken into account in the comparison, cost reduction still would be about 15%. Of course, other advantages result from the installation of the smaller dryer. These include reduced radiation and convection losses and a savings of approximately 40% in the floor area occupied.

This type of plant does not involve high installation costs, and both maintenance and operating labor requirements are minimal. Since they generally are built on a zonal principle, with each zone having an integral heater and fan, a good measure of process control can be achieved.

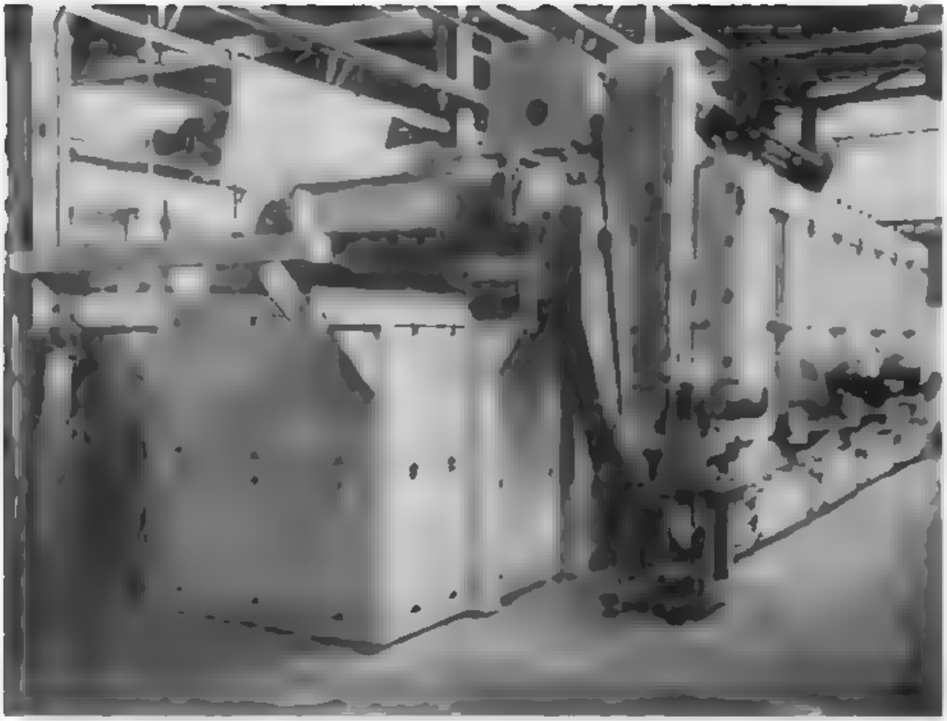


Figure 12. Band dryer and extruder with dye stuffs.

Furthermore, they provide a high degree of flexibility because of the provision of variable speed control on the conveyor.

SELECTION

The application ratings given in Table 1 in which are listed the approximate mean evaporative rates for products under a generic classification are based on APV experience over a number of years in the design and selection of drying equipment. It should be appreciated, however, that drying rates vary considerably in view of the variety of materials and their widely differing chemical and physical characteristics. Furthermore, drying conditions such as temperature and the moisture range over which the material is to be dried have a very definite effect on the actual evaporative rate. It is important, therefore, when using the figures quoted that an attempt is made to assess carefully the nature of the product to be handled and the conditions to which it may be subjected in order to achieve greater accuracy. With these factors in mind, it is hoped that the foregoing observations on drying techniques along with the appropriate tables and curves will provide a basis for making an assessment of the type, size, and cost of drying equipment.

In making a preliminary assessment for dryer selection, there are a number of further points to consider:

1. What is the nature of the upstream process? Is it feasible to modify the physical properties of the feed, eg, mechanical dewatering to reduce the evaporative load?
2. Does the quantity to be handled per unit time suggest batch or continuous operation?
3. From a knowledge of the product, select the type(s) of dryer that it appears would handle both the wet feedstock and the dried product satisfactorily and relate this to the equipment having the highest application rating in Table 1.
4. From a knowledge of the required evaporative duty, ie, the total mass of water to be evaporated per unit time and by the application of the approximate E_{av} figure given in Table 1, estimate the size of the dryer.
5. Having established the size of the dryer on an area or volumetric basis, refer to the appropriate curve in Figures 13 and 14 and establish an order of cost for the particular type of unit.

Although a great deal of fundamental work has been carried out into the mechanics of drying, which provides a basis for recommendations, it is most desirable for pilot plant testing to be done. This is necessary not only to support theoretical calculations but also to establish whether a particular dryer will handle the product satisfactorily. In the final analysis, it is essential to discuss the drying application with the equipment manufacturer who has the necessary test facilities to examine the alternatives objectively and has the correlated data and experience from field trials to make the best recommendation.

EFFICIENT ENERGY UTILIZATION IN DRYING

It generally is necessary to employ thermal methods of drying in order to reach what is termed a "commercially dry"

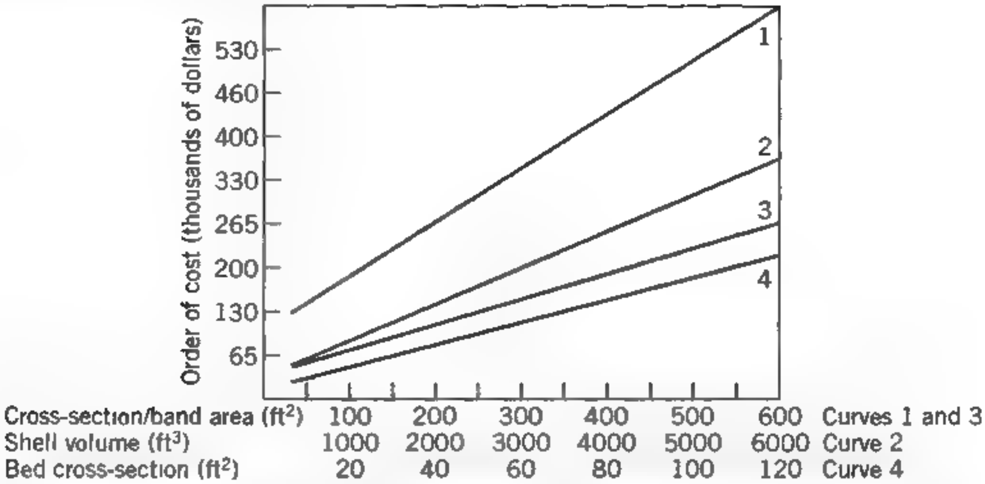


Figure 13. Approximate capital costs of (1) spray dryers, (2) rotary dryers (direct-oil-fired, mild steel, excluding product collection system), (3) continuous-band dryers steam-heated with roller extruder), (4) continuous-fluid-bed dryers (direct-oil-fired with primary cyclone).

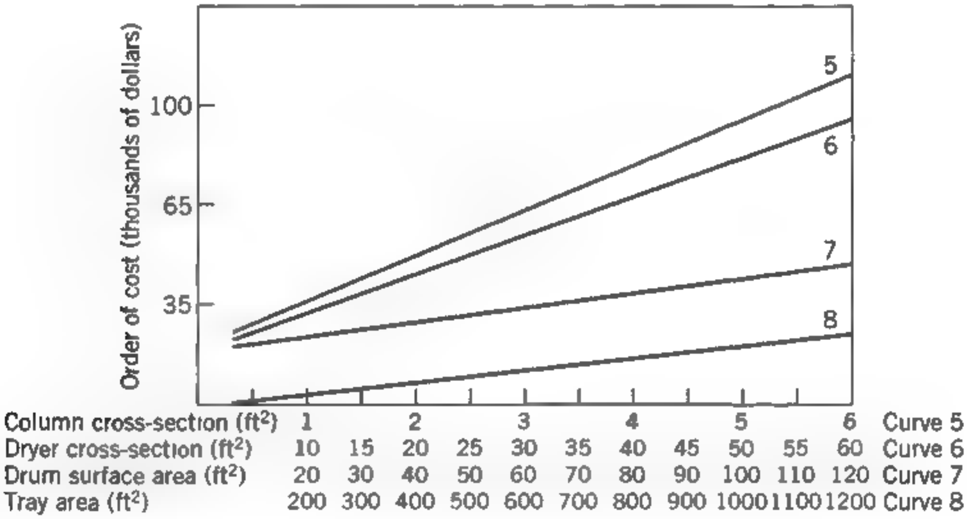


Figure 14. Approximate capital costs of (5) pneumatic dryers (direct-fired, stainless steel, including primary cyclone), (6) double-cone dryers (stainless steel, excluding vacuum equipment), (7) drum dryers (cast-iron drums, dip feed), (8) tray dryers (steam/electric-heated).

condition. As a result, drying forms an important part of most food and chemical processes and accounts for a significant proportion of total fuel consumption.

The rapid escalation of fuel costs over the past 10 yr, together with the prevailing uncertainty of future availability, cost, and possible supply limitations, highlights the continued need to actively engage in the practice of energy conservation. Some of the factors affecting dryer efficiency and certain techniques designed to reduce the cost of the drying operation are discussed in the next few sections.

In making an appraisal of factors affecting dryer efficiency, it is useful to draw up a checklist of those items that have a significant bearing on both operation and economy. It also is appropriate prior to examining various options to emphasize that in the final analysis, the primary concern is the "cost per unit weight" of the dried product. This single fact must largely govern any approach to dryer selection and operation. Additionally, there is an increasing necessity to consider the unit operation of drying in concert with other upstream processes such as mechanical dewatering and preforming techniques in a "total energy" evaluation.

In considering which factors have a bearing on dryer efficiency and what can be done to maximize that efficiency, the following aims should be kept in mind:

1. Maximum temperature drop across the dryer system indicating high energy utilization. This implies maximum inlet and minimum outlet temperature.

2. Employ maximum permissible air recirculation, ie, reduce to an absolute minimum the quantity of dryer exhaust, having due regard for humidity levels and possible condensation problems.
3. Examine the possibility of countercurrent drying, ie, two-stage operation with exhaust gases from a final dryer being passed to a predryer, or alternatively, preheating of incoming air through the use of a heat exchanger located in the exhaust gases.
4. Utilize "direct" heating wherever possible in order to obtain maximum heat release from the fuel and eliminate heat-exchanger loss.
5. Reduce radiation and convection losses by means of efficient thermal insulation.

While the above clearly are basic requirements, there are a number of other areas where heat losses occur in practice, including sensible heat of solids. Furthermore, other opportunities exist for improving the overall efficiency of the process. These are related to dryer types, methods of operation, or possibly the use of a combination of drying approaches to obtain optimum conditions.

Types of Dryers

Considering the requirements for high inlet temperature, the "flash" or pneumatic dryer offers great potential for economic drying. This stems from the simultaneous flash cooling effect that results from the rapid absorption of the latent heat of vaporization and allows the use of high inlet temperatures without thermal degradation of the product. This type of dryer also exhibits extremely high evaporative rate characteristics, but the short gas-solids contact time can in certain cases make it impossible to achieve a very low terminal moisture. However, a pneumatic dryer working in conjunction with a rotary or a continuous fluidized-bed dryer provides sufficient residence time for diffusion of moisture to take place. Such an arrangement combines the most desirable features of two dryer types and provides a compact plant and conceivably, the optimum solution.

As a further example, it is common practice in the process industries to carry out pretreatment of filter-cake materials using extruders or preformers prior to drying. The primary object is to increase the surface area of the product in order to produce enhanced rates of evaporation and smaller and more efficient drying plants. It is interesting to examine the improvements in energy utilization in a conveyor-band dryer resulting from a reduction in overall size of the dryer simply because of a change in the physical form of the feed material.

Consider a typical case. As a result of preforming a filter cake, the evaporative rate per unit area increases by a factor of 2, eg, 1.9 to 3.8 lb/ft^2 h. This permits the effective dryer size to be reduced to half that required for the nonpreformed material and, for a plant handling 1 ton/h of a particular product at a solids content of 60%, the radiation and convection losses from the smaller dryer enclosure show an overall reduction of some 140,000 Btu/h. Although it is necessary to introduce another processing item into the line to carry out the pretreatment, it can be shown that the reduction in the number of dryer sections and savings in horsepower more than offset the power required for the extruder. This differential is approximately 15 kW and the overall saving in total energy is approximately 10% ie, 466 kW compared with 518 kW. Furthermore, the fact that the dryer has appreciably smaller overall dimensions provides an added bonus in better utilization of factory floor space.

Drying Techniques

If, as stated, the prime concern is the cost per pound of dried product, then major savings can be achieved by reducing the amount of water in the feedstock to a minimum prior to applying thermal methods of drying. Again, since it generally is accepted that the mechanical removal of water is less costly than thermal drying, it follows that considerable economies can be made when there is a substantial amount of water that can be readily removed by filtration or centrifuging. This approach, however, may involve changing the drying technique. For example, whereas a liquid suspension or mobile slurry would require a spray dryer for satisfactory handling of the feed, the drying of a filter cake calls for a different type of dryer and certainly presents a totally different materials handling situation.

In approaching a problem by two alternative methods, the overall savings in energy usage may be considerable as illustrated in Figure 15, which is a plot of feed moisture content versus dryer heat load. As may be seen, the difference in thermal energy used in (A) drying from a moisture content of 35% down to 0.1%, or alternatively (B) drying the same material from 14% down to 0.1% is 24.3 × 10^6

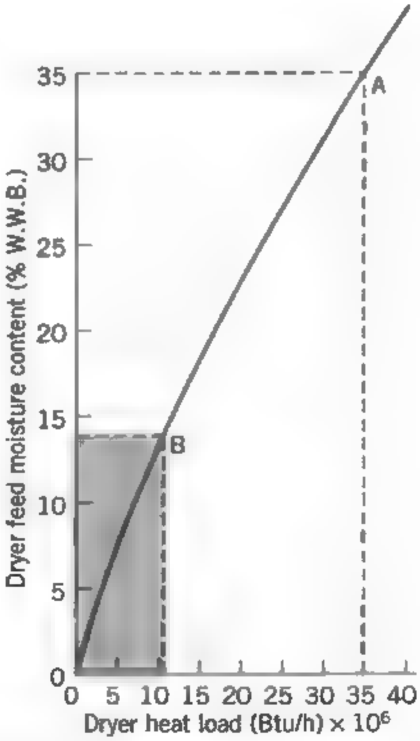


Figure 15. Thermal energy required for drying versus feed moisture content (duty as in Table 1).

Btu. Route A involves the use of a spray dryer in which the absolute weight of water in the feedstock is considerably greater than in route B, which involves the use of a thermoventuri dryer. Both types, incidentally, come under the classification of dispersion dryers and generally operate over similar temperature ranges. As a result, their efficiencies are substantially the same.

It will be apparent from Table 3 that the spray dryer is at a considerable disadvantage because of the requirements for a pumpable feed. This highlights the need to consider the upstream processes and, where a particular "route" to produce a dry product requires a slurried feed, to consider whether a better alternative would be to dewater mechanically and use a different type of dryer.

Comparing these two alternative methods for drying a mineral concentrate, the spray dryer uses a single-step atomization of a pumpable slurry having an initial moisture content of 35% and employs thermal drying techniques alone down to a final figure of 0.1%. The alternative method commences with the same feedstock at 35% moisture content but employs a rotary vacuum filter to mechanically dewater to 14%. From that point, a pneumatic dryer handles the cake as the second of two stages and thermally dries the product to the same final moisture figure.

While the difference in thermal requirements for the alternative routes already has been noted, it also is necessary to take into account the energy used for mechanical dewatering in accordance with assumptions outlined in the footnote to Table 3. From reference to this chart, it will be evident that the two-stage system requires approximately one-third of the energy needed by the single-stage operation but that the savings are not limited to operating costs. There also are significant differences in the basic air volumes required for the two dryers, which means that ancillaries such as product collection–gas cleaning equipment will be smaller and less costly in the case of the pneumatic dryer. The same applies to combustion equipment, fans, and similar items. Actually, the chart shows that capital cost savings are in the nature of 50% in favor of the filter and pneumatic drying system.

This illustration amply demonstrates the need for a more detailed consideration of drying techniques than perhaps has been the case in the past. It also points to the desirability of examining a problem on a "total energy" basis rather than taking the drying operation in isolation. Such a full evaluation approach often will prove it advantageous to change technology, ie, to use a different type of dryer than the one that possibly has evolved on the basis of custom and practice.

Operating Economies

Looking at dryer operations in terms of improving efficiency, it is interesting to see the savings that accrue if, for example, a pneumatic dryer is used on a closed-circuit basis, ie, with the recycle of hot gases instead of total rejection. Briefly, the "self-inertizing" pneumatic dryer consists of a closed loop as shown in Figure 16 with the duct system sealed to eliminate the ingress of ambient air. This means that the hot gases are recycled with only a relatively small quantity rejected at the exhaust and a correspondingly small amount of fresh air admitted at the burner. In practice, therefore, oxygen levels of the order of 5% by volume are maintained. This method of operation raises a number of interesting possibilities. It permits the use of elevated temperatures and provides a capability to dry products that under normal conditions would oxidize. The result is an increase in thermal efficiency. Furthermore, the amount of exhaust gas is only a fraction of the quantity exhausted by conventional pneumatic dryers. This is clearly important wherever there may be a gaseous effluent problem inherent in the drying operation.

A comparison of a self-inertizing versus a conventional pneumatic dryer is detailed in Table 4 with the many advantages clearly apparent. Drying with closed-circuit

Table 3. Comparison of Operating Conditions and Energy Utilization of Pneumatic-and-Spray Dryers Processing Concentrates

Parameter	Pneumatic dryer	Spray dryer
Feed rate	88,200 lb/h	88,200 lb/h
Filtrate rate	21,500 lb/h	—
Evaporative rate	9200 lb/h	30,700 lb/h
Production rate	57,500 lb/h	57,500 lb/h
Initial moisture content	14%	35%
Final moisture content	0.1%	0.1%
Air inlet temperature, °F	752	752
Air outlet temperature, °F	230	230
Total thermal input	21.43×10^6 Btu/h	66.2×10^6 Btu/h
Basic air volume (BAV) at NTP	28,100 NCFM	86,700 NCFM
Fuel consumption	1080 lb/h	3290 lb/h
Total dryer horsepower	187 kW	530 kW
Total filter horsepower	295 kW	—
Total system horsepower	482 kW	530 kW
Total thermal input expressed as kilowatts	6280 kW	19,400 kW
Total energy input to system	6762 kW	19,930 kW

Note: An assumed volumetric flow of 3 ft^3/min ft^2 of fitter area with approximately 1 hp10 ft^3 min has been used as being typical of the flow rates and energy requirements for the filtration or mechanical dewatering equipment.

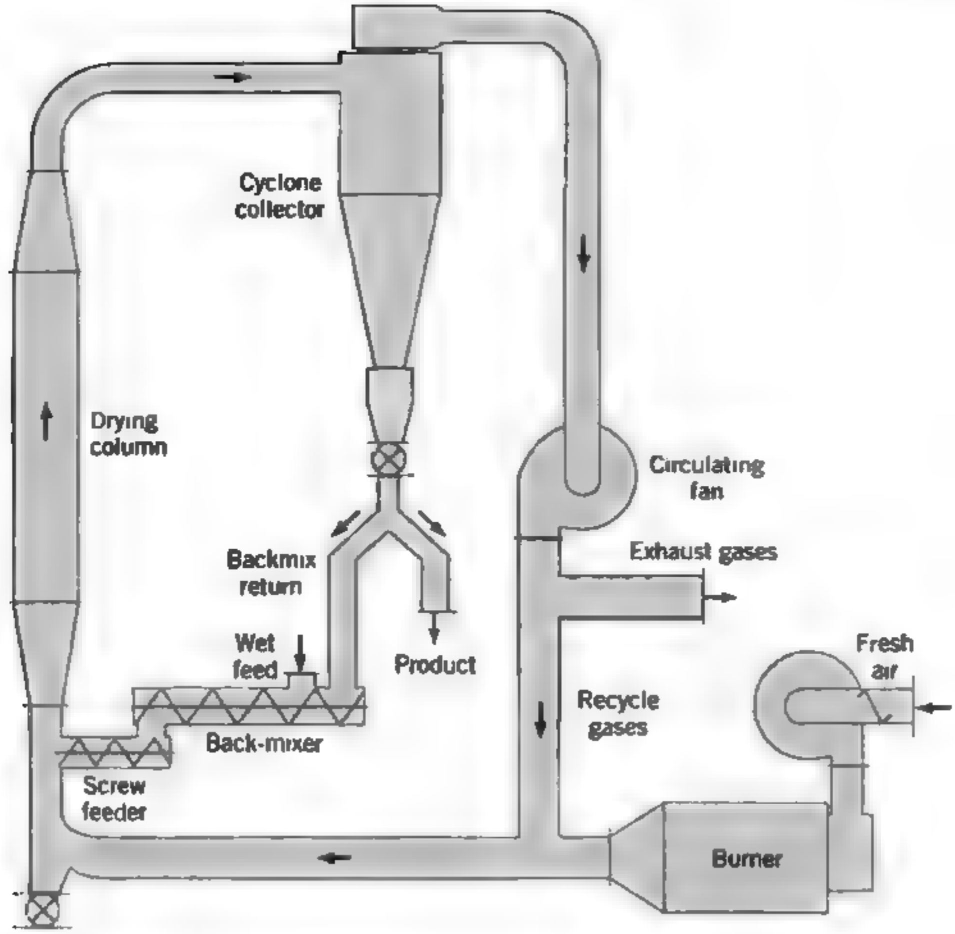


Figure 16. Self-inertizing pneumatic dryer with backmix facility.

Table 4. Comparison of Self-Inertizing Thermoventuri Dryer Versus Total Rejection Thermoventuri Dryer

Parameter	Closed circuit	Total rejection
Evaporation, lb/h	1,250	1,250
IMC, % wwb	85	85
FMC, % wwb	10	10
Feed, lb/h	1,500	1,500
Air inlet temperature, °F	932	662
Air outlet temperature, °F	302	248
Exhaust dry airflow, lb/h	1,368	16,400
Exhaust losses, Btu/h	79,700	744,000
Heat losses, Btu/h	120,000	150,000
Total heat input, Btu/h	1,680,400	2,375,700
Efficiency, %	87.9	62.4

operation is tending toward superheated vapor drying, and in practice 40–50% of the gases in circulation are water vapor. This suggests that the specific heat of the gas will be approximately 0.34 Btu/lb °F compared with about 0.24 Btu/lb °F in a conventional total rejection dryer. Since the mass of gas for a given thermal capacity is appreciably less than in a conventional dryer and, as previously mentioned, oxygen levels are low, much higher operating temperatures may be used. This permits a reduction in the size of the closed-circuit dryer. For the case given in Table 4 and comparing the two dryers on the basis of their cross-sectional areas, the total rejection dryer would have rather more than double the area of the closed-circuit system. There are obvious limitations to the use of such a technique, but the advantages illustrated are very apparent, especially the savings in thermal energy alone, viz, 695,000 Btu/h representing about 29.2% of the total requirements of the conventional drying system.

Effect of Changing Feed Rates

Since it has a significant bearing on efficiency, another factor of major importance to consider when designing dryers of this type is the possible effect of reducing feed rate. What variation in quantity of feed is likely to occur as a result of operational changes in the plant upstream of the dryer and as a result, what turndown ratio is required of the dryer? With spray dryers and rotary dryers, the mass airflow can be varied facilitating modulation of the dryer when operating at reduced throughputs.

This, however, is not the case with pneumatic and true fluidized-bed dryers since the gases perform a dual function of providing the thermal input for drying and acting as a vehicle for transporting the material. Since the mass flow has to remain constant, the only means open for modulating these dryers is to reduce the inlet temperature. This clearly has an adverse effect on thermal efficiency. It therefore is of paramount importance to establish realistic production requirements. This will avoid the inclusion of excessive scale-up factors or oversizing of drying equipment and thereby maximize operating efficiency.

Figure 17 illustrates the effects on thermal efficiency of either increasing the evaporative capacity by increased inlet temperatures or alternatively, reducing the inlet temperature with the exhaust temperature remaining constant at the level necessary to produce an acceptable dry product. While the figure refers to the total rejection case of the previous illustration where design throughput corresponds to an efficiency of 62.4%, the curve shows that if the unit is used at only 60% of design, dryer efficiency falls to 50%. The converse, of course, also is true.

In this brief presentation, an attempt has been made to highlight some of the factors affecting efficiency in drying operations and promote an awareness of where savings can be made by applying new techniques. While conditions differ from one drying process to another, it is clear that economies can and should be made.

SPRAY DRYING: NEW DEVELOPMENTS, NEW ECONOMICS

In the field of spray drying, the past 10 years have witnessed many new developments initiated mainly to meet three demands from food and dairy processors—better energy efficiency, improved functional properties of the finished powder, and production of new, specialized dried products. This has been accompanied by progress made in the automation of drying systems and in stricter requirements for the reduction of environmental impacts resulting from the operation of spray dryers.

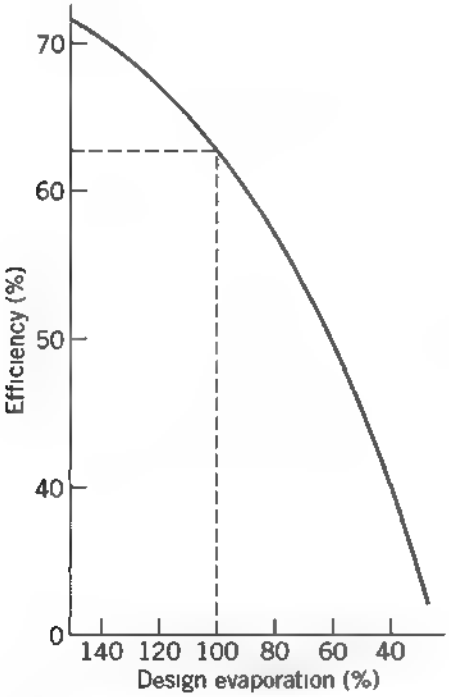


Figure 17. Variation of efficiency with dryer turndown.

While demands for energy efficient drying may have become somewhat relaxed recently with the stabilizing of energy costs, the fact remains that spray dryers and evaporators are among the most energy-intensive processing systems and the future of energy costs is still uncertain.

The extra functionality that may be required of the powders is to a large extent determined by the drying conditions, including pre- and posttreatment of the concentrate and final powder. Desirable characteristics are dust-free and good reconstitution properties that involve carefully controlled drying conditions, often with added agglomeration and lecithination steps.

The interest in the production of high-value specialty dried products instead of straight commodity products has been accentuated by the government's reduced support to dairy producers, whereby there will be less milk solids available. Processors naturally are looking into ways to better use these available supplies by producing products with improved functional, nutritional, and handling properties and, coincidentally, products with higher profit margins.

This is particularly noticeable in the utilization of whey and whey product derivatives. A larger percentage of the total whey produced today is handled and processed so that it will be suitable as food ingredients for human consumption. It also is finding new applications by being refined into whey protein concentrate and lactose through crystallization, hydrolyzation, and separation by ultrafiltration.

To handle the increase in plant size and complexity and to meet demands for closer adherence to exact product specifications, dryer instrumentation has been improved and operation gradually has evolved from manual to fully automated. Today, it is normal for milk drying plants to be controlled by such microprocessor-based systems as the APV ACCOS, with automation covering the increasingly complex startup, shutdown, and CIP procedures as well as sophisticated automatic adjustment of the dryer operating parameters to variations in ambient-air conditions.

Finally, more stringent demands governing environmental aspects of plant operations have resulted in that virtually all spray dryers today have to be equipped with exhaust air pollution control devices such as wet scrubbers or bag filters.

Principle and Approaches

Spray drying basically is accomplished in a specially designed chamber by atomizing feed liquid into a hot-air stream. Evaporation of water from the droplets takes place almost instantaneously, resulting in dried particles being carried with the spent drying air. The powder is subsequently separated from the air in cyclone separators or bag filters and collected for packaging. The powder particles being protected during the drying phase by the evaporative cooling effect results in very low heat exposure to the product, and the process is thus suitable also for heat-sensitive materials.

A number of different spray dryer configurations are available, distinguished by the type of feed atomization device and the drying chamber design.

therefore is of paramount importance to establish realistic production requirements. This will avoid the inclusion of excessive scale-up factors or oversizing of drying equipment and thereby maximize operating efficiency.

Figure 17 illustrates the effects on thermal efficiency of either increasing the evaporative capacity by increased inlet temperatures or alternatively, reducing the inlet temperature with the exhaust temperature remaining constant at the level necessary to produce an acceptable dry product. While the figure refers to the total rejection case of the previous illustration where design throughput corresponds to an efficiency of 62.4%, the curve shows that if the unit is used at only 60% of design, dryer efficiency falls to 50%. The converse, of course, also is true.

In this brief presentation, an attempt has been made to highlight some of the factors affecting efficiency in drying operations and promote an awareness of where savings can be made by applying new techniques. While conditions differ from one drying process to another, it is clear that economies can and should be made.

SPRAY DRYING: NEW DEVELOPMENTS, NEW ECONOMICS

In the field of spray drying, the past 10 years have witnessed many new developments initiated mainly to meet three demands from food and dairy processors—better energy efficiency, improved functional properties of the finished powder, and production of new, specialized dried products. This has been accompanied by progress made in the automation of drying systems and in stricter requirements for the reduction of environmental impacts resulting from the operation of spray dryers.

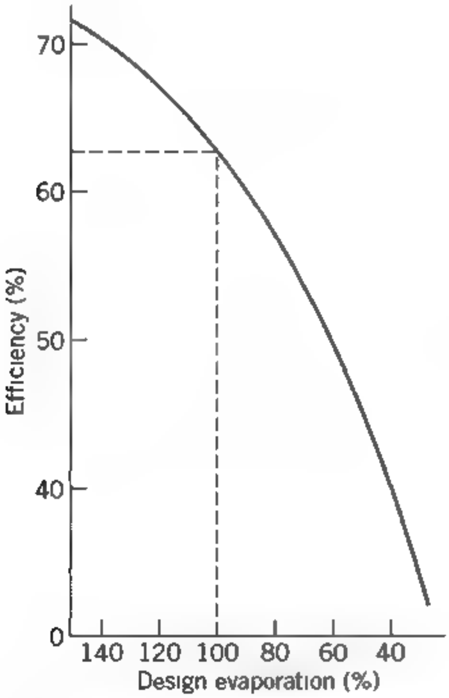


Figure 17. Variation of efficiency with dryer turndown.

While demands for energy efficient drying may have become somewhat relaxed recently with the stabilizing of energy costs, the fact remains that spray dryers and evaporators are among the most energy-intensive processing systems and the future of energy costs is still uncertain.

The extra functionality that may be required of the powders is to a large extent determined by the drying conditions, including pre- and posttreatment of the concentrate and final powder. Desirable characteristics are dust-free and good reconstitution properties that involve carefully controlled drying conditions, often with added agglomeration and lecithination steps.

The interest in the production of high-value specialty dried products instead of straight commodity products has been accentuated by the government's reduced support to dairy producers, whereby there will be less milk solids available. Processors naturally are looking into ways to better use these available supplies by producing products with improved functional, nutritional, and handling properties and, coincidentally, products with higher profit margins.

This is particularly noticeable in the utilization of whey and whey product derivatives. A larger percentage of the total whey produced today is handled and processed so that it will be suitable as food ingredients for human consumption. It also is finding new applications by being refined into whey protein concentrate and lactose through crystallization, hydrolyzation, and separation by ultrafiltration.

To handle the increase in plant size and complexity and to meet demands for closer adherence to exact product specifications, dryer instrumentation has been improved and operation gradually has evolved from manual to fully automated. Today, it is normal for milk drying plants to be controlled by such microprocessor-based systems as the APV ACCOS, with automation covering the increasingly complex startup, shutdown, and CIP procedures as well as sophisticated automatic adjustment of the dryer operating parameters to variations in ambient-air conditions.

Finally, more stringent demands governing environmental aspects of plant operations have resulted in that virtually all spray dryers today have to be equipped with exhaust air pollution control devices such as wet scrubbers or bag filters.

Principle and Approaches

Spray drying basically is accomplished in a specially designed chamber by atomizing feed liquid into a hot-air stream. Evaporation of water from the droplets takes place almost instantaneously, resulting in dried particles being carried with the spent drying air. The powder is subsequently separated from the air in cyclone separators or bag filters and collected for packaging. The powder particles being protected during the drying phase by the evaporative cooling effect results in very low heat exposure to the product, and the process is thus suitable also for heat-sensitive materials.

A number of different spray dryer configurations are available, distinguished by the type of feed atomization device and the drying chamber design.

Atomization Techniques

Proper atomization is essential for satisfactory drying and for producing a powder of prime quality. To meet varying parameters, APV offers a selection of atomizing systems: centrifugal, pneumatic nozzles, or pressure nozzles.

Centrifugal Atomization. With centrifugal or spinning-disk atomization (Fig. 18), liquid feed is accelerated to a velocity in excess of 800 ft/s to produce fine droplets that mix with the drying air. Particle size is controlled mainly be liquid properties and wheel speed. There are no vibrations, little noise, and small risk of clogging. Furthermore, the system allows maximum flexibility in feed rate, provides capacities in excess of 200 tons/h and operates with low power consumption.

Steam Injection. To produce a product with significantly increased bulk density and fewer fines, the APV steam injection technique (Fig. 19) has been refined to a point that allows its use with centrifugal atomizers in large drying operations.

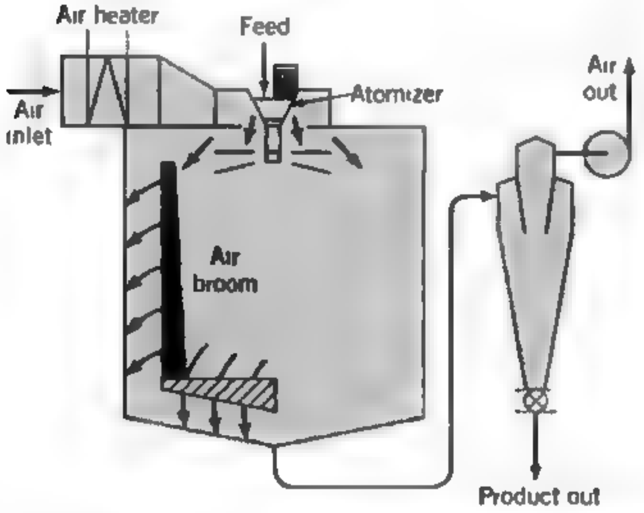


Figure 18. Flat-bottomed drying chamber incorporates rotating powder discharger and can be equipped with an air broom.

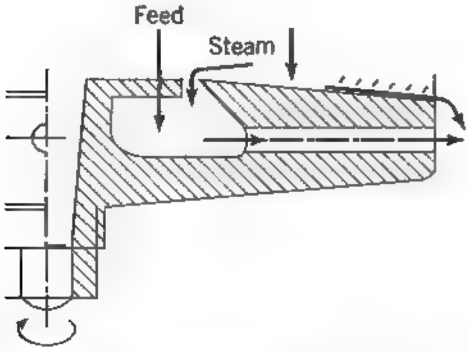


Figure 19. Steam is added around and into the atomizing disk to minimize air in the atomized liquid droplet.

During centrifugal atomization, air around the rotating wheel may be entrapped within the atomized droplets.

When heat is transferred from the drying air into the feed droplet, water evaporates and diffuses out through the surface, at the same time creating a hard shell around the particle and some hollow spaces inside. If the droplet already contains some air bubbles, this incorporated air will expand to fill out the created vacuoles and the particle will not shrink much during the drying process, resulting in porous particles as shown in Figure 20 microphoto of spray-dried autolyzed yeast.

In the steam injection process the air atmosphere around the spinning disk is replaced with a steam atmosphere, thus reducing the amount of air incorporated in the droplets. This means that there is no air that will expand within the particle and the created vacuoles will collapse, thus shrinking the particle and resulting in the type of dense, void-free particles illustrated in the Figure 21 microphoto. Control of the amount of steam injected permits a precise adjustment in powder bulk density. Furthermore, reduction of air-exposed surfaces often reduces product oxidation and prolongs powder shelf life.

Pressure Nozzles. With the pressure nozzle system (Fig. 22), liquid feed is atomized when forced under high pressure through a narrow orifice. This approach offers great versatility in the selection of the spray angle, direction of the spray, and positioning of the atomizer within the chamber. It also allows cocurrent, mixed-current, or countercurrent drying, with the production of powders having par-

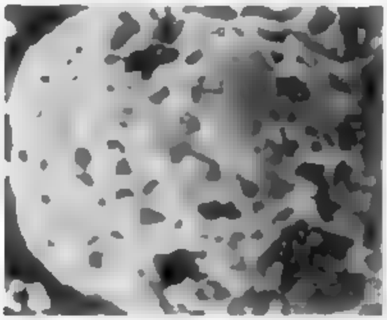


Figure 20. Spray-dried autolyzed yeast (2000× magnification). Particle is hollow and filled with crevices.

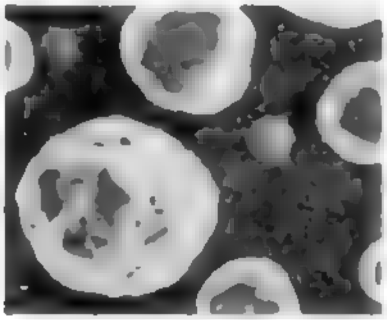


Figure 21. Spray-dried autolyzed yeast with steam injection (2000× magnification). Particle is solid and essentially void-free.

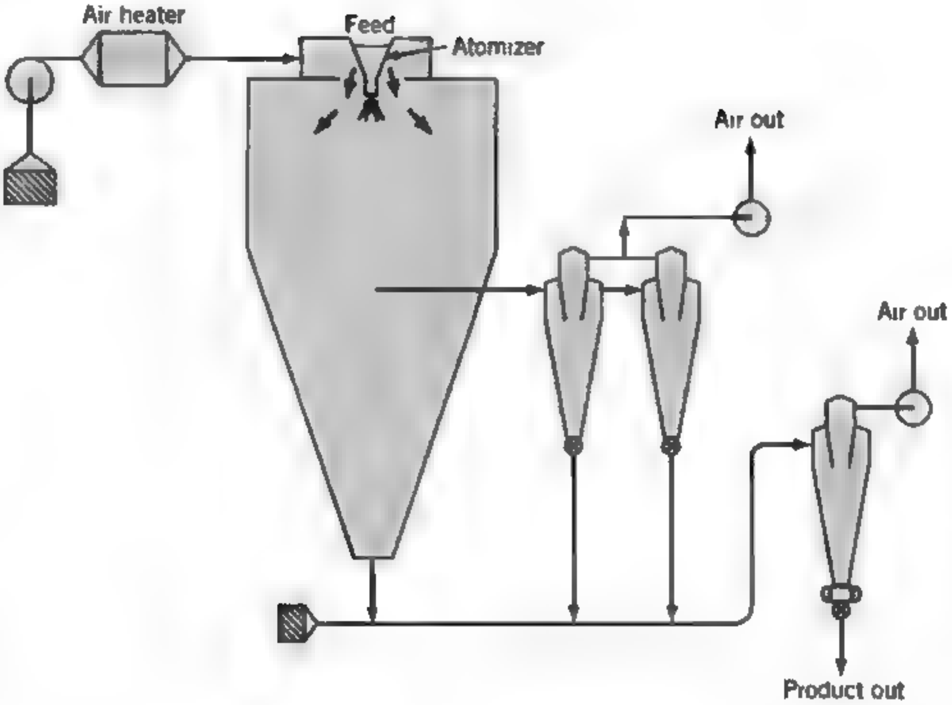


Figure 22. Typical spray dryer with conical chamber; arranged with high-pressure atomizer and air broom.

ticularly narrow particle size distribution and/or coarse characteristics. Since the particle size is dependent on the feed rate, dryers with pressure nozzles are somewhat limited relative to changing product characteristics and operating rates.

Pneumatic (Two-Fluid) Nozzles. This type of nozzle uses compressed air to accomplish the atomization of the feed product. The advantage with this type of atomizer is that it allows a greater flexibility in feed capacity than what can be obtained with pressure nozzles. However, particle size distribution is not as good as what can be obtained with the centrifugal or high-pressure nozzle-type atomizers, and because of the large quantities of compressed air required, operating costs tend to be quite high.

Single-Stage Drying

Defined as a process in which the product is dried to its final moisture content within the spray-drying chamber alone, the single-stage dryer design is well known throughout the industry, although some difference in airflow patterns and chamber design exist.

As illustrated, the drying air is drawn through filters, heated to the drying temperature, typically by means of a direct-combustion natural-gas heater, and enters the spray drying chamber through an air distributor located at the top of the chamber. The feed liquid enters the chamber through the atomizer, which disperses the liquid into a well-defined mist of very fine droplets. The drying air and droplets are very intimately mixed, causing a rapid evaporation of water. As this happens, the temperature of the air drops, as the heat is transferred to the droplets and used to supply the necessary heat of evaporation of the liquid. Each droplet thus is transformed into a powder particle. During this operation, the temperature of the particles will not increase much owing to the cooling effect resulting from water evaporation.

The rate of drying will be very high at first but then declines as the moisture content of the particles decreases. When the powder reaches the bottom of the drying chamber, it has attained its final moisture and normally is picked up in a pneumatic cooling system to be cooled down to a suitable bagging or storage temperature. Powder that is carried with the air is separated in cyclone collectors. As the powder is cooled in the pneumatic system, it also is subjected to attrition. This results in a powder that is fine, dusting, and has relatively poor redissolving properties.

Single-stage dryers are made in different configurations. The basic ones are described below.

Box Dryers. A very common type of dryer in the past, the drying air enters from the side of a boxlike drying chamber. Atomization is from a large number of high-pressure nozzles also mounted in the side of the chamber. Dried powder will collect on the flat floor of the unit, from which it is removed by moving scrapers. The exhaust air is filtered through filter bags that also are mounted in the chamber. In some instances, cyclones will be substituted for the filter bags.

Tall-Form Dryers. These are very tall towers in which the airflow is parallel to the chamber walls. The atomization is by high-pressure nozzles. This type of dryer is used mainly for the production of straightforward, commodity-type dried powders.

Wide-Body Dryers. While the height requirement is less than for the tall-form type, the drying chamber is considerably wider. The atomizing device can be either centrifugal or nozzle type. The product is sprayed as an umbrella-shaped cloud, and the airflow follows a spiral path inside the chamber. As shown in Figs. 18 and 22, this type of dryer is designed either with a flat or conical bottom.

Flat-Bottom Dryer. The flat-bottom dryer is not a new development, but the design has gained renewed interest in recent years.

The chamber has a nearly flat bottom from which the powder is removed continuously by means of a rotating pneumatic powder discharger. The dryer has the obvious advantage of considerably reduced installed height requirements compared to cone-bottom dryers, and it is also easy for operators to enter the chamber for cleaning and inspection. Another advantage is that the rotating powder discharger provides a positive powder removal from the chamber and a well-defined powder residence time. This has been shown to be important in the processing of heat-sensitive products such as enzymes and flavor compounds. Depending on the product involved, a pneumatic powder cooling system also may be installed.

Typically, this type of dryer is used for egg products, blood albumin, tanning agents, ice cream powder and toppings. The flat- and bottom chamber, incidentally, may be provided with an air broom, which is indicated by the colored section in Figure 18. By blowing tempered air onto the chamber walls while rotating, this device blows away loose powder deposits and cools the chamber walls to keep the temperature below the sticking point of certain products. Some items with which the air bottom technique has been successful are for fruit and vegetable pulp and juices, meat extracts, and blood.

Cone-Bottom Dryer. Figure 22, meanwhile, shows a conical-bottom chamber arrangement with a side air outlet, high-pressure nozzle atomization, and pneumatic product transport beneath the chamber. This single-stage dryer is very well suited to making relatively large particles of dairy products or proteins for cattle feed. If the product will not withstand pneumatic transport, it may be taken out unharmed directly from the chamber bottom.

Multiple-Stage Dryers

The best way to reduce energy usage in spray drying is, of course, to try and reduce the specific energy consumption of the process. With advances in atomizer and air distributor designs it has been possible with many products to operate with higher dryer inlet temperature and lower outlet temperature. While this procedure substantially cuts energy needs and does not harm most heat-sensitive products, care must be taken and proper balance struck. The nature of the product usually defines the upper limit, ie, the denaturation of milk protein or discoloring of other products. A higher inlet temperature requires close control of the airflow in the spray-drying chamber, and particularly around the atomizer. Furthermore, it must be noted that a lower outlet temperature will increase the humidity of the powder.

Generally speaking, the drying process can be divided into two phases: (1) the constant-rate drying period when drying proceeds quickly and when surface moisture and moisture within the particles that can move by capillary action are extracted and (2) the falling-rate period when diffusion of water to the particle surface becomes the determining factor. Since the rate of diffusion decreases with the moisture content, the time required to remove the last few percent of moisture in the case of single-stage drying takes up the major part of the residence time within the dryer. The residence time of the powder thus is essentially the same as that of the air and is limited to between 15 and 30 s. As the rate of water removal is decreasing toward the end of the drying process, the outlet air temperature has to be kept fairly high in order to provide enough driving force to finish the drying process within the available air residence time in the chamber.

In multiple-stage drying, the residence time is increased by separating the powder from the main drying air and subjecting it to further drying under conditions where the powder residence time can be varied independently of the airflow. Technically, this is done either by suspension in a fluidized bed or by retention on a moving belt. Since a longer residence time can be allowed during the falling-rate period of the drying, it is possible and desirable to reduce the drying air outlet temperature. Enough time to complete the drying process can be made available under more lenient operating conditions.

The introduction of this concept has led to higher thermal efficiencies. Fifteen years ago, the typical inlet and outlet temperatures of a milk spray dryer were 360–205°F. Today, the inlet temperature is often above 430°F, with outlet temperatures down to about 185°F.

Two-Stage Drying. In a typical two stage drying process as shown in Figure 23, powder at approximately 7% moisture is discharged from the primary drying chamber to an APV fluid bed for final drying and cooling. The fact that the powder leaves the spray dryer zone at a relatively high moisture content means that either the outlet air tem-

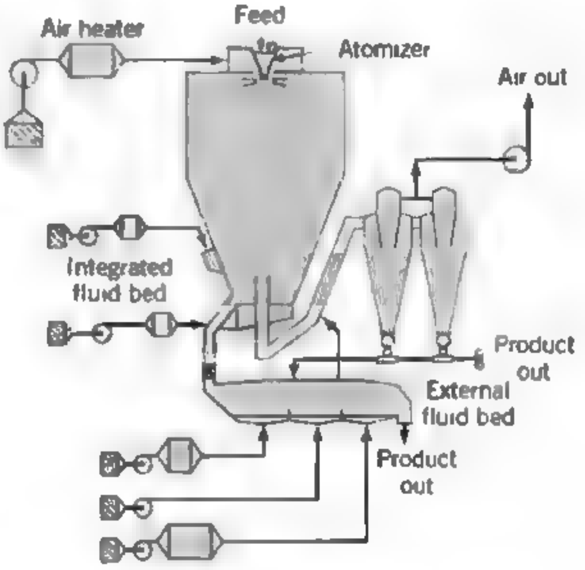


Figure 23. Two-stage drying with external fluid bed.

perature can be lowered or the inlet air temperature increased. Compared to single-stage drying, this will result in better thermal efficiency and higher capacity from the same size drying chamber. As the product is protected by its surrounding moisture in the spray-drying phase, there normally are no adverse effects on product quality resulting from the higher inlet air temperature.

The outlet air from the chamber leaves through a side air outlet duct, and the powder is discharged at the bottom of the chamber into the fluid bed. This prolongs the drying time from about 22 s in a typical single-stage dryer to more than 10 min, thus allowing for low-temperature use in the fluid bed.

In the development of this type of drying system, an initial difficulty was to provide a means to reliably fluidize the semimoist powders. This stems from the fact that milk powder products, and especially whey-based ones, show thermoplastic behavior. This makes them difficult to fluidize when warm and having high moisture content, a problem that was largely overcome by the development of a new type of vibrating fluid bed.

The vibrating fluid bed has a well-defined powder flow and typically is equipped with different air supply sections, each allowing a different temperature level for a optimum temperature profile. The last section normally is where the product is cooled to bagging and storage temperature.

While the specific energy consumption in the fluid-bed process may be relatively high, the evaporation is minor compared with the spray-drying process and the total energy use therefore is 15–20% lower.

Figure 24 shows the specific energy costs as a function of the water evaporated in the fluid bed or the residual moisture in the powder from the spray drying to the fluid-bed process. The curves are only shown qualitatively because the absolute values depend on energy prices, inlet temperatures, required residual moisture content, and other such parameters. The total cost curve shows a minimum that defines the quantity of water to be evaporated in the fluid bed to minimize the energy costs.

The introduction of two-stage drying also created the potential for a general improvement in powder quality, especially as far as dissolving properties are concerned. The slower and more gentle the drying process produces more solid particles of improved density and solubility and opens up the possibility of producing agglomerated powders in a straight-through process. If desired, the fluid bed can be designed for rewet instantizing or powder agglomeration and for the addition of a surface-active agent such as lecithin.

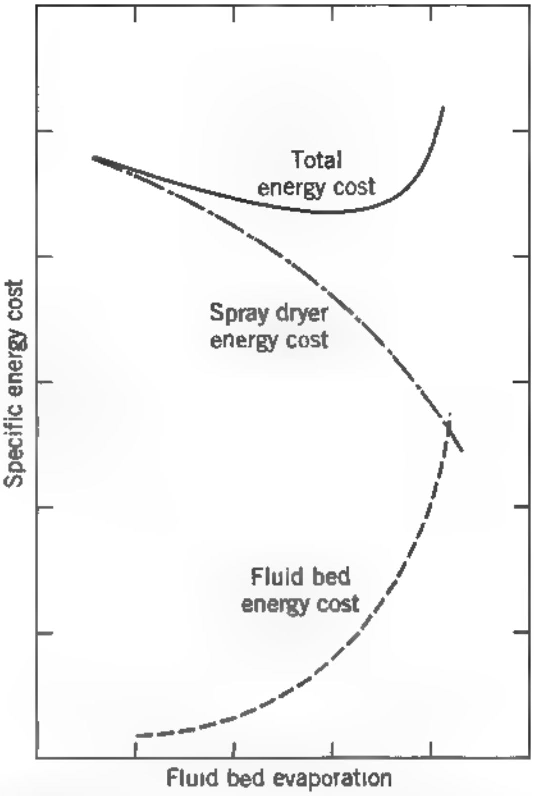


Figure 24. Specific energy cost as a function of the water evaporated in fluid bed.

Three-Stage Drying. This advanced drying concept basically is an extension of two-stage drying in which the second drying stage is integrated into the spray-drying chamber with final drying conducted in an external third stage (Fig. 25). The design allows a higher moisture content from the spray drying zone than is possible in a two-stage unit and results in an even lower outlet air temperature. An added advantage is that it improves the drying conditions for several difficult products. This is accomplished by spray drying the powder to high moisture content but at the same time avoiding any contact with metal surfaces by handling the powder directly on a fluidized powder layer in the integrated fluid bed. Moist powder particles thus are surrounded by already dry particles, and any tendency to stick to the chamber walls will be reduced.

In this drying concept, it is not necessary to vibrate the chamber fluid bed, although often the powders entering from the spray drying zone are difficult to fluidize because of their high moisture content and thermoplastic and hygroscopic characteristics. The fluidization characteristics are improved as the wet powder from the spray-drying zone is mixed and coated with dryer powder in the integrated fluid bed. The fluidization is further assisted by the use of a special type of perforated plate with directional airflow. The stationary fluid bed operates with high fluidizing velocities and high bed depths, both optimized to the product being processed.

The air outlet of the integrated fluid-bed system is located in the middle of the fluid bed at the bottom of the chamber. This forms an annulus around the air outlet duct and creates an aerodynamically clean design, completely eliminating the mechanical obstructions found in older two-point discharge designs. The dryer is equipped with tangential air inlets, so called Wall Sweeps, for conditioned air. These are important to the operation of the dryer in two ways: they will cool the wall and remove powder that may have a tendency to accumulate on the wall, and they also serve to stabilize the airflow within the chamber. The dryer thus operates with a very steady and well-defined airflow pattern that minimizes the amount of wall buildup.

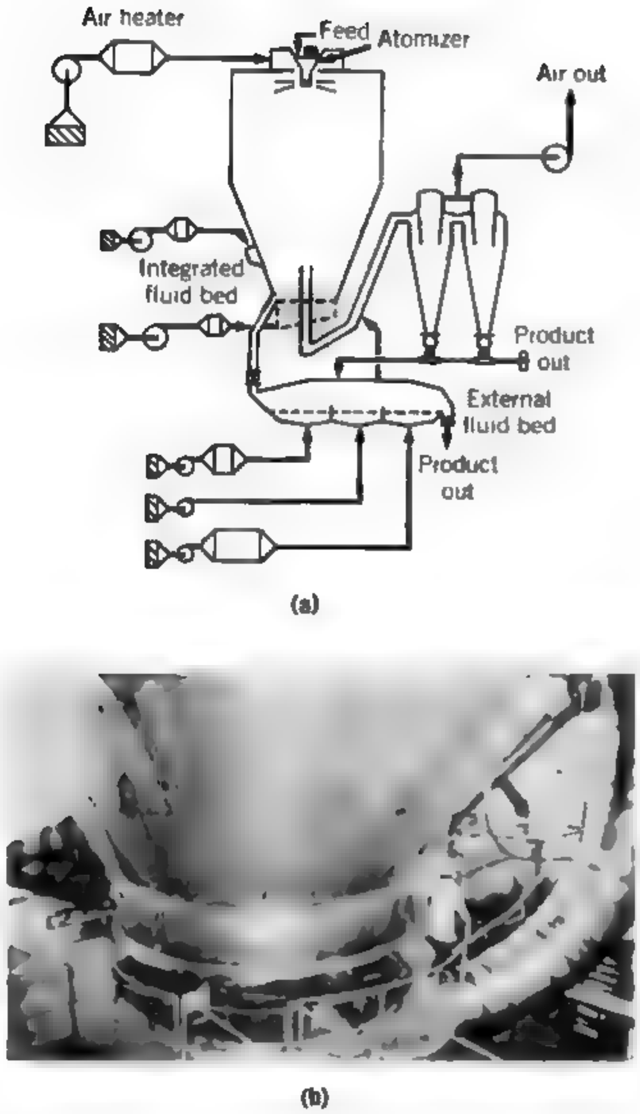


Figure 25. (**a**,**b**) Three-stage dryer combines conical drying chamber, integrated stationary fluid bed with directional air flow and external vibrating fluid bed. Provides optimum conditions for producing nondusty, hygroscopic, and high-fat-content products.

Atomization can be either by pressure nozzles or by a centrifugal-type atomizer.

The three-stage system is exceptionally suitable for the production on nondusty, hygroscopic, and high-fat-content products. The dryer can produce either high-bulk-density powder by returning the fines to the external fluid bed, or powder with improved wettability by straight-through agglomeration with the fines reintroduced into an atomizing zone. The concept also allows the addition of liquid into the internal fluid bed, thus opening the path to the production of very sophisticated agglomerated and instantized products, ie, excellent straight-through lecithinated powders. Whey powder from such a system shows improved quality because of the higher moisture level present when the powder enters the integrated fluid bed, providing good conditions for lactose after crystallization within the powder.

A three-stage dryer also offers a high production capacity in a small equipment volume. The specific energy requirement is about 10% less than for a two-stage dryer.

Spray-Bed Dryer

While the concept of a three-stage drying system with an integrated fluid evolved from traditional dryer technology, it served to spur the development of a modified technique referred to by APV as the "Spray Bed Dryer." This machine is characterized by use of an integrated fluid bed at the bottom of the drying zone but with drying air both entering and exiting at the top of the chamber. Atomization can be with either nozzles or a centrifugal atomizer.

The chamber fluid bed is vigorously agitated by a high fluidization velocity. Particles from the spray-drying zone enter the fluid bed with a moisture content as high as 10–15% depending on the type of product and are dried in the bed to about 5%. Final drying and cooling take place in an external fluid bed.

The structure of the powder produced in the Spray Bed differs considerably from the conventional. It is coarse, consists of large agglomerates, and consequently has low bulk density but exhibits excellent flowability. The dryer is very suited to the processing of complicated products having high contents of fat, sugar, and protein. Two of the many possible variations of this type drying are shown in Figures 26 and 27.

Spray-drying systems divided into two or more stages undoubtedly will be a characteristic of almost all future dryer installations. The advantages resulting from this technology will provide dairy and food processors with the necessary flexibility and energy efficiency required to meet today's uncertain market and whatever changes will be called for in the future.

Pollution-Control Devices

Virtually, all plants have a system to ensure clean exhaust air while collecting powder at an efficiency of about 99.5%, and may be supplied with secondary pollution-control collection equipment if necessary. In most new spray dryers this is accomplished using bag filter collectors after the primary collection device. In the case of such products as acid whey where the use of bag collectors is not practical, high-efficiency scrubbers are used. Some multipurpose plants have both plus an elaborate system of ducts and dampers to switch between the devices as different products are dried.

An added benefit from the use of sanitary bag collectors is an improvement in the yield of sale product. Although this increase typically is not more than 0.5%, it still can be of substantial value over a year of operation.

Heat-Recovery Equipment

Although it is possible to reduce the direct heating energy consumption by the use of multiple-stage drying, optimum thermal conditions generally require that heat recuperators be used as well. A few approaches are shown in Figure 28.

Despite the many recuperators available for recovering heat from drying air, only a few are suited for the spray-

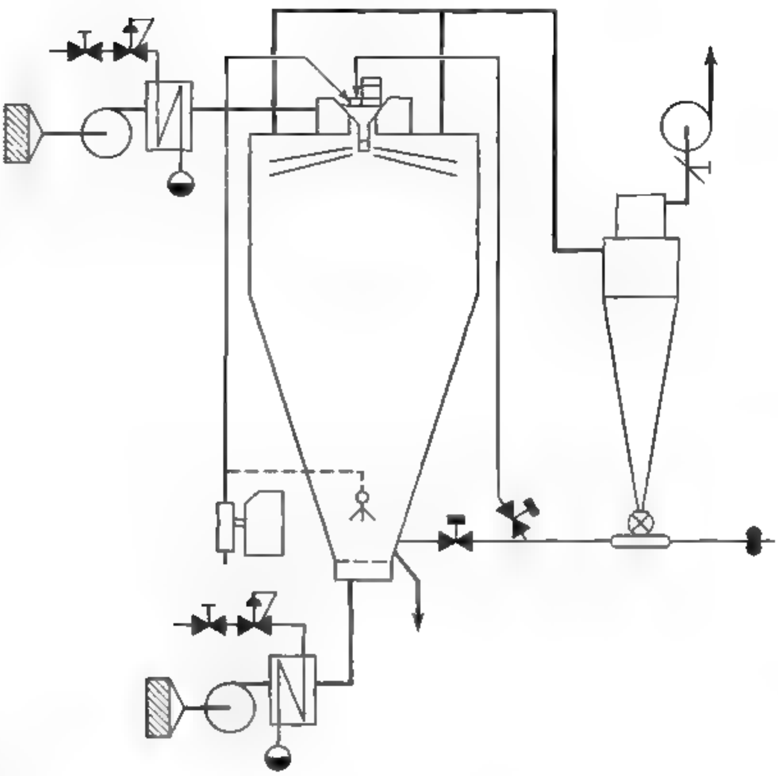


Figure 26. One variation of APV Crepaco Spray Bed Dryer arrangements (see Fig. 27 for other variation). Basic dryer with centrifugal atomizer and integrated fluid bed, which is agitated by high fluidation velocity.

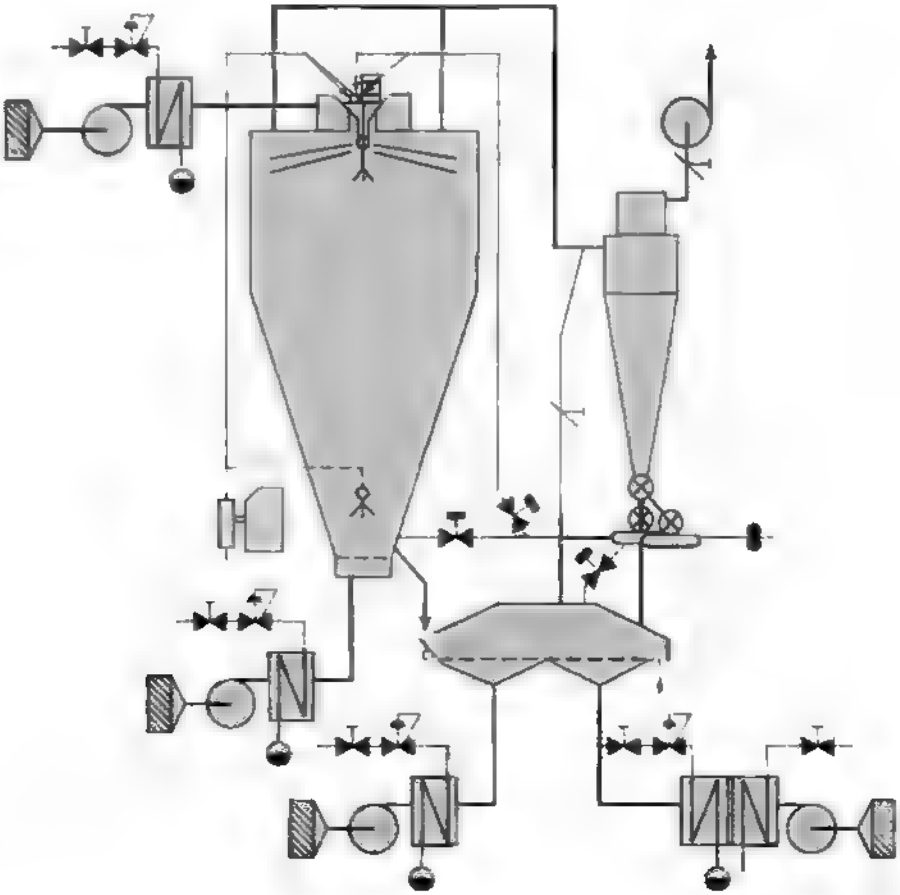


Figure 27. Spray bed dryer with added external fluid bed for final powder drying and cooling.

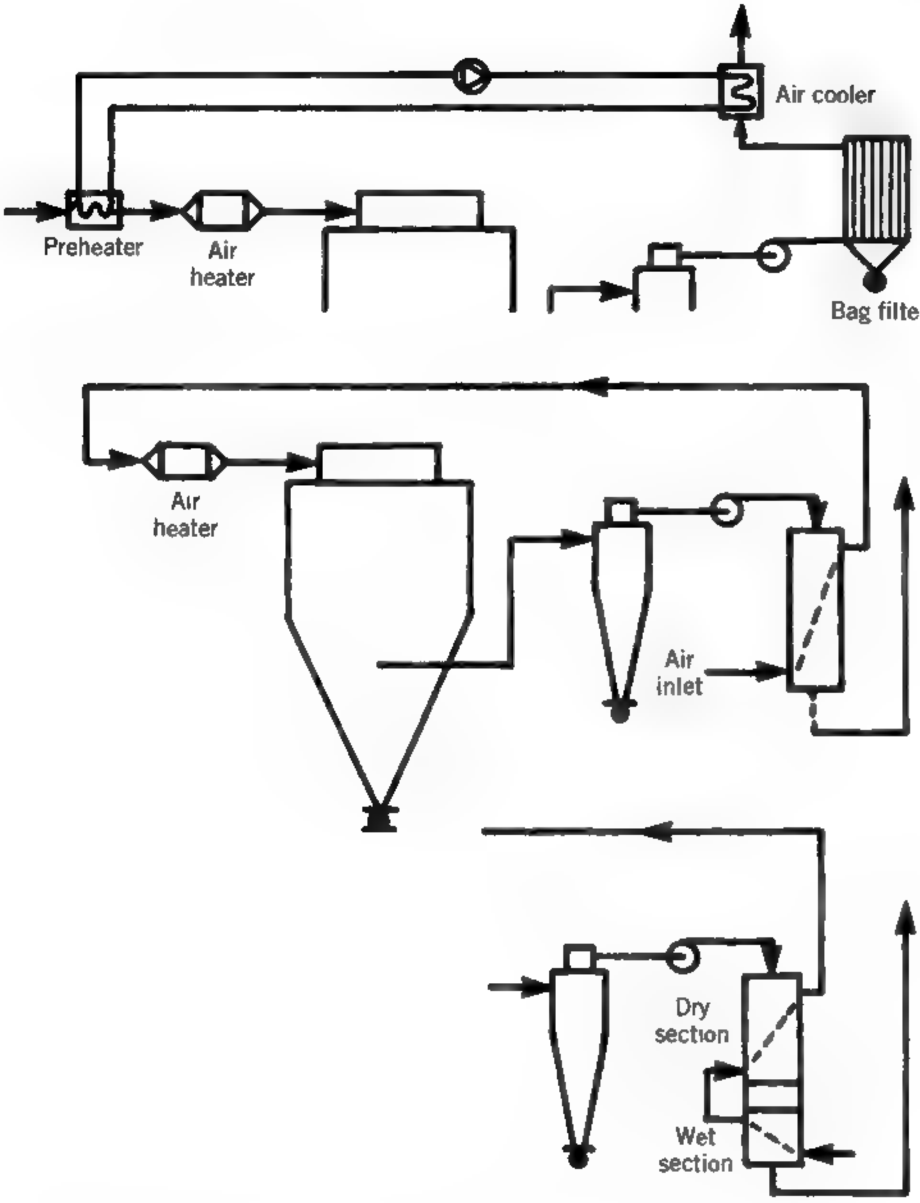


Figure 28. Spray-drying plant with (top) liquid coupled recuperator, (middle) single-stage recuperator, and (bottom) two-stage recuperator.

drying process. This is due to the dust-laden air that is involved in the process and that tends to contaminate heat exchange surfaces. To be effective, therefore, recuperators for spray-dryer use should have the following properties:

- Modular system
- High thermal efficiency
- Low pressure drop
- Automatic cleaning
- Large temperature range
- Stainless steel
- Large capacity
- Low price

Two such recuperators are the air-to-air tubular and the air-to-liquid plate designs.

Air-to-Air Heat Recuperators. For optimum flexibility, this heat exchanger is of modular design, each module consisting of 804 tubes welded to an end plate. Available in various standard lengths, the modules can be used for counterflow, cross-flow, or two-stage counterflow as sketched in Figure 29. While the first two designs are based on well-known principles, the two-stage counterflow (patent pending) system is an APV development. This recuperator consists of two sections, a long dry element and a shorter wet section. During operation, hot waste air is passed downward through the dry module tubes and cooled to just above the dew point. It then goes to the wet section where latent heat is recovered. The inner surfaces of these shorter tubes are kept wet be recirculating water. The system, therefore, not only recovers free and latent heat from the waste air but also scrubs the air and reduces the powder emission by more than 80%. Effectiveness and

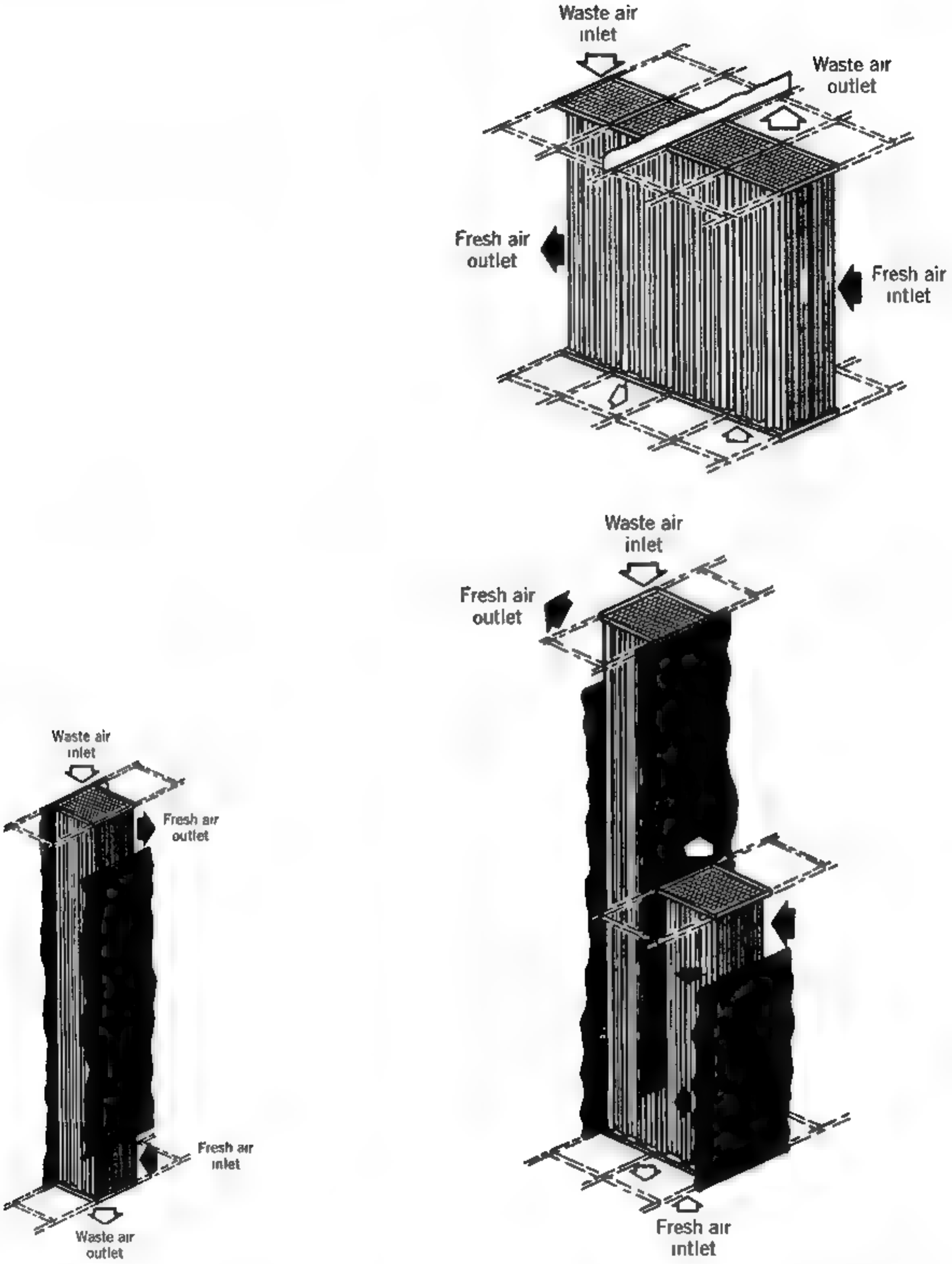

Waste air inlet
Waste air outlet
Fresh air outlet
Fresh air inlet
Waste air inlet
Fresh air outlet
Fresh air inlet
Waste air inlet
Fresh air outlet
Fresh air inlet
Waste air outlet

Figure 29. Air-to-air tubular heat recuperators.

economy are such that the system replaces a conventional recuperator-scrubber combination.

Air-to-Liquid Recuperator. In cases where an air-to-air recuperator is impractical or uneconomical because of space limitations or the length of the distance between the outlet and inlet air to be preheated, a liquid heat-recovery system is available. This plate recuperator as shown in Figure 30 uses various standard plates as the base with waste air being cooled in counterflow by circulating a water/glycol mixture. At high temperature, circulating thermal oils are used. Heat recovered by this means gen-

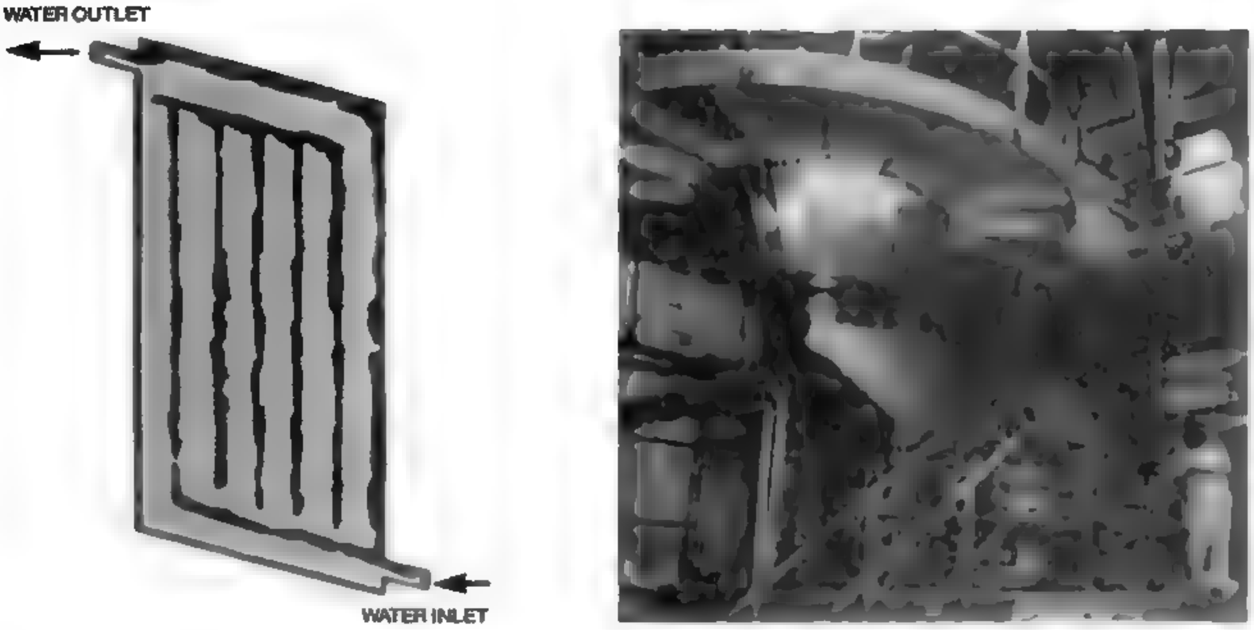


Figure 30. Thermoplate for air-to-liquid plate recuperator.

erally is recycled to preheat the drying air but can be used for other purposes such as heating water, clean-in-place (CIP) liquids, or buildings.

Other Heat-Recovery Methods. While many spray-drying plants are equipped with bag filters to minimize emissions to the environment and recover valuable powder, the filtration system can be coupled with a finned-tube recuperator for heat-recovery purposes. This type of recuperator is particularly effective when the air is not dust-loaded. It is compact, very flexible and normally, very inexpensive.

Some degree of heat recovery can also be accomplished by using available low-temperature waste streams to preheat the drying air through a finned-tube heat exchanger. Examples of such sources of waste heat include evaporator condensate and scrubber liquids.

SPRAY DRYERS: FLUID-BED AGGLOMERATION

Most powderlike products produced by spray drying or grinding are dusting, exhibit poor flow characteristics, and are difficult to rehydrate.

It is well known, however, that agglomeration in most instances will improve the redispersion characteristics of a powder. Added benefits of the agglomerated powder is that it exhibits improved flowability and is nondusting. All of these are characteristics for which the demand has increased in recent years.

Depending on the application or the area of industry where the process is being used, the process sometimes is also referred to as granulation or instantizing.

Instant Powders

Powders with particle size less than about 100 μm typically tend to form lumps when mixed with water and require strong mechanical stirring in order to become homogeneously dispersed or dissolved in the liquid. What is happening is that as water aided by capillary forces penetrates into the narrow spaces between the particles, the powder will start to dissolve. As it does, it will form a thick, gel-like mass that resists further penetration of water. Thus, lumps will be formed that contain dry powder in the middle and, if enough air is locked into them, will float on the surface of the liquid and resist further dispersion.

In order to produce a more readily dispersible product, the specific surface of the powder has to be reduced and the liquid needs to penetrate more evenly around the particles. In an agglomerated powder with its open structure, the large passages between the individual powder particles will assist in quickly displacing the air and allow liquid to penetrate before an impenetrable gel layer is formed. The powder thus can disperse into the bulk of the liquid, after which the final dissolution can take place.

Although there always is some degree of overlap between them, the reconstitution of an agglomerated product can be considered as consisting of the following steps:

1. Granular particles are wetted as they touch the water surface.
2. Water penetrates into the pores of the granule structure.
3. The wetted particles sink into the water.
4. The granules disintegrate into their original smallest particles, which disperse in the water.
5. The small dispersed particles dissolve in the water.

It is important to realize that it is the total time required for all these steps that should be the criterion in evaluating a product's instant properties. It is not unusual to see products characterized only on their wettability. This neglects the importance of the dispersion and disso-

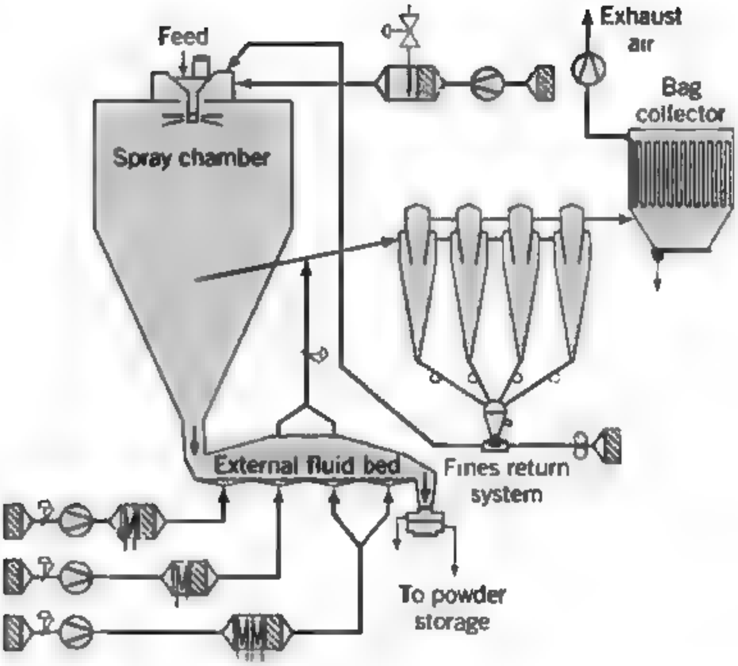


Figure 31. Straight-through agglomeration.

lution steps, the time for which may vary considerably with different agglomeration methods.

For powders that are produced by spray drying, there are a number of ways in which the agglomeration can be accomplished in the spray dryer itself. This often is referred to as the "straight-through" process and is illustrated in Figure 31. Note that fines powder from the cyclone is conveyed up to the atomizer, where it is introduced into the wet zone surrounding the spray cloud. Cluster formation will occur between the semimoist freshly produced particles and the recycled fines. The agglomerated product then is removed from the bottom of the drying chamber, cooled, and packaged. This method produces a degree of agglomeration that is sufficient for many applications.

An alternative approach to agglomeration is referred to as the "rewet method." This is characterized by processing an already existing fine dry powder into an agglomerate using fluidized-bed technology.

The Agglomeration Mechanism

Two particles can be made to agglomerate if they are brought into contact and at least one of them has a sticky surface. This condition can be obtained by one or a combination of the following means:

1. Droplet humidification whereby the surface of the particles is uniformly wetted by the application of a finely dispersed liquid.
2. Steam humidification whereby saturated steam injected into the powder causes condensation on the particles.
3. Heating—for the thermoplastic materials.
4. Addition of binder media, ie, a solution that can serve as an adhesive between the particles.

The steam condensation method usually cannot provide enough wetting without adversely heating the material and is used less frequently on newer systems.

After having been brought into a sticky state, the particles are contacted under such conditions that a suitable, stable agglomerate structure can be formed. The success of this formation will depend on such physical properties as product solubility and surface tension as well as on the conditions that can be generated in the process equipment.

For most products, possible combinations of moisture and temperature can be established as shown in Figure 32. Usually, the window for operation is further narrowed down by the specifications for product characteristics. Once the agglomerate structure is created, the added mois-

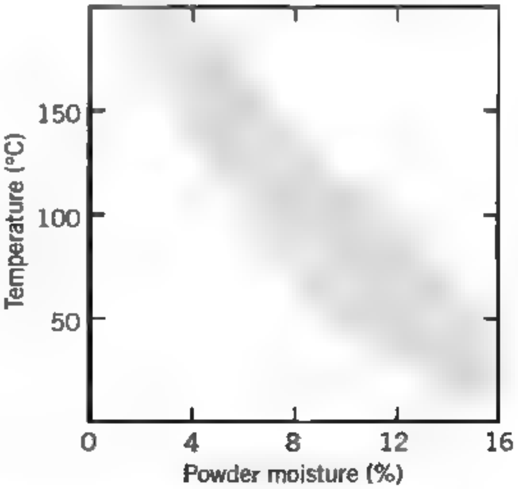


Figure 32. Typical combinations of conditions for agglomeration.

ture is dried off and the powder cooled below its thermoplastic point.

Agglomeration Equipment

While slightly different in equipment design and operation, most commercially available agglomeration processes fundamentally are the same. Each relies on the formation of agglomerates by the mechanism already described. This is followed by final drying, cooling, and size classification to eliminate the particle agglomerates that are either too small or too large. Generally, designs involve a re-wet chamber followed by a belt or a fluid bed for moisture removal. Such a system is shown in Figure 33.

It is obvious that this system is quite sensitive to even very minor variations in powder or liquid rates. A very brief reduction in powder feed rate will result in overwetting of the material with consequent deposit formation in the chamber. Conversely, a temporary reduction in liquid rate will result in sufficiently wetted powder and, therefore, weak agglomerates. Many designs rely on the product impacting the walls of the agglomeration chamber to build up agglomerate strength. Other designs include equipment for breaking large lumps into suitably sized agglomerates before the final drying. Obviously, deposit formation always will be a concern in agglomeration equipment as the process depends on the creation of conditions where the material becomes sticky.

Typically, equipment designs are very complicated, probably reflecting the fact that agglomeration actually is a complicated process. Despite the complexity of the process, however, it is possible to carry out agglomeration by means of comparatively simple equipment that involves the use of a fluidized bed for the rewetting and particle contact phase. This approach provides the following advantages:

1. There is sufficient agitation in the bed to obtain a satisfactory distribution of the binder liquid on the particle surfaces and to prevent lump formation.
2. Agglomerate characteristics can be influenced by varying operating parameters such as the fluidizing velocity, rewet binder rate, and temperature levels.
3. The system can accept some degree of variation of the feed rate of powder and liquid as the product level in the fluid bed always is constant, controlled by an overflow weir. Thus, the rewetting section will not be emptied of powder. Even during a complete interruption of powder flow, the fluidized material will remain in the rewet section as a stabilizing factor in the process.
4. By using fluid bed drying and cooling of the formed agglomerates, it is possible to combine the entire agglomeration process into one continuously operating unit.
5. Startup, shutdown, and operation of the fluid bed agglomerator are greatly simplified owing the stabilizing effect of the powder volume in the rewet zone.

Proper implementation of a fluid-bed agglomeration system requires a detailed knowledge of the fluidization technology itself. Fluidization velocities, bed heights, airflow patterns, residence time distribution, and the mechanical design of vibrating equipment must be known.

Features of Fluid-Bed Agglomeration

Figure 34 shows a typical agglomerator system where the process is implemented through the use of a vibrated, continuous fluid bed.

The powder is fed into the agglomerator by a volumetric screw feeder. As a result of the previously mentioned stabilizing effect of the material already in the fluid bed, the

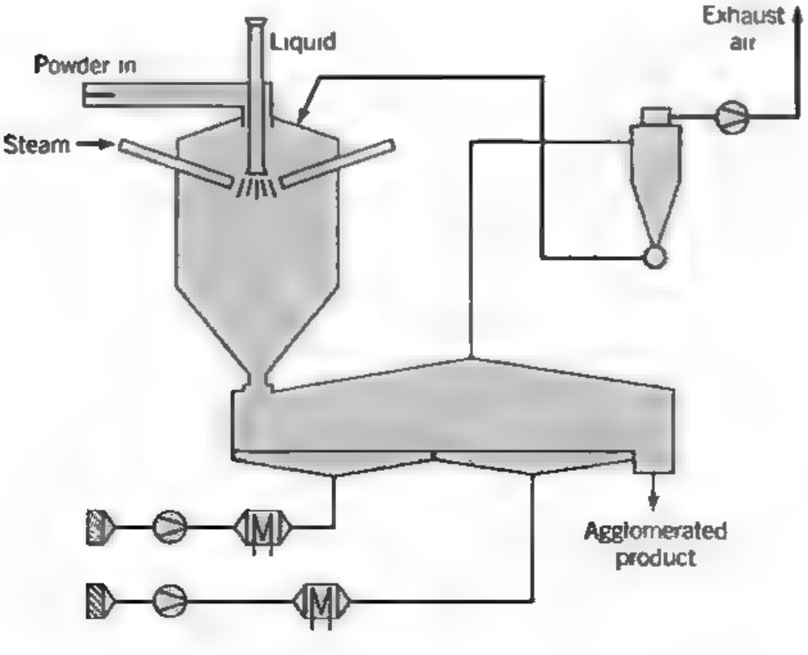


Figure 33. Typical agglomeration system.

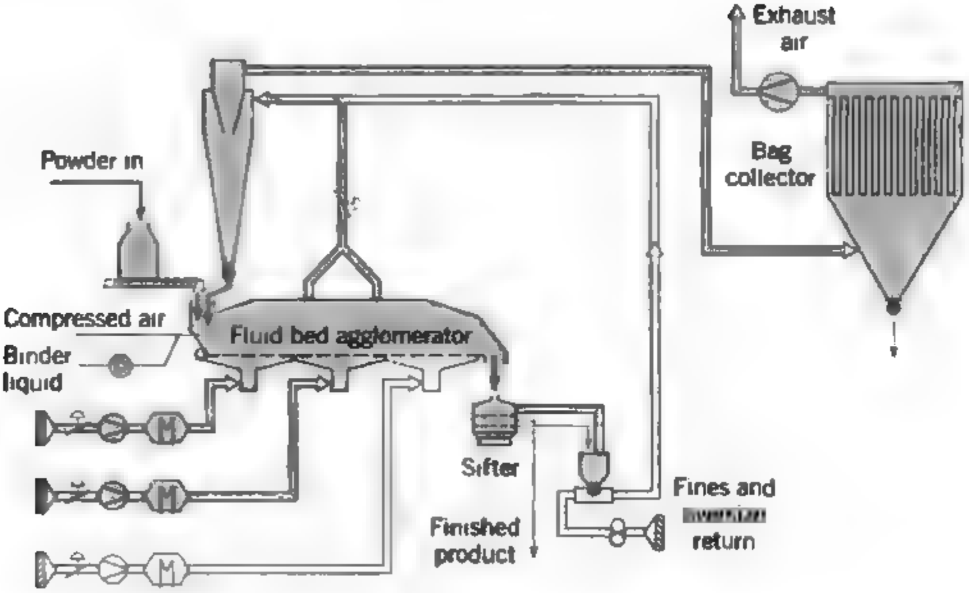


Figure 34. Fluid bed rewet agglomeration system.

Table 5. Frequently Used Binders

For food products	For chemical products
Malto dextrines	Lignosulfonates
Gum arabic	Poly(vinyl alcohol) (PVA)
Starch	Any of the food product binders
Gelatin	
Molasses	
Sugar	

reproducibility of a volumetric feeder is satisfactory and there is no need for a complicated feed system such as a loss-in-weight or similar type.

The fluid bed unit is constructed with several processing zones, each with a separate air supply system. The first section is the rewet and agglomeration section where agglomerates are formed. Here, the powder is fluidized with heated air in order to utilize its thermoplastic characteristics.

The binder liquid almost always is water or a water-based solution, whereas steam, as already explained, rarely is used. The binder liquid is sprayed over the fluidized layer using two fluid nozzles driven by compressed air. For large systems, numerous nozzles are used. Powder deposits are minimized by accurate selection of spray nozzle angles and nozzle position patterns. Powder movement is enhanced by the vibration of the fluid-bed unit itself and by the use of a special perforated air distribution plate with directional air slots. A proper detailed design is vital in order to have a trouble-free operation.

From the agglomeration zone, the powder automatically will flow into the drying area where the added moisture is removed by fluidization with heated air. In some instances, more than one drying section is required, and in such cases, these sections are operated at successively lower drying temperatures in order to reduce thermal exposure of the more heat-sensitive dry powder.

The final zone is for cooling where either ambient or cooled air is used to cool the agglomerates to a suitable packaging temperature.

During processing, air velocities are adjusted so that fine, unagglomerated powder is blown off the fluidized layer. The exhaust air is passed through a cyclone separator for removal and return of entrained powder to the inlet of the agglomerator. When there are high demands for a narrow particle size distribution, the agglomerated powder is passed through a sifter, where the desired fraction is removed while over- and undersize material is recycled into the process.

As with all rewet agglomeration equipment, the operation must be performed within certain operating parameters. Overwetting will lead to poor product quality, while underwetted powder will produce fragile agglomerates and an excessive amount of fines. However, fluid-bed agglomeration does offer a great degree of flexibility in controlling the final result of the process. The characteristics of the formed agglomerate can be influenced by operating conditions such as binder liquid rate, fluidizing velocity, and temperature. Typically, the fluid-bed rewet method will produce agglomerated products with superior redispersion characteristics.

As indicated by this partial list, this method has been used successfully with a number of products.

Dairy products
Infant formula
Calf milk replacer
Flavor compounds
Corn syrup solids
Sweeteners
Detergents
Enzymes
Fruit extracts

Malto dextrines
Herbicides
Egg albumin

In most cases, the agglomeration can be accomplished using only water as a rewet medium. This applies to most dairy products and to malto dextrine-based flavor formulations. For some products, increased agglomerate size and strength has been obtained by using a solution of the material itself as the binder liquid. In the case of relatively water-insoluble materials, a separate binder material has been used, but it must be one that does not compromise the integrity of the final product. The addition of the binder material may have a beneficial effect on the end product at times. This is seen, for example, in flavor compounds when a pure solution of malto dextrine or gum arabic may further encapsulate the volatile flavor essences and create better shelf life. In other instances, the added binder can become part of the final formulation as is the case with some detergents.

For some materials, the addition of a binder compounds is an unavoidable inconvenience. At such times, the selected binder must be as neutral as possible and must be added in small quantities so that the main product is not unnecessarily diluted. An example of this is herbicide formulations, which often have a very well defined level of active ingredients.

For products containing fat, the normal process often is combined with a step by which the agglomerates are coated with a thin layer of surface-active material, usually lecithin. This is done by mounting an extra set of spray nozzles near the end of the drying section where the surfactant is applied.

Variations of the fluid-bed rewet technology have been developed whereby the system serves as a mixer for several dry and wet products. An example of this may be seen in the APV fluid mix process for the continuous production of detergent formulations from metered inputs of the dry and wet ingredients. This provides an agglomerated end product that is produced with minimum energy input when compared to traditional approaches.

Spray-Bed Dryer Agglomeration

While the fluid-bed rewet agglomeration method produces an excellent product that is, in most respects, superior to that made directly by the straight-through process, a new generation of spray dryers has evolved that combines fluid-bed agglomeration with spray drying. These are referred to as "spray-bed" dryers. The concept was developed from spray dryers having a fluid bed integrated into the spray chamber itself and is depicted in Figure 35. What distinguishes the spray-bed dryer is that it has the drying air both entering and leaving at the top of the chamber. Atomization can be with nozzles or by a centrifugal atomizer.

During operation, the chamber fluid bed is vigorously agitated by a high fluidization velocity, and as the particles from the spray-drying zone, they enter the fluid bed with a very high moisture content and agglomerate with the powder in the bed. Fines carried upward in the dryer by the high fluidizing velocity have to pass through the spray cloud, thus forming agglomerates at this point as well. Material from the integrated fluid bed is taken to an external fluid bed for final drying and cooling.

The spray-bed dryer is a highly specialized unit that can produce only agglomerated powder. It is best suited for small to medium-sized plants since the efficiency of the agglomeration process unfortunately decreases as the plant increases in size. This is because the spray zone becomes too far removed from the fluid-bed zone as the size of the dryer increases.

Agglomerates from the spray-bed dryer exhibit excellent characteristics. They are very compact and show high agglomerate strength and good flowability.

Considerations and Conclusions

While the agglomeration process improves the redispersion, flowability, and nondustiness of most fine powders, it invariably decreases the bulk density. The comparison in Table 6 clearly shows that agglomeration improves the powder wettability and dispersibility. Individual powder particles with a mean diameter of less than 100 μm are converted into agglomerates ranging in size from 250 to 400 μm, with the rewet method being able to produce the coarser agglomerate. The powder bulk density will decrease from about 43 lb/ft^3 to approximately 28 lb/ft^3. Use of the rewet method will expose the product to one additional processing step that, in this case, will somewhat affect the proteins and result in a slightly poorer solubility.

Since fluid bed agglomeration can be operated as an independent process, it can be used with already existing power-producing equipment. It offers great flexibility and ease of operation, and provides a convenient way to add functionality, nondustiness, and value to a number of products.

SPIN FLASH DRYERS

Background Information

While mechanical dewatering of a feed slurry is significantly less expensive than thermal drying, this process results in a paste or filter cake that cannot be spray dried and can be difficult to handle in other types of dryers. The spin flash dryer is one option available for continuous powder production from pastes and filter cakes without the need for grinding.

Powders generally are produced by some form of drying operation. There are several generic types of dryers, but all must involve the evaporation of water, which can take anywhere from 1000 to 2500 Btu/lb depending on dryer type. The most common of these dryers probably is the spray dryer because of its ability to produce a uniform powder at relatively low temperatures. However, by its definition, a spray dryer requires a fluid feed material to allow its atomization device to be employed. Generally, there is a maximum viscosity limitation in the range of 1000–1500 SSU (Saybolt Seconds Universal viscosity unit) (see Fig. 36).

Figure 37 illustrates the amount of water that must be evaporated to produce 1 lb of bone dry powder from a range

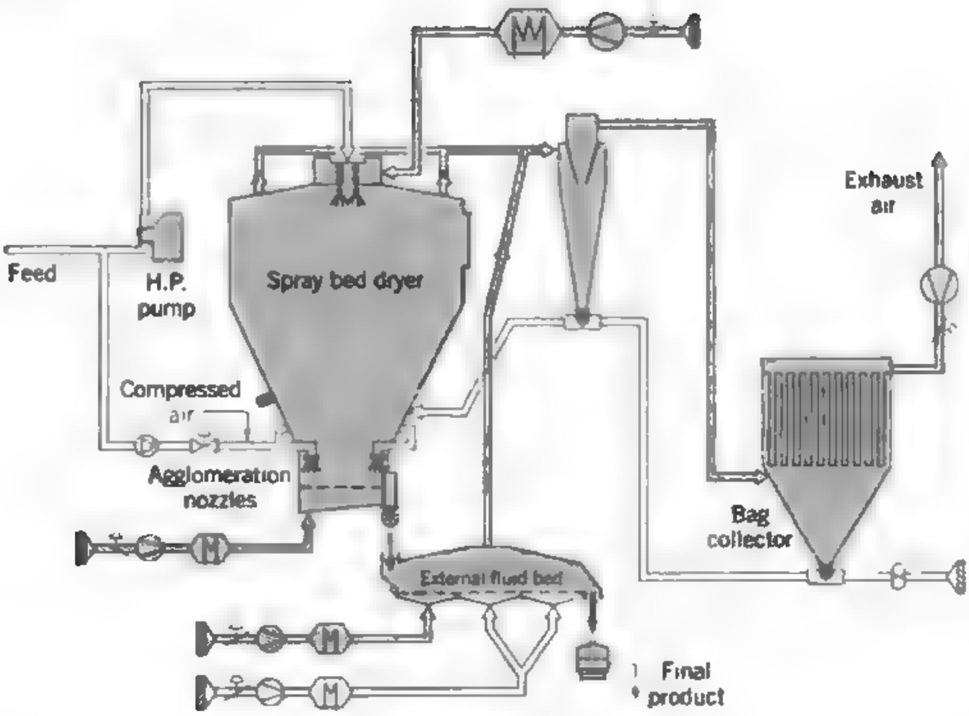


Figure 35. Spray bed-type agglomerating spray dryer.

Table 6. Reconstitutability and Physical Structure of Different Types of Skim Milk Powder

	Ordinary spray dried powder	Integrated fluid-bed agglomeration	Rewet agglomerated powder
Wettability, s	>1000	<20	<10
Dispersibility: %	60–80	92–98	92–98
Insolubility index	<0.10	<0.10	<0.20
Average particle size, μm	<100	>250	>400
Density, lb/ft^3	40–43	28–34	28–31

of different feed solids. It can clearly be seen that even a 5% increase in total solids will reduce the water evaporation and, hence, the dryer operating costs by about 20%. If this water removal can be done mechanically by, for example, filtration or centrifuging, the cost will be infinitely lower than that required to heat and evaporate the same water. The direct energy cost can be calculated as equivalent to 3–8 Btu/lb, compared to an average 1500 Btu/lb for evaporation. This increase in solids, however, inevitably will result in an increase in viscosity that may exceed the limitations of a spray dryer.

Options available for drying these higher-viscosity feed materials are listed in Table 7. The spin flash dryer is among the newest of the dryer options and has the capability of drying most materials ranging from a dilatent fluid to a cohesive paste.

Description

The spin flash dryer was developed and introduced in 1970 in response to a demand by the chemical industry to produce a uniform powder on a continuous basis from high-viscosity fluids, cohesive pastes, and sludges.

The spin flash dryer can be described as an agitated fluid bed. As shown in Figure 38, the unit consists primarily of a drying chamber (9), which is a vertical cylinder with an inverted conical bottom, an annular air inlet (7), and an axially mounted rotor (8). The drying air enters the air heater (4), is typically heated by a direct-fired gas burner (5), and enters the hot-air inlet plenum (6) tangentially. This tangential inlet, together with the action of the rotor, causes a turbulent whirling gas flow in the drying chamber.

The wet feed material, typically filter cake, is dropped into the feed vat (1) where the low-speed agitator (2) breaks up the cake to a uniform consistency and gently presses it down into the feed screw (3). Both agitator and feed screw are provided with variable speed drives.

In the case of a dilatent fluid feed, the agitated vat and screw would be replaced with a progressive cavity pump and several liquid injection ports at the same elevation as the feed screw.

As the feed material is extruded off the end of the screw into the drying chamber, it becomes coated in dried powder. The powder-coated lumps then fall into the fluid bed and are kept in motion by the rotor. As they dry, the friable

Figure 36. Typical APV Anhydro spin flash dryer with general dryer characteristics. Dryer characteristics: drying method—direct gas contact; flow—cocurrent; food material—dilatent fluids, cohesive paste, filter cake, moist granules; drying medium—air, inert gas, low-humidity waste gas; inlet temperature—up to 1800°F; capacity—up to 10 tons per hour of final product; product residence time—5–500 s.

Table 7. Dryer Options for High-Viscosity Materials

Direct suspension dryers
Pneumatic or flash dryers
Spin flash dryers
Fluid-bed dryers
Direct nonsuspension dryers
Tray dryers
Tunnel dryers
Belt dryers
Rotary dryers
Indirect dryers
Screw conveyor dryers
Vacuum pan dryers
Steam tube rotary dryers

surface material is abraded by a combination of attrition in the bed and the mechanical action of the rotor. Thus, a balanced fluidized bed is formed that contains all intermediate phases between raw material and finished product.

The dryer and lighter particles become airborne in the drying airstream and rise up the walls of the drying chamber, passing the end of the feed screw and providing, in effect, a continuous backmixing action within the heart of the dryer. At the top of the chamber, they must pass through the classification orifice, which can be sized to prevent the larger particles from passing on to the bag collector. These larger lumps tend to fall back into the fluid bed to continue drying.

Air exiting from the bag collector (10) passes through the exhaust fan (12) and is clean enough for use in a heat-recovery system. Dried powder is discharged continuously from the bottom of the bag collector through the discharge valve (11).

Two important features make the spin flash dryer suitable for products that tend to be heat-sensitive: (1) the dry powder is carried away as soon as it becomes light enough and therefore is not reintroduced into the hot-air zone, and (2) the fluid bed consists mainly of moist powder, which constantly sweeps the bottom and lower walls of the drying chamber and keeps them at a temperature lower than the dryer air outlet temperature. In addition to this self-cooling capacity, the lower edge of the drying chamber directly above the hot-air inlet can be provided with an auxiliary cooling ring.

Figure 39 illustrates the very rapid reduction in air temperature that occurs as a result of the high heat-transfer rate obtained in the fluid bed.

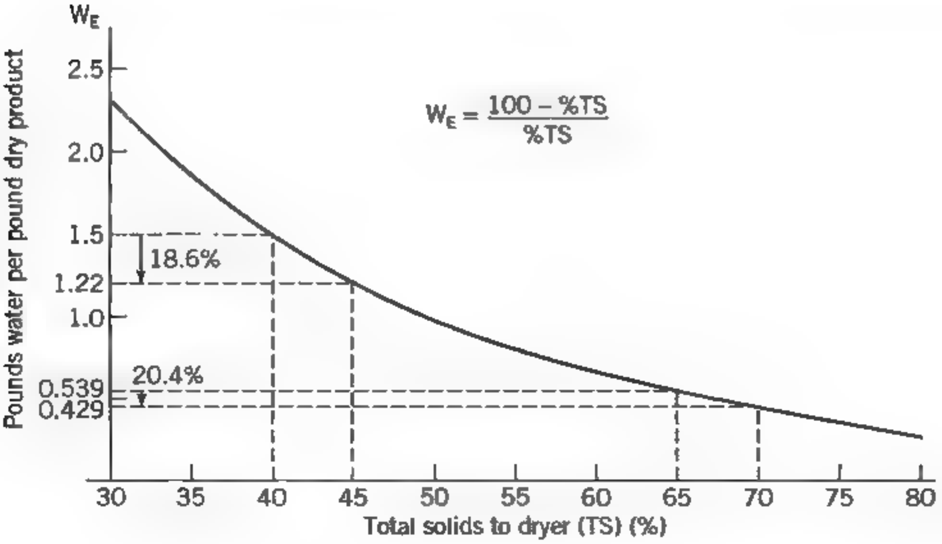


Figure 37. Water evaporation/total solids ratio.

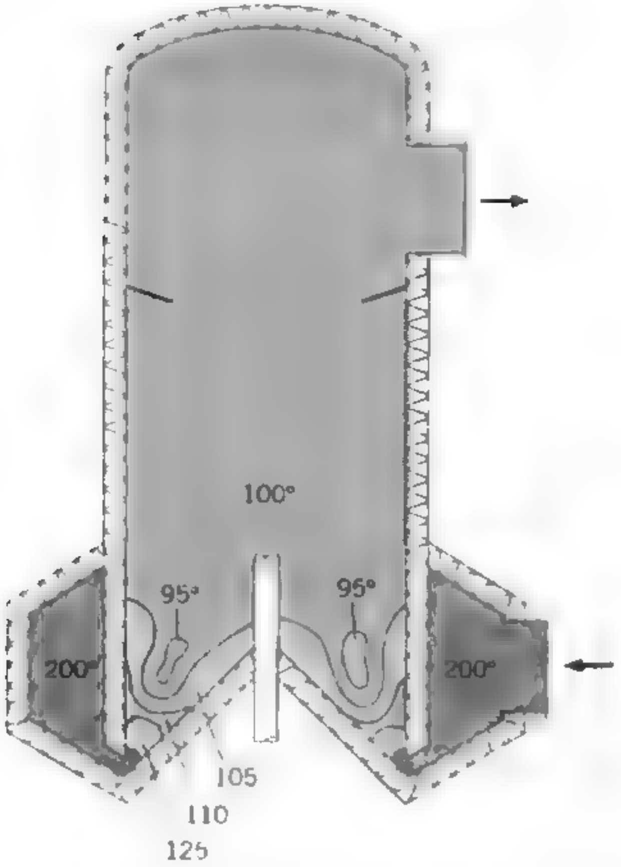


Figure 38. Fluid bed provides rapid air temperature reduction.

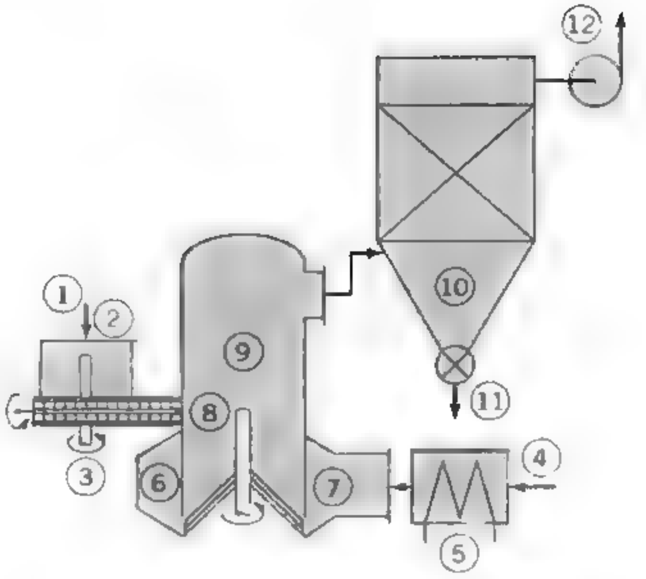


Figure 39. Standard spin flash dryer configuration.

Operating Parameters

Inlet temperature of the drying air introduced into the chamber is dependent on the particular characteristics of the product being dried but generally would be similar to that used on a spray dryer for the same product.

Outlet temperature is selected by test work to provide the desired powder moisture and is controlled by the speed of the feed screw. Since the spin flash dryer produces a finer particle size than does a spray dryer, it has been found that a slightly lower outlet temperature may be used to obtain

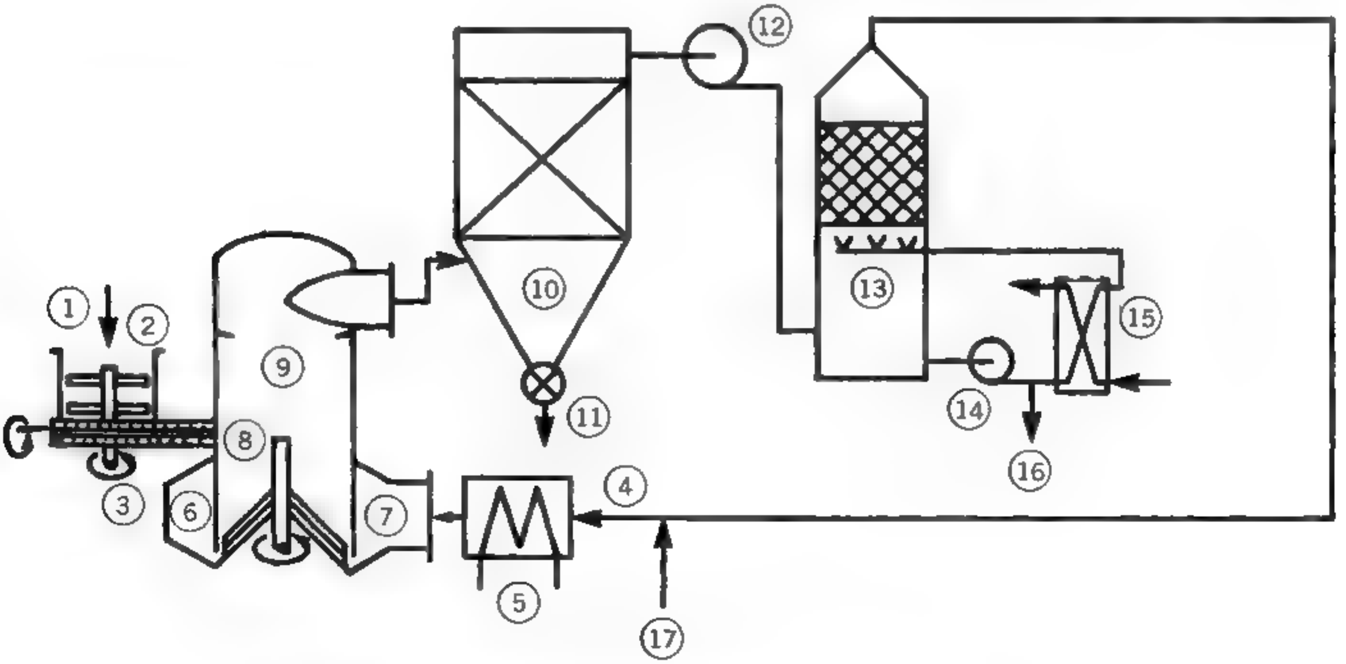


Figure 40. Spin flash dryer in closed-cycle arrangement.

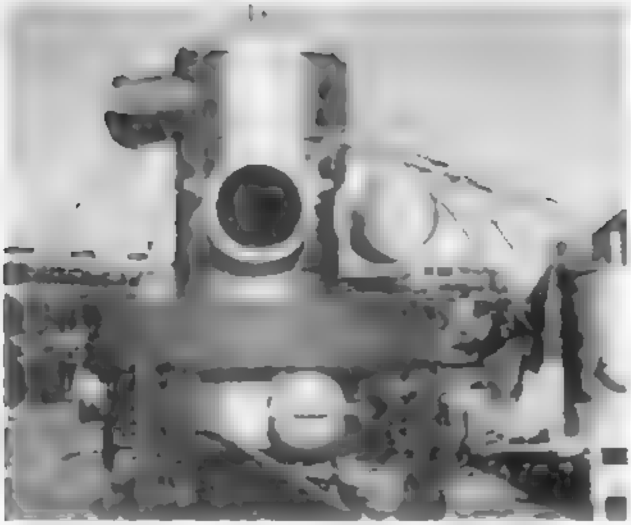


Figure 41. Spin flash dryer being prepared for shipment.

the same powder moisture. This provides an increase in thermal efficiency.

Air velocity through the cross section of the drying chamber is an important design factor and is determined in part by the final particle size that is required. A lower velocity will tend to reduce the final dried particle size carried out of the chamber. The major factor, however, is the stability of the very complex bed that must neither settle back into the air distributor nor blow out of the top of the chamber. Once the maximum velocity has been determined by test work for a given product, the diameter of a drying chamber can be selected to provide the desired water evaporation rate.

Capacity can be adjusted to suit the output from the preceding process equipment, which may be difficult to control and slow in response time. This is achieved by a cascade control from a feed vat level sensor to the inlet temperature controller set-point. The feed vat sometimes can be oversized to accept the batch discharge from a preceding filter press while allowing the dryer to operate continuously.

Closed-Cycle Drying

Once a decision has been made to increase feed solids prior to drying, the small size and lower airflow requirements of the spin flash dryer make it practical to design the system as a closed-cycle dryer with nitrogen as the drying medium. This type of system can be used to dry a solvent-based powder, allowing complete recovery of the solvent.

The simplified Figure 40 schematic shows a possible configuration of a closed-cycle spin flash dryer. The operating process as described earlier, is extended, with exhaust gas from the baghouse being scrubbed and cooled in a condenser (13) using cooled solvent from an external plate heat exchanger (15) as the scrubbing medium. Recovered solvent is bled off at (16), downstream from the scrubber recirculation pump (14) at a controlled rate based on scrubber liquid level.

Table 8. Products Dried on Spin Flash Equipment

	Unit	Sludge of various metal hydroxides	Dolomite	Yellow iron oxide	Alumina	Aluminum silicate	Calcium carbonate with binder	Calcium carbonate (pure)	Ferrite	Tartrazine (azo dye)	Food yellow (color index 15985)	Calcium stearate	Polyvanadate	Nickel catalyst on diatomaceous earth	Barium sulfate	Titanium dioxide
Inlet air temperature	F	482	590	617	482	842	536	968	932	410	437	212	914	662	1112	1292
Outlet air temperature	F	185	293	212	194	212	194	338	257	203	208	126	320	311	275	257
Temperature of the feed	F	60	60	55	65	60	55	32	55	50	70	70	55	70	60	60
Total solids in feed	%	70	34	35	29	20	58	67	62	50	28	43	35	33	78	65
Residual moisture	%	4.5	0.4	0.6	12.5	5.5	0.3	0	1.0	5.0	9	0.32	2.7	3	0.1	0.5
Mean particle size	μm	40	15	5	70	20	50	5	15	10	10	16	20	15	5	3
Bulk density	gr/cm^3	0.8	0.45	0.3	0.4	0.2	0.45	0.8	0.9	0.7	0.3	0.14	0 76	0.32	1.6	0.6

Table 9. Size and Cost Comparison: Spray Dryer vs. Spin Flash Dryer

Space requirements	Spray dryer type III-K no. 70/71	Spin flash type III no. 59
	Space requirements	
Heater type (gas)	Direct-fired	Direct-fired
Chamber diameter, ft	14′0″	2′7-1/2″
Building floor area, ft^2	650	325
Building height, ft	46′0″	16′6″
Building volume, ft^3	24,700	5,300
	Capacity	
Powder, lb/h	880	880
	Performance data	
Feed solids, %	30	45
Feed rate, lb/h	2,995	1,951
Water evaporation, lb/h	2,115	1,071
Powder moisture, %	0.4	0.4
Gas consumption, SCF/h[a] (1000 Btu/SCF)	4,416	2,192
Power consumption (kWh)	40	30
	Investment cost, U.S. $	
Building	75,000	20,000
Dryer equipment	174,000	147,000
Baghouse	22,000	18,000
Installation	35,000	22,000
	$306,000	$207,000
	Assumptions	
Gas cost, $/M Btu $4.32		
Electricity cost, $/kWh $0.07		
Salaries per hour $15.00		
Manpower required = 1 operator		
	Variable operating costs per hour	
Wages	$15.00	$15.00
Gas	19.08	9.47
Electricity	2.80	2.10
Total	$36.88	$26.57
Cost per lb of powder	$0.042	$ 0.03

Note: It can be seen that the investment in a spin flash dryer plant is about 32% lower than a spray dryer for the same capacity and that spin flash operating costs are approximately 29% lower. These figures, however, do not include the capital investment in filtration equipment.

[a] Standard cubic feet.

The drying chamber would be maintained at a pressure slightly higher than ambient using a pressurized nitrogen purge (17). The heater (5) would use either a steam coil or a thermal fluid system with an external heater.

A less expensive alternative to the closed-cycle approach is a "Lo-Ox" (low-oxygen) system where a low excess air burner is used in a direct-fired heater. The products of combustion are recirculated through the condenser, and the surplus gas is vented to atmosphere. The oxygen level in such a system can be controlled to less than 5%. (See also Figure 41.)

Spin-Flash-Dried Products

While Table 8 charts some typical products being dried on spin flash equipment, it should be noted that in some cases the inlet temperatures are limited by the heat source used and not by the spin flash process.

Other products test dried on a laboratory scale spin flash dryer include the following:

Product	Feed Solids, %	Powder Moisture, %
Iron oxide	70	2
Effluent treatment sludge	22	40
Lignin	49	15
Gum	40	13
Chitosan gel	6	40
Crab meat paste	30	9
Dutched cocoa cake	70	2

Cost Benefits

In comparison to a spray dryer, the spin flash dryer has a much shorter residence time and consequently is considerably smaller and requires less building space. Its ability to dry to even higher solids than a spray dryer results in operating cost savings. Table 9 shows a detailed size and cost comparison based on actual test drying of yellow iron oxide.

Conclusion

Despite its obvious size and cost advantages, there are many instances when a spin flash dryer cannot replace a spray dryer. Typically, such cases occur when a free-flowing spherical particle of a particular size range is required or when agglomeration is needed.

There are, however, many situations in both the food and chemical industries where the particular capabilities of the spin flash dryer to produce powders from paste warrant careful consideration of its use.

PARTIAL LIST OF PRODUCTS PROCESSED ON VARIOUS TYPES OF APV DRYERS

Spray Dryer

Albumen
Alcoholic extracts
Alfalfa
Alginates
Amino acids

Baby food
Bananas
Beef extract
Beer wort
Bile extract
Bouillon
Brain
Butter
Buttermilk

Calcium carbonate
Calcium salts
Carbon, active
Carboxymethylcellulose
Carrageenan
Casein hydrolysate
Caseinates
Catalysts
Cellulose acetate
Cellulose hydrate
Cheese
Chelates
Chlorophyll
Chocolate milk
Citrates
Colors
Cornstarch
Corn steep liquor
Corn syrup
Cream

Dextranes
Dextrin maltose

Fat-enriched milk
Fatty alcohol sulfonates
Fish meat, hydrolyzed
Fish pulp
Fish solubles
Flavors
Fruit juice
Fruit pulp fullers earth

Garlic
Gelatin
Glands
Glauber salt
Glazes
Glucoheptonate
Glucose
Gluten
Glyceryl monostearate
Gum arabic

Hormones
Hydrolysates
Hypochlorites

Ice cream mix
Lactates
Lactose
Licorice
Lignin
Liver extract

Magnesium carbonate
Magnesium oxide
Magnesium salts
Magnesium sulfate
Malted milk
Malt extract
Mango
Meat extract

Dextrose
Diastase
Diatomaceous earth
Dietetic products
Distillers waste

Egg white
Egg yolk
Emulsifiers
Enzymes
Pectin
Penicillin
Pepsin
Peptones
Phosphates
Pigments
Potassium sulfate
Potatoes
Potato waste liquor
Proteins

Quaternary salts
Rennet

Saponin
Seaweed extract
Senna
Sequestering agents
Silica-alumina gels
Silicates
Skim milk
Sodium adipate
Sodium phenate
Sorbate
Sorbose
Soybean milk
Soybean protein

Milk-cocoa
Molasses with filler

Nitrates

Olive paste

Pancreas
Papain
Peanut milk
Soy flour
Starch products
Stearic acid
Stearyl-tartrate
Sulfonates

Tannin extract
Tapioca
Tea extract
Thiamin
Thymus
Tomato
Vegetable extracts
Vegetable proteins

Wetting agents
Whey
Whey nonhygroscopic
Whole egg
Whole milk
Yeast
Yeast autolysate
Yeast hydrolysate

Zinc ammonium chloride
Zinc chromate
Zinc stearate

Film Drum Dyers

Agar
Apple pectin
Avocado pulp

Baby food
Beef, comminuted
Beef extract
Beef protein (hyrolyzed)
Beer concentrate
Blood
Buttermilk

Calcium acetate
Calcium carbonate
Calcium propionate
Caramel
Cattle food
Cellulose
Cereal (baby)
Chicken offal
Cider less
Corn syrup
Manure
Meat extract
Meat pieces
Milk crumb
Milk (skimmed)

Dextrine

Effluent
Essence

Fat
Fatty alcohol sulfates
Fermentation waste
Ferric ammonium syrup
Ferrous sulfate
Fish food
Fish stick liquor
Flavors
Flour

Gelatin
Glauber's salts

Herbicide
Hydrolyzed protein
Maize gluten
Mango flake
Single-cell protein
Sorghum and maize
Starch
Stearate
Stearic acid

Molasses

Oat flour

Onion pulp
Palm oil residue
Pectin
Phenylglycine casein
Potato (waste)
Press liquors
Protein (hyrolyzed)
Rice starch
Seaweed extract
Semolina

Steep liquor
Stick liquor
Sugar solution
Oats
Tapioca starch
Vegetable extract
Vegetable protein

Wax
Whale solubles
Wheat starch
Whey
Yeast cream
Yeast extract

Thermoventuri Dryer

Alginates
Alginic acid

Bread crumbs

Cocoa residue
Cornstarch
Coumarin

Dark grains
Demerara sugar
Dextrose
Fishmeal
Flour

Glucose starch
Groundnut residue
Lactose

Maize starch

Organic acids

Pectin
Potato

Spent grains
Sphagnum moss
Starches
Stearates
Sulfur waste
Tartaric acid

Wheat germ
Wheat gluten
Wheat starch
Yeast

Coveyor-Band Dryer

Almonds
Apples
Apricots
Assorted biscuits

Beans
Bran

Cabbage
Carrots
Celery
Chocolate milk crumb
Coconuts
Corn grit

Bread crumbs
Breakfast cereals
Filter board
Food concentrates
French fries

Garlic
Gelatin
Grapes

Iced biscuits
Jellies

Meat
Mints
Molding powders

Nougat
Nuts
Onions

Dog biscuits
Dog food
Pastilles
Peanuts
Peas
Pectin
Peppers
Pet food
Plums
Potatoes
Puffed cereals
Rice
Sausage rusk
Seaweed
Soya protein
Sugar dough
Sugar- and/or honey-coated cereals

Turnip
Wheat flakes

BIBLIOGRAPHY

Copyrighted APV Crepaco, Inc. Used with permission.

1. D. Noden, "Industrial Dryers Selection, Sizing, and Costs," *CPI Digest* 8(1), 6–23, (1985).
2. D. Noden, "Efficient Energy Utilization in Drying," *dfi News* 7(4), 8–14, (1985).
3. B. Bjarekull, "Spray Drying New Developments, New Economics," *dfi News* 8(2), 6–13 (1985).
4. B. Bjarekull, "Fluid Bed Agglomerization for the Production of Free-Flowing Dustless Powders," *dfi News* 8(2), 6–13 (1985).
5. S. G. Gibson, "Spin Flash Dryers for Continuous Powder Production from Pastes and Filter Cakes," *APV Dryer Handbook*, Oct. 1985, pp. 36–42.

APV Crepaco, Inc.
Lake Mills, Wisconsin

***E. COLI* 0157.** See FOODBORNE DISEASES.

EDIBLE FILMS AND COATINGS

Edible films and coatings are generally defined as thin layers of edible material that are applied on (or within) food products to extend shelf life by functioning as solute, gas, and vapor barriers. Also, they can modify product appearance, improve structural integrity, act as carriers of food additives (eg, antimicrobials, antioxidants, and flavorings) and, in certain instances, partially or completely replace synthetic packaging materials. The terms films and coatings are often used interchangeably. Generally, coatings are formed on foods after application by dipping, brushing, spraying, or tumbling, whereas stand-alone films are preformed by casting or extrusion and then placed on (or within) foods. Relevant commercial applications range from wax coatings for fresh produce, to extruded collagen casings for sausages, to sugar coatings for bakery and confectionery products, to water-soluble pouches for premeasured food additives. Interest in development, characterization, and application of edible films and coatings has recently intensified, resulting in voluminous peer-reviewed and patent literature on the subject. Several protein, polysaccharide, and lipid materials derived from plants and animals are known film-forming ingredients with current or potential edible packaging applications. Typically, protein- and polysaccharide-based films and coatings require incorporation of low molecular weight edible plasticizers such as glycerin, sorbitol, polyethylene glycol, propylene glycol, and fatty acids. The present summary is intended as a reference for various types of edible film-forming materials and not as an edible packaging applications guide.

PROTEIN FILMS

Collagen

One of the earliest uses of edible films is the use of natural casings made from animal intestines to restructure comminuted meat. Natural casings have the disadvantages of low mechanical strength, inconsistent quality, and limited availability. Therefore, natural casings have been largely supplanted by (artificial) collagen casings fabricated from swollen collagen fibers. Sausage casings hold and shape the meat batter while the meat protein is being heat-set. They have no real barrier properties; they allow moisture to pass when needed, and allow smoke and flavorings to enter the sausage. Compared with natural intestine casings, fabricated edible collagen casings offer improved uniformity, processibility, and sanitation. However, edible collagen casings are somewhat tough and tend to split when grilled.

The currently used extrusion process for fabricating collagen casings from regenerated bovine hide corium was developed in the late 1950s. The process involves dehairing hides by alkaline or acid treatment, decalcifying hide coria; grinding into small pieces; acidifying to produce a swollen slurry (4–5% solids); homogenizing; extrusion forming into tubular casings (8–10% solids); feeding extrudates into coagulating baths of brine or ammonium; washing casings free of salts; adding plasticizers and cross-linking agents; and drying (1). Perpendicular shear is applied to the collagen fibers as they exit the extruder to disrupt their laminar alignment to produce a more woven fiber structure. A collagen casing extrusion process developed in Germany in the 1930s employs collagen dispersions of higher solids contents (>12%) (1). Such dispersions require substantially higher extrusion pressures. This method has had limited acceptance outside Europe.

As an alternative to preformed collagen casings, a technology where the collagen casing is coextruded around the sausage meat batter was developed in the 1980s (2). The collagen dispersion is prepared *in situ* and pumped to a special extrusion nozzle. The meat emulsion is pumped through an inner opening in the nozzle and the collagen dispersion is applied to the emerging meat batter through counterrotating concentric cones that orient collagen fibers into a woven alignment. The continuous encased sausage rope is passed through a brine bath to dehydrate and set the collagen casing. The sausage rope is crimped into links, and the sausage is dried and smoked to further set the collagen casing. Coextruded sausage casings are more tender than preformed casings and, therefore, have better eating qualities, yet they remain more durable during grilling. The high initial cost of the coextrusion equipment has limited the acceptance of this method to large processing plants. Besides sausage casings, collagen-based edible films for use on netted roasts, boneless hams, fish fillets, and roast beef also have been commercialized since the late 1980s (3). Such films (trade name Coffi) can reduce cook shrink, increase product juiciness, and allow for easy removal of elastic stretch netting after heat processing. Reportedly, both refrigerated and frozen/thawed round beef steaks wrapped in collagen films prior to retail packaging (permeable film overwrap) or vacuum packaging exhibited substantially less fluid exudate than unwrapped steaks (4).

Gelatin

Gelatin is obtained by partial hydrolysis of collagen through alkaline or acidic treatments. It is an important hydrocolloid used in a number of food, photographic, pharmaceutical, and technical applications (5). In particular, gelatin is used widely in pharmaceutical and dietary supplement industries as an encapsulating agent (edible barrier) in hard (two-piece) capsules, soft capsules (softgels), and microcapsules (usually as coacervate with anionic polysaccharides). However, the film-forming properties of gelatin that have made it indispensable to the pharmaceutical and photographic industries have not been utilized by the food industry. This is mainly due to gelatin being too easily hydrated and solubilized to form an effective barrier in foods.

Cereal Proteins

Zein, the prolamin fraction of corn proteins has been studied extensively for its ability to form glossy, grease-resistant films that are not water-soluble. Protective coatings based on zein are commercially available for shelled nuts and confections (6) and have shown potential for extending the shelf life of tomatoes (7) and cooked turkey (8). Also, due to their grease resistance, zein-based coatings can reduce oil uptake by deep-fat fried foods (9). Wheat gluten also possesses good film-forming properties due to its cohesiveness and elasticity. At low moisture contents, wheat gluten films exhibited 10-fold lower aroma permeability than low-density polyethylene films (10). Typically, wheat gluten films are cast from aqueous alcohol solutions under alkaline (11) or acidic (12) conditions. The use of organic solvents (eg, ethanol or acetone) in zein and wheat gluten film-forming solutions has hindered the application of such films. Recently, a method was patented for preparing film-forming colloidal dispersions from dilute aqueous acid solutions of zein and wheat gluten (13). Also, zein-based moldable resins have been rolled into sheets (14). The relationship of wheat gluten (and other cereal proteins) to celiac disease, a dietary intolerance, should be considered when developing edible films, as well as allergies associated with various proteins. The film-forming properties of other cereal proteins, such as sorghum kafirin (15) and rice bran protein (16), have recently been investigated.

Oilseed Proteins

Soy protein-lipid films have traditionally been fabricated in East Asia on the surface of soy milk heated in open, shallow pans (6). Films are successively formed on and removed from the soy milk surface until protein in the solution is exhausted. Such films, known as "yuba" in Japan, can be used as meat and vegetable wrappers prior to cooking. Similar films have been prepared from peanut and cottonseed "milk." Also, heat-catalyzed protein polymerization through surface dehydration has been reported for heated solutions of soy protein isolates (17). This method is not suitable for large-scale commercial film production. Films have been cast from aqueous film-forming solutions of soy protein isolates (18). Soy protein films are generally less water resistant than zein or wheat gluten films.

Milk Proteins

Transparent and flavorless cast films have been prepared from both whey proteins and caseins (19). Reportedly, cross-linking with transglutaminase reduced water resistance and increased mechanical strength of milk protein films (20). Transglutaminase also catalyzed the formation of heterologous biopolymers from whey protein and 11S soy protein fraction (21). Besides film formation by casting, a modified wet spinning process was recently proposed for the continuous preparation of casein films (22). Edible sausage casings from casein have been prepared (23). Whey protein coatings have shown potential as oxygen barriers for dry roasted peanuts (24).

Other Proteins

Egg white (albumen) is a complex protein system with ovalbumin, ovotransferrin, ovomucoid, lysozyme, globulins, and ovomucin as the principal fractions. Films cast from aqueous alkaline solutions of desugared dried egg white (25) were clearer and more transparent than wheat gluten, zein, or soy protein films. Egg white films have limited water resistance and may find use as water-soluble packages (eg, pouches for premeasured food ingredients). Water-insoluble, transparent films with tensile strength close to those of polyethylene films have been developed from fish myofibrillar proteins (26). Other proteins that have received limited attention as film-formers are pea protein, feather keratin, and blood protein.

POLYSACCHARIDE FILMS

Alginates

Alginates are salts of alginic acid, a linear copolymer of D-mannuronic acid and L-guluronic acids that is extracted from brown seaweeds. The ability of alginates to gel in the presence of divalent or trivalent cations is utilized in forming alginate films. Calcium cations, which are more effective gelling agents than other polyvalent cations, "bridge" adjacent alginate chains via ionic interactions, after which interchain hydrogen bonding occurs (27). The application of alginate-based coatings on meat and seafood products has been discussed in several published studies and patent disclosures (3). A two-step procedure was used for forming such coatings. Application of aqueous sodium alginate solutions on food products, by dipping or spraying, was followed by treatment with a calcium salt solution (typically calcium chloride) to induce gelation (fixing). Calcium alginate coatings have reduced moisture losses from coated foods effectively, mainly due to their high moisture content. The coating acts as a sacrificing agent when moisture evaporates from the gel prior to any notable desiccation of the enrobed food (27). A bitter taste imparted on coated foods by calcium chloride has been a concern with alginate coatings. Patents (28,29) have been awarded for a fresh meat coating process in which a sodium alginate-oligosaccharide solution and a calcium chloride-thickening gum solution were successively applied on fresh meat by spraying or dipping. Based on these patents, an edible alginate-based coating for meats and other foods was developed and marketed (trade name Flavor-Tex) in the 1970s. Flavor-Tex's formulation included sodium alginate with maltodextrin in the first solution and calcium chloride with carboxymethyl cellulose in the second solution. Beef cuts coated with Flavor-Tex and stored at 5°C had reduced moisture loss but similar surface microbial counts compared with uncoated samples (30). Alginate coatings also extended shelf life and preserved texture of coated mushrooms by reducing the rate of water evaporation (31). In an active packaging application, lactic or acetic acids immobilized in calcium alginate gels were more effective in reducing viable counts of *Listeria monocytogenes* attached to lean beef surfaces after six days at 5°C than were acid treatments without alginate (32).

Carrageenan

Carrageenans are sulfated polysaccharides extracted from red seaweeds. The κ- and ι-carrageenan fractions form gels in the presence of monovalent and divalent cations. Locust bean gum and other mannans often are added to carrageenan gels to prevent syneresis and increase elasticity. Protective carrageenan coatings have been used to extend the shelf lives of meats and fish (3). A patented process described dipping precooked meat into an aqueous calcium carrageenan dispersion prior to freezing (33). The formed coatings extended product shelf life by preventing moisture loss and, thus, "freezer burn." Similar to alginate films, carrageenan films probably reduce moisture loss by serving as a reservoir of moisture that is sacrificed. A carrageenan-based active coating containing sorbic acid created a pH gradient on the surface of an intermediate moisture cheese analog product, thus enhancing the antimicrobial activity of sorbate (34).

Cellulose Derivatives

Water-soluble cellulose ethers including methyl cellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), and carboxymethyl cellulose (CMC) possess good film-forming properties. Their relative hydrophilicities increase in the order of HPC < MC < HPMC < CMC. Ethyl cellulose (EC) is another film-forming cellulose ether, which is water-insoluble. As discussed next, cellulose ethers often are used as matrices in composite films. Aqueous-based HPMC solutions are widely used in the pharmaceutical industry for tablet coating. MC and HPMC have been used as batter ingredients to reduce oil uptake during deep-fat frying of battered and breaded foods. Meatballs prepared from ground chicken breast and coated with HPMC absorbed up to 33.7% less fat than uncoated samples during deep-fat frying (35). HPMC coatings also reduced the number of viable *Salmonella montevideo* cells on the surface of tomatoes (36). Water-soluble, edible pouches from HPMC are used commercially to deliver preweighed minor manufacturing ingredients such as dough conditioners, leavening agents, vitamins, and minerals. Nature-Seal, a proprietary fresh produce coating formulation based on cellulose ethers and emulsifiers is used for delaying ripening of climacteric fruits (37).

Pectin

Pectins are composed primarily of the methyl esters of linear chains of 1,4-α-D-galacturonic acid units. They are extracted mainly from citrus peel and apple pomace. High-methoxyl (HM) and low-methoxyl (LM) pectins have degree of esterification above or below 50%, respectively. HM pectins gel at a soluble solids concentration of 55 to 80% and at acidic pH (2.8–3.7), whereas LM pectins gel in presence of divalent cations similarly to alginates (38). Applications of LM pectin coatings on oily or sticky food products such as shelled nuts and candied fruit have been proposed (39). A recent patent describes film casting from pectin or pectin/gelatinized starch mixtures (40).

Starch and Starch Derivatives

Starch, a glucose polymer, is composed of linear amylose and branched amylopectin. Both fractions can form films although amylose films have better mechanical and barrier properties than amylopectin films (41). Reportedly, tensile strength of nonplasticized starch films increased continuously as amylose content increased from 0 to 100% (42). As early as the 1950s, transparent films cast from water-butanol solutions of gelatinized amylose were evaluated for their functional properties (43). Extruded edible film wraps based on hydroxypropylated high amylose starch were commercially available (trade name Ediflex) in the late 1960s for application on frozen foods. High amylose starch coatings reduced moisture loss, maintained firmness, and reduced decay in coated, refrigerated strawberries (44). Also, various commercial modified starches, dextrins, and maltodextrins improved the quality of coated precooked dehydrated pinto beans (45).

Chitosan

Chitin, a glucosamine polymer, and chitosan (deacetylated chitin) are produced industrially from crustacean shell waste. Although abundantly available, chitin and chitosan have limited commercial use. They are approved in Japan and in Canada for various food applications but have not received approval by the FDA in the United States. Free-standing chitosan films can be cast from acetic, formic, or dilute hydrochloric acid solutions. For example, glycerin-plasticized films were cast from 3% w/w solutions of chitosan in 1% acetic acid (46). Protective chitosan coatings substantially reduced water loss in bell peppers and cucumbers (47). Also, chitosan coatings, due to the inherent antimicrobial properties of chitosan, inhibited growth of several spoilage microorganisms on raw refrigerated shrimp (48). Water-soluble *N,O*-carboxymethyl chitosan (NOCC) is produced by reacting chitin with chloroacetic acid under alkaline conditions. A commercially available NOCC coating (Nutri-Save) reportedly had some success as a selectively permeable postharvest coating for fresh fruit (38).

LIPIDS AND RESINS

Acetylated Glycerides

Acetylated glycerides can be obtained either by reacting glycerides with acetic anhydride or through the catalyzed interesterification of a fat or oil with triacetin (49). They solidify into stable α-form crystals that are waxy and relatively impermeable to moisture migration. Distilled acetylated monoglyceride coatings have been used in edible packaging applications since the 1960s. Claims have been made in patent disclosures that such coatings preserved meat freshness (50,51). However, acetylated monoglyceride coatings (trade name Dermatex) did not affect significantly the physical and sensory characteristics (eg, moisture, surface discoloration, and overall appearance scores) of vacuum-packaged beef steaks and roasts stored at 2°C for seven to eight weeks (52). More recently, acetylated monoglyceride (trade name Myvacet) coatings substantially reduced moisture loss and delayed the onset of lipid oxidation in frozen salmon pieces stored at −23°C (53). Certain application and sensory problems are associated with acetylated monoglyceride coatings including the tendency of highly saturated acetylated glycerides to crack and flake during storage. An acidic, bitter aftertaste also has been attributed to acetylated glycerides.

Waxes

Coating fresh produce with waxes to extend postharvest shelf life has a long history of use. Both natural waxes (eg, carnauba, beeswax, candelilla, and rice bran) and synthetic waxes (eg, paraffin and oxidized polyethylene) are used for this purpose. For example, commercial wax coating formulations include Primafresh 31 (carnauba wax), Shild-Brite PR-190 (natural waxes), Apple-Britex 559 (carnauba wax), and Fresh Wax 625 (oxidized polyethylene wax) (37). Generally, waxes are substantially more resistant to moisture transport than most other lipid or nonlipid edible films (27). Waxes applied to perishable produce replace natural surface waxes of the cuticle, prevent moisture loss, and improve surface luster. Application of wax coatings on the commodity surface may be accomplished by dipping, spraying, dripping, or controlled dropping (54). Waxes, by themselves, however, do not reduce decay. In fact, waxes can restrict oxygen and carbon dioxide gas exchange and cause uneven ripening or decay (55,56). For this reason, fungicides and growth regulators often are incorporated into waxes to control spoilage.

Resins

Resins are acidic substances that are secreted by many trees and shrubs in response to injury. Shellac resin is the alcohol soluble exudate of the insect *Laccifer lacca* and is the only commercially used natural resin of animal origin. Regular bleached shellac containing the natural shellac wax, or refined, dewaxed bleached shellac, which yields clearer solutions, are available. Shellac coatings are extensively used for candies (eg, candy corn), shelled nuts, and tablets (eg, multivitamins). Shellac-based enteric release coatings for solid dosage forms are utilized in the pharmaceutical industry (57). Fresh produce coatings from shellac or mixtures of shellac and waxes, such as carnauba wax, are commercially available (37,58). Besides shellac, other resins such as coumarone indene (petroleum-derived) and wood rosin are permitted for use in food coatings (59).

COMPOSITE FILMS

Combining various biopolymers is an obvious approach to tailor the functional properties of edible films and coatings to specific applications. Generally, polysaccharide and protein films provide limited resistance to moisture transmission due to the inherent hydrophilicity of such materials and to the notable amounts of incorporated hydrophilic plasticizers needed to impart adequate film flexibility. In contrast, hydrophobic lipids are effective moisture barriers. Composite films (prepared from emulsions or by lamination) made of lipids and polysaccharides or proteins combine the satisfactory structural and oxygen barrier properties of polysaccharide or protein films with the good moisture barrier characteristics of lipids. For example, bicomponent films prepared from cellulose ethers and lipids (eg, waxes, acetylated monoglycerides, and fatty acids) effectively restricted moisture migration in simulated food products containing major components differing substantially in water activity (60). Coatings comprised of MC or HPMC and fatty acids also may be used to control diffusion of antimicrobial agents (eg, potassium sorbate) into foods (61). Recently, highly refined cellulose dispersions made from fibrous agricultural by-products were copulverized at high pressure with various polysaccharides (guar gum, alginate, carrageenan, pectin, and xanthan gum) and coconut oil to form emulsions, which were cast into films (62). Such films had substantially lower water vapor permeability than those of polysaccharide-based films reported in the literature. Coatings comprised of sucrose esters of fatty acids and the sodium salt of CMC (trade names Semperfresh and Pro-long) effectively reduced ripening rate of fresh produce (63,64). Edible coating compositions for multicomponent food products combining shellac, HPC, and, optionally, fatty acids have been patented (65). Bicomponent chitosan-lauric acid films had 50% lower water vapor permeability than chitosan films (66). Composite protein-lipid films had lower water vapor permeability values than control protein films (19,67). Sodium caseinate-stearic acid coatings reduced white blush on peeled carrots by alleviating surface dehydration (68). Besides lipid-polysaccharide or lipid-protein films, composite films combining proteins and polysaccharides, such as starch, also have been studied recently (69,70). Blending various protein and polysaccharide film-formers with inexpensive starch can substantially reduce the cost of edible film formulations (71).

BIBLIOGRAPHY

1. L. L. Hood, "Collagen in Sausage Casings," in A. M. Pearson, T. R. Dutson, and A. J. Bailey, eds., *Advances in Meat Research*, Vol. 4, Van Nostrand Reinhold, New York, 1987, pp. 109–129.
2. J. W. Smits, "The Sausage Coextrusion Process," in B. Krol, P. S. van Roon, and J. H. Houben, eds., *Trends in Modern Meat Technology*, Pudoc, Wageningen, The Netherlands, 1985, pp. 60–62.
3. A. Gennadios, M. A. Hanna, and L. B. Kurth, "Application of Edible Coatings on Meats, Poultry and Seafoods: A Review," *Lebensm.-Wiss. Technol.* **30**, 337–350 (1997).
4. M. M. Farouk, J. F. Price, and A. M. Salih, "Effect of an Edible Collagen Film Overwrap on Exudation and Lipid Oxidation in Beef Round Steak," *J. Food Sci.* **55**, 1510–1512, 1563 (1990).
5. P. I. Rose, "Gelatin," in H. F Mark et al., eds., *Encyclopedia of Polymer Science and Engineering*, 2nd ed., Vol. 7, John Wiley & Sons, New York, 1987.

6. A. Gennadios et al., "Edible Coatings and Films Based on Proteins," in J. M. Krochta, E. A. Baldwin, and M. Nisperos-Carriedo, eds., *Edible Coatings and Films to Improve Food Quality*, Technomic Publishing Company, Lancaster, Penn., 1994, pp. 201–277.
7. H. J. Park, M. S. Chinnan, and R. L. Shewfelt, "Edible Coating Effects on Storage Life and Quality of Tomatoes," *J. Food Sci.* **59**, 568–570 (1994).
8. T. J. Herald et al., "Corn Zein Packaging Materials for Cooked Turkey," *J. Food Sci.* **61**, 415–417, 421 (1996).
9. U.S. Pat. 5,217,736 (June 8, 1993), R. D. Feeney, G. Haralampu, and A. Gross (to Opta Food Ingredients, Inc.).
10. F. Debeaufort and A. Voilley, "Aroma Compound and Water Vapor Permeability of Edible Films and Polymeric Packaging," *J. Agric. Food Chem.* **42**, 2871–2875 (1994).
11. U.S. Pat. 3,653,925 (April 4, 1972), C. A. Anker, G. A. Foster, Jr., and M. A. Loader (to General Mills, Inc.).
12. N. Gontard, S. Guilbert, and J.-L. Cuq, "Edible Wheat Gluten Films: Influence of the Main Process Variables on Film Properties Using Response Surface Methodology," *J. Food Sci.* **57**, 190–195, 199 (1992).
13. U.S. Pat. 5,736,178 (April 7, 1998), R. B. Cook and M. L. Shulman (to Opta Food Ingredients, Inc.).
14. H.-M. Lai, G. W. Padua, and L. S. Wei, "Properties and Microstructure of Zein Sheets Plasticized With Palmitic and Stearic Acids," *Cereal Chem.* **74**, 83–90 (1997).
15. R. A. Buffo, C. L. Weller, and A. Gennadios, "Films From Laboratory-Extracted Sorghum Kafirin," *Cereal Chem.* **74**, 473–475 (1997).
16. R. Gnanasambandam, N. S. Hettiarachchy, and M. Coleman, "Mechanical and Barrier Properties of Rice Bran Films," *J. Food Sci.* **62**, 395–398 (1997).
17. S. Okamoto, "Factors Affecting Protein Film Formation," *Cereal Foods World* **23**, 256–262 (1978).
18. A. H. Brandenburg, C. L. Weller, and R. F. Testin, "Edible Films and Coatings From Soy Protein," *J. Food Sci.* **58**, 1086–1089 (1993).
19. T. H. McHugh and J. M. Krochta, "Milk Protein-Based Edible Films and Coatings," *Food Technol.* **48**(1), 97–103 (1994).
20. H. Chen, "Functional Properties and Applications of Edible Films Made of Milk Proteins," *J. Dairy Sci.* **78**, 2563–2583 (1995).
21. M. Yildirim and N. S. Hettiarachchy, "Biopolymers Produced by Cross-linking Soybean 11S Globulin With Whey Proteins Using Transglutaminase," *J. Food Sci.* **62**, 270–275 (1997).
22. A. Frinault et al., "Preparation of Casein Films by a Modified Wet Spinning Process," *J. Food Sci.* **62**, 744–747 (1997).
23. U.S. Pat. 5,681,517 (October 28, 1997), W. Metzger (to Doxa GmbH)
24. J. I. Maté and J. M. Krochta, "Whey Protein Coating Effect on the Oxygen Uptake of Dry Roasted Peanuts," *J. Food Sci.* **61**, 1202–1206, 1210 (1996).
25. A. Gennadios et al., "Mechanical and Barrier Properties of Egg Albumen Films," *J. Food Sci.* **61**, 585–589 (1996).
26. B. Cuq, N. Gontard, and S. Guilbert, "Proteins as Agricultural Polymers for Packaging Production," *Cereal Chem.* **75**, 1–9 (1998).
27. J. J. Kester and O. R. Fennema, "Edible Films and Coatings: A Review," *Food Technol.* **40**(12), 47–59 (1986).
28. U.S. Pat. 3,395,024 (July 30, 1968), R. D. Earle (to R. D. Earle).
29. U.S. Pat. 3,991,218 (November 9, 1976), R. D. Earle and D. H. McKee (to Food Research, Inc.).
30. S. K. Williams, J. L. Oblinger, and R. L. West, "Evaluation of a Calcium Alginate Film for Use on Beef Cuts," *J. Food Sci.* **43**, 292–296 (1978).
31. A. Nussinovitch and N. Kampf, "Shelf-Life Extension and Conserved Texture of Alginate-Coated Mushrooms (*Agaricus bisporus*)," *Lebensm.-Wiss. Technol.* **26**, 469–475 (1993).
32. G. R. Siragusa and J. S. Dickson, "Inhibition of *Listeria monocytogenes* on Beef Tissue by Application of Organic Acids Immobilized in a Calcium Gel," *J. Food Sci.* **57**, 293–296 (1992).
33. U.S. Pat. 4,196,219 (April 1, 1980), C. P. Shaw, J. L. Secrist, and J. M. Tuomy (to The United States of America as represented by the Secretary of the Army).
34. J. A. Torres, J. O. Bouzas, and M. Karel, "Microbial Stabilization of Intermediate Moisture Food Surfaces II. Control of Surface pH," *J. Food Proc. Preserv.* **9**, 93–106 (1985).
35. V. M. Balasubramaniam et al., "The Effect of Edible Film on Oil Uptake and Moisture Retention of a Deep-Fat Fried Poultry Product," *J. Food Proc. Engr.* **20**, 17–29 (1997).
36. R. Zhuang et al., "Inactivation of *Salmonella montevideo* on Tomatoes by Applying Cellulose-Based Edible Films," *J. Food Prot.* **59**, 808–812 (1996).
37. M. O. Nisperos and E. A. Baldwin, "Edible Coatings for Whole and Minimally Processed Fruits and Vegetables," *Food Austr.* **48**, 27–31 (1996).
38. M. O. Nisperos-Carriedo, "Edible Coatings and Films Based on Polysaccharides," in J. M. Krochta, E. A. Baldwin, and M. O. Nisperos-Carriedo, eds., *Edible Coatings and Films To Improve Food Quality*, Technomic Publishing Company, Lancaster, Penn., 1994, pp. 305–355.
39. H. A. Swenson et al., "Pectinate and Pectate Coatings. II. Application to Nuts and Fruit Products" *Food Technol.* **7**, 232–235 (1953).
40. U.S. Pat. 5,451,673 (September 19, 1995), M. L. Fishman and D. R. Coffin (to The United States of America as represented by the Secretary of Agriculture).
41. Å. Rindlav-Westling et al., "Structure, Mechanical and Barrier Properties of Amylose and Amylopectin Films," *Carbohydr. Polym.* **36**, 217–224 (1998).
42. D. Lourdin, G. Della Valle, and P. Colonna, "Influence of Amylose Content on Starch Films and Foams," *Carbohydr. Polym.* **27**, 261–270 (1995).
43. J. C. Rankin et al., "Permeability of Amylose Film to Moisture Vapor, Selected Organic Vapors, and the Common Gases," *Ind. Engr. Chem. (Chem. & Engr. Data Series)* **3**, 120–123 (1958).
44. M. A. García, M. N. Martino, and N. E. Zaritzky, "Starch-Based Coatings: Effect on Refrigerated Strawberry (*Fragaria ananassa*) Quality," *J. Sci. Food Agric.* **76**, 411–420 (1998).
45. H. L. Su and K. C. Chang, "Dehydrated Precooked Pinto Bean Quality as Affected by Cultivar and Coating Biopolymers," *J. Food Sci.* **60**, 1330–1332 (1995).
46. B. L. Butler et al., "Mechanical and Barrier Properties of Edible Chitosan Films as Affected by Composition and Storage," *J. Food Sci.* **61**, 953–955, 961 (1996).
47. A. El Ghaouth et al., "Use of Chitosan Coating to Reduce Water Loss and Maintain Quality of Cucumber and Bell Pepper Fruits," *J. Food Proc. Preserv.* **15**, 359–368 (1992).
48. B. K. Simpson et al., "Utilization of Chitosan for Preservation of Raw Shrimp," *Food Biotechnol.* **11**, 25–44 (1997).
49. R. O. Feuge, "Acetoglycerides—New Fat Products of Potential Value to the Food Industry," *Food Technol.* **9**, 314–318 (1955).
50. U.S. Pat. 3,851,077 (November 26, 1974), M. Stemmler and H. Stemmler (to M. Stemmler & H. Stemmler).

51. U.S. Pat. 4,137,334 (January 30, 1979), C. Heine, R. Wüst, and B. Kamp (to Henkel KgaA).

52. D. B. Griffin et al., "Physical and Sensory Characteristics of Vacuum Packaged Beef Steaks and Roasts Treated With an Edible Acetylated Monoglyceride," *J. Food Prot.* **50**, 550–553 (1987).

53. Y. M. Stuchell and J. M. Krochta, "Edible Coatings on Frozen King Salmon: Effect of Whey Protein Isolate and Acetylated Monoglycerides on Moisture Loss and Lipid Oxidation," *J. Food Sci.* **60**, 28–31 (1995).

54. L. A. Grant and J. Burns, "Application of Coatings," in J. M. Krochta, E. A. Baldwin, and M. Nisperos-Carriedo, eds., *Edible Coatings and Films To Improve Food Quality*, Technomic Publishing Co., Lancaster, Penn., 1994, pp. 189–200.

55. L. A. Risse et al., "Volatile Production and Decay During Storage of Cucumbers Waxed, Imazalil-Treated, and Film-Wrapped," *HortScience.* **22**, 274–276 (1987).

56. C. H. Mannheim and T. Soffer, "Permeability of Different Wax Coatings and Their Effect on Citrus Fruit Quality," *J. Agric. Food Chem.* **44**, 919–923 (1996).

57. F. Specht et al., "The Application of Shellac as an Acidic Polymer for Enteric Coating," *Pharm. Technol.* **23**(3), 146, 148, 150, 152, 154 (1999).

58. R. D. Hagenmaier and R. A. Baker, "Layered Coatings to Control Weight Loss and Preserve Gloss of Citrus Fruit," *Postharv. Biol. Technol.* **30**, 296–298 (1995).

59. E. Hernandez, "Edible Coatings From Lipids and Resins," in J. M. Krochta, E. A. Baldwin, and M. Nisperos-Carriedo, eds., *Edible Coatings and Films To Improve Food Quality*, Technomic Publishing Co., Lancaster, Penn., 1994, pp. 279–303.

60. O. Fennema, I. G. Donhowe, and J. J. Kester, "Edible Films: Barriers to Moisture Migration in Frozen Foods," *Food Austr.* **45**, 521–525 (1993).

61. F. Vojdani and J. A. Torres, "Potassium Sorbate Permeability of Methylcellulose and Hydroxypropyl Methylcellulose Coatings: Effect of Fatty Acids," *J. Food Sci.* **55**, 841–846 (1990).

62. R. R. Ruan, L. Xu, and P. L. Chen, "Water Vapor Permeability and Tensile Strength of Cellulose-Based Composite Edible Films," *Appl. Engr. Agric.* **14**, 411–413 (1998).

63. F. H. Motlagh and P. C. Quantick, "Effect of Permeable Coatings on the Storage Life of Fruits. I. Pro-long Treatment of Limes (*Citrus aurantifolia* cv. Persian)," *Int. J. Food Sci. Technol.* **23**, 99–105 (1988).

64. C. R. Santerre, T. F. Leach, and J. N. Cash, "The Influence of the Sucrose Polyester, Semperfresh®, on the Storage of Michigan Grown 'McIntosh' and 'Golden Delicious' Apples," *J. Food Proc. Preserv.* **13**, 293–305 (1989).

65. U.S. Pat. 4,661,359 (April 28, 1987), J. Seaborne and C. Egberg (to General Mills, Inc.).

66. D. W. S. Wong et al., "Chitosan-Lipid Films: Microstructure and Surface Energy," *J. Agric. Food Chem.* **40**, 540–544 (1992).

67. N. Gontard et al., "Water Vapor Permeability of Edible Bilayer Films of Wheat Gluten and Lipids," *Int. J. Food Sci. Technol.* **30**, 49–56 (1995).

68. R. J. Avena-Bustillos et al., "Application of Casein-Lipid Edible Film Emulsions to Reduce White Blush on Minimally Processed Carrots," *Postharv. Biol. Technol.* **4**, 319–329 (1994).

69. N. Parris et al., "Water Vapor Permeability and Solubility of Zein/Starch Hydrophilic Films Prepared from Dry Milled Corn Extract," *J. Food Engr.* **32**, 199–207 (1997).

70. I. Arvanitoyannis and C. G. Biliaderis, "Physical Properties of Polyol-Plasticized Edible Films Made From Sodium Caseinate and Soluble Starch Blends," *Food Chem.* **62**, 333–342 (1998).

71. M. L. Fishman, "Edible and Biodegradable Polymer Films: Challenges and Opportunities," *Food Technol.* **51**(2), 16 (1997).

ARISTIPPOS GENNADIOS
Banner Pharmacaps, Inc.
High Point, North Carolina
CURTIS L. WELLER
MILFORD A. HANNA
University of Nebraska
Lincoln, Nebraska

EEL

Eel is a popular food fish in Europe and the Far East, especially in Japan. In some countries, however, eel is not so popular because of its snakelike appearance. In spite of this, the demand for eels has increased considerably during the last two decades.

Annual world production of eels is about 200,000 t. Twenty-five percent of these are wild eels captured mainly in Europe, North America, and Oceania, whereas the remaining 75% are cultured eels.

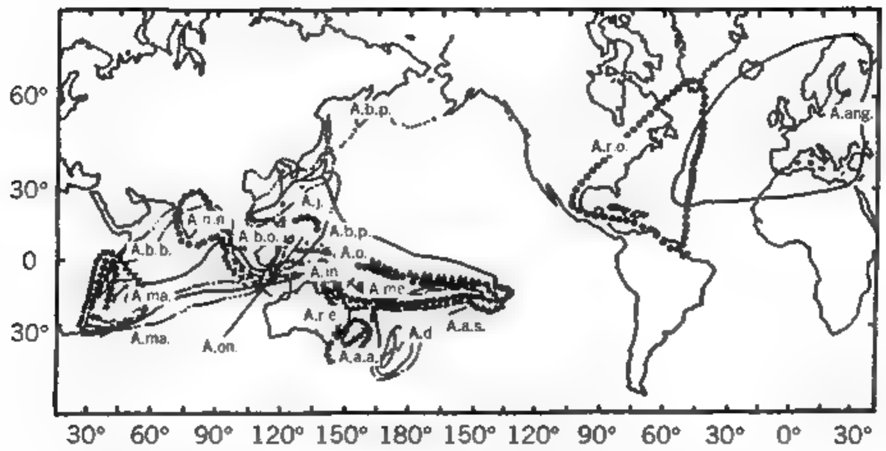
Japan pioneered the culture of eels more than 150 years ago. Its eel culture technique is now one of the most advanced in the world. Taiwan, which adopted Japanese culture methods, follows closely. In annual eel output, Taiwan, however, surpasses Japan, making it the world's largest supplier of eels (1). Meanwhile, several countries are also developing their own eel culture industry. Fluctuating natural eel stocks and the pollution of the eel's habitat have made world eel production unstable. The culture of eels may be the best way of ensuring the adequate supply of the fish.

BIOLOGY

Species and Characteristics

Freshwater eels are widely distributed throughout the world, mostly in the areas around the Atlantic and Indo-Pacific oceans (Fig. 1) (2). A total of 16 species and six subspecies are recorded, namely, *Anguilla anguilla* (European eel), *A. rostrata* (American eel), *A. japonica* (Japanese eel), *A. marmorata*, *A. bicolor* (subspecies: *A. bicolor pacifica* and *A. bicolor bicolor*), *A borneensis*, *A. celebesensis*, *A, ancestralis*, *A. obscura*, *A. australis* (subspecies: *A. australis australis* and *A. australis schmidti*), *A. megastoma*, *A. mossambica*, *A. nebulosa* (subspecies *A. nebulosa labiata* and *A. nebulosa nebulosa*), *A. reinhardti*, *A. interioris*, and *A. dieffenbachi*. However, *A. celebesensis* and *A. ancestralis* are classified as synonyms (3). There are several characteristics that distinguish the world's eel species, including dorsal fin length, vertebral number, color, size, head shape, habitat, and distribution (Table 1).

Dorsal Fin Length. Eels are generally divided into two types according to the proportion of the length from the



A. ang.	*Anguilla anguilla*	A. an.	*Anguilla ancestralis*	A. b.b.	*Anguilla bicolor*
A.ro.	*Anguilla rostrata*	A.me.	*Anguilla megastomaa*	A.b.p.	*Anguilla b. pacifica*
A. j.	*Anguilla japonica*	A. in.	*Anguilla interioris*	A. o.	*Anguilla obscura*
A. d.	*Anguilla dieffenbachi*	A bo.	*Anguilla borneensis*	A. a.a.	*Anguilla a. australis*
A. re.	*Anguilla reinhardti*	A. n.n.	*Anguilla nebulosa*	A. a.s.	*Anguilla a. schmidti*
A. ma.	*Anguilla marmorata*	A. n.l.	*Anguilla labiata*		
A. c.	*Anguilla celebesensis*	A. mo.	*Anguilla mossambica*		

Figure 1. Natural distribution of freshwater eels. *Source:* Ref. 2.

anterior base of the dorsal fin to the anus and the total body length. The long-fin type has a proportion of 7–17%, while the short-fin type has a proportion of 0–5%. Thirteen of the eel species belong to the long-fin category, three species belong to the short-fin type.

Vertebral Number. Another taxonomic criterion for eel types is the number of vertebrae. Eels have 100–119 vertebrae; the number varies among the different species. The number even varies within each species, which increases with higher latitudes.

Color. Eels can be mottled or plain. There are seven mottled and nine plain species.

Size. There is a considerable difference between female and male eels. The growth of female eels in inland water is much more superior than that of male eels. The females also stay longer in inland water before returning to the sea. Eels can grow at maximum sizes of 27 kg and 200 cm.

Head Shape. Eels are also classified according to the shape of their head. Those with a narrow head and thin, narrow lips are classified as narrow-head type while those with broad head and thick, broad lips are classified as broad-head type (4,5).

Distribution

Of the 16 species of eels, 14 are distributed in the Indo-Pacific areas. Some of these eels inhabit the tropical zone, while others abound in the temperate zone. Eels in the Indo-Pacific Ocean are distributed in both the southern and northern hemispheres. The northernmost boundary is at about 45° N in Hokkaido, Japan, while the southernmost limit is at about 50° S in the Auckland Islands, New Zealand. The two other species, the European eel and the American eel, inhabit the Atlantic Ocean and abound in the temperate zones of Europe and North America, respectively (Fig. 1).

LIFE HISTORY

The first successful attempt to understand the amazing life cycle and unusual traits of the eel has generally been accredited to two men who devoted most of their lives to studying eels: Danish biologist Johannes Schmidt and his student Vilhelm Ege. The discovery of a leptocephalus by Schmidt in 1904 started a 35-year study that helped establish a solid foundation to the ecological study of eels. Nevertheless, many questions concerning the life history of eels still remain unanswered. Although the life history of three species, European eel, American eel, and the Japanese eel are well investigated, little is known about the other species.

Eels are catadromous migrants, that is, they spawn at sea and spend most of their lives in inland waters. The spawning ground and migration route vary with each species.

European Eel

The European eel spawns at the Sargasso Sea, 22–30° N, 48–65° W, in the western Atlantic Ocean from early spring

Table 1. Major Details of the World's *Anguilla* Species

			Maximum size of females					
Dorsal fin length	Vertebral number	Color[a]	kg	cm	Species	Zone[b]	Distribution[c]	
Long	110–119	P	6.0	125[d]	*A. anguilla*	Temp	ATL	Europe, North Africa
Long	103–111	P	6.0	125[d]	*A. rostrata*	Temp	ATL	United States, Canada, Greenland
Long	112–119	P	6.0	125[d]	*A. japonica*	Temp	PAC	Japan, Korea, Taiwan, People's Republic of China
Long	109–116	P	20.0	150	*A. dieffenbachi*	Temp	PAC	New Zealand
Long	104–110	M	18.0	170	*A. reinhardti*	Trop	PAC	Australia, New Caledonia
Long	100–110	M	27.0	200	*A. marmorata*[e]	Trop	PAC	Africa, Madagascar, Indonesia, People's Republic of China, Japan, Pacific Islands, Sumatra
Long	101–107	M			*A. celebesensis*[f]	Trop	PAC	Philippines
Long	101–106	M			*A. ancestralis*[f]	Trop	PAC	Celebes
Long	108–116	M	22.0	190	*A. megastoma*	Trop	PAC	New Caledonia
Long	104–108	M			*A. interioris*	Trop	PAC	New Guinea, New Caledonia
Long	103–108	P	2.0	90	*A. borneensis*	Trop	PAC	Brunei
Long		M	10.0	150	*A. nebulosa*	—	—	—
Long	106–112				*A. n. nebulosa*	Trop	IND	Sri Lanka, Burma, Sumatra
Long	107–115				*A. n. labiata*	Trop	IND	South Africa
Long	100–106	P	5.0	125	*A. mossambica*	Trop	IND	Africa
Short		P	3.0	110	*A. bicolor*	—	—	—
	106–115	P			*A. b. bicolor*	Trop	IND	South Africa, Madagascar, Sri Lanka, Burma, Sumatra, Australia
Short	103–111				*A. b. pacifica*	Trop	PAC	Brunei
Short	101–107	P			*A. obscura*	Trop	PAC	New Caledonia
Short		P	2.5	95	*A. australis*			
Short	109–116				*A. a. australis*	Trop	PAC	Australia
Short	108–115				*A. a. schmidti*	Temp	PAC	New Caledonia, New Zealand

Source: Refs. 5 and 9

[a] Color abbreviations are P = plain, M = mottled.

[b] Zone abbreviations are Trop = tropical, Temp = temperate.

[c] Distribution abbreviations are ATL = Atlantic Ocean, IND = Indian Ocean, PAC = Pacific Ocean.

[d] *A. anguilla*, *A. rostrata*, and *A. japonica* are closely related (9).

[e] *A. marmorata* is the most widely distributed species (9).

[f] Synonymous (3).

through early summer. The prelarvae grow to about 25 mm in June. They then attenuate in shape, and gradually grow into transparent, leaf-shaped leptocephalus. The leptocephali drift with the current away from the Sargasso Sea to the coasts of Europe in about three years. The leptocephalus can attain a body length of 50 mm after a year and about 75 mm at two years old. They metamorphose into slim transparent elvers (Fig. 2) at 2.5 years old. The elvers then head for the coast and enter estuaries.

Both the female and male European eels stay in rivers until they grow to 6–12 and 9–19 years old, respectively. When they become sexually mature, the eels migrate downstream and back to the Sargasso Sea to spawn. After spawning, the adults die.

American Eel

The American eel also spawns in the Sargasso Sea. The elvers only take about 1.5 years to reach the coastal area of North America (6).

Japanese Eel

The spawning ground of the Japanese eel is estimated to be within the eastern part of Taiwan and the southern part of the Ryukyu islands, which may extend to the northeastern part of the Philippines. The spawning season is estimated to be between June and July. At the latter part of their development, the leptocephali drift with the Kuroshio current from the spawning ground and metamorphose into elvers when they reach the estuaries of Taiwan,

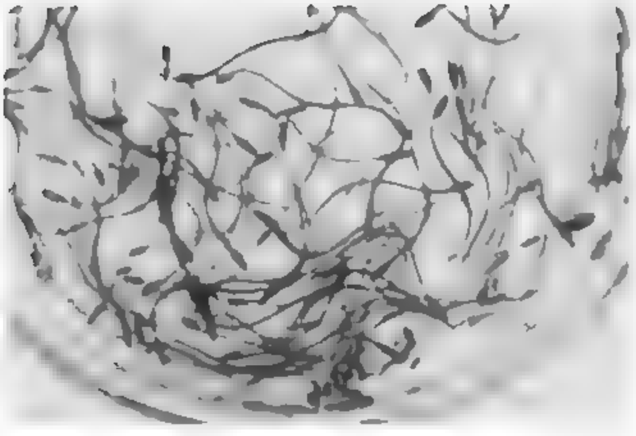


Figure 2. Elvers.

the People's Republic of China, Korea, and Japan after about 4–5 months. The migration time is estimated by the daily growth rings in the otolith. Using this index, the peak of elvers upstream migration is between December and January. The elvers enter the rivers and live and feed on small fish, shrimp, and aquatic insects for about 5–20 years until they reach adult size. They mature during autumn and winter (September–November). The pectoral fin, dorsal and ventral part of the mature males turn blackish and silvery. While mature males can weigh only about 500 g on maturity, the females can reach 2–3 kg. Sexually mature eels then migrate down the river into the ocean to spawn. Figure 3 illustrates the estimated spawning ground and life cycle of the Japanese eel (7). The Japanese eel is the most popular and most commonly eaten eel, particularly in Japan. Thus the following discussion focuses on the Japanese eel.

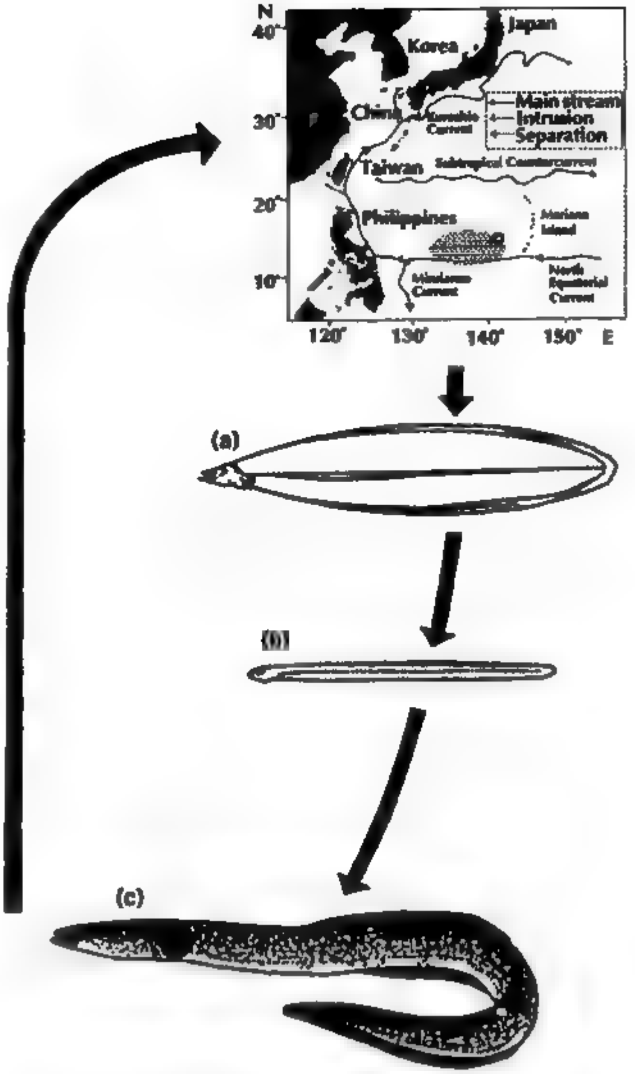


Figure 3. Estimated spawning ground and life cycle of the Japanese eel: (a) Leptocephalus, 5.5 cm T.L., drifted by current; (b) elver (ascending) migrates from estuary to river; (c) adult (descending) migrates from river to spawning ground. *Source:* Ref. 7.

ECOLOGY OF ELVERS

The amount of catch for elvers in coastal waters seems to be correlated with temperature. The maximum catch of elvers in the upstream areas has often been found during or several days after daily seawater temperature reaches its lowest in winter. The lowest water temperature ever recorded in an eel habitat is 15–16°C.

Maximum catch has also been observed to occur at the same time when salinity has leveled off and the flow of seawater has reached its maximum. The elvers also become most active during this period at night.

The biological rhythm of elver activity was found to follow the lunar cycle in the coastal waters. The peak catches occur only once a month, about the time of the new moon. On the other hand, a semilunar rhythm of the elvers in the rivers was observed, namely, two peak catches occurred in each lunar cycle, one around full moon, the other around new moon. The semilunar rhythm of upstream elvers also coincided with the spring tide (8).

CULTURE TECHNIQUES

Species Cultured

Of the different species of eels in the world, three are valued for its economic importance, namely, the European eel, the American eel, and the Japanese eel. From a marketing point of view, these species are similar in shape, growth, and behavior (8).

Elver Collection

The collection of the elvers is the starting point of eel culture. Attempts to propagate eels artificially have not been successful to date. In 1974, the Japanese succeeded in inducing artificial spawning of the eel and hatching the eel eggs. But attempts to rear the elver during the first three weeks of its life have not been as successful. Thus eel fry are still sourced entirely from the wild-caught elvers.

A growing concern within the eel culture industry in recent years is the diminishing number of large seaward running eels, as well as the elvers swimming upstream. This has been attributed mainly to pollution in estuaries, rivers, and streams and the overfishing of elvers. To remedy this, captured elvers are now widely used not only for culturing eels for consumption, but also for restocking in the lower reaches of rivers and streams.

Elvers are usually captured at nighttime. Light is used to attract them (Fig. 4) and they are caught in scoop nets in shallow water, in a fine-mesh net set across the width of the river, or in trap nets set in the estuaries (10).

Elvers are quite hardy; nevertheless, great care is taken when handling them to prevent mortality due to injury. They are not handled directly. They are placed in boxes lined with wet muslin or hung in mesh cages in the river. These boxes or cages are taken to eel farms within a few hours. The high demand coupled with a supply shortage has resulted in a phenomenal increase in the price of elvers.

CULTURE SYSTEMS

Eels can be cultured both extensively and intensively. Most eel farms, however, use the intensive culture system. This culture system is characterized by high stocking densities, stringent water-quality management, additional inputs such as formulated diets to increase fish production, and aggressive disease prevention.

Elver Rearing

There are various sizes and types of tanks being used in stocking elvers. The most commonly used, however, is the circular concrete tank, about 5 m in diameter and 60 cm in depth. Tanks are usually built under greenhouses and heated to above 25°C by thermostatically controlled electric immersion heaters (10). This is to protect the elvers from low temperatures.

The average stocking density of intensive culture is 1–2 kg of elvers/3.3m^2. Sorting and restocking are usually done after 20–30 days, when a significant difference in the size of eels is observed. The average density used for the second stocking phase is 1 kg of fish/3.3 m^2. Frequent sorting and restocking will bring about higher survival rate. These will also help maintain uniform growth and provide better feed conversion rates.

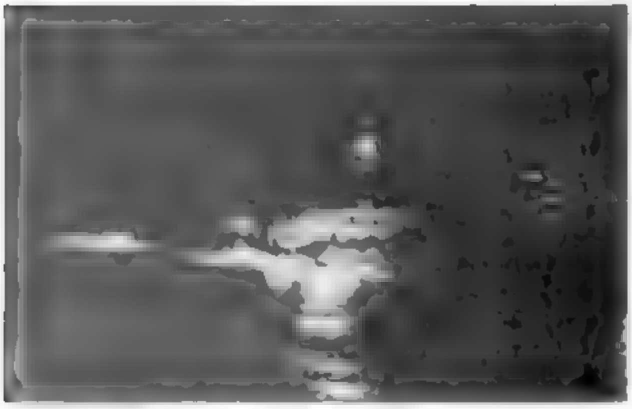


Figure 4. Offshore collection using boat and light to attract elvers.

The elvers have to be trained to take formulated feeds. The common method is to lower baskets containing tubifex worms or minced meat of oyster and clam twice a day, in the early morning and in the late evening under a wooden enclosure lit by a 20–40 W lamp. Elvers do not feed at temperatures below 13°C, thus temperatures must be kept above 13°C. Feeding time is gradually shifted to daytime.

Initially, elvers may not eat. In the long run, however, they learn to eat and eventually, attain rapid growth. After 100–150 days, a size of 100–200 fingerlings/kg is reached.

Feeding Management

Eels are carnivorous fish and thus require high animal protein input. The more animal protein that can be provided, the greater the eels' weight will be. Most eel farmers feed their eels with formulated feeds that are chiefly made of fish meal with added carbohydrates, minerals, vitamins, and other ingredients. Due to the high cost of these feeds, most eel farms supplement these with raw fish such as mackerel, sardine, and anchovy. Trash fish or scraps that are minced using electric grinders are also used widely as feeds. Eels, however, convert formulated feeds more efficiently than they convert raw fish.

Feeding is done once a day, at about 7:00 A.M. during summer and about 10:00 A.M. in winter. The usual feeding rate is 5–15% of the total weight of the eel for minced trash fish and 1.0–3.5% for formulated feeds (11). Nevertheless, factors such as the previous day's feeding condition and water and air temperature as well as water quality are considered when determining the feeding rate. Ideally, feeds should be consumed within 20–30 min.

Precautions must be taken to insure that no unnecessary organic debris are allowed to fall into the ponds. Thus eel farmers do not just broadcast the feeds into the ponds. Instead, a special feeding platform, usually shaded, within the pond is designated for feeding, and the eels learn to swim toward the area each feeding time. Feeding areas are preferably located where the oxygen level is high as this encourages the eels to eat well. Formulated feed is lowered on a perforated tray suspended just under the surface and lifted after feeding is done (Fig. 5). Raw fish, dipped for a few minutes in boiling water to soften the skin, are threaded through the eyes and also lowered and raised once eels have eaten all the flesh. Thus the only organic matter that goes into the pond is that which the eels eat.

Pond and Water Management

Growout ponds are either rectangular or square. In Japan, adult eels are raised in several types of ponds that make use of efficient drainage with water temperatures of about 28°C. Until recently, these eel ponds were between 5,000–20,000 m^2 in area. However, the practice of intensive culture has reduced pond size to about 500–1,000 m^2, and is expected to be further reduced in the future (10).

In Taiwan, two types of eel ponds are used, namely, the hard pond and the soft pond (Fig. 6). The hard pond has a concrete or red brick bottom and dikes and is small (1,000–1,650 m^2 and water depth of 0.8–1.2 m). The soft pond, on the other hand, has a mud bottom and concrete or brick dikes and is generally large (0.6–1.0 ha and water depth

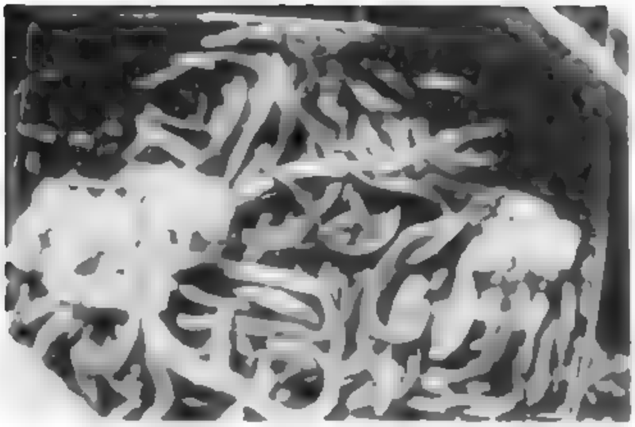


Figure 5. Eels feeding on formulated feed paste from a perforated tray lowered into the pond. The tray is lifted out once the eels stop feeding to avoid scraps fouling the water.

Figure 6. Soft ponds.

of 2.0–3.0 m). Recently, some farmers have reverted back to using earthen ponds (Fig. 7).

Most eel farms have many ponds of different sizes because eels of different sizes must be sorted to maintain uniform growth. The eels that grow fast are separated from those that grow slow and are transferred to larger ponds.

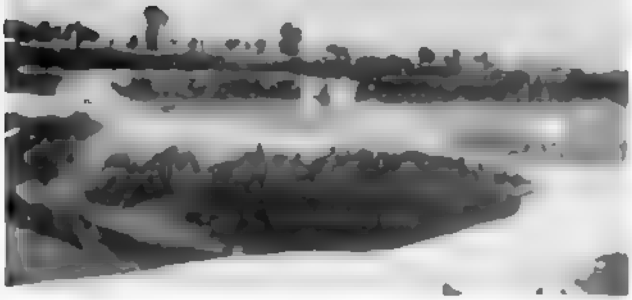


Figure 7. Earthen ponds.

Pond water quality is also an important aspect of eel culture because it could affect the health and growth of the cultured eels. A well-maintained pond could spell the difference between healthy and fast-growing eels and poor quality ones.

Pond water color can also be used to gauge water quality. The most favorable color is green because it can show the presence of zooplanktons, phytoplanktons, ideal pH value, high dissolved oxygen concentration, and other factors affecting water quality.

Eel ponds require large amounts of fresh water and are thus generally located in areas with good water supply both quantitatively and qualitatively. Water is kept free from pollution and within pH values of 6.5–8.0. Temperature is kept above 13°C because eels stop eating below this temperature. Oxygen, which is considered the most critical water characteristic, must be maintained above 1 mL/L because eels cannot sustain life below this level.

To ensure and improve the water quality in eel ponds, various methods may be adopted. These would include allowing more water to flow into the pond to increase the rate of water exchange, installing aerators to increase oxygen supply, and liming to improve water quality. Bottom sediments are also removed after harvest.

Disease Problems, Prevention, and Treatment

The eel's body surface is covered by thick mucus. Thus the eel generally is highly resistant to diseases, unless its skin is injured or the skin or gill is infected by parasites (12).

Various fungi and bacteria can infect eels. The most common of these are fungus disease, red disease, branchionephritis, swollen intestine disease, gill disease, and anchor worm disease.

Diseases usually infect eels during the changes in seasons. These may be caused by a wide variety of factors such as poor pond water quality and inefficient feeding management.

Prevention rather than medication should be the program followed in disease management. Prevention of diseases would include efficient feeding management and proper water management. Moreover, eels are handled with care to avoid skin damage, which is the primary cause of infections. In case of disease outbreak, medicines are added to the food or to the pond water.

Harvesting and Marketing

When eels reach marketable size, they are harvested daily or once every few days, usually at feeding time. The ideal marketable size is 5–6 fish/kg for the Japanese market and more than 6 fish/kg for other markets.

There are several ways of eel harvesting in the ponds. The common practice is by placing a net below the feeding platform. Other methods include draining the pond and catching the eels in long net bags or by drawing a seine across the pond.

Harvested eels are sorted into different sizes. Smaller ones are put back to the pond for further growth. The harvested eels are then starved for about two days by holding them in baskets in front of the water inlet of the pond or in midpond, or stacking perforated plastic baskets under showers of trickling water. They are then packed and sent to the market. Starvation removes undigested substances inside the intestine. This minimizes the risk of meat contamination with gastroenteric bacteria during cutting and deboning in processing plants.

Eels, when marketed live or quick frozen and glazed, fetch fair prices. When they are transported to the market live, eels are packed in containers with conditions that may vary, depending on the transport time and distance (Fig. 8).

CONSUMPTION AND PROCESSING

Different countries have different preferences and eating habits, and thus have different ways of preparing and eating eels. In Europe, the main eel consumers are the Germans, Dutch, Danes, and Swedes. These consumers commonly prefer smoked eel, which ranks as an expensive and luxurious food. The British also eat eels, but prefer the jellied type.

In the Far East, the main eel-eating country is Japan, where it is a custom to eat roasted eel (*kabayaki*) especially on the *Ushinohi* (a special eel-eating day near the end of July). The Japanese believe that the eel is nutritious and can make up for overexhaustion during the summer. Eel is also consumed widely in Taiwan, the People's Republic of China, Korea, and Hong Kong.

Preprocessing

Before eels are processed, they are graded according to size (Fig. 9). One way of preparing them is by putting them in a deep container and sprinkling them with salt. This treatment removes their slime until they die of asphyxiation. Another way is by putting them in fresh water and stunning them with an electric shock (10). Or they may simply be put in cold storage overnight. In this way, eel activities can be slowed down, and thus they can easily be handled for gutting or deboning.

Figure 8. Eels readied for the market. Eels are packed in polyethylene bags inflated with oxygen. (Note oxygen tank and corrugated cardboard boxes in background.)

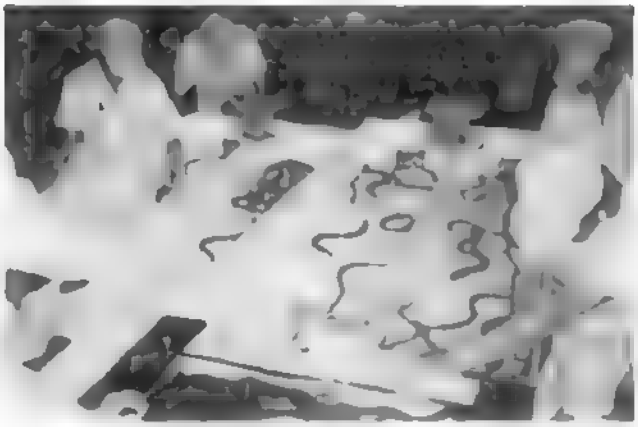


Figure 9. Eels being graded according to size.

Cutting Methods

There are generally two types of cutting, namely, *Kansai* style (cut abdominally) and *Kanto* style (cut dorsally). Newly cut eels are usually cleaned of slime by washing them in cold water and scraping. The eels are then gutted. This is done by slitting from the throat through either the belly (*Kansai* style) or up toward the back (*Kanto* style) to the tail, about 2.5 cm beyond the anus. The guts are then emptied and the backbone and head removed. The eel is washed thoroughly to remove traces of slime and blood. Machines specifically designed to eviscerate eels efficiently are also available.

Preparations

Fresh Eels. Fresh eels are cooked in several ways, such as braising and steaming. Steaming, however, is the more popular method. In both Taiwan and Mainland China, eel is either steamed with various vegetables and mushrooms or stewed with Chinese herbs, such as medlar, lovage, and dates. This herb-filled soup is considered as a revitalizing tonic soup for frail or disabled persons.

Smoked Eels. After the eels' are cut, they are brined in a saltwater solution, then hot smoked. In this process, the eels are dried and smoked. To allow uniform drying throughout the thickness of the fish, the temperature during smoking is increased gradually. The smoking process may vary from country to country. The finished product is then packed either in boxes or in cans (10).

Jellied Eels. Jellied eels is a traditional British way of preparing eels. First, gutted, cleaned, and cut eels are placed into boiling water and then simmered until the flesh becomes tender. Cooking time depends primarily on size. The cooked pieces marinated with hot liquor are then poured into large bowls containing gelatine dissolved in a small amount of water. The amount of gelatine solution is determined by considering the condition of the eels and their natural capacity to gel. Experience is thus necessary to get the right recipe. Once the mixture has cooled, the pieces in jelly are packed into cartons for immediate fresh consumption. Shelf life can reach two weeks at chilled temperatures. There are various other recipes for preparing jellied eels (10).

Roasted Eels. Roasted eel is a favorite Japanese cuisine. The Japanese prepare roasted eels in several ways, such as, *kabayaki* (roasted eel with seasoning), *shirayaki* (roasted eel without seasoning), *kimoyaki* (roasted eel viscera with seasoning), and *capitalyaki* (roasted eel head with seasoning). Until recently, these preparations were only available in Japanese specialty restaurants. Domestic production, however, has not kept up with the large demand for roasted eels in Japan. Japan thus imports frozen roasted eel from Taiwan, where the roasted eel processing industry has boomed in recent decades, and the People's Republic of China, where the industry is in its initial stages.

Live eels are processed into frozen roasted eel in processing plants. The ideal size of the raw material is between five and six eels per kilogram. Within this size range, the smaller size commands a higher price.

In the cut fillet style, the eel is cut crosswise into three equal sections, with the tail cut lengthwise into two. If the weight is not enough, each half of the tail is stretched together with one of the other sections by using bamboo sticks. This step is usually done using a stretching machine. Stretching is done to keep the meat flat during cooking. For the whole fillet style, the fish is not cut into sections after the degutting process; they are, however, also stretched.

After cutting, the fillets are arranged on a conveyor and pass through single-sided or double-sided roasting machines. The fillet may be roasted either seasoned or unseasoned (Figs. 10 and 11). In single-sided machines, the inside portion is roasted first, then the eel is turned and the other side is roasted. Liquefied petroleum gas is used as fuel. The appropriate roasting time is about 3–5 min. Roasting indicators are the color of the meat (it should become evenly light scorched), the scorch bubbles (some should appear on the skin; under 3% of the total area), and the central temperature of the meat (it should reach 78°C). The most delicate part in the whole procedure would be in the precooling step because this is where the fillet becomes most susceptible to recontamination. It is thus necessary to control the bacterial drop rate, until the total plate count is under three colony-forming units/min.

Figure 10. Frozen roasted eel without seasoning, cut fillet style.

Figure 11. Roasted eel dish with seasoning, cut whole fillet style.

Some manufacturers use precooling tunnels and spray cold air into the surface of the fillet: a shorter precooling time is maintained to minimize recontamination. The use of either individual quick freezing or contact freezing equipment is popular. In these types of equipment, the temperature drops to −18°C within 30 min. After the central temperature has reached −30°C to −35°C, the frozen fillet is then removed from the pan or conveyor, then packed and stored at a temperature of −20°C (13). Comparing the weight of the processed eel (eviscerated but not yet seasoned and roasted) to the total weight of the raw material, the yield for cut and whole fillet may reach as much as 58–60% and 68–70%, respectively.

Sanitary standards for the products include negative amounts of coliforms, total bacterial count of under 3,000/g, and negative presence of residues of contaminated chemicals in the meat, such as malachite green, methylene blue, oxolinic acid, antibiotics, nitrofurans, sulfamides, insecticides, and herbicides.

SKIN PROCESSING

Eel is not only used for food but also for other uses. Eel skin, for example, is processed into leather (Figs. 12 and 13). Although there is no fur on the eel skin, there are scales that are covered by follicles dispersed in the corium (14). The major steps in eel skin processing are liming, decoloring and removal of scales, removal of excess fat, tanning using alum, and glazing. Considering that the fat content of the eel skin is more than 25%, which is even higher than that of cattle, the removal of excess fat and scales are the more important steps in eel skin processing.

FUTURE DEVELOPMENT

The eel is a highly valued fish. Compared with other fish, the eel is rich in vitamins A and E, and calcium. The eel is recognized as one of the most nutritious cultured fishes. The fish is high in energy content, and calorific values,

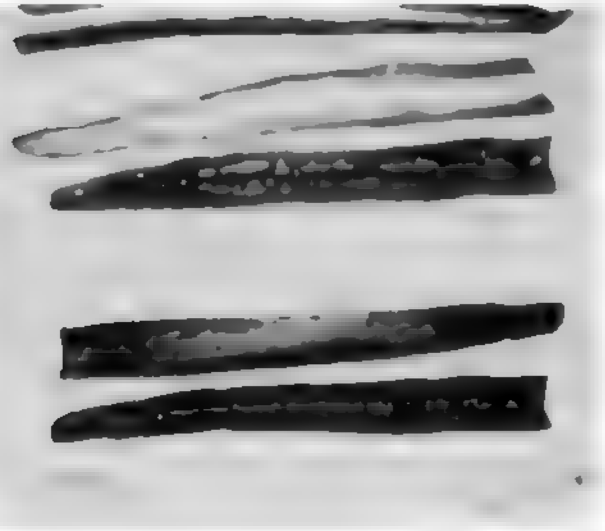


Figure 12. Tanned eel skin stained with different kinds of dye.

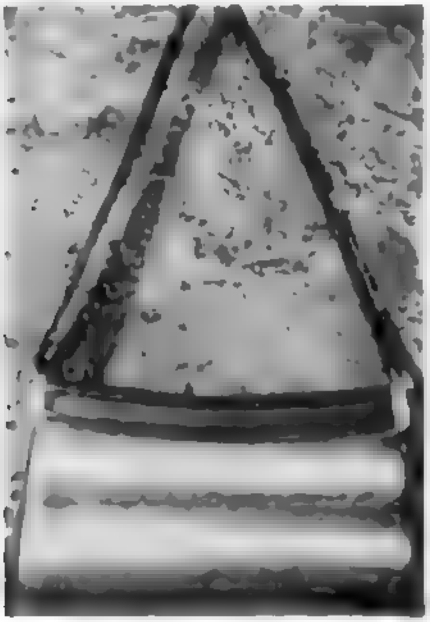


Figure 13. A shoulder bag made from tanned eel skin.

such that it is eaten to create appetite and stamina especially in hot and humid days. Its health benefits make the eel a valuable fish.

Eel skin, when processed, can be used in leathercrafts. More research, however, must be undertaken in finding ways to efficiently tan its skin to enable it to compete with other leathers.

Interest in the culture of eel has reached a high level in recent years because of its versatility and popularity, and high demand as gourmet food particularly in Japan, where it commands a good price. In eel-eating countries such as Japan, Taiwan, and some parts of Europe (Germany, Denmark, and the UK) enterprising activities concerning eels are in progress. These activities include research into new techniques of eel culture in cold climate, ie, Northern Europe.

Elvers used in eel culture are still sourced from the wild, and are diminishing in numbers. Thus investigations on eel propagation in captivity should be made. Attempts albeit unsuccessful have been made to propagate elvers artificially. The challenge for researchers now is to look for efficient and commercially successful ways to propagate them artificially. Researchers may gain hints from the lessons of past investigations and look for ways to unlock the mystery of the long life history of the eel. The fish's potentials are so great that the search will surely be worthwhile.

BIBLIOGRAPHY

1. C. C. Huang, "Taiwan Becomes World Leader in Exports of Eel," *Almanac of Food Industries in Taiwan, R.O.C.*, 56–57 (1990).
2. "Fisheries in Japan, Eels," *Jap. Mar. Prod.* 1977.
3. D. H. J. Castle and G. R. Williamson, "On the Validity of the Freshwater Eel Species, *Anguilla ancestralis* Ege, from Celebes," *Copeia* 2, 569–570 (1974).
4. L. Bertin, *Eels: A Biological Study*, Cleaver-Hume Press, London, 1956.
5. I. Matsui, *Eel Study: Biology*, Vol. 1, Kosei-Sha Kosei-Kaku, Tokyo, 1972 (in Japanese).
6. F. W. Tesch, *The Eel: Biology and Management of Anguillid Eels*, Chapman and Hall, London, 1977.
7. K. Tsukamoto, "Breeding Places of Freshwater Eels," in O. Tabeta, ed., *Early Life History and Prospects of Seed Production of the Japanese Eel* Anguilla japonica (in Japanese), Suisan Gaku Ser. 107, Koseisha Koseikaku, Japan, 1996, pp. 11–21.
8. I. C. Liao et al., "Investigation on *Anguilla japonica leptocephali* by Fishery Researcher I," in K. Aida and K. Tsukamoto, eds., *Studies on the Life Cycle of Eels* (in Japanese), Kaiyo Monthly Special Issue 18, 1999, pp. 27–33.
9. W. N. Tzeng, "Immigration Timing and Activity Rhythms of the Eel, *Anguilla japonica*, Elvers in the Estuary of Northern Taiwan, with Emphasis on Environmental Influences," *Bull. Jap. Soc. Fish. Oceanogr.* 47–48, 11–28 (1985).
10. A. Usui, *Eel Culture*, Fishing News (Books) Ltd., Surrey, UK, 1974.
11. T. P. Chen, *Aquaculture Practices in Taiwan*, Fishing News (Books) Ltd., Surrey, UK, 1976, pp. 17–28.
12. S. Egusa, *Epidemic Diseases of Fish*, Kosei-Sha Kosei-Kaku, Tokyo, Japan, 1978 (in Japanese).
13. H. C. Chen, "Frozen Roasted Eel Processing Industry in Taiwan," *in* J. L. Chuang, B. S. Pun and G. C. Chen, eds., *Fishery Product of Taiwan, JCRR Fisheries Series* **25B**, Joint Commission on Rural Reconstruction. Taiwan, R.O.C., 1977, pp. 20–26.
14. Y. S. Lai and Y. Y. Kuo, "Primary Report on the Eel Skin Tanning," *Bulletin of the Taiwan Fisheries Research Institute* **32**, 439–441 (1980) (in Chinese with English abstract).

I Chiu Liao
Taiwan Fisheries Research Institute
Keelung, Taiwan

EGGS AND EGG PRODUCTS

DEVELOPMENT OF THE EGG INDUSTRY

Eggs have been a human food source since the beginning of human residence on earth. The first usage of eggs was probably the taking of eggs from nests of wild birds. In primitive societies this continues to be significant source of high-quality protein, especially during the egg production season of wild birds. As civilization developed, the birds were domesticated and were kept in pens and shelters. In Western societies the chicken became the primary source of eggs. The growth of the egg industry in selected countries from 1965 to 1995 is shown in Table 1. The egg production in China amounted to almost 50% of total egg production in the world in 1995 and is continuing to increase at a rate greater than world average.

Until relatively recent times the keeping of chickens on farms was generally only a sideline to the main business of farm operation. Commercial egg farms became a primary source of farm income only in the last century. Large poultry farm operations for egg production did not become common until the last half of the twentieth century. The change in size of egg production units in the United States is shown in the data presented in Table 2. Data for recent years are not available in a form as presented in Table 2. However, a 1995 survey indicated that less than 1% of all eggs produced in the United States were from flocks of less than 1000 layers. In 1997 there were less than 350 egg-producing farms with more than 75,000 laying hens.

Table 2. Changes in Size of Laying Flocks in the United States from 1959 to 1974

	Percentage of all eggs sold		
Size of flock	1959	1964	1974
Less than 400	26.78	10.87	3.64
400 to 1,599	22.58	12.64	1.80
1,600 to 3,199	12.83	7.39	1.14
More than 3,200	37.81	69.10	93.43
More than 20,000		30 10	67.74
3,200 to 9,999			9.59
10,000 to 19,999			16 10
20,000 to 49,999			24 02
50,000 to 99,999			13.14
More than 100,000			30.58

Source Ref. 2.

EGG FORMATION AND STRUCTURE

The egg consists of four distinct parts: shell, shell membranes, albumen or white, and yolk. A schematic drawing of an egg is shown in Figure 1. The yolk is formed in the ovary. During embryonic development of the female chick, tiny ovules appear on the ovary, that may later develop into yolks of eggs to be produced by that hen. The number of immature ovules present in the chick at the time of hatching is far in excess of the number of eggs that will be produced by the hen during her lifetime.

Approximately 14 days prior to the laying of an egg, one of the rudimentary ovules in the ovary starts to increase in size. During the next 13 days the yolk develops in its follicular membrane. A suture line forms in the membrane

Table 1. Egg Production in Selected Countries

Country	1965	1975	1985	1995
United States	65,692	64,379	68,250	74,280
Canada	5,194	5,339	5,865	5,792
Mexico	5,000	7,446	18,092	21,200
Argentina	2,880	3,480	3,150	NA
Brazil	8,124	5,000	9,000	16,065
Venezuela	508	1,721	2,736	NA
France	9,220	13,120	14,910	16,991
Germany	16,110^{a}	20,050^{a}	18,746^{a}	13,847^{a}
Italy	9,990	11,400	10,900	12,017
The Netherlands	4,206	5,320	10,051	9,970
Spain	6,320	10,152	10,164	9,983
United Kingdom	7,840	13,861	13,117	10,644
Poland	6,264	8,013	8,631	6,500
USSR	29,000	57,700	77,000	43,220^{b}
Japan	18,625	29,798	35,700	42,167
Australia	2,196	3,384	3,825	NA
Turkey	1,448	2,597	5,700	8,000
China	NA	NA	NA	335,340
Republic of Korea	NA	NA	NA	8,317
Taiwan	NA	NA	NA	6,237
Thailand	NA	NA	NA	7,700

Note: All values in millions of eggs.

[a] Includes East and West Germany.

[b] Includes only Russia and Ukraine of former USSR.

Source: Ref. 1 (1967, 1977, 1987, 1997).

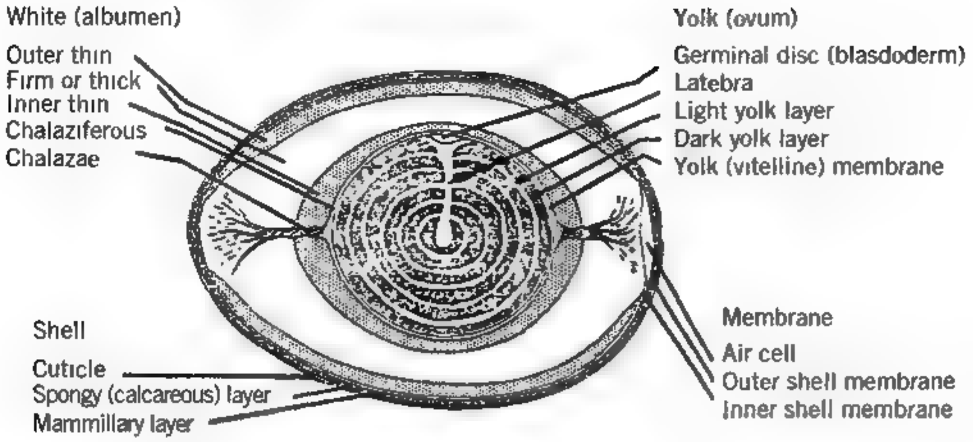


Figure 1. A schematic of the parts of an egg. *Source:* Ref. 4.

so that the matured yolk can be dropped into the infundibulum of the oviduct without the rupture of any blood vessels in the follicular membrane. A blood spot in an egg is generally caused by incomplete formation of the suture line.

The oviduct consists of five identifiable sections: infundibulum, magnum, isthmus, uterus, and vagina. The infundibulum, or funnel, collects the yolk, or ovum, and by peristaltic action moves it on to the magnum region, where the thick albumen is secreted and laid down in layers on the yolk. In the isthmus the shell membranes are formed around the yolk and thick albumen. The membranes, two in number, only loosely fit the enclosed material. Most of the time that the egg is in the oviduct is spent in the uterine section where thin albumen fills the shell membrane sack and the shell is laid down to form the rigid egg protective layer. The shell color is determined by the breed of the hen. Generally, hens with red earlobes produce brown-shelled eggs, and white-shelled egg producers have white earlobes. The total time spent in the oviduct is usually about 24 hs. Total time for the formation of the egg from the start of rapid growth of the ovule to time of laying varies from 12 to 15 days.

COMPOSITION OF THE EGG

The egg is composed of approximately 10% shell, 30% yolk, and 60% white or albumen. The egg is a very good source of high-quality protein and many minerals and vitamins. The exact levels of the minerals and vitamins is determined by the level of each in the diet of the hen. An average chemical composition of eggs, including shells, is summarized in Table 3.

The protein of egg white is complete; it contains all of the essential amino acids in well-balanced proportions. The white is made up mainly of the proteins ovalbumin, conalbumin or ovotransferrin, ovomucin, ovoglobulins, ovomucoid, and lysozyme. The structure of the thick white is the result of a complex of ovomucin and lysozyme.

The important yolk proteins are ovovitellin and ovolivetin. The lipid materials in the egg are all in the yolk. The fatty acid composition can easily be modified by changing the fatty acid makeup in the feed. In most commercial eggs where the hens are fed a corn and soybean meal diet the fatty acids are about one-third saturated and two-thirds mono- and polyunsaturated. Yolk color is controlled by the level of pigments, mostly xanthophyll, in the feed of the hens.

Some elements of the medical profession have emphasized the role of dietary cholesterol in human cardiovascular problems. The negative effect on egg consumption that this emphasis on cholesterol has had led to a number of research attempts to reduce the cholesterol content of eggs. Genetically it has been possible to achieve only slight reductions. Selection of hens for small yolk size has met with some success. A review on altering cholesterol by feeding (5) concluded that dietary modifications resulted in only minor changes in the cholesterol content of egg yolk. Another report (6) states that including 1.5 to 3.0% of menhaden oil in the laying hen's ration results in a temporary reduction of about 50% in the cholesterol concentration in the yolk. The fatty acid composition of the eggs was also modified in that the eicosopentenoic acid and docosahexanoic acid (omega-3 fatty acids) content of the yolks was significantly increased when the fish oil was included in the hen's ration. Levels of fish oil in excess of 3% in the ration resulted in fishy flavored eggs.

The relative ease with which egg composition can be modified has led to the development of a variety of "designer eggs." It has been reported that all vitamins and minerals content of eggs, except for choline and zinc, can be adjusted significantly by dietary modification for the hens (7).

PRODUCTION PRACTICES

As shown in Table 2, the majority of eggs at this time are produced on large egg production farms. With this change, the level of mechanization used in the production of eggs has increased. Over 95% of all laying hens in the United States and over 75% of all laying hens in the world are kept in cages (8). Legislation has been introduced in many

Table 3. Chemical Composition of the Egg

	Water (%)	Protein (%)	Fat (%)	CHO (%)	Ash (%)
Edible portion					
Whole egg	75.33	12.49	10.02	1.22	0.94
White	88.00	10.52	0	1.03	0.64
Yolk	48.80	16.76	30.87	1.78	1.77

	Calcium carbonate (%)	Magnesium carbonate (%)	Calcium phosphate (%)	Organic matter (%)
Shell	94.0	1.0	1.0	1.0

Source: Ref. 3 for edible portion; Ref. 4 for shell.

countries and passed in a few that would ban the use of cages for laying hens due to activities of animal welfare groups. Full details on production practices are given by North and Bell (8).

SHELL EGG PROCESSING

In a modern egg production unit hens are housed in cages with sloping floors so that eggs will roll from the cage onto an egg gathering belt. The eggs are transported by conveyor belts to a separate unit of the facility where they are washed, inspected for defects by passing them over an intense light source (candling), sized, and packed into cartons for retail or onto trays for bulk packaging. Throughout the operation individual eggs are not touched by any workers. The cartons or trays are packed into master containers by hand.

Some countries ban the washing of eggs to be sold at retail. In others, including the United States and Canada, almost all eggs are washed in a detergent solution at a temperature of about 45°C (113°F) and then sanitized with an approved sanitizing agent, frequently a chlorine-based compound. All of the washing should be done with water spray with or without brushes depending on the equipment.

After the eggs are washed and dried, they are conveyed to the candling area of the processing line. Here there is a strong light source under the eggs so that any defects such as cracked shells, dirty or stained shells, or inclusions in the egg might be detected and the defective eggs removed from the conveyor. Candling of eggs is used to segregate eggs into the several grades that are found in the marketplace. The retail grades in the United States are AA, A, and B. Quality factors used in grading include the shell (cleanliness and soundness), air cell (size), white (clarity and firmness), yolk (visibility of the outline), and freedom from defects. All grades of eggs are required to have a sound, not cracked, shell. Other quality factors are judgment calls, with smallness of the air cell, freedom from stains or dirt on the shell, firmness of the white, and dimness of the yolk outline being considered most desirable. For eggs that do not meet the grade standards two additional groupings are provided: dirty and checked or cracked. Details on the grading standards have been published (9).

There are six size classifications of eggs in the United States. Table 4 gives minimum weight requirements for each of the sizes. On the processing line the sizes are segregated by passing the eggs over a series of scales set so that the jumbo eggs are removed from the line first and the peewees are removed last.

After the eggs have been mechanically placed in cartons or on trays, many processors spray the tops of the eggs with a food grade mineral oil to preserve the interior quality of the egg during movement through market channels. The oil partially seals the pores of the shell to reduce loss of gases from the egg and to reduce the rate of pH change of the albumen. As a further aid in maintaining quality, eggs should be handled at refrigerated temperatures of less than 7°C (45°F) throughout market channels, and consumers are advised to keep eggs in the refrigerator after they purchase them. With good handling throughout market channels and in the home, eggs will remain high quality for several weeks and in a usable condition for several months. The factors that have the greatest effect on quality loss in eggs are time, temperature, humidity, and handling.

During the last decade a potential health risk in the usage of eggs has appeared. A new strain of *Salmonella enteritidis* is capable of infecting the reproductive system of the hen, so that some eggs are produced with the *S. enteritidis* in the egg at the time of production (10). The frequency of finding an egg positive for *S. enteritidis* in market eggs has been estimated to be between 1 in 10,000 and 1 in 20,000. Most eggs are heated to over 60°C (140°F)

Table 4. Weight Classes of Shell Eggs in the United States

Size name	Minimum ounces per dozen	Minimum average ounces per egg	Minimum average grams per egg
Jumbo	30	2.5	70.0
Extra Large	27	2.25	63.8
Large	24	2.0	56.7
Medium	21	1.75	49.6
Small	18	1.5	42.5
Peewee	15	1.25	35.4

Source: Ref. 4.

prior to eating, so the pathogen would be killed by cooking. Handling of eggs at refrigerated temperatures inhibits the increase in numbers of bacteria during marketing and holding in the home. With this threat to food safety from shell eggs, research has led to the development of systems for pasteurization of intact shell eggs to destroy all potential pathogens in the egg or on its shell (11).

CONVERSION OF SHELL EGGS TO LIQUID PRODUCT

An ever-increasing number of eggs are being broken at commercial egg breaking plants. The early growth of the egg products industry has been reviewed (12). In the first half of the twentieth century most of the egg breaking plants were located in the north central and western plains states. Since 1960 many of the new breaking plants have been built in the southeastern states. The yield of liquid products from a case (30 dozen) of large eggs is shown in Table 5. Data in this table also include the yields of solids that might be obtained if the egg products were dehydrated by either spray drying, pan drying, or freeze drying.

The growth of the egg products industry is summarized in Table 6. The shift from dried to liquid since 1970 was likely the result of mandatory pasteurization of all egg products since 1971 (14). The shift to liquid from 1988 to 1990 was likely the result of ultrapasteurization and aseptic packaging of liquid eggs that will give more than 4 weeks usable shelf life for the liquid product (15). The rapid expansion since 1986 is at least partially attributable to the potential incidence of *S. enteritidis* in the shell eggs. The savings in costs of freezing and in time saved by not having to thaw the products before using in food formulations are evident.

Table 5. Yields of Liquid Egg and Egg Solids per 30-Dozen Case of Large Size Eggs

	Liquid		Solids	
	Kilograms	Pounds	Kilograms	Pounds
White	13.8	30.4	1.67	3.68
Yolk	6.1	13.5	3.12	6.87
Whole egg	19.9	43.9	4.79	10 55

Source: Ref. 13.

Table 6. Processed Egg Product Production in the United States (in millions of pounds)

Year	Liquid	Frozen	Dried
1960	44	362	176
1965	44	368	216
1970	110	357	281
1974	301	367	73
1977	394	348	75
1980	422	331	81
1982	460	339	80
1984	528	322	76
1986	632	337	89
1988	797	371	106
1990	932	384	105
1992	1,276	418	130
1994	1,689	428	133
1996	2,033	406	139

Source. Ref. 1.

The pasteurization of liquid eggs was practiced for a number of years prior to the passage of the law making it mandatory for all egg products in the United States. The times and temperatures required for pasteurization in several countries are listed in Table 7. All of these values are for liquid whole egg. Details of pasteurization have been discussed (17).

In the processing of egg white it is necessary to remove the reducing sugars before the liquid can be dried. In early work on dehydration of egg white the process included a natural fermentation by whatever organisms were present in the egg white. A patent was issued in 1931 covering the use of lactic acid bacteria for egg white fermentation. The next development was the use a yeast, *Saccharomyces apiculatus*, and then the use of an enzyme, glucose oxidase. Each of these methods is still in use in some parts of the world.

The natural egg products of white and yolk are also supplied to the food processing industries in a number of blends and modifications often with the addition of sodium chloride or sugar. The chemical composition of some of the egg products is shown in Table 8. The difference in composition between pure yolk and commercial yolk should be emphasized. The difference is due to the inclusion of a significant amount of albumen with yolk in the commercial product.

NUTRITIONAL AND FUNCTIONAL PROPERTIES OF EGGS

Complete nutritional data on eggs and egg products prepared in several ways have been summarized (3). An abbreviated listing of nutrients in whole egg, yolk, and white is given in Table 9. As mentioned earlier, the content of various trace minerals, vitamins, and fatty acids in the egg can be modified significantly by varying the diet of the hen. The various uses for eggs in food products have been reviewed (19). The functional properties include whipping, emulsifying, coagulation, flavor, color, and nutrition. The foam produced during whipping should give a large volume and be stable. The whipping time required to get a maximum foam volume will vary for eggs of different qualities. Eggs with a high percentage of thick albumen require a longer time for whipping but the foam formed will be more

Table 7. Minimum Pasteurization Requirements for Whole Egg Products

	Temperature		Time,	
Country	°C	°F	minutes	Reference
United Kingdom	64.4	148	2.5	15
Poland	66.1–67.8	151–154	3.0	16
China (PRC)	63.3	146	2.5	16
Australia	62.5	144.5	2.5	16
Denmark	65–69	149–156.5	1.5–3.0	16
United States	60	140	3.5	13

Source: Ref. 16.

Table 8. Approximate Composition of Selected Egg Products (per 100 g)

		Composition				
Product	Calories	Water	Protein	Lipid	CHO	Ash
		Frozen eggs				
Whole egg	158	74.57	12.14	11.15	1.20	0.94
White	49	88.07	10.14	Trace	1.23	0.56
Yolk, pure[a]	377	48.20	16.10	34.10	—	1.69
Yolk, commercial[b]	323	55.04	14.52	28.65	0.36	1.43
Yolk, sugared	323	50.82	12.92	25.50	9.49	1.27
		Dehydrated eggs				
Whole egg	594	4.14	45.83	41.81	4.77	3.45
Whole egg, stabilized	615	1.87	48.17	43.95	2.38	3.63
White, stabilized, flakes	351	14.62	76.92	0.04	4.17	4.25
White, stabilized, powder	376	8.54	82.40	0.04	4.47	4.55
Yolk	687	4.65	30.52	61.28	0.39	3.16

[a] Contains approximately 17% white.
Source: Refs. 3 and 18.

stable. Cakes made using very low quality eggs generally do not have the volume of similar cakes made with higher-quality eggs. When egg white is dried, there is generally a loss in whipping properties. To overcome the decrease in foam volume, whipping aids such as triethyl citrate are frequently added to the dried egg white.

PRODUCTION OF EGG-RICH CONVENIENCE FOODS

Reference 20 outlines procedures for the manufacture of a number of value-added egg products, some of which will be described here.

Hard-Cooked Eggs

The preparation of hard-cooked eggs has been a function of kitchens for many years. In about 1970 the production of hard-cooked eggs for sale to salad bars, restaurants, and commercial food caterers was begun. The original products were cooked in hot water, partially cooled, peeled, and stored in a solution of citric acid and sodium benzoate. The concentration of the citric acid was initially 2% but has been lowered over the last 28 years to from 0.5 to 0.8%. The lower levels of acid produce eggs with softer cooked whites, much closer to freshly cooked eggs.

Cooking of hard-cooked eggs is now being done either in steam or hot water. Peeling of eggs was primarily a hand operation aided by equipment to crack the shells. Equipment has been developed that strips the shell from a cracked hard cooked egg so that efficiencies of production have been significantly increased. With improvements in packaging technology, some hard-cooked eggs are now marketed as a dry-packed, refrigerated product. It is estimated that almost 1% of all eggs are now being marketed as hard-cooked product.

Deviled Eggs

One use of hard-cooked eggs is the production of deviled eggs. A hard-cooked egg is cut in half through the middle either longitudinally or horizontally, and the yolk is removed. The yolk is removed and mixed with a salad dressing and other ingredients to produce a filling for the depression left in the white. Deviled eggs are a favorite for hospitality suites or buffets.

Scotch Eggs

This product was developed years ago but received very little publicity until relatively recently. A hard-cooked egg is wrapped in a thin layer of sausage meat. The sausage is then cooked in a deep fat fryer. In producing this item the sausage used should be very low in fat to get better adhesion of the surface of the hard-cooked egg. Scotch eggs are a snack food item frequently sold from refrigerated vending machines and are also used for hors d'oeuvres by cutting the finished product into quarters or halves.

Diced Egg Products

Diced eggs were first marketed as a means of utilizing hard-cooked eggs that were not perfectly smooth. With the popularity of hard-cooked diced eggs on salad bars, the demand was greater than the quantity available from the imperfect hard-cooked eggs. At this point innovative producers separated yolks and whites as liquids and steam-cooked trays of the two liquids. The cooked yolk and white were then diced. The product thus prepared gave yolk cubes that held together because of the residual albumen in commercially separated liquid yolk. The diced egg products have been marketed as individually quick frozen material in most instances. However, where market channels are short, nonfrozen, controlled atmosphere packaging is also being used.

Scrambled Eggs

Scrambled eggs are prepared as fully cooked, freeze-dried products for the camper; as fully cooked, frozen items for the microwaveable meal; and as frozen prepared liquid

Table 9. Distribution of Nutrients in Large Chicken Eggs (per egg, edible portion)

	Whole egg	Yolk[a]	White
Weight per egg, g	50	17	33
Water, g	37.28	8.29	29.06
Calories	79	63	16
Protein, g	6.07	2.79	3.35
Lipid, g	5.58	5.60	Trace
Carbohydrate, g	0.60	0.04	0.41
Fiber, g	0	0	0
Ash, g	0.47	0.29	0.18
Minerals			
Calcium, mg	28	26	4
Iron, mg	1.04	0.95	0.01
Magnesium, mg	6	3	3
Phosphorus, mg	90	86	4
Potassium, mg	65	15	45
Sodium, mg	69	8	50
Zinc, mg	0.72	0.58	0.01
Vitamins			
Ascorbic acid, mg	0	0	0
Thiamin, mg	0.044	0.043	0.002
Riboflavin, mg	0.150	0.074	0.094
Niacin, mg	0.031	0.012	0.029
Pantothenic acid, mg	0.864	0.753	0.080
Vitamin B_6, mg	0.060	0.053	0.001
Folacin, μg	32	26	5
Vitamin B_{12}, μg	0.773	0.647	0.021
Vitamin A, R.E.	78	94	0
Lipids			
Saturated fatty acids, g	1.67	1.68	0
14:0	0.02	0.02	0
16:0	1.23	1.24	0
18:0	0.43	0.43	0
Monounsaturated FA, g	2.23	2.24	0
16:1	0.19	0.19	0
18:1	2.04	2.05	0
Polyunsaturated FA, g	0.72	0.73	0
18:2	0.62	0.62	0
18:3	0.02	0.02	0
20:4	0.05	0.05	0
Cholesterol, mg	213	213	0
Amino acids			
Tryptophan, g	0.097	0.041	0.051
Threonine, g	0.298	0.151	0.149
Isoleucine, g	0.380	0.160	0.204
Leucine, g	0.533	0.237	0.291
Methionine, g	0.196	0.171	0.130
Cystine, g	0.145	0.050	0.083
Phenylalanine, g	0.343	0.121	0.210
Tyrosine, g	0.253	0.120	0.134
Valine, g	0.437	0.170	0.251
Arginine, g	0.388	0.193	0.195
Histidine, g	0.147	0.067	0.076
Alanine, g	0.354	0.140	0.215
Aspartic acid, g	0.602	0.233	0.296
Glutamic acid, g	0.773	0.341	0.467
Glycine, g	0.202	0.084	0.125
Proline, g	0.241	0.116	0.126
Serine, g	0.461	0.231	0.247

Note: Shell is 12% of weight of egg.

[a] Fresh yolk includes a small proportion of white.

Source: Ref. 3

mixes, packaged in cook-in-bag film. The basic formula for scrambled eggs consists of about 70% whole liquid egg and 30% milk with seasonings of salt and pepper. The most significant form of scrambled eggs is the frozen prepared mix packaged in film. This product is used extensively in hospitals and nursing home feeding because the cooked product remains hot for an extended period in the film bag. As some dieticians desire a reduced-cholesterol egg product for nursing home feeding, it is possible to use a blend of whites and yolk with only enough yolk for color as the egg source instead of whole liquid egg.

Omelets

The major difference between omelets and scrambled eggs is that in a true omelet no milk is included in the formula. Water is used instead of the milk. Omelets are produced for distribution as frozen product either fully cooked or as a premix. The formulation of omelet mixes frequently includes diced ham, bacon bits, mushrooms, onions, green or red peppers, and a number of other vegetables.

Crepes, Pancakes, and Waffles

Each of these products has a formula including flour and eggs. The crepe mixture is usually richer in eggs than the other two. Each of these products is fully cooked and frozen for distribution. There are also dry mixes for preparation of the products in the home.

Snack Foods from Eggs

A number of egg-rich snack foods have been proposed but, thus far, none have become significant users of eggs. A breaded, fried egg white ring that looks much like an onion ring was patented (21), and a procedure for producing an egg jerky flavored to taste like dried meat jerky was suggested (22). A cookie formulation was prepared so that each cookie contained one egg equivalent. Another snack food that is quite common is yogurt. It is possible to make a yogurt-type product using egg albumen as a partial replacement for the milk. In a sensory comparison of normal yogurt and the egg-substituted product, the latter was judged superior for mouth-feel and smoothness.

A number of egg-rich drinks have been produced. The drink sold in greatest quantity is eggnog. This is produced as a dried product as well as the refrigerated liquid drink. A number of fruit juices have been mixed with eggs to produce drinks of orange juice and egg, apple juice and egg, and cranberry juice and egg. When preparing any of the drinks in quantity, it is recommended that pasteurized liquid eggs be used. The prepared drinks should be kept at temperatures below 5°C (41°F).

MODIFYING THE COMPOSITION OF EGGS BY PROCESSING

The composition of egg products can be modified rather easily by utilizing yolk, white, or whole egg in various amounts. Much interest has been shown in reducing the amount of cholesterol in eggs. The cholesterol can be removed from the yolk by using supercritical extraction techniques with carbon dioxide as the extracting solvent. Much of the research has been with dried egg yolk, but it is possible to remove more than 80% of the cholesterol from the liquid yolk by supercritical extraction techniques (23).

EGGS AS A SOURCE OF PHARMACEUTICAL PRODUCTS

Lysozyme makes up 3.5% of the egg white. A cation exchange system consisting of a macroporous, weak acid resin can be used to recover most of the lysozyme (24). The system can be designed as a continuous operation. It was found that the residual lysozyme-free egg white possessed superior whipping, gelling, and emulsifying properties compared with native egg albumen. Lysozyme-free albumen has been approved for food product usage by federal agencies in the United States, providing the labeling is appropriate. Extracted lysozyme may be used in pharmaceutical products or as an antibacterial agent in the preservation of food products.

Fertile eggs are used extensively in the production of vaccines. Some recent work has shown promise for the egg to be used as a means of producing antibodies by manipulation of diet and management of the hen.

BIBLIOGRAPHY

1. USDA, *Agricultural Statistics*, U.S. Government Printing Office, Washington, D.C., 1961–1997.
2. W. J. Stadelman, and O. J. Cotterill, *Egg Science and Technology*, 3rd ed., AVI Publishing Westport, Conn., 1986.
3. USDA, "Composition of Foods, Dairy and Egg Products—Raw, Processed, Prepared," *Agricultural Handbook 8.1, USDA, ARS*, U.S. Government Printing Office, Washington, D.C., 1976; revised, 1989.
4. USDA, *Egg Grading Manual, USDA, AMS, Agriculture Handbook 75*, U.S. Government Printing Office, Washington, D.C., 1983
5. E. C. Naber, "The Effect of Nutrition on the Composition of Eggs," *Poultry Sci.* **58**, 518–528 (1979).
6. R. L. Adams et al., "Introduction of Omega-3 Polyunsaturated Fatty Acids into Eggs," *Abstracts of Papers of Southern Poultry Science Society*, Atlanta, Ga. (1989).
7. W. J. Stadelman and D. E. Pratt, "Factors Influencing Composition of the Hen's Egg," *World's Poultry Sci. J.* **45**, 247–266 (1989).
8. M. A. North, and D. D. Bell, *Commercial Chicken Production Manual*, 4th ed., Chapman & Hall, New York, 1990.
9. USDA, "Regulations Governing the Grading of Shell Eggs and United States Standards, Grades and Weight Class for Shell Eggs," *USDA, AMS, Poultry Division, 7 CFR, Part 56*, 1987.
10. R. G. Board and R. Fuller, *Microbiology of the Avian Egg*, Chapman and Hall, London.
11. W J. Stadelman et al., "Pasteurization of Eggs in the Shell," *Poultry Sci.* **75**, 1122–1125 (1996).
12. J. W. Koudele, and E. C Heinsohn, "The Egg Products Industry of the United States. I. Historical Highlights, 1900–1959," *Kansas Agric. Exp. Station Bull.* 423, 1960, pp. 1–48.
13. O. J. Cotterill and G. S. Geiger, "Egg Product Yield Trends from Shell Eggs," *Poultry Sci.* **56**, 1027–1031 (1977).
14. USDA, "Regulations Governing the Inspection of Eggs and Egg Products," *USDA, AMS, Poultry Division, 7 CFR, Part 59*, 1984.
15. H. R. Ball, Jr., et al., "Functions and Shelflife of Ultrapasteurized, Asceptically Packaged Whole Egg," *Poultry Sci.* (Suppl 1), 63 (1985).
16. "The Liquid Egg (Pasteurization) Regulations 1963," *Statutory Instruments, No. 1503*, H. M. Stationery Office, London, 1963.
17. F. E. Cunningham, "Egg Product Pasteurization," in W. J. Stadelman and O. J. Cotterill, eds., *Egg Science and Technology*, 4th ed., Haworth Publishing, Binghamton, N. Y., 1995.
18. O. J. Cotterill, and J. L. Glauert, "Nutrient Values for Shell, Liquid/Frozen and Dehydrated Eggs Derived by Linear Regression Analysis and Conversion Factors," *Poultry Sci.* **58**, 131–134 (1979).
19. Sheng-Chin Yang, and R. E. Baldwin, "Functional Properties of Eggs in Foods," in W. J. Stadelman and O. J. Cotterill, eds., *Egg Science and Technology*, 4th ed., Haworth Publishing, Binghamton, N. Y., 1995.
20. W. J. Stadelman et al., *Egg and Poultry Meat Processing*, Ellis Horwood, Chichester, England, 1988.
21. U.S. Pat. 4,421,770 (Dec. 20, 1983), J. M. Wicker and F. E. Cunningham (to Kansas State University).
22. U.S. Pat. 4,537,788 (August 28, 1985), V. Proctor and F E. Cunningham (to Kansas State University).
23. G. Zeidler, G. Pasin, and A. King, "Removing Cholesterol from Liquid Egg Yolk by Carbon Dioxide-Supercritical Fluid Extraction," in J. S. Sim and S. Nakai, eds., *Egg Uses and Processing Technologies—New Developments*, CAB International, Oxon, U.K.
24. E. Li-Chan et al., "Lysozyme Separation from Egg White by Cation Exchange Column Chromatography," *J. Food Sci.* **53**, 425–427 (1986).

William J. Stadelman
Purdue University
West Lafayette, Indiana

ELASTINS AND MEAT LIGAMENTS

Elastin and collagen are the principal components of connective tissue. They form a network that is responsible for the transmission of tension and the structure of muscle and other tissues. Elastin forms a lesser proportion of connective tissue than collagen and is not soluble during heating. The ratio of collagen to elastin in connective tissue is dependent on the tissue and its location. This ratio affects the tissue's mechanical properties and its physiological functions (1). Elastin is present in muscle only in small amounts, less than 3% of the total connective tissue. *Musculus semitendinosus*, however, contains more elastin, up to 37% of the total connective tissue. Elastin normally forms fibers and lamellae, is abundant in elastic ligaments, and elastic blood vessels, and is found to a small extent in the skin, lungs, and other organs.

Elastic tissue is often referred to as yellow connective tissue because of its color It contains the elastin fibers with filamentous, refractive, and fluorescent with a blue-white appearance under ultraviolet light. Because of the large number of nonpolar amino acids in the structure and their hydrophobic nature, the elastin fibers stain poorly with acid or basic dyes but do stain selectively with phenolic dyes such as orcein.

MORPHOLOGY

The basic morphological component of elastin is filament made up of a linear sequence of globular structures with a probable maximum diameter of 9–10 nm. Dehydrated elastin may be 4–6 nm in diameter (2). The freeze-etching technique reveals elastin's structure as a regular, three-dimensional network of filaments. The filaments cross their neighbors every 8–12 globular subunits, giving rise to a network with a large, less-dense central core (3). The filaments of elastin can be disordered and swollen when immersed in 25% glycerol for 8–10 days. The swollen elastin shows a much higher affinity for stains.

CHEMISTRY AND BIOCHEMISTRY

Elastin can be determined from the amount of desmosine or isodesmosine found in the tissue. Its amino acid composition is essentially identical as extracted from various tissues and are not affected by the age of the animal (4). However, compositions differ from animal to animal. Elastin contains 1–2% hydroxyproline. Almost 95% of its amino acids are nonpolar (Table 1). The sulfur amino acids, tryptophan, and tyrosine are present in small amounts or nonexistent. In contrast to collagen, elastin does not contain hydroxylysine. Elastin has about one-third of its amino acids as glycine, but their arrangement in the elastin molecule is unknown. Desmosine and isodesmosine, unique cyclic polymers, are made up of four lysine residues, which connect two to four elastin molecules. This reaction requires the oxidative deamination of three lysine ε-amino groups which give an intermediate followed by condensation with the ε-amino group of a fourth lysyl residue, and is catalyzed by a copper-containing enzyme, lysine oxidase. Therefore, the synthesis of elastin can be blocked by inhibition of this enzyme through dietary copper depletion, or by β-aminopropionitrile, the active lathyrogen of the sweet pea. The synthesis is age dependent; the amount of desmosine and isodesmosine increase and the lysine content decreases with advancing age. The elasticity of elastin is due to the exposure of nonpolar sequences of its molecules to water during the stretching, followed by spontaneous refolding of the molecule on removal of the applied force.

Elastin can be isolated as the residue remaining after the extraction of tissue with 0.1 *N* NaOH at 98°C for 45 min (4). Elastin is heat stable to a temperature of 140–150°C and is insoluble in a wide range of hydrogen bond breaking solvents at temperatures up to 100°C. It must be processed with harsh enzymatic or chemical treatments in order to solubilize it. This includes KOH/ETOH solubilized κ-2-elastin, oxalic acid solubilized α-elastin, elastase solubilized elastin, and various salt-soluble forms of elastin (9).

Using immunological techniques, two serologically distinct elastin antibody fractions in antiserum prepared against bovine ligamentum nuchae elastin were identified (9). One is a species-specific fraction that bound only to bovine elastin. The other population of antibodies has affinity for elastin from porcine aorta, bovine ligamentum nuchae and aorta, rabbit and hamster lung, and human aorta. The elastin radioimmunoassay can be modified to detect bovine or other elastins, depending on the choice of radiolabeled antigen, in meat products. This assay also permits the study and quantification of elastin synthesis and degradation in a wide range of meat-animal species without having to raise specific antibodies to each one.

Table 1. Amino Acid Compositions of Elastins from Selected Tissues of Various Meat Animals

	Residues per 1000 residues			
Amino Acid	Avian, insol[a]	Bovine, semitendinosus[b]	Sheep, vascular tissue[c]	Yellowfin tuna, dark muscle[d]
Asp	2	7.4	1.8	66.0
Hyp	22	12.4	0.0	6.0
Thr	3	8.2	9.2	51.0
Ser	5	8.3	9.0	53.0
Glu	12	17.1	20.4	134.6
Pro	128	110.9	105.3	65.1
Gly	352	325.9	241.1	128.5
Ala	176	223.1	288.7	91.7
Val	175	145.8	111.7	55.9
Cys/2	1	—	—	17.6
Met	—	—	—	22.3
Ile	19	26.5	17.5	38.2
Leu	47	61.4	57.5	82.9
Tyr	12	8.4	13.4	31.7
Phe	23	29.8	34.5	29.9
Hyl	22	12.4	—	0.0
Lys	4	5.3	30.7	62.2
His	1	0.6	13.6	14.8
Arg	5	5.7	6.8	48.0
isodes	3	2.1[a]	29.3[b]	0.5[a]
Des	3	1.2[a]	7.2[b]	0.3[a]

Source: [a]Ref. 4, [b]Ref. 5, [c]Ref. 6, and [d]Ref. 7.

The emission spectrum of elastin is similar to that of Type I collagen with peak excitation near 370 nm. Macroscopic ultraviolet fluorometry was used to measure the gristle contents, elastin, and collagen Type I of beef (10).

Fluorescence emissions were measured with a monochromator and a photomultiplier tube. Intact tendons and elastic ligaments had a strong fluorescence emission peak around 440–450 nm and only weak fluorescence around 510 nm. The 510:450 nm ratio was correlated to the amount of gristle in comminuted meats.

INDUSTRIAL APPLICATIONS

The visible gristle in meat is from a number of sources, including tendons (particularly from myotendon junctions), ligaments (such as the ligamentum nuchae in rib roasts), perimysium (particularly in the pennate extensors and flexors of the lower limbs), fasciae (such as the lumbodorsal fascia of the longissimus muscle), and intramuscular vessels (particularly arteries). All these sources consist mainly of Type I collagen or elastin. Elastin-contained gristle retains its tensile strength after typical cooking procedures, such as roasting and broiling (11,12). Fresh meat cuts containing gristle are generally perceived as lower quality products, because of the unpleasant sensation of gristle. However, the elastin concentration is not consistently related to variations in the tenderness of muscles, at least in the bovine species (13), nor is the total concentration of connective tissue components (collagen and elastin). Tenderness is actually more related to soluble collagen and the overall fiber arrangement in the meat.

Aging is the industrial practice of storing meat carcasses above the freezing point for a certain period of time to improve the tenderness. The changes are the result of the physical breakage of muscle and collagen protein fibers by rigor and enzymatic reactions. However, aging has no apparent effect on the structure of elastin tissue and its physical properties.

Tissues with high elastin content, such as elastic ligaments, blood vessels, and lung, are removed in the slaughtering operation. They are processed as offals with other organs and by-products in the rendering industry as a protein source for animal feed. In this application, they may be rendered at high or low temperature, depending on the planned use of the fat. Fat is separated from the solids by centrifugation. The solids are dried and batched with other protein sources for optimum amino acid content and distribution, and then used in animal feed. The make-up of the feed is determined by a linear program based computer program which takes account of the amino acids, fat, carbohydrate, vitamin, and mineral content required in feed formulations.

In the ready-to-eat processed meat industry, elastin tissues and meat ligaments retain their tensile strength and are not gelatinized under most heat-processing conditions. For this reason, elastin tissues in meat are removed, with other gristle, by mechanical means, by hand or mechanical gristle removers. Grinders equipped with gristle and bone removers are widely used in the processed meat industry for coarse-ground, nonemulsified products. In this case, the meat is put through a grinder and the bone chips and gristle are separated from the ground meat. For large-muscle products, such as ham, the meat must be hand trimmed The separated gristle with elastin and collagen tissue is discarded or chopped fine and used in emulsified products. It is used as a filler rather than functional proteins for economic reasons.

Meat cuts with a high gristle, collagen, and elastin content are not used in large-muscle products. They are used most often in emulsified products to minimize the toughness problem of the gristle. However, elastin and collagen tissues from gristle do not bind water or function as emulsifiers in meat emulsion; therefore, emulsion breakdown occurs more often in these products. To solve these problems, a computer-based, least-cost formulations program, which is widely used in the processed meat industry, has incorporated the connective tissue in individual meat cuts as a quality constraint in the computer simulation of product formulations. But no effort has been made to separate the elastin or ligament contribution as a constraint in the computer program, because of the low content of elastin in most meat cuts.

Unlike collagen, elastin does not swell at mild acid conditions and has no film-forming properties. It is not used in gelatin production because of its poor water solubility and gelation properties. It cannot be substituted for collagen in regenerated collagen casing, or collagen film and tissue. Elastin also has little use in the processed meat industry because it functions poorly as a binder and emulsifier. Because it is found in only small amounts in meats, because it is uneconomical to separate it from other meat tissues, and because it is poor nutritionally, elastin's technological development for use in foods and other industries has been hindered.

BIBLIOGRAPHY

1. R. J. Bagshaw, E. Weit, and R. H. Cox, "Aortic Connective Tissue Content in White Leghorn Females," *Poultry Science* **65**, 403 (1986).
2. C. Fornieri, I. P. Ronchetti, A. C. Edman, and M. Sjostrom, "Contribution of Cryotechniques to the Study of Elastin Ultrastructure," *Journal of Microscopy* (Oxford) **126**, 97 (1982).
3. M. Morocutti, M. Raspanti, P. Govoni, A. Kadar, and A. Ruggeri, "Ultrastructural Aspects of Freeze-Fractured and Etched Elastin," *Connective Tissue Research* **18**, 55 (1988).
4. H. R. Cross, G. C. Smith, and Z. L. Carpenter, "Quantitative Isolation and Partial Characterization of Elastin in Bovine Muscle Tissue," *Journal of Agricultural and Food Chemistry* **21**, 716 (1973).
5. J. A. Foster "Elastin Structure and Biosynthesis: An Overview," in, *Structural and Contractile Proteins. Part A. Extracellular Matrix*, L. W. Cunningham and D. W. Frederiksen, eds. Academic Press, New York, 1982, pp. 559–570.
6. J. R. Bendall, "The Elastin Content of Various Muscles of Beef Animals," *Journal of the Science of Food and Agriculture* **18**, 553 (1967).
7. S. Kanoh, T. Suzuki, K. Maeyama, T. Takewa, S. Watabe, and K. Hashimoto, "Comparative Studies on Ordinary and Dark Muscles of Tuna Fish," *Bulletin of the Japanese Society of Scientific Fisheries (NIHON SUISAN GAEFFAI-SHI)* **52**(10), 1807 (1986).

8. J. N Manning, P. F. Davis, N. S. Greenhill, A. J. Sigley, "Salt Soluble Cross-linked Elastin: Formation and Composition of Fibers," *Connective Tissue Research* **13**(4), 313 (1985).
9 R. P. Mecham and G. Lange, "Measurement by Radioimmunoassay of Soluble Elastins from Different Animal Species," *Connective Tissue Research* **7**, 247 (1980).
10. H. J. Swatland, "Fiber-Optic Reflectance and Autofluorescence of Bovine Elastin and Differences between Intramuscular and Extramuscular Tendon," *Journal of Animal Science* **65**, 158 (1987).
11 A. M. Pearson and F. W. Tauber, *Processed Meats*, AVI Publishing Co., Inc., Westport, Conn. 1984.
12. P. P. Purslow, "The Physical Basis of Meat Texture: Observations on the Fracture Behavior of Cooked Bovine *M. Semidendinosus*," *Meat Science* **12**, 39 (1985).
13. H. R. Cross, Z. L. Carpenter, and G. C. Smith, "Effects of Intramuscular Collagen and Elastin on Bovine Muscle Tenderness," *Food Science* **38**, 998 (1973).

Rudy R. Lin
Swift-Eckrich, Inc.
Downers Grove, Illinois

EMULSIFIER TECHNOLOGY IN FOODS

Most foods consumed by humans are emulsions, in that they contain either fats or oils dispersed in an aqueous phase, or they contain an aqueous phase dispersed in a fat or oil. An example of the prior emulsion would be milk; an example of the latter would be butter.

A food emulsion may be described as a multiple-phase system that contains at least two components that are normally immiscible. Oil and water are examples of such incompatible components. The water or aqueous phase may contain many other ingredients that are truly soluble in the aqueous phase such as sugars and salts. It may also contain colloidally dispersed components such as proteins and carbohydrate polymers such as cellulose. The fat or oil phase may contain other lipids such as phospholipids and glycolipids, and certain vitamins, colors, and sterols.

The preparation by humans of food emulsions not found in nature has been considered a culinary art and has been practiced as an art for centuries. A perfect example of an artful culinary emulsion has been mayonnaise. Such emulsions did not require long-term stability because they were consumed within a short period of time in the home or restaurant. Such problems as freeze/thaw stability, long-term storage, the physical rigors of transportation, and high degree of aeration did not concern the chef of bygone eras. His emulsions would be considered crude by today's standards. His emulsion—a sauce, for example—would be acceptable if stable for only a few hours, as compared with the months of stability required today in commercially produced emulsions such as salad dressings.

Milk and salad dressings are two of the primary examples of one type of emulsion—the oil-in-water emulsion. The oil or lipid phase is also known as the disperse, discontinuous, or internal phase. It is dispersed in the aqueous serum phase, also known as the external or continuous phase.

Butter and margarine are obvious examples of the opposite type of emulsion, the water-in-oil emulsion. The aqueous phase is dispersed in the fat or oil and is now known as the discontinuous or internal phase, as the lipid has become the continuous or external phase.

A gaseous phase can also exist and may be external, as in the case of an aerosol, or internal, as in whipped topping. Dual emulsions are commonly encountered; a cake batter is a good example of the latter. Air or leavening gasses are dispersed in the fat, which is, in turn, dispersed in the aqueous phase of the oil-in-water cake batter.

The viscosity of a food emulsion is a function primarily of the external phase. It is generally lower when the external phase is aqueous, as in the case of milk, and higher when the external phase is lipid, as in the case of margarine. Several other factors can affect emulsion viscosity. In the case of an oil-in-water type of emulsion, packing or clumping of the internal phase can affect yield value and viscosity. An example of such an emulsion is mayonnaise. The internal dispersed phase (oil) occupies over 70% of its volume, closely approaching its theoretical maximum when the oil globules are of uniform spherical shape. Such an emulsion exhibits little or no flow properties, and its yield value and viscosity are such as to lead the causal observer to assume it may be a water-in-oil emulsion.

Oil-in-water emulsions can contain higher than theoretical limits of lipid internal phase (74%) if globules are misshapen due to compression, and viscosity can be further affected by hydrogen bonding and van der Waals forces. Surface charges supplied by components such as phospholipids can reduce viscosity by imparting repellency.

When the internal phase of an oil-in-water emulsion is low, as in the case of dairy products, ingredients such as colloids, including cellulose, starch, and algins, may be added to increase viscosity. Homogenization of the lipid phase to attain more surface area per unit weight of disperse phase will increase viscosity. Indeed, when homogenization of dairy foods was introduced in the United States in the 1930s, it so improved perceived richness due to increased surface area and resultant viscosity that an attempt was made to ban the process on the basis that it deceived the consumer. As an example, using typical homogenization procedures, milk with an average 8 μ fat globule is converted to 512 globules of 1 μ providing an eightfold increase in surface area.

Most commercial food emulsions contain the disperse phase in small globules in the range of 0.1 to 0.2 μ. With water-in-oil emulsions such as margarine, the viscosity, or plasticity, is a function primarily of the crystalline solids contribution of the fat, as well as its melting point.

The aqueous phase included in the margarine lipid phase is dispersed using a scraped surface crystallizer, such as the votator, so that the water particles are as small as possible. Large particles explode into steam when the margarine is used for cooking with such violence as to cause spattering. A properly manufactured margarine will have the dispersed water particles in the 1 to 5 μ range.

Most oil-in-water emulsions such as dairy-type frozen desserts, because of very efficient modern homogenizers, possess fat globules with a diameter of less than 0.5 μ. The size of the fat globule will affect the light reflected to the viewers eyes in a simple oil in water emulsion. In fact, the size of the oil globule can be estimated from the appearance of the emulsion as given in Table 1.

The physical stability of an emulsion, that is, the tendency of the two phases not to separate, takes place in accordance with Stokes Law:

$$V = 2gr^2 \frac{d_1 - d_2}{9\eta}$$

where V is the velocity of the sphere, r the radius of the sphere, η the viscosity of the continuous phase, d_1 the density of the continuous phase and d_2, the density of the disperse phase, and g the force of gravity. In an oil-in-water emulsion, the oil globules of lower density will tend to rise through the more dense aqueous phase. They will rise more rapidly if they coalesce into larger globules, and their separation will be slowed by increased viscosity.

When an oil is finely divided in a aqueous phase, a tension is created due to the increased surface energy created by division. The oil tends to coalesce to reduce this tension by returning to the lowest free energy state. An emulsifier (surface active agent) will reduce this tension by adsorption at the interface between the two antagonistic phases, thus reducing the free energy (amphiphilic).

Some surfactants further stabilize food emulsions by inhibiting coalescence due to contributing an electrical charge (usually negative) at the fat globule surface. The likely charged globules repel one another, further reducing the opportunity for coalescence.

Most food emulsions produced today contain added surface active agents (emulsifiers). Modern food processing procedures are designed to produce food with long-term shelf life and to withstand the rigors of transportation, including temperature extremes and mechanical abuse. Added surfactants contribute significantly to long-term emulsion stability. As an example, salad dressings that would normally separate in a matter of hours are stable and acceptable to the consumer after months of storage.

Most surfactants used in food processing are esters of fatty acids, and although found in nature, they are in most cases not present in the food after processing in sufficient quantity to provide the desired emulsion stability. Emulsifiers are characterized by the fact that they are amphoteric; that is, they posses both an oil-loving and a water-loving moiety within the same molecule. Since they are neither completely lipophilic (oil loving) nor hydrophilic (water loving) they proceed to the interface between both components and orient themselves in a structure making the fat and water more compatible.

Table 1. Estimation of the Globule Size

Globule size (μm)	Appearance
>1	Milky white
0.1–1.0	Blue white
0.05–0.1	Gray, semitransparent
<0.05	Transparent

Source: Ref. 1.

Food emulsifiers can be manufactured by the esterification of a polyol such as glycerine or sorbitol with a source of fatty acid. Glycerine can be directly esterified with a fatty acid or, as is usually more economical, glycerine can be transesterified with a fat (glycerolysis). Both procedures will produce a surface active glyceryl monoester such as glyceryl monostearate. The resulting monoester will be surface active because it will possess the fat-loving stearic acid moiety and the water-loving hydroxyl groups of the unesterified polyol carbons within the same molecule (Fig. 1).

Although glyceryl monoesters are the most commonly used surfactants in the food industry and are found naturally occurring in most oil-bearing food (milk, nuts, olives, wheat, etc), other alcohols and aldehydes may be used to supply the hydroxyl groups. When a shortage of glycerine developed during World War II, it was found that a monoester of sorbitol produced by hydrogenation of glucose could be substituted in most cases. Not only were such esters found to be equivalent to those derived from glycerol, they were usually superior. Sorbitol differs from glycerine in that it contains six water-loving hydroxyl groups instead of three and is thus more hydrophilic (Fig. 2).

Other alcohols and aldehydes such as propylene glycol and sucrose may be used to supply the hydrophilic moiety of the surfactant. The hydrophilic character and functional value may be further enhanced by further esterification with organic acids such as lactic, citric, or acetic to produce the diester. The esters can be made even more hydrophilic by ethoxylation (Fig. 3) or by esterification with polymers such as polyglycerol. Because of its amphiphilic character, the surfactant will orient itself to the interface between the lipid and aqueous phase. The more hydrophilic the emul-

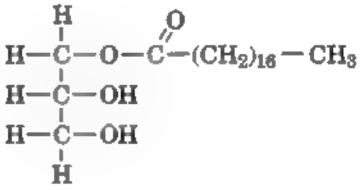


Figure 1. Glyceryl monostearate.

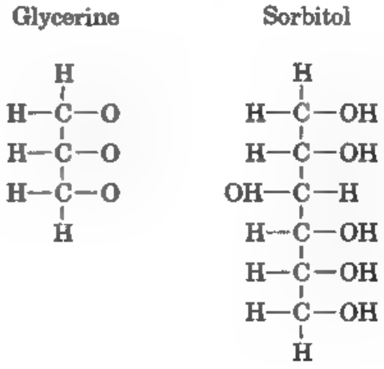


Figure 2. Structures of glycerine and sorbitol.

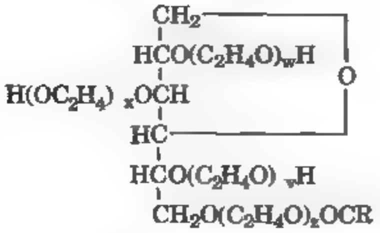


Figure 3. Structure of polysorbate 60 ethoxylated sorbitan monostearate.

sifier is, the greater will be the reduction in interfacial tension (Fig. 4). Its function is therefore threefold:

1. Reduction of interfacial tension
2. Formation of a cohesive film, encapsulating the fat or oil globule
3. Imparting an electrical charge to further aid in preventing coalescence through repellency

Although the functional value of a surfactant in forming stable emulsions is most valuable, these amphiphiles have other valuable physical and chemical attributes useful in food technology. Surfactants improve the texture and shelf life of starch-containing foods by forming complexes with starch components such as amylose, modifying the rheological properties of protein-containing foods such as wheat doughs, and improving the consistency and textural qualities of foods containing fats and other lipids by modifying their crystalline behavior (size and polymorphism) (2).

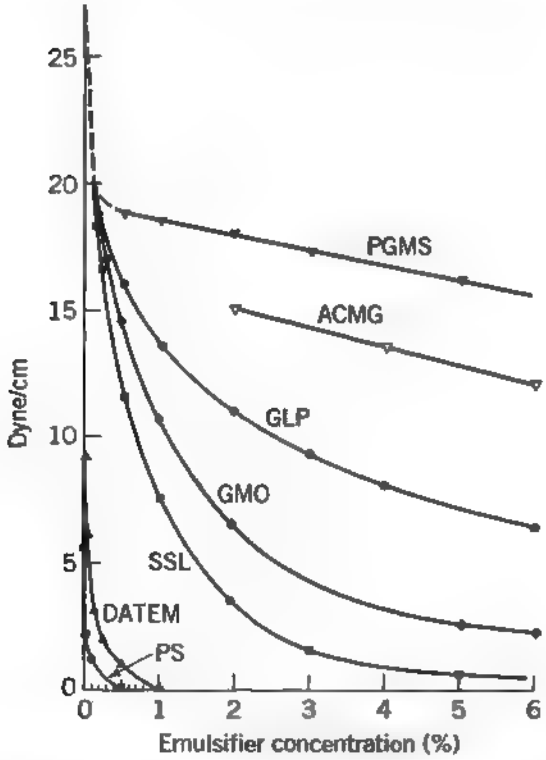


Figure 4. Interfacial tension between soya bean oil and water of 50°C. Measured by the Du Nouy technique. Surfactants tested are propylene glycol monostearate (PGMS), acetylated monoglycerides (ACMG), lactated glyceryl monopalmitate (GLP), distilled monoglyceride from sunflower oil (GMO), sodium stearyl-2-lactylate (SSL), diacetyltartaric acid ester of monoglycerides (DATEM), and polysorbate 60 (PS). *Source:* Ref. 2.

The reduction of surface tension improves the ease with which oil and water will mix. Reduction of surface tension will require less energy input during agitation when dispersing the oil through the aqueous phase. However, when it is realized that surface energy is increased by a factor of 10^6 in a fine emulsion and that the use of a surfactant will reduce surface tension by a factor of 20 to 25, it is apparent that the reduction of surface tension alone may be insufficient to provide a stable emulsion.

The formation of a cohesive film around the oil globule and between the interface of the oil and aqueous phase will make a major contribution to emulsion stability (Fig. 5). The α-tending surfactants, which are nonpolymorphic, are particularly effective encapsulating agents. These include propylene glycol monostearate, lactylated and acetylated monoglycerides, and sorbitan tristearate. These surfactants form a crystalline protective coating around the oil globule with great cohesive strength (4).

When a surfactant is adsorbed on the oil globule surface, the lipophilic hydrocarbon chain is oriented into the oil, with the polar moiety, for example, the OH group at

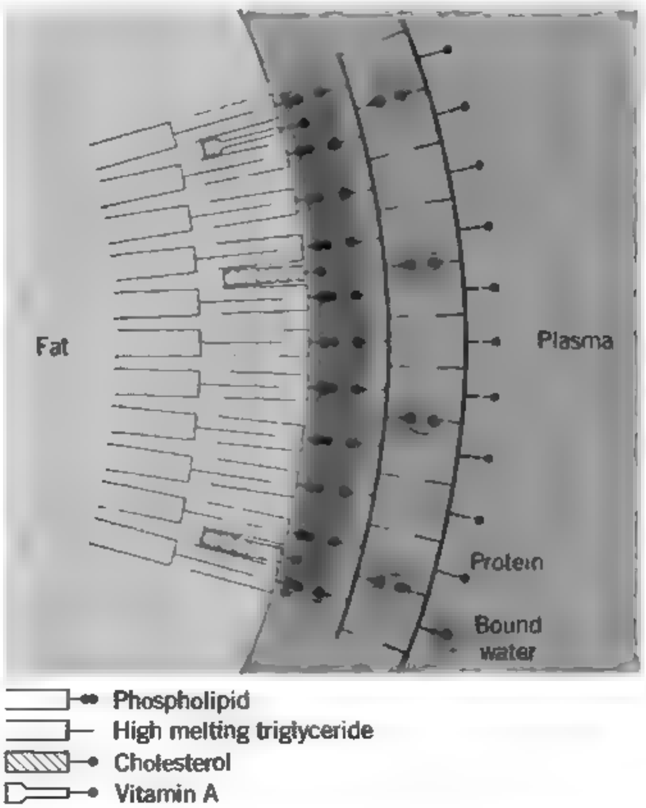


Figure 5. Fat globule surface of milk. *Source:* Ref. 3.

the interface with the aqueous phase. The globule surface is now relatively more hydrophilic with a vastly reduced tendency to coalesce with adjoining fat globules. The tendency to coalesce is further reduced when the fatty acid chains of the lipophilic moiety are high melting because the oil globule surface now takes on a melting point approaching that of the surfactant.

If the surfactant imparts an electrical charge to the surface of the oil globule, it further aids in contributing to emulsion stability because the likely charged globules tend to repel each other, further reducing the opportunity for coalescence. In addition, repellency aids in slowing the separation of the phases (migration of the internal phase). The presence of a surface electrical charge improves the stability of an emulsion when frozen because the emulsified globules are less likely to coalesce following compression and rupture of the surface film, which occurs as a result of concentration into the reduced unfrozen aqueous phase.

Most surfactants added to improve food emulsions are nonionic and do not impart an electrical charge to the globule surface. Lecithin is an exception. Lecithin, with its component phosphatides, although amphoteric, is relatively anionic.

The relative propensity of a surfactant for oil or water is known as its hydrophile–lipophile balance, or HLB. The HLB value may be expressed as the weight percentage of the hydrophilic moiety of the surfactant molecule divided by 5 to reduce the magnitude of the numbers with which we work. For example, if 50% of the molecular weight is hydrophilic, the HLB value would be 50 ÷ 5, or 10. The HLB values of some surfactants used in food emulsification are given in Table 2. HLB values will vary significantly depending on monoester and fatty acid content. The surfactants with lower HLB values tend to induce the formation of water-in-oil emulsions, such as margarine; those with higher values (>10) tend to induce the formation of oil-in-water emulsions. Some relationships between HLB value and the application of the surfactant to food emulsions are given in Table 3. It should be remembered that food emulsions usually contain a significant quantity of naturally occurring surfactants such as phospholipids; the added emulsifiers are merely supplementing the activity of the naturally occurring ones. HLB may be estimated from the solubility of the surfactant, as seen in Table 4.

Obviously, surfactants with high HLB values will tend to disperse more readily in aqueous media while those with low HLB values will be more easily dispersed in the lipid phase. However, there are important other factors that significantly affect surfactant dispersibility in any phase; most surfactants, especially those most effective when used in food processing, are lipophilic and solids at room temperature. As such, they disperse in aqueous media with difficulty. When first introduced as cake emulsifiers, the lipophilic monoglycerides were dispersed in the shortening, which was then added to the batter or cake mix. Monoglycerides and other lipophilic surfactants such as the sorbitan esters, when used in dairy products, were melted into the mix during pasteurization and dispersed to the interface of the fat and water on a molecular basis with the aid of hydrophilic surfactants naturally occurring in the cream phase. When lipophilic or high-melting surfactants such as monoglycerides were required for inclusion in a bread dough at room temperature, it was found necessary to "hydrate" them into a paste emulsion form with water to disperse them in dough or include less effective unsaturated fluid monoesters to form a soft, dispersible plastic.

The first monoglycerides used for the production of hydrates were fully saturated mono/diglycerides. They formed stable emulsions in paste form with about 75% water. Small quantities (ca 0.5%) of hydrophilic coemulsifiers such as sodium stearate or lecithin were added to improve emulsion stability.

When monoglycerides were later distilled to provide a higher monoester content of about 90%, it was found that such products formed gel-like lumps with water, making dispersion in the aqueous phase impossible (4). It was found that distilled monoglycerides and some other surfactants formed mesophases with water and that temperature and water content had to be carefully controlled to obtain adequate dispersion. Some mesophases are dispersible in the aqueous phase, others are more compatible with the lipid phase.

Surface active agents, when dispersed in an aqueous phase, can exist in essentially six mesomorphic or liquid

Table 2. Calculated or Determined HLB Number of Some Surfactants Used in Foods

Chemical name	HLB number
Glycerol monooleate	2.8
Propylene glycol monostearate	1.8
Glycerol monostearate	3.7
Lecithin	4.2
Sorbitan monostearate	4.7
Glycerol monostearate, self-emulsifying	5.5
Polyoxyethylene (20) sorbitan tristearate	10.5
Polyoxyethylene (20) sorbitan monostearate	14.9
Polyoxyethylene (20) sorbitan monooleate	15.0
Polyoxyethylene (40) stearate	16.9

Table 3. Relationship Between HLB and Surfactant End Use

HLB number	Application
4–6	Emulsifiers for w/o systems
7–9	Wetting agents
8–18	Emulsifiers for o/w systems
13–15	Detergents
15–18	Solubilizers

Table 4. Estimation of HLB by Water Solubility

Application in water	HLB range
Not dispersible	1–4
Poor dispersibility	3–8
Milky dispersion after vigorous agitation	6–8
Stable milky dispersion	8–10
Translucent to clear dispersion	10–13
Clear	13 +

crystalline phases. These phases are anhydrous crystals, fluid isotropic, neat lamellar, gel, dispersion, hexagonal and cubic or viscous isotropic (Fig. 6). When a liquid crystal results from heating the anhydrous crystal, it is known as a thermotropic mesomorph; when resulting from a crystallization from solvent such as water, it is known as a lyotropic mesomorph.

When existing as a pure crystal, the polar heads of the surfactant adjoin one another head to head, and the rigid hydrocarbon tails also adjoin. When heat is applied in the presence of water, the hydrocarbon tails liquify because the weak energy of the van der Waals forces between them is overcome. However, the hydrogen bonding between the polar heads remains, resulting in a semiliquid crystalline structure. The contraction of the disordered hydrocarbon chains opens a gap between the polar heads, allowing water to enter. A lamellar mesophase is formed. When this phase is cooled below the temperature where the hydrocarbon chains resolidify (known as the Krafft Point), a gel phase is formed with the water remaining between the polar groups. The gel is metastable; the water is eventually expelled, and a coagel of fine *β* crystals dispersed in water is formed.

When some surfactant crystals, such as monoglycerides, are heated to relatively high temperature with little water, the water is dispersed in the fluid lipid as micellar aggregates, cylinders at low water content, discs at higher. This phase is known as fluid isotropic.

When some surfactants are heated to higher temperature in the presence of water, they may form hexagonal cylindrical aggregates. Two types may be formed. The hexagonal I phase consists of cylinders with the polar heads on the outside and the hydrocarbon chains oriented inward as a core. Such phases are infinitely dilutable in aqueous media. The hexagonal II cylinder is the reverse of the first, with the polar moiety in the interior, surrounding a core of water, with the hydrocarbon chains oriented to the exterior. This phase is found only at low water content, usually less than 30%. At higher water content, this phase will separate from the aqueous media as a viscous mesophase.

Another lipophilic phase, somewhat associated with the hexagonal II phase, is the liquid crystalline cubic phase, frequently encountered with unsaturated monoglycerides. It is viscous and isotropic. Larsson (6) has described a cubic phase of monoglyceride as a polygon aggregate of water cylinders enclosed in a matrix of hexagonal II configura-

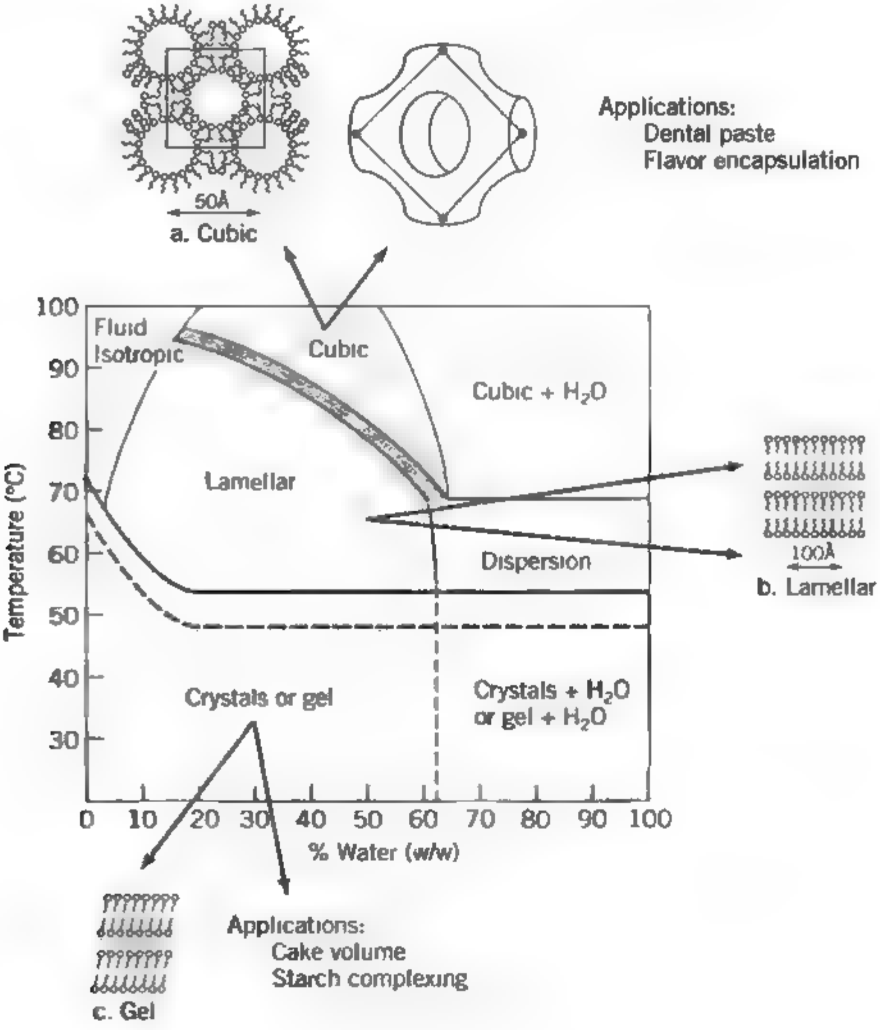


Figure 6. Phase diagram of glyceryl monopalmitate depicting molecular packing of cubic, lamellar, and gel mesophases. *Source:* Ref. 5.

tion, associated as a three-dimensional cubic lattice (Fig. 6). It can absorb up to about 40% water. When dispersed in excess water, surrounded by a lamellar phase, Larsson (7) has referred to the phase as a "cubosome."

When the lamellar mesophase is further diluted with water at the proper temperature, a dispersion phase is formed. The dispersion phase is not merely a dilution of the neat or lamellar phase. The particles are spheres of bimolecular layers alternating with water, and the hollow center can be filled with an aqueous phase. The lamellar bilayer is not stable because the hydrophobic edges resist the aqueous media, and the lamellar bilayer rolls up to form a sphere, with the polar groups external, protecting the internal hydrophobic tails ("liposome"). The physical form of a surfactant drastically affects its functionality. In some cases, the surfactant is completely nonfunctional due to nondispersal on a molecular basis. In other cases, its functional value can be doubled simply by changing the physical form to provide full dispersion.

Although there had been some reference to the use of monodiglycerides in margarine as early as 1921 (4), the first significant commercial use of a surfactant in food was the inclusion of lecithin to improve the viscosity of molten chocolate in the early 1930s (8).

Lecithin was found to lower the viscosity of chocolate, reducing the level of expensive cocoa butter normally required to do so. Lecithin was found to form a monolayer over the hydrophilic constituents of chocolate (sugar, milk, etc) with the lipophilic hydrocarbon tail extended into the molten cocoa fat, facilitating the flow of the vehicle by reducing friction (9).

The incorporation of sorbitan monostearate in chocolate inhibited the formation of bloom (10). Bloom is the result of migration to the surface of chocolate of unstable polymorphs of cocoa butter. As the polymorphs of fat migrate, they leave behind the cocoa fibers, which impart color and ultimately resolidify on the surface of chocolate as light-colored blotches. Sorbitan monostearate forms a monolayer on chocolate nonfat solids, impeding the capillary migration of the unstable cocoa fat polymorph to the surface, thus, inhibiting bloom (11).

About 1933, the so-called superglycerinated shortenings were introduced in the United States for use in the production of cakes (12,13). These contained a significant amount of monoglycerides, for example, 3%. These were included either by production *in situ* by alcoholisis of the fat with excess glycerine during refining or by direct addition of a monodiglyceride.

Shortenings with added surface-active monoglycerides were found to impart greater structural stability to the cakes, allowing for the inclusion of higher ratios of sugar to flour. Cakes with improved texture, volume, and symmetry, as well as keeping quality, resulted from the use of such shortening.

In 1968 the following definition for cake was proposed (14): Cake is a protein foam stabilized with gelatinized wheat starch and containing fat, emulsifiers, mineral salts, and flour and aerated principally by gases evolved by chemical reaction *in situ*.

It is well known that oil is an antifoam that tends to destroy any foam structure, including cake, by weakening the protein film. Plastic shortenings are composed principally of an oil fraction, usually 70 to 75%, blended with solid fats for solidity at room temperature. One would expect the shortening to act as an antifoam, and indeed it does, unless properly encapsulated within an emulsifier. The α-tending surfactants such as propylene glycol monostearate and glyceryl lactopalmatate are exceptionally effective emulsifiers, especially when liquid oil is used as the shortening, because of their ability to form a crystalline membrane around the oil globules, preventing the oil from migrating into the protein lamellae (15). Monoglycerides are also very effective encapsulating agents.

The dispersion of the emulsifier in the batter is most important from the standpoint of obtaining functional improvement. Dispersion by inclusion within the shortening is adequate if the shortening is fully dispersed. Complete dispersion of a plastic fat in an aqueous medium may require extensive art and still might not produce optimum results.

Wren (16) has described six possible physical states for the inclusion of surface active lipids (Table 5). Inclusion of the surfactant in shortening or margarine would represent the anhydrous state. However, it is often necessary to include the emulsifier in the aqueous phase. As an example, the traditional sponge cake formula contains no shortening. To obtain the benefits of an emulsifier, it must be added as an aqueous dispersion. Krog (17) compared the effectiveness of six different mesophases of a distilled glyceryl monostearate in the aqueous state (Table 6). The best results were obtained with the dispersion phase, followed closely by the crystalline gel. Poorest results were obtained with the coagel of β crystals in water. There is no doubt that the dispersion phase was more effective because it was more intimately dispersed in batter. The viscous isotropic cubic phase, poorly dispersible, provided very poor results as is but provided vastly improved results when dispersed in water.

Hydrophilic surfactants (eg, polysorbate 60) are added to cake batters to further reduce surface tension, improving the dispersibility of the lipid phase (eg, shortening) and

Table 5. Possible Physical States of a Surface-Active Lipid

Anhydrous	Polymorphic crystal
	Mixed crystal
	Liquid
	Solution in oil
Aqueous	Molecular solution
	Micellar solution
	Liquid crystalline phase
	Gel phase
	Coagel (crystals + water)
Lipoprotein	Insoluble
	Soluble
Inclusion compound with amylose	
Other macromolecular complex	
Surface film between air–oil–water–solid	"Simple" (monolayer)
	Lipoprotein
	Other complex
	Liquid crystalline phase

Source: Ref. 15.

Table 6. The Effect of Various Aqueous Preparations of DGMS in Sponge Cake

Type of DGMS preparations added	DGMS–water proportion	Temperature of preparation (°C)	Specific volume (cm³/kg) Cake batter	Specific volume (cm³/kg) Cake
Neat, lamellar phase	60:40	65	1140	3400
Viscous isotropic cubic phase	60:40	80	1112	3320
Viscous isotropic + water	10:90	80	1560	5060
Dispersion (pH 7.0)	10:90	65	2970	6900
Gel, α crystalline	10:90	25	2700	6000
Coagel, β crystals in water	10:90	25	1020	2400

Source: Ref. 16.

promoting aeration of the batter. They are usually added in hydrated paste or gel form (25–50% solids) to aid in dispersion. Naturally occurring surfactants such as phospholipids from egg also contribute to emulsification of the batter.

As early as 1925, it had been confirmed that the addition of eggs improved the whipping quality of ice cream mixes (18). It was assumed that the albumin protein fraction of egg was the principal aerating agent. In 1928 it was demonstrated that egg albumin had no beneficial effect on the ice cream mix and that the improved aerating quality was being contributed by the yolk (19). It was later determined that the improvement was due to the emulsification imparted by phospholipids and lipoprotein in the yolk (20,21). Inclusion of yolk in the mix was also shown to increase the electrical charge (anionic) carried by the fat globule (22).

In 1936 a patent was granted on the use of glyceryl monostearate as an emulsifier and whipping aid for ice cream (23). It was found that the monoester was as effective at 0.1 to 0.2% as egg yolk was at 0.5%. It was also found that the use of the monoester provided an ice cream that was drier and stiffer on freezing and thus extruded and packaged with greater facility.

Surfactants based on saturated fatty acids were found to be more effective as aerating agents when used as ice cream emulsifiers, as compared with those based on unsaturated fatty acids (Figs. 7 and 8). The more hydrophilic ethoxylated surfactants are more effective than those of lower HLB value. Those approved for use in food contain an average of 20 mol of ethylene oxide and have an HLB in the 14 to 16 range.

In the production of whippable emulsions, such as ice cream or whipped toppings, it is desirable to obtain good volume and stiffness. Contrary to published hypothesis, it was demonstrated that a partial breakdown of the emulsion during whipping to produce agglomerated (but not coalesced) fat globules provides maximum product stiffness (24,25). It was found that the more hydrophilic ethoxylated surfactants based on saturated fatty acids such as stearic provided maximum whippability, whereas those based on unsaturated fatty acids such as oleic provided maximum dryness and stiffness to the aerated product. A turbidimetic procedure was used to prove that surfactants actually accelerated the breakdown of the ice cream mix emulsion to provide agglomerated fat globules (Fig. 9) (24).

Combining a lipophilic saturated surfactant such as glyceryl monostearate with a hydrophilic unsaturated surfactant such as ethoxylated sorbitan monooleate resulted in a synergistic effect in frozen aerated desserts whereby the combination produced improved whippability and stiffness (26).

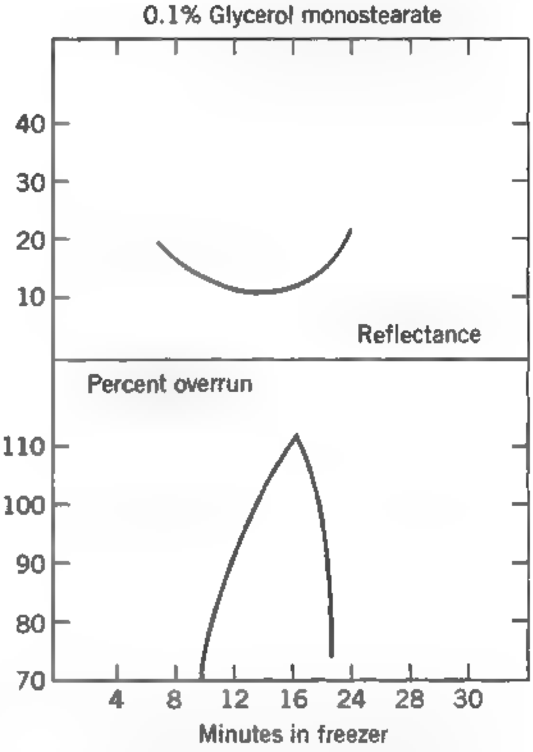


Figure 7. Performance characteristics, ice cream emulsifier glyceryl monostearate.

As with cake batter foams, α-tending surfactants form strong protective films around oil globules and are especially effective in promoting agglomeration of fat globules in dairy-type emulsions. The α-tending surfactants are adsorbed onto the globule surface, interfering with strong protein bonding and making the protective protein film more easily swept off the surface during whipping, resulting in increased agglomeration of globules with resulting firm texture and stiffness in whipped foams (eg, toppings).

Margarine is probably the simulated dairy product most widely consumed. It is a water-in-oil emulsion usually containing 80% fat and 20% aqueous phase. A lipophilic emulsifier system of 0.5 to 1.0% is used to stabilize the emul-

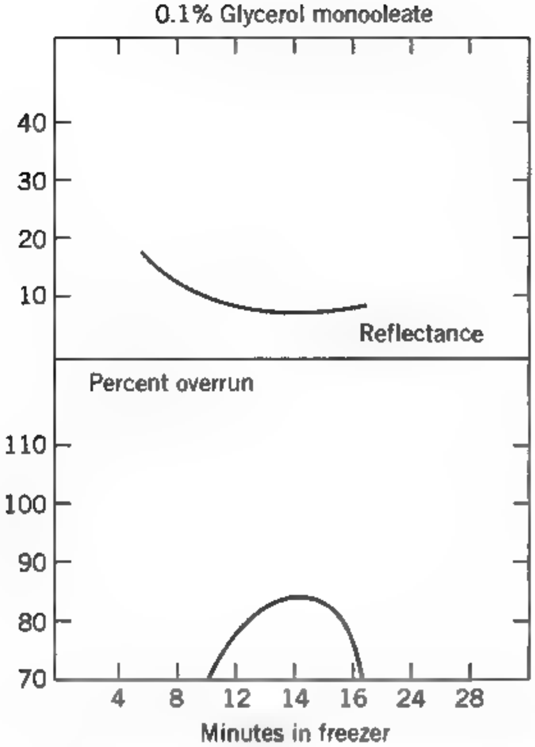


Figure 8. Performance characteristics ice cream emulsifier glyceryl monooleate.

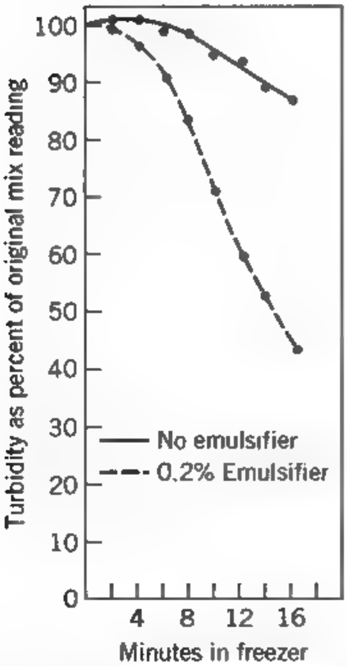


Figure 9. Effect of emulsifier on fat destabilization in the freezer. *Source:* Ref. 24.

sion, preventing leakage of the aqueous phase (weeping) and reducing spattering when the margarine is used for frying.

Surface-active agents with a high degree of unsaturation are usually preferred for margarine, especially the low-fat/high-aqueous types as they more tenaciously incorporate the high-level water into the oil phase.

Another category of simulated dairy products with a large market is milk replacers for young animals. Such emulsions are usually spray-dried in such a manner as to retain the emulsion potential intact, and the emulsion reforms instantly on rehydration.

The largest consumption of surfactants within the food industry is by the baking industry. Aside from the use of surfactants for cake-batter emulsification previously discussed, surfactants are used in yeast-raised products such as bread and rolls for the purpose of reducing the rate of crumb firming, the principal factor associated with staling, and to strengthen gluten (dough conditioning).

As long ago as 1852, the French chemist Boussingault (27) proved that bread did not stale because of moisture loss simply by hermetically sealing it in cans, where the crumb firmed as rapidly as that of unsealed loaves. It is now generally conceded that bread crumb firms primarily owing to the retrogradation of the gelatinized wheat starch. The linear amylose fraction (Fig. 10) retrogrades almost immediately after the baked product cools, thereby supplying physical structure to the baked food. It is not further involved in an ongoing firming. The branched amylopectin fraction (Fig. 10), however, continues to retrograde slowly over a period of days, causing the crumb to become firmer with aging.

Certain surfactants can retard crumb firming because they are able to form complexes with the glucose polymers of starch. Although these surfactants will form complexes with both the straight-chain amylose and the branched-chain amylopectin, it is now understood that it is most important to complex with the amylopectin since it is responsible for the progressive firming of crumb. It has been shown that the starch fractions will form a helix around the hydrocarbon chain of surfactants possessing the proper steric configuration (Fig. 11). The inside of the helix is lipophilic owing to the CH groups, and the exterior is hydrophilic owing to the presence of OH groups. Straight hydrocarbon chains such as stearic acid can accommodate the core of the helix with its diameter of 4.5 to 6.0 A. On the other hand, unsaturated fatty acids of the *cis* configuration cannot enter the helix, as they are not straight.

The complexing ability of a number of surfactants has been evaluated (28); some of these are given in Table 7. A correlation has been established between the ability of a surfactant to complex with amylose and the ability to also complex with amylopectin and retard crumb firming (29,30). The starch components are not as prone to retrograde, owing to interference with hydrogen bonding when the helical clathrates form. For complex formation to take place, the surfactant must possess not only the proper steric configuration but must be dispersible on a molecular basis in the aqueous phase of the dough or batter.

Surfactants such as monoglycerides are used because of their starch-complexing ability in other foods containing

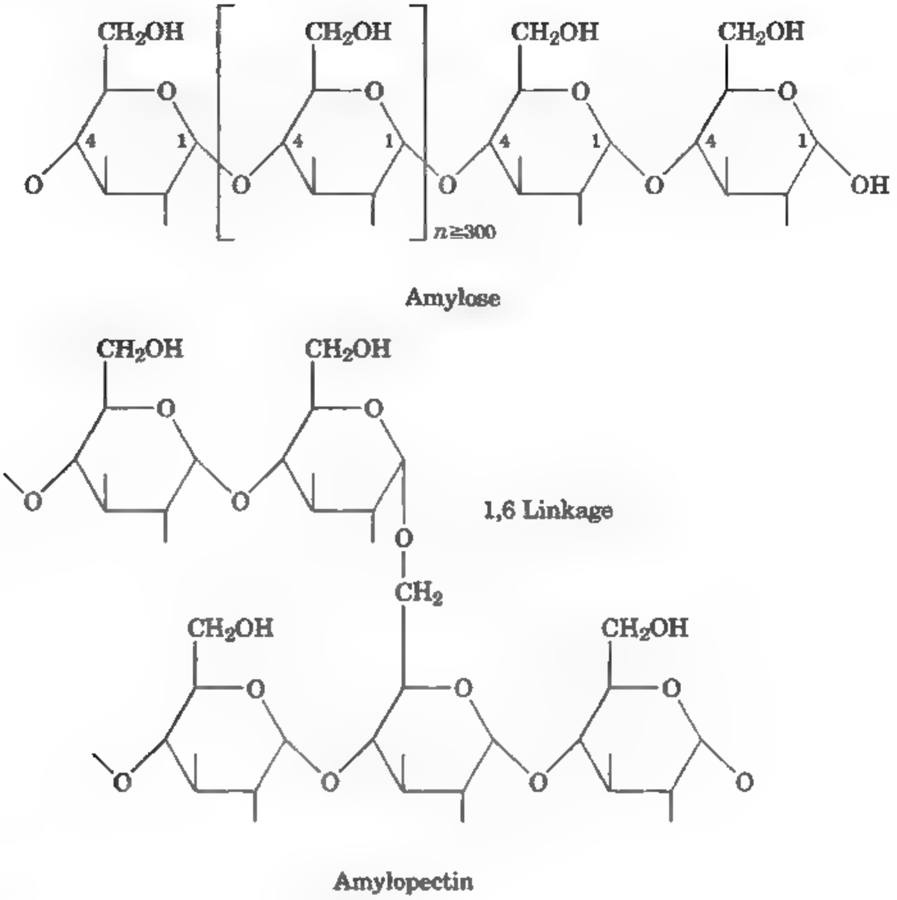


Figure 10. Structure of amylose and amylopectin.

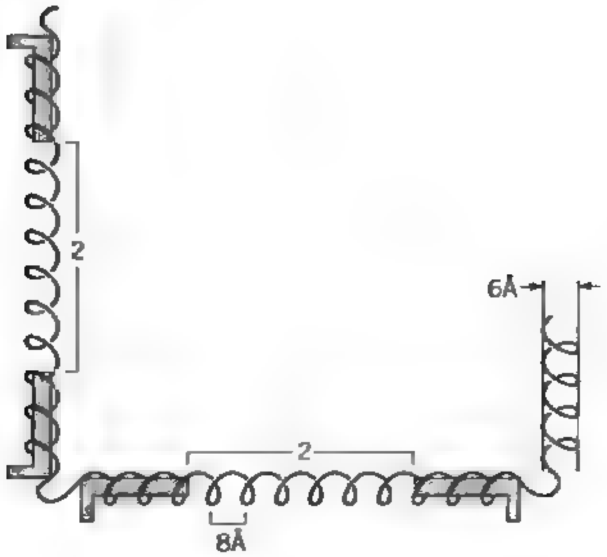


Figure 11. Schematic diagram of glyceryl monopalmitate/amylose complex. a = "Free" space.

Table 7. Complex Formation Between Amylose and Food Emulsifiers

Materials	Amylose complexing index
Distilled monoglycerides, 90–92% 1 monoester	
Lard, hydrogenated	92
Lard, unhydrogenated, 45% monoolein	35
Soya bean oil, hydrogenated, 85% monostearin	87
Soya bean oil, unhydrogenated, 55% monolinolein	28
Monodiglycerides, 45% monoester	28
Organic acid esters of monoglycerides	
Lactic acid esters	22
Succinic acid esters	63
Diacetylated tartaric acid esters	49
Distilled propylene glycol monostearate, 90%	15
Sucrose monostearate (commercial sample)	26
Polyoxylethylene-sorbitan-(20)-monostearate (commercial sample)	32
Stearoyl-2-lactylate	79
No stearoyl-2-lactylate	72
Ca stearoyl-2-lactylate	65
Na stearyl-fumarate	67

Source: Ref. 27.

starch, such as pasta, instant potato products, and starch-based desserts. By complexing with the starch, surfactants improve the texture, and cohesive strength and prevent stickiness in cooked pasta; they also, for the same reason, prevent lumpiness and stickiness in rehydrated instant potatoes and starch-based desserts, which would normally occur with the presence of free amylose.

Certain surfactants that are either anionic or ethoxylated have the ability to react with gluten and other proteins, thereby improving their film-forming ability and cohesive strength. They are known as dough strengtheners or dough conditioners.

Their ability to strengthen protein is the result of the surfactant forming a complex between glutinous protein fractions, together with nonglutinous protein and native polar lipids (31).

Because of the improved cohesive strength of the gluten, the dough has improved tolerance to mixing and increased tolerance to the addition of nonwheat proteins. These changes result in improved loaf volume and symmetry, better crumb texture and cell structure, and greater resistance to staling. The best known of these dough conditioners are the sodium and calcium salts of stearoyl lactylic acid, the ethoxylated glyceryl and sorbitan monostearates, and datem esters. Hydrophilic lecithins have also been shown to possess gluten-strengthening properties (32).

Surfactants are used to stabilize salad-dressing emulsions and to emulsify flavor oils. They are used in chewing gum and certain other confections such as caramel to prevent wrapper sticking and sticking to false dentures. Surfactants find use as extrusion aids in snack foods and meat analogues. Surfactants based on unsaturated fatty acids such as glyceryl monooleate are used as antifoams in sugar processing and confectionery manufacture.

The market for surfactants in foods is now about 400,000,000 lb annually in the United States. About 275,000,000 lb are consumed in foods in Europe and 180,000,000 in Japan.

BIBLIOGRAPHY

1. M. J. Lynch and W. C. Griffin, "Food Emulsions," in K. J. Lissant, ed., *Emulsions and Emulsion Technology*, Marcel Dekker, New York, 1974, p. 249.
2. N. Krog, *J. Am. Oil Chem. Soc.* **54**, 124 (1977).
3. N. King, *The Milk Fat Globule Membrane*, Commonwealth Agr. Bureau, Farnham Royal, Bucks, United Kingdom, 1955.
4. N. Krog, "Food Emulsifiers," in S. Friberg, ed., *Food Emulsions*, Marcel Dekker, New York, 1976, pp. 67–139.
5. E. Boyle, *Food Technol.* **51**, 52 (1997).
6. K. Larsson, *Chem. Phys. Lipids* **9**, 181 (1972).
7. K. Larsson, *J. Phys Chem.* **93**, 7304 (1989).
8. German Patent 530187 (May 15, 1930), Hansea Muhle, A.G.
9. W. Duck, *Pennsylvania Manufacturing Conference Progress Report 10*, Pennsylvania Manufacturing Confectioners Association, Lancaster, Pa., 1955.
10. N. Easton et al., *J Food Technol.* **6**, 21 (1952).
11. W. H. Knightly and J. W. DuRoss, *Proceedings of the 19th Annual Production Conference*, Pennsylvania Manufacturing Confectioners Assn. (Sect. 15), Lancaster, Pa., 1965.
12. U.S. Patent 2,132,393–2,132,398 (October 11, 1938), N. S. Goeth, A. S. Richardson, and V. M. Votaw (to Proctor and Gamble).
13. U.S. Patent 2,132,406 (October 11, 1938), A. K. Epstein and B. R. Harris (to Proctor and Gamble).
14. W. H. Knightly, "Surface Active Lipids in Foods," *Society of Chemical Industry Monograph* **32**, 141–157 (1968).
15. I. S. Shepherd and R. W. Yoell, "Cake Emulsions," in S. Friberg, ed., *Food Emulsions*, Marcel Dekker, New York, 1976, pp. 270–273.
16. J. J. Wren, "Surface Active Lipids in Foods," *Society of Chemical Industry Monograph* **32**, 158–170 (1968).
17. N. Krog, "The Effect of Various Mesomorphic Phases of Monoglyceride-Water Systems in Starch Products," Paper No. 268, AACC-ACS, 1968 Joint Meeting, Washington D.C., March 31–April 4, 1968.
18. H. F. DePew and S. W. Dyer, *Creamery and Milk Plant Monthly* **14**, 87 (1925).
19. D. A. Pettee, *Ice Cream Manufacturing* **2**, 56 (1929).
20. C. C. Walts and C. D. Dahle, *J. Agric. Res.* **47**, 967–977 (1933).
21. C. D. Dahle and D. V. Josephson, *Ice Cream Review* **20**, 60 (1937).
22. G. C. North, Ph.D. Thesis, University of Wisconsin, 1931.
23. U.S. Patent 2,065,398 (Dec. 22, 1936), W. D. Roth (to Industrial Patents Corp.).
24. P. G. Keeney, *Ice Cream Field* **71**, 20 (1958).
25. W. H. Knightly, *Ice Cream Trade Journal* **55**, 24 (1959).
26. U.S. Patent 3,124,464 (March 10, 1964), W. H. Knightly and G. P. Lensack (to Atlas Chemical Industries, Inc.).
27. J. B. Boussingault, *Annals of Chemistry and Physics* **36**, 470 (1852).
28. N. Krog, *Die Staerke* **23**, 206 (1971).
29. N. Krog and B. N. Jensen, *J. Food Technol.* **5**, 77 (1970).
30. N. Krog et al., *Cereal Foods World* **34**, 281–285 (1989).
31. O. K. Chung, C. C. Tsen, and R. J. Robinson, *Cereal Chem.* **58**, 220 (1981).
32. W. H. Knightly, "Lecithin in Baking Applications," in B. F. Szuhaj, ed., *Lecithins—Sources, Manufacture and Uses*, American Oil Chemists Society, Champaign, Ill., 1989, pp. 174–196.

WILLIAM H. KNIGHTLY
Emulsion Technology, Inc.
Wilmington, Delaware

See also EMULSIFIERS, STABILIZERS, AND THICKENERS.

EMULSIFIERS, STABILIZERS, AND THICKENERS

The food additives that will be discussed in this article fall into three functional categories. Although the definition of each function will be clarified in the discussion, the definitions given in 21 *Code of Federal Regulations* are as follows:

> §170.3(o)(8). Emulsifiers and emulsifier salts: Substances which modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.

starch, such as pasta, instant potato products, and starch-based desserts. By complexing with the starch, surfactants improve the texture, and cohesive strength and prevent stickiness in cooked pasta; they also, for the same reason, prevent lumpiness and stickiness in rehydrated instant potatoes and starch-based desserts, which would normally occur with the presence of free amylose.

Certain surfactants that are either anionic or ethoxylated have the ability to react with gluten and other proteins, thereby improving their film-forming ability and cohesive strength. They are known as dough strengtheners or dough conditioners.

Their ability to strengthen protein is the result of the surfactant forming a complex between glutinous protein fractions, together with nonglutinous protein and native polar lipids (31).

Because of the improved cohesive strength of the gluten, the dough has improved tolerance to mixing and increased tolerance to the addition of nonwheat proteins. These changes result in improved loaf volume and symmetry, better crumb texture and cell structure, and greater resistance to staling. The best known of these dough conditioners are the sodium and calcium salts of stearoyl lactylic acid, the ethoxylated glyceryl and sorbitan monostearates, and datem esters. Hydrophilic lecithins have also been shown to possess gluten-strengthening properties (32).

Surfactants are used to stabilize salad-dressing emulsions and to emulsify flavor oils. They are used in chewing gum and certain other confections such as caramel to prevent wrapper sticking and sticking to false dentures. Surfactants find use as extrusion aids in snack foods and meat analogues. Surfactants based on unsaturated fatty acids such as glyceryl monooleate are used as antifoams in sugar processing and confectionery manufacture.

The market for surfactants in foods is now about 400,000,000 lb annually in the United States. About 275,000,000 lb are consumed in foods in Europe and 180,000,000 in Japan.

BIBLIOGRAPHY

1. M. J. Lynch and W. C. Griffin, "Food Emulsions," in K. J. Lissant, ed., *Emulsions and Emulsion Technology*, Marcel Dekker, New York, 1974, p. 249.
2. N. Krog, *J. Am. Oil Chem. Soc.* **54**, 124 (1977).
3. N. King, *The Milk Fat Globule Membrane*, Commonwealth Agr. Bureau, Farnham Royal, Bucks, United Kingdom, 1955.
4. N. Krog, "Food Emulsifiers," in S. Friberg, ed., *Food Emulsions*, Marcel Dekker, New York, 1976, pp. 67–139.
5. E. Boyle, *Food Technol.* **51**, 52 (1997).
6. K. Larsson, *Chem. Phys. Lipids* **9**, 181 (1972).
7. K. Larsson, *J. Phys Chem.* **93**, 7304 (1989).
8. German Patent 530187 (May 15, 1930), Hansea Muhle, A.G.
9. W. Duck, *Pennsylvania Manufacturing Conference Progress Report 10*, Pennsylvania Manufacturing Confectioners Association, Lancaster, Pa., 1955.
10. N. Easton et al., *J Food Technol.* **6**, 21 (1952).
11. W. H. Knightly and J. W. DuRoss, *Proceedings of the 19th Annual Production Conference*, Pennsylvania Manufacturing Confectioners Assn. (Sect. 15), Lancaster, Pa., 1965.
12. U.S. Patent 2,132,393–2,132,398 (October 11, 1938), N. S. Goeth, A. S. Richardson, and V. M. Votaw (to Proctor and Gamble).
13. U.S. Patent 2,132,406 (October 11, 1938), A. K. Epstein and B. R. Harris (to Proctor and Gamble).
14. W. H. Knightly, "Surface Active Lipids in Foods," *Society of Chemical Industry Monograph* **32**, 141–157 (1968).
15. I. S. Shepherd and R. W. Yoell, "Cake Emulsions," in S. Friberg, ed., *Food Emulsions*, Marcel Dekker, New York, 1976, pp. 270–273.
16. J. J. Wren, "Surface Active Lipids in Foods," *Society of Chemical Industry Monograph* **32**, 158–170 (1968).
17. N. Krog, "The Effect of Various Mesomorphic Phases of Monoglyceride-Water Systems in Starch Products," Paper No. 268, AACC-ACS, 1968 Joint Meeting, Washington D.C., March 31–April 4, 1968.
18. H. F. DePew and S. W. Dyer, *Creamery and Milk Plant Monthly* **14**, 87 (1925).
19. D. A. Pettee, *Ice Cream Manufacturing* **2**, 56 (1929).
20. C. C. Walts and C. D. Dahle, *J. Agric. Res.* **47**, 967–977 (1933).
21. C. D. Dahle and D. V. Josephson, *Ice Cream Review* **20**, 60 (1937).
22. G. C. North, Ph.D. Thesis, University of Wisconsin, 1931.
23. U.S. Patent 2,065,398 (Dec. 22, 1936), W. D. Roth (to Industrial Patents Corp.).
24. P. G. Keeney, *Ice Cream Field* **71**, 20 (1958).
25. W. H. Knightly, *Ice Cream Trade Journal* **55**, 24 (1959).
26. U.S. Patent 3,124,464 (March 10, 1964), W. H. Knightly and G. P. Lensack (to Atlas Chemical Industries, Inc.).
27. J. B. Boussingault, *Annals of Chemistry and Physics* **36**, 470 (1852).
28. N. Krog, *Die Staerke* **23**, 206 (1971).
29. N. Krog and B. N. Jensen, *J. Food Technol.* **5**, 77 (1970).
30. N. Krog et al., *Cereal Foods World* **34**, 281–285 (1989).
31. O. K. Chung, C. C. Tsen, and R. J. Robinson, *Cereal Chem.* **58**, 220 (1981).
32. W. H. Knightly, "Lecithin in Baking Applications," in B. F. Szuhaj, ed., *Lecithins—Sources, Manufacture and Uses*, American Oil Chemists Society, Champaign, Ill., 1989, pp. 174–196.

WILLIAM H. KNIGHTLY
Emulsion Technology, Inc.
Wilmington, Delaware

See also EMULSIFIERS, STABILIZERS, AND THICKENERS.

EMULSIFIERS, STABILIZERS, AND THICKENERS

The food additives that will be discussed in this article fall into three functional categories. Although the definition of each function will be clarified in the discussion, the definitions given in 21 *Code of Federal Regulations* are as follows:

> §170.3(o)(8). Emulsifiers and emulsifier salts: Substances which modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.

> §170.3(n)(28). Stabilizers and thickeners: Substances used to produce viscous solutions or dispersions, to impart body, improve consistency, or stabilize emulsions, including suspending and bodying agents, setting agents, jellying agents, and bulking agents, etc.
>
> §170.3(o)(29). Surface-active agents: Substances used to modify surface properties of liquid food components for a variety of effects, other than emulsifiers, but including solubilizing agents, dispersants, detergents, wetting agents, rehydration enhancers, whipping agents, foaming agents, and defoaming agents, etc.

Generally emulsifiers and surface-active agents are relatively small molecules (molecular weight less than 1,000 Da), while stabilizers and thickeners are polymers such as gums and proteins. There are exceptions to this; calcium stearate is listed as a GRAS (generally recognized as safe) stabilizer and thickener while gum ghatti is approved for use as a GRAS emulsifier.

FUNCTIONS OF EMULSIFIERS, STABILIZERS, AND THICKENERS

As shown in Figure 1 surfactants have a lipophilic (fat-loving) portion and a hydrophilic (water-loving) portion; for this reason they are sometimes called amphiphilic (both-loving) compounds. The lipophilic part of food surfactants is usually a long-chain fatty acid obtained from a food grade fat or oil. The hydrophilic portion is either nonionic (glycerol is shown in Fig. 1), anionic (negatively charged, such as lactate), or amphoteric, carrying both positive and negative charges (phosphorylcholine is shown in Fig. 1). Cationic (positively charged) surfactants are usually bactericidal and somewhat toxic; they are not used as food additives. Examples of the three types indicated are a monoglyceride (nonionic), stearoyl lactylate (anionic), and lecithin (amphoteric). The nonionic surfactants are relatively insensitive to pH and salt concentration in the aqueous phase, while the functionality of the ionic types may be markedly influenced by pH and ionic strength.

Emulsifiers

The formation of an oil-in-water emulsion is outlined in Figure 2. If 100 mL of pure vegetable oil plus 500 mL of water are mixed vigorously to obtain an emulsion in which the average diameter of the oil globules is 1 μm, 600 m^2 (slightly more than 6,400 ft^2) of oil-water interface is generated. Using a purified vegetable oil, the interfacial tension (γ) versus water is about 3×10^{-6} J/cm^2. To form the emulsion, 18 J of the energy input is converted into interfacial energy. The addition of 1% glycerol monostearate (GMS) to the oil phase will lower γ to about 3×10^{-7} J/cm^2 so that the excess interfacial energy is only 1.8 J. This excess interfacial energy is the driving force behind coalescence of the oil globules and also the force resisting subdivision of oil droplets during mixing (each division increases the amount of interface present in the system). In a series of emulsification experiments in which the amount of mixing energy was constant and γ was changed by adding emulsifier, it was found that the average oil droplet diameter paralleled γ; that is, as more emulsifier was added, γ decreased and so did the average droplet size. The total excess interfacial energy was roughly constant.

Stabilizers

The phenomenon just described is the promotion of emulsion formation; this is not the same as stabilization of emulsions. The difference is seen by reference to Figure 2. After the emulsion is formed, if it is allowed to stand, the oil droplets will rise to the top or cream (assuming the volume ratio of oil to water is low enough for flotation to occur). The rate of creaming is inversely related to droplet diameter and to the viscosity of the aqueous phase: large droplets rise faster than small droplets, and faster in water than in a viscous gum solution. Emulsifiers that promote formation of smaller diameter drops and additives that increase viscosity give emulsions in which the rate of separation is slower, so in that sense only the emulsion is stabilized.

When two oil droplets make contact (facilitated by the creaming process), they may either clump (stick together, but retain their individual identity) or coalesce into one larger droplet, reducing total surface area and total excess interfacial energy. Clumped droplets may be readily redispersed by simply stirring the system; inverting a bottle of creamed (nonhomogenized) milk a few times redistributes the clumped milkfat droplets. In coalescence the emulsifier

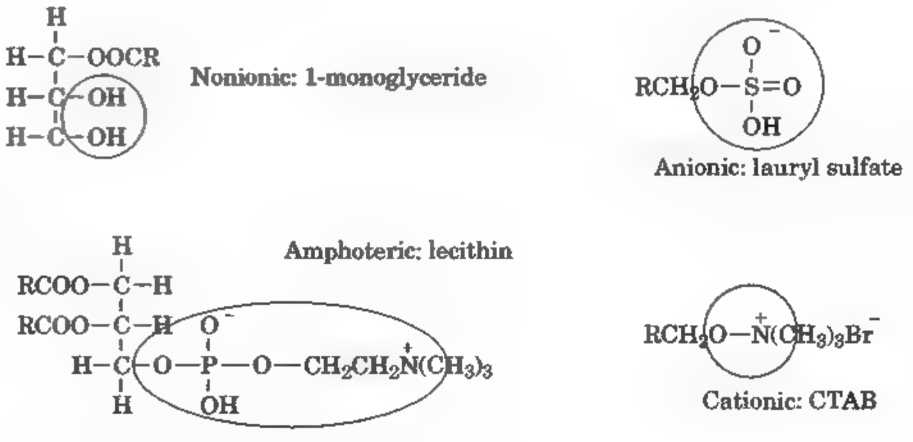


Figure 1. Generalized structure of amphiphilic molecules. R represents a lipophilic hydrocarbon (a fatty acid or fatty alcohol). The hydrophilic groups are glyceryl, (nonionic), sulfate (anionic), phosphorylcholine (amphoteric) and quaternary ammonium (cationic).

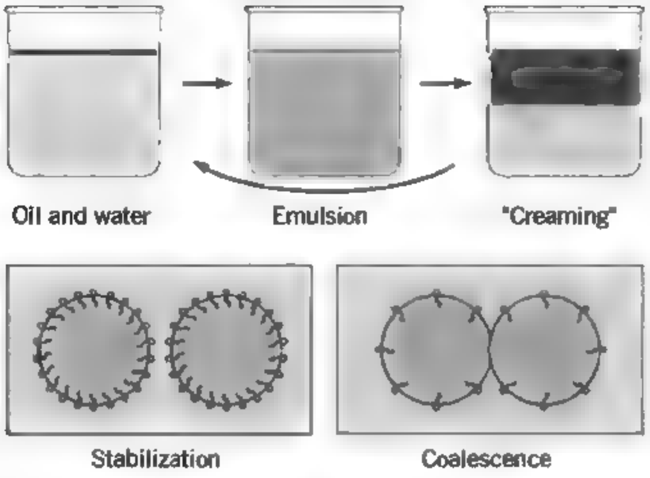


Figure 2. States of emulsion formation and breakdown. In the upper figures the dark area represents oil, the clear areas water. In the lower figures, shaded circles represent oil surrounded by water.

used may be efficient at reducing γ even at a low interfacial concentration but does not prevent the oil droplets from touching and coalescing during creaming.

A true emulsion stabilizer prevents coalescence (Fig. 2). In essence, the thin layer of water between the oil droplets is stabilized by various mechanisms. If the surfactant is anionic, then the surfaces of both oil droplets carry a negative charge and they are mutually repelled by electrostatic effects. This sort of stabilization is sensitive to ionic strength, and a high salt concentration will suppress the electrostatic repulsion, promote contact and coalescence, and lead to rapid emulsion breakdown.

A second kind of stabilization is shown by surfactants in which the hydrophilic portion is quite large; for example, the polyoxyethylene chain of the Tweens or ethoxylated monoglyceride. In this case the chain is anchored at the surface of the oil droplet by the lipophilic tail, but it is strongly hydrated and generates a layer of bound water around the droplet, preventing contact and coalescence. This functionality is relatively insensitive to salt concentration.

A third kind of stabilization is due to simple steric hindrance of contact. The alpha-tending emulsifiers such as propylene glycol monostearate (PGMS) form an actual solid layer at the oil-water interface (1,2). This film physically prevents the contents of oil droplets from coalescing even though their surfaces may be touching. Gums such as gum arabic and gum ghatti stabilize oil-in-water emulsions by a similar mechanism, forming a film of adsorbed polymer around the oil droplet, while some water-soluble proteins perform the same function in mixtures of ground meat and fat for making sausage.

It should be noted that emulsion stabilization is not directly related to the ability to lower interfacial tension. For example, 1% GMS in the oil phase lowers γ to 3×10^{-7} J/cm^2 but has little effect on coalescence rate, whereas 12% PGMS yields a γ of 9×10^{-7} J/cm^2 but gives an emulsion with excellent long-term stability.

Foam Stabilizers

The generation and stabilization of a foam is subject to the same thermodynamic energy considerations as emulsions; lowering the interfacial (surface) tension favors foam formation. Small surfactant molecules dissolved in the aqueous phase will promote foaming, and the stability of the foam is dependent on the maintenance of a film of water between air bubbles. The lipophilic portion of the surfactant enters the gas phase (rather than an oil phase) but in all other respects the situation is analogous to that where both phases are liquid (ie, emulsions).

Proteins are amphiphilic molecules in that many amino acids (eg, leucine, isoleucine, and valine) contain hydrophobic (lipophilic) side chains while others (glutamic, aspartic, lysine, and arginine) have ionic, hence hydrophilic, side chains. Normally, proteins such as egg albumin in solution are folded in such a way that the hydrophobic side chains are buried in the interior of the molecule in a nonpolar environment, while the hydrophilic side chains are on the surface of the molecule and interact with the polar aqueous environment. Introduction of air bubbles into the solution presents a new possibility for the lowest energy state of the protein, namely for it to unfold with the hydrophobic side chains entering the air phase and the hydrophilic chains remaining in the water phase. The portion of the proteins located in the aqueous phase hold water, preventing it from draining away from this region, hence stabilizing the foam. Whipping aids enhance the ability of the protein to unfold at the air-water interface; the energetics of protein unfolding become more favorable and the ease of foam formation increases. Solutes that increase the viscosity of the water phase, for example, gums, slow the rate of draining and thus increase foam stability.

Thickeners

Thickeners may function in four different ways: (*1*) by stabilizing an emulsion with a high volume percentage of internal phase, (*2*) by increasing the viscosity of the external phase, (*3*) by forming an elastic network in the external phase, and (*4*) by removing a portion of the external phase. An example of the first kind is mayonnaise, where egg yolk lipoprotein stabilizes an oil-in-water emulsion in which the internal phase (oil) represents more than 70% of the total volume. As the particle size of the oil droplets decreases, the relative amount of water that is immobilized around the surface increases, and the amount of mobile external phase (free water, in a nonthermodynamic sense) decreases, contributing to the high viscosity and body of the final product.

Certain gums form aqueous solutions that have a high viscosity (Table 1). When such a solution is used as the water component of a food formula, the viscosity of the final product reflects the viscosity of the solution. In a simple salad dressing formulation, increased viscosity in the aqueous phase slows down the flotation rate of the oil droplets formed during shaking and (in this sense) stabilizes the emulsion. Commercial batters (ie, cake, cake donut, and fish breading) require a certain viscosity for optimum functionality; gums may be used to impart and control batter viscosity.

Table 1. Properties of Some Gums

Gum	1% solution Viscosity, mPa·s	*21 CFR*
Low viscosity gums		
Arabic	2–5	184.1330
Ghatti	4–10	184.1333
Larch	2–10	172.610
Medium-viscosity gums		
Sodium alginate	25–800	184.724
Tragacanth	200–500	184.1351
Xanthan	800–1400	172.695
High-viscosity gums		
Guar	2000–3500	184.1339
Karaya	2500–3500	184.1349
Locust bean	3000–3500	184.1343
Cellulose gums		
Sodium carboxymethylcellulose	50–5000	182.1745
Hydroxypropyl methylcellulose	20–50,000	172.874
Methylcellulose	10–2000	182.1480
Gel formers		
Agar	Gel	184.1115
Calcium alginate	Gel	184.1187
Carrageenan	Gel	172.620
Furcelleran	Gel	172.655
Pectin	Gel	184.1588
Gellan	Gel	172.665

Gums and many modified starches in aqueous solution form gels under the proper conditions (Table 1). Gelation is usually dependent on some additional factor (temperature change and presence of divalent cations), and these properties are used during production to generate the desired degree of elasticity in the final food product. The elastic gel network prevents separation of dispersed oil and solids (eg, fruit) as well as giving a pleasing texture to the food. In most food products the external phase is water or an aqueous solution and the internal phase is a mixture of solid material or emulsified lipid. The viscosity of the product depends in part on the volume ratio of internal to external phases. Insoluble fibrous materials can physically adsorb several times their weight in water, so the addition of a small percentage of a material such as cellulose (either alpha or microcrystalline) or hemicellulose (cereal bran) will markedly reduce the amount of water, raising the internal to external phases ratio and hence the viscosity. As an example, a sauce might contain 20% solids (80% water) for an internal to external ratio of 20:80, or 0.25. Adding 5% powdered cellulose will convert about 30% of the water to the solid phase via adsorption, raising the internal to external ratio to 55:50, or 1.1, producing a marked difference in the viscosity of the sauce.

TYPES OF EMULSIFIER

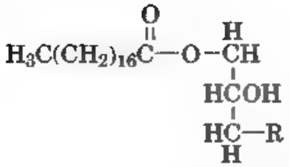
Emulsifiers may be divided into four classes as mentioned earlier: (*1*) nonionic, (*2*) anionic, (*3*) amphoteric, and (*4*) cationic. The actual item of commerce is seldom exactly like the organic chemical structures but is usually a mixture of similar compounds derived from natural raw materials. As a simple comparison, dextrose is one single, relatively pure chemical entity, described by the formula $C_6H_{12}O_6$. On the other hand, glycerol monostearate (GMS) is made from a hydrogenated natural fat or oil and the saturated fatty acid composition may well be something like 1% C_{12} 2% C_{14} 30% C_{16} 65% C_{18} and 2% C_{20}. In addition, the monoglyceride will be approximately 91% 1-monoglyceride and 9% 2-monoglyceride, which represents the chemical equilibrium.

Nonionic Emulsifiers

Monoglycerides and Derivatives. The manufacture of monoglycerides and derivatives used by the food industry were estimated to be more than 200 million lb in 1981 (3–5). The use of monoglycerides in food products first began in the 1930s when superglycerinated shortening became commercially available. Glycerine was added to ordinary shortening along with a small amount of alkaline catalyst, the mixture was heated causing some interesterification of triglyceride with the glycerine, and the catalyst was removed by neutralization and washing with water. The resulting emulsified shortening contained about 3% monoglyceride and was widely used for making cakes, particularly with high sugar levels. Subsequent use of monoglyceride to retard staling (crumb firming) in bread used plastic monoglyceride made by altering the ratio of glycerine to fat to achieve a higher final concentration of monoglyceride, with most of the remainder being diglyceride (commercial mono- and diglycerides may contain from 42 to 60% α-monoglyceride). Later developments in monoglyceride technology include (*1*) distilled monoglyceride, consisting of a minimum of 90% monoglyceride; (*2*) hydrated monoglyceride, which contains roughly 25% monoglyceride, 3% sodium stearoyl lactylate (SSL), and 72% water, forming a lamellar mesophase for better water dispersibility; and (*3*) powdered distilled monoglyceride in which the composition of the original feedstock fat is balance between saturated and unsaturated fatty acids so that the resulting powder hydrates fairly rapidly during mixing in an aqueous system such as bread dough.

The monoglyceride structure shown in Figure 3 is for 1-monostearin, also called α-monostearin. If the fatty acid is esterified at the middle hydroxyl, the compound is 2-monostearin, or β-monostearin. In technical specifications manufacturers usually give the monoglyceride content of their product as a percentage of α-monoglyceride. The routine analytical method for monoglyceride (6) detects only the 1-isomer; quantitation of the 2-isomer is much more tedious. The total monoglyceride content of a product is about 10% higher than the reported α-monoglyceride content. In a practical sense, however, when the functionality and cost effectiveness of various products are being compared, the α-monoglyceride content is a useful number because for all products it equals about 91% of the total monoglyceride present.

The fatty acid composition of monoglyceride reflects the makeup of the triglyceride fat from which it is made. Com-



$H_3C(CH_2)_{16}\overset{O}{\overset{\|}{C}}-O-\overset{H}{C}H-HCOH-\underset{H}{HC}-R$

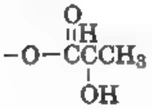
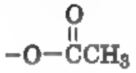
R	Emulsifier
$-OH$	Glycerol monostearate (GMS)
$-O(CH_2CH_2O)_nH$	Polyoxyethylene monoglyceride (EMG)
 $-O-\overset{O}{\overset{\|}{C}}\underset{OH}{\overset{H}{C}}CH_3$	Lactylated monoglyceride (LacMG)
 $-O-\overset{O}{\overset{\|}{C}}CH_3$	Acetylated monoglyceride (AcMG)
H	Propylene glycol monostearate (PGMS)

Figure 3. Nonionic emulsifiers based on glycerol esters and derivatives.

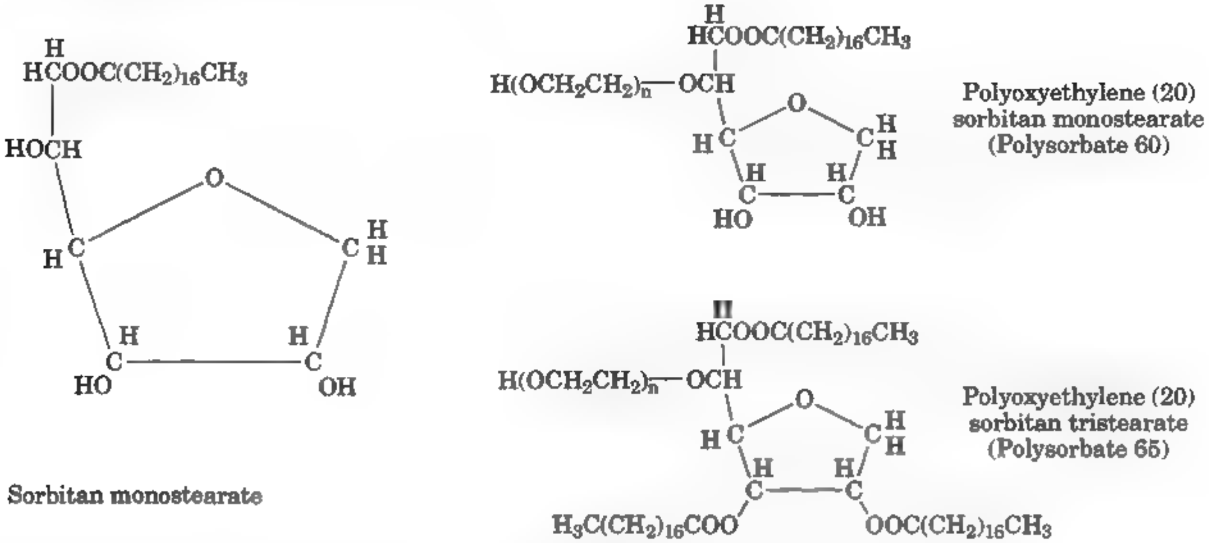
mercial GMS, for example, may contain as little as 65% stearate, if made from fully hydrogenated lard, or as much as 87% stearate, if made from fully hydrogenated soybean oil. The other principal saturated fatty acid will be palmitic, and because hydrogenation is practically never carried out to the extent that all unsaturation is removed (iodine value of zero), a small percentage of unsaturated (oleic and elaidic) acid is also usually present. Iodine values for powdered distilled monoglycerides (monoglyceride in a beaded form that hydrates readily when incorporated in a bread dough) are in the range of 19 to 36, and for plastic monoglycerides a typical range is 65 to 75. The unsaturated fatty acids are a mixture of oleic and linoleic and the *trans* isomers of these acids.

The product of the manufacture of ethoxylated monoglyceride (EMG) is somewhat random in structure. Monoglyceride is treated with ethylene oxide gas under pressure in the presence of alkaline catalyst at elevated temperatures. Ethylene oxide polymerizes via a series of ether linkages and also forms ether bonds with the free hydroxyl groups on the monoglyceride. The average number of ethylene oxide units per monoglyceride molecule is about 20 units ($n = 20$ in Fig. 3), distributed between two chains if the monoglyceride is doubly substituted. Chains may be attached to hydroxyls at both the two and three positions of the monoglyceride, although many more chains will be located at the α (three) position than at the β (two) position because of the difference in their chemical reactivities. The exact distribution of polymer chain lengths and distribution between α and β positions are functions of reaction conditions, for example, catalyst type and concentration, gas pressure, temperature, agitation, and length of reaction time.

The second group of monoglyceride derivatives, the α-tending emulsifiers (lactylated monoglyceride, acetylated monoglyceride, and PGMS), find their main use in cake production. These emulsifiers are dissolved in the shortening phase of the cake formulation and contribute to the emulsification of the shortening in the water phase as well as promoting incorporation of air into the fat phase. The particular property of these emulsifiers that makes them valuable in liquid shortening cakes is that they form a solid film at the oil water interface, not only stabilizing the emulsion but also keeping the lipid phase from preventing air incorporation (protein-stabilized foam formation) during cake batter mixing.

Sorbitan Derivatives. When the sugar alcohol sorbitol is heated with stearic acid in the presence of a catalyst, two reactions occur: sorbitol cyclizes to form the five-membered sorbitan ring and the remaining primary hydroxyl group is esterified by the acid. The resulting sorbitan monostearate (Fig. 4) is oil soluble, has a rather low hydrophilic lipophilic balance (HLB) value, and is the only one of the many sorbitan esters explicitly approved for food use in the United States (sorbitan tristearate is marketed as a crystal modifier, as a self-affirmed GRAS material). Other sorbitan esters of importance are the monooleate and the tristearate. Any of the three esters may be reacted with ethylene oxide to give polyoxyethylene derivatives, as indicated in Figure 4 which are water soluble and have a relatively high HLB. The monostearate derivative is known as Polysorbate 60, the tristearate is Polysorbate 65, and the monooleate is Polysorbate 80. The remarks made in connection with EMG regarding the length and location of the polyoxyethylene chains apply to these compounds. The average number of oxyethylene monomers per sorbitan ester molecule is 20 ($n = 20$), and in the case of the monoesters, chains of varying length (but with about 20 ethylene oxide units in aggregate) may be attached to more than one hydroxyl group of the sorbitan ring (triester has only one hydroxyl group available for derivatization).

Sorbitan monostearate is used in applications where fat is the continuous phase, that is, in fat-based confectionery



Sorbitan monostearate

Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60)

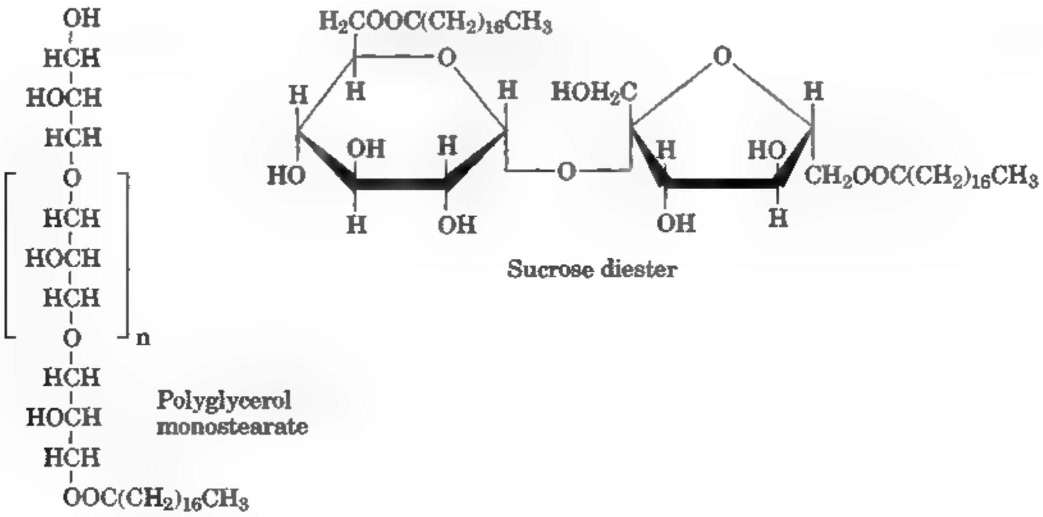
Polyoxyethylene (20) sorbitan tristearate (Polysorbate 65)

Figure 4. Nonionic emulsifiers based on sorbitan esters.

coatings, in bakery icings, and in coffee creamers. The polyoxyethylene derivatives have found wider usage. Polysorbate 60 is used in a variety of applications where a high-HLB emulsifier is required, and is used in fluid oil cake shortening systems (4,5,7), generally in combination with GMS and PGMS. The various sorbitan derivatives are often used in combination to obtain a specific desired HLB; the regulations regarding the permitted levels of each emulsifier in the final product are rather complicated: details can be found in 21 *CFR*.

Esters of Polyhydric Compounds. Polyglycerol esters (Fig. 5) have a variety of applications as emulsifiers in the food industry (3,8). The polyglycerol portion is synthesized by heating glycerol in the presence of an alkaline catalyst; ether linkages are formed between the primary hydroxyls of glycerol. In Figure 5 *n* may take any value, but for food emulsifiers the most common ones are *n* = 1 (triglycerol), *n* = 4 (hexaglycerol), *n* = 6 (octaglycerol), and *n* = 8 (decaglycerol) (in all cases *n* is an average value for the molecules present in the commercial preparation). The polyglycerol backbone is then esterified to varying extents, either by direct reaction with a fatty acid or by interesterification with a triglyceride fat. Again, the number of acid groups esterified to a polyglycerol molecule varies around some central value, so an octaglycerol octaoleate really should be understood as (approximately octa)glycerol (approximately octa)oleate ester. By good control of feedstocks and reaction conditions manufacturers do manage to keep the properties of their various products relatively constant from batch to batch.

The HLB of these esters depends on the length of the polyglycerol chain (the number of hydrophilic hydroxyl groups present) and the degree of esterification. As ex-



Sucrose diester

Polyglycerol monostearate

Figure 5. Nonionic emulsifiers based on polyhydric hydrophilic groups.

amples, decaglycerol monostearate has an HLB of 14.5, while triglycerol tristearate has an HLB of 3.6. Intermediate species have intermediate HLB values, and any desired value may be obtained by appropriate blending. The wide range of possible compositions and HLB values make these materials versatile emulsifiers for food applications.

Sucrose has eight free hydroxyl groups that are potential sites for esterification to fatty acids. Compounds containing six or more fatty acids per sucrose molecule have been approved for use as noncaloric fat substitutes under the name Olestra; this material acts like a triglyceride fat and has no surfactant properties. Compounds containing one to three fatty acid esters (Fig. 5) act as emulsifiers and are approved for food use in that capacity (9). They are manufactured by the following steps. First, an emulsion is made of fatty acid methyl ester in a concentrated aqueous sucrose solution. The water is then removed under vacuum at elevated temperature. An alkaline catalyst is added and the temperature of the dispersion is slowly raised to 150°C under vacuum, distilling off methanol formed on transesterification. Finally, the reaction mixture is cooled and purified. The degree of esterification is controlled by the reaction conditions, especially the sucrose to methyl ester ratio, and the final product is a mixture of esters. The HLB value of a particular product is lower (more lipophilic) as the degree of esterification increases, as would be expected.

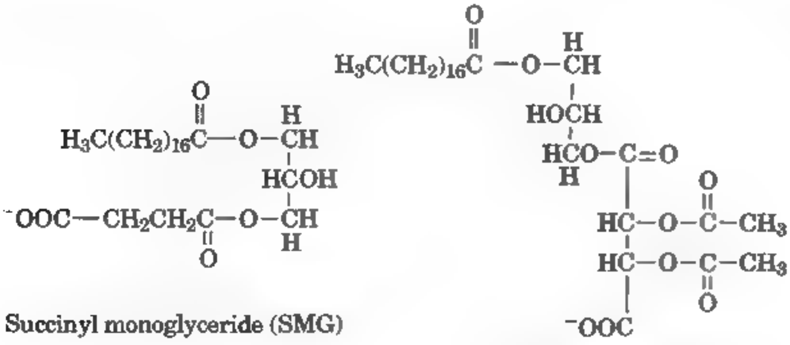
Anionic Emulsifiers

Monoglyceride Derivatives. A large number of anionic derivatives of monoglyceride have been synthesized and tested, but the two shown in Figure 6 are the most widely used. Others listed in Table 2 include stearyl monoglyceridyl citrate, succistearin (the succinate monoester of propylene glycol monostearate) monoglyceride citrate), and monoglyceride phosphate. Succinyl monoglyceride (SMG) is manufactured by reacting succinic anhydride with monoglyceride, forming the succinate monoester. Diacetyltartrate ester of monoglyceride (DATEM) is made by mixing monoglyceride, tartaric acid, and acetic anhydride in a defined ratio and reacting under closely controlled conditions. The product is a mixture of the possible esters, with the one shown in Figure 6 forming the largest proportion. The most usual application of these compounds is as dough conditioners (ie, surfactants added to bread dough), which increase the strength and elasticity of gluten in the dough. Bread and other yeast-leavened baked foods made with the addition of dough conditioners typically have larger volume and finer internal texture than products made without such conditioners.

Other Anionic Surfactants. In addition to SMG and DATEM, some other anionic surfactants that have been tried as dough conditioners are shown in Figure 7. Currently SSL is the one most widely used in the United States; sodium stearyl fumarate (10) has been offered but did not find acceptance, and sodium lauryl sulfate is used only as a whipping agent with egg whites. Lactic acid, having both a carboxylic acid and a hydroxyl function on the same molecule, readily forms an ester with itself. When stearic acid is heated with polylactic acid under the proper reaction conditions and then neutralized with sodium hydroxide, a product having the structure shown in Figure 7 is obtained. The monomer lactylic acid shown represents the predominant product; the lactylic dimer is also present, as well as lactylic trimers and tetramers (11). As with all compounds based on commercial stearic acid derived from fats, some percentage of the fatty acid is palmitic, with small portions of myristic and arachidic acids also found. Although SSL is readily water soluble, the calcium salt is practically insoluble. In this respect it mimics a soap; for example, sodium stearate is water soluble but calcium stearate is oil soluble. Either form may be used, depending on the details of the intended application. The free (unneutralized) form is also approved for certain uses.

Stearyl fumarate is a half ester of fumaric acid with stearyl alcohol (octadecanol). Although it might be expected to have dough-strengthening properties similar to SSL, this was not found to be so in practice and the product was not a commercial success.

The anionic structure shown in Figure 1 is sodium dodecyl sulfate (SDS), a sulfate ester of the C_{12} alcohol dodecanol. Commercially lauryl alcohol is produced by reduction of coconut oil. The alcohol portion of sodium lauryl sulfate is a mixture of chain lengths; the approximate composition is 8% C_8, 7% C_{10}, 48% C_{12}, 20% C_{14}, 10% C_{16}, and small amounts of longer chains. The most common use of


H$_3$C(CH$_2$)$_{16}$C—O—CH
HCOH
$^-$OOC—CH$_2$CH$_2$C—O—CH
H$_3$C(CH$_2$)$_{16}$C—O—CH
HOCH
HCO—C=O
HC—O—C—CH$_3$
HC—O—C—CH$_3$
$^-$OOC

Succinyl monoglyceride (SMG)

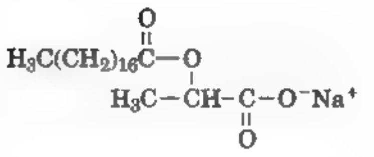
Diacetyltartrate ester of monoglyceride (DATEM)

Figure 6. Anionic derivatives of monoglycerides.

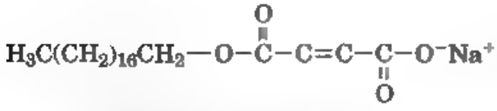
Table 2. Regulatory Status of Emulsifiers

Emulsifier	US *21 CFR*	Canadian[a]	EU No.[b]
Mono- and diglycerides (GRAS)[c]	182.4505	M 4, M.5	E 471
Succinyl monoglyceride	172.830		
Lactylated monoglyceride	172.852	L.1	E 472
Acetylated monoglyceride	172.828	A.2	E 472
Monoglyceride citrate	172.832		E 472
Monoglyceride phosphate (GRAS)	182.4521	A.94, C.7	
Stearyl monoglyceride citrate	172.755		E 472
Diacetyl-tartrate ester of monoglyceride (GRAS)	182.4101	A.3	E 472
Polyoxyethylene monoglyceride	172.834		
Polyoxyethylene (8) stearate		P.5	
Propylene glycol monoester	172.854	P.14	E 477
Lactylated propylene glycol monoester	172.850		
Succinylated propylene glycol monoester (succistearin)	172.765		
Sorbitan monostearate	172.842	S.18	E 491
Sorbitan tristearate		S.18B	
Polysorbate 60	172.836	P.3	E 435
Polysorbate 65	172.838	P.4	E 436
Polysorbate 80	172.840	P.2	E 433
Calcium stearoyl lactylate	172.844		E 482
Sodium stearoyl lactylate	172.846	S.15A	E 481
Stearoyl lactylic acid	172.848	L.1A	
Stearyl tartrate			E 483
Stearyl monoglyceridyl citrate	172.755	S.19	
Sodium stearyl fumarate	172.826		
Sodium lauryl sulfate	172.822		
Dioctyl sodium sulfosuccinate	172.810		
Polyglycerol esters	172.854	P.1A	E 475
Sucrose esters	172.859	S.20	E 473
Sucrose glycerides			E 474
Lecithin (GRAS)	184.1400	L.2	E 322
Hydroxylated lecithin	172.814	H.1	E 322
Triethyl citrate (GRAS)	182.1911		

[a] Canadian Food and Drug Regulations Table IV, Div. 16.
[b] European Parliament and Council Directive No 95/2/EC - 20 Feb 95
[c] Generally recognized as safe.



Sodium stearoyl lactylate (SSL)



$$H_3C(CH_2)_{16}CH_2{-}O{-}\overset{O}{\overset{\|}{C}}{-}C{=}C{-}\underset{\underset{O}{\|}}{C}{-}O^-Na^+$$

Sodium stearyl fumarate

Figure 7. Sodium salts of two anionic emulsifiers. The third one, SDS, is shown in Fig. 1.

sodium lauryl sulfate is as a whipping aid. It is added to liquid egg whites at a maximum concentration of 0.0125%, or to egg white solids at a level of 0.1%. It promotes the unfolding of egg albumin at the air-water interface and foam stabilization as discussed earlier. (Triethyl citrate, not strictly an emulsifier, is also used as a whipping aid.)

Amphoteric Emulsifiers

Lecithin. During the purification of crude oil extracted from soybean, safflower, or corn germ a phosphatide-rich gum is obtained that is treated and purified to give the various commercial lecithin products available to the food processor today (12–14). The dark crude material is bleached to give a more acceptable light brown color. Treatment with up to 1.5% hydrogen peroxide gives the product known as single-bleached lecithin, and further addition of up to 0.5% benzoyl peroxide yields double-bleached lecithin (14). Reaction with even higher levels of hydrogen peroxide plus lactic acid hydroxylates unsaturated fatty acid side chains at the double bond (yielding, eg; dihydroxystearic acid from oleic acid) giving hydroxylated lecithin, which is more dispersible in cold water than the other types and is more effective as an emulsifier for oil-in-water emulsions

(15). Normally some vegetable oil is added to the lecithin to reduce the viscosity to 7.5 to 10 Pa·s; this is called standardized fluid lecithin and contains roughly one-third oil and two-thirds phosphatides.

Figure 8 shows the structure of the main surface active components of lecithin. The phosphatidyl group is a phosphate ester of diglyceride. The fatty acid composition of the diglyceride is similar to that of the basic oil (16) so a number of different fatty acids are found, not just the stearic and oleic acids depicted. There is little phosphatidylserine present in soybean lecithin, and the other three derivatives are found in approximately equal amounts. Phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) are amphoteric surfactants, whereas phosphatidyl inositol (PI) is anionic. The HLB values of the three species are varied, with PC having a high, PE an intermediate, and PI a low value. The HLB of the natural blend is around 9 to 10, and emulsifier mixtures having this value will tend to form either oil-in-water or water-in-oil emulsions, although neither one is highly stable. On the other hand, intermediate HLB emulsifiers are excellent wetting agents and this is a principal application for lecithin.

The emulsifying properties of lecithin can be improved by ethanol fractionation (15). PC is soluble in ethanol, PI is rather insoluble, and PE is partially soluble. Adding lecithin to ethanol gives a soluble and an insoluble fraction. The phosphatide compositions of the two are (*1*) ethanol soluble—60%, PC; 30%, PE; 2%, PI; and (*2*) ethanol insoluble—4%, PC; 29%, PE; 55%, PI (17). The soluble fraction is effective in promoting and stabilizing oil-in-water emulsions, whereas the insoluble portion promotes and stabilizes water-in-oil emulsions. At least one company (Lucas Meyer) is using this process to produce industrial food-grade emulsifiers.

Cationic Emulsifiers

Quaternary Ammonium Surfactants. Cationic emulsifiers are not used as food additives. Some of them, such as the quaternary ammonium compound cetyltrimethylammonium bromide (CTAB) (Fig. 1), have bactericidal properties and are used for sanitation of food-processing equipment. The structure is shown to indicate the chemical nature of this group of surfactants and to complete the description of classes of emulsifiers and surfactant.

USES OF EMULSIFIERS

The regulatory status of these food additives falls into one of three groups. The first group, Section 172 additives, includes those additives that have been examined and judged to be allowable for certain uses at concentrations not to exceed a set level. As an example, stearyl monoglyceridyl citrate (21 *CFR* 172.755) is allowed for use to stabilize shortenings containing emulsifiers. To use this additive as a wetting agent in a dry gelatin dessert mix, the manufacturer would have to submit a petition to the Food and Drug Administration (FDA) seeking clearance for this application and could not sell it for this purpose until such clearance was given. Several of the additives listed in Section 172 are cleared for general use in foods and therefore may be used in any food where they are functional, at a level not to exceed that amount that produces the desired functional effects.

Sections 182 and 184 of 21 *CFR* deal with additives that are generally recognized as safe (GRAS) and thus can be used in any food. In some cases the regulation allows use at any level that is consistent with good manufacturing practices, while for other additives a tolerance or maximum use level is established. Section 182 (Substances Generally Recognized as Safe) lists additives that are recognized as safe based on a history of use without reported safety problems. If an additive undergoes a specific detailed evaluation and its safety is affirmed, it is then moved to Section 184 (Direct Food Substances Affirmed as Generally Recognized as Safe).

The relevant U.S., Canadian, and European Union regulatory references are listed in Table 2 for most food emulsifiers. In many cases the maximum permissible amount of emulsifier is specified. In other instances, the manufacturer is expected to use no more than the amount necessary to provide the desired technological effect.

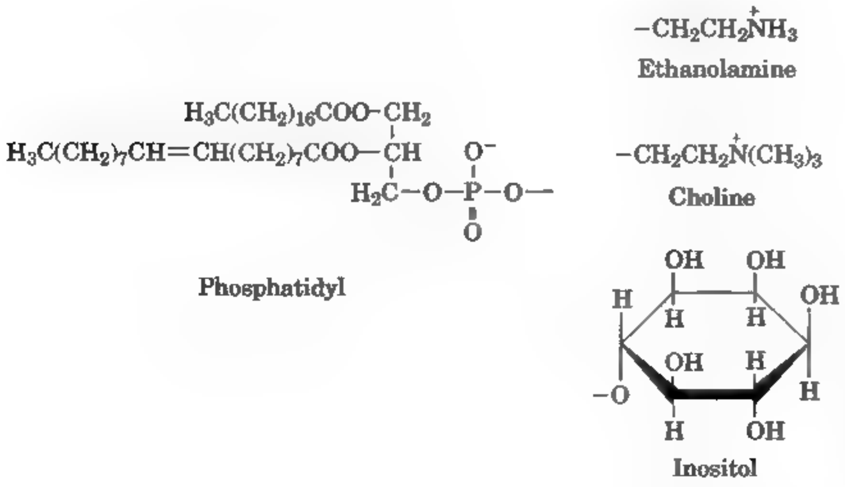


Figure 8. Surface active components of commercial lecithin. Phosphatidyl ethanolamine and phosphatidyl choline are amphoteric emulsifiers, while phosphatidyl inositol is anionic.

Many emulsifiers have functions that are not directly related to emulsification. For example, monoglycerides are widely used to retard firming (staling) of bread and similar baked goods. In this use the monoglyceride forms a complex with gelatinized starch in the bread crumb decreasing the rate at which it recrystallizes. Lecithin is added to melted chocolate intended for coating confectionery goods. The lecithin decreases the viscosity of the chocolate, improving coverage of the confectionery piece at lower use levels of the chocolate. Some emulsifiers (succistearin and polyglycerol esters) are used in oils to inhibit or modify fat crystal formation when the oils are cooled. The emulsifier deposits on the surface of the fat microcrystals (nuclei) and interferes with normal crystal growth.

CONCLUSION

Amphiphilic compounds (surfactants and emulsifiers) promote the formation of emulsions by lowering the interfacial free energy (surface tension γ) between the two phases, thus facilitating the subdivision of the internal phase into droplets of smaller diameter. They may be nonionic (monoglyceride), anionic (sodium lauryl sulfate), amphoteric (phosphatidyl choline), or cationic (cetyltrimethylammonium bromide); cationic surfactants are not used as food additives.

Emulsifiers stabilize emulsions by preventing the contact between the droplets that would lead to coalescence. This may occur by three mechanisms: (*1*) establishing electrostatic charges on the droplet surfaces, (*2*) generating a layer of bound water (in oil-in-water emulsions) around the surface of the droplet, and (*3*) forming a solid film at the interface.

Materials that stabilize foams usually perform both functions. They lower the surface tension between the liquid and air phases and also stabilize the thin liquid film between the occluded air bubbles. For food purposes, effective foaming agents are usually proteins (egg white and gelatin), but certain whipping agents can enhance this functionality.

Compounds that act as thickeners or bodying agents function in one of two ways. They may increase the viscosity of the aqueous phase, or they may form a gel network within that phase. Viscosity builders are usually gums or modified starches, while gelling agents can be gums, modified starches, or proteins. Some solid materials (eg, microcrystalline cellulose) increase the viscosity in a formulated product by adsorbing water and thus increasing the ratio of internal phase (solids, oil) to external phase (mobile water).

BIBLIOGRAPHY

1. J. C. Wootton et al., "The Role of Emulsifiers in the Incorporation of Air into Layer Cake Batter Systems," *Cereal Chem.* **44**, 333–343 (1967).
2. C. E. Stauffer, "The Interfacial Properties of Some Propylene Glycol Monoesters," *J. Colloid Interface Sci.* **27**, 625–633 (1968).
3. H. Birnbaum, "The Monoglycerides: Manufacture, Concentration, Derivatives and Applications," *Bakers Digest* **55**, 6–18 (1981).
4. W. H. Knightly, "Surfactants in Baked Foods: Current Practice and Future Trends," *Cereal Foods World* **33**, 405–412 (1988).
5. D. T. Rusch, "Emulsifiers: Uses in Cereal and Bakery Foods," *Cereal Foods World* **26**, 111–115 (1981).
6. American Association of Cereal Chemists, *Approved Methods of the American Association of Cereal Chemists*, 8th ed., AACC, St. Paul, Minn., 1983, Method 58–45.
7. D. I. Hartnett, "Cake Shortenings," *J. Am. Oil Chem. Soc.* **54**, 557–560 (1977).
8. V. K. Babayan, "Polyglycerol Esters: Unique Additives for the Bakery Industry," *Cereal Foods World* **27**, 510–512 (1982).
9. C. E. Walker, "Food Applications of Sucrose Esters," *Cereal Foods World* **29**, 286–289 (1984).
10. B. A. Brachfeld, J. J. Geminder, and C. P. Hetzel, "Sodium Stearyl Fumarate: A New Dough Improver," *Bakers Digest* **40**, 53–58, 86 (1966)
11. J. B. Lauridsen, "Food Surfactants, Their Structure and Polymorphism," *AOCS Short Course on Physical Chemistry of Fats and Oils*, May 11–14, 1986, Hawaii.
12. W. J. Wolf and D. J. Sessa, "Lecithin," in M. S. Peterson and A. H. Johnson, eds., *Encyclopedia of Food Technology and Food Science*, AVI Publishing, Westport, Conn., 1978, pp. 461–467.
13. C. R. Scholfield, "Composition of Soybean Lecithin," *J. Am. Oil Chem. Soc.* **58**, 889–892 (1981).
14. B. F. Szuhaj, "Lecithin Production and Utilization," *J. Am. Oil Chem. Soc.* **60**, 258A–261A (1983).
15. W. Van Nieuwenhuyzen, "Lecithin Production and Properties," *J. Am. Oil Chem. Soc.* **53**, 425–427 (1976).
16. J. Stanley, "Production and Utilization of Lecithin," in K. S. Markley, ed., *Soybeans and Soybean Products*, Interscience Publishers, New York, 1951, pp. 593–647
17. O. L. Brekke, "Oil Degumming and Soybean Lecithin," in D. R. Erickson et al., eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Mo., 1980, pp. 71–88.

CLYDE E. STAUFFER
Technical Food Consultants
Cincinnati, Ohio

See also EMULSIFIER TECHNOLOGY IN FOODS.

ENCAPSULATION TECHNIQUES

DEFINITION AND TECHNOLOGY DEVELOPMENT

Encapsulate is most commonly defined in its verb form as the act of enclosing or when used as a noun, it is synonymous with capsule. Microencapsulate applies to enclosure, or encasement in microcapsules.

Terms that are often used interchangeably with encapsulate are coated, or protected, eg, coated leavening, protected salt, and coated acids. All three terms are used when referring to practically any core material that is encased or enclosed in an outer shell. However, there are subtle distinctions that often dictate which term is more properly used. Very small capsules, perhaps 100 μ and less, are practically always called capsules or microcapsules. The adjectives coated or protected usually imply a cheaper, more crudely manufactured capsule. Coated or protected particles are generally larger than 100 μ.

In the late 1800s a pharmacist named Upjohn patented a process still referred to as pan coating (1). Relatively large solid particles were tumbled in a cylindrically shaped mixer as liquid coating material was sprayed on. Typically a sugar in water solution was the liquid used and the process involved alternately spraying and evaporating water, until through deposition of sugar, the rounded polished capsules achieved the desired size, shape, and shell thickness. The process was originally applied to drugs and pharmaceuticals for the purpose of taste masking—sugar coated pills—and is still being used extensively. Its application has expanded into several well-known brands of candies and confections.

Most texts establish pioneer work in microencapsulation as having been done in the 1930s by the National Cash Register Company (Dayton, Ohio) (2). Using a chemical process often termed coacervation, National Cash Register developed a means of occluding a colorless dye in a cross-linked gelatin shell. The particles were deposited (less than 20 μ in a cross section) in a thin layer on the underside of a sheet of paper in contact with a second sheet impregnated with a colorless reagent. Pressure exerted on the laminate by the point of a pen or pencil fractured the microencapsulate releasing the reagent-sensitive dye, producing a colored image. The process, which became commercial in 1954, produced the world's first carbonless duplicating paper. In 1981 production of carbonless copy business forms exceeded 500,000 tons per year (3,4).

The National Cash Register invention and the new coacervation process created interest during the mid-1950s in encapsulation in a wide variety of fields from agriculture to cosmetics and toiletries, from electronic parts to food processing (5). The coacervation process itself was examined and, for some applications, was found technically suitable and economical. For many others, of the myriad potential uses that were conceived, coacervation had technical limitations and was much too costly. However, the interest in coacervation spawned development of new encapsulation technologies; some of which have been found entirely suitable for food ingredient applications.

SPRAY DRYING

Spray drying is probably the oldest encapsulation technique and the most often used for preparation of dry, stable, food additives, particularly flavors. Spray-dried flavors have been available since the mid-1930s (6). The process is economical, flexible, and adaptable to commonly used, readily available processing equipment. Generally it is accomplished in three stages. A flavor, commonly an oil, although water-soluble flavors are also fixed using this technique, is mixed with an edible, food-grade polymeric material, such as gelatin, vegetable gum, modified starch, dextrin, or nongelling proteins often in the ratio of one part flavor to four parts fixatives (5). An emulsifier is added and the mixture is homogenized to produce an oil-in-water emulsion with a small micelle structure (7). The emulsion is atomized using one of several different methods, introducing an aerosol into a column of heated air in a drying chamber. The small droplets develop a spherical shape with the flavor oil gravitating to the center, or core and the aqueous–hydrocolloidal phase forming the outside coating. Rapid evaporation of water from the coating's surface maintains a temperature below 100°C (212°F) even in air columns of much higher temperatures. Fortunately the dwell time (exposure to heat in the chamber) is relatively short, usually only a few seconds (6).

Water-soluble flavors (or other food ingredients) are also fixed or entrapped in edible hydrocolloids but the particles do not have a clearly defined core and coating. Rather the spray-dried particle tends to be homogeneous.

Spray-dried particles are usually produced in particle sizes less than 100 μ, an optimum range for easy water solubility, or dispersability. Where necessary spray dried particles may readily be agglomerated or granulated for dry mixes where greater uniformity of particle size is desirable.

Before the introduction of spray drying, dry flavors were produced by plating out liquid flavors on dry substrates, typically sucrose, dextrose, starches, salt, or gelatin, depending principally on the composition of the mix being flavored. Although this crude method provided good flavor rendition and was obviously inexpensive (really nothing more than mixing), it provided no protection against loss of volatile components and oxygen-sensitive fractions were readily degraded (5).

With the advent of spray-dried flavors, oxygen degradation was significantly reduced as was loss of volatiles through evaporation. The colloidal, polymeric coatings provided at least some measurable protection. Some commercial products began to be referred to as locked-in flavors or protected. Even encapsulated began to be used to describe some spray-dried flavors in the early 1950s.

While spray drying offered a step forward in obtaining stability, the process created a problem in obtaining good flavor rendition (from wet to dry). Air, hot enough to instantly vaporize water, is going to drive off at least some low-boiling molecules, and large volumes of hot air will cause some instant oxidation particularly with sensitive terpene-rich citrus oils. Some answers to the problems associated with spray drying flavors came from the flavor chemist who began tailoring liquid flavors using less oxygen-sensitive molecules and fortifying liquids with excess volatiles to allow for rounding off during drying.

Spray drying remains the most common method for producing dry flavors. Some highly sophisticated flavor labs believe emphatically that they can accomplish anything with spray dehydration that can be done with more recently developed encapsulation techniques. However, the inadequacies of the process are generally recognized and it is the desire for superior dry flavors that have spurred the search for new encapsulation techniques and spread these concepts into many other food ingredient applications.

SOLVENT DEHYDRATION

This encapsulation method involves the preparation of a flavor emulsion or solution with the same polymeric hydrocolloidal coatings used in making spray-dried flavors. The emulsions are homogenized and atomized directly into polar solvents such as isopropenol, ethanol, glycerin, or polyglycols, which act to dehydrate the aerosol particles. Resulting microcapsules are recovered by filtration and vacuum dried at low temperatures (8). The technique offers the benefit of tighter protected flavor capsules, somewhat higher payloads than spray drying, and excellent flavor rendition. On the down side processing equipment is expensive and specialized and processing costs are significantly higher than with spray drying.

ENCAPSULATION BY EXTRUSION

Encapsulation of flavors by extrusion incorporated the basic techniques of solvent dehydration with a novel method for providing excellent protection to the core materials. The process involves the preparation of a low-moisture (5–10%) melt (100°–130°C) of low DE malto dextrin, sugar, and modified edible starch. The flavor to be encapsulated is combined along with an emulsifier and optionally an antioxidant, and mixed with a melt under extreme agitation. The molten emulsion is extruded through holes about 1/64 in. into a cold, isopropanol bath. The melt solidifies and is broken into small rods by agitation. The flavor particles are recovered by filtration, or centrifugation, often mixed with a flow agent and packaged for sale. The literature discusses many variations in the process. Some are designed to increase the payload (typically 8–10% flavor); often flavor capsules are given additional solvent washes to remove traces of surface oils further improving the exceptional stability this process affords to deterioration by oxidation. As such it is particularly useful with citrus-flavored oils (9).

Development of today's encapsulation by extrusion solvent dehydration began more than 30 years ago. It really comes from a process developed in 1956 involving adding flavor oils to a molten solution of sucrose and dextrose, cooling the solution to a hard slab, and subsequently grinding the rock-candy-like product to the desired particle size (10,11). From the development of pulverized rock-candy flavor to the current state-of-the-art flavor encapsulation by extrusion, significant processing and product improvements have been made by a number of inventors covered by at least six important patents. At one point there were three companies in the United States selling what was advertised as extruded flavor capsules. The volume, however, although significant never reached the size that the quality of these encapsulates would seem to merit. High process cost (perhaps as much as six times the cost of spray drying) is the major factor. Improvements in spray-drying technology, particularly improvements in formulating flavors designed for spray dehydration have narrowed the quality gap. When a dry flavor is required spray drying is still the technology of choice unless the need for the superior quality of extruded capsules is great enough to warrant the added costs

AIR SUSPENSION COATING

Perhaps more commonly termed fluid-bed processing, this technology probably accounts for the second largest commercial production of encapsulates for the food industry—second only to spray dehydration. Solid particles to be spray coated are suspended in a column of moving air of a controlled temperature. The coating material, which may be molten or dissolved in an evaporable solvent, can be a wide range of water-soluble or insoluble polymeric material, including starches, emulsifiers, dextrins, protein derivatives, and lipids atomized through spray nozzles into the air chamber and deposited on the particles of suspended core material. The movement from the bottom of the chamber through the aerosol to the top of the chamber is random allowing for a rather uniform coating of core material. The constant flow of air allows the molten lipid coating material impinging on the particle to cool and harden, or in the case of a solubilized coating, the solvent to evaporate. The partially coated capsule falls to the bottom of the chamber and the cycle is continued until the parameters for complete or near complete encapsulation have been achieved. The finished product is removed from the chamber, cooled, or perhaps put through a final drying procedure prior to packaging (2,4,12).

Fluid-bed encapsulation has achieved outstanding success as applied to food ingredients, probably because of its flexibility in being suitable for many different core materials. For a wide variety of coatings, it is cost-effective for many ingredient applications, although it is not inexpensive. Credit for the development process in the 1950s is generally given to Wurster, who, while a professor of pharmacy at the University of Wisconsin, used the technique for coating pharmaceutical tablets (13). The Wurster process has become popular, aided perhaps by the commercial availability of suitable solid particle fluidizing equipment. There have been many modifications, mostly centering around different methods to dispense coatings to achieve improved encapsulation or special product characteristics. In principle, however, they all adhere to the basic concept of Wurster, spraying aerosol droplets to impinge on and coat a solid particle (12).

CENTRIFUGAL EXTRUSION

The Southwest Research Institute (SWRI) is responsible for developing several generations of a unique encapsulating concept extending back into the 1960s. Two tubes are arranged concentrically to receive a flow of core material through the center tube and coating material through the outer orifice (the liquids are pumpled). The tubes converge in a common orifice (nozzle) through which is extruded a liquid column within a liquid column. The SWRI equipment actually includes multiple nozzles mounted on a rotating shaft so that they spin around a verticle axis of the shaft. Centrifugal force acts to break off the liquid shaft into small particles as they emerge from the nozzles. The process relies on surface tension to form the spherical core and for the outer liquid to form a continuous coating around the core.

Particles are collected in either solids (starches) or liquid solvents, which tend to cushion the impact and protect the particles and may also perform additional functions. Starches absorb excess moisture and coatings. Gelatin coatings may be hardened or solubility reduced through emersion in solvents containing suitable cross-linking agents (2,14).

COACERVATION

Coacervation is a colloidal chemical phenomenon first used as a microencapsulation technique in the early 1940s by Green of the National Cash Register Corp. It is considered by many to be the first true microencapsulation process.

Simple coacervation involves dispersing a colloid, eg, gelatin, in an appropriate solvent, eg, water. A core material, eg, a hydrophobic citrus oil, is dispersed in the mixture with agitation. By one or more method, such as the addition of sodium sulfate and lowering the temperature, the solubility of gelatin in water is reduced, creating a two-phase system. The colloid-rich phase appears as an amorphous cloud in the colloid-poor, more aqueous phase. If left to stand, the minute droplets would coalesce forming a separate liquid layer. However, under proper conditions (and most literature refers to coacervation as an art as well as a science), the coalescence of the polymeric colloid occurs around the suspended core particles of citrus oils, creating small, still unstable microcapsules. Final steps in the process include adding a suitable cross-linking, or hardening, agent such as glutaraldehyde; adjusting the pH; and subsequent collecting, washing, and drying of the now-stable citrus oil encapsulate.

Coacervation as described is also referred to as aqueous-phase separation, or oil-in-water encapsulation. Complex coacervation is possible only at pH values below the isoelectric point of gelatin. It is at these pH values that gelatin becomes positively charged, but gum arabic continues to be negatively charged. A typical complex coacervation process begins with the suspension, or emulsification, of core material in either gelatin or gum arabic solution. Then the gelatin or gum arabic solution (whichever was not used to suspend the core material) is added to the system while mixing continuously. The pH is adjusted to 3.8–4.3, the system is cooled to 5°C (41°F) and the gelled coacervate capsule walls are insolubilized with glutaraldehyde, or another hardening agent. Microcapsules are collected, washed, and dried.

It is possible to microencapsulate hydrophilic core material in oil-soluble coatings. A polar core is dispersed in an organic, nonpolar solvent at an elevated temperature. The coating material is then dissolved in the solvent. Encapsulation is achieved by lowering the temperature and allowing the polymeric coating material to emerge as a separate coacervate phase microencapsulating the core particles. The coating gradually solidifies and remains insoluble in cold solvent. This process is called either water-in-oil microencapsulation, or organic-phase separation. It is used typically in the pharmaceutical industry for encapsulation with ethylcellulose. However, ethylcellulose does not have general approval for use in the food industry.

Coacervation is an efficient, but expensive, microencapsulation technique. When small particle sizes are requred it is probably the only process that can produce submicron-size particles. With typical payloads in the range of 85–95%, it might be expected that the process would be economical to allow for its application in the food industry for myriad ingredients, such is not the case. Aside from a few specialized flavor applications there are no current uses for encapsulated food ingredients by the coacervation process; costs are much too high (2–4).

INCLUSION COMPLEXATION

B-Cyclodextrin is a cyclic glucose polymer consisting of seven glucopyranose units linked in a 1 → 4 position. The molecule is a doughnut-shaped structure with a hollow center, which enables it to form complexes with many flavors, colors, and vitamins. The *B*-cyclodextrin molecule forms inclusion complexes in the presence of water with compounds that can fit dimensionally within its cavity. Water molecules within the nonpolar center of the *B*-cyclodextrin molecules are readily substituted by less-polar guest molecules. The resulting complex precipitates from the solution and is recovered by filtration and a subsequent drying step. Core material ranges from 6 to 15% (w/w) (15). The odorless, crystalline complexes will tenaciously bind the guest molecules up to temperatures of 200°C (392°F) without decomposition. The same complexes easily release the bound molecule in the temperature and moisture conditions of the mouth (16).

Inclusion complexes are stable to oxygen, light, and radiation, and even onion and garlic oil complexes are almost odorless. Cyclodextrins—while approved for food applications in Eastern Europe, where they were developed, and in Japan—are not approved for use in foods in Western Europe, or in the United States (17,18).

SPRAY CHILLING

Material to be encapsulated, usually dry solids of a relatively small particle size, are suspended in molten coating material, most commonly fractionated and hydrogenated vegetable oils. The mixture is atomized through spray nozzles into a chamber of tempered air (usually refrigerated) where the droplets solidify into small spherical particles. Spray chilling is applied principally to solid food additives including vitamins and minerals, and also to spray-dried flavors to retard volatilization during thermal processing. Spray-chilled products are commonly used in dry mixes. The process is relatively inexpensive. Spray-chilled capsules provide a controlled release of core material. For systems in which a more positive barrier or shell is required, other encapsulation techniques may be preferable (3,19,20).

BASIC CRITERIA FOR ENCAPSULATING FOOD INGREDIENTS

Economics

When microencapsulation was in its infancy in the early 1970s and food technologists were searching for scraps of information about the concept from any source they could find, there was no lack of apparently good ideas for developing new encapsulated ingredients. Few were so foolish to ignore the cost of encapsulating, but most inventive minds operated on the basis that the real problem was to develop the technology, ie, to make the encapsulate and adapt it to the system. It was generally known that once a prototype is made, the reduction in costs is just a matter of time.

Since 1970 the development of encapsulation technology has been startling; however, the cost of encapsulating has not decreased, but has kept pace with inflation. Some relatively crude, lower—cost methods of encapsulation have been developed, but these have been limited in application where a poor encapsulate was good enough.

Most FDA-approved food ingredients have been encapsulated on a laboratory or small pilot-plant basis, but the real world exists outside the laboratory and there commercial viability of an encapsulate revolves around economics in its broadest sense.

Encapsulated ingredient projects begin with a need or a problem. Sometimes the problem is a matter of poor shelf life. More often a project begins with the concept of an encapsulate that would allow the development of an entirely new product that cannot be available without it. Whatever the need for encapsulation, the wisest first step is not to analyze the various methods of encapsulation that might work, but to look at the project, really a processed food system, in its entirety. Assume that the encapsulate is available and technically satisfactory in all respects. Estimate the cost of the encapsulate, ie, ingredient, coating, and both minimum and maximum processing costs.

Then make a judgment: Under the best technical and economic conditions should this project be taken to commercialization? The answer may be no, and for a variety of reasons having nothing to do with encapsulation. The maze of approvals and evaluations that are prerequisites for taking new processes or products to commercialization can be formidable. But assume that in the best-case scenario the answer is yes. It is then just as important to take a look at the worst case, ie, a product that is more costly than desirable and technical requirements that are not quite fulfilled. A definite yes answer in this case makes the project much more attractive, but a no answer, although perhaps disappointing, at least more clearly defines what is really necessary. Projects having long odds may be abandoned, avoiding an investment and a high-risk gamble.

The evaluation of projects prior to embarking on costly research and development is obviously necessary for all new ideas regardless of whether encapsulation is required. However, there is particular wisdom in carefully evaluating encapsulation projects. All too often problems are solved technically with an encapsulate that never becomes commercial for reasons that may have been obvious from the outset. This is particularly true of systems where low-cost food ingredients have been encapsulated. When a formulator must work with a $0.75/lb item that in its raw form costs only $0.10/lb, it is amazing how many other solutions to the problem can be developed in place of encapsulation.

Most encapsulation companies charge for development and preparation of encapsulated prototypes; not to make a profit or even to recover expenses, but primarily to provide that extra insurance that a project has been well thought out and will have some reasonable chance of becoming commercial. Once technical objectives are met, commitment to any financial investment requires consideration and approvals at a relatively high managerial level.

ENCAPSULATED INGREDIENTS—COMMERCIAL TYPES AND APPLICATIONS

The encapsulated food ingredients that are commonly discussed in the literature may represent just the tip of the iceberg. There are more than 20 companies advertising services in encapsulation, granulation, and agglomeration (21). Much of their work is done on a custom basis, and volumes and applications are unknown. There is also a significant amount of encapsulation being done by companies for captive use on a proprietary basis. However, there has developed since the late 1960s a major market for encapsulated ingredients, in addition to the dry flavor business, that by that time had already been established.

Flavors

In 1971 it was estimated that more than 15,000,000 lb of dry flavors were produced domestically (5). This figure has at least tripled. The primary reason for flavor encapsulation is still to provide a dry form for liquid materials, but as technology has advanced, encapsulated flavors have been developed to provide outstanding flavor rendition, excellent protection from degradation through oxidation, and reduced or controlled volatility to the extent that significant economies can be achieved by reducing high temperature losses through evaporation of expensive flavoring ingredients. Technology has been applied to flavors as diverse as citrus oils, menthol, oleoresins of spices and herbs, cinnamaldehyde, peppermint, natural and artificial cheese and seafood flavors, and many other (2,5).

Acidulants

Encapsulated acidulants were first offered commercially in the early 1970s. Initially dry, granular acids including fumaric, malic, citric, tartaric, and adipic were encapsulated to improve stability of acid-sensitive ingredients in dry mixes. By encapsulating acids with coatings such as hydrogenated vegetable oils, or malto dextrin, it was possible to reduce contact between acids and starches in puddings and pie fillings, thereby reducing potential prehydrolysis and significantly extending shelf life. An attendant benefit with encapsulated citric acid was that it reduced hygroscopicity. Core acids were released with addition of water or milk to a mix or, in the case of products employing hydrogenated vegetable oil coatings, on heating (2,5,22).

Encapsulated acids have been used to prevent undesirable discoloration of acid-sensitive food colors and dyes, degradation of aspartame, control of dusting, as means of regulating the gelling effect of acidulants on acid-sensitive pectins in fruit-based products, and alteration of bulk density. The most recent, and by now the largest application, for encapsulated acidulants is in the meat industry where the use of encapsulated citric acid, lactic acid, and glucono-*delta*-lactone, a gluconic acid precursor, provide a means of direct acidification of processed meat. In the manufacture of fermented sausage the industry has long relied on naturally occurring microorganisms or on *Lactobacillus* starter cultures whose activity on carbohydrates produces lactic acid, thereby lowering the pH to a level adequate to ensure protection against pathogenic microorganisms. The fermentation process can take as long as four to five days, and although it has been used for hundreds of years, it is not without problems, primarily the length of fermentation time and the variability from batch to batch. The potential for direct acidification was obvious, but not easily accomplished. Contact of ground meat with acid causes rapid separation of fat from protein, discoloration, and a change in texture from a soft uniform emulsion to hard, brittle, crumbly particles that are no longer usable. Use of encapsulated acidulants having hydrogenated vegetable oil coatings with appropriate melting points offer a mechanism for releasing acidulants into meat slowly and uniformly throughout the mass as temperature is elevated during cooking. The fermentation step is eliminated. The concept has been expanded to meat systems where acidification is possible to replace high-temperature retorting with short-term pasteurization (23–26). The desirability of using lactic acid as the acidulant by some technologists has extended the creativity of the encapsulators to include liquid lactic acid, which is first absorbed on solid calcium lactate particles prior to tight encapsulation in hydrogenated vegetable oil.

Dough-Conditioning Agents

Encapsulation technology has been applied to conditioners for protection and to control activity until the optimum point in the baking cycle is reached. Ascorbic acid, which is used as an oxidizing agent in strengthening and conditioning bread doughs, is unstable in the presence of water and degrades rapidly. Encapsulated ascorbic acid can be added to typical prefermenting broths with minor loss of potency, through the fermentation stage. During baking the hydrogenated vegetable oil coating melts, releasing the ascorbic acid for its conditioning–strengthening effect at the stage where it is most effective (27).

Leavening

Shelf life of chemically leavened doughs is restricted by reaction prior to proofing or baking. Dry, packaged mixes lose leavening activity particularly in high-moisture systems. The same is true of chemically leavened frozen doughs. Refrigerated and microwaveable frozen doughs offer unique leavening problems in that activity needs to be controlled, not simply prevented. Refrigerated doughs require some leavening initially when the dough is formed, but ideally no more reaction through the packaging stage. Once in the closed containers and held under suitable proofing conditions, rapid leavening is desirable to purge the seal and provide maximum can pressure. Microwaveable frozen products require initial protection from reaction, with rapid, intense leavening during the very brief time that a rise may occur within the microwaving process.

Encapsulated sodium bicarbonate with precise release characteristics in combination with selected leavening acids offers a commercial means of providing protection, and optimizing activity in a wide variety of baking systems. Encapsulation has also been applied to leavening acids, particularly glucono-*delta*-lactone for similar applications.

Protection of Yeast-Leavened Baked Goods

Sensitivity of yeast cells to a variety of ingredients used in baked products has created a highly specialized need for encapsulates. Even at low flavor levels acidulants, eg, citric, lactic, and acetic acids, will reduce the viability of yeast and may further affect the cellular structure of dough by reducing the total effect of yeast leavening. Preservatives such as sorbic acid and calcium proprionate, and even salt may have a similar killing effect on yeast as on the molds they are intended to inhibit. Citric, lactic, and acidic acids encapsulated in hydrogenated vegetable oil (HVO) are being used on a commercial basis to prevent the effect on yeast of low pH, as is HVO-coated sorbic acid. Other encapsulated additives that affect yeast negatively in the raw form have been produced and evaluated on a developmental basis with promising results. Potentially dried yeast cells themselves may one day be commercially available in an encapsulated form. Work has been done along this line, but apparently with limited success. The coating may be extremely useful in maintaining the viability of the cells, but encapsulation itself can have an inhibiting effect on yeast, and certainly the benefits of encapsulation would have to be major to warrant the added cost.

Vitamins and Minerals

A variety of dry mixes are fortified with vitamins and minerals including cereals, infant formulas, pet foods, and beverages. Encapsulation with both lipids and water soluble coatings are used to reduce off-flavors, permit controlled release in the digestive tract, improve flow properties, facilitate tableting, and enhance stability, particularly of vitamins, to extremes of moisture and temperature. Iron is encapsulated as are other minerals to control bulk density, reduce stratification in combination with lighter mix components, and to retard catalytic oxidative rancidity.

One use for an encapsulated vitamin provides such a major product improvement with an accompanying economic advantage that it merits special comment. The aquaculture industry has grown enormously in the last decade. The key to maintaining the health of pond-grown fish is in proper feeding. A number of nutrient supplements are added to fish rations, but one stands out prominently in any discussion of the applications for microencapsulation technology. Fish must ingest vitamin C in an adequate dosage for survival. Fish along with humans, apes, guinea pigs, and the fruit bat lack the enzymes required to synthesize ascorbic acid from protein consumed in their diet. Using raw ascorbic acid for feed fortification requires 5–10 times the level recommended in the normal diet of fish because oxygenated water is used in all three of the basic processes for producing fish feed, which rapidly reduces the potency of ascorbic acid. This reduction is accelerated with temperature and pressure elevation, which is required in all three processing techniques.

Encapsulation of vitamin C in a HVO coating prevents contact of the oxygen-sensitive ascorbic acid with water. The half-life of raw ascorbic acid in fish feed is measured in days. The half-life of the digestible, bioavailable, encapsulated version of vitamin C is measured in months (28–30).

Sodium Chloride

There has been a significant, although not major market, for coated salt as a food ingredient since the late 1960s, primarily in the meat industry where it is used in ground pork sausage where contact with raw salt will affect texture and turns the bright pink color to a dull bluish gray. Fat-coated salt, even when crudely applied, extends shelf life and the improvement is worth the additional cost at least to many manufacturers. As technology has improved so has the quality of HVO-encapsulated salt. Although still restricted from becoming a principal ingredient in the food industry because of the economics involved, applications have been extended to baked products to protect yeast and to retard the stiffening effect induced by raw salt through water absorption. Use in comminuted meat products has also increased to control changes in viscosity and texture produced by water absorption. Maintenance of a free-flowing viscosity of ground beef allows the cost of encapsulation to be offset by savings provided by increased throughput.

FUTURE DEVELOPMENT

A new and better technique to microencapsulate food ingredients may be developed in the next 10 years, and perhaps the new process will be more economical than the current state of the art. However, if the available technology stays the same, the market for encapsulates in the food industry will certainly keep growing at an accelerating rate. The benefits are significant.

The industry is becoming accustomed to what encapsulation can do and as the confidence level grows, so do the opportunities. What is perhaps equally important is knowing what the new technology can do and knowing what it can't do. Fortunately the food industry no longer looks at encapsulation as some far out technology that might be considered if all else fails. The industry has matured to the extent that products are tailored to fit specific applications. It is interesting to observe that with one common core ingredient—citric acid—one of the major ingredient producers offers 11 different grades, the other producer offers 8. Each one differs in variables that are fundamental to encapsulation, eg, payload, particle size, coatings, water solubility, or fat coatings. Other important differences include the coatings, melting points, origins, and costs. Each product is designed to be useful in a specific food ingredient system.

While commercialization of encapsulation technology covers many diverse fields within the broad term Food Industry, the majority of products and the greatest volume is focused on two segments, ie, meat processing and baking. It is interesting to observe that in both, encapsulation has been treated not as a tool to be tried and abandoned if it doesn't perform adequately the first time, but rather as a part of the answer to a food-processing problem, albeit an important part. Most initial attempts to solve problems with encapsulates are only partially successful. It is in the fine tuning, not only of the encapsulates to fit a system but, where possible, of the system's design to fit what can be achieved through encapsulation. The meat and baking industries have been doing this. Other industries such as the dairy and microwaveable entree industry would seem to be candidates for a similar developmental approach.

A significant part of encapsulation technology is in the selection or development of optimum edible Food and Drug Administration approved coatings to enclose and protect and still release under the desired conditions. There is room for new coating materials, particularly ones that will have higher melting points or will remain intact under higher temperatures than what is currently available. Edible coatings having release points of ca 74°C (165°F) would be particularly interesting.

BIBLIOGRAPHY

1. R. J. Versic, "Flavor Encapsulation, An Overview," *ACS Symposium Flavor Encapsulation, Series 370*, ACS, Washington, D.C., 1988, Chap. 1, p. 5.
2. J. D. Dziezak, "Microencapsulation and Encapsulated Ingredients," *Food Technology* **42**(4), 137 (Apr. 1988).
3. R. J. Versic, "Coacervation for Flavor Encapsulation," in Ref. 1, Chap. 14, pp. 126–131.
4. R. E. Sparks, "Microencapsulation," in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 15, 3rd ed., John Wiley & Sons, Inc., New York, 1981.
5. R. E. Graves, "Uses for Microencapsulation in Food Additives," *Cereal Science Today* **17**(4), 107, 1972.
6. G. A. Reineccius, "Spray-Drying of Food Flavors," in Ref 1, Chap. 7.
7. J. Brenner, "The Essence of Spray Dried Flavors: The State of the Art," *Perfumer and Flavorist* **8**, 40 (Apr.–May 1983).
8. R. Zilberboium, I. J. Kopelman, and Y. Talmon, "Microencapsulation by a Dehydrating Liquid: Retention of Paprika Oleoresin and Aromatic Esters," *Food Science* **1**(5), 1301 (1986)
9. S. J. Risch, "Encapsulation of Flavors by Extrusion," in Ref. 1, pp. 103–109.
10. U.S. Pat. 2,809,895 (Oct. 15, 1957), H. E. Swisher (to Sunkist Growers, Inc.).
11. T. H. Schultz, K. P. Dimick, B. Makower, "Incorporation of Natural Fruit Flavors into Fruit Juice Powder," *Food Technology* **10**, 57–60 (1956).
12. D. M. Jones, "Controlling Particle Size and Release Properties," in Ref. 1, pp. 158–176.
13. U.S. Pat. 2,648,609 (Aug. 11, 1953), D. E Wurster (to Wisconsin Alumni Research Foundation).
14. R. E. Lyle, D. J. Mangold, and W. W. Harlow, "The Making of Microcapsules," *Technology Today* **3**(3), 13–16 (Sept. 1904).
15. H. B. Heath and G. A. Reineccius, "Flavor Production," in *Flavor Chemistry and Technology*, AVI Publishing Co., Inc., Westport, Conn., 1986.

16. J. S. Pagington, "Molecular Encapsulation with *B*-Cyclodextrin Food Flavor Ingredients," *Process Packaging* 7(9) 50 (Sept. 1985).
17. G. A. Reineccius and S. J. Risch, "Encapsulation of Artificial Flavors with *B*-Cyclodextrin Food Flavor Ingredients," *Process Packaging* **8**(9), 1 (Aug.–Sept. 1986).
18. L. Szente and J. Szejtli, "Stabilization of Flavors by Cyclodextrins," in Ref 1, Chap. 16, pp. 148–157.
19. J. A. Bakan and J. S. Anderson, "Microencapsulation," in L. Lachman, H. A. Lieberman, and J. L. Kanig, eds., *The Theory and Practice of Industrial Pharmacy*, Lea & Febiger, Philadelphia, 1970, p. 384.
20. D. Blenford, "Fully Protected Food Flavor Ingredients," *Process Packaging* **8**(8), 43 (July 1986).
21. H. Weiss, president, Balchem Corp., unpublished data.
22. L. E. Werner, "Encapsulated Food Acids," *Cereal Food World* **25**(3), 102 (1980).
23. R. E. Graves, "Sausage Fermentation: New Ways to Control Acidulations of Meat," *National Provisioner* **198**(21), 32–34, 49 (May 1988).
24. J. Bacus, "Fermenting Meat, Part I," *Meat Processing* **24**(2), 26–31.
25. J. Bacus, "Fermenting Meat, Part II," *Meat Processing* **24**(3), 32–35 (Mar. 1986).
26. G. J. Jedlicka, "Chemical Acidulation of Semi-Dry Sausage." *paper presented at American Meat Institute Convention*, New Orleans, 1984
27. U.S. Pat. 3,959,496 (May 25, 1976) S. Jackel and R. V. Diachuk (to Baker Research and Development Co.).
28. C. Andres, "Encapsulated Ingredients," *Food Processing* **38**(12), 44 (Nov. 1977).
29. L. A. Gorton, "Encapsulation Stabilizes Vitamin C in Cupcakes," *Baking Industry* **148**(1805), 20–21 (Jan. 1981).
30. C. J. Pacifico, "A Novel Approach to Vitamin C Nutrition for Fish Rations," *Aquaculture Today* **2**(4), 20–22 (1989).

Bob Graves
Herb Weiss
Balchem Corporation
Slate Hill, New York

ENERGY USAGE IN FOOD PROCESSING PLANTS

There is a continuing concern about the energy demand by our food system. It is estimated that 17% of U.S. energy consumption is attributed to the food system (1). This figure includes energy used for production through processing, distribution, out-of-home preparation, and in-home preparation. The food industry requires energy for a variety of equipment such as gas fired ovens; dryers; steam boilers; electrical motors; refrigeration units; and heating, ventilation, and air-conditioning systems.

Natural gas is the predominant source of energy used by the U.S. food industry. In the last few decades about 50% of the gross energy used in food processing was from natural gas; 15%, from fuel oil, 13%, from electricity; and about 22%, from propane, butane, other petroleum products, coal, and some renewable energy sources (2). Within the food industry, the principal types of energy use include direct fuel use, steam, and electricity. Nearly 50% of energy use is in the form of direct fuel use. Almost 30% of energy is used to process steam and 10% to heat water. Almost 67% of electrical energy consumption is for generating mechanical power to operate conveyors, pumps, compressors, and other machinery. Refrigeration equipment consumes about 17% of electricity; lights consume about 10%; and heating, ventilation, and air-conditioning use approximately 4% of electricity.

Between 1973 and 1986, the cost of energy escalated dramatically. This trend promoted the installation of heat recovery equipment to conserve energy (3). It also focused the attention of scientists and engineers on the economic feasibility of alternative energy sources in various food-processing situations like cheese processing (4), food dehydration (5), and water heating (6). Energy consumption patterns of various food industries are briefly discussed.

MILK PROCESSING

Considerable variations in energy requirements for milk processing have been reported for different plants. Fuel requirements for producing pasteurized bottled milk ranges from 0.25 to 2.65 MJ/L of milk (7). The energy used for processing cheese during the regular plant operation is approximately 2.13 kJ/kg. Excluding drying and evaporation, energy required to produce 1 kg of cheese is in the range of 3.37 to 17.53 MJ (4). Some of this variation is due to a lack of concern to energy conservation, but there are also operational factors that may explain variations among plants.

Considering energy allocation to individual unit operations separately in a typical processing of pasteurized bottled milk, most of the energy is used in pasteurization step (approximately 23% of electricity and 35% of fuel). In the production of pasteurized bottled milk, bottle washing consumes approximately 58% of the fuel energy and 6% of the electricity used in the process.

In butter production pasteurization consumes approximately 30% of the fuel energy. The churning process and cold storage of butter consume approximately 50% of the electricity required for butter processing.

In yogurt production energy for heating and cooling per kilogram of product averages 1,146 kJ/kg (8). The heating of the base yogurt from 10 to 87.8°C uses 80% of the energy, and the electrical equipment used in product handling and packaging consumes only 6%. The manufacturing process of sour cream is similar to that for yogurt, except the amount of heating is considerably less because the highest product temperature is 22.2°C for sour cream compared to 87.8°C for yogurt. In packaging, the shrink wrap machine is not used for holding the sour cream cartons. The thermal energy ratio is 273 kJ/kg of sour cream and packaging uses less electrical energy primarily because of the lack of the shrink wrap operation. Product handling and packaging uses 49 kJ/kg of sour cream compared to 78 kJ/kg of yogurt. Total energy, thermal plus electrical, is 287 kJ/kg for sour cream compared to 1,224 kJ/kg for yogurt. The above values of energy to manufacture sour cream and yogurt exclude the following: the energy required to pasteurize

16. J. S. Pagington, "Molecular Encapsulation with *B*-Cyclodextrin Food Flavor Ingredients," *Process Packaging* 7(9) 50 (Sept. 1985).
17. G. A. Reineccius and S. J. Risch, "Encapsulation of Artificial Flavors with *B*-Cyclodextrin Food Flavor Ingredients," *Process Packaging* 8(9), 1 (Aug.–Sept. 1986).
18. L. Szente and J. Szejtli, "Stabilization of Flavors by Cyclodextrins," in Ref 1, Chap. 16, pp. 148–157.
19. J. A. Bakan and J. S. Anderson, "Microencapsulation," in L. Lachman, H. A. Lieberman, and J. L. Kanig, eds., *The Theory and Practice of Industrial Pharmacy*, Lea & Febiger, Philadelphia, 1970, p. 384.
20. D. Blenford, "Fully Protected Food Flavor Ingredients," *Process Packaging* 8(8), 43 (July 1986).
21. H. Weiss, president, Balchem Corp., unpublished data.
22. L. E. Werner, "Encapsulated Food Acids," *Cereal Food World* **25**(3), 102 (1980).
23. R. E. Graves, "Sausage Fermentation: New Ways to Control Acidulations of Meat," *National Provisioner* **198**(21), 32–34, 49 (May 1988).
24. J. Bacus, "Fermenting Meat, Part I," *Meat Processing* **24**(2), 26–31.
25. J. Bacus, "Fermenting Meat, Part II," *Meat Processing* **24**(3), 32–35 (Mar. 1986).
26. G. J. Jedlicka, "Chemical Acidulation of Semi-Dry Sausage." *paper presented at American Meat Institute Convention*, New Orleans, 1984
27. U.S. Pat. 3,959,496 (May 25, 1976) S. Jackel and R. V. Diachuk (to Baker Research and Development Co.).
28. C. Andres, "Encapsulated Ingredients," *Food Processing* **38**(12), **44** (Nov. 1977).
29. L. A. Gorton, "Encapsulation Stabilizes Vitamin C in Cupcakes," *Baking Industry* **148**(1805), 20–21 (Jan. 1981).
30. C. J. Pacifico, "A Novel Approach to Vitamin C Nutrition for Fish Rations," *Aquaculture Today* **2**(4), 20–22 (1989).

BOB GRAVES
HERB WEISS
Balchem Corporation
Slate Hill, New York

ENERGY USAGE IN FOOD PROCESSING PLANTS

There is a continuing concern about the energy demand by our food system. It is estimated that 17% of U.S. energy consumption is attributed to the food system (1). This figure includes energy used for production through processing, distribution, out-of-home preparation, and in-home preparation. The food industry requires energy for a variety of equipment such as gas fired ovens; dryers; steam boilers; electrical motors; refrigeration units; and heating, ventilation, and air-conditioning systems.

Natural gas is the predominant source of energy used by the U.S. food industry. In the last few decades about 50% of the gross energy used in food processing was from natural gas; 15%, from fuel oil, 13%, from electricity; and about 22%, from propane, butane, other petroleum products, coal, and some renewable energy sources (2). Within the food industry, the principal types of energy use include direct fuel use, steam, and electricity. Nearly 50% of energy use is in the form of direct fuel use. Almost 30% of energy is used to process steam and 10% to heat water. Almost 67% of electrical energy consumption is for generating mechanical power to operate conveyors, pumps, compressors, and other machinery. Refrigeration equipment consumes about 17% of electricity; lights consume about 10%; and heating, ventilation, and air-conditioning use approximately 4% of electricity.

Between 1973 and 1986, the cost of energy escalated dramatically. This trend promoted the installation of heat recovery equipment to conserve energy (3). It also focused the attention of scientists and engineers on the economic feasibility of alternative energy sources in various food-processing situations like cheese processing (4), food dehydration (5), and water heating (6). Energy consumption patterns of various food industries are briefly discussed.

MILK PROCESSING

Considerable variations in energy requirements for milk processing have been reported for different plants. Fuel requirements for producing pasteurized bottled milk ranges from 0.25 to 2.65 MJ/L of milk (7). The energy used for processing cheese during the regular plant operation is approximately 2.13 kJ/kg. Excluding drying and evaporation, energy required to produce 1 kg of cheese is in the range of 3.37 to 17.53 MJ (4). Some of this variation is due to a lack of concern to energy conservation, but there are also operational factors that may explain variations among plants.

Considering energy allocation to individual unit operations separately in a typical processing of pasteurized bottled milk, most of the energy is used in pasteurization step (approximately 23% of electricity and 35% of fuel). In the production of pasteurized bottled milk, bottle washing consumes approximately 58% of the fuel energy and 6% of the electricity used in the process.

In butter production pasteurization consumes approximately 30% of the fuel energy. The churning process and cold storage of butter consume approximately 50% of the electricity required for butter processing.

In yogurt production energy for heating and cooling per kilogram of product averages 1,146 kJ/kg (8). The heating of the base yogurt from 10 to 87.8°C uses 80% of the energy, and the electrical equipment used in product handling and packaging consumes only 6%. The manufacturing process of sour cream is similar to that for yogurt, except the amount of heating is considerably less because the highest product temperature is 22.2°C for sour cream compared to 87.8°C for yogurt. In packaging, the shrink wrap machine is not used for holding the sour cream cartons. The thermal energy ratio is 273 kJ/kg of sour cream and packaging uses less electrical energy primarily because of the lack of the shrink wrap operation. Product handling and packaging uses 49 kJ/kg of sour cream compared to 78 kJ/kg of yogurt. Total energy, thermal plus electrical, is 287 kJ/kg for sour cream compared to 1,224 kJ/kg for yogurt. The above values of energy to manufacture sour cream and yogurt exclude the following: the energy required to pasteurize the raw milk, the energy required to pump the base material into the processing vat, and the energy required in temporary cold storage prior to shipping. Yogurt in the processing vat is cooled from 87.8 to 42.2°C partially with well water. Measurements of the energy taken from the yogurt by the well water showed an average of 212 kJ/L of yogurt in 4.16 m^3 batch. The cooling could be considered free, except for the cost of the water and pumping energy (8).

Spray drying is the common means of converting fluid feedstocks into solids in the form of powders, granules or agglomerates. It is widely used for dehydration in the manufacture of a wide variety of food, pharmaceutical, and chemical products. Among the foods that have been successfully spray dried are milk, whey, cheese, coffee whitener, eggs, soups, baby foods, and fruits. Energy costs constitute a significant fraction of the operating costs. A number of methods have been suggested to decrease energy related costs during the spray-drying process by using insulation to decrease heat losses to the environment and heat recovery from exhaust gases (8). Typically, in the manufacture of spray-dried milk powder, air heating for the spray-drying operation requires between 50 and 80% of total fuel consumption and approximately 30–35% of total electricity consumption in the whole process. The variation in the primary fuel input results from heat recovery steps.

Cleaning in place (CIP) in milk processing plants is one of the operations that takes significant input of energy. In the manufacturing of Cheddar cheese, CIP consumes approximately 31% of the total fuel energy and 18% of the total electricity required for the whole process. In other processing operations such as milk pasteurization, creamery butter, and acid casein processing, the fuel consumption in CIP operation does not exceed 3% of total fuel used in the whole process, and the use of electricity is up to approximately 1% of total electric energy used for the whole process. In spray-dried milk powder production, CIP operation costs approximately 5–8% of the total fuel consumption and 4–5% of the total electricity used in milk powder processing.

LIQUID FOOD CONCENTRATION

Evaporation of liquid foods is most commonly accomplished using multieffect evaporators. The word *effect* indicates vapor flow in the evaporator. The total evaporation costs depend on the steam consumption, the specific area of the evaporator, and the specific product losses (9). The energy efficiency of an evaporator is usually expressed by thermal or steam economy, which is defined as a ratio of quantity of water evaporated to quantity of steam consumed (10). The steam economy of an evaporator increases with the increase in number of evaporator effect, because subsequent effects use vapor from the previous effect as the heating medium. As an example, a single-effect evaporator takes 1.5 kilogram of steam to evaporate 1 kg of water, whereas a double-effect evaporator only consumes 0.75 kg of steam to evaporate the same amount of water (11).

In conventional multieffect evaporators, those without mechanical or thermal recompression, the steam evaporated from the first effect is used as the heating medium for the next effect and so on. A mechanical vapor recompression evaporator is very much like any other evaporator except that steam is recycled. A compressor is installed, which takes the vapor off an effect and pressurizes it to a higher pressure for use as the heating medium. The major energy expense in such a system is electricity. The operating economy of a mechanical vapor recompression unit is equivalent to an 8–20-effect evaporator. All vaporization is carried out at the same temperature. Steam from the boiler and water are only needed for startup, which accounts for the tremendous savings.

The dairy industry carries out the evaporation of whole milk, skim milk, buttermilk, and whey. The steam economy of two-stage falling film plate evaporator with mechanical vapor recompression, when used to concentrate buttermilk and skim milk to 25% solids, was reported to be 6, which was claimed by the evaporator manufacture (12) to be equivalent to an 8–30-effect conventional evaporator, depending on the cost of fuel. The estimated fuel savings were expected to be enough to amortize the rotary compressor in less than one year (12). A 7-effect mechanical vapor recompression evaporator can remove 17.5 kg of water from the feed for each kilogram of steam supplied to the turbine, which gives the steam economy equal to 17.5 (13) while concentrating whey, skim milk, or whole milk.

Frozen concentrated orange juice (FCOJ) is consumed widely in the United States, European Common Market countries, Japan, Venezuela, and other countries. São Paulo, Brazil, and Florida are the two leading producers of FCOJ. Temperature accelerated short-time evaporator (TASTE) evaporators are used extensively for concentrating orange juice (14).

Energy consumption in a four-effect, seven-stage (the word *stage* indicates the flow of orange juice in the evaporator) TASTE evaporator with a capacity of evaporation of 18.140 kg H_2O/h ranged between 840 and 1,000 kJ/kg H_2O evaporated (15). By introducing one effect and one stage after the fourth stage, the magnitude of energy consumption was reduced to 570–640 kJ/kg of water evaporated. In most orange juice evaporators, the flow of orange juice and the steam pressure are controlled manually to obtain concentrated juice of the desired degree Brix (percent sucrose equals degree Brix). By means of automatic control, the energy consumption in a six-effect, eight-stage evaporator with a capacity to evaporate 9,070 kg H_2O/h was reduced by 6.7%

The data on energy consumption during the 1978–1981 processing season in a concentrated orange juice plant in Brazil were presented for a plant with a capacity to produce 70,000 t/yr of 65° Brix of frozen concentrated orange juice and 86,000 t/yr of citrus pulp pellets made from orange residues. The plant had on-site storage facilities for 17,600 t/yr of frozen orange juice at −8°C. At a separate location away from the plant, 13,650 t/yr of concentrated orange juice were stored at −25°C. For the plant as a whole, electricity was accounted for only 10% of the total energy consumed in the plant. Most of the steam generated was used to concentrate orange juice in evaporators; a small portion (16%) was used in the pelletizing operation of citrus pulp (16).

Evaporators are also used to produce tomato products with varying degrees of concentration. The primary products are classified as purées (solids content from 11% to 22%) and paste (solids content from 28% to 45%). A number of energy audits on tomato product evaporators have been performed (17). Daily average performance data are given for single-, double-, and triple-effect evaporators. Average daily steam economies for the single-effect evaporator averaged 0.84 compared to a theoretical average of 0.95. Two double-effect evaporators had average daily steam economies from 0.79 to 2.03. The average daily steam economy measured was 1.45 while the theoretical average was 1.91. As expected, the triple-effect evaporators showed the best steam economies ranging from 1.66 to 3.06 The lower ranges of steam economy were caused by frequent fouling of the heat exchangers by the burning product. The recent evaporators installed in tomato processing plants have four effects. In the multieffect evaporators the energy cost is directly proportional to the temperature difference across each effect. If the temperature difference is cut in half, twice as many effects can be put in, cutting the energy consumption approximately in half. It will also approximately double the equipment cost (18).

Beets are approximately 15% sugar of which about 88% is extracted giving 132 kg sugar/t of beets. The various sugar beet processors in California provided data that showed that for each ton of beets, 3.19 GJ of primary energy is consumed directly in processing (19). Dried pulp, a by-product of sugar beet processing, accounted for large amounts of energy used. Pulp drying requires 0.73 GJ/ton of sugar beets. This value is nearly 23% of the total direct energy consumed during processing of sugar beets. The evaporators used in the sugar concentrating process consumed nearly 44% of the total direct energy required.

Energy requirements were monitored during the production of a beet colorant on a pilot scale (20). Colorant production involved centrifugation, concentration of ferment, vacuum concentration, and spray drying. The first operation was juice extraction. The beets were blanched, abrasion peeled, and washed. They were then dried and immediately comminuted. The product was pumped to a centrifugal separator for separation. The clarified juice was sent to a plate heat and exchanger pasteurized at 88°C for 2 min, then cooled to 2°C. Extraction of the juice from 1 t of beets used 69.3 ± 0.2 kWh of electricity and 226 ± 13 kg of steam. It took 95 ± 5 min and yielded 550 ± 45 kg of juice at 7% solids. Then the juice was vacuum concentrated to 14% total solids. This used 343 ± 14 kg of steam and 5.1 ± 0.2 kWh of electricity.

The second operation in the production of beet colorant was the fermentation of the clarified beet juice. The temperature was maintained at 30 ± 2°C during the continuous fermentation. The juice extracted from 1 t of beets used 10.2 ± 0.2 kWh of electricity. The fermentation removed about 80% of the solids (20).

In the third operation, the fermented juice was centrifically separated. The clarified juice was vacuum concentrated in a single-effect falling film evaporator to 30% soluble solids, cooled to 16°C, and spray dried. The yeast cell slurry was also spray dried. The vacuum evaporation used 396 + 14 kg of steam and 4.6 ± 0.2 kWh of electricity to concentrate juice from 1 t of beets. Spray drying the juice took 29.6 ± 1.5 m^3 of natural gas and 2.8 + 0.2 kWh of electricity per ton of beets. Spray drying of yeast cells took 26.6 ± 1.5 m^3 of natural gas and 2.5 ± 0.2 kWh of electricity per ton of beets.

FOOD BLANCHING

Blanching is an important unit operation in the pretreatment of fruits and vegetables. It is used prior to freezing, canning, and dehydration. The blanching process is a relatively fast process. Blanching time is a function of piece size, heating medium, and temperature as well as material packing in the blancher. The most common heating media are steam and water. Design of a blancher has a significant influence on the energy used. The open steam blanchers are approximately 10% less efficient than the sealed units (21). Steam losses from the unsealed entrance and exit account for almost 80% of the energy input to the blancher. The steam blancher with end seals is similar in design to the conventional open steam blancher. Water sprays are positioned inside the ends of the blancher to liquify steam that might escape. They also serve to help cool the product at the exit. In such a design the steam losses are up to approximately 51% of the thermal energy input to the blancher. The thermal energy required to blanch spinach using a conventional steam blancher is 2.12 MJ/kg of product (21). Spinach processed in a blancher with hydrostatic seals requires only 0.95 MJ/kg of product (21). In a commercial blancher, at a spinach processing plant, energy used is 6.5 MJ of natural gas and fuel oil and 0.072 MJ of electricity per 1 kg of raw spinach (22).

Energy consumption data for three commercial water blanchers (tubular, screw conveyor, and water tank) indicate that type of product and type of blancher dictate the energy usage (21,23). For lima beans processed in a tubular water blancher, thermal energy usage is 0.543 MJ/kg of processed beans and thermal efficiency is 44.5%, the highest of all three water blanchers tested (23).

The screw conveyor water blancher processed cauliflower cuts. Thermal energy consumption averages 0.91 MJ/kg of product and its thermal efficiency is 31 2%. Energy accounting data on a commercial blancher of a water tank type at the spinach processing plant indicate that 31% of the energy input goes to heating the product and 69% is lost through incomplete condensation of steam, hot water discharge to a drain, and heat losses by convection and radiation (22).

POULTRY PROCESSING

Poultry processing consists of several operations: live bird holding, hanging, slaughtering, scalding, defeathering, eviscerating and chilling, grading, cutup, and packaging. All processes require some energy either as fossil fuel or electricity. Electricity is used for conveyors, refrigeration, lighting, air-conditioning, pumps, and the mechanical drives. Fossil fuels are used for space heating, production of steam or hot water, and feather singeing. The electrical demand for three selected poultry processing plants indicates that the refrigeration and other mechanical drives account for more than 80% of the total calculated electrical use. Lighting requires 6.8% and fans 7.8% of the total electricity. The rest of the total electrical energy is used for heating and air-conditioning of the plants (24). Calculating the electrical use by major area in those three processing plants, the average electrical use in the eviscerating and chilling areas together is 6.7% of the total. This percentage does not include electricity used for ice making and water chilling. Packing and shipping uses 1.6% of the total electricity, which is about 3.8 kWh/1,000 head (total electricity consumption is 237.5 kWh/1,000 head); but this does not include refrigeration for storage areas. Offal and waste handling equipment and waste treatment use 12.7% of the total electricity, and shops and services account for 11.9% of the total electricity use (24).

In another study (25), the electrical energy use per 1,000 broiler equivalents processed was 212 kWh. The cost of ice making, used for a water chiller, and in transportation accounted for approximately 45% of the total electricity consumption. It was noted that 7.2% of the electricity was used in clean-up operations.

FISH PROCESSING

The fresh and frozen packaged fish industry consumes 0.6–0.8% of the energy required by the food-processing industry (26). The fish plant manufactures fillets; dressed, gilled, and puffed fish; and ground fish scrap, which is sold as animal feed. Electrical energy consumption makes up 80% of the plant's total energy requirements (26). Natural gas is used for space heating purposes. Electrical energy is primarily used to operate the refrigeration equipment for the chilling and freezing of whole fish and processed fish products. In addition, it is used to a lesser extent to power motor drives and air-conditioning equipment, to generate hot water for cleaning purposes and to supply lighting. The average energy used ranges between 3.93 and 4.89 MJ/kg of fish product (26).

Ammonia reciprocating compressors are the largest single electricity consumers in the ice production plant. Electrical energy is the main source of energy in that production, representing approximately 85% of its energy requirements. The reported annual energy use in the ice industry was between 581 and 697 kJ/kg in the mid-1970s. This ratio has decreased in recent years due to more energy-efficient equipment and a shift to higher production rates of fragmentary ice and is reported as 380–420 kJ/kg of ice (26).

CANNING

The energy use for canning of tomato products was obtained from the daily amount of tomato received by the plant and the total daily energy consumption (2). Tomato processing consists of several operations. At the receiving station the tomatoes are removed from the gondolas with the use of water. The water containing field dirt of the tomatoes is pumped to a mud-settling tank. The tomatoes are conveyed in a hydraulic flume for additional washing and initial inspection. Water is recirculated in the hydraulic flume. The majority of the electrical energy at the receiving station was consumed by the pump conveying water to the mud-settling tank. The energy use of the receiving operation was 0.32 Wh/kg of raw tomatoes. Using data from six days of processing a thermal energy intensity value of 1,251.4 kJ/kg of tomatoes was calculated. This value represents processing of tomatoes into three products: tomato juice, canned peeled tomatoes, and tomato paste. The electrical energy intensity value is 0.025 kWh/kg of raw tomatoes.

Production of tomato juice involves crushing tomatoes, heating them rapidly to inactivate enzymes, filling juice into cans, and retorting the cans. Most operations consume electricity. Hot-break heaters and retorts use steam. Combining the thermal energy and electrical energy data to a similar base unit of kilojoules, the hot-break heaters and retorts account for almost 97% of the energy consumed in the processing line and the energy intensity of tomato juice production was 1,086.2 kJ/kg of raw tomatoes (2).

Processing of canned, peeled tomatoes requires lye-bath peelers to facilitate peel removal and retorts to sterilize the canned product and conveying equipment. Lyebath peelers and retorts consume steam. These two operations account for almost 99% of the total energy consumed (2). All other equipment operates with electricity. The energy intensity is 1,300.2 kJ/kg of raw tomatoes.

In tomato paste production, several operations such as sorting, pulping, and finishing are similar to tomato juice production. Steam is used in vertical heat exchangers to preheat tomato juice. A large quantity of steam is used in the evaporator. The evaporator and the heat exchanger used in preconcentration account for the majority of steam consumption. The energy intensity of tomato paste production in the cannery industry is 1,307.2 kJ/kg of raw tomatoes (2).

As a consequence of the seasonal vegetable or fruit production, most of the energy consumption is also seasonal. Energy is also consumed during the nonprocessing season, which adds to the energy cost of processed fruits and vegetables. A study of four vegetable canneries located in Western, New York (27) producing apple sauce, canned beans and carrots, indicate that, on the average, blanching and sterilization consume 9% (theoretical estimate); lighting and electric motors, 21%; and movement of vehicles, 4.1%. The rest of the energy is lost in different ways. A significant portion, 22.6%, is lost in steam generation; 21% is lost to the environment after steam generation; and 22.3% is spent on other activities (losses to start up and operation of process equipment prior to actual processing and inefficiencies of process equipment).

Peach canning involves receiving peaches in bins, dumping fruit into a water tank and then elevating it out with a conveyor. The peaches are then graded for size, rinsed with water and pitted. After pit removal, the peach halves are oriented cup down on a belt conveyor and conveyed to a lye-bath peeler. After exposure to caustic solution for a predetermined time, the fruit are again rinsed. The fruit are allowed to orientate cup up; are reinspected, are size-graded, and filled into cans along with syrup. The canned fruit are then heated in retorts to achieve the desired sterilization (2) Within various operations, transport of fruit and waste consume the most electricity. It was determined that 23% of the total electrical energy consumption occurs in dry conveying whereas 38% is consumed in pumping water to convey fruit and waste products (2). Retorting and lye-bath peeling are the most energy-consumptive unit operations, accounting for 89% and 10%, respectively, of total energy consumption. The energy used by the three lye-bath peelers is 135.7 kJ/kg of canned peaches.

There are four energy-intensive operations in citrus packing: degreening–precooling, wash–wax–drying, holding, and storage. Energy analysis of four citrus packing plants in California showed that electricity and natural gas are two major types of energy source (28). Orange and lemon packing differ primarily in the duration of storage required before shipment. Oranges are shipped as soon as they are harvested and packed in a plant. Storage is used mainly to chill and hold the fruit until shipment. This holding time does not exceed a few days. Lemons might be stored from two weeks to six months. Another important distinction between an orange and a lemon packing plant is in the degreening operation. Lemons degreen naturally in storage, oranges are put into a chamber with air containing ethylene at 18–21°C (28).

In the unload–dump unit, field bins of fruit are conveyed from a receiving area to the dump station where the fruit drop into a water tank. Fruit are conveyed in a water flume to the wash–was–dry operation, which consists of four steps: manual sorting and removal of rotten fruit, washing and rinsing, waxing and surface moisture removal by heated air, and manual sorting of fruit for juice processing. The above sequence is followed when water-based waxes are used. In cases when solvent-based waxes are used, the surface moisture is removed both prior to and after waxing.

The total energy intensities for orange and lemon packing vary between 0.64 and 1.03 MJ/kg (28). Electrical energy intensities are about the same in citrus packing plants (0.55–0.82 MJ/kg). Natural gas intensities, however, vary from 0.07 to 0.3 MJ/kg. Another study on consumption of energy in lemon, orange and grapefruit packing plants indicates that total energy intensities for plants have a range of 362.6–702.4 kJ/kg (29). Electrical power intensities vary from 163.3 to 583.3 kJ/kg. Natural gas use intensities for citrus packing vary from 53.8 to 159.8 kJ/kg. The possible reasons for the differences in energy used for packing are different harvest times, the refrigerated holding costs, and storage time.

BAKING INDUSTRY

The largest consumers of energy in a bakery are the ovens, heating boilers, steam generators, and refrigerators. The differences among the energy balances of the bakeries can be large. They are influenced, for example, by the size of the bakery, production structure, amount of production, location, and apparatus.

Based on energy measurement in two bakeries in Sweden, energy consumption of 13.96 MJ/kg of bread for a bakery with a capacity of 250,000 kg of bread per year and 4.88 MJ/kg bread for another bakery with a capacity of 3,500,000 kg of bread per year was reported (30). An investigation conducted in the United States (31) reported an energy consumption for the baking industry of 7.26 MJ/kg bread baked. This is based on measurements on a bakery with a capacity of 35,000 kg of bread per day. In another study, an energy consumption of three bakeries on an average was found to be 6.99 MJ/kg bread baked (32).

The difference in the energy consumption figures may be due to several factors including the size of the bakery. A small baker uses more energy per unit production than a large one. If the bakery has many different products, this will also cause an increase in the energy use. The transportation costs are also important. It was shown (32) that 13–21% of the total energy use in the bakeries was for the delivery vans. Thus the distribution range causes differences in energy use between different bakeries.

A German oven manufacturer reported that the energy use of their oven (a tunnel oven with convective heat transfer) was as low as 0.6 MJ/kg baked bread. This low figure is probably explained by an optimal use of the oven and the convective heat transfer. Much less energy consumption was mostly due to less ventilation.

Considering the energy distribution in bread making, the heating of pans and lids used 26% of the energy. The rest of the energy use lost either through ventilation (31%), exhaust gases (13%), or radiation and convection losses from the walls (30%) (33). These figures show that there are some major energy-conserving opportunities in the baking industry. These steps include minimization of the ventilation of the oven, use of materials with lower heat capacities in the pans and lids and use of heat exchangers to recover heat from the hot exhaust gases.

The British-style crumpet is a small, round bakery product made of unsweetened batter and cooked on a griddle. Traditionally, the product is marketed in the frozen form. In 1982, a modified-atmosphere technique was introduced to package crumpets (34). The aim of modified-atmosphere packaging was to reduce energy consumption without adversely affecting quality during storage and marketing of the product. The production of the crumpets involves mixing, pumping, baking, cooling, packaging, freezing, and storage. Electricity for mixing and pumping of batter and power for running associated motors of the griddle, refrigeration units, and packaging machines together total 92.1 kWh for every 1,000 kg of crumpets produced (35). Thermal energy requirements for process heating (baking) and cooling of 1,000 kg crumpets are 1,050 and 160 MJ, respectively. The results (35) indicate that the freezing process requires 440 MJ, an additional 29% energy to produce and cool the crumpets.

A major advantage of modified-atmosphere packaging over frozen crumpets is that the former requires no special conditions for storage, whereas, for the latter, refrigeration is necessary. Storage of 1,000 kg of crumpets uses 7.4 kWh of electricity and 642.8 MJ of refrigeration for 30 days in storage. In terms of total thermal equivalents, frozen storage adds approximately 43% more to the consumption of process energy for crumpets above that of the modified-atmosphere packaging system.

BIBLIOGRAPHY

1. *Energy Consumption in the Food System* report XIV, 13392-007-001, Federal Energy Administration, Washington, D.C., 1975.
2. R. P. Singh, "Energetics of an Industrial Food System," in R. P. Singh, ed., *Energy in Food Processing*, Vol. 1, Elsevier Science Publishing Co., Inc., New York, 1986.
3. D P. Donhowe, C. H. Amundson, and C. G. Hill, Jr., "Performance of Heat Recovery System for a Spray Dryer," *Journal of Food Process Engineering* **12**, 13–32 (1989).
4. R. K. Singh, D. B. Lund, and F. H. Buelow, "Applications of Solar Energy in Food Processing I Cheese Processing," *Transactions of the American Society of Agricultural Engineers* **26**, 1562–1569 (1983).
5. R. K. Singh, D. B. Lund, and F. H. Buelow, "Application of Solar Energy in Food Processing II: Food Dehydration," *Transactions of the American Society of Agricultural Engineers* **26**, 1569–1574 (1983).
6. R. K. Singh, D. B. Lund, and F. H. Buelow, "Application of Solar Energy in Food Processing IV· Effect of Collector Type and Hot Water Storage Volume on Economic Feasibility," *Transactions of the American Society of Agricultural Engineers* **26**, 1580–1583 (1983).
7. B. Elsy, "Survey of Energy and Water Usage in Liquid Milk Processing," *Milk Industry* **82**(10), 18–23 (1980).
8. G. H. Brusewitz and R. P. Singh, "Energy Accounting and Conservation in the Manufacture of Yogurt and Sour Cream," *Transactions of the American Society of Agricultural Engineers* **24**, 533–536 (1981).
9. S. Bouman, D. W. Brinkman, P. de Jong, and R. Waalewijn, "Multistage Evaporation in the Dairy Industry: Energy Savings, Product Losses and Cleaning," in S. Bruin, ed., *Preconcentration and Drying of Food Materials*, Elsevier Science Publishing Co., Inc., New York, 1988, pp. 51–60.
10. A. H. Zaida, S. C. Sarma, P. D. Grover, and D. R. Heldman, "Milk Concentration by Direct Contact Heat Exchange," *Journal of Food Process Engineering* **9**, 63–79 (1986).
11. D. P. Lubelski, S. L. Clark, and M. R. Okos, "Process Modifications to Reduce Energy Usage," in *Energy Management and Membrane Technology in Food and Dairy Processing, Proceedings from the Special Food Engineering Symposium Held in Conjunction with Food and Dairy Expo 83*, American Society of Agricultural Engineers, St. Joseph, Mich., 1983, pp. 7–13.
12. "MVR Evaporator Provides $60,000 Annual Fuel Cost Savings," *Food Process* **40**(1), 92 (1979).
13. P. Standford, "A Milestone in Evaporation Systems," *Dairy Rec.* **84**(7), 89–90, 92, 94, 96, 98 (1983).
14. C. S. Chen, "Citrus Evaporator Technology," *Transactions of the American Society of Agricultural Engineers* **25**, 1457–1463 (1982).
15. C. S. Chen, R. D Carter, and B. S. Buslig, "Energy Requirements for the TASTE Citrus Juice Evaporator," in *Energy Use Features*, Vol. 4, Pergamon Press, New York, 1979, p. 1841.
16. J. Filho, A. Vitali, C. P. Viegas, and M. A. Rao, "Energy Consumption in a Concentrated Orange Juice Plant," *J. Food Process Eng.* **7**, 77–89 (1984).
17. T. R. Rumsey, T. T. Conant, T. Fortis, E. P. Scott, L. D. Pederson, and W. W. Rose, "Energy Use in Tomato Paste Evaporation," *Journal of Food Process Engineering* **7**, 111–121 (1984).
18. R. W. Cook, "Multiple Effect Evaporation," in R. F. Matthews, ed., *Energy Conservation and Its Relation to Materials Handling in the Food Industry*, Proceedings of the 15th Annual Short Course for the Food Industry, 1975, pp. 78–82.
19. P. K. Avlani, R. P. Singh, and W. J. Chancellor, "Energy Consumption in Sugar Beet Production and Processing in California," *Transactions of the American Society of Agricultural Engineers* **23**, 783–787, 782 (1980).
20. J. E. Block, C. H. Amundson, and J. H. Von Elbe, "Energy Requirements of Beet Colorant Production," *Journal of Food Process Engineering* **5**, 67–75 (1981).
21. E. P. Scott, P. A. Carroad, T. R. Rumsey, J. Horn, J. Buhlert, and W. W. Rose, "Energy Consumption in Steam Blanchers," *Journal of Food Process Engineering* **5**, 77–88 (1981).
22. M. S. Chhinnan, R. P Singh, L. D. Pedersen, P A. Carroad, W. W Rose, and N. L. Jacob, "Analysis of Energy Utilization in Spinach Processing," *Transactions of the American Society of Agricultural Engineers* **23**, 503–507 (1980).
23. T. R. Rumsey, E. P. Scott, and P. A. Carroad, "Energy Consumption in Water Blanching," *Journal of Food Science* **47**, 295–298 (1981).
24. W. K. Whitehead and W. L. Shupe, "Energy Requirements for Processing Poultry," *Transactions of the American Society of Agricultural Engineers* **22**, 889–893 (1979).
25. L. E. Carr, "Identifying Broiler Processing Plant High Electrical Use Area," *Transactions of the American Society of Agricultural Engineers* **24**, 1054–1057 (1981).
26. L. G. Enriquez, G. J. Flick, and W. H. Mashburn, "An Energy Use Analysis of a Fresh and Frozen Fish Processing Company," *Journal of Food Process Engineering* **8**, 213–230 (1986).
27. W. Vergara, M. A. Rao and W K. Jordan, "Analysis of Direct Energy Usage in Vegetable Canneries," *Transactions of the American Society of Agricultural Engineers* **21**, 1246–1249 (1978)
28. M. Naughton, R. P. Singh, P. Hardt, and T. R. Rumsey, "Energy Use in Citrus Packing Plants," *Transactions of the American Society of Agricultural Engineers* **22**, 188–192 (1979).
29. L. P. Mayou and R. P. Singh, "Energy Use Profiles in Citrus Packing Plants in California," *Transactions of the American Society of Agricultural Engineers* **23**, 234–236, 241 (1980).
30. C. Trägårdh and co-workers, "Energy Relations in Some Swedish Food Industries," in P. Linko and co-workers, eds., *Food Process Engineering*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1980.
31. L. A. Johnson and W. J. Hoover, "Energy Use in Baking Bread," *Bakers Digest* **51**, 58 (1977).
32. G. A. Beech, "Energy Use in Bread Baking," *Journal of the Science of Food and Agriculture* **31**, 289 (1980).
33. A. Christensen, and R. P. Singh, "Energy Consumption in the Baking Industry," in B. M. McKenna, ed., *Engineering and Food*, Vol. 2, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1983, pp. 965–973
34. B. Ooraikul, "Gas-packaging for a Bakery Product," *Canadian Institute of Food Science Technology Journal* **15**, 313 (1982)
35. N. Y. Aboagye, B. Ooraikul, R. Lawrence, and E. D. Jackson, "Energy Costs in Modified Atmosphere Packaging and Freezing Processes as Applied to a Baked Product," in M. Le Maguer and P. Jelen, eds., *Food Engineering and Process Applications*, Vol. 2, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1986, pp. 417–425.

S. CENKOWSKI
D. S. JAYAS
University of Manitoba
Winnipeg, Manitoba
Canada

ENTERAL FORMULAS AND FEEDING SYSTEMS

Using a feeding tube to deliver nutrients directly into the stomach or upper gastrointestinal (GI) tract can be a lifeline for people who are unable to consume an adequate oral diet. Nutrient administration by vein (parenteral nutrition) is another option. Recent research results showing the superior physiologic benefits of enteral feeding over parenteral and favorable third party reimbursement have encouraged clinicians to use enteral tube feeding whenever possible, reserving parenteral nutrition for those with totally dysfunctional GI tracts or in whom enteral feeding tubes cannot be placed (1). Further, the availability of specially designed enteral feeding tubes that can be placed at the bedside, during surgery, or percutaneously using radiologic, endoscopic, or laparoscopic techniques has simplified feeding tube placement and management. These advances plus the availability of a wide variety of standard and condition-specific enteral formulas have made it possible to enterally feed many patients who previously would not have been considered candidates for enteral nutrition support (Table 1).

Table 1. Some Conditions When Tube Feeding Can Be Used

Dysphagia
Upper GI tract obstruction
Malabsorption syndromes
GI disease
Critical illness or injury
Pancreatitis
Short bowel syndrome
Glucose intolerance
Renal dysfunction
Cancer
Pulmonary dysfunction

ENTERAL FORMULAS

Enteral formulas can be categorized as standard or condition-specific, based on their nutrient composition. Generally, the caloric distribution of standard enteral formulas are consistent with recommendations for a healthy diet for the general population (2). Carbohydrate contributes 50 to 60% of calories, protein contributes 12 to 20% and fat about 20 to 30% of calories. Standard enteral formulas are designed to also provide 100% of recommended intakes (RDI) of vitamins and minerals in 1 to 2 liters. They are comprised of intact nutrients similar to those occurring in a usual oral diet (Table 2) that require digestion and absorption just as solid food would. Standard enteral formulas are also called polymeric formulas to indicate that they contain long-chained macronutrients that require digestion prior to absorption. The most widely used standard enteral formulas also contain fiber.

Table 2. Common Components of Enteral Solutions

Carbohydrates	Proteins	Lipids
Maltodextrin	Casein	Corn oil
Corn syrup	Whey	Canola oil
Sucrose	Soy protein isolate	Safflower oil
Fructose	Egg white solids	Soybean oil
Cereal solids	Skim milk	Marine oil
Fruit/vegetable purees	Pea protein	Fractionated coconut oil
		Butterfat

Initially fiber was added to help moderate bowel function in chronically tube-fed patients. Dietary fiber increases stool bulk as a result of the water-holding capacity of the fiber, the osmotic effect of the short-chain fatty acids produced by bacterial fermentation, and the bacterial mass itself (3). Dietary fiber also moderates transit time; that is, the time taken by undigested particles to move through the GI tract. An increase in dietary fiber hastens transit time in people with slow transit and can prolong it in people with too rapid a transit rate (3). Because of this moderating effect, adding fiber to the diet can be effective for both diarrhea and constipation. To supply fiber in enteral products, soy polysaccharide was found to be the most compatible with liquids and well tolerated by patients.

In 1987, the Federation of American Societies of Experimental Biology prepared a review of the known physiological effects of fiber (4). Based on their findings, they recommended an intake of dietary fiber of 10 to 13 g/Cal comprised of 70 to 75% insoluble fiber and 25 to 30% soluble fiber. This was felt to best represent the types of fiber that would be found in a healthy diet containing a variety of foods. Within the past several years, new fibers have become available for use in enteral nutrition products. A recent advance in enteral formulations then has been the addition of fiber blends that provide both insoluble and soluble fibers at these recommended levels. These fiber blends may include soy fiber, oat fiber, carboxymethylcellulose, hydrolyzed guar gum, gum arabic, or pectin. Some indigestible oligosaccharides, such as the fructooligosaccharides, although not technically classified as a fiber, have recently been added to enteral products because they are completely soluble, physically compatible with the product components, and provide fiberlike benefits (5).

People being fed standard enteral formulas must have adequate GI function to process nutrients and fiber. People who have malabsorption syndromes or other medical conditions (eg, short bowel syndrome, pancreatitis, inflammatory bowel disease) that compromise the gut's digestive capability may benefit from use of partially hydrolyzed or elemental formulas. The caloric distribution of these formulas is similar to that of standard enteral formulas. The nutrient sources, however, are partially hydrolyzed, rendering them smaller and easier to digest. For example, amino acids and/or peptides may be used instead of intact proteins, glucose polymers instead of complex carbohydrates. Medium chain triglycerides, fatty acids that contain 8- or 10-carbon fatty acids, often comprise at least part of the lipid component of hydrolyzed formulas. Because they are well absorbed and transported directly into the portal blood, they bypass the complex steps required for digestion and absorption of long-chain triglycerides, thus overcoming problems associated with inability to absorb long-chain fatty acids (6).

Standard formulas are available in caloric densities ranging from 1.0 to 2.0 kcal/mL. Caloric requirements or medical condition dictates the caloric concentration of the formula to be used. For example, a relatively stable adult could be provided with an adequate daily intake of protein, essential fatty acids, minerals, electrolytes, vitamins, and water in about 2,000 mL of a 1 kcal/mL product. Contrast this with an elderly patient with congestive heart failure who has a much lower daily calorie requirement and needs fluid restriction, but still must have a full complement of protein, vitamins, and minerals. In this case, a more concentrated formula would be desirable.

Generally the amount of water contained in a formula is inversely related to its caloric density. Most 1.0 kcal/mL formulas are approximately 80% water; 2.0 kcal/mL formulas may contain as little as 50% water. Ordinarily, most people need about 1 mL water/kcal fed (7) or a minimum of 1,500 mL of water unless medically contraindicated for people who need fluid restrictions (8). In most cases, all tube-fed patients need to have water in addition to formula.

Standard enteral formulas meet the metabolic needs of most patients. There are some conditions, however, when special formulations are needed. Some of these special formulations, called condition- or disease-specific formulas, are discussed in the following section.

CONDITION-SPECIFIC NUTRITIONALS

Hypermetabolic Stress or Trauma

Severe injury causes abrupt changes in metabolism. Critically ill patients experience a metabolic response to injury that is divided into what is known as an ebb phase and a flow phase (9). During the ebb phase, blood pressure, cardiac output, body temperature, and oxygen consumption are reduced, usually in conjunction with hemorrhage. This depression in metabolic rate may be a protective mechanism during this period of hemodynamic instability.

As blood volume stabilizes, the ebb phase is replaced by the flow phase. The acute response is a hypermetabolic state characterized by increased cardiac output and urinary nitrogen losses, altered glucose metabolism, and accelerated catabolism. Levels of the catabolic stress hormones, glucocorticoids (10,11), glucagon (12–14), and catecholamines (15–16) are increased. These hormonal conditions favor the breakdown of muscle tissue to provide amino acids for gluconeogenesis and hepatic protein synthesis. The physiologic consequence is a rapid loss of nitrogen and lean body tissue after severe injury. The levels of catabolic hormones decrease gradually during the adaptive flow phase. Recovery continues throughout this response, which is associated with the predominance of anabolic processes such as wound healing.

Historically, parenteral nutrition predominated in the care of critically ill patients because of the routine availability of central venous access and standardized techniques. Health care professionals preferred parenteral nutrition because it bypassed the GI tract, which they assumed to be totally dysfunctional in the critically ill. However, evidence indicates that enteral nutrition support confers benefits not seen with parenteral nutrition such as reduced incidence of infection and maintenance of gut structure and function. As a result, clinicians are increasingly advocating tube feeding for critically ill patients, whenever possible.

Recent nutrition research has focused on the clinical benefits of enterally feeding hypermetabolic patients. Several trials have reported significant decreases in infections and/or wound healing complications (17–20), hospital length of stay (17,20,21), and multiple organ failure (19) in patients fed specialized enteral formulas compared with patients fed standard enteral formulas. These specialized enteral formulas contain ingredients shown to enhance the immune response, improve wound healing, reduce free radical formation, and alter the inflammatory response. Included are the amino acids arginine (22) and glutamine (23) as conditionally essential nutrients during trauma, omega-3 fatty acids (24), and antioxidants (25–27).

Pulmonary Disease

Patients with chronic obstructive pulmonary disease (COPD) develop various degrees of respiratory muscle fatigue, hypoventilation, carbon dioxide retention (hypercapnia), and oxygen depletion (hypoxemia). In the majority of patients who experience difficulty in breathing, the obstruction of airflow is due to chronic bronchitis or emphysema (28). Typically, these patients experience marked weight loss, which may result both from decreased caloric intake (29,30) and from increased energy expenditure, presumably because of increased work of breathing (29). Because the oxidation of fat results in less carbon dioxide production than the oxidation of a equal caloric load of carbohydrate, formulas high in fat and low in carbohydrate have been developed for pulmonary patients. Such formulas have been shown to benefit patients with COPD, leading to an increase in general activity (31).

Acute respiratory distress syndrome (ARDS) is an often fatal form of acute lung injury that results from direct (eg, drowning) or indirect (eg, sepsis, trauma, pneumonia) injury to the lungs. Patients with ARDS have distinctive nutritional requirements due to their hypermetabolic status and presence of pulmonary inflammation. ARDS patients typically have elevated energy needs due to trauma and/or sepsis (32). However, overfeeding must be avoided as it may contribute to respiratory failure by increasing minute ventilation and dead space ventilation (33). On the other hand, inadequate nutrition can actually prolong the need for use of a ventilator if the respiratory muscle mass is broken down to provide energy and protein substrates for the body (34,35). This further contributes to increased complication rates, mortality, and prolonged length of hospital stay among patients on mechanical ventilation (34).

To address the nutritional needs of these patients, a formula was designed containing ingredients that could help control or reduce lung damage from inflammation and oxidation. Preclinical studies have shown that high-fat, low-carbohydrate diets containing omega-3 fatty acids from fish oil and γ-linolenic acid from borage oil rapidly modulate the fatty acid composition of inflammatory cell membranes (36–38), reduce the synthesis of important proinflammatory mediators of lung injury (36,39,40), attenuate lung permeability (36,40) and improve cardiopulmonary hemodynamics and respiratory gas exchange in models of ARDS (36,39). In a multicenter clinical trial, patients receiving this formula experienced a significant improvement in gas exchange and clinical outcome (41).

Cancer

Cachexia or wasting as a result of altered metabolism or decreased intake is a hallmark of cancer. The presence of a malignant tumor can cause a number of physiologic changes that can affect nutritional status. Some tumors cause an increase in basal metabolic rate (42), and changes in glucose (43) and protein (44) metabolism have been reported. Fluid and electrolyte imbalances (45), malabsorption (45), and anorexia (46) are other reported problems. There also can be serious nutritional consequences of cancer therapy: surgery, chemotherapy, or radiation therapy can cause nausea and vomiting, which can sometimes be treated with antiemetic drugs (45,47–49). Creative approaches are needed to maintain food intake, such as offering small high-calorie meals to anorexics or pureed foods to patients with painful swallowing (50,51). If oral intake is not satisfactory, tube feeding may be necessary; patients with obstructions of the nasopharynx or the esophagus can be fed in different sites (see "Delivery Systems").

The maintenance of adequate dietary intake in cancer patients does have benefits. Oral supplementation and tube feeding can help prevent weight loss during therapy (52,53), which is strongly related to the positive outcome of therapy. Aggressive tube feeding during chemotherapy can improve hematopoietic status (54), which may be related to more active chemotactic and phagocytic function (55). Peptide elemental diets have been shown to provide protection against radiation damage to the intestinal mucosa (56).

Diabetes

Although controversy continues to exist regarding the optimal diet for individuals with diabetes, most health care diabetes specialists use the nutrition recommendations issued by the American Diabetes Association. These latest guidelines (57) specify that protein supply 10 to 20% of total caloric intake, saturated fatty acids less than 10% of total calories, and polyunsaturated fatty acids less than 10% of total calories. Thus, 60 to 70% of total calories remain to be divided between carbohydrate (CHO) and monounsaturated fatty acid (MUFA) intake. The distribution is individualized based on nutritional assessment and treatment goals. Nevertheless, individuals who are at a healthy weight and have normal lipid levels are encouraged to use the nutrition recommendations of the National Cholesterol Education Program, in which individuals over 2 years of age are advised to limit fat intake to ≤30% of total calories (58,59). The rationale for these recommendations is the desire to lower saturated fat and cholesterol intake to reduce cardiovascular risk (60), and this is often difficult to achieve in a "typical" diet without lowering total fat intake. Most standard enteral formulas now available comply with these recommendations. However, tube feeding or dietary supplementation with standard liquid medical nutritional products can compromise the metabolic control of patients with abnormal glucose tolerance. This occurs because rapid gastric emptying of liquid formulas and efficient absorption of nutrients supplied by these products result in a rapid elevation of blood glucose. Standard enteral formulas empty from the stomach at least twice as fast as an isocaloric solid-food meal (61) and have been shown to produce a peak blood glucose response equivalent to that seen when an equivalent solution of pure glucose is fed (62). Rapid gastric emptying of formulas and rapid absorption of nutrients can complicate metabolic control of hyperglycemic patients. A number of clinical studies have shown that high-carbohydrate, low-fat diets may be inappropriate for many patients with diabetes (63–65). Moreover, recent research has demonstrated that substitution of MUFA for CHO can significantly improve glycemic control, lower serum triglycerides, and maintain or increase high density lipoprotein (HDL)-cholesterol, and raise the HDL-cholesterol to low density lipoprotein (LDL)-cholesterol ratio (66–68). Therefore, carbohydrate-reduced, high-monounsaturated fat formula seems a reasonable choice for patients with abnormal glucose tolerance.

Renal Disease

Once an initial injury to the kidneys causes chronic renal failure, renal disease typically progresses toward end-stage renal disease until dialysis or kidney transplantation are required, even if the cause of the initial damage is mitigated. Accordingly, nutritional management of people with renal disease depends on the level of residual renal function. The goals of nutritional management are to optimize nutritional status, minimize the burden of the diet on the person with renal failure, and to the extent possible, modify the nutrient profile to compensate for the nutritional and metabolic abnormalities associated with the loss of renal function (69). For persons with residual renal function, there is an additional goal of delaying progression of renal disease (69).

One of many features of chronic renal failure is a gradual increase in toxic nitrogenous wastes, the by-products of protein and amino acid metabolism. Protein restriction is advocated to optimize nutritional status (69) and help compensate for the metabolic effects of the loss of renal function (70–72). Although somewhat controversial, a moderate protein restriction also may limit the rate of disease progression (73), though nutritional status must be monitored closely to prevent undernutrition (74). Typical recommendations for protein intake are 0.7 to 0.8 g protein/kg/d for normally nourished people with chronic renal failure (69). Protein requirements are elevated in persons with end-stage renal disease on either hemodialysis or peritoneal dialysis due to increased nitrogen loss during dialysis. Typical recommendations are 1.0 to 1.5 g protein/kg/d for stable persons receiving dialytic therapy (69,75,76).

Energy requirements of people with chronic or end-stage renal disease are comparable to those of healthy adults, approximately 35 kcal/kg/d (76–78). Inadequate energy intake is one of the primary causes of wasting in this population (77). In addition to protein and energy, loss of renal function alters metabolism and excretion of many other nutrients. Recommendations for dietary fiber and fat intakes correspond to those for normal populations (69). Fluid and electrolyte restrictions typically are required for people treated with hemodialysis and are sometimes necessary during peritoneal dialysis (69). With the loss of renal function, phosphate excretion is impaired, necessitating both dietary phosphate restriction and administration of phosphate binders, typically in the form of calcium salts. Enteral products used in end-stage renal disease should contain nutrient levels tailored to meet the distinctive requirements of this population (79). Supplemental levels of vitamins A, C, and D typically are limited to RDI levels due to abnormalities in their metabolism (hypervitaminosis A (69,80–83), oxalosis (84–87), and loss of the ability to activate vitamin D (88), respectively). In contrast, supranormal levels of folic acid (89–91) and pyridoxine (92–94) are required. Metabolism of taurine (95,96), carnitine (97–99), selenium (100–102), and magnesium (69) is altered by loss of renal function. Deficiencies of the first three may develop, and supplements containing levels found in typical diets may be used. In contrast, hypermagnesemia is common and recommended intakes are approximately half of the RDI (69). Clinical research demonstrates that people on dialysis tolerate sole-source enteral nutrition using disease-tailored products and that these products may offer some biochemical advantages over standard nutritionals in the management of this population (79).

Pediatrics

Until 1988, the only enteral products available for feeding to children were infant formulas and adult nutritional products. Neither were seen as nutritionally ideal for this population. Infant formulas were too dilute for older children. Products for adults were perceived as too concentrated in some nutrients (eg, protein, sodium) and too low in others when the concentrations of protein and sodium were diluted to acceptable levels. This stimulated the development of products specifically for children aged 1 to 10 years, designed to provide complete, balanced nutrition for the support of both growth and tissue maintenance as well as the increased nutrient demands during acute and chronic illness.

Most of the products on the market have been designed for both oral and tube feeding. They have a caloric density of 1.0 Kcal/mL. The caloric distribution for pediatric products are typically 10 to 12% of calories from protein, 25 to 44% from fat, and 44 to 63% from carbohydrate. Like adult formulas, these formulas are generally lactose-free and gluten-free. They consist of intact milk protein sources, blends of vegetable oils, hydrolyzed starches, and sucrose, and some have added fiber. All have been formulated to meet the nutrient standards set forth by the National Academy of Sciences–National Research Council Recommended Dietary Allowances (RDAs) (7). These RDAs differ from the RDIs used for adult nutritionals in that they are more age-specific. In most cases, certain nutrients have been increased above these standards to account for increased needs during stress and illness. Recently, elemental formulas with hydrolyzed proteins or free amino acids have been developed for children who have severe food allergies or conditions resulting in maldigestion or malabsorption.

For most cases of failure to thrive, poor appetite, or when weight gain is desired, oral supplementation is usually sufficient. Conditions that require tube feeding in children are similar to those that necessitate tube feeding in adults (eg, neurologic disease, burns, severe infections, anorexia from disease, cancer, esophageal injury or obstruction, trauma, and surgery). Guidelines for attending to the nutritional needs of these children are available (103,104). The feedings may be for days, weeks, or years, depending on the nature and severity of the condition. When a child cannot receive adequate nutrition by tube or has impaired GI function, parenteral nutrition (nutrition infused by the vein) is required.

DELIVERY SYSTEMS

Nasoenteric tube feeding dates back as far as the fifteenth century; modern use began in the early 1950s with the development of fine polyethylene tubes and feeding pumps (105). In the 1960s, the development of total parenteral nutrition (TPN), which was viewed as more sophisticated and problem free, contributed to reduced use of enteral tube feeding in the 1970s and early 1980s. However, tube feeding is now resurging because of a number of factors. Enteral nutrition is less expensive than TPN (106) and results in fewer serious complications (107). Research shows that enteral feeding maintains gut mass, integrity, and function better than parenteral nutrition (1). The use of small-bore tubes made of soft biocompatible materials has led to increased patient tolerance of nasally placed tubes.

Most feeding tubes are now made of polyurethane or silicone, which does not disintegrate or become brittle over time and therefore does not need frequent replacement. Feeding tubes are mostly easily inserted into the stomach by way of the nasopharaynx. If the patient is at high risk for aspiration, the tube can be passed further down into the duodenum or jejunum, although it is sometimes difficult to get the tube through the pylorus (105). If the transversal route is not possible or long-term feeding is desired (more than six weeks) tubes can be inserted directly into the esophagus or into the stomach or duodenum through the abdominal wall. (Tube placements are depicted in Figure 1.) Cervical pharyngostomies, or more rarely, esophagostomies are performed when the access site has been obtained during surgery for head and neck cancer or surgical repair of the maxillofacial area (108). Feeding gastrostomies can be placed surgically or percutaneously (109); the advantage of percutaneous endoscope placement is that it can be done as an outpatient procedure by gastroenterologist with local anesthesia. Feeding jejunostomies are usually performed in conjunction with other intestinal surgery, facilitating early nutrition support. The diameter of feeding tubes is described in French (F) units (F unit = 0.33 mm). Large-diameter tubes (≥16 F) tend to cause

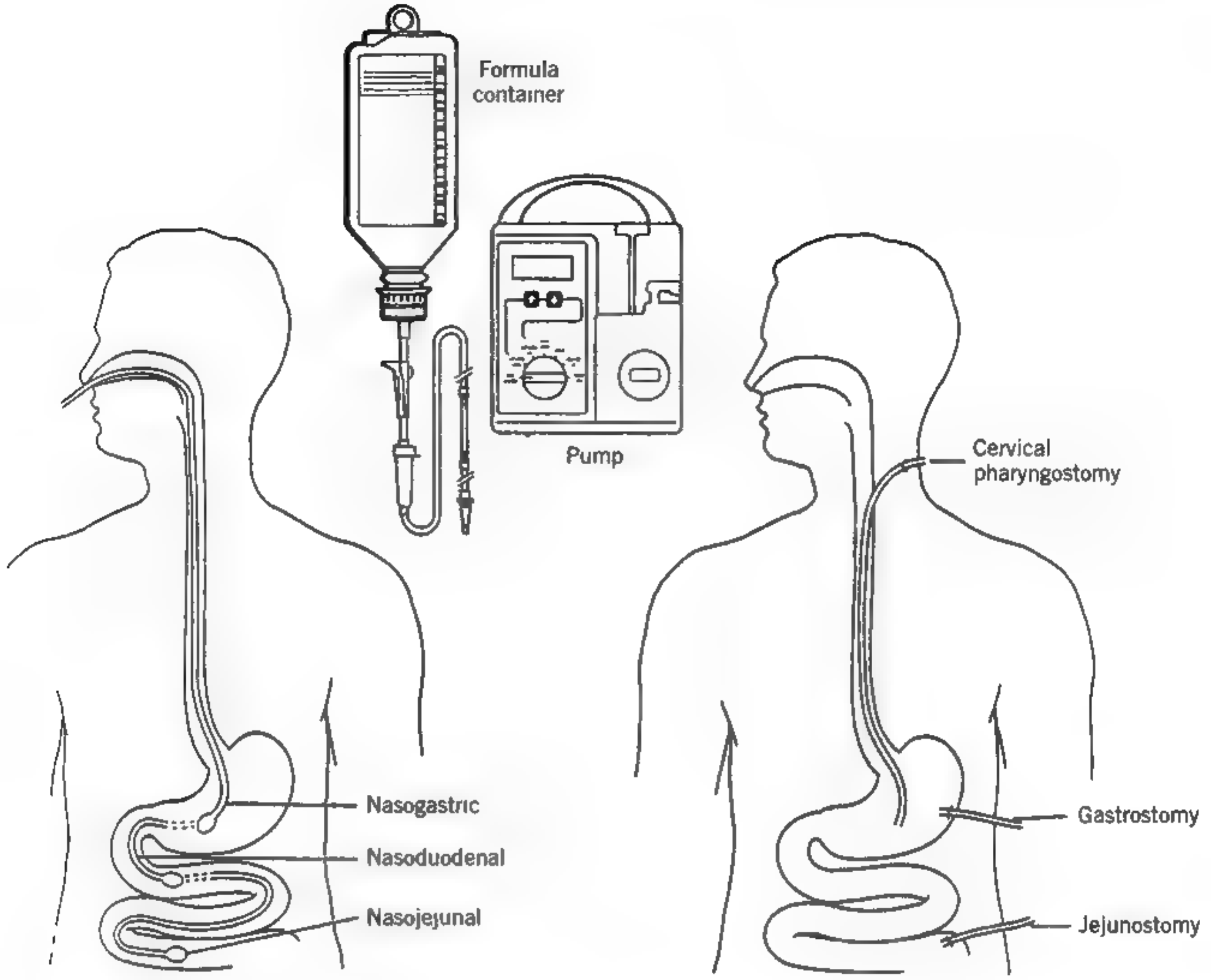


Figure 1. Tube feeding sites and devices. *Source:* Courtesy of Applied Therapeutics, Inc , Vancouver, Washington.

pressure necrosis in the nose as well as inflammation of throat and esophagus. They may compromise the function of the esophageal sphincter, increasing the potential for pulmonary aspiration. Large tubes may also interfere with swallowing, discouraging oral consumption of liquid supplements. Most adults tolerate nasal tubes ranging between 8 and 12 F. High viscosity formulas or those supplemented with fiber require at least a 10 F tube unless a feeding pump is being used. Extremely small caliber tubes (<6 F) require very low viscosity formulas such as elemental formulas.

Once the tube is in place, formula is usually administered at a slow rate and increased in increments depending on the condition of the patient until the patient's needs are met. Formula can be poured into 500 or 1,000 mL rigid containers or bags. To provide the safest environment for tube-fed patients, formulas are available in commercially prefilled containers that decrease the risk of bacterial contamination from handling and reduce staff time. The formula flows from the container through a feeding set, by gravity or by a pump (a small portable pump is shown in Figure 1), into the feeding tube (110). Formula can be delivered by a continuous or intermittent administration schedule. A continuous schedule is preferred when tube feeding is initiated, when patients have not been fed for several days, when patients are critically ill, and when duodenal or jejunal feeding sites are used (105,107). Intermittent feedings given five to eight times a day can simulate a meal pattern. Ambulatory tube-fed patients frequently use an intermittent or 16 to 18 h continuous feeding schedule because it permits more freedom of movement than does 24-h continuous feeding. Intermittent feeding may also be preferable in patients with diabetes because it simplifies insulin dosing. Most often delivery is controlled by means of an enteral feeding pump thus ensuring an even and controlled flow of formula, but it is also possible to infuse by gravity or with the use of a syringe (bolus feeding).

Enteral feeding pumps have been improved greatly since first being introduced. Enteral feeding pumps work via one of two mechanisms; rotary peristaltic or cassette technology. The rotary peristaltic pump works by looping the tubing of the administration set around the pumping mechanism and pressure against the tubing forces the formula out in a constant sequence of motions. The delivery is regulated by a drop-counting sensor. The cassette pump has a cassette that holds a specific volume of fluid that works with a pumping mechanism to discharge precise volumes at a specified rate. The cassette fits easily into place and eliminates the need to loop tubing for proper pump operation. Pumps have very sophisticated features and alarms. There are pumps that automatically flush the feeding tube at set intervals; this has been proven to reduce tube clogging. Safety features and alarms vary by pump manufacturer; however, basic safety features and alarms include memory, free-flow alarm, no flow alarm, and low battery alarm. Additional features may include select rate alarm, select run alarm, system self-check, lockout, and hold.

Enteral feeding pumps are used to control the rate of feeding delivery and provide increased safety in providing enteral nutrition support. Controlling the rate of formula delivery is imperative in the following patient care situations: (*1*) when feeding into the small bowel, (*2*) when feeding patients who are fluid restricted (eg, cardiac, renal), (*3*) when feeding patients intragastrically who have a history of gastroesophageal reflux or aspiration, or (*4*) when feeding patients who may have delayed gastric emptying secondary to their underlying disease (eg, diabetes). Enteral feeding pumps can have very positive outcomes as well as lower overall cost of patient care.

PROCESSING TECHNOLOGY

Enteral nutrition can be a patient's sole source of nutrition for extended periods. Therefore, high standards of care in the manufacturing process are necessary to assure consistent nutrient content and product sterility. Enteral nutrition manufacturers maintain rigid specifications for ingredient quality, processing conditions, and package integrity. The combination of specifications and documentation comprise a thorough quality assurance program to demonstrate the batch-to-batch consistency of the finished product. The specific processing parameters and quality assurance programs used in the manufacture of enteral formulas are proprietary, but are described in general terms in this section.

Ingredients

The ingredients for enteral nutritional products are selected following examination of attributes such as nutritional quality, safety for use, functional properties (eg, viscosity), and processing ease (eg, dispersibility in water). Following receipt of ingredients at the manufacturing facility, a rigid testing, storage, and handling program are used to assure ingredient quality. The testing program includes identity, microbiological and infestation testing, organoleptic testing, and nutritional potency. Storage conditions are carefully monitored to assure that extremes of temperature and moisture do not damage the ingredients. Water quality in enteral nutritional processing is assured through use of treated potable water supplies and water quality monitoring programs.

Ingredients are added during the manufacturing process at various points dictated by factors such as manufacturing convenience and assurance of distribution of the ingredient into the product. For example, it is most convenient to add carbohydrate sources (eg, maltodextrins) to the water phase to assure dispersion. Incorporation of ingredients into the product varies from use of direct addition to use of pumps such as triblenders.

Liquids

The ingredients in the oil and water phases are blended together in a concentrated mixture, heated, and the pH adjusted to stabilize the protein (Fig. 2). This mixture is subjected to a combination of processes which are similar to typical dairy processes: homogenization, pasteurization, UHTST (ultrahigh-temperature short-time sterilization), HTST (high temperature short time sterilization), deaeration, and clarification. Heat-labile nutrients and flavors are added after these processing steps. Samples are taken for measurement of pH and total solids at this point, and the mix is adjusted to final total solids and pH.

Shelf-stable, liquid enteral products are commercially sterilized using either retort or aseptic processes. In the retort process, the final product is filled in either metal, glass, or plastic containers and sealed, normally under a slight vacuum. The filled containers are subjected to rapid heating with superheated steam. In most cases, the containers are rotated or shaken to assure even distribution of heat throughout the product. In the aseptic filling process, the product undergoes a UHTST treatment just prior to filling. The containers and lids are independently treated with a sterilant (eg, hydrogen peroxide) to assure sterility. The product is filled in the package in a sterile filling zone in the filler. Aseptic packages include plastic, glass, aluminum, steel, and fiberboard containers.

In the case of both retort and aseptic sterilization processes, the manufacturer must establish that the product and package receive treatment that is adequate to reduce pathogenic microorganisms in the finished product to a nonviable level for the shelf life of the product. Typically, the manufacturer subjects the product and package to testing protocols to assure sterilization processes are adequate. Before commercial release, the product is incubated at typical storage temperatures to assure that no microbial contamination occurs postprocess.

Powders

Powdered products are sometimes the preferred form for certain products or specific settings. For a given volume of final reconstituted liquid product, powders have less bulk and weigh less than processed liquid products and, therefore, are easier and less expensive to store and transport. Powders generally have a longer shelf life than liquid forms. Some products are available only as powders because certain components cannot be exposed to thermal processing or are not stable for long periods in aqueous solution.

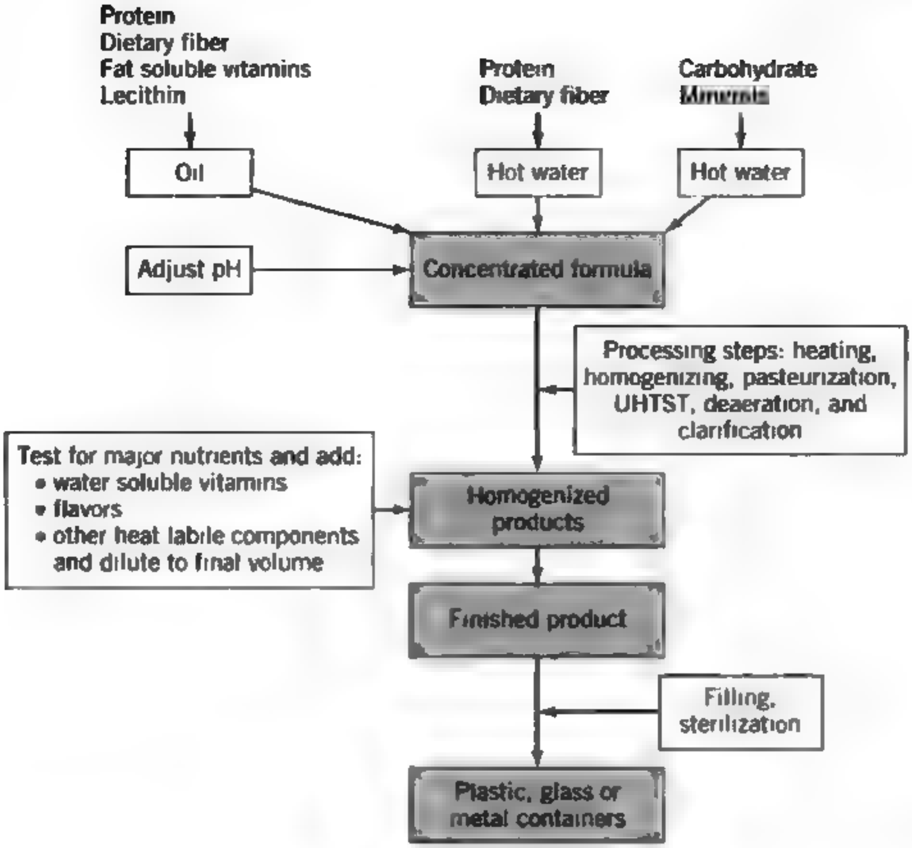

Protein
Dietary fiber
Fat soluble vitamins
Lecithin
Protein
Dietary fiber
Carbohydrate
Minerals
Oil
Hot water
Hot water
Adjust pH
Concentrated formula
Processing steps: heating, homogenizing, pasteurization, UHTST, deaeration, and clarification
Test for major nutrients and add:
• water soluble vitamins
• flavors
• other heat labile components and dilute to final volume
Homogenized products
Finished product
Filling, sterilization
Plastic, glass or metal containers

Figure 2. Formulation and processing of a liquid enteral formula.

Two methods of manufacturing are commonly used for powdered enteral products: spray-drying and dry-blending, either separately or in combination. Spray-dried product is UHTST or HTST processed as a concentrated liquid prior to the drying step. In the drying step over 95% of the water is removed to bring the powder moisture content to a level that does not promote microbiological growth. Dry-blending is a process where dry ingredients are mixed in a chamber by rotating ribbons or plows. Dry-blending is desirable when there is heat- or moisture-sensitive ingredients in the product formulation. Occasionally, base powders are spray-dried, followed by dry-blending of sensitive ingredients.

The process of spray-drying or dry-blending can produce a powder that does not disperse easily in liquid. The process of agglomeration is used to improve powder dispersability in liquid by binding small, dense particles together to provide a larger surface area to "wet."

Packaging

Enteral products are packaged in glass, metal (steel and aluminum), fiberboard, and plastic packages. Packaging materials are selected for ease and cost of manufacturing, convenience for the customer, and ability to protect the product from environmental conditions such as light, moisture, and oxygen. Glass and metal containers offer excellent protection from moisture or oxygen. Plastic containers are normally multilayer with one or more of the layers possessing properties that act as a barrier to oxygen, moisture, or light. The product label, outer carton, and shipping containers are also designed to provide protection to the product and help increase the package's durability. Packages are subjected to physical tests to assure that the rigors of shipping and handling do not adversely affect the integrity of the package's hermetic seal.

Product Shelf Life

A minimum of a one year room temperature storage shelf life for enteral nutrition products is normally the requirement of institutional health care providers for reasons of convenience and storage. Assurance of the product's nutritional and physical quality and the package's durability under normal storage conditions is accomplished by shelf life studies prior to product introduction. Ongoing product monitoring programs provide assurance that the product and package meet specifications throughout the product's life cycle.

REGULATORY ACTIVITIES

Enteral diets that are formulated simply as adult meal replacements are considered to be foods by the U.S. FDA and are not regulated any differently than standard foods. Webster's Dictionary defines food as "material consisting essentially of protein, carbohydrate, and fat used in the body of an organism to sustain growth, repair, and vital processes, and to furnish energy; also such food together with supplementary substances (as minerals, vitamins, and condiments)." Like regular foods, enteral diets are formulated with items that are generally recognized as safe (GRAS) or items approved as food additives by the FDA.

The FDA has a role in approving or advising food manufacturers regarding the health messages for conventional foods. There are two opposing views about the potential effects of regulating such messages: if health claims are not allowed, then the public may not be informed of the potential benefit of consuming certain foods. On the other hand, allowing health messages on foods opens the door for false or exaggerated claims to be made. Recently, the policy of the FDA has been changing toward the allowance of certain health messages. Regulations or food labeling are detailed in two sections of the Code of Federal Regulations (111).

The development of more specialized enteral formulas for specific diseases has placed these formulas under increasingly intense secrutiny by the FDA. In 1988, the orphan drug amendment created a new category of food called medical food, which was defined as follows: "The term medical food means a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements based on recognized scientific principles, are established by medical evaluation" (112). Implicit in this definition is the notion that disease conditions can change an individual's nutritional requirements or food intake leading to a deficiency or overabundance of a nutrient. Although the amendment did issue the definition of a medical food under the current federal regulations that implement the Food, Drug, and Cosmetic Act, there is no procedural framework for the evaluation of medical food products. This contrasts with the detailed regulations for the development and approval of new drugs and food additives as well as the regulations for the formulation and manufacture of infant formulas specified in the Infant Formula Act of 1980 (113).

ACKNOWLEDGMENTS

The authors would like to express their appreciation to Pamela Anderson, Sheila Campbell, David Cockram, Lisa Craig, Joyce Hall, Jeffrey Nelson, and Sean Moore for their invaluable contributions.

BIBLIOGRAPHY

1. ASPEN Board of Directors, "Guidelines for Use of Parenteral and Enteral Nutrition in Adult and Pediatric Patients," *JPEN, J. Parenter. Enteral Nutr.* **17**, 1SA–52SA (1993).
2. A. Chait et al., "Rationale of the Diet-Heart Statement of the American Heart Association. Report of the Nutrition Committee," *Circulation* **88**, 3008–3029 (1993)
3. J. H. Cummings, "Consequences of the Metabolism of Fiber in the Human Large Intestine," in G. V. Vahouny and D. Kritchevsky, eds., *Dietary Fiber in Health and Disease*, Plenum Press, New York, 1982, pp. 9–22.
4. Federation of American Societies for Experimental Biology, *Physiological Effects and Health Consequences of Dietary Fiber*. Washington, D.C., U.S. Dept. of Health and Human Services, 1987, pp. 160–163.
5. J. E. Spiegel et al., "Safety and Benefits of Fructooligosaccharides as Food Ingredients," *Food Technol.* **48**, 85–89 (1994).
6. A. C. Bach and V. K. Babayan, "Medium-Chain Trigylcerides: An Update," *Am. J. Clin. Nutr.* **36**, 950–962 (1982).
7. National Research Council, *Recommended Dietary Allowances*, 10th ed., Washington, D.C , National Academy Press, 1989.
8. S. Mobarhan and L. S. Trumbore, "Nutritional Problems of the Elderly," *Clin. Geriatr Med.* **7**, 191–214 (1991).
9. D. Cuthbertson and W. J. Tilstone, "Metabolism During The Post-Injury Period," *Adv. Clin. Chem.* **12**, 1–55 (1969).
10. J W. Bane, R. E. McCaa, and C. S McCaa, "The Pattern of Aldosterone and Cortisone Blood Levels in Thermal Burn Patients," *J. Trauma* **14**, 605–611 (1974).
11. G. M. Vaughn et al., "Cortisol and Corticotrophin in Burned Patients," *J. Trauma* **22**, 263–273 (1982).
12. D. W. Wilmore et al., "Hyperglucanemia After Burns," *Lancet* **1**, 73–75 (1974).
13. J. M. Shuck et al., "Dynamics of Insulin and Glucagon Secretions in Severely Burned Patients," *J. Trauma* **17**, 706–713 (1977).
14. R. R. Wolfe et al., "Glucose Metabolism in Severely Burned Patients," *Metabolism* **28**, 1031–1039 (1979).
15. D. W. Wilmore et al., "Catecholamines: Mediator of the Hypermetabolic Response to Thermal Injury," *J. Trauma* **15**, 697–703 (1975).
16. K. N. Frayn, P. F Maycock, and H. B. Stoner, "The Relationship of Plasma Catecholamines to Acute Metabolic and Hormonal Responses to Injury in Man," *Circ. Shock* **16**, 229–240 (1985).
17. J. M. Daly et al., "Enteral Nutrition With Supplemental Arginine, RNA, and Omega-3 Fatty Acids in Patients After Operation: Immunologic, Metabolic and Clinical Outcome," *Surgery* **112**, 56–67 (1992).
18. R. O. Brown et al., "Comparison of Specialized and Standard Enteral Formulas in Trauma Patients," *Pharmacology* **14**, 314–320 (1994).
19. F. A. Moore et al., "Clinical Benefits of an Immune-Enhancing Diet for Early Postinjury Enteral Feeding," *J. Trauma* **37**, 607–615 (1994).
20. J. M. Daly et al., "Enteral Nutrition During Multimodality Therapy in Upper Gastrointestinal Cancer Patients," *Ann. Surg.* **221**, 327–338 (1995).
21. K. A. Kudsk et al., "A Randomized Trial of Isonitrogenous Enteral Diets After Severe Trauma: An Immune-Enhancing Diet Reduces Septic Complications," *Ann. Surg.* **224**, 531–543 (1996).
22. A. Barbul, "Arginine: Biochemistry, Physiology and Therapeutic Implications," *J. Parenter Enteral Nutr.* **10**, 227–238 (1986).
23. R. J. Smith and D. W. Wilmore, "Glutamine Nutrition and Requirements," *J. Parenter. Enteral Nutr.* **14**, 94S–99S (1990).
24. M. M. Gottschlich, "Selection of Optimal Lipid Sources in Enteral and Parenteral Nutrition," *Nutr. Clin. Prac.* **7**, 152–165 (1992).
25. C. L. Pirani and S. M. Levenson, "Effect of Vitamin C Deficiency on Healed Wounds," *Proc. Soc. Exp. Biol. Med.* **82**, 95 (1953).
26. T. W. Goodwin, "Metabolism, Nutrition and Function of Carotenoids," *Annu. Rev. Nutr.* **6**, 273–297 (1986).

27. E. G. Maderazo et al., "A Randomized Trial of Replacement Antioxidant Vitamin Therapy for Neutrophil Locomotory Dysfunction in Blunt Trauma," *J. Trauma* **31**, 1142–1150 (1991).
28. R. S. Mitchell and T. L. Petty, "Chronic Obstructive Pulmonary Disease (COPD)," in R. S. Mitchell and T. L. Petty, eds., *Synopsis of Clinical Pulmonary Disease*, 3rd ed, CV Mosby, St. Louis, Mo., 1982, pp 97–113.
29. S. E. Brown and R. W. Light, "What Is Know Known About Protein-Energy Depletion: When COPD Patients Are Malnourished," *Journal of Respiratory Diseases*, 36–50 (May 1983).
30. R. J. Browning and A. M. Olsen, "The Functional Gastrointestinal Disorders of Pulmonary Emphysema," *Mayo Clinic Proceedings* **26**, 537–543 (1961).
31. J. D. Frankfort et al., "Comparison of Ensure-HN Plus and Pulmocare on Maximum Exercise Performance in Patients With Chronic Airflow Obstruction (CAO)," *American Review of Respiratory Diseases* **135**, A361 (1987).
32. F. B. Cerra et al., "Applied Nutrition in ICU Patients: A Consensus Statement of the American College of Chest Physicians," *Chest* **111**, 769–778 (1997).
33. P. Amene et al., "Hypercapnia During Total Parenteral Nutrition With Hypertonic Dextrose," *Crit. Care Med.* **15**, 171–172 (1987).
34. S. A. McClave, "The Consequences of Overfeeding and Underfeeding," *J. Resp. Care Pract.* **10**, 57–64 (1997).
35. K. Ideno et al., "Nutrition in the ICU," *J. Resp. Care Pract.* **9**, Apr/May, 41–50 (1996).
36. M. J. Karlstad et al., "The Anti-inflammatory Role of Gamma-Linolenic and Eicosapentanoic Acids in Acute Lung Injury," in Y. S. Huang and D. E. Mills, eds., *Gamma-Linolenic Acid: Biochemistry and Role in Medicine and Nutrition*, AOCS Press, Champaign, Ill., 1996, pp. 137–167.
37. J. D. Palombo et al., "Rapid Modulation of Lung and Liver Macrophage Phospholipid Fatty Acids in Endotoxemic Rats by Continuous Enteral Feeding with n-3 and Gamma-Linolenic Fatty Acids," *Am. J. Clin. Nutr.* **63**, 208–219 (1996).
38. J. D. Palombo et al., "Cyclic vs Continuous Enteral Feeding with n-3 and Gamma-Linolenic Fatty Acids: Effect on Modulation of Phospholipid Fatty Acids in Rat Lung and Liver Macrophages," *J. Parenter Enteral Nutr* **21**, 123–132 (1997).
39. M. J. Murray et al., "Select Dietary Fatty Acids Attenuate Cardiopulmonary Dysfunction During Acute Lung Injury in Pigs," *Am. J. Physiol.* **269**, H2090–H2099 (1995)
40. P. Mancuso et al., "Dietary Fish Oil and Fish and Borage Oil Attentuate Proinflammatory Eicosanoids in Bronchoalveolar Lavage Fluid and Pulmonary Neutrophil Accumulation in Endotoxic Rats," *Crit. Care Med.* **25**, 1198–1206 (1997).
41. J. E. Gadek et al., "Specialized Enteral Nutrition Improves Clinical Outcomes in Patients With or at Risk of Acute Respiratory Distress Syndrome (ARDS): A Prospective, Blinded, Randomized, Controlled Multicenter Trial," *Am. J. Resp. Crit. Care Med.* **157**, A677 (1998).
42. J. Warnold, K. Lundolm, and T. Scherstein, "Energy Balance and Body Composition in Cancer Patients," *Cancer Res.* **38**, 1801–1907 (1976).
43. C. P. Holroyde et al., "Altered Glucose Metabolism in Metastatic Carcinoma," *Cancer Res.* **35**, 3710–3714 (1975).
44. M. F. Brennan and M. E. Burt, "Nitrogen Metabolism in Cancer Patients," *Cancer Treatment Report* **65** (Suppl 5), 67–68 (1981).
45. M. E. Shils, "Nutritional Problems Induced by Cancer," *Med. Clin. North Am.* **63**, 1009–1025 (1979).
46. A. Theologides, "Anorexia in Cancer: Another Speculation on Its Pathogenesis," *Nutr. Cancer* **2**, 133–135 (1981).
47. J. G. Harris, "Nausea, Vomiting and Cancer Treatment," *Cancer* **28**, 194–201 (1978)
48. J. Medoff, "A Double-Blind Evaluation of the Anti-Emetic Efficacy of Benzquinamide, Prochlorperazine, and Trimethobenzamide in Office Practice," *Current Theoretical Research* **12**, 706–710 (1970).
49. R. A. Harrington et al., "Metoclopramide: An Updated Review of Its Pharmacological Properties and Clinical Use," *Drugs* **25**, 451–494 (1983)
50. U.S. Department of Health, Education, and Welfare, *Eating Hints: Recipes and Tips for Better Nutrition During Cancer Treatment*, National Institutes of Health, Rockville, Md., 1980.
51. E. H. Rosenbaum et al., *Nutrition for the Cancer Patient*, Bull Publishing, Palo Alto, Calif., 1980.
52. B. C. Walike and J. W. Walike, "Relative Lactose Intolerance. A Clinical Study of Tube-Fed Patients," *J. Am. Med. Assoc.* **238**, 948–951 (1977).
53. H. L. Greene et al., "Nasogastric Tube Feeding at Home: A Method for Adjunctive Nutritional Support of Malnourished Patients," *Am. J. Clin. Nutr.* **34**, 1131–1138 (1981).
54. D. A. Lipschitz and C. O. Mitchell, "Enteral Hyperalimentation and Hemoatopoietic Toxicity Caused by Chemotherapy of Small Cell Lung Cancer [Abstract]," *J. Parenter. Enteral Nutr.* **4**, 593 (1980).
55. L. H. Brubaker et al., "Serum Opsonic Defect in Malnourished Cancer Patients and Improvement Following Nutritional Therapy [Abstract]," *Clin. Res.* **27**, 774A (1979).
56. A. H. McArdle et al., "Prophylaxis Against Radiation Injury," *Arch. Surg.* **121**, 879–885 (1986).
57. American Diabetes Association, "Position Statement. Nutrition Recommendations and Principles for People with Diabetes Mellitus," *Diabetes Care* **21**(Suppl 1), S32–S35 (1998).
58. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, "Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. (Adult Treatment Panel II)," *JAMA, J. Am. Med. Assoc.* **269**, 3015–3023 (1993).
59. Expert Panel on Blood Cholesterol Levels in Children and Adolescents, "Treatment Recommendations of the National Cholesterol Education Program. Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents," *Pediatrics* **89**(Suppl) 525–584 (1992).
60. U.S. Department of Health and Human Services, *The Surgeon General's Report on Nutrition and Health. Summary and Recommendations*, U.S. Government Printing Office, Washington, D.C., 1988.
61. C. Stevens, D. L. Costill, and B. Maxwell, "Impact of Carbohydrate (CHO) Source and Osmolality on Gastric Emptying Rates of Liquid Nutritionals [Abstract]," *J. Parenter Enteral Nutr.* **3**, 32 (1979).
62. K. A. Cashmere et al., "Serum Endocrine and Glucose Response Elicited from Ingestion of Enteral Feedings [Abstract]," *Federation Proceedings* **40**, 440A (1981).
63. A. Peters, M. Davidson, and R. M. Isaac, "Lack of Glucose Elevation After Simulated Tube Feeding With a Low-Carbohydrate, High-Fat Enteral Formula in Patients with Type 1 Diabetes," *Am. J. Med.* **87**, 178–182 (1989).
64. W. Sturmer et al., "Favorable Glycaemic Effects of a New Balanced Liquid Diet for Enteral Nutrition. Results of a Short Term Study in 30 Type II Diabetic Patients," *Clin. Nutr.* **13**, 221–227 (1994).

65. L. D. Craig et al., "Use of a Reduced-Carbohydrate, Modified-Fat Enteral Formula for Improving Metabolic Control and Clinical Outcomes in Long-Term Care Residents With Type 2 Diabetes: Results of a Pilot Trial," *Nutrition* **14**, 529–534 (1998).
66. L. V. Campbell et al., "The High-Monounsaturated Fat Diet as a Practical Alternative for NIDDM," *Diabetes Care* **17**, 177–182 (1994).
67. A. Garg et al., "Effects of Varying Carbohydrate Content of Diet in Patients With Non-Insulin Dependent Diabetes Mellitus," *J Am. Med. Assoc.* **271**, 1428–1428 (1994).
68. B. Gumbiner, C C. Low, and P. D. Reaven, "Effects of a Monounsaturated Fatty Acid-Enriched Hypocaloric Diet on Cardiovascular Risk Factors in Obese Patients with Type 2 Diabetes," *Diabetes Care* **21**, 9–15 (1998).
69. J. D. Kopple, "Nutrition, Diet, and the Kidney," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., Lea & Febiger, Philadelphia, Penn. 1994, pp. 1102–1134.
70. W. E. Mitch and B. J. Maroni, "Nutritional Considerations in the Treatment of Patients With Chronic Uremia," *Miner. Electrolyte Metab.* **24**, 285–289 (1998).
71. E. Lusvarghi et al , "Natural History of Nutrition in Chronic Renal Failure," *Nephrol Dial Transplant* **11**(Suppl 9), 75–84 (1996).
72. W. E. Mitch, "Robert H. Herman Memorial Award in Clinical Nutrition Lecture, 1997: Mechanisms Causing Loss of Lean Body Mass in Kidney Disease," *Am. J. Clin. Nutr.* **67**, 359–366 (1998).
73. "Effects of Dietary Protein Restriction on the Progression of Moderate Renal Disease in the Modification of Diet in Renal Disease Study," *J. Am. Soc. Nephrol* **7**, 2616–2626 (1996).
74. J. S. Park et al., "Protein Intake and the Nutritional Status in Patients With Predialysis Chronic Renal Failure on Unrestricted Diet," *Korean J. Intern. Med.* **12**, 115–121 (1997).
75. M. J. Blumenkrantz et al., "Metabolic Balance Studies and Dietary Protein Requirements in Patients Undergoing Ambulatory Peritoneal Dialysis," *Kidney Int.* **21**, 849–861 (1982).
76. L. A. Slomowitz et al., "Effect of Energy Intake on Nutritional Status in Maintenance Hemodialysis Patients," *Kidney Int.* **35**, 704–711 (1989).
77. B. Schneeweiss et al., "Energy Metabolism in Acute and Chronic Renal Failure (see comments)," *Am. J. Clin. Nutr.* **52**, 596–601 (1990).
78. J. D. Kopple, "McCollum Award Lecture, 1996: Protein-Energy Malnutrition in Maintenance Dialysis Patients," *Am. J. Clin. Nutr.* **66**, 1544–1557 (1997).
79. D. B. Cockram et al., "Safety and Tolerance of Medical Nutritional Products as Sole Sources of Nutrition for People on Hemodialysis," *J. Ren. Nutr.* **8**, 25–33 (1998).
80. N. Cano et al., "Prealbumin-Retinol Binding Protein-Retinol Complex in Hemodialysis Patients," *Am. J. Clin. Nutr.* **47**, 664–667 (1988).
81. J. Kelleher et al., "Vitamin A and Its Transport Proteins in Patients with Chronic Renal Failure Receiving Maintenance Haemodialysis and After Renal Transplantation," *Clin. Sci.* **65**, 619–626 (1983).
82. F. R. Smith and D. S. Goodman, "The Effects of Diseases of the Liver, Thyroid and Kidneys on the Transport of Vitamin A in Human Plasma," *J. Clin. Invest.* **50**, 2426–2436 (1971).
83. H. Yatzidis, P Digenis, and P. Fountas, "Hypervitaminosis A Accompanying Advanced Chronic Renal Failure," *Br. Med. J.* **3**, 352–353 (1975).
84. M. A. Allman et al., "Vitamin Supplementation of Patients Receiving Hemodialysis," *Med. J. Aust.* **150**, 130–133 (1989)
85. C. Pru, J. Eaton, and C. Kjellstrand, "Vitamin C Intoxication and Hyperoxalemia in Chronic Hemodialysis Patients," *Nephron* **39**, 112–116 (1985).
86. P. Balcke et al., "Ascorbic Acid Aggravates Secondary Hyperoxalemia in Patients on Chronic Hemodialysis," *Ann. Intern. Med.* **101**, 344–345 (1984).
87. W. R. Salyer and D. Keren, "Oxalosis as a Complication of Chronic Renal Failure," *Kidney Int.* **4**, 61–66 (1973).
88. K. Satomura et al., "Renal 25-hydroxyvitamin D3-1-hydroxylase in Patients With Renal Disease," *Kidney Int.* **34**, 712–716 (1988).
89. J. C. Jeanette and I. D. Goldman, "Inhibition of the Membrane Transport of Folates by Anions Retained in Uremia," *J. Lab. Clin. Med.* **86**, 834–843 (1975).
90. V A. Skoutakis et al., "Folic Acid Dosage for Chronic Hemodialysis Patients," *Clin. Pharmacol. Ther.* **18**, 200–204 (1975).
91. V. W. Dennis and K. Robinson, "Homocysteinemia and Vascular Disease in End-Stage Renal Disease," *Kidney Int. Suppl.* **57**, S11–S17 (1996).
92. E. A. Ross et al., "Vitamin B6 Requirements of Patients on Chronic Peritoneal Dialysis," *Kidney Int.* **19**, 694–704 (1989).
93. J. D. Kopple et al., "Daily Requirement for Pyridoxine Supplements in Chronic Renal Failure," *Kidney Int.* **19**, 694–704 (1981).
94. A. G. Bostom et al., "High Dose B-Vitamin Treatment of Hyperhomocysteinemia in Dialysis Patients", *Kidney Int.* **49**, 147–152 (1996).
95. M. E. Suliman et al., "Total, Free and Protein-Bound Sulphur Amino Acids in Uraemic Patients," *Nephrol. Dial. Transplant* **12**, 2332–2338 (1997).
96. M. E. Suliman, B. Anderstam, and J. Bergstrom, "Evidence of Taurine Depletion and Accumulation of Cysteinesulfinic Acid in Chronic Dialysis Patients," *Kidney Int.* **50**, 1713–1717 (1996).
97. Y. Sakurauchi et al., "Effects of L-Carnitine Supplementation on Muscular Symptoms in Hemodialyzed Patients," *Am. J Kidney Dis* **32**, 258–264 (1998).
98. A. S. Alhomida et al., "Influence of Sex and Chronic Haemodialysis Treatment on Total, Free and Acyl Carnitine Concentrations in Human Serum," *Int. Urol. Nephrol.* **29**, 479–487 (1997).
99. B. de los Reyes et al., "L-Carnitine Normalizes the Reduced Carnitine Palmitoyl Transferase Activity in Red Cells From Haemodialysis Patients [letter]," *Nephrol. Dial. Transplant* **12**, 1300–1301 (1997).
100. T. H. Lin et al., "Trace Elements and Lipid Peroxidation in Uremic Patients on Hemodialysis," *Biol. Trace Elem. Res.* **51**, 277–283 (1996).
101. B. Dworkin et al., "Diminished Blood Selenium Levels in Renal Failure Patients on Dialysis: Correlations With Nutritional Status," *Am. J. Med. Sci.* **293**, 6–12 (1987).
102. G. Kallistratos et al., "Selenium and Haemodialysis: Serum Selenium Levels in Healthy Persons, Non-Cancer and Cancer Patients With Chronic Renal Failure," *Nephron* **41**, 217–222 (1985).
103. P. M. Queen and C. E. Lang eds. *Handbook of Pediatric Nutrition*, Aspen Publishers, Gaithersburg Md., 1993.
104. S. B. Baker, R. D. Baker, Jr., and A. Davis, eds. *Pediatric Enteral Nutrition*, Chapman and Hall, New York, 1994.

105. J. L. Rombeau and D. O. Jacobs, "Nasoenteric Tube Feeding," in J. L. Rombeau and M. D. Caldwell, eds., *Enteral and Tube Feeding*, W. B. Saunders, Philadelphia, Penn. 1984. pp. xx–xx.

106. A. H McArdle et al., "A Rationale for Enteral Feeding as the Preferable Route for Hyperalimentation," *Surgery* **90**, 613–623 (1981).

107. T. Jones, "Enteral Feeding: Techniques of Administration," *Gut* **27**, 47–49 (1986).

108. C Page, R. Andrassay, and J. Sandler, "Techniques in Delivery of Liquid Diet: Short-Term and Long-Term," in M. Deitel, ed., *Nutrition in Clinical Surgery*, Williams & Wilkins, Baltimore, Md., 1985, pp 60–87.

109. J. Rombeau et al., "Feeding by Tube Enterostomy," in J. L. Rombeau and M. D. Caldwell, eds., *Enteral and Tube Feeding*, W.B. Saunders, Philadelphia, Penn., 1984, pp. xx–xx.

110. L. Y. Young and M. A. Joda-Kimble, eds., *Applied Therapeutics: The Clinical Use of Drugs*, 4th ed., Applied Therapeutics, Vancouver, Wash., 1988.

111. *Regulations for Food Labeling: Conventional Foods*, 21 CFR PART 101, *Foods for Special Dietary Use*, 21 CFR Part 105.

112. *Definition of Medical Food*, Orphan Drug Amendments of 1988, 21 USC 360 e(b)(3).

113. Infant Formula Act of 1980, Public Law 96-359.

DEBRA ROONEY
DAVID DEIS
Abbott Laboratories
Columbus, Ohio

ENZYME ASSAYS FOR FOOD SCIENTISTS

REASONS FOR ASSAYING ENZYMES

In designing enzyme assays it is important to determine the level of sophistication required of the data obtained. One may be led astray by answers that are too simplistic; conversely, excessive time and effort may be expended in obtaining highly precise data to meet "quick-and-dirty" needs. Understanding the basis of various kinds of assays will help in choosing the proper level of assay sophistication to avoid these two kinds of errors.

Characterization of Enzymes for Applications

In industrial food applications the usual requirement of an enzyme is that it produce the desired functionality for the minimum cost. This often implies that offerings by alternate suppliers are assayed to find the best enzyme source for the process in hand. The characterizing factors are (*1*) rate (activity per gram of enzyme); (*2*) pH optimum; (*3*) temperature optimum; (*4*) stability under conditions of use; and (*5*) presence (or absence) of potentially deleterious side activities.

Rate. By rate, the food processor usually means the amount of modification obtained during the time allowed for enzyme action in the process. This may be different from the initial rate of conversion of substrate to product as defined by an enzymologist. An assay that measures the latter rate may be misleading if the modification occurs during extended incubation in the process.

pH, Temperature. The pH and temperature optimum curves published by suppliers usually confound the true influence of these factors on enzyme catalytic properties with the effect on enzyme stability. Enzyme denaturation is influenced by numerous factors, is usually irreversible, and occurs with first-order kinetics. Optimum curves are constructed using assays that measure the amount of substrate modification over a period of time and represent a summation of true rate effects plus denaturation during that time. They should be used with caution.

Stability. The presence of substrate stabilizes enzyme against denaturation; thus, the real optimum of interest is the stability of enzyme under the conditions of use: time, pH, temperature, substrate concentration, inorganic ions, organic solvents, and so on. An assay used to screen enzymes for a particular application should mimic as nearly as possible the actual use conditions, to give a reliable estimate of cost benefit.

Side activities. Preparing a pure isolated enzyme is cost-prohibitive for food applications. All commercial enzymes contain some activities other than those declared on the label; for example, fungal amylase usually contains some proteolytic activity, a bacterial protease may also contain some xylanase, and so on. These side activities may present a problem when the enzyme is used in certain food processing situations, and assays should be applied that will detect them, as well as the main enzyme of interest.

Characterization of Enzymes in Raw Materials

Specificity. Often enzymes with similar activities (ie, proteases) are obtained from different sources and assays are used to characterize them in terms of units per gram. If the different enzymes have varying specificity requirements, the results may be misleading. For example, if two proteases have specificities corresponding to elastase and trypsin, an assay based on azocollagen substrate will give a much higher value for the former enzyme, while one based on casein will favor the latter. If the protein to be modified is something quite different, for example, wheat gluten, neither assay will give a reliable comparison. Substrate specificity may be of major importance, as with proteases, or a negligible factor, as with lipoxygenase. It is best to assume that it is important until the contrary is established.

If an assay is used to monitor production of enzyme from biofermentation, specificity may be overlooked. Here the ideal is the quickest possible assay consistent with reasonable accuracy, making the assumption that enzyme specificity is constant. (This also depends on the conditions of biofermentation remaining constant, particularly the purity of the microbiological innoculum being used.) A quick assay based on azocollagen may suffice even if the protease being made is similar to trypsin in its specificity.

105. J. L. Rombeau and D. O. Jacobs, "Nasoenteric Tube Feeding," in J. L. Rombeau and M. D. Caldwell, eds., *Enteral and Tube Feeding*, W. B. Saunders, Philadelphia, Penn. 1984. pp. xx–xx.
106. A. H McArdle et al., "A Rationale for Enteral Feeding as the Preferable Route for Hyperalimentation," *Surgery* **90**, 613–623 (1981).
107. T. Jones, "Enteral Feeding: Techniques of Administration," *Gut* **27**, 47–49 (1986).
108. C Page, R. Andrassay, and J. Sandler, "Techniques in Delivery of Liquid Diet: Short-Term and Long-Term," in M. Deitel, ed., *Nutrition in Clinical Surgery*, Williams & Wilkins, Baltimore, Md., 1985, pp 60–87.
109. J. Rombeau et al., "Feeding by Tube Enterostomy," in J. L. Rombeau and M. D. Caldwell, eds., *Enteral and Tube Feeding*, W.B. Saunders, Philadelphia, Penn., 1984, pp. xx–xx.
110. L. Y. Young and M. A. Joda-Kimble, eds., *Applied Therapeutics: The Clinical Use of Drugs*, 4th ed., Applied Therapeutics, Vancouver, Wash., 1988.
111. *Regulations for Food Labeling: Conventional Foods*, 21 CFR PART 101, *Foods for Special Dietary Use*, 21 CFR Part 105.
112. *Definition of Medical Food*, Orphan Drug Amendments of 1988, 21 USC 360 e(b)(3).
113. Infant Formula Act of 1980, Public Law 96-359.

DEBRA ROONEY
DAVID DEIS
Abbott Laboratories
Columbus, Ohio

ENZYME ASSAYS FOR FOOD SCIENTISTS

REASONS FOR ASSAYING ENZYMES

In designing enzyme assays it is important to determine the level of sophistication required of the data obtained. One may be led astray by answers that are too simplistic; conversely, excessive time and effort may be expended in obtaining highly precise data to meet "quick-and-dirty" needs. Understanding the basis of various kinds of assays will help in choosing the proper level of assay sophistication to avoid these two kinds of errors.

Characterization of Enzymes for Applications

In industrial food applications the usual requirement of an enzyme is that it produce the desired functionality for the minimum cost. This often implies that offerings by alternate suppliers are assayed to find the best enzyme source for the process in hand. The characterizing factors are (*1*) rate (activity per gram of enzyme); (*2*) pH optimum; (*3*) temperature optimum; (*4*) stability under conditions of use; and (*5*) presence (or absence) of potentially deleterious side activities.

Rate. By rate, the food processor usually means the amount of modification obtained during the time allowed for enzyme action in the process. This may be different from the initial rate of conversion of substrate to product as defined by an enzymologist. An assay that measures the latter rate may be misleading if the modification occurs during extended incubation in the process.

pH, Temperature. The pH and temperature optimum curves published by suppliers usually confound the true influence of these factors on enzyme catalytic properties with the effect on enzyme stability. Enzyme denaturation is influenced by numerous factors, is usually irreversible, and occurs with first-order kinetics. Optimum curves are constructed using assays that measure the amount of substrate modification over a period of time and represent a summation of true rate effects plus denaturation during that time. They should be used with caution.

Stability. The presence of substrate stabilizes enzyme against denaturation; thus, the real optimum of interest is the stability of enzyme under the conditions of use: time, pH, temperature, substrate concentration, inorganic ions, organic solvents, and so on. An assay used to screen enzymes for a particular application should mimic as nearly as possible the actual use conditions, to give a reliable estimate of cost benefit.

Side activities. Preparing a pure isolated enzyme is cost-prohibitive for food applications. All commercial enzymes contain some activities other than those declared on the label; for example, fungal amylase usually contains some proteolytic activity, a bacterial protease may also contain some xylanase, and so on. These side activities may present a problem when the enzyme is used in certain food processing situations, and assays should be applied that will detect them, as well as the main enzyme of interest.

Characterization of Enzymes in Raw Materials

Specificity. Often enzymes with similar activities (ie, proteases) are obtained from different sources and assays are used to characterize them in terms of units per gram. If the different enzymes have varying specificity requirements, the results may be misleading. For example, if two proteases have specificities corresponding to elastase and trypsin, an assay based on azocollagen substrate will give a much higher value for the former enzyme, while one based on casein will favor the latter. If the protein to be modified is something quite different, for example, wheat gluten, neither assay will give a reliable comparison. Substrate specificity may be of major importance, as with proteases, or a negligible factor, as with lipoxygenase. It is best to assume that it is important until the contrary is established.

If an assay is used to monitor production of enzyme from biofermentation, specificity may be overlooked. Here the ideal is the quickest possible assay consistent with reasonable accuracy, making the assumption that enzyme specificity is constant. (This also depends on the conditions of biofermentation remaining constant, particularly the purity of the microbiological innoculum being used.) A quick assay based on azocollagen may suffice even if the protease being made is similar to trypsin in its specificity.

Characterization of Enzyme Rate Parameters

For a study made for the purpose of establishing basic enzymatic parameters (catalytic rate constant, affinity for substrate, inhibitor binding, etc) the assay must provide appropriate velocity estimates. In general this means the catalytic rate at time zero, that is, when the enzyme and substrate are first combined. Assays involving incubation for a fixed length of time present some difficulties for this purpose. Progress curves, in which the concentration of product is measured at intervals during the incubation, are valid and are not used as often as they might be to determine enzymatic parameters.

THEORETICAL ASPECTS OF ENZYME ASSAYS

Properly judging the nature of the desired assay requires a certain amount of theoretical understanding. This does not have to be in great depth; the requisite fundamentals are easily grasped and applied. The effort will be repaid by improved enzyme assays for routine work and process design (1).

Assay Characteristics

A sound assay, regardless of the level of sophistication required, will have (*1*) linear dependence on enzyme concentration, (*2*) adequate consideration of pH and temperature effects, (*3*) appropriate accuracy, (*4*) adequate sensitivity, and (*5*) speed and ease of performance. Unfortunately, many assays emphasize the last factor at the expense of the other four.

Enzyme Linearity. Enzyme linearity is paramount. A curved plot of assay response versus amount of enzyme used indicates that some chemical, physical, and/or enzymological factors have been overlooked. In fixed-time assays the formation of product is often not strictly linear with time because of substrate depletion or product inhibition and this nonlinearity is more pronounced at higher enzyme concentrations. Occasionally the chemical reaction used to quantitate the amount of product formed is not stoichiometric, leading to a nonlinear plot of, for example, spectrophotometric absorbance versus enzyme amount. Numerous nonlinear assays found in the literature indicate an incomplete understanding of the system being used for the assay.

Many assays are linear over a limited range of activity. These may be used (particularly if they meet the requirement for ease of use) if care is taken to ensure that measurements are made only within the linear range.

Temperature, pH. Elevated temperatures and extremes of pH contribute to enzyme denaturation during the assay. Temperature optimum curves are always due to this phenomenon, and activity decreases at high or low pH also may be due to enzyme instability. The pH also may affect enzyme catalytic activity, influencing the ionization state of the active site and possibly the substrate (2–4). A sound assay will take these factors into account.

Accuracy, Sensitivity. The required levels of accuracy (more properly, precision) and sensitivity should be carefully considered. Measurements of α-amylase activity may have a coefficient of variation of 2% or of 10%, depending on whether the assay is a replicated colorimetric one using a modified substrate (5) or a viscometric one using gelatinized ground grain (6). For standardizing a purified amylase for use in bread production, the former would be appropriate, while for identifying bins of wheat that has been subjected to sprouting, the latter method is quite adequate. Likewise, assays at almost any level of sensitivity may be constructed. Using a fluorescent substrate, picomolar concentrations of trypsin may be assayed (7), while in monitoring the production of microbial protease a simple protein-based assay (8) will do the job, although it is some five orders of magnitude less sensitive.

Convenience. Speed and ease of performance should be the last factors considered. These are important in many industrial contexts, but a fast, easy, inadequate assay will only result in the rapid generation of much useless data. After linearity, precision, enzyme stability, and sensitivity are established, then steps may be taken to increase output.

Initial Rates. Most assays measure the rate of formation of product from substrate, that is, $d[P]/dt$. This measurement may be of the initial rate, $d[P]/dt$, at the initiation of the reaction, or of the amount of product formed during a fixed time of incubation of substrate with enzyme. Fixed-time assays are convenient, in that a large number of samples may be run simultaneously. Unfortunately, they are also more prone to complications leading to the nonlinearity mentioned above. Accurate initial rate measurements are not contaminated by effects such as substrate depletion, product inhibition, or enzyme denaturation. However, they are not always easy to obtain. A relatively simple method of finding the initial rate is the following. Set up the assay system, take samples at various times t, and measure product concentration $[P]$ at each time. Usually there is a slight downward curve of the plot of $[P]$ versus t (Fig. 1) that makes the determination of the tangent ($d[P]/dt$) at zero time difficult. Using a simple program (Basic, or even most spreadsheet programs today are capable of this), fit the data points with a least squares quadratic curve:

$$[P] = a + b \times t + c \times t^2$$

The first derivative gives the desired initial rate with reasonable accuracy:

$$d[P]/dt = b + 2c \times t; \text{ and } d[P]/dt_{(t=0)} = b.$$

Determining Rate Parameters

Michaelis-Menten Parameters. In designing assays it is useful to know the maximum rate obtainable with a given amount of enzyme, $V_{\max}$, and the concentration of substrate that gives half that rate, K_M. These are the fundamental parameters in the Michaelis-Menten (M-M) rate

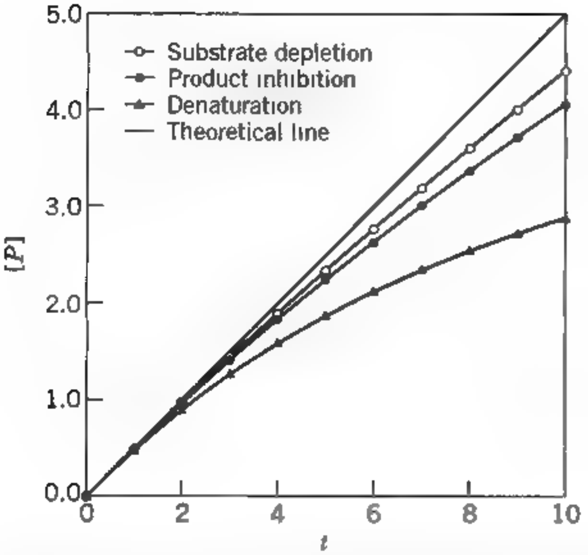


Figure 1. Nonlinearity of product formation with time, due to various factors. Initial rate for the theoretical reaction is 0.5. Fitting the nonlinear rates with a second degree polynomial, the calculated initial rates are: Substrate depletion, 0.51; Product inhibition, 0.51; Enzyme denaturation, 0.44. If the curves are fitted to a third degree polynomial, the calculated rates are 0.50, 0.50, and 0.49, respectively. *Source:* Ref. 1, p. 93.

equation: $v = V_{max}[S]/(K_M + [S])$, where $[S]$ is substrate concentration and v is the actual rate $d[P]/dt$. The usual procedure is to measure v at several concentrations $[S]$ and then calculate V_{max} and K_M, either by applying a computer program (9) such as HYPER (Fig. 2a) or by fitting a straight line to one of the linear transforms of the M-M equation. The usual transform is the double reciprocal or Lineweaver-Burk plot: $1/v = 1/V_{max} + (K_M/V_{max})(1/[S])$, where $1/v$ is plotted versus $1/[S]$ (Fig. 2b). From statistical considerations, this is the least desirable transform to use (11).

A better plot is the Hanes plot of $[S]/v$ versus $[S]$ (Fig. 2c): $[S]/v = K_M/V_{max} + (1/V_{max})[S]$. The points are spaced along the x axis at the same intervals as in the M-M plot rather than being crowded together near the y axis. The larger experimental errors inherent in the smaller values of v (at low $[S]$) have less influence on the linear least squares regression line. The Hanes plot should be used in treating v, $[S]$ data graphically; the use of the unsatisfactory Lineweaver-Burk plot should be discontinued.

The M-M equation is a differential equation, that is, v equals $d[P]/dt$ at the instantaneous value of $[S]$, usually taken as the initial substrate concentration. If v is only available as $[P]/t$ from a fixed-time assay, then the value taken for $[S]$ for the above calculations should be the average of the substrate concentration at the beginning and end of the incubation period, $([S]_0 + [S]_t)/2$. This approximation gives estimates of V_{max} and K_M that are much closer to the true values than if the initial value of $[S]$ is used (12).

Inhibitors

Two types of enzyme inhibitors are of interest to food scientists: (*1*) low-affinity inhibitors and (*2*) high-affinity inhibitors. The former are effective in the millimolar to micromolar concentration range and readily dissociate from the enzyme; an example is inorganic phosphate inhibiting phytase. The latter are effective in the nanomolar to picomolar concentration range and are bound tightly to the enzyme; an example is soybean trypsin inhibitor. For low-affinity inhibitors the dissociation constant K_i is a useful parameter to know; for high-affinity inhibitors the amount present is usually of more concern.

Inhibition Model. A general equilibrium model of inhibition is shown in Figure 3. If the parameter α is very large, so that the species EIS does not exist, the inhibition is termed competitive (inhibitor competes with S for the enzyme). The effect, shown in Figure 4a, is that V_{max} is unchanged, and K_M increases. If $\alpha = 1$ and $\beta = 0$, EIS is formed but does not proceed to product P, noncompetitive inhibition results. As shown in Figure 4a, V_{max} decreases and K_M is unchanged. If α is greater than 1 but not extremely large, mixed inhibition occurs. These types are diagnosed by comparing the Hanes plots in the absence and presence of inhibitor (Figure 4b).

Inhibitor Constant. If the inhibition is competitive, K_M is determined from the ratio of K_{app} (the apparent value of K in the presence of inhibitor of concentration $[I]$) to K_M (no inhibitor present): $K_{app}/K_M = 1 + [I]/K_i$. If inhibition is noncompetitive, the ratio of true maximum velocity to apparent maximum velocity in the presence of inhibitor gives $V_{max}/V_{app} = 1 + [I]/K_i$. Determining K_i, α, and β in the cases of partial and mixed inhibition is too complex to be discussed here.

High-Affinity Inhibitors. Measuring the concentration of high-affinity inhibitors (eg, soy trypsin inhibitor) is relatively straightforward (13). Trypsin is mixed with aliquots of soy meal extract and after a brief incubation (for formation of the inhibitor-enzyme complex) the amount of uninhibited enzyme remaining is measured by a simple assay. The rate of reaction is plotted versus the size of the extract aliquot; the straight line through the data obtained at lower levels of inhibition intersects the x axis at a point where the amount of enzyme equals the amount of inhibitor present (Fig. 5) (14). In the example shown, 1.2 mL of soy meal extract contained a molar amount of trypsin inhibitor equal to the number of moles of trypsin used in each assay tube. If the absolute amount of trypsin is established by a titration assay, the amount of soy trypsin inhibitor can be expressed in absolute molar units rather than arbitrary trypsin inhibitor units (15). Conversely, if the absolute concentration of high-affinity inhibitor is known, this method serves to measure the molar amount of enzyme present.

Endogenous Inhibitors. Endogenous inhibitors may be present in crude extracts of materials containing the enzyme being assayed, resulting in a marked nonlinearity in the assay (Fig. 6a). The uninhibited rate may be found as follows (1). Let $[e]$ be the amount of enzyme in the largest aliquot of extract used in making the plot of Figure 6. X is the fraction of that aliquot used for each of the other points.

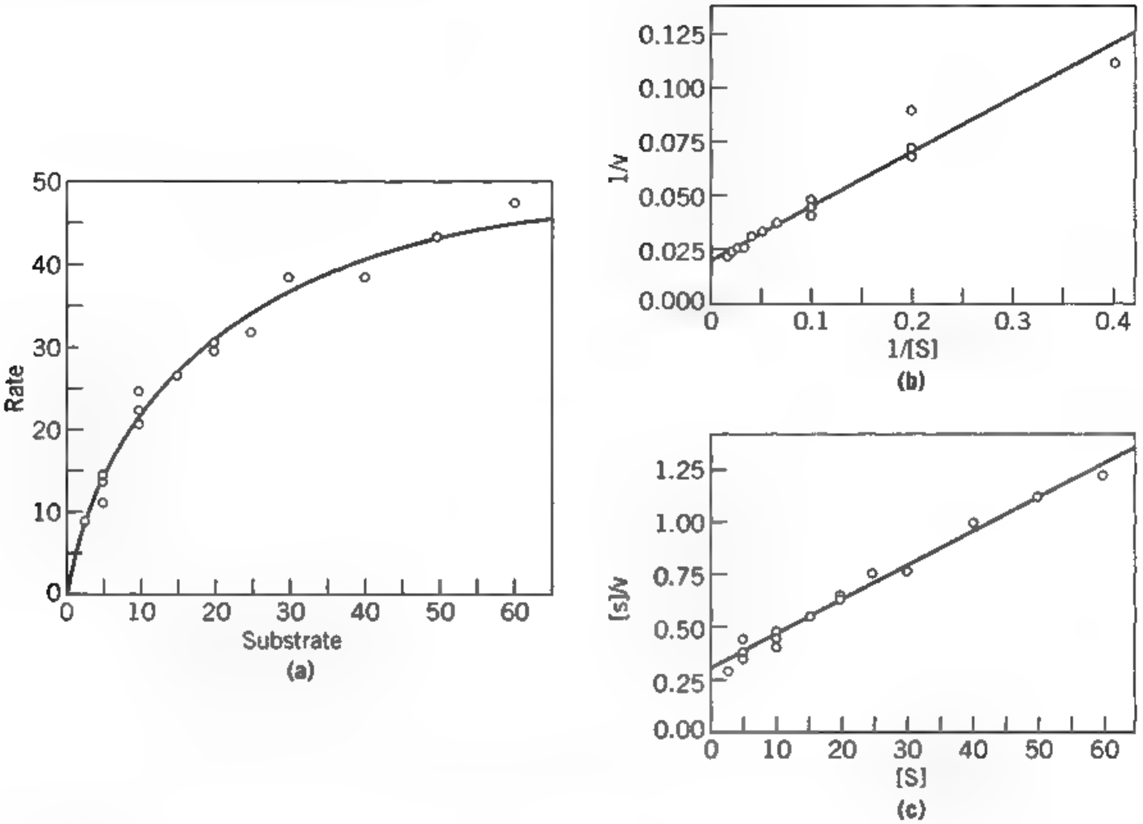


Figure 2. Determining V_{max} and K_M from experimental data using three different methods: (a) HYPER computer program: $V_{max} = 58.55$, $K_M = 17.44$, Std. Dev. 1.65; (b) Lineweaver-Burk plot: $V_{max} = 49.60$, $K_M = 12.85$, Std. Dev. 2.54; (c) Hanes plot: $V_{max} = 58.96$, $K_M = 17.56$, Std. Dev. 1.66. *Source:* Data from Ref. 10

$$\begin{tikzcd}
\mathrm{E} + \mathrm{S} \arrow[r, leftrightarrow, "K_M"] & \mathrm{ES} \arrow[r, leftrightarrow, "k"] & \mathrm{E} + \mathrm{P} \\
+ & + & \\
\mathrm{I} \arrow[d, leftrightarrow, "K_i"] & \mathrm{I} \arrow[d, leftrightarrow, "\alpha K_i"] & \\
\mathrm{EI} + \mathrm{S} \arrow[r, leftrightarrow, "\alpha K_M"] & \mathrm{EIS} \arrow[r, leftrightarrow, "\beta k"] & \mathrm{EI} + \mathrm{P}
\end{tikzcd}$$

Figure 3. General equilibrium model for reversible enzyme inhibition. *Source:* Ref. 1, p. 38.

The measured rate at each point is v_i. A plot is made of X/v_i versus X (Fig. 6b). From the y-axis intercept calculate the uninhibited rate; that is, 1/intercept equals the true rate due to enzyme concentration $[e]$. This method is useful for comparing enzyme amounts from different sources, and during enzyme purification until the inhibitor has been removed.

SPECIFIC ENZYME ASSAYS

Several assays for enzymes most commonly measured by food scientists will be described. These are only examples of the wide range of ingenious methods that have been reported for following the rate of product formation by food-related enzymes. In most cases factors such as pH, temperature, activating ions, time of reaction, and detection methods may be adjusted to fit the specific needs of the project in hand. Thus, these should be considered as starting points for designing assays to meet particular requirements, not the only way to measure the enzyme activity under investigation.

Proteases and Peptidases

Substrates. Protein substrates for proteases are most often hemoglobin or casein and must be completely soluble in buffer. Casein for protease assays is designated "nach Hammarsten," while hemoglobin "for protease assay" is usually of high quality. Casein precipitates below pH 6, so it is used at neutral to alkaline pH. Hemoglobin must be denatured before use, either by treatment with acid (if the assay is at acidic pH) (16) or urea (neutral to alkaline pH assay) (17). Gelatin, sometimes used in viscometric assays, is quite heterogeneous, so lot-to-lot reproducibility is a concern.

Proteins may be derivatized to fit assay needs. Diazotized protein allows measurement of solubilized peptide with a visible-range colorimeter (18). If the amino groups freed during hydrolysis are quantitated using, for example,

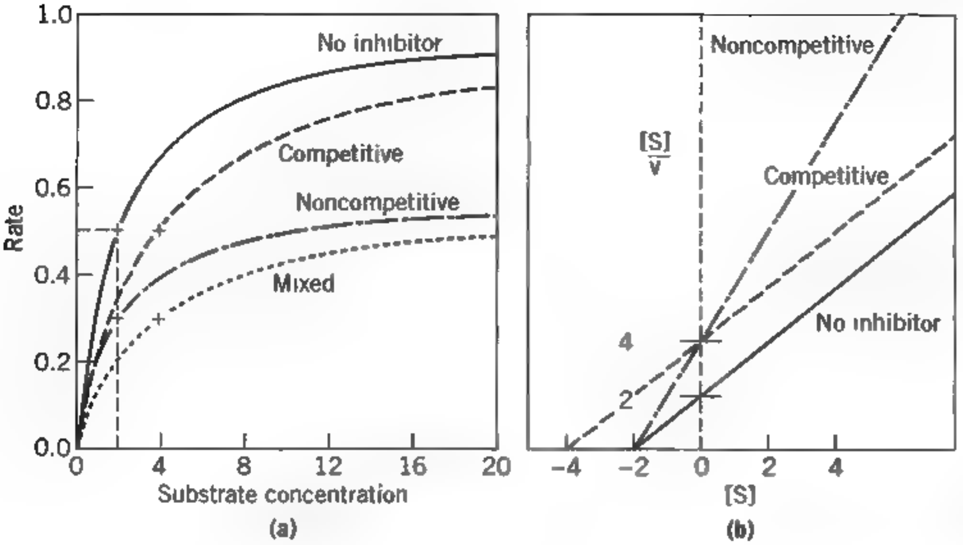


Figure 4. Hyperbolic and Hanes plots showing different modes of inhibition.

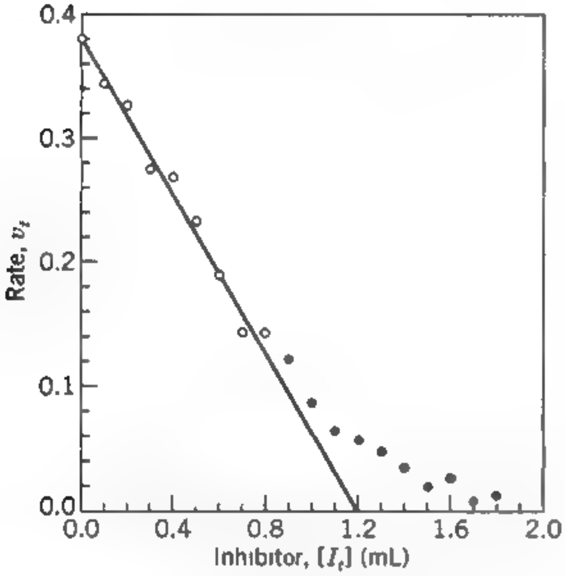


Figure 5. Plot of enzyme rates in presence of high-affinity soy trypsin inhibitor. *Source:* Data from Ref. 14.

TNBS (trinitrobenzenesulfonic acid), the ε-amino groups of lysine give a high blank value that may be removed by making the succinyl (19) or *N,N*-dimethyl (20) derivative of the protein. The latter is preferable for trypsinlike proteases.

Small synthetic molecules are also useful for assaying proteases. These give a change in spectrophotometric absorbance as they are hydrolyzed, so a continuous assay with its advantages is possible. Table 1 lists a number of small molecule substrates. Most of these are applicable to serine and/or sulfhydryl proteases, with two exceptions: FAGLA is a substrate for metalloprotease (neutral protease), and Z-Gly-Phe is a carboxypeptidase substrate. Acidic protease may be assayed using a chromogenic peptide. Aminopeptidase is usually assayed using an amino acid derivative such as L-leucine-β-naphthylamide.

Protease Assays. Most assays with protein substrates involve incubation for a set length of time, stopping the reaction with TCA (trichloroacetic acid), and measuring the amount of soluble peptide. Buffered TCA (0.11 MTCA, 18 g/L; 0.22 M sodium acetate, 18 g/L; and 0.33 M acetic acid, 19.8 g/L) gives superior enzyme linearity compared

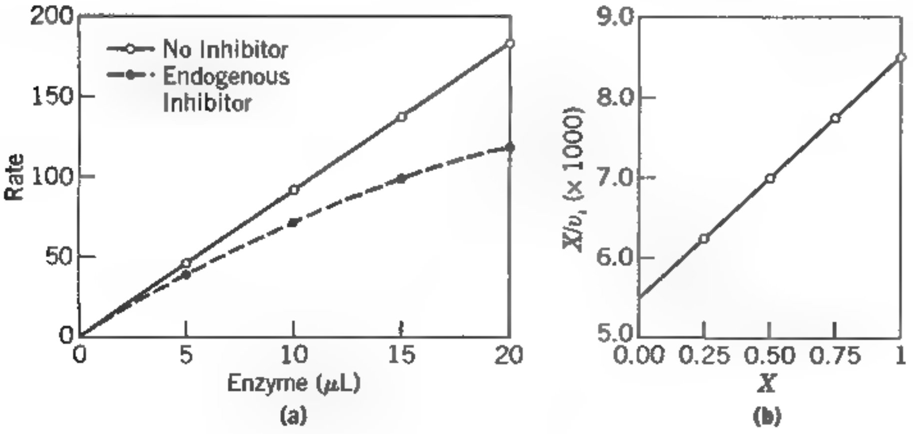


Figure 6. Method for finding uninhibited rate in the presence of endogenous inhibitor. *Source:* Ref. 1, p. 109.

Table 1. Absorbance Changes for Some Synthetic Substrates

Substrate	Acronym	λ, nm	Δε, mM	Reference
N-acetyl-L-tyrosine ethyl ester	ATEE	237	+0.23	21
		275	−0.17	21
N-benzoyl-L-arginine ethyl ester	BAEE	253	+1.15	21
N-benzoyl-L-arginine-*p*-nitroanilide	BAPA	410	+8.8	22
N-benzoyl-L-tyrosine ethyl ester	BTEE	254	+1.03	23
		256	+0.96	24
N-tosyl-L-Arginine ethyl ester · HCL	TAME	244	+0.80	23
		247	+0.41	25
3-(2-furylacryloyl)-glycyl-L-leucinamide	FAGLA	345	−0.32	26
N-carbobenzoxy-glycyl-L-phenylalanine	Z-Gly-Phe	224	1.0	27
N-carbobenzoxy-L-tyrosine-*p*-nitrophenyl ester	Z-Tyr-pNP	400	+18.8	23

with a simple aqueous TCA solution (8). Quantitation may be direct (absorbance of filtrate at 275 nm) or colorimetric (Folin-Lowry [28], bicinchoninic acid [29], or TNBS [30]). An example of a casein-based assay is the following (8). To 5 mL casein (12 mg/mL in 0.03 M phosphate buffer, pH 7.5), add 1 mL enzyme solution and after 10 min reaction add 5 mL of buffered TCA. After 30 min the mixture is filtered and absorbance at 275 nm is measured. For Folin-Lowry quantitation, to 1 mL of filtrate add 1 mL alkaline buffer (1 M Na_2CO_3, 0.25 M NaOH), 0.4 mL copper reagent (0.1% $CuSO_4 \cdot 5H_2O$, 0.2% NaK tartrate), mix, and allow to stand 10 min. Then add 0.75 mL diluted phenol reagent (Folin-Ciocalteau reagent diluted with 3 volumes H_2O), mix, wait 10 min, and measure absorbance at 700 nm versus an appropriate reagent blank. A plot of log absorbance versus log protein is linear over the range 3 to 400 μg of protein (eg, bovine serum albumin standard) (31).

An assay using TNBS to measure freed amino groups (30) uses *N,N*-dimethyl casein (20) (1 mg/mL) in buffer as substrate. To 1 mL in reaction tube, add 0.1 mL enzyme solution, incubate for the desired time, and heat the tube briefly in boiling water to stop the reaction. Add 1 mL 0.4 M phosphate buffer, pH 8.2 (containing 0.25% sodium dodecyl sulfate), 2 mL freshly made TNBS (1 mg/mL in water), and incubate in the dark 1 h at 60°C. Add 4 mL 0.1 N HCl, cool to room temperature, and measure absorbance at 340 nm. The reagent blank contains only buffer and TNBS; a complete assay with zero incubation time gives the correction for free amino groups in substrate and enzyme.

The absorbance change during the reaction of synthetic substrates with enzyme is recorded on a stripchart recorder, giving a continuous trace from which the initial velocity or the first-order reaction rate is obtained. Two assays will be described as examples.

1. TAME a substrate for trypsin (23). The reaction solution is 1.04 mM TAME (0.394 mg/mL) in 0.04 M Tris buffer, pH 8.1, 10 mM in CaCl. To 2.9 mL, add 0.1 mL enzyme solution and record the increase in absorbance at 244 nm.
2. FAGLA is a useful substrate for metalloproteases such as thermolysin (26). To 100 mL 0.05 M phosphate buffer, pH 7.2, add 76.9 mg FAGLA to give a 2.5 mM solution. This is well below the K_M of thermolysin for this substrate, so the reaction is kinetically first order. In the cuvette place 1.5 mL substrate, 1.4 mL buffer, and 0.1 mL enzyme, and measure the decrease in absorbance at 345 nm from 0.96 (zero time) toward an infinite-time value of 0.56. Plot log(absorbance − 0.56) versus time; the slope divided by 2.303 gives the first-order rate for the reaction, which is directly proportional to the concentration of enzyme. Preferably, a simple Basic computer program (1) may also be used to calculate the first-order rate constant.

Peptidase Assays. Carboxypeptidase is assayed by incubation with an *N*-acylated dipeptide, followed by measurement of the amino group freed (32). Substrate solution is 1 mM dipeptide (eg, 35.6 mg Z-Phe-Gly per 100 mL) in 0.025 M phosphate buffer, pH 7.2. To 200 μL add 50 μL enzyme, incubate for 1 h, stop by heating in boiling water, and measure free amino groups by reaction with TNBS as described previously. Carboxypeptidase may also be assayed continuously using Z-Gly-Phe in 0.05 M Tris buffer, pH 7.5, following the decrease in absorbance at 224 nm (27).

Aminopeptidase assays usually employ the β-naphthylamide of an amino acid. After incubation the freed β-naphthylamine is quantitated by diazotization (33). Substrate stock solution is 2 mM amino acid naphthylamide in 0.01 N HCl. Buffer is 0.025 M phosphate, pH 7.2. Color reagent is the diazonium salt Fast Garnet GBC (1 mg/mL) in 1M acetate buffer, pH 4.2, containing 10% (v/v) Tween 20. To the assay tube, add 1.6 mL buffer, 0.2 mL substrate, 0.2 mL enzyme, and incubate 3 h. Then add 1 mL color reagent, allow 5 min for color development, and read absorbance at 525 nm.

Carbohydrases

Substrates. Starch, the substrate for amylases, consists of a linear polymer, amylose, and a highly branched polymer, amylopectin. The ratio of these two components varies in starches obtained from different plant sources. They may be separated by treating a solution of gelatinized starch with thymol (34). In certain assays for α-amylase the use of one or the other fraction is preferable. If the assay involves colorimetric quantitation of freed reducing groups, the blank due to reducing ends of the substrate chain may be removed by reduction with $NABH_4$ (5).

Soluble cellulose substrates for cellulase are the carboxymethyl, hydroxypropyl, or hydroxyethyl derivatives (35). These are available as commercial products but should be characterized before use to ensure comparability between lots. Insoluble complexes of dyes with starch (36,37) or cellulose (38) are also available; enzyme activity is assayed by measuring the amount of dye that is solubilized during an incubation period.

Small synthetic substrates, usually the *p*-nitrophenylglycosides, are available for some glycosidases (39). Specific oligosaccharide derivatives have been used for assays of α-amylase and β-amylase (40,41).

Amylase Assays. For α-amylase (5), use 1 mL of a 1% solution of reduced starch in 0.02 M acetate buffer, pH 4.7, containing 1 mM $CaCl_2$. Add 1 mL enzyme solution, incubate for the time desired, then add 4 mL each of Reagent A and Reagent B of the neocuproine system (42). Heat in boiling water 12 min, make up to 25 mL, and read absorbance at 450 nm. Reagent A: in 600 mL water, dissolve 40 g anhydrous Na_2CO_3, 10 g glycine, and 0.45 g $CuSO_4 \cdot 5H_2O$. Make up to 1 L. Reagent B: dissolve 0.12 g neocuproine (2,9-dimethyl-1,10-phenanthroline-HCl) in 100 mL water. Store in a brown bottle.

Amylopectin forms a complex with I_2 which absorbs at 570 nm. As it is hydrolyzed by α-amylase the color decreases, the basis for a fixed end-point assay frequently used in cereals-related industries (43). A continuous assay based on this phenomenon is the following (44). Suspend 1 g soluble starch in 10 mL water, then slowly add this to 50 mL boiling water. Boil gently for 2 min, cool, and make up to 100 mL volume. To 2.5 mL buffered iodine solution (32 mg I_2, 330 mg KI, and 23.4 mg NaCl in 100 mL of 0.01 M phosphate buffer, pH 7.0), add 10 μL enzyme followed by 10 μL starch. Record the decrease in absorbance at 570 nm. This assay was developed for pancreatic α-amylase; for cereal α-amylases, the buffer system would be 0.02 M acetate, pH 4.7, 1 mM in $CaCl_2$.

α-Amylase is an *endo* glycosidase, hydrolyzing internal bonds in α-1,4 linked glucose polymers (40). A colorimetric assay using a blocked substrate takes advantage of this fact. The substrate is maltoheptaose, with a *p*-nitrophenyl group attached to the reducing end and a blocking agent (45) at the nonreducing end. α-Amylase cleaves this molecule in the middle. The assay mixture also contains glucoamylase and α-glucosidase, which combine to hydrolyze the short maltose (or maltotriose) derivative, freeing the chromogen, *p*-nitrophenol. The reaction is stopped by the addition of Tris buffer at pH 10, and the absorbance due to ionized *p*-nitrophenol is read at 410 nm.

β-Amylase hydrolyzes soluble starch to yield maltose, which is quantitated by colorimetric reaction with DNS (dinitrosalicylic acid) reagent (46). A 1% soluble starch solution is prepared as already described, except that 10 mL 0.16 M acetate buffer, pH 4.8, is added before adjusting to 100 mL total volume. Mix 0.5 mL substrate with 0.5 mL enzyme solution, incubate at 25°C for 3 min, then add 1 mL DNS reagent (50). Heat in boiling water 5 min, cool, add 10 mL water, and read absorbance at 540 nm. DNS reagent: in 500 mL water dissolve 10 g NAOH, 10 g 3,5-dinitrosalicylic acid, 2 g phenol, 0.5 g Na_2SO_3, and 200 g NaK tartrate, then make up to 1 L volume.

A chromogenic substrate, *p*-nitrophenyl maltopentaose, is used to assay this enzyme (41). β-Amylase removes two maltose units from the substrate, and α-glucosidase in the assay mixture then frees the chromogen. The reaction is stopped with alkaline buffer, and absorbance is read at 410 nm.

Cellulase Assays. Cellulase activity may be monitored using the DNS reagent (47). Dissolve 11.4 g CMC (carboxymethylcellulose) in 700 mL water with stirring, then add 7 g citric acid monohydrate, 19.6 g sodium citrate dihydrate, 0.1 g merthiolate, and 0.1 g glucose and make up to 1 L. To 1 mL substrate, add 1 mL enzyme solution, incubate 20 min at 50°C, add 3 mL DNS reagent, heat 15 min in boiling water, cool, and read absorbance at 540 nm. Cellulase activity may also be assayed by following the decrease in viscosity of a solution of CMC during incubation with enzyme (48,49). This requires a great deal of care and preparation and will not be discussed here.

Glycosidase. As an example of assays using nitrophenyl glycosides, that for β-galactosidase is given (50). In the reaction tube, combine 50 μL 0.1 M citrate buffer, pH 4.3, 50 μL 0.4% bovine serum albumin in water, 50 μL 20 mM pNP-β-galactoside in water, and 50 μL enzyme solution. After 30 min at 37°C, add 2 mL 0.25 M glycine, pH 10, and read the absorbance at 410 nm.

Xylanase. Interest has been growing recently in the use of xylanases in the food and feed industries. Xylans are pentose polymers, present primarily in cell walls of plants (an older name for them, based on this origin, is hemicellulose). Both soluble and insoluble types are found. The substrate for xylanases is usually derived either from a cereal grain (rye is the most abundant source) or wood (beech or larch gums). Assays may involve reducing-sugar techniques, viscometry, or chromogenic substrates (51). The chromogenic substrates are available in both soluble and insoluble (cross-linked) versions.

Xylanases, like amylases, may have either *endo* or *exo* specificities (similar to α- and β-amylases). Recently the two types have been separated and shown to have different kinds of improving actions in baked foods. Unfortunately, the specific assays that differentiate them have not been published. Using a soluble xylan as substrate, the *endo*-xylanase would show a rapid decrease in solution viscosity with a relatively slow increase in reducing groups, while the *exo*-xylanase would display the opposite behavior. The comparison of results from a viscometric assay and a reducing-sugar assay would readily differentiate the two types.

Ester Hydrolases

Lipase. Lipase acts on esters at the interface between water and oil (52); the substrate is a liquid vegetable oil (olive oil, soy oil) purified by percolation over a column of activated alumina or silica gel. It is emulsified with the help of vegetable gum or bile salt. The release of fatty acid is monitored by addition of base to keep the pH constant (continuous titrimetry, or pH stat, method) or by extraction and colorimetric measurement of the copper soap (53). For the latter assay, make substrate emulsion by adding 2 mL vegetable oil solution (1% in absolute EtOH) to 100 mL 0.025 M Tris buffer, pH 8.8, containing 0.6% sodium deoxycholate. To 5 mL substrate, add 0.1 mL enzyme, incubate 5 min, add 1 mL 1 N HCI and 10 mL isooctane, shake, and allow phase separation. To 5 mL of the upper (organic solvent) layer, add 1 mL copper reagent (5% cupric acetate in water, pH adjusted to 6.1 with pyridine), shake vigorously for 90 s, and allow the phases to separate. Read the absorbance at 715 nm of the upper phase.

Esterase. The best assay for esterase activity is the pH stat method. The substrate in water or salt solution is adjusted to the desired pH, enzyme is added, and base is continually added to keep the pH constant. The plot of base consumption versus time gives the rate of the reaction. For a spectrophotometric assay the system is lightly buffered at the desired pH, an indicator dye is included, and the change in absorption is measured. An assay for pectin methylesterase (54) uses 0.5% citrus pectin in water adjusted to pH 7.5, an indicator/buffer system of 0.01% bromthymol blue in 3 mM phosphate buffer, pH 7.5, and enzyme in the same buffer. To 2 mL of pectin solution, add 0.15 mL indicator/buffer and 0.83 mL water. Record absorbance at 620 nm briefly to establish a baseline, then add 20 μL enzyme and record the rate of absorbance change due to esteratic action. The system (without enzyme) is titrated with galacturonic acid to establish the correspondence between absorbance change and acid concentration.

Phosphatase. Phosphatase activity is monitored by colorimetric quantitation of P_i (inorganic phosphate) formed (55). An example is the assay for acid phosphatase using glucose-6-phosphate (G-6-P) (56). Substrate is 0.2 M maleate buffer, pH 6.7, containing 60 mM G-6-P, 8 mM KF, and 8 mM EDTA. To 1 mL, add 1 mL enzyme extract, incubate 20 min at 37°C, and add 1 mL cold 10% TCA to stop. To 1 mL, add 2 mL P_i reagent, hold 20 min at 45°C, and read absorbance at 820 nm. Reagent A: 10% (w/v) ascorbic acid in water. Reagent B: 4.2 g ammonium molybdate tetrahydrate in 1 L 1 N H_2SO_4. P_i reagent: 1 part A plus 6 parts B, made fresh daily, and kept in an ice bath until used.

Oxidases

Polyphenoloxidase. Polyphenoloxidase enzymes (eg, tyrosinase) catalyze two reactions: (*1*) oxygenation of a phenol to *o*-diphenol (cresolase) and (*2*) oxidation of a diphenol to o-quinone (catecholase). Substrates commonly used for assays are *p*-cresol (4-methylphenol), L-tyrosine (4-hydroxy phenylalanine), and *p*-coumaric acid (4-hydroxycinnamic acid). The corresponding diphenols are 4-methylcatechol, L-Dopa, and caffeic acid. Rates are monitored by absorbance changes at specific wavelengths as oxygenation or oxidation occurs. An assay system that differentiates the two kinds of activities is the following (57). For cresolase activity, mix 1.5 mL 0.05 M acetate, pH 4.8, 0.4 mL 1 mM *p*-cresol, 0.1 mL 1 mM 4-methylcatechol and 10 μL enzyme. Record increase in absorbance at 291 nm. The change is due only to oxygenation; oxidation of diphenol to quinone produces no absorbance change at 291 nm. For catecholase activity, mix 1.6 mL buffer, 0.4 mL 1 mM 4-methylcatechol, and 10 μL enzyme, recording the decrease in absorbance at 280 nm.

Lipoxygenase. Native lipoxygenase requires activation by hydroperoxydecadienoic acid, the product of its reaction with linoleic acid and oxygen; there is a lag period in the reaction if enzyme is simply mixed with a solution of substrate (58). The delay is removed by including a small amount of product in the stock substrate solution (168 mg linoleic acid and 14 mg hydroperoxylinoleic acid in 100 mL ethanol) (59). For assay, to 2.9 mL 0.2 M borate buffer, pH 9.0, add 50 μL substrate stock, followed by 50 μL enzyme solution. Mix and record the increase in absorbance at 235 nm.

Ascorbic Acid Oxidase (AAO). A simple spectrophotometric assay for AAO is based on the loss of absorbance at 265 nm when ascorbate is oxidized to dehydroascorbate (60,61). To 2.8 mL buffer (0.025 M citrate, 0.05 M phosphate, pH 5.6), add 100 μL substrate solution (0.05% ascorbate, 1% NA_2EDTA, neutralized), 50 μL 1% bovine serum albumin solution, and finally 50 μL enzyme solution. Record the rate of disappearance of absorbance at 265 nm.

Catalase, Peroxidase. For peroxidase, the reaction mixture is 0.01 M phosphate, pH 7.0, containing 25 mg/L H_2O_2 and 2.5 g/L pyrogallol (62). After adding enzyme, record the increase at 430 nm due to the formation of purpurogallin. The reaction of catalase with H_2O_2 is a first-order reaction (63). Hydrogen peroxide has a broad absorption band in the far ultraviolet; molar absorbance is 120 at 200 nm and 30 at 250 nm. To 2 mL assay solution (0.05 M phosphate, pH 7.0, containing 2 g/L H_2O_2), add 1 mL diluted catalase solution and record the ultraviolet absorbance. Plot log absorbance versus time; the slope divided by 2.303 equals the first-order rate constant, which is directly proportional to the amount of catalase in the assay (or use a computer program to calculate the rate constant).

Other Enzymes

Lysozyme. Hydrolysis of bacterial cell walls by lysozyme causes the cells to lyse; a turbid suspension of cells will slowly clear. The turbidimetric assay (64) uses a suspension of *Micrococcus lysodeikticus* (0.5 g/L) in 0.06 M phosphate, pH 6.2. To 1.5 mL, add 0.5 mL 0.3 M NaCl and 1 mL enzyme, place the cuvette in a colorimeter, and record % transmittance (not absorbance) at 540 nm. A plot of %T versus time is linear from about 10 to 40%, and the rate %T/min is proportional to enzyme in the range of 0 to 10 μg.

BIBLIOGRAPHY

1. C. E. Stauffer, *Enzyme Assays for Food Scientists*, Van Nostrand Reinhold, New York, 1989.

2. K. F. Tipton and H. B. F. Dixon, "Effects of pH on Enzymes," *Methods Enzymol.* **63**, 183–233 (1979).
3. W. W. Cleland, "The Use of pH Studies to Determine Chemical Mechanism of Enzyme-Catalyzed Reaction," *Methods Enzymol.* **87**, 390–404 (1982).
4. R. L. Van Etten, "Human Prostatic Acid Phosphatase: A Histidine Phosphatase," *Ann. N.Y. Acad. Sci.* **390**, 27–51 (1982).
5. D H. Strumeyer, "A Modified Starch for Use in Amylase Assays," *Anal. Biochem.* **19**, 61–71 (1967).
6. H. Perten, "Application of the Falling Number Method for Evaluating Alpha-Amylase Activity," *Cereal Chem.* **41**, 127–140 (1964).
7. M Monsigny, C. Kieda, and T. Maillet, "Assay for Proteolytic Activity Using a New Fluorogenic Substrate (Peptidyl-3amino-9-ethylcarbazole)," *EMBO J.* **1**, 303–306 (1982).
8. B. Hagihara et al., "Crystalline Bacterial Proteinase. 1. Preparation of Crystalline Proteinase of Bacillus subtilis," *J. Biochem.* **45**, 185–194 (1958).
9. W. W. Cleland, "Statistical Analysis of Enzyme Kinetic Data," *Methods Enzymol.* **63**, 103–137 (1979).
10. J -M Caillat and R. Drapron, "La lipase du blé. Caracteristiques de son action en milieux aqueux et peu hydrate," *Annales de Technologie Agricole* **23**, 273–286 (1974).
11. G. L. Atkins and I. A. Nimmo, "Current Trends in the Estimation of Michaelis-Menten Parameters," *Anal. Biochem.* **104**, 1–9 (1980).
12 H.-J. Lee and I. B. Wilson, "Enzymic Parameters: Measurement of V and K_m" *Biochim. Biophys. Acta* **242**, 519–522 (1971).
13. M. L. Kakade et al., "Determination of Trypsin Inhibitor Activity of Soy Products: A Collaborative Analysis of an Improved Procedure," *Cereal Chemistry* **51**, 376–382 (1974).
14. G. E. Hamerstrand, L. T. Black, and J. D. Glover, "Trypsin Inhibitors in Soy Products: Modification of the Standard Analytical Procedure," *Cereal Chemistry* **58**, 42–45 (1981).
15. C. E. Stauffer, "Measuring Trypsin Inhibitor in Soy Meal: Suggested Improvements in the Standard Method," *Cereal Chemistry* **67**, 296–302 (1990).
16. American Association of Cereal Chemists (AACC), *Approved Methods of the American Association of Cereal Chemists*, 8th ed., AACC, St. Paul, Minn., 1983, Method 22-62.
17. M. L. Anson, "The Estimation of Pepsin, Trypsin, Papain and Cathepsin with Hemoglobin," *J. Gen. Physiol.* **22**, 79–89 (1938).
18. R. M. Tomarelli, J. Charney, and M. L. Harding, "The Use of Azoalbumin as a Substrate in the Colorimetric Determination of Peptic and Tryptic Activity," *J. Lab. Clin. Med.* **34**, 428–433 (1949).
19. C. Schwabe, "A Fluorescent Assay for Proteolytic Activity," *Anal. Biochem.* **53**, 484–490 (1973).
20. Y. Lin, G. E. Means, and R. E. Feeney, "The Action of Proteolytic Enzymes on *N,N*-Dimethyl Proteins," *J. Biol. Chem.* **244**, 789–793 (1969).
21. G W. Schwert and Y. Takenaka, "A Spectrophotometric Determination of Trypsin and Chymotrypsin," *Biochim. Biophys. Acta* **16**, 570–575 (1955).
22. B. F. Erlanger, N. Kowosky, and W. Cohen, "The Preparation and Properties of Two New Chromogenic Substrates of Trypsin," *Arch. Biochem. Biophys.* **95**, 271–278 (1961).
23. B. C. W. Hummel, "A Modified Spectrophotometric Determination of Chymotrypsin, Trypsin and Thrombin," *Can. J. Biochem. Physiol.* **37**, 1393–1399 (1959).
24. K. A. Walsh and P. E. Wilcox, "Serine Proteases," *Methods Enzymol.* **19**, 31–41 (1970).
25. K. A. Walsh, "Trypsinogens and Trypsins of Various Species," *Methods Enzymol.* **19**, 41–63 (1970).
26. J. Feder, "A Spectrophotometric Assay for Neutral Protease," *Biochem. Biophys. Res. Commun.* **32**, 326–332 (1968).
27. P. H. Petra, "Bovine Procarboxypeptidase and Carboxypeptidase A," *Methods Enzymol.* **19**, 460–503 (1970).
28. O. H. Lowry et al., "Protein Measurement With the Folin Phenol Reagent," *J. Biol. Chem.* **193**, 265–275 (1951).
29. P. K. Smith et al., "Measurement of Protein Using Bicinchoninic Acid," *Anal. Biochem.* **150**, 76–85 (1985).
30. J. Adlers-Nissen, "Determination of the Degree of Hydrolysis of Food Protein Hydrolysates by Trinitrobenzenesulfonic Acid," *J. Agric. Food Chem.* **27**, 1256–1262 (1979).
31. C. E. Stauffer, "A Linear Standard Curve for the Folin-Lowry Determination of Protein," *Anal. Biochem.* **69**, 646–648 (1975).
32. J. E. Kruger and K. Preston, "The Distribution of Carboxypeptidases in Anatomical Tissues of Developing and Germinating Wheat Kernels," *Cereal Chem.* **54**, 167–174 (1977).
33. J. E Kruger and K. R. Preston, "Changes in Aminopeptidases of Wheat Kernels During Growth and Maturation," *Cereal Chem.* **55**, 360–372 (1978).
34. H. V. Street and J. R. Close, "Determination of Amylase Activity in Biological Fluids," *Clinica Chimica Acta* **1**, 256–268 (1956).
35. J. J. Child, D. E. Eveleigh, and A. S. Sieben, "Determination of Cellulase Activity Using Hydroxyethylcellulose as Substrate," *Can. J. Biochem.* **51**, 39–43 (1973).
36. M. Ceska, E. Hultman, and B. Ingleman, "The Determination of Alpha-amylase," *Experientia* **25**, 555–556 (1969).
37. B. Klein, J. A. Foreman, and R. L. Searcy, "The Synthesis and Utilization of Cibachron Blue-Amylose: A New Chromogenic Substrate for Determination of Amylase Activity," *Anal. Biochem.* **31**, 412–425 (1969).
38. T. K. Ng and J. G. Zeikus, "A Continuous Spectrophotometric Assay for the Determination of Cellulase Solubilizing Activity," *Analytical Biochemist* **103**, 42–50 (1980).
39. S. Matsubara, T. Ikenaka, and S. Akabori, "Studies on Takaamylase A. VI. On the α-Maltosidase Activity of Takaamylase A," *J. Biochem.* **46**, 425–431 (1959).
40. B. V. McCleary and H. Sheehan, "Measurement of Cereal α-Amylase: A New Assay Procedure," *J. Cereal Sci.* **6**, 237–251 (1987).
41. B. V. McCleary and R. Codd, "Measurement of β-Amylase in Cereal Flours and Commercial Enzyme Preparations," *J. Cereal Sci.* **9**, 17–33 (1989).
42. S Dygert et al., "Determination of Reducing Sugar With Improved Precision," *Anal. Biochem.* **13**, 367–374 (1965).
43. American Association of Cereal Chemists (AACC), Approved Methods of the American Association of Cereal Chemists, 8th ed., AACC, St. Paul, Minn., 1983, Method 22-01.
44. L. B. Marshall and G. D. Christian, "A Rapid Spectrophotometric Method for Iodimetric α-Amylase Assay," *Anal. Chim. Acta* **100**, 223–228 (1978).
45. U.S. Pat. 4 649 108 (March 10, 1987) H. E. Blair (to Genzyme Corporation).
46. P. Bernfeld, "Amylases, α and β," *Methods Enzymol.* **1**, 149–158 (1955).
47. G. L. Miller et al., "Measurement of Carboxymethyl-cellulase Activity," *Anal. Biochem.* **1**, 127–132 (1960).

48. M. A. Hulme, "Viscometric Determination of Carboxymethylcellulase in Standard International Units," *Arch. Biochem. Biophys.* **147**, 49–54 (1971).
49. K. Manning, "Improved Viscometric Assay for Cellulase," *J. Biochem. Biophys. Methods* **5**, 189–202 (1981).
50. J. J. Distler and G. W. Jourdian, "The Purification and Properties of α-Galactosidase From Bovine Testes," *J. Biol. Chem.* **248**, 6772–6780 (1973)
51. B. V. McCleary, "Measurement of *endo*-1,4-β-D-Xylanase", in J. Visser, ed., *Xylans and Xylanases*, Elsevier Science Publishers B. V., Amsterdam, 1992, pp. 161–170.
52. L. Sarda and P. Desnuelle, "Action de la lipase pancreatique sur les esters en emulsion," *Biochim. Biophys. Acta* **30**, 513–521 (1958).
53. D. Y. Kwon and J. S. Rhee, "A Simple and Rapid Colorimetric Method for Determination of Free Fatty Acids for Lipase Assay," *J. Am. Oil Chem. Soc.* **63**, 89–92 (1986).
54. A. E. Hagerman and P. J. Austin, "Continuous Spectrophotometric Assay for Plant Pectin Methylesterase," *J. Agric. Food Chem.* **34**, 440–444 (1986).
55. P. S. J. Chen, T. Y. Toribara, and H. Warner, "Microdetermination of Phosphorus," *Anal. Chem.* **28**, 1756–1758 (1956).
56. A. Rossi et al., "Properties of Acid Phosphatase From Scutella of Germinating Maize Seeds," *Phytochemistry* **20**, 1823–1826 (1981).
57. M. H. Keyes and F. E. Semersky, "A Quantitative Method for the Determination of the Activities of Mushroom Tyrosinase," *Arch. Biochem. Biophys.* **148**, 256–261 (1972).
58. B. Axelrod, T. M. Cheesbrough, and S. Laakso, "Lipoxygenase from Soybeans," *Methods Enzymol.* **71**, 441–451 (1981).
59. M. J. Gibian and R. A. Galaway, "Steady-State Kinetics of Lipoxygenase Oxygenation of Unsaturated Fatty Acids," *Biochemistry* **15**, 4209–4214 (1976).
60. E. Racker, "Spectrophotometric Measurements of the Metabolic Formation and Degradation of Thiol Esters and Enediol Compounds," *Biochim. Biophys. Acta* **9**, 577–578 (1952).
61. T. Tono and S. Fujita, "Determination of Ascorbic Acid by Spectrophotometric Method Based on Difference Spectra. II. Spectrophotometric Determination Based on Difference Spectra of L-Ascorbic Acid in Plant and Animal Foods," *Agric. Biol. Chem.* **46**, 2953–2959 (1982).
62. B. Chance and A. C. Maehly, "Assay of Catalases and Peroxidases," *Methods Enzymol.* **2**, 764–775 (1955).
63. R. M. J. Parry, R. C. Chandan, and K. H. Shahani, "A Rapid and Sensitive Assay of Muramidase," *Proc. Soc. Exp. Biol. Med.* **119**, 384–386 (1965).

CLYDE E. STAUFFER
Technical Food Consultants
Cincinnati, Ohio

ENZYMES IN FOOD PRODUCTION

The use of enzymes in the formulation and processing of food products is frequently thought of in terms of the addition of commercially available enzymes from an industrial source, that is, using enzymes as additives from an external or exogenous source. However, it should be recognized that virtually all food materials are, or were, living organisms and, as such, may contain a number of enzymes normally utilized in the metabolic pathways of the organism. These endogenous enzymes can play important roles in food processing. In some cases these enzymes provide an improving function, whereas in others their effects can be deleterious either to the processing itself and/or to the final product characteristics. This article will provide a background describing endogenous enzymes from a number of food sources and briefly describe how these enzymes may influence processing of the food material.

GENERAL

The amount and type of endogenous enzyme present in the fruit of a plant, whether a cereal grain seed or a fruit such as an apple or orange, depends, to a large extent, on the maturation stage of the plant. As a seed or fruit ripens, its enzyme content changes. As ripening proceeds, more hydrolytic enzymes are produced. In the case of a cereal grain, these enzymes begin the process of breaking down carbohydrate and protein stored in the seed in order to provide energy and amino acids necessary for the synthesis of new plant parts. While necessary for plant growth, these enzymes can have quite detrimental effects when flour from germinated wheat kernels is used in the production of foods, particularly in baked goods.

In ripening fruits, analogous enzymes begin a similar process of cellular breakdown that leads to characteristic loss of firmness normally associated with overripe, less desirable produce. The freshness of fruits and vegetables is often judged by the color and texture of the produce such as, for example, the bright green color and firm texture of fresh peas, beans, or peppers. When many products such as these ripen, their color changes to red, orange, yellow, brown, or even black. This is due, to a large degree, to endogenous enzymes, some of which degrade the green chlorophyll, thus changing the color of the product. Other enzymes such as lipoxygenase attack triglycerides forming free radicals and hydroperoxides. These free radicals can also lead to loss of green color due to oxidative degradation of chlorophyll and also changes in reds and orange colors due to reactions with carotenoid compounds. Oxidative reactions from these compounds can also lead to nutritional damage in the food product through detrimental action on vitamins as well as some proteins. Other color changes are also found to result from enzymatic browning reactions involving polyphenol oxidase (see "Browning Reactions—Color and Flavor") (1).

Texture is also affected by endogenous enzymes that hydrolyze cellular material, most of which is composed of various forms of carbohydrate material. Most commonly found in plants, these include cellulose, hemicellulose, starch, pectic material, and lignin. These carbohydrates are involved in cell structure of most plants and are responsible for the firm texture in young, preripe fruits and vegetables.

Meat color is also influenced by endogenous enzymes involved in oxidation–reduction reactions that alter the oxidation state of several forms of myoglobin as well as the amount of available oxygen. Changes in the oxidation state of oxymyoglobin and deoxymyoglobin can lead to production of metmyoglobin, which is responsible for the brown coloration in meat (1).

BAKED GOODS

Although a number of cereal grains may be used in the production of baked goods, the primary source for such products is wheat. Wheat is utilized in the production of baked goods such as breads, cakes, rolls, and pastries. The enzymes of greatest interest for this discussion will be those involved in protein and carbohydrate metabolism, specifically, the amylases, pentosanases, and proteases. Amylases are starch-degrading enzymes that act on the linkages between the glucose monomers making up the starch polymer. In starch, these linkages are either α-1,4 or α-1,6 glucosidic bonds connecting adjacent glucose residues. β-Amylase (EC 3.22.1.2) is an exo-type hydrolase that is able to hydrolyze only the penultimate α-1,4 bond, each cleavage producing a molecule of maltose. α-Amylase (EC 3.2.1.1) is an endo-type hydrolase that, while specific for the α-1,4 glucosidic bond, is capable of hydrolyzing such bonds anywhere along the internal portion of the glucose chain, producing small amounts of glucose as well as many different glucose oligomers. The difference between the two enzymes is important because they have different effects on the rheology of a starch system. β-Amylase has relatively little effect on the molecular weight of the starch molecule because it removes only one maltose at a time from starch molecules having molecular weights as large as several million. α-Amylase, however, can drastically alter the molecular weight because it can hydrolyze anywhere along the chain. That is, it can hydrolyze an internal α-1,4 glucosidic bond about in the middle of the glucose chain and immediately reduce the molecular weight by half. This action can greatly affect (decrease) the viscosity of the starch system.

Under ideal harvest conditions, wheat is in a dormant storage stage and contains comparatively few active enzymes. There may be significant amounts of β-amylase, but α-amylase levels are quite low. Thus, the amount of potentially detrimental endogenous starch-degrading enzyme in the sound wheat kernel is low. However, it is not unusual, particularly in the Pacific Northwest, that harvest conditions are not ideal. The most serious consequence from the standpoint of enzyme content is when harvest is preceded by humid, rainy conditions. In this environment, the seed breaks its dormancy and begins to grow. This phenomenon is commonly referred to as "sprouting" in the grain industry. This growth requires that the stored carbohydrate and protein be utilized for energy and synthesis of new plant parts. Thus, enzymes such as those which hydrolyze carbohydrate and protein material are synthesized and act on the stored material in the plant seed. It is the plant seed that is then processed into a food material such as wheat flour.

Whether the endogenous enzymes are a factor in utilizing the food material depends on a number of factors. First, how extensive was the sprouting? It may range from slight, in which case little enzyme was synthesized, to extensive, in which case a large amount of carbohydrase and proteinase enzymes have probably been formed. Second, what are the processing conditions for the manufacture of the food material? In general, high-moisture products such as breads will be much more susceptible to the action of these hydrolytic enzymes than will low-moisture products such as crackers. In bread processing, the dough may contain approximately 35 to 40% moisture, and the final baked product retains much of that moisture. Since processing involves a lengthy proofing period as well as the bake time, the presence of such high levels of water (most with a water activity [a_w] of 0.95–0.98) may lead to extensive hydrolysis if significant levels of endogenous enzymes are present.

In the dough stage, only that portion of the starch (both amylose and amylopectin) that is physically damaged in the milling process is susceptible to amylolytic attack. Depending on the type of wheat, the amount of starch damage may vary from about 3 to 8%. The amylase enzymes can attack the damaged starch and will release some water that was associated with the starch; however, the effect on the dough consistency will be relatively minor. However, the starch will gelatinize during the baking process as the internal temperature of the loaf reaches about 60 to 65°C (104°F). Both amylase enzymes may then rapidly degrade the gelatinized starch. However, it should be noted that endogenous amylase enzymes are relatively unstable above 65°C, and so are less effective as the temperature increases. The overall effect of the starch-degrading enzymes will depend on the amount of endogenous enzyme present and how long it has to act on the gelatinized starch.

It was thought for many years that α-amylase was the enzyme from sprouted wheat that was most responsible for the detrimental effects observed during baking of high-moisture bread products, but this is not the case. The most damaging of the hydrolytic enzymes in such a process are the proteases. Sprouted wheat contains proteolytic enzymes that can act on the protein fraction throughout the dough stage and into the baking stage. The result of excessive hydrolysis by proteolytic enzymes is a loss of gluten structure. This is manifest as a slack dough that has lost the viscoelasticity characteristic of a well-developed wheat flour dough. The finished product will have poor volume and texture characteristics. If hydrolysis proceeds to an extreme, all gluten structure may be lost, resulting in a very sticky, slack semiliquid. Some effects of these enzymes may be observed even when the degree of sprouting is relatively low. An assay for this type of enzyme would serve as a good diagnostic indicator of the quality of the flour used in an industrial food process.

In low-moisture baked products such as crackers where the final moisture content of the product is only about 3%, endogenous enzymes usually have less potential for detrimental effects. Because the processing conditions require less time compared with breads, and water bakeout in the oven during baking is more rapid, starch-degrading enzymes usually have relatively little effect. The rapid loss of water in the initial stage of baking leads to more rapid increases in the temperature of the dough piece, thus tending to inactivate the enzymes faster. In addition, because the available water is driven off rapidly, the starch gelatinization temperature increases such that relatively little starch is gelatinized in most cracker products. Thus, the amylolytic enzymes have much less of an opportunity to hydrolyze the starch in its most susceptible condition. Proteolytic enzymes may have an effect even in low-moisture products, but this will depend on proof time (long or short), since they will normally be rapidly inactivated in the baking process.

Unsprouted wheat flour also contains endogenous protease enzyme(s). These enzymes are activated only at relatively low pH (<4.0) and so are not normally active in most baked product processes. However, processing of fermented products such as soda crackers utilize long periods during which lactobacilli-based fermentation leads to low pH in the sponge stage. These low-pH endogenous proteases can be an important processing factor under these conditions, softening the dough and helping to "mellow" the final cracker dough-handling characteristics. Many chemically leavened cracker doughs have relatively basic pH values, so the endogenous low-pH proteases will not be activated.

Lipoxygenase enzymes in cereals, especially soybeans and wheat, may have significant consequences, both good and adverse. The desirable effect is that of bleaching the carotenoid pigments, resulting in whiter products made from the flour. This is a desirable attribute in products such as white bread and rolls. The amount of the lipoxygenase enzymes is greater in soy compared with wheat; thus, wheat flour may be supplemented with soy flour to achieve the bleaching effect. The downside to this activity is that too much lipoxygenase activity may lead to oxidation of native flour lipids, producing an undesirable rancid flavor/aroma characteristic. The type I lipoxygenase enzyme acts only on free fatty acids while type II lipoxygenase can act on triglycerides as well.

The pentosan fraction of the flour is an important functional participant in the formation of the dough. Though normally only about 2 to 3% of the flour, it is capable of absorbing up to 25% of the moisture in the dough. Hydrolysis of the pentosan fraction would have a significant effect on dough viscosity. However, at present, while pentosanase activity has been detected in germinated wheat, the levels appear to be quite low and of relatively little practical significance in terms of processing effects. Exogenous pentosanases, available commercially, may be used to greatly alter dough viscosity by destroying the water-holding capacity of the pentosan network.

Flour may also be used in nonbaking applications. For example, flour is often added to soup as a thickening agent. Endogenous enzymes, which may be present in that flour, are normally inactivated during the retorting step in the manufacture of the soup. If the flour is sound (unsprouted) containing relatively low levels of hydrolytic enzymes, the enzymes have little effect prior to retorting, and the desired thickening effect is maintained in the canned soup for long periods. However, if a sprouted wheat flour is used in this application, the endogenous enzymes can, prior to retorting, hydrolyze both starch and protein components, producing a thin, watery soup consistency.

FRESH FRUITS AND VEGETABLES

As mentioned in the "General" section, most plants contain various forms of carbohydrate, either as part of their cellular structure or as components of their "flesh." These include starch, cellulose, hemicellulose, pectins, and lignins. Several endogenous pectin-degrading enzymes contribute to the softening of many fruits and vegetables. Pectin methylesterase (EC 3.1.1.11) hydrolyzes the methyl ester from the methylated galactose units of pectin to form pectic acid and methanol. In the presence of sufficient calcium ion (Ca^{+2}) a complex is formed that can actually lead to an increase in firming of the plant tissue. However, this reaction can also lead to some problems in the fruit juice industry (see "Fruit juices"). Another enzyme, polygalacturanase, splits the α-1,4 glycosidic bond between adjacent galacturonosyl units. There are actually several forms of this enzyme, an endo-acting form (EC 3.2.1.15) and two exo-acting forms, EC 3.2.1.67 and EC 3.2.1.82 (2), which release D-galacturonic acid. These enzymes lead to extensive degradation of the substrate carbohydrate, leading to significant loss of structural integrity and a concomitant loss of firmness in the product. There is also a pectate lyase (EC 4.2.2.10) found, so far, only in microorganisms, which also splits the galacturonosyl chain at the α-1,4 glycosidic linkage leading to a loss of structural integrity. Although not strictly an enzyme endogenous to the plant, the microorganisms containing the enzyme may often be found to be present, thereby affecting the processing conditions in the absence of added commercial enzymes.

Another common plant cell wall constituent is cellulose. Cellulases (EC 3.2.1.4) are found in many plants, primarily in trees, but also in fruits and vegetables. This enzyme acts on the β-1,4 glucosidic bonds found in cellulose. Though cellulose is closely related to starch in being a polymer of glucose monomers, amylase enzymes cannot hydrolyze cellulose, and cellulases have no effect on starch. This is due to the different geometry of the β-1,4 bond in cellulose as compared with the α-1,4 bond found in starch. The loss of firmness attributable to the enzyme found in fruits and vegetables is still unconfirmed, but cellulases of microbial origin, often found with the plant, are known to be effective at hydrolyzing plant cellulose.

Pentosans, sometimes referred to as hemicellulose, are polymers of xylose, arabinose, or both. Generally the material is an arabinoxylan with β-1,4 linkages between the backbone xylose units. These polymers are commonly found in higher plants and are particularly important in cereal products because the pentosan polymer is capable of absorbing very large amounts of water, thus affecting formulations. There seems to be relatively little endogenous pentosanase enzyme active in cereals.

BEVERAGES

Alcoholic

Beer is a virtually universal libation of quite ancient origin. The methods used today are cleaner and more efficient, but the fundamental approach remains similar to that developed thousands of years ago. By accident or design, brewing beer represents one of the first systematic uses of enzyme technology in the production of a food product.

The basic goal of brewing is to convert carbohydrate to alcohol and protein to peptides and amino acids. Most often, the source of these substrates is the seed of the barley plant, though other seeds are also used. In many areas of Africa, for example, grain sorghum seed is utilized in the brewing process. As described for wheat kernels, these seeds also store energy as starch and amino acids as proteins. The first step in the brewing process involves "malting" of the barley seed. This is a process of controlled germination resulting in the synthesis of hydrolytic enzymes. The barley kernels are steeped in water under controlled temperature to a moisture content of about 45% and then allowed to germinate, usually on large floor areas where good aeration is maintained by turning and mixing the grain. After a suitable period at controlled temperature (about 15–20°C) the germinated grain is dried at 35 to 40°C for about 12 hours and subsequently ground to a course powder (3). This material then contains much increased levels of hydrolytic enzymes, primarily amylase and protease activity, which are essential to the brewing process.

As described previously in the baking section, the amylase enzymes (both alpha and beta) hydrolyze starch-producing sugars (maltose and glucose) and some oligosaccharides. With the addition of yeast (normally a strain of *Saccharomyces*) in a process known as "pitching," the glucose is converted to alcohol and CO_2 according to the well-known equation:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH$$

Yeast utilizes a maltase enzyme to convert the maltose to glucose. Thus, this step in the brewing process is facilitated through the action of endogenous enzymes from both the barley seed as well as a series of enzymatic steps utilizing enzymes endogenous to the yeast.

The same basic process of converting carbohydrate to alcohol is utilized in the production of distilled spirits. Such products have an ethanol content of about 20% and higher (beer having about 3–6% and wine having about 10–15% alcohol). While the variety of such alcoholic beverages is quite large, those derived from cereals will be the focus of this discussion. Many different cereal grains, including wheat, rye, barley, corn, and others, are used in the production of alcohol spirits. The source grains are ground, mixed with water, and heated to affect the gelatinization of the starch. The conversion of the starch to sugar is then accomplished by the addition of a malt containing high levels of saccharifying enzymes (α- and β-amylase). The "mash" is mixed and stirred under controlled conditions producing high levels of fermentable maltose and glucose. The mash is heated to inactivate the enzymes, cooled and fermented. This mixture is then distilled to produce the elevated alcohol content characteristic of such beverages. Again it is the use of the endogenous malt enzymes that are central to the production of these products.

Fruit Juices

Fruit juices are another category of beverage in which endogenous enzymes may play a role in the production process. Unlike cereals, the polysaccharides of fruits are primarily cellulose, hemicellulose, and pectic material. These compounds tend to cause a number of production problems, most of which are addressed by the addition of exogenous enzymes. This is the usual approach because most fruits intended for human consumption are harvested prior to achieving biological ripeness in order to maintain the characteristics (color and firmness) desired by the consumer. The native enzymes, which would be expressed with advanced ripening, are not available. Thus, the problems of clouding and poor filtration associated with the presence of pectic material and cellulose and hemicellulosic compounds are normally treated with microbially derived enzymes added as processing aids.

One interesting problem, normally characteristic of orange juice, is the loss of cloud stability in the juice. Orange juice is normally produced by pressing the juice from the fruit. Rather than being clear, the juice is meant to be an opaque liquid. The insoluble material that accompanies the juice as the fruit is pressed contributes desirable color, opaqueness, and flavor characteristics. Loss of this cloudiness is associated with the action of an endogenous enzyme, a pectin methylesterase (EC 3.1.1.11), which deesterifies the pectin to pectic acid. The ionized pectic acid is then able to form a complex with normally present calcium ions (Ca^{+2}), which results in a gel-like complex. This complex will precipitate over time as an undesirable layer of sediment leaving a clarified juice above it. There are several approaches to preventing this troublesome condition. One involves the heating of the juice to a temperature sufficient to inactivate the offending pectin methylesterase. This approach, however, has a deleterious effect on the flavor components of the juice and thus represents an approach of last resort. A more common and less detrimental solution is the addition of an exogenous polygalacturonase (EC 3.2.1.15), which depolymerizes the pectic acid prior to significant formation of the calcium complex (2).

Although the formation of this calcium–pectic acid complex presents problems for the orange juice industry, it can be used to advantage in other circumstances. For example, many vegetables tend to lose their firmness after harvest, particularly after canning. The addition of calcium chloride to canned tomatoes results in firming of the fruit. The firming has been attributed to the formation of the calcium–pectic acid complex. This phenomenon has been used to achieve desirable firming in many vegetables as well, including potatoes, peppers, and beans.

DAIRY FOODS

Cheeses are derived from the milk of cows, goats, and other ruminant animals. The textural characteristics of cheeses are determined, to a large extent, by the methods of curd manufacturing, but the flavors and aromas of cheeses are developed primarily in the ripening process. Cheese ripening is an enzymatic process involving enzymes endogenous to the milk, to the ruminants from which the milk is taken, and to microorganisms that are an essential part of the cheese-making process.

Milk protein is composed primarily from a set of proteins collectively called casein. These proteins form an association in milk to form micelles that remain suspended. These suspended micelles, along with fat globules and calcium phosphate, give milk its characteristic opaque white color. The first step in making cheese is the formation of a curd. Usually, the curd results from treatment of whole milk with acid or with enzyme to precipitate the casein fractions. Traditionally, this curd formation was accomplished using rennin (EC 3.4.23.4), an enzyme from the stomach of ruminants. The enzyme has a restricted specificity such that the hydrolysis proceeds to the extent necessary to precipitate the milk protein without causing extensive further protein hydrolysis. A commercially available substitute for rennin derived from microorganisms is called rennet. Other commercially available proteases may be used, but many cause too much hydrolysis, resulting in the formation of "bitter peptides" that contribute undesirable flavor characteristics. Protease treatment accomplishes the concentration of the milk protein into a dense curd, but it also initiates reactions that contribute to flavor development. This flavor development is enhanced by protease enzymes from the milk itself, primarily plasmin (EC 3.4.21.7), as well as enzymes endogenous to microorganisms used in the cheese process. These microorganisms may be bacterial and/or fungal depending on the specific cheese.

A number of processes occur during ripening, all enzyme mediated, which contribute to textural and flavor characteristics. They can be described by three categories of enzymatic activity. A more detailed description of each of these stages may be found in Reference 4. First, probably the most important enzymatic process involves proteolysis of the milk protein by rennin, plasmin, and microorganism-derived enzymes. Proteolysis results in the formation of peptides, amino acids, thiols, and other compounds, all of which contribute significantly to the flavor of the cheese.

A second lipolytic enzymatic process also contributes to the essence of cheeses. Although some lipolysis may lead to a perceived rancidity in some cheeses, the free fatty acids released by lipase (EC 3.1.1.3) activity do influence flavor positively as well. For the most part, the lipases involved are endogenous to microorganisms, although there is some evidence for participation of milk lipases as well. Esterases (EC 3.1.1.1) are also thought to contribute to the process by hydrolyzing mono- and diglycerides to ester compounds that are known to provide various flavor and aroma attributes.

A third process involves glycolysis, in which the lactose in milk is enzymatically converted to lactic and other acids through microorganism metabolism. The acids contribute significantly to the character of the cheese. In modern cheese making, these enzymatic reactions are accelerated by the addition of exogenous esterases and lipases to produce stronger flavors in a shorter period of time while providing addition control over the process.

ENDOGENOUS ENZYMES AS PROCESSING INDICATORS

Enzymes present in food materials may be used as diagnostic indicators of processing steps that would be difficult to assess otherwise. The following are several examples of the use of endogenous enzymes as indicators of the effectiveness of a processing step, often involving heat treatments to inactivate organisms or enzyme activity.

Pasteurization of Milk

The purpose of pasteurization is to destroy potentially harmful microorganisms in the milk through a process of rapid heating to about 85°C for a very short time (several seconds). It is sometimes difficult to determine whether the processing has effectively eliminated the targeted microorganisms. One way to assess the effectiveness of the treatment is to test the viability of the organisms. This, however, is a lengthy process not well suited for remedial heating. It happens that endogenous milk alkaline phosphatase (EC 3.1.3.1) is heat inactivated in a temperature range similar to that required to destroy the potentially harmful organisms. Thus, a simple colorimetric test (5) for alkaline phosphatase can quickly indicate whether the temperature of the milk has been raised sufficiently to inactivate the enzyme and, by inference, the microorganisms.

Blanching of Vegetables

Vegetables are often placed in boiling water to inactivate certain hydrolytic enzymes that would otherwise cause detrimental effects in terms of flavor and/or texture. Although certainly less a problem in the home, the proper degree of heating required to inactivate these enzymes, while not adversely affecting texture is critical to industries involved in the longer-term storage of such products in cans or bottles. Inadequate blanching in this case can result in seriously degraded product by the time the consumer opens the container for consumption of the product. Thus, it is important to know when a sufficient amount of heating has been achieved. A convenient way to assess the blanching process is to look at the activity of endogenous peroxidase (EC 1.11.1.7). While complete inactivation of peroxidase is not desirable, the proper degree of blanching is achieved when about 5 to 10% of the peroxidase activity remains. The assay method usually utilized for this test involves the use of gulacol and is based on the colorimetric determination of the enzymatic product tetragulacol (6) This methodology has been used to assess the degree of blanching in products such as peas, corn, beans, and other vegetables.

Oat Rancidity

Oats contain lipolytic enzymes, the action of which can lead rapidly to rancidity. In this case, as discussed for vegetables, heat inactivation of those lipolytic enzymes is often used to extend the shelf life of oats and products formulated with oats. Since the assay of the lipolytic enzymes is problematic, endogenous oat peroxidase activity serves as a more convenient indicator of sufficient thermal inactivation of the lipolytic enzymes.

Soybean Treatment

Soybeans are very high in protein and are therefore used to supplement other foodstuffs that are lower in protein quantity as well as quality. However, soybean meal is usually heated to alter the bitter flavor and to inactivate some antinutritional components such as trypsin inhibitors as well as lipoxygenase activity. Applying too much or too little heat in this process can produce undesirable effects. The endogenous urease enzyme (EC 3.5.1.5) is used as a convenient assay (7) to indicate the correct degree of thermal treatment.

BROWNING REACTIONS—COLOR AND FLAVOR

Maillard Browning

Several types of chemical reactions can affect color and flavor in food products. The Maillard reaction is a nonenzymatic reaction between a sugar and an amino function, often one on an amino acid. Although the reaction itself can proceed without the participation of an enzyme, the reaction is influenced by the concentration of the reactants, which in turn may be influenced by the presence of exoproteases and exo-amylases. Thus, endogenous enzymes such as β-amylase, a carboxypeptidase, or a leucineaminopeptidase can contribute to Maillard browning in food products, baked goods in particular. These reactions lead to significant color formation (evidenced by the color of the crust on a loaf of bread) as well as many flavor characteristics.

Enzymatic Browning

Another source of color in foods comes from a reaction usually referred to as enzymatic browning. This reaction is mediated by polyphenol oxidase (EC 1.10.3.1). This enzyme is able to interact with a large number of phenolic compounds and thus had been referred to by several names including tyrosinase, catecholase, polyphenolase, and cresolase. The basic oxidation reaction normally results in the formation of an unstable quinone, which then proceeds through a number of steps to form melanins. Melanins are brown to black in color and are responsible for undesirable spots on many fruits and vegetables such as bananas, apples, mushrooms, and potatoes. Because of the widespread presence of polyphenol oxidase and the damage it causes in many food products, numerous methods have been developed to try to prevent this reaction from occurring. Clearly, eliminating as much oxygen as possible will help. In addition, reducing agents such as ascorbic acid, sodium sulfite, and thioi compounds can also help by reacting with the quinone compound. These reactions do not, however, inactivate the enzyme itself, so when the oxidizing agents are depleted, the browning reaction may still proceed. Under the proper conditions, ascorbic acid can inactivate polyphenol oxidase reacting with an active-site histidine residue. Likewise, thiol compounds can also affect the enzyme activity by chelating the required metal cofactor (Cu^{++}) (1).

Other Flavor Reactions

Members of the plant genus *Allium*, which includes onions, shallots, garlic, and leeks, have strong, often irritating redolence that is the result of endogenous enzymatic activity. The characteristic odor of these plants is generally unnoticeable in the whole, undamaged bulb because the enzyme involved, allinase, is compartmentalized and unable to catalyze the odor-causing reaction. When tissue damage occurs, such as slicing the onion or garlic bulb, the enzyme is released and proceeds to react, producing the thiol compounds responsible for the offending (to some) aroma. For more detailed discussion of similar reactions in related food materials, the reader is referred to Reference 8.

BIBLIOGRAPHY

1. J. R. Whitaker, "Enzymes," in O. R. Fenema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, pp. 431–530.
2. W. Pilnik and G. J. Voragen, "The Significance of Endogenous and Exogenous Pectic Enzymes. In Fruit and Vegetable Processing," in P. F. Fox, ed., *Food Enzymology*, Vol. 1. Elsevier Applied Science, London, United Kingdom, pp. 303–333.
3. T. Godfrey, "Brewing," in T. Godfrey and J. Reichelt, eds., *Industrial Enzymology—The Application of Enzymes in Industry*, The Nature Press, New York, 1983, pp. 221–259.
4. P. F. Fox and J. Law, "Enzymology of Cheese Ripening," *Food Biotechnol.* **5**, 239–262 (1991).
5. D. A. Schiemann and M. H. Brodsky, "Studies of Scharer's Original Method for Alkaline Phosphatase in Milk With a Modification Utilizing an Organic Buffer," *J. Milk Food Technol.* **39**, 191–195 (1976).
6. P. Varoquaux et al., "Automatic Measurement of Heat Destruction and Regeneration of Peroxide," (in French) *Lebensm. Wiss. Technol.* **8**, 60–63 (1975).
7. K. Brocklehurst, "Electrochemical Assays: The pH-Stat," in R. Eisenthal and M J. Danson, eds. *Enzyme Assays: A Practical Approach*, Oxford University Press, Oxford, U.K., 1992, pp. 191–216.
8. R. C. Lindsay, "Flavors," in O. R. Fenema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, pp. 723–765.

GENERAL REFERENCES

H.-D. Belitz and W. Grosch, *Food Chemistry*, Springer-Verlag, New York, 1987

S. Schwimmer, *Sourcebook of Food Enzymology*, AVI Publishing, Westport, Conn., 1981.

C. Zapsalis and R. A. Beck, *Food Chemistry and Nutritional Biochemistry*, John Wiley & Sons, New York, 1985

PAUL R. MATHEWSON
Food Technology Resource Group
Park City, Utah

See also ENZYMOLOGY.

ENZYMOLOGY

Enzymes can be defined as proteins with very specific powers of catalysis. The enhancement in reaction rate is very high and may be as much as 10^{14}-fold in some instances. Although all enzymes are proteins, some require an additional small molecule, called a coenzyme or cofactor (cofactors can be metal ions or small organic molecules), to function. The role of enzymes in cells means that growth, maturation, storage, processing, and consumption–digestion of food all depend on various enzyme activities. In plants, enzymes are responsible for changes associated with ripening, including alterations in color, flavor, and texture. Enzymatic changes continue after harvesting of plants and also after the death of animals, for example, in the conversion of muscle to meat. These changes affect subsequent food quality. In many cases the action of endogenous enzymes is arrested or controlled by processing and storage.

Enzymes are also widely used to provide desirable changes in food quality attributes through changes in the chemical structure of food components. Enzymes have significant advantages over chemical catalysts, of which the most important are specificity and the ability to work at moderate temperatures. As a consequence, side reactions are minimized, and undesirable changes caused by harsh conditions are averted. Enzymes have traditionally been obtained from their natural sources: animals, plants, and microorganisms. Advances in molecular biology have led to the cloning and expression of enzymes from many sources into microorganisms (primarily yeasts and fungi) that are efficient producers and regarded as safe hosts. The value of enzymes used in food processing is already several hundred million dollars per annum and will likely expand as new applications are discovered or improved enzymes become available, for example, by genetic engineering to improve operating characteristics such as the pH-activity profile or thermostability.

KINETICS

The activity of an enzyme is determined by many factors, including enzyme, substrate, and cofactor concentrations; ionic strength; pH; and temperature. For conversion of substrate (S) to product (P) by an enzyme (E) the reaction scheme can be simply represented as

$$E + S \overset{k_s}{\rightleftharpoons} ES \xrightarrow{k_{\text{cat}}} E + P$$

where ES is the enzyme–substrate complex, K_s is the dissociation constant, and K_{cat} is the turnover number/rate constant for the breakdown of ES.

The initial reaction velocity (V) is then given by the Michaelis–Menton equation

$$V = \frac{k_{\text{cat}}(E)(S)}{K_m + (S)}$$

where K_m is the substrate concentration at which V equals one-half the maximum velocity ($V_{\max}$). Integration of the equation with respect to time gives

$$V_{\max} = K_m \ln \frac{(S)}{(S_t)} + [(S) - (S_t)]$$

where S and S_t are the substrate concentrations at zero time and time t, respectively. This equation is particularly useful in industrial situations where a reaction is allowed to proceed to near completion or equilibrium.

APPLICATIONS IN FOOD PROCESSING

α-Amylase (EC 3.2.1.1) catalyzes random hydrolysis of α 1–4 linkages in amylose and amylopectin to form straight- and branched-chain dextrins, oligosaccharides, and monosaccharides. Bacteria α-amylase is used to partially hydrolyze or "thin" gelatinized starch, often prior to further degradation to glucose syrups. The enzyme from *Bacillus* spp. is very thermostable and is used at 85 to 105°C. The enzyme can also be used to hydrolyze starch in sugar cane juice and in the brewing mash.

Fungal α-amylases, derived from *Aspergillus niger* and *A. oryzae* are much less heat stable. They produce large amounts of maltose and maltotriose and some glucose. Their principal applications are production of maltose syrups used in jam and confectionery, as brewing aids to improve fermentability of the mash and in removal of starch haze in beer, and in supplementation of endogenous α-amylase in bread flour to enhance the rate of fermentation by yeast and reduce dough viscosity, thereby improving loaf volume. Fungal α-amylase has largely replaced malt α-amylase previously used in these applications.

β-Amylase (EC 3.2.1.2) splits off β-maltose from the nonreducing ends of starch molecules. In the case of amylopectin, this produces β-limit dextrins since the enzyme cannot bypass the β-1-6 branch points. Complete hydrolysis of liquified starch produces about 80% maltose and 20% dextrins. Unlike fungal α-amylase, β-amylase does not produce maltotriose. The enzyme is produced from cereal or microbial sources and is used for production of maltose syrup from starch, and in both brewing and baking to produce maltose for fermentation by yeast to CO_2 and to alcohol.

Amyloglucosidase (glucoamylase) (EC 3.2.1.3) is an exoamylase that catalyzes the stepwise hydrolysis of α 1–4 linkages in starch, thereby releasing glucose molecules from the nonreducing end. It is used mainly to produce glucose syrups from liquified starch previously treated with α-amylase. Since the α 1–6 linkages in amylopectin are also slowly hydrolyzed, the final conversion to glucose reaches 95 to 97% w/w with the remainder being mostly maltose and higher saccharides. The glucose produced is usually used as a syrup, crystallized out, or converted to fructose by glucose isomerase. The enzyme can also be used to hydrolyze residual oligosaccharides in high-fructose corn syrup and in the analysis of the starch content of foods. Glucoamylase is produced commercially from *Aspergillus* or *Rhizopus* spp. It is typically used to process liquified starch at 60°C. Immobilization of the enzyme has been widely studied to provide the benefits of a continuous process. However, the soluble enzyme is relatively cheap so it continues to be used mostly in that form.

Catalase (EC 1.11.1.6) specifically catalyzes the decomposition of hydrogen peroxide to water and oxygen. In the dairy industry, a low concentration of H_2O_2 (up to 0.05% w/w) is used to "cold pasteurize" milk destined for cheese making, and to preserve milk and whey in some instances where refrigeration is not practical. The H_2O_2 is then destroyed by catalase. Commercial sources include beef liver and *A. niger*. The enzyme is frequently used in conjunction with glucose oxidase (see later) to remove H_2O_2 produced by that enzyme.

Cellulase (EC 3.2.1.4) is the name given to a complex of several enzymes that, acting together, hydrolyze cellulose into β-dextrins and glucose. The native structure of cellulose is a major impediment to enzyme action, so pretreatment by milling, or with alkali or steam, is often necessary to make the substrate accessible. The fungus *Trichoderma reesie* is a good source of cellulase. It contains (*1*) endocellulases that randomly split internal β 1–4 linkages to produce dextrins and cellobiose, (*2*) exocellulases that act from the nonreducing end of the polymer to produce cellobiose, and (*3*) cellobiase (or β-glucosidase) that converts cellobiose to glucose. The latter is especially important since it relieves end-product inhibition of the other enzymes by cellobiose. Cellulase is used primarily to turn cellulosic wastes into glucose that can be fermented to ethanol. It is also used on a small scale to degrade cellulose in foodstuffs.

β-Glucanase (EC 3.2.1.6) hydrolyzes β 1–3 or β 1–4 bonds in β-D-glucans. The products are oligosaccharides and glucose. The enzyme is used primarily in brewing, where it is added, along with other enzymes, to malted barley at the mash stage. The β-glucanase degrades and solubilizes barley gums (β-glucan polymers), including those contributed by dying yeast cells, which would otherwise increase the viscosity of the wort. These residual glucans can contribute to haze problems in the final product. Commercial sources of β-glucanase include *Bacillus subtilis* and *A. niger*.

Glucose isomerase (EC 5.1.3.5) catalyzes conversion of glucose to fructose, thereby increasing sweetness and value. The enzyme is actually a xylose isomerase, which also acts on glucose and requires magnesium as a cofactor. The reaction is readily reversible and at equilibrium, a fructose/glucose ratio of 52/48 is achieved. However, in practice it is uneconomical to take the conversion beyond 42% fructose, when the syrup is isosweet with glucose on a solids basis. The product (high-fructose corn syrup) is produced at a rate of several million tons per annum and has displaced sucrose and glucose syrups from many products, especially in soft drinks in the United States.

The enzyme is produced commercially from several microbial sources including *Bacillus, Actinoplanes*, and *Streptomyces* spp. and is readily immobilized by a variety of methods (1).

Glucose oxidase (EC 1.1.3.4) catalyses the reaction:

$$\text{glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2.$$

It is often used in conjunction with catalase, which breaks down H_2O_2, thereby sparing the glucose oxidase from denaturation as well as providing oxygen for use by glucose oxidase. Commercial preparations are derived from *A. niger* or *Penicillum* spp. Its main uses are to remove glucose from foods and as an antioxidant to prevent changes in color and flavor, particularly during food storage. It is used for removal of glucose from egg whites and whole eggs. Significant browning and off-flavor development due to Maillard reactions occurs if eggs are not desugared prior to drying. Other applications include removal of either dissolved or headspace oxygen from citrus drinks, canned soft drinks, beer, and wine, thereby preventing oxidative deterioration, and as an antioxidant in mayonnaise and production of gluconic acid. It is often the method of choice for assay of glucose since it is highly specific and sensitive.

Invertase (β-fructofuranosidase, EC 3.2.1.6) hydrolyzes sucrose into an equimolar mixture of glucose and fructose. Although the same result can be achieved by acid hydrolysis, the syrup produced by enzyme action is more pure and free from discoloration. Invert sugar syrup has several advantages over sucrose syrup: it is slightly sweeter, it does not crystallize at higher concentrations, and the sweetness intensity is stable in acidic foods. Until the advent of high-fructose corn syrup, it was used extensively to replace sucrose in jams and confectionery.

Other applications of invertase include its use in the production of liquid or soft centers in chocolate-coated sucrose candies, in recovery of scrap candy, in artificial honey, and as a humectant to hold moisture in foods. Invertase is produced commercially from yeasts, usually *Saccharomyces* or *Candida* spp., and is relatively cheap to produce. Consequently, it is used as a soluble enzyme and there is little commercial impetus for its application in an immobilized form.

Lactase (β-galactosidase, EC 3.2.1.23) hydrolyzes lactose into glucose and galactose. Small quantities of oligosaccharides containing galactose may also be formed as byproducts. Compared with lactose, the main products are, in combination, three to four times more soluble, about twice as sweet, easier to ferment, and directly absorbed from the intestine. Applications of lactase take advantage of these changes (2).

Hydrolysis of lactose in milk makes the milk more digestible for those who are lactose intolerant (especially infants) due to low levels of intestinal β-galactosidase. It also prevents crystallization of lactose in concentrated or frozen milk products, such as ice cream. Cheese whey is a major disposal problem, mostly because of its high lactose content. After separating the valuable whey protein, the lactose can be hydrolyzed by soluble or immobilized lactase. The product is then concentrated to give a stable, sweet syrup, which may be used in a variety of foods or fermented to ethanol (3).

Lactases produced commercially from yeasts such as *Kluyveromyces marxianus* have a neutral pH optimum and are used in milk processing. Lactases from molds such *A. niger* or *A. oryzae* have an acid pH optimum and are more suitable for whey processing. Lactoses are readily immobilized on a variety of supports for industrial use and for analysis of lactose in dairy products.

Lipases (EC 3.1.1.3) hydrolyze ester linkages of triglycerides to give free fatty acids, diglycerides, monoglycerides, and eventually glycerol. The enzyme is widely distributed in food tissues and belongs to the general class of esterases. Lipase action has traditionally been regarded as a problem in food science because the fatty acids produced may be unpalatable or susceptible to oxidation. This is a particular problem in stored cereals such as wheat and rice and in milk, where the release of short-chain fatty acids from milk fat by endogenous lipase leads directly to off-flavors. However, it is recognized that lipase action is also responsible for some of the desirable flavor in matured cheeses. Consequently, impure preparations (containing both lipase and esterase activities) are produced from molds such as *Mucor, Rhizopus*, and *Aspergillus* spp. for use in accelerated cheese ripening, often in conjunction with proteases. Concentrated cheese flavors produced in this fashion can be used in a variety of snack products. Lipase can also be used in directed transesterification of fats to improve functional properties and uses.

Pectinases are a group of enzymes that act on various pectic substances (pectins) in higher plants. There are three major types of pectinase: polygalacturonase (PG), pectin lyase (transeliminase) (PL), and pectin esterase (PE). PG (EC 3.2.1.15) splits glycosidic bonds within (endo-) or at the end of (exo-) the pectin molecule. Endo-PG action leads to a large decrease in viscosity of pectin solutions. PL (EC 4.2.2.10) also splits endoglycosidic bonds but the transelimination reaction yields a C^4-C^5 double bond on the nonreducing end. PE (EC 3.1.1.11) cleaves methanol from carboxyl groups, yielding low-methoxy pectin and polygalacturonic acid.

Since pectin is a major structural element in and between plant cell walls, alteration of the size or esterification of pectins can change the texture of fruits and vegetables during ripening and storage. Endogenous pectinases may soften texture during ripening. However, degradation of pectin can be inhibited by antisense RNA technology and this has been successfully exploited in tomatoes (4). Microbial pectinases are often responsible for postharvest rotting and decay. However, fungal pectinases, mostly from *Aspergillus* spp. are widely used as processing aids, mainly for extraction and clarification of fruit juices.

Polyphenol oxidase (PPO) (EC 1.10.3.1) is also known as phenolase tyrosinase, or catecholase. The enzyme is widely distributed in plants and fungi and in some mammals. In fruits and vegetables it is separated from phenolic substrates in the intact tissue. However, on cutting or other injury and exposure to oxygen, phenolase activity results in rapid browning due to polymerization of the quinones to give brown pigments (melanins).

Enzymatic browning is a major problem in handling and storage of fresh produce. However, in foods such as tea, coffee, and cocoa and in dried fruits such as raisins and dates, it improves color and flavor. Methods for control of enzymatic browning include: inactivation of the enzyme by heat, sulfites, or proteases; use of acidulants to lower pH and inhibit the enzyme; and use of chelators to remove the copper that is essential for activity and exclusion (or removal) of oxygen by appropriate packaging or glucose oxidase activity. Polyphenol oxidase activity in shrimp and other crustacea leads to blackening during harvest and storage. This can be prevented by use of the substrate analogue 4-hexylresorcinol (5).

Proteases hydrolyze peptide bonds in proteins and polypeptides to produce smaller peptides and amino acids. Many proteases also have esterase activity. These enzymes vary widely in their substrate specificity and optimum pH range. Enzymatic hydrolysis of protein is employed in a number of industries to create changes in product taste, texture, and appearance as well as in waste recovery. Plant proteases such as papain and ficin have broad substrate specificity and good thermal stability. They are used to tenderize meat, to chillproof beer, and also to recover protein hydrolyzate from scrap fish and bones. Animal proteases are generally more specific than the plant proteases. Trypsin has been used to inhibit the development of an oxidized flavor in stored milk and to solubilize heat-denatured whey protein (6). Pepsin is used primarily as an extender for calf rennet. Fungal proteases from *A. niger* and *A. oryzae* are use to modify gluten in bread flour, so as to reduce dough viscosity and improve color, texture and loaf volume. Bacterial proteases from *Bacillus* spp. are widely used in biscuit, cookie, and cracker dough since hydrolysis of gluten yields very elastic doughs that can be spread thinly without rupture. Other uses of microbial proteases include modification of soy and other food proteins to give improved functionality, decolorization of red blood cells to facilitate use of waste blood plasma protein from animal slaughter and production, and modification of gelatin from collagen. Recently there has been interest in using proteases in reverse to synthesize peptides. An example of this is the use of the enzyme thermolysin to produce the dipeptide sweetner aspartame (7).

Pullulanase (EC 3.2.1.41) and *isoamylase* (3.2.1.68) act specifically on the α 1–6 bonds of amylopectin in liquified starch to produce maltose and maltotriose. These enzymes improve the yield of mono- and disaccharides from starch when used in conjunction with other amylases. Pullulanase is produced commercially from *Klebsiella pneumonia* and is used in brewing to remove limit dextrins and allow the production of specialty beers such as "high alcohol" or "low calorie."

Rennet is obtained from the fourth stomach of unweaned calves and contains several enzymes including pepsin and chymosin (EC 3.44.3). Chymosin is an acid protease used in cheese making to coagulate the casein in milk. It catalyzes very specific and limited proteolysis of *k*-casein, thereby destabilizing the casein micelle and causing subsequent formation of curd. Calf rennet is expensive and is increasingly being replaced by microbial proteases with a high ratio of milk-clotting activity to general proteolytic activity. Suitable proteases have been obtained from *Endothia parasitica, Mucor meihei* and *M. pusillus*. Microbial proteases are now used in about one-half of all cheese production worldwide. Their main disadvantage is that they tend to be heat stable and cause proteolysis in whey products. However, a second generation of modified microbial rennets with lower stability is now available. The gene for chymosin has now been successfully cloned and expressed in several microorganisms (8). Products from this source may well replace both traditional and microbial rennets in the near future.

BIBLIOGRAPHY

1. S. A. Barker and G. S. Petch, "Enzymatic Processes for High-Fructose Corn Syrup," in A. Laskin, ed., *Enzymes and Immobilized Cells in Biotechnology*, Benjamin/Cummings, Menlo Park, Calif., 1985, pp. 93–105.
2. R. R. Mahoney, "Lactose: Enzymatic Modification," in P. F. Fox, ed., *Advanced Dairy Chemistry*, Vol. 3, Chapman and Hall, London, United Kingdom, 1997, pp. 77–105.
3. J. Barry, "A New Source for Alcohol," *Food Manufacture* **58**, 63–67 (1983).
4. G. Tucker, "Improvement of Tomato Fruit Quality and Processing Characteristics by Genetic Engineering," *Food Science and Technology Today* **7**, 103–108 (1993).
5. A. J. McEvily, R. Iyengar, and W. S. Otwell, "Sulfite Alternative Presents Shrimp Melanosis," *Food Technol.* **45**, 80–86 (1991).
6. J. C. Monti and R. Jost, "Solubilization of Cheese Whey Protein by Trypsin and a Process to Recover the Active Enzyme from the Digest," *Biotechnol. Bioeng* **20**, 1173–1185 (1981).
7. K. Nakanishi and R. Matsuno, "Enzymatic Synthesis of Aspartame," in R. D. King and P. S. J. Cheetham, eds., *Food Biotechnology*, Vol. 2, Elsevier Applied Science, London, United Kingdom, 1988, pp. 219–249.
8. D. Jackson, "Cost Reduction in Food Processing Using Biotechnology," in S. K. Harlander and T. B. Lubuza eds., *Biotechnology in Food Processing*, Noyes Press, Park Ridge, N.J., 1986, pp. 285–295.

GENERAL REFERENCES

P. S. J. Cheetham, "The Application of Enzymes in Industry," in A. Wiseman, ed., *Handbook of Enzyme Biotechnology*, 2nd ed., Ellis Horwood, Chichester, United Kingdom, 1985, pp. 274–373.

P. F. Fox, ed., *Food Enzymology*, Vols. 1 and 2, Elsevier Applied Science, London, United Kingdom, 1991.

T. Nagodawithana and G. Reed, eds., *Enzymes in Food Processing*, 3d ed., Academic Press, San Diego, Calif., 1993.

G. A. Tucker and L. F. J. Woods, eds., *Enzymes in Food Processing*, 2nd ed., Blackie Academic and Professional, London, United Kingdom, 1995.

RAYMOND R. MAHONEY
University of Massachusetts
Amherst, Massachusetts

See also ENZYMES IN FOOD PRODUCTION.

EVAPORATED MILK

HISTORY

Evaporated milk, like other processed canned foods, originated with the experiments of the French scientist Nicholas Appert. Appert, whose work on food preservation began in 1795, was the first to evaporate milk by boiling it in an open container and preserve it by heating the product in a sealed container. Fifty years later, Louis Pasteur laid the scientific foundation for heat preservation by demonstrating that food spoilage could be caused by bacteria and other microorganisms.

Patents for the preservation of milk after evaporation in a vacuum were granted to Gail Borden by the United States and England in 1856. Although these patents applied to concentrating milk without the addition of sugar, Borden's first commercial process was for the manufacture of sweetened condensed milk. The original vacuum equipment developed by Borden is now on display at the Smithsonian Institution.

Borden produced sweetened condensed milk at Wassaic, New York, in 1861. By 1865, new plants were opened in Brewster, New York, and Elgin, Illinois. In 1866, Charles A. Page and his three brothers built Europe's first commercial sweetened condensed milk plant in Switzerland. They later built plants in England and in the United States.

In 1884, a U.S. patent was issued for "an apparatus for preserving milk," and in 1885, the world's first commercial evaporated milk plant was opened in a converted wool factory in Highland, Illinois, where evaporated cream was manufactured and sold.

The first advertisement for evaporated milk appeared in 1893, calling the product "a perfect instant food." In 1894, the first recipe booklet for evaporated milk was distributed, and by 1895, the product was popular both in western mining areas of the United States, where fresh milk was scarce, and in the South, where there was little refrigeration. Consumers quickly recognized the value of evaporated milk as a safe, wholesome, and convenient food as well as a nutritional ingredient for cooking that had the added benefit of storage stability.

THE PRODUCT

Evaporated milk is a canned whole milk concentrate to which a specified quantity of vitamin D has been added and to which vitamin A may be added. It conforms to the Food and Drug Administration (FDA) Standard of Identity (21 CFR 131.130), having a minimum of 6.5% milkfat, 16.5% of nonfat milk solids, 23.0% total milk solids, and 25 IU vitamin D per fluid ounce. Related evaporated milk products are evaporated nonfat milk, evaporated lowfat milk, evaporated filled milk, and evaporated goat milk. Standards of Identity do not exist for these related evaporated milk products. Their typical compositions are the following:

Evaporated nonfat milk	Less than 0.5% milkfat, 20.0% total milk solids, 25 IU vitamin D and 125 IU vitamin A per fluid ounce added
Evaporated lowfat milk	2.0% milkfat, 18% nonfat milk solids, vitamins A and D added
Evaporated filled milk	6.0% vegetable fat, 17.5% nonfat milk solids, vitamins A and D added
Evaporated goat milk	Not less than 7.0% milkfat and 15.0% nonfat milk solids, vitamin D added

Table 1 shows the nutritional content of evaporated, evaporated nonfat, and evaporated lowfat milks.

Table 1. Nutritional Content per One-Half Cup Evaporated Milk

	Original	Nonfat	Lowfat
Calories	170	100	110
Protein (g)	8	9	8
Carbohydrates (g)	12	14	12
Fat (g)	10	Less than 1	3
Percentage of U S. Recommended Daily Allowance (U.S. RDA)			
Protein	20	20	20
Vitamin A	4	10	10
Vitamin C	[a]	[a]	[a]
Thiamine	2	2	2
Riboflavin	20	20	20
Niacin	[a]	[a]	[a]
Calcium	30	30	30
Iron	[a]	[a]	[a]
Vitamin D	25	25	25
Phosphorus	25	25	25

[a] Contains less than 2% of the U.S. RDA of these nutrients.

PRODUCT PROCESSING

A typical processing scheme for evaporated milk begins with high quality, fresh whole milk, which is standardized to produce the exact composition desired in the final product (addition or removal of cream or nonfat milk). The product is heated, concentrated under reduced pressure in an evaporator, homogenized, and cooled. Vitamins (A or D, or both) are added, and the final composition is verified. Final standardization is accomplished, when necessary. After cans are filled and sealed, they are sterilized in a three-phase continuous system consisting of preheater, retort, and cooler, cooled; labeled; and packed for shipment. In the United States, evaporated milk is packed in 5-, 12-, 20-, and 97-fluid ounce lead-free cans.

INDUSTRY PRODUCTION

Table 2 reflects U.S. production of evaporated milk and related products during the period 1975 to 1998. The data are based on manufacturers' production reported to the American Dairy Products Institute. Before 1983, data were not collected on the production of related evaporated milk products.

Table 2. Evaporated Milk Production

Year	Evaporated milk	Evaporated milk and related products[a]
1975	20,796,000	
1976	20,362,000	
1977	18,347,000	
1978	17,340,000	
1979	17,110,000	
1980	15,500,000	
1981	15,840,000	
1982	15,050,000	
1983	14,300,000	16,612,000
1984	13,700,000	15,892,000
1985	14,360,000	16,537,000
1986	12,996,000	14,881,000
1987	12,966,000	14,759,000
1988	13,357,000	15,164,000
1989	12,451,000	14,193,000
1990	13,169,000	14,895,000
1991	11,313,000	12,776,000
1992	12,153,000	13,595,000
1993	11,561,000	13,114,000
1994	10,585,000	11,771,000
1995	10,847,000	12,462,000
1996	9,853,000	11,271,000
1997	10,656,000	12,204,000
1998	10,275,000	11,843,000

Notes. Figures reported in cases; 48-tall equivalent. Product weight/case since 1985 40 lb.

[a] Includes evaporated milk plus evaporated nonfat milk, evaporated lowfat milk, and evaporated filled milk. Data not available before 1983.

GENERAL REFERENCES

C. W. Hall and T. I. Hedrick, *Drying of Milk and Milk Products*, 2nd ed., AVI, Westport, Conn., 1971.

E H. Parfitt, "The Development of the Evaporated Milk Industry in the United States," *Journal of Dairy Science* **39**, 838 (1956).

R. Seltzer, *The Dairy Industry in America*, Magazines for Industry, New York, 1976.

U.S. Food and Drug Administration, Department of Health and Human Services, "Evaporated Milk," *Code of Federal Regulations* **21**, 131.130 (1999).

Warren S. Clark, Jr.
American Dairy Products Institute
Chicago, Illinois

EVAPORATION

The primary objectives of evaporation, as a unit operation in food processing, are to reduce the volume of the product by some significant amount with minimum loss of nutrient components, and to preconcentrate liquid foods such as fruit juice, milk, and coffee before the product enters a dehydration process, thus saving energy in subsequent operations and reducing handling (transport, storage, and distribution) costs. Evaporation is accomplished by boiling liquid foods until the desired concentration is obtained, as result of the difference in volatility between the solvent and solutes. In the food industry, the solvent to withdraw is almost always water (1). Evaporation increases the solids content of a food and hence preserves it by a reduction in water activity; however, the flavor and color of a food may be changed during the process. The technical simplicity of evaporation gives it an obvious advantage compared to other methods such as reverse osmosis and freeze concentration. The removal of unpleasant volatile substances, for instance, during evaporation of milk, may also be an advantage. Evaporation of heat-sensitive materials is often accomplished under relatively high vacuum (low pressures), lower temperatures, and short residence time.

The first commercial use of evaporating equipment was made by Borden in 1856 (2). Since then, equipment for evaporation has evolved from open kettles through rotating steam coils to the modern natural or forced circulation evaporators. A wide range of different types of evaporator are available and evaporation can take place under different processing conditions. Commercial evaporation capacities range from 100 kg/h for pilot plants to over 200,000 kg/h for industrial installations (for instance, FMC, San Jose, Calif.; APV, Crawley, UK; Alfa-Laval, Lund, Sweden, and Dedert, Olympia Fields, Ill.).

Evaporation involves a sequence of processes. Computers can be used in the design of individual components and the overall system; however, to compromise between vastly opposed factors, the choice of the optimum design solution must be based on acquired experience and sound judgment. The intensive energy use of the evaporation process poses a continuing challenge in minimizing energy costs while maintaining product quality, especially at times of escalating energy costs. Multiple-effect evaporators and vapor recompression are measures that can be employed to improve the energy efficiency of the evaporation plant.

THEORY

The complete design of evaporator systems requires engineering calculations that involve combined heat and mass balances, and is essentially an application of the first law of thermodynamics. In an evaporator, the steam condenses at constant pressure, and the condensate leaves at this pressure and corresponding equilibrium temperature. The heat from the condensing steam boils water from a liquid that is being concentrated. The water vapor leaving the evaporator is at a lower temperature and pressure than the original steam supply, and can give up its latent heat in a double-effect evaporator to evaporate a lower boiling temperature liquid. This necessary lower boiling temperature can be maintained by operating a second effect of evaporation at a lower pressure than the first, usually under a vacuum. The same principles can be extended to more than two effects in industrial applications.

Steady-state mathematical models with equations that represent heat and mass transfer may be used for design purposes. Steam requirement, heat transfer surface area, and system capacity (production rate) can be determined for a given degree of concentration and processing time. A double-effect evaporator, with feed and steam or vapor streams moving in counterflow arrangement (Fig. 1) will be used here to illustrate the theory.

For the first effect, mass balance that relates solids entering and leaving the evaporator gives

$$\dot{m}_2 X_2 = \dot{m}_1 X_1 \tag{1}$$

and the rate of heat transfer to the evaporator from the steam is

$$\dot{m}_s \lambda_s = U_1 A_1 (T_s - T_1) \tag{2}$$

(The terms in equations are defined in the notation list at the end of the article). Assuming that there are negligible heat losses from the evaporator, the heat balance states that the amount of heat given up by the condensing steam equals the amount of heat used to raise the feed temperature to boiling point and then to boil off the vapor. In other words, the input energy of condensing vapor and feed equals the output energy of vapor and product, thus

$$\dot{m}_2 h_2 + \dot{m}_s \lambda_s = (\dot{m}_2 - \dot{m}_1) H_1 + \dot{m}_1 h_1 \tag{3}$$

Similarly, for the second effect, mass and energy balances are represented by the equations

$$\dot{m}_F X_F = \dot{m}_2 X_2 \tag{4}$$

$$(\dot{m}_2 - \dot{m}_1)\lambda_1 = U_2 A_2 (T_1 - T_2) \tag{5}$$

$$\dot{m}_F h_F + (\dot{m}_2 - \dot{m}_1)\lambda_1 = (\dot{m}_F - \dot{m}_2) H_2 + \dot{m}_2 h_2 \tag{6}$$

The overall heat-transfer coefficient U in equation 2 is defined by the sum of three thermal resistance terms, as represented by

$$\frac{1}{UA_m} = \frac{1}{h_s A_s} + \frac{1}{kA_m} + \frac{1}{h_p A_p} \tag{7}$$

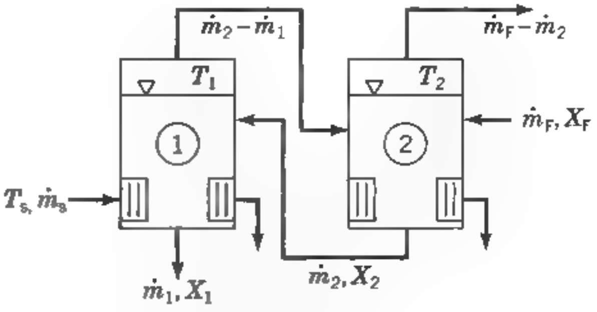


Figure 1. Schematic of double-effect evaporator, backward feed.

These resistances include resistance in the boundary layer (heat-transfer film) on the heating medium side, resistance to heat conduction in the material making up the heat-transfer surface, and resistance in the heat-transfer film on the product side.

In most evaporators, the heating medium will be steam or some other condensing vapor. The resistance to heat transfer on the heating medium side of the heat-transfer surface is normally created by a condensation film, and an expression for condensation heat-transfer coefficient as presented in one study (3) was

$$h_c = 0.94\left(\frac{k_f^3 \rho_f^2 g \lambda}{L \mu_f (T_s - T_w)}\right)^{1/4} \tag{8}$$

for vertical tubes and

$$h_c = 0.725\left(\frac{k_f^3 \rho_f^2 g \lambda}{D \mu_f (T_s - T_w)}\right)^{1/4} \tag{9}$$

for horizontal tubes. Both expressions have accounted for heat transfer involved in phase change. The heating-surface material is usually stainless steel, and it is relatively easy to compute the corresponding resistance knowing the thickness and the thermal conductivity of the material. The most complex resistance to heat transfer is due to the resistance film on the product side.

The heat transfer from a heated surface to a boiling liquid is by convection, but the heat flux varies with the temperature difference between the surface and the bulk liquid, as illustrated in Figure 2. When the temperature difference is very small (less than 5°C), the heat transfer is by natural convection in a regime called pool boiling. By increasing the temperature difference, vapor bubbles tend to form at selected locations on the surface, and the regime is called nucleate boiling, which continues until the temperature difference is near 55°C. Heat-transfer coefficient from surface to boiling liquid is increased. Beyond 55°C, a film of vapor bubbles forms and collects near the heat-transfer surface, resulting in a reduced heat flux. At temperature differences greater than 55°C, the film becomes stable; however, radiation heat-transfer mode begins to contribute to the heat flux and leads to greater heat-transfer coefficients. Either pool or nucleate boiling will probably describe the majority of physical situations that may exist in liquid food evaporation (4).

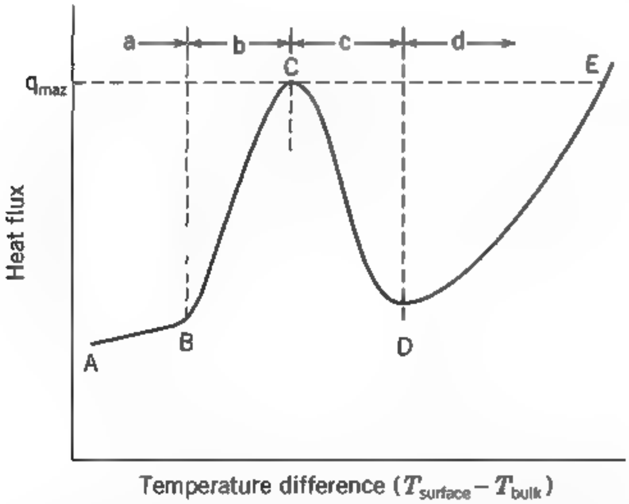


Figure 2. Typical boiling curve illustrating various regimes of boiling near heated surfaces. a, natural convection; b, nucleate boiling; c, transition boiling; d, film boiling. *Source:* Reprinted with permission from Ref. 4.

Heat transfer to boiling liquids is normally expressed in terms of dimensionless groups in the same manner as other types of heat transfer (5). Experimental studies with evaporation systems have resulted in a number of empirical expressions for convective heat-transfer coefficients to the product. For the case of natural circulation evaporators, the following correlation was developed (6):

$$h = 0.0086 \frac{k_f}{D}\left(\frac{u_m D \rho_f}{\mu_f}\right)^{0.8}\left(\frac{c_f \mu_f}{k_f}\right)^{0.6}\left(\frac{\sigma f}{\sigma}\right)^{0.33} \tag{10}$$

For the case of forced convection systems, the following expression was suggested (7):

$$h_b = 3.5h\left(\frac{1}{Z}\right)^{0.5} \text{ for } 0.25 < \frac{1}{Z} < 70$$

$$\frac{1}{Z} = \left(\frac{y}{1-y}\right)^{0.9}\left(\frac{\rho_f}{\rho_v}\right)^{0.5}\left(\frac{\mu_v}{\mu_f}\right)^{0.1} \tag{11}$$

where h_b and h are for convective heat transfer with and without boiling, respectively.

In falling-film evaporation three different flow regimes normally exist, depending on the Reynolds number (Re) of the film: laminar, wavy laminar, and turbulent. The truly laminar regime takes place for Re < 20–30, the wavy laminar regime starts at Re = 30–50, and at 1,000–3,000 a fully turbulent flow is established. The Reynolds number for a film is calculated according to the following equation (8):

$$\text{Re} = \frac{4G}{\mu} \tag{12}$$

where G is the mass flow rate per unit width of the wall.

For the laminar region, the local heat-transfer coefficient may be written as (8):

$$h = a\phi\left(\frac{G}{\mu}\right)^{-0.33}$$

$$\phi = \left(\frac{k_f^3 \rho_f^3 g}{\mu^2}\right)^{0.33} \tag{13}$$

Table 1 gives formulas for local heat-transfer coefficients according to different investigators.

Experimental product-side heat-transfer coefficients h_p were determined (9) for thin-film wiped-surface evaporators, which are extensively used for concentrating high-viscosity or heat-sensitive products. Product degradation is minimized by vigorous agitation and continuous removal of liquid films on the heat-transfer wall. The correlation obtained for the transition-flow regime (Re = 100–1,000) was

$$\text{Nu} = 0.0483\text{Re}^{0.586}\text{Pr}^{1.06}\text{Fr}^{0.118}\left(\frac{\mu}{\mu_w}\right)^{-2.93} \tag{14}$$

Table 1. Local Heat-Transfer Coefficients

Flow regime	A	ϕ_1	Exponent for Re	Notes
Laminar (Re < 20–30)	0.69	ϕ	0.33	Nusselt theory
Wavy laminar (Re < 1,000–3,000)	0.61	ϕ	−0.223	
Turbulent (Re > 1,000–3,000)	0.067	$\phi \cdot \mathrm{Pr}$	0.2	Colburn theory based on pipe friction
	0.030	$\phi \cdot \mathrm{Pr}$	0.33	
	0.19	$\phi \cdot \mathrm{Pr}$	0.067	
	0.0078	$\phi \cdot \mathrm{Pr}$	0.4	Pr ~ 5
	0.0122	$\phi \cdot \mathrm{Pr}$	0.33	Pr ~ 5

Source Ref. 8.

which is valid for liquid feed rate of 58–87 kg/h and agitation speed of 150–500 rpm. The correlation obtained for the turbulent-flow regime (Re > 1,000) was

$$\mathrm{Nu} = 4.137\mathrm{Re}^{0.283}\mathrm{Pr}^{3.325}\mathrm{Fr}^{0.032}\left(\frac{\mu}{\mu_w}\right)^{-0.758} \tag{15}$$

which is valid for liquid feed rate of 87–133 kg/h and agitation speed of 500–1,300 rpm. The authors found that unstable operation of the evaporator occurred when the speed of the wiper blades was below 150 rpm, and rapid increase in h_p was observed for rpm range 250–350. Under similar conditions of evaporation, heat-transfer coefficients were lower for high-viscosity liquids.

A detailed expression for the overall heat-transfer coefficient in multiple-effect falling-film evaporators was worked out (10). This coefficient U was given as a function of the enthalpy and temperature of the liquid and vapor phases, local heat-transfer coefficients, and mass fractions of solute in the inlet and outlet liquid streams. The fundamental mathematical model of multiple-effect evaporators was further applied (11) along with an accurate estimate of U and fouling factor R_d to design a five-effect evaporation unit capable of treating the input citrus juice flow rates indicated in (Figure 3). Results of the calculations are shown in Table 2 for orange and lemon juices that were concentrated from 11.7 to 65°Brix, and from 9.3 to 40°Brix, respectively.

According to one study (12), factors influencing the rate of heat transfer can be summarized as

1. Temperature difference between the steam and the boiling liquid.
2. Fouling of evaporator surfaces.
3. Boundary film thickness.

Factors influencing the economics of evaporation include

1. Loss of concentrate due to foaming and entrainment.
2. Energy expenditure.

The total evaporation depends on the steam consumption and the specific surface area of the evaporator. Capital costs increase with the size of the evaporator whereas energy cost decrease with it. If the fouling of the heating surface is expressed as specific product losses and is reduced from 1.0 to 0.2 kg thin milk/m², for modern evaporators with a specific surface area of 0.12–0.14 m²(kg/h), the production costs will decrease by about 40% for whole milk evaporation and 30% for skim milk evaporation (13). Increased fouling is caused by a nonuniform distribution of liquid to individual heating tubes in falling-film evaporators such that some groups of tubes have more deposit than the others.

Steam economy, defined as the mass of water evaporated per unit mass of steam utilized, is often used as a measure of evaporator performance. A similar definition could be used if another condensing vapor (ammonia, Freon, or diphenyl) is employed as the heating medium.

Steam requirement for double-effect is computed directly from solving the heat and mass balances presented earlier. Equations 1–6 can be used to compute the heat-transfer surface area after the overall heat-transfer coefficients U_1 and U_2 have been evaluated for both effects. The usual procedures for computing steam requirement involve assumptions, such as equal heat flux, equal heat-transfer area, or equal temperature gradient in each effect of the system.

For plants with increasing number of effects resulting in decreased potential temperature difference in each effect, the boiling point rise is of importance when calculating the temperature program of an evaporation plant. For well-defined solutions such as water, the elevation of boiling points is proportional to the molar concentration of the solute, thus

$$\Delta = \frac{RT_{A0}^2X^1}{\lambda} \tag{16}$$

Food liquids are normally more complicated and the boiling point rise has to be determined experimentally. For milk, the following values have been given (8):

Concentration	% DM	16	27.5	39	49	62	69	73
Temperature	°C	0.5	1	1.5	2	3	4	5

The Duhring plot for sucrose for various conditions are presented in Figure 4.

EQUIPMENT

Evaporators are basically heat exchangers, that transfer heat from steam or other heating medium to the food. Vapors produced must be separated. The selection of an evaporator should include the following factors (12):

1. Production rate (kilograms of water removed per hour).

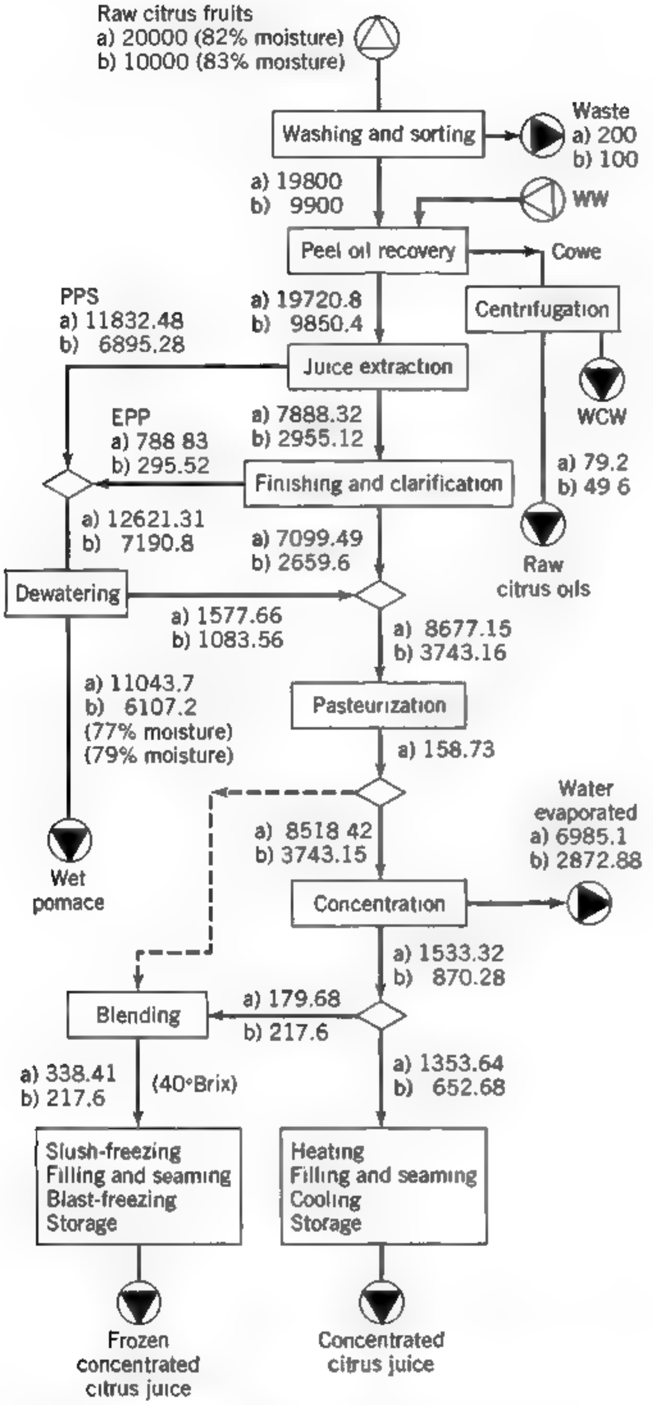

Raw citrus fruits
a) 20000 (82% moisture)
b) 10000 (83% moisture)
Washing and sorting
Waste
a) 200
b) 100
a) 19800
b) 9900
WW
Peel oil recovery
Cowe
Centrifugation
WCW
a) 79.2
b) 49 6
Raw
citrus oils
PPS
a) 11832.48
b) 6895.28
a) 19720.8
b) 9850.4
Juice extraction
EPP
a) 788 83
b) 295.52
a) 7888.32
b) 2955.12
Finishing and clarification
a) 12621.31
b) 7190.8
a) 7099.49
b) 2659.6
Dewatering
a) 1577.66
b) 1083.56
a) 8677.15
b) 3743.16
a) 11043.7
b) 6107.2
(77% moisture)
(79% moisture)
Pasteurization
a) 158.73
Wet
pomace
a) 8518 42
b) 3743.15
Water
evaporated
a) 6985.1
b) 2872.88
Concentration
a) 1533.32
b) 870.28
Blending
a) 179.68
b) 217.6
a) 338.41
b) 217.6
(40°Brix)
a) 1353.64
b) 652.68
Slush-freezing
Filling and seaming
Blast-freezing
Storage
Heating
Filling and seaming
Cooling
Storage
Frozen
concentrated
citrus juice
Concentrated
citrus juice

Figure 3. Flow diagram and material balance flow sheet for a concentrated citrus juice production plant with working capacity of 20,000 kg of raw oranges per hour and 10,000 kg of raw lemons per hour. The data reported here are expressed as kgh $^{-1}$; symbol (a) refers to orange, while symbol (b) to lemons. Stream identification symbols: COWE, citrus oils and water emulsion; EPP excess pulp and peel; PPS, peel, pulp, and seeds; WCW, water and citrus waste; WW, washing water. *Source:* Reprinted with permission from Ref. 11.

2. Degree of concentration required (percentage dry solids in the product).
3. Heat sensitivity of product in relation to the residence time and temperature of evaporation.
4. The requirement for volatile recovery facilities.
5. Ease of cleaning.
6. Reliability and simplicity of operation.
7. Size of evaporator.
8. Capital and operating costs.
9. Product quality.

Evaporators are classified as natural circulation evaporators and forced circulation evaporators.

Natural Circulation Evaporators

These may be open or closed pan evaporators, short-tube evaporators (also known as calandria vacuum pans in the food industry) (Fig. 5), or long-tube evaporators. Natural circulation evaporators operate by the thermo-syphon principle. The density difference between the boiling liquor and the circulation leg produces the driving force for liquid circulation. Typical applications include beet sugar, low to moderately viscous liquors, and nonsalting materials.

Pan evaporators are heated directly by gas or electrical resistance wires or indirectly by steam. They have relatively low rates of heat transfer, low energy efficiencies, and cause damage to heat-sensitive foods. However, they have low capital costs and are easy to construct and maintain. They have found wide applications in the preparation of sauces, gravies, and jam or other preserves (12).

Short-tube evaporators consist of a vessel or shell that contains a bundle of tubes. The feed solution is heated by steam condensing on the outside of the tubes. Liquor rises through the tubes, boils, and recirculates by flowing back down through a wide central bore. The vertical arrangement of tubes promotes natural convection currents and, therefore, higher heat transfer rates. The flow velocity is typically 0.3–1 m/s (15). These evaporators have low capital costs and are flexible, although generally unsuited to high-viscosity solutions. They are widely used for concentrating syrups, salt, and fruit juices.

The long-tube evaporators consist of a vertical bundle of tubes, each up to 50 mm in diameter and 5–15 m high. The length-to-diameter ratio is 70:130 (15). Liquid is preheated almost to boiling point (evaporation temperature) before entering the evaporator. For low-viscosity (less than 0.1 N · s/m^2) foods such as milk, the thin film of liquor is forced up the evaporator tubes and this arrangement is known as the climbing-film evaporator (Fig. 6). A great deal of foaming does not matter, but they are susceptible to caking (15). As steam is admitted to the chest of the evaporator, the liquid reaches the boiling point and evaporation starts; a lot of small vapor bubbles appears in the liquid and starts the two-phase flow. The bubbles expand and push the mixture of liquid and vapor upward, further heat is applied resulting in more vapor formed (plug flow). Farther upward the remaining liquid is maintained as a film on the tube wall with vapor flowing as a core (annular flow). In the final part of the tube, the film is bursting and the liquid appears as droplets in a flow of vapor (mist flow).

Table 2. Material and Energy Balance for the Evaporation Unit of the Concentrated Citrus Process

Parameter		1st effect	2nd effect	3rd effect	4th effect	5th effect	Unit
Internal pressure	a[a]	73.5	63.8	54.2	44.0	6.0	kPa
	b	57.0	39.2	25.0	14.2	6.0	
Juice temperature	a	91.7	88.1	84.2	79.6	41.2	°C
	b	85.9	76.9	66.9	55.5	40.8	
Boiling point rise	a	0.44	0.55	0.77	1.3	4.9	°C
	b	1.21	1.45	1.84	2.57	4.5	
Clean heat-transfer coefficient	a	3,764	3,416	2,971	2,259	130	Wm^{1} °C^{1}
	b	4,061	3,470	2,902	2,188	799	
Input liquid flow rate	a	8,518	7,140	5,765	4,392	3,014	kgh^{-1}
	b	3,743	3,233	2,694	2,123	1,517	
Input liquid concentration	a	11.7	14.0	17.3	22.7	33 1	°Brix
	b	9.3	10.8	12.9	16 4	23 0	
Output liquid flow rate	a	7,140	5,765	4,392	3,014	1,533	kgh^{-1}
	b	3,233	2,694	2,123	1,517	870	
Output liquid concentration	a	14.0	17.3	22.7	33.1	65.0	°Brix
	b	10.8	12.9	16.4	23.0	40.0	
Recirculation ratio	a	0	0	0	0	6	
	b						
Thermal loss	a	0.03	0.03	0.03	0.03	0.03	
	b						
Heat-transfer surface	a	193.3	193.3	193.3	193.3	193.3	m^2
Fouling factor ($\times 10^3$)	a	0.43	0.43	0.43	0.43	0.43	m^2 °CW^{-1}
	b	4.5	4.5	4.5	4.5	4.5	
Live steam consumption (95°C)	a			1,484			kgh^{-1}
	b			625			
Cooling water consumption (21–33°C)	a			72.3			m^{2}h^{-1}
	b			29.7			

Source: Adapted with permission from Ref. 11.

[a] Orange juice = a; lemon juice = b.

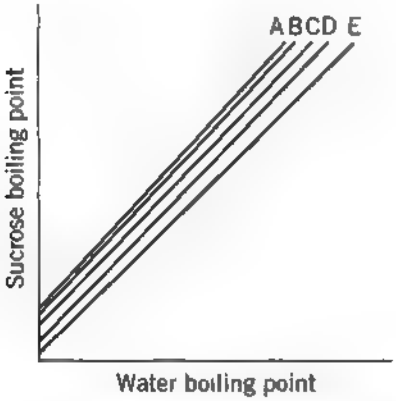


Figure 4. Duhring plot for sucrose for various sucrose concentrations per 100 g of water. A, 1,000 g; B, 800 g; C, 600 g; D, 400 g; E, 200 g. *Source:* Reprinted with permission from Ref. 12.

Pressure drop increases as more vapor is formed, and hence the evaporation temperature drops. This temperature drop must be as small as possible because it leads to higher energy consumption. The flow regime and temperature profile are depicted in Figure 7.

For more viscous foods, or those that are very heat sensitive, the feed is introduced at the top of the tube bundle, and the concentrate and the vapor are leaving the tubes at their bottom. Normally the liquid is superheated when entering the tube, thus the liquid is already at its boiling point and no part of the heating surface is used for preheating. This type of equipment is called the falling-film evaporator (Fig. 8). Only a small amount of product is in the tube, compared with the column of liquid in the climbing-film evaporator. Hydrostatic head loss found in climbing-film evaporators is absent in falling-film evaporators. Thus little temperature drop is seen during the passage through the tube (14). The gravitational force enhances the forces due to expansion of the steam, to produce high flow velocities of up to 200 m/s at the end of the tubes (12). Falling-film evaporators were developed to increase the rate of removal of water and decrease the danger of localized overheating.

A falling-film evaporator consists of the following major components: vertical heating tube bundle with surrounding heating jacket, calandria upper section with product distribution device, and calandria base section with separator. The liquid to be concentrated is fed into the upper part of the calandria where it is distributed evenly on the tube sheet, from which it flows down the interior walls of the vertical tubes as a thin boiling film. The most important factor in the trouble-free operation of this evaporator is the liquid coverage of the heating tubes, that is, the liquid flow rate required to cover each heating tube. Without enough liquid, the liquid film will break near the bottom of the tube. Dry spots develop on the tube, the heating surface becomes coated with a deposit, and eventually the tube may get clogged with burned-on product. The minimum liquid coverage depends on the type of product, required concentration, and operating time. If the coverage

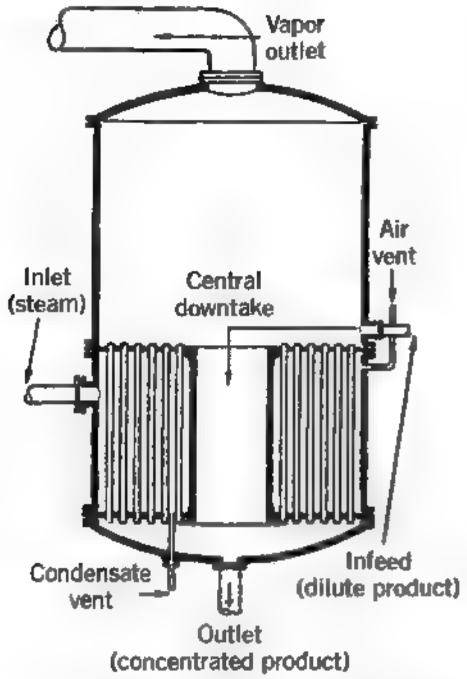


Figure 5. Calandria evaporator. *Source:* Reprinted with permission from Ref. 14.

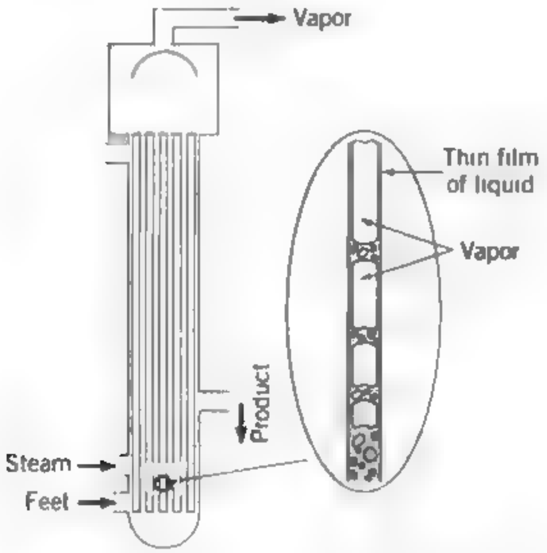


Figure 6. Climbing-film evaporator. *Source:* Reprinted with permission from Ref. 12.

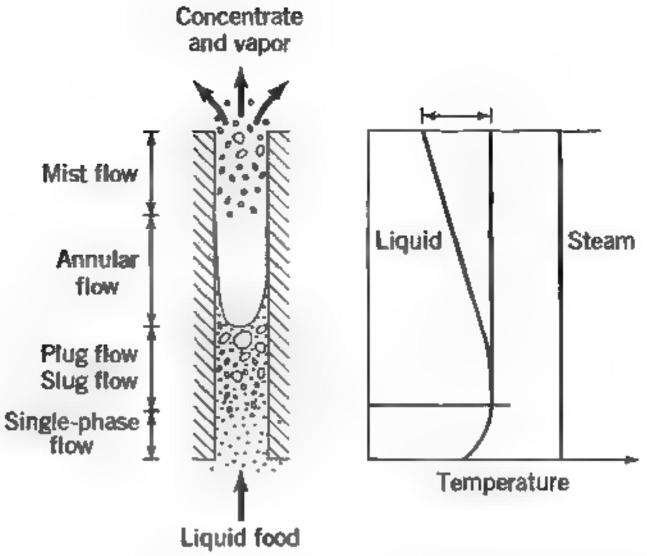


Figure 7. Flow regimes and temperature distribution in a climbing-film evaporator. *Source:* Reprinted with permission from Ref. 16.

falls short of a minimum value, fewer but longer heating tubes may be used, thus giving more liquid per tube while maintaining the same total heating surface area. The next choice is to subdivide the calandria to create more passes on the product side; recirculation of product within a calandria also improves coverage, however, it increases the residence time. Tube length design shall be a compromise between adequate liquid coverage and pressure drop. Short tubes with larger diameters have smaller pressure loss, whereas small diameter tubes lead to high velocity of the two-phase flow, causing high pressure loss and boiling point elevation. To this effect, it has been recommended that tubes between 8 and 12 m in length and with a diameter between 30 and 50 mm be used (16).

Product distribution in the upper part of the calandria can be done in two ways; dynamic distribution or static distribution. Dynamic distribution may be done by a full-cone nozzle in which the kinetic energy of the liquid is used to atomize the fluid into droplets and distribute them on the upper tube sheet. This distribution device, although simple and economic, does not guarantee even distribution. Static distribution devices (Fig. 9) are generally preferred. Even distribution is achieved by having uniform liquid static pressure over all openings in the base of a predistribution bowl, giving uniform wetting of all tubes. The system adapts readily to varying feed conditions by varying the liquid level. The falling-film evaporator is widely used for sugars and syrups, yeast extracts, fruit juices, dairy products and starch processing.

It has been pointed out that both types of long-tube evaporator are characterized by short residence times, high-heat transfer coefficients, and efficient energy use (steam economy = 2.5 to 3.3) in multiple-effect systems (12).

Forced Circulation Evaporators

Forced circulation evaporators have a pump or scraper assembly to move the liquor, usually in thin layers, to enhance heat-transfer rates and shorten residence times. The forced circulation design is generally used in applications where a solid is precipitated during evaporation or a scaling constituent is present.

Recirculated falling-film configurations are used when insufficient feed liquor is available to use the heat-transfer surface with single-pass operation (natural circulation method). A portion of the product liquor is combined with the feed stream and is pumped to the upper liquor cham-

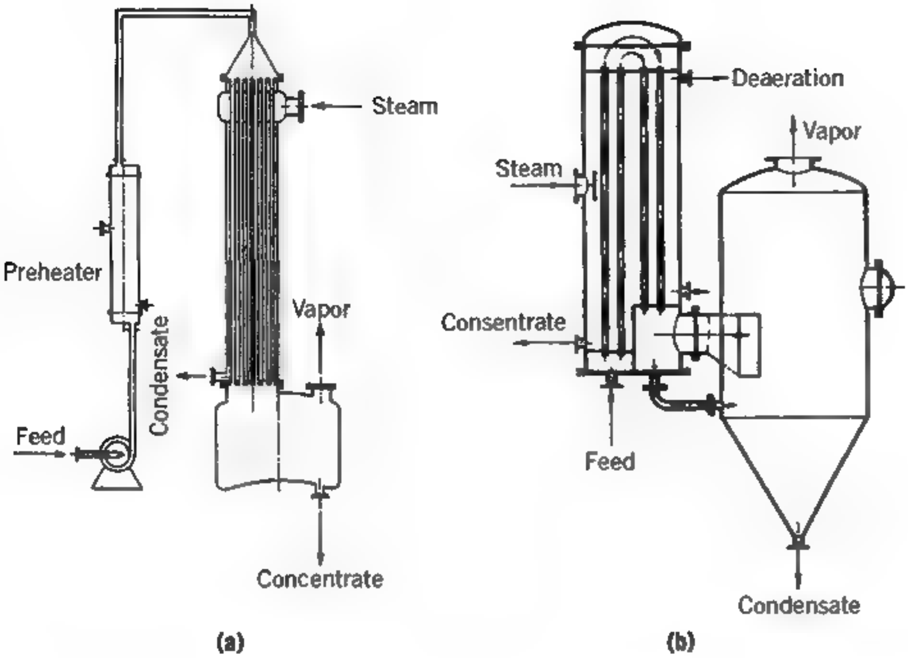


Figure 8. (a) Falling-film evaporator. (b) Falling-film evaporator, with falling and climbing sections. *Source:* Reprinted with permission from Ref. 15.

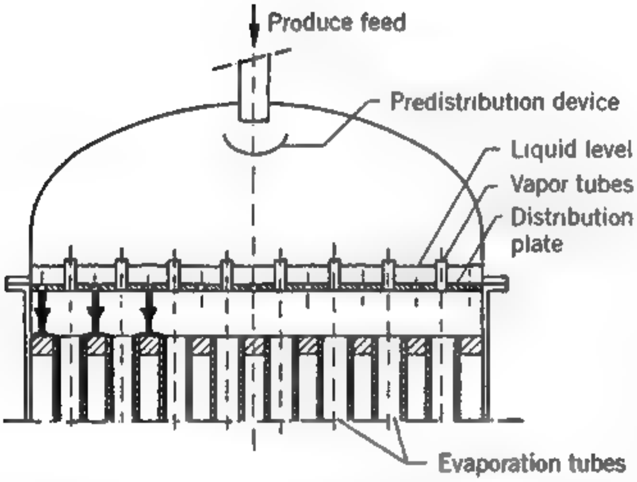


Figure 9. Liquid distribution in a falling-film evaporator. *Source:* Reprinted with permission from Ref. 8.

ber. Product retention time is greater than for the single-pass evaporator but is still relatively short as the operating volume is small. These evaporators find applications in moderately heat-sensitive foods.

The plate type or AVP evaporator, developed in the UK in the 1950s, is suitable for viscous liquids (0.3–0.4 N · s/m^2), because the food is pumped through the plate stack, consisting of a series of climbing-film and falling-film sections. The heating medium is steam. They are compact and are advantageous in having a small amount of product in the system at any one time. They have the additional advantage of ease of maintenance and inspection.

An example of a high-temperature, short-time (HTST or flash) evaporator is one with rotating heating surface and is shown in Figure 10. It is a mechanical thin-film evaporator. Feed flows to the underside of a stack of rotating hollow cones from a central shaft. The feed liquor is kept in rapid motion, and the centrifugal force (750–3,000 N) causes the formation of a very thin film (about 0.1 mm thick) in which the liquid remains for only a very short time of 0.6 to 1.6 s (17). Steam condenses on each cone, and heat is conducted rapidly through the thin metal to evaporate the liquor. High rates of heat transfer are obtained as steam condensate is flung from the rotating cones as soon as they are formed. A high degree of flexibility can be achieved by changing the number of cones. This evaporator is used for coffee and tea extracts, meat extract, fruit juices and enzymes for use in food processing.

A schematic cross section of an agitated-film (also called scraped-surface or wiped-surface) evaporator is shown in Figure 11. The product film near the heat-transfer surface is continuously agitated by a rotor to increase the heat-transfer rate on the product side. The evaporator may also consist of a steam jacket surrounding a highspeed rotor, fitted with short blades along its length (Fig. 12). The liquid enters the wide end and is forced through the precise space between the rotor and the wall (heated surface) toward the outlet for the concentrate. The blades agitate the thin film of liquid vigorously, thus promoting high heat-transfer rates and preventing the product from burning onto the hot surface. If the viscosity increases greatly during evaporation, it is better to feed the liquid at the narrow end. This type of equipment is highly suited to viscous (up to 20 N · s/m^2), heat-sensitive foods, or to those that are liable to foam or foul evaporator surfaces (fruit pulps and juices, tomato paste, meat extracts, honey, cocoa mass, coffee, and dairy products). Only single effects are possible, thus giving low steam economy. It is more intended for finishing highly viscous products after preconcentration in other equipment (12).

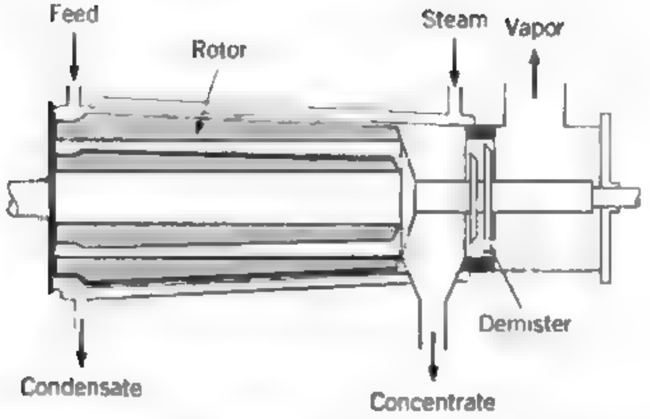


Figure 10. Mechanical thin-film evaporator. *Source:* Reprinted with permission from Ref. 15.

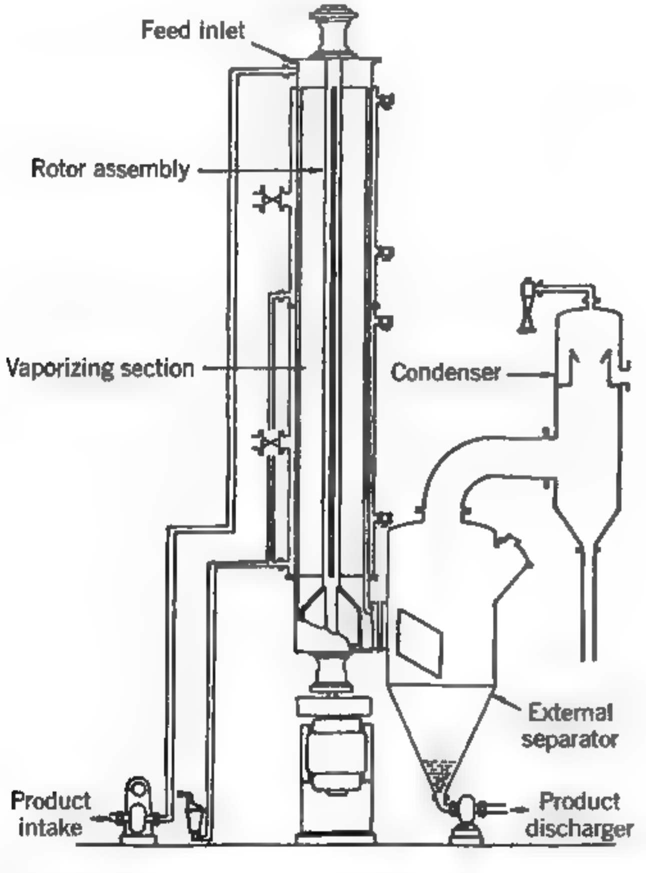


Figure 11. Schematic cross-section of agitated film evaporator. *Source:* Reprinted with permission from Ref. 4.

Energy Efficiency

The energy efficiency of evaporation systems may be improved by the following approaches (19): multiple-effect, vapor recompression, or refrigerant cycle evaporators.

Multiple-Effect Evaporating System. For the evaporation of temperature-sensitive materials it is important to keep the temperature as low as possible. This is achieved by working at a lower pressure. Practically all the evaporators mentioned above can be used at pressures below atmospheric.

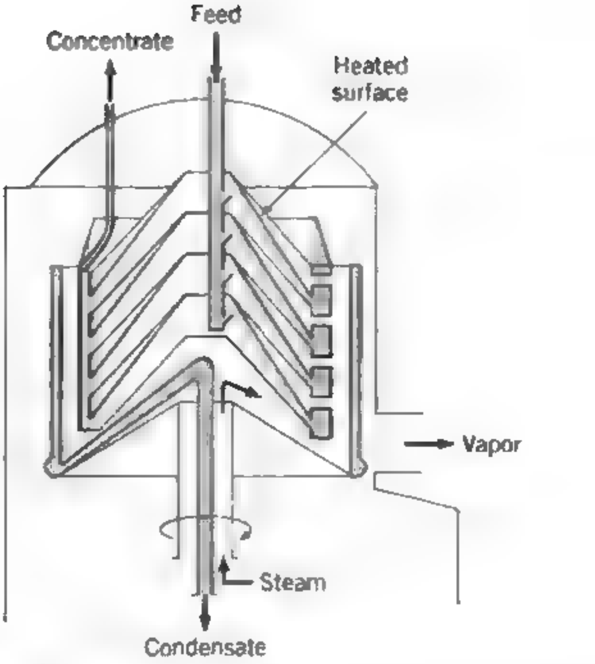


Figure 12. Centri-term evaporator. *Source:* Reprinted with permission from Ref. 18.

To make use of the large amount of heat in the vapor driven off the liquid food, the water vapor can be used as the heating medium in a following evaporator. This second evaporator must then operate at a lower pressure than the first. This is called double-effect evaporation. Repetition of this step produces multiple-effect evaporation. The effects then have progressively lower pressures to maintain the temperature difference between the feed and the heating medium. A multiple-effect evaporating system can be designed in several ways in regard to the direction of flow of the solution. Figure 13 illustrates, with a triple-effect evaporator, the arrangements of forward feed, backward feed, parallel feed, and mixed-feed system.

The liquid in the forward feed system enters the highest temperature evaporator first and exits as a concentrated liquid from the third evaporator that operates at the lowest temperature and pressure. The liquid is usually preheated by steam or vapor from one of the effects before it enters the first evaporator. The liquid leaving effect 1 has a higher temperature than the liquid in effect 2, and in crossing the throttle valve to effect 2, a small amount of the liquid water will flash to water vapor and the temperature of the liquid will drop to the operating temperature of effect 2.

In the backward feed system, the liquid flows in opposite direction of the forward feed evaporator. This system allows the concentrated liquid to exit at the highest temperature, which is advantageous for products having a high viscosity in the concentrated form. Because the liquid leaving effect 3 has a lower temperature than the liquid in effect 2, a pump is needed to provide additional energy to increase its temperature to the saturation temperature

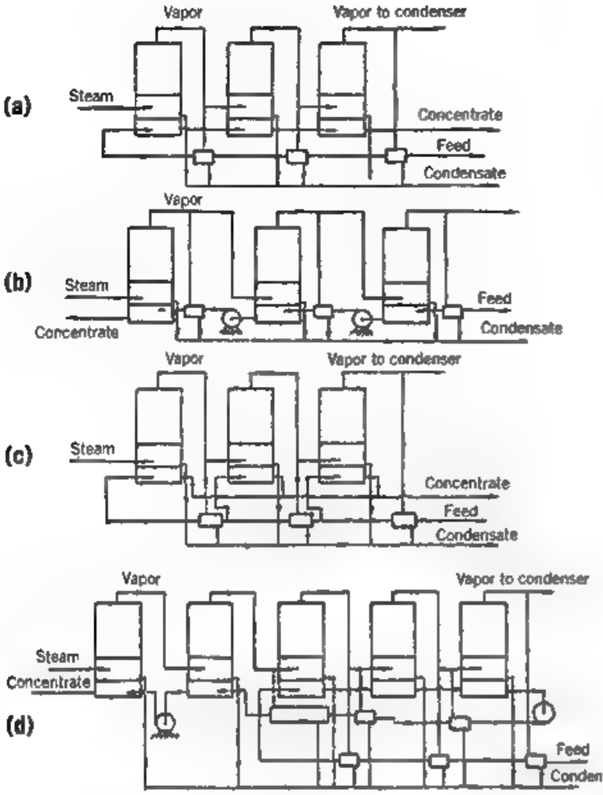


Figure 13. Multiple-effect evaporation. (a) Forward feed; (b) backward feed; (c) parallel feed; (d) mixed feed. *Source:* Reprinted with permission from Ref. 15.

corresponding to effect 2. A similar situation exists between effect 2 and effect 1.

The parallel feed evaporator essentially acts like three separate evaporators, each operating at a different pressure. It has the advantage of simple operation. However, it is the most expensive arrangement, because extraction pumps are required for each effect. Mixed-feed evaporators are useful for viscous food, such as distillery by-products (20). They have the simplicity of forward feed and economy of backward feed. The merits and shortcomings of each arrangement are described in Table 3.

In each evaporator of a multiple-effect installation it is necessary for the temperature of the heating medium to be higher than the boiling temperature; the number of stages is, therefore, limited. Savings in energy consumption decreases with every effect added. Ideally, 1 kg of steam vaporizes 1 kg of water in all effects, so that the steam economy would be 1, 2, 3, and 4, respectively, for one to four effects. In practice, the steam consumption is somewhat higher because of heat losses. Actual steam economy for evaporating milk is 0.8, 1.7, 2.5, and 3.3 (2). The steam economy for evaporating tomato paste was calculated (22); the values were 0.95, 1.91, and 2.60 compared to measured values of 0.84, 1.45, and 2.20 (on average), respectively, for one, two, and three effects.

Many HTST evaporators, under the trade name TASTE (thermally accelerated short time evaporator) have been installed in Florida and elsewhere (23). The initial feed is preheated to about 90°C in a conventional heat exchanger and flashed through a series of evaporators with progressively higher vacuums (15). A typical unit has seven stages plus a vacuum flash cooler arranged so there are four effects. The word *effect* indicates vapor flow in the evaporator, while the word *stage* indicates the flow of product (24). Temperature in the various stages range from 41 to 96°C. These units are small for their capacity and lack the vapor–liquid separation chambers of the low-temperature evaporators. There is more tendency for hesperidin to build up on the heat exchange surface of the TASTE evaporator in comparison with low temperature units, but because of the low retention time, it can be emptied rapidly. It can be cleaned in place, without dismantling, in minutes.

The steam economy of two tubular TASTE evaporators with four effects and seven stages (Fig. 14) and with nominal evaporation capacity of 27,211 kg (60,000 lb) and 45,352 kg (100,000 lb) of water per hour is 0.85 *N*; *N* being the number of effects of the evaporator (24). More stages are incorporated in the last effect because of reduction in product volume as it is concentrated. Similarly, it was found that the steam economy for two plate evaporators (four effects, five stages) (Fig. 15) with a capacity of 13,605 kg (30,000 lb) per hour was 0.82 *N*.

Vapor Recompression. Thermal recompression with a steam-jet compressor (25) is illustrated in Figure 16. A fraction of the vapor produced is combined with steam entering the heat exchanger in the low-pressure section of the Venturi. The remaining vapor is used in subsequent effects. This system is sometimes advantageous when the temperature of steam supplied by the boiler is too high for the foods. By regulating the size of the Venturi the pressure can be controlled and hence condensing temperature of the steam. Part of the kinetic energy associated with the high-velocity flow in the Venturi is used to compress the recirculated vapor. Vapor recompression will decrease steam consumption and increase the steam economy. When steam costs are moderate and electricity costs comparatively high, thermal compression is recommended. This simple method is relatively inexpensive, has a long operating life, and requires little maintenance. However, its characteristic operating curve has only one optimum operating point, thus restricting flexibility between design and actual operating conditions (16).

In a mechanical vapor recompression system (Fig. 17) all of the vapor produced is compressed to a slightly higher pressure by a mechanically driven compressor. The compressed vapor then enters the heat exchanger and on condensation provides heat to drive the evaporation process. This method can virtually eliminate steam requirement, as only a small amount of make-up steam is needed. Yet both the high capital and energy costs to operate the compressor need to be considered; it is about 10 times more expensive than the thermal type. Also, when evaporation pressures become too low, the volume of vapor to be handled increases dramatically, and mechanical compression is not economical. An evaporator operating at 5.0 kPa (33°C) would produce almost four times as much vapor volume as an evaporator operating at 20 kPa (60°C) on the same production rate basis (19). Nevertheless, mechanical

Table 3. Advantages and Limitations of Various Methods of Multiple-Effect Evaporation

Arrangement of effects	Advantages	Limitations
Forward feed	Least expensive, simple to operate, no feed pumps required between effects, lower temperatures with subsequent effects and, therefore, less risk of heat damage to more viscous product.	Reduced heat-transfer rate as the feed becomes more viscous, rate of evaporation falls with each effect, best quality steam used on initial feed, which is easiest to evaporate; feed must be introduced at boiling point to prevent less of economy (if steam supplies sensible heat, less vapor is available for subsequent effects)
Reverse feed	No feed pump initially, best-quality steam used on the most difficult material to concentrate, better economy and heat-transfer rate as effects are not subject to variation in feed temperature and feed meets hotter surfaces as it becomes more concentrated thus partly offsetting increase in viscosity	Interstage pumps necessary, higher risk of heat damage to viscous products as liquor moves more slowly over hotter surfaces, risk of fouling
Mixed-feed	Simplicity of forward feed and economy of backward feed, useful for very viscous foods	More complex and expensive
Parallel	For crystal production, allows greater control over crystallization and prevents the need to pump crystal slurries	More complex and expensive of the arrangements, extraction pumps required for each effect.

Source: Adapted with permission from Ref. 21.

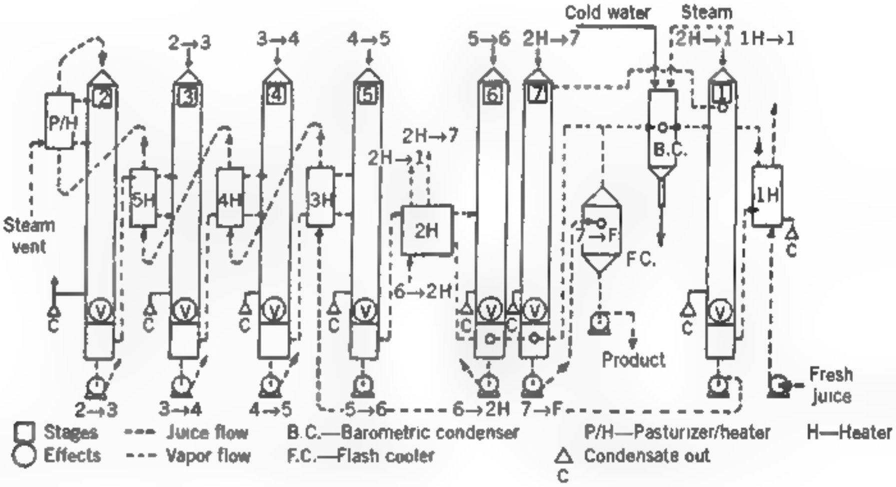


Figure 14. Schematic of a 5-effect, 7-stage TASTE Evaporator. *Source:* Reprinted with permission from Ref. 24.

vapor recompressors have more or less the same limited flexibility as thermal recompressors. This applies particularly to the high-speed radial turbo compressor, which reaches its surge limit if the actual vapor flow is too small, thus rendering the system unstable (16). Mechanical recompressors are complicated in design, and need careful maintenance and supervision of mechanical vibration.

Refrigerant Cycle Evaporators. Figure 18 shows a schematic system of juice concentration using a technique that combines a vapor compression refrigeration cycle with the evaporation system (25). Condensing water vapor boils liquid refrigerant to form a refrigerant vapor, which is then compressed to a higher temperature level and acts as a heat source to boil the liquid being concentrated (19). The evaporator, for simplicity, is shown as a single-effect design with means for recirculating the juice. Variations may employ multiple effects. The pressure in the system can be maintained by a relatively small steam ejector or a mechanical vacuum pump. Its advantage over a mechanical vapor recompression system is that the volume of vapor being compressed is much reduced at a given evaporator temperature. However, the refrigerant must be compressed through a wider temperature range because an additional heat exchanger is required.

Auxilliary Equipment

Preheaters. After feeding the product into the evaporation plant via a float balance tank and feed pump, the first process step is to preheat the product. In general, liquid food is stored at between 5 and 20°C for quality preservation; therefore, it must be preheated to at least the evaporation temperature of the first effect, most commonly between 40 and 110°C. Three types of preheater are used: plate, spiral tube, and straight tube heat exchangers (16). If there are solid particles in the product, a plate heat exchanger should not be used, because the particles may block the narrow product passages. The same problem may

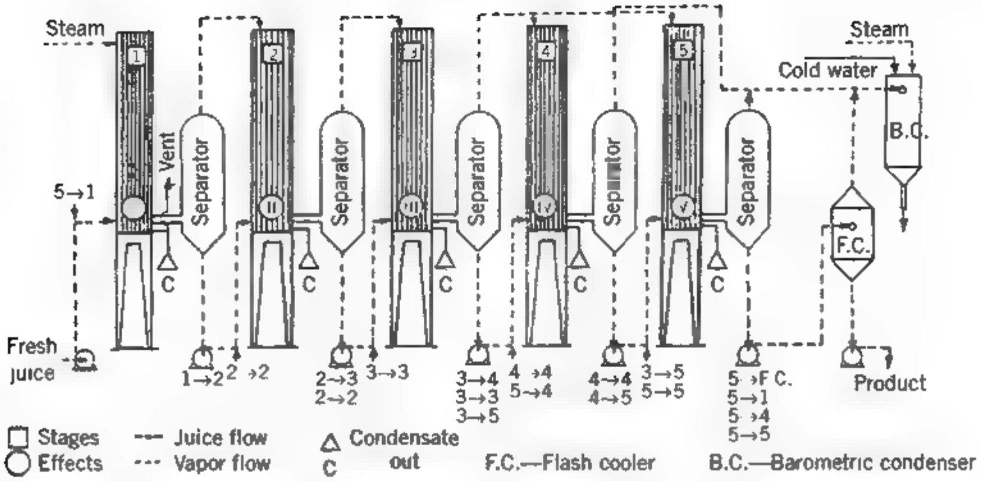

Steam
1
2
3
4
5
Vent
Separator
Separator
Separator
Separator
Separator
5→1
I
II
III
IV
V
C
C
C
C
C
Steam
Cold water
B.C.
F.C.
Fresh juice
1→2
2→2
2→3
2→2
3→3
3→4
3→3
3→5
4→4
5→4
4→4
4→5
3→5
5→5
5→F.C.
5→1
5→4
5→5
Product
Stages
Effects
Juice flow
Vapor flow
Condensate out
F.C.—Flash cooler
B.C.—Barometric condenser

Figure 15. Schematic of a 4-effect, 5-stage plate evaporator. *Source:* Reprinted with permission from Ref. 24.

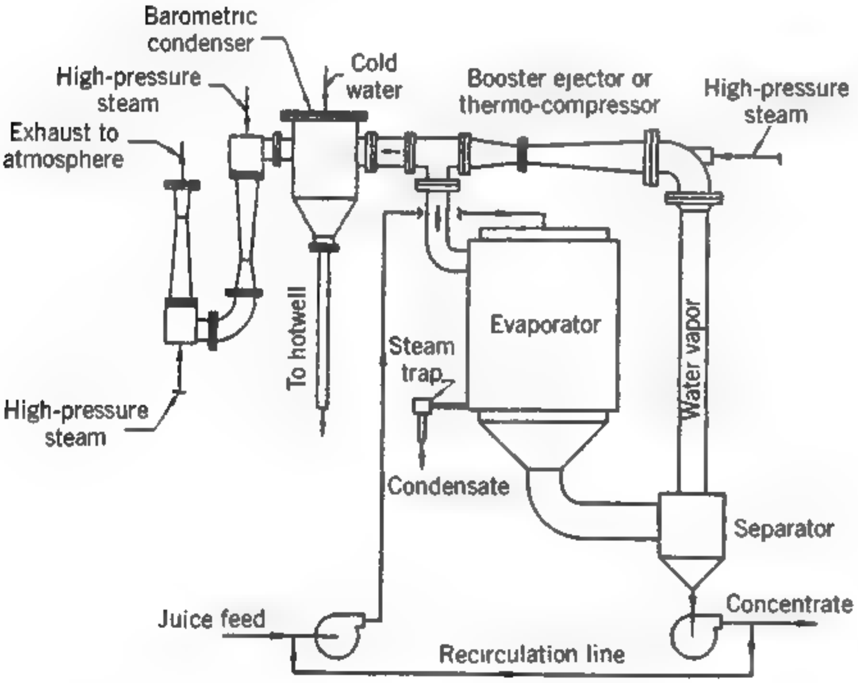

Barometric condenser
High-pressure steam
Exhaust to atmosphere
Cold water
Booster ejector or thermo-compressor
High-pressure steam
To hotwell
Evaporator
Steam trap
Water vapor
High-pressure steam
Condensate
Separator
Concentrate
Juice feed
Recirculation line

Figure 16. System of concentration using thermal recompression with a steam-jet compressor. *Source:* Reprinted with permission from Ref. 25.

also occur with protein-containing products, due to the formation of deposits if the preheating temperature exceeds the denaturalization temperature. Secondary flows developed in the spiral tube leads to higher heat-transfer coefficients than straight tube, and a reduced tendency to foul. The disadvantages are that inspection and replacement of the tube is very difficult. Straight tube preheaters are especially useful as intermediate preheaters following first step preheating with plate heat exchanger, where product of high concentration is required, and where equipment inspection is essential.

Separators. Separators are used to separate the mixture of vapor and concentrated product produced in the calandria. A separator should have high separation efficiency, small pressure loss, and short residence time. Because a fine mist of concentrate is produced during vigorous boiling and carried over in the vapor, separators should be designed to minimize entrainment. Large pressure loss is to be avoided lest extra heating surface must be installed in the next effect. The many different separators in use can be classified according to three operating principles: gravity separator, baffle separator, and centrifugal separator.

Condensers. Surplus vapor produced in evaporation can be condensed and reused where possible. Condenser cooling water is normally provided from a cooling tower or the city main; in certain cases, it may be extracted from rivers, lakes, or the sea. The tasks of a condenser are to maintain the heat balance of an evaporation plant, to stabilize the temperature in each effect, and to produce and maintain vacuum.

EFFECT ON FOODS

Nutritional changes take place during processing, the extent varying with the type of food, the process, the plant in use, and the degree of control exercised. Many losses are inevitable, particularly if the process involves heating. Part of the water-soluble nutrients such as the B vitamins, together with lesser and less-important amounts of mineral salts, protein, and even carbohydrate will be precipitated out. Vitamin C is oxidized in air and accelerated by heat, whereas vitamins A and D and niacin are unaffected (20). A comparison of nutrient losses in milk preserved by evaporation and UHT sterilization is shown in Table 4.

Aroma compounds that are more volatile than water will be lost during evaporation. With some products such as fruit juices, the retention of taste and aroma is important, yet in other foods such as cocoa and milk, the loss of unpleasant volatiles improves the product quality. The color of foods darkens, partly due to an increase in the solids content and partly because the reduction in water activity promotes chemical changes (for example, Maillard browning) (12). As these changes are time and temperature dependent, short residence times and low boiling points produce concentrates with higher qualities. For in-

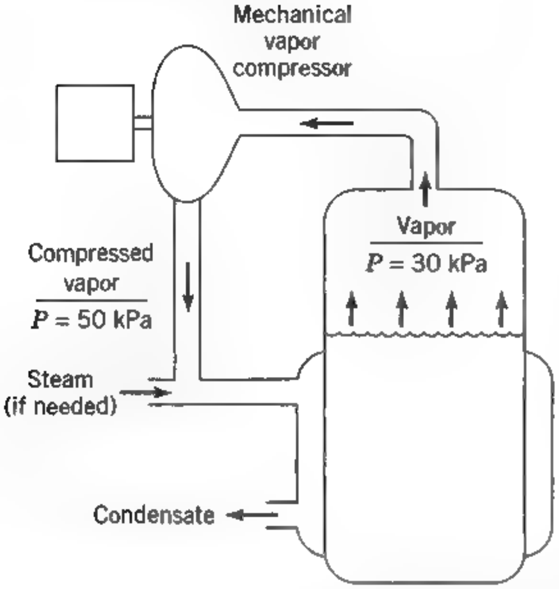

Mechanical vapor compressor
Vapor
P = 30 kPa
Compressed vapor
P = 50 kPa
Steam (if needed)
Condensate

Figure 17. A mechanical vapor recompression system. *Source:* Reprinted with permission from Ref. 19.

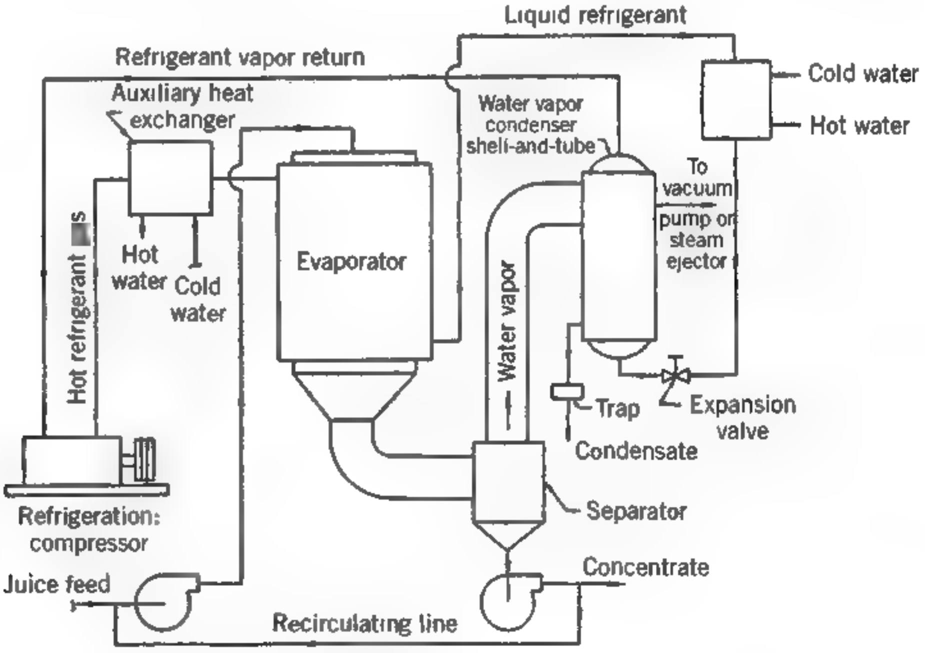

Liquid refrigerant
Refrigerant vapor return
Cold water
Hot water
Auxiliary heat exchanger
Water vapor condenser shell-and-tube
To vacuum pump or steam ejector
Hot refrigerant
Hot water
Cold water
Evaporator
Water vapor
Trap
Expansion valve
Condensate
Separator
Refrigeration: compressor
Juice feed
Concentrate
Recirculating line

Figure 18. System of concentration using refrigerant cycle evaporators. *Source:* Reprinted with permission from Ref. 25.

Table 4. Vitamin Losses in Concentrated and UHT Sterilized Milk

	Loss (%)				
Product	Thiamin	Vitamin B_6	Vitamin B_{12}	Folic acid	Ascorbic acid
Evaporated milk	20	40	80	25	60
Sweetened condensed milk	10	<10	30	25	25
UHT sterilized milk	<10	<10	<10	<10	<25

Source: Adapted with permission from Ref. 26.

stance, the Centri-therm mechanical thin-film evaporator produces a concentrate that, when diluted, has sensory and nutritional qualities that are virtually unchanged from those of the feed material (12).

NOMENCLATURE

A	Area, m^2
D	Diameter, mm
DM	Dry matter content, %
H	Enthalpy, J/kg
G	Mass flow rate per unit width of wall, kg/m · s
R	Gas constant = 8,314 N · m/g · mol °K
T	Temperature, °C
U	Overall heat transfer coefficient, W/m^2 °C
X	Mass fraction of solute in the liquid
X^1	Molar concentration
Fr	Froude number
Nu	Nusselt number
Pr	Prandtl number
Re	Reynolds number
a	Coefficient used in eq. 13
c	Specific heat, J/kg °C
g	Gravitational constant, m/s^2
h	Enthalpy of liquid, J/kg
h_c	Convective heat-transfer coefficient, W/m^2 °C
k	Thermal conductivity, W/m °C
$\dot{m}$	Mass flow rate for evaporator, kg/s
u	Liquid–vapor velocity, m/s
x	Thickness, mm
y	Vapor quality
Δ	Boiling point elevation, °C
λ	Latent heat of vaporization, J/kg
μ	Viscosity, kg/m · s
ρ	Density, kg/m^3
σ	Surface tension, N/m

Subscripts

1	First effect in a double-effect evaporator
2	Second effect in a double-effect evaporator
A	Solvent component of product (water)
F	Feed
b	Boiling
c	Concentration
f	Fluid, or liquid state
m	Log-mean value
p	Product
s	Steam (or other heating medium)
v	Vapor
w	Wall condition
0	Pure state

BIBLIOGRAPHY

1. H. A. Leniger, "Concentration by Evaporation," in T. Hoyem and O. Kvale, eds., *Physical, Chemical and Biological Changes in Food Caused by Thermal Processing*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1980, pp. 54–76.
2. A. W. Farrall, *Food Engineering Systems. Vol. 1, Operations*, AVI Publishing Co., Inc., Westport, Conn., 1976.
3. F. Kreith and W. Z. Black, *Basic Heat Transfer*, Harper & Row, 1980.
4. D. R. Heldman and R. P. Singh, *Food Process Engineering*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1980.
5. *Handbook of Fundamentals*, American Society of Heating, Refrigeration and Air Conditioning Engineers, 1989
6. E. L. Piret and H. S. Isbin, "Natural Circulation Evaporators," *Chemical Engineering Progress* **50**, 305–310 (1954).
7. J. M. Coulson and J. F. Richardson, *Chemical Engineering*, Vol. 2, 3rd ed., Pergamon Press, Elmsford, N.Y., 1976.
8. B. Hallstroem, "Heat Exchange," in *Evaporation, Membrane Filtration and Spray Drying in Milk Powder and Cheese Production, North European Dairy Journal*, 1985, pp. 37–41.
9. K. Stankiewicz and M. A. Rao, "Heat Transfer in Thin-Film Wiped-Surface Evaporation of Model Liquid Foods," *J. Food Process Eng.* 10, 113–131 (1988).
10. S. Angeletti and M. Moresi, "Modeling of Multiple-Effects Falling-Film Evaporators, " *Journal of Food Technology* **18**, 539–563 (1983).
11. M. Moresi, "Economic Study of Concentrated Citrus Juice Production," in B. M. McKenna, ed., *Engineering and Food*, Vol. 2, *Processing Applications*, 1984, pp. 975–991.
12. P. Fellows, *Food Processing Technology—Principles and Practice*, VCH Publishers, New York, 1988.
13. S. Bouman, D. W. Brinkman, P. de Jong, and R. Waalewijn, "Multistage Evaporation in the Dairy Industry: Energy Savings, Product Losses and Cleaning," in S. Brun, ed., *Preconcentration and Drying of Food Materials*, 1988, pp. 51–60.
14. M. A. Joslyn and J. L. Heid, *Food Processing Operations—Their Management, Machines, Materials, and Methods*, Vol. 3, AVI Publishing Co., Inc., Westport, Conn., 1964.
15. H. A. Leniger and W. A. Beverloo, *Food Process Engineering*, Reidel Publishing, Boston, 1975.
16. G. Hahn, "Evaporator Design," in D. MacCarthy, ed., *Concentration and Drying of Foods*, 1987, pp. 113–132.
17. P. P. Lewicki and R. Kowalczyk, in P. Linko, Y. Malkki, J. Olkku and J. Larinkari, eds., *Food Processing Systems*, Elsevier Applied Science Publishers, Barking, UK, 1980, pp. 501–505.
18. C. H. Mannheim and N. Passy, "Aroma Retention and Recovery During Concentration of Liquid Foods," Proceedings of the Third Nordic Aroma Symposium, Hemeelinna, 1972.
19. J. C. Batty and S. L. Folkman, *Food Engineering Fundamentals*, John Wiley & Sons, Inc., New York, 1983.
20. A. E. Bender, "Food Processing and Nutritional Values," in S. M. Herschdoerfer, ed., *Quality Control in the Food Industry*, Academic Press, Inc., Orlando, Fla., 1984.
21. J. G. Brennan, J. R. Butters, N. D. Cowell and A. E. V. Lilly, *Food Engineering Operations*, Applied Science, London, 1976.
22. T. R. Rumsey, T. T. Conant, T. Fortis, E. P. Scott, L. D. Pedersen and W. W. Rose, "Energy Use in Tomato Paste Evaporation," *J. Food Process Eng.* 7, 111–121 (1984).
23. B. S. Luh and J. G. Woodroof, *Commercial Vegetable Processing*, 2nd. ed. van Nostrand Reinhold, N.Y, 1988.

24. J. G. Filho, A. A. Vitali and F. C. P. Viegas, Energy Consumption in a Concentrated Orange Juice Plant. *J. Food Process Eng.* 7, 77–89, 1984.

25. *HVAC Systems and Applications*, Amer. Soc. Heating, Refrigeration and Air Conditioning Engineers, 1987.

26. J. W. G. Porter and S. Y. Thompson. "Effects of Processing on the Nutritive Value of Milk," Vol. 1 *Proc. Fourth International Conference on Food Science and Technology*, Madrid, 1976.

A. K. Lau
University of British Columbia
Vancouver, British Columbia
Canada

EVAPORATORS: TECHNOLOGY AND ENGINEERING

TYPES OF EVAPORATORS

In the evaporation process, concentration of a product is accomplished by boiling out a solvent, generally water, so that the end product may be recovered at the optimum solids content consistent with desired product quality and operating economics. It is a unit operation that is used extensively in processing foods, chemicals, pharmaceuticals, fruit juices, dairy products, paper and pulp, and both malt and grain beverages. It also is a unit operation, which, with the possible exception of distillation, is the most energy-intensive.

Although the design criteria for evaporators are the same regardless of the industry involved, the question always arises as to whether evaporation is being carried out in the equipment best suited to the duty and whether the equipment is arranged for the most efficient and economical use. As a result, many types of evaporators and many variations of processing techniques have been developed to take into account different product characteristics and operating parameters.

The more common types of evaporators include the following:

1. Batch pan
2. Natural circulation
3. Rising film tubular
4. Falling film tubular
5. Rising-falling film tubular
6. Forced circulation
7. Wiped film
8. Plate equivalents of tubular evaporators

Batch Pan

Next to natural solar evaporation, the batch pan as shown in Figure 1 is one of the oldest methods of concentration. It is somewhat outdated in today's technology but still is used in a few limited applications such as the concentration of jams and jellies where whole fruit is present and in processing some pharmaceutical products. Up until the early 1960s, it also enjoyed wide use in the concentration of corn syrups.

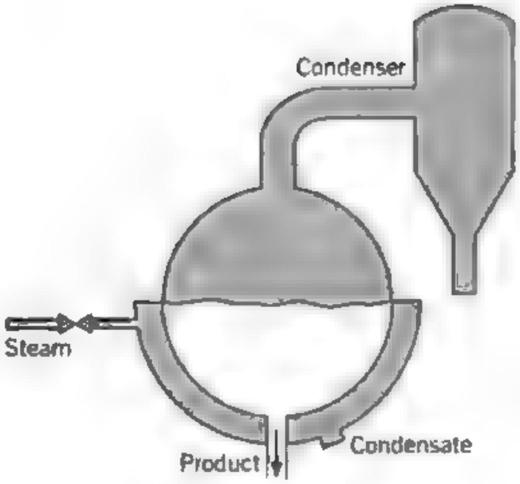


Figure 1. Jacketed batch pan.

With a batch pan evaporator, product residence time normally is many hours. Therefore, it is essential to boil at low temperatures and high vacuum when a heat-sensitive or thermodegradable product is involved. The batch pan is either jacketed or has internal coils or heaters. Heat-transfer areas normally are quite small as a result of vessel shapes, and heat-transfer coefficients tend to be low under natural convection conditions. Heat transfer is improved by agitation within the vessel. Low surface areas together with low HTCs (heat-transfer coefficients) generally limit the evaporation capacity of such a system. In many cases, large temperature differences cannot be used for fear of rapid fouling of the heat-transfer surface. Relatively low evaporation capacities, therefore, limit its use.

Tubular Evaporators

Natural Circulation. Evaporation by natural circulation is achieved through the use of a short tube bundle within the batch pan or by having an external shell and tube heater outside the main vessel as illustrated by Figure 2.

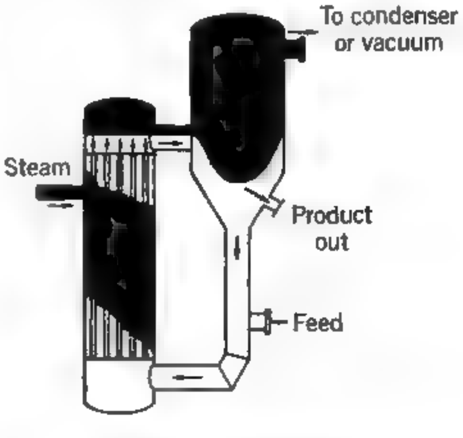


Figure 2. Natural circulation.

24. J. G. Filho, A. A. Vitali and F. C. P. Viegas, Energy Consumption in a Concentrated Orange Juice Plant. *J. Food Process Eng.* 7, 77–89, 1984.
25. *HVAC Systems and Applications*, Amer. Soc. Heating, Refrigeration and Air Conditioning Engineers, 1987.
26. J. W. G. Porter and S. Y. Thompson. "Effects of Processing on the Nutritive Value of Milk," Vol. 1 *Proc. Fourth International Conference on Food Science and Technology*, Madrid, 1976.

A. K. Lau
University of British Columbia
Vancouver, British Columbia
Canada

EVAPORATORS: TECHNOLOGY AND ENGINEERING

TYPES OF EVAPORATORS

In the evaporation process, concentration of a product is accomplished by boiling out a solvent, generally water, so that the end product may be recovered at the optimum solids content consistent with desired product quality and operating economics. It is a unit operation that is used extensively in processing foods, chemicals, pharmaceuticals, fruit juices, dairy products, paper and pulp, and both malt and grain beverages. It also is a unit operation, which, with the possible exception of distillation, is the most energy-intensive.

Although the design criteria for evaporators are the same regardless of the industry involved, the question always arises as to whether evaporation is being carried out in the equipment best suited to the duty and whether the equipment is arranged for the most efficient and economical use. As a result, many types of evaporators and many variations of processing techniques have been developed to take into account different product characteristics and operating parameters.

The more common types of evaporators include the following:

1. Batch pan
2. Natural circulation
3. Rising film tubular
4. Falling film tubular
5. Rising-falling film tubular
6. Forced circulation
7. Wiped film
8. Plate equivalents of tubular evaporators

Batch Pan

Next to natural solar evaporation, the batch pan as shown in Figure 1 is one of the oldest methods of concentration. It is somewhat outdated in today's technology but still is used in a few limited applications such as the concentration of jams and jellies where whole fruit is present and in processing some pharmaceutical products. Up until the early 1960s, it also enjoyed wide use in the concentration of corn syrups.

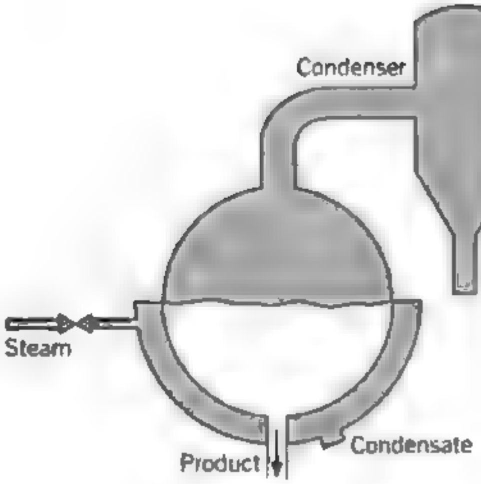


Figure 1. Jacketed batch pan.

With a batch pan evaporator, product residence time normally is many hours. Therefore, it is essential to boil at low temperatures and high vacuum when a heat-sensitive or thermodegradable product is involved. The batch pan is either jacketed or has internal coils or heaters. Heat-transfer areas normally are quite small as a result of vessel shapes, and heat-transfer coefficients tend to be low under natural convection conditions. Heat transfer is improved by agitation within the vessel. Low surface areas together with low HTCs (heat-transfer coefficients) generally limit the evaporation capacity of such a system. In many cases, large temperature differences cannot be used for fear of rapid fouling of the heat-transfer surface. Relatively low evaporation capacities, therefore, limit its use.

Tubular Evaporators

Natural Circulation. Evaporation by natural circulation is achieved through the use of a short tube bundle within the batch pan or by having an external shell and tube heater outside the main vessel as illustrated by Figure 2.

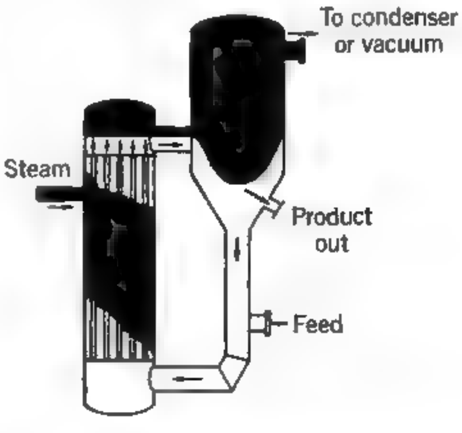


Figure 2. Natural circulation.

The external heater has the advantage that its size is not dependent on the size or shape of the vessel itself. As a result, larger evaporation capacities may be obtained. The most common application for this type of unit is as a reboiler at the base of a distillation column.

Rising Film Tubular. The first of the "modern" types of evaporators is the rising film unit, although its use by industry goes back to the early 1900s. The rising film principle was developed commercially by using a vertical tube with steam condensing on its outside surface (Fig. 3). Liquid inside the tube is brought to a boil, the vapor generated forming a core in the center of the tube. As the fluid moves up the tube, more vapor is formed, resulting in a higher central core velocity that forces the remaining liquid to the tube wall. Higher vapor velocities, in turn, result in thinner and more rapidly moving liquid film. This provides higher HTCs and shorter product residence time.

The development of this principle was a giant step forward in the evaporation field, particularly from the viewpoint of product quality. Its further advantage of high HTCs resulted in reduced heat-transfer area requirements and consequently, in a lower initial capital investment.

Falling Film Tubular. Following development of the rising film principle, it took almost a further half century for a falling film evaporation technique to be perfected (Fig. 4). The main problem was to design an adequate system for the even distribution of liquid to each of the tubes. Distribution in its forerunner, the rising film evaporator, was easy as the bottom bonnet of the calandria always was pumped full of liquid, thus allowing equal flow to each tube.

While all manufacturers have their own techniques, falling film distribution generally is based on the use of a perforated plate positioned above the top tube plate of the calandria. Spreading of liquid to each tube sometimes is further enhanced by generating flash vapor at this point. The falling film evaporator does have the advantage that the film is "going with gravity" instead of against it. This results in a thinner, faster moving film and gives rise to even shorter product contact time and a further improvement in the value of HTC.

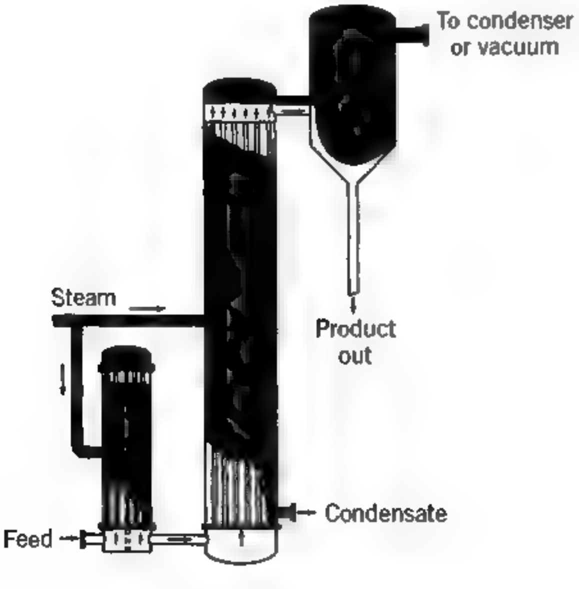


Figure 3. Rising film tubular.

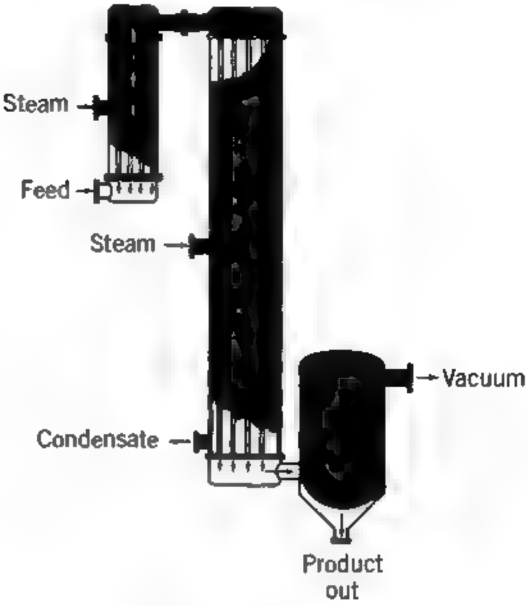


Figure 4. Falling film tubular.

The rising film unit normally needs a driving force or temperature difference across the heating surface of at least 25°F to establish a well-developed film, whereas the falling film evaporator does not have a driving force limitation. This permits a greater number of evaporator effects to be used within the same overall operating limits, in other words, if steam is available at 2.5 psig corresponding to 220°F and the last effect boiling temperature is 120°F, the total available ΔT is equal to 100°F. This would limit a rising film evaporator to four effects, each with a ΔT of 25°F. It would be feasible, meanwhile, to have as many as ten or more effects using the falling film technique.

Rising-Falling Film Tubular. As illustrated by Figure 5, the rising-falling film evaporator has the advantages of the ease of liquid distribution of the rising film unit coupled with lower head room requirements. The tube bundle is approximately half the height of either a rising or falling film evaporator and the vapor-liquid separator is positioned at the bottom of the calandria.

Forced Circulation. The forced circulation evaporator (Fig. 6) was developed for processing liquors that are susceptible to scaling or crystallizing. Liquid is circulated at a high rate through the heat exchanger, boiling is prevented within the unit by virtue of a hydrostatic head maintained above the top tube plate. As the liquid enters the separator, where the absolute pressure is slightly less than in the tube bundle, the liquid flashes to form a vapor.

The main applications for a forced circulation evaporator are in the concentration of inversely soluble materials,

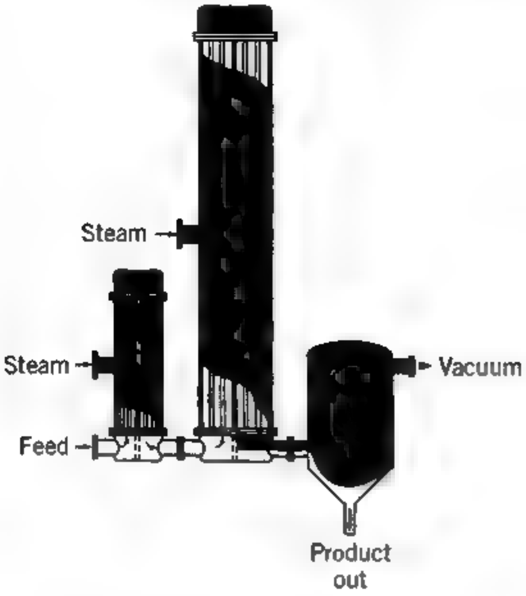

Steam
Steam
Feed
Vacuum
Product out

Figure 5. Rising-falling film tubular.

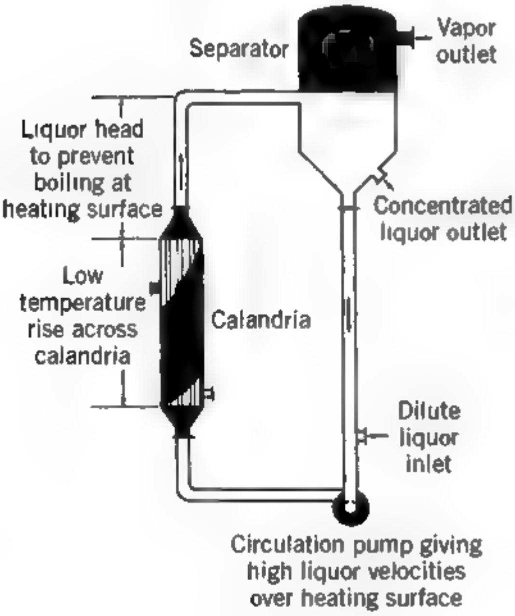

Separator
Vapor outlet
Liquor head to prevent boiling at heating surface
Concentrated liquor outlet
Low temperature rise across calandria
Calandria
Dilute liquor inlet
Circulation pump giving high liquor velocities over heating surface

Figure 6. Forced circulation.

which results in the deposition of solids. In all cases, the temperature rise across the tube bundle is kept as low as possible, generally in the region of 3–5°F. This results in a recirculation ratio as high as 200–330 lb of liquor per pound of water evaporated. These high recirculation rates result in high liquor velocities through the tubes, which helps to minimize the buildup of deposits or crystals along the heating surface. Forced circulation evaporators normally are more expensive than film evaporators because of the need for large-bore circulating pipework and large recirculating pumps generally of the axial flow type. Operating costs of such as unit also are considerably higher.

Wiped Film. The wiped or agitated thin film evaporator depicted in Figure 7 has limited applications and is confined mainly to the concentration of very viscous materials and the stripping of solvents down to very low levels. Feed is introduced at the top of the evaporator and is spread by wiper blades on to the vertical cylindrical surface inside the unit. Evaporation of the solvent takes place as the thin film moves down the evaporator wall. The heating medium normally is high-pressure steam or oil. A high-temperature heating medium generally is necessary in order to obtain a reasonable evaporation rate since the heat-transfer surface available is relatively small as a direct result of its cylindrical configuration.

The wiped film evaporator is very satisfactory for its limited applications. However, in addition to its small surface area, it also has the disadvantage of requiring moving parts such as the wiper blades, which, together with the bearings of the rotating shaft, need periodic maintenance. Capital costs in terms of dollars per pound of solvent evaporated also are very high.

Plate Evaporators

The plate equivalent of the tubular evaporator is available in four configurations: rising-falling film, falling film, Parvap, and Parflash plate evaporators. All have been developed by APV to handle the concentration of products that have varying characteristics.

Plate-type evaporators now have been sold commercially for nearly 30 years, and during that time over 1600 units manufactured by APV have been installed for concentrating hundreds of different products.

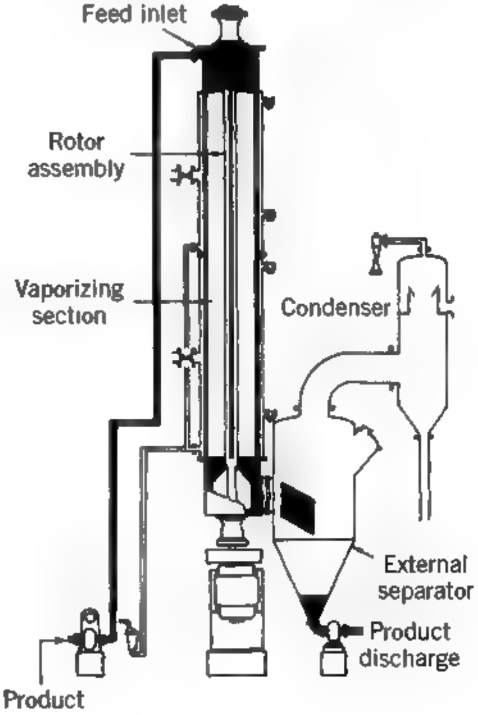

Feed inlet
Rotor assembly
Vaporizing section
Condenser
External separator
Product discharge
Product

Figure 7. Wiped film.

Rising/Falling Film Plate. The original plate type evaporator, the rising-falling plate system basically is a flexible, multiduty unit engineered for medium production runs of heat-sensitive products under sanitary conditions and at the lowest possible capital investment.

Operating on a single-pass rising and falling film principle, as shown in the exploded view of Figure 8, the evaporator consists of a series of gasketed plate processing units within a compact frame. As a thin layer of feed liquor passes over the rising and falling film section in each evaporation unit, it is vaporized on contact with adjacent steam heated plates and is discharged with its vapor to a vapor-liquid separator. All evaporation is accomplished in a matter of seconds within the plate pack. The product then is extracted and the vapor passed to a condenser, the next evaporator effect or a mechanical compressor. Product quality is given maximum protection against thermal degradation by means of high heat-transfer rates, low liquid holding volume, and minimum exposure to high temperatures.

The plate evaporator has a number of advantages over its tubular counterpart. Since it is designed to be erected on a single level with minimum headroom requirements, in many cases it will fit within an existing building with overhead restrictions as low as 12 or 13 ft. It also can be arranged as single or multiple effects without extensive building modifications or structural steel supports and can handle expanded duties by the addition of more plate units. Systems are available for evaporation rates to 35,000 lb/h with efficient in-place cleaning of all stainless, steel product contact surfaces.

Falling Film Plate. The success of the rising-falling film plate evaporator prompted the development of the falling film plate system. This design has several advantages over its predecessor, including even lower residence times and higher evaporation capabilities. While the rising-falling film plate unit is restricted to a maximum of 30,000–35,000 lb/h of water removal, the falling film plate evaporator with its larger vapor ports can accommodate 59,000–60,000 lb/h of evaporation. One of this unique design features as illustrated in Figure 9 is that each side of the plate may be used independently of the other, thus permitting two pass operation within the same frame for even shorter residence time and improved product quality.

Paravap. Especially designed to concentrate foaming liquids or those with high solids content or non-Newtonian viscosity characteristics, the APV Paravap replaces the wiped film evaporator in many cases. It successfully concentrates corn syrups and soap to better than 97–98% total solids or strips hexane and other solvents from vegetable oils and similar products. Figure 10 shows this type of unit in a simple schematic.

Note that the key element in the system is a plate heat exchanger (Fig. 11). Although specifically designed for liquid-liquid applications, it has been found that if a fluid is allowed to vaporize within the plate pack, the small plate gap and corrugated plate pattern create high vapor velocities (Fig. 12). This causes atomization of the liquid within the high-velocity vapor stream, resulting in a greater liquid surface area for mass transfer and enabling low residual solvent concentrations to be realized. Although the final product may be extremely viscous after separation from the vapor, the apparent viscosity within the plate pack is very low since only droplets are being transported in the vapor.

Paraflash. Similar to its tubular counterpart, the APV Paraflash (Fig. 13) is a suppressed boiling forced-circulation evaporator used mainly for concentrating products subject to fouling that is too excessive for a film evaporator. While this system uses a plate heat exchanger, vaporization is not allowed to take place within the exchanger itself. Boiling is suppressed either by a liquid static head above the heat exchanger or by the use of an

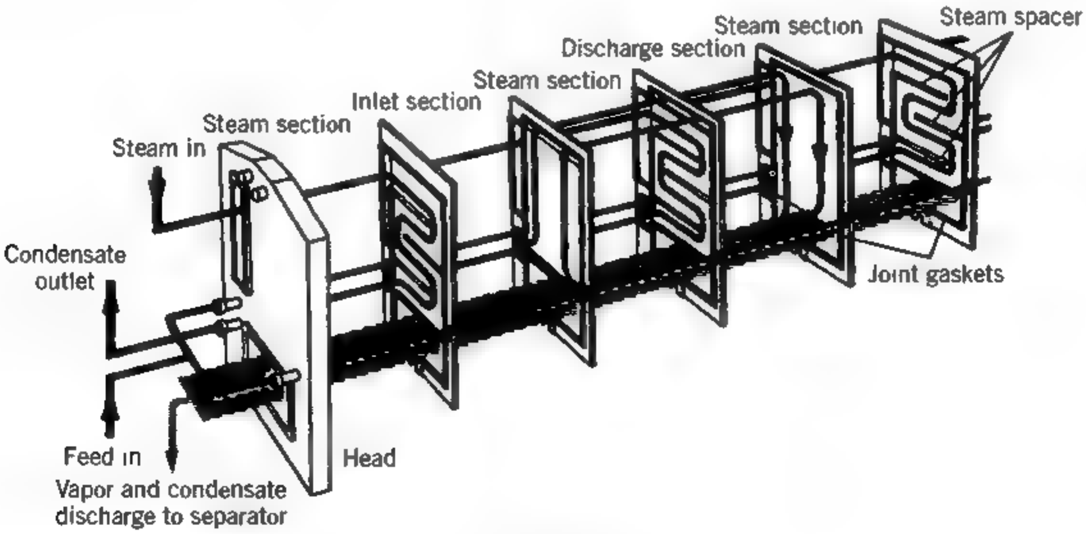


Figure 8. Rising-falling film plate evaporator.

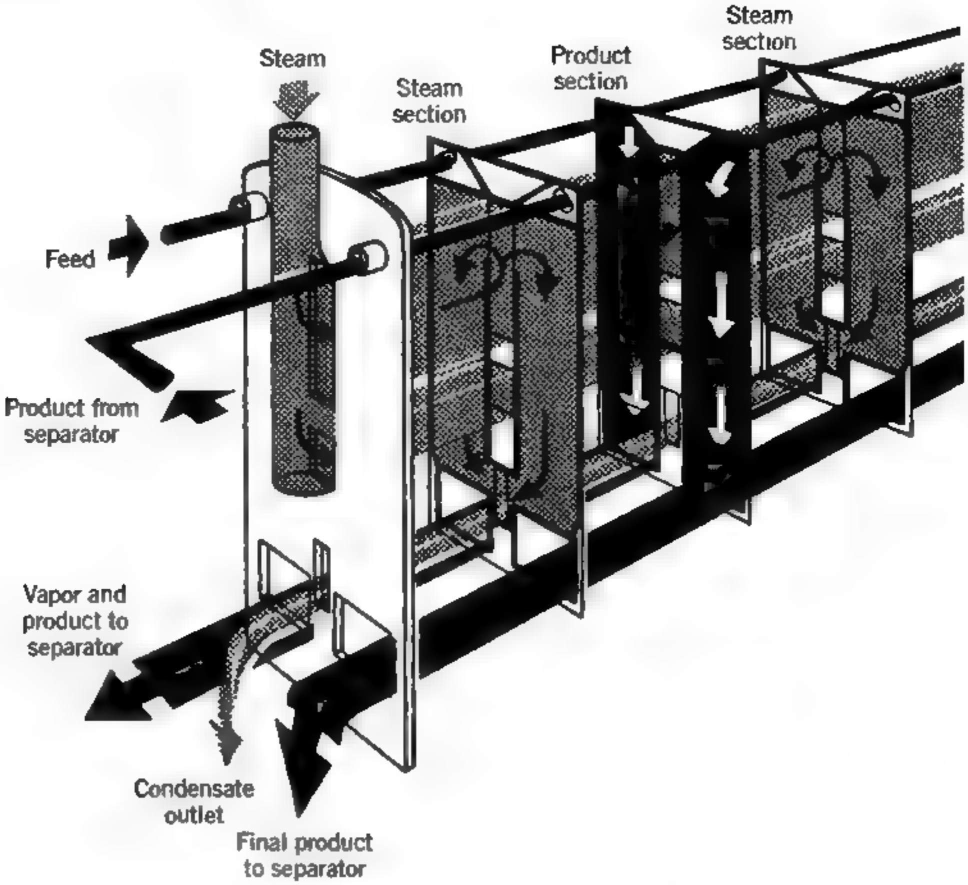

Steam
Steam section
Product section
Steam section
Feed
Product from separator
Vapor and product to separator
Condensate outlet
Final product to separator

Figure 9. Falling film plate evaporator.

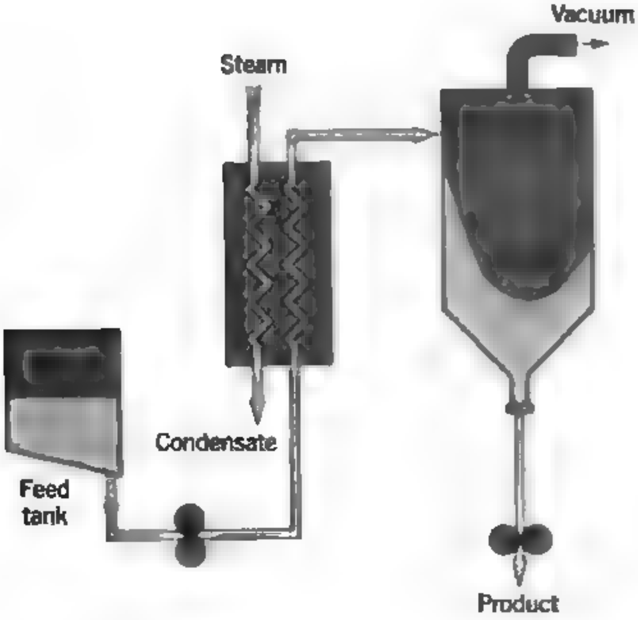

Vacuum
Steam
Condensate
Feed tank
Product

Figure 10. Paravap evaporator.

orifice piece in the discharge line. From a heat-transfer standpoint, the forced-circulation Paraflash is far more efficient than a tubular unit but suffers from a smaller equivalent diameter when large crystal sizes are present.

SELECTION OF EVAPORATORS

In choosing the evaporator best suited to the duty on hand, the following factors should be weighted.

Heat-Transfer Coefficients

The evaporator with the highest heat-transfer coefficients (HTCs) generally is the falling film type. Higher HTCs lead to smaller surface areas, resulting in minimum capital cost. Wherever possible, therefore, a film evaporator should be considered first.

While the biggest single disadvantage of the film evaporator is its susceptibility to scale buildup on the heat-transfer surface, its lower cost offsets this inconvenience on mildly scaling duties, particularly when high evaporative capacities are involved. A typical example of this is spin bath liquors. This solution, which results from the production of rayon and cellulose, consists mainly of sulfuric acid, sodium sulfate and water plus various impurities such as zinc sulfate. Scale builds up over a period of about 1 week and the evaporator then is boiled out.

In food related industries where the nature of most products is heat sensitive, it generally is desirable or nec-

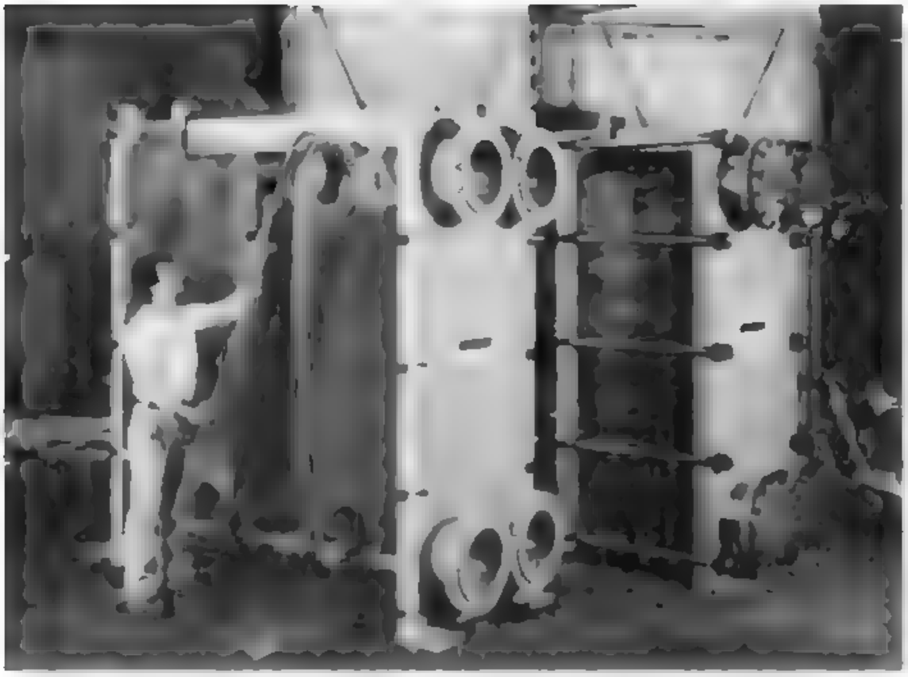


Figure 11. Plate heat exchanger.

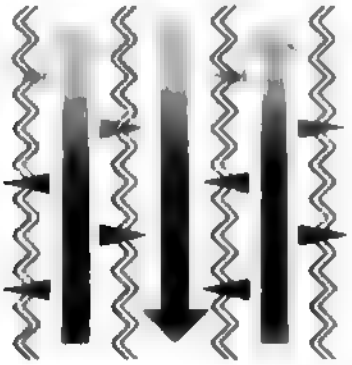


Figure 12. Corrugated plate pattern.

essary to clean out an evaporator at least once per day for sanitary reasons. In the chemical field, however, a minimum run of a week normally is required, and, in many cases, an evaporator will run for months.

Capacities

For evaporation capacities of up to about 60,000 lb/h of water removal, the possibility of using either a plate or tubular system should be considered. The capital investment is relatively close, but the lower cost of installing a plate evaporator together with its compactness, expendability, and reduced building requirements generally makes it extremely attractive. Above 60,000 lb/h evaporation, the

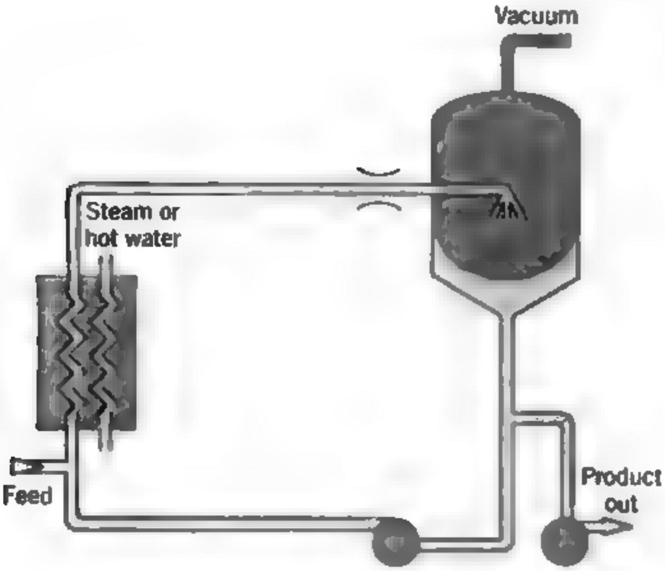


Figure 13. Suppressed boiling Paraflash evaporator.

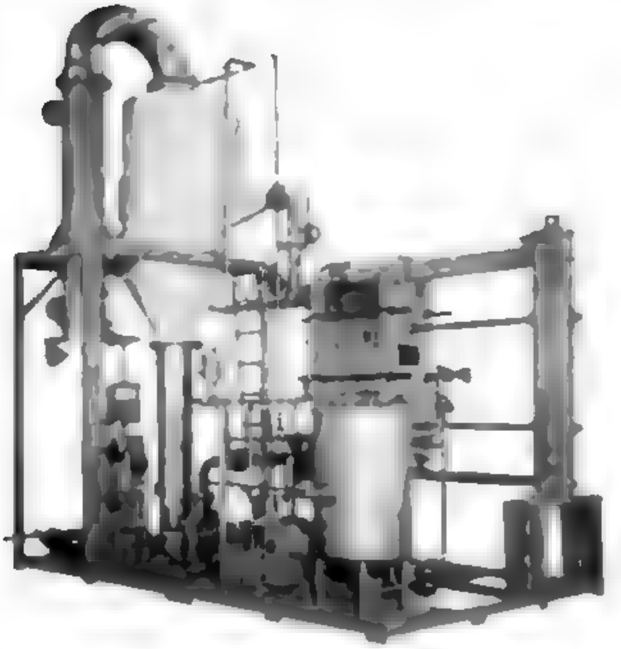


Figure 14. Single-effect R56 Paraflash concentrates brewers yeast from 13.

choice lies among various types of tubular evaporators. Available configurations are multieffect or mechanical vapor recompression.

Materials of Construction

In many cases, the choice of evaporator is determined by the necessary materials of construction (Fig. 15). An example would be a sulfuric acid evaporator, where, for product concentrations of up to 50%, the heating surface normally would be graphite. This immediately indicates use of a tubular evaporator as opposed to plate unit. With satisfactory temperature and concentration conditions, Incoloy and Hastelloy could be employed using either a tube or plate but would be much more expensive than graphite. Separators and piping would be either a fiber-filled phenolic resin or rubber-lined carbon steel. Sulfuric acid between 50 and 72% concentration again would normally use graphite as the heating surface providing temperatures are kept below 230–240°F, but piping and vessels would be lead-lined steel, tantiron, or cast lead. For construction reasons, a graphite tube bundle has to arranged as a rising film or forced-circulation evaporator with the vapor-liquid separator positioned at the top.

Multiple Evaporators

More than one evaporator may be used to concentrate a particular product. For example, there may be a main or bulk evaporator followed by a finishing unit. The main evaporator would be used to remove the majority of the water or solvent to a point where the liquid concentration gives rise to restrictions. Among these are boiling-point elevation, which reduces the overall temperature difference of a multieffect or MVR evaporator, product viscosity resulting in low HTCs, or saturated liquor resulting in crystallization. A forced-circulation crystallizing evaporator very often is the last product stage of a multieffect evaporator.

Consider the example of concentrating a solution from 4 to 60% total solids, a 15:1 concentration ratio. At the 40% total solids level, the viscosity is too high for a film evaporator and the product has to be processed in a Paravap or wiped film unit. However, in concentrating from 4 to 40% or a 10:1 ratio, 90% of the feed has been evaporated in the initial evaporator. The finishing unit now has to concentrate only from 40 to 60% or 1.5:1 ratio. This means that 96.43% of the total evaporation has been achieved in a multieffect or mechanical vapor recompression bulk evaporator at high thermal economy and probably while obtaining good HTCs. Only 3.57% of the duty is confined to a small single-effect evaporator, which handles the most difficult part of the concentration.

Crystallization

When the requirement is for a crystallizing evaporator, there never is any doubt that the stage where crystalliza-

Product		Materials of Construction
Most dairy products		304/316 stainless steel
Most food products		304/316
Most fruit juices		304/316
Most pharmaceuticals		304/316
if NaCl in any of above		Monel or titanium
Sulfuric acid	<50%	FFPR/graphite
		RLCS/graphite
	<72%	Lead lined/tantiron or cast lead
Hydrochloric acid		FFPR/graphite/RLCS
Phosphoric acid	(dilute/pure)	316
	(with impurites)	FFPR/graphite/RLCS
Caustic soda	<40%	Stress relieved C.S.
Caustic soda	(with NaCl)	Monel/nickel/NiLCS
Ammonium nitrate		304/304L
Ammonium sulfate		316

FFPR—fiber-filled phenolic resin; RLCS—rubber-lined carbon steel; NiLCS—nickel-lined carbon steel.

Figure 15. Guide to materials of construction.

tion takes place has to be a forced-circulation evaporator and, in most cases, a tubular unit. However, when the problem is not one of crystallization but a general scaling of the heat-transfer surface instead, the Paraflash is much more efficient. As shown in Fig. 16, the circulation rate through the plate is considerably less than through a tube and results in much lower horsepower requirements.

If crystals are formed and are too large for a plate evaporator to handle, the designer has to determine whether the tubular forced circulation unit should be a single- or multiple-pass configuration. Normally, forced-circulation tubulars are designed with tube velocities of 6–8 ft/s or higher to minimize fouling. In the Figure 17 comparison between single- and three-pass configurations, three-pass units are shown to have lower circulation rates directly resulting in higher temperature rises, lower log mean temperature differences, and, therefore, greater surface area. Even though the circulation rate is less, the pressure drop on the three-pass versus one-pass is more because of greater overall tube length. This results in larger absorbed horsepower. It generally is concluded, therefore, that a single-pass arrangement is less expensive from both the capital and operating cost standpoints.

HTCs, Forced Circulation, and Film

Estimation of HTCs from physical properties is easier and more reliable for forced-circulation units than for film evaporators. As a general rule, HTCs used for the latter are determined by experience or test work.

EVAPORATION CONFIGURATION AND ENERGY CONSERVATION

Engineers can help cut energy consumption even when evaporation requirements seem to become greater every year. This can be done by

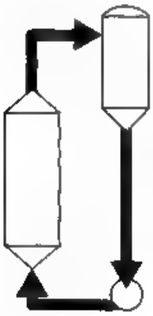
Solution properties:		
	Specific gravity	1.35
	Specific heat	0.62
	Thermal conductivity	0.26 Btu units
	Viscosity	100 cps

	Tubular Unit	**Plate Unit**
Heat duty, Btu/hr	5×10^6	5×10^6
Element	1¼ in. OD, 16 SWG, 25-ft tube	APV R10 plate
Total surface, ft^2	2620	2622
Fluid temperature in, °F	160	160
Fluid temperature out, °F	161.08	170
Service temperature, °F	190	190
Recirculation rate, gpm	10,975	1610
Total ΔP, psi	16.9	10.8
BHP absorbed	107.7	10.1

SWG = standard wire gauge; OD = outer diameter; gpm = gallons per minute.

Figure 16. Comparison between forced circulation tubular and plate evaporators.

Duty: Triple effect forced circulation evaporator
Feed: 102,000 lb/h, Cornsteep 6.14% → 51%



	1st Effect		**2nd Effect**		**3rd Effect**	
	1 Pass	3 Pass	1 Pass	3 Pass	1 Pass	3 Pass
Circulation rate (GPM)	12,275	6,190	12,275	6,190	14,500	4,800
Heat transfer area (ft)	4,434	5,541	4,434	5,541	4,434	4,656
Absorbed BHP	50	91	66	113	156	192

	One Pass	**Three Pass**
Equipment	$491,280	$540,960
Absorbed BHP	272	396

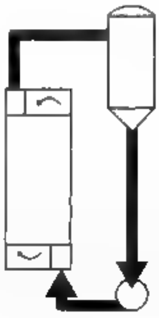


Figure 17. Comparison between single- and triple-pass calandria.

1. Installing a greater number of steam effects to an evaporator.
2. Making use whenever possible of steam thermocompression or mechanical vapor recompression.
3. Making sure that the feed to an evaporator is preheated to the boiling point in the most efficient manner.
4. Using low-grade heat from the evaporator whenever possible.
5. Insulating equipment to keep losses to a minimum.

Some of these points are illustrated by the Figure 18 schematic of a three-effect evaporator with steam thermocompression that uses evaporator condensate and intereffect vapor for feed preheating. Note that even this vapor has been used efficiently in prior effects and that condenser water also may be used if the feed temperature is low. Figure 19, meanwhile, outlines a four-effect evaporator with thermocompression and the use of a slightly different technique with a spray heating loop.

While a greater number of effects increases initial capital investment, savings in operating costs will justify the expenditure if evaporation capacities are large enough and the operating period long enough. A thermocompressor generally adds the equivalent of one or more effects at relatively little capital cost, although the surface area in the effects between thermocompressor suction and discharge must be increased. The HTC of these effects generally is very good so the increase in surface area is minimized. Care should be taken, however, to design with a low ΔT across the thermocompressor to ensure high entrainment ratios of vapor to steam.

It is also important when using a steam thermocompressor not to have a high-boiling-point elevation in those effects. This will cut down the (ΔT) available for heat transfer. Thermocompressors are somewhat inflexible and do not operate well outside their design conditions. They should not be used when the product may foul the evaporator surface and cause the design pressures and pressure boost across the thermocompressor to rise. Under these conditions, the amount of vapor entrained is reduced and a fall-off in evaporator capacity results.

Effective preheating of the feed also can reduce energy consumption considerably. The capital cost of preheaters is fairly small with the plate heat exchangers being particularly effective owing to their capability for realizing a very close temperature approach to the heating medium, whether it is condenser water, waste evaporator condensate, or intereffect vapor.

Although all of these features reduce energy consumption, still better methods are available. In less than a decade and probably within the next 5 yr, the majority of new large-capacity evaporators installed, particularly for bulk duties, will be of the mechanical vapor recompression (MVR) type. The mechanical vapor compressor can be driven by electricity from coal, hydropower, or nuclear

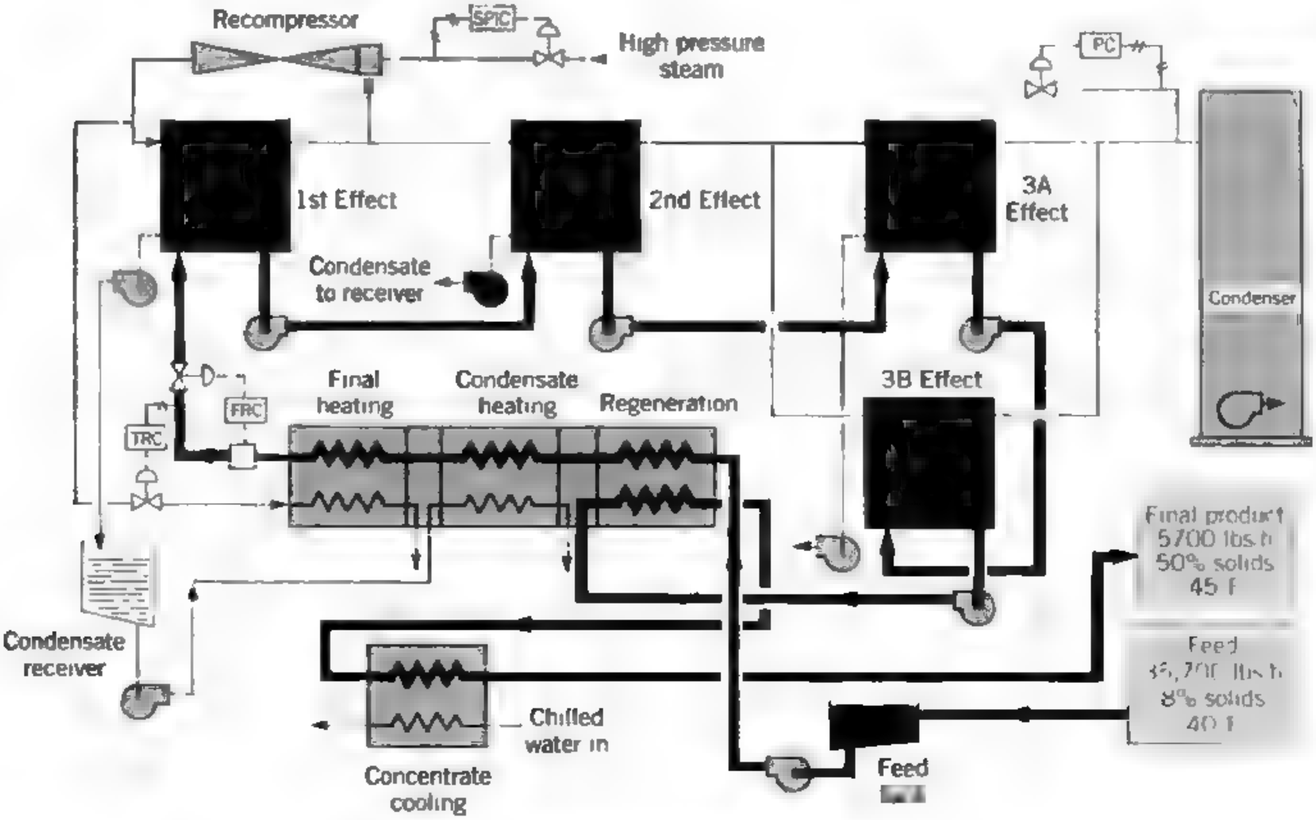


Figure 18. Three-effect steam jet recompression.

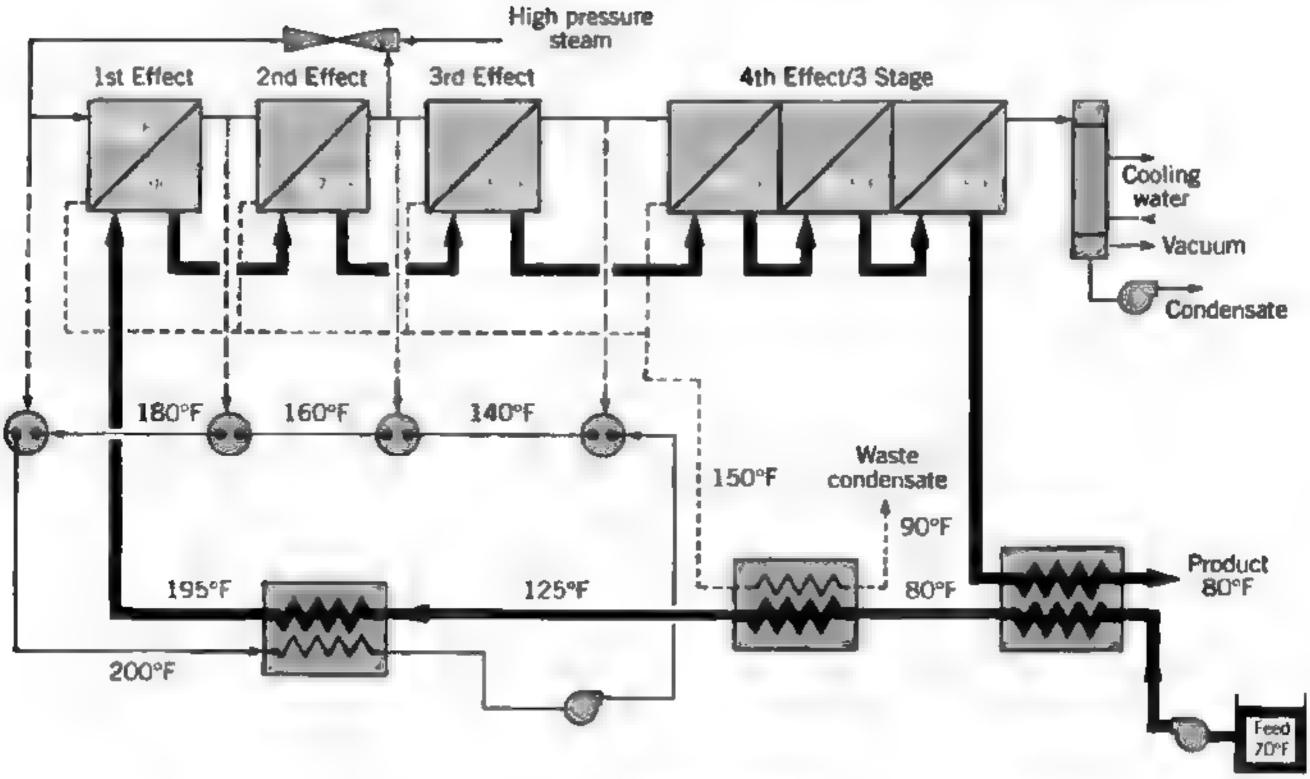
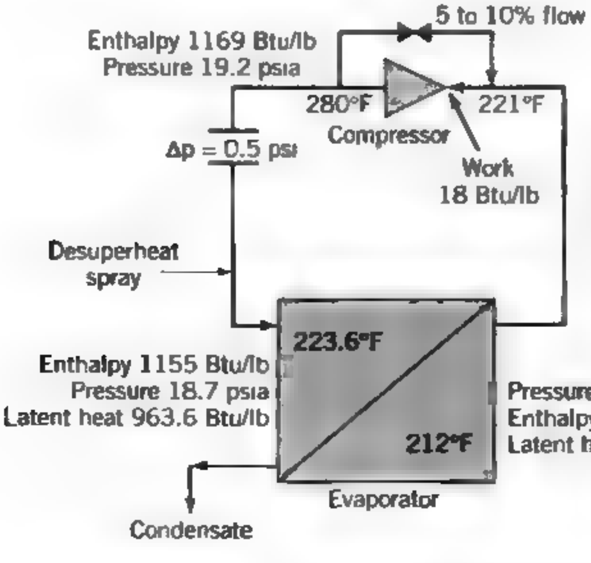

High pressure steam
1st Effect
2nd Effect
3rd Effect
4th Effect/3 Stage
Cooling water
Vacuum
Condensate
180°F
160°F
140°F
150°F
Waste condensate
90°F
195°F
125°F
80°F
Product 80°F
200°F
Feed 70°F

Figure 19. Four-effect thermocompression.

power even when all the gas and oil has been exhausted or is too expensive to use.

Briefly examining the thermodynamics involved with MVR, the Figure 20 schematic shows an evaporator with a liquid boiling point of 212°F (atmospheric pressure). All of the water vapor that is boiled off passes to a compressor. In order to keep the energy input to the system as low as possible, the pressure boost across the compressor is limited. In the majority of cases, this pressure boost will correspond to a saturated temperature rise in the region of 15°F or less.

In this example, there is a pressure boost of 4.5 psi across the compressor. Assuming that there is a pressure low of 0.5 psi in the system, the effective pressure on the


5 to 10% flow
Enthalpy 1169 Btu/lb
Pressure 19.2 psia
280°F
221°F
Compressor
Δp = 0.5 psi
Work
18 Btu/lb
Desuperheat spray
223.6°F
Enthalpy 1155 Btu/lb
Pressure 18.7 psia
Latent heat 963.6 Btu/lb
212°F
Pressure 14.7 psia
Enthalpy 1151 Btu/lb
Latent heat 970.6 Btu/lb
Evaporator
Condensate

Effective steam economy	970 + 18 = 54
Efficiency of drive & compressor	65 → 72%
Inefficiency due to by-pass	5 → 10%
∴Actual effective steam economy	32 → 35 Effects

Figure 20. Mechanical recompression evaporator.

steam side of the evaporator is 18.7 psia. This compressed water vapor condenses and gives up its latent heat, thus vaporizing more water from the liquid that is being concentrated. The latent heat, thus vaporizing more water from the liquid that is being concentrated. The latent heat of vaporization of water at atmospheric pressure is 970 Btu/h. Note that it requires a theoretical energy input of only 18 Btu/lb to raise the water vapor from 14.7 to 19.2 psia. The theoretical steam economy, therefore, is 970/18 = 54. When compressor efficiency is taken into account, this figure is brought down to between 32 and 35, which is another way of saying that the MVR system is equivalent to a 32–35-effect steam evaporator. Related to energy costs of 4 cents per kilowatt-hour for electricity for the compressor drive and $6.00 per 1000 lb of steam for a conventional steam evaporator, the MVR system then becomes the economic equivalent of just under a 19-effect evaporator.

The MVR has another definite advantage over steam. The condensate is available at high temperature and is ideal for evaporator feed preheating, particularly if the condensate rate is as high as 90% of the feed rate, ie, a 10:1 concentration ratio within the evaporator. There are many such evaporators in operation where the sole energy input to the system is through the compressor with steam requirements limited to approximately 15 min during startup.

Although Figure 20 illustrates an evaporator system that uses a compressor, it will be shown that the use of a turbo fan in place of the centrifugal compressor not only yields lower boost but also provides more surface area and better operating economics.

RESIDENCE TIME IN FILM EVAPORATION

Since many pharmaceutical, food and dairy products are extremely heat-sensitive, optimum quality is obtained when processing times and temperatures are kept as low as possible during concentration of the products. The most critical portion of the process occurs during the brief time that the product is in contact with a heat-transfer surface that is hotter than the product itself. To protect against possible thermal degradation, the time—temperature relationship therefore must be considered in selecting the type and operating principle of the evaporator to be used.

For this heat-sensitive type of application, film evaporators have been found to be ideal for two reasons: (1) the product forms a thin film only on the heat-transfer surface rather than occupying the entire volume, and thus residence time within the heat exchanger is greatly reduced, and (2) a film evaporator can operate with as low as a 6°F steam-to-product temperature difference. With both the product and heating surfaces close to the same temperature, localized hot spots are minimized.

As described previously, there are rising film and falling film evaporators as well as combination rising—falling film designs. Both tubular and plate configurations are available.

Rising Film Evaporators

In a rising film design, liquid feed enters the bottom of the heat exchanger, and when evaporation begins, vapor bubbles are formed. As the product continues up either the tubular or plate channels and the evaporation process continues, vapor occupies an increasing amount of the channel. Eventually, the entire center of the channel is filled with vapor while the liquid forms a film on the heat-transfer surface.

The effect of gravity on rising film evaporator is twofold. It acts to keep the liquid from rising in the channel. Further, the weight of the liquid and vapor in the channel pressurizes the fluid at the bottom and with the increased pressure comes an increase in the boiling point. A rising film evaporator therefore requires a larger minimum ΔT than does a falling film unit.

The majority of the liquid residence time occurs in the lower portion of the channel before there is sufficient vapor to form a film. If the liquid is not preheated above the boiling point, there will be no vapor and since a liquid pool will fill the entire area, the residence time will increase.

Falling Film Evaporators

As liquid enters the top of a falling film evaporator, a liquid film formed by gravity flows down the heat-transfer surface. During evaporation, vapor fills the center of the channel and as the momentum of the vapor accelerates the downward movement, the film becomes thinner. Since the vapor is working with gravity, a falling film evaporator produces thinner films and shorter residence times than does a rising film evaporator for any given set of conditions.

Tubular and Plate Film Evaporators

When compared to tubular designs, plate evaporators offer improved residence time since they carry less volume within the heat exchanger. In addition, the height of a plate evaporator is less than that of a tubular system.

ENGINEERING CONVERSION

Table 1 provides data for engineering conversion applicable to the use of an evaporation in food processing.

Estimating Residence Time

It is difficult to estimate the residence time in film evaporators, especially rising film units. Correlations, however, are available to estimate the volume of the channel occupied by liquid. Table 2 describes a number of applicable equations. Equation 1 is recommended for vacuum systems.

For falling film evaporators, the film thickness without vapor shearing can be calculated by equation 2.

Since the film is thin, this can be converted to liquid volume fraction in a tubular evaporator by equation 3.

For a falling film plate evaporator, equation 4 is used. As liquid travels down the plate and evaporation starts, vapors will accelerate the liquid. To account for this action, the rising film correlation is used when the film thickness

Table 1. Engineering Conversions

To convert from	to	Multiply by
	Heat capacity	
Calories/(gram)(°C)	Calories/(Gram)(mole)(°C)	Molecular weight
	Btu/(pound)(°F)	1.0
	Density	
Gram/milliliter	Pounds/gallon	8.33
	Pounds/ft^3	62.42
	Thermal conductivity	
Kilocalorie/(hr)(m)(°C)	Btu(h)(ft)(°F)	0.6719
W/(m)(°C)		0.5778
	Viscosity	
Centistokes	Centipoise	Specific gravity
Dynamic viscosity pound-mass (ft)(s)	Centipoise	1488.2
Kinematic viscosity cm^2/s	Centistokes	100
	Pressure	
Kilopascal	psi	0.14504
Bar		14.504
Inches Hg absolute	psia	0.4912
Atmosphere		14.696
Torr		0.01908
mmHg		0.01908
	Enthalpy	
Calorie/gram	Btu/pound-mass	1.8
	Work / Energy	
(Kilowatt)(hr)	Btu	3412.1
(Horsepower)(hr)		2544.4
Calorie		0.003968
	Heat-transfer coefficient	
Kilocalorie/(h)(m^2)(°C)	Btu(h)(ft^2)(°F)	0.2048
W/(cm^2)(°C)		1761.1

Table 2. Estimating Residence Time

1.[a]	$R_L = 1 - \dfrac{1}{1 + \left(\dfrac{(1-y)}{y}\right)\left(\dfrac{2\rho_v}{\rho_L}\right)^{1/3}}$
2.	$m = \left[\dfrac{3\Gamma\mu}{g\rho_L(\rho_L - \rho_v)}\right]^{1/3}$
3.	$R_L = 4m/d$
4.	$R_L = 2m/z$
5.	$t = AL/R_L q_L$

[a] Symbols. A = cross-sectional area, ft^2; d = tube diameter, ft; g = 32.17 ft/s^2, L = tube length, ft; m = film thickness, ft; R_L = liquid volume fraction (dimensionless); q_L = liquid rate, ft^3/s; t = time, s; z = plate gap, ft; Γ = liquid wetting rate, lb/s · ft; ρ_L = liquid density, lb/ft^3, ρ_v = vapor density; lb/ft^3; μ = liquid viscosity, lb/ft s; y = local weight fraction of vapor (dimensionless).

Source: Refs. 1 and 2.

falls below that of a falling film evaporator. In practice, the film thickness may be less than estimated by either method because gravity and vapor momentum will act on the fluid at the same time.

Once the volume fraction is known, the liquid residence time is calculated by equation 5. In order to account for changing liquid and vapor rates, the volume fraction is calculated at several intervals along the channel length. Evaporation is assumed to be constant along with channel length except for flash due to high feed temperature.

Table 3 shows a comparison of contact times for typical four-effect evaporators handling 80 GPM of feed. The tubular designs are based on 2-in.-diameter by 30 ft. Designs using different tube lengths, incidentally, do not change the values for a rising film tubular system.

The given values represent total contact time on the evaporator surface, which is the most crucial part of the processing time. Total residence time would include contact in the preheater and separator as well as additional residence within interconnecting piping.

Table 3. Residence Time Comparison

Contact time	Rising film tubular	Rising film plate[a]	Falling film tubular	Falling film plate
1st effect	88	47[b]	23	16[b]
2nd effect	62	20	22	13
3rd effect	118	30[b]	15[b]	9
4th effect	236[b]	78[b]	123[b]	62[c]
Total contact time	*504*	*175*	*183*	*100*

[a] Plate gap of 0.3 in assumed.

[b] Two stages.

[c] Three stages.

While there are no experimental data available to verify these numbers, experience with falling film plate and tubular evaporators shows that the values are reasonable. It has been noted that equation 2 predicts film thicknesses that are too high as the product viscosity rises so, in actuality, four-effect falling film residence times probably are somewhat shorter than charted.

Summary

Film evaporators offer the dual advantages of low residence time and low temperature difference that help assure a high product quality when concentrating heat-sensitive products. In comparing the different types of film evaporators that are available, falling film designs provide the lowest possible ΔT and the falling film plate evaporator provides the shortest residence time.

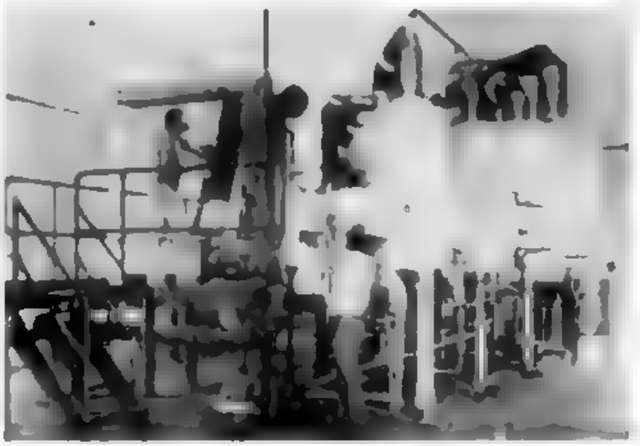


Figure 21. Rising-falling film plate evaporator in final stages of fabrication.

PLATE-TYPE EVAPORATORS

To effectively concentrate an increasing variety of products that differ by industry in such characteristics as physical properties, stability, or precipitation of solid matter, equipment manufacturers have engineered a full range of evaporation systems. Included among these are a number of plate-type evaporators.

Plate evaporators initially were developed and introduced by APV in 1957 to provide an alternative to the tubular systems that had been in use for half a century. The differences and advantages were many. The plate evaporator, for example, offers full accessibility to the heat-transfer surfaces. It also provides flexible capacity merely by adding more plate units, shorter product residence time (resulting in a superior quality concentrate), a more compact design with low headroom requirements and low installation cost.

These APV plate evaporation systems are available in four arrangements—rising-falling film, falling film, Paravap, and Paraflash—and may be sized for use in new produce development or for production at pilot plant or full-scale operating levels.

Rising-Falling Film Plate

The principle of operation for the rising-falling film plate evaporator (RFFPE) involves the use of a number of plate packs or units, each consisting of two steam plates and two product plates. As shown in Figure 21, these are hung in a frame resembling that of a plate heat exchanger. The first product passage is a rising pass and the second, a falling pass. The steam plates, meanwhile, are arranged alternately between each product passage.

The product to be evaporated is fed through two parallel feed ports and is equally distributed to each of the rising film annuli. Normally, the feed liquor is introduced at a temperature slightly higher than the evaporation temperature in the plate annuli, and the ensuing flash distributes the feed liquor across the width of the plate. Rising film boiling occurs as heat is transferred from the adjacent steam passage with the vapors that are produced helping to generate a thin, rapidly moving turbulent liquid film.

During operation, the vapor and partially concentrated liquid mixture rises to the top of the first product pass and transfers through a "slot" above one of the adjacent steam passages. The mixture enters the falling film annulus where gravity further assists the film movement and completes the evaporation process. The rapid movement of the thin film is the key to producing low residence time within the evaporator as well as superior heat-transfer coefficients. At the base of the falling film annulus, a rectangular duct connects all of the plate units and transfers the evaporated liquor and generated vapor into a separating device. Steam and condensate ports connect to all the steam annuli (Fig. 22).

The plate evaporator is designed to operate at pressures extending from 10 psig to full vacuum with the use of any

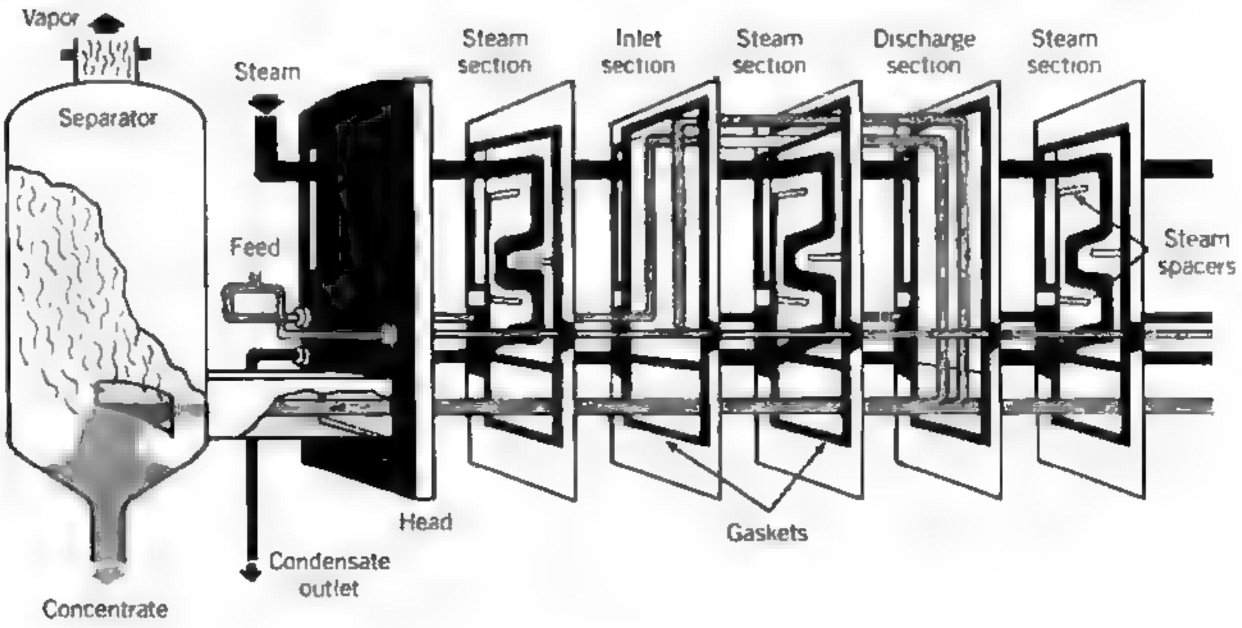


Figure 22. Rising-falling film plate evaporator arranged for one complete pass.

number of effects. However, the maximum pressure differential normally experienced between adjacent annuli during single effect operation is 15 psig. This, and the fact that the pressure differential always is from the steam side to the product side, considerably reduces design requirements for supporting the plates. The operating pressures are equivalent to a water vapor saturation temperature range of 245°F downward and thus are compatible with the use of nitrile or butyl rubber gaskets for sealing the plate pack.

Most rising-falling film plate evaporators are used for duties in the food, juice, and dairy industries where the low residence time and 100–200°F operating range temperatures are essential for the production of quality concentrate. An increasing number of plate evaporators, however, are being operated successfully in both pharmaceutical and chemical plants on such products as antibiotics and inorganic acids. These evaporators are available as multi-effect and/or multi-stage systems to allow relatively high concentration ratios to be carried out in a single pass, nonrecirculatory flow.

The rising-falling film plate evaporator should be given consideration for various applications.

- That require operating temperatures between 80 and 210°F
- That have a capacity range of 1000–35,000 lb/h water removal
- That have a need for future capacity increase since evaporator capabilities can be extended by adding plate units or by the addition of extra effects
- That require the evaporator to be installed in an area that has limited headroom
- Where product quality demands a low time-temperature relationship.
- Where suspended solid level is low and feed can be passed through 50 mesh screen

A "junior" version of the evaporator is available for pilot plant and test work and for low-capacity production. If necessary, this can be in multieffect-multistage arrangements such as the system illustrated in Figure 23.

Falling Film Plate

Incorporating all the advantages of the original rising—falling film plate evaporator system with the added bene-

Figure 23. Three-effect, four-stage, "junior."

fits of shorter residence time and larger evaporation capabilities, the falling film plate evaporator has gained wide acceptance for the concentration of heat sensitive products. With its larger vapor ports, evaporation capacities typically are up to 50,000–60,000 lb/h.

The falling film plate evaporator consists of gasketed plate units (each with a product and a steam plate) compressed within a frame that is ducted to a separator. The number of plate units used is determined by the duty to be handled.

As shown in Figure 24, one important innovation in this type of evaporator is the patented feed distribution system. Feed liquor first is introduced through an orifice (1) into a chamber (2) above the product plate where mild flashing occurs. This vapor/liquid mixture then passes through a single product transfer hole (3) into a flash chamber (4), which extends across the top of the adjacent steam plate. More flash vapor results as pressure is further reduced and the mixture passes in both directions into the falling film plate annulus through a row of small distribution holes (5). These ensure an even film flow down the product plate surface where evaporation occurs. A unique feature is the ability to operate the system either in parallel or in series, giving a two-stage capability to each frame. This is particularly advantageous if product recirculation is not desirable.

In the two-stage method of operation, feed enters the left side of the evaporator and passes down the left half of the product plate, where it is heated by steam coming from the steam sections. After the partially concentrated product is discharged in the separator, it is pumped to the right side of the product plate where concentration is completed. The final concentrate is extracted while vapor is discharged to a subsequent evaporator effect or to a condenser.

Paravap

The operating principle of the Paravap represents an application of the thin film, turbulent path evaporation process and in many cases, replaces the wiped or agitated film evaporator. Since it has no mechanical moving parts within the heat transfer area, costly maintenance repairs common to wiped film systems are eliminated. It is especially designed to concentrate liquids with high solids contents or non-Newtonian viscosity characteristics (Fig. 25).

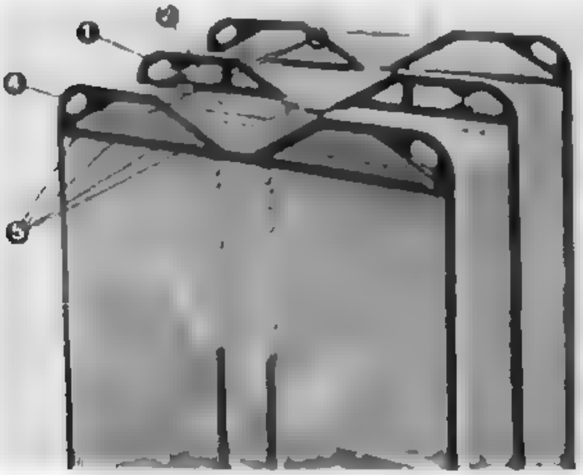


Figure 24. Typical product and steam plate unit for falling film plate evaporator.

With feed liquor and the heating medium of steam, hot water or hot oil directed into alternate passages within a plate heat exchanger, boiling begins as the liquid contacts the heated plates. The small plate gap and high-velocity flow pattern (Fig. 26) atomizes the feed and provides a greater liquid surface area for mass transfer. Since the vapor carries the feed in the form of minute particles, the apparent viscosity within the heat exchanger plate pack is

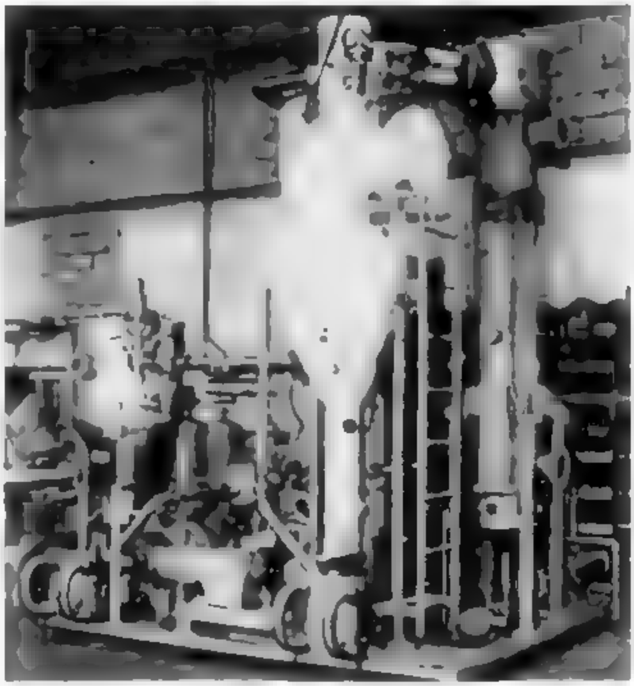


Figure 25. Single-effect R86 Paravap.

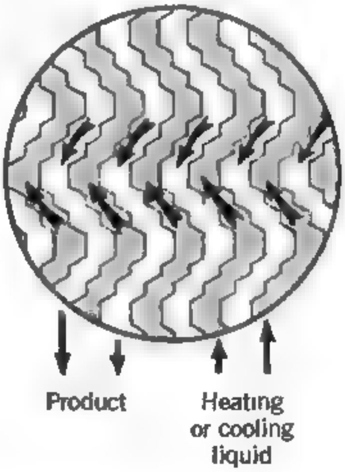


Figure 26. Small plate gap and high-velocity flow pattern.

very low. The final product, however, may be extremely viscous after separation from the vapor.

Advantages include low residence time to minimize thermal degradation, low rates of fouling, and economical operation.

Typical applications: concentrating soap to final product consistency; concentrating apple puree from 25 to 40° brix, grape puree to 50° brix, cherry puree to 60° brix, fruit juices to 95% solids, and sugar or corn syrup solutions to over 97%; solvent stripping duties such as hexane from oil (See also Figs. 25 and 27.)

Paraflash

Operating under a suppressed boiling, forced circulation principle, the APV Paraflash is used to concentrate products subject to fouling too excessive for film evaporators. Unlike the Paravap, vaporization does not occur within the heat-exchanger plate pack. Instead, liquor flashes as it enters a separator, crystallization takes place, and a suspended slurry results. Suppressed boiling combined with high liquid velocities deters scaling on the heat-transfer surface, minimizes cleaning downtime, and promotes longer production runs.

The Paraflash can be used in single or multiple effects for such products as grape juice (tartrate crystals), coffee, wheat starch, distillery effluent, and brewer's yeast (suspended solids). (See Fig. 27).

MECHANICAL VAPOR RECOMPRESSION EVAPORATORS

In recent years, MVR technology has been introduced and widely accepted as an effective approach to powering medium to large-capacity evaporators for both chemical and sanitary applications. While experience has shown that the higher capital cost of this equipment relative to that of steam-driven evaporation systems has been offset by significant energy savings, advances in MVR technology now have reduced energy requirements even further.

Definition

Simply stated, with mechanical recompression the water vapor boiled off in the evaporator is passed to an electrically powered compressor and is compressed through 1–3 psi. This raises the temperature of the vapor, which then is used as the heating medium. The difference in enthalpy between the vapors on the heating and process sides is comparatively small, with a resultant reduction in energy consumption and, depending on regional steam and power costs, an operating cost equivalent to at least an 8–30-effect evaporator. Theoretical thermal efficiency may exceed that of a 60-effect steam evaporator.

Typically, Figure 28a shows a single-effect steam evaporator operating at atmospheric pressure (14.7 psia) with vapor being produced at 212°F and sent to a condenser. The heat source for this system is steam at 17.2 psia, condensing at 220°F.

Figure 27. Single-effect, forced circulation Paraflash.

In Figure 28b, the same evaporator is shown operating with mechanical vapor compression. In this case, the vapor at 212°F, 14.7 psia, is sent to a compressor, where its pressure is raised to 17.2 psia. It then is charged to the steam side of the evaporator as the heating medium, condensing at 220°F. Savings are realized in two areas: no steam is required for evaporation, although nominal amounts are required for startup and compressor seals, and since the vapor from the evaporator is not sent to a condenser, cooling water requirements are dramatically reduced.

It will be shown later that excess vapor is produced gas a result of the inefficiencies in the compression cycle. This excess vapor is used to compensate for vent and radiation losses and at times, as an assist to preheating.

Development

To a large extent, the design of MVR evaporators has been dictated by the capabilities of the compressors available at the time. The primary limitation has been the compression ratio (absolute discharge pressure divided by absolute suction pressure) that can be achieved since this determines the temperature difference available for the evaporator. For example, if an evaporator is run at 212°F and atmospheric pressure and a compression ratio of 1.4 is used, the steam pressure at the discharge of the compressor is 1.4 × 14.7 psia or 20.58 psia. Since water vapor at 20.58 psia condenses at 229.5°F, the allowable temperature difiference ΔT for the evapo-

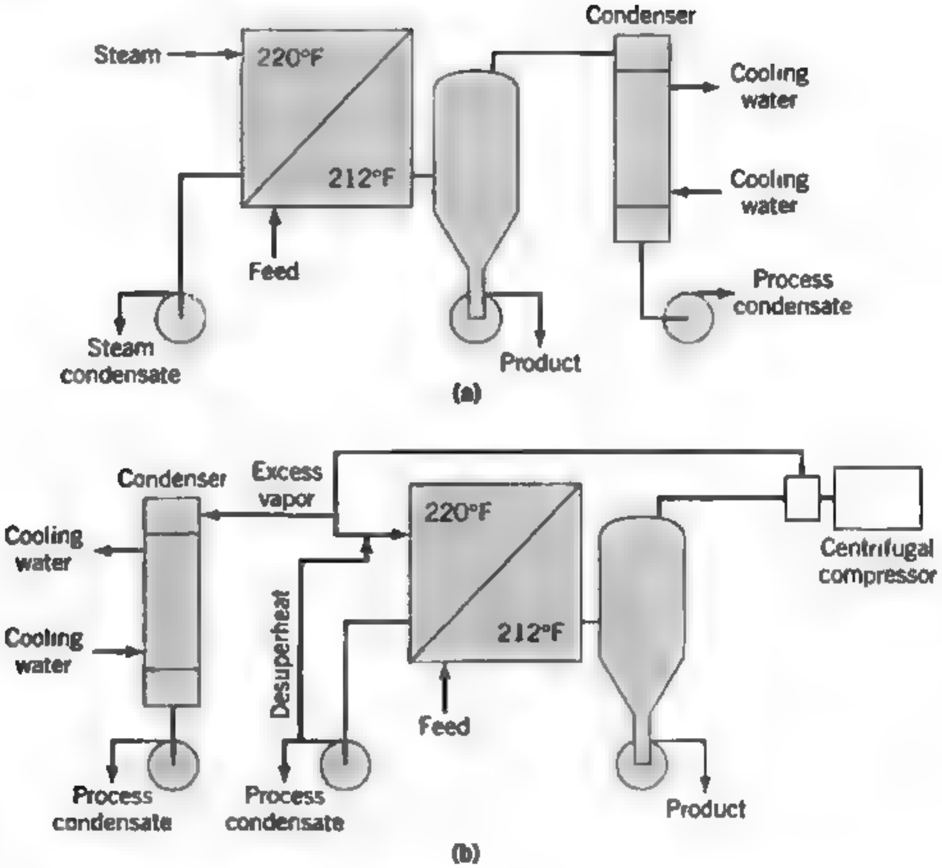

Condenser
Steam
220°F
212°F
Cooling water
Cooling water
Feed
Process condensate
Steam condensate
Product
(a)
Condenser
Excess vapor
Cooling water
Cooling water
220°F
212°F
Centrifugal compressor
Desuperheat
Feed
Process condensate
Process condensate
Product
(b)

Figure 28. (a) Single-effect evaporator. (b) Single-effect MVR evaporator.

rator is 17.5°F before losses are considered. Table 4 shows the total temperature differences for a number of conditions.

Centrifugal Compressors

The relatively high volume of vapor encountered in evaporators requires the use of centrifugal compressors in most cases. Initially, centrifugal compressors on steam were limited to a compression ratio of 1.4, which, in turn, limited the available temperature difference to between 13 and 17.5°F depending on boiling temperature. Only film evaporators could use MVR since there was not sufficient temperature difference to consider forced-circulation designs. Furthermore, products having significant boiling point elevation were excluded for this technique.

Two trends developed in recent years, however, have increased MVR capabilities and applications.

First improved design allows centrifugal compressors to run at higher speeds, increasing compression ratios approximately to 2.0 to 1. Consequently, a (ΔT of 27–36°F now can be obtained and thus, MVR can be used with forced-circulation systems and for the evaporation of products with boiling-point elevations.

Secondly, advances in evaporator design allow the operation of film evaporators with a lower (ΔT), thereby minimizing energy requirements. For some products, film evaporators can be calculated with a (ΔT (excluding losses) in the range of 6°F.

By increasing the ΔT available and decreasing the ΔT required, it also became possible to design multiple—effect MVR evaporators. Figure 29 illustrates a double-effect MVR system with a forced-circulation finisher operating across all effects. With this arrangement, the flow to the compressor is reduced and the finisher, operating at the higher concentration, takes advantage of the full ΔT available.

Fans

The next development in MVR design was an apparent reversal of prior advances.

Table 4. Saturated ΔT(°F) at Various Compression Ratios

	Boiling temperatures		
Compression ratio	130°F	170°F	212°F
1.2	6.9	8.0	9.3
1.4	12.9	15.0	17.5
1.6	18.2	21.2	24.7
1.8	23.0	26.8	31.2
2.0	27.3	31.9	37.2

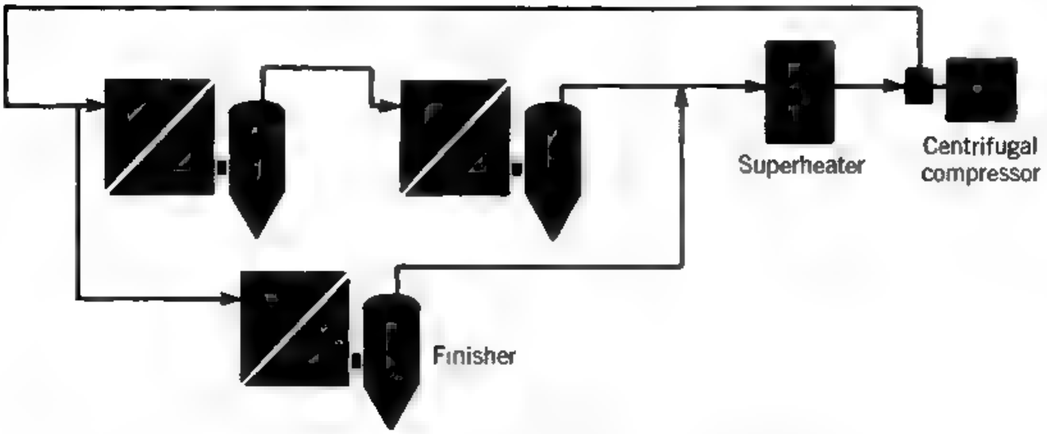


Figure 29. Multiple-effect MVR, centrifugal compressor with finisher.

As the required ΔT across a film evaporator decreased, it became possible to provide that ΔT by using a fan. This produces a compression ratio on the order of 1.2 to 1, providing approximately 7 or 8°F ΔT (before losses) for evaporation. The MVR system previously discussed and shown in Figure 30 is typical of fan use. It should be noted that the fan MVR technique can be used only on single-effect film evaporators handling product without significant boiling point elevation. Where the heat-transfer coefficient of the product is low, it sometimes is better to make use of the higher ΔT available from a centrifugal compressor.

Where fans can be used, however, the horsepower requirement usually is less than in centrifugal compressor designs. Furthermore, fans do not require that the inlet vapor be superheated. Instead, this is done either by a separate heat exchanger using steam or by recycling some of the compressor discharge vapor to the compressor suction.

It is, incidentally, a common practice to use a fan evaporator to concentrate a product up to a point where a large ΔT is required and then to switch to a small steam or MVR finishing evaporator for final concentration.

Other Designs

Rotary blowers are low-capacity positive compressors that occasionally are used with small MVR evaporators of up to approximately 15,000-lb/h capacity. This generally is a finishing-type evaporator.

In some cases, two compressors will be arranged in series as is shown in Figure 30. Here, the bulk of the evaporation is done in a single effect MVR system having a centrifugal compressor. Some of the compressor discharge vapor then is further compressed in a blower in order to operate a finishing evaporator.

Estimating Compressor Power Requirements

To calculate estimated power requirements for an MVR compressor power and ΔT values for both centrifugal compressors and fans have been plotted in Figures 31 and 32, respectively. These values compare reasonably well with installed MVR systems.

Table 5, meanwhile, compares the power requirements for different MVR designs. Note that it is possible to calculate an equivalent steam economy by converting horsepower to Btu/h and multiplying by 2545. This value then is divided by the latent heat to arrive at the equivalent steam used.

Thermodynamics

To comprehend the potential of MVR evaporation fully, it is important that the thermodynamics of this technique be

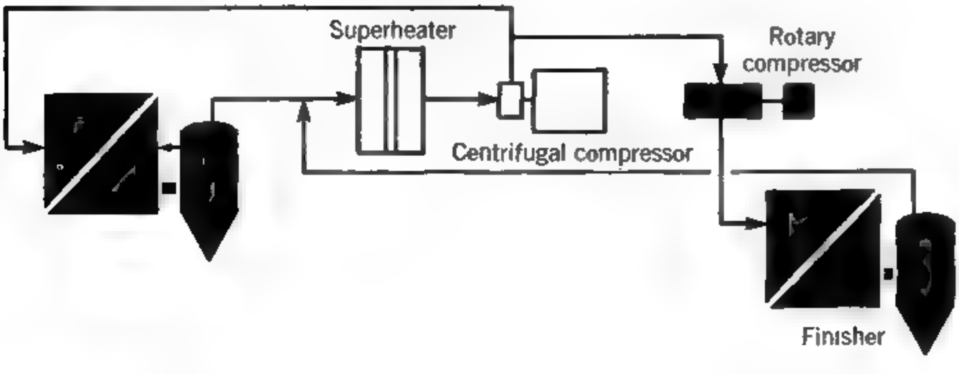
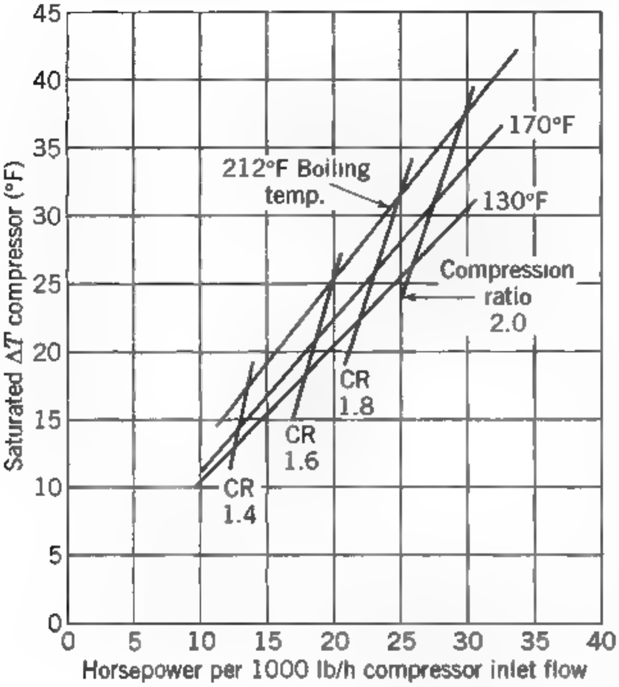


Figure 30. Single-effect MVR evaporator with centrifugal compressor and rotary-blower-driven finisher.



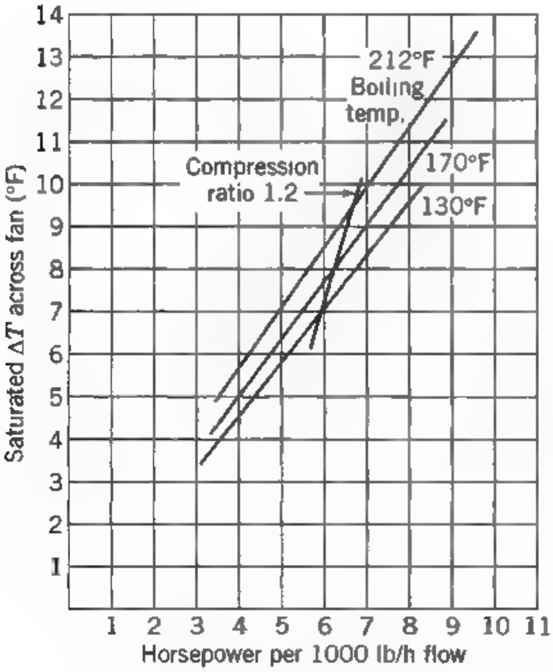
Compressors								
Boiling temperature 130°F			Boiling temperature 170°F			Boiling temperature 212°F		
CR	ΔT	HP	CR	ΔT	HP	CR	ΔT	HP
1.3	10.00	9.39	1.3	11.65	10.01	1.3	13.53	10.67
1.4	12.91	12.18	1.4	15.03	12.99	1.4	17.47	13.85
1.6	18.21	17.38	1.6	21.23	18.54	1.6	24.69	19.76
1.8	22.98	22.15	1.8	26.81	23.63	1.8	31.20	25.18
2.0	27.31	26.57	2.0	31.89	28.34	2.0	37.16	30.20
2.2	30.30	30.69	2.2	36.57	32.74	2.2	41.31	34.88

Figure 31. Horsepower versus ΔT for centrifugal compressors.

examined. This analysis involves Table 6 as well as the steam and MVR evaporator schematics shown in Figures 28a and 29b.

The first two columns of Table 6 give the properties of saturated water vapor 14.7 psia, 212°F and at 17.2 psia, 220°F. These values can be used to estimate the energy requirements for an evaporator operating at these conditions.

Looking first at the steam evaporator shown in Figure 29a, water at 17.2 psia, 220°F has an enthalpy (heat con-



Fans								
Boiling temperature 130°F			Boiling temperature 170°F			Boiling temperature 212°F		
CR	ΔT	HP	CR	ΔT	HP	CR	ΔT	HP
1.1	3.59	2.99	1.1	4.17	3.19	1.1	4.85	3.41
1.2	6.91	5.79	1.2	8.04	6.18	1.2	9.34	6.60
1.3	10.00	8.43	1.3	11.65	9.00	1.3	13.53	9.60

Figure 32. Horsepower versus ΔT for fans. *Note:* Vapor assumed to be superheated 10°F by external steam.

Table 5. Comparison of Typical MVR Designs (Approximate)[a,b]

	Single effect fan	Double effect centrifugal	Triple effect centrifugal
Compression ratio	1.2	1.6	2.0
Total ΔT available, °F	6.9	18.2	27.3
Vapor to compressor, lb/h	60,000	30,000	20,000
Horsepower/1000 lb h vapor flow	5.79	17.38	26.57
Total horsepower	374.4	521.4	531.4
Equivalent Btu	884,133	1,326,963	1,352,413
Equivalent steam, lb/h	870	1315	1347
Equivalent steam economy	69	45.6	44.5
Average ΔT per effect before losses, °F	6.9	9.1	9.1

[a] Boiling temperature 130°F, evaporation rate 60,000 lb/h.

[b] *Note:* In this example, the fan horsepower is lower than either of the centrifugal designs, but the lower ΔT required the greater the surface area.

Table 6. Properties of Water Vapor

Pressure, psia	14.7	17.2	17.2
State	Saturated	Saturated	Saturated
Temperature, °F	212	220	243
H-enthalpy vapor, Btu/lb	1150.5	1153.4	1164.6
Latent heat, Btu/lb	970.3	965.2	—
H-enthalpy liquid, Btu lb	180.2	188.2	—
S-entropy Btu/lb	1.7568	1.7442	1.7596

tent) of 188.2 Btu/lb while steam at the same conditions has an enthalpy of 1153.4 Btu/lb. The difference between the vapor and liquid is the latent heat or 965.2 Btu/lb. In other words, in order to produce one pound of steam at 220°F, 17.2 psia, from water at 220°F, 17.2 psia, the addition of 965.2 Btu/lb of energy is required.

In the case of the MVR evaporator, however, the vapor at 17.2 psia, 220°F (enthalpy 1153.4 Btu/lb) is produced from vapor at 212°F, 14.7 psia (enthalpy 1150.5 Btu/lb). Theoretically, only 2.9 Btu/lb of energy must be added.

The compressor, a fan in this case, operates such that the entropy of the discharge vapor be at least as high as the entropy of the inlet vapor. Since inefficiencies in the compression cycle will result in an exit entropy above that of the inlet entropy, the temperature of the existing vapor and energy input to the compressor must be increased.

Figure 33 is an enthalpy—entropy diagram for water vapor. The vapor at 14.7 psia, 212°F, is shown at point *A*. During compressions the entropy remains constant (ideally) or increases (actually). With typical efficiencies for this duty, the discharge temperature may be expected to rise to 243°F where the enthalpy is 1164.6 Btu/lb. The energy input from the compressor is 14.1 Btu/lb.

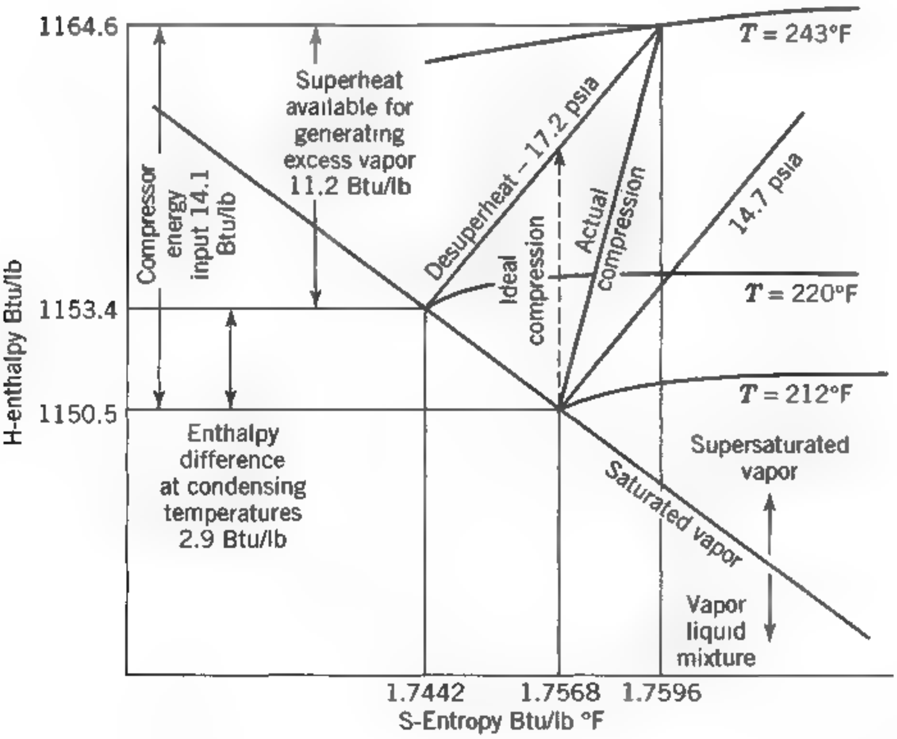

1164.6
1153.4
1150.5
H-enthalpy Btu/lb
T = 243°F
Superheat available for generating excess vapor 11.2 Btu/lb
Compressor energy input 14.1 Btu/lb
Desuperheat – 17.2 psia
Ideal compression
Actual compression
14.7 psia
T = 220°F
T = 212°F
Enthalpy difference at condensing temperatures 2.9 Btu/lb
Supersaturated vapor
Saturated vapor
Vapor liquid mixture
1.7442
1.7568
1.7596
S-Entropy Btu/lb °F

Figure 33. Enthalpy/entropy diagram.

In order to cool the vapor to its condensing temperature of 220°F, 11.2 Btu/lb of heat is removed. This can be done by introducing condensate at 220°F from the steam side of the evaporator. The 11.2 Btu/lb of heat removed from the vapor is absorbed into the condensate, some of which will vaporize and give off what is known as "excess vapor." For every pound of vapor cooled, 11.2 Btu of heat is absorbed in the condensate, which requires 965.2 Btu/lb to boil. Therefore, for each point of vapor leaving the compressor, 11.2/965.2 or 0.0116 lb of excess vapor is available.

This excess vapor is used in several ways. Since there is a slight difference in latent heat between the steam and the vapor, slightly more steam is required than the vapor generated. Other excess vapor is used to cover losses due to radiation and venting, is made available in some instances for preheating, or is sent to a condenser. It is significant to note that the condenser on an MVR evaporator is responsible only for vent and excess vapors. This results in a much lower cooling requirements than is necessary for steam evaporators.

It is possible to calculate an equivalent steam economy for an MVR system. In this example, for every pound of water evaporated, 970.3 Btu is absorbed. The compressor supplies 14.1 Btu but with motor and gear losses, probably requires 14.5 Btu of energy. The equivalent economy (970.3/14.5) is 67 to 1. Since one horsepower is equivalent to 2545 Btu/h, the compressor in the example requires 14.5/2545 or 0.0057 hp/lb · h of evaporation.

It should be noted that pressure losses through the evaporator that must be absorbed by the compressor have not been considered in this example. These losses would be taken into account by either higher compressor horsepower or lower ΔT over the heat-transfer surface.

BIBLIOGRAPHY

Adapted from:

P. Worrall, "Selection and Design of Evaporators," *CPI Digest* 4(4), 2–10 (Feb. 1977); 5(4), 2–9 (March 1979).

P. Worrall, "Residence Time in Film Evaporation," *CPI Digest* 8(3), 17–19 (Dec. 1986).

P. Worrall, "Plate Type Evaporators," *CPI Digest* 8(4), 12–15 (July 1987).

P. Worrall, "Tubular Evaporators and 3A Sanitary Standards," *APV Evaporator Handbook*, Nov. 1977, pp. 24–26

P. Worrall, "Advances in MVR Evaporator Technology," *APV Evaporator Handbook*, Nov. 1989, pp. 24–26.

Copyrighted APV Crepaco, Inc. Used with permission

1. HTR1 report, May 1978, p. 7.
2. *Perry's Chemical Engineer's Handbook*, 5th ed., pp. 5–57.

APV Crepaco, Inc.
Lake Mills, Wisconsin

EXTRACTION

All engineering studies of food processes are related to the basic unit operations of mass transfer and/or heat transfer. These principles are applied to the numerous procedures of transporting, preparing, processing, packaging, and distributing of basic and value-added foods. Secondary unit operations include techniques of separating components, reducing particle size, mixing ingredients, and concentrating desired components. The unit separation operations, distillation, evaporation, dehydration, and filtration all involve the extraction of a component from a liquid, gas, or solid by physical or chemical means. However, each category listed has such different applications of scientific and engineering principles that it is too unwieldy to place them all under a combined unit operation. Thus, due to these complexities, the procedures have been divided into numerous categories related to the mechanisms of separation. This greatly simplifies the scientific and engineering studies of various unit operations and the subsequent engineering design, manufacture, and integration of the process facilities into an overall food process. Various separations of components in a basic harvested food or a partially processed product are the most important sequences of processing a food to a finished product for the market. The unit operation of extraction is considered to be the removal or separation of a component by material that has greater affinity for the component being removed.

Extraction, the separating of a component from a liquid or solid by another liquid is handled as a separate unit operation since the process is based on diffusion of one component from the base material to an extracting liquid. Since the mechanics of these processes are based on diffusion, study of the processes and design of extracting equipment is based on the diffusivities of the solute being removed from the base material. This differs from other separation processes such as distillation that involve a change of phase in one or more components requiring different scientific principles. The original applications of extraction processes began many years ago with application of gas absorption and solvent extraction in the industrial chemical industry. It has been in relatively recent years that the technology has been extensively applied to separating components in food (1).

Extraction as practiced in the food industry is essentially the operation of removing or separating a component from the food to ensure food safety or to alter the properties. A rudimentary form of extraction begins with the basic separation or removal of components from an as-received food. With the growing problems of microbiological contamination in harvested fruits, vegetables, and animals, the extraction operation of washing as-received raw materials has received much emphasis over the past few years. The simple washing of a vegetable or fruit is necessary to ensure that contamination from soil, fertilizers, living organisms, pesticides, and so on is removed or at least reduced to an acceptable level, making the vegetable safe to eat. Proper washing is particularly important in slaughtered animals since extremely dangerous microorganisms such as *Escherichia coli* and *Salmonella* contaminate many of them. A much more complex procedure for removing or extracting a component is found in the process for producing vegetable oils. For example, many vegetable and seed oils are extracted from the base material by solvent extraction. A solvent in which the corn oil is soluble

In order to cool the vapor to its condensing temperature of 220°F, 11.2 Btu/lb of heat is removed. This can be done by introducing condensate at 220°F from the steam side of the evaporator. The 11.2 Btu/lb of heat removed from the vapor is absorbed into the condensate, some of which will vaporize and give off what is known as "excess vapor." For every pound of vapor cooled, 11.2 Btu of heat is absorbed in the condensate, which requires 965.2 Btu/lb to boil. Therefore, for each point of vapor leaving the compressor, 11.2/965.2 or 0.0116 lb of excess vapor is available.

This excess vapor is used in several ways. Since there is a slight difference in latent heat between the steam and the vapor, slightly more steam is required than the vapor generated. Other excess vapor is used to cover losses due to radiation and venting, is made available in some instances for preheating, or is sent to a condenser. It is significant to note that the condenser on an MVR evaporator is responsible only for vent and excess vapors. This results in a much lower cooling requirements than is necessary for steam evaporators.

It is possible to calculate an equivalent steam economy for an MVR system. In this example, for every pound of water evaporated, 970.3 Btu is absorbed. The compressor supplies 14.1 Btu but with motor and gear losses, probably requires 14.5 Btu of energy. The equivalent economy (970.3/14.5) is 67 to 1. Since one horsepower is equivalent to 2545 Btu/h, the compressor in the example requires 14.5/2545 or 0.0057 hp/lb · h of evaporation.

It should be noted that pressure losses through the evaporator that must be absorbed by the compressor have not been considered in this example. These losses would be taken into account by either higher compressor horsepower or lower ΔT over the heat-transfer surface.

BIBLIOGRAPHY

Adapted from:

P. Worrall, "Selection and Design of Evaporators," *CPI Digest* 4(4), 2–10 (Feb. 1977); 5(4), 2–9 (March 1979).

P. Worrall, "Residence Time in Film Evaporation," *CPI Digest* 8(3), 17–19 (Dec. 1986).

P. Worrall, "Plate Type Evaporators," *CPI Digest* 8(4), 12–15 (July 1987).

P. Worrall, "Tubular Evaporators and 3A Sanitary Standards," *APV Evaporator Handbook*, Nov. 1977, pp. 24–26

P. Worrall, "Advances in MVR Evaporator Technology," *APV Evaporator Handbook*, Nov. 1989, pp. 24–26.

Copyrighted APV Crepaco, Inc. Used with permission

1. HTR1 report, May 1978, p. 7.
2. *Perry's Chemical Engineer's Handbook*, 5th ed., pp. 5–57.

APV Crepaco, Inc.
Lake Mills, Wisconsin

EXTRACTION

All engineering studies of food processes are related to the basic unit operations of mass transfer and/or heat transfer. These principles are applied to the numerous procedures of transporting, preparing, processing, packaging, and distributing of basic and value-added foods. Secondary unit operations include techniques of separating components, reducing particle size, mixing ingredients, and concentrating desired components. The unit separation operations, distillation, evaporation, dehydration, and filtration all involve the extraction of a component from a liquid, gas, or solid by physical or chemical means. However, each category listed has such different applications of scientific and engineering principles that it is too unwieldy to place them all under a combined unit operation. Thus, due to these complexities, the procedures have been divided into numerous categories related to the mechanisms of separation. This greatly simplifies the scientific and engineering studies of various unit operations and the subsequent engineering design, manufacture, and integration of the process facilities into an overall food process. Various separations of components in a basic harvested food or a partially processed product are the most important sequences of processing a food to a finished product for the market. The unit operation of extraction is considered to be the removal or separation of a component by material that has greater affinity for the component being removed.

Extraction, the separating of a component from a liquid or solid by another liquid is handled as a separate unit operation since the process is based on diffusion of one component from the base material to an extracting liquid. Since the mechanics of these processes are based on diffusion, study of the processes and design of extracting equipment is based on the diffusivities of the solute being removed from the base material. This differs from other separation processes such as distillation that involve a change of phase in one or more components requiring different scientific principles. The original applications of extraction processes began many years ago with application of gas absorption and solvent extraction in the industrial chemical industry. It has been in relatively recent years that the technology has been extensively applied to separating components in food (1).

Extraction as practiced in the food industry is essentially the operation of removing or separating a component from the food to ensure food safety or to alter the properties. A rudimentary form of extraction begins with the basic separation or removal of components from an as-received food. With the growing problems of microbiological contamination in harvested fruits, vegetables, and animals, the extraction operation of washing as-received raw materials has received much emphasis over the past few years. The simple washing of a vegetable or fruit is necessary to ensure that contamination from soil, fertilizers, living organisms, pesticides, and so on is removed or at least reduced to an acceptable level, making the vegetable safe to eat. Proper washing is particularly important in slaughtered animals since extremely dangerous microorganisms such as *Escherichia coli* and *Salmonella* contaminate many of them. A much more complex procedure for removing or extracting a component is found in the process for producing vegetable oils. For example, many vegetable and seed oils are extracted from the base material by solvent extraction. A solvent in which the corn oil is soluble contacts a product, such as ground corn. This results in an oil-rich solvent with little of the solvent-insoluble products remaining in the oil fraction. The oil is then removed from the solvent by other separation operations such as distillation. Extraction processes can be carried out on a batch basis or as continuous steady-state operation. As is the case with most unit operations, the continuous operation is the easiest to analyze and design. Continuous operations are particularly important to maximize the operating costs and efficiency of extraction processes used in the food industry (1–3).

The basis for success of extraction processes is the difference in affinity for one component or material over another. For example, water has little affinity for vegetable oil, but the oil is completely soluble in an organic solvent. Hence, when an oil-containing food raw material, such as soybeans or corn, is placed in contact with water, the water will not absorb any oil. Oil will only be released if the food is in hot water, such as during cooking, when the structure of the food is changed so that oil is released. In this case the oil is not extracted by water but the water acts as a vehicle for washing out the oil and floating it to the surface.

Extraction during food processing is involved with the diffusion mass transfer of one component of the food being extracted, or leached, in the solvent phase. Although the extraction is enhanced by thorough contact or mixing, the controlling factor is the diffusion as measured by the diffusivity of the component being removed from the base material. There are two primary unit operations to consider under extraction of foods. The first is a liquid–solid extraction in which a soluble component is removed from the solid by a liquid. This process is known as leaching. The second, liquid–liquid extraction involves the removal of a component in a liquid by another liquid.

There are several special extraction processes whereby the extraction liquid changes phase during the process. However, the basic diffusivity of the condensing extracting material is still the basis for the component transfer rate. For example, steam is used for stripping volatile materials from both solids and liquids. Thus, the condensing steam is actually the solvent phase that either remains a vapor or changes from a vapor to a liquid during the extraction process. Some extractions, such as the use of hexane in extracting oil from soybeans, utilize a vapor solvent that condenses during the extraction and is recovered as an oil-rich liquid solvent.

PRINCIPLES OF EXTRACTION

The extraction rate at which a component (solute) is transferred from the material (solid or liquid) phase being treated to a solvent phase is the controlling factor involved in extraction. This is related to the mass-transfer coefficient (K_LA) as determined by the following conditions of the process and properties of the raw material, solute, and solvent:

The properties of the solvent as related to its affinity for the solute

The immiscibility of the raw material with the solvent

The particle size of the solid and thus the exposed surface area and depth of solvent penetration required (for leaching only)

Temperature of the liquids

Degree of contact between liquids, which is a function of the agitation or equipment design

The driving force is the difference between the saturation concentration of the solute in the raw material and the solvent ($C_s - C$). Hence, the rate of extraction (amount per unit time, dN/dt) would be

$$dN/dt = K_LA(C_s - C) \tag{1}$$

Extraction involves three separate operations: (*1*) mixing the raw material and the solvent to bring them into intimate contact, (*2*) separating the two phases following contact, and (*3*) removing the solute from the solvent so that the solvent can be recycled. Often the economic viability of a process depends on the third step of removing the solute and reclaiming the solvent. In many cases the solvent is relatively expensive (eg, organic solvent) and good separation and recycling is imperative. When the solvent in leaching is water and because the solute is often a waste material, the solvent would not be recycled.

LIQUID–SOLID EXTRACTION (LEACHING)

As a solvent comes in contact with the solid material, the solvent becomes richer in the solute that is being removed from the solid. The rate at which the solvent becomes

Table 1. Examples of Commercial Leaching Operations

Raw material	Solvent	Solute	Product
Sugar beets	Water	Sugar	Refined sugar
Sugar beets	Water	Sugar	Refined sugar
Corn, rapeseed, cottonseed, soybeans	Organic solvent, nonpolar (hexane)	Oil	Edible oil (vegetable)
Vanilla beans	Supercritical carbon dioxide	Vanilla	Vanilla extract
Coffee beans	Supercritical Carbon Dioxide	Caffeine	Decaffeinated coffee
Roasted coffee beans	Water	Insoluble fraction	Soluble coffee constituents
Vegetables	Steam	Debris	Cleaned vegetables
Fish and meat flesh	Nonpolar solvent (dichloroethane)	Oil	Refined fish oil
Fish and meat flesh	Polar solvent (isopropyl alcohol)	Water-oil	Oil, protein concentrate
Fish and meat flesh, enzyme hydrolysis	Water plus enzymes	Proteins	Water-soluble proteins

Table 2. Examples of Commercial Liquid–Liquid Extraction Operations

Raw material	Solvent	Solute	Product
Oil seed for oil recovery (ie, cotton seed, soybeans)	Organic solvent (ie, propane, hexane)	Fatty acid (oleic)	Refined vegetable oil
Fish oil	Hot water	Low-molecular-weight odor and taste components	Semirefined fish oil
Fish oil	Supercritical carbon dioxide	Cholesterol	Low-cholesterol high omera-3 refined fish oil
Butterfat in milk or cream	Supercritical carbon dioxide	Cholesterol	Low-cholesterol milk or cream

richer in the solute depends on the factors in equation 1: contact time, mass-transfer coefficient for the specific system, area of the solid exposed to the solvent, and concentration driving force. Diffusion in the solid is most often the rate-controlling factor. Table 1 shows examples of typical commercial processes in which a food is leached by a solvent to remove a specific solute. Note that the product or products can be the removed solute (eg, leaching sugar beets, vegetable oil, etc), the solid material remaining after extraction (eg, decaffeinated coffee, cleaned vegetables, etc), or both the remaining solid and solute (eg, fish oil and protein concentrate).

A diagram of a semicontinuous solid–liquid extraction system is shown in Figure 1. The pure solvent enters the extractor and leaves rich in solute while the solid that has been placed in the extractor decreases in solute soluble components. The rate of reaction decreases during the process as the solid is depleted in solute. Because fresh solvent is passing over the solid, the extraction can continue until essentially all solute is removed from the food. If the process is made continuous by conveying the solid into and out of the reactor in one stage, the extraction normally will not be complete because the solid–solvent exposure time is reduced and equilibrium is not reached. Throughput can be increased and extraction can be more complete when two or more extractors are used in series. Usually, a series of batch-continuous reactors are connected and fresh solvent is used for each reactor while the solid is moved down the reactor series. Figure 2 shows this multiextraction process in which three reactors are in series. If the remaining solid is one of the end products, it is necessary to remove the residual solvent. Often this secondary process turns out to be another extraction process such as steam stripping. If the solvent is water, the normal process is to remove it from the solid by a drying operation.

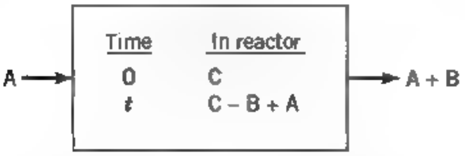


Figure 1. Batch solid–liquid extraction (leaching). A, Incoming pure solvent; A, outgoing solvent rich in solute (B); B, solute leached from solid (C); C, original solid rich in solute (B); C, extracted solid depleted of solute with retained solvent (A); *t*, extraction time.

A process whereby components of a food material are concurrently extracted while being made soluble by microbial and chemical action complicates leaching. A good example of this type of process is the batch hydrolysis of minced fish flesh processing waste by enzyme action. The water-insoluble components are changed into soluble (digested) materials. The resultant water-based protein solution can be dried to produce a high-quality protein product for both animal and human consumption. This technique is a good means for using the tremendous amounts of unused industrial fish, by-catch, underutilized edible species, and waste from processing whole fish (4).

LIQUID–LIQUID EXTRACTION

Liquid–liquid extraction operations are like solids leaching except the food, solvent, and solute are all in the liquid state. Most commercial processes are normally operated on a continuous basis. Table 2 shows the solvent, solute, and product produced from extracting some liquid raw materials. As shown in Figure 3, the ordinary extraction process involves countercurrent flow to maintain the maximum driving force. However, the relationship between the liquids sometimes makes a cocurrent operation more advisable.

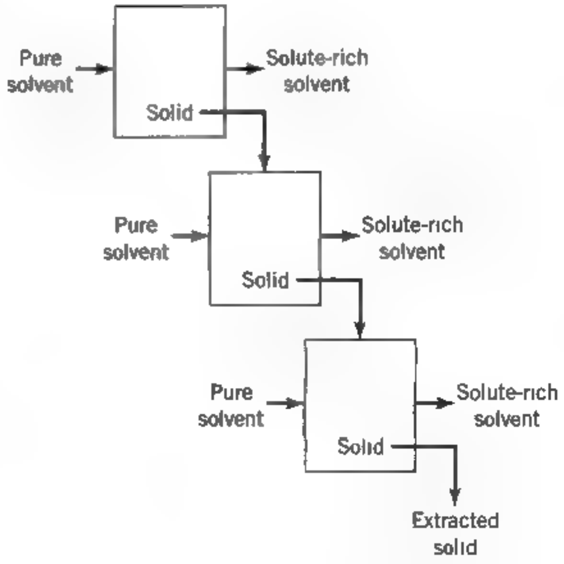


Figure 2. Batch-continuous three-phase solid–liquid extraction.

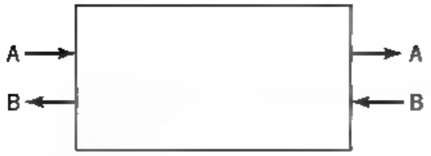


Figure 3. Continuous liquid–liquid extraction. A, Incoming food product rich in solute (S); A, outgoing food depleted of solute (S); B, incoming pure solvent; B, outgoing solvent rich in solute (S); S, solute originally in food.

The amount of solvent remaining in the food stream is dependent on the partial miscibility of the two liquids. Often the solute is recovered from the solvent as a viable product, or economics and environmental factors dictate that the solvent must be recycled. These goals are accomplished by steam stripping, evaporation, distillation, adsorption or chemical means, depending on the requirements and the materials.

SPECIAL EXTRACTION OPERATIONS

Over the past two decades, a considerable amount of scientific and applied research effort has been applied to the development of supercritical extraction. Many uses have been found for application of this technology (see the article SUPERCRITICAL FLUID TECHNOLOGY). Processes have been developed for extraction using supercritical fluids as the solvent. Carbon dioxide has been chosen as the supercritical fluid because it is nontoxic, less expensive than many organic solvents, leaves no residue of chemicals, and has a molecular weight and reaction behavior that is desirable. The extraction process using CO_2 is carried out at approximately 35 to 49°C and several thousand pounds per square inch (psi).

Decaffeination of coffee and tea were the first commercial processes carried out using CO_2 in a supercritical state, reducing initial caffeine content of 0.7 to 3% to as low as 0.02%. This was followed by processes for extracting hops and high-quality flavorings such as vanilla, ginger, paprika, rosemary, sage, and celery. Spices from supercritical extraction have been found to have superior antioxidant properties to some of the synthetic products in current use (5).

There is much interest in replacing hexane for extracting and refining oils from plant, animal, and seed sources. Interest ranges from eliminating the possible carcinogenicity of hexane residues to concentrating desired omega-3 fatty acids in fish oils. As development of critical extraction proceeds, new uses and improved economics of operation should see considerable increase in the commercial application of this technology.

BIBLIOGRAPHY

1. D. R. Heldman, *Food Process Engineering*, AVI Publishing, Westport, Conn., 1977, pp. 325–337.
2. G. M. Pigott, "Fish Processing," in *Encyclopaedia Britannica*, Vol. II, Encyclopaedia Britannica, Chicago, Ill., 1996.
3. G. M. Pigott, "Marine Oils," in Y. H. Hui, ed., *Baileys Industrial Oil and Fat Products*, Vol. III, 5th Ed., John Wiley & Sons, New York, 1996, pp. 225–254.
4. G. M. Pigott, "The Status and Future of Aquatic Food Research in the United States," *Proceedings of the International Seafood Research Meeting*, Dept. of Chemistry of Fishery Resources, Mie University, Tsu, Mie, Japan, September 30, 1994.
5. D. D. Duxbury, "High-Quality Flavor and Color Extracts Derived by Supercritical Extraction," *Food Processing* **50**, 50–54 (1989).

GEORGE PIGOTT
Sea Resources Engineering, Inc.
Kirkland, Washington

EXTRUSION

DEFINITION AND HISTORY OF USAGE

Extrusion is a process that combines several unit operations that include mixing, kneading, shearing, heating, cooling, shaping, and forming. This process also involves compressing and working a material to form a semisolid mass under a variety of controlled conditions, and then forcing it to pass through a restricted opening such as a shaped hole or slot at a predetermined rate (1). The first known record of extrusion was in 1797 when Joseph Bramah used a piston-driven device to manufacture seamless lead pipes (2). This innovation was adapted later by the food industry for the production of macaroni products. The first twin-screw extruder was developed in 1869 by Follows and Bates for sausage manufacture (2). A single-screw extruder, with shallow flights and rotating in a stationary cylindrical barrel, was developed for the Phoenix Gummiwerke A.G. in 1873 (3).

In the mid-1930s, forming extruders were used to mix semolina flour and water to form pasta products. A few years later, extrusion technology that combined transport, mixing, and shaping operations was used to produce the first ready-to-eat breakfast cereal from precooked oat-flour dough (4). In the late 1930s, Robert Colombo and Carlo Pasquetti developed a corotating and intermeshing twin-screw extruder for mixing cellulose without the use of a solvent (5). In the middle of the 1940s, the first extrusion-cooked, expanded food products, corn snacks, were commercially produced using single-screw extruders (6). Dry expanded pet food, dry expanded ready-to-eat breakfast cereals, and textured vegetable protein, produced by single-screw extruders, were introduced in the 1950s, 1960s, and 1970s, respectively (4,6). In the early 1970s, Creusot-Loire had developed the twin-screw plastics extruder for food applications. Finally, the 1980s have brought rapid commercialization of the production of feeds for aquatic species using the process of extrusion (6).

FOOD APPLICATIONS

Many different food products have been produced directly or indirectly by extrusion. Applications of extrusion have been classified into two categories: semifinished products and finished products (7). For semifinished products, extrusion cooking has been shown to offer economic advantages over the traditional process such as drum drying for producing pregelatinized cereal flours, potato starch, and cereal starches. By controlling the processing conditions to achieve the desired balance of gelatinization and molecular degradation, starches, and chemically modified starches with a wide range of cold water solubility values can be produced (8). Extensive patent literature has been covered on extruded breakfast cereals (9), snack foods (10) and textured foods (11–13). Other applications include dry and soft-moist pet foods, precooked and modified starches, flat bread, breadings, croutons, full-fat soy flour, precooked noodles, beverage bases, soup and gravy bases, and confections such as licorice, fruit gums, and chocolate (4,14).

GENERAL DESCRIPTION OF EQUIPMENT

Extrusion equipment can be classified thermodynamically or by pressure development in the extruder (15). According to the former classification, there are (*1*) autogenous extruders, which convert mechanical energy into heat energy during the flow process; (*2*) isothermal extruders where constant temperature is maintained throughout the extruder; and (*3*) polytropic extruders that operate between the extreme conditions of (*1*) and (*2*). If classified by the manner in which pressure is developed in the extruder, there are (*1*) direct or positive displacement types, include the ram or piston-type extruders and the intermeshing counterrotating twin-screw extruders; and (*2*) indirect or viscous drag types, which include roller extruders, single-screw extruders, intermeshing corotating twin-screw extruders, and nonintermeshing multiple-screw extruders.

The components of an extruder have been well described (4,16). They consist of drive, feed assembly, screw, barrel, die head assembly, cutters, and take-away system. Figure 1 shows a twin-screw extruder system manufactured by Werner & Pfleiderer with various components. Either AC or DC types of motors can be used to rotate the extruder screw in the barrel. Depending on the capacity, the size of the motors can be as small as a few horsepower for a laboratory-type extruder or several hundred horsepower for a commercial extruder used for full-scale production of extruded foods. Except for simple extruders such as the collet type, most extruder drive motors have a transmission and a speed variation device for controlling the screw rotation speed. Since the screw pushes food forward against the die, which builds pressure that could exceed hundreds of atmospheric pressure, a thrust bearing is required to support and center the screw and absorb the thrust exerted by the screw.

The feed assembly consists of dry ingredient and liquid ingredient feeders. Preblended dry ingredients are held in hoppers or bins above feeders, which can be vibratory, variable speed auger, or loss-in-weight type. Liquid ingredients are metered through positive displacement pumps, variable orifice, variable head, or water wheels. If a preconditioner is used, dry ingredients are mixed with water, steam, or other ingredients in a closed vessel, which can be operated under pressure if needed. The uniform feeding of dry and liquid ingredients is imperative for the consistent operation of an extruder.

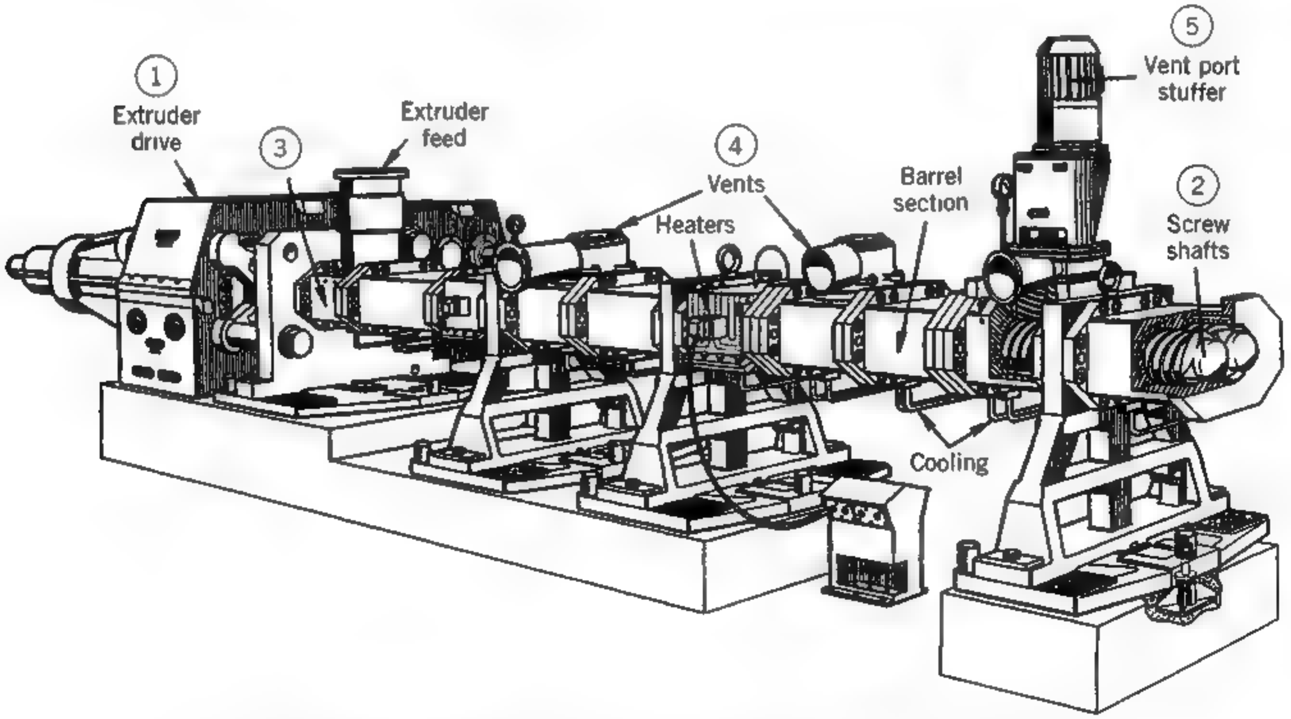


Figure 1. A Werner-Pfleiderer corotating, intermeshing twin-screw extruder. *Source:* Courtesy Krupp Werner & Pfleiderer, Ramsey, N.J.

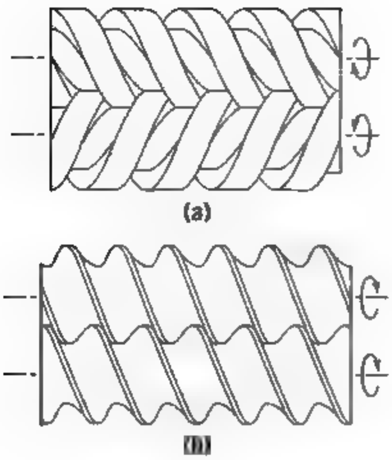


Figure 2. Schematic diagram of fully intermeshing counter- and corotating extruder screws. (a) Counterrotating screws and (b) corotating screws.

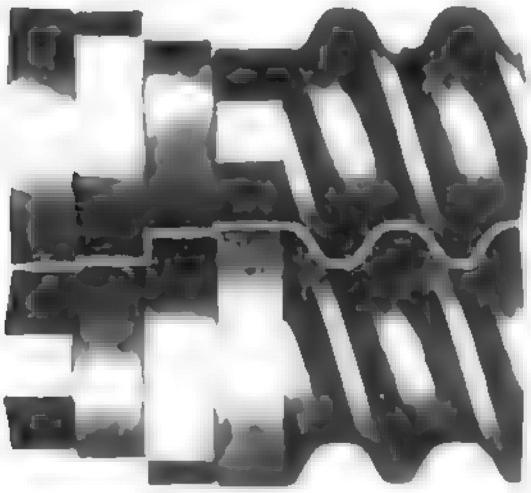


Figure 3. Four pairs of kneading disks are shown with corotating twin-lead screws.

The screw can be one piece or consist of many individual screw elements assembled in a screw shaft. The screw is usually divided into feed, transition, and metering sections for single-screw extruders. The feed section has deeper flights to accept the food ingredients from the feeder. In the transition section, the food ingredients are mixed, heated, and worked into a continuous mass. Thus, the transition section is also called the compression section because the materials are changed from a loose, powdery state into a plasticized dough. This is accomplished by (*1*) gradually decreasing flight depth or pitch in the direction of discharge, (*2*) heating from the barrel, and (*3*) working of the feed material to generate frictional heat. The metering section has shallow flights that increase the shear rate; therefore, the temperature of material increases rapidly in this section.

Twin-screw extruders are classified by the direction of screw rotation, corotating or counterrotating, and by the depth of screw engagement—fully intermeshing, partially intermeshing, and nonintermeshing (16,17). The schematic diagram of fully intermeshing counter- and corotating extruder screws is shown in Figure 2. For fully intermeshing twin-screw extruders, the flight depth is always constant and equal to the distance of screw overlap. The screw design consists of single-, twin-, and triple-lead types, which refers to the number of helical-shaped flights parallel along the length of the screw. To improve mixing and increase the conversion of mechanical energy into heat, another type of screw design, kneading disks (Fig. 3), is usually used.

The barrel fits tightly around the rotating screw and is assembled by bolting or clamping several segments together. An important parameter for the specification of the extruder is its L/D ratio, which is the extruder barrel length divided by the extruder barrel bore diameter. The barrels and their liners, in particular, are usually constructed of special hardened alloy to become wear resistant. The interior surface of barrel is either smooth or grooved. The presence of grooves increases the pumping capability of the extruder against high back pressure (4).

The die head assembly is located at the end of the extruder barrel and holds the extruder die plate and sometimes serves as support for the cutter. The die plate can hold many dies, which shape the food product before it emerges from the extruder. The dies are small openings that can be round, annular, slit, or of specially designed shape, such as alphabets or animals, and so on.

BIBLIOGRAPHY

1. J. D. Dziezak, "Single- and Twin-Screw Extruders in Food Processing," *Food Technol.* **43**, 164 (1989).
2. L. P. B. M. Janssen, *Twin Screw Extrusion*, Elsevier Science, New York, 1978.
3. P. Linko, P. Colonna, and C. Mercier, "High-Temperature, Short-Time Extrusion Cooking," in Y. Pomeranz, ed., *Advances in Cereal Science and Technology*, Vol. IV, AACC, St. Paul, Minn., 1981, pp. 143–235.
4. J. M. Harper, *Extrusion of Foods*, Vol. I & II, CRC Press, Boca Raton, Fla., 1981.
5. F. G. Martelli, *Twin-Screw Extruders*, Van Nostrand Reinhold, New York, 1983.
6. B. W. Hauck and G. R. Huber, "Single Screw vs Twin Screw Extrusion," *Cereal Foods World* **34**, 930 (1989).
7. W. Wiedmann, and E. Strobel, "Technical and Economic Advantages of Extrusion Cooking," *Technische Mitteilungen Krupp* **2**, 95 (1987).
8. M. G. Fitton, "Extruded Starches—Product Analysis, Structure and Properties," in G. O. Philips, D. J. Wedlock, and P. A. Williams, eds., *Gums and Stabilizers for the Food Industry 3*, Elsevier Applied Science, New York, 1985, pp. 213–220.
9. R. Daniels, *Modern Breakfast Cereal Processes*, Noyes Data Corporation, Park Ridge, N.J., 1970.
10. M. H. Gutcho, *Prepared Snack Foods*, Noyes Data Corporation, Park Ridge, N.J., 1973.
11. M. H. Gutcho, *Textured Foods and Allied Products*, Noyes Data Corporation, Park Ridge, N.J., 1973.

12. M. H. Gutcho, *Textured Food Products*, Noyes Data Corporation, Park Ridge, N.J., 1977.
13. M. H. Gutcho, *Textured Protein Foods*, Noyes Data Corporation, Park Ridge, N.J., 1977
14. O. B. Smith, "Extrusion Cooking of Corn Flours and Starches as Snacks, Breadings, Croutons, Breakfast Cereals, Pastas, Food Thickeners, and Additives," in G. E. Inglett, ed., *Maize: Recent Progress in Chemistry and Technology*, Academic Press, New York, 1982, pp. 193–219.
15. J. L. Rossen, and R. C Miller, "Food extrusion," *Food Technol.* **27**, 46 (1973).
16. J. M. Harper, "Food Extruders and Their Applications," in C. Mercier, P. Linko, and J. M. Harper, eds., *Extrusion Cooking*, AACC, St. Paul, Minn., 1989, pp. 1–15.
17. J. Fichtali and F. R. van de Voort, "Fundamental and Practical Aspects of Twin Screw Extrusion," *Cereal Foods World* **34**, 921 (1989).

FU-HUNG HSIEH
University of Missouri
Columbia, Missouri

EXTRUSION COOKING

BACKGROUND

Extrusion cooking generally refers to the combination of heating food products in an extruder to create a cooked and shaped product. Raw materials, such as flours, starches, proteins, salt, sugar, and other minor ingredients, are mixed, kneaded, cooked, and worked into a plasticlike dough mass. Heat can be applied directly by steam injection or indirectly through heated barrel or through the conversion of mechanical energy. The final process temperature in the cooking extruder can be as high as 200°C, but the residence time is relatively short, 10 to 60 s (1). Thus, extrusion cooking is also called a high-temperature, short-time (HTST) process.

The first cooking extruder was developed in the late 1940s and led to a great expansion of the application of extruders in the food field (2). Cooking extruders have many different sizes and shapes, which allow a wide range of moisture contents (10–40%), feed ingredients, cooking temperatures, and residence times. In addition, the ability for extruders to vary the screw, barrel, and die configurations for the cooking and shaping requirements of different food product makes extrusion cooking one of the most versatile food processes.

EFFECTS OF PROCESSING PARAMETERS

The process parameters for extrusion generally include screw speed, feed moisture, feed rate, and barrel temperature. Changes in these processing parameters will change the motor torque, die pressure, and product temperature. Visual product characteristics such as expansion ratio, specific length (length of extrudate per unit mass), and bulk density also will change.

Increases in screw speed at constant feed rate will cause a quick initial increase in die pressure and motor torque, followed by a sharp decrease to the new equilibrium value (3). The initial quick increase in die pressure and motor torque is due to the effort of the motor drive to overcome the inertia of the screw shaft and the momentary increase in the output. Because of a rapid decrease in the degree of fill due to screw speed increase, both torque and die pressure fall sharply after the initial increase (4,5). The specific energy input increases with increasing screw speed at constant feed rate; therefore, the product temperature increases. Extrudates are usually lower in expansion ratio, higher in specific length, and lower in bulk density with increasing screw speed (4,5).

Increasing feed moisture lowers the torque, specific mechanical energy input, and product temperature (3). Die pressure drops initially due to a lower dough viscosity caused by moisture increase. However, as the product temperature gradually becomes lower, the dough viscosity rises, causing die pressure to recover. Final equilibrium die pressure will depend on the relative effect of feed moisture and product temperature on the dough's viscosity. Extrudates are generally lower in expansion ratio and specific length, but higher in bulk density with a higher feed moisture (6).

An increase in feed rate causes rapid increases in motor torque and die pressure and a slower decrease in product temperature (3,7). Increases in feed rate while maintaining constant screw speed increase the degree of fill in the extruder barrel (8) and, thus, raise motor torque and die pressure (9). Since the specific energy input is decreased with increasing feed rate, the product temperature decreases. Increasing feed rate usually increases both expansion ratio and specific length, resulting in a lower bulk density (10).

Decreasing the barrel temperature causes the product temperature to decrease. A lower product temperature corresponds to a lower dough temperature or a higher dough viscosity and, therefore, a higher motor torque and die pressure (3). The physical characteristics of extrudates from lower barrel temperatures are usually similar to those from higher feed moistures.

CHANGES IN FOOD AFTER EXTRUSION

Since extrusion cooking is an HTST process, it changes the physical and chemical properties of food. Many methods have been developed and applied to characterize extruded products. Expansion of extrudates in both longitudinal and lateral directions can be evaluated by direct physical measurements. Apparent bulk density is determined by weighing the extrudates held in a container with a fixed volume. Bulk density or specific volume can be obtained using sand or rapeseed displacement methods. Scanning electron micrographs have been used to examine the extrudate's internal porous structure. Crispness, crunchiness, hardness, and other texture attributes can be determined objectively with a texture analyzer such as Instron or subjectively with human sensory evaluation methods. The color of extrudates can be assessed with a colorimeter. Flavor compounds formed can be determined with a GC-MS.

Many analytical tests have been developed to evaluate extruded products. For tests on cereal or starchy-type products, there are the water absorption index (WAI), water solubility index (WSI), amylograph, and gelatinization tests. The WAI is the weight of gel obtained per gram of dry product after dispersing the dry product into water, centrifuging, and decanting the supernatant. The WSI is the amount of dried solid recovered by evaporating the supernatant from the WAI test. The amylograph test determines the viscosities of a slurry from the finely ground, extruded product through a preprogrammed heating and cooling cycle.

Gelatinization of starches is one of the most important changes that occurs in cereal types of foods during extrusion. Starch, in the raw or native state, exists in the form of granules with many different shapes, ranging from round, oval to irregular, with sizes between 1 and 100 μm depending on the sources of starch. As water and heat are added during extrusion, the starch granules swell and the amylose in the granules begins to diffuse out. Eventually, the starch granules, consisting mostly of amylopectin, collapse and become a colloidal gel held in a matrix of amylose. For different types of starch, the temperature range at which gelatinization occurs differs according to the sources of starch. Many methods have been developed to determine the degree of starch gelatinization after extrusion. Some commonly used methods are (*1*) loss of birefringence (crystallinity) in polarized light, (*2*) X-ray diffraction patterns, (*3*) differential scanning calorimetry (DSC), and (*4*) enzyme susceptibility.

For proteinaceous-type products, two indices are of interest: the protein dispersibility index (PDI) and the nitrogen solubility index (NSI). While the latter measures the percentage of total nitrogen in extrudate that is soluble in water, the former determines the amount of protein in the extrudate that is dispersible in water; both are conducted under controlled conditions of extraction (2). As the amount of heat treatment during extrusion increases, more protein in the extrudate will be denatured, which will result in decreases in both the PDI and NSI. Similar to a cereal-type product, texture is also an important quality attribute for an extruded proteinaceous product. Both sensory and instrumental methods have been developed. The extrudate can also be examined by utilizing scanning electron microscopy to reveal the structural integrity of the protein matrix. Changes in proteins during extrusion can be briefly summarized as follows: (*1*) physically, extrusion converts protein bodies into a homogeneous matrix; and (*2*) chemically, the process recombines storage proteins in some way into structured fibers (11).

Besides starch gelatinization and protein denaturation, many other changes may occur during an extrusion process, such as nonenzymatic browning, enzyme inactivation, destruction of antinutritional factors, and microorganisms.

HTST EXTRUSION COOKERS

HTST extrusion cookers can be classified into two main categories: single-screw and twin-screw cooking extruders. The latter is further divided into corotating and counterrotating twin-screw extruders. Within the same type of cooking extruders, the mechanical features and operating characteristics may differ substantially from each other since there are many manufacturers supplying each type of cooking extrusion equipment. The following is a partial list of manufacturers that supply the HTST extrusion cookers:

Single-screw cooking extruders: Anderson, Bonnot, Buss, Egan, Johnson, Mapimpianti (Mapa), Sprout-Waldron, and Wenger

Corotating twin-screw cooking extruders: APV Baker, Buhler, Clextral, Wenger, and Werner-Pfleiderer

Counterrotating twin-screw cooking extruders: Brabender, Cincinnati Milacron, and Textruder

A more complete list can be found in a recent article (12).

METHODOLOGY FOR HTST EXTRUSION COOKING

Before beginning the HTST extrusion cooking process, the extruder and its related equipment must be assembled first. All fasteners should be tight and all parts aligned. If there is a torque specification for the retaining bolts, it must be closely followed using a torque wrench. After the barrel and screw(s) are assembled, the screw should be turned manually to ensure that it moves freely. The die plate with its die inserts is then bolted to the end of the extruder barrel. The rotating cutter assembly is installed and the cutting knives are adjusted to provide a cleanly cut product and an extended life for the blades. Water and steam lines are then connected to the extruder barrel as required. Condensate in the steam line must be removed. All feeders and regulators should be checked. If a volumetric feeder is used for dry ingredients, it should be calibrated first for each batch of feed materials. A typical Wenger extrusion cooking system is shown in Figure 1.

To minimize the amount of waste produced during the start-up, the cooking extruder is usually brought to operating condition and equilibrium as quickly as possible. This can be accomplished by preheating the barrel and die plate to the desirable operation temperature. The water pump is then started at a rate about 1.5 times the target operating rate. Once the water is in the extruder, start the extruder and the feed stream at a low rate. Increase the screw speed and feed rate, but reduce the water rate to operating levels gradually. When the barrel and die have reached stable operating temperatures, adjust the water rate, feed rate, and screw speed to obtain the desired product at a moderate torque level (90% or less motor load).

Similar to the start-up procedures, shutdown procedures for HTST cooking extruders must also be followed in sequence. Water is gradually increased first to allow uncooked material to purge the cooked material from the system. Barrel temperatures are reduced to stop cook reactions, which can prevent burning on after shutdown. When the percent load meter readings are below 25%, decrease the screw speed and shut off the extruder; in addition, shut off the feeder and water pump. The die plate is then removed by gradually and carefully undoing the bolts because some pressure may still exist behind the die plate.

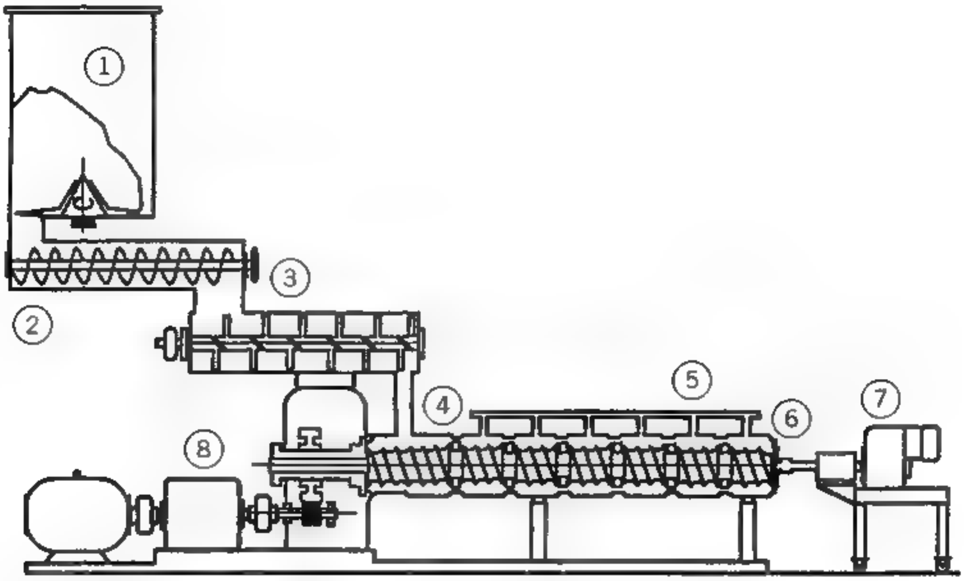


Figure 1. A Wenger extrusion cooking system. 1, Bin discharger; 2, feeder; 3, blender/preconditioner; 4, extruder, 5, steam injection ports; 6, die and variable speed cutter; 7, variable speed drive for cutter; 8, gear drive. *Source:* Courtesy of Wenger, Sabetha, Kansas.

APPLICATIONS AND DESCRIPTION FOR EXTRUSION COOKING OF DIFFERENT CATEGORIES OF PRODUCTS

Breakfast Cereals

There are four types of breakfast cereals that can be produced by HTST extrusion cooking process: (*1*) extrusion flaked cereals, (*2*) extrusion gun-puffed cereals, (*3*) extrusion shredded cereals, and (*4*) extrusion expanded cereals (13). Traditional flaked cereals are produced by flaking cooked grits. Extruded flaked cereals are different from traditional flaked cereals in that the grit for flaking is formed by extruding the mixed ingredients with a feed moisture of 25 to 35%. After exiting from the die holes, the product is cut off at the desired size and forms pellets. The pellets are then dried slightly and tempered before flaking. This process allows additional flexibility in the ingredients, such as multigrains or brans, used for the manufacture of flaked breakfast cereals.

Similar to extrusion flaked cereals, the first step of manufacturing extrusion gun-puffed and extrusion shredded cereals is to form pellets with the HTST cooking extruders. The pellets are dried and tempered before gun-puffing or tempered only before shredding. Thus, the pellet moisture content for flaked and gun-puffed cereals is usually lower than that for shredded cereals. After flaking, gun-puffing, or shredding, cereals may be sugarcoated if desired and then dried or toasted in an oven to impart cereals with a desirable toasted color and crisp or crunchy texture.

Extrusion expanded cereals differ from the other three types of extruded cereals in that the cereals are expanded into a porous matrix during the HTST extrusion cooking process. A typical moisture content in feed is about 15 to 25%, depending on the type of extruder and the extrusion conditions used. The extruded cereals are then sugarcoated, if desired, and then dried or toasted in an oven as with the other three types of extruded cereals.

Snacks

Many advances have been made over the years since corn collet was commercially developed in the 1940s. For example, extruded snacks now come in many different shapes other than the common collets or curls and in many different flavors other than basic cheese/oil such as products from potato, wheat, rice flour, or brans along with starches or modified starches (2).

Collet snacks can be divided into two types, baked or fried, depending on the moisture of the collets (14). Baked corn collets are extruded using a collet extruder at low moisture (<15%) to produce highly expanded products from degerminated corn grits. After extrusion, the collets are dried (baked) in an oven and coated with flavor and oil. Fried collets are extruded from corn meal that is moistened to a higher moisture (20–30%). Product expansion takes place during the deep fat frying step instead of during extrusion. The latter partially cooks and forms the product shape.

In addition to corn meal or corn grits, potato and other grain-based extruded snacks have been developed. Some examples are French fried potato stick, flatbread, rice cake, and multigrain snacks. Similar to collet snacks, they can be divided into either baked or fried types. Although the collet extruder can be used in some cases, other snacks, such as crispbread, may require more sophisticated extruders such as the HTST single-screw or twin-screw cooking extruders.

Extruded half-products are another type of snack products. Potato and other starches or modified starches are extruded to form very dense and thin pellets that are dried at a low temperature and controlled humidity environment for storage stability. The half-products need to be expanded by deep fat frying or baking and then enrobed with flavorings before consumption.

The latest development in the extruded snacks involves dual or coextrusion of two different components to form a single piece of snack using a specially designed die. Two extruders may be needed although, occasionally, one of the extruders may be substituted by a high-pressure gear pump if the second component is pumpable. This allows the development of many new snacks, for example, dual-textured snack products. The flow rates of the two extruders and the viscosity of the two components must be well regulated.

Texturized Protein Meatlike Products

According to Harper (2), two types of texturized meatlike products are derived from extrusion processes. The first is the meat extender, which can be produced by a single HTST extrusion cooking step using defatted soy flours or soy grits mixed with a variety of additives. The resulting product is highly expanded and shows distinct fiber formation. Once hydrated, the products can be used to extend ground meat or meat products. This product has found extensive applications in pizza toppings, meat sauces, and fabricated food formulations.

The second type of texturized meatlike product is a meat analog that can be used to replace meat. The finished product must be dense and have a layered fiber formation similar to that found in real meat. Additional requirements include maintaining a meatlike character after extensive cooking or retorting and giving the appearance and taste of real meat. The manufacture of meat analogs usually requires multiple extrusion processes or specially cooled dies (15).

Breadings and Croutons

The manufacture of breadings by either single-screw or corotating twin-screw extruders has been described (16,17). The starting materials are corn flour, wheat flour, or the second clears of wheat flour. Leavening agents, dry skim milk, salt, emulsifier, and other food additives may be added. Processing conditions during extrusion can be varied to obtain breadings that are functionally similar to traditional breadings. The extrudate is wet milled and sized prior to drying. Croutons are made in a similar manner. The small cubes are sized at the die of the extruder using a variable speed knife. Thus, the wet milling stage for the extrudate is not needed.

Pasta

The raw materials used in pasta products is semolina, the purified middlings of durum wheat, or durum flour, water, eggs and other ingredients (18). Commercially, pasta products are formed by the extruder or continuous press with a deep flighted screw. The screw not only forces the dough through the die, but it also kneads the dough into a homogeneous mass. A very accurate control of semolina flour and water are needed. Traditional pasta product uses low-temperature extrusion (<50°C). If the temperature of the dough exceeds 60°C, the cooking quality of the finished product will be damaged (2,18). Thus, excessive heat generated from mechanical energy input must be removed by circulating cooling water around the extruder barrel.

BIBLIOGRAPHY

1. E. W Schuler, "Twin-Screw Extrusion Cooking Systems for Food Processing," *Cereal Foods World* 31, 413 (1986).
2. J. M Harper, *Extrusion of Foods*, Vol. I and II, CRC Press, Boca Raton, Fla., 1981.
3. Q. Lu et al., "Model and Strategies for Computer Control of a Twin-Screw Extruder," *Food Control* 4, 25 (1993).
4. F. Hsieh, I. C. Peng, and H. E. Huff, "Effects of Salt, Sugar and Screw Speed on Processing and Product Variables of Corn Meal Extruded With a Twin-Screw Extruder," *J. Food Sci.* **55**, 224 (1990).
5. F. Hsieh et al., "Twin-Screw Extrusion of Rice Flour With Salt and Sugar," *Cereal Chem.* **70**, 493 (1993).
6. B. W. Garber, F. Hsieh, and H. E. Huff, "Influence of Particle Size on the Twin-Screw Extrusion of Corn Meal," *Cereal Chem.* **74**, 656 (1997).
7. M. K. Kulshreshtha and C. A. Zaror, "An Unsteady State Model for Twin Screw Extruders," *Trans. I. ChemE., Part C, Food and Bioproducts Proc.* **69**, 189 (1992)
8. G. Della Valle, J. Tayeb, and J. P. Melcion, "Relationship of Extrusion Variables With Pressure and Temperature During Twin-Screw Extrusion Cooking of Starch," *J. Food Eng.* **6**, 423 (1987).
9. F. Martelli, *Twin-Screw Extruders*, Van Nostrand Reinhold, New York, 1983.
10. M. Liang et al., "Barrel-Valve Assembly Affects Twin-Screw Extrusion Cooking of Corn Meal," *J. Food Sci.* 59, 890 (1994).
11. D. W. Stanley, "Protein Reactions During Extrusion Processing," in C. Mercier, P. Linko, and J. M. Harper, eds., *Extrusion Cooking*, American Association of Cereal Chemists, St. Paul, Minn., 1989, pp. 321–341.
12. "Extruder Directory," *Feed Tech* **2**, 32 (1998).
13. R. B. Fast, "Manufacturing Technology of Ready-to-Eat Cereals," in R. B. Fast and E. F. Caldwell, eds., *Breakfast Cereals and How They Are Made*, American Association of Cereal Chemists, St. Paul, Minn., 1990, pp. 15–42.
14. S. A. Matz, "Extruding Equipment," in S. A Matz, ed., *Snack Food Technology*, AVI, Westport, Conn., 1984, pp. 203–230.
15. A. Noguchi, "Extrusion Cooking of High-Moisture Protein Foods," in C. Mercier, P. Linko, and J. M. Harper, eds., *Extrusion Cooking*, American Association of Cereal Chemists, St. Paul, Minn., 1989, pp. 343–370.
16. O. B. Smith, "Extrusion Cooking of Corn Flours and Starches as Snacks, Breadings, Croutons, Breakfast Cereals, Pastas, Food Thickeners, and Additives," in G. E. Inglett, ed., *Maize: Recent Progress in Chemistry and Technology*, Academic Press, New York, 1982, pp. 193–219.
17. P. L. Noakes and W. A. Yacu, "Extrusion Cooking of Wheat Flour to Process Breadings," *Cereal Foods World* **33**, 687 (1988).

18. O J Banasik, "Pasta Processing," *Cereal Foods World* **26**, 166 (1981).

FU-HUNG HSIEH
University of Missouri
Columbia, Missouri

See also EXTRUSION PROCESSING: TEXTURE AND RHEOLOGY.

EXTRUSION PROCESSING: TEXTURE AND RHEOLOGY

In recent years extruders have been widely used in the human food and animal feed industry to manufacture products such as textured proteins, snack foods breakfast cereals, pasta, confectioneries, and pet foods. A list of products presently produced using the extrusion process is given in Table 1. The history of food extrusion has been reviewed in Ref. 2.

The two major functions of a food extruder are cooking and forming. In the cooking process, the product is heated through the transfer of heat energy, which is applied by steam injection or heaters and/or by the dissipation of viscous energy through shearing action between the rotating screw and the material. As a result of the cooking process, starches are gelatinized, proteins are denatured, undesirable enzymes are inactivated, and antinutritional substances (eg, trypsin inhibitors in soybeans) are destroyed. Because the temperature reached during the process can be quite high (ca 200°C) and the material takes a relatively short time to travel through the extruder (5 s to a few min), the cooking process is often referred to as high-temperature, short-time (HTST) extrusion. The forming extruder is used to produce special shapes from precooked material. Typically, the operating temperature of forming extruders are lower than that of cooking extruders.

Two major types of extruders are used in the food industry: single-screw and twin-screw extruders. The twin-screw extruders can be further classified according to the type of screws, ie, nonintermeshing or intermeshing and the type of rotation, ie, corotating or counter-rotating. Use of single-screw extruders began in the early years of the industry and they are still used today, though the twin-screw machines are becoming increasingly popular. Twin-screw extruders are comparatively more expensive than single-screw machines of the same production capacity. The flow of material in single-screw extruders depends on the friction between the barrel wall and the material. Hence the type of material that can be used is limited by its properties; proper conditioning of the material is necessary to reduce or eliminate process instability. The corotating, intermeshing twin-screw extruder uses the positive pumping characteristics created by the two intermeshing screws to convey the material forward. This type of mechanism reduces the need to precondition material. Other potential advantages of the corotating twin-screw extruder over the single-screw extruder include (1) narrow residence time distribution, leading to uniform processing, (2) better mixing capabilities, and (3) wide formulations of product, particularly low bulk density and high fat material that cannot be processed using a single-screw machine. The twin-screw extruder provides better control of the process and more uniform product characteristics than does the single-screw extruder. These advantages of the twin-screw machine compensates to some extent for the

Table 1. Extrusion Application in the Food and Feed Industry

Human food		Extrusion processes	Animal feed	
Starch-based	Protein-rich	Agrochemicals	Cattle and pig feed	Fish feeds
Pasta (2 types)	Animal protein	Fertilizers	Partial precooked cereals/seeds	Precooked flours (starch and protein) with different bulk densities
Tortilla flours	Sausages	Vegetables/soup		
Bakery products	Scrap meat/fish	Bagasse and other cellulosic material	Enzyme-engineered feed	
Confectioneries	Blood protein		Complete gelatinized cereals	
Candies	Milk protein	Cocoa waste material		
Licorices	Germ extrusion	Sucrose-based chemicals	Yeast wastes	
Caramels	Co-extruded meat/starch	Spices	Breaking up of cellulose	
Chocolate	Soft cheese	Maltodextrins	Protein hydrolysates	
Starches	Vegetable protein	Pulp for paper production	Pet foods	
Partly cooked	Soya/cottonseed/rapeseed			
Gelatinized	Oilseed meals	Proteins chemically modified		
Malt	Health foods			
Cereal snacks		Cross-linked starches		
Health foods		Lactose-based chemicals		
Breakfast cereals				
Flat breads/cookies				
Bran extrusion				
Crumb				
Instant-cooked foods				
Potato products				
Baby foods				

Source. Ref. 1. Courtesy of Elsevier.

18. O J Banasik, "Pasta Processing," *Cereal Foods World* **26**, 166 (1981).

FU-HUNG HSIEH
University of Missouri
Columbia, Missouri

See also EXTRUSION PROCESSING: TEXTURE AND RHEOLOGY.

EXTRUSION PROCESSING: TEXTURE AND RHEOLOGY

In recent years extruders have been widely used in the human food and animal feed industry to manufacture products such as textured proteins, snack foods breakfast cereals, pasta, confectioneries, and pet foods. A list of products presently produced using the extrusion process is given in Table 1. The history of food extrusion has been reviewed in Ref. 2.

The two major functions of a food extruder are cooking and forming. In the cooking process, the product is heated through the transfer of heat energy, which is applied by steam injection or heaters and/or by the dissipation of viscous energy through shearing action between the rotating screw and the material. As a result of the cooking process, starches are gelatinized, proteins are denatured, undesirable enzymes are inactivated, and antinutritional substances (eg, trypsin inhibitors in soybeans) are destroyed. Because the temperature reached during the process can be quite high (ca 200°C) and the material takes a relatively short time to travel through the extruder (5 s to a few min), the cooking process is often referred to as high-temperature, short-time (HTST) extrusion. The forming extruder is used to produce special shapes from precooked material. Typically, the operating temperature of forming extruders are lower than that of cooking extruders.

Two major types of extruders are used in the food industry: single-screw and twin-screw extruders. The twin-screw extruders can be further classified according to the type of screws, ie, nonintermeshing or intermeshing and the type of rotation, ie, corotating or counter-rotating. Use of single-screw extruders began in the early years of the industry and they are still used today, though the twin-screw machines are becoming increasingly popular. Twin-screw extruders are comparatively more expensive than single-screw machines of the same production capacity. The flow of material in single-screw extruders depends on the friction between the barrel wall and the material. Hence the type of material that can be used is limited by its properties; proper conditioning of the material is necessary to reduce or eliminate process instability. The corotating, intermeshing twin-screw extruder uses the positive pumping characteristics created by the two intermeshing screws to convey the material forward. This type of mechanism reduces the need to precondition material. Other potential advantages of the corotating twin-screw extruder over the single-screw extruder include (1) narrow residence time distribution, leading to uniform processing, (2) better mixing capabilities, and (3) wide formulations of product, particularly low bulk density and high fat material that cannot be processed using a single-screw machine. The twin-screw extruder provides better control of the process and more uniform product characteristics than does the single-screw extruder. These advantages of the twin-screw machine compensates to some extent for the

Table 1. Extrusion Application in the Food and Feed Industry

Human food		Extrusion processes	Animal feed	
Starch-based	Protein-rich	Agrochemicals	Cattle and pig feed	Fish feeds
Pasta (2 types)	Animal protein	Fertilizers	Partial precooked cereals/seeds	Precooked flours (starch and protein) with different bulk densities
Tortilla flours	Sausages	Vegetables/soup	Enzyme-engineered feed	
Bakery products	Scrap meat/fish	Bagasse and other cellulosic material	Complete gelatinized cereals	
Confectioneries	Blood protein	Cocoa waste material	Yeast wastes	
Candies	Milk protein	Sucrose-based chemicals	Breaking up of cellulose	
Licorices	Germ extrusion	Spices	Protein hydrolysates	
Caramels	Co-extruded meat/starch	Maltodextrins	Pet foods	
Chocolate	Soft cheese	Pulp for paper production		
Starches	Vegetable protein	Proteins chemically modified		
Partly cooked	Soya/cottonseed/rapeseed	Cross-linked starches		
Gelatinized	Oilseed meals	Lactose-based chemicals		
Malt	Health foods			
Cereal snacks				
Health foods				
Breakfast cereals				
Flat breads/cookies				
Bran extrusion				
Crumb				
Instant-cooked foods				
Potato products				
Baby foods				

Source. Ref. 1. Courtesy of Elsevier.

high initial capital investment required. The advantages and disadvantages of different types of extruders have been reviewed in Refs. 2–4. The engineering principles of extruders have also been reviewed (5).

During the HTST process, material typically enters the extruder in a powdered form. The heat, shear, and pressure generated during the process melts the material into a dough-like mass. The material flows from the barrel to a die of a smaller cross section. The pressure at the barrel exit, in the extruder-die setup, can be as high as 3000 psi, depending on the product and process conditions. In the extruder, no water vapor formation occurs at the elevated temperatures attained inside because of high pressures. The material expands as it exits from the die to the atmosphere. This expansion is caused partly by the stresses that are free to relax and partly by the moisture in the material that is flashed-off as vapor, owing to the pressure drop between the die exit and the atmosphere. The expanded product imparts textural and functional characteristics that determine the acceptability of the product. This article reviews the existing literature regarding the textural and rheological properties during extrusion cooking and addresses the relationship between the two.

TEXTURE

The term texture describes a wide range of physical properties of the food product. A product of acceptable texture is usually synonymous with the quality of a product. Table 2 is a glossary of terminology used to describe textural attributes. Textured has been defined as "the attribute of a substance resulting from a combination of physical properties and perceived by senses of touch (including kinaesthesis and mouthfeel), sight and hearing (6)." Texture as defined by the International Organization for Standardization is "all of the rheological and structural (geometric and surface) attributes of a food product perceptible by means of mechanical, tactile and where appropriate, visual and auditory receptors (7)." The following terms have been used to describe product characteristics of extrudates: tender, chewy, soft, tough, brittle, crunchy, crisp, smooth, fine, coarse, puffed, flaky, fibrous, and spongy. One or more of these terms may describe the same behavior.

A variety of properties have been used to quantify or measure the texture and functional properties of extrudates. Some of these are:

1. Bulk density, or density, defined as the mass per unit volume of the extrudate.
2. Breaking strength, defined as the stress required to shear a material. This stress is the ratio of the breaking force to the area of the material. In certain instances it is referred to as the force required to break or shear the sample. The breaking strength is an indirect measure of the stress–strain relationship of the extrudate. It is an important parameter that determines the final quality of the extrudate and can be related to such textural attributes as toughness, chewiness, crunchiness, and brittleness.

Table 2. List of Terms and Groups

Structure	Texture	Consistency
Terms of relating to the behavior of the material under stress or strain		
Firm	Rubbery	Brittle
Hard	Elastic	Friable
Soft	Plastic	Crumbly
Tough	Sticky	Crunchy
Tender	Adhesive	Crisp
Chewy	Tacky	Thick
Short	Gooey	Thin
Springy	Glutinous	Viscous
	Glutenous	
Terms relating to the structure of the material		
Smooth	Gritty	
Fine	Coarse	
Powdery	Lumpy	
Chalky	Mealy	
Relating to shape and arrangement of structural elements		
Flaky	Cellular	Glassy
Fibrous	Aerated	Gelatinous
Stringy	Puffed (puffy)	Foamed (foamy)
Pulpy	Crystalline	Spongy
Terms relating to mouthfeel characteristics		
Mouthfeel	Watery	Creamy
Getaway	Juicy	Mushy
Body	Oil	Astringent
Dry	Greasy	Hot
Moist	Waxy	Cold
Wet	Slimy	Cooling

Source: Ref. 6.

3. Viscosity of extrudate paste is measured using amylographs.
4. The extrudate dimension, particularly for snack foods, is another important characteristic. It is often referred to as the expansion ratio or the puff ratio and is defined as the ratio of the area of cross section of the extrudate to the area of cross section of the die. In this article, the swelling of extrudate due to elastic and moisture effects is referred to as extrudate expansion. Extrudate swell is referred to as swelling from all effects other than moisture. Puffing is referred to as the swelling believed to be predominantly from the moisture effect.
5. Water absorption index (WAI) is the weight of sediment formed per unit mass of sample, after the sample is centrifuged and the supernatant is removed. Water absorption capacity (WAC) is the ratio of the weight gained over the original weight after a known mass of powdered extrudate is soaked in water for a fixed period of time at a given temperature. Water solubility index (WSI) is the percentage of the initial sample present in the supernatant obtained from the WAI test.

The methods used to obtain these properties are not standardized. Because different methods have been used to obtain a property by the same name, procedures used to determine the properties should be reviewed before drawing conclusions.

The textural properties of the extrudate are affected by the process variables, including temperature, screw speed, screw and barrel dimensions, and product variables (eg. moisture content, composition, and particle size). The properties are evaluated as a function of different parameters, the most common of which includes product moisture, process temperature, and extruder screw speed. Other parameters, some of which may be interrelated, include residence time, die dimensions, screw dimensions, shear rate or shear stress, and product composition. When a number of process conditions are to be evaluated at different levels, the experimental size could be very large. To reduce the experiments to a manageable scale without increasing the experimental error, the response surface methodology (RSM) has been used to develop relationship between dependent and independent variables. According to Meyers (8), "response surface procedures are a collection involving experimental strategy, mathematical methods, and statistical inference which, when combined, enable the experimenter to make an efficient empirical exploration of the system in which he is interested." The RSM helps in finding an approximate function to enable one to predict future response and to determine conditions of optimum response.

In the RSM approach, the entire system is modeled as a black box. The mathematical functions that are developed to correlate the dependent and independent variables are empirical in nature. An advantage of this approach is that a detailed understanding of the changes that occur during the process is not necessary. The disadvantage is that a general relationship is not available, which requires studies for different machines and different formulations of raw material.

Proteins

The basic constituents of all proteins are amino acids. The linear protein chain consists of amino acids linked together by peptide bonds between amino and carboxylic sites. Proteins can have different levels of structure—primary, secondary, and tertiary—that result in a three-dimensional shape. Texturization of proteins can be used to produce two types of products (9). The first are meat analogues that are sold in place of meat. Such products have found acceptance in some countries, such as Japan (10). The second type of textured plant proteins (TPP) is as a meat extender. Several reviews on protein texturization using extruders can be found (9,11–15). During the extrusion process in the presence of water, globular proteins and aluerone granules unravel and align themselves in the presence of the shear field that exists in the extruder (14). Thermal denaturation (loss of native structure) is also expected to occur and is thought to be necessary for texturization (9), although some investigators contend that denaturation is not a necessary step in the texturization process (16).

The mechanism of texturization has been the subject of intense research. It is generally agreed that intermolecular disulfide bonding of proteins is responsible for the structure formation of spun soy fiber (17). It has been reported that the presence of heat and pressure cause the protein molecules in defatted soy meal to disassociate into subunits and become insoluble (18). The mechanism of bond formation has been investigated during extrusion (19). Results indicated that disulfide bonding had no role in extrusion texturization, suggesting that intermolecular amide bonds are instead responsible for texture formation during extrusion processing. The importance of amide bonds in protein texturization was also reported (20).

However, other researchers disagree with these findings. One study reported the formation of intermolecular disulfide bonding when defatted soy meal was extruded at temperatures between 110–150°C and found no evidence of intermolecular peptide bond formation (21). Another study used a single-screw extruder without a die and reported the formation of intermolecular disulfide bond for blends of defatted corn gluten and defatted soy flour extruded at 145°C (22). Others have also refuted the idea that intermolecular peptide bonds are responsible for texturization of extruded soy protein (23). The reason for this disagreement is not clear. The temperatures used by the first investigators (20) were higher than 175°C whereas the latter investigators (21,23) used lower temperatures. It was reported that thermal polymerization of peptide bond formation requires a minimum temperature of 180°C (24). This could be one possible reason for the conflicting results obtained by these researchers.

Most of the work evaluating product quality has used soy proteins. One study extruded defatted soy meal of moisture content 30% (dry basis) at a constant screw speed of 100 rpm with temperatures ranging from 107 to 200°C (25). The product density decreased with temperature (Fig. 1). Shear force and work increased with increasing temperature, but breaking strength increased as temperature increased to 160°C and then decreased with further increase in temperature. Another study used a three-

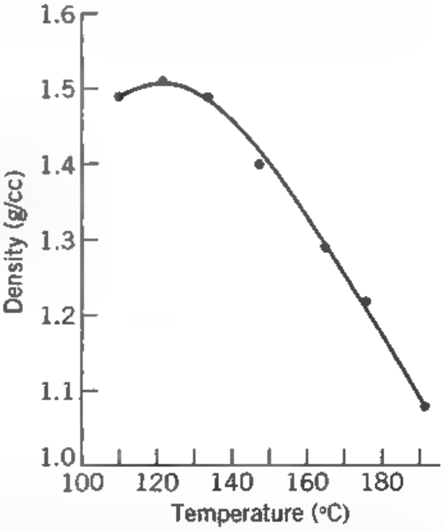


Figure 1. Effect of process temperature on product density. *Source:* Adapted with permission from Ref. 25.

variable, three-level fractional factorial design to measure the effect of extrusion of defatted soy flakes on residual trypsin inhibition activity (TIA), Warner Bratzler shear (WBS), and water absorption capacity (WAC) (26). Lower product moisture decreased residual TIA. Higher temperatures and lower moistures increased WAC, but increased feed moisture content decreased WBS.

A third study used both sensory and mechanical means to evaluate the texture of an extruded soybean meal product (27). A five step temperature range of 135–180°C was used in this experiment. A high correlation between the instrumental and sensory parameter was obtained. With increases in temperature, the product became less compact and spongy in appearance and had increased aligned fibers as reflected by cohesiveness. In a subsequent work, these investigators used a four-factor (temperature, screw speed, product moisture, and protein content) three-level RSM to evaluate the textural properties of extruded defatted soybean meal (28). Results from this study indicated that protein level and extrusion temperature were the most important factors affecting WBS values.

The effect of pH (5.5–10.5) of defatted soybean meal and soybean isolate on WBS values (20) indicated that at the extremes of the pH values, the extrudate appeared to have suffered a loss of structural integrity. The maximum shear force and sensory values were obtained at about a pH of 8, as indicated in Figure 2a and b. At pH < 5.5, the extrusion of the product becomes quite difficult, as pH is increased from 5.5 to 8.5 the product becomes tender, less chewy, and rehydrates rapidly (29). These effects have been attributed to the lower solubility of the proteins at acidic pH, which produced a denser product due to the formation of aggregates in the extruder (14).

Independent variables such as moisture content, temperature, and screw speed affect shear strain, stresses, and shear rate. The effect of shear environment on the textural properties of unroasted defatted soy flour was reported (30). Increasing strain enhanced cross-linking in the dough, as indicated by a higher work required to shear the sample. Higher product moisture reduced the density. A high shear rate (or shear stress) in the die achieved by increasing flow rate led to a denser and less absorbant product, caused by the disruption of cross-linking. When low levels of hydrocolloid (sodium alginate or methylcellulose) were added to defatted soy flour, sodium alginate increased chewiness, maximum force, WAC, and bulk density, whereas the addition of the same amount of methylcellulose decreased maximum force and chewiness (31).

A seven-factor, five-level experiment using RSM to optimize the process variables of temperature, screw speed, screw compression ratio, die diameter, and product moisture content during extrusion cooking of defatted soy grits studied the individual and interactive effects of the independent variables (32). Results indicated that high screw compression ratio and maximum temperature decrease between the barrel and the die favored good texture. In an investigation of the effects of product moisture, barrel temperature, and die temperature on the properties of extruded soy isolate and soy flour, it was found that soy isolate required higher pressure to extrude and had higher expansion and a narrower range of textures than did soy flour (33). The peak force measured using a Ottawa texture machine was found to correlate well with protein solubility, as illustrated in Figure 3. The higher barrel and low die temperature resulted in a better texture for both materials.

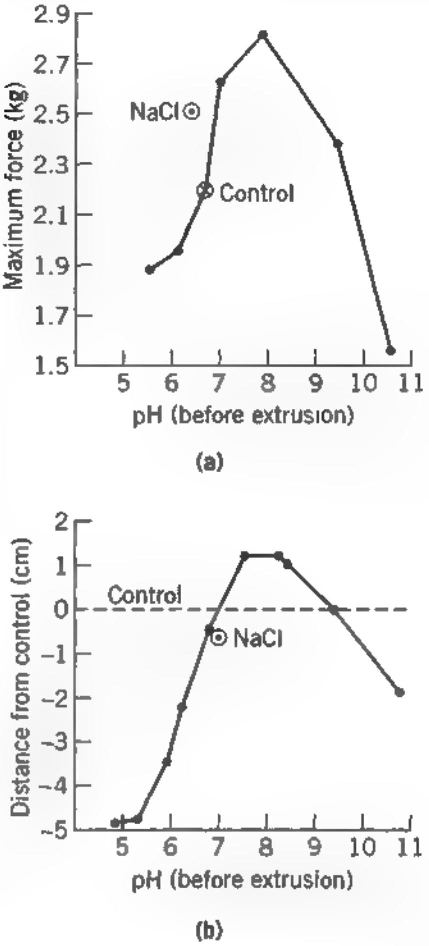


Figure 2. (a) Influence of pH adjustment on Warner-Bratzler shear values of extruded soy protein; (b) influence of pH adjustment on sensory response of extruded soy protein. *Source:* Adapted with permission from Ref. 20.

Although most research has been on soy proteins because of the potential to simulate meat products, some research has evaluated the properties of other high-protein materials after extrusion. These included studies of the effect of extrusion on glandless cottonseed flour and soy meal mixtures (34,35) and on cowpea meal (36) and the effect of process temperature, screw speed, moisture content, and pH on the WAC and nitrogen solubility index of cowpea, mung bean, defatted soybean, and air-classified mung bean (37). Protein type and amount were found to affect properties. Results from extrusion of blends of corn and soy proteins have been reported (22,38–40). Increasing the amount of soy protein in the corn–soy mixture led to products with better textural and functional properties.

Starch

Much literature is available on extrusion cooking of starch-based materials. Reference 41 is an excellent review of

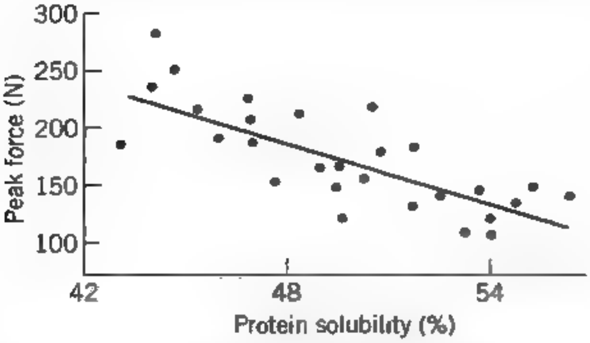


Figure 3. Correlation between OTMS texture and protein solubility for soya isolate. *Source:* Adapted with permission from Ref. 33.

starch extrusion. Starch consists of α-D-glucose units that are linked to form large macromolecules. Native starch is a mixture of two different glucose polymers—the linear amylose and the branched amylopectin. Under the severe shear environment inside the extruder, the starch molecule loses its granular and crystalline structure (42) and undergoes macromolecular degradation (43–48). The insoluble native starch is partially solubilized after extrusion at room temperature. The magnitude of the transformation is a function of multiple processing parameters and their interactions as well as the type of starches.

The extrudate characteristics of several varieties indicated that wheat starch had the highest expansion ratio at 135 and 225°C (Fig. 4) (49). Increasing product moisture content decreased the expansion ratio (49–54). Increasing temperature increased the expansion ratio up to a point after which it decreased with further increase in temperature (49,55). This decrease can be attributed to the degradation of starch molecules at higher temperatures. Expansion is affected by the dimensions of the die or nozzle (56,57) and product composition. Increasing the length-to-diameter ratio of the nozzle increased the expansion ratio. Increasing the amylose content decreased the expansion ratio (49,53,58). Presence of lipids, proteins, salts, and sugar can also affect the puff ratio (50,54,59–61). Decreasing the lipid content increased the expansion ratio of the product whereas increasing the salt and sugar concentration increased the expansion ratio.

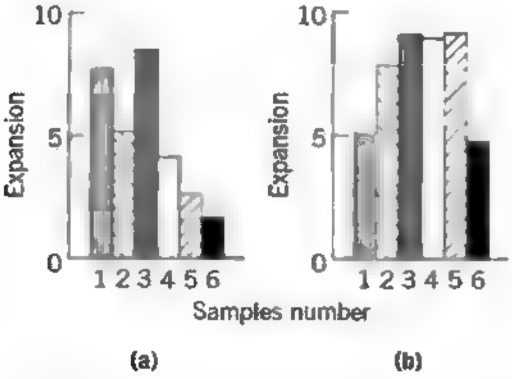


Figure 4. Expansion of products extruded at (a) 135°C and (b) 225°C. Starches are (1) waxy corn; (2) corn; (3) common wheat; (4) rice; (5) amylon 5 (6) amylon 7. Initial moisture content was 22% by weight. *Source:* Adapted with permission from Ref. 49.

Increasing moisture content increased the breaking strength of extrusion-cooked cornmeal (53). Increasing temperature was found to decrease the breaking strength for nonwaxy corn, but the reverse was true for waxy corn. Increasing the concentration of salt and sugar in the extrudate reduced the breaking strength (54). Results of two studies indicated an inverse relationship between expansion ratio and shear strength (54,55).

Native starches are insoluble in water at room temperature. However, on extrusion these materials are soluble in water and could be added as ingredients in other food mixes to provide an acceptable consistency. The solubility of starches increase with increasing severity of treatment. At lower moisture content, the solubility index is higher (49,62–64). Higher temperature also increases solubility.

A small portion of a considerable body of literature that addresses the effect of process and product variables on properties of extrudates has been cited. Some researchers have used the RSM to locate optimum conditions for the best product texture. Empirical predictive models have been developed to relate product quality to independent parameters. These models or equations suffer from two major drawbacks: (1) they are machine-dependent in that the data obtained from one extruder cannot be extrapolated to other machines, and (2) they do not explain the physical phenomena. Understanding of how the basic physical properties control texture formation is limited.

RHEOLOGY

Although, rheology is defined as the science of deformation and flow of matter, it typically is used to describe the flow of non-Newtonian fluids (65). The flow properties of the fluid can affect important extrusion parameters such as velocity and pressure profiles in the extruder, heat transfer between the walls and the fluid, pressure drop in the die, and energy requirements. These parameters determine the product quality, extruder and die design, and production rate.

During the extrusion process the flow patterns are complex (Fig. 5) and involve six major flow regions (66). These regions are:

1. Metering section, where the material is fully plasticized.
2. The entrance region to the die, where a converging flow field is developed generating large stresses.
3. The region within the die, where the disturbance caused by the entry flow gradually disappears.
4. The steady flow region in the die, where a fully developed flow exists.
5. The exit region, where the vapor bubble nucleation and growth and velocity profile rearrangement occur within the die followed by the expansion of the extrudate outside the die.

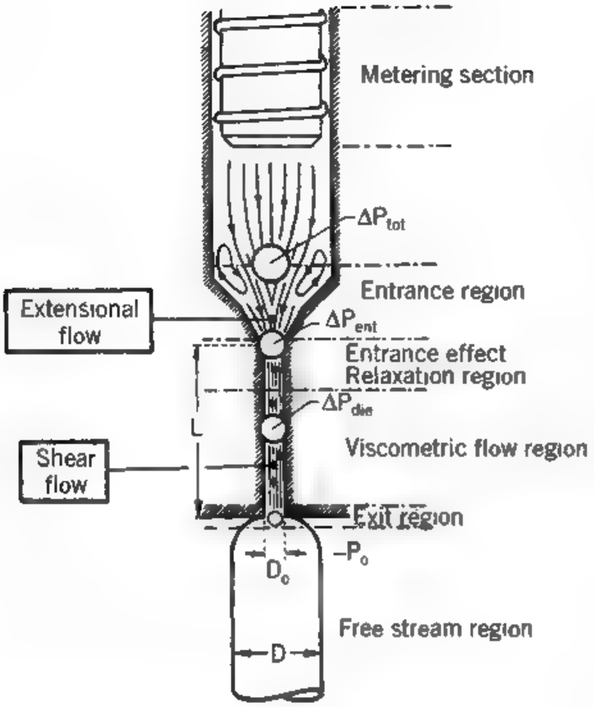


Figure 5. Flow pattern in an extruder barrel and die system. *Source:* Adapted with permission from Ref. 66.

6. The free stream region, where the extrudate expansion reaches an equilibrium value.

These flow situations of viscoelastic fluids necessitates the measurement of rheological parameters such as steady shear viscosity, primary and secondary normal stress differences, and elongational viscosity.

Two types of flows occur during extrusion cooking. The first is the shear flow that occurs owing to the presence of the walls, as in the screw channel and the die. The second is the extensional flow that is present farther away from the wall where the stream lines converge or diverge, such as at the entrance and exit to the die. The stress (σ) components in shear flow, are given by:

$$\sigma_{12} = \eta_s\dot{\gamma},\ \sigma_{23} = \sigma_{13} = 0,\ \sigma_{11} - \sigma_{22} = \mathrm{N}_1,\quad \sigma_{22} - \sigma_{33} = \mathrm{N}_2 \qquad (1)$$

where 1 is the direction in which the fluid flows, 2 is the direction of the velocity gradient, and 3 is the remaining neutral direction. The shear viscosity (η_s) and the normal stress differences (N_1, N_2) are functions of shear rate. For Newtonian fluids the shear viscosity is a constant and under simple shear flow N_1 and $\mathrm{N}_2 = 0$.

For extensional flows, extensional viscosity is defined by:

$$\sigma_{11} - \sigma_{22} = \eta_e\epsilon \qquad (2)$$

Extensional viscosity is constant for a newtonian fluid, but it may be a function of extensional rate for non-Newtonian fluids.

The three types of extensional flows are (1) uniaxial, (2) biaxial, and (3) planar. A cylindrical sample when stretched along its axis results in uniaxial extension. Compression of a cylindrical sample along its axis results in biaxial extension. Stretching a rectangular sheet of a sample along one direction, while keeping one of the remaining dimensions constant, results in planar extension (there are two viscosities in planar extension; see Ref. 67 for details). The ratio of the elongational viscosity (η_e) to the shear viscosity (η_s) is defined as the Trouton ratio. For Newtonian fluid, this ratio is a constant and equal to 3 for uniaxial extension, 4 for planar extension, and 6 for biaxial extension. For non-Newtonian fluids, this ratio is generally much higher and may vary with extensional rate.

The measurement of these rheological parameters during extrusion processing poses special problems. The rotational instruments used in the measurements of rheological parameters of liquids foods cannot be used because the shear rates obtained in these rheometers are several orders of magnitude below those encountered during extrusion and it is difficult to reproduce extrusion-like conditions in the rheometer. Thus on-line measurements of rheological parameters are necessary to avoid these problems.

Shear Viscosity

The rheological property that has received the most attention by scientists is shear viscosity. Some of the published viscosity studies are summarized in Table 3. A die or a viscometer (capillary or slit cross section with multiple pressure transducers located along the wall) attached to the extruder is often used to measure the viscosity. Alternatively, a single pressure transducer mounted near the exit of the barrel, along with capillary dies of different lengths but having the same radius and entry geometry, can be used to obtain the shear viscosity (77).

The first investigators to evaluate the viscosity of doughs during extrusion found that doughs exhibited shear thinning behavior (68). The viscosity decreased exponentially with temperature and was found to follow Arrhenius kinetics. Moisture was found to act as a plasticizer. Increased moisture content (M) decreased the dough viscosity. An empirical model of the form below was proposed:

$$\eta_s(\dot{\gamma}, T, M) = \eta^*\dot{\gamma}^{n-1}\exp(\Delta E/RT)\exp(KM) \qquad (3)$$

This model is one of the simplest and the most popular. The following model was proposed for constant moisture dough (69):

$$\eta_S(T, \dot{\gamma}, t) = \eta^*\dot{\gamma}^{n-1}\exp(\Delta E/RT)\exp\int_0^t k\exp(\Delta E_k/RT(t))dt \qquad (4)$$

In equation 4 it is assumed that the temperature is a function of time is known. Reactions such as gelatinization and denaturation can lead to network formation and affect viscosity (parameters k and ΔE_k control the reactions). A log polynomial model was used to express viscosity as a function of moisture content, shear rate, and temperature (71).

Table 3. Summary of Viscosity Studies Conducted During Extrusionlike Processes

Product	Method	Moisture (% w.b.)	Temperature (°C)	Reference
Cooked cereal dough	CDV	25–30	67–100	68
Defatted soy flour	CR	32	35–60	69
Pregelatinized corn flour dough	CD	22–35	90–150	70
Soy fluff	CD	25.5–35.5	100–160	71
Soy flour	CR	25–60	25–120	72
Corn grits	SDV	20–32	100–140	73
Potato grits	SDV	18–42	80–140	73
Corn starch	CDV	20–40	100–170	74
Corn grits	CDV	15–30	130–180	75
Corn grits	SDV	25–45	150–180	76

Note. CD, capillary die attached to the extruder; CR, capillary rheometer; CDV, capillary die viscometer is capillary die with multiple transducers along its length; SDV, slit die viscometer is slit die with multiple transducers along its length.

To account for the shear history in the extruder, an equation was suggested of the form (73).

$$\eta_S(\dot{\gamma}, T, M, N) = \eta^* \dot{\gamma}^{n-1} \exp(\Delta E/RT) \exp(KM) N^{-\alpha} \quad (5)$$

The flow curves obtained using a capillary rheometer (9) and slit-die viscometer were significantly different for food doughs (corn grits and potato flour), whereas for low-density polyethylene the flow curves were the same whether obtained using a capillary rheometer or slit-die viscometer. The flow behavior index was found to be affected by screw speed and temperature. This would indicate that shear history is varying, due to different screw speeds. Hence, the Bagley procedure for obtaining the viscosity during extrusion cooking is invalid because the fluid entering the die is rheologically not the same for each condition. A model has been developed that accounts for the thermal and mechanical energy imparted to the product (shear history) before the viscosity is measured (78).

In addition to these viscosity models, two recent models are worth mentioning. They present almost identical expression for starch and protein doughs based on reaction kinetics (79,80). The models are cumbersome because of the large number of constants (10 or more). One notable feature of these models is the inclusion of yield stress. Another study indicated that soy doughs exhibited yield stress (72). The magnitude of yield stress was found to be a function of temperature.

There are several drawbacks to the existing viscosity models. The model of Harper and co-workers (68) assumes that the decrease in viscosity is due to shear thinning only. Most plastic polymers are modeled by a network of macromolecular entanglements. Shear thinning flow is associated with the decrease of the entanglement density under the influence of deformation of the polymer (81). For food doughs the decrease in viscosity is due to the sum of shear thinning process history (screw configuration, residence time), and molecular degradation. A complete model that quantifies the effect of parameters other than product moisture and temperature on shear viscosity is still lacking. These effects are discussed in Ref. 82, though no attempt has been made to quantify them. Another drawback of these models are that the fluid is assumed to be inelastic, ie, normal stress effects are neglected. Because doughs are viscoelastic fluids, inelastic models are inadequate. Evidence has been presented that the pressure drop experienced at the entrance of the die is much greater than that predicted by shear viscosity alone, indicating that elasticity is important (79). As will be discussed later, elasticity is important in the manufacture of products at temperatures below 100°C.

Extruded starches are used as thickening agents or as ingredients in instant foods. The viscosity of powdered extrudate in solvent (water) has also received attention. Typically, shear thinning behavior is observed in all cases. Increasing the concentration of wheat starch from 5 to 9% in solution resulted in the shifting of flow behavior from almost Newtonian to shear thinning (83). For plastic polymers it is known that when the molecular weight is greater than a certain critical molecular weight, non-Newtonian flow behavior is observed (81). Extruded cornstarch in solution exhibited a constant viscosity at high and low shear rates (Fig. 6) (84). Increasing barrel temperature decreased the values of the constant viscosities, whereas increasing moisture content was found to increase the values of constant viscosity, ie, depended on the severity of the extrusion environment.

The effect of emulsifiers on dough viscosity has also been studied (70). The presence of sodium stearoyl-2-lactylate (SSL) was found to affect the viscosity, whereas diacetyltartaric acid ester of monoglyceride did not affect the viscosity. Fat did not affect the viscosity when SSL was present, but it was found to increase the viscosity in the absence of additives.

Normal Stress Difference

It is seen from equation 1 that when N_1 and N_2 are not equal to zero the normal stresses are unequal. The presence of unequal normal stresses can create some interesting phenomena (85). The reason for nonzero values of N_1 and N_2 can be attributed to the anisotropy of fluid microstructure in the flow field and is observed in elastic fluids (86). The ratio of primary normal stress difference to the wall shear stress is an indirect measure of the elasticity of the fluid, ie, the higher the ratio, the more elastic is the fluid.

Measurement of normal stress differences on-line has been a subject of intense research among polymer engi-

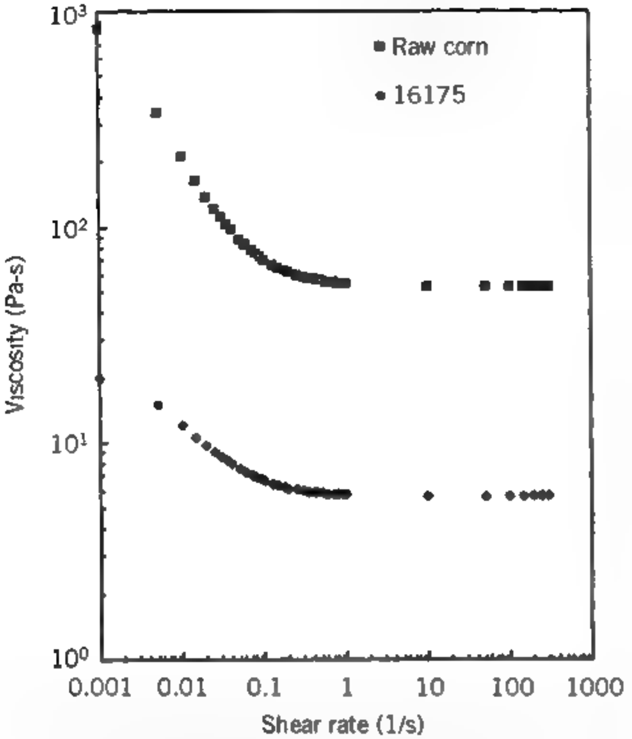


Figure 6. Flow behavior of extruded (16% moisture, 175°C and 150 rpm) and unextruded corn starch at 6% concentration and 60°C (84).

neers and rheologists. The two methods used to measure primary normal stress difference are the exit pressure method and the hole-pressure method. Exit pressure is the residual stress at the die exit. For inelastic fluid this pressure is zero, but positive for a viscoelastic fluid. Hole pressure is defined as the difference between the pressure measured by a flush-mounted transducer and a transducer located at the bottom of a slot directly opposite to it (Fig. 7). For a Newtonian fluid, under creeping flow conditions (Reynolds number ~ 0), the hole pressure is zero.

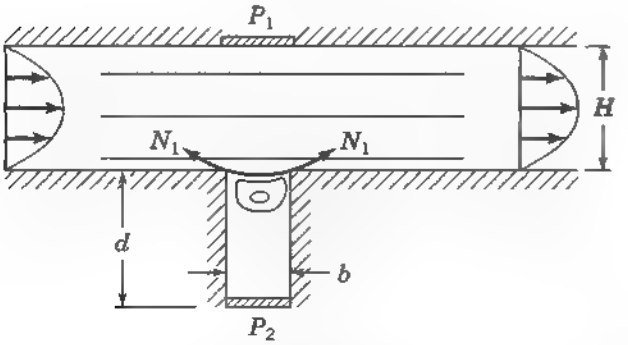


Figure 7. Schematic of the hole-pressure geometry for a transverse slot. The curvature of the streamlines near the mouth of the slot is shown. The fluid elasticity (N_1) results in a tension along the curved streamlines that tends to lift out of the slot.

The exit pressure technique has been used to calculate the normal stress differences (87,88). As it is difficult to measure the pressure at the die exit directly, exit pressure is usually obtained by linear extrapolation of the pressure profile from the region of fully developed flow in the die. The expressions for the normal stress differences for a slit die are (89):

$$N_1 = P_{ex}\left[1 + \frac{d \ln P_{ex}}{d \ln \tau_w}\right] \tag{6}$$

However, this method is controversial. Critical analyses of the method have been done (88–90). The origin of the controversy lies in the fact that for polymers, positive (87,88,91), zero (92), and negative (90,93–97) exit pressures have been obtained. There has been very few published studies using this method on foods. Both positive and negative exit pressures were reported for corn grits, but positive exit pressures only for potato grits (98). Another study examining this method for corn grits showed that products having lower moisture (25–35% db) content gave mostly negative exit pressures, whereas higher moisture values (40 and 45% db) gave positive exit pressures (99). Positive exit pressure was obtained using a capillary die (100). As it is impossible to flush-mount transducers on the walls of the capillary, the presence of hole pressure could lead to erroneous exit pressure values, so the use of a capillary die for this procedure is not recommended.

One assumption in obtaining the expression for the normal stress differences from the exit pressure theory (eq. 6) is that the flow remain fully developed till the die exit. There is evidence that for polymers, the flow does not remain fully developed till the exit (101,102), owing to the presence of exit disturbance. The exit disturbance will lead to a rearrangement of the velocity profile. The velocity rearrangement would lead to an overestimation of the exit pressure (89). Beyond a critical shear stress, the exit disturbance is negligible (88). The value of this stress would depend on the material. For low-density polyethylene this value is 25 kPa. However, there are data in the literature where negative exit pressures were obtained for experiments conducted above the recommended 25 kPa value for wall shear stress. A problem during the extrusion cooking of foods is that at temperatures greater than 100°C the presence of moisture flash at the die exit could cause an additional exit disturbance. Other problems with the exit-pressure method are viscous dissipation in the die and pressure dependence of viscosity of the material. Both these effects could lead to a concave pressure profile and would result in erroneous estimates of exit pressure.

The hole pressure was originally observed as an error in the measurement of normal stress difference using standard geometry rheometers (103). Studies subsequently established the presence of significant hole-pressure errors (104). The interest in the hole pressure arises from the claim that this property can be used for reliable prediction of the elastic properties of viscoelastic fluids. As the fluid passes over a hole or a slot, the stream line for a viscoelastic fluid tends to dip and this leads to the development of normal stresses that tend to lift the fluid (Fig. 8), thus creating a lower pressure at the bottom of the slot or hole

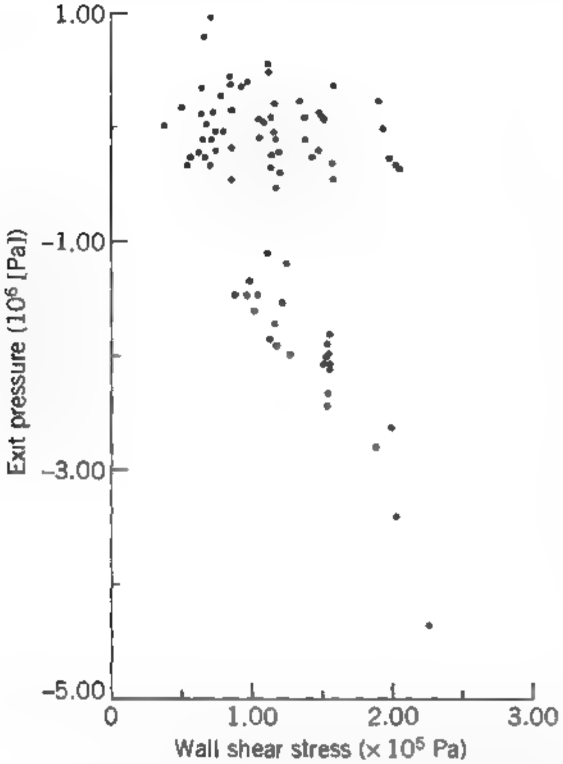


Figure 8. Exit pressure values for corn grits extruded under different conditions. *Source:* Ref. 76.

(105). This method has been used successfully to measure the normal stress difference for polymer solutions and melts (90,96,106–111). Typically, a slit die with holes or slots of different geometries is used to estimate the normal stress differences. The common hole geometry is a rectangular slot with its length transverse to the direction of the flow. This geometry is known as the transverse slot.

The original equations relating hole pressure and the normal stress difference (112) were later modified by Baird (109) to give the HPB equations. The HPB equations were reinterpreted to give the HPBL equations (90). Depending on the geometry of the hole, the HPBL equations are given by:

$$N_1 = 2P_{\mathrm{HE}} \frac{d \ln (P_{\mathrm{HE}})}{d \ln (\tau_w)} \quad \text{(Transverse slot)} \qquad (7a)$$

$$N_2 = -P_{\mathrm{HE}} \frac{d \ln (P_{\mathrm{HE}})}{d \ln (\tau_w)} \quad \text{(Parallel slot)} \qquad (7b)$$

$$N_1 - N_2 = 3P_{\mathrm{HE}} \frac{d \ln (P_{\mathrm{HE}})}{d \ln (\tau_w)} \quad \text{(Circular hole)} \qquad (7c)$$

where P_{HE} signifies the elastic contribution to the hole pressure. Despite violations of some assumptions, reasonably good comparisons of N_1 obtained from standard geometries and slit die were obtained (90,96,111). However, recent numerical studies have shown the HPBL equations to be valid (113,114).

This method is presently under evaluation in the authors' laboratory and has yielded promising results. A potential problem with this method is that for a fluid with yield stress, an error in pressure transmission could occur. For a fluid that has yield stress, higher hole pressure values will be obtained (95). Thus the effect of yield stress on hole-pressure values will have to be accounted for.

Extensional Viscosity

When a fluid flows from a larger to a smaller diameter tube (eg, from the barrel of the extruder to the die), a pressure drop is encountered. For a polymer melt or solution the magnitude of this pressure drop is significantly higher than that obtained for a Newtonian fluid of the same viscosity (87,115). Similar results were reported for corn grits during extrusion cooking (75). The excess pressure drop at the entrance was originally believed to be due to the elastic properties of the fluid. Recent studies for plastic polymers indicate that the flow in the die entry region cannot be explained by melt elasticity alone (116) and that the extensional viscosity is an important parameter that must be considered.

Cogswell (117) was the first to propose a method for obtaining extensional viscosity from the entrance pressure drop method. The entrance pressure drop is calculated by linear extrapolation of the pressure readings along the walls from the fully developed flow region of the capillary or slit to the die entrance. This reading is subtracted from that of the transducer located at the barrel exit or a reservoir. The basis for Cogswell's analysis is that the large entrance pressure drop in the converging flow region is due to the extensional nature of the flow. In addition, the presence of the wall introduces a shearing component. Thus, the entrance pressure drop is assumed to consist of two components: due to shear flow and due to extensional flow. The expression for the shear component of the pressure drop due is given by:

$$\Delta P_{\mathrm{Shear}} = \frac{2B}{3n \tan \alpha} \left(\frac{1 + 3n}{4n} \right)^n \dot{\gamma}^n \left(1 - \left(\frac{r_1}{r_0} \right)^{3n} \right) \qquad (8)$$

and the extensional component for the pressure drop is:

$$\Delta P_{\mathrm{Ext}} = \frac{2A}{3m} \left(\frac{\tan \alpha}{2} \right)^m \dot{\gamma}^m \left(1 - \left(\frac{r_1}{r_0} \right)^{3m} \right) \qquad (9)$$

These equations have been derived assuming a power law behavior in both shear and extensional flow. Expressions for sudden contraction in cylindrical and rectangular flow can also be found in Cogswell's paper. Several other expressions have been suggested using differing assumptions (118,119). There is no significant difference from the values of strain rate obtained from these expression for low die angles (<30°). A spherical coordinates system was assumed to arrive at a slightly modified expressions for equations 8 and 9 (120). An alternative analysis for determining the extensional viscosity in planar and uniaxial extension has been proposed from entrance pressure-drop data (121–123).

Studies on extensional viscosity of food doughs are scarce. A stretch-thinning behavior for corn grits using a capillary rheometer has been reported (124). Similar behavior for corn grits has been noted during extrusion cooking (76). Using Gibson's analysis, these authors found that the extensional viscosity decreased with increasing moisture content. The shear component of the entrance pressure was less than 5%. The Trouton ratio for the doughs ranged from 80–100. Not all materials exhibit shear thinning and stretch-thinning behavior. Several polymeric liquids exhibit stretch-thickening behavior while displaying shear thinning behavior in shear flows.

The entrance pressure-drop method for obtaining extensional viscosity has not been universally accepted. The shear viscosity parameters (B and n in eq. 8) are assumed to be known a priori. These parameters can be calculated from the pressure profile and flow-rate data in the die. At large die entrance angles, poor data fit is observed. The assumption used in the calculation of the shear component of the entrance pressure drop can lead to error. However, the magnitude of this error is expected to be small because extensional flow dominates. Also, only average quantities are computed, whereas in reality the stress and the rate of strain will change with position. It should be noted that this method gives only qualitative comparisons and not definitive data. Hence it is important that a comparison be made between the magnitude of the extensional viscosity obtained from entrance pressure calculations with those obtained by other methods. Such a comparison has been made for polymers, and the values obtained were reported to be of the same order of magnitude (125,126).

Dynamic Oscillatory Flow

The dynamic properties of rice dough have been studied using an on-line rheometer (127). Storage and loss moduli increased with increasing frequency and decreasing moisture content. However, these authors reported significant problems with the equipment and recommended several modifications.

TEXTURE AND RHEOLOGY

The relationship between product structure and its texture is well recognized (9). The structure achieved is dependent on the product composition, processing condition, and molecular configuration. Rheological properties are sensitive to changes in product composition, processing condition, and molecular configuration. Thus, rheological properties present themselves as a measure of product structure and hence its texture.

One of the most important characteristics of an extrudate is the expansion ratio. To some extent, extrudate expansion is indirectly related to other textural properties such as breaking strength, bulk density, and WAI. The expansion phenomena can be characterized in part from rheological parameters. Two recent articles discuss the expansion of extrudate from a rheological point of view (100,128).

During HTST (>100°C) extrusion, the moisture that is unable to escape due to the high pressures in the die is free to vaporize at or near the die exit and is assumed to be responsible for the expansion of the extrudate. Expansion ratios as high as 15 have been obtained. Experiments where the extrudates were cooled to below 100°C (as in the forming or half-product process) before exiting the die were reported (100). Extrudate swells (diameter of extrudate to that of the die) of 1.5, 2.27, and 3.00 were obtained for wheat flour, wheat starch, and manioc starch, respectively. When temperatures are below 100°C, no water vapor is released. In such instances the expansion is due to the elasticity of the material. A Newtonian fluid flowing out of a tube would expand at low Reynolds numbers (<16) but shrink at higher Reynolds numbers (85).

An approach commonly used to explain the extrudate swell is the elastic recoil mechanism. When an elastic fluid is flowing through the tube, it is subjected to extra tension along the stream lines. Once the fluid exits, the walls are no longer present to constrain the flow, which causes the fluid to contract along the stream lines, resulting in radial expansion. The magnitude of this tension is represented by the primary normal stress difference. Expressions for obtaining the extrudate swell have been derived (129). Other factors affecting extrudate swell include surface tension, gravity, inertia, wall slip, temperature effects, flow behavior index, and extensional viscosity. In general, the effect of inertia, surface tension, gravity, and wall slip reduce the extrudate swelling, whereas temperature effects, flow behavior index, elastic recovery, and extensional viscosity increase extrudate swell.

The rheological phenomena involved during extrusion cooking of foods have received some attention. The first attempt to incorporate the effect of elasticity on extrudate expansion qualitatively used pore volume as an indication of elasticity, ie, if the pore volume was small, elastic effects was responsible for the expansion (58). Product morphology was used to estimate dough elasticity (130). These studies, along with that of Alvarez-Martinez and coworkers (131), have speculated that dough elasticity is an important parameter that contributes to the magnitude of the radial expansion, although no quantification has been attempted.

At temperatures above 100°C the extrudate expansion contains contributions from both the elasticity and the moisture flash. A two-stage expansion occurs under these conditions. This phenomenon has been observed and photographed (132). The first stage happens immediately after the extrudate emerges from the die and is due to the elastic effect. A few seconds after the first effect and further downstream, bubble formation due to moisture vapor trying to escape can be observed. For high forces due to moisture effects, the moisture vapor quickly finds its way out. At these conditions the swelling occurs almost instantaneously. Depending on the process conditions, elastic or moisture effects may dominate.

Several investigators (58,131,133) have speculated that radial expansion alone is an insufficient parameter to quantify extrudate expansion. It was proposed that expansion occurs both radially and longitudinally and that a negative correlation exists between the two (58). Longitudinal expansion was defined as the mean length of unit mass of extrudate, or as the ratio of extrudate velocity to that of the velocity of fluid inside the die (58,131). Both these researchers report that longitudinal expansion is inversely proportional to the shear viscosity. However, these authors do not give a reason why shear viscosity should be important in longitudinal expansion.

Consider the flow inside the die, where the melt adheres to the die but flows at a maximum velocity at the center (parabolic profile for a Newtonian fluid). Downstream of the die exit the velocity profile approaches that of a plug flow, with all points having a uniform velocity. This means that the surface layer must be accelerated from rest, and it has been shown that conservation of mass and momentum demands that this acceleration cause an extensional flow in the surface layer while the center is decelerated and compressed (134). This could be a possible reason for the longitudinal expansion. Owing to the extensional nature of the flow, this effects to account for extrudate swelling are necessary (135). Another reason for longitudinal expansion may be the moisture vapor formed in the extrudate. As the vapor tries to escape from the extrudate, it stretches the dough. The magnitude of the expansion due to moisture loss would depend on the extensional viscosity (biaxial extension) of the fluid. This phenomenon is similar to the rise of bread during baking. The vapor pressure of the water will stretch the filament of dough that is cooling in the ambient temperature. If the vapor pressure is sufficient to rupture the extrudate, open structures on the extrudate surface are produced. On cooling, the material dries and solidifies to its final shape.

One must caution against the use of shear viscosity to explain the expansion phenomenon. It has been speculated that lower shear viscosity is favorable for greater expansion (41,136). Data contrary to the findings of these researchers have been presented (132). An increase in dough moisture causes a decrease in shear viscosity, but also causes a reduction in extrudate expansion. Shear viscosity is the least sensitive to molecular weight changes. From a rheological point of view, primary normal stress difference and elongational viscosity are probably far more important. These parameters may be able to better explain some of the conflicting results that exist in the literature and provide an insight as to why lipids, salts, and sugars behave the way they do. Moreover, both primary normal stress difference and extensional viscosity are significantly more sensitive to average molecular weight and molecular weight distribution (and hence to the structure of the material) than shear viscosity and could serve as an effective tool in process control.

CONCLUSION

Food texture as practiced in the industry has remained more of an art than a science. The ingredients of dough components, ie, proteins, starches, and lipids interact in a manner that is not fully understood. Evaluating the rheological parameters of the dough during extrusion may help in providing an understanding of the effect of product and process conditions on the texture of the extrudates. Techniques such as the hole pressure and entrance pressure drop for the measurement of elastic and extensional material functions could help provide a better understanding of the relationship between rheology and texture. These parameters could also be useful in developing a complete rheological equation of state (one that will predict both the viscous and elastic properties). Such equations of state would be useful in numerical modeling of the food extrusion process and help in developing a better understanding of the flow patterns in the extruder and aid in process control and product development.

NOMENCLATURE

A, B	Constants in equations 8 and 9
b	Slot width
d	Slot height
ΔE	Activation energy of flow
ΔE_k	Activation energy for cooking reaction
H	Slit height
k	Specific reaction velocity constant
K	Coefficient for moisture in equations 3 and 5
M	Moisture content
m	Power law index in extensional flow
N_1	Primary normal stress difference
N_2	Secondary normal stress difference
n	Power law index in shear flow
P_{ex}	Pressure drop in the exit
ΔP_{Shear}	Pressure drop due to viscous effects
ΔP_{Ext}	Pressure drop due to elongational flow
ΔP_{tot}	Total pressure drop
ΔP_{ent}	Entrance pressure drop
ΔP_{die}	Pressure drop across the die
P_{HE}	Elastic hole pressure
Q	Volume flow rate
r_0	Radius of the barrel
r_1	Radius at the die
T	Temperature
t	Time
α	Half-cone angle of the die
$\dot{\epsilon}$	Elongational strain rate
$\dot{\gamma}$	Shear rate
η_s	Shear viscosity
η_e	Elongational viscosity
η^*	Reference viscosity
σ_{ij}	Total stress
τ_{ij}	Extra stress components

BIBLIOGRAPHY

1. D. J. Van Zuilichem and W. Stolp in C. O'Connor, ed., *Extrusion Technology for the Food Industry*, Elsevier Science Publishing, New York.
2. J. D. Dziezak, "Single and Twin-Screw Extruders in Food Processing," *Food Technology* **43**, 164–174 (1989).
3. J. M. Harper, "Food Extruders and Their Applications," in C. Mercier, P. Linko, and J. M. Harper, eds , *Extrusion Cooking*, AACC, St. Paul, Minn., 1989, pp. 1–5.
4. B. W. Hauck and G. R. Huber, "Single Screw vs Twin Screw Extrusion," *Cereal Foods World* **34**, 934–939 (1989).

5. L. P. B. M. Janssen, "Engineering Aspects of Food Extrusion," in Ref. 3, pp. 17–38.
6. R. Jowitt, "The Terminology of Food Texture," *Journal of Texture Studies* **5**, 351–358 (1974).
7 International Organization for Standardization, "Sensory Analysis Vocabulary, Part IV," Geneva, Switzerland, 1981.
8. R. H. Meyers, *Response Surface Methodology*, Allyn & Bacon, Boston, 1976.
9. J M. Harper, *Extrusion of Foods*, Vols. 1 and 2, CRC Press, Boca Raton, Fla., 1981.
10. N. Noguchi, "Extrusion Cooking of High-Moisture Protein Foods," in Ref. 3, pp. 343–370.
11. F. F. Huang and C. K. Rha, "Protein Structures and Protein Fibers—A Review," *Polymer Engineering Science* **14**, 81–89 (1974).
12. J. P. Clark, "Texturization by Extrusion," *Journal of Texture Studies* **9**, 109–123 (1978).
13. D. W. Stanley and J. M. deMan, "Structural and Mechanical Properties of Textured Proteins," *Journal of Texture Studies* **9**, 59–76, 1978.
14. J. E. Kinsella, "Texturized Proteins: Fabrication, Flavorings and Nutrition," *CRC Reviews in Food Science and Nutrition* **10**, 147–207 (1978).
15. D. A. Ledward and J. R. Mitchell, "Protein Extrusion—More Questions Than Answers," in J. M. V. Blanshard and J. R. Mitchell, eds., *Food Structure—Its Creation and Evaluation*, Butterworths, Boston, 1988, pp. 219–229.
16. P. R. Sheard et al., "Macromolecular Changes Associated with the Heat Treatment of Soy Isolate," *Journal of Food Technology* **21**, 55–60 (1986).
17. J. J. Kelley and R. Pressey, "Studies with Soybean Protein and Fiber Formation," *Cereal Chemistry* **43**, 195–205, 1966.
18. D. B. Cumming et al., "Fate of Water Soluble Soy Protein During Thermoplastic Extrusion," *Journal of Food Science* **38**, 320–323 (1973).
19. L. D. Burgess and D. W. Stanley. "A Possible Mechanism for Thermal Texturization of Soybean Protein," *Journal of the Institute of Canadian Science and Technology—Alimentation* **9**, 228–231 (1976).
20. R. W. Simonsky and D. W. Stanley, "Texture-Structure Relationships in Textured Soy Protein. V. Influence of pH and Protein Acylation on Extrusion Texturization," *Canadian Institute of Food Science Technology* **15**, 294–301 (1982).
21. D. F. Hager, "Effects of Extrusion upon Soy Concentrate Solubility," *Journal of Agricultural and Food Chemistry* **32**, 293–296 (1984).
22. P. E. Neumann et al., "Uniquely Textured Products Obtained by Co-Extrusion of Corn Gluten Meal and Soy Flour," *Cereal Chemistry* **61**, 439–445 (1984).
23. P. R. Sheard et al., "Role of Carbohydrates in Soya Extrusion," *Journal of Food Technology* **19**, 475–483 (1984).
24. P. Melius, "Thermal Polymerization of Amino Acids Mixtures," *Federation Proceedings, Federation of American Society of Experimental Biologist* **34**, 573, (1975).
25. D. B. Cummings et al., "Texture–Structure Relationships in Texturized Soy Protein. II. Textural Properties and Ultrastructure of an Extruded Soybean Product," *Journal of the Institute of Canadian Science and Technology—Alimentation* **5**, 124–128 (1972)
26. J. M. Aguilera and F. W. Kosikowoski, "Soybean Extruded Product: A Response Surface Analysis," *Journal of Food Science* **41**, 647–651 (1976).
27. T. J. Maurice et al., "Texture—Structure Relationship in Textured Soy Protein. III. Textural Evaluation of Extruded Products," *Canadian Institute of Food Science Technology* **9**, 173–176 (1976).
28. T. J. Maurice and D. W. Stanley, "Texture–Structure Relationships in Texturized Soy Protein, IV. Influence of Process Variables on Extrusion Texturization," *Canadian Institute of Food Science Technology* **11**, 1–6 (1978).
29. O B. Smith, "Extrusion and Forming: Creating New Foods," *Food Engineering* **47**, 48–50 (August 1975).
30. S. H. Holay and J. M Harper, "Influence of the Extrusion Shear Environment on Plant Protein Texturization," *Journal of Food Science* **47**, 1869–1874 (1982).
31. G. Boison et al., "Extrusion of Defatted Soy Flour-Hydrocolloid Mixtures," *Journal of Food Technology* **18**, 719–730 (1983).
32. P. J. Frazier et al., "Optimization of Process Variables in Extrusion Texturing of Soya," *Journal of Food Engineering* **2**, 79–103 (1983).
33. P. R. Sheard et al., "Comparison of the Extrusion Cooking of a Soya Isolate and a Soya Flour," *Journal of Food Technology* **20**, 763–771 (1985).
34. M. V. Taranto et al., "Textured Cottonseed and Soy Flours: A Microscopic Analysis," *Journal of Food Science* **43**, 767–771 (1978).
35. M. V. Taranto et al., "Morphological, Ultrastructural and Rheological Evaluation of Soy and Cottonseed Flours Texturized by Extrusion and Nonextrusion Processing," *Journal of Food Science* **43**, 973–979, 984 (1978).
36. M. B. Kennedy and co-workers, "Effects of Feed Moisture and Barrel Temperature on the Rheological Properties of Extruded Cowpea Meal," *Journal of Food Processing and Engineering* **8**, 193–212 (1986).
37. C. B. Pham and P. R. Del Rosario, "Studies on the Development of Texturized Vegetable Products by the Extrusion Process," *Journal of Food Technology* **19**, 535–547 (1984).
38. J. A. Maga and K. Lorenz, "Sensory and Functional Properties of Extruded Corn-Soy Blends," *Lebensmittel Wissonschalf Technologie* **11**, 185–187 (1978).
39. Y. C. Jao et al., "Evaluation of Corn Protein Concentrate: Extrusion Study," *Journal of Food Science* **50**, 1257–1259, 1288 (1985).
40. M. Bhattacharya et al., "Textural Properties of Plant Protein Blends," *Journal of Food Science* **51**, 988–993 (1986).
41. P. Colonna and co-workers, "Extrusion Cooking of Starch and Starchy Products," in Ref. 3, pp. 247–319.
42. P. Colonna et al., "Flow, Mixing and Residence Time Distribution of Maize Starch Within a Twin Screw Extruder with a Longitudinally-Split Barrel," *Journal of Cereal Science* **1**, 115–125 (1983).
43. C. Mercier, "Effect of Extrusion Cooking on Potato Starch Using a Twin Screw French Extruder," *Staerke* **29**, 48–52 (1977).
44. P. Colonna and C. Mercier, "Macromolecular Modifications of Manioc Starch Components by Extrusion Cooking With and Without Lipids," *Carbohydrate Polym* **3**, 87–108 (1983).
45. P. Colonna et al., "Extrusion Cooking and Drum Drying of Wheat Starch. I. Physical and Macromolecular Modifications," *Cereal Chemistry* **61**, 538–543 (1984).

46. V. J. Davidson et al., "Degradation of Wheat Starch in a Single Screw Extruder: Characteristics of Extruded Starch Polymers," *Journal of Food Science* **49**, 453–458 (1984).

47 V. J. Davidson et al., "A Model for Mechanical Degradation of Wheat Starch in a Single Screw Extruder," *Journal of Food Science* **49**, 1154–1157 (1984).

48. L. L. Diosady et al., "Degradation of Wheat Starch in a Single Screw Extruder: Mechanico-kinetic Break Down of Cooked Starch," *Journal of Food Science* **50**, 1697–1699, 1706 (1985).

49. C Mercier and P. Feillet, "Modification of Carbohydrate Components by Extrusion-Cooking of Cereal Products," *Cereal Chemistry* **52**, 283–297 (1975).

50. J. M. Faubion and R. C. Hoseney, "High Temperature Short-Time Extrusion Cooking of Wheat Starch and Flour. I. Effect of Moisture and Flour Type on Extrudate Properties," *Cereal Chemistry* **59**, 529–533 (1982).

51. M. Gomez and J. M. Aguilera, "A Physicochemical Model for Extrusion of Corn Starch," *Journal of Food Science* **49**, 40–43, 63 (1984).

52. J. Owusu-Ansah et al., "Textural and Microstructural Changes in Corn Starch as a Function of Extrusion Variables," *Canadian Institute of Food Science Technology* **17**, 65–70 (1984).

53. M. Bhattacharya and M. A. Hanna, "Textural Properties of Extrusion Cooked Corn Starch," *Lebensmittel Wissenschaft und Technologie* **20**, 195–201 (1987).

54. F. Hsieh et al., "Effects of Salt, Sugar and Screw Speed on Processing and Product Variables of Corn Meal Extruded with a Twin-Screw Extruder," *Journal of Food Science* **55**, 224–227 (1990).

55. R. Chinnaswamy and M. A. Hanna, "Optimum Extrusion-Cooking Conditions for Maximum Expansion of Corn Starch," *Journal of Food Science* **53**, 834–836, 840 (1988).

56. R. Chinnaswamy and M. A. Hanna, "Die-Nozzle Dimension Effects on Expansion of Corn Starch," *Journal of Food Science* **52**, 1746–1747 (1987).

57. A. L. Hayter et al., "The Physical Properties of Extruded Food Foams," *Journal of Materials Science* **21**, 3729–3736 (1986).

58. B. Launay and J. M. Lisch, "Twin-Screw Extrusion Cooking of Starches: Flow Behavior of Starch Pastes, Expansion and Mechanical Properties of Extrudates," *Journal of Food Engineering* **2**, 259–280 (1983).

59. D Paton and W. A. Spratt, "Component Interactions in the Extrusion Cooking Process. I. Processing of Chlorinated and Untreated Soft Wheat Flour," *Cereal Chemistry* **55**, 973–980 (1978).

60. J. M. Faubion and R. C. Hoseney, "High Temperature Short-Time Extrusion Cooking of Wheat Starch and Flour. II. Effect of Protein and Lipid on Extrudate Properties," *Cereal Chemistry* **59**, 533–537 (1982).

61. M. Bhattacharya and M. A. Hanna, "Effect of Lipids on the Properties of Extruded Products," *Journal of Food Science* **53**, 1230–1231 (1988).

62. R. A. Anderson et al., "Gelatinization of Corn Grits by Roll and Extrusion Cooking," *Cereal Science Today* **14**, 4–7, 11–12 (1969).

63. R. A. Anderson et al., "Roll and Extrusion Cooking of Grain Sorghum Grits," *Cereal Science Today* **14**, 372–376 (1969).

64. H. F. Conway, "Extrusion Cooking of Cereals and Soybeans," *Food Product Development* **5**, 14–17, 27–29 (1971).

65. S. Middleman, "Advances in Polymer Science and Engineering: Application to Food Rheology," in C. K. Rha, ed., *Theory, Determination and Control of Physical Properties of Food Materials*, Riedel Publishing, Dordrecht, Holland, 1975, pp. 39–53.

66. J. L. Leblanc, "Recent Progress in Understanding Rubber Processing Through a Rheological Approach," *Progress and Trends in Rheology* **2**, 32–43 (1988).

67. C. J. S. Petrie, "Some Asymptotic Results for Planar Extension," *Journal of Non-Newtonian Fluid Mechanics* **34**, 37–62 (1990).

68. J. M. Harper et al., "Viscosity Model for Cooked Cereal Doughs," *American Institute of Chemical Engineering Symposium Series*, No. 108, 67, 40–43 (1971).

69. C. H. Remsen and J. P. Clark, "A Viscosity Model for a Cooking Dough," *Journals of Food Process Engineering* **2**, 39–63 (1977).

70. N. W. Cervone and J. M. Harper, "Viscosity of an Intermediate Moisture Dough," *Journal of Food Processing Engineering* **2**, 83–95 (1978).

71. Y. C. Jao et al., "Engineering Analysis of Soy Dough Rheology in Extrusion," *Journal of Food Process Engineering* **2**, 97–112 (1978).

72. L. A. Luxenburg et al., "Background Studies in the Modeling of Extrusion Cooking Processes for Soy Doughs," *Biotechnology Progress* **1**, 33–38 (1985).

73. A. Senouci and A. C. Smith, "An Experimental Study of Food Melt Rheology. I. Shear Viscosity Using a Slit Die Viscometer and a Capillary Rheometer," *Rheologica Acta* **27**, 546–554 (1988).

74. L. S. Lai et al., "On Line Rheological Properties of Amylose and Amylopectin Based Starch. The Role of Viscosity on Extrudate Expansion," *Proceedings of the Xth International Congress on Rheology* **2**, 55–57, (1988)

75. M. Padmanabhan and M Bhattacharya, "Analysis of Pressure Drop in Extruder Dies," *Journal of Food Science* **54**, 709–713 (1989).

76. M. Bhattacharya and M. Padmanabhan, "Elongational Viscosity During Extrusion Cooking Using a Converging Flow Analysis," Paper 243, Presented at the Institute of Food Technology Annual Meeting, Anaheim, Calif., 1990.

77. E. B. Bagley, "End Corrections in the Capillary Flow of Polyethylene," *Journal of Applied Physics* **28**, 624–627 (1957).

78. B. Vergnes and J. P. Villemaire, "Rheological Behavior of Low Moisture Molten Maize Starch," *Rheologica Acta* **26**, 570–576 (1987).

79. R. G. Morgan et al., "A Generalized Viscosity Model for Extrusion of Protein Doughs," *Journal of Food Process Engineering* **11**, 55–78 (1989).

80. K. L. Mackey et al., "Rheological Modeling of Potato Flours During Extrusion Cooking," *Journal of Food Process Engineering* **12**, 1–11 (1989).

81 G. V. Vinogradov and A. Y. Malkin, *Rheology of Polymers*, Mir Publishers, Moscow, 1980.

82. B. van Lengerich, "Influence of Extrusion Processing on In-Line Rheological Behavior, Structure, and Function of Wheat Starch," in H. Faridi and J. M. Faubion, eds., *Dough Rheology and Baked Product Texture*, Van Nostrand Reinhold, New York, 1989, pp. 421–471.

83. J. L. Doublier et al., "Extrusion Cooking and Drum-Drying of Wheat Starch. II. Rheological Characterization of Wheat Starch," *Cereal Chemistry* **63**, 240–246 (1986).

84. B. Launay and T. Kone, "Twin-Screw Extrusion Cooking of Corn Starch: Flow Behavior of Starch Pastes," in P. Zeuthen et al., eds., *Thermal Processing and Quality of Foods*, Elsevier Applied Science, London, 1984, pp. 54–61.

85. R. B. Bird and co-workers, *Dynamics of Polymeric Liquids*, Vol. 1, John Wiley & Sons, New York, 1987.

86. H. A. Barnes et al., *An Introduction to Rheology*, Elsevier Science Publishing, Amsterdam, The Netherlands, 1989.

87. C. D. Han, *Rheology in Polymer Processing*, Academic Press, New York, 1976.

88. C. D. Han, "Slit Rheometry," in A. A. Collyer and D. W. Clegg, eds., *Rheological Measurement*, Elsevier Applied Science, New York, 1988, pp. 25–48.

89. D. V. Boger and M. M. Denn, "Capillary and Slit Methods of Normal Stress Measurements," *Journal of Non-Newtonian Fluid Mechanics* **6**, 163–185 (1980).

90. A. S. Lodge and L. de Vargas, "Positive Hold Pressures and Negative Exit Pressures Generated by Molten Polyethylene Flowing Through a Slit Die," *Rheologica Acta* **22**, 151–170 (1983).

91. C. Rauwendaal and F. Fernandez, "Experimental Study and Analysis of a Slit Die Viscometer," *Polymer Engineering Science* **25**, 765–771 (1985).

92. R. Eswaran et al., "A Slit Viscometer for Polymer Melts," *Rheologica Acta* **3**, 83–91 (1963).

93. J. L. Leblanc, "New Slit Die Rheometer. Some Results with a Butadiene-Styrene Block Copolymer," *Polymer* **17**, 235–240, (1976).

94. L. Choplin and P. J. Carreau, "Excess Pressure Losses in Slit," *Journal of Non-Newtonian Fluid Mechanics* **9**, 119–146 (1981).

95. H. M. Laun, "Polymer Melt Rheology with a Slit Die," *Rheologica Acta* **22**, 171–185 (1983).

96. D. G. Baird et al., "Comparison of the Hole Pressure and Exit Pressure Methods for Measuring Polymer Melt Normal Stresses," *Polymer Engineering Science* **26**, 225–232 (1986).

97. N. Y. Tuna and B. A. Finlayson, "Exit Pressure Experiments for Low Density Polyethylene Melts," *Journal of Rheology* **32**, 285–308 (1988).

98. A. Senouci and A. C. Smith, "An Experimental Study of Food Melt Rheology. II. End Pressure Effects," *Rheologica Acta* **27**, 649–655 (1988).

99. M. Bhattacharya and M. Padmanabhan, "On-line Rheological Measurements of Food Dough During Extrusion Cooking," in *Proceedings of the Second International Conference in Extrusion and Rheology of Foods*. Rutgers University, New Brunswick, N.J., 1990.

100. R. C. E. Guy and A. W. Horne, "Extrusion and Co-Extrusion of Cereals," in Ref. 15, pp. 331–349.

101. B. A. Whipple and C. T. Hill, "Velocity Distributions in Die Swell," *American Institute of Chemical Engineering Journal* **24**, 664–678 (1978).

102. M. Gottleib and R. B. Bird, "Exit Effects in Non-Newtonian Liquids. An Experimental Study," *Ind. Eng. Chem. Fund.* **18**, 357–368 (1979).

103. J. M. Broadbent et al., "Possible Systematic Errors in the Measurement of Normal Stress Differences in Polymer Solution in Steady Shear Flow," *Nature* **217**, 55–57 (1968).

104. A. Kaye et al., "Determination of Normal Stress Differences in Steady Shear Flow. II. Flow Birefringence, Viscosity and Normal Stress Data for Polyisobutene Liquid," *Rheologica Acta* **7**, 368–379 (1968)

105. R. I. Tanner and A. C. Pipkin, "Intrinsic Errors in Pressure-Hole Measurement," *Transactions of the Society of Rheology* **13**, 471–484 (1969).

106. A. S. Lodge, "Low-Shear Rate Rheometry and Polymer Quality Control," *Chemical Engineering Communications* **32**, 1–60 (1985).

107. A. S. Lodge, "A New Method of Measuring Multigrade Oil Shear Elasticity and Viscosity at High Shear Rates," *SAE Technical Paper*, Series 872043 (1987).

108. A. S. Lodge et al., "Measurement of the First Normal-Stress Difference at High Shear Rates for a Polyisobutylene/Decalin Solution D2," *Rheologica Acta* **26**, 516–521 (1987).

109. D. G. Baird, "A Possible Method for Determining Normal Stress Differences from Hole Pressure Error Data," *Transactions of the Society of Rheology* **19**, 147–151 (1975).

110. D. G. Baird, "Fluid Elasticity from Hole Pressure Error Data," *Journal of Applied Polymer Science* **20**, 3155–3173 (1976).

111. R. D. Pike and D. G. Baird, "Evaluation of the Highashitani and Pritchard Analysis of the Hole Pressure Using Flow Birefringence," *Journal of Non-Newtonian Fluid Mechanics* **16**, 211–231 (1984).

112. K. Higashitani and W. G. Pritchard, "A Kinematic Calculation of Intrinsic Errors in Pressure Measurements Made with Hole," *Transactions of the Society of Rheology* **16**, 687–696 (1972).

113. R. I. Tanner, "Pressure-Hole Errors—An Alternative Approach," *Journal of Non-Newtonian Fluid Mechanics* **28**, 309–318 (1988).

114. M. Yao and D. S. Malkus, "Error Cancellation in HPBL Derivation of Elastic Hole-Pressure Error," *Center of the Mathematical Science*, Technical Summary Report 90-18, University of Wisconsin, Madison (1989).

115. W. Philippoff and F. H. Gaskins, "The Capillary Experiment in Rheology," *Transactions of the Society of Rheology* **2**, 263–284 (1958).

116. S. A. White and D. G. Baird, "The Importance of Extensional Flow Properties on Planar Entry Flow Patterns of Polymer Melt," *Journal of Non-Newtonian Fluid Mechanics* **20**, 93–101 (1986).

117. F. N. Cogswell, "Converging Flow of Polymer Melts in Extrusion Dies," *Polymer Engineering and Science* **12**, 64–73 (1972).

118. D. R. Oliver, "The Prediction of Angle of Convergence for the Flow of Viscoelastic Liquids into Orifices," *The Chemical Engineering Journal* **6**, 265–271 (1973).

119. A. B. Metzner and A. P. Metzner, "Stress Levels in Rapid Extensional Flows of Polymeric Fluids," *Rheologica Acta* **9**, 174–181 (1970).

120. A. G. Gibson "Converging Flow Analysis," in Ref. 88, pp. 49–82.

121. D. M. Binding, "An Approximate Analysis for Contraction and Converging Flows," *Journal of Non-Newtonian Fluid Mechanics* **27**, 173–189 (1988).

122. D. M. Binding and K. Walters, "On the Use of Flow Through a Contraction in Estimating the Extensional Viscosity of Mobile Polymer Solution," *Journal of Non-Newtonian Fluid Mechanics* **30**, 233–250 (1988).

123. D. M. Binding and D. M. Jones, "On the Interpretation of Data from Converging Flow Rheometers," *Rheologica Acta* **28**, 215–222 (1989).

124. A. Senouci et al., "Extensional Rheology in Food Processing," *Progress and Trends in Rheology* **2**, 434–437 (1988).

125. R. N. Shroff et al., "Extensional Flow of Polymer Melts," *Transactions of the Society Rheology* **21**, 429–446 (1977).

126. H M Laun and H. Schuch, "Transient Elongational Viscosities and Drawability of Polymer Melts," *Journal of Rheology* **33**, 119–175 (1989).

127. J. F. Steffe and R. G. Morgan, "On-Line Measurements of Dynamic Rheological Properties During Food Extrusion," *Journal of Food Process Engineering* **10**, 21–26 (1987).

128. M. Padmanabhan and M. Bhattacharya, "Extrudate Expansion During Extrusion Cooking of Foods," *Cereal Foods World* **34**, 945–949 (1989).

129. R. I. Tanner, "A Theory of Die Swell," *Journal of Applied Polymer Science* A-2, **8**, 2067–2078 (1970).

130. R. C. Miller, "Low Moisture Extrusion: Effect of Cooking Moisture on Product Characteristics," *Journal of Food Science* **50**, 249–253 (1985).

131. L. Alvarez-Martinez et al., "A General Model for Expansion of Extruded Products," *Journal of Food Science* **53**, 609–615 (1988).

132. R. Chinnaswamy and M. A. Hanna, "Relationship Between Viscosity and Expansion Properties of Variously Extrusion-Cooked Corn Grain Components," *Food Hydrocolloids* **3**, 423–434 (1990).

133. K. B. Park, "Elucidation of the Extrusion Puffing Process," Ph.D. Thesis, Department of Food Science, University of Illinois, Urbana, Il., 1976.

134. S. Richardson, "The Die Swell Phenomenon," *Rheologica Acta* **9**, 193–199 (1970).

135. R. J. Tanner, "Recoverable Elastic Strain and Swelling Ratio," in Ref. 88, pp. 93–117.

136. B. Vergnes et al., "Interrelationships Between Thermomechanical Treatment and Macromolecular Degradation of Maize Starch in a Novel Rheometer with Preshearing," *Journal of Cereal Science* **5**, 189–202 (1987).

GENERAL REFERENCES

D. Q. Ada and S. S. Wang, "Modeling Shearing Resistance of Powdery Starch for Simulation Studies of Extrusion Cooking Processes," *Starch / Staerke* **50**, 147–153 (1998).

E. S. M. Aal Abdel et al., "Effect of Extrusion Cooking on the Physical and Functional Properties of Wheat, Rice and Fababean Blends," *Lebensm.-Wiss. Technol.* **25**, 21–25 (1992).

E. D. Beecher and M. S. Starer, "Direct-Expanded Cereals Produced on High-Speed, High-Torque Twin Screw Extruders," *Cereal Foods World* **43**, 753–757 (1998).

H. H. Ben et al., "Extrusion-Cooking of Pea Flour: Structural and Immunocytochemical Aspects," *Food Structure* **10**, 203–121 (1991).

S. Bhajmohan and S. J. Mulvaney, "Modeling and Process Control of Twin-Screw Cooking Food Extruders," *Journal of Food Engineering* **23**, 403–428 (1994).

S. Bhatnagar and M. A. Hanna, "Starch-Stearic Acid Complex Development Within Single and Twin Screw Extruders," *J. Food Sci.* **61**, 778–782 (1996).

M. Bhattacharya, M. Padmanabhan, and K. Seethamraju, "Uniaxial Extensional Viscosity During Extrusion Cooking From Entrance Pressure Drop Method," *J. Food Sci.* **59**, 221–226, 230 (1994).

J. C. Cheftel, M. Kitgawa, and C. Queguiner, "New Protein Texturization Processes by Extrusion Cooking at High Moisture Levels," *Food Rev. Int.* **8**, 235–275 (1992).

Q. Di and S. S. Wang, "Kinetics of the Formation of Gelatinized and Melted Starch at Extrusion Cooking Conditions," *Starch / Staerke* **46**, 225–229 (1994).

W. Fangzhi, "Study of Single-Screw Extruders for Continuous Feeding of Canola Paste for Supercritical Extraction," *Dissertation Abstracts International B* **55**, 512 (1994).

S. Ilo et al., "The Effect of Extrusion Operating Conditions on the Apparent Viscosity and the Properties of Extrudates in Twin-Screw Extrusion Cooking of Maize Grits," *Lebensm.-Wiss. Technol.* **29**, 593–598 (1996).

A. S. Jaber Ba et al., "Texturization of Hand- and Mechanically-Deboned Poultry Meat Combinations With a Soy Protein Isolate by Extrusion Cooking," *Lebensm.-Wiss. Technol.* **25**, 153–157 (1992).

J. L. Kokini, "The Effect of Processing History on Chemical Changes in Single- and Twin-Screw Extruders," *Trends Food Sci. Technol.* **4**, 324–329 (1993).

M. K. Kulshreshtha, C. A. Zaror, and D. J. Jukes, "Simulating the Performance of a Control System for Food Extruders Using Model-Based Set-Point Adjustment," *Food Control* **6**, 135–141 (1995).

L. Levine and E. Boehmer, "The Fluid Mechanics of Cookie Dough Extruders," *Journal of Food Process Engineering* **15**, 169–186 (1992).

S. Lue, F. Hsieh, and H. E. Huff, "Extrusion Cooking of Corn Meal and Sugar Beet Fibre: Effects on Expansion Properties, Starch Gelatinization, and Dietary Fiber Content," *Cereal Chem.* **68**, 227–234 (1991).

K. L. Mackey et al., "Rheological Modeling of Potato Flour During Extrusion Cooking," *Journal of Food Process Engineering* **12**, 1–11 (1990).

M. Padmanabhan and M. Bhattacharaya, "Rheological Measurement of Fluid Elasticity During Extrusion Cooking," *Trends Food Sci. Technol.* **2**, 149–151 (1991).

G. J. Rockey, "RTE Breakfast Cereal Flake Extrusion," *Cereal Foods World* **40**, 422–424, 426 (1995).

W. Seibel and H. Ruguo, "Gelatinization Characteristics of a Cassava/Corn Starch Based Blend During Extrusion Cooking Employing Response Surface Methodology," *Starch / Staerke* **46**, 217–224 (1994).

M. Thiebaud, E. Dumay, and J. C. Cheftel, "Influence of Process Variables on the Characteristics of a High Moisture Fish Soy Protein Mix Texturized by Extrusion Cooking," *Lebensm.-Wiss. Technol.* **29**, 526–535 (1996).

S. M. Wang, J. M. Bouvier, and M. Gelus, "Rheological Behaviour of Wheat Flour Dough in Twin-Screw Extrusion Cooking," *International Journal of Food Science and Technology* **25**, 129–139 (1990).

S. Wang, J. Casulli, and J. M. Bouvier, "Effect of Dough Ingredients on Apparent Viscosity and Properties of Extrudates in Twin-Screw Extrusion Cooking," *International Journal of Food Science and Technology* **28**, 465–479 (1993).

W. Yacu, "Process Instrumentation and Control in Food Extruders," *Cereal Foods World* **35**, 919–926 (1990).

MRINAL BHATTACHARVA
MAHESH PADMANABHAN
University of Minnesota
St. Paul, Minnesota

See also EXTRUSION COOKING.

F

FATS AND OILS: CHEMISTRY, PHYSICS, AND APPLICATIONS

Fats and oils are the commercially important group of substances classified as lipids. Lipids are compounds usually associated with solubility in nonpolar solvents. They are mostly esters of long-chain fatty acids and alcohols and closely related derivatives. The most important aspect of lipids is the central position of the fatty acids. The scheme of Figure 1 illustrates this (1). The basic components of the lipids (also known as derived lipids) are listed in the central column. In the left-hand column are the phospholipids, most of which contain phosphoric acid groups. The right-hand column includes the compounds most important from a quantitative standpoint in fats and oils. These are mostly esters of fatty acids and glycerol. Fats are solid at room temperature, oils are liquid.

Food fats can be divided into visible and invisible fats (2). More than half of all the fats consumed are in the latter category, that is, those contained in dairy products (excluding butter), eggs, meat, poultry, fish, fruits, vegetables, and grain products. The visible fats include lard, butter, shortening, frying fats and oils, margarines, and salad oils.

Fats and oils may differ considerably in composition, depending on their origin. Fatty acid as well as glyceride composition will influence many of the properties of fats and oils. These properties may be further modified by appropriate processing methods. The fats and oils can be classified broadly into the following groups: animal depot fats, ruminant milk fats, vegetable oils and fats, and marine oils. The processing of fats and oils serves several purposes: to separate the oils or fats from other parts of the raw materials, to remove impurities and undesirable components, to improve stability, to change physical properties, and to provide desirable functional properties.

COMPONENT FATTY ACIDS

Even-numbered straight-chain saturated and unsaturated fatty acids make up the largest proportion of fatty acids in fats and oils. Minor amounts of odd-carbon-number acids, branched-chain acids, and hydroxy acids may also be present. Processed fats, especially hydrogenated fats, may contain a variety of geometric and positional isomers. The division of fatty acids into saturated and unsaturated groups is important because it generally reflects on the melting properties of the fat of which they are a part. There are some exceptions to this rule. Short-chain saturated fatty acids such as those present in milk fats and lauric fats have

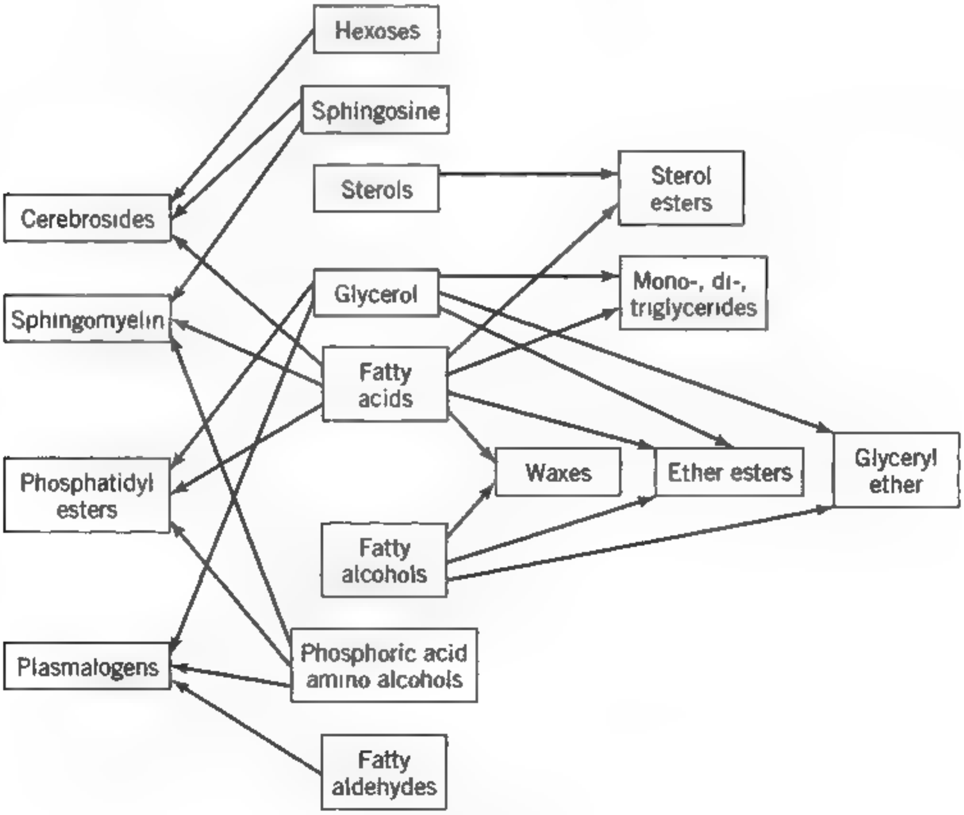


Figure 1. Interrelationship of the lipids.

very low melting points; unsaturated trans isomers have much higher melting points than the cis isomers and are therefore comparable to saturated fatty acids in their effect on melting characteristics.

Some of the important saturated fatty acids are listed in Table 1 with their systematic and common names. The unsaturated fatty acids are listed in Table 2. The naturally occurring unsaturated fatty acids are almost exclusively in the cis form. Trans acids are abundant in ruminant milk fats and in hydrogenated fats.

The depot fats of higher land animals consist mainly of palmitic, oleic, and stearic acids and are high in saturation. The fats of birds are somewhat more complex. The fatty acid compositions of the major food fats of this group are listed in Table 3 The kind of feed consumed by the animals greatly influences the fatty acid composition of the depot fats. For example, the high linolenic acid content of the horse fat in Table 3 is the result of pasture feeding. Animal depot fats are generally low in polyunsaturated fatty acids. The iodine value of beef fat is about 50 and of lard about 60.

Ruminant milk fat has an extremely complex fatty acid composition. The following fatty acids are present in cow's milk fat (3): even and odd saturated acids from 2:0 to 28:0; even and odd monoenoic acids from 10:1 to 26:1 with the exception of 11:1, and-including positional and geometric isomers; even unsaturated fatty acids from 14:2 to 26:2 with some conjugated trans isomers; monobranched fatty acids 9:0 and 11:0 to 25:0; some iso and some anteiso (iso acids have a methyl branch on the penultimate carbon, anteiso on the next to penultimate carbon; multibranched acids from 16:0 to 28:0, both odd and even with three to five methyl branches; and, finally, a number of keto, hydroxy, and cyclic acids.

Marine oils also contain a large number of different fatty acids. As many as 50 to 60 fatty acids have been reported (4). The 14 major ones consist of few saturated fatty acids (14:0, 16:0, and 18:0) and a larger number of unsaturated fatty acids with 16 to 22 carbon atoms and up to 6 double bonds. This provides the possibility for many positional isomers. It is customary to number carbon atoms of

Table 1. Saturated Even- and Odd-Carbon-Numbered Fatty Acids

Systematic name	Common name	Formula	Shorthand description
n-Butanoic	Butyric	$CH_3 \cdot (CH_2)_2 \cdot COOH$	4:0
n-Hexanoic	Caproic	$CH_3 \cdot (CH_2)_4 \cdot COOH$	6:0
n-Octanoic	Caprylic	$CH_3 \cdot (CH_2)_6 \cdot COOH$	8:0
n-Decanoic	Capric	$CH_3 \cdot (CH_2)_8 \cdot COOH$	10:0
n-Dodecanoic	Lauric	$CH_3 \cdot (CH_2)_{10} \cdot COOH$	12:0
n-Tetradecanoic	Myristic	$CH_3 \cdot (CH_2)_{12} \cdot COOH$	14:0
n-Hexadecanoic	Palmitic	$CH_3 \cdot (CH_2)_{14} \cdot COOH$	16:0
n-Octadecanoic	Stearic	$CH_3 \cdot (CH_2)_{16} \cdot COOH$	18:0
n-Eicosanoic	Arachidic	$CH_3 \cdot (CH_2)_{18} \cdot COOH$	20:0
n-Docosanoic	Behenic	$CH_3 \cdot (CH_2)_{20} \cdot COOH$	22:0
n-Pentanoic	Valeric	$CH_3 \cdot (CH_2)_3 \cdot COOH$	5:0
n-Heptanoic	Enanthic	$CH_3 \cdot (CH_2)_5 \cdot COOH$	7:0
n-Nonanoic	Pelargonic	$CH_3 \cdot (CH_2)_7 \cdot COOH$	9:0
n-Undecanoic		$CH_3 \cdot (CH_2)_9 \cdot COOH$	11:0
n-Tridecanoic		$CH_3 \cdot (CH_2)_{11} \cdot COOH$	13:0
n-Pentadecanoic		$CH_3 \cdot (CH_2)_{13} \cdot COOH$	15:0
n-Heptadecanoic	Margaric	$CH_3 \cdot (CH_2)_{15} \cdot COOH$	17:0

Table 2. Unsaturated Fatty Acids

Systematic name	Common name	Formula	Shorthand description
Dec-9-enoic		$CH_2=CH \cdot (CH_2)_7 \cdot COOH$	10.1
Dodec-9-enoic		$CH_3 \cdot CH_2 \cdot CH=CH \cdot (CH_2)_7 \cdot COOH$	12:1
Tetradec-9-enoic	Myristoleic	$CH_3 \cdot (CH_2)_3 \cdot CH \cdot CH \cdot (CH_2)_7 \cdot COOH$	14:1
Hexadec-9-enoic	Palmitoleic	$CH_3 \cdot (CH_2)_5 \cdot CH=CH \cdot (CH_2)_7 \cdot COOH$	16:1
Octadec-6-enoic	Petroselinic	$CH_3 \cdot (CH_2)_{10} \cdot CH=CH \quad (CH_2)_4 \cdot COOH$	18:1
Octadec-9-enoic	Oleic	$CH_3 \cdot (CH_2)_7 \cdot CH=CH \cdot (CH_2)_7 \cdot COOH$	18:1
Octadec-11-enoic	Vaccenic	$CH_3 \cdot (CH_2)_5 \cdot CH=CH \cdot (CH_2)_9 \cdot COOH$	18:1
Octadeca-9:12-dienoic	Linoleic	$CH_3(CH_2)_4 \cdot (CH{:}CH \cdot CH_2)_2 \cdot (CH_2)_6 \cdot COOH$	18:2ω6
Octadeca-9:12:15-trienoic	Linolenic	$CH_3 \cdot CH_2 \cdot (CH=CH \cdot CH_2)_3 \cdot (CH_2)_6 \cdot COOH$	18:3ω3
Octadeca-6:9:12-trienoic	Gamma linolenic	$CH_3 \cdot (CH_2)_4 \cdot (CH=CH \cdot CH_2)_3 \cdot (CH_2)_3 \cdot COOH$	18:3ω6
Octadeca-9:11:13-trienoic	Elaeostearic	$CH_3 \cdot (CH_2)_3 \cdot (CH=CH)_3 \cdot (CH_2)_7 \cdot COOH$	20:3
Eicos-9-enoic	Gadoleic	$CH_3 \cdot (CH_2)_9 \cdot CH=CH \cdot (CH_2)_7 \cdot COOH$	20:1
Eicosa-5:8:11:14-tetraenoic	Arachidonic	$CH_3 \cdot (CH_2)_4 \quad (CH=CH \cdot CH_2)_4 \quad (CH_2)_2 \cdot COOH$	20:4ω6
Eicosa-5:8:11:14:17-pentaenoic acid	EPA	$CH_3 \cdot CH_2 \cdot (CH=CH \cdot CH_2)_5 \cdot (CH_2)_2 \cdot COOH$	20:5ω3
Docosa-13-enoic	Erucic	$CH_3 \cdot (CH_2)_7 \cdot CH=CH \cdot (CH_2)_{11} \cdot COOH$	22:1
Docosa-4:7.10:13:16:19-hexaenoic acid	DHA	$CH_3 \cdot CH_2(CH=CH \cdot CH_2)_6 \cdot (CH_2) \cdot COOH$	22:6ω3

Table 3. Component Fatty Acids of Animal Depot Fats

	Fatty acids, % of total									
Animal	12:0	14:0	16:0	18:0	20:0	16:1	18:1	18:2	18:3	20·1
Cow		6.3	27.4	14.1			49.6	2.5		
Pig		1.8	21.8	8.9	0.8	4.2	53.4	6.6	0.8	0.8
Sheep		4.6	24.6	30.5			36.0	4.3		
Goat	3.5	2.1	25.5	28.1	2.4		38.4			
Horse	0.4	4.5	25.9	4.7	0.2	6.8	33.7	5.2	16.3	2.3
Chicken	1.9	2.5	36.0	2.4		8.2	48.2	0.8		
Turkey	0.1	0.8	20.0	6.4	1.3	6.2	38.4	23.7	1.6	

fatty acids starting from the carboxyl end; however, for biological activity it is interesting to number from the methyl carbon, which is done by using the symbol ω. Three different types of fatty acids can be distinguished, the oleic type with one double bond removed nine carbons from the methyl end (18:1ω3). Three different types of unsaturated fatty acids can be distinguished: the oleic type with one double bond removed nine carbon atoms from their methyl end (18:1ω9 or oleic acid type); the linoleic type with two double bonds removed six carbon atoms from the methyl end (18:2ω6 or linoleic acid type); and the linoleic type with three double bonds removed three carbon atoms from the methyl end (18:3ω3 or linoleic acid type). The end structure is usually retained even if additional double bonds are introduced or if additional carbon atoms are added. Thus, linoleic acid (18:2ω6) may be changed into arachidonic acid (20:4ω6) while retaining the ω6 structure which confers essential fatty acid character to the molecule. The latter two types are now often referred to as *n*-6 and *n*-3 fatty acids. The high content of polyenoic fatty acids makes fish oils highly susceptible to autoxidation. The component fatty acids of some marine and freshwater fish oils are listed in Table 4 (5).

Considerable interest has developed recently in the health effect of certain *n*-3 fatty acids, especially eicosapentaenoic acid (EPA) (20:5ω3) and docosahexaenoic acid (DHA) (22:6ω3). These fatty acids can be produced slowly from linoleic acid by herbivore animals, but not by humans. EPA and DHA occur in major amounts in fish from cold deep waters, such as cod, mackerel, tuna, swordfish, sardines, and herring (6,7). Arachidonic acid is the precursor in the human system of prostanoids and leukotrienes.

The vegetable oils and fats can be divided into three groups on the basis of their fatty acid composition. The first group comprises oils containing mainly 16- and 18-carbon fatty acids and includes most of the seed oils—cottonseed oil, peanut oil, sunflower oil, corn oil, sesame oil—as well as palm oil. The second group comprises seed oils containing erucic acid (docos-13-enoic), and includes rapeseed and mustard oil. The third group is that of the vegetable fats, comprising coconut oil and palm kernel oil, which are highly saturated and also known as lauric fats, as well as cocoa butter. The component fatty acids of some of the common vegetable oils and fats are listed in Table 5. Palmitic is the most common saturated fatty acid. Oils containing high levels of linolenic acid are susceptible to rapid oxidative deterioration.

The Crucifera seed oils, including rapeseed and mustard oil, are characterized by high levels of erucic acid (docos-13-enoic) and smaller amounts of eicos-11-enoic acid. Plant breeders have succeeded in replacing virtually all of these fatty acids by oleic acid, resulting in what is now known as canola oil (8).

Cocoa butter is unusual in that it contains only three major fatty acids, palmitic, stearic, and oleic, in approximately equal proportions.

COMPONENT GLYCERIDES

When a fat or oil is characterized by the determination of its component fatty acids, there still remains the question of how these acids are distributed among and within the glycerides. The stereospecific numbering (sn) of glycerol for a triacid glyceride is as follows:

Table 4. Major Component Fatty Acids of Some Marine and Freshwater Fish Oils

	14:0	16·0	18:0	16:1	18:1	20:1	22:1	18:2ω6	18:3ω3	18:4ω3	20:4ω6	20.5ω3	22:5ω3	22.6ω3
Herring	6.4	12.7	0.9	8.8	12.7	14.1	20.8	1.1	0.6	1.7	0.3	8.4	0.8	4.9
Turbot	6.5	12.0	0.9	15.4	17.4	18.6	17.8	0.5	0.2	0.8	0.1	3.0	0.6	1.9
Sablefish	6.7	11.1	1.9	6.6	29 0	18.1	14.8	0.7	0.2	0.3	0.3	1.4	0.5	1.0
Cod	1.4	19.6	3.8	3.5	13.8	3.0	1.0	0.7	0.1	0.4	2.5	17.0	1.3	29.8
Sole	4.3	16.5	2.4	14.4	12.2	3.9	Tr	0.3	2.0	1.6	4.0	11.9	10.6	7.0
Halibut	0.8	9.6	9.0	2.5	12.3	4.0	5.0	Tr	Tr		1.4	13.0	2.5	37.6
Carp	3.1	16.8	4.3	17.1	28.3	3.9		13.2	2.3		2.5	3.2		
Trout	2.7	20.9	8.3	3.9	18.4			7.3	1.6	3.2	1.7	5.8	Tr	7.0
Catfish	1.0	15.2	3.9	2.9	29.7	0.9		10.0	0.5	0.4	0.8	0.2	0.2	0.6

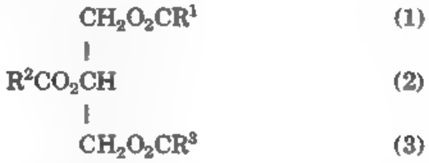
Source. Ref. 5.

Note: Values are percentage of total: Tr = trace.

Table 5. Component Fatty Acids of Some Vegetable Oils

	Fatty acid									
Oil	14:0	16:0	18:0	20:0	22:0	16:1	18:1	18:2	18:3	Total C_{18}
Cottonseed	1	29	4	Tr		2	24	40		68
Peanut	Tr	6	5	2	3	Tr	61	22		88
Sunflower		4	3				34	59		96
Corn		13	4	Tr	Tr		29	54		87
Sesame		10	5				40	45		90
Olive	Tr	14	2	Tr		2	64	16		82
Palm	1	48	4				35	9		51
Soybean	Tr	11	4	Tr	Tr		25	51	9	89
Safflower	Tr	8	3	Tr			13	75	1	92

Source: Ref. 10
Note: Values are weight %. Tr = trace.



$$\begin{array}{rl} & \mathrm{CH_2O_2CR^1} \qquad (1)\\ & | \\ \mathrm{R^2CO_2CH} & \qquad\qquad (2)\\ & | \\ & \mathrm{CH_2O_2CR^3} \qquad (3)\end{array}$$

The molecule is shown in the Fisher projection with the secondary hydroxyl pointing to the left. The location of fatty acids in the various positions on the glycerol molecule can be determined by stereospecific analysis (9). Several theories of glyceride composition have been proposed, such as even distribution, random distribution, and restricted random distribution. The distribution of fatty acids in the glycerides is of utmost importance for the physical properties of a fat. This is illustrated by pig fat and cocoa butter, which have similar fatty acid composition. In pig fat most of the unsaturation is located in the 1- and 3-positions, in cocoa butter it is in the 2-position (Table 6).

PHOSPHOLIPIDS

All fats and oils and fat-containing foods contain a number of phospholipids. The lowest amounts are present in animal fats such as lard and beef tallow. In some crude vegetable oils, such as cottonseed, corn, and soybean oils, phospholipids may be present at levels of 2 to 3%. Phospholipids are surface active, because they contain a lipophilic and a hydrophilic portion. Since they can be easily hydrated, they can be removed from fats and oils during the refining process. The structure of the most important phospholipids is given in Figure 2. The phospholipids removed from soybean oil are used as emulsifiers in foods. Soybean phospholipids, also known as soy lecithin, contain about 35% lecithin and 65% cephalin. The acyl groups in phospholipids are usually more unsaturated than those of the triglycerides in which they are present. Saturated fatty acids are found mostly in position 1 and unsaturated fatty acids in position 2.

UNSAPONIFIABLE COMPONENTS

The unsaponifiable portion of fats consists of sterols, terpenic alcohols, squalene, and hydrocarbons. In most fats the major unsaponifiable component is sterols. Animal fats contain cholesterol, and in some cases, minor amounts of the other sterols, such as lanosterol. Plant fats and oils contain phytosterols, usually at least three and sometimes four (10). The predominant phytosterol is β-sitosterol; others are campesterol and stigmasterol. The sterols are solids with high melting points; part of the sterols in natural fats are present as esters of fatty acids, part in free form. Cholesterol makes up 99% of the sterols of fish. The sterol content of some fats and oils is given in Table 7.

PROCESSING

In the commercial production of fats and oils, processing is used to separate, purify, and modify the oils and fats to make them suitable for the various functions they fulfill in the food system. A large portion of the seed oils produced in the temperate regions of the world are used in the form of solid fats: margarine, shortening, and frying and baking

Table 6. Positional Distribution of Fatty Acids in Pig Fat and Cocoa Butter

		Fatty acid, mole %					
Fat	Position	14:0	16:0	16:1	18:0	18:1	18:2
Pig fat	1	0.9	9.5	2.4	29.5	51.3	6.4
	2	4.1	72.3	4.8	2.1	13.4	3.3
	3	0	0.4	1.5	7.4	72.7	18.2
Cocoa butter	1		34.0	0.6	50.4	12.3	1.3
	2		1.7	0.2	2.1	87.4	8.6
	3		36.5	0.3	52.8	8.6	0.4

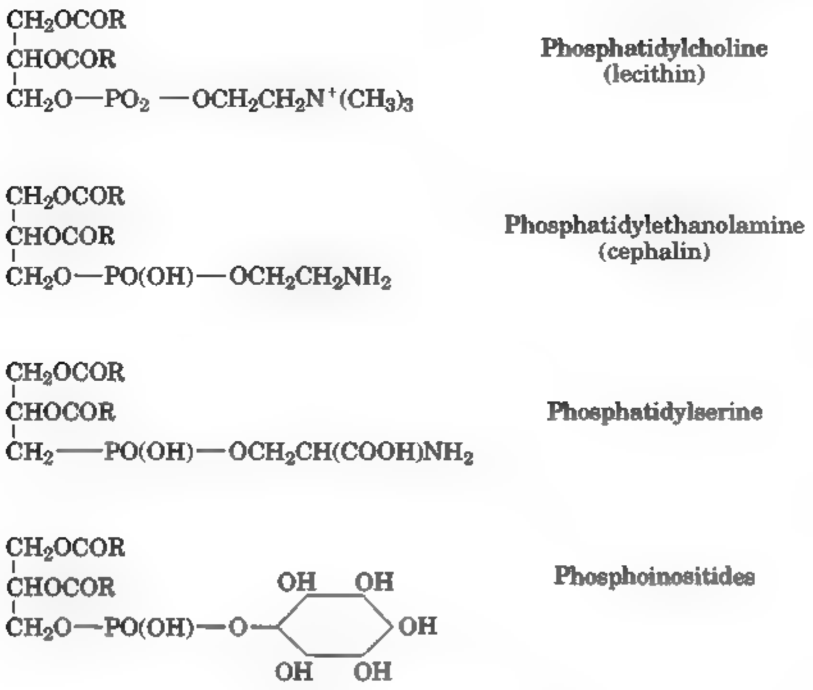


Figure 2. Structure of the major phospholipids.

fats. Hydrogenation is most often used to change oils into fats. Another use for hydrogenation is to improve the oxidative stability by partial hydrogenation to remove most of the linolenic acid.

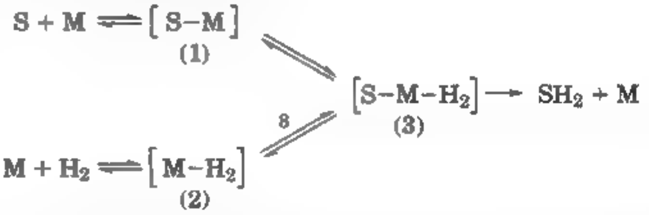
The separation of oils and fats from animal tissues is done by rendering, either dry rendering or steam rendering. The separation of oils from oilseeds usually involves a pretreatment, crushing or flaking of the seeds, followed by pressing (11). This is usually followed by solvent extraction to remove the remainder of the oil and yield a residue with less than 1% residual oil (12).

The crude oils obtained by rendering, pressing, and/or extraction are purified by a series of operations designed to remove impurities that may detract from the quality of the oil. Removal of phospholipids is achieved by degumming (13). The crude oils are treated with steam, which hydrates the phospholipids and makes them settle out. Degumming can also be achieved by using solutions of phosphoric or organic acids. The soybean "gums" are purified and used as food emulsifiers, known as soy lecithin.

Free fatty acids in crude oils are removed by alkali refining (13). Solutions of caustic soda are used to reduce the level of free fatty acid to 0.01 to 0.03%. Care is required to prevent saponification of neutral oil. Removal of free fatty acids can also be achieved by physical refining. This involves treatment of the oils under vacuum with steam. The advantage of physical refining is that the process is similar to deodorization and these processes can be combined. A possible disadvantage of physical refining relates to the high temperatures (up to 270°C) employed in this process. This may cause randomization of the glyceride structure, formation of dimers and conjugated fatty acids (positional isomerization) and *cis–trans* isomerization (14).

Bleaching is used to remove colored impurities, such as carotenoids and chlorophyll. In the bleaching process the oils are treated with bleaching earth or activated carbon. The yellow-red color of most vegetable oils, mostly carotenoids, is easily removed by bleaching earth. The green and brown pigments are more difficult to remove.

After refining and bleaching, vegetable oils are further processed into margarines, shortenings, and frying and baking fats. Two-thirds of all liquid oils produced in North America are used in the form of fats. Hydrogenation is used to change oils into fats, and involves the reaction of gaseous hydrogen, liquid oil, and solid catalyst under pressure and at high temperature (15). The catalyst used for edible oil hydrogenation is invariably of the activated nickel metal type (16). The hydrogenation reaction can be represented by the following scheme, in which the reacting species are the olefinic substrate (S), the metal catalyst (M), and hydrogen:



The intermediates **1**, **2**, and **3** are organometallic species and are labile and short-lived and cannot usually be isolated. In heterogeneous catalysis the metal surface performs the catalytic function. In theory, the finer the particle size, the more active the catalyst will be. In practice, however, particle size has to be balanced against filterability, since removal of the catalyst at the end of the process should not be too difficult.

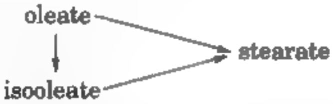
When hydrogen is added to double bonds in natural fats and the reaction is not carried to completion, a complex

Table 7. Sterol Content of Fats and Oils

Fat	Sterol, %
Lard	0.12
Beef tallow	0.08
Milk fat	0.3
Herring	0.2–0.6
Cottonseed	1.4
Soybean	0.7
Corn	1.0
Rapeseed	0.4
Coconut	0.08
Cocoa butter	0.2

mixture of reaction products results. Hydrogenation may be selective or nonselective. Selectivity means that hydrogen is added first to the most unsaturated fatty acids. Selectivity is increased by increasing hydrogenation temperature and decreased by increasing pressure and agitation. Selectively hydrogenated oil is more resistant to oxidation because of the preferential hydrogenation of the linolenic acid.

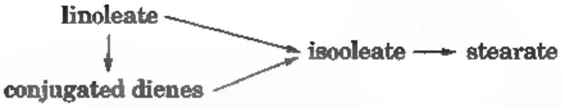
Another important factor in hydrogenation is the formation of positional and geometric isomers. Formation of trans isomers is rapid and extensive. The isomerization can be understood by the reversible character of chemisorption. When the olefinic bond reacts, two carbon-metal bonds are formed as an intermediate stage. The intermediate may react with an atom of adsorbed hydrogen to yield the half-hydrogenated compound, which remains attached by only one bond. Additional reaction with hydrogen would result in formation of a saturated compound. There is also the possibility that the half-hydrogenated olefin may again attach itself to the catalyst surface at a carbon on either side of the existing bond, with simultaneous loss of hydrogen. Upon desorption of this species a positional or geometric isomer may result. The proportion of trans isomers is high because this is the more stable configuration. Double bond migration occurs in both directions, but probably more extensively in the direction of the terminal methyl group. The hydrogenation of oleate can be represented as follows:



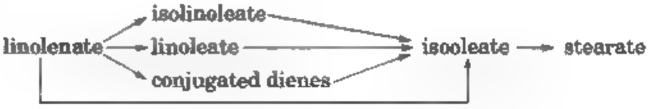
The change from oleate to isooleate involves no change in unsaturation but does result in a considerably higher melting point. This is why the hardening effect of hydrogenation is only partly the result of saturating double bonds; trans-isomer formation has a major effect on hardness.

For example, olive oil with an iodine value of 80 is liquid at room temperature. When soybean oil is hydrogenated to the same iodine value it is a fat with the consistency of lard.

Hydrogenation of linoleate first produces some conjugated dienes, followed by the formation of positional and geometric isomers of oleic acid, and finally stearate:



Hydrogenation of linolenate is more complex and is greatly dependent on reaction conditions. The possible reactions can be summarized as follows:



In the nonselective hydrogenation of seed oils, polyunsaturated fatty acids are rapidly isomerized or reduced and trans isomers increase to high levels (Fig. 3).

Interesterification is a process whereby fatty acid radicals can be made to move from one hydroxyl of a glycerol moiety to another one, either within the same glyceride or to another glyceride. The reaction pattern has been described (17):

$$RCOOR^2 + R^1COOR^3 \rightarrow RCOOR^3 + R^1COOR^2$$

The reaction is used in industry to modify the crystallization behavior and the physical properties of fats. The catalysts are usually alkaline and consist of sodium methoxide or alloys of sodium and potassium. At temperatures above the melting points of the reactants, several raw materials may be interesterified together so that new products are produced. If the reaction is carried out below the melting point, so that only the liquid fraction reacts, the process is called directed interesterification. Industrially, lard has been interesterified to improve its properties. Lard has a narrow plastic range, creams poorly, and gives poor cake volume. After interesterification these properties are greatly improved. Acetoglycerides can be prepared

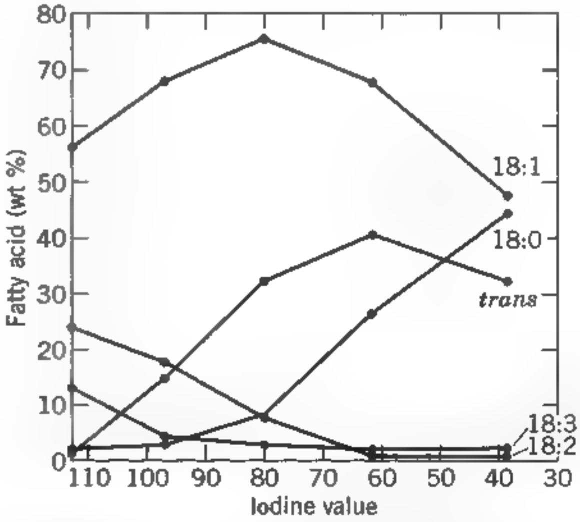


Figure 3. Change in fatty acid composition during hydrogenation of canola oil.

by interesterification of natural fats with glyceryl triacetate. The resultant products are waxy, translucent materials that can be used as edible coatings and plasticizers.

Interesterification may provide an alternative to hydrogenation for the production of margarine and shortening fats. Hydrogenation has the disadvantage of forming trans isomers and of losing essential fatty acids. Interesterification of liquid oils with highly saturated fats, obtained by complete hydrogenation or by fractionation, may result in nutritionally more desirable fats.

Ester interchange of fats with a large excess of glycerol, at high temperature, under vacuum, and in the presence of a catalyst, results in an equilibrium mixture of mono-, di-, and triglycerides. After removal of excess glycerol, the mixture is called technical monoglyceride. Technical monoglycerides are used as emulsifying agents in foods. Molecular distillation yields products with well over 90% 1-monoglycerides; these are also widely used in foods.

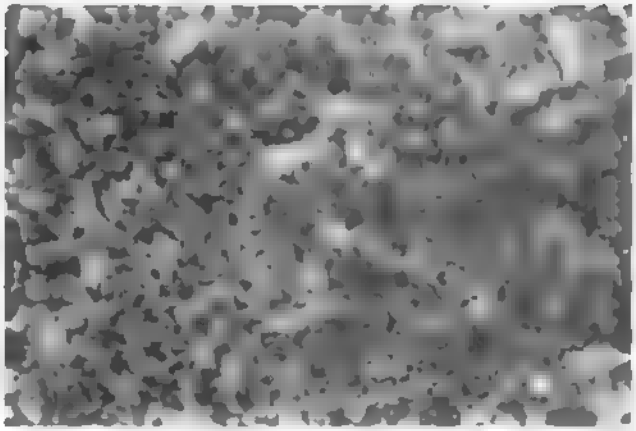


Figure 4. Fat crystals in a fat as seen in the polarizing microscope.

PHYSICAL PROPERTIES

Fats and oils are long-chain compounds that have particular physical properties of importance in processing and final use. Properties of surface activity, viscosity, solubility, and melting behavior are important in formulating emulsions and fat products such as shortenings and margarines.

The most important aspect of the physical properties of fats and oils is related to the solid-liquid and liquid-solid phase changes; in other words, melting and solidification. In fact, fats can be defined as partially solidified oils. When oils solidify they form crystals, usually of a size in the range of 1 to 10 μm. These fat crystals form a three-dimensional network that lends solid properties to the fat. The nature of this crystal network determines the rheological or textural properties of the product (18). The amount and size of the crystals in a fat determine its physical properties by influencing the density of junction points and, therefore, the strength of the crystal network. The crystals are held together by weak van der Waals forces and these can be disrupted by working or kneading. Upon resting, many of these bonds may be reestablished and the product is said to be thixotropic. Not all bonds are reformed after working, leading to what is known as work softening. The crystals in fats can be observed by polarized light microscopy as shown in Figure 4 (19).

The proportion of solids in a fat is of major importance in determining the rheological properties of a product. Fats may retain their solid character with solid fat content as low as 10%. Desirable spreadability occurs in a narrow range of solid fat content, roughly 15–35%. This is called the plastic range of fats.

Solidification of liquid oil results in a volume contraction and a positive (exothermic) heat effect. Melting of a fat results in volume expansion and a negative (exothermic) heat effect. Traditional methods of determination of solid fat depend on specific volume measurement at a given temperature (dilatometry). The heat effect is used in differential scanning calorimetry to determine melting and solidification properties. Modern methods of solid fat determination are based on nuclear magnetic resonance. Protons in solid fat behave differently in a magnetic field after being excited by radio-frequency energy than protons in liquid fat. This enables rapid and accurate estimation of solid fat content. The dilatometric method is empirical and the results are expressed as solid fat index (SFI).

In addition to crystal content and crystal size and shape, an important factor in the solidification of fats is polymorphism (the existence of different crystal modifications). Polymorphism results from different patterns of molecular packing in fat crystals. Triglycerides may occur in three main forms named α, β′, and β, in order of increasing stability. After crystals of a lower melting form have been produced, change into a higher melting form may take place. The change is monotropic, that is, it always proceeds in the sequence from lower to higher stability. When a fat is crystallized in an unstable form and heated at a temperature slightly above its melting point, it may resolidify in a more stable form (18). The melting point of tristearin in the α form is 54.7°C; in the β′ form, 64.0°C; and in the β form, 73.3°C. The cross-sectional structures of long-chain compounds in the different polymorphic forms is shown in Figure 5. In the α form the chain axes are randomly oriented and the crystals are of the hexagonal type. In the β′ form alternate rows are oriented in opposite directions and the crystal type is orthorhombic. In the β form the rows are oriented in the same direction and the crystal type is triclinic. The polymorphic forms are also distinguished by differences in the angle of tilt of the unit

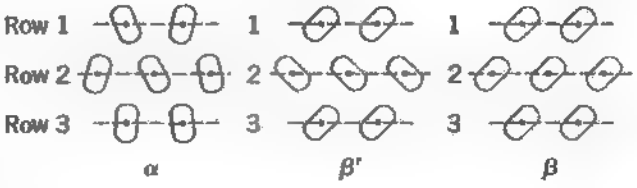


Figure 5. Cross-sectional structures of long-chain compounds. *Source:* Ref. 18, courtesy of *Journal of the American Oil Chemists' Society*.

cell in the crystal. X-ray diffraction analysis enables measurement of the short spacings and long spacings of the unit cell. Principal short spacings of the polymorphic forms are: α, 4.15 Å; β', 4.2 and 3.8 Å; β, 4.6, 3.85, and 3.7 Å (20). Fat crystals of different polymorphs have different crystal habit. That is, they appear to have different shapes when seen in the microscope: α crystals are fragile, transparent platelets of about 5-μm size; β' crystals are tiny needles about 1 μm long; β crystals are large and coarse, averaging 25 to 50 μm in size (21). These crystals can now be made visible by scanning electron microscopy (22).

For the production of margarines and most shortenings, the fat should be present in the β' form. Some fats have a tendency to convert slowly from the β' to the β form and this results in a coarsening of the crystal structure, making the product unacceptable. Fats which contain a very high level of fatty acids with identical chain lengths are especially prone to this effect. Canola and sunflower oils are notorious in this respect and have well over 90% of C_{18} fatty acids (Table 5).

The melting point of a fat is basically determined by the melting points of its constituent fatty acids. When we speak of the melting point of a fat we mean the end of the melting range, since only simple substances have a sharp melting point. The end of the melting range of a fat is dependent on the method used. Chain length and unsaturation of fatty acids determine their melting point. In addition, the configuration around the double bond is important, as is the arrangement of fatty acids at the different positions of the glycerides. Trans-unsaturated fatty acids have much higher melting points than their cis counterparts, making them more comparable with saturated fatty acids. For example, oleic acid (*cis*) has a melting point of 13°C and elaidic acid (*trans*) has a melting point of 44°C.

AUTOXIDATION AND ANTIOXIDANTS

The unsaturated bonds present in all oils and fats represent active centers, which may react with oxygen. This reaction leads to the formation of primary, secondary, and tertiary oxidation products which may result in the fat or fat-containing food becoming unfit for consumption. Oxidation of fats and oils may occur by a free-radical chain reaction known as autoxidation. Another process of oxidative deterioration involves the presence of a sensitizer and exposure to light. This is known as photosensitized oxidation (23,24).

The process of autoxidation and the resulting deterioration in flavor of fats and fatty foods are often described by the term rancidity. Lundberg (25) distinguishes several types of rancidity. When fats are exposed to oxygen, common oxidative rancidity will result in sweet but undesirable odors and flavors which will progressively become more intense and unpleasant. Flavor reversion is the term used for the objectionable flavors that develop in oils containing linolenic acid, especially soybean oil. This type of oxidation is produced with considerably less oxygen than common oxidation.

Among the many factors which affect the rate of oxidation are the following: amount of oxygen present, degree of unsaturation of the oil, presence of pro- and antioxidants, presence of heme-containing molecules and lipoxidase, light exposure, nature of packaging material, and storage temperature.

The autoxidation reaction can be divided into the following three parts: initiation, propagation, and termination. During the initiation, hydrogen is abstracted from an olefinic compound to yield a free radical:

$$RH \rightarrow R^{\cdot} + H^{\cdot}$$

The removal of hydrogen takes place at the carbon atom next to the double bond. The dissociation energy of hydrogen in various olefinic compounds has been listed by Ohloff (26). The value for an isolated double bond is 103 kcal/mol; for two double bonds separated by a methylene group it is only 65 kcal/mol. Once a free radical is formed, it will combine with oxygen to form a peroxy free radical, which can in turn abstract hydrogen from another unsaturated molecule to yield a peroxide and a new free radical, thus starting the propagation reaction. This may be repeated up to several thousand times and has the nature of a chain reaction:

$$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot}$$

$$RO_2^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$$

The propagation reaction can be followed by termination if the free radicals react with themselves to yield nonactive products:

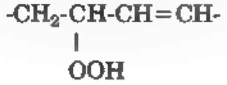
$$R^{\cdot} + R^{\cdot} \rightarrow R\text{-}R$$

$$R^{\cdot} + RO_2^{\cdot} \rightarrow RO_2R$$

$$nRO_2^{\cdot} \rightarrow (RO_2)_n$$

The hydroperoxides formed in the propagation part of the reaction are the primary oxidation products. The hydroperoxide mechanism of autoxidation has been described (27) and reviewed (23). The primary oxidation products are unstable and decompose into secondary oxidation products, mostly carbonyls. The peroxides are not important in flavor deterioration, which is caused wholly by secondary oxidation products. In the initial stages of the reaction there is a slow increase in the amount of hydroperoxides formed; this is the induction period. At the end of the induction period there is a sudden and rapid increase in peroxide content. The induction period is measured in accelerated tests to determine the storage stability of a fat or oil.

The rate of oxidation depends greatly on the degree of unsaturation. In the series of 18-carbon-atom fatty acids 18:0, 18:1, 18:2, 18:3, the relative rates of oxidation have been reported to be in the ratio of 1:100:1200:2500. In addition to the degree of unsaturation, the position of the double bonds in a polyunsaturated fatty acid may affect its oxidation rate. Zhan and Chen (28) found that conjugated linolenic acid oxidized considerably faster than linolenic acid. The reaction of unsaturated compounds proceeds by the abstraction of hydrogen from a carbon α to the double bond, resulting in a free radical stabilized by resonance. These free radicals are then transformed into a number of isomeric hydroperoxides of the general structure



$$\underset{\displaystyle OOH}{\text{-}CH_2\text{-}CH\text{-}CH{=}CH\text{-}}$$

In addition to the changes in double bond position, there are isomerizations from cis to trans and 90% of the peroxides formed may be in the trans configuration (25).

The decomposition of hydroperoxides has been described (29). It involves decomposition to alkoxy and hydroxy aldehydes, which are in great part responsible for the oxidized flavor of fats. They are powerful flavor compounds with very low flavor thresholds. For example, 2,4-decadienal has a flavor threshold of less than one part per billion.

Trace metals, especially copper, and to a lesser extent iron, will catalyze fat oxidation; metal deactivators, such as citric acid, can be used to counteract the effect of metals.

Antioxidants may be present naturally or added to fats and oils. Many foods contain natural antioxidants; the tocopherols are the most important of these. They are present in greater amounts in vegetable oils than in animal fats, which may explain the greater stability of the former. Antioxidants react with free radicals, thereby terminating the chain reaction. The synthetic antioxidants are phenolic compounds and limited in number. The four most widely used are BHA (butylated hydroxy anisole), BHT (butylated hydroxy toluene), PG (propyl gallate), and TBHQ (tertiary butyl hydroquinone). BHT is volatile with steam, which makes it less suitable for use in frying oils and baked products. Nonvolatile antioxidants are said to have carry-through properties for these applications.

EMULSIONS AND EMULSIFIERS

An emulsion is a heterogeneous system of two immiscible liquids, one of which is intimately dispersed in the other. The droplets of the dispersed phase generally have a diameter of over 0.1 μm. The emulsions are stabilized by the presence of a third component, the emulsifier, a surface-active agent which is partly soluble in both phases (30). Food emulsions usually contain water and oil as the immiscible phases, giving rise to emulsions of the oil-in-water (O/W) type or water-in-oil (W/O) type. The action of emulsifiers can be enhanced by the presence of stabilizers. Emulsifiers are surface-active compounds that have the ability to reduce the interfacial tension between liquid-liquid and air-liquid interfaces. This ability is the result of an emulsifier's molecular structure: the molecules contain two distinct sections, one having a polar or hydrophilic character, the other having nonpolar or hydrophobic properties. Most surface-active agents reduce the surface tension from about 50 dynes/cm to less than 10 dynes/cm when used in concentrations below 0.2%.

The relative sizes of the hydrophilic and hydrophobic sections of an emulsifier molecule mostly determine its behavior in emulsification. To select the proper emulsifier for a given application, the so-called HLB system was developed (HLB — hydrophile-lipophile balance). It is a numerical expression for the relative simultaneous attraction of an emulsifier for water and for oil. The HLB of an emulsifier is an indication of how it will behave but not how efficient it is. Emulsifiers with low HLB tend to form W/O emulsions, those with intermediate HLB form O/W emulsions, and those with high HLB are solubilizing agents. The HLB value can be either calculated or determined experimentally (31). The scale goes from 0 to 20, at least in theory, since at each end of the scale the compounds would have little emulsifying activity. The HLB value of some commercial nonionic emulsifiers is given in Table 8 (32).

Foods contain many natural emulsifiers, of which phospholipids are the most common.

Emulsions are stabilized by a variety of compounds, mostly macromolecules such as proteins, starches, and gums.

NOVEL FATS AND OILS

The modification of the composition of fats and oils to improve or change their functional or nutritional properties is a well-established practice. The process of hydrogenation was invented in 1897 and is still used widely in the fats and oils industry. Other means of modifying the properties of fats and oils include interesterification, fractionation, blending, and combinations of these techniques.

Plant breeding has been used to not only produce improved yields of oilseeds but also to change the composition of many oilseeds, the first example of which was low–erucic acid rapeseed oil (LEAR), or canola oil; it is now one of the

Table 8. HLB Values of Some Commercial Nonionic Emulsifiers

Trade name	Chemical designation	HLB
Span 85	Sorbitan trioleate	1.8
Span 85	Sorbitan trioleate	1.8
Span 65	Sorbitan tristearate	2.1
Atmos 150	Mono- and diglycerides from the glycerolysis of edible fats	3.2
Atmul 500	Mono- and diglycerides from the glycerolysis of edible fats	3.5
Atmul 84	Glycerol monostearate	3.8
Span 80	Sorbitan monooleate	4.3
Span 60	Sorbitan monostearate	4.7
Span 40	Sorbitan monopalmitate	6.7
Span 20	Sorbitan monolaurate	8.6
Tween 61	Polyoxyethylene sorbitan monostearate	9.6
Tween 81	Poly(oxyethylene sorbitan monooleate)	10.0
Tween 85	Polyoxyethylene sorbitan trioleate	11.0
Arlacel 165	Glycerol monostearate (acid stable, self-emulsifying)	11.0
Myrj 45	Polyoxyethylene monostearate	11.1
Atlas G-2127	Polyoxyethylene monolaurate	12.8
Myrj 49	Polyoxyethylene monostearate	15.0
Myrj 51	Polyoxyethylene monostearate	16.0

Source: Ref. 30.

world's most widely used vegetable oils. In LEAR oil, the level of erucic acid is less than 1%, and erucic acid has been replaced by oleic acid. This oil is still about 10% linolenic acid by weight, similar to the level in soybean oil. Plant breeders have developed low-linolenic-acid varieties of canola and soybean oil. These oils have improved oxidative stability and are better suited for the purpose of frying (33). Both sunflower and safflower oils have been modified by plant breeding to attain high levels of oleic acid. These are commercially available as Sunola (about 85% oleic acid) and Saffola (about 75% oleic acid) (34).

Genetic engineering has developed into a powerful technique to modify oilseed plants. In addition to changing agronomic properties such as yield and resistance to pests and herbicides, major changes in fatty acid and glyceride composition have been achieved (35). The rapeseed plant is easily amenable to genetic manipulation and has been the basis for the development of laurate canola, which can be used as a replacement of the tropical oils obtained from coconut and palm kernel. Because of its special glyceride structure, with no lauric acid in the 2-position, this is a truly novel oil. When partially hydrogenated, this oil may have many applications in the food industry (confectionery coatings, baked goods, coffee whitener, icings, toppings, etc.).

Fat replacers are substances that are meant to partially or completely replace the calories provided by fats (9 kcal/g). They may consist of fat-like substances that are not or only partly absorbed by humans. One example is Olestra, a sucrose polyester with six to eight acyl groups. The fatty acids are derived from soybean, corn, cottonseed, or sunflower oil. Olestra is not absorbed in the human digestive system and therefore yields no calories. Other fat replacers are based on the fact that the level of 9 kcal/g does not apply when short-chain fatty acids are present in the triglycerides. It is also known that long-chain saturated fatty acids, such as stearic acid, are incompletely metabolized and yield less than 9 kcal/g. By combining these two types of fatty acids into glycerides, fats are obtained that have energy values of 5 kcal/g. A product range of this type is Salatrim (an acronym for short and long acyl triglyceride molecules). It contains at least one stearic acid and one short-chain acid in each of the glycerides. It is produced by fully hydrogenating a liquid oil (canola or soybean) and then interesterifying with short-chain fatty acids (acetic, propionic, or butyric). By varying the composition, a family of products is obtained that range from solid to liquid at room temperature (36).

Medium-chain triglycerides (MCTs) are another type of structured fat made by transesterification of caprylic and capric fatty acids derived from coconut or palm kernel oils (37).

BIBLIOGRAPHY

1. J. M. de Man, *Principles of Food Chemistry*, 3rd ed., Aspen Publishers Inc., Gaithersburg, Md., 1999.
2. L. M. Smith, "Introduction to the Symposium on Milk Lipids," *Journal of the American Oil Chemists' Society* **50**, 175–177 (1973).
3. R. G. Jensen, "Composition of Bovine Milk Lipids," *Journal of the American Oil Chemists' Society* **50**, 186–192 (1973).
4. R. G. Ackman, "The Analysis of Fatty Acids and Related Materials by Gas-Liquid Chromatography," in R. T. Holman, ed., *Progress in the Chemistry of Fats and Other Lipids*, Vol. 12, Pergamon Press, Oxford, 1972.
5. R. G. Ackman, "Marine Lipids and Fatty Acids in Human Nutrition," *FAO Technical Conference on Fishery Products, Japan*, 1973.
6. R. G. Ackman, "The Year of the Fish Oils," *Chemical Industries* March, 139–145 (1988)
7. A. P. Simopoulos, "ω-3 Fatty Acids in Growth and Development and in Health and Disease," *Nutrition Today*, 10–19 (March/April 1988).
8. J. K. Daun. "Composition and Use of Canola Seed, Oil, and Meal," *Cereal Foods World* **29**, 291–293 (1984).
9. A. Kuksis. "Newer Developments in Determination of Structure of Glycerides and Phosphoglycerides," in R. T. Holman, ed., *Progress in the Chemistry of Fats and Other Lipids*, Vol. 12., Pergamon Press. Oxford, 1972.
10. E. Fedeli and G. Jacini, "Lipid Composition of Vegetable Oils," *Advances in Lipid Research* **9**, 335–382 (1971).
11. D K. Bredeson. "Mechanical Oil Extraction," *Journal of the American Oil Chemists Society* **60**, 211–213 (1983).
12. P. L. Christensen, "Solvent Extraction: Recent Developments," *Journal of the American Oil Chemists' Society* **60**, 214–215 (1983).
13. G. Haraldsson, "Degumming, Dewaxing and Refining," *Journal of the American Oil Chemists' Society* **60**, 251–256 (1983).
14. G. Hoffmann, *The Chemistry and Technology of Edible Oils and Fats and their High Fat Products*, Academic Press, London, 1989.
15. R. Larsson, "Hydrogenation Theory: Some Aspects," *Journal of the American Oil Chemists' Society* **60**, 275–281 (1983).
16. M. J. Beckman, "Hydrogenation Practice," *Journal of the American Oil Chemists' Society* **60**, 282–290 (1983).
17. M. W. Formo. "Ester Reactions of Fatty Materials," *Journal of the American Oil Chemists Society* **31**, 548–549 (1954).
18. J. M. de Man and A. M. Beers, "Fat Crystal Networks: Structure and Rheological Properties," *Journal of Texture Studies* **18**, 303–318 (1987).
19. E. S. Lutton. "Technical Lipid Structures." *Journal of the American Oil Chemists' Society* **49**, 1–9 (1972).
20. J. M. de Man, "Microscopy in the Study of Fats and Emulsions," *Food Microstructure* **1**, 209–222 (1982).
21. C. W. Hoerr, "Morphology of Fats, Oils and Shortenings," *Journal of the American Oil Chemists' Society* **37**, 539–546 (1960).
22. A. N Mostafa, A. K. Smith, and J. M. de Man, "Crystal Structure of Hydrogenated Canola Oil," *Journal of the American Oil Chemists' Society* **62**, 760–762 (1985).
23. E. N. Frankel. "Lipid Oxidation: Mechanisms. Products and Biological Significance," *Journal of the American Oil Chemists' Society* **61**, 1908–1918 (1984).
24. A. Sattar and J. M. de Man, "Photo-oxidation of Milk and Milk Products: A Review," *Critical Reviews in Food Science and Nutrition* **7**, 13–38 (1975).
25. W. O. Lundberg, *Autoxidation and Antioxidants*, John Wiley & Sons, Inc., New York, 1961.
26. G. Ohloff, "Fats are Precursors," in J. Solms, ed., *Functional Properties of Fats in Foods*, Forster Publishing Ltd., Zürich, 1973.

27. E. H. Farmer, "Peroxidation in Relation to Olefinic Structure," *Transactions of the Faraday Society* 42, 228–236 (1946).
28. A. Zhang and Z. Y. Chen, "Oxidative Stability of Conjugated Linoleic Acids Relative to Other Polyunsaturated Fatty Acids," *Journal of the American Oil Chemists' Society* 74, 1611–1613 (1997).
29. M. Keeney, "Secondary Degradation Products," in H. W. Schultz, E. A. Day and R. O. Sinnhuber, eds., *Lipids and Their Oxidation*, AVI Publishing Co., Inc., Westport, Conn., 1962.
30. P. Becher, "Emulsions—Theory and Practice," Van Nostrand Reinhold Co., New York, 1965.
31. S. Friberg, *Food Emulsions*, Marcel Dekker Inc., New York, 1976.
32. W. C. Griffin, "Emulsions," in *Kirk-Othmer Encyclopedia of Chemical Technology*, 2nd ed., Vol. 8, 1965, pp. 117–154.
33. M. D. Erickson and N. Freg, "Property Enhanced Oils in Food Applications," *Food Technology* 48, 63–68 (1994).
34. F. D. Gunstone, "Movements toward Tailor-Made Fats," *Progress in Lipid Research* 37, 277–305 (1998).
35. M. Lassner, "Transgenic Oil Crops: A Transition from Basic Research to Product Development," *Lipid Technology* 9, 5–9 (1997).
36. R. Kosmark, "Salatrim: Properties and Applications," *Food Technology* 50, 98–101 (1996).
37. C. C. Akoh, "Making New Structured Fats by Chemical Reaction and Enzymatic Modification," *Lipid Technology* 9, 61–66 (1997).

GENERAL REFERENCES

T. P. Hilditch and P. N. Williams, *The Chemical Constitution of Natural Fats*, 4th ed., John Wiley & Sons Inc., New York, 1964.

A. Kuksis, ed., *Handbook of Lipid Research*, Vol. 1. *Fatty Acids and Glycerides*, Plenum Press, New York, 1988.

V. C. Mehlenbacher, *The Analysis of Fats and Oils*, The Garrard Press, Champaign, Ill., 1960.

H. B. W. Patterson, *Hydrogenation of Fats and Oils*, Applied Science Publishers, London, 1983.

C. Ratledge, P. Dawson, and J. Rattray, eds., *Biotechnology for the Oils and Fats Industry*, American Oil Chemists' Society, Champaign, Ill., 1984.

D. M. Small, *Handbook of Lipid Research, Vol. 4. The Physical Chemistry of Lipids*, Plenum Press, New York, 1986.

D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, 4th ed., 3 Volumes, John Wiley & Sons Inc., New York, 1979.

J. M. DE MAN
University of Guelph
Guelph, Ontario
Canada

FATS AND OILS: FLAVORS

Fats and oils comprising the bulk of foods such as shortenings, mayonnaise, and cooking oils often contribute unique flavors to the product. They are chemically referred to as lipids and occur in food mainly in the form of triglycerides. Fats and oils can either increase or decrease the flavor quality of foods, depending on the chemical reactions taking place during processing and storage. Under the right conditions fats and oils can be susceptible to oxidation, which produces undesirable volatile compounds and causes detrimental flavor effects to foods. The rate of oxidation in fats and oils is influenced by a wide variety of factors including oxygen, light, trace metals, antioxidants, sensitizers, enzymes, temperature, and fatty acid composition. Although thermal oxidation in deep-fat frying may provide good flavors, the volatile flavor compounds produced through autoxidation are mainly responsible for the deterioration of the flavor quality of oils during storage. Some of the most common volatile flavor compounds produced by fats and oils include esters, aldehydes, alcohols, ketones, lactones, and hydrocarbons. Unsaturated aldehydes and ketones, which have the lowest sensory threshold, are principally responsible for the undesirable oxidized flavor of food. The separation and identification of these volatile flavor compounds by gas chromatography and mass spectroscopy have increased the knowledge base for fats and oils flavor chemistry.

The isolation, separation, and identification of volatile compounds from fats and oils is difficult due to their chemical complexity, variations in concentrations and volatilities, interactions between the volatile compounds themselves, and also reactions between the volatile compounds and food components such as proteins and carbohydrates (1). The mechanisms responsible for the production of volatile flavor compounds during oxidation must be thoroughly studied and understood to improve the flavor quality of fats and oils.

MECHANISMS OF AUTOXIDATION

Autoxidation is a free-radical chain reaction that includes initiation, propagation, and termination steps:

$$
\begin{array}{rl}
\text{Initiation} & \mathrm{RH} \rightarrow \mathrm{R}\cdot + \mathrm{H}\cdot \\
\text{Propagation} & \mathrm{R}\cdot + \mathrm{O_2} \rightarrow \mathrm{ROO}\cdot \\
 & \mathrm{ROO}\cdot + \mathrm{RH} \rightarrow \mathrm{ROOH} + \mathrm{R}\cdot \\
\text{Termination} & \mathrm{ROO}\cdot + \mathrm{R}\cdot \rightarrow \mathrm{ROOR} \\
 & \mathrm{R}\cdot + \mathrm{R}\cdot \rightarrow \mathrm{RR} \\
 & \mathrm{ROO}\cdot + \mathrm{ROO}\cdot \rightarrow \mathrm{ROOR} + \mathrm{O_2}
\end{array}
$$

Unsaturated fatty acids undergo oxidation by the above free-radical chain reaction to form hydroperoxides. The hydroperoxides are decomposed to form off-flavor compounds, which are called secondary oxidative products (2,3). Though the reaction of singlet-state fatty acids with triplet-state oxygen is thermodynamically difficult, fatty acid free radicals can react with triplet-state oxygen molecules fairly easily. Heat, metal catalysts, and both ultraviolet and visible light can accelerate the formation of free radicals of unsaturated fats during the initiation step of autoxidation. Typically, a hydrogen atom is removed from the methylene group alpha to the double bond of the fatty acid to form a fatty acid free radical, which has a short lifetime due to its tendency to achieve a stable electron pair. The alkyl radical reacts with oxygen to form a peroxy radical. This peroxy radical then reacts with a hydrogen atom that was abstracted from another unsaturated fatty acid to form a hydroperoxide. The formation of hydroperoxides in this manner is usually accompanied by a shift in the position of the double bonds, due to resonance stabilization of the fatty acid free radical and resulting formation of isomeric hydroperoxides. A mixture of four isomeric hydroperoxides that have -OOH groups at the 8, 9, 10, and 11 carbon positions are produced in the autoxidation of oleic acid. Hydroperoxides themselves are generally tasteless, colorless, and odorless; however, they are relatively unstable and therefore readily decompose to form hydrocarbons, alcohols, esters, acids, aldehydes, and ketones, each having differing molecular weights and flavor thresholds. The principal aldehyde compounds formed from the decomposition of hydroperoxides at the 8, 9, 10, or 11 carbon atom positions are 2-undecenal, 2-decenal, nonanal, and octanal, respectively. Oxidized linoleic acid produces hydroperoxides at either the 9 or 13 position, and a new conjugated double bond system is formed at either the 10 or 11 position (4). The autoxidation of linolenic acid typically results in the formation of hydroperoxides at either the 9, 12, 13, or 16 position on the hydrocarbon.

MECHANISMS OF PHOTOSENSITIZED OXIDATION

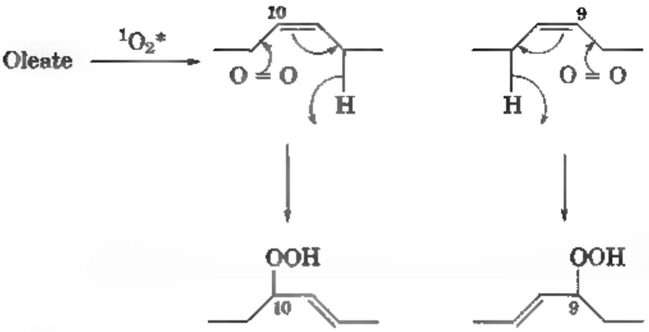
Although free-radical autoxidation is the primary mechanism for the formation of volatile flavor compounds from fats and oils, photosensitized oxidation initiated by sensitizers such as chlorophyll in the presence of light should be included as oxidation mechanisms that contribute to the formation of off-flavor volatile compounds (2,5). The reaction of fatty acids with oxygen to produce hydroperoxides requires a change in their electron spin states because the unsaturated fatty acid and hydroperoxide are in singlet-states, whereas oxygen is in a triplet-state. The reaction between singlet-state fatty acids and triplet-state oxygen to form singlet-state hydroperoxides can only occur if enough energy is present to overcome the spin barrier. Electrophilic singlet-state oxygen can directly react with the electron-rich, singlet-state unsaturated fatty acids to form singlet-state hydroperoxides. Naturally occurring pigments such as chlorophyll in vegetable oils can absorb ultraviolet and visible light and act as photosensitizers (sens) (6). The sensitizer transfers energy to triplet-state oxygen to form singlet-state oxygen, which reacts with unsaturated fatty acids to form singlet-state hydroperoxides:

$$\text{sens} \rightarrow {}^1\text{sens}^* \rightarrow {}^3\text{sens}^*$$

$${}^3\text{sens}^* + {}^3O_2 \rightarrow {}^1O_2 + {}^1\text{sens}$$

$${}^1O_2 + RH \rightarrow ROOH$$

Singlet-state oxygen reacts directly with singlet-state double bonds of unsaturated fatty acids by an "ene" reaction to form hydroperoxides:



The singlet-state oxygen molecule is joined onto one end of a double bond simultaneously as it abstracts an allylic proton, and a new double bond is formed between the allylic position and the other end of the original C = C bond (7). A mixture of conjugated and nonconjugated hydroperoxides is formed by the reaction of singlet oxygen with linoleic acid. Because the flavor stability and quality of vegetable oils can be decreased by singlet oxygen oxidation, attempts have been made to inhibit singlet oxygen oxidation. Antioxidants such as *β*-carotene, lycopene, and tocopherols, which have been found to be able to deactivate singlet-state oxygen back to its low-energy triplet-state (6,8,9), are effective at minimization of singlet oxygen oxidation.

THERMAL OXIDATION OF FATS AND OILS

Although autoxidation is the principal mechanism for the development of off-flavors in fats and oils, foods can retain desirable flavors from fats and oils during deep-fat frying (10). Thermal oxidative decomposition of fats and oils occurs as foods are continuously heated in oil at about 185°C in the presence of air. The lipid decomposition mechanism involved is virtually the same as in autoxidative processes, where a hydrogen atom is abstracted from fatty acids to form a fatty acid free radical, after which molecular oxygen reacts with the fatty acid free radical to form first the peroxy radical, and then the hydroperoxide. The hydroperoxide eventually decomposes to form volatile flavor compounds. Flavor compounds formed at deep-fat frying temperatures are different from those produced at room temperature, where each particular pathway of flavor compound formation has its own activation energy. When the reaction rate ($\ln K$) versus temperature ($1/T$) is plotted for specific flavor compounds, one compound might be produced at a higher rate at deep-fat frying temperatures, but when the temperature is decreased to room temperature, another flavor compound might be produced at a higher rate. Therefore, the formation of flavor compounds from fats and oils is strongly dependent on the processing temperature (11). Different fats and oils produce different volatile compounds during deep-fat frying due to differences in fatty acid composition. Corn and soybean oils produce relatively large quantities of decadienals and unsaturated aldehydes, whereas coconut oil produces more saturated aldehydes, methyl ketones, and γ and δ lactones during deep-fat frying (12). Coconut oil, which contains 90 to 94% saturated fatty acids, has extremely high thermal oxidative stability. It has been reported that γ lactones with unsaturation at the 2 or 3 position are of particular significance in deep-fat-fried flavors (13).

FACTORS AFFECTING OXIDATION

The rate of autoxidation in vegetable oils is affected by many factors including degree of unsaturation of fatty acids, metals, and antioxidants. For example, linoleic acid, which contains two double bonds, is oxidized at a faster rate than oleic acid, which contains only one double bond. The relative rates of autoxidation of oleic to linoleic to linolenic (3 double bonds) acid has been reported as 1:40–50:100 on the basis of oxygen uptake (14). Reasons for differences in autoxidative susceptibility include the fact that the bond strength of linoleic acid (52 kcal/mole) is much lower than oleic acid (77 kcal/mole), and linoleic acid has a higher reactivity with oxygen than oleic acid. Metals such as copper and iron that exist in two different valence states play an important catalytic role in the oxidation of fats and oils. Metals differ in their ability to catalyze the autoxidation of fats and oils depending on the concentration, reaction temperature, and polarity of the reaction medium. The addition of chelating agents such as ethylenediaminetetraacetic acid (EDTA), phospholipids, and/or free fatty acids to oils can improve their oxidative flavor stability by sequestering the potentially catalytic metal atoms (15).

Natural and synthetic compounds that directly reduce the oxidation of fats and oils in one of three ways: (*1*) by becoming oxidized themselves, (*2*) by donating hydrogen to fatty acid free radicals to terminate free-radical chemical chain reactions, or (*3*) by forming a complex between the lipid radical and the antioxidant radical. Examples of each type of reaction can be seen here:

$$\text{Antioxidant (A)} + O_2 \rightarrow \text{Oxidized Antioxidant} \quad (1)$$

$$R\cdot + AH \rightarrow RH + A\cdot \quad (2)$$

$$R\cdot + A\cdot \rightarrow RA \quad (3)$$

Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tocopherols, and propyl gallate are commonly used in the food industry to minimize the oxidation of fats and oils. The presence of light, oxygen, and high storage temperatures should be controlled to help antioxidants most effectively minimize oxidative deterioration in fats and oils.

FLAVOR FORMATION BY ENZYMATIC REACTIONS

Flavors associated with fats and oils sometimes originate through the activity of enzymes that first create fatty acid hydroperoxides and then volatile secondary products (16). The release of free fatty acids through lipolytic processes usually occurs before lipoxygenase, a major lipolytic enzyme, can catalyze the oxidation of the polyunsaturated fatty acids linoleic and linolenic acid to hydroperoxides. Lipoxygenase is limited to reactions with polyunsaturated fatty acids because of its substrate specificity for a *cis,cis* penta-1,4-diene unit with a methylene group at the ω-8 position, a structure that is only present in fatty acids containing at least two nonconjugated double bonds. Therefore, the higher the polyunsaturated fatty acid content in a vegetable oil, the more susceptible the oil is to enzymatic oxidation and degradation.

Vegetable oils may also contain the enzyme hydroperoxide lyase, which is responsible for catalyzing the formation of aldehydes directly from the fatty acid hydroperoxides formed by lipoxygenase (17). The "grassy" or "beany" flavors often attributed to fatty acid autoxidation in vegetable oils may also originate from the formation of such volatile compounds as *cis*-3-hexenal and hexanal through the cleavage of hydroperoxides by hydroperoxide lyase.

FLAVOR PROPERTIES OF FATS AND OILS

The types of volatile compounds produced from the oxidation of fats and oils are strongly influenced by the composition of the hydroperoxides formed as well as the extent of oxidative cleavage of double bonds contained in the fatty acids (18–20). Due to an abundance of possible reaction pathways, a variety of volatile compounds such as hydrocarbons, alcohols, furans, aldehydes, ketones, and acids are formed during oxidation. Most of the compounds responsible for oxidized flavors, however, are the aliphatic carbonyl compounds (21). The flavor characteristics and corresponding threshold values of aldehydes vary as shown in Table 1.

CONTRIBUTION OF SPECIFIC OILS TO FLAVOR

Soybean oil, the most abundant vegetable oil in the United States, contains approximately 7% linolenic acid. The relatively unsaturated hydrocarbon of linolenic acid results in it being highly susceptible to oxidation and often

Table 1. Flavor Characteristics and Threshold Values of Aldehydes

		Threshold value in oil (ppm)	
Aldehyde	Description	Odor	Taste
3:0	Sharp-irritating	3.60	1.00
5:0	Sharp-bitter	0.24	0.15
6:0	Green	0.32	0.08
7:0	Oily-putty	3.20	0.05
8:0	Fatty-soapy	0.32	0.04
9:0	Tallowy-soapy	13.50	0.20
10:0	Orange peels	6.70	0.70
5:1	Sharp-paint-green	2.30	1.00
6:1	Green	10.00	0.60
7:1	Putty-fatty	14.00	0.20
8:1	Woodbugs-fatty	7.00	0.15
9:1	Tallowy-cucumber	3.50	0.04
10:1	Tallowy-orange	33.80	0.15
7:2	Frying odor	4.00	0.04
9:2	Fatty-oily	2.50	0.46
10:2	Deep-fried	2.15	0.10

responsible for the oxidation of oil to produce off-flavor compounds. Soybean oil also contains photosensitizers such as chlorophyll that produce singlet oxygen in the presence of light (22). Singlet oxygen can directly react with fatty acid double bonds and oxidize the fatty acids at a rate 1,500 times that of autoxidation (23,24). Analysis of headspace has shown that 2,4-decadienals, 2-heptenal, 2,4-heptadienal, pentane, and hexanal are the principal volatile compounds formed from oxidized soybean oil (25). The flavor characteristics of oxidized soybean oil have been described as grassy or fishy, but a single volatile flavor compound that gives unique grassy or fishy flavor in oxidized soybean oil has not been identified (26). However, *trans*-2-hexenal and 2,6-nonadienal have been reported to give general grassy flavors, whereas deca-*trans*-2,cis-4,*trans*-7-trienol and oct-1-en-3-one have been linked to fishy flavors (13).

Vegetable oils such as corn, cottonseed, peanut, coconut, safflower, and olive oil, as well as animal fats such as lard and beef tallow provide unique flavor characteristics to foods. Oil processing, which involves the three steps of refining, bleaching, and deodorization, removes impurities that contribute to the development of undesirable flavors. Olive oil, which contains 50 to 83% oleic acid, is popular in the United States as a result of the positive nutritional implications of monounsaturated fats. The flavor compounds of olive oil have been separated and identified by gas chromatography–mass spectrometry. Hexanal, *trans*-2-hexenal, 1-hexanol, and 3-methylbutan-1-ol, which are formed by autoxidation, are the principal volatile compounds of olive oil. Its flavor can be influenced by many factors such as climatic and soil conditions, the maturation process of the olive, and storage conditions (27). Although many volatile compounds have been identified, specific chemical compounds that are responsible for olive oil's flavor have not been determined. Safflower oil, which contains 78% linoleic acid, possesses the highest content of polyunsaturated fatty acid of all the commercial oils. The high content of linoleic acid increases the susceptibility of the oil to oxidation, resulting in the formation of off-flavor compounds (28). Cottonseed oil, which contains 18% oleic acid and 53% linoleic acid, is mainly used in restaurants for frying potato chips, seafood, and snacks. The bland flavor of cottonseed oil reportedly does not mask the flavor of the product nor does it revert during deep-fat frying (29).

DETERMINATION OF FLAVOR COMPOUNDS

The isolation, separation, and identification of volatile flavor compounds is important to determine the chemical mechanisms of flavor compound formation from fats and oils during storage, to identify chemical compounds that are responsible for specific desirable and undesirable flavors, and to develop optimum processing and storage methods that can minimize the undesirable oxidation of fats and oils. Analysis of the flavor compounds present in fats and oils is difficult because of their volatility, presence in low concentrations, and likelihood of undergoing chemical alteration during isolation. Food flavors are extremely complex mixtures of volatile compounds that act individually or in combination to produce unique tastes and aromas. Any modification of the chemical composition of these compounds could drastically change the flavor characteristics of food. Solid phase microextraction (SPME) is a relatively new technology that holds great promise for aiding in the evaluation of flavors by gas chromatography (30). SPME is rapidly gaining popularity as the technique of choice in the isolation and identification of volatile compounds because of its ability to integrate sampling, extraction, concentration, and sample introduction in a single step. Both the cost and time involved with sample analysis is reduced, while at the same time efficiency and selectivity are increased (31).

Threshold values are significantly different among flavor compounds, thus the presence of a small quantity of a compound with a low threshold value could have a more significant influence on the overall flavor than the presence of a large quantity of a particular compound with a high threshold value (Table 2). Hydrocarbons, for example, have a threshold value of 90 to 2,150 ppm; alkanals, 0.04 to 1.0 ppm; and vinyl ketones, 0.00002 to 0.007 ppm (2). This wide range of threshold values for flavor compounds illustrates how one specific flavor compound could have a significant influence on the overall flavor regardless of the concentration. Several volatile compounds have been proven to be responsible for specific described flavors in oxidized fats, as shown in Table 3 (13).

SENSORY EVALUATION

Sensory evaluation is considered the most important test to determine the flavor quality and stability of oils and is therefore used to evaluate the effects of processing conditions, storage time and periods, and packaging environments on the flavor of fats and oils. The most common sensory evaluation method involving fats and oils is a hedonic scale of 1 to 10; with 1 indicating a "repulsive" flavor and 10 a "completely bland" flavor, as shown in Table 4 (32).

Sensory evaluation can be expensive, subjective, difficult to reproduce, time-consuming, and not readily available. As a result, chemical and physical methods such as peroxide value, thiobarbituric acid (TBA), conjugated diene determination, fluorescence, Schaal oven test, and active oxygen methods have been developed to more efficiently evaluate the flavor quality and stability of fats and oils. The identification of volatile compounds that are responsible for specific flavors such as fishy or grassy has been made possible by correlating sensory evaluations with instrumental analysis such as gas chromatography.

FLAVOR EVALUATION BY GAS CHROMATOGRAPHY

With recent advancements in isolation techniques for flavor compounds, gas chromatography, and statistical analyses, several evaluations of oil flavor quality by gas chromatographic methods have been published (32–36). These papers have reported excellent correlation coefficients of better than 0.9 between sensory scores and predicted sensory scores by gas chromatography of corn oil, soybean oil, sunflower oil, and hydrogenated soybean oil. When com-

Table 2. Effect of Threshold Values on Flavor Significance of Soybean Oil Volatiles

Major volatiles	Relative %	Threshold value[a] (ppm)	Weighted % (1-octen-3-ol)	Relative order
t,t-2,4-decadienal	33.7	0.10	2.5	2
t,c-2,4-decadienal	17.9	0.02	6.7	1
t,c-2,4-heptadienal	11.1	0.04	2.1	3
2-heptenal	5.6	0.2	0.21	8
t,t-2,4-heptadienal	4.5	0.1	0.34	7
n-hexanal	4.5	0.08	0.42	6
n-pentane	3.1	340	6.8×10^{-5}	16
n-butanal	1.5	0.025	0.45	5
2-pentenal	1.2	1.0	0.009	13
1-octen-3-ol	0.9	0.0075	0.9	4
2-pentyl furan	0.8	2.0	0.9	14
n-pentanal	0.7	0.07	0.075	10
2-hexenal	0.7	0.6	0.009	13
n-nonanal	0.7	0.2	0.026	11
n-heptanal	0.6	0.055	0.082	9
1-penten-3-ol	0.5	4.2	8.9×10^{-4}	15
2-octenal	0.5	0.15	0.025	12

Source: Ref. 23.
[a] Ref. 19.

Table 3. Compounds Responsible for Specific Flavors in Oxidized Fats

Flavor	Source compound(s)
Cardboard	Non-*trans*-2,-*trans*-6-dienal
Oily	Aldehydes
Painty	Pent-2-enal, aldehydes
Fishy	Deca-*trans*-2,*cis*-4,*trans*-7,trienol, oct-1-en-3-one
Grassy	*Trans*-hex-2-enal, nona-2,6-dienal
Deep-fried	Deca-*trans*-2,*trans*-4-dienal

Table 4. Sensory Evaluation of Oil

Flavor score		Description of flavor
10		Completely bland
9	(good)	Trace of flavor, but not recognizable
8		Nutty, sweet, bacony, buttery
7	(fair)	Beany, hydrogenated
6		Raw, oxidized, musty, weedy, burnt, grassy
5	(poor)	Reverted, rubbery, butter
4		Rancid, painty
3	(very poor)	Fishy, buggy
2		Intensive flavor and objectionable
1	(repulsive)	

pared with sensory evaluation, gas chromatography has many advantages including rapid analysis time, convenience, low cost, and high reproducibility. A preliminary gas chromatographic analysis of flavor compounds of mayonnaise has been done to predict the sensory quality of mayonnaise during storage (37). Various flavor compounds have been identified in mayonnaise including allyl isothiocyanate, acetic acid, ethyl acetate, pentane, and isomers of 2,4-decadienal. As storage time increased, levels of pentane and 2,4-decadienal increased, whereas other volatile concentrations stayed the same. The flavor compound allyl isothiocyanate, the major flavor constituent of mustard, and acetic acid and ethyl acetate, the flavor compounds in vinegar, did not change during the six-month storage period. There was a correlation coefficient of greater than 0.9 between actual sensory scores of mayonnaise and predicted sensory scores by gas chromatography. Therefore, the shelf life of mayonnaise can be accurately predicted by analyzing specific compounds associated with off-flavors such as pentane and 2,4-decadienal during storage periods.

Gas chromatography can also be used to study the effects of storage conditions and processing methods on the oxidation of fats and oils. Soybean and corn oils were exposed to different periods of light to produce a wide range of flavor quality (38). As the period of light storage increased, the amount of 2,4-decadienal (as measured by gas chromatography) increased and the sensory scores decreased. The correlation of sensory evaluation and instrumental analysis using gas chromatography was greater than 0.95.

BIBLIOGRAPHY

1. M. Supran, *Lipids as a Source of Flavor*, American Chemical Society, Washington, D.C., 1978.
2. E. N. Frankel, "Chemistry of Autoxidation: Mechanism, Products and Flavor Significance," in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, American Oil Chemists' Society, Champaign, Ill., 1985, pp. 1–37.
3. H. W.-S. Chan, "The Mechanism of Autoxidation," in H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, Orlando, Fla., 1987, pp. 1–16.
4. H. W. Chan and G. Levett, "Autoxidation of Methyl Linoleate. Separation and Analysis of Isomeric Mixtures of Methyl Linoleate Hydroperoxides and Methyl Hydroxylinoleates," *Lipids* **12**, 99–104 (1977).
5. I. Lee et al., "Quantitation of Flavor Volatiles in Oxidized Soybean Oil by Dynamic Headspace Analysis," *J. Am. Oil Chem. Soc.* **72**, 539–546 (1995).

6. M. Y. Jung and D. B. Min, "Effects of Quenching Mechanisms of Carotenoids on the Photosensitized Oxidation of Soybean Oil," *J. Am. Oil Chem. Soc.* **68**, 653–658 (1991).
7. H. W.-S. Chan and D. T. Coxon, "Lipid Hydroperoxides," in H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, Orlando, Fla., 1987, pp. 17–50.
8. S. Kaiser et al., "Physical and Chemical Scavenging of Singlet Molecular Oxygen by Tocopherols," *Arch. Biochem. Biophys.* **277**, 101–108 (1990).
9. H. Sies and W. Stahl, "Vitamins E and C, β-carotene, and other Carotenoids as Antioxidants," *Am. J. Clin. Nutr.* **62**, 1315S–1321S (1995).
10. W. W. Nawar, "Chemistry of Thermal Oxidation of Lipids," in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, Champaign, Ill., 1985, pp. 39–60.
11. S. S. Chang, R. J. Peterson, and C.-T. Ho, "Chemistry of Deep Fried Flavor," in M. K. Supran, ed., *Lipids as a Source of Flavor*, ACS Symposium Series 75, American Chemical Society, Washington, D.C., 1978, p. 18–22.
12. M. W. Formo et al., "Composition and Characteristics of Individual Fats and Oils," in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, John Wiley & Sons, New York, 1979, p. 289–478.
13. W. Grosch, "Lipid Degradation Products and Flavour," in I. D. Morton and A. J. Macleod, eds., *Food Flavours, Part A, Introduction*, Elsevier, Amsterdam, The Netherlands, 1982, pp. 325–398.
14. R. T. Holman and O. C. Elmer, "The Rates of Oxidation of Unsaturated Fatty Acids and Esters," *J. Am. Oil Chem. Soc.* **24**, 127–129 (1947).
15. J. Pokorny, "Major Factors Affecting the Autoxidation of Lipids," in H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, Orlando, Fla., 1987, pp. 141–206.
16. H. Brockerhoff and R. G. Jensen, *Lipolytic Enzymes*, Academic Press, New York, 1974.
17. R. Tressl and F. Drawert, "Biogenesis of Banana Volatiles," *J. Agric. Food Chem.* **21**, 560–565 (1973).
18. E. Selke, W. K. Rohwedder, and H. J. Dutton, "Volatile Components from Triolein Heated in Air," *J. Am. Oil Chem. Soc.* **54**, 62–67 (1977).
19. E. N. Frankel, J. Nowakowska, and C. D. Evans, "Formation of Methyl Azelaaldehydrate on Autoxidation of Lipids," *J. Am. Oil Chem. Soc.* **38**, 161–162 (1961).
20. A. C. Noble and W. W. Nawar, "Identification of Decomposition Products from Autoxidation of Methyl 4,7,10,13,16,19-Docosahexaenoate," *J. Am. Oil Chem. Soc.* **52**, 92–95 (1975).
21. D. A. Forss, "Odor and Flavor Compounds from Lipids," *Prog. Chem. Fats and Other Lipids* **13**, 177–258 (1972).
22. Y. A. Tan, C. L. Chong, and K. S. Low, "Crude Palm Oil Characteristics and Chlorophyll Content," *J. Sci. Food Agric.* **75**, 281–288 (1997)
23. E. N. Frankel, "Soybean Oil Flavor Stability," in D. R. Erickson, et al., eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Mo., and American Oil Chemists' Society, Champaign, Ill., 1980, pp. 229–244.
24. H. R. Rawls, and P. J. Van Santen, "A Possible Role for Singlet Oxygen in the Initiation of Fatty Acid Autoxidation," *J. Am. Oil Ch. Soc.* **47**, 121–125 (1970).
25. E. N. Frankel et al., "Comparison of Gas Chromatographic Methods for Volatile Lipid Oxidation Compounds in Soybean Oil," *J. Am. Oil Chem. Soc.* **62**, 620 (1985).
26. T. Mounts, "Processing of Soybean Oil for Food Uses," *Cereal Foods World* **34**, 268 (1989).
27. G. Montedoro, M. Bertuccioli, and G. Anichini, "Flavor of Foods and Beverages," Academic Press, Orlando, Fla., 1972.
28. T. J. Weiss, *Food Oils and Their Use*, AVI Publishing, Westport, Conn., 1970.
29. J. D. Dziezak, "Fats, Oils, and Fat Substitutes," *Food Technol.* **7**, 65–74 (1989).
30 C. L. Arthur and J. Pawliszyn, "Solid Phase Microextraction with Thermal Desorption using Fused Silica Optical Fibers," *Anal. Chem.* **62**, 2145–2148 (1990).
31. Z. Zhang, M. J. Yang, and J. Pawliszyn, "Solid Phase Microextraction," *Anal. Chem.* **66**, 844A–853A (1994).
32. D. B. Min, "Analyses of Flavor Qualities of Vegetable Oils by Gas Chromatography," *J. Am. Oil Chem. Soc.* **60**, 544–545 (1983).
33. H. P. Dupuy, S. P. Fore, and L. A. Goldblatt, "Direct Gas Chromatographic Examination of Volatiles in Salad Oils and Shortenings," *J. Am. Oil Chem. Soc.* **54**, 340–342 (1973).
34. J. L. Williams and T. H. Applewhite, "Correlation of the Flavor Scores of Vegetable Oils with Volatile Profile Data," *J. Am. Oil Chem. Soc.* **54**, 461–463 (1977).
35. K. Warner, E. N. Frankel, and K. J. Moulton, "Flavor Evaluation of Crude Oil to Predict the Quality of Soybean Oil," *J. Am. Oil Chem. Soc.* **65**, 386–391 (1988).
36. K. Robards et al., "Headspace Gas Analysis as a Measure of Rancidity in Corn Chips," *J. Am. Oil Chem. Soc.* **65**, 1621–1626 (1988).
37. D. B. Min and D. B. Tickner, "Preliminary Gas Chromatographic Analysis of Flavor Compounds on Mayonnaise," *J. Am. Oil Chem. Soc.* **59**, 226–228 (1982).
38. D. B. Min, "Correlation of Sensory Evaluation and Instrumental Gas Chromatographic Analysis of Edible Oils," *J. Food Sci.* **46**, 1453–1456 (1981).

DAVID B. MIN
DONALD F. STEENSON
The Ohio State University
Columbus, Ohio

FATS AND OILS: PROPERTIES, PROCESSING TECHNOLOGY, AND COMMERCIAL SHORTENINGS

Fats and oils are key functional ingredients in a large variety of food products. Common product applications are baked goods, snacks, icings, confections, fried food, and imitation dairy products. Fats and oils are also key parts of the human diet providing concentrated sources of energy and essential nutrients. This article briefly covers the chemistry, processing, physical characteristics, formulation, nutrition, and consumption of fats and oils in food applications.

CHEMISTRY AND STRUCTURE

Fats and oils are predominately mixtures of triglycerides. Triglycerides are the triesters of fatty acids and glycerol formed as a condensation product.

6. M. Y. Jung and D. B. Min, "Effects of Quenching Mechanisms of Carotenoids on the Photosensitized Oxidation of Soybean Oil," *J. Am. Oil Chem. Soc.* **68**, 653–658 (1991).
7. H. W.-S. Chan and D. T. Coxon, "Lipid Hydroperoxides," in H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, Orlando, Fla., 1987, pp. 17–50.
8. S. Kaiser et al., "Physical and Chemical Scavenging of Singlet Molecular Oxygen by Tocopherols," *Arch. Biochem. Biophys.* **277**, 101–108 (1990).
9. H. Sies and W. Stahl, "Vitamins E and C, β-carotene, and other Carotenoids as Antioxidants," *Am. J. Clin. Nutr.* **62**, 1315S–1321S (1995).
10. W. W. Nawar, "Chemistry of Thermal Oxidation of Lipids," in D. B. Min and T. H. Smouse, eds., *Flavor Chemistry of Fats and Oils*, Champaign, Ill., 1985, pp. 39–60.
11. S. S. Chang, R. J. Peterson, and C.-T. Ho, "Chemistry of Deep Fried Flavor," in M. K. Supran, ed., *Lipids as a Source of Flavor*, ACS Symposium Series 75, American Chemical Society, Washington, D.C., 1978, p. 18–22.
12. M. W. Formo et al., "Composition and Characteristics of Individual Fats and Oils," in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, John Wiley & Sons, New York, 1979, p. 289–478.
13. W. Grosch, "Lipid Degradation Products and Flavour," in I. D. Morton and A. J. Macleod, eds., *Food Flavours, Part A, Introduction*, Elsevier, Amsterdam, The Netherlands, 1982, pp. 325–398.
14. R. T. Holman and O. C. Elmer, "The Rates of Oxidation of Unsaturated Fatty Acids and Esters," *J. Am. Oil Chem. Soc.* **24**, 127–129 (1947).
15. J. Pokorny, "Major Factors Affecting the Autoxidation of Lipids," in H. W.-S. Chan, ed., *Autoxidation of Unsaturated Lipids*, Academic Press, Orlando, Fla., 1987, pp. 141–206.
16. H. Brockerhoff and R. G. Jensen, *Lipolytic Enzymes*, Academic Press, New York, 1974.
17. R. Tressl and F. Drawert, "Biogenesis of Banana Volatiles," *J. Agric. Food Chem.* **21**, 560–565 (1973).
18. E. Selke, W. K. Rohwedder, and H. J. Dutton, "Volatile Components from Triolein Heated in Air," *J. Am. Oil Chem. Soc.* **54**, 62–67 (1977).
19. E. N. Frankel, J. Nowakowska, and C. D. Evans, "Formation of Methyl Azelaaldehydrate on Autoxidation of Lipids," *J. Am. Oil Chem. Soc.* **38**, 161–162 (1961).
20. A. C. Noble and W. W. Nawar, "Identification of Decomposition Products from Autoxidation of Methyl 4,7,10,13,16,19-Docosahexaenoate," *J. Am. Oil Chem. Soc.* **52**, 92–95 (1975).
21. D. A. Forss, "Odor and Flavor Compounds from Lipids," *Prog. Chem. Fats and Other Lipids* **13**, 177–258 (1972).
22. Y. A. Tan, C. L. Chong, and K. S. Low, "Crude Palm Oil Characteristics and Chlorophyll Content," *J. Sci. Food Agric.* **75**, 281–288 (1997)
23. E. N. Frankel, "Soybean Oil Flavor Stability," in D. R. Erickson, et al., eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Mo., and American Oil Chemists' Society, Champaign, Ill., 1980, pp. 229–244.
24. H. R. Rawls, and P. J. Van Santen, "A Possible Role for Singlet Oxygen in the Initiation of Fatty Acid Autoxidation," *J. Am. Oil Ch. Soc.* **47**, 121–125 (1970).
25. E. N. Frankel et al., "Comparison of Gas Chromatographic Methods for Volatile Lipid Oxidation Compounds in Soybean Oil," *J. Am. Oil Chem. Soc.* **62**, 620 (1985).
26. T. Mounts, "Processing of Soybean Oil for Food Uses," *Cereal Foods World* **34**, 268 (1989).
27. G. Montedoro, M. Bertuccioli, and G. Anichini, "Flavor of Foods and Beverages," Academic Press, Orlando, Fla., 1972.
28. T. J. Weiss, *Food Oils and Their Use*, AVI Publishing, Westport, Conn., 1970.
29. J. D. Dziezak, "Fats, Oils, and Fat Substitutes," *Food Technol.* **7**, 65–74 (1989).
30. C. L. Arthur and J. Pawliszyn, "Solid Phase Microextraction with Thermal Desorption using Fused Silica Optical Fibers," *Anal. Chem.* **62**, 2145–2148 (1990).
31. Z. Zhang, M. J. Yang, and J. Pawliszyn, "Solid Phase Microextraction," *Anal. Chem.* **66**, 844A–853A (1994).
32. D. B. Min, "Analyses of Flavor Qualities of Vegetable Oils by Gas Chromatography," *J. Am. Oil Chem. Soc.* **60**, 544–545 (1983).
33. H. P. Dupuy, S. P. Fore, and L. A. Goldblatt, "Direct Gas Chromatographic Examination of Volatiles in Salad Oils and Shortenings," *J. Am. Oil Chem. Soc.* **54**, 340–342 (1973).
34. J. L. Williams and T. H. Applewhite, "Correlation of the Flavor Scores of Vegetable Oils with Volatile Profile Data," *J. Am. Oil Chem. Soc.* **54**, 461–463 (1977).
35. K. Warner, E. N. Frankel, and K. J. Moulton, "Flavor Evaluation of Crude Oil to Predict the Quality of Soybean Oil," *J. Am. Oil Chem. Soc.* **65**, 386–391 (1988).
36. K. Robards et al., "Headspace Gas Analysis as a Measure of Rancidity in Corn Chips," *J. Am. Oil Chem. Soc.* **65**, 1621–1626 (1988).
37. D. B. Min and D. B. Tickner, "Preliminary Gas Chromatographic Analysis of Flavor Compounds on Mayonnaise," *J. Am. Oil Chem. Soc.* **59**, 226–228 (1982).
38. D. B. Min, "Correlation of Sensory Evaluation and Instrumental Gas Chromatographic Analysis of Edible Oils," *J. Food Sci.* **46**, 1453–1456 (1981).

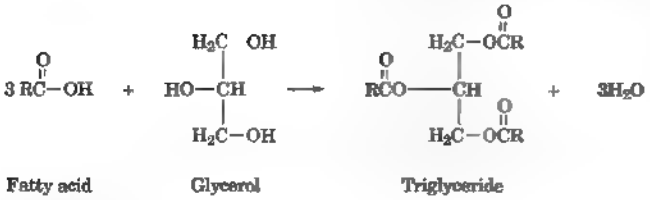
DAVID B. MIN
DONALD F. STEENSON
The Ohio State University
Columbus, Ohio

FATS AND OILS: PROPERTIES, PROCESSING TECHNOLOGY, AND COMMERCIAL SHORTENINGS

Fats and oils are key functional ingredients in a large variety of food products. Common product applications are baked goods, snacks, icings, confections, fried food, and imitation dairy products. Fats and oils are also key parts of the human diet providing concentrated sources of energy and essential nutrients. This article briefly covers the chemistry, processing, physical characteristics, formulation, nutrition, and consumption of fats and oils in food applications.

CHEMISTRY AND STRUCTURE

Fats and oils are predominately mixtures of triglycerides. Triglycerides are the triesters of fatty acids and glycerol formed as a condensation product.



Triglycerides may range from solid to liquid consistency at room temperature depending on the fatty acid esters they contain. Solid triglycerides are commonly called fats, and liquid triglycerides, oils. If all three fatty acids that comprise the triglyceride are the same, it is called a single triglyceride; conversely, if the fatty acids differ, the triglyceride is mixed.

Triglycerides typically comprise about 95% of fat or oil. The other components include free fatty acids, monoglycerides, diglycerides, phosphatides, sterols, vitamins, natural antioxidants, color-causing pigments, minerals, flavor-odor compounds, and other substances. Free fatty acids are the uncombined or unesterified fatty acids present in a fat or oil. Crude oils may contain several percent fatty acids, which result from hydrolysis. The formation of free fatty acids via hydrolysis leaves the partial glycerides (mono- and diglycerides) behind. Monoglycerides and diglycerides are frequently used in foods as emulsifiers. They are often found in functional cake and icing shortenings. They are produced commercially by the alcoholysis reaction of triglycerides or fatty acids with glycerol or by esterification of glycerol with fatty acids (1).

Phosphatides are found in fat and oil from plant and animal sources. These materials consist of a polyhydric alcohol (usually glycerol) that is esterified with fatty acids, phosphoric acid, and a nitrogen-containing compound. The most common phosphatide is a family of compounds found in soybean oil referred to as lecithin, which includes chemical lecithin (phosphatidyl choline), cephalin (phosphatidyl ethanolamine), lipositols (phosphatidyl inositol) (2). Sphingomyelins are a group of phosphatides that contain no glycerol (3). Lecithin is typically used as a food additive for its emulsification properties. Phosphatides are essentially removed in the refining process.

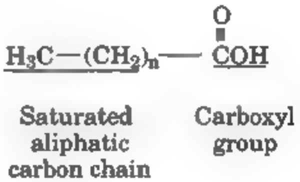
Colored materials occur naturally in fats and oils and generally are a result of two classes of pigments. Carotenoids and chlorophylls are the yellow-red and green colors, respectively, found in fats. Level and type of pigment vary greatly with the source, from the orange color of crude palm oil due to its carotene content to the dark green color of canola (or rapeseed) oil due to its high chlorophyll content. Vegetable oils also contain tocopherols that serve as natural antioxidants, retarding oxidation, and as a source of the essential nutrient vitamin E. Other antioxidants may be added to processed fats to preserve freshness (4,5). In contrast, meat fats contain negligible levels of tocopherols.

Sterols are common minor components of all natural fats and oils. Cholesterol, the infamous sterol associated with heart disease, is the principal sterol found in animal fats. Vegetable oils only contain trace quantities of cholesterol. Vegetable oils contain other sterols that vary in type and quantity with the vegetable oil source (6–13).

Fats and oils may contain a number of other minor constituents such as waxes, gums, hydrocarbons, and fatty alcohols. These materials are generally of little importance and are typically handled during processing.

Fatty Acids

The key to understanding the chemical and physical characteristics of a fat is understanding the component fatty acids that comprise the triglyceride. These can vary in chain length (number of carbon atoms), type of carbon–carbon bonding (saturated or unsaturated with hydrogen), and stereochemistry both within the fatty acid and its position in the triglyceride. The most common edible fatty acids are aliphatic, straight chain, saturated and unsaturated, containing an even number of carbon atoms and ending in a carboxyl group.

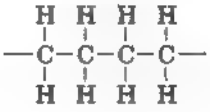


$$\underbrace{H_3C-(CH_2)_n}_{\text{Saturated aliphatic carbon chain}}-\underbrace{\overset{O}{\overset{\|}{C}}OH}_{\text{Carboxyl group}}$$

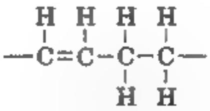
Small amounts of odd-numbered, straight-chain acids, branched chains, and cyclical acids are also present in edible vegetable oils (9,14).

The length of the chain can vary in edible fatty acids from as few as 4 carbons to as many as 24. Most fats predominately contain fatty acids that are 16 or 18 carbons long, although there are some exceptions like coconut oil, palm kernel oil, and butterfat, which contain high percentages of shorter (8–14 carbons) chain fatty acids.

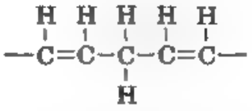
The fatty acids are classified by structure:



Saturated

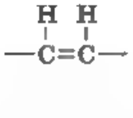


Monounsaturated



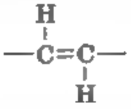
Polyunsaturated

The more unsaturated the fatty acid, the more chemically reactive a fatty acid will be. Saturated fatty acids have a higher melting point than the corresponding unsaturated fatty acids of the same chain length. The more unsaturated, the lower the melting point. Fatty acids with double bonds may differ in geometric form. Orientation of the hydrogen atoms around a double bond determines whether the fatty acid is *cis* or *trans*.



$-\overset{H}{C}=\overset{H}{C}-$

Cis



$-\overset{H}{C}=\underset{H}{C}-$

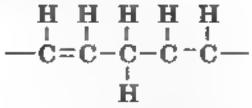
Trans

The *trans* form of a fatty acid is more stable and has a higher melting point. The double bond may also vary by position on the chain, although most natural fatty acids have double bonds that commonly occur in fixed positions.

There are two accepted systems of nomenclature to identify the position of double bonds in the chain. The Geneva system numbers the carbons in the chain consecutively starting from the carbon in the carboxyl group, which is numbered 1. The carbon with the lower number is used to identify the location of the double bond, that is, the carbon closest to the carboxyl group.

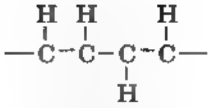
Biochemists typically identify the position of the double bond by the omega or *n* minus classification. This system refers to the position of the carbon in the double bond closest to the methyl end of the chain; the carbon of the end methyl group is number 1. Linoleic acid, a fatty acid 18 carbons long with two double bonds may be identified as 9,12-octadecenoic acid by the Geneva system. This same fatty acid would be an omega-6 or *n*-6 fatty acid by the alternate system. The second double bond would be an omega-9 or *n*-9. All of these structural variations can affect the chemical and physical properties of a fatty acid and the resulting triglyceride. The effects of chain length, unsaturation, and geometric isomerism on the melting point are shown in Table 1.

Polyunsaturates may also be described by the relative position of the double bonds.



$-\overset{H}{C}=\overset{H}{C}-\underset{H}{\overset{H}{C}}-\overset{H}{C}-\overset{H}{C}-$

Nonconjugated
(*cis-cis*)



$-\overset{H}{C}-\overset{H}{C}-\underset{H}{C}-\overset{H}{C}-$

Conjugated
(*cis-trans*)

Table 1. Fatty Acid Melting Points

Common name	Number of carbon atoms	Number of double bonds	Melting point, °F
Caproic	6	—	26
Caprylic	8	—	62
Capric	10	—	89
Lauric	12	—	112
Myristic	14	—	130
Myristoleic	14	1	65
Palmitic	16	—	145
Margaric	17	—	142
Stearic	18	—	157
Oleic	18	1	61
Elaidic	18	1 (*trans*)	111
Linoleic	18	2	20
Linolenic	18	3	9
Arachidic	20	—	168
Behenic	22	—	176
Erucic	22	1	92

The double bond locations in unhydrogenated fats and oils usually occur in characteristic positions. Processing, particularly hydrogenation, may alter these locations forming conjugates and other positional isomers.

The physical properties of a triglyceride will be affected by the individual fatty acids it contains and the location of these fatty acids on the glycerine backbone. Some typical source oil fatty acid compositions are shown in Table 2. A triglyceride composed of a single fatty acid type will have uniform, distinct physical properties such as a sharp melting point. Fats and oils are mixtures of triglycerides. These mixtures result in a less distinct, broader range for a property, e.g., a melting point range. The molecular configuration gives rise to characteristic physical properties typical of a given source, which may be quite unique like cocoa butter. Monoglycerides and diglycerides have higher melting points than the corresponding triglycerides (Table 3).

Solid fats can also orient themselves in different crystal forms or polymorphs depending on how the fat crystals orient themselves in the solid state (15,16). Fats containing a diverse mixture of triglycerides are more likely to remain in lower melting crystal forms. The more singular triglycerides will readily transform successively from lower-melting to higher-melting forms. The extent and rate of transformation are a function of the triglyceride structure and mixture, temperature of crystallization, and the duration of crystallization at a given temperature. Mixtures of fats with different polymorphic tendencies can be directed to a particular polymorphic form by control of the composition and crystallization conditions. β prime crystal formation of partially hydrogenated soybean oil in bakery shortenings and margarines is promoted by inclusion of small amounts of β prime tending, partially hydrogenated palm oil (17–23).

Processing

Animals or oilseeds are the sources of edible fat in the United States. Animal fats are obtained by separating the fat from the tissues in a process called rendering. Heat is applied to separate the fat from the tissue protein. Vegetable fats are obtained by crushing the oil source and extracting the fats by direct compression and filtration. Modern processing utilizes solvent (typically hexane) extraction to improve efficiency and yield. The solvent is recycled after recovery and reused. Oils and fats obtained from this process are called crude because they contain small amounts of nontriglyceride components. Crude fats and oils are processed to remove the undesired material and modify the physical properties to obtain useful, functional products.

Two types of refining processes are commonly in use· chemical or physical (steam) refining. Both employ similar steps, but with different objectives Most vegetable oils and

Table 2. Approximate Fatty Acid Composition, Percent Total Fatty Acids

Composition	Canola oil	Coconut oil	Corn oil	Cottonseed oil	Palm oil	Soybean oil	Tallow
C 6:0		<1					
C 8:0		8					
C10:0		6					
C12:0		47					
C14:0		19		<1	1		3
C14:1							1
C16:0	4	9	11	22	44	11	24
C16:1				<1			4
C17:0							2
C17:1							<1
C18:0	2	3	2	3	5	4	19
C18:1	61	6	27	19	40	24	43
C18:2	21	2	59	54	10	54	3
C18:3	9		1	1		7	<1
C20:0	1						
C20:1	1						
C22:0							
C22:1	<1						
C24:0							

Table 3. Fatty Acid Ester Melting Points, °F

Fatty acid	Monoglyceride	Diglyceride[a]	Triglyceride
C12:0 Lauric	145	136	116
C14:0 Myristic	159	152	135
C16:0 Palmitic	171	169	146
C18:0 Stearic	179	175	164
C18:1 Oleic	95	71	42
C18:1 Elaidic	137	131	108
C18:2 Linoleic	54	27	8
C18:3 Linolenic	60	10	−12

[a] 1,3 diglyceride

meat fats are chemically refined, but for some vegetable oils, notably coconut and palm, physical refining is more economical. The choice is generally determined by the convenience and economics of the process.

Chemical, or caustic refining, is intended to remove most of the free fatty acids present in the crude oils. The process consists of treating the crude with an alkali solution, which converts the free fatty acids to soap. These soaps are removed by centrifugation and water washing. Phospholipid, mucilagenous, and proteinaceous materials are also removed in this step. Caustic strength, temperature, mixing, contact time, and removal are critical process parameters.

The residual moisture is removed by vacuum drying prior to bleaching. The natural pigments, carotenes and chlorophylls, are removed by physical absorption on acid-activated clay or bleaching earth. Other absorbent materials such as activated carbon may also be used in specific situations. The critical variables are absorbent level, contact time, temperature, and removal of the spent absorbent. Typically, hydrogenation follows bleaching. Hydrogen is added to unsaturated fatty acids in this heterogeneous reaction with gaseous hydrogen and catalyst, usually nickel although other precious metals may be used. Critical parameters are reaction time, temperature, pressure, catalyst type and amount, and gas purity. Vessel design to provide effective mass and heat transfer is also critical. Less commonly, the oil may be bleached again after hydrogenation.

Hydrogenation raises the melting point of the triglyceride mixture, thus altering physical properties. It also improves oxidative and thermal stability by reducing the number of labile, polyunsaturated reaction sites in the fatty acids of the triglyceride. Hydrogenation allows the processor to differentiate products for their various applications. A processor may make a full range of products, from salad oils to solid frying shortenings, margarines to bakery shortenings, by hydrogenating and blending various oils.

Deodorization typically follows hydrogenation or bleaching if natural liquid oil is desired. The process is a high-temperature, vacuum, steam distillation that removes residual fatty acids as well as undesirable flavor and odor compounds. This step will also remove a small portion of the natural antioxidants. The high-temperature exposure also reduces the color due to transformation of the red and yellow pigments into nonchromophores. Key elements are temperature, vacuum, steam rate, and exposure time. Again, vessel design is critical to provide effective and efficient heat and mass transfer.

Liquid oils may be packaged directly after deodorization, but fluid or plastic shortenings are usually votated, or crystallized, after deodorization. The blended, melted product is fed under pressure to a scraped-surface heat exchanger at slightly above its melting point. The product is chilled creating crystal nuclei, which are allowed to grow slowly for a controlled time in a mildly agitated vessel, commonly referred to as a B unit. Nitrogen may be added to gasify a solid shortening, providing a whiter, more plastic product. Proper crystal conversion may require tempering. Liquid products may be tempered in process, whereas solids are typically stored for 24 to 48 h at their tempering temperature. Key parameters are flow rate, temperature, and tempering conditions. Control of crystal structure is essential for functional bakery shortenings.

Other Processes

There are other specialized processing steps. The most notable are winterization, fractionation, transesterification, interesterification, and directed interesterification. Winterization and fractionation separate melting fractions by cooling the shortening and separating the solids that crystallize. Processors use winterization to provide stable salad oils. Fractionation consists of cooling the oil and separating the solids a number of times to obtain various melting fractions. These are used to create hard butters (cocoa butter substitutes) for coating applications and high stability oils. These processes may employ a solvent (hexane or acetone) to improve the separation, which is commonly referred to as solvent winterization or solvent fractionation (24).

Transesterification, a type of interesterification, is a process that rearranges or redistributes the fatty acids on the glycerine backbone of the triglyceride. Processors may select conditions to distribute the fatty acids more randomly, thus modifying the physical properties of the shortening. This process does not create a significant level of fatty acid isomers (*trans*) like hydrogenation. Hydrogenation may be combined before or after the process to allow further modification of the shortening.

Interesterification involves two or more oil sources, randomizing the fatty acids of the source triglycerides. By cooling and removing the higher-melting triglycerides the reaction can continue with the remaining components redirecting the interesterification. This process, used to provide relatively pure triglycerides, is called directed interesterification (25–27). If excess glycerol is present, monoglycerides and diglycerides are formed. The relative amounts of each at equilibrium are a function of the ratio of fatty acids available to glycerol (28). This is commonly called glycerolysis.

PROCESSING AIDS AND ADDITIVES

A number of food-approved materials may be added at low levels to improve processing or to protect the oil quality during processing (Table 4). These materials are removed or reduced to minimal levels in the final product. Other additives may also be used to provide a functional effect in the final product. These uses must comply with Food and Drug Administration (FDA) regulations covering addition levels, methods of addition, permissible residual levels, and labeling.

FATS AND OILS FUNCTIONALITY

Fats and oils provide several functional characteristics in a variety of food products. Shortening products can provide lubricity, structure, a heat-transfer medium, a moisture barrier, and aeration. Fat systems can also act as a carrier for flavor, color, and vitamins. Oils themselves provide vital nutrition.

Table 4. Additives Used in Fats and Oils

Additive	Effect
Antioxidants	
Tocopherols	Retard oxidation
Butylated hydroxyanisole (BHA)	
Butylated hydroxytoluene (BHT)	
tert-Butylhydroquinone (TBHQ)	
Propyl gallate	
Colors	
β-carotene	Provides yellow color to finished product.
Tumeric and annatto	
Flavors	
Various acids, aldehydes, and ketones	Provide flavor and odor to the finished product
Phosphoric acid	Metal chelators, process hydrating agents, neutralizing agents.
Citric acid	
Dimethylpolysiloxane (methyl silicone)	Antifoaming agent, high-temperature antioxidant
Polyglycerol esters	
Oxystearin	Crystal inhibitors, crystal modification
Emulsifiers	
Monoglycerides, diglycerides	Emulsification, aeration, dough conditioning, wetting, antispattering, antisticking, separation and viscosity control
Lecithin	
Polyglycerol esters	
Calcium/sodium steroyl lactylate	
Lactylated monoglycerides and diglycerides	
Sorbitan esters	
Propylene glycol esters	

Fat is multifunctional in most applications. Frying and cooking fats provide lubricity, a heat-transfer medium, and a flavor medium in some applications. Fats provide lubricity and aeration in cakes and additionally structure in icings, cream fillers, and the like.

SALAD AND COOKING OILS

Salad and cooking oils are usually prepared from refined, bleached, and deodorized vegetable oils, or they are lightly hydrogenated and winterized for stability. The oil may be used for flavor (eg, peanut and olive oils), nutrition (eg, corn and canola oils), and stability. Antioxidants and crystal inhibitors may be added to salad oil (29,30). Oils may be blended for flavor and stability, if they are intended for heating and reuse, and dimethylpolysiloxane may also be added. Oils used in salad dressings typically have light color and bland flavor and must resist clouding. Salad dressing must contain a minimum of 30% oil by federal regulation (31). Mayonnaise is an emulsified, semisolid dressing that must contain 65% vegetable oil and whole eggs or egg yolks (32). Reduced-calorie dressings must contain at least one-third fewer calories than the conventional product. This is usually accomplished by reducing the fat content and stabilizing the increased water content with gums and thickeners to provide emulsion stability.

FRYING SHORTENINGS

Frying fats must maintain stability under abusive conditions that include high temperature, extractable materials from the fried food, and oxygen from the air. Stability of fried products (snack foods) is also important. The shortening imparts textural and flavor effects to the fried food. The key is the type of oil used and its chemical and physical properties. There are now new varieties of common vegetable oils with modified fatty acid compositions through breeding and genetic modification to expand the variety of properties available.

Frying shortenings are available as meat fats, meat fat–vegetable blends, and solid or liquid vegetable shortenings. Meat fat and solid vegetable products provide excellent stability, particularly under abusive conditions. But these products have poor nutritional attributes (cholesterol in meat fat, saturated fat, and *trans* fatty acids). Pourable, vegetable, liquid shortenings offer convenient handling with good frying stability. There is some compromise on oxidative stability, but fry stability is acceptable for most applications. Liquid products have lower saturated and *trans* fatty acid levels than solid products.

Fry life is a function of the chemical and physical characteristics of the frying shortening. These depend on the source oil(s) and processing. Fry life is also a function of many operational factors such as the type and amount of food fried, the size of the fryers, the time and temperature of frying, frequency of filtration, and cleaning procedures. Analytical characteristics vary with the product (Table 5).

Table 5. Typical Fly Shortening Analysis

	Solid		Liquid
Analysis	Meat fat–vegetable blend	Partially hydrogenated soybean	Partially hydrogenated soybean
Iodine value	52	69	101
Mettler dropping point, °F	117	103	95
Solid fat Index			
at 50 F	40	50	5
at 70 F	27	36	4
at 80 F	23	30	3
at 92 F	17	14	2
at 104 F	12	2	2
Fatty acid composition, %			
C16	24	11	11
C18	18	11	7
C18.1	41	70	46
C18:2	8	2	28
C18:3	1	trace	3

Lovibond color (1.5 red maximum), free fatty acid (0.05 wt% maximum) and peroxide value (1.0 meq/kg maximum) are identical for all products.

GRIDDLE AND PAN FRYING SHORTENINGS

Specialty colored and flavored shortenings are designed to replace butter or margarine in grilling, pan frying, and other applications (soups, sauces, gravies, basting, and bun wash). These may be liquid or solid, resist darkening, reduce waste (no water), and eliminate scorching (no milk solids). Other ingredients may be added. These include salt (flavor enhancement), flavorings (garlic), and even small amounts of butter itself (33–36). Coconut oil may be added for abusive, high-temperature applications (37).

BAKERY SHORTENINGS

A variety of general and specialized shortenings are available for baking applications (24,38). The principal products are bread, rolls, biscuits, cakes, icings, cookies, pastry, doughnuts, and pies. All-purpose shortening is intended to function adequately in a broad variety of applications from baking to frying. It is formulated to have stability, a wide plastic range for proper mixing, and some structure for applications like icings.

Cake and icing shortening is designed to meet the dual needs of cake and icing applications. The shortening must entrap and disperse air as an emulsion that is a cake batter or icing. In a cake, the fineness and amount of the air bubbles determine the finished height and cell structure. The shortening also lubricates the batter components and tenderizes the cake. In icings, the shortening must provide the structural properties (body and firmness) as well as its creaming or aerating ability. Monoglyceride and diglyceride emulsifiers are typically added to cake and icing shortening to improve the emulsifying properties. Table 6 shows the typical analytical data for all-purpose and cake and icing shortenings.

The development of emulsifier technology has allowed the formulation of specialized cake or icing shortenings and the development of prepared mixes. Their formulation is a function of their application: continuous versus batch

Table 6. Typical Shortening Analyses

Analysis	All-purpose (soy base)	Cake and icing (soy base)
Iodine value	74.00	74.00
Mettler dropping point, °F	120.00	120.00
Solid fat index		
at 50 F	24.00	24.00
at 70 F	20.00	20.00
at 80 F	19.00	19.00
at 92 F	16.00	16.00
at 104 F	10.00	10.00
Lovibond color, red, maximum	1.00	2.00
Free fatty acid, wt%, maximum	0.05	0.15
Peroxide value, meq/kg, maximum	1.00	1.00
α-monoglyceride, wt%, maximum	—	3.50

mixed, rich or lean formulation, liquid content, and sugar content. The shortening base is formulated and processed to obtain optimum emulsifier performance. Emulsifiers generally permit a richer, moister cake due to their capability to incorporate high levels of moisture in the batter (Table 7). The specialized mix shortenings contain even higher levels of emulsification and are used for consumer cake mixes, leaner snack-type cake applications, or cake products where long shelf life is required (39–41).

Similarly, emulsifier technology has allowed incorporation of high levels of moisture and air into the icing. The shortening must provide structure to allow the icing to hold this moisture and air as well as retain its shape. Processing of the shortening, particularly formulation and votation, is critical to provide this structure. Generally, cooler votation conditions improve the icing's ability to aerate, as measured by specific gravity, and resistance to separation and slumping.

Different levels and types of emulsification are required for formulations with increased moisture and aeration requirements (Table 8). Flat or thick fudge icings require little, if any, aeration. However, butter cream and some decorator icings require some emulsification ability, usually accomplished with monoglycerides and diglycerides or low levels of polysorbates. Specialty icing products are formulated to produce fluffier and higher moisture-type icings. Polyglycerol esters or combinations of monoglyceride and diglyceride, poylsorbate emulsifier systems are used. As the level of emulsification increases, the structure provided by the shortening becomes more critical to icing performance.

Breads and Rolls

Shortening is added at relatively low levels to breads for lubricity, softening, and shelf life. Structure is primarily obtained from starch and protein complexes. Emulsifiers are employed as dough conditioners to resist staling (drying) (Table 9). These may be added either as part of the shortening or hydrated first by heating in water until a liquid crystalline phase (mesophase) is formed and maintained by cooling the mixture. Biscuit shortenings are formulated to blend as small, discrete chunks into the cold dough, yet still provide sufficient solids for a flaky, tender texture.

Pie Crust Shortening

Pie shortenings are also mixed loosely into the cold dough to maintain separate fat layers for a flaky crust. Overmixing, or mixing too warm, incorporates the shortening intimately, which reduces flakiness, increases toughness, and increases shrinkage. The shortening system contains a loosely bound oil fraction to provide lubricity and tenderness to the crust.

Lard is the classic choice for pie shortening due to its solids content, plasticity at cool temperatures, and crystal structure. Vegetable shortenings have been formulated to provide similar functional properties with an improved nutritional (cholesterol and saturates) profile.

Puff Pastry and Danish Shortening

Puff pastry and Danish shortenings are referred to as roll-in products because they are formed from a laminated dough sheet, consisting of alternating layers of dough and shortening, that is traditionally formed by rolling the coated dough sheet and folding it to form layers.

Danish has a higher fat content than puff pastry, and softer-consistency products are used to achieve lubricity. Long refrigerated retarding (dough-relaxing) steps are used between folds to minimize toughness and maximize lubricity while maintaining structural integrity of the fat film. Puff pastry shortening contains higher solids for flakiness due to the lower fat content used. This shortening must be carefully formulated and processed so it remains workable, not stiff, to properly apply to the dough sheet. Dough and shortening temperature are critical. Puff pastry has larger expansion because of the number of layers and the water vapor sealed by the shortening between the dough layers.

Butter was originally used for these applications. Puff pastry and Danish are formulated to have similar composition (fat, moisture, and salt) and functionality and may be butter flavored as well.

OTHER APPLICATIONS

There are a number of other specialized shortening applications. Confectionery fats are primarily intended to be substitutes or extensions for cocoa butter in confections and coatings. These consist of lauric-based products (coconut and palm kernel oils), which may be hydrogenated, fractionated, or interesterified to better match the solids profile and melting point of cocoa butter. Domestic substitutes may be hydrogenated or fractionated as well (18–42–45). These do not generally have the same crystal type as cocoa butter, and they do not require tempering. Eating quality, snap, mouth melt, and glossy appearance are key performance factors. Coatings provide a moisture barrier

Table 7. Typical Cake Shortenings

Shortening	Emulsifiers	Moisture incorporation	Fat content
Cake and icing	Monoglyceride and diglyceride	Low	Moderate
Specialty cake			
Plastic solid	Propylene glycol monoesters and monoglycerides	Moderate	Moderate
Liquid	Lactylated monoglycerides and monoglycerides	Moderate	Moderate
Specialized mix shortenings	Higher levels of propylene glycol monoesters and monoglycerides	High	High

Table 8. Typical Icing Shortenings

Shortening type	Emulsifier	Icing applications
All-purpose	None	Flat, fudge icings
Cake and icing	Monoglycerides and diglycerides	Butter cream and decorator icings, and cream fillers
Specialty icings	Monoglycerides, diglycerides, and polyglycerol esters	Butter cream, fluffy decorator icings, and cream fillers

Table 9. Bread Emulsifiers and Dough Conditioners

Monoglycerides and diglycerides	Sodium stearoyl lactylate
Distilled monoglycerides	Calcium stearoyl lactylate
Ethoxylated monoglycerides	Lactylated stearate
Succinylated monoglycerides	Diacetyl tartaric acid esters

and are typically high-solids, low-melting-point products. These are often referred to as hard butters.

Similar shortenings may also be used in imitation dairy products like sour cream, dips, nondairy creamers, and whipped toppings. Texture and stability are keys to successful shortening formulation for these products.

Peanut butter stabilizers are partially hydrogenated, high-melting fats, or emulsifiers, which are added to fluid peanut butter to prevent oil separation.

BUTTER AND MARGARINES

Butter contains at least 80 wt% butterfat (46), which serves as the matrix sponge for the aqueous phase. The aqueous phase consists of water, casein, milk solids, and minerals. The solids comprise about 1 to 2 wt% of the butter, and salt may typically be added at concentrations from 1.5 to 3 wt%. Margarines are butter replacements made from shortening or shortening and oil blends with other ingredients to match butter's aqueous phase. Coloring, flavoring, and vitamins A and D may also be added. Margarine must contain 80% fat by federal regulation (47). Margarines are available in solid form, whipped, and as pourable liquids. They are also available in reduced-calorie, diet, or imitation spreads, which have a lower fat content.

U.S. FAT CONSUMPTION

The U.S. Department of Agriculture provides data on the availability of food fat for consumption per capita in the United States. These data measure the amount of fat available and do not consider the quantities wasted or discarded. Fats are separated into visible and invisible categories. The visible portion consists of easily identified fat like butter, margarine, shortening, and salad oils. The invisible category is ingested as part of other foods and is not easily recognized as fat such as dairy products.

Visible fat consumption has increased 16 lb per capita from 1970 to 1994 (Table 10). Visible fat usage accounts for this increase. This is to some degree a reflection of the increase in fast-foods and convenience foods, meals not made from scratch at home. Fat consumption appears not to be affected by dietary and health concerns, except that saturated fat consumption is down (48). Meat consumption is up, but the fat content contributed by meat has declined due to the use of leaner meats.

The amount of vegetable fat consumption has increased from 1970 to 1996 while animal fat consumption has decreased (Table 11). The amount of fat consumed in the diet

Table 10. Edible Fat Availability per Capita

Source	1970	1980	1990	1994
		Visible fats		
Butter, fat content	4.3	3.6	3.5	3.9
Lard and tallow	4.6	3.7	2.4	4.7
Margarine and spreads	8.7	9.0	8.7	7.9
Shortening and other fats	17.3	18.2	22.2	24.1
Salad and cooking oils	15.4	21.2	24.8	26.3
Other	2.3	1.5	1.2	1.6
Animal source	14.1	12.3	9.7	11.4
Vegetable source	38.5	44.8	53.1	57.2
Total visible fats	*52.6*	*57.2*	*62.8*	*68.6*
		Invisible fats		
Dairy products, excluding butter	15.6	14.9	15.4	15.7
Eggs, meats, poultry, fish	46.3	42.1	36.1	34.0
Fruits, vegetables, legumes, nuts, soy, and grains	7.2	6.7	8.8	8.7
Other	2.1	2.0	3.0	2.9
Animal source	61.9	57.0	51.5	49.7
Vegetable source	9.3	8.7	11.8	11.6
Total invisible fats	*71.2*	*65.7*	*63.3*	*61.4*
Total fats and oils	*123.8*	*122.9*	*126.2*	*129.0*

Source: Ref. 48.

Table 11. Fats and Oils Used in Foods, mm Pounds

Source	Year			
	1970–71	1980–81	1990–91	1995–96
		Vegetable oils		
Soybean	5,780	8,610	10,772	11,877
Corn	403	624	1,143	595
Cottonseed	805	555	777	497
Palm	160	291	98[a]	Not avail
Coconut	220	338	169	221
Peanut	181	119	129[a]	129[a]
Sunflower		79	166[b]	90[b]
		Meat fats		
Tallow	508	740	501	341
Lard	1,612	415	314	297
Total fats	*9,669*	*11,908*	*14,491*	*14,706*

[a] Incomplete data.

[b] Total edible and inedible.

Source: Ref. 49.

has decreased as a percentage of total calories, yet total fat consumption is higher. This is somewhat misleading since total caloric intake (carbohydrate and protein) has been increasing at a greater rate than fat consumption, thus reducing the relative amount of fat consumed when expressed as a percentage of total calories. The reality is that fat consumption remains high with the trend of a lower proportion of saturated fatty acid and a higher proportion of polyunsaturated fat intake.

NUTRITION

Fats are an important part of the human diet along with proteins and carbohydrates. Fats are a concentrated source of energy (9 kcal/g vs 4 kcal/g for proteins and carbohydrates), a source of essential fatty acids, and a source of fat-soluble vitamins (50).

Certain fatty acids cannot be synthesized by the body and are essential for our health. These acids, linoleic and linolenic, must be supplied by the diet. About 2% of linoleic acid is necessary in the human diet to prevent a deficiency, and 3% is considered to be a more adequate intake. The minimum requirement for linolenic acid has been estimated at a little over 0.5% of total calories (50,51).

Estimates of total fat in the U.S. diet have dropped to around 35% of calories and will vary depending on the source of the data. Reduction in fat intake is an important part of any weight loss-control diet because fat is such a dense source of calories. Most recent dietary health concerns center on cardiovascular disease, including heart attack and stroke. Diet is just one, and not necessarily the major, risk factor identified from epidemiological studies. Other factors include smoking, high blood pressure (hypertension), obesity, elevated serum cholesterol, male sex, sedentary lifestyle, diabetes, excessive stress, and a positive family history of cardiovascular disease. No direct cause-and-effect relationship has been established for these risk factors; only an increased statistical risk has been shown.

Most of the recent studies involving dietary fat focus on the specific type of fatty acid (saturated, monounsaturated, polyunsaturated, and *trans* isomers) and its relationship to serum cholesterol and lipoproteins (cholesterol carriers). There have been studies that have shown a relationship between carbohydrates, proteins, fiber, and trace minerals and cardiovascular disease (52).

Vegetable fats do not directly contain a significant amount of cholesterol. Animal and dairy fats do contain cholesterol and thus may directly affect serum cholesterol. Much recent work has been done relating specific fatty acid types with their effect on the lipoprotein carriers and, indirectly, serum cholesterol (53,61). Two general classes of lipoprotein carriers are largely involved. Low-density lipoproteins (LDL) contain the largest portion of total cholesterol in the blood. High levels of LDLs are associated with increased risk of cardiovascular disease, and they can be thought of as the bad cholesterol. High-density lipoproteins (HDL) transport cholesterol from the blood, and elevated levels of HDL are associated with decreased risk of coronary heart disease. Thus HDLs can be viewed as the good cholesterol (62).

Increased levels of saturated fat in the diet have been shown to increase total serum and LDL cholesterol, thus increasing the risk of heart disease (46). Much negative publicity has been associated with tropical fats (coconut, palm kernel, and palm oil) due to their high saturated fat content. Recent studies show palm oil may be an exception, despite its high saturated fat content (56,60,61,63–65). Similarly meat and dairy fats suffer because of their relatively high saturated fat content as well as their cholesterol content.

Diets high in monounsaturated fat and low in saturated fats, the so-called Mediterranean diet, have been shown to decrease total and LDL cholesterol while not affecting HDL cholesterol (53,55). Studies suggest this type of diet may be equal to, or better than, a low-fat diet for lowering blood cholesterol. Olive oil and canola oil are common sources of monounsaturates. Diets high in polyunsaturated fats beneficially decrease total and LDL cholesterol, but also decrease HDL cholesterol (53,55). Soybean and corn oil are common polyunsaturated fat sources.

Some animal studies have suggested a relationship between diets high in polyunsaturates and certain types of cancer, particularly breast cancer. Other animal studies indicate a high caloric diet due to increased fat may be related to increased cancer incidence, particularly colon and breast cancer (66,67). Any direct relationship between total caloric intake, fat unsaturation, and cancer incidence has not been proved.

Specific fatty acid isomers have also been studied. *Trans* isomers, which result from the hydrogenation process, are present at significant levels, 10 to 35%, in most processed shortenings. Most are present as monounsaturated, *trans* fatty acids. Early concerns about the safety of *trans* fatty acids in the U.S. diet were not supported by reliable epidemiological and animal data (68,69). Subsequent studies indicate that moderate *trans* fatty acid intake results in cholesterol response similar to saturated fat (70–73). Higher *trans* intake levels may also reduce HDL (70). *Trans* fatty acids do not appear to be associated with increased cancer risk (74).

Diets high in omega-3 polyunsaturates, like fish oils, have shown a reduced incidence of coronary heart disease. However, other data show an increased risk of stroke and bleeding tendency with this type of diet (54). More studies are needed to determine the long-term effects of diets supplemented with fish oil. Some vegetable oils (soybean and canola) are sources of omega-3 fatty acids without the cholesterol and saturated fatty acids associated with fish oil.

There is still controversy concerning nutritional and health effects of fat in our diets. Given the current state of knowledge, prudence would dictate moderation in the diet.

BIBLIOGRAPHY

1. M. Gupta, "Manufacturing Process for Emulsifiers," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 4, 5th ed., John Wiley & Sons, New York, 1996, pp. 569–572.
2. O. L. Brekke, "Oil Degumming and Soybean Lecithin," in D. R. Erickson et al., eds., *Handbook of Soy Oil Processing and Utilization*, American Soybean Association, St. Louis, Mo., and

American Oil Chemists' Society, Champaign, Ill., 1980, pp. 77–79.

3. W. W. Nawar, "Chemistry," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 5th ed., John Wiley & Sons, New York, 1996, pp. 401–402.
4. *Oils & Fats International* **2**, 31 (1986).
5. D F. Buck, "Antioxidant Applications," *Manufacturing Confectioner* **64**, 45–49 (1984).
6. N. A. M. Eskin et al., "Canola Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 53–54.
7. L. R. Strecker et al , "Corn Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed , John Wiley & Sons, New York, 1996, pp. 131–133
8. L. A. Jones and C. C. King, "Cottonseed Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 194–195.
9. D. Firestone, E. Fedeli, and E. W. Emmons, "Olive Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 258–259.
10. Y. Basiron, "Palm Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 280–282.
11. C. T. Young, "Peanut Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, p. 386.
12. F. T. Orthofer, "Rice Bran Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 404–406.
13 E. F. Sipos and B. F. Szuhaj, "Soybean Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 499–502.
14. D. Kitts, "Toxicity and Safety of Fats and Oils," in Y. H Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 5th ed., John Wiley & Sons, New York, 1996, pp 221–226.
15. C. W. Hoerr, *J. Am. Oil Chem. Soc* **37**, 539–546 (1960).
16. E. S. Lutton, "Lipid Structures," *J. Am. Oil Chem. Soc.* **49**, 1–9 (1972).
17. U.S. Pat. 2,530,596 (November 21, 1950), N. W. Zills and W. H. Schmidt (to Lever Bros. Co.).
18. U.S. Pat. 2,625,478 (January 13, 1953), K. F. Mattil and F. A. Norris (to Swift & Co.).
19. U.S. Pat. 2,521,242 (September 5, 1950), P. J. Mitchell, Jr. (to Procter & Gamble Co.).
20. M. M. Chrysam, "Margarines and Spreads," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., John Wiley & Sons, New York, 1996, pp. 79–82.
21. D. J. Metzroth, "Shortening: Science and Technology," in Y. H. Hui ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., John Wiley & Sons, New York, 1996, pp. 122–123.
22. R. D O'Brien, "Shortenings: Types and Formulations," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., John Wiley & Sons, New York, 1996, pp. 181–184.
23. U.S. Pat. 4,127,597 (November 28, 1978), J. C. Craig, M. F. Kozemple, and S. Elias (to U.S. Secretary of Agriculture).
24. R. Krishnamurthy and M. Kellens, "Fractionation and Winterization," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 4, 5th ed., John Wiley & Sons, New York, 1996, pp. 314–315.
25. U.S. Pat. 3,232,961 (February 1, 1966), W. Stein, H. Rutzen, and E. Sussner (to Henkel & Cie)
26. U.S. Pat. 2,875,066 (February 24, 1959), G. W. Holman and L. H. Going (to Procter & Gamble Co.).
27. U.S. Pat. 2,875,067 (February 24, 1959), G W. Holman and L. H. Going (to Procter & Gamble Co.)
28. M. Naudet, "Composition of Partial Esters of Glycerol and Fatty Acids," *Rev. Fr. Corps Gras* **17**, 97–104 (1970).
29. E. R. Sherwin, "Antioxidants for Vegetable Oils," *J. Am. Oil Chem. Soc.* **53**, 430–436 (1976).
30. *U.S. Code of Federal Regulations*, Title 21, Part 172, Sections 172.818 and 172.854, U.S. Government Printing Office, Washington, D.C., 1994
31. *U.S. Code of Federal Regulations*, Title 21, Part 169, Section 169.150, U.S. Government Printing Office, Washington, D.C., 1994.
32. *U.S. Code of Federal Regulations*, Title 21, Part 169, Section 169.140, U.S. Government Printing Office, Washington, D.C., 1994.
33. U S. Pat. 4,385,076 (May 24, 1983), T. G. Crosby (to Procter & Gamble Co.).
34. U.S. Pat. 4,384,008 (May 17, 1983), D. H. Millisor (to Procter & Gamble Co.).
35. U.S. Pat. 4,960,606 (October 2, 1990), T. G. Crosby (to Bunge Foods Corporation).
36. U.S. Pat. 4,962,951 (October 9, 1990), T. G. Crosby (to Bunge Foods Corporation).
37. U.S. Pat. 4,359,482 (November 16, 1982). T. G. Crosby (to Procter & Gamble Co.).
38. E. J. Pyler, *Baking Science and Technology*, Vols. 1 and 2, 3rd ed., Sosland Publishing Co., Kansas City, Mo., 1988.
39. U.S. Pat. 4,310,556 (January 12, 1982), J. L. Suggs, D. F. Buck, and H. K. Hobbs (to Eastman Kodak Co.).
40. U.S. Pat. 4,320,557 (January 12, 1982), J. L. Suggs, D. F. Buck, and H. K. Hobbs (to Eastman Kodak Co.).
41. U.S. Pat. 4,242,366 (December 30, 1980), J. E. Morgan, A. J. Del Vecchio, B. L. Brooking, and D. M. Laverty (to Pillsbury Co.).
42. U.S. Pat. 4,201,718 (May 6, 1980), J. T. Marsh.
43. U.S. Pat. 4,205,905 (May 27, 1980), M. Pike, I. G. Barr, and F. Tirtaux.
44. U.S. Pat. 4,268,534 (May 19, 1981), T. Kawada, and Y. Tanaka (to Kao Soap Co., Japan).
45. U.S. Pat. 4,108,879 (August 22, 1978), S. Minowa, Y. Tokoshima, N. Yasuda, and T. Tanaka (to Ashai Denko Kogyo KK, Japan).
46. *U.S. Code of Federal Regulations*, Title 21, Chapter 9, Section 321a (Butter Act), U.S. Government Printing Office, Washington, D.C., 1994
47. *U.S. Code of Federal Regulations*, Title 21, Part 166, Section 166.110, U.S. Government Printing Office, Washington, D.C., 1994.
48. Economic Research Service, *Food Review*, U.S. Department of Agriculture, Washington, D.C., September–December, 1997.
49. Economic Research Service, *Oil Crops Situation and Outlook*, U.S. Department of Agriculture, Washington, D.C., October 1998.
50. J. E. Kinsella, "Food Lipids and Fatty Acids: Importance in Food Quality, Nutrition, and Health," *Food Technol.* **42**, 124–145 (1988).
51. Food and Nutrition Board, National Research Council, *Recommended Daily Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989.
52. E. H. Ahrens, Jr., and W E Connor, "Report of the Task Force on the Evidence Relating Six Dietary Factors to the Nation's Health," *Am. J. Clin. Nutr.* **32** (Suppl.), 2621–2748 (1982).
53. S. M. Grundy, "Cholesterol and Coronary Disease. A New Era," *J. Am Med. Assoc.* **256**, 2849–2858 (1986).

54. A. Leaf and P. C. Weber, "Cardiovascular Effects of N-3 Fatty Acids," *N. Engl J. Med.* **318**, 549–557 (1988).
55. F. H. Mattson and S. M. Grundy, "Comparison of Effects of Dietary Saturated, Monounsaturated, and Polyunsaturated Fatty Acids on Plasma Lipids and Lipoproteins in Man," *J. Lipid Res.* **26**, 194–202 (1985).
56. A. Bonahome and S. M. Grundy, "Effect of Dietary Stearic Acid on Plasma Cholesterol and Lipoprotein," *N. Engl J. Med.* **318**, 1244–1248 (1988).
57. N. B. Carter, H. J. Heller, and M. A. Denkar, "Comparison of the Effects of Medium-Chain Triacylglycerols, Palm Oil, and High Oleic Sunflower on Plasma Triacylglycerol Fatty Acids and Lipid and Lipoprotein Concentrations in Humans," *J. Clin. Nutr.* **65**, 41–45 (1997).
58. K. Sundram, K. C. Hayes, and O. H. Siru, "Both Dietary 18:2 and 16:0 May Be Required To Improve the Serum LDL/HDL Cholesterol Ratio in Normocholesterolemic Men," *Nutr. Biochem.* **6**, 179–187 (1995).
59. K. C. Hayes, "Saturated Fats and Blood Lipids. New Slant on an Old Story," *Can. J. Cardiol.* **11** (Suppl. G), 16–86 (1995).
60. K. Sundram, K. C. Hayes, O. H. Siru, "Dietary Palmitic Acid Results in Lower Seum Cholesterol Than Does Lauric-Myristic Acid Combination in Normolipemic Humans," *Am. J. Clin. Nutr.* **59**, 841–846 (1994).
61. P. Kholsa and K. C. Hayes, "Dietary Palmitic Acid Raises Plasma LDL Cholesterol Relative to Oleic Acid Only at High Intake of Cholesterol," *Biochim. Biophys. Acta* **1210**, 13–22 (1993).
62. B. Dolan and J. M. Nash, "Searching for Life's Elixir," *Time*, 62–66 (Dec. 12, 1988).
63. J. T. Anderson, F. Grande, and A. Keys, "Independence of the Effects of Cholesterol and Degree of Saturation of Fat in the Diet on Serum Cholesterol in Man," *Am. J. Clin. Nutr.* **29**, 1184–1189 (1976).
64. Anonymous, "New Findings on Palm Oil," *Nutr. Rev.* **45**, 205–207 (1987).
65. M. Sugano, *Lipids* **40**, 48–51 (1987).
66. C. Ip et al., eds., *Dietary Fat and Cancer*, Alan R. Liss, New York, 1986.
67. W. C. Willet and B. MacMahon, "Diet and Cancer—Overview," *New Engl J. Med.* **310**, 697–703 (1985).
68. J. E. Hunter, C. Ip, and E. J. Hollenbach, "Isomeric Fatty Acids and Tumerigenesis: A Commentary on Recent Work," *Nutrition and Cancer* **7**, 199–209 (1985).
69. F. R. Senti, ed., *Health Aspects of Dietary Trans Fatty Acids*, Federation of the American Society for Experimental Biology, Bethesda, Md., 1985
70. J. T. Judd et al., "Dietary Trans Fatty Acids: Effects of Plasma Lipids and Lipoproteins on Healthy Men and Women," *Am. J. Clin. Nutr.* **59**, 861–868 (1994).
71. P. H. Nestel et al., "Plasma Lipoprotein and Lp[a] Changes With Substitution of Elaidic Acid for Oleic Acid in the Diet," *J. Lipid Res.* **33**, 1029–1036 (1992).
72. D. Kromhout et al., "Dietary Saturated and Trans Fatty Acids and Cholesterol and 25-Year Mortality From Coronary Heart Disease: The Seven Countries Study," *Prev. Med.* **24**, 308–315 (1995).
73. ASCN/AIN Task Force on Trans Fatty Acids, "Position Paper on Trans Fatty Acids," *Am. J. Clin. Nutr.* **63**, 663–670 (1996)
74. C. Ip and J. R. Marshal, "Trans Fatty Acids and Cancer," *Nutr. Rev.* **54**, 138–145 (1996).

GENERAL REFERENCES

E. Bernadini, *Vegetable Oils and Fat Processing*, Vol. 2, B. E. Oil, Rome, Italy, 1983.

E. W. Eckey, *Vegetable Fats and Oils*, Reinhold Publishing, New York, 1964.

M. Gupta, "Manufacturing Process for Emulsifiers," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol 4, 5th ed., John Wiley & Sons, New York, 1996, pp. 569–601.

T. P. Hilden and P. N. Williams, *The Chemical Constitution of Natural Fats*, 4th ed., John Wiley & Sons, New York, 1964.

Institute of Shortening and Edible Oils, *Food Fats and Oils*, 7th ed., Institute of Shortening and Edible Oils, Washington, D.C., 1994.

H. W. Lawson, *Standards For Fats and Oils*, AVI Publishing, Westport, Conn., 1985.

K. S. Markley, *Fatty Acids*, 2nd ed., John Wiley-Interscience, New York, 1960.

T. J. Wiess, *Food Oils and Their Uses*, 2nd ed., AVI Publishing, Westport, Conn., 1983.

THOMAS CROSBY
Bunge Foods Corporation
Bradley, Illinois

FATS AND OILS: SUBSTITUTES

A growing public awareness about the relationship between food and wellness has kept dietary fat at the top of the list of consumer nutritional concerns for at least a decade (see Table 1 Ref. 1). According to the U.S. Department of Agriculture's (USDA's) Continuing Survey of Food Intakes by Individuals (CSFII) (1994–1996), almost half of Americans think their diets are too high in fat (2). Research by Susan M. Krebs-Smith and colleagues, reported in the *American Journal of Clinical Nutrition*, supports the CSFII findings. Of the six most prevalent nutrient intake patterns identified by these researchers, not one meets the 30% calories-from-fat guideline. In fact, only 1% of the population actually fulfills all of the U.S. government's Food Guide Pyramid recommendations (3).

At 9 kcal/g, fat is the most concentrated source of energy among the macronutrients. According to the *Surgeon General's Report on Diet and Health*, high intake of dietary fat is "associated with increased risk for obesity, some types of cancer, and possibly gallbladder disease. Epidemiologic, clinical and animal studies provide strong and consistent evidence for the relationship between saturated fat intake, high blood cholesterol, and increased risk for coronary heart disease. Excessive saturated fat consumption is the major dietary contributor to total blood cholesterol levels" (4). Reflecting national health policy, the Surgeon General, the National Academy of Sciences, the American Dietetic Association, the American Heart Association, the National Cholesterol Education Project, the American Cancer Society, the National Institutes of Health, the USDA, and the U.S. Department of Health and Human Services are among the many health and government authorities that recommend limiting dietary fat to 30% of calories.

ROLE OF FAT REPLACERS IN FOOD

In its April 1998 position paper on fat replacers, The American Dietetic Association stated:

Table 1. Nutritional Content of Food: Top Five Concerns 1994–1998

	Percentage of consumers concerned				
Concern	1994 (%)	1995 (%)	1996 (%)	1997 (%)	1998 (%)
Fat content, low fat	59	65	60	56	59
Salt/sodium content, less salt	18	20	28	23	24
Cholesterol levels	21	18	26	20	20
Sugar content, less sugar	14	15	12	11	12
Food/nutritional value	4	8	6	11	12

Source. Ref 1.

> Fat replacers may be used for multiple purposes. One motivation is to facilitate a reduction in total and saturated fat consumption by maintaining the appeal of foods reduced in fat content. The goal of fat reduction is to decrease the incidence of obesity and chronic diseases. A second purpose may be to reduce total energy consumption to improve health and enhance physical appearance and self-concept. A third option entails using fat replacers to increase the volume of palatable foods that may be consumed without increasing fat or energy intake. (5)

According to Calorie Control Council research, the primary reason people use reduced-fat foods is to stay in better overall health (see Table 2). Not surprisingly, a food's fat content is the first item shoppers look for when reading a nutrition label (6). Yet even though consumers seem increasingly committed to lowering their dietary fat intake, they continue to rank taste as their top consideration when selecting food (see Table 3). And few nutrients affect the taste and overall sensory experience of food more than fat.

Fat not only adds aroma and flavor to food, but also influences palatability, flakiness, creaminess, and crispness. And to most people, the texture—or mouth-feel—of food ranks alongside taste as an important selection criterion. From the creaminess of ice cream, to the moist tenderness of cake, to the crispness of potato chips, fat is a critical component. Thus, removing fat from a food can affect far more than taste. Research has shown that most consumers, when given a food from which the fat has been removed and no replacement added, find the food unacceptable (7). The delicate balance between fat and a pleasant sensory experience can be seen in the home-cooking arena as well. Home bakers can replace *some* of the fat or oil in muffins and cakes with applesauce or pureed prunes. But removing all the fat will result in unacceptable appearance, texture, and taste.

Responding to a market that is ripe for low- and reduced-fat foods (see Table 4 and 5), manufacturers have made fat modification an increasingly important aspect of their research and development. Their objective is threefold: to create food products that qualify for nutrient content/health claim status (see Table 6); to capture the sensory attributes and functionality of fat; and to achieve all of this cost effectively. In 1990 Healthy People 2000 called on the food industry to create 5000 reduced-fat foods over the next decade. By 1992 manufacturers had exceeded that goal by 600 (8). In 1990 alone, the food industry rolled out more than 1400 products bearing a reduced- or low-fat label claim—up 70% from the number of such products the year before. By 1995 the number of fat-modified products had risen to 8500 (9,10).

Table 2. Why People Used Reduced-Fat Products

Reason	Percentage of adult Americans citing
To stay in better overall health	77
To eat or drink healthier food and beverages	71
To reduce fat	68
To reduce cholesterol	61
To maintain current weight	57
To reduce calories	56
To maintain an attractive physical appearance	52
To reduce weight	43
For refreshment or taste	39
To help with a medical condition	31

Source: Calorie Control Council 1998 National Survey.

COMPOSITION OF FAT REPLACERS

Focus groups conducted by the International Food Information Council suggest that there is a lack of knowledge concerning fat replacers among consumers as well as among health professionals. For example, very few consumers realized that the fat removed from low- and reduced-fat foods is replaced with another ingredient. And although health professionals understood that product reformulation replaced the taste and texture of fat, they typically had very little specific information (11).

Usually a fat replacer is not itself a fat. Some fat replacers, however, are lipids, but because of their structure, they may be undigestible or only partially digestible. Most fat replacers are limited in their use. Some are good thickening agents; others provide creaminess; still others make food moist. Having a variety of fat replacement options available allows manufacturers to use a "systems approach" to formulating low-fat and fat-free foods with the functional and sensory characteristics of their full-fat counterparts. The combination of fat replacers used in a product depends on which properties of that product are being duplicated. Combinations may include proteins, starches, dextrins, maltodextrin, fiber, emulsifiers, and flavoring agents. Some fat replacers are themselves blends of various ingredients (see Table 7).

Types

Fat replacers fall into three functional categories (5,7,12):

Table 3. Importance of Various Factors in Food Selection: 1994–1998

	Percentage of consumers saying "very important"				
Factor	1994 (%)	1995 (%)	1996 (%)	1997 (%)	1998 (%)
Taste	90	90	88	87	89
Nutrition	76	74	78	77	76
Product safety	69	69	75	73	75
Price	70	69	66	66	64

Source Ref. 1.

Table 4. Percentage of Consumers Who Eat Various Reduced-Fat Foods

		Among shopper segment	
Food	% Total who eat	% Healthy eaters	% Nutritionally apathetic
Salad dressing	68	78	49
Mayonnaise	55	60	39
Crackers	53	61	35
Ice cream	52	60	35
Chips	51	57	40
Cookies	51	54	40

Source. Ref. 6.

Table 5. Most Popular Reduced-Fat Products

Product	Percentage of adult Americans who consume
Skim or low-fat milk	62
Salad dressings/sauces/mayonnaise	56
Margarine	44
Chips/snack foods	40
Meat products	39
Ice cream/frozen desserts	36
Cakes/baked goods	32
Dinner entrees	30
Candy	18

Source: Calorie Control Council 1998 National Survey.

Table 6. Food Label Nutrient Content Claims

Claim[a]	Criterion
Fat free	Less than 0.5 fat
Low fat	3 g or less of fat
Reduced or less fat	At least 25% less fat[b]
Light	One-third fewer calories or 50% less fat[b]

[a] The Food and Drug Administration and the U.S. Department of Agriculture have established specific regulation concerning allowable nutrient content claims. The following claims are defined for one serving.

[b] As compared with a standard serving size of the traditional food.

1. Fat mimetics replace the bulk, body, and mouth-feel of fat. They reduce calories both because they are less calorically dense than fat and because they contain water, which itself replaces some fat. Mimetics based on gums, cellulose, and seaweed are noncaloric; those based on starch and protein provide 4 kcal/g.
2. Low-calorie fats include products such as caprenin and salatrim, both of which provide 5 kcal/g. They are metabolized more like carbohydrate and are rapidly used as energy rather than stored as fat.
3. Fat replacers, substitutes, or analogs are similar to fats and oils in function and in sensory properties. Theoretically, they can replace fat on a one-to-one weight basis. Generally, they are heat stable and can be used for frying. Fat replacers provide 0 to 3 kcal/g. Olestra is a zero-calorie fat replacer.

Derivations

Fat replacers are also classified according to the substances from which they are derived (see Table 8). Carbohydrate- and protein-based fat replacers are fat mimetics; fat-based fat replacers are low-calorie fats or fat replacers (5,7,13):

- *Carbohydrate-based.* Carbohydrates such as starches, fibers, cellulose, and gums are commonly used in low-fat and fat-free foods as texturing agents, bulking agents, moisturizers, and stabilizers. Carbohydrate-based ingredients provide 0 to 4 calories per gram.
- *Protein-based.* Milk, egg, or whey protein is heated and blended at high speed to produce tiny particles that feel creamy to the tongue. This process is called microparticulation. Protein-based ingredients also help to stabilize emulsions in sauces, spreads, and salad dressings. These ingredients are fully absorbed by the body and range in calories from 1 to 4 kcal/g, depending on degree of hydration.
- *Fat-based.* Some fat-reduction ingredients are actual fats designed to contribute fewer calories and less fat to foods. Others, such as olestra, are structurally modified to be calorie-free.

NUTRITIONAL IMPACT OF FAT REPLACERS

Some observers have speculated that the availability of reduced-fat and fat-free foods is contributing to the increasing rate of obesity among adults and children. To characterize fat replacers as a magic formula for weight loss is to misrepresent their optimal dietary role. To prevent or slow weight gain, calories eaten cannot exceed cal-

Table 7. Selected Applications and Functions of Fat Replacers

Application	Fat replacer	General functions[a]
Baked goods	Lipid based	Emulsify, provide cohesiveness, tenderize, carry flavor, replace shortening, prevent staling, prevent starch retrogradation, condition dough
		Retain moisture, retard staling
	Carbohydrate based	Texturize
	Protein based	
Frying	Lipid based	Texturize, provide flavor and crispness, conduct heat
Salad dressing	Lipid based	Emulsify, provide mouth-feel, hold flavorants
	Carbohydrate based	Increase viscosity, provide mouth-feel, texturize
	Protein based	Texturize, provide mouth-feel
Frozen desserts	Lipid based	Emulsify, texturize
	Carbohydrate based	Increase viscosity, texturize, thicken
	Protein based	Texturize, stabilize
Margarine, shortening, spreads, butter	Lipid based	Provide spreadability, emulsify, provide flavor and plasticity
	Carbohydrate based	Provide mouth-feel
	Protein based	Texturize
Confectionery	Lipid based	Emulsify, texturize
	Carbohydrate based	Provide mouth-feel, texturize
	Protein based	Provide mouth-feel, texturize
Processed meats	Lipid based	Emulsify texturize, provide mouth-feel
	Carbohydrate based	Increase water-holding capacity, texturize, provide mouth-feel
	Protein based	Texturize, provide mouth-feel, water holding
Dairy products	Lipid based	Provide flavor, body, mouth-feel and texture; stabilize
	Carbohydrate based	Increase viscosity, thicken, aid gelling, stabilize
	Protein based	Stabilize, emulsify
Soups, sauces, gravies	Lipid based	Provide mouth-feel and lubricity
	Carbohydrate based	Thicken, provide mouth-feel, texturize
	Protein based	Texturize
Snack products	Lipid based	Emulsify, provide flavor
	Carbohydrate based	Texturize, aid formulation
	Protein based	Texturize

Source: Food Technology, March 1998.

[a] Functions are in addition to fat replacement.

ories expended. Fat-modified foods are but one tool that can be used to create this energy balance.

The theory is that people may be cutting down on fat consumption, but by overindulging in fat-modified foods, they are consuming extra calories and dramatically tipping the energy balance toward obesity. But the fact is that despite the proliferation of low-fat and fat-free foods, Americans are actually eating *more* fat than in the past. Although between the 1970s and the 1990s, Americans decreased their intake of fat from approximately 37% of calories to approximately 34% of calories, this drop in *percent of calories from fat* represents neither a decrease in *calories* nor a decrease in *fat grams*. Over the same 20-year time span, the average adult's daily calorie intake increased by 300, and total fat gram consumption increased from about 81 g/day to 83 g/day (13). At 9 calories per gram, this 2-g/day increase in consumption adds up to about 540 extra calories per month, or almost 6500 extra fat calories per year.

In addition, the 1996 National Institutes of Health Consensus Conference Statement on Physical Activity and Cardiovascular Health identified physical inactivity as a major U.S. public health crisis. Inactivity reduces muscle mass, which translates into fewer calories burned, even at rest. When physical activity is low, a reduction in dietary fat is needed simply to *prevent* weight gain, even if fat intake is moderate. But increased fat consumption coupled with stagnant-to-falling exercise rates is the magic formula for positive energy balance and weight gain.

Another often overlooked fallacy in the theory that fat-modified foods have led to obesity concerns timing. Recently released population figures on obesity were compiled as of 1990—when 33% of the American public was identified as overweight. The market influx of fat-modified foods, however, didn't peak until 1995 (14). In addition, first-generation fat-modified foods of the 1980s were far less palatable than today's low-fat and fat-free products. A proliferation of good-tasting fat-modified foods in the 1990s can't be blamed for obesity statistics based on 1980s data.

Satiety

Bell and colleagues have demonstrated that people eat to a certain *volume*, rather than to a calorie count (15). Diets high in fat are calorically dense and highly palatable, but they are generally *less* satiating than diets high in carbohydrates. Although once in the intestine, fat seems to create strong signals of satiety, the "I'm full" feeling is delayed because of the time it takes time for fat to reach the intes-

Table 8. Example Fat Replacers: Ingredients and Applications

Type	Brand name(s)	Ingredients	Applications
Protein-based fat replacers			
Microparticulated protein	Simplesse K-Blazer	Milk and/or egg/white proteins, sugar, pectin, citric acid	Imparts fatlike creaminess and richness but no fat flavor; not heat stable; 1–2 kcal/g; digested as protein; used in ice cream, butter, sour cream, cheese, yogurt
Modified whey protein	Dairy-Lo	Whey or milk protein concentrate	Improves texture, flavor, stability of low-fat foods; 4 kcal/g; used in frozen dairy desserts, hard and processed cheese, sour cream, dips, yogurt, sauces, baked goods
Isolated soy protein	Supro ProPlus Supro Plus	Used in foods for 35 years; added to meat to reduce fat	
Fat-based fat replacers			
Sucrose polyester (olestra)	Olean	Sucrose core with 6 to 8 fatty acids	Looks, tastes, and feels like fat, calorie-free; passes through body unabsorbed; may lower cholesterol; only approved fat replacer that can be used for frying; used in savory snacks
Salatrim	Benefat	Soybean and canola oil	Used in confections, baked goods, dairy; 5 kcal/g
Caprenin	Caprenin	caprylic, capric and behenic acids, and glycerine	Good substitute for cocoa butter; can be used in confections; 5 kcal/g
Mono- and diglycerides	Dur-Em, Dur-Lo		Used to replace all or part of the shortening in cake mixes, cookies, icings, and some dairy products; emulsifiers that disperse fat in watery mediums so less fat is used; 9 kcal/g
Carbohydrate-based fat replacers			
Carrageenan	Carrageenan	Extract of red seaweed	First used as an emulsifier, stabilizer, thickener; provides gel-like mouth-feel; used to replace part of the fat in ground beef, hot dogs, processed cheese, low-fat desserts
Microcrystalline cellulose	Avicel (cellulose gel), Methocel		Used in salad dressings, mayonnaise, processed cheese, frozen desserts; forms gel in water; acts like fat and has the mouth-feel and appearance of fat; used as a stabilizer; calorie-free
Hydrolyzed oat flour	Oatrim, Beta-Trim, TrimChoice	Enzyme-treated oat flour containing β-glucan soluble flour	Used in baked goods, fillings and frostings, frozen desserts, dairy beverages, cheese, salad dressings, processed meat, confections; 1 to 4 kcal/g
Powdered cellulose	Solka-Floc		Often used in fried foods; causes less oil to be absorbed
Maltodextrins	CrystalLean; Lorelite; Lorelite; Lycadex; MALTRIN, Paselli D-LITE, EXCEL, SA2; STAR-DRI	Gel or powder extracted from tapioca, corn, potato, rice	Mimic fat's mouth coating, melting sensation, richness; used in salad dressings, pudding, spreads, dairy desserts, meat products; can be used in some cooked foods; 4 kcal/g
Polydextrose	Litesse, Sta-Lite	Water-soluble polymer of dextrose containing minor amounts of sorbitol and citric acid	Used as fat replacer and bulking agent; used in baked goods, chewing gum, confections, salad dressings, frozen dairy desserts, gelatins, puddings; 1 kcal/g
Gums	KELCOGEL, KELTROL, Slendid		Have creamy mouth-feel; provide thickening effect; pass through body virtually unmetabolized, common gums include guar gum, gum arabic, locust bean gum, modified carbohydrate gum/vegetable gum; used in a wide variety of foods

Table 8. Example Fat Replacers: Ingredients and Applications *(continued)*

Type	Brand name(s)	Ingredients	Applications
Inulin	Raftiline, Fruitafit, Fibruline	Chicory root extract	Used in yogurt, cheese, frozen desserts, baked goods, icings, fillings, dairy products, fiber supplements, processed meats, 1 to 1 2 kcal/g
Pectin	Splendid	Citrus peel and table sugar	Forms a gel that can replace up to 100% of fat in some foods; mouth-feel and melting sensation of fat; used in soups, sauces, gravies, cakes and cookies, dressings and spreads, frozen desserts, frosting
Z-Trim	Z-Trim	Processed hulls of oats, soybeans, peas, rice, or from bran of corn or wheat	Provides smooth mouth-feel of fat; can be used in a variety of foods; calorie-free

Sources. Refs. 5, 17, 13, and Fat Reduction in Foods, Calorie Control Council, 1997.

tinal tract. Large amounts of energy-dense fat can be ingested before satiety signals kick in. Thus, eating high-fat foods leads to what researchers call "passive consumption" or high-fat hyperphagia—that is, a tendency to consume *excess* energy (16).

Because of the weak effect it has on satiety, removing some fat or substituting for it does not substantially decrease the satiating effect of a food, but it can result in fewer calories being eaten. Consequently, lower-fat diets including use of fat-modified foods, rather than contributing to obesity, actually have the ability to *reduce* the passive consumption of excess calories. CSFII data from 1995 show that adults who used more fat-modified foods also consumed less energy overall (17). In addition, research by Arsenault and colleagues and by Sigman-Grant demonstrates that individuals who consume fat- and calorie-reduced foods have a more nutrient-dense diet than those who do not (18–20).

Compensating Behaviors

Probably the most common argument used to support the notion that overindulging in fat-modified foods is responsible for obesity is the compensation theory—that people equate "fat-free" with "low-calorie" and therefore eat more. Most of the research on dietary compensation and fat replacers has been done on olestra, available under the brand name Olean®.

Among individuals *who did not know* a fat replacer had been added to their diet, researchers have observed two behaviors, depending on participants' ability to regulate calories. The research has shown that children and young adult males, who generally regulate calories well, did compensate for the energy deficit created by fat replacement (21,22). In both groups, however, compensation was not 100% nor was it fat-specific. Consequently, although these children and lean young men did compensate for calories, they nevertheless consumed a diet that was lower in fat.

Researchers also studied a group of 105 individuals who do not regulate calories well. Among these participants, they observed both a decrease in the percentage of calories from fat and in total calories consumed. These preliminary findings indicate that for people who need to control body weight, fat replacers may be a useful tool for helping manage both fat and calorie intake (23–25).

When research participants *did know* that a fat replacer had been added to their food, compensation behavior remained negligible. In a 10-day study of 96 habitual potato-chip eaters, some individuals who knew they were eating fat-free potato chips ate a few more chips. Most participants however, ate the same amount of chips regardless of whether or not they knew they were eating full-fat chips or chips made with a fat replacer. It is important to note that all participants eating chips made with a fat replacer benefited from both a fat and a calorie reduction (26).

In another study examining the behavioral aspects of compliance with a low-fat diet, two groups totaling 44 women attempted to maintain a diet having less than 30% of calories from fat. Women who were allowed to use products with fat replacers reported feeling less deprived because they had a wider range of foods from which to choose (27). A six-month study conducted by Westerterp and colleagues tracked 217 free-living, nonobese men and women who received either reduced-fat or full-fat products. At the end of the intervention, study participants in the reduced-fat group had consumed fewer calories and had not gained weight. The control group, however, showed both an increase in calorie intake and weight (28). Similarly, in a recent three-month trial, conducted by Kelly and colleagues, study participants consuming a full-fat diet gained weight, whereas those consuming foods made with a fat replacer did not (29).

REGULATING FAT REPLACERS

The 1958 Food Additives Amendment to the Federal Food, Drug and Cosmetic Act requires that the Food and Drug Administration (FDA) approve all food additives prior to their use. Manufacturers must submit a food additive petition (FAP) to FDA, detailing the additive's production, its expected consumption level (average as well as 90th percentile); exposure by age group; and its safety profile, including for potentially sensitive subpopulations. When Congress enacted this legislation, it exempted (with some qualifications) two groups of substances:

- "Prior-sanctioned substances" are those which the FDA or the USDA had determined prior to 1958 as safe for use in food—for example, sodium nitrate and potassium nitrate used to preserve luncheon meats.
- GRAS substances are substances in use before 1958 that are "generally recognized as safe" by scientific experts based on the substances' history of use in food or on scientific procedures. If a substance was not in use in foods before 1958, its safety must be determined based on scientific procedures. Common GRAS substances—among the several hundred used in food—are sugar, baking soda, and vitamins.

Establishing GRAS status and filing a FAP require similar scientific support. One key difference between seeking GRAS status for a food component and filing a petition for approval of a food additive, however, is that the safety information about a GRAS substance is available in the scientific literature and readily accessible to the manufacturer, whereas safety information for the food additive may not yet be published. Acting independently from the FDA, manufacturers can convene a panel of experts to review the scientific information and determine whether or not a substance should be considered GRAS for a specific use.

The majority of fat replacers have garnered approval through the GRAS procedure (eg, various carbohydrate polymers, gums, gels and starches; microparticulated proteins; whey proteins; and fat emulsifiers). Procter & Gamble's caprenin offers a good example of an independent

Table 9. Dietary Impact of Fat Replacers

Regular diet				Diet with fat replacers			
Food	Portion	Kcal	Fat g	Food	Portion	Kcal	Fat g
Dietary impact of fat replacers: breakfast							
Coffee	10 oz	6	0	Coffee	10 oz	6	0
Creamer	1 t	16	1	Low-fat creamer	1 t	8	0
Bagel	1	195	1	Bagel	1	195	1
Cream cheese	1 oz	100	10	Light cream cheese	1 oz	60	5
Orange	1	65	0	Orange	1	65	0
Total		*382*	*12*	*Total*		*334*	*6*
Dietary impact of fat replacers: lunch							
Lean ground beef	4 oz	286	18	Lean ground beef	4 oz	286	18
Roll	1	114	2	Roll	1	114	2
Cheese	2 oz	120	9				
Low-fat cheese	2 oz	90	2				
Lettuce	1 oz	5	0	Lettuce	1 oz	5	0
Tomato	1 slice	4	0	Tomato	1 slice	4	0
Mayonnaise	1T	57	5	Fat-free mayonnaise	1T	10	0
Potato chips	1 oz	150	10	Fat-free potato chips	1 oz	0	0
Water	12 oz	0	0	Water	12 oz	0	0
Total		*736*	*44*	*Total*		*509*	*22*
Dietary impact of fat replacers: dinner							
Tossed salad	1-1/2 c	35	0	Tossed salad	1-1/2 c	35	0
Salad dressing	2 T	138	14	Low-fat salad dressing	2 T	70	6
Chicken breast	6 oz	284	6	Chicken breast	6 oz	284	6
Baked potato	1	220	0	Baked potato	1	220	0
Sour cream	1 oz	61	6	Fat-free sour cream	1 oz	17	0
Green beans	1/2 c	25	0	Green beans	1/2 c	25	0
Butter	2 pats	71	8	Low-fat margarine	2 pats	36	4
Ice cream	1/2 c	150	12	Low-fat ice cream	1/2 c	100	1.5
Iced tea	12 oz	99	0	Iced tea	12 oz	99	0
Total		*1,083*	*46*	*Total*		*886*	*19.5*
Dietary impact of fat replacers: snack							
Tortilla chips	1 oz	141	7	Fat-free tortilla chips	10 oz	98	0
Salsa	1 T	25	0	Salsa	1T	25	0
Soda	12 oz	148	0	Soda	12 oz	148	0
Total		*314*	*7*	*Total*		*271*	*0*
Grand Total		*2515*	*109*	*Grand Total*		*2000*	*45.5*

Source. Adapted from *The Weight Control Digest*, May/June 1996.

GRAS determination. Caprenin is a triglyceride formed of three fatty acids that are natural components of the diet. Procter & Gamble asked an independent expert panel of scientists to review caprenin for GRAS status. In 1991 the panel determined that caprenin should indeed be generally recognized as safe.

If the FDA chooses to disagree with a GRAS determination, it may take regulatory action. Companies can also petition FDA to affirm their independent GRAS determinations. In these cases, FDA publishes a notice in the *Federal Register* requesting public comment.

The FDA approval process for a *new* food ingredient begins with a food additive petition in which the manufacturer must supply extensive scientific data documenting intended use and safety. A food additive is defined as "an ingredient not previously found in food whose intended use results, or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food" (5). FDA may recommend intake limits and may require postmarket surveillance. Procter & Gamble's sucrose polyester fat replacer, olestra (Olean), went through the FAP process.

CONCLUSION

Currently, there is no single fat replacer that "does it all." The most successful approach to fat replacement may prove to be the "systems approach" that combines the features of various fat replacers to achieve the optimal taste and function in a particular food. In the future, the ideal fat replacer—whether a single entity or a blend of ingredients—will be one that captures the functionality and sensory qualities of fat; is calorie-free, inexpensive, and suitable for frying and cooking; and that does not interfere with drug utilization or with the absorption of other nutrients. Manufacturers may seek to promote other health benefits of certain fat replacers. For example, Oatrim and the soon-to-be-available Nu-trim, both developed by the USDA, contain β-glucan, the soluble fiber in oats and barley that lowers blood cholesterol.

Although an impressive number of fat-modified products are introduced every year, relatively few succeed. Until consumers are content with the taste and cost of fat-modified foods, their use will not be widespread, and their long-term impact on everyday eating habits and the health of the U.S. population will be difficult to measure. It is clear, however, that when used properly, fat-modified foods have the potential to play an important role in reducing both calories and fat (see Table 9). As the American Dietetic Association notes in its 1998 position paper on fat replacers:

> "Reduced-fat products can . . . be used to expand the diversity and volume of foods that can be consumed while maintaining a given level of energy consumption. . . . Safe and effective use of products containing fat replacers should be facilitated through efforts to improve consumer understanding of labeling information, the importance of food preparation practices, portion size and eating frequency control, and the relationship between energy expenditure and total energy balance." (5)

BIBLIOGRAPHY

1. *Trends in the United States: Consumer Attitudes and the Supermarket 1998*, Food Marketing Institute, Washington, D.C., 1998.
2. *What We Eat in America Survey 1994–1996. Continuing Survey of Food Intake in America (CSFII)*, U.S. Department of Agriculture, Washington, D.C., 1997.
3. S. M. Krebs-Smith et al., "Characterizing Food Intake Patterns of American Adults," *Am. J. Clin. Nutr.* **65**, 1264S–1268S (1997).
4. *U. S. Surgeon General's Report on Nutrition and Health*, U S. Department of Health and Human Services, Washington, D.C., 1988.
5. "Position of the American Dietetic Association Position: Fat Replacers," *J. Am. Dietet. Assoc.* **98**, 463–468 (1998).
6. *Shopping for Health*, Food Marketing Institute, Washington, D.C., 1997.
7. K. Napier, *Fat Replacers: The Cutting Edge of Cutting Calories*, American Council on Science and Health, New York, 1997.
8. *Healthy People 2000 Review: 1995–1996*, U.S. Department of Health and Human Services, Hyattsville, Md., 1996.
9. Calorie Control Council home page, URL: *www.caloriecontrol.com* (last accessed November 1998).
10. L. Dornblaser, "New Product News," *Prepared Foods* **4**, 37 (1996).
11. Internal memo to International Food Information Council Task Force on Fat Replacers, April 24, 1995.
12. E. E. Ziegler and L. J. Filer, eds., *Present Knowledge in Nutrition*, 7th ed., ILSI Press, Washington, D.C., 1996.
13. *IFIC Review: Use and Nutritional Impact of Fat Reduction Ingredients*, International Food Information Council, Washington, D.C., 1997.
14. International Food Information Council, "Fat Facts and Fiction," *Food Insights* Sept./Oct., 2 (1997).
15. E. A. Bell et al., "Energy Density of Foods Affects Energy Intake in Normal-Weight Women," *Am. J. Clin. Nutr.* **67**, 412–420 (1998).
16. J. E. Blundell and J. I. Macdiarmid, "Fat as a Risk Factor for Overconsumption: Satiation, Satiety, and Patterns of Eating," *J. Am. Dietet. Assoc.* **97**, S63–S69 (1997).
17. A. H. Lichtenstein et al., "Dietary Fat Consumption and Health," *Nutr. Rev.* **56**, S3–S18 (1998)
18. M. Sigman-Grant, "Can You Have Your Low-fat Cake and Eat It Too? The Role of Fat Modified Products," *J. Am. Dietet Assoc.* **97**, S76–S81 (1997).
19. J. E. Arsenault, "Nutritional Intake of Women Consuming Fat- and Calorie-Reduced Foods," poster session, American Dietetic Association Annual Meeting, Oct. 23, 1997.
20. M. Sigman-Grant, S. Poma, and K. Hsieh, "Update on the Impact of Specific Fat Reduction Strategies on Nutrient Intake of Americans," *FASEB J.* **12**, 3079A (1998).
21. L. L. Birch et al., "Effects of a Nonenergy Fat Substitute on Children's Energy and Macronutrient Intake," *Am. J. Clin. Nutr.* **58**, 326–333 (1993).
22. B. J. Rolls et al., "Effects of Olestra, a Noncaloric Fat Substitute, on Daily Energy and Fat Intakes in Lean Men," *Am. J. Clin. Nutr.* **56**, 84–92 (1992).
23. G. Bray et al., "Effects of Two Weeks of Fat Replacement by Olestra on Food Intake and Energy Metabolism," *FASEB J.* **9**, 2 (1995).

24. J. O. Hill et al., "Effects of 14 Days of Covert Substitution of Olestra on Spontaneous Food Intake," *Am. J. Clin. Nutr.* **67**, 1178–1185 (1998)
25 C. deGraff et al., "Nonabsorbable Fat (Sucrose Polyester) and the Regulation of Energy Intake and Body Weight," *Am. J. Physiol.* **270**, R1386–R1393 (1996).
26. D. L. Miller et al., "Effect of Fat-Free Potato Chips With and Without Nutrition Labels on Fat and Energy Intakes," *Am. J. of Clin. Nutr.* **68**, 282–290 (1998).
27. M. P. Bolton, P. W. Pace, and R. S. Reeves, "Use of Olestra Products in a Nutrition Education Program," *Journal of Nutrition in Recipes and Menu Development* **2**, 33–42 (1996).
28. K. R. Westerterp et al , "Dietary Fat and Body Fat: An Intervention Study," *International Journal of Obesity* **20**, 1022–1026 (1996).
29. S M. Kelly et al., "A 3-Month, Double-Blind, Controlled Trial of Feeding With Sucrose Polyester in Human Volunteers," *Br. J. Med.* **30**, 41–49 (1998)

SUZETTE MIDDLETON
Procter & Gamble Co.
Cincinnati, Ohio

FIBER, DIETARY

Dietary fiber is a general term for plant polysacharides and lignin that are not broken down by the digestive enzymes of human beings. Dietary fiber is found in plant cell walls, mucilages, gums, and algal polysaccharides. Synthetic polysaccharides used in the food industry are also considered dietary fiber. Resistant starch, starch that is not digested in the small intestine, is chemically and physically similar to other nondigestable polysaccharides and also may be included in the definition of fiber. Many compounds with a wide range of physical and chemical properties are referred to as dietary fiber. In a single plant, the fiber composition of the stem or leaves is very different from the fiber in the fruit or seeds. Dietary fiber is not a uniform substance and every plant has a unique dietary fiber makeup. Because dietary fiber includes many compounds with very different physicochemical properties, it is difficult to characterize. Division of fibers by their solubility in water is the most common method of classification, and is convenient because many of the physiological properties of fiber seem to be based on this physical property. As a rule, water-soluble fibers form viscous solutions and are used in the food industry as emulsifiers, food thickeners, and gelling agents. In the body, soluble fibers are associated with lipid and carbohydrate metabolism. Insoluble dietary fibers, such as those found in wheat bran, tend to contribute to fecal bulk and decreased intestinal transit time.

COMPONENTS

Complex polysaccharides from plants are divided into several distinct groups based on chemical structure. The most common structural component of the cell wall is *cellulose*. Cellulose is composed of long linear chains of glucose molecules (approx. 10,000) with beta 1–4 links, which cannot be broken down by digestive enzymes. The *hemicelluloses* found in plants are a heterogeneous group of compounds that contain a variety of different sugars arranged in a primary chain or backbone, with numerous side chains. The specific names of the hemicelluloses are based on their sugar content, such as xyloglucans (xylose and glucose), glucomannans (glucose and mannose), and arabinoxylans (arabinose and xylose). Solubility in water is dependent on the sugar components and the physical structure of the individual hemicellulose, but most compounds in this group are soluble in dilute alkali. *Pectin* from plants refers to a complex mixture of substances that is composed of a galacturonic acid core esterfied with methyl groups on the uronic acid residues. Rhamnose is also found in the backbone of pectins. Pectin is water soluble and used to produce highly viscous gels, whereas protopectins are insoluble. The only nonpolysaccharide included in the definition of dietary fiber is *lignin*. The lignins consist of chains of phenyl propane residues that cement and anchor the cell wall matrix and stiffen the plant tissue. As plants grow and age they undergo a process of lignification, and the dietary fiber makeup changes over time.

ANALYSIS

The different chemical properties of the various components of dietary fiber make it quite challenging to determine the fiber content of various plants and foods. Early methods for isolating dietary fiber commonly destroyed many of the water-soluble components of the fiber matrix. The term crude fiber is used to describe fiber content determined by an acid and alkali extraction. A rapid, reliable method of determining total dietary fiber content (water soluble and insoluble) has been recommended by the Association of Official Analytical Chemists (AOAC). This methodology combines gravimetric and enzymatic processes and is appropriate for measuring dietary fiber content of foods for labeling purposes. Analysis of the individual components of dietary fiber is much more complex. The best known procedure, developed by Southgate (1), removes the individual fractions of dietary fiber through a series of extractions. The addition of gas liquid or liquid chromatography allows for the identification of individual sugars present in each fraction.

PHYSICOCHEMICAL PROPERTIES

Every fiber source has unique physicochemical properties that elicit variable physiological responses in the body and allows for a wide range of uses in the food industry. These properties are variable and are influenced by factors such as particle size (coarsely ground wheat bran versus finely ground bran), cooking or freezing, and breakdown of the matrix during digestion. Properties such as water-holding capacity or the hydration of dietary fibers to form a gel matrix influence the viscosity of the gastrointestinal contents and can increase fecal bulk and stool softness. Adsorption and binding of organic molecules is also an important trait of dietary fibers. Polysaccharides with an electrical charge, such as pectin and lignin, are known to sequester or bind bile acids and increase their excretion.

24. J. O. Hill et al., "Effects of 14 Days of Covert Substitution of Olestra on Spontaneous Food Intake," *Am. J. Clin. Nutr.* **67**, 1178–1185 (1998)
25 C. deGraff et al., "Nonabsorbable Fat (Sucrose Polyester) and the Regulation of Energy Intake and Body Weight," *Am. J. Physiol.* **270**, R1386–R1393 (1996).
26. D. L. Miller et al., "Effect of Fat-Free Potato Chips With and Without Nutrition Labels on Fat and Energy Intakes," *Am. J. of Clin. Nutr.* **68**, 282–290 (1998).
27. M. P. Bolton, P. W. Pace, and R. S. Reeves, "Use of Olestra Products in a Nutrition Education Program," *Journal of Nutrition in Recipes and Menu Development* **2**, 33–42 (1996).
28. K. R. Westerterp et al , "Dietary Fat and Body Fat: An Intervention Study," *International Journal of Obesity* **20**, 1022–1026 (1996).
29. S. M. Kelly et al., "A 3-Month, Double-Blind, Controlled Trial of Feeding With Sucrose Polyester in Human Volunteers," *Br. J. Med.* **30**, 41–49 (1998)

SUZETTE MIDDLETON
Procter & Gamble Co.
Cincinnati, Ohio

FIBER, DIETARY

Dietary fiber is a general term for plant polysacharides and lignin that are not broken down by the digestive enzymes of human beings. Dietary fiber is found in plant cell walls, mucilages, gums, and algal polysaccharides. Synthetic polysaccharides used in the food industry are also considered dietary fiber. Resistant starch, starch that is not digested in the small intestine, is chemically and physically similar to other nondigestable polysaccharides and also may be included in the definition of fiber. Many compounds with a wide range of physical and chemical properties are referred to as dietary fiber. In a single plant, the fiber composition of the stem or leaves is very different from the fiber in the fruit or seeds. Dietary fiber is not a uniform substance and every plant has a unique dietary fiber makeup. Because dietary fiber includes many compounds with very different physicochemical properties, it is difficult to characterize. Division of fibers by their solubility in water is the most common method of classification, and is convenient because many of the physiological properties of fiber seem to be based on this physical property. As a rule, water-soluble fibers form viscous solutions and are used in the food industry as emulsifiers, food thickeners, and gelling agents. In the body, soluble fibers are associated with lipid and carbohydrate metabolism. Insoluble dietary fibers, such as those found in wheat bran, tend to contribute to fecal bulk and decreased intestinal transit time.

COMPONENTS

Complex polysaccharides from plants are divided into several distinct groups based on chemical structure. The most common structural component of the cell wall is *cellulose*. Cellulose is composed of long linear chains of glucose molecules (approx. 10,000) with beta 1–4 links, which cannot be broken down by digestive enzymes. The *hemicelluloses* found in plants are a heterogeneous group of compounds that contain a variety of different sugars arranged in a primary chain or backbone, with numerous side chains. The specific names of the hemicelluloses are based on their sugar content, such as xyloglucans (xylose and glucose), glucomannans (glucose and mannose), and arabinoxylans (arabinose and xylose). Solubility in water is dependent on the sugar components and the physical structure of the individual hemicellulose, but most compounds in this group are soluble in dilute alkali. *Pectin* from plants refers to a complex mixture of substances that is composed of a galacturonic acid core esterfied with methyl groups on the uronic acid residues. Rhamnose is also found in the backbone of pectins. Pectin is water soluble and used to produce highly viscous gels, whereas protopectins are insoluble. The only nonpolysaccharide included in the definition of dietary fiber is *lignin*. The lignins consist of chains of phenyl propane residues that cement and anchor the cell wall matrix and stiffen the plant tissue. As plants grow and age they undergo a process of lignification, and the dietary fiber makeup changes over time.

ANALYSIS

The different chemical properties of the various components of dietary fiber make it quite challenging to determine the fiber content of various plants and foods. Early methods for isolating dietary fiber commonly destroyed many of the water-soluble components of the fiber matrix. The term crude fiber is used to describe fiber content determined by an acid and alkali extraction. A rapid, reliable method of determining total dietary fiber content (water soluble and insoluble) has been recommended by the Association of Official Analytical Chemists (AOAC). This methodology combines gravimetric and enzymatic processes and is appropriate for measuring dietary fiber content of foods for labeling purposes. Analysis of the individual components of dietary fiber is much more complex. The best known procedure, developed by Southgate (1), removes the individual fractions of dietary fiber through a series of extractions. The addition of gas liquid or liquid chromatography allows for the identification of individual sugars present in each fraction.

PHYSICOCHEMICAL PROPERTIES

Every fiber source has unique physicochemical properties that elicit variable physiological responses in the body and allows for a wide range of uses in the food industry. These properties are variable and are influenced by factors such as particle size (coarsely ground wheat bran versus finely ground bran), cooking or freezing, and breakdown of the matrix during digestion. Properties such as water-holding capacity or the hydration of dietary fibers to form a gel matrix influence the viscosity of the gastrointestinal contents and can increase fecal bulk and stool softness. Adsorption and binding of organic molecules is also an important trait of dietary fibers. Polysaccharides with an electrical charge, such as pectin and lignin, are known to sequester or bind bile acids and increase their excretion.

It is hypothesized that in the intestine dietary fiber is responsible for binding or adsorbing toxic and mutagenic substances, thus enhancing their removal from the body. Dietary fiber also has the capacity to act as a cation exchanger in the intestine. Minerals and electrolytes can bind to fiber, potentially decreasing their absorption in the digestive tract. An additional characteristic of fiber is the ability to undergo degradation or fermentation by microflora of the large intestine. Water-soluble fiber sources such as pectin or gums may be totally degraded in the colon, whereas cellulose, being a tightly packed linear insoluble polysaccharide, is almost totally resistant to fermentation by the human colonic flora. In plant tissues a mixture of polysaccharides and lignin is produced making it difficult to accurately predict their physicochemical properties and their potential physiological effects in the body. This explains why the effects of oat bran are so different than those of wheat bran and why the overall characteristics of a fiber source may not be related to the physicochemical properties of the individual polysaccharides it contains.

PHYSIOLOGIC AND METABOLIC EFFECTS

Burkitt and Trowell (2) were the first to report the physiological importance of dietary fiber consumption. Based on epidemiological studies, they showed associations between low-fiber diets and chronic disorders such as constipation, diverticulosis, colon cancer, diabetes, and cardiovascular disease. Since the 1970s research has been carried out that, for the most part, confirms the role of dietary fiber in disease prevention. Normal laxation is an important health benefit of dietary fiber consumption. Certain varieties of dietary fiber have been shown to increase stool weight and frequency, soften feces, increase fecal bulk, and reduce gastrointestinal transit times. This is particularly true of insoluble dietary fibers such as cellulose, found in large quantities in wheat bran, and of soluble but nonfermentable fibers such as psyllium gum. Constipation may be prevented, or successfully treated, by increasing dietary fiber intake. Various hypotheses have been suggested as to how dietary fiber affects the composition of fecal material and the frequency of defecation. A likely explanation is the sponge theory. Dietary fiber in the gastrointestinal tract acts as a "water-laden sponge" (3). The sponge entraps or adsorbs water, ions, and nutrients, altering intestinal contents and changing the rate of peristalsis. The presence of fiber also affects the microfloral activity of the colon, the end result being decreased intestinal transit time with increased fecal bulk and water. Stools tend to be softer and larger accompanied by increased frequency of defecation.

Consumption of soluble dietary fibers has been shown to lower blood cholesterol levels in both laboratory animals and humans. Fibers such as oat bran, guar gum, and pectin all have a hypocholesterolemic effect. Several mechanisms of action appear to be responsible for this physiological response. Soluble fibers increase the viscosity of intestinal contents, which alters the mixing and diffusion of nutrients in the intestine and changes rates of nutrient absorption. Impaired bile acid and/or cholesterol absorption from the intestine is thought to lead to lowered blood cholesterol levels. In the colon, most soluble fibers are degraded by the microflora and produce short chain fatty acids (SCFA). It has been hypothesized that SCFA absorbed from the colon may directly inhibit cholesterol synthesis in the liver. High-fiber diets also alter the structure of the intestine. In experimental animals, intestinal length is increased and there is a marked increase in mucosal and muscle mass following fiber consumption (4). It is assumed that morphological changes in the intestine alter absorption patterns thus influencing metabolic processes in the body.

Many varieties of soluble fibers influence the rate of carbohydrate digestion and absorption. It has been shown that when certain fibers are added to test meals, postprandial glucose and insulin levels are decreased (5). In addition, intake of high-fiber foods tends to modulate glucose excursions following meal consumption. This helps to maintain glucose homeostasis and is of particular importance to individuals with impaired glucose tolerance or diabetes.

Prevention of cancer is also considered a health benefit of high-fiber diets. Epidemiological studies indicate that dietary fiber, and in particular a diet rich in cereals and vegetables, helps to prevent colon cancer (6). It is thought that the insoluble fibers that promote laxation and decrease intestinal transit times are responsible for this effect. If toxins and mutagens in foods are moved rapidly through the intestine, exposure of the mucosa to these harmful materials is minimized. Although it is thought that dietary fiber may have a role in preventing numerous cancers including mammary and prostate, this hypothesis has yet to be proven.

RECOMMENDATIONS

The numerous types of dietary fiber and their many components have led to difficulties in establishing recommended daily allowances. However, several professional groups have issued guidelines for levels of dietary fiber intake that should promote good health and have little or no negative side effects. The United States Department of Agriculture (USDA) has suggested a guideline of 11.5 g dietary fiber per 1,000 calories consumed ranging from approximately 20 to 35 g/day for adults (7). Recent recommendations of the American Health Foundation suggest a goal of minimal intake of dietary fiber for children and young adults 3 to 20 years of age be the equal to age plus 5 g of dietary fiber per day (age plus 5). This recommendation suggests that 3-year-old children consume no less than 8 g/day of dietary fiber and a 20-year-old consume 25 g/day (8).

NUTRITIONAL VALUE

Dietary fiber has been called a nonnutritive bulking agent. This is not an accurate description because dietary fiber does indeed contribute energy to the body. In cases where the fiber undergoes fermentation by the intestinal microflora, the SCFAs that are formed and absorbed into the blood contribute kilocalories to the body. Not all fibers are degraded by bacteria, so an average caloric value of 2 kcal/g fiber has been suggested by the British Nutrition Foundation Task Force. Actual values for different fiber sources range from 0 to 4 kcal/g.

USES IN THE FOOD INDUSTRY

The physicochemical properties of individual fiber sources determine their usefulness in the food industry. Many synthetic or modified fibers are used as thickening agents, texturizers, or stabilizers. Gums, also called hydrocolloids, are considered food additives. To achieve desired properties in food systems, they are used at levels of less than 2%. Some examples of gums include seaweed extracts such as alginates, agar, and carrageenan. Gums are also isolated from seeds (locust bean, guar) or dried resins that are expelled from trees (gum arabic, gum ghatti, gum karaya, gum tragancanth).

Derivatives of cellulose are also used in the food industry. Chemical modification of cellulose produces water-soluble fibers such as carboxymethylcellulose, methylcellulose, and hydroxypropylmethylcellulose. These compounds have a wide variety of uses such as incorporation into baked goods to increase shelf life and to improve crumb texture. Pectin, which is commercially derived from citrus peels and apple pomace, is used as a thickener, gelling agent, and suspending agent. The degree of methylation of the main polygalacuronic acid backbone determines the gel strength and gelling time of the pectin. Numerous other fibers are used in industry to improve food quality and produce new products.

Table 1. Dietary Fiber Content of Commonly Eaten Foods

Product	Dietary fiber (g/100 g)
Fruits	
Apple (fresh)	1.9
Banana	2.4
Orange	2.4
Grapes	1.0
Peach	2.0
Watermelon	0.5
Vegetables	
Broccoli	3.0
Cucumber	0.7
Corn	2.7
Lettuce	1.4
Tomato	1.1
Potato	1.6
Peas	2.3
Grains and legumes	
Wheat flour (white)	2.7
Wheat bran	42.8
White bread	2.3
Whole wheat bread	6.9
All Bran (Kellogg's)	32.3
Lentils (cooked)	7.9

Source. USDA Nutrient Data Base.

DANGERS OF HIGH-FIBER DIETS

A major concern regarding the safety of high-fiber diets is the effect of dietary fiber on the bioavailability of vitamins and minerals. This remains one of the few possible adverse effects of increasing dietary fiber consumption. Dietary fiber can bind or adsorb ions and act as a physical barrier, potentially interfering with absorption of important micronutrients from the intestine into the bloodstream. In young children and the elderly, where only small quantities of foods are consumed and variety may be limited, dietary fiber consumption should conform to the recommended daily intakes. Despite studies that suggest that high-fiber diets may have deleterious effects, many researchers firmly believe that there is no convincing scientific evidence that dietary fiber, even when consumed in large amounts, has any adverse effects on nutrition in humans. Deficiencies have not developed in long-term vegetarian adults who consumed more than 50 g of dietary fiber per day (9).

BIBLIOGRAPHY

1. D. A. T. Southgate, "The Chemistry of Dietary Fibre," in G. A. Spiller and R. J. Amen, eds., *Fiber in Human Nutrition*, Plenum Press, New York, 1976; pp. 31–72.
2. D. P. Burkitt and H. C. Trowell, *Refined Carbohydrate Foods and Disease. Some Implications of Dietary Fibre*, Academic Press, London, 1975.
3. M. A. Eastwood and E. R. Morris, "Physical Properties of Dietary Fiber that Influence Physiological Function: A Model for Polymers Along the Gastrointestinal Tract," *Am. J. Clin. Nutr.* **55**, 436–442 (1992).
4. A. Stark, A. Nyska, and Z. Madar, "Metabolic and Morphometric Changes in Small and Large Intestine in Rats Fed a High Fiber Diet," *Toxicol. Path.* **24**, 166–171 (1996).
5. A. Stark and Z. Madar, "Dietary Fiber," in I. Goldberg, ed., *Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals*, Chapman & Hall, New York, 1994, pp. 183–201.
6. M. J. Hill, "Cereals, Dietary Fibre and Cancer," *Nutr. Res. (N.Y.)* **18**, 653–659 (1998).
7. U.S. Department of Agriculture. *Food Guide Pyramid: A Guide to Daily Food Choices.* U.S. Department of Agriculture, Washington, D.C., 1992.
8. C. L. Williams, M. Bollella, and W. L. Wynder, "A New Recommendation for Dietary Fiber in Childhood," *Pediatrics* **96**, 985–988 (1995).
9. J. Rattan et al., "A High Fiber Diet Does Not Cause Mineral and Nutrient Deficiencies," *J. Clin. Gastroenterol.* **3**, 390–393 (1981).

ALIZA STARK
Hebrew University of Jerusalem
Rehovot, Israel

FILTH AND EXTRANEOUS MATTER IN FOOD

THE FEDERAL FOOD LAWS

Soon after the new century began in 1900, the efforts of that great champion of food purity, Dr. Harvey Wiley, began to pay off. The U.S. Congress passed the Pure Food Law in June of 1906 (1–3). A stronger and more detailed law emerged from Congress in 1938 (4). Since then the food law has been amended and expanded on several occasions. Today, the Federal Food, Drug, and Cosmetic Act (FD&C Act) stands as a major achievement in the protection of consumer health (5).

As great as that achievement was, however, it should be noted that protection of consumer health was not the only objective of the Pure Food Law. The courts have consistently ruled that it was the intention of Congress to keep filth, whether harmful or not, out of food. Congress wanted food held to the highest standards of hygiene and aesthetics (6,7). The hygienic standard has, however, received the greater amount of attention by the Food and Drug Administration (FDA) in its mission to protect the public's health (8).

Two Sections on Filth

The FD&C Act (9) contains two paragraphs that are especially pertinent to the topic of filth. The first, 402(a)(3), states that a food shall be deemed to be adulterated if it consists in whole or in part of any filthy, putrid, or decomposed substance or if it is otherwise unfit for food. The second, 402(a)(4), states that a food shall be deemed to be adulterated if it has been prepared, packed, or held under insanitary conditions whereby it may have been contaminated with filth, or whereby it may have been rendered injurious to health. There have been numerous challenges to FDA's interpretation of the "filth sections" of the Act.

ENFORCEMENT OF THE ACT

Enforcement actions fall roughly into two categories: actions against products and actions against persons (10).

The Butter Oil Case

In a precedent-setting case, the "butter oil case," decided in 1947, federal agents seized a large quantity of butter because the cream from which it had been made was contaminated with insect and rodent filth. The owner of the butter wished to recoup his losses by making strained butter oil out of the butter. FDA objected to this procedure on the grounds that the resulting butter oil would still contain soluble insect and rodent filth. The court upheld this objection, thus making soluble (invisible) filth just as much an adulterant as particulate (visible) filth (11).

Reconditioning

What the owner of the butter had proposed is a process called reconditioning; by this procedure a substandard product may be brought into compliance if the offending filth in the product is removed. Reconditioning processes are often approved by FDA (12–14).

"Too Small to See" Defense

Another landmark case involved some tomato paste that contained bits and pieces of larval corn earworms (*Helicoverpa zea*). The owner of the tomato paste claimed that the insect fragments could not be considered filth—and the consumer would not so perceive them—because the fragments were too small to see. The court rejected that argument (15).

Actions Against Persons: The *Park-Acme* Case

A series of inspections (1970–1972) of Acme Markets warehouses in Philadelphia and Baltimore resulted in charges against both the company and its president (Mr. Park) for violations of Section 402(a)(4) of the FD&C Act. The president claimed to be innocent on the grounds that the violative acts were committed by subordinates to whom he had delegated authority and that he had no knowledge of or responsibility for the violations. When the Supreme Court decided the case, it vitiated the excuse of "delegation" and determined that top executive officers do indeed bear complete responsibility for violations that occur in their organizations (7,13,16–18).

Filth: "Too Little to Matter"

The bold statements in the two principal filth sections of the FD&C Act would seem to totally preclude the presence of any filth in or around food. The courts, however, have never held to the letter of the law; in other words, there is such a thing as de minimis filth—too little to matter (12,13,15). Some judges have been remarkably lenient in their interpretations of what constitutes significant filth in or around foods, whereas other judges have been less forgiving. In any case, it must be admitted that, without the unconscionable waste of raw food materials, it is impossible to produce foods that are 100% free of filth. The Act, on the other hand, prohibits filth.

Food Defect Action Levels

To bridge the gap between this practical reality and the absolute ban on filthy food called for in the Act, the FDA has established a regulatory tool not mentioned in the FD&C Act, namely, the food defect action levels (DALs) (19,20). The DALs, which apply to about 90 categories of foods, mainly refer to field infestations and other unavoidable contamination. Infestations of stored foods and related insanitary conditions get no relief from the DALs.

The food DALs are administrative guidelines that are set on the basis of no hazard to human health (21). Any products that might be harmful to consumers are acted against on the basis of their hazard to health, whether or not they exceed the action levels. DALs are resorted to because it never has been possible to grow, harvest, and process foods that are totally free of natural defects. Insanitary manufacturing practices will prompt regulatory action whether or not the resulting product is above or below the pertinent DAL.

Recalls

To maintain their good reputations, many food firms are willing to act promptly to initiate a recall to protect both themselves and the public. Even though recalls are not mentioned in the FD&C Act and in spite of the fact that recalls pertaining to food are technically voluntary on the part of industry (10), the FDA can still be instrumental in prompting a recall when circumstances warrant it (7,22–24).

Extraneous Matter

The courts have always defined filth in its ordinary sense (ie, the dictionary definition) rather than giving the term a technical meaning (19). The same can be said for extraneous matter, that is, things that do not belong in food such as filth or any foreign matter that comes to be in food as a result of objectional conditions or practices in production, storage, or distribution of food. Included within the meaning of extraneous matter are filth (any objectionable matter contributed by animal contamination such as rodent, insect, or bird matter), decomposed material (decayed tissues due to parasitic or nonparasitic causes), and miscellaneous matter such as sand, soil, glass, rust, or other foreign substances (but not bacteria) (25).

The more common kinds of filth encountered during food inspections and analyses are whole and fragmented insects and mites, mammalian hairs and feces (mostly of rodent origin), feather barbules, urea (from mammalian urine), and uric acid (from avian excretions). Other kinds of filth and extraneous matter include molds and other fungi and weed seeds (26,27).

Light Filth

Since the 1930s the principal method for separating light filth such as rat hairs, feather barbules, and bits of insect exoskeleton from food has been by flotation. When mineral oil is added to a sample of food mixed in water, the oleophilic particles (ie, the light filth) float to the surface while the hydrophilic particles (ie, most foods) settle to the bottom. When the oily portion is passed through filter paper, the filth elements remain on the surface of the paper; the particles of filth are then identified and enumerated by a food analyst (28). Analysts often use reference manuals to assist them in identifying filth elements (26,29–35).

Although flotation methods are still the mainstay of food analysts, other kinds of analytical techniques have been and continue to be developed. These newer methods use biochemical analyses to search out contaminants or indicators of contamination that cannot be detected by direct visual inspection (36–42).

Heavy Filth

Most hard foreign objects (eg, pebbles, sand, glass, pieces of metal or plastic, ball bearings, pits), strictly speaking, are not filth; nevertheless they are often referred to as "heavy filth." These hard foreign objects may be considered adulterants if, when chewed, they cause a gritty mouthfeel (21), or if the particles are large enough or sharp enough to be hazardous to chew or swallow (43,44). The food DALs permit very low levels of natural hard objects (usually pit or shell fragments) in a few kinds of foods (21). When hard foreign objects occur in foods for which there is no applicable DAL, each alleged violation is treated on a case-by-case basis.

MODES OF CONTAMINATION

On the Farm

The circumstances by which foods may become adulterated by filth and extraneous matter are legion. The place where the food is grown may be the first source of filth. All field-grown crops are to some degree infested with or affected by insects, mites, rodents, birds, and molds (45). The process of harvesting or gathering the food from the field may inadvertently involve also bringing along live or dead animals, molds, weed seeds, pebbles, and particles of soil. The feces of animals or humans that live in or frequent the field may also be accidentally collected.

This kind of contamination is becoming increasingly important in crops such as lettuce, strawberries, and raspberries that are marketed with minimal after-harvest cleaning (46–48). Foods grown and harvested from aquatic habitats may also be subject to adulteration by a wide array of contaminants (49,50). Foodborne disease outbreaks associated with cross-contamination at retail outlets or in the home are at least as common, or perhaps more common, than outbreaks linked to contamination that occurs where the food is harvested.

In Storage

If the crop is temporarily stored on the farm or placed in longer-term storage off the farm, it may be attacked by insects, mites, molds, rodents, or birds (51,52). These adulterating entities produce mostly surface contamination, but some insects and molds are capable of penetrating food materials and living inside the seeds of beans, corn, wheat, and other crops.

Many kinds of manufactured foods that are stored in warehouses are very susceptible to molds and pests (insects, mites, rodents, birds). Food items stored in metal, glass, or plastic containers are generally safe from the penetrating sorts of infestations or contaminations (eg, urine), but these containers and all other kinds of packaging are susceptible to surface contamination by the urine and feces of rodents and birds. Rats and mice have little difficulty chewing their way into most kinds of plastic, paper, foil, or cloth packaging; some insects also have great penetrating capabilities (53).

In Processing

Standards of sanitation are supposed to be maintained at a high level in food manufacturing facilities, and this is often the case. However, it is difficult under even the best of circumstances to keep all pests out of food processing plants; any laxity of sanitary standards can lead to infestations by pests, and some of these pests may find their way into the foods being manufactured.

In Transportation

Opportunities for pests to infest foods during transport by railcars, trucks, and ships are numerous. The longer the journey in terms of time, the greater the likelihood that pest populations, especially of stored-grain pests (beetles, moths, mites), will dramatically increase.

In Retail

Every retail food outlet (eg, restaurants, grocery stores, sidewalk food stands) is to some extent the target of attack by flies, ants, and cockroaches and rats and mice, too, when the habitat is right for rodent pests. When these insects and rodents come into contact with food or food-contact surfaces, they may add to the food the bacteria and molds that they are carrying around with them. Occasionally a whole insect or rodent fecal pellet becomes entrapped in or falls into the food; the insect body or fecal pellet may remain largely intact or it may be broken up into small pieces as the food material is stirred during preparation. If the insect or fecal pellet had been carrying pathogenic bacteria, the food thus contaminated becomes hazardous to health if it is not subsequently heated to a high enough temperature to kill the pathogens. The Retail Food Code (54) provides guidance to managers on how to prevent foodborne diseases in retail food outlets.

At Home

Homes are no less a target for attack by pests than are retail food outlets. Unlike retail food managers, home occupants are under no legal obligation to keep pests under control. Many householders, if not for sanitary reasons, at least for aesthetic ones, attempt to keep pests under control. Both the obvious pests, such as mice, ants, cockroaches, and flies, and the more cryptic ones, such as stored-food mites, beetles, and moths, are capable of carrying pathogens and spoilage molds and leaving them behind on foods or food-contact surfaces. When homes and restaurants as sources of foodborne illness are compared, the home kitchen is the far more common source. Sanitation in the home kitchen is at least as important as sanitation in the commercial kitchen (55,56).

KEEPING FILTH OUT OF FOOD

Integrated Pest Management

Ever since DDT (a pesticide) came to be widely used after World War II, pesticides have been used as the major (sometimes, the only) answer to pest problems. In recent years, a somewhat different approach, integrated pest management (IPM), has come to be applied to pest problems (57–59). The IPM system first identifies the pest, then tries to suppress the pest population by capitalizing on weaknesses in the lifestyle of the pest. IPM may be applied to crops in the field; to stored, raw food grains; to transportation systems; to food facilities of all kinds (eg, warehouses, food factories, groceries, restaurants); and to the home kitchen.

Pesticides may play a greater or lesser role in IPM, depending on local circumstances. The goal of IPM is not to eliminate the use of pesticides (although the trend is in that direction), but to suppress or exclude pest populations (60). This goal requires a comprehensive approach that involves an array of techniques, often including application of pesticides and often emphasizing pest-exclusion practices. IPM merges with and becomes indistinguishable from those aspects of the Hazard Analysis and Critical Control Points (HACCP) system that deal with pest prevention and pest control (61).

For the food-facility manager, pest management is but one link in the chain of food sanitation that begins at the receiving door (62) and ends with the consumer. The important operational guidelines for the manager are to buy only from reliable suppliers, inspect all incoming materials, reject any substandard items, and maintain pest-free premises.

No matter how closely standard cleaning and sanitizing protocols are followed, if pests are tolerated in a food facility, they will inevitably deposit spoilage microorganisms and, occasionally, pathogens on food and food-contact surfaces. A major goal of the food-facility manager must therefore be: zero tolerance for pests. IPM makes it possible to reach this goal.

Hazard Analysis and Critical Control Points

Development of the HACCP system began in the early 1970s. The system has been widely adopted by the worldwide food industry; eventually, it will be mandatory for the entire food industry in the United States. The HACCP system emphasizes the identification, prevention, and elimination of microbiological hazards that could lead to foodborne illnesses (63–67). Extraneous materials such as filth and hard foreign objects do not figure prominently in HACCP, it is true. However, any HACCP program that proposes to be successful must take place in an environment where preventive maintenance protocols and sanitary standards are set at very high levels.

Food-manufacturing protocols often call for a biocidal step such as pasteurization or commercial sterilization that inactivates microorganisms and parasites. Cans or bottles that are aseptically sealed after the biocidal step may still contain hard foreign objects (eg, metal fragments, glass shards) that may be hazardous to health. Foods that go through additional handling after the biocidal step may be exposed to food-contact surfaces contaminated by pests, or the pests may actually come into direct contact with, and sometimes become incorporated into, the food. Such events would be of concern in a HACCP program because the pest may have introduced parasites, pathogens, or spoilage fungi that may proliferate and cause foodborne illness or spoilage of the food material.

Even if very high sanitary standards are maintained in a food-manufacturing facility, those standards will be compromised unless comparable standards extend backward along the food chain to the origin of the food on farms or in aquatic habitats. A system for the protection of the food from adulteration by filth must be in operation at all the steps from farm to table—harvesting, storing, transporting, manufacturing, warehousing, retailing. Defects in sanitation present challenges to the success of the HACCP system wherever it is applied along the food chain.

HOW DOES FILTH IN FOOD AFFECT HUMAN HEALTH?

No Effect

If the amount of filth in a food is so small that it falls within the limits set by the DALs, then that filth is assumed to have no adverse effect on human health (21). This conclusion is admittedly an assumption; the fact that this assumption is based on extensive practical experience rather than on scientific experimentation does not make it any less valid.

Indirect Effects

The presence of food-associated pests—insects, mites, rodents, birds—in a food facility is an indicator of unacceptable sanitation practices. The HACCP system works well only when high standards of environmental sanitation are maintained. The presence of food-associated pests compromises the gains in food safety that would otherwise be achieved by the HACCP program in a food facility.

There may be another indirect effect on human health that involves the production of an unhealthy or unstable emotional state when one discovers recognizable insects in or around one's food. This situation may occur anywhere, but it is especially significant if it occurs in a hospital or other patient-care facility when a person already debilitated from injury, illness, surgery, chemotherapy, or advanced age experiences revulsion and loss of appetite upon sighting obvious filth in his or her food.

Direct Effects

The accidental ingestion of insects and mites infesting food may result in the production of antibodies to the proteins of the ingested arthropods (68,69), but this rarely results in overt indications of disease. Matsumoto et al. (70) report on two patients who experienced anaphylaxis after eating foods made from ingredients contaminated with storage mites of a common species, *Tyrophagus putrescentiae* (the mold mite), and they comment on a third case involving anaphylaxis after ingestion of doughnuts made from ingredients infested by American house dust mites, *Dermatophagoides farinae* (71).

The potential for direct physical injury resulting from ingestion of insect-infested food was pointed out in the report of a case of colitis in an infant who had eaten baby cereal infested by dermestid beetles (72).

FILTH IN FOOD IN PERSPECTIVE

A Different World

Over the past 30 years or so, some very significant changes have transpired in the area of public health, especially as it relates to foodborne diseases (73). Widespread resistance to antibiotics by pathogens poses a major threat to human health. The population of surgical patients who undergo invasive procedures has burgeoned (such surgeries provide greater opportunities for colonization by pathogens, even some of those that may more usually be associated with food). The number of immunocompromised persons has increased enormously, not just because of infections by the human immunodeficiency virus, but also because more people are receiving chemotherapy and radiation treatments, and because that portion of the general population considered to be elderly (and therefore more susceptible to infections) is steadily increasing. All of these immunocompromised populations are regarded as being more susceptible to foodborne pathogens and toxins than are immunocompetent persons (74). Some kinds of opportunistic pathogens that never stood a chance of replicating in healthy hosts now find no constraints in immunocompromised hosts.

Emerging Pathogens

The classic foodborne pathogens that have long been known and well studied are quite capable of causing disease in immunocompromised persons and, to a lesser extent, in the immunocompetent. But now there are new pathogens and more virulent strains of classic pathogens to which virtually everyone is susceptible. Not only are the immunocompromised gravely challenged by the newer pathogens, but so also are the immunocompetent. No one is exempt from the threat of foodborne pathogens and toxins. Names and strains that were rarely or never heard of in the 1960s, are now in the news media on a daily basis. Enteropathogenic *Escherichia coli, E. coli* O157:H7 (and other highly virulent serotypes of this species), *Giardia lamblia, Cryptosporidium parvum, Salmonella typhimurium* Definitive Type 104, *Cyclospora cayetanensis*, and others are listed among dangerous emerging pathogens (75,76).

THE PARADOX OF FILTH IN FOOD

The Caricature

Some food safety experts and even at least one government have taken the position that looking for filth in food is simply not worth the required effort and expense. The small benefit to the public gained from occasionally finding a food item that is disgustingly filthy cannot, they contend, justify the expenditure of the time and money needed to make that discovery. To justify this position, these experts make a caricature of people's aversion to filth in food—according to them, everyone in a lifetime is going to "eat a peck of dirt" and, besides, it is harmless anyway. Apparently, even in this camp of experts there is still some interest in the maintenance of sanitation around food, if not in food, and that position continues to yield modest benefits to the consumer.

The Promise

The position just described reflects a misunderstanding of the significance of filth in food, especially if the filth was added to the food after the final biocidal step in food processing. The occasional rat hair that is extracted from a food sample probably does not represent the shedding of a single hair from a rat moving about above a food processing line. More likely that single rat hair represents two things—it is probably just one of a much larger number of hairs, most of which escaped detection; and it more probably represents a remnant of a fecal pellet, now disintegrated and dispersed throughout the food material.

When rats and mice groom themselves or each other, they remove and swallow hairs which then become incorporated into the feces of the rodent. Such pellets are never sterile, but only a small percentage of them carry pathogens that cause human disease. But some do, and here is where risk enters the picture, especially if the contamination occurs after the last biocidal step in processing and especially if the consumer of the food is immunocompromised.

What is said of the rat hair can be said in principle of all the filth elements that make their way into food—they all carry spoilage organisms, some of them carry pathogens, rarely do they emerge intact from the food production line, they are all more dangerous if they enter the food manufacturing process after the last biocidal step, and their danger to human health is related to the virulence of any pathogens they might carry and to the susceptibility status of the consuming host.

It is admirable for food safety monitors to say that action will be taken against a food manufacturer or warehouser or retailer if food pests or filthy conditions are found in a food facility, but it makes no sense to suggest that food emerging from that facility does not need to be examined for filth. The experience of countless food safety inspectors and food analysts testifies to the fact that food emerging from a filthy, pest-infested facility will bear filth far above the standard action levels and stands a good chance of becoming associated with a foodborne disease outbreak (77–79).

Early Warning

The analysis of food for adulteration by filth may serve as an early warning of lapses in sanitary protocols in the food facility and the nearly inevitable associated compromise of the HACCP program. Examination of food for light filth is a rapid, cost-effective, and efficient way to evaluate the status of sanitation in a food facility. This kind of ongoing quality assurance program gives the consumer confidence that the food as it leaves the production line is safe for consumption by virtually all persons, regardless of physical or emotional health, except for the small percentage of the population that suffers from specific food allergies. In view of the widespread occurrence of newly emerging, highly virulent pathogens, a laissez faire attitude toward filth in food is no longer tenable.

The reader may find a more detailed discussion of this topic in the first edition of this encyclopedia (80).

BIBLIOGRAPHY

1. R. C. Litman and D. S. Litman, "Protection of the American Consumer: The Congressional Battle for the Enactment of the First Federal Food and Drug Law in the United States," *Food Drug Cosmetic Law J.* **37**, 310–329 (1982).
2. R. A. Merrill and P. B. Hutt, *Statutory Supplement to Food and Drug Law—Cases and Materials*, The Foundation Press, Mineola, N.Y., 1980.
3. J. H. Young, *Pure Food—Securing the Federal Food and Drugs Act of 1906*, Princeton University Press, Princeton, N.J., 1989.
4. J. E. Hoffman, "FDA's Administrative Procedures," in *Seventy-fifth Anniversary Commemorative Volume of Food and Drug Law*, Food and Drug Law Institute, Washington, D.C., 1984.
5. W. F. Janssen, "Golden Anniversary of the FD&C Act: Consumers 'Never Had it so Good,' " *J. Assoc. Food Drug Off.* **52**, 59–60 (1988).
6. P. B. Hutt, "The Basis and Purpose of Government Regulation of Adulteration and Misbranding of Food," *Food Drug Cosmetic Law J.* **33**, 505–540 (1978).
7. J. T. O'Reilly, *Food and Drug Administration* (Regulatory Manual Series), Shephard's, Colorado Springs, Colo., 1979.
8. J. P. Hile, "New Theories of Enforcement," *Food Drug Cosmetic Law J.* **41**, 424–428 (1986).
9. U.S. Food and Drug Administration, *Federal Food, Drug, and Cosmetic Act, as Amended, and Related Laws*, HHS Publication No. (FDA) 89-1051, U.S. Department of Health and Human Services, Rockville, Md., 1989.
10. E. M. Pfeifer, "Enforcement," in *Seventy-fifth Anniversary Commemorative Volume of Food and Drug Law*, Food and Drug Law Institute, Washington, D.C., 1984.
11. V. A. Kleinfeld and C. W. Dunn, *Federal Food, Drug, and Cosmetic Act, Judicial and Administrative Record 1938–1949*, Commerce Clearing House, New York, 1953.
12. T. W. Christopher and W. W. Goodrich, *Cases and Materials on Food and Drug Law*, 2nd ed., Commerce Clearing House, New York, 1973.
13. R. A. Merrill and P. B. Hutt, *Food and Drug Law—Cases and Materials*, The Foundation Press, Mineola, N.Y., 1980.
14. Office of Enforcement, "Reconditioning of Foods Adulterated under 402(a)(4)," *Compliance Policy Guide 7153.04*, U.S. Food and Drug Administration, Washington, D.C., 1989.
15. V. A. Kleinfeld and C. W. Dunn, *Federal Food, Drug, and Cosmetic Act, Judicial and Administrative Record 1949–1950*, Commerce Clearing House, New York, 1951.
16. Y. H. Hui, *United States Food Laws, Regulations, and Standards*, John Wiley & Sons, New York, 1979.
17. J. S. Kahan, "Criminal Liability under the Federal Food, Drug, and Cosmetic Act—The Large Corporation Perspective," *Food Drug Cosmetic Law J.* **36**, 314–331 (1981).
18. J. A. Levitt, "FDA Inspections and Criminal Responsibility," *Food Drug Cosmetic Law J.* **36**, 469–477 (1981).
19. P. M. Brickey, Jr., "The Food and Drug Administration and the Regulation of Food Sanitation," in J. R. Gorham, ed., *Ecology and Management of Food-Industry Pests*, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 491–495.
20. J. M. Taylor, "Establishment and Use of Defect Action Levels," in *Association of Food Industries 82*, Association of Food Industries, Matawan, N.J., 1982.
21. Industry Activities Staff, *The Food Defect Action Levels*, U.S. Food and Drug Administration, Washington, D.C., 1998.
22. M. H. Bozeman, "Recalls—On Making the Best of a Bad Thing," *Food Drug Cosmetic Law J.* **33**, 342–359 (1978).
23. J. R. Phelps, "Actions in the Courts," *Food Drug Cosmetic Law J.* **35**, 502–510 (1980).
24. J. Bressler, "What FDA Expects During a Recall," *Food Product Development* **14**, 64–65 (Apr. 1980).
25. P. A. Cunniff, ed., *Official Methods of Analysis of AOAC International*, 16th ed., 3rd rev., 2 vol., AOAC International, Arlington, Va., 1997.

26. J. R. Gorham, ed., *Principles of Food Analysis for Filth, Decomposition, and Foreign Matter*, FDA Technical Bulletin 1, 2nd ed., Association of Official Analytical Chemists, Arlington, Va., 1981.
27. J. R. Gorham, ed., *Training Manual for Analytical Entomology in the Food Industry*, FDA Technical Bulletin 2, Association of Official Analytical Chemists, Arlington, Va., 1978.
28. E. C. Washbon and K. R. Halcrow, "Laboratory Procedures," in A. R. Olsen, T. H. Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 241–260.
29. O. L. Kurtz and K. L. Harris, *Micro-analytical Entomology for Food Sanitation Control*, Association of Official Analytical Chemists, Arlington, Va., 1962.
30. D. McClymont-Peace, *Key for Identification of Mandibles of Stored-food Insects*, Association of Official Analytical Chemists, Arlington, Va., 1985.
31 J. P. Sutherland, A. H. Varnam, and M. G. Evans, *A Colour Atlas of Food Quality Control*, Wolfe Publishing, London, 1986.
32. J. W. Gentry, K. L. Harris, and J. W. Gentry, Jr., *Microanalytical Entomology for Food Sanitation Control*, 2 vol., published by J. W. Gentry and K. L. Harris, Melbourne, Fla., 1991.
33. J. R. Gorham, ed., *Insect and Mite Pests in Food: An Illustrated Key*, Agriculture Handbook 655, U. S. Department of Agriculture, Washington, D. C., 1991.
34. D. H. Ludwig and J. R. Bryce, "Hairs and Feathers," in A. R. Olsen, T. H. Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 157–216.
35. G. Domenichini, ed., *Atlante delle Impurità Solide negli Alimenti—Manuale per il Riconoscimento dei Materiali Estranei*, Chiriotti Editori, Pinerolo, Italy, 1997.
36. J. J. Thrasher, "Detection of Metabolic Products, in J. R. Gorham, ed., *Principles of Food Analysis for Filth, Decomposition, and Foreign Matter*, FDA Technical Bulletin 1, 2nd ed., Association of Official Analytical Chemists, Arlington, Va., 1981, pp. 201–216.
37. H. R. Gerberg "Chemical Test for Mammalian Feces in Grain Products: Collaborative Study," *J. Assoc. Off. Anal. Chem.* **72**, 766–769 (1989).
38. P. E. Kauffman and D. B. Shah, "Enzyme Immunoassay for Detection of *Drosophila melanogaster* Antigens in the Juice of Various Foods," *J. Assoc. Off. Anal. Chem.* **71**, 636–642 (1988).
39. F. A. Quinn, W. Burkholder, and G. B. Kitto, "Immunological Technique for Measuring Insect Contamination of Grain," *Journal of Economic Entomology* **85**, 1463–1470 (1992).
40. B. Brader, "Are Food Sanitation Assays Meaningful?," *Cereal Foods World* **42**, 759–760 (1997).
41 P. A. Valdes, G. C. Ziobro, and R. S. Ferrera, "Use of Urease-Bromothymol Blue-Agar Method for Large-scale Testing of Urine on Grain and Seeds," *J. AOAC Int.* **79**, 866–873 (1996).
42. P. Valdes-Biles and G. C. Ziobro, "The Identification of the Source of Reagent Variability in the Xanthydrol/Urea Method," *J. AOAC Int.* **81**, 1155–1161 (1998).
43. F. N. Hyman, K. C. Klontz, and L. Tollefson, "Food and Drug Administration Surveillance of the Role of Foreign Objects in Foodborne Injuries," *Public Health Reports* **108**, 54–59 (1993).
44. J. R. Gorham, "Hard Foreign Objects in Food as a Cause of Injury and Disease A Review," in Y. H. Hui et al., eds., *Foodborne Disease Handbook*, Vol. 3, Marcel Dekker, New York, 1994, pp. 615–626.
45. D. T. Wicklow, "Preharvest Origins of Toxigenic Fungi in Stored Grains," in E. Highley et al., eds., *Stored Product Protection, Proceedings of the 6th International Working Conference on Stored-product Protection*, Vol. 2, CAB International, Wallingford, United Kingdom, 1994.
46. Office of Enforcement, "Recall of Frozen Strawberries Linked to Outbreak of Hepatitis A," in *The Enforcement Story Fiscal Year 1997*, U.S. Food and Drug Administration, Rockville, Md., 1998, pp. 113–115.
47. Office of Enforcement, "Import Detention of Raspberries from Guatemala," in *The Enforcement Story Fiscal Year 1997*, U S. Food and Drug Administration, Rockville, Md , 1998, pp. 116–117.
48. L. R. Beauchat and J-H. Ryu, "Produce Handling and Processing Practices," *Emerging Infectious Diseases* **3**, 459–465 (1997).
49. Office of Enforcement, "Import Detentions for Salmonella in Seafood," in *The Enforcement Story Fiscal Year 1997*, U.S. Food and Drug Administration, Rockville, Md., 1998, p. 127.
50. E. S. Garrett, C. Lima dos Santos, and M. L. Jahnacke, "Public, Animal, and Environmental Health Implications of Aquaculture," *Emerging Infectious Diseases* **3**, 453–457 (1997).
51. W. Stein, *Vorratsschädlinge und Hausungeziefer—Biologie, Ökologie, Gegenmaßnahmen*, Eugen Ulmer GmbH & Co., Stuttgart, 1986.
52. J. R. Gorham, ed , *Ecology and Management of Food-Industry Pests*, Association of Official Analytical Chemists, Arlington, Va., 1991.
53. H. A. Highland, "Protecting Packages Against Insects," in J. R. Gorham, ed., *Ecology and Management of Food-Industry Pests*, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 345–350.
54. *Food Code*, U.S. Food and Drug Administration, Washington, D C., 1999.
55. J. E. Collins, "Impact of Changing Consumer Lifestyles on the Emergence/Reemergence of Foodborne Pathogens," *Emerging Infectious Diseases* **3**, 471–479 (1997).
56. R. L. Hall, "Foodborne Illness: Implications for the Future," *Emerging Infectious Diseases* **3**, 555–559 (1997).
57. E. G. Thompson, "The Integrated Pest Management Approach to Food Protection," *Cereal Foods World* **29**, 149–154 (1984).
58. J. V. Osmun, "Insect Pest Management and Control," in F. J. Baur, ed., *Insect Management for Food Storage and Processing*, American Association of Cereal Chemists, St. Paul, Minn., 1984, pp. 17–24.
59. D. F. Jones and E. G. Thompson, "Integrated Pest Management for the Food Industry," in J. R. Gorham, ed., *Ecology and Management of Food-Industry Pests*, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 551–556.
60. J A. Gibson, "Review of Rodent and Insect Damage to Stored Products and Non-pesticidal Methods of Control," in D R. Houghton, R. N. Smith, and H. O. W. Eggins, eds., *Biodeterioration 7*, Elsevier Applied Science Publishers, Barking, United Kingdom, 1988.
61. J. R. Gorham, "HACCP and Filth in Food," *J Food Prot.* **52**, 674–677 (1989).
62. "The Right Way to Accept Deliveries," *Best Practices* **2**, 10 (1998).
63. O. P. Snyder, "HACCP in the Retail Food Industry," *Dairy, Food and Environmental Sanitation* **11**, 73–81 (1991).

64. E. J. Rhodehamel, "Overview of Biological, Chemical, and Physical Hazards," in M. D. Pierson and D. A. Corlett, Jr., eds., *HACCP—Principles and Applications*, AVI Book/Van Nostrand Reinhold, New York, 1992, pp. 8–28.

65. O P. Snyder, Jr., "HACCP—An Industry Food Safety Self-Control Program, Part III," *Dairy, Food and Environmental Sanitation* **12**, 164–167 (1992)

66. F. K. Käferstein, Y. Motarhemi, and D. W. Bettcher, "Foodborne Disease Control: A Transnational Challenge," *Emerging Infectious Diseases* **3**, 503–510 (1997).

67. National Advisory Committee on Microbiological Criteria for Foods, "Hazard Analysis and Critical Control Point Principles and Application Guidelines," *J. Food Prot.* **61**, 762–775 (1998).

68. H. M. Johnson et al., "Antigenic Properties of Some Insects Involved in Food Contamination," *J. Assoc. Off. Agric. Chem.* **56**, 63–65 (1973).

69. R. A. Wirtz, "Food Pests as Disease Agents," in J R. Gorham, ed., *Ecology and Management of Food-Industry Pests*, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 469–475.

70. T. Matsumoto et al., "Systemic Anaphylaxis after Eating Storage-mite-contaminated Food," *Int. Arch. Allergy Immunol.* **109**, 197–200 (1996).

71. A. M. Erben et al., "Anaphylaxis after Ingestion of Beignets Contaminated with *Dermatophagoides farinae*," *J. Allergy Clin. Immunol.* **92**, 846–849 (1993).

72. G. T. Okumura, "A Report of Canthariasis and Allergy Caused by *Trogoderma*," *Calif. Vector Views* **14**, 19–22 (1967).

73. E. M. Foster, "Historical Overview of Key Issues in Food Safety," *Emerging Infectious Diseases* **3**, 481–482 (1997).

74. J. G. Morris, Jr., and M. Potter, "Emergence of New Pathogens as a Function of Changes in Host Susceptibility," *Emerging Infectious Diseases* **3**, 435–441 (1997).

75. J. Lederberg, "Infectious Disease as an Evolutionary Paradigm," *Emerging Infectious Diseases* **3**, 417–423 (1997).

76. R. V. Tauxe, "Emerging Foodborne Diseases: An Evolving Public Health Challenge," *Emerging Infectious Diseases* **3**, 425–434 (1997).

77. A. R. Olsen, "Introduction," in A. R. Olsen, T. H. Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 1–10.

78. R. A. Baldwin, "Quality Assurance for Regulatory Science," in A. R. Olsen, T. H Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 11–20.

79. J. R. Gorham, "Reflections on Food-borne Filth in Relation to Human Disease," in A. R. Olsen, T. H. Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 269–275.

80. J. R. Gorham, "Filth and Extraneous Matter in Food," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, 1st ed., Wiley-Interscience, New York, 1991, pp. 847–868.

J. RICHARD GORHAM
Uniformed Services
University of the Health Sciences
Bethesda, Maryland

FISH AND SHELLFISH MICROBIOLOGY

Humans consume over 1,000 species of fish and shellfish that grow in diverse habitats and geographic regions all over the world (1). These fish and shellfish carry a variety of microorganisms from both aquatic and terrestrial sources. The high levels of moisture, rich nutrients, including free amino acids, other extractable nitrogenous compounds, digestible proteins, and psychrophiles, render seafood easily perishable, often spoiling in a short period of time even under refrigeration. In addition to spoilage microorganisms, seafood may contain various potential pathogens that can threaten the public health.

It is often difficult to maintain the quality of seafood products because there is a considerable distance between consumers and the harvesting areas, which provides opportunities for microbial growth and recontamination. To process fish and shellfish into stable products, low temperature, heat, curing, fermentation, and irradiation can be applied. This article covers the quantitative and qualitative aspects of microorganisms found in fish and shellfish and the factors affecting seafood quality. Organisms involved during the seafood processing are also described and discussed. Emphases are placed on spoilage bacteria, which cause the degradation of products and organisms that present risks to the public health.

MICROORGANISMS IN FINFISH

In healthy fish, muscle tissue or flesh is generally considered sterile. However, the fish surface and certain organs contain various levels of microorganisms: skin, 10^2–10^7/cm^2; intestinal fluid, 10^3–10^8/mL; and gill tissue, 10^3–10^6/mL (2,3). Cold marine water fish mainly carry psychrophilic gram-negative bacteria including *Moraxella*, *Acinetobacter*, *Pseudomonas*, *Flavobacterium*, and *Vibrio* (4,5). Both *Moraxella* and *Acinetobacter* were designated as *Achromobacter* in the past (6–8). The levels of these bacteria vary somewhat depending on season and food ingested (2,5,9). Fish intestines normally contain *Vibrio*, *Moraxella*, *Acinetobacter*, *Pseudomonas*, and *Aeromonas*, in addition to a small number of anaerobic bacteria, including *Clostridium* and *Bacillus*. Warm-water fish carry large numbers of gram-positive, mesophilic bacteria such as *Corynebacterium*, *Bacillus*, *Micrococcus*, and sometimes *Enterobacteriaceae* or even *Salmonella* (4,5).

The flora of fish depends on intrinsic factors (season, fish ground, and species) and extrinsic ones (fishing method, fish handling on board, storage condition, sampling technique, medium, and incubation temperature). Trawled fish usually carry bacterial loads 10–100 times greater than those of lined fish, because fish are dragged for a long time along the sea bottom (4). The physiological condition of fish prior to death has an effect on postharvest quality. When tuna, the fastest swimming fish, are captured in a highly stressful state, the buildup of lactic acid combined with elevated muscle temperature degrade the muscle quality, although the tuna is still acceptable for canning. Salmon harvested by gill netting die after an exhausting struggle, resulting in a shorter period of rigor mortis and deterioration during icing (10). The fish should be handled as soon as possible after being landed on the vessel. Careful handling of fish with gaff hook or forks and avoiding severe physical damage are crucial. Any breaks

64. E. J. Rhodehamel, "Overview of Biological, Chemical, and Physical Hazards," in M. D. Pierson and D. A. Corlett, Jr., eds., *HACCP—Principles and Applications*, AVI Book/Van Nostrand Reinhold, New York, 1992, pp. 8–28.
65. O P. Snyder, Jr., "HACCP—An Industry Food Safety Self-Control Program, Part III," *Dairy, Food and Environmental Sanitation* **12**, 164–167 (1992)
66. F. K. Käferstein, Y. Motarhemi, and D. W. Bettcher, "Food-borne Disease Control: A Transnational Challenge," *Emerging Infectious Diseases* **3**, 503–510 (1997).
67. National Advisory Committee on Microbiological Criteria for Foods, "Hazard Analysis and Critical Control Point Principles and Application Guidelines," *J. Food Prot.* **61**, 762–775 (1998).
68. H. M. Johnson et al., "Antigenic Properties of Some Insects Involved in Food Contamination," *J. Assoc. Off. Agric. Chem.* **56**, 63–65 (1973).
69. R. A. Wirtz, "Food Pests as Disease Agents," in J R. Gorham, ed., *Ecology and Management of Food-Industry Pests*, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 469–475.
70. T. Matsumoto et al., "Systemic Anaphylaxis after Eating Storage-mite-contaminated Food," *Int. Arch. Allergy Immunol.* **109**, 197–200 (1996).
71. A. M. Erben et al., "Anaphylaxis after Ingestion of Beignets Contaminated with *Dermatophagoides farinae*," *J. Allergy Clin. Immunol.* **92**, 846–849 (1993).
72. G. T. Okumura, "A Report of Canthariasis and Allergy Caused by *Trogoderma*," *Calif. Vector Views* **14**, 19–22 (1967).
73. E. M. Foster, "Historical Overview of Key Issues in Food Safety," *Emerging Infectious Diseases* **3**, 481–482 (1997).
74. J. G. Morris, Jr., and M. Potter, "Emergence of New Pathogens as a Function of Changes in Host Susceptibility," *Emerging Infectious Diseases* **3**, 435–441 (1997).
75. J. Lederberg, "Infectious Disease as an Evolutionary Paradigm," *Emerging Infectious Diseases* **3**, 417–423 (1997).
76. R. V. Tauxe, "Emerging Foodborne Diseases: An Evolving Public Health Challenge," *Emerging Infectious Diseases* **3**, 425–434 (1997).
77. A. R. Olsen, "Introduction," in A. R. Olsen, T. H. Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 1–10.
78. R. A. Baldwin, "Quality Assurance for Regulatory Science," in A. R. Olsen, T. H Sidebottom, and S. A. Knight, eds., *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 11–20.
79. J. R. Gorham, "Reflections on Food-borne Filth in Relation to Human Disease," in A. R. Olsen, T. H. Sidebottom, and S. A. Knight, eds , *Fundamentals of Microanalytical Entomology. A Practical Guide to Detecting and Identifying Filth in Foods*, CRC Press, Boca Raton, Fla., 1996, pp. 269–275.
80. J. R. Gorham, "Filth and Extraneous Matter in Food," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, 1st ed., Wiley-Interscience, New York, 1991, pp. 847–868.

J. RICHARD GORHAM
Uniformed Services
University of the Health Sciences
Bethesda, Maryland

FISH AND SHELLFISH MICROBIOLOGY

Humans consume over 1,000 species of fish and shellfish that grow in diverse habitats and geographic regions all over the world (1). These fish and shellfish carry a variety of microorganisms from both aquatic and terrestrial sources. The high levels of moisture, rich nutrients, including free amino acids, other extractable nitrogenous compounds, digestible proteins, and psychrophiles, render seafood easily perishable, often spoiling in a short period of time even under refrigeration. In addition to spoilage microorganisms, seafood may contain various potential pathogens that can threaten the public health.

It is often difficult to maintain the quality of seafood products because there is a considerable distance between consumers and the harvesting areas, which provides opportunities for microbial growth and recontamination. To process fish and shellfish into stable products, low temperature, heat, curing, fermentation, and irradiation can be applied. This article covers the quantitative and qualitative aspects of microorganisms found in fish and shellfish and the factors affecting seafood quality. Organisms involved during the seafood processing are also described and discussed. Emphases are placed on spoilage bacteria, which cause the degradation of products and organisms that present risks to the public health.

MICROORGANISMS IN FINFISH

In healthy fish, muscle tissue or flesh is generally considered sterile. However, the fish surface and certain organs contain various levels of microorganisms: skin, 10^2–10^7/cm^2; intestinal fluid, 10^3–10^8/mL; and gill tissue, 10^3–10^6/mL (2,3). Cold marine water fish mainly carry psychrophilic gram-negative bacteria including *Moraxella, Acinetobacter, Pseudomonas, Flavobacterium*, and *Vibrio* (4,5). Both *Moraxella* and *Acinetobacter* were designated as *Achromobacter* in the past (6–8). The levels of these bacteria vary somewhat depending on season and food ingested (2,5,9). Fish intestines normally contain *Vibrio, Moraxella, Acinetobacter, Pseudomonas*, and *Aeromonas*, in addition to a small number of anaerobic bacteria, including *Clostridium* and *Bacillus*. Warm-water fish carry large numbers of gram-positive, mesophilic bacteria such as *Corynebacterium, Bacillus, Micrococcus*, and sometimes *Enterobacteriaceae* or even *Salmonella* (4,5).

The flora of fish depends on intrinsic factors (season, fish ground, and species) and extrinsic ones (fishing method, fish handling on board, storage condition, sampling technique, medium, and incubation temperature). Trawled fish usually carry bacterial loads 10–100 times greater than those of lined fish, because fish are dragged for a long time along the sea bottom (4). The physiological condition of fish prior to death has an effect on postharvest quality. When tuna, the fastest swimming fish, are captured in a highly stressful state, the buildup of lactic acid combined with elevated muscle temperature degrade the muscle quality, although the tuna is still acceptable for canning. Salmon harvested by gill netting die after an exhausting struggle, resulting in a shorter period of rigor mortis and deterioration during icing (10). The fish should be handled as soon as possible after being landed on the vessel. Careful handling of fish with gaff hook or forks and avoiding severe physical damage are crucial. Any breaks in the skin and flesh quickly introduce spoilage bacteria, which deteriorate fish quality. Fish should be carefully cleaned, not exposed to sunlight or to the drying effects of wind, and cooled down to the temperature of melting ice (0°C) as quickly as possible (11,12).

Gutted and well-cleaned fish contain fewer bacteria than whole fish. Bleeding and gutting should be done as soon as fish arrive on deck (13). However, bleeding and gutting are not helpful in all fish harvesting operations; blue fish and dogfish are not benefited by this operation (13) if in-plant processing occurs shortly after harvest.

The method of stowing fish on the vessel can have different impacts on fish quality (14,15). Boxing, commonly used in Asia, Norway, and Iceland, provides good fish quality and quicker unloading at dockside, although it results in more labor needed in handling fish on the deck and less storage capacity. Bulking, commonly used in North America, allows for quick operation and maximum storage capacity; however, the pressure often causes poorer quality and decreases shelf life (11,16).

During the transport of fish from fish ground to fish pier, bacteria grow in the fish pen or hold board at various rates depending on fish handling and storage temperature. In the bulking stow in cold weather, a slime accumulates on the bottom of the fish hold. This fish hold slime is constituted mainly of various bacteria with a level ranging from 10^9 to 10^{10}/g (17). The bacterial flora includes *Moraxella*, *Acinetobacter*, *Flavobacter*, pseudomonads, and heavily mucoid corynebacteria, which are the major organisms responsible for the slimy deposits and are critical problems in boat sanitation (17,18). Thorough sanitation of the fish hold after unloading the catch is necessary to insure high quality of raw fish on future trips.

FISH SPOILAGE AND CHANGES IN BACTERIAL FLORA OF FISH DURING COLD STORAGE

The fish's regulatory mechanisms, which prevent invasion of the tissues by bacteria, cease to function after death. Bacteria then invade the fish body through the skin, enter the body cavity and belly walls via intestines, and penetrate the gill tissue and kidney by way of the vascular system. The low molecular substances and soluble proteins yielded from fish body during autolysis after rigor mortis provide rich nutrients for bacterial growth.

Various proteases and other hydrolytic enzymes secreted by psychrophilic and psychrotrophic organisms can act on the fish muscle even at low temperatures (17,19). The factors that influence microbial contamination and growth include fish species and size, method of catch, onboard handling, fishing vessel sanitation, processing, and storage condition (12,20,21). Fish are subject to rapid microbial spoilage if fish handling and storage are inadequate. It is estimated that about 10% of the total world catch is lost due to bacterial spoilage (22). Various microorganisms involved in spoilage are listed in Table 1 in descending order of spoilage activity. Some organism cause spoilage in different degrees depending on the total microbial flora, fish quality, handling and packaging methods, and storage temperature.

Table 1. Microorganisms Associated with Spoilage of Fresh Seafood

Spoilage activity	Microorganism
High	*Pseudomonas, (Alteromonas), putreijaciens, Pseudomonas, (Alteromonas), fluorescens,* other fluorescent pseudomonads, and other pseudomonads
Moderate	*Moraxella, Acinetobacter,* and *Alcaligenes*
Low or active only in specific conditions	*Aerobacter, Lactobacillus, flavobacterium, Micrococcus, Bacillus,* and *Staphylococcus*

Refrigerated fresh haddock fillets contain about 10^5 g of initial bacteria, predominated by *Moraxella-Acinetobacter* and *Corynebacterium*. After storage at 1°C for 14 days the bacterial number reaches 2.1×10^8/g and seafood enters the spoilage stage. *Pseudomonas* (*Alteromonas*) *putrefaciens* and fluorescent pseudomonads are organisms responsible for the spoilage of haddock at refrigerated temperatures (23). These spoilers account for only about 1% of the total count at the beginning but increase to at least 30% at the stage of spoilage. In other words, whenever *Pseudomonas putrefaciens* and fluorescent pseudomonads reach 30% of the total bacterial count, fish spoilage will result regardless of total bacterial level. When cod is stored at 20°C *Alteromonas* and *Vibrionaceae* will cause spoilage in one day (24).

As *Pseudomonas putrefaciens*, fluorescent pseudomonads, and other potential spoilers increase rapidly in initial spoilage stage; they produce vast amounts of proteolytic and other hydrolytic enzymes (5,25). Various macromolecules of fish body are degraded. Proteins are decomposed by proteases to peptides and amino acids and then further broken down to indole, amines, acids, sulfide compounds, and ammonia (26). Lipases break down lipids to form fatty acids, glycerol, and other products. Nucleotides are decomposed into nitrogenous compounds. Many enzymatic tests can determine microbial spoilage activity in fish, including hydrogen sulfide, gelatin hydrolysis. DNase, RNase, amylase, lipase, and trimethylamine oxide reductase tests and inoculation test on fish juice or fillets (20).

MICROORGANISMS IN SHELLFISH

Shellfish is composed of crustaceans (shrimp, crabs, lobster, crawfish, etc) and mollusks (bivalves, squids, snails, etc). Shellfish normally contains more moisture, greater amount of free amino acids, and more extractable nitrogenous compounds than finfish. These biochemical characteristics facilitate bacterial growth and deteriorative reactions resulting in the rapid spoilage of shellfish (27). Many shellfish grow in estuarine, coastal waters, and aquacultural ponds near residential areas and are hence susceptible to contamination by potential pathogenic organisms. Deterioration of shellfish quality results from enzymatic action from both the tissue and the contaminating organisms. Microorganisms that spoil shellfish are similar to those responsible for finfish spoilage. However, such organisms as *Moraxella-Acinetobacter* and *Lactobacillus* are more active in shellfish than in finfish.

Crustaceans

Shrimp. Among shellfish, shrimp ranks first in value and second in quantity next to crabs (28). Immediately after shrimp death, the tissue enzymes phenolases become active oxidizing tyrosine to bluish black zones or spots at the edges of the shell segments. The dark color is produced by melanin pigments that form on the internal shell surfaces on the underlying shrimp meat. At the same time a variety of bacteria start to proliferate and the growth can be accelerated if the storage temperature is not kept low enough. Removing the heads can reduce 75% of the bacteria. Gulf of Mexico shrimp contain mainly *Moraxella-Acinetobacter, Bacillus, Micrococcus*, and *Pseudomonas* (29). Most of these bacteria produce hydrolytic enzymes: 62% proteolytic, 35% lipolytic, 18% TMA-O reductive, and 12% indole positive (29–31). Shrimp unloaded from the trawlers have an average bacterial load of 6.0×10^5/g and market shrimp, 3.2×10^6/g. Bacterial counts used for indicating shrimp quality are 1.3×10^6/g, acceptable; 4.5×10^6/g, good; 1.1×10^7/g, fair; and 1.9×10^7/g, poor (32). During iced storage for 16 days, *Moraxella-Acinetobacter* increase from 27 to 82% of the total bacterial count while *Flavobacterium* decrease from 18 to 1.5%; *Micrococcus*, from 34 to 0%; and *Pseudomonas*, from 19 to 17% (33).

There are two putrefactive types of spoilage in shrimp. One is the production of indole, presumably from tryptophan by bacterial action before icing when exposed at a temperature favorable for bacterial growth. After commencement, the decomposition proceeds fairly rapidly even under ice. Indole is heat resistant and is a reliable spoilage indicator of raw material prior to processing. The other type of ammoniacal decomposition is slow and is characterized by an odor of free ammonia (34–36). The reaction is attributed to both microbial and tissue enzymatic activities depending on storage temperature and bacterial composition.

Crabs. The dominant crabs harvested in the United States are blue crab, king crab, Dungeness crab, and tanner-snow crabs. The bacterial flora of freshly caught crabs reflect that of the growing water, season, and geographic location. The hemolymph of healthy blue crabs from Chincoteague Bay, Va., is about one-fifth sterile according to 290 freshly caught crabs tested (37). The organisms found in the hemolymph of blue crabs are *Acinetobacter, Aeromonas, Pseudomonas, Flavobacterium, Vibrio, Bacillus*, coliforms, and *Clostridium* (38).

Greater numbers of bacterial species are found in Dungeness crabs from Kodiak Island and the Columbia River, waters close to human habitation, whereas the least number of species are found in the tanner crab from the Bering Sea, an area far from human habitation (39). The highest levels of bacteria occur in the gills, 10^3 to 10^7/g, as compared to 1×10^1 to 4×10^2/g in muscle tissue. Gills of Dungeness crabs from the Bering Sea contain *Moraxella, Acinetobacter, Alcaligenes, Micrococcus*, and *Staphylococcus*, whereas the muscle carries *Micrococcus* and *Staphylococcus* (39)

Crawfish. Spoilage of crawfish is caused by the potential spoilers similar to those detected in other crustaceans. In a total of 280 isolates found from spoiled crawfish, 22.1% were shown to be rapid spoilers; 16.4%, low spoilers; and 61.5%, nonspoilers (40). In the group of rapid spoilers over half were pseudomonads and less than half were *Moraxella-Acinetobacter*. Slow spoilers include *Pseudomonas, Moraxella-Acinetobacter, Alcaligenes, Flavobacterium, Aerobacter, Lactobacillus, Micrococcus*, and *Staphylococcus*. Organisms considered as nonrapid spoilers are *Aerobacter, Bacillus, Flavobacterium, Micrococcus, Sarcina*, and *Staphylococcus* (40,41). It is clear that organisms belonging to the same genus have different activities in spoilage.

Bivalves

Bivalves are mollusks, including oysters, clams, and mussels. They are soft-bodied animals that are enclosed by two rigid, bilaterally symmetrical shells. Bivalves are filter feeders and pass a large volume of water through their gills to obtain oxygen and food. Particulate matter, including microorganisms, from the water is trapped in mucus on the gills, then conveyed to the mouth, and finally to the digestive system. Bivalves, particularly oysters, ingest many microorganisms that can survive the digestive process and accumulate in the animals (42–44). The concentration of microorganisms in bivalves can be tens to hundreds of times as high as that in their growing water (42,45). Consumers and public health regulatory agencies are concerned about the pathogenic organisms found in bivalves that are affected by sewage pollution.

Fecal coliforms are generally used as indicators for bivalve quality and for domestic pollution in shellfish-growing waters (46,47). Following harvest, two microbiological guidelines are applied to determine the acceptability of shellfish meats. Bivalves at wholesale market level should have a 35°C standard plate count (SPC) of $<500{,}000$ g and a most probable numbers (MPN) fecal coliforms of $\leq 230/100$ g (46,47).

The microflora of bivalves at harvest is composed of both organisms that are symbiotic with the bivalves and organisms that are filtered from the water and ingested as food. These microorganisms vary qualitatively and quantitatively, depending on the nutrient level, salinity, temperature, and water quality. The commensal microflora include *Cristispira pectineus*, which colonizes the crystalline style of oysters, and spirochetes such as *Saprospira*, found in the crystalline style, stomach, and intestine of eastern oysters. These commensal organisms are difficult to culture and have no pathological significance to humans (48). Bivalves at harvest normally carry a total plate count of 10^3 to 10^5/g. Soft-shell clams harvested from different growing areas in the Chesapeake Bay contain a geometric mean of SPC, 2.0×10^4–7.2×10^4/g: total coliforms, 1.5 $\times 10^3$–6.3×10^3 100 g: fecal coliforms, 29–62/100 g: and *E. coli*, 14–27 100 g (49).

The common microflora of bivalves at harvest consists primarily of gram-negative rods including *Pseudomonas* and *Vibrio* species (50,51). The *Flavobacterium–Cytophaga* group occasionally exists in a certain level in oysters. Other organisms in oysters include *Acinetobacter, Corynebacterium, Moraxella, Alcaligenes, Micrococcus,* and *Bacillus*. The microflora of Gulf of Mexico's oysters is dominated by *Vibrio, Aeromonas, Moraxella*, and *Pseudomonas* (52). Low levels of yeasts such as *Rhodotorula rubra, Trichosporon, Candida*, and *Torulopsis* are also frequently encountered in eastern oysters. Several potential pathogenic strains of *Vibrionaceae* are naturally occurring in nonpolluted estuarine waters and may be encountered in bivalves. Coliforms, fecal coliforms, and *E. coli* are the most common contaminating organisms in bivalves at harvest (45,46,49)

Reducing a high microbiological load in bivalves can be accomplished by placing the animals in clean water that is free of undesirable microorganisms and under conditions in which the bivalves will actively feed. This process is called relaying and usually requires about 15 days to reach the satisfactory microbiological quality (53,54). The relaying process is often applied by transferring bivalves harvested from moderately polluted water into approved shellfish-growing water until the animals clean themselves. A second approach is called depuration in which the shellfish are maintained in tanks of clean water with controlled salinity and temperature (55). The water is often recycled through a biofilter to control water quality. The bivalves in this condition can reach the satisfactory microbiological quality within two to three days (53,55). Nevertheless, removal of viruses often does not correlate with fecal coliform elimination even if fecal indicators have a similar reduction rate as do enteric bacterial pathogens (56,57). Ultraviolet irradiation can facilitate the reduction of contaminating bacterial flora in oysters (56). Because hepatitis A may not be eliminated as readily as other enteroviruses during dupuration, hepatitis may still occur by consuming raw depurated shellfish (58).

MICROBIOLOGY AND QUALITY

Fish Products

Fish carries a variety of organisms and should be handled adequately and processed as soon as possible. Refrigeration, freezing, canning, pasteurization, salting, drying, fermentation, curing, and a combination of these methods are commonly used to process seafood into relatively stable, marketable products. Other methods such as irradiation and modified atmospheric preservation (59) have been extensively studied and proved to have potential application.

Refrigerated and Frozen Fish. Refrigeration at 5°C ceases the growth of the mesophiles and as the temperature is further lowered, psychrophiles are eliminated. During refrigeration storage a gradual killing off of the microorganisms occurs. Gram-negative asporogenous pseudomonads are cold sensitive, whereas gram-positives such as micrococci, lactobacilli, and streptococci are more resistant (60,61).

Cooling rate affects the survival of microorganisms. The maximum survival of *E. coli* is at a cooling rate of about 6°C/min, and the minimum at about 100°C/min (62). A similar minimum survival rate has been found for *Streptococcus faecalis, Salmonella typhimurium, Klebsiella aerogenes, Pseudomonas aeruginosa*, and *Azotobacter chroococcum*, but these organisms have an optimum survival rate varying from 7°C/min for *A. chroococcum* to 11°C/min for *P. aeruginosa* (63).

Frozen fish should be stored at or below −20°C and preferably at −30°C. During freezing the number of cells that are inactive ranges from 50 to 90% of the initial bacterial population. Gram-positive bacteria are more resistant to freeze injury than gram-negative ones. Some pathogenic organisms can also have a certain survivability at freezing temperatures (65). Spores are the most resistant microbial entity to freeze damage. Poliovirus inoculated into oysters showed a gradual decline in plaque-forming units during frozen storage at −36°C (64).

Reasons for the cryoinjury of cells are thermal shock, concentration of extracellular solutes, toxic action of concentrated intracellular solutes, dehydration, internal ice formation, and attainment of a minimum cell volume (62). Although cryoinjury results in the cell death, survival of microorganisms occurs and is greater in a supercooled environment than in a frozen one. Some *V. parahaemolyticus* cells, inoculated into oysters, sole fillets, and crabmeat, can persist at −15 or −30°C with a greater survival at −30°C although there is a sharp reduction in viability during freezing (66). Some pathogens such as *Listeria* can survive in freezing temperatures (60).

Cryoprotective agents are substances that can protect bacterial cells during freezing and thawing. Glycerol, dimethyl sulfoxide, egg white, carbohydrates, peptides, serum albumin, meat extract, milk, glutamic acid, malic acid, diethylene glycol, dextran, Tween 80, glucose, polyethylene glycol, and erythritol have cryoprotective functions probably due to reduction of damage to the cell wall and membrane (60).

Canned Fish. Most canned fish are fully processed products such as canned tuna, salmon, sardines, mackerel, fish balls, and other fish. These canned fish are commercially sterile with 12D process to destroy all pathogenic and other organisms, allowing a satisfactory shelf life at room temperature. However, some problems may arise due to the presence of heat-resistant spore formers in the underprocessed products or can leakage from improper seam closure and cross-contamination through cooling water. In oil pack, the oil may protect bacterial spores against heat resulting in nonsterile canned products (67). Flat sour spoilage may occur due to thermophiles, such as *B. stearothermophilus*, which survive processing and multiply during slow cooling and storage at high temperatures. Swollen cans are occasionally encountered when clostria such as *C. sporogenes* survive inadequate processing

Dried, Salted, and Smoked Fish. Dehydration is an old process that reduces the water activity (A_w) of the fish products below that required for the growth of microorganisms. The process involves drying with or without other preservatives to form dried, salted, or smoked fish.

Dried salted fish, fish bits (fried shredded fish), katsuobushi (dried and smoked skipjack stick), dried shark fins, and dried mullet roe are popular in Asia (68). Smoked salmon, herring, dogfish, and other fish are common fishery products in Europe. Growth of halophilic bacteria or molds may occur, resulting in the spoilage of salted fish. *Aspergillus* and *Penicillium* are major species associated with the color deterioration of salted round herring (68). Halophilic bacteria such as *Halobacterium* and *Halococcus* commonly present in solar salts are most troublesome during the salting and drying process. These bacteria cause red and pink discoloration and induce softness in salted fish.

Surimi-Based Products. Surimi is a mechanically deboned, water-washed frozen fish paste containing cryoprotectants. This high-protein, gel-forming material can be chopped and then mixed with salt, starch, and flavor compounds. The surimi mix is colored, textured, and cooked in two stages to set the gel to process into seafood analogues such as imitation crab, shrimp, lobster, or scallop. Freshly processed crab leg and flaked crab leg analogues contain only 10^2–10^3/g bacteria. During storage bacteria grow and SPC reaches 2×10^8/g at 10°C in 25 days and 10^4–10^6/g at 5°C in six weeks (69). SPC in flaked crab leg increases rapidly to 10^9/g after two and four weeks at 5 and 0°C, respectively. Spoilage and quality deterioration of crab analogues are indicated by number of bacteria (10^7/g), visible slime, odor, and appearance. Slime formation, softened texture, sour odor, and discoloration are consequences of spoilage. *Bacillus* is predominant initially but *Pseudomonas* gradually grows and finally outnumbers other genera at two weeks of storage at 0–5°C. *Bacillus*, which is possibly derived from the ingredient starch, is the major organism throughout the six-day storage at 15°C (69). The spoilage of other fish cake products such as kamaboko can be attributed to *Streptococcus*, *Leuconostoc*, and *Micrococcus* (70,71).

Shellfish Products

After being harvested, shellfish should be kept refrigerated or at low temperature and processed as soon as possible. Shrimp should be beheaded, peeled, or left unshelled and frozen.

Crabmeat. Blue crabs rank first in U.S. crab landings, and their major products are fresh and pasteurized meat. Other crabs caught are king, Dungeness, and tanner-snow crabs from which frozen section, claws and meat, and canned meat are commonly made (72,73).

To process blue crabmeat, live crabs are steam cooked at 121°C for 10 min. Cooked crabs are refrigerated overnight and the meat is removed by hand or machine. The meat is packed in plastic cups for fresh crabmeat or sealed in tin cans to process for pasteurized crabmeat (74). Three kinds of meat are available: lump meat taken from the back fins, claw meat extracted from claws, and regular meat collected from main body (72). In good plant sanitation, fresh crabmeat usually has a geometric mean SPC of 1.5×10^4–4.5×10^4/g, which increases to 1.4×10^5–3.2×10^5/g under poor plant sanitation (75). Cooked crabs should be stored in refrigeration (<2°C), separated from the live crabs. Refrigerated cooked crabs before picking usually contain bacteria of $<10^4$/g while cooked sponge crabs (gravid females carrying an egg mass) taken from the picking table contain bacterial levels as high as 10^6/g of whole crabs (76,77). Cooked sponge crabs have consistently been found to harbor greater numbers of bacteria than crabs without a sponge (77). Similarly, cooked green crabs, blue crabs that have recently molted and contain a higher level of water than fat crabs, carry higher levels of bacteria than normal crabs into the picking room. They contain higher moisture, which encourages bacterial growth during overnight refrigerated storage (76).

The commercial machine Quik Pik, which mechanically removes the body meat of blue crabs was started on a trial basis in 1978 and now operates successfully in Maryland under proper procedures (76,78). One quick Pik machine can pick 150 lb meat per hour, a rate equal to the work capacity of 30 hand pickers. The cooked crabs are placed in a round, rotating slotted cage to remove legs, fins, and claws, which are dropped through the slots. The crabs then pass through the debacking machine and cleaning device. The cores are loaded on racks for steam heating and then placed in the quik Pik shaker, which vigorously vibrates at 70 oscillations/s for 4 s. All the meat from cores will fall on the collecting belt for further inspection for broken shells and packaging (76).

Machine picking requires constant attention to cleanliness and sanitation to produce a meat product with a satisfactory bacterial quality. The machine should be disassembled and thoroughly cleaned and sanitized at the end of the day. Liquid on the machine during operation creates an aerosol that can greatly contaminate the meat (79). The meat conveyor belt needs continual washing with a tap water spray and sanitation in a chlorine (>200 ppm) bath. A comparison of the bacterial levels indicates that both the SPC and coagulase positive *S. aureus* for machine-picked meat are lower than for the hand product. However, a higher *E. coli* count is found in machine-picked meat than in hand-picked meat. This is not surprising because machine picking is processed under wet and warm environment whereas hand picking is operated in cool and dry conditions.

The normal shelf life of fresh crabmeat is 7–10 days and may last up to 14 days if meat with a low initial bacterial count (80) is stored under optimum refrigeration temperature. To extend the shelf life of crabmeat, the pasteurization process of holding crabmeat for 1 min at 171°–210°F, depending on the desired shelf life from 1 to 12 months, was patented (81). A process of 185°F for 1 min in the center of a 1-lb can (401 × 301) was found to sufficiently reduce an inoculated 10^6/100 g of *C. botulinum* type E spores to <6/100 g and to keep the meat nontoxic for 6 months at 40°F (82,83). A recommendation has been made to increase the time at 185°F to 3 min to provide for 12D cook based on the thermal death time studies of type E *C. botulinum* (84–86). Table 2 shows the thermal resistance characteristics of *C. botulinum* type E. For a complete process, an *F*-value of 31 based on an *F* 16/185 value and a cooling

Table 2. Decimal Reduction Time (D_{10}) for Heating *C. botulinum* Type E Spores

Strain	Heating medium	Temperature range (°C)	D_{10}-value minimum at 82.2°C	*Z*-value (°C)	Reference
Beluga	Blue crab meat	73.9–85.0	0.75	6.5	85
Beluga	Crabmeat	73.9–85.0	0.84	6.5	85
Sarotoga	Crabmeat	80.0–82.2	1.90	6.3	87
Alaska	Whitefish chubs	73.9–85.0	2.21	7.6	88
Sarotoga	Sardines in tomato sauce	76.7–82.2	2.9	6.3	87
Sarotoga	Tuna in oil	72.2–82.2	6.6	6.1	87
Strain 202	Crabmeat	76.7–85.0	1.16	6.38	85

meat temperature of 55°F within 180 min of heat process following storage at 35°F have been recommended (87).

If storage temperature of pasteurized crabmeat rises above 38°F, surviving bacteria may grow and bacterial spores may germinate leading to spoilage. Any leakage due to can defects can introduce psychrotrophic bacteria from cooling water and result in spoilage during refrigerated storage (89). Poor meat quality with a high level of initial bacteria will increase the chance of survival of potential spoilage bacteria. The spoiled pasteurized crabmeat usually contain both aerobic and anaerobic bacteria ranging from 4.0×10^2 to 5.0×10^8/g. Spoiled crabmeat prepared from machine picking has more anaerobic organisms than hand-picked crabmeat (79).

Bivalves. Heat shock has been attempted to facilitate oyster shucking by quickly passing the stocks through a steam tunnel to slightly open the shells. Although this process increases the shucking rate, disadvantages are yield reduction and high risk of bacterial contamination (90). Canning bivalves serves as a long-term preservation method. When recommended processing times and temperatures are followed, few microbiological problems are encountered (88). However, loss of texture and economical infeasibility are problems with this processing.

Pasteurization of oysters at 72–74°C for 8 min in a flexible pouch results in a relatively stable product with organoleptic properties similar to raw oysters. The products stored at 0.5°C for three months have a low level of both aerobic and anaerobic bacteria (91). Immediately after pasteurization, all the surviving bacteria in the oysters are *Bacillus* sp. At storage of five months *Bacillus* continues to dominate the aerobic bacteria while *Clostridium, Corynebacterium, Listeria, Peptostreptococcus,* and *Staphylococcus* constitute the facultatively anaerobic bacteria (92). Combining safe preservatives with the pasteurization can provide a safe and longer shelf life of oysters.

SEAFOOD IRRADIATION

Irradiation is a process extending seafood shelf life by exposing seafood to a certain level of ionizing radiation. Studies on the utilization of ionization radiation in food processing began in the early 1940s, and an extensive program implementing this process was launched in the early 1950s, in the United States (93,94). Ionization radiations are a group of corpuscular and electromagnetic radiations of extremely short wavelengths that can cause ejection of electrons from atoms or molecules. Only radiation with strong penetration, including gamma rays, x-rays, and electron beams, is useful in food preservation Gamma rays from radioactive isotopes such as Co-60 have been the most acceptable radiation source due to its availability, price, and properties. One rad, a unit of irradiation dosage, is equal to 100 ergs of radiation energy absorbed per gram of substrate. A megarad (Mrad) is a million rads, and a kilorad (krad) is a thousand rads.

There are several advantages in using ionization radiation for seafood preservation. The extension of shelf life of seafood by using low-dosage irradiation allows the expansion of markets farther inland where fresh seafood is otherwise unavailable. The long shelf life of irradiated seafood can adjust the seasonal production providing a year-round supply and unfluctuating prices. Irradiation of high-quality catches produces top-quality seafood thus reducing the need to discard or process deteriorating fish to fish meal (94,95). Ionization radiation directly affects living cells by causing breaks in DNA and indirectly affects other cell components. The presence of water increases the degree of the DNA damage. Complex cells are more sensitive to irradiation than simple ones. The death or injury of microorganisms is due to the direct hit on DNA and the indirect effects from ionization and diffusion of free radicals and peroxides produced around the cells.

Food irradiation can be classified into three classes depending on the level of microorganisms to be destroyed (60). Radappertization or Radiation sterilization is the use of high dose with 12D usually more than 2 Mrad for complete destruction of all or practically all of the organisms. Radurization or radiation pasteurization is using low dose of radiation to destroy a sufficient number of organisms to enhance the shelf life of foods. This process usually uses 100–1,000 krad to destroy 90–99% of the organisms. Radicidation is a low-level irradiation treatment that kills nonspore-forming pathogens to reduce or eliminate the food-borne illness problem. This kind of irradiation treatment normally uses 400–600 krad dose for destruction of salmonellae from poultry and red meat or feed but not for spores of *C. botulisum* or *C. perfringens*.

In seafood irradiation, radiation resistance is expressed as the decimal reduction dose (D_{10}), the dose (krad) required for a 90% or 1-log reduction in bacterial count. The radiation resistance of microorganisms varies, depending on strains and species and the D_{10}-values of various organisms are shown in Table 3. The resistance in descending order is yeast, *Micrococcus, Moraxella-Acinetobacter, Flavobacterium*, and *Pseudomonas* (60). The gram-positive

Table 3. Decimal Reduction Doses (D_{10}-Values) for Irradiation of Some Microorganisms and Toxins

Organism or toxin	Suspending medium	D_{10} (krad)	Ref.
Vibrio parahaemolyticus	Crabmeat	5–12	96
Proteus vulgaris	Oysters	20	95
	Crabmeat	10	95
Aeromonas hydrophila	Blue fish	14–22	96
Escherichia coli	Soft-shells clams	39–42	96
	Mussel	41–48	96
	Oysters	35	95
	Crabmeat	14	95
Salmonella typhimurium	Soft-shell clams	60	96
	Mussel	58–63	96
S. paratyphi A	Oysters	75	95
	Crabmeat	50	95
	Shrimp	85	95
S. paratyphi B	Shrimp	61	97
Shigella flexneri	Mussel	24–34	96
S. sonnei	Oysters	27	95
Staphylococus aureus	Oysters	150	95
	Crabmeat	80	95
	Shrimp	190	95
Adenovirus, echovirus	Eagles medium	410–490	60
Toxins, botulinum type E			
Washed cells	Buffer	1,700–2,100	60
Purified	Buffer	40	60
Staphylococcal			
Enterotoxin B purified	Buffer	2,700	60

bacteria are more resistant than gram-negative ones. Coccoid forms are more radioresistant than rod-shaped cells. Bacterial spores exhibit the most resistance to radiation with the exception of some gram-positive cocci. Compared to bacterial spores, viruses are more or equally resistant to irradiation. Although the amount of radiation needed to inactivate microbial toxins is similar to that for bacterial spores, the radiation level for inactivation of botulinum toxin is much higher than that needed to kill the bacterial cells (98) (Table 3).

In general, D_{10}-values for bacterial vegetable cells (excluding cocci), bacterial spores, *M. radiodurans*, viruses, and bacterial toxins are 20–100, 150–450, 200–600, 400–800, and 200–2,000 krad, respectively (99–104).

Low-dose irradiation treatment of seafoods usually does not induce detectable changes in flavor, texture, and appearance with the exception of a slight loss of flavor in a few products (105,106). The level of irradiation is one factor in determining the type of microorganisms remaining that cause spoilage of irradiated seafood. The spoilage microflora of irradiated fish after a low dose of $\leq$100–150 krad treatment consists mainly of pseudomonads. At dose levels higher than 100 krad, *Maraxella-Acinetobacter* represented the dominant group of organisms in fish spoilage. Irradiation of packaged fish favors the growth of lactic acid bacteria that will be the major spoilers. In general, nonirradiated fish spoils at an aerobic plate count (APC) of 10^6/g whereas low-dose irradiated fish spoils at about 10^8/g (94).

Under the optimum dose of 100–300 krad, fish and shellfish can be maintained three to seven weeks at refrigeration without altering the fresh-product characteristics (Table 4). Compared with the regular 10- to 14-day shelf life of the unirradiated products, low-dose irradiated products have a shelf life two to three times longer (94,109). To insure efficiency and successful processing, the seafood should initially be of high quality and irradiated products should be maintained as near 0°C as possible without freezing.

The safety of irradiated seafood must be evaluated based on the absence of microorganisms and microbial toxins harmful to man, the nutritional contribution of the product, and the absence of any significant amount of toxic compounds formed in the irradiated products (101). Low-dose irradiated seafood, particularly lean fish species, have been found to pose no potential health hazards (94,110). Despite the approval of over 35 countries irradiation in foods and the USDA clearance of low-dose application for poultry irradiation, seafood irradiation has not been permitted. This process can become a successful technique for seafood preservation once it is approved in the United States.

SEAFOOD MICROORGANISMS OF PUBLIC HEALTH SIGNIFICANCE

Seafood, particularly shellfish, may contain a variety of pathogenic microorganisms that impose a threat to the consumers' health. These potential pathogens include both indigenous organisms and contaminating organisms. Pathogens may contaminate the seafood after harvest or during processing. Some indigenous pathogens found are *Vibrio, Clostridium botulinum* type E, *Aeromonas*, and poisonous phytoplankton such as dinoflagellates (111,112). Extraneous pathogens include *Salmonella, Shigella, Listeria, Campylobacter, Staphylococcus aureus, E. coli, Bacillus cereus*, Hepatitis A, and Norwalk virus (64,112).

The consumption of bivalves is of greatest concern to the public due to several factors. Bivalves, especially oysters, are frequently consumed raw. The whole animal is consumed rather than just the muscle tissue, as in the case

Table 4. Shelf Life at 0°C of Selected Seafood after Optimal Dose Irradiation Treatment

Seafood	Shelf life (weeks)	Optimal dose (Mrad)	Ref.
Haddock fillets	3–5	0.15–0.25	107
Cod fillets	4–7	0 10	108
Ocean perch fillets	4	0.15–0.25	107
Mackerel fillets	4–5	0.25	107
English sole fillets	4–5	0.20–0.30	107
Smoked chub	6	0.10	107
Petrale sole fillets	2–3	0.20	107
Shrimp	4	0.15	107
Lobster meat (cooked)	4	0.15	107
King crab meat (cooked)	4–6	0.20	107
Dungeness crab meat (cooked)	3–6	0.20	107
Oyster meat	3–4	0 20	107

Note: Fish or shellfish were packed aerobically in hermetically sealed cans or oxygen-impermeable plastic bags.

of other raw crustaceans and raw fish. Bivalves are filter feeders that can trap microorganisms from the growing water into their bodies. Because the animals grow in nearshore estuarine waters that may encounter sewage pollution, bivalves may become a vector for disease transmission. Based on past records of bivalve shellfish borne disease between 1900 and 1986, there were 12,376 cases of food-borne illnesses in the United States excluding cases of paralytic shellfish poisoning (112). This has been estimated to be only 5–10% of the actual number of cases. These documented cases include 43% gastroenteritis, 26% typhoid, 11% infectious hepatitis, 11% Norwalk virus, and 2% *Vibrio*. The specific etiologic agents have been identified as *Salmonella typhi, Vibrio cholera* 01, *V. cholera* non-01, *V. vulnificus, V. parahaemolyticus, V. fluvialis, V. mimicus, V. hollisae, V. furnissii, E. coli, Salmonella* sp., *Shigella* sp. *Bacillus cereus, Staphylococcus* sp., *Campylobacter* sp., *Aeromonas hydrophila. Pleisomonas shigelloides*, hepatitis A, Norwalk virus, and other viral agents (112–115).

Food poisoning outbreaks reportedly have often been due to the consumption of seafood. The most common agent involved is *V. parahaemolyticus* (114,116). This species was first isolated in 1950 in Japan from seafood (117,118). Since then it has been implicated in more than 1,000 outbreaks per year in Japan and accounts for over 50% of that country's bacterial food poisoning. *V. parahaemolyticus* strains require a low concentration of salt for growth, but some cultures isolated from fresh water have been reported (119–124). Raw seafood is the major vehicle for the organism in Japan; cooked seafoods that have been recontaminated are the source of implication in the United States (117). Kanagawa reaction is commonly used to differentiate between virulent and avirulent isolates by testing whether a strain can produce a heat-stable hemolysin to lysis a blood agar containing 7% NaCl and mannitol. Cultures isolated from stools of patients are always Kanagawa positive (99%). On the contrary only about 1% of isolates from waters or seafoods is Kanagawa positive (125,126). The mechanism for this discrepancy is still unknown. One of the hypotheses is that there may be a transformation of Kanagawa-positive strains on passage through the intestines. There is a possible competitive advantage for Kanagawa positive strains to proliferate more readily in the intestines. The current method of isolating and identifying Kanagawa-positive strains in seafoods and waters may also leave some undetected (116,126).

The infective dose of *V. parahaemolyticus* for humans ranges from 10^5 to 10^7 viable cells, and a decrease in stomach acidity may lower the infective dose. The incubation period for symptoms is 5–90 h and the duration of the illness is normally 2–10 days. The frequencies of symptoms are diarrhea, 98%; abdominal cramps, 82%; nausea, 71%; vomiting, 52%; headache and fever, 27%; and chills, 24% (116,126). The organism has three biologically active hemolysins, substances that can lyse the animal's blood cells: a heat-stable peptide with 45,000 mol. wt., a heat-labile hemolysin, and a phospholipase (127,128). *V. parahaemolyticus* are heat sensitive and most food-poisoning cases result from cross-contamination or poor sanitation (129–131).

V. cholerae causes a gastrointestinal illness in humans called cholera (132). This species is usually divided into two groups, serotype 01 and non-01: both are found in aquatic environment. The 01 serotype contains two biotypes: classical and El Tor. The classical biotype prevailed worldwide until the 1960s and the El Tor biotype presently predominates, including in the United States (111,133,134). Cholera in the United States is relatively rare but occasional outbreaks occur in southern states such as Texas, Louisiana, and Florida due to consumption of raw or partially cooked molluscs, cooked crabs, and other shellfish (133,134). The infective dose is estimated to be about 10^6 cells. Taking antiacids or medication to lower gastric acidity will lower the infective dose (115). The incubation time varies from 6 h to 5 days. Severe symptoms include profuse watery diarrhea, dehydration, and death in the absence of prompt treatment. In the beginning, the stool is brown with fecal material and quickly acquires the classic rice-water appearance. Enormous amounts of fluid leave the body resulting in dehydration and difficulties in circulation. The stool is high in potassium and bicarbonate (111,133). Besides severe diarrhea, victims suffer from thirst, leg cramps, weakness, hoarse speech, and rapid pulse (132,133). In emergency treatment, prompt replacement of fluid and electrolyte losses by intravenous injection is often used. After an initial recovery, oral intake of glucose and electrolytes can improve the condition. In general, *V. cholerae* is sensitive to heat and cold, but it can survive at low temperatures for a certain time (135).

Non-01 cholera is a group of nonagglutinable (NAG) cholerae, which exist naturally in estuarine and coastal waters and also in rivers and brackish waters (128). Only about 5% of this group from seafood and patients isolates in the United States produce cholera toxin. However, the nontoxigenic strains cause gastrointestinal illness with principal symptoms of abdominal cramps, fever, bloody stools, nausea, and vomiting (133). Some human isolates of this group in the United States are from extraintestinal sources including wound infection, ear infection, and primary and secondary septicemia (133). Eating raw oysters has been found to be the major cause of most non-01 *V. cholerae* infection cases in the United States. Other seafood, egg and asparagus salad, or potatoes can occasionally be a vehicle for these organisms (134).

V. vulnificus is widely distributed in the environment and has been found in most U.S. estuarine and coastal waters (136–138). This water and seafood organism is found most frequently at water temperatures >20°C and low salinity of 0.5–1.6%. Environmental isolates are phenotypically identical to clinical isolates. Some strains show bioluminescence and may also be pathogenic (138). This organism can cause illness and infection through the consumption of contaminated raw or undercooked seafood, particularly mollusks such as oysters and clams. The incubation period for this illness is 16–48 h after ingestion. Symptoms include weakness, chills, fever, hypotension, and fatigue with occasional vomiting and diarrhea. Infection occurs, progresses rapidly, and may cause death in 40–60% of patients (128). Patients may have greater risk of infection if they have skin cuts or suffer chronic liver disease, gastric disease, or hemochromatosis (111,128).

Other potential *Vibrio* pathogens such as *V. hollisae, V. mimicus* and *V. furnissi* have been implicated in seafood-borne illnesses (111). Vehicles for these organisms are shellfish including oysters, clams, shrimp, and crawfish. Common symptoms are diarrhea, nausea, vomiting, and abdominal cramps. Both toxigenic and nontoxigenic strains of *V. mimicus* have been isolated but food-poisoning cases have mostly occurred with nontoxogenic ones. Some strains of these organisms have been newly isolated in the last few years, and more information will be available after further studies (111).

Clostridium botulinum type E is another intrinsic pathogen in seafood causing botulism type E intoxication. Based on the serological classification of the neurotoxin, *C. botulinum* is composed of eight types: A, B, C_1, C_2, D, E, F, and G (139). These types are divided into four groups according to proteolytic activity (140,141). Groups I and II are of particular importance for causing botulism in humans. Group I includes type A and proteolytic strains of types B and F. Group II contains all type E and nonproteolytic strains of types B and F. Type E is distributed in sediments of marshes, lakes, and coastal ocean waters and is common in the intestines of fish and shellfish. The organism does not proliferate in living fish but it may multiply in bottom deposits and in aquatic vegetation when the growth of algae reduces the oxygen level of the water to conditions suitable for *C. botulinum*. Type E can grow and produce toxin at 4°C and is heat sensitive (142,143). It is inhibited by water activity of <0.975 (5% NaCl) and pH <5.3 (144). Most outbreaks of botulism associated with fishery products have been with semipreserved products. Smoked, salted, fermented, and canned products are eaten without further cooking and can involve the risk of botulism due to inadequate processing (89,143). Satisfactory heat processing is critical in assuming quality safety of pasteurized crabmeat described above.

Plesiomonas shigelloides is found in fresh surface water and possibly in seawater and is more often isolated in the summer months. This organism has been implicated in human gastroenteritis for 40 yr (145). Seafood that may carry this organism are cuttlefish, raw oysters, salt mackerel, and undercooked oysters. Incubation time for symptoms is one to two days after ingestion of the food. Symptoms include diarrhea, abdominal pain, nausea, chills, fever, vomiting, and headache (145).

Enteric viruses are those animal viruses that are excreted in feces and discharged into domestic sewage. These viruses are more resistant than enteric bacteria to sewage treatment process and various environmental stresses. Several human enteric viruses may be implicated in the viral disease by seafood consumption, such as hepatitis, fever, diarrhea, paralysis, meningitis, and myocarditis. Shellfish may play a significant role as vectors in the transmissions of viral diseases. A study of cockles-borne disease indicated that about one-quarter of the hepatitis A cases in the southeast UK could be caused by shellfish consumption (146,147). In Frankfurt, Germany, about one-fifth of infectious hepatitis was associated with the consumption of contaminated oysters (148). Molluskan shellfish is the major seafood involved in the outbreak of hepatitis A incidence (58) and also attributes to 12.5% of non-A, non-B hepatitis in the United States (114). Outbreaks of Norwalk virus gastroenteritis by shellfish ingestion must be documented in Australia and the United States (97).

According to the Centers of Disease Control annual documentation, nearly 90% of all reported outbreaks of seafood-borne illness are associated with consumption of molluskan shellfish and a very few species of fish (148,149). In geographic distribution, Hawaii has the most cases accounting for 35% of all these seafood-borne illnesses. About half of all these seafood-borne illness cases are found in four states and territories: Hawaii, Puerto Rico, Virgin Islands, and Guam. Another third of these seafood-related illnesses occur in the continental states of New York, California, Washington, Connecticut, and Florida (149).

Chemical intoxication resulting from the harmful metabolites of microorganisms are often implicated in seafood. An allergic food poisoning after eating deteriorated scombroid fish is caused by formation of histamine by microbial enzyme histidine decarboxylase when fish have been exposed to environments favorable to bacterial growth. The organisms involved in histamine poisoning include *Pseudomonas putrefaciens, Aeromonas hydrophila, Proteus vulgaris, Clostridium perfringens, Enterobacter aerogenes*, and *Vibrio alginolyticus* (150).

MICROBIOLOGICAL CRITERIA AND INSPECTION

Microbiological criteria are an important but controversial issue in establishing a regulatory food-control program, particularly for seafoods in the United States. The purposes of microbiological criteria are to insure food safety, adhere to good manufacturing practice, and provide established measure for food inspection and marketing. According to the degree of compliance, microbiological criteria include three categories: standard, guideline, and specification (151). A standard is part of a law or ordinance and is a mandatory criterion. A guideline is a criterion for assessing microbiological quality during processing, distribution, and marketing of foods. A specification is used in purchase agreements between buyers and vendors (152,153).

As more countries are recognizing the need to assess the safety and quality of foods due to food-poisoning outbreaks and international trade disputes, several agencies have formed organizations to formulate microbiological criteria. The International Committee on Microbiological Specifications for Foods (ICMSF) was established by the International Association of Microbiological Society (IAMS) in 1962 for this and related purposes (154). The committee assists in establishing microbiological standards implementing method of examination, promoting safe movement of foods in international trade, and mediating disputes caused by disparate criteria. The committee also publishes books of its reports and recommendations (153–155). Many of the ICMSF recommendations were incorporated into the work of Code Alimentarius Commission to implement the Joint FAO/WHO Food Standard Program of the United Nations World Health Organization. The goal of the Joint Food Standard Program is to protect the health of consumers, ensure fair practices in the food trade, guide the preparation of draft standards and codes of practice, and promote the incorporation of all food standards by different governments. The program has also published recommended international codes of practice for many common seafood products such as shrimps or prawns and frozen fish (156,157).

To ensure consumer safety, inspection systems are enforced by the regulatory agencies in most of the countries. In the United States, the inspection program is carried out by different levels: local, state, and federal government. The federal regulatory seafood control systems include the Food and Drug Administration (FDA), U.S. Department of Agriculture (USDA), U.S. Department of Commerce (USDC), U.S. Department of Defense (USDD), and the Environmental Protection Agency (EPA). The FDA is in charge of imported seafood inspection, a mandatory surveillance system. The National Marine Fisheries Service of USDC operates the voluntary Fishery Product Inspection Program mainly for domestic seafood industry on a fee-for-service basis. The USDA and other federal agencies are responsible for some inspection systems related to their domain of authority. Much of the domestic seafood products are inspected by the states and municipalities in their respective territories. In recent years, the public concern in seafood safety and confusion among various inspection systems have led to the proposal of a national seafood surveillance system. At the time of this writing the USDA will likely be chosen to head this mandatory seafood inspection program.

Microbiological criteria have been controversial and confusing because of the variation in agency objectives, legislative authorities, unsatisfactory definitions, questions in sampling procedures, methodology, quality requirements, and uninsured consumer protection. In recent years a hazard analysis critical control point (HACCP) approach has been regarded as an effective, practical, and economic program in microbiological monitoring and safety of the products (152,158). The industry should develop the actual details of a HACCP program and insure the implementation of the complete process. In conducting hazard analysis of the products each step of the processing operation should be defined to specify the hazards associated with each step. The preventative measures at each processing step will be identified for elimination of the hazards. The critical control points are thus defined, bringing the hazards under control. The theory behind the HACCP concept, if properly implemented, is to reduce the number of governmental inspections, and overcome many of the weaknesses inherent in traditional inspection schemes (158). When plant personnel have been well trained in HACCP concepts, the quality and safety of products can be enhanced with efficient cost inspection.

Seafood microbiology is a difficult subject because of the many obstacles in this area. A broad spectrum of microorganisms is involved, including indigenous microbial flora organisms that contaminate the catch after it is landed and those that multiply during the process from the water to the dining table. The ambiguous taxonomy of many seafood microorganisms is confusing and impedes the study of these organisms. The problems with growth conditions, such as inadequate growth temperature and media composition, often result in disputable results of seafood sample analysis. The large number of nonculturable viable cells in marine bacteria greatly limits the study of the role of these organisms in the natural aquatic ecosystem and the relation to fish and shellfish.

The fast growth of psychrophilic and psychrotrophic bacteria cause fish and shellfish to spoil rapidly even in refrigeration. In addition to spoilers the potential pathogenic microorganisms present in seafood can proliferate expeditiously and threaten consumer safety. Prompt seafood handling and adequate processing are critical in the control of both spoilage and pathogenic organisms to provide safe and stable products. Good quality control and inspection, particularly the HACCP program, can ensure a high quality of seafood products.

Application of modern basic microbiology, biochemistry, and biotechnology can advance fish and shellfish microbiology. DNA probe, immunoassay, monoclonal antibody, and other rapid-detection methods provide accurate and expeditious procedures for determination of seafood pathogens. The study of bacterial cell envelope, a new field that has arisen in the last two decades, can provide information about the functions and physiochemical properties of cell envelope components, which are important in understanding bacterial survival and interaction with other organisms in the aquatic ecosystem. This field of knowledge will also be helpful in establishing new technology to control bacterial growth during processing. Study of nonculturable viable or dormant cells may lead to a new field of study: bacterial survival in the natural environment and its impact on seafood. This can provide another avenue in exploring the key factors behind many nondetectable food pathogens. The progress in seafood microbiology will greatly benefit the seafood industry, providing safe, better quality seafoods.

BIBLIOGRAPHY

1. R. E. Martin, W. H. Doyle, and J. R. Brooker, "Toward an Improved Seafood Nomenclature System," *Mar. Fish. Rev.* **45**, 1–20 (1983).
2. D. C. Georgala, "The Bacterial Flora of the Skin of North Sea Cod," *Journal of General Microbiology* **18**, 84–91 (1958).
3. J. Liston, "Qualitative Variations in the Bacterial Flora of Flatfish," *Journal of General Microbiology* **15**, 305–314 (1956).
4. J. M. Shewan, "Some Bacteriological Aspects of Handling, Processing and Distribution of Fish," *J Roy. Sanit. Inst.* **59**, 394–421 (1949).
5. J. M. Shewan, "The Microbiology of Sea-Water Fish," in G. Borgstrom, ed., *Fish as Food*, Vol. 1. Academic Press, Inc. Orlando, Fla., 1961.
6. P. Baumann, M. Doudoroff, and R. Y. Stanier, "A Study of the *Moraxella* Group. I. Genus *Moraxella* and the *Neisseria catarrhalis* Group," *Journal of Bacteriology* **95**, 58–73 (1968).
7. P. Baumann, M. Doudoroff, and R. Y. Stanier, "A Study of the *Moraxella* Group. II. Oxidative-Negative Species (Genus *Acinetobacter*)," *Journal of Bacteriology* **95**, 1520–1541 (1968).

8. G. L. Gilardi, "Morphological and Biochemical Differentiation of *Achromobacter* and *Moraxella* (DeBord's Tribe Mineae)," *Applied Microbiology* **16**, 33–38 (1968).
9. J. Liston, "A Quantitative and Qualitative Study of the Bacterial Flora of Skate and Lemon Sole Trawled in the North Sea," Ph.D. dissertation, Aberdeen University, Aberdeen, U.K. 1955.
10. B. R. Botta, B. E. Squires, and J. Johnson, "Effect of Bleeding, Gutting Procedures on the Sensory Quality of Fresh Raw Atlantic Cod (*Gadus morhua*)," *Canadian Institute of Food Technology Journal* **19**, 186 (1986).
11. R. M Brian and D. R. Ward, "Microbiology of Finfish and Finfish Processing," in D. R. Ward and C. R. Hackney, eds., *Microbiology of Marine Food Products*, Van Nostrand Reinhold, Co., Inc., New York.
12. D. R. Ward and N. J. Baj, "Factors Affecting Microbiological Quality of Seafoods," *Food Technology* **42**, 85–89 (1988).
13. W. K. Rodman, "On Board Fish Handling Systems for Offshore Wetfish Trawlers, Work Smarter Not Harder," in W. T. Otwell, compiler, *Proceedings of the First Joint Conference of the Atlantic Fisheries Technology Society*, Florida Sea Grant, University of Florida, 1987.
14. J. J. Connell, *Control of Fish Quality*, Fishing News Books Ltd., Surrey, UK, 1975.
15. R. Nichelson II, *Seafood Quality Control—Boats and Fish Houses: Processing Plant*, Marine Advisory Bulletins, Texas A & M University, Texas, 1972.
16. J. A. Dassow, "Handling Fresh Fish," in M. E. Stansoy, ed., *Industrial Fishery Technology*, Robert Krieger, New York, 1976.
17. T. Chai, "Studies on the Bacterial Flora of Fish Pen Slime," M. S. Thesis, University of Massachusetts, Amherst, 1970.
18. T. Chai, "Usefulness of Electrophoretic Pattern of Cell Envelope Protein as a Taxonomic Tool for Fish Hold Slime *Moraxella* Species." *Applied Environmental Microbiology* **42**, 351–356 (1981).
19. V. Venugopal, "Extracellular Proteases of Contaminant Bacteria in Fish Spoilage: A Review." *J. Food Prot.* **53**, 341–350 (1990).
20. T. Chai, *Fishery Bacteriology*, Joint Commission Rural Reconstruction, Taipei, Taiwan, 1979.
21. H. C. Chen and T. Chai, "Microflora of Drainage from Ice in Fishing Vessel Fish Holds," *Applied Environmental Microbiology* **43**, 1360–1365 (1982).
22. D. G. James. "The Prospects for Fish for the Undernourished Food and Nutrition," *FAO* **12**, 20–27 (1986).
23. T. Chai et al., "Detection and Incidence of Specific Species of Spoilage Bacteria in Fish. II. Relative Incidence of *Pseudomonas putrefaciens* and Fluorescent Pseudomonads on Haddock Fillets," *Applied Microbiology* **16**, 1738–1741 (1968).
24. E. M Ravesi, J. J. Licciardello, and L. D. Racicot, "Ozone Treatments of Fresh Atlantic Cod, *Gadus morhua*," *Mar. Fish. Ref.* **49**, 37–42 (1987)
25. J M Shewan, G. Hobbs, and W. Hodgkiss, "A Determination Scheme for the Identification of Certain Genera of Gram-Negative Bacteria, with Special Reference to the *Pseudomonadeceae*," *Journal of Applied Bacteriology* **23**, 379–390 (1960).
26. J. Liston, "Microbiology in Fishery Science," in J. J. Connel, ed., *Advances in Fish Science and Technology*, Fishing News Books Ltd., Surrey, UK, 1980.
27. E. A. Fieger and A. F. Novak, "Microbiology of Shellfish Deterioration," in Ref. 5.
28. National Marine Fisheries Service, *Fisheries of the United States 1988*, NMFS, U.S. Department of Commerce, Washington, D.C., 1989.
29. O. B. Williams and H. B. Rees, Jr., "The Bacteriology of Gulf Coast Shrimp. III. The Intestinal Flora," *Texas J. Sci.* **4**, 55–58 (1952).
30. O. B. Williams. "Microbiological Examination of Shrimp," *J. Milk and Food Technol.* **12**, 109–110 (1949).
31. O. B. Williams et al., "The Bacteriology of Gulf Coast Shrimp. II. Qualitative Observations on the External Flora," *Texas J. Sci.* **4**, 53–54 (1952).
32. M Green, "Bacteriology of Shrimp. II. Quantitative Studies of Freshly Caught and Iced Shrimp," *Food Res.* **14**, 372–383 (1949).
33. L. L. Campbell, Jr., and O. B. Williams, "The Bacteriology of Gulf Coast Shrimp. IV. Bacteriological, Chemical, and Organoleptic Changes with Ice Storage," *Food Technology* **6**, 125–126 (1952).
34. B. F. Cobb et al., "Effect of Ice Storage upon the Free Amino Acid Contents of Tails of White Shrimp *(Panaeus satiferus)*," *Journal of Agriculture and Food Chemistry* **22**, 1052–1056 (1974).
35. C. Vanderzant, E. Roz, and R. Nickelson, "Microbial Flora of Gulf of Mexico and Pond Shrimp," *J. Milk Food Technol.* **33**, 346–350 (Aug. 1970).
36. G. Finne, "Enzymatic Ammonia Production in Penaied Shrimp Held on Ice," in R. E. Martin and co-eds., *Chemistry & Biochemistry of Marine Food Products*, AVI Publishing Co., Inc., Westport, Conn., 1982.
37. H. S. Tubiash, R. K. Sizemore, and R. R. Colwell, "Bacterial Flora of the Hemolymph of the Blue Crab, *Callinectes sapidus*: Most Probable Numbers," *Applied Microbiology* **29**, 388–392 (1975).
38. R. K. Sizemore et al., "Bacterial Flora of the Hemolymph of the Blue Crab. *Callinectes sapidus*: Numerical taxonomy," *Applied Environmental Microbiology* **29**, 393–399 (1975).
39. M. A. Faghri et al., "Bacteria Associated with Crabs from Cold Waters with Emphasis on the Occurrence of Potential Human Pathogens," *Applied Environmental Microbiology* **47**, 1054–1061 (1984).
40. N. A. Cox and R. T. Lovell, "Identification and Characterization of the Microflora and Spoilage Bacteria in Freshwater Crayfish, *Procambarus clarkii*," *Journal of Food Science* **38**, 679–681 (1973).
41. R. J. Miget, "Microbiology of Crustacean Processing: Shrimp, Crawfish, Prawns," in Ref. 11
42. D. W. Cook, "Microbiology of Bivalve Molluskan Shellfish," in Ref. 11.
43. D. W. Cook, "Fate of Euteric Bacteria in Estuarine Sediments and Oysters Feces," *J. Miss. Acad. Sci.* **29**, 71–76 (1984).
44. A. R. Murchelano and J. L. Bishop, "Bacteriological Study of Laboratory-Reared Juvenile American Oysters *(Crassostrea virginica)*," *J. Muertebr. Pathol.* **14**, 321–327 (1975).
45. T. Chai et al., "Comparison of Microbiological Quality between Soft-Shell Clams and Growing Waters," *Journal of Applied Environmental Microbiology*, submitted.
46. Food and Drug Administration, *National Shellfish Sanitation Program Manual of Operation. Part II. Sanitation of the Harvesting and Processing of Shellfish*, FDA U.S. Department of Health and Human Services, Washington, D.C., 1989.
47. J. D. Clem, *Status of Recommended National Shellfish Sanitation Program Bacteriological Criteria for Shucked Oysters*

at the Wholesale Market Level, FDA. Washington, D.C., (1983)

48. V. T. Dimitroff, "Spirochaetes in Baltimore Market Oysters," *Journal of Bacteriology* **12**, 135–177 (1926).
49. T. Chai et al., "Microbiological Studies of Chesapeake Bay Soft-Shell Clams *(Mya arenaria)*," *J. Food Prot.* **53**, 1052–1057 (1990).
50. R. R. Colwell and J. Liston, "Microbiology Shellfish: Bacteriological Study of the Natural Flora of Pacific Oysters (*Crassostrea gigas*)," *Applied Microbiology* **8**, 104–109 (Feb. 1960).
51. T. E. Lovelace, "Quantitative and Qualitative Commensal Bacterial Flora of *Crassostrea virginica* in Chesapeake Bay," *Proc. Nat. Shellfish Assoc.* **58**, 82–87 (1968).
52. C. Vanderzant et al., "Microbial Flora and Level of *Vibrio parahaemolyticus* of Oysters *(Crassostrea virginica)*, Water and Sediment from Galveston Bay," *J. Milk Food Tech.* **36**, 447–452 (Sept. 1973).
53. G. P. Richards, "Microbial Purification of Shellfish: A Review of Depuration and Relaying," *J. Food Prot.* **51**, 218–251 (Mar. 1988).
54. D. W. Cook and R. D. Ellender, "Relaying to Decrease the Concentration of Oyster-Associated Pathogens," *J. Food Prot.* **49**, 196–202 (Mar. 1986).
55. G. H. Fleet, "Oyster Depuration—A Review," *Food Tech. Aust.* **30**, 444–454 (1978).
56. G. J. Vasconcelos and J. S. Lee, "Microbial Flora of Pacific Oysters *(Crassostrea gigas)* Subjected to Ultraviolet-Irradiated Seawater," *Applied Microbiology* **23**, 11–16 (1972).
57. J. G. Metcalf et al., "Bioaccumulation and Depuration of Enteroviruses by the Soft-Shelled Clam, *Mya arenaria*," *Applied Environmental Microbiology* **38**, 275–282 (1979).
58. G. P. Richards, "Outbreaks of Shellfish-Associated Interic Virus Illness in the United States: Requisite for Development of Viral Guidelines," *J. Food Prot.* **48**, 815 (1985).
59. K. L. Parkin and W. D. Brown, "Preservation of Seafood with Modified Atmospheres," in Ref. 36.
60. G. J. Banwart, *Basic Food Microbiology*, AVI Publishing Co., Inc., Westport, Conn., 1979
61. K. Schroder et al., "Psychrotrophic *Lactobacillus plantarum* from Fish and Its Ability to Produce Antibiotic Substance," in J. J. Connell, ed., *Advances in Fish Science and Technology*, Fishing News Books Ltd., Surrey, UK, 1980.
62. P. H. Calcott and R. A. Macleod, "Survival of *Escherichia coli* from Freeze-Thaw Damage: A Theoretical and Practical Study," *Canadian Journal of Microbiology* **20**, 671–681 (1974).
63. P. H. Calcott et al., "The Effect of Cooling and Warm Rates on the Survival of a Variety of Bacteria," *Canadian Journal of Microbiology* **22**, 106–109 (1976).
64. S. W. Weagant et al., "The Incidence of *Listeria* species in Frozen Seafood Products," *J Food Prot.* **51**, 655–657 (1988).
65. R. Digirolamo et al., "Survival of Virus in Chilled, Frozen, and Processed Oysters," *Applied Microbiology* **20**, 58–63 (1975).
66. H. C. Johnson and J. Liston, "Sensitivity of *Vibrio parahaemolyticus* to Cold in Oysters, Fillets and Crabmeat," *Journal of Food Science* **38**, 437–441 (1973).
67. N Neufeld, "Influence of Bacteriological Standards on the Quality of Inspected Fisheries Products," in R. Kreuzer, ed., *Fish Inspection and Quality Control*, Fishing News Ltd., London, 1971
68. B. S. Pan, "Low Moisture Fishery Products," in J-L. Chuang, B. S. Pan, and G-C. Chen, eds., *Fishery Products of Taiwan*, JCRR Fisheries Series **25B**, Taipei, Taiwan, 1977.
69. I. H. Yoon, J. R. Matches, and B. Rasco, "Microbiological and Chemical Changes of Surimi-Based Imitation Crab during Storage," *Journal of Food Science* **53**, 1343–1346 (1988).
70. E. L. Elliot, "Microbiological Quality of Alaska Pollock Serimi," in D. E. Kramer and J. Liston, eds., *Developments in Food Sciences: Seafood Quality Determination*, Elsevier, Amsterdam, The Netherlands, 1987.
71. J. R. Matches et al., "Microbiology of Surimi-Based Products," in Ref. 70.
72. R. R. Cockey and T. Chai, "Microbiology of Crustacea Processing: Crabs," in Ref. 11
73. J. S. Lee and D. K. Pfeifer, "Microbiological Characteristics of Dungeness Crabs *(Cancer magister)*," *Applied Microbiology* **30**, 72–78 (1975).
74. J. W. Duersch, M. W Paparella, and R. R. Cockey, *Processing Recommendation for Pasteurization Meat from the Blue Crabs*, Marine Products Laboratory, University of Maryland, Crisfield, Md., 1981.
75. F. A. Phillips and J. T. Peeler, "Bacteriological Survey of the Blue Crab Industry," *Applied Microbiology* **24**, 958–966 (1972).
76. M. W. Paparella, *Information Tips 78-4*, Marine Products Laboratory, University of Maryland, Crisfield, Md., 1978.
77. J. Pace, R. R. Cockey, and T. Chai, "Sources of Spoilage of Pasteurized Crabmeat," *Proceedings of the Interstate Seafood Seminars*, 314–323 (Oct. 1989).
78. R. R. Cockey and T. Chai, "Quik-Pik Machine Processing of Crabmeat and Quality Control," *Proceedings of the Interstate Seafood Seminars 1990*.
79. R. R. Cockey, "Bacteriological Assessment of Machine Picked Meat of the Blue Crabs," *J. Food Prot.* **43**, 172–174 (1980).
80. M. A. Benarde and R. A. Littleford, "Antibiotic Treatment of Crab and Oyster Meats," *Applied Microbiology* **5**, 368–372 (1957).
81. U.S. Pat. 2,546,428 (1951), G. C. Byrd.
82. R. R. Cockey and M. C. Tatro, "Survival Studies with Spores of *Clostridium botulinum* Type E in Pasteurized Meat of the Blue Crabs *Callinectes sapidus*," *Applied Microbiology* **27**, 629–633 (1974).
83. Maryland State Department of Health, *Regulations Governing Crabmeat*, Maryland State Department of Health, Baltimore, Sept. 3, 1957.
84. D. A. Kautter et al., "Incidence of *Cl. botulinum* in Crabmeat from the Blue Crabs," *Applied Microbiology* **28**, 722 (Oct. 1974).
85. R. K. Lynt et al., "Thermal Death Time of *Clostridium botulinum* Type E in Meat of the Blue Crabs," *Journal of Food Science* **42**, 1022–1025 (1977).
86. R. K. Lynt, D. Kautter, and H. Solomon, "Differences and Similarities among Proteolytic and Nonproteolytic Strains of *Clostridium botulinum* A, B, E, and F. A Review," *J. Food Prot.* **45**, 466–474 (Apr. 1982).
87. D. R. Ward et al., *Thermal Processing Pasteurization Manual*, Department of Food Science and Technology. VPI State University, Blacksburg, Va., 1982.
88. E. Tanikawa and S. Poka, "Heat Processing of Shellfish," in G. Borgstrom, ed., *Fish as Food*, Vol. IV. Academic Press, Inc., Orlando, Fla., 1965.

89. M. W. Eklund, "Significance of *Clostridium botulinum* in Fishery Products Preserved Short of Sterilization," *Food Technology* **36**, 107–115 (Dec. 1982).

90. F. Huand and C. E. Hebard, *Proceedings of Engineering and Economics of the Oyster Steaming Shucking Process*, VPI & SU, Hampton, Va., 1980.

91. T Chai, J. Pace, and T. Cossaboom, "Extension of Shelf-Life of Oysters by Pasteurization in Flexible Pouches," *Journal of Food Science* **49**, 331–333 (Feb. 1984).

92. J. Pace, C. Y. Wu, and T. Chai, "Bacterial Flora in Pasteurized Oysters after Refrigerated Storage," *Journal of Food Science* **53**, 325–327 (Feb. 1988).

93. G. G. Giddings, "Radiation Processing of Fishery Products," *Food Technology* **38**, 61–6, 94–97 (1984).

94. J. J. Licciardello and L. J. Ronsivalli, "Irradiation of Seafoods," in Ref. 36.

95. J. D. Quinn et al., "The Inactivation of Infection and Intoxication Microorganisms by Irradiation in Seafood," in FAO/IAEA, eds., *Microbiological Problems in Food Preservation by Irradiation*, IAEA, Vienna, 1967.

96. J. J. Licciardello, D. L. Dentremont, and R. C. Lundstrom, "Radio-Resistance of Some Bacterial Pathogens in Soft-Shell Clams *(Mya arenaria)* and mussels *(Mytilus edulis)*," *J. Food Prot.* **52**, 407–411 (1989).

97. C. W. Hung and B. S. Pan, "Effect of Gamma Irradiation Dosage on Frozen Shrimp," *Proceedings of the Atlantic Fisheries Technology Conference and International Symposium of Fisheries Technology*, Boston, Mass., 1985.

98. G. Hobbs, "*Clostridium botulinum* in Irradiated Fish," *Fd. Irrad. Inf.* **7**, 39–54 (1977).

99. A. Hobbs, "Toxin production by *Clostridium botulinum* Type E in Fish," in Ref. 97.

100. T. Miura et al., "Radiosensitivity of Type E Botulinum Toxin and Its Protection by Proteins, Nucleic Acids and Some Related Substances," in Ref. 97.

101. *Wholesomeness of Irradiated Food*, Joint FAO/IAEA/WHO Committee Report, WHO Technological Report Series **604-FAO**, Food and Nutrition Series 6, World Health Organization, Geneva, 1977.

102. R. B. Read, Jr., and J. G. Bradshaw, "γ-Irradiation of Staphylococcal Enterotoxin B," *Applied Microbiology* **15**, 603–605 (1967).

103. R. Sullivan et al., "Inactivation of Thirty Viruses by Gamma Radiation," *Applied Microbiology* **22**, 61–65 (1971).

104. T. Miura et al., "Radiosensitivity of Type E Botulinum Toxin and Its Protection by Proteins, Nucleic Acids, and Some Related Substances," in M. Herzberg, ed., *Proceedings of the First United States—Japan Conference on Toxic Microorganisms*, USDI, Washington, D.C., 1970.

105. E. F. Reben et al., "Biological Evaluation of Protein Quality of Radiation-Pasteurized Haddock, Flounder and Crab," *Journal of Food Science* **33**, 335–337 (1968).

106. R. O. Brook et al., "Preservation of Fresh Unfrozen Fishery Products by Low-Level Radiation. V. The Effects of Radiation Pasteurization on Amino Acids and Vitamins in Haddock Fillets," *Food Technology* **20**, 99–102 (1966).

107. J. W. Slavin et al., "The Quality and Wholesomeness of Radiation-Pasteurized Marine Products with Particular Reference to Fish Fillets," *Iso. Radiat. Technol.* **3**, 365–381 (1966).

108. L. J. Ronsivalli et al., *Study of Irradiated Pasteurized Fishery Products, Maximum Shelf Life Study. B. Radiation Chemistry*, U.S. Atomic Commission Contract No. AT(49-112)-1889, Bureau of Commercial Fisheries Technological Laboratories, Gloucester, Mass., 1970.

109. B. L. Middlebrooks et al., "Effects on Storage Time and Temperature on the Microflora and Amine Development in Spanish Mackerel *(Scomberomorus maculatus)*." *Journal of Food Science* **53**, 1024–1029 (1988).

110. J. H. Skala, E. L. McGown, and P. P. Waring, "Wholesomeness of Irradiated Foods," *J. Food Prot.* **50**, 150–160 (Feb. 1987).

111. C. R. Hackney and A. Dicharry, "Seafood-Borne Bacterial Pathogens of Marine Origin," *Food Technology* **42**, 104–109 (Mar. 1988).

112. S. R. Rippey and T. L. Verber, *Shellfish Borne Disease Outbreaks*, FDA. Shellfish Sanitation Branch. Northeast Technical Services Unit, Davisville, R.I., 1986

113. J. M. Janda et al., "Current Perspectives on the Epidemiology and Pathogenesis of Clinically Significant *Vibrio* spp.," *Clinical Microbiology Reviews* **1**, 245–267 (1988).

114. M. J. Alter et al., "Sporatic Non-A, Non-B Hepatitis: Frequency and Epidemiology in an Urban U.S. Population," *Journal of Infectious Diseases* **145**, 886–893 (1982).

115. P. Blake, "Vibrio on the Half Shells; What the Walrus and the Carpenter Didn't Know," *Annals of Internal Medicine* **99**, 558–559 (1987).

116. L. R. Beuchat, "*Vibrio parahaemolyticus*: Public Health Significance." *Food Technology* **36**, 80–92 (1982).

117. T. Fujino. "International Symposium on *Vibrio parahaemolyticus*" Saikon Publishing Co., Tokyo, Japan, 1974.

118. J. Sakurai, A. Matsuzaki, and T. Miwatani, "Purification and Characterization of Thermostable Direct Hemolysin of *V. parahaemolyticus*," *Infection and Immunity* **8**, 775–780 (1973).

119. L. R. Beuchat, "Interacting Effects of pH. Temperature, and Salt Concentration on Growth and Survival of *Vibrio parahaemolyticus*," *Applied Microbiology* **25**, 844–846 (May 1973).

120. D. Covert and M. Woodburn. "Relationship of Temperature and Sodium Chloride Concentration to the Survival of *Vibrio parahaemolyticus* in Broth and Fish Homogenate," *Applied Microbiology* **23**, 321–325 (Feb 1972).

121 D. Golmintz, R. C. Simpson, and D. L. Dubrow, "Effect of Temperature on *Vibrio parahaemolyticus* in Artificially Contaminated Seafood," *Developments in Industrial Microbiology* **15**, 288 (1974).

122 C. Vanderzant and R. Nickelson, "Survival of *Vibrio parahaemolyticus* in Shrimp Tissue under Various Environmental Conditions," *Applied Microbiology* **23**, 34–37 (Jan. 1972).

123 K. Venkateswaran et al., "Characterization of Toxigenic *Vibrio* Isolated from the Freshwater Environment of Hiroshima, Japan," *Applied Environmental Microbiology* **55**, 2613–2618 (Oct. 1989).

124. R. R. Colwell and J. Kaper, "*Vibrio cholerae, V. parahaemolyticus*, and Other Vibrios: Occurrence and Distribution in Chesapeake Bay," *Science* **198**, 394–396 (1978).

125. G. Spite, D. Brown, and R. Twedt, "Isolation of One Enteropathogenic, Kanagawa-Positive Strain of *V. parahaemolyticus* from Seafood Implicated in Acute Gastroenteritic," *Applied Environmental Microbiology* **35**, 1226–1227 (1978).

126. R. M. Twedt and D. F. Brown, "*V. parahaemolyticus*: Infection or Toxicosis?" *J. Milk Food Technol.* **36**, 129–134 (1973).

127. T. Honda et al., "Identification of Lethal Toxin with the Thermostable Direct Hemolysin Produced by *V. parahaemolyticus*, and Some Physiochemical Properties of the Purified Toxin," *Infection and Immunity* **13**, 133–139 (1976).

128. J. Oliver, "Vibrio: On Increasingly Troublesome Genus," *Diagn. Med.* 8, 43–49 (1985).

129. R. Delmore and P. Crisley, "Thermal Resistance of *Vibrio parahaemolyticus* in Clam Homogenate," *Journal of Food Science* 41, 899–902 (1979).

130 L. R. Beuchat and R. E Worthington, "Relationships Between Heat Resistance and Phospholipid Fatty Acid Composition of *Vibrio parahaemolyticus*." *Applied Environmental Microbiology* 31, 389–394 (Mar. 1976).

131. R. P. Delmore, Jr., and F. D. Crisley, "Thermal Resistance of *Vibrio parahaemolyticus* in Clam Homogenate," *J. Food Prot.* 42, 131–134 (1979).

132. R. Sakazaki, "Vibrio Infections," in H. Rieman and F. Bryon, eds., *Foodborne Infection and Intoxication*, Academic Press, Inc., Orlando, Fla., 1979.

133. J. Morris and R. Black, "Cholera and Other Vibrioses in the United States," *New England Journal of Medicine* 312, 343–350 (1985).

134. Centers for Disease Control, "Cholera in Louisiana—Update," *Morbidity Mortality Weekly Report* 35, 687 (1986).

135. L. Shultz et al., "Determination of the Thermal Death Time of *Vibrio cholerae* in Blue Crabs *(Callinectes sapidus)*," *J. Food Prot.* 49, 4–6 (Jan. 1984).

136. J. Oliver, R. Warner, and D. Cleland, "Distribution of *Vibrio vulnificus* and Other Lactose Fermenting Vibrios in the Marine Environment," *Applied Environmental Microbiology* 45, 985–987 (1983).

137. R. Tilton and R. Ryan, "Clinical and Ecological Characteristics of *Vibrio vulnificus* in the Northeastern United States," *Diagnostic Microbiology Infectious Disease* 6, 109–117 (1987).

138. J. Oliver et al., "Bioluminescence in a Strain of the Human Pathogenic Bacterium *Vibrio vulnificus*," *Applied Environmental Microbiology* 52, 1209–1221 (1986).

139. G. Sakagrechi, "Botulism," in Ref. 132.

140. L. Smith, *Botulism: The Organism, Its Toxins, the Disease*. Charles C. Thomas, Springfield, Ill., 1977.

141. J. Simunovic, J. Oblinger, and J. Adams, "Potential for Growth of Nonproteolytic Types of *Clostridium botulinum* in Pasteurized Meat Products. A Review." *J. Food Prot.* 48, 265–276 (Mar. 1985).

142. NCA. *Thermal Destruction of Type E Clostridium botulinum*, NCA Research Foundation. Washington, D.C., 1973.

143. G. Hobbs, "*Clostridium botulinum* and Its Importance in Fishery Products," *Adv. Food Res.* 22, 135–156 (1976).

144. A. Emodi and R. Lechowich, "Low Temperature Growth of Type E. *Clostridium botulinum* Spores. I. Effect of Sodium Chloride and pH," *Journal of Food Science* 34, 78–81 (1969).

145. M. Miller and S. Koburger, "*Plesiomonas shigelloides*: An Opportunistic Food and Waterborne Pathogen," *J. Food Prot* 48, 449–457 (May 1985).

146. C P. Gerba, "Viral Disease Transmission by Seafoods," *Food Technology* 42, 99–103 (1988).

147. M. C. O'Mahony et al., "Epidemic Hepatitis A from Cockles," *Lancet* 1, 518–520 (1983).

148. W. Stille et al., "Oyster-Transmitted Hepatitis," *Deutsche Medizinische Wochenschrift* 97, 145 (1972).

149. Centers for Disease Control, *Annual Summary of Foodborne Diseases*, CDC. U.S. Department of Health and Human Services, Atlanta, Ga., 1979–1986.

150. S. L. Taylor, "Marine Toxins of Microbial Origin," *Food Technology* 42, 94–98 (1988).

151. MCCFP. National Research Council *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*. National Academic Press, Washington, D.C., 1985.

152. E. S. Garrett III. "Microbiological Standards, Guidelines, and Specifications and Inspection of Seafood Products," *Food Technology* 42, 90–93 (1988)

153. International Committee on Microbiological Specifications for Foods. *Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications*. ICMFS, University of Toronto Press, Toronto, 1978.

154. International Committee on Microbiological Specifications for Foods, *Microorganisms in Foods. 1. Their Significance and Methods of Enumeration*, ICMFS. University of Toronto Press, Toronto, 1978.

155. International Committee on Microbiological Specifications for Foods, *Microorganisms in Foods. 2. Sampling for Microbiological Analysis: Principles and Specific Applications*. ICMFS, University of Toronto Press, Toronto, 1986.

156. Codex Alimentarius Commission, *Recommended International Code of Practice for Shrimps or Prawns*, Joint FAO/WHO, Rome, Italy, 1980

157. Codex Alimentarius Commission, *Recommended International Code of Practice for Frozen Fish*, Joint FAO/WHO, Rome, Italy, 1980.

158. National Academy of Sciences, *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, National Academy Press, Washington, D.C., 1985.

TUU-JYI CHAI
University of Maryland
Cambridge, Maryland

See also MICROBIOLOGY OF FOODS.

FISH AND SHELLFISH PRODUCTS

THE SEAFOOD CHAIN

It is known that humans have used rather advanced techniques for obtaining and processing seafood throughout recorded history. A main source of protein for the ancient Egyptians was fish from the Nile, Mediterranean, and pond culture. Fish was consumed fresh and salted for preservation by the Greeks. Dried fish became a major source of animal protein in Europe when the Roman church banned meat consumption on Fridays and during Lent.

It is known that American Indians used fish as an important part of their diets 10,000 years ago. The early European settlers coming to the New World brought processing and preservation practices such as drying, salting, pickling, and cooling. During colonial times salted dried cod was exported back to England. A major part of the diet of American northwest Indians and Eskimos was salmon that was sun dried and smoked over a wood fire.

It was not until the 19th century in the Great Lakes region that fish were frozen by using the combination of salt and ice to lower temperatures below freezing. The growth of commercial freezing processes over the last 100 years has made freezing the leading means of preserving

128. J. Oliver, "Vibrio: On Increasingly Troublesome Genus," *Diagn. Med.* **8**, 43–49 (1985).
129. R. Delmore and P. Crisley, "Thermal Resistance of *Vibrio parahaemolyticus* in Clam Homogenate," *Journal of Food Science* **41**, 899–902 (1979).
130. L. R. Beuchat and R. E. Worthington, "Relationships Between Heat Resistance and Phospholipid Fatty Acid Composition of *Vibrio parahaemolyticus*." *Applied Environmental Microbiology* **31**, 389–394 (Mar. 1976).
131. R. P. Delmore, Jr., and F. D. Crisley, "Thermal Resistance of *Vibrio parahaemolyticus* in Clam Homogenate," *J. Food Prot.* **42**, 131–134 (1979).
132. R. Sakazaki, "Vibrio Infections," in H. Rieman and F. Bryon, eds., *Foodborne Infection and Intoxication*, Academic Press, Inc., Orlando, Fla., 1979.
133. J. Morris and R. Black, "Cholera and Other Vibrioses in the United States," *New England Journal of Medicine* **312**, 343–350 (1985).
134. Centers for Disease Control, "Cholera in Louisiana—Update," *Morbidity Mortality Weekly Report* **35**, 687 (1986).
135. L. Shultz et al., "Determination of the Thermal Death Time of *Vibrio cholerae* in Blue Crabs *(Callinectes sapidus)*," *J. Food Prot.* **49**, 4–6 (Jan. 1984).
136. J. Oliver, R. Warner, and D. Cleland, "Distribution of *Vibrio vulnificus* and Other Lactose Fermenting Vibrios in the Marine Environment," *Applied Environmental Microbiology* **45**, 985–987 (1983).
137. R. Tilton and R. Ryan, "Clinical and Ecological Characteristics of *Vibrio vulnificus* in the Northeastern United States," *Diagnostic Microbiology Infectious Disease* **6**, 109–117 (1987).
138. J. Oliver et al., "Bioluminescence in a Strain of the Human Pathogenic Bacterium *Vibrio vulnificus*," *Applied Environmental Microbiology* **52**, 1209–1221 (1986).
139. G. Sakagrechi, "Botulism," in Ref. 132.
140. L. Smith, *Botulism: The Organism, Its Toxins, the Disease*. Charles C. Thomas, Springfield, Ill., 1977.
141. J. Simunovic, J. Oblinger, and J. Adams, "Potential for Growth of Nonproteolytic Types of *Clostridium botulinum* in Pasteurized Meat Products. A Review." *J. Food Prot.* **48**, 265–276 (Mar. 1985).
142. NCA. *Thermal Destruction of Type E Clostridium botulinum*, NCA Research Foundation. Washington, D.C., 1973.
143. G. Hobbs, "*Clostridium botulinum* and Its Importance in Fishery Products," *Adv. Food Res.* **22**, 135–156 (1976).
144. A. Emodi and R. Lechowich, "Low Temperature Growth of Type E. *Clostridium botulinum* Spores. I. Effect of Sodium Chloride and pH," *Journal of Food Science* **34**, 78–81 (1969).
145. M. Miller and S. Koburger, "*Plesiomonas shigelloides*: An Opportunistic Food and Waterborne Pathogen," *J. Food Prot* **48**, 449–457 (May 1985).
146. C. P. Gerba, "Viral Disease Transmission by Seafoods," *Food Technology* **42**, 99–103 (1988).
147. M. C. O'Mahony et al., "Epidemic Hepatitis A from Cockles," *Lancet* **1**, 518–520 (1983).
148. W. Stille et al., "Oyster-Transmitted Hepatitis," *Deutsche Medizinische Wochenschrift* **97**, 145 (1972).
149. Centers for Disease Control, *Annual Summary of Foodborne Diseases*, CDC. U.S. Department of Health and Human Services, Atlanta, Ga., 1979–1986.
150. S. L. Taylor, "Marine Toxins of Microbial Origin," *Food Technology* **42**, 94–98 (1988).
151. MCCFP. National Research Council *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*. National Academic Press, Washington, D.C., 1985.
152. E. S. Garrett III. "Microbiological Standards, Guidelines, and Specifications and Inspection of Seafood Products," *Food Technology* **42**, 90–93 (1988)
153. International Committee on Microbiological Specifications for Foods. *Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications*. ICMFS, University of Toronto Press, Toronto, 1978.
154. International Committee on Microbiological Specifications for Foods, *Microorganisms in Foods. 1. Their Significance and Methods of Enumeration*, ICMFS. University of Toronto Press, Toronto, 1978.
155. International Committee on Microbiological Specifications for Foods, *Microorganisms in Foods. 2. Sampling for Microbiological Analysis: Principles and Specific Applications*. ICMFS, University of Toronto Press, Toronto, 1986.
156. Codex Alimentarius Commission, *Recommended International Code of Practice for Shrimps or Prawns*, Joint FAO/WHO, Rome, Italy, 1980
157. Codex Alimentarius Commission, *Recommended International Code of Practice for Frozen Fish*, Joint FAO/WHO, Rome, Italy, 1980.
158. National Academy of Sciences, *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, National Academy Press, Washington, D.C., 1985.

TUU-JYI CHAI
University of Maryland
Cambridge, Maryland

See also MICROBIOLOGY OF FOODS.

FISH AND SHELLFISH PRODUCTS

THE SEAFOOD CHAIN

It is known that humans have used rather advanced techniques for obtaining and processing seafood throughout recorded history. A main source of protein for the ancient Egyptians was fish from the Nile, Mediterranean, and pond culture. Fish was consumed fresh and salted for preservation by the Greeks. Dried fish became a major source of animal protein in Europe when the Roman church banned meat consumption on Fridays and during Lent.

It is known that American Indians used fish as an important part of their diets 10,000 years ago. The early European settlers coming to the New World brought processing and preservation practices such as drying, salting, pickling, and cooling. During colonial times salted dried cod was exported back to England. A major part of the diet of American northwest Indians and Eskimos was salmon that was sun dried and smoked over a wood fire.

It was not until the 19th century in the Great Lakes region that fish were frozen by using the combination of salt and ice to lower temperatures below freezing. The growth of commercial freezing processes over the last 100 years has made freezing the leading means of preserving seafood for human consumption. As will be seen, the early fresh fish handling and freezing technology was not developed with good knowledge of the biological and physical factors that must be considered. Hence, the fishy odor of fish along with certain rancid and spoilage off-tastes were considered normal in fresh, frozen, and dried products.

Today it is realized that quality control of fish, as much or more than any other food, must be practiced from harvest to table. The seafood chain (Fig. 1) begins with the boat builder or hatchery designer and ends with the consumer. All must practice good techniques of sanitation, temperature control, and packaging protection. As the fish story unfolds, it will be seen that any break in this chain can be disastrous to the quality of seafood. If a processing plant, market, restaurant, or home smells fishy, then there has been a break in the chain. Good fish looks good, has a neutral fresh odor, and has firm flesh. Good fish is a healthful, highly nutritious protein food that has an unlimited market.

U.S. FISHERY

There are more than 2,000 species of finfish within the U.S. coastal marine waters, of which about ca 2% are consumed as food fish (1). In fact, the U.S. consumer is familiar with only a few of the some 200 species of finfish and 40 species of shellfish that are consumed throughout the world. This is not surprising when one compares the geographical annual per capita consumption of fish and shellfish as shown in Table 1 (2). Based on live weight of the landed animal, North America consumed 85.6 lb while consumption in other areas of the world ranged from 5 to 15 times that amount. There was little change in the amount of fish consumed by the average U.S. citizen from the early 1900s to 1970s (Table 2). Over the last 15 years, although still considerably below that of the rest of the world, the U.S. per capita consumption has risen about 30%. Most of this increase has been during the last 5 yr. With the increasing emphasis on eating lighter and leaner foods, this U.S. consumption is continuing to rise.

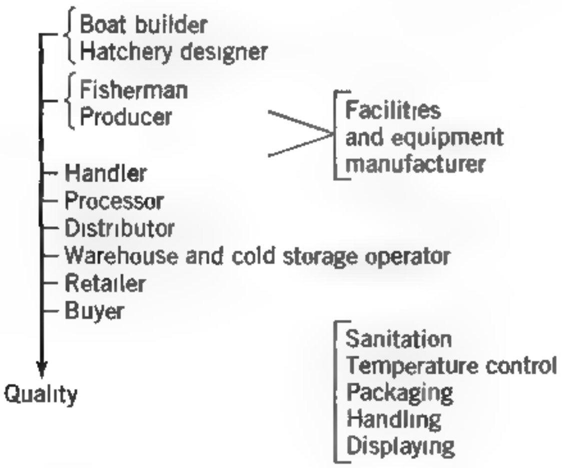


Figure 1. The seafood chain from the harvest to the table.

Table 1. Annual Per Capita Consumption of Live Weight Fish and Shellfish for Human Food, Selected Countries, 1982–1984

Country	Estimated live weight equivalent: Kilograms	Estimated live weight equivalent: Pounds
North America		
Canada	21.4	47.2
United States	17.4	36.4
Caribbean		
Barbados	33.6	74.1
Dominican Republic	6.8	15.0
Grenada	19.4	42.8
Haiti	3.7	8.2
Jamaica	17.0	37.5
Latin America		
Argentina	5.7	12.6
Bolivia	1.6	3.5
Brazil	6.2	13.7
Chile	18.7	41.2
Equador	14.2	13.1
Mexico	9.9	21.8
Panama	14.4	31.7
Europe		
Denmark	22.0	48.5
France	24.8	54.7
Iceland	88.4	194.9
Norway	46.0	101.4
USSR	27.3	60.2
Near East		
Egypt	5.5	12.1
Israel	14.2	31.3
Lebanon	0.5	1.1
Oman	35.5	78.3
Far East		
China	4.9	10.8
India	3.1	6.8
Japan	74.5	164.2
Philippines	35.7	78.7
Africa		
Congo (Brazaville)	33.4	73.6
Ethiopia	0.1	0.2
Kenya	4.7	10.4
Republic of South Africa	9.6	21.2
Oceania		
Australia	16.0	35.3
New Zealand	12.2	26.9

Source. Ref. 2.

Table 2. U.S. Annual Per Capita Consumption of Commercial Fish and Shellfish, 1953–1985

Year	Civilian resident population, July 1[a]	Fresh and frozen[b]	Per capita canned[c]	Consumption cured[d]	Total
1909[e]	90.5	4.3	2.7	4.0	*11.0*
1910	92.4	4.5	2.8	3.9	*11.2*
1915	100.5	5.8	2.4	3.0	*11.2*
1920	106.5	6.3	3.2	2.3	*11.8*
1925	115.8	6.3	3.2	1.6	*11.1*
1930	122.9	5.8	3.4	1.0	*10.2*
1935	127.1	5.1	4.7	0.7	*10.5*
1940	132.1	5.7	4.6	0.7	*11.0*
1945	128.1	6.6	2.6	0.7	*9.9*
1950	150.8	6.3	4.9	0.6	*11.8*
1955	163.0	5.9	3.9	0.7	*10.5*
1960	178.1	5.7	4.0	0.6	*10.3*
1965	191.6	6.0	4.3	0.5	*10.8*
1970	201.9	6.9	4.5	0.4	*11.8*
1975	213.8	7.5	4.3	0.4	*12.2*
1980[f]	225.6	8.0	4.5	0.3	*12.8*
1981[f]	227.7	7.8	4.8	0.3	*12.9*
1982[f]	229.9	7.7	4.3	0.3	*12.3*
1983[f]	232.0	8.0	4.8	0.3	*13.1*
1984[f]	234.4	8.3	5.0	0.3	*13.6*
1985[f]	237.0	9.0	5.2	0.3	*14.5*
1986[f]	239.4	9.0	5.4	0.3	*14.7*
1987[f]	241.5	10.0	5.1	0.3	*15.4*
1988[f]	243.9	9.6	5.1	0.3	*15.0*

Source. Ref. 2.

[a] Resident population for 1909–1929 and civilian resident population for 1930 to date.

[b] Fresh and frozen fish consumption from 1910 to 1928 is estimated. Beginning in 1973, data include consumption of artificially cultivated catfish.

[c] Canned fish consumption for 1910–1920 is estimated. Beginning in 1921, it is based on production reports, packer stocks, and foreign trade statistics for individual years.

[d] Cured fish consumption for 1910–1928 is estimated.

[e] Data for 1909 estimate based on the 1908 census and foreign trade.

[f] Domestic landing data used in calculating these data are preliminary.

Fishery Landings

The 1988 commercial domestic landings, by species, of marine fish and shellfish in the United States is shown in Table 3 (2). Near-shore landings (0–3 mi offshore) totaled 1,762,059 t (value $2,026,147,000), offshore landings (3–200 mi offshore) totaled 2,953,034 t (value $1,671,279,000), and international water catches totaled 259,735 t (value $313,642,000), for a grand total of 4,974,828 t (value $4,010,068,000). Almost 60% of the total fish and shellfish landings by the United States are caught 3–200 mi offshore. Much of the harvest in these waters is in the Fisheries Conservation Zone, which extends fishing jurisdiction beyond that normally considered international waters.

There is a large variation in the value of different species based on the consumer acceptance and the economics of harvest. Two relatively low-cost species, menhaden and pollock, accounted for 48.3% of the total fish and shellfish landed but had a value amounting to only 8.2% of the total. Menhaden is reduced to fish meal for animal consumption, whereas pollock is the basis for the rapidly growing surimi and pollock fillet industry. Conversely, high-value salmon accounted for only 5.5% of the catch but represented 22.7% of the total value, Shellfish also accounted for a relatively small volume of the total landings in the United States (less than 12%) but represented 37.5% of the total value.

It is important to include the production of fish and shellfish from the rapidly growing aquaculture industry when considering total fish and shellfish availability. Although long established in other parts of the world (particularly Asia) aquaculture is a relatively new and rapidly growing industry in the United States. As shown in Table 4, aquaculture-raised fish accounted for 281,800 t (620 million lb) in 1986 (3).

For many years trout was the leading aquaculture finfish grown in the United States. However, catfish became the dominant species in the 1970s and salmon production passed trout in 1985. Catfish farming in the United States has increased from 1,004 t (22.1 million lb) in 1977 to 134,090 t (295 million lb) in 1988 (4). This represents about 53% of the total aquaculture fish and shellfish and 5% of the total combined wild and aquaculture fish and shellfish.

Imports and Exports

Tables 5 and 6 show the imports and exports of fishery products to and from the United States (2). In 1988 1,350,000 t (2,971 million lb) of edible fishery products were imported while only 36% of that amount, 483,550 t (1,064 million lb), were imported.

Harvesting Gear

Static Gear. Although early fishing depended on spear fishing and later was the basis for sophisticated harpoon

Table 3. Commercial Landings of Fish and Shellfish by U.S. Fishing Craft, 1988

Species	Total	
	Metric tons	Thousand dollars
	Fish	
Alewives		
Atlantic and Gulf	2,561	626
Great Lakes	4,856	191
Anchovies	5,636	2,615
Bluefish	7,644	3,012
Bonito	4,178	1,827
Butterfish	2,468	3,407
Cod		
Atlantic	34,506	42,941
Pacific	232,727	68,858
Croaker	4,810	4,596
Cusk	1,072	1,021
Flounders		
Atlantic and Gulf:		
Blackback	8,211	21,533
Fluke	16,334	44,345
Yellowtail	5,041	13,187
Other	11,616	27,022
Pacific	392,612	87,818
Total flounders	*433,814*	*193,905*
Groupers	5,547	21,703
Haddock	2,916	7,030
Hake		
Pacific (whiting)	142,919	15,820
Red	1,739	618
White	5,446	3,639
Halibut	37,017	72,718
Herring, sea		
Atlantic	41,003	5,229
Pacific	59,617	57,431
Jack mackerel	10,227	1,685
Lingcod	2,965	2,385
Mackerel		
Atlantic	12,377	2,722
King	1,954	5,043
Pacific	45,514	7,498
Spanish	1,922	1,479
Menhaden		
Atlantic	360,462	35,162
Gulf	638,721	73,259
Total menhaden	999,183	108,421
Mullets	14,806	11,218
Ocean perch		
Atlantic	1,066	1,467
Pacific	5,406	2,546
Pollock		
Atlantic	14,992	11,071
Alaska	1,396,833	211,354
Rockfish	58,360	39,368
Sablefish	48,817	91,793
Salmon, Pacific		
Chinook or king	20,716	117,551
Chum or keta	66,436	134,689
Pink	80,053	127,297
Red or sockeye	86,199	437,630
Silver or coho	21,539	93,506
Total salmon	*274,943*	*910,673*
Scup or porgy	6,513	9,572
Sea bass		
Black	2,188	5,144
White	49	218
Sea trout		
Gray	9,314	7,948
Spotted	1,403	3,169
White	168	229
Sharks		
Dogfish	4,568	975
Other	6,622	8,454
Snapper		
Red	1,884	9,496
Other	3,083	11,319
Striped bass	185	517
Swordfish	5,814	42,703
Tilefish	2,066	7,222
Tuna		
Albacore	8,643	16,278
Bigeye	2,472	15,149
Bluefin	1,699	17,305
Skipjack	136,077	149,052
Yellowfin	127,140	182,202
Unclassified	354	546
Total tuna	*276,385*	*380,532*
Whiting	16,134	8,621
Other marine finfishes	119,697	74,953
Other freshwater finfishes	13,271	18,534
Total fish	*4,389,185*	*2,505,515*
	Shellfish	
Clams		
Hard	5,616	67,818
Ocean quahog	21,006	14,921
Soft	3,091	18,717
Surf	28,824	29,183
Other	1,225	4,142
Total clams	*58,757*	*134,781*
Crabs		
Blue, hard	99,184	84,357
Dungeness	21,518	54,771
King	9,513	84,153
Snow (tanner)	66,372	137,052
Other	10,082	23,227
Total crabs	*206,669*	*383,560*
Lobsters		
American	22,064	145,236
Spiny	3,250	23,030
Oysters	14,466	78,498
Scallops		
Bay	258	3,414
Calico	5,383	12,462
Sea	13,860	128,243
Shrimp		
New England	3,078	7,497
South Atlantic	10,997	51,667
Gulf	102,416	414,469
Pacific	33,590	32,401
Total shrimp	*150,081*	*506,034*
Squid		
Atlantic	21,200	16,220
Pacific	36,481	7,689

Table 3. Commercial Landings of Fish and Shellfish by U.S. Fishing Craft, 1988 *(continued)*

	Total	
Species	Metric tons	Thousand dollars
Other shellfish	52,174	65,386
Total shellfish	*585,643*	*1,504,553*
Grand total, 1988	*4,974,828*	*4,010,068*

Source. Ref. 2.

Note: Landings are in round (live) weight for all items, except clams, oysters, and scallops, which are reported in weight of meat.

systems, modern commercial fishing methods use either static gear or moving gear when a vessel is used for towing or dragging. There are three basic types of static gear used in the commercial fisheries. The hook-and-line technique is familiar to all sports fishermen. The fish is attracted to the hook by an edible bait or by an attractive device such as a feather. As the fish tries to take the food from the bait or attacks the lure, it becomes attached to the barbed hook and cannot shake the hook from its mouth. A technique called long-lining is the major commercial use of hook-and-line fishing. A series of baited hooks are suspended from a buoyed horizontal line. This use of multiple hooks greatly increases the chances of catching fish and makes the fishing a viable commercial venture.

There are two basic types of trap used to catch fish and shellfish. One is an enclosure placed in the pathway of a moving or migrating animal while the other is a container or pot in which bait is placed. An animal crawls or swims into the tunnel entrance and then cannot escape because it is unable to find its way out of the narrow opening. Traps usually have a one-way wire door that can be easily pushed aside to enter but prevents regression

Static nets, called gill nets, are a form of static gear closely related to traps. Nets are suspended from a few fathoms up to 50 fathoms into the water from a buoyed line. They are placed in the water where fish are known to be passing. The moving fish hits and penetrates the net. As it realizes that it has hit an obstruction, it tries to pull back. This causes its gills to become entangled in the webbing where the fish is securely held until the fishermen pull the net to the surface. Figures 2–7 show the types of static gear described above (5).

Towed or Dragged Gear. It is often necessary to sweep large areas of the ocean to catch sufficient volumes of fish or shellfish. This is done by trawling, in which large nets are towed through the water, or dredging, in which gear is towed along the bottom.

A trawl net is constructed like a large net bag that has a restricted end called the cod end. As a fishing vessel pulls the funnellike nets, fish are swept into the nets and collected in the bottom or cod end. The vessel must travel at sufficient speed to insure that the fish being collected into the net cannot swim out of the opening. The goal of a trawl operation, lasting from less than an hour to several hours is to fill the cod end. A typical trawl net and a dredge are shown in Figures 8–10 (5).

Encircling Gear. Encircling gear is used for catching large amounts of fish that are schooled or densely concentrated. One end of the net, called a seine net is pulled from the fishing vessel and completes the operation by encir-

Table 4. U.S. Private Aquaculture Production and Value

Species	1980	1983	1984	1985	1986
Baitfish	22,046	22,046	23,598	24,807	25,247
	(44,000)	(44,000)	(47,045)	(51,280)	(51,522)
Catfish	76,849	220,000	239,800	271,357	326,979
	(53,572)	(132,000)	(191,840)	(189,194)	(228,886)
Clams	561	1,689	1,689	1,588	2,506
	(2,295)	(9,500)	(4,178)	(4,717)	(8,307)
Crayfish	23,917	60,000	59,400	64,999	97,500
	(12,951)	(30,000)	(27,700)	(32,500)	(48,750)
Freshwater prawns	300	275	317	267	178
	(1,200)	(1,500)	(1,698)	(1,540)	(893)
Mussels	—	775	917	928	1,206
	—	(1,500)	(1,584)	(1,248)	(1,725)
Oysters	23,755	23,300	24,549	22,473	24,090
	(37,085)	(31,500)	(38,970)	(39,997)	(42,797)
Pacific salmon	7,616	20,600	45,086	84,305	74,398
	(3,400)	(6,800)	(17,252)	(25,439)	(32,751)
Shrimp	—	255	528	440	1,354
	—	(874)	(1,566)	(1,687)	(3,408)
Trout	48,141	48,400	49,940	50,600	51,000
	(37,474)	(50,000)	(54,435)	(55,154)	(55,590)
Other species	—	7,000	9,900	14,000	15,500
	—	(7,000)	(9,900)	(20,000)	(21,700)

Source: Ref. 3.

Note. Production is in 1,000 lb, and value, in parentheses, is in $1,000.

Table 5. U.S. Fishery Products Imports by Principal Items, 1987 and 1988

	1987		1988	
Item	Thousand pounds	Thousand dollars	Thousand pounds	Thousand dollars
	Edible fishery products			
Fresh and frozen				
Whole or eviscerated				
Cod, cusk, haddock, and flounder	105,158	65,198	85,139	52,717
Halibut	9,295	23,138	11,952	25,432
Salmon	41,902	113,008	50,144	155,173
Tuna				
Albacore	201,988	171,988	195,991	202,967
Other[a]	370,517	158,771	354,156	169,331
Other	214,628	183,022	222,696	203,399
Fillets and steaks				
Flounder	73,003	148,734	58,534	119,996
Groundfish	315,418	570,065	253,187	431,126
Other	232,564	396,324	205,988	358,766
Blocks and slabs	403,577	539,358	303,237	382,482
Shrimp	461,173	1,676,844	489,740	1,725,971
Crabmeat	12,571	67,427	10,821	59,639
Lobster				
American (includes fresh-cooked meat)	38,974	178,069	39,732	183,482
Spiny	41,949	397,854	37,806	363,195
Scallops (meats)	39,934	162,273	32,039	115,706
Analogue products with shellfish	30,539	51,197	24,516	41,569
Other fish and shellfish	98,996	169,855	92,128	163,444
Canned				
Herring, not in oil	5,617	8,726	6,541	10,264
Sardines				
In oil	27,352	35,106	22,813	30,824
Not in oil	37,670	25,470	30,546	23,154
Tuna				
In oil	329	869	318	744
Not in oil	211,356	206,051	244,186	297,922
Balls, cakes, and puddings				
Analogue products without shellfish	4,737	6,786	2,574	4,361
Other	8,797	13,130	5,650	8,376
Abalone	2,790	19,867	2,434	22,487
Clams	13,974	15,288	11,268	13,993
Crabmeat	7,967	20,626	7,720	19,622
Lobsters				
American	637	4,184	594	5,342
Spiny	136	748	52	280
Oysters	32,668	36,144	27,524	39,817
Shrimp	17,132	33,380	14,138	28,730
Other fish and shellfish	57,579	89,499	52,350	80,952
Cured				
Pickled or salted				
Cod, haddock, hake, etc	31,893	60,542	31,361	52,665
Herring	22,213	9,594	20,333	8,618
Other	9,991	22,792	11,784	27,718
Other fish and shellfish	16,108	29,306	10,966	29,119
Total edible fishery products	*3,201,132*	*5,711,233*	*2,970,958*	*5,459,383*
	Inedible fishery products			
Meal and scrap	393,730	52,508	265,310	49,567
Fish oils	30,509	18,930	27,688	9,666
Other	—	3,035,026	—	3,353,379
Total inedible fishery products	—	*3,106,464*	—	*3,412,612*
Grand total	—	*8,817,697*	—	*8,871,995*

Source Ref. 2.

Note: Data include imports into the United States and Puerto Rico and include landings of tuna by foreign vessels at American Samoa. Statistics on imports are the weight of individual products as exported, ie, fillets, steaks, whole, headed, etc.

[a] Includes loins and disks.

Table 6. U.S. Domestic Fishery Products Exports, by Principal, 1987 and 1988

Item	1987		1988	
	Thousand pounds	Thousand dollars	Thousand pounds	Thousand dollars
Edible fishery products				
Fresh and frozen				
Whole or eviscerated				
Cod, cusk, haddock, and flounder	105,158	65,198	85,139	52,717
Halibut	9,295	23,138	11,952	25,432
Salmon	41,902	113,008	50,144	155,173
Tuna				
Albacore	201,988	171,988	195,991	202,967
Other[a]	370,517	158,771	354,156	169,331
Other	214,628	183,022	222,696	203,399
Fillets and steaks				
Flounder	73,003	148,734	58,534	119,996
Groundfish	315,418	570,065	253,187	431,126
Other	232,564	396,324	205,988	358,766
Blocks and slabs	403,577	539,358	303,237	382,482
Shrimp	461,173	1,676,844	489,740	1,725,971
Crabmeat	12,571	67,427	10,821	59,639
Lobster				
American (includes fresh-cooked meat)	38,974	178,069	39,732	183,482
Spiny	41,949	397,854	37,806	363,195
Scallops (meats)	39,934	162,273	32,039	115,706
Analogue products with shellfish	30,539	51,197	24,516	41,569
Other fish and shellfish	98,996	169,855	92,128	163,444
Canned				
Herring, not in oil	5,617	8,726	6,541	10,264
Sardines				
In oil	27,352	35,106	22,813	30,824
Not in oil	37,670	25,470	30,546	23,154
Tuna				
In oil	329	869	318	744
Not in oil	211,356	206,051	244,186	297,922
Balls, cakes, and puddings				
Analogue products without shellfish	4,737	6,786	2,574	4,361
Other	8,797	13,130	5,650	8,376
Abalone	2,790	19,867	2,434	22,487
Clams	13,974	15,288	11,268	13,993
Crabmeat	7,967	20,626	7,720	19,622
Lobsters				
American	637	4,184	594	5,342
Spiny	136	748	52	280
Oysters	32,668	36,144	27,524	39,817
Shrimp	17,132	33,380	14,138	28,730
Other fish and shellfish	57,579	89,499	52,350	80,952
Cured				
Pickled or salted				
Cod, haddock, hake, etc	31,893	60,542	31,361	52,665
Herring	22,213	9,594	20,333	8,618
Other	9,991	22,792	11,784	27,718
Other fish and shellfish	16,108	29,306	10,966	29,119
Total edible fishery products	*3,201,132*	*5,711,233*	*2,970,958*	*5,459,383*
Inedible fishery products				
Meal and scrap	393,730	52,508	265,310	49,567
Fish oils	30,509	18,930	27,688	9,666
Other	—	3,035,026	—	3,353,379
Total inedible fishery products	—	*3,106,464*	—	*3,412,612*
Grand total	—	*8,817,697*	—	*8,871,995*

Source: Ref. 2.

Note. Data include imports into the United States and Puerto Rico and include landings of tuna by foreign vessels at American Samoa. Statistics on imports are the weight of individual products as exported, ie, fillets, steaks, whole, headed, etc.

[a] Includes loins and disks.

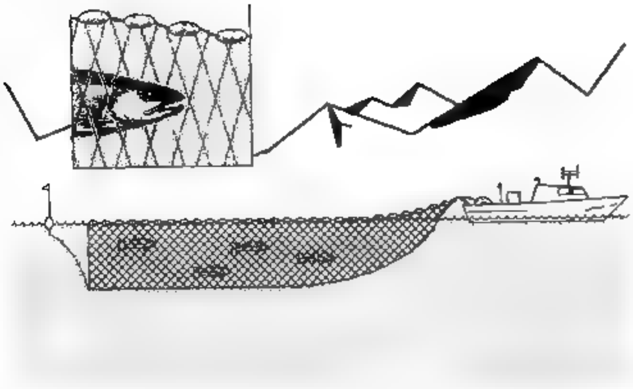


Figure 2. Static fishing gear. Gillnetting—a method of fishing in which fish swim into a suspended net and become entangled by their gills in the webbing. The net can be placed at various depths, depending on the fishery and locale. *Source:* Courtesy of *Seafood Leader*.

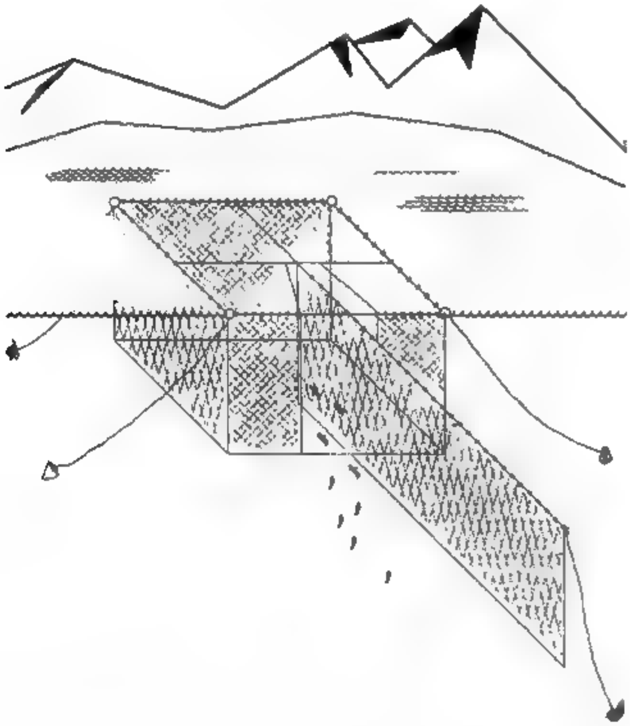


Figure 3. Static fishing gear. Cod traps—set on the ocean floor close to shore, these open-trapped box nets contain a door facing the shore, where seashore feeding cod are deterred by a net fence that directs them to the trap; once inside, they tend to swim in circles. *Source:* Courtesy of *Seafood Leader*.

cling the school of fish and bringing the end back to the vessel. Most seines (Fig. 11) have a line on the bottom that can be pursed to close the net and prevent fish from escaping. Typical fish that can be exploited by this method of harvest include anchovy, pilchard, sardine, salmon, herring, and tuna.

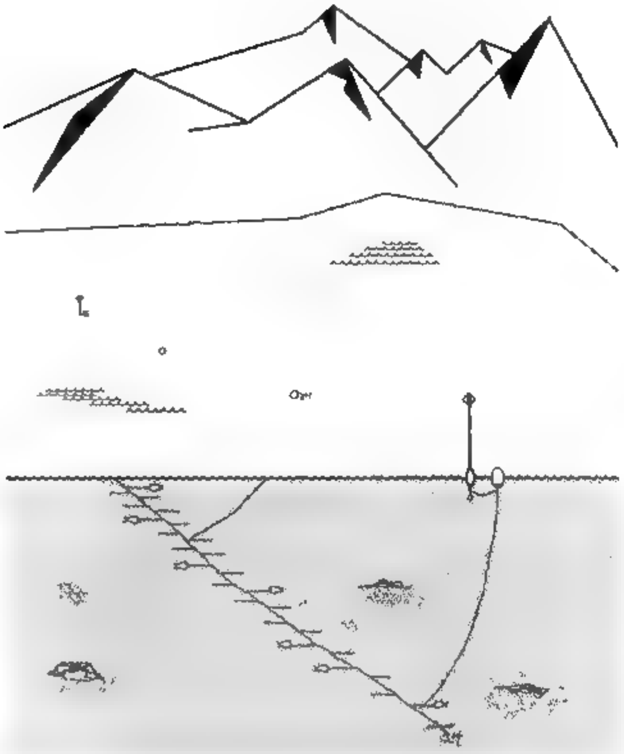


Figure 4. Static fishing gear. Longlining—a method of fishing involving one main line to which a series of shorter lines (gangions) with baited hooks are attached. Used at various depths; ie, surface longlining for pelagic species such as tuna and swordfish, bottom longlining for demersal species as halibut and cod. *Source:* Courtesy of *Seafood Leader*.

Fishing Vessels

The subject of fishing vessel design, construction, and operation is a most complex subject and will only be mentioned here. Fishing vessels range from small outboard motor boats operated by artisan fishermen to large ocean-going vessels, which require a sophisticated crew for efficient operation. The smaller vessels operate close to shore and return with the catch each day.

The length and sophistication of modern near-shore fishing vessels depends on the fishery, the fishing location and distance from shore, the distance that must be covered going to and from the fishing grounds, and the value of the catch. It is common to find vessels 20–50+ ft in length operating in the near-shore fishery and catcher-processor vessels of several hundred feet in length operating offshore on the high seas. The crews number from one to five on near-shore vessels and up to several hundred (including process crew) on the large catcher-processors. Likewise, the cost of fishing vessels varies tremendously, from about $200,000 for a small near-shore vessel to approximately $30,000,000 for the large offshore catcher-processors.

MAINTAINING QUALITY OF THE CATCH

Quality is a term that has many definitions, depending on the background and interests of those queried. To some,

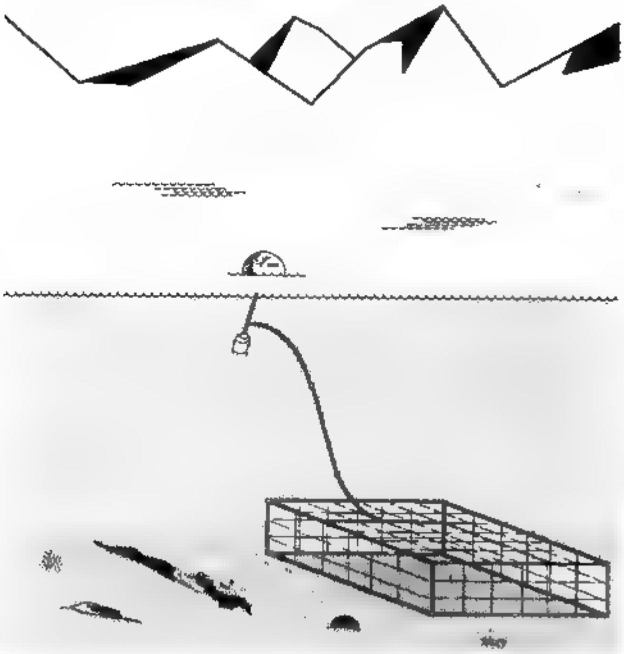


Figure 5. Static fishing gear. Crab traps—framed with iron rods and covered with polyethylene rope webbing, crab trays are usually fished on single lines. Size varies according to fishery; ie, small traps are used for blue crab, large traps are used for king crab. *Source.* Courtesy of *Seafood Leader*.

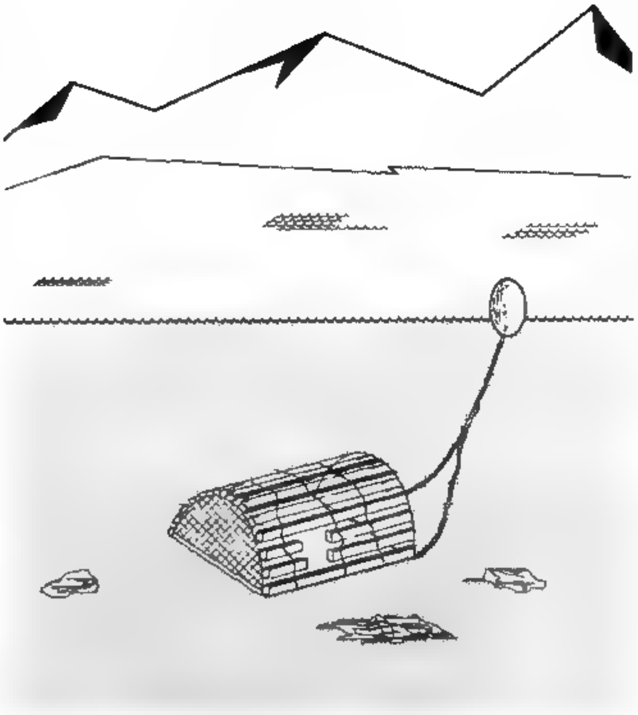


Figure 7. Static fishing gear. Inshore lobster fishing—wooden or wire traps with cotton or nylon twine are set on the ocean floor at various depths, either individually or in strings on a line, baited with either fresh or salted fish. *Source:* Courtesy of *Seafood Leader*.

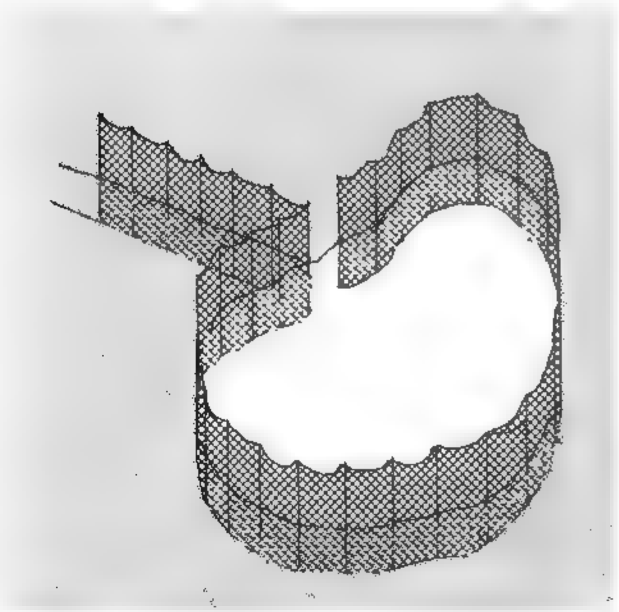


Figure 6. Static fishing gear. Weir fishing—rigid poles are driven into the mud bottom to form a heart-shaped configuration; a straight line of poles, leading from the shoreline to the weir, acts as a barrier directing the fish into the weir. Using skiffs, fishermen first seine the catch, then use a brailer or dip-net to collect them. *Source:* Courtesy of *Seafood Leader*.

the measuring of the product degradation by biological factors such as microorganisms or enzymes is the only means of determining quality. To others the aesthetic values that make a product look good are just as important in defining quality. In reality the biological factors determining safety and nutritional values and the physical factors used in a grading system (ie, size and weight uniformity, color, and blemished surfaces) are integrated to mean quality to most people.

The maintenance of quality or the fresh nature of landed seafood depends on many operations from the catching, landing, and shipboard handling to the transporting, storing, processing, and distributing. A biological specimen can only decrease in quality as it travels through the various steps of a commercial venture. Enzymes and microorganisms cause spoilage and degradation that are irreversible. Physical damage not only affects the appearance of a product, but such damage as skin ruptures allow microorganisms to invade the tissue and cause earlier deterioration. Therefore, all participants in the commercial seafood chain are important for maintaining high-quality products.

The Impact of Fishing Methods

Fishing gear is usually designed to give maximum efficiency in catching fish. The total cost of catching a given amount of fish includes vessel cost and operation, fishing gear cost and maintenance, manpower required, and other

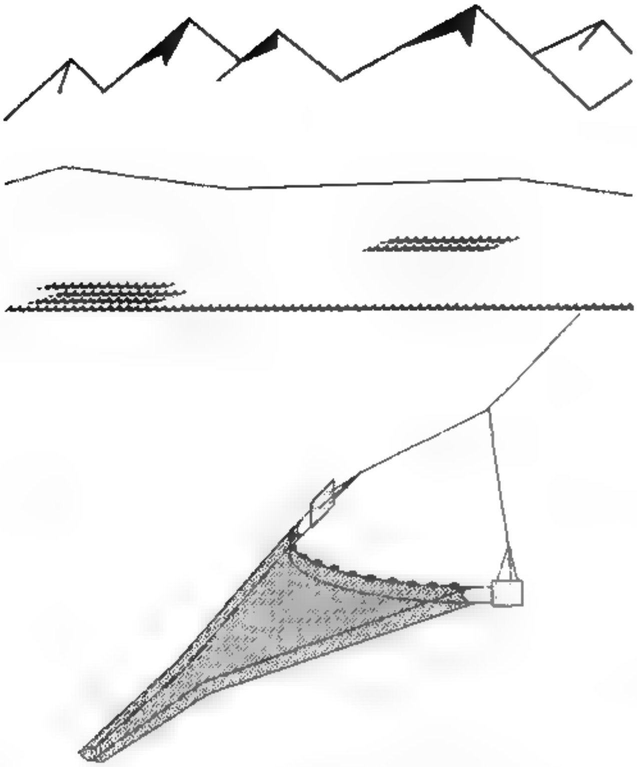


Figure 8. Towed or dragged fishing gear. Otter trawling—a method of fishing in which a large wedge-shaped net is dragged along the ocean bottom; an otter door is attached to each side of the net to hold the net open and keep it horizontal. Fish collect in the cod end (the back) of the net. *Source:* Courtesy of *Seafood Leader*.

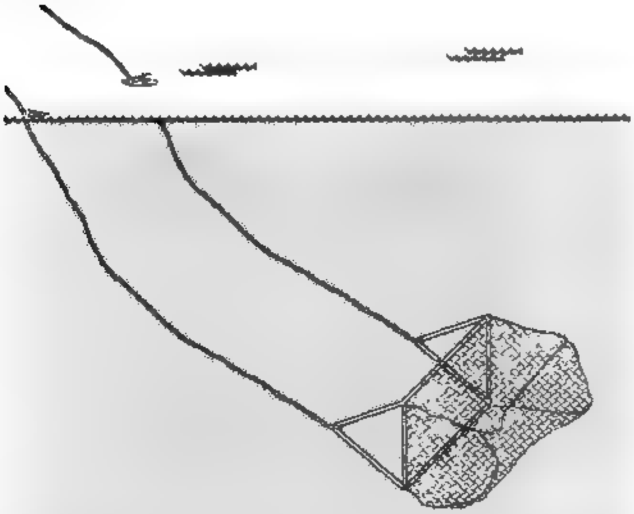


Figure 9. Towed or dragged fishing gear. Scallop dredging—a method of fishing which involves raking a metal frame with teeth and a chainmesh bag across the ocean bottom. *Source:* Courtesy of *Seafood Leader*.

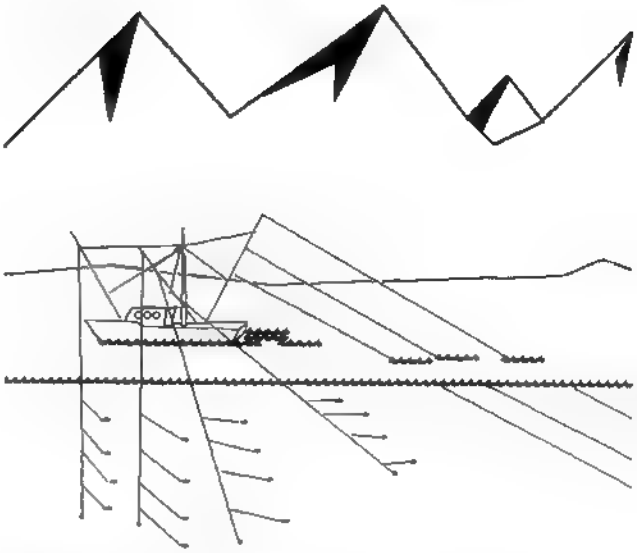


Figure 10. Towed or dragged fishing gear. Trolling—a method of fishing in which several fishing lines with numerous lures are dragged slowing through the water.

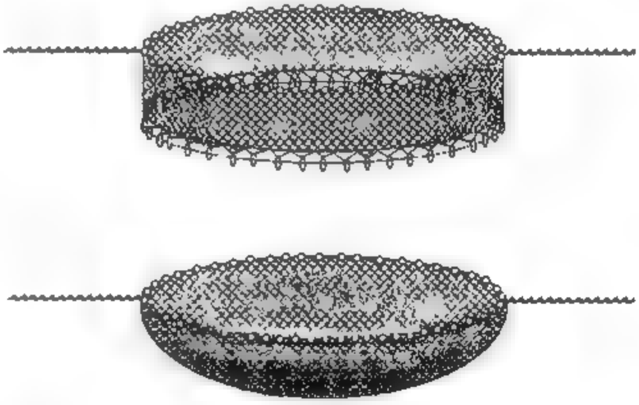


Figure 11. Encircling gear. Purse seining—a method of fishing involving a long, deep net that stands like a fence in the water, supported at the surface by floats and held down by lead lines at the bottom. A person in a skiff takes one end of the net around the school of fish and joins it at the other end, and the vessel hauls in the wire purse line strung through the bottom of the net, forming a purse under the fish. *Source:* Courtesy of *Seafood Leader*.

costs involving machinery and equipment. All factors are combined to give the cost of a given harvest known as the catch per unit effort (CUE). However, the best CUE does not insure good quality in the fish landed, and a processor is somewhat at the mercy of the fishing operation for initial quality of raw materials from the sea.

The type of gear used in fishing has a definite bearing on the quality. When a fish dies after or during vigorous exercise, such as struggling on a hook and line, metabolic activity including that of protein and lipid degrading enzymes, adversely affects the subsequent spoilage rate of the dead animal. If a fish has been feeding and has food in its stomach, the increased metabolic activity greatly accelerates the loss of quality in the slaughtered animal. The so-called soft belly of a fish is caused by this enzyme activity after the fish is dead. Because the amount of time that a hooked fish struggles is directly related to the subsequent spoilage rate, troll-caught fish are often of better quality than longline-caught fish that are allowed to die thrashing in the water.

The quality of gill net-caught fish is extremely variable depending on the length of time that the net is in the water. Hence, there can be a tremendous difference in the quality of fish taken from a given set, the last caught often being of higher quality. This factor is certainly realized by the wholesale buyers in that troll-caught fish consistently command a higher price than gill net-caught fish.

Certain visual or aesthetic factors also affect the market price of fish even though there may not be any real difference in the quality of the flesh. Marks from the web of a gill net are often caused when the fish struggles back and forth to release its entrapped gills. Unless the web causes cuts that allow bacteria to enter the flesh, these marks normally do not adversely affect the biological quality of the flesh. Hence, the word *quality* has different definitions, depending on whether it refers to the biological state of the edible portion or the visual appearance of the fish.

There are several types of fishing gear that cause abrasions and punctures during the catching and subsequent handling. Trawling, dredging, spearing or harpooning, and gillnetting all cause different degrees of damage. Trawling, which accounts for ca 40% of the world's fish catch, exerts extreme pressure on the fish as the cod end becomes full. In addition to scale abrasion, ruptures in the skin and internal portions release gut bacteria and decrease shelf life of the subsequent product.

The best quality is maintained in fish that are caught by trapping. As long as the trapping device is emptied on a reasonable cycle, the fish remains alive and is quickly killed prior to sale or processing. Some species, such as crabs, must be kept alive prior to butchering or there will be a blue color in the meat. This is due to the blood chemistry of crabs; they have a copper complex instead of the heme, or iron, complex found in most animals. If the crab is not butchered live and the blood removed prior to processing, the copper will oxidize, giving a blue color to the meat. Although the aesthetic value of the crab is impaired, the eating quality is not affected. However, as in the case of abraided fish skin, the consumer is not willing to consider blue crabmeat as anything but a low-grade, poor-quality product.

Aquaculture is somewhat akin to catching wild fish in trapping devices. The fish or shellfish are raised in an enclosed area and then removed when ready for market. As in the case of trap-caught fish, farmed fish are live when harvested.

Shipboard Handling of the Catch

The proper handling of fish during harvesting and on shipboard can minimize the adverse effects of gear. Of utmost importance when fish are first landed is that they are segregated and placed in a sanitary chilled environment. Minimizing the bacterial and enzymatic activity by fast reduction of temperature in freshly landed finfish and shellfish is probably the most important step in the entire chain of events that takes a fish from the water to the table.

Figure 12 shows the extreme variation in storage life of fresh and frozen commercial fish prepared for the market. This curve has been compiled from many published sources that give the shelf life of fish as related to the handling methods (6). It has been shown that landed high-quality fish that is chilled rapidly and carefully handled, processed, and packaged can be acceptable for up to three weeks after being caught. On the other end of the scale, a fish that has undergone poor handling on shipboard (eg, 70% of as caught quality) and subsequent marginal handling during the processing and marketing stages is inedible after four days.

Fish that has been properly handled, processed, packaged, and frozen can be held up to one year without significant deterioration. However, there are many factors that must be considered in discussing shelf life of a fresh or frozen product. The species, the oil content, the catching technique, and the state of the fish when harvested are all uncontrollable factors that have a major bearing on the shelf life of a seafood product.

PROCESSING SEAFOOD

Inspecting As-Received Seafoods

Seafoods received in the processing area of a vessel or in a shore-based plant vary tremendously in the state and form. This is the situation when batches of product from different sources or catching vessels are mixed in the received lot. A sensory inspection must be made to insure that the raw material passes the criteria specified by the buyer or processor.

A sensory evaluation utilizes touch, odor, and sight to determine the acceptability of a given lot of seafood (7). An on-site inspection should concentrate on microbial contamination, enzymatic degradation, and other chemical and physical factors that reduce the marketability of the seafood. A faint fresh, nonfishy odor; firm and elastic flesh; bright and full translucent eyes; bright pink gills; and bright and moist skin surface with no heavy deposits of mucus or slime are all properties of a good fresh fish.

In addition to microbial and enzymatic degradation that can take place in improperly handled seafood, the oil in fatty fish that have not been chilled rapidly or adequately protected from the environment are subject to oxidative rancidity. This is both an aesthetic and nutritional problem. Oxidation from the air (and sometimes autooxidation within the seafood) not only affects the odor and taste acceptability of the product but also destroys the omega-3 ($n - 3$) fatty acids in the oil that are so important for human nutrition.

Preprocessing As-Received Seafoods

Preprocessing begins on shipboard where the seafood is, at the minimum, segregated and chilled. Many fish destined for the fresh market are butchered and washed. The minimal butchering operation consists of removing the visceral portion and often the gills. The ultimate in shipboard celerates the loss of quality in the slaughtered animal. The so-called soft belly of a fish is caused by this enzyme activity after the fish is dead. Because the amount of time that a hooked fish struggles is directly related to the subsequent spoilage rate, troll-caught fish are often of better quality than longline-caught fish that are allowed to die thrashing in the water.

The quality of gill net-caught fish is extremely variable depending on the length of time that the net is in the water. Hence, there can be a tremendous difference in the quality of fish taken from a given set, the last caught often being of higher quality. This factor is certainly realized by the wholesale buyers in that troll-caught fish consistently command a higher price than gill net-caught fish.

Certain visual or aesthetic factors also affect the market price of fish even though there may not be any real difference in the quality of the flesh. Marks from the web of a gill net are often caused when the fish struggles back and forth to release its entrapped gills. Unless the web causes cuts that allow bacteria to enter the flesh, these marks normally do not adversely affect the biological quality of the flesh. Hence, the word *quality* has different definitions, depending on whether it refers to the biological state of the edible portion or the visual appearance of the fish.

There are several types of fishing gear that cause abrasions and punctures during the catching and subsequent handling. Trawling, dredging, spearing or harpooning, and gillnetting all cause different degrees of damage. Trawling, which accounts for ca 40% of the world's fish catch, exerts extreme pressure on the fish as the cod end becomes full. In addition to scale abrasion, ruptures in the skin and internal portions release gut bacteria and decrease shelf life of the subsequent product.

The best quality is maintained in fish that are caught by trapping. As long as the trapping device is emptied on a reasonable cycle, the fish remains alive and is quickly killed prior to sale or processing. Some species, such as crabs, must be kept alive prior to butchering or there will be a blue color in the meat. This is due to the blood chemistry of crabs; they have a copper complex instead of the heme, or iron, complex found in most animals. If the crab is not butchered live and the blood removed prior to processing, the copper will oxidize, giving a blue color to the meat. Although the aesthetic value of the crab is impaired, the eating quality is not affected. However, as in the case of abraided fish skin, the consumer is not willing to consider blue crabmeat as anything but a low-grade, poor-quality product.

Aquaculture is somewhat akin to catching wild fish in trapping devices. The fish or shellfish are raised in an enclosed area and then removed when ready for market. As in the case of trap-caught fish, farmed fish are live when harvested.

Shipboard Handling of the Catch

The proper handling of fish during harvesting and on shipboard can minimize the adverse effects of gear. Of utmost importance when fish are first landed is that they are segregated and placed in a sanitary chilled environment. Minimizing the bacterial and enzymatic activity by fast reduction of temperature in freshly landed finfish and shellfish is probably the most important step in the entire chain of events that takes a fish from the water to the table.

Figure 12 shows the extreme variation in storage life of fresh and frozen commercial fish prepared for the market. This curve has been compiled from many published sources that give the shelf life of fish as related to the handling methods (6). It has been shown that landed high-quality fish that is chilled rapidly and carefully handled, processed, and packaged can be acceptable for up to three weeks after being caught. On the other end of the scale, a fish that has undergone poor handling on shipboard (eg, 70% of as caught quality) and subsequent marginal handling during the processing and marketing stages is inedible after four days.

Fish that has been properly handled, processed, packaged, and frozen can be held up to one year without significant deterioration. However, there are many factors that must be considered in discussing shelf life of a fresh or frozen product. The species, the oil content, the catching technique, and the state of the fish when harvested are all uncontrollable factors that have a major bearing on the shelf life of a seafood product.

PROCESSING SEAFOOD

Inspecting As-Received Seafoods

Seafoods received in the processing area of a vessel or in a shore-based plant vary tremendously in the state and form. This is the situation when batches of product from different sources or catching vessels are mixed in the received lot. A sensory inspection must be made to insure that the raw material passes the criteria specified by the buyer or processor.

A sensory evaluation utilizes touch, odor, and sight to determine the acceptability of a given lot of seafood (7). An on-site inspection should concentrate on microbial contamination, enzymatic degradation, and other chemical and physical factors that reduce the marketability of the seafood. A faint fresh, nonfishy odor; firm and elastic flesh; bright and full translucent eyes; bright pink gills; and bright and moist skin surface with no heavy deposits of mucus or slime are all properties of a good fresh fish.

In addition to microbial and enzymatic degradation that can take place in improperly handled seafood, the oil in fatty fish that have not been chilled rapidly or adequately protected from the environment are subject to oxidative rancidity. This is both an aesthetic and nutritional problem. Oxidation from the air (and sometimes autooxidation within the seafood) not only affects the odor and taste acceptability of the product but also destroys the omega-3 ($n - 3$) fatty acids in the oil that are so important for human nutrition.

Preprocessing As-Received Seafoods

Preprocessing begins on shipboard where the seafood is, at the minimum, segregated and chilled. Many fish destined for the fresh market are butchered and washed. The minimal butchering operation consists of removing the visceral portion and often the gills. The ultimate in shipboard

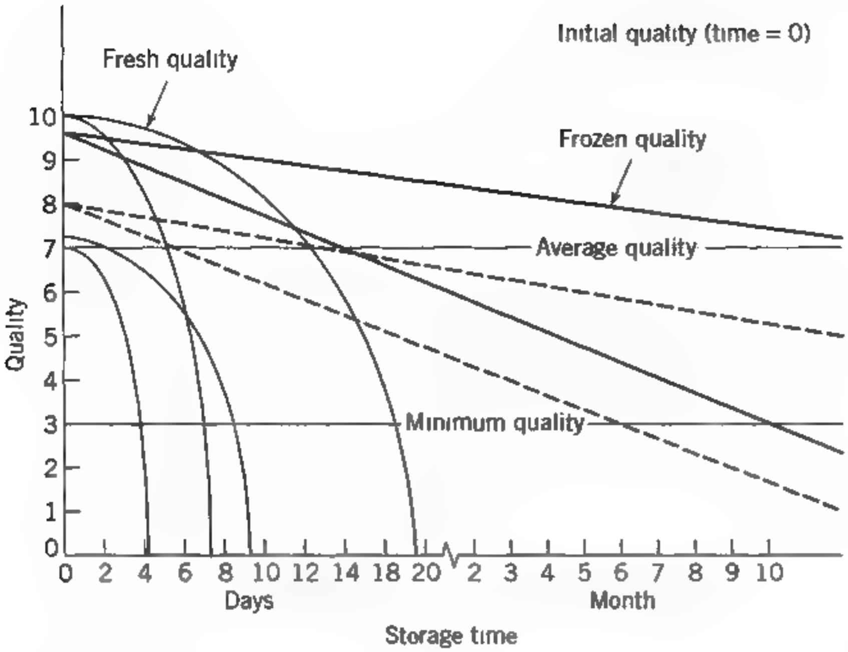


Figure 12. Shelf life of fishery products. *Source:* Ref. 12.

butchering is heading and gutting, which consists of removing the viscera, head, and often the tail and fins. Shrimp are normally iced but sometimes the head is removed on shipboard. Crab are either delivered live directly to the shore plant or are kept alive in seawater tanks until delivery. In all shipboard preprocessing operations the most important procedure is to lower the temperature of the catch as soon as possible after it is taken from the water.

Preprocessing operations not carried out on shipboard are completed in the processing plant. After a second visual inspection, fish are butchered. This primarily consists of eviscerating but also can include scaling, trimming, and further cleaning when necessary for a specific processing operation.

Finfish are portioned for processing or direct marketing by filleting, steaking, or dressing a whole fish or section for roasting or broiling. Depending on the size and sophistication of the processing plant, these operations are carried out either by hand or machine. Crustacea (eg, crayfish, lobster, and crab) and mollusks (eg, clams, oysters, and mussels) are handled and processed quite differently from finfish.

Shrimp are iced on shipboard and unloaded by basket and conveyor into the plant receiving area. In the plant they are segregated and graded as to size by machine, passed over a visual inspection table for removing substandard specimens, cooked, and then headed and peeled (normally by machine). Large prawns are sometimes headed and handled individually on shipboard or in the plant. Those destined for market in an unpealed condition are then frozen. Prawns with head off and not cooked are called headed green prawns in the trade. Shrimp, regardless of whether they are marketed cooked and peeled, green fresh or frozen, or in other forms are sold by the count to designate size. For example 21–25 count shrimp means that there are 21–25 shrimp per lb.

Crab is cooked prior to processing or marketing. It is important that the crab are alive when butchered just before being cooked whole. This is due to the high copper content in the crab blood, which oxidizes to a blue color if allowed to remain in the meat of a dead crab. Crab, depending on the species, are sold in the shell whole (eg, blue and dungeness), segregated with shell on into legs and body portions (eg, king and snow), or as leg and body meat. Meat is removed or shaken from the cracked shell portions by hand.

Bivalves must be alive when purchased and subsequently cooked in a plant, restaurant, or home. A healthy live oyster, clam, or mussel will have a tightly closed shell. Any gapers, eg, those with an open shell, must be discarded. This requirement for handling only live molluscs is important because they normally are not iced after being harvested by digging, picking, dredging, or tonging, but are delivered directly to the receiving station or plant.

Problems involving toxins (eg, paralytic shellfish poisoning) or communicable diseases being transmitted to the consumer by mollusks have become increasingly more prevalent as the coastal waters become more polluted (8). These animals are static so they are particularly vulnerable to any fluctuating environmental pollution problem that may exist. Furthermore, the conditions causing the meat to be inedible cannot be detected by simple in-plant inspection. This has resulted in an intricate system involving surveillance of mollusk-growing areas by federal and local government agencies who are responsible for closing harvest areas when there is a potential problem.

Total Utilization

There is a growing emphasis on improving the total utilization of seafood raw material (8,9). For many years, only the most desirable portion of the fish, often accounting for 20 or 30% (eg, fillets) of the fish, was used. The remainder was considered waste or a raw material for preparing cheap animal feeds. Environmental and economic considerations dictate that this gross misuse of base raw material must be stopped. Hence, the modern attitude is that there is no such thing as fish waste. The portions remaining after the initial edible portion is removed should be considered secondary raw materials that can often equal or exceed the amount of the primary edible portion. The initial preprocessing operation must consider the ultimate total utilization destination of all portions of the raw material. Some of the products that can be prepared or manufactured from secondary raw materials are outlined in Table 7.

The developing operations and markets that use minced flesh for human consumption are beginning to have an impact on the economics of operating and the market for seafood products. Minced fish is used in engineered and formulated foods, the fastest growing segment of the food industry. Sources of minced flesh include

1. Frames (remaining skeleton) from a fillet operation.
2. Industrial fish presently being used for meal and oil.
3. Small fish currently being discarded or converted into fish meal that cannot be economically filleted or otherwise processed.
4. Freshwater fish presently being underutilized.
5. Low-fat fish that do not have good keeping properties due to rapid enzyme action (eg, pollock).

Table 7. Total Utilization of Secondary Raw Materials from the Sea

	Product	
Raw material	Edible	Industrial
Finfish[a]	Steaks Roasts Fillets Minced flesh, surimi, formed foods, and extracted protein Specialty foods Refined oil Roe and milt Food additives	Meal, oil, pet foods, pharmaceutical raw materials, and leather (skins)
Industrial fish[a]	Minced flesh, surimi, formed foods, and extracted protein Specialty foods Refined oil Roe and milt Food additives	Meal, oil, pet foods, pharmaceutical raw materials, and leather (skins)
Shellfish	Whole (as caught) Portions Whole meat Minced meat Specialty products Food additives	Crustacea shell, extracted protein, chitin products, and calcium salts Bivalve shell land fill Meal and oil
Seaweed	Food Food additives	Pharmaceutical raw materials Extracted products for industry

Source. Ref. 8.

[a] Process residues are an important source of recyled high-protein ingredients for on-site preparation of aquaculture-raised fish and shellfish feed.

Minced flesh can be used in many products that require a protein base or binder. Such items include sausage (much lower saturated fat and calories than those made from pork), wieners, other cased meats, extruded meat products, nutrition-controlled foods (eg, low fat, high protein, and low sugar or carbohydrate), and other foods requiring ingredients with highly functional properties.

In the past, fish oils have been by-products from the production of fish meal. This low-grade oil has been used for industrial purposes or for making margarine. The large amount made into margarine has been manufactured in foreign countries because fish oil is not allowed for this purpose in the United States. The recent interest in fish oils for health has created a challenge for researchers to develop satisfactory refining techniques and subsequent edible products from the refined oils.

Some 30 yr ago it was recognized that fish oil has beneficial fatty acids that are active in preventing or minimizing the effects of certain cardiac problems and other diseases. However, it was not until the early 1980s that highly publicized work demonstrated that diets of Greenland Eskimos, high in marine fish and mammals, were associated with greatly reduced numbers of deaths due to ischemic heart disease when compared to populations in more developed countries consuming low fish and high animal fat diets (10). Fish oil, through the consumption of more fish and formulated foods containing fish oil are now considered a valuable contribution to a more healthful diet (11–13).

Heat Processing

Methods of Heating. Seafood, like any other food is cooked or heated to make it taste good by changing the texture and bringing out flavors and odors. If sufficient heat is added to pasteurize the food, microorganisms that can cause public health diseases and illnesses are destroyed or inactivated. Enzymes are also inactivated by heat so that protected cooked food stored under refrigeration is subjected to a minimum of degradation by hydrolysis.

The ultimate in heat processing is sterilization whereby the product is heated for a sufficient time at a given temperature. If the product is hermetically sealed so that there is no postprocessing contamination, it will have an indefinite shelf life free from degradation by microorganisms and enzymes.

Seafood is cooked and pasteurized by standard radiant energy baking ovens, infrared heating ovens, and microwave ovens. The heating in any oven is due to a combination of conduction, convection, and radiation. Food in a standard oven receives conduction heating from the pan or oven rack, convection heating from the air that is heated by the walls of the oven, and radiant heating from the exposed heating units. Infrared ovens do not have open heating elements because there is little convection heating from the air being heated by conduction.

Microwave heating is a specialized form of dielectric radiant heating that has certain advantages over other dielectric methods. Because the molecular polarizations are reversed many millions of times per second, the friction of molecules contacting each other cause heat and subsequent rapid, uniform heating of a food. Microwave heating has risen rapidly over the past decade. There are now microwave ovens in about 80% of U.S. households. Restaurants and institutional kitchens are rapidly increasing the use of this fast way of heating and cooking foods. The microwave oven with additional convection heating is becoming popular in that it combines the advantages of fast, uniform heating with the advantages of a conventional convection oven.

The growing popularity of microwave ovens has greatly increased the demand for microwavable foods. The major challenge to the food scientist developing microwavable foods is lack of radiant heat that causes the surface of a food to brown. Hence, cooked nondeep-fried potatoes, chicken, white fish, etc remain white on the surface and do not take on the normally expected desirable browning on the surface. Microwavable batter and breadings have been developed that are the color of deep-fried products. Hence, the future of microwaved foods will indeed be insured if low-fat, consumer-acceptable microwavable batter and breaded foods can be developed that take the place of deep-fried products. Fish fillets and formed patties from minced fish base stand to benefit as much from this development as any other food product. Fish sandwiches and batter and breaded fish have been growing in popularity, especially in fast-food restaurants. However, as shown in Table 8, the desirable omega-3 fatty acids suffer and the fat calories are tremendously increased when a fish is deep fried (9).

Commercial sterilization is carried out in steam retorts, which can process at temperatures above that of boiling water, normally at or above 117°C (242°F). The time and temperature required for sterilization must be sufficient to kill *Clostridium botulinum* spores that, when viable, can grow in an anaerobic (nonoxygen) atmosphere and produce lethal toxins. These spores must be held for 32 min at 110°C (230°F) to insure total destruction. Many low pH (high acidity) products such as certain fruits, vinegar-packed foods, and highly acid formulated foods prevent *C. botulinum* spores from growing. Many of these foods do not have to be sterilized at the temperatures required for high pH (low acidity). However, near neutral or high pH vacuum-packed products, such as seafood, are particularly vulnerable to anaerobic spore growth and sterilization must be insured.

Table 8. Effect of Processing on Omega-3 $(n - 3)$ Fatty Acids^a

Species	Product	Lipid, %	Fatty acid in oil EPA, %	 DHA, %
Cod	Fresh	0.29	18.67	27.98
	Batter and breaded	0.47	12.53	12.25
	Batter and breaded, deep-fried in liquid vegetable oil	5.53	0.03	0.09
	Batter and breaded, deep-fried in solid vegetable fat	9.14	0.21	0.65
	Batter and breaded, deep-fried in beef shortening	7.07	0.66	1.51
Salmon, Sockeye	Fresh	—	6.25	9.73
	Canned	—	5.0	5.35
	Canned (added salmon oil)	—	5.76	8.61

Source: Ref. 9.

To ensure a margin of safety, the sterilization requirement for canned fish is that the geometric center of a can or pouch must be held for 32 min at 116°C (240°F) (14). Each food and each different geometric form of container requires a different total processing time to accomplish sterilization. These processing times, determined by thermal death time laboratory studies, are mandatory for each processing company that is canning hermetically sealed food. Each batch of canned food must be coded and retort processing records kept to prove that the product was sterilized.

It should be emphasized that anaerobic conditions often prevail in a canned food even though an incomplete or no vacuum is drawn on the can prior to sealing. This is a result of the subsequent oxidation of components in a food by the remaining oxygen in the air.

Another precaution that must be practiced by processors is the venting of a steam retort prior to beginning the official retorting time. This is to prevent air pockets from insulating some of the cans so that there is nonuniform temperature in the retort. Of course, hydrostatic retorts that heat containers in a column of water kept above normal atmospheric boiling temperature by hydrostatic water pressure do not have a problem with entrapped air.

Effects of Heating. Fresh or frozen seafood being cooked for a meal should be heated for the minimum time required to improve the texture and the taste for the consumer. The normally dangerous microorganisms, those known as public health disease organisms are destroyed at relatively low temperatures. As shown in Table 9, the most heat resistant of the group, thermophiles, have an optimum growth at 50–60°C (122–150°F). Hence, a seafood is normally safe to eat if the geometric center has been raised above 150°F when it is actually pasteurized.

Table 9. Optimum Growth Temperature Range for Bacterial Group

Bacterial group	Optimum temperature range
Psychotrophs	14–20°C (58–68°F)
Mesophiles	30–37°C (86–98°F)
Faculative thermophiles	38–46°C (100–115°F)
Thermophiles	50–66°C (122–150°F)

Source: Ref. 14.

Overcooking causes heat degradation of nutrients, oxidation of vitamins and oils, and leaching of water-soluble minerals and proteins. The retention of B vitamins, zinc, and iron is particularly important for populations that consume fish as the major source of meat in their diet. In addition, overcooking causes too much water to be released and the drying effect causes flesh to become tough, thus nullifying the desired effect of texture improvement.

Refrigeration and Freezing Technology

As has already been stressed, the most important factor in handling fresh fish is to lower the temperature to just above freezing as soon as it is removed from the water. Because the condition of the harvested fish and the subsequent handling determines the shelf life of a fresh fish, it is not possible to state exact times that a seafood can be held in ice or under refrigeration and be considered a high-quality food. This is shown in the wide range of shelf life that has been published in the literature (Fig. 12). In general, fish with a high oil content or enzyme activity have greatly reduced shelf life and are often of marginal quality after a few days.

High-quality seafood, when frozen properly soon after being removed from the water, is often far superior to fresh fish available on the market. This is due to the fact that all microbial action is stopped and enzyme action is significantly reduced in frozen fish. However, the initial quality of the fish being frozen, the rate of freezing, the temperature at which the frozen seafood is held, and the uniformity of the freezing temperature are all important to maintaining a high-quality product. It is surprising how many people involved in the seafood chain do not understand some of these basic factors in insuring the high quality of a seafood. Thus the constant challenge of those involved in seafood technology is the continual education and reeducation of everyone involved in the commercial seafood chain.

Freezing Seafood. During the freezing of seafood, structural changes take place in the cells and cell walls as well as in components that are between the cells. Many of these adverse changes are caused by water crystals that expand and rupture the cell walls. This allows liquid within the cells to leak out when the flesh is thawed. Hence, free liquid, called drip, exudes from seafood when it is thawed. This loss of free water reduces the water content of the seafood causing economic loss to the seller and greatly reduces the fresh qualities of the thawed product.

As seafood is cooled above and below approximately 28°F in a constant-temperature environment there is a near linear relationship between the temperature decrease and the time. However, as the water in the flesh begins to freeze, there is a long period of time during which the temperature remains almost constant. This period is a critical range for freezing and is caused by heat (heat of fusion) being removed from the fish to freeze water rather than to lower the temperature of the flesh. This relationship is shown in Figure 13.

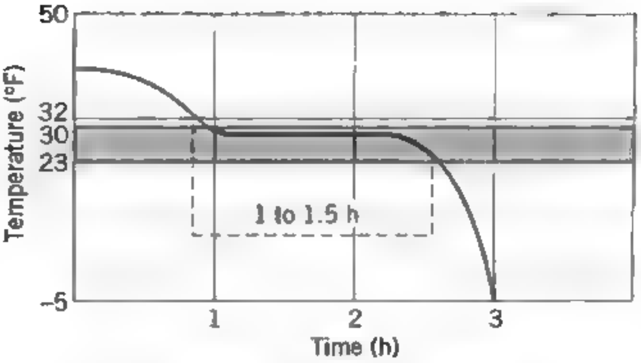


Figure 13. Idealized freezing curve for fish muscle.

The longer a product remains in the critical zone, the larger the ice crystals formed in the cells will be. When water is frozen rapidly, small crystals form and do not have time to increase in size due to the nucleation characteristics of a water molecule. Experience has shown that if seafood passes through the critical zone in ca 1.5 h, there will be little damage to cell structure as a result of large crystals formed during freezing. This is demonstrated by microphotographs of cells frozen under different conditions. Figure 14 showns cells (magnified 640 ×) of a rainbow trout that was rapidly frozen and passed through the critical zone in less than 1 h (15). Note the well-defined intact cell structure is similar to that of high-quality fresh flesh. Figure 15 is a similar microphotograph of cells from the other half of the same rainbow trout, which took approximately 5 h to pass through the critical zone. In this case extreme damage to the cell structure caused by large ice crystals can be noted. This is a dramatic demonstration of why fast freezing of seafood is essential to maintaining the quality of frozen products.

There is wide variation in commercial freezing facilities available to the seafood industry, and it is most important that commercial operators carefully study their options when purchasing and installing new equipment. The most efficient methods of freezing involve both conduction and convection freezing in an immersion system. Liquid refrigerants such as the freons have been used for this purpose

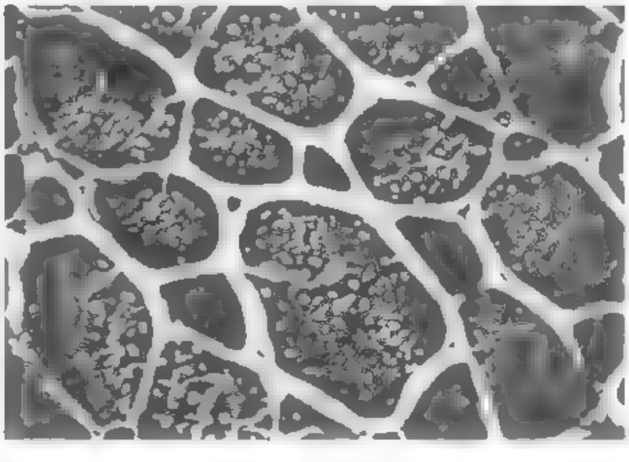


Figure 14. Cell structure of rainbow trout frozen rapidly (× 640). *Source:* Ref. 15.

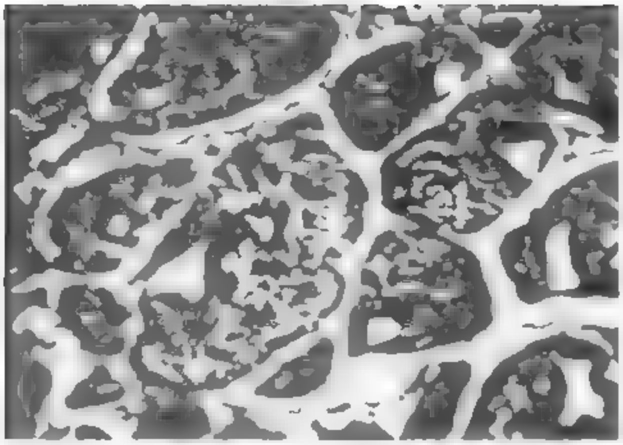


Figure 15. Cell structure of rainbow trout frozen slowly (×640). *Source:* Ref. 15.

but have proven too costly. Immersion freezing using refrigerated brine is fast and efficient but a considerable amount of salt is absorbed into the flesh during freezing and can be a problem to product marketing. Refrigerated seawater is used extensively, especially in Alaska for rapidly chilling and holding the fresh catch on shipboard.

Plate freezers rapidly freeze, by conduction, products that are in contact with the plates. Plate freezers are used to their best advantage when the plates can be brought together to contact both sides of a package. This is the case for rectangular packaged seafood portions or prepared formulations. A disadvantage is that irregular items, particularly large fish, can only contact the plate on one side and the exposed side must be frozen by convection. Another disadvantage is using plate freezers for whole fish, because the plate only contacts one side of the large irregular shaped item and causes one side of the finished product to be flat while the other side is the nice rounded shape of the live fish. However, unless the fish are suspended by hooks, this same condition prevails in any freezer where the fish are placed on shelves during freezing.

Convection blast freezing, where cold air is circulated over the products, is less efficient than conduction freezing but can be used for larger volumes. A disadvantage to blast freezing is that the rapidly moving dry air can dehydrate a product that is exposed for any length of time. This can be eliminated by proper packaging protection, but it also greatly decreases the heat transfer rate and lengthens the time of freezing and the subsequent time at which the product is in the critical range.

The problems with blast freezing can often be minimized or eliminated when a combined blast-plate-freezing system is used. This can be demonstrated by comparing Figures 16 and 17 in which chum salmon weighing 4 lb were frozen in two commercial units (16). Figure 16 is the freezing curve for a conventional blast freezer in which whole (headed and gutted) fish were placed on racks and frozen. Note that the time in the critical range was about 4 h and the total time to reduce the temperature to 0°F was about 8 h. The freezing curve shown in Figure 17 was that of a 4-lb chum salmon in a combined blast and plate freezer. The time in the critical range for this system was about 40 min, and the total freezing time to reach 0°F was slightly over 1.5 h. The fish frozen in the combined blast-plate freezer were far superior to those frozen in the blast freezer alone. Furthermore, the reduction in weight due to drip loss gave a significant economic advantage to the more rapidly frozen fish.

Holding Seafood in Cold Storage. Good freezing practices involving fast freezing and minimal dehydration of fresh seafood insures that high-quality products are delivered to the cold storage for holding. Equally important to the continuing maintenance of high quality is the environment under which the products are kept during the cold storage period. Unless frozen seafood is kept at extremely low temperatures, there is a certain amount of free water (not frozen) remaining in the product. This is a result of the antifreeze effect of soluble salts in the cell, which become more concentrated as water freezes. Depending on the specific food, the physical and chemical conditions of the food, and the composition (including water content), the point at which all of the water is frozen is in the range of −45°F. Because few, if any commercial cold storages are held at that low temperature, there is a certain amount of free water remaining in all frozen food (9). This is depicted in Figure 18, indicating that the normal commercial cold storage temperatures are well above −45°F. In fact, different commercial cold storage warehouses in the United States range in temperature from slightly above 0°F to a low that seldom is below −20°F.

Not only is the average commercial cold storage facility maintained at a temperature at which several percent of the water remain unfrozen but there is normally a significant fluctuation in the base cold storage temperature. Thus when the temperature fluctuates above and below the average, some of the water in the product is continually frozen, thawed, and refrozen. The effect of this fluctuation is to greatly increase the effect of enzyme action that essentially digests the protein in the same manner that it is digested in the gut of an animal. This is emphasized by the fact that continual thawing and refreezing is not limited to the original free water and causes the enzyme action to spread throughout the flesh. This is why the meat in seafood held for long periods can be extremely soft when thawed. This effect is even more noticeable in many home freezer units where temperature fluctuations are greater than those found in commercial facilities. Hence, holding a product at a higher but constant cold storage temperature can result in a better product than when it is held at a fluctuating lower temperature. This concept is depicted in Figure 19.

It can be important to know how much fish a given size cold storage unit will hold. The true density of a seafood ranges from 70 to 80 lb/ft^2. Because frozen fish blocks are essentially composed of solid fish flesh, they have about the same density as the natural flesh. At the other end of the spectrum, individually frozen and loosely packed fish range from 30 to 50 lb/ft^2. A well-run cold storage, allowing for the average distribution of product forms and allowing for air spaces and movement within the room, usually holds about 20–30 lb/ft^2.

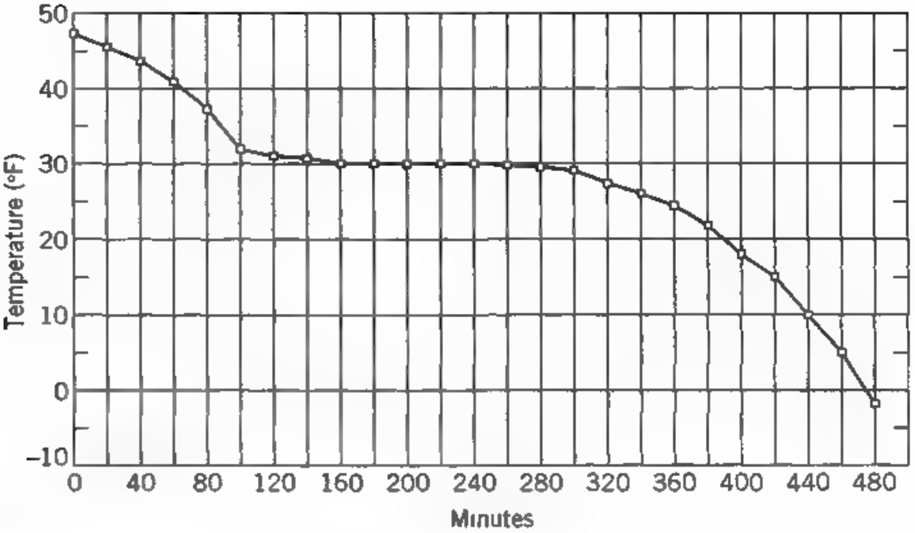


Figure 16. Freezing curve for blast freezing of chum salmon (4 lb).

Refreezing Seafood. Often it is necessary for a processing plant to receive frozen fish (either whole, butchered, or partially processed portions) for subsequent final processing or reprocessing. This occurs when fish are frozen at sea or other remote areas where total processing is not practical due to limited facilities or economic considerations. A typical example is when fish blocks are shipped to a plant for thawing, trimming, battering and breading, and refreezing. A similar situation occurs when frozen fish or preprocessed raw materials are held in cold storage until they are processed in response to a specific market demand. For example, if fresh salmon are cut into steaks and frozen, there is no opportunity to sell the fish in the fillet form if the demand or price of fillets takes a sudden increase.

It is often said that seafood cannot be thawed and refrozen. This is based on the too-often encountered situation whereby the fresh fish has been abused, the products have not been frozen rapidly, or have been held at high or fluctuating cold storage conditions. However, if the fish have been properly frozen and held in cold storage as described above, there will be little cell damage and the thawed product will have minimum water loss and reduction in quality from that of the original fresh raw material. Thus it can be thawed, reprocessed, and refrozen without significantly altering the quality of the finished product from that of the fresh fish.

The increase of high-seas processing vessels encouraged by the 200-mi limit (Fishery Conservation Zone) has greatly accelerated the final processing of frozen products prepared at sea. The future will probably bring an even greater acceleration of this trend as such products as batter and breaded (nondeep-fried) portions, whole fish, and products engineered and formulated from minced flesh or chunk portions are being developed for microwave cooking. These are inexpensive low-fat products with good sensory properties especially acceptable to the increasingly large number of nutrition-conscious consumers.

Commercial Refrigeration Systems

There is a distinct difference between selecting facilities for freezing or for cold storage for a commercial seafood operation.

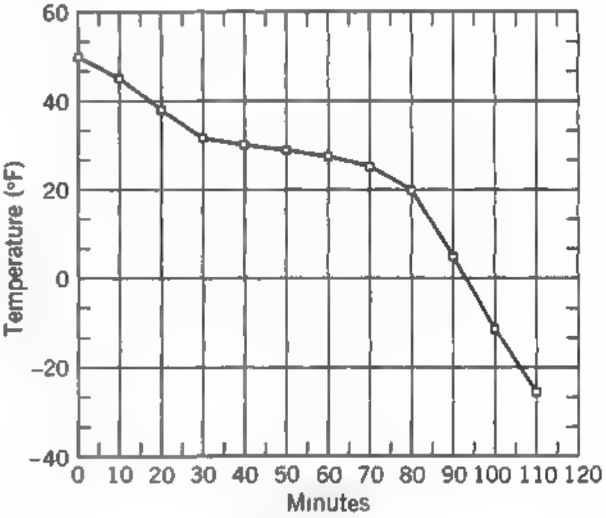


Figure 17. Freezing curve for blast-plate freezing of chum salmon (4 lb).

Freezing Facilities. The freezing facility is related to the products that will be processed, the throughput of product, and the space availability in the processing area. The differences between various freezing techniques lie in the control of the type of heat transfer between the product and the refrigerant. The types of freezer as related to the products being frozen are as follows.

Natural Convention Freezing. This facility is a room or chamber in which the product is frozen by natural convection. There are minimum problems with dehydration but the freezing rate is slow. Only products not affected by slow freezing should be frozen in this type of freezer. These would include formulated products or very small items where the water loss or cell damage through slow freezing is not a problem.

Combined Conduction and Natural Convection. The addition of freezer plates on which the product is placed

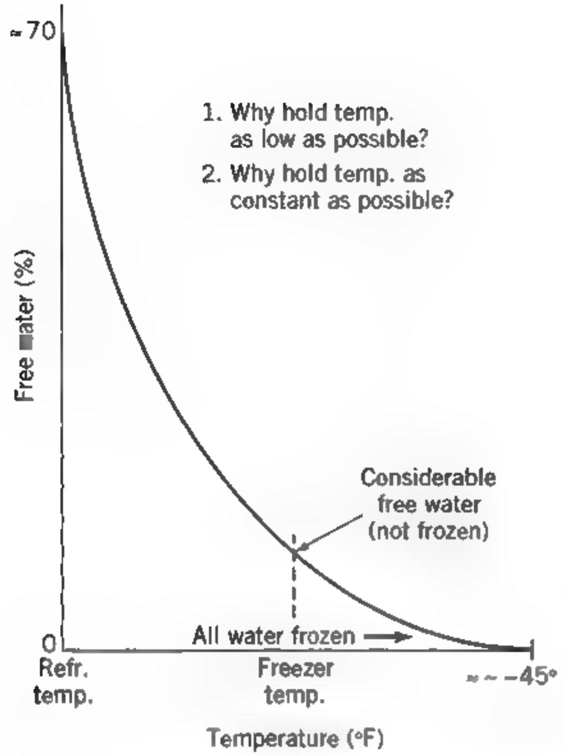


Figure 18. Remaining free water in frozen food held at different temperatures.

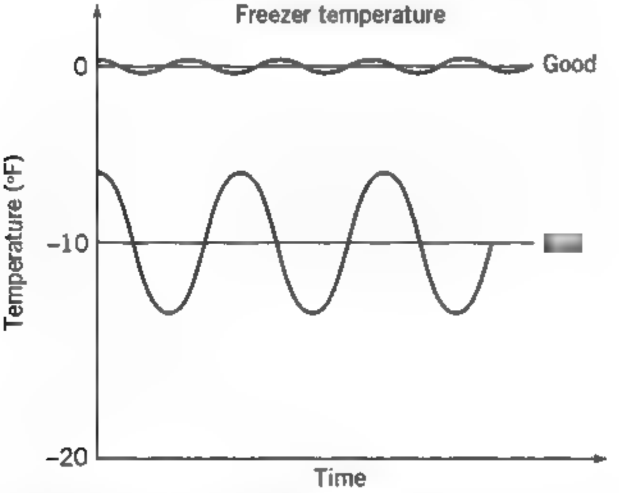


Figure 19. Time-temperature relationship of food during cold storage holding.

greatly accelerates the freezing rate and maintains the advantage of minimum hydration during freezing.

Blast Convection Freezing. Blast freezing is a popular method of freezing irregularly shaped seafood items such as whole or partially dressed fish. A major disadvantage of this facility is that considerable loss of water through hydration can cause an unsightly surface condition known as freezer burn. This hydration, if allowed to continue, can remove a considerable amount of water from the flesh, making the fish inedible. Such is the case when a product is frozen and then allowed to remain (actually to be stored) in the freezer for some length of time before being removed.

When unpackaged products are destined for storage in a cold room with fast moving air, they are glazed to protect against dehydration. This consists of immersing the frozen product in cold water and then allowing the water film to freeze on the surface. The layer of ice protects the fish, because the air removes water from the glaze rather than from the product.

Combined Conduction and Blast Convection Freezing. This facility normally consists of refrigerated plates in a forced-air cold room. This freezer has the advantage of fast conduction freezing assisted by convection air on portions of the seafood that are not in contact with the plate surface. The tremendous increase in freezing rate by this combination of freezing techniques as compared to that of a blast freezer were previously discussed and compared in Figures 16 and 17. Normally the freezing rate is high enough to eliminate the problem of surface dehydration.

Conduction Freezing. When small rectangular products or packages with two parallel surfaces are placed between two refrigerated plates in a cold room or chamber, fast freezing takes place with minimum harm to the products. These types of facility have mechanisms that allow the plates to be vertically adjusted. This allows the plates to open, or separate, during loading and then they come in contact with the product for freezing.

Immersion and Cryogenic Freezing. Immersion in a cold liquid that will not affect the safety of the product (eg, brine, liquid nitrogen, or freon) is the fastest method of freezing. Freezing takes place by both convection from the circulating fluid and conduction from being in direct contact with the liquid. Immersion freezing in extremely cold refrigerants such as nitrogen or freon is called cryogenic freezing. Brine freezing utilizes saturated salt solutions that freeze rapidly due to the combined convection and conduction but takes place at about 0°F.

Cryogenic freezing has two limiting factors for extensive use in freezing seafood products. The first is that the freezing is so rapid that it causes extreme internal tension due to the freezing of the fiberous materials at a differential rate. This causes the flesh to rupture. It can be minimized by allowing the product to temper at room temperature before further handling, packaging, and placing in cold storage. The splitting problem can be fairly well overcome for small items such as fillets and steaks but large fish cannot be satisfactorily frozen by this means. Brine, on the other hand does not have the splitting problem and is a major means of freezing whole tuna fish on high-seas catcher vessels.

The second problem with cryogenic freezing is that it is uneconomical to operate unless the freezer is used continuously, because a considerable amount of heat from the cryogen is lost each time a processing unit is shut down and restarted. Most seafood-processing plants do not operate on an extended basis so that the cost per pound for intermittent freezing limits cryogenic freezing to a few fish processing plants.

Modern cryogenic freezing no longer uses liquid immersion true freezing. It is more economical to spray the liquid refrigerant on the food as it moves along on a conveyor belt. This uses less refrigerant and still gives the same advantages as immersion. Furthermore, when extremely cold liquids are used for freezing, especially in immersion freezing, vapor bubbles are formed that insulate the liquid from the product and prevent the rapid heat transfer expected from such a large temperature difference between the product and the liquid.

Cold Storage Facilities. Cold storage facilities must meet the requirements for long-term storage of commercial seafood products ranging from large whole glazed fish to cases of packaged retail products. Of particular importance in purchasing or contracting for such facilities in a plant or choosing a public cold storage for use include the following:

1. A design with proper insulation and construction materials that will insure a minimum of heat loss through the walls, ceiling, and floor.
2. Properly designed protection to minimize heat loss through doors and other openings while the product is being taken into or out of the cold storage room.
3. Refrigeration machinery that will have sufficient capacity to hold the cold storage at the desired temperature.
4. Sufficient refrigeration capacity to insure that the temperature in the cold storage rooms does not fluctuate to the extent of adversely affecting the products being stored. This is probably the major problem encountered with commercial cold storage facilities. So often a low bid is awarded to a contractor who is the lowest due to cutting back on the amount of refrigeration machinery and thus the ability to maintain constant storage temperature during variable and seasonal outside weather conditions.

Cured and Dried Seafood Products

Control of water activity, defined as the ratio of the vapor pressure of water in a product at any given temperature divided by the vapor pressure of pure water at the same temperature, not only applies to smoking and drying but also to salting, pickling, and product formulation. Product stability, the growth of microorganisms, and chemical reactions occurring during processing and storage are all dependent on the water activity, that is, the ability of water to move and interact with other ingredients in the food. The importance of water activity (a_w) in foods is shown in Table 10. The many facets of water activity and its relation to the preservation, safety, and shelf life of foods has been summarized by a group of internationally recognized experts (17).

Dehydration. Drying or dehydration is a means of controlling water activity by reducing the water content of a product. Many dried products, such as cereal grains, legumes, and many nuts and fruits, are dried by nature in the field prior to harvesting. In the past humans dried seafood products in the sun, long before they were aware that they were controlling water activity. In fact, today many developing countries located in tropical parts of the world use the sun as a major means of drying seafood and other food products.

The principal cured fishery products produced in the United States are shown in Table 11 (2). It should be noted that these statistics do not differentiate between smoked, salted, dried, and pickled products due to the fact that all of the processes are based on controlling or reducing water content. It is often difficult to distinguish between process classifications. For example, salted and salted-dried fish have a different final moisture content but the mechanism of removing the water and stabilizing the product are the same, control of water activity

The most efficient means of drying a seafood product is through dehydration by forced-air drying, vacuum drying, or vacuum freeze-drying. In each case the drying mechanism is a combination of adding heat to increase the temperature and vapor-driving forces between the product and the environment. The drying time is divided into two distinct periods: constant drying rate and falling drying rate. During the constant rate period, all of the heat added to the product is used to evaporate water from the surface and near surface of the product. In this case, there is free water in contact with the environment and drying occurs similarly to that of an open container of water. During the falling rate period, part of the heat energy is imparted to the product to cause water to migrate to the surface. Therefore, the product is heated during this period of the drying cycle.

Excellent highly nutritious dried formulated fish-base products can be prepared from the minced flesh of seafood. Shaped into forms such as patties and air dried, these items have a long and stable shelf life (18).

Curing. Whereas dehydration removes sufficient water to inhibit growth of microorganisms, curing consists of adding sufficient chemicals (eg, sodium chloride, sugars, and acetic acid) to prevent degradation of a product by microorganisms. Although sufficient water is not removed to accomplish this objective, the water activity is reduced to the point where growth is prevented.

Curing methods currently practiced include dry salting, where split fish is covered with salt and the brine liquor is allowed to escape, and pickling where products are immersed in a strong brine, or pickle, allowing salt to penetrate the product and water to be exuded into the brine solution. Low-fat white fish such as cod are dry salted by the heavy (hard) cure and fatty fish are cured in airtight barrels by the Gaspe (light) cure. Figure 20 shows the process for the hard cure; the last step is air drying, which results in a salted-dried product with long-term, room temperature shelf life and a water activity of between 0.75 and 0.85. The Gaspe or light-cured product remains edible only a few days in the wet-stack stage ($a_w = 0.85$–0.90) at room temperatures and must be pressed and mechanically dried for longer-term storage.

Smoking. The age-old practice of smoking has changed drastically over the last few decades. The process as originally practiced by Eskimos and American Indians to pre-

Table 10. Importance of Water Activity in Foods

a_w	Phenomena	Food examples
1.0		Water-rich foods (a_w = 0.90–1.0): foods with 40% sucrose or 7% NaCl, cooked sausages, bread crumbs, and kippered fish
0.95		
0.90	General lower limit for bacterial growth	Foods with 55% sucrose or 12% NaCl, dry ham, medium-age cheese, and hard-smoked fish
0.85	Lower limit for growth of most yeast	Intermediate-moisture foods (a_w = 0.55–0.90): foods with 65% sucrose or 15% NaCl, salami, old cheese, and salt fish
0.80	Lower limit for activity of most enzymes	Flour, rice (15–17% water), fruitcake, and sweetened condensed milk
0.75	Lower limit for halophilic bacteria	Foods with 26% NaCl (satd), marzipan (15–17% water), and jams
0.70	Lower limit for growth of most xerophilic (dry loving) molds	
0.65	Maximum velocity of Maillard reactions	Rolled oats (10% water)
0.60	Lower limit for growth osmphilic or xerophilic yeasts and molds	Dried fruits (15–20% water), toffees, and caramels (8% water)
0.55	DNA becomes disordered (lower limit for life to continue)	Dried foods (a_w 0–0.55)
0.50		Noodles (12% water), spices (10% water), and fish protein concentrate (10% water)
0.40	Minimum oxidation velocity	Whole egg powder (5% water)
0.30		Crackers and crusts (3–5% water)
0.25	Maximum heat resistance of bacterial spores	
0.20		Whole mild powder (2–3% water); dried vegetables (5% water), and cornflakes (5% water)
0.00	Maximum oxidation velocity	

Source: Ref. 17

serve fish for the winter months was essentially a drying process whereby heat from a fire was used to reduce the moisture content sufficiently for extended storage. The smoke flavor was somewhat incidental to the process. In fact in some areas the fish were dried by the sun and the natural air currents and the smoke was used to prevent flies and insects from consuming and contaminating the product. This dried smoked fish, which takes days to cure, is known as hard smoked.

Today, most smoked fish is smoked for the flavor and there is relatively little loss of water in the process. The change in processing is a reaction to the consumer, who prefers a soft, moist texture rather than the tough texture of a dried product, and to the processor, who cannot afford to tie up large processing areas for longer-term smoking. Furthermore, the minimizing of moisture loss greatly improves the economics of processing and marketing smoked products. Hence, although smoked fish are considered to be processed in the same manner as fish in which water activity is altered, in reality the modern product is a partially or wholly cooked fish that has smoke added as a condiment (19).

Most of the smoked fish prepared in the United States has little shelf life stability beyond that of a fresh fish. The commercial process of smoking involves splitting and cleaning the fish, salting or brining (soaking in a brine solution) to firm the texture of the meat, draining to remove excess moisture, and smoking. Smoking is carried out as a cold smoke, the temperature of the smoke does not rise above 85°F, or as a hot smoke, the smoke is hot enough (eg, 250°F) to raise the center temperature of the fish to above 140°F. It is also common to smoke the fish with colder smoke and then to raise the smoke and air temperature during the terminal part of the smoking to pasteurize the fish.

Today, much of the smoking is carried out in commercially constructed smoking facilities, or kilns, that have smoke generators (using sawdust), controlled temperature forced air, and humidity control of the air. There are as many specific smoking procedures as there are processors in the business. Each processor has a favorite method, including the use of certain additives in the brine, controlled-temperature drying, smoking, cooking, and cooling. Modern kiln smoking has allowed precise control of the variables in smoking and has removed much of the artisan approach that was so prevalent when all smoking was carried out using open wood fires.

Specialty hard-smoked fish, known as jerky, is prepared by smoking and drying fish to a hard, chewy consistency. This product is popular in the bar and tavern trade and for hikers who wish to carry a meat product that will not spoil during a several-day outing.

Irradiation

The United States is out of step with the rest of the world when considering the use of irradiation for preserving food. During the early 1980s, the World Health Organization gave ionizing radiation its blessing after an extensive review of many years of scientific work and investigation (20). Since that time, many countries in the world have been using irradiation on a wide variety of foods (9). Al-

Table 11. U.S. Production of Principal Cured Fishery Products

Item	Thousand pounds	Thousand dollars
Salted and pickled		
Cod	2,633	2,509
Halibut	45	73
Herring		
Lake	460	185
Sea	12,190	18,102
Mullet	133	79
Sablefish	1,032	1,404
Salmon	11,386	32,024
Total	*27,819*	*54,376*
Smoked and kippered		
Carp	153	134
Chubs	2,151	3,971
Cod	210	588
Eels	184	669
Halibut	281	630
Herring		
Lake	38	52
Sea	963	1,876
King mackerel	13	52
Lake trout	84	167
Marlin	9	43
Mullet	137	212
Paddlefish	257	469
Pollock, Pacific	900	875
Sablefish	1,880	4,157
Salmon	13,071	63,532
Sturgeon	377	1,338
Trout, unclassified	242	406
Tuna	48	108
Tunalike fish, bonita, yellowtail	4	3
Whitefish	2,910	5,526
Whiting	1,028	485
Unclassified fish and crustaceans	293	746
Total	*25,233*	*86,039*
Dried		
Cod	579	971
Shrimp	368	2,064
Total	947	3,035

Source: Ref. 2.

though there has been steady progress in the United States toward approval of the process, there is still a widespread disagreement between scientific, government, and consumer groups regarding the pros and cons of using this form of energy for processing foods (21).

Two factors probably have equally contributed to this battle, namely the association in the minds of people between ionizing radiation and nuclear warfare, and the DeLaney Clause of the 1958 Food Additive Amendment to the Food, Drug and Cosmetic Law. The amendment, best known for the prohibition of a food additive that is shown to be carcinogenic in a test animal at any dose level, states that irradiation is a food additive and not a food-processing technique. It is interesting to note that the U.S. Department of Agriculture has issued an extensive list of publications that show the safety and wholesomeness of irradiated foods (22).

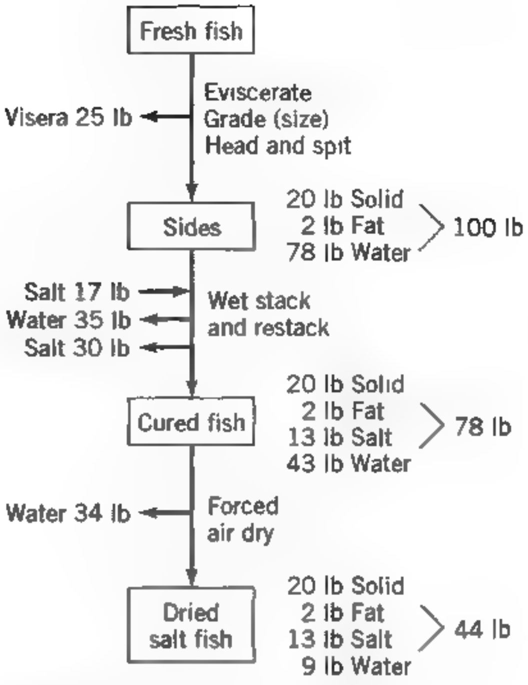


Figure 20. Process for hard curing and drying of fish.

Some confusion exists about the units used for measuring radiation doses that a product receives. This is because the original unit of measurement, the rad, was changed to kilograys (kGy); 100,000 rad (100 krad or 1 Mrad) is equivalent to 1 kGy (100,000 Gy). As shown in Table 12, the doses for processing range from 0.75–2.5 kGy for pasteurization to 30–40 kGy for sterilization. Note that specific process terminology has been suggested for the various operations. Pasteurization is radurization, sanitization is radicidation, deinfestation is the process for destroying eggs and larvae, and sterilization is radapertization. Two radioisotopes, Co-60 and Ces-137, and electron-accelerator-generated electron beams meet the requirements for producing sufficient energy and intensity to penetrate food and accomplish the four basic processes.

The radurization of fresh seafood has an excellent potential for extending shelf life. Being in the low-dose range, this process should not meet the resistance that is found for the publicized high doses that accomplish sterilization, but result in some off-flavor (23). The extra week of shelf

Table 12. Dose Ranges for Food Irradiation Processes

Process	Dose range	
Radurization (pasteurization)	75–250 krad	0.75–2.5 kGy
Radicidation (sanitization)	250–1,000 krad	2.5–10 kGy
Destroy eggs/larvae (deinfestation)	below 100 krad	below 1 kGy
Radappertization (sterilization)	3–4 Mrad	30–40 kGy

Source: Ref. 23.

life that can be given to a fresh fish by radurization would be of economic benefit to the entire industry. The extra shelf life would eliminate product loss at the retail level and would extend the market range for delivering fresh fish.

There is every reason to believe that irradiation will eventually become an accepted and extensively used processing technique for seafood, especially seafood for the fresh market, where all parties will benefit. Perhaps during the next decade the radurization of seafoods will be commonplace. After all, 20 yr ago there was the same consternation over the use of microwave ovens, and today they are in 80% of U.S. homes.

Packaging

Proper packaging of products is necessary to protect food from adverse losses and changes in weight, texture, flavor, nutritional components, and protection against contamination and physical damage. With the growing demand for high-quality seafood, the major emphasis is on packaging for fresh and frozen products. Basic types of packaging, in order of increasing ability to protect fresh or frozen food, include paper, coated paper, fiber, foil, films, laminates, and combinations.

One of the primary considerations in selecting packaging for fresh and frozen foods is the gas and water permeability of the material, usually plastic films, plastic containers, and treated paper products. There is always a balance between the cost of the package and the degree of protection given to the seafood. A very low cost uncoated cellophane has a moisture permeability that is so high that frozen and even cold fresh products are rapidly dessicated during holding. A film such as poly(vinyl chloride) (PVC) costs more but gives moisture-barrier protection that improves the overall economics of the processing and marketing chain.

The same economic analysis must be made for gas permeability, because oxygen entering the package tends to oxidize lipids and give off-flavors while some of the desirable volatile taste and odor components may be lost from the product. The fact that some films give good vapor-barrier protection but allow moisture to pass while others act in a reverse manner has encouraged the development of laminated films for food packaging. Laminates for seafood packaging often consist of polyethylene (which is cheap, is heat sealable, is usable over a wide range of temperatures, and is a good moisture barrier) and another film that has a low permeability for gas (eg, CO_2, O_2, and N_2). Examples of laminates used in frozen seafood products include polyethylene laminated with low-gas-permeability poly(vinylidene chloride) (PVDC) or saran), polyester, cellulose, aluminum, or nylon.

Packaging not only protects the food but is also an important factor in the sales appeal of the product. Packaging that shrinks film to give better evacuation of air from the package, overwraps for multiple packages and trays, overwraps to give rigidity to a package, allows see-through to the product, uses vacuum to minimize oxidation of the product, and uses gas flushing to expel further air are all popular methods of protecting processed seafoods. Packaging materials have become quite sophisticated and the accompanying use technology has been developed to meet the requirements for economic applications, long-term storage of products (particularly frozen), and enhanced consumer appeal.

Over the last decade, the market for fresh food products has become increasingly popular. A system combining closely controlled combinations of low temperature, high humidity, and proper ventilation has allowed extensive increase of the shelf life of perishable fresh foods. This system, hypobarics, was awarded the Institute for Food Technology's Technology Industrial Achievement Award in 1979 (24). Although hypobarics is a container system rather than a package, it can be considered a package in that it is used to contain a fresh product during the plant storage and transport time required to move the product to market. Originally developed for fruit, the system was extended to all fresh food items including seafood. Although the system has not been used extensively on seafood, it has been very successful with numerous products and should certainly be mentioned as a potential use for extending the shelf life of fresh seafood.

Another important area involving packaging technology is modified-atmosphere packaging (MAP), which has been shown to increase the shelf life of many perishable foods and formulated products by reported values of from 50 to 400% (25). However, there has been considerable concern by both regulatory authorities and researchers over maintaining the safety of MAP foods as they travel through the channels of commerce. The system, consisting of modifying the gaseous environment with a package, results in various combinations of carbon dioxide, nitrogen, and oxygen. Carbon dioxide is the important factor replacing much of the oxygen and nitrogen in the air (eg, 75% CO_2, 15% N_2, and 10% O_2). Of concern is maintenance of the proper refrigeration temperatures and the gas ratios for a given product so that microbial growth does not present an undue safety hazard.

A considerable amount of research has been carried out in the MAP of seafood products (26). A significant amount of effort is being expended on developing the processor-distributor combination to improve quality and value of fresh seafoods.

SEAFOOD SAFETY

The discussion of MAP is a good place to end the packaging discussion because it leads to the subject of general safety of foods as presented to the consumer. Often packaging is a major factor involved in these concerns, because the environment created by the combination of processing and packaging often creates potential for adverse microbial growth. There are two programs, one specifically for shellfish and one for refrigerated foods, that are important in reducing the microbiological risks associated with harvesting, handling, processing, and distributing to the consumer.

The National Shellfish Sanitation Program

The National Shellfish Sanitation Program (NSSP) is designed to prevent harvesting of shellfish in polluted waters containing pathogenic organisms or other contaminants (27). The program, begun in 1925 following illnesses culminating in typhoid fever outbreaks caused by contaminated sewage, is administered cooperatively among the federal government, states, and industry through the interstate Shellfish Sanitation Conference. The Food and Drug Administration is responsible for appraising each state's shellfish program to insure that they are complying with the specified requirements. NSSP gives each state the responsibility of defining or classifying its waters from which shellfish are harvested. The waters are tested and analyzed on a continual basis and classified as follows:

1. *Approved.* These waters may be harvested for the direct marketing of shellfish at all times.
2. *Conditionally Approved.* These waters do not meet the criteria for the approved waters at all times but may be harvested when criteria are met.
3. *Restricted.* Shellfish may be harvested from restricted waters if subjected to a suitable purification process.
4. *Prohibited.* Harvest for human consumption cannot occur at any time.

The term harvest limited is used to refer to conditionally approved, restricted, or prohibited waters. A closure area is an area in which some restriction on harvest has been placed (eg, a harvest-limited area).

Hazard Analysis and Critical Control Point Principles

As the popularity and volume of refrigerated foods have been significantly increasing over the last decade, there has been an accompanying increase in the risks of foodborne disease. The overall safety record of a wide variety traditional refrigerated fresh fruits and vegetables; raw meats, poultry, and seafood; pasteurized milk; cured, ready-to-eat meats and seafood; high-moisture cheeses, yogurt, and pickles; and perishable delicatessan products has been good. However, the complexity of food-distribution systems presents risk factors that are increasing. This is especially the case of a new generation of engineered and formulated foods including frozen or restaurant dinners; frozen or delicatessan entrées; dry, frozen, or canned pasta; fresh, refrigerated salads; canned, frozen, or dry gravies; canned or dry soups; and frozen, canned, or refrigerated cooked meats and seafood (28).

In response to this increasing hazard, the National Academy of Science has studied the problem and recommended a system, the hazard analysis and critical control point (HACCP) system, which "provides a more specific and critical approach to the control of microbiological hazard in foods than that provided by traditional inspection and quality control approaches" (29). The system, similar to the critical control point system used by engineers in planning design, construction, and startup of a building or operation, include the following principles:

1. Describing the product and how the consumer will use it.
2. Preparing a flow diagram for intended manufacturing and distribution of the product.
3. Conducting risk analysis for ingredients, product, and packaging; reducing the risks by making changes to the design; and incorporating these changes into the processing and packaging schemes.
4. Selecting critical control points (CCP) and designating their location on the flow diagram and describing CCP, establishing monitoring.
5. Implementing HACCP in routine activities.

The assurance of food safety is a complex subject that impacts all phases of the commercial food chain, ending with the consumer. HACCP undoubtedly will have a strong influence on the future of many food operations. This impact will especially affect the seafood industry with its myriad safety and sanitation problems, many of which are specific to products from the marine and fresh-water environments.

FUTURE DEVELOPMENT

Fish is health food is not a marketing gimmick but truly states the merits of seafood. The demand for this highly nutritious protein food, much avowed for its healthful omega-3 fatty acids, will continue to grow in demand. This pressure on a limited resource will encourage better biological management of the natural wild stocks (including international cooperation), faster expansion of aquaculture operations, and the total utilization of the raw material.

Much of the so-called industrial fish and the waste from present processing operations, currently being reduced to cheap animal foods, can and will be upgraded to human foods. Headed and gutted industrial fish can be deboned to give a highly acceptable minced flesh for engineered and formulated foods A fish frame from which fillets have been removed contains as much meat as the fillets. This meat can be removed by deboning, and the minced flesh, nutritionally equal to the fillet or to any other minced flesh, is a tremendous source of base protein materials for formulated foods. Modern processing machinery, growing knowledge about the technology of formulated foods, and the demand for high-quality prepared foods support the trend to use previously underutilized seafood and seafood portions for this market.

As has been discussed, the technology of handling, packaging, and transporting fresh seafoods is rapidly developing and the future will see more fresh seafood on the market. In conclusion there is an exciting future for seafood in the United States. The new generation of refrigerated and frozen seafood and formulated products, as well as better quality traditional items, will continue to encourage increased consumption of both wild and farmed fish and shellfish.

BIBLIOGRAPHY

1. S. N. Jhavari, P. S. Karakoltsidis, J. Montecalvo, Jr., and S. M. Constantinides, *Journal of Food Science* **49**, 110 (1984).

2. National Marine Fisheries Service, *Current Fishery Statistics No. 8800*, United States Department of Commerce, Washington, D.C., 1988.
3. U.S. Department of Agriculture, "Aquaculture Situation and Outlook Report," *AQUA 1* **31**, (Oct. 1988).
4. "Farm-Raised Catfish," *Aquaculture Magazine* **18**, 81 (1989).
5. L. A. Nielsen and D. L. Johnson, *Fisheries*, American Fisheries Society, Bethesda, Md., 1983.
6. K. S. Hildebrand, unpublished data, 1984.
7. G. M. Pigott, "Total Utilization of Raw Materials from the Sea," *Proceedings of the Conference on Formed Foods*, Brigham Young University, Brighton, Utah, Apr. 1–2, 1985.
8. J. J. Sullivan, M. G. Simon, and W. T. Iwaoka, "Comparison of HPLC and Mouse Bioassay Methods for Determining PSP Toxins in Shellfish," *Journal of Food Science* **48**, 1321 (1983).
9. G. M. Pigott and B. W. Tucker, *Seafood: The Effect of Technology on Nutrition*, Marcel Dekker, Inc., New York, 1990.
10. H. O. Bang and J. Dyerberg, "Plasma Lipid and Lipoprotein in Greenlandic West Coast Eskimos," *Acta Medica Scandinavica* **192**, 85–94 (1972).
11. G. M. Pigott and B. W. Tucker, "Science Opens New Horizons for Marine Lipids in Human Nutrition," *Food Reviews International* **3**, 105–138 (1987).
12. W. E. M. Lands, "Fish and Human Health: A Story Unfolding," *World Aquaculture* **20**, 59–62 (1989).
13. B. W. Tucker, "Sterols in Seafood: A Review," *World Aquaculture* **20**, 69–72 (1989).
14. *Canned Foods*, The Food Processors Institute, Washington, D.C., 1980.
15. R. A. Bello and G. M. Pigott, "Ultrastructural Study of Skeletal Fish Muscle after Freezing at Different Rates, *Journal of Food Science* **47**, 1389–1394 (1982).
16. G. M. Pigott, insert in Ref. 9.
17. L. B. Rockland and L. R. Beuchet, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, Inc., New York, 1987.
18. R. A. Bello and G. M. Pigott, "Dried Fish Patties: Storage Stability and Economic Considerations," *Journal of Food Science* **4**, 247–260 (1980).
19. G. M. Pigott, "Smoking Fish—Special Considerations," *Proceedings of the Smoked Fish Conference Symposium*, University of Alaska and University of Washington Sea Grant, Seattle, Wash., Apr. 27–29, 1981.
20. Codex Alimentarius Commission, *Microbiological Safety of Irradiated Foods*, FAO/World Health Organization Joint Office, Rome, Italy, 1983.
21. G. Giddings, "Irradiation: Progress or Peril?" *Prepared Foods* **158**, 62–67 (1989).
22. U.S. Department of Agriculture, *Safety and Wholesomeness of Irradiated Foods*, The National Agricultural Library, Beltsville, Md., 1986.
23 G. M. Pigott, "Radurization of Aquaculture Fish: A Value-Added Processing Technology of the Future," *The Proceedings of Aquaculture International Congress and Exposition*, Vancouver, B.C., Sept. 6–9, 1988.
24. N. H. Mermelstein, "Hypobaric Transport and Storage of Fresh Meats and Produce Earns 1979 IFT Food Technology Industrial Achievement Award," *Food Technology* **33**, 32–40 (1979).
25. J. H. Hotchkiss, "Experimental Approaches to Determining the Safety of Food Packaged in Modified Atmospheres," *Food Technology* **42**, 55–64 (1988).
26. M. F. Layrisse and J. P. Matches, "Microbiological and Clinical Changes in Spotted Shrimp Stored under Modified Atmosphere," *Journal of Food Protection* **47**, 453–457 (1984).
27. D. L. Leonard, M. A. Broutman, and K. E. Harkness, *The Quality of Shellfish Growing Waters on the East Coast of the United States*, Ocean Assessments Division, NOAA, Rockville, Md., 1989.
28. D. A. Corlett, Jr., "Refrigerated Foods and Use of Hazard Analysis and Critical Control Point Principles," *Food Technology* **43**, 91–94 (1989).
29. Food Protection Committee, Subcommittee on Microbiological Criteria, *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, National Academy of Sciences, National Research Council, National Academy Press, Washington, D.C., 1985.

George M. Pigott
University of Washington
Seattle, Washington

FISH CAKES. See Fish and shellfish products.

FISHES: ANATOMY AND PHYSIOLOGY

The diversity of forms found among the species of fishes is matched only by the diversity of aquatic environments on the earth. Aside from the shared characteristics of possessing a backbone, being cold blooded, and having gills and fins, the variation in anatomy, physiology, and ecology of all the fish species is extensive indeed. In size, they range from a 1-cm goby to the 15-m whale shark. They are found in waters from nearly 4,000 m above sea level to 7,000 m below sea level as well as in water temperatures from below freezing to 44°C. They are also found in environments that are nearly distilled to those that are very salty. Fish inhabit surface waters in full sunlight as well as very deep waters in complete darkness where pressures are extremely high. While most are strict water breathers, some have adapted to breath air where oxygen in water might be lacking. It may not be surprising, therefore, that there may be more than 25,000 species of fishes, accounting for more than half of all vertebrate species in the animal kingdom. Fishes also play an important role in human life by providing food and recreation. The traditional commercial fishery and now, to an increasing degree, aquaculture provide a high-quality protein source for human consumption. The sport fishery and hobby aquarium industry are both very important sectors of the economy. Feed for agricultural animals and fish oil supplements to human diets are examples of secondary uses of fish products for human benefit.

The purpose of this article is to outline some of the fundamental aspects of the anatomy and physiology of the bony fishes. The reference list at the end of the article serves as a guide to more detailed literature on various subjects covered here. The anatomy and physiology of eight major systems in fish are briefly described. These are the digestive, renal, cardiovascular, respiratory, nervous, endocrine, reproductive, and immune systems. The infor-

2. National Marine Fisheries Service, *Current Fishery Statistics No. 8800*, United States Department of Commerce, Washington, D C., 1988.
3. U S. Department of Agriculture, "Aquaculture Situation and Outlook Report," *AQUA 1* **31**, (Oct. 1988).
4. "Farm-Raised Catfish," *Aquaculture Magazine* **18**, 81 (1989).
5. L. A. Nielsen and D. L. Johnson, *Fisheries*, American Fisheries Society, Bethesda, Md., 1983.
6. K. S. Hildebrand, unpublished data, 1984.
7. G. M. Pigott, "Total Utilization of Raw Materials from the Sea," *Proceedings of the Conference on Formed Foods*, Brigham Young University, Brighton, Utah, Apr. 1–2, 1985.
8. J. J. Sullivan, M. G. Simon, and W. T. Iwaoka, "Comparison of HPLC and Mouse Bioassay Methods for Determining PSP Toxins in Shellfish," *Journal of Food Science* **48**, 1321 (1983).
9. G. M. Pigott and B. W. Tucker, *Seafood: The Effect of Technology on Nutrition*, Marcel Dekker, Inc., New York, 1990.
10. H. O. Bang and J. Dyerberg, "Plasma Lipid and Lipoprotein in Greenlandic West Coast Eskimos," *Acta Medica Scandinavica* **192**, 85–94 (1972).
11. G. M. Pigott and B. W. Tucker, "Science Opens New Horizons for Marine Lipids in Human Nutrition," *Food Reviews International* **3**, 105–138 (1987).
12. W. E. M. Lands, "Fish and Human Health: A Story Unfolding," *World Aquaculture* **20**, 59–62 (1989).
13. B. W. Tucker, "Sterols in Seafood: A Review," *World Aquaculture* **20**, 69–72 (1989).
14. *Canned Foods*, The Food Processors Institute, Washington, D.C., 1980.
15. R. A. Bello and G. M. Pigott, "Ultrastructural Study of Skeletal Fish Muscle after Freezing at Different Rates, *Journal of Food Science* **47**, 1389–1394 (1982).
16. G. M. Pigott, insert in Ref. 9.
17. L. B. Rockland and L. R. Beuchet, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, Inc., New York, 1987.
18. R. A. Bello and G. M. Pigott, "Dried Fish Patties: Storage Stability and Economic Considerations," *Journal of Food Science* **4**, 247–260 (1980).
19. G. M. Pigott, "Smoking Fish—Special Considerations," *Proceedings of the Smoked Fish Conference Symposium*, University of Alaska and University of Washington Sea Grant, Seattle, Wash., Apr. 27–29, 1981.
20. Codex Alimentarius Commission, *Microbiological Safety of Irradiated Foods*, FAO/World Health Organization Joint Office, Rome, Italy, 1983.
21. G. Giddings, "Irradiation: Progress or Peril?" *Prepared Foods* **158**, 62–67 (1989).
22. U.S. Department of Agriculture, *Safety and Wholesomeness of Irradiated Foods*, The National Agricultural Library, Beltsville, Md., 1986.
23. G. M. Pigott, "Radurization of Aquaculture Fish: A Value-Added Processing Technology of the Future," *The Proceedings of Aquaculture International Congress and Exposition*, Vancouver, B.C., Sept. 6–9, 1988.
24. N. H. Mermelstein, "Hypobaric Transport and Storage of Fresh Meats and Produce Earns 1979 IFT Food Technology Industrial Achievement Award," *Food Technology* **33**, 32–40 (1979).
25. J. H. Hotchkiss, "Experimental Approaches to Determining the Safety of Food Packaged in Modified Atmospheres," *Food Technology* **42**, 55–64 (1988).
26. M. F. Layrisse and J. P. Matches, "Microbiological and Clinical Changes in Spotted Shrimp Stored under Modified Atmosphere," *Journal of Food Protection* **47**, 453–457 (1984).
27. D. L. Leonard, M. A. Broutman, and K. E. Harkness, *The Quality of Shellfish Growing Waters on the East Coast of the United States*, Ocean Assessments Division, NOAA, Rockville, Md., 1989.
28. D. A. Corlett, Jr., "Refrigerated Foods and Use of Hazard Analysis and Critical Control Point Principles," *Food Technology* **43**, 91–94 (1989).
29. Food Protection Committee, Subcommittee on Microbiological Criteria, *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, National Academy of Sciences, National Research Council, National Academy Press, Washington, D.C., 1985.

GEORGE M. PIGOTT
University of Washington
Seattle, Washington

FISH CAKES. See FISH AND SHELLFISH PRODUCTS.

FISHES: ANATOMY AND PHYSIOLOGY

The diversity of forms found among the species of fishes is matched only by the diversity of aquatic environments on the earth. Aside from the shared characteristics of possessing a backbone, being cold blooded, and having gills and fins, the variation in anatomy, physiology, and ecology of all the fish species is extensive indeed. In size, they range from a 1-cm goby to the 15-m whale shark. They are found in waters from nearly 4,000 m above sea level to 7,000 m below sea level as well as in water temperatures from below freezing to 44°C. They are also found in environments that are nearly distilled to those that are very salty. Fish inhabit surface waters in full sunlight as well as very deep waters in complete darkness where pressures are extremely high. While most are strict water breathers, some have adapted to breath air where oxygen in water might be lacking. It may not be surprising, therefore, that there may be more than 25,000 species of fishes, accounting for more than half of all vertebrate species in the animal kingdom. Fishes also play an important role in human life by providing food and recreation. The traditional commercial fishery and now, to an increasing degree, aquaculture provide a high-quality protein source for human consumption. The sport fishery and hobby aquarium industry are both very important sectors of the economy. Feed for agricultural animals and fish oil supplements to human diets are examples of secondary uses of fish products for human benefit.

The purpose of this article is to outline some of the fundamental aspects of the anatomy and physiology of the bony fishes. The reference list at the end of the article serves as a guide to more detailed literature on various subjects covered here. The anatomy and physiology of eight major systems in fish are briefly described. These are the digestive, renal, cardiovascular, respiratory, nervous, endocrine, reproductive, and immune systems. The information is basic and is presented here with the assumption that the reader has some knowledge about vertebrate anatomy and physiology but very little or no knowledge about fish. The intention is to present some basic information and stimulate interest in this important food animal.

GROSS ANATOMY AND ORIENTATION

The anatomy of the salmonid is emphasized here because they are one of the most widely studied genera in terms of physiology and aquaculture science. Figure 1 presents the general orientation and terminology that will be used throughout this article. It also points out the major external features of the animal.

Figure 2 illustrates the general features of the internal organs of the salmonid. The muscle mass is, by far, the largest component of the body. The muscle mass, relative to the body, is larger in fish than in other terrestrial or aerial vertebrates. The dense medium of water requires a large force for locomotion compared to air. That same medium, however, provides substantial structural support such that the fish can maintain neutral bouyancy with a swim bladder or body lipids. It is also this great mass of muscle that makes the fish uniquely desirable as a food animal. There are two types of muscle in the fish: red and white. In most species of fish less than 10% of the total muscle mass is made up of red muscle. The color-oriented terminology is based on the visual appearance of two types of muscle. The red muscle appears darker because there is up to a 10-fold greater blood capillary to muscle fiber ratio in the red muscle compared to the white muscle. Red muscle is used for sustained swimming, fueled by aerobic glycolysis and lipolysis, while the white muscle is used for burst swimming, fueled by anaerobic glycolysis. The approximate weight distribution, in percent, of the major body parts of a trout are listed below.

Liver	1.2
Spleen	0.1
Intestine	4.7
Heart	1.2
Swim bladder	0.2
Kidney	0.9
Muscle	56.9
Skin	8.7
Axial skeleton	13 5
Gills	2.8
Head	11.8

DIGESTIVE SYSTEM

The anatomy and function of the digestive system in fish is basically the same as in other vertebrates. It is made up of a combination of mechanical and chemical processes. Food is ingested, broken down, and either absorbed into the blood or remains in the gut and is eventually expelled as feces. Differences arise between fish species in feeding habits, and the environment may influence the presence, position, shape, and size of a particular organ. Fish may be divided into three categories according to the food they eat. Herbivores are specialized and eat detritus and plants. Omnivores eat both plants and animal material, and carnivores feed on larger invertebrates and other fish.

Digestive Organs

The oral cavity is made up of lips, mouth, teeth, and tongue. Fleshy lips are common to herbivores, designed for grazing and straining. Carnivore lips are generally unmodified and thin. Bottom feeders often have barbels, sensory appendages that help guide them when feeding and grazing.

Mouths are large or tubular. Large mouths are usually associated with predatory fish, such as a barracuda, whereas tubular mouths such as that of a seahorse, are better adapted for plankton eaters due to the enhanced ability to suck water and food. Mouth position can be variable depending on the types of feed normally consumed. Bottom feeders have mouths on the ventral surface, surface feeders have a dorsal mouth and bony fish (teleosts) have a terminal mouth.

Teeth are thought to have originally arisen from scales covering the lips. Fish generally have small teeth for gripping and capturing prey. There are four possible types of teeth present in fish, also depending on feeding habits: (*1*) jaw teeth, (*2*) palate teeth, (*3*) tongue teeth, and (*4*) throat teeth. There is a strong correlation between dentition, feeding habits, and food eaten. Jaw teeth are more developed in carnivores, but they are poorly developed or absent in herbivores, which usually have well-developed throat teeth attached to the gill arches.

The tongue of a fish is very rudimentary. It has no salivary glands but does bear sensory taste buds. Any lubricant fluid in the mouth originates from mucous cells scattered throughout the inner lining, not from the tongue. Plankton eaters also have well-developed gill rakers. This is a sieve-type apparatus attached to the inner edges of the gill arches and serves to filter out phytoplankton, crustaceans, and, in some cases, even diatoms.

A short, broad, and muscular esophagus connects the mouth and elastic stomach. It functions in the transport and taste of food. The musculature of the esophagus tends to be more developed in freshwater fish than in saltwater fish, as it plays a role in minimizing water intake while ingesting food.

The stomach is muscular and motile. The inner surface is lined with gastric glands that secrete digestive enzymes, necessary for the breakdown of food. The size of the stomach is related to the size of food particles and the interval between meals, ie, large food size and infrequent feeding are usually associated with large stomachs, and smaller food particles ingested more constantly are associated with fish with small or no stomachs. The pyloric valve, a ring of muscle, controls the passage of food between the stomach and intestine.

The intestine is a relatively simple tube that has digestive glands and an abundant supply of blood vessels. There are two valves, one at each end. The pyloric valve is at the anterior end and the ilioceacal valve is at the posterior end. Here is where much of the absorption of nutrients takes place. Digestive products are picked up in solution. Intes-

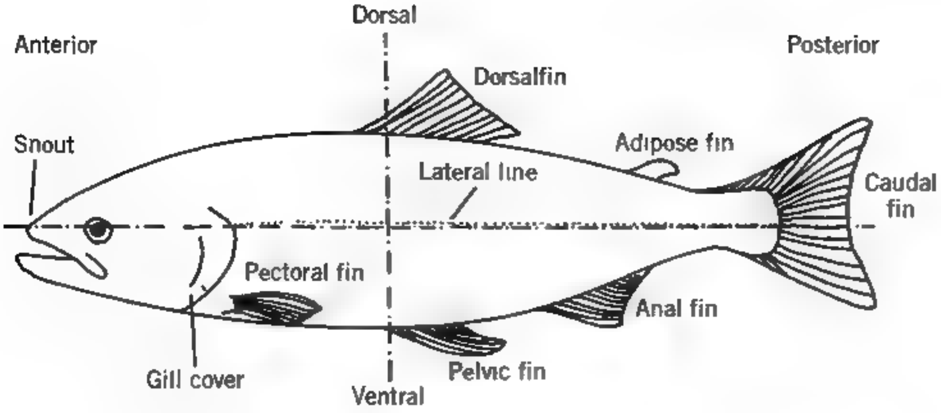

Dorsal
Anterior
Posterior
Dorsalfin
Snout
Adipose fin
Lateral line
Caudal fin
Pectoral fin
Anal fin
Gill cover
Ventral
Pelvic fin

Figure 1. External anatomy.

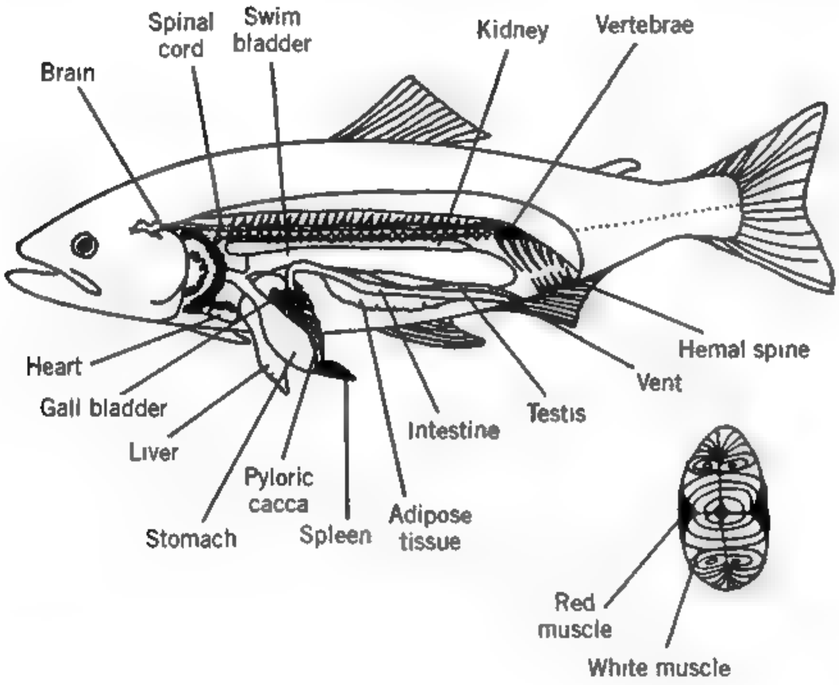

Spinal cord
Swim bladder
Kidney
Vertebrae
Brain
Heart
Gall bladder
Liver
Hemal spine
Vent
Testis
Intestine
Pyloric caeca
Stomach
Spleen
Adipose tissue
Red muscle
White muscle

Figure 2. Internal auditing and cross section of trunk.

tine length varies with feeding habits: carnivores have short straight intestines, herbivores have long and coiled intestines. Gut length seems more correlated to the amount of indigestible material in the food rather than the nature of the food. A large surface area is realized by extensive infoldings and ridges. The intestine leads to the rectum where vascularization and secretory cells are sparse, but where more mucous cells are present. The anal opening is called a vent and is also the terminal location for the urinary and reproductive ducts. While the common collection of these ducts suggest a corollary with the avian cloaca, it is not the case. The three ducts in fish all empty to the external environment and not to a common internal chamber. Absorption of plant material is probably poor; probably about 20% or less. Hatchery diets for salmonids may be absorbed up to 80%.

Food Movement

Movement of food through the digestive system is aided by peristaltic waves of muscle contraction. Cilia also line the tract and are especially useful in stressful situations when peristalsis may become reduced or even stop. This suggests that peristalsis is under some nervous control. Mucous membranes in the mouth, esophagus, and stomach provide lubrication to aid the passage of the food along the digestive tract.

Associated Organs

The pyloric caeca is an important appendage that opens into the intestine. It has the same epithelium as the intestine and its purpose is to increase the effective surface area. It may have both an absorptive and digestive function. A two or more lobed liver, which serves several metabolic and energetic functions, also produces bile, which aids in digestion. The gallbladder, which opens into the intestine, stores excess bile, which is secreted when required. The pancreas, which is large and distinct in the elasmobranch but diffuse in teleosts, secretes insulin and glucagon in response to nutritional intake, but also secretes digestive enzymes into the intestine. In teleosts, small beads of pancreatic tissue are scattered in the mesentaries near the digestive tract and are supplied with artery, vein, nerve, and pancreatic ducts. Little is known about the pancreatic secretions and most knowledge is based on histological evidence.

Digestive Enzymes

While goblet cells secrete mucus, gland cells throughout the digestive system contribute digestive enzymes necessary for the breakdown of food. The stomach is lined with secretory cells (gastric glands) that secrete hydrochloric acid (HCl) and pepsinogen. These chemicals break down protein molecules into amino acids and are, therefore, especially important to carnivorous fish. The stomach is very acidic (pH 2–4). Pepsin activity is dependent on pH and temperature, whereas HCl secretion is dependent on temperature and meal size. Optimum pepsin activity occurs at pH values of 2 and 4. Stomach distension stimulates gastric secretions.

Both the pyloric caeca and intestinal mucosa are sources of lipase, which breaks down fats into fatty acids and glycerine. Absorption of fat may occur in the anterior of the intestine. Intestinal secretions include proteases such as trypsin, lipase, and carbohydrase. These enzymes work best at neutral to alkaline pH. Bicarbonate is secreted (possibly from the pancreas) to raise the pH of the food coming from the stomach. Bile salts, secreted from the gallbladder may also play a role in adjusting the digestive juices to the proper pH to facilitate the digestive process. Bile salts are detergentlike substances formed from the decomposition of cholesterol and other steroids that function in fat emulsification aiding in the digestion of lipids. Fat soluble vitamins (A, D, E, K) are digested by bile. Digestive proteases are also secreted from the pancreatic tissue.

Most of the food absorption takes place in the intestine and pyloric caeca with the undigested material passing on to the rectum. Factors such as temperature, fat content, and presence of indigestible material affect the passage and digestion and absorption of food.

Innervation of Digestive Organs

Innervation of the digestive organs is not well understood, although it is thought that there is sympathetic innervation from paired ganglia, lateral to the spinal cord to the stomach, intestine, and rectum. This adrenergic system has an inhibitory effect. Parasympathetic innervation is through three cranial nerves to various parts of the digestive tract. Glossopharyngeal (IX) and facial (III) nerves innervate the mouth and esophagus areas. The vagus (X) innervates all visceral portions. These cranial nerves are cholinergic and have a stimulatory effect. Intrinsic nerves form a network inside the tissue of the digestive system. These nerves do not originate in the brain or the spine. They may assist in peristaltic movement. Intestinal and pancreatic secretions appear to be also under both nervous and hormonal control.

Feeding Types

Predators have well-developed grasping and holding teeth, ie, sharks, pike, and gar, and well-defined stomachs with strong acid secretions and short intestines (relative to herbivores). They depend on their senses of vision or the lateral line sensory organ to detect their prey. Grazers browse on plants and organisms and sometimes on each other. The trout is an example of this, and in crowded ponds they often nip at the fins of the other fish. Strainers usually have well-developed gill rakers that strain out the plankton and crustaceans in the water that flows through the mouth and gills. Suckers suck in their food. They have mouths on the ventral surface of their heads but adaptive lips. An example of this type of feeder is the sturgeon. Parasites live off the body fluids of other fish. An examples of this type of feeder is the lamprey.

Stimuli for Feeding

Internal motivation is driven by factors such as season and temperature, diurnal light, light intensity, and the time and nature of the last feeding. Fish have been known to feed better at dusk and early morning. Due to patterns of growth, fish also feed in spring and through the summer much more voraciously than in the winter. The hypothalamus is also thought to be involved especially where hunger and satiation triggers are concerned. Stimuli for feeding also comes through the senses, such as smell, taste, sight, and the lateral line system.

RENAL SYSTEM

The kidneys are important in filtering undesirable materials out of the blood as well as serving a role in water and ion balance. Active transport, together with epithelial permeability are adjusted in order that ions and other materials vital to the body can be conserved and control over body water content can be exercized.

Basic Anatomy and Physiology

The kidney in salmonidae is made up of two parts running along the anterior-posterior axis between the body cavity and vertebral column. Anterior and posterior portions, also known as head and trunk, respectively, have slightly different functions but there is no visual distinction between them. The anterior kidney is associated with inter-renal tissue and chromaffin cells, which are involved in blood cell and hormone production; the posterior kidney is associated with filtration and urine production.

Kidney tissue is made up of individual units called nephrons (Fig. 3). Each nephron is made up of a renal corpuscle, consisting of the glomerulus and Bowman's capsule and a kidney tubule. The glomerulus is a network of afferent and efferent arterioles whose blood supply comes from

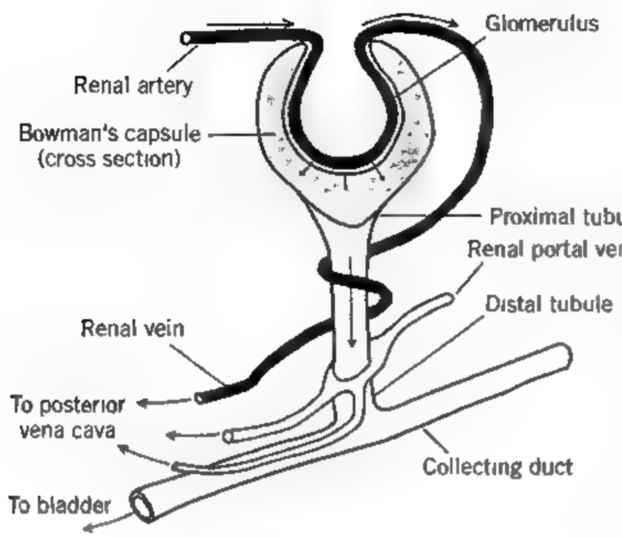


Figure 3. Kidney nephron.

the dorsal aorta or a venous supply called the renal portal system. The Bowman's capsule encapsulates the glomerulus and nonselectively filters solutes and molecules less than 70,000 mol. wt. from the blood. The filtrate is carried through the renal tubule, which is made up of the proximal, intermediate, and distal segments. It is in the tubule that reabsorption of selected electrolytes, minerals, amino acids, glucose, and other plasma organics takes place via a capillary network back into the blood. The remaining fluid containing unreabsorbed constituents flows to the collecting tubule and gathers in the urinary bladder, if present, where reabsorption of more ions may take place before being excreted out the urinary duct.

The process of nonselective filtration and subsequent selective reabsorption is an efficient process, because the body reabsorbs only what it needs. It is an adaptable system, because needs may change over time and through changing circumstances. Although efficient, this system requires energy as many ions are reabsorbed by active transport. Control of kidney function is accomplished by a variety of hormonal mechanisms and includes tissues of the thyroid, kidney, gonads, hypothalamus, and possibly the pituitary. In mammals, it is well established that a renin-angiotensin system and antidiuretic hormone (ADH) regulate renal function for ionic and water regulation. While the exact role of such a system in fishes is not well established, there is evidence that similar mechanisms are found in fishes. In this model, renin is secreted under nervous and hormonal control from a juxtaglomelular apparatus, located near the glomerulus, in response to decreased Na^+ concentration or blood pressure as well as under stressful conditions. Renin is then converted to angiotensin, which increases blood pressure through the constriction of blood vessels and causes aldosterone to be release from the adrenal cortex (of mammals). Aldosterone stimulates ionic uptake from the distal tubule of the nephron. ADH, from the pituitary, controls the amount of water leaving the nephron by controlling the permeability of the epithelium in the collecting duct. An increase in blood osmolarity causes an increase in ADH, which increases the permeability of the epithelium, resulting in the increased absorption of water out of the filtrate, back into the blood.

Freshwater fishes are hyperosmotic regulators. That means that the concentration of ions and other solutes is greater in the blood than in their surrounding water, which in most cases is very dilute. The fish will thus absorb water osmotically from their environment through all permeable epithelia such as the gills, skin, and gut. The regulatory problem is one of getting rid of the excess water and the kidney plays that important role. A large amount of urine is produced, which is dilute and contains creatine, uric acid, and some ions. The volume of urine produced must balance the quantity of water entering the body. Sodium (Na^+) and chloride (Cl^-) ions passively diffuse out of the body across permeable epithelia and are actively taken up, to a large extent, across the gill epithelium.

Electrolyte reabsorption out of the urine takes place across the renal tubule. Na^+ is actively extracted and it appears that Cl^- passively follows. Calcium (Ca^{2+}), magnesium (Mg^{2+}), and other divalent ions must also be reabsorbed, because they are normally absent in the urine of freshwater fish. The reabsorption of these ions is usually accomplished without the osmotic absorption of water. The distal segment, collecting duct and urinary bladder appears to be relatively impermeable to water. Macromolecule reabsorption, including glucose, amino acids, and other plasma organic constituents takes place in the first segment of the proximal tubule. Salts are reabsorbed in the distal segment, and any remaining in the filtrate may be reabsorbed from the urinary bladder. Only a small proportion of total organic nitrogen is excreted via the kidneys, although this appears to be an important excretory pathway for minor nitrogenous products, such as creatine and uric acid. Major nitrogenous products such as ammonia and urea are excreted via the gills.

The kidney in marine fishes plays a crucial role in hypoosmotic osmoregulation. Converse to the freshwater fish, the blood concentrations of ions and solutes in fish blood relative to the concentrated ionic environment of the marine environment is rather dilute. The osmotic problem created by such a gradient is one of dessication, which the fish counteracts by actively taking in water from the environment by drinking and reducing water loss at the kidneys. Between 60 and 80% of ingested water is absorbed through the gut, along with the monovalent ions Na^+, Cl^-, and potassium (K^+). Those excess salts are actively excreted. The gills play a major role in that function, although the kidney excretes Mg^{2+}, sulfate, and other divalent ions. Although less than 20% of the ingested divalent ions are actually absorbed most are passively eliminated via the intestines, that which is absorbed is handled by the kidney tubules.

The kidney nephron of marine fishes often lack the distal segment of the tubule and has less glomeruli. In some cases, such as the goosefish, glomeruli are absent. Because glomeruli are suited to the excretion of large volumes of water the importance of the glomerulus in marine teleosts is greatly reduced. Urine from a marine kidney thus has high osmolality and is low in volume. Much of the water entering the glomerulus reabsorbed to help prevent dehydration, a case opposite to the freshwater kidney. Nitrogenous excretion is similar to the pathway in freshwater fish, with ammonia and urea excreted through the gills and minor nitrogenous products such as creatine and uric acid excreted by the kidney.

Anadromous and catadromous fish must be capable of adjusting their osmotic balance to survive the changes in salinities that they experience in their life cycle. They have glomerular kidneys that can adjust to changing urine volumes, and possess gills and oral membranes capable of both uptake and secretion of certain ions against the prevailing diffusion gradients. The kidneys in those animals are capable of adjusting urine volume and composition on demand. When those fish, for example, are transferred or voluntarily move from fresh water to salt water, the urine composition gradually changes after a few days. Urine flow decreases and osmolality increases as divalent ions are excreted. Sodium and chloride content in the urine decreases as the chloride cells (specialized salt excreting cells on the gills) take over this function. Glomelular filtration rate may temporarily slow down and tubular reabsorption increases.

CARDIOVASCULAR SYSTEM

The cardiovascular system moves the blood around the body. It is basically composed of the heart (cardio-) and network of blood vessels (-vascular) throughout the body. The purpose of blood circulation is the transport of materials between certain locations within the body. Gases such as oxygen are taken up from the water and move from gills to the tissues and others such as carbon dioxide move from tissues back to the gills for excretion. End products of metabolism such as lactate, originate in the muscles and are transported to the liver for breakdown. Glucose made in the liver must be moved to the tissues where it will be used. Materials that are foreign to the body are excreted by gills, released in the urine, or engulfed and destroyed by specialized cells in the blood. Amino acids and other nutrients from digested materials must move from gut to the tissues. Blood cells that are produced in the anterior kidney and spleen must be distributed throughout the body. Because of the vast range of environments in which fish are found, the diversity in the form and function of the cardiovascular systems are as wide ranging. The basic conformity among salmonid fishes are emphasized here.

Heart

The teleost heart has four chambers: the sinus venosus, the atrium, the ventricle, and the bulbus (Fig. 4). The venous blood from the body is received in the sinus venosus. This is a rather flat bag anterior to the transverse septum separating the heart and head compartments from the visceral cavity. The atrium is a thin-walled bag that feeds the blood to the muscular ventricle. The ventricle is the powerhouse that contracts and propels the blood throughout the body. The bulbus is rather muscular as well and helps to dampen pressure waves as the blood pulses out of the ventricle. There are one-way valves that prevent the blood from flowing backward with each contraction. Cardiac output in teleosts ranges from 5 to 100 mL/kg per minute with a general mean about 15–30 mL/kg per minute.

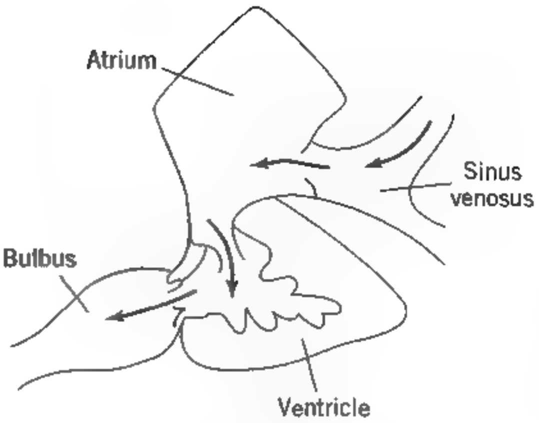


Figure 4. Cross section of a trout heart. *Source:* Ref. 1.

Blood flow is accomplished primarily by the heart, which is a pump for the system. The basic route is from the heart to the gills, where gas and ionic exchange with the environment occurs, and then to the rest of the body (Fig. 5). The blood is then pumped back to the heart through a venous network where the cycle begins over again. About 4–6% of body weight is a reasonable approximation of the blood volume. Blood pressures in teleosts are in the range of 30–70 mm Hg. Because the resistance to blood flow increases as the diameter of the blood vessels decreases, blood flow declines from the point where it leaves the heart. For example, the blood vessel network in the gills reduces the pressure found in the ventral aorta by about 40–50%. That resistance may increase with stresses such as low oxygen levels in the water, whereas other conditions such as exercise or elevated levels of adrenaline dilate the blood vessels and decrease the resistance to blood flow.

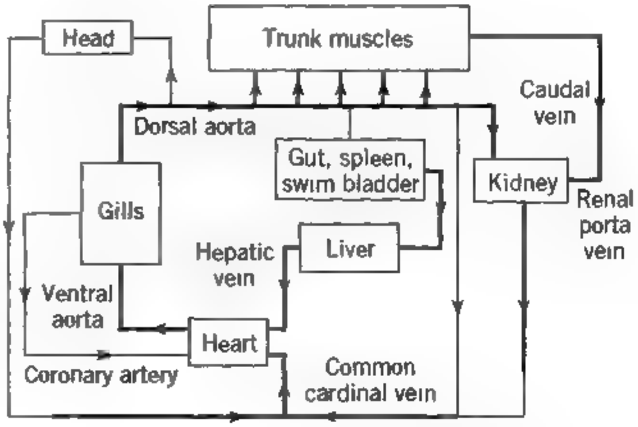


Figure 5. General flow of blood in fish.

The Secondary Circulation

The primary circulation in fish comprises the blood vessels that contain red and white blood cells. There is a secondary circulation, sometimes known as the lymphatic or venolymphatic system, that is characterized by low blood cell content and by low pressure. There are direct connections between the primary and secondary circulation through small vessels, often at right angles to the blood vessel of the primary circulation. This physical configuration causes plasma to be skimmed out of the primary circulation and into the secondary circulation. The secondary circulation feeds this fluid, low in cells as a result of the skimming, and fluid from tissue beds back to the primary circulation via the heart. Salmonids have major ducts just under each lateral line and along the dorsal midline. These may serve to collect fluid from the secondary circulation and return it to the heart. Fin and trunk movements may play an important role in pumping venous fluids back to the heart. There is a tail heart in some fishes that aids the movement of this secondary fluid back to the heart.

RESPIRATORY SYSTEM

The respiratory system is extremely varied among the 25,000 species of fishes. This, once again, reflects the wide range of habitats to which fish have managed to adapt. The primary function of the respiratory system is to transport gases between the environment and the tissues of the body that consume oxygen as well as excrete ammonia and carbon dioxide. The vital components in this system are the water flow over the gills outside the fish and the circulatory system inside the body. The consumption of oxygen (O_2) as well as the excretion of carbon dioxide (CO_2) and ammonia (NH_3 and NH_4^+) take place across the gill.

Anatomy

The fish gill has four gill arches on each side of the midline and two rows of primary or gill filaments per arch (Fig. 6). Arranged perpendicularly to the filament are rows of secondary lamellae on both side of each filament. The plates of secondary lamellae form narrow channels through which the water flows. This space is approximately 0.02–0.05 mm wide, 0.2–1.6 mm long, and 0.1–0.5 mm high. This width is particularly important in that one-half of that width is the minimum distance for gases and dissolved materials such as ions to diffuse between water and blood. The surface of the secondary lamellae is the primary surface across which gases, ions, and other dissolved materials pass between water and blood.

Water Flow over the Gills

The water flow over the gills is directed in an anterior to posterior direction. More importantly, it flows in the opposite direction to the flow of blood through the secondary lamellae in the gills. This countercurrent flow maintains the maximum gradients between blood and water for the gases throughout the transit of water through the gills. The mouth or buccal pump is driven by skeletal muscles that control the mouth of the floor and opercular covers.

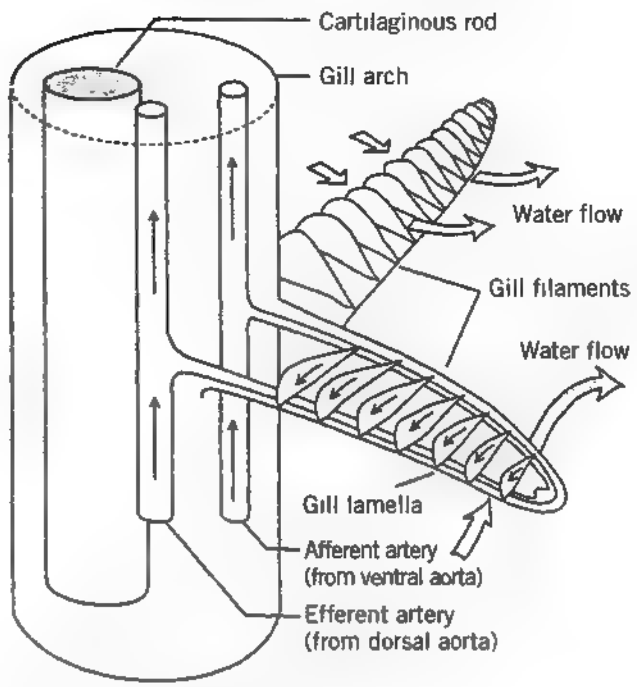


Figure 6. Fish gill with patterns of blood and water flow. *Source:* Ref. 2.

Water, therefore, flows from mouth, over gills, and out the operculum. When the floor of the mouth is lowered, negative pressure is created in the buccal cavity and water is sucked into the mouth. As the mouth is closed and the floor of the buccal cavity raised, the water is forced posteriorly, across the gills, through the opercular cavity. This cycle is repeated continuously and the result is a unidirectional flow of water through the mouth and gills.

While this rhythmic ventilation is the general rule, some fish are ram ventilators, that is, they ventilate the gills by keeping their mouths open and swimming forward through the water. Salmonids also do this at moderate to high swimming velocities. There are also other variations on how fish move water across the respiratory surface of the gill for respiration. Some of these variations such as ram ventilation may reduce the energetic costs of ventilation.

Blood

Blood is a suspension of various cells in a solution of proteins and electrolytes, which make up the plasma. It is the vehicle for transporting materials from different locations in the body, as discussed above. The volume of blood in teleosts ranges between 3 and 6% of wet body weight. Fish do not have bone marrow and the origin of the blood cells is the anterior kidney (anterior 30%) and the spleen. These are termed hematopoietic tissues. The production of blood cells is stimulated by conditions such as bleeding and inhibited by starvation. There is an erythropoesis stimulating factor, found in the plasma of fish that stimulates blood cell production. Very little is found in plasma of resting fish.

White blood cells or leukocytes, are involved in the clotting of blood and play an important part in the immune system. The red blood cell, or erythrocyte is important in the transport of oxygen in the body. The packed red cell volume, or hematocrit, is expressed in percent and varies between 20 and 35%. The erythrocyte contains the respiratory pigment, hemoglobin (Hb), which binds oxygen and enables the oxygen content of the blood to be higher than that of simple dissolved oxygen. The concentration of Hb in blood is approximately 7–12 g % (equal to grams/100 mL). The total oxygen content is about 9–14 vol %.

Blood Proteins

The proteins in the plasma serve to maintain the blood osmolarity. It is the source of colloid osmotic pressure. Some other functions are to buffer pH changes in the blood and to transport vitamins, hormones, and inorganic ions, that attach to these molecules in blood, to tissues. Another important function of special plasma proteins are the antibody proteins that play an important role in the specific immune system of the body. In carp, for example 4.15% of the blood weight are protein. Of that weight, 2.82 g is albumin, 0.79 g is globulin, and 0.23 g is fibrinogen.

Response to Environmental Change

Fish respond to environmental changes in different ways. Some fish are known as oxygen regulators because they maintain O_2 consumption at lower O_2 levels by increasing ventilation. Oxygen conformers, on the other hand, adjust O_2 consumption according to ambient O_2 levels. Salmonids are regulators. The following are some effects of environmental changes on the respiratory system and the response of fish.

Temperature increase presents a three-pronged problem for respiration. It increases the O_2 demand with increased metabolism, lowers the solubility of O_2 in water, and lowers the affinity of O_2 to the haemoglobin molecule. Both heart rate and ventilation increase in response to an increase in temperature. The resistance of the peripheral circulation also decreases.

Hypoxia, or lowered environmental O_2, causes an increase in the frequency and amplitude of ventilation in most fish. While there is a decrease in heart rate, there is an increase in the volume of each stroke of the heart (stroke volume), thus maintaining the output of blood from the heart. This results in a large increase in the ratio of water pumped over the gills to the blood perfused through the gills (ventilation-to-perfusion ratio). Oxygen uptake is, therefore, maintained in the face of lowered water O_2 concentration.

Exercise increases cardiac output due to an increase in stroke volume. Ventilation increases and there is a fourfold to eightfold increase in O_2 uptake. The ventilation-to-perfusion ratio remains relatively constant. Acidic conditions generally increase ventilation.

NERVOUS SYSTEM

The nervous system, acting with the endocrine system, is the way a body coordinates its bodily functions. There are sensory input from external as well as internal sources, and nervous output that sends coordinated commands to various parts of the body as nervous impulses and hormones, which can be stimulatory or inhibitory. Finally, there is integration between those two components at various levels including simple automatic reflexes; integration of vital processes, such as breathing; and higher levels, which include complex learning such as in the homing mechanisms of the salmon returning to its freshwater spawning site. The fish nervous system is not completely understood and it is complicated by the many diverse adaptations present. Generalization is difficult but the basics of the fish nervous system are presented here with an emphasis on the bony fish.

Central Nervous System

The central nervous system constitutes the brain and the spinal cord (Fig. 7). The configuration of the fish brain resembles that of other aquatic vertebrates. All the typical nerves and paired lobes are present. While this basic form is consistent among fish species, the relative sizes of the lobes reflect the adaptive response of the particular species to its physical and biological environment. The optic lobes, therefore, in fish with large eyes that live in dimly lit environments are larger than in fish with small eyes. The basic anatomy and function of the brain of a bony fish (including teleosts) is described here.

The Prosencephalon is made up of the telencephalon and diencephalon. The telencephalon is known as the forebrain and contains the paired olfactory lobes. The forebrain is predominantly involved with reception and conduction of smell impulses. The size of the lobes indicates the importance the role of smell has in the fish species. Elasmobranchs (sharks) and bony fishes have quite pronounced olfactory lobes. The forebrain also appears to play a role in fish behavior. The diencephalon is sometimes known as the between brain. It lies in between the olfactory lobes of the telencephalon and contains the pineal organ. There are two possible functions of the pineal organ. It has been suggested that the pineal body is either a photosensory structure or plays a secretory role. The pineal gland may react to the chemical composition of the cerebrospinal fluid or brain tissue by external or internal (endocrine) secretion. Another important component of the diencephalon is the hypothalamus, which is an important area of interaction between the nervous and endocrine systems. Nervous inputs into this area from the brain result in the production and release of many regulatory hormones of the body (more details in the endocrine section). The diencephalon appears to be an important center for incoming and outgoing messages relating to internal homeostasis.

The optic lobes lie in the Mesencephalon and are the central control for vision. The optic tectum, which is the nervous center for the optic lobes, is also found in the mesencephalon. The tectum is made up of neurons and the fibers from the optic lobe end here. The tectum correlates visual impressions with muscle reaction such as darting for prey or avoiding objects in the water. The choroid

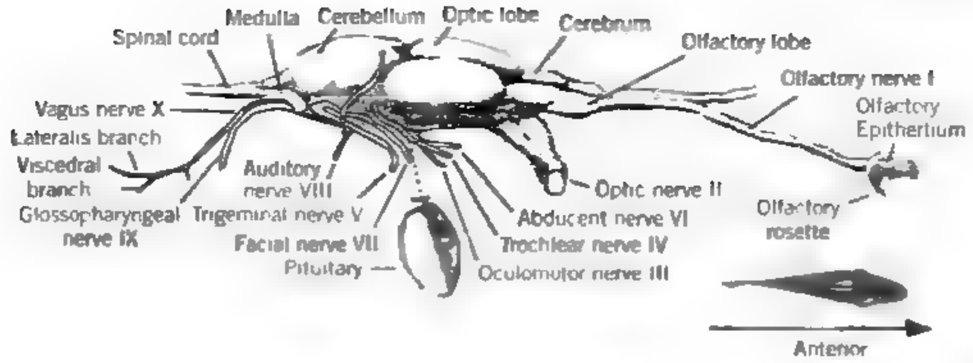


Figure 7. Brain. *Source:* Ref. 3.

plexus, which serves to nourish the central nervous system, is found in the midbrain. It is filled with cerebrospinal fluid and is richly supplied with blood vessels.

The cerebellum has a role in swimming equilibrium, muscle tone maintenance, and fish orientation. The lateral line and sense organs are associated with the cerebellum. The division between the medulla oblongata and the spinal cord is not distinct. All sensory nerves except smell (I) and sight (II) lead to this center. This acts as a relay center between the spinal cord and the higher brain areas. It also controls certain somatic and visceral functions including respiration, osmoregulation, and swimming equilibrium.

As in other vertebrates the gray matter makes up the central region of the spinal cord and consists of nerve cells. White matter, which surrounds this core, contains the myelinated nerve fibers. The spinal cord receives sensory, afferent (to the brain) fibers by way of the dorsal roots of the spinal nerves and gives off the efferent (from the brain) motor fibers to the ventral roots of the spinal nerves.

Peripheral Nervous System

The peripheral nervous system provides a means of communication from the environment where stimuli are received by sensory organs, to the central nervous system and from the central nervous system to the proper effector organs in the body, muscles, or glands. The spinal nerves are paired and carry sensory and motor fibers from and to the fish body. Ganglia act as relay centers along the spinal nerves. Fish have 10 cranial nerves (higher vertebrates have 12). They are similar to spinal nerves except they do not have a ventral or dorsal root and they emerge from the skull. Some cranial nerves are strictly sensory (afferent) and some are strictly motor (efferent).

The autonomic nervous system is part of the peripheral nervous system that innervates smooth muscle, heart muscle, and glands. Within this system is the sympathetic nervous system, made up mostly of spinal nerves, and the parasympathetic nervous system, made up of cranial nerves. The autonomic nervous system controls many of the vegetative (slow, routine, and automatic) systems of the body. As in mammals, the innumerable voluntary functions of the body are controlled in various centers of the brain.

Coordination within the nervous system involves matching the input stimuli to appropriate actions. Those actions may involve the regulation of heart rate, for example, with various feedback of important information from the cardiovascular system. It involves the coordination of gross behavior such as schooling, where inputs from visual and acoustical sensors are coordinated with gross muscular action involved in swimming, which in itself involves a balancing act involving the labyrinths, fin positions, and trunk movement.

Sensory Organs

Fish have an acute sense of smell and rely on the olfactory organ to find food, avoid predators, and to help guide migratory fish to their spawning grounds. Some fish utilize pheromones, chemical substances that can be smelled, to signal alarm to the rest of the school. Catfish, which have an excellent sense of smell, use the specific odor of the slime of individual fish to differentiate fish in a school hierarchy. As the water passes through the nostril over a transverse septum, olfactory epithelium connected to the olfactory nerve signals the smell response to the olfactory lobes in the brain.

Taste buds are the gustatory organs and can be found in the epithelium of the mouth, lips, esophagus and snout. Some fish, mainly those that are not sight feeders, may have taste buds at other places on the body. Free nerve endings from the cranial nerves appear in sensitive regions, suggesting that touch works with taste to stimulate the feeding response. External taste receptors respond to monovalent ions and, to a lesser degree, divalent ions. Internal receptors respond to amino acids, sugars, strong salts, and acids. Both taste and smell are based on organic chemoreception. It also appears that tactile, chemical, and temperature sensations are closely integrated in fish skin.

Sight is extremely important to salmonids and other fish that depend on this sense to detect movements and color changes that signal prey, predator, and mate. The eyes of most bony fishes have a spherical lens, the most powerful shape for a single lens, that in some cases can be changed in convexity and in distance from the retina, the visual layer of the eye (Fig. 8). This is how the fish focus, because very little focusing is done by the cornea. There is little difference in the refractive index, the source of light diffraction, between the cornea and water and, therefore, little focusing is necessary. The cornea does play a role in protecting the rest of the eye from physical pollutants and harm and may help prevent dehydration. The iris controls the amount of light getting through to the retina. Most fish

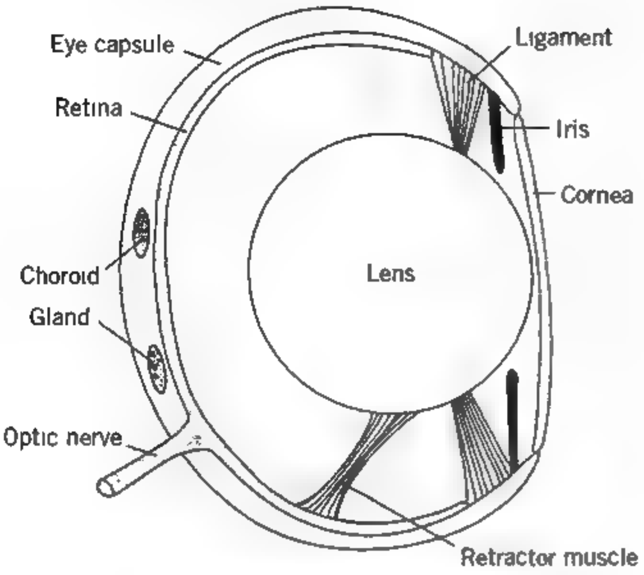


Figure 8. Cross section of a fish eye. *Source:* Ref. 2.

(eg, elasmobranchs) have a fixed iris that contains guanine and melanin. Sight feeders, such as the salmonids, have a well-developed lens muscle and focus by actually moving the lens. The eyeball is ellipsoid and lends a great depth of field, that is the sharp simultaneous focus of objects that are distant and near. This is due to a wide-angle configuration of the components: the flattened eyeball, the round lens, and the protrusion of the lens out of the iris. Light travels through the lens and is focused on the retina. Retinal layers such as the optic nerve fibers, ganglion cells, bipolar cells, and photoreceptor cells, (the cones and rods) all have a role in converting the light to sight images in the brain. The rod cells are sensitive to low light levels but not to color. The cone cells react to strong light and are color sensitive. Photochemical reactions in these cells are transmitted as electrical impulses to the optic nerve and eventually to the optic lobe in the brain. The sensitivity of the retina to particular colors of light depends on the pigments found in the rods and cones in the retina.

The small pineal organ serves a visual as well as a secretory role in fish. It is a small fingerlike organ is located just under the cranium on the dorsal midline of the brain. It serves possible functions in the detection of day length. While it is not vital to life, it may serve an important role in the adjustment of body color to the environment as well as in the synchronization of physiological changes associated with smoltification, in anadromous salmonids, with day length.

The inner ear functions in both equilibrium and hearing. It is found on either side of the medulla oblongata of the brain. The inner ear is made up of two sections, the pars superior and the pars inferior. The pars superior has three semicircular canals filled with fluid and has an ampullae and utriculus. The ampullae contains the receptor tissue, which has sensory cells with long sensory hairs similar to taste buds, and the lateral line. Displacement of the body causes the sensory cells to react and the proper righting mechanism to take place. The utriculus of the pars superior contains an otolith; a calcarious stone. This stone lies on the sensory hairs and responds to the force of gravity. This system works with the retina for maintenance of balance. The pars inferior is the structure of actual sound reception and it is composed of the two vesicles, sacculus and lagena. These vesicles, containing otoliths, are innervated by the auditory nerve. The otoliths will vibrate at a different resonance then the rest of the body when presented with sound waves. This triggers the sensory hairs to send a nervous impulse up the cranial nerve.

The lateral line is a set of sense organs found only in fishes and the aquatic stages of amphibians. The lateral line is innervated by cranial nerves and is closely associated with the auditory centers. The brain relays secondary impulses from the lateral line to the hypothalamus, thalamus, the optic tectum, and the cerebellum. The lateral line helps in the detection of prey, predators or mates by sensing hydrodynamic displacement. The lateral line receptors respond to pressure waves and low frequency vibrations and other localized disturbances.

ENDOCRINE SYSTEM

The endocrine system coordinates physiological processes with each other as well as with the external environment by way of chemical messengers called hormones. The hormones are released in response to changes such as light, temperature and stress. They ellicit and coordinate the appropriate responses from parts of the body, whether it be by a generalized stress response, a fright reaction, maturation of the gonads, smoltification, migration or spawning. Hormones thus play important roles in many physiological processes such as reproduction, osmoregulation, mineral metabolism, and growth. Endocrine glands are ductless tissues that release hormones into the blood.

The effects of hormones may be slow but are persistent in contrast to the rapid and transient action of nerves. It is important to realize the close relationship between the endocrine system and the nervous system. These two systems work together to create a communication network with each system dependent on the other for complete integration. Neurohormones are released by nerve cells, not endocrine glands, but have similar actions. They are carried by the blood to a target organ or to an endocrine gland, which can, in turn, release its own hormone. Neurohormones act as a link between the endocrine and nervous system.

Pituitary Gland

The pituitary gland lies close to the hypothalamus in the brain. It is made up of the infundibulum, the down growth of tissue from the brain, and the hypophysis, the upgrowth of tissue from the roof of the mouth. The hypophysis is differentiated into the adenohypophysis and the infundibular tissue. The adenohypophysis is the site of synthesis, storage, and release into the blood of several different peptide hormones. The infundibular, or neural, tissue forms the neurohypophysis, which is connected to the brain by the infundibular stalk. The neurohypophysis is not a discrete tissue in teleosts and appears to be a storage-release center for the materials that are synthesized in the hypothalamus and then transported to the neurohypophysis along neurosecretory axons.

Growth hormone increases appetite, improves food conversion, increases protein synthesis, decreases nitrogen loss, stimulates fat mobilization and oxidation, and stimulates insulin synthesis and release. Prolactin is necessary for electrolyte and water balance in fresh water. It reduces water absorption and reduces sodium movement through the gill, kidney and urinary bladder. It may also cause the dispersal of xanthophore pigment in the skin of some fish. It is involved in lipid metabolism fat storage and reduces thyroxine levels of the serum. Thyroid stimulating hormone (TSH) regulates thyroid function and is controlled by negative feedback from the levels of circulating thyroid hormones. Adrenocorticotropic hormone regulates the release of adrenocorticoids (steroids) from the interrenal tissue of the kidney. Melanophore stimulating hormone has a small effect on external body pigmentation in teleost. Fish also have sympathetic innervation of melanophores. Gonadotropic hormone, luteinizing hormone, and, to a minor extent, follicle stimulating hormone, have crucial roles to play in gonad development and spawning. Oxytocin is involved in reproduction: egg formation, mating, and spawning. Vasopressin affects diuresis and, therefore, assists in osmoregulation.

It is clear that the pituitary gland is the major gland in the endocrine system, exerting a large influence on processes of the body such as growth, osmoregulation, and reproduction. Target organs of the pituitary hormones are the thyroid gland, interrenal tissue, testis, and ovary. The synthesis of stimuli from the environment and subsequent hormonal release demonstrates the link between the central nervous system and the endocrine system of the body. That link is the hypothalamus–pituitary axis. The hypothalamus receives external stimuli and communicates changes to the adenohypophysis of the pituitary, in close proximity, where the release of the appropriate hormone is elicited.

Thyroid Gland

Thyroid tissue is generally scattered throughout the pharyngeal area, in the head kidney, and in some cases around the eye. The hormones secreted by the thyroid are thyroxine (T4) and triiodothyronine (T3). Thyroid action is linked closely to the pituitary, which secretes TSH. Secretion of TSH is under negative feedback control depending on the levels of circulating thyroid hormones. There also appears to be a strong relationship between thyroid activity and the lunar cycle. Peak thyroxine levels have been shown at the period of the new moon, which is also when peaks in the outward migration of coho salmon have been observed. The surge of thyroxine influences growth, migratory restlessness, and seawater preference.

Ultimo Branchial Gland

This gland is equivalent to the parathyroid gland in terrestrial mammals. It is generally located between the abdominal cavity and sinus venosus. Calcitonin is produced by this gland, which regulates calcium by controlling its conservation and mobilization.

Pancreas

The pancreas of teleost is usually diffuse and scattered around the intestine and spleen, and often extends into the liver. Insulin and glucagon are the two hormones associated with this gland. The action of the pancreas appears to be modified by different nutritional states, particularly in response to changes in protein metabolism. Insulin affects amino acid metabolism and incorporates amino acids into skeletal muscle and may not be as important to glucose homeostasis in teleosts as it is in mammals. It decreases the rate of gluconeogenesis. It may have some role in saltwater survival. Glucagon simulates gluconeogenesis from muscle amino acids.

Renal Tissue and Interrenal Tissue

A number of hormones and tissues are involved in kidney function. Those include renin, angiotensin, and antidiuretic hormone. Their functions are covered in the section on the renal system. Interrenal tissue is located in the anterior kidney tissue, or the head kidney. The primary hormones released are corticosteroids. Under control of the pituitary through the action of adrenocorticotropic hormone (ACTH), the corticosteroids are released in response to appropriate stimuli. Chromaffin cells, the functional equivalents of medullary tissue of the adrenals in mammals, secrete the catecholamines, adrenaline, and noradrenaline.

Corticosteroids such as cortisol are important hormones produced in the interrenal glands. Interrenal tissue response increases with stressful conditions, such as temperature change, exposure to toxic elements, and spawning. This elicits a subsequent increase in cortisol levels. Cortisol is important in regulation of water and electrolyte balance by affecting ATPase activity and sodium flux across the gills. It is known as the saltwater adapting hormone, because it increases renal sodium retention, reduces glomerular filtration rate, and increases permeability of urinary bladder. It is important in smoltification and subsequent saltwater acclimation. Cortisol is also involved in metabolism, by promoting gluconeogenesis and related carbohydrate and protein metabolism. It also plays a role in preventing exhaustion by mobilizing energy reserves. Corticosteroids influence oocyte maturation and seem to be influenced by pituitary gonadotropins.

Catecholamines, such as adrenaline and noradrenaline, respond immediately to stress. Catecholamines cause hyperglycemia (high blood glucose), with a reduction in liver and muscle glycogen in some cases. They also cause systemic vasoconstriction, vasodilation in the gills, and increase the force and rate of the heartbeat.

REPRODUCTIVE SYSTEM

The reproductive systems and strategies in fishes are extremely varied. Examples of the salmonid family are emphasized here. Salmonids reproduce sexually. Pacific salmon spawn only once in their life cycle, whereas the trouts and Atlantic salmon spawn repeatedly during their life span. Unlike other fish, lipid levels have little to do with initiating spawning activity or gamete development in the salmon. Lipid levels are, however, correlated to the distance that animals have to migrate up freshwater streams to reach their spawning grounds. Lipids as well as body proteins are consumed in this migration. The primary environmental factor involved in sexual maturation is day length. The changes in day length are probably more important than absolute day length in the processes that affect reproduction. Temperature also plays an important role in gonadal development in some fishes.

Both the testes and ovaries are located along the midline of the fish, ventral to the swim bladder, extending from the anterior to the posterior end, terminating at the vent. Eggs are commonly released into the peritoneal cavity and reach the outside through a funnel and ovarian duct. Sperm almost always stay inside ducts until they are released.

Egg Size and Fecundity

In general, fish that lay smaller eggs lay many of them. Cod, for instance, lay millions of eggs that incur a high mortality rate. Smaller eggs also tend to hatch in a relatively short time and the hatched young require microscopic food almost immediately because the stored nutrients in the way of yolk is limited or nonexistent. Conversely, fish that lay larger eggs lay fewer eggs that take longer to hatch and have higher survival rates. The young also can survive on the yolk for days or weeks and can ingest large food particles when feeding begins.

There is a correlation between the length of parental care and egg size. That is, the larger and fewer the eggs, the greater the parental care of the young. Herring spawn millions of eggs in kelp beds and abandon them. Salmon lay fewer, larger eggs and bury them in the gravel but still die and do not tend to their young. In the other extreme, some fish produce only a few eggs that are incubated in the body and live young are born that are more or less ready to fend for themselves. The yolk material is solid in salmonids. Their eggs sink in water because they are very dense. The yolk material influences the density and the development time. There is only about 30% water in the yolk material of salmon, whereas the herring egg has oil droplets in the yolk, thus allowing the egg to float. Oviparous fish are egg layers such as salmon, viviparous fish are placental live-bearers as mammals, and ovoviviparous fish are live-bearers but are passive parents.

Hormones

Gametogenesis, the formation of gametes, is dependent on pituitary hormones called gonadotropins. The gondadotropins are leutenizing hormone (LH) and follicle stimulating hormone (FSH). Blocking gonadotropins reduces gametogenesis. These names are taken from mammalian sources. Although both, or analogues of both, may be present in fish, it is LH that predominates. This hormone appears to regulate the maturation and release of the egg. The follicles probably produce the estrogens, estrone, and estradiol under hormonal control from the pituitary gonadotropin. These hormones control the development of secondary sex characteristics in the female fish. They also serve an important function in the production of vitellogenin in the liver. Vitellogenin is then taken up from the blood and incorporated into the yolk proteins. Progesterone, which maintains the uterine development during pregnancy in mammals, may be absent in fish. The corticosteroids testosterone and androstenodione may control the secondary sex characteristics in the male fish.

Gonads and Gamete Formation

The early development of gametes is similar to other vertebrates. The processes of spermatogenesis, (sperm development) and oogenesis (egg development) proceed in the following manner. In spermatogenesis a diploid spermatogonium, through meiosis, gives rise to haploid spermatocytes, which then become spermatids. A process called spermiogenesis is the final step of differentiation of sperm from spermatids. As Figure 9 shows, the sertoli cells serve as nutritive cells. During this process, the cysts swell and finally rupture, releasing the sperm to the lumen, which is continuous with the sperm duct. The interstitium between the cysts contain interstitial cells, fibroblasts, and blood vessels. In oogenesis, cells destined to become eggs proliferate, enlarge, and each becomes surrounded with follicular, or nutritive cells. The granulosa layer of the follicle cells is thought to give rise to the yolk. The proteins in the yolk are derived from vitellogenin as discussed above. The development of all eggs in the salmonid occurs synchronously, that is all at once.

Ovulation

Ovulation, or the release of all the eggs from the follicles, is triggered by the completion of yolk deposition. Once the ova leaves the follicle, they lose the supply of nutrients provided by the follicular cells. They depend on the ovarian

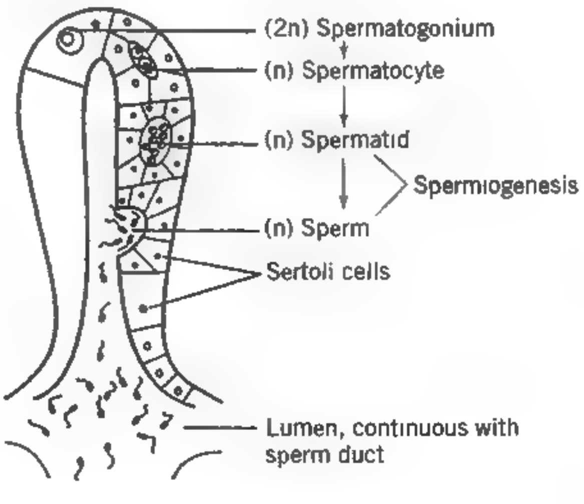


Figure 9. Spermatogenic cyst with several spermatogonial cells.

fluid for nutrients and oxygen. The origin, quantity, and turnover rate of the ovarian fluid are unknown, although it could be a dilute ultrafiltrate of plasma.

Spawning

Spawning follows soon after ovulation. The range of spawning behavior as well as the spawning itself in fish is probably wider than that in all land vertebrates put together. This event is probably controlled by a combination of hormones and environmental factors. There may be a special spawning hormone acting directly on the central nervous system. Mammalian reproduction involves both the pituitary gonadotropins, LH and FSH, affecting the gonads and the ovary, which respond by producing estrogen and progesterone, whereas the LH hormone activity in fish predominates and FSH activity is absent or weak in some species.

Fertilization

Fertilization is also varied among species. Generally, one sperm enters the egg via a micropyle, which closes after entry (Fig. 10). This prevents multiple fertilization. Thereafter, the two nuclei unite. The chorion, the tough outer shell, or protective coating, of the egg swells by the egg imbibing water. This water hardening provides the egg with a protective shell permeable to dissolved substances, gases, and water. Water hardening produces a perivitelline space between the cell membrane and the external environment. Eggs and sperm survive only a short time if fertilization does not take place.

Development

Developmental time depends on environmental conditions. The important factors are temperature, salinity, oxygen, and light. All conditions have varying effects, depending on species. The following outline shows the major steps in the development of the fish, from embryonic to adult stages (Fig. 11). It is similar in pattern to the general pattern in vertebrate development.

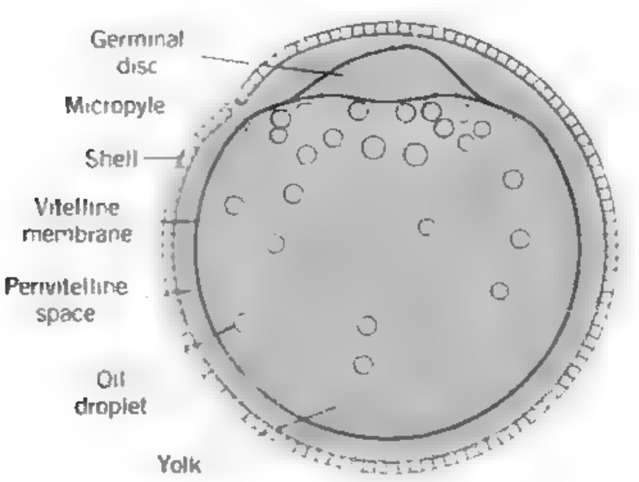


Figure 10. Cross section of a salmonid egg.

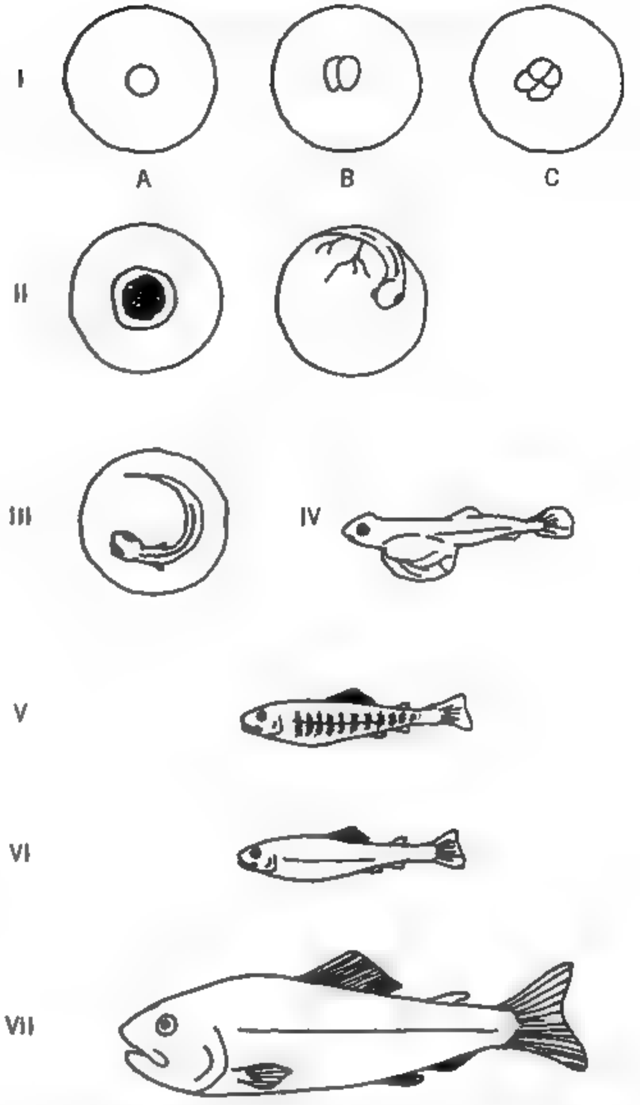


Figure 11. Development stages of a salmonid. I = blastic cleavage; cell division is shown in A, B and C. Cell division continues. II = gastrulation, III = organogenesis, IV = hatching, V = parr, VI = smolt, VII = juvenile to adult.

I. Blastodisc cleavage (2 cells)
II. Gastrulation
  Specialization of cells
  Body tissues develop and grow over the yolk
  Eyed stage
  Basic body form is evident
III. Organogenesis
  Internal organs form
  Fins appear
  Circulatory system develops connection between yolk and internal vasculature
IV. Hatching
  Hatching glands develop around head of embryo and secrete chorionase, an enzyme that dissolves

the tough chorion, or egg shell, for the embryo to escape.

V. Parr
  General body form resembles that of the adult
  Dark vertical bars along the lateral body wall
VI. Smolt
  Parr marks disappear
  Body coloration is silver
  Body shape is slightly more elongate than smolt
VII. Juvenile to adult
  General body shape and color is like the smolt
  Size increases as it grows and matures at sea

IMMUNE SYSTEM

The immune system comprises the defense mechanisms of the body that prevent or control disease-producing organisms from entering the body and causing disease. The primary problem of the immune system is to recognize self from nonself. Self refers to all normal physical and chemical components of the body and nonself is everything else. The latter may include bacteria, parasites, viruses, and tumors.

Before discussing immune system in detail, a few key terms should be defined. Immunocompetence is the ability of a fish to mount a specific immune response against a foreign body or agent. The first stages of immunocompetence in salmonid fishes start at 1–2.5 g body weight. It is fully developed at 4 g. Immunological tolerance refers to a condition where the host does not recognize an antigen as being foreign and, therefore, tolerates its presence. Vaccination should not be conducted before immunocompetence is established, or immunotolerance may result. An antibody is a protein manufactured by white blood cells (WBC) that are designed to bind to an antigen for which it was made. An antigen is any substance, usually foreign to the body that is capable of causing the activation of an immune response.

The immune system is made up of two kinds, or lines, of defenses. The first is the non-specific defense and the second is the specific defense. The nonspecific defense mechanisms are also divided into two categories of those involved in preventing nonself substances from entering the body and those that deal with substances that manage to enter the body. Nonspecific defense mechanisms are mounted against any foreign substance, whereas the specific defense mechanisms are designed to attack very specific antigens. Usually the term immune system refers to this last category.

Nonspecific Defense Mechanisms

Physical and chemical barriers to pathogen entry include the mucus on the skin and the skin itself. Mucus is continuously produced from goblet cells and it provides both a physical and chemical barrier to pathogen entry. It sloughs away pathogens and contains antibacterial proteins such as lysozymes, agglutinins, and lysins. Both the epithelium and the scales that make up the skin provide an effective physical barrier to pathogens. Some pathogens, however, do enter the body. Once the pathogens are inside, the following components of the nonspecific immune system work to limit growth and multiplication of the pathogen. Serum transferrin is a protein that keeps free iron concentrations low. Growing and replicating bacteria need iron. C-reactive protein is a nonspecific agglutinating protein that coats the surface of the antigen and allows phagocytosis to occur more readily. Interferon is a soluble substance that acts against viruses. It prevents viral DNA replication in an infested host and cell. Cellular nonspecific defenses are performed by certain WBC such as neutrophils, macrophages and natural killer cells.

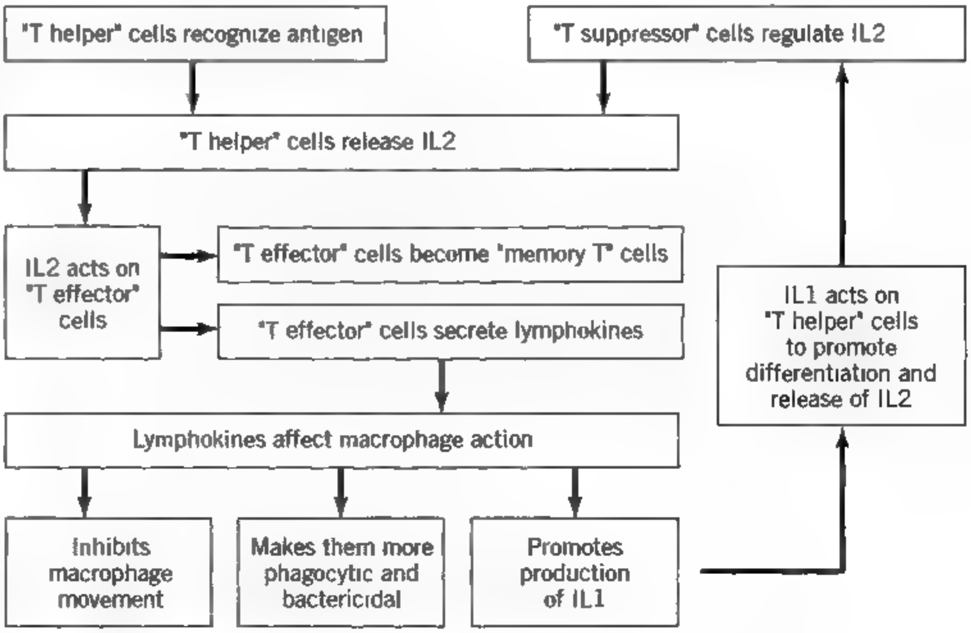

"T helper" cells recognize antigen
"T suppressor" cells regulate IL2
"T helper" cells release IL2
IL2 acts on "T effector" cells
"T effector" cells become "memory T" cells
"T effector" cells secrete lymphokines
IL1 acts on "T helper" cells to promote differentiation and release of IL2
Lymphokines affect macrophage action
Inhibits macrophage movement
Makes them more phagocytic and bactericidal
Promotes production of IL1

Figure 12. 1. Recognition of antigen by T helper (Th) cell. 2. Th cell releases a soluble messenger cell called interleukin (IL2). 3. Interleukin 2 acts on T effector cells. 4(a). T effector cells secrete lymphokines. 4(b). T effector cells begin differentiating to memory T cells. 5. Lymphokines mediated macrophage action, which results in antigen or antigen-host cell destruction. 6(a). Lymphokine promotes macrophage production of another interleukin (IL1). 6(b). Migration inhibition factor, a lymphokine, inhibits macrophage movement away from the infection locus where antigen was recognized. 6(c). Another lymphokine activates macrophages and makes them more rapidly phagocytic and more strongly bactericidal. 7. IL1 acts on Th cells to promote their differentiation and the release of more IL2 This action is regulated by T suppressor cells.

Specific Defense Mechanisms

There are two types of specific defense mechanism. Humoral immunity defense involves the production of specific immunoglobulins (Ig), which are globulin proteins that have antibody activity. The cellular immunity involves the stimulation of certain white blood cells to destroy specific antigens. The organs involved include the anterior kidney, thymus and spleen. The head kidney and the spleen have antibody-producing cells as well as phagocytes and macrophages. The spleen produces lymphocytes. Fishes lack bone marrow, and the above tissues act, in place, to supply the necessary elements for a functional immune system. Fish immunoglobins are tetramer molecules as opposed to the pentamers in humans and there is only one class of immunoglobins in fish compared to the two to five classes in mammals. These proteins are found in lymph, blood, mucus, and interstitial fluids.

Humoral Immunity. In this type of specific immune response, antigens are selectively removed from the circulation by the use of antibody-producing white blood cells, which are specific to particular antigen. There are two types of cell involved in humoral immunity. Some of the cells produce the antibody and some act as memory cells. Once exposed to a particular antigen, the memory cells can rapidly grow and multiply when they encounter that antigen again.

Cellular Immunity. In this type of specific immunity, the antigen causes certain groups of primed white blood cells to act specifically against the invading antigen. These WBCs can ingest or destroy the antigen as well as proliferate chemical messengers signaling the presence of nonself material. It seems that immersion-vaccination results in eliciting a particularly strong cellular immunity. This type of immunity is also referred to as cell mediated immunity. The following are various cell types involved in cell mediated immunity.

Neutrophils are the first line of defense after antigen invasion. These cells are phagocytic in humans and some fish such as catfish, but they are not in salmonids. They may release a toxic substance in the vicinity of the antigen. They are abundant in the WBC picture. Macrophages have a phagocytic function and also play a significant role as a secretory immune cell. They are more like the human neutrophil in their phagocytic and bactericidal action. Special processing macrophages process and present the antigen to the lymphocytes. The antigen is taken up by these macrophages and processed internally. The antigen is then brought to the surface for lymphocytes to destroy. T-cell lymphocytes are antigen-specific lymphocytes that send chemical messengers that enhance phagocytic and bactericidal activity of macrophages. T-cells do not engulf pathogens, although they do have receptors for antigens. They also enhance macrophage activity. The activities of T cells are restricted by the fact that the antigen must be first processed by a macrophage and presented to this lymphocyte. They are unable to respond directly to free antigen. Their response to an antigen presented to them is to differentiate into effector T-cells and memory T-cells. The coordinated action of the various components of the specific immune system is demonstrated in Figure 12.

BIBLIOGRAPHY

1. D. J. Randall, *American Zoologist* 8, 179–189 (1968)
2. G. A. Wedemeyer, F. P. Meyer, and L. Smith, *Diseases of Fishes, Book 5: Environmental Stress and Fish Diseases*, TFH Publications, Neptune, N.J., 1976.
3. L. Smith and G. Bell, *A Practical Guide to the Anatomy and Physiology of Pacific Salmon*, Misc. special publication of Environment Canada, Fisheries and Marine Service, Ottawa, 1975.

GENERAL REFERENCES

Reference 3 is a good general reference.

P. J. Bentley, *Comparative Vertebrate Endocrinology*, Cambridge University Press, London, 1976.
P. J. Bentley, *Endocrines and Osmoregulation*, Springer-Verlag, New York, 1971.
C. E. Bond, *Biology of Fishes*, W. B. Saunders Co., Toronto, 1979.
C. P. Hickman, Jr., and B. F. Trump, *Excretion, Ionic Regulation, and Metabolism*, in W. S. Hoar and D. J. Randall, eds., *Fish Physiology*, Vol. 1, Academic Press, Inc., Orlando, Fla., 1969.
W. S. Hoar and D. J. Randall, eds., *Fish Physiology, Vol. IV: The Nervous System, Circulation and Respiration*, Academic Press, Orlando, Fla., 1970.
W. S. Hoar and D. J. Randall, eds., *Fish Physiology, Vol. II: The Endocrine System*, Academic Press, Orlando, Fla., 1969.
K. F. Lagler, J. E. Bardach, R. R. Miller, and D. R. May Passino, *Ichthyology*, John Wiley & Sons, Inc., New York, 1977.
A. J Matty, *Fish Endocrinology*, Croom Helm, London, 1985.
P. Moyle and J. J. Cech, Jr., *Fishes: An Introduction to Ichthyology*, Prentice-Hall Press, Englewood Cliffs, N.J., 1982.
C. Pincher, *A Study of Fish*, Duell, Sloan and Pearce, New York, 1948.
G. H. Satchell, *Circulation in Fishes*, Cambridge Press, 1971.
L. Smith, *Introduction to Fish Physiology*, F. F. H. Publications, 1982.

GEORGE KATSUSHI IWAMA
University of British Columbia
Vancouver, British Columbia
Canada

FISHES: SPECIES OF ECONOMIC IMPORTANCE

This article consists of brief descriptions of fish species of importance to either the commercial or sport fisheries in North America. The description of the each species consists of information about their physical appearance, notes about how they are caught and what food value they hold, their distribution, and their reproductive biology. The common and scientific names used in this article comply with published information (1).

In some cases, limiting the selection of species to those found in North America and of commercial or sport importance restricted the representatives of a particular genus or family. In most cases, however, the omitted species of any genus share most of the major characteristics of those

Specific Defense Mechanisms

There are two types of specific defense mechanism. Humoral immunity defense involves the production of specific immunoglobulins (Ig), which are globulin proteins that have antibody activity. The cellular immunity involves the stimulation of certain white blood cells to destroy specific antigens. The organs involved include the anterior kidney, thymus and spleen. The head kidney and the spleen have antibody-producing cells as well as phagocytes and macrophages. The spleen produces lymphocytes. Fishes lack bone marrow, and the above tissues act, in place, to supply the necessary elements for a functional immune system. Fish immunoglobins are tetramer molecules as opposed to the pentamers in humans and there is only one class of immunoglobins in fish compared to the two to five classes in mammals. These proteins are found in lymph, blood, mucus, and interstitial fluids.

Humoral Immunity. In this type of specific immune response, antigens are selectively removed from the circulation by the use of antibody-producing white blood cells, which are specific to particular antigen. There are two types of cell involved in humoral immunity. Some of the cells produce the antibody and some act as memory cells. Once exposed to a particular antigen, the memory cells can rapidly grow and multiply when they encounter that antigen again.

Cellular Immunity. In this type of specific immunity, the antigen causes certain groups of primed white blood cells to act specifically against the invading antigen. These WBCs can ingest or destroy the antigen as well as proliferate chemical messengers signaling the presence of nonself material. It seems that immersion-vaccination results in eliciting a particularly strong cellular immunity. This type of immunity is also referred to as cell mediated immunity. The following are various cell types involved in cell mediated immunity.

Neutrophils are the first line of defense after antigen invasion. These cells are phagocytic in humans and some fish such as catfish, but they are not in salmonids. They may release a toxic substance in the vicinity of the antigen. They are abundant in the WBC picture. Macrophages have a phagocytic function and also play a significant role as a secretory immune cell. They are more like the human neutrophil in their phagocytic and bactericidal action. Special processing macrophages process and present the antigen to the lymphocytes. The antigen is taken up by these macrophages and processed internally. The antigen is then brought to the surface for lymphocytes to destroy. T-cell lymphocytes are antigen-specific lymphocytes that send chemical messengers that enhance phagocytic and bactericidal activity of macrophages. T-cells do not engulf pathogens, although they do have receptors for antigens. They also enhance macrophage activity. The activities of T cells are restricted by the fact that the antigen must be first processed by a macrophage and presented to this lymphocyte. They are unable to respond directly to free antigen. Their response to an antigen presented to them is to differentiate into effector T-cells and memory T-cells. The coordinated action of the various components of the specific immune system is demonstrated in Figure 12.

BIBLIOGRAPHY

1. D. J. Randall, *American Zoologist* 8, 179–189 (1968)
2. G. A. Wedemeyer, F. P. Meyer, and L. Smith, *Diseases of Fishes, Book 5: Environmental Stress and Fish Diseases*, TFH Publications, Neptune, N.J., 1976.
3. L. Smith and G. Bell, *A Practical Guide to the Anatomy and Physiology of Pacific Salmon*, Misc. special publication of Environment Canada, Fisheries and Marine Service, Ottawa, 1975.

GENERAL REFERENCES

Reference 3 is a good general reference.

P. J. Bentley, *Comparative Vertebrate Endocrinology*, Cambridge University Press, London, 1976.

P. J. Bentley, *Endocrines and Osmoregulation*, Springer-Verlag, New York, 1971.

C. E. Bond, *Biology of Fishes*, W. B. Saunders Co., Toronto, 1979.

C. P. Hickman, Jr., and B. F. Trump, *Excretion, Ionic Regulation, and Metabolism*, in W. S. Hoar and D. J. Randall, eds., *Fish Physiology*, Vol. 1, Academic Press, Inc., Orlando, Fla., 1969.

W. S. Hoar and D. J. Randall, eds., *Fish Physiology, Vol. IV: The Nervous System, Circulation and Respiration*, Academic Press, Orlando, Fla., 1970.

W. S. Hoar and D. J. Randall, eds., *Fish Physiology, Vol. II: The Endocrine System*, Academic Press, Orlando, Fla., 1969.

K. F. Lagler, J. E. Bardach, R. R. Miller, and D. R. May Passino, *Ichthyology*, John Wiley & Sons, Inc., New York, 1977.

A. J Matty, *Fish Endocrinology*, Croom Helm, London, 1985.

P. Moyle and J. J. Cech, Jr., *Fishes: An Introduction to Ichthyology*, Prentice-Hall Press, Englewood Cliffs, N.J., 1982.

C. Pincher, *A Study of Fish*, Duell, Sloan and Pearce, New York, 1948.

G. H. Satchell, *Circulation in Fishes*, Cambridge Press, 1971.

L. Smith, *Introduction to Fish Physiology*, F. F. H. Publications, 1982.

George Katsushi Iwama
University of British Columbia
Vancouver, British Columbia
Canada

FISHES: SPECIES OF ECONOMIC IMPORTANCE

This article consists of brief descriptions of fish species of importance to either the commercial or sport fisheries in North America. The description of the each species consists of information about their physical appearance, notes about how they are caught and what food value they hold, their distribution, and their reproductive biology. The common and scientific names used in this article comply with published information (1).

In some cases, limiting the selection of species to those found in North America and of commercial or sport importance restricted the representatives of a particular genus or family. In most cases, however, the omitted species of any genus share most of the major characteristics of those species represented in this review. The amount of information given here about a particular species is largely a function of the body of published knowledge about that fish. This is the reason for any uneven treatment among species. The salmonlike fishes, for example, are probably the most intensively studied family of fishes in the world. The body of knowledge is consequently much larger than a group of fishes such as the ocean perches. Measured units such as body size, weight, fecundity, and age at sexual maturity are estimates in many cases, and are based on measurements on a few individuals. They should, therefore, be considered as rough estimations of the average value. The reference list at the end of the article contains reviews articles and books relevant to the species covered in this article.

ANCHOVY

The anchovies belong to the family Engraulidae. They belong to the same order as the herrings, order Clupeiformes. The anchovies are a schooling fish that are almost exclusively marine, except for some populations that migrate into or become landlocked in freshwater systems. The three species found in North American waters are the northern anchovy, the striped anchovy, and the silver anchovy. The northern anchovy is found off the Pacific Coast and the latter two are Atlantic species that are relatively rare in North America. The anchovies are typically found in temperate to tropical waters of Pacific, Atlantic, and Indian Oceans. They are a small and silver fish that usually measure less than 15 cm, although they have been reported as large as 50 cm. They have characteristically silvery sides with greenish blue to gray backs and a bright strip along the midlateral flank. The anchovies have no lateral line.

The northern anchovy (*Engraulis mordax mordax*) is presently of commercial importance in California. Their distribution extends from Cape San Lucas in Baja California to the Queen Charlotte Islands off the British Columbia coast. The preferred temperature range seems to be between 14.5 and 18.5°C. Although there was a sufficient abundance of northern anchovy off the British Columbia coast in the 1940s, stocks have declined to a point that a viable fishery cannot be sustained there. Populations off the California coast have been increasing in the last 20 yr, where they are captured in schools with nets for the fresh, cured, or canned markets. They have also been caught for the bait and fish meal markets.

Evidence from California, shows that the mature anchovy may spawn several times each year. Spawning takes place at temperatures between about 13 and 17.5°C near the surface and at night. Several thousand eggs are broadcast and fertilized in the open waters. The eggs are ellipsoid and measure about 1.4 mm along the long axis and slightly less than 1 mm in diameter. As the embryo develops, it hangs upside down during development. At those temperatures, the eggs hatch in about two to four days and the alevins are 2.5–3 mm long. The warm waters also enhance the utilization of yolk, taking only about three days for the yolk sac to be absorbed. The young resemble the adults by the time they are about 25 mm long. They become sexually mature when they are about 13 cm. While about half of the two- to three-year-old fish will be mature, all at four years of age are sexually mature. They may live to be seven years old. Like other coastal fishes, the anchovies move to deeper waters during the winter and migrate toward the inshore during the summer. They also spend the daylight hours near the darker bottom and rise toward the surface at night.

The striped anchovy (*Anchoa hepsetus*) is particularly abundant from Chesapeake Bay to the West Indies and Uruguay. Only strays from those areas have ended up in the northern United States and Nova Scotia, Canada. The silver anchovy (*Engraulis eurystole*) is normally found in the Atlantic Ocean from Woods Hole, Mass. to Beaufort, N.C.

STRIPED BASS

The name bass is usually used in reference to the largemouth bass (*Micropterus salmonides*), which has the reputation for being the most popular game fish in North America. The striped bass (*Morone saxatilis*), while also being an extremely popular game fish, is classified under a different family (Fig. 1). Like groups of other fishes, the basses include a number of species that are quite different from each other. The striped bass is not hermaproditic as the sea bass is and it has only two spurs on the opercular bone instead of three. It, therefore, belongs to the Percichthyidae family instead of the Serranidae to which the sea bass belong.

There is no predictable way in which this animal can be caught because of its sporadic feeding habits. The fisherman may be lucky with a particular lure or bait one day but be frustrated without a bite on the identical tackle and bait the next day. The animal is caught with a wide variety of sport fishing tackle. The most common tackle is the spinning rod with a lure that resembles the shad. The striped bass can also be caught on the fly rod with streamers or casting with bait. While it may be caught in open lakes and reservoirs, the most popular locations are at the outfalls of hydroelectric power plants or at the foot of dams. The elusive fish attracts the avid fisherman of inland waters. The present record is a 26.9 kg striper taken from the Colorado River in 1977. In addition to its large size, the popularity of the striped bass as a sport fish is due to the fact that it feeds actively in warm as well as cold temperatures. It also actively feeds in daylight as well as in the dark. This popularity, however, has added to the plight of dwindling populations of this species in Atlantic coastal waters. The effects of pollution and mechanical destruction of their free-floating eggs in dams in combination with the increasing catch by sport fishing has imposed great pressures on populations of this fish.

Although the striped bass is found on both coasts of North America, its native habitat is the Atlantic Coast. The populations in California and Oregon are decedents of a handful of animals that were transplanted from the East Coast into the San Francisco Bay area between 1879 and 1881. The construction of dams, which trapped migrating

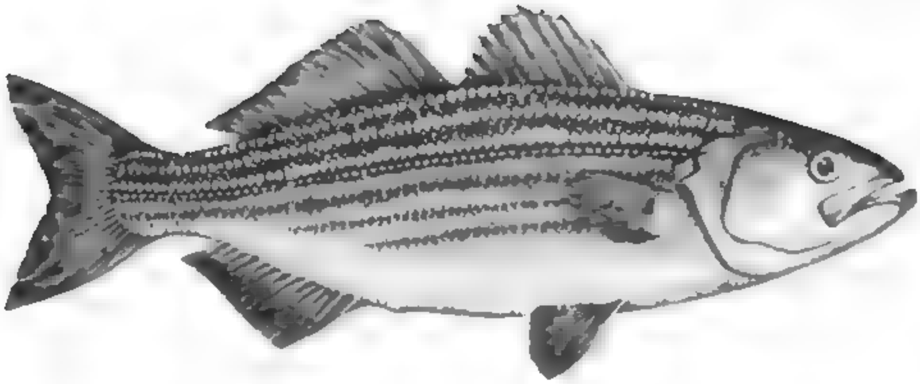


Figure 1. Striped Bass (*Morone saxatilis*). *Source:* Copyright 1990 by B. Guild-Gillespie.

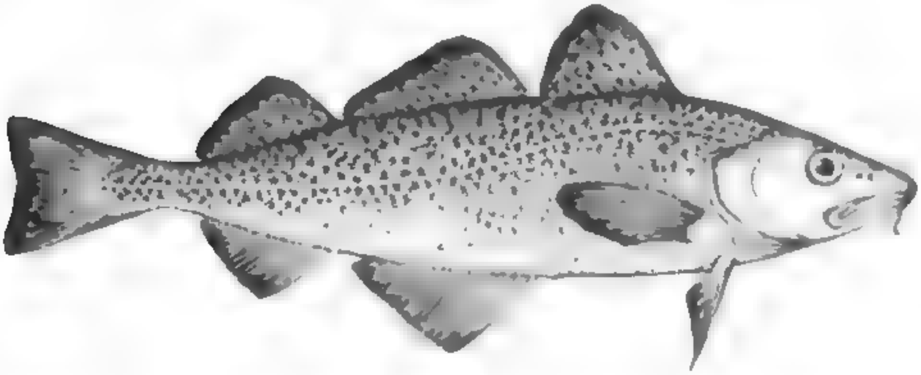


Figure 2. Atlantic cod (*Gadus morhua*). *Source:* Copyright 1990 by B. Guild-Gillespie.

populations of striped bass, demonstrated that these animals can adapt to a wholly freshwater existence. They can thrive without the saltwater phase of their life cycle. This, along with the development of successful rearing technology in hatcheries, led to the transplantation of populations to inland states throughout North America and to the establishment of successful fisheries in 17 inland states.

The striped bass is omnivorous. The juvenile fish feeds on planktonic organisms, such as copepods, and on insect larvae. After the animals are between 7.6 and 12.7 cm in length, they begin to capture and feed on smaller fishes. The adult will feed on a wider range of organisms including fish, worms, shrimps, and shellfish. The striped bass is an anadromamous fish. That means that, like the costal salmon species, it spends its adult life in salt water and migrates into freshwater streams to spawn. Unlike the salmon that migrate great distances in salt water, however, the bass's saltwater residence is confined to coastal waters.

The female produces about a million eggs, 1 mm in diameter, for every 4.5 kg of body weight. The females are always larger than the males and also live longer. The females can live up to 40 yr, reaching sizes of over 4.5 kg. All animals that are greater than 13.6 kg are almost exclusively females. With the increasing day length and warming temperatures of spring, the migration into freshwater streams occurs. Spawning generally takes place between 4-yr-old females and 2-yr-old males. The spawning takes place in open waters of the stream. The female broadcasts her eggs, which are fertilized by several males. Due to the oil content of the eggs, they are bouyant and float freely in the current of the river and later in the estuary. After a rather short incubation period of two or three days, the alevins hatch with yolk sacs. The alevins obtain nourishment from the yolk for the first four to six days, after which they resemble a small adult. Very much like the salmon young, the 5.1- to 7.6-cm young show vertical parr marks, which are eventually replaced by the characteristic horizontal stripes when they are about 6 in. in length. The juvenile then spends its growing and maturing period in the coastal marine environment until sexual maturity.

COD FISHES

The cods belong to the family Gadidae, which has three subfamilies, the Lotinae, the Phycinae, and the Gadinae. As Figure 2 shows, the cods have elongate bodies with large heads and mouths. They have well-formed caudal fins and may have two or three dorsal fins as well as one or two anal fins. They are normally dark and colored brown to gray. The cods produce free-floating eggs that do not contain oil globules. There are approximately 55 species of cods belonging to 21 genera. They are primarily marine and inhabit the cooler waters, near the bottom of northern seas. Most migrate inshore during the summer and move to deeper waters in the fall and winter. There are exceptions such as the burbot (*Lota lota*), which is a freshwater fish, and landlocked populations of the Atlantic tomcod (*Microgadus tomcod*). Furthermore, there are species that reside in southern seas. The 7 species discussed here were selected on the basis of their commercial importance as food fishes.

Atlantic Cod

The Atlantic cod (*Gadus morhua*) is one of the most important food fishes of the world. It is Canada's most important commercial species in terms of net value. Atlantic cod is caught commercially in a many ways, including trawl gear, seine, and gill nets, hand lines and jiggers. In 1984, the 463,100 tons of Atlantic cod landed in Canada was valued at $168.6 million. The Atlantic cod is also caught for sport with hook and line. The Atlantic cod cheek is a delicacy, served in the finest restaurants. By-products of the cod include fish meal, cod liver oil, and glue. Between these two extremes, the Atlantic cod is present in almost every food niche. It is sold fresh, frozen, smoked, salted, and canned. Fish and chips and fish sticks are prime market products for the cod.

The Atlantic cod is distributed throughout the cooler northern waters of the Atlantic Ocean. On the eastern shore, the Atlantic cod is found from Iceland, south to the Baltic Sea and to the Bay of Biscay. On the western side of the Atlantic, it is found in the North Atlantic from Greenland and southern Baffin Island, to Cape Hatteras, N.C. The majority of the populations are found on the continental shelf.

The Atlantic cod can be found spawning during most months of the year, from February to December, throughout their extensive range. The northern populations spawn earlier. As an example, spawning in the Grand Bank, which is one of the major fishing grounds for the Atlantic cod, begins in April, peaks in May, and ends in June. Spawning takes place in the open ocean at varying depths. While some may spawn at depths less than 110 m, other Atlantic cod populations will spawn deeper than 182 m. Both eggs and milt are broadcast into the water. Fecundity is high and increases with age. Although a fish 51 cm in length may produce 200,000 eggs, a female 140 cm may produce 12 million eggs. Like the haddock, the eggs are about 1.5 mm in diameter, spherical, transparent, and bouyant. They rise and float freely under the surface during incubation. As for all fish eggs, incubation time depends on temperature. Due to the colder waters that characterize their environment, the eggs take from 40 to 60 days to hatch (−1.5 to 1°C). The newly hatched young are about 3–6 mm long and remain pelagic until a length of about 25–30 mm. They then descend to the bottom to feed and mature to adult sizes. The average adult caught by the commercial fishery is about 5–6 yr old, 50–60 cm long, and about 1–2 kg. They can live to be over 20 yr old and the largest recorded animal was 95.9 kg.

Pacific Cod

The Pacific cod (*Gadus macrocephalus*) is known as the common or gray cod. Like its counterpart on the Atlantic Coast, it is the most important trawl-caught bottom fish in western Canada. This trawl catch increased to a peak of about 9.1 million kg in 1966. It decreased after that to about 2.3 million kg in 1970. The Pacific cod is sold fresh and frozen. The flesh is filleted, and products such as fish sticks or fillet blocks are made for the domestic and export markets.

The Pacific cod is similar in appearance to the Atlantic cod. In general, it may be more slender and may have a longer barbel under the lower jaw that equals or exceeds the diameter of the eye. The Pacific cod is found on both sides of the Pacific Ocean. Its distribution extends from Santa Monica, Calif. north to Alaska and around into the Okhotsk and Japan seas. It is also found off Korea and in the Yellow Sea to Port Arthur.

The Pacific cod may be found in shallow waters in the spring but migrates into deep water in the fall. They spawn in the winter months. Spawning females range from 40 to 60 cm in length and are two to three years old when they spawn the first time. A 60-cm female may produce about 1.2 million eggs, which measure about 1 mm in diameter. The eggs and milt are broadcast into open waters and the fertilized eggs may float near the surface, depending on the salinity. In the cold northern waters, the eggs may take four weeks to hatch at 2°C. The incubation time is reduced to about 8 days at about 10°C. At 5°C, the yolk sac is absorbed in about 10 days. The young will grow to about 20–50 cm in one year and to about 45–75 cm in about two years.

Haddock

The haddock (*Melanogrammus aeglefinus*) is a commercially valuable food fish of the cod family. Haddock are caught commercially with the otter trawl. They are sold as a fresh, frozen, smoked, and canned product. The major stocks are presently recovering from the overexploitation of the 1960s.

Like the other cods, the haddock is rather dark in color. The color fades from the purplish gray on the dorsal surface, laterally to a light pink below the lateral line, and eventually to a white on the ventral surface. There is a characteristic black blotch on the lateral side posterior to the head and just dorsal to the pectoral fins.

The haddock is found on both sides of the Atlantic. On the eastern side of the North Atlantic, it is distributed from Iceland south to the English Channel. It is found in the White, Kara, Norwegian, and North seas. On the western side of the Atlantic, the haddock is found from southwestern Greenland, south along the east coast of Canada and the United States to about Cape Cod. They migrate between the shallower (30–37 m) warm waters of the banks during the summer and the deeper (55–125 m) waters in the winter.

The haddock spawns in waters up to 90 m in depth, primarily on the Grand, Emerald, Browns, and Georges banks. They spawn between January and May, depending on location. The spawning adults are between 3 and 5 yr old. While many features of the reproductive process in the haddock are characteristic of the cods, observations of aquarium specimens suggest that the reproductive behavior of the male fish is complex, involving color changes, sound production, and courtship displays. The spawning female measures between 40 and 60 cm long and may produce between 230,000 and 1.77 million eggs, respectively.

The eggs resemble that of the Atlantic cod. Like the other cods, the eggs and milt are broadcast into the open waters. After fertilization, the eggs float to the surface and float freely until they hatch. In the cooler waters of the North Atlantic, incubation may take between one (10°C) and four (2°C) weeks. The newly hatched alevins are about 4 mm long and grow to young larvae of about 25 mm before they resemble the adult in body form. They start to descend to deeper waters between 40 and 50 mm. Although the average 5-yr-old haddock may measure about 50 cm in length, the fish can live to be over 15 yr in age and an individual 112 cm long and 16.8 kg has been recorded.

Pollock

The pollock (*Pollachius virens*) is also known by other names such as Boston bluefish, coalfish, and green cod. The flesh, which is of a darker color than the cods and haddock, has a richer flavor than its relatives. The adult weighs about 2–5 kg and is fished commercially as an important food fish. The pollock is caught commercially in a number of ways, including trawling, longline, hand line, weirs, and traps. It is sold in fresh, frozen, and smoked forms. It is also fished for sport in the shallower waters with artificial lures.

Like the haddock, the pollock are distributed throughout the North Atlantic. On the east side, they are found from Iceland, south to the Bay of Biscay. On the west side of the Atlantic, the pollock are found from southwestern Greenland to Cape Hatteras, N.C.

At maturity, the male and female are about 3 yr old and are about 50 cm long; although records show that they can live up to 14 yr, weight 70 kg, and measure over 100 cm. The pollock shares much of the reproductive features of other cod fishes. They produce about 225,000 eggs each; the eggs are about 1 mm in diameter, spherical, bouyant, and remain pelagic until hatching. Eggs and milt are released into the open waters at a depth of about 100–200 m. Unlike other cod fishes, the pollock spends more time swimming throughout the water column as opposed to being a primarily bottom fish. Its preferred depth is about 110–180 m. Like the cod fishes, however, the pollock migrates toward shore and shallow waters in the summer and offshore to deeper waters in the winter.

Walleye Pollock

The walleye pollock (*Theragra chalcogramma*) has come to be one of the most important commercial fisheries in the world. It has been described as the most productive single-species fishery in the world. While it once had a limited market for human consumption due to its soft flesh, it is fished intensively today for the roe, frozen fish block and fillets, and processed fish flesh markets. Surimi, a processed minced fish flesh product, is also one of the prime products from the flesh of walleye pollock. These fishery products that derive from the walleye pollock are taken by separate fisheries, although trawling and seining are the common methods of catch. The surimi fishery consists of large factory trawlers or large factory ships with fleets of smaller catcher vessels; walleye pollock of all sizes are taken from the bottom and midwater depths. The freezer fishery consists of large factory trawlers and smaller independent fisherman that harvest walleye pollock off the bottom, along with other species such as turbot and the Pacific cod. The spawning adults are caught for the roe fishery from the midwaters.

The walleye pollock has a typical codlike appearance with three, well-separated dorsal fins and very small or no barbels projecting from the lower jaw. It usually has an olive green to brown color on its back with irregular blotches or mottled appearance. It is lighter on its ventral surface and has silvery sides.

The walleye pollock is distributed from central California, north through the Bering Sea, around to Asian waters to the southern reaches of the Sea of Japan. It has a wide distribution vertically in the water column. It has been found from the surface to depths below 380 m.

Walleye pollock begin to mature sexually at about 2–3 yr of age, when they measure about 25 cm. By the time they are 5–6 yr old and 45 cm, more than 90% are mature. The fecundity of a 50-cm female is between about 200,000 and 220,000 eggs. Estimates of fecundity range from about 100,000 to 1 million eggs for females between 30 and 70 cm in length, and 225 to 2,000 g, respectively. While spawning occurs during one season of the year, the female will spawn a number of times within that season. The eggs develop and mature in batches over time to allow multiple spawnings. Spawnings takes place mostly between March and April. The eggs measure about 1.4 mm in diameter and float freely in the water, once they are spawned. They are found mostly in the upper 20 m of the water column. Hatching may take place between 14 (5°C) and 24 (2°C) days. The hatched young measure about 4 mm in length and resemble tadpoles, with characteristic markings. They are born with yolk sacs, which are absorbed by the time they attain about 7 mm in length. At 22 mm and about 50 days after hatching, they have all the adult fin rays and are considered juveniles. Adults can live to 15 yr, although most are between 1 and 7 yr of age.

Hake

The red hake (*Urophycis chuss*) and the white hake (*U. tenuis*) are often considered as good food fishes. The white hake grows to a larger size and is distributed over a broader range than the red hake. Characteristics of the white hake are presented here. Similar in size to other cod fishes, the hake is distinguished by the smaller head, large eyes, long dorsal and anal fins and the numerous barbels on the ventral side of the chin They have a reddish brown color on their back, which fades to lighter shades on the side and white on the belly. The lateral line is pale. While some hake fisheries exist, this species is mostly taken incidental to other fisheries.

The hake live on mud bottoms, 200–1,000 m in depth. They are distributed on the continental slopes in the North Atlantic from Iceland south to North Carolina. Some have strayed as far south as Florida in deep water. Like the other cod fishes, they move to deeper waters in the fall and winter.

The average size of an adult at maturity is between 60 and 70 cm, although specimens over 100 cm have been recorded. Spawning takes place at different times depending on the location, although most takes place in the winter and early spring. Fecundity is high relative to other cods. A 70-cm-long female may produce about 4 million eggs, and one 90 cm long may produce 15 million eggs. The eggs, therefore, are smaller than those of the other cod fishes and measure about 0.75 mm in diameter. The eggs, which are transparent and bouyant, float about the sea until the young alevins, about 2 mm long, hatch. The young grow up to about 80 cm as pelagic fish, after which they migrate to deeper waters to feed and grow to maturity.

Whiting

The name whiting can refer to any one of three species of fish. It was a name originally given to the walleye pollock (*Theragra chalcogramma*) (1). The Pacific tomcod (*Microgadus proximus*) is also commonly called the whiting. While the Pacific tomcod is highly regarded as a tasty food fish, it is not abundant enough for a commercial fishery. The blue whiting (*Micromesistius poutassou*) on the other hand is fished in Europe for food. The blue whiting is rather rare in North America.

The blue whiting is normally found in the northeast Atlantic, off the coast of Greenland and off southern Iceland. It may also be found off Europe between Norway, south to western Mediterranean. Records of the blue whiting in North American range from Sable Island, south to Woods Hole, Mass. The blue whiting is a deep-water fish, caught by otter trawls at depths beyond 183 m off the continental slope during the spring or early summer. The scarce data on the reproductive features of this fish suggest that they resemble, verly closely, reproduction in other species of the Gadidae. It has been suggested that spawning occurs between mid March to mid May (2). Spawning probably occurs in deep waters, deeper than 1,000 m, and the eggs are about 1–1.3 mm in diameter. The few specimens inspected from the northwest Atlantic suggest that the blue whiting is smaller than other Gadids, measuring less than 40 cm.

FLATFISHES

As the name implies, the flatfishes include the fishes that are flat in the dorsoventral axis (Fig. 3). They have both eyes on one side of the body and are often found lying on the bottom of the ocean covered in layer of sand or mud. All the flatfishes are classified in the order Heterosomata and include the soles of the family Soleidae and the flounders in the family Pleuronectidae. This latter family includes the commercially important species of the true halibuts in the genus *Hippoglossus*, the turbots in the genus *Reinhardtius*, and the right-eyed flounders in the genus *Platichthys*. The commercially important species tend to be right-sided; that is the side that is visible to the observer is the right side of the fish. The left eye migrates, along with appropriate skeletal, muscular and neural rearrangements, so that both eyes end up on the right, uppermost side of the animal. Although these fishes are well known as benthic animals, fish in the juvenile stages are also pelagic. The reading of rings in the otoliths, or ear stones is sometimes used to age flatfishes. Nine commercially important species are discussed here.

Halibut

Although it seems reasonable at this time to regard both the Atlantic and Pacific halibuts as belonging to the same species *Hippoglossus hippoglossus*, the Pacific halibut is considered in current literature to be a separate species *Hippoglossus stenolepis* (Schmidt) (3,4). There were declining levels of global commercial catches during the 1960s and 1970s. The large increases in bottom trawling in the 1960s and 1970s were significant contributions to the decline. These global trends were even more exaggerated in some local fisheries such as the Atlantic landing in New England. The average annual landing was 4.83 million kg (eviscerated and heads off) in 1879. In 1930–1939 it was 802,858 kg and by the period 1970–1975, it had fallen to 75,296 kg. Regulation and stock management have curtailed this trend and Pacific stocks, for instance, seem to be recovering.

The chief way in which commercially caught halibut is preserved for storage and transport to market is freezing. The animal is eviscerated and has the head off before the intact trunk is frozen. While earlier methods included salting and chilling with ice, current methods of quick freezing and glazing or vacuum packaging in plastic provides a more reliable and consistent method.

Atlantic Halibut. The Atlantic halibut (*Hippoglossus hippoglossus*) is a highly prized food fish. The Atlantic hal-

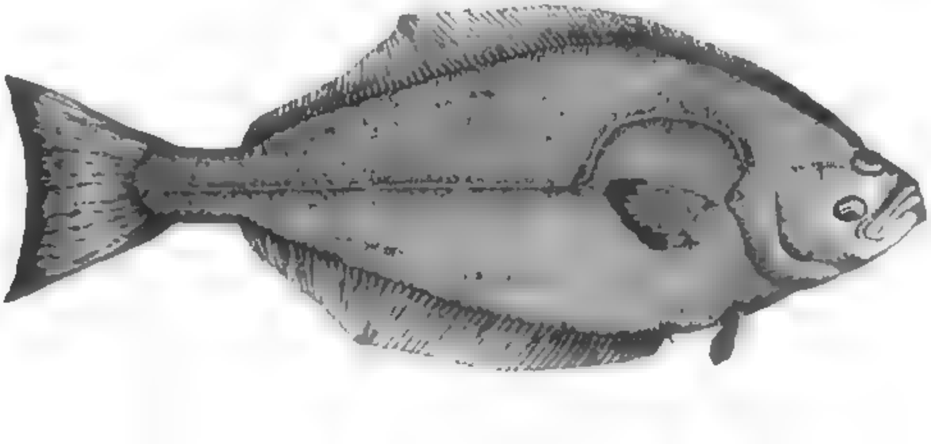


Figure 3. Halibut (*Hippoglossus hippoglossus*). *Source:* Copyright 1990 by B. Guild-Gillespie.

ibut is commercially taken by otter trawls and longlines, with smaller fish being taken by trawls and the larger fish being caught on longlines. Both sizes are taken by fisheries specifically directed at their catch or as incidental catches when trawling or long-lining for cod, haddock, or other ground fishes. The flesh is marketed fresh or frozen. The high price it commands enables fishermen to take relatively small quantities. In spite of this fact, there is evidence suggesting that certain populations are being overfished. The average length of the mature male and female halibut was 84 cm and 98 cm, respectively, in the period 1959 to 1969. Those lengths dropped to 66 cm and 70 cm, respectively, in the years 1970 to 1979. This reduction in average size at maturity is characteristic of populations subjected to heavy harvesting of the larger individuals.

The Atlantic halibut has a typical appearance of a flatfish. It has a flattened body, lying on its left side with its right side showing. The lower side is characteristically white and the right uppermost side is colored various shades of brown. The lateral line is prominent and curved over the pectoral fins. It is a very large fish, with record weights of over 100 kg. The larger fishes are between 30 and 35 yr old. It is the largest of the flatfishes. The females, in particular, have the highest growth rates as well as growth potential among the flatfishes. Females, therefore, become larger than males.

The Atlantic halibut is found on the east and west shores of the north Atlantic Ocean. The distribution on the east shore spans the Bay of Biscay to the Barents Sea. On the west side, the Atlantic halibut is found off the west coast of Greenland, south along the Canadian coast to the coast of Virginia. It prefers cooler waters between 3 and 9°C, at depths of 200–300 m.

Knowledge about reproduction in the halibut is relatively new. Atlantic halibut spawn between February and March around the distribution in Canada. They spawn in deep waters ranging from about 180 m on the western side and about 1,000 m or deeper in the northeastern Atlantic. The mature female, which weighs about 90 kg, may produce over 2 million eggs. The eggs are about 3 mm in diameter, spherical, and neutrally buoyant. They float during development at depths greater than 54 m but the hatched young sink as they develop. These free-floating eggs are most abundant at temperatures between 4.5 and 7°C and salinities between 33.8 and 35.0 ppt. Incubation takes about 16 days at 6°C. Little else is known about the details of the early life stages of the Atlantic halibut.

Pacific Halibut. The Pacific halibut (*Hippoglossus stenolepis*) has had a long commercial history on the Pacific coast of North America. The transacontinental railways across North America facilitated the Pacific halibut catches to be shipped across to eastern destinations and contributed to the near demise of the Pacific populations of this species. At the turn of the century, the total Canadian and U.S. catches was more than 4.54 million kg. Additional technological advancements in fishing vessels and gear increased harvests to a point that by 1930, increases in fishing efficiency no longer yielded greater catches. Through regulation under the International Fisheries Commission, the Pacific halibut stocks have rebuilt over time. The Pacific halibut is commercially caught mainly on hook and line or on other gear that does not involve nets of any kind. Numerous baited hooks are set on lines and rest on the bottom. Those sets can be made by dories from larger mother ships. Longlines of baited hooks can be set and retrieved by the mother ship.

The Pacific halibut is distributed throughout both western and eastern coasts of the Pacific. On the western side, it is found from southern California, north along the western coast to the Bering Sea. It is found off Anadyr, Kuril Islands, and Kamchatka, down to the northern areas of Japan. Like other flatfishes, the Pacific halibut is benthic (living on the bottom) at depths of about 1,100 m.

Reproduction and details of the early life stages in the Pacific halibut are better understood than the Atlantic halibut. Spawning takes place in the winter between November and January from 275 to 412 m. It is not entirely clear whether females spawn every year or whether they spawn every other year. Both may be possible. Pacific halibut are known to migrate as far as 1,600 km to spawn. The mature female is about between 8 and 16 yr old and about 100–150 cm long. Males are considerably smaller. The female may produce between 600,000 and 1.5 million spherical eggs of about 3–3.5 cm in diameter, which are neutrally bouyant between 100 and 200 m. The egg surface has a honeycombed appearance due to the presence of many small holes. The eggs as well as the newly hatched young are pelagic for four to five months after spawning. Like the Atlantic halibut, the newly hatched alevins start to sink to deeper waters and are found below about 200 m. The alevins are transparent, except for the eyes, and about 8–15 mm long with very large yolk sacs. The young are still symmetrical at this point. When they reach a size of about 18 mm, the left eye starts to migrate to the right side and the yolk sac is barely evident. The young appear as small adults by the time they are about 30 mm. By the time the young are about three to five months old, they rise in the water column to about 100 m. Currents carry the young inshore and they become benthic fish at about six to seven months in age. As they mature, they gradually move to deeper waters. By the time they are about 5–7 yr old, they are established at depths of about 100 m and available to the commercial fishery.

Greenland Halibut. The Greenland halibut (*Reinhardtius hippoglossoides*) is a moderately flat fish that has a rich-tasting flesh. It is also known commonly as the Greenland turbot in the marketplace. Greenland halibut are caught commercially by longlines, gill nets, and otter trawls. It is only moderately flat because both sides of the fish are equally muscled. While it is primarily sold as frozen fillets, it is also sold as a smoked product. It is a moderate-size fish that grows to about 25 kg and 120 cm in Atlantic waters; the Pacific specimens are somewhat smaller. The underside is usually white in a young fish but gray in older fish. The visible side is dark brown to black. It is distinguished by its prominent teeth along a protruding lower jaw.

The Greenland halibut is distributed in cold waters in both the Atlantic and Pacific Oceans. In the northeast Atlantic, it is found from around Iceland and the Greenland

Sea to the Arctic Ocean, and both Barents and Norwegian Seas. From there, it is found southward to the Faroe-Shetland Ridge. In the northwest Atlantic, it is distributed from western Greenland, south to Georges Bank. In the Pacific, it is found from northern California to Japan through the Bering and Okhotsk Seas, Sakhalin, and Kamchatka.

The limited knowledge about the details of spawning in this species is, no doubt, due in part to the fact that it spawns in the winter in the far north and at depths of about 650–1,000 m, at temperatures of about 0–3°C, making observation difficult. Spawning takes place in the winter or early spring, depending on location. The Greenland halibut can live to an age between 15 and 20 yr old. Until they are about 5–7 yr old, the males and the females grow at about the same rate. After that, the females grow larger and at a faster rate. All fish over 90 cm are females. The mature female may produce between 30,000 and 300,000 eggs, depending on size. The eggs are larger than the halibut eggs, measuring about 4–4.5 mm in diameter. The eggs are transparent and the newly hatched young rise to depths of about 30 m below the surface, where they live as pelagic fish until they are about 70 mm long. At that stage, they move to deeper waters but do not become benthic like the halibuts; although they are associated close to the bottom.

Yellowtail Flounder. The yellowtail flounder (*Limanda ferrunginea*) is valued as a tasty product that is often sold fresh but that may also be sold frozen. It is either fished directly with otter trawls or as an incidental catch on longlines in the American plaice fishery. While it is fished commercially, it is usually just the largest of the population that gets caught because of its small mouth. While it has the lateral line that curves over the pectoral fins, like the halibuts, the yellowtail flounder has a small mouth and the body size is much smaller. An average adult caught in the commercial fishery is about 30 cm long. This fish gets its name from the yellow markings anterior to the tail and at the bases of dorsal and anal fins. It has a white underside and the color of the visible right side may range from a reddish brown to an olive green.

The yellowtail flounder has a relatively limited distribution, compared to other flatfishes. It is found only in the western North Atlantic Ocean, from southern Labrador down to the Chesapeake Bay. The time of spawning depends on location, but mainly occurs in the spring to summer months. The female produces a large number of small eggs. Females that measure 40–45 cm may produce between 1 and 2 million eggs that are slightly less than 1 mm in diameter. The eggs and milt are deposited on, or near the bottom. Fertilization occurs there and the bouyant eggs then float to near the surface and drift during incubation. The incubation time is about five days at about 10°C. The newly hatched alevins are about 2–3.5 mm long and grow to about 11.5–16 mm before they metamorphose from the anatomically symmetrical young to a smaller version of the adult form. The yellowtail flounder may live to 12 yr of age, and individuals have been recorded up to about 60 cm. The typical fish caught commercially is between 4 and 10 yr old, depending on the location of the catch. The females grow at significantly faster rates than the males.

American Plaice. The American plaice (*Hippoglossoides platessoides*) is a white-fleshed fish that is highly enjoyed as a food fish. It is a very important commercial fish that constitutes about one-half of the commercially caught flatfish species in Canada. Most of the northwest Atlantic fishery is Canadian. American plaice is commercially caught with otter trawls, gill nets, and with the Danish seine. Most of the commercial fishing takes place along the Labrador Shelf, Grand Bank, along the southern Gulf of St. Lawrence, and on the banks of the Scotian Shelf. The flesh is normally sold as frozen fillets. It is colored white on the underside and reddish to grayish brown on the visible right side. The maximum body size rarely reaches over 60 cm.

The American plaice is found on both sides of the North Atlantic Ocean. It is distributed from Iceland, south to the English Channel, on the eastern side and from Baffin Island, south to Rhode Island on the western side It is normally found between about 75 and 275 m of water, preferring temperatures around freezing. It is benthic and prefers the fine sand or mud bottom. It has a seasonal migratory pattern, which brings it to deeper waters in the winter and to shallower waters in the spring.

Spawning take place between early April and June, depending on location. Spawning takes place at depths of about 90–180 m, at temperatures between 0 and 2.5°C. The mature female produces many small eggs. A 40-cm-long female, 8 yr old, may produce between 250,000 and 300,000 eggs, while a 70-cm-long female has been recorded to produce 1.5 million eggs. Males mature earlier at 4–5 yr old. The eggs measure about 1.5–2.8 mm in diameter. They are bouyant, without oil droplets and float near the surface during incubation. They, therefore, drift widely. At surface temperatures of about 5°C, the young alevins of about 4–6 mm, hatch in about 11–14 days. They transform from the symmetrical young to a small fish resembling the adult form at about 18–34 mm in length. They can live to be 25 yr old, and the record size is 81.2 cm long and 6.3 kg dressed.

Petrale Sole. The petrale sole (*Eopsetta jordani*) is a large flatfish that is prized as an excellent food fish, especially along the west coast of North America. The livers of this fish are a rich source of vitamin A. It is distributed from Bering Sea, south to Baja California. Like other flatfishes, it migrates seasonally and is found at depths of around 70–130 m throughout most of the year, except during the winter, when it moves to deeper waters, around 300–460 m. The petrale sole is white on the underside and olive brown on the visible right side. It has a large mouth and lacks free spines at the origin of the dorsal fin.

The oldest female petrale sole recorded was 25 yr old, whereas the eldest male was 19 yr old. The average spawning female is about 44 cm and the male is about 38 cm. The female produces a large number of relatively large eggs, the number depending on size. Records show a 42-cm female produced 400,000 eggs and a 57-cm female produced 1.2 million eggs. The eggs measure about 1.3 mm in diameter. Spawning takes place between late winter and early spring. Spawning takes place at depths of about 350 m and the bouyant eggs float to shallower depths where they are carried by prevailing currents during incubation. The eggs hatch into 3 mm alevins in about 8–9 days at 7°C and the yolk sac is absorbed within a further 10 days. Samples that measured 22 mm in length showed metamorphosis complete. Other evidence suggests that they settle to their adult benthic existence by the time they are 1–2 yr old.

Rock Sole. The rock sole (*Lepidopsetta bilineata*) is the most commonly used food fish of the smaller flatfishes. There have been several subspecies identified in the Pacific Ocean. At 15 yr of age, the females may reach a length of 60 cm, and the males, 50 cm. It is recognized by a canal formed along the lateral line that arches, typically, over the pectoral fin. It is a right-sided flatfish. Unlike other flatfishes, it tends to inhabit shallower waters. It is commercially taken at depths up to about 200 m, although it is scarce from about 100 m and deeper. Like other flatfishes, however, it does migrate to even shallower waters during the summer. Its distribution extends to both sides of the Pacific Ocean from southern California, up along the North American west coast to the Bering and Okhotsk seas and around to Korea and the Sea of Japan.

Observation off the North American west coast show that spawnings takes place between February and April. The fecundity of females measuring 35 cm and 46 cm in length were estimated at 400,000 and 1.3 million eggs, respectively. The eggs are pigmented yellowish orange and are adhesive. They measure about 1 mm in diameter. Incubation is a function of temperature and may take between 6 and 25 days, corresponding to a temperature range of about 8 to 3°C, respectively. Hatched alevins are about 5 mm long and yolk absorption takes about 10–14 days, influenced again on ambient temperature. Fully metamorphosed young have been observed at about 20 mm in length. The oldest recorded female was 25 yr old, and the eldest male on record was 15 yr old.

Dover Sole. The Dover sole (*Microstomus pacificus*) is highly prized for its quality flesh and excellent keeping qualities in the frozen state. It was originally dismissed as a viable commercial species because of its softness and sliminess. It may be uniformly brown on the visible right side and the underside may range in color from a light to a dark gray. It is characterized by having a lateral line canal that is almost straight as well as the excessive production of slime. While the body is also extraordinarily flaccid, it is known as a very hardy fish. The Dover sole is distributed from northern Baja California, up to the Bering Sea, found mainly on soft substrates. The average body size is about 70 cm.

Spawning in California takes place from November to February. Mature females are approximately 45 cm and males mature at about 40 cm. The female produces a wide range of eggs depending on size. Samples from Oregon showed a 42.5-cm female produced 52,000 eggs, while another 57.5-cm female had 266,000 eggs. The eggs are large, measure 2–2.6 mm in diameter, and have a wrinkled surface. After hatching, the young remain bilaterally symmetrical for several months. Flatfishes usually metamorphose and settle to a near benthic existence at about 20 mm. Dover sole specimens up to 100 mm in length have been observed in a pelagic life stage.

English Sole. The English sole (*Parophrys vetulus*) is characterized by its pointed head and, like the Dover sole, a lateral line canal that is almost straight. It is a moderate-size flatfish that grows to about 60 cm as a female and up to 50 cm as a male. It is colored a uniform brown on the uppermost right side and pale yellow to white on the underside. It has had a long history of being a commercially important species in North America. Its particular iodine flavor, found in some inshore populations, has identified its place in the marketplace. It is typically fished commercially at depths shallower than about 130 m, although its distribution extends to about 300 m.

Spawning off British Columbia occurs between January and March. The range in fecundity is extreme. Records show that a 30-cm female produced 150,000 eggs, and one measuring 44 cm produced 1.9 million eggs. The size of the maturing male and female are rather similar. Mature females measure about 30 cm, whereas the males measure about 26 cm. The eggs are small and measure slightly less than 1 mm in diameter. The eggs float due to the presence of oil droplets of various sizes in the yolk of the egg, start to sink just before hatching. The surface of the egg is covered with small wrinkles and pores. Observations in California show that the incubation time is about 90 days and the new alevins are about 2.8 mm long. Due to the oil droplets, the alevins hang upside down until the yolk is absorbed, in about 10 days, at which time they can swim. They remain pelagic for 6–10 weeks, after which they metamorphose to their adult form and seek the bottom. Young English sole are found in shallow waters but as they mature, the larger fish move to deeper waters. Like the other flatfishes, it tends to inhabit deeper waters in the winter and seek the shallower waters closer to shore in the spring. Another rather peculiar characteristic of this flatfish is that it can migrate long distances. There are records of English sole traveling over 1,000 km, between Vancouver Island and California.

HERRINGS AND SARDINES

The herrings are commercially important in that they support a variety of food fish markets (Fig 4). Until the late 1960s, the herring was reduced and utilized heavily as a source of oil and fish meal. The herring has and continues to be used for pet food. Herring as food for humans has had a long history. Today, they are sold fresh, frozen, smoked (kippers), and pickled. The smaller Atlantic herring, which are canned, is well known around the world as a sardine. The larger Atlantic herring are canned as kipper snacks and fillets. The roe from herring has recently found a market in Japan as a delicacy item known as kazunoko, as their local herring populations have declined. The herrings belong to the family Clupeidae. Both Atlantic and Pacific herrings are subspecies of the species *Clupea harengus*. The two fish look similar, characterized by a silvery body

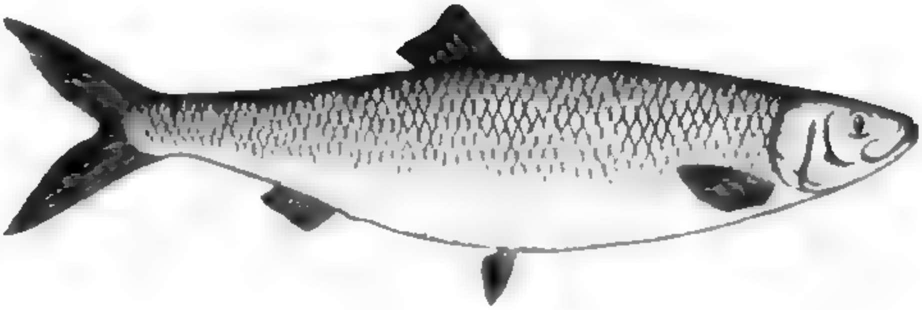


Figure 4. Herring (*Clupea harengus harengus*). *Source:* Copyright 1990 by B. Guild-Gillespie.

that is highly compressed laterally, large cycloid scales and large eyes. The pearl essence from the scales was in high demand during the 1940s for use in high-quality paints for aircrafts.

The Pacific sardine (*Sardinops sagax*) is commonly referred to as the pilchard. It is not the fish that North Americans commonly refer to as the sardine. While the pilchard has some commercial value for its oil and for fish meal, very little is canned.

Pacific Herring

The Pacific herring is fished today for its roe and for reduction purposes. Both herring and herring spawn have been fished by the North American natives since 800 BC. The commercial herring fishery that began in the late 1800s was based on the salted herring market. This was replaced by a fishery based on the reduction industry in which the herring carcasses were reduced for oil and meal for commercial feeds for poultry and fish culture. The relatively new market of herring roe for the Japanese market started in the early 1970s. Today, the herring roe goes to Japan, and the carcasses are reduced for oils and meal.

The Pacific herring (*Clupea harengus pallasi*) is distributed throughout the coastal regions of both eastern and western shores of the Pacific Ocean. On the eastern shore, it is found from northern Baja California up to the Beaufort Sea. On the western side, it is found from Korea, north to the Arctic Ocean. The adults are found at depths of 100–150 m. Juveniles are found between 150 and 200 m of water.

The Pacific herring spawn in the late winter through to April, with peak spawning occurring in March. This exclusive spring spawning distinguishes the Pacific herring from their Atlantic counterpart. Most are able to spawn by the time they are about three years of age. The spawning process is very dramatic as the fish broadcast eggs and sperm near shore. The water in which they spawn turns white with the milt from the males, covering as much as 257 km of shore line and at depths from the surface to about 10 m. The spawning area is usually in the intertidal zone, in sheltered bays or on open sand beaches, but not on exposed coastal areas. The texture of the substrate seems to be an important factor in determining the exact location of spawning.

The female produces between 9,000 and 38,000 eggs depending on her size, which can range from about 20 to about 30 cm. The relative fecundity is about 200 eggs per gram body weight per year. In extreme cases, fish can grow to 50 cm and produce over 100,000 eggs. The eggs are about 0.9 mm in diameter before fertilization but expands to about 1.2–1.5 mm in diameter after fertilization and the absorption of water. They are also very sticky once they are exposed to water. The eggs commonly adhere to aquatic plants such as eelgrasses or rockweed. The incubation time is about 10 days and the newly hatched alevins measure about 7.5 mm in length. The yolk is absorbed within the following two weeks and the fish then begin to feed on planktonic organisms. At that stage, the young do not resemble the adults. They are white, thin, and have large eyes. In about two months following yolk absorption, the young start to resemble the adult form and begin to form schools. By the late summer, the young are about 2.5–4.0 cm long and move to deeper waters in the fall. The Pacific herring can live to be 10 yrs old. During growth and maturation, they may be about 15 mm by the end of their second year, about 20 mm at the end of their fourth year, and about 23 cm at the end of the eighth year of life. They return to shallow waters close to shore as they mature sexually and prepare to spawn. They also tend to have a diurnal migration as rise to the surface in the evening and swim to deeper waters at dawn.

Atlantic Herring

The Atlantic herring (*Cuplea harengus harengus*) looks like its Pacific counterpart with iridescent blue or bluish green back and sides and a silver belly. Like the Pacific herring, its abundance has been declining steadily over time. The Canadian landings, for example, were 528,000 tons in 1968; 250,000 tons in 1975; 177,000 tons in 1980; and 147,000 tons in 1982. The advent of the highly efficient purse seine net has contributed to the harvest pressures on populations of herring. Gill nets, trap nets, and weirs have traditionally been used to catch herring. It is distributed on both sides of the North Atlantic Ocean. On the west side, it is found from Greenland south along the east coast of North America to Cape Hatteras. On the east side of the Atlantic, its distribution extends from Iceland, south to Europe between the White Sea to the Strait of Gibraltar.

Atlantic herring mature to spawn at three to five years of age. There seems to be several discrete stocks that spawn at different times of the year; there are spring, summer, and fall spawning populations. There may be stocks spawning every month between April and November throughout its distribution. Spring spawning occurs in shallower inshore areas, whereas summer and fall spawning occurs in deeper offshore waters. Like the Pacific herring, eggs and milt are broadcast into the open water where fertilization occurs. The eggs then sinks to adhere to bottom plants such as Irish moss and several algal species at depths of 1–4 m. The fecundity can range an order of magnitude from 23,000 to 261,000 eggs, depending on body size and age. The fecundity increases with body size and age up to a certain age, after which egg numbers decline with further aging. Fecundity is also a function of when spawning occurs. Spring spawners produce up to about half the egg numbers of summer and fall spawner. The eggs, however, have a larger yolk mass. This may reflect a strategy to survive the colder months of spring when food supply might be less abundant than in the summer and fall months. The small eggs, which measure from 1 to 1.5 mm in diameter, take from 10 to 30 days to hatch, depending on the ambient water temperature. The hatched alevins are about 4 mm long. They are light sensitive and avoid bright light, seeking deeper waters during the day. Unlike the Pacific herring that stay relatively close to their spawning grounds, Atlantic herring migrate offshore extensively.

MENHADEN

The Atlantic menhaden (*Brevoortia tyrannus*) is a bony, oily fish that is of commercial importance for oil, fish meal, and fertilizer products. It belongs to the family Cludeidae. It is a major protein component in commercial feed fed to cultured fishes. Livestock and poultry feed may also contain menhaden meal. The production of paints, soaps, and certain lubricants may use the menhaden oil. It is not consumed by humans because it has a lot of bones and its oily nature gives off an unpleasant odor when cooked. It is a particularly important fishery from Massachusetts to the Carolinas. It resembles the herring in appearance except for several features. The menhaden is deeper in the body, being more elliptical from a lateral view. It has a large head and lacks teeth. It also has a distinctive black spot posterior to the gill covers with more spots of irregular shapes and sizes along the ventral halves of the flanks. It has silvery sides with a back that can have a blue, brown, or green hue to it. Menhaden are harvested almost exclusively by the purse seine net. Purse seine fishing depends almost completely on the sighting of schools of menhaden at the surface, from aircraft or large vessels. The schooling behavior is an outstanding characteristic of this species, from larval to adult stages.

The menhaden is distinct in its feeding habits in that it is one of few fish that can feed on planktonic organisms. While it is rather common for juvenile fishes to feed on plankton, most fishes use feed higher up the food chain. The menhaden has very fine gill rakers that filter out phytoplankton such as diatoms as well as planktonic crustaceans.

The menhaden is euryhaline. The Atlantic menhaden is mainly an ocean fish that schools off coastal waters of the Atlantic, although it has been reported in fresh water as well. It is distributed from the Gulf of St. Lawrence to southern Florida. Freshwater populations have been reported in the St. John River, New Brunswick, Canada.

The Atlantic menhaden can live up to 12 yr, although specimens over seven yr old are rare. While a few mature at 1 yr of age, 80% are sexually mature at 2 yr of age, and all are sexually mature by the time they are 3 yr old. The body size at 3 yr is approximately 25 cm. Reproduction of the menhaden is similar to the herring. They spawn at sea or in large bays, throughout their distribution. They may also be able to spawn in the St. John River, where they are found year-round. The spawning period for Maine to Massachusetts, its northern distribution, occurs between May and October. Populations south of that area spawn during the other half of the year The male and female broadcast eggs and sperm into the water where fertilization occurs. The fertilized eggs measure about 1.3–1.9 cm in diameter. Fecundity of females measuring 20–35 cm were 38,000–631,000. The eggs are spherical and bouyant, the latter being due to the presence of an oil globule in the yolk. The young are approximately 2.4–4.5 mm in length at hatching.

MULLET

Distributed mainly in the Atlantic Ocean, Black Seas, and the Mediterranean, the order Mullus includes several species of mullet that frequently move up to the Norwegian coast (Fig. 5). Two species, the red mullet (*Mullus barbatus*) and the striped mullet (*Mullus surmuletus*) generally spawn off the coast in the summer and reach sexual maturity in two to three years. They prefer sandy or muddy bottoms. The oil bubbles in the eggs permit them to float in the water. With the arrival of fall season, the juveniles seek greater depths. Preferring sandy or muddy ground, the red mullet has a steep forehead whereas the striped mullet, usually found above sandy ground, has a less-steep forehead. Although teeth are not present in the upper jaw, they are located on the vomer and the gums. Mullets had been an edible delight since Roman times. Although they are small, they commanded a high price and were commonly brought in the dining hall alive.

Nowadays, the commercially important mullet species is another striped mullet (*Mugil cephalus*). It is large and measures 90 cm and weighs 7 kg. This ash gray fish has a dark blue shimmer with 9–10 light longitudinal stripes on the sides of the body. The striped mullet resides in warm seas including the Mediterranean and frequents river mouths or lagoons. Young striped mullets are nourished in salt water or brackish water ponds until they have grown to acceptable size for the consumer market.

The suborder Mugiloidei, family Mugilidae includes several species of mullet that inhabit coastal waters and can acclimatize to brackish water, fresh water, or salt water. They prefer soft ground with a rich plant source located in tidal zones. The generic name *Mugil* (sucker) derives from their feeding habit. They prey on detritus and tiny organisms on the floor. Otherwise, these mullet feed on mussels, snails, planktons, and little organisms that frequent algal populations.

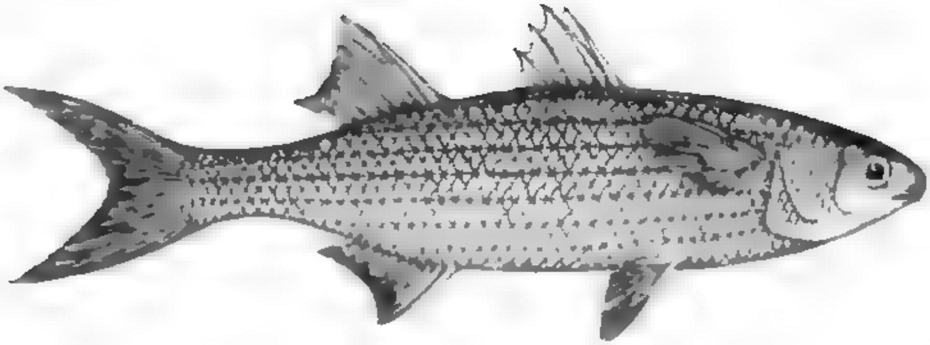


Figure 5. Striped mullet (*Mugil cephalus*). *Source:* Copyright 1990 by B. Guild-Gillespie.

Ocean Perch, Rockfish, and Redfish

The fishes, commonly known as ocean perches, rockfishes, and redfishes belong to the family Scorpaenidae, the scorpion fishes (Fig. 6). In general, these are highly valued food fishes that resemble the freshwater bass in appearance. They are found in all tropical and temperate marine environments around the world. There are 60 genera with over 300 species in this family. The representatives of the popular food fishes in North America belong to the genus *Sebastes*. Fishes in this genus are ovoviviparous, that is they bear live young with some passive nuturing from the mother. The commercially important representatives comprise two species in the Pacific and three species in Atlantic waters.

Pacific Ocean Perch

The Pacific ocean perch (*Sebastes alutus*) constitutes the major species of the Pacific *Sabastes* that is caught commercially for food. The fish may be caught by trawl or hand lines offshore and is mainly sold as fresh and frozen fillets. It can be recognized by a prominent knob, or overhanging tissue, off of the lower lip. It is colored a bright red over the entire body, including fins. The ventral side is usually lighter red. There are olive brown patches below the dorsal fin and on the dorsal side of the caudal penduncle. The Pacific ocean perch is found from southern California north to the Bering Sea. A close relative, the *Sebastes paucispinosus*, is found in the western shores of the Pacific, mainly around northern Honshu in Japan. It is usually found at depths below 130 m and fished most often between about 160 and 300 m.

The mature Pacific ocean perch can be between 30 and 50 cm in length. As for most species, the male is generally smaller, lives longer, and grows more slowly than the female. Spawning occurs in the winter months, and the young are born between January and February. Fecundity can vary an order of magnitude. Fish about 32 cm long and 7 yr old produce about 30,000 eggs, and a female 44 cm long and 20 yr old produces about 300,000 eggs. Unlike the bottom-dwelling adults, the younger are pelagic. Ocean perch have low growth rates. Because of this, the young stay pelagic until the second or third year of life before heading to the bottom to grow to maturity. Adults may live to be 30 yr old.

Yelloweye Rockfish

The yelloweye rockfish (*Sebastes ruberrimus*) are named for their brilliant yellow eyes. They have a characteristic orange-yellow color to their body with a pink tint along the back and sides. The fins are also pink with black margins. The bellies are white with black dots. They are found around reefs from Baja California up the west coast of North America to the Gulf of Alaska. These fish are mostly caught commercially by setlines with live or dead herring bait. They contribute significantly to the whitefleshed fillet market in fresh or frozen states. The yelloweye rockfish can grow to be about twice the size of the ocean perch. They can grow to be about 90 cm. They are normally found at depths between about 50–550 m. The fecundity of a 9 kg

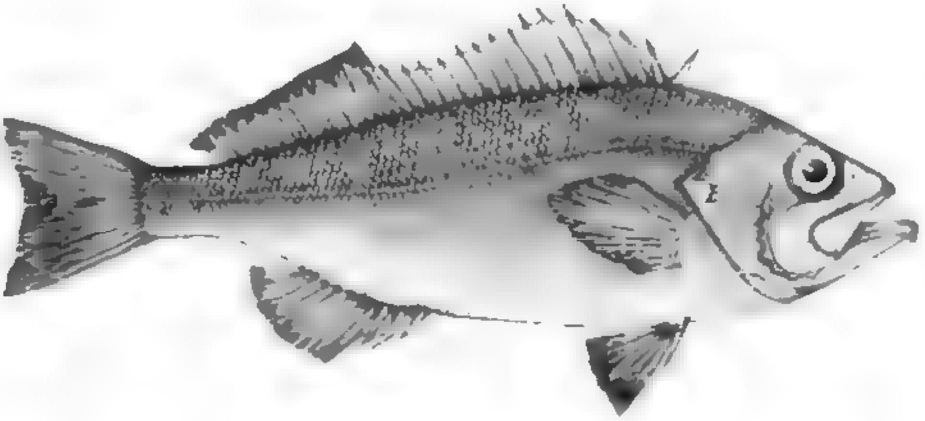


Figure 6. Golden redfish (*Sebastes marinus*). *Source:* Copyright 1990 by B. Guild-Gillespie.

female can exceed 2.5 million young. The young off the Washington coast are born in June.

Atlantic Redfishes

There are several species that belong to *Sebastes* in the Atlantic. Four occur in the North Atlantic Ocean, three in the western North Atlantic and one (*Sebastes viviparous*) in the eastern North Atlantic. The three species of Atlantic redfishes found off the North American coast are the Acadian redfish (*Sebastes fasciatus*), the golden redfish (*Sebastes marinus*), and the deepwater redfish (*Sebastes mentella*). They are all commercially valuable as food fishes, although specific fisheries for each species do not exist. They are, rather, fished as a group and marketed as fresh and frozen fillets under the name of ocean perch. The deepwater redfish probably makes up the majority of the catch off the Newfoundland-Labrador coast, while the Acadian redfish is the main species in the commercial catch of redfish off the Georges Bank-Gulf of Maine region. These fishes are caught with otter trawls.

The Acadian redfish has an orange-red body with red fins, with the pelvic and anal fins having a particularly deep red color. There are green-black blotches below the dorsal fin and on the posterior part of the gill covers. There may be green iridescent flecks on the body above the lateral line. The deepwater redfish is bright red all over. The golden redfish can be colored either orange-yellow or gold-yellow.

The distribution of the Atlantic redfishes extends from Iceland, south to about Virginia on the U.S. coast. The golden redfish is more common in the northern range of this distribution but less common in North American waters compared to the Acadian redfish or the deepwater redfish. The golden redfish is common off Greenland, Iceland, Norway, and in the southern Barents Sea. The deepwater redfish has the broadest distribution of the three redfishes and occurs throughout the North Atlantic range of this genus except the North Sea and the Gulf of Maine. It is also found farther offshore than the other two species. The Acadian redfish may be considered the North American redfish because it is most common along the Canadian continental shelf and southward, particularly along the Georges Bank and in the Bay of Fundy and Gulf of Maine waters. The Acadian redfish occupies the shallowest waters of the three species, found most often at depths between about 130 and 370 m. The golden redfish is found from about 300 to about 370 m. The deepwater redfish is found in waters about 350–700 m.

About half of the populations of the Acadian and deepwater redfishes mature at about 19 cm and 30 cm for males and females, respectively. The female golden redfish matures at about 41 cm. There is evidence suggesting the young hatch inside the female golden redfish in April and are born between April and May. The Acadian and deepwater redfishes hatch their young between March and June and release their young between March and June. A study in the Gulf of Maine showed that the Acadian redfish about 30 cm retains about 50,000 fertilized eggs and releases about 15,000 to 20,000 living young. The young of the Acadian redfish grow to be about 8 cm in the first year and grow an average of about 2.5 cm each year up to about 10 yr of age, declining in rate after that. The growth rates in males and females seem to be about the first 20 yr of life.

The Atlantic redfishes, like the Pacific *Sebastes*, grow very slowly. Sections of the otoliths have been used to age these fishes. That data show that the golden and deepwater redfishes can live to be 48 yr old. This depends on the population. The populations of Acadian redfish in the Gulf of Maine may live up to 20 yr and grow to a maximum size of about 46 cm and 1.4 kg.

SALMON

The salmon family Salmonidae comprises numerous landlocked and anadromous species (Fig. 7). They are found in most waters of the northern hemisphere and dominate the northern waters of North America, Europe, and Asia. This family is made of of three subfamilies, the Salmoninae (salmon, trout, char), the Coregoninae (whitefish), and the Thymallinae (grayling). A common feature among all species is the adipose fin located on the dorsal surface between the dorsal and caudal fins. While representatives of the Coregoninae and Thymallinae belong to this family, the term salmon is usually applied to the salmon and trout of the Salmoninae. This article deals with only those species that are commonly referred to as salmon, which includes species in the genera *Oncorhynchus* and *Salmo*. Even this restriction does not eliminate the confusion between common names and the scientific classification. The Atlantic salmon, for example, is strictly classified as a trout in the genus *Salmo*. The rainbow trout, on the other hand, has just recently been transferred from the genus *Salmo* to *Oncorhynchus*, but it continues to be called a trout. The species covered here include the Pacific salmon species, the rainbow trout, and the Atlantic salmon.

The anadromous species spawn in fresh water where the eggs incubate and where the young spend varying lengths of time. It is common for adults to return to the streambed where they were hatched. During the sexual maturation process, the typically silver fish begins to darken and takes on various colors including black, brown, orange, and red. There is usually a dramatic transformation of the shape of the head and in some instances, the whole trunk. The head of the male usually elongates and there is a pronounced development of teeth. The chum salmon, for instance, develops a large hump back during this process. Many species do not feed once they enter fresh water and begin their migration to their spawning grounds. The spawning process culminates a long journey, sometimes covering thousands of miles, and normally ends in death for the Pacific salmon. The trouts, on the other hand, usually recuperate and may return to spawn a number of times. The Pacific salmon, Atlantic salmon, and steelhead trout are anadromous. The landlocked salmonids discussed in this article include the rainbow trout and the kokanee salmon. While these are the natural life cycles, some species can be grown entirely in fresh water. For example, rainbow trout can be grown quite successfully in seawater netpens and the coho and pink salmon can spend their entire life cycle in fresh water.

female can exceed 2.5 million young. The young off the Washington coast are born in June.

Atlantic Redfishes

There are several species that belong to *Sebastes* in the Atlantic. Four occur in the North Atlantic Ocean, three in the western North Atlantic and one (*Sebastes viviparous*) in the eastern North Atlantic. The three species of Atlantic redfishes found off the North American coast are the Acadian redfish (*Sebastes fasciatus*), the golden redfish (*Sebastes marinus*), and the deepwater redfish (*Sebastes mentella*). They are all commercially valuable as food fishes, although specific fisheries for each species do not exist. They are, rather, fished as a group and marketed as fresh and frozen fillets under the name of ocean perch. The deepwater redfish probably makes up the majority of the catch off the Newfoundland-Labrador coast, while the Acadian redfish is the main species in the commercial catch of redfish off the Georges Bank-Gulf of Maine region. These fishes are caught with otter trawls.

The Acadian redfish has an orange-red body with red fins, with the pelvic and anal fins having a particularly deep red color. There are green-black blotches below the dorsal fin and on the posterior part of the gill covers. There may be green iridescent flecks on the body above the lateral line. The deepwater redfish is bright red all over. The golden redfish can be colored either orange-yellow or gold-yellow.

The distribution of the Atlantic redfishes extends from Iceland, south to about Virginia on the U.S. coast. The golden redfish is more common in the northern range of this distribution but less common in North American waters compared to the Acadian redfish or the deepwater redfish. The golden redfish is common off Greenland, Iceland, Norway, and in the southern Barents Sea. The deepwater redfish has the broadest distribution of the three redfishes and occurs throughout the North Atlantic range of this genus except the North Sea and the Gulf of Maine. It is also found farther offshore than the other two species. The Acadian redfish may be considered the North American redfish because it is most common along the Canadian continental shelf and southward, particularly along the Georges Bank and in the Bay of Fundy and Gulf of Maine waters. The Acadian redfish occupies the shallowest waters of the three species, found most often at depths between about 130 and 370 m. The golden redfish is found from about 300 to about 370 m. The deepwater redfish is found in waters about 350–700 m.

About half of the populations of the Acadian and deepwater redfishes mature at about 19 cm and 30 cm for males and females, respectively. The female golden redfish matures at about 41 cm. There is evidence suggesting the young hatch inside the female golden redfish in April and are born between April and May. The Acadian and deepwater redfishes hatch their young between March and June and release their young between March and June. A study in the Gulf of Maine showed that the Acadian redfish about 30 cm retains about 50,000 fertilized eggs and releases about 15,000 to 20,000 living young. The young of the Acadian redfish grow to be about 8 cm in the first year and grow an average of about 2.5 cm each year up to about 10 yr of age, declining in rate after that. The growth rates in males and females seem to be about the first 20 yr of life.

The Atlantic redfishes, like the Pacific *Sebastes*, grow very slowly. Sections of the otoliths have been used to age these fishes. That data show that the golden and deepwater redfishes can live to be 48 yr old. This depends on the population. The populations of Acadian redfish in the Gulf of Maine may live up to 20 yr and grow to a maximum size of about 46 cm and 1.4 kg.

SALMON

The salmon family Salmonidae comprises numerous landlocked and anadromous species (Fig. 7). They are found in most waters of the northern hemisphere and dominate the northern waters of North America, Europe, and Asia. This family is made of of three subfamilies, the Salmoninae (salmon, trout, char), the Coregoninae (whitefish), and the Thymallinae (grayling). A common feature among all species is the adipose fin located on the dorsal surface between the dorsal and caudal fins. While representatives of the Coregoninae and Thymallinae belong to this family, the term salmon is usually applied to the salmon and trout of the Salmoninae. This article deals with only those species that are commonly referred to as salmon, which includes species in the genera *Oncorhynchus* and *Salmo*. Even this restriction does not eliminate the confusion between common names and the scientific classification. The Atlantic salmon, for example, is strictly classified as a trout in the genus *Salmo*. The rainbow trout, on the other hand, has just recently been transferred from the genus *Salmo* to *Oncorhynchus*, but it continues to be called a trout. The species covered here include the Pacific salmon species, the rainbow trout, and the Atlantic salmon.

The anadromous species spawn in fresh water where the eggs incubate and where the young spend varying lengths of time. It is common for adults to return to the streambed where they were hatched. During the sexual maturation process, the typically silver fish begins to darken and takes on various colors including black, brown, orange, and red. There is usually a dramatic transformation of the shape of the head and in some instances, the whole trunk. The head of the male usually elongates and there is a pronounced development of teeth. The chum salmon, for instance, develops a large hump back during this process. Many species do not feed once they enter fresh water and begin their migration to their spawning grounds. The spawning process culminates a long journey, sometimes covering thousands of miles, and normally ends in death for the Pacific salmon. The trouts, on the other hand, usually recuperate and may return to spawn a number of times. The Pacific salmon, Atlantic salmon, and steelhead trout are anadromous. The landlocked salmonids discussed in this article include the rainbow trout and the kokanee salmon. While these are the natural life cycles, some species can be grown entirely in fresh water. For example, rainbow trout can be grown quite successfully in seawater netpens and the coho and pink salmon can spend their entire life cycle in fresh water.

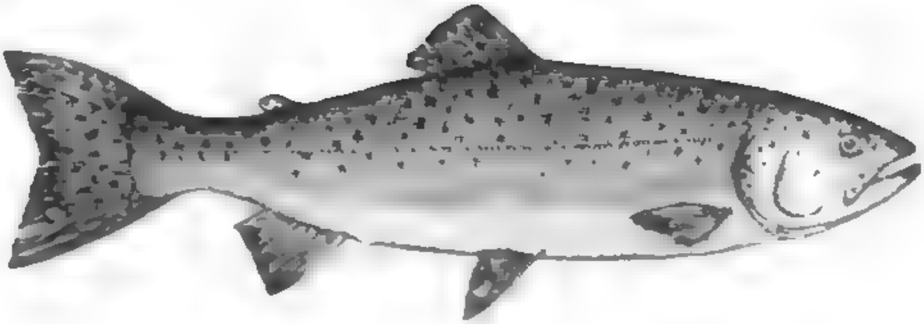


Figure 7. Rainbow trout (*Oncorhynchus mykiss*). *Source:* Copyright 1990 by B. Guild-Gillespie.

Spawning behavior typically involves a high degree of territoriality and aggression between individuals as mates selection takes place. The female will usually create a depression, called a redd, in the gravel bottom by beating the rocks with the side of her tail. After eggs and milt are deposited simultaneously for fertilization to take place, the female will cover the eggs with gravel by moving upstream from the redd and making another redd, causing the displaced gravel to cover the first redd that contains the eggs. The fertilized eggs then incubate in fresh water, which percolates through the gravel, supplying oxygen and carrying away metabolic products such as ammonia and carbon dioxide. The developing embryos as well as the hatched alevins live off yolk carried in a yolk sac while they are still under the gravel. After the yolk is consumed, their mouths open and they begin to emerge from the gravel and feed on organisms in the open waters of the stream. The young of anadromous fishes spend varying lengths of time in fresh water. This fresh water residence ends when the animal undergoes the process of smoltification. This is the total of behavioral, morphological, and physiological changes that occur in the juvenile fish to prepare it for life in salt water. After the process of smoltification, the young migrate to sea to spend the rest of their life cycle growing to sexual maturity.

Pacific Salmon

Chinook Salmon. The chinook salmon (*Oncorhynchus tshawytscha*) is one of the most prized fishes on the west coast of North America. It is also one of the most important commercial species. The chinook salmon is known as the tyee or king salmon when the body size reaches greater than 13.6 kg. This fish has the largest body size of the Pacific salmons. While 13.6 kg is more common as the maximum size, the world's record is 57.3 kg. It is this characteristic combined with its high market value that has made it a popular choice for aquaculture in the northwestern states and on the west coast of Canada. There are two varieties of chinook salmon, the red chinook and the white chinook, which are named according to their flesh color. The red flesh commands the higher price. This segregation is rather unique among the salmon. Chinook salmon are caught commercially with trolling gear, purse seine or gill nets, long lines, and fish wheels. The majority are caught by trollers. Chinook salmon are sold as fresh, frozen, and canned products, with the canned products coming from the net fishery. They are caught for sport with a variety of lures including artificial lures, bait, and fiys. Dead herring serve as a desirable bait, trolled deep in the water column.

Chinook salmon adults migrate extensively and are primarily found in the Pacific Ocean from the Ventura River in southern California, north to Point Hope, Alaska. Limited evidence suggests, for instance, that the Canadian shinook migrate north to the Gulf of Alaska but remain within 160 km of that area until they return south to spawn. This species is less common in the Arctic Ocean and in the Bering and Okhotak seas and the Sea of Japan. The largest numbers come from the largest rivers such as the Fraser River in British Columbia. Substantial numbers of chinook also come from the Yukon River. While there have been numerous attempts to transplant this species to many locations throughout North America and around the world, it seems that only success was on South Island, New Zealand, where a self-supporting population was established.

The average age of an adult returning to spawn varies from four to seven years, depending on the location. Three- to five-year-old returning adults are more common in southern streams, whereas five- and six-year-old adults are more common in northern streams. It is also quite common for river systems to have more than one stock of chinook salmon returning to it, where there may be spring, fall, and winter runs of returning adults.

The time of spawning depends on the location and the distance the adults must swim upriver to reach the spawning grounds. In the Fraser River, for example, the chinooks spawn between July and November and the adults may travel up to 965 km from the mouth of the river to their spawning grounds. The chinooks that return to the Yukon River spawn between July and August and may travel up to 1930 km to reach their spawning grounds. Other stocks that travel only a few miles, if that, in British Columbia spawn in September and in October. Chinook salmon tend to spawn in deeper waters and in larger gravel than other Pacific salmon species. Each female may carry from 4,000 to 13,000 eggs. The eggs are large for fish eggs and measure about 7 mm in diameter. The eggs hatch the following spring and the alevins spend two to three weeks in the gravel with their yolk sacs. The emergent young remain in the freshwater rivers for varying lengths of time, depending primarily on the water temperature. The young will smolt and migrate to sea after about three months in warmer southern areas such as in southern British Columbia, while chinook spend at least one year in fresh water in northern areas. Chinook juveniles may spend two years in fresh water in the Yukon River. The maturing adults may spend two to three years at sea before returning to fresh water to spawn. Spawning females are, therefore, about four to five years old.

Coho Salmon. The coho salmon (*Oncorhynchus kistuch*) is also an important commercial and sport fish in North America. It is the mainstay of the saltwater sport fishery for salmon. Most of the coho are commercially caught in August by trolling with plugs, spoons, or feathered jigs, although purse seines and gill nets are also used. The trolled catch is sold as the fresh or freshly frozen product, while the netted fish are either canned or smoked. Sport fish are caught in the late summer to early fall months of July to October. The gear used by sport anglers is similar to the commercial trolling gear except it is smaller in size. Coho are also caught on the fly with bucktail flies and with bait such as frozen or pickled herring. Because coho remain silver and continue to feed while traveling upstream to spawn, they are pursued by the sport angler in many freshwater rivers and streams.

The adult distribution extends from southern California to the Gulf of Alaska. The juveniles are found in the fresh waters from Monterey Bay, Calif., to Point Hope, Alaska. Coho salmon are also found in the Anadyr River in the former USSR and south from that point to Hokkaido, Japan. This species has been transported to other parts of the world such as Argentina and Chile, with Chile reporting some success in the establishment of naturally reproducing stocks. Coho have also been introduced into the Great Lakes by both Canadians and U.S. citizens. While there are reports of natural reproduction, the maintenance of those stocks are heavily dependent on the annual planting of cultured stocks.

The full-grown adult can weigh from 1.8 to 5.5 kg. It enters fresh waters to spawn from late September to October and may travel up to about 240 km upriver to spawn. Asian populations exhibit more of the separation of summer and fall, or fall to winter runs than those populations returning to North American river systems. Spawning takes place in October or November. The eggs are rather large, measuring about 6–7 mm in diameter. The range in fecundity is about 2,000–3,000 eggs. After the eggs have hatched, the young spends between one and two years growing in fresh water. This again, depends on the location. Coho juveniles in British Columbia spend about one year in fresh water while those in the Yukon spend two years growing in fresh water. After their freshwater residence, the young smolts migrate to sea. The saltwater residence usually lasts about 18 months, although the phenomenon of precocious sexual maturation, or jacking, is predominant in this species. While normal adults will return between three and four years of age, jacks return to spawn at only two years of age. Coho salmon normally stay within about 40 km of the coast.

Chum Salmon. While the chum salmon (*Oncorhynchus keta*), or dog salmon, is a less desirable species for the commercial or sport fisheries, it has maintained an important position in the diets of the native people of North America who capture the fish with nets and fish wheels. It is a preferred species for smoking because of its low fat content. Its pale to white flesh has the lowest fat content of the Pacific salmons. This species has been used the least for transplantations to other geographic areas, perhaps due to its relatively low commercial and sport value. Most of the chum salmon are harvested while they are migrating toward their home streams to spawn. Commercial catches are harvested mainly between August and October with purse seines and gill nets. Some are also caught by trolling gear. The trolled catch is sold fresh. Some are smoked or dry salted for preservation. The bulk of the commercial catch, however, is canned.

The marine adults are found in Pacific and Arctic oceans, in the Sea of Japan, and in the Okhotsk and Bering seas. They probably have the widest distribution among the Pacific salmons. The major spawning areas in North America lie between Puget Sound in Washington State to Kotzebue, Alaska. The geographic range for their spawning covers the west coast of North America from Oregon to the Mackenzie River. The time frame for adults arriving at the spawning grounds ranges from July, in northern British Columbia to September or even as late as January for streams further south. The location of the spawning grounds, reflecting the degree to which the chum salmon swim into fresh water to spawn, varies considerably. Most arrive at the mouths of rivers in an advanced state of sexual development and spawn relatively close to the ocean. In general, few chum salmon migrate more than 160 km from the mouth of a river to spawn. The Yukon River, however, is an exception; the population ascends over 1,930 km to spawn.

The mature adult is usually between two and four years old. While the adult chum salmon can weigh from 3.6 to 5.5 kg, fish up to 13.7 kg have been reported. The fecundity ranges from about 2,000 to 3,000 eggs and each egg measures about 5–6 mm in diameter. The hatched young spend a period in the gravel feeding off the yolk until the spring when they emerge and migrate directly to sea. Depending on the distance to the ocean, the young may take from a single night up to several months until they reach sea water. The migratory behavior of the young chum are similar to the pink salmon and the young of both species may be found making the seaward journey together. They usually travel by night and hide in the gravel during the day. They are also not dependent on schooling to make the trip, although they do school once they have reached the estuary. Once in the ocean, the young chum will usually spend a few months, until mid to late summer, in the coastal areas before migrating further out to sea. Chum salmon from North America have been captured as far as 4,180 km off the west coast in the North Pacific. Most chum salmon spend two or three years in the ocean before returning to their home streams to spawn. They begin to appear at the mouths of rivers around May or June of their final year at sea.

Pink Salmon. The pink salmon (*Oncorhynchus gorbuscha*) is the most abundant of the Pacific salmon species. It gets its name from the pink color of its flesh. Like the chum salmon, this species is not regarded as being highly desirable compared to the deep red flesh of the sockeye salmon. The size range of the adult is about 1.4–2.3 kg, although animals as large as 6.4 kg have been recorded. A unique characteristic of this species is its fixed, two-year life cycle.

There is no overlap between the reproducing stock of one year to the next. This allows two separate stocks to utilize any stream for reproduction. Where there are odd-year stocks as well as even-year stocks using the same river, one often dominates. The Fraser River pink salmon run in British Columbia, for example, is primarily an odd-year stock, while the run in the Queen Charlotte Islands is an even-year stock.

Pink salmon is caught commercially with purse seine and gill nets as well as with trolling gear, the latter contributing only a minor part of the total catch. Fish caught by trolling is sold fresh, but 95% of the commercial catch is canned. Pink salmon is also fished for sport by trolling artificial lures.

Pink salmon is found in the Pacific and Arctic oceans, the Bering and Okhotsk seas, and the Sea of Japan. Its distribution in North America extends from the Sacramento River in California, north to the delta of the Mackenzie River, being most abundant around the central area of this range. It can be found from the surface to depths of about 36.6 m. Transplantations have established self-sustaining populations of pink salmon in northern Europe and in fresh water in Lake Superior. There are transplanted populations along the Atlantic Coast of North America that seem to be self-sustaining.

Pink salmon may spawn in a wide range of locations from tidal areas of certain rivers up to 483 km upstream from the mouths of large rivers. The pink salmon are also known as humpbacks because the sexually mature males develop a large hump behind the head. The snout elongates dramatically and large teeth emerge from the jaws. The adults will appear at the mouths of rivers from June to September to begin their migration to their spawning beds. Pink salmon spawn from mid-July to late October and the eggs hatch from late December to late February. Like the chum salmon, the young emerge from the gravel after the yolk supply is consumed and move directly to sea. The emergent young, which measure about 3.8 cm in fork length, may travel 16 km in a single night to reach the ocean. Their appearance is distinctive for salmon in that they lack the vertical parr marks on their sides. They are colored blue-green on their back and have silver sides. If the journey takes more than a night, the young will hide during the day in the gravel and emerge again at night to be carried by the current to the sea.

Sockeye Salmon. The anadromous sockeye was the first Pacific salmon species to be fished commercially. The sockeye has long been the mainstay of the Pacific salmon fishery. Its deep red flesh and high oil and protein contents have always brought it the highest price among the Pacific salmon species on the market. The sockeye is mainly caught with purse seine and gill nets, although trolling is also used. The product is mainly canned, although it may be sold fresh when the fishery is open. Native people use traditional methods of nets, weirs, and gaffs to harvest the fish. Although the sockeye salmon are not an important sport fish, effective artificial lures have been developed that have served both sport and commercial trollers at the mouth of major sockeye rivers such as the Fraser in British Columbia.

The sockeye salmon (*Oncorhynchus nerka*) is represented by an anadromous form and a landlocked form known as the kokanee. Specific recognition as subspecies has been given to each. The anadromous form i *O. nerka nerka* Walbaum and the kokanee is designated *O. nerka kennerlyi* Suckley. The anatomy of the two subspecies is very similar except for the total body size. The average weight of an adult anadromous sockeye is about 2.7–3.2 kg, whereas the adult kokanee weighs less than 455 g. Adult sockeye have been recorded to be up to 6.8 kg.

The distribution of this species extends from the Klamath River in California to Point Hope in Alaska. They are also found in Asia from northern Hokkaido, Japan, to the Anadyr River in the former USSR. The distribution of the kokanee certainly follows that of the anadromous sockeye. It occurs in lakes where the anadromous salmon must have had access at one time. Both kokanee and most anadromous sockeye return to the spawning grounds as four- or five-year-old fish. Both species spawn in the fall. The actual time depends on location. Kokanee, for example, spawn in September and October in Kootenay Lake in British Columbia but in November and December in Boulter Lake, Ontario. The major spawning grounds for this species are the watersheds draining into the Fraser, Skeena, and Nass rivers in British Columbia. Anadromous sockeye spawn between July and December, again depending on location.

Sockeye become sexually mature from three to eight years in age. While a one-year freshwater residence and a two- to three-year saltwater residence is normal, precocious males may return to spawn after only one year at sea. Fecundity is highly variable in this species. It ranges from 370 to 1760. While the kokanee egg is naturally small, the egg of the anadromous sockeye is large, measuring about 4.5–5 mm in diameter. After hatching and consuming all the yolk, the young sockeye will spend at least one year in fresh water, although some will spend two or even three years in fresh water before migrating to sea. This variability is, to some extent, responsible for the range in size of adult sockeye. These years in fresh water are spent in nursery lakes. The fry feed on planktonic organisms. Anadromous sockeye smolts will migrate to sea in the spring of their second and fifth year of life. At sea, the maturing sockeye migrate north and northwest, spreading out to the distribution outlined above.

Rainbow Trout. The rainbow trout, steelhead trout, and kamloops trout all belong to the same species, *Oncorhynchus mykiss* formerly *Salmo gairdneri*. This species represents one of the most highly prized game fishes in North America. The names rainbow and kamloops trout refer to the nonanadromous populations of this species, while the steelhead is normally used to refer to the large, anadromous variety of this species. The kamloops trout usually refers to the larger variety of the nonanadromous trout. All varieties of this species are highly prized sport fishes. The flesh is red to pink in fish that have been feeding on planktonic organisms and it tends to be more pale in fish from larger lakes where they feed on fish more than plankton. They take the bait or lure aggressively and fight the line, jumping out of the water many times in the process.

Most of the steelhead are caught in fresh water in coastal areas. The commercial catch is usually incidental to the salmon fishery and are almost exclusively canned. The commercial gear involved in this fishery, therefore, consists of gill nets.

Although the native range of this species is the eastern Pacific Ocean and for the freshwater forms, west of the Rocky Mountains from Mexico to Alaska, all forms of the rainbow trout can be found throughout North America in suitable habitats. It has also been introduced successfully in New Zealand, Australia, Tasmania, South America, Africa, Japan, southern Asia, Europe, and Hawaii. It is a very plastic species that adapts to different environments readily and may show variations in its physical and behavioral characteristics throughout its range. It is probably the most studied fish in terms of fish physiology and anatomy.

The nonanadromous rainbow and kamloops trout are naturally spring spawners. They move from the lakes to inlet or outlet streams from about mid-April to June and spawn in streams with beds of fine gravel. This is the timing for North America. Because their distribution spans the globe, the actual month of spawning will depend on when spring occurs at a particular location. The fecundity is about 1,400–2,700 eggs, but this is extremely variable; the range extends from 200 to 12,750. The eggs are 3–5 mm in diameter. The eggs usually hatch in about four to seven weeks and the yolk is consumed in about three to seven days. The young emerge from the gravel between mid June and mid August and may reside in the stream until fall of that year or they may spend as long as three years in the stream. The young of the rainbow and kamloops trout will then migrate upstream or downstream to the lake to feed and mature until they are ready to spawn.

The anadromous steelhead trout populations may spawn in the spring, fall or winter. An established population for a given stream, however, will maintain a consistent pattern from year to year. The fecundity and egg size are similar to the rainbow and kamloops trouts. Most of the young will spend two to three years in fresh water before migrating to sea as smolts in the spring, where they may spend an equal amount of time maturing in the ocean. Many will survive the spawning and return for a second or third spawning.

Atlantic Salmon

The Atlantic salmon (*Salmo salar*) has attracted both commercial and sport fisherman like no other species throughout history; so much so that populations of Atlantic salmon disappeared from the Thames in 1833 and from Lake Ontario in 1890 due to overexploitation. The Atlantic salmon are caught commercially with net and troll gear. They are caught in the sport fishery with live bait as well as on the fly and artificial lures. The intensive interest on this species has, unfortunately, eliminated entire populations of this species from certain watersheds over time. The commercial fishery for the Atlantic salmon off Greenland places a particularly heavy pressure on the Atlantic salmon population at large, because those waters represent the major feeding grounds for all the stocks in the Atlantic Ocean.

The native distribution of the Atlantic salmon is the basin of the North Atlantic Ocean, from the Arctic Circle to Portugal and from northern Quebec south to the Connecticut River. While fertilized eggs and live fish transplantations to the Pacific waters have occurred since 1905, there is no record of the establishment of self-sustaining populations. Like several of the Pacific salmons, there are established landlocked populations of this species in North America. The landlocked Atlantic salmon are sometimes called the ouananiche. There are self-sustaining populations in Sebago Lake, Maine; Lake Ontario; and Lake St. John, Quebec.

The Atlantic salmon is a typical anadromous fish. They spend 1–3 yr in fresh water as juveniles and migrate to sea as smolts where they may spend 1–2 yr growing and maturing. While mortalities in spawning are high, significant numbers of spawners return to spawn two or three times. The normal spawning time is from October to late November. The fish in the southern regions tend to spawn later. The female will produce about 1,540 eggs per kg body weight. The size of returning adults ranges from 2.7 to 6.8 kg. The returning adult spawner will normally have spent 2 yr at sea. The Atlantic salmon generally have longer life spans than the Pacific salmon. Ages up to 11 yr have been reported. While the eggs may hatch in April of the following year, the young alevins will stay in the gravel and consume their yolk until May or June, at which time they emerge into the water column. The period of freshwater residence is variable, depending on the location. The younger parr in North America will spend 2–3 yr in fresh water until they smolt, at about 15.2 cm in length. Populations in Greenland, however, have been recorded to spend as long as 4–8 yr in fresh water, attaining about the same body size.

Unlike the slow growth in fresh water, the Atlantic salmon undergo rapid growth at sea. An exception to the average size of returning adults, stated above, are the precociously matured fish that return after one year at sea. These are often males and are in the 1.4–2.7 kg range. There are many reports of individuals exceeding the normal size range. While the average Atlantic salmon caught commercially is about 4.6 kg, record sizes of 35.9 and 37.7 kg have been reported. While the average landlocked Atlantic salmon weighs about 0.9–1.8 kg, individual fish weighing 16.1 and 20.3 kg have been caught in North American lakes.

SMELT

The smelts are small, slender fishes that belong to the family Osmeridae. This group of fish probably got its name from one characteristic, their smell. The smell, which may be described as that of freshly cut cucumbers, is particularly noticeable when large numbers are caught during spawning runs. The six genera and 10 species are found in the northern hemisphere in the Atlantic, Arctic, and Pacific oceans. They are circumpolar in distribution. Some species in this family spend their entire life cycle in fresh water and others in sea water. There are species, furthermore, that are anadromous. The four species found in

North America are the pond smelt (*Hypomesus olidus*), the longfin smelt (*Spirinchus thaleichthys*), the Eulachon (*Thaleichthys pacificus*), and the rainbow smelt (*Osmerus mordax*). The latter two species are discussed here because the existence of both sport and commercial fisheries for them. The former two species play a minor part in any commercial fishery and in normal consumption.

Rainbow Smelt

As Figure 8 shows, the rainbow smelt is a slender fish that may reach lengths of 17.8–20.3 cm, although 35.6-cm fish have been recorded. As the name implies, it is a colorful silver fish with a pale green back and iridescent sides. The rainbow smelt has supported a commercial fishery for over 100 yr. It has always been abundant throughout its distribution. Most fish are caught in the spawning migration by trawl nets. The Atlantic rainbow smelt is also a popular sport fish. It is caught on hook and line through the ice as well as by dip netting or seine netting during the spawning run. It is an anadromous fish but like other anadromous fishes, it can successfully spend its entire life cycle in fresh water. The landlocked forms tend to have a darker color than the marine counterparts. As with other anadromous fishes, they lose the silver appearance and darken when they begin to spawn.

There are two subspecies of the *Osmerus mordax*: the Arctic rainbow smelt (*O. mordax mordax*) and the Atlantic rainbow smelt (*O. mordax dentex*). The Arctic rainbow smelt includes those populations that are in the Pacific Ocean and are distributed from Vancouver Island, north around the state of Alaska, and into the Arctic Ocean to Cape Bathurst. The distribution of the Atlantic smelt was originally restricted to the marine waters from New Jersey to Labrador. The Atlantic smelt today is represented by both anadromous forms and landlocked forms in the Great Lakes.

The anadromous and landlocked forms behave in a similar way in spawning. The spawning adults ascend streams to lay and fertilize their eggs. They begin their ascent in the spring when the ice cover disappears, usually in the months between March and May. Spawners can be up to six years old, but are commonly between two and three years old. Spawning, which usually occurs at night, involves two or more males to each female. While spawning normally takes place in streams, fish will spawn on the shoreline if the currents are too strong to permit migration. Eggs and milt are released into the moving current and the eggs quickly stick within seconds to anything they come in contact with. The fertilized eggs, which measure about 1 mm in diameter, eventually end up resembling balloons on stems as the sticky outer coat washes off except at the point of attachment. Most of the spawners, like many other anadromous fishes, die after spawning. Although reports of fecundity vary, about 494–530 eggs per gram of body weight are produced. The eggs incubate for about two to four weeks in water temperatures between 6 and 10°C. The hatched young, which are about 5 mm long are quickly swept down to the lake or estuary where they continue to feed and grow to a length of about 5 cm. They spend the remaining period up to spawning in the middle of the water column of lakes or in coastal waters close to shore.

Eulachon

Thaleichthys pacificus, the eulachon, means oily fish of the Pacific. Aside from the characteristic odor the oiliness of this fish is its outstanding feature. The oil content of the body is so high that the dried carcass can be lit like a candle. Another common name for this fish is candlefish, because in the days before the luxuries of candles and inexpensive oils, small strips of rags were inserted into the mouths of dried eulachons and lit for light. The rich oils lend a flavorful taste to this fish, which probably accounts for its high value as a food fish. The eulachon resembles the rainbow smelt in appearance, although it is darker in coloration and lacks the iridescence in the body walls. It is typically brown to blue-black on the dorsal surface. The coloration on the ventral surface is white and the sides are a light color, intermediate between the two extremes.

The eulachon is caught by the native Indian community as well as by the commercial fishery. The volume caught by the native fishery has exceeded, and probably still exceeds, the commercial catch. The commercial fishery utilizes drift gill nets and occurs principally in the Fraser River as the eulachon migrates to spawn. The commercial catch is used for human consumption but mostly for feed at fur farms. The native catch has always been based on the oil from the eulachon. It is used for cooking and for curing. Traditionally, the grease trails got their name because they were used to carry the extracted eulachon oil from the fishing grounds to the points of trade.

The eulachon is found only on the west coast of North America, from the Kalamath River in California up to the panhandle of Alaska and to the Pribilof Islands. Like the

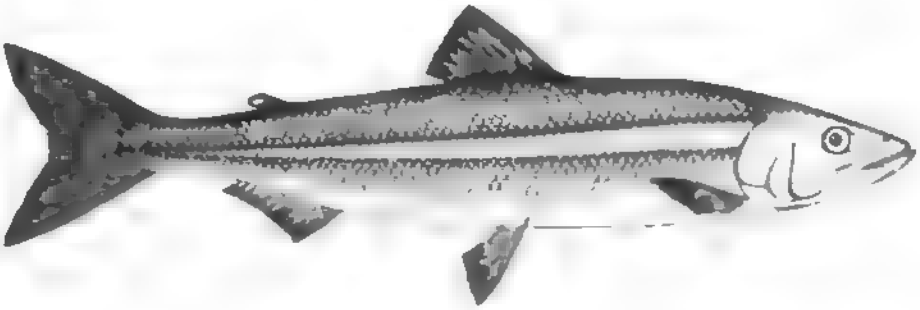


Figure 8. Rainbow smelt (*Osmerus mordax*). *Source:* Copyright 1990 by B. Guild-Gillespie.

rainbow smelt, the eulachon is an anadromous fish that migrates from the sea up freshwater streams, soon after the ice cover breaks, to spawn. This occurs from mid March to mid May. The spawning females, which measure about 15.2–17.8 cm in length, produce an average of 25,000 eggs and shed them in the free current along with the milt from the male partners. They are at least three years old when they spawn. Unlike the small rainbow smelt egg, the eulachon produces a relatively large egg of 8–10 mm in diameter, which is irregular in shape. The eulachon eggs have two outer layers. The outer, adhesive, layer breaks on spawning and everts to anchor the egg to coarse particles such as gravel. The outer and inner layers remain attached at one point. While most of the adults die after spawning, some survive to spawn a second time. Like the rainbow smelt, the eggs take about two to three weeks to hatch and the just-hatched young resemble young herring. They measure about 5 mm in length and are slender and nearly transparent. The small young are swept to sea with the current. They move to deeper waters farther from the shore as they mature. Although they normally measure 10.2–17.8 cm in length, individuals up to 30.5 cm have been recorded.

TUNA AND SWORDFISH

Tuna

The true tuna belong to the tribe Thunnini, which belongs to the family Scombridae of the suborder Scombroidei, order Perciformes (Fig. 9). The bullet, frigate, bluefin, albacore, yellowfin, skipjack, blackfin longtail, and the slender tuna are all true tunas. The other tribes in this family include the Sardini (bonitos), the Scomberomorini (seerfish), and the Scombrini (mackerel). The smallest tunas (bullet and frigate) usually weigh less than 3 kg while the largest (bluefin) tunas can exceed 700 kg. The large bluefin tunas can live to 38 yr. This family of fishes possesses remarkable limits for growth. The billfishes, such as the black marlin, grow even larger than the tuna. Commercial fisherman have reported taking black marlin in excess of 3,000 kg, no doubt among the largest fishes in all the seas.

The tunas have been fished for food for thousands of years. The skipjack, yellowfin, and bigeye tunas dominate the commercial catches. Most of the tuna harvests occur in the Pacific Ocean. About 50% of the world catch of tuna is caught by Japan and the United States. The balance is caught by almost every nation in the areas where the tuna are found. They are caught with almost every kind of gear including the pole and line method as well as giant purse seines and long-lining. About 40% of the tuna are caught by the pole and line method where large schools of fish are first baited close to the boat with live anchovies or sardines that are thrown overboard and then caught with barbless lures on single poles. This process is called chumming. The balance of the commercial catch are harvested by purse seines (30%) and by long lines (30%). The purse seiner encircles the school of fish up to about a kilometer in diameter while letting out a special net that can be gathered at the bottom, like a purse or bag. As the boat completes the circle, the purse is gathered up to the boat along with the fish. This technique had caused high mortalities in dolphins (also called porpoises by fisherman) that often congregate above schools of yellowfin tuna. Many of the dolphins die because they become entangled in the net and are prevented from surfacing to breath. After this came to public attention in the mid-1960s, purse seine nets and seining methods have been modified to prevent this entanglement and to allow the dolphins to escape from the net. The long-line vessel will let out a line up to 130 km in length with as many as 2,000 baited hooks dangling from this main line, supported by floats. In addition to the commercial catch, the tuna represent one of the most desirable sport fish in the world. It is well known for attracting avid sport fishermen who spend thousands of hours and dollars in pursuit of this challenging and exciting catch.

Tuna is a very expensive food item. It is at least as expensive as beef, relative to protein content. Over 90% of the world catch is consumed by the United States, Japan, France, Spain, Italy, and West Germany. A typical tuna fish sandwich is probably made of albacore, yellowfin, skipjack, or bigeye tuna. Certain species, such as the skipjack, are sold as a dried product in Japan. The flesh of the bluefin and bigeye tunas are valued as raw sashimi in Japan. Record prices for tuna come from Japan where the flesh of such large tuna, with a high oil content, has sold for as much as $26,000 U.S. a ton.

The tuna and the billfishes represent some of the most interesting fishes in the world in that they deviate from the norm. The tuna are found in every temperate and tropical ocean around the world. They are most commonly found in the Atlantic, Pacific, and the Indian oceans. Tuna and billfishes are not only among the largest fishes in the oceans, but are also the fastest swimmers in the world. At high speeds, their body forms represent an extremely high degree of streamlining an hydrodynamic efficiency. At

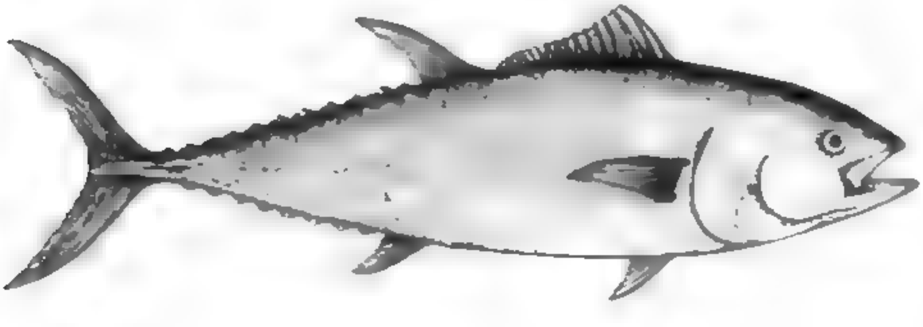


Figure 9. Tuna (*Thunnus thunnus*). *Source*: Copyright 1990 by B. Guild-Gillespie.

those speeds, their fins retract into grooves and their eyes become flush with the surface of the body. Even the slowest pace for the tuna represents the top speed any human could hope to achieve. Relatives of the tuna, the wahoo and sailfish have been recorded at swimming speeds over 75 and 100 km/h. Not only are these animals most remarkable for their speed but they are also known for the incredible distances they travel. Tagging studies, which tend to yield conservative estimates, have shown individual bluefin tuna and black marlin to travel over 10,000 km, at a rate of over 30 km/day. While most of the distance records are held by the larger bluefin tuna, the skipjack tuna has been recorded to travel over 9,500 km and the yellowfin tuna, over 5,000 km. There are numerous records of individually tagged members of this group of remarkable fish crossing every major ocean in the world.

As stated above, the general body shape of the tuna is one designed for hydrodynamic efficiency. The body is rather round in cross section and laterally oval. The tail is lunate (shaped like a crescent moon), which provides a high degree of forward thrust, with minimum drag. Like many other fishes, the upper body is dark and the ventral side is light in color to aid in camouflage. Body markings change with age and may include vertical bars or markings of different shapes. These markings may serve to identify sex, age, species, and particular stages in the reproductive cycle.

One need for the efficiency in swimming is their mode of respiration. Tuna are ram ventilators. That is, they must swim with their mouths open to pass sufficient water over their gills. One result of this swimming activity is the high energy requirements of this fish. A tuna can consume up to one-quarter of its body weight in food each day. Such a high metabolic rate naturally produces a great amount of heat. The tuna are rather unique among fishes in that four genera of the family regulate body temperature above that of the ambient water. The bluefin tuna, for example, maintains a visceral temperature 10–15°C above ambient water temperature. Maintaining a higher body temperature presumably allows quicker digestion as well as a more rapid mobilization of stored energy reserves. It may be more accurate to state that those tunas that regulate body temperature have specialized mechanisms for dissipating the heat from metabolism. Special circulatory structures enable this heat to be transferred to the external surface.

As stated above, the tuna is highly active fish. The need for high rates of oxygen uptake by the fish to feed this high metabolic rate is met by a larger gill surface area and higher hemoglobin content than most other fishes. The constant swimming motion in combination with pectoral fins that act like hydrofoils maintains the fish at a certain position in the water column. This greatly reduces the need for the swim bladder, which functions in other fishes as a buoyancy organ. The swim bladder is greatly reduced or absent in species of tuna. Unlike most other fishes, there is a greater proportion of red muscle to white muscle mass in the body. White muscle is used for short bursts of anaerobic activity while the red muscle is used for routine swimming in fishes. This makeup provides the machinery with which the tuna can accomplish the incredible journeys over long distances.

Spawning takes place in various regions, depending on the species. For example, the Atlantic mackerel spawns in the northwest Atlantic and the large bluefins spawn in the Straits of Florida and the Gulf of Mexico. Both eggs and sperm are broadcast into the water, near the surface. The eggs of the Atlantic mackerel are found mainly in the top 10 m of water. The female tuna lays about 100,000 eggs per kg body weight; there might be about 10 million eggs from a 100-kg animal. This is not unreasonable because the bluefin tuna can reach a maximum size of about 700 kg. These tiny eggs of about 1 mm float about in the upper layers of the ocean, held bouyant by an oil droplet inside the egg. While the various tuna species are found in waters of a wide range of temperatures, most eggs take only a few days to hatch. As in many other fishes, only about two of those individuals survive to maturity.

Swordfish

The swordfishes belong to the family Xiphidae (Fig. 10). The Xiphidae are related to other giant mackerellike fishes such as the tunas (Scombridae) and the spearfishes (Istiophoridae), in that they all belong to the suborder Scombroidei. The single representative of this species (*Xiphias gladius*) is known as the common swordfish or broadbill. It is highly prized as a game fish and as a food item. Like the tunas, there is evidence that the swordfish has been fished for thousands of years. Its dominant physical feature is the large sword protruding from the upper jaw. While this feature was thought to serve a predatory function, it may function more in the enhancement of swimming speed by reducing the resistance of water to the body, much like the pointed head of a rocket. There are reports, however, of many whales with broken bills of swordfish impaled in their bodies, some through their vertebrae. This fish may be referred to by sport anglers as the broadbill swordfish. The teeth and the spines of the first dorsal fin are lost with age. Its large body is scaleless.

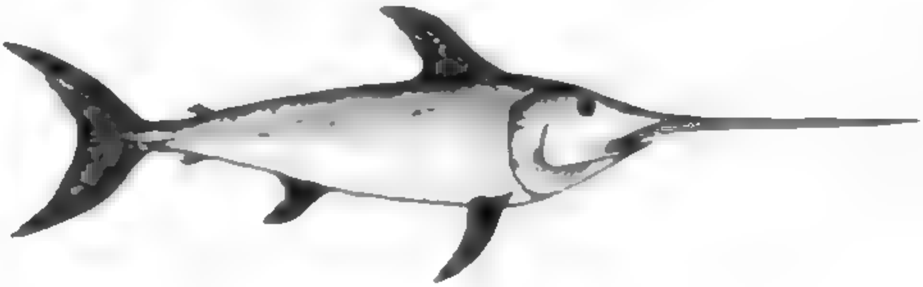


Figure 10. Swordfish (*Xiphias gladius*). *Source*: Copyright 1990 by B. Guild-Gillespie.

Although an individual weighing 537 kg was caught in 1953 off the Chilean coast, most other record sizes are closer to half that weight. This is relatively small compared to members of the other families in the Scombroidei. The billfishes also represent a minor part of the commercial catch, relative to the tunas. The average annual catch for the swordfish between 1982 and 1985 was estimated at 49,000 t, about 1.6% of the total catch for the Scombroidei.

Unlike the tunas and other billfishes that avoid deep waters, the swordfish moves widely in the water column. When it dives to great depths and returns to the surface, it is often found in semiconsciousness at the surface until it regains equilibrium. It is in this stupor that fishermen have used the harpoon to catch this prize. While this was the popular method of catch until the early 1960s, the majority of today's commercial catch of swordfish is by long line. This billfish also contributes to the specialized market of big game fishing around the world where spending $1,000/day in the pursuit of a sailfish or billfish is not uncommon. The local communities where big-game fishing ports are located may depend heavily on the economy of that sport.

The distribution of the swordfish extends throughout the temperate, subtropical, and tropical waters in all oceans of the world. They are found in coastal as well as oceanic areas. Like the related tunas and spearfishes, the swordfish are highly migratory and have body forms that are very streamlined. The basic physiological and reproductive features of the swordfish are similar to those of the tuna. Spawning is thought to occur throughout the year in the Caribbean Sea, Gulf of Mexico, and off the Florida coast. Although the swordfish egg is somewhat larger than the tuna egg, and the fecundity is slightly lower, the major features of the reproductive system and process are the same as those for the tuna. The eggs are approximately 1.7 mm in diameter and contain a large oil globule. Young hatch out at about 4 mm in length and may grow rapidly, up to 2 mm per day.

BIBLIOGRAPHY

1. *List of Common and Scientific Names from North America and Canada*, 4th ed., American Fisheries Society, 1980
2. W. A. Clemens and G. V. Wilby, *Fishes of the Pacific Coast of Canada*, 2nd ed., Fish. Res. Board Can. Bulletin **68**, 1961.
3. D. Miller, "The Blue Whiting, *Micromesistius poutassou*, in the Western Atlantic, with Notes on Its Biology," *Copeia* **2**, 301–305 (1966).
4. F. H. Bell, *The Pacific Halibut: The Resource and the Fishery*, Alaska Northwest Publishing Co., Anchorage, 1981.
5. W B. Scott and M G. Scott, "Atlantic Fishes of Canada," *Can. Bull. Fish. Aquat. Sci.* **219**, 1–731 (1988).

GENERAL REFERENCES

References 1–4 are good general references.

D. L. Alverson, A. T. Pruter, and L. L. Ronholt, *A Study of Demersal Fishes and Fisheries of the Northeastern Pacific Ocean, H R. MacMillan Lecture Series in Fisheries*, Inst. Fish. University British Columbia, 1964.

G. Godson, *Fishes of the Pacific Coast*, Stanford University Press, Stanford, Calif., 1988.

J. L. Hart, "Pacific Fishes of Canada," *Bull. Fish. Res. Board Can.* **180**, 1973.

J. G. Hunter, S. T. Leach, D. E McAllister, and M. B Steigerwald, "A Distributional Atlas of Records of the Marine Fishes of Arctic Canada in the National Museums of Canada and Arctic Biological Station," *Syllogeus* **52**, 1–35 (1984).

H. Kasahara, *Fisheries Resource of the North Pacific Ocean. Part 1. H. R. MacMillan Lectures in Fisheries*, Institute of Fisheries, the University of British Columbia, Vancouver, 1960

P. A. Larkin and W. E. Ricker, "Canada's Pacific Marine Fisheries. Past Performance and Future Prospects," in *Inventory of the Natural Resources of British Columbia*, 1964, 194–268.

Y. Okada, *Fishes of Japan*, Maruzen Co. Ltd., Tokyo, 1955.

F. S. Russell, *The Eggs and Planktonic Stages of British Marine Fishes*, Academic Press, Inc., Orlando, Fla., 1976.

D. J. Scarratt, ed., "Canadian Atlantic Offshore Fishery Atlas," *Can. Spec. Publ. Fish. Aquat. Sci.* **47**, 1982.

W. B. Scott and E. J. Crossman, "Freshwater Fishes of Canada," *Bull. Fish. Res. Board Can.* **184**, 1973.

A. Wheeler, *The Fishes of the British Isles and North-west Europe*, Michigan State University Press, East Lansing, 1969.

N. J. Wilimovsky, "List of the Fishes of Alaska," *Stanford Ichthyology Bull.* **4**, 279–294 (1954).

Specific to SpeciesS. F. Hildebrand, "Family Engraulidae," in *Fishes of Western North Atlantic, Mem. Sears Found. Mar Res.*, Vol. 3, Yale University, New Haven, Conn., 1963, pp. 151–249.

J. L. McHugh and J. E. Fitch, "An Annotated List of the Cluepoid Fishes of the Pacific Coast from Alaska to Cape San Lucas, Baja California, *Calif Fish Game* **37**, 491–495 (1951).

R. H. Boyle, *Bass*, W. W. Norton & Co., New York, 1980.

R. H Boyle, *The Hudson River, A Natural and Unnatural History*, New York, 1979.

J. N. Cole, *Striper*, Boston, 1978.

N. Karas, *The Complete Book of the Striped Bass*, New York, 1974.

E C. Raney, E. F. Tresselt, E H Hollis, V. D. Vladykov, and D. H. Wallace, *The Striped Bass.* Bulletin of the Bingham Oceanographic Collection 14, Peabody Museum of Natural History, Yale University Press, New Haven, Conn., 1952.

M. P. Fahay, "Guide to the Early Stages of Marine Fishes Occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf," *J. Northw. Atl. Fish. Sci.* **4**, 1–423 (1983).

L. S. Incze, C. M. Lynde, S. Kim, and R. Strickland, "Walleye Pollock, *Theragra chalcogramma*, in the Eastern Bering Sea," in N. J. Wilimovsky, L. S. Incze, and S. J. Westerheim, eds., *Species Synopsis: Life Histories of Selected Fish and Shellfish of the Northeast Pacific and Bering Sea*, Washington Sea Grant Program and Fisheries Research Institute, University of Washington, Seattle, 1988, pp. 55–69.

H. A. Innis, *The Cod Fisheries, The History of an International Economy*, Yale University Press, New Haven, Conn., 1940.

H. A. Innis, *The Cod Fisheries*, University of Toronto Press, Toronto, 1954, pp. 1–10.

A. C. Jensen *The Cod*, Fitzhenry and Whiteside, Toronto, 1972

S. Kim and D. R. Gunderson, "Walleye Pollock, *Theragra chalcogramma*, in the Gulf of Alaska," in H. J. Wilimovsky, L. S Incze, and S. J. Westrheim, eds., *Species Synopsis: Life Histories of Selected Fish and Shellfish of the Northeast Pacific and Bearing Sea*, Washington Sea Grant Program and Fisheries Research Institute, University of Washington, Seattle, 1988, pp. 70–82.

D. H. Steele, "Pollock (*Pollachius virens* (L.)) in the Bay of Fundy," *J. Fish. Res. Board Can.* **20**, 1267–1314 (1963).

C. R. Forrester, "Life History Information Some Groundfish Species," *Fish. Res. Board Can. Tech. Rep.* **105**, 1969.

F. B. Hagerman, *The Biology of the Dover Sole, Microstomus pacificus (Lockinton)*, Calif. Div. Fish. Gam Fish. Bull. **78**, 1949.

International Pacific Halibut Commission, *The Pacific Halibut: Biology, Fishery, and Management*, IPHC Technical Report No. **22**, 1987.

K. S. Parker, "Pacific Halibut, *Hippoglossus stenolepsis*, in the Gulf of Alaska," in N. J. Wilmovsky, L. S. Incze, and S. J. Westrheim, eds., *Species Synopsis: Life Histories of Selected Fish and Shellfish of the Northeast Pacific and Bering Sea*, Report of the Washington Sea Grant Program and Fisheries Research Institute University of Washington, Seattle, 1988, pp. 94–111.

J. H. S. Blaxter, "The Herring: A Successful Species," *Can. J. Fish. Aquat. Sci.* **42**(Suppl. 1), 21–30 (1985).

J. H. S. Blaxter and F. G. T. Holliday, "The Behavior and Physiology of Herring and Other Clupeoids," in F. S. Russell, ed. *Adv. Mar. Biol.*, Vol. 1, Academic Press, Orlando, Fla., 1963, pp. 261–393.

J. H. S. Blaxter and J. R. Hunter, "The Biology of the Clupeoid Fishes," in J. H. S. Blaxter, F. S. Russell, and M. Yonge, eds., *Advanced Marine Biology*, Vol. 20, Academic Press, Orlando, Fla., 1982, pp. 1–223.

D. J. Grosse and D. E. Hay, "Pacific Herring *Clupea harengus pallasi*, in the Northeast Pacific and Bering Sea," in N. J. Wilimovsky, L. S. Incze, and S. J. Westrheim, eds., *Species Synopsis: Life Histories of Selected Fish and Shellfish of the northeast Pacific and Bering Sea.* Washington Sea Grant Program and Fisheries Research Institute, University of Washington Press, Seattle, 1988, pp. 34–54.

S. Morita, "History of the Herring Fishery and Review of Artificial Propagation Techniques for Herring in Japan," *Can. J. Fish. Aquat. Sci.* **42**(Suppl 1), 22–229 (1985).

J. W. Reintjes, *Synopsis of Biological Data on the Atlantic Menhaden, Brevortia Tyrannus*, FAO Species Synop. **42**, Cir. 320, Washington, D.C. 1969.

W. Templeman, *Redfish Distribution in the North Atlantic*, Bull. Fish. Res. Board Can. **120**, 1959.

B. A. Branson, "Sockeye Salmon," *Oceans* **9**, 25–29 (1976).

R. E. Foerster, "The Sockeye Salmon, *Oncorhynchus nerka*," *Bull. Fish. Res. Board Can.* **162**, 1968.

W. S. Hoar, "The Chum and Pink Salmon Fisheries of British Columbia, 1917–1947," *Bull. Fish. Res. Board Can.* **90**, 1951.

F. Neave, "The Origin and Speciation of *Oncorhynchus*," *Trans. Roy. Soc. Can.* **52**, 25–39 (1958)

S. D. Sedgwick, *The Salmon Handbook*, Andre Deutsch Ltd, 1982.

L. Shapovalov and A. C. Taft, "The Life Histories of the Steelhead Rainbow Trout (*Salmo gairdneri gairdneri*) and Silver Salmon (*Oncorhynchus kistuch*)," *Calif. Fish Bull.* **98**, 1985.

N. J. Wilimovsky, ed., *Symposium on Pink Salmon*, Institute of Fisheries, University of British Columbia, Vancouver, 1962.

M. Fish, "A Review of the Fishes of the Genus *Osmerus* of the California Coast," *Proc. U.S. Nat. Mus.* **46**(2027), 29–297 (1913).

J. L. Hart, "Pacific Fishes of Canada," *Bull. Fish. Res. Board Can.* **180**, 1973

J. L. Hart and J. L. McHugh, "The Smelts (*Osmeridae*) of British Columbia," *Bull. Fish. Res. Board Can.* **64**, 1944.

M. J. A. Butler, "Plight of the Bluefin Tuna," *National Geographic* **169**, 220–239 (1982).

J. Joseph, W. Klawe and P. Murphy, *Tuna and Billfish-Fish Without a Country*, Inter-American Tropical Tuna Commission, Scripps Institution of Oceanography, LaJolla, Calif. 1988.

G. D. Sharp, and A. E. Dizon, eds., *The Physiology Ecology of Tunas*, Academic Press, Inc., Orlando, Fla , 1978.

George Katsushi Iwama
University of British Columbia
Vancouver, British Columbia
Canada

FISH, MINCED. See Surimi: Science and Technology.

FLAVOR CHEMISTRY

OVERVIEW OF FLAVOR COMPOUNDS

Flavors

Flavors possess a variety of chemical groups and structures. They can be heterocyclic, carbocyclic, terpenoid, aromatic, and so on. The overall flavor of foods is due to carbohydrates, lipids, and proteins; however, specific flavors can be elicited by numerous other classes of compounds, such as alcohols, aldehydes, ketones, and various heterocyclic compounds (pyrazines, pyrroles, pyridines etc). Flavor components in food range in number from 50 to 250 compounds in fresh products such as fruits and vegetables and to more than double this number in foods subjected to heat or enzyme treatment; for example, more than 700 compounds have been reported in roast coffee aroma (1). The investigation of around 200 different food products has led to the identification of nearly 5000 compounds (2,3), the vast majority of them identified by gas chromatography/mass spectrometry (GC/MS) analysis.

Flavor sensation may be due to a single compound (4) or to a group of compounds. Single compounds are called flavor notes; examples are 4-hydroxy-3-methoxybenzaldehyde (vanillin) (Fig. 1), the character note for vanilla flavor, and 3-phenyl-2-propenal (cinnamaldehyde) (Fig. 2) for cinnamon flavor. On the other hand, a group of compounds representing a particular flavor is called the flavor profile

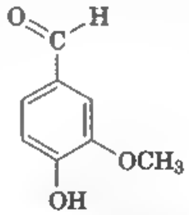


Figure 1. 4-Hydroxy-3-methoxybenzaldehyde (vanillin).

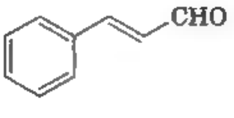


Figure 2. 3-Phenyl-2-propenal (cinnamaldehyde).

F. B. Hagerman, *The Biology of the Dover Sole, Microstomus pacificus (Lockinton)*, Calif. Div. Fish. Gam Fish. Bull. **78**, 1949.

International Pacific Halibut Commission, *The Pacific Halibut: Biology, Fishery, and Management*, IPHC Technical Report No. **22**, 1987.

K. S. Parker, "Pacific Halibut, *Hippoglossus stenolepsis*, in the Gulf of Alaska," in N. J. Wilmovsky, L. S. Incze, and S. J. Westrheim, eds., *Species Synopsis: Life Histories of Selected Fish and Shellfish of the Northeast Pacific and Bering Sea*, Report of the Washington Sea Grant Program and Fisheries Research Institute University of Washington, Seattle, 1988, pp. 94–111.

J. H. S. Blaxter, "The Herring: A Successful Species," *Can. J. Fish. Aquat. Sci.* **42**(Suppl. 1), 21–30 (1985).

J. H. S. Blaxter and F. G. T. Holliday, "The Behavior and Physiology of Herring and Other Clupeoids," in F. S. Russell, ed. *Adv. Mar. Biol.*, Vol. 1, Academic Press, Orlando, Fla., 1963, pp. 261–393.

J. H. S. Blaxter and J. R. Hunter, "The Biology of the Clupeoid Fishes," in J. H. S. Blaxter, F. S. Russell, and M. Yonge, eds., *Advanced Marine Biology*, Vol. 20, Academic Press, Orlando, Fla., 1982, pp. 1–223.

D. J. Grosse and D. E. Hay, "Pacific Herring *Clupea harengus pallasi*, in the Northeast Pacific and Bering Sea," in N. J. Wilimovsky, L. S. Incze, and S. J. Westrheim, eds., *Species Synopsis: Life Histories of Selected Fish and Shellfish of the northeast Pacific and Bering Sea*. Washington Sea Grant Program and Fisheries Research Institute, University of Washington Press, Seattle, 1988, pp. 34–54.

S. Morita, "History of the Herring Fishery and Review of Artificial Propagation Techniques for Herring in Japan," *Can. J. Fish. Aquat. Sci.* **42**(Suppl 1), 22–229 (1985).

J. W. Reintjes, *Synopsis of Biological Data on the Atlantic Menhaden, Brevortia Tyrannus*, FAO Species Synop. **42**, Cir. 320, Washington, D.C. 1969.

W. Templeman, *Redfish Distribution in the North Atlantic*, Bull. Fish Res. Board Can. **120**, 1959.

B. A. Branson, "Sockeye Salmon," *Oceans* **9**, 25–29 (1976).

R. E. Foerster, "The Sockeye Salmon, *Oncorhynchus nerka*," *Bull. Fish. Res. Board Can.* **162**, 1968.

W. S. Hoar, "The Chum and Pink Salmon Fisheries of British Columbia, 1917–1947," *Bull. Fish. Res. Board Can.* **90**, 1951.

F. Neave, "The Origin and Speciation of *Oncorhynchus*," *Trans. Roy. Soc. Can.* **52**, 25–39 (1958)

S. D. Sedgwick, *The Salmon Handbook*, Andre Deutsch Ltd, 1982.

L. Shapovalov and A. C. Taft, "The Life Histories of the Steelhead Rainbow Trout (*Salmo gairdneri gairdneri*) and Silver Salmon (*Oncorhynchus kistuch*)," *Calif. Fish Bull.* **98**, 1985.

N. J. Wilimovsky, ed., *Symposium on Pink Salmon*, Institute of Fisheries, University of British Columbia, Vancouver, 1962.

M. Fish, "A Review of the Fishes of the Genus *Osmerus* of the California Coast," *Proc. U.S. Nat. Mus.* **46**(2027), 29–297 (1913).

J. L. Hart, "Pacific Fishes of Canada," *Bull. Fish. Res. Board Can.* **180**, 1973

J. L. Hart and J. L. McHugh, "The Smelts (*Osmeridae*) of British Columbia," *Bull. Fish. Res. Board Can.* **64**, 1944.

M. J. A. Butler, "Plight of the Bluefin Tuna," *National Geographic* **169**, 220–239 (1982).

J. Joseph, W. Klawe and P. Murphy, *Tuna and Billfish-Fish Without a Country*, Inter-American Tropical Tuna Commission, Scripps Institution of Oceanography, LaJolla, Calif. 1988.

G. D. Sharp, and A. E. Dizon, eds., *The Physiology Ecology of Tunas*, Academic Press, Inc., Orlando, Fla , 1978.

GEORGE KATSUSHI IWAMA
University of British Columbia
Vancouver, British Columbia
Canada

FISH, MINCED. See SURIMI: SCIENCE AND TECHNOLOGY.

FLAVOR CHEMISTRY

OVERVIEW OF FLAVOR COMPOUNDS

Flavors

Flavors possess a variety of chemical groups and structures. They can be heterocyclic, carbocyclic, terpenoid, aromatic, and so on. The overall flavor of foods is due to carbohydrates, lipids, and proteins; however, specific flavors can be elicited by numerous other classes of compounds, such as alcohols, aldehydes, ketones, and various heterocyclic compounds (pyrazines, pyrroles, pyridines etc). Flavor components in food range in number from 50 to 250 compounds in fresh products such as fruits and vegetables and to more than double this number in foods subjected to heat or enzyme treatment; for example, more than 700 compounds have been reported in roast coffee aroma (1). The investigation of around 200 different food products has led to the identification of nearly 5000 compounds (2,3), the vast majority of them identified by gas chromatography/mass spectrometry (GC/MS) analysis.

Flavor sensation may be due to a single compound (4) or to a group of compounds. Single compounds are called flavor notes; examples are 4-hydroxy-3-methoxybenzaldehyde (vanillin) (Fig. 1), the character note for vanilla flavor, and 3-phenyl-2-propenal (cinnamaldehyde) (Fig. 2) for cinnamon flavor. On the other hand, a group of compounds representing a particular flavor is called the flavor profile

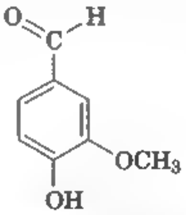


Figure 1. 4-Hydroxy-3-methoxybenzaldehyde (vanillin).

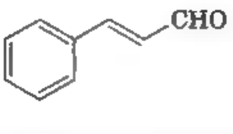


Figure 2. 3-Phenyl-2-propenal (cinnamaldehyde).

of that food product (5). Although a large number of flavor compounds are usually identifiable in a flavor profile, in general only a small number have a significant effect on the overall flavor, these compounds are often called character impact compounds. An example is 2-isobutylthiazole (Fig. 3), the character impact compound found in tomato. The relative concentration of each component in a flavor profile is crucial for its imitation. Flavors can be biosynthesized naturally in foods, such as apples, bananas, and other fruits and vegetables, or they can be produced from precursors during processing or thermal treatment such as baking, roasting, and frying. Furthermore, flavors can be generated by enzymatic modifications such as in cheese or by microbial fermentation as in butter.

The origin of natural flavors and their precursor compounds is found in animal and plant tissue, so flavor research interfaces with other disciplines in its effort to extract and identify these compounds (6,7).

Flavor Enhancers

Monosodium glutamate (MSG), 5′-ribonucleotides such as 5′-inosine monophosphate (5′-IMP) and 2-methyl-3-hydroxy-4-(4*H*)-pyrone (maltol) (Fig. 4) are called flavor enhancers. The actual mechanism of flavor enhancement is not known. These substances contribute a delicious or umami taste to foods when used at levels in excess of their detection limits and enhance flavors of food at levels below their detection thresholds. Their effects are prominent and desirable in the flavors of vegetables, dairy products, meats, poultry, and fish.

Essential Oils

Essential oils are also known as volatile oils, or essences. When exposed to air they evaporate at room temperature. They are usually complex mixtures of a wide variety of organic compounds (such as hydrocarbons, terpenes, alcohols, ketones, phenols, aldehydes, esters, etc). Essential oils are obtained by steam distillation or solvent extraction from many odorous plant sources such as clove, cinnamon, orange, lemon, jasmine, rose, and so on. The organic constituents of essential oils are synthesized by the plant during its normal growth.

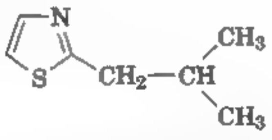


Figure 3. 2-*iso*-Butylthiazole.

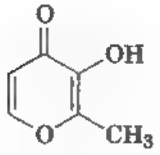


Figure 4. 2-Methyl-3-hydroxy-4(4*H*)-pyrone (maltol).

Resins

Resins are natural products that can be obtained either directly from the plant as exudates or are prepared by alcohol extraction of plants that contain resinous materials. Naturally occurring resins are solids or semisolids at room temperature. They are soluble in alcohol or basic solutions but insoluble in water. They are usually noncrystalline and soften or melt on heating. Chemically, they are complex oxidation products of terpenes. They rarely occur in nature without being mixed with gums and/or volatile oils forming oleoresins.

SENSORY BASIS OF FLAVOR

Flavor can be defined as the combined perception of taste and smell. It involves receptors in both the oral and nasal cavities. In the oral cavity the taste buds are mainly distributed on the surface of the tongue. Each taste bud consists of a barrel-like structure in which the taste cells are packed. Taste receptors, which are found on the surface of the taste cells, are linked to the brain by way of cranial nerves, which carry the nerve impulses to the brain after the neurotransmitters are released from the taste cells. This process is initiated by the formation of the taste compound–receptor complex. Olfactory cells on the other hand are situated in the upper part of the nasal cavity. Their receptors perform a similar function to that of the taste cells through their own nerve fibers, which transmit neural impulses from receptors directly to the olfactory bulb in the brain There is a common view that there are four primary taste qualities—sweet, sour, bitter and salty—and seven primary odors—camphoraceous, musky, floral, peppermint, ethereal, pungent, and putrid (8).

Sweet Taste

Sweet taste is produced by several different classes of compounds (Fig. 5), such as sugars, polyhydric alcohols, α amino acids, proteins, and synthetic sweeteners.

Sour Taste

Sour taste results from the presence of hydrogen ions on the tongue; however, sourness and acidity (pH) are not directly related, but there is some correlation. Two acids having the same pH do not produce the same degree of response.

Salty Taste

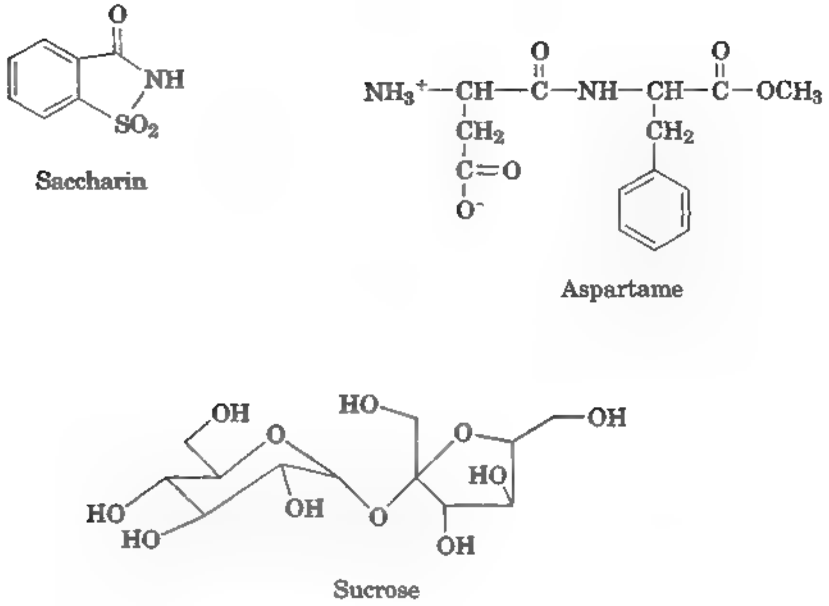
Salt taste is stimulated by most soluble salts having low molecular weights.

Bitter Taste

Three major classes of organic compounds encountered in food materials are associated with bitterness: alkaloids, glycosides (Fig. 6), and peptides.

Astringency

A taste-related phenomena perceived as a dry feeling in the mouth along with a coarse puckering of the oral tissue,


Saccharin

Aspartame

Sucrose

Figure 5. Examples of sweet-tasting compounds.

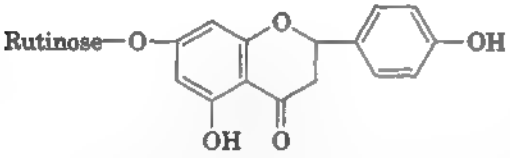


Figure 6. Naringin, a bitter-tasting glycoside.

astringency is due to tannins or polyphenols interacting with the proteins in the saliva to form precipitates or aggregates. Astringency may be a desirable flavor property, such as in tea and red wine.

Pungency

Certain compounds (Fig. 7) found in several spices and vegetables cause characteristic hot, sharp, burning, and stinging sensations that are known collectively as pungency.

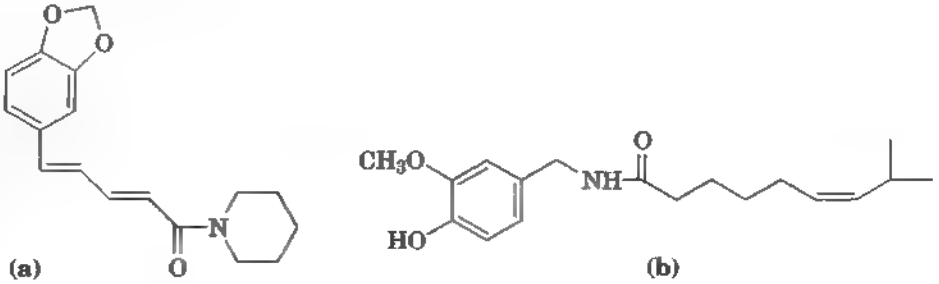
Cooling Effect

Cooling sensations occur when certain chemicals (Fig. 8) contact the nasal or oral tissues and stimulate a specific receptor. This effect is most commonly associated with mintlike flavors, including peppermint, spearmint, and wintergreen.

CLASSIFICATION AND ORIGIN OF FLAVOR COMPOUNDS

Classification of Flavor Compounds Based on Their Mode of Formation: Biogenetic and Thermogenetic

One way of classifying flavor compounds is based on their mode of formation; flavors can be generated either naturally (9,10) or by heat treatment during food processing (11,12). Natural flavors are mainly the secondary metabolites of the living tissue, formed during the natural growth cycle of the organism by the action of enzymes, whereas flavors produced by heat treatment are the result of thermal degradation and oxidation of various food ingredients and their complex interactions. The initial or primary precursors of flavors are the polymers found in food such as proteins, lipids, polysaccharides, and DNA, which can undergo either enzymatic or thermal hydrolysis to produce the intermediate precursors, mainly dimers and monomers. These, in turn, can undergo different biotransformation reactions during normal metabolic growth and produce metabolites that have specific flavor qualities that remain *in situ* when the plant is harvested and produce the perceived flavor effect when consumed. On the other hand, during thermal processing of food products, the intermediate precursors formed by thermal hydrolysis can undergo complex chemical transformations, and further


(a)

(b)

Figure 7. Examples of compounds causing pungency. (a) Piperine, component responsible for the sensation of black pepper; (b) capsaicin, the pungent principal of "hot" pepper.

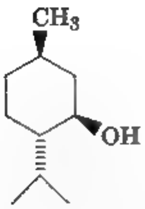


Figure 8. (−)-Menthol causes a cooling sensation.

degradations, to generate cooked flavor (Fig. 9). The type of flavor effect depends on the conditions of processing and the type of the initial precursors found in the particular food product. In terms of composition, thermally generated flavors are richer in heterocyclic compounds compared with enzymatically produced flavors.

Precursors of Flavor Compounds in Food

Meat and Related Products. Raw meat has no particular appealing flavor. However, during various processes of cooking, the initial precursors in meat produce water-soluble intermediate precursors, such as glycopeptides, free nucleotides, peptides, amino acids, amino-sugars, free sugars, fatty acids, and so on. The meat flavor, therefore, is the combined effect of the chemicals produced from the thermal degradation of these intermediates and the products formed from the amino–carbonyl interactions, such as between the reducing sugars and amino acids; this reaction is known as the Maillard reaction or nonenzymatic browning as it is responsible also for the brown color produced when foods are heated (11–13). During thermal degradation amino acids and sugars produce complex mixture of compounds. Sulfur-containing amino acids are specially important for the generation of meat aroma; cysteine, cyctine, and methionine produce sulfur-containing small molecules, such as hydrogen sulfide, 3-(methylthio)-propionaldehyde, and 2-mercaptoethylamine, that play an important role in the aroma of meat products (Fig. 10). Thermal degradation of thiamine produces furans, thiophenes, and thiazoles, important heterocyclic compounds with meatlike flavor. Thermal oxidation of unsaturated fatty acids leads to the formation of different aldehydes.

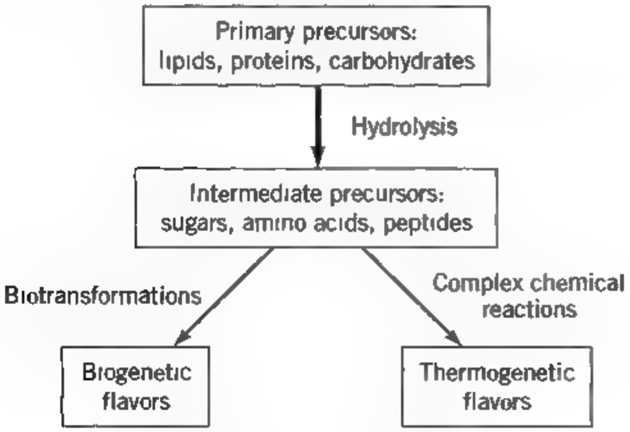

Primary precursors:
lipids, proteins, carbohydrates
Hydrolysis
Intermediate precursors:
sugars, amino acids, peptides
Biotransformations
Complex chemical reactions
Biogenetic flavors
Thermogenetic flavors

Figure 9. Origin of flavor compounds in food.

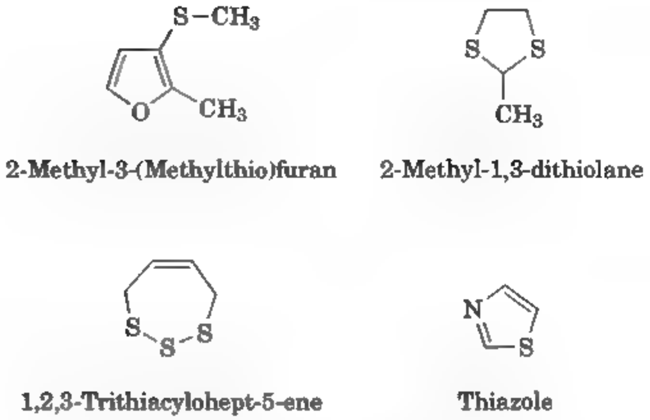

S−CH3
O
CH3
2-Methyl-3-(Methylthio)furan
S
S
CH3
2-Methyl-1,3-dithiolane
S
S
S
1,2,3-Trithiacylohept-5-ene
N
S
Thiazole

Figure 10. Aroma compounds identified in cooked beef.

Nonalcoholic Beverages: Tea and Coffee. Tea and coffee are examples of food products in which both modes of flavor formation, thermal and enzymatic, play a role in the generation of flavor. During the initial fermentation of green tea leaves, for example, important intermediate precursors are produced by the action of endogenous enzymes to produce polyphenols, carotenoids, and unsaturated fatty acids, such as lenolenic acid, which under the action of lipoxygenase enzymes can produce important flavor aldehydes such as (*Z*)-3-hexenal and (*E*)-2-hexenal. In the heat-treatment stage tea leaves are dried at 85°C and the coffee beans are roasted at >180°C to produce numerous volatile aroma compounds (Fig. 11) by thermal degradation and Maillard reaction processes. The main intermediate precursors involved during roasting of coffee are sugars, amino acids, fatty acids, peptides, amines, phenolic acids such as 3-(3′,4′-dihydroxyphenyl)propenoic acid (caffeic acid), 1-methylpyridinium-3-carboxylate (trigonelline), and so on. In addition, tea and coffee contain alkaloids such as 1,3,7-trimethylxanthine (caffeine) and 3,7-dimethylxanthine (theobromine) that impart a bitter taste to the beverage.

Alcoholic Beverages: Wine. There are three main sources of flavor compounds in wine; some are already present in the grape, some are formed during fermentation process, and some may form during aging by Maillard-type reactions. The type of wood in which the wine is stored can

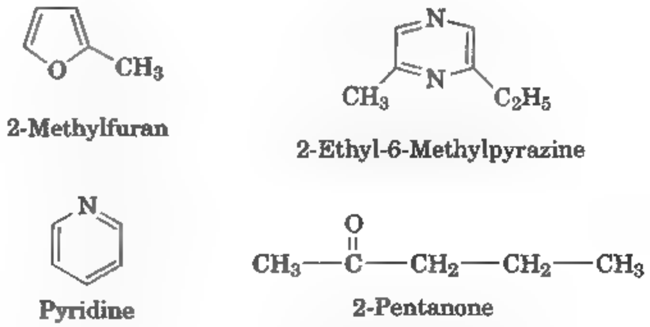

O
CH3
2-Methylfuran
N
N
CH3
C2H5
2-Ethyl-6-Methylpyrazine
N
Pyridine
O
CH3—C—CH2—CH2—CH3
2-Pentanone

Figure 11. Some volatiles identified in coffee aroma.

also contribute to the overall flavor of the wine. Most of the 300 or so flavor compounds identified in different wines such as 2-methyl-1-propanol, 1-hexanol, 2-phenyl-ethanol, ethyl acetate, and ethyl lactate, are formed during fermentation. The natural aroma of some grape varieties results from a mixture of different terpenes such as (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol), 3,7-dimethyl-1,6-octadien-3-ol (linalool), (*Z*)-3,7-dimethyl-2,6-octadien-1-ol (nerol) (Fig. 12). Sugars such as arabinose, xylose, mannose, and rhamnose produce furans during the aging process by reacting with the amino acids such as proline, alanine, and asparagine by the Maillard reaction (14,15).

Fruits and Vegetables: Tomato. Generally, the aroma of all fresh fruits and vegetables is produced by the action of enzymes on the hydrolysis products of proteins, lipids, and carbohydrates. Free fatty acids in tomato and other vegetables are generated by the action of phospholipases. Then, lipoxygenase enzymes convert the unsaturated fatty acids into important flavor aldehydes such as (*Z*)-3-hexenal, hexanal and alcohols such as 3-hexenol. Amino acids can also be converted into aldehydes and alcohols by the action of enzymes for example leucine is converted into 3-methyl-butanal and 3-methyl-butanol; carotenoids in tomato can be converted into flavor ketones such as 2,6-dimethyl-2,6-undecadien-10-one.

Milk and Dairy Products. More than 200 aroma compounds have been identified in differently processed milk. Some of these compounds have their origin in the raw milk, and some of them are formed during processing due to the degradation of the main precursors found in the raw milk such as fats, carbohydrates (mainly lactose), and proteins. These precursors undergo two types of transformations: (*1*) chemical, such as oxidations, and (*2*) biochemical with the participation of the enzymes and the microbial flora contained in the milk or by exogenous bacteria and microbial flora, such as during preparation of cheese and yogurt. The most important precursor responsible for the production of milk aroma is the lipid. Milk triglycerides are hydrolyzed by bacterial lipases to produce free fatty acids, which in turn are oxidized into different carbonyl compounds, among them many methyl ketones responsible for the milk aroma. Other important carbonyl compounds include oct-1-en-3-one (responsible for the metallic aroma), (*Z*)-4-heptenal (characteristic odor of cream), and γ and δ lactones formed by the thermal oxidation of fatty acids (8).

OCCURRENCE AND ORGANOLEPTIC PROPERTIES OF HETEROCYCLIC COMPOUNDS IN FOOD

Although heterocyclic compounds are present only in minute amounts in foods, they constitute the most important character impact compounds because of their low threshold values. They are extremely important as compounding ingredients and in the development of new flavors. Almost half of the 5000 flavor compounds identified till now are heterocyclic in nature (16).

Furans

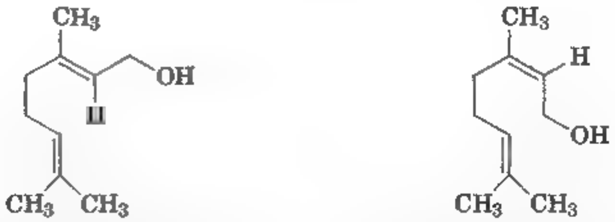
Furans are mainly associated with caramel-like, sweet, fruity, nutty, meaty, and burnt odor impressions. Because of their olfactory properties, many furans are commercially important flavoring chemicals. Furans are formed in food by thermal degradation of carbohydrates and by the Maillard reaction. They are almost present in all food aromas and essential oils. The most abundant furans are 2- and 2,5-disubstituted, such as 2-methylfuran, which is found in coffee aroma. The 2-substituted furans with aldehyde, ketone, or alcohol functional groups generally have fruity aromas with the mild flavor of caramel when added in small amounts to nonalcoholic beverages and ice creams. 3-Acetyl-2,5-dimethylfuran is used in imitation nut flavors (Fig. 13).

Thiophenes

Thiophenes can significantly contribute to the sensory properties of foods, but they are not as numerous as furans; they have been detected in boiled and canned beef, cooked chicken, asparagus, leeks roasted peanuts, popcorn, rice, bread, and coffee. Of the 46 sulfur-containing compounds identified in pressure-cooked lean ground beef, 20 were thiophenes. The most frequently found thiophenes are the parent compound itself, 2-alkylated, 2- and 3-acylated derivatives; they are described as being pungent and green-sweet. 2-Acetyl-3-methylthiophene (Fig. 14) is described in a patent as imparting a honeylike flavor to syrup bases, and 3-acetyl-2,5-dimethylthiophene is recommended in another patent for improving the aroma of tobacco and perfumes.

Pyrroles

Pyrroles are among the most widespread heterocyclic compounds found in food flavors such as coffee, roasted peanuts, popcorn, and tobacco smoke. Few pyrroles have also



(*E*)-3,7-Dimethyl-2,6-octadiene-1-ol (Gereniol)

(*Z*)-3,7-Dimethyl-2,6-octadiene-1-ol (Nerol)

Figure 12. Terpenoids in wine.

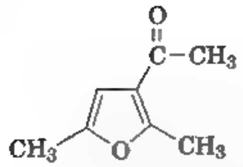


Figure 13. 3-Acetyl-2,5-dimethylfuran.

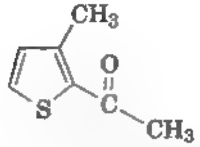


Figure 14. 2-Acetyl-3-methylthiophene.

been identified in cooked beef, cooked asparagus, leeks, and bread. They are mainly formed via the Maillard reaction during cooking. N- and 2-substituted pyrroles are more common, and they impart a burnt character note to the food product. 1-Pyrroline significantly enhances butter flavor of manufactured margarine (Fig. 15).

Thiazoles

Thiazoles possess extraordinary potent sensory properties that can be described as green, roasted, or nutty. They play an important role in the flavors of meat products, vegetables, passion fruit, roasted products, milk, coffee, rum, and whisky. Thiazoles can be formed in food products either naturally or through the Maillard reaction. 2-Isobutylthiazole (Fig. 3), the characteristic aroma of fresh tomato, when added to canned tomato can produce a more intense flavor. 2,4,5-Trisubstituted thiazoles usually have roasted and meaty flavor characteristics. 2-Hydroxymethyl-4-methylthiazole is a patented flavor compound to produce woody and burnt flavor notes.

Pyrazines

Pyrazines represent 4% of all aroma compounds used as flavoring agents. They are the most widely distributed heterocyclic compound in food flavors, found in more than 50 food products of vegetable and animal origin whether processed or not. Pyrazines are described as having nutty, roasted, green, and fruity flavors. They principally occur in cooked beef, potato, mushroom, roasted nuts, bread, cheese, coffee, and some alcoholic beverages. 2-Alkyl-3-methoxypyrazines are widespread in vegetables; the characteristic aroma of green pepper is due to 2-*i*-butyl-3-methoxypyrazine (Fig. 16). 2-Methoxy-3-ethylpyrazine imparts a potato flavor to food products; 2,5-dimethylpyrazine has the flavor of fried chicken, whereas 2-ethoxy-6-methylpyrazine gives the strong aroma and flavor of fresh pineapple.

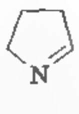


Figure 15. 1-Pyrroline.

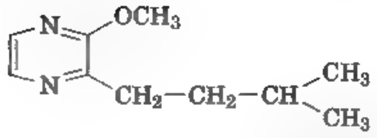


Figure 16. 2-*iso*-butyl-3-methoxypyrazine.

MAILLARD REACTION AND FORMATION OF HETEROCYCLIC FLAVOR COMPOUNDS IN FOOD

Maillard Reaction

Flavors can be produced either naturally by the action of enzymes or by thermal processing due to the interaction of different food components or their pyrolytic degradation. The interaction of reducing sugars with amino-containing components and their subsequent reactions is termed nonenzymatic browning or Maillard reaction (17–21). This reaction is considered to be the most important reaction in food chemistry because in addition to flavors, it is also responsible for the formation of color, antioxidants, carcinogens, and so on. It can also reduce the nutritional value of foods by effectively decreasing the concentration of essential amino acids. The Maillard reaction results in the formation of distinctive brown color and aroma of broiled, baked, and roasted food products. Consequently, many unpleasant-tasting raw foods can be transformed by Maillard reactions into desirable products via processes such as bread baking, coffee roasting, and chocolate manufacture. The Maillard reaction is initiated by the interaction of the open chain form of the reducing sugars with amino acids resulting in the formation of a Schiff base that exists in equilibrium with glycosylamino acid. The equilibrium constant for this reaction is unfavorable, but the glycosylamino acid slowly undergoes a rearrangement reaction to yield a relatively stable derivative. The type of derivative formed depends on the reducing sugar; aldoses undergo Amadori rearrangement (22) to produce 1-(amino acid)-1-deoxy-2-ketoses, whereas ketoses undergo Heyns rearrangement to produce 2-(amino acid)-2-deoxy-1-aldose. Both rearrangements are acid catalyzed; the carboxyl group of the amino acids provides the internal acid catalyst. The net result of this reaction is the transformation of an aldose into a ketose, and vice versa, via the formation of N-glycosides (Fig. 17). This initial stage of the Maillard reaction is well documented and understood; however, the subsequent reactions of these rearrangement products that produce flavors and colors are not very well defined. The rearrangement products themselves are colorless, nonvolatile compounds that do not impart to the food products any specific flavor qualities. However, during the thermal processing of foods they decompose to produce different reactive intermediates that interact further with other food components to produce, among others, a wide range of heterocyclic compounds. These heterocyclic compounds possess potent flavor qualities at very low concentrations

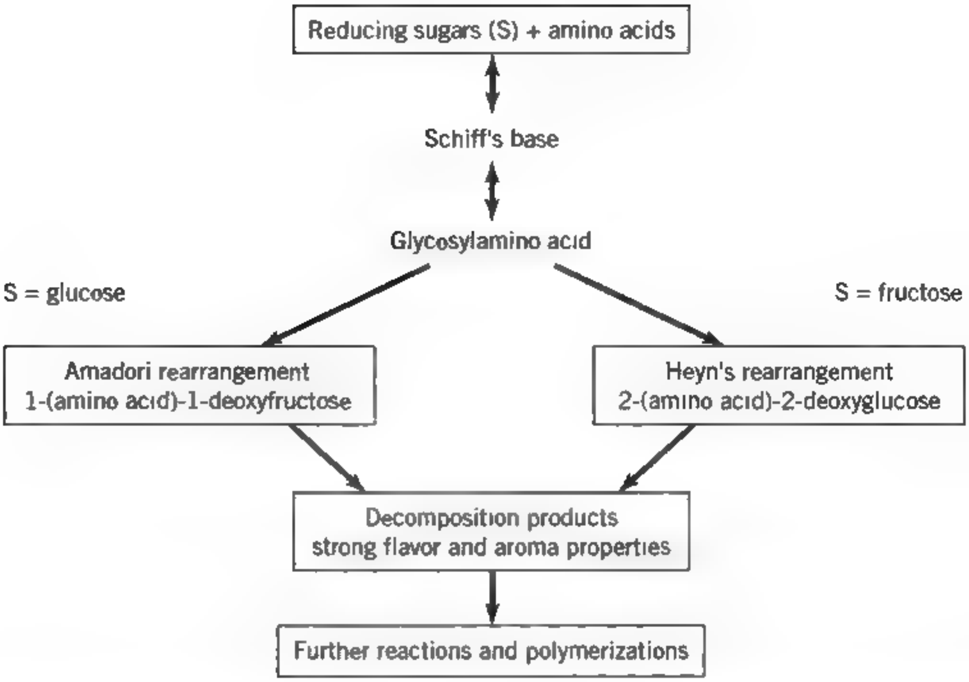

Reducing sugars (S) + amino acids
Schiff's base
Glycosylamino acid
S = glucose
S = fructose
Amadori rearrangement
1-(amino acid)-1-deoxyfructose
Heyn's rearrangement
2-(amino acid)-2-deoxyglucose
Decomposition products
strong flavor and aroma properties
Further reactions and polymerizations

Figure 17. Maillard reaction.

that makes them important contributors to the flavor of baked, roasted, and cooked food. Eventually some of the intermediates formed will polymerize to form brown colored melanoidins that are characteristic of cooked food products.

Further Reactions of Amadori and Heyns Rearrangement Products

The mechanism of decomposition of Amadori and Heyns rearrangement products (AHRP) to produce heterocyclic flavor compounds is not well understood due to the complexity of the food matrix. However, based on the chemistry of carbohydrates, some decomposition pathways have been proposed that involve 1,2- and 2,3-enolizations of the open chain form of the AHRPs followed by β-eliminations to produce 1,2- and 2,3-dicarbonyl compounds. These reactive intermediates can undergo many reactions, including dehydrations, cyclizations to produce furans, retroaldolizations, and further reactions with nitrogen and sulfur nucleophiles to produce N- and S-heterocyclic compounds, such as pyrazines by the Strecker degradation (17).

FLAVOR FORMATION BY ENZYMES AND MICROORGANISMS

In contrast to thermally produced flavors, which comprise a large number of heterocyclic compounds, flavors generated by enzymes in vegetables and fruits consist mainly of aldehydes, ketones, esters, alcohols, terpens, terpenoids, and S-containing aliphatic and aromatic volatile compounds. They can be produced from nonvolatile precursors by the action of different enzymes in the intact tissue before harvest or due to the disruption of the cell tissue so that the compartmentalized endogenous enzymes and the substrates interact. Alternatively, flavors can be produced by the action of exogenous enzymes or microorganisms during fermentation (8).

Origins of Enzymatically Produced Flavors in Vegetables

Aldehydes. Aldehydes containing less than five carbon atoms usually are associated with the development of off-flavors. However, unsaturated alkenals containing 5 to 10 carbon atoms impart desirable flavor attributes to fresh vegetables; *cis*- and *trans*-2-hexenals are both potent aroma compounds of "green leaf" character. The melonlike odor of cucumbers are due to *cis*-2-nonenal (Fig. 18). Aliphatic aldehydes may arise and accumulate in vegetables from precursors such as esters, amino acids, and free fatty acids. Esters can be hydrolyzed by esterases and followed by enzymatic oxidation (NADP-dependent dehydrogenases) to produce aldehydes. Amino acids can be converted into α-keto acids by the action of transaminases, followed by enzymatic decarboxylation to produce flavor aldehydes. Triglycerides in plant tissue can be hydrolyzed by lipases to produce free fatty acids, which in turn can produce long-chain aldehydes by the action of lipoxygenases and lyases.

Sulfur Volatiles. Sulfur-containing volatiles are produced mainly by alliaceous (onion, garlic, leek, etc) and cruciferous (cabbage, mustard, broccoli, horseradish, etc) plants. Alliaceous vegetables produce disulfides and re-

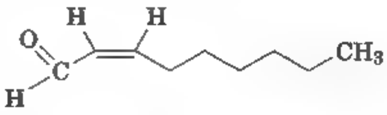


Figure 18. *cis*-2-Nonenal.

lated volatiles, whereas cruciferous vegetables generate isothiocyanates. The main precursor of disulfides in alliaceous plants are S-substituted L-cysteine sulfoxides, which on the action of allinases produce thiosulfinates (Fig. 19), the principal component responsible for the odor of fresh alliaceous plants. On heating, the thiosulfinates are converted into corresponding disulfides, which are responsible for the cooked flavor. The pungent taste of cruciferous vegetables, on the other hand, are caused by isothiocyanates (Fig. 20). The enzymes responsible for the release of these compounds are thioglucosidases, which act on different thioglucosides found in these vegetables to produce the corresponding isothiocyanates. The enzymes and the substrates in these vegetables are compartmentalized; the tissue of the plant should be disrupted to bring the substrate close to the enzyme for the generation of the flavor compounds.

Origins of Enzymatically Produced Flavors in Fruits

About 2000 distinct fruit volatiles have been isolated till now. They consist mainly of esters, aromatic aldehydes, lactones, alcohols, terpenoids, and some thioesters. Unlike fresh vegetables, the aroma is more frequently preformed and arises directly from the intact fruit.

Esters and Aromatic Aldehydes. Esters make the most important contribution to what we usually perceive as fruit flavors. They are present in higher levels than any other class of compound, and also they are present in a greater variety compared with any other class. Most of the esters contribute to the character impact of the resultant aroma. Certain esters have been associated with the aroma of specific fruits, such as methyl butyrate with apple, isopentyl acetate with banana, and ethyl butyrate with orange (Fig. 21). Esters are biosynthesized by enzymatic esterification of carboxylic acids with alcohols through the action of such enzymes as esterases and acyl CoA-alcohol transacetylase.

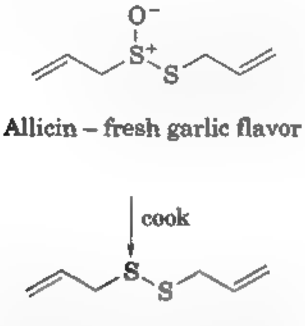
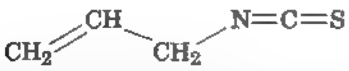
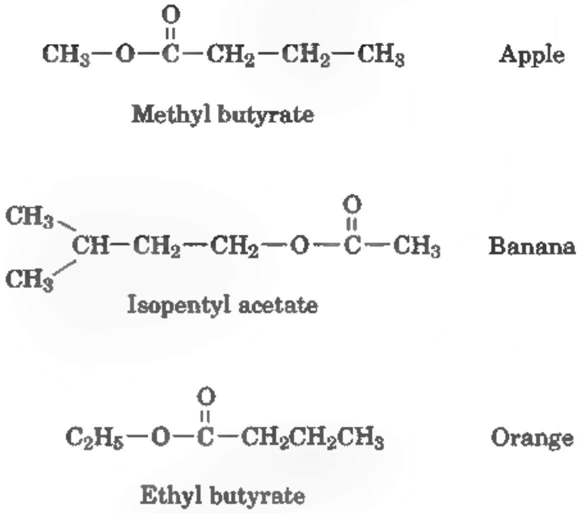

Allicin – fresh garlic flavor
cook
Diallyldisulfide – cooked garlic flavor

Figure 19. Components responsible for the garlic flavor.



$CH_2{=}CH{-}CH_2{-}N{=}C{=}S$

Figure 20. Allylisothiocyanate, principal "hot" component in mustard.



$CH_3{-}O{-}C({=}O){-}CH_2{-}CH_2{-}CH_3$

$(CH_3)_2CH{-}CH_2{-}CH_2{-}O{-}C({=}O){-}CH_3$

$C_2H_5{-}O{-}C({=}O){-}CH_2CH_2CH_3$

Figure 21. Esters with specific fruit flavors.

In general, the most abundant alcohol in the volatile fraction of a fruit is usually present in the most abundant ester. Aromatic aldehydes sometimes constitute the main flavor component of certain fruits. Benzaldehyde (Fig. 22), for example, is the principal flavor compound in bitter almond. It is also present in the volatiles of peaches, apricots, cherries, and plums. They are generally produced from cyanogenic glycosides by the action of β-glucosidases, which hydrolyzes the glycoside into the free sugar and (R)-mandelonitrile. The latter then undergoes an elimination reaction in the presence of hydroxynitrile lyase to produce the benzaldehyde. 4-Hydroxy-3-methoxybenzaldehyde (vanillin), another aromatic aldehyde, is also produced by the same series of enzymes.

Origins of Enzymatically Produced Flavors in Dairy Products: Milk and Cheese

In contrast to vegetables and fruits, in which the endogenous enzymes are responsible for the release of aroma compounds, dairy flavors are generated by in addition to endogenous enzymes, added microbial enzymes that play a predominant role in the formation of flavors. In general, the flavor of dairy products originates from microbial, enzymatic, and chemical transformations (mostly oxidations)—the relative importance of which is not always understood. These transformations give rise to a series of volatile and nonvolatile compounds, some of which have been shown to correlate well with some typical dairy flavor notes and some have little indication of their real contri-

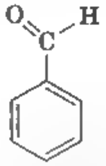


Figure 22. Benzaldehyde.

bution. For any dairy aroma, the total number of compounds identified is far less than in those foods subjected to thermal treatment. Thousands of heterocyclic compounds present in such foods are missing in fermented ones. The characteristic dairy food flavors are usually related to the occurrence of relatively few key components.

Milk furnishes the flavor precursors for all the fermented products (cheese, yogurt etc) made from it. The milk lactose, in addition to being a nutrient for the growth of the starter microorganisms, is also the principal precursor of 2,3-butadione (diacetyl), an important constituent of cultured products. It imparts characteristic creamy note to most cheeses and other dairy products. It is found in butter, unripened soft cheeses such as cottage cheese, and also in Swiss cheese. Milk proteins (caseins) are the source of bitter peptides that contribute to the aroma of different cheeses. The sweet flavor of Swiss cheese is due to a complex formed between calcium (or magnesium) and peptides. The burned note of Gruyère cheese is attributed to peptides complexed with 2,5-dimethyl-4-hydroxy-3-[2*H*]furanone; these peptides and amino acids are produced during the ripening of cheese by the action of proteolytic enzymes on the milk caseines. Some amino acids can be oxidized into corresponding carboxylic acids, such as alanine, and into propionic acid, which is responsible in part for the aroma and taste of Swiss cheese. Although carbohydrates and proteins play an important role as precursors of cheese aroma, fatty acids are the key precursors for both desirable and undesirable aroma. C4–C10 fatty acids impart well-known pungent notes to the cheese, whereas 2-methylketones, lactones, and dairy-associated aldehydes such as *cis*-4-heptenal are important precursors to other flavors. In addition, they act as a medium for the action of enzymes responsible for the formation of aroma, and they serve as a repository for all the other aroma compounds. As such, they determine the relative composition of the aroma vapor above the cheese.

ANALYSIS OF FLAVOR COMPOUNDS

The analysis of food flavors is complicated because of several factors. The flavor-active compounds are found in very low concentrations, ranging from a few 100 ppm for strongly flavored food products to less than 10 ppm for weakly flavored foods. In addition, they represent a wide range of functional groups with widely differing physical and chemical properties. Moreover, some of the components are thermally labile whereas others are highly reactive and may be lost if great care is not exercised during their isolation and concentration as well as in the subsequent analysis. A further complication that puts great demands on the analytical methodology is that the trace components, even when present at levels of ppm or less, may sometimes make a greater contribution to the flavor than components present in vastly greater amounts. The economic importance of such a low threshold value flavor compound lies in the reduced cost of production of highly flavored food products. Despite impressive data available on the chemical composition of food flavors, there is still lack of complete understanding of what determines the flavor of many foods. This may be due, for some products at least, to the inability of the analytical techniques to identify important trace components. However, major advances both in GC and MS technology—such as the introduction of inert thermostable capillary columns; gentle nonvaporizing on-column injection methods; improved interfaces between GC and MS; and sophisticated facilities for spectral acquisition, storage, data manipulation, and library search—make the modern GC/MS an analytical tool of outstanding powers, capable of acqusition of useful mass spectral data from the narrowest of GC peaks.

Establishing the chemical composition and the structure of flavor compounds found in food is important for correlating structures to sensory properties and for understanding the mechanisms by which flavors are formed from their precursors. Although analysis of food flavors is similar in many respects to analysis of any other mixtures, there are special problems associated with it, such as the low concentration of flavor components and their extreme complexity, frequently containing several hundred components of different functional groups. In some cases, trace components in an aroma have far greater sensory importance than other components present in larger amounts; hence, sample preparation and concentration is specially important for analysis of flavor compounds.

Sample Preparation

There are two different approaches to prepare samples for flavor analysis. The first approach is total volatile analysis, which attempts to isolate and concentrate from target food products all the chemicals that could possibly contribute to the flavor. This can be achieved by different distillation and extraction methods. The most appropriate method is largely dictated by the nature of the product under study and by the extent to which its flavors are affected adversely by heat. Fresh fruits and other heat-sensitive products, for example, can be distilled at low temperatures under vacuum, whereas heat-stable products can be steam distilled and simultaneously extracted into an organic solvent by using Likens- and Nickerson-type apparatuses. The second approach is headspace analysis, which aims at analysis of volatile compounds found at equilibrium in the vapor phase above the food. The second approach is faster and simpler and requires small quantities of the food product for analysis. In addition, the headspace contains volatiles in the same relative concentrations as one actually inhales. The headspace volatiles can be analyzed by injecting a small volume of the volatiles directly into a gas chromatograph (GC) or, after its concentration, by cryogenic trapping or by the use of porous polymers such as Porapak and Chromosorb or by activated charcoal (23).

Chemical Methods of Analysis

The identification of the flavor components present in a sample can be established rapidly by GC/MS analysis. However, in complex mixtures, important trace components are often either poorly separated from or totally masked by other components. Consequently, interpretation of their mass spectra may be difficult to perform. To overcome this problem the sample can be modified prior to, during, or after GC/MS analysis to simplify its complexity. Modification of the sample prior to GC/MS analysis can be achieved by preliminary fractionation of its components into polar, nonpolar, and weakly polar fractions or into acidic, basic, and neutral fractions by column chromatography using silica gel. Carbonyl compounds as a group are of special significance since they occur in a wide range of food products of plant and animal origin. As a group they can be isolated by treating the sample with an acidified solution of 2,4-dinitrophenylhydrazine and analyzed as their 2,4-dinitrophenylhydrazone derivatives by GC, HPLC, TLC, and so on. Modification of the sample injected onto a GC/MS can be performed on-line by means of chemical abstractors deposited on deactivated solid supports contained in a coiled tubing and placed before the analytical column of the GC. The function of the abstractors is to remove components bearing specific functional groups by reacting with them. The absence of a particular peak is an indication of the presence of the functional group when the resulting chromatograms are compared with those obtained in the absence of the abstractor. Alternatively, to achieve greater resolution for incompletely separated peaks, components of each chromatographic peak can be recovered by different trapping techniques and analyzed again using different columns. Trapping techniques may be very simple, such as collection of peaks in cooled glass capillary tubes inserted into the GC column or by use of porous-layer open-tubular (PLOT) glass capillaries containing a layer of diatomacous earth support permanently fused to the wall of the capillary tube (23).

Gas Chromatography/Mass Spectrometry

The combined GC/MS remains the most powerful technique available to the chemist for the separation and identification of complex mixtures, because it generates the maximum amount of structural information for the smallest amount of sample in the shortest time. For more than 20 years it has been the mainstay technique in the analysis of flavor components in food and still is, despite recent advances in complementary techniques such as GC/IR (24).

The separation of the complex mixture into its components is achieved on the gas chromatographic column, which can be divided into two types: packed and open tubular (or capillary). The separated components are then introduced through the GC/MS interface into the ionization chamber of the mass spectrometer, where the molecules are bombarded with a beam of energetic electrons (electron impact mode), causing them to ionize and fragment in a way that is characteristic of the molecule The resulting mixture of ions are then separated on the basis of their mass/charge ratio (m/z) and their relative abundances are recorded. The results are then displayed as a plot of ion abundance versus m/z, which is called the mass spectrum. This represents the characteristic fingerprint of the molecule. The mass spectrum of an unknown compound then can be used to identify its structure by comparison to other known mass spectra; this can be done conveniently by computers.

Gas Chromatography–Olfactometry (GCO)

GC is used mainly in the quantitative analysis of flavor mixtures. However, when the instrumental detector is replaced with a human nose—through the use of a sniffing port—GC can also be used to perform sensory analysis. The technique is known as GCO (25). The main application of GCO is to establish and identify the odor-active components and odor quality of individual components present in a mixture and separated on a GC column. The most common techniques that utilize GCO include CharmAnalysis® and Aroma Extract Dilution Analysis (AEDA). Both techniques are based on dilution analysis and produce quantitative estimates of relative potency of compounds eluting from a GC column. In dilution analysis, the aroma extracts are serially diluted, and each dilution is analyzed until no significant odor can be detected. In AEDA, the number of dilutions required to eliminate the odor is used to estimate the potency of that particular compound. In CharmAnalysis®, retention times are included in the calculation of the potency factors that are known as "charm values."

Purge and Trap–Thermal Desorption

Low concentration of aroma volatiles can be isolated and concentrated on porous polymer traps such as poly[2,6-diphenyl-*p*-phenylene oxide] (Tenax-GC) using purge and trap (P&T) apparatus (26). Subsequently, the trapped volatiles can be *thermally* desorbed into a GC column using thermal desorption (TD) devices mounted on a GC. Both solid and liquid samples can be analyzed, if proper sampling apparatus is used. In both cases, samples are purged with purified inert carrier gas for a specific period of time (20–90 min), at a flow rate in the range of 10 to 80 mL/min. The temperature, which can range from subambient to few hundred degrees Centigrade, depends on the nature of the sample. The adsorbant trap is then removed and fitted with a syringe needle and attached to the thermal desorber. The traps are thermally desorbed for a period of 3 to 10 min at temperatures depending on the nature of the sample (50–350°C). During the desorption period, the temperature of the GC column is maintained at subambient values to cryofocus the aroma volatiles. After the desorption phase, the volatiles can be separated and eventually identified by initiating the temperature programming of the column.

Solid-Phase Microextraction

Solid-phase microextraction (SPME) is a simple adsorption/desorption technique (26) that eliminates some of the problems associated with solvent extraction of aroma volatiles. The device used to perform SPME consists of a 1-cm fused silica fiber, coated with a specific stationary phase (such as polydimethylsiloxane, carboxene, etc) and bonded to a stainless steel plunger and encased in a holder. The fused silica fiber can be drawn into a hollow needle attached to the holder by using the plunger. The device can be used to adsorb aroma compounds either from a solution or from the headspace above the solution, by inserting the needle through the septum that seals the sample vial. After sample adsorption, the needle can be introduced into the GC injector where the adsorbed volatiles are thermally desorbed into the GC column and analyzed. The adsorption equilibrium is usually attained in 2 to 30 min, depending on the concentration of the sample and the thickness and the type of the coating of the fiber.

BIBLIOGRAPHY

1. S. Van Straten, F. De Vrijer, and J. C. de Beauveser, eds., *List of Volatile Compounds in Food*, 3rd ed., supplements 1–8, Central Institute for Nutrition and Food Research, TNO, Zeist, The Netherlands, 1977–1980.
2. S. Van Straten et al., eds., *Volatiles in Food, Qualitative Data*, 5th ed. supplement I, Division for Nutrition and Food Research, TNO, Zeist, The Netherlands, 1983.
3. H. Maarse, and C. A. Visscher, eds., *Volatile Compounds in Food. Qualitative Data*, 5th ed., supplement I, Division for Nutrition and Food Research, TNO, Zeist, The Netherlands, 1984.

[illegible]. P. Z. Bedoukian, *Perfumary and Flavoring Synthetics*, Allured, Wheaton, Ill., 1986.

5. L. B. Sjoström, *The Flavor Profile*, A.D. Little, Cambridge, Mass., 1972.
6. H. B. Heath, *Source Book of Flavors*, AVI, Westport, Conn., 1981.
7. H. B. Heath and G. Reineccius, *Flavor Chemistry and Technology*, AVI, Westport, Conn., 1986.
8. G. G. Birch and M. G. Lindley, eds., *Developments in Food Flavors*, Elsevier Applied Science, London, United Kingdom, 1986.
9. T. H. Parliment and R. Croteau, eds., *Biogeneration of Aroma*, ACS Symposium Series 317, American Chemical Society, Washington, D.C., 1986
10. P. R. Ashurst, ed., *Food Flavourings*, Blackie, United Kingdom, 1991.
11. T. H. Parliment, R. J. McGorrin, and C. T. Ho, eds., *Thermal Generation of Aromas*, ACS Symposium Series 409, American Chemical Society, Washington, D.C., 1989.
12. T. H. Parliment, M. J. Morello, and R. J. McGorrin, eds., *Thermally Generated Flavors*, (ACS Symposium Series 543), American Chemical Society, Washington, D.C., 1994.
13. W. G. Moody, "Beef Flavor—A Review," *Food Technol.* **37**, 227–232 (1983).
14. M. A. Amerine and C. S. Ough, *Wine and Must Analysis*, John Wiley and Sons, New York, 1980.
15. J. R. Piggott and A Paterson, eds., *Distilled Beverage Flavour*, VCH, Ellis Horwood Ltd., Chichester, United Kingdom, 1989.
16. G. Vernin, ed., *Chemistry of Heterocyclic Compounds in Flavours and Aromas*, Ellis Horwood Ltd., Chichester, United Kingdom, 1982.
17. C. Eriksson, ed., *Maillard Reactions in Food: Chemical, Physiological, and Technological Aspects*, Prog. Fd. Nutr. Sci. Vol. 5, Pergamon Press, Oxford, United Kingdom, 1981.
18. G. R. Waller and M. S. Feather, eds., *The Maillard Reaction in Foods and Nutrition*, ACS Symposium Series 215, American Chemical Society, Washington, D.C., 1983.
19. M. Fujimaki, M. Namiki, and H. Koto, eds., *Amino Carbonyl Reactions in Food and Biological Systems*, Dev. Food Sci. No 13, Elsevier, Amsterdam, The Netherlands, 1986.
20. P. A. Finot et al., eds., *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, Birkhauser Verlag, Basel, Switzerland 1990.
21. T. P. Labuza et al., eds., *Maillard Reaction in Chemistry, Food, and Health*, The Royal Society of Chemistry, Cambridge, U.K., 1994.
22. V. A. Yaylayan and A. Huygues-Despointes, "Chemistry of Amadori Rearrangement Products," *Crit. Rev. Food Sci. Nutr.* **34**, 321–369 (1994).
23. I. D. Morton and A. J. Macleod, eds., *Food Flavours—Part A*, Elsevier Scientific, New York, 1982.
24. J. Gilbert, ed., *Applications of Mass Spectrometry in Food Science*, Elsevier Applied Science, London, United Kingdom, 1987.
25. C-H. Ho and C. H. Manley, eds., *Flavor Measurement*, Marcel Dekker, New York, 1993.
26. Z. Zhang and J. Pawliszyn, "Headspace Solid-phase Microextraction," *Anal. Chem.* 1843–1852 (1993).

V. A. YAYLAYAN
McGill University
St. Anne de Bellevue, Quebec
Canada

FOAMS AND SILICONES IN FOOD PROCESSING

Potato chips, jams and jellies, beers and wines, fruits and vegetables. What do all these foods have in common? The manufacturing processes for all these products commonly experience unwanted foaming.

FOAMS

Foam can be defined as a gas dispersed in a liquid at a ratio such that the mixture's bulk density approaches that of a gas rather than a liquid (1). Foaming can occur for several reasons, stemming from both mechanical and chemical origins. Examples of mechanical contributors of foam formation are agitation near a gas–liquid interface, excessive vacuums or pressure gradients, or the free fall of liquids (2). The addition of certain chemicals that act as surface-active materials, or surfactants, may also produce foaming within a process. The existence of starch in a food process is an example of this. Foam formation can cause many problems within a process, including reducing the capacity of open vats, increasing the need for head space in closed systems, slowing the time needed to drain liquids or dried foods, interfering with process instruments, or it can increase housekeeping costs by introducing the need for maintenance personnel to deal with safety hazards such as slippery floors and the overflowing of process tanks (2).

Two basic actions can be taken to stop the formation of foam in a food process. The first involves mechanical means of eliminating the foam, such as heating, centrifuging, spraying, or ultrasonic vibration (3). The other option is to introduce chemical antifoams or defoamers to the process. This article will focus on the chemical method of foam control. Defoamers are materials that specialize in destroying existing foam immediately. Antifoams are materials that are designed as long-lasting means of preventing the formation of foam. Examples of foam control products range from liquid products such as hydrocarbons (kerosene, vegetable oils, organic phosphates, and acetylenic glycols), polyethers, fluorocarbons, and silicones, to solid products such as fatty amides, hydrocarbon waxes, solid fatty acids, and esters (3). The remainder of this article will focus on silicone-based antifoam products.

SILICONE ANTIFOAMS

Two theories exist to describe how antifoams work. Both are based on the fact that pure liquids do not foam. The presence of some stabilizing material is necessary in the surface layer of foam films to allow them to remain stable. These theories are also based on the premise that antifoaming agents either replace or modify these stabilizing materials. The first theory states that the antifoam disperses in the form of fine drops into the liquid film between the bubbles and spreads as a thick duplex film, ie, a film that is thick enough to have two definite surfaces. The tension created by the spreading duplex film leads to the rupture of the original liquid film (bubble wall), destroying the foam.

The second theory is similar to the first and states that the antifoam produces a less cohesive, mixed monolayer on the surface of the liquid film. Because this monolayer is of less coherence than the original film stabilizing monolayer, it will cause the destabilization of the bubble wall (3). In either case, it is important to note that antifoams must be surface active, that they must be able to spread to the entire foaming surface of the process, and that they must remain insoluble to continue to be active.

Since the discovery of polydimethylsiloxane in 1943 by scientists of what is now Dow Corning Corp., there have been many applications found for this unique substance. Applied as antifoams since shortly after their discovery, these silicone-based products are able to provide benefits that most organic defoamers cannot (4). Due to the inert nature of the polydimethylsiloxane molecule, silicone-based antifoams will not react with most process media. This inert nature also allows the antifoam to remain effective longer than reactive organic material. Silicones exhibit a low surface tension (<20 dynes/cm) (5), allowing them to spread quickly and evenly over the foaming surface, an important requirement for an antifoam already mentioned.

Both of these attributes contribute to the most important benefit of using silicone antifoams: their efficiency. Silicone-based antifoams are effective usually in amounts less than 100 parts active silicone per million parts foaming media in industrial applications and in as little as 5–10 ppm in food-grade systems.

Silicone antifoams are available as three basic types of product. First, the polydimethylsiloxane, or silicone oil, can be used as an effective antifoam for many nonaqueous-type processes. Second, the silicone oil can be compounded with hydrophobic silica to form a product that can be useful in both aqueous and nonaqueous systems. It has been theorized that the addition of this silica gives an added benefit of physically disrupting the foam interlayer. The third and most effective product for aqueous systems is an emulsion of the silicone–silica compound. An emulsion is the most effective carrier of a compound in a water environment, allowing for ease in dilution and dispersion.

Silicones can be used in many processes. There are silicone-based antifoams that are permissible for use in direct contact with food, as per the Food and Drug Administration's regulation #173.340, which allows for up to 10 parts of active silicone per million parts of food in the final product. Certain silicones also meet the U.S. Department of Agriculture's specifications for federally inspected foods. Many are also kosher approved. Silicone antifoams usually will not impart any taste or smell to a product. Because these antifoams are essentially inert and are used in such low amounts, they rarely affect process reactions.

BASIC GUIDELINES

There are several basic guidelines to follow when using silicone antifoams to be the most effective as possible. Silicone antifoams should be prediluted and mixed in with a small amount of the foaming medium prior to introduction into the system. This allows the antifoam to disperse evenly and can prevent shocking of an emulsion, which could cause the emulsion particles to coalesce, or stick together. The antifoam should be dispensed in such a manner as to allow it to disperse evenly and reach the surface of the foaming site. This could involve spraying the antifoam onto the surface, or providing slight agitation when adding the antifoam to allow it to reach the surface. The antifoam should be added prior to the point where foaming occurs. This allows the product to act as it was designed; that is, as an antifoam rather than a defoamer.

When using silicone emulsified antifoams, there are additional precautions that should be taken that involve the amount of shear that the antifoam is subjected to. Because this oil-based antifoam is emulsified using surfactants and thickeners, the product can be destroyed by disturbing the emulsion particles, causing coagulation or separation. High levels of mechanical shear can do this through turbulent agitation, violent shaking, or pumping through gear type, centrifugal pumps. Care should be taken to provide gentle agitation when prediluting, or when adding the antifoam to the foaming process.

FUTURE DEVELOPMENT

Significant advancements continue to be made in the development of silicone-based antifoams. Recent developments have focused on areas involving organic–silicone blends. Many newly commercialized products use this new technology and may impart even more efficiency to current antifoam product lines. Work is also being conducted on varying the types of delivery systems for silicone antifoams. Instead of water-based emulsions, solid media are being investigated as delivery systems for the antifoam compound to the foaming interface. This could provide benefits to separation processes using ultrafiltration. Typically, emulsifiers used in antifoam emulsions have shown a tendency to clog and even damage many types of cross-flow ultrafiltration membranes.

As the food industry continues to expand with quicker, more convenient processed foods, so too will the opportunities grow for foam control. Currently, an area experiencing growth is the microwavable foods segment. This is also an area where foaming has been a problem. Many manufacturers desire microwaved food to resemble food that has been prepared by conventional means. Although the formation of foam on food or drinks that have been microwaved does not affect the taste of these products, it is not aesthetically pleasing. The use of silicone antifoams can alleviate these types of problem.

BIBLIOGRAPHY

1. A. P. Kouloheris, "Foam: Friend and Foe," *Chemical Engineering* (1987).
2. J B. McGee, "Selecting Chemical Defoamers and Antifoams," *Chemical Engineering* (1989).
3. M. J. Owen, "Antifoaming Agents," in *Encyclopedia of Polymer Science and Engineering*, Vol. 2, 2nd ed., John Wiley & Sons, Inc., New York.
4. C. C Currie, "What Silicones Can Do for the Food Processor," *The Canner* (1952).
5. Internal Dow Corning Surface Tension Studies, TIS #1952-I0030-119.

GENERAL REFERENCES

F. LaBelle, "Taming Foam Problems in Formulations and Products," *Prepared Foods* **163**, 75 (1994).

W H. B. Denner, "Food Additives: Recommendations for Harmonization and Control," *Food Control* **1**, 150–162 (1990).

P. R. Garrett, "Defoaming: Theory and Industrial Application," Marcel Dekker, New York, 1993.

E. G. Gooch, "Determination of Traces of Silicone Defoamer in Fruit Juices by Solvent Extraction/Atomic Absorption Spectroscopy," *J. AOAC Int* **76**, 581–583 (1993).

S. Hegenbart, "Processing Aids: The Hidden Helpers," *Prepared Foods* **159**, 83–84 (1990).

M. Holland, "Effective Foam Control in Beet Processing," *International Sugar Journal* **98**, 347–348 (1996).

A. Monferrer and J. Villalta, "Practical Aspects of Frying II," *Alimentacion Equipos y Tecnologia* **12**, 85–90 (1993).

K. Yasunaga et al., "Stabilization Mechanism for Bubbles Formed in Frying Foods," *Journal of the Japanese Society for Food Science and Technology* **44**, 400–406 (1997).

A. A. Zotto, "Antifoams and Release Agents," in J. Smith, ed., *Food Additive's User's Handbook*, Blackie and Sons Ltd., Glasgow, Scotland, 1991, pp. 236–241.

Chris Combs
Dow Corning Corporation
Midland, Michigan

FOOD ADDITIVES

The broadest practical definition of a food additive is any substance that becomes part of a food product either directly or indirectly during some phase of processing, storage, or packaging.

Direct food additives are those that have been intentionally added to food for functional purpose, in controlled amounts, usually at low levels (from parts per million to 1–2% by weight). Basic foodstuffs are excluded from the definition, although ingredients that are added to foods (eg, high-fructose corn syrup [HFCS], starches, and protein concentrates) are often included among food additives.

Indirect or nonintentional additives, on the other hand, are those entering into food products in small quantities as a result of growing, processing, or packaging. Examples of these are lubricating oils from processing equipment or components of a package that migrate into the food before consumption.

The legal definition of a food additive is much more complex and often the subject of controversial interpretation; it is addressed in the sections of this article dealing with government regulations.

The practice of adding chemicals (eg, salt, spices, herbs, vinegar, and smoke) to food dates back many centuries. In recent years, however, the ubiquitous presence of chemical additives in processed foods has attracted much attention and public concern over the long-term safety of additives to humans.

Although the safety issue is far from subsiding, the scientific consensus is that food additives are indispensable in the production, processing, and marketing of many food products, and that the judicious use of chemical additives—typically in the range from a few parts per million (ppm) to less than 1% by weight of the finished food—contributes to the abundance, variety, stability, microbiological safety, flavor, and appearance of the food supply. While food additives offer a major contribution to the palatability and appeal of a wide variety of foods, their level of use is relatively insignificant in the total human diet.

There is much discussion about whether a food additive or food product is natural or synthetic. This classification, in many instances, has become somewhat arbitrary. Many food additives synthesized in chemical laboratories are also found naturally occurring in normal food. Monosodium glutamate, a flavor-enhancing food additive, for instance, is the sodium salt of glutamic acid, an amino acid found in many foods such as mushrooms and tomatoes and metabolized by the human body using the same biochemical pathways of digestion. Vitamin C (ascorbic acid) and its isomer, erythorbic acid, are the same chemicals that are found in oranges. Similarly, citric acid, which is today produced commercially by enzymatic fermentation of sugars, is the same chemically as the naturally occurring chemical that has been found to make lemons and limes tart.

Direct food additives serve several major functions in foods. Many additives, in fact, are multifunctional. The basic functions are as follows:

- *Preservation*. Food preservation techniques have advanced in the past hundred years and now include thermal processing, concentration and drying, refrigeration and freezing, modified atmosphere, and irradiation. However, the use of chemical preservatives frequently augment these basic preservation techniques and represent the most economical way for food manufacturers to ensure a reasonable shelf life for the product. Antioxidants and antimicrobial agents perform some of these functions as well.
- *Processing*. Food processors are increasingly using food additives to ensure the integrity and appeal of finished products. Emulsifiers maintain mixtures and improve texture in breads, dressings, and other foods. They are used in ice cream when smoothness is desired, in breads to increase shelf life and volume and to distribute the shortening, and in cake mixes to achieve batter consistency. Sorbitol, a humectant and sweetener, is used to retain moisture and enhance flavor. With the removal of sugar from many

BIBLIOGRAPHY

1. A. P. Kouloheris, "Foam: Friend and Foe," *Chemical Engineering* (1987).
2. J B. McGee, "Selecting Chemical Defoamers and Antifoams," *Chemical Engineering* (1989).
3. M. J. Owen, "Antifoaming Agents," in *Encyclopedia of Polymer Science and Engineering*, Vol. 2, 2nd ed., John Wiley & Sons, Inc., New York.
4. C. C Currie, "What Silicones Can Do for the Food Processor," *The Canner* (1952).
5. Internal Dow Corning Surface Tension Studies, TIS #1952-I0030-119.

GENERAL REFERENCES

F. LaBelle, "Taming Foam Problems in Formulations and Products," *Prepared Foods* **163**, 75 (1994).

W H. B. Denner, "Food Additives: Recommendations for Harmonization and Control," *Food Control* **1**, 150–162 (1990).

P. R. Garrett, "Defoaming: Theory and Industrial Application," Marcel Dekker, New York, 1993.

E. G. Gooch, "Determination of Traces of Silicone Defoamer in Fruit Juices by Solvent Extraction/Atomic Absorption Spectroscopy," *J. AOAC Int* **76**, 581–583 (1993).

S. Hegenbart, "Processing Aids: The Hidden Helpers," *Prepared Foods* **159**, 83–84 (1990).

M. Holland, "Effective Foam Control in Beet Processing," *International Sugar Journal* **98**, 347–348 (1996).

A. Monferrer and J. Villalta, "Practical Aspects of Frying II," *Alimentacion Equipos y Tecnologia* **12**, 85–90 (1993).

K. Yasunaga et al., "Stabilization Mechanism for Bubbles Formed in Frying Foods," *Journal of the Japanese Society for Food Science and Technology* **44**, 400–406 (1997).

A. A. Zotto, "Antifoams and Release Agents," in J. Smith, ed., *Food Additive's User's Handbook*, Blackie and Sons Ltd., Glasgow, Scotland, 1991, pp. 236–241.

CHRIS COMBS
Dow Corning Corporation
Midland, Michigan

FOOD ADDITIVES

The broadest practical definition of a food additive is any substance that becomes part of a food product either directly or indirectly during some phase of processing, storage, or packaging.

Direct food additives are those that have been intentionally added to food for functional purpose, in controlled amounts, usually at low levels (from parts per million to 1–2% by weight). Basic foodstuffs are excluded from the definition, although ingredients that are added to foods (eg, high-fructose corn syrup [HFCS], starches, and protein concentrates) are often included among food additives.

Indirect or nonintentional additives, on the other hand, are those entering into food products in small quantities as a result of growing, processing, or packaging. Examples of these are lubricating oils from processing equipment or components of a package that migrate into the food before consumption.

The legal definition of a food additive is much more complex and often the subject of controversial interpretation; it is addressed in the sections of this article dealing with government regulations.

The practice of adding chemicals (eg, salt, spices, herbs, vinegar, and smoke) to food dates back many centuries. In recent years, however, the ubiquitous presence of chemical additives in processed foods has attracted much attention and public concern over the long-term safety of additives to humans.

Although the safety issue is far from subsiding, the scientific consensus is that food additives are indispensable in the production, processing, and marketing of many food products, and that the judicious use of chemical additives—typically in the range from a few parts per million (ppm) to less than 1% by weight of the finished food—contributes to the abundance, variety, stability, microbiological safety, flavor, and appearance of the food supply. While food additives offer a major contribution to the palatability and appeal of a wide variety of foods, their level of use is relatively insignificant in the total human diet.

There is much discussion about whether a food additive or food product is natural or synthetic. This classification, in many instances, has become somewhat arbitrary. Many food additives synthesized in chemical laboratories are also found naturally occurring in normal food. Monosodium glutamate, a flavor-enhancing food additive, for instance, is the sodium salt of glutamic acid, an amino acid found in many foods such as mushrooms and tomatoes and metabolized by the human body using the same biochemical pathways of digestion. Vitamin C (ascorbic acid) and its isomer, erythorbic acid, are the same chemicals that are found in oranges. Similarly, citric acid, which is today produced commercially by enzymatic fermentation of sugars, is the same chemically as the naturally occurring chemical that has been found to make lemons and limes tart.

Direct food additives serve several major functions in foods. Many additives, in fact, are multifunctional. The basic functions are as follows:

- *Preservation*. Food preservation techniques have advanced in the past hundred years and now include thermal processing, concentration and drying, refrigeration and freezing, modified atmosphere, and irradiation. However, the use of chemical preservatives frequently augment these basic preservation techniques and represent the most economical way for food manufacturers to ensure a reasonable shelf life for the product. Antioxidants and antimicrobial agents perform some of these functions as well.
- *Processing*. Food processors are increasingly using food additives to ensure the integrity and appeal of finished products. Emulsifiers maintain mixtures and improve texture in breads, dressings, and other foods. They are used in ice cream when smoothness is desired, in breads to increase shelf life and volume and to distribute the shortening, and in cake mixes to achieve batter consistency. Sorbitol, a humectant and sweetener, is used to retain moisture and enhance flavor. With the removal of sugar from many

foods for dietetic reasons, a substitute-bulking agent is growing in importance.

- *Appeal and convenience.* The changing eating habits of the consumers, partly brought about by the large increase in the percentage of women who work outside the home, is creating a growing need for convenience foods. In many of these types of foods, it is essential that a variety of additives be used to provide the taste, color, texture, body, and general acceptability that are required. This need for convenience, while maintaining aesthetic appeal and taste, is becoming extremely important. Most food additives such as gums, flavoring agents, colorants, and sweeteners are included by food processors because consumers in the developed countries demand that food look and taste good as well as being easy and safe to serve.
- *Nutrition.* There have been tremendous advances in knowledge of human nutrition, and consumers are increasingly aware of the value of good nutrition. Vitamins, antioxidants, proteins, and minerals are added to foods and beverages as supplements in an attempt to ensure proper nutrition for those who do not eat a well-balanced diet. In addition, additives such as antioxidants are often used to prevent deterioration of natural nutrients during processing. Recently there is more importance attributed to disease prevention through proper nutrition, as well as to increasing performance through sport nutrition products. On the other hand, the medically based desire for good nutrition through a balanced diet may adversely affect consumer demand for some food additives, such as fat substitutes.

Substances that come under the general definition of direct food additives number in the thousands and include both inorganic and organic chemicals, natural products, and modified natural and synthetic or artificial materials. Most food additives have a long history of use; others are the result of recent research and development to fill particular requirements of modern food processing. Some are common chemicals of industry that are upgraded in terms of purity to allow their use in food.

Food additives comprise more than 30 segments. With well over 2300 food additives currently approved for use in the United States (and more petitioning for approval), it would be impossible in a brief article such as this to discuss each and every substance.

The U.S. Code of Federal Regulations (CFR) provides classification for food additives. In the CFR, direct food additives are divided into the following eight categories:

1. Food preservatives (eg, sodium nitrate, sorbates)
2. Coatings, films, and related substances (eg, polyacrylamide)
3. Special dietary and nutritional additives (eg, vitamins)
4. Anticaking agents (eg, sodium stearate, silicon dioxide)
5. Flavoring agents and related substances (eg, vanillin)
6. Gums, chewing gum bases, and related substances (eg, xanthan gum)
7. Other specific usage additives (eg, calcium lignosulfonate)
8. Multipurpose additives (eg, glycine)

Secondary direct food additives permitted in food for human consumption are divided into four different types in the CFR, as follows:

1. Polymer substances for food treatment (eg, acrylate acrylamide resins)
2. Enzyme preparations and microorganisms (eg, rennet, amylase)
3. Solvents, lubricants, release agents, and related substances (eg, hexane, trichloroethene)
4. Specific usage additives (eg, boiler water additives, defoaming agents)

Indirect food additives included in the CFR are divided into eight categories, as follows:

1. Components of adhesives (eg, calcium ethyl acetoacetate 1,4-butanediol modified with adipic acid)
2. Components of coatings (eg, acrylate ester copolymer coatings and polyvinyl fluoride resins)
3. As components of paper and paperboard (eg, slimicides, sodium nitrate/urea complex, and alkyl ketene dimers)
4. As basic components of single- and repeated-use food contact surfaces (eg, cellophane, ethylene-acrylic acid copolymers, isobutylene copolymers and nylon resins)
5. As components of articles intended for repeated use (eg, ultrafiltration membranes and textiles and textile fibers)
6. Controlling growth of microorganisms (eg, sanitizing solutions)
7. Antioxidants and stabilizers (eg, octyltin stabilizers in vinyl chloride plastics)
8. Certain adjuvants and production aids (eg, animal glue, hydrogenated castor oil, synthetic fatty alcohols, and petrolatum)

FUNCTIONALITY OF FOOD ADDITIVES

Adopted from the National Academy of Sciences national survey of food industries the Food and Drug Administration (FDA) uses the following terms to describe the physical or technical effects for which direct human food ingredients may be added to foods:

- *Anticaking agent or free flow agent.* Substance added to finely powdered or crystalline food product to prevent caking, lumping, or agglomeration

- *Antimicrobial agent*. Substance used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats, mold, and rope inhibitors; also includes antimicrobial agents, antimyotic agents, preservatives, and mold-preventing agents (indirect additives)
- *Antioxidant*. Substance used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation
- *Boiler water additive*. Substance used in a steam or boiler water system as an anticorrosion agent, to prevent scale or to effect steam purity
- *Color or coloring adjunct*. Substance used to impart, preserve, or enhance the color or shading of a food; includes color fixatives, color-retention agents, and so on
- *Curing or pickling agent*. Substance imparting a unique flavor and/or color to food, usually producing an increase in shelf-life stability
- *Dough strengthener*. Substance used to modify starch and gluten, thereby producing more stable dough
- *Drying agent*. Substance with moisture-absorbing ability; used to maintain an environment of low moisture
- *Emulsifier or emulsifier salt*. Substance that modifies surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion
- *Enzyme*. Substance used to improve food processing and the quality of finished food
- *Firming agent*. Substance added to precipitate residual pectin, thus strengthening the supporting tissue and preventing its collapse during processing
- *Flavor enhancer*. Substance added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own
- *Flavoring agent or adjuvant*. Substance added to impart or help impart a taste or aroma in food
- *Flour treating agent*. Substance added to milled flour at the mill to improve its color and/or baking qualities, inducing bleaching and maturing agents
- *Formulation aid*. Substance used to promote or promote or to produce a desired physical state or texture in food. Including carriers, binders, fillers, plasticizers, film formers, and tableting aids, and so on
- *Freezing or cooling agent*. Substance that reduces the temperature of food materials through direct contact
- *Fumigant*. Volatile substance used for controlling insects or pests
- *Humectant*. Hygroscopic substance incorporated in food to promote retention of moisture; includes moisture retention agents and antidusting agents
- *Leavening agent*. Substance used to produce or stimulate production of carbon oxide in baked goods to impart a light texture; includes yeast, yeast foods, and calcium salts
- *Lubricant or release agent*. Substance added to food contact surfaces to prevent ingredients and finished products from sticking to them (direct additives); includes release agents, lubricants, surface lubricants, waxes, and antiblocking agents (indirect additives)
- *Malting or fermenting aid*. Substance used to control the rate or nature of malting or fermenting process; includes microbial nutrients and suppressants and excluding acids and alkalis
- *Masticatory substance*. Substance that is responsible for the long-lasting and pliable property of chewing gum
- *Nonnutritive sweetener*. Substance having less than 2% of the caloric value of sucrose per equivalent unit of sweetener capacity
- *Nutrient supplement*. Substance necessary for the body's nutritional and metabolic process
- *Nutritive sweetener*. Substance having greater than 2% of sucrose per equivalent unit of sweetening capacity.
- *Oxidizing or reducing agent*. Substance that chemically oxidizes or reduces another food ingredient, thereby producing a more stable product.
- *pH control agent*. Substance added to change or to maintain active acidity or basicity; includes buffers, acids, alkalis, and neutralizing agents
- *Processing aid*. Substance used as a manufacturing aid to enhance the appeal or utility of a food or component; Includes clarifying and clouding agents, catalysts, flocculents, filter aids, crystallization inhibitors, and so on
- *Propellant*. Gas used to supply force to expel a product or to reduce the amount of oxygen in contact with the food in packaging
- *Sequesterant*. Substance that combines with polyvalent metal ions to form a soluble metal complex to improve the quality and stability of products.
- *Solvent or vehicle*. Substance used to extract or dissolve another substance
- *Stabilizer or thickener*. Substance used to produce viscous solutions or dispersions; to impart body, to improve consistency, or to stabilize emulsions; includes suspending and bodying agents, setting agents, and bulking agents
- *Surface-active agent*. Substance used, other than emulsifiers, to modify surface properties of liquid food components for a variety of effects; includes solubilizing agents, dispersants, detergents, wetting agents, rehydrating enhancers, foaming agents, defoaming agents, and so on
- *Surface finishing agent*. Substance used to increase palatability, preserve gloss, and inhibit discoloration of foods; includes glazes, polishes, waxes, and protective coatings
- *Synergist*. Substance used to act or react with another food ingredient to produce a total effect different from or greater than the sum of the effects produced by the individual ingredients
- *Texturizer*. Substance that affects the appearance or feel of the food.

- *Tracer.* Substance added as a food constituent (as required by regulation) so that levels of this constituent can be detected after subsequent processing and/or combination with other food materials
- *Washing or surface removal agent.* Substance used to wash or assist in the removal of unwanted surface layers from plant or animal tissues

GOVERNMENT REGULATIONS

The application of food additives is highly regulated worldwide, although regulatory philosophy, the approval of specific product, and the level of enforcement differ from country to country. Basic regulations in the United States, Western Europe, and Japan are described in this section. These three major industrial regions are the largest consumers of food additives. With only 13% of the world's population, these countries account for more than two-thirds of the food additive market.

United States

The U.S. Food and Drug Administration (FDA) is the principal U.S. regulatory body controlling the use of food additives. It does so through the 1958 Food Additives Amendment to the Food, Drug & Cosmetic (FD&C) Act of 1938. The amendment was enacted with the threefold purpose of

1. protecting public health by requiring proof of safety before a substance could be added to food,
2. advancing food technology, and
3. improving the food supply by permitting the use of substances in food that is safe at the levels of intended use.

According to the legal definition, food additives that are subject to the amendment include "any substance the intended use of which results or may reasonably be expected to result directly or indirectly in its becoming a component or otherwise affecting the characteristics of any food." This definition includes any substance used in the production, processing, treatment, packaging, transportation, or storage of food.

If a substance is added to a food for a specific purpose, it is referred to as a direct additive. For example, the low-calorie sweetener aspartame, which is used in beverages, puddings, yogurt, chewing gum, and other foods, is considered a direct additive.

Indirect food additives are those that become part of the food in trace amounts due to its packaging, storage, or other handling. For example, minute amounts of packaging substances may find their way into foods during storage. Food packaging manufacturers, therefore, must prove to the FDA that all materials coming in contact with food are safe, before they are permitted for use in such a manner.

For regulatory purposes, all food additives fall into one of three categories:

1. generally recognized as safe (GRAS) substances,
2. prior sanctioned substances, or
3. regulated direct/indirect additives.

GRAS substances (of which there are approximately 700 in all) are a group of additives regarded by qualified experts as "generally recognized as safe." These substances are considered safe because their past extensive use has not shown any harmful effects.

Prior sanctioned substances (approximately 1400) are products that already were in use in foods prior to the 1958 Food Additives Amendment to the federal FD&C Act and are therefore considered exempt from the approval process. Some prior sanctioned substances also appear on the GRAS list. This is the grandfather clause of the amendment. The FDA is involved in an ongoing review of the GRAS and prior sanctioned lists to ensure that these substances are tested by means of the latest scientific methods. Likewise, the FDA also reviews substances that are not currently included on the GRAS list to determine whether they should be added.

All other additives are regulated; that is, a specific food additive petition must be filed with the FDA, requesting approval for use of the additive in any application not previously approved. A food or color additive petition must provide convincing evidence that the proposed additive performs as it is intended. Moreover, animal studies using large doses of the additive for long periods are often necessary to show that the substance would not cause harmful effects at expected levels of human consumption.

In deciding whether an additive should be approved, the agency considers the composition and properties of the substance, the amount likely to be consumed, its probable long-term effects, and various other safety factors. Absolute safety of any substance can never be proven. Therefore, the FDA must determine if the additive is safe under the proposed conditions of use, based on the best scientific knowledge available.

In addition, FDA operates an Adverse Reaction Monitoring System (ARMS) to help serve as an ongoing safety check of all additives. The system monitors and investigates all complains by individuals or their physicians that are believed to be related to specific foods, food additives, or nutrient supplements.

All color additives are subject to the Color Additive Amendment of 1960. Colors permitted for use in foods are classified either as certified or exempt from certification. Certified colors are man-made, with each batch being tested by the manufacturer and the FDA (certified) to ensure that they meet strict specifications for purity.

Color additives that are exempt from certification include pigments derived from natural sources. However, color exempt from certification also must meet certain legal criteria for specifications and purity.

The FD&C Act also authorizes the establishment of reasonable standards of identity and quality for food products. The standards of identity specify in detail what can and cannot be packaged under a given product name. Standards of identity exist for milk, cream, cheese, frozen dessert, bologna products, cereal products, cereal flours, pasta, canned and frozen fruits and vegetables, juices, eggs, fish, nuts, nonalcoholic beverages, margarine, sweeteners, dressings, and flavorings. An approved food additive in the United States may be precluded from use in certain foods characterized by the standards of identity unless the additive is specifically required by or is listed as an optional ingredient in the standards. The standards of identity establish the ingredient composition of a given food, which can then be labeled by its common name. If the manufacturer does not adhere to the standard composition, the food must be labeled "imitation."

Flavor substances are regulated somewhat differently, and the rules are less restrictive. However, the use of aroma chemicals as flavor ingredients is regulated also under laws that may differ from country to country. Following the lead of the United States, inclusion in a positive list that spells out which chemicals are permitted for food use has become the prevalent legislation for regulating flavor chemicals worldwide. The United States has a list of flavor substances that are deemed GRAS based on history of use, review of available toxicology, and the opinion of experts. These GRAS lists (through GRAS 18 issued in 1998) have been compiled since 1977 by the expert panel of Flavor Extracts Manufacturers Association (FEMA) of the United States. Over the years, more than 1800 materials have appeared on FEMA lists. Formed in 1909, FEMA is an industry association that originally started pursuing voluntary self-regulation and later was granted quasi-official status on regulatory matters regarding flavor chemicals by the FDA. The FEMA expert panel was formed in 1960. This independent panel, composed of eminently qualified experts recruited from outside the flavor industry, has expertise in human nutrition, physiology, metabolism, and toxicology and chemical structure-activity relationships. Most industrial countries more or less follow the U.S. system.

In the United States, the FDA has primary jurisdiction over food additives, although clearance for use of additives in certain products must be obtained from other government agencies as well. For example, the U.S. Department of Agriculture (USDA) through the Meat Inspection Division (MID) exercises jurisdiction over additives and ingredients for meat and poultry, and the Bureau of Alcohol, Tobacco, and Firearms (BATF) of the U.S. Department of the Treasury controls the ingredients used in alcoholic beverages.

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which was issued in 1972 and amended in 1988, covers pesticides used on raw agricultural products. The FDA, however, is responsible for enforcing tolerances for pesticide residues that end up in food products, such as ethylene dibromide used in grain products.

Under FDA, USDA, and BATF regulations, the ingredients of a food or beverage must be stated on the product label in decreasing order of predominance. For many direct additive categories, chemical constituents must be identified by their common names and the purpose for which they were added.

One of the recent regulations involving the food industry as well as food additive manufacturers came with the passing of the Nutrition Labeling and Education Act of 1990 (NLEA), which amends the federal FD&C Act, to make nutrition labeling mandatory for most FDA-regulated foods. The nutrition labeling regulations issued by the FDA and the USDA's Food Safety and Inspection Service (FSIS) required compliance by August 8, 1994. The FDA found that by the end of May 1995, more than 80% of the domestic and imported food products checked were in compliance with the regulations.

The FDA's Food Additives Amendment also contains what is known as the Delaney Clause, which mandates the FDA to ban any food additive found to cause cancer in humans or animals, regardless of dose level or intended use. The clause applies not only to new food additives but also to those in use prior to 1958. The Delaney Clause is totally inflexible in that it does not recognize any threshold level below which the additive might not present a health hazard. Thus, it has caused a number of problems for the food industry and for food additives.

Certain additives, such as the sweetener cyclamate, FD&C Red #2 food color, and so on, have been banned after they were found to be potential carcinogens, although many experts believe that feeding tests in animals at massive dose levels may not bear any correlation to the potential risk to humans of chronic ingestion at very low levels. Were it not for a moratorium mandated by Congress, saccharin would have also been banned in the United States several years ago by the FDA in compliance with current food laws.

Approval Process

A new substance gains approval for food use through the successful submission of a Food Additive petition that must document the following:

- Safety, including chronic feeding studies in two species of animals
- Intended use
- Efficiency data at specific levels in specified food systems
- Manufacturing details and product specifications
- Methods for analysis of the substance in food
- Environmental impact statement

Quite frequently, this process can be lengthy—up to 10 years in the case of aspartame and Olestra®—and costly in terms of production hours and dollars. There is little doubt that every phase of the U.S. food additives business is affected by regulations and operates with a constant awareness of the importance of FDA decisions. Not only is the introduction of a new food additive impossible without FDA approval, but also the additives in use are under constant scrutiny by the regulatory agency and remain always vulnerable to new unfavorable toxicological findings. Although the barring of an additive may create opportunities for suppliers to develop new or substitute materials, the potential market is often too small to create sufficient incentive, and the potential loss of the ingredient may raise excessive havoc within affected sectors of the food industry. For example, the ban on cyclamates, followed by the close call on saccharin, almost caused the demise of a strong diet soft drink industry. The well-recognized need for alternative safe sweeteners that would command a high price undoubtedly acted as a stimulus for G. D. Searle (now Monsanto NutraSweet/Kelco division) to engage in a 10 year effort to have aspartame cleared for food use.

European Union

Food additives are regulated by Directive 89/107/EEC of December 21, 1988, on the approximation of the laws of member states concerning food additives authorized for use in foodstuffs intended for human consumption. The European Union (EU) food additives law recognizes 106 food additives. Later, several amendment and adaptations of Directive 89/107/EEC were introduced or proposed, including the following:

- A list of additives, the use of which is authorized to the exclusion of all others.
- The list of foodstuffs to which these additives may be added, the conditions under which they may be added and, where appropriate, a limit on the technological purpose of their use.
- The rules on additives used as carrier substances and solvents, including where necessary their purity criteria.
- The proposal in 1990 by the European Commission of a first specific directive relating to sweeteners and on food additives other than colors and sweeteners. The Sweetener Directive took effect in 30 July 1994. Member countries were asked to adopt the directive by December 31, 1995, which did not occur. The sweetener guidelines are expected to open up new markets for low-calorie food products and will simplify the logistical matters.

Efforts have been toward a uniform registration process so that a registration obtained in one country would be valid in all EU member countries. The new EU food additive law, however, will not prevent individual countries from asking for additional or country-specific requirements for new product registrations. At the EU level, several institutions and groups are involved in the development of food additives law, including the Scientific Committee for Food (SCF), one of the institutions of the European Commission that deals with safety issues; it includes representatives from different national professional organizations, representatives from the food industry, retailers, and so on.

The EU rules for the evaluation, marketing, and labeling of novel foods such as genetically modified foods are also being developed. The new marketing rules would also oblige manufacturers to obtain permission before placing new foods or ingredients on the market, with the exceptions for products that are substantially equivalent to existing foods. The new rules have still to be cleared by the European Parliament, which has the power to veto under the new codecision procedure introduced in 1995.

In many countries, additives must be declared in the labeling. Within the European Community, some additive groups have been uniformly codified with "E" numbers for the orientation of consumers. Some countries, such as Germany, have gone further, adopting regulations on an acceptable daily intake (ADI) basis that build on the newest toxicological knowledge. Some examples of "E" numbers are shown in Table 1.

Japan

In Japan, the Food Chemistry Division of the Ministry of Health and Welfare (MHW) has jurisdiction over food additives through the Food Sanitation Law, which was enacted in January 1948 with several amendments adopted since then. Amendments to the regulations as well as additions or deletions to Kohetisho (the Japanese Codex of Food Additives) were mostly influenced by two major objectives: (1) protection of food sanitation and customer safety, and (2) harmonization with international regulatory requirements.

Most discussions on regulating food additives in Japan have been related to defining what food additives should be under legal restriction and on labeling requirements. Very often in these discussions, differentiating "synthetic" and "natural" food additives had been at issue. In Japan,

Table 1. Selected Food Additives and their European Codes

	Antioxidants
E300	L-Ascorbic acid (vitamin C)
E307	α-Tocopherol, synthetic (vitamin E)
E311	Propyl gallate (PG)
E320	Butylhydroxyanisole (BHA)
	Colorants
E100	Curcumin
E101	Riboflavin
E102	Tartrazin (Yellow No. 5)
E150	Caramel
E160	Annatto
E160a	Beta-carotene
E162	Beetroot red (Betanin; Betanidin)
	Emulsifiers
E322	Lecithin
E471	Mono- and diglycerides of fatty acids
E475	Polyglycerol esters of fatty acids
	Preservatives
E200	Sorbic acid
E202	Potasium sorbate
E210	Benzoic acid
E211	Sodium benzoate
E212	Potassium benzoate
E282	Potassium propionate
	Thickeners and stabilizers
E401	Sodium alginate
E415	Xanthan
E420	Sorbitol
E440a	Pectin

those two generally used terms have often misled customers into a blind belief in natural food additives. However, regulatory bodies as well as the food additive industry no longer distinguish additives with these terms. The latest amendment of the Food Sanitation Law (May 24, 1995) includes deletion of the term "chemically synthesized substances" from the law. Thus, "natural" food additives are regulated under the law amended (being enacted from May 24, 1996), unless they are listed as "existing food additives."

The following short summary of the history of food additive regulations and their effect shows why a distinction had been made between synthetic and natural and why those terms are now obsolete.

It was in 1948 that the term food additive appeared in the Food Sanitation Law and a positive list of food additives was created in Japan. It was the first positive list created in the world, and it did not distinguish between synthetic or natural additives. In the Food Sanitation Law, the term additive means anything added to, mixed into, permeating, or otherwise put in or upon food for the purpose of processing or preserving it.

Since 1989 regulations such as labeling requirements cover natural additives as well, and other requirements for natural additives are planned for the future. Legal coverage of natural additives was indicated by General Agreement on Tariffs and Trade (GATT) officials as a part of the move toward international harmonization of regulations. It was in 1987, when the Japanese government gave notification to GATT officials about a new labeling regulation, that officials pointed out that the Japanese regulation only covered synthetic additives, creating a large inconsistency with regulations of other nations. Today, when new natural food additives are used in Japan, suppliers also need to report them to the MHW. However, in general, data requirements for natural additives are still not as strict as those for chemical substances. There are about 1200 items in the list of natural additives while the conventional list contained 349 compounds as of 1992.

Another important regulation that strongly affects this industry is labeling. The Enforcement Regulation of the Food Sanitation Law, Chapter II (amended on June 10, 1981 and enacted in 1983), defines the term label, and it requires that both synthetic and natural food additives be declared on the food label.

DESCRIPTION OF MAJOR FOOD ADDITIVES

Practically every food manufacturing operation depends in some degree on the use of food additives, but the range of additives necessary for the formulation varies (Table 2). Of the variety of ways food additives have been classified, most involve functional grouping. Although chemical classifications may be useful, because they place molecules of similar structure and physical-chemical properties in comparable categories, compounds of a single chemical family may perform different functions in the food industry. Therefore, the following brief description of the major food additives is based on functional classification.

Anticaking Agents

Food products that contain hygroscopic substances require the addition of an anticaking agent to inhibit formation of aggregates and lumps and thus retain the free-flowing characteristic of the products. Such chemicals as calcium aluminum silicate, calcium phosphate tribasic, magnesium carbonate, magnesium silicate, calcium stearate magnesium stearate, microcrystalline cellulose, and sodium aluminum silicate at a level of 1.0% or less have been suggested as effective anticaking agents in free-running salt and in dry powdered mixes. Colloidal silicon dioxide is also used at a level of 1.0% as an anticaking agent in salt (NaCl). The decahydrate of sodium ferrocyanide or Yellow Prussiate of soda at the extremely low level of 5 ppm causes the formation of star-shaped crystals of sodium chloride rather than the usual cubical ones. The star-shaped crystals known in the trade as dendritic salt possess nonsegregating properties; hence, they are less likely to cake.

Antifoaming (Defoaming) Agents

Antifoaming agents are substances used to reduce foaming caused by proteins or gases that may interfere with processing. Foaming may be largely suppressed or completely eliminated by the use of small quantities, generally about 10 ppm, of dimethylpolysiloxane, mono- and diglycerides, oleic acid, silicon dioxide, white mineral oil, and a number of other fatty acids. These compounds have been suggested for use in the preparation of certain comminuted meat products, bakery products, confections, dairy products, vegetable oils, alcoholic and nonalcoholic beverages, jams and jellies, molasses, soups, starches, syrups, and pickles.

Antioxidants

Antioxidants are described in the "Preservatives" section of this article.

Bleaching, Maturing, and Dough-Conditioning Agents

Because some chemicals serve as both bleaching and maturing agents, and others are referred to as dough-conditioning agents or bread improvers, it is perhaps desirable to consider all of them under one heading.

Bleaching agents are used in the production of certain cheeses, processed fruits, crude fats and oils, and meat products to neutralize color that may be present naturally. As an example, after crude oils are refined to remove impurities, they must be further treated by bleaching to remove coloring materials that are usually present in crude fats and oils. Sulfuric and metaphosphoric acids and hydrogen peroxide have been used for this purpose. Calcium hypochlorite is used in bleaching sugar syrup prior to crystallization.

Bleaching plays special importance in the flour milling and baking industries. Freshly milled wheat flour has a yellowish color, caused by the presence of small quantities of carotenoid and other pigments. Such flour also lacks the quality necessary to make elastic, stable dough. When such flour is stored and allowed to age for several months, it gradually becomes whiter because of oxidation and matures, making it acceptable for baking. For many years,

Table 2. Selected Food Additives and their Major Uses

	Processed/cured meats	Dairy products	Jams/ Jellies	Pickles/ sauces	Breakfast cereals	Cookies/ crackers	Baked goods	Mayonnaise/ dressings
Anticaking agents					X			
Antifoaming agents								X
Antioxidants	X				X			X
Bleaching/maturing agents						X	X	
Colors	X	X	X	X	X	X	X	X
Emulsifiers		X						
Enzymes		X					X	
Fat substitutes		X				X	X	X
Firming agents				X				
Flavors		X		X	X	X	X	X
Glazing compounds							X	
Humectants							X	
pH-adjusting compounds	X	X	X	X				X
Preservatives	X	X	X	X		X	X	
Release agents						X	X	
Sequesterants								X
Sweeteners		X	X	X	X	X	X	
Thickeners/stabilizers	X	X	X				X	X
Vitamins		X			X			

	Carbonated/still beverages	Snacks/chips	Dessert mixes	Frozen entrees	Fats/oils	Margarine	Candies/chewing gums
Anticaking agents			X				X
Antifoaming agents	X				X		X
Antioxidants	X	X			X		X
Bleaching/maturing agents					X		
Colors	X	X	X			X	X
Emulsifiers	X		X	X		X	X
Enzymes							
Fat substitutes		X	X	X		X	
Firming agents							
Flavors	X	X	X	X			X
Glazing compounds							
Humectants							
pH-adjusting compounds	X						
Preservatives	X	X		X			
Release agents		X					X
Sequesterants					X		
Sweeteners	X		X	X			X
Thickeners/stabilizers	X	X	X	X			
Vitamins	X		X			X	

the natural bleaching and aging was the only means millers and bakers had of producing the desired material. These natural processes were slow and costly and did not always yield a consistently satisfactory product. Then it was discovered that certain oxidizing agents incorporated into the flour in small amounts brought about rapid improvements in its color and bread-making properties. Oxidizing agents such as ammonium persulfate, potassium bromate, nitrogen peroxide, and chlorine dioxide is recommended for flour treatment.

Colorants

Colorants, both synthetic and natural derivatives, are used in many foods to enhance their appearance and thereby influence the perception of texture and taste.

Colors can be divided into two types: FD&C certified and noncertified colors. The term FD&C color is a carryover from the federal FD&C Act of 1938. It refers to synthetic colors that were regarded as "harmless and suitable for use" in foods, drugs, and cosmetics. To market their products, U.S. producers must submit product samples from each batch of material for certification.

Certified food colorants can be divided into dyes and lakes. Dyes are water-soluble compounds that impart color to a substance through dissolution. Lakes are both water and oil insoluble and impart color through dispersion in food. Because of their high tinctorial strength, synthetic colorants of the azo and triphenyl-methane class are used at levels of few ppm to about 300 to 500 ppm, whereas naturally derived colorants require much higher levels, often a few percent as in the case of caramel color.

Noncertified colors can be either natural origins, such as vegetables (eg, carrot oil, red beet juice, paprika, etc), fruits (eg, grape skin extract, cranberry juice concentrate, etc), and insects (cochineal extract), or produced by chemical synthesis (eg, ferrous gluconate, riboflavin, synthetic carotenoid, etc). Although a great number of noncertified food colorants are used by the industry, demand is concentrated in a few products; namely, caramel, annatto, paprika, and synthetic carotenoids. Food color additives and their sources are listed in Table 3.

Emulsifiers

Emulsifiers are additives that allow normally immiscible liquids, such as oil and water, to form a stable mixture. They are widely used in foods to achieve the texture, taste, appearance, fat reduction, and shelf life desired in foods.

The most common and commercially important emulsifiers are monoglycerides and diglycerides of fatty acids and their esters (eg, glyceryl monostearate), lactylated esters (eg, sodium stearyl lactylate), propylene glycol mono- and diesters (eg, propylene glycol monostearate), lecithin, sorbitan esters (eg, sorbitan monostearate), and polysorbates (eg, polyoxyethylene sorbitan monolaurate). These products are usually formulated for specific applications so that the combination provides both enhanced performance and ease of use. A great number of other emulsifiers are also used by the food industry, but in smaller volumes (Table 4).

Enzymes

Enzymes are catalysts used during food processing to make chemical changes to the food. They are biological catalysts that make possible or greatly speed up chemical reaction by combining with the reacting chemicals and bringing them into the proper configuration for the reaction to take place. They are not affected by the reaction. All enzymes are proteins and become inactive at temperatures above 40°C or in unfavorable conditions of acidity or alkalinity. Food enzymes perform some of following functions:

- Speed up reactions
- Reduce viscosity
- Improve extractions
- Carry out bioconversions
- Enhance separations
- Develop functionality
- Create/intensify flavor

Table 3. Selected Food Color Additives

	Color	Source
Allura Red No. 40	Red	Synthetic
Amaranth, Red No. 2[a]	Red	Synthetic
Annatto extract	Yellow	Vegetable
Beet juice	Red	Vegetable
Brilliant blue FCF, Blue No. 1	Blue	Synthetic
Beets, dehydrated (beet powder)	Purple	Vegetable
Canthaxanthin	Red	Synthetic
Caramel	Brown	Semisynthetic
Apo-carotenal	Orange	Synthetic
Beta-carotene	Yellow	Synthetic
Carrot oil	Yellow	Vegetable
Chlorophyll	Green	Vegetable
Cochineal Extract, (carmine)	Red	Insect
Erythrosine, Red No. 3	Red	Synthetic
Fast Green, Green No. 3	Green	Synthetic
Fruit juice (grape and cranberry)	Red	Fruit
Grape skin extract (enocyanin)	Red	Fruit
Paprika	Red	Vegetable
Paprika oleoresin	Red	Vegetable
Ponceau 4R, (Red No. 4)[a]	Red	Synthetic
Riboflavin	Yellow	Synthetic
Saffron	Yellow	Vegetable
Sodium Indigo Disulfonate, Blue No 2	Blue	Synthetic
Sunset Yellow, Yellow No. 6	Yellow	Synthetic
Tartrazine, Yellow No. 5	Yellow	Synthetic
Titanium dioxide	White	Synthetic
Turmeric (curcumin)	Yellow	Vegetable
Turmeric oleoresin	Yellow	Vegetable
Vegetable juice	Red	Beet and red cabbage juice

[a] Approved in Europe and Japan; banned in the United States.

Table 4. Emulsifiers and their Regulatory Status in the United States and Europe

Emulsifier	USA[a]	European no.
Mono- and diglycerides (GRAS)	182.4505	E 471
Succinyl monoglyceride	172.830	
Lactylated monoglyceride	172.852	E 472
Acetylated monoglyceride	172.828	E 472
Monoglyceride citrate	172.832	E 472
Monoglyceride phosphate (GRAS)	182.4521	
Stearyl-monoglyceride citrate	172.755	E 472
Diacetyl-tartrate ester of monoglyceride (GRAS)	182.4101	E 472
Polyoxyethylene monoglyceride	172.834	
Polyoxyethylene (8) stearate	172.838	
Propylene glycol monoester	172.854	E 477
Lactylated propylene glycol monoester	172.850	
Sorbitan monostearate	172.842	E 491
Sorbitan tristearate	172.842	
Polysorbate 60	172.836	E 435
Polysorbate 65	172.836	E 436
Polysorbate 80	172.840	E 433
Calcium stearoyl lactylate	172.844	E 482
Sodium stearoyl lactylate	172.846	E 481
Stearoyl lactylic acid	172.848	
Stearyl tartarate		E 483
Stearyl monoglyceridyl citrate	172.755	
Sodium stearyl fumarate	172.826	
Sodium lauryl sulfate	172.822	
Dioctyl sodium sulfosuccinate	172.810	
Polyglycerol esters	172.854	E 475
Sucrose esters	172.859	E 473
Sucrose glycerides		E 474
Lecithin (GRAS)	184.1400	E 322
Hydroxylated lecithin	172.814	E 322
Triethyl citrate (GRAS)	182.1911	

[a] Code of Federal Regulation (CFR) Title 21.

- Synthesize chemicals

Food enzymes are usually classified into the following categories:

- Carbohydrases (amylases are commercially the most important subgroup hydrolyzing 1,4 glycosidic bonds in carbohydrates)
- Proteases, hydrolyze peptide bonds in proteins
- Lipases, split hydrocarbons from lipid
- Pectic enzymes and cellulases, hydrolyzing the plant cell wall material
- Specialty enzymes

These enzyme categories can be divided further into around 15 to 20 subgroups. Traditional roles of enzymes in the food industry have been in the processing of bakery goods, alcoholic beverages, and starch conversion. But interest is now focused on newer and more varied applications, such as hydrolysis of lactose, the preparation of modified fats and oils, the processing of fruit juices, and other processes where newer enzymes are being identified. Important food applications of enzymes are listed in Table 5.

The commercially important food enzymes are α amylase, glucoamylase, and glucose isomerase, which are especially significant in the United States because of their widespread use in the conversion of cornstarch into high-fructose corn syrup (HFCS). Rennin, which is used in cheese making, is also of significant volume, followed by a host of other enzymes, including pectinases, invertase, lactase, and maltase (used for the modification of starches and sugars), catalase, pepsin, glucoseoxidase (an antioxidant for canned foods), and bromelin, ficin, and papain (plant proteases used for tenderizing meat and producing easily digestible foods). Enzymes are extremely specific and can act only on a single class of chemicals such as proteins, carbohydrates, or fats.

Table 5. Enzyme Applications

Dairy products	Milk coagulation (rennet), milk protein modification, cheese flavor development, enzyme modified cheeses, and removal of hydrogen peroxide
Baking and cereals	Antistaling, dough improvement, improved crust color, and gluten hydrolysis
Sugar processing	Removal of starches and processing from cane sugar
Starch conversion	Starch modification, liquefaction, isomerization, saccharification, modification, and increasing yield
Oils and fats	Improving yields, interesterification, oil extraction, and lecithin production
Flavors	Synthesis of flavors, production of natural esters
Alcohol fermentation	Starch liquefaction, improving yeast growth
Brewing	Adjunct liquefaction, enhanced fermentability, filtration improvement, production of light beer, and removal of protein haze
Fruit juices, and wines	Increasing press yields, juice clarification, shelf-life extension
Coffee processing	Separation of bean, viscosity control of concentrate
Chemical processes	Biotransformation, synthesis (eg, emulsifiers)
Analytical	Tests for dietary fiber, sugars
Waste treatment	Breakdown of cellulose, lignin, oil residues, and other solid waste material

Enzymes are produced from animal tissues (eg, pancreatin, tripsin, lipase), plant tissues (eg, ficin, bromelin), and most frequently by microorganisms (eg, pectic, or starch enzymes). Microbial production from a variety of species of molds, yeast, and bacteria is increasingly becoming the predominant source of enzymes.

Application of genetic engineering to the development of enzymes has already made a significant impact. The first food ingredient produced by genetic engineering was chymosin, "Chy-Max®," a microbial rennet developed by Pfizer (now Cultor Food Science), which has been approved by the regulatory agencies in the United States, Canada, the United Kingdom, Australia, Italy, and several other countries.

Firming Agents

Fruits and vegetables contain pectin components that are relatively insoluble and form a firm gel around the fibrous tissues of the fruit and prevent its collapse. Addition of calcium salts causes the formation of calcium pectate gel, which supports the tissues and affords protection against softening during processing. The calcium salt is sometimes added to the canned vegetable in the form of a tablet containing both sodium chloride and calcium chloride.

Canned vegetables, canned apples, frozen apples, and tomatoes are sometimes treated during processing with calcium chloride, calcium citrate, monocalcium dihydrogen phosphate, or calcium sulfate to prevent them from becoming soft and disintegrating. Suggested level of use of these calcium salts is 0.02%, calculated as calcium in the final food product. In canned potatoes, calcium chloride and calcium citrate at a level of 0.5% (calculated as calcium) are used.

Aluminum sulfate, ammonium aluminum sulfate, potassium aluminum sulfate, and sodium aluminum sulfate are used as firming agents in pickles and relishes. A more recently introduced firming agent is aluminum sulfate for canned crabmeat, lobster, salmon, shrimp, and tuna. Calcium chloride acts as a firming agent in cheddar and cottage cheese.

Flavors

The word *flavor* describes a complex sensation provided by compositions of many defined aromatic ingredients. Flavorings are concentrated preparations used to impart a specific aroma to food or beverage. Flavoring ingredients are the most numerous single group of intentional additives utilized by the food industries. Flavors should not be viewed as a single homogeneous class of food additives but as a composite of closely interrelated and somewhat overlapping sectors with differentiated characteristics, as discussed next.

Essential oils and natural extracts are usually defined as the volatile material obtained from a particular plant species by the process of distillation, expression (cold pressing), and maceration. Essential oils represent complex aroma mixtures containing as many as hundreds of chemical constituents. Included are vanilla, cocoa, cola, spice oleoresins, and so on. Essential oils may be used for imparting scent or aroma to consumer products, as raw materials for compounding flavor compositions, or as the source of isolated aroma chemicals, also used in compounding.

Aroma chemicals comprise organic compounds with a defined chemical structure that are isolated from microbial fermentation, plants, or animal sources or are produced by organic synthesis. Included are anethole, vanillin, citronellol, geraniol, and so on. Aroma chemicals may be added directly to foods and beverages or used as raw materials in flavor compositions.

Flavor compositions consist of complex mixtures of various aromatic materials from a few to 100 or more constituents. Compounded flavors may contain aroma chemicals, natural extracts, essential oils, solvents, and in some cases other functional additives (eg, antioxidants, acidulants, emulsifiers, etc).

Flavor compositions are added to foods and beverages to

- Create a totally new taste;
- Enhance, extend, round out, or increase the potency of flavors already present;
- Supplement or replace flavors to compensate for losses during processing;
- Simulate other more expensive flavors or replace unavailable flavors; and
- Mask less desirable flavors—to cover harsh or undesirable tastes naturally present in some food, other than hide spoilage.

Flavoring substances may be classified as follows:

- *Natural flavoring substance.* Obtained by physical separation, enzymatic process, or microbial process from vegetable or animal sources, either in the raw state or after processing (including drying, torrefaction, and fermentation)
- *Nature-identical flavoring substance.* Obtained by synthesis or isolated by chemical processes, chemically identical to substances naturally present in vegetable or animal sources (this classification is used in Europe, but not allowed in the United States)
- *Artificial flavoring substance.* Obtained by chemical synthesis and not found to occur in nature
- *Flavoring preparation.* Products other than natural substances, whether concentrated or not, with flavoring properties, obtained by physical separation, or enzymatic or microbial processes, from material of vegetable or animal origin, either in the raw state or after processing (including drying, torrefaction, and fermentation)
- *Process flavorings.* Products obtained by heating to a temperature not exceeding 180°C for a period not exceeding 15 min using a mixture of ingredients, not necessarily themselves having flavoring properties, of which at least one contains nitrogen (amino) and another is a reducing sugar.
- *Smoke flavorings.* Smoke extracts used in traditional foodstuff smoking processes
- *Flavor enhancers.* Some amino acids and nucleotides, as well as sodium salts (such as monosodium glutamate, sodium inositate and sodium guanylate), have only a weak taste by themselves but have the power to considerably enhance the taste sensation caused by other ingredients in savory flavors.

The types of flavor compositions, their manufacturing processes, the starting materials for manufacturing them, and the common product forms are summarized in Table 6.

Glazing and Polishing Agents

Glazes and polishes are used on coated confections to give luster to the otherwise dull coating. Chemicals that are used for this purpose include acetylated monoglycerides, beeswax, carnauba wax, gum arabic, magnesium silicate, mineral oil, petrolatum, shellac, and zein. Generally these compounds are used at levels of about 0.4%, with the exception of mineral oil and petrolatum, which are used at 0.15%, and zein at 1.0%.

Humectants

Substances that have affinity to water, with stabilizing action on the water content of a material, are termed humectant or moisturizing agents. These are hygroscopic materials that prevent loss of moisture when incorporated into foodstuffs. Ideally, a humectant maintains within a rather narrow range of moisture content caused by humidity fluctuations. Among commonly used humectants are the following substances: glycerin, mono- and diglycerides of fatty acids, propylene glycol, pectin, molasses, potassium polymethaphosphate, and sorbitol.

pH-Adjusting Agents

A large group of chemical additives that are widely used in foods might be considered under the broad heading of pH-adjusting agents. Other terms that describe these chemicals include acidulants, acids, alkalis, buffers, and neutralizers. These chemicals are used in most segments of the food processing industries, including:

- the baking industry as chemical leavening agents,
- in soft drinks to provide tartness,
- in certain dairy products to adjust the acidity,
- in cheese spreads for emulsification,

Table 6. Commercial Flavor Compositions

Type of flavor	Classification	Manufacturing process	Raw materials	Product form
Compounded flavors	Natural or synthetic	Blending, mixing	Essential oils, natural extracts, fruit juice concentrates, aroma chemicals	Liquid Spray-dried, encapsulated
Natural extracts	Natural	Extraction, enzymatic treatment	Food substrates (eg, plants, fish, meat, etc)	Liquid Paste
Reaction flavors (thermally processed)	Natural	Heating/pressure cooking	Amino acids and sugars, hydrolyzed proteins	Paste powder
Enzymatically modified flavors	Natural	Enzymatic/microbial reaction	Food substrates (eg, cheese)	Paste powder

- in confectionery products as flavoring, to control the degree of inversion of sugars, and to control the texture in the processing of chocolates,
- in jams and jellies to provide proper gel formation.

Citric acid is the most versatile and widely used of the food acidulants, and very large volumes of phosphoric acid are required for cola beverages. Other acidulants frequently used in processed foods include acetic, adipic, fumaric, hydrochloric, lactic, malic and tartaric acids, and glucono-δ-lactone.

Important alkalies used in the food field include ammonium bicarbonate, ammonium hydroxide, calcium carbonate, calcium oxide, potassium bicarbonate, potassium hydroxide, sodium bicarbonate, sodium carbonate, sodium hydroxide, and sodium sesquicarbonate.

Quite often, the pH may be difficult to adjust or to maintain after adjustment. Stability of pH can be accomplished by the addition of buffering agents that, within limits, effectively maintain the desired pH even when additional acid or alkali may be added. Examples of representative buffer solutions are as follows:

- Phosphoric acid : dibasic potassium phosphate
- Formic acid : sodium formate
- Acetic acid : sodium acetate
- Sodium bicarbonate : sodium carbonate
- Dibasic sodium phosphate : sodium hydroxide

Preservatives

Chemical preservatives play a very important role in the food industry, from manufacture through distribution to the ultimate consumer. The choice of a preservative takes into consideration the product to be preserved, the type of spoilage organism endemic to it, the pH of the product, period of shelf life, and ease of application. No one preservative can be used in every product to control all organisms, and therefore combinations are often used. In certain foods, specific preservatives have very little competition. In the concentrations used in practice, none of the preservatives discussed here is lethal to microorganisms in foods. Rather, their action is inhibitory.

Preservatives may be divided into two main groups: (1) antioxidants and (2) antimicrobials. Antioxidants are food additives that retard atmospheric oxidation and its degrading effects, thus extending the shelf life of foods. Examples of food oxidative degradation include products that contain fats and oils in which the oxidation would produce objectionable rancid odors and flavors, some of which might even be harmful. Antioxidants are also used to scavenge oxygen and prevent color, flavor, and nutrient deterioration of cut or bruised fruits and vegetables.

Food antioxidants may be divided into oil- and water-soluble compounds (Table 7) and also classified as natural or synthetic.

The most frequently used natural antioxidants are ascorbic acid (vitamin C), its stereo isomer erythorbic acid, and their sodium salts (sodium ascorbate, sodium erythorbate), plus the mixed δ- and γ-tocopherols. Vitamin C finds more major use as a nutritive supplement or in pharmaceutical preparations. Smaller amounts, however, are intentionally used for antioxidant purposes. Erythorbic acid (*iso*-ascorbic acid) is virtually devoid of vitamin C activity (only 5% that of ascorbic acid). Citric acid and tartaric acid are also natural antioxidants (and antioxidant synergists) but are predominantly added to foods as acidulants.

Synthetic antioxidants used as direct food antioxidants include butylated hydroxyanisole (BHA), butylated hy-

Table 7. List of Major Food Antioxidants

Oil-soluble antioxidants
Butylated hydroxyanisole (BHA)
Butylated hydroxytoluene (BHT)
tert-Butyl hydroquinone (TBHQ)
Propyl gallate (PG)
Tocopherols
Thiodipropionic acid
Dilauryl thiodipropionate
Ascorbyl palmitate
Ethoxyquin
Water-soluble antioxidants
Ascorbic acid
Sodium ascorbate
Erythorbic acid
Sodium erythorbate
Glucose Oxidase/catalase enzymes
Gum Guaiac
Sulfites
Rosemary extract

droxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ), and propyl gallate (PG). These antioxidants are oil-soluble compounds. Their primary application is in fats and oils to retard the development of rancid taste.

To improve the performance of antioxidants, two other types of food additives, sequestrants (eg, EDTA, citric acid) and synergists (eg, mixtures of antioxidants and lecithin), are frequently used with them. Antioxidants may also be present in food packaging as indirect food additives.

Recently, definitive studies have shown and widely publicized in the news media that antioxidant nutrients such as ascorbic acid (vitamin C) and tocopherols (vitamin E) can protect against harmful cell damage and thus prevent certain human diseases. Foods formulated with antioxidants and other vitamins are now recommended to prevent and cure cancer, cardiovascular diseases, and cataracts. The same antioxidants that are used to prevent oxidative deterioration of food may be used in functional foods (also called nutraceuticals, designer foods, etc) to create products that prevent or cure certain chronic diseases.

Antimicrobials are capable of retarding or preventing growth of microorganisms such as yeast, bacteria, molds, or fungi and subsequent spoilage of foods. The principal mechanisms are reduced water availability and increased acidity. Sometimes these additives also preserve other important food characteristics such as flavor, color, texture, and nutritional value. The primary food additives used for this function are sorbic acid, potassium and sodium sorbates, calcium and sodium propionates, benzoic acid, sodium and potassium benzoates, and parabens.

In addition to these compounds, several organic acids such as citric, malic, lactic, ascorbic, phosphoric, and tartaric also act as antimicrobial agents, but because of their greater use as acidulants, they are covered elsewhere.

Sulfur dioxide and sulfites are also applied extensively for controlling undesirable microorganisms in soft drinks, juices, wine, beer, and other products. Salt, sugar, alcohol, spices, essential oils, and herbs also inhibit growth of microorganisms, but usually their primary function is different when added to food.

Major uses of antimicrobial preservatives by food industry include the following:

- Sorbates are used as mold and yeast inhibition in processed cheese and spreads, salad dressings, and dried fruits, and are effective in the acidic pH range up to pH 6.5.
- Benzoates are used in beverages, fruit juice, and pickles, and are effective in the pH range between 2.5 and 4.0.
- Propionates are used as mold and rope inhibitors in bread and baked goods.
- Parabens are effective in low-acid foods (pH greater than 5.0), such as meat and poultry products.
- Sulfur dioxide and sulfite salts (eg, potassium bisulfite, potassium metabisulfite, sodium bisulfite, sodium metabisulfite, and sodium sulfite) are the most effective inhibitors of deterioration of dried fruits and fruit juices. Sulfur compounds also used in the fermentation industry to prevent spoilage by microorganisms and as a selective inhibitor of undesirable organisms.

Release Agents

Release agents are used to prevent confectionery, and to a lesser extent baked goods, from sticking to equipment or to the container in which they are heated. They are also used to prevent pieces of confection from adhering to each other. Included are acetylated monoglycerides, calcium stearate, magnesium carbonate, magnesium silicate, magnesium stearate, mannitol, mineral oil, mono- and diglycerides, sorbitol, and stearic acid.

Sequestering Agents

Also called chelates, sequestering agents combine with polyvalent metal ions to form a soluble metal complex to improve the quality and stability of products as free metallic ions promote oxidation of food. They are used in various aspects of food production and processing chiefly to obviate undesirable properties of metal ions without the necessity of precipitating or removing these ions from solutions.

Ethylenediaminetetraacetic acid (EDTA) is the most commonly used sequestering, or metal-complexing, agent used in the food industry. This compound, as well as the disodium or calcium disodium salts of tetraacetic acid retards discoloration of dried bananas, beans, chickpeas, canned clams, pecan pie filling, frozen potatoes, and canned shrimp. Also, these compounds improve flavor retention in canned carbonated beverages, salad dressings, mayonnaise, margarine, and sauces; retard struvite formation in canned crabmeat and shrimp; and protect against rancidity in dressings, mayonnaise, sauces, and sandwich spreads. EDTA is used at concentrations of 33 to 800 ppm.

Other chemicals that may be included in this category are calcium acetate, calcium gluconate, calcium sulfate, citric acid, stearyl citrate, tartaric acid, sodium tartarate, calcium monoisopropyl citrate, sodium hexametaphosphate, phosphoric acid, potassium citrate, and various calcium, potassium, and sodium phosphates.

Sweeteners

Sweeteners are used in formulated foods for many functional reasons as well as to impart sweetness. They render certain foods palatable and mask bitterness; add flavor, body, bulk, and texture; change the freezing point and control crystallization; control viscosity, which contributes to body and texture; and prevent spoilage. Certain sweeteners bind the moisture in food that is required by detrimental microorganisms. Alternatively, some sweeteners can serve as food for fermenting organisms that produce acids that preserve the food, thus extending shelf life by retaining moisture. These auxiliary functions must be kept in mind when considering applications for artificial sweeteners.

Sweeteners may be classified in a variety of ways:

Table 8. Polyols: Their Calorie Value and Relative Sweetness to Sugar

Polyol sweetener	Relative sweetness, sucrose = 100	Calorie value (U.S. FDA allowance), Kcal/g
Erythritol	60–70	0.2
Isomalt	45–65	2.0
Lactitol	40	2.0
Maltitol	90	3.0
Mannitol	70	1.6
Sorbitol	50–70	2.6
Xylitol	100	2.4

- *Nutritive or nonnutritive.* Materials either are metabolized and provide calories or are not metabolized and thus are noncaloric.
- *Natural or synthetic.* Commercial products that are modifications of a natural product (eg, honey or crystalline fructose is considered natural; saccharin is a synthetic compound).
- *Regular or low-calorie/dietetic/high-intensity.* Although two sweeteners may have the same number of calories per gram, one may be considered low-calorie or high-intensity if less material is used for equivalent sweetness.
- *As foods.* For example, fruit juice concentrates can impart substantial sweetness.

Sweetness is a subjective perception influenced by a multitude of variables, including temperature of the food being tasted, pH, other flavors and ingredients in the food, physical characteristics of the food sweetener, concentration, rate of sweetness development, and permanence of sweetness and flavor. Sweetness is measured via sensory methods by taste panels. Results can vary depending on foods consumed prior to testing (even several hours before testing), the flavors to which the taster is accustomed, tasting experience of the panelist, time of day, and physical surroundings in the test room.

Sucrose, commonly known as table sugar (or refined sugar), is the standard against which all sweeteners are measured in terms of quality of taste and taste profile. It is consumed in the greatest volume of all sweeteners. However, sucrose, high-fructose corn syrup (HFCS), and other natural sweeteners, such as molasses, honey, maple syrup, and lactose sweeteners, are not considered additives and are not covered in this report.

Polyols (sugar alcohols) are a group of sweeteners that provide the bulk of sugars, without as many calories as sugar (Table 8). Polyols are important sugar substitutes and utilized where their different sensory, special dietary, and functional properties make them feasible. Polyols are obtained from their parent sugars by catalytic hydrogenation. In most European countries and in the United States polyols are utilized in low-calorie food formulations. Polyols are absorbed more slowly in the digestive tract than sucrose; therefore, they are useful in certain special diets. However, when consumed in large quantities some of them have a laxative effect. Polyols offer the same preservative benefit and a similar bodying effect to food than sucrose. Polyols are more resistant to either thermal breakdown or hydrolysis than sugar. Moreover most polyols are resistant to fermentation by oral bacteria, and therefore prime ingredients for tooth-friendly confectioneries (eg, “sugarless chewing gums).

High-intensity sweeteners, once used mainly for dietetic purposes, are now used as food additives in a wide variety of products. They are termed high-intensity because they are many times sweeter than sucrose and closely mimic its sweetness profile. Because of the very low use levels, however, high-intensity sweeteners cannot perform other key auxiliary functions in food and often must be used in conjunction with other additives such as low-calorie bulking agents. The relative sweetness of high-intensity sweeteners to sucrose sugar and their regulatory status are summarized in Table 9.

In 1988, the U.S. FDA approved the use of Hoechst AG’s *acesulfame K* (Sunette®) for use in chewing gum, dry beverage mixes, instant coffee and tea, gelatins, puddings, and nondairy creamers. In 1998 its approval was extended to

Table 9. High Intensity Sweeteners: Their Regulatory Status and Sweetness Relative to Sugar (1998)

Sweetener	Sweetness, sucrose = 1	United States	Canada	Europe	Japan
Cyclamate, Na Salt	30	P	A	A	N
Aspartame	200	A	A	A	A
Acesulfame K	200	A	A	A	N
Saccharin	300	A	N[d]	A	A
Sucralose[a]	600	A	A	P	N
Thaumatin (Talin)	3,000	N	N	N	A
Alitame[b]	2,000	P	P	P	P
Neohesperidin DC	2,000	N	N	A	N
Stevioside	300	N	N	N	A
Glycyrrhizin	50	N[c]	N	N	A

Note. A = approved; P = petition filed; N = not approved.

[a] Sucralose is approved in Australia, Russia, Brazil, New Zealand, Quasar, Romania, and Mexico.

[b] Alitame is approved in Australia, New Zealand, People’s Republic of China, Indonesia, Colombia, and Mexico.

[c] Glycyrrhizin is approved as a flavoring, but not as a sweetener in the United States.

[d] Saccharin in Canada is limited for use in personal care products and pharmaceutical, but it is banned in foods and beverages.

Table 10. Functions of Major Food Thickeners and Stabilizers

	Thickening	Emulsion stabilization	Suspending properties	Gelation	Crystallization control	Water binding	Mouth-feel	Foam stabilization	Flavor fixation	Protective film forming	Synergistic effect	Fat substitution
Agar				X								
Unmodified starches	X	X	X	X								
Modified starches	X	X	X	X		X	X					X
Casein						X		X		X		
Gelatin	X	X		X				X	X	X	X	
Carboxymethylcellulose (CMC)	X	X			X	X	X		X	X		X
Methylcellulose (MC)	X	X		X				X		X	X	
Guar gum	X	X				X					X	
Alginates	X	X	X	X		X	X	X		X	X	
Xanthan gum	X	X	X		X		X					X
Pectin	X			X			X			X		X
Locust bean gum	X	X										
Gum arabic		X	X		X			X		X		
Carageenan	X	X	X	X	X	X	X				X	X

liquid beverages. Acesulfame K has a rapidly perceptible sweet taste 200 times as potent as sucrose.

Alitame is a dipeptide made of two amino acids, L-aspartic and D-alanine. The sweetener is 2000 times as sweet as sugar with the same taste as sugar. Its potential market applications include bakery products, snack foods, candies and confectionery, ice cream, and frozen dairy products.

Aspartame was approved in the United States in 1981 for use in prepared foods and dry beverage mixes, as a tabletop sweetener, and in 1983 in liquid soft drinks. Aspartame is about 200 times as sweet as sucrose. Unlike most low-calorie sweeteners, aspartame is digested by the body to amino acids, which are metabolized normally. However, because of its intense sweetness, the amounts ingested are small enough that aspartame is generally considered noncaloric.

Cyclamate is 30 times sweeter than sugar. It has a sugarlike taste, a good shelf life, and a synergistic effect when combined with other high-intensity sweeteners. Currently it is approved in Canada and Europe, but it has been banned in the United States since 1970.

Neohesperidin dihydrochalcone (DHC) is derived from bioflavonoids of citrus fruits. It is 1500 to 2000 times sweeter than sucrose, leaves a licorice aftertaste, and gives a delayed perception of sweetness. DHC is produced from bitter Seville oranges by hydrogenation of natural neohesperidin, the main flavonoid constituent of some oranges.

Saccharin, used primarily as its sodium salt, is approximately 300 times as sweet as sucrose. Saccharin is the most widely used nonnutritive sweetener worldwide and the least expensive on a sweetness basis. It combines well with other sweeteners and has excellent shelf life. However, it has several disadvantages: it is bitter, has metallic aftertaste, and there is concern over its safety. Saccharin has been used primarily in soft drinks, but also as a tabletop sweetener and in a wide range of other beverages and foods.

Sucralose is a selectively chlorinated derivative of sucrose, the only high-intensity sweetener based on sucrose. It is approximately 600 times sweeter than sucrose. Sucralose is approved for food use in the United States, Canada, and several other countries.

Stevia (Stevia rebaudiana), a plant native to South America, is the source of the stevia extract, which is the natural sweetener. It can be used in food products that require baking or cooking because of its stability in high temperatures. Stevia is approved for use as a sweetener in Japan.

Thaumatin, a mixture of sweet-tasting proteins from the seeds of *Thaumato coccusceus daniellii*, a West African fruit, is about 2000 to 2500 times sweeter than sucrose. Its taste develops slowly and leaves a licorice aftertaste. Thaumatin acts synergistically with saccharin, acesulfame K, and stevioside; potential applications include beverages and desserts. It has been permitted in Japan as a natural food additive since 1979.

Thickeners and Stabilizers

Thickeners and stabilizers (also called as hydrocolloids, gums, or water-soluble polymers) provide a number of useful functions to food products (Table 10). The technical base for these effects results from the ability of these materials to modify the physical properties of water. Most food and beverage products largely consist of water. Thickener and stabilizer materials

- function as rheology modifiers, affecting the flow and feel (mouth) of food and beverage products,
- act as suspension agents for food products containing particulate matter,
- stabilize oil/water mixtures,
- act as binders in dry and semidry food products, and
- create both hard and soft gels in food products that require this physical form.

During the 1990s fat replacement became a major application for modified starches and gums as these additives provide unique texturing, bulking, and emulsifying properties of the displaced fat. Also, natural gums were offered as good sources of dietary fiber. Thickeners and stabilizers are generally used in very small amounts in most food products, in concentrations of 0.15% in jam, 0.35% in ice cream, and 1 to 2% in salad dressings.

Two principal classes of these materials are recognized (a third class known as "synthetic polymers," obtained from petroleum or natural gas precursors, are not used in food):

1. Natural materials obtained from plants or animals, including gum arabic, locust bean gum, guar gum, alginates, carrageenan, pectin, starches, casein, gelatin, and so on.
2. Semisynthetic materials that are manufactured by chemical derivatization of natural organic materials (generally based on a polysaccharide), or by microbial fermentation. This group includes carboxymethylcellulose (CMC) and other modified cellulose compounds, dextran, and gellan and xanthan gums.

Vitamins

Vitamins are nutritive substances required for normal growth and maintenance of life. They play an essential role in regulating metabolism, converting fat and carbohydrates into energy, and forming tissues and bones. Vitamins are typically divided into two groups: fat-soluble and water-soluble vitamins (Table 11). The fat-soluble group is

Table 11. Vitamins Consumed as Food Additives and U.S. RDI Values

Vitamin	Principal synonyms	Major market form	U S. RDI value
Vitamin A			
A_1	Retinol	Vitamin A acetate Vitamin A palmitate	5000 IU
A_2	Dehydroretinol		
Vitamin B			
Niacin	Vitamin B_3	Nicotinic acid	20 mg
Thiamin	Vitamin B_1	Thiamin hydrochloride	1.5 mg
Riboflavin	Vitamin B_2	Riboflavin	1.7 mg
Pantothenic acid	Vitamin B_5	Calcium pantothenate	10 mg
Pyridoxine	Vitamin B_6	Pyridoxine hydrochloride	2 mg
Cyanocobalamin	Vitamin B_{12}		6 mcg
Folic acid, biotin	Vitamin B_c	Folate	0.4 mg, 0.3 mg
Vitamin C			
	Ascorbic acid	Ascorbic acid Sodium ascorbate Calcium ascorbate	60 mg
Vitamin D			
D_2	Ergocalciferol		400 IU
D_3	Cholecalciferol		
Vitamin E			
	Tocopherols	DL α-tocopherol acetate D-α-tocopherol D-α-tocopheryl acid succinate	30 IU
Vitamin K			
K_1	Phytonadione	Phylloquinone	65 mcg
K_3	Menadione		

Table 12. Fortified Food Groups

Food	Vitamin	Use level	Remarks
Milk	Vitamin D	420 IU/L	Optional but generally added
Beverages (noncarbonated)	Vitamin C	15–100% of US RDI per serving	Optional; also added as an antioxidant
Cereals	Most essential vitamins	25–100% of US RDI per serving	Optional; added to 90% of cold cereals
Flour	Thiamin, riboflavin, niacin	8–15% of US RDI per 2-oz serving	Mandatory
Margarine	Vitamin A	33,100 IU per kilogram	Optional, but generally added
Miscellaneous foods (eg, instant breakfast, energy bars, etc)	Most essential vitamins		Added to position food as complete meal replacement

usually measured in International Units (IU). The water-soluble group is usually measured in units of weight.

Thirteen vitamins are recognized as essential for human health, and deficiency diseases occur if any one is lacking. Because the human body cannot synthesize most vitamins, they must be added to the diet. Most vitamins are currently consumed as pharmaceutical preparations, or over-the-counter vitamin supplements. Some, like vitamins B, C, D, and E, however, are added directly to food products (Table 12). Ready-to-eat breakfast cereals are a successful example of fortification. Because the primary use of these cereals is as a complete breakfast entree, they are commonly formulated to provide 25% or more of the Daily Value (% DV) per serving of 10 to 12 important vitamins and minerals common to cereals.

Vitamins are added to processed foods for several related reasons:

- To restore vital nutrients lost during processing; this is important with dried milk, dehydrated vegetables, canned foods, and refined and processed foods
- To standardize nutrient levels in foods when they fluctuate because of seasonal variations, soil differences, and methods of preparation
- To fortify fabricated foods that are low in nutrients and promoted as substitutes for traditional products; this includes complete breakfasts, breakfast drinks, meat extenders, and imitation products such as eggs, milk, cheese, and ice cream
- To fortify a major staple, such as bread, with a nutrient known to be in short supply
- For the preparation of designer food (nutraceuticals) containing vitamins that are shown to be useful in preventing chronic diseases.

In addition, vitamins may be used as functional ingredients in foods. Vitamin E (tocopherol) and vitamin C (ascorbic acid) protect foods by serving as antioxidants to inhibit the destructive effects of oxygen. This helps protect the nutritive value, flavor, and color of food products. In addition, ascorbic acid enhances the baking quality of breads, increases the clarity of wine and beer, and aids color development and inhibition of nitrosamine formation in cured meat products. Beta-carotene and β-apo-8'-carotenal are vitamin A precursors, which are brightly pigmented and may be added to foods such as margarine and cheese to enhance their appearance. The roles of these substances outside their nutritional functions are discussed elsewhere in this article (see the sections "Antioxidants," "Preservatives," and "Colorants").

Water-Correcting Agents

Water used in the brewing industry is often corrected to a uniform mineral salt content that corresponds to water known to give the most satisfactory final product. A wide variety of salts are used for this purpose, including mono- and diammonium phosphates, calcium chloride, calcium hydroxide, calcium oxide, calcium dihydrogen phosphate, calcium sulfate, magnesium sulfate, potassium aluminum sulfate, potassium chloride, potassium sulfate, sodium bisulfate, and mono-, di-, and trisodium phosphates. Some of the chemicals, in addition to standardizing the salt content, also control the acidity, thus providing uniform conditions for yeast fermentation.

GENERAL REFERENCES

A. T. Brannen, *Food Additives*, Marcel Dekker, New York, 1980.

Code of Federal Regulations, *Title 21—Food and Drugs. Subchapter B—Food For Human Consumption*, Parts 100–199, U.S. Government Printing Office, Washington, D.C., 1998.

J. M. Concon, *Food Toxicology, Contaminants, and Additives*, Vols. 1 and 2, Marcel Dekker, New York, 1987.

F. J. Francis, *Colorants*, American Association of Cereal Chemists, St. Paul, Minn., 1998.

T. E. Furia, *Handbook of Food Additives*, 2nd ed., CRC Press, Boca Raton, Fla., 1980.

M. Glicksman, *Food Hydrocolloids*, CRC Press, Boca Raton, Fla., 1982.

R. J. Lewis, *Food Additives Handbook*, Van Nostrand Reinhold, New York, 1989.

T. Nagodawithana and G. Reed, *Enzymes in Food Processing*, Academic Press, San Diego, Calif., 1993.

P. Newberne, "GRAS Flavoring Substances, 18th List," *Food Technol.* **52**, 65–92 (1998).

L. O'Brien Nabors and R. C. Geraldi, *Alternative Sweeteners*, 2nd ed., Marcel Dekker, New York, 1991.

G. Reineccious, *Source Book of Flavors*, 2nd ed., Chapman & Hall, New York, 1994.

L. P. Somogyi, "Direct Food Additives in Fruit Processing," in L. P. Somogyi, H. S. Ramaswamy, and Y. H. Hui, eds., *Processing Fruits, Biology, Principles, and Applications*, Technomic Publishing, Lancaster, Penn., 1996.

Laszlo P. Somogyi
Consulting Food Scientist
Kensington, California

FOOD ALLERGY

The ancient Roman poet and philosopher Lucretius was quoted as saying: "One man's food may be another man's poison." Although Lucretius was not referring to food allergies, his quote applies nicely to these illnesses. Most foodborne illnesses have the potential to affect everyone in the population. However, food allergies affect only a few individuals in the population. Food allergies involve an abnormal immunologic response to a particular food or food component, usually a naturally occurring protein component of the food. The same food or food component would be safe and nutritious for the vast majority of consumers.

True food allergies can occur through two different immunologic mechanisms, the antibody-mediated mechanism and the cellular mechanism. The most common type of food allergies involve mediation by immunoglobulin E, or IgE.

IGE-MEDIATED FOOD ALLERGIES

In IgE-mediated food allergies, allergen-specific IgE antibodies are produced in the body in response to exposure to a food allergen, usually a protein. These IgE antibodies are highly specific and will recognize only a specific portion of the protein that they are directed against. Occasionally, IgE antibodies produced against one particular protein in a specific food will confer sensitivity to another food either because the food is closely related or because it shares a common segment with the allergenic protein. Some food proteins are more likely to elicit IgE antibody formation than others. Although exposure to the food is critical to the development of allergen-specific IgE, exposure will not invariably result in the development of IgE antibodies even among susceptible people. Many factors, including the susceptibility of the individual; the immunogenic nature of the food and its constituent proteins; the age of exposure; and the dose, duration, and frequency of exposure, are likely to influence the formation of allergen-specific IgE antibodies.

Allergen-specific IgE antibodies are produced by plasma cells and attach themselves to the outer membrane surfaces of two types of specialized cells: mast cells, which are found in many different tissues, and basophils, which are found in the blood. In this so-called sensitization process, the mast cells and basophils become sensitized and ready to respond to subsequent exposure to that specific food allergen. However, the sensitization process itself does not result in any symptoms. No adverse reactions will occur without subsequent exposure to the specific allergenic protein or some closely related protein. Sensitization to a particular allergen distinguishes allergic individuals from nonallergic individuals.

Once the mast cells and basophils are sensitized, subsequent exposure to the allergen results in the allergen cross-linking two IgE molecules on the surface of the mast cell or basophil membrane. This interaction between the allergen and the allergen-specific IgE triggers the release of a host of mediators of allergic disease, which are either stored or formed by the mast cells and basophils. Several dozen different mediators have been identified, including histamine, prostaglandins, and leukotrienes. Histamine is one of the primary mediators of IgE-mediated allergies. Histamine is responsible for many of the early symptoms associated with allergies. Many of the other mediators are involved in the development of inflammation. The interaction of a small amount of allergen with the allergen-specific IgE antibodies results in the immediate release of comparatively large quantities of the various mediators into the bloodstream and tissues. Thus, exposure to extremely small amounts of allergens can elicit symptoms. This mechanism of IgE-mediated reactions is involved in many different types of allergies to foods, pollens, mold spores, animal danders, bee venom, and pharmaceuticals. Only the source of the allergen is different.

IgE-mediated food allergies are sometimes called immediate hypersensitivity reactions because of the short onset time (a few minutes to a few hours) between the ingestion of the offending food and the onset of symptoms. Since the mediators released from the mast cells and basophils can interact with receptors in a number of different tissues in the body, a rather wide variety of symptoms can be associated with IgE-mediated food allergies (Table 1). The most common symptoms associated with food allergies are those involving the skin and the gastrointestinal tract. Respiratory symptoms are less frequently involved with food allergies than with various inhalant allergies such as pollen and animal dander allergies. However, asthma is a very serious, though uncommon, respiratory manifestation of food allergies. Fortunately, most food-allergic individuals suffer from only a few of the many possible symptoms.

Most of the symptoms of IgE-mediated food allergies are not particularly definitive, which can make clinical diagnosis rather difficult. For example, the gastrointestinal manifestations of food allergies can also be associated with many other foodborne illnesses and a variety of other diseases as well. Additionally, there are millions of asthmatics, but only a few are allergic to foods.

Anaphylactic shock is, by far, the most serious manifestation of food allergies. Anaphylactic shock involves gastrointestinal, cutaneous, and respiratory symptoms in combination with a dramatic fall in blood pressure and cardiovascular complications. Death can ensue within minutes of the onset of anaphylactic shock. Fortunately, very few individuals with food allergies are susceptible to such severe reactions after the ingestion of the offending food.

The severity of an allergic reaction will depend to some extent on the amount of the offending food that is ingested. Severe reactions are more likely to occur when an allergic individual inadvertently ingests a large amount of the offending food, especially if that individual happens to be

Table 1. Symptoms of IgE-Mediated Food Allergies

Gastrointestinal symptoms	Cutaneous symptoms
Vomiting	Urticaria (hives)
Diarrhea	Dermatitis
Nausea	Angioedema
Respiratory symptoms	Other symptoms
Rhinitis	Anaphylactic shock
Asthma	Laryngeal edema

exquisitely sensitive. However, exposure to even trace quantities can elicit noticeable reactions due to the large release of mediators.

The most common allergenic foods are peanuts, tree nuts (almonds, walnuts, pecans, cashews, etc), soybeans, cows' milk, eggs, fish, crustacea (shrimp, crab, lobster, etc), and wheat. Peanut allergy is the most common food allergy, especially in the United States where peanuts are a popular dietary item and peanut butter is introduced at an early age. Throughout the world, cows' milk allergy is the most common food allergy among infants due to the widespread ingestion of milk during the first months of life. Any food that contains protein has the potential to elicit an allergic reaction in someone. The most common allergenic foods tend to be foods with high protein content that are frequently consumed. The exceptions are beef, pork, chicken, and turkey, which are uncommonly allergenic despite their frequent consumption and high protein content.

The prevalence of IgE-mediated food allergies is not precisely known. The overall prevalence of food allergies in the developed countries of the world ranges from 4 to 8% in infants to perhaps 1% in adults. Thus, many infants and young children outgrow their IgE-mediated food allergies. The reasons for the development of tolerance to previously allergenic foods are not understood but may involve the development of blocking antibodies of other types, especially IgG and IgA. Allergies to some foods, such as cows' milk and eggs, are more frequently outgrown than allergies to other foods, such as peanuts.

The diagnosis of food allergies is typically approached in a stepwise fashion. The diagnosis of food allergies by an allergist is often critical because parental diagnosis and self-diagnosis are often incorrect, leading to identification of the wrong incorrect foods or the identification of too many foods as allergens. Most individuals with IgE-mediated food allergies are allergic to one or two foods; only on rare occasions do coexistent allergies occur to more than three foods. Thus, the goal of medical diagnosis is to establish a cause-and-effect relationship between one or a few foods and the onset of allergic symptoms. Most physicians begin the diagnosis by taking a careful history of the patient's adverse reactions taking note of the foods eaten immediately before the onset of symptoms, the amount of various foods consumed, the type, severity, and consistency of symptoms, and the time intervals between eating and the onset of symptoms. Sometimes histories are needed from several episodes to reach a probable diagnosis. Challenge tests with the suspected food(s) can be used to establish with certainty the role of a specific food in the reaction. The double-blind, placebo-controlled food challenge (DBPCFC) is considered the most reliable procedure. In the DBPCFC, neither the patient nor the medical personnel know when the food (in capsules or disguised in another food or beverage) is going to be administered and when the placebo is to be administered. Thus, the DBPCFC is free of bias. Single-blind and open challenge tests also have value in some situations. History alone can be sufficient to make the diagnosis in some situations if the cause-and-effect relationship is particularly compelling. Challenge tests are seldom used on individuals who experience life-threatening allergic reactions for rather obvious reasons.

Once the adverse reaction has been clearly linked to the ingestion of a specific food, an assessment of the possible role of IgE must be conducted to determine if the adverse reaction has an allergic mechanism. The diagnosis of an IgE-mediated mechanism can be made with either the skin prick test (SPT) or radioallergosorbent test (RAST). The SPT is the simpler of the two procedures. In the SPT, a small amount of a food extract is applied to the patient's skin, the site is pricked with a needle to allow entry of the allergen, and the site is observed for the development of a wheal-and-flare (basically a hive) response. A wheal-and-flare response at the skin prick site demonstrates that IgE affixed to skin mast cells has reacted with some protein in the food extract releasing histamine into the surrounding tissue and resulting in the formation of the hive. The RAST is an alternative procedure that uses a small sample of the patient's blood serum. In the RAST, the binding of serum IgE to food protein bound to some solid matrix is assessed using radiolabeled or enzyme-linked antihuman IgE. While the RAST is more expensive than the SPT, it is equally reliable and can be conducted in a specialized laboratory in the absence of the patient. The RAST is preferred for patients with extreme sensitivities because of the risk associated with severe reactions to the SPT. It should be emphasized that a positive SPT or RAST in the absence of a history of allergic reactions to that particular food is probably meaningless. The SPT and RAST are the most frequently used and reliable tests to assess the role of IgE in an adverse food reaction.

The specific avoidance diet is the primary means of treatment for IgE-mediated food allergies. For example, if allergic to peanuts, don't eat peanuts. With IgE-mediated food allergies, very low amounts of the offending food can be tolerated by most allergic individuals. Thus, the construction of a safe and effective avoidance diet can be quite difficult. Food-allergic patients must have considerable knowledge of food composition. For example, casein, whey, and lactose are common food ingredients that are derived from cows' milk. These milk ingredients would likely be hazardous for milk-allergic individuals. The ingredient must contain the specific allergenic protein to be hazardous to the allergic consumer. For example, peanut oil and soybean oil, despite being derived from allergenic sources, do not contain protein and would not be hazardous for peanut-allergic or soy-allergic individuals unless the oils had become contaminated during use. The careful reading and complete understanding of food labels is critical to the implementation of a safe and effective avoidance diets. Of course, the manufacturers of packaged foods have the responsibility to ensure that the label statements on packages are accurate. Occasionally, errors are made by food processors that result in the presence of undeclared residues of allergenic foods in a packaged food. The contamination of one food with another from the use of shared food processing equipment is one of the most common errors occurring in food manufacturing. However, restaurant and other food service meals can present an even bigger challenge for food-allergic individuals. Residues of allergenic foods can arise from the use of shared food preparation equipment (utensils, cooking surfaces, pots and pans, etc). Additionally, the accurate identification of all of the ingredients in food service and restaurant meals can sometimes be quite difficult, and such foods are not labeled. As a result, many inadvertent exposures occur among allergic consumers who are attempting to avoid their offending food(s).

Cross-reactions are another perplexing issue for food-allergic consumers as they attempt to develop effective avoidance diets. Cross-reactions can occur but do not inevitably occur between closely related foods. For example, many individuals are allergic to peanuts, but most of these individuals are not allergic to other legumes such as soybeans, peas, green beans, and so on. A few of these individuals are cross-reactive with one or more other legumes. Alternatively, cross-reactions frequently occur among the various crustacea (shrimp, crab, lobster, and crayfish). Cows' milk and goats' milk invariably cross-react as do the eggs of various avian species. Cross-reactions can also occur between foods and other environmental allergens. The most common examples are the cross-reactions that occur between some fresh fruits and vegetables and certain pollen allergies in some individuals and the cross-reaction that occurs in a few individuals with allergies to natural rubber latex with several foods, including bananas and kiwis. The basis for such cross-reactions is frequently not known.

Infants born to parents with histories of allergic disease are much more likely than other infants to develop food allergies. Prevention of the development of food allergies in such infants is quite difficult. The avoidance of commonly allergenic foods such as cows' milk, eggs, and peanuts primarily through breast feeding appears to delay but not prevent the development of food allergies.

A few specialized hypoallergenic foods are available in the marketplace. These foods are intended for infants who have developed allergies to infant formula made with cows' milk. The most effective hypoallergenic infant formulae are based on extensively hydrolyzed casein. Although casein is a common milk protein and a major milk allergen, the hydrolysis of its peptide bonds renders it safe for cows' milk–allergic infants.

Other approaches to the treatment of food allergies are considered controversial. Immunotherapy (eg, allergy shots), sublingual food drops, and the use of rotation diets would be examples of such controversial approaches.

Pharmacological approaches can be used to treat the symptoms of allergic reactions. In particular, epinephrine (also known as adrenalin) is prescribed for individuals who experience life-threatening food allergies. The early administration of epinephrine after inadvertent exposure to the offending food can be life-saving for such patients. Antihistamines can also be used to treat the less-serious symptoms of food allergies.

CELL-MEDIATED FOOD ALLERGIES

Cell-mediated food allergies are sometimes called delayed hypersensitivity reactions, because the symptoms of these reactions typically appear 6 to 24 hours after consumption of the offending food. Cell-mediated allergies involve the interaction of food allergens with sensitized lymphocytes, usually in the intestinal tract. These reactions occur without the involvement of IgE or other antibodies. The interaction between the allergen and the sensitized lymphocyte results in lymphokine production and release, lymphocyte proliferation, and the generation of cytotoxic T lymphocytes. Lymphokines are soluble proteins that exert profound effects on tissues and cells resulting in localized inflammation. Lymphocyte proliferation increases the number of reactive cells thus magnifying the inflammatory process. The generation of cytotoxic or killer T cells results in the destruction of other intestinal cells including the critical absorptive epithelial cells.

The T lymphocytes responsible for cell-mediated allergies abound in the gut-associated lymphoid tissue. Intestinal T lymphocytes are likely to be very critical in food-related, delayed hypersensitivity. However, the inaccessibility of these lymphocytes has hampered experimental studies of the role of cell-mediated reactions in food allergies. As a result, the prevalence and importance of cell-mediated food allergies remains unknown.

Celiac disease, also known as gluten-sensitive enteropathy, is the one illness that seems likely to involve a cell-mediated mechanism. Celiac disease occurs in certain individuals following the ingestion of wheat, rye, barley, triticale, and perhaps oats. Although the mechanism of celiac disease is not completely understood, an immunocytotoxic reaction mediated by intestinal lymphocytes is probable. On ingestion of proteins from the offending grains, the absorptive cells of the small intestinal epithelium are damaged and the absorptive function of the small intestine is severely compromised, resulting in a malabsorption syndrome. The symptoms of celiac disease include diarrhea, bloating, weight loss, anemia from inadequate iron absorption, bone pain from impaired calcium absorption, chronic fatigue, weakness, muscle cramps, and, in children, failure to gain weight and growth retardation. The prevalence of celiac disease in the U.S. is not precisely known but is thought to be about 1 in every 2000 individuals. Celiac disease appears to occur more frequently in Europe and Australia. Celiac disease rarely occurs in individuals of Chinese or African heritage. Celiac disease may manifest at any age. Environmental factors, such as viral illness, may possibly contribute to the onset of celiac disease in some cases.

Celiac disease is triggered by the ingestion of the protein fractions, specifically the gliadin proteins, of wheat and related grains. Other grains, such as corn and rice, do not contain similar gliadin proteins. As with IgE-mediated food allergies, most consumers do not react adversely to the ingestion of these particular grain proteins. In susceptible individuals, a particular segment of the gliadin protein interacts with the sensitized T lymphocytes to elicit a cell-mediated immune response.

The most definitive diagnosis of celiac disease requires taking a small bowel biopsy. The biopsy material is examined for evidence of flattened intestinal villi, a feature that is characteristic of the disease. A normal appearance of the biopsy material is restored after avoidance of wheat and related grains. Alternatively, a blood sample from the patient can be examined for the presence of antiendomysial antibodies, which are elevated in celiac patients. Because of the invasiveness of the biopsy procedure, the diagnosis is often made tentatively on the basis of symptomatic improvement after avoidance of wheat and related grains.

Celiac disease is also treated with thorough implementation of an avoidance diet. In the case of celiac disease, the total avoidance of wheat, rye, barley, triticale, and perhaps oats is usually advocated. Some, but not all, patients can tolerate oats. Ingredients derived from wheat, rye, barley, and oats that contain protein must also be avoided. The tolerance of celiac sufferers for these grains is not precisely known, but it is thought that small amounts of these foods can provoke reactions.

CONCLUSION

Food allergies affect a small percentage of the population. The reactions can range in severity from mild to life-threatening. Food allergies are caused primarily by naturally occurring substances in foods. Food allergies can be triggered by ingestion of very small amounts of the offending food.

GENERAL REFERENCES

J. Brostoff and S. J. Challacombe, eds., *Food Allergy and Intolerance*, Bailliere Tindall, London, United Kingdom, 1987.

P. J. Lemke and S. L. Taylor, "Allergic Reactions and Food Intolerance" in F. N. Kotsonis, M. Mackey, and J. Hjelle, eds., *Nutritional Toxicology*, Raven Press, New York, pp. 117–137.

D. D. Metcalfe, H. A. Sampson, and R. A. Simon, eds., *Food Allergy-Adverse Reactions to Foods and Food Additives*, Blackwell Scientific Publications, Boston, Mass., 1991.

J. E. Perkin, ed. *Food Allergies and Adverse Reactions*, Aspen Publishers, Gaithersburg, Md., 1990

W. Strober, "Gluten-Sensitive Enteropathy: A Nonallergic Immune Hypersensitivity of the Gastrointestinal Tract," *J. Allergy Clin. Immunol.* **78**, 202–211 (1986).

R. S. Zeiger and S. Heller, "The Development and Prediction of Atopy in High-Risk Children: Follow-up at Age Seven Years in a Prospective Randomized Study of Combined Maternal and Infant Food Allergen Avoidance," *J. Allergy Clin. Immunol.* **95**, 1179–1190 (1995).

STEVE L. TAYLOR
SUSAN L. HEFLE
University of Nebraska
Lincoln, Nebraska

FOOD ANALYSIS

Food scientists analyze foods to obtain information about composition, appearance, texture, flavor, shelf life, safety, processibility, and microstructure (1–4). Nevertheless, the term food analysis is often reserved for determination of food composition, and so this will be the emphasis of this article. Knowledge of the composition of foods is important for several reasons, including compliance with legal standards, quality assurance, determination of nutritional value, detection of adulteration, safety, processing, and research and development (2,3). An understanding of the methods of food analysis is important to food scientists working in academia, industry, and government.

The diversity and complexity of food materials mean that a large number of different analytical techniques have been developed to analyze their properties. These techniques can be conveniently divided into either classical methods (such as gravimetry, titration, distillation, and solvent extraction) or instrumental methods (such as spectroscopy, chromatography, and electrophoresis).

INFORMATION SOURCES

Food analysts often have to identify the various types of analytical methods available for determining the concentration of a specific component and then select the one that is most suitable for their particular application. A knowledge of the various sources where information about analytical methods is available is therefore extremely important to the food analyst.

Books

A number of textbooks and reference books have been written on the subject of food analysis (1–8). These books cover topics such as sampling, data analysis, principles of analytical techniques, and procedures for analyzing specific food components (eg, water, fat, protein, carbohydrates, and minerals). This type of book usually provides a general overview of analytical methods, rather than the specific details needed to actually carry out an analysis.

A variety of other types of book contain information that is also useful to food analysts (eg, monographs, conference proceedings, encyclopedias, and handbooks). Monographs are books that deal with a particular specialized subject area, such as spectroscopy or chromatography. Conference proceedings contain papers that were presented at a scientific or technical meeting on a particular topic and are usually published by the sponsoring organization, for example, the American Chemical Society. Encyclopedias provide information about a wide range of different analytical techniques, for example, *Kirk-Othmer Encyclopedia of Chemical Technology* (9). Some handbooks contain information about the physicochemical properties of materials (molecular weight, structure, density, refractive index, solubility, specific heat capacity), which are useful for the identification and determination of specific food components (8,10–12).

Official Methods

In many circumstances it is important for a food analyst to use a standard analytical method that has been approved by a professional association, such as the Association of Official Analytical Chemists, the American Association of Cereal Chemists, or the American Oil Chemists Society (13–15). These methods are compiled in volumes that are periodically updated as the methods are improved or as new methods are accepted. Standard methods have been developed after years of collaborative testing and are considered reliable and official.

Periodicals

Analytical methods developed by other scientists are often reported in scientific journals. Useful information about analytical methods may be obtained from food science journals (eg, *Food Chemistry, Food Microstructure, Food Testing and Analysis, Journal of Food Science*) or from journals published in other areas (eg, *Analytical Chemistry, Journal of the Chemical Society, Applied Spectroscopy*). For a detailed overview of many of the useful periodicals for food analysts see chapter 1 of Pomeranz and Meloan (1).

Reviews

Critical reviews are usually written by one or more experts in the field and are intended to offer a comprehensive survey of the current state of the knowledge on a specific topic. A critical review may be written about the application of a specific analytical instrument, recent developments in instrumentation, or the determination of a specific component. Critical reviews are usually published in scientific journals or book series (eg, *Advances in Analytical Chemistry and Instrumentation, Advances in Food Research, Critical Reviews in Food and Nutrition*).

Theses

Details about analytical methods can often be located in master's and doctoral theses. Since 1938 theses from U.S. universities have been processed for microfilming by University Microfilms of Ann Arbor, Michigan. Abstracts of up to 600 words of these theses are published in *Dissertation Abstracts*. An annual list of U.S. master's theses in the pure and applied sciences has been published since 1955–1956 by the Thermphysical Properties Research Center, Purdue University, Lafayette, Indiana.

Modern Information Retrieval Systems

During the past few decades, computerized systems for storing and retrieving scientific information have developed rapidly. Many of these can be accessed from a computer terminal in a library or via the Internet. An awareness of the systems available and their proper use can significantly reduce the time and effort involved in a literature search on a particular subject.

Several databases of particular interest to food analysts are available: AGRICOLA (USDA, National Agricultural Library, Beltsville, Md.), BIOSIS PREVIEWS (Biosciences Information Services, Philadelphia, Pa.), CA Search (Chemical Abstracts Service, Columbus, Ohio), CAB Abstracts (Commonwealth Agricultural Bureau, Slough, U.K.), CRIS (USDA, Washington, D.C.), Dissertation Abstracts Online (University Microfilms International, Ann Arbor, Mich.), Science Citation Index (Institute for Scientific Information, Philadelphia, Penn.), and Food Science and Technology Abstracts (International Food Information Service, Reading, U.K.).

Trade Publications

Many companies that manufacture chemicals or analytical instruments produce publications that are valuable sources of information for food analysts. These include bibliographies and abstracts of technical/scientific articles in a specific area, or detailed handbooks giving specifications, properties, and details of analytical procedures. The instruction manuals normally provided by companies with the instrumentation they sell are indispensable for installing, using, and servicing equipment. Several scientific and trade journals periodically prepare lists of major commercial providers of chemicals and instrumentation (eg, *Science, Prepared Foods, and Laboratory Equipment Directory*).

SAMPLING

Sample selection, preparation, and labeling are extremely important aspects of any food analysis procedure, which can lead to large errors if not carried out correctly. Ideally, the sample analyzed in a laboratory should have exactly the same properties as the total population it is supposed to represent. To achieve this a food analyst would have to analyze every sample, which is rarely possible because many analytical techniques are either destructive, time-consuming, expensive, or labor intensive. The analyst must therefore select a limited number of samples from the total population using a sampling plan that will ensure that their properties are a good representation of the true value. A sampling plan is a set of rules that an analyst uses to decide the number of samples of a given population to test, the location from which the samples should be selected, and the method used to collect them. The choice of a particular sampling plan depends on the purpose of the analysis, the physical property to be measured, the nature of the total population and of the individual samples, and the type of analytical technique used to characterize the samples. For certain products and types of populations, sampling plans have already been developed and documented by various organizations that authorize official methods.

Once a representative sample has been selected, it must be prepared for analysis in the laboratory. This step must be done very carefully in order to make accurate and precise measurements. Typical preparation steps involve: making the sample homogeneous, reducing the sample to a manageable size, and preventing sample deterioration (eg, due to evaporation, enzyme activity, lipid oxidation, or microbial growth). It is important to carefully label samples with information such as the nature of the sample, the place it was selected from, the date it was taken, and the person who collected and prepared it. It is also important to report the results of any analysis in a clear fashion, stating the procedures used, the number of replications performed, and the estimated reliability of the measured value.

SELECTION OF AN ANALYTICAL METHOD

A food analyst often has a large number of different analytical procedures that can be used to analyze a particular food product. Selection of the most suitable procedure is often the key to the success of an analysis. Some of the most important factors that should be considered are as follows (4):

1. *Precision.* A measure of the ability to reproduce an answer between determinations performed by the same scientist (or group of scientists) using the same equipment and experimental approach.
2. *Reproducibility.* A measure of the ability to reproduce an answer by scientists using the same experimental approach but in different laboratories using different equipment.
3. *Accuracy.* A measure of how close one can actually measure the true value of the parameter being measured.
4. *Simplicity of operation.* A measure of the ease with which the analysis may be carried out by relatively unskilled workers.
5. *Economy.* The total cost of the analysis, including the reagents, instrumentation, and time.
6. *Speed.* The time needed to complete the analysis.
7. *Sensitivity.* A measure of the lowest concentration of material that can be detected or quantified by a given technique.
8. *Specificity.* A measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components.
9. *Safety.* A measure of the potential hazards associated with reagents and procedures used in the analysis.
10. *Destructive/nondestructive.* Whether the sample is destroyed during the analysis or remains intact.
11. *Official approval.* Various international bodies have given official approval to methods that have been comprehensively studied by independent analysts and shown to be acceptable to the various organizations involved, for example, AOAC or AOCS.

ANALYTICAL METHODS

It is convenient to classify analytical methods as being either classical or instrumental (15). Classical methods include gravimetry, titration, distillation, evaporation, and solvent extraction. Instrumental methods include various spectroscopic (infrared, visible, ultraviolet, nuclear magnetic resonance, mass spectroscopy), chromatographic (thin-layer chromatography, gas chromatography, high-performance liquid chromatography), electrical (potentiometry, conductiometry, electrophoresis), and optical (polarimetry and refractive index) methods.

Classical Methods

The classical methods of food analysis were developed more than a century ago to give reliable and reproducible results using chemicals and equipment that were readily available in most laboratories (eg, balances, glassware, ovens, heaters). Typically, classical methods involve procedures such as weighing, volume determination, titration, filtering, evaporation, distillation, and solvent extraction. Although these classical methods are still widely used today, they are often being replaced by more convenient instrumental methods.

Instrumental Methods

A variety of instrumental methods have been developed to analyze the properties of food materials (1–5). These can be categorized according to the physical principles on which they are based.

Spectroscopy. Many instrumental methods utilize interactions between some form of radiation (electromagnetic, acoustic, electron beam, or neutron beam) and matter to obtain information about the properties of foods (1,2,3,16). Radiation beams may interact with materials in a variety of ways, including absorption, emission, reflection, refraction, scattering, and polarization. Instrumental methods based on each of these interactions are utilized in food analysis, although those based on absorption and emission tend to be the most common.

Atomic spectroscopy utilizes electromagnetic radiation in the ultraviolet and visible region and relies on the transition of outer-shell electrons between different energy levels. In atomic emission spectroscopy (AES), a sample is heated to atomize it and to excite the electrons to a higher energy state. The emission of radiation by the excited atoms is then measured using suitable detectors. In atomic absorption spectroscopy (AAS), the absorption of radiation when a beam of electromagnetic radiation is passed through an atomized sample is recorded. Each element has its own unique set of energy levels and so the wavelengths of emitted or absorbed radiation can be used to identify it, whereas the extent of the absorption/emission can be used to quantify it. Atomic spectroscopy is principally used to determine the concentration of mineral elements in foods.

UV-visible absorption spectrophotometry is one of the most common types of spectroscopic technique used by food analysts. Like atomic spectroscopy, it relies on transitions of outer-shell electrons. A beam of radiation is transmitted through the sample, and the reduction in its amplitude due to absorption is measured. By carrying out measurements over a range of wavelengths a spectra is obtained that contains peaks corresponding to the absorbing groups. The concentration of a substance can be determined by measuring the height of one of these absorption peaks (provided there is no interference from other molecules). A number of more sensitive and specific methods based on luminescence are available for materials that fluoresce or phosphoresce.

Infrared (IR) techniques rely on electromagnetic radiation absorbed or emitted by a sample due to the vibration or rotation of molecules. The interaction between infrared radiation and a sample leads to a spectra that contains peaks that correspond to different types of chemical groups. The location and magnitude of a peak provides information about the type and concentration of components present. IR techniques are available to simultaneously determine moisture, protein, lipids, and carbohydrates in foods.

Nuclear magnetic resonance (NMR) techniques rely on transitions of nuclei between different energy levels when they are placed in a static magnetic field. Only nuclei that have a property referred to as spin can be detected by NMR, the most common being ^{1}H. NMR is an extremely powerful analytical technique that can be used to determine concentrations, molecular structures, phase transitions, and images.

A number of analytical instruments have been developed to provide information about the composition or structure of food materials that utilize the absorption, diffraction, emission or scattering of X rays.

Optical Methods. Analytical techniques that utilize the rotation of monochromatic light (polarimeters) or the refractive index (refractometers) of a material are frequently used to determine the concentration of components in liquids. These techniques are simple to use and provide rapid measurements, so they are often used for quality control purposes.

Chromatography. Chromatography is a term used to designate a variety of analytical techniques that can separate mixtures of molecules into different fractions. It is mainly used in the food industry for two purposes: (*1*) to purify materials, and (*2*) to determine the types and amounts of specific substances present. Chromatography involves passing a solution of molecules (usually in a gas or liquid carrier) through a matrix. The carrier is called the mobile phase because it moves through the matrix, whereas the matrix is called the stationary phase because it remains stationary. Molecules are separated according to their interaction with the matrix: the stronger the interaction, the slower they move. The strength of the interaction depends on the physicochemical properties of the substance and the matrix. Molecules can be separated according to their size, polarity, electrical charge or molecular interactions. It is convenient to divide chromatographic methods into different categories according to the physicochemical basis of the separation (Table 1).

Table 1. Physicochemical Basis of Separation in Chromatographic Techniques

Basis	Physical principle	Examples
Molecular size	Differences in the size and shape of molecules	Gel filtration, size exclusion
Polarity	Differences in partition coefficient between polar and nonpolar solvent	Partition
Electrical charge	Differences in electrostatic interaction between charged groups on molecules in the mobile phase and the matrix	Ion-exchange
Molecular affinity	Specific interactions between molecules in the mobile phase and the matrix	Affinity

Chromatographic separations can be carried out using a variety of different experimental arrangements. In paper chromatography the sample is spotted on to the bottom of a sheet of paper. The paper is usually impregnated with a liquid, that acts as the stationary phase. The bottom of the paper is placed in a vessel that contains a solvent. The solvent acts as the mobile phase because it carries the sample with it as it moves up the paper due to capillary forces. The molecules are separated according to the strength of their interaction with the stationary phase: the stronger the interaction with the stationary phase, the slower they move. More-sophisticated separations can be carried out using two-dimensional paper chromatography.

Thin-layer chromatography (TLC) has largely replaced paper chromatography because it is quicker, more sensitive, has greater resolution, and has better reproducibility. The general principles of TLC are similar to paper chromatography, except that the paper is replaced by a glass plate coated with a thin layer of porous material that acts as the stationary phase.

Column Chromatography. Originally, column chromatographic techniques used glass columns with either gravity or a slight vacuum to move the mobile phase through them. Steel columns and high pressures are now widely used to speed up this process. In high-pressure (or performance) liquid chromatography (HPLC) the sample to be analyzed is dissolved in a liquid mobile phase and passed through a column containing the stationary phase (which may be either solid or liquid). In gas chromatography the sample is usually volatilized and then carried by a gaseous mobile phase through a thin tube that is coated with the stationary phase. The efficiency of the separation can be manipulated by carefully selecting the most appropriate combination of stationary and mobile phases.

Electrical Techniques. A number of analytical instruments utilize measurements of the electrical properties of foods to obtain information about their composition. Potentiometric methods rely on measurements of the potential difference between an indicator electrode and a reference electrode. The magnitude of the potential difference is related to the concentration of a specific component within a solution. Coulometric methods are based on the measurement of the electrical charge required to completely electrolyze the substance being analyzed: the greater the electrical charge, the higher the concentration of the substance. The composition of some foods can be determined by measuring their electrical conductivity. The current passing between a pair of electrodes is measured and then related to the composition of a specific component within the food.

Electrophoresis relies on differences in the migration of charged macromolecules or colloidal particles in a solution when an electrical field is applied across it. It is used to separate molecules and particles on the basis of their size, shape, or charge. A buffered solution containing the substance to be analyzed is poured onto a porous matrix (usually a strip of paper or a gel) and a voltage is applied across it. The charged substance moves through the gel in a direction that depends on the sign of their electrical charge, and at a rate that depends on the magnitude of the charge and the friction to their movement. The friction of a substance is a measure of its resistance to movement through the matrix and is largely determined by the relationship between the effective size of the substance and the size of the pores in the matrix. The smaller the size of the substance, or the larger the size of the pores in the matrix, the lower the resistance and therefore the faster the substance moves through the matrix. Matrices with different porosities can be purchased from chemical suppliers, or made up in the laboratory, so that substances with different sizes can be analyzed. Electrophoresis techniques can be carried out in two dimensions to improve resolution. Substances are separated in one direction on the basis of their size, and then in a perpendicular direction on the basis of their charge.

Biochemical Analysis. A number of biochemical techniques are available that can be used to determine the concentration or identity of specific food components. Enzymic techniques utilize specific enzyme reactions, whereas immunological techniques utilize specific interactions between an antigen and an antibody. Biochemical assays can be purchased commercially for many important food components. These kits contain the chemicals and instructions required to carry out the analysis and are usually easy to use, rapid, extremely sensitive, and specific.

Because some of the nutritional requirements of microorganisms and experimental animals are similar, it is possible to employ analytical microbiology to determine some substances that are essential constituents of living cells. Microorganisms, as reagents, have been used to determine amino acids, vitamins, nucleic acids, heavy metals, growth factors, nutritional value of proteins, and antibodies. The basic principle is that in the presence of limiting amounts of certain compounds, the amount of microbial growth is a function of the amounts of the compounds. The microorganisms used for assay are primarily bacteria, but yeasts, fungi, and protozoans also have been used. The assay methods include diffusion in a gel, turbidimetric and dilution methods, gravimetric methods, and metabolic response methods.

MAJOR FOOD COMPONENTS

A knowledge of the concentration of the major components present in a food material are important to food scientists for a variety of reasons, including legal requirements, labeling, quality assurance, processing, food safety, nutrition, and so on. In this section, methods for analyzing the major components in foods are reviewed: moisture, carbohydrates, protein, lipids, and minerals. The analytical techniques described in the previous section often form part of these methods.

Moisture

Moisture determination is one of the most important and widely used measurements in the processing and testing of foods. The moisture content ($M\% = 100m_{\text{water}}/m_{\text{sample}}$), where m_{water} is the mass of the water and m_{sample} is the mass of the whole food) is simply related to the total solids ($T\% = 100 - M\%$).

The thermal drying method relies on the evaporation of water from a sample and is one of the most commonly used procedures for determining moisture content (eg, conventional oven, forced air oven, vacuum oven, microwave oven, and infrared oven). The mass of a sample is weighed before and after it is dried, and the moisture content is then calculated. Care must be taken to ensure that the water is removed in a consistent fashion and that there are no interferences from chemical degradation or evaporation of other substances. In distillation methods, the food sample is mixed with an organic solvent and then placed in a specially designed glass vessel with a collection arm. The glass vessel is heated to evaporate the water, which is collected in the graduated collection arm, where its volume is determined. The most commonly used chemical methods for moisture determination are the Karl Fischer titration and the gas production method. These methods rely on specific chemical reactions between water and another substance that lead to some quantifiable parameter. Several instrumental methods based on differences in the physical properties of water compared with the other components are widely used to determine moisture content, including infrared, gas chromatography, NMR, and several electrical methods. These instruments are simple to use and provide rapid and reliable measurements and are therefore particularly suitable for routine quality control applications.

Carbohydrates

Carbohydrates are the most abundant and widely distributed food components. They include mono-, di-, oligo- and polysaccharides (17). The latter can be divided into structural polysaccharides (cellulose, hemicellulose, lignin) and nutrient polysaccharides (starch, glycogen). Assay methods of mono-, di- and oligosaccharides include chemical, colorimetric, chromatographic, electrophoretic, optical, and biochemical techniques. Many procedures involve preliminary separation by chromatographic or electrophoretic techniques prior to quantification by classical chemical or optical assays. The use of enzymatic assays is gaining popularity because of their high specificity, sensitivity, and ease of use.

Starch can be extracted from foods using perchloric acid or calcium chloride and precipitated with iodine, and the starch liberated from the complex can be determined colorimetrically.

The determination of structural polysaccharides is fraught with many difficulties because the materials contain many undefined polymers, varying in size and composition. In food composition tables, carbohydrates are often reported as the difference after subtracting from 100% the moisture, protein, oil, and ash content. In some cases, fiber (crude, total, and dietary) is determined separately.

Protein

Methods of determining the total protein content of foods (crude protein) are empirical in nature. Proteins are polymers of varying length and molecular structure that may contain up to 24 different kinds of amino acid (17). Amino acids can be determined, after hydrolysis of proteins, by colorimetric, enzymatic, microbiological, and chromatographic methods. The most powerful methods of amino acid analysis in protein hydrolysates are based on ion-exchange and gas chromatography.

The most common procedures for determination of crude protein rely on a measurement of a specific element or chemical group in the proteins. The protein content is then calculated using an experimentally established calibration factor. For example, the approximate protein content of a food can be estimated by measuring the nitrogen content and then multiplying by 6.25 (because on average 16% of a protein's mass is nitrogen). The elements or groups most commonly used are nitrogen, aromatic amino acids, or the peptide linkage. It is assumed that the constituent determined is present entirely in the protein fraction and that the empirical conversion factor is a constant. Determination of the protein content based on the nitrogen content can be made by the Kjeldahl method (based on digestion, neutralization, and titration) or the Dumas method (based on pyrolysis). A variety of spectrophotometric methods that rely on the presence of specific groups are also available for determining the protein content. These include the biuret method (formation of a purple complex between copper salts in alkali solutions and compounds containing at least two peptide bonds), the Lowry method (color formed after interaction of proteins with a phenol reagent and copper under alkaline conditions), direct method (measurement of absorbance at 280 nm, 210 nm, or two wavelengths), turbidimetric methods (based on scattering of light from protein aggregates formed when a certain reagent is added), and dye-binding methods (add excess dye to protein solution and measure unbound dye). A variety of other instrumental methods can also be used to determine protein content, the most popular being NMR and IR.

The concentrations of specific types of proteins in foods can be determined using liquid chromatography or electrophoretic techniques. The proteins are first separated on the basis of their molecular weight or electrical charge and are then identified by comparison with standards of known properties.

Lipids

The most common methods of lipid analysis are based on their sparing solubility in water and their considerable solubility in organic solvents. Successful lipid analysis often requires that covalent bonds between lipids and other constituents (such as proteins or carbohydrates) are broken before the solvent extraction is carried out, so that the lipids are free to be solubilized. The efficiency of extraction is usually greatest when the polarities of the lipid and solvent are similar. Ethyl ether and petroleum ether are two of the most common extraction solvents. Combinations of alcohol and ether, ternary mixtures of chloroform–methanol–water, and water-saturated butanol are other examples of effective solvents. Solvent extraction techniques may be batch (eg, an extraction funnel), semicontinuous (eg, Soxhlet), or continuous (eg, Goldfish). The fat content of milk and other dairy products is often determined by nonsolvent extraction techniques, such as the Babcock, Gerber, or detergent methods. Numerous instrumental methods are available for determining the lipid content of certain foods, including IR, NMR, densitometry, X-ray absorption, and dielectric measurements. The identity of the molecules in the lipid phase can be determined by chromatography, mass spectrometry, or NMR.

The processing and quality of food oils are often determined by physical characteristics such as their melting point, smoke point, flash point, fire point, solid fat content, and degree of oxidation. Consequently, analytical methods have been developed to characterize these quantities.

Mineral Components

Ash is the inorganic residue remaining after the incineration of organic matter. It is a measure of the total mineral components in a food. Depending on the nature of the mineral components, various ashing procedures can be employed, such as dry ashing (in a muffle furnace) or wet ashing (by acid digestion). The individual elements within an ash can be determined by titrimetric methods, colorimetric methods, or atomic spectroscopy techniques. Atomic spectroscopy techniques can provide a complete profile of the different types of element in a food material, whereas the titrimetric and colorimetric methods are usually designed to determine a particular element. In addition, atomic spectroscopy techniques have a much higher sensitivity and specificity.

BIBLIOGRAPHY

1. Y. Pomeranz and C. E. Meloan, *Food Analysis, Theory and Practice*, 3rd ed., Chapman and Hall, New York, 1994.
2. S. S. Nielsen, ed., *Introduction to the Chemical Analysis of Foods*, Jones and Bartlett, Boston, Mass., 1994.
3. D. W. Gruenwedel and J. R. Whitaker, eds., *Food Analyses, Principles and Techniques*, Vols. I–V, Marcel Dekker, New York, 1984.
4. C. S. James, *Analytical Chemistry of Foods*, Blackie Academic and Professional, London, United Kingdom, 1994.
5. D. King, ed., *Developments in Food Analysis*, Elsevier Applied Science, London, United Kingdom, 1984.
6. G. C. Birch and K. J. Parker, eds., *Control of Food Quality and Analysis*, Elsevier Applied Science, London, United Kingdom, 1984.
7. W. Diemair, ed., *Handbook of Food Chemistry, Food Analysis*, Springer-Verlag, Berlin, Germany, 1967
8. *CRC Handbook of Chemistry and Physics*, 58th ed., CRC Press, Cleveland, Ohio, 1998.
9 J. I. Kroschwitz and M. Howe-Grant, eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., Wiley-Interscience, New York, 1991–1998
10. R. M. C. Dawson et al., *Data for Biochemical Research*, 3rd ed., Clarendon Press, Oxford, United Kingdom, 1986.
11. S. Budavari, *The Merck Index*, 12th ed., Merck, Whitehouse Station, N.J., 1996.
12. *Approved Methods of Analysis*, 8th ed., American Association of Cereal Chemists, St. Paul, Minn., 1983.
13. *Official Methods of Analysis*, 15th ed., Association of Official Analytical Chemists, Washington, D.C., 1990.

14. *Official Methods of Recommended Practices*, 4th ed., American Oil Chemists Society, Chicago, Ill., 1990.
15. D. A. Skoog, D. M. West, and F. J. Holler, *Analytical Chemistry: An Introduction*, 6th ed., Harcourt Brace, Philadelphia, Pa., 1994.
16. R. H. Wilson, ed., *Spectroscopic Techniques for Food Analysis*, Wiley-VCH, New York, 1994.
17. O. R. Fennema, ed., *Food Chemistry*, Marcel Dekker, New York, 1996.

JULIAN MCCLEMENTS
University of Massachusetts
Amherst, Massachusetts

FOOD AND NUTRITION SCIENCE ALLIANCE (FANSA)

The Food and Nutrition Science Alliance, FANSA, is a partnership of four professional scientific societies whose members have joined forces to speak with one voice on food and nutrition science issues. FANSA's combined membership includes more than 100,000 food, nutrition, and medical practitioners and scientists.

FANSA's statements help consumers sort through the confusing array of information about food and nutrition and offer tips for evaluating the validity of such information. For instance, FANSA alerts consumers to be cautious about claims made about dietary supplements:

1. When buying dietary supplements, caveat emptor applies.
2. If it sounds too good to be true, it probably is not true.
3. Dietary supplements encompass vitamins and minerals as well as herbals and botanicals.
4. Multivitamins may help some people.
5. Less is known about herbals and botanicals than vitamins and minerals.
6. High doses of some dietary supplements may be harmful.
7. "Natural" is not synonymous with "safe."
8. For good health, eat a variety of foods.

Other FANSA statements address the following topics:

- Interpreting nutrition research
- Making sense of scientific research about diet and health
- Making sense of risks associated with diet
- Folic acid: a reminder for women before and during pregnancy

FANSA's "10 Red Flags of Junk Science" help consumers think twice before reacting to the latest news bulletin about nutrition. Any combination of the following signs should send up a red flag of suspicion about the accuracy of the information:

1. Recommendations that promise a quick fix
2. Dire warnings of danger from a single product or regimen
3. Claims that sound too good to be true
4. Simplistic conclusions drawn from a complex study
5. Recommendations based on a single study
6. Dramatic statements that are refuted by reputable scientific organizations
7. Lists of "good" and "bad" foods
8. Recommendations made to help sell a product
9. Recommendations based on studies published without peer review
10. Recommendations from studies that ignore differences among individuals or groups

FANSA member organizations include the American Dietetic Association (ADA), American Society for Clinical Nutrition (ASCN), American Society for Nutritional Sciences (ASNS), and the Institute of Food Technologists (IFT).

As the nation's largest group of food and nutrition professionals with 68,500 members, ADA serves the public by promoting optimal nutrition, health, and well-being. With 1300 members, ASCN is the scientific society for clinical nutritionists in medicine and the health sciences. With 3400 members, ASNS (formerly American Institute of Nutrition) is the principal professional organization of nutrition research scientists in the United States. And as the society for food science and technology, IFT represents 28,000 food scientists and others in related professions, working in all facets of the food system, in academia, industry, and government (IFT's website can be accessed at *http://www.ift.org*).

ELLEN J. SULLIVAN
Institute of Food Technologists
Chicago, Illinois

FOOD AND NUTRITION SCIENCE ALLIANCE ORGANIZATIONS

The Food and Nutrition Science Alliance (FANSA) is a partnership of four professional scientific societies whose members have joined forces to speak with one voice on food and nutrition science issues. FANSA's combined membership includes more than 100,000 food, nutrition, and medical practitioners and scientists.

FANSA member organizations are as follows:

The American Dietetic Association (ADA)
American Society for Clinical Nutrition (ASCN)
American Society for Nutritional Sciences (ASNS)
Institute of Food Technologists (see the article INSTITUTE OF FOOD TECHNOLOGISTS).

THE AMERICAN DIETETIC ASSOCIATION

As the nation's largest group of food and nutrition professionals with nearly 70,000 members, ADA (founded in

1917) serves the public by promoting optimal nutrition, health, and well-being.

ADA members in the United States and abroad help shape the food choices and impact the nutritional status of the public. The membership includes dietitians, dietetic technicians, students, and others holding baccalaureate and advanced degrees in nutrition and dietetics. ADA members play a key role in shaping the public's food choices, thereby improving its nutritional status, and in treating persons with illnesses. Members offer preventive and therapeutic nutrition services in a variety of settings.

The ADA is a collective partnership of members, staff, and allied organizations. A number of networks provide the framework for member involvement, including state and district associations and 26 dietetic practice groups. The practice groups include members who specialize in public health, education, research, long-term care, foodservice management, private practice, sports nutrition diet counseling for diabetes, weight management, and heart disease, among other areas.

ADA members provide expert testimony at hearings, lobby Congress and other government bodies, comments on proposed federal and state regulations, and develop position statements on critical food and nutrition issues. The Commission on Accreditation and Approval for Dietetics Education accredits and approves more than 600 entry-level dietetics education programs for entry into the profession. Through its National Center for Nutrition and Dietetics, ADA offers programs to educate consumers about the links between food and nutrition and health.

The *Journal of the American Dietetic Association* and the *ADA Courier* supply members with current food and nutrition research and practice information, as well as Association news. As an important player in the International Committee of Dietetic Associations, ADA helps promote information sharing and professional cooperation among dietetics practitioners around the world.

Further information is available at *http:www.eatright.org*.

AMERICAN SOCIETY FOR CLINICAL NUTRITION

With 1300 members, ASCN is the premier scientific society for clinical nutritionists in medicine and the health sciences.

The goals and objectives of ASCN are as follows:

- To encourage and implement undergraduate and graduate education in basic and clinical nutrition, particularly in medical schools
- To expand research and clinical training opportunities in nutrition for health professionals
- To provide opportunities for investigators to present and discuss current research in human nutrition
- To offer continuing education programs in clinical nutrition for physicians, basic scientists, and allied health professionals
- To provide a journal for publication of meritorious work in clinical and experimental nutrition
- To promote and publicize the professional application of nutrition science in health promotion, disease prevention, and patient care
- To facilitate and promote the issuing of credentials for practitioners of clinical nutrition
- To participate in developing and implementing public policies and procedures concerned with clinical nutrition
- To expand federal and private funding of clinical nutrition research and training
- To maintain integrity and ethical behavior among nutrition societies through adherence to the society's formalized code of professional responsibility

ASCN sponsors two major forums for continuing education of physicians, basic scientists, and allied health professionals: the annual meeting and the postgraduate course. The postgraduate course, held annually since 1985, addresses current nutrition research findings and their clinical applications in health and disease. ASCN also cosponsors meetings with other nutrition societies, such as ASNS, the American Society for Parenteral and Enteral Nutrition, the Canadian Society for Nutritional Sciences, and the International Union of Nutritional Sciences, which organizes the International Nutrition Congress every four years.

The *American Journal of Clinical Nutrition* (AJCN), the society's official journal, publishes peer-reviewed original research contributed by scientists throughout the world and provides perspectives on nutrition, editorials, special articles, meeting reports, book reviews, and letters to the editor. Special supplements are devoted to symposia, workshops, and other long reports.

Government and the news media turn to ASCN members for authoritative information on issues related to clinical nutrition and its role in maintaining health and preventing and treating disease. ASCN also works with other professional societies to disseminate accurate information on current nutrition and dietary issues.

General management of the society is vested in an elected council. The executive officer, as the society's chief administrative officer, implements financial, scientific, publication, and education programs under policies established by the council. In addition, the executive officer interacts with legislators, the National Academy of Sciences, the National Institutes of Health, and other government bodies to enhance the role of ASCN as a national source of clinical nutrition expertise; it also maintains liaison with Federation of American Societies for Experimental Biology (FASEB) constituent societies and with national and international nutrition societies.

Further information is available at *http://www.faseb.org/ascn*.

AMERICAN SOCIETY FOR NUTRITIONAL SCIENCES

With 3400 members, ASNS is the principal professional organization of nutrition research scientists in the United States. It is dedicated to improving the quality of life through the science of nutrition.

The Society fulfills its mission by

- fostering and enhancing research in animal and human nutrition;
- providing opportunities for sharing, disseminating, and archiving peer-reviewed nutrition research results (at its annual meeting and in its official publication, the *Journal of Nutrition*);
- fostering quality education and training in nutrition;
- upholding standards of ethical behavior in research, the protection of human research participants, and the care and treatment of research animals;
- providing opportunities for fellowship and support among nutritionists; and
- bringing scientific knowledge to bear on nutrition issues through communication and influence in the public domain.

The ASNS Secretariat and its staff, housed in Bethesda, Maryland, administer the affairs of the Society. The executive officer interacts with the elected officers of the Society to manage the business affairs of the Society and the *Journal of Nutrition*, to maintain a relationship with the Federation of American Societies for Experimental Biology (FASEB), to coordinate public affairs activities, and to provide support for the selection and scheduling of the scientific program at the annual meeting (Experimental Biology).

Further information is available at *http://www.faseb.org/asns*.

Kristen McNutt
Consumer Choices, Inc.
Winfield, Illinois

FOOD CHEMISTRY AND BIOCHEMISTRY

The Institute of Food Technologists (IFT; *http://www.ift.org*) is one of the largest scientific societies representing professionals in food science and technology. A food scientist is defined by the IFT as a person who studies the physical, microbiological, and chemical makeup of food. In an accreditation program, the IFT has defined minimum standards for undergraduate food science degrees. These standards highlight the importance of chemistry in a food scientist's education, with a greater minimum requirement for chemistry than any other single discipline. Four introductory chemistry courses are required: two in general chemistry, one in organic chemistry, and one in biochemistry. Building on this base are a lecture and laboratory course in food chemistry and a food analysis lecture and laboratory course that utilizes chemical and microbiological techniques. Over the last few decades, however, food chemistry has evolved into a field of such scope that these courses are barely adequate to serve as an introduction to the field.

EDUCATION

One indication of the rapid evolution of food chemistry is the increase in specialized food chemistry and food chemistry analysis books. In the early 1970s there were virtually no general food chemistry text books. From the mid 1970s on, however, there was an explosive growth in such books (Table 1).

Most of the books have chapters on water, carbohydrates, lipids, amino acids and proteins, enzymes, vitamins and minerals, color, flavor, texture, toxic compounds and additives. Besides the trend to more general textbooks in the food chemistry area, there is a trend to increased complexity and proficiency in these books. Also, new food

Table 1. Some General Food Chemistry Textbooks

Author or editor	Title	Publishing company	Year
F A. Lee	*Basic Food Chemistry*	AVI, Westport, Conn.	1975
O. R. Fennema (ed.)	*Food Chemistry* (3rd ed)	Marcel Dekker, New York	1996 (original version, 1976)
C. Zapsalis and R. A. Beck	*Food Chemistry and Nutritional Biochemistry*	Originally John Wiley and Sons, New York, now Macmillan, London	1985
H. D. Belitz and W. Grosch	*Food Chemistry* (translation from the German version by D. Hadziyev)	Springer-Verlag, Berlin	1986
J. M. deMan	*Principles of Food Chemistry* (3rd ed.)	Aspen Publishers, Gaithersburg, Md.	1999 (original version 1980)
D. W. S. Wong	*Mechanism and Theory in Food Chemistry*	Van Nostrand Reinhold, New York	1989
D. Pearson	*The Chemical Analysis of Foods* (7th ed.)	Churchill Livingstone, New York	1976 (original version 1926)
Y. Pomeranz and C. E. Meloan	*Food Analysis: Theory and Practice*	Chapman and Hall	1978
S. S. Nielsen	*Food Analysis* (2nd ed.)	Aspen Publishers, Gaithersburg, Md.	1998 (original version 1994)
C. M. Weaver	*Food and Chemistry Laboratory*	CRC Press, New York	1996
L. M. L. Nollet	*Handbook of Food Analysis* (2 volumes)	Marcel Dekker, New York	1996

Table 2. Food Chemistry Topics

Water

Physical properties, pH
Hydrogen bonding, solubility of food components
Water activity

Carbohydrates

Basic structures and naming conventions
Chemistry of reducing and nonreducing sugars
Nonenzymatic browning
Natural homo and heteropolysaccharides, sources, biosynthesis and uses
Chemical modification of natural polysaccharides and their uses
New carbohydrate polymers from genetic engineering

Lipids

Basic structures and naming
Lipid reactions, hydrolytic and oxidative rancidity
Crystal structure and physical properties
Refining, hydrogenation, and interesterification reactions
Chemistry of artificial fats
Modified fat composition from genetic engineering

Amino acids and proteins

Basic structure and naming
Protein structure
Denaturation conditions
Protein chemical changes in processing

Enzymes

Catalytic action, stereochemical specificity
Food enzymes, action, kinetics, stability, uses, and sources
Enzymatic browning and rancidity
Enzyme immobilization
Postharvest physiology of plants
Postmortem changes in animals
Modified enzyme activity through genetic engineering

Vitamins and minerals

Chemistry and interaction of vitamins and minerals with food components under processing conditions
Biosynthesis and biodegradation, metabolism

Color

Natural food pigments and their chemistry
Artificial colors and their metabolism
Color measurement

Flavor

Biosynthesis of flavor components
Sweetening and the flavor theories
Physiology of taste and smell
Chemistry of artificial sweeteners

Texture

Food colloids
Gel formation
Emulsifiers and foaming agents
Viscosity

Table 2. Food Chemistry Topics *(continued)*

Toxic compounds

Natural microbial, plant, and animal toxins
Synthetic food toxins
Radionucleotides and toxic metals
Residues
Metabolism and excretion of toxic compounds

Food additives

Sequestrants and their chemistry
Artificial and natural sweeteners
Antioxidants
Acidulants
Bases, leavening agents
Antibiotic and probiotic agents
Gases and propellants
Other food additives

Food analysis

Analysis of any of the above food components using:
  Wet chemical and physical methods
  Chromatography techniques including high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis
  Spectroscopy techniques, visible (absorption and reflectance), ultraviolet, fluorescent, infrared, atomic absorption and emission, mass spectrometry (MS), nuclear magnetic resonance (NMR), electron spin resonance (ESR).
  Electroanalytical techniques, selective electrodes, coulometry and voltammetry, electrophoresis
  Enzymatic methods, enzymatic electrodes, enzyme immunoassays
  Thermal methods, differential scanning calorimetry, cryoscopy
  Rheological methods

chemistry analysis books have recently been emphasized. Food chemistry analysis borrows techniques from many other disciplines and has therefore become very complex. An indication of this increased complexity is the two-volume, 3000-plus-page series entitled, *Handbook of Food Analysis*. Included in the two volumes are 48 chapters with each chapter written by a group of experts in their field.

With the increase in intricacy of the discipline, increased specialization is necessary. As early as 1944 the need for specialization in food science and technology was recognized by educators in the field (1). Nowadays food chemistry itself covers such a variety of topics (see Table 2) that further specialization is an inevitable trend.

PUBLICATIONS

There is no doubt that parallel with the increase in educational material, publications in the food chemistry discipline have increased. As in the educational area there is indication that the discipline is becoming increasingly specialized and complex.

It is instructive to look at trend-setting journals in the food chemistry area. Although it is difficult to define a journal as solely dealing with food chemistry, leading journals in food science and technology have been rated by the In-

Table 3. 1996 Food Science and Technology Journals Rated By Impact Factor

Rank	Journal title	Impact factor
1	*CRC Critical Reviews in Food Science*	2.173
2	*Journal of Agricultural and Food Chemistry*	1.732
3	*Biotechnology Progress*	1.588
4	*Trends in Food Science and Technology*	1.430
5	*Food and Chemical Toxicology*	1.398
6	*International Journal of Food Microbiology*	1.387
7	*Journal of Dairy Research*	1.374
8	*Journal of Food Protection*	1.372
9	*Journal of Cereal Science*	1.354
10	*Journal of American Oil Chemists Society*	1.241
11	*Journal of Food Science*	1.225
12	*Cereal Chemistry*	1.160
13	*Journal of Dairy Science*	1.139
14	*Food Microbiology*	1.127
15	*Food Technology*	1.100
16	*Netherlands Milk and Dairy Journal*	1.056
17	*Meat Science*	1.048
18	*Food Hydrocolloids*	1.034
19	*Journal of Texture Studies*	0.985
20	*Journal of the Institute of Brewing*	0.955

Source Ref. 2.

stitute for Scientific Information (2) on the basis of impact factor (the top 20 journals out of a list of 73 are given in Table 3). Impact factor is defined as the number of yearly citations of journal articles divided by the number of yearly articles. Therefore, an impact factor of 1.00 means that if a journal contained 50 papers in a given year, there would also be 50 citations of former journal papers for the same year.

Review journals such as *CRC Critical Reviews in Food Science, or Trends in Food Science and Technology* will always have somewhat higher impact factors because reviews are generally larger, well-referenced papers and preferentially cited over many smaller papers in a field. Also, some journals are not be considered primarily food technology journals and may not appear on the list for this reason. An example of this latter case is the *Journal of the AOAC International* (1.256 impact factor). However, impact factors do give a measure of the influence of various journals independent of the number of papers that appear in a journal (ie, large journals that have more total citations are rated on an equivalent scale to small journals because of the per-article nature of impact factors).

From Table 3 it can be seen that two leading food chemistry journals are the *Journal of Agricultural and Food Chemistry* (*JAFC*), and *Food and Chemical Toxicology*. The latter journal covers an important food chemistry specialization. The first of these is published by the American Chemical Society, which is the world's largest scientific society. *JAFC* is also rated by impact factor as the second most influential agriculture journal and the fifth most influential applied chemistry journal. Mirroring the growth of food chemistry and related fields, this journal has grown from 1548 pages in 1989 to almost 5000 pages in 1996. With this expansion the journal has developed subheadings (Table 4) to organize the research areas.

Table 4. Subheadings Used by the *Journal of Agricultural and Food Chemistry*

Analytical Methods
Biotechnology
Chemical Changes During Processing/Storage
Chemistry of Crop and Animal Protection
Composition of Foods/Feeds
Environmental Chemistry
Flavors and Aromas
Food Chemistry/Biochemistry
Nutrition Toxicology

Other leading food science and technology journals are often commodity publications such as the *Journal of Dairy Research* and the *Journal of Cereal Science*. All of these commodity publications contain a large proportion of food chemistry articles.

Food chemistry has now grown and matured to a point that it has influenced other disciplines such as nutrition and medicine. Two examples are nonenzymatic browning reactions and antioxidant protection of lipids to prevent oxidative rancidity. The former is now known to be a very general reaction that impacts conditions such as diabetes, aging, and even Alzheimer's disease (3). The nonenzymatic browning products of concern in these conditions have been called advanced glycated end products. Food antioxidants, long known to play a role in preventing off-flavors or rancidity in food, now also find an important role in human health. The search for new and better natural antioxidants is an area of intense research interest, with scientists still unsure of the best protective agents found in mostly plant foods (4). In fact, the interest in foods and ingredients that may have health benefits has developed into a large new interdisciplinary research area dealing with functional foods or nutraceuticals.

Even though food chemistry has developed a great deal over the last few decades, clearly there is still room for tremendous growth in the discipline because there is still a great deal that we do not understand about the chemistry of an increasingly diverse food supply.

BIBLIOGRAPHY

1. O. Fennema, "Educational Programs in Food Science: A Continuing Struggle for Legitimacy, Respect, and Recognition," *Food Technol.* **43**, 170–182 (1989).
2. E. Garfield, *SCI Journal Citation Reports*, Institute for Scientific Information, Philadelphia, Penn., 1996, p. 83.
3. M P. Vitek, et al., "Advanced Glycation End Products Contribute to Amyloidosis in Alzheimer's Disease," *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4766–4770 (1994)
4. B. Halliwell, "Antioxidants in Human Health and Disease," *Annu. Rev. Nutr.* **16**, 33–50 (1996).

PETER SPORNS
University of Alberta
Edmonton, Alberta
Canada

FOOD CHEMISTRY: MECHANISM AND THEORY

Food chemistry deals with the chemical identity of food components and chemical reactions governing the changes and performance of individual or interacting food components during handling, processing, and storage. Although the individual constituents may often be readily identified, the interactions of food components are extremely complex.

Food chemistry is a branch of chemistry with its foundation built on chemical principles and reaction mechanisms, and a comprehension of the subject often requires thorough understanding and application of knowledge from various chemistry and chemistry-related disciplines. In this regard, food chemistry is quite similar to biochemistry, except that the former relates chemistry to food systems and the latter to biological systems. For example, a biochemist may be interested in elucidating the biosynthetic pathways of wheat storage proteins, but for a food chemist, it is more relevant to relate the chemical structures of these proteins to functional properties, such as changes and effects in dough quality. Another example is found in the Maillard reaction. An organic chemist may investigate the chemical reactions and their mechanism in the formation of melanoidin compounds. A biochemist is likely more interested in the reactions because they are related to the aging process of certain vital proteins, such as lens crystalline, collagen, and elastin. A food chemist is also interested in the Maillard reaction, but more in linking the reaction mechanism to physical and chemical changes in food systems such as flavor development, browning, and nutritional loss.

In this article reaction mechanisms of sufficient importance in food systems and their current developments are presented. A number of good background references have been published (1–5).

CARBOHYDRATES

The Maillard Reaction

In 1912, the French chemist Louis-Camille Maillard first observed that yellow-brown pigments formed in the reaction among sugars and amino acids, peptides, and proteins in a heated solution. Food chemists have recognized the practical relevance of this reaction to many chemical and physical changes during processing and storage of food. The first review (in English) on the Maillard chemistry in food systems was published in 1951 (6). Since then, numerous reviews on this subject have appeared (eg, 7–9,15). The biological importance of this reaction has been recognized only in the last 20 years. It is now well established that the reaction is linked to glycosylated hemoglobin (HbA_{1c}) in diabetes, hardened lens crystallins in cataract disease, and a number of other aging proteins (10,11).

The Maillard reaction comprises a series of reactions: (*1*) formation of glycosylamine via a Schiff-base formation between a reducing sugar and the amino group of an amino acid, (*2*) Amadori rearrangement in which glycosylamine is converted to ketosamine, (*3*) enolization (C1–C2 or C2–C3), followed by cyclodehydration. In general, under acidic conditions, the nitrogen is protonated, and 1,2-enolization is assisted by the positively charged nitrogen acting as an electron sink. Alkali and strong basic amine favor 2,3-enolization (Fig. 1).

The actual reactions are far more complicated than those outlined here, and there are many variations in the pathway. The initial step in the Amadori rearrangement is suggested as N-protonation. However, addition of the proton to the ring oxygen has also been proposed. Most discussions on the Maillard reaction concern the monosubstituted amines, but the ketosamine formed in the reaction can also react with another molecule of an aldose resulting in disubstitution. Another variation is the Heynes rearrangement in the conversion of D-fructosylamine (a ketosamine) to 2-amino-2-deoxy-D-glucose (an aldosylamine).

A pathway that involves sugar fragmentation and free-radical formation prior to the Amadori rearrangement has been suggested (12,13) (Fig. 2). The radical has been structurally identified to be N,N′-disubstituted pyrazine cation radical and it is formed by the dimerization of a two-carbon enaminol product from the cleavage of glycosylamine.

Direct dehydration of the Amadori compound has been proposed as an alternative to enolization (14,15) (Fig. 3). In this mechanism, the Amadori compound undergoes a trans-elimination at C2–C3, followed by a second dehydration at C3–C4, to form a hydroxypyran and finally a pyrylium ion. The highly electrophilic pyrylium ion can undergo various nucleophilic additions, ring opening, and recyclization.

Maillard reaction products have very diverse structures and are involved in various secondary reactions. A compilation of 450 volatile Maillard reaction products and related compounds (16) and reviews on this subject are available (7–9,15). Dicarbonyl compounds, such as 3-deoxyglycosulose generated by the 1,2-enolization pathway and the glycosulos-3-ene formed via the 2,3-enolization are the key intermediates for subsequent degradative reactions relating to color and flavor production. One of the well-known pathways is the Strecker degradation in which the carbonyl forms a Schiff base with the α-amino group of an amino acid. Enolization, decarboxylation, and hydrolysis yield an aldehyde corresponding to the original amino acid with one fewer carbon. The aldehydes derived from this degradative pathway constitute many important flavor compounds in food systems. Compounds generated by degradation of the dicarbonyl compounds include pyrroles, pyrazines, oxazoles and derivatives, pyrrolines, pyrrolidines, pyrones, thiazole, and thiazoline.

Sulfite inhibition of nonenzymatic browning also involves the dicarbonyl intermediates reacting with sulfur oxoanion to form stable sulfonate product (17). The sulfur oxoanion may replace the C4 hydroxy group of the deoxyglycosulose or undergoes 1,4-addition to the double bond of the α,β-unsaturated glycosulos-3-ene.

Formation of melanoidins is caused by polymerization of unsaturated carbonyl compounds. Condensation between 3-deoxyglycosulose and its enamine, which is a reductone, has been suggested to be the structural unit of the polymer (18,19) (Fig. 4), although polymers of repeating units of furan or pyrrole have also been proposed.

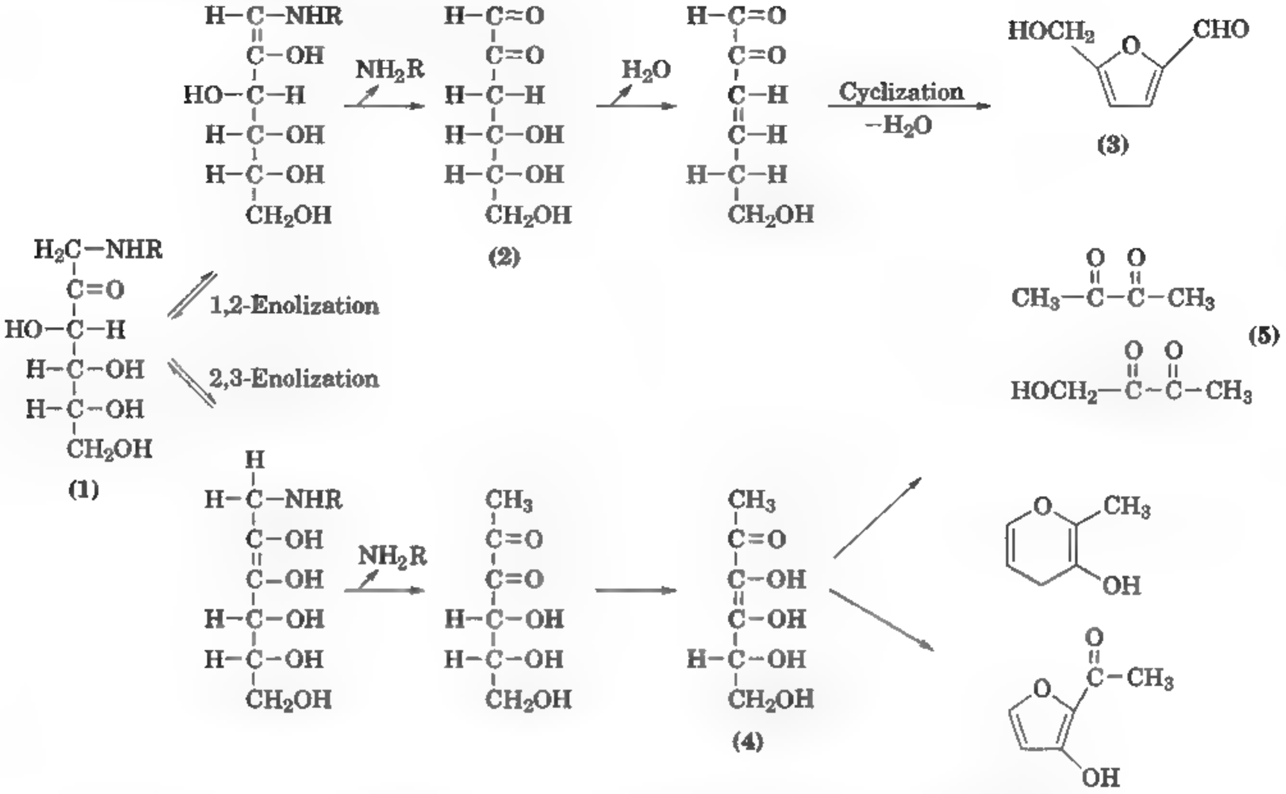


Figure 1. Reaction pathways of Amadori product: (1) Amadori product, (2) deoxyglycosulose, (3) furaldehyde, (4) glycosulos-3-ene, (5) reductones.

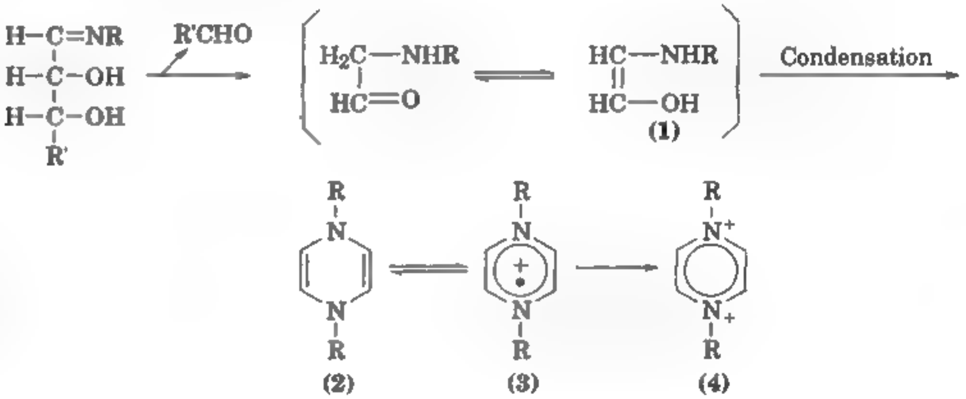


Figure 2. Fragmentation and free radical formation in the Maillard reaction: (1) enaminol, (2) dialkyldihydropyrazine, (3) dialkylpyrazine cation radical, (4) dialkylpyrazinium compound. *Source:* Ref. 12.

The Maillard reaction offers a perfect example of how basic organic chemistry—studies of the reaction mechanism between sugar and amino acids and the structural identification of possible intermediate compounds—has made it possible to explain some of the most interesting and important interactions in food systems.

Polysaccharide Polymers

The past two decades have witnessed an increasing interest in the relationship between molecular structure and functional mechanism of food polysaccharides. Growth in the knowledge of polymer chemistry has made a dramatic change in the way food scientists interpret structure and function of food polysaccharides.

One milestone in this area is the elucidation of the gelling mechanism of alginate in the early 1970s (20). The mechanism of gelation has been shown to be the interaction between calcium ions and the polyguluronate blocks of alginate polymer. Such interactions provide junctions that cross-link alginate polymers into a three-dimensional network, known as the "egg box" model (Fig. 5).

The concept of junction-zone linking long-chain polymers has since been included in explaining various kinds of gelation. For example, carrageenan polymers are known to associate by intermolecular double helices. Gelation oc-

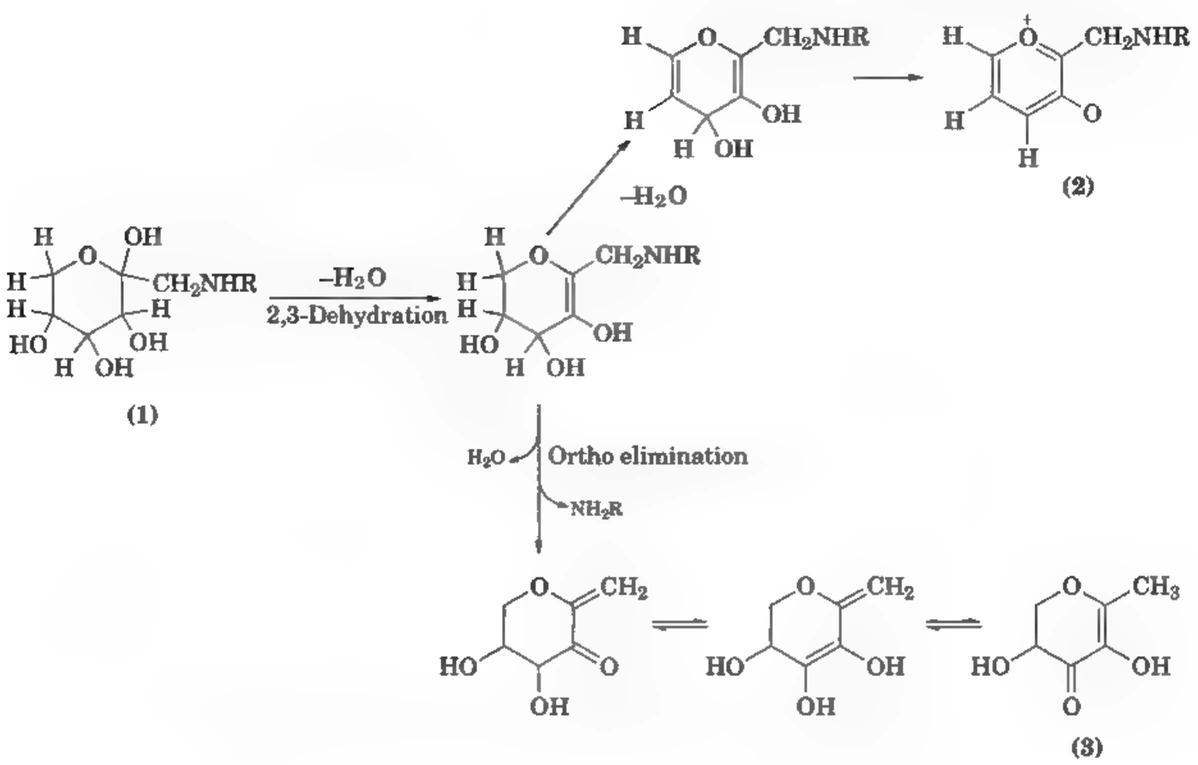


Figure 3. Direct sequential dehydration of Amadori product: (1) Amadori product, (2) pyrylium ion, (3) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. *Source:* Refs. 14 and 15.

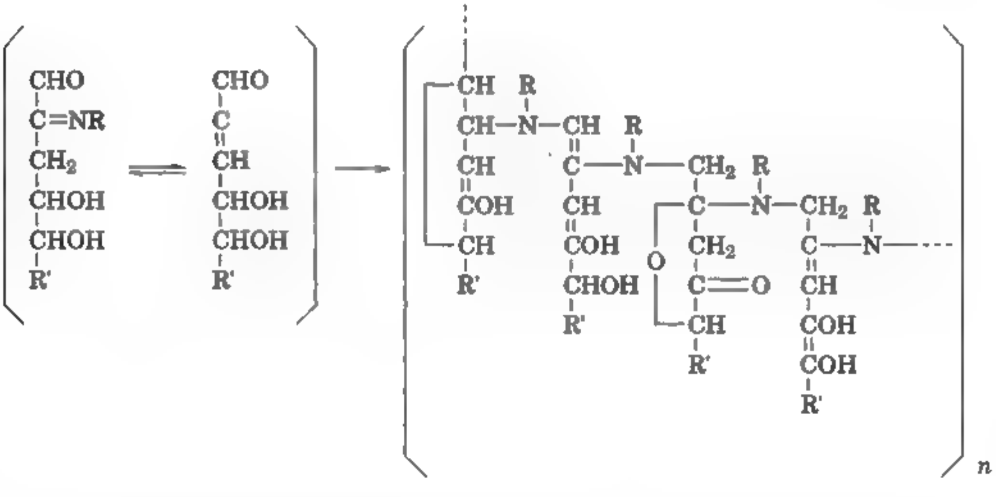


Figure 4. Repeating unit of melanoidin. R-NH_2 = amine, R′ = H or CH_2OH *Source* Ref 18

curs with the subsequent aggregation of these helices providing cross-linking junctions to build a continuous network (21,22). The number of charged sulfate groups along the polymer contributes to the degree of aggregation and the characteristics of the gel.

Carrageenan forms stable complexes with κ-casein via the interaction between the sulfate anions and the highly positively charged region of the protein. The synergistic interaction between κ-carrageenan and locust bean gum has also been interpreted on the basis of junction cross-linking.

High methyoxy pectin gels at low pH and in the presence of a cosolute such as sucrose. At low pH, the carboxyl groups are protonated, causing a decrease in electrostatic repulsion. Addition of a cosolute lowers the water solvation of the polymer. Both factors increase hydrophobic interaction and association of the polymers into cross-linking junction zones (23).

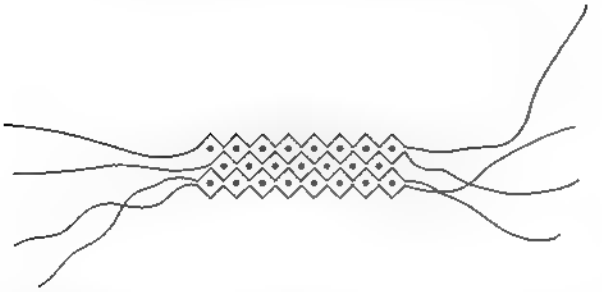


Figure 5. The "egg box" model. Line = alginate polymer; dark circle = calcium ion. *Source:* Ref. 20.

Starch gelation represents a more complex system, which has received continuous attention from various disciplines. The structure of crystalline amylose (of B-form starch) as originally elucidated by X-ray diffraction consists of two parallel strands of right-handed sixfold helices packed in an antiparallel double helix (24). More recent work has suggested a parallel packing of left-handed, parallel-stranded double helices for crystalline amylose from both A- and B-form starch (25,26). In solution, the conformation of amylose assumes a random coil containing short segments of loose and irregular helical structure (27,28).

Various models have been proposed for the structure of amylopectin. Most investigations seem to support a cluster-type model in that amylopectin is composed of clusters of oriented chains with the branching points collected together toward the reducing end (29).

Gelation occurs when starch granules in suspension are heated above the gelatinization temperature and cooled. Heating causes the granules to swell irreversibly, accompanied by the solubilization of amylose while most of the amylopectin is retained. On cooling, the starch gel formed has a composite structure of swollen amylopectin granules distributed in a matrix of amylose gel (30). Retrogradation involves crystallization of both the amylose in the gel phase and the amylopectin in the granules. The amylose molecules associate through hydrogen bonding into an insoluble precipitate. Amylopectin also exhibits interchain association. However, amylopectin molecules have an average chain length of 20 to 25 and a degree of polymerization of approximately 10^3. Interchain association between the polymers can only extend for 15 to 20 glucose units before being interrupted by the branching points. Crystallization of the amylopectin fraction is slow and results in a gradual increase in the rigidity of the granules and hence the amylose gel matrix. Reversion of a starch gel to the granular crystalline state is termed retrogradation, a process chiefly responsible for the staleness of bread.

It is important to note that in a gel the intermolecular association of polymers usually involves extensive segments of the polymeric chains held together by hydrogen bonding, electrostatic forces, hydrophobic or ionic interactions to form cross-linking junctions. Depending on the gel, a variety of rheological properties can exist. The entire field of polysaccharide gels has gone through a rapid expansion in the scope of basic research and intensified investigation in the relationship between the molecular arrangement and gelling characteristics.

LIPIDS

Lipid Oxidation

Lipid autoxidation is a free-radical chain reaction involving hydroperoxides. The mechanism has been extensively investigated since the first systematic study by Bateman and Bolland in the 1940s. In 1970, Rawls and van Santen proposed a possible role for singlet oxygen in the initiation of fatty acid autoxidation (31). In the presence of a suitable sensitizer, lipid oxidation proceeds via the "ene" reaction in which the dioxygen molecule is added to the olefinic carbon with a subsequent shift in the position of the double bond, and both conjugated and nonconjugated hydroperoxides are formed. In contrast, the classical free-radical mechanism produces only the conjugated hydroperoxides. The distribution of hydroperoxides are different from that found in the free-radical reaction.

In linoleate autoxidation, the product is composed predominantly of the 9- and 13-hydroperoxides, because the stability of the conjugated diene system favors the oxygen attack at the end position. Experimentally, however, four hydroperoxides are obtained: two with *trans, cis* and the other two with *trans, trans* stereochemistry (13-hydroperoxy-9-*cis*, 11-*trans*-octadecadienoic; 13-hydroxyperoxy-9-*trans*, 11-*trans*-octadecadienoic; 9-hydroxyperoxy-10-*trans*, 12-*cis*-octadecadienoic, and 9-hydroperoxy-10-*trans*, 12-*trans*-octadecadienoic). A unified mechanism has been proposed to account for the formation of these products (32) (Fig. 6). In this scheme the peroxy radicals initially formed have the *trans, cis* configuration and exist in two conformational isomers. The normal pathway involves the abstraction of hydrogen from another linoleate by either isomer to yield the *trans, cis* hydroperoxides. The alternative pathway involves the loss of oxygen from the conformers to give either the original pentadienyl radical or a new carbon radical in which the stereochemistry of the partial double bond is inverted. Oxygen addition to this new pentadienyl radical yields a *trans, trans*-diene peroxy radical which ultimately gives the *trans, trans*-conjugated hydroperoxide.

Because autoxidation involves free radicals, radical scavengers should effectively terminate the chain reaction. Most antioxidants are substituted phenolic compounds that act by transferring hydrogen to lipid peroxy radicals. The resulting aryloxy radical of the antioxidant is stable and unreactive in oxidative reactions. The resonance stability of the aryloxy radical depends on the substitution groups. Electron-releasing groups decrease the transition energy for the formation of the aryloxy radical. Bulky substituents stabilize the aryloxy radical but also create steric hindrance making the antioxidant less accessible to the lipid peroxy radical (33).

Physical Chemistry of Lipids

Another area of great interest to food chemists concerns the physical chemistry of lipids—polymorphism, crystal

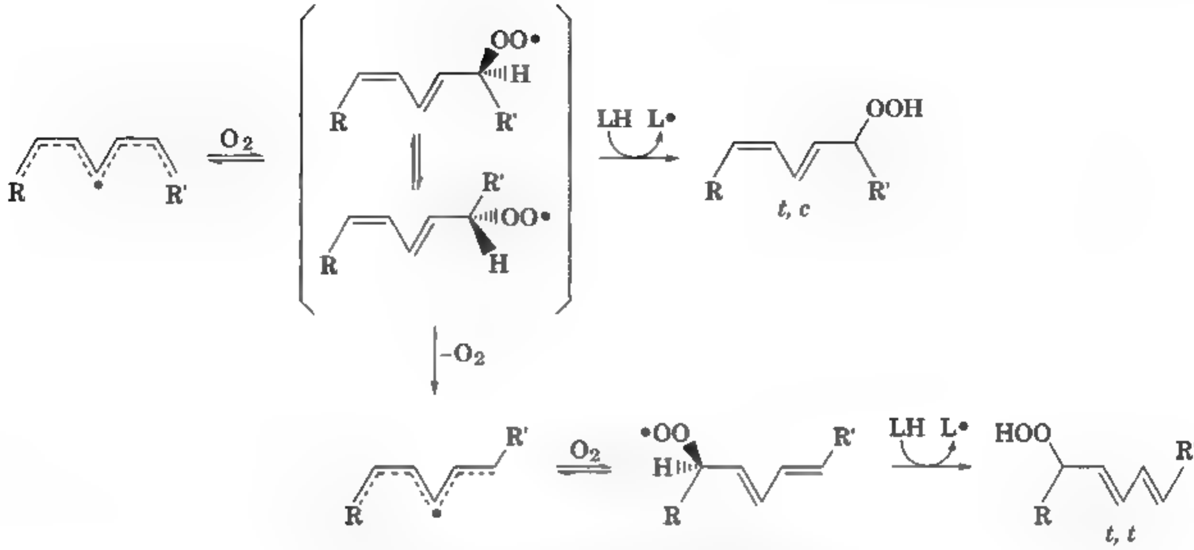


Figure 6. A unified mechanism for lipid autoxidation. *Source:* Ref. 32.

habit, emulsion, etc. Triglycerides exhibit multiple crystalline structures. For example, tristearin melts at 54.7, 64.0, and 73.3°C, representing the transition from the less stable α form to the more stable forms, β′ and β. The α form has a hexagonal crystal subcell. The intermediate melting β′ form is orthorhombic and the highest melting polymorph; the β form has a triclinic subcell. This multiplicity of molecular conformation and packing influences the fluidity, texture, and appearance of the product. Alpha crystals are fragile platelets of 5 μm in size. Beta crystals tend to be large and coarse with 25–50 μm in diameter. Fats in the β′ form appear as tiny needles of ~1 μm in length. The β′ crystals can incorporate large numbers of air bubbles providing a smooth texture to an oil product such as margarine and shortening. The fatty acid composition, as well as the position of a particular fatty acid in the glyceride, affects the crystal habit of a particular fat. Interesterification usually causes a β′ to β conversion.

The current concept of emulsion stability came with the understanding of the factors controlling attractive/repulsive forces and their interaction with distance between disperse particles. Flocculation and coalescence of oil droplets in an emulsion is dependent on the balance between van der Waals attraction and electrostatic repulsion. The combination of these two gives a net potential energy vs distance curve with a potential minimum at certain intermediate ranges, where repulsion may be greater than attraction and a measure of stability exists (34). At long and short ranges, the net potential is always attraction.

An emulsifier consists of both hydrophilic and hydrophobic segments in the same molecule. Food grade emulsifiers are usually partial esters of fatty acids, polyols, and water-soluble organic acids. When an emulsifier is dispersed in water and heated, a liquid crystalline mesophase is formed (35). The mesophase assumes a lamellar, hexagonal, or cubic structure depending on the type of emulsifier and temperature. In a ternary system such as an emulsifier in an oil/water system, similar types of mesophases are formed (36). The ordered layer of this liquid-crystalline phase stabilizes the oil-in-water emulsion by forming a film at the interface. It decreases the attractive forces between the oil droplets and provides a steric barrier against coalescence between the droplets.

PROTEINS

Chemical Reactions in Processing

Because proteins contain many amino acids with reactive side-chain groups, it is expected that a variety of reactions may occur during food processing. One of the most extensively studied reactions is alkali degradation. Alkaline treatment is used in food industry for peeling, solubilization, and texturization of food proteins and manufacture of gelatin, sausage casings, and tortillas.

The most well-studied reaction is β-elimination in which the α-hydrogen of an amino acid residue is abstracted by the hydroxide ion (37) (Fig. 7). In protein-bound cysteine, the resulting product is a dehydroalanine which is an α,β-unsaturated compound. Nucleophilic side groups, such as the lysyl ε-amino and cysteinyl sulfur, react with dehydroalanine via Michael addition, leading to new cross-linkings in the proteins and loss of certain essential amino acids.

Alkaline treatment also causes hydrolysis of the amide groups in asparagine and glutamine, and the guanidino group in arginine. Racemization of amino acids is also detected. Protein-bound amino acids are more susceptible to α-hydrogen abstraction and hence recemization to the D-form (38). Prolonged heating results in isopeptide formation between the ε-amino group of lysine and the carbonyl group of aspartic or glutamic acid, or the amide groups of glutamine or asparagine.

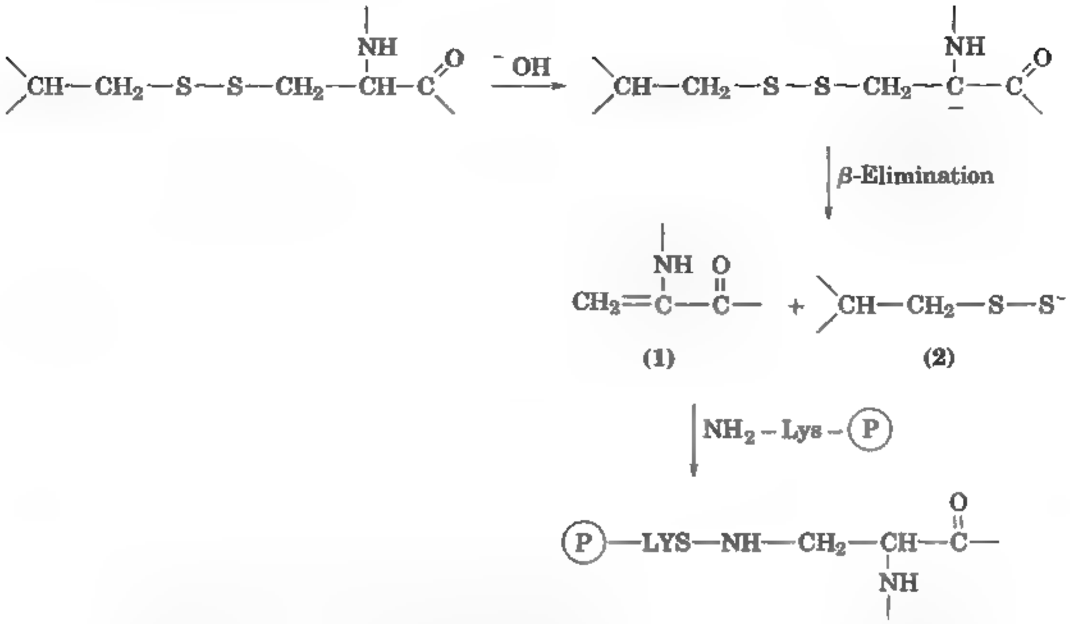


Figure 7. Reaction of β-elimination in alkali degradation of proteins: (1) dehydroalanine, (2) persulfide product, (3) lysinoalanine.

Protein Structure and Functionality

For a food chemist the structure of proteins is quite often viewed in the context of functionality in a food system. For example, the chemistry of muscle fibers and the mechanism of muscle contraction are related to rigor mortis and postmortem tenderness of meat (39). The onset of rigor mortis follows rapid depletion of ATP and breakdown in the regulatory system that controls the calcium level in muscle. Increasing concentration of calcium in the sarcoplasm induces contraction, whereas lack of ATP in the system stops the dissociation of the actin-myosin complex formed. The muscle loses its natural extensibility and this postmortem change is known as rigor mortis. Postmortem tenderness, however, is related to proteolysis of the muscle proteins. The acid proteases, such as the cathepsins, have received much attention in this respect. The calcium-dependent proteinase, calcium activated factor, has been linked to the causes of postmortem tenderization (40). In the last decade, there have been significant advances in our understanding of the molecular structure as well as the morphology and mechanism of muscle cytoskeletal proteins (41–43). These new developments in the basic knowledge of muscle proteins will inevitably affect the way their functionality in food systems is interpreted.

Despite the importance of cereal seed proteins, until recently, their molecular structure has been little understood. In the last several years, the complete amino acid sequence (or sequence deduced from cDNA) of gliadin (α-type) has been determined (44). The low molecular weight (LMW) glutenin subunits have been mapped by two-dimensional gel electrophoresis and sequenced (45). The high molecular weight (HMW) glutenin genes have been sequenced and expressed in *Escherichia coli* (46). Most recently, wheat transformation with genes for HMW subunits has been achieved, and the expression of one or two transgenic proteins in wheat results in stepwise increase in dough elasticity (47). Structural analysis of the amino acid sequence of the HMW glutenins is especially revealing. The protein molecule contains a large central repetitive region rich in glutamine, forming a loose spiral structure (48). Several cysteine residues are located at the α-helical region near the *N*- and *C*-terminal ends. Intermolecular disulfide bonds between the terminal cysteine residues cross-link the glutenin subunits into gluten polymers with the spiral regions in between. Hydrogen bonding could form among the side chains of glutamine residues, as well as the peptide backbone. However, attempt to relate the elastic mechanism of HMW subunits to a model consisting of spiral motifs that can extend and reform has not been entirely convincing (49). The continuing efforts on the investigation of protein sequences and structures in combination with genetic engineering studies are of immense value in providing the molecular basis for the specific role of HMW glutenins.

Bovine casein micelles exist in large spherical colloidal particles of 500 to 3000 Å in diameter and 10^7 to 3×10^{10} in particle weight. It has long been postulated that a micelle is assembled from submicelles containing a mixture of various casein molecules. However, the supramolecular structure of casein submicelles and micelles is unclear. One model suggests that submicelles are bound by electrostatic interaction via colloidal calcium phosphate through their ester phosphate groups (50). Because κ-casein is almost phosphate free, binding occurs only among the other caseins in the submicelle. Submicelles with a low level of κ-casein are oriented in the interior, and the surface of the micelle is covered entirely with submicelles having a high content of κ-casein. A more recent model depicts the structure of micelles as a protein gel without the formation of discret submicelles (51). The core of a micelle is a gel matrix with casein proteins held by microgranules of calcium phosphate. On the surface of the micelle is a "hairy" layer composed of a uniform density of macropeptide segments of κ-casein. The highly flexible and hydrated polar polypeptide chains provide steric stabilization to the micelle. Cleavage of the macropeptides by chymosin changes the surface characteristics of the micelle resulting in aggregation.

The cDNA sequences for the four major caseins (α_{s1}, α_{s2}, β, and κ) are known. Suggestions have been made to improve the functionality of casein using genetic engineering techniques (52). These include (*1*) alteration of the proportion of κ-casein to enhance the stability of casein micelles, (*2*) construction of an additional cleaving site in casein for chymosin, resulting in a change of rheological effects of proteolysis, (*3*) dephosphorylation of casein, creating additional phosphate groups for stabilization, (*4*) deletion of a polar segment from the otherwise nonpolar *N*-terminus of κ-casein, thereby enhancing its amphiphilicity.

Similar strategy has been applied to the whey protein β-lactoglobulin. It has been postulated that the thermal instability of this protein is due to the unfolding of the polypeptide segment (residues 115–125) containing the free cysteine-121, and the subsequent sulfhydryl-disulfide exchange with the disulfides (65–160 and 106–143) or with κ-casein in a milk system. Hence, deletion or substitution of the cys-121 by site-directed mutagenesis is expected to enhance the thermal stability and the functional properties of β-lactoglobulin.

COLORANTS

There are two basic categories of color compounds: conjugated polyenes and metalloporphyrins. The former includes carotenoids, annatto, anthocyanins, betanain, dyes, and lakes. The effect of conjugation is lowering the π-π^* transition energy from the highest occupied molecular orbital to the lowest unoccupied molecular orbital. Increased conjugation in the molecule shifts the absorption maximum to a higher wavelength. Substituent groups with lone pairs of electrons tend to increase π conjugation by resonance.

Carotenoids consist of a basic structure of eight repeating isoprene units that are highly conjugated. In the conjugated system, the terminal double bond has the highest electron density and is most susceptible to oxidative attack. Degradation proceeds from the end to the center of the molecule, resulting in a progressive shortening of the polyene chain (53).

Anthocyanins are flavonoid compounds characterized by the flavylium nucleus. The flavylium form is stabilized by resonance with the positive charge delocalized throughout the entire structure, making the anthocyanin molecule intensely colored. The structural transformation of anthocyanins in aqueous medium was thoroughly investigated in the early 1980s. Flavylium salts exist in equilibrium in different forms: flavylium cation (AH^+), quinoidal base (A), carbinol pseudobase (B) and chalcone (C). The equilibrium between AH^+ and A involves the transfer of proton from the C5, C7, or C4′ hydroxyl groups to a water molecule. In the hydration reaction, the water molecule is preferentially added to the C2 of the pyrylium ring of AH^+, resulting in colorless B. The conversion of B to C is a base-catalyzed tautomerization (Fig. 8). Because most isolated natural anthocyanins when placed in slightly acidic medium (pH 3–6) exist largely in the colorless forms (B and C), these fundamental studies should have practical implications. Evidence indicates that substitution pattern at various positions of the anthocyanin molecule influences the equilibrium constants of these reactions and hence the distribution of the colored (AH^+, A) and colorless (B, C) species (54).

The two best known examples of metalloporphyrins found in food are the myoglobin and chlorophylls. A porphyrin metal complex possesses 19 π-electrons in an 18-atom ring. The main effect of the metal on the transitions is the conjugation of the metal $p\pi$ orbital with the porphyrin π orbital (55). The splitting of the δ orbitals of the metal ion (due to the porphyrin) exhibits additional loss of degeneracy from the theoretically predicted octahedral symmetry. In oxymyoglobin heme complex the iron coordination positions are directed to the four porphyrin nitrogens, and in the fifth and the sixth positions, to histidine F8 and O_2 (or H_2O), respectively. The role of the protein globin is to stabilize the steric and electronic configuration of the iron heme, and to facilitate the back-bonding of electrons from the iron to the π^* orbital of the oxygen (Fig. 9). The hydrophobicity of the heme pocket excludes the binding of ionic ligands such as CN^-, OH^-, and the closely packed amino acid side chains restrict the size and orientation of the ligand (56).

Chlorophylls, unlike myoglobin which contains a transition metal ion, are porphyrins complexed with the alkali earth metal Mg^{++}. The compounds are hydrophobic because of the a long chain C_{20} phytol esterified to the propionic acid side chain at C7. The magnesium atom of chlorophyll can be readily replaced by weak acids or other metals such as copper, zinc, and iron. The free base obtained after removal of the metal is pheophytin, with the transition intensity shifted to a lower wavelength. Hydrolysis of the phytyl chain by alkali or enzyme yields the chlorophyllides, which are water-soluble and green-colored, with spectral characteristics similar to those of the chlorophylls. Removal of magnesium from the chlorophyllides yields pheophorbides, which have the same spectral properties as those of the pheophytins. These types of conversion have been frequently implicated as causes of color loss in processed green vegetables (57,58). Copper complexes of pheophytin and clorophyllide are stable to acid and are used as food colors in some European countries (59).

There is a considerable interest in colorants from natural resources. Cape jasmine (*Gardenia jasminoides*) have been investigated for the production of carotenoid and crocin. The flower is also rich in flavonoids. Another example is the mold *Monascus purpureus*, which has been used in Asian countries for centuries. The pigments produced are a mixture of red, yellow, and purple polyketides (60). Pigments extracted from algae, yeasts, and insects have also

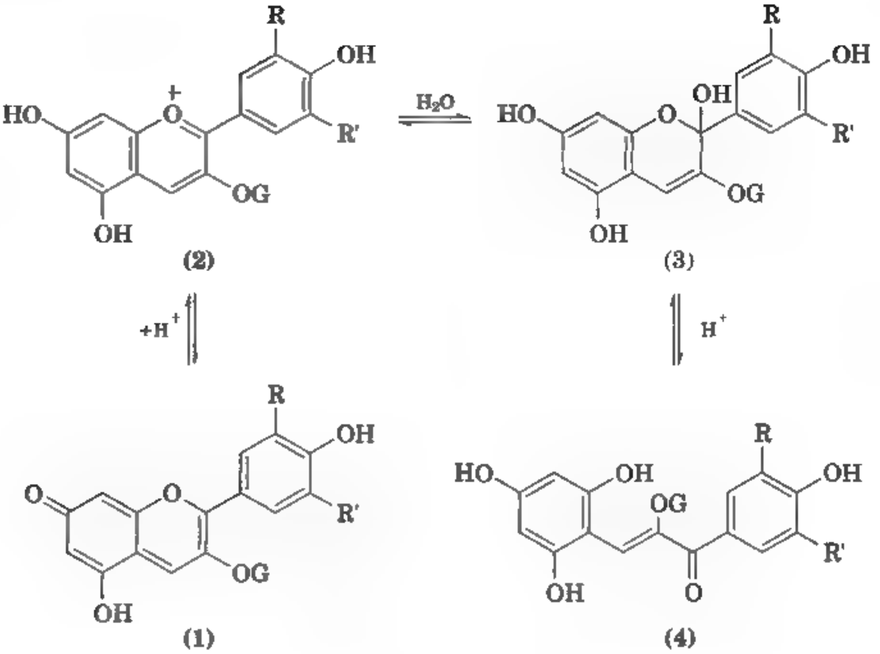


Figure 8. Resonance stabilization of flavylium cation: (1) flavylium cation (red), (2) carbinol pseudobase (colorless), (3) chalcone (colorless), (4) quinoidal base (blue). *Source:* Ref. 54.

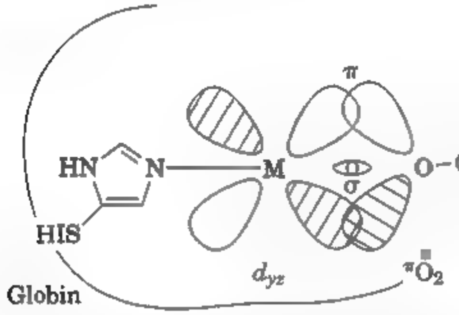


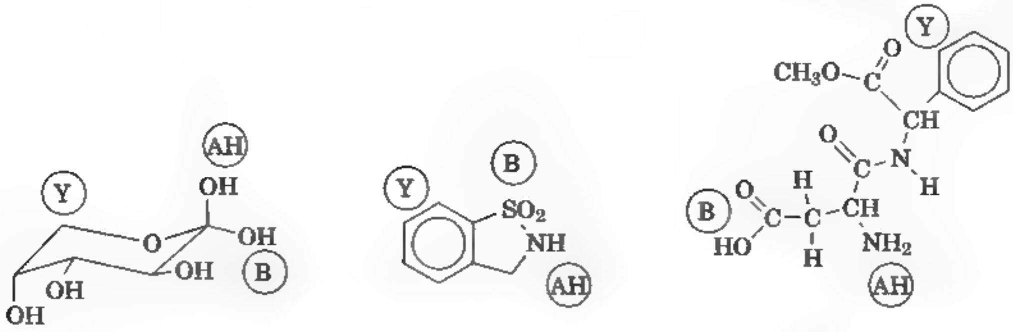
Figure 9. Back-bonding of electrons from the transition metal to π^* orbital of oxygen.

been investigated for potential use as natural colorants (61). Attempts have also been made to produce derivatives of anthocyanins and other pigments in plant tissue cultures (62).

FLAVORS

Flavor is the sensation produced by a material and perceived principally by the senses of taste and smell. Over the years, scientists have attempted to relate chemical senses to the molecular structures of various compounds in an effort to develop a coherent theory. In 1967 it was proposed the glucophore unit, which is responsible for sweetness in compounds, should consist of an AH, B hydrogen bonding system where H is an acidic proton and B is an electronegative atom or center (63). In 1972 the two-component system was extended to include a dispersion bonding component designated as γ component. The distance parameters of the resulting tripartite structure are A, B = 2.6, B, γ = 5.5, and A, γ = 3.5 Å. The orbital distance between the AH proton and B is 3.0 Å. In a three-dimensional picture, the glucophore binds to the receptor site and the sweet taste is initiated by intermolecular hydrogen bonding between the glucophore and a similar AH, B unit on the receptor. The γ component acts to align the molecule to the receptor. The locations of AH, B, γ components in many sweet compounds are known. For example, in aspartame, the protonated α-amino group and the ionized β-carboxyl group of the aspartyl residue represent the AH and B unit, respectively (Fig. 10). The phenylalanine end of the molecule represents the γ component. Only the L-L isomer of aspartame is sweet, and this type of enantiomeric effect in sweetness is also shown in several simple amino acids. To account for this fact, the receptor AH, B site is believed to consist of a spatial barrier. Hence, the L-D isomer of aspartame has the methyl ester group so positioned that the molecule cannot fit into the pocket for interactions (64). A similar concept is also applied to the aminosulfonates, such as saccharin and acesulfame K. Ring substitution experiments on acesulfame K indicate a loss of sweetness when the length of the hydrophobic group on the nitrogen exceeds 0.7 Å, suggesting that a bulky substituent would create a steric hindrance (65).

Another group of sweeteners worthy of attention are the sweet proteins, which are approximately 100,000 times sweeter than sucrose on a molar basis and several times on a weight basis. Thaumatin I, the most studied sweet protein, consists of a single polypeptide of 207 amino acids (M_W 22,000), with 8 disulfides and no histidine. The largest domain is a flattened β barrel formed by 11 antiparallel β strands, with 6 residues per strand on the average. Attached to this are domains II and III, with loops linked and stabilized by disulfide bonds (66). Monellin, the other known sweet protein, consists of subunits I and II of 50 and 44 amino acid residues, respectively. The protein consists of a 5-stranded, antiparallel β sheet (2 β strands from



(1) (2) (3)

Figure 10. Assignment of glucophore unit in sweet compounds: (1) fructose, (2) saccharin, (3) aspartame. *Source:* Ref. 64.

subunit I and 3 from subunit II) where each strand contains an average of 10 residues (67). There is only one sulfhydryl group (in subunit II), and in contrast to thaumatin I, no disulfide bond. Despite the intense sweetness of both proteins, there is little similarity in their three-dimensional structures. However, antibodies raised against thaumatin also cross-react with monellin (68). Five homologous amino acid sequences are found in thaumatin I and monellin; two are topologically similar and one of these is located in a β bend protruding from the surface of the molecule. This tripeptide sequence (-Glu-Tyr-Gly-) has been postulated to be the active site responsible for the sweet taste (69,70). The structure–function relationship of these proteins is not clear.

A similar AH, B concept has been employed to explain bitterness with little success. The structural requirements for bitter taste have been proposed, and it has been shown that, in contrast to the AH/B system for sweeteners, bitter compounds need only one polar group (electrophilic or nucleophilic with a negative charge) and a hydrophobic group, with the intensity of bitter taste depending on the size and shape of the hydrophobic part of the molecule (71,72). Although the monopolar–hydrophobic concept for bitter taste requires more research, the importance of hydrophobicity to bitterness has been well established. Introduction of an additional hydrophobic group onto a sweet compound often results in modifying the taste from sweet to bitter.

Several theories have been presented over the years to correlate the molecular structure with the perceived odor quality of a chemical compound. The infrared theory has had some success in correlating odor quality with low-energy molecular vibrations (73). The site-fitting theory attempts to link the size, shape, and electronic status of a molecule to a complementary receptor site (74). It has been suggested that although the overall shape and size of an odorous compound is responsible for the sensory perception, the functional group determines the orientation and the affinity of the molecule to the receptor (75). Molecular connectivity terms have been derived to relate the quantitative description of certain aspects of molecular topology to several classes of odor compounds and found significant correlations between the two (76). Another study on the qualitative structure activity relationship has been conducted using computerized pattern recognition techniques (77). Both chemical composition and the shape of the molecule seem to be important contributing factors. In addition to the properties mentioned, it is also evident that the chirality and hydrophobicity of the compound are important for odor intensity.

Current knowledge about olfactory reception suggests the initial interaction occurs in the olfactory epithelium between the odorant with receptor proteins that extend from the dendrites of the olfactory sensory neurons (78,79). In humans, there are around 1,000 genes encoding 1,000 different odor receptors, each expressed in thousands of olfactory sensory neurons. A particular odor would bind to a characteristic set of multiple receptors rather than individual receptor cells, depending on the chemical groups of the odor molecule. Binding of an odorant to receptors activates a G protein–adenylate cyclase cascade, which results in the generation of cAMP. The cAMP causes the opening of an ion channel either directly or by phosphorylation via a cAMP-dependent protein kinase (80,81). The signals are transmitted to the localized glomeruli in the olfactory bulb, but exactly how the cortex decodes and reconstructs the signals into sensory responses is not known. There is still lack of a clear relationship between chemical structure and odor quality, and it is impossible to predict odor quality of a compound with a known structure.

NATURAL TOXICANTS

There are a large number of natural toxic chemicals in our daily diet. Toxicants may be chemical constituents of the food itself, contaminants from microbial infestation, or degradation products formed during food storage and processing. Toxic chemicals are commonly present in all plants, in which they act as protective agents against microbial attack, insects, and predators (82). Although the generally low level of these compounds together with a varied diet usually eliminate the risk of intoxification, the presence of many of these natural pesticides in our food seems to be receiving very little attention from the general public.

One well-known example of natural pesticides is the glycoalkaloids, solanine and chaconine, found in potatoes

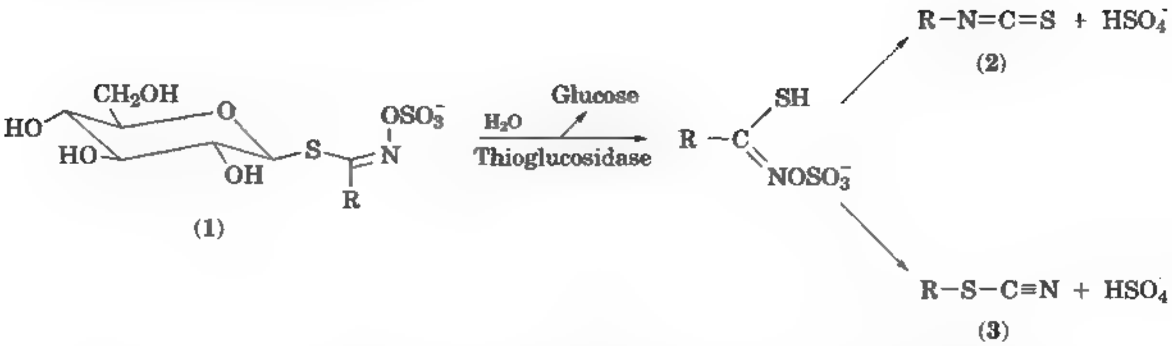

CH2OH
HO
HO
OH
S
OSO3-
N
R
(1)
Glucose
H2O
Thioglucosidase
SH
R
C
NOSO3-
R−N=C=S + HSO4-
(2)
R−S−C≡N + HSO4
(3)

Figure 11. Degradation of glucosinolate: (1) glucosinolate, (2) isothiocyanate, (3) thiocyanate. *Source*. Ref. 83.

at the level of approximately 10 mg/100 g fresh weight (83). These glycoalkaloids are strong inhibitors of cholinesterase causing neurological disorder symptoms. Lethal doses for humans range from 3 to 6 mg/kg body weight, and doses of greater than 2 mg/kg are normally considered toxic.

Another class of toxicants, glucosinolates, occurs predominantly in vegetables such as cabbage, cauliflower, radish, mustard greens, brussels sprouts, and broccoli. Glucosinolates are hydrolyzed by the enzyme thioglucoside glycohydrolase when the plant tissues are crushed. The unstable aglycone produced is converted to isothiocyanate in a process similar to the Lossen rearrangement or thiocyanate (84) (Fig. 11). The latter compound is a goitrogen.

A number of amino acids, peptides, and proteins constitute an interesting class of toxicants. There are more than 250 nonprotein amino acids found in plants, many of which are structurally similar to the protein amino acids. For example, β-pyrazol-1-ylalane, pipecolic acid, homoarginie, α,β-diaminobutyric acid are analogs of histidine, proline, arginine, and lysine, respectively. Toxicity of these nonprotein amino acids is usually caused by the competition with structurally similar protein amino acids for proteins, resulting in functionally inactive products, or in interference with protein synthesis.

The notorious toxins produced by *Clostridium botulinum* are neurotoxins that block the release of acetylcholine

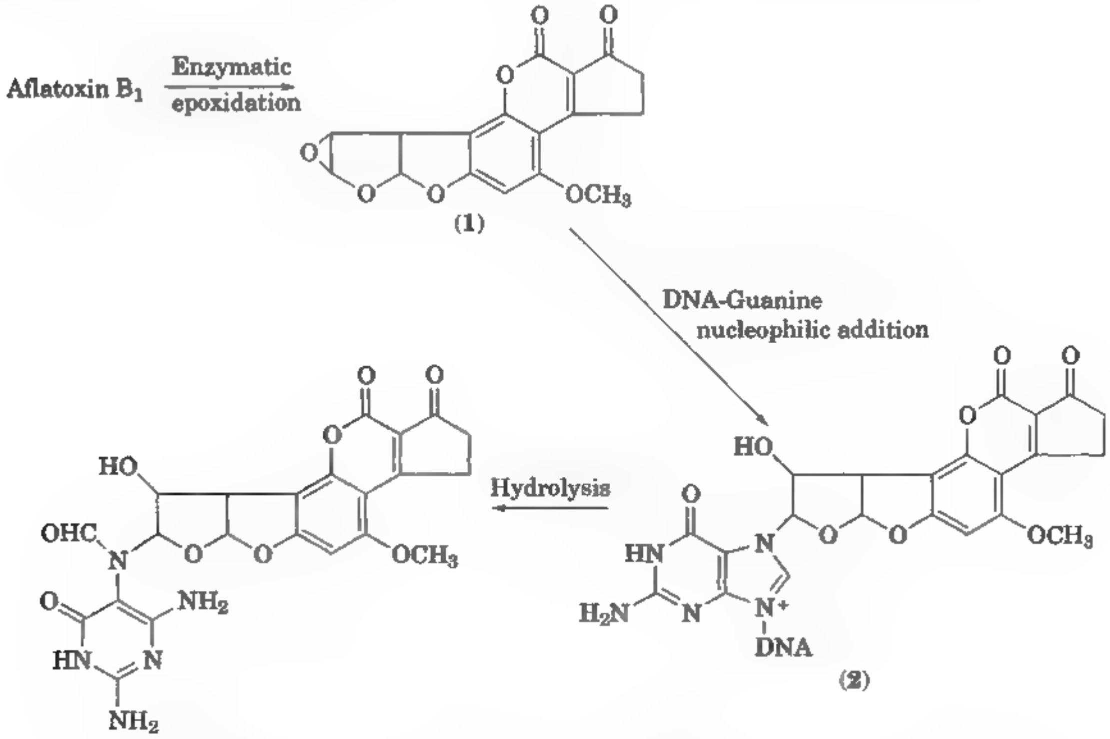

Aflatoxin B1
Enzymatic epoxidation
OCH3
(1)
DNA-Guanine nucleophilic addition
Hydrolysis
HO
OHC
N
NH2
HN
N
NH2
HN
H2N
N
N+
DNA
(2)

Figure 12. Formation of conjugate between aflatoxin B_1 and DNA: (1) AFB_1-8,9-oxide, (2) AFB_1-Guanine-DNA. *Source:* Ref. 85.

in the peripheral nervous system, resulting in paralysis. The lethal dose is in the range of 1 to 5 ng/kg body weight. *Botulinum* toxins are synthesized as an inactive precursor, which is a single polypeptide with a molecular weight of ~150,000 Da. The protein is cleaved by an endogenous protease into an active molecule consisting of a heavy chain and a light chain linked by a disulfide bond. The toxin initially binds to gangliosides (sialic acid–containing glycolipids) on the plasma membrane of cholinergic nerve. The toxin–ganglioside complex undergoes endocytosis, and the low pH in the vesicle activates the insertion of the toxin into the membrane (85).

Mycotoxins are toxic fungal metabolites that often contaminate peanuts, cereals, and dairy products. The most extensively studied mycotoxins are the aflatoxins produced by *Aspergillus* and *Penicillium*. The toxicity of aflatoxin B_1 is caused by their metabolism to the reactive AFB_1-8,9-oxide, which forms covalently linked conjugates with DNA and proteins (86) (Fig. 12), or undergoes hydrolysis to AFB_1-8,9-dihydrodiol. The latter product is a dialdehyde phenolate ion stabilized by resonance and forms Schiff base with proteins.

Polycyclic aromatic hydrocarbons are known carcinogens found in grilled meat products. These compounds include benzo[a]pyrene, benzo[a]fluroanthene, benzo[a]anthracene, and chrysene. They are metabolized by enzymatic activation to form a dihydrodiolepoxide that binds DNA. Heterocyclic amines found in high temperature cooking are enzymatically converted to *O*-acyl derivative, which covalently binds DNA, resulting in mispairing in replication and transcription (87).

Many *N*-nitrosamines are found in cured meat and fish, including *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopyrrolidine, and *N*-nitrosopiperidine. Metabolically, nitrosamines undergo enzymatic α-hydroxylation, followed by C-N bond cleavage to yield alkydiazohydroxide, which further breaks down to alkyldiazonium ion. Both alkydiazohydroxide and alkyldiazonium ion react with DNA to form covalent conjugates (88).

CONCLUSIONS

This brief overview clearly indicates the dynamic interactions among various interrelated disciplines. The very fact that interactions among food components are complex reflects the scope and diversity of the underlying chemistry. It is often inadequate to treat food chemistry in a descriptive manner. Approaches must be taken to analyze the kind of chemical mechanisms involved and to follow the sequence of steps in terms of chemical equations and principles. Food chemistry encompasses reaction mechanisms involving free radicals, transition metals, coordination chemistry, stereochemistry, molecular structures, biopolymers, and colloids, in addition to common organic chemical reactions. The process of searching for a fundamental understanding of how food components react and interact is indeed an exciting challenge for food scientists.

DISCLOSURE STATEMENT

Reference to a company and/or products is only for the purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

BIBLIOGRAPHY

1. D. W. S. Wong, *Mechanism and Theory in Food Chemistry*, Chapman & Hall, New York, 1989.
2. H. Belitz and W. Grosch, *Food Chemistry*, 2nd ed , Springer-Verlag, Berlin, Heidelberg, 1982, Translated in English by D. Hadziyev, 1987.
3. O. R. Fennema, *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996
4. J. M. deMan, *Principles of Food Chemistry*, 2nd ed., Van Nostrand Reinhold, New York, 1990.
5. D. W. S. Wong, *Food Enzymes: Structure and Mechanism*, Chapman & Hall, New York, 1995.
6. J. P. Danehy and W. W Pigman, "Reactions Between Sugars and Nitrogenous Compounds and Their Relationship to Certain Food Problems," *Adv. Food Res.* **3**, 241–290 (1951).
7. E. Dworschak, "Nonenzymatic Browning and Its Effect on Protein Nutrition," *Crit. Rev. Food Sci. Nutr.* **13**, 1–40 (1980).
8. J. P. Danehy, "Maillard Reactions: Nonenzymatic Browning in Food Systems With Special Reference to the Development of Flavors," *Adv. Food Res.* **30**, 77–138 (1986).
9. M. Namiki, "Chemistry of Maillard Reactions: Recent Studies on the Browning Reaction Mechanism and the Development of Antioxidants and Mutagens," *Adv. Food Res.* **32**, 115–184 (1988).
10. A. Cerami, H. Vlassara, and M Brownlee, "Glucose and Aging," *Sci. Am.* **256**, 90–96 (1987).
11. A. Cerami, "Aging of Proteins and Nucleic Acids: What Is the Role of Glucose?" *Trends Biochem. Sci.* **11**, 311–314 (1986).
12. M. Namiki and T. Hayashi, "A New Mechanism of the Maillard Reaction Involving Sugar Fragmentation and Free Radical Formation," in G. R. Waller and M. S. Feather, eds., *The Maillard Reaction in Foods and Nutrition*, ACS Symposium Series 215, American Chemical Society, Washington, D.C., 1983, pp. 21–46.
13. T. Hayashi, and M Namiki, "Role of Sugar Fragmentation in an Early Stage Browning of Amino-Carbonyl Reaction of Sugar With Amino Acid." *Agric. Biol. Chem.* **50**, 1965–1970 (1986).
14. V. Yaylayan and P. Sporns, "Novel Mechanisms for the Decomposition of 1-(Amino acid)-1-deoxy-D-fructoses (Amadori Compounds): A Mass Spectrometer Approach," *Food Chem.* **26**, 283–305 (1987).
15. V. Yaylayan and A. Huyghues-Despointes, "Chemistry of Amadori Rearrangement Products: Analysis, Synthesis, Kinetics, Reactions and Spectroscopic Properties," *Crit. Rev. Food Sci. Nutr.* **34**, 321–369 (1994).
16. S. Fors, "Sensory Properties of Volatile Maillard Reaction Products and Related Compounds," in G. R. Waller and M. S. Feather, eds., *The Maillard Reaction in Foods and Nutrition*, ACS Symposium Series 215, American Chemical Society, Washington, D.C., 1983, pp. xx–xx.
17. B. L. Wedzicha and D. J. McWeeney, "Non-enzymatic Browning Reactions of Ascorbic Acid and Their Inhibition. The Production of 3-Deoxy-4-sulphopentosulose in Mixtures of Ascor-

bic Acid, Glycine and Bisulphite ion," *J. Sci. Food Agric.* **35**, 577–587 (1974).

18 H. Kato and H. Tsuchida, "Estimation of Melanoidin Structure by Pyrolysis and Oxidation," *Prog. Food Nutr. Sci.* **5**, 147–156 (1981).

19 H. Kato, S. B. Kim, and F. Hayase, "Estimation of the Partial Chemical Structures of Melanoidins by Oxidative Degradation and ^{13}C CP-MAS NMR," *Dev. Food Sci.* **13**, 215–223 (1986).

20. G. T. Grant et al., "Biological Interactions Between Polysaccharides and Divalent Cations: The Egg Box Model," *FEBS Lett.* **32**, 195–198 (1973).

21. E. R. Morris, D. A. Rees, and G. R. Robinson, "Cation-specific Aggregation of Carrageenan Helices; Domain Model of Polymer Gel Structure," *J. Mol. Biol.* **138**, 349–362 (1980).

22. G. Robinson, E. R. Morris, and D. A. Rees, "Role of Double Helices in Carrageenan Gelation: The Domain Model," *J. Chem. Soc. Chem. Comm.* **1980**, 152–153 (1980).

23. D. G. Okenfull, "The Chemistry of High Methoxyl Pectins," in R. H. Walter, ed., *The Chemistry and Technology of Pectin*, Academic Press, New York, 1991, pp. 87–108.

24. H.-C. H. Wu and A. Sarko, "The Double-helical Molecular Structure of Crystalline β-Amylase," *Carbohydr. Res.* **61**, 7–25 (1978).

25. A. Imberty and S. Perez, "A Revisit to the Three Dimensional Structure of β-Type Starch," *Biopolymers* **27**, 1205–1221 (1988).

26. A. Imberty, H. Chanzy, and S. Perez, "The Double-helical Nature of the Crystalline Part of A-starch," *J. Mol. Biol* **201**, 365–378 (1988).

27. R. C. Jordan, D. A. Brant, and A. Cesaro, "A Monte Carlo Study of the Amylose Chain Conformation," *Biopolymers* **17**, 2617–2632 (1978).

28. A. Neszmelyi and J. Hollo, "Some Aspects of the Structure of Starch—A 3-D Molecular Modelling Approach," *Starch / Starke* **41**, 1–3 (1988).

29. D. J. Manners, "Recent Development in Our Understanding of Amylopectin Structure," *Carbohydr. Polym.* **11**, 87–112 (1989).

30. M. Miles, et al., "The Role of Amylose and Amylopectin in the Gelation and Retrogradation of Starch," *Carbohydr. Res.* **135**, 271–281 (1985).

31. H. R. Rawls and P. J. Van Santen, "A Possible Role for Singlet Oxygen in the Initiation of Fatty Acid Autoxidation," *J. Am. Oil Chem. Soc.* **47**, 121–125 (1970).

32. N. A. Porter, "Mechanisms for the Autoxidation of Polyunsaturated Lipids," *Acc. Chem. Res.* **19**, 262–268 (1986).

33. G. Scott, "Antioxidants *in vitro* and *vivo*," *Chem. Br.* **21**, 648–653 (1985).

34. S. Friberg, "Emulsion Stability," in S Friberg, ed., *Food Emulsions*, Marcel Dekker, New York, 1976, pp. 1–37.

35. E. Boyle, and B. German, "Monoglycerides in Membrane Systems," *Crit. Rev. Food Sci. Nutr.* **36**, 785–805 (1996).

36. L. Hernquist, "Polar Lipids in Emulsions and Microemulsions," in E. Dickinson, ed., *Food Emulsions and Foams*, Royal Society of Chemistry, London, 1987, pp. 110–157.

37. J. R. Whitaker and R. E. Feeney, "Chemical and Physical Modification of Proteins by the Hydroxide Ion," *Crit. Rev. Food Sci. Nutr.* **19**, 173–212 (1983).

38. R. Liardon and S. Ledermann, "Racemization Kinetics of Free and Protein-bound Amino Acids Under Moderate Alkaline Treatment," *J. Agric. Food Chem.* **34**, 557–565 (1986).

39. Y. L. Xiong, "Structure–Function Relationships of Muscle Proteins," in S. Damodoran and A. Paraf, eds., *Food Proteins and Their Applications*, Marcel Dekker, New York, 1997, pp. 341–392.

40. D. E. Goll et al., "Role of Muscle Proteinases in Maintenance of Muscle Integrity and Mass," *J. Food Biochem.* **7**, 137–177 (1983).

41. I. Rayment et al., "Structure of the Actin–Myosin Complex and Its Implications for Muscle Contraction," *Science* **261**, 58–65 (1993).

42. M. Irving et al., "Tilting of the Light-Chain Region of Myosin During Step Length Changes and Active Force Generation in Skeletal Muscle," *Nature* **375**, 688–632 (1995).

43. R. D. Vale, "Getting a Grip on Myosin," *Cell* **78**, 733–737 (1994).

44. D. D. Kasarda, et al., "Nucleic Acid (cDNA) and Amino Acid Sequences of α-Type Gliadins From Wheat (*Triticum aestivum*)," *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4712–4716 (1984).

45. H. P. Tao and D. D. Kasarda, "Two-dimensional Gel Mapping and *N*-Terminal Sequencing of LMW-Glutenin Subunits," *J. Exp. Bot.* **40**, 1015–1020 (1989).

46. G. Galili, "Heterologous Expression of a Wheat High Molecular Weight Glutenin Gene in *Escherichia coli*," *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7756–7760 (1989).

47. F. Barro et al., "Transformation of Wheat With High Molecular Weight Subunit Genes Results in Improved Functional Properties," *Nature Biotechnology* **15**, 1295–1299 (1997).

48. P. R. Shewry et al., "Biotechnology of Breadmaking: Unraveling and Manipulating the Multiprotein Gluten Complex," *Bio / Technology* **13**, 1185–1190 (1995).

49. D. D. Kasarda, G. King, and F. Kumosinski, "Comparison of Spiral Structures in Wheat High Molecular Weight Glutenin Subunits and Elastin by Molecular Modeling," in T. F. Kumosinski and M. N. Liebman, eds., *Molecular Modeling: From Virtual Tools to Real Problems*, American Chemical Society, Washington, D.C., 1994, pp. 209–220.

50. D. G. Schmidt, "Association of Caseins and Casein Micelle Structure," in P. F. Fox, ed., *Developments in Dairy Chemistry*, Applied Science Publishers, London and New York, 1982, pp. 61–86.

51. C. Holt, "Structure and Stability of Bovine Casein Micelles," *Adv. Protein Chem.* **43**, 63–151 (1992).

52. Y Kang and T. Richardson, "Genetic Engineering of Caseins," *Food Technol.* **39**, 89–94 (1985).

53. C. Marty and C. Berset, "Factors Affecting the Thermal Degradation of All-*trans*-β-carotene," *J. Agric. Food Chem.* **38**, 1063–1067 (1990).

54. R. Brouillard, G. A. Iacobucci, and J. G. Sweeny, "Chemistry of Anthocyanin Pigments. 9. UV-Visible Spectrophotometric Determination of the Acidity Constants of Apigeninidin and Three Related 3-Deoxyflavylium Salts," *J. Am. Chem. Soc.* **104**, 7585–7590 (1982).

55. D. J. Livingston and W. D. Brown, "The Chemistry of Myoglobin and Its Reactions," *Food Technol.* **35**, 244–252 (1981)

56. D. Ladikos and B. L. Wedzicha, "The Chemistry and Stability of the Haem–Protein Complex in Relation to Meat," *Food Chem.* **29**, 143–155 (1988).

57. H. K. Lichtenthalev, "Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes," *Methods Enzymol.* **148**, 350–382 (1987).

58. S. J. Schwatz and T. V. Lorenzo, "Chlorophylls in Foods," *Crit. Rev. Food Sci. Nutr.* **29**, 1–27 (1990).

59. A. H. Humphrey, "Chlorophyll," *Food Chem.* **5**, 57–67 (1980).

60. A. J. Taylor, "Natural Colors in Food," in J. Walford, ed., *Developments in Food Colors—II*, Applied Science Publishers, London, 1984, pp. 31–49.
61. K. Spears, "Developments in Food Colourings: The Natural Alternatives," *Trends in Biotechnol.* **6**, 283–288 (1988).
62. D. K. Dougall et al., "Biosynthesis and Stability of Monoacylated Anthocyanins," *Food Technol.* **51**, 69–71 (1997).
63. R. S. Shallenberg and T. E. Acree, "Molecular Theory of Sweet Taste," *Nature* **216**, 480–482 (1967).
64. F. Lelj et al., "Interaction of α-L-Aspartyl-L-phenylalanine Methyl Ester With the Receptor Site of the Sweet Taste Bud," *J. Am. Chem. Soc.* **98**, 6669–6675 (1976).
65. F. Pautet and C. Nofre, "Correlation of Chemical Structure and Taste in the Cyclamate Series and the Steric Nature of the Chemoreceptor site," *Z. Lebensm.-Unters.-Forsch.* **166**, 167–170 (1978).
66. C. M. Ogata et al., "Crystal Structure of a Sweet Tasting Protein Thaumatin I, at 1.65 Å Resolution," *J. Mol. Biol.* **228**, 893–908 (1992).
67. J. R. Somoza et al., "Two Crystal Structures of a Potently Sweet Protein: Natural Monellin at 2.75 Å Resolution and Single-Chain Monellin at 1.7 Å Resolution," *J. Mol. Biol.* **234**, 390–404 (1993).
68. C. A. M. Hough and J. A. Edwardson, "Antibodies to Thaumatin as a Model of the Sweet Taste Receptor," *Nature* **271**, 381–383 (1978).
69. H. van der Wel, "Physiological Action and Structure Characteristics of the Sweet-tasting Proteins Thaumatin and Monellin," *Trends Biochem. Sci.* **5**, 122–123 (1980).
70. S. H. Kim, A. deVos, and C. Ogata, "Crystal Structures of Two Intensely Sweet Proteins," *Trends Biochem. Sci.* **13**, 13–15 (1988).
71. A. van der Heijden. "Sweet and Bitter tastes," in T. E. Acree and R. Teranishi, eds., *Flavor Science: Sensible Principles and Techniques*, American Chemical Society, Washington D.C., 1993, pp. 67–115.
72. H. D. Belitz et al., "QSAR of Bitter Tasting Compounds," *Chem. Ind. (London)* **1**, 23–26 (1983).
73. R. H. Wright, *The Science of Smell*, Basic Books, New York, 1964.
74. J. E. Amoore, *Molecular Basis of Odor*, Charles C. Thomas, Springfield, Ill., 1970.
75. M. G. J. Beets, "Relationship of Chemical Structure to Odor and Taste," in *Proceedings, Third International Congress on Food Science and Technology*, Institute of Food Technologists, Chicago, Ill., 1971, pp. 379–395.
76. L. M. Kier, T. D. Paolo, and L. H. Hall, "Structure–Activity Studies on Odor Molecules Using Molecular Connectivity," *J. Theor. Biol.* **67**, 585–595 (1977).
77. G. Ohloff, "Chemistry of Odor Stimuli," *Experientia* **42**, 271–279 (1986).
78. L. Buck and R. Axel, "A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Reception," *Cell* **65**, 175–187 (1991).
79. K. Mori and Y. Yoshihara, "Molecular Recognition and Olfactory Processing in the Mammalian Olfactory System," *Progress in Neurobiology* **45**, 585–619 (1995).
80. S. H. Snyder, P. B. Sklar, and J. Pevsner, "Molecular Mechanisms of Olfaction," *J. Biol. Chem.* **263**, 13971–13974 (1988).
81. W. Pickenhagen, "Enantioselectivity in Odor Perception," in R. Teranishi, R. G. Buttery, and F. Shahidi, eds., *Flavor Chemistry, Trends and Developments*, ACS Symposium Series 388, American Chemical Society, Washington, D.C., 1989, pp. 151–157.
82. B. N. Ames, "Chemicals, Cancers, Causalities and Cautions," *CHEMTECH* **19**, 590–598 (1989).
83. S. F. Osman, "Glycoalkaloids in Potatoes," *Food Chem.* **11**, 235–247 (1983).
84. G. R. Fenwick, R. K. Heaney, and W. J. Mullin, "Glucosinolates and Their Breakdown Products in Food and Food Plants," *Crit. Rev. Food Technol.* **18**, 123–200 (1983).
85. E. Schantz and E. A. Johnson, "Properties and Use of Botulinum Toxin and Other Microbial Neurotoxins in Medicine," *Microbiol. Rev.* **56**, 80–89 (1992).
86. R. G. Croy and G. N. Wogan, "Temporal Patterns of Covalent DNA Adducts in Rat Liver After Single and Multiple Doses of Aflatoxin B_1," *Cancer Res.* **41**, 197–203 (1981).
87. Y. Hashimoto, K. Shudo, and T. Okamoto, "Activation of a Mutagen, 3-Amino-methyl-5H-pyrido[4,3-b]indole. Identification of 3-Hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole and Its Reaction With DNA," *Biochem. Biophys. Res. Comm.* **96**, 355–362 (1980).
88. M. C. Archer, "Reactive Intermediates From Nitrosamines," in R. Snyder, ed., *Biological Reactive Intermediates—II, Advances in Experimental Medicine and Biology*, Vol. 136B, Plenum Press, New York, 1982, pp. 1027–1035.

DOMINIC W. S. WONG
USDA
Albany, California

FOOD CONSUMPTION SURVEYS IN THE U.S. DEPARTMENT OF AGRICULTURE

One mission of the U.S. Department of Agriculture (USDA) is to encourage the production and availability of a sufficient, safe, and nutritionally adequate supply of food for Americans. In support of this mission, USDA conducts surveys to monitor food use and food consumption patterns in the U.S. population. Early studies on food and nutrition were begun at the end of the last century. These small-scale studies aimed to help people in the working class achieve good diets at low cost. As time went on, recognition of the need for nationally representative food and nutrient intake data resulted in the development of larger surveys. The USDA's most recent national food consumption survey was the 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII), which measured the kinds and amounts of foods eaten by Americans. Conducted as a telephone follow-up to the CSFII, the 1994–1996 Diet and Health Knowledge Survey (DHKS) measured attitudes and knowledge about diet and health among Americans. The CSFII and DHKS were designed so that individuals' attitudes and knowledge about healthy eating could be linked with their food choices and nutrient intakes. Both surveys address the requirements of the National Nutrition Monitoring and Related Research Act of 1990 (P.L. 101-445) for continuous monitoring of the dietary status of the American population, including the low-income population. See the section "The National Nutrition Monitoring and Related Research Program."

USES OF THE DATA

As shown in Table 1, evaluations of diet quality and tracking changes in the diet over time have many useful federal,

60. A. J. Taylor, "Natural Colors in Food," in J. Walford, ed., *Developments in Food Colors—II*, Applied Science Publishers, London, 1984, pp. 31–49.
61. K. Spears, "Developments in Food Colourings: The Natural Alternatives," *Trends in Biotechnol.* **6**, 283–288 (1988).
62. D. K. Dougall et al., "Biosynthesis and Stability of Monoacylated Anthocyanins," *Food Technol.* **51**, 69–71 (1997).
63. R. S. Shallenberg and T. E. Acree, "Molecular Theory of Sweet Taste," *Nature* **216**, 480–482 (1967).
64. F. Lelj et al., "Interaction of α-L-Aspartyl-L-phenylalanine Methyl Ester With the Receptor Site of the Sweet Taste Bud," *J. Am. Chem. Soc.* **98**, 6669–6675 (1976).
65. F. Pautet and C. Nofre, "Correlation of Chemical Structure and Taste in the Cyclamate Series and the Steric Nature of the Chemoreceptor site," *Z. Lebensm.-Unters.-Forsch.* **166**, 167–170 (1978).
66. C. M. Ogata et al., "Crystal Structure of a Sweet Tasting Protein Thaumatin I, at 1.65 Å Resolution," *J. Mol. Biol.* **228**, 893–908 (1992).
67. J. R. Somoza et al., "Two Crystal Structures of a Potently Sweet Protein: Natural Monellin at 2.75 Å Resolution and Single-Chain Monellin at 1.7 Å Resolution," *J. Mol. Biol.* **234**, 390–404 (1993).
68. C. A. M. Hough and J. A. Edwardson, "Antibodies to Thaumatin as a Model of the Sweet Taste Receptor," *Nature* **271**, 381–383 (1978).
69. H. van der Wel, "Physiological Action and Structure Characteristics of the Sweet-tasting Proteins Thaumatin and Monellin," *Trends Biochem. Sci.* **5**, 122–123 (1980).
70. S. H. Kim, A. deVos, and C. Ogata, "Crystal Structures of Two Intensely Sweet Proteins," *Trends Biochem. Sci.* **13**, 13–15 (1988).
71. A. van der Heijden. "Sweet and Bitter tastes," in T. E. Acree and R. Teranishi, eds., *Flavor Science: Sensible Principles and Techniques*, American Chemical Society, Washington D.C., 1993, pp. 67–115.
72. H. D. Belitz et al., "QSAR of Bitter Tasting Compounds," *Chem. Ind. (London)* **1**, 23–26 (1983).
73. R. H. Wright, *The Science of Smell*, Basic Books, New York, 1964.
74. J. E. Amoore, *Molecular Basis of Odor*, Charles C. Thomas, Springfield, Ill., 1970.
75. M. G. J. Beets, "Relationship of Chemical Structure to Odor and Taste," in *Proceedings, Third International Congress on Food Science and Technology*, Institute of Food Technologists, Chicago, Ill., 1971, pp. 379–395.
76. L. M. Kier, T. D. Paolo, and L. H. Hall, "Structure–Activity Studies on Odor Molecules Using Molecular Connectivity," *J. Theor. Biol.* **67**, 585–595 (1977).
77. G. Ohloff, "Chemistry of Odor Stimuli," *Experientia* **42**, 271–279 (1986).
78. L. Buck and R. Axel, "A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Reception," *Cell* **65**, 175–187 (1991).
79. K. Mori and Y. Yoshihara, "Molecular Recognition and Olfactory Processing in the Mammalian Olfactory System," *Progress in Neurobiology* **45**, 585–619 (1995).
80. S. H. Snyder, P. B. Sklar, and J. Pevsner, "Molecular Mechanisms of Olfaction," *J. Biol. Chem.* **263**, 13971–13974 (1988).
81. W. Pickenhagen, "Enantioselectivity in Odor Perception," in R. Teranishi, R. G. Buttery, and F. Shahidi, eds., *Flavor Chemistry, Trends and Developments*, ACS Symposium Series 388, American Chemical Society, Washington, D.C., 1989, pp. 151–157.
82. B. N. Ames, "Chemicals, Cancers, Causalities and Cautions," *CHEMTECH* **19**, 590–598 (1989).
83. S. F. Osman, "Glycoalkaloids in Potatoes," *Food Chem.* **11**, 235–247 (1983).
84. G. R. Fenwick, R. K. Heaney, and W. J. Mullin, "Glucosinolates and Their Breakdown Products in Food and Food Plants," *Crit. Rev. Food Technol.* **18**, 123–200 (1983).
85. E. Schantz and E. A. Johnson, "Properties and Use of Botulinum Toxin and Other Microbial Neurotoxins in Medicine," *Microbiol. Rev.* **56**, 80–89 (1992).
86. R. G. Croy and G. N. Wogan, "Temporal Patterns of Covalent DNA Adducts in Rat Liver After Single and Multiple Doses of Aflatoxin B_1," *Cancer Res.* **41**, 197–203 (1981).
87. Y. Hashimoto, K. Shudo, and T. Okamoto, "Activation of a Mutagen, 3-Amino-methyl-5H-pyrido[4,3-b]indole. Identification of 3-Hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole and Its Reaction With DNA," *Biochem. Biophys. Res. Comm.* **96**, 355–362 (1980).
88. M. C. Archer, "Reactive Intermediates From Nitrosamines," in R. Snyder, ed., *Biological Reactive Intermediates—II, Advances in Experimental Medicine and Biology*, Vol. 136B, Plenum Press, New York, 1982, pp. 1027–1035.

DOMINIC W. S. WONG
USDA
Albany, California

FOOD CONSUMPTION SURVEYS IN THE U.S. DEPARTMENT OF AGRICULTURE

One mission of the U.S. Department of Agriculture (USDA) is to encourage the production and availability of a sufficient, safe, and nutritionally adequate supply of food for Americans. In support of this mission, USDA conducts surveys to monitor food use and food consumption patterns in the U.S. population. Early studies on food and nutrition were begun at the end of the last century. These small-scale studies aimed to help people in the working class achieve good diets at low cost. As time went on, recognition of the need for nationally representative food and nutrient intake data resulted in the development of larger surveys. The USDA's most recent national food consumption survey was the 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII), which measured the kinds and amounts of foods eaten by Americans. Conducted as a telephone follow-up to the CSFII, the 1994–1996 Diet and Health Knowledge Survey (DHKS) measured attitudes and knowledge about diet and health among Americans. The CSFII and DHKS were designed so that individuals' attitudes and knowledge about healthy eating could be linked with their food choices and nutrient intakes. Both surveys address the requirements of the National Nutrition Monitoring and Related Research Act of 1990 (P.L. 101-445) for continuous monitoring of the dietary status of the American population, including the low-income population. See the section "The National Nutrition Monitoring and Related Research Program."

USES OF THE DATA

As shown in Table 1, evaluations of diet quality and tracking changes in the diet over time have many useful federal, state, and local applications, including policy formation, program planning, and nutrition education. Users of the survey data include federal government agencies, such as the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), the Federal Trade Commission, and numerous USDA agencies; state agencies and larger county health departments; food and agricultural industries; and universities. The data are used to determine the food choices Americans make and to evaluate the content and adequacy of their diets in relationship to the Dietary Guidelines for Americans (1); other federal government statements of dietary policy, such as the year 2000 nutrition objectives (2); and the National Academy of Sciences' Recommended Dietary Allowances (3). Health interventions such as the National Cancer Institute's "5-A-Day for Better Health Program" (4) and nutrition education materials such as the Food Guide Pyramid (5) are developed and targeted based on survey results. The data are also used to assess the nutritional impact of the USDA's food assistance programs; to estimate exposure to pesticide residues, food additives, and contaminants; to develop food fortification, enrichment, and food labeling policies; and to assess the demand for agricultural products and marketing facilities.

Table 1. Uses of Data from Nationwide Food Surveys

Current nationwide food surveys conducted by USDA's Agricultural Research Service encompass two types of surveys: surveys of foods eaten by individuals both at home and away from home and surveys of attitudes and knowledge about healthy eating, about diet and health relationships, and about dietary guidance. A third type of survey that measured food used by households and the costs of those foods has been discontinued although results from those surveys are still used. Results from these three types of surveys are used for a variety of purposes.

Assessment of dietary intakes

Provide detailed benchmark data on food and nutrient intakes and eating patterns of the population
Monitor the nutritional quality of diets and determine the size and nature of populations at risk of having diets low in certain nutrients
Identify socioeconomic and attitudinal factors associated with diets
Identify changes in food and nutrient consumption that would reduce health risks

Economics of food consumption

Predict demand for agricultural products and marketing facilities
Determine the effects of socioeconomic factors on the demand for food
Determine the demand for food away from home and its effects on the nutritional quality of diets

Food policy, programs, and guidance

Determine appropriate levels of enrichment or fortification based in part on food use
Track use of food labels and their effect on dietary intakes
Monitor food security, hunger, and diet quality
Identify populations that might benefit from intervention programs
Determine the amounts of foods that are suitable to offer in food distribution programs
Identify factors affecting participation in some food programs and estimate the effect of participation on diet quality
Estimate the effect of food programs on demand for food
Develop food plans that reflect food consumption practices and meet nutritional and cost criteria
Develop food guides and dietary guidance materials that target nutritional problems in the U.S. population
Identify educational strategies to increase the knowledge of nutrition and to improve the eating habits of Americans

Food safety

Estimate exposure to pesticide residues, food additives, contaminants, and naturally occurring toxic substances
Predict food items in which a food additive can safely be permitted in specified amounts
Determine the need to modify regulations in response to changes in consumption

Historical trends

Correlate food consumption and dietary status with incidence of disease over time
Follow food consumption through the life cycle
Predict changes in food consumption and dietary status as they may be influenced by economic, technological, and other developments

RECENT USDA FOOD CONSUMPTION SURVEYS

The 1994–1996 CSFII and the 1994–1996 DHKS, popularly known as the *What We Eat in America* survey, are the most recent surveys conducted by the USDA (6). The 1994–1996 survey collected dietary data on individuals of all ages in the United States. A survey of food and nutrient intakes by children under 10 years of age was conducted in 1998 as a supplement to the CSFII 1994–1996. The Supplemental Children's Survey (SCS) was conducted in response to the 1996 Food Quality Protection Act of 1996 (P.L. 104–170), which required the Secretary of Agriculture to provide the EPA with information on food consumption patterns of a statistically valid sample of infants and children. This requirement follows a 1993 report of the National Academy of Sciences entitled *Pesticides in the Diets of Infants and Children* in which concern was raised that current food consumption data did not provide sufficient sample sizes for adequate estimation of exposure to pesticide residues in the diets of children (7).

In the CSFII/DHKS 1994–1996, a nationally representative sample of individuals of all ages was asked to provide, through in-person 24-h recall interviews, information about their food intakes on two nonconsecutive days as well as socioeconomic and health-related information. About two weeks after the food intake interview, a subsample of individuals age 20 years and over was asked to answer a series of questions about knowledge and attitudes toward dietary guidance and health. The sample design included oversampling of low-income individuals to yield a national sample of the low-income population. The final survey data set includes information on two days of food and nutrient intake data for more than 15,000 individuals and information on diet, health, and food safety issues from nearly 6000 adults who also responded to the DHKS (6). The method of data collection for the SCS is identical to that used in the 1994–1996 CSFII; the SCS includes two days of dietary intake on approximately 5000 children birth through 9 years of age.

Methodology research has always been important in planning the increasingly complex food consumption surveys at the USDA. Most surveys have contained a component to improve methods in future surveys as well as to evaluate food consumption of the sample under study. Such studies build on earlier experience and findings (8). To meet the objectives of improving the accuracy and comprehensiveness of dietary intake data and developing a more cost-effective method of data collection for national surveys of food consumption to be conducted in the year 2000 and later, the USDA's Food Surveys Research Group (part of the Beltsville Human Nutrition Research Center of the Agricultural Research Service) has a dietary survey methods research program. The objective of this research program is to develop and test new dietary intake methodology, utilizing a telephone mode of data collection. This research includes the development and testing of a computer-assisted telephone interview (CATI) for collecting dietary data and the selection and testing of food measurement aids to improve portion size estimation. A critical component of the research program is a large-scale validation study of 400 individuals to compare estimates of energy intake as determined by the 24-hour dietary recall method against estimates of actual energy expenditure by individuals as determined by the doubly labeled water technique (9). The doubly labeled water technique provides precise measures of energy expenditure in free-living individuals and may be used to validate the assessment of energy intake by other methods (10).

HISTORY OF USDA FOOD CONSUMPTION SURVEY METHODOLOGY

Household Food Consumption Surveys

The USDA has conducted household food consumption surveys for about 100 years. In 1894 Congress mandated that human nutrition investigations be conducted by the USDA Office of Experiment Stations. W. O. Atwater, the first director of the Experiment Stations, is credited with the first food consumption studies in the United States in the late 1800s. He recognized the essential links between such studies and research on food composition, nutritional requirements, and dietary guidance, and he pioneered studies in all of these areas. Atwater (11,12) sought food consumption information that would help him develop recommendations on what a working man should eat and how families could spend their food money wisely. By 1898 USDA investigators had made studies of food consumption by more than 300 families (13). In early studies, participants were "willing families." Researchers used a food inventory-record to collect data by determining the weight and cost of food used by the family from inventories of food on hand at the start and end of the survey period and records of foods brought into the home during the period (14).

Because this complex procedure was found to be too intrusive, too time-consuming, and too costly, it was replaced in the 1930s by the food list-recall (or food list). The new technique required only an interview with the household respondent (usually the homemaker) who recalled, using the food list, the quantities of listed foods used by the household during the preceding week and the amounts paid for purchased items. Although the list-recall procedure was introduced with little preliminary study, response rates for the food list-recall were later shown to be much higher than for the food inventory-record method (8).

Nationwide Household Food Consumption Surveys

The USDA began periodic nationwide surveys of households in the 1930s using the list-recall method along with statistical sampling techniques that permitted the collection of data from large numbers of households in relatively short periods of time. In the 1930s and 1940s, nationwide surveys included the 1935–1936 Consumer Purchases Study, the 1942 Spending and Saving in Wartime Survey, and a survey of food consumption of urban families in 1948. The surveys in 1935–1936, 1942, and 1948 preceded the advent of probability sampling in surveys (15) and were less than fully representative of the U.S. population (8).

Later household surveys attempted to represent the total U.S. population. Their results indicated the way U.S. food supplies were distributed among households of different sizes, income, and location. Because each food was reported as brought into the household, its cost could be obtained and an estimate made of the money value of food used in households. The 1955 Household Food Consumption Survey (HFCS) was the first of the USDA's food consumption surveys in which a commercial firm selected the sample and collected and edited the data. The USDA retained responsibility for overall planning and monitoring of all survey operations, provision of support data for analysis, and the interpretation and publication of results. The 1965–1966 HFCS was the first to cover all four seasons of the year and to collect information on food intake by individuals (see later), as well as the food used by the household as a whole.

In addition to the periodic nationwide surveys of households, the USDA has conducted smaller methodological or special-purpose surveys of food consumption. More precise methods evolved as various problems were investigated (8). For example, several studies addressed survey methodology issues such as food discard measurement, questionnaire design and wording, and interviewer training and compared the food inventory-record and food list-recall methods of data collection, confirming the decision to adopt the list-recall technique for use in future surveys (16–21). Other studies (22,23) explored techniques for collecting dietary data from individuals. The 1950s and early 1960s saw widespread concern for disadvantaged and low-income families, and the Pilot Food Stamp Program was initiated in 1961 in eight economically depressed areas. A before-and-after study of food consumption and dietary levels in an urban and a rural area showed that the Food Stamp Program increased the purchase of more nutritious foods by needy families and also expanded the market for agricultural products—a major government objective being to utilize farm surpluses (24). The Food Stamp Program became permanent with the Food Stamp Act of 1964.

Two additional nationwide household food consumption surveys were conducted—in 1977–1978 and in 1987–1988.

The 1977–1978 Nationwide Food Consumption Survey (NFCS) included five supplemental surveys: low-income households, households with elderly persons, and households in Puerto Rico, Alaska, and Hawaii. Some of these supplemental surveys facilitated studies of factors affecting food consumption and diet quality among groups eligible for some of the USDA's food assistance programs; others provided information on food consumption in locations outside the conterminous states that had not previously been studied (13). In 1979–1980, a follow-up survey to the 1977–1978 low-income survey was conducted to assess whether shifts had occurred in food consumption and dietary quality that might be associated with rising food costs and changes in the Food Stamp Program since the survey of similar households in 1977–1978.

As food supplies increased and became more varied, the number of foods on the food list-recall form increased rapidly: about 200 items in 1948, about 2300 items in 1977, and nearly 3000 items by 1987 (8). In the NFCS of 1987–1988, one innovation was the use of a laptop computer programmed to handle the burden of a growing food list. The computer was intended to improve processing time also.

The collection of two types of information—household use of food and individual intake—in the nationwide surveys of 1965–1966, 1977–1978, and 1987–1988 created large respondent burden and, in the 1987–1988 survey, low response rates. Because of these concerns and increasing national awareness of the relationship between diet and health, the collection of information on household use of food was discontinued after the 1987–1988 survey, completing the shift in emphasis from collecting household food use data to individual intake data.

Dietary Intake by Individuals

Information on food intakes by individuals is more precise than household food use data for the assessment of diet quality (13). For several reasons, household data were less than ideal for analyses of diet quality relative to the Recommended Dietary Allowances (RDA), which were the only standards available (25). Household food consumption data included food discard, resulting in overestimates of nutritional quality. To compare household intake levels with the RDAs, it was necessary to adjust for the consumption of food away from home, which was not surveyed, as well as to make various assumptions related to the apportionment of food among household members and their differing nutritional needs. Individual intake data represent foods as eaten, excluding food discard and including both food eaten at home and food away from home. Individual intakes can appropriately be compared with sex- and age-specific RDAs. The first USDA nationwide survey of food intakes by individual members of households was conducted in spring 1965 as a supplement to the 1965–1966 HFCS. One day of intake for about 14,500 individuals in the United States was collected using a one-day (24-hour) recall.

The 1965 individual intake data were found to be very useful as baseline data, resulting in many requests for enlarging their scope—more intake days per individual, all seasons, and more questions on dietary practices. Consequently, the scope of the second nationwide survey of dietary intakes by individuals was greatly expanded in 1977–1978 and the name changed from the *Household* Food Consumption Survey to the *Nationwide* Food Consumption Survey. Three consecutive days of food intake were collected in four seasons for more than 30,000 individuals in the 48 conterminous states. In the NFCS 1977–1978, individuals provided information on their own intakes, unlike in the HFCS 1965 when the household respondent had provided information for all household members. In 1987–1988, another NFCS was conducted, which included the collection of three consecutive days of food intake data from about 15,000 individuals. Most of the procedures used to obtain food intake reports were similar in the two NFCS surveys. Intake data were collected using a 24-hour recall and a two-day record. Interviewers collected the first day of data in the home and instructed the individuals in the households on filling out the two-day records. The interviewer then returned after the third intake day to answer any questions and collect the records.

In 1985 the first national USDA survey of dietary intake by individuals independent of a household food use component began. Its purpose was to collect data more frequently than every 10 years, thus providing up-to-date information on the adequacy of diets of selected population groups and early indications of dietary changes—important considerations for data that are used in planning food assistance and educational programs and in administering a variety of public programs affecting the supply, safety, and distribution of the nation's food. The CSFII was repeated in 1986. In both years the survey included two nationally representative samples—an all-income sample and a low-income sample. In both years, the all-income sample included about 1500 women 19 to 50 years of age and about 500 of their children 1 to 5 years of age. The low-income sample included about 2100 women and about 1200 children 1 to 5 years of age in 1985 and about 1300 women and 800 children in 1986. A sample of men age 19 to 50 years of age was included in 1985 only. Food intake data were collected using a panel approach: Collection from each individual took place on up to six nonconsecutive days at intervals of approximately two months over a one-year period. The first day of intake was collected using an in-person interview; subsequent days of data were collected by telephone when possible. A 24-hour dietary recall was used for all intakes.

In 1989 the panel aspect of the 1985 and 1986 CSFII was dropped and the CSFII 1989–1991 was conducted using the same methodology as the individual intake portion of the NFCS 1987–1988. Individuals who took part in the CSFII 1989–1991 were asked to provide three consecutive days of dietary data. The first day's data were collected in an in-home interview using a one-day (24-hour) dietary recall. The second and third days' data were collected using a self-administered two-day dietary record.

Diet and Health Knowledge Survey

In 1989 the DHKS was initiated to improve understanding of factors that affect food choices and provide a link between an individual's knowledge and attitudes and his or her dietary behavior. Individuals who were identified as the main meal-planners/preparers in the CSFII were contacted by telephone, if possible, about six weeks after collection of the dietary data and were asked to answer a series of questions about knowledge and attitudes toward diet, health, and food safety. In the 1994–1996 DHKS, adults 20 years and over replaced main meal-planners/preparers as the unit of observation.

An overview of USDA nationwide food surveys is provided in Table 2.

TRENDS IN FOOD CONSUMPTION

In the depression years of the 1930s, concern about the quality of American diets was high. To address this and other issues related to family economics, the USDA and the Bureau of Labor Statistics conducted several national studies (13). The comprehensive picture of household food consumption and dietary levels obtained in the Consumer Purchases Study of 1935–1936 indicated that one-third of the nation's families had diets that were poor by nutritional standards (26). These findings gave impetus to the enrichment of white flour and bread with iron and three B vitamins, establishment of the National School Lunch Program, and expansion of nutrition education and research. Also, USDA economists used results to project food consumption in the United States and to develop food budgets to help families select good diets. A later version of the least costly of these food budgets, the Thrifty Food Plan, is still used in the federal formulas for counting the nation's poor (27) and for setting benefit levels in the Food Stamp Program (28).

The 1942 Spending and Saving in Wartime Survey measured the early effects of World War II on food consumption in urban, rural, and farm families at different income levels (29). The survey found marked improvement from the 1930s in diets overall, but many families' intakes of several nutrients were low in comparison with the new standards, the RDAs, first issued in 1941. Between the 1935–1936 survey and the 1948 survey of urban areas, great strides were made in the distribution and storage of food products, most notably in home refrigeration. These changes affected the way people purchased and used food.

Between the household food consumption surveys of 1955 and 1965–1966, the availability and consumer acceptance of many new, more convenient food products changed the cooking practices in many American households. For example, the use of mixes for baked products such as cakes and muffins and the availability of ready-made baked products led to a decrease in baking "from scratch," and household consumption of flour, sugar, and other basic baking ingredients decreased.

Between 1965–1966 and 1977–1978, the proliferation of new products was especially marked. Technological changes, such as freeze-dried coffee, and the increasing variety of commercially frozen foods reflected breakthroughs in food processing and packaging. Lifestyle changes, such as increases in the proportion of women employed outside the home, may have decreased the time spent in meal preparation and increased the demand for convenience foods and fast-food restaurants.

Between 1977 and 1985, when the CSFII was initiated, substantial changes occurred in food intakes—shifts to lower fat milk, less meat eaten separately (not as part of a mixture), and more grain products. These shifts, most prominent among higher income, more educated respondents, may have reflected concerns about diet and health issues. Nutrient intakes were at least as good, if not better in some respects, in 1985 than in 1977. However, the intakes of some nutrients were still below the 1980 RDAs (30); these observations were apparent at all levels of income and in all geographic regions.

The NFCS 1987–1988 showed a continuation of the dietary trends observed between 1977 and 1985 toward lower-fat milk, less meat eaten separately, and more grain products. Total fat intakes as a percentage of calories fell from 40% in 1977–1978 to 36% in 1987–1988. More of the household food dollar was spent away from home, and fewer meals were consumed from household food supplies in 1987–1988 than in 1977–1978. These changes may have resulted from a desire for increased convenience and variety. The food industry responded in a number of ways: more and varied restaurants, more microwavable packaging, and more bakeries, delicatessens, and salad bars in supermarkets.

In 1989–1991 survey data indicated that eating habits more closely followed national dietary guidelines than in the past, but the amount of fat in the average diet was still higher than the 30% recommended by the Dietary Guidelines for Americans (1), and Americans were eating lower amounts of fruits, vegetables, grains, and low-fat dairy products than recommended. In 1989–1991, results from the DHKS showed that only about one-fourth of main meal-planners/preparers met dietary recommendations for fat and saturated fat, and that their perceptions about their diets did not always match reality.

Data from the 1994–1996 CSFII indicated a continued decline in the percentage of calories from fat—from 36% in 1987–1988 to 33% in 1994–1996. However, only about one-third of adults had fat intakes that provided 30% or less of calories. Consumption of grain-based products, especially grain mixtures such as pizza and lasagna, continued to rise between the late 1980s and the mid-1990s. Among young children, consumption of fluid milk decreased by 16% since the late 1970s, while consumption of carbonated soft drinks increased by 16%.

PYRAMID SERVINGS DATA

Along with improvements in data collection methods, another methodological advance during the 1990s involves the way information on food intakes by individuals is reported to the public. Since the 1965–1966 HFCS, when information on the intakes of food by individuals was collected for the first time, average quantities of foods consumed have been reported in grams or as the percentages of individuals consuming food from selected food groups or subgroups.

Information expressed as percentages of individuals using food or as gram amounts of food has numerous uses, including comparing food consumption over time. Also, nutrient intakes could be calculated from food intakes and then compared with standards such as the RDAs (3). These comparisons gave important information about the ade-

Table 2. Overview of USDA Nationwide Food Surveys, 1936 to 1998

Survey	Population	Sample	Type of data collected	Dietary method
1935–36 Consumer Purchases Study	Farm, village, and city households in five geographic regions	Husband and wife families, white and native born	Household food use	Seven-day list-recall, seven-day food inventory record
1942 Family Spending and Saving in Wartime	Cities, rural nonfarm areas, and farms	Housekeeping families and single persons	Household food use	Seven-day list-recall
1948 Food Consumption of Urban Families	Urban families nationwide in spring plus surveys in four cities	Housekeeping families of two or more persons	Household food use	Seven-day list-recall
1955 Food Consumption of Households	48 states plus a supplement of farm households	National, self-weighting probability sample of housekeeping households	Household food use	Seven-day list-recall
1965–66 Household Food Consumption Survey (HFCS)	48 states	Two separate samples (basic and low income); selected household members were asked to provide intake information	Household food use Individual intake	Seven-day list-recall 24-h dietary recall in spring only
1977–78 Nationwide Food Consumption Survey (NFCS)	48 states	Two separate samples (basic and low income); all household members were asked to provide intake information	Household food use Individual intake	Seven-day list-recall Three consecutive days: 24-h dietary recall and two-day diet record
1985–86 Continuing Survey of Food Intakes by Individuals (CSFII)	48 states	Two separate samples (basic and low income); women 19–50 years and their children 1–5 years in both years and men 19–50 years in 1985 only	Individual intake	Women and children: six nonconsecutive 24-h dietary recalls; day one in person and remaining days by telephone Men: day one only
1987–88 NFCS	48 states	Two separate samples (basic and low income); all household members were asked to provide intake information	Household food use Individual intake	Seven-day list-recall Three consecutive days: 24-h dietary recall and two-day diet record
1989–91 CSFII	48 states	Two separate samples (basic and low income); all household members were asked to provide intake information	Individual intake	Three consecutive days: 24-h dietary recall and two-day diet record
1989–91 Diet and Health Knowledge Survey (DHKS)	48 states	Main meal-planners/preparers with a completed day one intake in CSFII	Dietary knowledge, behavior, and attitudes	Telephone followup to CSFII
1994–96 CSFII	50 states	Oversampling of the low-income population; only selected household members were asked to provide intake information	Individual intake	Two nonconsecutive 24-h dietary recalls
1994–96 DHKS	50 states	Adults 20 years and over with a completed day one intake in CSFII	Dietary knowledge, behavior, and attitudes	Telephone followup to CSFII
1998 Supplemental Children's Survey to CSFII 1994–96	50 states	Children 0–9 years	Individual intake	Two nonconsecutive 24-h dietary recalls

quacy of diets but were difficult to interpret to the public, especially in light of more recent dietary recommendations that specified recommendations as the number of servings from specified food groups to eat each day. These recommendations are from the Dietary Guidelines for Americans and the USDA Food Guide Pyramid (1,5). To meet the need for a more easily understood way to report on the adequacy of food intakes, USDA developed a method for converting data from the CSFII into servings (31). This method facilitates comparisons of food intakes by Americans to dietary recommendations in the Food Guide Pyramid. The Pyramid shows a recommended range of servings to eat each day from five major food groups—grain, vegetable, fruit, dairy, and meat. In general, the number of servings that is right for a person depends on calorie needs; people who need more calories should eat more servings. The Pyramid also provides guidance to help people choose diets low in fat and moderate in sugars.

The method developed by the USDA adheres to Pyramid principles, uses the serving sizes specified by the Pyramid, and strictly categorizes foods according to Pyramid criteria (5). Since many foods people eat—foods like pizza, soups, and pies—count toward more than one food group, the method separates foods into their ingredients before servings are counted. Table 3 compares the average American diet against Food Guide Pyramid recommendations. The average number of servings from the fruit, dairy, and meat groups are below minimums recommended. Those from the grain and vegetable groups are near the bottom of recommended ranges.

The Pyramid recommends that Americans limit the fat in their diets to 30% of calories. People will consume about half of this amount as nondiscretionary fat if they eat the recommended number of servings from each food group, select the lowest fat choices within each group, and add no fat to their foods in preparation or at the table. Additional fat, up to 30% of calories, is considered discretionary in that people can decide whether to get it from higher-fat food choices or from additions to their foods during preparation or at the table. Examples of discretionary fat are the fat absorbed by french-fried potatoes during preparation, the fat from margarine spread on bread at the table, and the fat in whole milk. In 1994–1996, the average fat intake of Americans, at 33% of calories, exceeded the recommendation. Only 8% of calories were from nondiscretionary fat sources. Discretionary fat accounted for 25% of calories.

The Pyramid suggests that Americans try to limit their added sugars (defined as any sugar that is not naturally occurring in the food) to 6 tsp a day if they eat about 1600 calories, 12 tsp at 2200 calories, or 18 tsp at 2800 calories. In 1994–1996, Americans consumed an average of 20 tsp of added sugars a day in a diet that provided about 2000 calories. Added sugars accounted for 16% of calories.

THE NATIONAL NUTRITION MONITORING AND RELATED RESEARCH PROGRAM

"Growing recognition of the relation of food to health and its consequent social implications has created widespread interest in estimates of the adequacy of diets of different population groups." That statement, written in 1939 by Hazel K. Stiebeling and Esther F. Phipard in the introduction to an early survey report from the USDA, still rings true (32). Although tremendous progress in evaluating dietary adequacy occurred during the first hundred years of nutrition research, many research needs remain. In contrast with W. O. Atwater's landmark work in the late 1880s and early 1890s (11), which yielded information only on dietary energy, protein, fat, and carbohydrate, national food consumption surveys at the end of the twentieth century provide information on about 40 additional nutrients and dietary components. Yet, as we face the new millennium, there are still nutrients for which the analytical methodology continues to evolve, and there are still blanks in our understanding of the relationships between diet and health.

Nutrition research encompasses many different fields, ranging from agriculture and economics to biochemistry and medicine. The missions of many different agencies of the U.S. government include responsibility for monitoring or promoting the health of different population subgroups. In the 1990s, with passage of the National Nutrition Monitoring and Related Research Act of 1990 (P.L. 101-445), the federal government set in place a comprehensive plan to link all the agencies engaged in food and nutrition monitoring activities.

The 1990 Act required the federal government to develop the National Nutrition Monitoring and Related Research Program (NNMRRP) along with a Ten-Year Comprehensive Plan for Nutrition Monitoring and Related Research. As required by law, the plan was transmitted by the President to Congress and was published in the *Federal Register* on June 11, 1993 (33). The NNMRRP in the United States is a complex system of coordinated activities that provides information about the dietary, nutritional, and related health status of Americans; the relationships between diet and health; and the factors affecting dietary and nutritional status. The Ten-Year Plan serves as the basis for planning and coordinating the activities of the more than 20 federal agencies responsible for nutrition monitoring and related research activities. The primary goals of the plan are to ensure that the agencies participating in the NNMRRP collect data that are continuous, timely, and reliable; coordinate data collection with other member agencies; use comparable methods for data collection and reporting of results; and conduct research on the issues and topics relevant to monitoring the nutritional and health status of the population and subgroups at nutritional risk.

Prior to the NNMRR Act of 1990, nutrition monitoring research was conducted as a part of the National Nutrition Monitoring System (NNMS). The history of the goals and milestones of federal nutrition monitoring in the United States can be found in Refs. 34 to 37.

Two comprehensive reports on nutrition monitoring activities conducted as part of the NNMS were published in the late 1980s. The first report, *Nutrition Monitoring in the United States: A Progress Report from the Joint Nutrition Monitoring Evaluation Committee*, was prepared by a federal advisory committee jointly sponsored by the U.S. Department of Health and Human Services (HHS) and the USDA (36). The report provided an overview of the dietary and nutritional status of the U.S. population and was intended to serve as a reference, or baseline, for subsequent reports. The two major data sources for that report were the National Health and Nutrition Examination Survey of 1976–1980 conducted by HHS and the NFCS of 1977–1978 conducted by the USDA. The second report, *Nutrition Monitoring in the United States: An Update Report on Nutrition Monitoring* (37), used data produced or released since publication of the 1986 report to provide an update on the dietary and nutritional status of the U.S. population and on selected health conditions and behaviors.

Table 3. Comparison of the Average American Diet Against Food Guide Pyramid Recommendations

Food group	Recommended range of servings	Average number of servings consumed per day, two-day average	Percentages of individual consuming servings recommended based on caloric intake, two-day average[a]
Grain group	6 to 11	6.7	38
Vegetable group	3 to 5	3.3	41
Fruit group	2 to 4	1.5	23
Dairy group	2 to 3	1.5	23
Meat group (ounces)[b]	5 to 7	4.7	32

Source. The Food Guide Pyramid (5). USDA's Continuing Survey of Food Intakes by Individuals, 1994–1996; individuals 2 years and older (6).

[a] Recommended amounts were derived from sample patterns in the Food Guide Pyramid (5). For the grain, vegetable, fruit, and meat groups, individuals consuming less than 2200 calories met the recommendation if they consumed at the bottom of the range of servings, individuals consuming 2200 up to 2,800 calories meet the recommendation if they consumed in the middle of the range, and individuals consuming 2800 calories or more met the recommendation if they consumed at the top of the range. For the dairy group, women who were pregnant or lactating and individuals 11 through 24 years of age were counted as meeting the recommendation if they consumed at least three dairy servings a day; all other individuals were counted as meeting the recommendation if they consumed at least two dairy servings a day.

[b] The Pyramid recommends that servings of the meat group should be the equivalent of 5 to 7 oz of cooked lean meat, poultry, or fish. According to the Pyramid, 1 egg, 1/2 c of tofu, 2 tb of peanut butter, 1/3 c of nuts, and 1/4 c of seeds are each equivalent to 1 oz of cooked lean meat.

A third report was published following passage of the 1990 Act. The *Third Report on Nutrition Monitoring in the United States* was based on data gathered by the NNMRRP since the 1989 report, giving special emphasis to low-income and high-risk population subgroups (38). This report concluded with a chapter on 33 recommendations spanning all the component areas. Future years will no doubt see the achievement of many of those recommendations as well as the emergence of new opportunities for the expansion of nutrition knowledge.

BIBLIOGRAPHY

1. U.S. Department of Agriculture and U.S. Department of Health and Human Services, *Nutrition and Your Health: Dietary Guidelines for Americans*, 4th ed., Home and Garden Bull. No. 232, U.S. Dept. of Agriculture, Washington, D.C., 1995.
2. U.S. Department of Health and Human Services, Public Health Service, *Healthy People 2000: National Health Promotion and Disease Prevention Objectives*, DHHS Publication No. (PHS) 91-50212, U.S. Government Printing Office, Washington, D.C., 1991.
3. Food and Nutrition Board, National Research Council, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
4. A. S. Subar et al., *5-a-Day for Better Health: A Baseline Study of Americans' Fruit and Vegetable Consumption*, National Institutes of Health, National Cancer Institute, Washington, D.C., 1992.
5. *The Food Guide Pyramid*, Home and Garden Bul. No. 252, U.S. Dept. of Agriculture, Washington, D.C., 1992.
6. *The 1994–96 Continuing Survey of Food Intakes by Individuals and the 1994–96 Diet and Health Knowledge Survey*, CD-ROM, NTIS Accession No. PB98-500457, U.S. Dept. of Agriculture, Washington, D.C., 1998.
7. National Research Council, *Pesticides in the Diets of Infants and Children*, National Academy Press, Washington, D.C., 1993.
8. E. M. Pao, K. E. Sykes, and Y. S. Cypel, *USDA Methodological Research for Large-Scale Dietary Intake Surveys*, 1975–88, Home Econ. Res. Rep. No. 49, U.S. Department of Agriculture, Washington, D.C., 1989.
9. D. A. Schoeller, "Measurement of Energy Expenditure in Free-Living Humans by Using Doubly Labeled Water," *J. Nutr.* **118**, 1278–1289 (1988).
10. A. E. Black et al., "Measurements of Total Energy Expenditure Provide Insights Into the Validity of Dietary Measurements of Energy Intake," *J. Am. Dietetic Assoc.* **93**, 571–579 (1993).
11. W. O. Atwater, *Foods: Nutritive Value and Cost*, Farmers' Bul. No. 23, U.S. Dept. of Agriculture, Washington, D.C., 1894.
12. W. O. Atwater, *Methods and Results of Investigations of the Chemistry and Economy of Food*, Expt. Sta. Office Bull. No. 21, U.S. Dept. of Agriculture, Washington, D.C., 1895.
13. B. B. Peterkin, "USDA Food Consumption Research: Parade of Survey Greats," *J. Nutr.* **124**, 1836S–1841S (1994).
14. A. P. Bryant, "Some Results of Dietary Studies in the United States," in *USDA Yearbook of Agriculture 1898*, U.S. Dept. of Agriculture, Washington, D.C., 1899, pp. 439–452.
15. W. G. Cochran, *Sampling Techniques*, John Wiley and Sons, New York, 1953.
16. S. Adelson, E. Asp, and I. Noble, "Household records of food used and discarded," *J. Am. Dietetic Assoc.* **39**, 578–584 (1961).
17. S. F. Adelson and E. C. Blake, *Diets of Families in the Open Country, a Georgia and an Ohio County, Summer 1945*, Misc. Pub. No. 704, U.S. Dept. of Agriculture, Washington, D.C., 1950.
18. F. Clark and L. Fincher, *Nutritive Content of Homemakers' Meals, Four Cities, Winter 1948*, Info. Bul. No. 112, U.S. Dept. of Agriculture, Washington, D.C., 1954.
19. F. Clark et al., *Food Consumption of Urban Families in the United States . . . With an Appraisal of Methods of Analysis*, Ag. Info. Bull. No. 132, U.S. Government Printing Office, Washington, D.C., 1954.
20. B. B. Reagan and E. Grossman, *Rural Levels of Living in Lee and Jones Counties, Mississippi, 1945, and a Comparison of Two Methods of Data Collection*, Ag. Inf. Bull. No. 41, U.S. Department of Agriculture, Washington, D.C., 1951.

21. J. Murray et al., *Collection Methods in Dietary Surveys. A Comparison of the Food List and Record in Two Farming Areas of the South*, S. Coop. Series Bull. 23, S.C. Ag. Expt. Sta, Clemson, S. Car., 1952.
22. S. F. Adelson, "Some Problems in Collecting Dietary Data From Individuals," *J. Am. Dietetic Assoc.* **36**, 453–461 (1960).
23. S. F Adelson, and A. Keys, *Diet and Some Health Characteristics of 123 Business and Professional Men and Methods Used to Obtain Dietary Information*, Ag. Res. Serv. Pub. No 62-11, U.S. Dept. of Agriculture, Washington, D.C., 1962.
24. R. B Reese and S. F. Adelson, *Food Consumption and Dietary Levels Under the Pilot Food Stamp Program, Detroit, Michigan and Fayette County, Pennsylvania*, Ag. Econ. Rep. No. 9, U.S. Dept. of Agriculture, Washington, D.C., 1962.
25. *Dietary Levels: Households in the United States, Spring 1977. Nationwide Food Consumption Survey 1977–78*, Rep. No. H-11, U.S. Dept. of Agriculture, Washington, D C., 1985.
26. H. Stiebeling, *Are We Well Fed?*, USDA Pub. No 430, U.S. Dept. of Agriculture, Washington, D.C., 1941.
27. M Orshansky, "How poverty is measured," *Monthly Labor Review* **92**, 37–41 (1969).
28. B. B. Peterkin et al., *The Thrifty Food Plan, 1983*, Human Nutrition Info. Serv. Admin. Rep. No 365, U.S. Dept. of Agriculture, Washington, D.C., 1983.
29. *Family Food Consumption in the United States*, USDA Misc. Pub. No. 550, U.S. Dept. of Agriculture, Washington, D.C., 1944.
30. Food and Nutrition Board, National Research Council, *Recommended Dietary Allowances*, 9th ed., National Academy Press, Washington, D.C., 1980.
31. L. E. Cleveland et al., "A Method for Assessing Food Intakes in Terms of Servings Based on Food Guidance," *Am. J. Clin. Nutr.* **65** (Suppl), 1254S–1263S, (1997).
32. H. K. Stiebeling and E. F. Phipard, *Diets of Families of Employed Wage Earners and Clerical Workers in Cities*, USDA Circular No. 507, U.S. Dept. of Agriculture, Washington, D.C., 1939.
33. U.S. Department of Health and Human Services and U.S. Department of Agriculture, "Ten-year Comprehensive Plan for the National Nutrition Monitoring and Related Research Program," *Federal Register* **58**, 32751–32806 (1993).
34. M. F. Kuczmarski and R. J. Kuczmarski, "Nutrition Monitoring in the United States," in M. E. Shils, J. S. Olson, and M. Shike, eds, *Modern Nutrition in Health and Disease*, Vol. 2, 8th ed., Lee & Febiger, Philadelphia, Penn., 1994, pp. 1506–1516.
35. M. F. Kuczmarski, A. Moshfegh, and R. Briefel, "Update on Nutrition Monitoring Activities in the United States," *J. Am. Dietetic Assoc.* **94**, 753–760 (1994).
36. U.S. Department of Health and Human Services and U.S. Department of Agriculture, *Nutrition Monitoring in the United States—A Report From the Joint Nutrition Monitoring Evaluation Committee*, DHHS Publication No. (PHS) 86-1255, U.S. Government Printing Office, Washington, D.C., 1986.
37. Federation of American Societies for Experimental Biology, Life Sciences Research Office, *Nutrition Monitoring in the United States—An Update Report on Nutrition Monitoring*, DHHS Pub No. (PHS) 89-1255, Public Health Service, U.S. Government Printing Office, Washington, D.C., 1989.
38. Federation of American Societies for Experimental Biology, Life Sciences Research Office, *Third Report on Nutrition Monitoring in the United States*, U.S. Government Printing Office, Washington, D.C., 1995.

KATHERINE S. TIPPETT
CECILIA WILKINSON ENNS
ALANNA J. MOSHFEGH
USDA/ARS
Beltsville, Maryland

FOOD CROPS: NONDESTRUCTIVE QUALITY EVALUATION

Quality of food crops is not a single characteristic but comprises many properties or attributes. Quality encompasses sensory properties (appearance, texture, and flavor), nutritive values, chemical constituents, mechanical properties, functional properties, and defects. It is convenient to think of *quality* as the composite of intrinsic characteristics of the commodity and *acceptability* as people's perceptions of and reactions to those characteristics. Instrumental methods for measuring quality of food crops were introduced in the 1920s, with improvements and new technologies being introduced continuously. Instrumental methods generally measure chemical constituents or physical properties of the commodity that can be related directly or indirectly to functional behavior or sensory acceptability. Measurement by some instrumental methods involves preparation that destroys the sample, which restricts use to a limited number of random samples for predicting quality of the population. Nondestructive methods leave the product intact and undamaged so it can be stored or marketed after analysis. Nondestructive methods are preferred over destructive methods for commercial purposes and for monitoring quality over time in research programs. Various methods are available for measuring specific quality attributes nondestructively; a few are used commercially, some are used in postharvest research, and others are in the developmental stages.

PRINCIPLES USED FOR NONDESTRUCTIVE INSTRUMENTAL QUALITY EVALUATION

Nondestructive instrumental methods for measuring quality involve electromagnetic (visible and near infrared light, X-ray, and magnetic resonance), electrochemical, or mechanical (force, deformation, or vibration) responses. These principles are applied in the following methods of measurement.

DESCRIPTION AND APPLICATION OF METHODS

Electromagnetic Radiation

Electromagnetic wavelengths encompass, from longest to shortest, radio waves, microwaves, light, X-ray, and gamma waves. Light here refers to ultraviolet, visible, near infrared, and infrared wavelengths. Visible light is that portion of the electromagnet spectrum that humans can see. The visible and near infrared ranges have been most used for measuring quality, but X-ray and magnetic resonance permit deeper penetration of the tissues.

Light

Visible and Near Infrared Wavelengths. Each chemical bond in the chemical constituents of the tissue absorbs energy in a characteristic manner that causes the absorption of only certain wavelengths of light. Analysis of the relative amount of each wavelength of light that is reflected or transmitted through the tissues can inform us of the concentration of pigments, sugars, oils, and certain other chemical constituents within the tissue. Simple measurements may involve the difference or ratio between two specific wavelengths of light. Some defects are presently detected by using reflectance images taken at two wavelengths where sound and damaged tissue have different relative absorbances. Recently whole-spectrum data reduction techniques using partial least squares (PLS) or similar mathematical treatments are being implemented to reduce spectral data to a small number of basic *factors* that are then used in multiple regression equations to predict components. PLS has been particularly useful for near infrared data.

Reflectance, Transmittance, and Interactance. Light can be measured in reflectance, transmittance, or interactance (body reflectance) modes. Food crops are cellular tissues that are highly light scattering. Most of the light impinging on a food crop penetrates its surface and interacts with the chemical constituents and physical structure of the tissue. The small portion of light that is reflected directly at the surface is called specular reflectance or gloss and is not generally useful for measuring quality. The portion that is reflected after very shallow penetration is usually also called reflectance but is more properly termed diffuse reflectance. Reflectance from deeper within the tissues may be called interactance or body reflectance. Energy that passes entirely through the product is called body transmittance or direct transmittance, depending on whether the material does or does not scatter the light internally ("clear" juices or oils pressed from some fruits and vegetables cause little scattering).

The principle of light absorption by specific chemical bonds applies whether the measurement is reflectance or transmittance. The measurement of transmittance requires that the illuminating light must not reach the detector except by passing through the product. Such a light seal is difficult to achieve with irregularly shaped products like fruits and vegetables, especially at high speeds. Therefore body reflectance is preferred over transmittance measurement for on-line sorting operations that must handle each sample in a fraction of a second (about 5 to 12 fruit per second is currently desired for various products). However, the distribution within the product of pigments or other constituents of interest determines whether reflectance can be used or if transmittance is required.

The reactions of visible light with pigments result in the color we perceive when we view the product: chlorophyll looks green; anthocyanins give us shades of reds, purples, and blues; carotenoids are yellow, orange, or red. The pigment contents of many fruits and vegetables change as the product ripens, so ripeness often can be estimated by measuring pigment content or color. Additionally, color is often associated with consumer acceptability of the product. Reflectance from the outer few cells of the product is used in grading lines of packing houses to sort apples, carrots, citrus, nectarines, peaches, pears, plums, potatoes, and tomatoes according to defined color categories (1). Diffuse reflectance in the 640- to 750-nm wavelength range is used to predict maturity of lettuce, oranges, and peaches (2,3) based on the chlorophyll content, which has peak absorption at 680 nm. The difference in absorbance (optical density) at 690 nm and 740 nm (noted as ΔOD [690–740 nm]) was associated with ripeness of apple (4). Maturity of tomatoes is based on chlorophyll and lycopene contents, which can be predicted by ΔOD (710–780 nm) and ΔOD (570–780 nm) values, respectively (5). Maturity of blueberries, papayas, and peaches can also be predicted by transmittance based on pigment content. The effectiveness of ripeness sorting by these methods depends on the composition of the product, interfering absorbance by other components, and the relationship of pigment content to ripeness. In peaches the density of the stone as well as the amount of anthocyanin around the stone must be considered when using transmittance measurements of chlorophyll content. With apples, watercore may affect chlorophyll readings.

Near-infrared diffuse reflectance spectra are used to predict starch, protein, oil, and moisture contents of grains and oil seeds (6) and sugars in fruit (7,8). Dry matter content of onions and potatoes, mainly carbohydrates, is determined by near infrared wavelengths (9).

The composition and therefore the absorbance characteristics of healthy tissue differ from those of damaged or decayed tissues. Internal defects that can be detected by transmittance include hollow heart in potato tubers, core breakdown in pears, watercore in apples, freeze damage in citrus, and smut content on wheat. Hollow heart detection is based on ΔOD (800–710 nm), core breakdown on ΔOD (690–740 nm), watercore on ΔOD (810–760 nm), and smut on wheat on ΔOD (890–930 nm). Surface defects that can be quantified by reflectance (R) include bruise ($R_{400-450\ \text{nm}}$, $R_{770-890\ \text{nm}}$, and others) (10,11), scald, russet, and other defects of apples; rind defects on lemons ($R_{560-650\ \text{nm}}$); mold, scab, crack, and exposed pit on dried prunes (near infrared wavelengths) (12), and blight, soft rot, and scab in potato tubers (13).

Fluorescence and Delayed Light Emission. Fluorescence is light that is instantaneously emitted by an illuminated sample at a wavelength longer (lower energy) than the illuminating wavelength. Delayed light emission (DLE) is similar to fluorescence but is emitted after a time delay that allows chemical reaction to occur. In fruits and vegetables, chlorophyll exhibits fluorescence and DLE, both with characteristic kinetics (time courses). Fluorescence emission peaks characteristic of the photosystem II antennae (686 nm), reaction center (696 nm), and photosystem I antenna (730–740 nm) are used to study chlorophyll function during the growth and ripening periods of fruit (14). Because chlorophyll content is often related to maturity in fruits and vegetables, chlorophyll fluorescence and DLE have been proposed for measuring maturity of apricots, citrus, melons, papaya, persimmons, tea leaves, and tomatoes (15–18). Total, maximum, or changes in fluorescence or DLE can be used to detect exposure to physiological stress injury such as caused by chilling temperatures, high temperatures, air pollutants, and mechanical stress (15,16,19,20). Equilibration in the dark for up to 10 min may be required to obtain stable values of chlorophyll fluorescence or DLE; however, a relatively recent chlorophyll fluorescence technique, pulse amplitude modulated (PAM) fluorometry, does not require the dark period. PAM fluorescence has been used to follow development of chilling injury (21) and responses to heat treatments (22).

A fluorescence application *not* based on chlorophyll detected mechanical injury of oranges by measuring fluorescence from oils that leaked from damaged oil cells (23).

X ray. X-ray technology has been modified to recognize rapidly internal characteristics of commodities on grading lines or on mechanical harvesters. Absorption of X rays varies directly with density and water concentration. Commercial uses include detection of hollow heart of potatoes (24) and freeze damage in citrus (25). X ray is being studied for automated detection of bruises and watercore in apples (26), split pits in peaches, presence of pits in processed stone fruits, and insect infestation in fruits and nuts (27). In research, X ray computed tomography shows density changes in tomato locules during maturation (28) and internal sprouting and ring separations due to microbial rot in onion (29).

Magnetic Resonance. Nuclear magnetic resonance (NMR) detects concentration and mobility of hydrogen nuclei (and certain other nuclei) and indicates amounts of water, oil, and sugar. Of particular interest in horticultural applications, areas of greater free water content produce a stronger NMR signal than surrounding tissues, so that disorders involving water distribution can be detected (30). Magnetic resonance imaging (MRI) has been used to show morphology, ripening, seeds or pits, voids, pathogen invasion, worm damage, bruises, and other defects, as well as changes due to ripening, heat, chilling, and freezing (30–34). The major disadvantages of MRI are cost and speed. In a less expensive adaptation, a limited volume of tissue is averaged to detect concentrations of sugars or oils within a product (35,36). Currently, MR and MRI are not practical for routine quality testing; but, like all technologies, they are becoming cheaper, faster, and more feasible for research and specialized applications. Magnetic resonance detectors have great potential for evaluating internal quality of products.

Imaging. The electromagnetic technologies—light, X ray, and MR—can be applied using sensors that integrate a limited area or volume of the object being measured or using imaging technology (machine vision). Imaging provides spatial information. Most imaging is two-dimensional, much like the image on a computer monitor or television screen; X-ray CT and MRI can be three-dimensional. The more dimensions, the more expensive is the equipment and the more computationally intensive is the measurement. At present, light imaging is being used on-line for sorting apples, peaches, citrus, and some other commodities according to color, for some surface defects, and for sizing some commodities (11,37–39). Research is directed at improving speed, accuracy, and cost.

Electrochemical

The relative concentrations of volatile compounds (nonaromatic and aromatic) within a fruit or vegetable increase during maturation and ripening. The electrical conductivity of semiconductor gas detectors, based on different polymers and metal oxides, decreases on exposure to volatiles. A battery of several detectors can produce a profile that may indicate maturity or presence of some disorders. The electronic sniffer concept (40,41) has been tested on apples, blueberries, melons, and strawberries. Further research is needed to explore the selection of semiconductors and to relate the profiles to quality categories (42).

Mechanical

Quasi-Static Force and Deformation. Most force/deformation measurements are destructive, for example, the familiar Magness-Taylor fruit firmness test (a penetrometer) or the Kramer shear test (multiblade shear widely used in the processed foods industry), or too slow for on-line use, such as the Cornell firmness tester (a creep tester) (42). A nondestructive, noncontact firmness detector was recently patented (43) that uses a laser to measure the deflection caused by a short puff of pressurized air, similar to some devices used by ophthalmologists to detect glaucoma. Under fixed air pressure, firmer products deflect less than softer ones. This appears to be a quite localized measurement; that is, a very small portion of the total fruit or vegetable is actually tested. In early tests, laser-puff readings correlated fairly well with destructive Magness-Taylor firmness values for apple, cantaloupe, kiwifruit, nectarine, orange, pear, peach, plum, and strawberry.

Dynamic Force and Deformation

Impact. The impact response of a product is directly related to its mechanical properties, mass, and shape. Impact is the rapid collision of two objects, whether one or both objects are in motion. Impact testing techniques include drop, falling mass (or impact probe), and impact ram. A number of impact parameters have been proposed to measure horticultural product firmness, including peak force and time in contact with the impacting object. Impact responses have been studied for apples, blueberries, cherries, kiwifruit, peaches, pears, potatoes, and tomatoes (44–46). One potential problem with impact tests on fruits and vegetables is that some bruising may occur.

Vibration. Sonic (or acoustic) vibrations encompass the audible frequencies from 20 Hz to about 15 kHz and ultrasonic vibrations are above that range. When an object is excited by periodic forces, such as at sonic frequencies, or by certain types of impact it vibrates. Resonance (maximum vibration) occurs at certain frequencies that depend on the mechanical properties, size, shape, and density of the product. The firmer the flesh, the higher the resonant frequency for products of the same size and shape. The traditional watermelon ripeness test is based on the acoustic principle, where one thumps the melon and listens to the pitch (frequency) of the resonance. Resonant frequency, wave propagation velocity, attenuation, and reflection are the important parameters for evaluating texture of horticultural commodities. The sonic vibration method is nondestructive and is suitable for rapid firmness measurement. Sonic measurement generally represents the mechanical properties of the entire product, unlike puncture or compression tests that sample localized tissues. Sonic measurements are excellent for following changes in individuals over time in research applications and are suitable for determining average firmness of grower lots of fruit. Sonics may not be suitable for sorting operations as they have not always proved capable of predicting firmness of individual fruit as determined with a penetrometer (47–53). Some of the fruit tested have been apples, avocados, bananas, grapes, kiwifruit, head lettuce, mangoes, melons, peaches, pears, pineapples, and tomatoes.

Ultrasonic measurement has generally not been successful for quality measurement on fruits and vegetables despite its success in medicine and animal studies. The structure and air spaces in fruits and vegetables make it difficult to transmit sufficient ultrasonic energy through them to obtain useful measurements (54). Wave propagation velocity, attenuation, and reflection are the important ultrasonic parameters for quality evaluation of horticultural commodities. Skin texture of oranges and cracks in tomatoes could be evaluated by reflectance and backscatter, respectively (54). Hollow heart in potatoes was detectable (55), but bruises in apples could not be readily detected (56). Ultrasonic measurements correlated well with firmness or ripeness of melon and avocado (57). However, a more powerful ultrasonic source is required to penetrate most fruits and vegetables.

Sensor Fusion

There is growing recognition that quality is a multifaceted attribute, leading to a need for "sensor fusion" or the combining of several measurements into a quality classification (38,58). The measurements may be from multiple sensors examining different parts of the same fruit but more likely will be from different kinds of sensors that detect different characteristics. Sorting requires high-speed, nondestructive sensing of each piece of fruit or vegetable and a statistical procedure to combine the measurements into a classification decision. Currently, automated sorting is mainly by size, color, and, for some commodities, surface defects. Fruit packinghouses in the United States require sorter speeds of 5 to 12 fruit per second. Statistical combination of measurements will increase the likelihood of predicting overall quality. However, it is important to understand what each sensor measures and to understand the relationship of that characteristic to quality, so the limitations of the sorting method are appreciated.

FUTURE DEVELOPMENTS

Engineers and horticulturists continuously develop new, nondestructive methods for evaluating quality of fruits and vegetables. Some of the methods are incorporated into grading lines to sort the individual fruit or vegetables by one or several quality attributes. Some methods, such as MRI, are too costly or too slow for commercial, on-line implementation at present, but these methods are useful for horticultural research. And new sensing and computing technologies will likely render these sensors commercially economical in the future.

BIBLIOGRAPHY

1. M. J. Delwiche et al., "Surface Color Measurement of Fruits and Vegetables," in G. Brown and Y. Sarig, eds., *Nondestructive Technologies for Quality Evaluation of Fruits and Vegetables*, American Society of Agricultural Engineers, St. Joseph, Mich., 1994, pp. 63–71.
2. E. J. Brach et al., "Lettuce Maturity Detection in the Visible (380–720 nm), Far Red (680–750) and Near Infrared (800–1950) Wavelength Band (*Lactuca sativa*)," *Agron. Sci. Prod. Veg. et de l'Environ* **2**, 685–694 (1982)
3. Y. Chuma, T. Shiga, and K. Morita, "Evaluation of Surface Color of Japanese Persimmon Fruits by Light Reflectance Mechanized Grading Systems," *J. Soc. Agric. Machinery, Japan* **42**, 15–120 (1980)
4. K. L. Olsen, H. A. Schomer, and R. D. Bartram, "Segregation of Golden Delicious Apples for Quality by Light Transmission," *Proceedings of the American Society for Horticultural Science* **91**, 821–828 (1967).
5. A. E. Watada et al., "Estimation of Chlorophyll and Carotenoid Contents of Whole Tomato by Light Absorbance Technique," *J. Food Sci.* **41**, 329–332 (1976).
6. K. H. Norris, "Instrumental Techniques for Measuring Quality of Agricultural Crops," in M. Lieberman, ed., *Postharvest Physiology and Crop Preservation*, Plenum Press, New York, 1983, pp. 471–484
7. S. Kawano, H. Watanabe, and M. Iwamoto, "Determination of Sugar Content in Intact Peaches by Near Infrared Spectroscopy With Fiber Optics in Interactance Mode," *Journal of the Japanese Society for Horticultural Science* **61**, 445–451 (1992).
8. D. C. Slaughter, D. Barrett, and M. Boersig, "Nondestructive Determination of Soluble Solids in Tomatoes Using Near Infrared Spectroscopy," *J. Food Sci.* **61**, 695–697 (1996).
9. G S. Birth et al., "Nondestructive Spectrophotometric Determination of Dry Matter in Onions," *J. Am. Soc. Hortic. Sci.* **110**, 297–303 (1985)
10. B. L. Upchurch et al., "Spectrophotometric Study of Bruises on Whole Red Delicious Apples," *Transactions of the American Society of Agricultural Engineers* **33**, 585–589 (1990).
11. D. J. Aneshansley, J. A. Throop, and B. L. Upchurch, "Reflectance Spectra of Surface Defects on Apples," in *Sensors for Nondestructive Testing*, Proceedings of Sensors for Nondestructive Testing International Conference, Orlando, Fla., Feb. 18–21, 1997. NRAES (Northeast Reg. Agr. Eng. Svc.), Coop. Extn., Ithaca, N.Y., 1997, pp. 143–160.
12. M J. Delwiche, S. Tang, and J. F. Thompson, "A High-speed Sorting System for Dried Prunes," *Transactions of the American Society of Agricultural Engineers* **36**, 195–200 (1993).
13. R. L. Porteous, A. Y. Muir, and R. L. Wastie, "The Identification of Diseases and Defects in Potato Tubers From Measurements of Optical Spectral Reflectance," *Journal of Agricultural Engineering Research* **26**, 151–160 (1981).
14. J. Gross and I. Ohad, "In vivo Fluorescence Spectroscopy of Chlorophyll in Various Unripe and Ripe Fruit," *Photochemistry and Photobiology* **37**, 195–200 (1983).
15. J. A. Abbott et al., "Technologies for Nondestructive Quality Evaluation of Fruits and Vegetables," *Horticultural Reviews* **20**, 1–120 (1997).
16. J. A. Abbott, W. R. Forbus, Jr., and D. R. Massie, "Temperature Damage Measurement by Fluorescence or Delayed Light

Emission from Chlorophyll," in G. Brown and Y. Sarig, eds., *Nondestructive Technologies for Quality Evaluation of Fruits and Vegetables*, American Society of Agricultural Engineers, St. Joseph, Mich., 1994, pp. 44–49.

17. Y. Chuma, M. Ohura, and A. Tagawa, "Delayed Light Emission as a Means of Sorting Tomatoes, DLE Characteristics of Tomatoes Excited by Flash Light," *Journal of the Faculty of Agriculture, Kyushu University* **26**, 159–167 (1982).
18. W. R. Forbus, Jr., G. G. Dull, and D. A. Smittle, "Nondestructive Measurement of Canary Melon Maturity by Delayed Light Emission," *Journal of Food Quality* **15**, 119–127 (1992).
19. R. M. Smillie, and R. Nott, "Assay of Chilling Injury in Wild and Domestic Tomatoes Based on Photosystem Activity of the Chilled Leaves," *Plant Physiol.* **63**, 796–801 (1979).
20. J. A. Abbott, A. R. Miller, and T. A. Campbell, "Detection of Mechanical Injury and Physiological Breakdown of Cucumbers Using Delayed Light Emission," *J. Am. Soc. Hortic. Sci.* **116**, 52–57 (1991).
21. S. Lurie, R. Ronen, and S. Meier, "Determining Chilling Injury Induction in Green Peppers Using Nondestructive Pulse Amplitude Modulated (PAM) Fluorometry," *J. Am. Soc. Hortic. Sci.* **119**, 59–62 (1994)
22. A. B. Woolf and W. A. Laing, "Avocado Fruit Skin Fluorescence Following Hot Water Treatments and Pretreatments," *J. Am. Soc. Hortic. Sci.* **121**, 47–151 (1996).
23. J. L. Uozumi et al., "Spectrophotometric System for Quality Evaluation of Unevenly Colored Food" [in Japanese], *Nippon Shokuhin Kogyo Gakkaishi* [Journal of the Japanese Society of Food Science and Technology] **34**, 163–170 (1987).
24. E. E. Finney and K. H. Norris, "X-ray Scans for Detecting Hollow Heart in Potatoes," *American Potato Journal* **55**, 95–105 (1978).
25. B. L. Upchurch et al., "Detection of Internal Disorders," in G. Brown and Y. Sarig, eds., *Nondestructive Technologies for Quality Evaluation of Fruits and Vegetables*, American Society of Agricultural Engineers, St. Joseph, Mich., 1994, pp. 80–85.
26. E. W. Tollner, J. K. Brecht, and B. L. Upchurch, "Nondestructive Evaluation: Detection of External and Internal Attributes Frequently Associated with Quality or Damage," in S. E. Prussia and R. L. Shewfelt, eds., *Postharvest Handling: A Systems Approach*, Academic Press, New York, 1993, pp. 225–255.
27. T. F. Schatzki et al., "Defect Detection in Apples by Means of X-ray Imaging," in *Sensors for Nondestructive Testing*, Proceedings of Sensors for Nondestructive Testing International Conference, Orlando, Fla., Feb. 18–21, 1997, NRAES (Northeast Reg. Agr. Eng. Svc.), Coop. Extn., Ithaca, N.Y., 1997, pp. 161–171.
28. J. K. Brecht et al., "Using X-ray Computed Tomography (X-ray Ct) to Nondestructively Determine Maturity of Green Tomatoes," *HortScience* **26**, 45–47 (1991).
29. E. W. Tollner et al., "Nondestructive Testing for Identifying Poor Quality Onions," in J. A. DeShazer and G. E. Meyer, eds., *Optics in Agriculture, Forestry, and Biological Processing*, Proceedings of the Society of Photo-Optical Instrumentation Engineers 2345, International Society for Optical Engineering, Bellingham, Wa., 1995, pp. 392–397.
30. C. J. Clark et al., "Application of Magnetic Resonance Imaging to Pre- and Post-Harvest Studies of Fruit and Vegetables," *Postharvest Biology and Technology* **11**, 1–21 (1997).
31. M. Saltveit, Jr., "Determining Tomato Fruit Maturity with Nondestructive in Vivo Nuclear Magnetic Resonance Imaging," *Postharvest Biology and Technology* **1**, 153–159 (1991).
32. J. S. MacFall and G. A. Johnson, "The Architecture of Plant Vasculature and Transport as Seen with Magnetic Resonance Microscopy," *Canadian Journal of Botany* **72**, 1561–1573 (1994).
33. K. Akimoto, W. F. McClure, and K. Shimizu, "Non-destructive Evaluation of Vegetable and Fruit Quality by Visible Light and MRI," *Proceedings of Automation and Robotics in Bioproduction and Processing* **1**, 117–124 (1995).
34. B. Zion, M. J. McCarthy, and P. Chen, "Real-time Detection of Pits in Processed Cherries by Magnetic Resonance Projections," *Lebensm.-Wiss. Technol* **27**, 457–462 (1994).
35. P. Chen et al., *Development of a High-speed NMR Technique for Sensing Fruit Quality*, Paper 95-3613, American Society of Agricultural Engineers, St. Joseph, Mich., 1995.
36. S. I. Cho et al., "Nondestructive Sugar Content Measurements of Intact Fruit Using Spin-spin Relaxation Times (T_2) Measurements by Pulsed ^{1}H Magnetic Resonance," *Transactions of the American Society of Agricultural Engineers* **36**, 1217–1221 (1993).
37. P. H. Heinemann et al., "Machine Vision Inspection of Golden Delicious Apples," *Applied Engineering in Agriculture* **11**, 901–906 (1995).
38. M. J. Delwiche, S. Tang, and J. F. Thompson, *Prune Defect Detection by Line-scan Imaging*, Paper 88-3024, American Society of Agricultural Engineering, St. Joseph, Mich., 1988.
39. B. K. Miller and M. J. Delwiche, *A Color Vision System for Peach Grading*, Paper 88-6025, American Society of Agricultural Engineering, place of publication 1988.
40. M. Benady et al., "Fruit Ripeness Determination by Electronic Sensing of Aromatic Volatiles," *Transactions of the American Society of Agricultural Engineers* **38**, 251–257 (1995).
41. J. E. Simon "Electronic Sensing of Aromatic Volatiles for Quality Sorting of Blueberry Fruit," *J. Food Sci.* **61**, 967–969, 972 (1996).
42. E. Kress-Rogers, ed., *Handbook of Biosensors and Electronic Noses: Medicine, Food, and the Environment*, CRC Press, Boca Raton, Fla., 1997
43. A. R. Hamson, "Measuring Firmness of Tomatoes in a Breeding Program," *Proceedings of the American Society for Horticultural Science* **60**, 425–433 (1952).
44. U.S. Pat. 5,372,030 (Dec. 13, 1994), S. E. Prussia, J. J. Astleford, B. Hewlett, and Y. C. Hung (to University of Georgia Research Foundation).
45. M. J. Delwiche, S. Tang, and J. J. Mehlschau, "An Impact Force Response Fruit Firmness Sorter," *Transactions of the American Society of Agricultural Engineers* **32**, 321–326 (1989).
46. V. A. McGlone and P. N. Schaare, *The Application of Impact Response Analysis in the New Zealand Fruit Industry*, Paper 93-6537 American Society of Agricultural Engineers, St. Joseph, Mich., 1993.
47. J. Sugiyama, K. Otobe, and Y. Kikuchi, *A Novel Firmness Meter for Fruits and Vegetables*, Paper 94-6030, American Society of Agricultural Engineers, St. Joseph, Mich., 1994.
48. J. A. Abbott et al., "Nondestructive Sonic Firmness Measurement of Apples," *Transactions of the American Society of Agricultural Engineers* **38**, 1461–1466 (1995).
49. J. A. Abbott and D. R. Massie, "Nondestructive Sonic Measurement of Kiwifruit Firmness," *J. Am. Soc. Hortic. Sci.* **123**, 317–322 (1998).
50. P. R. Armstrong, H. R. Zapp, and G. K. Brown, "Impulsive Excitation of Acoustic Vibrations in Apples for Firmness Determination," *Transactions of the American Society of Agricultural Engineers* **33**, 1353–1359 (1990).
51. K. Peleg, U. Ben-Hanan, and S. Hinga, "Classification of Avocado by Firmness and Maturity," *J. Texture Stud.* **21**, 123–129 (1990).
52. M. L. Stone et al., *Watermelon Maturity Determination in the Field With Acoustic Impedance Techniques*, Paper 94-6024, American Society of Agricultural Engineers, St. Joseph, Mich., 1994.

53. I. Shmulevich, N. Galili, and N. Benichou, "Development of a Nondestructive Method for Measuring the Shelf-life of Mango Fruit," *Proceedings Food Processing Automation IV Conference*, Chicago, Ill., Nov. 3–5, 1995.
54. H. Yamamoto, M. Iwamoto, and S. Haginuma, "Nondestructive Acoustic Impulse Response Method for Measuring Internal Quality of Apples and Watermelons," *Journal of the Japanese Society for Horticultural Science* **50**, 247 261 (1981).
55. N. Sarkar and R. R. Wolfe, "Potential of Ultrasonic Measurements in Food Quality Evaluation," *Transactions of the American Society of Agricultural Engineers* **26**, 624–629 (1983).
56. Y. Cheng and C. G. Haugh, "Detecting Hollow Heart in Potatoes Using Ultrasound," *Transactions of the American Society of Agricultural Engineers* **37**, 217–222 (1994).
57. B. L. Upchurch et al., "Ultrasonic Measurement for Detecting Apple Bruises," *Transactions of the American Society of Agricultural Engineers* **30**, 803–809 (1987).
58. N. Galili, A. Mizrach, and G. Rosenhouse, *Ultrasonic Testing of Whole Fruit for Nondestructive Quality Evaluation*, Paper 93-6026, American Society of Agricultural Engineers, St. Joseph, Mich., 1993.
59. N. Ozer, B. Engel, and J. Simon, "Fusion Classification Techniques for Fruit Quality," *Transactions of the American Society of Agricultural Engineers* **38**, 1927–1934 (1995).

JUDITH A. ABBOTT
USDA/ARS
Beltsville, Maryland

FOOD CROPS: POSTHARVEST DETERIORATION

Harvest is a major event for any food that is derived from plants. As a plant part is severed from the plant it loses its source of supply of nutrients and its repository for metabolic waste products. Until the detached plant part undergoes conventional food processing, it continues to live, respire, transpire, and senesce, ultimately leading to death. Postharvest deterioration continues until the item is either processed or consumed. Handling techniques have been developed to slow the physiological processes to provide a product that is satisfactory to the consumer.

Food crops are categorized as agronomic or horticultural. Agronomic (field) crops are primarily the grains and oilseeds that are harvested in a dry to semidry state and tend to be relatively stable to handling and storage as long as they are protected from moisture and insects. Horticultural (garden) crops, which comprise fruits, vegetables, and nuts, tend to be much more perishable, requiring sophisticated handling systems to transport them from field to consumer.

Estimates vary widely on how much of a crop is actually consumed (1,2). Processing techniques such as canning, freezing, and drying are designed to minimize these losses and extend the length of the season they are available for consumption. Postharvest handling techniques that manipulate the storage environment extend the life of the product while keeping it in a fresh state. Minimal processes such as cutting, slicing, and dicing increase the appeal and convenience of the item frequently at the expense of greater perishability (3). Losses of edible product begin in the field during harvesting and loading; continue during transport to the processing plant, packinghouse, or market; during storage at any point in the distribution scheme; and during food preparation or even by the consumer at the point of consumption. Losses may be complete resulting from the discarding of part (removal of outer leaves) or all (discarding a rotten fruit) of a given item. Frequently losses are more subtle and less tangible such as loss of acceptability, nutritional quality, or economic value. An understanding of the scope of losses incurred for a particular fruit or vegetable requires an understanding of the complexity of the handling and distribution system for that item (4,5).

Key concepts provide insight into the perishability of most fresh horticultural crops. Physiological deterioration of a fresh item begins at harvest and continues until processing or consumption. Respiration is the metabolic breakdown of food constituents to release the necessary energy to sustain the healthy tissue. Transpiration is the release of moisture from the surface of the fruit or vegetable. Senescence is genetically programmed deterioration that leads to cell and tissue death (4). Quality refers to the properties of a particular fruit or vegetable that make it unique and influence its purchase and consumption by the consumer. Shelf life is the time period a product can be maintained at an acceptable level of quality (5).

Although most harvested products are at their peak of quality at harvest, climacteric fruits continue to ripen after detachment from the plant. These fruits (eg, apples, bananas, pears, and tomatoes) will develop color, flavor, and textural attributes during postharvest storage. In many cases climacteric fruits do not develop full flavor off the plant, but the perishability of the fully ripe fruits precludes distribution.

CAUSES OF LOSSES

Many factors contribute to losses of fresh products during postharvest handling and storage. Mechanical injury results in cuts or bruises that decrease purchase acceptability. Such damage may not be immediately evident as softening and discoloration associated with bruising takes time to develop. Thus it is frequently difficult to determine the cause of the injury and how to take corrective action.

Microbes can invade plant tissue, particularly when it has been mechanically damaged. Until recently, postharvest pathologists were primarily interested in plant pathogens present in the field or introduced during handling that lead to decay and spread from item to item within bulk containers. More recently, introduction of human pathogens by contamination from irrigation water, untreated animal manure, or association with raw meats or their exudates has led to safety concerns in fruits and vegetables that are not thoroughly washed or cooked prior to consumption (5).

As mentioned, physiological processes occur in the fruit or vegetable after detachment from the plant. Increased respiration accelerates tissue degradation and can lead to flavor development, which may be desirable in the form of ripe-fruit flavor or may be undesirable off-flavors. Excess transpiration results in shriveling, wilting, or loss of turgor causing rejection of squash, lettuce, or broccoli, and so on.

53. I. Shmulevich, N. Galili, and N. Benichou, "Development of a Nondestructive Method for Measuring the Shelf-life of Mango Fruit," *Proceedings Food Processing Automation IV Conference*, Chicago, Ill., Nov. 3–5, 1995.
54. H. Yamamoto, M. Iwamoto, and S. Haginuma, "Nondestructive Acoustic Impulse Response Method for Measuring Internal Quality of Apples and Watermelons," *Journal of the Japanese Society for Horticultural Science* **50**, 247 261 (1981).
55. N. Sarkar and R. R. Wolfe, "Potential of Ultrasonic Measurements in Food Quality Evaluation," *Transactions of the American Society of Agricultural Engineers* **26**, 624–629 (1983).
56. Y. Cheng and C. G. Haugh, "Detecting Hollow Heart in Potatoes Using Ultrasound," *Transactions of the American Society of Agricultural Engineers* **37**, 217–222 (1994).
57. B. L. Upchurch et al., "Ultrasonic Measurement for Detecting Apple Bruises," *Transactions of the American Society of Agricultural Engineers* **30**, 803–809 (1987).
58. N. Galili, A. Mizrach, and G. Rosenhouse, *Ultrasonic Testing of Whole Fruit for Nondestructive Quality Evaluation*, Paper 93-6026, American Society of Agricultural Engineers, St. Joseph, Mich., 1993.
59. N. Ozer, B. Engel, and J. Simon, "Fusion Classification Techniques for Fruit Quality," *Transactions of the American Society of Agricultural Engineers* **38**, 1927–1934 (1995).

JUDITH A. ABBOTT
USDA/ARS
Beltsville, Maryland

FOOD CROPS: POSTHARVEST DETERIORATION

Harvest is a major event for any food that is derived from plants. As a plant part is severed from the plant it loses its source of supply of nutrients and its repository for metabolic waste products. Until the detached plant part undergoes conventional food processing, it continues to live, respire, transpire, and senesce, ultimately leading to death. Postharvest deterioration continues until the item is either processed or consumed. Handling techniques have been developed to slow the physiological processes to provide a product that is satisfactory to the consumer.

Food crops are categorized as agronomic or horticultural. Agronomic (field) crops are primarily the grains and oilseeds that are harvested in a dry to semidry state and tend to be relatively stable to handling and storage as long as they are protected from moisture and insects. Horticultural (garden) crops, which comprise fruits, vegetables, and nuts, tend to be much more perishable, requiring sophisticated handling systems to transport them from field to consumer.

Estimates vary widely on how much of a crop is actually consumed (1,2). Processing techniques such as canning, freezing, and drying are designed to minimize these losses and extend the length of the season they are available for consumption. Postharvest handling techniques that manipulate the storage environment extend the life of the product while keeping it in a fresh state. Minimal processes such as cutting, slicing, and dicing increase the appeal and convenience of the item frequently at the expense of greater perishability (3). Losses of edible product begin in the field during harvesting and loading; continue during transport to the processing plant, packinghouse, or market; during storage at any point in the distribution scheme; and during food preparation or even by the consumer at the point of consumption. Losses may be complete resulting from the discarding of part (removal of outer leaves) or all (discarding a rotten fruit) of a given item. Frequently losses are more subtle and less tangible such as loss of acceptability, nutritional quality, or economic value. An understanding of the scope of losses incurred for a particular fruit or vegetable requires an understanding of the complexity of the handling and distribution system for that item (4,5).

Key concepts provide insight into the perishability of most fresh horticultural crops. Physiological deterioration of a fresh item begins at harvest and continues until processing or consumption. Respiration is the metabolic breakdown of food constituents to release the necessary energy to sustain the healthy tissue. Transpiration is the release of moisture from the surface of the fruit or vegetable. Senescence is genetically programmed deterioration that leads to cell and tissue death (4). Quality refers to the properties of a particular fruit or vegetable that make it unique and influence its purchase and consumption by the consumer. Shelf life is the time period a product can be maintained at an acceptable level of quality (5).

Although most harvested products are at their peak of quality at harvest, climacteric fruits continue to ripen after detachment from the plant. These fruits (eg, apples, bananas, pears, and tomatoes) will develop color, flavor, and textural attributes during postharvest storage. In many cases climacteric fruits do not develop full flavor off the plant, but the perishability of the fully ripe fruits precludes distribution.

CAUSES OF LOSSES

Many factors contribute to losses of fresh products during postharvest handling and storage. Mechanical injury results in cuts or bruises that decrease purchase acceptability. Such damage may not be immediately evident as softening and discoloration associated with bruising takes time to develop. Thus it is frequently difficult to determine the cause of the injury and how to take corrective action.

Microbes can invade plant tissue, particularly when it has been mechanically damaged. Until recently, postharvest pathologists were primarily interested in plant pathogens present in the field or introduced during handling that lead to decay and spread from item to item within bulk containers. More recently, introduction of human pathogens by contamination from irrigation water, untreated animal manure, or association with raw meats or their exudates has led to safety concerns in fruits and vegetables that are not thoroughly washed or cooked prior to consumption (5).

As mentioned, physiological processes occur in the fruit or vegetable after detachment from the plant. Increased respiration accelerates tissue degradation and can lead to flavor development, which may be desirable in the form of ripe-fruit flavor or may be undesirable off-flavors. Excess transpiration results in shriveling, wilting, or loss of turgor causing rejection of squash, lettuce, or broccoli, and so on.

Physiological disorders can develop during storage from preharvest nutritional deficiencies, postharvest stress conditions, or an interaction of preharvest and postharvest factors leading to unacceptable quality or reduced shelf life.

Some fruits and vegetables are susceptible to chilling injury, which develops at low temperatures above the freezing point. Chilling injury produces different symptoms in different crops including abnormal ripening, surface lesions or pitting, increased susceptibility to decay, browning discoloration, and off-flavor development. Chilling-susceptible products include bananas, beans, cucumbers, grapefruit, melons, and tomatoes (6). Other physiological disorders can result from improper mineral nutrition during growth and development; during exposure to low levels of oxygen (O_2), high levels of carbondioxide (CO_2), or high levels of ethylene (C_2H_4) during storage; or an interaction of these and other factors.

As consumer demand increases for more fresh fruits and vegetables to meet nutritional concerns and provide more convenience to fit them into a busy lifestyle, more fresh items are sold in a cut or otherwise minimally processed form. Cutting tends to break apart cells, leading to loss of cell material; accelerate respiration, leading to more rapid degradation of the tissue; and increase surface area, resulting in surface evaporation. Composition of the gaseous atmosphere that is most likely to preserve quality of these products can result in the production of off-odors and flavors. Cutting appears to decrease the losses due to chilling injury in susceptible fruits and vegetables.

PREVENTION OF LOSSES

The goal of postharvest handling is to minimize the loss of product quality between harvest and either processing or consumption. The first line of defense is prevention of physical injury. Environmental conditions during storage and handling represent the next line of defense. Addition of chemical compounds can prevent losses. Finally, sophisticated packaging techniques are being developed and used that employ one or more of the preceding lines of defense.

Physical protection of products from injury is used to minimize mechanical damage. Reduction of mechanical injury can be achieved by decreasing the number and height of falls of individual items or containers during handling. When falls are unavoidable, damage due to impact can be minimized by cushioning, which can be achieved by foam padding, liquid foam, water, or even product that will be discarded. Impact damage of one fruit on another can be reduced by decreasing dumping operations and using spacer bars in conveyor lines to minimize fruit-to-fruit contact. Vibration damage tends to increase with an increase in the size of bulk handling and the distance from field (or orchard) to packing facility. Packing the product in wholesale or retail packages close to the field, reducing or eliminating tractor-drawn vehicles from field to packinghouse, and use of paved roads for transport will reduce vibration damage.

Likewise, microbial decay and insect damage can be reduced by physical protection. Since mechanical damage frequently provides a route for invasion, any means of mechanical protection will help decrease microbial and insect damage. Once endogenous protective barriers such as rind or peel are penetrated, chances for future losses are increased. A greater incidence of pests and disease in the field will be reflected in greater problems during postharvest handling. By leaving these problems in the field, similar problems during handling and storage will be reduced. Physical removal of diseased or infected items during sorting or grading to prevent the spread of pathogens reduces subsequent infections. To prevent cross-contamination from human pathogens, all areas in which fresh fruits and vegetables are handled should be kept free from insects, birds, their droppings, and any raw animal products. The relatively short handling periods of fruits and vegetables are such that insect infestation is not usually a major problem. With grain products, however, storage times are long, and insects, and the microbes they deposit during their visits, pose a more serious threat. Physical barriers are an important part of an insect-protection plan.

In addition, screening products from light affects quality. Light enhances chlorophyll breakdown and thus speeds yellowing of green vegetables. Light also enhances chlorophyll synthesis in nongreen vegetables such as potatoes. This greening is a quality defect as it is an indicator of light-catalyzed synthesis of toxic alkaloids such as solanine. Light enhances β-carotene synthesis in tomatoes, but the effect on color quality is much more significant preharvest than postharvest.

Manipulation of environmental conditions is also an important tool available to postharvest handlers. In general, lowering the temperature while maintaining high relative humidity increases the shelf life of a product by reducing the rates of respiration and transpiration. Composition of gaseous atmosphere can be either modified or controlled in the storage room, container, or consumer package to slow ripening and senescence.

Proper temperature control is the most important tool in preventing postharvest losses. As the temperature is lowered, rates of respiration and transpiration decrease. The growth of microorganisms is also slowed by lower temperatures. For most fresh products, storage at temperatures as close to freezing as possible will extend shelf life. Freezing should be avoided as inadvertent freezing and thawing of fresh items leads to breakage of cell membranes and loss of desirable texture. Quick cooling after harvest to remove field heat is imperative in items like strawberries and green vegetables that respire rapidly and perish quickly. Hydrocooling and icing are used for products that can withstand water, but water is an excellent vehicle for spreading microorganisms. Forced-air cooling is another effective method, while vacuum-cooling is used for high-value items with a large surface area like lettuce. Slower cooling such as room cooling is permissible for products being stored for a longer time such as apples, but the final temperature should be as close to optimal as possible. When calculating refrigeration requirements, it must be remembered that respiring plant material evolves heat, known as the heat of respiration.

Prevention of chilling injury can be achieved by storing susceptible commodities at temperatures above the critical storage temperature, which ranges from 4°C for snap beans to 15°C for bananas. A complete list of optimal storage temperatures is available (7). In commercial practice a compromise temperature between 5 and 10°C is frequently used to store most fresh items. At this temperature it is assumed that damage to chilling-susceptible product will be minimal while the decrease in shelf life to nonsusceptible items will not be economically significant. Ice is usually added to green vegetables to lower the temperature and increase relative humidity (RH) without changing room temperature. The success of these strategies depends on a rapid turnaround of fresh product to minimize losses.

Maintenance of a high RH lowers transpiration of heavily transpiring products. Just as each commodity has an optimal storage temperature, it also has an optimal RH. If the RH is too high, microbial growth is enhanced. If it is too low, shriveling or wilting can result. Rapid changes in temperature of a product can lead to condensation on the surface and increased susceptibility to microbial decay.

Food additives are effective agents for the protection of plant products, but they are coming under greater scrutiny as consumers become more wary of chemicals. Many currently used compounds are under regulatory review and the approval of new compounds is unlikely. Microbial inhibitors help prevent the growth of spoilage microorganisms. Fumigants have been used to disinfest products from insects. External waxes are applied to porous fruit such as citrus fruits and cucumbers to slow water loss. These waxes also enhance appearance by providing gloss.

Although not a chemical as such, food irradiation induces chemical changes similar to conventional processing and is considered a food additive by the FDA. Low-dose irradiation has been approved in many countries for the inhibition of sprouting in potatoes and onions, insect disinfestation, and shelf-life extension. In some crops like strawberries, irradiation is effective in extending shelf life, but in others damage is induced at doses lower than effective for extension. Irradiation appears to be a safer technique for disinfestation than chemical fumigation, but questions of consumer acceptance of irradiated product have limited willingness of the food industry to adopt it as a widespread technique.

Shelf life of fresh products may also be extended by modification of the composition of atmospheric gases. Respiration and other metabolic processes are slowed with a decrease in oxygen and an increase in carbon dioxide. In some crops such as apples, pears, and onions, long-term storage is enhanced by controlling the atmosphere. Other crops such as lettuce and most root crops are susceptible to CO_2. Optimal storage atmospheres for crops have been published (7). Controlled-atmosphere storage usually occurs in large storage rooms where the gaseous atmosphere is monitored and changed to maintain the desired composition. In modified-atmosphere storage the initial gaseous composition is established but changes as respiration leads to decreased O_2 and increased CO_2 in the container. Atmosphere modification is very effective at maintaining texture and appearance but can lead to the development of off-flavors. Modified atmosphere packaging (MAP) is the major technique used to preserve fresh-cut products. MAP is most effective when used in conjunction with temperature reduction. In fresh-cut vegetable products like lettuce, the consumer has shown a willingness to sacrifice some losses in fresh flavor for added convenience. It is not clear that the consumer is willing to make the same sacrifice for fresh-cut fruits.

Advances in film technology have introduced greater sophistication in packaging of fresh products. Packaging protects the product by confining it, preventing contamination, shielding from mechanical damage and pests, permitting atmosphere modification, and providing instructions for optimal handling. The type of container used and its function can change as the item moves through handling and distribution. In general, the fewer handling steps and product transfers, the less opportunity for damage.

Plastics are being widely used in the packaging of fresh fruits and vegetables. They are employed at the pallet level for containerization and prevention of moisture loss around as well as used for MAP within shipping cartons, retail packages, or even individual items. Barrier films have different transmission properties to permit or exclude specific gases such as water vapor, CO_2, O_2 and C_2H_4, depending on specific requirements of the individual items. These films permit in-package atmosphere modification, extending the advantages of the technology to the supermarket shelves. Determination of the best initial composition of gases has been limited by the variation of the individual items in response to differing atmospheres. While accumulation of CO_2 and C_2H_4 could be detrimental to product quality, absorbers of these compounds can be included in sachets enclosed in the package or imbedded in the packaging material itself (8).

A logical extension of MAP is shrinking of the film tightly around the individual produce item. Although generally considered a type of modified atmosphere, individual plastic films are really more analogous to externally applied waxes, which change the diffusion properties of the item with the external atmosphere. These films slow transpiration while modifying the internal gaseous composition. Shelf life of some products like lemons is dramatically extended while other products develop off-odors and off-flavors due to altered metabolism. Edible films such as sucrose polyesters and proteins permit moisture control and changes in respiration of whole and cut products either as a retail item or within a retail package (9).

BIBLIOGRAPHY

1. M. C. Bourne, *Post Harvest Food Losses—The Neglected Dimension in Increasing the World Food Supply*, Cornell International Agriculture Mimeograph 53, Ithaca, N.Y., 1977.
2. E. B. Pantastico and O. K. Bautista, "Postharvest Handling of Tropical Vegetable Crops," *HortScience* **11**, 122–124 (1976).
3. R. C. Wiley, ed., *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman & Hall, New York, 1994.
4. A. A. Kader et al., *Postharvest Technology of Horticultural Crops*, University of California, Davis, Calif., 1992.
5. R. L. Shewfelt and S. E. Prussia, eds., *Postharvest Handling: A Systems Approach*, Academic Press, San Diego, Calif., 1993.
6. C. Y. Wang, "Chilling Injury of Tropical Horticultural Commodities," *HortScience* **29**, 986–988 (1994).
7. R. E. Hardenburg, A. E. Watada and C. Y. Wang, *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*, USDA Agriculture Handbook 66, Washington, D.C., 1986.

8. T. P. Labuza, "An Introduction to Active Packaging for Foods," *Food Technol.* **50**, 68–71 (1996).
9. J. Krotcha, E. A. Baldwin, and M. O. Nisperos-Carriedo, *Edible Coatings and Films to Improve Food Quality*, Technomic Publishing Co., Lancaster, Penn., 1994.

GENERAL REFERENCES

J. K. Brecht, "Physiology of Lightly Processed Fruits and Vegetables," *HortScience* **30**, 18–22 (1995).

A. C. Cameron, P. C. Talasila, and D. W. Joles, "Predicting Film Permeability Needs for Modified-Atmosphere Packaging of Lightly Processed Fruits and Vegetables," *HortScience* **30**, 25–34 (1995).

I. J. Church and A. L. Parsons, "Modified Atmosphere Packaging Technology—A Review," *J. Sci. Food Agric.* **67**, 143–152 (1995).

R. B How, *Marketing Fresh Fruits and Vegetables*, Chapman and Hall, London, United Kingdom, 1991.

S. J. Kays, *Postharvest Physiology of Perishable Plant Products*, Chapman and Hall, London, United Kingdom, 1991.

B. Ooraidul and M. E. Stiles, *Modified Atmosphere Packaging of Food*, John Wiley & Sons, New York, 1991.

D. S. Smith et al., eds., *Processing Vegetables: Science and Technology*, Technomic Publishing Co., Lancaster, Penn., 1997.

C. Y. Wang, *Alleviation of Chilling Injury of Horticultural Crops*, CRC Press, Boca Raton, Fla., 1990.

ROBERT L. SHEWFELT
University of Georgia
Athens, Georgia

FOOD CROPS: STORAGE

This article describes various factors to be considered during the storage of fresh fruits and vegetables and some supplemental methods used with cold storage. Space limitations preclude detailed prescriptions of storage for specific crops. This information is available in other publications (1–4).

Tissues of fruits and vegetables after harvest are still alive and continue to respire, metabolize, and change. Successful storage of fruits and vegetables extends the marketing period of these commodities and minimizes economic losses because most of the crop is utilized. Good storage conditions will maintain crop quality, reduce deterioration, render excellent salable commodities, and avoid causing injury to tissues by chilling, freezing or ammonia fumes.

FACTORS AFFECTING STORAGE LIFE

The most important factor affecting storage life is the quality of the product at harvest. Other factors to be considered during storage are temperature, humidity, and sanitation. Optimal storage conditions for fresh food crops will change from time to time, as changes in technology and popularity of different cultivars occur.

Product Quality

The quality of produce at harvest greatly affects the length of storage. The commodity should be harvested at the recommended stage of maturity (which differs with commodity) for maximal storage and should be free of physical damage, as skin breaks and bruises can lead to increased susceptibility to water loss and to bacterial and fungal attack. The extent of incipient infections by pathogens should be determined prior to storage, because such infections can cause rot and decay during storage.

Other factors to be considered in the determination of storage life and conditions are cultivar, environmental conditions during growth, cultural practices, handling practices before storage, and transit conditions. Allowances for improper handling, unfavorable growing conditions, and poor or long-distance transit conditions should be made in determining optimal storage conditions and predicting storage life. Furthermore, different parts of plants are used as food, including roots, stems, fruits, flowers, and leaves, and these parts have different storage requirements.

Respiration

Plant tissues respire, using oxygen in the conversion of carbohydrates to carbon dioxide, water, and energy. The energy is primarily given off as heat. Respiration rates and the amount of heat released increase as storage temperature increases, about two to three times for every 10°C (18°F) rise in temperature. The storage life of a commodity is inversely proportional to the rate of respiration. Therefore, as temperature increases, respiration rate increases, and storage life decreases. Respiration rates differ with plant species, cultivar, morphological tissue, and growing conditions. Rates gradually decrease after harvest for many fruits and vegetables, but in certain crops respiration rates gradually increase as they ripen, peak to a climacteric, and then decrease (5).

Temperature

Temperature affects the rates of produce metabolism and respiration, spore germination and pathogen growth, softening, moisture loss, and undesirable growth, such as sprouting. For every 10°C increase in temperature, the rate of deterioration or undesirable growth increases two to three times. Refrigerated storage is therefore recommended for many perishable commodities to slow the changes listed above (6). For optimal storage, storage room temperatures should be held constant, with variations of less than 1–2°C. The recommended storage temperature for many crops is 0°C, while temperatures below 0°C will cause freezing damage. Crops sensitive to chilling should be stored at 12.5°C; temperatures below 12.5°C but above 0°C will injure these commodities. Storage temperatures higher than optimal will shorten the expected shelf life of the produce. Temperature fluctuations can cause condensation on the product, leading to an increase in pathogen growth and subsequent decay.

Precooling

The first step in good temperature management of perishable commodities is precooling after harvest and before shipment, storage, or processing (7–10). Prompt precooling can reduce commodity respiration, water loss, metabolism, and pathogen growth. This is done using special equipment and rooms, when rapid cooling is essential and economically beneficial. Precooling can be accomplished in refrigerated storage rooms equipped with proper control of airflow.

Precooling methods quickly transfer heat from the produce to a cooling medium, such as water, ice, or air. The rate of transfer depends on the availability of the cooling medium to the commodity, the temperature difference between the medium and the commodity, the type of medium, and the flow rate of the medium. The commercial methods used include hydrocooling, vacuum cooling, air cooling, and icing. The methods used depend on the type of produce, the availability of equipment, facilities, containers, cost, and the proximity to market. See Table 1 for a list of methods of cooling for different commodities.

Humidity

Maintenance of proper humidity around the commodity is necessary during precooling and storage. Fruits and vegetables transpire and lose water during precooling, storage, and transit. They will wilt or shrivel if humidity is too low. Humidity is generally expressed as percent relative humidity (% RH), the ratio of water vapor pressure in the air to saturation vapor pressure at a certain temperature (11). As temperature increases, the capacity of air to hold water increases. For most vegetables, a relative humidity of 98–100% are recommended to prevent wilting. Water may be sprinkled on the floor of a storage room or misted in the air if it is necessary to increase the relative humidity around a commodity. Other practices to ensure adequate humidity include using good room insulation, preventing air leaks, and providing sufficient cooling surface to keep the difference between refrigerant surface temperature and commodity temperature as small as possible.

Sanitation

Sanitary conditions must be maintained in storage facilities to prevent the growth of contaminating organisms. Under high humidity conditions, molds grow on the surfaces of packages, walls, and ceilings, and although they may not directly decay produce, they may produce unwanted substances that accelerate decay, give off-flavors to the commodity, or create favorable environments for decay organisms. Periodic and thorough cleaning of storage surfaces and good air circulation help prevent the growth of surface molds. Whitewash can be sprayed on ceilings and walls when storage rooms are empty and before a new load comes in. Surfaces may also be scrubbed with sodium hypochlorite or trisodium phosphate, with subsequent rinsing and airing before new commodities are loaded. Fungicidal paint or fumigation with a mixture of 85% carbon dioxide and 15% ethylene can also be used. If odors have developed in a room, air purification can be accomplished by absorbing the odors with 6–14 mesh-activated coconut-shell carbon or washing the air with water (12).

Table 1. Methods of Cooling for Different Commodities

Cooling method	Commodities cooled
Hydrocooling	Asparagus, cantaloupes, celery, cucumbers, green peas, peaches, peppers, radishes
Vacuum cooling	Asparagus, artichokes, broccoli, Brussels sprouts, cabbage, cauliflower, celery, endive, escarole, head lettuce, parsley, spinach, sweet corn
Air cooling	Cauliflower, cucumbers, grapes, melons, peppers, strawberries, tomatoes
Icing	Broccoli, Brussels sprouts, cantaloupes, carrots, green onions, kale, radishes

INJURIES

Certain injuries to produce can occur during refrigerated storage under suboptimal conditions and include those due to chilling, freezing, or ammonia. Chilling injury usually occurs to commodities of tropical origin at temperatures below 10–13°C (50–55.4°F), although certain temperate commodities are also susceptible (13–15). Table 2 lists some commodities susceptible to chilling injury and their symptoms. Often symptoms cannot be detected at low temperatures and only become visible several days after the commodity has been removed to warmer temperatures. The extent of damage depends on length of exposure and chilling temperature. It may occur in a commodity exposed a short amount of time to a temperature considerably below the danger zone, but not if it is exposed longer to a higher temperature in the danger zone. Also, chilling injury may be cumulative, and chilling during transit or in the field before harvest may add to the effects of storage chilling. Chilling injury has been reduced by treatments such as intermittent warming, temperature preconditioning, controlled atmosphere or hypobaric storage, waxing, and film packaging.

Freezing injury occurs when ice crystals form in produce tissues, and is manifested by a mushy, limp, and water-soaked appearance. The extent of freezing injury depends on exposure time and temperature and varies with different commodities.

Ammonia injury occurs when ammonia escaping from direct-expansion refrigeration units come in contact with the commodity. Injury is evident as brown to greenish black discoloration of the outer tissues and, in severe cases, as discoloration and softening of inner tissues (16). Daily

Table 2. Commodities Susceptible to Chilling Injury and Their Symptoms

Commodity	Lowest safe temperature (°C)	Symptoms
Avocados	4.5–13.0	Grayish brown flesh discoloration
Bananas	11.5–13.0	Dull color when ripe
Cantaloupes	2–5	Pitting, surface decay
Cucumbers	7	Pitting, decay
Eggplants	7	Surface scald, *Alternaria* rot, black seeds
Lemons	11–13	Pitting, red blotch
Peppers	7	Sheet pitting, *Alternaria* rot on pods and calyxes, seed blackening
Potatoes	10	Browning, sweetening
Tomatoes, ripe	7–10	Water soaking, softening, decay

Source: Ref. 1.

odor checks or installation of ammonia detection systems help prevent ammonia injury to produce. Fumes can be removed by aeration and washing the air of the storage room with water.

SUPPLEMENTAL METHODS TO COLD STORAGE

Although refrigeration is the most effective method for retarding decay of food crops, supplements can be used to control the growth of pathogens, slow respiration rates, control physiological disorders and sprouting, reduce moisture loss, or retard ripening and senescence. Supplemental methods in use include chemical treatments, controlled- and modified-atmosphere storage, waxes, irradiation, and protective packaging.

Chemical treatments are used if they are nontoxic to humans and the commodity, cost-effective, effective for their intended purpose, and approved by federal regulatory agencies, for example, the use of growth regulators to control sprouting of potatoes and onions during storage. Calcium chloride is used to control bitter pit and maintain firmness in apples. Fungicides and bactericides, such as chlorine and sodium *o*-phenylphenate, are often added to wash water, soak tanks, and hydrocoolers to reduce growth of pathogens prior to and during refrigerated storage (17).

In controlled-atmosphere (CA) storage, commodities are held in atmospheres of specified proportions of oxygen, carbon dioxide, or nitrogen, different from ambient air. This is accomplished by adding or scrubbing oxygen or carbon dioxide in airtight containers or rooms (18–21). In modified-atmosphere storage, storage atmospheres are different from that of ambient air, but are not controlled precisely.

Waxing is used to reduce moisture loss and improve the appearance of the commodity. Fungicides can also be mixed with the wax to retard decay. The effectiveness of the wax depends on the thickness of the coat and the uniformity of application.

Gamma irradiation has been used to disinfect papayas, mangoes, and grapefruit. Commercial use is limited because of the cost and size of necessary equipment and consumer acceptance. Dosages of 1.5–2 kilogray can control decay and sprouting in certain commodities (22), but can also cause discoloration, pitting, softening, abnormal ripening, and flavor loss (23).

Protective packaging safeguards produce against physical damage during packing, such as that acquired from dropping of containers, overfilling of containers, or movement of the commodity within containers (24). Protective packaging must be sturdy and moisture tolerant. Packaging materials include plastic foam, waxed fiberboard, film box liners, wooden pallet bins, nailed wooden boxes, wirebound veneer crates, and perforated polyethylene or polypropylene bags.

FUTURE DEVELOPMENTS

Technologies are continually being researched and developed to improve the storage life of food crops. Future developments should result in more economical and energy-effective cooling methods, more efficient transportation and distribution systems, improved shipping containers, and increased usage of controlled- and modified-atmosphere storage.

BIBLIOGRAPHY

1. R. E. Hardenburg, A. E. Watada, and C. Y. Wang, *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Crops*, USDA Agricultural Handbook, 66, rev., U S. Government Printing Office, Washington, D.C., 1986.
2. A. A. Kader and co-workers, *Postharvest Technology of Horticultural Crops*, Cooperative Extension Publication 3311, University of California, Berkeley, 1985.
3. A. L. Ryall and W. J. Lipton, *Handling, Transportation and Storage of Fruits and Vegetables*, Vol. 1, *Vegetables and Melons*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1979.
4. A. L. Ryall and W. T. Pentzer, *Handling, Transportation and Storage of Fruits and Vegetables*, Vol. 2., *Fruits and Tree Nuts*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1982.
5. J. B. Biale and R. E. Young, "Respiration and Ripening in Fruits—Retrospect and Prospect," in J. Friend and M. J. C. Rhodes, eds., *Recent Advances in the Biochemistry of Fruit and Vegetables*, Academic Press, Inc., Orlando, Fla., 1981.
6. J. A. Bartsch and G. D. Blanpied, "Refrigerated Storage for Horticultural Crops," *Agric. Eng. Ext. Bull.* **448**, Cornell University, Ithaca, N.Y., 1984.
7. F. E. Henry, A. H. Bennett, and R. H. Segall, "Hydroaircooling: A New Concept for Precooling Pallet Loads of Vegetables," *ASHRAE Trans.* **82**(part 2), 541–547 (1976).
8. F. M. R. Isenberg, R. F. Kasmire, and J. Parson, *Vacuum Cooling Vegetables*, Cornell University Cooperative Extension Information Bulletin **186**, 1982.
9. F. G. Mitchell, R. Guillou, and R. A. Parson, *Commercial Cooling of Fruits and Vegetables*, California Agricultural Experimental Station Extension Service Manual **43**, 1972.
10. I. J. Pflug and co-workers, "Precooling of Fruits and Vegetables," *ASHRAE Symp.* SF-4-70, 1970.
11. J. J. Gaffney, "Humidity: Basic Principles and Measurement Techniques," *HortScience* **13**, 551–555 (1978).
12. H. Hansen and J. Kuprianoff, "Some Experiences with Air-Washing in Cold and Gas Stores of Pears and Apples," *Internat. Inst. Refrig. Bul. Sup.* 1, 337–340 (1961).
13. H. M. Couey, "Chilling Injury of Crops of Tropical and Subtropical Origin," *HortScience* **17**, 162–165 (1982).
14. J. M. Lyons, "Chilling Injury in Plants," *Annual Review of Plant Physiology* **24**, 445–466 (1973).
15. L. L. Morris, "Chilling Injury of Horticultural Crops: An Overview," *HortScience* **17**, 161–162 (1982).
16. E. Brennan, I. Leone, and R. H. Daines, "Ammonia Injury to Apples and Peaches in Storage," *Plant Dis. Rptr.* **46**, 792–795 (1962).
17. J. W. Eckert, "Postharvest Diseases of Fresh Fruits and Vegetables—Etiology and Control," in N. F. Haard and D. K. Salunkhe, eds., *Symposium: Postharvest Biology and Handling of Fruits and Vegetables*, AVI Publishing Co., Inc., Westport, Conn., 1975.
18. P. E. Brecht, "Use of Controlled Atmospheres to Retard Deterioration of Produce," *Food Technology* **34**, 45–50 (1980).
19. F. M. R. Isenberg, "Controlled-Atmosphere Storage of Vegetables," *Hortic. Rev.* 1, 337–394 (1979).
20. D. G. Richardson and M. Meheriuk, eds., *Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities, Proceedings of the Third National CA Research Conference, Beaverton, Oreg., July 1981.*

21. R. M. Smock, "Controlled Atmosphere Storage of Fruits," *Hortic. Rev.* **1**, 301–336 (1979).
22. J. H. Moy, "Potential of Gamma Irradiation of Fruits: A Review," *Journal of Food Technology* **12**, 449–457 (1977).
23. R. A. Dennison and E. M. Ahmed, "Irradiation Treatment of Fruits and Vegetables," in Ref. 17.
24. F. G. Mitchell, *Packaging Horticultural Crops*, Univ. Calif. Coop. Ext. Perishables Handling 52, 1983, pp. 2–4.

GENERAL REFERENCES

F. Artes and A. J. Eseriche, "Intermittent Warming Reduces Chilling Injury and Decay of Tomato Fruit," *J. Food Sci.* **59**, 1053–1056 (1994).

J. R. Birewar, "Development of Improved On-farm Grain Drying Facility in Nigeria," *Agricultural Mechanization in Asia and Latin America* **27**, 51–53 (1996).

J. Dincer, "Precooling of Cylindrically Shaped Grapes: Experimental and Theoretical Heat Transfer Rates," *Journal of Food Processing and Preservation* **17**, 57–71 (1994).

I. Edeogu, J. Feddes, and J. Leonard, "Comparison Between Vertical and Horizontal Air Flow for Fruit and Vegetable Precooling," *Canadian Agricultural Engineering* **39**, 102–112 (1997).

E. A. Estes, "Feasibility and Affordability Considerations in Precooling Fruits and Vegetables," *American Society for Agricultural Engineers Publication* **1-95**, 390–396 (1995).

S. L. Gillies and P. M. A. Toivonen, "Cooling Method Influences the Postharvest Quality of Broccoli," *HortScience* **30**, 313–315 (1995).

A. L. Kyung and Y. J. Yong, "Effects of Low Temperature and CA on Quality Changes and Physiological Characteristics of Chilling Injury During Storage of Squash (*Circubitai imoschatai*) *Journal of the Korean Society for Horticultural Sciences* **39**, 402–407 (1998).

S. Lurie and A. Sabehat, "Prestorage Temperature Manipulations to Reduce Chilling Injury in Tomatoes," *Postharvest Biology and Technology* **11**, 57–62 (1997).

A. Nagao, T. Indou, and H. Dohi, "Effects of Curing Condition and Storage Temperature on Postharvest Quality of Squash Fruit," *Journal of the Japanese Society for Horticultural Science* **60**, 175–181 (1991).

A. Picon et al., "Effects of Precooling, Packaging Film, Modified Atmosphere and Ethylene Absorber on the Quality of Refrigerated Chandler and Douglas Strawberries," *Food Chem.* **48**, 189–193 (1993).

A. C. Purvis, "Interaction of Waxes and Temperature in Retarding Moisture Loss From and Chilling Injury of Cucumber Fruit During Storage," *Proceedings of the Florida State Horticultural Society* **107**, 257–260 (1994).

T. B. Puttaraju and T. V. Reddy, "Effects of Precooling on the Quality of Mango (cv. Mallika)," *Journal of Food Science and Technology India* **34**, 24–27 (1997).

J. M. Sala, "Involvement of Oxidative Stress in Chilling Injury in Cold-Stored Mandarin Fruits," *Postharvest Biology and Technology* **13**, 255–261 (1998)

B. D. Shukla and R. T. Patil, "Dryers and Drying Technology for Food Crops," *Indian Journal of Agricultural Science* **62**, 579–589 (1992)

R. Turk and E. Celik, "The Effect of Vacuum Precooling on the Half Cooling Period and Quality Characteristics of Iceberg Lettuce," *Acta Horticulture* **343**, 321–324 (1993).

C. Y. Wang, "Chilling Injury of Fruits and Vegetables," *Food Rev. Int.* **5**, 209–236 (1989).

C. Y. Wang, "Heat Treatment Affects Postharvest Quality of Kale and Kollard, but not Brussel Sprouts," *HortScience* **33**, 881–883 (1998).

A. B. Woolf, "Reduction of Chilling Injury in Stored Hass Avocado Fruit by 38 Degree Water Treatment," *HortScience* **32**, 1247–1251 (1997).

A. B. Woolf et al., "Reducing External Chilling Injury in Stored Hass Avocados With Dry Heat Treatments," *J. Am. Soc. Hortic. Sci.* **120**, 1050–1056 (1995).

A. B. Woolf et al., "Reduction of Chilling Injury in Sweet Persimmon Fuyu During Storage by Dry Air Heat Treatments," *Postharvest Biology and Technology* **11**, 155–164 (1997).

Cindy B. S. Tong
Alley E. Watada
USDA/ARS
Beltsville, Maryland

FOOD CROPS: VARIETAL DIFFERENCES, MATURATION, RIPENING, AND SENESCENCE

Nutritional labeling of packaged food products conveys a misleading impression that similar foods are uniform in nutrient composition. Raw agricultural crops are particularly susceptible to wide variations in nutrient content, sensory quality, and suitability as ingredients in prepared or processed food products. Differences in composition result from many factors, including cultivar, growing condition, maturation, ripening, and senescence (1). Terms critical to an understanding of this subject are defined in Table 1.

VARIETAL DIFFERENCES

Humans eat a wide variety of plant products, thanks to the large number of plant species cultivated for food. Most individual fruits, vegetables, nuts, oilseeds, and grains represent separate species. A notable exception is *Brassica oleracea*, a species comprising the crops of broccoli, brussels sprouts, cabbage, cauliflower, collards, kale, and kohlrabi. Although not as diverse as *B. oleracea*, most species have variations in cultivars that are apparent in differences in growth response, pest and disease resistance, stress response, nutritional quality, and shipping stability.

The concept of varietal differences is based on Mendel's classic study of genetics in peas. Varietal differences are evident in the growth patterns of the crop in the field, such as the height of the plant, its branching characteristics, color and morphology of flower and fruit, and yield potential for edible product at harvest. Some cultivars offer greater resistance to pests and disease at harvest. Resistance can be achieved as a result of removal of chemical compounds that attract insects or synthesis of compounds that are toxic to the disease microorganisms or pests. Unfortunately, the same compounds that are toxic to the pest may adversely affect quality or may even be allergenic or toxic to humans (4).

Cultivars adapt in different ways to changes in growing locations and environmental conditions. Some cultivars will thrive in one location and suffer in others. Cultivars of fruits and vegetables vary in their response to temperature, cultural practices, and soil conditions, such as fertility, soil compaction, and moisture content. Certain cultivars within a species may show greater resistance to

Table 1. Definition of Terms

Cultivar (cultivated variety)	Group of plants within a particular cultivated species that is distinguished by a character or group of characters and that maintains its identity when propagated either asexually or sexually (2).
Horticultural maturity	The stage of development when a plant or plant part possesses two prerequisites for use by consumers for a particular purpose (3).
Maturation	The stage of development leading to the attainment of physiological and horticultural maturity (3).
Physiological maturity	The stage of development when a plant or plant part will continue to ontogeny even if detached (3)
Ripening	The composite of the processes that occur from the latter stages of growth and development through the early stages of senescence and result in characteristic aesthetic quality and food quality, as evidenced by changes in composition, color, texture, and other sensory attributes (3)
Senescence	Those processes that follow physiological maturity or horticultural maturity and lead to death of tissue (3).
Species	A group of plants (within a genus) that often exhibit many more morphological similarities than to members of the genus (2).

water, salt, and low-temperature or high-temperature stresses. The response to stress by plants is often reflected in changes in the edible portion of the tissue (eg, tomato plants resistant to low-temperature stress tend to produce fruit resistant to low temperatures during storage). Varietal differences have also been noted in the nutritional composition of crops at harvest and in the ability of a crop to maintain marketable quality during handling, shipping, and storage.

Crops may be improved by selecting for desired traits from existing cultivars or wild strains. The most celebrated modern example of crop improvement was the development of high-yield rice cultivars by Borlaug that resulted in the Green Revolution. In addition to conventional breeding techniques, biotechnology offers powerful tools and recombinant DNA in the development of better crops. Cell culture techniques permit rapid screening of potential strains if a reliable index for desirable trait(s) is available. Genetic engineering permits the direct manipulation of genetic material within the plant, promising the development of designer plants with the characteristics desired. These techniques are effective when the trait is clearly defined and a single gene is involved, such as disease resistance (4) or high-linoleic oilseeds (5). Much less effective has been the genetic modification of plants to improve quality that involves modification of texture or flavor where the physiological determinants of the characteristic are not clearly defined.

Many examples demonstrate the importance of varietal differences in the marketplace. Differences in color and sweetness in fresh apple cultivars help satisfy regional and individual tastes. Other cultivars are preferred for specific processing or home applications. The length of the peach season in a particular growing area is the result of a succession of early-, middle-, and late-maturing cultivars; different cultivars adapt to different climatic regions. Clingstone cultivars provide a firmer peach for canning and a better appearance, but many consumers prefer the flavor of freestone peaches in the fresh market. The cultivar of choice for a grape grower depends on the intended application: fresh, juice, jelly, raisins, or wine. Supersweet cultivars of corn were developed to increase sugar content by slow hydrolysis of sucrose during postharvest handling and storage. Processing cultivars of tomatoes must have a high-solids content, commercial cultivars that are shipped across the country must be able to withstand the rigors of handling and storage, and cultivars grown in backyard gardens must have high flavor impact. Hard cultivars of wheat are higher in protein and are used in breads, whereas soft cultivars are used in other applications, such as cookies and cakes.

Mechanization of harvesting operations has been achieved for several commodities. Certain species are more amenable to mechanical harvesting than others, and cultivars for processing are more likely to have been adapted for mechanization than fresh-market cultivars. Certain cultivars are more amenable to mechanical harvest than others. Characteristics important in ease of mechanical harvest include the uniformity of size and maturity at harvest, decreased susceptibility to bruising and other damage, and adaptability to fully mechanized production and handling systems. Successful mechanization requires an integration of breeding, quality, and engineering considerations.

MATURATION

Harvest is a critical point for the quality of any product derived from plants. The optimum harvest time varies with the period of growth and development of a plant from crop to crop. For example, celery (stems), cabbage (leaves), and broccoli (inflorescences) are harvested during vegetative growth, and cucumber and beans are harvested as partially developed fruits. Maturity or stage of development at harvest can be viewed from either a biological or market perspective. Full development biologically is called physiological maturity, and satisfactory sensory quality is related to horticultural maturity (3).

Many crops present a narrow window for optimal harvest. Maturity at harvest affects yield, stability during storage and handling, susceptibility to bruising or other damage, nutrient composition, and the quality of an item at purchase and consumption. All these factors should be considered in determining the best time to harvest. With most vegetables, this decision requires a compromise between harvesting early enough to obtain tender, succulent quality and waiting to obtain better yield.

RIPENING

The desirable attributes associated with high-quality fruit develop during ripening. Nonclimacteric fruits develop these attributes while still attached to the plant and are characterized by low levels of carbon dioxide (CO_2) and ethylene (C_2H_4) evolution. Little further ripening occurs in nonclimacteric fruit after harvest. Nonclimacteric fruits should be harvested as close to the flavor peak as possible because flavor will not develop after detachment.

Climacteric fruits are characterized by a rapid respiratory burst of greatly increased CO_2 and C_2H_4 at the initiation of ripening. The climacteric rise, triggered by C_2H_4, can occur on or off the plant. The fruit will generate its own C_2H_4 from aminocyclopropane carboxylic acid (ACC) by ACC oxidase, and ACC is generated from *S*-adenosyl methionine (SAM) by ACC synthase (6). If the fruit is harvested in a physiologically mature state before the increased generation of ACC and is kept in an environment free of C_2H_4, it can be maintained in a mature, unripe state during the transportation and storage. If climacteric fruits are harvested too early, they will not develop full flavor potential during storage. If harvested too late, they will become unacceptably soft before purchase or consumption. The marketplace offers greater incentives to the grower who harvests too early than to the one who harvests too late. In crops such as peaches and tomatoes where this window between too early and too late is narrow, premium quality product is difficult to obtain. Ripening can be artificially triggered by using an external source of C_2H_4. This process permits the shipment of green bananas from the tropics to geographically distant markets where ripening is triggered close to the consumer, long-distance truck shipment of green tomatoes for ripening close to the distribution point, and controlled-atmosphere storage of apples before ripening to permit year-round consumption.

Desirable changes associated with ripening include changes in appearance, flavor, texture, and nutritive value. Color changes are the result of the degradation of chlorophyll (green), which unmasks xanthophylls and carotenes (yellow and orange), and the biosynthesis of anthocyanins (red, blue, or purple) and the carotenoid lycopene (red). The development of sweetness in fruits is the result of a disappearance of sour (acids) and bitter (including tannins) compounds and the accumulation of sweet (sugars) compounds. Flavor is the combination of taste (sweet, sour, bitter) and aroma that results from the accumulation of volatile compounds during ripening, producing characteristic flavors associated with specific fruits. Softening during ripening is the result of degradation of cell walls, primarily pectins, by a series of hydrolase enzymes (5). Vitamins (particularly A and C) accumulate during ripening, thereby enhancing the nutritional quality of the product.

SENESCENCE

Plants senesce by predictable, controlled patterns that lead to death. Senescence is distinct, although not always distinguishable, from aging (longevity). Plant organs also senesce, which is frequently enhanced by detachment. Ripening, as described earlier and defined in Table 1, is a special case of the early phase of senescence. In somewhat oversimplified terms, ripening tends to lead to improvement of quality attributes of fruits, whereas other senescence processes tend to lead to loss of quality. The primary objective of postharvest handling and storage is to control ripening and slow senescence, thus providing the consumer with a product of optimum quality at a reasonable price.

The most obvious signs of senescence in vegetables are loss of color and tissue softening. Unlike similar changes noted in ripening fruits that are considered quality improvements, yellowing and softening of green vegetables is considered detrimental. Yellowing, as in ripening, involves the unmasking of yellow pigments by chlorophyll depletion as chloroplasts are converted to chromoplasts. Likewise, softening is associated with breakdown of cell wall components, such as pectin and cellulose.

The cellular physiology of senescence is complex. Although it was previously believed that senescence resulted from the release of organic acids and hydrolases within the cell, it is now generally recognized that senescence is controlled by genetics. Senescence affects gene expression, protein and nucleic acid degradation, chemical composition and physical properties of membranes, and the structure and function of plant organelles (7). Although predominantly a degradative process, it is misleading to view senescence solely in the context of degradation. Metabolic processes function as a series of delicate balances between biosynthetic and degradative reactions. During growth and development as well as ripening, accumulation of a compound is the net result of biosynthesis exceeding degradation. Likewise, during senescence, net loss of a compound is the result of increased degradation, decreased synthesis, or a change in the balance between the two.

Early changes observed in the ultrastructure of senescing plant tissue include swelling of thylakoids in chloroplasts. Mitochondria and vacuoles are affected later in the process. Changes in the nucleus are generally observed late in senescence before disruption of the plasma membrane, leading to cell and tissue death. Of particular interest in the study of senescence are the increase in protease enzymes and loss of membrane integrity. Membranes serve as internal barriers within the cell and regulate the flow of ions and metabolites. During senescence and aging, these membranes lose their integrity, which leads to increased permeability. A current theory suggests that membrane lipids are degraded by hydrolytic and peroxidative mechanisms, resulting in increased permeability and decreased function of membrane-bound proteins.

Not all degradation of plant tissue is attributable to senescence. Mechanical damage can cause physical rupture of membranes and lead to mixing of enzymes and substrates, which results in the browning and discoloration called a bruise. Inadequate nutrition of the plant during growth and development can lead to deficiencies within a detached plant organ and result in a physiological disorder. Low temperatures and controlled atmospheres slow the respiration and senescence of many plant organs, but certain species are susceptible to physiological disorders that result from exposure to low temperatures, low O_2, high CO_2, or elevated C_2H_4. In addition, excess water loss can lead to loss of turgor pressure, resulting in undesirable textural properties.

BIBLIOGRAPHY

1. R. L. Shewfelt, "Sources of Variation in the Nutrient Content of Agricultural Commodities from the Farm to the Consumer," *Journal of Food Quality* **13**, 37–54 (1990).
2. J. Janik, *Horticultural Science*, 4th ed., W. H. Freeman, New York, 1986.
3. A. E. Watada et al., "Terminology for the Description of the Developmental Stages of Horticultural Crops," *HortScience* **19**, 20–21 (1984).
4. S. L. Franck-Oberaspach and B. Keller, "Consequences of Classical and Biotechnological Resistance Breeding for Food Toxicology and Allergenicity," *Plant Breeding* **116**, 1–17 (1997).
5. D. S. Brar, T. Ohtani and H. Uchimiya, "Genetically-Engineered Plants for Quality Improvement," *Biotechnology and Genetic Engineering Reviews* **13**, 167–179 (1996).
6. J-M. Lelievre et al., "Ethylene and Fruit Ripening," *Physiol. Plant* **101**, 727–739 (1997).
7. L. D. Nooden, "The Phenomena of Senescence and Aging," in L. D. Nooden and A. C. Leopold, eds., *Senescence and Aging in Plants*, Academic Press, Orlando Fla., 1988, pp. 1–50.

GENERAL REFERENCES

M. J. Chrispeels and D. E. Sadava, *Plants, Genes, and Agriculture*, Jones and Bartlett, Boston, Mass., 1994

J. Gross, *Pigments in Vegetables: Chlorophylls and Carotenoids*, Chapman and Hall, London, United Kingdom, 1991.

R. C. Hoseney, *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1994.

A. A. Kader, ed., *Postharvest Technology of Horticultural Crops*, University of California Press, Oakland, Calif., 1992.

S. J. Kays, *Postharvest Physiology of Perishable Plant Products*, Chapman and Hall, London, United Kingdom, 1991

R. Lal and S. Lal, *Genetic Engineering of Plants for Crop Improvement*, CRC Press, Boca Raton, Fla., 1993

G. Mazza and E. Maniati, *Anthocyanins in Fruits, Vegetables and Grains*, CRC Press, Boca Raton, Fla., 1993.

G. B. Seymour, J. E. Taylor, and G. A. Tucker, eds., *Biochemistry of Fruit Ripening*, Chapman and Hall, London, United Kingdom, 1993.

L. P. Somogyi, H. S. Ramaswamy, and Y. H. Hui, eds., *Processing Fruits: Science and Technology*, Vol. 1, Technomic, Lancaster, Penn., 1996.

ROBERT L. SHEWFELT
University of Georgia
Athens, Georgia

FOOD ENGINEERING

The largest business activity in the world is the supplying of food to an ever-increasing population. This business includes growing and harvesting, transportation and handling, storing, processing and preservation, packing, distribution, and marketing. During the past 50 to 75 years, the various phases of the food business have grown from small family-type enterprises to gigantic, increasingly sophisticated and integrated food supply systems. The need for this change has been dictated by increasing concentrations of people in large urban areas, where the livelihood of large segments of the population depend on huge quantities of foodstuffs being readily available. Because large-volume production of foods and food materials is often remote from dense concentrations of consumers, efficient mass production and transportation of food supplies are absolutely necessary.

The vertically integrated food industry that has grown from these demands, probably more than any other human activity, requires the support of diversified, well-rounded teams of scientists, engineers, economists, and marketing specialists. Food engineering is a relatively new professional and scientific field defined in the 1950s when several engineering-educated food scientists and technologists employed by educational institutions and various segments of the food industry recognized that few engineers were educated and trained for the increasingly complex world food industry.

Foods are composed of a large variety of physically and chemically complex materials. A powerful analysis and design concept, called unit operations, originated and was extensively developed by those in the field of chemical engineering. This concept has been immensely useful in food engineering operations ranging from raw material assessment to finished product evaluation. Unit operations permits a myriad of processing steps to be seen as relatively few basic physical and chemical transformations. For example, crushing, mixing, and filtering are physical unit operations; reduction and polymerization are chemical unit operations.

Engineers in other industries are almost exclusively physical-science oriented. Functional engineers in the food industry must be knowledgeable in the biological sciences as applied to the food industry, including sanitation, spoilage, public health, environmental control, and biological process engineering, in which microorganisms are used to drive or mediate processes to produce food materials or food products.

The food requirements of the modern world can no longer be met by small, isolated, and nonintegrated food production systems, each involved in a single phase of the food industry. The logistic requirements and the complexity of feeding a world in which many countries are unable to produce sufficient food for their own populations have created a demand for a more science- and engineering-based approach. Today, and increasingly in the future, the food engineer must have the ability to play an important role in the integration of all phases of food production, preservation, and distribution into a smoothly functioning industry.

PROFESSIONAL RESPONSIBILITIES OF A FOOD ENGINEER

Some principal responsibilities of food engineering professionals, whether in industry or academia, are to develop and improve the basic steps involved in the production of food materials, process these materials into finished products, and apply this knowledge to commercial operations. Considerations during all phases must include consumer acceptability (aesthetic and sensory) and safety of the product for human or animal consumption through good manufacturing practices, as prescribed by the U.S. Food and Drug Administration.

Food engineers are involved in a wide spectrum of activities, including operations, engineering feasibility, and pre-engineering studies for updating existing facilities and planning new operations. Other responsibilities include designing process machinery and equipment layouts and integration, developing and improving unit operations and processes, ensuring food sanitation and safety, and maintaining and upgrading facilities. Planning operations also incorporate logistics of supplying, storing, and transporting raw materials and ingredients as well as finished products.

Food engineers are increasingly required to have knowledge of computers and computer applications, especially methods and techniques relating to process control in manufacturing operations. The feedback of information in closed-loop systems is particularly important because the biological state is the primary factor in controlling the quality of the final product.

EDUCATIONAL REQUIREMENTS OF A FOOD ENGINEER

The first collegiate year is about the same for all engineering curricula. The second year is likely to be similar, but with some departures for beginning specialization by field. Even in the third and fourth years, commonality for related specialities is not unusual.

The first year for all engineering students is primarily devoted to core curriculum requirements and beginning foundation courses in the natural sciences and mathematics. Most foundation science courses have decidedly engineering slants; the subject matter is presented and used quantitatively, rather than descriptively. Courses directed toward engineering specialities usually begin in the second year and increase markedly in number and complexity in the third and fourth years.

Engineering curricula tend to require more prescribed courses as well as a greater number of courses than other curricula. There are two important results from this: (*1*) it is not so unusual for engineering students to take an extra term to complete a degree, and (*2*) the options for taking nonrequired courses are limited, meaning that elective courses may have to be taken as overloads.

There is no opportunity at present to pursue a 4-year accredited collegiate program leading to a baccalaureate in food engineering in any American college or university. Furthermore, no such program will exist until a food engineering program meeting the requirements of the Engineers Council for Professional Development (ECPD) has enrolled and graduated students and then has been examined and approved by an accreditation committee of the ECPD. The procedure briefly outlined here is the one followed by more than a dozen separately identified engineering degree programs that have received accreditation.

At present, the best opportunity to prepare for a career in food engineering after a baccalaureate is to select a college or university that provides goal-compatible, ECPD-accredited programs in engineering; formal instruction for majors and nonmajors in related scientific and technical areas; and existing procedures for permitting significant student and advisor input into the planning of programs tailored to meet student aims. Goal-compatible engineering curricula might include agricultural, chemical, mechanical, civil, bio- and biochemical engineering. Related scientific and technical areas might include food science, nutrition, microbiology, sanitary engineering, pollution control, and public health engineering.

An alternative might be to pursue a program in food science, with technical electives selected from appropriate engineering courses (perhaps with advisors in food science and engineering). The minimum grounding in engineering should include fluid mechanics, mechanics of materials, heat and mass transfer, thermodynamics, unit operations and processes, process control, computer science for engineers, and applied mathematics for engineers.

The opportunities mentioned in the previous paragraphs for study in or related to food engineering might require an extra term or two of academic work. Because of this, a third alternative might be worth considering. With careful planning from both the engineering and food science viewpoints, and with institutional cooperation, it is conceivable that a B.S. degree in engineering and an M.S. degree in food science could be earned in 5 academic years.

RESEARCH AND DEVELOPMENT AREAS IN FOOD ENGINEERING

In the United States, there has been relatively little financial support given to food engineering programs for fundamental investigation of the complex composition and physical properties that must be considered in the design, construction, and operation of food processing operations. European countries have taken a world leadership position in the design, manufacturing, and marketing of food processing equipment. The support given to process engineers parallels the development of innovative new technologies, such as aseptic processing and controlled atmosphere storage and packaging.

The challenge of engineering practice is to find usefully precise and economically feasible solutions to real problems. The basic laws of physics, chemistry, and biology, expressed in the language of mathematics, show the relationships among the elements composing a system, whether it be a piece of machinery or the planet on which we live. However, because of many shortcomings of the precise knowledge of these relationships, balanced mathematical expressions are not determinable without the use of experimentally designed constants and secondary relationships that balance the equations and make them useful for application to practical situations. Hence, the application to sound engineering principles implies the use of experimentally determined information combined with scientific knowledge. These relationships can be practically applied to design a successful plant, to design and build machinery and equipment for successful and economical operations, and to complete records and information relating to production performance so that present operations can be improved and new ones developed. The problems associated with using an engineer's tools in the food industry are all too common. Food processing and food handling machinery and equipment are often based on previous practice rather than efficient functioning, easy maintenance, and meeting sanitary requirements. Modern-day food manufacturing requires reliance on sound engineering data, which often are not available or are in need of updating or improvement to evaluate and commercialize manufacturing methods and techniques. Some of the important food engineering areas in need of further study include

1. The changes in mass and composition that occur in foods during processing.
2. The pack densities, specific gravities, and compressibilities that are brought about in loading bins, storage tanks, and vats.
3. The safe loading depths for fresh or raw products during transportation and storage.
4. The flow properties for various foods in different forms and process states.
5. The properties of viscous material such as purées, pulps, slurries, etc.
6. The specific heat, heat capacity, and heat conductivity of foods in all states of preservation, especially when simultaneous mass and heat transfer occur.
7. The effect of particle size on various processes, which has a bearing on many processes such as spray drying, grinding, filtering slurries and miscella, and disintegration operations.

Both mechanical and chemical unit operations and processes are necessary in food production. Mechanical operations include washing, sizing, sorting, grinding, mixing, polishing, grading, packaging, and materials handling. Processes for preserving (and often to improve texture and flavor) include a combination of chemical and physical unit operations and processes. These include (*1*) adding heat for cooking, pasteurizing, or sterilizing; (*2*) removing heat for cooling (refrigerating), freezing, or storing in a frozen state; (*3*) adding chemicals to control water activity, aid in fermentation or other processes (eg, enzyme hydrolysis), and improve sensory characteristics and acceptance; (*4*) removing water to control water activity; and (*5*) irradiating to pasteurize or sterilize. Most food processing uses a combination of these steps. For example, drying is normally carried out by adding heat to vaporize the water, whether it be under vacuum or atmospheric pressure. Of course, the primary purpose of these operations is to control or destruct microorganisms and their metabolic products and to reduce certain detrimental chemical and physical reactions. However, many undesirable chemical and physical relationships within foods, such as oxidative rancidity, vitamin deterioration, and certain textural changes, are often caused or accelerated by processing methods.

FOOD ENGINEERING AS RELATED TO OTHER DISCIPLINES AND PROFESSIONAL SOCIETIES

Food science and food technology are related and overlapping disciplines that have similar basic science requirements, such as general and organic chemistry, physics, mathematics through calculus, and microbiology. Food science courses emphasize the relationships between basic science courses and the various aspects of food production, processing, and preservation. Separations—differentiation among career aims and goals—usually occur after college graduation and the entrance into graduate school or the industrial and commercial job market. Food scientists ordinarily concentrate on the basic chemistry and microbiology of foods; food technologists usually are more involved in the applied aspects of handling and processing foods. Many basic researchers specialize in the biological relationships of foods and must have particularly strong backgrounds in biology, biochemistry, and microbiology.

The third member of the team is the food engineer. Engineers professionally involved in the food industry must have the food science and technology background to actively and productively participate in maintaining an adequate food supply for the world's burgeoning population.

The close work and professional relationships among food scientists, food technologists, and food engineers are demonstrated by the extent to which they maintain memberships in cross-disciplinary professional societies. The Institute of Food Science and Technology (IFT) was founded in 1939. Several decades later, a food engineering division was formed in the IFT. Many of the founding members of the division were food technologists who, through experience or further training, had learned applied engineering related to food processing. Today, the food engineer has found more direct relationships with fellow engineers in professional engineering societies, including the American Society of Agricultural Engineers, the American Society of Chemical Engineers, the American Society of Mechanical Engineers, and other scientific and professional organizations. The food engineer is now established as an indispensible contributor to the further scientific and technological development and growth of our most important scientific and industrial enterprise.

J. T. Clayton
University of Massachusetts
Amherst, Massachusetts

FOOD, DRUG, AND COSMETIC COLORANTS.

See Colorants: food, drug, and cosmetic colorants.

FOOD FERMENTATION

DEFINITION

Food fermentation is the study of microbial activity, usually anaerobic, on suitable substrates under controlled or uncontrolled conditions resulting in the production of desirable foods or beverages that are characteristically more stable, palatable, and nutritious than the raw substrate.

food industry are all too common. Food processing and food handling machinery and equipment are often based on previous practice rather than efficient functioning, easy maintenance, and meeting sanitary requirements. Modern-day food manufacturing requires reliance on sound engineering data, which often are not available or are in need of updating or improvement to evaluate and commercialize manufacturing methods and techniques. Some of the important food engineering areas in need of further study include

1. The changes in mass and composition that occur in foods during processing.
2. The pack densities, specific gravities, and compressibilities that are brought about in loading bins, storage tanks, and vats.
3. The safe loading depths for fresh or raw products during transportation and storage.
4. The flow properties for various foods in different forms and process states.
5. The properties of viscous material such as purées, pulps, slurries, etc.
6. The specific heat, heat capacity, and heat conductivity of foods in all states of preservation, especially when simultaneous mass and heat transfer occur.
7. The effect of particle size on various processes, which has a bearing on many processes such as spray drying, grinding, filtering slurries and miscella, and disintegration operations.

Both mechanical and chemical unit operations and processes are necessary in food production. Mechanical operations include washing, sizing, sorting, grinding, mixing, polishing, grading, packaging, and materials handling. Processes for preserving (and often to improve texture and flavor) include a combination of chemical and physical unit operations and processes. These include (*1*) adding heat for cooking, pasteurizing, or sterilizing; (*2*) removing heat for cooling (refrigerating), freezing, or storing in a frozen state; (*3*) adding chemicals to control water activity, aid in fermentation or other processes (eg, enzyme hydrolysis), and improve sensory characteristics and acceptance; (*4*) removing water to control water activity; and (*5*) irradiating to pasteurize or sterilize. Most food processing uses a combination of these steps. For example, drying is normally carried out by adding heat to vaporize the water, whether it be under vacuum or atmospheric pressure. Of course, the primary purpose of these operations is to control or destruct microorganisms and their metabolic products and to reduce certain detrimental chemical and physical reactions. However, many undesirable chemical and physical relationships within foods, such as oxidative rancidity, vitamin deterioration, and certain textural changes, are often caused or accelerated by processing methods.

FOOD ENGINEERING AS RELATED TO OTHER DISCIPLINES AND PROFESSIONAL SOCIETIES

Food science and food technology are related and overlapping disciplines that have similar basic science requirements, such as general and organic chemistry, physics, mathematics through calculus, and microbiology. Food science courses emphasize the relationships between basic science courses and the various aspects of food production, processing, and preservation. Separations—differentiation among career aims and goals—usually occur after college graduation and the entrance into graduate school or the industrial and commercial job market. Food scientists ordinarily concentrate on the basic chemistry and microbiology of foods; food technologists usually are more involved in the applied aspects of handling and processing foods. Many basic researchers specialize in the biological relationships of foods and must have particularly strong backgrounds in biology, biochemistry, and microbiology.

The third member of the team is the food engineer. Engineers professionally involved in the food industry must have the food science and technology background to actively and productively participate in maintaining an adequate food supply for the world's burgeoning population.

The close work and professional relationships among food scientists, food technologists, and food engineers are demonstrated by the extent to which they maintain memberships in cross-disciplinary professional societies. The Institute of Food Science and Technology (IFT) was founded in 1939. Several decades later, a food engineering division was formed in the IFT. Many of the founding members of the division were food technologists who, through experience or further training, had learned applied engineering related to food processing. Today, the food engineer has found more direct relationships with fellow engineers in professional engineering societies, including the American Society of Agricultural Engineers, the American Society of Chemical Engineers, the American Society of Mechanical Engineers, and other scientific and professional organizations. The food engineer is now established as an indispensible contributor to the further scientific and technological development and growth of our most important scientific and industrial enterprise.

J. T. CLAYTON
University of Massachusetts
Amherst, Massachusetts

FOOD, DRUG, AND COSMETIC COLORANTS.

See COLORANTS: FOOD, DRUG, AND COSMETIC COLORANTS.

FOOD FERMENTATION

DEFINITION

Food fermentation is the study of microbial activity, usually anaerobic, on suitable substrates under controlled or uncontrolled conditions resulting in the production of desirable foods or beverages that are characteristically more stable, palatable, and nutritious than the raw substrate.

HISTORY

The history of food fermentation paralleled the development of microbiology and food microbiology. Traditionally many foods were prepared by fermentation, but the reasons behind the success or failure of the processes were not known. After the work of Pasteur in the 1850s and others, who demonstrated that a specific microorganism (eg, yeast) acting on a suitable substrate (grape juice) will produce a desirable product (wine), the science of food fermentation began. Now many food fermentation principles and practices are well established, and food companies can predictably produce consistently good-quality fermented products. With the advances in genetic engineering, old processes are being improved and new ones are being discovered. Also, many indigenous fermented foods (such as some Oriental foods and African tribal foods) and their processes are not well known and are areas for future investigation.

Although the principles of fermentation in many foods are understood in a laboratory setting, the scaling-up of these processes to commercially successful operations is complicated. A detailed account of all aspects of anaerobic fermentation, including methodology of anaerobic cultivation; mutation and genetic engineering of anaerobic bacteria; industrially important strains and pathways, biochemistry, kinetics, and transport in anaerobic fermentation; bioenergetics of anaerobic processes; data collection and analysis; mixed culture interactions; and design and application of anaerobic systems has been published by Erickson and Fung (1).

MICROORGANISMS USED IN FERMENTED FOODS

The number of microbial species used in fermented foods is surprisingly small. Of the thousands of species of microorganisms in nature, only the following genera are well utilized by the food fermentation industry (2):

Bacteria: *Acetobacter, Streptococcus, Lactococcus, Leuconostoc, Pediococcus, Lactobacillus, Propionibacterium, Brevibacterium, Bacillus, Micrococcus*, and *Staphylococcus*. There has been a major renaming of some key bacteria used in fermentation as follows:

Streptococcus cremois to *Lactococcus lactis* subsp. *cremoris*

Streptococcus lactis to *Lactococcus lactis* subsp. *lactis*

Streptococcus diacetylactis to *Lactococcus lactic* subsp. *lactis biovar diacetylactis*

Leuconostoc citrovorum to *Leuconostoc mesenteroides* subsp. *cremoris*

Lactobacillus bulgaricus to *Lactobacillus delbrueckii* subsp. *bulgaricus*

Streptococcus thermophilus unchanged

Lactobacillus helvticus unchanged

Yeast: *Saccharomyces* (especially *S. cerevisiae* and *S. carlsbergensis*), *Candida, Torulopsis*, and *Hansenula*

Mold: *Aspergillus, Penicillium, Rhizopus, Mucor, Monascus*, and *Actinomucor*

In many tradition or indigenous fermented products, the cultures are not known or identified.

TYPES OF FERMENTATION PROCESSES

Single-Culture Fermentation

The key to the success of single-culture fermentation is to provide the culture with a sterile substrate and environment with no contamination during the fermentation process. Examples include wine making, beer making, bread making, production of single-culture fermented dairy products, and vinegar production. In this type of fermentation the viable cells increase in a typical growth curve sequence of lag phase, log phase, stationary phase, and death phase. Primary metabolites (alcohol, acid, etc) are made during the log phase, and secondary products (antibiotics, toxins, etc) are made after the culture reaches the stationary phase.

An ideal culture for fermentation should possess the following attributes:

1. Organisms must be pure.
2. Organisms must grow and reproduce quickly.
3. Organisms must be genetically stable yet amiable to manipulation for better performance.
4. Organisms must produce uniform product in a short time.
5. Organisms must not produce undesirable by-products.
6. Organisms must have a protective mechanism (eg, acid or bacteriocin production) against other undesirable contaminants.

Mixed Pure Culture Fermentation

Some products need a mixture of known cultures. The mixed pure cultures can be a controlled mixture of bacteria or bacteria with a combination of yeast and mold. Yogurt making and cheese making are good examples of this type of mixed fermentation. It is necessary to provide a balance of the two cultures for maximum performance. For example, in yogurt making the desired ratio of cocci (*S. thermophilus*) to rod (*L. bulgaricus*) is 5:1 in cell numbers, but by weight the ratio should be 1:1.

Mixed Natural Culture Fermentation

In many parts of the world, especially in developing countries, the flora of indigenous fermented foods are mixed natural cultures. The interactions of those microbes are exceedingly complex, and the success of such fermentations depends on following traditional processes and not on scientific principles, because many of the responsible cultures have not been isolated and studied. The principal fermentation reactions in foods are listed in Table 1. This list does not include reactions in industrial fermentation of solvents, acids, and so on.

Microbial Interactions

The interactions of mixed cultures are of great interest in studying the kinetics of population development in liquid

Table 1. The Principal Fermentation Reactions in Foods

Lactic acid fermentation

Homofermentative: $C_6H_{12}O_6$ (glucose) to $2CH_3CHOHCOOH$ (lactic acid)

Heterofermentative: $C_6H_{12}O_6$ (glucose) to $CH_3CHOHCOOH$ (lactic acid) + CO_2 + C_2H_5OH (ethyl alcohol)

Propionic acid fermentation

$3C_6H_{12}O_6$ (glucose) to $6CH_3CHOHCOOH$ (lactic acid)
$3CH_3CHOHCOOH$ (lactic acid) to $2CH_3CH_2CO_2H$ (propionic acid) + CH_3COOH (acetic acid) + CO_2 + H_2O

Citric acid fermentation

$CH_2COOHHOCCOOHCH_2COOH$ (citric acid) to $2CH_2COCOOH$ (pyruvic acid) to $CH_2COCHOHCH_3$ (acetylmethylcarbinol) + $2CO_2$

Acetylmethylcabinol can be oxidized to $CH_3COCOCH_3$ (diacetyl) or reduced to $CH_3CHOHCHOHCH_3$ (2,3, butylene glycol)

Alcoholic fermentation

$C_6H_{12}O_6$ (glucose) to $2C_2H_5OH$ (ethyl alcohol) + $2CO_2$

Butyric acid fermentation

$C_6H_{12}O_6$ (glucose) to CH_3COOH (acetic acid) + $CH3CH_3CH_2COOH$ (butyric acid) + CH_3CH_2OH (ethyl alcohol) + $CH_3(CH_2)_2CHOH$ (butyl alcohol) + CH_3COCH_3 (acetone) + CO_2 + H_2

Gassy fermentation

$2C_6H_{12}O_6$ (glucose) + H_2O to $2CH_2CHOHCOOH$ (lactic acid) + CH_3COOH (acetic acid) + C_2H_5OH (ethyl alcohol) + $2CO_2$ + $2H_2$

Acetic acid formation (oxidative)

C_2H_5OH (ethyl alcohol) + H_2O O_2 to CH_3COOH (acetic acid)

and solid fermented foods. The following is a discussion of the study on the interactions between an important fermentative yeast (*S. cerevisiae*) and an important industrial bacterium (*Acetobacter suboxydans*) and an important environmental contaminant (*Escherichia coli*).

Figure 1 shows the interaction between *S. cerevisiae* and *A. suboxydans*. For the yeast, both direct count (under the microscope) and electronic count (Coulter Electronic Counter, which can differentiate particles sizes) increased with time. The direct count registered about 10 times more cells than the electronic count. For the bacterium, the direct count increased with time, but the electronic count increased to about 7.8 log cell/mL and then started to decrease. This decrease in number is due to the adhesion of many of the bacterial cells to yeast cells. The electronic counter cannot differentiate these two populations when they are in clumps. However, microscopic observations can ascertain the different counts. The concentration of glucose completely disappeared in the first 10 h of yeast and bacterial interactions. The pH of the medium first decreased and then stabilized at pH 3. This is due to oxidation of alcohol (produced by *S. cerevisiae*) by *A. suboxydans*.

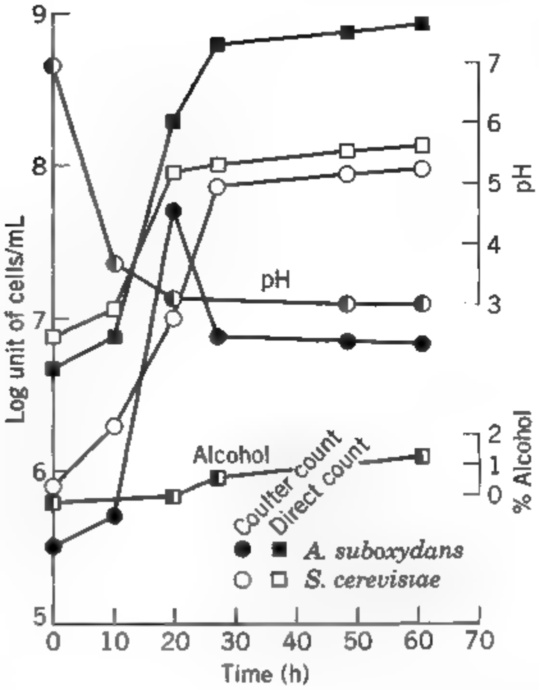


Figure 1. Interaction of mixed cultures of *S. cerevisiae* and *A. suboxydans* in terms of cell numbers monitored by direct count and electronic count as well as product formation (alcohol and acid production). *Source*: Ref. 3, used with permission.

The interactions of yeast and *E. coli* (Fig. 2) showed interesting contrasts compared with the yeast–*Acetobacter* interactions. Growth of yeast as monitored by viable cell count, direct cell count, and electronic count all increased with time. After reaching a stationary phase, viable yeast count decreased, but direct count and electronic counts registered no reduction in numbers. A likely explanation is that the latter two biomass measurements counted both live and dead cells, but the viable cell count method only registered living cells. After the stationary phase, some of the yeast cell went through the death cycle. The growth of *E. coli* showed an increase in the viable cell count and direct cell count. The electronic count reached 8 log cells/mL and then declined, exhibiting a trend similar to that observed in the yeast and *Acetobacter* interactions. Alcohol contents increased to about 2%, and the pH first dropped to around 4 and then rose to 6. This pattern differed from that obtained from the interaction of yeast and *Acetobacter*. *E. coli* cannot oxidize alcohol, and this did not create large amounts of acid to counteract the basic metabolites in the reaction vessel. This resulted in a medium that reverted to a more alkaline state.

This study indicated that different methods of estimating biomass may provide different results in mixed culture fermentation. The electronic counting method is useful when a single culture is monitored. In mixed culture interaction that involves clumping, this method is not suitable for differentiating mixed populations.

The following sections are synopses of major fermented food. Detailed treatment of these subjects can be found in books listed in the reference section.

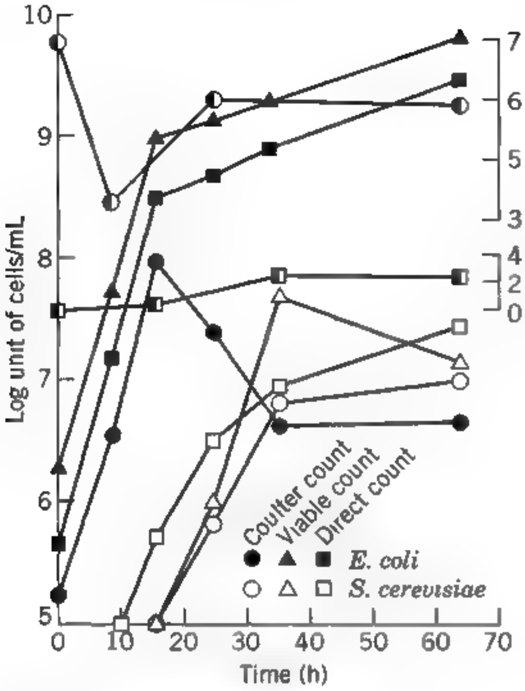


Figure 2. Interaction of mixed culture of *S. cerevisiae* and *E. coli* in terms of cell numbers monitored by direct count, electronic count, and viable cell count as well as product formation (alcohol and acid production). *Source:* Ref. 3, used with permission.

Fermented Liquid and Semisolid Dairy Products

Cultured Buttermilk. Pasteurized skim milk inoculated with 0.5% mixture of *Streptococcus (Lactococcus) lactis, S. (L.) cremoris, Leuconostoc cremoris*, or *L. dextranicum* incubated at 22°C for 14 to 18 h, results in a product with pH 4.5 acid curds with pleasant aroma and flavor. It has acid tastes.

Sour Cream. Milk with 19% milk fat and 0.2% citric acid inoculated with 1% *S. (L.) lactis* and *S. (L.) cremoris*, incubated at 22°C for 14 to 16 h results in a product with pH 4.5 that is creamy and sour and good for baked potatoes and other foods.

Acidophilus Milk. Skim milk inoculated with 5% *Lactobacillus acidophilus* incubated at 38°C for 18 to 24 h results in an acidic (1% acid) sour milk product. It is claimed to have health benefits due to large numbers of live cultures in the product.

Sweet Acidophilus Milk. Skim milk inoculated with a large percentage of *L. acidophilus* results in 5×10^6; *L. acidophilus* per mL of *live* cultures in milk. The product *is not fermented* and must be kept cold (4°C) until sold. Contrary to regular acidophilus milk, objected to by some consumers due to the high acidity, this product has no acid taste but is claimed to relieve gastrointestinal problems.

Bulgaricus Milk. Whole milk inoculated with 2% *L. bulgaricus* incubated at 38°C for 10 to 12 h results in a sour (2–4% acid) product, claimed to relieve gastrointestinal problems. It is consumed in large quantities by people in the Georgia region of Europe.

Yogurt. Milk with added 2 to 4% nonfat dry milk powder inoculated with 5% combination of *S. thermophilus* and *L. bulgaricus* (1:1 ratio, by weight, or 5:1 ratio by cocci to rod) incubated at 45°C for 3 to 6 h or 32°C for 12 to 14 h results in a smooth, viscus gel with a delicate walnutlike flavor. The product must be chilled immediately. A variety of fruits, berries, and flavors can be added to the product. This is the fastest-growing product in the dairy industry.

Kefir. Goat, sheep, or cow's milk inoculated with kefir grain (a mixture of *Saccharomyces kefir, Torula, Lactobacillus caucasicus, Leuconostoc*, etc) incubated at 22°C for 12 to 18 h results in a product with 1% alcohol and 0.8% acid. The product is strained through cheesecloth. The milk foams and fizzles like beer.

Koumiss. Mare's milk (which has five times more vitamin C than cow's milk) inoculated with *L. bulgaricus* plus *Torula* yeast incubated at 22°C for 12 to 18 h results in a drink with 1 to 2.5% alcohol and 0.7 to 1.8% acid.

THERAPEUTIC VALUE OF CULTURED MILK

In 1907 Metchnikoff wrote the book *Prolongation of Life* and claimed that drinking sour milk regularly gave people in southeast Europe long life and good health, because premature old age was a result of absorption of toxins produced by bacteria in the large intestines. Large volumes of sour milk with live cultures changed the balance of microflora in the intestines so that toxic compounds were not produced and people lived longer. At a minimum, drinking live cultures of *L. acidophilus* or *L. bulgaricus* is not harmful. For people taking antibiotics after major surgery, which tends to sterilize the intestines, these live organisms might very well be beneficial. People with gastrointestinal problems may find sour milk helpful in suppressing diarrhea. This is still a controversial area.

FERMENTED SOLID DAIRY PRODUCTS—CHEESE

Cheese is made by the coagulation or precipitation of milk protein by acid produced by starter cultures (*S. [L.] lactis* and *S. [L.] cremoris*) with the aid of rennin (from the stomach of calves) added to the milk. Milk is usually pasteurized at 62.8°C for 30 min or 72.0°C for 15 s. Raw milk has been used to make cheese but is not advisable due to the potential of survival of foodborne pathogens such as *Listeria monocytogenes*. Regulations indicate the cheese made with raw milk cannot be sold until a 60-day aging period. The assumption is that in 60 days pathogens, if present in the cheese, will be killed by the starter cultures and the acidity involved in the product. A food-grade dye, annatto, is often added to give the cheese the familiar yellow color. Some cheese makers are now not using the dye so that the cheese will not have additives in the product. When milk proteins coagulate, the resultant liquid portion is called whey. From 10 lb of milk, only 1 lb of cheese is made and 9 lb are discarded as whey. Whey utilization is an important area of research because whey has very high biological oxygen demand (BOD) (30,000–60,000 ppm) and can be an important environmental contaminant if the BOD is not reduced. Also, whey contains about 50 to 70% of the nutrients of milk, and this rich food source should be effectively utilized. After the curd is formed, it can be poured into perforated molds lined with cheesecloth, and the extra whey is drained. For harder cheese, the curd is cooked, stirred, cut, and pressed. The more whey expressed from the protein mass, the harder the resultant cheese will be. Salt is often added to the curd to give flavor as well as to prevent undesirable microorganisms from growing. Cheese is then ripened for several months or even years before consumption.

Classification of Cheese

There are literally hundreds of cheese varieties being made in the world. Most of the names are from the town or city in which the cheese originated. From a texture point of view, cheese can be classified into the following.

Soft, Unripened or Ripened Cheeses. Cottage cheese is an example of soft, unripened cheese. It can be made by acid produced by the starter cultures (*Lactococcus lactics* and *L. cremoris*) along with rennin or by direct set using food-grade acid such as mesolactide and D-glucono-δ-lactone. For the direct set method, the cottage cheese will coagulate very fast—within seconds at 4°C. For starter cultures, one can use a "short set" method by inoculating the milk with 5% starter cultures, and the process will be completed in 5 h. For the "long set" method, a 3% percent inoculation can be used, and it will take 10 to 14 h to complete the process. After setting the curd, it be cut using 1/4″ wire for small-curd cottage cheese and 3/4″ wire for large-curd cottage cheese.

Soft, Ripened Cheeses. Limburger cheese is an example of soft, ripened cheese. The soft cheese is placed on wooden shelves, and a surface bacterium (*Brevibacterium linens*) grows on the surface of the cheese and produces a brownish red surface growth. Protein is broken down into ammonia and gives the strong flavor to this cheese. Camembert cheese is another soft cheese, but it is ripened by a surface mold, *Penicillium camemberti*, with a mixture of bacteria. This famous French cheese is called the queen of cheese.

Semisoft, Ripened Cheeses. Several mold-ripened cheeses are classified under this category. They are all ripened by *Penicillium roqueforti*, a mold that grows throughout the cheese mass. They are also called blue-veined cheese. Roquefort cheese is called the king of cheese. Other cheeses in this group are Stilton, Gorgonzola, and bleu.

Hard Cheeses. These cheeses are well ripened and hard. Among the most important cheeses are cheddar, a bacterial-ripened cheese, and Swiss cheeses, which are cheeses with eyes. The characteristics eyes of Swiss cheeses are caused by CO_2 produced by *Propionibacterium shermanii* during anaerobic fermentation in the ripening stage.

Cheese Varieties and Descriptions

Principal cheese varieties and descriptions are as follows (4).

American. This term is used to identify the group that includes cheddar, Colby, and so on, popularized in the United States.

Bleu. The French name, bleu, is used for cheese similar to Roquefort but either not made in Roquefort, France, or not made from ewe's milk.

Blue. Roquefort-type cheese made in the United States and Canada is referred to as blue. Brick cheese, of American origin, is made from whole milk, with a mild but pungent and sweet flavor.

Cheddar. This is the most important cheese and accounts for 75% of cheese made in the United States. The name came from the town Cheddar in the UK where it was first made. In addition, cheddaring is the name of a step in the manufacturing process of piling and repiling of curd. This will provide better adhesiveness of the protein and also allow starter cultures to produce more acid to control coliforms. Most cheddar cheeses are ripened for 60 days and some for a year or more (sharp and very sharp cheddar).

Colby. Similar to cheddar, Colby has a softer body and more open texture.

Cottage. A soft unripened cheese, cottage cheese is made from skim milk. Flavoring materials such as peppers, olives, pimientos, or garlic may be added. When more than 4% of fat is added, it is called creamed cottage cheese.

Cream. Cream cheese is a soft, rich, unripened cheese made of cream or a mixture of cream and milk.

Edam. Made from whole milk, Edam has a mild flavor and firm body. It is usually shaped like a flattened ball and covered with red coloring or red paraffin.

Gouda. Gouda is similar to Edam, except that it contains more fat and is usually packaged like Edam.

Limburger. A soft, surface-ripened cheese, Limburger has a characteristic strong flavor and aroma.

Parmesan. A very hard cheese that will keep almost indefinitely, Parmesan is used as grated cheese on salads, soups, and with pasta.

Process Cheese. Process cheese is made by grinding and mixing together by heating and stirring one or more naturally fermented cheese of the same or different varieties, together with an emulsifying agent into a homogenous mass at very high temperature (130°C) for a few seconds. After the mixture has been properly prepared, the cheese can be made into various thickness, shapes, and forms. Also, a variety of ingredients such as bacon, spices, port wine, and so on can be incorporated into the cheese. At least one-third of all cheese marketed in the United States, except soft, unripened cheese, is process cheese.

Process Cheese Food. Process cheese food is made in the same way as process cheese, except that certain dairy products (cream, milk, skim milk, cheese whey, or whey albumin) may be added. At least 51% of the weight of the finished product must be cheese.

Process Cheese Spread. This is made in the same way as process cheese food, except that it contains more moisture and less fat and must be spreadable at a temperature of 21.1°C (70°F). Fruits, vegetables, or meats may be added.

Roquefort. A cheese made only in the Roquefort area of France from ewe's milk, Roquefort is characterized by sharp, peppery flavor and blue-green veins throughout the cheese. It is ripened principally by blue mold in the interior.

Swiss. A hard cheese with an elastic body, nutlike flavor, Swiss cheese is best known by the holes or eyes that develop as the cheese ripens.

An excellence reference set—*Cheese and Fermented Milk Foods Vol 1: Origins and Principles* and *Vol 2: Procedures and Analysis*—that covers all aspects of fermented diary foods was published by Kosikowski and Mistry (5).

ALCOHOLIC BEVERAGES

The per capita consumption of alcohol beverages in the United States is about 2.4 gal of wine, 24 gal of beer, and 1.6 gal of spirits. In 1995 alcoholic beverages accounted for $103.9 billion in sales, with beer being $62.6 billion, spirit being $29.5 billion, and wine being $11.8 billion. Alcoholic beverages can be classified as fermented, not distilled, and distilled.

Fermented, Not Distilled Beverages

Wine. By definition, wine is a fermented product from fruits. By far the most important wine is produced from grapes, although it can be produced from apples, pears, berries, and other fruits. *Vitis vinifera* and *V. labrusca* are the most important species. Grape growing is a science itself (viticulture), and wine making is called enology.

To obtain a good wine, the enologist must consider the species and varieties used, the climate and soil conditions that dictate the vintage of a good wine, and the time to pick the grapes to make the wine. Europe produces about 80% of the world's wine, with North and South America producing about 14%; Africa, 4%; Asia, 1%; and Oceania, 1%. Whereas in Europe people consume about 30 gal of wine a year per person, the U.S. population consumes 2 to 3 gal per year per person. Grapes are first picked in autumn, when the amount of sugar in the grape stabilizes, then the grapes are stemmed and crushed to separate and remove the leaves and stems. The resultant materials, juice, skin, pulp, and so on, are called must (*L. mustum*, "new wine"), which is the source of the wine.

Next SO_2 is added at 50 to 100 ppm to condition the must for its ability to inhibit undesirable organisms and its antifungal and antioxidative properties. The correct amount of sugar is added to define the final concentration of alcohol in the wine. The rule is when one wants to make a wine of X % alcohol one would add 2 X % of sugar. This is because 1 g of sugar will be converted to half a gram of alcohol and another half a gram of carbon dioxide, which is released into the environment. The conditioned must will then be inoculated with a wine yeast (usually *S. cerevisiae* var. *ellipsoideus* Montrachet strain) at 1% inoculum level. For red wine the must can be fermented on the skin, which allows extraction of the red anthocyanin by alcohol to give the red color to the wine. Alternatively, the must can be hot-pressed (the must is heated to 62.7°C, and the juice is pressed out while hot), which also extracts the red color. To make white wine, white grapes can be used or, if red grapes are used, a cold press will yield a white juice. Pressing of the wine is an important step. In a traditional wine press, when the must is poured into the press, some juice flows through the system without pressing. This "free-run" juice yields the best wine. The harder the grapes are pressed, the poorer the quality of the wine produced. At this point it is important to check the acidity of the must. The liquid should be about pH 3.6 and 0.7 to 0.9 g acid/100 mL of juice. If adjustment is needed, tartaric acid is used to acidify the must, and water is used to dilute the juice. This is the amelioration step. The juice is now ready for secondary fermentation.

Secondary fermentation involves putting the fermenting must into a closed container (cooperage) and applying an airlock to prevent oxygen from entering the system and to allow carbon dioxide to escape. Because heat is produced (exothermic reaction), the fermenting must is kept at 15.5°C to 21°C for red wine and 12.8°C to 18.3°C for white wine.

Fermentation will continue for two to six weeks depending on the amount of dryness (reduction of sugar) desired in the finished wine. Before the completion of fermentation, several racking steps may be done. Racking is a process in which the wine is separated from lees (dead yeast cells and insoluble materials at the bottom of the cooperage). The first racking is done after fermentation to desired dryness (an appropriate amount of sugar is fermented to alcohol), followed by racking after dropping (crystallizing) the insoluble tartaric in cold temperature (ca 0°C), and the final racking is done after clarification and before bottling. Clarification can be achieved by the addition of fining agents such as gelatin, casein, bentonite, and polyclar AT. Enzymes may also be added to degrade pectin. The new wine is put into a wooden cooperage for aging. The biochemistry involved in aging is complex, but at least one year of aging is necessary for a good wine to be produced. The exact time for bottling of wine is determined by the wine master of the particular winery. Wine can be pasteurized (55.5–65.5°C for a few seconds), but more often it is bottled without heat treatment, because heat tends to destroy flavor compounds. To remove any residues after aging, a filtration step can be made before bottling. Wine continues to improve in the bottle. Ten years of aging is optimum.

Classification of Wines

Hundreds of varieties of wines are produced around the world, and no classification can cover all of them. The following is a functional classification of wines.

Dry or Sweet Wines. In dry wine all fermentable sugar is converted to alcohol. Typically the wine has about 8 to 12% alcohol. Examples are Chablis, Riesling, Burgundy, and Chianti. Sweet wine has some unfermented sugar. The alcohol is about 13 to 15%. Examples are Tokay and sauterne.

Fortified or Unfortified. In fortified wine more alcohol is added to the product. Some examples are sherry, port, muscatel, and champagne. Unfortified wine is a wine in which all the alcohol resulted from fermentation.

Sparkling or Still. Sparkling wine is bottled before fermentation has ceased and liberates effervescence after the bottle is opened. Champagne made in France is the true example of fermentation in the bottle. To be able to show that the gas is generated in the bottle, a label can be printed as "fermented in this bottle." Injection of CO_2 into a wine to achieve effervescence is called crackling. In this case the manufacturer cannot claim that the wine is fermented in this bottle. Still wines are those in which fermentation ceased before bottling and no CO_2 exists.

Red or White. Fermentation of the skin of red grape will result in red wine such as claret, Burgundy, port, and chianti. Reisling is a good example of a white wine.

Generic Wine. Wines blended from several varieties of grapes are called generic wines. Examples are Burgundy, chianti, Chablis, and claret.

Variety Wine. Wines from predominately (at least 75%) one type of grape variety are variety wines. Examples are cabernet, pinot noir, zinfandel, and chardonnay.

Vintage Wine. Vintage wine must have at least 95% of the juice coming from the year claimed.

Prevention of Wine Spoilage

Oxygen is the worst enemy of wine because it promotes the growth of *Acetobacter*, which oxidizes alcohol to acetic acid. Oxygen also oxidizes wine color from white to heavy amber or from rich purple to tawny brown and affects the flavor of the wine. Dirty equipment and environment contributes to spoilage of wine directly by introducing microorganisms or indirectly due to residues of unwanted chemicals. Undesirable microorganisms during the fermentation process or in aging and storage can also contaminate wine.

Champagne Processing

Champagne processing is a fascinating subject and deserves some discussions. About 250 years ago a monk named Don Perignon in the Champagne region of France discovered the process of making champagne. He exclaimed, "Come and see I am drinking stars." Champagne making is now a highly regulated manufacturing process under the control of the French government. At the perfect time of maturity of the grapes (chardonnay, pinot meunier, and/or pinot noir), they are picked by hand. Great care is taken not to bruise the delicate skin of the grapes during the harvesting stage. The grapes are then transported to the winery to be pressed to obtain the grape juice. Four thousand kg or 8800 lb of grapes are placed in a tub called marc. Each marc is pressed several times, yielding a total of 2550 L as laid down by the rules of *appellation*, which calls for 1.6 kg (3.52 lb) of grapes per liter of juice. The first three pressings yield 2050 L of first-quality juice known as the cuvee. A final pressing then yields 500 L of taille or lesser-quality juice.

After pressing, the vin de cuvee and vin de taille are allowed to stand for the solids to settle. After that time the must will be allowed to ferment and is made into wine. Only wine made from vin de cuvee is used for champagne making. After fermentation, the cuvee will have about 10% alcohol. Into this cuvee more sugar and yeast culture will be added, and a strong cap is applied to the bottle to start a secondary fermentation in the bottle. The amount of added sugar will dictate the amount of pressure generated in the bottle. This is why champagne bottles are made with very strong and thick glass. During this fermentation, the yeast will convert the added sugar to alcohol and carbon dioxide. It is this carbon dioxide that causes champagne to bubble. An important step in champagne making is to remove the yeast cells from the enclosed bottle so that the champagne will not be cloudy. To achieve this, a very tedious procedure is used in which a worker will turn the champagne bottle one quarter time per day for two weeks (riddling). Each day after riddling, the bottle will be tilted a little more toward the neck such that yeast will slowly migrate to the neck of the bottle by gravity. After completion of the riddling process, all the yeast will be at the neck of bottle. The neck is then frozen. This will cause a column of ice to be formed, which traps the yeast. The bottle is then opened quickly, and the pressure of the gas in the bottle will expel the column of ice with the yeast. Quickly the worker will add some sterile wine into the bottle to compensate for the lost liquid, and the bottle is tightly closed. A champagne is then made. The champagne can be stored in caves for years before shipment for sale. In the Meot & Chandon company in Epernay, France, something like 5 million bottles of champagne are stored in 7.1 mile of caves in this lime country. Current research work on champagne includes: (*1*) development of disease-resistant strains of grape vine; (*2*) use of alginate pellets to immobilize yeast cell in the champagne for secondary fermentation; after fermentation, it would take only 40 s to settle all the alginate pellets with yeast to the neck and remove the yeast a frozen column—this can take the place of the tedious "riddling" process by hand; and (*3*) measurement of amount of bubble and foam by use of robots and camera to examine champagne quality.

Beer

Although beer is more complicated to produce, it was developed earlier in history than wine at around 6000 B.C. By definition beer is an alcoholic drink derived from grains. The most common source of beer is barley, with rice and corn used as adjuncts. The per capita consumption in Europe is about 32 gal of beer a year, whereas in the United States the per capita consumption is about 25 gal. Beer is made by malting barley to obtain α- and β-amylases, then by mashing, which is a process in which more grain (adjuncts) and water are added to the malt. The mixture is heated slowly to first achieve proteolysis (35°C for 1 h) and then starch hydrolysis (67–68°C for 20–30 min); finally, the enzymes are inactivated (mashing off at 75–80°C). The main purpose is to allow α- and β-amylase to degrade amylose and amylopectin to yield glucose and maltose for fermentation by yeast, because yeast cannot degrade starch directly. The liquid (which can be considered as nutrient broth for yeast) is separated from the husks of the grains by lautering and sparging. Basically these are filtration and rinsing steps to obtain clear liquid for fermentation. The resultant clear fluid is called wort. Before addition of yeast for fermentation, the wort is boiled and hops (*Humulus lupulus*) is added to give the bitter flavor of beer. After cooling to 8.8°C, yeast is added (pitching). *Saccharomyces carlsbergensis* (bottom yeast) is the yeast of choice for lager beer making. Fermentation continues for three to four days, at which time a lot of foam is developed in the fermentation vessel (Krausen formation). After five days, the Krausen collapses and after 10 to 12 days fermentation ceases. At this stage the beer is called green beer. Yeast cells can be recovered for animal feed, and CO_2 is collected and later injected into finished beer for foam formation. The green beer is aged for about three months before being bottled for sale. Beer can be sold in unpasteurized form (draft beer), which has a short shelf life. Most of the beer on the market is pasteurized at 57.2 to 60°C for 15 to 20 min in bottles and cans. Some beers are filtered and not pasteurized (draft canned beer). Beer made in the preceding manner is called lager beer, and the alcohol content is about 3.5 to 4%. Using *S. cerevisiae* (top yeast), ale can be made that has about 6% alcohol. European beers are "heavier" because they use only barley, which has more protein than barley used in the procedure in the United States, where barley is mixed with rice and corn adjuncts that decrease the total protein contents.

Light Beer. Light beer is made by using enzymes such as glucoamylase and pullulanase along with the α- and β-amylases to convert all the starch to fermentable sugar; thus, no sugar is left in the beer after fermentation. The beer has fewer (lighter) calories than the regular beer, which has residual starch. Light beer still has calories from alcohol since 1 g of alcohol provides 7 cal of energy.

Dry Beer. Dry beer was started in Japan. Japanese beer has a sweeter taste than Western beer and is not blended. By a process called *superattenuated fermentation*, special yeast strains are used for prolonged fermentation, resulting in the utilization of all the sugars. In dry beer making, no enzyme is added to reduce the carbohydrate.

Ice Beer. This is the newest entry into the beer market. Beer is first made and then quickly frozen to obtain small ice crystals. The beer is then filtered through a membrane that retains the ice crystals. The reason behind this process is that the ice crystal will capture undesirable compounds of the beer with a resultant smoother beer. Some people think that this is just another commercial effort to keep consumers entertained by having another style of beer. Because the water is removed from the beer during the freezing and filtration processes, ice beer has a higher alcohol content of about 6% compared with regular beer.

Sake Making

Sake, a pale white drink, is closely related to Sinto religion and is often called rice wine. Technically it is a beer because it is made of rice. The rice is first inoculated with spores of *Aspergillus oryzae* (Koji), which is the source of amylase to degrade starch. The Koji is then added to more rice along with yeast to achieve fermentation. The resultant liquid has a 14 to 20% alcohol content with no gas.

Beverage from Distilled Fermented Liquids

Because natural fermentation can achieve only about 12 to 15% alcohol and in extreme cases like sake, 20% drinks of higher alcoholic content are achieved by concentrating alcohol through the process of distillation.

Whiskey includes rye, bourbon, Scotch, Irish, Canadian Pisco, and vodka, which are distilled from fermented grains. Rums (Cuba, Puerto Rico, Philippines, Jamaica, Barbados, Martinique, and New England) are distilled from sugarcane and molasses. Tequila is distilled from agave. Brandies are distilled from a variety of fermented fruit juices such as grape (cognac, Armagnac, and brandy), apple (calvados and apple jack), cherry (kirsch and cherry brandy), and plum (slivovitz and Micabelle). In addition, distillation with flavored compounds provide such drinks as gins, liqueurs, absinthe, aquavit, and bitters.

Fermented Products from Vegetables

Vegetables have low buffering capacity. After harvest, vegetables have a heterogeneous population, including *Pseudomonas, Bacillus, Chromobacterium*, and a variety of enteric organisms; lactic acid bacteria (the organisms responsible for fermentation) exists in relatively small numbers. However, once the material is placed under anaerobic conditions with the addition of 2.5% salt, lactic acid bacteria quickly predominates and initiates a favorable fermentation process. Such is the case in sauerkraut making. Shredded cabbage has 3 to 6% sugar (glucose, fructose, and sucrose). The first group of organisms growing are *Enterobacter cloacae, Erwinia herbicola*, and *Leuconostoc mesenteroides* (a heterofermentative lactic). As the lactic acid increases, the enterics die off and *Leuconostoc mementeroides* predominates, producing favorable compounds for the development of *Lactobacillus plantarum* (a homofermentative lactic). This organism continues to produce lactic acid to a level of 1.5 to 2.0% and completes the fermentation process. Vegetable fermentation represents a form of natural fermentation because, regardless of the type of starter culture used, *L. plantarum* always ends up as the principal organism in a properly treated fermentation vet. A popular fermented vegetable in Korea is Kimchi. Hundreds of varieties of Kimchi are made by anaerobic fermentation of mixed vegetable along with meat, spices, and other ingredients. It is interesting to note that in the West, vegetable fermentation is usually by use of one type of vegetable, but in the Orient the rule is to use several kinds of vegetable in the same fermentation vessel.

The fermentation of cucumbers in the making of dill pickles and salt stock pickles is another example of vegetable fermentation. The microbial succession of cucumber fermentation closely follows the pattern achieved in sauerkraut fermentation. Almost any kind of vegetable can be fermented.

Fermented Products from Meats

Since ancient times many types of fermented meat product have been developed. However, scientific understanding of fermented sausage resulted only after the advancement of meat microbiology in the last 100 years. Currently most meat is consumed unfermented. However, many varieties of meat products are preserved in the form of sausages (such as frankfurters, bologna, Vienna sausage, loaves and luncheon meats, bratwurst, brockwurst, and braunschweiger). Only 6% of U.S. sausages are fermented. They are either fermented sausages (pepperoni and salami) or semidry fermented sausages (cervelat and Thuringer). The basic ingredients for fermented sausage are ground meat (one type or a mixture of a few types), salt (2.5–3.5%), sugar (1%, for fermentation), spices, nitrites, ascorbate and erythorbate, and lactic acid bacteria. The traditional process of applying cultures is to let chance contaminants start the fermentation. This process is highly unreliable because ground meat contains many types of microorganism other than lactic acid bacteria. The second process is by back-slopping, when the batter from a previous successful batch is added to a fresh sausage batch to initiate the fermentation. This method is more reliable than the traditional method but is still subject to unwanted variations. The third and most reliable process is by the addition of starter cultures. Lactic acid bacteria such as *Pediococcus* and *Lactobacillus* have been successfully used to make fermented sausages. These bacteria in the presence of sugar and the anaerobic environment provided by the casing of the sausage quickly produce lactic acid and also develop the tangy flavor typical of the taste of fermented sausage. Fermented sausages can also be smoked to add distinctive flavors.

Fermented Cereal Foods—Bread

According to many people of the world, bread is the staff of life. Bread is only one type of fermented cereal product. Other products include rolls, Danish pastry, crackers, doughnuts, pretzels, and so on. Bread and bakery products are a multibillion dollar business in the United States, the majority of which is bread. The definition of a bread is a product of moistened, kneaded, fermented, and baked meal of flour (mainly from wheat) with appropriate added ingredients. Yeast (*S. cerevisiae*) is the organism to facilitate the fermentation process to make bread. Yeast can be supplied to the baking industry in a compressed, bulk, or active dried yeast form. Bread is made by many different processes, but the most popular method is the sponge-dough method. After all the ingredients are mixed for the sponge part (65% of total flour, water, yeast, and yeast nutrients), the sponge is kept at 25°C for 4.5 h for the first fermentation. The volume increases to four to five times, and the temperature increases to 30°C due to the exothermic reaction of fermentation. The rest of the ingredients (flour, water, sweetener, fat, dairy product, crumb softener, rope and mold inhibitor, dough improver, and enrichment) are then added to make the final dough. The dough is mixed at 72 rpm to produce a smooth cohesive dough that has a glossy sheen. The mixed dough is allowed to rest for 20 to 30 min, then the dough is divided and rounded and put into molds. In the proof box (35–43 °C at 80–90% humidity for 60 min) the dough expands to the desired volume due to the fermentation of sugar with the development of CO_2, which is trapped by the elastic gluten of the dough. Baking is the last step in bread making. During baking the heat of the process expands the trapped gas in the dough matrix and causes the dough fabric to oven-spring. Enzymes are active until the dough reaches 75°C, when the gluten matrix coagulates and dough structure is set into the form of the bread. When the bread surface reaches 130 to 140°C, sugar and soluble protein react chemically to give the crust color and textures. The center of the bread does not exceed 100°C. After cooling, slicing, wrapping, and distributing, the bread reaches consumers. In many places in Europe, bread is baked a couple of times a day and consumers eat bread fresh from the oven. In the United States, breads in supermarkets are usually half a day to a day old before consumers purchase the products. One of the problems of the science of bread making is staling. After years of research, the exact cause of staling is still unresolved.

Production of Vinegar

Vinegar is literally a result of souring (aigre) of wine (vin). The origin of vinegar no doubt followed the production of wine, because a bad batch of wine will result in some form of vinegar. Vinegar has been used as a flavoring agent, food preservation agent, and even as medicine. Both Eastern and Western cultures have records of vinegar in ancient history. Although vinegar production is always treated as a part of food fermentation, it is, in reality, an oxidative process. In the presence of molecular oxygen and *Acetobacter aceti*, or related species, alcohol is oxidized to acetic acid. Historically vinegar was made by the let-alone process or the field process; poor quality wine was allowed to sit in the open air for oxidation to occur. Currently the methods to make vinegar are the trickling process and the submerge culture generator. In the trickling process, wine is trickled into a Frings-type generator where the liquid comes in contact with wood shavings, thereby exposing it to large amounts of oxygen. The alcohol is then oxidized by the *Acetobacter* in the system. At the bottom of the system, the collected liquid contains about 12% vinegar. This process takes about two weeks. The submerged culture system takes about 35 h to achieve the same concentration of vinegar. In this closed system, air is pumped into the generator containing wine, and the organisms in the mixture actively oxidize alcohol to vinegar. Since *Acetobacter* can overoxidize acetic acid to hydrogen and carbon dioxide, *Gluconobacter* is used in submerged systems because this organism does not have enzymes to further oxidize acetic acid to other compounds. Vinegar can be pasteurized to render it more stable in storage.

Mold-Modified Foods

In many parts of the world the indigenous fermented products are modified by molds along with yeast and bacteria. These products are usually the result of uncontrolled, naturally mixed culture fermentation. Much research needs to be done on these foods to ascertain the microbes involved in the process, the safety of the products, and the economical feasibility of large-scale production in modern industrial plants. Some of the more important foods in this category are listed next.

Soy Sauce. A product of the Orient, soy sauce is made from fermentation of whole or defatted soybean and soybean products along with roasted wheat, using a combination of *Aspergillus oryzae, Pediococcus halophilus, Lactobacillus delbrueckii, Torulopsis versatilus*, and *Saccharomyce rouxii*, resulting in a dark reddish liquid, salty taste, and an important flavoring agent.

Miso. This is a product of the Orient from fermentation of whole soybeans mixed with rice or barley using *Aspergillus oryzae, S. rouxii, Torulopsis etchellsii*, and *P. halophilus* to produce a dark reddish, smooth paste with a strong flavor and salty taste.

Hamanatto. A product of the Orient, hamanatto is made from fermentation of whole black soybeans and wheat flour by *Aspergillus, Streptococcus*, and *Pediococcus*. These black soft beans have a salty flavor and are used as a condiment.

Sufu. Sufu is a product of China obtained by fermentation of soybean curd (tofu) using *Actinomucor elegans* and *Mucor dispersus*, resulting in salty and strongly flavored cream cheese–type cubes.

Tempeh. Tempeh is a product of Indonesia and vicinity using whole soybeans inoculated with *Rhizopus oligosporus*, resulting in a soft bean cake bound by mycelia of the mold. It has a clean taste and can be fried, cooked, or eaten as is.

Natto. A product of Japan, natto is obtained by fermentation of whole soybeans with *Bacillus subtilis* var. *natto*, which results in beans covered with viscous, sticky glutamic acid polymers produced by the bacteria. It has a strong ammonia odor and is eaten with or without further cooking.

Other mold-modified foods include *bongkrek* (coconut cake, Indonesia), *ontjom* (peanut cake, Indonesia), *lao-chao* (glutinous rice, China and Indonesia), *ang-kak* (red rice, China and the Philippines), *idli* (rice, India), *doza* (rice, India), *trahana* (parboiled wheat, meat, and yogurt, Turkey), *injera*, (teff, Ethiopia), *kishk* (wheat and milk, Egypt and Middle East), *gari* (cassava roots, West African and Nigeria), *ogi* (maize, Benin and Nigeria), *mahewu* (maize, South Africa), *pozol* (maize, Mexico), and many others.

Other food products are also fermented, such as coffee, cacao, vanilla, tea, citron, ginger, mead, fish sauces (*nu'oc mari*), poi, olives, *pidan* (hundred-year-old egg), and so on. The field of food fermentation can even include such processes as production of single-cell protein, lactic acid, citric acid, glutamic acid, lysine, antibiotics, vitamins, lipids, ascorbic acid, and so on. Many areas of food fermentation overlap with industrial fermentation and biochemical engineering.

THE ROLE OF GENETIC ENGINEERING IN FOOD FERMENTATION

Most of the cultures used in food fermentation are naturally occurring cultures. Selection of special strains allow the manufacturers to produce special aromas, flavors, textures, and odors of foods. By selection, mutation, and crossing of genes, much improved cultures have been developed to produce better fermented food. Now with the advent of genetic engineering scientists can insert genes of almost any living things into the chromosomes or plasmids of another organism to produce desirable products. For example, the rennin gene of calf has been cloned into *E. coli* to produce rennin that is chemically and biochemically exactly like the rennin extracted from the calf, but the enzyme is much cheaper. A lot of work has been done in developing phage-resistant dairy starter cultures. These types of development will no doubt help advance the science and productivity of starter culture technology and, it is hoped, provide more delicious, nutritious, plentiful, less expensive, and healthy fermented food products.

In conclusion, food fermentation is an important field of study. Although much has been learned about some well-known fermentation processes such as beer, wine, cheese, and bread, much needs to be studied concerning mold-modified foods, indigenous foods, and improvement of existing processes. With the rapid advancement of genetic engineering, some dramatic changes in the field of food fermentation should occur in the near future.

BIBLIOGRAPHY

1. L. E. Erickson and D. Y. C. Fung, eds., *Handbook on Anaerobic Fermentations*, Marcel Dekker, New York, 1988.
2. S. E. Gilliland, *Bacterial Starter Cultures for Food*, CRC Press, Boca Raton, Fla., 1985.

3. D. Y. C. Fung et al., "Mixed Culture Interactions in Anaerobic Fermentations," in L. E. Erickson and D. Y. C. Fung, eds. *Handbook on Anaerobic Fermentations*, Marcel Dekker, New York, 1998, pp. 501–536.
4. G. Sauder, *Cheese Varieties and Descriptions*, USDA Agricultural Handbook No. 54, Washington, D.C., 1978.
5. F. V. Kosikowski, and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed. Vols. 1 and 2, Edwards Brothers, Ann Arbor, Mich., 1997.

GENERAL REFERENCES

M. A. Amerine et al., *Technology of Wine Making*, 4th ed., AVI Westport, Conn., 1980.

G. J. Banwart, *Basic Food Microbiology*, 2nd ed., Chapman and Hall, New York, 1989.

L. R. Beuchat, *Food and Beverage Mycology*, AVI, Westport, Conn., 1978.

C. M. Bourgeois, J. Y. Leveau, and D. Y. C. Fung *Microbiological Control of Food and Agricultural Products* (English ed.), VCH, New York, 1995.

D. R. Buege and R. C. Cassens, *Manufacturing Summer Sausage*, Extension. No. A 3058, University of Wisconsin, Madison, Wis., 1980.

R. A. Copeland, *Enzyme*, Wiley-VCH, New York, 1996.

W. Crueger and A. Crueger, *Biotechnology: A Textbook on Industrial Microbiology*, Sinauer, Sunderland, Mass., 1984.

M P. Doyle, L. R. Beuchat, and T. J. Montville, *Food Microbiology· Fundamentals and Frontiers*, ASM, Washington, D.C., 1997.

D. Y. C. Fung and K. Vicheinroj, *Introduction to Food Fermentation*, Kansas State University, Manhattan, Kans. 1998.

Cultures for the Manufacture of Diary Products, Chr. Hansen, Inc., Milwaukee, Wis., 1997.

ICMSF, *Microorganisms in Foods 5: Microbiological Specifications of Food Pathogens*, Blackie Academics and Professionals, New York, 1996.

J. M. Jay, *Modern Food Microbiology*, 5th ed., Van Nostrand Reinhold, New York, 1996.

P. Z. Margalith, *Flavor Microbiology*, Charles C Thomas, Springfield, Ill., 1981.

R. T. Marshall, *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington D.C., 1992.

D. A. A. Mossel et al., *Essentials of the Microbiology of Foods*, John Wiley, New York, 1995.

C. S. Pederson, *Microbiology of Food Fermentation*, 2nd ed., AVI, Westport, Conn., 1979.

H. J. Peppler and D. Perlman, *Microbial Technology*, Vol. 11, Academic Press, Orlando, Fla., 1979.

R. K. Robinson, *Dairy Microbiology*, Vols. 1 and 2, Elsevier Applied Science, Barking, United Kingdom, 1976.

J. H. Silliker et al., *Microbial Ecology of Foods*, Vols. 1 and 2, Academic Press, Orlando, Fla., 1980.

P. F. Stanbury and A. Whitaker, *Principles of Fermentation Technology*, Pergamon Press, Elsmford, N.Y., 1984.

K. H. Steinkraus, *Handbook of Indigenous Fermented Foods*, Marcel Dekker, New York, 1983.

R. P. Vine, *Commercial Winemaking: Processing and Controls*, AVI, Westport, Conn., 1981.

D. I. C. Wang et al., *Fermentation and Enzyme Technology*, John Wiley, New York, 1979.

DANIEL Y. C. FUNG
Kansas State University
Manhattan, Kansas

FOOD FREEZING

Freezing has long been established as an effective method for the long-term preservation of foods. It has the potential to maintain quality and nutritional characteristics close to those of fresh foods. Although refrigerated storage has been developed to a stage where high quality can be maintained for a few weeks, when extended shelf life is required of a preservation technology, the most commonly used methods are freezing, thermal processing, and canning. It is generally accepted that of these three methods, freezing is superior (1). Properly conducted freezing retains much of the flavor, color, texture, and nutritive value of food. The activity of microorganisms is much reduced, and, while frozen, many enzymes also have reduced activity. However, the freezing process can disrupt the control systems that influence enzyme action, leading to undesirable changes during thawing. Where this is a problem, special techniques may be used to destroy or reduce enzymic activity before freezing. The consequences of thawing are mentioned because, with the exception of frozen commodities that are consumed directly (such as ice cream), normal freezing preservation requires that products be thawed before they are used.

Long before commercial application, refrigeration was used as a preservation technique. An early report is found in the *She King*, Chinese poetry written in 1100 B.C. (2). The earliest refrigeration agent was natural ice from frozen rivers and lakes and mountain snows. Natural refrigeration was used to preserve meat, game, fish, and, more recently, eggs, vegetables, and fruits. It has long been accepted that low temperatures reduce the rate of spoilage of food products compared to higher temperatures, and those who reside in colder climates have tended to expect food to store for longer times than those from hot climates. Modern progress in the refrigerated storage of foods, however, required the development of systems capable of producing low temperatures on demand.

REFRIGERATION HISTORY

The early history of the development of refrigeration has been reviewed and summarized (1–4). Table 1 presents some of the important milestones in the development of refrigeration.

The use of cool conditions for food storage dates back before the dawn of recorded history. Closer to modern times, in 1683, Robert Boyle reported the effects of refrigeration and freezing on foods and insects. Ice was reportedly used by a Massachusetts fisherman to preserve his catch until it could be brought to port and marketed (5). Natural ice, harvested in winter and stored in special facilities, was the primary source of refrigeration until the development of mechanical systems for ice making in the mid 1800s, based on the developing science of thermodynamics.

The early development of mechanical refrigeration was targeted to medical needs rather than to food preservation. John Gorrie developed a mechanical system, using air compression and expansion and the Joule–Thomson effect, to

3. D. Y. C. Fung et al., "Mixed Culture Interactions in Anaerobic Fermentations," in L. E. Erickson and D. Y. C. Fung, eds. *Handbook on Anaerobic Fermentations,* Marcel Dekker, New York, 1998, pp. 501–536.
4. G. Sauder, *Cheese Varieties and Descriptions*, USDA Agricultural Handbook No. 54, Washington, D.C., 1978.
5. F. V. Kosikowski, and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed. Vols. 1 and 2, Edwards Brothers, Ann Arbor, Mich., 1997.

GENERAL REFERENCES

M. A. Amerine et al., *Technology of Wine Making*, 4th ed., AVI Westport, Conn., 1980.

G. J. Banwart, *Basic Food Microbiology*, 2nd ed., Chapman and Hall, New York, 1989.

L. R. Beuchat, *Food and Beverage Mycology*, AVI, Westport, Conn., 1978.

C. M. Bourgeois, J. Y. Leveau, and D. Y. C. Fung *Microbiological Control of Food and Agricultural Products* (English ed.), VCH, New York, 1995.

D. R. Buege and R. C. Cassens, *Manufacturing Summer Sausage*, Extension. No. A 3058, University of Wisconsin, Madison, Wis., 1980.

R. A. Copeland, *Enzyme*, Wiley-VCH, New York, 1996.

W. Crueger and A. Crueger, *Biotechnology: A Textbook on Industrial Microbiology*, Sinauer, Sunderland, Mass., 1984.

M P. Doyle, L. R. Beuchat, and T. J. Montville, *Food Microbiology· Fundamentals and Frontiers*, ASM, Washington, D.C., 1997.

D. Y. C. Fung and K. Vicheinroj, *Introduction to Food Fermentation*, Kansas State University, Manhattan, Kans. 1998.

Cultures for the Manufacture of Diary Products, Chr. Hansen, Inc., Milwaukee, Wis., 1997.

ICMSF, *Microorganisms in Foods 5: Microbiological Specifications of Food Pathogens*, Blackie Academics and Professionals, New York, 1996.

J. M. Jay, *Modern Food Microbiology*, 5th ed., Van Nostrand Reinhold, New York, 1996.

P. Z. Margalith, *Flavor Microbiology*, Charles C Thomas, Springfield, Ill., 1981.

R. T. Marshall, *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington D.C., 1992.

D. A. A. Mossel et al., *Essentials of the Microbiology of Foods*, John Wiley, New York, 1995.

C. S. Pederson, *Microbiology of Food Fermentation*, 2nd ed., AVI, Westport, Conn., 1979.

H. J. Peppler and D. Perlman, *Microbial Technology*, Vol. 11, Academic Press, Orlando, Fla., 1979.

R. K. Robinson, *Dairy Microbiology*, Vols. 1 and 2, Elsevier Applied Science, Barking, United Kingdom, 1976.

J. H. Silliker et al., *Microbial Ecology of Foods*, Vols. 1 and 2, Academic Press, Orlando, Fla., 1980.

P. F. Stanbury and A. Whitaker, *Principles of Fermentation Technology*, Pergamon Press, Elsmford, N.Y., 1984.

K. H. Steinkraus, *Handbook of Indigenous Fermented Foods*, Marcel Dekker, New York, 1983.

R. P. Vine, *Commercial Winemaking: Processing and Controls*, AVI, Westport, Conn., 1981.

D. I. C. Wang et al., *Fermentation and Enzyme Technology*, John Wiley, New York, 1979.

DANIEL Y. C. FUNG
Kansas State University
Manhattan, Kansas

FOOD FREEZING

Freezing has long been established as an effective method for the long-term preservation of foods. It has the potential to maintain quality and nutritional characteristics close to those of fresh foods. Although refrigerated storage has been developed to a stage where high quality can be maintained for a few weeks, when extended shelf life is required of a preservation technology, the most commonly used methods are freezing, thermal processing, and canning. It is generally accepted that of these three methods, freezing is superior (1). Properly conducted freezing retains much of the flavor, color, texture, and nutritive value of food. The activity of microorganisms is much reduced, and, while frozen, many enzymes also have reduced activity. However, the freezing process can disrupt the control systems that influence enzyme action, leading to undesirable changes during thawing. Where this is a problem, special techniques may be used to destroy or reduce enzymic activity before freezing. The consequences of thawing are mentioned because, with the exception of frozen commodities that are consumed directly (such as ice cream), normal freezing preservation requires that products be thawed before they are used.

Long before commercial application, refrigeration was used as a preservation technique. An early report is found in the *She King*, Chinese poetry written in 1100 B.C. (2). The earliest refrigeration agent was natural ice from frozen rivers and lakes and mountain snows. Natural refrigeration was used to preserve meat, game, fish, and, more recently, eggs, vegetables, and fruits. It has long been accepted that low temperatures reduce the rate of spoilage of food products compared to higher temperatures, and those who reside in colder climates have tended to expect food to store for longer times than those from hot climates. Modern progress in the refrigerated storage of foods, however, required the development of systems capable of producing low temperatures on demand.

REFRIGERATION HISTORY

The early history of the development of refrigeration has been reviewed and summarized (1–4). Table 1 presents some of the important milestones in the development of refrigeration.

The use of cool conditions for food storage dates back before the dawn of recorded history. Closer to modern times, in 1683, Robert Boyle reported the effects of refrigeration and freezing on foods and insects. Ice was reportedly used by a Massachusetts fisherman to preserve his catch until it could be brought to port and marketed (5). Natural ice, harvested in winter and stored in special facilities, was the primary source of refrigeration until the development of mechanical systems for ice making in the mid 1800s, based on the developing science of thermodynamics.

The early development of mechanical refrigeration was targeted to medical needs rather than to food preservation. John Gorrie developed a mechanical system, using air compression and expansion and the Joule–Thomson effect, to

Table 1. Important Events in the Development of the U.S. Frozen Food Industry

Year	Event
500 B.C.–1800 A.D	Cooling achieved by use of snow, natural ice, air in cold climates, evaporative cooling of water, and radiative cooling
1820	Natural ice had come into general use as an article of commerce and was used on a large scale for food preservation.
1851	Jacob Fussell of Baltimore first sold ice cream on a significant commercial scale in the United States.
1861	Thomas S. Mort established what is believed to be the world's first cold-storage plant in Darling Harbor, Australia.
1864	Ferdinand Carre patented an ammonia compression machine in France.
1870	Ammonia compression machines were brought to a level of practicality almost simultaneously by Dr. Carl Linde in Germany and David Boyle in the United States.
ca 1880	Ammonia compression machines and insulated rooms began to be used in the United States
ca 1916	Scientific work on the methodology of freezing foods began in earnest.
1923	The quick freezing industry began with the founding of a freezing company by Clarence Birdseye.
1929	MA Joslyn and W.V Cruess reported on the need to blanch vegetables before freezing.
	The Birdseye organization was bought by Postum Co. (now General Foods Corp.), marking the real beginning of marketing of frozen foods through retail stores in the United States.
1930	Mechanical refrigeration had assumed an almost indispensable part of the food distribution system in the United States.
1949	Mechanically refrigerated railroad cars came into use about this time.
Early 1960s	Fluidized-bed freezers and individually quick frozen foods began to assume a position of importance in the United States
1962	Liquid nitrogen food freezers were first used commercially.
1968	Freon®-12 freezers were first used commercially.

Source. Ref. 1.

cool air in a cylinder and produce ice for application in the treatment of malaria (2). Commercial ice making began about 1876. The number of mechanical refrigerated warehouses began increase about 1890, but it was not until about 1915 that large-scale construction began. These early refrigeration systems were much too large to be installed in individual homes.

In the mid to late 1930s and the early 1940s, small-scale compressors became readily available. This allowed for the design and manufacture of relatively cheap and compact refrigeration units, which meant that home refrigeration was now practical. The dangers of ammonia refrigerant led to the development of an alternative, safer group of refrigerants, the fluorocarbons (Freon), by the DuPont company. In recent years, the undesirable environmental consequences of these refrigerants has led to continued development of more environmentally acceptable refrigerants.

In 1923, Clarence Birdseye created the first modern frozen food business, which set in motion a revolution in food preservation that has had significant consequences to the American diet. By 1930, the list of frozen foods consisted mainly of fish, small fruits, poultry, meat, eggs, and a few vegetables. Quality was variable. Joslyn and Cruess recognized the role of enzymes in quality degradation and introduced the blanching process for the destruction of enzymes. This led to significant quality improvement. During the past 60 years, the research efforts of frozen food processors, packaging manufacturers, and university and government scientists have led to further improvements in the quality and stability of frozen foods.

When describing the technologies of food freezing, it is best to consider the food freezing process from two perspectives: (*1*) the methodologies for removal of heat, that is, the cooling process, and (*2*) the effects of removal of heat on the properties of the system undergoing cooling.

THE REMOVAL OF HEAT (REFRIGERATION)

Principles of Refrigeration

Heat flows from a high temperature to a lower temperature. To remove heat from an object by convection, conduction, or radiation, there must be a heat sink at a lower temperature. If the object is at ambient temperature, then the heat sink must be at a temperature below ambient to achieve cooling. There are several ways to reduce temperature below ambient. Early cooling methods involved evaporative cooling, which is what one experiences when a cooling breeze causes the evaporation of perspiration. Evaporative cooling has long been used for cooling water with porous pots and for air conditioning with swamp coolers. The principles behind evaporative cooling will be described later. Another simple method is to use ice, but if natural ice is not used, ice must how be produced in refrigerated cooling systems capable of producing temperatures below the freezing point of ice.

Two observations are key to the understanding of refrigeration methods. First, a gas increases in temperature after being compressed with no heat removed and, similarly, reduces in temperature after expansion. Second, heat has to be supplied to a liquid in order for it to vaporize at constant temperature, and the condensation of a vapor yields heat. The two most commonly used refrigeration methods employ the *vapor compression refrigeration cycle* and the *absorption refrigeration cycle* (6,7). Mechanical energy drives the vapor compression system, which is commonly found in warehouses. Thermal energy drives the absorption cycle, which benefits from greater mechanical simplicity and the ability to use a variety of sources for thermal energy. In both cycles, the primary heat sink is a liquid boiling at low temperature and pressure, and the primary heat rejection is a vapor at higher temperature and pressure condensing to a liquid. In the vapor compression system, work is used to compress the vapor; in the absorption system, heat is used to raise the temperature of the absorbent and boil off the vapor at a higher temperature.

To better understand the operation of these refrigeration systems, it is necessary to learn the principles of thermodynamics. Thermodynamics is the name given to the study of energy, its transformations, and its relation to the states of matter. The units of measurement representing amount of heat or work are the calorie (cal), the joule (J), and the British thermal unit (Btu). These are defined as follows (8):

> One calorie is the heat required to raise the temperature of 1g of pure water from 14.5°C to 15.5°C.
>
> One joule is the work done when a force of 1 N acts through a distance of 1m.
>
> One Btu is the quantity of heat required to raise the temperature of 1lb of water from 63°F to 64°F.

Refrigeration is the process of removal of heat from an object or area. As has already been stated, heat moves from a warm to a cold body. In addition, two thermodynamic laws must always be obeyed. The first law of thermodynamics, the law of conservation of energy, states that energy cannot be created or destroyed. This law may be restated in a variety of forms, all of which imply that although energy may assume many forms, the total amount of energy must remain constant. It follows that if energy should disappear in one form it must simultaneously appear in other forms (8,9). Consequentially, different forms of energy are interconvertible and equivalent. For each possible transformation, there is a definite conversion ratio.

In one form, the second law of thermodynamics states that no system can receive heat at a given temperature and reject it to a higher temperature without the input of work from the surroundings. Because a refrigeration system performs such a transfer of heat, from a lower temperature region to a higher temperature region, this transfer necessarily requires the input of work in some form.

To properly describe a thermodynamic process, it is necessary to maintain a careful bookkeeping system that accounts for energy in all the forms that are interconverting. This permits keeping track of the transformations quantitatively. There are some standard thermodynamic functions that are used for these bookkeeping purposes. In addition, the conditions of the process must be defined to make the bookkeeping feasible. Considering equilibrium states, such characteristic thermodynamic properties as internal energy (U), entropy (S), and enthalpy (H) are defined functions of easily measurable properties, such as pressure (P), temperature (T), and volume (V). Also, for equilibrium states, there are relationships among P, PV, and T. For an ideal gas, for example, the relationship $PV = RT$ holds, where R is a constant. Real gases have pressure–volume–temperature relationships that are extensions of this simple equation. Several standard process routes can be to describe the thermodynamic relationships of a system, including:

1. *Constant temperature (isothermal) process*. In this process, temperature is held constant and volume varies with pressure.
2. *Constant pressure (isobaric) process*. In this process, pressure is held constant and volume varies with temperature.
3. *Constant entropy (adiabatic) process*. In this process, no heat transfer occurs between the system and its surroundings.

The bookkeeping can then be carried out by using some standard thermodynamic diagrams, which are discussed in the next section.

Thermodynamic Diagrams

In a thermodynamic diagram, the temperature, pressure, volume, enthalpy, and entropy relationships of substances are represented. The most frequently used diagrams are temperature–entropy and pressure–enthalpy diagrams. In these diagrams, it is important to know whether the system being described is one phase or multiphase and which phases are present. This is most readily achieved by plotting the saturation lines, which represent, in a system capable of existing as liquid or as vapor (ie, a fluid), the point at which the first bubble of vapor appears in the liquid and the point at which the last drop of liquid evaporates to vapor. Thus, the saturation lines delineate the boundary between the one-phase and the two-phase regions. The one phase may be liquid or vapor, or the two phases may be liquid and vapor in coexistence. At some temperature and pressure, known as the critical point, liquid and vapor can no longer be distinguished.

To calculate heat and work in a mechanical refrigeration system, it is necessary to have knowledge of the thermodynamic properties of the working fluid, or refrigerant. This can readily be represented by means of a pressure–enthalpy diagram. A schematic pressure–enthalpy diagram is shown in Figure 1. The line that describes the property of saturated liquid (g), the line that describes the property of saturated vapor (h), and the critical point (*) are marked. To the left of g is a single-phase region of liquid. The g describes the liquid at the point when vapor just begins to boil off. The h describes the vapor as the last drop of liquid vaporizes. Between g and h is a two-phase region of mixed liquid and vapor. Beyond h, the single phase is vapor. Vertical lines describe conditions of constant enthalpy, where the heat content of the refrigerant is unchanging. Horizontal lines describe conditions of constant pressure. The line c describes a constant temperature path. Note that in the two-phase region, this line is horizontal, that is, at constant pressure. At constant pressure, a pure liquid boils at constant temperature. As pressure increases, the boiling point increases. The line e describes a path of constant entropy. Additional lines, such as constant quality (ie, constant liquid/vapor ratio) and constant density lines can also be plotted. Diagrams such as these are readily available for all common refrigerants. Similar

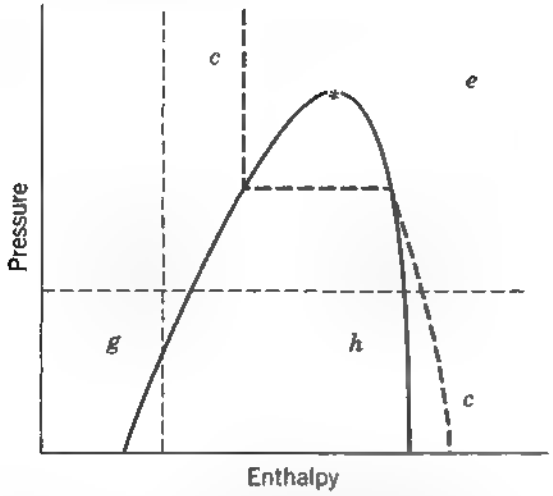


Figure 1. A schematic pressure–enthalpy diagram.

diagrams for temperature–entropy relations can be drawn. The key lines for understanding refrigeration systems are those that describe the saturated liquid and the saturated vapor.

Compression Refrigeration Cycles

Figure 2 schematically shows a mechanical refrigeration system. In the evaporator, a liquid under low pressure boils as it absorbs heat entering from the surroundings. The vapor travels to a mechanical compressor, which raises its pressure. The temperature increases. The high-pressure vapor passes through a condenser, where it rejects the heat to the surroundings. As heat is lost, the vapor condenses. The resulting liquid is held in a reservoir. As required, liquid from the reservoir passes through an expansion valve into the low-pressure side and enters the evaporator, where the cycle begins again. An operating principle of such a device, which uses work to remove heat in a cyclical process, may be described by a reversed Carnot cycle. Figures 3 and 4 are the basic thermodynamic diagrams that describe the process. In Figure 3, the changes in unit mass of refrigerant fluid are as follows. Point *e* represents the saturated liquid in the reservoir. It has a heat content of H_1, pressure of P_2, and temperature of T_2. As it passes through the expansion valve, the pressure drops, and some vaporization occurs, lowering the temperature and bringing the fluid to *a*, at temperature T_1, pressure P_1 and heat content still H_1. As the refrigerant takes in heat from the surroundings (at a temperature greater than T_1), the heat content increases. At *b*, the heat content is now H_2, with the pressure still P_1 and the temperature T_1. This point is on the saturation line and describes the boiling of the last drop of the liquid. From *b* to *c* describes the compression of the initially saturated vapor, an adiabatic (or isentropic) step. The increase in heat content is the work supplied by the compressor to increase the pressure. At *c*, the temperature is T_3, the heat content is H_3, and the pressure is P_2. The vapor enters the condenser and loses heat to the surroundings. At *d*, on the saturation line, condensation just starts to occur. The pressure is still P_2, the temperature has dropped to T_2 (still above the surroundings), and the heat content is now H_2. As heat continues to leave the refrigerant, it enters the two-phase region, until the saturated liquid is reached at *e*. At *e*, the condition of the liquid reservoir, the heat content is H_1, the pressure is P_2, and the temperature is T_2. Thus, in one pass through the cycle, the unit mass of refrigerant has picked up an amount of heat, $H_2 - H_1$, in the evaporator at temperature T_1 and has rejected this heat in addition to the heat representing

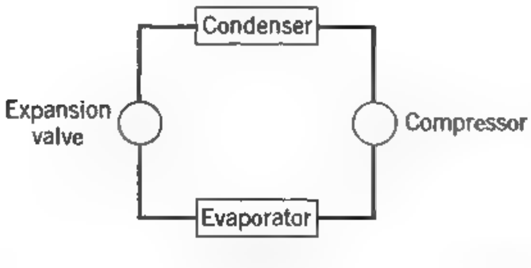


Figure 2. A mechanical refrigeration system, schematic.

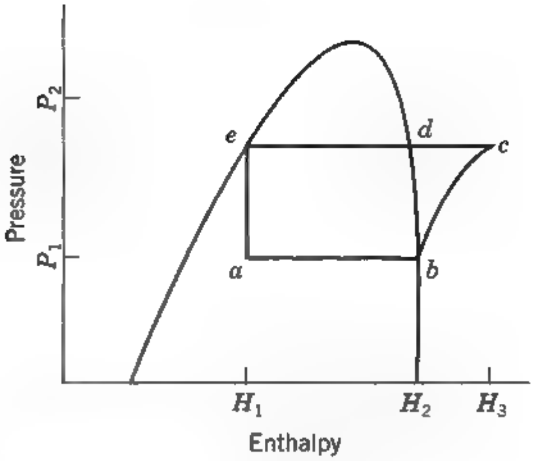


Figure 3. Pressure–enthalpy diagram for the mechanical refrigeration system shown in Figure 2.

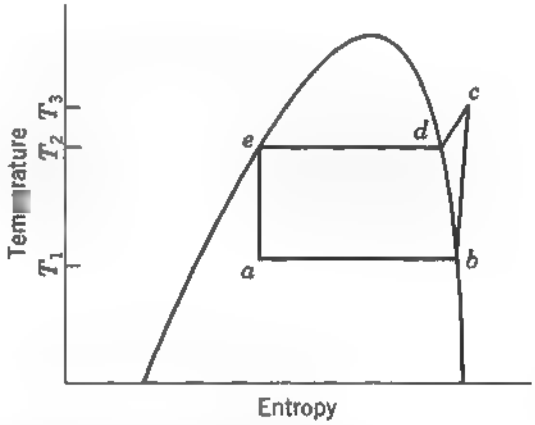


Figure 4. Temperature–entropy diagram for the mechanical refrigeration system shown in Figure 2.

the work of compression, $H_3 - H_2$ in the condenser. Work is being used to enable heat to be transferred from a lower temperature to a higher temperature. A refrigerant is chosen so that the pressure required for a boiling point at or below the refrigeration temperature is not too low and so that the pressure required to have a boiling point high enough to reject heat into the surroundings of the condenser is not too high. Table 2 lists appropriate temperatures and pressures for some common refrigerants. The major components of a vapor compression system are the compressor, the condenser, the evaporator, and the controls. Any standard type of compressor is satisfactory; reciprocating compressors are commonly used, but rotary and centrifugal compressors can also be used. Condensers and evaporators are simply heat exchangers. The condenser, which serves to reject heat from the system, may use air cooling, water cooling, or evaporative cooling. Water-cooled condensers flow the water through one of three configurations: shell and tube, shell and coil, and tube in tube. An air-cooled condenser circulates the high-temperature, high-pressure refrigerant through the condenser coil and uses air moved by free convection, wind, or fans to remove the heat. An evaporative condenser transfers heat from the coil to a film of water that evaporates into an air stream passing over it. In the evaporator, the liquid refrigerant boils at low temperature and pressure within the coil, and the medium to be cooled is in contact with the exterior of the coil.

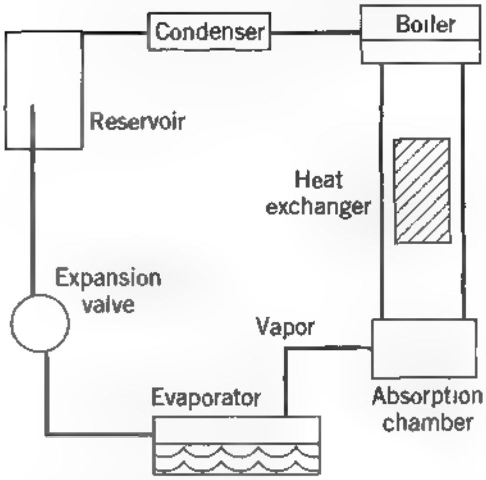


Figure 5. An absorption refrigeration system, schematic.

Absorption Refrigeration Cycle

Figure 5 illustrates a typical absorption refrigeration system. Once again, heat is picked up from the surroundings by the boiling of the low-pressure refrigerant liquid. As the vapor exits the evaporator, it contacts the absorbent and dissolves. The solution is pumped around to the boiler, where it is heated. The refrigerant boils off from the still-liquid absorbent and is transferred to the condenser, where it loses heat and is liquefied. The liquid refrigerant is stored in a reservoir until required, when it passes through an expansion valve into the evaporator. The absorption at low temperature and pressure, heating, and boiling off of the vapor at high temperature and pressure are the equivalent of the mechanical compression step. In each case, the refrigerant is taken from the T_1, P_1 condition to the T_2, P_2 condition. The ammonia absorption refrigeration system requires some additional components because the absorbent fluid—water—is itself volatile; therefore, there is a need to separate a significant amount of water vapor from the ammonia vapor exiting from the boiler. This is achieved by using a distillation column.

For the absorption system, the special components are the absorption chamber, the boiler, and the heat exchangers that transfer the heat in the returning "stripped" absorbent to the refrigerant-loaded absorbent being pumped to the boiler. As mentioned earlier, for an ammonia-water system, a distillation coil is required to separate water from ammonia in the exiting vapor stream from the boiler. The evaporator and condenser can be similar to those of the vapor compression systems, although special modifications may be required to deal with residual water in the ammonia-water system.

To compare the capabilities of different refrigeration systems, some definitions are appropriate.

Ton of refrigeration. The standard unit of refrigeration capacity is known as the ton of refrigeration. It is the

Table 2. Properties of Some Important Refrigerants

	Vapor pressure (MPa)		
Temperature (°C)	Ammonia	R22	R134
−50	0.0408	0.0299	0.0645
−30	0.119	0.164	0.085
−10	0.290	0.355	0.201
+10	0.613	0.681	0.415
+30	0.855	1.192	0.770
+50	2.028	1.943	1.318

Properties at −40°C evaporator, +20°C condenser

Refrigerant	Evaporator P (MPa)	Condenser P (MPa)	Compression ratio	Net refrigeration (kJ/kg)	Refrigerant circulation (kg/s)
Ammonia	0.071	0.853	11.99	1131.45	0.0031
R22	0.105	0.910	8.65	164.21	0.00609
R134	0.051	0.569	11.23	146.95	0.02392

rate at which heat would have to be removed to convert 1,000 kg of water at 0°C into ice at the same temperature in a 24-hour period. The latent heat of fusion of water is 79.68 kcal/kg. Hence, a ton of refrigeration is equal to a rate of heat removal of 3,320 kcal/h.

Enthalpy. Enthalpy, a measure of the heat quantity in a product, is a relative quantity. For most refrigerants, the enthalpy of the saturated liquid at 0°C is by convention set at 200 kcal/kg. For frozen foods, the enthalpy at −40°C is by convention set at 0 kcal/kg.

Refrigeration effect. The net refrigeration effect for a system under given operating conditions can be calculated from the pressure–enthalpy diagram as

$$RE = H_g - H_f$$

where RE is the refrigerating effect (kcal/kg), H_g is the enthalpy of vapor refrigerant leaving the evaporator, and H_f is the enthalpy of the refrigerant entering the evaporator. In the example of the mechanical refrigeration cycle in Figure 4, RE is given by $H_2 - H_1$.

Quantity of refrigerant circulated per ton of capacity. This quantity is self-defining.

Heat of compression. This is the enthalpy difference in the refrigerant between compressor discharge and inlet. In Figure 4, it is represented by $H_3 - H_2$. It can be expressed as kcal/kg.

Work of compression. This is the product of refrigerant mass flow and heat of compression. It can be expressed as kilocalories per minute. Standard conversions allow it to be expressed in other units where necessary.

Condenser heat load. This is determined by subtracting the enthalpy of the saturated liquid leaving the condenser from the enthalpy of the superheated vapor entering the condenser: $H_3 - H_1$.

Coefficient of performance. This is the ratio of output to input. The theoretical value is given by the refrigeration effect divided by the heat of compression. This, however, ignores friction losses in the compressor, which cause the heat input from the compressor (ie, the work of compression) to be less than the energy required to operate the compressor. The practical coefficient of performance is often only 60% of the theoretical value.

The choice of refrigerant is made on the basis of several criteria. These include (10):

1. The latent heat of evaporation. A high latent heat is preferred.
2. The pressure that would be experienced in the condenser at the condensing temperature. Excessively high pressures are to be avoided.
3. The freezing temperature of the refrigerant must be below the temperature of the evaporator.
4. The critical temperature of the refrigerant must be sufficiently high to give an effective working diagram for the temperatures experienced in the high-pressure side.
5. The refrigerant should ideally be nontoxic, noncorrosive, and chemically stable.
6. It should be easy to detect leaks in the system.
7. For industrial applications, where large quantities of refrigerant are used, low-cost refrigerants are preferred.

Clearly, not all these criteria can be satisfied by any one refrigerant. Ammonia has a high latent heat of evaporation, and leaks are easily detected. However, it is toxic and corrosive. On balance, the ease of leak detection renders it safe for use despite its toxicity. The fluorocarbons are also commonly used in refrigeration units. They have a wider range of applicable temperatures than ammonia, but environmental concerns are associated with their use.

Where lower temperatures are required, it is possible to use multistage refrigeration, where essentially two or more refrigeration cycles are coupled—the low stage of one is the high stage of the next. An alternative approach is to have two-stage compression (Figure 6).

To freeze a product, it must in some way be coupled to the refrigeration effect. Freezing equipment uses a variety of solutions. Where mechanical refrigeration is used, the cooling source is the evaporator. It is possible to put the product in direct contact with the evaporator, as is the case in some designs of plate freezers, where the plates themselves are machined internally as the evaporator. It is more common, however, to use some form of secondary refrigerant, which is cooled by passing through the heat exchanger of the evaporator, and then to contact this fluid, directly or indirectly, with the object. The secondary cooling medium may be a variety of fluids or air. The ability of this fluid to transport heat has an influence on the freezer design. Fluids based on aqueous solutions have a high volumetric heat capacity, so the volume of fluid required to transport a given quantity of heat is not too high. Air has a much smaller volumetric heat capacity (given the much lower density), so the volume of air required to transport the same quantity of heat is much greater. Freezers that use air as the transfer fluid need powerful fans to blow large volumes of air past the product to be frozen if heat

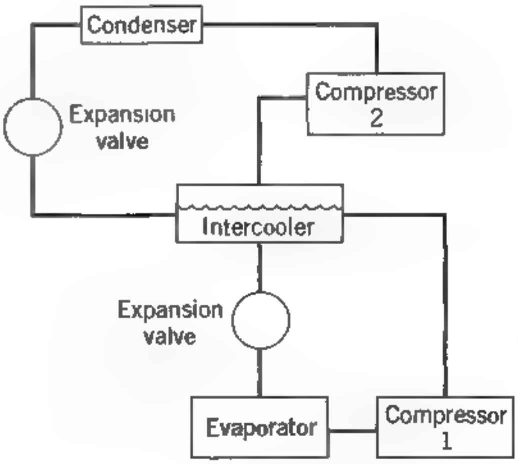


Figure 6. Two-stage refrigeration, schematic.

removal is to be rapid. A variety of freezer designs exist (11). In plate freezers, the product is in contact with metal plates, cooled either directly (ie, the plates are themselves the evaporator coil) or indirectly, by circulating a secondary refrigerant (usually an aqueous solution such as glycol solution or brine of sufficiently low freezing point) through the coolant channels. This gives effective cooling of the product if the geometry of the product results in good thermal contact with the plates, but poorer cooling occurs if air spaces interrupt the thermal contact. An alternative cooling method is direct immersion of the product in the secondary cooling fluid. Using aqueous secondary refrigerants with high volumetric heat capacity, immersion results in excellent cooling rates, but the potential for contamination of the product exists. Using air as refrigerant, the potential for contamination is much less, but the volumetric heat capacity is much lower, so the heat transfer capabilities depend on the volume of cold air that can be brought into close contact with the product in unit time. A wide variety of freezer designs exist that use air as the heat transfer fluid. Static freezers use natural air circulation, and forced air (blast) freezers use a high-velocity cold air stream that passes by the product. Many mechanisms have been evolved for ensuring product throughput, and batch and continuous processes exist. In either case, air temperature and air velocity are critical to the rate of heat removal.

The common characteristic of the mechanical refrigeration systems so far discussed is their ability to produce cooling at the throw of a switch. This characteristic is also found in thermoelectric cooling systems (12). Thermocouples are devices that produce an electrical voltage as a result of temperature difference. Thermoelectric coolers are the reverse concept—devices that produce a temperature difference on passage of an electric current. If the higher temperature node is at ambient temperature, the lower temperature node is a heat sink. Using modern semiconductor materials, effective thermoelectric cooling devices have been manufactured that can be used to provide refrigeration. These are used primarily in special situations. They do not have the high capacities of mechanical refrigeration systems.

Mechanical or thermoelectric refrigeration is not the sole means of providing a heat sink. Just as ice has traditionally been used as a cooling source, cryogenic media may be used as a cooling source. As with ice, these are not turnkey systems to produce cooling at the throw of a switch. Rather, these are systems where cooling capacity is stored in a convenient form. Special cooling processes are used to produce the cryogenic medium. In food freezing, common cryogenic coolants are liquid nitrogen or solid carbon dioxide. These are produced centrally and distributed and stored in special insulated containers before use. The properties of the cryogens are summarized in Table 3. These cryogens can be used directly, bringing them into contact with the product to be frozen. The vaporization of the cryogen is an important component of the heat sink capacity, but the heat required to raise the temperature of the cryogen is also an important source of cooling capacity. Because the three primary drivers in rates of heat transfer are the volumetric contact rate between coolant and product, the volumetric heat capacity of the coolant, and the temperature difference between the coolant and the object to be cooled, the rates of heat transfer tend to be higher than for mechanical refrigeration systems, in which the refrigeration temperature is higher, and the volumetric heat capacity of the secondary refrigerant is lower. Refrigeration systems are capable of providing a large range of cooling rate capabilities for products to be frozen. The design of a refrigeration system should take into account the length of the operating season, the refrigeration load, the amplitude of fluctuations in this load, the buildup of frost on the evaporator surfaces and the need to remove it to maintain cooling efficiency, the target temperatures, and the cooling medium of choice. Other factors to be considered are the type of refrigeration, capital cost, and ease of maintenance (13,14). The characteristics of the product often determine the most appropriate freezing system for the product. The next section describes the freezing process within a product and its dependence on the external cooling conditions and the characteristics of the product.

Table 3. Thermal Properties of Cryogenic Refrigerants

Property	Nitrogen	Carbon Dioxide
Boiling point (°C)	−195.4	−78.6 (sublimes)
Specific heat of vapor (kJ/kg K)	1.03	0.837
Heat of vaporization (kJ/kg)	199	573
Heat removal (bp to −18°C, kJ/kg)	384	623

THE FOOD FREEZING PROCESS

What Is Freezing?

At its simplest, freezing can be taken to refer to a process that reduces the temperature of a product to 0°C or below. However, it also implies that ice should form. In addition, when considering food freezing, it is normally required that the temperature be reduced to −18°C if extended storage life is to be achieved (15). The primary component of food is water. The use of the term freezing implies that some, or most, of this component enters the solid state, that is, becomes ice. In order for ice to form, heat must be removed. As heat is removed, the temperature of a food will begin to reduce. When it reaches the freezing temperature, ice does not necessarily form at first. Often, the temperature continues to reduce below the freezing point, resulting in a supercooled system. At some point, the creation of in-ice nucleus initiates ice formation. Some ice can now form very rapidly from supercooled water, as very small crystals. The heat released as this supercooled water converts to ice raises the temperature back toward the freezing point. Once the ice formation has begun, further ice crystals form in concert with heat removal. The extent of initial supercooling determines the depth to which the initial burst of crystallization penetrates. Beyond that, the rate of heat removal has the primary influence on ice crystal size. If heat removal is rapid, the propagation of a few crystals is insufficient to match the heat flux. Hence, incipient supercooling occurs, which results in an increased probability of nucleation. As a result, a larger number of ice crystals are formed (15). If the cooling rate is slower, propagation can keep pace with heat removal, and fewer larger crystals are the result. Figure 7 illustrates the relationships between concentration and ice content that govern the freezing process. As ice forms, removing water from the unfrozen phase, the concentration of solutes in the unfrozen phase increases. Figure 7 shows the concentration of the unfrozen phase as a function of temperature or, alternatively, shows the concentration dependence of freezing point. These two statements are equivalent. The two curves illustrate schematically the differences that exist for different materials. The important point to grasp is that for two systems with the same initial water content, freezing can result in different amounts of ice at the same temperature. Also, addition of more solute can result in a lower freezing point. Both of these observations are germane to the understanding of the behavior of frozen foods.

When cellular systems are frozen, ice may or may not form within cells (16,17). In freezing, there is a driving force to keep the concentration of the unfrozen phase matched to the requirements of the phase diagram (Figure 7). Within a cell, ice must form or water will translocate to the external medium for this to happen. If cooling is rapid, there may not be time for translocation, and ice will form within cells. Slow cooling may leave enough time for translocation, so that ice forms only in external locations. Product quality is better if translocation of the water has not occurred; therefore, fast freezing tends to result in higher initial quality.

Frozen food quality is influenced not only by the initial freezing conditions, but also by the conditions of storage. Chemical and physical change is possible under frozen storage conditions, at rates that depend on the temperature (17,18). In general terms, the lower the temperature, the slower the change, although it should be recognized that, depending on the storage life required, there will be a threshold temperature that is low enough. However, even brief exposure to higher temperatures, especially above −10°C, significantly reduces quality and shelf life. Rate of freezing, which is related to rate of cooling, is an important determinant of initial quality. Figure 8 shows a typical plot of the enthalpy of a product as a function of temperature. Freezing involves the removal of heat. The amount of heat to be removed to go from ambient temperature to the storage temperature can be determined from Figure 8. However, it is not only the changed heat content that will influence the rate of the freezing process. The size of the object will also influence the rate of the process. The amount of heat to be removed depends on the mass and volume of the object. Because heat removal is through the surface, the rate of heat removal depends on the surface area and the effectiveness of heat transfer between the surface and the refrigerating medium. It also depends on the internal rates of heat transport within the object. For small products, the surface heat transfer is most important, and the cooling rate is influenced primarily by the heat transfer characteristics of the freezer. For larger products, the internal characteristics of the product assume greater importance, and the heat content and internal heat transfer characteristics are the factors that have the greatest influence on the freezing rate and the rate of change of temperature. More simply, small products can be frozen slowly or rapidly, depending on the choice of freezer conditions. Large objects take much longer to freeze because the internal properties control the freezing rate, and the freezer characteristics are no longer the controlling factor. Only in the surface zone do the freezer characteristics markedly influence freezing rates. Beyond some critical surface heat transfer condition, the maximum possible surface heat transfer rate will exceed the rate of heat transfer from the interior of the product. Because of the mechanism of heat transfer, there exists a gradient in temperature between the surface of the product and the center. This temperature gradient is one determinant of the rate of internal heat transfer. As Figure 8 shows, the change in

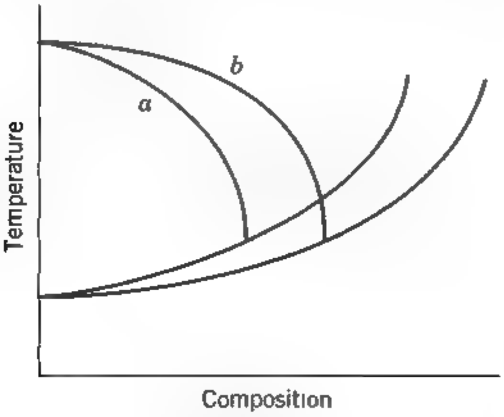


Figure 7. A typical phase diagram for freezing.

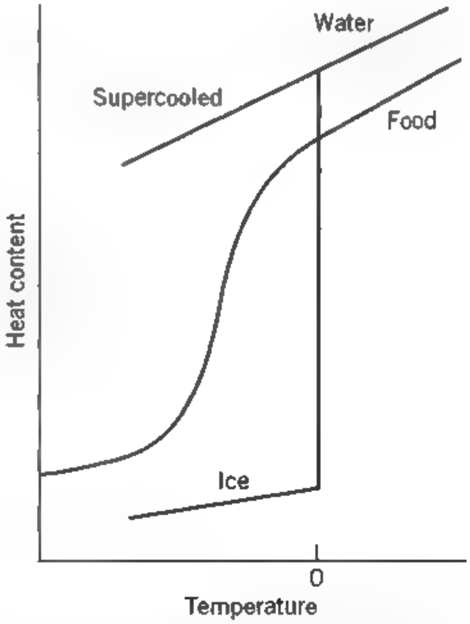


Figure 8. The enthalpy–temperature diagram for aqueous systems in the region of the freezing temperature.

heat content for a 1°C drop in temperature is dependent on temperature. This is a consequence of the two different processes that result in a change in heat content: temperature change and phase change. The heat associated with temperature change is called sensible heat (ie, heat that can be sensed). When phase change occurs in a pure system at constant pressure, there is no temperature change. The heat associated with phase change is called latent heat. In Figure 8, the slope of the plot for pure water (sensible heat) is approximately 1 kcal/kg/K. For ice, it is approximately 0.5 kcal/kg/K. The conversion of water to ice is accompanied by a change in heat content of approximately 80 kcal/kg. Thus, cooling of water to below freezing temperatures will result in a steady drop in temperature, followed by a region of steady temperature during the phase change, and then a further steady drop in temperature. Because nucleation is necessary, there is a region of supercooling where the temperature drops below the freezing point before freezing initiates, and the temperature returns to that of the freezing plateau. This is illustrated in Figure 9, which shows the cooling profile at the surface and the center of a pure water sample. In a real product, with heat content as illustrated in Figure 8, the freezing curves appear as in Figure 10. Supercooling is still apparent, but now the temperature declines slowly as ice forms. Once most of the ice has formed, the rate of temperature change increases again. The trajectory of Figure 10, except for the supercooling dip, can be estimated from the phase diagram of Figure 7, because the reason for the plateau region is the need to remove latent heat, and the amount of latent heat to be removed depends on the change in ice content as a function of temperature. It is also possible to determine Figure 8 from Figure 7, and vice versa. The enthalpy–temperature profiles of Figure 8, traversing from ambient temperature, show first the sensible heat zone. The slope is the specific heat of the product and depends on water content and solids content. Water typically has a specific heat of 1 kcal/kg/K, and food solids typically have a specific heat of about 0.3 kcal/kg/K. In the freezing zone, the enthalpy change is a combination of sensible heat for the unfrozen material and the latent heat released by the formation of ice. Once most of the freezing is over, the slope is characteristic of the sensible heat of the frozen system, in part influenced by the amount of unfrozen water that remains. Figures 9 and 10 illustrate the difference in freezing profile for different positions in the sample, which are in part a consequence of the internal temperature gradients that accompany heat conduction.

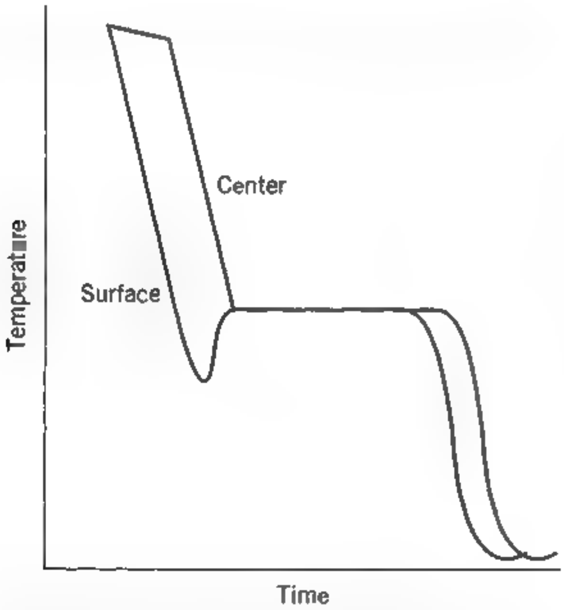


Figure 9. A temperature–time cooling diagram for a pure water sample (surface and center).

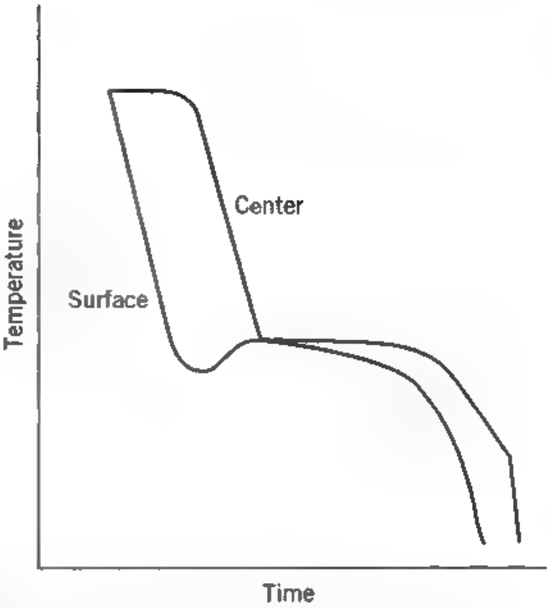


Figure 10. A temperature–time cooling diagram for a food sample (surface and center).

Freezing is normally considered complete when the center of the product reaches a predefined temperature. To estimate the freezing time, it is necessary to factor in the external conditions and the internal product properties. Successful design of a refrigeration system requires an estimate of the freezing time, because this helps define the refrigeration requirement. The alternative International Institute of Refrigeration (IR) definitions of freezing time (19) are (*1*) nominal freezing time, the time between the surface reaching 0°C and the thermal center reaching a temperature 10K colder than the temperature of initial ice formation at the thermal center; and (*2*) effective freezing time, the time to lower the temperature of the product from its initial temperature to a given temperature at its thermal center. Because of the complex dependence of enthalpy on temperature, prediction is very complex. The anisotropic structure of food also adds complexity. Heat transfer coefficients are not well known. A variety of analytical and numerical predictive methods, are available and are discussed in the literature (20–24).

A simple method to reach a rough estimate of freezing time uses Plank's equation:

$$t_f = \frac{\rho L}{T_f - T_a}\left(P\,\frac{a}{h} + R\,\frac{a^2}{k}\right)$$

where t_f is the freezing time, L is the latent heat of the water fraction, ρ is the density, T_f is the initial freezing temperature, T_a is the temperature of the refrigerating medium, a is the characteristic dimension, h is the surface heat transfer coefficient, k is the thermal conductivity, and P and R are constants that depend on the geometry of the sample. This equation clearly illustrates the effect of size, which was discussed previously. For a small object, the relative importance of the a and a^2 terms highlights the term involving h. As a increases, a^2 increases in significance, and the term in k increases in importance. Thus, the freezing time of small objects is determined primarily by the surface heat transfer, and that for large objects is determined primarily by the thermal conductivity of the object. One purpose of estimating freezing time is to determine the heat load for the refrigeration system. If the heat to be removed and the freezing time are known, then the rate of heat removal required can be estimated. This then gives an indication of the required capacity of the refrigeration system.

Many different types of food products are preserved by the freezing process, but they do not exhibit the same characteristics in the freezing process or require the same process conditions. For example, plant tissues, on freezing, tend to undergo disruption of the cell structure (16). In fresh plant tissue, the cell wall complex can act as an effective barrier to ice propagation during freezing, with the result that ice may form only externally under slow cooling conditions. Water will tend to transfer from the cell. The resulting textural quality is often inferior. If rapid cooling is used, greater supercooling is produced in the cells and internal freezing becomes possible. Water transfer is minimized, and the textural properties are improved. Freezing in plant tissues often produces structural disruptions of the cells, which allows for unwanted enzymic activity during storage or thawing. The results of this enzyme activity significantly change flavor and other attributes and lead to lowered quality or complete unacceptability of the product. To prevent this, it is often advised to thermally treat (blanch) the material before freezing to inactivate the enzymes responsible for the unwanted changes. This procedure was first introduced by Joslyn and Cruess. Because blanching is a thermal treatment, it causes textural changes that are acceptable in products normally consumed cooked, but they are often unacceptable in products normally consumed raw. Blanching is most often used for vegetables and less often for fruits, which are frozen as is. For high-water-content plant tissues, freezing damage can cause significant textural alteration. Where texture is important, this might mean that freezing is an unacceptable process. A good example is lettuce, where the high turgor pressure, essential to the eating quality, is lost by freezing. The individual material has to be examined to determine whether freezing and thawing cause unacceptable changes and whether blanching can be used to prevent these changes. Animal tissues exhibit different characteristics from plant tissues (16). First, the resistance to internal cell freezing is much less, and so cell dehydration during freezing, with consequential drip loss on thawing, is less of a problem. Also, the effect of freezing on the enzymatic processes is less marked than for plant systems. The biochemical system most at risk from freezing is the muscle contraction system. The structural disruption of freezing causes uncoupling of the control of the contractile mechanism. If ATP is present, uncontrolled contraction can occur, resulting in extremely tough material. This is obviated by ensuring that rigor has been passed. Because rigor is a time-consuming process, methods to accelerate the depletion of the ATP reserve have been developed. These allow for the freezing process to be speeded up without causing unacceptable textural changes in the meat.

Storage Temperatures

Because the purpose of freezing is to extend the shelf life of food products, the influence of storage temperature on storage life and thawed quality is a concern. Traditionally, it has been assumed that storage at −18°C (0°F) is adequate for all products. Although it is true that storage at lower temperatures is preferred to storage at higher temperatures, −18°C does not always result in an acceptable shelf life. Some products require lower storage temperatures for extended storage. Ice cream and fish, for example, are much better stored at temperatures below −30°C if unacceptable changes in quality are to be avoided. The influence of temperature on storage life is now much better understood. At temperatures close to −5°C, change tends to be accelerated, compared to temperatures above or below. This is because of the reaction-enhancing effect of increased solute concentration in the unfrozen phase outweighing the general slowing effect of lowered temperature (18). This is of special significance in the careful handling of frozen foods. Beyond the zone of maximum ice formation, the influence of temperature is the primary determinant of reaction rates. However, it is now understood that it is the relative temperature, rather than some absolute temperature, which is significant. As a system freezes and ice forms, the properties of the unfrozen aqueous phase change. Increasing concentration and decreasing temperature lower the mobility of the solute molecules. At some characteristic temperature, solute mobility becomes severely constrained, and reaction rates decrease markedly. Although it may not be economically feasible to use temperatures below this characteristic temperature for storage, it also serves as a reference temperature against which the actual temperature of storage can be compared. For products where this temperature is lower, storage temperatures also should be lower to achieve extended shelf life.

As previously indicated, freezing is necessarily followed by thawing. The thermal properties of water and ice are such that the freezing process and the thawing process are not mirror images. Thawing follows a different profile (18). Because ice is a better conductor of heat, heat transfer into a frozen product is more rapid than heat removal from a similar unfrozen product. As ice begins to melt at the surface, a thermal barrier is formed, because liquid water is a less effective heat transfer medium. As a result, on thawing the temperature rapidly rises into the zone around

−5°C, and then heat transfer becomes less effective. The latent heat plateau takes longer to traverse than in freezing, where the heat transfer is through an external layer of ice. As a consequence of this property, exposure of frozen products to higher temperatures even for a short time can lead to significant quality loss. The thawing process, too, if not well controlled, can result in loss in quality, as a consequence of the potential for significant reaction at −5°C.

Freezing is an effective method for food preservation. The deceptively simple technology requires care to be exercised in temperature control for optimum quality of product to be maintained to consumption.

BIBLIOGRAPHY

1. O. Fennema, "The U.S. Frozen Food Industry: 1776–1976," *Food Technol.* **30**, 56–68 (1976).
2. R. V. Enochian and W. R. Woolrich, "The Rise of Frozen Foods," in N. W. Desrosier and D. K. Tressler, eds., *Fundamentals of Food Freezing*. AVI, Westport, Conn., 1977, pp. 1–24.
3. T. P. Labuza and A. E. Sloan, "Forces of Change: From Osiris to Open Dating," *Food Technol.* **35**, 34–43 (1981).
4. C. P. Mallett, "Editorial Introduction," in C. P. Mallett, ed., *Frozen Food Technology*. Blackie, London, United Kingdom, 1993, pp. xix–xxv.
5. H. T. Cook, "Refrigerated Storage," in C. W. Hall, A. W. Farrall, and A. L. Rippen, eds., *Encyclopedia of Food Engineering*. AVI, Westport, Conn., 1986.
6. W. R. Woolrich and A. F. Novak, "Refrigeration Technology," in N. W. Desrosier and D. K. Tressler, eds., *Fundamentals of Food Freezing*. AVI, Westport, Conn., 1977, pp. 25–53.
7. F. W. Sears, M. W. Zemansky, and H. D. Young. *University Physics*, 5th Ed., Addison-Wesley, Reading, Mass., 1976.
8. J. M. Smith and H. C. Van Ness, *Introduction to Chemical Engineering Thermodynamics*, 3rd ed., McGraw-Hill, New York, 1975.
9. R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, Orlando, Fla., 1986.
10. P. O. Persson and G. Londahl, "Freezing Technology," in C. P. Mallett, ed., *Frozen Food Technology*, Blackie, London, United Kingdom, 1993, pp. 20–57.
11. E. R. Hollowell, *Cold and Freezer Storage Manual*, 2nd ed., AVI, Westport, Conn., 1980.
12. H. J. Goldsmith, "Thermoelectric Refrigerators," in D. M. Rowe, ed., *CRC Handbook of Thermoelectrics*, CRC Press, Boca Raton, Fla., 1995.
13. *Engineered Refrigeration Systems (Industrial and Commercial)*, American Society of Heating, Refrigeration and Air Conditioning Engineers, Atlanta, Ga., 1994.
14. D. S. Reid, "Fundamental Physicochemical Aspects of Freezing," *Food Technol.* **37**, 110–115 (1983).
15. D. S. Reid, "The Freezing of Food Tissues," in B. W. W. Grout and G. J. Morris, eds., *The Effects of Low Temperatures on Biological Systems*, Arnold, London, United Kingdom, 1987, pp 478–479.
16. D. S. Reid, "Physical Phenomena in the Freezing of Tissues," in C. P. Mallett, ed., *Frozen Food Technology*, Blackie, London, United Kingdom, 1993, pp. 1–19.
17. O. R. Fennema, W. D. Powrie, and E. H. Marth, *The Low Temperature Preservation of Food and Living Matter*, Dekker, New York, 1973.
18. International Institute of Refrigeration, *Recommendation for the Processing and Handling of Frozen Foods*, 3rd ed., International Institute of Refrigeration, Paris, France, 1986.
19. Y.-C. Hung, "Prediction of Cooling and Freezing Times," *Food Technol.* **44**, 137 (1990).
20. "Cooling and Freezing Times of Foods," in *ASHRAE Handbook Fundamentals*, American Society of Heating, Refrigeration and Air Conditioning Engineers, Atlanta, Ga., 1997.
21. A. C. Cleland, *Food Refrigeration Process-Analysis, Design and Simulation*, Elsevier Applied Science, United Kingdom, London, 1990.
22. R. Z. Plank, 1913, cited by A. J. Ede, "The Calculation of the Rate of Freezing and Thawing of Foodstuffs," *Modern Refrigeration* **52**, 52–55 (1949).
23. D. J. Cleland, A. C. Cleland, and R. L. Earle, "Prediction of Freezing and Thawing Times for Multi-Dimensional Shapes by Simple Formulae. Part 2: Irregular Shapes," *International Journal of Refrigeration* **10**, 234 (1987).
24. Q. T. Pham, "Simplified Equation for Predicting the Freezing Time of Foodstuffs," *J. Food Technol.* **21**, 209 (1986).

GENERAL REFERENCES

A. D. Althouse, S. H. Turnquist, and A. F. Bracciano, *Modern Refrigeration and Air Conditioning*, The Goodheart-Wilcox Co., South Holland, Ill., 1988.

American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE), *Refrigeration Systems and Applications*, ASHRAE, Atlanta, Ga., 1994.

American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE), *Equipment*, ASHRAE, Atlanta, Ga., 1996.

American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE), *Fundamentals*, ASHRAE, Atlanta, Ga., 1997.

O. R. Fennema, W. D. Powrie, and E. H. Marth, *The Low Temperature Preservation of Food and Living Matter*, Dekker, New York, 1973.

W. B. Gosney, *Principles of Refrigeration*, Cambridge University Press, Cambridge, United Kingdom, 1982.

L. E. Jeremiah, ed., *Freezing Effects on Food Quality* Marcel Dekker, New York, 1996

M. K. Karel, O. R. Fennema, and D. B. Lund, *Principles of Food Science, Part II. Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975.

C. P. Mallett, ed., *Frozen Food Technology*, Blackie, London, United Kingdom, 1993.

M. R. Okas, *Physical and Chemical Properties of Food*, ASAE Publication No. Q0986, St. Joseph, Mich., 1987.

S. Rahman, *Food Properties Handbook* CRC Press, Boca Raton, Fla., 1995.

R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, Orlando, Fla., 1986.

R. Thevenot, *History of Refrigeration*, International Institute of Refrigeration, Paris, France, 1979.

D. S. Reid
University of California
Davis, California

FOOD MARKETING

Most of the efforts and resources in food science and technology are devoted to making food production faster, more efficient, of higher quality, and, of course, less expensive. Food marketing is concerned with a number of other important activities such as the identification of consumer needs; the design of need-satisfying food products and services; the pricing, distribution, and communications (including advertising and promotion) of food and allied products. Most people think of food marketing as synonymous with advertising, and while this is the most obvious activity, it is by no means the most important. Marketing is more than just a set of techniques to entice people to buy a product, it is a philosophic approach to doing business.

MARKETING PHILOSOPHY

The marketing philosophy has been described by numerous authors, in literally hundreds of textbooks. Some of the more classic definitions are "marketing is the performance of activities that seek to accomplish an organization's objectives by anticipating customer or client needs and directing a flow of need-satisfying goods and services from producer to customer or client" (1) and "marketing is a social and managerial process by which individuals and groups obtain what they need and want through creating and exchanging products and value with others" (2). In 1985, the American Marketing Association approved this definition: "Marketing is the process of planning and executing the conception, pricing, promotion, and distributing of ideas, goods, and services to create exchanges that satisfy individual and organizational objectives." (3). A simpler definition that captures the essence of marketing is as follows: "Marketing is the anticipation, understanding, and satisfaction of customer needs while realizing the organization's objectives." The key to all of these definitions is that marketing involves activities that begin with assessing what the consumer needs and wants and translates that into food products that can be sold for a profit. Food marketers believe that the most profitable way to sell products is to offer the consumer the products that they value the most. Food marketers understand that what they make in the factories may not be the same thing consumers are buying at the checkout line. Although not involved in the food industry, Charles Revson, of Revlon Cosmetics, summed up this concept as follows: "In the factories we make cosmetics, at the counters we sell hope." McDonald's makes hamburgers but its customers are buying quick and convenient appetite satisfaction. Food marketers must understand and focus on what consumers want to buy and not simply what food marketers make. But producing the right product is not enough. Food marketers are also concerned with moving that product to the point of consumption (distribution) and making sure that intermediate (wholesalers and retailers) as well as final (consumers) buyers know all the differential benefits derived from product usage. This differential advantage represents the primary basis for persuading intermediate and final buyers to purchase and use a particular food product. The tools available for persuasion include advertising, personal selling, consumer, and trade promotions.

Production vs Marketing vs Sales

After World War II, consumers had considerable spending power, pent-up demand, and too few goods to purchase. Firms found instant success by producing for the mass market. During the production era, food marketers focused on low-cost products to satisfy excess demand. An example is TV dinners. The earliest versions were frequently bland and cheap. Companies that made these products focused on cost reduction and just getting the product to market. However, as demand decreased and competition increased, firms shifted to a sales orientation. While the company made only minor changes to the TV dinners, they increased sales promotions and gave price deals to increase sales.

One point of confusion is the difference between sales and marketing. In the 1960s when marketing came into vogue, many companies promoted their sales managers to marketing managers. Marketing to many was just modern-day sales. Nothing could be further from the truth marketing is a way of doing business. Marketing focuses on the customer and profits, and it sets the stage so that when the sales force is asked to sell, the odds are in their favor. Marketing ensures that the product the sales force is selling is one that the customer wants, in the type of package, in the proper stores, and in the right sizes, etc. The sales force is responsible for getting the volume. Marketing makes sure that the volume is profitable.

As the TV dinner people found that increased selling activity increased sales, it also meant lower margins and profits. Food marketers began to ask the question; "What do consumers really want from frozen prepared foods?" It was not the traditional TV dinner that they had been pushing on the public in the previous years. Food marketers, not the production department, defined what should be done to make the product more attractive (and, therefore, more profitable). Today's frozen entrees and dinners are much better, and many consumers say a delicious, product choice. By taking a marketing approach, the company wins with higher profits and the consumer wins with better products.

THE MARKETING MANAGEMENT PROCESS

The marketing management process consists of analyzing market opportunities, researching and selecting target markets, developing marketing strategies, planning marketing tactics, and implementing and controlling the marketing effort.

The steps in the marketing management process are as follows:

1. Identify market opportunities.
2. Select target market(s) and position the product in the target market.
3. Develop a strategy for each target market.
4. Plan the tactics to accomplish the strategy.
5. Provide a procedure to implement the strategy.

6. Put in place a system for monitoring performance.
7. Evaluate and modify as needed.

MARKETING STRATEGY

Marketing strategy is how food marketers get from where they are to where they want to be. Many people confuse marketing strategy with simply beating the competition. However, marketing strategy is serving customers' real needs. Competitive realities are what possible strategies are tested against. Marketing strategy involves manipulating the marketing mix in the context of environmental variables to satisfy the needs of the target market. The target market is a fairly homogeneous (similar) group of customers to whom a company wishes to appeal. The marketing mix represents the controllable variables the company manipulates to satisfy the target market. The marketing mix is made up of four basic variables generally known as the four *P*'s product, place, promotion, and price.

MARKETING ENVIRONMENT

The marketing environment consists of forces that are not controllable but that influence both the consumer and the food marketer. Although the list of such environmental, uncontrollable or external forces is endless, the most common factors confronting the marketer are as follows.

1. *Competition.* Competitive analysis has been in vogue for the last five years. Understanding competitive strengths and weaknesses is a major input to marketing strategy today. A number of books have focused on just this topic (4,5).

2. *Demographics (Population Descriptors).* Changes in demographics has also had a major impact on food marketing. Baby boomers and the aging of America are just two examples of demographic trends that will lead to new food marketing techniques. *American Demographics* is one journal that provides an excellent overview of changes in American demographics.

3. *Politics.* No area of business is unaffected by politics. The implication to the food industry is through the USDA, FDA, FTC, FCC, and more recently the DEP.

4. *The Economy.* Recession, depression, inflation, a booming economy, unemployment, etc all affect the ability of food marketers to carry out their mission. Even more important to the food business is Wall Street, which frequently determines corporate policies based on current stock prices. Companies attempting to raise stock prices by cutting costs and raising profits often find competitors taking market share from them.

5. *The Physical Environment.* This usually refers to available resources. Probably the most important variables are oil prices and availability of farmland.

6. *Technology.* Little needs to be said about technology. It has influenced every aspect of food marketing. At retail, computerized shelf space management, talking shopping carts, and in-store videos will change the nature of the shopping experience. Distribution is already taking advantage of satellite dishes on trucks to monitor the exact location and contents of the trucks. Warehouses are using scanning technology to position and control product movement. Food manufacturers have similarly benefited from technology. The *Journal of the International Food Technologists (IFT)* addresses technology in food processing.

7. *Social—Cultural Factors.* Of all factors changing food marketing, the changing American family is number one. Women leaving home to join the work force has made convenience a priority. More meals are being purchased from alternative outlets than ever before. No segment of the food industry has been unaffected by these changes (6)

Food marketers employ the controllable variables, the four *P*'s, to address the uncontrollable forces that influence the success of a firm's marketing strategy.

MARKETING MIX (THE FOUR *P*'s)

Product

In the area of food marketing, a product can be described as the need-satisfying offering of a firm. Because consumers are buying satisfaction, companies must be concerned with product quality. From a marketing perspective, quality means the ability of a product to satisfy a customer's needs or requirements. The product area of the four *P*'s is concerned with developing the right product for a specific target market. A product can either be a physical good, a service, or a blend of both. Products in the food industry are usually limited to consumer or industrial products. Consumer products are those products meant for the final consumer. Industrial products are products meant for use in producing other products.

Product Development

Product development involves offering new or improved products for a present market. In the food industry, competition is strong and dynamic, making it essential for companies to keep developing new product. New product planning is not optional, it must be done just to survive in today's changing markets. A new product is one that is new in any way for the company concerned. According to the Federal Trade Commission (FTC), a product can only be called new for six months and only if it is entirely new or changed in a functionally significant or substantial respect. The cost of new product introductions can be high. Experts estimate that consumer-product companies spend at least $20 million to introduce a new brand and 70–80% of these new brands are flops (7).

Place (Distribution)

The place component of the four *P*'s can be thought of as making products available in the right quantities and locations when the customers want them. A product is not much good to a consumer if it is not available when and where it is wanted. Place requires the selection and use of marketing specialists to provide target consumers with the product. Most consumer goods in our economy are distributed through multiple institutions, or middlemen, which are commonly referred to as marketing channels, or channels of distribution. A channel of distribution is any series of firms or individuals who participate in the flow of goods and services from producer to final user or consumer.

The issue of distribution in food marketing has historically fallen into either food retail such as supermarkets or food service such as restaurants or school lunch programs. In the old model, food was either sold as components, taken home, and made into meals for supermarkets or sold as complete meals for the food service. Customers either bought meat, potatoes, and vegetables and made dinner or went to a restaurant for dinner.

Recently this model of distribution has become less clear. Time-starved American consumers want and are willing to pay for more prepared meals. Each year the percentage of the food dollar spent on traditional supermarket food has dropped and food service has increased. In 1997 the percentage of the food dollar spent on traditional supermarket products has fallen to less than 50%, while it was as high as 70% in the 1950s and 1960s.

This consumer shift in demand for prepared foods has led to two major shifts in the distribution channel. First, a new form of food service supplier has appeared. These stores specialize in providing inexpensive complete prepared meals to be consumed either at home or at the store. Unlike the fast-food restaurants, they offer more traditional meals such as meat loaf, roasted chicken, baked ham, and macaroni and cheese. These dishes are referred to as comfort food and are sold in outlets called Boston Market, Kenny Rogers Roasters, or Chili's. These outlets look more like restaurants than supermarkets and are frequently called family restaurants.

To compete against the family restaurants, many food retailers have actually changed into a new format called a "grocerant." Grocerants look more like supermarkets than restaurants but have significantly more space dedicated to prepared meals. In many cases the grocerant will have a number of chefs in the store preparing the food as it is ordered. These meals are sold in outlets called Eatzi's of Dallas, or Zagarra's of Philadelphia. The grocerant still sells various food components and groceries, but this is just a small portion of total sales. Eatzi's reported selling 38% ready-to-heat, 30% ready-to-eat, 12% raw prepared, and 20% conventional groceries.

Both the traditional supermarket and restaurant have changed to stop the loss of sales to the two new formats. Restaurants are providing more attention to takeout, and take home. Once just a nuisance, restaurants now see that they can make larger margins and increase total sales by not being limited to the number of tables available. Many restaurants are allocating space to a take-out section and have comfortable waiting areas for the take-out patrons to wait. The second defense against the new formats is to add entertainment to the traditional dining experience. Rather than compete just for the food, the new restaurants are creating themes that draw customers for fun as well as food. These theme restaurants called "eatertainment" include Rain Forest Café, Planet Hollywood, King Henry's Feast, and many others.

Supermarkets have also tried to appeal to the time-starved consumer by providing prepared food in the stores. They are often called Meal Solutions or Home Meal Replacement and offer complete meals in the deli sections. In some cases these meals are prepared in-house, sometimes in a central commissary for the chain, and in some other cases an outside supplier is used. At the time of this writing no single format has been demonstrated to be superior.

Regardless of the end point, traditional supermarket, restaurant, grocerant, eatertainment, or new supermarket, the people who supply those companies fit into one of the following categories. Marketing intermediaries fit into one of the following categories.

1. *Merchant wholesalers* take title to (own) the goods they sell and sell primarily to other resellers (retailers), industrial, and commercial customers rather than to individual consumers.
2. *Agent middlemen*, such as manufacturer's representatives and brokers, also sell to other resellers and industrial or commercial customers, but they do not take title to the goods they sell. They usually specialize in the selling function and represent client manufacturers on a commission basis.
3. *Retailers* sell goods and services directly to final consumers for their personal, nonbusiness use.
4. *Facilitating agencies*, such as advertising agencies, marketing research firms, collection agencies, and railroads, specialize in one or more marketing functions on a fee-for-service basis to help their clients perform those functions more effectively and efficiently (8).

Choosing the correct channel of distribution is critical in getting products to the target market's place. Because in the food industry physical goods are almost always involved, place requires physical distribution (PD) decisions. Physical distribution is the transporting and storing of goods to match target customers' needs with a firm's marketing mix (9). From the customer point of view, the concern is not how the product was stored or moved, but rather what is the customer service level, how rapidly and dependably a firm can deliver what the customer wants. It is important for food marketers to understand the customer's point of view. Physical distribution is usually the invisible part of marketing and only gets the customer's attention if something goes wrong.

Promotion

Promotion is concerned with telling the target market about the right product. A promotion is a direct inducement that offers an extra value or incentive for the product to the sales force, distributors, or the consumer with the primary objective of creating an immediate sale (10). Promotions are aimed at both consumers and middlemen for the purpose of keeping goods moving smoothly through the pipeline. This is accomplished by using a combination of push and pull strategies. A push strategy provides incentives to the middlemen to buy and resell the product, therefore pushing the goods on to the next stage of the pipeline to the ultimate consumer. A pull strategy provides incentives to the consumer to pull the product out of the end of the pipeline at the retailer level. Food marketers should use a combination of both strategies so that the product will flow easily from the manufacturer to the consumer. The ultimate goal of promotions is to induce behavior. Sales promotions refer to promotional activities other than advertising, publicity, and personal selling that stimulate interest, trial or purchase by final customers or others in the channel.

Consumer Promotion. Examples of consumer promotions are coupons, sweepstakes, contests, product samples, refunds, rebates, tie-ins, premiums, bonus packs, trade-ins, and exhibitions. These promotions are directed at consumers who purchase products at the retail level and are designed to provide them with an inducement to purchase the marketer's brand. Consumer promotions are part of a promotional pull strategy and work along with advertising to encourage consumers to create a demand for a particular brand.

Trade Promotion. Trade promotions in the food industry are sometimes known as promotions to the retailer. This term may be misleading as these promotions are targeted to distributors, wholesalers, and retailers. Trade promotions are critical because product cannot be sold to consumers if it is not first sold to the middlemen. Because the average supermarket stocks more than 10,000 items, promotions need creativity to break through the clutter. Trade promotions include activities such as promotional allowances, dealer incentives, point-of-purchase displays, sales contests and sweepstakes, and trade shows. Trade promotions are designed to motivate distributors and retailers to carry a product and make an extra effort to promote it to their customers (push strategy).

One of the most commonly used trade promotions in the food industry is the promotional allowance. These are payments made by a manufacturer to resellers (often off-invoice) for merchandising its products or running in-store promotional programs such as reduced shelf prices, special displays, or in-store advertising. Another common but controversial promotion is the slotting allowance (sometimes known as a stocking allowance or street money). Slotting allowances are the fees that are often demanded by the retailers to gain admission into their stores. The position taken by the retailer is that with increased competition, a proliferation of new products, and small profit margins, they are required to ask for these fees, claiming that they will be used to promote the products, redesign shelves, and reprogram computers. Most manufacturers feel differently about how slotting fees are used. One food industry source estimated that 70% of all slotting fees go directly to the retailers' bottom line (11). Many food manufacturers view slotting allowances as little more than corporate bribery.

Traditionally, food companies have spent more of their promotional dollars on trade promotions than on consumer promotions, in an effort to win shelf space in the midst of a rising tide of new products. Recently, however, expenditures on promotions to consumers have been growing while expenditures on promotions to the trade have been declining slowly. Of the three major areas of a company's promotional spending, consumer promotions, trade promotions, and media spending, the three-year trend shown in Table 1 has appeared (12).

As an example, Kellogg Co., the world's leading cereal manufacturer, is one of the companies that intends to tilt its marketing strategy away from the trade and back toward the consumer. This comes on the heels of sluggish domestic sales. In addition to Kellogg Co., Kraft General Foods has decided to step up advertising for its Maxwell House coffee, a brand that stopped advertising in 1987 and lost its number one position in the coffee business (13) (Table 2).

Advertising

Simply stated, advertising tells the target market that the right product is available, at the right price, and at the right place and time. Advertising is defined as any paid form of nonpersonal presentation of ideas, goods, or services by an identified sponsor. It includes the use of such media as magazines, newspapers, radio and television, signs, and direct mail (14). The most common vehicles of advertising used by food companies are network and spot television, radio, and magazines. According to one source

> the food industry is expected to be the biggest ad spender in 1990 with a budget of $8.4 billion, according to a study by Schonfeld & Associates of Evanston, Ill. The 107 publicly owned restaurant and fast-food chains are forecast to spend 8% more this year, to more than $1.5 billion. Supermarket chains will raise spending by 5.6% to $1.8 billion, with Kroger Co. the biggest at $225 million. Ad spending for health and beauty aids is expected to rise by 11% to more than $11.2 billion with Bristol-Myers Co. leading the way at $1.1 billion (15).

Personal Selling

Personal selling is defined as direct communication between sellers and potential customers. Personal selling is usually face-to-face, but communication can take place over the telephone. The strength of personal selling in food

Table 1. Annual Survey of Promotional Practices

	Consumer Promotions	Trade Promotions
1987	26.1%	39.9%
1988	26.3%	39.6%
1989	27.1%	39.4%

Table 2. Biggest Ad Spenders in the Prepared Food Category

Rank	Company
1	Kellogg Company
2	General Mills Inc.
3	Philip Morris Companies, Inc.
4	Quaker Oats Company
5	Campbell Soup Company

Source. Ref. 16.

Note. For a one-year time period ending in June 1989. Total advertising expenditures for prepared foods for this time period was $1.2 billion.

marketing is its flexibility, as it provides immediate feedback that helps salespeople adapt to the current situation.

In total there are more than 11 million consumer salespersons in the United States, with another 9 million salespersons in the industrial marketing area. How important are salespersons? One study, for example, found that executives of industrial firms rated the sales function as 5 times more important than advertising in their marketing mixes; for consumer durables marketers, sales was rated 1.8 times as important as advertising, while for consumer nondurables, advertising and person selling were rated as about equally important (17).

The basic steps of personal selling are as follows; prospecting, planning sales presentations, making sales presentations, and following up after the sale. Prospecting involves following all the leads in the target market to identify potential customers. Once the prospect has been located, it is necessary to make a sales presentation that is the salesperson's effort to make a sale.

Price

The fourth *P* a food marketer must be concerned with is price. When a company sets a price, they must take into consideration the kind of competition in the target market and the cost of the whole marketing mix. Other considerations to be made in pricing decisions are customer reaction to possible prices, current practices as to markups, discounts, and other terms of sale, and finally, legal restrictions on pricing. Price is an important consideration in food marketing because if a customer will not accept the price, all of the planning decisions and efforts could be wasted.

MARKETING RESEARCH

Finally, food marketing usually encompasses marketing research. Marketing researchers represent the information-gathering arm of the firm and helps to determine exactly what products consumers want as well as how to best distribute, advertise, and promote those products. Marketing research is also used extensively to measure performance. Do consumers really understand the benefits of the product, how much is being sold and to whom, etc? Simply stated, marketing research is intended to provide timely and relevant information to improve marketing decision making.

In attempting to meet the needs of consumers, food marketers need to keep up with all of the changes taking place in their markets. Most food marketers rely on marketing research to help them make decisions about the marketing plan. The American Marketing Association adopted this definition of marketing research in 1987:

> Marketing Research is the function which links the consumer, customer, and public to the marketer through information—information used to identify and define marketing opportunities and problems; generate, refine, and evaluate marketing actions; monitor marketing performance; and improve understanding of marketing as a process. Marketing Research specifies the information required to address these issues; designs the method for collection information; manages and implements the data collection process; analyzes the results; and communicates the findings and their implications (19).

Food marketers must use marketing research to help them make good decisions. One way is to use the scientific method—a decision-making approach that focuses on being objective in testing ideas before accepting them. This way a marketer does not assume his or her intuition is correct without evidence to support it. The marketing research process is a five-step application of the scientific method. It includes

1. Defining the problem.
2. Analyzing the situation.
3. Getting problem-specific data.
4. Interpreting the data.
5. Solving the problem.

CONCLUSION

In order for the food industry to maintain its preeminence, all the parts must not only work individually, but work together. While the engineers and food technologists are developing new products, food marketers must ensure that the research and development is market directed. Making food less expensive to produce is only valuable if people want and will buy the less-expensive product. Food marketing is the discipline that brings the consumer into the corporate planning process. It ensures that the products desired will not only be available, but that consumers will know about the benefits of the product and where to get it. Food marketing is no more important than any other food business function, but it is essential in today's food business environment.

BIBLIOGRAPHY

1. E. J. McCarthy, *Basic Marketing*, Richard D. Irwin, Inc., Homewood, Ill., 1990, p. 8.
2. P. Kotler, *Principles of Marketing*, Prentice Hall, Inc., Englewood, N.J., 1989, p. 5.
3. *Marketing News*, 1 (Mar. 1, 1985).
4. A. Ries and J. Trout, *Marketing Warfare*, McGraw-Hill Inc , New York, 1983.
5. M. Porter, *Competition in Global Industries*, Harvard Business School Press, Boston, 1986.
6. J. Stanton, "2001· A Food Odyssey," *HEIB*, 1990
7. Ref. 1, p. 261.
8. H. Boyd, *Marketing Management*, Richard D. Irwin, Inc., Homewood, Ill., 1990, p. 22.
9. Ref. 1, p. 344.
10. L. Haugh, "Defining and Redefining," *Advertising Age*, M-44 (Feb. 14, 1983).
11. J. Dagnoli and L. Freeman, "Marketers Seek Slotting-Fee Truce," *Advertising Age*, 12 (Feb. 22, 1988).

12. Donnelly Marketing, *12th Annual Survey of Promotional Practices*, Stamford, Conn., 1990, p. 3.
13. R. Gibson, "Kellogg Shifts Strategy to Pull Consumers In," *The Wall Street Journal*, Jan. 22, 1990, p. 131.
14. Ref. 1, p. 366.
15. *Ad Week's Marketing Week*, July 3, 1989, p. 6.
16. B. Bagot, "1990 Industry Outlooks," *Marketing and Media Decisions*, 33 (Jan. 1990).
17. W. Wilkie, *Consumer Behavior*, John Wiley & Sons, Inc., New York, 1990, p. 492.
18. D. Lehmann, *Market Research and Analysis*, Richard D. Irwin, Inc., Homewood, Ill., 1989, p. 3.

JOHN L. STANTON
RICHARD J. GEORGE
CAROL A. GALLAGHER
Saint Joseph's University
Philadelphia, Pennsylvania

See also UNITED STATES FOOD MARKETING SYSTEM in the Supplement section.

FOOD MICROSTRUCTURE

The microstructure (location, distribution, size, morphology, porosity, etc) of a food material is closely correlated to its texture (1), component functionality (2), and nutritional quality (3). The challenge lies in finding the appropriate methodology (or combination of such) for microstructural characterization. This review will focus mainly on the various techniques available for such characterization with special emphasis on their applications and limitations.

A basic approach for visualizing the microstructure of a sample is to obtain a magnified view of its components through microscopic techniques. Excellent examples of the relationship of microscopic observations to changes observed during processing can be found in the literature (4,5). Our attempt will be to summarize the differences of some basic microscopy techniques and their limitations and advantages.

LIGHT MICROSCOPY

The most routinely applied microscopy techniques are those collectively categorized under light microscopy. At the heart of these techniques is a microscope equipped with a light source and a series of lenses: the condenser, objective, and ocular. Figure 1a shows the basic principles of image magnification in a light microscope. A light source provides illumination, that passes through first the condenser, then the specimen, followed by the objective lens producing a reversed upside-down image, which is then further magnified by the ocular lens (6,7). The resolution of light microscopy is between 200 and 500 nm (5). The proper use and limitation of each of these components has been described elsewhere (7).

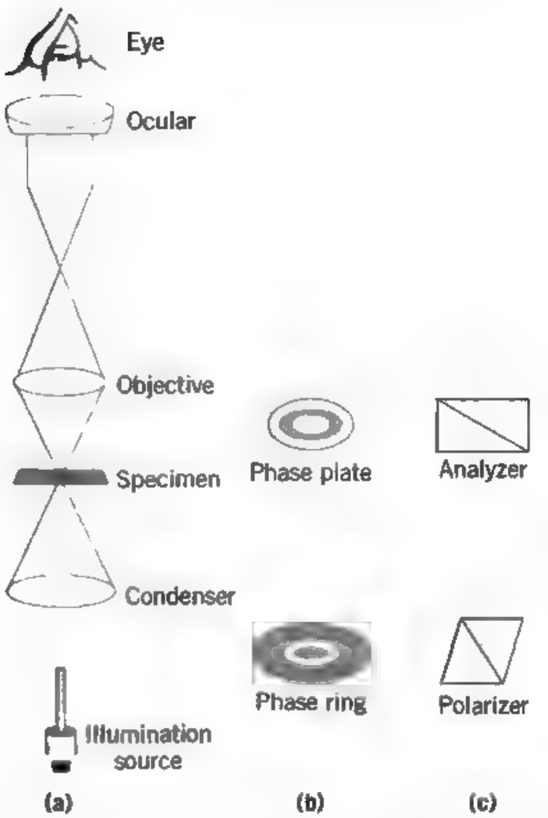


Figure 1. (a) Schematic of image magnification in light microscopy; (b) phase plates required for phase contrast microscopy (see text for details); (c) polarizers required for polarized light microscopy (see text for details).

A specimen's microstructure can only be seen in the microscope when there is some form of contrast. Contrast is usually established through the staining (chemically or physically attaching specific dyes) of particular components. Some commonly used stains in food research are presented in Table 1. Vaughan (4) described in detail the use of various stains to locate specific components in a variety of commodities. An example of the relationship of microstructural changes occurring during processing looked at the effect of mixing on the structure of dough made from different quality flours by observing uranyl-acetate stained gluten fractions (8). Proper dough development required a matrix network of protein strands. For living cells, which may be harmed by stains, a phase ring and plate may be added to achieve phase contrast (7) as shown in Figure 1b.

Table 1. Stains of Food Components Used in Light Microscopy Work

Compound	Stain
Starch	Iodine
Protein	Fast green, acid fuchsin
Plant cell walls, muscle tissue	Toluidine Blue O
Fats	Oil Red O, Sudan III

Source: Ref. 6.

12. Donnelly Marketing, *12th Annual Survey of Promotional Practices*, Stamford, Conn., 1990, p. 3.
13. R. Gibson, "Kellogg Shifts Strategy to Pull Consumers In," *The Wall Street Journal*, Jan. 22, 1990, p. 131.
14. Ref. 1, p. 366.
15. *Ad Week's Marketing Week*, July 3, 1989, p. 6.
16. B. Bagot, "1990 Industry Outlooks," *Marketing and Media Decisions*, 33 (Jan. 1990).
17. W. Wilkie, *Consumer Behavior*, John Wiley & Sons, Inc., New York, 1990, p. 492.
18. D. Lehmann, *Market Research and Analysis*, Richard D. Irwin, Inc., Homewood, Ill., 1989, p. 3.

JOHN L. STANTON
RICHARD J. GEORGE
CAROL A. GALLAGHER
Saint Joseph's University
Philadelphia, Pennsylvania

See also UNITED STATES FOOD MARKETING SYSTEM in the Supplement section.

FOOD MICROSTRUCTURE

The microstructure (location, distribution, size, morphology, porosity, etc) of a food material is closely correlated to its texture (1), component functionality (2), and nutritional quality (3). The challenge lies in finding the appropriate methodology (or combination of such) for microstructural characterization. This review will focus mainly on the various techniques available for such characterization with special emphasis on their applications and limitations.

A basic approach for visualizing the microstructure of a sample is to obtain a magnified view of its components through microscopic techniques. Excellent examples of the relationship of microscopic observations to changes observed during processing can be found in the literature (4,5). Our attempt will be to summarize the differences of some basic microscopy techniques and their limitations and advantages.

LIGHT MICROSCOPY

The most routinely applied microscopy techniques are those collectively categorized under light microscopy. At the heart of these techniques is a microscope equipped with a light source and a series of lenses: the condenser, objective, and ocular. Figure 1a shows the basic principles of image magnification in a light microscope. A light source provides illumination, that passes through first the condenser, then the specimen, followed by the objective lens producing a reversed upside-down image, which is then further magnified by the ocular lens (6,7). The resolution of light microscopy is between 200 and 500 nm (5). The proper use and limitation of each of these components has been described elsewhere (7).

A specimen's microstructure can only be seen in the microscope when there is some form of contrast. Contrast is usually established through the staining (chemically or physically attaching specific dyes) of particular components. Some commonly used stains in food research are presented in Table 1. Vaughan (4) described in detail the use of various stains to locate specific components in a variety of commodities. An example of the relationship of microstructural changes occurring during processing looked at the effect of mixing on the structure of dough made from different quality flours by observing uranyl-acetate stained gluten fractions (8). Proper dough development required a matrix network of protein strands. For living cells, which may be harmed by stains, a phase ring and plate may be added to achieve phase contrast (7) as shown in Figure 1b.

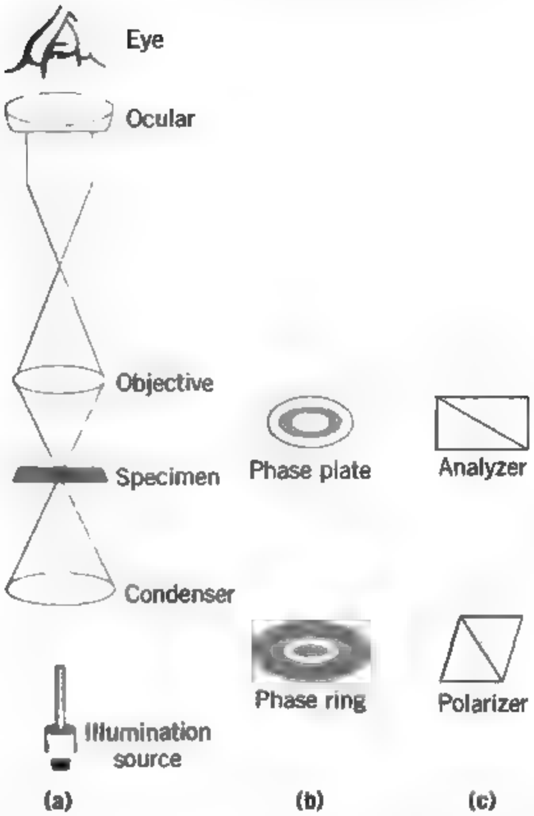


Figure 1. (a) Schematic of image magnification in light microscopy; (b) phase plates required for phase contrast microscopy (see text for details); (c) polarizers required for polarized light microscopy (see text for details).

Table 1. Stains of Food Components Used in Light Microscopy Work

Compound	Stain
Starch	Iodine
Protein	Fast green, acid fuchsin
Plant cell walls, muscle tissue	Toluidine Blue O
Fats	Oil Red O, Sudan III

Source: Ref. 6.

Polarizers are common modifications added to the compound microscope to view highly ordered (crystalline) material. The polarizers are placed between the illumination source and the condenser (polarizer) and between the specimen and objective (analyzer) as shown in Figure 1c. Many organic crystals are birefringent: entering light is split into two component and polarized in two perpendicular directions (7). Birefringence is the measure of the differences in refractive indices of the two components (9). Polarized microscopy has been used in food to measure the sacromere length in muscle fiber and subsequently related to meat quality (5), to identify the botanical origin of starch grains and in turn follow the specific patterns of gelatinization and recrystallization (9), and to differentiate the three polymorphic forms of triglycerides in fats, oils, and emulsions and relate each to lipid stability (5).

A powerful light microscopy technique relies on the ability of objects to fluoresce. Fluorescence is described as a process in which light energy is absorbed at a particular wavelength that excites the molecule to a higher electron energy state and higher vibrational state. Subsequently, this energy is re-emitted in the form of light in a range of wavelengths that can be quantified (10). Various food components are autofluorescent, whereas others require the attachment of specific fluoropores for them to be observed under fluorescence (Table 2). Selecting the appropriate fluorescence probes and/or filters to minimize background signals is critical for improved sensitivity of the detected fluorescence. The two types of filters used to obtain adequate fluorescence are the exciter and barrier filters. The exciter filter transmits only those wavelengths that are near the excitation maximum of the fluorescent compound while the barrier filter transmits only the wavelengths emitted by the sample (11). Vodovotz et al. (12) discussed the environmental factors that affect the efficacy of a specific probe including solvent polarity, proximity, and concentrations of quenching species and pH of the aqueous medium. Various examples of the use of fluorescence in food research include the effect of processing on starch as well as cell wall structure, enzyme activity, and nutritional quality of cereal grains (3,11).

The main drawbacks of light microscopy are limited resolution and the need to obtain thin sections for analysis. A specimen with considerable thickness yields a blurred or diffused image because the depth of field in conventional microscopy at high power is 2 to 3 μ while the resolving power is 0.2 μ (13). The confocal microscope circumvents this problem by focusing a point light source on a small volume within the specimen, rendering an image of in-focus plane only, with the out-of-focus parts appearing as black background (14). The confocal microscope functions by focusing a light source on an x-y plane at a specific depth (z). The illumination is often coherent (laser) light since its greater intensity allows for additional applications such as observing fluorescence in a specimen. The specific fluorescent probes can aid in the localization of different components depending on the number of laser lines available in the instrument. Figure 2 shows the principal parts and light path of a confocal microscope. The laser light passes through an objective lens (which acts as a condenser as well as a collector; hence, the term confocal), bounces off a diachroic mirror, and is then projected onto a pinhole (confocal aperture) in front of a detector (photomultiplier tube). To obtain more than just a pinhole image, an x-y plane is scanned (rastered), and the image is then collected and reconstructed in a computer (12,14,15).

Table 2. Fluorescent Probes and Stains Used with Food Components in Fluorescence Microscopy

Food Component	Fluorescence probe
Collagen	Autofluorescence
Elastin	Autofluorescence
Pigments (chlorophyll, carotenoids)	Autofluorescence
Phenolic compounds (lignin and ferulic acid)	Autofluorescence
Cartilage, bone	Autofluorescence
β-D-Glucan	Calcofluor white, Congo Red
Periodate/Schiff's	Starch
Proteins	Acid fuchin, Thiazine Red R
Lipids	Nile Blue A, Sudan III

Source: Refs. 5, 6, and 12.

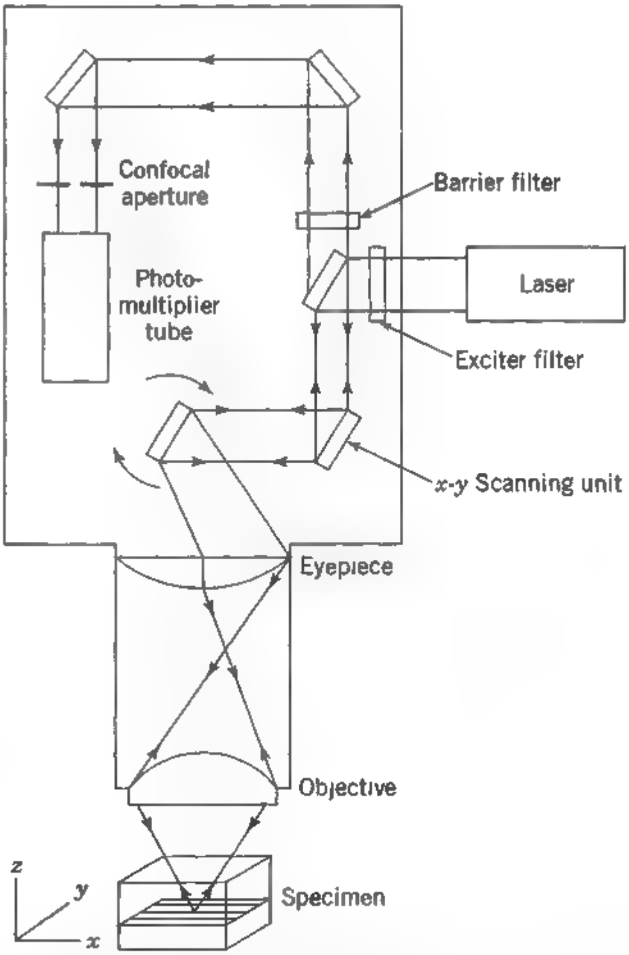


Figure 2. Schematic of the light path in a confocal microscope. *Source:* Ref. 12, used with permission.

An important application of the confocal microscope is the ability to observe a three-dimensional image without the need to physically section the sample, allowing a disturbance-free observation of the specimen (12). Although transmission electron microscopy (TEM) can provide a three-dimensional view of the specimen, the sample must be dried or frozen (or replicated with platinum and carbon) since TEM is carried out in vacuum. Additionally, confocal microscopy requires less sample preparation such as dehydration and fixation, since thin sections are obtained optically. For example, beef actomyosin solutions mixed with glutaminase were viewed directly at different time intervals as a gel network was formed (16). Dynamic processes such as dough formation (17) are also possible.

The combination of two powerful techniques such as laser scanning confocal microscopy (LSCM) and fluorescence microscopy can be used to localize and identify different components. Heertje and coworkers (18) followed the rising of dough containing fluorescein isothiocyanate solution at room temperature. They were able to distinguish various locations of gluten, starch, and air pockets. A similar study by Blonk and van Aalst (15) looked at the influence of various additives and the gluten network in dough on bread loaf volume. In their study, gluten was stained with rhodamine and was shown to have a finite expandability, whereas inclusion of an additive with surfactant capabilities aided in the formation of a continuous gluten film. No difference was detected in the location and distribution of starch (stained with periodic Acid-Schiff's reaction) and gluten (stained with FuoroLink Cy3) when comparing fresh and aged bread (19).

LSCM has been most commonly used in the fields of medicine and microbiology/biochemistry. The main benefit of LSCM is the elimination of diffuse light by the use of a pinhole. However, this requires greater illumination, which may result in photobleaching and photodamage to live specimen. To circumvent this problem, a microscope with an efficient light path from the specimen to the detector is required as well as its operation at maximal sensitivity (highest gain) (20). Some relevant applications include the study of pH imaging in cells (21,22), detection of single molecules (such as DNA) in solution (23), the determination of the intracellular 3-D (or 4-D) structure of individual cell components by microinjecting dyes into living cells (24), and observing the location and distribution of *Salmonella enteritidis* in egg shells held at various conditions (25).

ELECTRON MICROSCOPY

Electron microscopy (EM) provides better resolution and higher magnifications compared with conventional light microscopy (26) because the illumination source in EM is electrons focused with magnetic lenses rather than photons focused with glass lenses. Electrons are absorbed by air; therefore, operation is carried out under vacuum. The sample must not release any volatile substances when placed in the microscope, and thus it is dried or frozen or replicated with platinum and carbon. In the latter case the replica is examined (6).

EM can be carried out in two modes: scanning electron microscopy (SEM) and TEM. SEM is used to examine surfaces. Nonconductive materials such as food specimens need to be made electrically conductive. This can be achieved by depositing a metal layer such as gold palladium onto the sample using a spatter coater. The sample is scanned by a focused electron beam. In conventional SEM the sample needs to be completely dry because the vacuum within the microscope would cause the water to evaporate. In addition, lipids in the sample would melt under the electron beam (27). Careful specimen preparation is critical to avoid artifacts and essentially determines the value of information obtained (28). The preparation methods are dictated mainly by the type of sample. Moisture and fat content as well as structural differences should be considered. For example, fixation, dehydration, and coating may require longer time for compact structures such as low-fat cheeses compared with full-fat cheeses (6). Critical point drying after chemical fixation with osmium tetroxide and a low concentration of gluteraldehyde is an effective sample preparation method for protein systems such as gluten (29). Dry cereal grains and starch have been examined with SEM with relatively simple sample preparation, to assess relations among components and extent of damage due to milling (30,31), action of amylolytic enzymes on starch granules (32) and to relate granule size and surface morphology to the genetic background of the source plant (33). Observation of bread by SEM can provide information on gas cell size. However, the use of fixatives and removal of moisture can cause the protein matrix to shrink away from the starch granules, altering the appearance of the bread components (34,35).

High-moisture samples, as most foods are, and high-fat systems can be examined with cryo-SEM. This technique involves freezing the specimen and examining it in the SEM at a temperature at which neither water is lost nor fat is melted (36). Allan-Woajtas (37) has discussed methods to freeze samples in preparation for SEM. Cryo-SEM is very useful in the study of food materials that are easily damaged by conventional SEM, such as baked products and emulsions. A significant advantage of the technique is the ability to study dynamic phenomena by freezing samples at time intervals, during emulsion destabilization, for instance, or dough formation and baking (38). Sargent (36) has reviewed some of the numerous applications of cryo-SEM in food systems. Dairy products such as cheese, ice cream, and full- and low-fat spreads have been examined in great detail. Low-temperature SEM has helped reveal the mechanism of emulsion and foam destabilization in products such as salad dressings, meringues, and mayonnaise. Cocoa powders, butter, and chocolate have been extensively characterized as well. Structure organization in cereals and changes during heating or baking (35,39), dough formation and effect of mixing on the gluten network (28,40), and examination of cake batters (41) are some of the many applications of cryo-SEM in the study of cereal products (42).

Despite the extent of applications, cryo-SEM is not free of artifacts. The most common are due to formation of ice crystals, which can displace structural elements and destroy the initial structure (6). Some of the limitations of the conventional and cryo-SEM can be resolved by an emerging technique, the environmental SEM (ESEM) (39,43), that can produce images at ambient temperature without drying or metal coating. This technique could have great impact in the direct observation of food systems.

TEM visualizes the internal structure of food samples. Thin sections of samples embedded in epoxy resin or platinum-carbon replicas of the sample are placed in the path of the electron beam, and the electrons are transmitted through the sample (6). Cryo-fixation followed by replication produces superior TEM images of high resolution. The image quality depends on the quality of the replica, which requires high skill and experience. TEM has been used to identify protein and lipid structures in grains (30), to determine structure and stability of cake batters (41), to study the properties of microencapsulated droplets in starch/water gels (44), to assess enzyme activity on flours (45), to monitor changes in plant cell walls as a result of processing (46), and to obtain images of food-related proteins (47).

EM techniques in general provide qualitative information on the structure and microscopic organization of food products. Quantitative information can be obtained by image analysis techniques that employ sophisticated statistical analysis. Computer-aided digitization of the micrographs can be used to obtain, for instance, to study air cell size distributions (48) or even two- and three-dimensional reconstructions of protein structures (47). Image analysis will be described in more detail later.

ATOMIC FORCE MICROSCOPY

The light and electron microscopes helped to cross many boundaries in the world of molecules through elegant methods of sample preparation that have enabled microscopists to approach the theoretical resolution of their instruments. Still, to pass from the subcellular level to the molecular domain, the ability to resolve objects smaller than 1 nm is needed. This task can be accomplished by the scanning probe microscopes (49), a family of microscopes that make it possible to examine molecules or groups of molecules without the heavy metal coatings that are often necessary to produce contrast in optical and high-vacuum systems and without the many of the other constraints inherent to optical and electron microscopes.

The first probe microscope, the scanning tunneling microscope, was invented in 1986 and led to the development of the atomic force microscopy (AFM) (50). AFM is more suitable for nonconductive samples such as biological specimen (51).

The major difference between AFM and light or electron microscopy is in the way the image is created. Conventional microscopy employs lenses and relies on optics to create an image. In AFM the image is created by scanning a sharp tip, which is attached to a flexible cantilever, across a sample surface (Figure 3), much like a record stylus plays a record. When the tip is in close proximity to the sample, repulsive forces cause the cantilever to bend away from the surface. By measuring the extent of cantilever bending, a detailed topography can be recorded. The most common detection system is the deflection of a laser beam (52) reflected off a mirror mounted on the back of the cantilever mechanism. This system is very sensitive and can detect cantilever deflections produced by the tip scanning over individual atoms and molecules. The force exerted by the tip on the sample is very important for precision imaging. Too low a force causes the tip to mistrack and lose detail, whereas too high a force can displace or damage the sample. To control the force exerted by the tip, the cantilever assembly is mounted on a piezoelectric device that also controls the precision with which the tip is positioned relative to the surface of the sample. The precision of this mechanism is the key to the high resolution of the AFM (53).

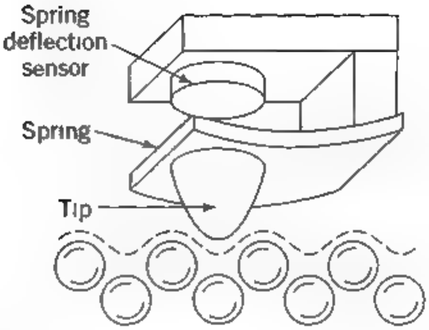


Figure 3. Schematic of the AFM cantilever-tip mechanism. *Source:* Ref. 51, used with permission.

One of the major advantages of the AFM is the minimal sample preparation required to obtain high-resolution images. Specimens are applied to a substrate as a dilute solution or thin film. No coating, extensive drying, or other preparation is needed. The substrate can be any flat surface such as a glass slide or freshly cleaved mica.

AFM can be carried out in three modes: contact, noncontact, and tapping mode (6). In the contact mode, the tip touches the sample. As the cantilever bends in response to surface features, a variable force is exerted on the sample. This force is compared with a preset value, and the piezoelectric device moves up or down to meet that value. The whole scan is then performed at constant force. The distance that the piezo needs to travel to achieve that constant force is recorded and amplified to create the image (53). Higher scan speeds can be attained with the contact mode, which is the only mode that can obtain "atomic resolution" images. Contact mode is the technique of choice for very rough samples with extreme changes in vertical topography; for example, deep grooves or valleys. The main disadvantage is that contact mode can be damaging to very soft samples due to the high forces, a result of capillary interactions from the absorbed fluid layer on the sample surface (54).

Most of the AFM imaging in food systems has been done with the contact mode. Aggregates of β-lactoglobulin, a whey protein, were imaged and characterized after deposition on mica and graphite (55). This level of detail cannot be accomplished with electron microscopy because of the restrictions imposed by the size of the metal coatings necessary to obtain the EM image. Kirby et al. (56) obtained high-resolution images of xanthan gum that showed extreme stiffness of individual molecules entangled in a network. Images of pectin (57), another thickening agent, appeared to be a more flexible structure that can be easily distorted. This can explain the relatively lower viscosity attained by pectin as compared with xanthan gum. Round et al. (58) studied an unusual branched structure for tomato pectins that could not be predicted from enzymatic hydrolysis, showing that AFM can be used to differentiate among polysaccharides from different plant sources. Interactions between α-gliadin layers were also investigated (59). It was shown that the molecules in the protein film have a very compact conformation that was affected by the presence of additives, demonstrating the potential use of AFM in characterization of edible protein-based films. Kirby et al. (53) obtained images of cellulosic gelled structures exuded by *Acetobacter xylinum* that show a network of flat ribbons enclosing the bacteria. Baker's yeast strains along with polysaccharide molecules from the cell wall were imaged (60) with minimal sample preparation. The action of α-amylase on starch granules has been captured in real time (61), demonstrating the ability of AFM to directly observe enzymatic reactions and structure changes on the substrate (Figure 4).

In the noncontact mode the cantilever is vibrated close to its resonance frequency, and the tip does not touch the sample. The image is created by detecting the damping effect on the cantilever vibrations caused by long-range attractive forces between the atoms of the tip and the sample. The main advantage of using the noncontact mode is that the shearing forces imposed on the sample by the tip are reduced or even eliminated, making imaging of very soft specimens possible. The main disadvantage is that the noncontact mode usually only works on extremely hydrophobic samples where the absorbed fluid layer at the surface of the sample is at minimum.

The tapping mode overcomes this limitation (54). It is similar to the noncontact mode, but, in this case, the cantilever is strongly vibrated and the tip intermittently touches the sample. The major advantage of this technique is that the adhesive forces arising from absorbed water on the surface are reduced. Shearing is also minimized since the tip touches the surface only momentarily. As a result, this mode provides high-resolution images that could otherwise be obtained only with both the sample and the cantilever immersed in a liquid (53) and is very suitable for soft hydrophilic specimens. Tapping mode is gaining momentum in studying food systems. Tsoubeli and Taub (62) obtained high-resolution images of bovine myofibrils in air and followed aggregation and gel formation as a result of thermal processing.

DYNAMIC LIGHT SCATTERING

Dynamic light scattering (DLS) is an alternative method for determining food microstructure that is based on scattering of light by moving particles. Details of this noninvasive and relatively rapid method are given elsewhere (63). In the food industry, DLS is used for determining particle sizes in the range of 1 to 300 μm in such diverse products as chocolate, wheat and soy flour, confectionery sugar, spices, and mayonnaise. More specific instruments have been developed to detect particles outside this range (64). The main application of this technique has involved very dilute systems (milk and oil in water emulsions), because the concentration of scatters had to be sufficiently low that an incident photon of light is scattered only once by the sample (63). Therefore, DLS can rarely be used directly on most foods, since particulate concentration is too high and native structure is difficult to maintain upon dilution. Nevertheless, DLS has been used to look at the changes in the average size of casein micelles with addition of calcium

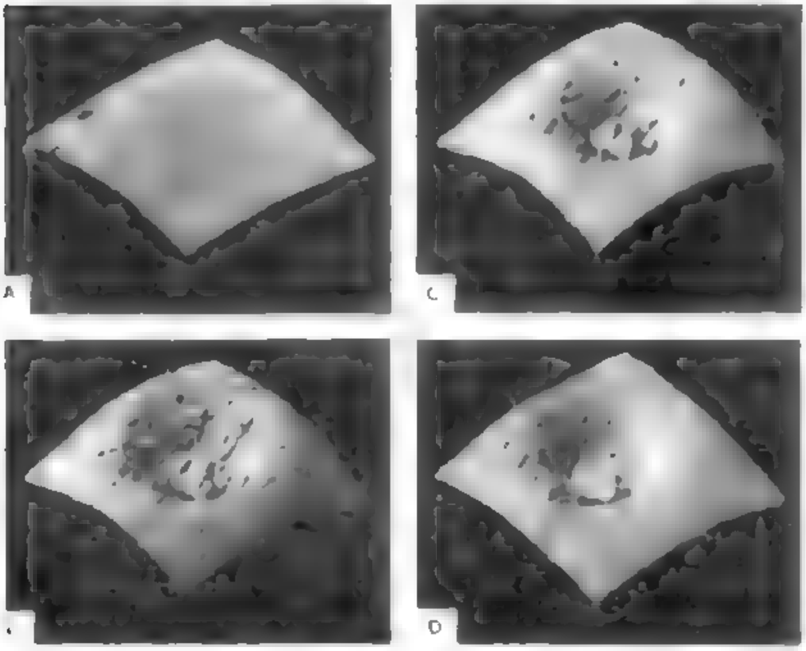


Figure 4. α-Amylase action on a single starch granule obtained by contact mode AFM. A–D: 0, 105, 420, and 735 s, respectively. *Source:* Ref. 61, used with permission.

phosphate, increased temperature, and different pHs (63). These changes (reflecting processing protocols) could be related to the functional properties of milk. Additionally, DLS has been used to detect size distribution (65) and adsorbed monolayers (66) in emulsions.

A modification of DLS, diffusion wave spectroscopy (DWS), in which incident and back-scattered light are conducted through fiber optics, can be used to provide estimates of mean particle size in concentrated suspensions. For example, the particle-size changes in rennet (67) and acid-induced (68) gelation of milk were studied by DWS. However, to obtain accurate particle sizes with DWS, the instrument needs to be calibrated with separate samples with known size scatterers, which may not have the same optical characteristics as the food samples used, making the interpretation of results difficult (63).

IMAGE ANALYSIS

To obtain microstructural information from microscopic or other imaging techniques, data need to be collected and analyzed. The first step would require a computer rendering of the image, which may be accomplished by photographing and digitizing the data. In the case of LSCM, this would be done automatically. Once microstructural data are collected, subjective analysis may not be sufficient. Quantification of objects observed in an image may be as simple as measuring its length with an eyepiece micrometer or counting individual components. These measurements, if performed in a large number of samples, could be extremely time-consuming; therefore, automated image analysis techniques may be preferable. However, the automated system used is required to recognize the desired object(s), and differentiate it (them) from artifacts such as overlapping or touching cells, debris, and dirt, so as to exclude them from the measurements (7). Regardless of the method used, analysts need to be sure that their observations are not due to artifacts arising from sample preparation, such as fixing and embedding, or instrumental limitations such as the optics used.

Various objects display irregular shapes, which are difficult to characterize quantitatively. Fractal analysis can be employed to estimate the degree of irregularity in a sample. Unlike Euclidean geometry in which dimensions are expressed in whole numbers (eg, 2 for area and 3 for volume), fractal dimensions consist of fractional numbers (such as 1.3, etc). However, for true fractal objects they must exhibit self-similarity, at least over the range being described (69). This technique can be used to follow changes in the irregularity of a sample due to processing. For example, the extent of agglomeration of coffee particles can be quantified and related to their dispersibility in a liquid (69). The difficulty with fractal analysis lies with the ability to obtain accurate data from imaging technique, such as the edge pixels in a digitized image.

These techniques represent a few, but not all, of the methods available to obtain microstructural information of food products. It is imperative to understand the limitation of each technique, especially when interpreting the results. Proper microstructural analysis can be extremely beneficial in characterizing changes occurring in food products, which manifest themselves in a macroscopic level.

BIBLIOGRAPHY

1. D. W. Stanley, "Food Texture and Microstructure," in R. Moskowitz, ed., *Food Texture: Instrumental and Sensory Measurement*, Marcel Dekker, New York, 1987, pp. 35–64.
2. A. Hermansson, "Protein Functionality and Its Relation to Food Microstructure," *Qualitas Plantarum, Plant Foods for Human Nutrition* **32**, 369–388 (1983).
3. S. H. Yiu, "Food Microscopy and the Nutritional Quality of Cereal Foods," *Food Structure* **12**, 123–133 (1993).
4. J. G. Vaughan, *Food Microscopy*, Academic Press, New York, 1979.
5. J. M. Aguillera and D. W. Stanley, *Microstructural Principles of Food Processing and Engineering*, Elsevier Science, New York, 1990.
6. M. Kalab, P. Allan-Wojtas, and S. S. Miller, "Microscopy and Other Imaging Techniques in Food Structure Analysis," *Trends Food Sci. Technol.* **6**, 177–186 (1995).
7. J. James and H. J. Tanke, *Biomedical Light Microscopy*, Kluwer Academic Publishers, Dordecht, The Netherlands, 1991.
8. D. B. Bechtel, Y. Poranz, and A. de Francisco, "Breadmaking Studied by Light and Transmission Electron Microscopy," *Cereal Chem.* **55**, 392–401 (1978).
9. E. Varriano-Marston, "Polarization Microscopy: Applications in Cereal Science," in D. B. Bechtel, ed., *New Frontiers in Food Microstructure*, American Association of Cereal Chemists, St. Paul, Minn., 1983, pp. 71–104.
10. R. G. Fulcher and P. J. Wood, "Identification of Cereal Carbohydrates by Fluorescence Microscopy," in D B. Bechtel, ed., *New Frontiers in Food Microstructure*, American Association of Cereal Chemists, St Paul, Minn., 1983, pp. 111–147.
11. R. G. Fulcher, P. J. Wood, and S. H. Yiu, "Insight Into Food Carbohydrates Through Fluorescence Microscopy," *Food Technol.* **1**, 101–106 (1984).
12. Y. Vodovotz et al., "Bridging the Gap: Use of Confocal Microscopy in Food Research," *Food Technol.* **6**, 74–82 (1996).
13. J. J. Lemasters et al., "Laser Scanning Confocal Microscopy of Living Cells," in B. Herman and J. J. Lemasters, eds., *Optical Microscopy: Emerging Methods and Applications*, Academic Press, San Diego, Calif., 1993.
14. H. D. Cavanagh, W. M. Petroll, and J. V. Jester, "Confocal Microscopy: Uses in Measurement of Cellular Structure and Function," *Progress in Retinal and Eye Research* **14**, 527–565 (1995).
15. J. C. G. Blonk and H. van Aalst, "Confocal Scanning Light Microscopy in Food Research," *Food Res. Int* **26**, 297–311 (1993).
16. S. H. Kim et al., "Polymerization of Beef Actomyosin Induced by Transglutaminase," *J. Food Sci.* **58**, 473–474, 491 (1993).
17. G. J. Brakenhoff et al., "3-Dimensional Imaging of Biological Structures by High Resolution Confocal Scanning Laser Microscopy," *Scanning Microscopy* **2**, 33–40 (1988).
18. I. Heertje et al., "Confocal Scanning Laser Microscopy in Food Research: Some Observations," *Food Microstructure* **6**, 115–120 (1987).
19. Y. Vodovotz and C. Chinachoti, "Confocal Microscopy of Bread," in M. H. Tunick, S. A. Palumbo, and P. M. Fratamico, eds., *New Techniques in the Analysis of Foods*, Plenum Press, New York, 1998, pp. 9–17

20. J. J. Lemasters et al., "Laser Scanning Confocal Microscopy of Living Cells," in B. Herman and J. J. Lemasters, eds., *Optical Microscopy, Emerging Methods and Applications*, Academic Press, San Diego, Calif., 1993, pp. xx–xx

21. R. Sanders et al., "Quantitative pH Imaging in Cells Using Confocal Fluorescence Lifetime Imaging Microscopy," *Anal. Biochem.* **227**, 302–308 (1995).

22. A. N. Hassan et al., "Formation of Yogurt Microstructure and Three-Dimensional Visualization as Determined by Confocal Scanning Laser Microscopy," *Journal of Dairy Science* **78**, 2629–2636 (1995).

23. S. Nie, D. T. Chiu, and R. N. Zare, "Real-Time Detection of Single Molecules in Solution by Confocal Fluorescence Microscopy," *Anal. Chem.* **67**, 2849–2857 (1995).

24. S. J. Wright et al., "Introduction to Confocal Microscopy and Three-Dimensional Reconstruction," *Methods in Cell Biology* **38**, 1–45 (1993).

25. J. W. Wong Liong, J. F. Frank, and S. Bailey, "Visualization of Eggshell Membranes and Their Interaction With *Salmonella enteritidis* Using Confocal Scanning Laser Microscopy," *J. Food Prot.* **60**, 1022–1028 (1997).

26. M. T. Postek et al., *Scanning Electron Microscopy—A Student's Handbook*, Laboratory Research Industries, Burlington, Vt., 1980

27. M. Kalab, "Electron Microscopy of Foods," in E. B. Bagley and M. Peleg, eds., *Physical Properties of Foods*, AVI, Westport, Conn., 1983, pp. 43–104.

28. K. Autio and T. Laurikainen, "Relationships Between Flour/Dough Microstructure and Dough Handling and Baking Properties," *Trends Food Sci. Technol.* 8(6), 181–185 (1997).

29. A. M. Hermansson and W. Buchheim, "Characterization of Protein Gels by Scanning and Transmission Electron Microscopy," *Colloid Interface Sci* **81**, 519–530 (1981).

30. E. Varriano-Marston, "Intergrating Light and Electron Microscopy in Cereal Science," *Cereal Foods World* **26**, 558–561 (1981).

31. T. Otto, B. K. Baik, and Z. Czuchajowska, "Microstructure of Seeds, Flours and Starches of Legumes," *Cereal Chem.* **74**, 445–451 (1997).

32. P. M. Mathias et al., "Studies of Amylolytic Breakdown of Damaged Starch in Cereal and Non-cereal Flours Using High Performance Liquid Chromatography and Scanning Electron Microscopy," in *Starch: Structure and Functionality*, Royal Society of Chemistry, Cambridge, United Kingdom, 1997, pp. 129–140.

33. M. Obanni and J. N. BeMiller, "Ghost Microstructures of Starch From Different Botanical Sources," *Cereal Chem.* **73**, 333–337 (1996).

34. J. F. Chabot, L. F. Hood, and M. Liboff, "Effect of Scanning Electron Microscopy Preparation Methods on the Ultrastructure of White Bread," *Cereal Chem.* **56**, 462 (1979).

35. T. P. Freeman and D. R. Shelton, "Microstructure of Wheat Starch: From Kernel to Bread," *Food Technol.* **45**, 162–168 (1991).

36. J. A. Sargent, "The Application of Cold Stage Scanning Electron Microscopy to Food Research," *Food Microstructure* **7**, 123–135 (1988).

37. P. Allan-Wojtas, "A Simple Carrier for Freezing Difficult Food Samples in Preparation for Scanning Electron Microscopy," *Food Structure* **9**, 75–76 (1990).

38 B. E. Brooker, "Low-Temperature Microscopy and X-Ray Analysis of Food Systems," *Trends Food Sci. Technol.* **1**, 100–103 (1990).

39. C. M. McDonough et al., "Microstructure Changes in Wheat Flour Tortillas During Baking," *J. Food Sci.* **61**, 995–999 (1996).

40. L. G. Evans, A. M. Pearson, and G. R. Hooper, "Scanning Electron Microscopy of Flour-Water Doughs Treated With Oxidizing and Reducing Agents," *Scanning Electron Microscopy* **III**, 583–592 (1981).

41. S. I. Hsieh, E. A. Davis, and J. Gordon, "Cryofixation Freeze-Etch of Cake Batters, Cereals and Cereal Products, Electron Microscopy," *Cereal Foods World* **26**, 562–564 (1981).

42. S. H. Yiu, "Food Microscopy and the Nutritional Quality of Cereal Foods," *Food Structure* **12**, 123–133 (1993).

43. E. Doehne and D. C. Stulik, "Applications of the Environmental Scanning Microscope to Conservation Science," *Scanning Microscopy* **4**, 275–286 (1990)

44. K. Eskins et al., "Ultrastructural Studies on Microencapsulated Oil Droplets in Aqueous Gels and Dried Films of a New Starch-Oil Composite," *Carbohydr. Polym.* **29**, 233–239 (1996).

45. M. P. Oria, B. R. Hamaker, and J. M. Shull, "Resistance of Sorghum Alpha-, Beta-, and Gamma-Kafirins to Pepsin Digestion," *J. Agric. Food Chem.* **43**, 2148–2153 (1995).

46. J. T. Marle et al., "Chemical and Microscopic Characterization of Potato Cell Walls During Cooking," *J. Agric. Food Chem.* **45**, 50–58 (1997).

47. R. Y. Yada et al., "Visions in the Mist: The *Zeitgeist* of Food Protein Imaging by Electron Microscopy," *Trends Food Sci. Technol.* **6**, 265–270 (1995).

48. J. Tan, H. Zhang, and X. Cao, "SEM Image Processing for Food Structure Analysis," *J. Texture Stud.* **28**, 657–672 (1997).

49. V. J. Morris and T. J. McMaster, "Molecular Microscopy—Probing Biological Structure," *Trends Food Sci. Technol.* **2**, 80–84 (1991).

50. G. Binnig, C. F. Quate, and C. Gerber, "Atomic Force Microscope," *Phys. Rev. Lett.* **59**, 930–933 (1986).

51 P. K. Hansma et al., "Scanning Tunneling Microscopy and Atomic Force Microscopy: Application to Biology and Technology," *Science* **242**, 209–216 (1988).

52. G. Meyer and N. M. Amer, "Novel Optical Approach to Atomic Force Microscopy," *Appl. Phys. Lett.* **53**, 1045–1047 (1988).

53. A. R. Kirby, A. P. Gunning, and V. J. Morris, "Atomic Force Microscopy in Food Research: A New Technique Comes of Age," *Trends Food Sci. Technol.* **6**, 359–365 (1995).

54. *Scanning Probe Microscopy Training Notebook*, Digital Instruments, Santa Barbara, Calif., 1998.

55. M. N. Tsoubeli, E. A. Davis, and J. Gordon, "Exploring Whey Proteins at the Molecular Level With Atomic Force and Scanning Tunneling Microscopy," *Scanning 96 Abstracts* **18**, 254 (1996).

56. R. Kirby, A. P. Gunning, and V. J. Morris, "Imaging Xanthan Gum by Atomic Force Microscopy," *Carbohydr. Res.* **267**, 161–166 (1995).

57. A. R. Kirby, A. P. Gunning, and V. J. Morris, "Imaging Polysaccharides by Atomic Force Microscopy," *Biopolymers* **38**, 355–366 (1996).

58. A. N. Round et al., "Unexpected Branching in Pectin Observed by Atomic Force Microscopy," *Carbohydr. Res.* **303**, 251–253 (1997).

59. L. Wannerberger et al., "Interaction Between Alpha-Gliadin Layers," *Journal of Cereal Science* **26**, 1–13 (1997).

60. R. de S. Pereira, N. A. Parizotto, and V. Baranauskas, "Observation of Baker's Yeast Strains Used in Biotransformation by Atomic Force Microscopy," *Appl. Biochem. Biotechnol.* **59**, 135–143 (1996).

61. P. R. Shewry et al., "Scanning Probe Microscopes—Application in Cereal Science," *Cereal Chem.* **74**, 193–199 (1997).
62. M N. Tsoubeli and I. A. Taub, "ESR and AFM Studies of Bovine Myofibrils and Restructured Meat Systems," *IFT Book of Abstracts*, 58 (1998).
63. D G. Dalgleish and F. R. Hallett, "Dynamic Light Scattering: Applications to Food Systems," *Food Res. Int.* **28**, 181–193 (1995).
64. P. E. Plantz, "Laser Light Scattering," *Cereal Foods World* **28**, 252–253 (1983).
65. F. R. Hallett et al., "Particle Size Analysis: Number Distributions by Dynamic Light Scattering," *Canadian Journal of Spectroscopy* **34**, 63–70 (1989).
66. D. G. Dalgleish, "Conformations and Structures of Milk Proteins Adsorbed to Oil-Water Interfaces," *Food Res. Int.* **29**, 541–547 (1996).
67. D. S. Horne and C. M. Davidson, "The Use of Dynamic Light-Scattering in Monitoring Rennet Curd Formation," *Milchwissenschaft* **45**, 712–725 (1990).
68. D. G. Dalgleish and D. S. Horne, "Studies of Gelation of Acidified and Renneted Milks Using Diffusion Wave Spectroscopy," *Milchwissenschaft* **46**, 417–420 (1991).
69. M. Peleg, "Fractals and Foods," *Crit. Rev. Food Sci. Nutr.* **33**, 149–165 (1993).

Y. VODOVOTZ
NASA Johnson Space Center
Houston, Texas
M. TSOUBELI
Campbell Soup Co.
Camden, New Jersey

FOOD PLANT DESIGN AND CONSTRUCTION

Design and construction of a new food plant or a major expansion is a relatively rare event in the normal career of a food professional. When it does occur, it is important to have an understanding of critical issues and to have a well-planned approach. This article provides the nonexpert food professional with enough information to interact effectively with the engineers, architects, and constructors who are usually involved in any large project.

CRITICAL ISSUES

Nearly every food plant design must face the following issues:

- *Sanitary design.* Hazard analysis and critical control point (HACCP) plans must be accommodated to ensure human safety and product quality. Certain aspects of sanitary design are dictated by government regulation, whereas others are voluntary but highly recommended.
- *Efficient material and people flow.* Flow of material and of people dictates plant layout and thus overall design. It also strongly influences expansion opportunities, sanitation, and safety.
- *Environmental impact.* A food plant discharges wastes that can require treatment. A plant can be affected by its neighbors' discharges, and it must be a good neighbor aesthetically.
- *Minimum capital and operating cost.* Food plants must be efficient competitors. Resources must be invested wisely in light of life-cycle costs and designs that minimize such costs as maintenance and sanitation.

Other more specific issues and further details on these issues are addressed in the description of the overall approach in the balance of this article.

PROJECT APPROACH

The approach outlined in this section has proved successful in the design and construction of new or renovated production facilities. It is adapted from one used by a large equipment and engineering firm focused on the food industry. It includes three steps or phases: a preliminary study of feasibility; an engineering and design phase (which itself may have some divisions); and a construction phase. Table 1 describes the phases in outline form.

PHASE I—FEASIBILITY STUDY

The primary objective of the feasibility study is to establish a financial analysis, specifically the capital and operating costs that can be compared with the estimated benefits to test whether the proposed project meets investment criteria. The feasibility study also imposes a discipline on the various interested parties, such as production, engineering, management, and marketing. They must agree on specific assumptions and design criteria. Once established and proved satisfactory, these criteria are the basis for all subsequent designs.

Develop Process Parameters

Present and Historical Sales. The most important decisions about a new plant are what it should make and how much. These are dictated by corporate strategy, but sizing has such an influence on design and cost that it requires special analysis. The typical trade-off is line capacity against inventory levels, especially for seasonal products. Minimum inventory level consistent with efficient customer service must be established. Modern practice is to minimize inventory as much as possible, even to the extent of importing raw materials when necessary.

Projected Sales Data. The plant should satisfy the projected sales requirement for some future identified date. This forecast is based on growth rate projections for each product category. The design basis year is often designated as the fifth year after completion of construction. Expansions beyond the year used as the design basis are provided by physically expanding the building or adding production capacity.

Raw Ingredient Utilization and Procurement. Usage levels and purchase options of raw ingredients are evaluated to determine liquid and dry ingredient storage and receiving requirements. Issues include whether ingredients are received in bulk, semibulk containers, or discrete packages (bags, drums, and boxes). Minimum inventory levels depend on ordering quantities, time for delivery, safety stock, and stability of the materials. These factors influence equipment and space requirements.

Table 1. Three Phases of the Design and Construction of a Food Plant

Phase I –Feasibility study

- Develop process parameters
  - Present and historical sales
  - Projected sales data
  - Raw ingredient utilization and procurement
  - Operations and production
  - Packaging and filling
  - Automation
  - Packaging material utilization
  - Materials handling methods
  - Finished product storage
- Develop utility requirements
  - Water utilization
  - Fuel utilization
  - Electrical utilization
  - Sewage and wastewater
  - Solid wastes
- Develop site parameters
  - Area needed
  - Projected plant configuration
  - Owner's building style
- Cost estimate and schedule

Phase II—Engineering and architectural design

- Process specifications and engineering
  - Piping and instrumentation diagram
  - Process equipment arrangement
  - Process piping
  - Process equipment specifications
  - Process installation specifications
- Utility specifications and engineering
  - Mechanical flow diagrams
  - Mechanical equipment arrangements
  - Mechanical equipment specifications
  - Mechanical piping
  - Mechanical installation
  - Electrical single-line diagram
  - Electrical equipment arrangement
  - Electrical equipment details
  - Electrical equipment specifications
  - Electrical equipment installation
- Site and facility specifications and engineering
  - Site layout
  - Architectural design
  - Architectural details
  - Building and zoning permits
- Cost estimate and financial analysis

Phase III—Project and construction management

- Bidding
  - Contract type
  - Site
  - Building
  - Process equipment
  - Mechanical
  - Electrical
  - Process installation
- Site management
  - Scheduling of site activities
  - Receiving storing, and releasing material and equipment
  - Verification of work
  - Accounting and payments
  - Project documents
  - Start-up and commissioning
  - Close out

Operations and Production. Decisions must be made about production rates, operating days, and shift arrangements. Traditionally, food plants have operated five days per week, with two shifts of production and one for cleanup per day, but there is a growing trend toward operating plants for 24 hours per day and seven days per week as the ideal to minimize capital costs. There are significant challenges in achieving such a schedule in many food plants, including finding qualified workers willing to keep the required schedule. Allowance must also be made for swings in required production rates, and equipment must be chosen that can accommodate such swings. Many types of food processing equipment have only a 2:1 turndown ratio, meaning they can only run over a range of 50% to 100% of nominal capacity. Some types are more flexible but many are less.

An important document resulting from this step is the process flow diagram, which identifies schematically each unit operation with associated flow rates and important process conditions.

Packaging and Filling. The type and rate of commercially available filling equipment must be evaluated for present and projected capacities. There may need to be several packaging lines for some process lines. Packaging lines are typically highly automated because packaging often is labor intensive. Flexibility in packaging in the sense of package size changes, material changes, and case configurations must be considered.

Automation. The level of plant automation must be established during this phase. Plant floor control systems are typically integrated with management information systems even up to the corporate level so that production requirements can be transmitted to the factory and production costs and performance can be transmitted to accounting. This is a fast-changing area, but some assumption must be made for the purpose of cost estimating. A typical automation structure includes PLCs (programmable logic controllers) on major equipment, PCs (personal computers) as operator workstations controlling segments of a process, and somewhat larger computers at the plant level, all connected by local area networks (LANs). The plant and the corporate computers are typically connected by dedicated high-speed communication channels.

Packaging Material Utilization. The usage levels and purchasing methods for packaging must be evaluated to determine storage and receiving requirements. Unique storage requirements such as temperature or environment control must be defined. For example, some paper and polymer packaging material must be equilibrated with the relative humidity of the plant to perform well. Glass containers received during cold weather must be warmed to prevent condensation of moisture. The packaging materials storage capacity will reflect such factors as proximity to supplier, long-term purchasing agreements, and volume discounts.

Materials Handling Methods. Materials handling methods for raw materials, work-in-process, and finished goods must be established. Choices include pneumatics for bulk powders, pipe for liquids, conveyors, totes, automated vehicles, person-driven vehicles, overhead rail, and manually powdered carts. Common issues include delivery of packaging material to point of use, where to put unitizing (palletizing), and how to handle trash such as empty ingredient bags and drums. It is desirable to minimize trash generation in food processing areas. Glass and wood should not be used (except for glass jars and bottles used as packages).

Finished Product Storage. An important issue is whether the plant site is also a distribution center for a wider line of products or simply ships its output to another site. This has a significant impact on storage requirements, traffic patterns, information systems, and overall design. If the plant is not to also be a distribution center, then the requirement for on-site finished goods storage may be minimal, and costs can be saved. If the plant is also to be a distribution center, then issues of receiving from other sites, order receiving and picking, and order shipping must be faced.

Develop Utility Requirements

Critical utilities include water, fuel, electricity, wastewater treatment, and solid waste disposal. If the process involves refrigeration, uses refrigerated ingredients, or produces refrigerated products, then it will probably require a central ammonia system. Utility requirements are estimated from the process flow diagram and material and energy balances. Local providers of water, fuel, waste disposal, and power are sources of information on rates, characteristics, and limitations. It is important that capacity be sufficient for current and future requirements and that costs be stable and predictable. Disposal of liquid and solid wastes is normally best handled by municipal facilities, but in some locations, a food plant must become self-sufficient in these matters. When permitted, land disposal of liquid wastes can be satisfactory, but it requires large amounts of land. Often, local farmers will accept food solid waste as animal feed and remove it at their own cost. Disposing of packaging waste can be a challenge in some areas.

Develop Site Parameters

To develop an accurate cost estimate, it is important to have a specific site in mind, but before one can be identified, it is necessary to understand the requirements. It is not unusual for land area to be 4 to 5 times the building area and sometimes much more, such as when land disposal of liquid wastes is included. Ideal sites are generally level and well-drained, have utilities supplied or within relatively short distances, are close to desired transportation, have no undesirable neighbors, are removed from residential areas, have safe and efficient traffic patterns, and permit designs with room for expansion in the future.

Site location at a broader scale is dictated largely by marketing considerations, but it is also influenced by other factors such as labor availability and cost, utility availability and cost, taxes, regulatory environment, raw materials, transportation access (including air, for visits from headquarters), and quality of life (to help in recruiting management).

Sadly, the ideal piece of land probably does not exist, at least not in the ideal location. This means various compromises must be made, which usually have cost implications. Generally, in the feasibility phase, a time zone or region of the country is known, perhaps even a specific state or community. Rarely is a specific plot of land identified at this stage, because the feasibility is still under study. Although it is tempting to assume an ideal plot of land (large enough, solid-bearing soil, easy access to utilities, great location), it is prudent to test the sensitivity of costs to various adverse assumptions, such as the need for pilings because of weak soil, the need to pay for utility access, or the need to pave an access road. These alternatives can be identified with assumed costs as subcases of the ideal. They rarely are the sole cause of unsatisfactory cost projections, but they can contribute.

In economic evaluation, if an ideal case is not feasible, then no worse case will be. However, if an ideal case is marginal, then one or two adverse circumstances can make a difference, and the project may be shaky by corporate standards. It is important to discover this early before large sums are spent on design and construction.

Projected Plant Configuration. A preliminary plan illustrating the production facility on the assumed lot is developed. This helps identify site-related costs and assists in presenting the concept to management, municipalities, and designers.

Owner's Building Style. The final design of a process facility must suit the owner's needs, which may include using the facility in marketing. Visits by the public are relatively rare, but it is common to welcome customers to food plants, and some provision for safe touring might be appropriate. Good design can make a utilitarian facility an attractive place to work and a good neighbor in its community at very little cost.

Cost Estimate

An estimate of the capital cost of process equipment and services can be determined from the preliminary design. Operating costs depend on the quantities and costs of utilities. The facility cost is estimated from the preliminary layout and the site plan, often by applying an experience-based unit cost to the estimated area. For many food plants in the late 1990s, facility costs were about $80 to $100 per square foot. Facility costs for food plants are typically 35 to 50% of the total cost, depending on the complexity of the process involved.

The feasibility study summarizes all the assumptions, projected costs, and operating procedures as well as the parameters on which the final design will be based. The study report is a reference document for the balance of the project and also can support such requirements as environmental impact statements.

The financial analysis includes a projection of capital requirements during design, construction, and the first five years of operation to meet projected production requirements. Pro forma operating statements, staffing requirements, inventory costs, and other scheduled operating costs are the basis of operating statements as well as profit and loss calculations. Modern financial analysis computes the net present value (NPV) of future cash flows using a predetermined hurdle or discount rate, often close to 20%. If the estimated NPV is positive over the expected life of the plant, the project is feasible and should be funded. Cash flow is the sum of profits after taxes plus depreciation. Capital investments are treated as negative cash flows when they occur. Clark discusses cost estimating and economic analysis in greater detail (1).

A schedule for the project is prepared that shows the major activities and their expected duration. It is important to recognize the dependence of certain activities on others—construction cannot begin until the relevant design is complete; steel cannot be erected until foundations are laid; it takes time for structural steel to be fabricated; earthwork is difficult when the ground is frozen; obtaining permits, as mentioned later, may require a long time; and equipment cannot be installed until the building is largely complete.

PHASE II—ENGINEERING AND ARCHITECTURAL DESIGN

The new facility should be designed around the process. It should accommodate the projected growth in production and future expansions, keeping in mind that new products will be developed and existing products discontinued. Layout should consider how changes could be made with minimal disruption to existing operations.

Structural steel, foundations, and other elements should be designed to support equipment and piping and to facilitate expansion. Floor drains are important in wet areas, and floors should be sloped to drain. Layout of equipment to permit access for maintenance and easy changes is critical.

Room, floor, and ceiling finishes are dictated by process conditions and should be chosen with an understanding of life-cycle costs. Ceilings should be avoided in process areas if possible because they collect dust and moisture and are hard to clean. Heating, ventilating,and air conditioning (HVAC) requirements are also set by the process and can be stringent in food plants, which may be dusty, humid, and require extremes of heating and cooling. Meat and poultry plants, for instance, are routinely cooled to 50°F to reduce microbial growth, and some cereal processing plants are heated to 140°F on occasion to eliminate insect infestations. Employee comfort is achieved by adequate ventilation and appropriate cooling or heating.

Process Specifications and Engineering

Piping and Instrumentation Diagram. The piping and instrumentation diagram is based on the process flow diagram but adds much more detail. All equipment, piping, and instruments are shown with indications of size, operating rates, and expected conditions of temperature, flow rate, and pressure. Each device is identified with a number that is used to track it through procurement, receiving, installation, and operation. It is good practice to determine if the corporation has an existing asset accounting system and to use numbers from the beginning of the project that are compatible with the system. Thus, the new assets are easily incorporated into the corporation's records with little effort. The same identification system can be extended to a preventive maintenance system. If cleaning-in-place is involved in the process, and it often is, then there should be a separate cleaning-in-place piping and instrumentation diagram to avoid confusion. The cleaning-in-place system in some aspects nearly duplicates the process and in others simply connects with the process equipment. Trying to show both on one drawing is difficult.

Process Equipment Arrangement. The major process equipment is located on scale drawings. These often rely on vendor shop drawings that typically are prepared late in a project, yet the arrangement drawings are needed early to establish building size and layout. One solution to this dilemma is to make generous space allowances, because equipment always seems to grow; it rarely shrinks. Also, supports, platforms, chutes, conveyors, motors, and control cabinets are often overlooked early in design and added later, so additional space is always needed.

Process Piping. Typical piping details, isometrics, pipe support methods, and piping routes are identified. As with the equipment, piping seems to grow during a project as needs are refined and detail is developed. Piping can encounter conflicts with structural steel, lighting, fire protection, electrical conduit, and HVAC ducting because typically each of these engineering disciplines develops independently within a project. It falls to project management and sometimes to the owner to identify and resolve early such interferences and conflicts. One solution is to identify chases or spaces reserved for certain purposes, such as electrical, process piping, or utility piping. Even with this precaution, there will be conflicts that must be resolved. It is much less expensive to do so in the design stage than in the field.

Process Equipment Specifications. Each piece of equipment is defined by a specification that describes its performance, special features, regulatory issues if any, and desired spare parts and support, such as assistance during start-up. The specifications are used primarily for purchasing but also become an important part of the documentation of the project.

Process Installation Specifications. The primary purpose of this document is to define responsibility for procurement, receipt, and installation of the equipment. A typical issue is defining responsibility for supplying electrical power to a unit. Often, the electrical power is installed up to a distribution panel by one contractor but then must be delivered to each piece of equipment, which can only be done after the equipment is set in place. Various parties may become involved, but it is best to establish uniform standards, routes, details, and practices.

Utility Specifications and Engineering

Mechanical Flow Diagrams. The mechanical flow diagrams will indicate all mechanical equipment type, size, and operating rates. Instrumentation for mechanical control and alarm are identified. Each device is assigned a unique item number. The relationship of the mechanical equipment with the process must be shown and defined. Typical mechanical systems included in the mechanical flow diagram include steam, hot water, cold water, compressed air, fuel gas, inert gas, vacuum, chilled water, ammonia, glycol coolant, and conveyor chain lubricant. There can be several pressures of steam and several levels of ammonia refrigerant.

Mechanical Equipment Arrangements. The mechanical equipment arrangement drawings locate all mechanical equipment.

Mechanical Equipment Specifications. Each piece of mechanical equipment is defined by an equipment specification, which includes its performance, rates, features, and regulatory requirements.

Mechanical Piping. Typical mechanical piping details, pipe support methods, insulation, and mechanical piping routes are identified. The distinction between process piping and mechanical piping is that process piping often is stainless steel, sanitary tubing, whereas the mechanical piping often is carbon steel, fiberglass, copper, or other materials. Routing, hangers, and other details should be compatible for both; that is, the same sanitary design practices should be followed in food processing areas. Some utility piping may exist outside the food processing areas, and there less expensive practices may be followed. However, an area that initially is not a processing area, such as a storage area, may later be converted into a processing area, so it is wise to anticipate such possibilities when specifying utility hangers, insulation, and routing.

Mechanical Installation. The installation responsibility for each piece of mechanical equipment and piping system is defined, including responsibility for procurement, receipt, and installation.

Electrical Single-Line Diagram. Electrical distribution for all process, mechanical and facility equipment drives, and lighting is indicated on the single-line diagram.

Electrical Equipment Arrangement. The electrical equipment, switchgear, distribution, motor control centers, low-voltage panels, and lighting panels are located. Normally, these do not occupy prime space, but they should be accessible, and space for future expansion should be provided. The relationship of the switchgear to incoming power and transformers is defined. Reliability of local power must be assessed to determine if two independent sources are needed, and provisions for future requirements should be made.

Electrical Equipment Details. Typical electrical details and conduit arrangements are identified. In food plants, electrical conduit should be mounted away from walls so that the space between can be cleaned. Perforated channel (Unistrut) and all-thread rods should not be used as hangers in food processing areas and probably should not be used at all because they are hard to clean.

Electrical Equipment Specifications. Each major electrical device—transformer, switchgear, motor control center, mechanical control panel, process control panels and lighting panels—are defined for purchase and installation. It is common for corporations to establish preferred suppliers for such equipment in an attempt to standardize and simplify maintenance.

Electrical Equipment Installation. The installation responsibility for each piece of electrical equipment is defined, including responsibility for procurement, receipt, and installation.

Site and Facility Specifications and Engineering

Site Layout. A survey, topographical study, and appropriate soil borings are obtained for the specific site. These help guide placement of the building on the site and influence foundation design. The site plan or layout shows building location, access routes for trucks, visitors and employees, and provisions for expansion (typically dotted in). Location of underground utilities is also shown. Identification of bordering neighbors is common.

Architectural Design. Each floor or level of the building is shown in plan view, and elevations (side views) are prepared. Because it is common to put support equipment on the roof, a roof plan is also needed. Overall people, material, and traffic flow patterns are shown. A perspective rendering, which is a drawing showing what the building will look like when finished, is sometimes prepared for presentations, especially to management and local governments.

Architectural Details. Architectural details include materials of construction, wall finishes, floor finishes, doors, security systems, lighting (exterior and interior), and various amenities such as cafeteria, if any, lounges, offices, rest rooms, and health centers. Modern plants commonly contract for food service, but may only provide vending machines and a place to eat. Rest rooms and lockers must be convenient to work areas but separated, usually by corridors. Exterior lighting should be directed at the plant, not mounted on the walls, and should not use mercury vapor lamps because they attract insects. Mercury vapor lamps can be used on the perimeter of the property to attract insects away from the plant.

Floors, walls, and ceilings (if any) in food processing areas should be impervious, easy to clean, and resistant to moisture and strong cleaning solutions often used in food plants. There are many possibilities, but epoxy-based coatings properly selected for the service are economic choices. Epoxy-based floor coatings can be harmed by thermal shock, such as exposure to live steam, unless they are filled with the correct amount of inert material. Such fillers also provide some texture to the coating, which may improve traction on the floor. Fiberglass-reinforced epoxy panels are often used for walls and ceilings. Insulated sandwich panels are often used for refrigerated spaces and can be a sanitary and economic choice for walls and ceilings. Each architectural detail in a food plant must be selected with an understanding of the environment and hazards to which it is exposed. The environment is often wet or dusty; there are strong chemicals (acids and caustics) used for cleaning; there can be oils and abrasive materials; and the area must be protected against insect, rodent, and bird infestation. Common commercial and industrial materials and details are not adequate for a food plant.

Building and Zoning Permits. The site layout, architectural details, and layouts are used to obtain construction and building permits. These are issued by local government agencies after review of the plans for compliance with site zoning and building requirements. Normally, the procedure for obtaining permits begins early in the design phase because it can influence design. Setback requirements, for instance, affect building location on a site. Final permits require final design plans, usually signed and sealed by a registered architect and engineers licensed to practice in the state. Exceptions to some requirements can be obtained, but it is usually prudent to comply with most requirements. Obtaining permits can be time-consuming, especially if public hearings are required for zoning changes, but areas seeking economic development and investment will usually expedite the process if asked.

Cost Estimate and Financial Analysis

The cost estimate and financial analysis prepared in the feasibility phase is revised in light of the completed design and any subsequent information, such as revised sales forecasts, costs, or other developments. The cost estimate prepared at this point becomes the control budget for construction and should always be preserved as the basis for any future changes. A detailed schedule is also prepared, adding detail to those prepared earlier.

PHASE III—PROJECT AND CONSTRUCTION MANAGEMENT

The critical issues during this phase are control of the budget, schedule, and quality of construction. The budget is based on the estimate made at the end of design. As bids are received, the budget is adjusted to reflect reality. If good estimating practice is followed by experienced engineers and architects working from a reasonably complete design, the total cost should not change during bidding and construction *if the design does not change*. The biggest risk to the budgeted cost is design changes during construction. These arise for a number of reasons: new information, new people with new opinions, unforeseen circumstances and events, and changes in strategy. Good project management strives to control design changes, for whatever reason. The contingency allowance always included in any cost estimate is not intended to cover design changes; it exists to account for errors in the estimate corresponding to the original scope. Knowing that there is almost always some uncertainty about scope, some owners provide a separate allowance, called the owner's contingency, to cover such incidents. The schedule is a living document that is revised as information is received, as estimated duration of activities are converted into actual duration, and as activities are added. It is common for management to insist on a fixed completion date because of market obligations and other constraints, even in the face of delays in approval, design, and construction. Reconciling the inevitable conflicts is a significant challenge. Techniques include overlapping construction activities, starting construction before design is complete (fast-track approach), paying premium wages to work extra shifts and weekends, and, as a last resort, compromising design intent, by using more easily available materials, for example.

Maintaining quality during construction requires constant vigilance by the owner and the construction manager. There is always a temptation to cut corners on materials or procedures to save money, but once details have been specified, they must be followed unless prior approval is received for a substitute. Tests are common for concrete, coatings, electrical wire, pipe, and other materials. Experience and care are required in sanitary welding and application of coatings. Before allowing a contractor to proceed, the manager should make sure that the tradesmen are skilled, and the manager should routinely inspect the work as it proceeds. Construction can rarely improve on a design, but it can easily compromise the intent if permitted to do so.

Bidding

There are several approaches to organizing and managing construction. The entire project can be managed by a general contractor, who may also have performed the design (design–build approach), or the design may have been performed by a different firm, typically an architecture and engineering company. The owner can engage a construction manager, who may or may not have performed the design, but who advises the owner on the selection of subcontractors but does not perform any of the work himself. In the construction management approach, the owner directly engages each subcontractor, and the Construction Manager maintains the budget and schedule and monitors construction quality. Finally, the owner can serve as his or her own construction manager. Because of the relatively unique skills needed for construction, as compared with those of the typical food professional, it is a rare corporation that can perform this task on a large project successfully.

Depending on the project approach taken, there may be just one bid (or negotiated price) or many. Typically, it is easier to get competitive bids on smaller and more specialized packages than on a smaller number of larger packages. On the other hand, the more parties involved the more complex the management task. A common solution is to have bid packages for the following major components: site, building shell, mechanical, electrical, process equipment installation (millwright), and process and packaging equipment (multiple packages). The mechanical and electrical contractors often install the utility and service equipment and connect the process equipment. Some mechanical contractors install process equipment, but because much of it is highly specialized, often the vendors assume this responsibility.

Some types of process and packaging equipment have long lead times and so must be ordered early in the project to ensure delivery at the proper time. Ideally, equipment is delivered when its correct space is available so that handling is minimized.

Lead times for structural steel, process and packaging equipment, and large mechanical and electrical equipment have significant impact on the schedule. Once ordered, their progress through manufacturing must be monitored to ensure adherence to the plan, which may require a full-time person or more.

Site Management

Typically, the owner, the contractor or construction manager, and the major subcontractors have staff members on site, usually in trailers. A good practice followed by many owners is to hire or assign the future plant manager and key staff members early in a project and make them responsible during all phases of the construction project. Alternatively, project management specialists may be assigned. In any event, the following activities occur on site.

Scheduling of Site Activities. Coordinating the various trades and subcontractors is a critical, ongoing effort. It is common to have a daily meeting of all parties to report attendance, revise schedules, resolve interferences and conflicts, and predict needed material and people.

Receiving, Storing, and Releasing Material and Equipment. As equipment and material are received, they are inspected, verified against specifications and orders, and stored securely, protected against weather and unauthorized access. As needed, equipment and material are released for use. Contractors are generally responsible for their own materials, but the owner or the construction manager may handle process equipment and piping.

Verification of Work. Contractors routinely file liens against owners to ensure payment for their work. As work is completed, the owner or construction manager secures waivers of liens and approves payment, upon inspection and satisfaction. It is normal to retain a portion of payment against correction of faults, usually documented in a punch list. The punch list is maintained as a living document, and responsibility for and disposition of each item are noted.

Accounting and Payments. Accurate, fair, and timely payment for completed and accepted equipment, material, and services is critical. This requires documentation in the form of purchase orders, contracts, receipts, invoices, inspection reports, and the punch list. Typically, contractors expect to be paid as they incur expenses subject to a retained portion, often 10%, which is paid at completion and satisfaction of any defects. To document progress, a contractor may submit time sheets supporting the labor costs and receipts for materials. If the contract is a lump sum, these are compared to an estimate of the total; if the contractor is being paid for time and materials, these are the costs plus an agreed profit and overhead, if any. As costs are actually incurred for the project, the control budget is adjusted, and projections are made for amounts to complete. The owner must be kept informed of the budget status almost continuously.

Project Documents. In addition to the cost and disbursement documents, schedules, and inspection reports, there are important permanent records that accumulate during construction. These include operations and maintenance manuals for all equipment, equipment and facility drawings as designed and as built, and all the permits and certificates issued. These must be collected, cataloged, and preserved.

Start-up and Commissioning. Once process, packaging, and mechanical equipment is installed and utilities are provided, each unit can usually be tested in isolation to be sure motors rotate correctly, instruments read correctly, and controls work as expected. This testing is usually the responsibility of the party installing the device. After all components of a system, such as one process line, are in place, then it can be tested as a whole. This may require coordination of speeds, alignment of conveyors, and integration of controls. Vendors of process and packaging equipment routinely provide assistance in this phase, during which operators are trained in safe and effective operation and maintenance of the system. Commissioning occurs with the initial operation of a system using realistic raw materials and attempting to produce acceptable final product. It is wise not to expect design efficiency at this point, and in fact, any salable product should be seen as a dividend. Each process unit operation must be assessed over its expected range of operating rates and conditions and compared with process design and equipment specifications. Deficiencies, if any, must be corrected or otherwise accommodated, perhaps by lowering expectations, if necessary. The limiting components of the system must be identified. These may well be different than was intended or expected in design. Ideally, at the end of commissioning, which may take a month of effort, the new process line is operating at expected design rates and efficiencies, the operators are trained and self-sufficient, the vendor technicians have departed, and the plant or line is ready to accept orders routinely.

Close Out. It is wise to have a formal project completion conference among the owner, the construction manager, the designer, and major vendors to transfer any remaining documents, resolve any outstanding invoices, and transfer accumulated knowledge and experience. Sometimes this can correspond with dedication of a new facility and involve some modest ceremony and souvenirs; on other occasions, it can be a simple business meeting, but it is important to somehow mark the official end of a project.

BIBLIOGRAPHY

This article was adapted from A. Cukurs, *Food Plant Design and Construction*, APV Crepaco, Lake Mills, Wisc., 1989.

1. J. P. Clark, "Cost and Profitability Estimating," in K. J. Valentas, E. Rotstein, and P. Singh, ed., *Handbook of Food Engineering Practice*, CRC Press, Boca Ranton, Fla., 1997, pp. 537–557.

GENERAL REFERENCES

T. J. Imholte, *Engineering for Food Safety and Sanitation*, 2nd ed., Technical Institute of Food Safety, Crystal, Minn., 1998.

J. PETER CLARK
Oak Park, Illinois

FOOD PRESERVATION

Matching the supply of food as produced by the agricultural sector with the demand for food by consumers in both time and space necessitates the use of a variety of preservation techniques. Food is being produced in larger quantities by fewer people in rural areas often very distant from the urban consumer. The production of agricultural commodities follows cyclical patterns of supply, dictated by such things as cropping times and yearly fluctuations. The requirements on the food supply by the consumer include safety, quality, adequate shelf life, variety, and convenience. Thus, the demand for food tends to be more constant than its production, food needs to be transported from the production sector to the consuming markets, food needs to be transformed from its raw state as produced by the agricultural sector into a vast array of consumer goods, and throughout this process quality must be maintained, safety must be guaranteed, and the economics must be favorable, minimizing losses and waste. This matching of supply and demand in both space and time both defines and introduces the field of food preservation.

Food in its many forms is subject to very rapid deterioration beginning soon after harvest. The factors contributing to this process include biological deterioration and postharvest loss from bacteria, yeasts, molds, insects, and rodents, and chemical breakdown of food components catalyzed by enzymes, light, or oxygen. Preservation techniques attempt to control these deterioration processes through the destruction of microorganisms present in the food, through the manipulation of factors essential for the continued growth of microorganisms, or through the control of factors responsible for chemical deteriorations. It is difficult to define the time frame necessary to consider a food preserved. Milk can be preserved through pasteurization and refrigeration to attain a shelf life of 10 to 14 days. Milk can also be preserved through spray drying to attain a shelf life of a year or longer. Thus a food might be considered preserved when it is effectively moved from production to consumption, maintaining safety and quality, minimizing losses, and being delivered in a form which is convenient and acceptable to the consumer.

Although new preservation processes have been developed and traditional processes have been modified, food preservation has been practiced for millennia. Since the beginning of man the need has existed to gather food. Processes such as sun-drying, salting, smoking, or food fermentations are considered to be early forms of food preservation. The technology of food preservation increased dramatically with the discovery of microorganisms late in the eighteenth century and the appreciation of their role in food deterioration that followed. The reader is referred to Ref. 1 for a complete history of food preservation.

CAUSES AND MANIFESTATIONS OF FOOD DETERIORATION

Microorganisms

Microorganisms are ubiquitous living organisms that need nutrients, moisture, appropriate oxygen conditions, and favorable pH ranges to grow. Microorganisms can contaminate our food supply from the point of production or harvest until the time of consumption. Many microbial food contaminants are native to the soil or animal environment from which the food is derived, and many are added unintentionally through handling practices. The components of most foodstuffs (carbohydrates, proteins, etc) serve as ideal nutrients for the growth of microorganisms and this growth leads to potentially harmful populations of microorganisms, potentially harmful buildup of microbial metabolites, for example, toxins, enzymes, polysaccharides, or pigments, and a deterioration in food quality resulting from the metabolism of food constituents, for example, amino acid or fatty acid release from proteins or lipids. Microorganisms important to food deterioration include bacteria, yeasts, and molds. Some bacteria can produce spores, a dormant form that is capable of withstanding long periods under conditions in which the vegetative form could not grow. These spores can then germinate when conditions once again become favorable. Spores are much more heat resistant than their vegetative form, and hence during processing, if elimination of all bacteria is a goal, then the heat resistance of the spore must be targeted.

It is important to distinguish between pathogenic and nonpathogenic species. Pathogens are organisms that cause disease, and a number of human pathogens can be transmitted through food. They include *Salmonella* spp., *Staphylococcus aureus, Clostridium botulinum, Listeria monocytogenes*, and many others. Mycotoxins are fungal metabolites, some of which are potentially toxic or carcinogenic to humans, for example, aflatoxin from *Aspergillus flavus*. It is essential that all pathogenic species be controlled in preserved foods to eliminate foodborne disease. This is done by providing measures to eliminate what may be present in raw materials (eg, those of animal origin) through processing, and by providing measures to eliminate the entry of contaminating pathogens to the food supply through hygienic manufacturing practices. Most microorganisms, however, are not pathogenic but can cause food spoilage if allowed to grow, primarily as a result of off-flavors and odors from metabolic by-products. These, too, need to be controlled as they account for diminished shelf

BIBLIOGRAPHY

This article was adapted from A. Cukurs, *Food Plant Design and Construction*, APV Crepaco, Lake Mills, Wisc., 1989.

1. J. P. Clark, "Cost and Profitability Estimating," in K. J. Valentas, E. Rotstein, and P. Singh, ed., *Handbook of Food Engineering Practice*, CRC Press, Boca Ranton, Fla., 1997, pp. 537–557.

GENERAL REFERENCES

T. J. Imholte, *Engineering for Food Safety and Sanitation*, 2nd ed., Technical Institute of Food Safety, Crystal, Minn., 1998.

J. PETER CLARK
Oak Park, Illinois

FOOD PRESERVATION

Matching the supply of food as produced by the agricultural sector with the demand for food by consumers in both time and space necessitates the use of a variety of preservation techniques. Food is being produced in larger quantities by fewer people in rural areas often very distant from the urban consumer. The production of agricultural commodities follows cyclical patterns of supply, dictated by such things as cropping times and yearly fluctuations. The requirements on the food supply by the consumer include safety, quality, adequate shelf life, variety, and convenience. Thus, the demand for food tends to be more constant than its production, food needs to be transported from the production sector to the consuming markets, food needs to be transformed from its raw state as produced by the agricultural sector into a vast array of consumer goods, and throughout this process quality must be maintained, safety must be guaranteed, and the economics must be favorable, minimizing losses and waste. This matching of supply and demand in both space and time both defines and introduces the field of food preservation.

Food in its many forms is subject to very rapid deterioration beginning soon after harvest. The factors contributing to this process include biological deterioration and postharvest loss from bacteria, yeasts, molds, insects, and rodents, and chemical breakdown of food components catalyzed by enzymes, light, or oxygen. Preservation techniques attempt to control these deterioration processes through the destruction of microorganisms present in the food, through the manipulation of factors essential for the continued growth of microorganisms, or through the control of factors responsible for chemical deteriorations. It is difficult to define the time frame necessary to consider a food preserved. Milk can be preserved through pasteurization and refrigeration to attain a shelf life of 10 to 14 days. Milk can also be preserved through spray drying to attain a shelf life of a year or longer. Thus a food might be considered preserved when it is effectively moved from production to consumption, maintaining safety and quality, minimizing losses, and being delivered in a form which is convenient and acceptable to the consumer.

Although new preservation processes have been developed and traditional processes have been modified, food preservation has been practiced for millennia. Since the beginning of man the need has existed to gather food. Processes such as sun-drying, salting, smoking, or food fermentations are considered to be early forms of food preservation. The technology of food preservation increased dramatically with the discovery of microorganisms late in the eighteenth century and the appreciation of their role in food deterioration that followed. The reader is referred to Ref. 1 for a complete history of food preservation.

CAUSES AND MANIFESTATIONS OF FOOD DETERIORATION

Microorganisms

Microorganisms are ubiquitous living organisms that need nutrients, moisture, appropriate oxygen conditions, and favorable pH ranges to grow. Microorganisms can contaminate our food supply from the point of production or harvest until the time of consumption. Many microbial food contaminants are native to the soil or animal environment from which the food is derived, and many are added unintentionally through handling practices. The components of most foodstuffs (carbohydrates, proteins, etc) serve as ideal nutrients for the growth of microorganisms and this growth leads to potentially harmful populations of microorganisms, potentially harmful buildup of microbial metabolites, for example, toxins, enzymes, polysaccharides, or pigments, and a deterioration in food quality resulting from the metabolism of food constituents, for example, amino acid or fatty acid release from proteins or lipids. Microorganisms important to food deterioration include bacteria, yeasts, and molds. Some bacteria can produce spores, a dormant form that is capable of withstanding long periods under conditions in which the vegetative form could not grow. These spores can then germinate when conditions once again become favorable. Spores are much more heat resistant than their vegetative form, and hence during processing, if elimination of all bacteria is a goal, then the heat resistance of the spore must be targeted.

It is important to distinguish between pathogenic and nonpathogenic species. Pathogens are organisms that cause disease, and a number of human pathogens can be transmitted through food. They include *Salmonella* spp., *Staphylococcus aureus, Clostridium botulinum, Listeria monocytogenes*, and many others. Mycotoxins are fungal metabolites, some of which are potentially toxic or carcinogenic to humans, for example, aflatoxin from *Aspergillus flavus*. It is essential that all pathogenic species be controlled in preserved foods to eliminate foodborne disease. This is done by providing measures to eliminate what may be present in raw materials (eg, those of animal origin) through processing, and by providing measures to eliminate the entry of contaminating pathogens to the food supply through hygienic manufacturing practices. Most microorganisms, however, are not pathogenic but can cause food spoilage if allowed to grow, primarily as a result of off-flavors and odors from metabolic by-products. These, too, need to be controlled as they account for diminished shelf life and economic loss. In this latter case, however, it is important to recognize that spoilage organisms are not eliminated during processing, but rather kept below a critical number to provide adequate shelf life to the product. An example would be pasteurized milk, in which pasteurization eliminates all pathogens, but a population of spoilage organisms remains and limits the shelf life to 14 to 16 days in most cases. For further reading on food microbiology, the reader is referred to Refs. 2 to 5.

Enzymes

Enzymes are naturally present in most raw food commodities and a number of enzymes catalyze reactions that are detrimental to product quality. These include off-flavor production by lipoxygenases, lipases, and proteases; textural changes due to pectic enzymes and cellulases; color changes due to polyphenol oxidase, chlorophyllase, and peroxidase; and nutritional changes due to ascorbic acid oxidase or thiaminase. A mild heating process such as blanching or pasteurization is usually sufficient to destroy any harmful enzymes that may be present in a food.

Pests

Insect and rodent infestations and losses can occur readily in open fields during growth and production and in bulk stores of food exposed or partially exposed to the environment, and this is especially prevalent in developing countries where storage conditions are less than adequate. Insects can attack grains, reducing their nutritive value and imparting a sour taste to flour, peas, beans, meat, fish, cheese, and so on. Insects damage far more food than they consume due to the deposition of larvae in webbings, rendering much of the food store as waste. Rodents are also a serious concern in food storage, also damaging far more than they consume through adulteration with droppings, filth, and hair and potential transmission of disease.

Temperature

Uncontrolled heat and cold can cause detrimental reactions to occur in foods. Increased but moderate temperatures, 30 to 40°C, accelerate the rates of reactions such as oxidation, which lead to the production of off-flavors and odors, color changes, and nutrient loss and significantly decrease the generation times of microorganisms, leading to enhanced populations. Decreased temperatures, in the 0 to 15°C range, can lead to detrimental physiological reactions in certain susceptible products. These include chill injury in tomatoes or bananas, which results in color and texture changes and increase in microbial susceptibility; cold shortening in meats resulting in tough carcasses after slaughter; chill sweetening in potatoes resulting in increased glucose contents and enhanced browning during frying; and staling of bread, which occurs much more readily at these temperatures.

Moisture

The water present in a food plays a critical role in supporting microbial growth and chemical reactions. The moisture is an integral part of the structure of a food, both at the molecular level (associated with macromolecules as bound or plasticizing water) and at the structural level (eg, intracellular or extracellular). Water activity is defined as the equilibrium relative humidity of the food, or a measure of free water in the food, that water which is able to participate in reactions. It has been shown that detrimental reactions in foods are often related more to the water activity of the food than its moisture content, and water activity has been used widely over the last several decades as a measure of food stability. It is increasingly being recognized, however, that the physical state of the solids in the food and their interaction with water is also a determining factor in reaction rates. Both rates of microbial growth and chemical reactions are also influenced by water and solute diffusion. In recent years, the glass transition of both small molecules (eg, sugars) and macromolecules (eg, starches) has been recognized as a key event in the occurrence of solute diffusion-controlled deteriorative reactions, and hence another measure of food stability related specifically to water relations in the food. A good review of water activity and glass transitions related to food preservation can be found in Ref. 6. Thus the gain or loss of moisture from a food due to its conditions of storage, particularly relative humidity, may influence the water activity and solute diffusion rates and thus the microbial and chemical stability of the product. Additionally, changes in the relative humidity of storage can also affect structure and texture of the food. High relative humidities of storage can favor splitting or cracking of the skins of some fruits, while low relative humidities can favor wilting and shriveling of fruits and vegetables, leading to a loss of weight and thus economic value.

Oxygen

The oxygen requirements for both the storage of foods and for microorganisms vary considerably. Bacteria have very strict oxygen requirements and are classified accordingly as aerobic, anaerobic, or facultative organisms with wider tolerances. Oxygen is also an initiator of chemical reactions that are detrimental to various food constituents, including lipids, vitamins, pigments, and some amino acids. However, reduced oxygen contents can impair the physiological function of food tissues, such as fruits, vegetables, or meat. Hence control of oxygen contents through modified atmospheres and protective packaging can help to reduce food deterioration.

Light

Exposure to light can be detrimental to some food products. Light can promote oxidative rancidity of lipids; oxidation of milk leading to breakdown of proteins and formation of unpleasant volatiles; changes in various pigments (eg, myoglobin in red meats); breakdown of vitamins such as vitamin A, riboflavin, and ascorbic acid leading to nutrient loss; or the development of potentially toxic light-induced glycoalkyloids such as solanine in potatoes that have turned green due to increased chlorophyll content (from light exposure).

Time

Time itself plays a very great role in food deterioration. While some preservation methods such as canning are intended to produce food for years of storage, other preservation methods such as pasteurization and refrigeration produce shelf lives of days to weeks. Such processes aim to slow but not eliminate deteriorations, and recontamination becomes possible unless protective packaging is employed or after package integrity is broken. Food deteriorations described earlier will substantially reduce the shelf life of a product, but virtually all foods will become unconsumable over time.

PRESERVATION PRINCIPLES AND APPLICATIONS

Preservation techniques put into practice control mechanisms for reducing food deterioration. Often, various methods are combined to ensure safety and preservative action while maintaining maximal quality and stability. For example, fluid milk is preserved through a combination of pasteurization and subsequent refrigeration, aseptic thermal processing is usually combined with aseptic packaging, and freezing is usually accompanied by a blanching pretreatment to reduce enzymatic activity. For more detailed general information on preservation principles and techniques, the reader is referred to Refs. 7 to 15.

Physical Preservation

Thermal Methods. A number of preservation processes use heat to extend the shelf life of foods. High-temperature preservation methods performed commercially are controlled processes that include canning, aseptic processing, pasteurization, and blanching. Microorganisms differ in their heat resistance and are classified as psychrotrophs, mesophiles, or thermophiles according to their tolerance to heat. However, all microorganisms can be destroyed by the application of heat, and each organism has a certain time/temperature relationship associated with it to ensure a given reduction in its population. The most intense heating preservation process would render the food sterile. Sterilization refers to the complete destruction of all microorganisms. Complete sterility is difficult to achieve and often leads to a reduction in the quality attributes of the food, since most food components such as proteins or vitamins are also heat sensitive. Commercial sterility has been defined as the destruction of all pathogens and spoilage organisms in a food. Canning, thermal retorting in aluminum cans or flexible pouches, and aseptic processing, thermal processing prior to packaging followed by aseptic packaging techniques, target commercial sterility as their goal. Provided the food is maintained in the commercially sterile state after processing, a shelf life of two years or more can be achieved.

Canned food is typically packaged and maintained under a vacuum to eliminate oxygen and to act as an indicator for the consumer of loss of can seal integrity and hence sterility. As a result, the growth of anaerobic microorganisms, those that cannot grow in the presence of oxygen, would be favored if present. *C. botulinum* is an anaerobic, pathogenic, spore-forming microorganism that can cause severe illness and death; hence, it is essential that all spores of *C. botulinum* have been destroyed during the canning operation. *C. botulinum* is also pH sensitive, with a minimum growth requirement of pH 4.6. Thus, foods to be sterilized are characterized as either low-acid or high-acid foods, with the latter requiring a less severe (eg, boiling water) process than the former (must be processed under pressure to achieve temperatures in excess of 121°C). As the food is thermally processed after being packaged, the heat transfer characteristics of the food itself also become of importance in determining the processing time required at a specified temperature. Thus the calculations required to ensure lethality of all *C. botulinum* spores become very complex. The reader is referred to Refs. 16 to 19 for further reading on the subject of canning. Aseptic processing also targets commercial sterility but relies on continuous methods of heat treatment of the food prior to being packaged. Such heat treatment methods either use heat indirect heat exchangers, in which the food is separated from the heating medium by a barrier, or direct methods such as steam injection or steam infusion, where the heating medium and food are in direct contact. Such processes need to account for any added water from steam condensation, and hence are usually followed by a vacuum treatment for water removal, which also provides rapid cooling to the processed product. Once processed, there must be no contamination before packaging, implying sterile processing environments, and aseptic packaging must follow, to provide a package that will maintain the product in its commercially sterile condition until consumption. Details of aseptic processing techniques can be found in Refs. 20 to 23.

Pasteurization is a low-order, time- and temperature-dependent heating process that is designed to destroy all pathogens present in the food; to reduce the bacterial load in the case of milk and eggs; to reduce the yeast and mold count in the case of beer, wine, and fruit juices; and to extend the shelf life. Pasteurization can be performed prior to packaging in either batch or continuous heat exchangers, as in milk, or subsequent to packaging in either continuous or batch processes, as in bottles or cans of beer. Blanching is another low-order heat process used primarily as a pretreatment step prior to either freezing or canning. In a freezing operation, blanching is performed primarily to destroy enzymes that may continue to be active during freezing, or after thawing, when tissue rupture due to the freezing process may have allowed for enzymes and their substrates to come together, promoting fast enzymic deterioration. Prior to canning, blanching is performed primarily to cleanse and wilt tissue for ease of packaging, and to expel tissue gas that may interfere with an appropriate can vacuum.

Low-temperature methods include both refrigeration and freezing. Storage below 15°C but above freezing retards growth of microorganisms, retards metabolic activities of animal tissues postslaughter and plant tissues postharvest, retards deteriorative chemical reactions such as oxidation and enzyme-catalyzed reactions, and retards moisture loss. Unlike heating preservation, cold preservation does not destroy microorganisms, only retards their growth. The foods are still perishable and organisms will grow more rapidly once conditions become favorable. The shelf life can be extended from less than one week for highly perishable products such as raw milk or ripe tomatoes to more than six months for more durable products such as onions or smoked meats. Freezing preservation is achieved through both low temperatures that inhibit microbial growth and rates of reactions and reduction in water content as a result of ice crystallization. A reduction to subzero temperatures will cause water in the food to crystallize to ice, but ideally there should be little or no other changes to food components as a result. Shelf life of frozen foods can range from three months to a year or longer, depending on storage temperature, but is still limited by enzymatic activity, oxidation, and freeze dehydration (sublimation, "freezer burn"), especially if packaging has not been adequate to provide an oxygen or moisture barrier. Freezing will reduce the population of microorganisms that may be present, but will not eliminate them, so thawed foods again become very perishable, often more so than their fresh form due to damage of cellular and tissue integrity caused by ice formation, which may also bring enzymes in closer contact with their substrates, a process referred to as decompartmentalization. Commercial food freezing facilities utilize several methods, including air freezing through sharp (natural convection), blast, or fluidized-bed (forced convection) techniques; indirect contact systems such as plate freezers or scraped surface freezers; or direct contact freezing systems utilizing low-temperature liquids or cryogens. Packaging may be done either prior to or after a freezing process, depending on the commodity and equipment utilized. Detailed information on food freezing can be found in Refs. 24 to 26.

Reduction of Water Content. Microorganisms need favorable moisture conditions within the food, water activity greater than 0.6 to 0.8, for their growth. It is therefore possible to manipulate the water activity of the food to inhibit microbial action. Concentration of liquid food products through thermal evaporation, freeze concentration, osmotic dehydration, and membrane processes achieves lowered water contents, and provided that water activity or solute diffusion rates have been lowered sufficiently, offers preservation action. Thermal concentration removes water in the form of vapor from the liquid. It is usually performed in multiple effect falling-film vacuum evaporators designed for thermal efficiency and product quality. Freeze concentration removes water from liquid foods in the form of ice and is particularly suited to foods such as fruit juices with depressed freezing points due to high sugar concentrations within the food. Osmotic dehydration has been used for fruit slices in sugar solutions whereby water will migrate from the fruit slice into the sugar solution due to the high osmotic pressure of the solution. Membrane processes such as reverse osmosis, ultrafiltration, or microfiltration remove water from foods in the form of liquid water, due to the presence of a semipermeable membrane and the imposition of a pressure gradient. Solvent and low molecular weight solute, depending on the membrane pore size, pass through the membrane in the permeate stream while the higher molecular weight solutes are concentrated in the retentate stream.

The nearly complete removal of water through dehydration by solar, cabinet, tunnel, drum, or spray-drying methods also offers a form of preservation by reduction of available water for microbial growth or chemical or enzymatic reactions. Dehydration occurs under controlled conditions that cause minimal changes in the food properties. The food can then be consumed dried, as in some dried fruits or meats, but is more likely to be rehydrated prior to consumption, as in dried milk and eggs, instant potato flakes, or instant coffee. The reconstituted product should resemble as closely as possible the quality of the original food. In addition to preservation, the drying of foods decreases the weight and bulk of the original food and adds a measure of convenience to the product. Sun drying has been practiced for centuries and is still used for the dehydration of grains, seaweeds, raisins, and other foods, particularly in developing countries. Most commercial fruit and vegetable operations employ continuous tunnel or belt dehydration systems that utilize heated air as the drying medium. The majority of liquid foods, such as skim milk, cake and soup mixes, flavors, purees, juices or instant coffee, are dried in spray dryers that atomize the usually preconcentrated liquid product into tiny droplets that dry rapidly in the surrounding heated environment, being recovered from the air stream as fine powder particles. This spray-dried powder is capable of further conditioning to render it more soluble for easy dispersion/dissolution, a process called instantizing. Such a process is frequently conducted on powders destined for the consumer market, but rarely on powders destined for commercial processing. Freeze drying removes water from a frozen food through sublimation under vacuum and is particularly suited to thermally sensitive products, such as instant coffee or convenience-type prepared entrees. See Refs. 27 to 29 for more information on food dehydration and Ref. 30 for detailed information on freeze drying.

Oxygen Control. Because of the strict oxygen requirements for bacterial growth and the participation of oxygen in a number of chemical reactions, oxygen control can act as a means of food preservation. Controlled and modified atmospheric storage of foods are techniques to maintain gaseous atmospheres with strictly controlled oxygen contents. The controlled or modified atmosphere can be maintained in warehouses for bulk foods (eg, apples), often prior to further processing, or can be maintained at the microatmospheric level within a food package. Food packaging also offers protective barriers to food against the action of contaminating microorganisms, pests, moisture, oxygen, and light. The packaging necessary to maintain preservation is usually chosen to accompany the particular process. Examples include multilaminate flexible packaging for aseptically processed foods, or rigid aluminum cans for retorting. Information on modified atmospheres can be found in Refs. 31 to 36 and on food packaging in Refs. 37 to 41.

Radiation. The use of nuclear energy in the form of gamma radiations, short wavelengths emitted by unstable isotopes of cobalt 60, or cesium 137, to inactivate microorganisms has been a developing technology since 1945. The main goal of irradiation is to extend the shelf life of foods where heat or chemical means are unfeasible due to the nature or geographic location of the food. Major potential applications of this process include spices due to the heat-sensitive volatile flavor components; insect disinfestation of grains and fruit; extended shelf life of fruits, vegetables, fish, shellfish, and meat products; sterilized diets for military, space, and medical uses; and animal feeds and moist pet foods. The irradiation occurs in an enclosed chamber in which the product can be exposed to an even distribution of the penetrating gamma rays for the necessary time to accomplish microorganism inactivation. The safety of irradiated foods has been extensively studied and proved. Further details on food irradiation can be found in Refs. 42 to 45.

Chemical Preservation

Intermediate Moisture Foods. An intermediate moisture food (IMF) is one that can be eaten as is, without rehydration, and yet is shelf stable without refrigeration or thermal processing. Whereas most foods have water activities in the range of 0.9 to 1.0, IMF rely on water activities in the range of 0.65 to 0.85, below that required for the growth of the most tolerant organisms, for their preservation effects. The aqueous portion of such foods is also very high in viscosity, due to the high concentration of dissolved solutes present in a low amount of water, and hence water and solute diffusion is also a limiting factor in moisture availability to sustain growth and promote chemical reactions. Included in this category of foods are jams and jellies, fruit cakes, pepperoni, sweetened condensed milk, marshmallows, soft cookies, and many others. Sugar and other humectants, water-absorbing compounds such as sorbitol, glycerol, starches, or gelatin, can be used to formulate these foods. Although the technology can produce a range of products with acceptable texture, many of which have been in existence since historical times, the flavor profile created by the various humectants has been a limiting factor in new IMF product development. Another factor to consider in the preservation of IMF is the heterogeneity of the product. There are many multicomponent foods consisting of two or more distinct parts (eg, bakery product and fruit or icing, pasta and sauce). Not only does the stability of each component need to be considered separately, but the stability of the components after moisture equilibration (based on water activity or solute diffusion properties) also needs to be considered. See Refs. 6 and 46 for further information on IMF.

pH Control. Acids can be used to lower the pH of foods to below the tolerable range for microorganisms. Acid can also enhance the lethality of heating processes. *C. botulinum*, the organism of concern in commercial canning processes, will not grow at less than pH 4.5 and thus it is not necessary to thermally process high-acid foods ($pH < 4.5$) under the same rigid time/temperature standards as is the case with low-acid foods ($pH > 4.5$). The addition of acid to such foods as soft drinks and the production of acid in some food fermentations are effective controls of microbial growth. However, pH control is normally associated with some other means of preservation as well since the palatability of many foods and chemical stability of their constituents, for example, proteins, also decrease at low pH levels.

Chemical Addition. Salt can be added to foods for its contribution to the preservation of the food, for example, butter, fish, or cured meat products such as bacon. The action of salt results from the osmotic pressure created in the aqueous environment surrounding the microbial cell, in an analogous manner to the addition of sugar in intermediate moisture foods or the use of sugar syrups for osmotic dehydration of fruits. Plasmolysis, the partial dehydration of the cell, results, and the viability of the microorganism is thus destroyed. Smoke is also a type of chemical preservative and has been used since historical times to preserve foods, especially meat and fish products. The action of smoke results from the formation of small amounts of preservative chemicals, and the internal temperatures and dehydration of tissues associated with the hot-smoking process. Cold smoking at temperatures less than 30°C relies solely on the formation of bactericidal chemicals and is usually associated with other means of preservation such as salting, refrigeration, or packaging. Chemical preservatives, such as benzoic acid or sodium benzoate, sorbic acid or potassium sorbate, sodium nitrite or nitrate, and sulfur dioxide are permitted at low levels in some foods as preservative agents against microbial growth. The use of chemical preservatives and other food additives are closely regulated by governmental agencies. Chemical preservatives have been reviewed in Refs. 47 to 49.

Biological Preservation

Fermentations. Unlike the processes described previously, food fermentations have as their goal an increase in the numbers of microorganisms present in a food. The traditional foods of many countries rely on fermentation processes, and fermentation is a historical but important means of food preservation throughout the world. Fermented foods are preserved through the action of a particular organism, unique to each given commodity, on a particular substrate within the food product, primarily carbohydrates but also proteins and lipids. The conditions of fermentation favor the growth of the desirable organism, which is often added in the form of a pure culture, and cause the competitive disappearance of undesirable spoilage or pathogenic organisms. The metabolic by-products of the fermentation change conditions, such as pH or oxygen content within the food, that also act to inhibit the undesirable organisms and include lactic and other acids, ethanol and other alcohols, gases such as CO_2, and a variety of other compounds at low levels that are responsible for the unique flavor characteristics of the particular product. Examples of food fermentations include the production of alcohols by yeasts in wine, cider, and beer and the production of lactic acid by bacteria in fermented milks, sour cream, yogurt, fermented meats, pickles, sauerkraut, and vinegar. Further information of food fermentations can be found in Refs. 50 to 52.

Others

There is much interest from food technologists in developing new and novel food preservation methods, to improve all aspects of food preservation from energy efficiency to food quality and convenience. Examples of these methods include the so-called hurdle technology, which is a sequence of processing steps, each designed to address a particular deterioration source, but none used to the extent that alone it would provide sufficient preservation. There is also recent interest in the use of natural antimicrobial agents, such as nisin, produced by one group of microorganisms (lactic acid bacteria) that controls growth of another group. The use of nonthermal processes such as microfiltration, which would filter out bacteria from a liquid food without relying on heat to destroy the vegetative cell, are being utilized; such a process is being used for "cold pasteurization" of beer or milk. Nontraditional thermal methods such as microwave or ohmic heating processes are also being investigated and exploited. See Refs. 53 and 54 for further details of some of these novel food preservation methods.

BIBLIOGRAPHY

1. S. Thorne, *The History of Food Preservation*, Barnes and Noble Books, Totowa, N.J., 1986.
2. W. C. Frazier and D. C. Westhoff, *Food Microbiology*, 4th ed., McGraw-Hill, New York, 1988.
3. J. M. Jay, *Modern Food Microbiology*, 5th ed., Chapman and Hall, New York, 1996.
4. G. J. Mountney and W. A. Gould, *Practical Food Microbiology and Technology*, 3rd ed., Van Nostrand Reinhold, New York, 1988.
5. B. Ray, *Fundamental Food Microbiology*, CRC Press, Boca Raton, Fla., 1996.
6. H. Levine and L. Slade, "Beyond Water Activity: Recent Advances Based on an Alternative Approach to the Assessment of Food Quality and Safety," *Crit. Rev. Food Sci. Nutr.* **30**, 115–360 (1991).
7. P. Fellows, *Food Processing Technology: Principles and Practice*, VCH Publishers, New York, 1988.
8. G. W. Gould, ed., *Mechanisms of Action of Food Preservation Procedures*, Elsevier Applied Science, New York, 1989.
9. P. Jelen, *Introduction to Food Processing*, Reston Publishing, Reston, Va., 1985.
10. M. Karel, O. R. Fennema, and D. B. Lund, *Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975.
11. E. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., Van Nostrand Reinhold, New York, 1988.
12. V. Kyzlink, *Principles of Food Preservation*, Elsevier Science, New York, 1990.
13. N. N. Potter and J. H. Hotchkiss, *Food Science*, 5th ed., Chapman and Hall, New York, 1995.
14. I. A. Taub and R. P. Singh, *Food Storage Stability*, CRC Press, Boca Raton, Fla., 1997.
15. S. Thorne, *Developments in Food Preservation*, Elsevier Applied Science, New York, 1982.
16. A. C. Hersom and E. D. Hulland, *Canned Foods: Thermal Processing and Microbiology*, 7th ed., Chemical Publishing, New York, 1980.
17. S. D. Holdsworth, *Thermal Processing of Packaged Foods*, Blackie Academic and Professional, New York, 1997.
18. J. Larousse and B. E. Brown, eds., *Food Canning Technology*, Wiley-VCH Publishers, New York, 1997.
19. A. Lopez, *A Complete Course in Canning*, 11th ed., Canning Trade, Baltimore, Md., 1981.
20. J. R. David, R. H. Graves and V. R. Carlson, *Aseptic Processing and Packaging of Food: A Food Industry Perspective*, CRC Press, Boca Raton, Fla., 1996.
21. A. C. Hersom, "Aseptic Processing and Packaging of Food," *Food Rev. Int.* **1**, 215–270 (1985).
22. S. D. Holdsdworth, *Aseptic Processing and Packaging of Food Products*, Elsevier Applied Science, New York, 1992.
23. E. M. A. Willhoft, ed., *Aseptic Processing and Packaging of Particulate Foods*, Blackie Academic and Professional, New York, 1993.
24. M. C. Erickson and Y.-C. Hung, eds., *Quality in Frozen Food*, Chapman and Hall, New York, 1997.
25. L. E. Jeremiah, ed., *Freezing Effects on Food Quality*, Marcel Dekker, New York, 1996.
26. C. P. Mallett, ed., *Frozen Food Technology*, Blackie Academic and Professional, New York, 1993.
27. G. V. Barbosa-Canovas and H. Vega-Mercado, *Dehydration of Foods*, Chapman and Hall, New York, 1996.
28. D. MacCarthy, ed., *Concentration and Drying of Foods*, Elsevier Applied Science, New York, 1986.
29. A. S. Mujumdar, *Handbook of Industrial Drying*, Marcel Dekker, New York, 1987.
30. S. H. Goldblith, L. R. Rey, and W. W. Rothmayr, eds., *Freeze Drying and Advanced Food Technology*, Academic Press, New York, 1975.
31. B. A. Blaikstone, ed., *Principles and Applications of Modified Atmosphere Packaging of Foods*, 2nd ed., Blackie Academic and Professional, New York, 1998.
32. A. L. Brody, ed., *Controlled / Modified Atmosphere / Vacuum Packaging of Foods*, Food and Nutrition Press, Trumbull, Conn., 1989.
33. M. Calderon and R. Barkai-Golan, eds., *Food Preservation by Modified Atmospheres*, CRC Press, Boca Raton, Fla., 1990.
34. J. M. Farber and K. L. Dodds, eds., *Principles of Modified-Atmosphere and Sous Vide Product Packaging*, Technomic, Lancaster, Pa., 1995.
35. B. Ooraikul and M. E. Stiles, eds., *Modified Atmosphere Packaging of Food*, E. Horwood, New York, 1991.
36. R. T. Parry, ed., *Principles and Applications of Modified Atmosphere Packaging of Foods*, Blackie Academic and Professional, New York, 1993.
37. J. F. Hanlon, *Handbook of Package Engineering*, 2nd ed., McGraw-Hill, New York, 1984.
38. T. Kadoya, ed., *Food Packaging*, Academic Press, San Diego, Calif., 1990.
39. M. Mathlouthi, ed., *Food Packaging and Preservation: Theory and Practice*, Elsevier Applied Science, New York, 1986.
40. F. A. Paine and H. Y. Paine, *Handbook of Food Packaging*, L. Hill Co., Glasgow, Scotland, 1983.
41. G. L. Robertson, *Food Packaging: Principles and Practice*, Marcel Dekker, New York, 1993
42. Council for Agricultural Science and Technology, *Ionizing Energy in Food Processing and Pest Control. I. Wholesomeness of Food Treated with Ionizing Energy*, Task Force Report No. 109, 1986, *II. Applications*, Task Force Report No. 115, Council for Agricultural Science and Technology (CAST), Ames, Iowa, 1989.

43. P. S. Elias and A. J. Cohen, eds., *Recent Advances in Food Irradiation*, Amsterdam, Elsevier Biomedical Press, 1983.
44. J. Farkas, *Irradiation of Dry Food Ingredients*, CRC Press, Boca Raton, Fla., 1988.
45. E. S. Josephson and M. S. Peterson, eds., *Preservation of Food by Ionizing Radiation*, CRC Press, Boca Raton, Fla., 1982.
46. T. M. Hardman, ed., *Water and Food Quality*, Elsevier Applied Science, New York, 1989.
47. E. Lueck, *Antimicrobial Food Additives: Characteristics, Uses, Effects*, 2nd ed., Springer-Verlag, New York, 1997.
48. B. Ray and M. Daeschel, eds., *Food Biopreservatives of Microbial Origin*, CRC Press, Boca Raton, Fla., 1992.
49. R. H. Tilbury, ed., *Developments in Food Preservatives*, Applied Science, London, United Kingdom, 1980.
50. A. H. Rose, ed., *Fermented Foods*, Academic Press, Toronto, Ontario, 1982.
51. K. H. Steinkraus, ed., *Handbook of Indigenous Fermented Foods*, Marcel Dekker, New York, 1983
52. B. J. B. Wood, ed., *Microbiology of Fermented Foods*, Elsevier Applied Science, New York, 1985.
53. G. V. Barbosa-Canovas et al., *Nonthermal Preservation of Foods*, Marcel Dekker, New York, 1998.
54. G. W. Gould, ed., *New Methods of Food Preservation*, Blackie Academic and Professional, London, United Kingdom, 1995.

H. DOUGLAS GOFF
University of Guelph
Guelph, Ontario
Canada

FOOD PROCESSING

Individuals, companies, and corporations in the business of food processing have three basic responsibilities: (*1*) to ensure food safety, (*2*) to enhance food stability, and (*3*) to alter the form of feedstocks and ingredients to fit consumer demands. Food safety is paramount. The hazards associated with processed foods can be microbial, physical, or chemical. Microbial hazards are from pathogenic microorganisms or viruses that either invade the consumer or produce toxins in food during growth and dormancy phases. Examples of physical hazards are broken glass, rocks, or metal pieces, which can inflict serious harm if consumed inadvertently. Chemical hazards can come from cleaning compounds or other equipment maintenance fluids or can be associated with overuse of regulated food ingredients (such as sulfites in wine that control oxidative discoloration and growth of undesirable microorganisms but that can lead to bronchospasms in some individuals). Many of the processes used in food production are specifically used to ensure food safety.

Simply ensuring food safety does not, however, guarantee stability for the product shelf life desired by consumers. For example, retorting canned low-acid food through 12 decimal reductions for *Clostridium botulinum* to prevent botulism will only result in approximately a two-decimal reduction in *Bacillus stearothermophilus*, a thermophilic organism that causes flat sour spoilage if canned foods are stored above 38°C. Therefore, canned low-acid foods generally receive a more severe heat treatment than is required for ensuring food safety. In another example, refrigeration can prevent the growth of most pathogenic microorganisms but will not prevent enzyme activity or the growth of spoilage psychrophiles (some pathogens, such as *Listeria monocytogenes* or *Yersinia entercolitica*, are now being found to be psychrophilic). Food spoilage can result from nonpathogenic microorganisms, enzyme activity, chemical deterioration (eg, oxidation of lipids), contamination from pests, and mechanical damage.

In addition to safety and stability, much of modern food processing is designed to provide food to the consumer in a form convenient for use and desirable for taste. For example, while potatoes can be stored safely for long periods, precooking, mashing, and drum drying renders them in a form that can be consumed simply by adding hot water. Addition of dried spices or cheese flavoring may further enhance the appeal of the product. Ice cream can safely be packaged in one-gallon tubs and stored for months, but an individual serving on a stick with a chocolate coating will be much more convenient and appealing.

Conventional food processing, regardless of type of food, can be divided into three classes: separation, assembly, and preservation. These can occur at harvest, at the food-processing plant, or even at the point of retail sales. Increasing emphasis is being placed on field processing as exemplified by the widespread use of mechanical harvesters fitted with cleaning, sorting, color measurement, and size-grading systems. The known food-processing operations are grouped in Tables 1, 2, and 3.

Foods can be categorized as living tissue, or raw foods, and nonliving tissue. Living tissue foods include fresh fruits, vegetables, meats, and grains. Processes for preserving living tissue are considered separately from those used for the preservation of nonliving tissue, for example, canned, frozen, and dried foods.

FOOD COMPOSITION

Chemical Composition

Virtually all foods are derived from living tissues, although individual nutrients and additives, including lipids, carbohydrates, amino acids, and vitamins, can be synthesized. A nutritionally adequate dietary regimen of pure nutrients would be neither economically nor aesthetically useful except for medical applications such as intravenous feeding. Foods consist of hundreds of compounds because they are derived from the life processes of living tissues. The components of food composition are the nutrients that are essential to sustain life processes. Information on specific compounds in foods can be obtained from the literature, as can tables listing the nutrient composition of commodities, refined food components (eg, sucrose and gelatin), and processed as well as standardized formulated foods (eg, bread and margarine). Tables of nutrient composition are available for most foods found worldwide and contain quantitative data on moisture and caloric value (kJ or kcal × 4.184) and on lipid, protein, carbohydrate, fiber, ash, mineral, and vitamin contents. Tables of food composition are useful only as a first approximation to the actual nutritive value and gross chemical composition of

Table 1. Separation Unit Operations of the Food Industry Arranged by Mode of Separation and Phases Being Separated

Phases to be separated	Mode of separation		
	Physical	Chemical	Mechanical
Gas–gas			
Gas–liquid	Condensation and blanching		Deaeration
Gas–solid	Blanching		Controlled atmosphere, deaeration, compression, and densifying
Liquid–liquid	Distillation and membrane filtration	Solvent extraction	Centrifugation
Liquid–solid	Foaming, coagulation, and drying	Ion exchange, solvent extraction, and flocculation	11 Unit operations[a]
Solid–solid	Dialysis, freeze concentration, and sublimation		34 Unit operations[b]

[a] Mechanical separations include churning, centrifugation, clarification, draining, expelling, filtration, flotation, pressing racking, rendering, and skimming.

[b] Mechanical separations include abrading, boning, cutting, coring, crushing, dividing, defeathering, eviscerating, flaking, finishing, filleting, grinding, harvesting, husking, hulling, inspection, milling, peeling, picking, pitting, pulverizing, slicing, sieving, sorting, shredding, sizing, sifting, scarifying, sampling, shucking, stemming, vining, and winnowing.

Table 2. Assembly Unit Operations of the Food Industry Arranged by Mode of Assembly and Phases Being Assembled

Phases to be assembled	Mode of assembly		
	Physical	Chemical	Mechanical
Gas–gas			
Liquid–gas			Aeration, foaming, carbonation, and whipping
Solid–gas	Baking, puffing, and agglomeration	Humidification, oxidizing, and proofing	Extrusion and aeration
Liquid–liquid		Neutralization and acidification	Emulsification, homogenization, dispensing, mixing, and pumping
Liquid–solid	Crystallization	Soaking, malting, rehydration, and acidification	Dispersing, dissolving, immersion, mixing, and pumping
Solid–solid	Braising and roasting	Aging	7 Unit operations[a]
Gas–liquid–solid		Sprouting	Weighing and blending

[a] Mechanical assembly operations for solid–solid mixtures, include coating, enrobing, filling, forming, molding, pelleting, and stuffing.

Table 3. Preservation Unit Operations of the Food Industry Arranged by Mode of Preservation and Spoilage Vectors

Spoilage vector	Mode of preservation					
	Physical					
	Heat	Cold	Dehydration	Nonthermal	Chemical	Mechanical
Microbes	Frying, boiling, pasteurizing retorting	Cooling and freezing	Dehydration, desiccation, evaporation, and lyophilization	Irradiation, ultrahigh hydrostatic pressure, and high-intensity electromagnetic and ultrasonic fields	Acidification, brining, fermentation, isomerization, pickling, and smoking	Cleaning, centrifugation, and washing
Enzymes	Blanching, boiling, and scalding	Hydrocooling		Irradiation and ultrahigh hydrostatic pressure	Acidification	
Chemical	Exhausting and deodorizing	Chilling			Hydrogenation esterification, and smoking	Cleaning, degassing, and washing
Economic pests	Pasteurization	Freezing	Dessication	Irradiation	Fumigating	Cleaning, compressing, and washing
Mechanical damage		Freezing				Aspiration, cleaning, centrifugation, sorting, and washing

specific foods. Foods derived from living tissue are of extremely variable composition. The concentration of any nutrient is affected by horticultural, genetic, harvesting, handling, storage, and distribution factors. Furthermore, chemical analytical procedures may not correlate with the biological activity of available nutrients in the food.

Functional and Conformational Data

Foods and food ingredients are selected not only on the basis of nutritional content but for their functional and conformational attributes, which include toughness and tenderness; fiber content; style of cut; color and surface appearance; odor and flavor; microbial content; defects and extraneous matter; adaptability to freezing or heat treatment; genetic and varietal factors; geographical or regional production area; date of production (vintage); portion of plant or animal used; method of preservation; key ingredients; emulsifying capacity; water-binding capacity; and foaming capacity.

FOOD LAWS AND REGULATIONS

In the United States, a number of food laws have been passed with the primary objective to prevent food adulteration, either economic or hazardous. Some of the more prominent laws are the 1938 Food, Drug, and Cosmetic Act (FDCA), the 1958 Food Additives Amendment to the FDCA (including the Delaney clause, which prohibited the use of any known human or animal carcinogen in foods), the 1966 Fair Packaging and Labeling Act, and the 1990 Nutrition Labeling and Education Act. Food regulations promulgated by the Food and Drug Administration (FDA) for the purpose of enforcing these laws are found in Title 21 of the Code of Federal Regulations, while regulations promulgated by the U.S. Department of Agriculture (USDA) (for meat, poultry, eggs, and their products) are in Title 9. In addition to these national regulations, food processors dealing in international commerce may have to follow international regulations, such as those defined by the Codex Alimentarius.

Currently, regulations exist pertinent to current good manufacturing practices (CGMPs), nutritive requirements, food standards of identity, labeling, use of Hazard Analysis and Critical Control Point (HACCP) plans, and others. The most recent and important regulations are the mandating of HACCP by the FDA in 1995 for domestic and imported fish and fishery products and in 1996 by the Food Safety and Inspection Service of the USDA (FSIS-USDA) covering domestic and imported meat and poultry products. HACCP is a systematic approach to identification, evaluation, and control of food safety hazards. The systematic approach is based on the following seven points:

1. Conduct a hazard analysis.
2. Determine the critical control points.
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective actions.
6. Establish verification procedures.
7. Establish record keeping and documentation procedures.

The regulations require as a prerequisite to the HACCP systems of an individual food processor the establishment of sanitation standard operating procedures (SSOPs), which are written procedures determining how a food processor will meet sanitary conditions and practices in a food plant. SSOPs are based on the CGMP regulations.

Grades and Grading

The USDA also provides grading services for fruits, vegetables, meats, poultry, dairy products, and grains. Services may be obtained on a contractual basis for inclusive grading or for grading of selected lots of product. Grades are not mandatory as are identity standards. Grades provide a rational basis for the evaluation of the worth of processed foods for trading, financial, or contractual purposes where the buyer or seller cannot sample or inspect the products themselves. All grades and grading procedures, fees, labeling and identification procedures, and sampling plans (including those for canned, frozen, and dried fruits and vegetables) are specified. Because changes in regulations take place frequently, the regional or the Washington, D.C., office of the FDA or the USDA should be consulted for the latest regulations.

PRESERVATION OF LIVING TISSUE

The genetically controlled taste, color, nutritive value, cell structure and conformation of fresh foods are usually best at harvest. Protection of these qualities is achieved by retarding or inhibiting the detrimental action of microbes, enzymes, and other chemical degradation while maintaining the integrity of the membranes, enzyme systems, and gross structure of the food. Specifically, the following must be accomplished:

1. The growth rate of microbes normally associated with the product at harvest or during handling and transport must be inhibited or reduced.
2. Production of deteriorative enzymes or enzyme systems responsible for softening, color loss, or flavor changes must be inhibited or their rate of action must be reduced. Enzymes may be associated with the product or with incidental microbial contamination.
3. Loss of moisture and deterioration by chemical contamination must be prevented. Volatile organic compounds generated through respiratory action or present in storage often must be removed.
4. Higher life forms, insects, mites, and so on, must be inactivated or eliminated.
5. Bruising and various forms of mechanical damage must be prevented.

Processing operations required for the distribution of living tissue depend on the following factors: inherent storage potential of the tissue (this can be improved through genetic engineering, eg, by improving skin strength, resistance to microbes, or reducing respiration rate), desired storage life, degree of handling and shipping required, and intended use of the tissue (eg, further processing, food service, or retail consumption).

The intensity of processing operations is limited by the tissue itself in terms of temperature range, water activity, respiratory gas composition, mechanical stresses, and concentrations of chemicals. Temperature has the greatest effect on shelf life, because activation energies (and hence reaction rates) for microbial growth and enzyme activity are two to five times greater than those of most deteriorative chemical reactions. Except for certain fruits and vegetables, storage should be to 0°C without ice formation (Table 4). When shipping distances are great, expensive and highly perishable commodities are routinely shipped by air freight. Many fruits and vegetables are stored in atmospheres with controlled or modified O_2, CO_2, or C_2H_4 compositions.

A clear distinction is now made between processing whole, intact living tissue and minimally processed products that have arisen largely due to consumer demand in developed countries for freshlike, high-quality convenience foods. Minimally processed products include living tissues that have been cut, peeled, or shredded and generally require refrigeration in combination with other treatments, such as pH control, antioxidant addition, or chlorinated water dips, to extend the shelf life of the products. The application of "hurdle" technology to ensure safety and stability refers to synergistically applying several preserving treatments in series to minimize or eliminate microbial and enzymatic activity while preserving the freshlike nature of the products.

PRESERVATION OF NONLIVING TISSUE

Most processed foods, for example, canned, frozen, and dried products, are marketed as nonliving tissue or man-

Table 4. Storage Conditions and Approximate Useful Storage Life for Selected Living-Tissue Foods

Food	Temperature (°C)	Relative humidity (%)	Atmosphere composition (vol. %)	Useful storage (days)
Apples	0	90–95	air	100–250
			1–5% CO_2, 2–3% O_2	>180
Bananas	14	90–95	air	7–10
Beans, snap	6	95	air	7–10
Beets (topped)	0	>95	air	90–150
Broccoli	0	>95	air	10–14
	0	>95	1–5% CO_2, 3.5% O_2	40
Cabbage	0	>95	air	30–180
Carrots (topped)	0	>95	air	30–270
Celery	0	95	air	30–60
Cherries	0	>90	air	15
Corn, sweet	0	95	air	4–8
Cranberries	3	90–95	air	60–120
Cucumbers	10	90–95	air	10–14
Eggs	0	85–92	air	150–180
Grapefruit	10–15	85–90	air	40–60
Grapes	0	80–95	air	20–180
Lettuce	0	>95	air	15–20
Meat	0	95	air	5–15
Melons	7–10	90–95	air	3–15
Onions	0	60–75	air	200
Oranges	0	85–90	air	50–80
Oysters (shucked)	0	100 (wet ice)	—	7–10
Papaya	10	85–90	air	5–20
Peaches	0	90	air	15–30
Pears	0	90–95	air	60–180
Peas	0	90–95	air	5–15
Peppers (green)	10	92–95	air	15
Potatoes	4	90	air	150–250
Poultry	0	>95	air	15
Pumpkin	10–12	70–75	air	90
Radishes	0	>90	air	60–120
Raspberries	0	>90	air	2
Shrimp	0	ice	air	10–12
Spinach	0	>90	air	10
Strawberries	0	>90	air	5
Sweet potato	12–16	85–90	air	90–200
Tomatoes (green)	12–16	90	air	15–20
Turnips	0	>90	air	100–150

ufactured foods. Separation, assembly, and preservation operations determine the final quality of the product. Typical processing temperatures, pressures, and pH values are shown in Table 5. Because most preservation operations allow little or no residual microbial or enzyme activity, deterioration during storage is chemical. The relatively low activation energies of deteriorative chemical reactions compared with enzyme reactions present little advantage to low-temperature storage and make practical the storage of sterile foods at 25°C for months without appreciable loss of original nutrients and quality factors. Often foods are manufactured with a specified economic shelf life. Expiration dates are used to ensure adequate quality while minimizing inventory costs. The effect of various processing operations on the nutritive content of foods has been reported. Measurable changes in the vitamin content of foods usually indicate significant changes in flavor, color, and structure.

HEAT TREATMENT

Populations of all life forms show characteristic death rates when exposed to elevated temperatures, pressures, and concentrations of certain chemicals. Enzymes show similar inactivation kinetics. The benefits of heat preservation, for example, the high inactivation rates of microbes and deteriorative enzymes, considerably outweigh the

Table 5. Typical Temperatures, Pressures, and pH Values in Food-Processing Operations

Temperature	Example of use	Limits of applications
	Heat-transfer media	
Cryogenic freezing—liquid nitrogen	Rapid freezing to minimize moisture loss	Cost, stress cracking during freezing
Cryogenic freezing—solid carbon dioxide (−78.5°C)	Rapid freezing	Cost
Cryogenic freezing—air, plate and aqueous base freezants (−40 to −5°C)	Commercial freezing; parasite and insect destruction; storage; freeze drying	Foods not suitable for freezing
Refrigerated storage (0–10°C)	Commercial refrigerated storage	Microbial growth possible
Room temperature storage, air (20–40°C)	Canned and dry food storage	Suitable only for preserved and packaged foods
Water, air, atmospheric steam (50–100°C)	Pasteurization of milk and eggs; blanching; sterilization of acid foods (pH 4.5); air drying; cooking;	Excessive times will cause poor color, flavor, and structure
Steam (110–130°C)	Thermal sterilization of nonacid foods; destruction of antinutritive factors	Rapid thermal degradation of nutrients, pigments, structure, and flavors
Oil, steam, infrared radiation (180°C)	Frying, roasting, baking, generation of browning reaction products	Short duration surface treatments
Various infrared radiation sources (180°C)	Surface heating for flash drying, peeling	Charring, pyrolysis without precision control
	Pressure[a]	
<0.5 kPa	Freeze drying	Cost
0.5–4 kPa	Hypobaric storage, deaeration, vacuum concentration, vacuum cooling	Cost
101 kPa	Most processing operations	
0.1–1 MPa	Steam sterilization, over pressure for glass and pouch packs; carbonated and aerosol packaged food	Cost
>1 MPa	Extruders, hydraulic pressing, homogenization, microbial and enzyme inactivation at 1 GPa (10,000 atm) and higher	Cost, specialized products
	Hydrogen ion concentration	
pH 1	Acid hydrolysis	Must be neutralized
pH 2.5	Lemons, limes, vinegar, organic acids	Taste
pH 3–4.5	Fruits, acidified foods	Noncompatability of foods; protein denaturation
pH 3.5–7	Normal pH of most foods	
pH 8–9	Solubilization of certain proteins for extraction, alkali process cocoa	Must neutralize to normal pH for consumption
pH 12	Limed corn	Color, flavor, nutrient loss
33% sodium hydroxide	Peeling of fruits and vegetables	Surface treatment only, must neutralize to normal pH for consumation

[a] To convert kPa to mmHg, multiply by 7.5; to convert MPa to atm, divide by 0.101.

drawbacks, for example, heat-induced losses of desirable food nutrient, structures, colors, and flavors.

The heat treatment needed to inactivate microbes or enzymes can be calculated from the energy for activation and the known rate of inactivation at a given temperature, usually 121°C. These values depend on the food system and its pH, water activity, and chemical profile. Typical activation energies and inactivation rates at 121°C for heat-resistant microbes are 209–335 kJ/mol (50–80 kcal/mol) and 0.1 to 10 decimal reductions/min. Commercial heat preservation operations assume a starting concentration of *C. botulinum* spores (mixed varieties) of 10 to 12/g and an inactivation rate of 5 to 10 decimal reductions/min at 121°C.

Preservation heating times at any temperature can be determined by integrating the lethal temperature–time effects at the slowest heating point in the package. Thus if the geometry of the container and the thermophysical properties of the food are known, it is possible to calculate the heating time necessary to ensure a safe level of microbes or residual enzyme activity in the food and the residual concentration of desired nutrients. In general, higher life forms (eg, insect eggs, mites, etc) are killed by even mild heat treatments. Computer programs are available for determining safe heat-preservation operations when given information about the product, including its initial temperature, container characteristics, fill, and heat-exchange system (pure steam, steam–air, water, etc).

The hydrogen ion is extremely toxic to most microbes. Foods having a pH below 4.5 can be commercially sterilized by heating to 100°C with a limited holding period.

Aseptic preservation involves performing separate heat-preservation operations on the food and the containers prior to assembly. Liquid products can be heated and cooled rapidly under optimum conditions of heat transfer in specialized heat exchangers. The sterile product is packaged under sterile filling conditions into sterile unit retail packages, drums, or bulk-storage tanks.

NONTHERMAL PROCESSES YIELDING MICROBIAL INACTIVATION

Thermal preservation processes such as retorting canned foods and pasteurizing milk and eggs have the disadvantage of degrading product quality as perceived by the consumer as well as nutritional quality. In recent years, much research has been conducted with success to develop nonthermal food processes that destroy microorganisms. These processes include irradiation, ultrahigh hydrostatic pressurization, pulsed electric fields, oscillating magnetic fields, high-intensity visible and UV light, and ultrasonics (manothermosonication). In all of these processes, microbial inactivation increases with the duration and intensity of the treatment, analogous to thermal processes.

Food irradiation has gained prominence worldwide as a nonthermal method. Irradiation refers to ionizing electromagnetic radiation that inactivates microorganisms by producing high-energy electrons within the food product. Irradiation has been approved for use in the United States on a number of food products (Table 6). Specifically, the FDA has approved irradiation from sealed units of radioactive nuclei (^{131}Cs or ^{60}Co), electron beams generated from machine sources at energies not to exceed 10 MeV, or X rays generated from machine sources not to exceed 5 MeV.

The legal basis for regulation of food irradiation by the FDA is that irradiation is included in the definition of "food additive" in the 1958 Amendment to the FDCA. The primary concern is that the radiation, in the process of destroying microorganisms and enzymes, will produce toxic substances. However, the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI) concluded in 1980 after review of extensive research that irradiation of any commodity up to an overall average dose of 10 kGy presents no toxicological hazard. Despite its safety and potential benefits for a safe food supply, food irradiation still remains a controversial food process with many food processors unwilling to install irradiation systems.

The other nonthermal preservation methods have not found widespread usage and are still under development. Ultrahigh hydrostatic pressure (UHP) has been shown to be effective in inactivating microorganisms (vegetative bacteria, parasites, yeasts, and molds) at pressures between 300 and 600 MPa (43,000 to 87,000 psi). Processes can be made continuous for liquid foods, and processing times range from one minute to one hour at room temperature. The UHP process can also be enhanced by increasing temperatures up to 90°C, to the point of inactivating enzymes and spores. The other nonthermal processes involve exposing the food to high-intensity field strengths for short durations, sometimes including up to 100 rapid pulses. The degree of microbial inactivation depends on the strength of the field, its duration, and the number of pulses. Increasing temperature during these processes also enhances inactivation.

FREEZING

The rate of loss of color, flavor, structure, and nutrients in foods is a function of temperature; thus, lower storage temperatures prolong the useful life of foods. However, below 0°C, the free water in food forms ice crystals as a function of moisture content, solute composition, and storage temperature. Ice formation is both beneficial and detrimental. Benefits include strengthening of structures and removal of free moisture, which reduces water activity. Benefits, however, are often far outweighed by the deleterious effects of ice crystal formation, the partial dehydration of the tissue surrounding the ice crystal, and the freeze concentration of potential reactants. Ice crystals disrupt cell structures mechanically, and the increased concentration of cell electrolytes can result in the chemical denaturation of proteins.

The technology of food freezing emphasizes as short a passage of time through the temperature zone of maximum ice crystal formation as possible. The formation of as small an ice crystal as possible minimizes the mechanical disruption of cells and possibly reduces the effects of solute concentration damage. Rapid freezing can only be accom-

Table 6. Approved Uses of Food Irradiation in the United States

Food product	Purpose of irradiation	Dose (kGy)	Date approved[a]
Fresh fruits and vegetables	Inhibition of growth and maturation	1 (max)	April 1986 (FDA)
Dehydrated aromatic vegetable substances (herbs, seeds, spices, teas, vegetable seasonings)	Control of foodborne pathogens	30 (max)	April 1986 (FDA)
Food	Anthropod pest control	1 (max)	April 1986 (FDA)
Enzyme preparations, dehydrated	Control of foodborne pathogens	10 (max)	April 1986 (FDA)
Pork	Control of *Trichinella*	0.3 to 1.0	January 1986 (USDA) July 1985 (FDA)
Hawaiian papaya	Fruit flies	0.15 (min)	February 1989 (USDA)
Packaged poultry products, fresh or frozen, including ground, hand-boned and skinless products	Control of foodborne pathogens	1.5 to 3.0	September 1992 (USDA) May 1990 (FDA)
Mechanically deboned poultry meat	Control of foodborne pathogens	1.5 to 3.0	September 1992 (USDA) May 1990 (FDA)
Meat, meat by-products, and certain meat food products	Control of foodborne pathogens	4.5 (max) for refrigerated products 7.0 (max) for frozen products	Proposed by the USDA in February 1999 Approved by the FDA in December 1997

[a] Final rules published in the Federal Register by the Food and Drug Administration (FDA) or the United States Department of Agriculture (USDA) unless otherwise indicated.

plished by large temperature differences and high heat-transfer coefficients.

Many processed foods and certain animal products tolerate freezing and thawing because their structures can accommodate ice crystallization, movement of water, and the related changes in solute concentrations. Starches can be modified to form gels that accept several freezing and thawing cycles without breakdown. By contrast, most fruits and vegetables lose significant structural quality on freezing because their rigid cell structures fail to accommodate ice crystal formation. However, it is not possible to store foods at temperatures low enough to ensure complete conversion of all water to ice; as a result, commercial frozen food storage temperatures represent an economic balance between storage costs (time, energy, and capital investment) and projected shelf life.

The freezing process disrupts tissue structures and allows cell contents to become mixed so that undesirable enzyme reactions can take place at significant rates even at storage of −18°C. These reactions can generate off-flavors, reduce nutrient concentrations, and cause major changes in the structure and appearance of foods. The amount of free liquid or drip found after a freeze–thaw cycle is a good indication of the structural damage.

Heat treatment (blanching) prior to freezing eliminates enzyme-mediated changes in color, flavor, and structure. Most deteriorative enzymes are inactivated by exposure to a temperature of 100°C for 1 to 5 min. The enzymatic oxidative deterioration of frozen fruits can be inhibited with sulfur dioxide, sucrose, and combinations of citric acid, sodium chloride, and ascorbic acid (preceded by vacuum removal of oxygen if heat is not used).

Most frozen foods have a useful storage life of one year at −18°C; however, foods high in fat (eg, sausage products) may become rancid in two weeks. Frozen storage can result in moisture loss from the food through a freeze-drying process, because the heat-transfer surfaces used to maintain storage temperatures are at a lower temperature than the storage area. For this reason, frozen foods must be protected against drying by a moisture barrier. In addition, foods subject to oxidative deterioration must be protected from air.

Freezing-preservation equipment can be classified by method of heat transfer. Usually air is the heat-exchange medium for freezing foods. Foods are loaded on a belt or vibrating conveyor and passed through air flowing upward at up to 5 m/s at temperatures as low as −40°C. The air is recycled through coils and fans located next to the conveyor and returned through the conveyor. Because the air has a partial water vapor pressure lower than the food, freeze drying can occur, and some of the water in the product is removed and deposited as ice on the heat-exchange coils. As a freezing medium, air has other drawbacks. The low gas–solid heat-transfer coefficient and the heat capacity of air require either low temperatures or high velocities to obtain needed high heat fluxes. Low air temperatures increase refrigeration costs and high air velocities generate additional fan heat loads. For these and other reasons, freezing by conduction or by liquid heat-transfer methods are less costly in terms of capital investment and energy.

Liquid heat-transfer media that are used for direct-immersion freezing include food-grade dichlorodifluoromethane, nitrous oxide, and water solutions of various edible salts, sugars, alcohols, acid, and esters. Liquid heat-transfer agents offer a high-heat-transfer coefficient and reduced pumping costs, eliminate product desiccation, and allow operation at high low-side equipment temperatures. Drawbacks include possible changes in food flavor and costs of processing. Although dichlorodifluoromethane offers major operating advantages as compared with other heat-transfer media, cost and environmental concerns have reduced its potential usefulness as an ideal direct-immersion freezant. There is a need for a direct-immersion liquid freezant that is safe, low cost, thermodynamically efficient, and compatible with foods with respect to flavor, color, and odor.

Conduction freezing between chilled plates is a cost-effective method of heat removal provided the product can be assembled in a geometry compatible with the plate surfaces. Packages having semiinfinite slab geometry are loaded between stacks of platens through which refrigerant is circulated. Good heat transfer is maintained by maintaining a pressure on the stack of platens.

Other freezing methods use direct immersion in liquid nitrogen, exposure to solidified carbon dioxide, and immersions of packaged products in liquid freezants, for example, sodium or calcium chloride brines, methanol, or propylene glycol solutions.

The quality of frozen food is related to storage temperature; however, because constant storage temperatures are not always feasible, the shelf life is often determined by the highest temperature and total length of time that food is exposed to that temperature before use. Maintaining a −18°C storage environment from time of freezing until use continues to be a major technical problem facing the frozen-food industry.

REDUCTION OF WATER ACTIVITY (DEHYDRATION)

Microbes require a specific minimum level of water activity (a_w), defined by the relative humidity (measured in equilibrium with the food) for growth and reproduction at a given temperature and substrate composition. Foods possess characteristic equilibrium relationships between water activity and moisture content at given temperatures, which are known as sorption isotherms. Preservation against microbial spoilage by dehydration requires a moisture content equal to a water activity below 0.65 (Fig. 1). Dehydration contrasts with food concentration where water is removed for reasons other than for effective reduction in water activity.

Living tissue, when dried to a water activity below 0.97, suffers irreversible disruption of metabolic processes. Deteriorative chemical reactions, enzyme-catalyzed or not, are generally a function of water activity and reactant concentration. Thus, nonenzymatic browning (Maillard reaction), oxidation, and internal rearrangements (eg, staling and protein cross-linking) can increase in rate as water activity is reduced because of the increase in concentration of reactants. Many reactions show a minimum rate in the range of $a_w = 0.4$.

Prior to dehydration, foods are usually heat treated to inactivate enzyme systems. Those foods susceptible to rapid nonenzymatic browning resulting from high concentrations of reducing sugars are treated with sulfur dioxide, and products subject to oxidative rancidity can be treated with antioxidants and packaged to prevent exposure to oxygen. Low-temperature storage (5°C) reduces chemical deterioration. Blends of dehydrated products must be assembled from ingredients having the same water activity.

High-quality dried foods can be obtained only if the drying system is designed to match heat penetration with the rate of release of moisture from the food. Typically, continuous-belt dryers are staged to provide three or more zones into which the product is reapplied into progressively deeper beds. Each zone operates at a dry- and wet-bulb temperature, a through-flow (upflow and downflow through a bed of materials) air velocity, and a bed depth that optimize product quality, energy use, and production rate.

Liquids and pastes are commonly dried in spray, drum, or freeze dryers. In spray drying, product is atomized through a nozzle (rotary, pneumatic, or high pressure) into a drying chamber in a continuous operation with hot air entering at typically 200 to 300°C. Evaporative cooling provides rapid cooling of the inlet air and prevents heating of the product until in a dry, stable state. Drum drying is generally a much more severe heat treatment unless done under vacuum. Freeze drying preserves original product qualities (flavor, structure, nutrients, etc) to the greatest extent, but it is a slow and costly process and therefore finds application mostly with high-value products such as spices and coffee. Particulate foods can be dried in continuous conveyor systems, fluidized beds, freeze dryers, or batch tunnel systems. Fluidized beds use pneumatic flow to fluidize particulate product and have the advantage of optimizing drying surface area for heat and mass transfer per unit volume of dryer. Some fruits, salted animal products, nuts, berries, and many field crops are sun-dried. Other food processes that produce dehydration include osmotic dehydration (in which intact fruits and vegetables are exposed to hypertonic solutions), vacuum dehydration, microwave dehydration, extrusion cooking, pneumatic dehydration, foam-mat dehydration, and gun puffing.

MECHANICAL FOOD PROCESSES ENHANCING PRESERVATION

Mechanical food-preservation methods are characterized by separation operations, which tend to affect food quality factors less than other methods, as exemplified by the production of sterile draft beer by filtration.

Packaging is the primary mechanical preservation method; barrier materials protect preserved foods from external spoilage factors during storage and distribution. Other mechanical food-processing methods include sieving, filtration, air classification, washing, sorting, ultrafiltration, microfiltration, centrifugation, extraction, stripping, flotation, and so on. Size grading, defect sorting, and washing are routinely used to reduce or eliminate microbes, enzymes, chemicals, insects, and other matter. Virtually all food materials require repetition of these operations between harvest and preservation.

Washing equipment is designed to accommodate the type of food being washed. Leafy vegetable washers, for example, use a series of rotating paddles that transfer the material along the water surface in a tank so that sand can settle to the bottom of the tank. Fruit washers have reels or belts fitted with high-pressure water sprays that mechanically loosen dirt and debris. For tomatoes, tree fruit,

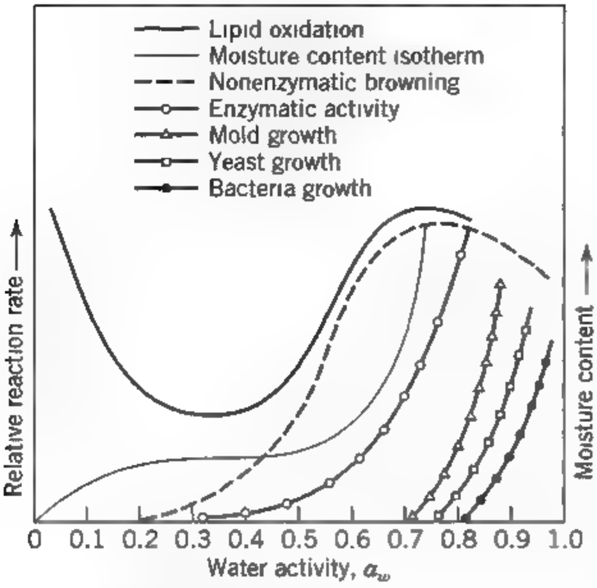


Figure 1. Relative rate of food deterioration factors as a function of water activity a_w.

and root vegetables washing units have spinning soft-rubber disks that are mounted in rows to give an intensive wiping action at the surface of the food as it rolls over the disk array. Disk washers reduce water consumption in tomato washing from 1,500 L/t (360 gal/short ton) to as little as 20 L/t (5 gal/short ton). Other food washing equipment uses combinations of sprays, brushes, and mechanical agitators. For example, froth flotation removes foreign particulate organic matter from cut corn and peas. Approved wetting agents (eg, sodium lauryl sulfate) are often incorporated in wash water to improve the efficiency of the washing operation. The proper chlorination of wash and transport water also is extremely important in maintaining low levels of microbes. Break-point chlorination ensures adequate but not excessive residual free chlorine (because >5 ppm of free chlorine can spoil flavors of some foods). This method of chlorination is implemented by adding sufficient chlorine to oxidize organic materials in the water and leave a residual concentration to ensure antimicrobial activity. Water used in the final washing of food materials processed under FDA jurisdiction must be suitable for drinking.

Liquid foods free of suspended solids may be sterilized by filtering spoilage microbes. The filtered product, for example, wine, beer, and certain fruit juices, is packaged under aseptic conditions in a sterilized container. Centrifugation can be used to reduce microbial contamination and to remove extraneous organic matter in liquid foods such as milk.

CHEMICAL PRESERVATION METHODS

Addition of chemicals usually occurs in conjunction with other preservation methods because they enhance the effectiveness of heat, refrigeration, drying, or packaging. Examples include the addition of antioxidants to fried foods to reduce the need for more expensive oxygen-impermeable flexible packaging and the use of sulfur dioxide to retard the development of brown color in frozen or dried apples.

The extreme toxicity of the hydrogen ion toward food-spoilage microbes and its tolerance by humans has made it a preferred food preservative. Studies have shown that various organic acids exert a strong inhibitory effect on the growth of microbes beyond the activity of the hydrogen ion itself. Lactic, propionic, acetic, sorbic, and benzoic acids; their sodium or potassium salts; and certain derivatives of benzoic acid find extensive use as yeast, mold, and bacterial inhibitors in bread, beverages, and sauces. Combinations of hydrogen ion, organic acids, mild heat treatment, and exclusion of oxygen have allowed the simple and safe preservation of foods not tolerant of conventional heat sterilization above 100°C, freezing, or dehydration.

Traditional food-preservation methods were based largely on drying in conjunction with preservation with locally available chemical preservatives. These included salt, organic acids generated by a lactic or acetic acid fermentation (as in pickling), wood smoke, and ethanol derived from the fermentation of substances containing sugar. Many traditionally preserved products are available in the marketplace, although modern preservation methods predominate because they are convenient.

Chemical preservatives and chemical preservation methods used in conjunction with other preservation methods for meat and poultry are controlled by USDA regulations. The FDA has jurisdiction over the use of chemical preservatives in other foods. Additives are reviewed continually for heat safety.

TOXICOLOGICAL IMPLICATION OF PROCESSED FOOD

A primary objective of food processing is to ensure a safe food supply. Certain harvested foods can contain naturally occurring poisonous substances, compounds with pharmacological effects, or compounds that can interfere with the utilization of nutrients present in other foods when they are consumed together.

Foods just harvested may contain or become contaminated with spore or vegetative forms of microbes, parasites, or the wastes of higher life-forms. Careless postharvest handling, storage, processing, and distribution operations provide additional opportunities for microbial growth or contamination. In addition to the hazard associated with pathogenic microorganisms, microbial growth can result in the release of toxins that the consumer has no way of identifying, consequently, food poisoning outbreaks occur. Fortunately, some toxins are heat sensitive and are inactivated during the cooking process. However, only good manufacturing, handling, and distribution practices ensure the prevention of microbial growth. These practices must be carried out from the time of harvest to the time of consumption.

A third source of potential health hazards in food supplies are toxic compounds formed during the chemical degradation of processed and stored foods. Oxidative breakdown products of unsaturated fatty acids have been implicated as potential carcinogens, for example, malonaldehyde.

In addition to the preceding, foods must be protected from contamination by pesticides and other chemicals used for crop protection; from exposure to hazardous chemicals and extraneous materials, for example, asbestos in air and water supplies; and radioactive isotopes. Many toxic compounds can be found as soil contaminants or as contaminants in lakes, oceans, or groundwater supplies and can be concentrated in the edible tissues of plants and animals.

FOOD-PROCESSING FACILITIES

Factors important in the selection of sites for food-processing operations include availability of raw materials, abundant potable water supply, low-cost waste disposal facilities, adequate low-cost energy supply, adequate seasonal and nonseasonal labor supply, ease of access to rail and truck transportation, proximity to consumers, and adequate storage areas. The relative importance of each of these factors depends on the length of the processing season, the ease of storage and handling of raw materials, the perishability of the finished products, and the complexity of the processing operation.

Highly perishable salad vegetables can be trimmed, inspected, bagged, and vacuum cooled using mobile equipment in or at the edge of the growing area. Processing of frozen french fries requires raw potato storage capacities of up to 10 months; thus plants tend to be located near principal potato-growing areas, for example, the northwestern United States. Continued developments in mechanical harvesting and mobile harvester separation operations for fruits and vegetables will cause further processing plant shifts toward concentrated growing areas where irrigation, climate, and level terrain make mobile processing feasible.

As processing technology improves, an increasing number of plants are relocating away from eastern U.S. population centers toward sources of raw materials. This shift is particularly noticeable in the meat industry and has occurred concurrently with the development of vacuum-packed cut beef, which provides more than a 50% reduction in storage and shipment space over conventional quarter or sides.

Certain food products are highly perishable, have marginal value, or have only regional acceptance. Dairy products, bread, soft drinks, beer, glass-packed items, and certain cured-meat products are usually manufactured from more stable raw materials or are locally produced starting materials and are distributed daily. With such distribution, production schedules are determined by local weather forecasts, by day of the week and holidays, and by seasonal factors so that returns of stale or overage products can be minimized. Considerable progress has been made in the analysis of perishable food distribution practices. A universal product code applicable to all processed foods for retail sale has further improved distribution efficiencies by allowing automatic retail checkout and continuous inventory review.

FOOD PACKAGING

Glass, woven, natural fiber, and ceramic containers have been used to store food since antiquity. However, the invention of tin-plated steel containers (ca 1800) provided the food industry with its first functional and disposable packaging system. Advances in metal container manufacturing continue to maintain the status of the cylindrical can as the primary package for heat-processed, shelf-stable foods and beverages. Notable developments include substitution of electrolytic tin plating for hot dipping, new organic linings to greatly reduce the need for tin, reduced steel thickness, and improved steel composition for better strength and corrosion resistance. Developments in coating technology have resulted in the availability of two-piece drawn aluminum and steel containers and of numerous easy-open systems. New glass containers have increased strength and have continued to reduce package weight and cost.

Rigid glass or metal containers have a major processing advantage: they can be filled and sealed at speeds in excess of 1000/min. Thus the relatively higher container cost can be recovered to some degree by higher production rates. Their rigidity (ie, sturdiness) facilitates stacking and handling during distribution and storage.

Paper, plastics, and aluminum have provided the food industry with container material that can be tailored to specific barrier needs. Ease of forming, sealing, opening, and decoration; strength; and low weight have made them preferred materials for refrigerated, dry, and frozen products.

Resistance to thermal treatment and desired barrier and mechanical qualities are obtained by laminating or coextruding appropriate combinations of paper, aluminum foil, and plastic. Lamination and coextrusion seal transparent packages against moisture and gas permeation and strengthen the packaging.

Pouches formed by heat-sealing paper–foil–plastic laminates on three sides, filling, and then heat-sealing the fourth side have found extensive use with moisture- and oxygen-sensitive dry foods as well as with high-moisture thermally processed foods. Pouches capable of withstanding saturated steam or steam–air mixtures at 121°C for several hours are finding greater use as substitutes for metal and glass containers. The advantage of pouches for heat sterilization is their slab rather than cylindrical shape. Their thinner cross section allows a shorter heat-processing time than cylindrical containers of the same capacity. Mechanical advances in forming, filling, sealing, and handling pouches will result in continued substitution of laminates for rigid metal and glass containers.

Plastics and plastic-coated papers are widely used for beverages, including carbonated drinks. Several aseptic systems are marketed for bulk packing of liquids in multiliter plastic bags.

Semirigid containers are also gaining increased acceptance, particularly as replacements for the #10 can (2.72 kg or 96 oz). Initial developments have produced a lightweight drawn-metal container measuring 24 × 30 × 6 cm with a double-seamed top. The thickness of this container (6 cm) is such that during heat sterilization, heat travels only 3 cm by conduction versus about 8 cm in the conventional #10 can. Shorter heat treatments allow the preservation of a greater variety of specialty products. The half-steam-table size eliminates cleaning of pans, because the opened unit serves as a serving tray. Other semirigid systems have been developed to replace the steel can for single-portion service. These containers generally use a heat-sealed or glued paper–foil–plastic laminated closure.

Aerosol containers have played a specialized role in the food industry as dispensers for such foods as whipped toppings (using nitrous oxide as a propellant), cake frosting, and barbecue sauces. Special filling techniques and product formulations are required because the aerosol package cannot be heat sterilized once it has been sealed.

Packaging forms an integral part of most food processing. For this reason, studies of storage conditions over a range of temperatures, relative humidities, and handling conditions must be made to determine the suitability of a package. Packaging in contact with foods must be tested for migration of packaging components under actual conditions of use, and pickup of off-flavor must be evaluated on a product-by-product basis. Because of potential migration of packaging materials into foods, current food and drug regulations should be consulted prior to marketing foods packaged in nonstandard materials.

BIBLIOGRAPHY

This article was adapted from M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 11, 3rd ed., John Wiley & Sons, New York, 1979. Note: All specific citations have been removed. A user must refer to the original text to obtain specific references.

GENERAL REFERENCES

G. V. Barbosa-Cánovas and H. Vega-Mercado, *Dehydration of Foods*, Chapman and Hall, New York, 1996.

D. A. Corlett, *HACCP User's Manual*, Aspen Publishers, Gaithersburg, Md., 1998.

J. F. Diehl, *Safety of Irradiated Foods*, 2nd ed., Marcel Dekker, New York, 1995.

W. A. Gould, *CGMP's/Food Plant Sanitation*, CTI Publications, Baltimore, Md., 1994.

W. A. Gould, *Unit Operations for the Food Industries*, CTI Publications, Baltimore, Md., 1996

D. R. Heldman and D. B. Lund, *Handbook of Food Engineering*, Marcel Dekker, New York, 1992.

S. D. Holdsworth, *Aseptic Processing and Packaging of Food Products*, Elsevier, New York, 1992.

P. Jelen, *Introduction to Food Processing*, Prentice-Hall, Englewood Cliffs, N.J., 1989.

N. G. Marriott, *Essentials of Food Sanitation*, Chapman and Hall, New York, 1997.

N. N. Potter and J. H. Hotchkiss, *Food Science*, 5th ed., Chapman and Hall, New York, 1995.

G. L. Robertson, *Food Packaging. Principles and Practice*, Marcel Dekker, New York, 1993.

R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, 2nd ed., Academic Press, New York, 1993.

K. J. Valentas, E. Rotstein, and R. P. Singh, *Handbook of Food Engineering Practice*, CRC Press, New York, 1997.

R. C. Wiley, *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman and Hall, New York, 1994.

James Faller
University of Illinois
Urbana, Illinois

FOOD PROCESSING: EFFECT ON NUTRITIONAL QUALITY

The definition of *processing* is "a natural phenomenon marked by gradual changes that lead toward a particular result"; "a series of actions or operations conducing to an end"; or "a continuous operation or treatment especially in manufacture" (1). *Food processing*, therefore, refers to the series of actions involved in order to prepare and preserve a food supply by some continuous operation or treatment to achieve as a goal a safe, high-quality product with extended shelf life.

Changes that occur to food quality may be thought of as two types: deterioration and spoilage. Deterioration involves changes in quality induced by physicochemical and/or biochemical reactions taking place with or without the intervention of a physical environment (such as oxygen, carbon dioxide, water, light, heat, etc). Spoilage, on the other hand, generally refers to changes in quality due to action of biological agents such as bacteria, molds, or insects. Effects of deteriorative reactions and spoilage agents on food quality result in changes in both sensory properties (ie, appearance, flavor, texture) and nutritive value (vitamin content, protein value, etc). The extent to which these reactions can occur depends on the sensitivities and types of food products considered. For example, certain fruits such as strawberries or raspberries may be readily spoiled by the presence of molds. Green peas or beans, on the other hand, lose flavor and result in undesirable textures due to enzyme-induced reactions.

Through various types of food processing, quality factors can be maintained or extended, the extent of which depends on the food product to be considered. By canning fruits and vegetables (eg, pears, peaches, peas, beets) with proper heat sterilization and packaging, these products will not suffer spoilage, although they may deteriorate to some extent in terms of color, flavor, and texture. The extent of these changes depends on a wide array of factors, including storage conditions after packaging. Factors affecting quality of food products include: (*1*) initial quality of the raw materials and handling from harvesting to the manufacturing plant; (*2*) pretreatment (including cleaning, sanitizing, washing, aspiration, screening, filtration, chlorination, fumigation, etc); (*3*) sorting (removal of extraneous materials); (*4*) peeling, coring, dehairing, defeathering, husking, stemming, and so on; (*5*) disintegration/physical separation in cases where grinding, pulping, pressing, or expelling are needed, generally followed by screening, filtering, or centrifugation; and finally, of course (*6*) the final finished food manufacturing step. These final manufacturing steps comprise a myriad of different types of food processes including clarification/filtration; crystallization; curing/smoking; dehydration; evapoonly 3 cm by conduction versus about 8 cm in the conventional #10 can. Shorter heat treatments allow the preservation of a greater variety of specialty products. The half-steam-table size eliminates cleaning of pans, because the opened unit serves as a serving tray. Other semirigid systems have been developed to replace the steel can for single-portion service. These containers generally use a heat-sealed or glued paper–foil–plastic laminated closure.

Aerosol containers have played a specialized role in the food industry as dispensers for such foods as whipped toppings (using nitrous oxide as a propellant), cake frosting, and barbecue sauces. Special filling techniques and product formulations are required because the aerosol package cannot be heat sterilized once it has been sealed.

Packaging forms an integral part of most food processing. For this reason, studies of storage conditions over a range of temperatures, relative humidities, and handling conditions must be made to determine the suitability of a package. Packaging in contact with foods must be tested for migration of packaging components under actual conditions of use, and pickup of off-flavor must be evaluated on a product-by-product basis. Because of potential migration of packaging materials into foods, current food and drug regulations should be consulted prior to marketing foods packaged in nonstandard materials.

BIBLIOGRAPHY

This article was adapted from M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 11, 3rd ed., John Wiley & Sons, New York, 1979. Note: All specific citations have been removed. A user must refer to the original text to obtain specific references.

GENERAL REFERENCES

G. V. Barbosa-Cánovas and H. Vega-Mercado, *Dehydration of Foods*, Chapman and Hall, New York, 1996.

D. A. Corlett, *HACCP User's Manual*, Aspen Publishers, Gaithersburg, Md., 1998.

J. F. Diehl, *Safety of Irradiated Foods*, 2nd ed., Marcel Dekker, New York, 1995.

W. A. Gould, *CGMP's/Food Plant Sanitation*, CTI Publications, Baltimore, Md., 1994.

W. A. Gould, *Unit Operations for the Food Industries*, CTI Publications, Baltimore, Md., 1996

D R. Heldman and D. B Lund, *Handbook of Food Engineering*, Marcel Dekker, New York, 1992.

S. D Holdsworth, *Aseptic Processing and Packaging of Food Products*, Elsevier, New York, 1992.

P. Jelen, *Introduction to Food Processing*, Prentice-Hall, Englewood Cliffs, N.J., 1989.

N. G. Marriott, *Essentials of Food Sanitation*, Chapman and Hall, New York, 1997.

N. N. Potter and J. H. Hotchkiss, *Food Science*, 5th ed., Chapman and Hall, New York, 1995.

G. L. Robertson, *Food Packaging. Principles and Practice*, Marcel Dekker, New York, 1993.

R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, 2nd ed., Academic Press, New York, 1993.

K. J. Valentas, E. Rotstein, and R. P. Singh, *Handbook of Food Engineering Practice*, CRC Press, New York, 1997.

R. C. Wiley, *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman and Hall, New York, 1994.

JAMES FALLER
University of Illinois
Urbana, Illinois

FOOD PROCESSING: EFFECT ON NUTRITIONAL QUALITY

The definition of *processing* is "a natural phenomenon marked by gradual changes that lead toward a particular result"; "a series of actions or operations conducing to an end"; or "a continuous operation or treatment especially in manufacture" (1). *Food processing*, therefore, refers to the series of actions involved in order to prepare and preserve a food supply by some continuous operation or treatment to achieve as a goal a safe, high-quality product with extended shelf life.

Changes that occur to food quality may be thought of as two types: deterioration and spoilage. Deterioration involves changes in quality induced by physicochemical and/or biochemical reactions taking place with or without the intervention of a physical environment (such as oxygen, carbon dioxide, water, light, heat, etc). Spoilage, on the other hand, generally refers to changes in quality due to action of biological agents such as bacteria, molds, or insects. Effects of deteriorative reactions and spoilage agents on food quality result in changes in both sensory properties (ie, appearance, flavor, texture) and nutritive value (vitamin content, protein value, etc). The extent to which these reactions can occur depends on the sensitivities and types of food products considered. For example, certain fruits such as strawberries or raspberries may be readily spoiled by the presence of molds. Green peas or beans, on the other hand, lose flavor and result in undesirable textures due to enzyme-induced reactions.

Through various types of food processing, quality factors can be maintained or extended, the extent of which depends on the food product to be considered. By canning fruits and vegetables (eg, pears, peaches, peas, beets) with proper heat sterilization and packaging, these products will not suffer spoilage, although they may deteriorate to some extent in terms of color, flavor, and texture. The extent of these changes depends on a wide array of factors, including storage conditions after packaging. Factors affecting quality of food products include: (*1*) initial quality of the raw materials and handling from harvesting to the manufacturing plant; (*2*) pretreatment (including cleaning, sanitizing, washing, aspiration, screening, filtration, chlorination, fumigation, etc); (*3*) sorting (removal of extraneous materials); (*4*) peeling, coring, dehairing, defeathering, husking, stemming, and so on; (*5*) disintegration/physical separation in cases where grinding, pulping, pressing, or expelling are needed, generally followed by screening, filtering, or centrifugation; and finally, of course (*6*) the final finished food manufacturing step. These final manufacturing steps comprise a myriad of different types of food processes including clarification/filtration; crystallization; curing/smoking; dehydration; evaporation and distillation; fermentation; foaming; mixing/shearing operations (whipping, kneading, blending); forming/shaping; heating/cooking; maturation/aging; or cooling/freezing.

To discuss the effects of food processing on nutritional quality, it is important to have an understanding of some of the basic types of food manufacturing available, their historical development and why they are used. Table 1 provides a very brief chronological sequence of events that have taken place in the development of food processing since the beginning of time. Although in a very subjective way there has been general interest in the effect of processing treatments on product integrity, most traditional processes have developed by accident over time. Any true acknowledgement of nutritional or overall quality of foods has only become more of a science and concern during the last half of the twentieth century. As knowledge expands on the effects of microorganisms and chemicals on man, the standards for purity and safety increase. The standards in the United States, for example, are largely determined by state and federal food and drug agencies as well as individual food industry standards.

In addition to introducing various types of manufacturing techniques and some of the processing parameters that influence quality of foods, it is also important to include a brief discussion on chemical kinetics, which encompasses the study of the rates at which chemical reactions proceed. It is through a basic understanding of kinetics of degradation of nutrients and general physical characteristics in combination with a thorough knowledge of how their reactions rates are affected by different processing conditions that a food technologist can optimize a food process to achieve the highest quality possible and predict a maximum shelf life for that product. The area of kinetics in food systems has received a great deal of attention in past years, primarily due to efforts to optimize or at least maximize the quality of food products during processing and storage. A good understanding of reaction kinetics provides a better idea of how to formulate or fortify food products to preserve and/or extend the existing nutrients or components in a food system or minimize the appearance of undesirable breakdown products. Although limited kinetic information is presently available for food systems or ingredients, several researchers have compiled kinetic data on various quality attributes, including enzyme and protein changes (2), flavor changes (3), physical/textural changes (4), and vitamins and pigments (5,6), in order to facilitate the development of mathematical formulas for the optimization of a given food process. The interaction of the study of kinetics and its relationship to food processing and maintaining optimum food quality will be discussed in a later section. Since a complete discussion of all types of food manufacturing and their effect on quality of foods is beyond the scope of this article, only a few selected processes that are representative of various influencing processing variables that may affect a food product will be presented.

THERMAL PROCESSING

One of the earliest commercially available and most prevalent methods of food preservation during the twentieth century has been thermal processing of foods. The term thermal processing generally refers to a process during which a food product is subjected to high temperatures with the objective of inactivating undesirable microorganisms and/or enzymes in order to comply with public health standards. It includes operations such as conventional and aseptic canning as well as pasteurization. Foods are by nature contaminated by microorganisms and contain indigenous enzymes that may cause undesirable changes in products during storage. Proper thermal processing can extend shelf life by weeks (eg, pasteurization) or months to years (eg, canning). Unfortunately, in addition to destruction of microorganisms, there is an associated undesirable degradation of heat-sensitive vitamins and other quality factors. It is, therefore, of critical importance to determine the appropriate heat treatment for individual food products when calculating process times to ensure high-quality and safe products.

Pasteurization

In the case with pasteurization, not all vegetative microorganisms are killed and thus must be stored under conditions that minimize growth. For example, with milk, pasteurization is used to kill pathogenic microorganisms; however, some vegetative spoilage microorganisms can still survive this heat treatment and, thus, milk requires refrigeration. In the case with beer, pasteurization serves mainly to kill spoilage microorganisms. Techniques used to extend shelf life include refrigeration; chemical additives that alter the microenvironment of food, reducing growth of microorganisms (eg, sweetened condensed milk, food acids in pickles and fruit juices); packaging (eg, maintain anaerobic conditions in bottled beer); and fermentations with desirable organisms (eg, cheese, yogurt, etc).

The individual heat treatment applied to a food product depends on the specific heat resistance of a particular microorganism that is used as a basis for the process. For instance, high-temperature, short-time (HTST) processing for milk is 161°F (71.7°C) for 15 s as compared with conventional pasteurization of 145°F (62.8°C) for 30 min. HTST is better due to less nutrient destruction and sensory quality changes. This is because destruction rates for nutrients and quality factors are less dependent on temperature than destruction rates for microorganisms. The process criteria for pasteurization of milk are the thermal destruction of *Coxiella burnetti*, the rickettsia organism responsible for Q fever. These process criteria vary according to food type and pH. For instance, in the case with high-acid fruits (cherries), the criteria are set for the destruction of yeasts and molds, and for fermented beverages (beer, wine), the criteria are set for the destruction of wild yeast. In the case with pasteurization of foods, since there is not established sterilization, these products need to have lowered storage temperatures.

Canning

Canning is a general term applied to processes in which a product is sealed in a hermetic container and contains no active microorganism or enzymes that could grow under conditions imposed during storage. A food product is not

Table 1. Historical Development of Processing and Preservation Techniques

Date	Event
30,000 B.C.	In-ground ovens /w/ hot stones, Europe
27,000 B.C	Clay ovens, Czech Republic
before 15,000 B.C. (Old Stone Age)	Roasting, pounding, drying
after 15,000 B.C (Middle Stone Age)	Dried fish, boiling, food storage, smoking, steaming
11,000 B.C.	First grain mills, North America
before 9000 B.C (New Stone Age)	Alcoholic fermentation, acetification, salting, baking, bread making, sieving, primitive pressing, seasoning
6000–5000 B.C	Olive oil press, Near East
4500 B.C	Dairy produce, butter, cheese, Near East
3500 B.C (Bronze Age)	Filtration, lactic acid fermentation, flotation, leavened bread, flavoring
1500 B.C. (Iron Age)	Refinement of flavoring, and cooking
1200 B.C.	Deep frying, Egypt
after 1000 B.C.	Ice used for refrigeration
600–400 B.C. (Roman Period)	Food adulteration common
A.D. 100s	Invention of mill /w/ vanes, Britain
A.D 300	Water mills
A.D 700s	Distillation of spirits, Iran
A.D. 1100	Distillation of alcoholic beverages, Italy
A.D. 1276	First official whiskey distillery, Ireland
1300s	Rice mill, Italy
end of Middle Ages	Preservation of fruits with sugar instead of honey
1500	Mechanical sieving
1588	Ramelli's grain mill
1678	Champaign, France
1795	Introduction to thermally processed foods (N. Appert)
1800–1870	Canning industry develops
1801	Beet sugar refinery, France
1835	Evaporated milk process patented
1851	High-pressure steam sterilizer patented by R. C. Appert
1850–1860	Pasteurization of wine
1853	Vacuum evaporated milk process developed (G. Borden)
1800–1850	Ice-making machines patented
1840	Roller mills for flour, Hungary
Civil War period	Expansion of canned food industry
late 1800's	Development of pure cultures for beer
1877	Laval's centrifugal cream separator
1899	First report on high-pressure preservation (B. H. Hite)
1902	Fat hydrogenation patented
1912	Discovery of vitamins, Poland
1915–1920	Large-scale refrigerated warehouses
1920	Electropure process for milk (electrical discharge for inactivation of microorganisms)
WW I	Introduction of dehydrated foods
1930s–1950s	Most work accomplished on isolation of individual vitamins
1940–1946 (WW II period)	Fortification of foods, expansion of dehydrated foods (eg, milk, eggs, vegetables)
1945	Introduction of radiation for preservation of foods
by end of 1950s	Irradiation ready for commercial processing of foods
1958	Irradiation of foods halted by FDA for three decades
1985	FDA approves irradiation for *Trichinella spiralis* control in pork
1986	FDA approves irradiation for microorganism control in dehydrated enzymes, herbs, spices, vegetable seasoning; disinfestation of vegetables and fruits; and ripening delay of fruits
1990	FDA approves irradiation for microorganism control in fresh/frozen poultry
1991	First commercially UHP-treated fruit products, Japan
1995	FDA approves irradiation for *Salmonella* control in animal feed and pet food
1997	FDA approves irradiation for microorganism control in fresh chilled/frozen red meats

necessarily sterile, however, since excessive storage temperatures (120–140°F [48.9–60°C]) could result in growth of certain microorganisms. To achieve commercial sterility, the following factors need to be considered: nature of the food (eg, pH); storage conditions following thermal processing; heat resistance of the microorganism or spore; heat-transfer characteristics of the food container and heating medium; and the initial load of microorganisms.

The nature of the food and its microenvironment during storage are extremely important for establishing guidelines for a thermal process. Since, generally, canned foods are void of oxygen, obligate aerobes do not pose health hazards or spoilage problems. Spores of anaerobic microorganisms are also less heat resistant than those of anaerobic organisms (facultative or obligative anaerobes). An exception would be the case of canned cured meat products where oxygen may not entirely be removed. Aerobes such as *Bacillus subtilis* and *Bacillus mycoides* have been identified in these cases as spoilage agents (7).

A major factor for consideration in anaerobic environments is the pH of the food system. For instance, there are extremely heat-resistant spores that may survive a typical commercial process but do not constitute a health or spoilage hazard because of the low pH of the food. For this reason, the protocol for thermal process design for foods has been set according to the pH of the food products as follows.

1. High-acid foods ($\text{pH} < 3.7$): spore-forming bacteria do not grow, and thermal inactivation is based on yeasts and molds
2. Acid foods with $3.7 < \text{pH} < 4.5$: processed to inactivate acid-producing spore formers (eg, *Bacillus coagulans*)
3. Low-acid foods with $\text{pH} > 4.5$: processed to inactivate *Clostridium botulinum*, which produces an extremely poisonous toxin and is assumed to be present in all products intended for canning; it cannot grow below pH 4.5.

Other spores that are more heat resistant than *C. botulinum* such as *Bacillus stearothermophilus*, a nonpathogenic thermophilic spore former 20 times more heat resistant but requires elevated storage temperatures (120–130°F [48.9–54.4°C]) to grow, are referred to as *flat sour* organisms. These could be potential problems if elevated storage temperatures were present. Often in qualifying a process, the organism used is another putrefactive anaerobe referred to as PA 3679 since it is less toxic, easy to assay, and exhibits a heat resistance slightly greater than *C. botulinum.*

Two main criteria must be considered in designing a thermal process to accomplish inactivation of spores or vegetative cells. The first is the understanding of thermal inactivation kinetics or rate of destruction of food-spoilage-causing organisms and the dependence of rate on temperature. The second is taking into consideration the temperature history of the product or the heat-transfer characteristics (heat penetration) that determine the temperature profiles achieved within the container during processing. Destruction of microorganisms and their spores generally obey first-order kinetics, although exceptions have been described by Stumbo (8). The rate of destruction is characterized by a first-order reaction rate constant. The general rate equation, $-dC/dt = kC$, as presented later in a short discussion on kinetics, applies to any first-order reaction process and is used here to describe the thermal inactivation of bacterial spores, where C = concentration of viable spores, k = the rate constant (t^{-1}) and t = time. After rearranging terms and integrating over time, this equation becomes $\ln [C_0/C] = kt$, where C_0 = initial concentration of viable spores. From a semilog plot of $\ln C$ versus time (t) of exposure to a constant lethal temperature, a straight line will result that intercepts the ordinate axis at $\ln C_0$ with a slope of $-k$. The temperature dependency of the rate constant is described by the Arrhenius equation. It is also an exponential function that is described by a straight line on a semilog plot $\ln k$ versus $1/T$. The equation is $\ln (k/k_0) = (E_a/R) \cdot [(T_0 - T)/T_0T]$, where T = temperature (K), k_0 = reference rate constant at a reference temperature (T_0), E_a = energy of activation (cal/mole), and R = universal gas constant (1.987 cal/mol · K).

In the canning industry, another term is used that is related to the first-order reaction rate constant, referred to as the D-value, defined as the time in minutes at a given temperature to reduce the population of microorganisms or spores by 90%, or the logarithmic order of death, using the common (base 10) logarithm. It has a similar time dependency as k, and the mathematical relationship between the two terms is $D = 2.303/k$. The temperature dependence of the rate of destruction (D-value) also follows a well-defined relationship: $\log (D)$ is linearly related to temperature (Fig. 1). It can be seen that a plot of $\log (D)$ versus temperature (°F) provides a z-value that is the reciprocal of the slope (Fig. 2). The z-value represents the temperature change (°F) necessary for the D-value to change by 90% (one log cycle). This plot is referred to as the thermal death time (TDT) curve. This z-value reflects the sensitivity of change of temperature on the destruction of the microorganism, similarly to the energy of activation (E_a). This provides the information required to equate the various time/temperature treatments in terms of thermal destruction of microorganisms and spores. In fact, it may be observed from Figure 2 that many different process designs could be selected to achieve the same thermal lethality.

The fact that thermal inactivation of microorganisms or spores follows a first-order reaction rate indicates that an

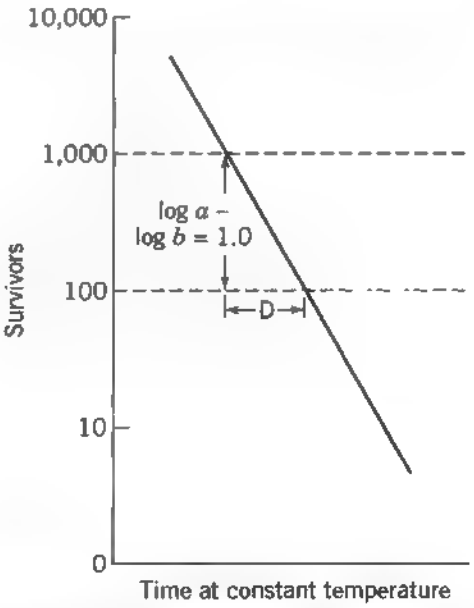


Figure 1. Graphical representation of a first-order kinetics for the destruction of bacterial spores.

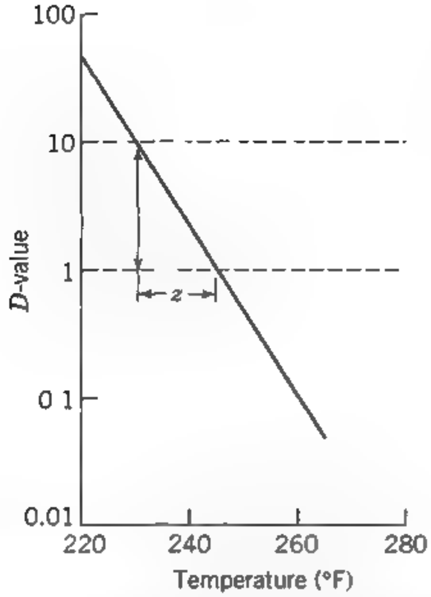


Figure 2. Graphical representation of thermal death time (TDT) for microorganisms.

absolute zero count theoretically cannot be reached. Each order of *D* minutes results in a reduction of 90%, which implies that 10% are left. Thus, this becomes the probability of survival and a number of *D*-values is used to define the order of the process. To specify a TDT curve, all that is needed is a slope (*z*-value) and a reference point providing a *D*-value at a reference temperature. For the sterilization of low-acid foods with highly resistant microorganisms, a reference temperature of 250°F (121°C) is generally specified and the order = 12*D*, or 12 decimal reductions. In the case of *C. botulinum*, in low-acid foods, the highest D_{250} value known for this organism is 0.21 min; therefore, the minimum process sterilizing value is $F = 0.21 \times 12 = 2.52$ min. Lethal rate may be calculated as $1/t = 10^{(T-250)/z}$. Actually, all low-acid foods are processed far beyond the minimum botulinum cook in order to deal with spoilage bacteria of much greater heat resistance. In the case of high-acid foods or pasteurization processes in which microorganisms have lower heat resistance, a reference temperature of 212° (100°C) or 150°F (65.6°C) is used. Factors affecting thermal resistance of microorganisms or spores are pH and buffer components (eg, salts), ionic environment, water activity (particularly concentrated-type products, such as condensed soups or evaporated milk), and overall medium composition.

This information in combination with the temperature history of the sample will provide the information required to design an appropriate thermal process. Foods processed in containers require that their temperature history be determined at the slowest heating point (8,9). Heat penetration depends on: (*1*) surface heat-transfer coefficient, (*2*) physical and thermal properties of the product, (*3*) ΔT between the steam and initial product temperature, (*4*) container size (overall dimensions), and (*5*) type of heat transfer within the container (ie, convection or conduction). A good review on determining heat penetration in canned foods, which is beyond the scope of this presentation, and the use of Ball's formula for calculating thermal process times is presented by Teixeira (10).

In optimizing a thermal process, it is not only important to inactivate microorganisms or spores but also to retain sensory and nutrient quality factors. Since reaction rate constants and dependency on temperature are very different for nutrient and sensory quality factors as compared with thermal destruction of microbes, an opportunity exists to optimize quality. Aseptic canning systems have become more and more prevalent in recent years to minimize quality losses that occur in slow heated foods processed in conventional retort systems and to allow more economical packaging systems. The basic concept allows sterilizing the product using either HTST or ultrahigh temperature (UHT) processing outside the container, followed by filling aseptically into separately sterilized containers. A good example of quality improvement with such aseptic systems has been presented by Josylyn and Heid (11) showing thermal inactivation of a bacteria ($F_0 = 6$ min) compared with thiamin destruction curves for various levels of loss (Fig. 3). The only criteria is that a time/temperature relationship be chosen along the TDT curve. It is clear that higher processing temperatures allow greater retention of thiamin than at lower processing temperatures at longer processing times. Thiamin is one of the most thermally labile of all the vitamins and provides a good illustration of how quality can be maximized without sacrificing microbiological safety. It should be kept in mind, however, that this particular example also assumes that a first-order rate re-

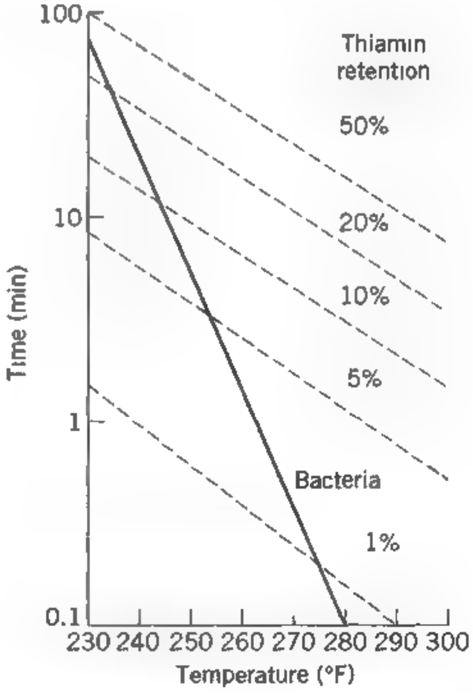


Figure 3. Time-temperature sterilization curve for bacteria ($F_0 = 6$ min) compared with time–temperature destruction curves for 1, 5, 10, 20, and 50% loss of thiamin. *Source:* Ref. 11.

action applies to the destruction of thiamin, which may or may not be the case with other nutrients.

Currently, most aseptically processed products are of fluid nature, although studies are constantly under way to apply aseptic processing to fluid products containing solid particulates, such as soups or stews with chunks of meat and vegetables. These particulates would be the cold spots in the process. Until recently, there has been no way to actually monitor the temperature at these points, and, thus, food engineers have relied on mathematical models to predict temperature changes. Mathematical models to account for heat-transfer coefficients have been demonstrated by Sastry (12). A joint task between the National Center for Food Safety and Technology (NCFST) and the Center for Aseptic Processing and Packaging Studies (CAPPS) resulted in establishing a protocol for use by any company that wants to produce a low-acid aseptic product containing particulates; their aseptic process filing was accepted by the FDA in 1997 (13). This milestone opened a new market for aseptically processed particulate foods, resulting in higher quality and safety, and better color, texture, and overall quality products. To further ensure safety and quality of thermally processed foods, specific guidelines have been set forth by the U.S. Code of Federal Regulations. All plants producing low-acid canned foods must be registered with the Food and Drug Administration (FDA), indicating name and place of business, location, processing method, and list of food products processed. There are four operational levels for low-acid foods: (*1*) adequacy of equipment and procedures to perform safe processing operations; (*2*) adequacy of record keeping to prove safe operations; (*3*) justification of the adequacy of time/temperature processes; and (*4*) qualifications of supervisory staff responsible for thermal processing and container closure operations.

Irradiation

Similarly to conventional thermal processing, the concept of irradiation is to temporarily raise the energy level of a system sufficient enough to result in the death of microorganisms, thus extending the shelf life of a product. In the case of irradiation, however, instead of raising the temperature of the system for a given period of time, the system is exposed to a source of radiation, resulting in ionization of individual atoms or molecules to produce an electron and a positively charged atom. The energy of radiation is often measured in electron volts (eV), where one electron volt is the energy acquired by an electron falling through a potential of 1 V (or $1/\text{eV} = 1.602 \times 10^{-12}$ erg). The total effect of the radiation on a given material depends on the energy of each photon, its source, as well as the total number of photons impinging on the material. The relative ionization of electrons varies with the depth of absorption in a given material (absorber). Using irradiation as a technique for preservation allows the foods to be kept cold or frozen during treatment, thus potentially allowing greater stability of quality factors of the food product.

In looking at units of measurement for irradiation of foods from an historical perspective, a variety of different units have been proposed. Since the amount of energy absorbed by a material and the amount of ionization produced, as a result of the interaction of radiations with a given material, depend on a number of variables—including the number of particles passing through the material, energy of the particles, and the nature of the absorber—measurement of the radiation dose becomes a complex issue. Early references mention the roentgen (R) as a unit of measure or the amount of energy delivered by X-rays or γ-rays to a gram of dry air or 83.3 erg. Since measurements in biological systems, however, were found to range from 93 erg/g in water or biological tissue up to 150 erg/g in bone, it was decided by the International Commission on Radiological Units that the term *rad* would be used. That is the quantity of radiation that results in the absorption of 100 erg/g at the point of interest (14). Now the radiation absorbed is conventionally reported in units of *grays* (Gy) or *kilograys* (kGy), where $1 \text{ Gy} = 1 \text{ J/kg}$ ($1 \text{ J} = 10^7$ erg).

Although the concept of using ionizing radiation for the preservation of foods has existed since about 1945 and near-commercialization since the end of the 1950s, its actual practice as a common food processing technique still has not taken a strong hold in the United States even at the close of the twentieth century. One of the reasons contributing to this delay was the passage of the Food Additives Amendment to the Food, Drug and Cosmetics Act in 1958, which classified radiation as a food additive rather than a preservation processing technique. This legislation resulted in extensive and time-consuming testing before acceptance could be considered (15). Although the first actual approval of irradiation of a food product in the United States was for insect disinfestation of wheat flour in 1963, approval for the first meat product for the control of *Trichinella spiralis* in pork was not until 1985. This was followed by approval in the United States of irradiation of chicken in 1990 and beef in 1997 (Table 1). A review by Pauli and Tarantino (16) presents the FDA's standards by which they determine safety of proposed applications of radiation. The major areas that they pursue to ensure safety include radiological, toxicological, and microbiological safety and nutritional adequacy. From a radiological point of view, there is no concern since the currently approved radiation sources are of too low an energy level to induce radioactivity. Assessment of the toxicological safety of irradiated foods was accomplished through the Bureau of Foods Irradiated Food Committee, set up by the FDA. After going through a series of assessments based on animal feeding and mutagenicity studies, the group concluded that radiation doses below 1 kGy present no evidence of possible toxicological risk. For larger radiation doses it was determined to assess the process on a case-by-case basis, particularly foods consumed in significant quantities.

There has been a great deal of controversy over irradiation of foods and its safety to both workers in the plants conducting the treatment as well as the handling and consumption of irradiated food products. To a large extent these concerns are still present, but with the education of consumers regarding safety and benefits of irradiation, opinions are gradually changing. Hashim and coworkers (17) made recommendations for increasing consumer acceptance of irradiated foods, including: (*1*) development of educational programs to increase consumer understanding of the role of irradiation of foods; (*2*) preparation of informative irradiation labels and/or posters to assure consumers of the safety of irradiated products; (*3*) conduct television shows, promoting children interaction along with pamphlets and informational brochures; and (*4*) conduct in-store sampling surveys of cooked irradiated foods. Since the FDA acceptance of irradiation to control growth of microorganisms in red meat in December of 1997, there has been a great deal of changing of public opinion over irradiation as a means to preserve foods. Resurreccion and Galvez (18) and Lusk et al. (19) have also discussed the need for consumer acceptance of irradiation as a safe processing method of preservation. At present the consumer's perception of this technique is still questionable but has been shown to greatly improve after conducting educational programs addressing the overall safety of the process.

From a microbiological safety viewpoint, some issues that are raised on the effects of irradiation of foods are whether irradiation may result in the mutation of microorganisms that may lead to more virulent pathogens and, if there is reduction of the spoilage microorganisms, whether, as a consequence, pathogens will be able to grow undetected without competition. The FDA, however, has indicated that radiation-induced mutation is not a concern with respect to increased virulence or increased heat resistance. In fact, Farkas (20) has indicated that radiation is more likely to reduce virulence of surviving pathogens. Other issues of concern involve losses of nutrients. There has been general evidence, however, that indicates conventional cooking alters the nutrient quality much more than irradiation. Macronutrients, including proteins, lipids, and carbohydrates, are not significantly affected up to doses of 10 kGy with only minor changes at sterilization doses of 50 kGy (21). There is evidence that vitamins may degrade with irradiation, which would be expected due to any process that elevates the energy level of the individual food constituents. The degree of degradation will depend on a number of factors such as the dose of radiation, the food type, the temperature at which irradiation occurs and the presence of oxygen. Generally, low-temperature radiation in the absence of oxygen reduces significantly the losses of vitamins as well as maintaining storage at low temperature and in sealed containers (22). Similarly to thermal processing, it has been found that thiamin is the most labile of the water-soluble vitamins in the presence of irradiation processing, whereas vitamin E has been shown to be the most susceptible of the oil-soluble vitamins to degradation from irradiation. The FDA requires that those vitamins most affected by irradiation are not a significant source from that particular processed food product in the overall diet.

Overall quality of irradiated foods may be affected by (*1*) radiation dose, (*2*) dose rate, (*3*) temperature and atmospheric conditions during irradiation (eg, presence of oxygen), (*4*) temperature and environmental conditions following irradiation during storage, and (*5*) development of radiolytic products (23). The presence of radiolytic products can result in oxidation of myoglobin and fat, which may result in discoloration and rancidity or other off-odor and/or off-flavor development. For instance, ozone, which is a strong oxidizer produced during irradiation in the presence of oxygen, may oxidize myoglobin, resulting in a bleached appearance. Color changes may be influenced by the packaging environment. It has been reported that irradiated vacuum-packaged meats develop a fairly stable bright pink or red color in turkey breasts, pork, or beef (24–26). This stresses the importance of elimination of oxygen before irradiation. Irradiation in the frozen state minimizes movement of free radicals to react throughout the food system and, thus, can minimize sensory quality issues. According to Kropf et al. (27) and Luchsinger et al. (28), any irradiation-induced off-odors may be removed during conventional cooking; however, studies are ongoing to investigate. Another issue that should be considered is the effect of the type of packaging that is used to prevent the evolution of hydrogen, low-molecular-weight hydrocarbons, and halogenated polymers (29). Packaging materials must be approved by the FDA according to the 21 CFR 179.45.

Flavor-transfer problems to the food could occur as a result of using conventional fresh meat overwrap (eg, polyvinylchloride). The development of detection methods for irradiation of foods is currently an active area of investigation. This is important from a regulatory compliance view point (15). With the development of these methods, there should be an acceleration of approval of irradiated foods as well as new applications and enhancement of international trade. Since irradiation involves no major chemical, physical, or sensory changes in foods, minute changes must be focused upon. Glidewell et al. (30) prepared a comprehensive review on detection methods for irradiated foods. For example, detection of hydrocarbon formation from irradiated lipid-containing foods offers a potential method of detection. Crone et al. (31) detected the formation of 2-alkyl-cyclobutanone formed from fatty acids in irradiated but not cooked foods. Other methods of detection include measurement of cell membrane damage through measurement of electrical impedance, electron spin resonance, and thermal and near-infrared analysis.

From a microbiological point of view, the predominant spoilage organisms are Gram-negative psychrotrophic microorganisms, which are very susceptible to irradiation (32). It has been shown that doses of about 1 kGy virtually eliminate Gram-negative microorganisms. However, it is not as effective on Gram-positive lactic acid–producing microorganisms. Nevertheless, refrigerated storage of meats has increased dramatically as a result of irradiation. Lambert et al. (33) found pork loin slices packaged under nitrogen and irradiated to 1 kGy had an extension of 21 days beyond the control at 5°C. As with TDT in conventional thermal processing, the death of a microorganism resulting from exposure to radiation can also be evaluated by plotting the logarithm of the surviving fraction, in this case, against dose. With thermal sterilization, the effect of kill is not solely dependent on the quantity of heat absorbed by the cell but also on the intensity factor (temperature) and on time. Radiation sterilization, on the other hand, is actually less complicated since the intensity factor is called *dose rate*, or the amount of radiation absorbed by the cell per unit time. Although dose rate has some lethal effects, it is possible to relate radiation effects to dose alone according to the following equation: $n = n_0e^{-D/D_0}$, where n = the number of live organisms after irradiation, n_0 = the initial number of microorganisms, D = dose of radiation received, and D_0 = constant dependent on organism type and environmental factors (Fig. 4).

Depending on the dose level of radiation energy applied, foods may be pasteurized to reduce or eliminate pathogens or they may be sterilized to eliminate all microorganisms. The approved sources of radiation for food use include γ-rays (produced by radioisotopes cobalt-60 or cesium-137) and machine-generated X rays (with a maximum energy of 5×10^6 electron volts [eV]) or electrons (with a maximum energy of 10 MeV).

FOOD PRESERVATION THROUGH CONTROL OF WATER

As mentioned in the general discussion of food preservation, the most serious limitation of shelf life and nutritional quality is from microbiological activity. This section covers briefly the concept of reducing the amount of water in food systems as a means of food preservation. By lowering the amount of water available for microorganisms to remain active and reducing mobility of potentially reactive chemical species in a food system, it is possible to extend quality of a product from both a microbiological and nutritional point of view. Food preservation processes falling in this category include concentration processes such as evaporation or freeze-concentration; dehydration including air drying, spray drying, freeze drying, and so on, and methods of adding salt or sugar and thereby lowering the available water by increasing osmotic pressure. Since all of these methods depend on lowering available water, instead of describing in complete detail each type of process, which is beyond the scope of this article, the overall general relationship between availability of water and chemical reactions or microbiological activity will be discussed in this section.

One of the most important concepts in understanding the relationship between moisture removal and shelf-life extension is that of *free* versus *bound* water in foods, which are terms that grew in importance particularly during the latter half of the twentieth century. It was realized that active water is more important to the stability of a food system than the total amount of water present, or water content (34). The term water activity (a_w) is a thermodynamic property defined as the ratio of the vapor pressure of water in a food system (p) to the vapor pressure of pure water (p_0) at a given temperature or the equilibrium relative humidity (ERH) of the air surrounding the food system at a given temperature, such that $a_w = p/p_0 = \%\ \text{ERH}/100$. Water activity is currently the most common term used by researchers and industry professionals in the food processing business. A sorption isotherm is a graphical illustration of the relationship of water activity, or relative humidity of the vapor space surrounding a food system, and the equilibrium moisture content of that material. An isotherm is generally divided into three major regions (Fig. 5). Region A represents strongly bound water with an enthalpy of vaporization considerably higher than that of pure water. This region represents the first layer of water molecules sorbed at hydrophilic, charged and polar groups of food constituents (proteins, polysaccharides) and includes structural water (hydrogen-bonded water), monolayer water, and hydrophobic hydration water (35). This bound water is generally considered unfreezable and unavailable for chemical reactions or acting as a plasticizer.

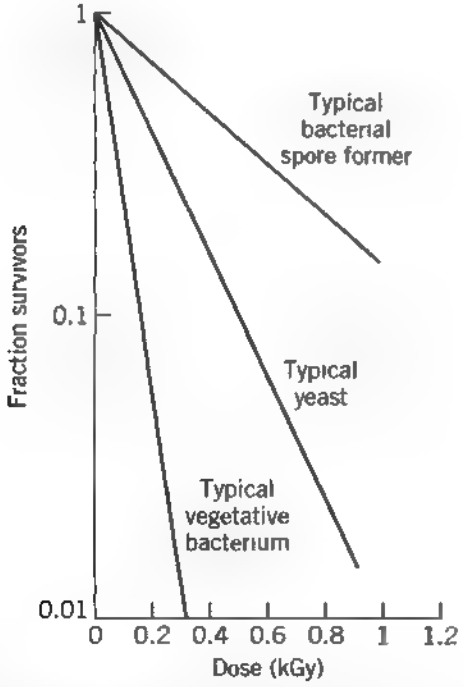


Figure 4. Representation of dose-response curves for different types of microorganisms. *Source:* Ref. 14.

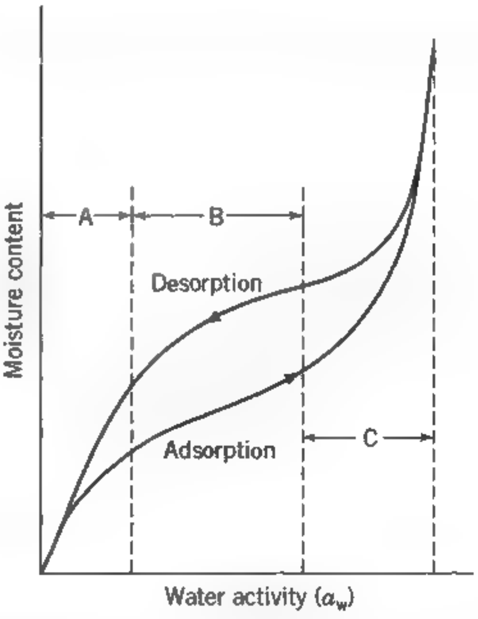


Figure 5. General type of sorption isotherm for food products. *Source:* Ref. 50.

Region B includes water molecules that are less firmly bound than those in region A with an enthalpy of vaporization only slightly more than that of pure water. This water may or may not be unfreezable and is available for low-molecular-weight solutes and some biochemical reactions. It may be considered a sort of transition zone between bound and free water. Region C consists of water molecules that have properties resembling more closely those of free water, often referred to as capillary water or that which is loosely bound with no excess heat of binding detected in this region.

Concerning the effect of temperature on water activity, as temperature increases, the amount of water adsorbed usually decreases. In fact, the effect of temperature on isotherms follows a Claussius–Clayperon relationship as depicted in an integrated form, holding moisture content constant: $\ln(a_2/a_1) = \Delta H_s/R(1/T_1 - 1/T_2)$, where ΔH_s = heat of sorption (cal/mole); $R = 1.987$ cal/mol · K; a_1 and a_2 are different water activities at their respective temperatures T_1 and T_2. ΔH_s refers to that amount of energy above and beyond that associated with the latent heat of vaporization. The average heat of sorption or the energy of binding of water (also referred to as E_b) can be determined by plotting the natural logarithm of a_w versus $1/T$ at different moisture contents. The resulting slope of the straight line is $-\Delta H_s/R$ (Fig. 6). Iglesias and Chirife (36) tabulated values for E_b for different food systems at different temperature ranges. Several techniques of measurement of water activity have been sited (37–41). Probably the most common approach to estimating the *amount* of bound water is through the Brunauer–Emmet–Teller (BET) isotherm or the "BET monolayer value" as determined from the following equation: $a/m(1 - a) = [1/m_1c] + [(c - 1)/(m_1c)]a$, where a = water activity, m = water content (g water/g solid), m_1 = monolayer value, and c = constant.

Information on sorption behavior is important in concentration and dehydration processes, particularly toward the end of a dehydration process where the total heat of vaporization (ΔH) plus heat of sorption are of concern. Because the heat of sorption depends on the partial pressure of water over the food and on the energy of water binding in the food, knowledge of these values is helpful in the design of these processes in calculating the amount of energy required for proper drying. Furthermore, since water activity affects stability, it must be brought to an appropriate level at the end of drying that is suitable for long-term storage. Different quality factors and microbiological growth all have different sensitivities to different water activities. Knowledge of the relative reaction rates in combination with heats of sorption and rates of moisture diffusivity in different types of food materials will provide a food technologist with the tools to design the most efficient process by which to remove water from a food in order for it to maximize drying and optimize quality with extended shelf life. It can be seen from Figure 7 that, depending on the type of food system, different levels of water activity will need to be achieved to best reduce degradation reactions of certain types. In general, for most foods, microbiological activity can be curtailed providing a water activity below 0.91 for bacteria and below 0.80 for most molds, although some xerophilic fungi have been found to grow as low as 0.65. On the other hand, oxidation-type reactions are minimal around 0.3 to 0.4 a_w, but nonenzymatic browning reactions are maximum around 0.4 to 0.60 a_w. It is clear that processing condition designs are highly dependent on the individual system under consideration. The importance of hysteresis should also be mentioned. By this it is meant that the upward (adsorption side) of the curve has a different path than the desorption side of the isotherm (Fig. 5). This in combination with the effect of temperature on hysteresis is also important in design and operating and controlling drying processes and reverse osmosis.

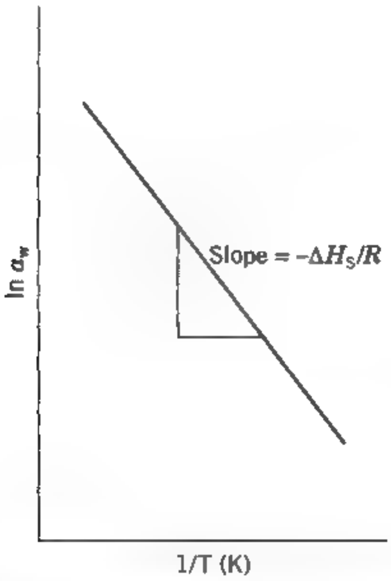


Figure 6. Graphical representation of determination of heat of sorption in food systems at constant moisture content.

Several types of deleterious changes may take place during dehydration, including nonenzymatic browning, lipid oxidation, vitamin degradation, pigment loss, reduced solubility and textural changes as well as flavor and aroma volatilization. Through an understanding of the influences of water activity on the rates of degradation of various food components, food technologists have the ability to design and regulate the processes during the different stages of drying to best maintain the integrity of the natural components of the system. For instance, it has been shown by several researchers that browning reactions follow zero-order kinetics after an induction period (42–44), and it is most severe toward the end of the drying period at low moisture where less evaporative cooling takes place (42). Franzen et al. (45) developed a model for nonenzymatic browning in skim milk within a temperature range of 35 to 130°C as a function of time, temperature, and moisture content. Ascorbic acid, on the other hand has been shown to be particularly sensitive at high moisture contents. To optimize vitamin C retention, a product should be dried at low temperatures during the constant rate period of drying, followed by increasing the temperature as the moisture content decreases (46). Rates of ascorbic acid degradation during drying have been reported in the literature (47–49). Different methods of de-

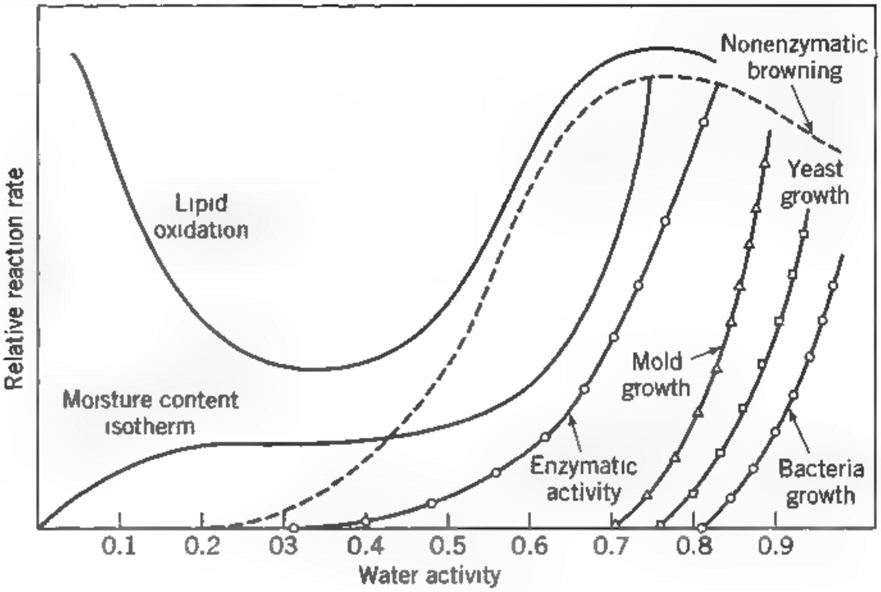


Figure 7. Relative rates of typical deteriorative reactions in foods as a function of water activity. *Source:* Ref. 99.

hydration and their major characteristics have been reviewed by Okos et al. (50).

EMERGING FOOD PROCESSING TECHNOLOGIES AND THEIR IMPLICATIONS ON QUALITY

High Pressure

As can be ascertained from the history of time in the developments made within the area of food processing and preservation and the gradual rise in concern over the nutritional benefits resulting from these processes, most advancement has occurred within the past half century. With the ever-increasing issues of a faster pace of life and the desire for more economical and better quality products for consumption, there has been greater interest in new processes to improve efficiencies. A few new technologies are beginning to emerge in the food industry that are worth mentioning. One area of recent interest is in using ultrahigh hydrostatic pressure (UHP) processing of foods. According to the Le Chatelier principle, any reaction, conformational change, or phase change that is accompanied by a decrease in volume will be favored at high pressure, while reactions involving an increase in volume will be inhibited (51). High-pressure treatments are independent of product size and geometry, and their effect is uniform and instantaneous, unlike conventional heat-transfer systems (52–54). It is somewhat difficult to predict, however, exactly the effects of increasing pressure on food systems due to their complexity. Pressure is applied via a pressure-transferring medium such as water or other fluid and can be effective at ambient temperature, thereby decreasing the amount of thermal energy needed during conventional processing (55,56). The following are considered the major effects of high pressure on food systems: (*1*) most important, the inactivation of microorganisms (57); (*2*) modification of biopolymers such as protein denaturation (58), enzyme inactivation or activation (59), or gel formation (60); (*3*) quality retention such as flavor and color (60,61); and (*4*) product functionality (eg, density changes, freezing and melting temperatures, or texture attributes (62,63). There have been reports that lipid oxidation may increase with application of high pressure in codfish and pork (64,65) but browning may decrease at elevated pressures (66). Due to the lower temperatures required for UHP treatment of foods, nutritional quality is subject to less degradation. UHP keeps covalent bond intact and affects only noncovalent ones, resulting in improvement in overall quality characteristics (61). For instance, Horie et al. (67) reported 95% retention of vitamin C in strawberry jam after UHP processing.

In high-pressure processing, foods are generally subjected to pressures in a range of 100 to 600 MPa at around room temperature. In April 1990, a Japanese company, Meidi-ya Food, introduced the first UHP-processed food, a high-acid fruit jam, and by May 1991 a variety of products including yogurts, fruit jellies, and sauces were UHP processed. The Japanese companies Pokka and Wakayama set up the first semicontinuous UHP processes for bulk treatment of citrus juices at a rate of 4000 to 6000 L/h (68). At present, however, there are no companies in the United States using this UHP process in food manufacture. The key to the success of this particular technology will be the inactivation of microorganisms with the simultaneous retention of nutritive and organoleptic characteristics of a given product. The attainment of reliable and consistent data on the destruction of microorganisms has yet to be accomplished. Great strides have been made by investigators; some have reported first-order kinetics on destruction of bacteria and yeast as a function of pressure (55,69–71). Some researchers, however, have reported a possible two-phase inactivation with the first population inactivated rapidly and the second found to be more resistant (72). It is important to determine inactivation kinetics as a function of pressure, temperature, and medium composition (73). There are many reports on the effect of UHP on reduction of microorganisms but few authors report kinetics, which as previously mentioned is essential to the prediction of destruction of pathogenic bacteria and spores. Those authors who have determined first-order kinetics have attempted to apply the traditional concepts of *D*- and *z*-values; however, other authors have shown evidence that death is not first order, indicating the invalidity of *D*- and *z*-values. As pointed out by authors Villota and Hawkes (5), a major drawback in thermal processing is the incomplete reporting of necessary conditions when collecting kinetic data. In the case of UHP processing, this is even more crucial with added variables, such as rate of pressure increase and decrease, the come-up time for the pressure, the actual isostatic pressure, and the initial population concentration. Their pressure resistance depends on the type of microorganism and suspension media composition, temperature of applied pressure, gas solubility, ionic strength, and pH. Recovery after release of pressure treatment is also an important consideration for proper assessment of the treatment.

Ohmic Heating/Pulsed Electric Field

Other areas of food processing that have received a great deal of attention include ohmic heating and pulsed electric field (PEF). Ohmic heating was one of the earliest forms of electricity applied to food pasteurization (74). Its mode of operation is by direct passage of electric current through the food product, which generates heat as a result of electrical resistance. Ohmic heating reduces the time of heating as compared with conventional heat transfer by convection or conduction. Its advantages are (*1*) rapid and uniform heating, (*2*) less thermal damage to product, (*3*) decreased operational costs, and (*4*) absence of hot surfaces and therefore reduced fouling, as occurs in UHT processing (75). Ohmic heating is of particular interest to aseptic processing, keeping in mind factors such as particle size, particle density, carrier viscosity and composition, and electrical conductivity (76). Although this technology was introduced in the early twentieth century, with the pasteurization of milk pumped between parallel plates with different voltages, the technology disappeared due to the lack of suitable electrode materials (ie, breakdown of carbon electrodes resulting in contamination of product) and adequate control systems. With the development of new electrode materials such as stainless steel, there has been renewed interest (77).

One of the earliest systems using electrical discharge for inactivation of microorganisms was the Electropure process for milk in the 1920s. This process involved passing an electric current through carbon electrodes and heating the milk to 70°C to inactivate *Mycobacterium tuberculosis* and *Escherichia coli*. An electrohydraulic treatment was introduced in the 1950s to inactivate microorganisms suspended in liquid foods. By increasing the electric field intensity, the frequency of the number of pulses and their duration, greater inactivation of microorganisms can be achieved (78–81). This, again, depends on other factors such as treatment temperature, pH, ionic strength, and conductivity of the fluid medium (ie, high-lipid-containing fluids are less conductive than aqueous media). Vega-Mercado et al. (82) have reviewed some of the most recent advances in the area of high-intensity PEFs. It is clear that this is still a developing technology but warrants mentioning as a new technology of the future in food processing. Nevertheless, a great deal of work needs to be accomplished in the area of determining the kinetics of destruction of microorganisms as a function of PEF; determination of uniform delivery of treatment; the impact of temperature, pH, moisture, and lipid content on the process; and the influence of food additives. Also, little is known about the influence of PEF on vitamin retention. It has been suggested that this methodology will have most of its potential in combination with other processing techniques.

REACTION KINETICS IN FOOD SYSTEMS

Advances in more efficient and versatile methods of food processing and preservation have been occurring exponentially over the past few decades in order to meet the continually increasing population and consumer's demands for quality foods, with particular focus on their nutritional aspects. Not only does quality of processed foods depend on the initial integrity of the raw materials, but also on the changes occurring during processing and subsequent storage. Emphasis is growing in the area of nutraceuticals and fortified high-energy foods (83–85). Not only nutritional quality is important to the food processor, however, but also the general appearance of the food, its flavor, color, and texture, many factors of which are highly dependent on the target consumer groups of interest, which are based on different cultural, geographical, and sociological backgrounds. It is, therefore, of critical importance to the food industry to minimize losses of quality in food products during processing, as well as subsequent storage. It is through the development of mathematical models to predict behavior of food components under a set of conditions and optimization of processes for maximum product quality that continued advancement can be achieved in processing techniques. To obtain these goals, extensive information is needed on the rates of destruction of quality parameters and their dependence on variables such as temperature, pH, light, oxygen, and moisture content. A food engineer can then develop new processing techniques to achieve optimum product quality based on an understanding of reaction rates and mechanisms of destruction of individual quality factors combined with the knowledge of the application of kinetics. There are three main areas of concern when dealing with reaction kinetics: (*1*) the stoichiometry, (*2*) the order and rate of reaction, and (*3*) the mechanism. For simple reactions, the stoichiometry is probably the first consideration. Once this is clarified or elucidated, the mechanisms involved in the reaction can be determined. It should be mentioned that based on actual kinetic data, our idea of the stoichiometry may change. In highly complex reactions, as is the case of many reactions occurring in food systems, a great deal of overlap exists among the three aforementioned areas. Thus, it is of critical importance to take a close and analytical look at the overall system to be able to characterize reaction pathways.

BASIC PRINCIPLES OF KINETICS

Order of Reaction

The first step in developing the basis for the kinetics of degradation of a particular nutrient is the determination of the order of the reaction. Understanding of the mechanisms involved in a reaction is important to properly obtain and report meaningful kinetic information, select reaction conditions leading to a desired end product, and/or minimize the appearance of undesirable compounds. Unfortunately, very seldom has effort been given to clearly understand the mechanisms involved in the reaction in complex systems, as in the cases with food and biological materials. Most information available has been oversimplified. In fact, most investigators have often tried to adapt fairly simple zero- or first-order reaction kinetics to complex situations without trying to understand the actual pathways involved. Although, from a practical point of view it is clear that simplifications may be taken, applicability of the information may be restricted only to the conditions encompassed by the experimental design, and thus, one may incorrectly predict trends by directly using reported information.

The reaction pathway, also called reaction mechanism, may be determined through proper experimentation. A chemical reaction may take place in a single step, as in the case of elementary reactions, or in a sequence of steps, as would be the case of most reactions occurring in food systems. Conditions such as temperature, oxygen availability, pressure, initial concentration, and the overall composition of the system may affect the mechanism of the reaction. For instance, the degradation of folic acid and ascorbic acid can be affected by the presence of oxygen, resulting in modification of the reaction pathway and thus, the type of breakdown products. Moreover, the rate at which these parent compounds disappear may be highly influenced by the presence and concentration of the breakdown products generated. It is true that the level of complexity involved in these reactions may be of such magnitude that a complete understanding of the mechanism of deterioration cannot always be easily determined or identified or, even more, may hinder the development of simple techniques to rapidly evaluate the stability of a given system. Nevertheless, it should be stressed that more reliable information is obtained when understanding of the reaction pathways is achieved. Reaction pathways for vitamin and pigment degradation kinetics have been reviewed (5).

The most common approach for the determination of the reaction order for a simple reaction, taking into consideration its initial rate, is as follows: $-dC/dt = k \cdot C^n$, where C = concentration, k = reaction rate constant, n = order of the reaction, and t = time. After taking the natural logarithm on both sides of the equation, a plot of the $\ln(-dC/dt)$ versus $\ln C$ will give a straight line, whose slope corresponds to the reaction order (n), as shown in Figure 8. Although the intercept should correspond to the reaction rate constant (k), it is normally considered that, for the sake of accuracy, this would not be the preferred approach for its estimation. Rather, once the reaction order has been determined, the rate constant can be calculated by applying the corresponding equation for that reaction order. Other methods can also be used to determine the order of the reaction with respect to the reactants and products involved in the reaction, depending on the stoichiometry (5).

Reaction Rate

A common approach in reporting reaction rates in food systems is as the change in concentration of a reactant as a function of time. The reaction rate thus provides a measurement of the reactivity and stability of a given component in a particular system. A number of factors that have been observed to influence the reaction rate include (*1*) concentration of reactants, products, and catalysts; (*2*) environmental factors such as temperature, pressure, and oxygen availability; (*3*) wavelength and intensity of light; and (*4*) physicochemical properties such as viscosity, pH, ionic strength, and conductivity. Depending on the type of reaction and the components, other factors will also be influential in controlling reaction kinetics.

Although traditionally one can apply reaction kinetics to monitor chemical changes occurring in a system, other physicochemical changes may also be described using a kinetic approach. For instance, textural and color changes occurring in food systems can be described using reaction rates. It is obvious that the numbers obtained represent the final effect caused by other complex reaction mechanisms leading to an overall result. For instance, color changes in a product containing carotenoids, may be an indication of the stability of the system, and in particular, stability of the carotenoids as related to environmental conditions. Another example is the textural changes in starch-based systems as a function of time, which may be the result of starch retrogradation mechanisms as well as lipid–amylose interactions as influenced by environmental conditions. Since reactions in food systems are normally complex and a combination of several elementary processes, additional basic information may be necessary to postulate reaction rate expressions. Identification of intermediates and previous knowledge of rate equations to fit data for other systems may provide assistance in properly characterizing a given reaction. The most commonly found reactions in food and biological systems are the zero-, first-, and second-order reactions.

Zero-Order Reactions. In zero-order reactions the rate is independent of the concentration. This may occur in two different situations: (*1*) when intrinsically the reaction rate is independent of the concentration of reactants and (*2*) when the concentration of the reacting compound is so large that the overall reaction rate appears to be independent of its concentration. Many catalyzed reactions fall in the category of zero-order reactions with respect to the reactants. On the other hand, the reaction rate may depend on the catalyst concentration or other factors unrelated to the concentration of the compound under investigation. Thus for a zero-order reaction at constant density, the overall expression would be as follows: $-dC/dt = k_0$, where C = concentration, k_0 = the zero-order reaction rate constant, and t = time. After integrating, this equation be-

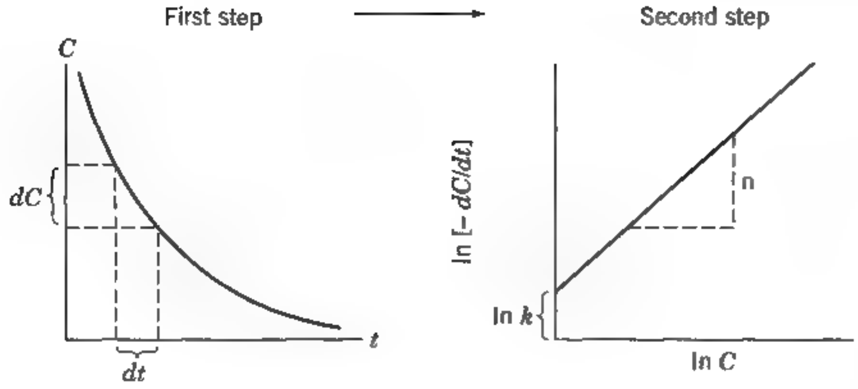


Figure 8. Graphical representation for determining reaction order (n) of a reaction.

comes $C_0 - C = k_0t$, where C = concentration at time t, and C_0 = initial concentration. According to this mathematical expression, a distinguishing feature for this type of reaction is a linear decrease in concentration as a function of time as illustrated in Figure 9. Typical reactions that have been represented by zero-order reactions include some of the autooxidation reactions. It is clear that zero-order reactions do not appear to occur as frequently in food systems as other reaction orders. In most cases, it is evident that the most common situation for this type of reaction is when the concentration of the reactants is so large that the system appears to be independent of concentration.

First-Order Reactions. A large number of reactions occurring in food systems appears to follow a first-order reaction. A mathematical expression for this behavior would be as follows: $-dC/dt = k_1C$, where k_1 = first-order reaction rate constant. By integration, this equation becomes $\ln[C/C_0] = k_1t$. Thus, according to this mathematical expression ln (C) versus time will be a linear function where the slope corresponds to $-k$ as shown in Figure 10. The term half-life ($t_{1/2}$) of a reactant is commonly used and may be described as $t_{1/2} = \ln 2/k_1$. These mathematical expressions clearly indicate that the half-life and the reaction rate for a true first-order reaction are independent of the initial concentration. Although in a number of systems this may be the case, often formulated products will not follow true first-order reaction kinetics, but rather a *pseudo-first-order* reaction. In fact, in formulated systems the presence of breakdown products may strongly influence the order of the reaction. However, for only a given value of initial concentration, the reaction may follow apparent first-order kinetics. To determine if a given reaction does indeed follow a pseudo-first-order kinetics, conditions for the kinetic study can be chosen to follow the technique of *flooding*. Through this approach, all but one of the concentrations are set sufficiently high that, compared with the one reagent present at lower concentration, the others are effectively constant during the time of the experiment. Since only one of the concentrations changes appreciably during the run, the effective kinetic order is reduced to the reac-

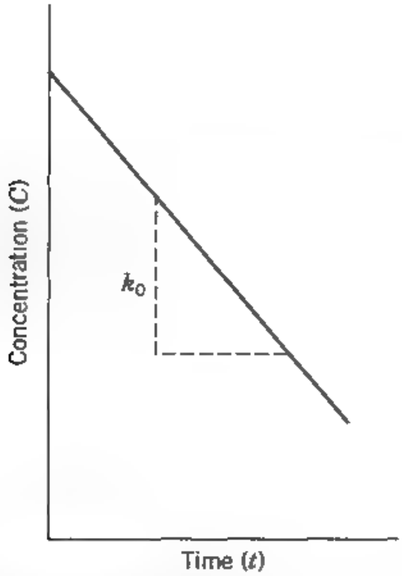


Figure 9. Graphical representation for the determination of a zero-order rate constant (k_0).

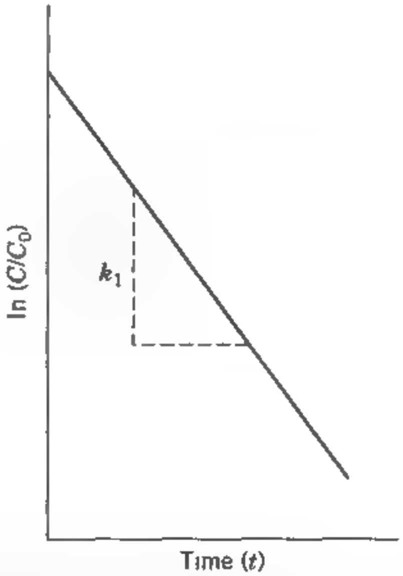


Figure 10. Graphical representation for the determination of a first-order rate constant (k_1).

tion order with respect to that one substance. If the order of the reaction is determined to be one, the reaction is said to follow a pseudo-first-order reaction. The degradation of ascorbic acid, for instance, has been primarily found to follow first-order kinetics in food systems. On the contrary, degradation of ascorbic acid in model systems has frequently been found to follow pseudo-first-order kinetics. It appears that the presence of breakdown products modifies the kinetics of deterioration of ascorbic acid, and, thus, its initial concentration will influence its rate of degradation. These factors, of course, serve to further complicate the prediction of nutrient retention.

Second-Order Reactions. Two types of second-order reaction kinetics are of importance. Type I, A + A → P, where A is a reactant and P is a product, may be mathematically described as $-dC_A/dt = k_2 \cdot C_A^2$. Type II reaction, A + B → P, where A and B are the reactants and P the product, may be mathematically described as $-dC_A/dt = k_2 C_A C_B$, where C_A = concentration of reactant species (A) at time (t), C_B = concentration of reactant species (B) at time (t) and k_2 = second-order reaction rate constant. For Type I, the integrated kinetic expression yields: $[1/C_A] - [1/C_{A_0}] = k_2 t$, which in terms of the half-life becomes $t_{1/2} = 1/k_2 \cdot C_{A_0}$. For Type II, the integrated form yields: $k_2 t = [1/(C_{A_0} - C_{B_0})] \cdot \ln [C_{B_0} \cdot C_A / C_{A_0} \cdot C_B]$, where C_{A_0} and C_{B_0} are the respective initial concentrations and C_A and C_B are the respective concentrations at time (t). It should be stressed, however, that Type II reactions do not have to necessarily follow a second-order reaction. For instance, for the particular case where component A is present in large amounts as compared with component B, the reaction may follow first-order kinetics with respect to B. A typical plot of second-order kinetics is presented in Figure 11.

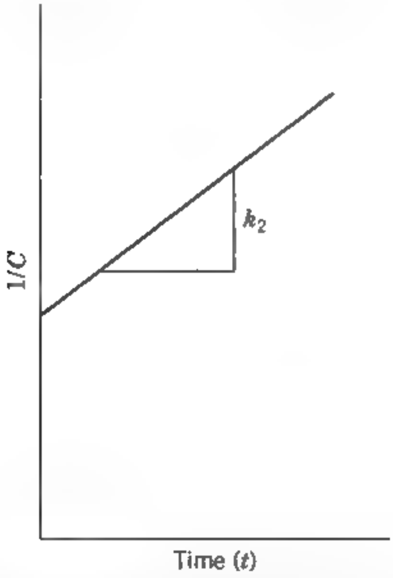


Figure 11. Graphical representation for the determination of a second-order rate constant (k_2) for a Type I reaction.

Nonelementary Reactions

Many different types of reactions fall under the category of nonelementary reactions, which will only be mentioned by name. To characterize the kinetics of nonelementary reactions, one can assume a series of individual elementary reactions taking place. In these reactions, intermediates may not be observed or quantitated, either because they are present in very small amounts or because they are unstable. Such reactions would fall under three main categories: (*1*) consecutive or series reactions, (*2*) reversible or opposing reactions that attain a finite equilibrium, and (*3*) parallel or competitive reactions. The types of intermediates postulated may fall in any one of the following categories, namely, free radicals, ions and polar substances, molecules, and transition complexes (chain reactions and nonchain reactions). It should be mentioned that a classical example of a transition complex-chain reaction is the degradation of β-carotene. It involves an autoxidation reaction involving three main periods: (*1*) induction (formation of free radicals), (*2*) propagation (free-radical chain reactions), and (*3*) termination (formation of nonradical products). A more in-depth analysis and mathematical approach to characterizing this reaction has been reported by several authors (86–89).

Another example of a transition complex reaction is that of a nonchain catalyzed type that may involve the interaction of a substrate with a catalyst to form a complex, followed by its decomposition to form a product. Upon decomposition, the catalyst is then regenerated and is capable of taking part in the reaction once again. An example of this type of transition complex reaction is that of enzyme-catalyzed reactions. It should be mentioned that most of the reactions occurring in biological systems are catalytic in nature. The basic principles for enzyme-catalyzed reactions have been presented by Michaelis–Menten, who proposed the theory of complex formation. Although the general principle of chemical kinetics may apply to enzymatic reactions, the phenomenon of saturation with substrate is unique to enzymatic reactions. In fact, at low substrate concentrations the reaction velocity is proportional to the substrate concentration, and thus the reaction is first order with respect to the substrate. As the substrate concentration increases, the reaction progressively decreases, being no longer proportional to the concentration of the substrate and deviating from any first-order kinetics. The reaction follows zero-order reaction kinetics, due to saturation with the substrate. For the particular case of enzyme kinetics, however, the cases of competitive and noncompetitive inhibition also add complications to the reaction and are thus not as easily defined by simple kinetics. A general description of enzyme-substrate kinetics can be found in most any classical biochemistry textbook (eg, 90, 91).

Temperature Effects

When considering reaction rates, it is clear that these values may be influenced by a large number of parameters, including temperature and pressure. In fact, equilibrium yields, chemical reaction rates, and product distribution may be drastically influenced by temperature. Since chemical reactions are accompanied by heat effects, if these are large enough to cause a significant change in temperature of the reaction mixture, these effects also need to be considered. This would be particularly important in reactor design. The effect of temperature for an elementary process may follow, in most cases, the Arrhenius equation: $k = k_0\exp(-E_aRT)$ where, k_0 = frequency or collision factor, E_a = activation energy (cal/mol), R = gas constant (1.987 cal/mol · K), and T = absolute temperature (K). It is obvious that if the frequency factor and the activation energy could be evaluated from molecular properties of the reactants, it would be possible to estimate the values corresponding to the reaction rate. Unfortunately, our knowledge of kinetics is limited, particularly for complex systems, as would be the case of food systems or products.

It is, however, important to mention the collision theory as an approach to deal with kinetics. In Figure 12, the energy levels involved in a reaction are illustrated. According to the collision theory, upon the collision of reactive molecules, enough energy is generated to provide the necessary activation energy. Such a theory was used as the foundation for the determination of rate expressions based on the frequency of molecular collision required to generate a minimum energy.

Another theory, the activated-complex or transition-state theory, has also been suggested. According to this approach, which still relies on reactions occurring due to collision between reactive molecules, an activated complex is formed from the reactants that eventually decomposes to generate products. The activated complex is in thermodynamic equilibrium with the reactants. Complex decomposition is, then, the limiting step. Regardless of the theories considered, they do not provide the means to rapidly and easily calculate activation energies from simple thermodynamic information. Thus in practical terms, one has to obtain basic kinetic information to be able to determine the effect of temperature as affecting reaction kinetics. Based on the Arrhenius equation, it is clear that if one plots the ln k versus $1/T$, the slope would correspond to the activation energy divided by the gas constant. Moreover, this value by itself will not provide any idea on the reactivity of a given system, only information on temperature dependence of the reaction.

Although the Arrhenius equation is commonly used to describe temperature dependence of the reaction rate in most food systems, deviations may occur as reported by several authors, including Labuza and Riboh (92). In fact, a large number of factors may contribute to deviations. Changes in reaction mechanisms may occur for a large temperature range. For instance, it is highly possible that mechanisms of deterioration may change at conditions below the freezing point due to a concentration effect. On the other hand, at high temperatures, changes in the physical state of some compounds, including fats and sugars, may occur. Lipids may change from a solid to a liquid state, while sugars may change from an amorphous to a crystalline or to a liquid state. Because of the high complexity of food systems, it is also possible that when various mechanisms of deterioration operate simultaneously, the effect of temperature may alter the rate of one, thus causing inhibition or catalysis in the other mechanisms. Finally, irreversible changes such as starch hydrolysis or protein denaturation may occur due to temperature, thus modifying the reactivity of the system. In fact, although enzyme-catalyzed reactions will have an increasing reaction rate upon an increase in temperature, a decrease will be observed beyond a certain temperature due to enzyme inactivation.

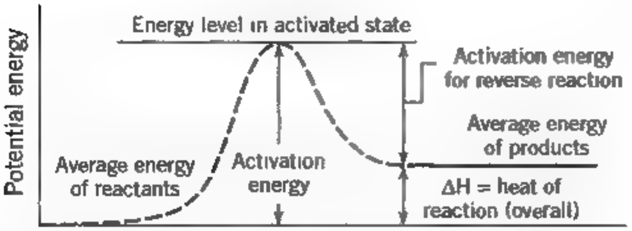

Energy level in activated state
Activation energy for reverse reaction
Potential energy
Average energy of products
Average energy of reactants
Activation energy
ΔH = heat of reaction (overall)

Figure 12. Representation of potential energy levels during the process of a given endothermic reaction.

Of particular importance to food processors is the determination of any nutritional and overall quality changes that may occur as a result of processing conditions encountered in operations such as dehydration, sterilization, extrusion, and so on. It is not only important to establish the extent of these undesirable changes, but it is also crucial to know the rate at which these undesirable changes take place. It is the ultimate goal of the food manufacturer to be able to optimize the quality of the final product while still maintaining an accurate perspective of the economics of this approach. Although a variety of methodologies have been proposed for the optimization of nutrient or quality retention during processing, only a few authors have actually tested the feasibility of these methods. Due to the complex nature of food materials, complete kinetics models are not easily attainable, resulting in one of the major obstacles in optimization in the food industry. Several researchers, however, have successfully applied optimization techniques to different types of food processes such as thiamin retention during sterilization (93) and ascorbic acid retention during air drying (94) using Pontryagin's maximum principle. Mishkin et al. (95) applied the complex method for optimization of ascorbic acid in air drying and minimizing browning in the dehydration of white potatoes. Saguy (96) published a practical text on computer-aided techniques for food technologists covering model building with applications and implementation of kinetics, simulation, heat transfer, and so on. With the fast-paced advancement in computer software, computer prototyping based on mathematical models rather than actual physical models has greatly aided product and process design. Datta (97) has reviewed some different commercially available computer-aided engineering (CAE) software programs and their sources with examples of computational fluid dynamics (CFD) and heat transfer for processes such as canning of liquid and solid foods, extrusion, and continuous sterilization of liquids.

FINAL REMARKS

It is important for the food technologist to attain a complete understanding of the principal processing variables involved in a general wide array of types of food processing techniques combined with a general knowledge of the kinetics of degradation of food quality characteristics. Although not discussed due to time constraints, the effect of storage conditions, whether ambient, refrigerated, frozen, or under controlled atmosphere, depending on its mode of preprocessing, is also an area of importance in maintaining quality of processed foods. Although generally many of the deleterious reactions occurring in foods are significantly slowed down at reduced temperatures, mechanisms of reaction can be altered such that prediction and optimization of these conditions becomes even more of a challenge. It should be kept in mind that some of the new emerging technologies in food processing will require more basic studies in order to predict retention of vitamins and organoleptic properties. Understanding of the mechanisms involved in reactions leading to the destruction of a compound or quality character should facilitate development of kinetics information with wider applicability. This will facilitate the optimization process of process and storage conditions and provide guidelines to determine formulation or fortification protocols of products leading to a higher nutritional value. It will also facilitate and encourage the development of new technologies for future food processing systems needed not only to feed the ever-expanding world population but also to ensure a high-quality nutritious food supply (98,99).

BIBLIOGRAPHY

1. *Merriam-Websters Collegiate Dictionary*, 10th ed., Merriam-Webster, Inc., Springfield, Mass., 1999.
2. D. R. Thompson and J. Norwig, "Microbial Population, Enzyme and Protein Changes During Processing," in M. R. Okos, ed., *Physical and Chemical Properties of Food*, ASAE, St. Joseph, Mich., 1986, pp. 202–265.
3. D. Lund, "Kinetics of Physical Changes in Foods," in M. R. Okos, ed., *Physical and Chemical Properties of Food*, ASAE, St. Joseph, Mich., 1986, pp. 367–381.
4. L. A. Wilson, "Kinetics of Flavor Changes in Foods," in M. R. Okos, ed., *Physical and Chemical Properties of Food*, ASAE, St. Joseph, Mich., 1986, pp. 382–407.
5. R. Villota and J. G. Hawkes, "Reaction Kinetics in Food Systems," in D. R. Heldman and D. B. Lund, eds., *Handbook of Food Engineering*, Marcel Dekker, New York, 1992, pp. 39–144.
6. R. Villota and J. G. Hawkes, "Kinetics of Nutrients and Organoleptic Changes in Foods During Processing," in M. R. Okos, ed., *Physical and Chemical Properties of Food*, ASAE, St. Joseph, Mich., 1986, pp. 266–366.
7. L. B. Jensen, *Microbiology of Meats*, 2nd ed., Garrard Press, Champaign, Ill., 1945.
8. C. R. Stumbo, *Thermobacteriology in Food Processing*, 2nd ed., Academic Press, New York, 1973.
9. A. López, ed., *A Complete Course in Canning, Book 1—Basic Information on Canning*, 11th ed., The Canning Trade, Inc., Baltimore, Md., 1981.
10. A. Teixeira, "Thermal Process Calculations," in D. R. Heldman and D. B. Lund, eds., *Handbook of Food Engineering*, Marcel Dekker, New York, 1992, pp. 563–619.
11. M. A. Joslyn and J. L. Heid, *Food Processing Operation: Their Management, Machines, Materials and Methods*, Vol II, AVI, Westport, Conn., 1963.
12. S. K. Sastry, "Mathematical Evaluation of Process Schedules for Aseptic Processing of Low-Acid Foods Containing Discrete Particles," *J. Food Sci.* **51**, 1323–1328 (1986).
13. S. Palaniappan and C. E. Sizer, "Aseptic Process Validated for Foods Containing Particulates," *Food Technol.* **51**(8), 60–68 (1997).
14. M. Karel, "Radiation Preservation of Foods," in M. Karel, O. R. Fennema, and D. B. Lund, eds., *Principles of Food Science. Part II. Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975, pp. 93–130.
15. D. G. Olson, "Irradiation of Food," *Food Technol.* **52**(1), 56–62 (1998).
16. G. H. Pauli and L. M. Tarantino, "FDA Regulatory Aspects of Food Irradiation," *J. Food Protect.* **58**, 209–212 (1995).
17. I. B. Hashim, A. V. A. Ressureccion, and K. H. MacWatters, "Consumer Attitudes Toward Irradiated Poultry," *Food Technol.* **50**(3), 77–80 (1996).
18. A. V. A. Resurreccion and F. C. F. Galvez, "Will Consumers Buy Irradiated Beef?," *Food Technol.* **53**(3), 52–55 (1999).
19. J. L. Lusk, J. A. Fox, and C. L. McIlvain, "Consumer Acceptance of Irradiated Meat," *Food Technol.* **53**(3), 56–59 (1999).
20. J. Farkas, "Microbiological Safety of Irradiated Foods," *Rev. Int. J. Food Microbiol.* **9**, 1–15 (1989).
21. J. F. Diehl, ed., "Nutritional Adequacy of Irradiated Foods," in *Safety of Irradiated Foods*, 2nd ed., Marcel Dekker, New York, 1995.
22. *Safety and Nutritional Adequacy of Irradiated Food*, World Health Organization, Geneva, Switzerland, 1994.
23. D. W. Thayer, "Food Irradiation: Benefits and Concerns," *J. Food Quality* **13**, 147–169 (1990).
24. J. A. Lynch, H. J. H. MacFie, and G. C. Mead, "Effect of Irradiation and Packaging Type on Sensory Quality of Chilled-stored Turkey Breast Fillets," *J. Food Sci. Technol.* **26**, 653–668 (1991).
25. N. Lebepe et al., "Changes in Microflora and Other Characteristics of Vacuum-packaged Pork Loins Irradiated at 3.0 kGy," *J. Food Sci.* **55**, 918–924 (1990).
26. J. G. Niemand, H. J. Van der Linde, and W. H. Holzapfel, "Shelf-life Extension of Minced Beef Through Combined Treatments Involving Radurization," *J. Food Protect.* **46**, 791–796 (1983).
27. D. H. Kropf et al., "Palatability, Color, and Shelf-life of Low-dose Irradiated Beef," in *Proc. of 1995 Int. Congr. of Meat Science and Technology*, San Antonio, Tex., 1995.
28. S. E. Luchsinger et al., "Sensory Analysis and Consumer Acceptance of Irradiated Boneless Pork Chop," *J. Food Sci.* **61**, 1261–1266 (1996).
29. M. Lee et al., "Irradiation and Packaging of Fresh Meat and Poultry," *J. Food Protect.* **59**, 62–72 (1996).
30. S. M. Glidewell et al., "Detection of Irradiated Food: A Review," *J. Sci. Food Agric.* **61**, 281–300 (1993).
31. A. V. J. Crone, J. T. G. Hamilton, and M. H. Stevenson, "Effects of Storage and Cooking on the Dose Response of 2-Dodecylcyclobutanone, a Potential Marker for Irradiated Chicken," *J. Sci. Food Agric.* **58**, 249–252 (1992).
32. J. D. Monk, L. R. Beuchat, and M. P. Doyle, "Irradiation Inactivation of Food-borne Microorganisms," *J. Food Protect.* **58**, 197–208 (1995).

33. A. D. Lambert, J. P. Smith, and K. L. Dodds, "Physical, Chemical and Sensory Changes in Irradiated Fresh Pork Packaged in Modified Atmosphere," *J. Food Sci.* **57**, 1294–1299 (1992).
34. W. J. Scott, "Water Relations of Food Spoilage Microorganisms," *Adv. Food Res.* **7**, 83–127 (1957).
35. J. E. Kinsella and P. F. Fox, "Water Sorption by Proteins: Milk and Whey Proteins," *Crit. Rev. Food Sci. Nutr.* **24**, 91– (1986).
36. H. A. Iglesias and J. Chirife, *Handbook of Food Isotherms*, Academic Press, New York, 1982.
37. J. A. Troller and J. H. B. Christian, *Microbial Survival, Water Activity and Food*, Academic Press, New York, 1978.
38. M. Karel, "Water Activity and Food Preservation," in M. Karel, O. R. Fennema, and D. B. Lund, eds., *Principles of Food Science. Part II. Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975, pp. 237–263.
39. T. P. Labuza, *Moisture Sorptions: Practical Aspects of Isotherm Measurement and Use*, American Association of Cereal Chemists, St. Paul, Minn., 1984.
40. S. S. H. Rizvi, "Thermodynamic Properties of Foods in Dehydration," in M. A. Rao and S. Rizvi, eds., *Engineering Properties of Foods*, 2nd ed., Marcel Dekker, New York, 1995, p. 123.
41. M. S. Rahman, *Food Properties Handbook*, CRC Press, Boca Raton, Fla., 1995.
42. C. E. Hendel, V. G. Silveira, and W. O. Harrington, "Rates of Nonenzymatic Browning of White Potato During Dehydration," *Food Technol.* **9**(9), 433–438 (1955).
43. C. Petriella et al., "Kinetics of Deteriorative Reactions in Model Food Systems of High Water Activity: Color Changes Due to Nonenzymatic Browning," *J. Food Sci.* **50**, 622–626 (1985).
44. R. K. Singh, *Kinetics and Computer Simulation of Storage Stability in Intermediate Moisture Foods*, Ph.D. Thesis, University of Wisconsin, Madison, Wis., 1983.
45. K. Franzen, R. K. Singh, and M. R. Okos, "Kinetics of Nonenzymatic Browning in Dried Skim Milk", *J. Food Eng.* **11**, 225–239 (1990).
46. M. Mishkin, I. Saguy, and M. Karel, "Optimization of Nutrient Retention During Processing: Ascorbic Acid in Potato Dehydration," *J. Food Sci.* **49**, 1262–1266 (1984).
47. R. Villota and M. Karel, "Prediction of Ascorbic Acid Retention During Drying. I. Moisture and Temperature Distribution in a Model System," *J. Food Process. Preserv.* **4**, 111–134 (1980).
48. R. Villota and M. Karel, "Prediction of Ascorbic Acid Retention During Drying. II. Simulation of Retention in a Model System," *J. Food Process. Preserv.* **4**, 141–159 (1980)
49. S. G. Haralampu and M. Karel, "Kinetic Models for Moisture Dependence of Ascorbic Acid and β-Carotene Degradation in Dehydrated Sweet Potato," *J. Food Sci.* **48**, 1872– (1983).
50. M. R. Okos et al., "Food Dehydration," in D. R. Heldman and D. B. Lund, eds., *Handbook of Food Engineering*, Marcel Dekker, New York, 1992, pp. 437–462.
51. A. Williams, "New Technologies in Food Preservation and Processing: Part II," *Nutr. Food Sci.* **1**, 16 (1994).
52. G. D. Alemán et al., "Pulsed Ultra High Pressure Treatments for Pasteurization of Pineapple Juice," *J. Food Sci.* **61**, 388 (1996)
53. D. Knorr, "Effects of High-Hydrostatic-Pressure Process on Food Safety and Quality," *Food Technol.* **47**(6), 156–161 (1993).
54. F. Zimmerman and C. Bergman, "Isostatic Pressure Equipment for Food Preservation," *Food Technol.* **47**(6), 162–163 (1993).
55. C. Hashizume, K. Kimura, and R. Hayashi, "Kinetic Analysis of Yeast Inactivation by High Pressure Treatment at Low Temperatures," *Biosci. Biotechnol. Biochem.* **59**, 1455 (1995).
56. E. Palou et al., "Combined Effect of High Hydrostatic Pressure and Water Activity on *Zygosaccharomyces bailii* Inhibition," *Lett. Appl. Microbiol.* **24**, 417 (1997).
57. D. G. Hoover et al., "Biological Effects of High Hydrostatic Pressure on Food Microorganisms," *Food Technol.* **43**(3), 99–107 (1989).
58. K. Heremans, "High Pressure Effects on Proteins and Other Biomolecules," *Ann. Rev. Bioeng.* **11**(1), 1–21 (1982).
59. E. Morild, "The Theory of Pressure Effects on Enzymes," in C. B. Anfinsen, J. T. Edsall, and F. M. Richards, eds., *Advances in Protein Chemistry*, Vol. 34, Academic Press, London, United Kingdom, 1981, pp. 93–166.
60. J.-C. Cheftel, "Applications des Hautes Pressions en Technologie Alimentaire," *Actualité des Industries Alimentaires et Agro-Alimentaires* **108**(3), 141–153 (1991).
61. R. Hayashi, "Application of High Pressure to Food Processing and Preservation: Philosophy and Development," in W. E. L. Spiess and H. Schubert, eds., *Engineering and Food*, Vol. 2, Elsevier Applied Science, London, United Kingdom, 1989, pp. 815–826.
62. D. Farr, "High Pressure Technology in the Food Industry," *Trends Food Sci. Technol.* **1**, 14–16 (1990).
63. T. Deuchi and R. Hayashi, "Pressure-Application to Thawing of Frozen Foods and to Food Preservation Under Sub-Zero Temperature," in R. Hayashi, ed., *High Pressure Science for Food*, San-ei Pub. Co., Kyoto, Japan, 1991, pp. 101–110.
64. P. B. Cheah and D. A. Ledward, "High Pressure Effects on Lipid Oxidation," *J. Am. Oil Chem. Soc.* **72**, 1059 (1995).
65. P. B. Cheah and D. A. Ledward, "High Pressure Effects on Lipid Oxidation in Minced Pork," *Meat Sci.* **43**, 123 (1996).
66. T. Tamaoka, N. Itoh, and R. Hayashi, "High Pressure Effect on Maillard Reaction," *Agric. Biol. Chem.* **55**, 2071–2074 (1991).
67. Y. Horie et al., "Jam Preparation by Pressurization," *Nippon Nogeikagaku Kaishi* **65**, 975–980 (1991).
68. B. Mertens and G. Deplace, "Engineering Aspects of High Pressure Technology in the Food Industry," *Food Technol.* **47**(6), 164–169 (1993).
69. P. Butz and H. Ludwig, "Pressure Inactivation of Microorganisms at Moderate Temperatures," *Physica* **139** & **140B**, 875 (1986).
70. A. Carlez et al., "High Pressure Inactivation of *Citrobacter freundii, Pseudomonas fluorescens* and *Listeria innocua* in Inoculated Minced Beef Muscle," *Lebensm.-Wiss. Technol.* **26**, 357 (1993).
71. J. Smelt and G. Rijke, "High Pressure Treatment as a Tool for Pasteurization of Foods," in C. Balny et al., eds., *High Pressure and Biotechnology*, Vol. 224, Colloque INSERM, John Libbey Eurotext, Montrouge, France, 1992
72. J.-C. Cheftel, "High Pressure, Microbial Inactivation and Food Preservation," *Food Sci. Technol. Int.* **1**, 75 (1995).
73. H. Ludwig et al., "Inactivation of Microorganisms by Hydrostatic Pressure," in C. Balny et al., eds., *High Pressure and Biotechnology*, Vol. 224, Colloque INSERM, John Libbey Eurotext, Montrouge, France, 1992.
74. K. Allen, V. Eidman, and J. Kinsey, "An Economic Engineering Study of Ohmic Food Processing," *Food Technol.* **50**(5), 269–273 (1996).
75. E. Scott, "Ohmic Heating Hits Commercial Scale," *Food Technol.* **NZ30**(7), 8 (1995).

76. P. Zoltai and P. Swearingen, "Product Development Considerations for Ohmic Processing," *Food Technol.* **50**(5), 263–266 (1996).
77. S. Palaniappan, S. K. Sastry, and E. R. Richter, "Effects of Electricity on Microorganisms: A Review," *J. Food Proc. Preserv.* **14**, 393 (1990)
78. R. Benz and U. Zimmermann, "Pulse-length Dependence of the Electrical Breakdown in Lipid Bilayer Membranes," *Biochim. Biophys. Acta* **597**, 637 (1980).
79. D. Knorr et al., "Food Application of High Electric Field Pulses," *Trends Food Sci. Technol.* **5**, 71 (1994).
80. T. Y. Tsong, "Review on Electroporation of Cell Membranes and Some Related Phenomena," *Biochim. Bioeng.* **24**, 271 (1990).
81. J. W. Larkin and S. H. Spinak, "Regulatory Aspects of New/Novel Technologies," in D. I. Chandarana, ed., *New Processing Technologies Yearbook*, National Food Processors Association, Washington, D.C., 1996.
82. H. Vega-Mercado et al., "Nonthermal Preservation of Liquid Foods Using Pulsed Electric Fields," in M. S. Rahman, ed., *Handbook of Preservation*, Marcel Dekker, New York, 1999, pp. 487–520.
83. A. E. Sloan, "The New Market: Foods for the Not-So-Healthy," *Food Technol.* **53**(2), 54–60 (1999).
84. M. Molyneau and C. M. Lee, "The U.S. Market for Marine Nutraceutical Products," *Food Technol.* **52**(6), 56–57 (1998).
85. J. Giese, "Vitamin and Mineral Fortification of Foods," *Food Technol.* **49**(5), 109–122 (1995).
86. E. V. Alekseev et al., "Kinetic Principles of the Oxidation of Polyenic Hydrocarbons. Communication 1. Decomposition of β-Carotene in the Presence of Free Radical Initiators," *Bulletin of the Academy of Sciences of the USSR. Division of Chemical Sciences* (translated from *Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya*), **11**, 2342–2347 (1968).
87. A. B. Gagarina, O. T. Kasaikina, and N. M. Émanuél, "Kinetics of Autooxidation of Polyene Hydrocarbons in Aromatic Solvents," *Doklady Akademii Nauk SSSR* **195**, 387–390 (1970).
88. E. I. Finkel'shtein, É. V. Alekseev, and É. I. Kozlov, "The Kinetics of β-Carotene Solid Film Autooxidation," *Doklady Akademii Nauk SSSR* **208**, 1408–1411 (1973).
89. E. I. Finkel'shtein, É. V. Alekseev, and É. I. Kozlov, "Kinetic Relationships of the Solid State Autooxidation of β-Carotene," *Zhurnal Organicheskoi Khimii* **10**, 1027–1034 (1974).
90. A. L. Lehninger, *Biochemistry*, 2nd ed., Worth, New York, 1975.
91. M. F. Mallette et al., *Introductory Biochemistry*, Williams & Wilkins, Baltimore, Md., 1971.
92. T. P. Labuza and D. Riboh, "Theory and Application of Arrhenius Kinetics to the Prediction of Nutrient Losses in Foods," *Food Technol.* **36**(10), 66–74 (1982).
93. I. Saguy and M. Karel, "Optimal Retort Temperature Profile in Optimizing Thiamine Retention in Conduction-type Heating of Canned Foods," *J. Food Sci.* **44**, 1485 (1979).
94. M. Mishkin, M. Karel, and I. Saguy, "Applications of Optimization of Food Dehydration," *Food Technol.* **36**(7), 101–109 (1982).
95. M. Mishkin, I. Saguy, and M. Karel, "Dynamic Optimization of Dehydration Processes. Minimizing Browning in Dehydrated Potatoes," *J. Food Sci.* **48**, 1617–1621 (1983).
96. I. Saguy, ed., *Computer-Aided Techniques in Food Technology*, Marcel Dekker, New York, 1983.
97. A. K. Datta, "Computer-Aided Engineering in Food Process and Product Design," *Food Technol.* **52**(10), 44–52 (1998).
98. M. Toussaint-Samat, *History of Food* (Anthea Bell, Trans.), Barnes & Noble, New York, 1998.
99. J. M. Flink, "Intermediate Moisture Food Products in the American Marketplace," *J. Food Proc. Preserv.* **1**, 324–339 (1977).

JAMES G. HAWKES
Continental Colloids, Inc.
Chicago, Illinois

FOOD PROCESSING: STANDARD INDUSTRIAL CLASSIFICATION

When one is in a grocery store, one is faced with an incredible selection of processed foods. For food manufacturers, this illustrates only one point: the amount of research and development that precedes their marketing. For regulatory agencies such as the Food and Drug Administration and the Department of Agriculture, this confirms the amount of work needed to assure the safety and wholesomeness of these products. However, for some other federal agencies, this poses an entirely different headache. For example, the Department of Labor (DOL) and the Environmental Protection Agency (EPA) must classify and give a name to each establishment that manufactures each product in order to enforce their legal mandates. To them it is a huge logistic nightmare. For example, the DOL must have access to a record of the number of employees suffering injuries in each type or category of food processing plants. To fill this need, the executive branch of the government has developed the Standard Industrial Classification (SIC) for food processing plants. The *SIC Manual* is one of the most useful federal publications available to both government agencies and the industries. One example of its usefulness is in assisting the allocation of resources. For example, a government agency can plan its budget accordingly by using the SIC to determine the number of food processing plants it has to inspect annually. Another application is the use of SIC to determine the employment status of the food processing profession. Such classification applies only to food processing establishments in the United States and this article discusses the SIC in details.

STANDARD INDUSTRIAL CLASSIFICATION MANUAL

The SIC Manual is issued by the Office of Management and Budget (OMB) of the Executive Office of the President of the United States. The manual divides industries in the United States into major groups. One major group 20 covers food and kindred products. This group includes establishments manufacturing or processing foods and beverages for human consumption, and certain related products, such as manufactured ice, chewing gum, vegetable and animal fats and oils, and prepared feeds for animals and fowls. Products described as dietetic are classified in the same manner as nondietetic products, eg, as candy, canned fruits, and cookies. Chemical sweeteners are classified in Major Group 28.

Major Group 20 is subdivided according to the following criteria (Table 1).

The following discusses each industry under food and kindred products. The abbreviations used are: * = The

76. P. Zoltai and P. Swearingen, "Product Development Considerations for Ohmic Processing," *Food Technol.* **50**(5), 263–266 (1996).
77. S. Palaniappan, S. K. Sastry, and E. R. Richter, "Effects of Electricity on Microorganisms: A Review," *J. Food Proc. Preserv.* **14**, 393 (1990)
78. R. Benz and U. Zimmermann, "Pulse-length Dependence of the Electrical Breakdown in Lipid Bilayer Membranes," *Biochim. Biophys. Acta* **597**, 637 (1980).
79. D. Knorr et al., "Food Application of High Electric Field Pulses," *Trends Food Sci. Technol.* **5**, 71 (1994).
80. T. Y. Tsong, "Review on Electroporation of Cell Membranes and Some Related Phenomena," *Biochim. Bioeng.* **24**, 271 (1990).
81. J. W. Larkin and S. H. Spinak, "Regulatory Aspects of New/Novel Technologies," in D. I. Chandarana, ed., *New Processing Technologies Yearbook*, National Food Processors Association, Washington, D.C., 1996.
82. H. Vega-Mercado et al., "Nonthermal Preservation of Liquid Foods Using Pulsed Electric Fields," in M. S. Rahman, ed., *Handbook of Preservation*, Marcel Dekker, New York, 1999, pp. 487–520.
83. A. E. Sloan, "The New Market: Foods for the Not-So-Healthy," *Food Technol.* **53**(2), 54–60 (1999).
84. M. Molyneau and C. M. Lee, "The U.S. Market for Marine Nutraceutical Products," *Food Technol.* **52**(6), 56–57 (1998).
85. J. Giese, "Vitamin and Mineral Fortification of Foods," *Food Technol.* **49**(5), 109–122 (1995).
86. E. V. Alekseev et al., "Kinetic Principles of the Oxidation of Polyenic Hydrocarbons. Communication 1. Decomposition of β-Carotene in the Presence of Free Radical Initiators," *Bulletin of the Academy of Sciences of the USSR. Division of Chemical Sciences* (translated from *Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya*), **11**, 2342–2347 (1968).
87. A. B. Gagarina, O. T. Kasaikina, and N. M. Émanuél, "Kinetics of Autooxidation of Polyene Hydrocarbons in Aromatic Solvents," *Doklady Akademii Nauk SSSR* **195**, 387–390 (1970).
88. E. I. Finkel'shtein, É. V. Alekseev, and É. I. Kozlov, "The Kinetics of β-Carotene Solid Film Autooxidation," *Doklady Akademii Nauk SSSR* **208**, 1408–1411 (1973).
89. E. I. Finkel'shtein, É. V. Alekseev, and É. I. Kozlov, "Kinetic Relationships of the Solid State Autooxidation of β-Carotene," *Zhurnal Organicheskoi Khimii* **10**, 1027–1034 (1974).
90. A. L. Lehninger, *Biochemistry*, 2nd ed., Worth, New York, 1975.
91. M. F. Mallette et al., *Introductory Biochemistry*, Williams & Wilkins, Baltimore, Md., 1971.
92. T. P. Labuza and D. Riboh, "Theory and Application of Arrhenius Kinetics to the Prediction of Nutrient Losses in Foods," *Food Technol.* **36**(10), 66–74 (1982).
93. I. Saguy and M. Karel, "Optimal Retort Temperature Profile in Optimizing Thiamine Retention in Conduction-type Heating of Canned Foods," *J. Food Sci.* **44**, 1485 (1979).
94. M. Mishkin, M. Karel, and I. Saguy, "Applications of Optimization of Food Dehydration," *Food Technol.* **36**(7), 101–109 (1982).
95. M. Mishkin, I. Saguy, and M. Karel, "Dynamic Optimization of Dehydration Processes. Minimizing Browning in Dehydrated Potatoes," *J. Food Sci.* **48**, 1617–1621 (1983).
96. I. Saguy, ed., *Computer-Aided Techniques in Food Technology*, Marcel Dekker, New York, 1983.
97. A. K. Datta, "Computer-Aided Engineering in Food Process and Product Design," *Food Technol.* **52**(10), 44–52 (1998).
98. M. Toussaint-Samat, *History of Food* (Anthea Bell, Trans.), Barnes & Noble, New York, 1998.
99. J. M. Flink, "Intermediate Moisture Food Products in the American Marketplace," *J. Food Proc. Preserv.* **1**, 324–339 (1977).

JAMES G. HAWKES
Continental Colloids, Inc.
Chicago, Illinois

FOOD PROCESSING: STANDARD INDUSTRIAL CLASSIFICATION

When one is in a grocery store, one is faced with an incredible selection of processed foods. For food manufacturers, this illustrates only one point: the amount of research and development that precedes their marketing. For regulatory agencies such as the Food and Drug Administration and the Department of Agriculture, this confirms the amount of work needed to assure the safety and wholesomeness of these products. However, for some other federal agencies, this poses an entirely different headache. For example, the Department of Labor (DOL) and the Environmental Protection Agency (EPA) must classify and give a name to each establishment that manufactures each product in order to enforce their legal mandates. To them it is a huge logistic nightmare. For example, the DOL must have access to a record of the number of employees suffering injuries in each type or category of food processing plants. To fill this need, the executive branch of the government has developed the Standard Industrial Classification (SIC) for food processing plants. The *SIC Manual* is one of the most useful federal publications available to both government agencies and the industries. One example of its usefulness is in assisting the allocation of resources. For example, a government agency can plan its budget accordingly by using the SIC to determine the number of food processing plants it has to inspect annually. Another application is the use of SIC to determine the employment status of the food processing profession. Such classification applies only to food processing establishments in the United States and this article discusses the SIC in details.

STANDARD INDUSTRIAL CLASSIFICATION MANUAL

The SIC Manual is issued by the Office of Management and Budget (OMB) of the Executive Office of the President of the United States. The manual divides industries in the United States into major groups. One major group 20 covers food and kindred products. This group includes establishments manufacturing or processing foods and beverages for human consumption, and certain related products, such as manufactured ice, chewing gum, vegetable and animal fats and oils, and prepared feeds for animals and fowls. Products described as dietetic are classified in the same manner as nondietetic products, eg, as candy, canned fruits, and cookies. Chemical sweeteners are classified in Major Group 28.

Major Group 20 is subdivided according to the following criteria (Table 1).

The following discusses each industry under food and kindred products. The abbreviations used are: * = The item has been made in the same establishment as the basic material; ** = The item has been made from purchased materials or materials transferred from another establishment; IGN = Industry Group No; IN = Industry No.

Table 1. Standard Industrial Classification (SIC) and Subdivisions for Major Group 20: Food and Kindred Products

Industrial group no.: Industrial group name
  Industrial no.: Industrial name

201: Meat products
  2011: Meat-packing plants
  2013: Sausages and other prepared meat products
  2015: Poultry slaughtering and processing
202: Dairy products
  2021: Creamery butter
  2022 Natural, processed, and imitation cheese
  2023: Dry, condensed, and evaporated dairy products
  2024: Ice cream and frozen desserts
  2026. Fluid milk
203: Canned, frozen, and preserved fruits, vegetables, and food specialties
  2032: Canned specialties
  2033: Canned fruits, vegetables, preserves, jams, and jellies
  2034: Dried and dehydrated fruits, vegetables, and soup mixes
  2035: Pickled fruits and vegetables, vegetable sauces and seasonings, and salad dressings
  2037: Frozen fruits, fruit juices, and vegetables
  2038: Frozen specialties, not elsewhere classified
204: Grain mill products
  2041: Flour and other grain mill products
  2043: Cereal breakfast foods
  2044: Rice milling
  2045: Prepared flour mixes and doughs
  2046: Wet corn milling
  2047: Dog and cat food
  2048: Prepared feeds and feed ingredients for animals and fowls, except dogs and cats
205: Bakery products
  2051: Bread and other bakery products, except cookies and crackers
  2052: Cookies and crackers
  2053: Frozen bakery products, except bread
206: Sugars and confectionary products
  2061: Cane sugar, except refining
  2062: Cane sugar refining
  2063: Beet sugar
  2064: Candy and other confectionery products
  2066: Chocolate and cocoa products
  2067: Chewing gum
  2068: Salted and roasted nuts and seeds
207: Fats and oils
  2074: Cottonseed oil mills
  2075: Soybean oil mills
  2076: Vegetable oil mills, except corn, cottonseed, and soybean
  2077: Animal and marine fats and oils
  2079: Shortening, table oils, margarine, and other edible fats and oils, not elsewhere classified
208: Beverages
  2082. Malt beverages
  2083: Malt
  2084: Wines, brandy, and brandy spirits
  2085: Distilled and blended liquors
  2086: Bottled and canned soft drinks and carbonated waters
  2087: Flavoring extracts and flavoring syrups, not elsewhere classified
209: Miscellaneous food preparations and kindred products
  2091: Canned and cured fish and seafoods
  2092. Prepared fresh or frozen fish and seafoods
  2095 Roasted coffee
  2096: Potato chips, corn chips, and similar snacks
  2097: Manufactured ice
  2098: Macaroni, spaghetti, vermicelli, and noodles
  2099: Food preparations, not elsewhere classified

MEAT PRODUCTS (IGN 201)

Meat Packing Plants (IG 2011)

Establishments primarily engaged in the slaughtering, for their own account or on a contract basis for the trade, of cattle, hogs, sheep, lambs, and calves for meat to be sold or to be used on the same premises in canning, cooking, curing, and freezing, and in making sausage, lard, and other products. Also included in this industry are establishments primarily engaged in slaughtering horses for human consumption. Establishments primarily engaged in slaughtering, dressing, and packing poultry, rabbits, and other small game are classified in Industry 2015; and those primarily engaged in slaughtering and processing animals not for human consumption are classified in Industry 2048. Establishments primarily engaged in manufacturing sausages and meat specialties from purchased meats are classified in Industry 2013; and establishments primarily engaged in canning meat for baby food are classified in Industry 2032.

1. Bacon, slab and sliced*
2. Beef*
3. Blood meal
4. Boxed beef*
5. Canned meats, except baby foods and animals feeds*
6. Corned beef*
7. Cured meats*
8. Dried meats*
9. Frankfurters, except poultry*
10. Hams, except poultry*
11. Hides and skins, cured or uncured
12. Horse meat for human consumption*
13. Lamb*
14. Lard*
15. Luncheon meat, except poultry*
16. Meat extracts*
17. Meat packing plants
18. Meat*
19. Mutton*
20. Pork*
21. Sausages*
22. Slaughtering plants: except animals not for human consumption
23. Variety meats edible organs*
24. Veal*

Sausages and Other Prepared Meat Products (IN = 2013)

Establishments primarily engaged in manufacturing sausages, cured meats, smoked meats, canned meats, frozen meats, and other prepared meats and meat specialties, from purchased carcasses and other materials. Prepared meat plants operated by packing houses as separate establishments are also included in this industry. Establishments primarily engaged in canning or otherwise processing poultry, rabbits, and other small game are classified in Industry 2015. Establishments primarily engaged in canning meat for baby food are classified in Industry 2032. Establishments primarily engaged in the cutting up and resale of purchased fresh carcasses, for the trade, (including boxed beef) are classified in Wholesale Trade, Industry 5147.

1. Bacon, slab and sliced**
2. Beef**
3. Bologna**
4. Calf's-foot jelly
5. Canned meats, except baby foods and animal feeds**
6. Corned beef**
7. Corned meats**
8. Cured meats: brined, dried, and salted**
9. Dried meats**
10. Frankfurters, except poultry**
11. Hams, except poultry**
12. Headcheese**
13. Lard**
14. Luncheon meat, except poultry**
15. Meat extracts**
16. Meat products: cooked, cured, frozen, smoked, and spiced**
17. Pastrami**
18. Pigs' feet, cooked and pickled**
19. Pork: pickled, cured, salted, or smoked**
20. Potted meats**
21. Puddings, meat**
22. Sandwich spreads, meat**
23. Sausage casings, collage
24. Sausages**
25. Scrapple**
26. Smoked meats**
27. Spreads, sandwich: meat**
28. Stew, beef and lamb**
29. Tripe**
30. Vienna sausage**

Poultry Slaughtering and Processing (IN 2015)

Establishments primarily engaged in slaughtering, dressing, packing, freezing, and canning poultry, rabbits, and other small game, or in manufacturing products from such meats, for their own account or on a contract basis for the trade. This industry also includes the drying, freezing, and breaking of eggs. Establishments primarily engaged in cleaning, oil treating, packing, and grading of eggs are classified in Wholesale Trade, Industry 5144; and those engaged in the cutting up and resale of purchased fresh carcasses are classified in Wholesale and Retail Trade.

1. Chickens, processed: fresh, frozen, canned, or cooked
2. Chickens: slaughtering and dressing
3. Ducks, processed: fresh, frozen, canned, or cooked
4. Ducks: slaughtering and dressing
5. Egg albumen
6. Egg substitutes made from eggs
7. Eggs: canned, dehydrated, desiccated, frozen, and processed
8. Eggs: drying, freezing, and breaking
9. Frankfurters, poultry
10. Game, small: fresh, frozen, canned, or cooked
11. Game, small: slaughtering and dressing
12. Geese, processed: fresh, frozen, canned, or cooked
13. Geese: slaughtering and dressing
14. Ham, poultry
15. Luncheon meat, poultry
16. Poultry, processed: fresh, frozen, canned, or cooked
17. Poultry: slaughtering and dressing
18. Rabbits, processed: fresh, frozen, canned, or cooked
19. Rabbits, slaughtering and dressing
20. Turkeys, processed: fresh, frozen, canned, or cooked
21. Turkeys: slaughtering and dressing

DAIRY PRODUCTS (IGN 202)

This industry group includes establishments primarily engaged in:

1. Manufacturing creamery butter; natural, processed, and imitation cheese; dry, condensed, and evaporated milk; ice cream and frozen dairy desserts; and special dairy products, such as yogurt and malted milk; and
2. Processing (pasteurizing, homogenizing, vitaminizing, bottling) fluid milk and cream for wholesale or retail distribution.

Independently operated milk-receiving stations primarily engaged in the assembly and reshipment of bulk milk for use in manufacturing or processing plants are classified in Industry 5143.

Creamery Butter (IN 2021)

Establishments primarily engaged in manufacturing creamery butter.

1. Anhydrous butterfat
2. Butter oil
3. Butter powder
4. Butter, creamery and whey
5. Butterfat, anhydrous

Natural, Processed, and Imitation Cheese (IN 2022)

Establishments primarily engaged in manufacturing natural cheese (except cottage cheese), processed cheese, cheese foods, cheese spreads, and cheese analogs (imitations and substitutes). These establishments also produce byproducts, such as raw liquid whey. Establishments primarily engaged in manufacturing cottage cheese are classified in Industry 2026, and those manufacturing cheese-based salad dressings are classified in Industry 2035.

1. Cheese analogs
2. Cheese products, imitation or substitutes
3. Cheese spreads, pastes, and cheeselike preparations
4. Cheese, except cottage cheese
5. Cheese, imitation or substitutes
6. Cheese, processed
7. Dips, cheese based
8. Processed cheese
9. Sandwich spreads, cheese
10. Whey, raw: liquid

Dry, Condensed, and Evaporated Dairy Products (IN 2023)

Establishments primarily engaged in manufacturing dry, condensed, and evaporated dairy products. Included in this industry are establishments primarily engaged in manufacturing mixes for the preparation of frozen ice cream and ice milk and dairy- and nondairy-based cream substitutes and dietary supplements.

1. Baby formula: fresh, processed, and bottled
2. Buttermilk: concentrated, condensed, dried, evaporated, and powdered
3. Casein, dry and wet
4. Cream substitutes
5. Cream: dried, powdered, and canned
6. Dietary supplements, dairy and nondairy based
7. Dry milk products: whole milk, nonfat milk, buttermilk, whey, and cream
8. Eggnog, canned: nonalcoholic
9. Ice cream mix, unfrozen: liquid or dry
10. Ice milk mix, unfrozen: liquid or dry
11. Lactose, edible
12. Malted milk
13. Milk, whole: canned
14. Milk: concentrated, condensed, dried, evaporated, and powdered
15. Milkshake mix
16. Skim milk: concentrated, dried, and powdered
17. Sugar of mix
18. Whey: concentrated, condensed, dried, evaporated, and powdered
19. Whipped topping, dry mix
20. Yogurt mix

Ice Cream and Frozen Desserts (IN 2024)

Establishments primarily engaged in manufacturing ice cream and other frozen desserts. Establishments primarily engaged in manufacturing frozen bakery products, such as cakes and pies, are classified in Industry 2053.

1. Custard, frozen
2. Desserts, frozen: except bakery
3. Fruit pops, frozen
4. Ice cream: eg, bulk, packaged, molded, on sticks
5. Ice milk: eg, bulk, packaged, molded, on sticks
6. Ices and sherbets
7. Juice pops, frozen
8. Millorine
9. Parfait
10. Pops, dessert: frozen-flavored ice, fruit, pudding, and gelatin
11. Pudding pops, frozen
12. Sherbets and ices
13. Spumoni
14. Tofu frozen desserts
15. Yogurt, frozen

Fluid Milk

Establishments primarily engaged in processing (eg, pasteurizing, homogenizing, vitaminizing, bottling) fluid milk and cream, and related products, including cottage cheese, yogurt (except frozen), and other fermented milk. Establishments primarily engaged in manufacturing dry mix whipped toppings are classified in Industry 2023; those producing frozen whipped toppings are classified in Industry 2038; and those producing frozen yogurt are classified in Industry 2024.

1. Buttermilk, cultured
2. Chocolate milk
3. Cottage cheese, including pot, bakers', and farmers' cheese
4. Cream, aerated
5. Cream, bottled
6. Cream, sour
7. Dips, sour cream based

[illegible]. Eggnog, fresh: nonalcoholic

9. Flavored milk drinks
10. Half and half
11. Milk processing (pasteurizing, homogenizing, vitaminizing, bottling)
12. Milk production, except farm
13. Milk, acidophilus
14. Milk, bottled
15. Milk, flavored
16. Milk, reconstituted
17. Milk, ultrahigh temperature
18. Sour cream

19. Whipped cream
20. Whipped topping, except frozen or dry mix
21. Yogurt, except frozen

CANNED, FROZEN, AND PRESERVED FRUITS, VEGETABLES, AND FOOD SPECIALTIES (IGN 203)

The canned products of this industry group are distinguished by their processing rather than by the container. The products may be shipped in bulk or in individual cans, bottles, retort pouch packages, or other containers.

Canned Specialties (IG 2032)

Establishments primarily engaged in canning specialty products, such as baby foods, nationality speciality foods, and soups, except seafood. Establishments primarily engaged in canning seafoods are classified in Industry 2091.

1. Baby foods (including meats), canned
2. Bean sprouts, canned
3. Beans, baked: with or without meat—canned
4. Broth, except seafood: canned
5. Chicken broth and soup, canned
6. Chili con carne, canned
7. Chinese foods, canned
8. Chop suey, canned
9. Chow mein, canned
10. Enchiladas, canned
11. Food specialties, canned
12. Italian foods, canned
13. Macaroni, canned
14. Mexican foods, canned
15. Mincemeat, canned
16. Nationality specialty foods, canned
17. Native foods, canned
18. Pasta, canned
19. Puddings, except meat: canned
20. Ravioli, canned
21. Soups, except seafood: canned
22. Ravioli, canned
23. Soups, except seafood: canned
24. Spaghetti, canned
25. Spanish foods, canned
26. Tamales, canned
27. Tortillas, canned

Canned Fruits, Vegetables, Preserves, Jams, and Jellies (IN 2033)

Establishments primarily engaged in canning fruits, vegetables, and fruit and vegetable juices; and in manufacturing catsup and similar tomato sauces, or natural and imitation preserves, jams, and jellies. Establishments primarily engaged in canning seafoods are classified in Industry 2091; and those manufacturing canned specialties, such as baby foods and soups, except seafood, are classified in Industry 2032.

1. Artichokes in olive oil, canned
2. Barbecue sauce
3. Catsup
4. Cherries, maraschino
5. Chili sauce, tomato
6. Fruit butters
7. Fruit pie mixes
8. Fruits, canned
9. Hominy, canned
10. Jams, including imitation
11. Jellies, edible: including imitation
12. Juice, fruit: concentrated-hot pack
13. Juices, fresh: fruit or vegetable
14. Juices, fruit and vegetable: canned or fresh
15. Ketchup
16. Marmalade
17. Mushrooms, canned
18. Nectars, fruit
19. Olives, including stuffed: canned
20. Pastes, fruit and vegetable
21. Preserves, including imitation
22. Purees, fruit and vegetable
23. Sauces, tomato based
24. Sauerkraut, canned
25. Seasonings (prepared sauces), tomato
26. Spaghetti sauce
27. Tomato juice and cocktails, canned
28. Tomato paste
29. Tomato sauce
30. Vegetable pie mixes
31. Vegetables, canned

Dried and Dehydrated Fruits, Vegetables, and Soup Mixes (IN 2034)

Establishments primarily engaged in sun drying or artificially dehydrating fruits and vegetables, or in manufacturing packaged soup mixes from dehydrated ingredients. Establishments primarily engaged in the grading and marketing of farm dried fruits, such as prunes and raisins, are classified in Wholesale Trade, Industry 5149.

1. Dates, dried
2. Dehydrated fruits, vegetables, and soups
3. Fruit flour, meal, and powders
4. Fruits, sulphured
5. Olives, dried
6. Potato flakes, granules, and other dehydrated potato products
7. Prunes, dried
8. Raisins

9. Soup mixes
10. Soup powders
11. Vegetable flour, meal, and powders
12. Vegetables, sulphured

Pickled Fruits and Vegetables, Vegetable Sauces and Seasonings, and Salad Dressings (IN 2035)

Establishments primarily engaged in pickling and brining fruits and vegetables and in manufacturing salad dressings, vegetable relishes, sauces, and seasonings. Establishments primarily engaged in manufacturing catsup and similar tomato sauces are classified in Industry 2033, and those packing purchased pickles and olives are classified in Wholesale or Retail Trade. Establishments primarily engaged in manufacturing dry salad dressing and dry sauce mixes are classified in Industry 2099.

1. Blue cheese dressing
2. Brining of fruits and vegetables
3. Cherries, brined
4. French dressing
5. Fruits, pickled and brined
6. Horseradish, prepared
7. Mayonnaise
8. Mustard, prepared (wet)
9. Olives, brined: bulk
10. Onions, pickled
11. Pickles and pickle salting
12. Relishes, fruit and vegetable
13. Russian dressing
14. Salad dressings, except dry mixes
15. Sandwich spreads, salad dressing base
16. Sauces, meat (seasoning): except tomato and dry
17. Sauces, seafood: except tomato and dry
18. Sauerkraut, bulk
19. Seasonings (prepared sauces), vegetable: except tomato and dry
20. Soy sauce
21. Thousand Island dressing
22. Vegetable sauces, except tomato
23. Vegetables, pickled and brined
24. Vinegar pickles and relishes
25. Worcestershire sauce

Frozen Fruits, Fruit Juices, and Vegetables (IN 2037)

Establishments primarily engaged in freezing fruits, fruit juices, and vegetables. These establishments also produce important byproducts such as fresh or dried citrus pulp.

1. Concentrates, frozen fruit juice
2. Dried citrus pulp
3. Frozen fruits, fruit juices, and vegetables
4. Fruit juices, frozen
5. Fruits, quick frozen and cold pack (frozen)
6. Vegetables, quick frozen and cold pack (frozen)

Frozen Specialties Not Elsewhere Classified (IN 2038)

Establishments primarily engaged in manufacturing frozen food specialties, not elsewhere classified, such as frozen dinners and frozen pizza. The manufacture of some important frozen foods and specialties is classified elsewhere. For example, establishments primarily engaged in manufacturing frozen dairy specialties are classified in Industry Group 202, those manufacturing frozen bakery products are classified in Industry Group 205, those manufacturing frozen fruits and vegetables are classified in Industry group 205, those manufacturing frozen fruits and vegetables are classified in Industry 2037, and those manufacturing frozen fish and seafood specialties are classified in Industry 2092.

1. Dinners, frozen: packaged
2. French toast, frozen
3. Frozen dinners, packaged
4. Meals, frozen
5. Native foods, frozen
6. Pizza, frozen
7. Soups, frozen: except seafood
8. Spaghetti and meatballs, frozen
9. Waffles, frozen
10. Whipped topping, frozen

GRAIN MILL PRODUCTS (IGN 204)

Flour and Other Grain Mill Products (IN 2041)

Establishments primarily engaged in milling flour or meal from grain, except rice. The products of flour mills may be sold plain or in the form of prepared mixes or doughs for specific purposes. Establishments primarily engaged in manufacturing prepared flour mixes or doughs from purchased ingredients are classified in Industry 2045, and those milling rice are classified in Industry 2044.

1. Bran and middlings, except rice
2. Bread and bread-type roll mixes*
3. Buckwheat flour
4. Cake flour*
5. Cereals, cracked grain*
6. Corn grits and lakes for brewers' use
7. Dough, biscuit*
8. Doughs, refrigerated or frozen*
9. Durum flour
10. Farina, except breakfast food*
11. Flour mills, cereals: except rice
12. Flour mixes*
13. Flour: buckwheat, corn, graham, rye, and wheat
14. Frozen doughs*
15. Graham flour
16. Granular wheat flour

17. Grits and flakes, corn: for brewers' use
18. Hominy grits, except breakfast food
19. Meal, corn
20. Milling of grains, dry, except rice
21. Mixes, flour: eg, pancake, cake, biscuit, doughnut*
22. Pancake batter, refrigerated or frozen*
23. Pizza mixes and prepared dough*
24. Semolina (flour)
25. Wheat germ
26. Wheat mill feed

Cereal Breakfast Foods (IN 2043)

Establishments primarily engaged in manufacturing cereal breakfast foods and related preparations, except breakfast bars. Establishments primarily engaged in manufacturing granola bars and other types of breakfast bars are classified in Industry 2064.

1. Breakfast foods, cereal
2. Coffee substitutes made from grain
3. Corn flakes
4. Corn hulled (cereal breakfast food)
5. Farina, cereal breakfast food
6. Granola, except bars and clusters
7. Hominy grits prepared as cereal breakfast food
8. Infants' foods, cereal type
9. Oatmeal (cereal breakfast food)
10. Oats, rolled (cereal breakfast food)
11. Rice breakfast foods
12. Wheat flakes

Rice Milling (IN 2044)

Establishments primarily engaged in cleaning and polishing rice, and in manufacturing rice flour or meal. Other important products of this industry include brown rice, milled rice (including polished rice), rice polish, and rice bran.

1. Flour, rice
2. Milling of rice
3. Polishing of rice
4. Rice bran, flour, and meal
5. Rice cleaning and polishing
6. Rice polish
7. Rice, brewers'
8. Rice, brown
9. Rice vitamin and mineral enriched

Prepared Flour Mixes and Doughs (IN 2045)

Establishments primarily engaged in preparing flour mixes or doughs from purchased flour. Establishments primarily engaged in milling flour from grain and producing mixes or doughs are classified in Industry 2091.

1. Biscuit mixes and doughs**
2. Bread and bread-type roll mixes**
3. Cake flour**
4. Cake mixes**
5. Dough, biscuit**
6. Doughnut mixes**
7. Doughs, refrigerated or frozen**
8. Flour: blended or self-rising**
9. Frozen doughs**
10. Gingerbread mixes**
11. Mixes, flour: eg, pancake, cake, biscuit, doughnut**
12. Pancake batter, refrigerated or frozen**
13. Pancake mixes**
14. Pizza mixes and doughs**

Wet Corn Milling (IN 2046)

Establishments primarily engaged in milling corn or sorghum grain (milo) by the wet process, and producing starch, syrup, oil, sugar, and byproducts, such as gluten feed and meal. Also included in this industry are establishments primarily engaged in manufacturing starch from other vegetable sources (eg, potatoes, wheat). Establishments primarily engaged in manufacturing table syrups from corn syrup and other ingredients, and those manufacturing starch-based dessert powders, are classified in Industry 2099.

1. Corn oil cake and meal
2. Corn starch
3. Corn syrup (including dried), unmixed
4. Dextrine
5. Dextrose
6. Feed, gluten
7. Fructose
8. Glucose
9. High fructose syrup
10. Hydrol
11. Meal, gluten
12. Oil, corn: crude and refined
13. Potato starch
14. Rice starch
15. Starch, instant
16. Starch, liquid
17. Starches, edible and industrial
18. Steep water concentrate
19. Sugar, corn
20. Tapioca
21. Wheat gluten
22. Wheat starch

Dog and Cat Food (IN 2047)

Establishments primarily engaged in manufacturing dog and cat food from cereal, meat, and other ingredients.

These preparations may be canned, frozen, or dry. Establishments primarily engaged in manufacturing feed for animals other than dogs and cats are classified in Industry 2048.

Prepared Feeds and Feed Ingredients for Animals and Fowls, Except Dogs and Cats (IN 2048)

Establishments primarily engaged in manufacturing prepared feeds and feed ingredients and adjuncts for animals and fowls, except dogs and cats. Included in this industry are poultry and livestock feed and feed ingredients, such as alfalfa meal, feed supplements, and feed concentrates and feed premixes. Also included are establishments primarily engaged in slaughtering animals for animal feed. Establishments primarily engaged in slaughtering animals for human consumption are classified in Industry Group 201. Establishments primarily engaged in manufacturing dog and cat foods are classified in Industry 2047.

1. Alfalfa, cubed
2. Alfalfa, prepared as feed for animals
3. Animal feeds, prepared: except dogs and cats
4. Bird food, prepared
5. Buttermilk emulsion for animal food
6. Chicken feeds, prepared
7. Citrus seed meal
8. Earthworm food and bedding
9. Feed concentrates
10. Feed premixes
11. Feed supplements
12. Feeds, prepared (including mineral): for animals and fowls—except dogs and cats
13. Feeds, specialty: mice, guinea pigs, minks, etc
14. Fish food
15. Hay, cubed
16. Horsemeat, except for human consumption
17. Kelp mean and pellets
18. Livestock feeds, supplements, and concentrates
19. Meal, bone: prepared as feed for animals and fowls
20. Mineral feed supplements
21. Oats: crimped, pulverized, and rolled: except breakfast food
22. Oyster shells, ground: used as feed for animals and fowls
23. Pet food, except dog and cat: canned, frozen, and dry
24. Poultry feeds, supplements, and concentrates
25. Shell crushing for feed
26. Slaughtering of animals, except for human consumption
27. Stock feeds, dry

BAKERY PRODUCTS (IGN 205)

Bread and Other Bakery Products, Except Cookies and Crackers (IN 2051)

Establishments primarily engaged in manufacturing fresh or frozen bread and bread-type rolls and fresh cakes, pies, pastries and other similar perishable bakery products. Establishments primarily engaged in producing dry bakery products, such as biscuits, crackers, and cookies, are classified in Industry 2052. Establishments primarily engaged in manufacturing frozen bakery products, except bread and bread-type rolls, are classified in Industry 2053. Establishments producing bakery products primarily for direct sale on the premises to household consumers are classified in Retail Trade, Industry 5461.

1. Bagels
2. Bakery products, fresh: bread, cakes, doughnuts, and pastries
3. Bakery products, partially cooked: except frozen
4. Biscuits, baked: baking powder and raised
5. Bread, brown: Boston and other—canned
6. Bread, including frozen
7. Buns, bread-type (eg, hamburger, hot dog), including frozen
8. Buns, sweet, except frozen
9. Cakes, bakery, except frozen
10. Charlotte Russe (bakery product), except frozen
11. Croissants, except frozen
12. Crullers, except frozen
13. Doughnuts, except frozen
14. Frozen bread and bread-type rolls
15. Knishes, except frozen
16. Pastries, except frozen: eg, Danish, French
17. Pies, bakery, except frozen
18. Rolls, bread-type, including frozen
19. Rolls, sweet, except frozen
20. Sponge goods, bakery, except frozen
21. Sweet yeast goods, except frozen

Cookies and Crackers (IN 2052)

Establishments primarily engaged in manufacturing fresh cookies, crackers, pretzels, and similar dry bakery products. Establishments primarily engaged in producing other fresh bakery products are classified in Industry 2051.

1. Bakery products, dry: eg, biscuits, crackers, pretzels
2. Biscuits, baked: dry, except baking powder and raised
3. Communion wafers
4. Cones, ice cream
5. Cookies
6. Cracker meal and crumbs
7. Crackers: eg, graham, soda

8. Matzoths
9. Pretzels
10. Rusk
11. Saltines
12. Zwieback

Frozen Bakery Products, Except Bread (IN 2053)

Establishments primarily engaged in manufacturing frozen bakery products, except bread and bread-type rolls. Establishments primarily engaged in manufacturing frozen bread and bread-type rolls are classified in Industry 2051.

1. Bakery products, frozen: except bread and bread-type rolls
2. Cakes, frozen, pound, layer, and cheese
3. Croissants, frozen
4. Doughnuts, frozen
5. Pies, bakery, frozen
6. Sweet yeast goods, frozen

SUGAR AND CONFECTIONERY PRODUCTS (IGN 206)

Cane Sugar, except Refining (IN 2061)

Establishments primarily engaged in manufacturing raw sugar, syrup, or finished (granulated or clarified) cane sugar from sugar cane. Establishments primarily engaged in refining sugar from purchased raw cane sugar or sugar syrup are classified in Industry 2062.

1. Cane sugar, made from sugarcane
2. Molasses, blackstrap: made from sugarcane
3. Molasses, made from sugarcane
4. Sugar, granulated: made from sugarcane
5. Sugar, invert: made from sugarcane
6. Sugar, powdered: made from sugarcane
7. Sugar, raw: made from sugarcane
8. Syrup, cane: made from sugarcane

Cane Sugar Refining (IN 2062)

Establishments primarily engaged in refining purchased raw cane sugar and sugar syrup.

1. Molasses, blackstrap: made from purchased raw cane sugar or sugar syrup
2. Refineries, cane sugar
3. Sugar, granulated: made from purchased raw cane sugar or sugar syrup
4. Sugar, invert: made from purchased raw cane sugar or sugar syrup
5. Sugar, powdered: made from purchased raw cane sugar or sugar syrup
6. Sugar, refined: made from purchased raw can sugar or sugar syrup
7. Syrup, made from purchased raw can sugar or sugar syrup

Beet Sugar (IN 2063)

Establishments primarily engaged in manufacturing sugar from sugar beets.

1. Beet pulp, dried
2. Beet sugar, made from sugar beets
3. Molasses beet pulp
4. Molasses, made from sugar beets
5. Sugar, granulated: made from sugar beets
6. Sugar, invert: made from sugar beets
7. Sugar, liquid: made from sugar beets
8. Sugar, powdered: made from sugar beets
9. Syrup, made from sugar beets

Candy and Other Confectionery Products (IN 2064)

Establishments primarily engaged in manufacturing candy, including chocolate candy, other confections, and related products. Establishments primarily engaged in manufacturing solid chocolate bars from cacao beans are classified in Industry 2066, those manufacturing chewing gum are classified in Industry 2067, and those primarily engaged in roasting and salting nuts are classified in Industry 2068. Establishments primarily engaged in manufacturing confectionery for direct sale on the premises to household consumers are classified in Retail Trade, Industry 5441.

1. Bars, candy: including chocolate-covered bars
2. Breakfast bars
3. Cake ornaments, confectionery
4. Candy, except solid chocolate
5. Chewing candy, except chewing gum
6. Chocolate bars, from purchased cocoa or chocolate
7. Chocolate candy, except solid chocolate
8. Confectionery
9. Cough drops, except pharmaceutical preparations
10. Dates: chocolate covered, sugared, and stuffed
11. Fruit peel products: candied, glazed glace, and crystallized
12. Fruits: candied, glazed, and crystallized
13. Fudge (candy)
14. Granola bars and clusters
15. Halvah (candy)
16. Licorice candy
17. Lozenges, candy: nonmedicated
18. Marshmallows
19. Marzipan (candy)
20. Nuts, candy covered
21. Nuts, glace
22. Popcorn balls and candy-covered popcorn products

Chocolate and Cocoa Products (IN 2066)

Establishments primarily engaged in shelling, roasting, and grinding cacao beans for the purpose of making chocolate liquor, from which cocoa powder and cocoa butter are derived, and in the further manufacturing of solid chocolate bars, chocolate coatings, and other chocolate and cocoa products. Also included is the manufacture of similar products, except candy, from purchased chocolate or cocoa. Establishments primarily engaged in manufacturing candy from purchased cocoa products are classified in Industry 2064.

1. Baking chocolate
2. Bars, candy: solid chocolate
3. Cacao bean products: chocolate, cocoa butter, and cocoa
4. Cacao beans: shelling, roasting, and grinding for making chocolate liquor
5. Candy, solid chocolate
6. Chocolate bars, solid: from cacao beans
7. Chocolate coatings and syrups
8. Chocolate liquor
9. Chocolate syrup
10. Chocolate, instant
11. Chocolate, sweetened or unsweetened
12. Chocolate butter
13. Chocolate mix, instant
14. Chocolate powdered: mixed with other substances

Chewing Gum (IN 2067)

Establishments primarily engaged in manufacturing salted, roasted, dried, cooked, or canned nuts or in processing grains or seeds in a similar manner for snack purposes. Establishments primarily engaged in manufacturing confectionery-coated nuts are classified in Industry 2064, and those manufacturing peanut butter are classified in Industry 2099.

1. Nuts, dehydrated or dried
2. Nuts: salted, roasted, cooked, or canned
3. Seeds: salted, roasted, cooked, or canned

FATS AND OILS (IGN 207)

Cottonseed Oil Mills (IN 2074)

Establishments primarily engaged in manufacturing cottonseed oil, cake, meal, and linters, or in processing purchased cottonseed oil other than into edible cooking oils. Establishments primarily engaged in refining cottonseed oil into edible cooking oils are classified in Industry 2079.

1. Cottonseed oil, cake, and meal: made in cottonseed oil mills
2. Cottonseed oil, deodorized
3. Lecithin, cottonseed

Soybean Oil Mills (IN 2075)

Establishments primarily engaged in manufacturing soybean oil, cake, and meal, and soybean protein isolates and concentrates, or in processing purchased soybean oil other than into edible cooking oils. Establishments primarily engaged in refining soybean oil into edible cooking oils are classified in Industry 2079.

1. Lecithin, soybean
2. Soybean flour and grits
3. Soybean oil, cake, and meal
4. Soybean oil, deodorized
5. Soybean protein concentrates
6. Soybean protein isolates

Vegetable Oil Mills, Except Corn, Cottonseed, and Soybean (IN 2076)

Establishments primarily engaged in manufacturing vegetable oils, cake and meal, except corn, cottonseed, and soybean, or in processing similar purchased oils other than into edible cooking oils. Establishments primarily engaged in manufacturing corn oil and its byproducts are classified in Industry 2046, those that are refining vegetable oils into edible cooking oils are classified in Industry 2079, and those refining these oils for medicinal purposes are classified in Industry 2833.

1. Castor oil and pomace
2. Coconut oil
3. Linseed oil, cake, and meal
4. Oils, vegetable: except corn, cottonseed, and soybean
5. Oiticica oil
6. Palm kernel oil
7. Peanut oil, cake, and meal
8. Safflower oil
9. Sunflower seed oil
10. Tallow, vegetable
11. Tung oil
12. Walnut oil

Animal and Marine Fats and Oils (IN 2077)

Establishments primarily engaged in manufacturing animal oils, including fish oil and other marine animal oils, and fish and animal meal; and those rendering inedible stearin, grease, and tallow from animal fat, bones, and meat scraps. Establishments primarily engaged in manufacturing lard and edible tallow and stearin are classified in Industry Group 201; those refining marine animal oils for medicinal purposes are classified in Industry 2833; and those manufacturing fatty acids are classified in Industry 2899.

1. Feather meal
2. Fish liver oils, crude
3. Fish meal

4. Fish oil and fish oil meal
5. Grease rendering, inedible
6. Meal, meat and bone: not prepared as feed
7. Meat and bone meal and tankage
8. Oils, animal
9. Oils, fish and marine animal: eg, herring, menhaden, whale (refined), sardine
10. Rendering plants, inedible grease and tallow
11. Stearin, animal: inedible
12. Tallow rendering, inedible

Shortening, Table Oils, Margarine, and Other Edible Fats and Oils, Not Elsewhere Classified (IN 2079)

Establishments primarily engaged in manufacturing shortening, table oils, margarine, and other edible fats and oils, not elsewhere classified. Establishments primarily engaged in producing corn oil are classified in Industry 2046.

1. Baking and frying fats (shortening)
2. Cottonseed cooking and salad oil
3. Margarine oil, except corn
4. Margarine, including imitation
5. Margarine-butter blend
6. Nut margarine
7. Oil, hydrogenated: edible
8. Oil, partially hydrogenated: edible
9. Oil, vegetable winter stearin
10. Olive oil
11. Peanut cooking and salad oil
12. Shortenings, compound and vegetable
13. Soybean cooking and salad oil
14. Vegetable cooking and salad oils, except corn oil: refined

BEVERAGES (IGN 208)

Malt Beverages (IN 2082)

Establishments primarily engaged in manufacturing malt beverages. Establishments primarily engaged in bottling purchased malt beverages are classified in Industry 5181.

1. Ale
2. Beer (alcoholic beverage)
3. Breweries
4. Brewers' grain
5. Liquors, malt
6. Malt extract, liquors, and syrups
7. Near beer
8. Porter (alcoholic beverage)
9. Stout (alcoholic beverage)

Malt (IN 2083)

Establishments primarily engaged in manufacturing malt or malt byproducts from barley or other grains.

1. Malt byproducts
2. Malt: barley, rye, wheat, and corn
3. Malthouses
4. Sprouts, made in malthouses

Wines, Brandy, and Brandy Spirits (IN 2084)

Establishments primarily engaged in manufacturing wines, brandy, and brandy spirits. This industry also includes bonded wine cellars that are engaged in blending wines. Establishments primarily bottling purchased wines, brandy, and brandy spirits but that do not manufacture wines and brandy, are classified in Wholesale Trade, Industry 5182.

1. Brandy
2. Brandy spirits
3. Wine cellars, bonded: engaged in blending wines
4. Wine coolers (beverages)
5. Wines

Distilled and Blended Liquors (IN 2085)

Establishments primarily engaged in manufacturing alcoholic liquors by distillation, and in manufacturing cordials and alcoholic cocktails by blending processes or by mixing liquors and other ingredients. Establishments primarily engaged in manufacturing industrial alcohol are classified in Industry 2869, and those only bottling purchased liquors are classified in Wholesale Trade, Industry 5182.

1. Applejack
2. Cocktails, alcoholic
3. Cordials, alcoholic
4. Distillers' dried grains and solubles
5. Eggnog, alcoholic
6. Ethyl alcohol for medicinal and beverage purposes
7. Gin (alcoholic beverage)
8. Grain alcohol for medicinal and beverage purposes
9. Liquors: distilled and blended—except brandy
10. Rum
11. Spirits, neutral, except fruit—for beverage purposes
12. Vodka
13. Whiskey: bourbon, rye, scotch type, and corn

Bottles and Canned Soft Drinks and Carbonated Waters (IN 2086)

Establishments primarily engaged in manufacturing soft drinks and carbonated waters. Establishments primarily engaged in manufacturing fruit and vegetable juices are classified in Industry Group 203; those manufacturing fruit syrups for flavoring are classified in Industry 2087; and those manufacturing nonalcoholic cider are classified in Industry 2099. Establishments primarily engaged in bottling natural spring waters are classified in Wholesale Trade, Industry 5149.

1. Beer, birch and root: bottled or canned
2. Carbonated beverages, nonalcoholic: bottled or canned
3. Drinks, fruit: bottled, canned, or fresh
4. Ginger ale, bottled or canned
5. Iced tea, bottled or canned
6. Lemonade: bottled, canned, or fresh
7. Mineral water, carbonated: bottled or canned
8. Soft drinks, bottled or canned
9. Tea, iced: bottled or canned
10. Water, pasteurized: bottled or canned

Flavoring Extracts and Flavoring Syrups, Not Elsewhere Classified (IN 2087)

Establishments primarily engaged in manufacturing flavoring extracts, syrups, powders, and related products, not elsewhere classified, for soda fountain use or for the manufacture of soft drinks, and colors for bakers' and confectioners' use. Establishments primarily engaged in manufacturing chocolate syrup are classified in Industry 2066.

1. Beverage bases
2. Bitters (flavoring concentrates)
3. Burnt sugar (food color)
4. Cocktail mixes, nonalcoholic
5. Coffee flavorings and syrups
6. Colors for bakers' and confectioners' use, except synthetic
7. Cordials, nonalcoholic
8. Drink powders and concentrates
9. Flavoring concentrates
10. Flavoring extracts, pastes, powders, and syrups
11. Food colorings, except synthetic
12. Food glace, for glazing foods
13. Fruit juices, concentrated: for fountain use
14. Fruits, crushed: for soda fountain use

MISCELLANEOUS FOOD PREPARATIONS AND KINDRED PRODUCTS (IGN 209)

Canned and Cured Fish and Seafoods (IN 2091)

Establishments primarily engaged in cooking and canning fish, shrimp, oysters, clams, crabs, and other seafoods, including soups: and those engaged in smoking, salting, drying, or otherwise curing fish and other seafoods for the trade. Establishments primarily engaged in shucking and packing fresh oysters in nonsealed containers, or in freezing or preparing fresh fish, are classified in Industry 2092.

1. Canned fish, crustacea, and mollusks
2. Caviar, canned
3. Chowders, fish and seafood: canned
4. Clam bouillon, broth, chowder, juice: bottled or canned
5. Codfish: smoked, salted, dried, and pickled
6. Crab meat, canned and cured
7. Finnan haddie (smoked haddock)
8. Fish and seafood cakes: canned
9. Fish egg bait, canned
10. Fish, canned and cured
11. Fish: cured, dried, pickled, salted, and smoked
12. Herring: smoked, salted, dried, and pickled
13. Oysters, canned and cured
14. Salmon: smoked, salted, dried, canned, and pickled
15. Sardines, canned
16. Seafood products, canned and cured
17. Shellfish, canned and cured
18. Shrimp, canned and cured
19. Soups, fish and seafood: canned
20. Stews, fish and seafood: canned
21. Tuna fish, canned

Prepared Fresh or Frozen Fish and Seafoods (IN 2092)

Establishments primarily engaged in preparing fresh and raw or cooked frozen fish and other seafoods and seafood preparations, such as soups, stews, chowders, fishcakes, crabcakes, and shrimpcakes. Prepared fresh fish are eviscerated or processed by removal of heads, fins, or scales. This industry also includes establishments primarily engaged in the shucking and packing of fresh oysters in nonsealed containers.

1. Chowders, fish and seafood: frozen
2. Crabcakes, frozen
3. Crabmeat picking
4. Crabmeat, fresh: packed in nonsealed containers
5. Fish and seafood cakes, frozen
6. Fish fillets
7. Fish sticks
8. Fish: fresh and frozen, prepared
9. Oysters, fresh: shucking and packing nonsealed containers
10. Seafoods, fresh and frozen
11. Shellfish, fresh and frozen
12. Shellfish, fresh: shucked, picked, or packed
13. Shrimp, fresh and frozen
14. Soups, fish and seafood: frozen
15. Stews, fish and seafood: frozen

Roasted Coffee (IN 2095)

Establishments primarily engaged in roasting coffee, and in manufacturing coffee concentrates and extracts in powdered, liquid, or frozen form, including freeze dried. Coffee roasting by wholesale grocers is classified in Wholesale Trade, Industry 5149.

1. Coffee extracts
2. Coffee roasting, except by wholesale grocers

3. Coffee, ground: mixed with grain or chicory
4. Coffee, instant and freeze dried

Potato Chips, Corn Chips, and Similar Snacks (IN 2096)

Establishments primarily engaged in manufacturing potato chips, corn chips, and similar snacks. Establishments primarily engaged in manufacturing pretzels and crackers are classified in Industry 2052; those manufacturing candy covered popcorn are classified in Industry 2064; those manufacturing salted, roasted, cooked or canned nuts and seeds are classified in Industry 2068; and those manufacturing packaged unpopped popcorn are classified in Industry 2099.

1. Cheese curls and puffs
2. Corn chips and related corn snacks
3. Popcorn, popped: except candy covered
4. Pork rinds
5. Potato chips and related corn snacks
6. Potato sticks

Manufactured Ice (IN 2097)

Establishments primarily engaged in manufacturing ice for sale. Establishments primarily engaged in manufacturing dry ice are classified in Industry 2813.

1. Block ice
2. Ice cubes
3. Ice plants, operated by public utilities
4. Ice, manufactured or artificial: except dry ice

Macaroni, Spaghetti, Vermicelli, and Noodles (IN 2098)

Establishments primarily engaged in manufacturing dry macaroni, spaghetti, vermicelli, and noodles. Establishments primarily engaged in manufacturing canned macaroni and spaghetti are classified in Industry 2032, and those manufacturing fried noodles, such as Chinese noodles, are classified in Industry 2099.

Macaroni and products, dry: eg, alphabets, rings, seashells

1. Noodles: egg, plain, and water
2. Spaghetti, dry
3. Vermicelli

Food Preparations, not Elsewhere Classified (IN 2099)

Establishments primarily engaged in manufacturing prepared foods and miscellaneous food specialties, not elsewhere classified, such as baking powder, yeast, and other leavening compounds; peanut butter; packaged tea, including instant; ground spices; and vinegar and cider. Also included in this industry are establishments primarily engaged in manufacturing dry preparations, except flour mixes, consisting of pasta, rice, potatoes, textured vegetable protein, and similar products that are packaged with other ingredients to be prepared and cooked by the consumer. Establishments primarily engaged in manufacturing flour mixes are classified in Industry Group 204.

1. Almond pastes
2. Baking powder
3. Bouillon cubes
4. Box lunches for sale off premises
5. Bread crumbs, not made in bakeries
6. Butter, renovated and processed
7. Cake frosting mixes, dry
8. Chicory root, dried
9. Chili pepper or powder
10. Chinese noodles
11. Cider, nonalcoholic
12. Coconut, desiccated and shredded
13. Cole slaw in bulk
14. Cracker sandwiches made from purchased crackers
15. Desserts, ready to mix
16. Dips, except cheese and sour cream based
17. Emulsifiers, food
18. Fillings, cake or pie: except fruits, vegetables, and meat
19. Frosting, prepared
20. Gelatin dessert preparations
21. Gravy mixes, dry
22. Honey, strained and bottled
23. Jelly, corncob (gelatin)
24. Leavening compounds, prepared
25. Marshmallow creme
26. Meat seasonings, except sauces
27. Molasses, mixed or blended**
28. Noodles, fried (eg, Chinese)
29. Noodles, uncooked: packaged with other ingredients
30. Pancake syrup, blended and mixed
31. Pasta, uncooked: packaged with other ingredients
32. Peanut butter
33. Pectin
34. Pepper
35. Pizza, refrigerated: not frozen
36. Popcorn, packaged: except popped
37. Potatoes, dried: packaged with other ingredients
38. Potatoes, peeled for the trade
39. Rice, uncooked: packaged with other ingredients
40. Salad dressing mixes, dry
41. Salads, fresh or refrigerated
42. Sandwiches, assembled and packaged: for wholesale market
43. Sauce mixes, dry
44. Sorghum, including custom refining
45. Spices, including grinding
46. Sugar grinding

47. Sugar, industrial maple: made in plants producing maple syrup
48. Sugar, powdered**
49. Syrups, sweetening: honey, maple syrup, sorghum
50. Tea blending
51. Tofu, except frozen desserts
52. Tortillas, fresh or refrigerated
53. Vegetables peeled for the trade
54. Vinegar
55. Yeast

BIBLIOGRAPHY

"Standard Industrial Classification Manual," Office of Management and Budget, Executive Office of the President. U.S. Government Printing Office, Washington, D.C., 1987.

Y. H. Hui
American Food and Nutrition Center
Cutten, California

FOOD PROCESSING: TECHNOLOGY, ENGINEERING, AND MANAGEMENT

The technology of food is intertwined with the business of selling food. Although this is not unique to food technology, having insight into the food business will provide some insight into the motivation it provides for the development of new technologies and the maintenance of old ones. Decision making in the food industry requires knowledge of both the business of selling food and the technology of producing, holding, and delivering food. The right information about the technology and the business, coupled with organized approaches to decision making, can yield important benefits to those responsible for making decisions in the industry.

Some generalizations about the food business are that it is high volume, low margin, multiple product, transportation intensive, and end-user marketing intensive. Because of the need for food to be ubiquitous, the business requires multiple distribution points and complicated distribution networks. The nature of food as material and as a perception of the consumer both defines and constrains the food business. The intent is to deliver safe, palatable, and profitable product to the purchaser. Technology constrains the business by specifying what can and cannot be done with food materials while maintaining a viable product. The reality is that the technology and the business of selling food are inseparable.

Because profit per sale is usually low, food businesses must make many sales to gain a reasonable profit. This emphasis on volume requires food technology to provide speed in production. This makes the production facility challenge one of turning out salable foodstuffs by the ton. In the food industry, great value is placed on technological innovation that enables automated handling of materials and scale-up of processes and procedures from small to large volumes. In such a high-volume business, small improvements in efficiency of the technology or distribution or the decision making can have important consequences for the profit (or even survival) of an enterprise.

Most food companies see opportunities for growth in the introduction of new products. This has led to varieties of products from single companies and much product differentiation within categories of products. The technology to develop, manufacture, and distribute these new product entries must be invented and applied in order for such growth to happen. This perception of how growth is attained, and the perception that growth is important, provide important spurs for technological development.

That food must be ubiquitous leads to situations in which the business and the technology cannot be separated. Although lengthening shelf life is a regular goal and concern of the food technologist, the distribution system must be designed around what the technology and the consumer will bear. Some fresh foodstuffs are flown to market, whereas other products are shipped by train or barge. The network of warehouses, transportation corridors, vehicles, and storage facilities all must accommodate to the realities of the food as material and to the requirement that consumer's expectations are matched in food products once delivered. The business accommodates to the realities of the product, whereas the technologist tries to change the product to achieve new advantages for the business. Because food must be everywhere there are people, the marketing of food must be ubiquitous—ultimately, food products are consumed teaspoon by teaspoon, and each individual consumer must be sold on the product.

The other technological reality that affects the food business is the need for uniform products when the inputs are variable. The biological systems that generate the inputs to food products require modification to yield uniform outputs. The modification is usually called processing. Besides turning out food by the ton, food technology must also provide a means to control the performance of the inputs so that the consumer's perception of the product is the same container to container and bite by bite.

The nature of the business of food has important consequences for the technologist, and the technology of food has important consequences for the businessperson. Decisions, whether they are viewed as business oriented or technology oriented, should not be seen in isolation from either arena.

CONTROLLING FOOD QUALITIES

Much of the application of food technology is for the purpose of producing uniform output from variable input. This is a significant difference from fabricators who assemble parts into a product. Such assembly presumes interchangeable inputs (parts). By contrast food processors assume that the inputs are variable. Milk from farm A is different from milk from farm B. Peas harvested on Monday are different from peas harvested on Tuesday. The challenge and opportunity is to control the quality of the output product by controlling the processing steps applied to the inputs.

Consistently reproducing a food quality implies that this quality is somehow measurable. From a consumer perspective this is usually not the case. Even articulating the nature of a food quality may be difficult for a consumer. Although there are words like *sweetness* and *saltiness*, more often the expressions describing quality are much more general—"it tastes good" or "it doesn't taste right." This problem for quality measurement is further complicated by the variability among consumers of what is considered a good quality for food to have. Preferences vary. For control to be possible the consumer's perception of quality must be separated from the measurable qualities of food materials. The food technologist seeks desirable effects through variables that are controllable. The underlying assumption is that controlling ingredient amounts, processing times and temperatures, holding times, and the like will produce a food product that meets the consumer's quality expectations. The connection between processing variables that can be measured and replicated and the consumer's perception of the qualities of a food product is real, but it is indirect. Many intervening factors influence this connection and most of them are not measurable.

For a given product the best hope of consistent qualities is to control what can be measured as carefully as possible. Keeping in mind that the inputs are intrinsically variable and that processes also vary, "control" must be defined statistically. If a quality measure of an ice cream product is its gross weight, then the ideal is that the gross weight of every container comes from the same distribution of such weights. Because we cannot eliminate variability entirely, the best we can do is to make sure that the underlying distribution does not change. In these situations a distribution is said to have "changed" if its center (mean) or its dispersion (standard deviation) changes. A manufacturing step is said to be "in control" if the measurements taken are scattered according to the same distribution.

Deciding whether an operation is in control or not can be done by routinely sampling the output from the operation and measuring the target quality for each item in the sample. Sample statistics can be computed, including typically the mean of the sample, the range of the sample, and the standard deviation of the sample. These numbers have their own distributions, but these distributions are related to the target quality measurement in well-known ways. The easiest connection to describe is the one between the mean of the measurements and the mean of the sample. The mean of the sample means has the same value as the mean of the target measurements, and the standard deviation of the sample means (also called the standard error of the mean) is the standard deviation of the measurements divided by the square root of the sample size. Furthermore, the distribution of the sample means is distributed approximately normally, provided the sample size is big enough (in practice big enough typically means four or more items in the sample). This fact is true even if the distribution of the measurements is not normal. This enables a method for making the in control decision.

W. A. Shewhart (1) developed a strategy and a graphic that made this decision making feasible for a wide variety of situations. He proposed that if the sample mean fell more than three standard deviations from the empirically established mean of the process (established when the process was in control), this was evidence that the operation producing the measurement was not in control. If the sample mean was less than three standard deviations from the established process mean, the operation was deemed in control. Because the distribution of the sample means is approximately normal, the probability of the sample mean falling beyond three standard deviations by chance (ie, without the distribution of the individual measurements changing) can be computed and is quite small (less than 0.003).

The graphic that Shewhart developed is called a control chart (Fig. 1). This consists of three equally spaced parallel lines (or sometimes two lines for the range chart). The centerline corresponds to the process mean that is also the mean of the sample distribution; the upper and lower lines correspond to the mean plus three standard deviations and the mean minus three standard deviations of the sample distribution, respectively. Sample means and sample

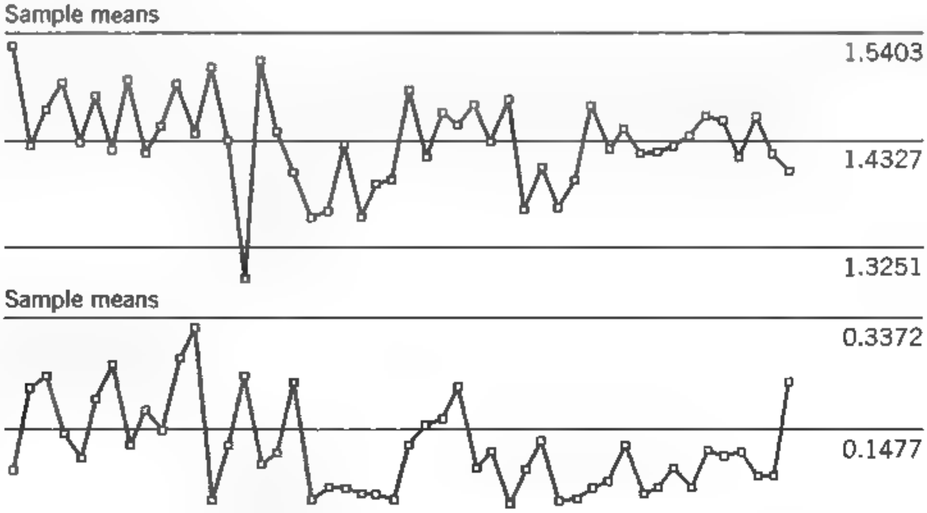


Figure 1. An example control chart for means and ranges of sample size four for gross weights of quarts of ice cream. There is one out-of-control point in the sample means chart.

ranges can be plotted successively from left to right to indicate a time sequence. Any time a plotted point falls outside the three standard deviation limits, the operation is assumed to be out of control.

Many of Shewhart's followers have made refinements of this device for decision making, but the essence of this method has remained the same since originally suggested. The main idea is that unusual values for the sample statistics indicate that the underlying distribution of measurements has changed. It is a signal that the cause of this change should be sought and found. Making decisions based on this method can produce two types of errors. On the average, three times in a thousand samples the sample mean will be beyond the "three sigma limits" even though the underlying distribution has not changed. Or the plotted point will fall between the control limits even though the distribution has changed. Such errors can be anticipated, and the use of this device can be adjusted to take into account the importance of these errors.

Control charts have been used to help propel the quality revolution. Emphasis on good quality products and on continuous quality improvement has had large impact on some industries. In automotive and electronic industries the commitment to constant quality improvement has indeed made products more reliable and convenient. The food industry has benefited less from the quality emphasis for two reasons. First, it is difficult to reduce the intrinsic variability in viable biological systems. The amount of this variability is unlikely to be reducible because genetic diversity is a strength of biological systems. This often leads to a level of variability in food materials that is hard to reduce. Second, distinguishing between a successful product and a failure is not clear-cut. This makes it difficult to define and detect quality improvement. For example, if a stereo or one if its components fail, the symptom is usually obvious to most users. In a food product, the wrong amount of an ingredient may be a failure, but the result may not be detectable by the consumer. Similarly, a food manufacturer may choose to change a product. While some customers may judge the altered product a failure, others may like it better.

This brief article cannot deal with the details of developing and using control charts, but there are many texts that can assist (2). The basic underlying idea is that patterns in variation of measurements can be used to determine the state of control (or lack of it) of the qualities of food products.

THE OPTIMIZING DECISION MAKER

Those responsible for the policy and assets of a food business are (usually without knowing it) optimizers. They try to achieve the best results in the business situation they face while recognizing the technological situation. This means conscientious managers will attempt to use resources efficiently. The usual procedure when optimizing is to array the alternatives, eliminate the infeasible ones from consideration, and then measure the remaining alternatives until a best one is determined (3). Sometimes this is an informal procedure, with little information gathering and minor mathematics, and sometimes it is very formal with structured information requirements and complex mathematics. The science associated with food materials often structures information requirements, the technology provides numbers and determines what is feasible, and the business objectives provide the measuring stick to choose among alternatives This kind of decision making links the business to the technology in important and useful ways. Not only is the approach structured and systematic, but also for many kinds of decisions there are formal (mathematical) methods that can assist the decision maker.

Decisions that affect the efficiency of a food industry enterprise are made at many levels in the business. Sometimes they are onetime occurrences (should we invest in a new production process?) and sometimes they are recurring (how long should we process that product and at what temperature?). Because some decisions become routine, they are sometimes dismissed as trivial or unimportant. This is not always the case, as routine decisions about food formulations, delivery, processing parameters, production scheduling, and the like have important impacts on costs, profits, and the safety of the food product. If a recurring decision is not optimal, the result is regular losses to the business. In many cases, formal models can be built for these decision situations, and formal methods can be applied to give the decision maker good information to act on. Although the optimization opportunity itself is constrained by the quality of the information available to the user, it still will be the best the food manager can do in the situation he or she faces.

An important piece of optimizing is the objective measure (4). This device allows the user to choose between alternatives—providing a measure of which one is better. There is sometimes a perceived conflict between the objectives of the food technologist and the food industry manager. If the technologist insists on some kind of quality objective—best product, highest-quality product—this may come in conflict with profit objectives of the business. The inputs required to achieve such extreme quality objectives are often so expensive that the necessary product price becomes more than the market will bear. The contribution of food technology in this business context must be to specify what is feasible. Otherwise, the decision will be driven by two (or more) conflicting objectives and will never be optimal.

EXAMPLES

Allocation of Milk Resources in Cheese Making

In a step in the manufacture of cheddar cheese, a variety of milk resources are blended in a cheese vat with other ingredients (5). Making the blend is called standardizing, and the resulting blend is called cheese milk. The blend is allowed to coagulate before other processing steps are taken. The milk resources used are valuable; the cheesemaker pays to acquire them. Furthermore, the cheese yield and cheese quality are affected by which combination and proportions of resources are used in the blend. In the United States, the composition of the resulting product (which is determined by the composition of the cheese milk) is also constrained by government regulations and definitions. A recurring decision that the cheesemaker faces is determining the constituent amounts for each of the inputs to the cheese milk. The decision is constrained in many ways, but it is made regularly with the ordinary business objective of making a profit.

Technological investigation has determined a cheese yield formula (6) that allows the cheesemaker to predict the cheese yield if enough detail is known about the casein, fat, salt retention, and water retention of this step in the process. This technological model requires that the casein-to-fat ratio of the cheese milk be restricted to a small range. If this is done, the formula can be used to predict pounds of output for a given set of inputs. The potential inputs to the cheese milk include milk, condensed skim milk, nonfat dry milk, whey protein concentrate, cream, frozen cream, water, and other ingredients. Almost all the inputs affect the casein-to-fat ratio and the cheese yield. Because each of these resources will have different prices at different times, the cheesemaker can optimize the cheese milk by making the optimal choice of resources for the current set of price and availability circumstances.

This optimization opportunity may be modeled as a linear program (7). The variables are the inputs to the cheese milk; the constraints may be constructed out of these variables to restrict the casein-to-fat ratio and to reflect government regulations, company policies, resource availability, and other restrictions on the cheese product. An objective measure may be constructed from the variables and corresponding costs coupled with the sales value of the resulting product. The advantage of casting this as a linear program is that the resulting model can then be solved by computer to quickly determine the optimal constituents of the cheese milk. The result will simultaneously reflect the technological, legal, and economic realities facing the cheesemaker. Solutions generated this way have been shown to have a profound impact on the profitability of the cheesemaker. Without such a model and the computer support, cheesemakers can make good guesses, but they cannot be assured of regularly choosing optimal inputs to the cheese vat.

In this example we have a high-volume product (the vats may hold 20,000 lb of cheese milk) and variable inputs (the fat and solids content of incoming milk varies from farm to farm and animal to animal) and the recurring decision of how to standardize the cheese milk to achieve uniform outputs. Because of its recurring nature, this decision has important economic consequences for the cheesemaker. Investigation of cheese technology provided the model (the cheese yield formula) that enables the application of optimization methods to assist with the decision of how to standardize the cheese milk.

Formulating a Cheese Topping

When a new product is fielded, there is the opportunity to establish its formulation. This decision affects the economics associated with the product and the consumer's perception of the product. The challenge is to devise a formulation that is technologically feasible and commercially viable. Because there are many alternatives to any formulation, some strategy has to be adopted to determine the desired formulation. An optimization strategy provides a good way to sift through alternatives and come up with an attractive formulation.

Cheese toppings may be manufactured by making processed cheese in such a way that the substance has many potential consistencies and textures. Such material may be mixed with other ingredients such as onions, chives, bacon bits, red pepper, and many more alternatives. The resulting product may be used by consumers as a topping for vegetables, potatoes, and salads. Properly formulated, it may be used hot or cold in a variety of ways. Details about the formulation of the plain cheese topping may be found in Hanrez-LaGrange (8). For this illustration we will consider the problem of determining what proportions of the noncheese ingredients added to the plain cheese toppings will make the best product. Preliminary studies have guided the decision makers to the point of determining what combination of red pepper pieces and nacho flavor will make the best cheese topping.

With a new product in the laboratory or pilot stage, determination of an acceptable objective measure for optimization is difficult. This measure will be used to compare alternative formulations, but it should indicate the performance of the product when it is ultimately marketed. Because this amounts to a forecast, the measure is beset with intrinsic variability problems of all forecasts. A procedure is to ask a sample of consumers to taste and then rate the product. Presuming this sample represents the intended market, the formulator may use the rating as a guide for adjusting the formulation. The connection between the formulator and the consumer panelist is a ballot requiring the panelist's responses. The questions used to solicit these responses can be used to fashion an objective measure. Although there are many ways to phrase such questions, care should be taken that the responses somehow relate to the potential market for the product. One possibility is ask the consumer to rate the product as acceptable or not acceptable. If the sample of tasters represents the target market for the product, the set of purchasers will be a subset of the set of acceptors. The bigger the set of acceptors, the larger the potential market. Actual sales will depend on many factors not controllable by manipulating the cheese topping formulation, such as advertising, distribution, price, and the competition. This acceptor set size measure can be taken for any potential formulation and becomes a way to compare formulations in order to discard the less desirable ones. Other measures are possible, but for this example, the acceptor set size will be used as the objective measure.

The strategy, then, is to ask a sample of consumers whether various formulations of this product with varying amounts of red peppers and nacho flavoring are acceptable. The objective is to find a formulation that will maximize the acceptor set size when the product is marketed. The acceptor set size is presumed to be a function of the formulation. Because the intention is that the product is to be sold in large volume, this function may be presumed to be continuous and differentiable over a reasonable range of values for input amounts of red pepper and nacho flavor. For such a function the mathematical construct called the gradient exists and may be used as a guide in seeking a better (bigger acceptor set size) formulation. The gradient of a function is a vector of partial derivatives of the function with respect to the variables. It has the property that it always points in the direction in which the function is increasing most rapidly. The advantage here is that we may estimate the partial derivatives of this function without knowing an explicit formula for the acceptor set size function. Thus, the gradient may be estimated and improvements to the acceptor set size function may be made by determining the gradient, taking a step in that direction, and retesting to determine how to adjust the formulation further. In symbols:

$A = f(x, y)$ is the acceptor set size function $\mathrm{grad}(A) = (f_x, f_y)$

where x is the amount of nacho flavor, and y is the amount of red pepper

If (x_0, y_0) is the initial formulation, then

$$(x_1, y_1) = (x_0, y_0) + k \times \mathrm{grad}(A)(x_0, y_0)$$

where k is the step size in the gradient direction. This process of estimating the gradient and determining the next test formulation ends when there is no more improvement possible (the gradient is at or near (0, 0)) or when the noise in the data overpowers the information in the estimate of the gradient.

Partial derivatives are measures of rates of change in the direction parallel to the axes of the chosen variable. To estimate the value of the partial derivative at a given formulation requires that functional estimates be generated at higher and lower values of the chosen variable while the other variables are held constant. In this example, the initial formulation was $x_0 = 3\%$ and $y_0 = 13.75\%$. To obtain estimates of the partial derivative required functional estimates for 2%, 3%, and 4% nacho flavor while holding the amount of red pepper in the mix at a constant 13.75%. To get the other partial derivative required holding nacho flavor at a constant 3% and getting functional estimates of the acceptor set size with red pepper at 8.75%, 13.75%, and 18.75%. The results of the initial test are shown in Figure 2.

The gradient vector for this test was estimated as:

$$\mathrm{grad}(A)I(x_0, y_0) = (0.50, -0.38)$$

which implies that a better formulation is possible with more nacho flavor and less red pepper. Choosing the step size depends on the product and ingredient circumstances and the judgment of the investigator. The next step in the optimization process was to test with the formulation set at

$$(x_1, y_1) = (4.35\%, 12.67\%)$$

The functional estimates for the acceptor set size are shown in Figure 3.

Further adjustments in this formulation are possible providing that big enough samples of consumers can be

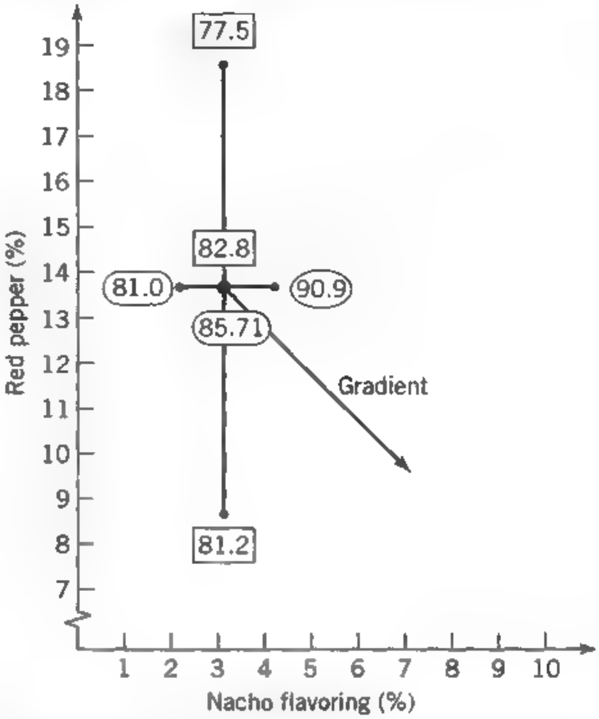


Figure 2. Determining the initial gradient in the ingredient space. Note that the gradient in the figure suggests the objective value will increase if the amount of red pepper decreases and the amount of nacho flavor increases.

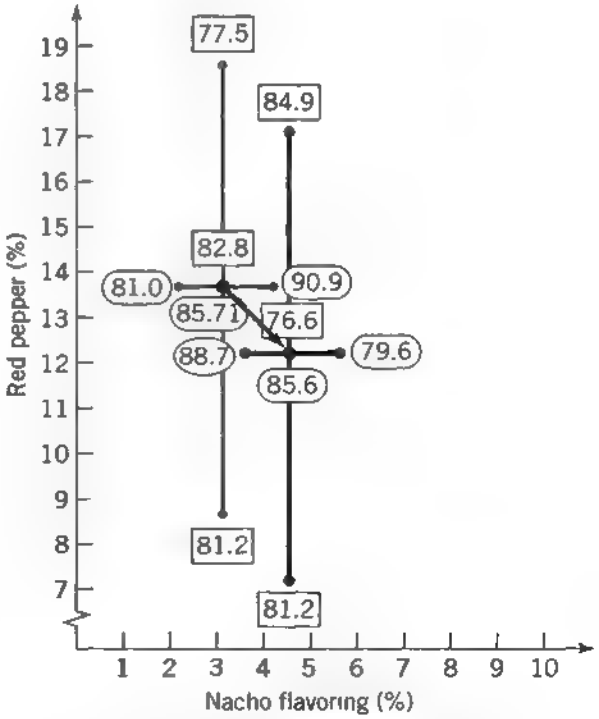


Figure 3. The values after one step in the gradient search process.

used to generate the accuracy necessary to make the results useful. This approach can be used for more than two variables, but the number of formulations for which data must be gathered also grows

This sketch of a gradient search method leaves out many details of how the estimates are generated and used to determine the next formulation. More information may be found in Hanrez-LaGrange (8) and Hanrez-LaGrange and Norback (9). This approach has some similarity to response surface methods (RSM). The main difference is that in RSM, a formula for the objective measure must be generated, and this estimation of the objective measure then may be searched for a maximizing value. Because this function is maximized over the entire range of feasible formulations, it usually requires more expensive data collection than direct estimates of the gradient for a specific formulation and is of little value beyond its use in finding a maximum. Besides finding the maximum, the gradient search approach shows the impact of different components on the objective measure.

The weaknesses in this method are in the determination of the step size at each iteration, in the noise intrinsic to sampling from a population of consumers, and the fact that it may be suboptimal if the objective measure is multimodal. The latter difficulty can be avoided by careful prescreening of the product and its potential formulations followed by restriction of the feasible formulations so that only one maximum may occur over the range of ingredient values being considered. In every test case, the procedure has yielded good solutions to formulation problems in two or three iterations. The gradient search procedure may be used for any functional objective measure, not just for the acceptor set size measure.

Making and adjusting formulations will have important economic consequences in high-volume situations. In this case, the food technology constrained the formulation, whereas the business objective of making the acceptor set size largest adjusted the formulation among the feasible alternatives. The situation could also be constrained by the cost of the inputs to the product, by company policy, or by government regulation. Decision support methods (gradient search) were applied to assist in determining the right formulation for the product. Such methods could be applied again if the product were to be reformulated for a substitute ingredient. Small variations in a formulation can have important consequences to the consumer's response to the product and to its profitability. With infinitely many formulations to choose from, these methods provide an organized means of choosing a good one.

Extending the Shelf Life of Fresh Seafood

An ongoing effort by the purveyors of fresh seafood is the opening of inland markets. This can be done by speedier transportation, but this turns out to be costly and sometimes hard to arrange if these products are to enter ordinary marketing channels. This business objective can also be advanced by improving the holding technologies for fresh seafood products (4). With such technologies the product can come through shipping and handling and still be desirable to the consumer. Perhaps the most common of these technologies is refrigeration, but this too has limits, which still leaves large markets untapped.

Two emerging technologies have application here: modified atmosphere packaging and the use of a sorbate dip on the product. With modified atmosphere packaging, the product is enclosed by a barrier material and the atmosphere over the product is replaced by one without oxygen. The permeability of the barrier material to oxygen is a controllable variable—the packaging material must be chosen (and paid for) by the seafood seller. The sorbate dip technology requires that the product be coated with a sorbate solution of variable concentration. Because these two technologies can be used simultaneously, a reasonable question is, what combination of both would be most beneficial to the seafood seller?

This was investigated by gradient search methods. Consumer response data were taken by asking consumers to taste and then to rate the sample tasted as acceptable or unacceptable. (Other data were taken as well.) The purpose was to determine the consumer response to applications of combinations of these technologies by estimating the acceptor set size over time. The system was constrained by the limits on the sorbate concentration that seemed acceptable and by available barrier materials. The acceptor set size function was assumed to be differentiable and the data taken were used to estimate the gradient of this function. The gradient of any differentiable function has the property that it points in the direction of the steepest ascent of the function. Thus, after each completed test, new information was available that indicated what adjustment in the technologies could be made to make the combined application better. This approach is an example of a gradient search method and is a decision-making tool that is broadly applied in optimization, often as part of a computer algorithm. The novelty here is that data collection must be done before each iteration of the algorithm, making the whole procedure considerably slower than most algorithms.

A starting permeability of the barrier film and concentration of sorbate is chosen. Tests are run for higher and lower permeabilities at this sorbate concentration and for higher and lower sorbate concentrations at this permeability. The resulting data allows the estimation of the gradient vector at the starting point. If the gradient vector is at or near zero, little improvement is possible, otherwise a step in the direction of the gradient will improve the performance of the combined technologies. New values for permeability and concentration are chosen and the test is repeated.

The application of this procedure portrayed consumer response through time to different combinations of technologies. An important question not addressed is, is the extra expense of implementing and using the technology worth the resulting extension in shelf life? Addressing this question would mean a more complicated model would have to be developed and perhaps different computational methods would be required.

Managing Material Flow and Batching

Production scheduling, production quality control and inventory management in the food industry lead to a focus on the flow of materials through the production facility. Many important decisions depend on accurate and timely information regarding the amount and quality of materials that are undergoing some step in the manufacturing sequence. This information is used by the manager to determine product costs, procurement, or harvest requirements, and to project output product amounts and to assist in setting prices. Product safety, good product qualities, and product profitability require that the manufacturing sequence conform to the requirements of the food as material and that the processing facilities be managed efficiently. Because of the typical low margin per sale, the throughput for such facilities must be large. This means that small improvements in efficiency will have important consequences for profitability. In many food production facilities, the manufacturing sequence is further complicated because either the technology or the economics require batch processing. Although the ideal may be "continuous processing," the technology may not be available as in the manufacture of cheddar cheese, the blending of processed cheese, or the processing of low-acid foods. In other cases, the continuous technology is too expensive or otherwise infeasible to be adopted as in the retort processing of low-acid foods. This leads to complications in achieving target productions, especially in cases where batches of intermediate materials are used in multiple products. The decision making surrounding procurement, production scheduling, and quality control is strongly connected to the technology of the food being processed. This technology once again determines the manufacturing sequence and constrains the amount of throughput.

The flow of materials in canning requires that incoming vegetables be washed, sorted by grades, placed in a container that is partially filled with brine, then retort processed before being cooled, labeled, and placed in cases. Although not all of these steps are used in every manufacturing sequence, brining and retort processing are both batch-processing steps. The flow of materials provides a structure to organize information regarding this manufacturing sequence. Such information can be fed forward to control subsequent steps in the processing. For example, the size of peas (as well as the variety of peas and the formulation of the brining solution) will affect the time and temperature settings for the subsequent retort processing. Amounts of materials and corresponding costs may be kept in this same flow of materials structure as well as information required by government regulation or company policy.

Using a flow of materials structure provides great organizational advantage. Although it is possible to do the arithmetic and track the resources by hand for a few products, with many products that use many intermediate products the mass of detail that accumulates is soon overwhelming. Using the flow of materials as a structure provides more than organizational convenience, however. The development of the so-called Gozinto matrices can be done from the flow of materials structure (10,11), and their connection to materials requirements planning (MRP) means that accurate projections of materials use, costs, and inventory amounts can be conveniently made. Although the time phasing of inventory and production is not as important a problem in the food industry as it is in fabrication industries, tracking the use of intermediate products, product costing, and batching are.

A Gozinto matrix is created through a procedure that organizes production information into a lower triangular, invertible matrix. The rows and columns of this matrix correspond to products and inputs to products, organized so that no row corresponding to an input to a product occurs above the row corresponding to the product in the matrix. This means that the bottom rows in the matrix correspond to ingredients purchased or harvested from outside the production facility, whereas the top rows correspond to the output products of the facility. The rows in between correspond to intermediate products that may find there way into many output products. More details may be found in Mize and coworkers (11).

Inverting this matrix and doing some elementary matrix algebra provides a means of anticipating and tracking inventories and production. For specified target production, the precise input needs of each ingredient or intermediate product may be determined. Inventories would be kept to a minimum if we could procure or manufacture exactly these quantities of input materials. But getting exactly the quantity of intermediate products required is complicated by batch processing and by the use of batch output in multiple products. The amount of brine needed for a target number of cases of canned vegetables is rarely a whole number of batches of brine. More likely, the target will require 12.37 batches of brine, or some other in between number. Is it better in these cases to make 12 batches or 13 batches or to make partial batches (ie, 0.37 batches)?

If we make more batches than the target, we will have excess quantity of batch output, which must be stored or discarded, or used in excess production of final product, which then must be stored. If we make less than the target production, we may not meet demand (causing stock outs) and opportunity costs associated with lost sales. If we make partial batches, we face the same labor and fixed costs associated with full batches but get less output. Furthermore, partial batches often imply important technological changes—especially in the food industry—since batch inputs may not scale linearly. Salt and spices are good examples of inputs for which it is not good enough to simply cut the amounts in half when the batch is half the size. If this technological information is not available, product quality will vary when the batch is rescaled.

In these circumstances, what is the best the production manager can do? If the costs just described are understood and can be estimated, an optimization model can be built and used to determine how many batches of input are best for target production amounts. The difficult part is the construction of an objective measure, which simultaneously takes into account the costs of the different alternatives as well as potential revenues from the products. Measuring profits for a production run will require determining the revenue from each of the products made and subtracting ingredient costs, batching costs, and the costs from overproduction and underproduction. The resulting detailed model can be found in Chung (12). For purposes of this review suffice it to say that it is possible and profitable to apply optimization in batching situations. The goal of the model is to find not only a product mix but also a batch mix that maximize the penaltied profits.

Although other models are possible, this example shows how such a model might be constructed. Such models may require that certain values be integers but still may be solved by computer methods. The result will be optimal choices regarding the number of batches produced that reflect the costs and revenues that the production facility faces, rather than the intuition of the manager.

CONCLUSION

The examples given here give some ideas how food technology may be incorporated in the structure of decision-making models. When there are many alternatives to choose from, the effort required to search for a best one often leads to more profit. The business of food technology and the food technologist in this decision-making realm is to constrain the set of alternatives. The objectives of the organization can then drive the decision-making process to the choice of an appropriate alternative. This optimization philosophy helps keep all participants in food industry decision making pulling in the same overall direction, even though interests and expertise of the individuals may be vastly different.

What is seldom investigated is this connection between the technology of food and the decision making done by food industry managers. If this connection is managed properly, important consequences for the efficiency and profitability of the business can be realized. The procurement of materials and the production, marketing, and distribution of food products all depend on the nature of food as material and the consumer's perception of that material as each spoonful is consumed. Approaching this from an optimization perspective—arraying the alternatives, measuring the alternatives, and choosing a desirable one—is a useful and profitable way to manage.

BIBLIOGRAPHY

1. W. A. Shewhart, *Economic Control of Quality of Manufactured Product*, D. Van Nostrand, Princeton, N.J., 1931. Republished by American Society for Quality Control, Milwaukee, Wis., 1980.
2. E. L. Grant and R. S. Leavenworth, *Statistical Quality Control*, 7th ed., McGraw-Hill, New York, 1996.
3. N. M. Gordon and J. P. Norback, "Choosing Objective Measures When Using Sensory Methods for Optimization and Product Positioning," *Food Technol.* **39**, 96, 98–101 (1985).
4. W. F. Sharp, J. P. Norback, and D. A. Stuiber, "Using a New Measure to Define Shelf Life of Fresh Whitefish," *J. Food Sci.* **51**, 936–939, 959 (1986).
5. G. L. Kerrigan and J. P. Norback, "Linear Programming in the Allocation of Milk Resources for Cheese Making," *Journal of Dairy Science* **69**, 1432–1440 (1986).
6. L. L. Van Slyke and W. V. Price, *Cheese*, Ridgeview, Independence, Ohio, 1979.
7. J. P. Norback and S. R. Evans, "Optimization and Food Formulation," *Food Technol.* **37**, 73–76, 78, 80 (1983).
8. V. Hanrez-LaGrange, *An Optimization Procedure with Sensory Analysis Data in the Development of Process Cheese Toppings*, Master's Thesis, University of Wisconsin, Madison, Wis., 1987.
9. V. Hanrez-LaGrange and J. P. Norback, "Product Optimization and Acceptor Set Size," *Journal of Sensory Studies* **2**, 119–136 (1987).
10. A. Vazsonyi, *Scientific Programming in Business and Industry*, John Wiley, New York, 1958.
11. J. H. Mize, C. R. White, and G. H. Brooks, *Operations Planning and Control*, Prentice-Hall, Englewood Cliffs, N.J. 1971.
12. H. K. Chung, *Planning and Optimization for Logistics Management in the Food Industry*, Ph.D. Thesis, University of Wisconsin, Madison, Wis., 1999.

JOHN NORBACK
University of Wisconsin
Madison, Wisconsin

FOOD REGULATIONS: INTERNATIONAL, CODEX ALIMENTARIUS

International food regulations govern the import and export of both raw agricultural commodities and processed food products to ensure food safety, control unwanted release of exotic species of plants and animals, and limit the spread of both plant and animal diseases. International food regulations also include standards for trade, including units of measure and minimum quality standards.

Until recently, there were essentially no unified international food regulations. Each nation negotiated with specific trading partners in cooperative agreements. This was a process driven by the standards of the importing country, since the exporter was responsible for ensuring that the product in question complied with the regulations of the importing country. Because national food regulations varied widely from nation to nation, this proved to be an inefficient process. This process also allowed countries to use food regulations as de facto barriers to foreign competition. Although there are still no legally binding international food regulations, increasing international trade and broadening participation under the World Trade Organization (WTO) is now forcing a rapid harmonization of international standards. A set of unified international food standards has emerged, with the force of the world's largest international trade organization behind it. More importantly, the WTO has established a process for the adoption of future standards, as well as for settling trade disputes involving food standards.

HISTORY

The General Agreement of Tariffs and Trade (GATT) was created in 1947 in an effort to foster international trade. From 1947 to 1994, the GATT served as the umbrella organization for international trade, establishing standards and rules to allow countries to trade with each other in an open and fair manner. Despite major success at fostering trade in nonfarm products, the GATT contained few effective rules for agricultural trade.

apply optimization in batching situations. The goal of the model is to find not only a product mix but also a batch mix that maximize the penaltied profits.

Although other models are possible, this example shows how such a model might be constructed. Such models may require that certain values be integers but still may be solved by computer methods. The result will be optimal choices regarding the number of batches produced that reflect the costs and revenues that the production facility faces, rather than the intuition of the manager.

CONCLUSION

The examples given here give some ideas how food technology may be incorporated in the structure of decision-making models. When there are many alternatives to choose from, the effort required to search for a best one often leads to more profit. The business of food technology and the food technologist in this decision-making realm is to constrain the set of alternatives. The objectives of the organization can then drive the decision-making process to the choice of an appropriate alternative. This optimization philosophy helps keep all participants in food industry decision making pulling in the same overall direction, even though interests and expertise of the individuals may be vastly different.

What is seldom investigated is this connection between the technology of food and the decision making done by food industry managers. If this connection is managed properly, important consequences for the efficiency and profitability of the business can be realized. The procurement of materials and the production, marketing, and distribution of food products all depend on the nature of food as material and the consumer's perception of that material as each spoonful is consumed. Approaching this from an optimization perspective—arraying the alternatives, measuring the alternatives, and choosing a desirable one—is a useful and profitable way to manage.

BIBLIOGRAPHY

1. W. A. Shewhart, *Economic Control of Quality of Manufactured Product*, D. Van Nostrand, Princeton, N.J., 1931. Republished by American Society for Quality Control, Milwaukee, Wis., 1980.
2. E. L. Grant and R. S. Leavenworth, *Statistical Quality Control*, 7th ed., McGraw-Hill, New York, 1996.
3. N. M. Gordon and J. P. Norback, "Choosing Objective Measures When Using Sensory Methods for Optimization and Product Positioning," *Food Technol.* **39**, 96, 98–101 (1985).
4. W. F. Sharp, J. P. Norback, and D. A. Stuiber, "Using a New Measure to Define Shelf Life of Fresh Whitefish," *J. Food Sci.* **51**, 936–939, 959 (1986).
5. G. L. Kerrigan and J. P. Norback, "Linear Programming in the Allocation of Milk Resources for Cheese Making," *Journal of Dairy Science* **69**, 1432–1440 (1986).
6. L. L. Van Slyke and W. V. Price, *Cheese*, Ridgeview, Independence, Ohio, 1979.
7. J. P. Norback and S. R. Evans, "Optimization and Food Formulation," *Food Technol.* **37**, 73–76, 78, 80 (1983).
8. V. Hanrez-LaGrange, *An Optimization Procedure with Sensory Analysis Data in the Development of Process Cheese Toppings*, Master's Thesis, University of Wisconsin, Madison, Wis., 1987.
9. V. Hanrez-LaGrange and J. P. Norback, "Product Optimization and Acceptor Set Size," *Journal of Sensory Studies* **2**, 119–136 (1987).
10. A. Vazsonyi, *Scientific Programming in Business and Industry*, John Wiley, New York, 1958.
11. J. H. Mize, C. R. White, and G. H. Brooks, *Operations Planning and Control*, Prentice-Hall, Englewood Cliffs, N.J. 1971.
12. H. K. Chung, *Planning and Optimization for Logistics Management in the Food Industry*, Ph.D. Thesis, University of Wisconsin, Madison, Wis., 1999.

JOHN NORBACK
University of Wisconsin
Madison, Wisconsin

FOOD REGULATIONS: INTERNATIONAL, CODEX ALIMENTARIUS

International food regulations govern the import and export of both raw agricultural commodities and processed food products to ensure food safety, control unwanted release of exotic species of plants and animals, and limit the spread of both plant and animal diseases. International food regulations also include standards for trade, including units of measure and minimum quality standards.

Until recently, there were essentially no unified international food regulations. Each nation negotiated with specific trading partners in cooperative agreements. This was a process driven by the standards of the importing country, since the exporter was responsible for ensuring that the product in question complied with the regulations of the importing country. Because national food regulations varied widely from nation to nation, this proved to be an inefficient process. This process also allowed countries to use food regulations as de facto barriers to foreign competition. Although there are still no legally binding international food regulations, increasing international trade and broadening participation under the World Trade Organization (WTO) is now forcing a rapid harmonization of international standards. A set of unified international food standards has emerged, with the force of the world's largest international trade organization behind it. More importantly, the WTO has established a process for the adoption of future standards, as well as for settling trade disputes involving food standards.

HISTORY

The General Agreement of Tariffs and Trade (GATT) was created in 1947 in an effort to foster international trade. From 1947 to 1994, the GATT served as the umbrella organization for international trade, establishing standards and rules to allow countries to trade with each other in an open and fair manner. Despite major success at fostering trade in nonfarm products, the GATT contained few effective rules for agricultural trade.

Since 1948 national food safety and animal and plant health measures that affect trade were subject to GATT rules. These rules required nondiscriminatory treatment of imported agricultural products from different foreign suppliers, and, in theory, imported products were supposed to be treated no less favorably than domestically produced agricultural goods. However, the agreement allowed that for purposes of protecting human, animal, or plant health, governments could impose more stringent requirements on imported products than they required of domestic goods. This clause was often intentionally misused to keep imported farm products out of a country to protect domestic producers. In 1979 the Tokyo Round of trade negotiations attempted to limit abuse of this clause and produced the Agreement on Technical Barriers to Trade. In this agreement, governments agreed to use relevant international food standards—except when they considered that these standards would not adequately protect health. Although the agreement established the precedent of using international standards, there remained a large loophole for using sanitary and phytosanitary measures as barriers to trade.

In April 1994 the GATT completed the final act of a series of international trade negotiations, commonly referred to as the Uruguay Round, including reforms of international food regulations. The negotiations culminated in a treaty that created the WTO (which superseded the GATT as the umbrella organization for international trade) and contained the WTO Agreement on Sanitary and Phytosanitary (SPS) Measures concerning the application of food safety and animal and plant health regulations.

The SPS agreement, now the centerpiece for international food regulations, stated that the WTO and its member countries would adopt the existing international standards, guidelines, and recommendations developed by specific international organizations as the basis for its food standards. The SPS agreement recognizes the standards of the Codex Alimentarius (Codex), the International Office of Epizootics (OIE), and the International Plant Protection Convention (IPPC). Importantly, the SPS agreement contains specific guidelines for the continual creation and adaptation of food standards to respond to new developments and knowledge in food safety and plant and animal health. The SPS also outlines a specific dispute settlement procedure, with effective timetables and requirements. In effect, the SPS agreement, administered through the WTO and based on the recommendations of independent organizations, is the first effective set of unified international food regulations.

The SPS agreement is an interesting and important success story. It demonstrates that voluntary, market-oriented collaboration can achieve difficult goals, and it endorses the existence and work of international technical advisory bodies. The three organizations to which the WTO defers for its food standards were created independently and prior to the creation of the WTO. None of these organizations had legal authority to impose their recommendations on any nation. But the recommendations and guidelines set by these organizations were well founded, as they represented the consensus opinions of scientists and experts from around the globe.

Although the adoption of the standards of these organizations created a much needed baseline for international trade, it is important to note that these standards do not supersede the ability of nations to maintain their own food safety standards. First, these rules apply only to member nations of the WTO. But even WTO member nations maintain control of their food safety standards. No member nation is required to adopt any international food standard as its own. However, if a member nation maintains a food standard stricter than an established international standard that results in restrictions on trade, WTO rules require that the nation demonstrate the need for the stricter standard using sound scientific principles. The SPS agreement also stipulates: "Members shall accept the sanitary or phytosanitary measures of other Members as equivalent, even if these measures differ from their own . . . if the exporting Member objectively demonstrates to the importing Member that its measures achieve the importing Member's appropriate level of sanitary or phytosanitary protection" (1). In other words, each country is free to devise its own sanitary and phytosanitary measures as long as those measures achieve the proper level of sanitary/phytosanitary protection for its exported product. The SPS agreement also states that sanitary and phytosanitary measures "shall not be applied in a manner which would constitute a disguised restriction on international trade" and standards must be applied "only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence" (1).

To ensure that the standards-setting process remains relevant to WTO member countries, the SPS agreement stipulates that "members shall play a full part, within the limits of their resources, in the relevant international organizations and their subsidiary bodies," with particular reference to the Codex, OIE, and IPPC "to promote within these organizations the development and periodic review of standards, guidelines and recommendations with respect to all aspects of sanitary and phytosanitary measures" (1). The SPS also created a Committee on Sanitary and Phytosanitary Measures to monitor the process of international harmonization and coordinate efforts in this regard with the relevant international organizations.

The Codex

The Codex Alimentarius (Codex), to which the WTO defers for food safety standards, was created in 1962 by the United Nation's Food and Agriculture Organization (UNFAO) and the World Health Organization (WHO) under the direction of the FAO/WHO Food Standards Program. The Codex Alimentarius, which means "food code" in Latin, was created "to guide and promote the elaboration and establishment of definitions and requirements for foods, to assist in their harmonization and, in doing so, to facilitate international trade" (2). The Codex is composed of more than 160 member countries, each represented by delegates who participate through the various committees that comprise the Codex (see Fig. 1).

There are several committee categories within the Codex: the executive committee, general subject committees

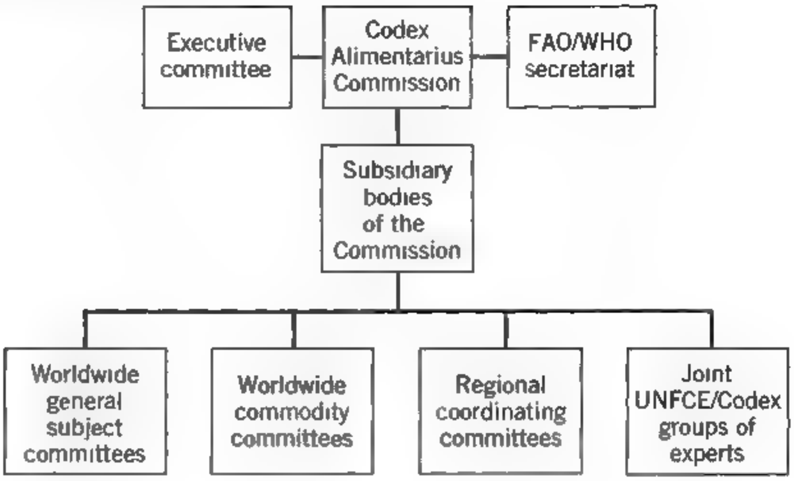

Executive committee
Codex Alimentarius Commission
FAO/WHO secretariat
Subsidiary bodies of the Commission
Worldwide general subject committees
Worldwide commodity committees
Regional coordinating committees
Joint UNFCE/Codex groups of experts

Figure 1. Subsidiary bodies of the Codex Alimentarius Commission.

and commodity committees. The executive committee is the executive organ of the Commission; that is, it is responsible for its functioning. It is comprised of a chair and three vice-chairpersons, as well as one member representing each of six geographic regions of the globe: Africa, Asia, Europe, Latin America and the Caribbean, North America, and the southwest Pacific. Actual standards and food safety recommendations are established either through a general subject committee, such as food labeling, or commodity committees, such as milk and milk products (see Table 1). There are 8 general subject committees and 14 commodity committees. As the Codex has operated for more than 30 years and has been establishing standards and guidelines throughout this time, most committees meet only on an irregular basis when developments require new or revised standards.

Table 1. Codex Commodity and General Subject Committees

Worldwide Codex Commodity Committees (and their host countries)

Cocoa products and chocolate (Switzerland)
Sugars (United Kingdom)
Processed fruits and vegetables (United States)
Fats and oils (United Kingdom)
Soups and broths (Switzerland)
Fresh fruits and vegetables (Mexico)
Milk and milk product (New Zealand)
Cereals, pulses, and legumes (United States)
Processed meat and poultry products (Denmark)
Meat hygiene (New Zealand)
Vegetable proteins (Canada)
Fish and fishery products (Norway)
Nutrition and foods for special dietary uses (Germany)
Natural mineral waters (Switzerland)

Worldwide Codex General Subject Committees

Food labeling (Canada)
Food additives and contaminants (Netherlands)
Food hygiene (United States)
Pesticide residues (Netherlands)
Veterinary drugs in foods (United States)
Methods of analysis and sampling (Hungary)
Food import and export inspection and certification systems (Australia)
General principles (France)

Examples of standards established by the Codex general committees include limits for allowable levels of pesticide residues in food products, guidelines for hygienic handling of food products, limits on food additives and contaminants (ie, preservatives, coloring agents, etc), veterinary drug residues, and even analysis and sampling methods.

Examples of commodity committees are cereals, pulses, and legumes; milk and milk products; cocoa products and chocolate; sugars; and natural mineral waters.

The Codex works closely with both its parent organizations, the UNFAO and the WHO, as well as the WTO to ensure that its work addresses the relevant concerns of these organizations.

The OIE

The OIE, the organization responsible for animal health, was created in 1924. The OIE is an intergovernmental organization headquartered in Paris, France. It consists of more than 150 member countries who, as with the Codex, are represented by delegates participating through various commissions and committees. The OIE is organized into three types of commissions: an administrative commission, regional commissions, and specialist commissions, as well as working groups.

The International Committee, the highest authority of the OIE, meets once a year. Its main function is to coordinate the work of the OIE commissions and adopt international standards for animal health, especially for international trade, as well as to adopt resolutions on the control of major animal diseases. Due to the geographic nature of animal health problems, regional commissions were created to address the specific animal health concerns for the five major geographic areas of the world: Africa, the Americas, Asia, Europe, and the Middle East.

To facilitate its role in harmonizing regulations for trade in animals and animal products, the OIE maintains the *International Animal Health Code*, prepared by the

International Animal Health Code Commission, as well as a *Manual of Standards for Diagnostic Tests and Vaccines* prepared by the OIE's Standards Commission. These publications establish minimum standards to protect animal health (both domesticated and farm animal as well as wild animals) and to prevent the spread of animal diseases that might result from the trade in animals or animal products.

Examples of standards established by the OIE are guidelines for assessing the health of animals before, during, and after transport of animals in international trade, guidelines for destroying pathogens in animal products, and recommended diagnostic procedures.

The International Plant Protection Convention

The IPPC is the phytosanitary (plant health) standard-setting organization of the SPS agreement and is the basis on which countries collaborate to prevent the spread and introduction of plant pests and plant products. The purpose of the Convention according to the IPPC preamble is "international cooperation in controlling pests of plants and plant products and in preventing their international spread, and especially their introduction into endangered areas." The scope of the Convention extends to the protection of both cultivated and natural flora and includes both direct and indirect damage by pests.

From its creation in 1951 until 1992, the IPPC existed as an international agreement administered through the FAO and implemented primarily through the cooperation of regional and national plant protection organizations. However, the FAO recognized the role that the IPPC would play in the then-developing WTO/SPS agreement and created an IPPC Secretariat in 1992. The FAO also established a standard-setting process and formed the Committee of Experts on Phytosanitary Measures (CEPM) in 1993. The IPPC currently has more than 100 member countries and is administered through the UNFAO's Plant Protection Service.

In addition to the Secretariat and CEPM, amendments to the Convention resulted in the creation of the Commission on Phytosanitary Measures to review the state of plant protection in the world, provide direction to the work of the IPPC Secretariat, and approve international standards.

The Secretariat is responsible for coordinating the work of the IPPC and, most importantly, the elaboration of International Standards for Phytosanitary Measures (ISPMs). These standards are developed by a process that begins with submission of proposed standards from the Secretariat, by expert groups organized by the Secretariat, or by regional or national plant protection organizations. Draft standards are reviewed by the CEPM and are sent to member governments for consultation before being submitted to the Commission for adoption.

IPPC standards fall into three categories: reference standards, concept standards, and specific standards. To date, the IPPC has produced primarily reference and concept standards to support the subsequent specific standards.

The IPPC also has a dispute settlement clause to help resolve international trade disputes, although this process is separate from the WTO dispute settlement process and is not legally binding.

CONCLUSION

The demand for agricultural products is expected to more than double worldwide over the next 40 years, through both population growth and dietary improvement in developing country populations. Many of these countries lack adequate land and agricultural resources to meet their expected food needs domestically without significant destruction of wildlife habitat, elevating the importance of international agricultural trade. Consumers throughout the world are also raising their food safety expectations, thus the need for viable international food safety standards.

BIBLIOGRAPHY

1. *Agreement on the Application of Sanitary and Phytosanitary Measures*, World Trade Organization, Geneva, Switzerland, 1995.
2. *Codex Alimentarius: FAO/WHO Food Standards*, United Nations FAO, Rome, Italy, 1996.

GENERAL REFERENCES

"Agreement on the Application of Sanitary and Phytosanitary Measures," available from the World Trade Organization at *www.wto.org/wto/goods/spsagr.htm*.

"Understanding the WTO Agreement on Sanitary and Phytosanitary (SPS) Measures," available from the World Trade Organization at *www.wto.org/wto/goods/spsund.htm*.

All background material used to prepare this article came from and is available at the following World Wide Web addresses:

World Trade Organization, www.wto.org
Codex Alimentarius, www.fao.org/waicent/faoinfo/economic/esn/codex/ and www.fsis.usda gov/OA/codex/
OIE, www.oie.int
IPPC, www.fao.org/ag/agp/agpp/pq/

ALEX A. AVERY
DENNIS T. AVERY
Hudson Institute
Churchville, Virginia

FOOD SAFETY AND RISK COMMUNICATION

Foods commonly contain a number of microorganisms (bacteria, fungi, viruses, etc) as well as potential chemical contaminants (food additives, pesticide residues, veterinary drug residues, metals, etc) that may pose some level of risk to consumers. Although the magnitudes of the risks may vary dramatically among the various risk agents, it is clear that significant public attention focuses on virtually all types of food safety risks, both large and small. Effective communication of such food safety risks to the general population as well as to lawmakers is an important goal of health scientists, government agencies, and food industry representatives.

The process of risk communication was defined by the National Research Council (NRC) to include an interactive process of exchange of information including information on the nature of the risk and other messages regarding concerns, opinions, or reactions to the risk messages (1). With respect to communication of food safety risks, common risk communication methods have been mostly one-way and technocratic and frequently involve regulatory agencies, industry, or government officials transmitting the results of risk assessments with the aim that the public accept the risk messages and act accordingly (2). In the last decade this view has been challenged, and alternative risk communication methods have been developed to improve dialogue with the public and affected or interested parties. It has been suggested that such a dialogue should provide effective public involvement in the decision-making process and not merely reflect transference of food safety risk information from experts to nonexperts.

RISK MESSAGES

Risk messages are only a part of the interactive risk communication process and are often designed to inform the public about food safety risks. Due to the highly technical nature of the subject matter, the task of formulating food safety risk messages that are clear, accurate, and not misleading is a challenging one. Complex language and technical jargon are commonly used in risk messages, leading to information that is often incomprehensible to the public. Effective risk messages should be developed using clear and plain language and should transmit relevant information in addition to recommending practical actions that the public can take (1).

Effective risk communication requires communicators to recognize and overcome several obstacles that are rooted in the limitations of scientific risk assessment and in public understanding. Technical barriers to effective risk communication include the need to make assumptions and subjective judgments in the risk assessment process. In the risk assessment of a food chemical there is a need to consider any possible gaps in knowledge that will lead to uncertainties in the risk estimates (3). Uncertainties frequently arise from the use of animal models in the risk assessment process. Typical human health risk assessments rely on results from laboratory animal studies that must be extrapolated to humans. Evaluating results from toxicology studies where chemicals are administered to laboratory animals at a very high dose requires extrapolation to humans exposed to a low dose, sometimes over a lifetime, generating different levels of uncertainty. According to Young (4), the greater the uncertainty about a given effect, the more likely it is to be overestimated. Experts often adopt measures based on conservative assumptions, which provides a tendency to dramatically overestimate the predicted levels of risk (5).

Risk messages should present a clear explanation of the scientific evidence, including data gaps, significance of uncertainties, and possible differences in opinion among experts. In formulating risk messages it is important to be truthful and to discuss practical factors to be considered for risk management/control. Risk communicators should explain steps to be followed for risk reduction or, when applicable, address possible consequences (health, economic) resulting from risk control measures. The common views of experts should be expressed, and consumer concerns need to be considered (6). Nonetheless, consumer reaction to food safety risk messages may often involve misunderstanding of the scientific facts and may lead to general distrust of those providing the messages.

RISK COMPARISONS

The sheer complexity and uncertainty inherent in risk assessment provides a significant barrier to public understanding and appreciation of the magnitude of risks. One method of explaining risk information is to make comparisons to other risks. Some feel that comparing and contrasting risks with similar assessments of commonplace situations help the consumer better understand risks (7), because comparisons are more meaningful to the public than unfamiliar magnitudes (probabilities) associated with risks. Examples of such an approach include comparing the risks of cigarette smoking with the risks of motor vehicle accidents, home accidents, background radiation levels, or hang gliding.

Although risk comparisons can be helpful in communicating the magnitude of risks, they are not by themselves adequate determinants of decisions. Despite the appeal of using risk comparisons to put results of risk assessments in perspective, such risk comparison practices have been subject to criticism, because they ignore critical elements concerning public values and acceptability of different types of risks. Moreover, some comparisons involving different types of risk often ignore different levels of uncertainty inherent in the risk estimates.

With regard to chemical hazards, Ames et al. (8) and Gold et al. (9) ranked the potential human carcinogenic risks of exposures to a variety of environmental pollutants, synthetic pesticide residues, naturally occurring toxins, and pharmaceutical products using an index that relates predicted human exposure levels for carcinogens to their carcinogenic potency in rodents. Their results indicated that the risks posed by residues of synthetic pesticides or environmental pollutants ranked low in comparison to risks of naturally occurring carcinogens.

RISK AND CONSUMER PERCEPTIONS

Perception of risks among members of society is very complex and is determined by both biological and nonbiological factors. Due to the often highly technological content of risk messages, such messages can generate frustration on the part of the public who ultimately complain of the inaccessibility of the scientific knowledge expressed in those messages. On the other hand, if risk messages are oversimplified, they can also result in negative reactions from the public, such as distrust or unnecessary fear. While many experts define a food safety risk as a probability of an adverse health effect caused by exposure to some potentially hazardous agent in food, perceived risk may be defined as

Table 1. Qualitative Factors Affecting Risk Perception and Evaluation

Factor	Conditions associated with increased public concern	Conditions associated with decreased public concern
Voluntariness of exposure	Involuntary	Voluntary
Controllability	Uncontrollable	Controllable
Fairness	Inequitable distribution of risks and benefits	Equitable distribution of risks and benefits
Familiarity	Unfamiliar	Familiar
Origin	Caused by human actions or failures	Caused by acts of nature
Memorability	Memorable	Not memorable
Dread	Dreaded effects	Effects not dreaded
Catastrophic potential	Fatalities and injuries grouped in time and space	Fatalities and injuries scattered and random
Benefits	Effects irreversible	Effects reversible
Effects manifestation	Delayed effects	Immediate effects
Effects on children	Children specifically at risk	Children not specifically at risk
Victim identity	Identifiable victims	Statistical victims
Trust in institutions	Lack of trust in responsible institutions	Trust in responsible institutions

Source. Ref. 3.

the sum of hazard, derived from the risk assessment process, and outrage, which involves nonbiological, qualitative factors (10). Table 1 lists a variety of factors related to public concern and conditions that provoke high or low levels of outrage among consumers. It has been pointed out that the public pays too little attention to hazard while the scientific experts pay little or no attention to outrage, which explains the common differences between public and expert opinions concerning risks. Some risks possessing high hazard but low outrage may be of less public concern than those with low hazard but high outrage (10). The acceptability of a risk depends on those outrage factors. Many major food safety–related disputes relate more to risk acceptability than to the actual risks calculated. It is common that a voluntary risk is more acceptable than one perceived as being involuntarily inflicted on consumers. Slovic (11) provides many examples of how hazard and outrage influence public perception of risks.

Pesticide residues in foods provide a good example of how outrage factors may significantly influence public perception of risks. Consumer attitude surveys revealed that between 72 and 82% of the public deem pesticide residues as a major concern in food safety (12), although this contrasts greatly with the opinion of most health scientists and regulatory agencies who contend that the dietary risks from exposure to pesticides in foods are minimal (13). Pesticide residues are associated with several conditions of increased public concern, such as being involuntary, (largely) uncontrollable, unfamiliar, and inequitable (it is commonly assumed that consumers may be the ones taking the risks while food producers may be reaping the benefits from pesticide use). Significant concern is also expressed by the public with regard to new technologies such as food irradiation, biotechnology, engineered foods, and the use of growth hormones given to cows with the aim of boosting milk production. The scientific processes of risk assessment and risk management provide information concerning the risks, costs, and benefits of policy choices, but the ultimate management of the risks is an issue of social policy that requires decisions to be made on the basis of value choices (6).

BIBLIOGRAPHY

1. National Research Council, *Improving Risk Communication*, National Academy Press, Washington, D.C., 1989.
2. C. W. Scherer, "Strategies for Communicating Risks to the Public," *Food Technol.* **45**, 110–116 (1991).
3. C. K. Winter and F. J. Francis, "Assessing, Managing and Communicating Chemical Food Risks," *Food Technol.* **51**, 85–92 (1997).
4. F. E. Young, "Weighing Food Safety Risks," *FDA Consumer* **23**, 8–13 (1989).
5. P. H. Abelson, "Health Risk Assessment," *Reg. Toxicol. Pharmacol.* **17**, 219–223 (1993).
6. E. Groth, "Communicating With Consumers About Food Safety and Risk Issues," *Food Technol.* **45**, 248–253 (1991).
7. R. Wilson and E. A. C. Crouch, "Risk Assessment and Comparisons: An Introduction," *Science* **23**, 267–270 (1987).
8. B. N. Ames and L. S. Gold, "Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis," *Science* **249**, 970–971 (1990).
9. L. S. Gold et al., "Rodent Carcinogens: Setting Priorities," *Science* **258**, 261–265 (1992).
10. P. M. Sandman, "Risk Communication: Facing Public Outrage," *EPA Journal* **13**, 21–22 (1987).
11. P. Slovic, "Informing and Educating the Public About Risk," *Risk Analysis* **6**, 403–415 (1986).
12. C. M. Bruhn et al., "Consumer Response to Pesticide/Food Safety Risk Statements: Implications For Consumer Education," *Dairy, Food and Environmental Sanitation* **18**, 278–287 (1998).
13. S. O. Archibald and C. K. Winter, "Pesticides in Our Food: Assessing the Risks," in C. K. Winter, J. N. Seiber, and C. F. Nuckton, eds., *Chemicals in the Human Food Chain*, Van Nostrand Reinhold, New York, 1990, pp. 1–50

ELISABETH L. GARCIA
CARL K. WINTER
University of California
Davis, California

See also FOOD SAFETY AND RISK MANAGEMENT;
TOXICOLOGY AND RISK ASSESSMENT.

FOOD SAFETY AND RISK MANAGEMENT

The emerging field of food safety risk analysis is composed of three dependent yet separate fields: risk assessment, risk management and risk communication. This trinity of risk-related factors forms the basis for the decisions about chemicals requiring regulation and the type of regulation needed. The ultimate goal of the process is food safety, the prevention of adverse health effects due to exposure to hazardous substances in food. Many different chemicals enter the food supply, including pesticides, natural toxins (such as aflatoxins), metals, and intentionally used food additives.

Risk management is a process to determine what level of risk is significant and to identify, select, and implement options to reduce or minimize levels of risk (1). Probabilistic risk information obtained through the risk assessment process is provided to risk managers who determine what action, if any, should be taken to manage (control) the risk in question. Representatives of both the government and industry sectors are frequently involved in risk management activities.

Risk management provides a regulatory decision-making process that involves consideration of political, social, economic, and technological information in addition to simply considering the results from the risk assessment process. As such, risk management requires the use of value judgments on issues such as acceptability of risk and the reasonableness of the costs of control (2).

Although risk assessment and risk management activities must be coordinated, these entities must also maintain some independence to guarantee the scientific integrity of the risk assessment process and to minimize potential conflicts of interest between risk assessors and risk managers (3,4). Nevertheless, it has been suggested that the separation between risk assessment and risk management may not be advantageous or possible, and some authors propose an integrated model, which combines elements of risk assessment and management (5).

Risk management considers risk characterization results, searches for viable options or strategies to minimize or reduce the potential risk, and identifies risk control strategies, if needed, to reduce the risks to acceptable levels. Ideally, a monitoring step should follow to allow an evaluation of the impact of regulatory decision on public health.

Since risk management considers political, economic, and social issues in addition to risk characterization results, optimal risk management decisions should include input from a variety of stakeholders that may be impacted by such decisions. Stakeholders may represent either the public or private sectors and include consumer and environmental organizations, the food industry (producers, processors, distributors, retailers, and restaurants), regulatory bodies, the legislative sector, and trade associations.

CONSIDERATION OF RISK CHARACTERIZATION

Once the risk assessment process has been completed to provide a mathematical characterization of the risk and a description of the various uncertainties inherent in the calculation of the risk, risk managers will consider this risk estimate as the starting point from which to develop an appropriate risk mitigation strategy, if it is deemed that the risk should be reduced. It should be emphasized that optimal risk management efforts require scientifically appropriate risk assessments.

An important aspect in the consideration of the risk characterization is the need for experienced judgment. Inherent in the risk assessment process are large gaps in knowledge that require many choices to be made among competing models and assumptions; this introduces considerable uncertainty into the risk estimates that must be appreciated in the risk management process. Optimally, risk managers should be allowed the flexibility to make risk-related decisions using a weight-of-evidence approach that allows for the consideration of all available valid scientific data. It has been suggested, however, that risk assessments are often conducted using a strength-of-evidence approach in which experiments demonstrating positive toxicological effects are given more weight than any number of negative experiments of equal quality (6). This may be particularly true in the case of carcinogen risk assessment, where conservative assumptions may exaggerate risks greatly and, therefore, may distort regulatory practices (7).

RISK, COSTS, AND BENEFITS

In food safety decision-making processes, benefit–cost analysis is frequently adopted to reduce risk to a single dimension (such as the risk of developing cancer, nervous system disorder, or death), to avoid subjectivity in the decision making, and as an aid in setting priorities. An important role of the risk manager is to identify various risk-control options and to assess the risks and benefits associated with each option. Costs and benefits may be calculated on both economic and health scales; such a process is highly controversial as it often assigns monetary values to human life, deaths, illnesses, and health issues. As an alternative to conventional cost–benefit analysis, other techniques have been developed to provide assistance in evaluating various options where costs and benefits may be expressed on nonmonetary scales. It should be emphasized that although benefit–cost analysis can be of some help in the decision-making process, it is fundamental to ensure that important qualitative factors are not underplayed by quantitative factors (2).

PREVENTION, INTERVENTION, AND CONTROL ACTIONS

Chemical hazards can be introduced into the food supply at all stages along the food chain. Standards for food safety are set, and the food industries are entrusted to comply with them. In addition to regulatory standards, consumer demands and purchasing habits may also require food industry representatives to consider implementing various risk control measures.

In the process of preparing a strategy to reduce potential risks, it is clear that considerable improvements can be achieved by appropriate education, training, and prevention. Implementation of Good Manufacturing Practices and Hazard Analysis Critical Control Point (HACCP) programs have led to the widespread adoption of several risk prevention measures in the production and processing of food products.

Legislative mandate largely determines the flexibility afforded risk managers in interpreting the results of risk assessments and in considering other factors before making regulatory decisions. A variety of models are often considered in the food safety risk management process and their uses are often prescribed by law. Some common models include:

Zero risk, which applies to food additives shown to induce cancer in humans or animals. For chemicals regulated in such a manner there is a zero tolerance.

Reasonable certainty of no harm or negligible risk, which typically deems acceptable a cancer risk of no more than one excess cancer above background per million people exposed, as calculated using conservative (risk-magnifying) risk assessment methods.

Risk balancing, which specifically requires that risk and benefits should be considered together before making a decision.

Technical feasibility, which considers both the level of risk and the availability of current technological methods to control the risk.

UNITED STATES REGULATIONS

Food additives are regulated by the U.S. Food and Drug Administration (FDA). Before an additive is approved for food use it must be demonstrated that it is safe under its intended conditions of use. Some food ingredients have been reviewed by qualified personnel and are granted the status of Generally Recognized as Safe (GRAS). Food additives that have been demonstrated to be noncarcinogenic are allowed for use if the exposure estimates are below the Acceptable Daily Intake (ADI), which is derived by applying conservative uncertainty factors to exposure levels that typically do not cause observed effects in laboratory animals.

Within the Federal Food, and Drug and Cosmetic Act, the Delaney Clause, adopted in 1958, states that no substance which has been shown to induce cancer in humans or animals can be used as a food additive (zero risk basis). As such, potentially carcinogenic chemicals are not allowed as food additives regardless of the expected level of exposure.

The FDA has applied the concept of a negligible risk to veterinary drugs while taking negligible risk and risk balancing approaches to regulate specific carcinogenic food contaminants, such as polychorinated biphenyls (PCBs) in fish and aflatoxins in peanuts and other products (8).

The U.S. Environmental Protection Agency (EPA) regulates drinking water contaminants under provisions of the Safe Drinking Water Act. For noncarcinogenic drinking water contaminants, allowable levels are set to ensure that a fraction of the ADI is not exceeded. For carcinogenic drinking water contaminants, it has been recognized that zero risk is not technologically attainable. As an alternative, maximum containment levels are established at the lowest technologically feasible levels; these typically result in lifetime cancer risks in the order of 1 in 100,000 or lower, but risks for some chemicals at the maximum containment level exceed 1 in 100,000 (8).

The major law regulating pesticides, the Federal Insecticide, Fungicide, and Rodenticide Act, provides the EPA with the authority to permit specific pesticide uses when it has been determined that the potential benefits of the uses of the pesticides outweigh their potential risks. Some benefits of pesticides may be directly related to health; an example concerns the use of a fungicide that may result in food residues yet may prevent the formation of naturally occurring fungal toxins of potentially greater health risk. Substitution effects are also important, since elimination of the use of a specific pesticide that may leave food residues could lead to an increase in the use of less-effective pesticides, resulting in greater potential for environmental disruption and worker-safety concerns. Another benefit considered by EPA is the pesticide's ability to produce an abundant, available, and affordable food supply by increasing crop yields and reducing production costs and consumer prices.

In August 1996 new legislation repealed the Delaney Clause with respect to pesticide residues. The legislation limited risk-balancing provisions concerning pesticide residues in foods and instituted a "reasonable certainty of no harm" standard. In addition, regulatory practices were prescribed to consider exposures and sensitivities of specific population subgroups such as infants and children, to consider the aggregate exposure of pesticides from dietary, water, and residential exposure, to consider the cumulative risks posed from exposure of families of pesticides that possess common toxicological mechanisms of action, and to consider other types of toxicological effects such as endocrine disruption.

As noted in the previous discussion, a variety of different risk management practices are prescribed and the choice of which practices to use depends on the classification of the chemical (eg, pesticide, food additive, veterinary drug) and the medium from which humans are exposed (eg, food, water). As such, an acceptable level of risk for one type of food chemical may differ greatly from what is considered legally acceptable for another type of food chemical, and the use of practices such as risk balancing (comparing risks with benefits and/or economic impact) and technical feasibility may be allowed under some laws and not allowed under others. A summary of the various regulatory models used in the United States is provided in Table 1. In essence, it is possible for the same chemical to be subjected to different allowable levels of risk depending on whether consumers eat it, breathe it, or drink it. It is common that higher risks are tolerated for workers who are exposed to chemicals during their job (such as pesticide manufacturers and applicators) than for the general population. In general, such risks are justified because the general population includes children, pregnant women, elderly, and ill people, whereas the workforce is on average healthier and frequently subjected to medical surveillance, and their work environment, in many cases, is monitored (8).

Table 1. U.S. Federal Laws Regulating Chemicals in Food and Water

Law	Regulatory agency	Regulated products	Regulatory model
Food, Drug and Cosmetic Act (1906, 1938, amended 1958, 1960, 1962, 1968)	FDA	Food and feed additives Veterinary drugs Natural toxins	Zero risk Negligible risk Negligible risk/risk balancing
Federal Insecticide, Fungicide, and Rodenticide Act (1948, amended 1972, 1975, 1978)	EPA	Pesticides	Reasonable certainty of no harm/limited risk balancing
Safe Drinking Water Act (1974, amended 1977, 1996)	EPA	Drinking water contaminants	Technical feasibility

Source: Ref 9.

INTERNATIONAL REGULATIONS

International food standards are set by the Codex Alimentarius Commission, which was established by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) Assembly in 1961–1962. The Codex Alimentarius Commission membership is open to all member nations and associate members of FAO and/or WHO, and currently comprises more than 160 members. The overall objective of the Codex is to ensure consumer protection and to facilitate international trade.

Scientific risk assessment provides the basis for Codex risk management decisions. Two FAO/WHO scientific advisory bodies, the Joint Expert Committee on Food Additives (JECFA) and the Joint Meeting on Pesticide Residues (JMPR) focus their activities primarily on risk assessment. These committees are independent of the Codex system and advise FAO and WHO and their members. Within Codex, risk management is defined as the process of considering risk assessment results, weighing policy alternatives, and, if required, selecting and implementing appropriate control options including regulatory measures. The outcome of the risk management process is the development of standards, guidelines, and other recommendations, which are developed by the Codex subsidiary bodies, such as the Committee on Food Additives and Contaminants (CCFAC), the Committee on Pesticide Residues (CCPR), and the Committee on Residues of Veterinary Drugs in Food (CCRVDF). In the case of food contaminants, the normal approach is for CCFAC to set levels of contaminants that are as low as reasonably achievable. The CCRVDF recommends maximum residue limits (MRLs) for residues of veterinary drugs in food, and the CCPR sets ADIs and MRLs for pesticide residues. Monitoring of both the effectiveness of the control measure (through periodic evaluation of the decision) and its impact on risk to the exposed population is expected following implementation of the control measure (3).

BIBLIOGRAPHY

1. V. T. Covello and M. W. Merkhofer, *Risk Assessment Methods: Approaches for Assessing Health and Environmental Risks*, Plenum, New York, 1993
2. National Research Council, *Improving Risk Communication*, National Academy Press, Washington, D.C., 1989.
3. United Nations Food and Agricultural Organization, *Risk Management and Food Safety*, FAO Food and Nutrition Paper 65, FAO/WHO, Rome, Italy, 1997.
4. U.S. Food and Drug Administration, "Food Safety From Farm to Table . . . A National Food Safety Initiative Report to the President," *Dairy, Food and Environmental Sanitation* **17**, 555–574 (1997).
5. M. J. DiBartolomeis, "Risk Assessment and Risk Management," in A. M. Fan and L. W. Chang, eds., *Toxicology and Risk Assessment*, Marcel Dekker, New York, 1996, pp. 775–776.
6. G. M. Gray, *Key Issues in Environmental Risk Comparisons: Removing Distortions and Insuring Fairness*, Reason Foundation, Los Angeles, Calif., 1996.
7. A. L. Nichols and R. J. Zeckhauser, "The Perils of Prudence: How Conservative Risk Assessments Distort Regulation," *Reg. Toxicol. Pharmacol.* **8**, 61–75 (1988).
8. J. V. Rodricks, *Calculated Risks: The Toxicity and Human Health Risks of Chemicals in Our Environment*, Cambridge University Press, New York, 1992.
9. C. K. Winter and F. J. Francis, "Assessing, Managing, and Communicating Chemical Food Risks," *Food Technol.* **51**, 85–92 (1997).

ELISABETH L. GARCIA
CARL K. WINTER
University of California
Davis, California

See also FOOD SAFETY AND RISK COMMUNICATION; TOXICOLOGY AND RISK ASSESSMENT.

FOOD SAFETY EDUCATION: CONSUMER

SOURCES OF CONSUMER INFORMATION

Consumers indicate that their primary source of food safety information is the media (1–4). Television newspaper stories alert the public to current food safety issues with magazines frequently providing more in-depth information. Labels and cookbooks also serve as sources of information.

Food industry and consumer groups, government agencies, educational institutions, and professional organizations provide information to the consumer through the media or directly via educational flyers, articles in magazines or newsletters, and meetings.

The Institute of Food Technologists (IFT), a professional society of food and nutrition scientists, has identified about 80 academic members who serve as food science communicators. An IFT media guide with food science communicator contact information organized by topic and geographic areas is distributed to major news services across the country. Communicators initiate or respond to almost 1000 media contacts a year.

The American Dietetic Association (ADA) has organized a similar media outreach activity. Although ADA spokespersons primarily address nutritional issues, food safety is also an area of inquiry. The association has published position papers on a variety of issues with safety components, such as food irradiation, biotechnology, and fat substitutes.

Other professional societies have developed food safety material for the public. For example, *Before Disaster Strikes, A Guide to Food Safety in the Home* can be ordered through the International Association of Milk, Food and Environmental Sanitarians Web site, *www.iamfes.org*.

The International Food Information Council (IFIC) Foundation communicates science-based information on nutrition and food safety issues to health professionals, media, educators, and government officials. The Foundation is supported primarily by the broad-based food, beverages, and agricultural industries. IFIC Foundation activities include publishing a bimonthly newsletter, "Food Insight"; placing science-based information on the Internet (www.ificinfo.health.org); sponsoring educational events and roundtables for opinion leaders; writing scientific white papers, consumer brochures, and school curricula; and preparing educational exhibits at scientific and professional meetings. IFIC Foundation has also prepared a comprehensive media guide with background and consumer publications on current issues and lists of scientific experts.

The American Council on Science and Health, Inc. (ACSH) is a consumer educational consortium concerned with food safety and other issues. The ACSH board, composed of 250 physicians, scientists, and policy advisers in a variety of fields, reviews the Council's reports and participates in ACSH educational activities. ACSH produces a wide range of publications (*www.acsh.org*), including peer-reviewed reports on important health topics. ACSH representatives appear regularly on television and radio, in public debates and in other forums, and hosts media seminars and press conferences. Publications related to food safety include: *Eating Safely: Avoiding Foodborne Illness; Irradiated Foods; Feeding Baby Safely: Facts, Fads and Fallacies; An Unhappy Anniversary: The Alar "Scare" Ten Years Later;* and *ACSH Holiday Dinner Menu*.

The U.S. Food Safety and Inspection Service established the U.S. Department of Agriculture (USDA) hot line to reach consumers with information on safe handling of meat and poultry. Since its inception the hot line has responded to more than one million calls for information on meat and poultry safety with more than 100,000 calls received on the toll-free line from January through December 1996 (5). Information is also available through a fast fax service and at the Web page *http://www.usda.gov.fsis*. In 1996 over 80% of hot line calls were from consumers, with the remainder coming from businesses and professional clients including educators, communicators, health and human service professionals, students, teachers, and the media. Through their professional capacity, many of these callers also extend information to the public.

The *FDA Consumer*, published 10 times per year and available electronically at *www.fda.gov/fdac/*, contains consumer-oriented articles on various health topics including food safety.

The USDA provides information to food and health educators and the land grant university cooperative extension system through material such as "Food Safety Focus" and "Food Safety Educator." The cooperative extension system in each state uses these materials or others developed within the state to provide food safety information to the public. Many of these are listed in the National Food Safety Database.

The National Food Safety Database serves as a management system of U.S. food safety materials used by the Cooperative Extension Service (CES), consumers, industry, and other public health organizations. The Web site *www.foodsafety.org* lists many of the food safety resources developed for various audiences, ranging from producer to consumer. In addition to printed resources, a growing amount of information now is accessible in electronic format, and on the Internet.

Food industry and consumer groups prepare consumer material on proper food handling or address areas of food safety concern. The Center for Science and the Public Interest reaches the public through its newsletter and interaction with the media. In recent years *Consumer Reports*, published by Consumer's Union, has addressed food safety and safe food handling.

CONSUMER CONCERNS

Better documentation of the consequences of microbiological-based foodborne illness and increased media coverage have probably led to increased consumer concern about microbiological hazards. Nationwide surveys indicate that more people volunteer concerns about microbiological hazards than any other potential food safety issue. Survey responses regarding volunteered concern about microbiological safety increased from 36% in 1992 to 69% in 1997 (6). When the question of concern about food contamination by bacteria or germs was specifically asked, 82% of respondents classified it as a serious hazard. This response indicates more concern about this type of food hazard than any other food safety risk.

Although pesticide residues continue to generate concern among a large segment of the population, only 65% ranked it a serious hazard in 1997, a significant decreased from 82% in 1989 (6). Concern about other food safety areas has also decreased. Those expressing serious concern with antibiotics and hormones used in poultry or livestock decreased from 61% in 1989 to 43% in 1997. Those rating the use of nitrites in food as a serious hazard decreased from 44% in 1989 to 28% in 1997. Those rating use of additives and preservatives as a serious hazard decreased from 30% in 1989 to 21% in 1997.

The USDA hot line calls indicate the food safety areas related to animal products where people sought information. In 1996 most calls, 74%, were inquiries about safe storage, handling, and preparation of meat and poultry products (5). Twelve percent of inquiries dealt with marketing and inspection issues, 6% addressed questionable products and practices, and 4% related to labeling, product dating, and basic nutrition. More than 6000 inquiries related to handling of turkey, followed by beef and veal with almost 5000 calls.

CONSUMER SAFE-HANDLING KNOWLEDGE

Although consumers recognized the potential seriousness of foodborne bacteria and believed they were well informed (1), many were unaware of safe-handling and storage practices, with those age 35 or younger demonstrating the lowest level of knowledge (7–8). A 1998 nationwide poll indicated more than 90% of consumers had heard of *Salmonella*, yet critical gaps existed in food safety knowledge (9).

Many factors have contributed to consumers' lack of familiarity with safe food handling. Increased participation in the paid labor force has resulted in less in-home training of young people in food handling; few schools offer or require food preparation classes; partially prepared foods may have different, less familiar handling requirements (7). Additionally, some historic food safety recommendations related to temperature and acidity do not eliminate risks from some pathogens.

Food safety experts have identified the most common food-handling problem by consumers at home. The top factors attributed to mishandling include contaminated raw food, inadequate cooking or heat processing, obtaining food from unsafe sources, improper cooling, intervals of 12 hours or more between preparation and eating, and colonized person handling implicated food or poor hygiene (10). Mishandling associated with specific pathogens included the same contributing factors (11).

In a nationwide mail survey, Williamson et al. (8) found specific safe-handling practices were not practiced by 15 to 30% of respondents. People failed to rapidly cool cooked food, with 29% indicating they would let roasted chicken sit on the counter until it cooled completely before refrigerating. Only 32% indicated they would use small shallow containers to refrigerate leftovers. People were not aware that failure to refrigerate may jeopardize safety, with 18% not concerned or uncertain about the safety of cooked meat and 14% about poultry left unrefrigerated for more than four hours. The need for sanitation was not recognized, with only 54% indicating they would wash a cutting board with soap and water between using it to cut raw meat and chop vegetables.

Similarly, in a California survey, only 63% indicated they clean the food preparation area with soap and water. The importance of temperature control was not fully understood, with 50% indicating they refrigerate leftovers in large containers. Of particular concern, more than half of consumers always or sometimes tasted leftovers to check whether they are still safe (1).

Even consumers who knew they were being watched and evaluated made mistakes. An audit of consumer food-handling practices among 106 U.S. and Canadian households found 96% had at least one critical violation. Households average 2.8 critical and 5.8 major violations per household (12). A critical violation was defined as one that, by itself, can potentially lead to a foodborne illness, whereas major violations are unlikely to cause foodborne illness but are frequently cited as contributing factors.

CONSUMER PERCEPTION OF THE SOURCE OF FOODBORNE ILLNESS

Many consumers misperceive the nature of foodborne illness and the most likely source of the pathogen (13). Consumer belief about the type of food responsible for foodborne illness—meat, poultry, seafood, eggs—is consistent with expert opinions. However, consumers believe foodborne disease is a minor illness without fever that occurs within a day of eating a contaminated food. Infections caused by *Salmonella* and *Campylobacter*, the most common foodborne illnesses in the United States (14), are not consistent with the symptoms consumers described, because of the longer latency and fever-causing properties.

Most consumers believe their illness is caused by food prepared somewhere other than the home. Williamson et al. (8) found about one-third of consumers thought food safety problems most likely occurred at food manufacturing facilities, and one-third blamed unsafe practices at restaurants. Only 16% thought the home was the most likely place for mishandling. Fein et al. (13) found 65% attributed foodborne illness to food prepared at a restaurant, with 17% believing the mishandling occurred at the supermarket and 17% at home. Similarly, a nationwide 1998 survey found 39% of consumers thought safety problems occurred at the food processor or manufacturer, 20% believed they occurred at the restaurant, and only 15% thought mishandling occurred in the home (15). In contrast, food safety experts believe sporadic cases and small outbreaks in home are far more common than those cases constituting recognized outbreaks (7).

If consumers misperceive the nature and origin of foodborne illness, they underestimate the frequency of serious consequences and thus are less motivated to change. Schafer et al. (16) found that motivation to practice safe food-handling behavior requires the belief that someone could be harmed by not following guidelines, and that new behavior could prevent foodborne disease. The failure to associate at-home food-handling practices with foodborne illness is a serious impediment to convincing people to discontinue potentially hazardous food-handling behavior (13).

U.S. NATIONAL FOOD SAFETY PROGRAM

A coalition of national industry, government, and consumer groups formed the Partnership for Food Safety Education to provide safe food-handling information to the public. In 1998 the Partnership launched the *Fight BAC* (bacteria) education campaign. The initial goal of this campaign was to covey four key principles of food safety: Clean: wash hands and cooking utensils; Separate: prevent cross-contamination; Cook: heat foods to proper temperature; and Chill: store foods correctly. The program encourages grassroots education in local communities through dissemination of a *Fight BAC* kit with implementation ideas and reproducible materials. Although it is too early to judge the success of the program, materials have been widely disseminated and *Fight BAC* symbols and flyers are in major supermarkets.

BIBLIOGRAPHY

1. C. M. Bruhn and H. G. Schutz, "Consumer Food Safety Knowledge and Practices," *J. Food Safety* **19**, 73–87 (1999).
2. T. Hoban, *Consumer Awareness and Acceptance of Bovine Somatotropin*, Grocery Manufacturers of America, Washington, D.C., 1994.
3. T. Hoban and P. A. Kendall, *Consumer Attitudes About the Use of Biotechnology in Agriculture and Food Production*, North Carolina State University, Raleigh, N.C., 1993.
4. J. C. Busby and C. Ready, "Do Consumers Trust Food Safety Information," *Food Rev.* **19**, 46–49 (1996).
5. *Making the Connection, USDA's Meat and Poultry Hotline, 1996.* U.S. Dept. of Agriculture, Washington, D.C., 1998.
6. Abt Associates, *Trends in the United States, Consumer Attitude and the Supermarket 1997*, Food Marketing Institute, Washington, D.C., 1997.
7. Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, "Scientific Status Summary, Food Borne Illness: Role of Home Food Handling Practices," *Food Technol.* **49**, 119–131 (1995).
8. D. M. Williamson, R. B. Gravani, and H. T. Lawless, "Correlating Food Safety Knowledge With Home Food-Preparation Practices," *Food Technol.* **46**, 94–100 (1992).
9. Food Safety and Inspection Service, "Consumers Are Changing," *Food Safety Educator* **3**, 2 (1998).
10. F. Bryan, "Risks Associated With Vehicles of Food Borne Pathogens and Processes That Lead to Outbreaks of Food Borne Diseases," *J. Food Protect.* **51**, 663–673 (1988).
11. N. H. Bean and P. M. Griffin, "Food Borne Disease Outbreaks in the United States, 1973–1987; Pathogens, Vehicles, and Trends," *J. Food Protect.* **53**, 804–817 (1990).
12. R. Daniels, "Home Food Safety," *Food Technol.* **52**, 54–56 (1998).
13. S. B. Fein, C. T. Jordan, and A. S. Levy, "Food Borne Illness: Perceptions, Experience, and Preventive Behaviors in the United States," *J. Food Protect.* **58**, 1405–1411 (1995).
14. R. V. Tauxe, "Epidemiology of *Campylobacter jejuni* Infections in the United States and Other Industrialized Nations, in J. Nachamkin, M. J Blaser, and L. S. Tompkins, eds., *Campylobacter jejuni: Current Status and Future Trends*, American Society for Microbiology, Washington, D.C., 1992, pp. 9–19.
15. Abt Associates, *Trends in the United States, Consumer Attitude and the Supermarket 1998*, Food Marketing Institute, Washington, D C., 1998.
16. R. B. Schafer et al., "Food Safety: An Application of the Health Belief Model," *J. Nutr. Educ.* **25**, 17–24 (1993).

CHRISTINE M. BRUHN
University of California
Davis, California

FOOD SCIENCE AND TECHNOLOGY: DEFINITION AND DEVELOPMENT

Food science and technology have been defined in various ways. All definitions basically specify that food technology is the application of the principles and facts of science, engineering, and mathematics to the processing, preservation, storage, and utilization of food. Food science, on the other hand, deals chiefly with the acquiring of new knowledge to elucidate the course of reactions or changes occurring in foods whether natural or induced by handling procedures. With respect to "natural," nearly all our foods are of biological origin. Foods consist of chemicals, contain enzymes of intrinsic origin, and generally are subject to the action of enzymes of extrinsic origin and to microorganisms. Furthermore, various physical phenomena affect their state. Foods are subject to a myriad of forces, many of which are known; a far greater number of influences are still unknown. There is thus a great need for food science, which, among its functions, is the acquiring of new knowledge. Although in theory food science deals with the acquiring of new knowledge without there being any intended benefit from the new knowledge, in practice there is usually some hoped-for benefit such as greater stability of the food, a gain in acceptability or nutritional value, or a reduction in the likelihood of spoilage.

Food science and technology arose as separate disciplines through the coalescing of those portions of other sciences that dealt with foods. Traditionally, chemists, microbiologists, and other kinds of scientists studied or taught about foods from their particular points of view, but food processing transcends any one of the traditional disciplines. The distinction between chemists and microbiologists who study foods and food scientists and technologists is that the former devote their efforts chiefly to determining chemical composition, or the microbial state of foods. They are concerned with only one aspect of food. There is a need for those whose vision and competency cuts across discipline lines. When food processing is involved, knowledge of only one branch of science is not enough. There is a need for scientists and technologists whose knowledge of the different fields germane to various aspects of food preservation enables them to couple principles from the traditional fields with principles of food preservation to effect the most suitable solution to a food processing problem. The same applies to food engineering. There are principles that apply to the solution of engineering problem. Sometimes the most efficient engineering solution is not the most suitable or, more serious, not even an acceptable solution from the point of view of handling a food.

There have been instances of having an individual trained in one of the traditional branches of engineering assigned to the task of devising a new procedure for the handling of food. The process designed often was efficient from an engineering standpoint but a disaster from a microbiological point of view because allowance was not made for proper cleaning of the equipment. Food retained in the system could act as an inoculum of spoilage organisms for portions of food subsequently passing through the equipment. An engineer trained as a food technologist would recognize that possibility and select the next best option, one less efficient from an engineering point of view but one least likely to permit spoilage organisms to flourish.

In effect, a food scientist or technologist is a generalist in science rather than being an adherent of only one branch of science. The same applies to a food engineer. Food engineers augment their knowledge of engineering principles with the same kind of multidisciplinary knowledge that characterizes the food scientist.

Because foods themselves are intrinsically complex and subject to various forms of change (biological, chemical, nutritional, physical, and sensory) it was quite logical that a science arose that was designed to pull together the wide range of knowledge that impinges in some way on food and food processing.

FOOD RESEARCH BEFORE THE TERM "FOOD SCIENCE" AROSE

Food science as a distinct discipline is not quite a century old, but aspects of it have existed for centuries. Appert's development in 1810 of the process of canning (1) was an epochal event. The process wasn't called canning then, and Appert himself did not really know the principle upon which his process depended, but canning has had a major impact on food preservation ever since its development. It was the first of the purposely invented processes. Other methods developed earlier, such as drying and fermentation, go back to antiquity and were a result of the evolution of procedures over centuries rather than the purposeful application of the scientific method. Pasteur's study on the spoilage of wine and his description in 1864 (2,3) of how to avoid such spoilage persists not only because of the scientific importance of his findings but also because the term, pasteurization, is so much a part of our vocabulary. There were other early studies on food spoilage. In 1897 Prescott and Underwood published a seminal study of the spoilage of canned clams and lobsters (4). There was also a 1898 paper of theirs that dealt with the spoilage of canned corn. Russell (5) carried out what is now believed to be the first scientific work on spoilage of canned foods. He studied the amount of heat needed to preserve canned peas. His study, published in 1895, was little noticed for some time because it was buried in a report of the Wisconsin Agricultural Experiment Station (5).

A study initiated in 1920 that led to a truly scientific breakthrough was that of the National Canners Association (NCA; now called the National Food Processors Association). The NCA study (6) was the first to result in sound mathematical expressions for the amount of thermal energy needed to destroy microbial spores, the amount of heat put into a can and removed from it during heating and cooling, and a means of calculating the least amount of heat needed to destroy some specified number of spores in the can. Actually, there is no such thing as attaining a complete kill. One has to content oneself with the probability of there being one surviving spore. The usual level specified is 12D, or the probability of one container in 100 billion containing a viable spore.

The NCA study is a good illustration of fundamental scientific knowledge being discovered as an outgrowth of an investigation carried on for a very practical reason: to avert food spoilage and the risk of a *Clostridium botulinum* spore being able to grow out later and cause a very serious form of food intoxication (poisoning). Throughout the food field, the pharmaceutical industry, and others where sterility must be attained, the NCA study is the source of the knowledge needed.

THE FIRST OF THE TEACHING DEPARTMENTS

Just a little bit earlier than investigations such as that of the NCA, universities began to teach phases of food preservation that would be called food science today. Dairy processing and meats processing were taught during the nineteenth century, but the subject matter of each tended to be taught from the point of view of that commodity alone. In the 1910s things changed. At four different universities, predecessor departments to food science and technology arose. Like dairy manufacturers, their first departmental names were descriptive of the commodity area that they represented, but the pioneer teachers of food preservation recognized that the principles and facts that applied to their products had application to other commodities; thus, they tended to broaden their approach by teaching food preservation across commodity and discipline lines. At the University of Massachusetts, the departmental designation was Horticultural Manufacturers; at the University of California, it was Fruit Products; at Oregon State, the departmental title, like that at Massachusetts, related to Horticulture; at the Massachusetts Institute of Technology (MIT), the first courses were taught in Biology, since that was the school in which Prescott was located.

Among those who instituted formal teaching of that which is called food science today were Walter Chenoweth (G. E. Livingston, unpublished) of the University of Massachusetts; William V. Cruess, at California; Ernest Weigand, at Oregon State University; and Samuel C. Prescott (7), at MIT. The early teachers found that they could not truly teach food preservation unless they looked at the food and processes from the points of view of more than one discipline or commodity, for example, bacteriology in Prescott's case (4). That kind of thinking led to the departments becoming the first of the present food science and technology departments.

In defense of the dairy departments and other commodity-oriented departments of that time, it must be pointed out that in the last century there was less of what would be called further processing than today, when one type of commodity is incorporated into another commodity to produce still a third form of food; consequently, there was less reason to look across commodity lines.

Before discussing the evolution of the terms food science and technology, two other pioneers merit special mention. One of those is Dr. Carl R. Fellers (G. E. Livingston, unpublished). He was one of the most influential of the early teachers and researchers (8). He spent most of his career at the University of Massachusetts. From the early 1920s on, he evinced both in this teaching and research the concept that science and technology as they relate to food are one, that there are particular facts associated with particular foods, but the same principles and methods apply whether the food be apples, confections, fish, tortillas, veal, or zucchini. Another individual of the same sort was Dr. Bernard E. Proctor (G. E. Livingston, unpublished), a professor at MIT. The book *Food Technology*, published in 1937 by Prescott and Proctor (9), was among the first to use that term. Incidentally, Prescott, Cruess, Fellers, and Proctor all were early presidents of the Institute of Food Technologists (IFT).

FOOD SCIENCE AND TECHNOLOGY AS A TITLE

The founding of the IFT in 1939 (10) led to general use of the term food technologist. Reference was made in the last section to Prescott and Proctor's book for its use of the term food technology; another who did so in the same year, 1937, was Bitting (11). He called himself a food technologist on the title page of his book. By 1945, the original four departments that had taught the subject under different titles had changed their departmental names to food science, food science and technology, food engineering, or some variant such as food science and nutrition. Several other food science departments were organized in the 15 years after World War II. A few of the departments started ex nihilo; most had their origins in some commodity department or represented amalgamations where phases of food science taught in various departments were transferred and combined to form a new department. A factor that also solidified the use of food technology was development of the IFT's curricular minimum standard for food science and technology (12). That subject may be found in the article INSTITUTE OF FOOD TECHNOLOGISTS: HISTORY AND PERSPECTIVES.

The coalescing of the interests of those who conduct research on foods and food processing came somewhat late in the history of science. Not until after World War II did the term food science/technology come into widespread use. The two disciplines are so interlinked they function as one and are generally taught that way. Food engineering has generally been taught under the rubric food technology. Sometimes it is taught in an agricultural engineering department. Today one thus finds departments designated as food science, food science and technology, and sometimes food science and nutrition; if nutrition is a part of the title, however, the designation is generally food science and human nutrition. The reason for specifying "human" is that nutrition is taught in animal science courses and in foods and nutrition departments, and a distinction had to be made between the types of nutrition taught in these older kinds of departments. Nutrition as taught in foods and nutrition departments often deals with the clinical side of nutrition, whereas that taught in food science departments is more likely to delve into the relations between the retention of nutrients as influenced by various processing techniques. Both types of department naturally have common ground in elucidating the functions the various nutrients perform, interrelations among nutrients, and the well-being of humans when foods are used wisely.

POSITIONS FILLED BY FOOD SCIENTISTS/ TECHNOLOGISTS

The types of industrial jobs available to food scientists and technologists cover the gamut of responsibilities from examination of raw products to final sales. Positions of responsibility are also available in government (the Food and Drug Administration, the U.S. Department of Agriculture, the Department of Defense, and among others, the Public Health Service). The same applies to state and local governments such as state departments of agriculture and state and local health departments. In the academic world, teachers and researchers find many opportunities. Entrepreneurs such as food consultants often establish a remunerative niche for themselves. The roles food scientists and technologists play will be more fully set forth in the article FOOD SCIENCE AND TECHNOLOGY: THE PROFESSION. So too will developments as far as food processes are concerned. The next topic here will deal with changes taking place in the makeup of members of the profession.

Mermelstein (13) prepared in 1998 an article demographic as to changes taking place in the profession and as to employment, salaries, and the locations of food scientists/technologists both geographically and by employment field. He pointed out that at present males constitute 61% of IFT members, whereas females constitute 39%. Predictive of the future is the fact that female members tend to be younger than males. That 39% of IFT members are females is significant in itself, for 30 years ago they constituted a very small part of IFT membership. Since they tend to be younger today than their male counterparts, this suggests that in not too many years they will be the dominant portion of the membership.

The ascendancy of women in the profession can be seen in the colleges, too. Three decades ago there were few women among food science/technology graduates. Today that situation has changed dramatically. Female students outnumber male majors in many food science departments. The same applies to the Student Division of the IFT. Female student members are as numerous as male students, and often they assume the dominant roles in running the various student chapters by being elected chair of their university's IFT student chapter. There is little question that women are occupying roles, some of which were closed to them in years past, and at far higher levels than heretofore. Throughout industry, there are several female vice presidents. In the academic world, professors are as likely to be female as male, and several of the teaching departments have female heads or chairs.

An interesting facet of the survey Mermelstein reported is that a far higher proportion of those who work in the research and development (R&D)/scientific/technical category are women. Overall the salaries for women were lower than for men, the median salary being $68,500 for men versus $50,000 for women, but part of that discrepancy is a reflection of the fact the women are younger and thus have worked for fewer years. For those with a bachelor's or master's degree and less than one year's experience, the discrepancy was substantial, that is, $4300 and $5500, respectively. At the Ph.D. level, the discrepancy was only $400.

For those in industry with all years of experience combined, those with the B.S., M.S., Ph.D. and MBA degrees earned median salaries of $54,000; $60,000; $72,000; and $75,000, respectively. For educational institutions, the salaries were $33,682; $34,150; and $64,400; the MBA degree was not listed since it is outside of food science. For the first three categories, government employees averaged $49,825; 50,000; and $70,500. For scientific/trade organizations, the median salaries were $55,000; $57,000; $76,351; and $75,500. At the Ph.D. level and that of the MBA, median salaries by form of employment were comparable.

In summary, food science became defined only in the fore part of the twentieth century, and the term did not come into common use until nearly midway in this century. It differs from many other sciences in the scope of its outlook. As most sciences have developed, they have looked inward toward greater and greater specialization. Notwithstanding that, most practitioners within a field must today keep abreast of developments elsewhere in the great area in which their science belongs. A microbiologist, for example, generally attempts to stay knowledgeable of development elsewhere in the biological and biochemical fields. The scientist thus looks outward to other areas of his/her science. Food scientists and technologists must look outward with exceptional vigor. They cannot confine themselves to one major area such as the biological, biochemical, or physical sciences or engineering. Because food and food processing encompass all these areas, the food scientist or technologist must stay abreast of developments in several other diverse areas to practice the profession most effectively. The profession is thus one of unusual challenge. Because so many sciences impinge on food processes and since food is processed in an increasingly number of different ways today, there is an even greater need for food science and technology than there was when the two were first constituted as a new and separate field. Food scientists and technologists are the individuals most fit by training to be able to recognize, study, use, and interpret the myriad of interrelated processes that occur whenever food is preserved, stored, or utilized.

BIBLIOGRAPHY

1. S. A. Goldblith, "The Science and Technology of Thermal Processing. Part I," *Food Technol* **25**, 1256–1262 (1971).
2. M. Frobisher, Jr., *Fundamentals of Bacteriology*, W B. Saunders Co., Philadelphia, Penn., 1937, pp. 443–448.
3. J. M. Jay, *Modern Food Microbiology*, 5th ed., Chapman and Hall, New York, 1996, pp 5, 7.
4. S. A. Goldblith, "The Science and Technology of Thermal Processing, Part 2," *Food Technol.* **26**, 64–69 (1972).
5. H. L. Russell, *Gaseous Fermentation in the Canning Industry, 12th Annual Report*, Wisconsin Agricultural Experiment Station, Madison, Wis., 1895, p. 227.
6. W. D. Bigelow et al., *Heat Penetration in Processing Canned Food*, Bulletin 16-L, National Canners Association, Berkeley, Calif., 1920.
7. S. A. Goldblith, *Pioneers in Food Science*, Vol. 1, Food and Nutrition Press, Trumbull, Conn., 1993.
8. R. L. Hall, "Pioneers in Food Science and Technology. 'Giants in the Earth'," *Food Technol* **43**, 192 (1989).
9. S. C. Prescott and B. E. Proctor, *Food Technology*, McGraw-Hill, New York, 1937.
10. N. H. Mermelstein, "History of the Institute of Food Technologists: The First 50 Years," *Food Technol.* **12**, 14–18, 35–52 (1989).
11. A. W. Bitting, *Appertizing or the Art of Canning Its History and Development*, The Trade Pressroom, San Francisco, Calif., 1937.
12. R. M. Schaffner, "What Training Should a Four-Year Food Technology Student Recieve," *Food Technol.* **12**, 7–14 (1958).
13. N. H. Mermelstein, "1997 IFT Membership Employment & Salary Survey," *Food Technol.* **52**, 63–73 (1998).

JOHN POWERS
University of Georgia
Athens, Georgia

See also FOOD SCIENCE AND TECHNOLOGY: THE PROFESSION.

FOOD SCIENCE AND TECHNOLOGY: THE PROFESSION

Food scientists and technologists are employed in academia, by government and industry, by consulting firms, in entrepreneurial endeavors of various sorts and as private consultants. Crucial to almost any profession is the education its members receive prior to entering into that profession. Food science and technology is no exception. For that reason, the development of the Institute of Food Technologists' (IFT) minimum undergraduate curriculum will be described first.

In 1958 the IFT sponsored a conference between industrial leaders and university personnel to devise an undergraduate curriculum that would answer industry's needs for graduates educated in food science (1). A model curriculum was devised. In essence it was a consensus between the course work industry thought graduates coming into their fields should have versus the very practical matter that there is a limit to the number of courses a curriculum can have and still permit students to graduate in four years.

In 1962 and again in 1965 additional conferences were held (2). They resulted in the IFT adopting its minimum undergraduate curriculum (2). It was revised again in 1977 (3). The eventual outcome was that the gradual refining of thought as to courses that are essential for a would-be food scientist to take became the IFT's minimum undergraduate standard curriculum.

The reason the specified curriculum became a standard arises from the fact the IFT put teeth into a related program. The IFT awards approximately 145 scholarships (4). IFT scholarships can go only to universities that have a curriculum conforming to IFT's minimum requirements, and, furthermore, not only must the scholarship holder be at a university conforming to the IFT's minimum undergraduate curriculum, s/he must be following that curriculum. Many food science and technology departments have more than one curriculum. At the University of Georgia, for example, there is an environmental public health program administered by the Food Science and Technology was not listed since it is outside of food science. For the first three categories, government employees averaged $49,825; 50,000; and $70,500. For scientific/trade organizations, the median salaries were $55,000; $57,000; $76,351; and $75,500. At the Ph.D. level and that of the MBA, median salaries by form of employment were comparable.

In summary, food science became defined only in the fore part of the twentieth century, and the term did not come into common use until nearly midway in this century. It differs from many other sciences in the scope of its outlook. As most sciences have developed, they have looked inward toward greater and greater specialization. Notwithstanding that, most practitioners within a field must today keep abreast of developments elsewhere in the great area in which their science belongs. A microbiologist, for example, generally attempts to stay knowledgeable of development elsewhere in the biological and biochemical fields. The scientist thus looks outward to other areas of his/her science. Food scientists and technologists must look outward with exceptional vigor. They cannot confine themselves to one major area such as the biological, biochemical, or physical sciences or engineering. Because food and food processing encompass all these areas, the food scientist or technologist must stay abreast of developments in several other diverse areas to practice the profession most effectively. The profession is thus one of unusual challenge. Because so many sciences impinge on food processes and since food is processed in an increasingly number of different ways today, there is an even greater need for food science and technology than there was when the two were first constituted as a new and separate field. Food scientists and technologists are the individuals most fit by training to be able to recognize, study, use, and interpret the myriad of interrelated processes that occur whenever food is preserved, stored, or utilized.

BIBLIOGRAPHY

1. S. A. Goldblith, "The Science and Technology of Thermal Processing. Part I," *Food Technol* **25**, 1256–1262 (1971).
2. M. Frobisher, Jr., *Fundamentals of Bacteriology*, W B. Saunders Co., Philadelphia, Penn., 1937, pp. 443–448.
3. J. M. Jay, *Modern Food Microbiology*, 5th ed., Chapman and Hall, New York, 1996, pp 5, 7.
4. S. A. Goldblith, "The Science and Technology of Thermal Processing, Part 2," *Food Technol.* **26**, 64–69 (1972).
5. H. L. Russell, *Gaseous Fermentation in the Canning Industry, 12th Annual Report*, Wisconsin Agricultural Experiment Station, Madison, Wis., 1895, p. 227.
6. W. D. Bigelow et al., *Heat Penetration in Processing Canned Food*, Bulletin 16-L, National Canners Association, Berkeley, Calif., 1920.
7. S. A. Goldblith, *Pioneers in Food Science*, Vol. 1, Food and Nutrition Press, Trumbull, Conn., 1993.
8. R. L. Hall, "Pioneers in Food Science and Technology. 'Giants in the Earth'," *Food Technol* **43**, 192 (1989).
9. S. C. Prescott and B. E. Proctor, *Food Technology*, McGraw-Hill, New York, 1937.
10. N. H. Mermelstein, "History of the Institute of Food Technologists: The First 50 Years," *Food Technol.* **12**, 14–18, 35–52 (1989).
11. A. W. Bitting, *Appertizing or the Art of Canning Its History and Development*, The Trade Pressroom, San Francisco, Calif., 1937.
12. R. M. Schaffner, "What Training Should a Four-Year Food Technology Student Recieve," *Food Technol.* **12**, 7–14 (1958).
13. N. H. Mermelstein, "1997 IFT Membership Employment & Salary Survey," *Food Technol.* **52**, 63–73 (1998).

JOHN POWERS
University of Georgia
Athens, Georgia

See also FOOD SCIENCE AND TECHNOLOGY: THE PROFESSION.

FOOD SCIENCE AND TECHNOLOGY: THE PROFESSION

Food scientists and technologists are employed in academia, by government and industry, by consulting firms, in entrepreneurial endeavors of various sorts and as private consultants. Crucial to almost any profession is the education its members receive prior to entering into that profession. Food science and technology is no exception. For that reason, the development of the Institute of Food Technologists' (IFT) minimum undergraduate curriculum will be described first.

In 1958 the IFT sponsored a conference between industrial leaders and university personnel to devise an undergraduate curriculum that would answer industry's needs for graduates educated in food science (1). A model curriculum was devised. In essence it was a consensus between the course work industry thought graduates coming into their fields should have versus the very practical matter that there is a limit to the number of courses a curriculum can have and still permit students to graduate in four years.

In 1962 and again in 1965 additional conferences were held (2). They resulted in the IFT adopting its minimum undergraduate curriculum (2). It was revised again in 1977 (3). The eventual outcome was that the gradual refining of thought as to courses that are essential for a would-be food scientist to take became the IFT's minimum undergraduate standard curriculum.

The reason the specified curriculum became a standard arises from the fact the IFT put teeth into a related program. The IFT awards approximately 145 scholarships (4). IFT scholarships can go only to universities that have a curriculum conforming to IFT's minimum requirements, and, furthermore, not only must the scholarship holder be at a university conforming to the IFT's minimum undergraduate curriculum, s/he must be following that curriculum. Many food science and technology departments have more than one curriculum. At the University of Georgia, for example, there is an environmental public health program administered by the Food Science and Technology

Department. A student in that program is not eligible for an IFT scholarship because the public health curriculum does not specify some of the courses the IFT considers to be critical in the training of a food scientist.

In the United States there are approximately 60 universities claiming they teach food science and technology. Of these, 43 meet the minimum standard (4). The standard recommended is just that. It is not accreditation. Accreditation does exist for some sciences and certain other fields, but the IFT has not sought to foster that kind of program conformity. In general, universities are not in favor of all types of programs being accredited because that tends to tie the hands of the university. Sometimes a university must supply funds to an accredited department to enable it to keep its accreditation. Loss of accreditation reflects unfavorably upon a university. To avoid that happening, a university may supply the funds to the accredited department even though the funds are needed more critically elsewhere.

For the purpose of awarding scholarships, the IFT's minimum standard applies throughout North America. There are seven Canadian universities and one Mexican university, the curricula of which meet the IFT's minimum standard. Students in those countries are eligible to apply for the IFT scholarships. Not every year, but often, a student in Canada or Mexico receives one of the IFT's scholarships. The Canadian Institute of Food Science and Technology has a scholarship program of its own. Students at some Canadian universities are fortunate in that they can seek to obtain a scholarship under either of these two North American programs.

The purpose of the minimum standard curriculum is to ensure that individuals graduating from a food science program bring to the job the kind of qualifications employers have a right to expect and the kind of education most conducive to the long-term interests of graduates in food science.

The most recent revision of the undergraduate minimum standard curriculum occurred in 1992 (5). The IFT reexamines the curriculum at intervals not greater than 10 years to take into account changes in the industry, use of new tools such as students developing computer literacy, and the institution of new kinds of courses. For example, a "capstone" course was not originally required. Today the IFT considers it important that students at the senior level take a course that incorporates and unifies principles the students should have learned in food chemistry, microbiology, and engineering; food processing; nutrition; sensory analysis; and statistics. How that unification is fostered is up to the faculty. The course can be one of food processing, product development, some combination thereof, or a wholly different form of instruction that requires the students to plan and think for themselves in carrying out a project.

Not only does the Education Committee revise the standard curriculum at intervals, it monitors conformance to the curriculum by evaluating each university's curriculum at intervals not greater than five years to be sure the university is still in compliance with the minimum standard.

Aside from the capstone course, the curriculum currently in place (5,6) requires one or more courses in food chemistry, food analysis, food microbiology, food processing, food engineering, written and oral communication skills, computer literacy, and courses that call for "critical thinking skills." These are courses in food science. Additional courses in food science depend on the student's interest, but allowance has to be made in the student's program so that required courses in basic science and mathematics may be taken. In fact, they *must* be taken. Among these required courses are two courses in general chemistry plus one course each in organic chemistry and biochemistry; two courses in calculas, one in general physics, one in statistics, two courses in communication skills (normally prior to the food science courses calling for development of such skills), and not less than four courses in history, ethics, economics, literature, art, sociology, philosophy, psychology, and foreign languages. The intent is that students, no matter their specializations, should have enough "general" education to be a well-rounded citizen. The necessity for the science and mathematics courses is obvious. If students are to secure the maximum benefit from instruction in food science, they must have a sufficient and sound background in the basic sciences underlying each of the different types of course. Food science courses can then be taught at the appropriate level of comprehensiveness and depth.

Additional requirements such as the food science and technology unit should preferably be administratively independent and have an adequate budget. The faculty members must be trained in the subjects they teach; they should be encouraged to participate in professional associations and to engage in research. Teaching loads should not be so high as to interfere with adequate preparation for teaching.

Not only has the Education Committee of the IFT been involved, but other individuals and groups have examined various aspects of the educational process (7–9). The International Union of Food Science and Technology (IUFoST) has considered the types of educational programs available, including a listing of food science and technology programs in various countries (10). The listing is quite extensive. It is not, however, complete. The University of Pretoria and the University of Stellenbosch in South Africa were not listed. Both universities have well-developed food science programs. In 1991 a symposium, covering food science teaching worldwide, was held in Europe (11). Educational systems—as is well known—are different there. The same applies to the developing countries. Netto (12) commented that in most developing countries, education does not come first, that in both industrialized and developing countries the expertise of food scientists and technologists is needed to win the fight against foodborne diseases and contaminants and to search for safer and more nutritious food products.

THE PROFESSION

Once trained, the question facing any professional is: Where can I use my education and what will be the opportunities? Those in food science and technology are fortunate. The food industry in the United States is large, sophisticated, and highly efficient. Since the end of World War II disposable income required for food purchases has declined precipitously. It was once above 22%. In 1985 food expenditures as a proportion of household disposable income had declined to 18% (13). In 1995 the percentage was approximately 11% (14). Much of the change came about because of the efficiency of American agriculture and that of the food industry. Roberts et al. (15) claim that the food industry, allowing for stimulation of other industries (transportation, packaging, printing, etc) is a $1.8 trillion industry.

Since the end of World War II the value-added part of the food industry has increased substantially. In 1980 it surpassed agriculture's contribution. The overtaking of agriculture is significant, for U.S. agriculture is known worldwide for its efficiency. Only about 2.5% of the U.S. population is required to produce the food we consume plus that exported. Developments in efficiency and in the value-added side of the food industry are largely a result of the efforts of food scientists and technologists. They are not always the originators of new ideas. Marketeers regularly initiate requests for new, value-added products. Whether or not food scientists or technologists originate the idea, they are the ones who bring hoped-for results to reality.

STRENGTHENING VALUE-ADDED COMPONENTS

This next immediate description involves a few illustrations of ways food scientists and technologists have strengthened the value-added components of the American food industry and point out in particular that all fundamental knowledge does not come from scientific projects and that applied projects generally have to have a scientific component before they come to full fruition.

In the companion entry, "Food Science and Technology: Definition and Development," reference was made to the National Canners Association (NCA) research on thermal processing of food. The reason the project was undertaken was because canned foods had acquired a bad reputation in the 1910s. They were the cause of botulism outbreaks. The outbreaks were the result of failure to heat sterilize the canned product adequately, but the underlying cause was the lack of fundamental knowledge to determine just how much heat needed to be applied. The food scientists, technologists, engineers, and a mathematician on the research team brought into being the knowledge required to prevent such outbreaks.

The NCA study illustrates the gamut of technological, scientific, and mathematical knowledge often brought to bear on a technological problem and the fact that basic scientific knowledge is frequently one of the outcomes of a very practical project. The mathematician on the research team, Ball, devised the mathematical procedure allowing safe thermal processing to be attained (16,17). The point to going into detail is to highlight the fact that the food science profession is many faceted. Science, engineering, and mathematics all may need to be brought into play to solve the problems at hand. In the case here, a trade organization was the agency that developed scientific knowledge so fundamental that its reach has been well beyond the food industry.

A second benefit attained from the NCA study is that knowledge of proper thermal processing methods enabled canned foods to shake off a reputation as an unsafe food product and to acquire one of almost complete safety. That in turn has been a major factor in canned foods, a value-added product, assuming a prominent role in the U.S. economy. Aside from convenience and being amenable to long-term storage, canned foods are "safe." Food technologists are the ones who have made that so.

Two other illustrations will be given to demonstrate that the profession works in various ways. Clarence Birdseye, an engineer and an inventor working on his own, brought about change because of innovative thought. He reasoned that food frozen quickly enough would retain its original qualities. He was close to being correct but not entirely so. The process worked for seafoods, but later on when freezing was extended to vegetable products, trouble arose. The flavor, color, and texture of the vegetables usually deteriorated within a month or so. Blanching had to be inserted as a processing step to inactivate the enzymes naturally present in the vegetables (18). Even at freezing temperatures, enzymes can cause changes deleterious to food quality. Researchers at the University of California demonstrated that peroxidase had to be destroyed to be sure that it and other enzymes would be inactivated (17). Here science had to step in to bring an application to fulfillment. The frozen foods industry has most certainly affected the eating habits of Americans and made a major contribution to the value-added portion of the food industry.

The third illustration will be that of research at a state government laboratory. Rare today is the homemaker who squeezes oranges for juice at breakfast. At the Florida Citrus Experiment Station scientists using fundamental knowledge reasoned that if orange juice were concentrated under a vacuum, the temperature would be low enough to avoid the heat-induced taste to which canned orange juice is so susceptible. Furthermore, they reasoned that if a small amount of fresh juice were added back to the concentrate, the top flavor notes could be restored to the concentrate. The process was successful until the volume produced began to cause the orange concentrate to be on the market for an appreciable time; then trouble arose as it had with frozen foods. As the juice was concentrated, calcium compounds in the juice were likewise concentrated. Upon storage even at freezing temperatures, the calcium compounds reacted with low methoxyl pectin produced by enzymes in the orange concentrate to cause curdling and gelation. Mild heating—not enough to affect the sensory quality—had to be added as a pasteurization step to inactivate the pectic enzymes (19,20). As with frozen foods, fruit and vegetable concentrates increased greatly in volume and went on to add materially to the value-added part of the food industry. In this instance, governmental scientists brought about a new industry, first by using scientific reasoning to devise a new application, then by using scientific research to overcome a limitation not anticipated.

NEWER PROCESSES

Enough illustrations have been given in detail to show that the profession functions in various settings: industry, trade organizations, universities, the government, and entrepreneurial enterprises. Within recent years, newer forms of processing have arisen Among them is extrusion processing (21), controlled atmosphere and modified atmosphere storage of fruits and vegetables; controlled atmosphere packaging of food products (22,23); minimal processing coupled with refrigeration and some forms of packaging to permit the marketing of fruits, vegetables, and other kinds of foods (24–25); and edible packaging of various sorts (26–28). Not identical to the fresh produce but generally close to it, minimal processed foods have made a decided niche for themselves. Aseptic processing has unquestionably developed for itself a major role in food preservation (29), and ohmic heating is coming to the fore (30).

Irradiation of foods is being considered anew. Outbreaks of food infections or intoxication from organisms on fresh produce has revived the idea of irradiating fruits to destroy pathogenic microorganisms (31).

An area where a major change is taking place in the food field is that of biotechnology or "genetic engineering" as it is sometime called (32–35). Katz (33) points out that the movement toward improved vegetable oils will be helped by the New Plant Genome Initiatives signed into law by President Clinton in 1997. Biotechnology research and other innovations are not without objection (36) or the risk of development of allergenicity (37).

NUTRACEUTICALS

A phase of food science coming to the fore is that of so-called nutraceuticals (38–41). They are often also referred to a phytochemicals. In many respects, this phase of science is at the same stage as was research into vitamins 70 to 80 years ago. Nutraceuticals are chemicals in the food that either upon theoretical grounds or by observation appear to possess physiological properties. The questions are (*1*) do nonnutritive compounds affect in any way the action or use of demonstrated nutritional substances; if so, how and to what extent, and (*2*) are such actions beneficial or detrimental to health? The task is to relate the presence of one or more of the nutraceuticals to specific effects. Today, of course, isolation and identification of unknown compounds is far more feasible than it was when the first of the vitamins began to be unequivocally identified. Testing for effects however is no less arduous.

GOVERNMENTAL ACTIVITIES

Very brief mention was made of a state governmental laboratory when the development of citrus concentrate was referred to, but that research was a very small part of the effect governmental agencies have on food science and technology. The research laboratories of the U.S. Department of Agriculture (USDA) employ many food scientists and technologists. Aside from the five major regional laboratories and the USDA laboratories in Beltsville, Maryland, there are numerous area laboratories such as the Southern Area Laboratory in Lane, Oklahoma, and the Tree Fruit Research Laboratory in Wenachee, Washington, which carry on research. The research is often oriented toward raw products, and livestock or poultry production, but at many of these laboratories, major effort today goes into food processing or utilization. There is also the joint state–federal program of the Agricultural Research Service and the Land-Grant Experiment Stations.

The Department of Defense (DOD) likewise employs a good number of food scientists and technologists. The RD&E Center of the U.S. Army Natick Facility conducts research on the stability of military rations, demographic acceptability of different kinds of food, and nutritional studies. The National Marine Fishery Service, Department of Commerce, also employs food scientists and technologists, though its operations are much smaller than those of the USDA or DOD.

One of the things that has added stature to the profession is that the *U.S. Federal Government's Directory of Occupational Titles* began to list both food science and food technologists as professional fields rather than lumping food scientists under some older category such as microbiologist or chemists (42).

GOVERNMENTAL REGULATION

A major employer of food scientists and technologists is the Food and Drug Administration (FDA). In spite of the matter of occupational titles just referred to, they may not be called food scientists or technologists because their duties are generally that of an analyst or an inspector. The same applies to the USDA and its Food Inspection Service. Of increasing importance today has been the institution of Hazard Analysis at Critical Control Points (HACCP) and Standard Operating Procedures (SOP) for sanitation, which are programs mandated by the federal government but implemented by the food firm according to specifications set down by the government (43) where the company's own quality control personnel are responsible for inspecting of the food at points along the processing line. HACCPs arise where there is the possibility of microbial organisms contaminating the food or increasing appreciably in number or where other forms of contamination might be the cause of a regulatory complaint.

Major effort is being directed toward solutions to the problem of pathogenic organisms infecting the food supply. Among the pathogens are protozoa (44), viruses (45); nor is the home exempt from such problems (46). In assessing dangers, recognition has to be taken of the fact that not all fecal coliforms are necessarily of public health significance (47).

Miller (48) pointed out that an integration of food science, toxicology, microbiology, nutrition, and genetics is needed to solve problems of food safety. Hutt (49) described several of the modifications made in the FDA Modernization Act of 1997. In *Food Technology* (50) the proceedings of a symposium to honor Peter Baron Hutt are described. Hutt himself explained and criticized the approval system for food additives. Articles on food additives have not been cited here. They really need to be considered in the light of risk assessment (51).

SUMMARY

The food industry is, as O'Conner said, an industrial powerhouse (13). It adds greatly to the gross domestic production of the United States. For many of the other industrial countries of the world, the same is true. Food production is a key part of the economy. Developments originated by food scientists, technologists, and engineers, while not the sole source of advances made by the food industry, have been among the foremost in bringing the United States to the enviable position it occupies. The roles food scientists and technologists assume have been illustrated here mostly by example. The very nature of the profession requires that its practitioners be individuals with a strong, broad background in basic science and mathematics and with a sufficiently broad knowledge of food science and engineering to be able to fit within anyone of the several areas the food industry encompasses. That is the reason the IFT promulgated an undergraduate minimum standard curriculum only. There is a general consensus that specialization should not commence until graduate studies are undertaken. The survey reported by Mermelstein (52) teems with information as to the demographics of job opportunities, degree level helpful, gender, types of employment, and salaries received by those in various fields of employment.

The food industry and food science are on the move in other countries, too (53). In a synopsis of a publication by the National Science Board (54), academic universities are stated to be the "manufacturing plants for new knowledge." They are the largest producers of basic research, but it is pointed out that Europe now leads North America in science and engineering doctoral production. Western and Eastern European universities now graduate about 60% more doctoral students than does North America. The report states further, contrary to popular opinion, that most doctoral graduates do not work in academia, only 28.4% do. There has been a rapid rise in R&D spending outside the United States, but there has also been a rapid rise in spending within the United States by foreign firms. The United States possesses 46% of the world's industrial R&D capacity.

BIBLIOGRAPHY

1. R. M. Schaffner, "What Training Should a Four-Year Food Technology Student Receive?" *Food Technol.* **12**, 7–14 (1958).
2. "Report of IFT Council," *Food Technol.* **20**, 1567–1599 (1966).
3. "Discussion Papers and IFT Undergraduate Curriculum Minimum Standard—1977 Revision," *Food Technol.* **44**, 32–61 (1990).
4. *IFT Administered Scholarship/Fellowship Program for the 1998–99 School Year*, No. 25, Institute of Food Technologists, Chicago, Ill 1997.
5. L. D. Satterlee, "Introduction to the 1992 IFT Undergraduate Minimum Standards," *Food Technol.* **46**, 155 (1992).
6. "IFT Undergraduate Curriculum. Minimum Standards for Degrees in Food Science (1992 Revision)," *Food Technol.* **46**, 156–157 (1992).
7. O. Fennema, "Education Programs in Food Science: A Continuing Struggle for Legitimacy, Respect and Recognition," *Food Technol.* **43**, 170–182 (1989).
8. T. P. Labuza, "Mission 2000: IFT's Future," *Food Technol.* **43**, 68–84 (1989).
9. T. P. Labuza and D. R. Lineback, "The University-Industrial Relationship in Food Science and Technology," *Food Technol.* **41**, 74–91 (1987).
10. International Union of Food Science & Technology, *Directory of Courses and Professional Organizations in Food Science and Technology*, 2nd ed. rev., CSIRO Division of Food Research, North Ryde, Australia, 1980
11. I. D. Morton and J. Lengee, eds., *Education and Training in Food Science, a Changing Scene*, Ellis Horwood-Simon and Schuster International Group, Hemel, Hempstead, Hertfordshire, England, 1992.
12. A. G. Netto, "Present and Future of Food Science and Technology in Developing Countries," *Food Technol.* **43**, 148–168 (1989).
13. J. M. O'Connor, *Food Processing: An Industrial Powerhouse in Transition*, D.C. Heath, Lexington, Mass., 1988.
14. A. Manchester and A. Clauson, "Spending for Food up Slightly in 1995," *Food Review* **19**, 2–5 (1996).
15. T. A. Roberts, C. R. Dillon, and T. J. Siebenmorgen, "The Impact of Food Processing on the U.S. Economy," *Food Technol.* **50**, 65–68 (1996).
16. C. O. Ball, "Mathematical Solution of Problems on Thermal Processing of Canned Foods," *University of California Publications in Public Health* **1**, 1–244 (1928).
17. C. O. Ball, "Thermal Process Time for Canned Food," *Bulletin of the National Research Council* **7**, Part 1, 5–76 (1923).
18. A. L. Arighi, M. A. Joslyn, and G. L. Marsh, "Enzyme Activity in Frozen Pack Vegetables," *Ind. Eng. Chem.* **26**, 595–598 (1936).
19. E. L. Moore, R. L. Huggart, and E. C. Hill, "Storage Changes in Frozen Concentrated Citrus Juices—Preliminary Report," *Proc. Florida State Hort. Soc.*, 63rd Annual Meeting, p. 165 (1950).
20. F. W. Wenzel et al., "Gelation and Clarification in Concentrated Citrus Juice. 1. Introduction and Present Status," *Food Technol.* **5**, 454–457 (1951).
21. S. Ilo and E. Berghofer, "Kinetics of Thermomechanical Destruction of Thiamin During Extrusion Cooking," *J. Food Sci.* **63**, 312–316 (1998).
22. M. A. Cliff, O. L. Lau, and M. C. King, "Sensory Characteristics of Controlled Atmosphere- and Air-Stored 'Gala' Apples," *Journal of Food Quality* **21**, 239–249 (1998).
23. M. M. Wall and R. D. Berghage, "Prolonging the Shelf-Life of Fresh Green Chile Peppers Through Modified Atmosphere Packaging and Low Temperature Storage," *Journal of Food Quality* **19**, 467–477 (1996).
24. D. G. Hoover, "Minimally Processed Fruits and Vegetables: Reducing Microbial Load by Nonthermal Physical Treatments," *Food Technol.* **51**, 66–71 (1997).
25. G. Gunes and C. Y. Lee, "Color of Minimally Processed Potatoes as Affected by Modified Atmosphere Packaging and Antibrowning Agents," *J. Food Sci.* **62**, 572–575 (1997).
26. E. A. Baldwin et al., "Use of Lipids in Coatings for Food Products," *Food Technol.* **51**, 56–62, 64 (1997).
27. J. M. Krochta and C. De Mulder-Johnson, "Scientific Status Summary. Edible and Biodegradable Polymer Films: Challenges and Opportunities," *Food Technol.* **51**, 61–74 (1997).

28. T. D. Cai and K. C. Chang, "The Effect of Coatings and Processing Methods on Dehydrated Precooked Whole Pinto Beans Quality," *Journal of Food Quality* **20**, 315–328 (1997).
29 D. M Dignan et al., "Safety Considerations in Establishing Aseptic Processes for Low Acid Foods Containing Particulates," *Food Technol.* **43**, 118–121, 131 (1989).
30. "Overview of Articles on Ohmic Heating," *Food Technol.* **50**, 241–273 (1996).
31. D. G. Olson, "Status Summary, Irradiation of Food," *Food Technol.* **52**, 56–62 (1998).
32. K. Liu and E. A. Brown, "Enhancing Vegetable Oil Quality Though Plant Breeding and Genetic Engineering," *Food Technol.* **50**, 67–71 (1996).
33. F. Katz, "The Move Towards Genetically Improved Oils," *Food Technol.* **51**, 66(1997).
34. J. Q. Wilkinson, "Biotech Plants: From Lab Bench to Supermarket Shelf," *Food Technol.* **51**, 37–42 (1997).
35. F. Katz, "Biotechnology—Newer Tools in Food Technology's Toolbox," *Food Technol.* **50**, 63–65 (1996).
36. D. E. Pszczola, "Activists Target Transgenic Soybeans," *Food Technol.* **50**, 29 (1996).
37. R. L. Fuchs and J. D. Astwood, "Allergenicity Assessment of Foods Derived from Genetically Modified Plants," *Food Technol.* **50**, 83–88 (1996).
38. "Articles on Nutraceuticals," *Food Technol.* **52**, 44–57 (1998).
39. M-T Huang et al., *Food Phytochemicals for Cancer Prevention. I Fruits and Vegetables*, ACS Symposium Series 546, American Chemical Society, Washington, D.C. 1994.
40. C-T Ho et al., *Food Phytochemicals for Cancer Prevention. II Teas, Spices and Herbs*, ACS Symposium Series 547, American Chemical Society, Washington, D.C. 1994.
41. P. A. LaChance, *Nutraceuticals: Designer Foods. III Garlic, Soy and Licorice*, Food and Nutrition Press, Trumbull, Conn. 1997.
42. *Dictionary of Occupational Titles*, 4th ed., U.S. Government Printing Office, Washington, D.C. 1991.
43. H. R. Cross, "HACCP: Pivotal Changes for the Meat Industry," *Food Technol.* **50**, 236 (1996).
44. G. J. Jackson et al., "Cyclospora—Still Another New Foodborne Pathogen," *Food Technol.* **51**, 120 (1997).
45. D. O. Cliver, "Scientific Status Summary: Virus Transmission Via Food," *Food Technol.* **51**, 71–78 (1997).
46. R. W. Daniels, "Home Food Safety," *Food Technol.* **52**, 54–56 (1998).
47. M. P. Doyle, "Fecal Coliforms in Tea: What's the Problem?" *Food Technol.* **50**, 104 (1996)
48. S. A. Miller, "Developing a New Food Wholesomeness Science to Ensure Food Safety," *Food Technol.* **51**, 62–65 (1997).
49. P B Hutt, "A Guide to the FDA Modernization Act of 1997," *Food Technol.* **52**, 54–60 (1998).
50. "Food Regulatory Policy· A Symposium to Honor Peter Barton Hutt," *Food Technol.* **50**, 101 128 (1996).
51. C. R. Winter and F. J. Francis, "Assessing, Managing, and Communicating Chemical Food Risks," *Food Technol.* **51**, 85–92 (1997).
52. N. H. Mermelstein, "1997 IFT Membership Employment and Salary Survey," *Food Technol.* **52**, 63–73 (1998).
53. "Setting the Course for Global Food Markets," *Food Review* **19** (1996).
54. "NSF Data Show Shifts in R&D," *Chem. Eng. News* **76**, 44–52 (1998).

JOHN POWERS
University of Georgia
Athens, Georgia

See also FOOD SCIENCE AND TECHNOLOGY: DEFINITION AND DEVELOPMENT.

FOOD SPOILAGE

Food is considered spoiled when an undesirable change in the color, flavor, odor, or texture of the product has occurred. Microbial activity, endogenous chemical activity, insect/rodent damage, or physical injury such as bruising and freezing are responsible for spoilage. Spoilage classifications will vary with the consumer; that is, one individual may consider a food spoiled where another does not. A perfect example is fermented food. To some individuals, the food may be considered delicious but to other individuals who have had limited exposure to that type of food, the unfamiliar odors associated with the product would be considered spoiled. Individuals also differ in their thresholds to various odors and flavors, which would affect their judgment of the food's quality. Less obvious changes in food composition may also classify the food as spoiled. For example, nutrient losses, presence of harmful bacteria, or the formation of toxic substances within the food would all render the product spoiled. In these and other cases where food has been labeled as spoiled, the food is removed from distribution and labeled unfit for human consumption. The significance of this action is that there are economic losses associated with removal of the products from distribution and consumption, and the producer, processor, or consumer must assume these losses.

ORGANOLEPTIC DESCRIPTION OF SPOILAGE

Changes in the visual, tactile, olfactory, and flavor characteristics of food constitute spoilage, symptoms varying with the commodity. As an aid to summarize the types of spoilage occurring in many of the major food commodities, Table 1 lists some of these spoilage descriptors and the associated factor/mechanism for their development. Examples of visual changes would include the surface darkening of red meats, greening of cooked and cured meats, surface darkening of cut fruits, and fuzzy areas being generated on the surface of fruit, bread, or cheese. Other visual changes that also overlap tactile perceptions include sliminess on meats and vegetables and ropiness and curdling in fluid milk products. Off-odors and off-flavors associated with spoiled foods may be described by the terms putrid, rancid, bitter, soapy, sour, stale, and earthy. These off-odors and off-flavors vary not only between food categories but may also vary within a food category. For instance, the spoilage of marine temperate water fish is characterized by development of fishy, rotten, and hydrogen sulfide notes, whereas tropical fish and freshwater fish

Table 1. Selected Spoilage Indicators of Various Food Commodities.

Spoilage descriptor	Spoilage factor
Meat	
Off-odors: fruity, sweet smelling esters, followed by the formation of putrid sulfur compounds (indole, methanethiol, dimethyl disulfide and ammonia)	*Pseudomonas* spp.
Slimy or tackiness: masses of bacterial growth and the softening or loosening of meat structural proteins	*Pseudomonas* spp.
Greening: discoloration of meat pigments in frankfurters	Heterofermentative species of lactobacilli and *Leuconostoc*
Discoloration: surface browning	Pigment oxidation
Fish	
Off-odors: hydrogen sulfide, methyl mercaptan; dimethyl sulfide, trimethylamine	*Pseudomonas* spp
Softening/mushiness: liquefaction of muscle tissue in Pacific whiting	Proteolytic enzyme activity
Discoloration: blackening of tissue in shrimp	Enzymic oxidation of polyphenols
Eggs	
Runny yolk and off-odors: H_2S and musty odors	*P. graveolens*
Discolored yolks	*Pseudomonas, Aeromonas, Acinetobacter, Proteus*
Pinspots: mycelial growth on inside of eggs	Molds—*Penicillium* and *Cladosporium*
Milk	
Off-flavors and odors: Bitter and occasionally putrid	*Pseudomonas* spp.
Clotting or curdling	Lactic acid bacteria
Butter	
Off-odors: putrid odors due to certain organic acids (ie, isovaleric acid)	*P. putrefaciens*
Off-odors: rancid odors from short-chain fatty acids hydrolyzed from fats	*P. fragi*
Cheese	
Holes or gas pockets	Gas-producing bacteria
Fuzzy areas	Molds
Vegetables	
Soft roll: initially sunken areas, followed by sunken area becoming soft, and wet and spreading to encompass the entire vegetable	*Erwinia* spp. and *Pseudomonas* spp.
Gray mold rot: Gray mycelium	*Botrytis cinerea*
Black semisoft blemishes	*Alternaria* spp.
Black rot: in potatoes	*Ceratocystis fimbriata*
Chilling injury (pitting and russeting; surface browning; improper ripening; decay)	Membrane lipid oxidation
Shriveling	Moisture loss
Citrus fruit	
Green rot: dense olive-colored layer of mycelium and powdery conidia	*Penicillium digitatum*
Noncitrus fruits	
Brown rot: brown discolorations in peaches, plums, and apples	*Monilinia fructicola* and *Penicillium expansum*
Downy mildew: in grapes	*Plasmapara viticola*
Gray mold rot: gray mycelium (fuzz) in strawberries	*Botrytis*
Legumes	
Reddish-brown spots/streaks	*Uromyces*
Off-flavors: beany, grassy, and rancid	Chemical lipid oxidation
Grains	
Toxic compound generation: mycotoxins	Molds

Table 1. Selected Spoilage Indicators of Various Food Commodities.

Spoilage descriptor	Spoilage factor
Bread	
Fuzzy areas: mycelium	*Rhizopus stolonifer*
Nuts	
Fuzzy areas: mycelium	Molds
Canned foods	
Swelling of can	Gas-producing organisms
Wine	
Off-flavors: grassy (*cis*-hexen-3-al; *trans*-hexen-2-al); cork taint (2,4,6-trichloroanisole); vinegary (acetic acid)	*Acetobacter* spp.
Beer	
Off-odor: buttery or honeylike due to presence of diacetyl	*Pediococcus cerevisiae*
Ropiness: liquid becomes viscous and pours as an "oily" stream	*Acetobacter, Lactobacillus, Pediococcus cerevisiae,* and *Gluconobacter oxydans*
Off-flavors: sourness due to oxidation of ethanol to acetic acid	*Acetobacter* spp.
Turbidity	*Zymomonas anaerobia* and *Saccharomyces* spp.
Browning and haze formation	Chemical oxidation of polyphenols
Chocolate	
Surface whitening	*Chrysosporium* spp. or fat recrystallization
Oils	
Off-flavors: rancid	Chemical lipid oxidation
Potatoes	
Sprouting: toxic formation of solanine	Physiological

spoilage is characterized by fruity and sulfhydryl notes. Development of spoilage flavors and odors are seldom abrupt but occur in successive steps. In the case of fish, the initial accumulation of inosine monophosphate (IMP) contributes to the pleasant flavor of the fish while subsequent breakdown of IMP results in loss of such flavor. Further storage leads to the microbial or chemical generation of putrid or rancid compounds, respectively, that are responsible for making the product unpalatable. It is important to note that oftentimes no specific volatile compound may be held responsible for the spoilage aromas. Instead the concentration of a class of volatile compounds, such as the sulfur class of volatiles, and the relative concentration of that class to other classes of volatiles may better describe differences in spoilage aromas.

FOOD SPOILAGE MECHANISMS

Microbiological Degradation

Organisms and Factors Affecting Growth. The types of microorganisms that are responsible for spoiling foods include bacteria, molds, and yeasts. Like other living things, microorganisms require nutrients, water, and minerals to survive and grow, and to accomplish these activities, the microorganisms degrade the foods. Many factors affect the growth and biochemical activities of microorganisms in foods and these are the chemical composition of the food, the conditions of processing and storage, and the inherent properties of the microbial species present. With regard to the latter point, different types of microorganisms have growth habitats for which they are best suited. For example, bacteria are adapted to fast growth in nonacidic (pH 5.0–8.0) and moist conditions, whereas molds grow more slowly but are better adapted to growth in relatively acidic (pH 2.5) or dry conditions. Yeasts are similar to molds, but many also tolerate conditions containing high concentrations of sugar.

The microbial flora in a food will be initially dictated by the product's environment or by contaminants picked up during processing, handling, packaging, and storage of that product. For example, freshly caught fish from warm or tropical waters carry a microbial population primarily of mesophilic bacteria (bacteria that grow best from 15 to 40°C) such as *Micrococcus coryneforms* and *Bacillus*. In contrast, cold and temperate water fish species harbor predominantly psychrotrophic bacteria (bacteria that grow at refrigeration temperatures) that includes the genera *Moraxella/Acinetobacter, Pseudomonas, Flavobacterium,* and *Vibrio*. Upon refrigerated storage, however, the psychrotrophic bacteria in both the warm and cold water fish become the predominant species as a result of natural competition. Since a greater concentration of psychrotrophic organisms are initially found in seafood harvested from temperate waters, their spoilage could be expected to be quicker than seafood harvested from tropical waters. Such conjecture has at least been substantiated with organoleptic evaluations of tropical and temperate shrimp stored in ice (1). Another important intrinsic factor in fish that has played an important role in determining the microbial flora during storage is the pH Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue, and thus only small amounts of lactic acid are produced postmortem. As a result, fish muscle contains a high postmortem pH (>6.0), and under these conditions the pH-sensitive spoilage bacteria *Shewanella putrefaciens* may grow. In the case of bivalves such as oysters and clams that store energy in their tissues as glycogen, lactic acid may be produced in much greater quantities. As a result, the reduction in pH favors multiplication by fermentative-type microorganisms such as Lactobacilli, Streptococci, and yeasts.

In other meat items stored at refrigeration temperatures, only approximately 10% of the initial bacteria present are able to grow, and of those microorganisms able to grow, the fraction causing spoilage is even lower. Hence, it is important to identify these "specific spoilage organisms" in each food so treatments can be optimized to minimize their growth. Influencing the composition of those specific spoilage organisms on meat, however, will be the available moisture. In a high humidity environment, bacterial spoilage in fresh meats is dominant, with coalescence of colonies producing a slime layer on the surface. When the surface of the meat is too dry, as it is with many dried and fermented meat products, mold tends to predominate as the spoilage organisms. On the other hand, the application of vacuum or modified atmosphere packaging to cooked meat products generates conditions conducive to dominance by lactic acid bacteria. Producing acids such as lactic acid, acetic acid, and formic acid, the bacteria generate spoiled products described as sour and acid. When these products are stored aerobically following their anaerobic storage, additional obnoxious odors may be produced that are described as slightly sweet and cheesy.

Molds are able to grow on many other kinds of food in addition to meat. These include cereals/grains, milk, fruit, vegetables, and nuts. As a result of the mold growth, several kinds of food spoilage may arise: off-flavors, toxins, discolorations, and rotting. Of these, the most important spoilage problem associated with molds is the formation of mycotoxins. Mycotoxins are secondary metabolites of molds that are toxic to vertebrate animals in small amounts when introduced via a natural route. The most important toxic effects are different kinds of cancer and immune suppression. While some mycotoxins are only present in the mold, most of them are excreted in the foods. The high resistance of mycotoxins to physical and chemical treatments makes them difficult to remove from foods once they are present. Hence, it is important to limit mold growth on food items.

The well-accepted conclusion that molds are mostly responsible for the spoilage of fruit is based on the visible presence of fungal mycelium and spores on the surface of these products at the time of rotting. Yeasts, however, commonly occur on the surface of freshly harvested fruits at populations of 10^3 to 10^5 cells per square centimeter (2). These yeasts in most circumstances are considered to remain relatively inactive as they do not produce the appropriate enzymes to degrade the skin of the fruit and establish infection. Physical damage of the skin, however, by overripening, mechanical injury, or fungal attack exposes the fruit tissue upon which yeast can rapidly grow to produce secondary spoilage.

In products, such as beer and wine, that are dependent on microbial action, spoilage may also ensue when growth of undesirable microorganisms occurs. Even in chocolate-covered confectionery products, spoilage may result from growth of foodborne *Chrysosporium* species. Growth of these xerophilic fungi has produced a white surface discoloration that for all purposes was similar in appearance to the effects observed from recrystallization of cocoa butter (3).

Enzymatic/Biochemical Activity of Microorganisms. During their growth, microorganisms utilize food constituents and generate metabolic end products. As a consequence, the physical, chemical, and sensory properties of the food are substantially changed. Generally, the nature of these changes is not well described, making it difficult to explain spoilage on a biochemical basis. These biochemical activities, however, often include enzyme activity of a pectinolytic, proteolytic, and/or lipolytic nature.

In fruits and vegetables, pectolytic fluorescent pseudomonads, mainly *P. fluorescens* and *P. viridiflava*, account for substantial proportions of postharvest rot in cold storage and at wholesale and retail markets. The ability of these pseudomonads to cause maceration of plant tissues is primarily due to their ability to produce an extracellular pectate lyase capable of degrading pectin components of plant cell walls.

Grain is also subject to breakdown by carbohydrases from spoilage fungi. These fungi have been shown through *in vitro* studies to produce cellulase, polygalacturonase, pectin methyl esterase, 1-4-β-glucanase, β-glucosidase, and β-xylosidase (4). Environmental conditions such as water activity (A_w) and temperature, however, influence the production of these enzymes.

A key metabolic reaction of most yeasts when cultured under favorable anaerobic conditions is the fermentation of sugars such as glucose, fructose, sucrose, and maltose to produce ethanol and carbon dioxide. In addition to the loss of sweetness, this activity produces gassy products with a distinctive alcoholic aroma and flavor. Many hundreds of other secondary end products are formed during fermentation, and these also have a significant impact on the sensory properties. Such products include higher alcohols, organic acids, esters, aldehydes, and ketogenic substances that, although produced in small concentrations, have very low flavor and aroma thresholds. Unfortunately, with the exception of *Saccharomyces cerevisiae*, the production and sensory relevance of these secondary metabolites by yeasts are not well known (2).

Fragmentary knowledge exists on yeast metabolism of nitrogen compounds. As one example where this type of biochemical activity is of significance, metabolism of lysine during the growth of the yeast *Brettanomyces intermedius* in wine produced mousy taints due to the production of substituted tetrahydropyridines (2). It is also well documented in the brewing and wine literature that some, but not all, strains of *S. cerevisiae* can produce objectionable concentrations of hydrogen sulfide and sulfur dioxide and that these properties are linked to their metabolism of amino acids and inorganic sulfur compounds in the growth medium.

Yeast can both synthesize and metabolize organic acids. These activities change the acidities and flavor profiles of the product. In addition, when oxidative utilization of organic acids by yeast occurs via the tricarboxylic acid cycle, the pH of the product may rise to values that allow the growth of spoilage bacteria.

In foods, microbial proteolysis is another key spoilage reaction. Initially, it leads to the development of bitter flavors, followed by the production of strong ammonia odors and putrefaction. These proteases, secreted into the food to break down the proteins, include both endoproteases and exoproteases (aminopeptidases and carboxypeptidases). Thus, volatile fatty acids originate from proteins by deamination of amino acids while volatile bases arise from decarboxylation of amino acids and deamination of adenylates in the food. The degradation products, in turn, serve as a nutrient source for the microorganisms. When ample concentrations of low molecular weight nitrogen constituents (free amino acids, creatine, taurine, etc) are already present in the food product, however, as they are in fresh fish, protease synthesis and excretion is inhibited. In later stages of spoilage when most of the free amino acids have been depleted, then proteolysis becomes important. Even so, it appears that some level of low molecular weight peptides must be present to activate the synthesis of the extracellular proteases in *P. fragi*, an active spoiler of muscle food (5). Other factors in the food medium have also been shown to affect synthesis and excretion. For example, in milk supplemented with iron, the size of the *Pseudomonas* population when extracellular enzymes were produced was 10 times larger in the supplemented milk than in the nonsupplemented milk (6). The types of proteases produced by a microorganism, however, will vary. Thus, *P. aeruginosa* secretes two proteases, *V. alginolyticus* secretes a collagenase and five alkaline serine proteases, and *Aeromonas hydrophila* secretes two proteinases and one aminopeptidase. In general, these proteases have molecular weights ranging from 20 to 50 kDa, consist of only a single polypeptide, and lack the cysteine amino acid. The latter two characteristics are especially important because they contribute to the high heat stability of the extracellular proteases. Hence, even though the vegetative cells may be killed by a heat treatment, extracellular microbial enzymes continue to exert their activity. To reduce the possibility of this type of activity in milk, thresholds have been set in raw milk at approximately 2×10^6 colony forming units/mL (7). Levels above this threshold would thus generate pasteurized milk that would spoil quickly despite bacterial counts being reduced greatly. Product deterioration by proteolytic enzymes generated from psychrotrophic sporeformers, such as *Bacillus* spp., however, is also receiving attention in the dairy industry. When in the spore state, these microorganisms easily survive the typical range of pasteurization conditions with subsequent germination and outgrowth of vegetative cells. Consequently, during their growth, activity by degradative enzymes, including proteases, leads to gelation of ultra-high temperature (UHT) milk, sweet curdling of milk, bitter and unclean off-flavors in cheese, decreases in cheese yield, and textural and body defects such as "wheying off" in cultured dairy products. Interestingly, it has been noted that the optimum temperature for production of degradative enzymes by these microorganisms is usually lower than the optimum temperature for cell division (8). Thus, it is possible for milk held at refrigeration temperatures for extended periods of time to develop off-flavors through microbially produced enzymes even though the observed microbial population might remain below that normally associated with formation of microbial defects.

Lipases are another group of hydrolytic enzymes released by microorganisms to break down the food. In this case, the enzymes cleave fatty acids from triglycerides (a molecule containing three fatty acids esterified to a glycerol backbone). For those lipases found in milk, a preference occurs for cleavage at the 3-position in the triglyceride where butyric and caproic fatty acids reside. Upon hydrolysis, these volatile fatty acids at very small concentrations (1.5 mEq/100 g of fat) have distinctly unpleasant smells and tastes, which is termed hydrolytic rancidity. Also, in milk, some thermoduric psychrotrophs have been shown to produce phospholipases, particularly phospholipase C, that hydrolyse the ester linkage between the glycerol backbone and the phosphoryl group of a phospholipid. In milk, this attack takes place at the fat globule membrane where phospholipids are found. The lipolysis at the fat globule membrane results in an increased susceptibility of the exposed milkfat to the subsequent action of lipases. It has also been suggested that the degradation of the fat globule membrane by phospholipase C results in the aggregation of fat globules that leads to the bitty cream defect frequently observed in cream products.

Not all lipolytic action results directly in flavor defects. Only when the free fatty acids (FFAs) are short to medium range (C4 to C12) do they contribute directly. In many cases, FFAs react with protein constituents in the food producing textural defects in the product, but rarely are these defects of a severity to render the product spoiled. In cases where the FFAs are of an unsaturated nature (contain one or more double bonds in their structure), they may increase or decrease the susceptibility of the food to undergo lipid oxidation. When the FFA is cleaved from a membrane phospholipid early in storage, it will increase the oxidative stability of the product, but release of FFA from membranes later in storage will decrease oxidative stability. FFAs released from storage triglycerides almost always are more susceptible to lipid oxidation than they were when esterified. This process of lipid oxidation, in turn, leads to the presence of off-flavors and off-odors in the product. Lipid oxidation will be described in more detail in the next section.

The last group of microbial enzymes to be discussed in this section is one that leads to trimethylamine oxide (TMAO) degradation. In marine fish, TMAO is believed to be involved in the fish's osmotic regulation, and levels in the fish will be dictated by the levels required for counteracting the osmotic pressure of seawater as well as amounts present in the food. Degradation of this compound by psychrotrophic bacteria, such as *Achromobacter*, apparently involves both a triamineoxidase for activation of the TMAO substrate and a dehydrogenase for reduction of the compound to trimethylamine (TMA). In turn, when TMA reacts with the fat in the muscle, it creates a characteristic fishy odor. Odors appear at TMA levels as low as 2.9 mM of muscle extract, while definite odors have been observed at levels of 7.2 mM in the muscle extract (9). Significant amounts of TMA, however, will not be observed until after the bacterial lag phase that extends from the onset of rigor to its resolution, due to a postulated bacteriostatic effect of rigor mortis.

Lipid Oxidation

Oxidative rancidity has long been recognized as a major cause of spoilage in food. In many products (potato chips, nuts, oil, cereals, etc) stored for varying lengths of time, the off-flavors and off-odors generated as a result of lipid oxidation are described as rancid, cardboardy, or painty. In meat that has been cooked, refrigerated, and then rewarmed, the off-flavors are described as warmed-over. In stored fish, the off-odors may be described as either rancid or fishy.

Lipid oxidation is a radical-driven process that begins with an activated oxygen species abstracting a hydrogen atom from an unsaturated fatty acid. The active oxygen species can be produced by light; or by interaction of the oxygen with enzymes such as lipoxygenase, peroxidase, or microsomal enzymes; or with transition metal ions, particularly iron. The oxygenated fatty acid, in turn, forms a hydroperoxide (primary reaction product) by abstracting a hydrogen atom from an adjacent fatty acid, which, in turn, can continue the process of abstracting hydrogen from a neighboring fatty acid. Hydroperoxides do not directly cause an adverse effect on the flavor and aroma of the food; rather, it is their breakdown to aldehydes and ketones (secondary reaction products). During this breakdown, additional free radical compounds may be generated that, in turn, could start another sequence of lipid oxidation reactions.

The susceptibility of a food to oxidize is dependent on both its chemical composition and its physical environment. The major chemical component in the food that will affect oxidation is the degree of unsaturation that exists in the fatty acids. The more double bonds in the fatty acid, the faster it will oxidize. For example, fish contain unsaturated fatty acids with one to six double bonds, and they will oxidize faster than chicken, which contains unsaturated fatty acids with one to four double bonds. Concentrations of catalysts in the food system are also important in determining the susceptibility of the food to oxidize. Of these catalysts, the nonenzymic catalysts (transition metal ions) play a key role, as they not only participate in the initiation process but also participate in the breakdown of hydroperoxides to low molecular weight off-flavors and off-odors. The fact that most foods, even immediately after harvest and slaughter, are believed to have hydroperoxides present, would lend credence to a dominant initiation role being played by transition metal-catalyzed hydroperoxide breakdown. Other compositional factors affecting the food's susceptibility to oxidize include pro-oxidants and antioxidants (tocopherol, flavonoids), as well as the microbial contamination. Illustrating this latter point, for instance, lipid oxidation was reduced in pork muscle stored at 2°C when lipolytic enzyme-producing bacteria were present (10). As for the physical environment, oxygen availability, temperature, and water activity play a role in the susceptibility of a food's lipid to oxidize.

Biochemical/Physiological Degradation

Chilling Injury. Chilling injury is a term used to describe the physiological damage that occurs in many plants and plant products, particularly those of tropical and subtropical origin, as a result of their exposure to low but nonfreezing temperatures. Visual symptoms vary with the product but may include surface pitting, water rot, poor color development or inability to ripen, and general loss of structural integrity. Appearance of these symptoms has been found to depend on the time and temperature of exposure to the chilling temperature as well as the maturity of the plant product. It primarily occurs only after transfer to nonchilling temperatures.

One of the most important visual symptoms of chilling injury is pitting, a general collapse of the fruit tissue induced by low-temperature dehydration. It is more evident in fruits such as limes, grapefruit, mango, or avocado in which the outermost covering is harder and thicker than the adjacent layer. In fruits with thin and/or soft peels such as peppers, eggplant, and tomato, water soaking, surface discoloration, and sheet pitting are more common. More specifically in eggplant, pitting begins with the deformation and browning of the cells located several layers beneath the epidermis followed by deformation and browning of the pericarp cells. In broccoli, chilling injury deterioration is manifested by chlorophyll loss and yellowing of flower buds.

Physiological and biochemical investigations into the nature of chilling injury have focused on membrane-induced damage in chilling-susceptible fruits and vegetables as a result of their inability to increase protective enzyme systems that would reduce and scavenge free radicals attacking the membranes. As evidence for this line of thought, it was found that while superoxide dismutase activity increased during cold storage in both chilling-sensitive and -tolerant cultivars, catalase, ascorbate oxidase, and glutathione reductase activities were higher in tolerant cultivars than nontolerant cultivars at low temperature (11). Further support for the free-radical membrane-damage hypothesis has been in the observation that free-radical-mediated membrane damage precedes the visual symptoms (12). Tolerance to low temperatures may be increased, however, by exposing the sensitive fruits to nonchilling low temperatures prior to refrigerated storage. The acclimation period apparently enables the material to activate and produce the necessary enzymes required for protection during low-temperature storage. Inactivation of these enzymes, on the other hand, would most likely occur during minimal processing (cutting, slicing, coring, peeling, trimming, or sectioning of agricultural produce), and this inactivation would account for the observation that minimally processed vegetables are more sensitive to chilling injury than the intact produce. In fact, spoilage as a result of chilling injury occurred sooner in minimally processed vegetables than microbial degradation (13), which demonstrates that changes in the processing and handling of the food will often shift what type of spoilage is observed.

Senescence. Senescence is an ill-defined collective term used by plant physiologists to describe deteriorative reactions occurring during the final stage of the life of a plant, its constituent tissues, or its cells. Although the senescence of different organs can vary in rate and with organ function, several consistent features are found in a range of senescence situations. For physical changes, there may be loss of green color or synthesis of new pigments such as carotenoids or flavonoids. In terms of texture, there may be softening, wilting, or drying. Increased development of infections and lesions are also signs of senescence that indicate the material has lost its resistance to pathogens. Bear in mind that fruits and vegetables continue to live, respire, and change after harvest, and although nonclimacteric fruits and vegetables, such as citrus fruits and beans, do not ripen or improve after harvest, climateric fruits and vegetables, such as tomatoes and bananas, continue to ripen after harvest. Thus, nonclimacteric fruits and vegetables would be considered to immediately start the process of senescence following harvest, whereas climacteric fruits and vegetables only undergo senescence during storage after development of desirable sensory characteristics. Studies designed to elucidate the mechanism of senescence point again to the role of membrane lipid oxidation. It appears that during storage, protective systems that exist in the tissue become disabled and thus provide increased opportunities for free-radical reactions to inactivate key metabolic pathways. Physiological changes accompanying senescence include: loss of chlorophyll; disassembly of chloroplast structure; degradation of cell walls; altered membrane composition; loss of membrane fluidity; loss of cellular compartmentation; release of vacuolar contents; altered sugar content; a switch to alternative substrates for respiration; net loss of RNA; increased protease activity; net loss of protein; and altered amino acid content. In simplified terms, however, these changes could be described as arising from a change in the balance between anabolic and catabolic processes.

Endogenous Enzymatic Degradation. Activity of enzymes endogenously present in the food product may also lead to quality deterioration and spoilage during storage. Examples of these types of enzymes include phenolases, lipoxygenases, proteases, dimethylases, and peroxidases.

Postmortem, polyphenoloxidases present in shrimp and lobsters catalyze oxidation of tyrosine to *o*-quinones, which then condense with other quinones or with amino acids or proteins to form melanin. Although tyrosine is not colored, melanins are dark brown to black pigments, and their formation in shrimp and lobster is described as blackspot development.

Tenderization or flesh softening is considered to be associated with the disappearance of Z-disks, dissociation of actomyosin complex, destruction of connectin, and general denaturation of collagenous tissue. Of the proteases indigenous to skeletal muscle, Ca^{2+}-dependent proteases and the lysosomal cathepsins have been reported to be the best candidates for bringing about the textural changes during postmortem storage. Whereas these activities are desirable, activities associated with proteases invading from an animal's abdominal cavity are not. For example, herring, mackerel, and capelin caught during periods of heavy feeding are very susceptible to autolytic tissue degradation, and it is highly recommended to gut these fish as soon after capture as possible (9).

Breakdown of trimethylamine N-oxide (TMAO) by the enzyme TMAO demethylase in gadoid fish species generates dimethylamine and formaldehyde (FA). The FA, in turn, is extremely reactive and cross-links with myofibrillar proteins, causing a toughening of the texture and a loss of water-holding capacity that may be described as a "cottony" and "spongy" texture. Generally this reaction is evident during frozen storage of the gadoid species; however, if fresh fish are stored for long periods of time, such as when the fish are treated with chemicals and/or modified atmospheres, the consequences of this enzyme activity may also be evident.

For those fish species where red pigmentation is desirable (eg, salmon), postharvest bleaching would be considered undesirable. Lipoxygenases associated with fish skin, however, are capable of bleaching carotenoid pigments in fish skin and/or flesh.

A common occurrence that foods undergo during harvesting and processing is tissue disruption. With fruits and vegetables, this action could be either intentional (cutting broccoli, harvesting asparagus) or unintentional (fruit bruising, nicking of potatoes). For grains and oilseeds, grinding is often applied to abstract the desirable fractions, whereas with muscle foods, it could be anything from cutting into portions to mincing or deboning the tissue. These actions have important consequences, since during tissue disruption, a loss of an essential control system, enzyme decompartmentation, occurs. Enzyme and substrate not normally in contact with each other are thrown together, and the resulting activity leads to deterioration in quality. Harvesting asparagus, for example, initiates a lignification process that can render the stalk tough and inedible in a short period. This reaction is thought to proceed by peroxidase-catalyzed polymerization of phenolic compounds that then couple to cell wall constituents in vascular tissue, creating a rigid network (14). In minced fish tissue, a more extreme example of tissue disruption, lipolytic enzymes are released from lysosomes and proceed to attack the muscle lipids, rendering changes in texture and water-holding capacity of the product.

Physical Degradation

Insects. During growth, harvesting, and storage, insects may invade and feed on plant products. In some cases, this may directly lead to extensive losses in the product. In other cases, the appearance of the product would be considered unacceptable to the consumer and in this sense could be considered spoiled. Cured meats like country-style hams may also be damaged when insects lay their eggs in the meat. The larvae arising from the eggs burrow and feed on the meat causing a corky appearance. Insects may also serve as the vector for subsequent spoilage of the product. For example, the common fruit fly will transfer the mold that causes soft rot in vegetables (15).

Dehydration. The control of water in foods during storage is essential for fresh and dry foods. In the case of many types of fresh produce, they must be wrapped to prevent transpiration that would cause wilting and shriveling. On the other hand, it is important that the water vapor transfer rate of the wrapping not be too low so as to cause condensation. At 100% relative humidity, an acceptable water vapor transfer rate for packaging of most produce would be between 50 and 100 g/m^2/day (16). When the relative humidity in the packaging is less than 80 to 95% of saturation, fruit and vegetables lose moisture, and reduction in quality occurs when as little as 3 to 6% of the produce moisture is lost (17).

Dehydration or loss of moisture is also a problem for food stored in the frozen state. In meats, such moisture loss is often evidenced as freezer burn, a glassy appearance produced by the presence of tiny cavities left behind by sublimated ice. Apart from appearance of the product, loss of moisture will also affect the food's juiciness and texture. Oftentimes this moisture is recrystallized and leads to an unattractive formation of ice within the package.

Absorption of Odors. Many foods high in fat and improperly packaged have the potential to absorb odors from other products. For instance, milk left opened in the refrigerator will pick up odors from other highly volatile opened products in the refrigerator. The presence of odors not normally associated with the product may thus be justification for it being labeled as spoiled.

TOOLS FOR DETECTION OF SPOILAGE

Sensory Evaluations

Since quality and acceptability in most cases is ultimately determined by the consumer, sensory evaluation employing humans is one of the standard criteria used for judgment of spoilage. Depending on the product and the projected method of spoilage in that product, participants may be asked to judge visual and/or organoleptic characteristics. Many different approaches (tests) may be taken during sensory evaluation, but, in general, there may be difference tests (panelist asked to judge which of several samples is different) or hedonic scaling tests (panelist asked to rate a characteristic on an arbitrary scale with defined end points). The difficulty with using these type of analyses on a routine basis is the expense and time involved to conduct such analyses.

Microbial Evaluations

When microbial degradation is the major source of spoilage in the food, it is common practice to analyze for total numbers of bacteria in the food. The food may be rinsed to determine surface count or it may be ground/mixed with a specially formulated liquid medium of which diluted portions are taken and spread onto an agar plate. One of the drawbacks with regard to this type of analysis is that it takes a minimum of one to two days for the bacteria to grow on the agar and generate visible colonies for counting. The second even more important drawback is the realization that total count of bacteria does not always correlate to degree of spoilage. When spoilage is evident as visible growth (mold, pigmented or nonpigmented, slimy bacterial colonies), there is a direct relationship between total numbers of microorganisms and degree of spoilage. More often, though, spoilage is a result of the production of off-odors and off-flavors caused by bacterial metabolism. In these cases, there is no correlation between total numbers of bacteria and spoilage since only a fraction of the total flora participates in the spoilage. Thus, it becomes advantageous to identify which organisms are responsible for the spoilage and concentrate on enumerating their populations by employing selective media. No specific spoilage bacteria, however, have been identified in the case of vacuum-packaged beef or with vacuum-packaged cold-smoked salami (18). Even when the specific spoilage organisms are well established, there are instances where their enumeration is of limited value. For example, spoilage occurs at lower cell densities on high pH (>6.0) meat than on normal pH (<5.8) meat (19). Therefore, in those cases, it has been more appropriate to concentrate on identifying and quantifying the metabolic products responsible for the spoilage.

Other more advanced methods exist to identify spoilage organisms, and these rely on the immunochemical and genetic characteristics of the microorganisms. For detecting molds in grains, for example, antibodies to specific fungi may be linked directly or indirectly to a fluorescent dye. This technique is suitable for work on individual grains, but difficulties arise in assessment of molds in large samples of grain and in quantifying levels of molds present. Genetic detection techniques, on the other hand, are generally based on the hybridization of genomic DNA with a specific DNA probe. Hybridization may be accomplished by a variety of methods, but the most popular is the polymerase chain reaction (PCR). Such tools have proven useful in the identification of wild yeast belonging to the genus *Saccharomyces* that are biochemically and physiologically very similar to the culture yeast used in beer. By and far, the largest number of PCR applications have been directed at specific food pathogens, where it is essential to confirm the causative agent from a public health standpoint. Spoilage organisms, however, are of concern primarily from an economic significance, hence it is not necessary to detect them individually in foods. Consequently, primers may be designed for a conserved region of the DNA that allows detection of the majority of spoilage organisms as a group for a given commodity. Using such an approach for the PCR-ELISA assay, a detection threshold of 10^2 cfu/cm_2 was found (20). The advantage to using PCR rather than traditional plating is that the detection of the microorganisms is not dependent on the organisms' state of growth or on environmental influences. Limitations of PCR, however, are the inability to differentiate between viable and nonviable microorganisms, and substances in the food variably affect the activity of the DNA polymerase.

Chemical Evaluations

The chemical methods used for evaluating spoilage basically embody the assumption that as foods undergo spoilage, some new product or products are created by spoilage. Some of the specific components for which analyses have been undertaken and related to spoilage include: chitin, ergosterol, D-amino acids, D-lactate, tyramine, volatile basic nitrogen, trimethylamine, hydroperoxides, thiobarbituric acid-reactive substances, and headspace volatiles.

Chitin (N-acetyl-glucosamine) is an important component of fungal cell walls and is assessed by hydrolysis of chitin to glucosamine, followed by deamination to an aldehyde that is measured colorimetrically. Chitin, unfortunately, is also a major cuticular component of grain storage insects. Because insect debris is common in stored grain, the levels of chitin may not always be related to the level of spoilage.

Ergosterol (ergosta-5-7, 22-trienol) is another predominant sterol found in most fungi belonging to the classes Ascomycetes and Deuteromycetes. The amount of ergosterol produced by the fungi, however, is influenced by substrate composition, extent of aeration, and growth phase of mycelium; thus, it may not always be a true indicator of the extent of spoilage.

D-amino acids are important components of bacterial peptidoglycans. The presence of >1 ppm D-alanine in a variety of fruit juices indicated bacterial as opposed to yeast contamination (21). Thus, the potential exists for this compound to be used as a spoilage indicator. Similarly the D-isomer of lactate is not found in fresh foods and can be a diagnostic feature for bacterial spoilage.

Biogenic amines (putrescine, spermine, spermidine, histamine, tyramine) are produced by a number of bacteria and represent a hazard due to their psychoactive or vasoactive effects. In terms of their usefulness as spoilage indicators, only tyramine has been detected prior to the appearance of a faint putrid smell (initial stage of putrefaction). Using a sensor, composed of a tyramine oxidase-immobilized column and an oxygen electrode, tyramine levels have been found useful for estimating bacterial spoilage in aging beef. (22)

Both trimethylamine (TMA) and total volatile basic nitrogen (TVBN) levels have been routinely measured during iced and refrigerated storage of fish products. A recent comprehensive study, however, using 115 specimens of marine teleosteans and examination after three, five, and eight days of chilled storage revealed a strong relationship of organoleptically detected spoilage with TMA content, but not with TVBN content (23).

Food spoilage due to lipid oxidation has commonly been monitored by analyzing foods for the presence of hydroperoxides (primary reaction products) and for thiobarbituric acid-reactive substances (TBARS, secondary reaction products). Which compound is targeted depends on the food being analyzed. For instance, in frying oils, hydroperoxides are the compound of choice, whereas in muscle foods, TBARS has been the most common substance measured. The latter compound is preferred in muscle because hydroperoxides tend to break down quickly from interaction with the iron in the muscle.

At the root of many of the spoilage problems in food is the presence of off-flavors and off-odors. It therefore stands to reason that these compounds would be the target for identification and quantification. One of the most common means to measure the food for off-flavors and off-odors has been to sample the headspace of the food for its volatile content and subject it to gas chromatography (GC), oftentimes in combination with mass spectrometry (MS). In this manner, the dominant volatiles, 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone, have been used as indicators of spoilage in stored grain (24). Another food item where GC has been used to correctly identify spoilage samples has been with shrimp. In this case, the data from 9 to 11 volatiles were required for identification of rancidity in shrimp meat (25). Similarly, GC/MS in combination with principal component analysis of the data was proven useful in rapid differentiation of control reduced-fat milk samples from reduced-fat milk samples abused by light, heat, copper, and microbial (*P. fluorescens, P. aureofaciens,* or *P. putrefaciens*) contamination (26).

Another instrumental method to analyze volatiles that just came on the scene in the early to mid 1990s is the electronic nose. This instrument consists of an array of nonspecific electronic chemical sensors that react differentially with the headspace volatiles of the food. Similar to the human nose, the electronic nose recognizes odor patterns rather than specific patterns. While the human brain processes the signals from our nose, a pattern recognition routine analysis program analyzes the sensor responses in the electronic nose. Thus, in conjunction with sensory panels, the instrument can be trained to recognize products that have changed significantly in aroma and flavor. The potential of electronic noses to serve as spoilage indicators has been demonstrated with products such as grain, ground meat, vacuum-packaged beef, and freeze-stored chicken (27). To further the use of this instrument for spoilage measurements, however, improvements will need to be made in the sensors' stability, susceptibility to humidity, and lifetime.

TOOLS FOR PREDICTION OF SPOILAGE

Although it is all well and good to be able to detect a spoiled food, it is even better if we have the tools to predict when spoilage will occur. With that information, preventative measures may in some cases be applied. More often, the information is used to determine the distribution chain that should be taken by the product in order that it be bought and consumed before spoilage takes place.

Two approaches can be taken for prediction of spoilage. In one approach, the food is subjected to conditions that would accelerate its degradation. The time it takes to reach a defined level of deterioration is then related to the time it would take for the food to spoil under common storage practices. The inherent assumption to this approach, however, is that the conditions used for acceleration (temperature, oxidative catalysts, etc) would lead mechanistically to the same type of reactions as would have occurred without the activation. Such is not always the case.

In the second approach for prediction of spoilage, the state of the food at some earlier time period is related to the time at which spoilage would occur. In this approach, either microbial or chemical attributes may be used to describe the state of the food. For example, it has been shown that a chicken carcass with a significantly higher number of *P. fluorescens* would not continue to be hygienically acceptable as long as one that had a fewer number of spoilage organisms at day of processing (28). On the other hand, headspace GC data for milk that underwent a preincubation (24°C, 18 h) was related to the shelf life of milk stored at refrigeration temperatures (29). In both these examples, however, the predictions would only be valid for the product and storage conditions that existed for the study developing the model. In contrast, predictive microbiology aims to take into account the variability that occurs in product and storage conditions. It is based on the premise that the responses of populations of microorganisms to environmental factors are reproducible and that by considering environments in terms of identifiable dominating constraints it is possible, from past observations, to predict the responses of those microorganisms. The necessary components to predictive microbiology therefore include determining the specific spoilage microorganisms, their spoilage domains, and their growth kinetics for which a mathematical model is derived and validated. Those factors that should be considered during the development of the models are the pH, water activity, temperature, atmosphere, preservatives, and interactions among microorganisms. Many of the models currently developed have been incorporated into a commercial software program (Food Micromodel Ltd., Leatherhead, UK) where they may be applied to broad categories of food.

MEASURES TO PREVENT SPOILAGE

To ensure that food items reach the consumer before spoilage takes place, many cost-effective treatments may be applied to delay spoilage. These treatments concentrate on modifying those factors known to affect the rate at which the changes contributing to spoilage would take place and may involve processing or storage operations. They may be grouped into treatments where temperature is modified, pressure is applied, additives are added, or atmospheres are modified.

High temperatures have traditionally been applied to a number of foods to inactivate microorganisms and enzymes. In applying such treatments, however, the temperatures used should not decrease the desirable properties of the food. Also, many high-temperature treatments do not absolutely ensure a stable product. As alluded to previously, some microorganisms (thermoduric and thermophilic) may be able to survive the heat treatment, and extracellular microbial hydrolytic enzymes are also extremely heat resistant.

Refrigeration and frozen storage is another common technique used to extend shelf life. In addition to inhibiting microorganisms, low temperatures work by slowing down metabolic and chemical reactions in the product. Unfortunately, some fruits and vegetables are sensitive to refrigeration temperatures in that they exhibit chilling-injury symptoms. For these items, the most successful means to avoid chilling injury is to simply refrain from exposing susceptible commodities to temperatures below their critical threshold. Alternatively, the severity of chilling injury in a few commodities may be reduced when temperature conditioning (lowering temperatures to slightly above the chilling range for various periods) is applied.

High-pressure processing has been a recent treatment for delaying spoilage in food in that vegetative cells, including yeast and molds, can be inactivated by pressures of ~300 to 600 MPa (30). An advantage of the new technology is that flavor and vitamins are unaffected or only minimally altered by high-pressure processing at room temperature. On the other hand, bacterial spores are highly pressure resistant, since pressures exceeding 1200 MPa may be needed for their inactivation.

The use of chemicals may also be used to prevent or delay spoilage. Examples include antimicrobials, antioxidants, and calcium, which inhibit, respectively, microbial, oxidative, and chilling-injury spoilage. For any chemical additive, however, it must be legally approved for use in the product of concern.

Modified atmospheres containing carbon dioxide, oxygen, and nitrogen in varying proportions are used commercially to delay or prevent the growth of spoilage organisms in foods. As the concentration of carbon dioxide increases in the atmosphere, the growth rate of most bacteria is more or less inhibited. Excessive concentrations of carbon dioxide, however, may cause physiological damage in fresh produce such as uneven ripening in tomatoes, surface pitting in asparagus, and discoloration in celery. Therefore, the usual concentrations of carbon dioxide used for extending shelf life are in the range of 5 to 20%.

Another variation of modifying the atmosphere of a food is to apply edible coatings. Such coatings are made of edible materials that are used to enrobe the food and provide a semipermeable barrier to gases and water vapor. These coatings have the potential to reduce moisture loss, restrict oxygen entrance, lower respiration, retard ethylene production, seal in flavor volatiles, and carry additives that retard discoloration and microbial growth.

The measures just listed to reduce or prevent spoilage represent only a handful of those available; however, these and other techniques are increasingly used together in combination with preservative or hurdle technologies. These are applied on the basis that organisms develop homeostatic mechanisms to survive environments and that by attacking different elements of the survival response, increased microbial inactivation will occur with application of milder treatments. Keep in mind that removal of one spoilage mechanism does not imply that the product would be spoilage-free. More often than not, other spoilage mechanisms would surface with the extension of storage that has been incurred. Whether the mechanisms are of concern, however, would depend on the typical length of time that the product takes to get to the consumer. Thus, while spoilage may be viewed as a very complex set of activities, it must ultimately be viewed in the context of the distribution chain and the point at which consumption would take place.

BIBLIOGRAPHY

1. I. N. A. Ashie, J. P. Smith, and B. K. Simpson, "Spoilage and Shelf-Life Extension of Fresh Fish and Shellfish," *Crit. Rev. Food Sci. Nutr.* **36**, 87–121 (1996).
2. G. Fleet, "Spoilage Yeasts," *Crit. Rev. Biotechnol.* **12**, 1–44 (1992).
3. J. L. Kinderlerer, "*Chrysosporium* Species, Potential Spoilage Organisms of Chocolate," *J. Appl. Microbiol.* **83**, 771–778 (1997).
4. N. Magan, "Early Detection of Fungi in Stored Grain," *International Biodeterioration & Biodegradation* **32**, 145–160 (1993).
5. V. Venugopal, "Extracellular Proteases of Contaminant Bacteria in Fish Spoilage: A Review," *J. Food Prot.* **53**, 341–350 (1990).
6. L. Fernandez et al., "Proteolytic and Lipolytic Activities of *Pseudomonas fluorescens* Grown in Raw Milk With Variable Iron Content," *Milchwissenschaft* **47**, 160–163 (1992).
7. D. D. Muir, "The Shelf-life of Dairy Products: 4. Intermediate and Long Life Dairy Products," *Journal of the Society of Dairy Technology* **39**, 119–124 (1996).
8. R. R. Meer et al., "Psychrotrophic *Bacillus* spp. in Fluid Milk Products: A Review," *J. Food Prot.* **54**, 969–979 (1991).
9. A. Pedrosa-Menabrito and J. M. Regenstein, "Shelf-Life Extension of Fresh Fish—A Review. Part I—Spoilage of Fish," *Journal of Food Quality* **11**, 117–127 (1988).
10. Y. J. Chung-Wang, M. E. Bailey, and R. T. Marshall, "Reduced Oxidation of Fresh Pork in the Presence of Exogenous Hydrolases and Bacteria at 2°C," *J. Appl. Microbiol.* **82**, 317–324 (1997).
11. J. M. Sala, "Involvement of Oxidative Stress in Chilling Injury In Cold-Stored Mandarin Fruits," *Postharvest Biology and Technology* **13**, 255–261 (1998).
12. H. Zhuang, D. F. Hildebrand, and M. M. Barth, "Senescence of Broccoli Buds is Related to Changes in Lipid Peroxidation," *J. Agric. Food Chem.* **43**, 2585–2591 (1995).
13. J. S. Kang and D. S. Lee, "Susceptibility of Minimally Processed Green Pepper and Cucumber to Chilling Injury as Observed by Apparent Respiration Rate," *International Journal of Food Science and Technology* **32**, 421–426 (1997).
14. D. W. Stanley, "Biological Membrane Deterioration and Associated Quality Losses in Food Tissues," *Crit. Rev. Food Sci. Nutr.* **30**, 487–553 (1991).
15. R. E. Brackett, "Vegetables and Related Products," in L. R. Beuchat, ed., *Food and Beverage Mycology*, 2nd ed., Van Nostrand, New York, 1982, pp. 129–154.
16. C. H. Mannheim, "Control of Water in Foods During Storage," *Journal of Food Engineering* **22**, 509–532 (1994).
17. C. A. Phillips, "Review: Modified Atmosphere Packaging and Its Effects on the Microbiological Quality and Safety of Produce," *International Journal of Food Science and Technology* **31**, 463–479 (1996).
18. Y. Blixt and E. Borch, "Using an Electronic Nose for Determining the Spoilage of Vacuum-Packaged Beef," *International Journal of Food Microbiology* **46**, 123–134 (1999).
19. R. H. Dainty, "Chemical/Biochemical Detection of Spoilage," *International Journal of Food Microbiology* **33**, 19–33 (1996).
20. R. Gutiérrez et al., "Quantitative Detection of Meat Spoilage Bacteria by Using the Polymerase Chain Reaction (PCR) and an Enzyme Linked Immunosorbent Assay (ELISA)," *Lett. Appl. Microbiol.* **26**, 372–376 (1998).
21. I. Gandolfi et al., "D-Alanine in Fruit Juices: A Molecular Marker of Bacterial Activity, Heat Treatments and Shelf-Life," *J. Food Sci.* **59**, 152–154 (1994).
22. Y. Yano et al., "Changes in the Concentration of Biogenic Amines and Application of Tyramine Sensor During Storage of Beef," *Food Chem.* **54**, 155–159 (1995).
23. T. Civera et al., "Sensory and Chemical Assessment of Marine Teleosteans—Relationship Between Total Volatile Basic Nitrogen, Trimethylamine and Sensory Characteristics," *Sciences Des Aliments* **13**, 109–117 (1993).
24. T. Borjesson, U. Stollman, and J. Schnurer, "Volatile Metabolites Produced by Six Fungal Species Compared With Other Indicators of Fungal Growth on Cereal Grains," *Applied and Environmental Biology* **58**, 2599–2605 (1992).
25. L. S. Bak, L. Jacobsen, and S. S. Jorgensen, "Characterisation of Qualitative Changes in Frozen, Unpeeled Cold-Water Shrimp (*Pandalus borealis*) by Static Headspace Gas Chromatography and Multivariate Data Analysis," *Z. Lebensm. Unters.-Forsch.* **208**, 10–16 (1999).
26. R. T. Marsili, "SPME-MS-MVA as an Electronic Nose for the Study of Off-Flavors in Milk," *J. Agric. Food Chem.* **47**, 648–654 (1999).
27. J-E. Haugen and K. Kvaal, "Electronic Nose and Artificial Neural Network," *Meat Science* **49**, S273–S286 (1998).
28. S. M. Russell, "A Rapid Method for Predicting the Potential Shelf Life of Fresh Broiler Chicken Carcasses," *J. Food Prot.* **60**, 148–152 (1997).
29. B. Vallejo-Cordoba and S. Nakai, "Keeping-Quality Assessment of Pasteurized Milk by Multivariate Analysis of Dynamic Headspace Gas Chromatographic Data. 1. Shelf-Life Prediction by Principal Component Regression," *J. Agric. Food Chem.* **42**, 989–993 (1994).
30. M. Hendrickx et al., "Effects of High Pressure on Enzymes Related to Food Quality," *Trends Food Sci. Technol.* **9**, 197–203 (1998).

Marilyn Erickson
University of Georgia
Griffin, Georgia

FOOD SURFACE SANITATION

Of paramount importance in food manufacture is the freedom of microbial (spoilage and pathogenic microorganisms) and foreign body contamination in the final product. Such contamination may arise from the constituent raw materials or the processing environment, which includes food contact surfaces, the air, people, and pests. Failure to control these factors may lead to product recalls, loss of sales or profits, adverse publicity, and, if regulatory requirements have been infringed, fines, sanctions, or ultimately site closure or loss of production/export license. For mechanisms would surface with the extension of storage that has been incurred. Whether the mechanisms are of concern, however, would depend on the typical length of time that the product takes to get to the consumer. Thus, while spoilage may be viewed as a very complex set of activities, it must ultimately be viewed in the context of the distribution chain and the point at which consumption would take place.

BIBLIOGRAPHY

1. I. N. A. Ashie, J. P. Smith, and B. K. Simpson, "Spoilage and Shelf-Life Extension of Fresh Fish and Shellfish," *Crit. Rev. Food Sci. Nutr.* **36**, 87–121 (1996).
2. G. Fleet, "Spoilage Yeasts," *Crit. Rev. Biotechnol.* **12**, 1–44 (1992).
3. J. L. Kinderlerer, "*Chrysosporium* Species, Potential Spoilage Organisms of Chocolate," *J. Appl. Microbiol.* **83**, 771–778 (1997).
4. N. Magan, "Early Detection of Fungi in Stored Grain," *International Biodeterioration & Biodegradation* **32**, 145–160 (1993).
5. V. Venugopal, "Extracellular Proteases of Contaminant Bacteria in Fish Spoilage: A Review," *J. Food Prot.* **53**, 341–350 (1990).
6. L. Fernandez et al., "Proteolytic and Lipolytic Activities of *Pseudomonas fluorescens* Grown in Raw Milk With Variable Iron Content," *Milchwissenschaft* **47**, 160–163 (1992).
7. D. D. Muir, "The Shelf-life of Dairy Products: 4. Intermediate and Long Life Dairy Products," *Journal of the Society of Dairy Technology* **39**, 119–124 (1996).
8. R. R. Meer et al., "Psychrotrophic *Bacillus* spp. in Fluid Milk Products: A Review," *J. Food Prot.* **54**, 969–979 (1991).
9. A. Pedrosa-Menabrito and J. M. Regenstein, "Shelf-Life Extension of Fresh Fish—A Review. Part I—Spoilage of Fish," *Journal of Food Quality* **11**, 117–127 (1988).
10. Y. J. Chung-Wang, M. E. Bailey, and R. T. Marshall, "Reduced Oxidation of Fresh Pork in the Presence of Exogenous Hydrolases and Bacteria at 2°C," *J. Appl. Microbiol.* **82**, 317–324 (1997).
11. J. M. Sala, "Involvement of Oxidative Stress in Chilling Injury In Cold-Stored Mandarin Fruits," *Postharvest Biology and Technology* **13**, 255–261 (1998).
12. H. Zhuang, D. F. Hildebrand, and M. M. Barth, "Senescence of Broccoli Buds is Related to Changes in Lipid Peroxidation," *J. Agric. Food Chem.* **43**, 2585–2591 (1995).
13. J. S. Kang and D. S. Lee, "Susceptibility of Minimally Processed Green Pepper and Cucumber to Chilling Injury as Observed by Apparent Respiration Rate," *International Journal of Food Science and Technology* **32**, 421–426 (1997).
14. D. W. Stanley, "Biological Membrane Deterioration and Associated Quality Losses in Food Tissues," *Crit. Rev. Food Sci. Nutr.* **30**, 487–553 (1991).
15. R. E. Brackett, "Vegetables and Related Products," in L. R. Beuchat, ed., *Food and Beverage Mycology*, 2nd ed., Van Nostrand, New York, 1982, pp. 129–154.
16. C. H. Mannheim, "Control of Water in Foods During Storage," *Journal of Food Engineering* **22**, 509–532 (1994).
17. C. A. Phillips, "Review: Modified Atmosphere Packaging and Its Effects on the Microbiological Quality and Safety of Produce," *International Journal of Food Science and Technology* **31**, 463–479 (1996).
18. Y. Blixt and E. Borch, "Using an Electronic Nose for Determining the Spoilage of Vacuum-Packaged Beef," *International Journal of Food Microbiology* **46**, 123–134 (1999).
19. R. H. Dainty, "Chemical/Biochemical Detection of Spoilage," *International Journal of Food Microbiology* **33**, 19–33 (1996).
20. R. Gutiérrez et al., "Quantitative Detection of Meat Spoilage Bacteria by Using the Polymerase Chain Reaction (PCR) and an Enzyme Linked Immunosorbent Assay (ELISA)," *Lett. Appl. Microbiol.* **26**, 372–376 (1998).
21. I. Gandolfi et al., "D-Alanine in Fruit Juices: A Molecular Marker of Bacterial Activity, Heat Treatments and Shelf-Life," *J. Food Sci.* **59**, 152–154 (1994).
22. Y. Yano et al., "Changes in the Concentration of Biogenic Amines and Application of Tyramine Sensor During Storage of Beef," *Food Chem.* **54**, 155–159 (1995).
23. T. Civera et al., "Sensory and Chemical Assessment of Marine Teleosteans—Relationship Between Total Volatile Basic Nitrogen, Trimethylamine and Sensory Characteristics," *Sciences Des Aliments* **13**, 109–117 (1993).
24. T. Borjesson, U. Stollman, and J. Schnurer, "Volatile Metabolites Produced by Six Fungal Species Compared With Other Indicators of Fungal Growth on Cereal Grains," *Applied and Environmental Biology* **58**, 2599–2605 (1992).
25. L. S. Bak, L. Jacobsen, and S. S. Jorgensen, "Characterisation of Qualitative Changes in Frozen, Unpeeled Cold-Water Shrimp (*Pandalus borealis*) by Static Headspace Gas Chromatography and Multivariate Data Analysis," *Z. Lebensm. Unters.-Forsch.* **208**, 10–16 (1999).
26. R. T. Marsili, "SPME-MS-MVA as an Electronic Nose for the Study of Off-Flavors in Milk," *J. Agric. Food Chem.* **47**, 648–654 (1999).
27. J-E. Haugen and K. Kvaal, "Electronic Nose and Artificial Neural Network," *Meat Science* **49**, S273–S286 (1998).
28. S. M. Russell, "A Rapid Method for Predicting the Potential Shelf Life of Fresh Broiler Chicken Carcasses," *J. Food Prot.* **60**, 148–152 (1997).
29. B. Vallejo-Cordoba and S. Nakai, "Keeping-Quality Assessment of Pasteurized Milk by Multivariate Analysis of Dynamic Headspace Gas Chromatographic Data. 1. Shelf-Life Prediction by Principal Component Regression," *J. Agric. Food Chem.* **42**, 989–993 (1994).
30. M. Hendrickx et al., "Effects of High Pressure on Enzymes Related to Food Quality," *Trends Food Sci. Technol.* **9**, 197–203 (1998).

MARILYN ERICKSON
University of Georgia
Griffin, Georgia

FOOD SURFACE SANITATION

Of paramount importance in food manufacture is the freedom of microbial (spoilage and pathogenic microorganisms) and foreign body contamination in the final product. Such contamination may arise from the constituent raw materials or the processing environment, which includes food contact surfaces, the air, people, and pests. Failure to control these factors may lead to product recalls, loss of sales or profits, adverse publicity, and, if regulatory requirements have been infringed, fines, sanctions, or ultimately site closure or loss of production/export license. For example, incomplete sanitation of a meat grinder was responsible for a large outbreak of *Salmonella typhirium* infected from ingestion of ground beef (1). Many food service operations are integrating hazard analysis and critical control point (HACCP) along with sanitation to ensure food safety (2).

The sanitation of surfaces, when undertaken correctly, is cost effective, easy to manage, and, if diligently applied, can reduce the risk of microbial or foreign body contamination of product. When incidents of such contamination occur, it is often not easy to trace and rectify the exact source and as surface sanitation is relatively cheap, it can provide management with a good tool to reduce this risk. In this context, surface sanitation:

1. Removes microorganisms, or material conducive to microbial growth. This reduces the chance of contamination by pathogens and extends the shelf life of some products.
2. Removes materials that could lead to foreign body contamination or could provide food or shelter for pests.

Surface sanitation is also implemented in food processing to provide a wide range of additional benefits including:

1. Reduces waste and improves the appearance and quality of product by removing product left on lines that may deteriorate and reenter subsequent production runs.
2. Increases process performance in some areas (eg, plate and scrape surface heat exchangers).
3. Extends the life of, and prevents damage to, equipment and services.
4. Provides a safe and clean working environment for employees and thus increases morale and productivity.
5. Presents a favorable image to customers and the public.

In this article, food surfaces are defined as both food contact and environmental surfaces. Environmental surfaces are included as, although they are not in direct contact with the product, contamination may be transferred from them to the product by people, pests, the air, or cleaning procedures. Only hard surfaces are considered eg, equipment, floors, walls, and utensils as other surfaces, eg, protective clothing or skin, would be traditionally dealt with under personal hygiene.

It is further assumed that the surfaces addressed have been designed hygienically. Poor hygienic design will restrict the efficiency of even the most effective cleaning procedure and may vitiate any subsequent disinfection programs. The principles of hygienic design are comprehensively described elsewhere (3–5). The basic hygiene design of equipment for open food processing has been reviewed recently (6).

FOOD SOILS

Debris will build up on surfaces throughout the production period; this will require subsequent removal by cleaning. This debris may be the result of normal production, spillages, line jams, maintenance, packaging, or general dust and dirt. Such undesirable material, which may include food residues, microorganisms, and foreign matter, is referred to as soil. In practical terms, a soil is anything in the wrong place at the wrong time; peas on a conveyor during production are product but after production or on the floor are soil.

Product soils are usually easy to visualize and are characterized primarily by the product type eg, protein, fat or carbohydrate. The process however is also important as a given product may present a variety of cleaning problems, depending on whether it is dry, wet, heat treated, frozen or the length of time it is left prior to cleaning.

Microbial soils cannot generally be observed by the eye but require microscopic examination. Bacterial attachment to surfaces is well documented (7–9), and the influence of bacterial growth on surfaces, termed biofilms, in the food industry has been discussed (10,11). Examination of stainless steel coupons attached to production lines, by epifluorescent microscopy, has been used to assess microbial levels in a range of food soils (12). An example of a heavy biofilm buildup over 16 h is shown in Figure 1 for a baked bean soil. After 12 h or so, this biofilm appeared to be of a brown vegetable appearance, but the photographs show clearly that it is bacterial in nature. A thorough understanding of a soil's characteristics is therefore required to ensure a successful and economic sanitation program.

FUNDAMENTALS

The process of sanitation is intended to remove all undesirable material (food residues, microorganisms, foreign bodies, and cleaning chemicals) from surfaces in an economical manner, to a level at which any residues remaining are of minimal risk to the quality or safety of the product. Sanitation is divided into two broad areas, the cleaning of open and closed surfaces, though the principles are essentially the same. Open surface sanitation refers to all equipment and environmental surfaces that are readily cleaned manually. Closed surface sanitation, generally referred to as clean-in-place or CIP, is undertaken where manual cleaning is difficult, impractical or impossible (e.g., tanks, homogenizers and pipelines).

Sanitation programs are a combination of four major factors:

- Mechanical or kinetic energy
- Chemical energy
- Temperature or thermal energy
- Time

Mechanical or kinetic energy is employed to physically remove soils and may include manual brushing (physical abrasion), pressure jet washing (fluid abrasion), or the circulation of fluid in CIP systems (turbulent flow). Of these

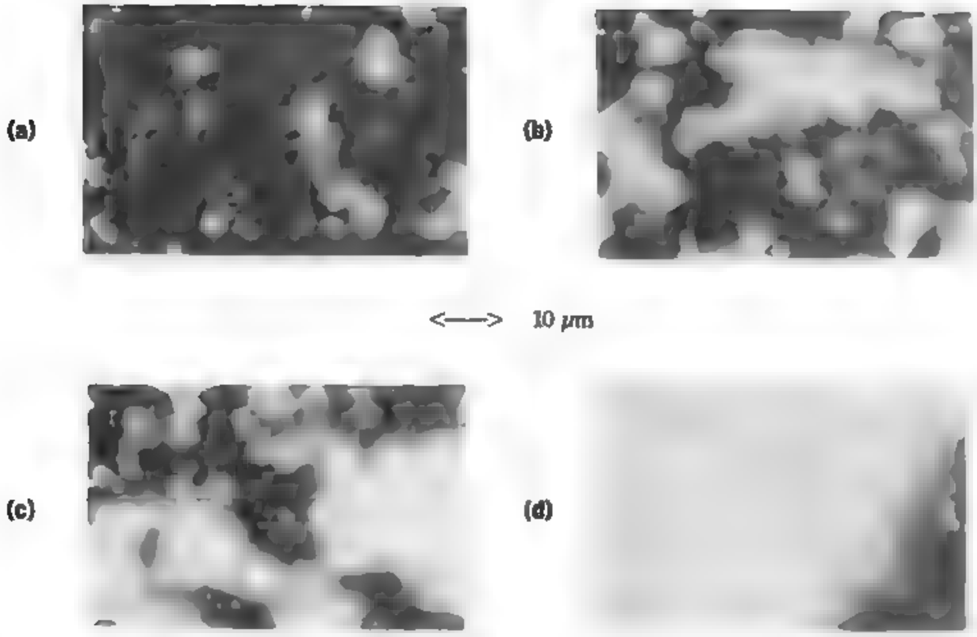


Figure 1. Build up of a bacterial biofilm on a baked bean production line. Photographs were taken using epiflourescent microscopy such that bacteria fluoresced orange while the background remained dark. (**a**) 4 h, (**b**) 8 h, (**c**) 12 h, (**d**) 16 h.

methods, physical abrasion is the most efficient in terms of energy transfer (13); for turbulent flow, a mean velocity of 1.5 m/s should be achieved (14).

Chemical energy is fundamental to both the cleaning and disinfection elements of sanitation. In cleaning, chemicals are used to break down soils so that the soils are less tenacious and to suspend them in solution to allow them to be rinsed away. In disinfection, chemicals are used to reduce the viability of microorganisms remaining on surfaces after cleaning.

Temperature or thermal energy is important for several reasons. Cleaning and disinfection chemical effects increase with temperature linearly and approximately double for every 10°C rise. Temperatures above the melting point of fatty or oily soils are used to break down and emulsify these deposits and high temperatures, particularly in CIP systems, have a disinfection effect in their own right.

Time is a factor that is often overlooked in sanitation systems. It is an essential prerequisite for the previously discussed energy forms and generally the longer the time period employed, the more efficient the process. Time can also be used to reduce the degree of energy input required from other sources when precleaning soaking is undertaken.

The combinations of these four factors varies for different cleaning systems such that if one energy source is restricted, this shortfall may be compensated for by utilizing greater inputs from the others. For example, in CIP cleaning, the energy that can be derived from mechanical energy is low but much higher temperatures and chemical concentrations are possible than can be safely used in open surface cleaning. The influence of chemical (detergents), temperature, and mechanical energy (pressure washing) has been described for open surfaces (15–18) and for CIP systems (19).

Soil removal from surfaces has been shown to basically follow first-order reaction kinetics (20,21) such that the decrease in the log of the mass of soil per unit area remaining is linear with respect to cleaning time (Fig. 2). This approximation is only valid in the central portion of the plot; it has been reported (22) that in practice, soil removal is initially faster and ultimately slower than that which a first-order reaction predicts (dotted line in Fig. 2). The reasons for this are unclear although initially, unadhered, gross soil is usually easily removed whereas ultimately, soils held within surface imperfections or otherwise shadowed from cleaning effects would be more difficult to remove.

As routine cleaning operations are therefore not 100% efficient over multiple soiling/cleaning cycles, soil deposits will accumulate on surfaces. During this phase, cleaning will become less efficient and attached microbial numbers will increase. This situation is usually controlled by the

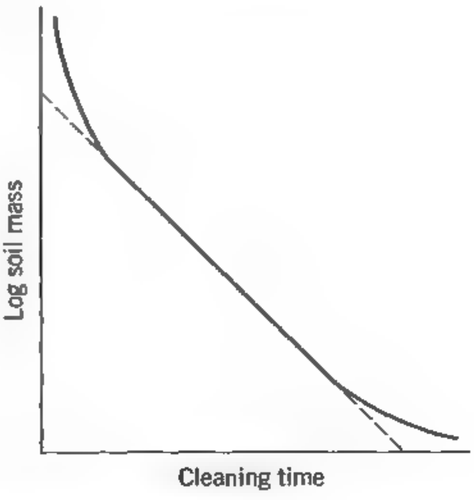


Figure 2. Removal of soil from surfaces with cleaning time.

application of a periodic clean (23), the object of which is to periodically return the surface bound soil accumulation to an acceptable base level (Fig. 3). This is achieved by increasing cleaning time and/or energy input (eg, higher temperatures, alternative chemicals, or manual scrubbing) and is the basis of many food processors weekend clean down.

SANITATION PROCEDURE

The principal stages involved in a typical sanitation procedure are as follows:

1. Preparation. Dismantle equipment as far as is practicable or necessary and/or remove unwanted utensils/equipment. Protect electric or other sensitive systems and/or screen off other lines or areas to prevent transfer of debris by the sanitation process.
2. Gross soil removal. Where appropriate, remove all loose or gross soil by eg, brushing, shoveling, scraping, or vacuuming. Wherever possible, soil on floors and walls should be picked up rather than washed to drains.
3. Prerinse. Rinse with low pressure cold water to remove loose small debris. Hot water can be used for fatty soils, but too high a temperature may coagulate proteins.
4. Cleaning. Apply cleaning chemicals, temperature, and mechanical energy to remove adhered soils.
5. Interrinse. Rinse with low pressure cold water to remove soil detached by cleaning operations and cleaning chemical residues.
6. Disinfection. Apply chemical disinfectants to remove or reduce the viability of remaining microorganisms to as low a level as possible.
7. Postrinse. Rinse with low pressure cold water to remove disinfectant residues if required.
8. Intercycle conditions. Remove excess water and/or do everything necessary to prevent the growth of microorganisms in the period up until the next production process or the next use of the equipment/area.

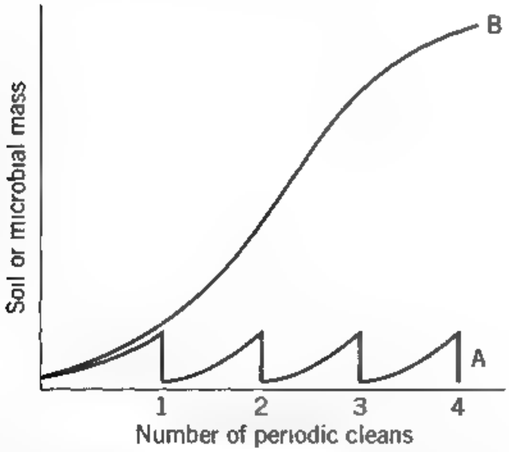


Figure 3. Build up of soil and/or microorganisms A, with periodic cleans and B, without periodic cleans (19).

Although broadly similar for all general sanitation procedures, alternatives may be used. In CIP systems a second cleaning cycle may be added if both an alkaline and acidic phase are incorporated. With only light soiling to be removed, it may be appropriate to combine stages 4–6 by using a detergent-sanitizer, a chemical with both cleaning and antimicrobial properties. This should only be used for light soils, however, as with normal or heavy soils the antimicrobial properties will be quickly lost. Stages 3, 5, and 7 may be omitted in dry cleaning operations where organic solvents replace water in the cleaning and, where appropriate, the disinfection stages. Because of the general nature of this article, specialist dry cleaning is not discussed further.

CHEMICALS

This section gives a brief and general introduction to the chemicals used in sanitation. For further information, readers are directed to the specific articles on detergents and disinfectants in this encyclopedia. Sanitation chemicals, because of the procedure outlined in the previous section, are usually employed as cleaning or disinfection agents. This section is therefore divided into cleaning and disinfection subsections. Some chemicals routinely used, such as quaternary ammonium compounds (QUATS) or iodophores, have both cleaning and biocidal properties, although in this section they are described for their primary function only.

Cleaning

Unfortunately no single cleaning agent is able to perform all the functions necessary to facilitate an efficient cleaning program, so a cleaning solution, or detergent, is blended from some of the following characteristic components: water, surface active agents inorganic alkalies inorganic and organic acid, and sequestering agents.

Water. Water is the basic ingredient of most cleaning systems as it provides the cheapest readily available transport medium for removing soils. It also has dissolving powers to remove ionic and water soluble compounds such as salts and sugars, will help emulsify fats at temperatures above their melting point, and can be used as an abrasive agent when high pressure washing. It is, however, a poor wetting agent and cannot dissolve nonionic compounds.

Surface Active Agents. Surface active agents, wetting agents or surfactants are composed of a long, nonpolar (hydrophobic or lyophilic) chain or tail and a polar (hydrophilic or lyophobic) head as illustrated in Figure 4. Surfactants may be anionic, cationic, or nonionic, depending on their ionic charge in solution. The polar end is able to penetrate into water as the ionic charges are greater in magnitude than the weaker hydrogen bonding between the water molecules. The nonpolar end is unable to easily break apart the water molecules' hydrogen bonding but can enter

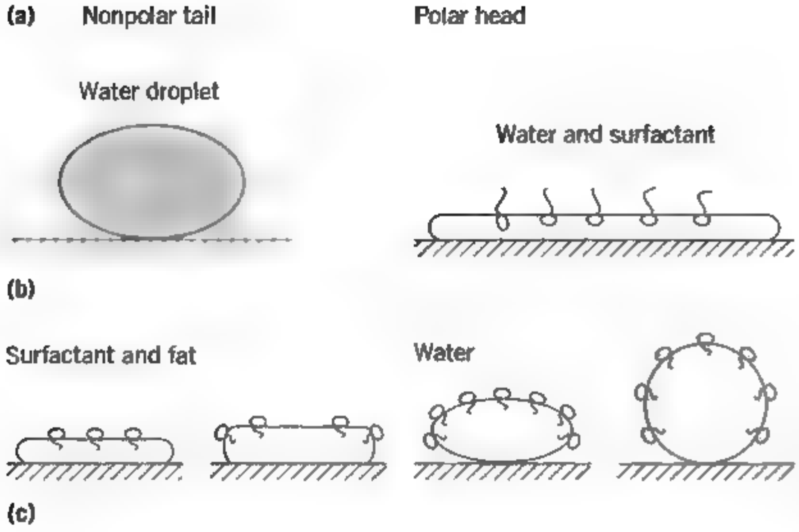
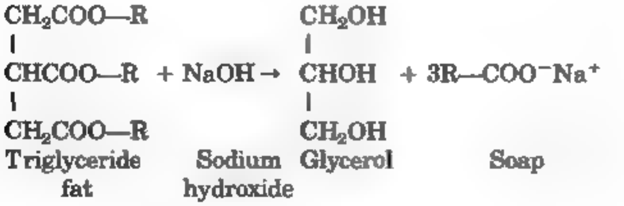


Figure 4. Schematic diagrams showing: (a) structure of surfactant molecule, (b) surfactant improving the wettability of water, and (c) surfactant removing fat from a surface and dispersing it in suspension.

other nonpolar compounds such as fats and oils. This aids cleaning in two ways: it reduces water surface tension and removes and suspends fats.

Surface tension is reduced as the polar head enters the water and breaks down hydrogen bonding. If a surfactant is added to a drop of water on a surface, the surface tension of the water is reduced and the drop collapses to wet the surface (Fig. 4). Increased wettability allows greater water penetration into soils and hence better cleaning action. Fats and oils are removed from surfaces as the polar head of the surfactant molecule dissolves in the water, whereas the hydrophobic end dissolves in the fat. Due to the forces acting on the fat-water interface, the fat particle tends to form a sphere, as this has the lowest surface area for a given volume. In so doing, the fat particle will roll up, become detached from the surface, and remain in suspension (Fig. 4).

Inorganic Alkalies. Alkalies are useful cleaning agents because they are cheap, break down proteins, saponify fats, and may be bactericidal. A typical saponification reaction is shown below in which a nonpolar R group is changed to a soluble form that has good surfactant properties.



$$\begin{array}{lcll} CH_2COO{-}R & & CH_2OH & \\ | & & | & \\ CHCOO{-}R & + NaOH \rightarrow & CHOH & + 3R{-}COO^-Na^+ \\ | & & | & \\ CH_2COO{-}R & & CH_2OH & \\ \text{Triglyceride fat} & \text{Sodium hydroxide} & \text{Glycerol} & \text{Soap} \end{array}$$

Generally, the stronger the alkali the greater the degree of saponification, although this is a compromise, as corrosiveness also increases with alkali strength. Alkaline compounds can also precipitate scum, a reaction of the soap with water hardness ions (Ca^{2+} and Mg^{2+}), and may have poor rinsability. For some applications, alkaline detergents are chlorinated, as at high pH chlorine increases peptizing of proteins and may reduce mineral deposition. Chlorine at high pH, however, is also very corrosive.

Inorganic and Organic Acids. Acids are not generally used in the food industry as they have little dissolving power for fats, oils, and proteins. They are useful, however, in making soluble mineral scales such as hard-water deposits, beer stone, and milk stone. A typical reaction would be as follows:

$$\underset{\text{Insoluble}}{CaCO_3} + 2HCl \rightarrow \underset{\text{Soluble}}{CaCl_2} + H_2O + CO_2$$

As with alkalies, the stronger the acid the more corrosive it is. Strong acids (mineral acids) include hydrochloric, nitric, and phosphoric acid, whereas weaker organic acids typically used include citric, lactic, and acetic acid.

Sequestering Agents. Sequestering agents are employed to sequester or chelate mineral salts by forming soluble complexes with them. Their primary use is in the control of water hardness ions although they are also useful in maintenance of alkaline conditions by buffering, emulsification of oils, and fats and increasing rinsability. Sequestrants can be both organic and inorganic, organic sequestrants being usually based on polyphosphates and inorganic chelating agents commonly being the potassium or sodium salts of ethylene diamine tetraacetic acid (EDTA).

A general-purpose food detergent may contain a strong alkali to saponify fats, weaker alkali builders to aid corrosion resistance, surfactants to improve wetting, dispersion, and rinsability and sequestrants to control hard water ions. Ideally, the detergent should also be safe, nontainting, stable, noncorrosive, biodegradable, and cheap. The detergent chosen for a particular application will depend on the soil to be removed; the solubility characteristics and cleaning procedure recommended for a range of food soils is shown in Table 1.

Table 1. Solubility Characteristics and Cleaning Procedures Recommended for a Range of Solid Types

Food (or soil)	Solubility characteristics	Cleaning procedure recommended
Sugars, organic acids, salt	Water soluble	Mildly alkaline detergent
High-protein foods (meat, poultry, fish)	Water soluble Alkali soluble Slight acid soluble	Chlorinated alkaline detergent
Fatty foods (fat meat butter, margarine, oils)	Water insoluble Alkali soluble	Mildly alkaline detergent, if ineffective, use strong alkali
Stone-forming foods, mineral scale (milk products, beer, spinach)	Water insoluble Acid soluble Alkali insoluble	Chlorinated cleaner or mildly alkaline cleaner, alternate with acid cleaner on each 5th day
Heat-precipitated water hardness	Water insoluble Alkali insoluble Acid soluble	Acid cleaner
Starch foods, tomatoes, fruits, vegetables	Partly water soluble Alkali soluble	Mildly alkaline detergent

Source. Ref. 24.

Disinfection

The cleaning portion of the sanitation cycle has been shown to reduce bacterial numbers on surfaces by up to 99.9% or three log orders (25). Work in our own laboratories has shown that with detergent soaks and pressure washing, up to 4–5 log orders may be removed. However, given that bacterial numbers on surfaces could be between 10^7 and 10^{10} organisms/cm (2,25), viable bacteria are likely to be present on surfaces after cleaning. The aim of disinfection procedures is to remove or reduce the viability of these remaining microorganisms.

If possible, temperature is used as a disinfectant as it penetrates into surfaces, is noncorrosive, is nonselective to microbial types, and is easily measured and leaves no residue (20). Whereas high temperatures are often used as disinfectants in CIP systems, their use on open surfaces is usually uneconomic, hazardous, or impossible. In such cases, chemical biocides are employed and, as with cleaning chemicals, no single disinfectant satisfies all the performance requirements. Biocides are rarely mixed, however, so a choice has to be made from a limited number of disinfectant types. Universally used biocides include chlorine releasing components, quaternary ammonium compounds, iodine compounds, amphoterics, and peracetic acid.

Chlorine is the most widespread and cheapest disinfectant used in the food industry and is available in fast-acting (chlorine gas, hypochlorites) or slow-releasing forms (e.g., chloramines, dichlorodimethylhydantoin). Quaternary ammonium compounds (QUATS or QAC's) are based on ammonium salts with substituted hydrogen atoms and a chlorine or bromine anion, whereas iodophores are soluble complexes between elemental iodine (active ingredient) and nonionic surface active agents. Amphoterics are based on the amino acid glycine, often incorporating an imidazole group. Peracetic acid may be used by itself or formulated with hydrogen peroxide. A range of characteristics for examples of these disinfectant types is shown in Table 2. Other disinfectants used to a limited extent include biguanides, formaldehyde, ozone, chlorine dioxide, and bromine; however, biocides successfully used in other industries, eg, phenolics or metal ion-based products, are not used for food applications due to safety or taint problems. Frank and Chmielewski (26) confirmed the effectiveness of quaternary ammonium compounds and chlorine in reducing *S. aureus* 1,000-fold on stainless steel and domestic food preparation surfaces.

Disinfectant concentration and contact time are important considerations in the reduction of microbial viability. The relationship between death and concentration is not linear but follows a sigmoidal curve dependent on the resistance of organisms within the population. Disinfectants do not, therefore, necessarily kill all microorganisms in a population, and increasing concentration may not enhance this effect. For disinfectants to be effective, they must find, bind to, and transverse microbial cell envelopes before they reach their target site (27). Chemical disinfectants are also effective in preventing cross-contamination from food contact surfaces (28). Sufficient contact time is therefore critical to give good results. Amphoterics and QUATS may be left on surfaces for extended periods between production runs without rinsing, as they are FDA approved as indirect food additives at low concentration.

The performance of the cleaning procedure may influence disinfection efficiency. Any soil or cleaning chemical residues remaining may protect microorganisms from disinfectant penetration or may react with the disinfectant and destroy its antimicrobial abilities. Biocides are best used within their specified pH range, although performance can generally be increased by increasing the temperature. A study by Best et al. (29) examined the efficacy of 14 disinfectants against *Listeria* spp. Their effectiveness varied depending on whether the test organism was dried on a steel surface, in suspension, or in the presence of organic material. Consequently, it is important to select the appropriate disinfectant for the particular contaminated surface, especially if organic material is present.

Table 2. Characteristics of Some Universal Disinfectants

Property		Chlorine	QUAT	Iodophore	Amphoteric	Peracetic acid
Microorganism control	Gram positive	+ +	+ +	+ +	+ +	− +
	Gram negative	+ +	−	+ −	+ +	− +
	Spores	+	−	+/−	−	− +
	Yeast	+ +	− −	+ −	+ +	+ +
Developed microbial resistance		−	−	−	+	−
Inactivation by	Organic matter	+ +	+	+	+	+
	Water hardness	−	+	−	−	−
Detergency properties		−	+ +	+	+	−
Residual film formation		−	− +	+/−	+ +	−
Foaming potential		−	+ +	+	+ +	−
Problems with	Taints	+/−	−	+/−	−	+/−
	Stability	+/−	−	−	−	+/−
	Corrosion	+	−	+	−	−
	Safety	+	−	+	−	+ +
	Other chemicals	−	+	+	−	−
Cost		−	+ +	+	+ +	+

Note: − No effect (or problem).
+ Effect.
+ + Large effect.

METHODS

Open Surfaces

After gross soil removal, open surfaces can be cleaned and disinfected in their normal position or dismantled and/or transferred to a separate area (cleaned-out-of-place, COP). Sanitation procedures can be undertaken by hand using simple tools eg, brushes or cloths (manual cleaning) or by using specialized equipment designed to cover larger areas more rapidly.

Manual cleaning is often undertaken because no specialist equipment is required and high levels of mechanical energy input can be used at exactly the right point. Sanitation procedures can also be undertaken quickly (over small surface areas) and in places that alternative techniques could not reach or may damage. Due to operator safety, however, only low levels of temperature and chemical energy can be applied and it is costly in time and labour to cover large surface areas. Only tools specifically designed for food sanitation should be used, eg, plastic-handled brushes with stiff, colored nylon bristles, and they should be regularly cleaned and stored in a disinfectant solution to reduce cross-contamination in use.

Manual cleaning of smaller, dismantled items can be improved using COP techniques. When soak tanks are used, items can be subjected to higher temperatures and chemical concentrations for an extended time period prior to manual cleaning and disinfection. For an automated approach, specialized equipment can be used that also applies the mechanical energy input, eg, tray washers and tunnel dish washers.

For large areas of open surface, sanitation equipment is designed to disperse chemicals and/or provide mechanical energy. Chemicals may be applied as mists, foams, or gels, whereas mechanical energy is provided by water jets or scrubbing actions. The use of high temperature is rarely used for cleaning or disinfections, because of the excessive energy requirements needed to heat soil and/or surfaces to suitable temperatures.

Mist spraying of chemicals onto surfaces is undertaken using small, hand-pumped containers, knapsack sprayers (Fig. 5) or pressure washing systems at low pressure. As for the other chemical application methods, pressure washing systems can be mobile units (Fig. 5), wall mounted serving one or more outlets, or centralized where one unit may supply many outlets via a ring main. Chem-

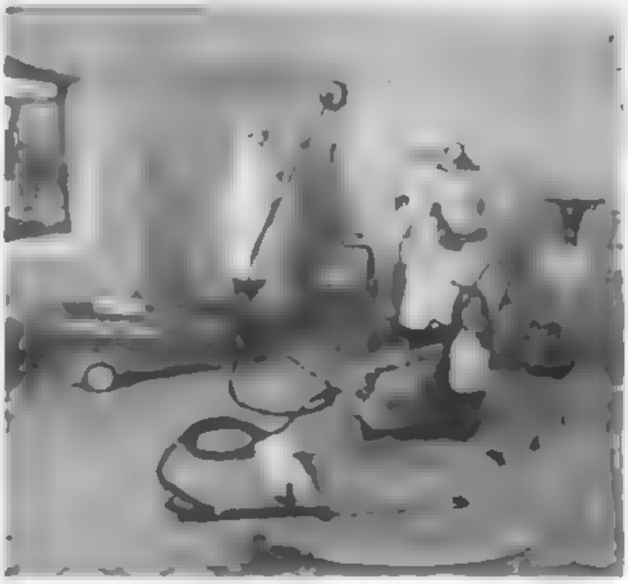


Figure 5. A range of cleaning equipment suitable for use in food hygiene. Clockwise from left: electrically driven scrubber brush, gel applicator, detergent or disinfectant mist sprayer, floor scrubber, mobile high-pressure/low-volume washer with standard lance connected, shrouded lance to reduce aerosol spread, low-pressure/high-volume gun.

icals are added, usually by venturi injectors, either at the pumps of mobile or wall-mounted units, at the outlets of high pressure water ring main systems or via separate low pressure chemical ring mains. Misting is only able to wet vertical smooth surfaces and therefore only small quantities can be applied. Only weak chemicals can be applied, as the technique tends to form aerosols, so for cleaning purposes, misting is only useful for light soiling. The detergent and loosened soil are removed via high or low pressure water rinsing. Once the surfaces have been cleaned, however, misting is a very useful and most widely applied technique for applying disinfectants.

If more tenacious soiling is to be removed, detergents may be applied as a foam. Foams are more viscous than mists, are not as prone to aerosol formation, and remain on vertical surfaces for around 5–20 min. This allows the use of more concentrated detergents, longer contact times, and because of the nature of the foam, a more consistent application of chemicals as it is easier to spot areas that have been missed. Foams can be generated and applied by the entrapment of air in high pressure systems or by the addition of compressed air in low pressure equipment, and are rinsed away by high or low pressure water. Although the visible nature of the foam should aid complete removal from surfaces, its foaming capabilities can make rinsing difficult.

The use of thixotropic gels has recently been introduced to further extend contact time over foams and improve rinsability. Gels are typically fluid at high and low concentrations but become thick and gelatinous at a concentration of around 5–10%. They are easily mixed and applied through high and low pressure systems, foaming equipment, or portable electrically pumped units (Fig. 5), remain on surfaces for extended periods (hours) and are easily diluted and rinsed away with high or low pressure water. Gels are usually more expensive than foams however.

The proportions of input from each energy source for a range of open surface cleaning techniques are shown in Figure 6. The figure illustrates the various methods' abilities to satisfactorily remove both light and heavy soiling from surfaces.

High pressure-low volume water systems, in which water is typically pumped at pressures up to 100 bar through a 15° nozzle, are widely used in the food industry. Water jets confer high mechanical energy, can be used on a wide range of equipment and environmental surfaces, are not limited to flat surfaces, will penetrate into surface irregularities, and can mix and apply sanitation chemicals. Soil removal does not necessarily increase linearly with pressure, as illustrated for a bacterial soil in Table 3, and may decrease. This is related to the water droplet size emerging from the nozzle, for which there will be an optimum size and hence impingement, for each soil type (24). Droplet size reduces with increasing pressure and pressures of around 50 bar are satisfactory for many operations. Care must be taken when using pressure washers as they are able to transfer soil and bacteria from one surface to another over large distances and may damage electrical installations or other delicate equipment.

For the cleaning of floors and other predominantly flat surfaces eg, walls, equipment that produces a mechanical scrubbing action may be used. Such equipment may include water-driven attachments to high pressure systems, electrically operated small diameter (23 cm) brushes, and floor scrubbers (Fig. 5). With these techniques, high mechanical input is combined with chemical energy after which surfaces may be rinsed with low pressure water. Larger and more sophisticated scrubber dryers, or floor automats, have a squeegee and vacuum unit mounted behind the scrubbing brush(es) to suck up the resulting soil and detergent emulsion. These devices provide a rapid and effective clean, and produce a dry surface that is safer and less conducive to microbial growth but can only be used efficiently in food processing areas designed for their use. All mechanical scrubbing units should be cleaned and disinfected after use to avoid cross-contamination.

Enclosed Surfaces, CIP

This section gives a brief and general introduction to CIP and readers are directed to the specific article on CIP in

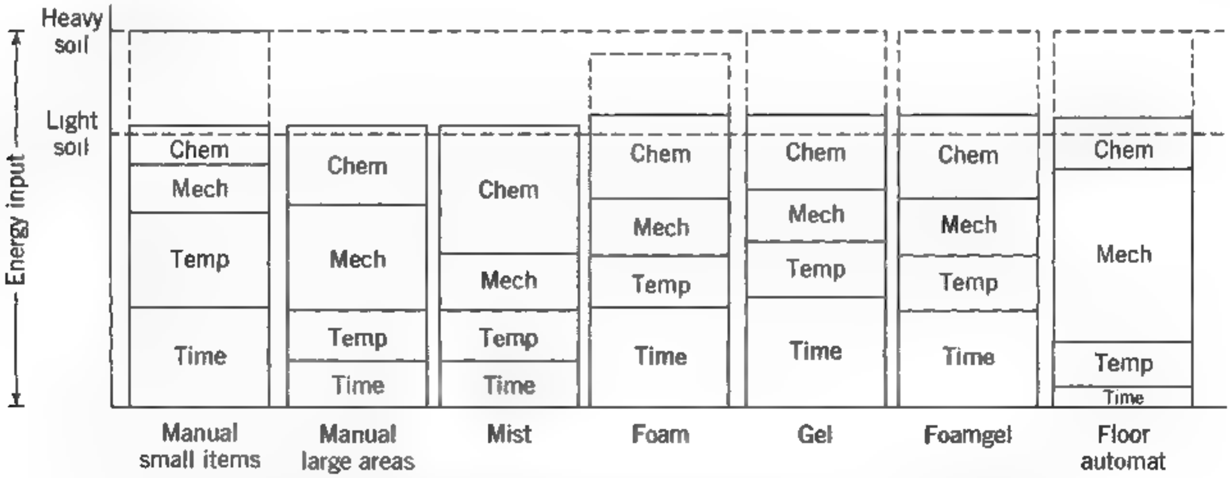


Figure 6. Relative energy inputs for a range of cleaning techniques. The dotted lines above each technique represent their abilities to cope with heavy soiling (13).

Table 3. The Effect of Water Pressure on Spray Cleaning Efficiency

Pressure (bar)	2	25	50	75	98
Microorganisms remaining cm^2 (as measured by DEM)	2.9×10^5	3.1×10^4	1.9×10^4	7.7×10^3	3.3×10^4

this encyclopedia, for further information. As a technique, CIP is well established, especially in liquid food processing, and is achieved by the sequential circulation of water, detergents, and disinfectants through processing equipment that remains assembled. Mechanical or kinetic energy is provided by turbulent flow in pipes and jet impingement in tanks and vessels. CIP systems are best employed to clean process equipment that has been designed specifically for CIP cleaning and should supply detergents at the correct temperature, concentration, and velocity in the right place for the required contact time.

It is fundamental with enclosed systems that hygienic design and product safety from sanitation chemicals is addressed. Attention must be paid to hygienic design so that areas where product and microorganisms can lodge, out of the flow of cleaning chemicals and disinfectants, are eliminated (3). Special devices should be built into the system, eg, key pieces, flow plates, swing bends, and electrical interlocks to ensure that product contamination by CIP solutions cannot occur, and a single valve must never be relied upon to safeguard product (30).

There are three main types of CIP systems: single use, reuse, and multiuse. Single-use systems are the most basic and are so called because the detergent is only used once and then drained. A simple system is shown in Figure 7. The system can be located close to the equipment to be cleaned, to eliminate long pipe runs, and is low on capital cost. It may be operated automatically or manually and the final rinse may be collected to use as the initial rinse in the following cleaning cycle. Although it is expensive on detergent, a single-use system may be the only system applicable if the product soil to be removed is such that detergent contamination occurs to a degree that detergent recovery is impractical.

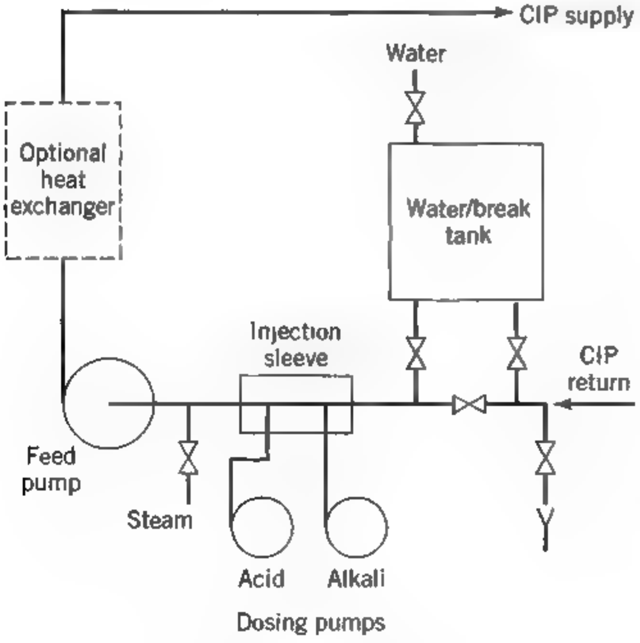


Figure 7. A simple, single-use CIP system.

Reuse systems are generally more complex and may be used to clean more than one process. They are designed to recover the cleaning fluids for reuse and are best employed where product soil is light or where much of the soil can be removed with the prerinse. Reuse systems are normally run automatically with programmed cleaning cycles for each circuit and there is, therefore, the need for timing, temperature and dosing control equipment, filters, storage vessels, and recording instrumentation. With large installations, the CIP unit can be placed out of the process area, or centralized, which may be advantageous for some applications. This can, however, lead to extensive pipe runs, which require large solution volumes and are prone to heat loss and expansion problems. This can be partially overcome in decentralized systems in which the detergent solution is mixed, heated, and distributed centrally, but rinse waters and other chemicals are heated and/or mixed and supplied close to the process equipment to be cleaned.

Multiuse systems combine the best design features of both single-use and reuse systems and are used where versatility in temperature, cleaning fluid type, concentration, and the ability to recover or drain cleaning fluids are required.

As a cleaning technique, CIP has many advantages. Cleaning schedules are adhered to automatically and use optimum cleaning fluids, concentrations, and temperatures to help achieve consistently satisfactory results. Processes can be cleaned as soon as production finishes to reduce down time and the life of equipment is extended due to reduced dismantling. Manual input can be reduced and operator safety increased through less contact with hazardous materials or work places. The major disadvantages are initial capital cost and the inflexibility of some systems if manufacturing processes are altered. Russel (31) reviewed some of the difficulties in cleaning processing equipment and emphasized the importance of designing safe, easy-to-use cleaning equipment as well as safer sanitation chemicals. More automated CIP systems will probably be introduced to reduce human error.

EVALUATION OF EFFECTIVENESS OF SANITATION SYSTEMS

As with other important aspects of safe and wholesome food production, sanitation programs require regular evaluation of their effectiveness as part of a specified quality assurance system. Traditionally, this has been undertaken on two levels—an immediate assessment of the performance of a sanitation program by sensory evaluation and a historical measurement of microbial surface populations if surfaces are visibly clean. Sensory evaluations are used as a process control to rectify immediately obviously poor sanitation. Bacterial evaluations may be used to optimize sanitation procedures and ensure compliance with microbial standards and in hygiene inspection and troubleshooting exercises. Recently, rapid methods have been developed to assess microbial surface populations in a time relevant to process control and in this context, only techniques that provide estimates in less than 15–20 min are considered.

As with the inherent faults of end product analysis in describing production quality, reliance should not be placed on assessment techniques that can only sample a very small proportion of plant surfaces. Assessment techniques should, rather, be designed to monitor the effectiveness of an integrated HACCP-type (Hazard Analysis and Critical Control Point) approach to sanitation in which such critical control points may include detergent and disinfection concentration, solution temperature, application procedures, and chemical stock rotation.

Sensory evaluation involves visual inspection of surfaces under good lighting, feeling for greasy or encrusted surfaces, and smelling for product and/or offensive odors. If these assessments indicate the presence of product residues, no further analysis is required and microbiological examination will be misleading. If no product residues are detected, microbiological techniques may be applied. All of the microbiological techniques appropriate for food factory use involve the sampling of microorganisms from contaminated surfaces and their culture using standard agar plating methods. Microorganisms may be collected via cotton or alginate swabs (after which they are resuspended by vortex mixing or dissolution), water rinses for larger enclosed areas, or directly using agar contact plates. Traditional techniques have been reviewed (32,34) since which time few improvements have occurred other than with contact methods which are now available commercially as premade sampling units.

Traditional microbiological techniques all require a minimum of 24–48 h to provide results and these only provide a historical view of the efficiency of sanitation programs. Rapid methodology was devised to ascertain sanitation efficiency and for some products, when to clean, in a time relevant to process control. The two most common techniques are epifluorescent microscopy and the ATP technique. With epifluorescent microscopy, microorganisms may be sampled by swabbing and filtering the resuspension media or filtering rinses (direct epifluorescent filter technique, DEFT) or by enumerating the surface of coupons attached to product contact surfaces (direct epifluorescent microscopy, DEM). After swabbing, estimates may be made of soil and/or microorganisms by analysis of total or microbial adenosine triphosphate (ATP) levels. ATP bioluminescence provides a reliable and rapid alternative to traditional microbiological methods (35). The efficiency and flexibility of this method makes it a unique hygiene-monitoring system for food plants (36). A recent study by Green et al. (37) showed that cleaning agents and sanitizers can affect ATP bioluminescence measurements differently. Consequently, it is important to select carefully the type and concentration of chemical cleaner or sanitizer used to clean the processing equipment when using this method to monitor hygiene. In the majority of practical applications, where an assessment of cleaning is required, analysis for total ATP is preferred on the assumption that any residues, soil or microorganisms, should have been removed. The use of DEM and DEFT has been described for assessing open surface hygiene (12,38), rinses (39), and the use of ATP (40,41).

The accuracy of a range of surface hygiene assessment techniques as compared to the most accurate method, DEM, has been described (42), and is shown in Table 4. The table shows the ability of a number of commercial contact, self-prepared (DIY) contact, traditional swabbing and rapid techniques (ATP and DEFT) to predict a DEM count of 10^4, 10^6 and 10^8 bacteria/cm^2. The results indicate that only above 10^6 bacteria/cm^2 are these methods accurate and they are therefore useful for indicating gross contamination only. For cotton swabs, the most widely used method in practice, a result of zero is more likely than a result of 10^4 when assessing surface populations of 10^4 bacteria/cm^2. The rapid methods available are at least as accurate as traditional methods and as shown in Table 5, which compiles a range of attributes for typical assessment methods, the choice between rapid and traditional techniques is a balance of cost and speed of result.

EVALUATION OF THE EFFECTIVENESS OF DISINFECTION

The effectiveness of a disinfectant can only be undertaken by assessing viable microbial levels before and after exposure. Ideally, this should be carried out in the field, but due to problems with reproducibility and scale (assessing only a small area of the total plant), this may take many weeks before satisfactory results are achieved. Disinfectant testing is, therefore, usually undertaken in the laboratory under strictly defined conditions to enable reproducible and rapid results.

At their simplest, disinfectant test methods consist of adding a known concentration of disinfectant to a known inoculum of bacteria for a defined time and temperature period and assessing surviving bacterial numbers. Microorganism survival may be assessed by inoculating a sample of the disinfectant/microorganism mixture into traditional culture medium and enumerating any microcolonies produced or by the use of a disinfectant concentration/microorganism mixture dilution range and recording the disinfectant dilution that just prevents microbial growth. These disinfectant tests, referred to as suspension tests, are simple to undertake but can only be used to screen potential products for disinfectant action or to confirm the biocidal abilities of known disinfectants as a quality control procedure.

More extensive suspension test methodologies are available that simulate some of the conditions under which disinfectants may have to work. Such conditions may include the presence of soil, different contact times, alternative temperatures, a variety of bacterial types and the degree of water hardness (43). The results of suspension tests have been used to ascertain recommended in-use dis-

Table 4. Accuracy in the Estimation of Surface Microbial Populations for Various Hygiene Assessment Methods as Compared to DEM

		Prediction of a DEM count (cm^2)			
Method used	Repeats	10^4	10^6	10^8	$2SD^2$
Commercial contact A	58	3.42	6.18	8.94	±1.11
DEFT swab	108	2.06	4.46	6.86	±1.22
Commercial contact B	30	3.57	6.01	8.45	±1.77
ATP swab	31	2.36	4.66	6.96	±2.22
DIY contact	30	4.76	6.15	7.55	±2.33
Commercial contact/swab	17	1.50	3.04	4.59	±2.81
Cotton swab	70	1.47	4.41	7.35	±2.88
Alginate swab	70	0.61	3.55	6.49	±4.06
Commercial contact C	46	2.81	5.35	7.89	±5.54

Table 5. Attributes of a Range of Surface Hygiene Assessment Techniques

Attribute commercial	DEM	DEFT Swab	ATP Swab	TVC Swab	CONTACT DIY	CONTACT
Analysis for	Total count	Viable/total	Viable/soil	Viable count	Viable count	Viable count
Preparation time	5 min	15 min	10 min	1 h	1h	1 min
Test time	5–10 min	15 min	2 min	25 min	1 min	1 min
Surface requirement	Coupon	Any	Any	Any	Flat only	Flat only
Field application	Research Yes	Yes	Yes	Yes	Yes	Yes
Degree of competence	Micro training	Micro training	Basic training	Basic micro	Basic micro	Little/none
Facilities required	UV microscope	UV microscope	Luminometer (portable)	Basic lab	Basic lab	Incubator
Speed of result	<5 min	<5 min	<1 min	>1–2 days	>1–2 days	>1–2 days
Test cost	50p/$1	50p/$1	£1/$2	50p/$1	50/$1	£1–4/$2–8
Initial cost	£2–5,000 $4–10,000	£3–15,000 $6–30,000	£2.5–10,000 $5–20,000	Low	Low	Very low

infectant concentrations, but in reality they only highlight conditions under which disinfectants that have failed should not be used.

Through the auspices of national standard organizations, many countries have standard disinfectant testing procedures, including Australia, New Zealand, South Africa, France, Germany, The Netherlands, the UK, and the United States (44). There is little standardization between countries, however, and to address this issue, with the planned harmonization of Europe in 1992, the European Suspension Test (EST) (45) was published with some agreement between European countries. To pass the EST, a disinfectant must reduce the viability of 5 test organisms by 5 log orders in 5 min at both low (0.03% bovine albumin) and high (1.0% bovine albumin) soil levels. Results for two examples each of a range of disinfectant types are shown in Table 6. These results illustrate a number of points including: the relative resistance of microbial species, the variation of disinfectant action within a single microbial species, the influence of organic load and hence the necessity to thoroughly clean prior to disinfection in practice, and the range of biocidal action between and within product types. In its early stages of development, a collaborative trial in 10 European countries found the EST to be sufficiently repeatable and reproducible to be adopted internationally (46).

Microorganisms are not primarily found in the food production environment in suspension. In an attempt to more closely simulate an in-use conditions surface, tests have been developed in which microorganisms are dried onto surfaces prior to disinfection (47–49). Although these tests provide a stronger challenge to the disinfectant (50), there are problems with the tests in that the drying process may alter microbial resistance, it is difficult to remove a consistent proportion of microorganisms for enumeration after disinfection and again, microorganisms are rarely dried onto surfaces in practice. A surface test has recently been described (51) in which disinfectants are tested against bacterial biofilms developed on stainless steel samples and in which the viability of the biofilm was assessed, while surface bound using a Malthus microbiological growth analyser (Malthus Instruments Ltd. (Radiometer), UK). The relative resistance of bacteria when attached to surfaces as compared to in suspension is shown in Table 7. A range of disinfectants was tested under the conditions of the EST at their manufacturers' recommended concentration (MRC) for bacteria in suspension and at the MRC and 10 and 100 times this concentration, when attached to a surface. The results showed that bacteria were 10 to 100 times more resistant to biocides when attached to a surface and that biocides that performed well in suspension did not necessarily perform well on surfaces. Care should therefore be exercised in transferring the results from suspension tests to in-use applications.

FUTURE DEVELOPMENT

Sanitation is likely to feature much more prominantly in the eyes of management in the future due to their desire

Table 6. European Suspension Test Results for a Range of Commonly Used Disinfectants Tested at their Manufacturers Recommended Concentrations (MRC)

			Log reduction					
Product	MRC(%)	Organic Load (%)	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Staphylococcus aureus*	*Enterococcus faecium*	*Saccharomyces cerevisiae*	Result
Peracid	0.1	0.03	7, 6	7, 7	7, 7	6, 5	5, 1, 1	Fail
		1.0	7, 1, 5	7, 6, 7	7, 1, 6	6, 0, 5	5, 0, 0	Fail
Peracid	0.1	0.03	5, 5	7, 7	7, 7	7, 7	>4, 5	Pass
		1.0	5, 5	7, 7	6, 7	7, 7	>4, 5	Pass
HOCl	0.25	0.03	7, 7	7, 7	7, 6, 6	5, 6	4, 5, 2	Fail
		1.0	4, 3	2, 2	2, 2	0, 0	3, 5, 3	Fail
HOCl	0.3	0.03	6, 6	5, 4, 4	6, 5	6, 6	>4, 3	Fail
		1.0	3, 3	0, 3, 2	1, 4	2, 2	0, 0	Fail
QUAT	0.2	0.03	2, 2	3, 5, 4	7, 7	6, 5	5, 3, 4	Fail
		1.0	2, 2	3, 1	7, 6	5, 5	4, 1	Fail
QUAT	1.0	0.03	5, 6	6, 5	5, 7	5, 5	5, 5	Pass
		1.0	5, 7	5, 6	7, 7	4, 6, 6	4, 5, 5	Pass
Biguanide	0.5	0.03	2, 5, 5	2, 5, 5	2, 6, 7	3, 6, 7	5, 4	Pass
		1.0	2, 1	2, 1	2, 2	1, 1	2, 1	Fail
Biguanide	0.5	0.03	6, 6	5, 6	7, 5	7, 7	1, 2	Fail
		1.0	6, 6	4, 3	4, 4	7, 7	1, 1	Fail
Iodophore	0.2	0.03	7, 7	7, 7	7, 6	7, 6	4, 4	Fail
		1.0	1, 1	3, 2	4, 2	1, 1	0, 0	Fail
Iodophore	1.0	0.03	4, 4	7, 7	5, 5	3, 4	2, 3	Fail
		1.0	2, 3	3, 5, 5	5, 5	3, 3	1, 1	Fail
Amphoteric	1.0	0.03	5, 5	4, 7, 7	7, 7	6, 7	4, 5, 5	Pass
		1.0	4, 5, 7	6, 4, 7	7, 7	4, 7, 6	2, 5, 3	Fail
Amphoteric	1.0	0.03	7, 6	7, 7	7, 7	6, 6	5, 5	Pass
		1.0	7, 6	6, 6	7, 7	6, 6	5, 5	Pass

to seek increased standards of hygiene. These have arisen from:

1. The realization of the importance of environmental routes of infection to product for both microbial and foreign body contaminants.
2. Pressure from customers and consumers for higher standards of hygiene.
3. Trends in food production towards short shelf-life products that intrinsically demand higher standards of hygiene.
4. The ability to sell product of a low microbiological load at a premium.
5. Increased plant size, where single mistakes will incur large financial losses.
6. Legislation.

Demand for increased standards of hygiene could lead to further advances in rapid methodology to determine surface hygiene in a time relevant to process control, chemical testing schemes that more closely simulate in-use conditions, improved application techniques that are more efficient and reduce cross-contamination via cleaning methods, the design of equipment and processing areas with more thought to ease of cleanability, and the increased use of automation and/or robotics for both production and sanitation operations.

Other trends may be influenced by environmental and energy restrictions. This will lead to the use of chemicals that leave no undesirable environmental residues. For ex-

Table 7. The Relative Resistance of Bacteria Attached to a Surface (Malthus) and in Suspension (EST)

		Result			
		EST		Malthus	
Product type	Conc. (%)	0.03	1.0	0.03	1.0
Peracid	0.1	Pass	Pass	Pass	Fail
	1.0			Pass	Pass
	10.0			Pass	Pass
Iodophore	0.2	Pass	Fail	Fail	Fail
	2.0			Pass	Pass
	20.0			Pass	Pass
Biguanide	0.5	Pass	Fail	Fail	Fail
	5.0			Pass	Fail
	50.0			Pass	Pass
HOCl	0.25	Pass	Pass	Fail	Fail
	2.25			Fail	Fail
	25.0			Pass	Pass
Amphoteric	1.0	Pass	Pass	Fail	Fail
	10.0			Pass	Fail
QUAT	1.0	Pass	Pass	Fail	Fail
	10.0			Fail	Fail

Note: For both the EST and Malthus tests, three organisms were used—*Pseudomonas aeruginosa, Proteus mirabilis,* and *Staphylococcus aureus;* a pass criteria of a 5 log reduction in 5 min for all three organisms was used.

ample, the formation of chemical by-products when chlorine is used as a disinfectant remains a great concern. Chlorination of drinking water has been shown to produce a number of cancer-causing chemicals (52). Consequently, there is a definite trend to increase the use of alternative disinfectants such as ozone, chlorine chloride, and chloramine for treating drinking water as they have fewer chlorinated disinfectant by-products (53). Further trends will include a reduction in water usage, and an associated move towards dry cleaning methods such as ring-main vacuum lines, and as for hygiene reasons, more efficient and less manual sanitation application techniques.

BIBLIOGRAPHY

1. T. H. Roels et al., "Incomplete Sanitation of a Meat Grinder and Ingestion of Raw Ground Beef: Contributing Factors to a Large Outbreak of *Salmonella typhyrium* Infection," *Epidemiology and Infection* **119**, 127–134 (1997).
2. M. Setiabuhdi, M. Theis, and J. Norback, "Integrating Hazard Analysis and Critical Control Point (HACCP) and Sanitation for Verifiable Food Safety," *Journal of the American Dietetic Association* **97**, 889–891 (1997).
3. "Hygienic Design of Equipment for Open Food Processing," *Trends Food Sci. Technol.* **6**, 305–310 (1995).
4. Technical Manual No. 7, *Hygienic Design of Food Processing Equipment*, Campden Food and Drink Research Association, Chipping Campden, UK, 1983.
5. Technical Manual No. 8, *Hygienic Design of Post Process Can Handling Equipment*, Campden Food and Drink Research Association, Chipping Campden, UK, 1985.
6. Technical Manual No. 17, *Hygienic Design of Liquid Handling Equipment for the Food Industry*, Campden Food and Drink Research Association, Chipping Campden, UK, 1987.
7. R. C. W. Berkeley, J. M. Lynch, P. R. Rutter, and B. Vincent, *Microbial Adhesion to Surface*, Ellis Horwood Ltd., Chichester, UK, 1980
8. K. C. M. Marshall, *Microbial Adhesion and Aggregation*, UK, Springer-Verlag, Berlin, 1984.
9. C. K. Bower, J. McGuire, and M. A. Daeschel, "The Adhesion and Detachment of Bacteria and Spores on Food-contact Surfaces," *Trends Food Sci. Technol.* **7**, 152–157 (1996).
10. S. Bouman, D. B. Lund, F. M. Driessen, and D. G. Schmidt, "Growth of Thermoresistant *Streptococci* and Deposition of Milk Constituents on Plates of Heat Exchangers during Long Operating Times," *Journal of Food Protection* **45**, 806–812 (1982).
11. S J. Lewis and A. Gilmour, "Microflora Associated with the Internal Surfaces of Rubber and Stainless Steel Milk Transfer Pipeline," *Journal of Applied Bacteriology* **62**, 327–333 (1987).
12. J. T Holah, R. P. Betts and R. H. Thorpe, "The Use of Epifluorescence Microscopy to Determine Surface Hygiene," *International Biodeterioration* **25**, 147–153 (1989).
13. M. T. Offiler, "Open Plant Cleaning: Equipment and Methods," in *Proceedings of Hygiene for the 90s* Campden Food and Drink Research Association, Chipping Campden, UK, November 7–8, 1990
14. D. A. Timperley, "The Effect of Reynolds Number and Mean Velocity of Flow on the Cleaning-in-Place of Pipelines," in *Proceedings of Fundamentals and Applications of Surface Phenomena Associated with Fouling and Cleaning in Food Processing*, Lund University, Lund, Sweden, April 6–9, 1981.
15. D. G. Dunsmore, "Bacteriological Control of Food Equipment Surfaces by Cleaning Systems. 1. Detergent Effects," *Journal of Food Protection* **44**, 15–20 (1981).
16. W. L. Shupe, J. S. Bailey, W. K. Whitehead, and J. E. Thompson, "Cleaning Poultry Fat from Stainless Steel Flat Plates," *Transactions of the American Society of Agricultural Engineers* **25**, 1446–1449 (1982).
17. M. E. Anderson, H. E Huff, and R. T. Marshall, "Removal of Animal Fat from Food Grade Belting as Affected by Pressure and Temperature of Sprayed Water," *Journal of Food Protection* **44**, 246–248 (1985).
18. N. E. Middlemiss, C. A. Nunes, J E Sorensen, and G. Paquette, "Effect of a Water Rinse and a Detergent Wash on Milkfat and Milk Protein Soils," *Journal of Food Protection* **48**, 257–260 (1985).
19. D. A. Timperley and C. N. M. Smeulders, "Cleaning of Dairy HTST Plate Heat Exchangers: Optimisation of the Single-Stage Procedure," *Journal of the Society of Dairy Technology* **41**, 4–7 (1965).
20. W. G. Jennings, "Theory and Practice of Hard-Surface Cleaning," *Advances in Food Research* **14**, 325–459 (1965).
21. H. J. Schlussler, "Zur Kinetik von Reinigungsvorgangen an festen Oberflachen," Symposium uber *Reinigen und Desinfizieren lebensmittel verarbeitender Anlagen*, Karlsruhe, West Germany, 1975.
22. M. Loncin, "Modelling in Cleaning, Disinfection and Rinsing," in *Proceedings of Mathematical Modelling in Food Processing*, Lund Institute of Technology, Lund, UK, 7–9 September, 1977.
23. D. G. Dunsmore, A. Twomey, W. G. Whittlestone, and H. W. Morgan, "Design and Performance of Systems for Cleaning Product Contact Surfaces of Food Equipment: A Review," *Journal of Food Protection* **44**, 220–240 (1981).
24. R. P. Elliott, "Cleaning and Sanitation," in *Principals of Food Processing Sanitation*, A. M. Katsuyama, ed., The Food Processors Institute, USA, 1980.
25. H. Mrozek, "Development Trends with Disinfection in the Food Industry," *Deutsche Molkerei-Zeitung* **12**, 348–352 (1982).
26. J. E Frank and R. A. N. Chmielewski, "Effectiveness of Sanitation with Quaternary Ammonium Compound or Chlorine on Stainless Steel and Other Domestic Food-Preparation Surfaces," *J. Food Prot.* **60**, 43–47 (1997).
27. R. Klemperer, "Tests for Disinfectants: Principles and Problems," in *Disinfectants: Their Assessment and Industrial Use*, Scientific Symposia Ltd., London, 1982.
28. S. F. Bloomfield and E. Scott, "Cross-Contamination and Infection in the Domestic Environment and the Role of Disinfectants," *J. Appl. Microbiol.* **83**, 1–9 (1997).
29. M. Best, M. E. Kennedy, and F. Coates, "Efficacy of a Variety of Disinfectants Against *Listeria* spp.," *Appl. Environ. Microbiol.* **56**, 377–380 (1990).
30. D. A. Timperley, "Cleaning in Place (CIP)," *Journal of the Society of Dairy Technology* **42**, 32–33 (1989).
31. M. Russell, "Coming Clean on Sanitation," *Food Engineering* **66**, 104–106 (1994)
32. J. T. Patterson, "Microbiological Assessment of Surfaces," *Journal of Food Technology* **6**, 63–72 (1971).
33. J. D. Baldock, "Microbiological Monitoring of the Food Plant: Methods to Assess Bacterial Contamination on Surfaces," *Journal of Milk Food Technology* **37**, 361–368 (1974).
34. S. M. Kulkarni, R. B. Maxy, and R. G. Arnold, "Evaluation of Soil Deposition and Removal Processes: An Interpretive View," *Journal of Dairy Science* **58**, 1922–1936 (1975).

35. J. M. Hawronskyj and J. Holah, "ATP: A Universal Hygiene Monitor," *Trends Food Sci. Technol.* **8**, 79–84 (1997).
36. L. S. Madl, "Sanitation in a Flash, ATP Bioluminescence Technology Makes its Way into Food Plants," *Food Processing* **58**, 100 (1997).
37. T. A. Green, S. M. Russell, and D. L. Fletcher, "Effect of Chemical Cleaning Agents and Commercial Sanitizers on ATP Bioluminescence Measurements," *J. Food Prot.* **62**, 86–90 (1999).
38. J. T. Holah, R. P Betts, and R. H. Thorpe, "The Use of Direct Epifluorescent Microscopy (DEM) and the Direct Epifluorescent Filter Technique (DEFT) to Assess Microbial Populations on Food Contact Surfaces," *Journal of Applied Bacteriology* **65**, 215–221 (1988).
39. C. H. McKinon and R. Mansell, "Rapid Counting of Bacteria in Rinses of Milking Equipment by a Membrane Filtration Epifluorescent Microscopy Technique," *Journal of Applied Bacteriology* **51**, 363–367 (1981).
40. P. Thompson, "Rapid Hygiene Analysis Using ATP Bioluminescence," *European Food and Drink Review*, 42–48 (Spring 1989).
41. W. J. Simpson, "Instant Assessment of Brewery Hygiene Using ATP Bioluminescence," *Brewers Guardian* **118**, 20–22 (1989).
42. J. T. Holah, "Monitoring the Hygienic Status of Surfaces," in *Proceedings of Hygiene for the 90s*, Campden Food and Drink Research Association, Chipping Campden, UK, 1990.
43. A. D. Russel, "Factors Influencing the Efficiency of Antimicrobial Agents," in A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe, eds., *Principles and Practice of Disinfection*, Blackwell Scientific Publications, London, 1982
44. *Review of Worldwide Disinfectant Test Methods*. British Association for Chemical Specialties, Sutton, UK, 1989.
45. Test Methods for the Antimicrobial Activity of Disinfectants in Food Hygiene. Council of Europe, Strasbourg, 1987.
46. B. Van Klingeren, A. B. Leussink, and W. Pullen, "A European Collaborative Study on the Repeatability and the Reproducibility of the Standard Suspension Test for the Evaluation of Disinfectants in Food Hygiene," *Report of the National Institute of Public Health and Environmental Hygiene*, No. 35901001, 1981.
47. *Tube Test for the Evaluation of Detergent-Disinfectants for Dairy Equipment*. International Standard FIL-1DF44. Brussels. International Dairy Federation, 1967.
48. "AOAC Use-Dilution Method," in S. Williams, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th Edition, Association of Official Analytical Chemists, Inc., Arlington, Va., 1984.
49. *AFNOR T72-190 Germ-Carrier Method*. Paris: l'Association Française de Normalisation, 1986.
50. S. F. Bloomfield, "Disinfectant Testing in Relation to the Food Industry," in *Proceedings of Hygiene for the 90s*, Campden Food and Drink Research Association, Chipping, Campden, UK, 1990
51. J. T. Holah, C. Higgs, S. Robinson, D. Worthington, and H. Spenceley, "A Conductance-based Surface Disinfectant Test for Food Hygiene," *Letters in Applied Bacteriology* **11**, 255–259 (1990).
52. S. D. Richardson, "Drinking Water Disinfection By-products," in R. A. Meyers, ed., *The Encyclopedia of Environmental Analysis and Remediation*, Vol 3, John Wiley & Sons, New York, 1998, pp. 1398–1421.
53. S. D. Richardson et al., "Chemical By-products of Chlorine and Alternative Disinfectants," *Food Technol.* **52**, 58–61 (1998).

GENERAL REFERENCES

P. R. Hayes, *Food Microbiology and Hygiene*, Elsevier Applied Science Publishers, New York, 1985.

A. M. Katsuyama, *Principles of Food Processing Sanitation*, The Food Processors Institute, Washington, D.C., 1980.

N. G. Marion, *Essentials of Food Sanitation*, Chapman and Hall, London, 1997.

N G. Marriot, *Principles of Food Sanitation*, AVI Publishing Co., Westport, Conn., 1985

A. J. D. Romney, *Cleaning in Place*, The Society of Dairy Technology, Huntingdon, Cambridgeshire, U.K., 1990.

FOOD TOXICOLOGY

Food toxicology is a specialized area of the discipline of toxicology that deals with toxic substances in foods. It is the study of the nature, sources, and formation of toxic substances in foods, their deleterious effects on consumers, the manifestations and mechanisms of these effects, and the identification of the limits of the safety and thus the regulation of these substances. Because the public is concerned about the quality and safety of foods, it is important to understand the principles of food toxicology and food safety. In this article, the general principles of toxicology are presented before the discussion of food toxicology.

TOXICOLOGY: PRINCIPLES, DEFINITIONS, AND SCOPE

Toxicology is the study of the adverse effects of substances on living organisms. It is a multidisciplinary field of study dealing with the detection, occurrence, properties, effects, and regulations of toxic compounds. It therefore involves an understanding of chemical reactions as well as biological mechanisms of toxic actions.

A toxicological study usually consists of four elements: (*1*) a chemical agent capable of causing a deleterious response; (*2*) a biological system with which the chemical agent may interact to produce the deleterious response; (*3*) a means by which the chemical agent and the biological system are permitted to interact; and (*4*) a response that can be used to quantitate the deleterious effect on the biological system.

Two aspects of interaction between substances and living organisms are of importance: the influence of the substances on the living organism and the influences of the organism on the substances. The chemical agent capable of causing a deleterious effect in the organism is defined as a poison or toxicant. A toxicant will exert toxicity, which is defined as the capacity to produce toxic injury to cells or tissues, only at appropriate conditions when the biological system is exposed to a certain dose of the toxicant. In effect, Paracelsus (1493–1541) noted, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." Thus, as a rule, a substance is a toxicant only in toxic doses; virtually any substance, even pure water or sugar, is poisonous when taken in great excess. The capability to detect subtoxic levels of toxicants in biological system, such as in the plasma or urine, is of particular importance because, once known,

35. J. M. Hawronskyj and J. Holah, "ATP: A Universal Hygiene Monitor," *Trends Food Sci. Technol.* **8**, 79–84 (1997).
36. L. S. Madl, "Sanitation in a Flash, ATP Bioluminescence Technology Makes its Way into Food Plants," *Food Processing* **58**, 100 (1997).
37. T. A. Green, S. M. Russell, and D. L. Fletcher, "Effect of Chemical Cleaning Agents and Commercial Sanitizers on ATP Bioluminescence Measurements," *J. Food Prot.* **62**, 86–90 (1999).
38. J. T. Holah, R. P Betts, and R. H. Thorpe, "The Use of Direct Epifluorescent Microscopy (DEM) and the Direct Epifluorescent Filter Technique (DEFT) to Assess Microbial Populations on Food Contact Surfaces," *Journal of Applied Bacteriology* **65**, 215–221 (1988).
39. C. H. McKinon and R. Mansell, "Rapid Counting of Bacteria in Rinses of Milking Equipment by a Membrane Filtration Epifluorescent Microscopy Technique," *Journal of Applied Bacteriology* **51**, 363–367 (1981).
40. P. Thompson, "Rapid Hygiene Analysis Using ATP Bioluminescence," *European Food and Drink Review*, 42–48 (Spring 1989).
41. W. J. Simpson, "Instant Assessment of Brewery Hygiene Using ATP Bioluminescence," *Brewers Guardian* **118**, 20–22 (1989).
42. J. T. Holah, "Monitoring the Hygienic Status of Surfaces," in *Proceedings of Hygiene for the 90s*, Campden Food and Drink Research Association, Chipping Campden, UK, 1990.
43. A. D. Russel, "Factors Influencing the Efficiency of Antimicrobial Agents," in A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe, eds., *Principles and Practice of Disinfection*, Blackwell Scientific Publications, London, 1982
44. *Review of Worldwide Disinfectant Test Methods*. British Association for Chemical Specialties, Sutton, UK, 1989.
45. Test Methods for the Antimicrobial Activity of Disinfectants in Food Hygiene. Council of Europe, Strasbourg, 1987.
46. B. Van Klingeren, A. B. Leussink, and W. Pullen, "A European Collaborative Study on the Repeatability and the Reproducibility of the Standard Suspension Test for the Evaluation of Disinfectants in Food Hygiene," *Report of the National Institute of Public Health and Environmental Hygiene*, No. 35901001, 1981.
47. *Tube Test for the Evaluation of Detergent-Disinfectants for Dairy Equipment*. International Standard FIL-1DF44. Brussels. International Dairy Federation, 1967.
48. "AOAC Use-Dilution Method," in S. Williams, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th Edition, Association of Official Analytical Chemists, Inc., Arlington, Va., 1984.
49. *AFNOR T72-190 Germ-Carrier Method*. Paris: l'Association Française de Normalisation, 1986.
50. S. F. Bloomfield, "Disinfectant Testing in Relation to the Food Industry," in *Proceedings of Hygiene for the 90s*, Campden Food and Drink Research Association, Chipping, Campden, UK, 1990
51. J. T. Holah, C. Higgs, S. Robinson, D. Worthington, and H. Spenceley, "A Conductance-based Surface Disinfectant Test for Food Hygiene," *Letters in Applied Bacteriology* **11**, 255–259 (1990).
52. S. D. Richardson, "Drinking Water Disinfection By-products," in R. A. Meyers, ed., *The Encyclopedia of Environmental Analysis and Remediation*, Vol 3, John Wiley & Sons, New York, 1998, pp. 1398–1421.
53. S. D. Richardson et al., "Chemical By-products of Chlorine and Alternative Disinfectants," *Food Technol.* **52**, 58–61 (1998).

GENERAL REFERENCES

P. R. Hayes, *Food Microbiology and Hygiene*, Elsevier Applied Science Publishers, New York, 1985.

A. M. Katsuyama, *Principles of Food Processing Sanitation*, The Food Processors Institute, Washington, D.C., 1980.

N. G. Marion, *Essentials of Food Sanitation*, Chapman and Hall, London, 1997.

N G. Marriot, *Principles of Food Sanitation*, AVI Publishing Co., Westport, Conn., 1985

A. J. D. Romney, *Cleaning in Place*, The Society of Dairy Technology, Huntingdon, Cambridgeshire, U.K., 1990.

FOOD TOXICOLOGY

Food toxicology is a specialized area of the discipline of toxicology that deals with toxic substances in foods. It is the study of the nature, sources, and formation of toxic substances in foods, their deleterious effects on consumers, the manifestations and mechanisms of these effects, and the identification of the limits of the safety and thus the regulation of these substances. Because the public is concerned about the quality and safety of foods, it is important to understand the principles of food toxicology and food safety. In this article, the general principles of toxicology are presented before the discussion of food toxicology.

TOXICOLOGY: PRINCIPLES, DEFINITIONS, AND SCOPE

Toxicology is the study of the adverse effects of substances on living organisms. It is a multidisciplinary field of study dealing with the detection, occurrence, properties, effects, and regulations of toxic compounds. It therefore involves an understanding of chemical reactions as well as biological mechanisms of toxic actions.

A toxicological study usually consists of four elements: (*1*) a chemical agent capable of causing a deleterious response; (*2*) a biological system with which the chemical agent may interact to produce the deleterious response; (*3*) a means by which the chemical agent and the biological system are permitted to interact; and (*4*) a response that can be used to quantitate the deleterious effect on the biological system.

Two aspects of interaction between substances and living organisms are of importance: the influence of the substances on the living organism and the influences of the organism on the substances. The chemical agent capable of causing a deleterious effect in the organism is defined as a poison or toxicant. A toxicant will exert toxicity, which is defined as the capacity to produce toxic injury to cells or tissues, only at appropriate conditions when the biological system is exposed to a certain dose of the toxicant. In effect, Paracelsus (1493–1541) noted, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." Thus, as a rule, a substance is a toxicant only in toxic doses; virtually any substance, even pure water or sugar, is poisonous when taken in great excess. The capability to detect subtoxic levels of toxicants in biological system, such as in the plasma or urine, is of particular importance because, once known, further exposure can be avoided. In toxicology, exposure is the total amount of toxicants received by the biological system of interest. It can be expressed as the product of concentration and duration. A chemical agent does not produce toxic effects in a biological system unless that agent or its biotransformation products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce the toxic manifestation.

The route or site of exposure affects the toxicity of a chemical agent to the biological system. In food toxicology, the route of exposure is through the gastrointestinal tract (ingestion). The duration of animal exposure to toxicants is usually divided into four categories: acute, subacute, subchronic, and chronic. Acute exposure is defined as exposure to a toxic chemical for less than 24 hours. Although acute exposure usually refers to a single administration, repeated exposures may be given within a 24-hour period for some slightly toxic or practically nontoxic chemicals. Subacute exposure refers to repeated exposure to a toxic chemical for 1 month or less; subchronic exposure, to repeated exposure for 1 to 3 months; and chronic exposure, to repeated exposure for more than 3 months.

The LD_{50} of a compound is commonly reported as a measure of the toxicity of that compound. The LD_{50} can be defined as the dose of a compound that causes 50% mortality in a population. However, limitations of the LD_{50} as a measure of toxicity are often not recognized. The LD_{50} is an indicator of acute toxicity, using death as the end point, and therefore is not indicative of the effect of the compound under low dose or long-term exposure, which is usually the case for food toxicants. Chronic toxicity studies are needed to establish the effects of long-term or repeated administration of a compound. Chronic toxicity studies are used to determine the no observed effect level (NOEL) or no observed adverse effect level (NOAEL). From these studies, the reference dose (RfD) can be established. The RfD is defined as the maximum dose (mg/kg body weight) of an agent that is assumed to be without an adverse noncancer health impact on the human population (1).

The biological systems used for toxicity testing can be whole animals, including humans, dogs, and rodents, or they can be tissues or organs in culture, cell cultures, cell-free systems, eukaryotes (such as yeast and *Aspergillus*) and prokaryotes (such as *Salmonella typhimurium* and *Escherichia coli*), and plants. The age, sex, strain, and nutritional and disease status of the animal species all affect the outcome of the toxic effect. The use of enzyme inducers or inhibitors to modulate drug metabolism enzyme systems also affects animal susceptibility and toxicity to environmental chemicals. Whole animals are used to determine LD_{50} and organ target toxicity. They are also used to assess whether a compound is carcinogenic (ie, capable of inducing cancers) or teratogenic (ie, capable of inducing defects in the developing embryo). Insects are used in conjunction with pesticide development and for the detection of environmental mutagens (ie, causing a change in the DNA). Eukaryotes and prokaryotes are now widely used for the determination of mutagenic and carcinogenic potencies of environmental toxicants, drug impurities, and compounds present in foods. Currently, there is a trend in toxicology to use cell cultures as an alternative model system to animals. In this case, the TC_{50} (the toxic concentration that will induce poisonous effects to 50% of the cell population) is used to indicate the potential toxicity of the test compound. Cell-free systems are used to study the biochemical mechanism of toxicity.

Thus, the occurrence of a toxic response is dependent on the chemical and physical properties of the agent, the duration of exposure, and the susceptibility of the biological system or subject.

The single-most important factor that determines the potential harmfulness of a toxicant is the relationship between the concentration of the toxic agent and the effect produced in the biological system. This is referred to as the dose–response relationship. Toxic responses will not occur unless the chemical interacts with the target site(s). The degree of the response is related to the concentration of the agent at the reactive site, which in turn is related to the dose administered. In addition, the toxic response should be quantifiable.

A graphic expression of the typical dose–response relationship is shown in Figure 1. A sigmoidal response curve is obtained when the dosage is plotted on a logarithmic scale. The response may be applied to an individual, a system, or a fraction of a population; it ranges from 0 to 100%. The lowest dose of any toxicant that evokes a stated all-or-none response is called the threshold dose. Below this dose, there is no response. It is through the use of this dose–response relationship that the toxicologist is able to obtain the LD_{50} of a toxicant if mortality is used as an end point. See Reference 2 for a discussion of dose–response and LD_{50}.

After administration to a test animal, toxicants usually undergo a series of complex processes, including absorption, distribution, metabolism, and excretion, before they exert their toxic effects (Fig. 2). An overview of these processes can be found in References 3 and 4. During the ex-

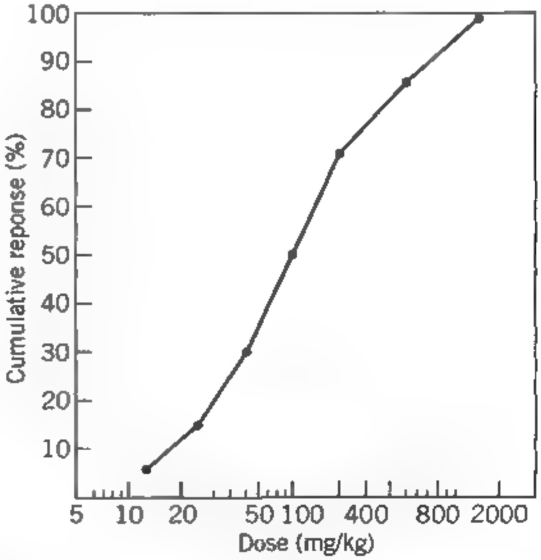


Figure 1. A typical sigmoid form of the dose-response relationship. Dosage is most often expressed as mg/kg and plotted on a log scale.

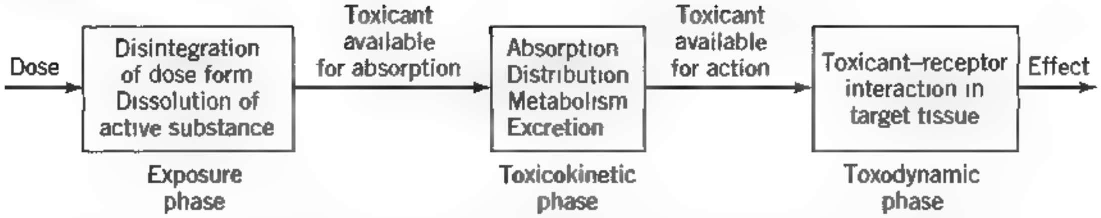

Dose
Disintegration of dose form Dissolution of active substance
Toxicant available for absorption
Absorption Distribution Metabolism Excretion
Toxicant available for action
Toxicant–receptor interaction in target tissue
Effect
Exposure phase
Toxicokinetic phase
Toxodynamic phase

Figure 2. The three phases of toxicant action: the exposure phase, the toxico-kinetic phase, and the toxodynamic phase.

posure phase, the toxicant may undergo chemical alteration to compounds that may be more or less toxic than the parent compound. For example, hydrolysis of esters can take place in the gastrointestinal tract through the action of intestinal microflora. In this connection, azo compounds can be reduced to the more toxic aromatic amines.

The toxicokinetic phase includes all the processes involved in the relationship between the effective dose of a toxicant and the concentration present at the various body fluid compartments and the target tissue. During the toxicokinetic phase, two types of processes play an important role:

1. *Distribution processes that involve absorption, distribution to the organs, and excretion*. Toxicants are transported and then may bind to protein carriers or tissue components. The principles of pharmacokinetics apply to this distribution process of toxicants.
2. *Biotransformation of toxicants*. This usually involves the bioactivation of the toxic agent. This metabolic biotransformation is accompanied by changes in chemical properties such as hydrophilicity and lipophilicity, which in turn affect their distribution in the organisms, the binding to macromolecules (such as proteins and DNA), and excretion.

Metabolism of toxicants mainly occurs in the liver but may also occur in other tissues, such as the lung, kidney, skin, and gonads. Through enzymatic biotransformation processes, the lipophilic compounds are converted to more water-soluble metabolites. Two types of enzymatic reactions are involved in toxicant metabolism: phase I reactions, which involve oxidation, reduction, and hydrolysis; and phase II reactions, which consist of conjugation reactions. Phase I reactions generally convert compounds to derivatives that are more water soluble than the parent molecule. The reactions occur mainly via two oxidative enzyme systems, the cytochrome P-450 system (the mixed-function oxygenase) and the mixed-function amine oxidase. More important than these particular conversions is that these two systems also add or expose functional groups such as —OH, —SH, —NH_2, and —COOH, which promote the compound's covalent conjugation with endogenous moieties such as glucuronic acid, sulfate, and amino acids through the actions of phase II reaction enzymes. These conjugated secondary metabolites possess increased water solubility and significant ionization properties at physiologic pH that in turn facilitate their secretion or transfer across hepatic, renal, and intestinal membranes.

The toxodynamic phase comprises the action of the toxicant molecules on the specific sites of action and the expression of the observed toxic effect. The target organ on which the toxicant acts and the effector organ in which the effect is induced, or on which the effect is observed, need not be identical. The concentration of the active toxicant metabolite reached in the target determines to what degree a biological action will be elicited. The toxic effect observed in the biological system can be the result of interference with the normal function of the enzyme systems; blockade of the oxygen transport by hemoglobin; interference with the general functions of the cell; interference with DNA, RNA, and protein synthesis; hypersensitivity reactions; and direct chemical irritation of tissues.

Many carcinogens undergo enzymatic activation to reactive ultimate carcinogens that are electrophilic and are capable of covalent interaction with cellular macromolecules, including DNA. In addition to these secondary carcinogens, there are also primary carcinogens that are reactive and do not require metabolic activation. If the damaged DNA is not repaired, the genome lesions are expressed in replicated cells that later will transform into abnormal cells. For a review of carcinogen metabolism, DNA adduct formation, and DNA repair, see reference 5. These abnormally altered or initiated cells may be removed through the process of programmed cell death (apoptosis) or may undergo proliferation to form preneoplastic lesions. The growth and progression of preneoplastic lesions into a neoplasm or cancer depends on the presence of promoting or inhibiting compounds or conditions in the animal's environment. For many types of cancers, dietary factors have been shown to play a major role in the promotion or inhibition of tumor development. For detailed discussions on carcinogenesis mechanisms, refer to References 5 and 6. The role of dietary factors in cancer development was summarized in Reference 7.

FOOD TOXICOLOGY

Food contains hundreds of thousands of substances, and most of these have not been characterized or tested. The recognition of the safety of these substances, at the level consumed in the diet, is based on a history of consumption of those foods. Substances added to foods that do not have this history, such as new food additives, must undergo specific toxicological testing to determine that the federal Food Drug and Cosmetic Act (FD&C Act) standard of "reasonable certainty of no harm" is met.

The toxicants present in food can be of biological origin, a direct or indirect food additive, a contaminant, or produced during cooking or processing. Exogenous factors, such as the nature of the compounds, the dose, the frequency of exposure, the route of exposure, the presence of other nutrients or drugs, and various environmental factors, affect the toxicity of food toxicants. Endogenous factors, such as the physiology and morphology of the gastrointestinal tract, the nature of the gastrointestinal microflora, and the metabolic activity of the body, can also affect toxicity. The intestinal microflora play important roles in inducing the formation of some toxicants, such as nitrosamines and cyanogenic glycosides. The toxicology of food compounds has been the subject of several books (8,9).

Foodborne Hazards of Microbial Origin

Foodborne disease agents are characterized by their diversity. Some produce their effects through toxic metabolites resulting from the growth of microorganisms in the food before ingestion and are classified as foodborne intoxications (eg, staphylococcal food poisoning and botulism). Others produce adverse effects through ingestion of living microorganisms and thus are called foodborne infections (eg, *Salmonella, Vibrio parahemolyticus, Listeria monocytogenes*, and *Clostridium perfringens* poisoning). A detailed description of these bacteria, their toxins, prevalence, and toxicity can be found in a review by Concon (10). Other pathogens are emerging as important public health threats, including *E. coli* O157:H7, *Campylobacter jejuni*, and *Yersinla* (11). The severity of the toxic effects ranges from temporary discomfort to the acute lethality of botulism. Infants, the elderly, and persons on immunosuppressive or chemotherapeutic drugs are thought to be more susceptible to the toxic effects of these microbial agents. The main source of these hazards may be on the farm, during food processing, or more likely, during food service preparation or preparation at home. The changing consumer lifestyle, including increased number of women in the workforce, limited time for food preparation, and an increase in the number of single heads of households, may also impact the emergence and reemergence of foodborne pathogens (12).

Environmental Contaminants

Toxicants included in this category are the trace elements and organometallic compounds (eg, mercury, lead, and cadmium) as well as a variety of organic compounds (eg, polychlorinated biphenyls [PCBs]). These toxicants tend to have common behavioral characteristics, although they are quite different in chemical structure. Environmental toxicants tend to be stable and thus persistent in the environment. They tend to bioaccumulate in the food chain and can be biotransformed with increasing toxicity in humans. Lead and mercury have been shown to cause major toxic effects in infants and young children because of the greater absorption of the compounds, increased sensitivity of developing systems, and their greater frequency of exposure to the toxicants.

The major source of lead exposure through food is in fruits, vegetables, and grains, which is primarily the result of deposition of environmental lead into the plants. The elimination of leaded gasoline, lead in soldered food cans, and lead in pottery glazes have all contributed to a decrease in the levels of lead in food. Fish, plants, and animals take up cadmium from the environment, and shellfish are the major source of cadmium in the diet. Mercury exposure via food most often occurs when fish and seafood are contaminated with mercury. The Food and Drug Administration (FDA) limits the amount of heavy metals in our foods. For example, cadmium in food colors is limited to 15 parts of cadmium per million parts of food color (15 ppm), and the maximum acceptable level of mercury in fish and seafood is set at 0.5 ppm

Because PCBs tend to be stored in the fat tissues of animals, the FDA has established maximum allowed levels of between 0.2 and 3 ppm PCBs in milk, eggs, animal fats, and shellfish. The used of PCBs was put under regulatory control in 1972, and production was ceased in the United States in 1977. As a result, the amount of PCBs in the U.S. diet has dramatically dropped since 1971. A possible role of PCBs in the development of breast cancer has been hypothesized. However, findings from epidemiological studies have been inconclusive. For a review of this issue as well as other health issues related to exposure to chlorinated contaminates, see the work by Holland (13). The toxicity of metals (14) and other food contaminants (8) has been reviewed.

Naturally Occurring Toxicants

Naturally occurring toxicants are products of the metabolic processes of animals, plants, and microorganisms from which the food products and nutrients are derived. Humans may be exposed to these naturally occurring toxicants through direct consumption of the foods or through secondary exposure from the edible by-products of food-producing animals. Most toxicants of animal origin come from fish and shellfish, including saxitoxin (paralytic shellfish poisoning), tetrodotoxin (puffer fish poisoning), ciguatoxin (ciguatera poisoning), and histamine (scombroid poisoning). The toxins produced by fungus on infected grains and other foods are also considered naturally occurring toxicants. They include aflatoxins, ergot toxins, ochratoxin, zearalenone, and the trichothecene toxins. Many of these mycotoxins (toxins produced by fungal cultures) are carcinogenic, mutagenic, and teratogenic. Many mushrooms also produce toxins with varying types of effects. See references 9 and 10 for further details on the toxicity of mycotoxins and toxic mushrooms.

Plants contain hundreds of biologically active compounds, which recently have been termed phytochemicals. Interest in phytochemicals has grown dramatically in recent years as a result of reports that they may offer potential health benefits (including cancer prevention) and agricultural benefits (increased resistance to plant pathogens). However, in many cases, these compounds have also been demonstrated to have toxic effects. Examples of toxic compounds found in plants consumed by humans are listed in Table 1 (9,10).

The concept of compounds having both beneficial and harmful effects, depending on the dose, has been well es-

Table 1. Examples of Toxic Compounds Found in Plants Consumed by Humans

Type of toxicity	Source of compounds involved
Cyanide poisoning	Cyanogenic glycosides in almonds, lima beans, cassava root, and sorghum
Goitrogens	Glucosinolates, isothiocyanates in the *Brassica* family (cabbages, brussels sprouts)
Favism	Fava beans
Lathyrism	Chick peas or garbanzos
Hemaglutinins	A wide variety of legumes
Enzyme inhibitors	Legumes, potatoes, eggplant, tomatoes
Carcinogenic	Pyrolizidine alkaloids in some herbs
Vasoactive amines	Bananas, avocado, cheeses
Interferes with hormone action	Phytoestrogens in soybeans
Antinutrient compounds	Phytic acid in cereals and legumes, oxalates in spinach and tea
Stimulants and other psychoactive effects	Caffeine in teas and coffee

tablished for various nutrients and pharmaceuticals. The phenomenon is called hormesis and is defined as the production of beneficial effects in a population at low exposures and adverse effects at high exposures to a given chemical. Data demonstrating that hormesis may occur with compounds that have previously been considered only as toxins, such as pesticides and heavy metals, has recently been reviewed (13). It appears likely that this holds true for many plant compounds as well. For example, tannins represent a group of natural plant compounds that have been shown to have both beneficial and toxic effects (15).

Further investigations into the mechanisms of action of these biologically active plant compounds are needed. In many cases, there is little information on the toxicity of these compounds, especially when compared to the amount of toxicology information needed for food additives and pesticides (16). Increased consumption of these compounds is likely because of the current development of "functional foods" that often contain various phytochemicals with the goal to improve health. However, the optimum levels of consumption for healthful effects and the levels that would result in toxicity are currently unknown for most phytochemicals.

Concerns regarding the safety of genetically engineered foods include the potential development of increased levels of plant toxins or of new allergens in these foods. In the United States, it is the responsibility of FDA to ensure that genetically engineered foods are safe. See reference 17 for a discussion of FDA's policy for foods developed by biotechnology.

Reaction Products

Toxic reaction products may be produced after cooking, processing, or charcoal broiling of foods. Carcinogenic nitrosamines are produced in cured meats such as bacon during cooking from the reaction of nitrite with secondary amines. Potent mutagenic and carcinogenic heterocyclic amines such as PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline), MeIQ(2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline), MeIQx(2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline) are found in cooked fish and meat owing to pyrolysis of amino acids, peptides, and proteins (18). Polycyclic aromatic hydrocarbons such as the carcinogen benzo(a)pyrene accumulates in foods during charcoal broiling and smoking. The production of reaction products from food irradiation, a method of food preservation, has been investigated in numerous studies. The safety of irradiated food produced in accordance with good manufacturing practices has been acknowledged by the World Health Organization (19). An evaluation of the safety of food irradiation was required for recent approval of food irradiation as a method of preservation of meat (20). See reference 21 for further information on the safety of irradiated foods.

Residues

Residues of chemicals occur in foods as a result of the use of pesticides, drugs in food-producing animals, and food packaging materials. To ensure a safe food supply, the Environmental Protection Agency (EPA) regulates the safety of food by setting safety standards to limit the amount of pesticide residues that legally may remain in or on food or animal feed that is sold in the United States. The FDA and the U.S. Department of Agriculture (USDA) ensure compliance with these safety standards by monitoring domestic and imported foods. Annual reports summarizing the findings of pesticide monitoring programs are available from the USDA. The regulation of pesticides in food production is currently undergoing major reform because of the passage of the Food Quality Protection Act (FQPA) in August 1996. Major changes in pesticide residue safety standards resulting from the FQPA include a new safety standard based on a "reasonable certainty of no harm"; consideration of aggregate exposure from all sources of pesticides (drinking water, residential, and dietary exposure); and consideration of all pesticides with a common mechanism of toxicity as a group to determine cumulative exposure. The EPA must consider children's special sensitivity and exposure to pesticides. Under the new law, the EPA is required to develop and use a screening and testing program for chemicals with the potential to disrupt the endocrine process (endocrine disruptors). The impact of this new law on the use of pesticides has yet to be determined because the regulations, which will enforce this law, are still under development.

Various drugs and antibiotics are used in the production of animal food products. The use of these drugs and the amount of residues allowed in the animal tissues are regulated by FDA. In most cases, tolerances range from 0 to

1 ppm. The unintentional transfer of compounds from packaging materials into foods is another source of food residues. Food residues from pesticides, drugs, and packaging materials are called unintentional food additives. Animal drug residues and food contact substances, which "may reasonably be expected to become a component of food," must be shown to be safe for humans under similar standards as food additives. Although public concern regarding food residues has always been the subject of the lay press, there is little evidence of health hazards from the small amount of residues present in foods produced in the United States (22). The use of pesticides in other countries may not be as well regulated, and therefore the safety of pesticide residues in imported foods continues to be a concern.

Food Additives

A food additive is defined as a substance or mixture of substances, other than a basic foodstuff, that is present in a food as a result of any aspect of production, processing, storage, or packaging. Today, more than 2,500 different additives are intentionally added to produce desired effects. They include preservatives, antioxidants, sweeteners, nutritional additives, flavoring agents, coloring agents, bleaching agents, texturizing agents, and miscellaneous additives. A comprehensive summary of food additive toxicology has recently been published (23). The majority of direct food additives, predominantly spices and flavors, are generally recognized as safe (GRAS) substances that have been used for many years and have been found to present no significant hazard with normal human food uses. However, the use of some food additives, such as cyclamates, red no. 2 and violet no. 1, is banned owing to their potential carcinogenic activity.

When a manufacturer submits a petition for a food additive approval, the FDA requires information on the chemical identity of the additive, the purpose and amount proposed for use, functionality testing to demonstrate effectiveness of the additive, methods of analysis in food, and safety data for the additive. Safety evaluation is discussed in the next section. The FDA operates an adverse reaction monitoring system (ARMS) to help serve as an ongoing safety check of all food additives. The system monitors and investigates all complaints by individuals or physicians that are believed to be related to specific foods, food and color additives, or vitamin and mineral supplements. The ARMS computerized database helps officials decide whether reported adverse reactions represent a real public health hazard associated with food, so that appropriate action can be taken. However, it is possible that postmarket surveillance may not be sensitive enough to identify a small subsection of the population that has severe adverse effects to a particular additive. This concern has been raised with the recent approval of the fat replacement Olestra. The purported benefit of fat replacers is possible prevention of obesity through calorie reduction. However, adverse gastrointestinal effects in some individuals have raised concern and resulted in a label requirement on foods containing this additive.

Dietary Supplements

A dietary supplement is any product that is taken by mouth and intended to supplement the diet and is labeled as a dietary supplement. Dietary supplements may contain vitamins, minerals, herbs, and amino acids as well as substances such as enzymes, organ tissues, metabolites, extracts, or concentrates. The Dietary Supplement Health and Education Act (DSHEA) of 1994 established new guidelines for the safety requirements of dietary supplements and dietary ingredients of supplements. One requirement is that "the dietary supplement contains only dietary ingredients which have been present in the food supply as an article used for food in a form in which the food has not been chemically altered." The alternative requirement is that there is a history of use or other evidence of safety establishing that the dietary ingredient when used under the conditions recommended or suggested in the labeling of the dietary supplement "will reasonably be expected to be safe." As a result, dietary ingredients used in dietary supplements are no longer subject to the premarket safety evaluations required of other new food ingredients.

The ARMS program for food additive postmarket surveillance also serves to monitor safety of dietary supplements. Recently, ARMS reports of adverse health effects associated with dietary supplements containing ephedrine alkaloids resulted in proposed safety measures by the FDA, including limits on the amount of ephedrine per serving and label warning statements for ephedrine-containing supplements.

SAFETY EVALUATION AND RISK ASSESSMENT

A number of different laws—the Food, Drug, and Cosmetic Act (FD&C Act); the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA); the recent FQPA and DSHEA; the Meat Inspection Act; and the Poultry Products Inspection Act—are routinely employed by the FDA, USDA, and EPA to govern the safety of food. For chemical food safety issues, a risk assessment process is used to determine the levels of a compound that will be allowed in foods. There are four steps in risk assessment: (*1*) hazard identification, (*2*) dose–response assessment, (*3*) exposure assessment, and (*4*) risk characterization. See reference 1 for an overview of risk assessment.

Hazard Identification

Hazard identification is the determination of the known or potential health effects of consumption of the compound. In some cases, the need for toxicological testing can be predicted from the chemical structure based on known structure–activity relationships. Several chemical structures, such as *n*-nitroso or aromatic amine groups, are potentially carcinogenic. In many cases, manufacturers use this information to decide whether to go forward with expensive animal testing of their products. *In vitro* and short-term animal tests are also used before investing in long-term animal testing. These can include tests for mutagenicity, carcinogenecity, developmental toxicity, reproductive toxicity, neurotoxicity, and immunotoxicity (3). However, for final approval of pesticides and food additives, the long-term animal bioassay is a key component in the hazard identification process. Table 2 lists the types of toxicological tests that are conducted using the animal model system. For food additives, the safety evaluation involves assigning a concern level to the additive, based on structural information and an estimate of exposure. The minimum toxicological testing required for each concern level is listed in Table 3.

Epidemiological studies, in which a positive association between exposure and disease has been observed, and case reports of accidental poisonings have provided convincing evidence of the human risk of various compounds. This has been the basis for the identification of many naturally occurring toxins, including ergot alkaloids, potato alkaloids, and toxic mushrooms.

Dose–Response Assessment

The dose–response assessment examines the relationship between the magnitude of the exposure and the probability of adverse effects. There are two approaches to dose–response assessments: a threshold approach for noncancer end points and a nonthreshold approach for cancer end points. The threshold is the level of exposure below which no adverse effects result. This approach is based on establishing a NOAEL, which is the highest dose in milligrams/kilogram body weight that results in no detectable damage to the animal during chronic or subchronic testing. Safety factors are applied to the NOAEL to determine the RfD and the acceptable daily intake (ADI). The ADI values are used by the World Health Organization for pesticides and food additives to define "the daily intake of a chemical, which during an entire lifetime appears to be without appreciable risk on the basis of all known facts at that time." The safety factors, also called uncertainty factors, allow for differences in species and individual susceptibility to toxicity. A factor of 10 is used for each extrapolation (ie, from animals to humans, from a small number of animals to a population, from a short-term study to a chronic study) to result in a total safety factor of 100 to 1,000. The FQPA requires an additional 10-fold safety factor for pesticides if adverse effects on infants and children are unknown or increased compared to adults. Therefore, the RfD or ADI = NOAEL ÷ safety factors.

Table 2. Types of Toxicological Tests

Acute tests (single exposure or dose)

Determination of median lethal dose (LD_{50})
Acute physiologic changes (blood pressure, pupil dilation, etc)

Subacute tests (continuous exposure or daily doses)

Three-month duration
Two or more species (one nonrodent)
Three dose levels (minimum)
Administration by intended or likely route
Health evaluation, including body weight, complete physical examination, blood chemistry, hematology, urinalysis, and performance tests
Complete autopsy and histopathology on all animals to determine the presence of any tissue damage or cancerous tissues

Chronic tests (continuous exposure or daily doses)

Two-year duration (minimum)
Two species selected for sensitivity from previous tests
Two dose levels (minimum)
Administered by likely route of exposure
Health evaluation including body weight, complete physical examination, blood chemistry, hematology, urinalysis, and performance tests
Complete autopsy and histopathology on all animals to determine the presence of any tissue damage or cancerous tissues

Special tests

Teratogenicity
Multigenerational reproduction feeding study (all aspects other than teratogenicity)
Skin and eye effects
Behavioral effects

Dose–response models for cancer, which is considered to be a nonthreshold end point, can be categorized into two types. One type is the statistical or probability model, such as the log–probit and logit models. The other type is a mechanistic-based model, such as the one-hit, multistage, and linearized multihit models. A discussion of these models and the use of pharmacokinetic models to improve cancer risk assessment can be found in reference 25.

Exposure Assessment

For exposure assessment, estimates of the likely levels of human consumption of the particular food substance or the estimate of daily intake (EDI) must be made. It is necessary to know the following: (*1*) the amount of substance in the foods; (*2*) daily intake of each food containing the substance by consumers; and (*3*) the portion of the population that are consumers. The amount of a direct food additive in the foods will be based on the amount needed to obtain the desired effects of the additive and this will have been determined by the manufacturer petitioning for approval of the additive. For pesticides, tests are conducted to determine the level of pesticide that remains on the agricultural commodities after the pesticide is used in the production of that commodity. Food consumption surveys, food disappearance figures, and market basket surveys can be used to determine the EDI. However, food consumption surveys are most often used. The anticipated use and the toxicity of the compound will affect whether values for average consumers or heavy consumers (at or above 95th percentile for food intake) and values for the whole population or consumers only are used in the EDI. The daily intake of food consumed (kilograms per day) multiplied by the amount of the compound in the food (milligrams/kilogram) provides an estimate of the amount of the compound (milligrams per day) that will be consumed. Guidelines on the estimations of exposure to food additives and chemical contaminants have been published by the U.S. FDA (26).

Table 3. Minimum Toxicological Testing of Food Additives Required by the U.S. Food and Drug Administration

Indirect food additives	
Virtually nil exposure (<0.05 ppm)	Acute oral study—rodent
Insignificant exposure (>0.05 ppm)	Acute oral study—rodent[c]
	Subchronic feeding study (90-day)—rodent with *in utero* exposure
	Subchronic feeding study (90-day)—nonrodent
	Multigenerational reproduction feeding study (minimum of 2 generations) with teratology phase—rodent[a]
	Teratology study
	Short-term tests for carcinogenic potential[b]
Significant exposure (>~1 ppm)	Acute oral study—rodent[c]
	Subchronic feeding study (90-day)—rodent[c]
	Lifetime feeding study (about 2 y)—rodent with *in utero* exposure for carcinogenesis and chronic toxicity
	Lifetime feeding study (about 2 y)—rodent for carcinogenesis
	Short-term feeding study (at least 1 year)—nonrodent
	Multigenerational reproduction feeding study (minimum of 2 generations) with teratology phase—rodent
	Teratology study[a]
	Short-term tests for carcinogenic potential[b]
	Metabolism studies[b]
Direct food additives	
Concern level I (lowest concern)	Short-term feeding study (at least 28 days)—rodent
	Short-term tests for carcinogenic potential
Concern level II	Acute oral study—rodent[c]
	Subchronic feeding study (90-day)—rodent
	Subchronic feeding study (90-day)—nonrodent
	Multigenerational reproduction feeding study (minimum of 2 generations) with teratology phase—rodent
	Teratology study[a]
	Short-term tests for carcinogenic potential
Concern level III (highest concern)	Acute oral study—rodent[c]
	Subchronic feeding study (90-day)—rodent[c]
	Lifetime feeding study (about 2 y)—rodent with *in utero* exposure for carcinogenesis and chronic toxicity
	Lifetime feeding study (about 2 y)—rodent for carcinogenesis
	Short-term feeding study (at least 1 year)—nonrodent
	Multigenerational reproduction feeding study (minimum of 2 generations) with teratology phase—rodent
	Teratology study[a]
	Short-term tests for carcinogenic potential
	Metabolism studies[b]

Notes: If carcinogenicity is suspected as a problem, carcinogenicity studies will be done no matter what the circumstances. References to current guides on these toxicology studies are contained in the "Redbook" (24).

[a] If indicated by available data or information.

[b] Suggested.

[c] If needed as preliminary to further study.

Risk Characterization

Risk characterization is the estimate of the incidence of health effects that are likely to occur under specific conditions of exposure. This is dependent on the exposure levels and the toxicity of the compound. Simply, risk = toxicity × exposure. Risk characterization requires a judgment of the applicability of the science to the potential human exposure conditions.

For noncarcinogens, the approach is to ensure that the estimated intake or exposure does not exceed the RfD. Intakes at levels below this level are considered to be without appreciable risk. The amount of additive or pesticide residue that is allowed in foods (ie, the tolerance) is based on the lowest amount needed for efficacy, not safety. As a result, the tolerance may be many folds lower than the safe level based on the RfD. See Table 4 for an example of the determination of tolerance levels.

For carcinogens, the risk assessment is based on the assumption that there is no threshold for carcinogenesis and that the dose–response relationship observed at high doses is similar to that at low doses. An acceptable level of risk has been set at a consumption level estimated to produce no more than one cancer in a million individuals. The insensitivity of the animal bioassay for detecting carcinogens has led to the use of high doses in order to generate a sufficiently high frequency of response to be statistically significant. Results from these studies then need to be ex-

Table 4. Example of Determination of Maximum Permissible Levels

1. The synthetic food color additive Rosy Red was tested in rats and dogs in a chronic feeding study. A NOAEL of 1,000 mg/kg body weight per day (mg/kg/day) was determined.
2. Safety factor = 10 (extrapolation to humans) × 10 (individual variation) = 100
3. RfD = 1,000 mg/kg/day ÷ 100 = 10 mg/kg/day
4. Rosy Red is being developed for use in licorice candy. Using food consumption survey data, it was found that the person with the highest intake would consume 100 g of candy per day.
5. The maximal permissible intake per day (MPI) = RfD × 60 kg (adult body weight) = 10 mg/kg × 60 kg = 600 mg per day.
6. Assuming that Rosy Red will not be added to any other foods, the maximum permissible level (MPL) (also called tolerance level) that would be approved would be MPL = MPI/food factor. Food factor represents an estimation of the amount of that food in the diet. MPL = 600 mg ÷ 100 g licorice = 6 mg Rosy Red/g licorice. However, if only 1 mg Rosy Red/g candy was needed for the desired color, the MPL would be 1 mg/g.
7. The RfD is usually well above the EDI of a food additive.

trapolated to the low-dose range that represents the human exposure levels. A very large number of animals would be required to obtain a statistically valid response level if low doses were used, resulting in increased testing costs. Therefore, the dose used may be up to the maximum tolerated dose (MTD), which is the highest dose that does not produce overt toxic responses in subchronic studies. Concerns with the use of the MTD are that this dose may result in depressed food intake and may saturate other physiological processes, such as activation and inactivation by enzyme systems, active transport, and DNA repair. Cytotoxicity caused by high doses may result in mitogenesis or increased cell proliferation, enhancing the carcinogenesis process. Thus, use of the MTD may result in the ability of a compound to induce cancer through mechanisms that do not occur at low doses.

In part to address this issue, the EPA proposed new guidelines for cancer risk assessment in 1996 (27). The 1986 cancer guidelines classified compounds as A, B1, B2, C, D, or E carcinogens. Classification as an A carcinogen was based on "sufficient evidence of carcinogenecity from human studies," and an E classification was based on "evidence of noncarcinogenecity." Tumor findings in animals or humans were the dominant components of the classification. The 1996 proposed guidelines will use a narrative description of the likelihood and conditions of human hazard, such as "not likely to be a human carcinogen, because animal data shown to be not relevant to humans." Therefore, consideration of the route of exposure, the dose–response relationship, and mode of action information are changes in the proposed guidelines. Further discussion on the proposed guidelines can be found in the Science Advisory Board review (28).

A major difference in the cancer risk assessment process for food additives and for pesticides is that the Delaney clause still applies to food additives, but has been removed for pesticides by the FQPA. The Delaney clause of the FD&C Act states that "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, to induce cancer in man or animal" (Food Additive Amendment of 1958, section 409). The Delaney clause therefore does not allow for consideration of dose or mechanism of action. Therefore, even if it is clearly demonstrated that the mechanism of cancer induction in animals is not relevant to humans, the Delaney clause would prohibit the use of the product in foods. This is one reason why the new FQPA eliminated the Delaney clause from pesticide regulations.

CONCLUSIONS

The safety of the food supply affects the entire population of a country. Current laws and regulations in the United States have resulted in the provision of one of the safest food supplies in the world. However, as production and processing methods of food continue to change, new challenges and issues in food toxicology continue to arise.

The level of exposure to naturally occurring toxins may increase in the near future as a result of the increased development of functional foods and pest-resistant crops. Functional foods or nutraceuticals represent a rapidly growing segment of the food industry. Functional foods are defined as foods that contain components with anticipated health benefits. Examples include beverages with added herbal extracts, cereals with added fiber, and vegetables and fruits bred to contain higher levels of antioxidants or other phytochemicals. Concerns regarding environmental and health impacts of pesticide use have resulted in the drive to lower pesticide use through the development of biological pesticides and pest-resistant crops. In many cases, pest resistance is achieved through increasing the plant's naturally occurring defense mechanisms, which may involve naturally occurring toxicants. In both cases, the goal is to improve overall health. However, as mentioned earlier, our current level of understanding of the dose–response curves for naturally occurring compounds is very limited, making it extremely difficult to determine when levels of exposure cease being beneficial and start to become harmful.

Understanding of the interactions between the many compounds in the diet and the interaction between diet and other environmental compounds is another area of needed research. The requirement of the FQPA to consider all pesticides with a common mechanism of action is one step toward recognizing the need to consider the impact of exposure to a variety of compounds. This is a very complex and difficult question to address, but research on this topic is beginning to increase.

Consumer understanding of the dose–response concept is critical to their understanding of how low levels of food additives and pesticides in foods can pose little to no risk, even though these compounds may have toxic effects at high doses. Consumer acceptance of pesticide residues and processes such as food irradiation has been shown to increase after educational programs to develop this type of understanding. The commonly held beliefs "natural is safe" and that "synthetic chemicals pose a greater risk than natural compounds" also need to be addressed through public educational programs. Greater understanding of these issues will prevent undue concern and overreaction to the presence of small amounts of synthetic chemicals and may prevent poisonings and adverse health effects caused by overzealous consumption of "natural" compounds with promised health benefits.

BIBLIOGRAPHY

1. E. M. Faustman and G. S. Omenn, "Risk Assessment," in C. D. Klaassen, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996, pp. 75–88
2. D. L. Eaton and C. D. Klaassen, "Principles of Toxicology," in C. D. Klaassen, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996, pp. 13–33.
3. C. D. Klaassen, *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996.
4. A. W. Hayes, *Principles and Methods of Toxicology*, 3rd ed., Raven Press, New York, 1994.
5. H. C. Pitot and Y. P. Dragan, "Chemical Carcinogenesis," in C. D. Klaassen, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996, pp. 201–267.
6. R. W. Rudon, *Cancer Biology*, 3rd ed., Oxford University Press, New York, 1995.
7. *Food, Nutrition and the Prevention of Cancer: A Global Perspective*, World Health Cancer Fund and American Institute for Cancer Research, Washington, D.C., 1997.
8. T. Shibamoto and L. F. Bjeidanes, *Introduction to Food Toxicology*, Academic Press, San Diego, Calif., 1993.
9. J. M. Concon, *Food Toxicology: Principles and Concepts*, Vol. 1, Marcel Dekker, New York, 1988.
10. J. M. Concon, *Food Toxicology· Contaminants and Additives*, Vol. 2, Marcel Dekker, New York, 1988.
11. L. Slutsker, S. F. Altekruse, and D. L. Swerdlow, "Foodborne Diseases. Emerging Pathogens and Trends," *Infect. Dis. Clin. North Am.* 12, 199–216, 1998.
12. J. E. Collins, "Impact of Changing Consumer Lifestyles on the Emergence/Reemergence of Foodborne Pathogens," *Emerg. Infect. Dis* 3, 471–479 (1997).
13. C. D. Holland, *Chemical Hormesis: Beneficial Effects at Low Exposures Adverse Effects at High Exposures*, Texas Institute for Advancement of Chemical Technology, Bryan, Tex., 1998.
14. F. K. Ennever, "Metals," in A. W. Hayes, ed. *Principles and Methods of Toxicology*, Raven Press, New York, 1994, pp. 417–446.
15. K. T. Chung, C. I. Wei, and M. G. Johnson, "Are Tannins a Double-Edged Sword in Biology and Health," *Trends Food Sci. Technol.* **9**, 168–175 (1998).
16. G. J. A. Speijers, "Toxicological Data Needed for Safety Evaluation and Regulation on Inherent Plant Toxins," *Natural Toxins* 3, 222–226 (1995).
17. J. H. Maryanski, "FDA's Policy for Foods Developed by Biotechnology," in K. Engel, G. R. Takeoka, and R. Teranishi, eds., *Genetically Modified Foods: Safety Issues*, American Chemical Society, Washington, D.C., 1995, pp 12–22.
18. H. A. J. Schut and E. G. Snyderwine, "DNA Adducts of Heterocyclic Amine Food Mutagens: Implications for Mutagenesis and Carcinogenesis," *Carcinogenesis* **20**, 353–368 (1999).
19. *Safety and Nutritional Adequacy of Irradiated Foods*, World Health Organization, Geneva, Switzerland, 1994.
20. U.S. Food and Drug Administration, "Irradiation in the Production, Processing and Handling of Food," *Federal Register* **62**, 64107–64121 (1997)
21. J. F. Diehl, *Safety of Irradiated Foods*, Marcel Dekker, New York, 1995.
22. National Research Council, *Carcinogens and Anticarcinogens in the Human Diet*, National Academy Press, Washington, D.C., 1996.
23. J. A. Maga and A. T. Tu, eds., *Food Additive Toxicology*, Marcel Decker, New York, 1995.
24. U.S. Food and Drug Administration, *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, U.S. Food and Drug Administration, Bureau of Foods (now Center for Food Safety and Applied Nutrition), Washington, D.C., 1982.
25. D. C. Rees and D. Hattis, "Developing Quantitative Strategies for Animal to Human Extrapolation," in A. W. Hayes, ed., *Principles and Methods of Toxicology*, Raven Press, New York, 1994, pp. 275–315.
26. M. J. DiNovi and P. M Kuznesof, *Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet*, U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, Office of Premarket Approval, Washington, D.C., 1995.
27. U.S. Environmental Protection Agency, *Proposed Guidelines for Carcinogen Risk Assessment*, Washington, D.C., 1996.
28. Science Advisory Board, *An SAB Report: Guidelines for Cancer Risk Assessment*, U.S. Environmental Protection Agency, Washington, D.C., 1997.

BERNADENE MAGNUSON
University of Idaho
Moscow, Idaho

See also TOXICOLOGY AND RISK ASSESSMENT.

FOOD UTILIZATION

Most people take the process of eating for granted, knowing that the body will take care of itself. But between eating and the cellular utilization of dietary nutrients, hundreds of thousands of metabolic processes take place. Food must first be digested, or broken down into particles of a size and chemical composition that the body can readily absorb. Absorption takes place mostly in the small intestine, where specialized cells transfer digested nutrients to the blood and lymph vessels. In some cases, special changes are needed so that the nutrients can be transported to the cells where they are to be used or further processed. Within the cells, the nutrients are either stored or metabolized, that is, broken down into simpler components for energy or excretion (catabolism), or used to synthesize new materials for cellular growth, maintenance, or repair (anabolism).

safe" and that "synthetic chemicals pose a greater risk than natural compounds" also need to be addressed through public educational programs. Greater understanding of these issues will prevent undue concern and overreaction to the presence of small amounts of synthetic chemicals and may prevent poisonings and adverse health effects caused by overzealous consumption of "natural" compounds with promised health benefits.

BIBLIOGRAPHY

1. E. M. Faustman and G. S. Omenn, "Risk Assessment," in C. D. Klaassen, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996, pp. 75–88
2. D. L. Eaton and C. D. Klaassen, "Principles of Toxicology," in C. D. Klaassen, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996, pp. 13–33.
3. C. D. Klaassen, *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996.
4. A. W. Hayes, *Principles and Methods of Toxicology*, 3rd ed., Raven Press, New York, 1994.
5. H. C. Pitot and Y. P. Dragan, "Chemical Carcinogenesis," in C. D. Klaassen, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996, pp. 201–267.
6. R. W. Rudon, *Cancer Biology*, 3rd ed., Oxford University Press, New York, 1995.
7. *Food, Nutrition and the Prevention of Cancer: A Global Perspective*, World Health Cancer Fund and American Institute for Cancer Research, Washington, D.C., 1997.
8. T. Shibamoto and L. F. Bjeidanes, *Introduction to Food Toxicology*, Academic Press, San Diego, Calif., 1993.
9. J. M. Concon, *Food Toxicology: Principles and Concepts*, Vol. 1, Marcel Dekker, New York, 1988.
10. J. M. Concon, *Food Toxicology: Contaminants and Additives*, Vol. 2, Marcel Dekker, New York, 1988.
11. L. Slutsker, S. F. Altekruse, and D. L. Swerdlow, "Foodborne Diseases. Emerging Pathogens and Trends," *Infect. Dis. Clin. North Am.* **12**, 199–216, 1998.
12. J. E. Collins, "Impact of Changing Consumer Lifestyles on the Emergence/Reemergence of Foodborne Pathogens," *Emerg. Infect. Dis* **3**, 471–479 (1997).
13. C. D. Holland, *Chemical Hormesis: Beneficial Effects at Low Exposures Adverse Effects at High Exposures*, Texas Institute for Advancement of Chemical Technology, Bryan, Tex., 1998.
14. F. K. Ennever, "Metals," in A. W. Hayes, ed. *Principles and Methods of Toxicology*, Raven Press, New York, 1994, pp. 417–446.
15. K. T. Chung, C. I. Wei, and M. G. Johnson, "Are Tannins a Double-Edged Sword in Biology and Health," *Trends Food Sci. Technol.* **9**, 168–175 (1998).
16. G. J. A. Speijers, "Toxicological Data Needed for Safety Evaluation and Regulation on Inherent Plant Toxins," *Natural Toxins* **3**, 222–226 (1995).
17. J. H. Maryanski, "FDA's Policy for Foods Developed by Biotechnology," in K. Engel, G. R. Takeoka, and R. Teranishi, eds., *Genetically Modified Foods: Safety Issues*, American Chemical Society, Washington, D.C., 1995, pp. 12–22.
18. H. A. J. Schut and E. G. Snyderwine, "DNA Adducts of Heterocyclic Amine Food Mutagens: Implications for Mutagenesis and Carcinogenesis," *Carcinogenesis* **20**, 353–368 (1999).
19. *Safety and Nutritional Adequacy of Irradiated Foods*, World Health Organization, Geneva, Switzerland, 1994.
20. U.S. Food and Drug Administration, "Irradiation in the Production, Processing and Handling of Food," *Federal Register* **62**, 64107–64121 (1997)
21. J. F. Diehl, *Safety of Irradiated Foods*, Marcel Dekker, New York, 1995.
22. National Research Council, *Carcinogens and Anticarcinogens in the Human Diet*, National Academy Press, Washington, D.C., 1996.
23. J. A. Maga and A. T. Tu, eds., *Food Additive Toxicology*, Marcel Decker, New York, 1995.
24. U.S. Food and Drug Administration, *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, U.S. Food and Drug Administration, Bureau of Foods (now Center for Food Safety and Applied Nutrition), Washington, D.C., 1982.
25. D. C. Rees and D. Hattis, "Developing Quantitative Strategies for Animal to Human Extrapolation," in A. W. Hayes, ed., *Principles and Methods of Toxicology*, Raven Press, New York, 1994, pp. 275–315.
26. M. J. DiNovi and P. M Kuznesof, *Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet*, U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, Office of Premarket Approval, Washington, D.C., 1995.
27. U.S. Environmental Protection Agency, *Proposed Guidelines for Carcinogen Risk Assessment*, Washington, D.C., 1996.
28. Science Advisory Board, *An SAB Report: Guidelines for Cancer Risk Assessment*, U.S. Environmental Protection Agency, Washington, D.C., 1997.

BERNADENE MAGNUSON
University of Idaho
Moscow, Idaho

See also TOXICOLOGY AND RISK ASSESSMENT.

FOOD UTILIZATION

Most people take the process of eating for granted, knowing that the body will take care of itself. But between eating and the cellular utilization of dietary nutrients, hundreds of thousands of metabolic processes take place. Food must first be digested, or broken down into particles of a size and chemical composition that the body can readily absorb. Absorption takes place mostly in the small intestine, where specialized cells transfer digested nutrients to the blood and lymph vessels. In some cases, special changes are needed so that the nutrients can be transported to the cells where they are to be used or further processed. Within the cells, the nutrients are either stored or metabolized, that is, broken down into simpler components for energy or excretion (catabolism), or used to synthesize new materials for cellular growth, maintenance, or repair (anabolism).

The complicated processes involved are different for each nutrient, although the paths that certain nutrients take intersect at various points. The chemical details of metabolism belong to the disciplines of biochemistry and physiology; standard texts in these subjects can be consulted for detailed information. This article provides a brief overview of what happens in the body to the foods that are eaten, with particular attention to the three major nutrients: carbohydrates, proteins, and fats.

THE ALIMENTARY SYSTEM

The alimentary or digestive system is a long tube that consists of the mouth, esophagus, stomach, small intestine, colon, rectum, and anus. Some important accessory organs connected to the digestive tract are the salivary glands, gallbladder, pancreas, and liver. Along this tract, foods are broken down into smaller units, both physically and chemically, and then absorbed for use by the body. Figure 1 shows the general outline of the entire human digestive system.

The Food Path

The food placed in the mouth is chewed, softened, and swallowed. In the stomach, it is churned and then propelled into the small intestine where it is mixed with the bile from the gallbladder and digestive enzymes from the intestinal walls. The products of this digestion are partly or completely absorbed into either the portal vein or the lacteal system.

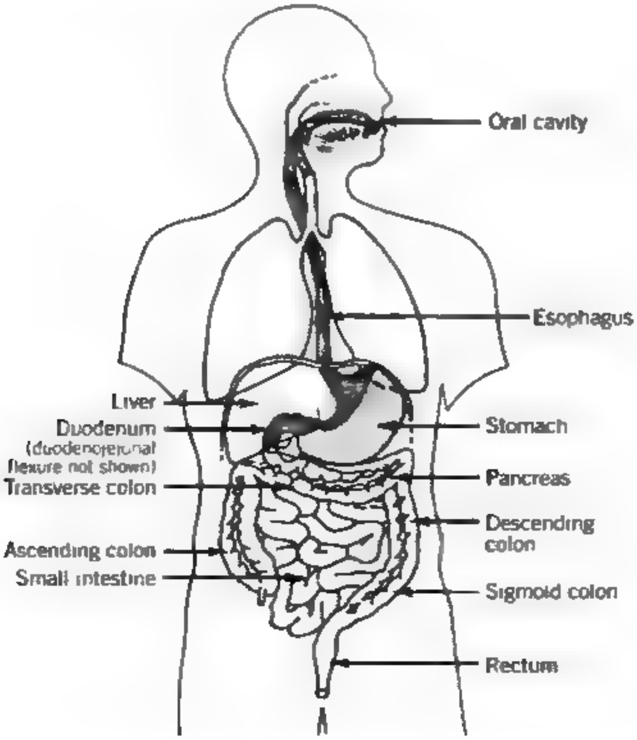


Figure 1. The digestive system of the human body.

In the mouth, chewing (mastication) reduces large food lumps into smaller pieces and mixes them with saliva. This wetting and homogenizing facilitates later digestion. In clinical dietetics, edentulous (toothless) patients or those with reduced saliva secretion have trouble eating dry foods and require a soft, moist diet. Saliva facilitates swallowing and movements of the tongue and lips, keeps the mouth moist and clean, serves as a solvent for taste bud stimulants, acts as an oral bugger, provides some antibiotic activity, and inhibits loss of calcium from the teeth by maintaining a neutral pH. Saliva contains ptyalin (salivary amylase, a digestive enzyme) and mucin (a glycoprotein). Mucin lubricates food and ptyalin digests carbohydrates to a small extent. Each day the salivary gland makes about 1,500 mL of saliva (ca 5.5–6 cups).

The bolus of food is propelled forward by rhythmic contractions of the entire intestinal system. These peristaltic waves move the food from the mouth, through the esophagus, and into the stomach. Certain individuals, especially nervous people, tend to swallow air when eating. When part of the air is expelled through the mouth, belching results; the remaining air is expelled as flatus. If too much air is swallowed, there will be abdominal discomfort.

From the mouth, food travels through the esophagus, the stomach cardia, the stomach body, the greater curvature, the pylorus, and the duodenum. These are all parts of the stomach, where food is well mixed. Figure 2 shows the general structure of this organ and the site of specific secretions. The acid, mucus, and pepsin cause partial digestion, and peristalsis mixes up the food. The food is then released gradually through the pylorus into the duodenum.

The gastrointestinal (GI) system, the stomach and intestines, breaks down complex carbohydrates, proteins, and fats into absorbable units, mainly in the small intestine. Vitamins, minerals, fluids, and most nonessential nutrients are also digested and absorbed to varying degrees. Foods are digested by enzymes secreted by different parts of the GI system. Table 1 summarizes the major digestive enzymes and their actions. After the digestive process is complete, nutrients are ready for absorption, which occurs mainly at the small intestine. The absorption of each nutrient is discussed later.

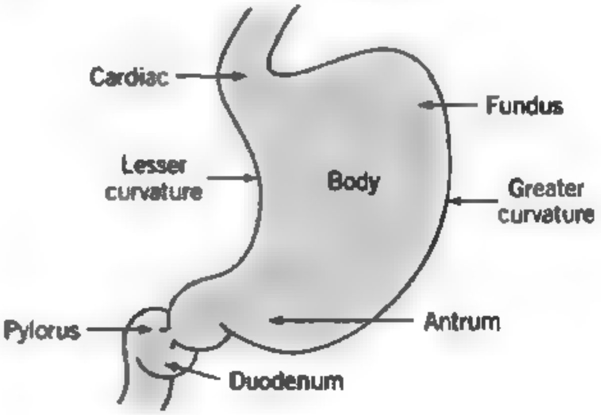


Figure 2. General structure of the stomach.

After the nutrients have been absorbed, they enter the circulation in two ways. Most fat-soluble nutrients enter the lacteal or lymphatic system, which eventually joins the systemic blood circulation at the thoracic duct. Other nutrients enter the hepatic portal vein and are received by the liver, which eventually releases them to the bloodstream.

Enzymes and Coenzymes

After digestion and absorption, the nutrients exist as hexoses (mainly glucose and fructose), fatty acids, glycerols, and amino acids and are then metabolized in various fashions. Many of the metabolic processes require the presence of a catalyst, a substance that can facilitate a chemical reaction. Although participating in the process, it may or may not undergo physical, chemical, or other modification itself. Nonetheless, the catalyst usually returns to its original form after the reaction.

In the body, most biological reactions require a special class of catalysts: the protein catalysts or enzymes. Each enzyme catalyzes only one or a small number of reactions. There are many enzymes, each with a specific responsibility. Without enzymes, most biological reactions would proceed at a very slow speed. Coenzymes are accessory substances that facilitate the working of an enzyme, mainly by acting as carriers for products of the reaction. In this case, the enzyme is composed of two parts: a protein (apoenzyme) and a nonprotein (cofactor or coenzyme). Many coenzymes contain vitamins or slightly modified vitamins as the major ingredient. A coenzyme can catalyze many types of reaction. Some coenzymes transfer hydrogens; others transfer groups other than hydrogens. Table 2 describes the characteristics of the former; Table 3, those of the latter. Because most of the metabolic reactions discussed in this article involve coenzymes, a knowledge of the biochemical role of vitamins is important.

CARBOHYDRATES

Starch, cellulose, and their derivatives are the only polysaccharides consumed to any extent by humans. The major simple sugars ingested include the monosaccharides (such as glucose and fructose in honey and fruit juices) and disaccharides (such as maltose in beer, lactose in milk, and sucrose in table sugar). Also ingested are dextrins, sugar alcohol, and trisaccharides and tetrasaccharides, although in very small quantities. The sections that follow discuss briefly the digestion and absorption pathways of these carbohydrates, the involvement of carbohydrates in energy formation and storage, the specific processes by which carbohydrates are broken down or synthesized by the body, and the regulation of glucose levels in the blood.

Digestion and Absorption

Starch is partially hydrolyzed by ptyalin in the mouth (Table 1 and Fig. 3). The short stay in the oral cavity permits only dextrines and small polysaccharide fragments to break off from the starch molecules, and the action of ptyalin is terminated by the acid in the stomach. In the small intestine, all digestible carbohydrates are reduced to monosaccharides, namely, glucose, fructose, galactose, mannose, and pentoses. Currently, it is believed that the final hydrolysis, or digestion, of disaccharides to monosaccharides occurs in the intestinal mucosal walls. In humans, all nondigestible carbohydrates such as lignin, hemicellulose, and cellulose are passed into the colon, where they are mainly fermented to release gas. By contrast, ruminants (animals such as cattle, sheep, and goats) have the ability to digest fiber.

Most of the monosaccharides are absorbed before the food residue reaches the end of the ileum. Absorption is principally carried out by active transport (requiring energy), although diffusion (passive movement) also occurs. Glucose and galactose enter the blood five times faster than mannose and pentoses, whereas fructose is absorbed about two to three times faster. Glucose entry into the blood may be as high as 129 g/h.

The monosaccharides traverse the intestinal and portal veins to reach the liver, from which they may eventually be released into blood circulation. In the liver, most of the fructose and galactose are converted to glucose, which is the main simple sugar in the blood, although fructose and galactose may be present throughout the bloodstream if a person consumes a large amount of them. An actively nursing mother also has some lactose in the blood, because this disaccharide is manufactured by the active mammary tissues. In the liver, part of the glucose is released to the circulation, part converted to glycogen for storage, part changed to other essential substances required by the body, and part oxidized to energy. Normally, a peak plasma glucose level of 120 to 140 mg/100 mL is reached within 60 min after a mixed meal.

After glucose has reached the bloodstream, some enters cells to give energy, and some is converted to glycogen in tissues such as muscle. Glycogen is found in many organs in the body, but the liver and muscle are the major storage sites. Most of the glucose is used to provide energy through a three-stage process: glycolysis, the citric acid cycle, and the respiratory chain.

Energy Formation and Storage

Everything a body does requires energy. Nature has provided the animal body with a wide spectrum of methods that permit energy either to be released to be stored and released to satisfy its energy need. There are five different energy systems known to operate in animal cells.

Direct Release of Energy (Mainly as Heat)

glucose (or fat or protein) + oxygen = carbon dioxide
+ water + energy to be stored + heat.

Energy Stored as Adenosine Triphosphate (ATP). One technique of storing part of the energy released from the oxidation of foodstuffs is incorporating it into ester bonds between certain organic compounds and phosphoric acid groups. The resulting substances are called high-energy phosphate compounds, the most important of which is probably adenosine triphosphate. This ubiquitous mole-

Table 1. Characteristics of the Enzymatic System of Digestion

			Enzyme(s) involved in digestion		
Location	Food or substrate	Products of digestion	Name(s)	Source(s)	Active in acid-base (pH)
Mouth	Starch	Maltose, dextrins, disaccharides, monosaccharides, branched oligosaccharides	Ptyalin, or salivary amylase	Salivary glands	Slightly acidic (6.7)
Stomach	Protein	Proteoses, peptones, polypeptides, dipeptides, amino acids	Pepsin	Peptic or chief cells of stomach	Acidic (1.6–2.4)
	Milk casein	Milk coagulation	Rennin	Stomach mucosa	Acidic (4.0); requires calcium for activity
	Fat	Triglycerides; some monoglycerides and diglycerides, glycerol, fatty acids	Gastric lipase	Stomach mucosa	Acidic
	Protein				
Small intestine (mainly duodenum and jejunum)[a]	proteoses, peptones, etc	Polypeptides, dipeptides, etc	Trypsin (activated trypsinogen)	Exocrine gland of pancreas	Alkaline (7.9)
	Proteoses, peptones, etc	Polypeptides, dipeptides, etc	Chymotrypsin (activated chymotrypsinogen)	Exocrine gland of pancreas	Alkaline (8.0)
	Polypeptides with free carboxyl groups	Lower peptides, free amino acids	Carboxypeptidase	Exocrine gland of pancreas	
	Fibrous protein	Peptides, amino acids	Elastase	Exocrine gland of pancreas	
	Carbohydrate				
	Starch, dextrins	Maltose, isomaltose, monosaccharides, dextrins	α-Amylase (amylopsin)	Exocrine gland of pancrease	Slightly alkaline (7.1)
	Fat				
	Triglycerides	Monoglycerides and diglycerides, glycerol, fatty acids	Lipase (steapsin)	Exocrine gland of pancreas	Alkaline (8.0)

	Cholesterol	Cholesterol esters	Cholesterol esterase	Exocrine gland of pancreas	
	Nucleic acids	Nucleotides			
	Ribonucleic acid	Ribonucleotides	Ribonuclease	Exocrine gland of pancreas	
	Deoxyribonucleic acid	Deoxyribonucleotides	Deoxyribonuclease	Exocrine gland of pancreas	
	Protein				
Small intestine (mainly jejunum and ileum)	Polypeptides	Amino acids	Carboxypeptidase, aminopeptidase, dipeptidase	Brush border of the small intestine	
	Carbohydrate				
	Sucrose	Glucose, fructose	Sucrase	Brush border of the small intestine	Acidic-alkaline (5.0–7.0)
	Dextrin (isomaltose)	Glucose	α-Dextrinase (isomaltase)	Brush border of the small intestine	
	Maltose	Glucose	Maltase	Brush border of the small intestine	Acidic (5.8–6.2)
	Lactose	Glucose, galactose	Lactase	Brush border of the small intestine	Acidic (5.4–6.0)
	Fat				
	Monoglycerides	Glycerol, fatty acids	Lipase (enteric)	Brush border of the small intestine	
	Lecithin	Glycerol, fatty acids	Lecithinase	Brush border of the small intestine	
	Nucleotides	Nucleosides, phosphate	Nucleotidase	Brush border of the small intestine	
	Nucleosides	Purines, pyrimides, pentose	Nucleosidase	Brush border of the small intestine	
	Organic phosphates	Free phosphates	Phosphatase	Brush border of the small intestine	Alkaline (8.6)

---

[a] The food is not grouped together (eg, all fat, all proteins, etc), instead the food is placed in an order that follows the sequence of digestion along the duodenum to jejunum. This attempts to present the digestive enzymes in their expected sequence of action.

Table 2. Characteristics of Coenzymes That Transfer Hydrogens

Enzyme system	Coenzyme	Vitamin component	Nonvitamin component
Dehydrogenase	Flavin adenine dinucleotide (FAD)	Riboflavin (vitamin B_2)	Adenine, ribose, phosphate
Dehydrogenase	Nicotinamide adenine dinucleotide (NAD)	Niacin	Adenine, ribose, phosphate
Part of dehydrogenase	Lipoic acid (thiotic acid)	None	Lipoic acid
Respiratory chain	Coenzyme Q	None	Quinone (vitamin E-related substance)

Table 3. Characteristics of Coenzymes That Transfer Nonhydrogen Groups

Enzyme system	Coenzyme	Vitamin component	Nonvitamin component
Transaminases, decarboxylase	Pyridoxal phosphate	Vitamin B_6	None
Part of dehydrogenase	Lipoic acid (lipoamide)	None	Lipolic acid
Dehydrogenase	Coenzyme A	Pantothenic acid	β-Mercaptoethylamine, adenine, ribose, phosphate
Cocarboxylase (decarboxylase, transketolase)	Thiamin pyrophosphate	Thiamin	Phosphate
Methyl transferase	5-Methyltetrahydrofolate	Folic acid	None
Transmethylase	Coenzyme B_{12}	Vitamin B_{12}	Adenine, ribose
Carboxylase	Carboxyl-biotin complex	Biotin	None

cule is considered the energy powerhouse of the body. It releases its energy in the following reactions:

$$\text{ATP} + H_2O = \text{ADP} + \text{P} + 7.5\ \text{kcal}$$
$$\text{ADP} + H_2O = \text{AMP} + \text{P} + 7.5\ \text{kcal}$$

where ATP = adenosine diphosphate, P = inorganic phosphate, and AMP = adenosine monophosphate. Theoretically, the conversion of 1 mol of ATP to AMP can produce 15 kcal. However, within the body ATP is normally changed only to ADP when energy is needed. The energy released from this process can be used for such work as organ building, heartbeat, transportation across cell membranes, and muscle contraction. Sometimes these compounds are called active phosphate carriers and dischargers. Figure 4 summarizes the role of ATP in body energy and metabolism.

Energy Stored in Creatine Phosphate, or Phosphocreatine. Creatine phosphate is another energy-rich phosphate compound found in muscle. It can contribute to muscle energy metabolism in two ways:

$$\text{creatine phosphate} + H_2O = \text{creatine} + \text{P} + 7.5\ \text{kcal}$$

$$\text{creatine phosphate} + H_2O \quad \overset{\underset{\smile}{\overset{\frown}{\text{ADP}\quad\text{ATP}}}}{\rightarrow} \quad \text{creatine}$$

In the first reaction the energy is released directly. In the second, the energy is transferred to ADP and later released for muscular or other work. Creatine phosphate is sometimes called an active phosphate carrier. Similar substances are 1,3-diphosphoglyceric acid and phosphoenolpyruvic acid.

Energy Stored in Active Acetate. The active acetate is the substance acetyl-CoA, which participates in intermediate metabolism. In terms of energy, formation of 1 mol of acetyl-CoA is equivalent to that of 1 mol of ATP.

Low-Energy Phosphate Compounds. Not all organic phosphates are of the high-energy type. Substances such as glucose-6-phosphate are phosphate compounds carrying a small amount of stored energy, such as 2–3 kcal/mol. If the above energy systems are summarized the oxidation or complete metabolism of glucose will yield the following:

$$C_6H_{12}O_6\ (\text{glucose}) + 6O_2 = 6CO_2 + 6H_2O + \text{heat} + 38\ \text{ATP}$$

Cellular Metabolism Processes

Within the cells, a series of complex biochemical processes is needed to degrade glucose (carbohydrate) to release the energy needed by the body. Three biological processes are involved: glycolysis, the citric acid cycle, and the respiratory chain. The glucose involved derives from food and internal production, which occurs mainly in the liver. In the cells of the liver, stored glycogen may be converted to glucose by the process of glycogenolysis; in the process of gluconeogenesis, glucose is synthesized from noncarbohydrate sources. However, as will be seen later, the muscles can also indirectly contribute the energy from cellular metabolism by participating in these processes. These processes are briefly defined in Table 4 and discussed in the following sections.

Glycolysis. The first step of carbohydrate metabolism is glycolysis, in which the six-carbon glucose is converted to a three-carbon substance (pyruvic or lactic acid), as indicated in Figure 5. Figure 6 illustrates the intermediate metabolic steps during the transformation of glucose to pyruvic acid. The interconversion between pyruvic and lactic acid occurs mainly in the muscle and will be discussed later. During the process of glycolysis, the conversion of 1

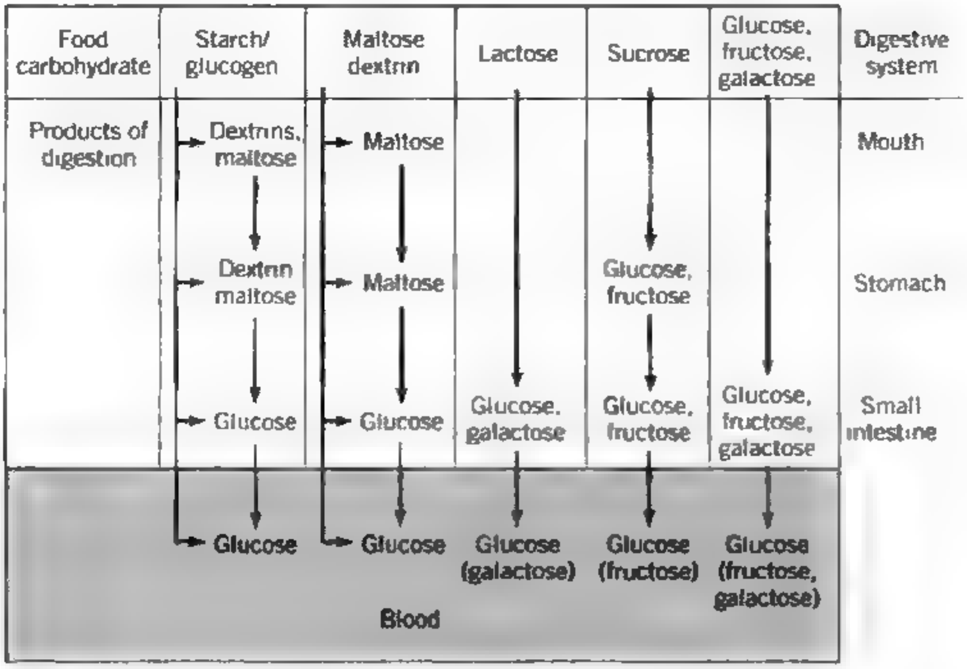

Food carbohydrate
Starch/ glucogen
Maltose dextrin
Lactose
Sucrose
Glucose, fructose, galactose
Digestive system
Products of digestion
Dextrins, maltose
Maltose
Mouth
Dextrin maltose
Maltose
Glucose, fructose
Stomach
Glucose
Glucose
Glucose, galactose
Glucose, fructose
Glucose, fructose, galactose
Small intestine
Glucose
Glucose
Glucose (galactose)
Glucose (fructose)
Glucose (fructose, galactose)
Blood

Figure 3. Carbohydrate digestion.

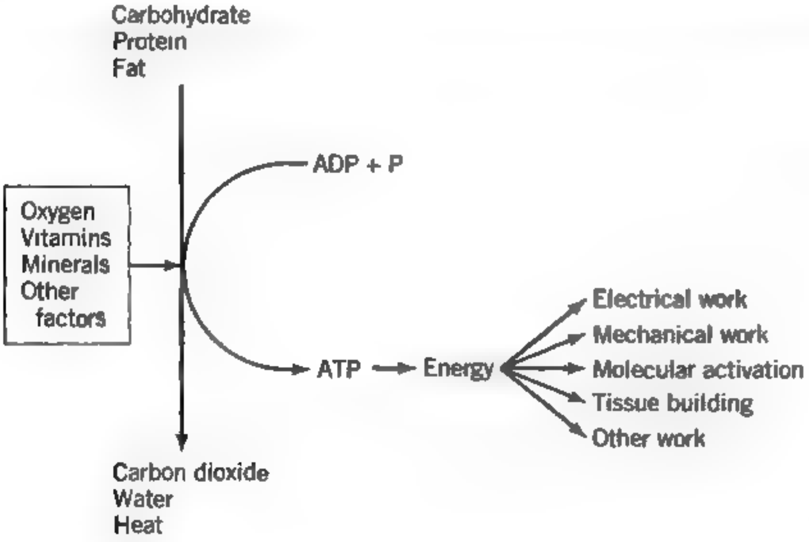

Carbohydrate
Protein
Fat
ADP + P
Oxygen
Vitamins
Minerals
Other factors
ATP
Energy
Electrical work
Mechanical work
Molecular activation
Tissue building
Other work
Carbon dioxide
Water
Heat

Figure 4. The role of ATP in body energy metabolism.

mol of glucose to 2 mol of pyruvic acid generates four hydrogen atoms and 8 mol of ATP. The hydrogen atoms released are eventually converted to water; the details are discussed below. Before the pyruvic acid can be converted to carbon dioxide, water, and energy, it must be transformed into a highly versatile metabolite, the two-carbon substance acetyl-CoA (Fig. 6). This transformation is irreversible.

Citric Acid Cycle. The citric acid cycle is a series of chemical reactions that metabolizes acetyl-CoA to carbon dioxide and hydrogen atoms, as indicated in Figures 7 and 8. In each cycle, two carbon dioxide molecules and four pairs of hydrogen atoms are put through the respiratory chain, together with the electrons, to generate 12 mol of ATP and 4 mol of water from oxygen. This cycle is the major link, or common path, in the transformation of carbohydrate, fat, and protein to carbon dioxide and water. Metabolites of the three nutrients enter the cycle at different strategic points. Because the cycle requires the respiratory chain to complete its work, it will not function in the absence of oxygen (anaerobically).

As indicated above, pyruvic acid may be removed from the glycolysis process by being converted to lactic acid. If so, there must be a source of hydrogen atoms, which are normally obtained from the production of phosphoglyceraldhyde (Fig. 6). In this case, glucose metabolism and energy (ATP) production can continue for a while without oxygen (that is, without going through the citric acid cycle). This anaerobic respiration occurs in muscle where an oc-

Table 4. Definitions of Some Metabolic Terms

Term	Definition
Glycolysis	The breaking down of hexoses (six carbon sugars), mainly glucose, into three-carbon substances (pyruvic or lactic acid); the process is sometimes termed the Embden-Meyerhof pathway
Glycogenesis	The formation of glycogen from glucose
Glycogenolysis	The breaking down of glycogen into glucose and its metabolites
Gluconeogenesis	The synthesis of glucose (and thus glycogen) from noncarbohydrate sources, such as lactate, glycerol, and amino acids
Citric acid cycle	Also termed the Krebs Cycle or tricarboxylic acid cycle; the process whereby carbohydrate, fat, and protein is completely oxidized to carbon dioxide, water, and energy; accomplished with the assistance of the respiratory chain
Respiratory chain	The transport of hydrogen atoms from biological oxidation for acceptance by oxygen atoms to form water molecules

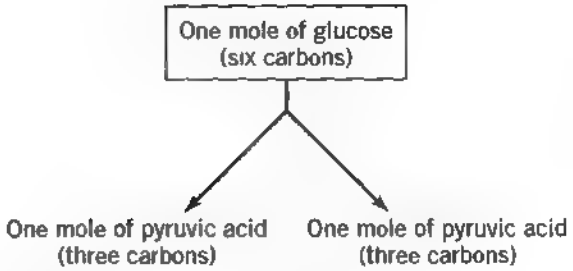

One mole of glucose
(six carbons)
One mole of pyruvic acid
(three carbons)
One mole of pyruvic acid
(three carbons)

Figure 5. The overall result of glycolysis.

casional burst of energy is needed. The lactic acid that accumulates is converted back to pyruvic acid when the oxygen supply is restored, in which case the citric acid cycle is reactivated. The soreness of muscle from heavy work or exercise results from the presence of a large amount of lactic acid.

Respiratory Chain. Biological oxidation, or the respiratory chain, is a very complicated process whereby hydrogen atoms released by substances through oxidation are transported by a number of intermediates until the hydrogen atoms are accepted by oxygen to produce water. The respiratory chain involves both oxidation and reduction. Ox-

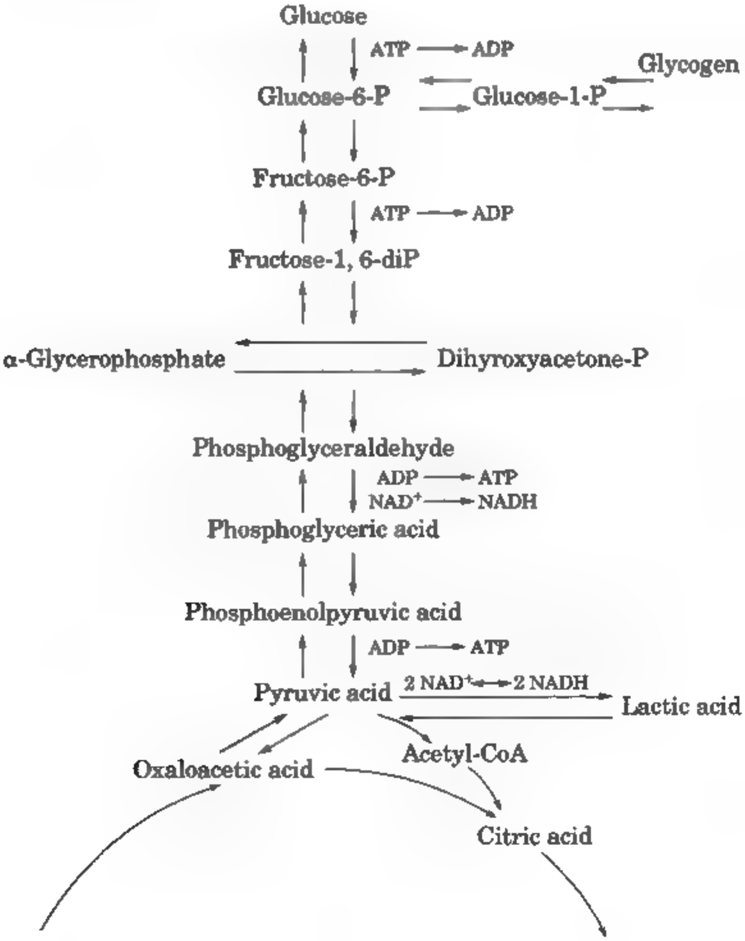

Glucose
ATP → ADP
Glycogen
Glucose-6-P
Glucose-1-P
Fructose-6-P
ATP → ADP
Fructose-1, 6-diP
α-Glycerophosphate
Dihyroxyacetone-P
Phosphoglyceraldehyde
ADP → ATP
NAD⁺ → NADH
Phosphoglyceric acid
Phosphoenolpyruvic acid
ADP → ATP
Pyruvic acid
2 NAD⁺ ← 2 NADH
Lactic acid
Acetyl-CoA
Oxaloacetic acid
Citric acid

Figure 6. Reactions involved in glycolysis.

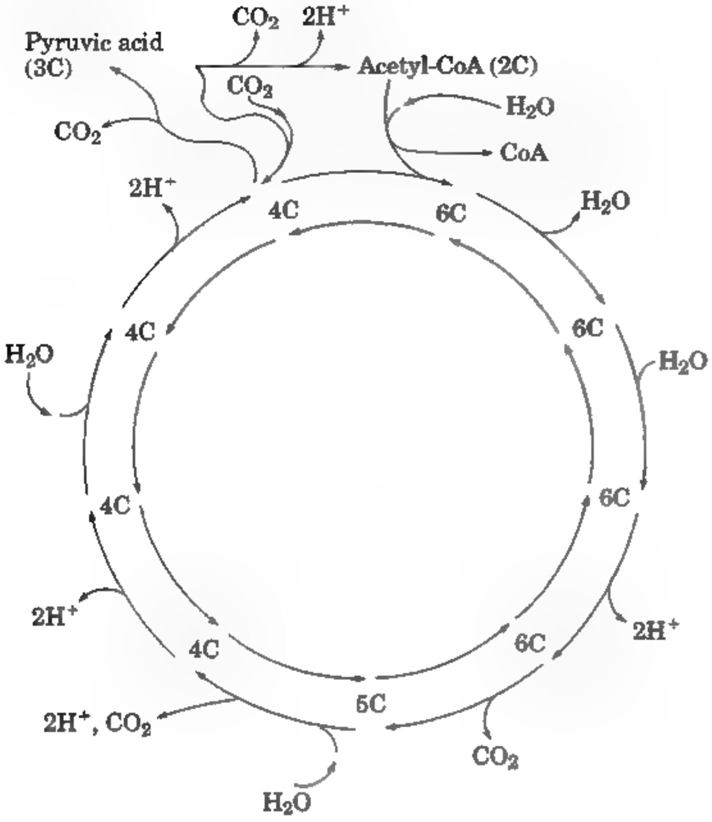

Pyruvic acid (3C)
CO₂
2H⁺
Acetyl-CoA (2C)
CO₂
H₂O
CoA
CO₂
2H⁺
4C
6C
H₂O
4C
6C
H₂O
H₂O
4C
6C
2H⁺
4C
6C
2H⁺
5C
2H⁺, CO₂
CO₂
H₂O

Figure 7. Outline of the citric acid cycle (C refers to a carbon atom).

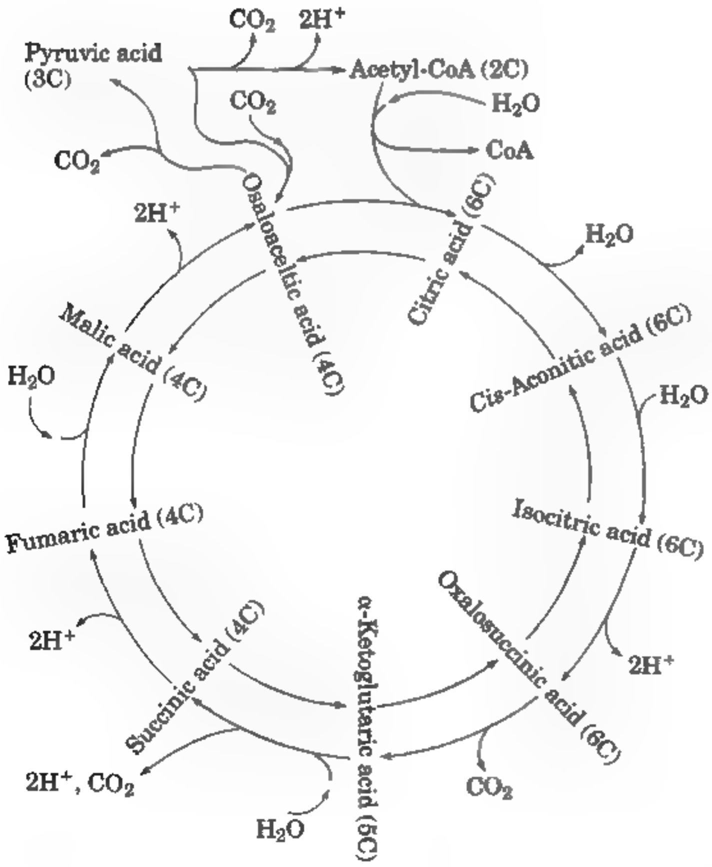

Pyruvic acid (3C)
CO₂
2H⁺
Acetyl-CoA (2C)
CO₂
H₂O
CoA
CO₂
2H⁺
Oxaloacetic acid (4C)
Citric acid (6C)
H₂O
Malic acid (4C)
Cis-Aconitic acid (6C)
H₂O
H₂O
Fumaric acid (4C)
Isocitric acid (6C)
2H⁺
Succinic acid (4C)
α-Ketoglutaric acid (5C)
Oxalosuccinic acid (6C)
2H⁺
2H⁺, CO₂
CO₂
H₂O

Figure 8. Components and products of the citric acid cycle.

idation is the process whereby a substrate either takes up oxygen or loses hydrogen. The substrate is oxidized, while the source substance that provides the oxygen or accepts hydrogen is the oxidizing agent. Reduction is the reverse process, whereby a substrate loses oxygen or accepts hydrogen. This substrate is reduced and the source substance that gains oxygen or loses hydrogen is the reducing agent.

The respiratory chain involves coenzymes (Tables 2 and 3) and consists of the following series of events:

1. Hydrogen atoms are released from a substrate, a process requiring an energy source.
2. The coenzyme NAD accepts hydrogen (NAD to $NADH_2$).
3. The hydrogen in NAD is accepted by FAD (FAD to $FADH_2$).
4. The hydrogen in $FADH_2$ is accepted by coenzyme Q.
5. The hydrogen in $CoQH_2$ is released as hydrogen by losing an electron, and a chain of cytochromes becomes reduced by accepting the electron.
6. The electron is transferred to molecular oxygen (O_2).
7. The negatively charged oxygen (O_2) reacts with two protons (H^-) to form water.

The entire process of the respiratory chain, including the formation of ATPs, is illustrated in Figure 9. The complete conversion of glucose to carbon dioxide, water, and energy is shown in Figure 10. The amount of energy used or stored is shown in Tables 5 and 6.

Glycogenesis and Glycogenolysis. In plants, carbohydrate is stored as starch, a polysaccharide of glucose. In animals, carbohydrate is stored in the form of glycogen, also a polysaccharide of glucose. The amount of glycogen stored in the body depends on the diet and the physiological status of the animal. In man, glycogen is produced and stored mainly in the liver and muscle. The process, known as glycogenesis, occurs readily when adequate glucose is present.

However, when the glucose concentration in the liver and muscle decreases, their glycogen content must be broken down to provide glucose for energy. This reverse process of glycogenesis is glycogenolysis. Its occurrence in the muscle is slightly different from that in the liver. In the liver, the glycogen can be directly degraded to glucose. However, in the muscle, the enzyme for the final step is missing so that glycogen is degraded only to glucose-6-phosphate, which has to be converted to pyruvic and lactic acids instead (Fig. 6). These can be converted to glucose via the citric cycle.

Gluconeogenesis. Gluconeogenesis is the synthesis of glucose (and thus glycogen) from noncarbohydrate sources such as amino acids, fatty acids, glycerol, and lactic and pyruvic acids. Figure 11 shows how muscle glycogen can be converted to glucose in the liver in spite of the muscle's lack of the appropriate enzyme. Figures 11 and 12 show how protein and fat can be converted to glucose. The interrelationship among the three nutrients, carbohydrate, protein, and fat, will be discussed at length in later sections.

Regulation of Blood Glucose

Another important aspect of what happens to carbohydrates in the body is the balancing of blood glucose (or blood sugar) levels. In a normal person, blood glucose fluctuates within narrow limits: between 70 and 100 mg/100 mL of blood. This is achieved by a balance between the supply and removal of blood glucose. If blood glucose drops below the norm, hypoglycemia occurs. In a healthy individual, the blood sugar is restored to normal by the provision of glucose from three sources. Simply eating additional carbohydrates increases the absorption of monosaccharides, and the liver can then release more glucose. Second, the glycogen in liver and muscle may be degraded (glycogenolysis) to form more glucose. Third, protein and fat may be degraded to provide glucose (gluconeogenesis).

If a person's blood glucose rises above the norm, hyperglycemia occurs. If the person is in normal health, the body spontaneously lowers the blood glucose levels in one or more of the following ways: (*1*) more insulin is released to drive glucose into cells for oxidation, (*2*) more glycogen is formed (glycogenesis) in the liver and muscle, (*3*) more glucose is changed to fat (lipogenesis) in fat cells, and (*4*) more glucose is excreted in the urine (glucosuria).

PROTEINS

Dietary protein exists in three forms. The major portion is conjugated with other substances, a small fraction is associated with fats and carbohydrates, and only a very small part exists as free protein, such as that in egg white. To be used by the cells, all proteins must be broken down into their constituent amino acids. In the sections that follow, the paths of protein digestion, absorption, and metabolism will be traced.

Digestion

Digestion of protein begins in the stomach (Table 1 and Fig. 13) where acid activates the pepsinogen (an enzyme) to release pepsin (another enzyme). The pepsin cleaves peptide linkages in the protein to produce polypeptides, each with two or more amino acids. When the stomach content reaches the duodenum, the pH is raised to about 6.5 by the presence of alkaline pancreatic juice. In the small intestine, chymotrypsin and trypsin hydrolyze most of the protein molecules to form small polypeptides and dipeptides. These are further digested to form free amino acids by the pancreatic enzyme carboxypeptidase and the intestinal enzymes aminopeptidase and dipeptidase. Those small peptides not split into individual amino acids may gain enter to mucosal cells to be digested later, for body tissues can utilize only amino acids.

Because protein molecules also contain nonpeptide linkages, they must be denatured first (by heat or stomach acid) before the appropriate enzymes can digest them. The denaturation process exposes the protein molecules, providing more surface area for enzymatic action. On the

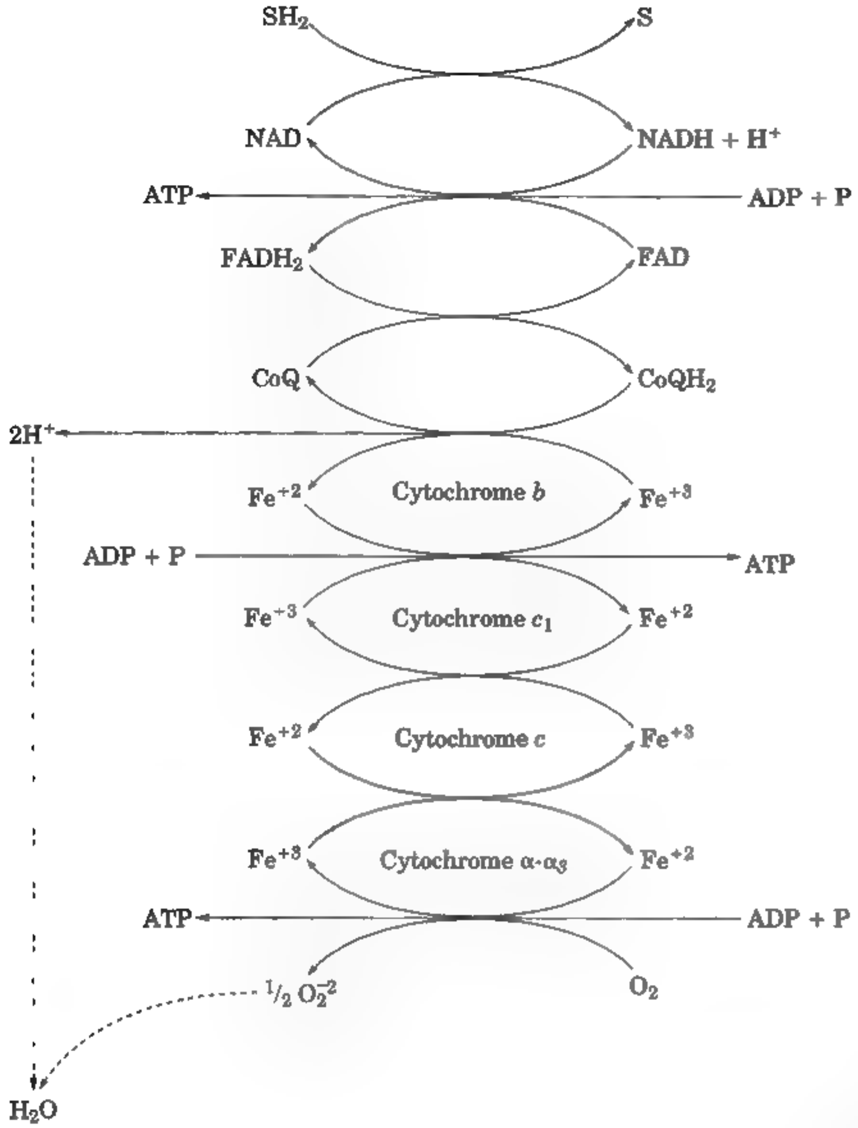


Figure 9. The respiratory chain or electron transport system (S = substrate).

other hand, excess heating or cooking can also reform some other linkages, making digestion more difficult.

Absorption

Normally only amino acids (and certain small peptides) are absorbed. In the small intestine all free amino acids, whether ingested or digested products, are absorbed via the mucosal cells into the hepatic portal vein. The D-amino acids are not absorbed as well as the L forms. The L-amino acids, the biologically active ones, are absorbed by active transport (a process requiring energy). Absorption occurs along the entire small intestine, with the slowest rate along the ileum. The stomach and colon may also absorb some amino acids. About 20–30% of ingested proteins are unabsorbed and excreted in the stools.

Although normally only amino acids are absorbed, it is well known that in small infants some undigested proteins are also absorbed. The subsequent antigen–antibody interaction causes the child to develop an allergic reaction when ingesting the same protein foods later. This explains the high allergic incidence among infants to foods such as eggs and cereals. If adults show allergy to ingested protein foods, they are still probably capable of absorbing whole protein molecules. For the majority of the population, this ability disappears with age.

Amino acids seem to be utilized best when they are absorbed in accordance with the body need for growth and function. Any excess absorbed will not be stored but excreted in the urine or metabolized to ammonia. Studies have shown that absorption and utilization of proteins are optimized when adults evenly distribute their protein intake throughout the day.

In some cases, erratic patterns of protein absorption may be due to stomach irregularities rather than irregular protein ingestion, for the stomach regulates the emptying of the nutrient into the small intestine. In patients with partially or completely removed stomachs, the rate of protein emptying is so disturbed that much of this valuable nutrient is lost in the fecal waste or degraded by intestinal

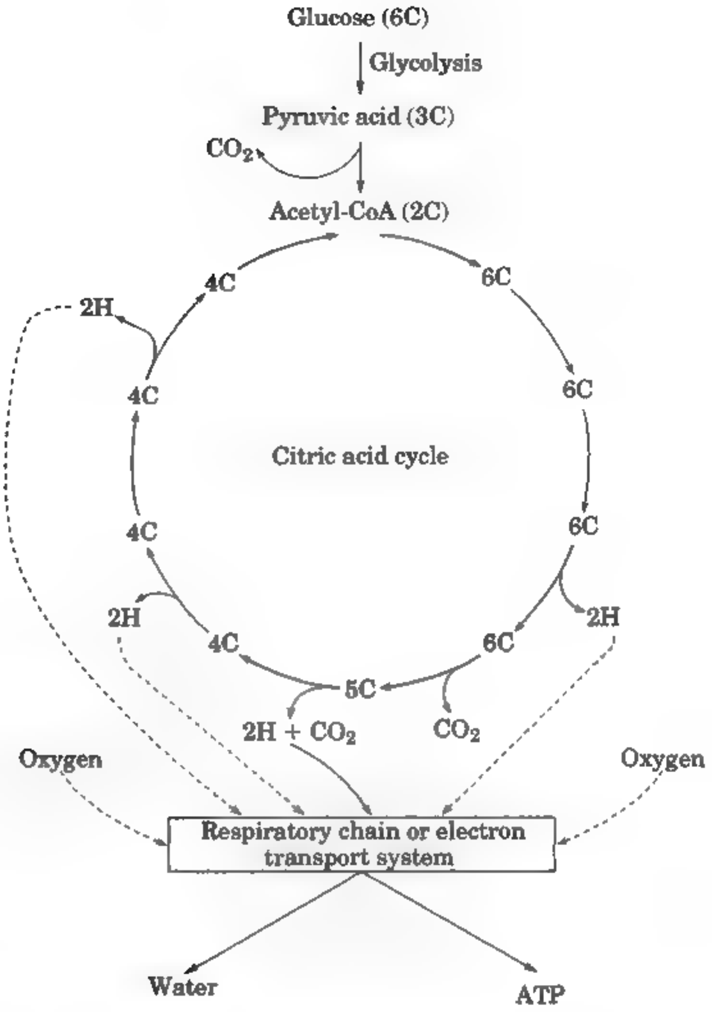

Glucose (6C)
Glycolysis
Pyruvic acid (3C)
CO2
Acetyl-CoA (2C)
6C
6C
6C
2H
6C
CO2
5C
2H + CO2
4C
2H
4C
4C
4C
2H
Citric acid cycle
Oxygen
Oxygen
Respiratory chain or electron transport system
Water
ATP

Figure 10. The three stages of converting glucose to carbon dioxide, water, and ATP.

Table 5. Energy Production during the Conversion of 1 Mol of Glucose to Carbon Dioxide and Water in the Presence of Oxygen

		Phosphorylation[a]			ATP	
Sequence	Product of specific reaction	SD	SP	OP	Loss	Gain
Glycolysis	Glucose-6-P	x			1	
	Fructose-1,6-diP	x			1	
	Phosphoglyceric acid		x			2
	Phosphoglyceraldehyde			x		6
	Pyruvic acid		x			2
Citric acid cycle	Acetyl-CoA			x		6
	Oxalosuccinic acid			x		6
	Succinyl-CoA			x		6
	Succinic acid		x			2
	Fumarate			x		4
	Oxaloacetate			x		6
Total number of ATP gained during the oxidation of 1 Mol of glucose under aerobic conditions						38

[a] SD = substrate dephosphorylation; SP = substrate phosphorylation; OP = oxidative phosphorylation.

Table 6. Energy Production during the Conversion of 1 Mol of Glucose to Carbon Dioxide and Water in the Presence of Oxygen

		Phosphorylation[a]			ATP		
Sequence	Product of specific reaction	SD	SP	OP	Loss	Gain	Hydrogen atoms
Glycolysis	Glucose-6-P	x			1		
	Fructose-1,6-diP	x			1		
	Phosphoglyceraldehyde			x			Released
	Phosphoglyceric acid		x			2	
	Pyruvic acid		x			2	
	Lactic acid						Accepted

[a] SD = substrate dephosphorylation; SP = substrate phosphorylation; OP = oxidative phosphorylation.

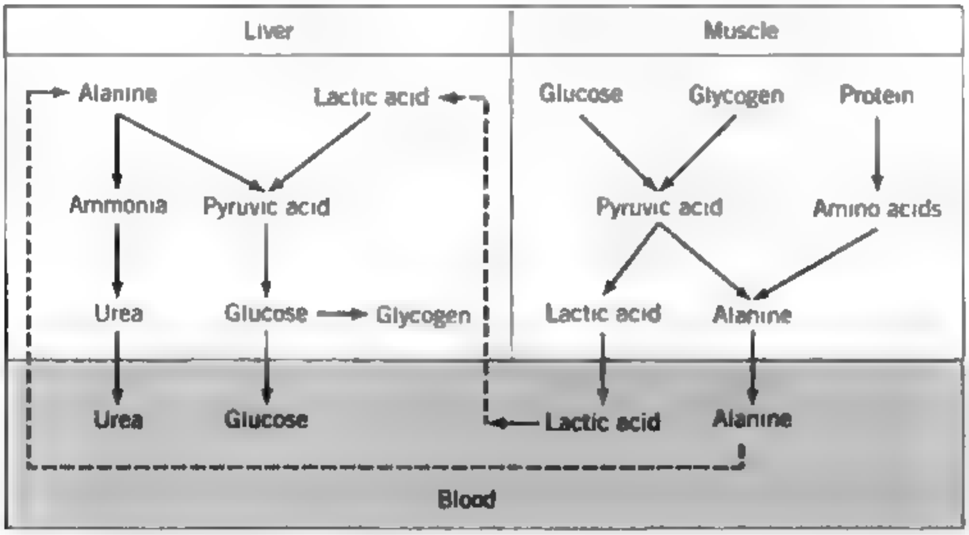

Liver
Muscle
Alanine
Lactic acid
Ammonia
Pyruvic acid
Urea
Glucose
Glycogen
Glucose
Glycogen
Protein
Pyruvic acid
Amino acids
Lactic acid
Alanine
Urea
Glucose
Lactic acid
Alanine
Blood

Figure 11. The role of liver and muscle in gluconeogenesis.

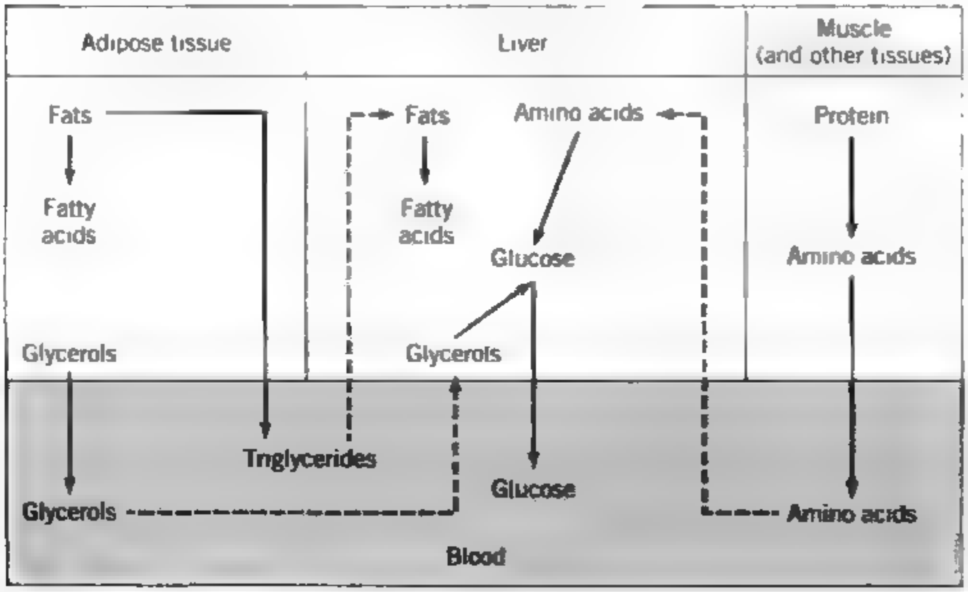

Adipose tissue
Liver
Muscle (and other tissues)
Fats
Fatty acids
Glycerols
Fats
Fatty acids
Amino acids
Glucose
Glycerols
Protein
Amino acids
Triglycerides
Glycerols
Glucose
Amino acids
Blood

Figure 12. An overview of glucose formation from fat and protein.

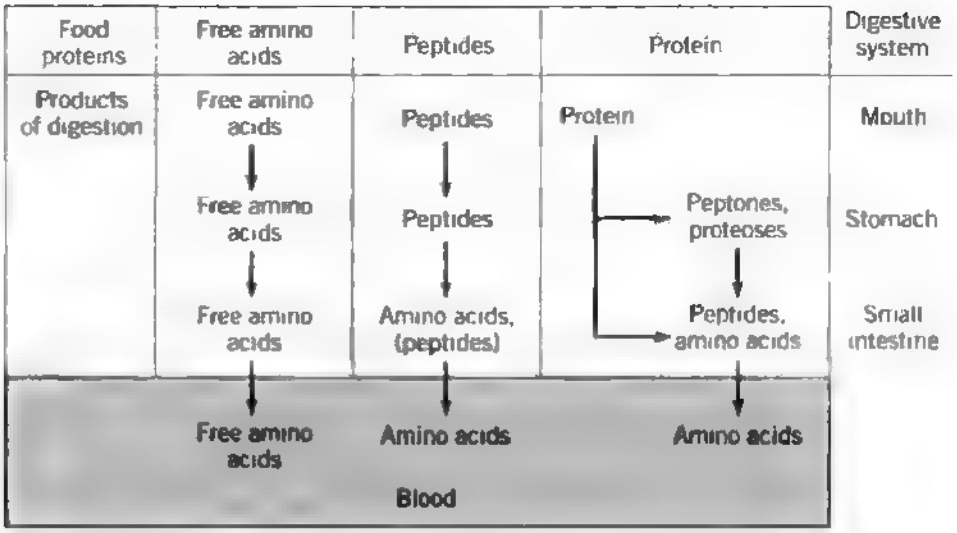


Figure 13. Protein digestion.

flora. When undigested amino acids are decarboxylated by intestinal bacteria, the important chemicals such as histamine, tyramine, ammonia, and similar substances are formed, many of which are absorbed. These substances are undesirable in excessive quantities. The ammonia formed by deamination plays a critical role in certain pathological conditions, for the absorbed ammonia can cause body deterioration. The absorption of amino acids is also impaired if the intestinal mucosa is damaged, as occurs in sprue, ulcerative colitis, and resection of a moderate amount of the small intestine. With time, however, there is a functional adaptation of the intestinal mucosa.

Metabolism

The liver may release some absorbed amino acids for body metabolism. Body amino acids may be catabolized or used in body repair, building, and maintenance. The importance of protein to the body is illustrated by its numerous functions and the complexity of its metabolism. If a normal person has a regular diet, three major factors determine the direction of protein metabolism: the quality and quantity of protein consumed, the amount of calories ingested, and the physiological and nutritional status of the body.

Protein metabolism revolves around a body pool of amino acids that are continuously released by protein hydrolysis and resynthesized. The amino acids ingested are the same as those released in the body. Body tissues can utilize only amino acids, not small peptides. About 50–100 g of body protein turns over daily, ranging from the slowest rate in the collagen to the fastest rate in the intestinal mucosa. Any amino acids filtered through the kidneys are reabsorbed, although certain congenital defects in the kidney tubules may interfere with this process. During pregnancy, infancy, childhood, and other conditions of growth, protein synthesis exceeds degradation. Individuals in these categories, therefore, require a large pool of amino acids.

Figure 14 illustrates the general catabolism, or degradation, of protein to amino acids and other metabolites. Figure 15 gives the general outline of protein formation in the body, and Figure 16 provides an overview of protein metabolism. The sections that follow discuss certain aspects of protein metabolism: degradation, protein synthesis, nitrogen balance, and metabolism of creatine and creatinine.

Protein Degradation. Protein is degraded to its individual amino acids in the muscle and other tissues, but the major site of actual destruction (catabolism) of each amino acid is the liver. The amino acids released from all other organs, especially the muscles, reach the blood and are diverted to the liver for degradation (Figure 14). Under normal circumstances, the catabolism of protein is balanced by its formation, although during stresses such as starvation and disease destruction can outstrip synthesis.

The procedure for the oxidation of an amino acid begins with deamination, a process in which the ammonia or amino group of the acid is removed so that only the carbon skeleton of the amino acid is left. Two metabolites are formed: the keto acids and ammonia (Fig. 14). The ammonia is changed to urea, which is released to the blood and eventually excreted in the kidney. Normally, we excrete about 20–30 g of urea in the urine each day. Because urea forms exclusively in the liver, advanced liver disorders raise the blood urea nitrogen level.

The keto acids formed from deamination enter the citric acid cycle to be oxidized. The exact process whereby keto acids and ammonia are formed varies with each individual amino acid, although the goal is the same. Figure 17 shows the points at which each of the acids and their keto acids enter the citric acid cycle or are transformed to pyruvate to form glucose. Within the citric acid cycle, the keto acids may be made to form glucose (gluconeogenesis). The keto acids are also tied to fat metabolism by the interconversion of keto acids and fatty acids. In sum, when protein is degraded amino acids are formed. Some amino acids circulate, some are oxidized, some are converted to glucose, and some are directed to other paths.

Protein Synthesis. Protein synthesis is the process of linking different amino acids by their amino groups (pep-

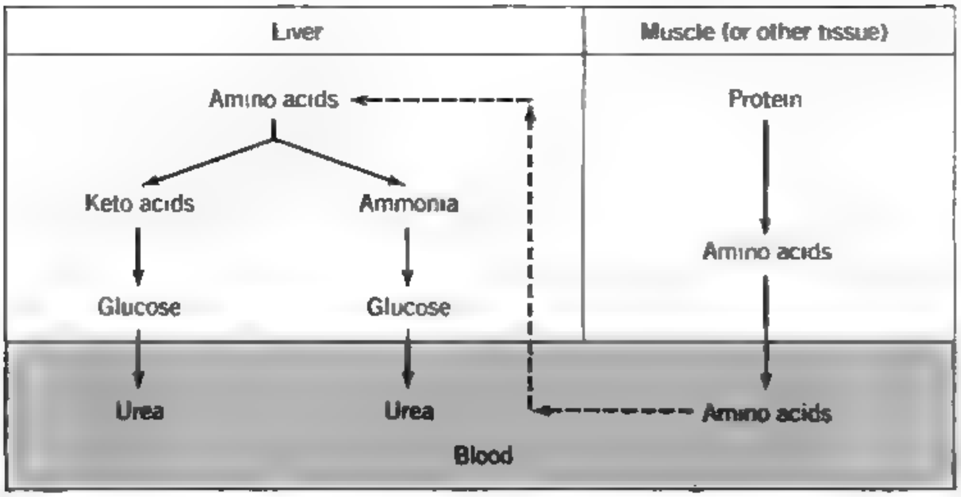

Liver
Muscle (or other tissue)
Amino acids
Keto acids
Ammonia
Glucose
Glucose
Urea
Urea
Protein
Amino acids
Amino acids
Blood

Figure 14. An overview of protein catabolism (degradation).

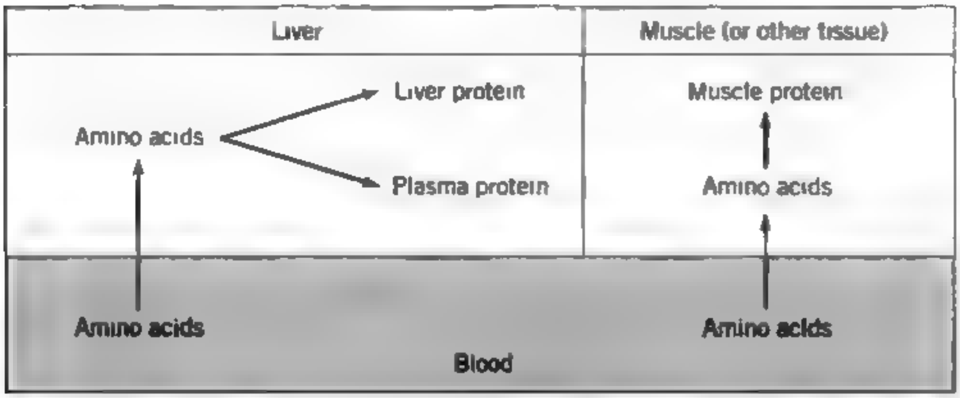

Liver
Muscle (or other tissue)
Liver protein
Amino acids
Plasma protein
Muscle protein
Amino acids
Amino acids
Amino acids
Blood

Figure 15. An overview of protein anabolism (synthesis).

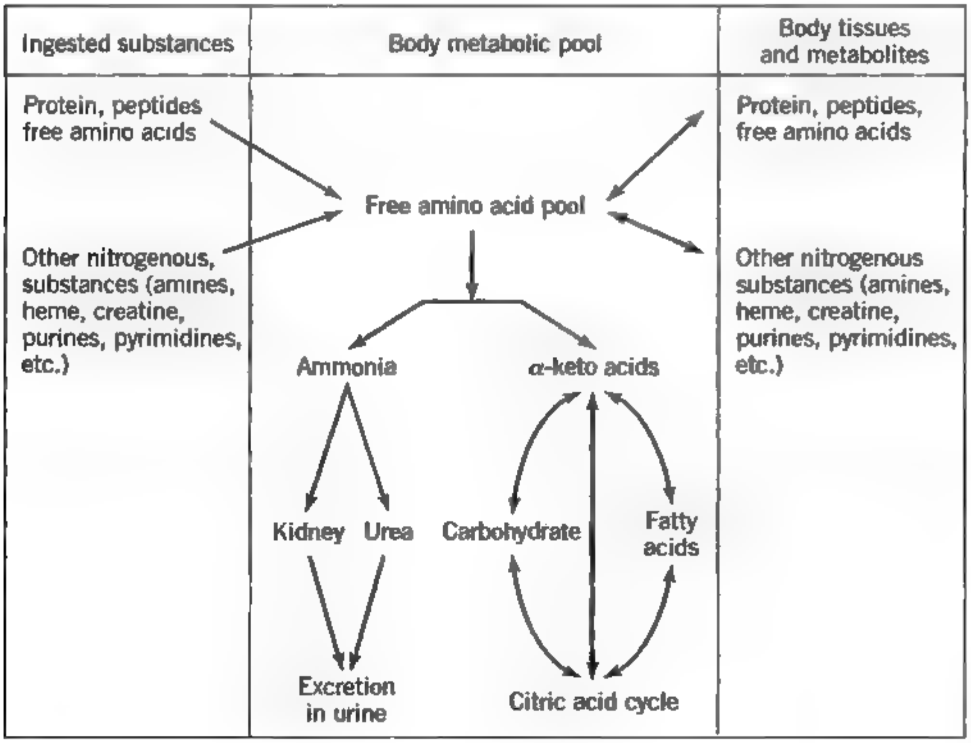

Ingested substances
Body metabolic pool
Body tissues and metabolites
Protein, peptides free amino acids
Other nitrogenous, substances (amines, heme, creatine, purines, pyrimidines, etc.)
Free amino acid pool
Protein, peptides, free amino acids
Other nitrogenous substances (amines, heme, creatine, purines, pyrimidines, etc.)
Ammonia
α-keto acids
Kidney
Urea
Carbohydrate
Fatty acids
Excretion in urine
Citric acid cycle

Figure 16. An overview of protein metabolism including nonprotein nitrogenous substances.

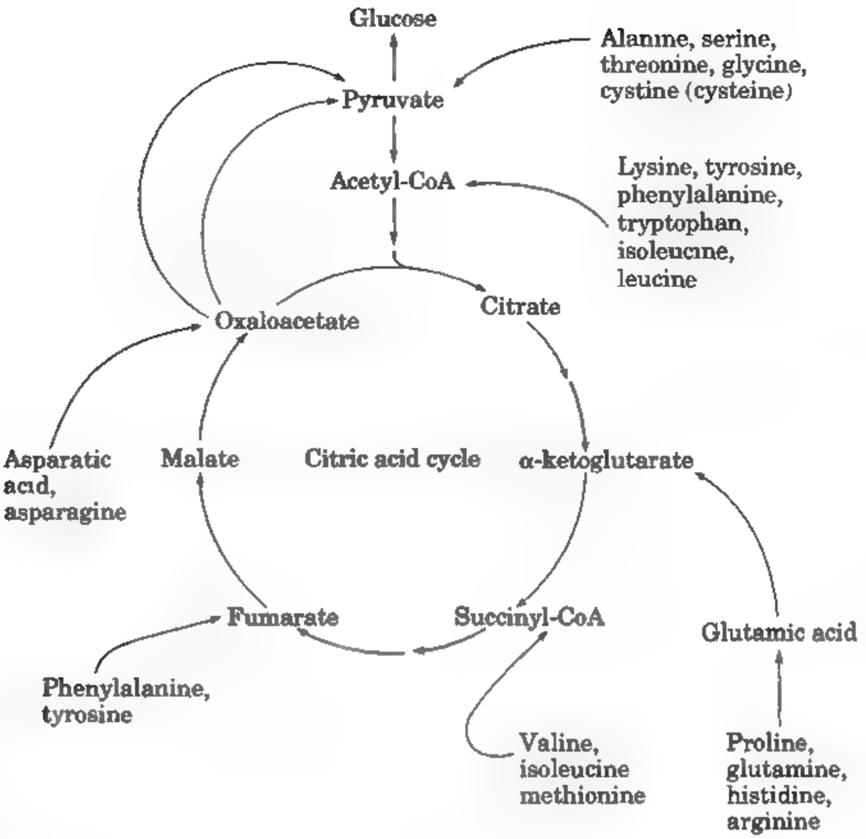

Glucose
Pyruvate
Alanine, serine, threonine, glycine, cystine (cysteine)
Acetyl-CoA
Lysine, tyrosine, phenylalanine, tryptophan, isoleucine, leucine
Citrate
Oxaloacetate
Asparatic acid, asparagine
Malate
Citric acid cycle
α-ketoglutarate
Fumarate
Succinyl-CoA
Glutamic acid
Phenylalanine, tyrosine
Valine, isoleucine methionine
Proline, glutamine, histidine, arginine

Figure 17. Introduction of the carbon structures of individual amino acids into the citric acid cycle.

tide linkages) to form a molecule of protein. This molecule must contain the right number of amino acids in the appropriate pattern and sequence needed for a specific body structure, such as hair, skin, muscle, tears, saliva, enzymes, or hormones. The nucleic acids DNA and RNA control this process, which takes place in cell nucleus cytoplasm. The formation of each specific protein requires a highly specific genetic code, the details of which may be obtained from a standard biochemistry or molecular biology text.

Although it occurs in nearly all cells, protein synthesis in some is more frequent and intense than in others. The organs that seem to synthesize the most protein are the liver, muscle, and those cells, tissues, and organs that manufacture or secrete enzymes, hormones, and other protein substances. One exception is the synthesis of plasma proteins by the liver. This occurs because red blood cells have no nuclei and are thus unable to synthesize protein themselves.

For a protein to be synthesized, the appropriate type and number of amino acids must be available. There are 8–10 amino acids whose carbon skeletons cannot be synthesized or manufactured by the body. They are called the essential amino acids and must be supplied in the diet. In addition, there are 10–15 amino acids that the body can manufacture (including their carbon skeletons), which are the nonessential amino acids. If the appropriate carbon skeleton of an amino acid is present, the body can add, subtract, and transfer the amino group until the right amino acid is formed. Protein synthesis is normally preceded by massive deamination, amination, and transamination to obtain the appropriate amino acids.

Nitrogen Balance. Of the three major nutrients protein is the most important in the sense that it makes up lean body mass. Another factor that makes protein important is that body protein turnover is tremendous. For example, the formation of hair, skin, saliva, and sweat all involve large losses of protein that must be constantly replaced. One acceptable technique to determine if there is enough protein in the diet is to measure the ingestion and excretion of nitrogen (nitrogen balance), because all protein has a relatively constant content of this element. Theoretically, if a young adult is in normal health and a proper stage of development, the amount of nitrogen consumed should be equal to the amount excreted. This is nitrogen equilibrium. However, depending on age, physiological condition, dietary intake, and other factors, some people are in positive nitrogen balance and others are in negative nitrogen balance.

Metabolism of Creatine and Creatinine. Body muscles perform work and may occasionally be required to provide a sudden burst of energy. They achieve this by the hydrolysis of two unique high-energy bonds. One is ATP and the other is phosphocreatine (or creatine phosphate), both of which were mentioned above. The body, mainly the muscles, contains about 100–150 g of phosphocreatine and creatine. Although not an amino acid, creatine is a unique nitrogenous chemical derived from three amino acids:

arginine, glycine, and methionine. Creatine, which is water soluble, is found in meat and meat products (such as extracts, soups, and gravies). Food contains little or no phosphocreatine, because of its easy degradation to creatine (or creatinine, another metabolite) and phosphoric acid (Fig. 18).

Phosphocreatine is a high-energy compound that provides instant energy when the muscles need it. If it is depleted, ATP is then used. Phosphocreatine seems to help maintain the ATP levels. After exertion and after all stored energy is used, creatine is rephosphorylated to form phosphocreatine.

Although an important body constituent, creatine is usually not excreted as such. Instead, the spontaneous, nonenzymatic dephosphorylation of phosphocreatine produces creatinine, in an irreversible process. Every healthy individual excretes a constant amount of creatinine, which is thus considered to be a normal waste product. The amount in the urine also reflects the amount of active muscle mass in the host. A woman excretes about 15–22 mg/kg, and a man about 20–26 mg/kg. Because creatinine is normally excreted rapidly, any increase in its level in the blood is a sign of kidney malfunction. Physicians attempting to make sure of the proper collection of urine frequently exploit the constancy of creatinine excretion. The volume of urine collected should reflect a 24-h excretion if the level of creatinine is within the normal range.

Although creatine is normally not excreted in the urine, children and women occasionally do dispose of the chemical in this manner. The excretion rate for women is especially high during and after pregnancy. The urinary level of creatine is also high in patients with diabetes, hyperthyroidism, and fever and those experiencing malnutrition or simple starvation. This reflects the degradation of musculature.

FATS

The digestion, absorption, transportation, and cellular metabolism of fats follow yet a third set of chemical and biological pathways in the body. However, they intersect the pathways of carbohydrates and proteins at several points. The following sections briefly describe the general fate of fats in the body.

Digestion, Absorption, and Transportation

Ingested fat meets its first significant digestive enzyme in the duodenum, where the exocrine gland of the pancreas provides the most important lipase (Table 1 and Fig. 19). The lipases in the saliva, stomach, and small intestine have only a small effect on fat digestion, as shown by the tremendous reduction in fat digestion when the pancreas is disabled. When the exocrine gland of the pancreas is not working properly, undigested and unabsorbed fat causes steatorrhea (bulky, clay-colored, fatty stools). The combined detergent actions of bile salts (from the gallbladder),

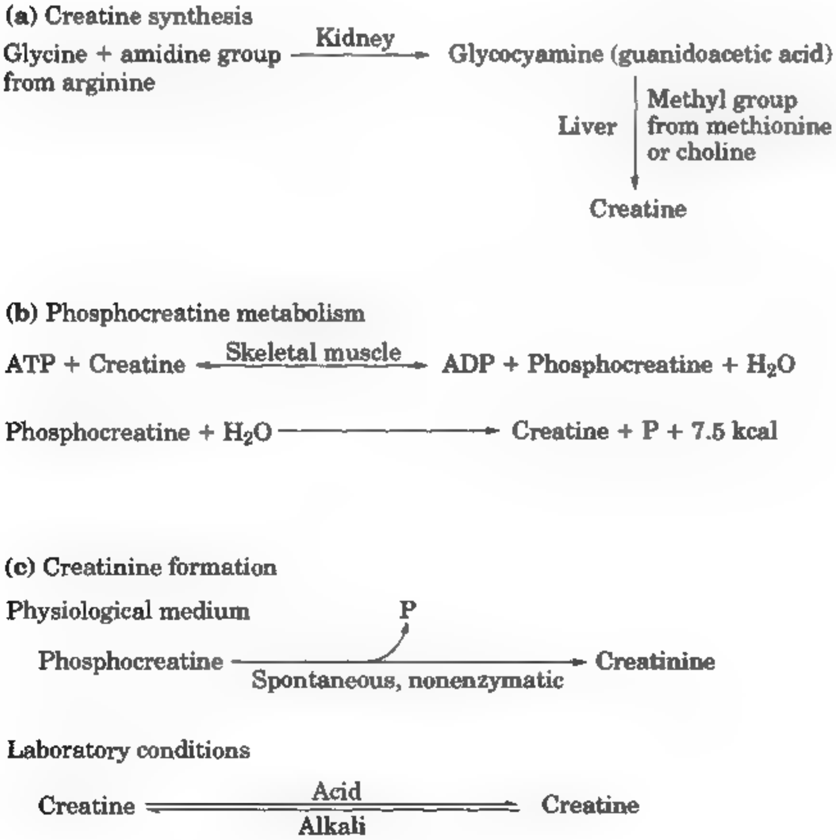

(a) Creatine synthesis
Glycine + amidine group from arginine
Kidney
Glycocyamine (guanidoacetic acid)
Liver
Methyl group from methionine or choline
Creatine
(b) Phosphocreatine metabolism
ATP + Creatine
Skeletal muscle
ADP + Phosphocreatine + H2O
Phosphocreatine + H2O
Creatine + P + 7.5 kcal
(c) Creatinine formation
Physiological medium
P
Phosphocreatine
Creatinine
Spontaneous, nonenzymatic
Laboratory conditions
Creatine
Acid
Alkali
Creatine

Figure 18. Metabolism of creatine and creatinine.

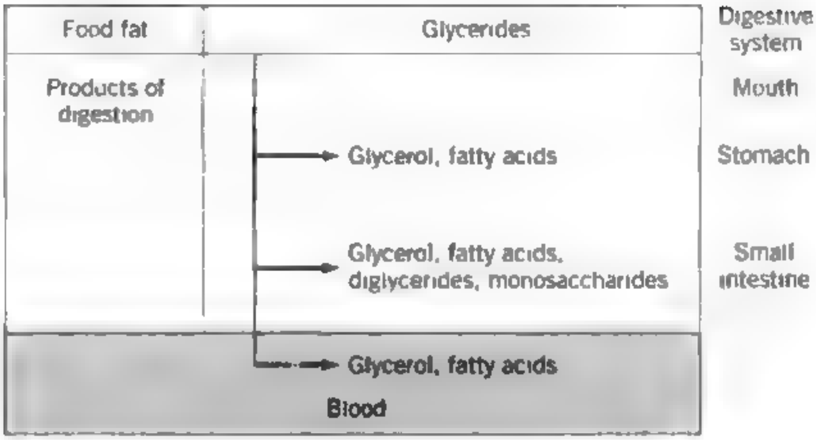


Figure 19. Triglyceride digestion.

fatty acids, and glycerides emulsify fat, thus facilitating its digestion by lipase.

In the small intestine, half of the ingested triglycerides are hydrolyzed by lipase to form free fatty acids and glycerols. The rest are changed to monoglycerides and a small amount of diglycerides. The monoglycerides are absorbed into the intestinal mucosa, where they are further hydrolyzed to glycerols and free fatty acids.

Free fatty acids in the intestine are absorbed in two ways. Fatty acids with less than 10–12 carbon atoms pass directly from the intestinal lumen, through mucosal cells, into the portal vein, and to the liver. Here some are released into circulation as free fatty acids, some are converted to triglycerides for deposition, and some are circulated in the blood as glycerides or as fatty acids that reside within the complex of lipoproteins. Fatty acids with more than 10–21 carbon atoms are absorbed into the mucosal cells, where they are regrouped with glycerols to form triglycerides. The triglycerides attach themselves to very low density lipoproteins to form chylomicrons, which enter the systemic circulation via the lymph and thoracic duct. Chylomicrons are fat globules 1 μm in diameter and visible under the microscope.

Other fatty substances are absorbed to varying degrees. For example, animal sterols are absorbed easier than plant sterols. Pancreatic secretion, fatty acids, and bile salts, which together emulsify and esterify cholesterol, are necessary for cholesterol absorption of cholesterol. It is currently believed that cholesterol is absorbed mainly in the ileum. Like the triglycerides, absorbed cholesterol is incorporated into the chylomicrons, which reach the systemic circulation.

Within two to three hours after the ingestion of food containing short-chain fatty acids, the blood level of chylomicrons remains unchanged, although it may rise sharply if the meal contains long-chain fatty acids. Normally, after a mixed meal, the plasma develops a milky appearance because of the presence of chylomicrons in the blood. This is sometimes known as lipemia. In the presence of the enzyme lipoprotein lipas, these plasma chylomicrons are cleared and their contents diverted to the liver and adipose tissue.

Blood plasma, therefore, contains fat in the following forms: fatty acids, glycerol, glycerides, cholesterol, cholesterol esters, and phospholipids. These forms are bound to the albumin, α-globulin, and β-globulin fractions of the plasma proteins. The resulting lipid-protein complexes have varying densities. The highest densities occur in those with the most protein and least lipid; the lowest densities occur in those with the least protein and most lipid. Consequently, the complexes are classified into high-density, low-density, and very low density lipoproteins. In general, very low density lipoproteins carry mainly triglycerides; low-density lipoproteins carry mainly cholesterol; and α-lipoproteins carry phospholipids, albumin, and free fatty acids. For a normal person, about 95% of ingested fat is absorbed, mainly in the duodenum and jejunum, with some absorption by the ileum. About 5% of fecal waste is fat, which comes from the diet, cell debris, and bacterial synthesis.

Although most of the fats are emptied into the lymphatic system after absorption and eventually reach the systemic circulation, the bile salts separate from the fats and travel through the portal vein into the liver. There they are reincorporated into the bile. Bile salts are thus cycled through the enterohepatic circulation (the liver, gallbladder, intestinal lumen, portal vein, and back to the liver). About 80–90% of bile salts in the intestinal lumen are reabsorbed in this way; the rest are lost in the stool.

Cellular Metabolism

The adult body distributes fats to two main locations: the membranes and other structural parts of cells (commonly called structural fats) and the fat cells (neutral fats), which are mainly white. Infants have some brown fat cells, which can regulate body temperature by producing heat to support the baby's higher metabolic rate. Neutral body fat contains mainly triglycerides, plus small amounts of diglycerides and monoglycerides, which are important metabolic intermediates. Consequently, triglycerides are the main form of stored energy.

Fat Degradation. Stored fat is degraded as needed to provide energy. Fat degradation occurs in two major stages: hydrolysis of glycerides and oxidation of fatty acids. In the adipose tissues, glycerides are hydrolyzed by a lipase to form fatty acids and glycerols. Both of these are released into the circulation for transport to the liver, where further hydrolysis may occur. When triglycerides are hydrolyzed, the released glycerols can be converted to phosphoglyceraldehyde in the liver (Fig. 6 and 20). This compound can in turn be converted to either carbon dioxide and water or glucose.

The process of oxidizing the fatty acids to carbon dioxide, water, and energy is called β-oxidation, or alternate oxidation. It occurs mainly in the mitochondria of liver cells. The carbon chain is broken down by the successive removal of two-carbon fragments from the carboxyl end to form acetic acids. These can combine with CoA to form acetyl-CoA, which can enter the citric acid cycle to be oxidized (Figs. 8, 17, and 20). When the fatty acids are reduced to acetyl-CoA, hydrogen atoms are also released, which can be passed on to the respiratory chain. When fatty acids are completely oxidized, they generate more ATP than the molecular equivalent of carbohydrate because less oxygen is present. This explains why fat has a higher caloric value. However, unsaturated fatty acids generate less energy than the molecular equivalent of saturated fatty acids because less hydrogen is present in the former.

Most of the naturally occurring fatty acids are even numbered, and thus their oxidation always produces acetyl-CoA at the end. However, if the fatty acids happen to be odd chained, propionyl-CoA is formed instead. Propionyl-CoA can also enter the citric acid cycle if the coenzyme with vitamin B_{12} is available.

Fat Synthesis. Fat synthesis takes place in two major stages: the formation of fatty acids and the formation of triglycerides. Fatty acids synthesis is achieved in two places: the mitochondria and the cytoplasm. Within the mitochondria, β-oxidation is reversed and two-carbon units are added until the appropriate fatty acids are formed. Outside the micochondria, in the cytoplasm, another form of fatty acid synthesis occurs. Here the starting compound is acetyl-CoA which serves as the end of the fatty acid molecule. The remaining carbons are incorporated as two-carbon units derived from the malonyl group. The incorporation is accompanied by simultaneous recarboxylation. The fatty acids formed are mainly 12–14 carbons long and rarely more than 16. The body can synthesize unsaturated fatty acids from the saturated ones by removing hydrogen, although it is unable to synthesize the essential ones.

In the adipose tissues, fatty acids combine with glycerol to form triglycerides, or neutral fats. This reaction occurs in the mitochondria. Figure 20 summarizes the information on fat synthesis.

As indicated earlier, glycerol can be converted to glucose (gluconeogenesis). However, acetyl-CoA cannot be converted to pyruvic acid (Fig. 10). Although keto acids can enter the citric acid cycle, there is very little net conversion

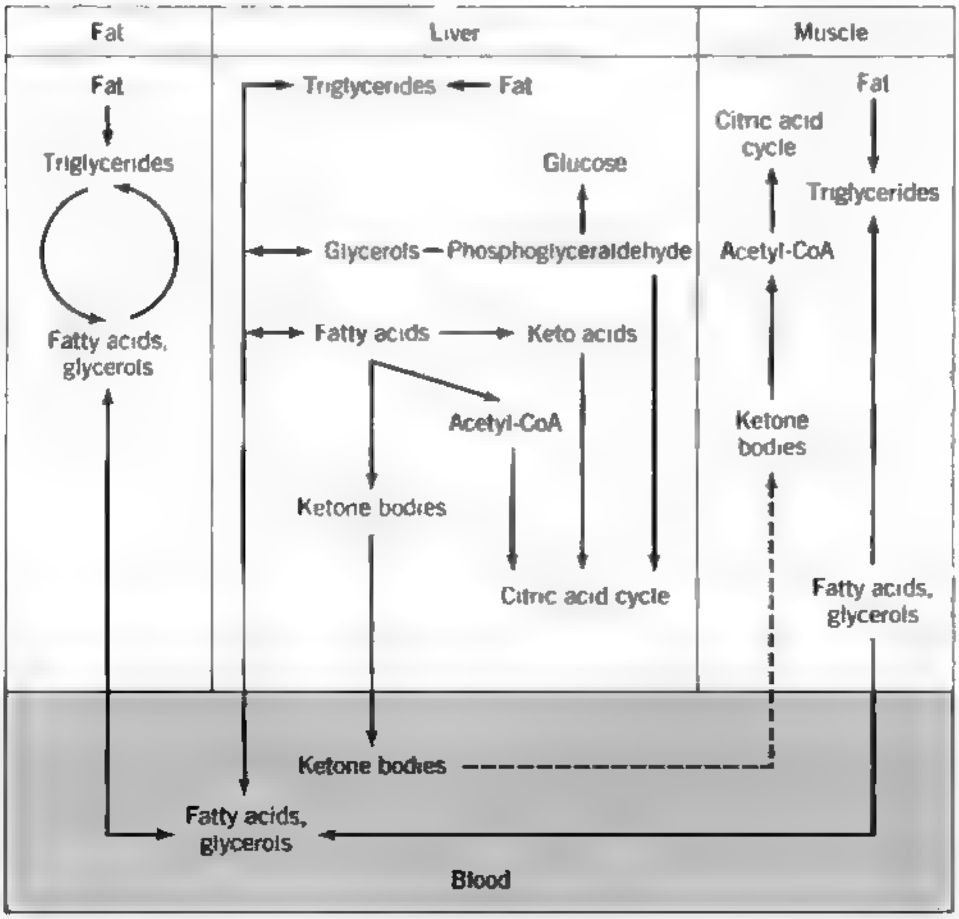


Figure 20. An overview of fat metabolism.

of fat to carbohydrate in the body, with the exception of the small amount of phosphogyceraldehyde formed from glycerol.

Ketone Bodies. During the normal process of β-oxidation of fatty acids, the liver has the appropriate enzyme to remove the CoA from acetoacetyl-CoA to form acetoacetic acid. Acetones and β-hydroxybutyric acids can be formed from acetoacetic acids. The last three compounds are collectively called ketone bodies. The small amount of ketone bodies normally made by the liver is transported by the circulation to the muscle for conversion to acetyl-CoA, which is put through the citric acid cycle (Fig. 20). Acetone is eliminated via urination and respiration. Because under normal circumstances the ketone bodies are metabolized as soon as they are formed, a person rarely excretes more than 1 mg of ketone each day, and blood levels are usually less than 1 mg/100 mL.

However, the ketones can accumulate under certain conditions, and the resulting clinical condition is known as ketosis. The main cause of ketosis is the accumulation of acetyl-CoA because the citric acid cycle in the liver is not operating at its normal or optimal efficiency. The most common cause is a sequence of events called intracellular carbohydrate starvation. First, decreased supply of glucose leads to a reduction in pyruvic acid, acetyl-CoA, and cellular energy supply. Second, for compensation, fatty acid oxidation is increased to provide energy with an accumulation of acetyl-CoA. Third, the oversupply of acetyl-CoA leads to the formation of ketone bodies.

Glucose supply to cells is reduced in people with diabetes mellitus and people who undergo dietary alterations

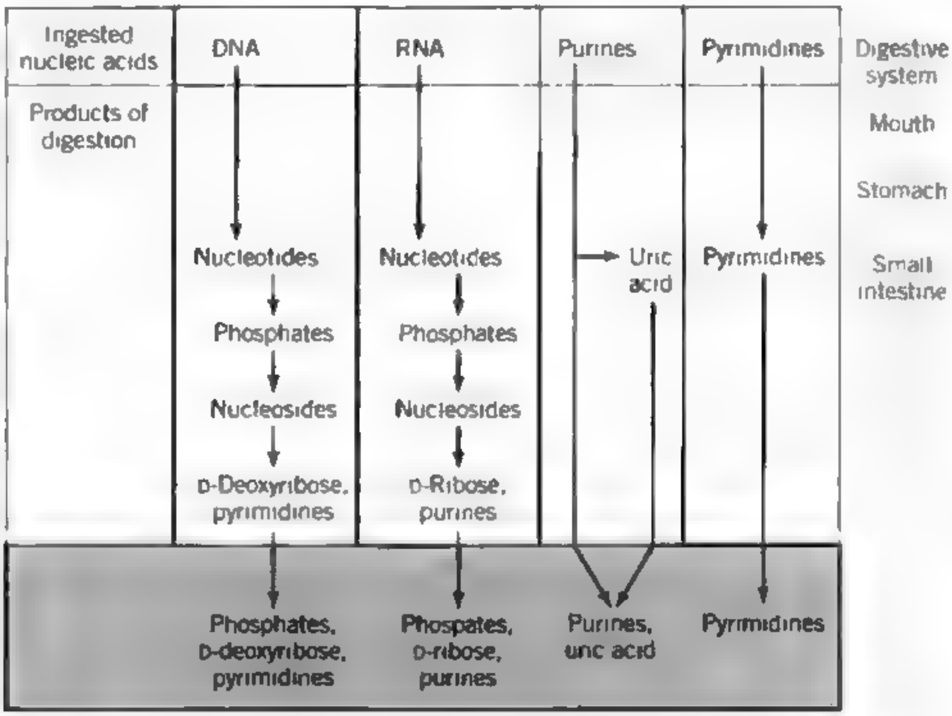

Ingested nucleic acids
DNA
RNA
Purines
Pyrimidines
Digestive system
Products of digestion
Mouth
Stomach
Nucleotides
Nucleotides
Uric acid
Pyrimidines
Small intestine
Phosphates
Phosphates
Nucleosides
Nucleosides
D-Deoxyribose, pyrimidines
D-Ribose, purines
Phosphates, D-deoxyribose, pyrimidines
Phospates, D-ribose, purines
Purines, uric acid
Pyrimidines

Figure 21. Digestion of nucleic acids.

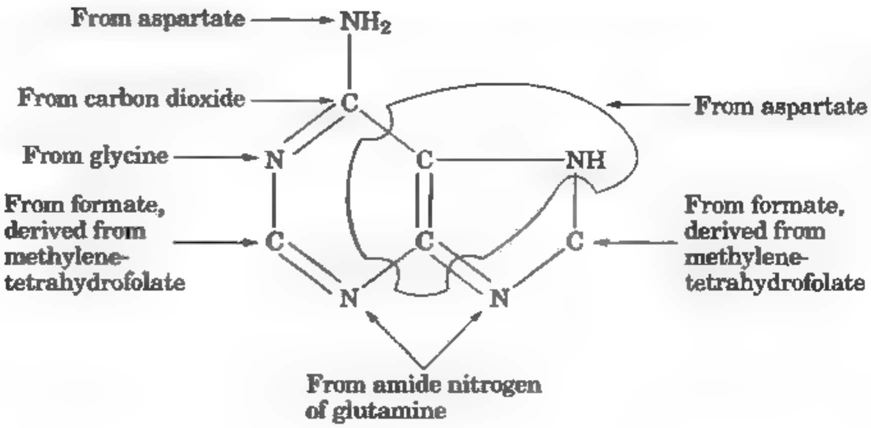

From aspartate
NH2
From carbon dioxide
C
From aspartate
From glycine
N
C
NH
From formate, derived from methylene-tetrahydrofolate
C
C
C
From formate, derived from methylene-tetrahydrofolate
N
N
From amide nitrogen of glutamine

Figure 22. Origins of the atoms in purine.

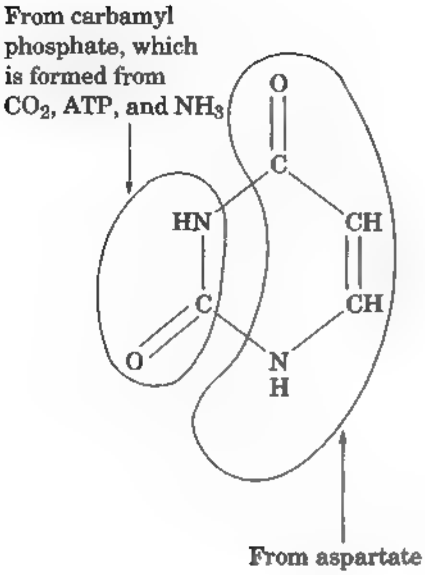


Figure 23. Origins of the atoms in pyrimidine.

such as high-fat—low-carbohydrate intake or simple starvation. In a diabetic patient, the lack of insulin prevents glucose from entering cells. When a persons's diet is low in calories, high in fat, or low in carbohydrate, a similar metabolic pattern takes place. The inadequate intake of carbohydrate means a low supply of glucose to cells, and ketosis may develop. However, an intravenous introduction of glucose counteracts ketosis, which is why carbohydrate is an antiketogenic agent.

Cholesterol Metabolism. Body metabolism of cholesterol is of special concern to nutritional scientists. Dietary cholesterol comes mainly from animal products such as fats, eggs, and organ meats. Sterols are obtained from plant foods, although they are not absorbed by the human digestive system. Ingested cholesterol is readily absorbed via the lymphatic system after esterification in the intestinal mucosa (with fatty acids). The body can also synthesize cholesterol, mainly in the intestinal mucosa and liver. It is currently believed that the amount of cholesterol synthesized by the body is inversely related to the quantity consumed. However, the problem of regulating serum cholesterol level by reducing dietary intake is a much debated issue.

Synthesis and degradation of cholesterol occur simultaneously and continuously. The body removes cholesterol by conjugating it with taurine or glycine in the liver and excreting it in the bile, although the enterohepatic circulation makes sure that some cholesterol is reabsorbed.

NUCLEIC ACIDS

Practically everything humans eat contains nucleic acids, which occur in cell chromosomes; nucleic acids are responsible for heredity. During digestion, ingested nucleic acids are initially cleaved into nucleotides by pancreatic nucleases (Fig. 21 and Table 1). Next, the small intestine secretes nucleotidase, which hydrolyzes nucleotides to form phosphoric acid and nucleosides. The latter are split by intestinal nucleosidases to form sugars, purine, and pyrimidine, all of which are absorbed by active transport. Undigested large molecules of nucleic acids are excreted in the stool.

Nucleic acids can also be synthesized. In the body, purines, pyrimidines, and sugars are put together to form ribonucleic acid (RNA), deoxyribonucleic acid (DNA), nicotinamide adenine dinucleotide (NAD), and other related substances. However, the liver can also make pyrimidines and purines. Figure 22 shows the origins of atoms in synthesized purine, and Figure 23 those of pyrimidine.

Within a cell, although DNA is stable throughout life, RNA is in constant equilibrium with a metabolic pool. Purines and pyrimidines may be excreted as such in the urine or be metabolized to uric acid (purines) or carbon dioxide and ammonia (pyrimidines).

Uric acid in the body comes from two sources: synthesis from glycine and degradation of purines. In humans, uric acid is excreted in the urine, although in most other mammals it is converted to allantoin before excretion. The normal blood level of uric acid is 4 mg/100 mL. The kidney reabsorbs much of the filtered uric acid, but the body excretes about 1 g of uric acid in 24 h. A standard reference text should be consulted for additional information on DNA, RNA, and molecular genetics.

WATER, VITAMINS, AND MINERALS

From the stomach to the colon, water passes freely and reversibly between the intestinal lumen and body compartments, although less so in the stomach than elsewhere in the gastrointestinal tract. Water moves in or out of the intestinal lumen to insure osmotic equilibrium on the two sides. In general, the osmolality of the contents of the small intestine resembles that of the plasma. After nutrients in the intestine have been absorbed, the excess water in the lumen is passed out with fecal waste to maintain osmotic equilibrium. Sodium moves freely according to the concentration gradient on the two sides of the mucosal cells. In the colon, sodium moves from body to luman in accordance with the osmotic gradient.

All water-soluble vitamins are absorbed along the small intestine. Except for vitamin B_{12}, a healthy person can absorb these vitamins rapidly. All fat-soluble vitamins require the presence of pancreatic enzyme, bile salts, glycerides, and fatty acids for absorption, as does fat itself. In a healthy person, all essential minerals are absorbed easily by the body, although the extent varies with individual minerals.

BIBLIOGRAPHY

This article has been adapted from Y. H. Hui, *Human Nutrition and Diet Therapy*. Jones and Bartlett, Boston, 1983. Used with permission.

GENERAL REFERENCES

D. A. Bender, *Amino Acid Metabolism*, 2nd ed., John Wiley & Sons, Inc., New York, 1985.

E. Braunwall and co-workers, *Harrison's Principles of Internal Medicine*, 11th ed., McGraw-Hill, Inc., New York, 1987.

M. D. Cashman, "Principles of Digestive Physiology for Clinical Nutrition," *Nutr. Clin. Pract.* 1, 241 (1986).

J. J. Cunningham, *Introduction to Nutritional Physiology*, G. F. Stickley, Philadelphia, 1983.

G L. Eastwood. *Core Textbook of Gastroenterology*, Lippincott, Philadelphia, 1984.

S. I. Fox, *Human Physiology*, 2nd ed., W. C. Brown, Dubuque, Iowa, 1987.

E. Goldberger, *A Primer of Water, Electrolyte and Acid-Base Syndromes*, 7th ed., Lea & Febiger, Philadelphia, 1986.

A. Guyton, *Textbook of Medical Physiology*, 3rd ed., Saunders, Philadelphia, 1986.

R. V. Heatley and co-workers, eds., *Clinical Nutrition in Gastroenterology*, Churchill, London, 1986.

Y. H Hui, *Human Nutrition and Diet Therapy*, Jones and Bartlett Publishers, Boston, 1983.

J O. Hunter and V. A. Jones, eds., *Food and the Gut*, Bailliere Tindall, London, 1985.

M. C. Linder, *Nutritional Biochemistry in Metabolism*. Elsevier Science Publishing Co., Inc , New York, 1985.

R. K. Murray and co-workers, *Harper's Biochemistry*. Appleton & Lange, Norwalk, Conn., 1988.

R. Pike and M. Brown, *Nutrition: An Integrated Approach*, 3rd ed., John Wiley & Sons, Inc., New York, 1985.

W. K. Stephenson, *Concepts in Biochemistry*, 3rd ed., John Wiley & Sons, Inc., New York, 1988.

L. Stryer, *Biochemistry*, 3rd ed., W. H. Freeman, New York, 1988.

G. V. Vahouny and co-workers, eds., *Dietary Fiber: Basic and Clinical Aspects*, Plenum Press, New York, 1986.

J. West, *Best and Taylor's Physiological Basis of Medical Practice*, 11th ed., Williams & Wilkins, Baltimore, Md., 1985.

P. C. Wilson and H. L. Greene, "The Gastrointestinal Tract: Portal to Nutrient Utilization," in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Disease*, 7th ed., Lea & Febiger, Philadelphia, 1988.

Y. H. Hui
American Food and Nutrition Center
Cutten, California

FOODBORNE DISEASES

All through history, human beings no doubt were affected by a great variety of foodborne diseases through consumption of water and food. No one knows for certain the number of cases of foodborne intoxications and infections occurring annually in the world. In the United States the Centers for Disease Control and Prevention reported that between 1988 and 1992 a total of 2423 outbreaks of foodborne diseases with 77,373 cases of illness. Among outbreaks for which the etiology is known, bacterial pathogens caused 79% of the outbreaks and 90% of the cases. Since many outbreaks and cases are not reported, the estimation of foodborne cases is between 6 and 33 million cases per year and from 525 to 9000 deaths. The total cost of productivity losses from a few major pathogens ranges from \$5 to \$6 billion per year. Class 1 recalls of food products for life-threatening bacteria increased from 79 recalls in 1988 to 378 recalls in 1995. In countries with poor sanitation, one can only surmise that the number of foodborne disease cases is much higher. There is a heightened awareness of the role of foodborne diseases by consumers in the United States due to some sensational outbreaks of foodborne diseases affecting a large number of people and the deaths of children after the consumption of undercooked hamburger or contaminated cheeses. As a result, the government has implemented greater monitoring programs and Hazard Analysis Critical Control Point (HACCP) procedures in the food industry to attempt to curtail the outbreaks and protect the safety of consumers. Consumers are much more aware of the great potential for large-scale foodborne outbreaks and demand a safer food supply. At the same time consumers are also demanding more fresh foods, minimally processed food, and organic foods where control of foodborne pathogens are more difficult. There is also a drastic demographic change in the society where more and more people live longer and thus have lower resistance to diseases in general, and more people are at risk due to immunocompromised diseases and conditions that make them more susceptible to foodborne diseases. Food distribution systems also have been greatly improved, and thus production of food in one location can be transported to hundreds and thousands of miles in a short time. When a problem occurs, the amount of food involved can be astronomical, such as a case of recalling 25 million pounds of ground beef due to one contamination source. The company involved is no longer in existence as a major player in food supply. Another important development is international trade. Vast amounts of food are regularly shipped from one country to another with minimal monitoring of the microbial safety of the food involved. To complicate matters further, there are actually now microorganisms that are emerging or reemerging in the food supply and make tracking and controlling of these organisms more difficult. Fortunately, with new developments in microbial detection methods and systems these microorganisms are being detected more frequently with better accuracy and rate (see the article RAPID METHODS OF MICROBIAL ANALYSIS). Also there are better and more efficient intervention strategies and methods in food processing to control unwanted microorganisms (see the articles FOOD FERMENTATION and MICROBIOLOGY OF FOODS). Thus food microbiologists, food scientists, epidemiologists, medical personnel, public health workers, and consumer educators are charged with the responsibility of studying the occurrence, enumeration, isolation, detection, characterization, prevention, reporting, education about, and control of foodborne microorganisms in and from food, water, and the environment nationally and internationally.

DEFINITIONS

Food intoxication is the ingestion of toxic compounds in foods, from chemical contamination or preformed by toxigenic microorganisms, by susceptible persons who later became ill.

Food infection is the ingestion of large numbers of viable, pathogenic microorganisms in food by susceptible persons who later became ill. Usually the number is around one million live organisms per gram of food, but

E. Braunwall and co-workers, *Harrison's Principles of Internal Medicine*, 11th ed., McGraw-Hill, Inc., New York, 1987.

M. D. Cashman, "Principles of Digestive Physiology for Clinical Nutrition," *Nutr. Clin. Pract.* 1, 241 (1986).

J. J. Cunningham, *Introduction to Nutritional Physiology*, G. F. Stickley, Philadelphia, 1983.

G L. Eastwood. *Core Textbook of Gastroenterology*, Lippincott, Philadelphia, 1984.

S. I. Fox, *Human Physiology*, 2nd ed., W. C. Brown, Dubuque, Iowa, 1987.

E. Goldberger, *A Primer of Water, Electrolyte and Acid-Base Syndromes*, 7th ed., Lea & Febiger, Philadelphia, 1986.

A. Guyton, *Textbook of Medical Physiology*, 3rd ed., Saunders, Philadelphia, 1986.

R. V. Heatley and co-workers, eds., *Clinical Nutrition in Gastroenterology*, Churchill, London, 1986.

Y. H Hui, *Human Nutrition and Diet Therapy*, Jones and Bartlett Publishers, Boston, 1983.

J O. Hunter and V. A. Jones, eds., *Food and the Gut*, Bailliere Tindall, London, 1985.

M. C. Linder, *Nutritional Biochemistry in Metabolism*. Elsevier Science Publishing Co., Inc , New York, 1985.

R. K. Murray and co-workers, *Harper's Biochemistry*. Appleton & Lange, Norwalk, Conn., 1988.

R. Pike and M. Brown, *Nutrition: An Integrated Approach*, 3rd ed., John Wiley & Sons, Inc., New York, 1985.

W. K. Stephenson, *Concepts in Biochemistry*, 3rd ed., John Wiley & Sons, Inc., New York, 1988.

L. Stryer, *Biochemistry*, 3rd ed., W. H. Freeman, New York, 1988.

G. V. Vahouny and co-workers, eds., *Dietary Fiber: Basic and Clinical Aspects*, Plenum Press, New York, 1986.

J. West, *Best and Taylor's Physiological Basis of Medical Practice*, 11th ed., Williams & Wilkins, Baltimore, Md., 1985.

P. C. Wilson and H. L. Greene, "The Gastrointestinal Tract: Portal to Nutrient Utilization," in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Disease*, 7th ed., Lea & Febiger, Philadelphia, 1988.

Y. H. Hui
American Food and Nutrition Center
Cutten, California

FOODBORNE DISEASES

All through history, human beings no doubt were affected by a great variety of foodborne diseases through consumption of water and food. No one knows for certain the number of cases of foodborne intoxications and infections occurring annually in the world. In the United States the Centers for Disease Control and Prevention reported that between 1988 and 1992 a total of 2423 outbreaks of foodborne diseases with 77,373 cases of illness. Among outbreaks for which the etiology is known, bacterial pathogens caused 79% of the outbreaks and 90% of the cases. Since many outbreaks and cases are not reported, the estimation of foodborne cases is between 6 and 33 million cases per year and from 525 to 9000 deaths. The total cost of productivity losses from a few major pathogens ranges from $5 to $6 billion per year. Class 1 recalls of food products for life-threatening bacteria increased from 79 recalls in 1988 to 378 recalls in 1995. In countries with poor sanitation, one can only surmise that the number of foodborne disease cases is much higher. There is a heightened awareness of the role of foodborne diseases by consumers in the United States due to some sensational outbreaks of foodborne diseases affecting a large number of people and the deaths of children after the consumption of undercooked hamburger or contaminated cheeses. As a result, the government has implemented greater monitoring programs and Hazard Analysis Critical Control Point (HACCP) procedures in the food industry to attempt to curtail the outbreaks and protect the safety of consumers. Consumers are much more aware of the great potential for large-scale foodborne outbreaks and demand a safer food supply. At the same time consumers are also demanding more fresh foods, minimally processed food, and organic foods where control of foodborne pathogens are more difficult. There is also a drastic demographic change in the society where more and more people live longer and thus have lower resistance to diseases in general, and more people are at risk due to immunocompromised diseases and conditions that make them more susceptible to foodborne diseases. Food distribution systems also have been greatly improved, and thus production of food in one location can be transported to hundreds and thousands of miles in a short time. When a problem occurs, the amount of food involved can be astronomical, such as a case of recalling 25 million pounds of ground beef due to one contamination source. The company involved is no longer in existence as a major player in food supply. Another important development is international trade. Vast amounts of food are regularly shipped from one country to another with minimal monitoring of the microbial safety of the food involved. To complicate matters further, there are actually now microorganisms that are emerging or reemerging in the food supply and make tracking and controlling of these organisms more difficult. Fortunately, with new developments in microbial detection methods and systems these microorganisms are being detected more frequently with better accuracy and rate (see the article RAPID METHODS OF MICROBIAL ANALYSIS). Also there are better and more efficient intervention strategies and methods in food processing to control unwanted microorganisms (see the articles FOOD FERMENTATION and MICROBIOLOGY OF FOODS). Thus food microbiologists, food scientists, epidemiologists, medical personnel, public health workers, and consumer educators are charged with the responsibility of studying the occurrence, enumeration, isolation, detection, characterization, prevention, reporting, education about, and control of foodborne microorganisms in and from food, water, and the environment nationally and internationally.

DEFINITIONS

Food intoxication is the ingestion of toxic compounds in foods, from chemical contamination or preformed by toxigenic microorganisms, by susceptible persons who later became ill.

Food infection is the ingestion of large numbers of viable, pathogenic microorganisms in food by susceptible persons who later became ill. Usually the number is around one million live organisms per gram of food, but there are cases that at low as 100 cells can become infectious.

Food poisoning is the ingestion of contaminated food containing either chemical preformed toxins or live microbes by susceptible persons who later became ill.

Foodborne outbreak is the consumption of contaminated food from one source by two or many people who later became ill. In the case of botulism one affected person will constitute one outbreak.

Foodborne disease case is the consumption of contaminated food by one susceptible person who later became ill. A foodborne outbreak can have 2 cases or 100,000 cases, with the exception of botulism, when one case is considered one outbreak.

Endemic is the usual cases of a particular illness in a community.

Epidemic is an unusual, large number of cases of a particular illness from a single source in a community.

Pandemic is a disease affecting the entire world.

Epidemiology is the study of diseases in a population using statistical methods. An epidemiologist studies patterns of diseases and their causative agents in terms of a population, whereas a physician treats individual patients.

Etiologic agent is the agent that caused a specific disease.

FOOD INTOXICATIONS

Chemical intoxications are usually the result of accidents. People have been poisoned by inorganic compounds such as antimony, arsenic, cyanide, cadmium, lead, selenium, and mercury. The symptoms usually occur rapidly (a few minutes or hours), and reactions are usually violent in cases of ingestion of large doses of the toxic compounds. Immediate medical assistance is essential for the victims in such cases.

Long-term chemical intoxication is also possible by the ingestion of a small amount of toxins in food or water over many years and later the person became ill. There are also many naturally occurring chemical toxins in foods as well as unintentionally added chemical toxins such as pesticides and chemical residuals from packaging materials and the environment.

BACTERIAL AND MICROBIAL INTOXICATION

This form of intoxication is the result of consumption of preformed toxic compounds in the food by susceptible persons. The effects can be rapid (within hours) but usually are longer than chemical intoxication.

Clostridium botulinum

The first recorded outbreak of botulism was in 1793 involving sausages (*botulus*) in Germany. Since that time many outbreaks all over the world have been reported. From 1899 to 1977 there were 766 outbreaks involving 1961 cases and 999 deaths. In the United States between 1971 and 1985, three outbreaks were recorded with 485 cases and 55 deaths. In 1990 there were 12 outbreaks, 22 cases, and 5 deaths and in 1994 there were 42 cases of foodborne botulism, 86 cases of infant botulism, and 11 cases of wound botulism with no fatality. The general public usually have panic in the report of botulism due to its high fatality rate. The organism is a Gram-positive, anaerobic, spore-forming rod that can grow at temperatures from 3.3°C to as high as 50°C. Most strains will grow well at 30°C, with optimum temperature at 37°C. To control the growth of the organism the pH must be below 4.6, salt content of 10% and above, and a water activity of less than 0.94. The vegetative cells of this organism are easily killed by heat, but the spores formed by this cell are far more resistant to heat, cold, acid and basic chemicals, radiation, and other forms of preservation methods. Thus control of botulism is geared to the destruction of the spores. Time and temperature combinations of canning of all foods are designed to kill the most heat-resistant spores of *Clostridium botulinum*. The spores formed by this organism can reside in soil, water, and the environment and can be transmitted to foods. Foods involved in botulism cases usually are improperly home-canned medium- or low-acid foods. Information since 1899 indicates that about 70% of the outbreaks can be traced to improperly processed home-canned foods and 9% to commercially processed food, with the other outbreaks from unknown sources. Symptoms develop from 18 to 96 h after ingestion of toxic foods. They include vomiting, nausea, fatigue, dizziness, vertigo, headache, dryness of mouth, muscle paralysis, and death by asphyxiation. Since the toxin affects peripheral nerves the patient is alert until the moment of death. There are several types of botulin toxins (types A, B, C_1, C_2, D, E, F, and G). These are large molecular weight proteins (about 1 million dalton). The important toxins affecting human beings are toxins A, B, and E, and rarely F. These toxins are among the most toxic materials produced by a biologic system. It was estimated that one pure ounce of toxin can kill 200 million people. Treatment is by administration of monovalent E, bivalent AB, trivalent ABE, or polyvalent ABCDEF antisera. Fortunately, the toxins are heat sensitive. Boiling of the toxin for 10 min will destroy it. The toxins can be detected by animal tests using mice as well as immunologic tests using specific antibodies (gel diffusion tests, ELISA, RIA tests, etc). Recently a rapid polymerase chain reaction (PCR) method has been employed to detect the botulin gene harbored by cultures isolated from foods. The information, however, does not directly imply that the food is toxigenic and harbors the toxin. The key to preventing botulism is to know the composition (pH, A_w oxidation-reduction potential, presence of inhibitory compounds, etc) of the food and to utilize proper time and temperature for processing as well as correct packaging and storage of the processed food. All high-moisture, low-acid foods processed and then stored under anaerobic conditions either in cans, glass bottles, or pouches should be subject to close scrutiny to avoid the possibility of *C. botulinum* surviving and later germinating and producing the toxins. Since there are proteolytic and nonproteolytic strains of *C. botulinum* the absence of off-odor of a suspected canned food cannot guarantee the safety of the food.

Never taste a suspected food or use food from swollen, dented, or deformed cans. When in doubt, always boil the suspected food for 10 min before discarding it.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive facultative anaerobic coccus occurring in clusters. The organisms is ubiquitous and can be found in human skin, nose, hair, and many food items. The organisms, when allowed to grow in food, may produce a class of low-molecular-weight (ca 30,000 daltons) protein toxins called staphylococcal enterotoxins (A, B, C_1, C_2, C_3, D, and E). These toxins, when ingested by a susceptible person, will cause severe nausea, vomiting, abdominal cramps, diarrhea, and prostration about 4 to 6 h after consumption. Recovery is about 24 to 72 h: Victims will not die, but may wish they had, as the reactions are very violent. Also there is no immunity against the toxin, thus a person can have staphylococcal intoxication repeatedly. Along with *Salmonella* and *Clostridium perfringens*, staphylococcal intoxication ranks among the top three agents of foodborne disease in the past 25 years. In 1992 there were six outbreaks involving 206 cases with no death. Since this is a nonreportable disease, many more outbreaks and cases occur regularly without being reported to public health officials. These toxins are heat stable. Once formed in food the toxin is very hard to destroy. Heating the toxins at 80°C for 5 h will not destroy the toxin. Boiling for 3 h will destroy the toxin and cooking under pressure (121°C) will inactive the toxin in 30 min. For practical purposes these toxins are not inactivated by normal cooking procedures.

Due to the fact that the enterotoxins are heat stable, detection of live *S. aureus* in foods has only limited value in terms of assessment of the potential of the food to cause staphylococcal food intoxication. For example, assume a food is contaminated with a toxigenic strain of *S. aureus* and the organisms grew to 1 million cells and released large amount of enterotoxins (in micrograms) in the food and the food is subsequently cooked. Even though live *S. aureus* cannot be found in this food item, the food is still capable of causing a case of staphylococcal intoxication due to the heat-stable toxins in the food. Several years ago there was a big concern regarding canned mushrooms that were shipped from a foreign country to the United States. Certainly no live *S. aureus* were found in canned mushrooms, yet the preformed enterotoxins in the canned mushrooms caused many cases of food intoxication.

The value of monitoring live *S. aureus* is to ascertain the hygienic quality of the food and the potential of the live organisms to grow and produce the enterotoxins in foods. Detection of staphylococcal enterotoxins has been a subject of much research in the past 25 years. Monkey, cats, and other animals have been used to detect toxins but are not practical for routine testing. Immunological methods such as the ELISA test, Latex agglutination tests, and gel diffusion tests are used to detect the toxin in foods. The commercial kits can detect enterotoxin A, B, C, D, and/or E either singularly or in combination.

Fortunately *S. aureus* is not a good competitor compared with other spoilage organisms (eg, *Pseudomonas*) in raw foods such as ground beef and fish, and so on. However, in the absence of competitors, such as in salty food (eg, ham) or processed foods (eg, processed cheese), the organism can grow and produce heat-stable toxins. Enough toxins can be produced in 4 h at room temperature to cause a problem. That is why this intoxication is called picnic food poisoning; during a picnic, food may be left nonrefrigerated for hours before consumption by partygoers after various activities. It is therefore essential to prevent *S. aureus* from growing in the food by proper refrigeration (4°C) of food or keeping hot food hot (60°C). This advice is applicable to all subsequent discussions on food intoxication and infections.

Aspergillus

Aspergillus flavus and *A. parasiticus* are molds that can produce a group of carcinogenic toxins called aflatoxins (*A-fla*-toxin). In 1960 in England, 100,000 turkeys died of unknown causes, and the disease was called Turkey X disease. After much work, the contaminant was found to have originated from peanut meals from Brazil. The organisms responsible for producing the toxic compounds were identified as *A. flavus*. Later, *A. parasiticus* was also found to be able to produce the toxin. Recently *A. nomius* has also been added to the list of cultures producing aflatoxin. The mold can grow between 7.5°C and 40°C, with optimal temperature at 24 to 28°C. The minimal water activity for growth is 0.82 and the optimal is 0.99. pH range of growth is from 2 to near 11. Production of the toxins generally parallel the growth of the organisms. Sporulation of the cultures seems to be a prerequisite to toxin production. In one to three days of growth, the organism can produce the toxins. The primary toxins are B_1, B_2, G_1, and G_2. B and G indicate that the toxins fluoresce blue or green under ultraviolet light, respectively. When cows consume B_1 and B_2 toxins, they can modify the toxins and excrete the toxins as M_1 and M_2 in milk.

Spores of these molds are ubiquitous; the organisms have been found to grow in rice, sorghum, peanut, corn, wheat, and soybean crops, as well as in animal feed. Human food shown to support growth and toxin production of this mold include peanut, peanut butter, pecans, beans, dried fruits, fish, and even cheese. Because the toxins are carcinogenic, they are under strict government scrutiny since the Delaney Clause of 1958 prohibits the presence of carcinogenic compounds in foods. Currently the allowed limit is 20 ppb for animal feed and all foods except milk, which has an action level of 0.5 aflatoxin M_1. Although no direct food-related aflatoxin cases have been reported in the United States, there are concerns that aflatoxin can affect the immune systems of patients. There were aflatoxin cases reported in Southeast Asia when people consumed food heavily contaminated with molds. The toxins can be detected by animal tests using ducklings or chick embryos. Thin-layer chromatography and high-performance liquid chromatography can also be used to detect these toxins. Recently, monoclonal antibodies have been employed to detect these toxins with great rapidity (10 to 30 min) and sensitivity (1 ppb and lower). Attempts to detoxify aflatoxin by ozone, peroxides, and ammonia have been met with limited success. Thus, the best preventive measure is not to allow the mold to contaminate the food and feed and to keep these commodities in a dry environment unfavorable for mold growth.

Exotoxins Versus Endotoxins

It is necessary to differentiate these toxins before a discussion on foodborne infections. *Exotoxins* are toxins produced by an organism and later released into the environment. The cell remains alive and intact. Ingestion of these preformed toxins causes foodborne intoxication. These toxins are protein toxins mainly produced by Gram-positive organisms. Because they are proteins, they can be neutralized by corresponding antibodies and detected by a variety of immunologic methods. These toxins are relatively heat sensitive (except the staphylococcal enterotoxins as described earlier). These toxins also have distinct pharmacology. Examples of exotoxins are staphylococcal enterotoxins (affecting the intestinal tracts) and botulinum neurotoxins (affecting the nervous system).

Endotoxins are part of the cell wall material of Gram-negative cells. Every Gram-negative bacterium examined has endotoxins. These are complex molecules containing protein, carbohydrate, and lipid. The protein moiety determines antigenicity, the carbohydrate moiety determines immunologic specificity, and the lipid moiety causes toxicity. Unlike exotoxins, antibodies will not neutralize toxicity because the toxic part is the lipid. All endotoxins have the same action and are released when the Gram-negative bacterium lysogenizes. These endotoxins cause fever by acting as *exogenous pyrogens*. The exogenous pyrogen, when absorbed into the bloodstream, causes injury to the leukocytes, which in turn releases an *endogenous pyrogen*. This endogenous pyrogen stimulates the thermoregulatory center of the brain at the hypothalamus and causes fever. Therefore, fever production by a patient is indicative of a foodborne infection case. Endotoxins can be detected by the limulus amebocyte lysate (LAL) test. In the presence of endotoxins, the LAL will form a gel. The reaction takes about 1 h. Currently, hospital materials should be pyrogen-free, and LAL is the standard test for pyrogens in the hospital supplies and environment. Because endotoxins are released upon lysis of the cell, it is advisable in certain cases that antibiotics not be administered in mild food infection cases. Lysis of cells by antibiotics such as *Escherichia coli* O157:H7 may allow the release of other harmful toxins in the intestinal tract and may case a more severe infection case.

BACTERIAL INFECTION

Clostridium perfringens

C. perfringens occupies an interesting position as being both a foodborne infection agent as well as a foodborne intoxication agent. On the one hand, the susceptible person has to ingest large numbers of viable *C. perfringens* before coming down with food poisoning, and on the other hand, the organism produces an enterotoxin to cause the illness. In 1990 there were 11 outbreaks and 1240 cases with no deaths in the United States. The estimate, however, is 652,000 cases with an average of 7.6 deaths per year at an annual cost of $123 million to the U.S. economy. *C. perfringens* is a Gram-positive anaerobic spore-forming rod and produces at least 13 different toxins that can cause diseases such as gas gangrene. One of the toxins is named *C. perfringens* enterotoxin (CPE), which is released in the intestinal tract and causes the infection/intoxication by this organism. The generation time (time for doubling of a population of cells) of *C. perfringens* in ideal conditions is as short as 7 min, making it the fastest-growing organism known. Spores of the organism distribute widely in nature and can easily contaminate foods. Most of the incidences of *C. perfringens* food poisoning involve meats prepared in large quantities one day and consumed the next day while the food is held at lukewarm temperatures. In such conditions, most vegetative cells of competitors die off while the spores of *C. perfringens* have a chance to survive, germinate, and grow into large numbers. The organisms in large numbers (about 10,000 to 1 million per gram), when ingested by a susceptible person, will start to sporulate in the small intestine due to the favorable anaerobic environment. The gene coded for sporulation also controls the release of an enterotoxin that is responsible for the diarrhea characteristics of *C. perfringens* food poisoning. It is noteworthy that *C. perfringens* does not sporulate in foods and therefore the CPE is not preformed in the food to cause the food poisoning cases. Symptoms occur between 8 and 20 h after ingestion of a large number of viable *C. perfringens* and include acute abdominal pain, diarrhea, and nausea, with rare vomiting. The symptoms are milder than those caused by *Salmonella*. Detection of this organism is by anaerobic cultivation of food using differential anaerobic agar such as tryptose sulfite cycloserine agar. *C. perfringens* forms black colonies in this agar medium. Immunologic methods such as reverse-passive agglutination assay and ELISA test have been developed to detect the CPE in food, culture fluid, and feces.

Salmonella

Salmonella is the classic example of foodborne infection. *Salmonella enteritidis* was isolated in 1884 and still is an important foodborne organism. In 1992 there were 80 outbreaks with 2834 cases and four deaths due to *Salmonella* in the United States. The organism is a Gram-negative, facultative anaerobic, non-spore-forming rod, motile by peritrichous flagella. It does not ferment lactose and sucrose but ferments dulcitol, mannitol, and glucose. There are exceptions to the general characteristics. For example, lactose-positive cultures have been found, and nonmotile species such as *S. pullorum* and *S. gallinarum* exist. The organism is heat sensitive but can tolerate a variety of chemicals, such as brilliant green, sodium lauryl sulfite, selenite, and tetrathionate. These compounds have been used for the selective isolation of this organism from food and water. To confirm the isolate as a *Salmonella*, one must perform serologic tests by use of polyvalent anti-O antiserum (against cell surface antigens) or polyvalent anti-H antiserum (against flagella antigens). This genus went through several revisions in classification and taxonomy of species and subspecies in the past 20 years due to advancement of genetic typing systems.

Currently there are two species, namely, *S. enterica* with six subspecies and 2356 serovars and *S. bongori* with 19 serovars Each serovar is potentially pathogenic. In the literature, many scientists still use the traditional genus and species nomenclature such as *S. typhimurium*, *S. typhosa*, and so on. *Salmonella* has been found in water, ice, milk, dairy products, shellfish, poultry, poultry meat products, eggs and egg products, animal feed, pets, and so on. Human beings can be healthy carriers of this organism. It has been estimated that 4% of the general public carries this organism. There are actually three types of diseases caused by *Salmonella*: (*1*) enteric fever caused by *S. typhosa* (typhoid fever), in which the organism, ingested along with food, finds its way into the bloodstream and disseminates to the kidney and is excreted in the stools; (*2*) septicemia caused by *S. cholerasuis*, in which the organism causes blood poisoning; and (*3*) gastroenteritis caused by *S. typhimurium* and *S. enteritidis*, a true foodborne infection. In the last case, large numbers of live *Salmonella* are ingested with food and, in one to three days, liberate the endotoxins that cause localized violent irritation of the mucous membrane with no invasion of the bloodstream and no distribution to other organs. Symptoms of salmonellosis occur 12 to 24 h after ingestion of food containing 1 to 10 million *Salmonella* per gram and include nausea, vomiting, headache, chills, diarrhea, and fever. The illness lasts for two to three days. Most patients recover; however, death can occur in the very old, the very young, and those with compromised immune systems.

Since no *Salmonella* is allowed in cooked food for interstate commerce and international trade, the detection of *Salmonella* has been a subject of much research and development. Detection of *Salmonella* by the classical method includes preenrichment of culture from food sample, enrichment or selective enrichment of the liquid culture, plating of liquid on selective agar to isolate cultures, biochemical tests of suspect colonies, and confirmation of isolates with typical biochemical profiles by serological tests. These procedures may take up to five days for completion. Recently a variety of methods and procedures have been developed and implemented for the effective isolation, enumeration, detection, identification, and characterization of *Salmonella*. Improvement of preenrichment and enrichment procedures have been made by the manipulation of incubation temperature (use 42°C instead of 37°C), addition of various stimulation compounds such as Oxyrase enzyme, and concentration of cells by immunomagnetic separation (Dynal system). A large number of biochemical diagnostic kits, such as API, MicroID, Enterotube, Biolog, Vitek, and so on, have been developed and marketed to conveniently and automatically identify isolates. Manual and automated sandwich ELISA tests, by EIA Assurance test, VIDAS, Tecra, and so on, and lateral immunomigration tests kits by BioControl VIP system and Neogen have been used widely. For the genetic tests, DNA/RNA probes system by Genetrak, PCR test by Perkin-Elmer, Probelia, and BAX system and ribotyping by Qualicon system and others are finding their ways into food microbiology laboratories (see the article RAPID METHODS OF MICROBIOLOGICAL ANALYSIS).

Because *Salmonella* is heat sensitive, proper cooking will destroy the organism. Also, proper chilling, refrigeration, and good sanitation will minimize the problem. *Salmonella* remains one of the most important food pathogens in our food supply.

Shigella

Shigella is a Gram-negative, facultative anaerobic non-spore-forming rod quite often confused with *Salmonella* in the bacteriologic diagnostic process. It is nonmotile and hydrogen sulfide negative. The colonies are smaller than *Salmonella*. In terms of foodborne infection, *Shigella* is not as prevalent as *Salmonella*, but this organism is very important in waterborne diseases, especially in tropical and subtropical countries where sanitation conditions are poor. The organism is transmitted by water, food, humans, and animals. The "4 Fs" involved in the transmission of *Shigella* are food, finger, feces, and flies. One to four days after ingestion of the organisms, there will be an inflammation of walls of the large intestines and ileum. Invasion of the blood is rare. Bloody stool will occur, owing to superficial ulceration. The cell wall of *Shigella*, when lysed, will release endotoxins. In addition, *S. dysenteriae* produces an exotoxin that is a highly toxic neurotoxin. This toxin can be neutralized by specific antibody. Mortality rate of shigellosis is higher than that of salmonellosis. Prevention of shigellosis can be achieved by sanitation, good hygiene, treatment of water, prevention of contamination, detection of carriers, and isolation of patients from the general public.

Vibrio cholerae

Vibrio cholerae was a very important disease-causing organism in the late nineteenth century and early twentieth century worldwide. The organism is under control in many industrialized countries; however, it is still a very important waterborne disease in places with poor sanitary conditions. The classical work of John Snow in 1854 showed the transmission of *V. cholerae* through poorly designed water delivery systems in London. His work led to the development of much improved water delivery systems and water treatment systems by public health official and environmental engineers in developed countries around the world. In the United States in 1990 there was one outbreak involving 26 cases and one death. The appearance of *V. cholerae* in industrialized countries often causes panic because this organism has the potential to start a pandemic infection. It is a Gram-negative, curved rod that looks like a comma under the microscope, thus the original name of *V. comma*. No spore is formed. *V. cholerae* grows well in alkaline medium and is actively motile by a single polar flagellum. The organism is endemic in India and Southeast Asia and is spread by person-to-person contact, water, milk, food, and insects. The organism produces enterotoxins and endotoxins in the intestines and causes severe irritation to the mucous membranes with resultant outflow of fluid and salts and impairs the sodium pump of mammalian cells, thus causing severe diarrhea, dehydration, acidosis, shock, and even death. The mortality rate may be as high as 25 to 50%. The most effective therapy is replacement of water and electrolytes to correct severe dehydration and salt depletion. *V. cholerae* remains a dreaded communicable disease in many parts of the world, and much education and public health work need to be done to reduce human suffering from this organism. Besides conventional biochemical tests, currently there are immunologic and DNA probes and PCR methods for rapid detection of this organism

Vibrio parahemolyticus

Vibrio parahemolyticus is an organism that has caused many cases of foodborne disease in Japan for many years. This is because citizens in Japan like to consume raw or undercooked seafood, which may be contaminated with the organisms, especially in the summer months when the water is warm in Northern Hemisphere. Most of the original reports and research works were in Japanese and not readily understandable or available to microbiologists in the West. U.S. scientists started working on the organism in earnest around 1969. In 1971 three outbreaks of this organism occurred in the United States. Since U.S. citizens do not regularly consume raw seafood, the sources of the illness were probably recontamination of cooked foods. In 1990 there were four outbreaks and 21 cases reported. The fact that no outbreaks were reported in 1988, 1989, 1991, and 1992 indicates that this organism is not a source of common foodborne infections in the United States.

The organism is a Gram-negative curved rod and is halophilic (salt loving); it grows best in 3 to 4% salt medium and can grow in 8% salt also. The growth temperature range is 15 to 40°C, and the pH range is 5 to 9.6. The organism is sensitive to streptomycin, tetracycline, chloramphenicol, and novobiocin but resistant to polymyxin and colistin. The Kanagawa positive strains hemolyze human blood. Environmental strains are negative for this test. The organism is distributed in fish and shellfish from seawater as well as from fresh water. Most of the outbreaks are recorded in the summer months when the water is warm in the Northern Hemisphere. Symptoms of the disease occur about 12 h after ingestion of a large number of viable cells (10^5/g) and include abdominal pain, diarrhea, vomiting, mild chills, and headache. The symptoms are similar to those of salmonellosis but more severe. It has been described that salmonellosis affects the abdomen of the patient, whereas *V. parahemolyticus* infection affects the stomach of the patient. Detection of the organism is best achieved by good selective medium such as BTB-salt-Teepol agar. Prevention of the occurrence of infection by this organism is by adequate cooking of seafood.

Vibrio vulnificus

This organism can be considered as an emerging pathogen. It causes more than 90% of all seafood-related deaths in the United States. The organism is widespread in estuarine environments and has been isolated from waters around the world. Consumption of raw oysters contaminated with this organism may lead to septicemia and death. Also the organism may invade wounds of victims as the persons wade or work in the contaminate water. The organism is a typical vibrio-shaped organism and is classified as biotype 1 and biotype 2. The organism grows well in common bacteriological agars such as MacConkey agar and blood agar. The incubation time of the illness is from one to seven days. The disease involves fever; chills; nausea and, to a lesser extent, vomiting; abdominal pain; and diarrhea. Development of secondary lesions can be serious and may result in vasculitis and necrotizing fasciitis necessitating surgical removal of tissues or even limb amputation. Since the organism is killed by common cooking practices, the problem is the consumption of raw seafood, especially raw oysters. People with liver damage and with immunocompromised conditions should definitely avoid eating raw seafood. There was one outbreak with two cases and one death reported in the United States in 1990.

Bacillus cereus

Bacillus cereus and other *Bacillus* species have been implicated in foodborne diseases only in recent years, although these organisms have been suspected as agents of foodborne illness for a long time. In 1991 there were five outbreaks, 253 cases, and no deaths reported in the United States. These are Gram-positive, aerobic, spore-forming rods occurring widely in nature and contaminating foods easily. Because of the general resistance of spores of this organism and the prolific biochemical activity of the vegetative cells, it can be considered one of the most important environmental bacterial contaminants of foods. Two distinct clinical symptoms are caused by this organism. The diarrheal syndrome occurs after 12 to 24 h of ingestion of large numbers (about 1 million) of viable *B. cereus* and includes abdominal pain, watery diarrhea, rectal tenesmus, and nausea without vomiting. The diarrheal enterotoxin is formed in the intestine of the host and causes the disease. The diarrheal syndrome is the result of consuming proteinaceous foods, pudding, milk and milk products, sauces, and vegetables. The emetic syndrome causes illness almost exclusively associated with cooked rice and noddles and is characterized by a rapid onset (1–5 h) with nausea, vomiting, and malaise. The toxin is preformed in the food by large numbers of *B. cereus* (1 to 10 million cells). A large number of viable *B. cereus* found in food indicates poor food handling and storage practices. To truly assess the foodborne illness potential, one has to detect the toxins involved. Currently no diagnostic kits are available for the detection of emetic toxin, but two kits available for diarrheal enterotoxin, one by OXOID that utilizes reverse passive latex agglutination tests and another by Tecra that uses the ELISA format.

Other *Bacillus* suspected of causing foodborne diseases include *B. licheniformis* and *B. subtilis*, in which large numbers (10^5–10^6 organisms/gram of food) of these organisms are ingested by susceptible persons. It should be noted that *B. subtilis var natto* is used to ferment a popular food item in Japan called Natto in which the organisms produce a polymer of glutamic acids as well as other flavor compounds. Control of *Bacillus* food poisoning is complicated by the ubiquitous nature of this organism. The best measures are to prevent the spore from germinating and to prevent multiplication of vegetative cells in cooked and ready-to-eat foods. Freshly cooked food eaten hot immediately after cooking should not be a problem. However, slow reheating of previously cooked rice products should be treated with caution. Refrigeration of leftover cooked rice products is highly recommended as a preventive measure.

Campylobacter jejuni

Campylobacter jejuni, recognized as an emerging pathogen in the past 10 years, has been reported as the most common bacterial cause of gastrointestinal infection in humans, even surpassing rates of illness caused by *Salmonella* and *Shigella*. In 1992 there were six outbreaks, 138 cases, and two deaths reported in the United States. *Campylobacter* was originally called *Vibrio fetus*, because it was first recognized as an agent of infertility and abortion in sheep and cattle. The organism is a member of the family *Spirillaceae* because of the physiologic and morphologic similarities to *Spirillum*. The organism is a Gram-negative, slender, curved bacteria that is motile by a single polar flagellum. It neither ferments nor oxidizes carbohydrates, is oxidase positive, reduces nitrates, but will not hydrolyze gelatin or urea and is methyl red and Voges-Proskauer reaction negative. It will grow between 25 and 43°C. The organism is an obligate microaerophile that grows optimally in 5% oxygen. This attribute has been used for isolation of the organism by applying appropriate gas mixtures into the headspace of cultivation media. Recently in D. Fung's laboratory the enzyme Oxyrase was found to greatly stimulate the growth of this organism, even in the absence of special gas mixtures, thus facilitating rapid and convenient detection and isolation of this organism. The incubation time of *C. jejuni* food poisoning ranges from two to five days; the duration of the sickness may be up to 10 days. The patient will exhibit enteritis, fever, malaise, abdominal pain, and headache. The stools become liquid and foul smelling. Blood, bile, and mucus discharge may occur in serious cases. The organism has a worldwide distribution, with outbreaks related to milk, poultry, eggs, red meat, pork, and water reported. It has been isolated from 50 to almost 100% of poultry carcasses in several studies. Competitive exclusion protocols have been devised to prevent the attachment and growth of *C. jejuni* by inoculating large numbers of natural intestine microorganisms in newly hatched chicks. Detection of this organism is by suitable liquid and solid growth media designed for the organism and rapid tests involving ELISA, PCR, ribotyping, and so on. One complication in studying this organism is the presence of a viable but nonculturable population of *C. jejuni* in the environment. Proper food processing techniques (heating, cooling, chemical treatment of foods, etc) will control this fragile organism. Its prevalence as a foodborne pathogen can be attributed to post-processing contaminations of food. Again, good sanitation and hygiene should reduce the incidence of this organism in our food supplies. Because of increased outbreaks and cases related to this organism much research worldwide is being conducted to monitor the organism. *C. jejuni* may be the next major foodborne disease-causing organism to be faced by food microbiologists nationally and internationally.

Escherichia coli

Escherichia coli is one of the most common bacteria in our environment. Most people do not think of *E. coli* as a foodborne pathogen; however, recent research and information indicates that some strains of *E. coli* can indeed cause severe foodborne diseases. The sensational outbreak of *E. coli* O157:H7 involving hundreds of people and four deaths of children by the consumption of undercooked hamburger served by a fast-food chain in the United States in 1993 awakened the general public to the importance of food safety. Food industries, academic communities, regulatory agencies, and consumer groups have been actively working on solving the problem of *E. coli* O157:H7 ever since that outbreak. Although much has been learned about this organism, far more needs to be done to determine the habitat, the detection, and the control of this organism. *E. coli* is a Gram-negative, facultative anaerobic, non-spore-forming rod that occurs widely in nature as well as in intestines of humans and animals. It is glucose and lactose positive, indole and methyl red positive, but Voges-Proskauer and citrate negative. The most useful way to classify the species is by serotyping, using antibodies against O, H, and K antigens of various strains of *E. coli*. Most *E. coli* isolated from the environment are not pathogenic. However, there are six classes of pathogenic and diarrheagenic *E. coli*. They are enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EaggEC), enteropathogenic (EPEC), and diffusely adherent (DAEC) *E. coli*.

EHEC, or enterohemorrhagic *E. coli*, was first identified as a human pathogen in 1982. The most important serotype is O157:H7. Other serotypes in this group are O26:H11, O103, O104, O111, and others. *E. coli* O157:H7 causes the most concern worldwide because of its unusual cultural characteristics and pathogenicity. Unlike most *E. coli*, this serotype does not ferment sorbitol within 24 h, does not possess β-glucuronidase activity, and does not have the ability to hydrolyze 4-methylumbelliferyl-β-D-glucuronide (MUG), which is an important diagnostic characteristic of most other *E. coli* strains. Because of these differences in routine microbiological manipulations, *E. coli* O157:H7 has been excluded in the protocol for common *E. coli*. The organism produces one or more Shiga-like toxins (SLT; also known as verotoxin, VT) and it possesses an attaching and effacing gene (*eae* gene) and a large plasmid (60 MDA). The organism causes severe illness, especially in children and immunocompromised patients. There are three manifestations of the disease: hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), or thrombotic thrombocytogenic purpura (TTP). Symptoms of HC occur, within one to two days after consuming contaminated foods. The initial symptoms are mild, nonbloody diarrhea followed by severe abdominal pain, and a short fever or no fever. The watery diarrhea will last for 24 to 48 h followed by 4 to 10 days of bloody diarrhea, severe abdominal pain, and dehydration. Patients with HC may develop more severe life-threatening complications such as HUS or TTP.

HUS symptoms are characterized by microangiopathic hemolytic anemia (pallor, intravascular destruction of red blood cells), thrombocytopenia (depressed platelet counts), and acute renal failure and may lead to death. TTP affects mostly adults and is a rare syndrome of *E. coli* O157:H7 infection. It causes neurological abnormalities such as nervous system deterioration, seizures, and strokes. Patients will often develop blood clots in the brain and may die. The infectious dose of *E. coli* O157:H7 is between 2 to 200 cells. Adhesion of the organisms to the intestinal wells is important, but it does not enter the circulatory system. The organism colonizes the intestinal tract where toxins are produced and then become active in the colon. For this reason much research is being conducted to have competitive exclusion by nonpathogenic organisms to attach to the epitheleal cells before *E. coli* O157:H7 can have a chance to interact with the epitheleal cells. Research in this area is in its infant stage.

Much information is now known about the characteristics of this organism. It grows well at 37°C but poorly at 44 to 45°C, a temperature usually used to isolate *E. coli*. It can grow between 8 and 10°C and can survive in ground beef at −20°C for nine months. It grows in neutral pH ranges of 5.5 to 7.5 but can grow in the pH 4.0 to 4.5 range, and more recent data indicated that it can survive in apple cider in the range of 3.6 to 4.0. The organism is heat sensitive. Proper cooking temperature of 160°F or 71°C will destroy the organism in foods. The organism is quite salt tolerant with the ability to grow at 8% NaCl at 37°C; however, at the lower incubation temperature of 10°C, growth was inhibited at 4 to 6%. This organism grows well in the water activity range of 0.99 with a minimum at 0.95.

Outbreaks of *E. coli* O157:H7 have been reported from water, meat, poultry, dairy products, salad, apple cider, and even fermented meats and mayonnaise. Detection methods include conventional culture procedures designed specifically for *E. coli* O157:H7, a variety of diagnostic kits, serologic tests, ELISA, PCR, ribotyping, and so on. The aim is to accurately and rapidly screen the presence or absence of the organisms in 25 g of food. A 24-h negative screening protocol is now available. One commercial company even suggested an 8-h protocol. This organism will continue to be very important in food microbiology for the foreseeable future.

ETEC, or enterotoxigenic *E. coli*, is a the major cause of infantile diarrhea in developing countries and is most frequently responsible for traveler's diarrhea. The serotypes involved include O8, O15, O20, O25, and others.

EIEC, or enteroinvasive *E. coli*, strains cause nonbloody diarrhea and dysentery by invading and multiplying within colonic epithelial cells. Serotypes include O28ac, O112, O124, and others.

EAggEC, enteroaggregative *E. coli*, affects infants and children with persistent diarrhea. These cells have the characteristic pattern of aggregative adherence on Hep-2 cells.

EPEC, or enterotoxigenic *E. coli*, has been defined as "diarrheagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens, but whose pathogenic mechanisms have not been proven to be related either to heat-liable enterotoxins (LT) or heat-stable enterotoxins (ST) or to *Shigella*-like invasiveness." The serotypes included in EPEC are O55, O86, O111ab, O119, O125ac, O126, and others.

DAEC, or diffusely adherent *E. coli*, have been associated with diarrhea in children in Mexico and can produce mild diarrhea without blood or fecal leukocytes.

A comprehensive treatment of *E. coli* O157·H7 and other *E. coli* strains as well as many foodborne pathogens can be found in the book by Doyle, Beuchat, and Montville (1997). Prevention and control of pathogenic *E. coli* is best done by education of food handlers, who should adhere to strict hygienic practices. Fecal and other waste materials from humans and animals should be decontaminated and not allowed in contact with water and food supplies. Kalamaki, Price, and Fung (1997) summarized screening and identification test kits for *E. coli* (see Table 1). Similar tables for the detection of *Enterobacteriaceae, Campylobacter, Salmonella, Listeria*, Rotavirus, *Staphylococcus aureus, Vibrio cholerae*, and *V. vulnificus* are presented in the same publication. Due to these developments it is possible now to have a negative screening of *E. coli* O157:H7 in about one day. However, when a food sample showed a positive screening result, the conventional methods must be used to confirm the presence or absence of *E. coli* O157:H7.

Yersinia enterocolitica

Yersinia enterocolitica is a Gram-negative, facultative anaerobic, non-spore-forming bacterium, sucrose positive, rhamnose negative, indole positive, motile at 20°C but not at 37°C, and highly virulent to mice. Serotyping is very important in separating this organism from other closely related Gram-negative bacteria. Although *Y. enterocolitica* has an optimal growth temperature at around 32 to 34°C, it is often isolated on enteric agars at 22 to 25°C. It grows slowly in simple glucose-salts medium but grows much better with supplements such as methionine or cysteine and thiamine. One important aspect of this organism is that it can grow in vacuum-packaged meat under refrigeration because it is a facultative anaerobe and is a psychrotroph. After ingestion of large numbers of this organism, the susceptible person can develop fever, abdominal pain, and diarrhea, with nausea and vomiting occurring less frequently. More serious intestinal disorders include enteritis, terminal ileitis, and mesenteric lymphadenitis. Extraintestinal infections of *Y. enterocolitica* have been reported, including septicemia, arthritis, erythema nodosum, sarcoidosis, skin infection, and eye infection.

Foods suspected of being a source of yersiniosis in the United States include chocolate milk, milk powder, chow mein, tofu, and pasteurized milk. Pork products have also been suspected.

Isolation of this organism typically goes through an enrichment step using nutrient broth or Rappaport broth and then through a plating medium using an enteric agar (SS, XLD, DCL, etc). The CIN agar (cefsulodin–irgasan–novobiocin agar) is commercially available for the isolation of this pathogen; however, many other organisms, such as *Salmonella* and *Serratia*, also grow on this agar. In D. Fung's laboratory a new agar named KV202 has been de-

Table 1. *E. coli* Screening and Identification Test Kits

Test kit	Supplier	Assay principle	Limit of detection	Sensitivity	Specificity	% Agreement	False negative rate	False positive rate	Total time	Cost per assay
ANTI *E coli* O157	Dynal, Inc., Lake Success, NY	Immunomagnetic separation	2 cells/g	NR[a]	NR	NR	NR	NR	30 h	$2 60
Assurance EIA EHEC[b]	BioControl	ELISA	<0.003–0.093 cfu/g	100%	98.4% (a) 99.5% (b) 100% (c)	100 (b) 99.4 (c)	0% (b) 1% (c)	1.1% (b) 0% (c)	<20 h	$5.15
Bactident *E. coli*	Merk	β-D-glucoronidase activity assay		96.30%	100%	NR	85%[c]	100%[d]	30 min for test only	NR
COLI ST EIA.ETEC STA	Denkaseiken Co Ltd, Tokyo, Japan	ELISA	NR	100%	100%	NR	0%	0%	24 h	NR
E. coli O157:H7 Kit	AMPCOR (Neogen Corp.)	Immunoprecipitation	10^4–10^9 cfu/mL	97.90% (c)	95% (a) 90.4% (c)	94.1 efficiency (c)	<1% (a) 2.1% (c)	<1% (a) 9.6% (c)	6–28 h	$9.50
E. coli O157.H7 VIA	TECRA	ELISA	1–5 cfu/25g	93%	>96%	NR	7%	<4%	20 h	$6.00
E coli O157 Latex Test	Unipath Ltd.	Latex agglutination	Pure colony	100%	79%	NR	0%	21%	2 min for test only	NR
EHEC-Tek	Organon Tecknika	ELISA	<1 cell/g	76.5% (c)[e]	NR	NR	NR	NR	24–26 h	$9.60
EZ COLI	DIPCO	ELISA	10^4 cfu/mL	100%	95 7%	97.8 accuracy	0%	<2%	24 h	$9.48
GENE-TRAK	GENE-TRAK	Nucleic acid hybridization	10^4 cfu/mL	97 40% (a)	100% (a) 100% (b)	NR	1.2% (a)	0% (a)	24–48 h	$7.00
HEC O157 ELISA[f]	3M Company	Blot ELISA	<1 cell/g	84.5% (e)	95.2% (e)	89 9% efficiency (e)	0% (c) 15.5% (e)	2% (c) 4.8% (e)	26–28 h	$5.00
Premier EHEC	Meridian Diagnostics	ELISA	Verotoxin detection	100%	99.6%	99.7	0%	0.4%	NR	NR
Rapidec coli[g]	bioMerieux	β-D-glucoronidase activity assay	Pure colony	91.30%	100%	NR	70.80%[c]	100%[d]	2–6 h for test only	NR
Rhabdebact ETEC-LT	Karo BioDiagnostics AB Hudding, Sweden	Immunofluorescence	Pure colony	96%	100%	59.20	4%	0%	25.5 h	NR
VIDAS	bioMerieux Vitek	ELFA	10^3 cfu/mL	NR	NR	NR	11.10%	0% (b) 1.7% (a)	24–48 h	$8.00
VIP EHEC[b]	BioControl	Immunoprecipitation	<0.003–2.4 cells/g	100% (b) 99.6% (c)	100% (b) 94.3% (c)	99.4 (a)	0% (b) 0.3% (c)	0% (b) 5.6% (c)	18 h	$8.50
VET-RPLA[h]	Unipath Ltd.	Latex Agglutination	titre 20–128	100%	100%	88.50	0%	0%	37 h	$11.04

[a] No reported.
[b] AOAC first action.
[c] Negative predictive value.
[d] Positive predictive value.
[e] Increases with IMS step.
[f] FSIS method.
[g] Not available in North America.
[h] Heat labile enterotoxin detection.
Source Ref 1, used with permission.

veloped that separates *Salmonella* and *Serratia* colonies from *Yersinia* by the development of black colonies. Control of yersiniosis is through proper handling of raw and cooked food of all types, especially pork products, and of water for food processing. There has been no reported outbreaks of *Y. enterocolitica* between 1988 and 1992.

Listeria monocytogenes

Listeria monocytogenes has developed into a very important food pathogen in the past 10 years from the standpoint of economic and public health impact. The organism is a small, short, Gram-positive non-spore-forming rod. It is motile by a characteristic tumbling motion or slightly rotating fashion. The organism grows on simple laboratory media in the pH range between 5 and 9. On solid agar, the colonies are translucent, dewdrop-like, and bluish when viewed by 45° incident transmitted light (Henry's illumination step). Biochemically, this organism can be confused with such organisms as *Lactobacillus, Brochothrix, Erysipelohrix*, and *Kurthia*. A variety of biochemical tests have been devised to separate *L. monocytogenes* from other *Listeria* species, such as *L. innocua, L. welshimeri*, and *L. murrayi*. Serotyping is also important in the identification of this organism, the most important ones being 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, and 4b. *Listeria* is a psychrotroph capable of growing at temperatures as low as 2.5°C and as high as 44°C. Because dairy products have been implicated in outbreaks of listeriosis, much research has been directed toward cheese and milk products. The organism has been found to survive the processing of cottage cheese, cheddar cheese, and Colby cheese. A question of great concern is whether *L. monocytogenes* can survive the current pasteurization temperature of milk (ie, 63°C for 30 min or 72°C for 15 s). Data on this issue are still inconclusive, and research on this topic is still ongoing. It is important to note that, at present, the time and temperature regulation for pasteurization of milk has not been affected by the possible heat resistance of *L. monocytogenes*. The disease starts with infection of the intestine—the infective dose is not known at this point. Patients may develop transitory flu-like symptoms such as malaise, diarrhea, and mild fever. In severe cases, virulent strains are capable of multiplying in macrophages and later producing septicemia. When this occurs, the bacteria can affect the central nervous system, the heart, the eyes, and may invade the fetus of pregnant women and result in abortion, stillbirth, or neonatal sepsis.

Several well-documented cases of listeriosis have been reported in Nova Scotia (1981), Massachusetts (1983), and the most well known one involving Mexican-style soft cheese in Southern California (1985). Due to concerted effort by the food industry and government agencies, the outbreaks of *L. monocytogenes* seemed to have subsided for about 10 years. Between 1998 and 1992 only one outbreak, involving two cases and one death, was recorded for *L. monocytogenes*, and it appeared that the problem of *L. monocytogenes* was under control. However, recently in 1998 and 1999 the organism was found in surfaces and equipment of production lines of frankfurters, air condition lines, hot dogs, lunch meat, and turkey breasts and caused many outbreaks and recalls. One company recalled 30 million lb of ready-to-eat products due to *L. monocytogenes*. Another company recalled 15 million lb of hot dog and deli meat products due to an outbreak of *L. monocytogenes* that included 20 deaths—14 adults and 6 miscarriages/stillbirths—and at least 97 illnesses in 22 states. There is a resurgence of concern about this organism due to the pathogenic nature of the illness involving miscarriages and stillbirth of fetuses.

L. monocytogenes has been isolated in a variety of commodities, including poultry carcasses, meat and chopped beef, dry sausages, milk and milk products, cheese, vegetables, and surface water. Control measures include eliminating occurrence of the organism in the raw food materials, transporting vehicles, and food-processing plants (especially in controlling cross-contamination of raw and finished products); in practicing good general sanitation of the entire food-processing environment; in regular monitoring of the occurrence of this organism in the food-processing facilities; and in preventing pregnant females from working in and around an environment that might have the possibility of exposing them to *L. monocytogenes*. Because the organism is killed by heat and is susceptible to sanitizing agents, proper cooking of food and decontamination of the food preparation environment will also help reduce risks. Much research has been devoted to the rapid isolation, enumeration, and identification of this organism. Many diagnostic kits, immunological systems, and genetic systems have been developed to rapidly screen this organism in the food supply.

Aeromonas hydrophia

Aeromonas hydrophia has been associated with foodborne infection, although the evidence is not conclusive. The organism is a facultative anaerobic, Gram-negative, motile rod. Biochemically, it is similar to *E. coli* and *Klebsiella*. The optimal temperature for growth is 28°C and the maximum is 42°C. Many strains can grow at 5°C, which is a temperature usually considered adequate to prevent growth of foodborne pathogens.

Diseases caused by *A. hydrophila* include gastroenteritis (cholera-like illness and dysentery-like illness) and extraintestinal infections such as septicemia and meningitis. This organism has been isolated from fish, shrimp, crabs, scallops, oysters, red meats, poultry, raw milk, vacuum-packaged pork and beef, and even bottled mineral water.

Because the organism is a psychrotroph, cold storage is not an adequate preventive measure. Proper heating of food offers sufficient protection against this organism. Consumption of undercooked food or raw food such as raw shellfish is discouraged.

Plesiomonas shigelloides

Plesiomonas shigelloides has been a suspect in foodborne disease cases. The organism is Gram-negative, facultative anaerobic, catalase negative, and fermentative. It is oxidase positive and can be differentiated from bacteria in the family *Enterobacteriaceae* by this test, since the latter is oxidase negative. The organism also resembles *Shigella* but can be differentiated from *Shigella* by being motile. It is capable of producing many diseases, ranging from enteritis to meningitis.

Gastroenteritis by *P. shigellosides* is characterized by diarrhea, abdominal pain, nausea, chills, fever, headache, and vomiting after an incubation time of one to two days. Symptoms last for a week or longer. All reported food involved with cases of gastroenteritis were from aquatic origin (salted fish, crabs, and oysters). The organism can be isolated from a variety of sources, including humans, birds, fish, reptiles, and crustaceans. The true nature of this organism as a foodborne agent is not fully known because the organism has not been well studied to date.

Miscellaneous Bacterial Foodborne Pathogens

Many other microbes are suspected of being foodborne pathogens. However, they are not currently being labeled as true foodborne pathogens owing to a lack of reports of these organisms, as well as a lack of isolation methods and research on these organisms. Many of these organisms may be identified as foodborne pathogens in the future. Among these organisms are the Gram-negative bacteria *Citrobacter, Edwardsiella, Enterobacter, Klebsiella, Hafnia, Kluyvera, Proteus, Providencia, Morganella, Serratia, Vibrios*, and *Pseudomonas* and the Gram-positive bacteria *Corynebacterium, Streptococcus*, and other species of *Bacillus* and *Clostridium*. Miscellaneous organisms include *Brucella, Mycobacterium* (T, B), *Coxiella burnetii* (Q-fever), and *Leptospirosis, Erysipelas*, and *Tularemia*.

Foodborne Viruses

Foodborne viruses are much less studied by food microbiologists than are bacteria and fungi owing to the difficulty of cultivating these entities, as conventional bacteriologic media will not allow these particles to grow. There are, no doubt, many foodborne outbreaks and cases caused by a variety of viruses, but scientists in many cases are not able to identify the sources of the infection. Viruses that have been incriminated in foodborne diseases include hepatitis A virus (oysters, clams, doughnuts, sandwiches, and salad), Norwalk virus (oysters), polio virus (milk and oysters), ECHO virus (oysters), enteroviruses (oysters), and coxsackievirus (oysters). Much more research needs to be done in the field of food virology to help reduce the incidences of foodborne diseases caused by viruses.

Protozoa and Related Organisms

Protozoans such as *Cryptosporidium, Cyclospora, Toxoplasma, Giardiasis, Entamoeba, Balantidium*, and others can also cause human foodborne diseases. The most sensational outbreak was the one involving *Cryptosporidium parvum*, which affected 400,000 people and caused several deaths in Milwaukee in 1993. *Cyclospora cayetanensis* also was in the news due to imported fruits and caused a foodborne outbreak. These organisms have complex life cycles and are studied by specialists in this area. Recently a organism named *Pfiesteria piscicida* was responsible for killing million of fish in the Eastern shores of the United States. The organism has 24 life stages ranging from a cyst stage to a toxic zoospore phase to an amoeba stage. People in contact with water infected with this organism complained of vomiting and liver problems, but no conclusive data are available on the pathogenicity of this organism on human.

Nonmicrobial Foodborne Disease Agents

Consumption of food containing other living organisms can directly and indirectly cause foodborne diseases as well. Among nonmicrobial foodborne disease agents are scombroid fish (associated with high level of histamine), cestodes (flatworms such as *Taenia saginata, T. solium*, and *Diphyllobothrium latum*), nematodes (hookworm such as *Trichinella spiralis*), trematodes (fluke such as *Clonorchis sinensis*), shellfish (indirectly toxin from the dinoflagellate *Gonyaulax catenella*), ciguatera (from eating fish such as barracudas, groupers, and sea basses that feed on toxic algae), and other poisonous fish (such as puffer fish and moray eel).

SUMMARY

Food safety is everybody's responsibility. Scientists are charged with identifying the agents causing foodborne infections and intoxications and studying the mechanisms of the intoxication and infection as well as working on the isolation, enumeration, characterization, and identification of the causative agents and their control by developing intervention strategies and preservation methods. The food industry uses this basic knowledge and applies it to good manufacturing practices to produce wholesome, nutritious, and safe foods by utilizing modern equipments, systems, processing techniques, and distribution systems. Government agents are charged with the responsibility of monitoring the safety of food supplies and enforcing regulation to ensure the production, distribution, and sales of wholesome foods. The consumer must also be educated in the handling of raw and cooked food at the point of purchase, as well as preparation of the food and final consumption. All parties are responsible for the food safety of all involved. The delightful book *Safe Eating* by Acheson and Levinson (1998) details the problems involving food safety and solutions to protect consumers in laypeople's terms and yet provides much scientific information about the entire issue of food safety and consumer protection. It is a book worth reading and studying by consumers concerned about food safety.

BIBLIOGRAPHY

1. M. Kalamaki, R. Price, and D. Y. C. Fung, "Rapid Methods for Identifying Seafood Microbial Pathogens and Toxins," *J. of Rapid Methods and Automation in Microbiol.* **5**, 87–138 (1997).

GENERAL REFERENCES

M. R. Adams and M. O. Moss, *Food Microbiology*, The Royal Society of Chemistry, London, United Kingdom, 1995.

G. J. Banwart, *Basic Food Microbiology*, AVI, Westport, Conn., 1981.

C. M. Bourgeois, J. Y. Levean, and D. Y. C. Fung, *Microbiological Control of Foods and Agricultural Products*, English ed., VCH, New York, 1995.

J E L. Corry, D. Roberts, and F. A. Skinner, *Isolation and Identification Methods for Food Poisoning Organisms*, Academic Press, New York, 1982.

M. P. Doyle, *Food Borne Bacterial Pathogens*, Marcel Dekker, New York, 1989.

M. P. Doyle, L. R. Beuchat, and T. J. Montville, *Food Microbiology: Fundamentals and Frontiers*, ASM Press, Washington, D.C., 1997

U.S. Food and Drug Administration, *Bacteriology Analytical Manual*, 8th ed., AOAC INTERNATIONAL, Arlington, Va., 1995.

D. Y. C. Fung, "Types of Microorganisms," in F. E. Cunningham and N. A. Cox, eds., *Microbiology of Poultry Meat Products*, Academic Press, New York, 1987, pp. 5–27.

D. Y. C. Fung, "What's Needed in Rapid Detection of Foodborne Pathogens," *Food Technol.* **49**, 64–67 (1995).

D. Y. C. Fung, "Overview of Rapid Methods of Microbiological Analysis," in M. C. Tortorello and S. M Gendel, eds., *Food Microbiology Analysis: New Technologies*, Marcel Dekker, New York, 1997, pp. 1–25.

D. Y. C. Fung, *Handbook of Rapid Methods and Automation in Microbiology Workshop*, Kansas State University, Manhattan, Kans., 1998.

D. Y. C. Fung et al., "Novel Methods to Stimulate Growth of Food Pathogens by Oxyrase and Related Membrane Fractions," in R. C. Spencer, E. P. Wright, and S. W. B. Newsome, eds., *Rapid Methods and Automation in Microbiology*, Intercept Limited, Andover, United Kingdom, 1994, pp. 313–326.

J. M. Jay, *Modern Food Microbiology*, 5th ed., Van Nostrand Reinhold, New York, 1996.

R. T. Marshall, ed., *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington, D.C., 1992.

M. D. Pierson and N. J. Stern, *Foodborne Microorganisms and Their Toxins: Developing Methodology*, Marcel Dekker, New York, 1985.

B. Ray, *Fundamental Food Microbiology*, CRC Press, Boca Raton, Fla., 1996.

A. N. Sharpe, *Food Microbiology: A Framework for the Future*, Charles C. Thomas, Springfield, Ill., 1980.

J. H. Silliker et al., *Microbial Ecology of Foods*, Vols. 1 and 2, Academic Press, New York, 1980.

C. Vanderzant and D. Splittstoesser, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, D C., 1991.

DANIEL Y. C. FUNG
Kansas State University
Manhattan, Kansas

FOODBORNE MICROORGANISMS: DETECTION AND IDENTIFICATION

The many methods that are used to enumerate microorganisms or to detect their toxic products in foods can be placed in two groups. One group includes those methods that require the organisms to be viable, while the other includes methods that detect cells that may be nonliving, or parts and products of cells, and the two groups are listed in Table 1 along with the reported minimum detectable numbers of cells or products for each. In both groups are methods that are used primarily for the detection and enumeration or microorganisms, whereas some are used primarily to identify microorganisms.

Table 1. Examples of the Two Broad Categories of Enumeration/Detection Methods and their Minimum Response Cell Numbers

	Minimum numbers
A. Viable / respiring cells required	
1. Standard plate count	1
2. Most probable number (MPN)	<1
3. Dye reductions	~10^{6a}
4. Hydrophobic grid membrane filter (HGMF)	<10
5. Microcolony-DEFT	10^3
6. Electrical impedance	10^6–10^7
7. Radiometry	10^4–10^5
8. Catalasemeter	~10^4
9. Microcalorimetry	~10^4
B. Viable cells are not needed	
1. Direct microscopic count (DMC)	10^4
2. Direct epifluorescent filter technique (DEFT)	10^3–10^4
3. Fluorescent antibody	10^6–10^7
4 ATP assay	10^5–10^6
5. *Limulus* amoebocyte lysate test (LAL)	~10^2
6. Radioimmunoassay	<10^3
7. ELISA, EIA	10^5–10^6
8. Thermostable nuclease (TNase)	10^7
9. DNA probes	10^6–10^7

[a] Four hours for resazurin reduction.

DETECTION METHODS THAT REQUIRE VIABLE CELLS

This group of methods includes some of the oldest in use along with some that were developed only in the last two decades. Because they all depend on dividing cells, their speed is dependant directly upon the growth rate of the organisms of interest. Their chief value is that they may be used to determine the number of viable or colony-forming units (cfu) in a food product, and the number of organisms found depends on the factors of culture media employed, temperature and time of incubation, incubation environment (aerobic or anaerobic), pH, and a_w of culture medium. Each of those listed in Table 1 is further discussed as follows.

Standard/Aerobic Plate Count (SPC, APC)

Along with direct microscopic count, this is one of the oldest methods for enumerating bacteria in foods and food specimens. Official methods for determining cfus are described in standard references (1,2) and no further details are presented here.

There are two basic ways to conduct an SPC: pour and surface plating of samples or diluents. By the former, diluent is placed in empty petri dishes followed by pouring of cooled molten agar and mixing with diluted sample. With surface plating, diluents are spread over the surface of dried agar media, typically with bent glass rods (hockey sticks). The following factors may be considered in deciding whether to use the pour or surface plate method, assuming the same time and conditions of incubation.

J. E. L. Corry, D. Roberts, and F. A. Skinner, *Isolation and Identification Methods for Food Poisoning Organisms*, Academic Press, New York, 1982.

M. P. Doyle, *Food Borne Bacterial Pathogens*, Marcel Dekker, New York, 1989.

M. P. Doyle, L. R. Beuchat, and T. J. Montville, *Food Microbiology: Fundamentals and Frontiers*, ASM Press, Washington, D.C., 1997

U.S. Food and Drug Administration, *Bacteriology Analytical Manual*, 8th ed., AOAC INTERNATIONAL, Arlington, Va., 1995.

D. Y. C. Fung, "Types of Microorganisms," in F. E. Cunningham and N. A. Cox, eds., *Microbiology of Poultry Meat Products*, Academic Press, New York, 1987, pp. 5–27.

D. Y. C. Fung, "What's Needed in Rapid Detection of Foodborne Pathogens," *Food Technol.* **49**, 64–67 (1995).

D. Y. C. Fung, "Overview of Rapid Methods of Microbiological Analysis," in M. C. Tortorello and S. M. Gendel, eds., *Food Microbiology Analysis: New Technologies*, Marcel Dekker, New York, 1997, pp. 1–25.

D. Y. C. Fung, *Handbook of Rapid Methods and Automation in Microbiology Workshop*, Kansas State University, Manhattan, Kans., 1998.

D. Y. C. Fung et al., "Novel Methods to Stimulate Growth of Food Pathogens by Oxyrase and Related Membrane Fractions," in R. C. Spencer, E. P. Wright, and S. W. B. Newsome, eds., *Rapid Methods and Automation in Microbiology*, Intercept Limited, Andover, United Kingdom, 1994, pp. 313–326.

J. M. Jay, *Modern Food Microbiology*, 5th ed., Van Nostrand Reinhold, New York, 1996.

R. T. Marshall, ed., *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington, D.C., 1992.

M. D. Pierson and N. J. Stern, *Foodborne Microorganisms and Their Toxins: Developing Methodology*, Marcel Dekker, New York, 1985.

B. Ray, *Fundamental Food Microbiology*, CRC Press, Boca Raton, Fla., 1996.

A. N. Sharpe, *Food Microbiology: A Framework for the Future*, Charles C. Thomas, Springfield, Ill., 1980.

J. H. Silliker et al., *Microbial Ecology of Foods*, Vols. 1 and 2, Academic Press, New York, 1980.

C. Vanderzant and D. Splittstoesser, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, D.C., 1991.

Daniel Y. C. Fung
Kansas State University
Manhattan, Kansas

FOODBORNE MICROORGANISMS: DETECTION AND IDENTIFICATION

The many methods that are used to enumerate microorganisms or to detect their toxic products in foods can be placed in two groups. One group includes those methods that require the organisms to be viable, while the other includes methods that detect cells that may be nonliving, or parts and products of cells, and the two groups are listed in Table 1 along with the reported minimum detectable numbers of cells or products for each. In both groups are methods that are used primarily for the detection and enumeration or microorganisms, whereas some are used primarily to identify microorganisms.

Table 1. Examples of the Two Broad Categories of Enumeration/Detection Methods and their Minimum Response Cell Numbers

	Minimum numbers
A. Viable/respiring cells required	
1. Standard plate count	1
2. Most probable number (MPN)	<1
3. Dye reductions	~10^{5a}
4. Hydrophobic grid membrane filter (HGMF)	<10
5. Microcolony-DEFT	10^3
6. Electrical impedance	10^5–10^7
7. Radiometry	10^4–10^5
8. Catalasemeter	~10^4
9. Microcalorimetry	~10^4
B. Viable cells are not needed	
1. Direct microscopic count (DMC)	10^4
2. Direct epifluorescent filter technique (DEFT)	10^3–10^4
3. Fluorescent antibody	10^6–10^7
4. ATP assay	10^5–10^6
5. *Limulus* amoebocyte lysate test (LAL)	~10^2
6. Radioimmunoassay	<10^3
7. ELISA, EIA	10^5–10^6
8. Thermostable nuclease (TNase)	10^7
9. DNA probes	10^6–10^7

[a] Four hours for resazurin reduction.

DETECTION METHODS THAT REQUIRE VIABLE CELLS

This group of methods includes some of the oldest in use along with some that were developed only in the last two decades. Because they all depend on dividing cells, their speed is dependant directly upon the growth rate of the organisms of interest. Their chief value is that they may be used to determine the number of viable or colony-forming units (cfu) in a food product, and the number of organisms found depends on the factors of culture media employed, temperature and time of incubation, incubation environment (aerobic or anaerobic), pH, and a_w of culture medium. Each of those listed in Table 1 is further discussed as follows.

Standard/Aerobic Plate Count (SPC, APC)

Along with direct microscopic count, this is one of the oldest methods for enumerating bacteria in foods and food specimens. Official methods for determining cfus are described in standard references (1,2) and no further details are presented here.

There are two basic ways to conduct an SPC: pour and surface plating of samples or diluents. By the former, diluent is placed in empty petri dishes followed by pouring of cooled molten agar and mixing with diluted sample. With surface plating, diluents are spread over the surface of dried agar media, typically with bent glass rods (hockey sticks). The following factors may be considered in deciding whether to use the pour or surface plate method, assuming the same time and conditions of incubation.

1. Size of colonies: smaller on pour, larger on surface.
2. Spreading of colonies: less pronounced on pour.
3. Enumeration of psychrotrophs: better with surface.
4. Crowding of colonies on plate: more with surface.
5. Use of selective/differential media: better with surface.
6. Colonial features of colonies: better with surface.
7. Microaerophilic organisms: better with pour.
8. Strict aerobes. better with surface.
9. Colony pigmentation: better observed with surface.
10. Strict anaerobes: better with pour.
11. Subculturing of colonies: better with surface.

Reproducibility: Generally Better With Pour

Although surface plating has some advantages over pour plating, the latter method is much more widely used. The way in which food specimens are homogenized for cfu determinations is an important decision that affects the numbers of organisms found. Brisk shaking by hand or by use of mechanical shakers provides suitable results when comminuted meats or powdered food specimens are used, but reproducibility is not always good. Homogenizing with a Waring blender is a widely used method and this device allows one to homogenize chunks of food as well as comminuted products. One drawback to using this method is the heat build up that occurs in the container when blending is carried out for more than about 2 min. Other drawbacks to the use of the Waring blender have been discussed elsewhere (3). The Stomacher has emerged as the method of choice for food homogenizations. Developed by Sharpe and Jackson (4), this device has been compared to the Waring blender and other methods and found to give better results overall (5–7).

Spiral Plater. The use of the Spiral plating device is another way to achieve surface plating, and a large number of investigators have shown that the proper use of this device achieves results comparable to those by the more traditional methods (8–11). Among the advantages it offers is the use of only one petri dish to effect the enumeration of cfus over a wide range, and dilution blanks are not needed. The plating of solid foods can present problems unless care is taken to prevent particles from clogging the Spiral plater stylus.

Dry Film. A more recent surface-plating methodology is the use of dry film such as Petrifilm where an inoculum is spread over the surface of a prescribed area impregnated with culture medium. The colonies that develop are smaller than for the classical surface plate method, but their enumeration is aided by color development from tetrazolium in the film. This method has been shown to give results comparable to those by pour or surface plating in petri dishes (12,13).

Other Methods. Other variations of methods that allow one to enumerate cfus include roll tube methods that are especially useful for enumerating strict anaerobes, and Rodac or contact plates that may be used to determine cfus by their direct application to food plant surfaces. A common problem with contact plates employing nonselective media is the spreading of colony growth and also overgrowth by molds, but this can be minimized by using selective culture media for specific groups of organisms (14), or using properly dried contact plates when nonselective media are used.

Most Probable Numbers (MPN)

This is a statistical method that can be used to enumerate all viable cells in a food product or specific indicators, pathogens, or fungi, depending on the media employed. MPN is run either as a 3- or 5-tube method and results are generally higher than by either pour or surface Plating methods. Details of MPN methodology are presented in *Standard Methods* (2). Some of the problems encountered with plate count methods are avoided by the use of MPN. For example, problems presented by spreading colonies as well as those of inaccurate counts are avoided. On the down side, no information is provided on colony features or the types of organisms when nonselective media are employed. As noted above, MPN counts are generally higher than plate counts; and large quantities of glassware are needed (3). MPN is the method of choice for enumerating coliforms or *Escherichia coli* in foods and waters. This method has recently been reported for *Listeria* spp. (15) and *Salmonella* spp. (16) in raw meat and poultry.

Dye Reductions

Although these methods cannot be used to make precise determinations of microbial numbers, they can be used to make estimates of certain organisms within number ranges. The dyes most commonly used are methylene blue, resazurin, and tetrazolium, and detailed procedures for their use are described in *Standard Methods* (2).

Dye reduction methods are based on the fact that respiring microorganisms effect their reduction resulting in color changes. Methylene blue changes from blue to white, resazurin from slate blue to pink or white, and tetrazolium from colorless to its red formazan. In general, the color changes are essentially linear in the $\log_{10}$ 5 to 8 cfu/g range, although not all organisms are equal in their reductive abilities.

Dye reduction methods are normally employed as screens. Resazurin has been shown to be an excellent screen for $\log_{10}$ 5.0 cfu/g for raw milk (17,18), meats (19–21) poultry (22), and frozen shrimps (23). Methylene blue has a long history of use as a screen for raw milk (17) where the time (in hours) it takes for the blue color to be reduced to its colorless form is used as an approximation of overall microbial load. When compared to nitroblue tetrazolium and indophenyl nitrophenyl tetrazolium, resazurin produced faster results (20). Using surface samples from sheep carcasses, resazurin was reduced in 300 min by 18,000 cfu/cm^2, nitroblue in 600 min by 21,000 cfu/cm^2 whereas with indophenyl nitrophenyl tetrazolium 18,000 cfu/cm^2 effected reduction in 660 min (20).

These methods are both simple to use and inexpensive, but as noted above, some groups of organisms are more efficient in reducing the oxidized forms than others. Their use has been described (1) and discussed elsewhere (3).

Hydrophobic Grid Membrane Filter (HGMF)

A hydrophobic grid membrane filter consists of 1600 wax grids on a single membrane filter that restricts growth and colony size to individual grids (24–26). On one HGMF, from 10 to 9×10^4 cells can be enumerated by an MPN procedure, and enumeration can be automated. The method can detect as few as 10 cells/g and results can be achieved in 24 h or so, and it can be used to enumerate all cfu's or specific groups such as indicator organisms (27–29), yeasts and molds (30), or pathogens such as salmonellae (31). This method has been automated (30) and given AOAC approval (27). Hydrophobic grid membrane filter systems have been reported for enumerating *Escherichia coli* (32) and *Listeria monocytogenes* (33,34).

A typical application of HGMF consists of filtering 1 mL of a 1:10 homogenized sample through a HGMF membrane and placing the membrane on a suitable medium for incubation overnight to allow colonies to develop. The grids that contain colonies are enumerated and MPN is calculated.

Microcolony Direct Epifluorescent Filter Technique (Microcolony-DEFT)

The DEFT, described shortly is a method for determining viable and nonviable cells, whereas microcolony-DEFT is a variation used to determine viable cells only. Typically, food homogenates are filtered through DEFT membranes and the membranes are then placed on the surface of appropriate media and incubated for 3 to 6 h (3 h for gram-negative and 6 h for gram-positive bacteria). The microcolonies on the filters are stained and enumerated (35). The latter authors showed this method to be satisfactory for enumerating coliforms, pseudomonads, and staphylococci, and that as few as 10^3/g could be detected with results within 8 h.

In another variation of DEFT, Rodrigues and Kroll (36) devised a microcolony epifluorescence microscopy method that combines DEFT with the hydrophobic grid membrane filter (HGMF). By this method, nonenzyme detergent-treated samples are filtered through Nucleopore polycarbonate membranes. The membranes are transferred to the surface of selective agar and incubated for 3 h for gram-negative and 6 h for gram-positive bacteria. The membranes are then stained with acridine orange and the microcolonies are enumerated by epifluorescence microscopy. Results were achieved in less than 6 h without resuscitation for injured organisms, and in about 12 h when a resuscitation step was employed.

Electrical Impedance

Impedance does not lend itself to the enumeration of microbial numbers per se, but it can be used to indicate the presence of respiring cells in the range of about 10^4/g or above. The method is based on the measurement of impedance decrease caused by respiring microorganisms in suitable culture substrates, and it lends itself to the screening of food products for the presence or absence of a certain minimum number of cells (37,38). The lowest number of viable bacterial cells that can elicit an impedance response is in the 10^6–10^7/mL range. If a product contains a lower number of cells/milliliter, say, 10^3/g, the time it takes this number to reach the threshold level is the impedance detection time (IDT).

As a screen, impedance measurement may be used to determine how long it takes (in hours) for IDT where the longer the time the lower the number of cells in the food sample. By use of known numbers of organisms in appropriate substrates, one can determine cutoff times to reflect, eg, 10^5 cells/g. All aerobic bacteria (total numbers) may be detected as well as specific groups of organisms by using the appropriate growth media (39). Results can be obtained within hours, depending on initial numbers, and the method lends itself to automated data collection. This method has been used to detect coliforms in foods (40–42), to estimate the flora of frozen orange juice concentrate (43), raw meats (44–46), and milk (47). The potential food and nonfood applications for the technology were recently reviewed (48).

Radiometry

This method employs the use of ^{14}C-labeled fermentable substrates with the radiolabel on a carbon atom that respiring organisms can release as $^{14}CO_2$. In general, the larger the number of cells the more $^{14}CO_2$ released, and approximate numbers of cells are determined by the amount of radioactive carbon dioxide released. It normally requires 10^4–10^5 coliforms to release enough $^{14}CO_2$ from lactose to be detected.

Although radiometry is not widely used, it has been shown to be applicable to the detection of the microbial flora of foods (49,50) and to the specific detection of salmonellae (51).

Radiometry is used as a screen in much the same way as impedance and dye reduction methods, where the time required to produce a threshold quantity of $^{14}CO_2$ is a function of the number of cells that can release CO_2 from the labeled substrate. The radiolabel and the necessity for radioactivity counters make this method unpopular in the food industry.

Catalasemeter

This disk flotation device was designed by Gagnon et al. (52) and further developed at the University of Quebec (53) to detect the volume of oxygen released when microbially produced catalase acts on hydrogen peroxide. The catalasemeter consists of the preparation of food extracts that contain catalase (formed by catalase-positive organisms) followed by soaking a filter paper disk with the enzyme preparation. The filter paper disk is immediately placed in a tube containing 3% hydrogen peroxide. The disk-containing tube is placed in a photometer and the time in seconds that elapses between the initial fall of the disk to the bottom of the tube and its subsequent rise, due to the buoyancy caused by bubbles of oxygen at its surface, is recorded as flotation time. When large numbers of catalase-positive organisms are in the food preparation, more catalase is present to produce more oxygen from the hydrogen peroxide and subsequently the shorter the flotation time.

The Catalasemeter as described above was employed by Dodds et al. (54) to make rapid assessments of the microbial quality of vacuum-packaged cooked turkey products, and cfus of 10^4/g or higher could be detected accurately in 300 sec, with longer and less accurate flotation times required with lower numbers. Overall, the test correlated well with cfu's of catalase-positive Enterobacteriaceae with a correlation coefficient of 0.804.

Microcalorimetry

This method has received only limited study, but it offers potential as a screen for ca 10^4 microorganisms/g in canned foods as well as in ground beef. It is based on the fact that respiring cells emit heat and that accurate measurements of the heat correspond to cell numbers. Either a batch or flow type microcalorimeter is used, and some of these instruments are sensitive to a heat flow of 0.01 cal/h (55). The organisms that grow in canned foods can be detected by observing and measuring the thermogram produced when sensors are placed on the outside of cans (56). About 10^4 cells/g are necessary to give a detectable response (3), and specific groups of organisms, such as lactic acid bacteria (57) and yeasts (58,59) can be detected or characterized under appropriate conditions.

DETECTION OF ORGANISMS AND/OR THEIR PRODUCTS BY METHODS THAT DO NOT REQUIRE VIABLE CELLS

Unlike some of the enumeration methods that can be used to make rather precise determinations of microorganisms in foods, most of those that do not require cell viability can be used only to determine numbers at or above their respective threshold ranges. In addition to the latter, some of these methods lend themselves to the identification of certain groups of organisms, and they are discussed further in this regard in a later section. Their application for enumeration of cells or the detection of cell products is discussed as follows.

Direct Microscopic Count (DMC) Methods

In addition to being the oldest ways to determine the numbers of microorganisms in foods and other specimens, DMCs are the fastest. For food use, the two oldest and most widely used DMC methods are the Breed method for bacteria, and the Howard mold count slide for molds.

The Breed method consists of adding 0.01 ml of a sample to a 1-cm^2 area on a special microscope slide followed by drying, fixing, staining, and viewing with the oil immersion objective of a calibrated compound microscope (1). The method is widely used for raw milk, and it can be used for other food products such as powdered eggs. Results can be obtained in about 5 min. but a minimum of around 10^4 cells/mL are needed. Results obtained by this method are generally always higher than for viable cell methods such as APC since no distinctions can be made between viable and nonviable in cells (3). In spite of its drawbacks, this method is valuable when one wishes to know in the shortest possible time if a given level of microorganisms exists in a food product.

A slide method that may be used to detect and enumerate only viable cells was developed by Betts et al (60). Key to the method is the use of the tetrazolium salt 2-(*p*-iodophenyl-3-(*p*-nitrophenyl)5-phenyl tetrazolium chloride (INT). Cells are exposed to filter-sterilized INT for 10 min at 37°C in a water bath followed by filtration on 0.45 μm membranes. Following drying of membranes for 10 min at 50°C, the special membranes are mounted in cotton seed oil and viewed with coverslip in place. The method was found to be workable for pure cultures of bacteria and yeasts. Betts et al. found that the INT method underestimated APC by 1–1.5 log cycles when compared to APC using milk. By use of fluorescence microscopy and Viablue (modified aniline blue fluochrome), viable yeast cells could be differentiated from nonviable cells (61,62).

The Howard mold count is a slide method specifically developed for monitoring tomato products for molds (63). A similar method has been developed for *Geotrichum candidum* in fruits and vegetable products. These methods can be used to assess the prevalence of fungal contamination by observing their mycelia or mycelial fragments, but the values obtained rarely correspond to viable mold counts when plating methods are employed (3).

Direct Epifluorescent Filter Technique (DEFT)

DEFT is a more recent modification of the classical DMC method. A good review of this technique was published by Pettipher (64). By the basic method, a diluted food homogenate is filtered through a 5-μm nylon filter. The filtrate is collected and treated with 2 mL Triton X-100 and 0.5 mL trypsin. After incubation, the treated filtrate is passed through a 0.6-μm Nucleopore polycarbonate membrane and the filter is stained with acridine orange. Following drying, the stained cells are enumerated by epifluorescence microscopy and the number of cells/g calculated by multiplying the average number/field by the microscope factor (65–67). Results can be obtained in 25–30 min, and numbers as low as around 6,000 cfu/g can be obtained from meats and milk products.

DEFT has been used successively not only with milk but to estimate numbers of microorganisms on meat and poultry (67) and on food contact surfaces (68). It has been adapted to enumerate viable gram-negative and all gram-positive bacteria in milk in about 10 min (69). The further adaptation of DEFT to the enumeration of viable cells was previously discussed. A shortened procedure for DEFT developed by Champagne et al. (70) was used to assay the quality of raw milk in tanker deliveries.

Fluorescent Antibody (FA)

This is a microscope slide method that finds its widest use in food microbiology in the examination of foods for salmonellae (71,72), although the method can be used for any organisms to which an antibody can be made. By this method, an antibody is made to the organism of interest and made fluorescent by coupling it to a fluorescent compound such as fluorescein isocyanate. Following the preparation of slide smears, drying, and fixing, the coupled antibody–fluorescent dye is added. If the organism to which the antibody is made is present, it will react with the cells and evidence for the reaction is ascertained by observing for fluorescence under a microscope with oil immersion objective. The FA technique can be run as a direct or an indirect method, with the latter being preferred by many. By the latter, the homologous antibody is not coupled with fluorescent label but instead, an antibody to this antibody is prepared and coupled. This method obviates the need to prepare antibody for each organism of interest. More recent modifications of FA include the use of monoclonal antibodies, which appear to reduce false positive reactions (3). A modified antibody–DEFT technique has recently been reported by a number of researchers to provide a rapid and direct method for enumerating *E. coli* 0157:H7 in beef (73,74) and *Listeria* in vegetables (75).

This method is rapid but requires the presence of about 10^6–10^7 cells/ml for success in finding positive cells on the slide. It is official by AOAC (76), and it is more satisfactory in the hands of some than others. False positive results may run as high as 8–10% and false negatives may be as high as 1–3%, depending on the experience of the microscopist. The FA technique is more of a screen for the presence of targeted organisms in the 10^6–10^7/mL range than an enumeration method per se. It can be automated (77) and viable salmonellae cells can be enumerated when combined with a microcolony technique (78). Its utility as a method to identify given organisms is obvious.

ATP Assay

This method is based on the fact that all cells contain ATP and that the quantity detected in a specimen is referable to a given number of cells. The presence and amount of ATP are detected by use of the firefly luciferin-luciferase assay with the use of a photometer to measure emitted light (79,80). The problems that must be dealt with are that (1) the quantity of ATP varies for different bacterial cells, with the amount ranging from 0.1 to 4.0 fg/bacterial cell and for yeast cells from 13 to 100 fg/cell and (2) background ATP from food substances must be excluded from the analysis. By one commercially available system, background ATP can be eliminated and ATP assays can be run in an automated manner.

It generally requires 10^5–10^6 cells/g in order to have enough ATP to emit consistent detectable light, but results can be obtained within 1 h depending on the initial number of cells. The method has been shown to be comparable to APC for the aerobic flora of ground beef (81) over the APC range of 10^5–10^6 cells/g and to be applicable to seafoods (82), and yeasts in beverages (83). Sensitivity can be increased for beverages by filtration and concentration of cells. Recent studies by Ellerbroek et al. (84) found that the ATP assay was suitable for monitoring the hygiene of poultry carcasses in slaughterhouses by enumerating psychrotrophic and mesotrophic bacteria present. This method was also reported for assessing the microbial quality of milk samples (85).

Limulus Amoebocyte Lysate (LAL) Test

The LAL method is applicable to the determination of lipopolysaccharides (LPS) or endotoxins in foods, and as such the detection of gram-negative bacteria, both viable and nonviable. It employs the lysate from the amoebocytes of the horseshoe crab (*Limulus polyphemous*), which is the most specific substance known for detecting LPS (86). Because all gram-negative bacteria produce LPS while gram-positives do not, LAL is a test for gram-negative bacteria.

The LAL test can be run in several ways, and these have been presented and discussed elsewhere (87). The most commonly used is a tube gelation method where the food specimen is diluted in pyrogen-free water and 0.1 or 0.2 mL amounts are added to similar quantities of the LAL reagent in pyrogen-free tubes (all glassware and reagents must be pyrogen-free for use by this method). Following incubation at 37°C for 1 h, positive tests are indicated by the presence of firm gels. The chromogenic substrate method consists of using synthetic substrates that produce a color change in the presence of LPS and the LAL reagent, and the color change is read spectrophotometrically. The latter method can be run in 30 min, and it lends itself to automation (87,88).

Commercially available LAL reagents can detect 1.0 pg of LPS, and since a typical *Escherichia coli* cell contains about 3.0 fg of LPS, the LAL test should be able to respond to about 300 cells. Studies with pseudomonads from meats have shown the test to be capable of detecting 10^2 cfu/mL (89), and it has been shown to be effective for testing meats (90), raw milk (91–93), seafoods (94), sugars and some processed meats (54).

Since the total gram-negative flora of a food product can be determined within 1 h by use of LAL, this test has been adapted to the approximation of the total bacterial count using fresh ground beef (87,95). By this procedure, the gram-negative bacteria are represented by LAL values, whereas gram-positives are estimated by multiplying respective gram-negative numbers by predetermined ratio values of gram negatives to gram-positives. Estimated total bacteria by this procedure have been shown to compare favorably to the total count by use of APC methods (89,95).

Since the LAL test can be conducted in 1 h or less, it can be used to make rapid assessments of the microbial load or overall sanitation of a food product. In this application, low LAL values are more meaningful than high values since the latter could represent nonviable cells.

Radioimmunoassay (RIA)

This method does not lend itself to the enumeration of microbial cells per se, but it can be used to detect/quantitate toxic products of microbial cells. Briefly, RIA consists of adding a radioactive label (eg, ^{125}I) to a soluble antigen followed by reaction of the antigen with its homologous antibody bound to a solid such as polystyrene. After washing, the adherence of the radiolabel to the polystyrene is assessed by use of a radioactivity counter.

RIA is one of the most sensitive methods known for detecting staphylococcal enterotoxins (96,97), enterotoxins of *E coli* (98), *Vibrio cholerae* (99), mycotoxins (100,101) and other similar products (3). It is sensitive to 0.1 ng of staphylococcal enterotoxin A, 0.5 ng of aflatoxin B_1, and 20 ppb ochratoxin A. The most serious drawbacks to this method is the need for radioactive compounds and the consequent need for a radioactivity counter.

Enzyme Immunoassay, Enzyme-linked Immunoabsorbent Assay (EIA, ELISA)

In its simplest form, ELISA can be viewed as RIA without a radioactive element. Instead of the latter, an enzyme substrate reaction is used to indicate an homologous antigen—antibody reaction. Because of the absence of radioactivity, ELISA is much more widely used to detect microbial toxins in foods, and it has been used to detect salmonellae cells in food products (102–104). An automated ELISA test was recently reported for detecting *Salmonella* in chocolate (105). The ELISA test gave 15% fewer false negatives compared to conventional culture methods. For the latter, 10^4–10^5 cells/mL may be detected but the general minimum is around 10^6/mL. It can detect 0.1 ng/ml of *Clostridium perfringens* enterotoxin, and 10 pg/mL or less of aflatoxin M_1 (106) and other mycotoxins (107–109). ELISA tests to detect botulinal toxins Type A (110), Type E (111), and Type G (112) have been developed as well as tests for staphylococcal enterotoxins (113–115).

Although ELISA methods can be run with either polyclonal or monoclonal antibodies, the latter tend to give better results (116). A commercial method for detecting *Listeria monocytogenes* employing monoclonal antibodies is available (117). A collaborative study on the ELISA test proved it effective for detecting *L. monocytogenes* and other *Listeria* spp. in foods (118). This method was subsequently adopted by AOAC International for detecting *L. monocytogenes* in dairy, seafood, and meat products. A monoclonal method for salmonellae has been show capable of detecting as few as 10^6 cells/mL (119).

Thermostable Nuclease (TNase)

This test is specifically designed for *Staphylococcus aureus* and it can be run in 1–2 h (120). All strains of *S. aureus* that produce enterotoxins also produce this heat-stable nuclease, although many TNase-positive strains do not produce enterotoxins (121).

The microslide method consists of a layer of agar containing toluidine blue O and DNA into which holes are made for test samples. After heating to 100°C, food or food homogenates are placed in the holes and the slide is incubated at 37°C for 1–2 h. If TNase is present, the DNA is degraded and this is manifested by a change in color of the dye (122). It generally takes about 5×10^7 *S. aureus* cells to produce detectable amounts of TNase and this enzyme is generally produced before enterotoxins. Thus, this test can be used to screen foods for the presence of enterotoxins even in the absence of viable *S. aureus* cells (123,124). Occasional false positive tests may occur from *Streptococcus faecalis* strains that also produce TNase.

DNA Probes

Although DNA probes are most often used to detect specific organisms, their use may be viewed as screens for the organism in question since 10^6–10^7 cells/mL are needed for positive tests. In a typical application, one selects a unique DNA sequence of the organism of interest and tags a single strand with a radioisotope. DNA fragments of unknown organisms are prepared by use of restriction endonucleases. After separating the fragment strands, they are transferred to cellulose nitrate filters and hybridized to the radioactive probe. After gentle washing to remove unreacted probe DNA, the presence of the radiolabel is detected by autoradiography or by use of a radioactivity counter.

A number of investigators have developed and tested DNA probes for salmonella (125–127), listeriae (128–131), and other food-borne pathogens and found the method to be satisfactory for detecting the presence of target organisms in mixed culture. Commercial methods exist for salmonellae and listeriae, but they generally require 44 + h when used on products that contain low numbers of target organisms.

DNA probes are used in colony hybridization methods where micro- or macrocolonies of the target organism are allowed to develop directly on membranes that are incubated on suitable agar plates. Following treatment of the colonies to separate and fix DNA strands, the DNA probe is applied to the membrane and the radiolabel is tested for. Colony hybridizations have been employed with success to detect *Listeria monocytogenes*, enterotoxigenic strains of *E. coli* (132,133), and *Yersinia enterocolitica*. These developments have resulted in the polymerase chain reaction (PCR) technique which has found considerable application for detecting foodborne pathogens (134).

One of the drawbacks to the use of DNA probes in the food industry is the presence of radioactive elements. One of the most promising nonisotope probes employs biotin and its detection using a streptavidine-alkaline phosphatase conjugate to produce an insoluble color precipitate in the presence of a dye. One such method was developed (135) and shown to be workable for *E. coli* and to detect 2×10^7 cells/g in about 30 h. The nonisotopic detection system required about 3 h for results.

BIBLIOGRAPHY

1. E. H. Marth, ed., *Standard Methods for the Examination of Dairy Products*, 14th ed., American Public Health Association, Washington, D.C., 1978.
2. M. L. Speck, ed., *Compendium of Methods for the Microbiological Examination of Foods*, 2nd ed., American Public Health Association, Washington, D.C., 1984
3. J. M. Jay, *Modern Food Microbiology*, 3rd ed., Van Nostrand Reinhold, New York, 1986
4. A. N. Sharpe and A. K. Jackson, "Stomaching: A New Concept in Bacteriological Sample Preparation," *Applied Microbiology* **24**, 175–178 (1978).
5. B. S. Emswiler, C. J. Pierson, and A. W. Kotula, "Stomaching vs Blending. A Comparison of Two Techniques for the Homogenization of Meat Samples for Microbiological Analysis," *Food Technology* **31**, 40–42 (1977).
6. W. H. Andrews, C. R. Wilson, P. L. Poelma, A. Romero, R. A. Ruce, A. P. Duran, P. D. McClure, and D. E. Gentile, "Usefulness of the Stomacher in a Microbiological Regulatory Laboratory," *Applied Environmental Microbiology* **35**, 89–93 (1978).
7. J. M. Jay and S. Margitic, "Comparison of Homogenizing, Shaking, and Blending on the Recovery of Microorganisms and Endotoxins from Fresh and Frozen Ground Beef as As-

sessed by Plate Counts and the *Limulus* Amoebocyte Lysate Test," *Applied Environmental Microbiology* **38**, 879–884 (1979).

8. J. E. Gilchrist, J. E. Campbell, C. B. Donnelly, J. T. Peeler, and J. M. Delaney, "Spiral Plate Method for Bacterial Determination," *Applied Microbiology* **25**, 244–252 (1973).
9. C. B. Donnelly, J. E. Gilchrist, J. T. Peeler, and J. E. Campbell, "Spiral Plate Count Method for the Examination of Raw and Pasteurized Milk," *Applied Environmental Microbiology* **32**, 21–27 (1976)
10. B. Jarvis, V. H. Lach, and J. M. Wood, "Evaluation of the Spiral Plate Maker for the Enumeration of Micro-organisms in Foods," *Journal of Applied Bacteriology* **43**, 149–157 (1977).
11. Association of Official Analytical Chemists "Spiral Plate Method for Bacterial Count: Official First Action," *Journal of the Association of Official Analytical Chemists* **60**, 493–494 (1977).
12. C. L. Nelson, T. L. Fox, and F. F. Busta, "Evaluation of Dry Medium Film (Petrifilm VRB) for Coliform Enumeration," *Journal of Food Protection* **47**, 520–525 (1984).
13. L. B. Smith, T. L. Fox, and F. F. Busta, "Comparison of a Dry Medium Culture Plate (Petrifilm SM plates) Method to the Aerobic Plate Count Method for Enumeration of Mesophilic Aerobic Colony-forming Units in Fresh Ground Beef," *Journal of Food Protection* **48**, 1044–1045 (1985).
14. M. P. deFigueiredo and J. M. Jay, "Coliforms, Enterococci, and Other Microbial Indicators," in M. P. deFigueiredo and D. F. Splittstoesser, eds., *Food Microbiology: Public Health and Spoilage Aspects*, Avi Publishing, Westport, Conn., 1976.
15. L. S. L. Yu, R. K. Prasai, and D. Y. C. Fung, "Most Probable Numbers of *Listeria* Species in Raw Meats Detected by Selective Motility Enrichment," *Journal of Food Protection* **58**, 943–945 (1995).
16. E. S. Humbert et al., "Miniaturized Most Probable Number and Enrichment Serology Technique for the Enumeration of *Salmonella* spp. on Poultry Carcasses," *Journal of Food Protection* **60**, 1306–1311 (1997).
17. C. K. Johns, "Place of the Methylene Blue and Resazurin Reduction Tests in a Milk Control Program," *American Journal of Public Health* **29**, 239–247 (1939).
18. R. Dabbah, W. A. Moats, S. R. Tatini, and J. C. Olson, Jr., "Evaluation of the Resazurin Reduction One-Hour Test for Grading Milk Intended for Manufacturing Purposes," *Journal of Milk Food Technology* **32**, 44–48 (1969).
19. P. J. Dodsworth and A. G. Kempton, "Rapid Measurement of Meat Quality by Resazurin Reduction. II. Industrial Application," *Canadian Institute of Food Science Technology Journal* **10**, 158–160 (1977).
20. D. N. Rao and V. S. Murthy, "Rapid Dye Reduction Tests for the Determination of Microbiological Quality of Meat," *Journal of Food Technology* **21**, 151–157 (1986).
21. R. A. Holley, S. M. Smith, and A. G. Kempton, "Rapid Measurement of Meat Quality by Resazurin Reduction. I. Factors Affecting Test Validity," *Canadian Institute of Food Science Technology Journal* **10**, 153–157 (1977).
22. H. W. Walker, W. J. Coffin, and J. C. Ayres, "A Resazurin Reduction Test for Determination of Microbiological Quality of Processed Poultry," *Food Technology* **13**, 578–581 (1959).
23. R. Kummerlin, "Technical Note: Resazurin Test for Microbiological Control of Deep-Frozen Shrimps," *Journal of Food Technology* **17**, 513–515 (1982).
24. A. N. Sharpe and G. L. Michaud, "Hydrophobic Grid-Membrane Filters: New Approach to Microbiological Enumeration," *Applied Microbiology* **28**, 223–225 (1974).
25. A. N. Sharpe and G. L. Michaud, "Enumeration of High Numbers of Bacteria Using Hydrophobic Grid-Membrane Filters," *Applied Microbiology* **30**, 519–524 (1975).
26. A. N. Sharpe, M. P. Diotte, I. Dudas, S. Malcolm, and P. I. Peterkin, "Colony Counting on Hydrophobic Grid-Membrane Filters" *Canadian Journal of Microbiology* **29**, 797–802 (1983).
27. A. O. A. C., "Enumeration of Coliforms in Selected Foods, Hydrophobic Grid Membrane Filter Method, Official First Action," *Journal of the Association of Official Analytical Chemists* **66**, 547–548 (1983).
28. P. Entis, "Enumeration of Coliforms in Non-Fat Dry Milk and Canned Custard by Hydrophobic Grid Membrane Filter Method: Collaborative Study," *Journal of the Association of Official Analytical Chemists* **66**, 897–904 (1983).
29. M. H. Brodsky, P. Entis, A. N. Sharpe, and G. A. Jarvis "Enumeration of Indicator Organisms in Foods Using the Automated Hydrophobic Grid Membrane Filter Technique," *Journal of Food Protection* **45**, 292–296 (1982).
30. M. H. Brodsky, P. Entis, M. P. Entis, A. N. Sharpe, and G. A. Jarvis, "Determination of Aerobic Plate and Yeast and Mold Counts in Foods Using an Automated Hydrophobic Grid Membrane Filter Technique," *Journal of Food Protection* **45**, 301–304 (1982).
31. P. Entis, "Rapid Hydrophobic Grid Membrane Filter Method for *Salmonella* Detection in Selected Foods," *Journal of the Association of Official Analytical Chemists* **68**, 555 (1985).
32. J. R. Sage and S. C. Ingham, "Survival of *Escherichia coli* 0157:H7 After Freezing and Thawing Ground Beef Patties," *Journal of Food Protection* **61**, 1181–1183 (1998).
33. P. I. Peterkin, E. S. Idziak, and A. V. Sharpe, "Use of Hydrophobic Grid-Membrane Filter DNA Probe to Detect *Listeria monocytogenes* in Artificially-Contaminated Food," *Food Microbiology* **9**, 155–160 (1992)
34. W. Yan et al., "Comparison of the Hydrophobic-Grid Membrane Filter DNA Probe Method and the Health Protection Standard Method for the Detection of *Listeria monocytogenes* in Food," *International Journal of Food Microbiology* **30**, 379–384 (1996)
35. U. M. Rodrigues and R. G. Kroll, "Rapid Selective Enumeration of Bacteria in Foods Using a Micro-Colony Epifluorescence Microscopy Technique," *Journal of Applied Bacteriology* **64**, 65–78 (1988).
36. U. M. Rodrigues and R. G. Kroll, "Microcolony Epifluorescence Microscopy for Selective Enumeration of Injured Bacteria in Frozen and Heat-Treated Foods," *Applied Environmental Microbiology* **55**, 778–787 (1989).
37. D. Hardy, S. J. Kraeger, S. W. Dufour, and P. Cady, "Rapid Detection of Microbial Contamination in Frozen Vegetables by Automated Impedance Measurements," *Applied Environmental Microbiology* **34**, 14–17 (1977).
38. P. Cady, D. Hardy, S. Martins, S. W. Dufour, and S. J. Kraeger, "Automated Impedance Measurements for Rapid Screening of Milk Microbial Content," *Journal of Food Protection* **41**, 277–283 (1978).
39. J. M. Wood, V. Lach, and B. Jarvis, "Detection of Food-Associated Microbes Using Electrical Impedance Measurements," *Journal of Applied Bacteriology* **43**, xiv–xv (1977).
40. M. P. Silverman and E. F. Munoz, "Automated Electrical Impedance Technique for Rapid Enumeration of Fecal Coli-

forms in Effluents From Sewage Treatment Plants," *Applied Environmental Microbiology* **37**, 521–526 (1979).

41 S B Martins and M. J. Selby, "Evaluation of a Rapid Method for the Quantitative Estimation of Coliforms in Meat by Impedimetric Procedures," *Applied Environmental Microbiology* **39**, 518–524 (1980).

42. R. Firstenberg-Eden and C. S. Klein, "Evaluation of a Rapid Impedimetric Procedure for the Quantitative Estimation of Coli-Forms" *Journal of Food Science* **48**, 1307–1311 (1983).

43. J. L. Weihe, S. L. Seist, and W S. Hatcher, Jr., "Estimation of Microbial Populations in Frozen Concentrated Orange Juice Using Automated Impedance Measurements," *Journal of Food Science* **49**, 243–245 (1984).

44. R. Firstenberg-Eden, "Rapid Estimation of the Number of Microorganisms in Raw Meat by Impedance Measurement," *Food Technology* **37**, 64–70 (1983).

45. R. Andreine, "Automated Microbiological Techniques," *Latte* **19**, 456–463 (1994).

46. M. Campanini, S. Barbuti, and G. Camorali, "Quick Evaluation of Microbial Contamination of Fresh Meat by an Impediometric Procedure," *Industria-Conserve* **64**, 121–125 (1989).

47. R. Firstenberg-Eden and M. K. Tricarico, "Impedimetric Determination of Total, Mesophilic and Psychotrophic Counts in Raw Milk," *Journal of Food Science* **48**, 1750–1754 (1983).

48. P. Silley and S. Forsythe, "Impedance Microbiology—A Rapid Change for Microbiologists," *Journal of Applied Bacteriology* **80**, 233–243 (1996).

49. R. A. Lampi, D. A. Mikelson, D. B. Rowley, J. J. Previte, and R. E. Wells, "Radiometry and Microcalorimetry—Techniques for the Rapid Detection of Foodborne Microorganisms," *Food Technology* **28**, 52–55 (1974).

50. D. B. Rowley, J. J. Previte, and H P. Srinivasa, "A Radiometric Method for Rapid Screening of Cooked Foods for Microbial Acceptability," *Journal of Food Science* **43**, 1720–1722 (1978).

51. B. J. Stewart, M. J. Eyles, and W. G. Murrell, "Rapid Radiometric Method for Detection of *Salmonella* in Foods," *Applied Environmental Microbiology* **40**, 223–230 (1980).

52. M Gagnon, W. M. Hunting, and B. Esselen, "New Method for Catalase Determination," *Analytical Chemistry* **31**, 144–146 (1959).

53. R. Charbonneau, J. Therrien, and M. Gagnon, "Detection and Measurement of Bacterial Catalase by the Disk-Flotation Method Using the Catalasemeter," *Canadian Journal of Microbiology* **21**, 580–582 (1975).

54. K. L. Dodds, R. A. Holley, and A. G. Kempton, "Evaluation of the Catalase and *Limulus* Amoebocyte Lysate Tests for Rapid Determination of the Microbial Quality of Vacuum-Packed Cooked Turkey," *Canadian Institute of Food Science Technology Journal* **16**, 167–172 (1983).

55. W. W. Forrest, "Microcalorimetry," *Methods in Microbiology* **6B**, 285–318 (1972).

56 L. E. Sacks and E. Menefee, "Thermal Detection of Spoilage in Canned Foods," *Journal of Food Science* **37**, 928–931 (1972).

57. T. Fugita, P R. Mond, and I. Wadso, "Calorimetric Identification of Several Strains of Lactic Acid Bacteria," *Journal of Dairy Research* **45**, 457–463 (1978).

58. B. F. Perry, A. E. Beezer, and R. J. Miles, "Flow Microcalorimetric Studies of Yeast growth: Fundamental Aspects," *Journal of Applied Bacteriology* **47**, 527–537 (1979).

59. B. F. Perry, A. E. Beezer, and R. J. Miles, "Characterization of Commercial Yeast Strains By Flow Microcalorimetry," *Journal of Applied Bacteriology* **54**, 183–189 (1983).

60. R. P. Betts, P. Bankes, and J. G. Board, "Rapid Enumeration of Viable Micro-Organisms by Staining and Direct Microscopy," *Letters in Applied Microbiology* **9**, 199–202 (1989).

61. T. C. Hutcheson, T. McKay, L. Farr, and B. Seddon, "Evaluation of the Strain Viablue for the Rapid Estimation of Viable Yeast Cells," *Letters in Applied Microbiology* **6**, 85–88 (1988).

62. H. A. Koch, R. Bandler, and R. R. Gibson, "Fluorescence Microscopy Procedure for Quantification of Yeasts in Beverages," *Applied Environmental Microbiology* **52**, 599–601 (1986).

63. Association of Official Analytical Chemists, *Official Methods of Analysis*, 15th ed., Vol. 1, AOAC, Arlington, Va., 1990.

64. G. L. Pettipher, "The Direct Epifluourescent Filter Technique," in M R. Adams and C. F. A. Hope, eds., *Progress in Industrial Microbiology*, Vol. 26, Elsevier, Amsterdam, 1989, pp. 19–26.

65. J. E. Hobbie, R. J. Daley, and S. Jasper, "Use of Nucleopore Filters for Counting Bacteria by Fluorescence Microscopy," *Applied Environmental Microbiology* **33**, 1225–1228 (1977).

66. G. L. Pettipher, R. Mansell, C. H. McKinnon, and C. M. Cousins, "Rapid Membrane Filtration-Epifluorescent Microscopy Technique for Direct Enumeration of Bacteria in Raw Milk," *Applied Environmental Microbiology* **39**, 423–429 (1980).

67. B. G. Shaw, C. D. Harding, W. H. Hudson, and L. Farr, "Rapid Estimation of Microbial Numbers on Meat and Poultry by Direct Epifluorescent Filter Technique," *Journal of Food Protection* **50**, 652–657 (1987).

68. J. T. Holah, R. P. Betts, and R. H. Thorpe, "The Use of Direct Epifluorescent Microscopy (DEM) and the Direct Epifluorescent Filter Technique (DEFT) to Assess Microbial Populations on Food Contact Surfaces," *Journal of Applied Bacteriology* **65**, 215–221 (1988).

69. U. M Rodrigues and R. G Kroll, "The Direct Epifluorescent Filter Technique (DEFT): Increased Selectivity, Sensitivity and Rapidity," *Journal of Applied Bacteriology* **59**, 493–499 (1985).

70. C. P. Champagne et al., "Determination of Viable Bacterial Populations in Raw Milk Within 20 Minutes by Using a Direct Epifluorescent Filter Technique," *Journal of Food Protection* **60**, 874–876 (1997).

71. J. M. Goepfert, M. E. Mann, and R. Hicks, "One-day Fluorescent-Antibody Procedure for Detecting Salmonellae in Frozen and Dried Foods," *Applied Microbiology* **20**, 977–983 (1970).

72. B. M. Thomason, "Rapid Detection of *Salmonella* Microcolonies by Fluorescent Antibody," *Applied Microbiology* **22**, 1064–1069 (1971).

73. L. Restaino et al., "A 5-h Screening and 24-h Confirmation Procedure for Detecting *Escherichia coli* 0157·H7 in Beef Using Direct Epifluourescent Microscopy and Immunomagnetic Separation," *Letters in Applied Microbiology* **24**, 401–404 (1997).

74. M. L. Tortorello and D. S. Stewart, "Antibody-Direct Epifluourescent Filter Technique for Rapid Direct Enumeration of *Escherichia coli* 0157.H7 in Beef," *Applied and Environmental Microbiology* **60**, 3553–3559 (1994).

75. M. L. Tortorello, K. F. Reinecke, and D. S. Stewart, "Comparison of Antibody-Direct Epifluourescent Filter Technique With Most Probable Number Procedure for Rapid Enumeration of *Listeria* in Fresh Vegetables," *Journal of AOAC International* **80**, 1208–1214 (1997).

76. L. D. Fantasia, J. P. Schrade, J. F. Yager, and D. Debler, "Fluorescent Antibody Method for the Detection of *Salmonella*: Development, Evaluation, and Collaborative Study," *Journal of the Association of Official Analytical Chemists* **58**, 828–844 (1975).
77. T. E. Munson, J. P. Schrade, N. B. Bisciello, Jr., L. D. Fantasia, W. R. Hartung, and J. J. O'Connor, "Evaluation of an Automated Fluorescence Antibody Procedure for Detection of *Salmonella* in Foods and Feeds," *Applied Environmental Microbiology* **31**, 514–521 (1976).
78. U. M. Rodrigues and R. G. Kroll, "Rapid Detection of Salmonellas in Raw Meats using a Fluorescent Antibody-Microcolony technique," *Journal of Applied Bacteriology* **68**, 213–223 (1990).
79. A. N. Sharpe, M. N. Woodrow, and A. K. Jackson, "Adenosinetriphosphate (ATP) Levels in Foods Contaminated by Bacteria," *Journal of Applied Bacteriology* **33**, 758–767 (1970).
80. G. A. Kimmich, J. Randles, and J. S. Brand, "Assay of Picomole Amounts of ATP, ADP, and AMP Using the Luciferase Enzyme System," *Analytical Biochemistry* **69**, 187–206 (1975).
81. C. J. Stannard and J. M. Wood, "The Rapid Estimation of Microbial Contamination of Raw Meat by Measurement of Adenosinetriphosphate (ATP)," *Journal of Applied Bacteriology* **55**, 429–438 (1983).
82. D. R. Ward, K. A. LaRocco, and D. J. Hopson, "Adenosine Triphosphate Bioluminescent Assay to Enumerate Bacterial Numbers on Fresh Fish," *Journal of Food Protection* **49**, 647–650 (1986).
83. K. A. LaRocco, P. Galligan, K. J. Littel, and A. Spurgash, "A Rapid Bioluminescent ATP Method for Determining Yeast Contamination in a Carbonated Beverage," *Food Technology* **39**, 49–52 (1985).
84. L. Ellerbroek et al., "Hygiene Monitoring in Poultry Meat Production, Use of Bioluminescence ATP Assay," *Fleischwirtschaft* **7**, 486–487, 489 (1998).
85. S. Girotti et al., "Determination of Microbial Contamination in Milk by ATP Assay," *Czech Journal of Food Science* **15**, 241–248 (1997).
86. J. D. Sullivan, Jr., F. W. Valois, and S. W. Watson, "Endotoxins: The Limulus Amoebocyte Lystae System, in A. W. Bernheimer, ed., *Mechanisms in Bacterial Toxinology*, Wiley, New York, 1976.
87. J. M. Jay, "The *Limulus* Amoebocyte Lysate (LAL) Test, in M. R. Adams and C. F. A. Hope, eds., *Progress in Industrial Microbiology: Rapid Methods in Food Microbiology*, Elsevier, Amsterdam, 1989.
88. K. Tsuji, P. A. Martin, and D. M. Bussey, "Automation of Chromogenic Substrate *Limulus*Amebocyte Lysate Assay Method for Endotoxin by Robotic System." *Applied Environmental Microbiology* **48**, 550–555 (1984).
89. H. J. Fallowfield and J. T. Patterson, "Potential Value of the *Limulus* Lysate Assay for the Measurement of Meat Spoilage," *Journal of Food Technology* **20**, 467–479 (1985).
90. J. M. Jay, S. Margitic, A. L. Shereda, and H. V. Covington, "Determining Endotoxin Content of Ground Beef by the *Limulus* Amoebocyte Lysate Test as a Rapid Indicator of Microbial Quality," *Applied Environmental Microbiology* **38**, 885–890 (1979).
91. K.-J. Zaadhof and G. Terplan, "Der *Limulus*-test—ein Verfahren zur Buerteilung der Mikrobiologischen Qualität von Milch und Milchprodukten," *Deutsch Molkerzeit* **34**, 1094–1098 (1981).
92. J. Mottar et al., "Routine Limulus Amoebocyte Lysate (LAL) Test for Endotoxin Determination in Milk Using a Toxinometer ET-201," *Journal of Dairy Science* **60**, 223–228 (1993).
93. P. C. Vasavada, "Rapid Methods and Automation in Dairy Microbiology," *Journal of Dairy Science* **76**, 3101–3113 (1993).
94. J. D. Sullivan, Jr., P. E. Ellis, R. G. Lee, W. S. Combs, Jr., and S. W. Watson, "Comparison of the *Limulus* Amoebocyte Lysate Test with Plate Counts and Chemical Analyses for Assessment of the Quality of Lean Fish," *Applied Environmental Microbiology* **45**, 720–722 (1983).
95. J. M. Jay, "Rapid Estimation of Microbial Numbers in Fresh Ground Beef By Use of the *Limulus* Test," *Journal of Food Protection* **44**, 275–278 (1981).
96. B. A. Miller, R. F. Reiser, and M. S. Bergdoll, "Detection of Staphylococcal Enterotoxins A, B, C, D, and E in Foods By Radioimmunoassay, Using Staphylococcal Cells Containing Protein A as Immunoadsorbent," *Applied Environmental Microbiology* **36**, 421–426 (1978).
97. H. Robern, M. Dighton, Y. Yano, and N. Dickie, "Double-Antibody Radioimmunoassay for Staphylococcal Enterotoxin C_2," *Applied Microbiology* **30**, 525–529 (1975).
98. R. A. Giannella, K. W. Drake, and M. Luttrell, "Development of a Radioimmunoassay for *Escherichia coli* Heat-Stable Enterotoxin: Comparison With the Suckling Mouse Bioassay," *Infectious Immunology* **33**, 186–192 (1981).
99. D. B. Shah, P. E. Kauffman, B. K. Boutin, and C. H. Johnson, "Detection of Heat-Labile-Enterotoxin-Producing Colonies of *Escherichia coli* and *Vibrio cholerae* by Solid-Phase Sandwich Radioimmunoassay," *Journal of Clinical Microbiology* **16**, 504–508 (1982).
100. O. Aalund, K. Brunfeldt, B. Hald, P. Krogh, and K. Poulsen, "A Radioimmunoassay for Ochratoxin A: A Preliminary Investigation," *Acta Pathologic Microbiologica Scandinavica Section* **83**, 390–392 (1975).
101. J. J. Pestka, V. Li, W. O. Harder, and F. S. Chu, "Comparison of Radioimmunoassay and Enzyme-Linked Immunosorbent Assay for Determining Aflatoxin M_1 in Milk," *Journal of the Association of Official Analytical Chemists* **65**, 294–301 (1981).
102. S. A. Minnich, P. A. Hartman, and R. C. Heimsch, "Enzyme Immunoassay for Detection of Salmonellae in Foods," *Applied Environmental Microbiology* **43**, 877–883 (1982).
103. B. J. Robison, C. I. Pretzman, and J. A. Mattingly, "Enzyme Immunoassay in Which a Myeloma Protein is Used for Detection of Salmonellae," *Applied Environmental Microbiology* **45**, 1816–1821 (1983).
104. R. S. Flowers, K.-H. Chen, B. J. Robison, J. A. Mattingly, D. A. Gabis, and J. H. Silliker, "Comparison of *Salmonella* Bio-EnzaBead Immunoassay Method and Conventional Culture Procedure for Detection of *Salmonella* in Crustaceans," *Journal of Food Protection* **50**, 386–389 (1987).
105. B. Pacher-Beck, "Automated ELISA Test for Detection of *Salmonella* in Chocolate—A Note," *Lebensmittel & Biotechnologie* **13**, 59 (1996).
106. W. J. Hu, N. Woychik, and F. S. Chu. "ELISA of Picogram Quantities of Aflatoxin M_1 in Urine and Milk," *Journal of Food Protection* **47**, 126–127 (1984).
107. J. J. Pestka, P. K. Gaur, and F. S. Chu, "Quantitation of Aflatoxin B_1 and Aflatoxin B_1 Antibody by an Enzyme-Linked Immunosorbent Microassay," *Applied Environmental Microbiology* **40**, 1027–1031 (1980).

108. J. J. Pestka, B. W. Steinert, and F. S. Chu, "Enzyme-Linked Immunosorbent Assay for Detection of Ochratoxin A," *Applied Environmental Microbiology* **41**, 1472–1474 (1981).

109. J. J. Pestka and F. S. Chu, "Enzyme-Linked Immunosorbent Assay of Mycotoxins Using Nylon Bead and Terasaki Plate Solid Phases," *Journal of Food Protection* **47**, 305–308 (1984).

110. S. Notermans, J. Dufrenne, and M. van Schothorst, "Enzyme-Linked Immunosorbent Assay for Detection of *Clostridium botulinum* Type A," *Japanese Journal of Medical Science Biology* **31**, 81–85 (1978).

111. S. Notermans, J. Dufrenne, and S. Kozaki, "Enzyme-Linked Immunosorbent Assay for Detection of *Clostridium botulinum* Type E Toxin," *Applied Environmental Microbiology* **37**, 1173–1175 (1979).

112. G. E. Lewis, Jr., S. S. Kulinski, D. W. Reichard, and J. F. Metzger, "Detection of *Clostridium botulinum* Type G Toxin by Enzyme-Linked Immunosorbent Assay," *Applied Environmental Microbiology* **42**, 1018–1022 (1981).

113. G. C. Saunders and M. L. Bartlett, "Double-Antibody Solid-Phase Enzyme Immunoassay for the Detection of Staphylococcal Enterotoxin A," *Applied Environmental Microbiology* **34**, 518–522 (1977).

114. G. Stiffler-Rosenberg and H. Fey, "Simple Assay for Staphylococcal Enterotoxins A, B, and C: Modification of Enzyme-Linked Immunosorbent Assay," *Journal of Clinical Microbiology* **8**, 473–479 (1978).

115. S. Notermans, R. Boot, P. D. Tips, and M. P. DeNooij, "Extraction of Staphylococcal Enterotoxins (SE) from minced meat and subsequent detection of SE with Enzyme-Linked Immunosorbent Assay (ELISA)," *Journal of Food Protection* **46**, 238–241 (1983).

116. J. M. Farber and J. I. Speirs, "Monoclonal Antibodies Directed Against the Flagella Antigens of *Listeria* Species and Their Potential in EIA-Based Methods," *Journal of Food Protection* **50**, 479–484 (1987).

117. B. T. Butnam, M. C. Plank, R. J. Durham, and J. M. Mattingly, "Monoclonal Antibodies Which Identify a Genus-Specific *Listeria* Antigen," *Applied Environmental Microbiology* **54**, 1564–1569 (1988).

118. M. S. Curiale, W. Lepper, and B. Robinson, "Enzyme-Linked Immunoassay for Detection of *Listeria monocytogenes* in Dairy Products, Seafoods and Meats: A Collaborative Study," *Journal of the AOAC International* **77**, 1472–1489 (1994).

119. J. A. Mattingly, "An Enzyme Immunoassay for the Detection of All *Salmonella* Using a Combination of Myeloma Protein as a Hybridoma Antibody," *Journal of Immunological Methods* **73**, 147–156 (1984).

120. B. M. Hill, "The Thermostable Nuclease Test as a Method for Identifying *Staphylococcus aureus*," *Australian Journal of Dairy Technology* **38**, 95–96 (1983).

121. C. E. Park, H. B. El Derea, and M. K. Rayman, "Evaluation of Staphylococcal Thermonuclease (TNase) Assay as a Means of Screening Foods for Growth of Staphylococci and Possible Enterotoxin Production," *Canadian Journal of Microbiology* **24**, 1135–1139 (1978).

122. A. Koupal and R. H. Deibel, "Rapid Qualitative Method for Detecting Staphylococcal Nuclease in Foods," *Applied Environmental Microbiology* **35**, 1193–1197 (1978).

123. J. F. Kamman and S. R. Tatini, "Optimal Conditions for Assay of Staphylococcal Nuclease," *Journal of Food Science* **42**, 421–424 (1977).

124. B. S. Emswiler-Rose, R. W. Johnston, M. E. Harris, and W. H. Lee, "Rapid Detection of Staphylococcal Thermonuclease on Casings of Naturally Contaminated Fermented Sausages," *Applied Environmental Microbiology* **40**, 13–18 (1980).

125. R. Fitts, M. L. Diamond, C. Hamilton, and M. Neri, "DNA-DNA Hybridization Assay for Detection of *Salmonella* Spp. in Foods," *Applied Environmental Microbiology* **46**, 1146–1151 (1983).

126. R. S. Flowers, M. A. Mozzola, M. S. Curiale, D. A. Gabis, and J. H. Silliker, "Comparative Study of a DNA Hybridization Method and the Conventional Culture Procedure for Detection of *Salmonella* in Foods," *Journal of Food Science* **52**, 781–785 (1987).

127. J. R. Bandekar, K. K. Ussuf, and P. M. Nair, "Detection of *Salmonella* in Chicken and Fish Samples Using DNA Probes," *Journal of Food Safety* **15**, 11–19 (1995).

128. S. Notermans, T. Chakraborty, M. Leimeister-Wächter, J. Dufrenne, K. J. Heuvelman, H. Maas, W. Jansen, K. Wernars, and P. Guinee, "Specific Gene Probe for Detection of Biotyped and Serotyped *Listeria* Strains," *Applied Environmental Microbiology* **55**, 902–906 (1989).

129. A. R. Datta, B. A. Wentz, D. Shook, and M. W. Trucksess, "Synthetic Oligodeoxyribonucleotide Probes for Detection of *Listeria Monocytogenes*," *Applied Environmental Microbiology* **54**, 2933–2937 (1988).

130. R. F. Duvall and A. D. Hitchins, "Pooling Noncollaborative Multilaboratory Data for Evaluation of the Use of DNA Probe Test Kits in Identifying *Listeria monocytogenes* Strains," *Journal of Food Protection* **60**, 995–997 (1997).

131. L. Herman and H. de Ridder, "Evaluation of a DNA Probe Assay for the Identification of *Listeria monocytogenes*," *Milchwissenschaft* **48**, 126–128 (1993).

132. S. L. Moseley, P. Echeverria, J. Seriwatana, C. Tirapat, W. Chaicumpa, T. Sakuldaipeara, and S. Falkow, "Identification of Enterotoxigenic *Escherichia coli* by Colony Hybridization Using Three Enterotoxin Gene Probes," *Journal of Infectious Disease* **145**, 863–869 (1982).

133. W. E. Hill, J. M. Madden, B. A. McCardell, D. B. Shah, J. A. Jagow, W. L. Payne, and B. K. Boutin, "Foodborne Enterotoxigenic *Escherichia coli*: Detection and Enumeration by DNA Colony Hybridization," *Applied Environmental Microbiology* **45**, 1324–1330 (1983).

134. W. E. Hill, "The Polymerase Chain Reaction: Application for the Detection of Foodborne Pathogens," *CRC Reviews in Food Science and Nutrition* **36**, 123–173 (1996).

135. S. Dovey and K. J. Towner, "A Biotinylated DNA Probe to Detect Bacterial Cells in Artificially Contaminated Foodstuffs," *Journal of Applied Bacteriology* **66**, 43–47 (1989).

FOODSERVICE SYSTEMS

OVERVIEW

The foodservice industry is defined as the industry responsible for preparing food or meals outside the home. In the last part of the 20th century, the food dollar has moved away from traditional food shopping. In the late 1990s, approximately 50% of food dollars were spent for food prepared away from the home. It is predicted that by 2005, the foodservice industry will have more points of distribution, many new selections, and fresher foods.

In the 1960s, the development of new food technologies in food production, packaging, transportation, changes in lifestyles, increased mobility of the population, and development of computers made possible the growth of modern foodservice. Higher expenditure on food away from home is the result of higher incomes, several paychecks in a family, and busier lifestyles. Any foodservice operation that supplies prepared food to a customer, such as institutions, fast-food operations, restaurants, and espresso stands, can be classified as a system.

The external environment energizes this dynamic foodservice system. Without this external energy, the system fails. We can approach large or small foodservice systems as interrelated activities that make up the total system. As in any system, the basic operating activities influence the total system.

Inputs in the foodservice system are the operational, physical, and people required to produce the operation objectives, a food(s) product. The activity, procurement, and production of food are the finished product; the quality of food and service are the output of the system. An internal control is the menu. The external controls include the federal and state laws and regulations governing the operation. The responses from internal and external environments include the customer comments, plate waste, cost, and frequency of selection of a food item. All external feedback provides the energy that keeps the system going.

We can divide the foodservice industry into three groups: commercial, institutional, and military feeding. The commercial feeding is the largest segment of the foodservice industry. This industry includes those operations that are open to the public and supply meal service daily for profit, such as, on-site feeding from table service, amusement and ball park concessions, airlines, cruise ships, espresso stands, food courts, and casual theme eateries. The institutional noncommercial feeding groups include business, education, correctional institutions, and health care facilities that operate their own foodservice. Many of these establishments do not operate for profit but must stay within a break-even budget. Some institutional foodservice operations do make a profit, but profit is not the primary goal. For many of these organizations, nutritious food, nutrition education, and customer satisfaction are the primary goals. Military feeding includes the foodservice to the troops, noncommissioned officers and officers clubs, and military exchange foodservice. The military is now emphasizing healthy entrees and nutrition education. Menus are modified to reduce fat, salt, and cholesterol and to increase the use of fresh fruits and vegetables.

Foodservice systems are designed for efficient purchasing, storage, preparation, delivery of product, service, and transportation. Recipes are standardized for each type of operation. In some facilities, methods of preparation are controlled using assembly-line techniques in back-of-the-house and front-of-the-house operations to speed customer service. Customers in the 1990s do not have the patience or time to stand in line. Sophisticated, computerized equipment has added to the efficiency of many foodservice operations. Microwave ovens are used to reheat or reconstitute many food items in institutional and commercial operations.

Restaurants and large supermarkets prepare cooked-to-order food or use prepared food already portioned and shaped (eg, ready-cut potatoes for frying). They may also choose to use meat and desserts proportioned and ready to serve. Computer technology has increased efficiency of the institutional and commercial foodservice operations when ordering food, either from a central commissary or direct from a manufacturer. The larger restaurants and fast-food chains order food from a central location where whole meals are prepared and packaged so that they remain fresh. The preprepared food or meals are distributed to other establishments, sometimes at a great distance from the preparation facility. Preservation methods include fast freezing, precooking, and dehydrating. New food technologies and food irradiation add shelf life to food products, which increases efficiency and decreases cost.

Many successful foodservice operations have identified a particular segment of the population and focus on specific consumer desires. The take-out phenomenon of foodservice that grew from recognizing the needs of a particular consumer population is an example. In the past decade, more gourmet take-out foods from large supermarkets, specialty take-out, and fast food eateries have developed. The larger franchise chain operations use marketing research and advertising strategies to identify and increase customer sales.

Commercial foodservice includes eating and drinking operations, food contractors, recreation and sports centers, stores and gas station foodservice, and lodging establishments. The franchise foodservice provides more theme or specialty foodservice. Fast-food foodservice includes specialties in sandwiches or hamburgers, seafood, and ethnic foods. Local operators or fully franchised operations may own some fast-food operations. Hotels may have an inhouse managed foodservice operation or contract with a franchised operation. A trend in the hotel foodservice operations is to have independent coffee or espresso kiosks at customer traffic areas. Some large department stores combine a gourmet menu and take-out service with their restaurant foodservice.

In-house management or a food contractor may operate the foodservice operations in health care institutions. Increasing costs, competition, and change of population have provided challenges for the health care industry. The number of older patients who require complicated dietary regiments and modified diets, operating expenses of hospitals, convalescent homes, and long-term care facilities continues to increase. The philosophical change in feeding the older population in convalescent homes and long-term care facilities to provide a more liberalized diet has helped to lower the variety of foods prepared. This trend, along with new technologies, has helped the health care foodservice industry to become more efficient. Feeding facilities for employees, the public, and catering are potential revenue sources. Health care foodservice directors are encouraged to meet their customer's needs and have become very entrepreneurial.

Changes in the public's eating behaviors have influenced changes in college and university feeding. College costs, fewer residential colleges and universities, and more diverse college populations have had an impact on the foodservice operation. Nontraditional board plans, à la carte programs, cash operations, 24-hour service, take-out service, espresso kiosks, and a variety of menus are innovations that have developed. Foodservice is no longer obtained in just one location for those living in residence halls. Specialty dining halls for the faculty and guests and catering activities are other types of foodservice that may be provided.

Centralization of various functions has become a stable operational function in college and university foodservice operations. They may centralize food purchasing, storage, preparation, and some production. Foodservice employees may then transport the prepared item or raw material to a satellite unit. Recipe and menu standardization, preprepared purchased food items, and computerized operations have made college and university foodservice operations more efficient and cost effective.

School lunch in the United States has a long history. The rejection of young men for military service in World War II because of poor nutritional status led the impetus to the development of the National School Lunch Program. The National School Lunch Act in 1946 provided more than 23.5 million children with a school lunch, with approximately 50% receiving reduced price or free lunches. The National School Lunch Act had a dual purpose: "to safeguard the health and well-being of the nation's children and to encourage the domestic consumption of nutritious agricultural products." Federal regulations control the meal pattern and provide for joint administration of the program through a federal-state-local school district relationship.

The Federal Government has changed the Child Nutrition Act often since 1946. Legislation and amendments have changed the funding policy and circumstances for offering free or reduced-price meals to children. Legislation has reshaped the commodity distribution plan, provided for supplemental feeding programs, and authorized the School Breakfast Program. One of the biggest impacts on the federal school lunch program was the Omnibus Reconciliation Act of 1980. This legislation reduced the reimbursement rate to schools and changed the income eligibility standard for students who could receive free or reduced-price meals. Other legislation modifications were made in the 1990s to help achieve reductions in federal spending. School foodservice personnel work creatively to meet these legislation changes while maintaining a nutritious and satisfying meal program that will appeal to the student population.

School foodservice programs vary in type of organization and management from the one unit, one manager operation to a centrally managed unit in a large city school system. Many large school systems have centralized food preparation centers that distribute food in bulk or preplated, hot or refrigerated service to students in schools with satellite service centers. Since early 1970, program regulations have changed, allowing schools to negotiate with food contractors to provide meals.

Commercial and industrial organizations also offer employee feeding programs. The increase in white collar workers and suburban living increased foodservice sales in office buildings. The use of food and beverage vending machines with microwave ovens has simplified and reduced the cost of these foodservice operations.

Food contractors use marketing strategies to encourage sales, such as on-site bakeries, espresso coffee centers, and take-home food items. A variety of settings are used, from elegant executive dining rooms to the vending-machine cafeteria.

In the transportation segment of foodservice, food contractors provide most of the in-transit airline feeding. They adapt menus and food items to specifications of the various airline carriers. Sales in the transportation feeding for passenger and cargo liners and railroads continue to decline. The major thrust in transportation foodservice has been efficient productivity.

MENU SYSTEM

The foodservice operation begins and ends with the menu, a detailed list of foods served. Creative menu planning uses originality, imagination, and knowledge. The menu is a guide for the kitchen and wait person and a printed list for customers. The menu determines the foods purchased, equipment needed for production, personnel required for production and service, and work schedules of the employees. It is a marketing tool and an important factor in controlling costs.

The menu must be consistent with the goals of the organization and meet the food preferences of the identified customer. Considerations to use in menu planning for commercial foodservice include the goals of the organization, equipment and physical facilities, skill of employees, money available, and type of service. Most institutional foodservice operations include the consideration of serving nutritious foods. Commercial foodservice has moved to include healthy food and the caloric value of foods served on their menus. In the 1990s, many restaurants have included caloric values or notations identifying "healthy heart" food items. The public's avoidance of many of these healthy foods has led some foodservice establishments to discontinue the nutrition information menu. Managers planning menus for any foodservice operation must be aware of the food preferences, food habits, and cultural make-up of their customers. Monitoring current trends in eating behaviors is necessary. Balancing these food preferences of the clientele with the food budget, the constraints of the facility, and employees skills are important tasks for all foodservice managers.

To determine effectiveness of the menu, many foodservice operations prepare a method to evaluate menus. This may be a record of food purchased, customer preference surveys, plate waste checks, or verbal opinion from customers. Production employees can contribute to the evaluation of menus. Providing a routine employee menu evaluation allows the employees some ownership in the production of the menus.

PURCHASING, RECEIVING, AND STORAGE SYSTEMS

To maintain an effective and cost-efficient foodservice operation, the purchasing and inventory system must be carefully planned. Selecting the establishment's suppliers on an objective basis and developing specifications and formal buying procedures contribute to efficient management. Computers have made this part of the foodservice system more efficient and effective.

Effective purchasing procedures include the knowledge of availability of food, number of suppliers in the area, skill of production employees, organization's food and nonfood items budget, and the quality and value of food items. The purchasing manager must use appropriate buying methods, establish good ordering schedules, and develop an effective system of communicating needs from the production to the buyer. The purchasing manager must have a thorough knowledge of the menu, organization mission, food and nonfood specifications, and personnel resources.

Specifications for food and nonfood items should be detailed to ensure the continuous quality of products. Several federal agencies have established quality grades for the safety of many food products. Determination of the quality of a food or nonfood item to buy and whether to prepare or buy prepared foods will be governed by the organization's mission, menu, and identified customer.

Computer technology is used to develop an effective system for purchasing and recording the delivery of food and nonfood items. Employees must be trained to receive foods and store them at appropriate temperatures. All preplanning and savings can be lost by careless employees in the storage and receiving area.

Designing effective food-receiving procedures provides control and predictability. Receiving procedures that involve daily reports, a tagging system, and invoice stamps are appropriate management tools. Providing a checking system to evaluate the accuracy of the foodservice receiving system is important.

Adequate procedures for storage can reduce spoilage and product theft. Storage areas, including dry, refrigerated, and frozen, should be checked regularly. Procedures for checking dry, refrigerated, and frozen food items are essential for inventory and product control. Physical inventory systems are essential. Computer software has been developed for day-to-day inventories interfaced with ordering and purchasing. These systems give the foodservice organization a painless, perpetual inventory system.

PRODUCTION SYSTEM

Basic preparation standards and procedures are necessary in any foodservice system. A standard product can be developed consistently, and waste can be avoided. Using preprepared foods will reduce the need for skilled labor and reduce energy requirements. Because the cost of operating a foodservice establishment is still climbing, the production system has the potential to be the most expensive of all the systems. Skilled labor is expensive, but so is untrained, unskilled labor. Therefore, the use of standardized recipes, good quality ingredients, training, and proper supervision of production is essential.

The menu is the input and control, where production planning begins. The production system transforms the raw product into the salable output of the foodservice system. Accurate forecasting is the prediction of the food needs for the meal, day, or defined period based on established trends. Forecasting is a procedure that can limit food waste and maintain cost controls. Past sales trends can be used along with present and future developments. Food sales performance determines the quantities of food and portion size to be prepared. This information is included on the production schedule, along with special instructions for preparation and work assignments. New computer technologies and modern equipment can help in making the production equipment more efficient. To maintain quality and consistent foodservice, employees must follow the standardized recipes, procedures, and portion control. Quality preprepared and preplated food items have provided automation and decreased personnel expense in this area.

To maintain food quality, the purchase of quality food is the first step. Using standardized recipes, supervision of food production, and critical tasting techniques are other factor in producing quality items. The food needs to be safe, wholesome, and pleasing to the targeted customer for the foodservice operation to be successful.

THE SERVICE SYSTEM

The wait person or server communicates the establishment's attitude toward the customer. Some foodservice operations use the term guest to emphasize the value placed on their clientele. The server spends more time with the customer and may be the first and last person with whom the customer has contact. A good culture of service occurs when servers are empowered to respond positively to all customer requests. Management must promote this "culture of service" to all employees that have contact with the customer. Good servers are aware of the customer's discomfort and dissatisfaction. Having a positive regard for the customer is training by example. Continuing education for managers and employees is necessary.

A full-service restaurant practices one of the four basic service styles or a combinations of styles. Family-style service uses serving dishes placed on the table, and the customers serve themselves. This is also known as English service. Smorgasbords and self-serve salad bars are forms of the family-style service. Plate service, or American service, is generally used by most restaurants. All the food is put on plates in the kitchen and served in the dining room. Bread and butter may be served on a bread and butter plate. French service, or table-side service, is another style. This service is characterized by the service of food from a heated cart by a waiter and assistant. The waiter and assistant prepare finishing touches to the food on the cart at the table and serve the food to the customer. During platter service, or Russian service, the food is fully prepared and cut into portions in the kitchen. The waiter serves the food on platters directly to the customer. Both French and Russian types of service allow for showmanship and drama at the customer's table. More equipment, utensils, and skilled waiters are needed for this type of service. Portion control may also be a problem.

In the 1990, most full-service restaurants added another type of service: the take-out and delivery service. Take-out foods have become a vital part of the American's way of life. Restaurants and food retailers have become kitchens-on-the-go. A consumer survey revealed that approximately three-quarters (78%) of U.S. households make one carry-out or delivery purchase in a typical month. The take-out customer is "in a hurry," "tired," or has "no other place to go." Therefore, the customer is looking for speedy service, accurately filled orders, easy access, reasonable prices, and sometimes portion sizes that provide enough for leftovers. The restaurant's take-out contact person may be the only employee the customer sees. A good service culture is important.

HUMAN RESOURCE SYSTEM

Foodservice operations have had a negative production record in the past, but the trend is to reverse this image. The foodservice industry is learning to maximize its best resource—the people they employ. Increasing productivity and decreasing employee turnover should be objectives of a good foodservice manager. Effective recruitment, selection, orientation, and training will enhance these objectives.

A plan for recruiting and selecting new employees communicates the knowledge and skill needed for employment. Employee orientation and training programs developed by the fast-food chains have emphasized the need for training programs in all foodservice operations. Managers need to be trained for their positions and to understand the principles of learning to maximize training efforts. Managers who are not trained to use effective investigative methods, active listening, and conflict-resolution techniques when small employee problems occur may discover a larger employee problem. Failure to resolve small problems may contribute to an unhappy working environment and frequent employee changes. Unresolved employee problems open the door to labor unions to represent the employees better.

Government legislation affects human resources planning. Since the 1960s, several pieces of legislation have had significant impact on the hiring, disciplining, promotion, demotion, internal organization environment, and separation of employees.

Developing standards of performance to evaluate employee and staff can increase the employee's involvement and motivation. Management by objective can be used for staff personnel and is an effective personal motivator. "Management by walking around" can be an effective way to "catch" an employee doing something right. Management by walking around adds to more positive employee performance evaluations. Using this technique will allow the manager to correct inappropriate behavior or work procedures on the spot. An employee manual with the foodservice operation's policies and procedures is necessary for good employee–employer relationships A positive approach, humor, and a good self-image can be valuable assets for a foodservice manager. Providing a positive work environment and paying attention to the employee and the employee's contributions to the organization's goals can be the most effective motivator in the industry.

EQUIPMENT AND ENERGY SYSTEM

Knowledge of equipment and energy is necessary to provide efficient transformation of the raw food to quality food products. Training of personnel in the care and use of the equipment is important for preserving the equipment. Today's current use of computerized equipment requires better equipment care to reduce the cost of repair. On the other hand, many foods are purchased preprepared or precut and require less equipment and skill to prepare and cook. When purchasing a piece of equipment, consideration should be given to the initial cost, installation, maintenance, personnel using the equipment, and energy cost. Calculations can be used to determine these answers and the equipment cost over time.

In energy management, a planned and organized program is necessary for an efficient organization. An equipment maintenance checklist can provide a starting point. Many equipment manufacturers and utility companies have tips for energy conservation.

QUALITY ASSURANCE CONTROL SYSTEM

Quality assurance programs allow foodservice operations to be proactive rather than reactive. Satisfaction of the customer is the goal of the operation. Quality assurance programs ensure the attainment of that goal. The use of the hazard analysis critical control point concept has been recommended as a preventive approach to quality control in foodservice systems. Quality audits are an important component of a quality assurance program and provide feedback. These audits allow management to control the output of the system.

GENERAL REFERENCES

I. Obenaeur, "Foodservice Trends," *Restaurants USA* **18**, 39–46, 1998.

J. Payne-Palacio and M. Theis, *West and Wood's Introduction to Foodservice*, 8th ed., Prentice-Hall, Englewood Cliffs, N.J., 1997.

A. E. Sloan, "Food Industry Forecast: Consumer Trends to 2020 and Beyond," *Food Technol.* **52**, 37–44 (1998).

DOROTHY POND-SMITH
Washington State University
Pullman, Washington

FREEZE CONCENTRATION

Freeze concentration is a process in which aqueous solutions are concentrated by partially freezing them and separating the ice produced from residual unfrozen solution. Because nonaqueous volatile components (eg, volatile flavors and aromas in foods) are not removed, freeze concentration provides more selective water removal than evaporation. It is also gentler because no heat exposure is involved. Freeze concentrated liquid foods often taste measurably better than evaporative concentrates. Unfortunately, the taste improvement does not always translate into improved sales. In spite of process improvements and cost reductions achieved in recent years, freeze concentration remains much more costly than evaporative concentration.

PRODUCTS

Coffee extract, fruit juices, milk, beer, wine, and vinegar have been concentrated by freeze concentration. Freeze concentration is also used to recover potable water in the form of meltable ice from sea water and brackish water. A less-selective analogous process, partial solidification, is used to separate high-melting fats from low-melting fats.

Freeze concentration works best for clear solutions; any insoluble solids present are likely to reduce cleanness of separation and be trapped and lost when the concentrate is separated from the ice. Therefore, insoluble solids should be removed before freeze concentration begins. They can, if desired, be added back to the concentrate after the process is complete. Freeze concentration also does not work well if solutes in the solution precipitate as concentration occurs.

FREEZING POINT DEPRESSION

Solutes depress the freezing point of water. Therefore, as ice forms and solute concentrations increase, the freezing point decreases. Figure 1 shows how the freezing points of wine and some juices depend on concentration (1). The freezing point depression is roughly inversely proportional to the solute's molecular weight. Thus, at equal concentrations, freezing point depressions for juices (eg, apple juice) in which the main solutes are fructose and glucose are greater than those for juices (eg, orange juice) containing greater amounts of sucrose. Depending on the fruit juice involved, solute concentrations in the 50% range can be obtained by cooling juice to −8 to −14°C. Cooling to −13 to −18°C is required to obtain 60% concentration.

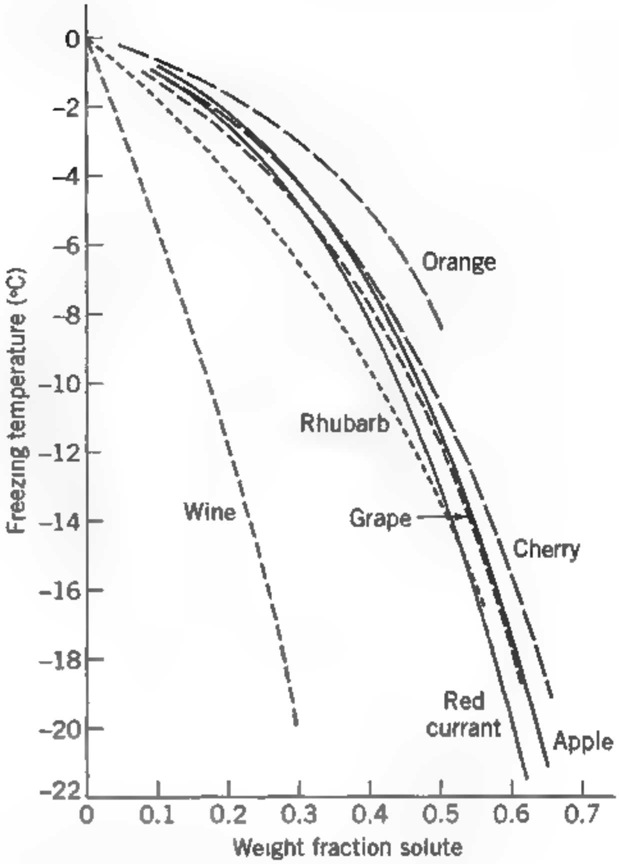


Figure 1. Freezing point versus concentration for wine and various fruit juices. *Source:* Ref. 1. Courtesy of Marcel Dekker.

When simple binary solutions (eg, NaCl in water) undergo freezing, the solute involved will precipitate at a certain temperature and corresponding concentration, the eutectic temperature and concentration. As the solute precipitates, the remaining water simultaneously freezes. It is extremely difficult to separate the components of the resulting solid mixture. For such solutions, freeze concentration beyond the eutectic concentration is impossible. Liquid foods contain complex mixtures of solutes that inhibit each others' crystallization. Therefore, eutectics rarely form when liquid foods freeze. Around 65% solute concentration, the remaining unfrozen water in foods so strongly associates with the solutes, or is otherwise prevented from freezing, that freezing stops or radically slows down. Further, at such concentrations, concentrates become so viscous that they cannot be readily separated from ice. Therefore, 65% represents the upper concentration limit for freeze concentration of liquid foods. Final concentrations obtained in most food freeze concentration systems are lower (45 or 50%). In some cases, only a modest increase in concentration is provided, such as when low-sugar-content wine grape juices are freeze concentrated to permit production of wine that contains adequate amounts of alcohol. Large increases in concentration are used when wine grape juice is freeze concentrated so that it can be stored for subsequent use in wine production.

CLEANNESS OF SEPARATION

Apple jack has been freeze concentrated on farms by slowly freezing it in a milk can and augering out the concentrate that accumulates along the can's axis. A great deal of solute is left behind, trapped in ice. Similar loss-prone procedures can be used to freeze concentrate small amounts of aqueous solution in laboratories. In commercial freeze concentration, solute losses must be minimized; nearly all

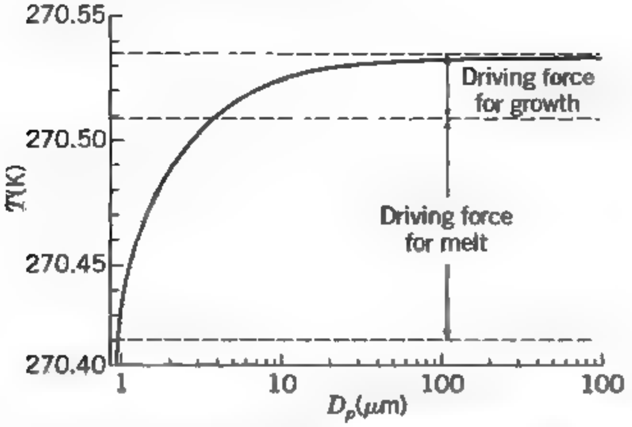


Figure 2. Freezing point versus crystal diameter for ice in a 30% sucrose solution. *Source:* Reprinted courtesy of Niro Process Technology B.V.

solute-containing liquid has to be cleanly separated from ice. This requires production of well-rounded, suitably large crystals of ice and efficient means of separation. Solute losses can be kept below 0.01% when food liquids are separated from suitably large ice crystals (200 to 250 μm diameter) in wash columns (the most efficient separation devices currently available). When centrifugation is used for ice-concentrate separation, losses are in the 1 to 3% range. For pressing, losses are around 5%. Losses are also bad for filtration. Losses for these less-efficient ice-concentrate separation systems can be reduced by using reverse osmosis to recover solutes from melted ice.

ICE CRYSTAL FORMATION AND GROWTH

Ice-free aqueous solutions have to be cooled below their equilibrium freezing point before ice crystal nuclei form and freezing starts. Ice can nucleate in several ways. Heterogeneous nucleation usually predominates in ice-free solutions and rapidly occurs roughly 6°C below the initial

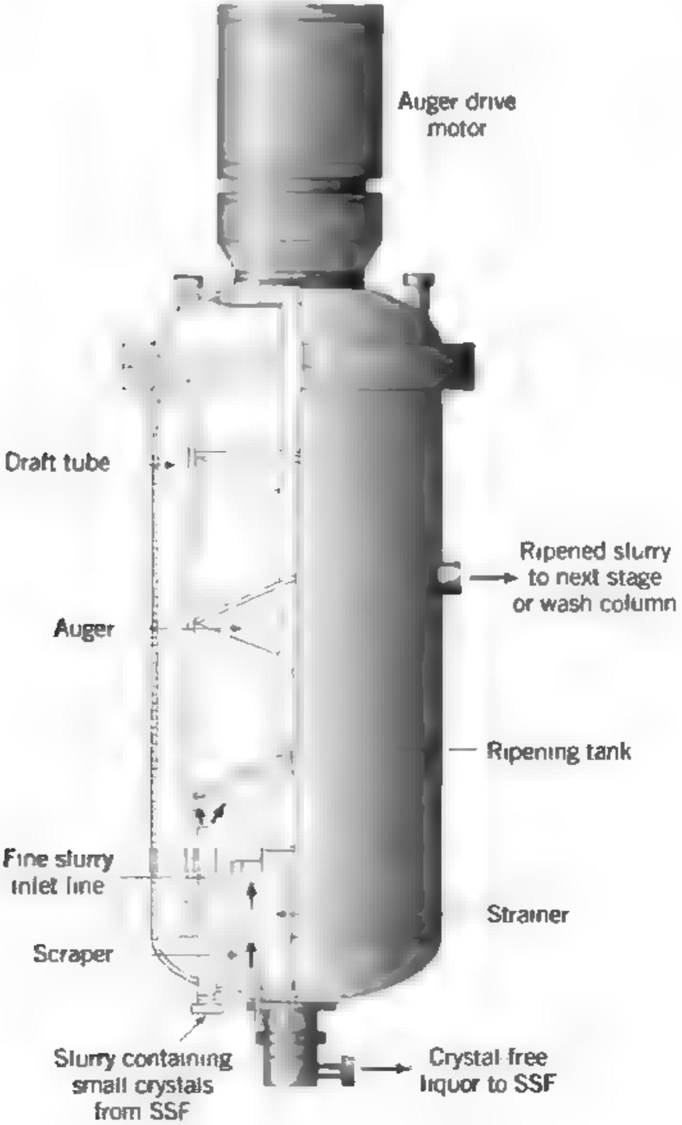

Auger drive motor
Draft tube
Ripened slurry to next stage or wash column
Auger
Ripening tank
Fine slurry inlet line
Strainer
Scraper
Slurry containing small crystals from SSF
Crystal-free liquor to SSF

Figure 3. A ripening tank. SSF = scraped-surface freezer. *Source:* Reprinted courtesy of Niro Process Technology B.V.

equilibrium freezing point of the solution. Once nucleation occurs, the solution's temperature rises and closely approaches its equilibrium freezing point at its current concentration. Ice nuclei are very small and have to be converted into much larger ice crystals for efficient separation. This can be done by bulk supercooling that causes existing crystals to grow by maintaining the solution below its equilibrium freezing point. The greater the supercooling, the greater the rate of growth. However, if the supercooling is too large, highly branched crystals from which solution does not separate cleanly will form. Further, new nuclei will continually form on cooling surfaces if temperature differences larger than 6°C are used to provide heat removal.

Therefore, it is preferable to form small ice crystals on sharply chilled, rapidly scraped surfaces and mix those crystals with larger crystals in tanks, in which the small crystals sacrificially melt and cause large-crystal growth. The process occurs because ice crystals with small radii have slightly lower equilibrium freezing points than crystals with larger radii (Fig. 2). Slurries containing mixtures of smaller and larger crystals assume a temperature between the respective equilibrium freezing temperatures of the small and large crystals. The small crystals tend to melt, and the large crystals tend to grow, a process known as Ostwald ripening (2). The temperature differences involved are very small, but the process is fairly rapid in stirred systems because the crystals provide an enormous amount of heat-transfer and mass-transfer surface.

Ripening in freeze concentration systems is frequently carried out in a large, enclosed, vertical, cylindrical tank fitted with a helical agitator and draft tube (Fig. 3). Liquid withdrawn from the mixing tank through a scraped screen at its bottom is fed to freezers and returns to the tank as an ice-containing slurry. The process runs best when the returning slurry contains very small crystals (10 μm diameter), and the bulk of the crystals in the tank are much larger (120 to 250 μm diameter).

Table 1. Local Conditions in a Four-Stage Freeze-Concentration System Converting 5,000 kg/h of a 10% Solute Feed into a 50% Solute Concentrate

Stage	1	2	3	4
Concentration (% s.s.)	16.0	26.1	36.4	50.0
Temperature (°C)	−2.0	−3.7	−6.5	−9.6
Ice-formation rate (kg/hr)	2,100	1,200	900	600
Mean ice crystal diameter (μm)	240	198	163	120

ICE PRODUCTION

Roughly 75% of a feed by volume may be converted into ice in a typical food freeze concentration system. Stirring is needed to provide rapid ice crystal ripening and growth, but ice-concentrate mixtures containing so much ice cannot be readily stirred or pumped. Therefore, enough concentrate is retained in the system to keep ice volume percentages in the system within the stirrable range (below 28% by volume).

FREEZE-CONCENTRATION SYSTEMS

The basic components in most freeze concentration systems are freezers, ripening tanks, and separators (2). Originally, single-stage systems,—systems containing one ripening tank or, in some cases, a bulk-growth tank—were

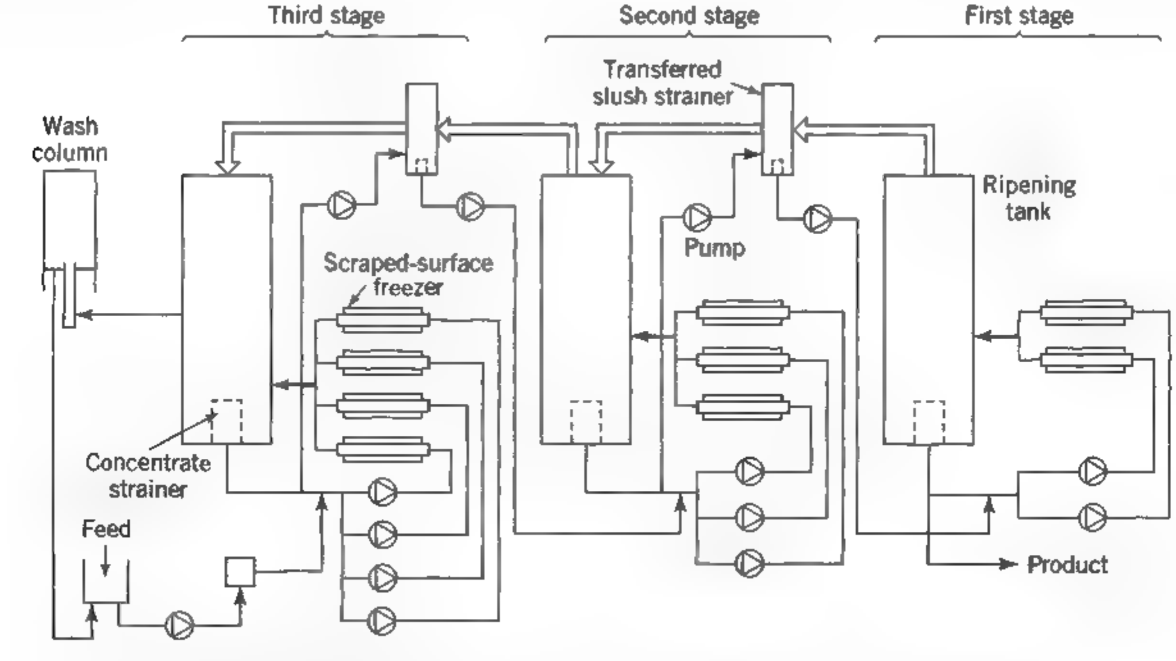


Figure 4. A three-stage freeze-concentration system. *Source:* Reprinted courtesy of Niro Process Technology B.V.

used and are still used when little concentration is required Countercurrent multistage systems that use wash columns as separators are used to provide high degree of concentration. Compared to single-stage operation, countercurrent multistage operation provides faster, cleaner separation; greater thermodynamic efficiency (reduced energy consumption); faster ripening and crystal growth; and higher concentrations. Figure 4 shows a modern three-stage countercurrent freeze-concentration system. Systems containing up to five stages are used. Each stage consists of a ripening tank served by several scraped-surface freezers operating in parallel (3). Clear liquid, drawn out of the tank through a scraped strainer, is pumped through the associated freezers and returns to the tank as an ice-containing slurry. Although high scraping rates and strong chilling are used, the liquid passes through the freezers so quickly that only 7.5 to 8% of the stream is converted into ice, and 5- to 10-μm diameter crystals are produced.

Flow within the system is arranged so that the liquid being concentrated and the ice produced move countercurrent with respect to each other in an overall sense. Part of the liquid drawn through the ice strainer at the bottom of a ripening tank is sent to the next tank in terms of concentrate flow, that is, to the tank with the next higher concentration. Ice-concentrate mix continuously transfers out of each stage. Most of the concentrate in the mix is mechanically expelled; much of what remains is displaced by more dilute concentrate from the stage the ice is about to enter. The ice enters the next stage in the direction of ice flow, which is the next-more-dilute stage. Ice-crystal size progressively increases as ice moves from the most concentrated stage to the most dilute stage.

Strained, ice-free product concentrate discharges from the most concentrated stage. Ice is removed in slurry form at the opposite end, the dilute end of the system, where separation is easiest. The discharged mixture of ice and dilute stage liquor is sent to a wash column, where it is propelled upward and slightly mechanically compressed. The compression expels most of the accompanying liquor. The ice is melted near the top of the column, forming melted water that displaces remaining solute-containing liquor from the pores in the advancing bed of ice. If the rate of ice advance is suitably slow, very clean displacement is obtained, and, as previously mentioned, less than 0.01% solute is lost. If it is too fast, very large amounts of solute are lost. The maximum rate of ice advance that can be safely used is roughly proportional to the ice crystal diameter squared and inversely proportional to the difference between viscosity of the displaced liquor and the viscosity of the water carrying out the displacement. Concentrations, temperatures, ice-formation rates, and mean ice crystal diameters in the stages of a four-stage freeze concentration system processing 5,000 kg/h of liquid feed are listed in Table 1.

The total rate of ice production, 4,800 kg/h, exceeds the net rate of ice removal, 4,000 kg/h, because some ice melts because of stirring-induced friction in the system. Roughly two hours of ice-holdup time in a multistage, freeze-concentration system may be required to achieve adequate ice crystal size growth. The average solute holdup is usually roughly twice as long. Less than a few minutes of holdup time are used in juice-concentration evaporators.

BIBLIOGRAPHY

1. H. G. Schwartzberg, "Food Freeze Concentration," in H. G. Schwartzberg and M. A. Rao, eds., *Biotechnology and Food Process Engineering*, Marcel Dekker, New York, 1990, pp. 127–202.
2. H. A. C. Thijssen, "Current Developments in the Freeze Concentration of Liquid Foods," in S. A. Goldblith, L. Rey, and W. W. Rothmayr, eds., *Freeze Drying and Advanced Food Technology*, Academic Press, London, United Kingdom, 1975, pp. 481–501
3. U.S. Patent 4,430,104 (Feb. 7, 1984). W. Van Pelt and J. Roodenrijs (to Grasso's Koniklijke Machine Fabrieken N V)

HENRY SCHWARTZBERG
University of Massachusetts
Amherst, Massachusetts

FREEZE DRYING

Freeze drying (lyophilization) is the drying of material in the frozen state. It is usually carried out under vacuum, at absolute pressures that readily permit ice to sublime (change directly from solid to vapor). Absolute pressures used in food freeze drying range between 50 μm Hg and 1,500 μm Hg.

Moist foods contain water that freezes (ie, forms ice) when suitably cooled, and smaller amounts of bound water that do not, even at -60°C, but instead form part of a solute-rich matrix that becomes extremely viscous and stiffens as temperature drops. In frozen foods, ice is embedded in that matrix Except for clear juices and extracts, frozen foods also contain other solid components, such as cell walls, membranes, and subcellular particles. As noted in the article FREEZE CONCENTRATION, food solutes depress the freezing point of water. The initial equilibrium–freezing points of foods usually lie between -0.5°C and -2°C. As foods freeze, the solutes they contain become more concentrated, and the freezing point depression increases. Therefore, successive portions of a food's water content freeze at lower and lower temperatures. Freezing is complete when the last freezable water changes into ice. In many foods, this effectively occurs between -20°C and -30°C.

During food freeze drying, ice sublimes and bound water desorbs as vapor. Most bound water remains when the last ice has sublimed; almost all has to be removed to provide stable, freeze-dried food. To do this, the product temperatures are raised, and added vacuum drying time is provided after the last ice sublimes. Ice-containing regions in foods remain fully frozen during freeze drying; temperatures well above freezing exist in parts of regions whose ice content has already sublimed.

PRODUCTS

Soluble coffee is the material freeze-dried in greatest volume. Vegetables for dried soup mixes, mushrooms, herbs, spices, cheese starter cultures, shrimp, fruits for ready-to-eat breakfast cereals and vegetables, meats, fish, and fruits for military, camping, and space-travel rations are or have been freeze-dried commercially. Freeze drying is used in making highly compressed, dry, military rations that expand to near-normal size and shape when rehydrated. Patents (1) or other technical literature describe the freeze drying of eggs, dairy products, powdered fish and shellfish, gelatin, bacon, cooked rice, emulsified peanut butter, soluble tea, puddings, jellies, pie fillings, salads coated with dressings, berries, avocado powder, potato-based products, fruit juices, and yeasts. Laboratory-scale freeze drying is used to preserve food samples.

ADVANTAGES AND DISADVANTAGES

Advantages of freeze drying include (*1*) low thermal damage; (*2*) good retention of volatile flavors (2); (*3*) good vitamin retention with minor exceptions (eg, loss of pantothenic acid in pork) (3); (*4*) rapid product rehydration (sometimes this requires slow freezing before drying); (*5*) low product shrinkage; (*6*) long product storage life if products are suitably packed; and (*7*) retention of biological activity (particularly if cryoprotectants are used during freezing). Disadvantages of freeze drying include (*1*) freeze drying costs much more than conventional drying (4); (*2*) certain products are damaged by freezing before drying; (*3*) freeze-dried products rapidly pick up moisture unless packed and maintained at low humidity; (*4*) freeze-dried products are very friable; and (*5*) some freeze-dried products (eg, carrots) bleach when stored (this can be prevented by suitable pretreatment).

SYSTEM COMPONENTS

Figure 1 shows basic components of a simple vacuum freeze dryer: a vacuum chamber containing a set of parallel heating plates, between which is a tray holding the frozen food to be dried; a vacuum pump for evacuating the chamber and removing inleaking air; a condenser where water vapor generated during drying condenses as ice; refrigeration for cooling the condenser; and a system that supplies hot heat-transfer fluid to the plates. Other elements not shown include a pressure-tight door that permits insertion and removal of loads; instruments for sensing plate, condenser and drying food temperatures, and for sensing pressure in the chamber; and the controller that regulates plate temperatures.

Figure 1 shows a single pair of heating plates and one tray of frozen food. Plant-scale freeze dryers contain many plates and many rows of trays holding many tray-loads of product. Large-scale freeze dryers are described later in this article. Reference 5 provides design details about such dryers.

Small-scale freeze dryers may contain up to five heating plates. They can process up to four rows of product, have a transparent plastic door that permits products to be observed during drying, and may be fitted with a balance or load cells that weigh food loads as they dry. Drying rates and temperatures measured in suitably instrumented small freeze dryers are used to help select operating conditions used in production-scale drying.

PREPARATION

Foods have to be suitably prepared (cleaned, peeled, trimmed, sliced, diced, and blanched or sulfited where necessary) and properly frozen before drying. To improve permeability, holes are punched in the skins of some foods, such as blueberries and peas. Meat is trimmed to remove fat (which interferes with drying) and, when sliced, is cut across the grain to maximize heat transfer and ease of vapor escape during drying. Liquid feeds may be evaporatively concentrated or freeze concentrated to reduce water removal loads in drying.

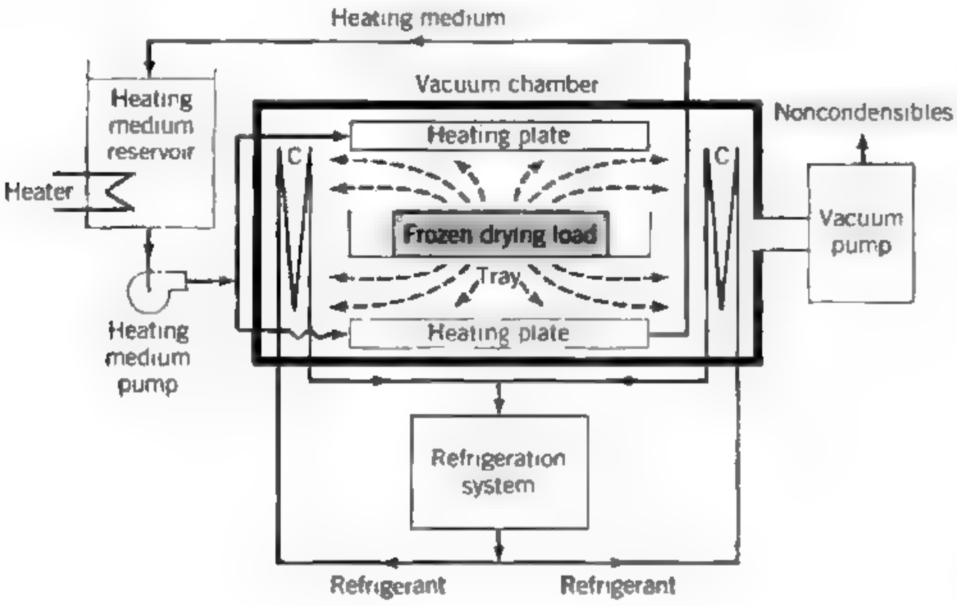


Figure 1. Basic components of a simple one-shelf vacuum freeze drier. C = condenser.

Freeze drying and the properties of freeze-dried foods are greatly affected by how foods are frozen. Slow freezing is used to produce freeze-dried coffee dark enough to be accepted by consumers and to improve the rehydratability of freeze-dried fruits. Rapid freezing permits more rapid desorption of bound water, but also causes greater resistance to vapor flow during sublimation.

BASIC PROCESS

Contact-based conductive heating has been used in some commercial freeze dryers. Microwave heating has been tested experimentally (6). In modern freeze dryers, radiant heating is by far the most commonly used method of providing heat input. Therefore, only drying based on its use will be discussed. Let us examine what happens when a slab of frozen food placed on a perforated tray is freeze dried in a simple dryer, like that shown in Figure 1. After the dryer door is closed and sealed, the refrigeration is turned on, cooling the condenser. The vacuum pump is turned on, and, as air leaves the dryer, pressure drops. When the pressure closely approaches the vapor pressure of ice at the set condenser temperature (eg, 97 μm Hg absolute for a −40°C condenser), hot fluid is pumped through the heating plates.

The plate temperatures rise until they reach a desired set value (eg, 120°C). Heat transfers radiantly from the plates to the slab, causing sublimation on both sides of the slab. Vapor produced at the bottom of the slab escapes through the perforations in the tray. Sublimation interfaces form and recede into the slab, leaving behind ice-free pores in the matrix containing the bound water. Thereafter, heat, radiantly transferred to outer surfaces of the slab, transfers conductively across the ice-free layers, causing further sublimation. Small amounts of bound water also evaporate. Vapor produced by both processes flows through the pores in the ice-free layers, causing a pressure drop that may be a large fraction of the absolute pressure in the dryer. The vapor leaves the slab and flows to the condenser, where it freezes.

The ice-free layers deepen as sublimation proceeds. Consequently, heat- and vapor-flow resistances increase, and the sublimation rate progressively decreases. After the last ice sublimes, that is, the sublimation interfaces meet near the center of the slab, the center temperature rises and bound water desorbs more rapidly. To prevent overdrying and overheating of outer parts of the slab, the plate temperature is reduced as desorption proceeds. At the end of drying, the plate temperature may be 50°C, and the center product temperature may be 30°C.

After drying is complete, the vacuum is broken by admitting air or, preferably, dry nitrogen. The product is quickly removed, rapidly transferred to a low humidity room, and quickly packed and sealed in an impermeable container. After all product is discharged from the dryer, ice is removed from the condenser by passing hot refrigerant gas through it or by spraying it with hot water. The melt produced drains from the dryer through a closeable outlet at its bottom.

HEAT TRANSFER

Heat that transfers across ice-free layers in a drying food causes a proportional amount of sublimation. Therefore, dZ/dt, the time rate of increase in the depths of ice-free layers in a slab, can be expressed in terms of the rate of heat transfer (7):

$$\frac{dZ}{dt} = \frac{k_t(T_o - T_s)}{\Lambda \rho X Z} \tag{1}$$

where T_o is the outer-surface temperature, T_s is the temperature at the sublimation interface, Z is the thickness of the ice-free layer, k_t is its thermal conductivity, Λ is the latent heat of sublimation (roughly 2,836 kJ/kg ice), and ρ and X are the initial density and weight fraction of ice in the slab, respectively.

The k_t is roughly proportional to the food's initial solids content and also depends on pressure. At absolute pressures greater than roughly 100 mm Hg, k_t is the same as at atmospheric pressure, that is, roughly 0.032 to 0.17 $\text{W} \cdot \text{m}^{-1} \cdot {}^{\circ}\text{C}^{-1}$. At lower pressures, k_t decreases sigmoidally as pressure decreases, and roughly around 100 μm Hg levels off at roughly one half to one quarter of its atmospheric pressure value. The respective pressures at which k_t shifts start and finish depend on pore diameter. In most cases, freeze drying is carried out at or close to the low-pressure k_t limit. Thermal conductivities encountered in freeze-drying foods are substantially lower than those of many thermal insulators.

PRESSURE DROP

The pressure drop caused by vapor flow across the ice-free layers in a slab is proportional to dZ/dt. Consequently, it can be shown that

$$(P_s - P_o) = \frac{k_t(T_o - T_s)}{\Lambda K} \tag{2}$$

where P_s is the pressure at the sublimation interface, P_o the pressure at the outer surface of the slab, and K is the permeability of the porous ice-free layers. K is proportional to the volume fraction of ice initially present, roughly inversely proportional to ice-crystal diameter, increases somewhat as the mean partial pressure of water in the pores increases, and decreases if excessive amounts of noncondensibles are present. Typical K range between 0.7×10^{-9} and 10×10^{-9} $\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot (\mu\text{m Hg})^{-1}$ (5×10^{-9} to 70×10^{-9} s^{-1} in SI units).

SUBLIMATION TEMPERATURE

The vapor pressure versus temperature relationship for ice, shown in Figure 2, fixes P_s in terms of T_s. In lightly loaded freeze dryers with wide vapor-transfer spaces, P_o equals the vapor pressure of the ice on the condenser. Figure 2 also depicts equation 2 solved to determine T_s and P_s for the freeze drying of a slab of coffee extract for which

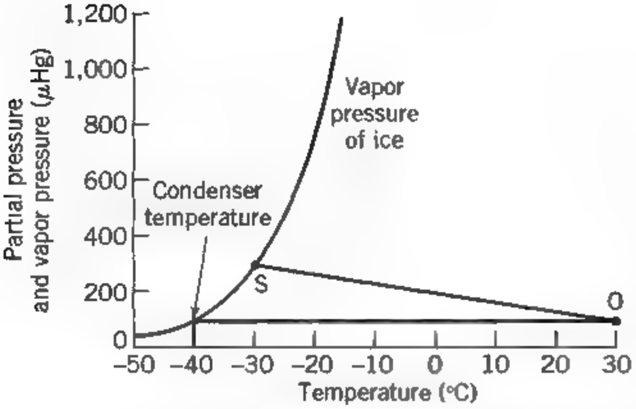


Figure 2. Vapor pressure of ice versus temperature and pressure and temperature conditions at the outer surface (O) and sublimation interface (S) of a slab of food undergoing freeze drying in a chamber where the condenser temperature is −40°C.

$k_t = 0.035\ \mathrm{W \cdot m^{-1} \cdot {}^\circ C^{-1}}$, $K = 3.5 \times 10^{-9}\ \mathrm{kg \cdot m^{-1} \cdot s^{-1} \cdot (\mu m\ Hg)^{-1}}$, and $T_o = 30°\mathrm{C}$. The condensed ice temperature is −40°C. Therefore, as shown by the dotted lines, $P_o = 97\ \mu\mathrm{m}$ Hg. T_o and P_o define point O. A line, drawn through O with slope $-k_t/(K \cdot A)$, intersects the ice vapor pressure curve at S, providing P_s and T_s, which respectively are 301 μm Hg and −29.4°C.

PRODUCT SURFACE TEMPERATURE

T_o is greatly affected by T_p, the heating plate temperature; higher T_p cause higher T_o. Usually, T_p is rapidly ramped up to a set value and then held there until sublimation is complete. If so, T_o initially will be only slightly higher then the condenser temperature. Then, as drying proceeds and the sublimation interfaces recede, that is, Z increases, T_o progressively rises. T_o can be calculated as a function of T_p during sublimation by relating the rate of radiant heat absorption by the food to the rate at which heat is conductively transferred through the ice free layers. Equation 2 and the vapor pressure versus temperature relationship for ice also have to be used in carrying out this calculation.

To prevent thermal damage, T_o must be kept below a specified upper limit or limits that depend on the product being dried. A fixed upper limit, such as 40°C for coffee extract, is usually used. Upper limits that depend on exposure time are used occasionally; higher T_o is allowed if the exposure time is short. Plate temperatures and drying rates must be reduced if excessively high T_o develop as Z increases. During the desorption phase of drying, plate temperatures are usually progressively reduced to prevent T_o from rising excessively.

DRYING TIME

Equation 1 can be rearranged and integrated, as shown below, to provide t_s, the time required to complete sublimation:

$$t_s = \int_{Z=0}^{L} \frac{(A\rho X)ZdZ}{k_t(T_o - T_s)} \tag{3}$$

where L is the final depth of the ice-free layer, that is, half the slab thickness for slabs where sublimation occurs at equal rates on both sides. T_o and, to a lesser extent, T_s vary as Z changes during drying. Therefore, equation 3 usually must be numerically integrated. The integrand in equation 3 is proportional to ZdZ. Therefore, t_s will be proportional to L^2 regardless of the particular way T_s and T_o change during drying. It appears that required desorption times and required total drying times are also proportional to L^2.

It usually takes 14 to 18 hours to freeze dry a 12.5-mm (0.5-in.) thick slab of food that initially contains 75 to 85% water. In freeze-drying a 12.5-mm thick slab of coffee extract containing 74% water, 60% of the drying time was used for sublimation, 40% for desorption. Concentrated extracts with lower water contents usually dry more rapidly, but as concentration increases, a larger proportion of the drying time is used for desorption. Less information is available about the portion of drying time used for desorption for other products.

COLLAPSE

During sublimation, T_s must be kept low enough to prevent pore structure collapse caused by partial melting (8). When collapse occurs, vapor escape is cut off, the product foams, and drying rapidly fails. Collapse will occur at T_s greater than −23°C for coffee extract. Collapse can occur at T_s as low as −46°C (for Concord grape juice). At the other extreme, T_s as high as −1.5°C can be safely used for potatoes. Collapse can occur if flow-pressure drop is excessive or if P_o is too large, but it can be prevented, if need be, by reducing the condenser operating temperature or by reducing the sublimation rate by decreasing T_p.

PARTICULATE PRODUCTS

Most foods are freeze-dried in particulate form. Heat and mass transfer during the freeze drying of particles is more complex than for the freeze drying of slabs. Sublimation occurs beneath all exposed surfaces, but is most rapid where temperature is greatest—at ice–vapor interfaces closest to the heating plate. Although sublimation takes place over a greater depth of product, the required drying time remains proportional to L^2 (the product bed depth squared). Because vapor flows readily through spaces between particles, trays with solid bottoms can be used without interfering with drying in lower parts of the load. Because t_s depends on L^2, thicker parts of particulate loads can take markedly longer to dry than parts of average thickness. Therefore, care must taken to load trays uniformly.

Excessive amounts of fines were produced when slabs of freeze-dried extract were ground to provide granules for consumer use. To prevent this, frozen coffee extract is ground to produce granules of desired size before drying and freeze-dried in granule form in shallow beds in trays. Fines produced during grinding are removed by screening and recycled by mixing them with extract about to be frozen. Vapor-flow resistance during freeze drying of extract granules is much smaller than for slabs, but heat-transfer resistance is greater. In drying extract granules, heating plates are often maintained at 130°C while sublimation occurs. Yet, because of low pressure drop, T_s is only slightly higher than the condenser temperature during this period. After sublimation is complete and desorption alone occurs, plate temperatures are gradually reduced to 50°C.

Excessive product loss caused by granule entrainment in high-velocity vapor used to occur when coffee extract granules were freeze-dried, but now, in modern, properly operated, well-designed dryers, losses are now less than 0.1%.

FOAMED PRODUCTS

Gas is injected or beaten into some liquid feeds to foam them before they are frozen and dried. This reduces bulk density, improves vapor permeability during drying, or permits creation of novel foamed products. Freeze-dried soluble coffee made from concentrated extract, in foamed, frozen, granular form has the same bulk density as freeze-dried granular soluble coffee made from unfoamed, normal concentration extract. Without foaming, it would be denser and less acceptable. At regularly used continuous production conditions, foamed granules dry in roughly 7 hours.

CONDENSATION

The largest energy cost for freeze drying is that for the refrigeration used to condense vapor as ice. This cost can be reduced by removing water before drying and by operating the condenser at the highest temperature that still reliably prevents collapse. Higher condenser temperatures can be safely used when the pressure drop caused by vapor flow is low, as occurs when extract granules are dried.

In addition to removing heat of condensation (roughly the same as the heat of sublimation), the refrigerator also removes heat the vapor picks up from the product and the heating plate and heat transferred to the condenser by radiation. Heat shields or condenser enclosures that do not significantly interfere with vapor flow are used to reduce transfer of radiant heat to the condenser.

The temperature at the outer surface of deposited condensed ice is higher than at the condenser wall itself. When air inleakage is small, transparent ice forms on the condenser and the temperature difference across the ice is small. If inleakage is moderate, milky ice forms, and the temperature difference is greater. When inleakage is excessive, porous frost that conducts heat poorly forms and the temperature on the outside of the frost is markedly higher than in the condenser. When this occurs, the condenser operating temperature may have to be reduced to prevent P_o from rising enough to cause collapse. This raises refrigeration costs. Accumulation of air around condensers is minimized by having the vapor contacting the condenser efficiently sweep air toward the vacuum pump.

The drier shown in Figure 3 has two condensers, an on-line one that condenses vapor as ice, and an off-line one undergoing de-icing. The two condensers cyclically change roles with the aid of a pressure-tight slide gate. Thus, de-icing takes place almost continually while drying occurs. Ice buildup on the active condenser is 2 to 3 mm, at most. Up to 17 mm of ice may deposit on condensers subjected to de-icing only at the end of drying.

BATCH FREEZE DRIERS

Plant-scale, batch, freeze drying of foods is usually carried out in cylindrical vacuum chambers available in various sizes. A very large unit may provide 90 m^2 of shelf area and up to 227 kg/h of sublimation capacity. In some systems, trays of frozen food are loaded on a rack of shelves that is pushed into a drier on an overhead rail. There, the tray-laden shelves intermesh with built-in heating plates, as shown in Figure 3. Seven pairs of shelves are shown; 15 pairs are usually used. In other driers, loaded trays sit on built-in guide rails that lie between built-in heating plates. In still another system, both shelves and heating plates form part of a cart that is pushed into the drier. Heating fluid flows through the plates through connections made after the cart enters the drier. In each system, the sequence of operations used and basic processes involved are, in most respects, essentially the same as those described for a one-shelf drier.

Groups of batch freeze driers operating on a staggered schedule are used to efficiently freeze-dry food on a sustained basis. Staggered operation evens out refrigeration, heating, and vacuum pumping loads; reduces manpower needs; and permits sharing of refrigeration, heating, and vacuum pumping systems.

CONTINUOUS FREEZE DRIERS

Freeze-dried foods produced year-round in large volume are often freeze-dried continuously in cylindrical chambers, up to 3.7-m wide and up to 25-m long, that process 15 rows of trays at a time (9). Generated vapor is removed by using multiple pairs of switchable condensers, similar to those shown in Figure 3. Trays of frozen food enter such driers one-at-a-time through a double-valve lock, without loss of vacuum. The injected trays are automatically elevated, arrayed in a vertical set, and then pushed forward as a set. The pushed trays move through the drier in separate rows between heated plates, whose local temperatures are programmed to provide heat input that varies properly with axial tray position. At the discharge end of the drier, trays and their now-dry contents are individually automatically lowered and exit, one-at-a-time, through a double-valve lock. Pairs of locks are used at each end of large driers. In smaller driers, individual locks are used at each end. Depending on drier size and feed moisture content, available continuous freeze driers can process 5,000 to 27,500 kg of feed per day.

ALTERNATIVE FREEZE DRYING METHODS

U.S. food freeze drying patents issued between 1961 and 1976, an important period of development, have been re-

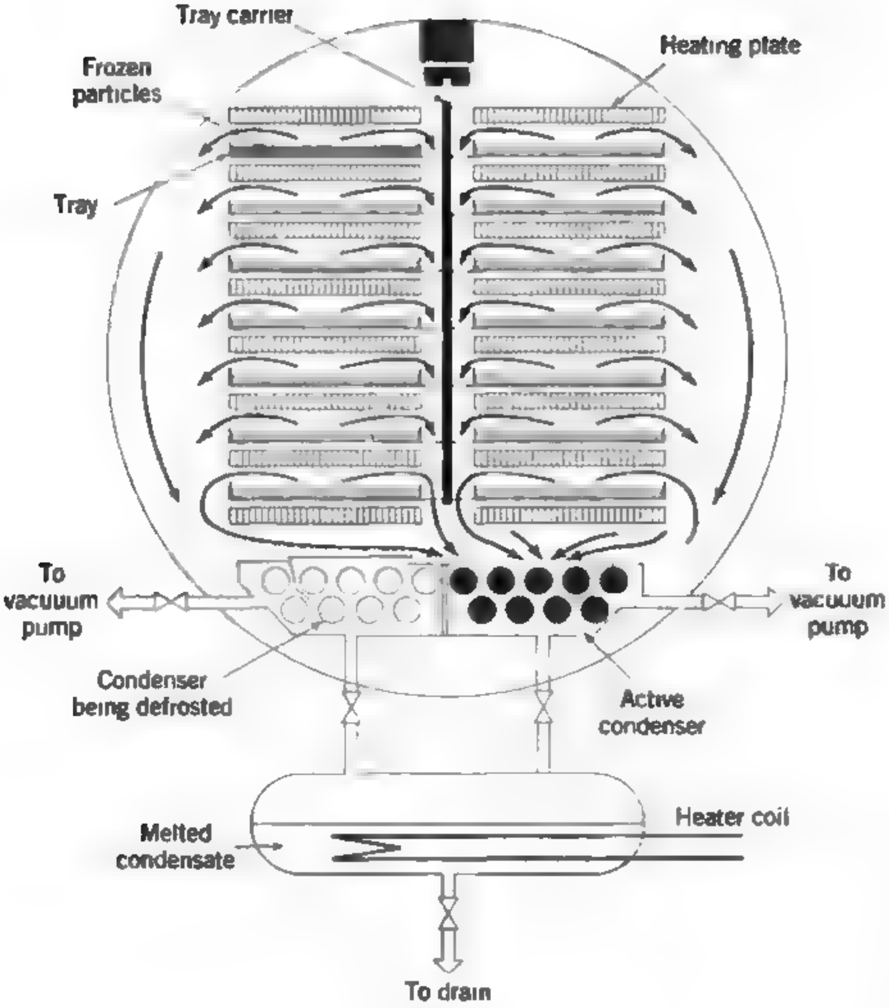


Figure 3. A batch freeze drier in which dual, switchable condensers are used and in which trays enter on a rack of shelves supported on an overhead track. *Source:* Reproduced with permission of Atlas-Stord Inc.

viewed by Gutcho (1). Some of these patents describe now-standard freeze drying methods and equipment, methods for freeze drying particular products, or methods for preparing such products for drying. Others describe freeze drying methods that for one reason or another have been abandoned or are rarely or infrequently used today. Some describe infrequently used heating methods: direct-contact heating; microwave heating; infrared heating; induction heating; use of layers of expanded metal mesh or of ribbed trays to facilitate heat transfer; or the use of heat pumping to couple heating and condensation. Others describe spray freeze drying, various types of atmospheric freeze drying, circulation of low-pressure gas through the drying material, or vapor removal by adsorption instead of condensation. Still other patents describe use of moving belts, vibrating conveyers, helical conveyors or vertical stacks of scraped trays as particle conveying devices in continuous freeze driers. Food freeze drying patents and literature for the years 1977 to 1998 were reviewed in preparing this article. Aside from development of the previously described continuous freeze drier, innovation in the field has slowed down. Atmospheric freeze drying in sorbent-containing fluidized beds and use of microwave heating in freeze drying are still of interest, and articles dealing with these methods continue to appear from time to time.

BIBLIOGRAPHY

1. M. H. Gutcho, *Freeze Drying Processes for the Food Industry*, Noyes Data Corp., Park Ridge, N.J., 1977.
2. M. Karel, "Fundamentals of Dehydration Processes," in A. Spicer, ed., *Advances in Preconcentration and Dehydration*, Elsevier Applied Science, London, United Kingdom, 1974, pp. 45–94.
3. J. M. Flink, "Effect of Processing on Nutritive Value of Food: Freeze-Drying," in M. Recheigl, ed., *Handbook of the Nutritive Value of Processed Food*, Vol. 1, CRC Press, Boca Raton, Fla., 1982, pp. 45–62.
4. J. Rolfgaard, "Freeze Drying: Processing, Costs and Applications," in A. Turner, ed., *Food Technology International Europe*, Sterling, London, United Kingdom, 1984, pp. 47–49.
5. J. Lorentzen, "Freeze Drying: The Process, Equipment and Products," in S. Thorne, ed., *Developments in Food Preservation*, Vol. 1, Elsevier Applied Science, London, United Kingdom, 1981, pp. 153–175.
6. U. Rosenberg and W. Bogl, "Microwave Thawing, Drying and Baking in the Food Industry," *Food Technol.* **41**, 85–91 (1987).
7. M. Karel, "Dehydration of Foods," in O. R. Fennema, ed., *Principles of Food Science, Part 2, Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975, pp. 359–395.

8. R. J. Bellows and C. J. King, "Freeze Drying of Aqueous Solutions: Maximum Allowable Operating Temperatures," *Cryobiology* **9**, 559 (1972).
9. H. G. A. Unger, "Revolution in Freeze Drying," *Food Processing Industry* **51**, 20 (April 1982).

HENRY SCHWARTZBERG
University of Massachusetts
Amherst, Massachusetts

FREEZING SYSTEMS FOR THE FOOD INDUSTRY

FUNDAMENTALS

During storage foods are subjected to changes that affect food quality and that sooner or later will lead to severe deterioration and eventually spoilage of the foods. These changes are caused by microorganisms and chemical and physical reactions. Often a combination or interaction of different reactions will cause changes, lowering the quality primarily by changing the sensory properties of the product.

The wide variety of circumstances, including type of food and ingredients, will determine the type of changes that will dominate. In meat and fish products or other foods rich in protein, changes caused by microorganisms will dominate while food rich in fat is more susceptible to chemical and biochemical change. Physical changes occur in all types of food.

The purpose of all food preservation methods is to inhibit or decrease the speed of reaction responsible for the deterioration. All of these reactions are among other factors influenced by temperature. The speed of reaction is decreased at lower temperatures. Cooling and chill storage therefore are perhaps the most important methods of enhancing the storage life of most food products. But even at temperatures near the freezing point some reactions, including growth of many microorganisms, continue at a rate that will limit the preservability to a relatively short period of time.

When the food temperature is $< -10°C$ microbiologic growth will cease. Chemical, biochemical, and physical reactions will still continue at very low temperatures but at a slow pace. Storage life is substantially enhanced as compared to storage at chilled or ambient temperature.

HISTORY OF REFRIGERATION

Refrigeration applied both above and below the freezing point of foods has been used for thousands of years to preserve foods and to increase comfort. Historians estimate that caves were used for food storage about 100,000 yr ago (1).

Temperature inside the caves is naturally low as a result of the evaporization of water, which is often present.

An Egyptian frieze from 3000 B.C. shows a slave waving a fan in front of a clay pot. The cooling effect from vaporization of water was also utilized in this case. Egyptians also utilized terrestrial radiation toward space at night under a clear sky to produce ice.

Ice was used locally when available. Later it was harvested during wintertime and stored for the summer season. In 1100 B.C. a Chinese poem mentioned ice houses. Ice and snow were transported over great distances, eg, from the Apennines to Rome, and caravans were transporting ice and snow from Lebanon to the palaces of the Califs of Damascus, Baghdad, and the sultans in Cairo.

In many countries ice was believed to be a gift of the gods to humans. This was confirmed as late as during the mid-nineteenth century, when the American John Goorie in 1844 managed to produce ice with an air compressor but did not dare to publish the invention under his own name. Instead, under a pseudonym he wrote a scientific article describing an ice machine as a future possibility. The New York newspaper, *The Globe*, shortly after published an article titled "Some lunatic in Florida believes his machine can make ice equally good as the All Mighty."

Obviously hunters and gatherers living in a cold climate did use the natural freezing of their food products in order to preserve them over long periods of time.

Exactly when the temperature-decreasing effect—with the addition of certain salts to water—was discovered is not known. There is, however, reason to believe that the method was used in India in the fourth century A.D. During the fourteenth and fifteenth century a number of European scientists were working with salt solutions and managed to achieve temperatures as low as -15 to $-20°C$. With those salt mixtures the stage between natural and artificial cooling was passed.

It was not until 1755, however, that the first apparatus for making ice was constructed by William Cullen. Vaporization of water at reduced pressure was utilized.

During the first half of the nineteenth century four events of fundamental importance for the refrigeration industry took place: (1) the systematic work on the liquefaction of gases, (2) the genesis of thermodynamics originated by Nicolas Carnot in 1824 on the invention of the refrigeration machine using compression of a liquefiable gas (often referred to as the Carnot engine), (3) Jacob Perkins's work in mechanical refrigeration in 1834, and (4) the air cycle machine (Goorie, 1844); the latter two inventions remained undeveloped for two decades.

The start of industrial freezing of food is often set at around 1880, even if the first industrial installations were made some 20 years earlier. During 1870–1880 frozen meat was transported from the southern hemisphere to Europe. Initially those endeavors were unsuccessful. In beef cargo from Buenos Aires to Rouen (port on Seine River) in France as much as 25% in weight was lost by sublimation and the quality was unacceptable. The breakthrough came in 1877 when a shipment of frozen meat was brought in from Buenos Aires to Marseilles on the steamer *Paraguay* (2). This shipment was followed by another one from South America to New York in 1879, and in 1880 the steamer *Strathleven* made the journey from Sydney and Melbourne to London.

As compared to the quality achieved today, much was to be wished for. The freezing was carried out very slowly and the storage temperatures were high compared to the

8. R. J. Bellows and C. J. King, "Freeze Drying of Aqueous Solutions: Maximum Allowable Operating Temperatures," *Cryobiology* **9**, 559 (1972).
9. H. G. A. Unger, "Revolution in Freeze Drying," *Food Processing Industry* **51**, 20 (April 1982).

HENRY SCHWARTZBERG
University of Massachusetts
Amherst, Massachusetts

FREEZING SYSTEMS FOR THE FOOD INDUSTRY

FUNDAMENTALS

During storage foods are subjected to changes that affect food quality and that sooner or later will lead to severe deterioration and eventually spoilage of the foods. These changes are caused by microorganisms and chemical and physical reactions. Often a combination or interaction of different reactions will cause changes, lowering the quality primarily by changing the sensory properties of the product.

The wide variety of circumstances, including type of food and ingredients, will determine the type of changes that will dominate. In meat and fish products or other foods rich in protein, changes caused by microorganisms will dominate while food rich in fat is more susceptible to chemical and biochemical change. Physical changes occur in all types of food.

The purpose of all food preservation methods is to inhibit or decrease the speed of reaction responsible for the deterioration. All of these reactions are among other factors influenced by temperature. The speed of reaction is decreased at lower temperatures. Cooling and chill storage therefore are perhaps the most important methods of enhancing the storage life of most food products. But even at temperatures near the freezing point some reactions, including growth of many microorganisms, continue at a rate that will limit the preservability to a relatively short period of time.

When the food temperature is <-10°C microbiologic growth will cease. Chemical, biochemical, and physical reactions will still continue at very low temperatures but at a slow pace. Storage life is substantially enhanced as compared to storage at chilled or ambient temperature.

HISTORY OF REFRIGERATION

Refrigeration applied both above and below the freezing point of foods has been used for thousands of years to preserve foods and to increase comfort. Historians estimate that caves were used for food storage about 100,000 yr ago (1).

Temperature inside the caves is naturally low as a result of the evaporization of water, which is often present.

An Egyptian frieze from 3000 B.C. shows a slave waving a fan in front of a clay pot. The cooling effect from vaporization of water was also utilized in this case. Egyptians also utilized terrestrial radiation toward space at night under a clear sky to produce ice.

Ice was used locally when available. Later it was harvested during wintertime and stored for the summer season. In 1100 B.C. a Chinese poem mentioned ice houses. Ice and snow were transported over great distances, eg, from the Apennines to Rome, and caravans were transporting ice and snow from Lebanon to the palaces of the Califs of Damascus, Baghdad, and the sultans in Cairo.

In many countries ice was believed to be a gift of the gods to humans. This was confirmed as late as during the mid-nineteenth century, when the American John Goorie in 1844 managed to produce ice with an air compressor but did not dare to publish the invention under his own name. Instead, under a pseudonym he wrote a scientific article describing an ice machine as a future possibility. The New York newspaper, *The Globe*, shortly after published an article titled "Some lunatic in Florida believes his machine can make ice equally good as the All Mighty."

Obviously hunters and gatherers living in a cold climate did use the natural freezing of their food products in order to preserve them over long periods of time.

Exactly when the temperature-decreasing effect—with the addition of certain salts to water—was discovered is not known. There is, however, reason to believe that the method was used in India in the fourth century A.D. During the fourteenth and fifteenth century a number of European scientists were working with salt solutions and managed to achieve temperatures as low as -15 to -20°C. With those salt mixtures the stage between natural and artificial cooling was passed.

It was not until 1755, however, that the first apparatus for making ice was constructed by William Cullen. Vaporization of water at reduced pressure was utilized.

During the first half of the nineteenth century four events of fundamental importance for the refrigeration industry took place: (1) the systematic work on the liquefaction of gases, (2) the genesis of thermodynamics originated by Nicolas Carnot in 1824 on the invention of the refrigeration machine using compression of a liquefiable gas (often referred to as the Carnot engine), (3) Jacob Perkins's work in mechanical refrigeration in 1834, and (4) the air cycle machine (Goorie, 1844); the latter two inventions remained undeveloped for two decades.

The start of industrial freezing of food is often set at around 1880, even if the first industrial installations were made some 20 years earlier. During 1870–1880 frozen meat was transported from the southern hemisphere to Europe. Initially those endeavors were unsuccessful. In beef cargo from Buenos Aires to Rouen (port on Seine River) in France as much as 25% in weight was lost by sublimation and the quality was unacceptable. The breakthrough came in 1877 when a shipment of frozen meat was brought in from Buenos Aires to Marseilles on the steamer *Paraguay* (2). This shipment was followed by another one from South America to New York in 1879, and in 1880 the steamer *Strathleven* made the journey from Sydney and Melbourne to London.

As compared to the quality achieved today, much was to be wished for. The freezing was carried out very slowly and the storage temperatures were high compared to the storage temperatures used today. In 1915 the German scientist Rudolf Plank showed the importance of rapid freezing in experiments on fish. This knowledge was soon applied for other products.

Although fish, meat, poultry, and berries have long been subjected to preservation by freezing, frozen-state deliveries to consumers did not take place until the development of "quick freezing" in the mid-1920s. The date is often set at the October 14, 1924, the day when Clarence Birdseye received a patent for a plate freezer. This apparatus was revolutionary, as it was now possible to quick freeze packaged foods for the retail outlets. In 1929 the American company Postum/General Foods bought Birdseye's Company, and in the following year the first consumer package of frozen foods were marketed in Springfield, Massachusetts (3).

In 1938 frozen foods were introduced to Europe and the year after, British companies were licensed to produce quick-frozen foods. After World War II the market expanded rapidly throughout all industrialized countries.

FUNDAMENTAL CHANGES DURING FREEZING

The importance of early freezing is related to the need to decrease the rate of the deterioration processes caused by chemical, biochemical, and physical reactions as well as microbiological activity. Chemical and biochemical reactions influence the product quality not during the freezing process, but during subsequent storage. The importance of quick freezing is some times argued from a sensory point of view (4). It must not, however, be forgotten that quick freezing is most important from a technical–economical–operational point of view. With reference to quality, the rate of freezing determines the size of weight loss and in some cases also the microbiologic quality of the product. The drip loss or loss of product juice on thawing is determined by the rate of freezing as well.

The freezing process may be seen as a lowering of the product temperature from its original value to the storage temperature in question. However, from a technical–economical–operational point of view a more strict definition is needed.

Ice Crystallization

The major component of most foods is water, which in the freezing process is transferred from a liquid to a solid state. This transfer obviously result in numerous changes.

Most food products consist of or contain animal and/or vegetable cells forming biological tissues. The water solution of the tissue is contained between the cells—intercellular fluid—and within the cells—intracellular fluid.

When the food product is cooled below 0°C ice begins to form at the initial freezing point. The temperature at which freezing starts depends on the concentration of dissolved substances—salts and other solubles—present. The concentration is higher within than outside the cells. The cell membrane acts as an osmotic barrier and maintains the difference in concentration.

When the product is frozen the first ice crystals are formed outside the cell since the freezing point is higher because of the more diluted fluid here than inside the cell. Once started, the rate of ice crystallization is a function of the speed of heat removal as well as the diffusion of water from within the cell to the intercellular space. If the freezing rate is low, few crystallization centers—nucelli—are formed in the intercellular space. During the freezing process the cell looses water by diffusion through the membrane, and this water crystallizes to ice on the surface on the crystals already formed outside the cell. As in slow freezing, there are few nucleins formed; those existing as crystals grow to a relatively large size.

As the cells loose water, the remaining solution within the cells becomes increasingly concentrated and the cell volume shrinks causing the cell wall to partly or entirely collapse. The large ice crystals formed outside the cell wall occupy a larger volume than does the corresponding amount of water, and therefore they will exert a physical pressure on the cell wall. In some cases this pressure can be sufficient to damage the cell wall and contribute to an increased drip loss on thawing.

By increasing freezing rates a larger number of ice crystallization nuclei are formed, which results in a much smaller size of the final crystals as compared to slow freezing. However, even in the case of high freezing rates most of the crystals are formed outside the cells. Only at extremely high freezing rates not obtainable in commercial freezing of food products, small crystals are formed uniformly throughout the tissue both externally and internally with respect to the cell.

As the food products are cooled down below the initial freezing point, an increasing amount of water is turned into ice and the residual solutions become more concentrated. The relation of water frozen out as ice and the concentration of the remaining solution have an impact on the preservability of a number of food products (5).

The size of the ice crystals has long been regarded as crucial for the quality of the frozen product. It appears, however, from experience as well as a number of investigations that the differences in ice crystal size and distribution have little effect on the sensory properties of the food product when presented to the consumer, provided up-to-date equipment and good commercial practice have been used (6).

The freezing rate is not negligible, however. On the contrary, in good commercial practice the freezing time must be determined for each product in order to safeguard against microbiologic growth, which is most important from a safety point of view and low controlled weight losses, which is important from an economic point of view.

The practical result of different freezing rates—size and locations of ice crystals—can be seen as a difference in drip loss of water or "product juice" when a product is thawed. Loss of juice results in a more or less pronounced loss of texture, flavors, and—in most cases—nutrients. For this reason the drip loss is often used as an indication of the quality loss during freezing and subsequent storage. The relation between speed of freezing and drip loss for two different foods is illustrated in Figure 1.

For strawberries it is rather obvious that the consumer will not readily accept the product with a 20% drip loss if a product with only 8–10% drip loss is available. The latter

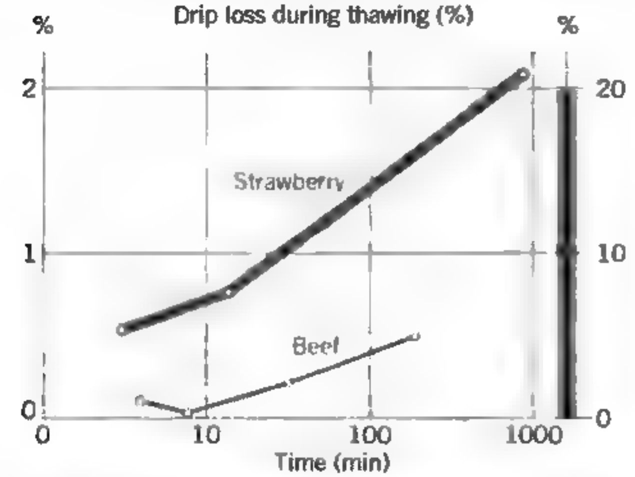


Figure 1. Drip loss during thawing of sliced beef and strawberries, frozen at various rates, to an equalization temperature of −18°C. *Source:* Ref. 6.

level of drip loss can be achieved in modern freezers usually employing the fluidization technique.

When comparing slices of beef even very slow freezing rates gave a small drip loss hardly noticeable to the consumer. The small improvement achieved by quick freezing is normally not observed.

Microbiology

With reference to temperature requirements, bacterias are divided into four basic groups according to type of growth: thermophilic, mesophilic, psychrophilic, and psychrotrophic. Of those the two latter groups are of special interest in food spoilage at low temperatures. Psychrophilics are often an important cause of spoilage of protein-rich foods such as meat, fish, poultry; psychrophilic bacteria grow well at temperatures above 0°C.

The optimum temperature for growth, however, is much higher. Psychrotrophics are also able to grow close to the freezing point but the optimum temperature is higher than that for psychrophilics. Lowering the temperature will slow down the growth rate of all bacteria, and at temperatures used in commercial storage of frozen foods all microbiologic growth ceases completely. The time to decrease the temperature to below the freezing point is critical. A product temperature of −10°C is normally considered safe with regard to microbiologic growth.

During freezing and frozen storage some bacteria are impaired and even destroyed. Under certain circumstances the death of bacteria will cause a considerable decrease in the total number of viable cells in the frozen products. Since some species are more susceptible to freezing injuries than others there may also be a change in the relation between various species (7).

From a microbiologic point of view the total flow from the production to the consumption must be regarded. During this flow, food products are subjected to various temperatures and other growth-affecting factors. Large variations occur from product to product; meat and meat products have been chosen as examples in the following discussion. The general concept is obviously valid for most food products.

During slaughter and subsequent handling the surface of the meat is infected by microorganisms originating from the animal itself and from the environment.

The number of organisms present is dependent on the hygienic conditions, but the flora consists to a great extent of spoilage organisms that thrive on the meat surface and multiply.

Rapid cooling reduces the rate of growth substantially, but many of the psychrophilic and psychrotropic organisms will grow even at chill temperatures. These organisms depend on free oxygen for their metabolism, and since oxygen is available in the surface layer only, no growth will occur in the interior of the meat.

Freezing of carcass meat therefore normally will not cause any serious problems from a microbiologic point of view. In commercial freezing the freezing rate is fast enough to stop the growth at the surface. As the microorganisms cannot develop under the surface layer, the freezing rate is of less importance microbiologically.

Processing, like cutting and mincing, increases the microbiologic contamination as the surface/volume ratio increases. The freezing rate becomes more critical. A common pack in the industry today is the 30-kg carton normally frozen in a traditional air-blast tunnel. It is then essential that a good air circulation be provided in order not to prolong the freezing time.

Freezing times for cartons subjected to both adequate and inadequate airflow are compared in Figure 2.

Inadequate airflow has been achieved by placing the meat cartons directly on pallets with only a small wooden spacer (30 mm) between each layer instead of being placed on freezing racks or with rigid layer separators with a minimum height of 50 nm.

If spacers used as in the improper airflow system do not cover the total carton area, some cartons may collapse, which will prevent airflow through the different layers.

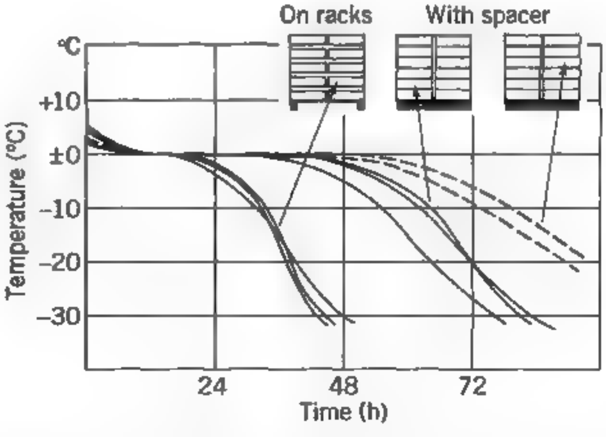


Figure 2. Recorded temperature decrease during the freezing of cartons in an air-blast tunnel (air temperature −38°C, front air velocity 1.5 m/s, size of cartons 160 × 400 × 600 mm). *Source:* Ref. 7.

The freezing time is obviously prolonged. There is also a risk that cartons are removed from the air-blast tunnel before the freezing is completed if blocking of the air channels is not visible from the outside. In the latter case there may be substantial growth of microorganisms during subsequent storage, ie, until a sufficiently low temperature has been reached.

In most cases a freezing time of 24–36 h down to −10°C in the center of wholesale cut meat will not cause any microbiologic problems. If the degree of cutting is increased to smaller cuts, such a long freezing time may become a major problem. Those products should preferably be frozen integrated in the processing line before packaging or in very small packages that allow for a much faster freezing.

As most prepared foods involve a high degree of processing as well as mixing of different ingredients the freezing becomes very important. The general pattern of the growth of microorganisms in the production of prepared foods is illustrated in Figure 3.

During storage of raw material as well as during handling and preparation, microorganism growth will take place. If the preparation is followed by heat treatment, the total number of microorganisms will be reduced.

At this point the product could be handled in two different ways: either packaged and frozen in batch-operated equipment or frozen in-line and then packed. If the products are placed on racks and then transported to a freezing tunnel for freezing, there may be a time lapse, resulting in a marked growth of microorganisms. If the product has been heat treated, it will pass through the temperature zone of optimum microbiologic activity.

A chilling operation immediately after the heat treatment—which means that the products are cooled down to below −10°C, then packaged and frozen—will reduce the growth of bacteria. However also in this case microbiologic growth can be recorded. If the products are instead frozen in-line immediately after the heat treatment and then packaged, there will be almost no increase in the number of bacteria present.

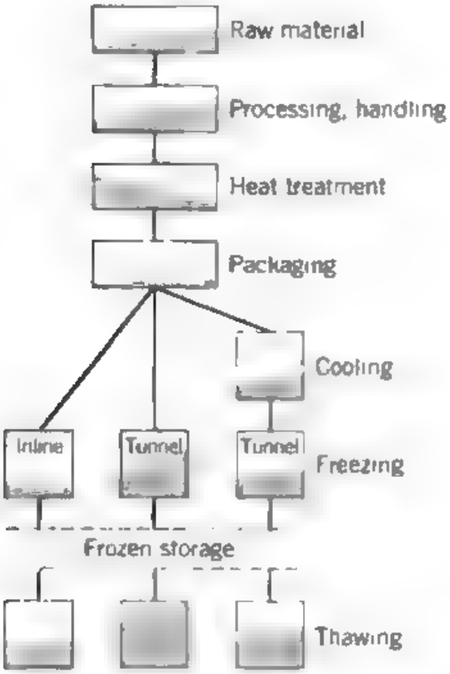


Figure 3. Growth pattern of bacteria during different steps in the processing of precooked frozen foods. *Source:* Ref. 7.

In-line freezing is, of course, even more essential when prepared foods are processed without heat treatment prior to the freezing process.

A low microbiologic load in the frozen state will directly influence the preservability of the food product after thawing. All measures taken to arrest growth of microorganisms prior to freezing are therefore beneficial.

Compared to batch freezing in a tunnel, the modern in-line freezer provides much faster freezing, and even more important, the in-line process itself minimizes the delays in product flow from preparation to freezing and through the temperature zone, which is critical from a microbiologic point of view.

Desiccation

During the freezing process it is unavoidable that a certain evaporation of water from the surface takes place, resulting in both a quality loss and a weight loss. Only if the product is tightly enclosed in a water vapor inpermeable packaging material, evaporation can be completely avoided. If there are small spaces between the product and the packaging material ice is deposited in these.

Freezers that are poorly designed and improperly used may cause a weight loss in the order of 5–7%, while properly designed and used equipment will cause no more than 0.5–1.5% weight loss. As the total freezing cost often is no greater than 3–5% of the product value, it is obvious that dehydration losses are of great importance in any comparison of different freezing methods.

There is a definite correlation between the degree of dehydration loss and the rate of freezing. In Figure 4, the temperature gradient in a product with a thickness of 2 b and in the air surrounding the product is plotted against the partial pressure of water vapor in moist air.

The size of the dehydration is influenced by a number of factors related to the biological materials as well as to physical handling, temperature of the heat-transfer medium, dimensions of the product, etc. The rate of evaporation from the product is determined by the conditions at the surface.

In a simplified consideration the evaporation rate may be regarded as proportional to the vapor pressure Δp^2. The curve corresponds to the "relative" humidity of the product surface. This concept is introduced in order to represent the diffusion resistance that may exists in cell walls, etc.

During infinitely slow freezing the surface temperature approaches the average temperature t_3. Then the evaporation rate increases to the value Δp_3. Hence it is clear that $t_3 - t_2$ should be as great as possible in order to obtain low dehydration losses. Three principal factors contribute to this:

- b large. In thick products the surface temperature will be low during most of the freezing process.

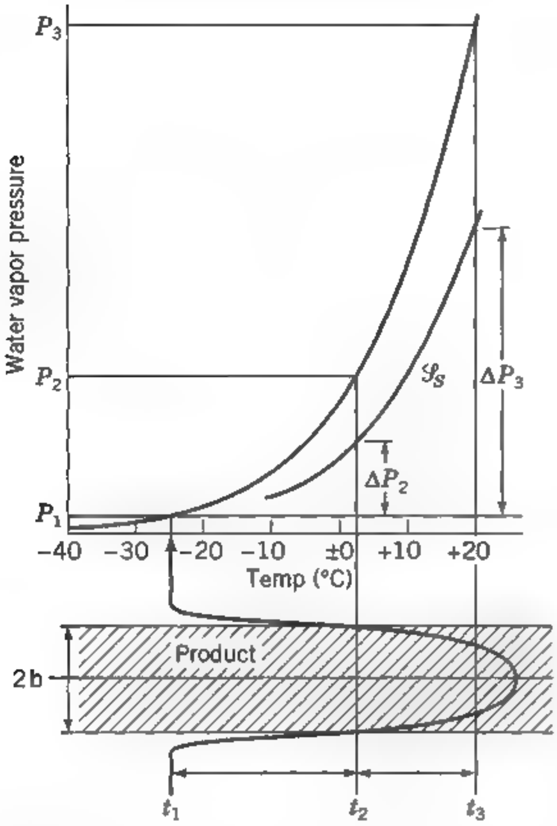


Figure 4. Dehydration mechanism during freezing. *Source:* Ref. 8.

- t_1 low. The lower the temperature of the ambient air, the more curved the temperature gradient in the product will be.
- High heat-transfer rate. A low air temperature has no appreciable effect if the heat-transfer rate is too low. Therefore, it is extremely important that the heat-transfer conditions be favorable. This is more important for thinner products.

Wet products generate water vapor at a rate proportional to the difference between the vapor pressure at the surface of the product and that of the surrounding air. In a product that has a more or less dry surface there is a resistance in the cell walls against diffusion of vapor from the interior of the product to the surface and the air. This results in a reduction of vapor pressure at the surface of the product.

The water vapor pressure decreases rapidly when the temperature is reduced, which means that the dehydration will be less the colder the heat-transfer medium is. The importance of a low temperature during freezing is well illustrated in Figure 5, where the accumulated weight loss during different freezing tests with a temperature varying from −13 to −35°C is plotted against the core temperature of the product. The diagram illustrates both the importance of the latent heat zone and the drastic influence of the air temperature.

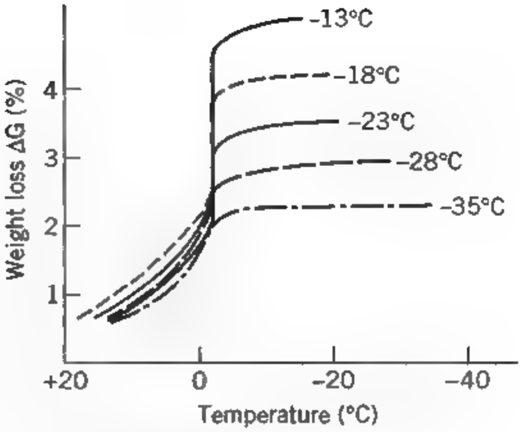


Figure 5. Accumulated dehydration during freezing at different air temperatures. *Source:* Ref. 9.

Glazing for Protection of Product Quality. In order to improve shelf life by preventing desiccation and oxidative changes, it has become standard practice to glaze certain individually frozen products, eg, shrimp, after freezing. The product quality is greatly improved as the thin ice layer prevents the product from the changes mentioned above. The glazing is carried out by spraying the frozen product with cold water, which immediately freezes on the surface. Even if the product leaves the freezer at a low outfeed temperature, the product temperature may increase considerably after the glazing operation. This high-end temperature may cause clumping of the product when packed, and the slow decrease in temperature during subsequent storage and distribution may lead to noticeable texture changes on thawing.

To avoid these problems special equipment has been developed—GLAZoFREEZE—that is designed to lower the product temperature of the glazed product immediately after the glazing operation. The equipment utilizes the fluidization technique. In GLAZoFREEZE the fluidization must be specially gentle to the product in order to avoid damage of the glazed surface.

DEFINITIONS

Definitions of the freezing process, freezing time, freezing rate, and speed of freezing are most useful in comparisons of different systems and equipment. The International Institute of Refrigeration gives the following definitions (10).

Freezing Process

During the freezing process different parts of a product will pass through the various stages at different times. If a particular location in the product is considered, three stages of temperature changes can be defined.

Prefreezing Stage

This is the interval between the time at which a high-temperature product is subjected to a freezing process and the time at which the water starts to crystallize.

Freezing Stage

The period during which the temperature at the considered location is almost constant because the heat that being extracted is causing the majority of water to change phase into ice.

Reduction to Storage Temperature

This is the period during which the temperature is reduced from the temperature at which most of the freezable water has been converted to ice to the intended final temperature. The final temperature can result when the storage temperature is reached in any part of the product, including the thermal center or the equalization temperature. The equalization temperature is the temperature achieved under adiabatic conditions—without heat exchange with the environment.

Freezing Time

The freezing time is defined as the time elapsed from the start of the prefreezing stage until the final temperature is reached. The freezing time depends not only on the initial and final temperature of the product and the quantity of heat removed but also on the dimensions—especially the thickness—and shape of the product as well as on the heat-transfer process and its temperature.

Freezing Rate

For a product or a package the freezing rate (degrees Celsius per hour) is the difference between the initial and the final temperatures divided by the freezing time. In a given point of a product the local freezing rate is equal to the difference between the initial temperature and the desired temperature divided by the time elapsed until the moment at which the latter temperature is achieved in this particular point.

Speed of Ice Front Movement

The freezing rate may be evaluated by the speed of movement of the ice (in centimeters per hour) through a product. This speed is faster near the surface and slower toward the center. As a result, reported freezing rates from different sources are not necessarily comparable.

Practical Freezing Time

Besides the above-mentioned definitions the industry has developed a common definition of freezing time for practical commercial purposes.

From a practical point of view the freezing time is defined as the time required to lower the temperature of a product to an equalization temperature of −18°C under adiabatic conditions. This definition determines the commercial capacity of the freezing equipment.

The time the product is held in the freezer is known as standard freezing time (SFT) or holding time. In Figure 6 ET stands for equalization time. In determining capacity of equipment there will be an equalization to −18°C. However, in practical life this temperature equalization takes place during subsequent handling and storage and the equalization temperature will be that of the surrounding environment. From a theoretical point of view the total freezing time (TFT) for a product is SFT + ET.

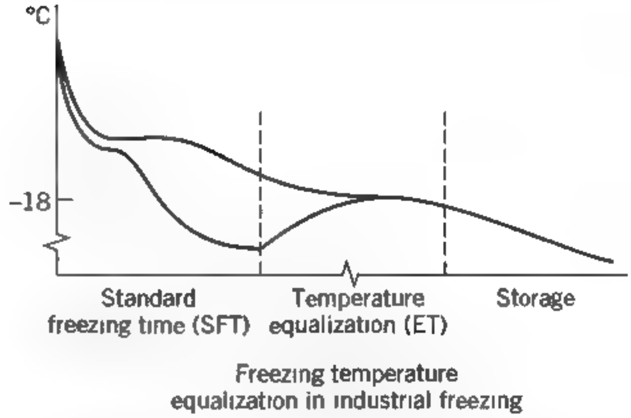


Figure 6. Practical commercial definition of the freezing process.

Important is that contrary to what is commonly believed the product temperature is not brought down to −18°C in the center of the product in the freezer. This temperature or preferably a lower temperature is achieved during subsequent handling, packaging, and storage.

Freezer Capacity

The investment in any freezing equipment is based on requirements to freeze a certain quantity of food per hour. As a principle the following relation is valid for any type of freezer:

$$C = Q/F = Vq/F$$

where C is the capacity of the freezer expressed as tons per hour, Q is the quantity, in tons, of the product that can be accommodated in the freezer, F is the holding time, in hours, of the product *in the particular freezer*, V is the volume, in cubic meters, of the product that can be accommodated in the freezer, and q is the volume weight, in tons per cubic meter, of the product.

The holding time refers to the temperature change that is required, ie, usually from incoming temperature to −18°C equalization temperature.

It is important to understand the fundamental importance of the holding time for the capacity of a freezer. Every product has different holding times. The amount of heat to be extracted per kilogram of product usually varies only little within a group of products, eg, vegetables, but the dimensions of each product particle have a drastic influence (see Fig. 7).

As all heat must be extracted from the product through its surface, the relation between surface and weight, the specific surface in square meters per kilogram, is of great interest. Volume and weight are entirely proportional to each other and therefore can substitute each other in this discussion. In Figure 7 can be seen that the freezing time is inversely proportional to the specific surface for particles.

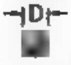
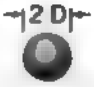
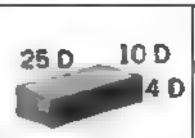
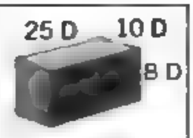
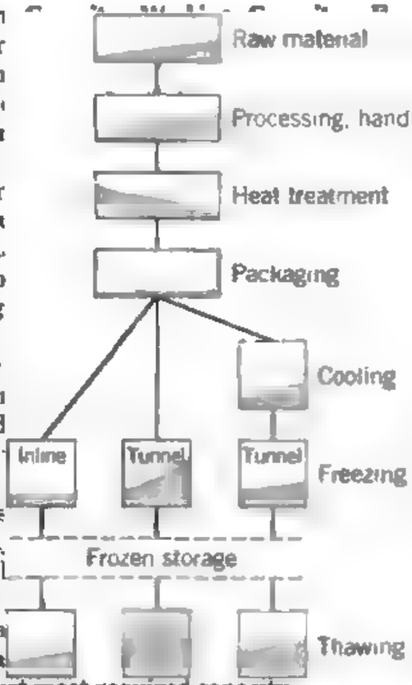
				
V =	$\frac{\pi \cdot D^3}{6}$	$8 \cdot \frac{\pi \cdot D^3}{6}$	$1000 \cdot 0{,}56\ D^3$	$2000 \cdot 0{,}56\ D^3$
F =	πD^2	$4\pi D^2$	$780\ D^2$	$1060\ D^2$
$\frac{F}{V}$ =	$6 \cdot \frac{1}{D}$	$3 \cdot \frac{1}{D}$	$1{,}40 \cdot \frac{1}{D}$	$0{,}94 \cdot \frac{1}{D}$
I =	1	2	4,3	6,4
T =	6 min	12 min	2,5h	5,5h

Figure 7. Heat to be extracted during freezing of different package sizes (where V = volume, F = surface, I = inverted specific surface, T = practical holding time, F/V = specific surface).

As a practical example a simple rack tunnel with a capacity of one ton per hour of spinach purée in 45-mm-thick packages can be taken. It may have the following capacities:

Spinach, packaged, 45-mm layer	1 ton/h
Peas, unpackaged, 30 mm layer	1.5 ton/h
Peas, packaged, 45 mm layer	0.6 ton/s
Parsley, packaged, 45 mm layer	0.4 ton/h
TV-dinner, packaged, 35 mm layer	0.2 ton/h

In the case of parsley and TV dinners the capacity is reduced primarily because of the low volume weight and secondarily because of the poor heat transfer inside the packages.



Design … in-line
operatior … in), it is
importan … city and
working …

Desigr … n in vol-
ume per … product
feed is er … the load
will be flu … fluenced
by fluctu. … feed will
have to b … 100% of
the desig … product
flow will

Other … inute of
every wor … peration
is started … he prod-
uct flow … eaks for
lunch or … ve to be
stopped s

Finall … flow be-
cause of l … ing line.
In total, … 70% of
design ca … product.
To obtain … ks in the
chain must meet required capacity.

Frequently a freezer may be used for several different products. For such products the above working capacity will have to be multiplied with the capacity factor, which relates the capacity of that particular product to the capacity of the product stated in the design specification. For products with very low capacity factor, the operating conditions usually differ so much that a separate working capacity must be established.

Precooling. Precooling is defined as the cooling of the product before it enters the freezer. The process of precooling has a positive effect on the energy required to freeze a product and generally reduces the frost load on the coils as well. Precooling can be done by blowing ambient air over the product, blowing ambient air that has been evaporatively cooled by water sprays, immersion in cold water (refrigerated or nonrefrigerated), or blowing refrigerated air over the product.

Sanitation during precooling processes is an important consideration because the conveyor system could be at a temperature for all or part of the cycle that can allow bacterial buildup.

Precooling reduces the load on the low-temperature refrigeration system, which reduces the power required by the low-temperature system. If a refrigerated precooler is used, the precooling heat is removed by a refrigeration system that consumes less power per unit of heat removed from the product, and a net power savings is achieved.

FREEZING EQUIPMENT

Today's freezing equipment can be divided into two main groups: integrated in the processing line and operating in batches.

According to the heat-transfer method, there are basically three main types of equipment:

- Air-blast freezers, which use air for heat transfer. Because air is the most common freezing media, this method of heat transfer has probably the largest range of designs.
- Contact freezers. Heat transfers occurs through conduction. A refrigerated surface is placed in direct contact with the product or package to carry away the heat. Alternatively, the product is immersed in a cold liquid—brine.
- Cryogenic freezers. These freezers use liquefiable gases, nitrogen or carbon dioxide to produce vapors that precool and freeze the products.

Combinations of these heat-transfer methods can be seen in special designs.

The freezing equipment can also be divided into two main groups with reference to the product. Individually quick-frozen (IQF) and packaged products.

All of these methods are used in the food processing industry; however, the preferred systems are those that can be operated in-line, integrated with the processing and packaging operations.

The freezing equipment must be designed to accommodate the three stages of the freezing process and should ideally optimize the total process.

The following design criteria are of special interest (see below under "Major Considerations in Freezer Design"):

Product quality
Hygiene
Minimum product losses
Reliable and simple operation
Simple maintenance
Economy, freezing cost

In the following sections the freezing equipment will be discussed with the basis on the method of heat transfer.

Air-Blast Freezers

Air is the most common freezing medium, and for that reason a number of designs can be found. Even if a storage room should never be considered as a piece of freezing equipment, it is sometimes used for this purpose. However, freezing in a storage room involves so many disadvantages that it should be used only in exceptional cases. The freezing is so slow that the quality of almost all products will be affected adversely.

Sharp Freezer, Blast Room

Basically, a sharp freezer or blast room is a cold storage room that has been especially constructed and equipped to operate at low temperatures for freezing. Even if this room is equipped with extra refrigeration capacity as well as fans for air circulation, there is normally no controlled airflow over the products, and for that reason freezing is normally slow. This type of equipment is, however, still used sometimes for bulk products such as beef quarters, but not for processed food products.

Tunnel Freezers

In tunnel freezers refrigerated air is circulated over the product, which is placed on trays or special spacers that stand in or pass through the tunnel in racks or trolleys.

The racks or spacers are arranged to provide an air space between each layer of trays. The racks or trolleys can be moved in and out of the freezer manually or by a fork-lift truck pushed through the tunnel by a pushing mechanism or slide-through. Tunnels are also used for freezing hanging meat carcasses carried on a suspension conveyor or in especially designed racks.

Practically all products can be frozen in a tunnel freezer. Whole, diced, and sliced vegetables may be frozen in cartons or unpacked in a 30–40-mm-deep layer on trays. Spinach, broccoli, meat patties, fish fillets, and prepared foods are frozen in packages in this type of equipment. It is, however, important to recognize that both refrigeration capacity and arrangements for air circulation have been designed for a specific product range. This means that if the tunnel freezer is designed for freezing meat carcasses or bulk meat cartons, the tunnel design is not appropriate for handling unpacked products such as vegetables. The result is often a increase in weight loss that influences both the sensory properties of the product and the economics of the freezing operation.

The flexibility of this type of equipment is balanced by high manpower requirements and a considerable weight loss if improperly used. The high manpower is caused by the handling, releasing, cleaning, and transportation of the trays and of the racks.

The tunnel should always be filled with product in such way that uniform airflow is obtained over all products to be frozen (see Fig. 8).

Mechanized Freezing Tunnels

A certain degree of mechanization is achieved when the racks are fitted with casters or wheels. The racks or trolleys are usually moved on rails by a pushing mechanism often powered hydraulically. Mechanized tunnel freezers are known as push-through tunnels, carrier freezers, and sliding-tray freezers. A typical design is shown in Figure 9.

As in any other tunnel freezer, the products are placed on trays that, in turn, are stacked on trolleys. The tunnel contains one or two rows of trolleys that are pushed forward stepwise by each other on rails in line with the product line. When a trolley leaves the freezer, the frozen prod-

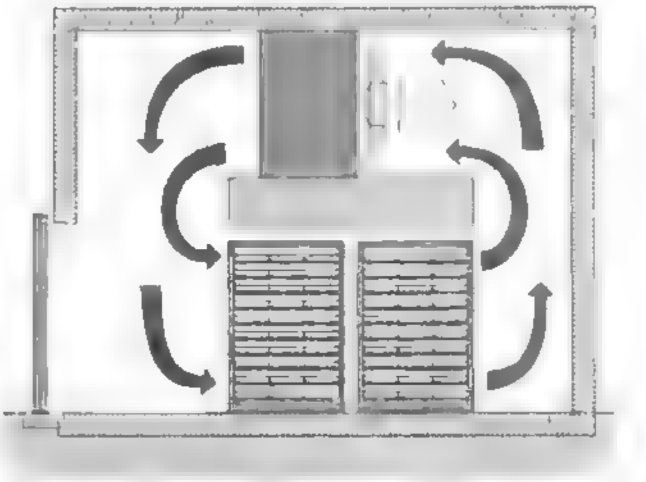


Figure 8. Stationary freezing tunnel.

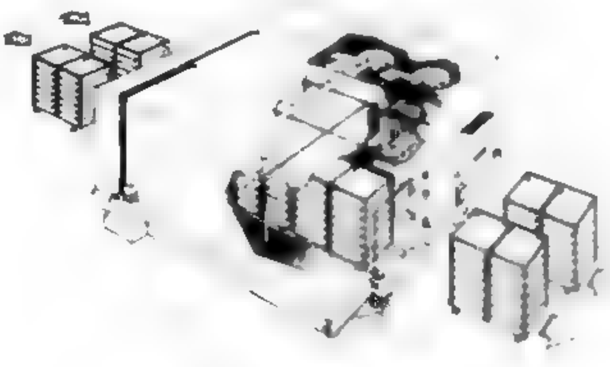


Figure 9. Mechanized freezing tunnel.

ucts are removed from the trays and the trolleys are returned to the loading station.

The design and construction of the freezer is common to most tunnel freezers. The evaporation coils are placed on a steel frame standing on the insulated floor. They have a thin spacing, which varies with the depth of the coils. The spacing is wide at the air inlet and narrow at the outlet. This ensures an even frost buildup on the coils without detriment to the airflow. The coils are furnished with liquid and suction headers and arranged for pump circulation of the refrigerant. In some installations arrangements are also made for gravity feed. Standard defrosting is effected by hot gas or by leaving the doors open and running the fans during the night.

The fans circulate the air down between the coil and the wall through the coil, through the trolleys and the products, and then the air is deflected up along the wall and back to the fans above the sheeting.

Tunnel freezers are built for capacities varying from a few hundred kilograms to several tons per hour. The freezing time in this type of equipment varies considerably from a few hours when freezing unpackaged vegetables in a thin layer to 48 h freezing meat carcasses.

The sliding-tray freezer is basically a tunnel consisting of one huge rack accommodating many large trays on each tier. At one end of the construction there is an elevating mechanism that lifts the arriving tray to the top tier, where it is pushed in, forcing all the other trays on this tier to advance one step. The tray at the far end is pushed onto an elevator that brings it down one tier where the tray is entered. Consequently, on every odd tier the trays will be advancing, and on every even tier they will be returning. For each tray that enters, all trays will advance one step.

All mechanisms are usually hydraulically powered. Outside the freezer enclosure automatic loading and unloading of the trays may be arranged.

The freezing trays are in the traveling-tray freezer connected to one or two sturdy roller chains at each end. These are arranged to move the trays forward to a set of sprockets elevating the trays one tier while maintaining their horizontal position. This type is constructed as large as those mentioned previously, but requires more space because the minimum pitch between the tiers is decided by the sprockets, which are 200–300 mm in diameter.

The carrier freezer may be regarded as two push-through tunnels, one on top of the other. At the stop section a row of carriers is pushed forward, while it is returned in the lower section. At both ends there are elevating mechanisms. A carrier is similar to a bookcase with shelves. When it is indexed up at the loading end of the freezer, the products on one tier at a time are pushed off the shelf onto a discharge conveyor.

When the carrier is indexed up the next time, this shelf is level with the loading belt from which new products are transferred to the carrier (see Fig. 10).

The carriers may be designed for almost any pitch between the tiers and for any length and width, giving maximum compactness. The loading and unloading may be manual or fully automatic.

Another automatic freezing tunnel is the reciprocating spiral freezer, which consists of two parallel sets of rails, one of which is fixed. In between there is a set of movable rails. Initially the products rest on the fixed set. The movable set lifts the products clear of the fixed set, advances one stroke, descends to leave the products resting on the fixed set again, and then returns to the initial position.

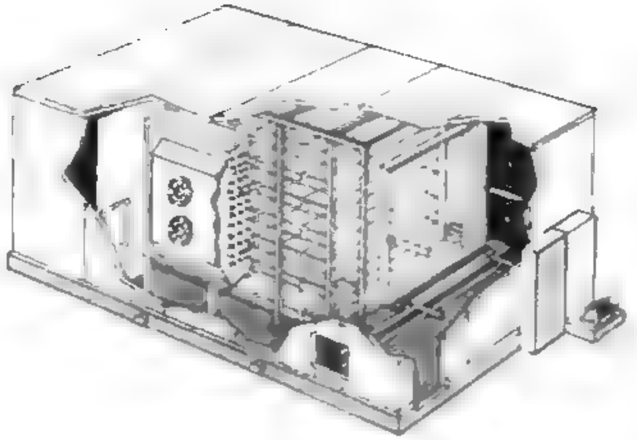


Figure 10. Carrier freezer.

Both sets are arranged to form a large spiral, the fixed set supported from an external steel structure and the movable set fitted to a central cylinder. This provides a reciprocating motion around the vertical axis as well as up and down.

The infeed can be arranged very easily for a range of carton sizes provided the utilization of the conveyor area is limited. Small items must be placed on trays that are loaded, unloaded, and transferred from outfeed to infeed separately.

As the total product load is accelerated and decelerated for each stroke, a relatively slow motion is necessitated, making this type of freezer suitable mainly for intermediate and large package sizes.

All freezer designs described above are intended primarily for packaged products. Attempts to freeze fish fillets, meat patties, etc individually on trays invariably have been only moderate successful because of a number of problems, primarily the following:

1. Products stick to the trays. This causes damage and weight losses if products are removed mechanically. An alternative is to heat the trays to release the products. This requires complicated equipment and causes reduced capacity.
2. Hygiene. The trays must be washed after removal of the frozen products if acceptable hygiene is to be obtained.
3. The handling of trays from outfeed to infeed is costly whether it is manual or automatic.

The principal advantages of automatic air-blast freezers in comparison with automatic plate freezers, which are usually the alternative, are the following:

4. Products of much varied thickness may be frozen simultaneously or immediately after another.
5. Products do not need to be square in shape.
6. Higher capacities per unit are possible.

The automatic or mechanized tunnel freezer generally has the same advantages and disadvantages as the classical tunnel except that it is slightly better suited as an inline freezer.

Labor costs can be reduced and the flexibility is somewhat better as different products can be handled at the same time by different tracks having different dwell times.

Belt Freezers

The first belt freezers basically consisted of a wire mesh belt conveyor in a blast room, which satisfied the need for a continuous product flow. In addition to the disadvantage of poor heat transfer in a blast room, many mechanical problems arose.

Modern belt freezers normally utilize vertical airflow, forcing the air through the product layer, which creates good contact with all product particles. A condition is, however, that the product be evenly distributed over the entire belt area. Where the product layer is thin or nonexisting, there is less resistance to the air, which will concentrate to these areas and bypass the thicker product layer. This phenomenon, called channeling, may result in poorly frozen products and thus must be avoided by careful and even spreading of the product across total belt width under all operating conditions. Single-belt freezers designed for freezing unpacked products can be designed to achieve a fluidized freezing.

In order to decrease the necessary floor space of a single-belt freezer the belts can be stacked above each other as in a multitier belt freezer or a spiral belt freezer. The latter being the most important modern belt freezer equipment. A typical multitier freezer is shown in Figure 11.

A multitier freezer can be used where the factory layout requires a straight-through product flow and where available space is narrow. A freezer consists of three conveyor systems positioned one above the other with fans and coils positioned above the top belt supported by the same steel structure that carries the conveyor system.

Products are fed onto the top belt and transported through the cooling zone into the freezing zone and to the opposite end of the freezer. Here the products are transferred from the top belt to the second via a stainless-steel transfer chute. On a second belt they are conveyed back through the freezing zone to the infeed, where they are transferred to the third belt. The third belt takes the products through the freezing zone and delivers the frozen products to the outfeed, from which the products leave the freezer. This arrangement has the advantage that the product, after being surface frozen on the first belt, may be stacked in a rather deep bed on the lower belts. Thus the total belt area required can be reduced.

By vertically stacking the belt in tiers a minimum of floor space is occupied by the freezer. This is the case in the modern spiral belt freezer (Fig. 12), which maximizes the belt surface area in a given floor space. This is achieved by using a belt that can be bend laterally around a rotating drum. The belt in supported by rails and driven by the friction against the drum. The most advanced and refined spiral freezers operate with a low tension drive system.

The continuous-belt design eliminates product transfer points where product damage can occur within the freezing system. The products are placed on the belt outside the freezer where they can be monitored and will remain in the same spot until leaving the freezer. The flexibility of the belt used allows for more than one infeed and outfeed with one and the same belt, and infeed and outfeed may be arranged in any direction desired to suit the layout of the processing line.

In the most modern version of spiral belt freezers a self-stacking belt—FRIGoBELT—where each tier rests directly on the vertical side links of the tier beneath is used. This construction eliminates the need for rails and runners and allows more tiers of belt to be installed in a given space. The whole stack turns as a unit. Products cannot roll and blow out of the closed freezing zone and cannot become stuck within freezer because there are no stationary structural parts to snag them. Equally important is the improvements of the hygienic conditions of the enclosed product zone. The belt in contact with the product is regularly cleaned and dried in an external washing unit outside the freezer.

The side links of the belt will also serve to channel the vertical airflow in the freezing zone. The air is blown down over the upward-moving products in a countercurrent flow, which is a very efficient form of heat transfer.

The spiral belt freezer provides great flexibility with regard to the product range to be handled. Both unpackaged and packaged products are frozen and typical products are meat patties, fish cakes, fish fillets, and bakery products, which all may be frozen raw or prepared.

Fluidized-Bed Freezers

Previously freezing of vegetables took place in a plate freezer or tunnel freezer, and the result was more or less a block frozen product that was hard to thaw and rather inconvenient in handling. The use of "cluster busters" in order to obtain a more free-flowing product caused considerable mechanical damage. Belt freezers were introduced soon after World War II, but in order to meet the high freezing demands those freezers became rather huge. In the early 1960s fluidized freezing was introduced after years of experiments and tests, and it was possible for the first time to quick-freeze vegetables individually very fast in a commercial application.

Fluidization occurs when particles of fairly uniform shape and size are subjected to an upward airstream. At a certain air velocity the particles will float in the airstream, each one separated from the other but surrounded by air and free to move. In this state the mass of particles can be compared to a fluid.

If a mass is held in a container that is fed in one end and the other end is lower, the mass will move toward the lower end as long as more products are added. By utilizing low-temperature air to achieve the fluidization the products are frozen and simultaneously conveyed by the same air without the aid of a conveyor (see Fig. 13).

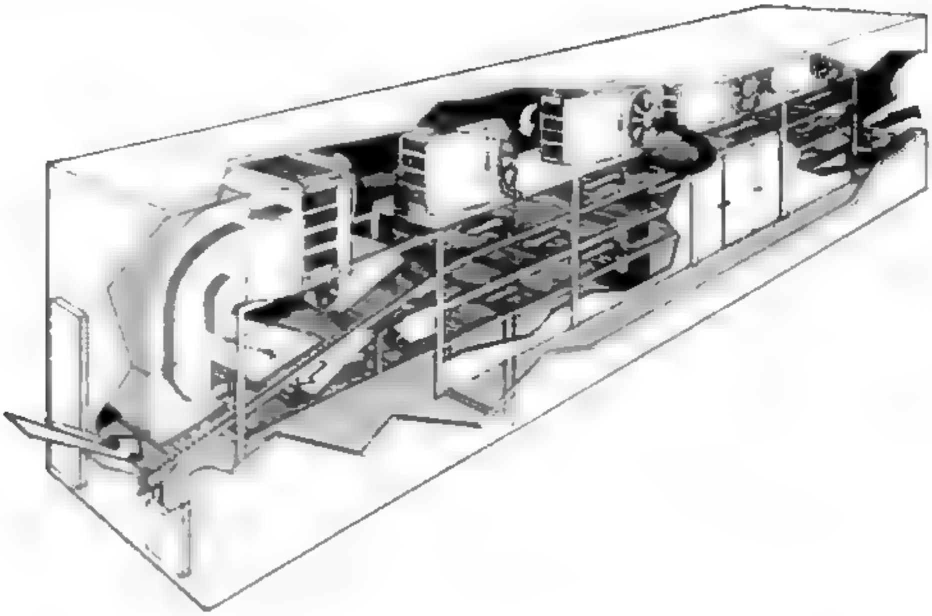


Figure 11. Multitier freezer (TRIoFREEZE).

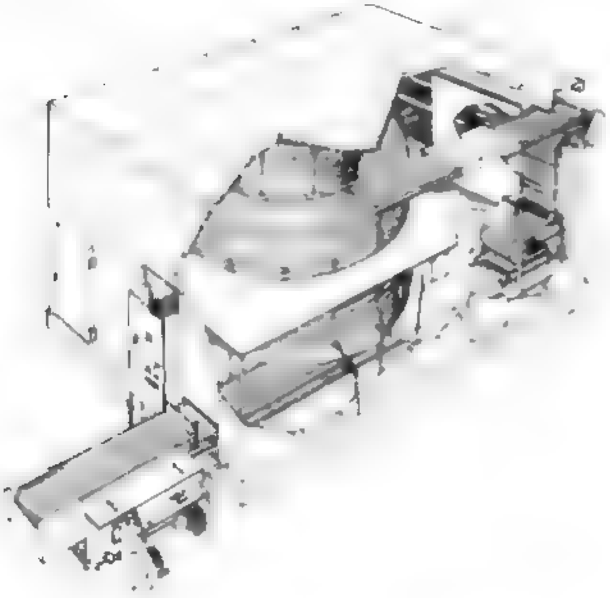


Figure 12. Spiral freezer (GYROCOMPACT).

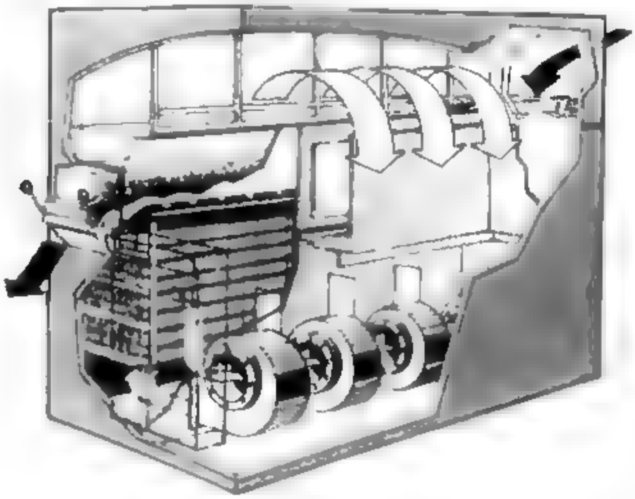


Figure 13. Fluidized-bed freezer (FLoFREEZE).

The use of the fluidization principle gives a number of advantages in comparison with the use of a belt freezer. The product is always individually quick-frozen (IQF); this applies also to products with a tendency to stick together, eg, French-style green beans, sliced carrots, and sliced cucumber.

The freezer is totally independent of fluctuations in load. If the freezer is partly loaded, the air distribution can be the same as for the full load, ie, with no hazard of channeling. The variability of freezing with products is greatly improved because a deep fluidized bed can accept products with more surface water. Consequently, there is no hazard of belt damage if a dewatering screen is broken down temporarily.

One type of fluidized-bed freezer combines the fluidized bed with a conveyor belt. The freezer operates in two stages: a crust freezing zone and a finishing freezing zone. In the former the product is carried on a fluidized bed that guarantees an efficient heat transfer, a quick crust freezing, and particle separation. The crust frozen product is then conveyed on a belt through the second freezing zone.

The fluidization technique achieves a very efficient air-product contact, which gives heat-transfer rates which are much higher than those for conventional air-blast freezing tunnels or belt freezers. The efficiency of the heat removal can also be seen in the physical dimensions of the equipment, which are generally one-third of the comparable belt freezer.

The fluidized-bed freezer is in-line equipment suitable not only for vegetables, berries, and other fruits but also for processed products such as French fried potatoes, peeled cooked shrimps, diced meat, and meatballs.

Contact Freezers

In a contact freezer the product is either in direct contact with the freezing media—immersed—or indirectly by being in contact with a belt or plate containing the freezing media.

Immersion Freezers

The immersion freezers consists of a tank with a cooled freezing medium, such as salt, sugar, or alcohol solution in water or other nontoxic mixtures of water and solutes. The product is immersed in this brine or sprayed while being conveyed through the tank.

This type of equipment has been quite commonly used for surface freezing of turkeys and other poultry on markets where a light color is demanded. Final freezing is accomplished in a separate blast tunnel or during cold storage. The latter, however, may jeopardize quality because of slow core freezing. It is necessary to protect the product from contact with the brine by using high-quality packaging materials with absolutely tight seal. Brine residues on the packages are washed off with water at the freezer exit.

A sodium chloride brine was earlier sometimes used in direct contact with the product in the fishing industry. For freezing tuna fish, for example, it is still used in some places.

Indirect-Contact Freezers

The most commonly contact freezer is the plate freezer, where the product is pressed between hollow metal plates that are positioned horizontally or vertically, with a refrigerant circulating through them.

Another type of freezer utilizes two belts with the refrigerant circulating outside the belts or alternatively placing the product on a single belt. All these arrangements provide a very good heat transfer, which is reflected in short freezing times, provided the product itself is a good heat conductor.

The advantage of good heat transfer at the surface is gradually reduced with increasing product thickness. For this reason it is often limited to a maximum of 50–60 mm. It is further important that the packages are well filled and if metal trays are used to carry the packs that these are not distorted.

The pressure from the plates or belts maintain throughout the freezing process practically eliminates what is known as "bulging" and the frozen packs will maintain their regular shape within very close tolerances.

Plate Freezers

There are two main types of plate freezer: the horizontal and the vertical plate freezer. Either type can be manual or automatic. The typical manual horizontal plate freezer contains 15–20 plates. The product is placed on metal trays or in other systems metal frames and transported to the freezer where they are manually loaded between the plates.

In order to obtain automatic operation of the horizontal plate freezer the whole battery of plates is moved up and down in an elevator system. At the loading conveyor level the plates are separated, and a row of packages that have accumulated on a transport conveyor from the processing and packaging are pushed in between the open plates, simultaneously discharging a row of frozen products at the opposite side. This cycle is repeated until all frozen packages at this level has been replaced. Then the space between the two plates are closed, all plates are indexed up, and the next set of plates are opened for loading and unloading. A typical automatic plate freezer is shown in Figure 14.

The vertical plate freezer is used mainly for freezing products in blocks weighing 10–15 kg and has been specially developed for freezing fish at sea. The freezer consists of a number of vertical freezing plates forming partitions in a container with an open top. The product is simply fed in from the top and the blocks after freezing is discharged either to the side, upwards, or down through the bottom. Usually this mechanism is automized. The discharge of the products is enhanced by a short period of gas defrost at the end of the freezing cycle and the use of compressed air or a hydraulic system to force out the product.

Band Freezers

Single-band and double-band freezers are designed to freeze thin product layers. The freezers can either be straight forward bands as shown in Figure 15 or as a drum (Fig. 16).

The band freezer illustrated in Figure 16 is designed to freeze and form liquids and semiliquids into individual pellets as an in-line operation.

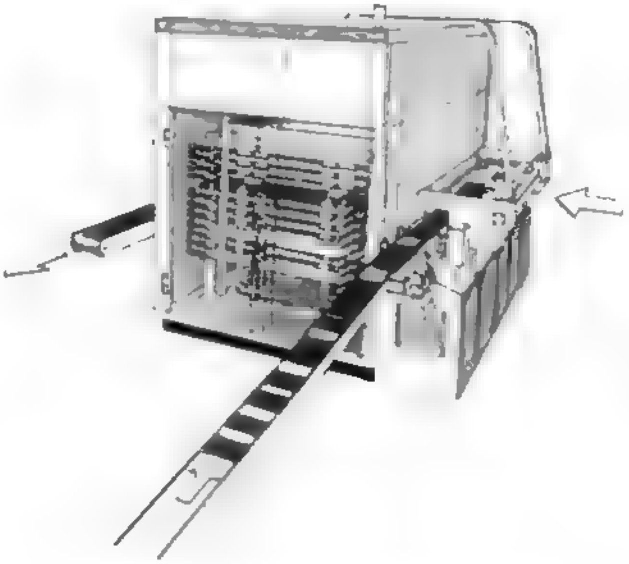


Figure 14. Automatic plate freezer (AUToPLATE).

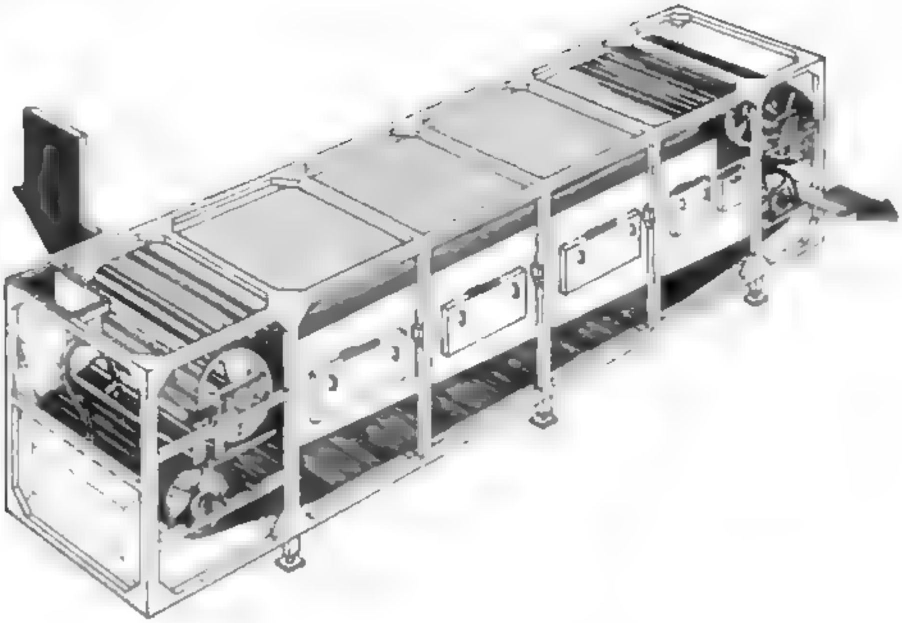


Figure 15. Contact band freezer (PELLoFREEZE).

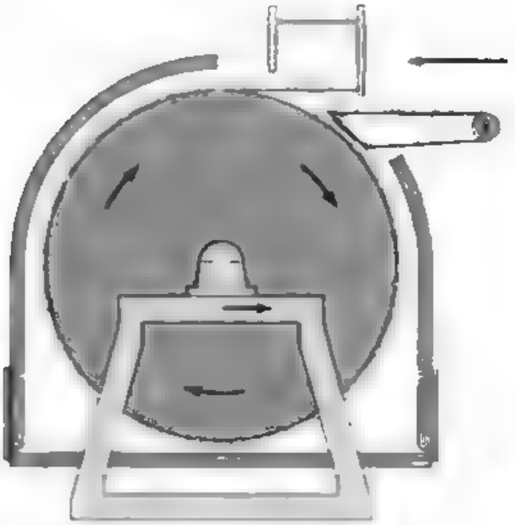


Figure 16. Direct-contact band freezeR—drum freezer.

The product is formed and frozen between two endless stainless-steel bands, of which the top band is flat and the lower band is corrugated with flexible seals on each side. The product is supplied on the corrugated band by a spreading device, after which the flat band is brought in contact with the product, thus totally enclosing it. After the freezing-forming zone the two bands are separated. The liquid is now frozen to a mat and passes through the final forming operation. The product finally enters the outfeed conveyor in an IQF form.

A monopropylene glycol–water brine is used as an intermediate freezing medium. The brine is circulated by pumps from a sump below the freezing pump via a brine cooler to the freezing zone, where it passes over the outer surfaces of the band with high velocity.

Typical products frozen in band freezers are chopped spinach purée, fruits pulps, egg yoke, sauces, and soups.

The drum freezer can be viewed as a more compact band freezer. Distinction is made between both vertical and horizontal drums. This type of freezer is also known as a rotary freezer.

Cryogenic Freezers

Cryogenic freezers differ from all other types of freezers in one fundamental respect: they are not connected to a refrigeration plant. The heat-transfer medium is nitrogen or carbon dioxide liquefied in large industrial installations and shipped to the food freezing factory in low-temperature well-insulated pressure vessels.

The design of cryogenic freezers has improved significantly in recent years. As for all types of freezers also this type can be found as straight-belt, multitier, and spiral belt as well as immersion designs. In principle the same basic equipment can be used for both gases, but with slight modifications. The size and mobility of cryogenic freezers allow for flexibility in design and redesigning a processing plant.

Key attributes of the equipment are high heat-transfer rates, low investment costs, and rapid installation and startup. Especially interesting applications are those for chilling, firming, or crusting products.

A typical belt liquid nitrogen (LIN) freezer is shown in Figure 17. Liquid nitrogen at −196°C is sprayed into the freezer in which the atmosphere is circulated by small fans. The freezant or liquid nitrogen partially evaporates immediately leaving the spray nozzles and on contact with the products. The cold gas is circulated by fans toward the infeed end precooling the products entering the freezer and thereafter extracted by an exhaust fan.

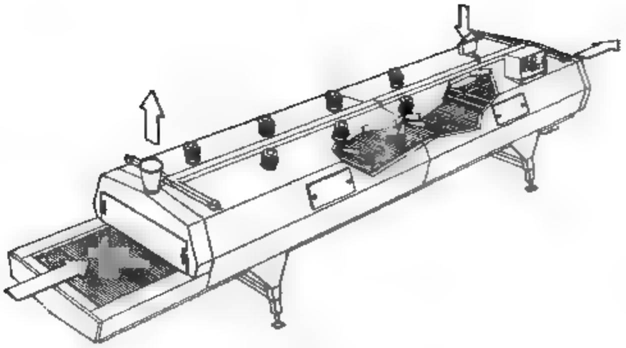


Figure 17. Cryogenic freezer (AGA FREEZE).

The freezant thus passes in countercurrent to the movement of products on the belt and giving a high heat-transfer efficiency, which is an advantage in terms of quality for some special products.

However, the quick freezing may also result in cracking of the product surface if sufficient precautions are not taken. The freezant consumption is in the range of 1.2–2.0 kg per kilogram of product. The capacity can vary from 150 to 1000 kg/h, and typical products are meat cuts, fish fillets, seafood, fruit, berries, pies, and pastries.

Prefreezer

A recent development utilizing liquid nitrogen as freezing medium is an apparatus designed for quick crust freezing of extremely wet, sticky, or sensitive products that can then be easily handled in a spiral belt freezer or a fluidized-bed freezer for completion of the freezing process without deformation or breakage. The freezers also offer a possibility of freezing products that are difficult to freeze in conventional systems (see Fig. 18).

The products are frozen by means of direct immersion in liquid nitrogen, which gives an almost instantaneous freezing of the surface. The product is fed vertically into an IQF tank with a continuous flow of liquid nitrogen.

In this stream the products are gently received and separated at the same time as a very thin layer of the product surface is frozen. From this first step the products drop down on a belt and are fed into a bath of liquid nitrogen that together with a spray completes the crust freezing. The liquid nitrogen is then separated from the product and collected in a sump from where it is recirculated by means of a specially designed pump.

Carbon Dioxide Freezer

Liquid carbon dioxide is normally stored under high pressure. At atmospheric pressure it exists only as a solid or a gas. When the liquid is released to the atmosphere, 50% of the liquid becomes dry-ice snow and 50% vapor both at −79°C. Because of these unusual properties, carbon dioxide freezer designs vary widely.

In a LIN freezer the cold gas phase is used to precool the product before it is exposed to the nitrogen spray. As liquid carbon dioxide forms snow that needs time for sublimation, the injection is moved closer to the product infeed as compared to the LIN freezer.

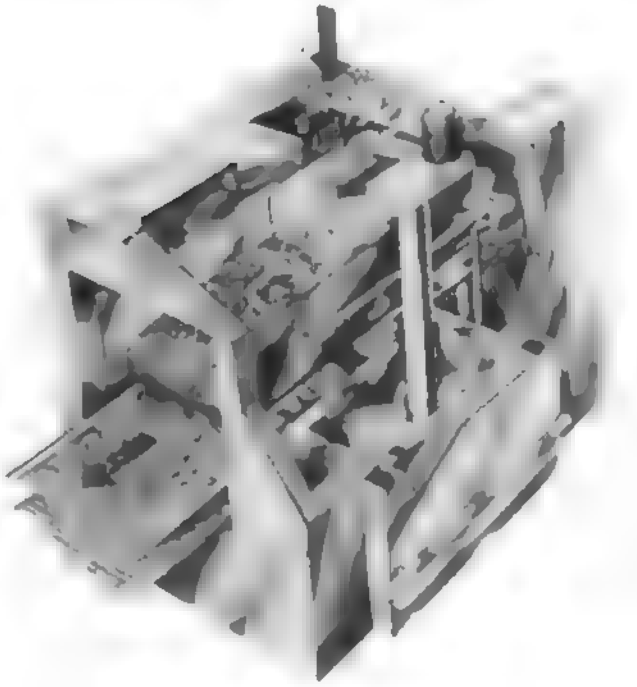


Figure 18. Prefreezer (CRUSToFREEZE).

THERMODYNAMIC PROPERTIES

An important consideration in all designs of food processing involving heat exchange is the thermal properties of the food products to be handled in the system. The variable composition and structure of foods influence those properties. During storage the chemical and physical properties, the time, and the temperatures are the most important factors. The composition of the food product varies with species, growth condition, age, feed, harvest, slaughter, catch, handling, and processing, as well as storage conditions. All of these factors influence the thermal properties.

For these reasons it is easily understood that the values of thermal properties are not exact values but most often estimates. The more detailed a description of the food product measured the more accurate the values given. Another important consideration when using experimentally found thermal properties is the difference in the methods of measurement, which may place limitations on the value of the data (4). Computer programs have been developed to estimate the thermal physical properties from the knowledge of the specifications of the products, such as chemical composition, temperature, and density.

Values of specific heat and latent heat of fusion are often calculated directly from the water content of the product. This is the case for the values given in Table 1.

The values of water content in Table 1 are averaged for the product. The water content for fruit and vegetables

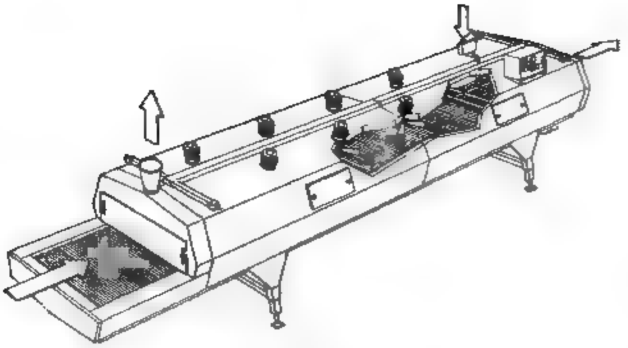


Figure 17. Cryogenic freezer (AGA FREEZE).

The freezant thus passes in countercurrent to the movement of products on the belt and giving a high heat-transfer efficiency, which is an advantage in terms of quality for some special products.

However, the quick freezing may also result in cracking of the product surface if sufficient precautions are not taken. The freezant consumption is in the range of 1.2–2.0 kg per kilogram of product. The capacity can vary from 150 to 1000 kg/h, and typical products are meat cuts, fish fillets, seafood, fruit, berries, pies, and pastries.

Prefreezer

A recent development utilizing liquid nitrogen as freezing medium is an apparatus designed for quick crust freezing of extremely wet, sticky, or sensitive products that can then be easily handled in a spiral belt freezer or a fluidized-bed freezer for completion of the freezing process without deformation or breakage. The freezers also offer a possibility of freezing products that are difficult to freeze in conventional systems (see Fig. 18).

The products are frozen by means of direct immersion in liquid nitrogen, which gives an almost instantaneous freezing of the surface. The product is fed vertically into an IQF tank with a continuous flow of liquid nitrogen.

In this stream the products are gently received and separated at the same time as a very thin layer of the product surface is frozen. From this first step the products drop down on a belt and are fed into a bath of liquid nitrogen that together with a spray completes the crust freezing. The liquid nitrogen is then separated from the product and collected in a sump from where it is recirculated by means of a specially designed pump.

Carbon Dioxide Freezer

Liquid carbon dioxide is normally stored under high pressure. At atmospheric pressure it exists only as a solid or a gas. When the liquid is released to the atmosphere, 50% of the liquid becomes dry-ice snow and 50% vapor both at −79°C. Because of these unusual properties, carbon dioxide freezer designs vary widely.

In a LIN freezer the cold gas phase is used to precool the product before it is exposed to the nitrogen spray. As liquid carbon dioxide forms snow that needs time for sublimation, the injection is moved closer to the product infeed as compared to the LIN freezer.

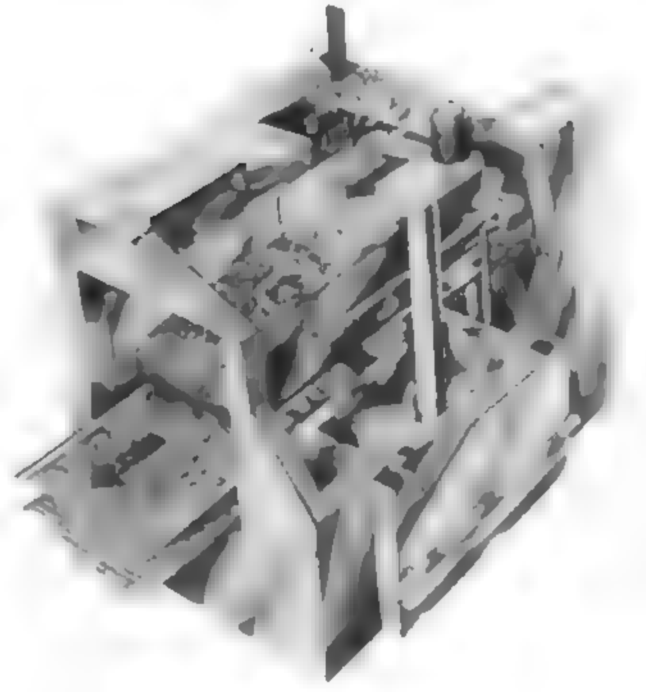


Figure 18. Prefreezer (CRUSToFREEZE).

THERMODYNAMIC PROPERTIES

An important consideration in all designs of food processing involving heat exchange is the thermal properties of the food products to be handled in the system. The variable composition and structure of foods influence those properties. During storage the chemical and physical properties, the time, and the temperatures are the most important factors. The composition of the food product varies with species, growth condition, age, feed, harvest, slaughter, catch, handling, and processing, as well as storage conditions. All of these factors influence the thermal properties.

For these reasons it is easily understood that the values of thermal properties are not exact values but most often estimates. The more detailed a description of the food product measured the more accurate the values given. Another important consideration when using experimentally found thermal properties is the difference in the methods of measurement, which may place limitations on the value of the data (4). Computer programs have been developed to estimate the thermal physical properties from the knowledge of the specifications of the products, such as chemical composition, temperature, and density.

Values of specific heat and latent heat of fusion are often calculated directly from the water content of the product. This is the case for the values given in Table 1.

The values of water content in Table 1 are averaged for the product. The water content for fruit and vegetables

Table 1. Thermal and Related Properties of Food and Food Materials

Food or food material	Water content % (mass)[a]	Highest freezing point, °C[c]	Specific heat[b]		Latent heat of fusion[d] kJ/kg
			Above freezing, kJ/kg · °C	Below freezing, kJ/kg °C	
		Vegetables			
Artichokes, Globe	84	−1.2	3.78	1.90	281
Artichokes, Jerusalem	80	−2.5	3.68	1.85	268
Asparagus	93	−0.6	4.00	2.01	312
Beans, snap	89	−0.7	3.90	1.96	298
Beans, lima	67	−0.6	3.35	1.68	224
Beans, dried	11	—	1.95	0.98	37
Beets, roots	88	−1.1	3.88	1.95	295
Broccoli	90	−0.6	3.93	1.97	302
Brussels sprouts	85	−0.8	3.80	1.91	285
Cabbage, late	92	−0.9	3.98	2.00	308
Carrots, roots	88	−1.4	3.88	1.95	295
Cauliflower	92	−0.8	3.98	2.00	308
Celeriac	88	−0.9	3.88	1.95	295
Celery	94	−0.5	4.03	2.02	315
Collards	87	−0.8	3.85	1.94	291
Corn, sweet	74	−0.6	3.53	1 77	248
Cucumbers	96	−0.5	4.08	2.05	322
Eggplant	93	−0.8	4.00	2.01	312
Endive (escarole)	93	−0.1	4.00	2.01	312
Garlic	61	−0.8	3.20	1.61	204
Ginger, rhizomes	87	—	3.85	1.94	291
Horseradish	75	−1.8	3.55	1.79	251
Kale	87	−0.5	3.85	1.94	291
Kohlrabi	90	−1.0	3.93	1.97	302
Leeks	85	−0.7	3.80	1.91	285
Lettuce	95	−0.2	4.06	2.04	318
Mushrooms	91	−0.9	3.95	1.99	305
Okra	90	−1.8	3.93	1.97	302
Onions, green	89	−0.9	3.90	1.96	298
Onions, dry	88	−0.8	3.88	1.95	295
Parsely	85	−1.1	3.80	1.91	285
Parsnips	79	−0.9	3.65	1.84	265
Peas, green	74	−0.6	3.53	1.77	248
Peas, dried	12	—	1.97	0.99	40
Peppers, dried	12	—	1.97	0.99	40
Peppers, sweet	92	−0.7	3.98	2.00	308
Potatoes, early	81	−0.6	3.70	1.86	271
Potatoes, main crop	78	−0.6	3.63	1.82	261
Potatoes, sweet	69	−1.3	3.40	1.71	231
Pumpkins	91	−0.8	3.95	1.99	305
Radishes	95	−0.7	4.06	2.04	318
Rhubarb	95	−0.9	4.06	2.04	318
Rutabagas	89	−1.1	3.90	1.96	298
Salsify	79	−1.1	3.65	1.84	265
Spinach	93	−0.3	4.00	2.01	312
Squash, summer	94	−0.5	4.03	2.02	315
Squash, winter	85	−0.8	3.80	1.91	285
Tomatoes, mature green	93	−0.6	4.00	2.01	312
Tomatoes, ripe	94	−0.5	4.03	2.02	315
Turnip greens	90	−0.2	3.93	1.97	302
Turnip	92	−1.1	3.98	2 00	308
Watercress	93	−0.3	4.00	2.01	312
Yams	74	—	3.53	1.77	248

Table 1. Thermal and Related Properties of Food and Food Materials *(continued)*

Food or food material	Water content % (mass)[a]	Highest freezing point, °C[c]	Specific heat[b]		Latent heat of fusion[d] kJ/kg
			Above freezing, kJ/kg · °C	Below freezing, kJ/kg °C	
		Fruits			
Apples, fresh	84	−1.1	3.78	1.90	281
Apples, dried	24	—	2.27	1.14	80
Apricots	85	−1.1	3.80	1.91	285
Avocados	65	−0.3	3.30	1.66	218
Bananas	75	−0.8	3.55	1.79	251
Blackberries	85	−0.8	3.80	1.91	285
Blueberries	82	−1.6	3.73	1.87	275
Cantaloupes	92	−1.2	3.98	2.00	308
Cherries, sour	84	−1.7	3.78	1.90	281
Cherries, sweet	80	−1.8	3.68	1.85	268
Cranberries	87	−0.9	3.85	1.94	291
Currants	85	−1.0	3.80	1.91	285
Dates, cured	20	−15.7	2.17	1.09	67
Figs, fresh	78	−2.4	3.63	1.82	261
Figs, dried	23	—	2.25	1.13	77
Gooseberries	89	−1.1	3.90	1.96	298
Grapefruit	89	−1.1	3.90	1.96	298
Grapes, American	82	−1.6	3.73	1.87	275
Grapes, Vinifera	82	−2.1	3.73	1.87	275
Lemons	89	−1.4	3.90	1.96	298
Limes	86	−1.6	3.83	1.92	288
Mangoes	81	−0.9	3.70	1.86	271
Melons, Casaba	93	−1.1	4.00	2.01	312
Melons, Crenshaw	93	−1.1	4.00	2.01	312
Melons, honeydew	93	−0.9	4.00	2.01	312
Melons, Persian	93	−0.8	4.00	2.01	312
Melons, watermelon	93	−0.4	4.00	2.01	312
Nectarines	82	−0.9	3.73	1.87	275
Olives	75	−1.4	3.55	1.79	251
Oranges	87	−0.8	3.85	1.94	292
Peaches, fresh	89	−0.9	3.90	1.96	298
Peaches, dried	25	—	2.30	1.16	84
Pears	83	−1.6	3.75	1.89	278
Persimmons	78	−2.2	3.63	1.82	261
Pineapples	85	−1.0	3.80	1.91	285
Plums	86	−0.8	3.83	1.92	288
Pomegranates	82	−3.0	3.73	1.87	275
Prunes	28	—	2.37	1.19	94
Quinces	85	−2.0	3.80	1.91	285
Raisins	18	—	2.12	1.07	60
Raspberries	81	−0.6	3.70	1.86	271
Strawberries	90	−0.8	3.93	1.97	302
Tangerines	87	−1.1	3.85	1.94	291
		Whole fish			
Haddock, cod	78	−2.2	3.63	1.82	261
Halibut	75	−2.2	3.55	1.79	251
Herring, kippered	70	−2.2	3.43	1.72	235
Herring, smoked	64	−2.2	3.28	1.65	214
Menhaden	62	−2.2	3.23	1.62	208
Salmon	64	−2.2	3.28	1.65	214
Tuna	70	−2.2	3.43	1.72	235
		Fish fillets or steaks			
Haddock, cod, perch	80	−2.2	3.68	1.85	268
Hake, whiting	82	−2.2	3.73	1.87	275
Pollock	79	−2.2	3.65	1.84	265
Mackerel	57	−2.2	3.10	1.56	191

Table 1. Thermal and Related Properties of Food and Food Materials (*continued*)

Food or food material	Water content % (mass)[a]	Highest freezing point, °C[c]	Specific heat[b] Above freezing, kJ/kg · °C	Specific heat[b] Below freezing, kJ/kg °C	Latent heat of fusion[d] kJ/kg
Shellfish					
Scallop, meat	80	−2.2	3.68	1.85	268
Shrimp	83	−2.2	3.75	1.89	278
Lobster, American	79	−2.2	3.65	1.84	265
Oysters, clams, meat, and liquor	87	−2.2	3.85	1.94	291
Oyster in shell	80	−2.8	3.68	1.85	268
Beef					
Carcass (60% lean)	49	−1.7	2.90	1.46	164
Carcass (54% lean)	45	−2.2	2.80	1.41	151
Sirloin, retail cut	56	—	3.08	1.55	188
Round, retail cut	67	—	3.35	1.68	224
Dried, chipped	48	—	2.88	1.44	161
Liver	70	−1.7	3.43	1.72	235
Veal, carcass (81% lean)	66	—	3.33	1.67	221
Pork					
Bacon	19	—	2.15	1.08	64
Ham, light cure	57	—	3.10	1.56	191
Ham, country cure	42	—	2.72	1.37	141
Carcass (47% lean)	37	—	2.60	1.31	124
Bellies (33% lean)	30	—	2.42	1.22	101
Backfat (100% fat)	8	—	1.87	0.94	27
Shoulder (67% lean)	49	−2.2	2.90	1.46	164
Ham (74% lean)	56	−1.7	3.08	1.55	188
Sausage, links or bulk	38	—	2.62	1.32	1.27
Sausage, country style, smoked	50	−3.9	2.93	1.47	168
Sausage, frankfurters	56	−1.7	3.08	1.55	188
Sausage, Polish style	54	—	3.03	1.52	181
Lamb					
Composite of cuts (67% lean)	61	−1.9	3.20	1.61	204
Leg (83% lean)	65	—	3.30	1.66	218
Dairy products					
Butter	16	—	2.07	1.04	54
Cheese, Camembert	52	—	2.98	1.50	174
Cheese, Cheddar	37	−12.9	2.60	1.31	124
Cheese, cottage (uncreamed)	79	−1.2	3.65	1.84	265
Cheese, cream	51	—	2.95	1.48	171
Cheese, Limburger	45	−7.4	2.80	1.41	151
Cheese, Roquefort	40	−16.3	2.67	1.34	134
Cheese, Swiss	39	−10.0	2.65	1.33	131
Cheese, processed American	40	−6.9	2.68	1.34	134
Cream, half-and-half	80	—	3.68	1.85	268
Cream, table	72	−2.2	3.48	1.75	241
Cream, whipping, heavy	57	—	3.10	1.56	191
Ice cream, (10% fat)	63	−5.6	3.25	1.63	211
Milk, canned, condensed, sweetened	27	−15.0	2.35	1.18	90
Milk, evaporated, unsweetened	74	−1.4	3.53	1.77	248
Milk, dried (whole)	2	—	1.72	0.87	7
Milk, dried (nonfat)	3	—	1.75	0.88	10
Milk, fluid (3.7% fat)	87	−0.6	3.85	1.94	291
Milk, fluid (skim)	91	—	3.95	1.99	305
Whey, dried	5	—	1.80	0.90	17

Table 1. Thermal and Related Properties of Food and Food Materials *(continued)*

Food or food material	Water content % (mass)[a]	Highest freezing point, °C[c]	Specific heat[b] Above freezing, kJ/kg · °C	Specific heat[b] Below freezing, kJ/kg °C	Latent heat of fusion[d] kJ/kg
		Poultry products			
Egg, whole (fresh)	74	−0.6	3.53	1.77	247
Eggs, white	88	−0.6	3.88	1.95	295
Eggs, yolks	51	−0.6	2.95	1.48	171
Eggs, yolks (sugared)	51	−3.9	2.95	1.48	171
Eggs, yolks (salted)	50	−17.2	2.93	1.47	168
Eggs, dried (whole)	4	—	1.77	0.89	13
Eggs, dried (white)	9	—	1.90	0.95	30
Chicken	74	−2.8	3.53	1.77	248
Turkey	64	—	3.28	1.65	214
Duck	69	—	3.40	1.71	231
		Miscellaneous			
Honey	17	—	2.10	1.68	57
Maple syrup	33	—	2.50	1.26	111
Popcorn, unpopped	10	—	1.92	0.97	34
Yeast, baker's compressed	71	—	3.45	1.73	238
		Candy			
Milk chocolate	1	—	1.70	0.35	3
Peanut brittle	2	—	1.72	0.87	7
Fudge, vanilla	10	—	1.92	0.97	34
Marshmallows	17	—	2.10	1.05	57
		Nuts, shelled			
Peanuts (with skins)	6	—	1.82	0.92	20
Peanuts (with skins, roasted)	2	—	1.72	0.87	7
Pecans	3	—	1.75	0.88	10
Almonds	5	—	1.80	0.90	17
Walnuts, english	4	—	1.78	0.89	13
Filberts	6	—	1.82	0.92	20

[a] Water contents of fruits and vegetables are from Lutz and Hardenburg (1968) except for Jerusalem artichokes; dried beans; and peas, yams, dried apples, figs, peaches, prunes, and raisins; the latter are from Watt and Merrill (1963). Water contents of meats, dairy, and poultry products, miscellaneous candy, and nuts are also from Watt and Merrill, water contents of eggs (yolks, salted) and fish are from ASHRAE (1972, 1974, and 1978).

[b] Freezing points of fruits and vegetables are from Whiteman (1957), and average freezing points of other foods are from ASHRAE (1972, 1974, and 1978).

[c] Specific heat was calculated from Siebel's formulas (1892).

[d] Latent heat of fusion was obtained by multiplying water content expressed in decimal form by 144, the heat of fusion of water in Btu/lb.

Source Reprinted with permission of the American Society of Heating; Refrigerating and Air-Conditioning Engineers from the 1989 *ASHRAE Handbook—Fundamentals*. Ref. 11.

varies with the stage of development or maturity when harvested and also with type of species, growing conditions, and the storage conditions after harvest. The values given in the table apply to mature product shortly after harvest. For meat the water content values are for the time of slaughter or after the aging period. In reality the water content varies considerably, not only between different animals but also between different muscles from the same animal. For processed products the water content depends on the specific process used.

The freezing points given in Table 1 are based on experiments in which the product has been cooled slowly until freezing occurred. For fruits and vegetables the highest temperature at which the product freeze are given and for other foods average freezing temperature is shown.

With reference to specific heat it should be observed that this is a function of temperature. The value given in Table 1 are from 0°C. In a unfrozen product the specific heat will be slightly lower as temperature rise, and in frozen foods there is a large change in specific heat as temperature decreases. The latter is, of course, related to the changes in composition, in particular the water content. When calculating specific heat of a frozen product it is assumed that the water is frozen to ice and that the specific heat involved is that of ice. This assumption is not totally correct. The freezing of most foods that is transferring from liquid water to ice is a gradual process that occurs over a wide temperature range.

With reference to the latent heat of fusion given in Table 1, these values are also subject to error because they do not consider the chemical composition other than water content. They are, in other words, the product of the heat of fusional water and the water content.

The variations that occur in values given on thermal properties should be taken into account in practical applications. Today a comprehensive work is carried out at several research institutes and universities, eg, at Campden Food and Drink Research Association, UK (12) to compare different data. Besides Table 1, a compilation of thermal properties presented by Polley and co-workers is of interest (13).

The definitions of the thermal properties normally listed are

Water content—the mass of water in the product divided by the total mass expressed in percentage.

Average freezing point—the temperature at which the liquid and solid state of a product are in equilibrium expressed in Celsius.

Latent heat—the quantity of heat necessary to change 1 kilogram of liquid to solid without change of temperature measured as kJ/kg.

Specific heat—the amount of heat needed to rise the temperature of 1 kg of a food product 1° measured as kJ/kg, °C.

Heat of respiration—the quality of heat generated per 24 h measured as kJ/24 h, kg.

The large variations that occur for thermal properties of food products explain why theoretical calculations on freezing time are difficult and should not be used to determine the requirements for a specific freezer. Those requirements should be determined by practical tests.

DETERMINATION OF HOLDING TIME

For calculation of the capacity of any freezer, the necessary holding time is essential. For bulk products such as peas, green beans, French fried potatoes, and fish sticks the capacity of standard equipment is usually specified separately. This is also the case with packaged products that are homogeneous such as spinach purée and fish fillet blocks. For other products holding time must be determined before capacity can be stated.

In the literature many different formulas can be found for accurate determination of holding times. In reality, however, they are of little use, because products differ so much in composition and form that the work to transform the characteristics of a product into mathematical terms is a lot more time-consuming than test freezing of the very product itself. This can usually be done in less time than it takes to analyze the composition of the product.

Of course, such test freezing should be carried out under controlled conditions that do correspond to those that can actually be achieved in production. A suitable pilot freezer for different airflow directions is shown in Figure 19.

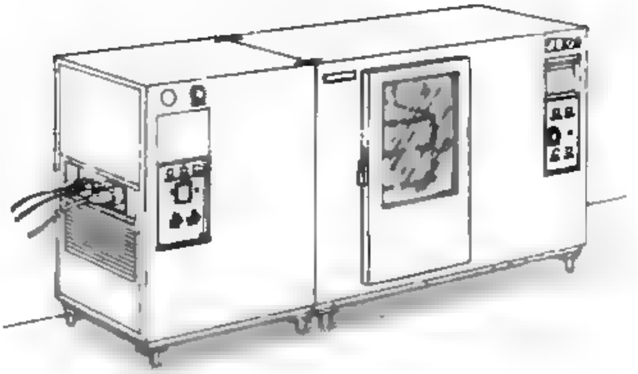


Figure 19. Test freezer.

MAJOR CONSIDERATIONS IN FREEZER DESIGN

Safety of Personnel

The safety of personnel that operate, clean, and service a freezer should be a main consideration in the freezer design. In too many instances, serious injuries have been the result of unsafe design and operation of freezing machinery.

Mechanical hazards exist in the conveyor drive systems, fans, and other areas. The machinery must be designed so that all areas of a freezer can be easily cleaned and inspected while ensuring that personnel are suitably protected. All drives and fans must be fully guarded so that no worker individual or worker's clothing can reach any part of the machine and get caught or crushed. Emergency stop switches need to be located throughout the machine so that if someone does get caught in the machinery the machine can be stopped quickly to minimize the injury. Fan guards must be designed sufficiently open to remain unrestricted by frost buildup while still preventing personnel from getting their hands or clothing into the fan.

The extreme cold found in most modern freezers represents a hazard to personnel in the form of hypothermia and frostbite. The high air velocities found in efficient freezers that use air as the product heat-transfer medium greatly increases heat transfer and the wind-chill factor, which will quickly freeze exposed skin and draw heat from the body at a very high rate. Exposure to these low temperatures must be limited to a tolerable period, and the fans must be shut off whenever a person needs to enter such a freezer.

Cryogenic freezers must not be entered during operation; even a very brief exposure to the cryogen can quickly cause frostbite as a result of the extremely high heat transfer between the boiling liquid or sublimating solid and warm flesh.

Cryogenic freezers, which generally employ either liquid nitrogen or carbon dioxide in snow form, must be evacuated and refilled with air prior to entry by personnel. The freezers purge all the air out during operation, which results in an oxygen content insufficient to sustain consciousness or life. Such machines must be locked out during operation to prevent access and risk of asphyxiation.

Noise can reach levels that damage hearing in some freezers. Care must be taken to avoid exposure to noise levels above 95 dB by wearing a suitable form of hearing protection such as earplugs or earmuffs.

Noise can be reduced to safe levels by proper selection of fans. Excessive noise is generally a result of using a fan at a pressure above which it is designed to operate, causing cavitation.

Machinery Protection

The hostile environment in which freezers operate render human surveillance of their operation almost impossible. The machinery therefore must operate in an extremely dependable manner and have a number of detection devices mounted and operational so that if something does go wrong, the damage will be minimized.

The most common problems encountered in freezers are caused by ice accumulation, product jams, and operational errors. Ice accumulation can occur as a result of poor defrost procedure, excessive moisture on the conveyor carrying the product into the freezer, or poor startup and shutdown procedures. Ice can accumulate in locations in the machine where it impedes the safe operation of the freezer by jamming a part of by forcing the conveyor or other parts out of their normal operating position. Product jams can occur because the products to be frozen is improperly loaded onto the conveyor or the conveyor can be blocked by an obstruction in the product path. In either case good operation of the freezer and sound design can alleviate the problem. Operational errors are symptomized by a wide variety of problems. Typical symptoms are frozen conveyors due to improper startup, unbalanced fans, or an ice-plugged coil due to improper defrost.

Excessive pressure in the refrigeration piping and the coils can lead to a failure and subsequent loss of refrigerant and potential safety hazard. During normal operation the refrigeration piping in the freezer is under low pressure, so the risk of a ruptured pipe is small at that time. When a freezer is defrosted, the temperature of the coil is raised in order to melt the frost of the coil. If the refrigeration piping is not properly designed and installed, potentially dangerous pressures can develop in the coil. It is imperative that suitable relief devices be installed in the coil so that if the operator makes an error the pressure of the refrigerant in the coil cannot go above a safe level.

Product Safety

In a typical freezer application the value of the product that passes through the freezer in a period of just a few weeks can exceed the cost of the freezer. It is therefore sound practice to ensure that the product is not damaged or contaminated by the freezer.

Contamination can occur as a result of improper cleaning, debris such as surface coatings, or particles created by wear. Even if the contaminant is not harmful or toxic, its presence can render the product unsalable.

Product contamination can be minimized by sound freezer design in which wear debris cannot be generated in a location where it can get into the product as it is being conveyed through the freezer. Typical sources of contamination are wear debris created between the conveyor belt and the wear strip supporting the belt, dripping of condensed water at entries and discharges from freezers, flaking coatings, and leaking fluids such as hydraulic oil.

Product handling within the freezer can physically damage the product. Symptoms of products that have been damaged within the freezer include clumping together of pieces intended to be separate and free-flowing (IQF), pieces of the product torn from the conveying device as a result of the product being frozen to the conveyor, collision damage as a result of the product itself, and buildup of ice on the product.

Clumping together of product is generally a result of improper handling by the freezer as a result of poor design or improper operation. It is important that relative motion between the pieces to be frozen is maintained while the surface of the product freezes. The relative motion between the particles can be achieved by fluidization, mechanical agitation, or immersion in a boiling liquid that boils at a temperature well below the product freezing point.

In order to avoid damage to a product as it is removed from the conveyor that is transporting it through the freezer, it is essential that the product be solidly frozen and that the product does not adhere to the conveyor excessively.

Excessive adhesion can be avoided by careful selection of the conveyor material or by transporting the product in a manner such that it does not rest on a solid conveyor during the period that the surface of the product is being frozen.

Collision damage is generally a result of excessive loading of product onto the conveyor, which can result in the product colliding with other products inside the freezer or colliding with stationary structures within the freezer. Such collisions frequently result in product jams, which can destroy a considerable amount of product at each occurrence. To avoid such problems even feed equipment should be used.

Ice buildup on products can result in an insightly product. This condition can occur when excessive free moisture is transported into the freezer and is transferred from unfrozen to frozen product in the early stages of freezing. This can be controlled by minimizing the free moisture going into the freezer with the product and by controlling the motion of the product once it is inside the freezer.

Hygiene in the Freezer Environment

Proper hygiene in the freezer environment must be defined by the user. Factors such as the sensitivity of the product to various degrees of contamination must be considered in setting sanitary standards. For example, if a cooked product is frozen that requires no additional cooking prior to consumption, extreme hygienic procedures and very sanitary equipment must be employed because of the high risks to the consumer. Contamination of the product can assume many forms. Typical forms of contamination that must be considered are bacterial, wear debris, pieces of a different product, and foreign debris.

Bacterial contamination is generally the result of bacterially contaminated food coming in contact with the product and attaching itself. This can occur if the conveying system has not been adequately cleaned and sanitized.

Wear debris will be created in all freezing and processing machinery. Whether or not the product is considered contaminated is a function of how obvious the debris is and what the composition is. Stainless steel rubbing on either a plastic such as polyethelyne or directly on another piece of stainless steel will generate a significant amount of dark-gray powder. If this gray powder is deposited on the product such that it is visible macroscopically, the product is considered contaminated.

Contamination can also occur if previously frozen food or any food particle is deposited onto a product that is being frozen. This can be the result of inadequate cleaning between changes in product changes on the freezer or production line. A clean design and thorough cleaning can alleviate this problem.

Automatic cleaning of freezing machinery permits cleaning with less labor and reduces the need to access difficult areas and dangerous locations while still providing adequate cleaning. Additionally, automatic cleaning reduces labor and the necessary cleaning time (see Fig. 20).

Cleaning solutions must be selected carefully to adequately clean the machinery without damaging the materials or surface coatings used on the freezer. After cleaning, a sanitizing agent is sometimes employed to sterilize the machine.

Materials for Freezing Machinery

Hygienic standards demand that all food machinery including freezers be constructed of nontoxic materials that permit use of aggressive cleaning agents such as mild caustics without corroding. Typical materials used presently are 300 series stainless steel, galvanized steel, aluminum, and various food-grade plastics.

Special considerations must be taken into account in selection of materials for specific functions in the freezer.

Surfaces that come in contact with the product must be smooth and totally noncorrosive and not adhere to the product, either frozen or thawed. Stainless steel and plastics are generally used for product contact.

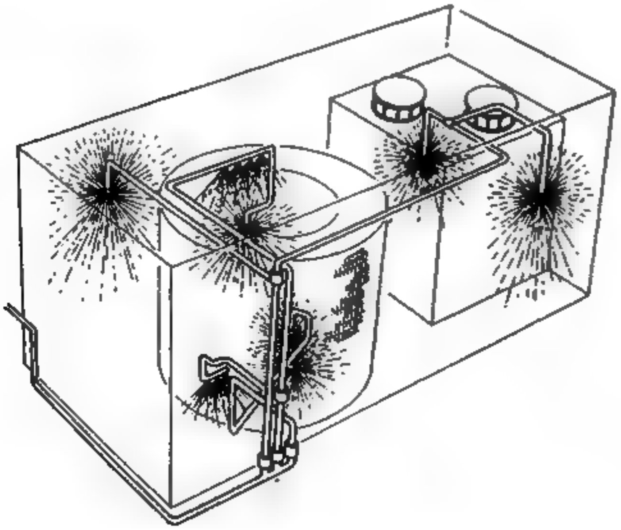


Figure 20. Cleaning in place (CIP).

Materials through which heat must pass must have a high thermal conductivity. Applications that fall under this category are heat-transfer coils, flat metal belts used on contact freezers, and plates used on horizontal and vertical plate freezers.

High-insulation properties are required in practically all freezers to separate the cold environment from the ambient air as well as to prevent the formation of condensation on the warm external walls. Generally the insulating walls are of a panel construction with either metal or fiberglass skins and a plastic low thermal conductivity core. The panel is generally bonded together to give suitable mechanical properties and also to prevent the ingress of moisture. If moisture were to enter the panel, deterioration of the panels thermal and mechanical properties could result because of the freeze-thaw cycles encountered in a typical freezer.

Lubricants selected for food freezer applications must be selected on the basis of both their low-temperature properties and their toxicity. Gearboxes in the freezer must be located so that any leakage of lubricant cannot contaminate the product. Greases and oils used in close proximity to the product or product-carrying surface must be edible as insurance against accidental contamination over the life of the freezer is impossible. Lubricants must remain viscous enough over the entire operating range they are to be exposed (even when the freezer is held at extremely low temperatures without product) to permit dependable operation of the machinery. This generally requires the use of synthetic lubricants at temperatures below 7°C. Lubricants should also have the ability to maintain their properties with considerable moisture content as the cleanup and thawing will result in significant water accumulation in the lubricant. Lubricants must generally be changed at short intervals as a result of water contamination.

Coatings used in freezers must be permanent, or contamination of the product can result. Painted coatings requiring meticulous preparation in application are frequently fragile and require routine maintenance. Metallic coatings such as galvanizing, flame spraying, and plating are generally durable but are not as durable as a component made entirely of noncorrosive material. When coating a material with zinc, consideration should be given the thickness of the coating desired as the zinc will be consumed over time depending on its thickness and the environment. Zinc may not be in contact with the food product.

Materials at freezer temperatures generally undergo large changes in their physical properties, such as brittleness and size. The property changes can result in large stress-induced distortions and breakage unless adequately accounted for.

Mechanical Efficiency

The mechanical efficiency of the freezer is a measure of how much work goes into the mechanical devices within the freezer. The mechanical devices typically consists of conveyor drives, fans, and other powered devices. It is important to realize that any energy put into the freezer either by the work performed in the freezer or by removing heat from the product must be removed by either the refrigeration system or the cryogen. Therefore, a significant multiplier must be applied to the cost of adding extra energy to the freezer environment.

The quantity, types, and power consumption of energy-consuming devices in a given freezer vary greatly between freezer types. Fans are generally the largest power-consuming component in fan-equipped freezers. Fan efficiencies are defined as the ratio of useful fan work to the actual power consumed by the fan motor and can vary from 40 to 70% depending on the selection of the fan. Conveyor drives are generally relatively small power consumers.

Coils

Coils are employed in almost all types of freezers that transfer heat by circulating cold air over the product and serve the function of transferring the heat from the air to the refrigerant. The efficiency of such a freezer is greatly influenced by the design of the coil as the design determines the difference between the air and refrigerant temperatures, which has a large bearing on the initial and operating cost of the refrigeration system. Coil efficiency is a function of materials of construction, configuration of the surface and refrigerant pipes, air velocity, and refrigerant circulation.

In addition to heat-transfer considerations, freezer coils must be designed with sanitation, corrosion, frost buildup, and speed of defrosting in mind.

In order to remain operational as long as possible, coils must be designed to accept frost without becoming plugged up prematurely. This is generally done by careful selection of the spaces between the heat-transfer surfaces or by continuous defrosting.

Continuous defrosting can be accomplished by installing multiple banks of coils and shutting off one bank and defrosting it while the others remain operational, blowing the frost from the coils with compressed air during operation, or rinsing the frost from an operating coil with a mixture of glycol and water. In such cases a glycol concentration is used to remove the water. A continuous (automatic) defrosting system is illustrated in Figure 21.

Fans

Fans for freezers should be selected to provide the highest possible economy over the life of the freezer taking into account dependability, efficiency, and initial costs.

Fans positioned such that the air flows over them before the coil are subject to large frost buildup. Frost buildup on a fan will result in imbalance, which will impose large stresses on the fan wheel and the motor. The fan must be designed to accept the resultant imbalance without failing.

Fan efficiency is determined by how the fan is selected and applied. Inlet and discharge effects can greatly alter a fan's performance. The air pressure and volume of air that pass through the fan are normally presented graphically by the fan producer. A fan must be selected to give the correct quantity of air over a fully normal operating range, which can vary significantly from the time when a freezer has been recently defrosted and lightly loaded with product to when the freezer is frosted up and heavily loaded with product.

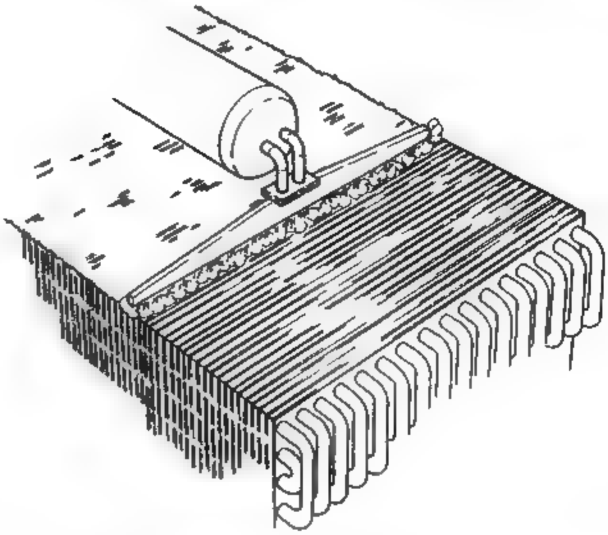


Figure 21. Automatic defrosting (ADF).

Fan motors must be selected and designed to take the mechanical stresses imposed on them as well as to power the fan wheel over a wide operating range. Fan motor bearings should be specially lubricated to run freely over a full range of operating temperatures. The bearings need to be adequate to tolerate a significant amount unbalance as discussed before.

Electrical Considerations

Dependable and safe electrical installations require special attention in a freezer environment because of the high moisture and large temperature changes that cause water to migrate and condensate on electrical wiring and components. Electrical devices and wiring need to be protected from moisture by ensuring that they always remain above the dewpoint under all operating conditions and by protecting them from water entry during defrosting and cleaning. Electrical panels external to the freezer need to be protected from the cleanup conditions found in most plants by taking special precautions in sealing and ventilation for cooling.

Airflow

In freezers employing air as the heat-transfer medium the design of the airflow has a major influence on the performance of the freezer and on the food product quality. The significant factors to consider in regard to airflow are quantity, evenness of distribution over the product, and power consumed by the fans.

The quantity of the air circulated influences the performance of the coils, the change in air temperatures through the freezer, and heat-transfer rates between the air and the product, all assuming that the areas through which the air flows remain constant. An efficient airflow is illustrated in Figure 22.

The distribution of the air over the product should be carefully controlled to give the designed freezing rate uniformly over all the product passing through the freezer.

The deterioration of the airflow through the freezer with increased coil frosting and heavy product loading needs to be taken into account when designing the air system so that the freezer maintains full performance under the full range of operating conditions.

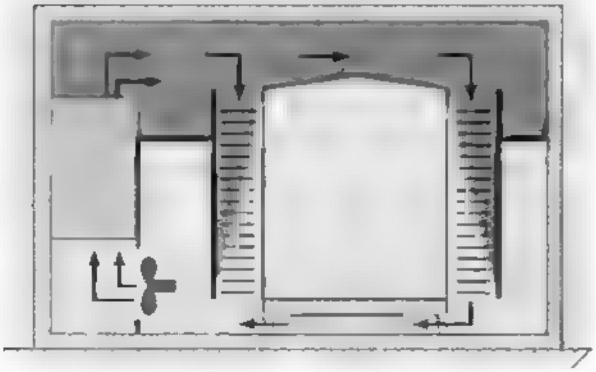


Figure 22. Example of efficient airflow in a freezer.

REFRIGERATION SYSTEMS

The Second Law of Thermodynamics states that heat can be raised from a low to a high temperature level only by expenditure of work. This means that energy inherently flows only from high to low temperatures. From the long list of refrigeration processes, only two are normally of importance for the food industry: (1) a closed mechanical refrigeration system containing a compressor, a condenser, an expansion valve, and an evaporator and (2) an open cryogenic refrigeration system using either liquid nitrogen (N_2), or carbon dioxide (CO_2).

Mechanical Refrigeration

The main elements in a mechanical refrigeration system are shown in Figure 23. The closed system is filled with a refrigerant like Freon or ammonia. Refrigerant as a gas is pulled from the evaporator (1) to the compressor (2) driven by a motor (5). The compressor discharge the gas to the condensing pressure and the gas condensates to liquid in the condenser as heat is removed from the condensor to the surrounding air or to water circulating through the condenser.

From the condenser the liquid goes through the expansion valve. The expansion valve is regulating the flow of refrigerant being evaporated in the evaporator and also keeping the pressure between high and low levels.

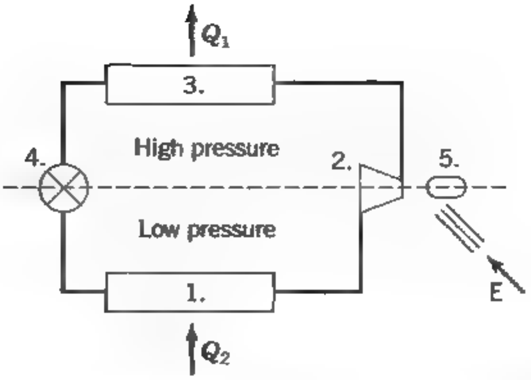


Figure 23. Mechanical refrigeration system: (1) evaporator; (2) compressor; (3) condenser; (4) expansion valve; (5) motor.

Energy picked up in the evaporator (Q_2) plus energy introduced to the compressor shaft (E) equals the energy delivered from the condensor (Q_1):

$$Q_2 + E = Q_1$$

The system described is the same as used in heat pumps. The main difference between the two systems is that in a refrigeration application the heat pickup in the evaporator is the objective, while in a heat pump the main goal is to produce heat in the condensor.

Heat recovery from refrigeration systems will be of greater importance as energy costs rice and environmental questions require an increasing amount of attention. Whenever a demand for heat exists near a refrigeration plant, one form or another of heat recovery from the refrigeration plant should be considered.

One- or Two-Stage Systems

The simple arrangement illustrated in Figure 23 is generally used for all installations where the purpose is to keep the temperature around the freezing point or higher.

For storage temperatures around −30°C and for freezing equipment it is normally economical to use a two-stage system. Such a system contains, in principle, two subsystems, as shown in Figure 23, working together. The condensing pressure for the low-stage compressor is about the same as the evaporation pressure for the high-stage compressor.

The vapor pressure (in megapascals) for refrigerants R12, R22, R134A, and R717 (ammonia, NH_3) as a function of the temperature in Celsius is shown in Table 2. Assuming that ammonia R717 is used, an evaporation temperature of −30°C corresponds to a pressure of 0.119 MPa and a condensing temperature of +35°C corresponds to a pressure of 1.36 MPa. This gives a compression ratio of 11.4 which is uneconomically high. For economical operation the ratio should not be more than 8–10; therefore, in this case a two-stage system should be used.

Table 2. Saturated Vapor Pressure of Some Refrigerants, MPa

Temperature, °C	Refrigerant[a]			
	R12	R22	R134A	R717
50	0.0391	0 0645	0.0299	0.0408
−40	0.0642	0.105	0.0516	0.0715
−30	0.100	0.164	0.0847	0.119
−20	0.152	0.245	0.133	0.190
−10	0.219	0.355	0.201	0.290
0	0.309	0.498	0.293	0.428
+10	0 424	0.681	0.415	0.613
+20	0.567	0.910	0.572	0.855
+30	0.745	1.192	0.770	1.164
+40	0.959	1.534	1.016	1.551
+50	1.217	1.943	1.318	2.028

[a] R12 = dichlorodifluoromethane, CCl_2F_2, R22 = dichlorodifluoromethane, $CHClF_2$, R134A = tetrafluoroethane, CF_3CH_2F, R717 = ammonia, NH_3.
Source: Reprinted with permission of ASHRAE from the 1990 *ASHRAE Handbook-Refrigeration*. Ref. 14.

The intermediate pressure is selected so that the two compressors will work with the same compression ratio. This means in this case that the intermediate pressure should be about 0.44 MPa, corresponding to a temperature of 0°C.

The Refrigerant

Refrigerants are the vital working fluid in a refrigeration system. They absorb heat from one area and dissipate heat into another. The design of the refrigeration equipment is influenced by the properties of the refrigerant selected.

A refrigerant must satisfy many requirements, some of which do not directly relate to its ability to transfer heat. The environmental consequences of a refrigerant that leaks from a system must also be considered. Because of their stability, fully halogenated compounds, called CFCs, persist in the atmosphere for many years and eventually diffuse into the statosphere. According to several scientists the existence of CFCs in the stratosphere has a detrimental effect on the ozone layer. The ozone layer protects the earth from too much ultraviolet radiation. A depletion of ozone might mean a dangerous increase of uv radiation on earth. This fact, coupled with the CFC contribution to the greenhouse effect, has led most nations to urge the industry to minimize the leakage of CFCs into the atmosphere and gradually to phase out their use.

Several of the large chemical companies are at present heavily engaged in finding good substitutes for the CFCs. One such substitute is refrigerant 134a, which is listed in Table 2.

The present available substitutes do not have the same properties as, for example, R12. The users therefore have to accept the fact that a plant designed for the use of R12 will have some limitations when operated with, eg, R134a, and in most cases modifications are necessary.

Most likely the use of R717 (ammonia) will be more and more frequent, particularly for large industrial applications.

For commercial installations such as those in department stores most likely we will see a central refrigeration plant using ammonia chilling brine being pump-circulated to the various locations where refrigeration is needed. By using a brine the risk of ammonia gas leaking and coming in contact with the public will be minimized.

Compressors

Piston compressors of various sizes dominated in the refrigeration industry until about 25 years ago, when screw compressors were first used also for refrigeration applications.

Today screw compressors are commonly used for industrial applications, while for small systems such as refrigerators and home freezers the use of hermetic piston compressors is still widespread.

Large compressors are often equipped with a capacity-control mechanism, which makes it possible to maintain constant evaporation temperatures even when the demand varies. It should, however, be noted that the efficiency generally decreases when the compressor works with reduced capacity.

The efficiency of refrigeration systems is influenced by many factors other than the compressor. Only when all details are well designed and tuned together can an optimal result be achieved.

Engine Room as Package Unit or Site-Built

In the early days of the refrigeration industry all engine rooms were site-built. Today, however, it is more common for the refrigeration plant to be delivered as a package unit (see Fig. 24). This offers the benefit of quick deliveries with very little work on-site. If the package unit is designed by an experienced supplier, it also means less risk for functional problems during startup. Finally it offers a greater flexibility than a site-built installation, which tends to be more important as the industry must accommodate changing demands.

Economical Operation Conditions

Two basic facts are important for anyone responsible for the operation of an industrial refrigeration plant.

Out of an existing refrigeration installation more refrigeration capacity is achieved if the evaporation temperature is elevated. This is clearly illustrated in Figure 25. Therefore, it is important to make sure that the evaporator surfaces are kept as clean as possible from ice and that good air circulation is obtained over the whole surface. It is also important that the refrigerant be in good contact with every part of the evaporator.

The condition of the condensor is also of importance for a good economy. As can be seen from Figure 26 the electrical power consumption goes down with lowering the condensing temperature. For efficient operation the evaporation temperature should be kept high and the condensing temperature low.

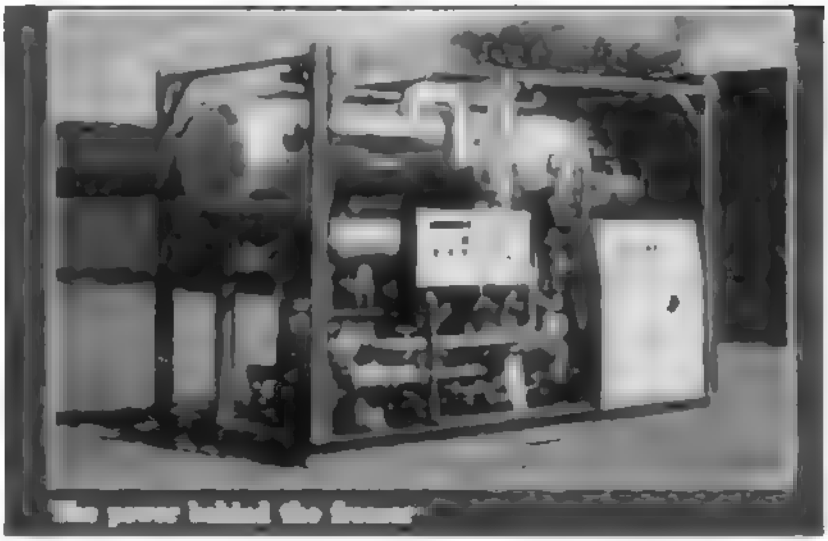


Figure 24. Package refrigeration unit. Refrigeration capacity = 400 kW at −40°C evaporation temperature and 35°C condensation temperature. Length = 12 m; width = 2.1 m; height = 3 m.

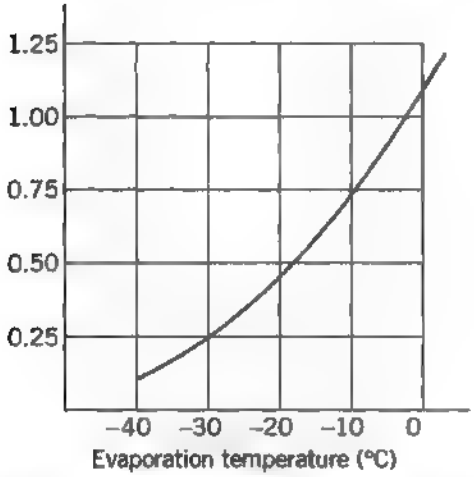


Figure 25. Refrigeration capacity as a function of evaporation temperature.

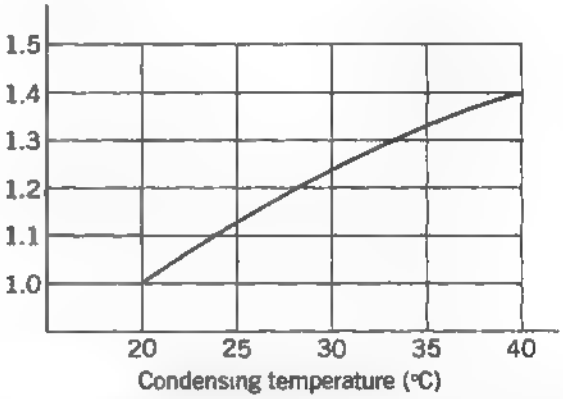


Figure 26. Electric power consumption as function of condensing temperature. Constant condensing temperature.

With the help of an automatic process control system it is possible to improve the efficiency of a refrigeration installation, including the operation of the freezing equipment.

Figure 27 illustrates a typical example of how a process line is shown on a data screen being part of an automatic process control system.

Cryogenic Refrigeration Systems

The second refrigeration process of importance for the food industry is the cryogenic system. In contrast to the mechanical refrigeration system described above, the cryogenic system is open, which means that the refrigerant is consumed and not recirculated. Liquid nitrogen or liquid or solid carbon dioxide (CO_2) are normally used as refrigerants.

In a cryogenic system the refrigeration machinery for production of LIN or liquid CO_2 are large industrial units normally located at a distance remote from the food processing factory. With help of special transport equipment the cryogenics are transported from the place of manufacturing to storage tanks at the food factories.

From an economic viewpoint the principal difference between a mechanical refrigeration system and a cryogenic system is that for the former the investment cost for the food producer is fairly high while the running cost is low. The opposite goes for cryogenic systems. This fact is illustrated in Figure 28.

THE ECONOMICS OF FREEZING

The freezer is usually the largest investment in a processing line. The freezer's impact on operating costs as well

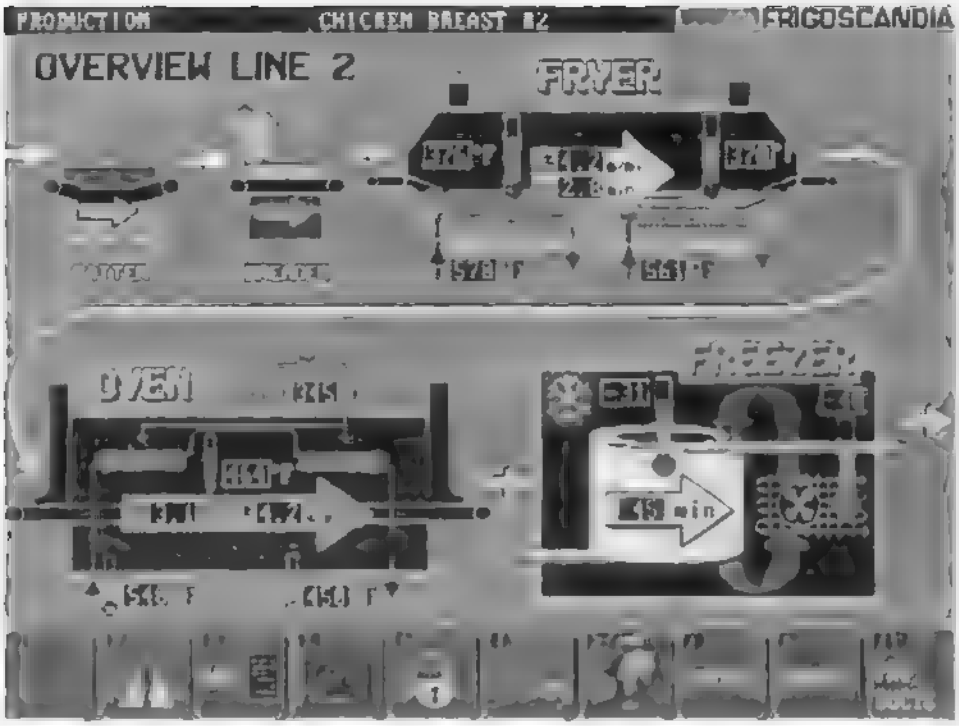


Figure 27. Screen illustration of part of a processing line being controlled automatically.

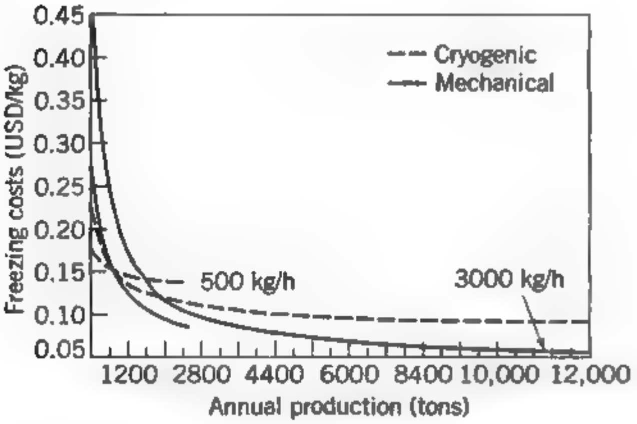


Figure 28. Freezing cost as function of utilization per year for mechanical freezing and cryogenic freezing. Constant evaporating temperature.

as on the final product quality and cost is similarly high. For the investor, it is essential to ensure that the design of the freezing system allows it to be properly integrated into the process and consistently operated at an optimal level.

Reliability

The value of the food products that pass through a freezer in a few weeks' time is often much higher than the investment cost of the freezer itself. This makes reliability a crucial consideration for the food processor.

Not only is the time the freezer is in operation important, but also the amount of time it is working to full capacity. A breakdown of any part of the line stops the whole line, and product that moves through a line without freezing properly may loose much or most of its market value.

With some systems, product blows out of the freezing zone, lodging in and freezing to other parts of the equipment. This can jam up the line and eventually cause damage.

Dehydration

Dehydration losses will always be present in any freezing system. The evaporation of water vapor from unpacked products during freezing becomes evident as frost builds up on evaporation surfaces. This frost is also caused by excessive infiltration of warm, moist air into the freezer. Still air inside the diffusion-tight carton often creates larger dehydration losses than does the unpackaged products frozen in an IQF freezer. Heat transfer is poor because no air circulation occurs within the package. The result is an evaporation of moisture that can be significant; however, the frost may stay inside the carton.

A poorly designed freezing tunnel may operate with dehydration losses of 3–4%, while a well-designed tunnel can be built to operate with losses of 0.5–1.5%. Liquid nitrogen tunnels normally operate with a dehydration loss of about 0.2–1.25%. This loss occurs when the nitrogen gas is circulated over the product at the infeed end of the freezer.

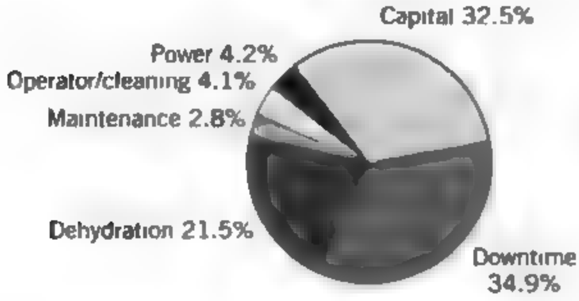
Freezing Cost Comparison

The cost of a freezing system comes both from the purchase price and the true cost of operation over the lifetime of the system. High operating costs can offset the advantages of a low initial purchase price. Consequently, it is essential to consider all the factors before making an investment, ie, to consider a freezer's "all-in economy."

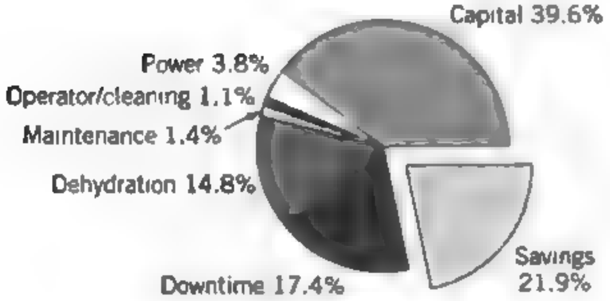
In making preinvestment analyses, a comparison should be made of all pertinent factors involved, eg, capital, power, operation, cleaning, and maintenance, dehydration, and downtime costs. Figure 29 shows the relative costs when the real-life data from a GYRoCOMPACT were compared with the data from a conventional spiral freezer.

The graphs in this cost study (based on freezing of 1500 kg/h of hamburgers) clearly illustrates that the real cost of a freezer is not merely a question of the initial capital outlay. All the other factors involved in the economics of freezing contribute to the all-in cost picture. Figure 30 shows the effect of an efficient operation over a 5-yr period.

With all the crucial factors taken into account, the somewhat higher purchase price of one freezer was more than offset by the unique module design concept of the same freezer, including a particular belt design indicating major overall cost savings. The extra investment has a payback time of about 1 yr. Thereafter, the continued cost savings means profit for the food processor.



Conventional spiral freezer



GYRoCOMPACT®

Figure 29. Relative freezing cost.

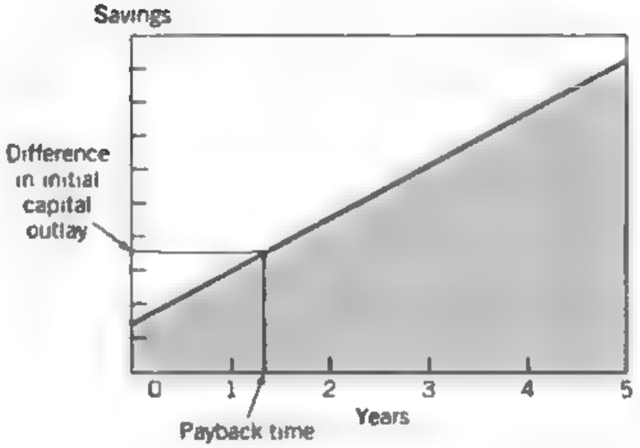


Figure 30. Accumulated annual savings.

Flexibility and Upgradability

With the competitiveness of today's consumer market, timing is of decisive importance. A system that is easy to modify will make it possible for the processor to respond quickly to the requirements of a new market and to expand or relocate the system easily. A freezing system built as a permanent fixture that cannot be readily expanded, easily removed, or conveniently traded in or resold could cost a great deal more at a later date as needs change.

Maintenance and Service Support

Freezers create an extremely harsh environment for mechanical parts. Optimum performance from even the most efficient freezer requires proper maintenance. Attention must be paid to how complicated and time-consuming such maintenance will be, and what effect this will have on valuable processing time.

Another important factor in the economics of freezing is backup support and a wide range of related services. In judging a freezer supplier's support capability, consideration should be given to the level of service offered in the planning and installation stages as well as after the deal is complete.

The processor will also need to consider where the supplier's service centers are located, and what commitment of service resources they are prepared to make. This will determine how quickly a service technician can respond in an emergency.

BIBLIOGRAPHY

1. R. Thévenot, *A History of Refrigeration throughout the World*, International Institute of Refrigeration, Paris, 1979.
2. W. R. Woolrich, *The Men Who Created Cold*, Exposition Press, New York, 1967.

3. O. E. Anderson, Jr., *Refrigeration in America*, Princeton University Press, Princeton, N.J., 1953.
4. M Jul, *The Quality of Frozen Foods*, Academic Press, Orlando, Fla., 1984
5. F Lindelöv, "Chemical Reactions in Frozen Cured Meat Products. TTT—Examination of Cured and Uncured Pork Bellies," in *Proceedings of Commissions C1 and C2 Meeting* (Karlsruhe, W. Germany, International Institute of Refrigeration, Paris, 1977.
6. S Åström and G Löndahl, "Air Blast In-line Freezing versus Ultra Rapid Freezing. A Comparison of Freezing Results with Some Various Vegetables and Prepared Foods," in *Proceedings of Commissions 4 and 5 Meeting* (Budapest), International Institute of Refrigeration, Paris 1969
7. G. Löndahl and T. Nilsson, "Microbiological Aspects of Freezing of Foods," *International Journal of Refrigeration* 1, 1978.
8. S. Åström, "Freezing Equipment Influence on Weight Losses," in *SOS / 70 Proceedings, 3rd International Congress of Food Science and Technology* (Washington, D.C., 1970), Institute of Food Technologists, Chicago, 1971.
9. G. Lundgren and K. Nilsson, "Vattenförluster vid infrysning av livsmedel" ("Water Loss during Freezing of Foods"), *Livsmedelsteknik*, No. 9, 1969 (Sweden).
10. *Recommendations for the Processing and Handling of Frozen Foods*, International Institute of Refrigeration, Paris, 1986.
11. American Society of Heating, Refrigeration and Air-Conditioning Engineers, *ASHRAE Handbook—Fundamentals*, 1989.
12. S. D. Holdsworth, CFDRA Technical Memorandum No. 452, Campden Food & Drink Research Association, Chipping Camden, UK, 1979.
13. S. L. Polley, O. P. Snyder, and P. Kotnour, "A Compilatision of Thermal Properties of Foods," *Food Technology* (Nov. 1980).
14. American Society of Heating, Refrigeration and Air-Conditioning Engineers, *ASHRAE Handbook—Refrigeration*, 1990.

JIM HEBER
GORAN LÖNDAHL
PER OSKAR PERSSON
LEIF RYNNEL
Helsingborg, Sweden

FRESH-CUT FRUITS AND VEGETABLES: MODIFIED ATMOSPHERE PACKAGING

Fresh and fresh-cut fruits and vegetables are living tissue that undergoes catabolic metabolism including respiration (1). Unlike their fresh, intact counterparts, fresh-cut or "minimally processed" fruits and vegetables are subjected to specific processing and preservation steps. Fresh-cut fruits and vegetables are prepared by one or more unit operations such as peeling, slicing, shredding, juicing, and so on. They also are subjected to a partial but not end-point preservation treatment that may include use of minimal heat, antioxidants and other preservatives, radiation, pH control, chlorinated water dips, or a combination of these treatments (2). The initial preparation and preservation treatments are usually followed by a controlled/modified atmosphere, vacuum-packaging step. The packaged product should be maintained at a reduced temperature above the freezing point (~1°C) throughout storage, distribution, and marketing up until the time of preparation for consumption.

Fresh-cut produce is a perishable food that is included in Brody's (3) minimally processed/reduced-oxygen-packaged/chilled-distribution category of commercial foods. A major advantage is that it offers freshlike, ready-to-eat convenience and quality for both institutional and retail markets.

The composition of the earth's atmosphere (commonly called air), exclusive of water vapor and trace contaminants, is 78.08% nitrogen, 20.94% oxygen, 0.93% argon, 0.34% carbon dioxide, and trace amounts of several other noble gases including neon and helium (4). In nature all fresh, unpackaged food not immersed in water is surrounded, and to some extent permeated, by air. Chemical interactions routinely occur between fresh, unpackaged food and the air's oxygen and carbon dioxide, which are highly reactive gases. These reactions result in significant short- and long-term changes in the chemical and physical characteristics of food that can have positive or negative impacts on a food's utility, quality, storability, and nutrient status.

Fresh-cut produce items in the marketplace include peeled, sliced carrot sticks; sliced/diced onions; celery sticks; peeled, sliced/diced Irish potatoes; melon balls; peeled, cored/sliced/diced pineapple; apple slices; cole slaw; sliced tomatoes; destemmed grapes; decapped, sliced strawberries; shredded lettuce; mixed salad greens; and even prepared Waldorf salad with sliced apples, raisins, diced celery, and walnut pieces.

PARTIAL PROCESSING OF FRESH PRODUCE

Postharvest processing operations on fresh produce are essentially limited to those that take place prior to blanching. Thus, enzyme systems remain functional, and abundant microflora are present on minimally processed produce at the time it is packaged. Food processing operations and treatments applied to fresh-cut produce include chilling, washing, peeling, coring, slicing/cutting, acidified chlorine solution immersion, applications of antioxidants/firming agents/antimicrobials, and dewatering via centrifugation. Obviously many of these operations cause stress or damage to the plant tissue, shortening its shelf life. Furthermore, despite the sanitizing treatments carried out during fresh-cut processing, surviving psychotrophic microorganisms can thrive at damaged tissue sites even under refrigeration.

THE RATIONALE FOR MODIFIED ATMOSPHERE PACKAGING OF FRESH-CUT PRODUCE

Damaged plant tissue usually demonstrates marked increases in aerobic respiration and catabolic metabolism and is accompanied by increased moisture loss and a decline in sensory and nutritional quality. Two primary means that are used to delay postprocessing quality degradation are refrigerated storage/distribution and alteration of the atmosphere surrounding the fresh-cut produce. Modified atmosphere packaging (MAP) of fresh-cut pro-

3. O. E. Anderson, Jr., *Refrigeration in America*, Princeton University Press, Princeton, N.J., 1953.
4. M Jul, *The Quality of Frozen Foods*, Academic Press, Orlando, Fla., 1984.
5. F Lindelöv, "Chemical Reactions in Frozen Cured Meat Products. TTT—Examination of Cured and Uncured Pork Bellies," in *Proceedings of Commissions C1 and C2 Meeting* (Karlsruhe, W. Germany, International Institute of Refrigeration, Paris, 1977.
6. S Åström and G Löndahl, "Air Blast In-line Freezing versus Ultra Rapid Freezing. A Comparison of Freezing Results with Some Various Vegetables and Prepared Foods," in *Proceedings of Commissions 4 and 5 Meeting* (Budapest), International Institute of Refrigeration, Paris 1969
7. G. Löndahl and T. Nilsson, "Microbiological Aspects of Freezing of Foods," *International Journal of Refrigeration* **1**, 1978.
8. S. Åström, "Freezing Equipment Influence on Weight Losses," in *SOS / 70 Proceedings, 3rd International Congress of Food Science and Technology* (Washington, D.C., 1970), Institute of Food Technologists, Chicago, 1971.
9. G. Lundgren and K. Nilsson, "Vattenförluster vid infrysning av livsmedel" ("Water Loss during Freezing of Foods"), *Livsmedelsteknik*, No. 9, 1969 (Sweden).
10. *Recommendations for the Processing and Handling of Frozen Foods*, International Institute of Refrigeration, Paris, 1986.
11. American Society of Heating, Refrigeration and Air-Conditioning Engineers, *ASHRAE Handbook—Fundamentals*, 1989.
12. S. D. Holdsworth, CFDRA Technical Memorandum No. 452, Campden Food & Drink Research Association, Chipping Camden, UK, 1979.
13. S. L. Polley, O. P. Snyder, and P. Kotnour, "A Compilatision of Thermal Properties of Foods," *Food Technology* (Nov. 1980).
14. American Society of Heating, Refrigeration and Air-Conditioning Engineers, *ASHRAE Handbook—Refrigeration*, 1990.

Jim Heber
Goran Löndahl
Per Oskar Persson
Leif Rynnel
Helsingborg, Sweden

FRESH-CUT FRUITS AND VEGETABLES: MODIFIED ATMOSPHERE PACKAGING

Fresh and fresh-cut fruits and vegetables are living tissue that undergoes catabolic metabolism including respiration (1). Unlike their fresh, intact counterparts, fresh-cut or "minimally processed" fruits and vegetables are subjected to specific processing and preservation steps. Fresh-cut fruits and vegetables are prepared by one or more unit operations such as peeling, slicing, shredding, juicing, and so on. They also are subjected to a partial but not end-point preservation treatment that may include use of minimal heat, antioxidants and other preservatives, radiation, pH control, chlorinated water dips, or a combination of these treatments (2). The initial preparation and preservation treatments are usually followed by a controlled/modified atmosphere, vacuum-packaging step. The packaged product should be maintained at a reduced temperature above the freezing point (~1°C) throughout storage, distribution, and marketing up until the time of preparation for consumption.

Fresh-cut produce is a perishable food that is included in Brody's (3) minimally processed/reduced-oxygen-packaged/chilled-distribution category of commercial foods. A major advantage is that it offers freshlike, ready-to-eat convenience and quality for both institutional and retail markets.

The composition of the earth's atmosphere (commonly called air), exclusive of water vapor and trace contaminants, is 78.08% nitrogen, 20.94% oxygen, 0.93% argon, 0.34% carbon dioxide, and trace amounts of several other noble gases including neon and helium (4). In nature all fresh, unpackaged food not immersed in water is surrounded, and to some extent permeated, by air. Chemical interactions routinely occur between fresh, unpackaged food and the air's oxygen and carbon dioxide, which are highly reactive gases. These reactions result in significant short- and long-term changes in the chemical and physical characteristics of food that can have positive or negative impacts on a food's utility, quality, storability, and nutrient status.

Fresh-cut produce items in the marketplace include peeled, sliced carrot sticks; sliced/diced onions; celery sticks; peeled, sliced/diced Irish potatoes; melon balls; peeled, cored/sliced/diced pineapple; apple slices; cole slaw; sliced tomatoes; destemmed grapes; decapped, sliced strawberries; shredded lettuce; mixed salad greens; and even prepared Waldorf salad with sliced apples, raisins, diced celery, and walnut pieces.

PARTIAL PROCESSING OF FRESH PRODUCE

Postharvest processing operations on fresh produce are essentially limited to those that take place prior to blanching. Thus, enzyme systems remain functional, and abundant microflora are present on minimally processed produce at the time it is packaged. Food processing operations and treatments applied to fresh-cut produce include chilling, washing, peeling, coring, slicing/cutting, acidified chlorine solution immersion, applications of antioxidants/firming agents/antimicrobials, and dewatering via centrifugation. Obviously many of these operations cause stress or damage to the plant tissue, shortening its shelf life. Furthermore, despite the sanitizing treatments carried out during fresh-cut processing, surviving psychotrophic microorganisms can thrive at damaged tissue sites even under refrigeration.

THE RATIONALE FOR MODIFIED ATMOSPHERE PACKAGING OF FRESH-CUT PRODUCE

Damaged plant tissue usually demonstrates marked increases in aerobic respiration and catabolic metabolism and is accompanied by increased moisture loss and a decline in sensory and nutritional quality. Two primary means that are used to delay postprocessing quality degradation are refrigerated storage/distribution and alteration of the atmosphere surrounding the fresh-cut produce. Modified atmosphere packaging (MAP) of fresh-cut produce results in a reduced oxygen concentration and an increased carbon dioxide concentration surrounding the product.

Living plant tissue takes up oxygen and liberates carbon dioxide during aerobic respiration. When this tissue is hermetically sealed inside a package that is selectively permeable to oxygen and carbon dioxide, the initial rate of oxygen uptake by the tissue exceeds the rate at which oxygen permeates by diffusion into the package from the surrounding air. When the oxygen concentration inside the package falls to around 12%, the respiration rate of the tissue begins to slow (5). At an 8% oxygen concentration, metabolic activity such as ripening of fruit tissue declines significantly. Aerobic respiration, and the rate of oxygen uptake by the tissue, reach a minimum when the in-package oxygen concentration attains a 1 to 5% level. If the package material is appropriately permeable, the minimum oxygen concentration is maintained at a steady state; that is, the rate of oxygen uptake by the tissue equals the rate of oxygen permeation into the package from surrounding air. At this minimum respiration rate, tissue quality loss due to catabolic metabolism is minimized and the onset of senescence is delayed.

Since the plant tissue also liberates carbon dioxide during respiration, the carbon dioxide concentration inside the package rises simultaneously as the oxygen level declines. Therefore, the MAP material must be sufficiently permeable to carbon dioxide to prevent excessively high levels from accumulating inside the package, which would injure the plant tissue. After the package is sealed, the interior relative humidity rapidly increases to nearly 100%. The package material is a good barrier to water vapor, so the rate of moisture vapor transmission through the package is much less than the rate at which moisture vapor accumulates inside the package via evapotranspiration from the plant tissue. Thus, shortly after the produce is sealed inside the package the moisture vapor pressure deficit between the tissue and the in-package atmosphere attains zero and further tissue moisture loss is arrested.

PROBLEMS AND CONCERNS ASSOCIATED WITH MAP OF FRESH-CUT PRODUCE

Considerable care must be taken in designing MAP for fresh-cut produce. Polymeric packaging materials having appropriate permeabilities for oxygen and carbon dioxide must be carefully selected to prevent the development of an anaerobic atmosphere within the package after filling and sealing. When the in-package atmospheric oxygen concentration declines to a low level (usually somewhere between <1 and 3%), the tissue aerobic respiration rate reaches a minimum termed the extinction point. If the oxygen level falls below the extinction point, anoxia ensues and the tissue begins anaerobic respiration, which is fermentative in nature. This event triggers the rapid destruction of the tissue's like-fresh sensory quality, generating off-flavors and negatively impacting texture and appearance; in short, the product becomes unmarketable.

The optimum atmosphere within the modified atmosphere (MA) package, with respect to oxygen and carbon dioxide concentration, depends on the variety of produce, the extent of tissue damage sustained during processing, and the product temperature. Maximum quality retention is achieved when product temperature is maintained either just above its freezing point or, for some items, just above the chill-injury temperature. Lowering produce temperature reduces respiration by a factor of two- to threefold for each 10°C decline (Q10 = 2–3), and use of an appropriate MA package can result in an additional fourfold reduction in respiration rate (6).

When the temperature is sufficiently cool and an appropriate MA package results in an in-package oxygen concentration slightly above the extinction point, aerobic microbial growth also is slowed, further retarding tissue decay. However, if the package is temperature abused or improperly designed or filled, causing the oxygen concentration to fall below the extinction point, the anoxic atmosphere can encourage the growth of anaerobic and/or facultative anaerobic, psychotrophic microorganisms (2), some of which are pathogenic. This potential for pathogenic microorganisms to develop within MA-packaged fresh-cut products is of considerable concern to food science professionals and regulatory authorities.

In many cases the steady-state (equilibrium) oxygen content in fresh-cut MA packages is 2 to 5%, and there is a potential that carbon dioxide will increase to as high as 16 to 19% (7). Maximum tolerable carbon dioxide concentrations for many fresh-cut or intact items, including cauliflower, lettuce, celery, kiwifruit, cabbage, radish, sweet pepper, banana, and carrot, are in the 2 to 5% range, and physiological damage can occur at levels in excess of 2 to 6% (5). Thus, the polymeric materials selected for use in MAP need to be four to six times more permeable to carbon dioxide than to oxygen. Sometimes it is necessary to use package parameters for fresh-cut produce that provide a compromise, with the equilibrium atmosphere somewhat higher in oxygen concentration than ideal, in order to avoid excessive accumulation of carbon dioxide. The package material selected also should have a moderate to high permeability to moisture vapor in order to minimize the chance of moisture condensation on the inside surface of the package due to minor temperature fluctuations during storage and distribution.

MODES OF MAP

There are two modes of MAP—passive and active (7). Passive MAP involves placing the plant tissue in a selectively gas-permeable polymeric package, hermetically sealing the package, and then allowing tissue aerobic respiration to reduce oxygen and increase carbon dioxide concentrations inside the package to a desired steady-state equilibrium. Active MAP involves placing the plant tissue in a selectively gas-permeable package, evacuating the package atmosphere, and replacing it by flushing the unsealed package with a specific mixture of oxygen, carbon dioxide, and nitrogen gases (8) followed by rapid sealing of the package. The flushing gas composition is usually selected to provide optimum levels of oxygen and carbon dioxide (with a balance of nitrogen) to immediately diminish aerobic respiration rate of the particular tissue system being packaged. Active MAP may also include the utilization of absorbers or adsorbers placed inside the package to scavenge oxygen, carbon dioxide, ethylene (5), and water vapor as well as the use of antimicrobial agents such as carbon monoxide. The goal of passive and active MAP design is to achieve a balance between the enclosed plant tissue respiration rate and package gas permeability to attain or maintain an acceptable equilibrium atmosphere within the package; that is, a MA that will delay ripening/senescence and thereby extend product shelf life.

SHELF-LIFE REQUIREMENTS OF MAP FRESH-CUT PRODUCTS

In the United States fresh-cut products are processed and packaged either at the source of production or at regional or local processing facilities. The postprocessing shelf life needed at each of these processing sites is 14 to 21 days, 7 days, and a short time (12–24 hours), respectively (9). The use of vacuum and gas flushing techniques in conjunction with differentially permeable packages (primarily flexible polymeric film bags) is essential for attaining a 21-day shelf life and desirable for achieving 7 days of postprocessing shelf life. Simpler packaging materials, combined with vacuum or gas flushing, are usually sufficient for attaining a short postpackaging shelf life.

SELECTION OF MAP MATERIALS

Determining the optimal atmosphere/packaging material requirements for MAP of fresh-cut produce is a complex task. Various researchers have worked to develop mathematical models to aid package material selection. Information about the various mathematical models can be obtained by referring to references provided by Zagory and Kader (7). However, use of these models to select MA package design criteria cannot be relied upon exclusively. Empirical testing of selected packaging is absolutely essential for ultimate successful use of MAP (10).

MAP of fresh-cut produce requires careful selection of polymeric packaging materials especially with regard to their permeability to oxygen, carbon dioxide, and water vapor. It is necessary that the polymeric material selected be three to six or more times more permeable to carbon dioxide than to oxygen. Polymeric materials potentially suitable for MAP fresh-cut produce include low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), medium-density polyethylene (MDPE), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), ethylene vinylacetate (EVA) copolymers, ionomers, and polybutylene (10).

SAFETY CONSIDERATIONS

The probability of a microbial foodborne disease resulting from consumption of MA-packaged fresh-cut produce is relatively low. Only a few pathogens are capable of growing at refrigerated temperatures, and normal spoilage organisms which are usually psychotrophic have a competitive advantage over most pathogens that may be present (11). Moreover, many fruits are sufficiently acidic to prevent pathogen growth. Nevertheless, foodborne disease does occur with fruits and vegetables. Some pathogens that have potential to develop in MA-packaged fresh-cut products are *Escherichia coli, Aeromonas hydrophila, Clostridium botulinum, Listeria monocytogenes, Salmonella* sp., and parasites including *Entamoeba histolytica* and *Giardia lamblia* can be present (11). Therefore, an intense commitment to sanitation and adherence to U.S. Food and Drug Administration (FDA) Good Manufacturing Practices (GMPs) as well as implementation of a Hazard Analysis Critical Control Point (HACCP) program should be a part of fresh-cut food processors quality assurance efforts (12).

Another important food safety consideration is the potential for migration of harmful/toxic chemicals from packaging materials in immediate contact with the fresh-cut produce into it. Commercial plastic packaging materials usually include nonplastic components that might migrate to contained foods. Thus, polymeric MAP materials must comply with all governmental regulations related to indirect food additives, and packaging suppliers must be able to certify such compliance (10).

BIBLIOGRAPHY

1. Institute of Food Technologists, "Quality of Fruits and Vegetables—A Scientific Summary by IFT's Expert Panel on Food Safety and Nutrition," *Food Technol.* **44**(6), 99–106 (1990).
2. R. C. Wiley, "Introduction to Minimally Processed Refrigerated Fruits and Vegetables," in R. C. Wiley, ed., *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman and Hall, New York, 1994, pp. 1–14.
3. A. L. Brody, "Minimally Processed Foods Demand Maximum Research and Education," *Food Technol.* **52**(5), 62–66, 204–206 (1998).
4. "Components of Atmospheric Air," in R. C. Weast and S. M. Selby, eds., *Handbook of Chemistry and Physics*, 48th ed., The Chemical Rubber Co., Cleveland, OH, 1967–1968, p. F-142.
5. A. A. Kader, D. Zagory, and E. L. Kerbel, "Modified Atmosphere Packaging of Fruits and Vegetables," *Crit. Rev. Food Sci. Nutr.* **28**, 1–30 (1989).
6. D. O'Beirne, "Modified Atmosphere Packaging of Fruits and Vegetables," in T. R. Gormely, ed., *Chilled Foods the State of the Art*, Elsevier Applied Science, New York, 1990, pp. 183–199.
7. D. Zagory and A. A. Kader, "Modified Atmosphere Packaging of Fresh Produce," *Food Technol.* **42**(9), 70–77 (1988).
8. J. P. Smith, H. S. Ramaswamy, and B. K. Simpson, "Developments in Food Packaging Technology, Part II: Storage Aspects," *Trends Food Sci. Technol.* **1**(5), 111–118 (1990).
9. M. Cantwell, "Postharvest Handling Systems: Minimally Processed Fruits and Vegetables," in A. A. Kader, ed., *Postharvest Technology of Horticultural Crops*, 2nd ed., University of California Division of Agriculture and Natural Resources, Oakland, Calif. 1992, pp. 277–281.
10. D. V. Schlimme and M. L. Rooney, "Packaging of Minimally Processed Fruits and Vegetables," in R. C. Wiley, ed., *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman and Hall, New York, 1994, pp. 135–182.

11. R. E. Brackett, "Microbiological Spoilage and Pathogens in Minimally Processed Refrigerated Fruits and Vegetables," in R. C. Wiley, ed., *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman and Hall, New York, 1994, pp. 269–312.
12. D. M. Dignan, "Regulatory Issues Associated with Minimally Processed Refrigerated Foods," in R. C. Wiley, ed., *Minimally Processed Refrigerated Fruits and Vegetables*, Chapman and Hall, New York, 1994, pp. 327–353.

DONALD SCHLIMME
MARK KANTOR
University of Maryland
College Park, Maryland

See also CONTROLLED ATMOSPHERES FOR FRESH FRUITS AND VEGETABLES.

FRUIT DEHYDRATION

Dehydration, or drying, is a preservation process that involves the reversible removal of water from the fruit tissues, thereby extending the storage life because microorganisms and the native enzymes of fruits are deprived of the moisture necessary for their activity. However, it is not a sterilizing process, and means must be provided to preserve the low-moisture equilibrium and prevent the fruit tissue from regaining moisture until such time as deliberate reconstitution is desired. Although preservation is usually the principal reason for dehydration, other considerations are often important; for example, significant reductions in the weight and bulk of fruits for economical transport and storage.

Drying fruits is one of the oldest techniques of food preservation; Persians, Greeks, and Egyptians have used it since ancient times. Sun drying was the method applied in early times, and it still accounts for a significant part of the dried fruit consumed in the world today. However, since the latter part of the nineteenth century mechanically dehydrated fruits are produced in increasingly larger quantities.

Today the food dehydration industry is large and extends to all countries throughout the world. The following countries are highly important dried fruit producers: Australia, Argentina, Chile, France, Greece, Portugal, Spain, South Africa, Turkey, and the United States (Table 1). In the United States more than 90% of the dried fruit output is produced in California, where apples, apricots, dates, figs, peaches, pears, prunes, and raisins represent the significant volume of dried fruits; raisins account for over 61% of the total volume (Table 2). Dried fruits, particularly apples, are also produced in Washington and Oregon. Consumption of dried fruits in the United States has remained almost constant during the past 20 years. Annual per capita consumption was 2.47 lb (1121 g) in 1977 and 2.94 lb (1335 g) in 1997 (Fig. 1). The small increase in consumption is largely attributed to the higher use of raisins. Recently the development of an increasing variety of convenience foods, such as instant beverages, breakfast cereal, healthy fruit snacks, and so on, often depend on the availability of high-quality dehydrated fruits. Development of new forms of dried fruit and successful preparation of intermediate-moisture products have led to products that are stable to store and pleasant to eat directly.

Table 1. Major Dried Fruit Producing Countries

Dried Fruit	Principal Countries of Origin
Apples	Chile, Italy, Spain, U.S.
Apricots	South Africa, Turkey, U.S.
Cranberries	U.S.
Currants	Australia, Greece, U.S.
Dates	Egypt, Iran, Iraq, Saudi Arabia, U.S.
Figs	Greece, Turkey, U.S.
Peaches	Australia, Chile, South Africa, U.S.
Pears	Australia, Chile, South Africa, U.S.
Prunes	Argentina, Chile, Croatia, South Africa, U.S.
Raisins	Afganistan, South Africa, U.S.

PRINCIPLES

Dehydration involves simultaneous heat transfer and moisture diffusion (mass transfer). The conversion of liquid (or solid in the case of freeze drying) to vapor demands the supply of latent heat to the product; this can be achieved by a variety of methods: conduction by contact with a heated metal plate, convection from heated air, radiation from an infrared source, or microwave energy. The process may be accelerated by the application of vacuum.

Dehydration Terms

Dried is the term applied to all dried products, regardless of the method of drying. Dehydration refers to the use of mechanical equipment and artificial methods under carefully controlled conditions of temperature, humidity, and airflow. Although the term dehydrated does not refer to any specific moisture content in the finished product, it is usually considered implying virtually complete water removal to a range of 1 to 5% moisture. Products with such low water content can be stored in a moisture-proof package at room temperature for periods well in excess of two years with no detectable change in quality.

Evaporation refers to the use of the sun or forced-air dryers to evaporate moisture from fruit to a fairly stable product. The moisture level of evaporated fruit is approximately 15 to 25%. In general, sun drying will not lower the moisture content of fruit below 15%. The shelf life of such fruit products does not exceed one year, unless they are held in cold storage. For the extended shelf life of most evaporated fruits, sulfur treatment (except for prunes, unbleached raisins, and dates) or the use of a chemical mold inhibitor is necessary.

Vacuum drying is a method of drying in a vacuum chamber under reduced atmospheric pressure to remove water from the fruit at less than the boiling point of water under ambient conditions.

Freeze drying is a method of drying in which the fruit is frozen and then dried by sublimation in a vacuum chamber under high vacuum (an absolute pressure of less than

Table 2. Production Volume and Value of Dried Fruits in California Five Year Averages, 1992–1996

Commodity	Drying ratio: fresh to dry fruit[a]	Average annual production (tons)	Average farm value (dollars)
Apples	7–10 to 1	2,720	3,168,800
Apricots	5.5–8.5 to 1	2,200	4,840,00
Dates		19,900	19,900,000
Figs	3 to 1	14,350	13,919,00
Peaches, Freestone	6–8.75 to 1	1,420	1,377,400
Pears, Bartlett	6–7 to 1	970	834,000
Prunes	2.75–3.25 to 1	174,730	167,740,800
Raisins	4–5 to 1	342,750	332,467,500
Total		*559,040*	*544,247,500*

[a] Pounds of fresh fruit required to yield one pound of dehydrated product.
Source: Dried Fruit Association of California, Sacramento, California.

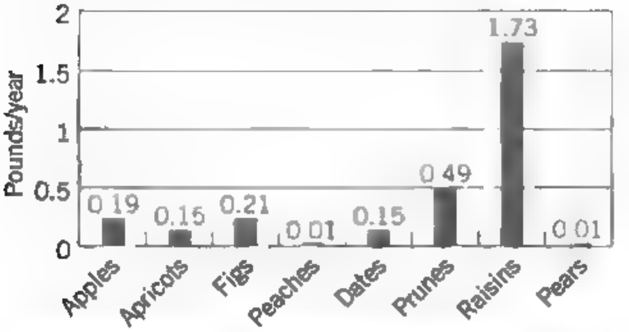


Figure 1. Per capita consumption of dried fruits in the United States, 1997.

4.6 mmHg, the vapor pressure of the ice and water at 0°C, is necessary) to around 2% moisture.

Intermediate-moisture fruits (IMF) are dried on the basis of water activity level rather than on the percentage of moisture. Restraint of the water molecules to a degree that prohibits spoilage by microorganisms occurs at different moisture contents depending on the amount and nature of the dissolved material present and to some degree on the insoluble components. Food may be classified as an IMF if it has water activity level greater than a common low-moisture fruit (0.2) and less than that of most fresh fruit (0.85). In practice, most IMF has a water activity in the range of 0.65 to 0.85 and contains 15 to 30% moisture.

Water activity (a_w) is defined as the ratio of the water vapor pressure in equilibrium with a food to the vapor pressure of water at the same temperature. Bacteria will not grow if a_w is below 0.9, and yeast and molds, are inhibited below a_w of 0.7.

Dehydrofreezing is a hybrid process that combines the best features of both drying and freezing. The process consists of drying fruit to about 50% of its original weight, then this intermediate material is quick-frozen by standard freezing techniques. The quality of dehydrofrozen fruit is equal to that of frozen products, as the drying process is discontinued at a stage where quality impairment usually does not occur. The advantages of the process are a 50% reduction in storage and freight charges and even greater savings in packaging costs in comparison with traditional frozen products.

DRYING METHODS

Quality requirements, raw material characteristics, and economic factors determine selection of a drying method as well as the operating conditions for a given fruit. Several drying methods are commercially used, each better suited for a particular situation.

The selection of drying methods depends on the following factors:

- Physical form of raw material: whole fruit (eg, cranberry, grape, plum, etc), sliced form (eg, banana, mango, kiwi, etc), liquid, paste, slurry, pulp, thick liquid, and large versus small aggregates
- Properties of raw material: sensitive to oxidation, sensitive to temperature damage, thermoplastic residues, and so on
- Susceptibility to microbial attack
- Sugar content
- Presence of a "skin" that acts as a barrier for water removal (eg, grapes, blueberries)
- Desired product characteristics: powder, instant solubility, retention of shape, and so on
- Value of the finished product: low, medium, or high price

The following three basic types of drying process are used for fruits:

1. Sun drying and solar drying are practiced for certain fruits, such as prunes, grapes, and dates.
2. Atmospheric dehydration processes, including:
    a. stationary, or batch, processes (using kiln, tower, and cabinet dryers)
    b. continuous processes (eg, tunnel, belt trough, conveyor, and fluidized bed)
    c. spray dryers, suitable for making powders from fruit juice concentrates, and drum, or roller, dryers, useful for drying fruit juice concentrates, slurries, or pastes
3. Subatmospheric dehydration techniques, including:
    a. vacuum dehydration processes, which are useful for the processing of low-moisture fruits with high

   sugar content, such as peaches, pears, and apricots

   b. freeze drying, which ensures high flavor retention and minimal damage to product structure and nutritional value; the finished product with open structure permits fast and nearly complete rehydration

More than 200 types of dryers are available commercially to process the diverse physical forms of foods and required product characteristics. For drying fruits, conventional types of dryers are commonly used because of their simplicity of construction and operation, as well as low cost. Most products are seasonal in nature, which means that dryers are operated only over a fraction of the year. Thus, the units must have low capital costs for economic reasons.

Sun Drying

Sun drying of fruit crops is still practiced unchanged from ancient times. This method is limited to climates with hot sun and dry atmosphere, and to certain fruits such as prunes, grapes, dates, figs, apricots, and pears. These crops are processed without much technical aid by simply spreading fruits on the ground, on racks, or roofs and exposing them to the sun until dry. Because sun-dried products generally have moisture levels no lower than 15 to 20%, they have a limited shelf life. It is a slow process, unsuitable for producing high-quality products, and dust, dirt, and insects often contaminate the finished products.

Solar Drying

In recent years considerable interest has been focused on the use of solar energy because of the rapid increase of fuel costs. In this method, solar energy is used alone or may be supplemented by an auxiliary energy source. A simple method of accelerating the sun-drying rate of fruit is to paint the trays black. A solar trough application of mirrors to increase solar energy and indirect solar dryers applying solar collectors are also recommended to improve the efficiency of this process.

Kiln Drying

The natural draft from rising heated air used for drying fruit is the oldest type of dehydration equipment still in commercial use. Kiln dryers generally have two levels: gas burners on the lower floor provide heat, and the warm air rises through a slotted floor to the upper level. Food materials such as apple slices are spread out on the slotted floor in a layer about 25 cm deep and turned over periodically by fork until they reach the desired moisture, usually between 16 and 24%.

Cabinet Drying

Cabinet dryers are arranged for batch operation and are usually held at constant temperature, though humidity may decrease during the drying process. They consist of an enclosed chamber fitted with a variable heater and a fan for air stirring, together with deflectors for airflow adjustment, outlet air louvers, and adjustable inlet air louvers. Cabinet drying is a particularly useful research tool for establishing the drying characteristics of a new product, prior to a large-scale commercial run.

Tunnel Drying

The equipment is similar to a cabinet dryer except that it allows a continuous operation along a rectangular tunnel through which move tray-loaded trucks. The tunnel dryer provides rapid drying without injury to fruits and permits a uniform drying process; therefore, it is widely used in drying fruit.

Tunnel dryers are classified by the direction in which the air traverses the product. In a parallel flow unit, the fresh material encounters the driest and warmest air initially, and leaves the drier at the coldest end; in a countercurrent flow unit, the air direction is opposite to the movement of the product, so the dry product leaving the drier encounters hot dry air. Multistage dryers consisting of three, four, or five dryer stages are also used. Such systems are flexible and can achieve close to optimum drying conditions for a wide variety of products.

Conveyor Drying

Conveyor dryers are continuous processing equipment and consist of an endless belt that carries the material to be dried through a tunnel of warm circulating air. The speed of the conveyor is variable to suit both the product and the heat conditions. Furthermore, process conditions are usually controlled by designing the system in sections, thus allowing different flow rates, humidities, and temperatures to be set in each section, and by rotating the product when it moves from one section of the belt to the next.

This drying method has the advantage of essentially automatic operation, which minimizes labor requirements. The conveyor dryer is best adapted to the large-scale drying of a single commodity for the whole operating season. It is not well suited to operations in which the raw material or the drying conditions are changed frequently, because extensive hours of start-up and shutdowns make it difficult to produce satisfactory products.

Spray Drying

Spray drying involves the dispersion of liquid or slurry in a stream of heated air, followed by the collection of the dried particles after their separation from the air. The process is widely used to dehydrate fruit juices.

The general construction of a spray dryer incorporates four main features:

1. a heater and at least one fan to produce air at the required temperature and velocity;
2. an atomizer or jet to produce liquid particles of the required size;
3. a chamber in which the liquid droplets are brought into intimate contact with the hot air; and
4. a means of removing the product from the air stream.

The final product is delivered as a free-flowing powder.

Drum Drying

In drum drying, which is suitable for a wide range of liquid, slurried, and pureed products, a thin layer of product is applied to the surface of a slowly revolving heated drum. In the course of about 300 degrees of one revolution, the moisture in the product is flashed off, and the dried material is scraped off the drum by a stationary or reciprocating blade at some point on the periphery. The residence time of the product in the drier is on the order of two to few minutes.

Drum drying is an inexpensive method; however, its commercial application is limited to less heat sensitive products. Its usefulness for fruit dehydration is quite limited because the high temperature required, usually above 120°C, imparts a cooked flavor and off-color to most fruit products. Also, the high sugar content of most fruit juices makes them difficult to remove from the drum dryers because of their high thermoplasticity.

Vacuum Drying

Dehydration under vacuum has special merits for certain fruits in terms of final quality. Drying can be carried out at lower temperatures than with air drying, and heat damage is minimized. Furthermore, as drying is carried out with virtual absence of air, oxidation of the fruit is virtually eliminated.

Vacuum-drying systems have the following components: (*1*) a vacuum chamber, (*2*) a heat supply, (*3*) a vacuum-producing unit, and (*4*) a device to collect water vapor as it evaporates from the food. The vacuum shelf dryer, the simplest type of vacuum drier, is used to process a wide range of fruit products, including liquid, pastes, discrete particles, chunks, slices, and wedges. The equipment consists of a vacuum chamber containing a number of shelves arranged to supply heat to the product and to support the trays on which the product is loaded into the chamber. The shelves may be heated electrically or, more often, by circulating heated fluid through them. The vacuum chambers connect to suitable vacuum-producing equipment, located outside the vacuum chamber, which may be a vacuum pump or a steam injector.

Another essential part of a vacuum dryer that has a vacuum pump is a condenser, which collects water vapor to prevent it from entering the pump. A steam injector, which is often used to create vacuum, is an aspirator in which high-velocity steam jetting past an opening draws air and water vapor from the vacuum chamber.

Because of the high installation cost and operating cost of vacuum driers, they are used only for high-value raw materials or products requiring reduction to extremely low levels of moisture without damage.

Freeze Drying

In freeze drying (also called lyophilization) the moisture is removed from the fruit by sublimation, that is, converting ice directly into water vapor. Therefore, no transfer of liquid occurs from the center of the mass to the surface. As drying proceeds, the ice layer gradually recedes toward the center, leaving vacant spaces formerly occupied by ice crystals.

The processing involves two basic steps: (*1*) the raw fruit is first frozen in the conventional manner followed by (*2*) drying to around 2% moisture in a vacuum chamber while still frozen. The most common type of freeze-drying equipment is a batch chamber system similar to a vacuum shelf dryer but with special features to meet the needs of the freeze-drying process.

Freeze dehydration produces the highest-quality fruit products obtainable by any drying method. The porous nonshrunken structure of the freeze-dried product facilitates rapid and nearly complete rehydration when water is added. The low processing temperatures and the rapid transition minimize the extent of various degradative reactions such as nonenzymatic browning and also help to reduce the loss of flavor substances.

The industrial application of freeze drying to a wide range of fruits has been limited because of the requirements of high capital investment, high processing costs, and the need for special packaging. This process is suitable only for high-value products.

PREDRYING TREATMENTS

Preparation of raw fruit for drying is similar to that of canning and freezing. It includes washing, sorting for size and maturity, peeling, and cutting into halves, wedges, slices, cubes, nuggets, and so on. Alkali dipping is used for raisins and prunes. Blanching is used for some fruits.

Sulfur is applied to help preserve the color of dried fruits such as apples, pears, peaches, and apricots. The FDA generally recognizes several sulfite salts and SO_2 gas as safe (GRAS) for use in foods. Both enzymatic and nonenzymatic browning of fruits could be inhibited by application of sulfites. However, sulfites have been associated with severe allergy-like reactions in some asthmatics, prompting the FDA to limit sulfites to certain categories of food products. The food regulation in the United States requires that the presence of sulfites must be declared on the label when the sulfiting agents have a functional effect or are present at a detectable level, defined as 10 ppm or more.

Although sulfites are allowed in dry fruits, their use is unpopular to many consumers. Therefore, alternative treatments to retard enzymatic browning and other oxidative reactions during drying have been investigated. Probably the best-known alternative to sulfite is ascorbic acid and its isomer erythorbic acid. These compounds are added to syrups or applied by dipping the fruit in solutions containing the browning inhibitor, sometimes in combination with an organic acid such as citric acid and a calcium salt. Browning inhibitor penetration may be enhanced by vacuum infiltration treatment, which also removes air from the fruit tissues.

Current alternatives to sulfites are not equivalent to sulfites in effectiveness, cost, or functionality. Because sulfites serve multiple functions, for example, inhibiting enzymatic and nonenzymatic browning and controlling growth of microorganisms, a sulfite replacement is likely to contain several components having different and complementary functions.

POSTDRYING TREATMENTS

Treatments of finished fruits vary with the kind of fruit and intended use of the products. The addition of an anticaking agent, usually calcium stearate at less than 0.1% concentration, is necessary to prevent caking of most dehydrated fruits.

Sweating of dehydrated fruits is a treatment to equalize moisture of the batch. It is usually accomplished by keeping the dried fruit in bins or boxes. Bins are also used for secondary drying to reduce moisture levels of particulate fruits from 10–15% to 3–5%, a range at which drying rates are limited by slow diffusion of water. Temperatures of about 40°C and airflow provided by a blower fan, of about 33 m/min, suit the nearly dry product. These conditions minimize the risk of heat damage at a stage when fruit products are most susceptible to degradation.

Screening is often required to remove the unwanted size portion of the dried product. Removal of unwanted size pieces, or fines, is usually accomplished by passing the dry product over a vibrating wire cloth or perforated metal screens and collecting the fractions separately. Fines may be used in other products, or sometimes represent a loss.

The acceptable fraction passes onto the final inspection operation to remove foreign materials, discolored pieces, or other imperfections such as skin, carpel, or stem particles. Manual and visual selection of most dehydrated products is necessary and is carried out by inspectors while the product is moving along on a continuous belt. In addition to inspectors, magnetic devices are usually installed over the belt to remove ferrous metal contaminants.

PACKAGING

The shelf life of a dehydrated fruit product is influenced to a large extent by its packaging, which must protect the dehydrated product against moisture, light, air, dust, microflora, foreign odor, insects, and rodents. To avoid caking, air-conditioned and dehumidified rooms (below 30% RH) are required to package low-moisture fruits with high sugar content.

Freeze-dried fruits must be packed in inert gas to ensure storage stability. Nitrogen gas is commonly used to extend the storage stability of oxygen-sensitive products. In inert gas packaging, a headspace oxygen level of 1 to 2% is targeted.

PROCEDURES FOR SELECTED COMMODITIES

Apples

Apples are either dried immediately after harvesting or after being stored in cold or controlled-atmosphere (CA) storage until a convenient processing time occurs. The best varieties for drying are Gravenstein, Pippin, and Golden or Red Delicious apples. Two types of dried apple products are recognized under U.S. standards: (*1*) evaporated apples and (*2*) dehydrated (low-moisture) apples.

Evaporated apples, also called regular moisture or dried, are cut to desired size and dried to average, not more than 24% moisture by weight. Unsulfured apples however, must average not more than 20% moisture. Only artificial dryers such as kiln, tunnel, or continuous belt dryers are commonly used to produce dried apples. The process involves sizing, coring, and slicing the fruit pieces to 0.95 to 1.3 cm (3/8–1.5 in.) in thickness. The fruit is usually dipped into a sodium sulfite solution, then dried in a kiln, tunnel, or continuous belt dryer to approximately 16 to 25% moisture. The fruit slices during drying are exposed to the fumes of burning sulfur. Drying in the kiln requires 14 to 18 h at 65 to 74°C (150–165°F).

If a tunnel dryer is used, the air entering the tunnel is at 74°C (165°F) with a relative humidity of 35%. At the outlet, the air is at about 54°C (130°F) with a relative humidity of 35%.

Evaporated apples can be stored for short periods of time, less than three months, at ambient room temperatures in a dry atmosphere. For prolonged storage, 7°C (45°F) or less is required. Unsulfured evaporated apples require 4 to 5°C (39–40°F) cold storage. Evaporated apples will generally reconstitute with one part apples in five parts water by weight.

If the product is processed to a low-moisture state (less than 5% moisture), the dried apples are cut to the desired size, usually 0.64 and 0.85 cm (1/4 and 3/8 in.), dice. Frequently the fruit pieces are instantized by compression or perforation prior to dehydration. Dehydrated (low-moisture) apples are processed in forced-air dryers, such as the continuous belt dryer, using evaporated fruit as raw material. Some apples are vacuum dried for snack or other specialty product applications.

Only 300 ppm SO_2 is necessary to prevent browning. To prevent caking, 0.5% maximum calcium stearate may be added. Packaging is generally in fiberboard boxes with a net weight of 15 to 40 lb (6.8–18 kg), depending on the product destiny.

Approximately 100 kg of fresh unpeeled fruit will yield about 10 to 11 kg of dehydrated apples. Dehydrated apples will generally fully reconstitute with one part of apple in six parts water by weight. The maximum allowable SO_2 level in dried apple products in the United States is 1000 ppm and a maximum of 500 ppm in the European Community.

Apricots

Apricots are picked for drying from mid-June to early July when they are fully tree-ripened. The best varieties for drying are Royal, Blenheim, and Moorparks. California produces more than 90% of the U.S. crop and 10% of the world crop.

Apricots are either sun-dried or dried in tunnel driers. The fruit is halved, the pit is removed, and the fruit is placed cup up on a flat wooden tray. The filled trays are exposed to sulfur dioxide fumes for about 12 h. After sulfuring, the trays are transferred to a field, exposing the fruit to full sun. Apricots are allowed to dry in this manner for one day, then the individual trays are transferred to a shady area and stacked 3 to 4 ft high. They are allowed to dry in the stack for approximately one week, then they are removed from the trays, placed into boxes or bins, and ultimately delivered to a packing plant.

Berries

Berry flavor and color degrades with exposure to prolonged elevated temperatures. Also, the juices of both strawberries and raspberries tend to drip during heating. Therefore, conventional air drying techniques, such as continuous belt driers are not used for drying berries.

Freeze drying has been the typical method for drying strawberry and raspberry pieces. The berries are individually quick-frozen and then spread onto drying trays. The freeze-dry chamber is filled with the trays, and the shelf temperatures during the cycle may be varied to maintain an optimal product temperature.

The packaging must be a high-moisture barrier film, such as foil-paper laminate or foil polyethylene laminate, because with exposure to air freeze-dried berries absorb moisture very quickly. Cereal and confection products often contain freeze-dried berries.

Drum dryers are also used to dry strawberry and raspberry purees. Because of high temperatures (>220°F, >105°C) for a time period of 1 to 5 min, the color is often browner and flavor is more caramelized than those of freeze-dried products.

Cranberries are being dehydrated and sold as a snack food and are being used in mixtures of other dehydrated fruit as a snack. The Ocean Spray Company has developed *Craisin*, which is a sugar-induced cranberry that is dried and used in baked and cereal products or sold as a snack in its own package.

Blueberries are dried by tunnel dryers and freeze-dryers. For both processes individually frozen berries are used as the raw material. The use of dried blueberries is gaining acceptance in cereals and baked products. The drying of currants and gooseberries is mainly a home industry due to lack of volume production of these fruits.

Dates

Dates are almost exclusively sun-dried, as a favorable dry climate exists in the areas where date palms are grown. Such areas as Southern Iran, Iraq, and Egypt have a very high daily temperature during the growing and harvesting seasons and thus are ideal for natural sun drying of dates. In Arizona and Southern California, where dates are also grown, they are seldom dehydrated except in the preparation of low-moisture date products. In such cases, the dates are cut to small pieces or extruded to paste and dried in vacuum dryers.

Figs

The principal varieties grown for drying are Calimyrna, Mission, Adriatic, and Kadota. Figs are usually allowed to dry partially on the trees. In some cases, the trees are lightly shaken at intervals. Figs are usually mechanically gathered from the ground and are typically dry enough to be loosely packed in boxes or bins. Sometimes they are further dried on trays in the sun to a moisture content of approximately 17 to 18%.

After being transported to the plant in sweat boxes or bins, the figs are normally screened, graded for size, and then inspected to remove insect-damaged fruits. The screening is necessary to divide the figs into the required finished product styles. After the first sorting and grading operation, the figs to be stored for later processing are packed in boxes and placed in an airtight chamber and fumigated. This operation is repeated several times over a two-week holding period.

The figs to be processed are conveyed through a cold-water reel washer to remove dust and foreign material. They are then directed to a processing unit where they are immersed in hot water for 5 to 10 min. Soak time depends on the size and variety of fruit being processed. The figs at this point have adsorbed some water and, because of increased susceptibility to mold, are sprayed with potassium sorbate. They are conveyed, typically, over a dewatering belt where they may either be put into small plastic tubs to equilibrate or placed into retorts directly. The retorting process includes exposure to live steam for 2 or 3 min, which further softens the figs. The fruit is air-cooled and packaged.

Peaches and Nectarines

The primary peach for drying is the Lovell, a highly colored freestone variety. Faye Elberta is also dried. Sungrade and Le Grande nectarines are preferred for drying; both are freestone fruits.

Freestone peaches and nectarines are harvested and processed similarly to apricots. After sulfuring, the fruits are placed in full sun for two to three days or longer, depending on weather conditions, at which time they are transferred to shady stack storage and dried for several more weeks. Finally, they are removed from the trays, transferred to boxes or bins, and delivered to the packing plant.

Dehydrated (low-moisture) apricots and peaches are processed from the sun-dried (evaporated) fruit to a limited extent. Because of the high sugar content and the sensitivity of the yellow pigment to heat, a vacuum process is necessary to dehydrate these fruits to less than 5% moisture. Vacuum shelf dryers are used for the process. The evaporated fruit halves are sliced or diced before loading on the drying trays.

Pears

Pears that are to be dried are allowed to ripen on the tree. The summer Bartlett variety is used for drying in California. The fruits are handpicked and transported to cutting sheds where they are cored and halved. Placed cup up on wooden racks, they are stored overnight in sulfur houses where they are exposed to burning sulfur to prevent browning. The pear halves are removed from the sulfur house, dried in the sun for four to eight days, and then transferred to stacked storage for an additional two to three weeks.

Once dried, the fruit is delivered to the packing plant, where it is usually processed to fill orders. The dried fruit from the field may sometimes be stored as long as several years before being repacked. Pears are little known as a dried fruit and most commonly appear in packages of mixed dry fruits.

Prunes

Although plums are one of the most widely distributed fruits in the United States, only one type of plum, the French type, is designated a prune. The La Petite d'Agen variety brought to the Santa Clara Valley of California by a French nurseryman in 1856, known as the California French prune, is used for drying and is grown almost entirely in California. Prunes are the second most important dried fruit crops. The California production accounts for 100% of the U.S. production and 70% of the world's supply.

Most of California's plums for prune production are harvested mostly in late August by machine. The soluble solids content of juice must reach 22% prior to harvest. Immediately after harvesting, the orchard-ripened fruit is taken to the dehydrator yard where it is washed, placed on large wooden trays, and dehydrated to about 18% moisture in forced-draft tunnel dehydrators. The drying process requires 24 to 36 h, depending on the size and solids content of the fruit. Three pounds of plum will yield 1 lb of prune.

Dried prunes are processed through a series of screening, grading, and washing steps. Grading involves separation according to size, ranging from 23 to 150 prunes per pound. Hand sorting for cull removal follows, after which the prunes are conveyed to a blancher where they are held from 8 to 20 min to inactivate enzymes and preserve color and flavor. Potassium sorbate and fresh water are then sprayed onto the prunes to maintain proper moisture content and to add a preservative. Fruit to be pitted is sent through automatic pitting machines that either squeeze the pit out with mechanical fingers or punch it out. The pitted or unpitted prunes are again handsorted for rejects, automatically weighed into boxes or sacks, sprayed with potassium sorbate preservative, and sealed. Other popular prune products include prune juice (a water leachate of the prune) and prune paste, used in baking and confections.

Raisins

The United States and Turkey produce over two-thirds of the world's supply of raisins. Approximately 25% of the U.S. grape production is made into raisins, processed almost entirely in the San Joaquin Valley of California. Raisins are the most important dried fruit crops. Cultivation for raisin grapes is little different than for fresh market grapes.

Thompson Seedless, Muscat, Black Corinth, and Sultan grapes are the principal varieties processed for raisins. The fruit is handpicked in August from the vine in bunches and set on paper to be dried in the sun for 7 to 10 days. At this point, fruit dehydration has not been uniform. Thus, turning of the berries is done to speed the drying process and allow raisins to dry more uniformly. Without turning, some raisins will be too dry, while others will be too moist, requiring a long curing period later on. Within approximately five to seven days after turning, the raisin trays are ready to be retrieved and loaded into sweat boxes to equalize and then to be shipped to the processing plant. The maximum moisture allowed at the packinghouse for incoming raisins is 16%, but usually they are in the 9 to 12% moisture range.

Golden Bleach raisins are dipped in 0.25% hot NaOH (lye) solution after harvest for 3 to 6 s, sulfured for 4 to 6 h, and dried in the sun or in forced-air dryers. Soda-dipped raisins are dipped for 30 to 60 s in a 4% solution of soda ash and Na_2CO_3 at 35 to 38°C and then processed in the same manner as Golden Bleach, but they are not sulfured. From this point, the processing is the same as for natural-dried raisins.

Raisins are processed by a series of screening, destemming, and air separations. These processes are repeated until lightweight particles are removed. Small raisins may then be utilized in distilled alcohol products or in cereal or bakery products.

The raisins are next washed and sent through a dewatering operation, which removes excess surface water. They go through another destemming operation, and their moisture content is adjusted to 18% by water sprays. The fruits are sorted by a mechanical sorter and packaged for storage or shipment. The packages used for raisins are 14-g (0.5-oz) miniboxes, 500-g (1.1-lb) canisters, and 1-kg (2.2-lb) bags, and bulk size packages that contain from 13 to 500 kg (28.6 to 1100 lb) each. Raisins are also sold to industrial users as raisin paste and raisin juice concentrate (70° Brix).

BIBLIOGRAPHY

1. J. G. Brennan, *Food Dehydration*, Technomic Publishing Co., Lancaster, Pa., 1994
2. L. Calvin, *Fruit and Tree Nuts. Situation and Outlook Report*, USDA Economic Research Service, Washington, D.C., 1996.
3. A. I. Liapis, "Freeze Drying," in A. S. Mujundar, ed., *Handbook of Industrial Drying*, 2nd ed., Marcel Dekker, New York, 1995, pp. 295–326.
4. M. R. Okos et al., "Food Dehydration," in D. R. Heldman and D. B. Lund, eds., *Handbook of Food Engineering*, Marcel Dekker, New York, 1992, pp. 437–562.
5. G. M. Sapers, "Browning of Foods: Control by Sulfites, Antioxidants, and Other Means," *Food Technol.* **47**, 75–84 1993.
6. L. P. Somogyi, D. M. Barrett, and Y. H. Hui, *Processing Fruits, Major Processed Products*, Technomic Publishing Co., Lancaster, Pa., 1996.
7. L. P. Somogyi and B. S. Luh, "Dehydration of Fruits," in J. G. Woodroof and B. S. Luh, eds., *Commercial Fruit Processing*, 2nd ed., AVI Publishing Co., Westport, Conn., 1986, pp 353–405.
8. L. P. Somogyi, H. S. Ramaswamy, and Y. H. Hui, *Processing Fruits, Biology, Principles, and Applications*, Technomic Publishing Co., Lancaster, Pa., 1996.

LASZLO P. SOMOGYI
Consulting Food Scientist
Kensington, California

FRUIT PRESERVES AND JELLIES

The fruit preserve and jelly categories are certainly a major milestone in food evolution. Their history dates back to ancient times, when a confection or a dessert has been documented as a part of the meal. The use of sugar widened the possibilities for preserving fruits. In fact, during colonial times, a jam sometimes formed while fruit was being boiled. This jam or gel formed when the correct proportions of pectin, sugars, and acids occurred. Jam and jelly production was once considered an art; now it is a science. More is known about the components necessary to produce this kind of gel. This knowledge has led to other applications such as stabilized fruit fillings and sauces, processed fruit juices, canned fruits, and frozen desserts and confections.

DEFINITIONS AND STANDARDS

The name preserves covers a broad range of products, including jams, butters, marmalades, and conserves, as well as ordinary preserves. Preserves contain the largest fruit pieces, whereas jams contain smaller pieces that are crushed or chopped with added acid. Fruit butters are made of fruit pulp cooked to a smooth consistency. They are pressed through a coarse strainer and are more concentrated than jams. Scorching can be a problem because of their high viscosity. Marmalades have the characteristics of both jellies and preserves. They contain thin citrus peel or fruit pieces and are chiefly made from citrus fruits, alone or in combination with other fruits. Conserves are similar to jams, except that two or more fruits are cooked together and raisins and nuts can be added. Jellies are in a class by themselves. They are clear sparkling spreads in which fruit juices as the source of flavor, and, in some cases, the thickening agent.

The Federal Standards and Definitions do not differentiate between preserves and jams (1,2). A preserve is minimally 45 parts prepared fruit with 55 parts of sugar and is concentrated to 65% or higher solids, resulting in a semisolid product. Jellies are similar to preserves, with 45 parts of clarified fruit juice and 55 parts of sugar, resulting in a minimum of 65% solids. Both categories can utilize a maximum of 25% corn syrup for sweetness as well as pectin and acid to achieve the gelling texture required. Fruit butters are prepared from mixtures containing not less than five parts by weight of fruit to two parts of sugar.

GELATION-PECTIN MECHANISM

Typical Gel Formations

Gelation, the formation of the polymer network that gives commercial fruit preserves and jellies their texture, depends on four essential ingredients—pectin, sugar, acid, and water—added in the correct proportions. A pectin gel is a system resembling a sponge filled with water. This polymer is in a partially dissolved, partially precipitated state. The chain molecules are locally joined by limited crystallization, forming a three-dimensional network in which water, sugar, and other solutes are held. Some fruits such as tart apples, red and black raspberries, oranges, and cranberries have enough pectin and acid present. Still others, such as ripe apples and plums, contain sufficient pectin but lack enough acid. Pectin or acid must be added when using most fruits. Sugar is always needed when high-methoxyl pectin is used.

Since fruits vary widely with regard to maturity, climatic conditions, and storage, it can be difficult to ensure the proper composition. Fruit should be picked just before processing to ensure taste and texture. It should be picked as ripe fruit in the early morning to ensure quality. Overripe fruit will have reduced sugar quality, and the pectin will suffer molecular breakdown from enzyme activity. If fresh fruit is not available, frozen, cold-pack, or canned fruit can be used for jams and preserves.

The juice of grapes, currants, lemons, sour oranges, and grapefruits contains sufficient pectin and acid for jelly manufacture. Strawberries, rhubarb, and apricots usually contain sufficient acid but may lack pectin. On the other hand, sweet cherries and quinces may lack acid yet have enough pectin. Commercial pectin, either liquid or powder, can be added as a supplement. The viscosity of a fruit juice is an index of its gelling power.

Pectin is found in the flesh, skins, and seeds of most fruits. It can be extracted when fruit is boiled. Pectin is a complex carbohydrate consisting of polygalacturonic acid chains having a wide variety of molecular weights. The chains contain some carboxyl groups that are partially methylated, forming the ester known as pectin.

Generally, a degree of methylation (DM) of 50% divides commercial pectin into two main groups: high-methoxyl (HM) pectins and low-methoxyl (LM) pectins. The LM-pectin group includes both conventional and amidated versions. The HM pectins are the predominate choice for the standard jellies and preserves. The LM pectins can be utilized in low-sugar fruit spreads.

The degree of methylation of pectin has a critical influence on the solution and gelation characteristics of preserves and jellies. The highest DM that can be achieved by extraction of the natural raw material is about 75%. Pectins of DM ranging from 0 to 70% are produced by demethoxylation in the manufacturing process.

The DM of HM pectins controls their relative speed of gelation; hence, the terms slow-set and rapid-set HM pectin. If a higher degree of methylation of pectin is used, the higher will be the pH required for a fast set. A fast set is necessary to suspend fruit pieces and prevent fruit flotation or sinking. A slow set is necessary for a clear jelly, so that air bubbles are removed. The pectin's quality is standardized on the basis of its 150° standard, which means that, under controlled conditions, 1 lb of 150-grade pectin will gel 150 lb of sugar. This method is known as SAG.

Another method for testing pectin gels is the Voland-Stevens LFRA texture, analyzer in which the gel's elasticity is exceeded. The Tarr-Baker gelometer was the original jelly strength tester. However, after it was discontinued in 1965, the Voland-Stevens analyzer was declared an acceptable replacement because of its operational similarity, portability, compactness, reproducibility, and ease of use and calibration (3). This flexible instrument can be attached to a printer to measure elasticity and other attributes of a gel. This method is comparable to the SAG method.

Jellies are usually produced at a pH of 3.1 and jams at 3.3. HM pectin can gel sugar solutions with a minimum of about 55% soluble solids within a pH range of approximately 2.0 to 3.4. For any soluble solids with a value above 55%, there is a pH value at which gelation is optimal for a particular HM pectin and a pH range within which gelation can be controlled.

Sugars have a general dehydration effect on HM-pectin solubility. At higher solids values, there is less water available to act as a solvent for the pectin. Hence, there is an increased tendency to gel. Because gelation relies on the proper balance of soluble solids and pH in the medium, it is possible to compensate for a reduction in soluble solids by reducing the pH. Any HM pectin can gel rapidly or slowly and the rate can be controlled by the soluble solids and pH.

An attempt was made to quantify fruit content in jams by combining chemical composition data, particularly the inorganic elements that are stable to processing such as ash, magnesium, and potassium, with the rheological forces such as yield stress and flow index in a regression analysis that could explain 90% of the variability in fruit content (4).

Novel Gel Systems

The typical manufacturing methods for jams and jellies use the four necessary components: fruit, pectin, sugar, and acid. Some combination pectin sources have been devised. In one (5), an emulsifier is added to the sugar surface and blended with the bulk of the sugar. A very fine pectin is then mixed with acid and combined with the emulsifier–sugar complex. The emulsifier acts not only as a glue, causing the fine pectin particles to adhere to the sugar surface, but it also acts as an antifoaming agent and a dispersing agent in the final gel production.

Another convenient product, one-step pectin gelling composition, has been developed (6). Pectin particles are mixed with moistened coarse sugar particles. Acid may be added in a dry form and will adhere to the sugar, or it can be predissolved in water and sprayed onto the mix. Much less pectin can be used in this method as compared with dry blending fine pectin, acid, and sugar. This is due to the use of larger sugar particles and smaller pectin particles, so that the pectin dissolves faster while the concentration of dissolved sugar solids is retarded.

Instant pectins are technologically optimized pectins with better dispersion and solubility properties. They dissolve rapidly even at low temperatures and work into jam mixes without prior mixing with sucrose or corn syrup (7).

The LM pectin offers another gel formation method for preserves and jellies. However, the gel formed does not conform to the federal standard of identity. The use of this pectin does result in reduced-sugar jams, jellies, and preserves, the LM-pectin group includes both conventional (acid demethuylated) and amidated types. These pectins require calcium ions for gelation, not sugar or acid.

The gelation of LM pectin is controlled primarily by the reaction of a divalent cation with the acid groups of the pectin chains. LM pectin can be used at solids levels as low as 10%. The pH range for LM pectin is 3.0 to 6.0 because the role of acid is minimized. For successful gelation to occur, 50 to 100% of the acid groups must be complexed with calcium. The amidated pectin, which has fewer free acid groups, requires less calcium for gelation and relies on hydrogen bonding between the amide and free acid groups. Amidated pectins form gels that are more rigid than those formed with conventional pectins. Conventional pectins produce a thickening effect and are aptly used for jams and preserves; amidated pectins are used for jellies.

CARBOHYDRATE SWEETENERS

In HM-pectin systems, sugar accounts for more than 50% of the total weight and 80% of the total solids in a jam. It contributes solids; maintains microbiological shelf life; provides sweetness, body, and mouth-feel; contributes to gelation; and adds color and shine to the jam.

Other sugars that can be used are glucose syrup, dextrose, invert sugar syrup, and honey. When other sugars are substituted for sucrose in jam, the effects on the HM-pectin gelation are as follows:

- Inversion of sucrose reduces gel strength and lowers the gelling temperature.
- Glucose syrup usually reduces the gel strength. High-dextrose-equivalent (DE) glucose syrups decrease gelling temperature; regular-DE syrups increase it.
- Sugar alcohols such as sorbitol and xylitol are used in dietary products. Sorbitol jams can be made with HM pectin, soluble solids of 65%, and a pH of 3.0 Xylitol has limited solubility. At the 39% limit of solubility, gelatin with HM pectin can be obtained when the pH is lowered to 2.7.
- Maltitol syrups are used in the manufacture of sugar-free jellies. Suitable selection of the maltitol content needs to complement the acidified and nonacidified gelatin to deliver the appropriate applications (8).

A study was developed to compare some effects of gelling agents and sweeteners in high- and low-sugar-content carbohydrate gels (9). HM pectin, LM pectin, carrageenan, and alginate gels were the gelling agents, while sucrose and high-fructose corn syrup (HFCS) were the sweeteners. Soluble solids ranged from 35 to 65% with polydextrose as the bulking agent. The properties compared were bound water, water activity, syneresis (35% soluble solids), closely simulated HM-pectin gels because of the comparable spreadability properties. The water-binding property of sucrose exceeded HFCS with most gel systems, except where a combination of LM pectin and carrageenan was used. The water binding served as an index for predicting syneresis, or weeping; spreadability; and shear. However, a synergy of carrageenan with pectin or alginate with pectin resulted in increased bound water compared with individual gums.

Another study to improve nonsugar jam systems used the addition of xylitol or saccharin to veltol to improve the color and taste of apricot jam with minimal changes during storage (10). The use of aspartame in fruit spreads was minimally documented until its stability and effectiveness were measured in 1986 (11). A high-performance liquid chromatographic method was the quality control tool for monitoring levels of aspartame. This study predicted an average half-life of 168 days for aspartame in a fruit spread kept at 25°C. It occurs only at higher temperatures.

Gel strength and gelling temperatures of both amide and conventional LM pectins are influenced by the type of sugar used in the gel. Gels prepared with HFCSs have significantly lower gel strengths at all calcium levels than gels prepared with sucrose. However the use of 42- and 62-DE corn syrups give higher gel strengths than sucrose in LM-pectin formulations.

PROCESSING TECHNIQUES

Traditional Process

The traditional process used for preserve and jelly manufacture is the open kettle, batch boiling technique. The boiling process, in addition to removing excess water, also partially inverts the sugar, develops the flavor and texture, and destroys yeast and mold. In jelly manufacture, the fruit is boiled to extract the pectin and destroy the pectin-hydrolyzing enzymes. The juice is then separated by straining or pressing, and the press cake is boiled with more water to obtain more pectin. Pectin deficiency is remedied by the addition of commercial pectin. Added pectin needs to be dispersed with sugar to ensure uniform distribution. Either liquid or dry sugar is added.

A second boiling step is necessary to concentrate the juice to the critical point for gel formation of the particular pectin–sugar–acid system being used (12). Extended boiling causes acid volatilization, pectin breakdown, and losses in flavor and color. Vacuum concentration (50–60°C) produces a higher-quality jelly than atmospheric pressure boiling (105°C). A refractive index reading indicating soluble-solids content is the point at which the concentration stops. A flow diagram for these processes can be found in Reference 13.

The pH of the jelly will determine the set temperature of the pectin. The setting temperature of a jelly at pH 3.0 can be lowered approximately 10°C with rapid-set pectin or 20°C with slow-set pectin by decreasing the acidity to pH 3.25 (14).

For preserves and jams, the same procedure is used except that the fruit pulp is not strained. Rapid-set pectin is preferred to suspend the fruit more evenly and to minimize settling out.

Both products are packed hot, at about 85°C, into containers that are then sealed. Hot sterilized jars with hot sterilized lids and caps can also be used. Once filled, the jars are turned over to heat the lids and then returned to the upright position. The hot water bath technique can be a water bath and boiled from 5 to 15 min, depending on the fruit. This is a better method for deterring mold growth for fruit preserves and jellies. The U.S. Department of Agriculture (USDA) sanctions the use of paraffin or a two-piece metal lid and screwband for sealing jellies, but it highly recommends processing them for extra safety precaution.

The continuous process utilizes a premix for its efficiency. The APV system uses a plate evaporator for jellies. The Alfa-Laval system uses a scraped-surface heat exchanger for preserves because of the fruit pieces involved. The soluble-solids content or Brix degree is monitored by either an in-line unit or an automatic unit with electrical feedback to control the evaporator (15,16).

The filling temperature for these processes should be 85 to 95°C. This will ensure proper setting, fruit distribution, and a sterile product. A rotary multiple-piston displacement machine is used for filling. Speeds range from 100 to 600 jars per minute. Jars are washed and preheated before filling. Capping occurs immediately afterward, ensuring a vacuum seal.

The pack will be sterile in most cases if it is filled at not less than 85°C and capped using a steam flow closure. If a steam flow closure is not used, the sterile pack will require the use of a steam-sterilizing unit to cool it. Jars can be cooled continuously using water sprays of about 60°C to avoid thermal shock. Subsequently, 20°C water is used to finish this process. The jar temperature should be above ambient. The vacuum seal is check by a nonvacuum detector to ensure a hermetic seal. Jars are passed through a visual inspection point to locate and remove jars with unfavorable attributes such as foreign material, floating fruit pieces, and bubble formation. The jars are then passed to labeling machines, packed in cases or trays, and shrink-wrapped.

The order of addition of ingredients is very important. As in vacuum cooking, a slow-set pectin is preferred to limit the change of preset with the pectin. Most manufacturers use pectin solutions that are easily prepared and dissolve much more effectively than powder. The pectin solution can be added before or after concentration of the batch. Addition of the pectin after concentration results in a faster cooking rate due to the lower viscosity of the batch during concentration. For jelly manufacture, it is best to add the pectin solution before cooking is completed. The addition of 15 to 25% corn syrup deters crystallization from occurring due to sugar inversion resulting from low-temperature vacuum cooking (17). Low-sugar jams require less cooking than jellies and can use larger quantities of HM pectin to improve gel quality.

Modern Processing Techniques

A process to replace canning has been developed in Sweden by Alfastar (16). This multitherm process is said to preserve food for several months without chemicals as well as to achieve a fresher-tasting product. The process is rapid, with even heating through the product. The processing temperature is 150°C and can be reached in less than a minute.

The use of enzymes has made the process more efficient. Serum separation can be reduced by using an effective amount of pectin esterase to the aqueous phase and incubating it in the presence of divalent cations (18).

Another modern technique being used today includes extraction of pectin by pretreating the fruit in an electromagnetic field of superhigh frequency (19).

The microwave oven is the latest method for processing jams and jellies for the homemaker (13). An oversized container must be used for this process to avoid boiling over. Fruit, sugar, and some butter are mixed and allowed to stand for 30 min. The butter will help to deter the frothiness that may develop. The mixture is microwaved until it boils, with frequent stirring. It is then cooked for 10 to 13 min more in the microwave. Jams produced from this mixture keep well in the refrigerator for several months. They can also be canned for greater safety.

The no-cooked freezer jams are by far the easiest of these processing techniques. The fruit is mixed with an appropriate amount of sugar. This technique does not rely on pectin as much, because pectin is not heated to form the gel bonding that cooking at high temperatures creates. Lemon juice, if any, is added to the pectin, and then stirred into the sugar–fruit mix. The mixture is placed in sterilized containers and covered with two-piece metal lids and kept at room temperature for 24 h before placing in the freezer. Once opened, it can be stored up to three weeks in the refrigerator.

QUALITY PARAMETERS

The overall quality of fruit preserves and jellies has increased because of improvements in processing techniques, increased knowledge of fruit characteristics, and competitive situations. Fruit quality control is most important because it affects flavor, odor and color of the preserves and jellies.

The following criteria are important for manufacturing a quality product:

- Fruit appearance, ripeness, and solids must be optimized.
- Fruit juice must be clarified properly to ensure a clear jelly.
- Appropriate pectin grade, 120 to 200, must be used.
- Sugar assay and appearance must be appropriate.
- Corn syrup buffering capacity, solids, and appearance must meet minimum specifications.

Processing must be monitored in the areas of appearance, flavor, color, viscosity, pH, and solids. Powder pectin use results in a dark, rich color and stiff gel. In contrast, liquid pectin, which is less concentrated, makes a less stiff product. Typically, high-DM pectin must be conditioned to increase its set time and to optimize the DM. This pectin follows first-order kinetics in its stability.

Jelly quality attributes are similar to those of jam except that jelly is clear and bright, does not contain fruit pieces, holds its shape when unmolded, and cuts easily with a spoon. A stiff jelly is so firm that the mold will retain the mold shape.

Some problems that can occur are the following:

Cloudy jelly. Unclarified juice, underripe fruit, or pouring so slowly into containers that gelling occurs can result in a cloudy jelly.

Color changes. Darkening at the top of the jars can be caused by storing them in too warm a place or by an imperfect jar seal.

Color fading. Fading can occur with red fruits if they are stored in too warm and too bright areas or stored too long. The natural colorants in the fruit are highly susceptible to high temperatures and light. Another possible cause of color fading could be that the processing was not sufficient to either destroy the enzymes that can affect color, or that the processing time elevated the temperature, causing color destruction. Trapped air bubbles can also contribute to the chemical changes caused by oxidation.

Crystal formation. An excess of sugar can "seed" the jelly when HM pectin is used. This excess sugar comes from overcooking, too little acid, or from undercooking the recipe. Tartrate crystals can form in grape jelly, if juice is left to stand in the cold for several hours before being used. Moreover, if the glass interior is scratched, seeding can occur.

Floating fruit. This can result either from undercooking or from not driving off enough water to create the viscous gel necessary to maintain even fruit distribution. Fruit pieces not properly cut or not ripe enough can also lead to floating fruit.

Gummy jelly. Gummy jelly can result from overcooking and creating invert sugar.

Mold. The appearance of mold can be the result of imperfectly sealed jars and airborne contamination, if the full sugar complement was not used. The water activity created makes a favorable environment for contamination from the jars if they were not properly sterilized or simply underprocessed. A change in the appearance and off-odor or fermented smell will not necessarily occur. However, mold is often seen before taste is affected.

Weeping jelly. Syneresis in jelly can be overcome by not overcooking, not storing in a warm place, and using the appropriate amount of pectin or acid.

Stiff or tough jelly. Overcooking or using too much added pectin delivers a tough jelly.

Jelly failures. An improper balance has occurred when a gel is not formed. Inaccurate measurement, insufficient cooking, overcooking, or increasing the recipe prevents the pectin from building its network.

In a study (20), reduced boiling time improved both the aroma and flavor of fruit preserves. However, it was noted that the retention of flavor and color can be protected during the shelf life by means of modified packaging and appropriate storage practices, thus eliminating light and oxygen and storing at 15°C.

CURRENT TRENDS WORLDWIDE

The jam, jelly, and preserve market is expected to reach $1.5 billion by 2000, with the upscale market showing the greatest potential for growth. Gourmet fruit spreads, preserves, jams, and jellies, including more imports and exotic flavors, are the new products. According to *Food & Beverage Marketing*, the estimated growth rate for these products is 3% per year. The nutritional and health benefits take the forms of less sugar and more fruit; no sugar is added because high-sugar fruit juice is used instead of 100% fruit (18,21). A more convenient packaged powdered pectin has sugar added to it to be used as a sugar and pectin mix for preparing jams and jellies at home. Argentina has a line of dietetic jams, the Netherlands markets lower-calorie jams and preserves, and Japan produces a jelly drink.

The packaging revolution has also affected this market in the form of squeezable plastic containers for convenience, trays and containers of layered foil materials that add longer shelf life to products, and contemporary plastic jelly jars and lids featuring dinosaurs.

SUMMARY

Fruit preserves and jellies have become segmented over the last few years and will continue to grow via the gourmet marketplace. They have grown right along with the new processes, continuous operation, aseptic processing, and microwaving. There is still opportunity for growth in refining current gel systems and developing more convenient ones for either commercial or home use.

BIBLIOGRAPHY

1. *United States Standards for Grades of Fruit Preserves (or Jams)*, 4th ed., Dept. of Agriculture Food Safety and Quality Service, Washington, D.C., Jan. 4, 1980.
2. *United States Standards for Grades of Fruit Jelly*, 2nd ed., Dept. of Agriculture, Food Safety and Quality Service, Washington, D.C., Sept. 3, 1979.
3. S. A. Angalet, "Evaluation of Voland Stevens LFRA Texture Analyzer for Measuring the Strength of Pectin Sugar Jellies," *J Texture Stud.* **17**, 87–96 (1986).
4. E. Costell, E. Carbonell, and L. Duran, "Chemical Composition and Rheological Behavior of Strawberry Jams," *Acta Alimentaria* **16**, 319–330 (1987).
5. U.S. Pat. 4,686,106 (Aug. 11, 1987), R. Ehrlich and R. Cox (to General Foods Corporation).
6. U.S. Pat. 4,800,096 (Jan. 24, 1989), D. DiGiovacchino, R. Carlson, R. Jonas, and S. Marion (to General Foods Corporation)
7. D. Hesse, "Instant Pectins for Use in Jams and Marmalades," *Food Marketing and Technology* **8**, 12–14 (1994).
8. "Sugar Free Jellies and Gums," *Kennedy's Confection* **2**, 9 (1995).
9. D. L. Gerdes, E. E. Burns, and L. S. Harrow, "Some Effects of Gelling Agents and Sweetners on High and Low Sugar Content Carbohydrate Gels," *Lebensm.-Wiss. Technol.* **20**, 282–285 (1987).
10. M. Ragab, "Characteristics of Apricot Jam Sweetened With Saccharin and Xylitol," *Food Chem.* **23**, 55–64 (1987)
11. M. C. Dever et al., "Measurement and Stability of Aspartame in Fruit Spread," *Canadian Institute of Food Science Technology Journal* **19**, 86–88 (1986).
12. M. Glocksman, "Pectins," in *Gum Technology in the Food Industry*, Academic Press, New York, 1969, pp. 159–190.
13. R. W. Broomfield, "Preserves," in *Food Industries Manual*, 22nd ed., AVI, Westport, Conn., 1988, pp. 335–355.
14. R. M. Ehrlich, "Controlling Gel Quality by Choice and Proper Use of Pectin," *Food Product Development* **2**, 36–42 (1968).
15. *Continuous Processing Systems*, Chilton Food Engineering, Chilton Co., Chicago, Ill , 1984, pp. 90–92.
16. U.S. Pat. 4,562,085 (Dec. 31, 1985), F. Ruggiero (to Alfa Laval, Inc.).
17. D. Tressier, "Jams, Jellies, Marmalades, and Preserves, Candied and Glaced Fruits, Fruit Syrups and Sauces," in *Fruit and Nut Products*, Vol. 3, AVI, Westport, Conn., 1976, pp. 76–98.
18. E. C. Nwanekezi and M. Kpolulu, "Characterization of Pectin Substances From Selected Tropical Fruits," *Journal of Food Science and Technology India* **31**, 159–161 (1994).
19. D. O'Beirne and S. Egan, "Some Effects of Reduced Boiling Time on the Quality of Fruit Preserves," *Lebensm.-Wiss. Technol.* **20**, 241–244 (1987).
20. M. Kratchanova et al., "Extraction of Pectin From Fruit Materials Pretreated in an Electromagnetic Field of Super High Frequency," *Carbohydr. Polym.* **25**, 141–144 (1994).
21. E. Kratz, "Jams, Jellies, Marmalades" *Food Marketing and Technology* **7**, 5–6, 8, 10, 13 (1993)

GENERAL REFERENCES

R. Baker, N. Berry, and Y. Hui, "Fruit Preserves and Jams," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology*, Technomic, Lancaster, Pa., 1996, pp. 117–133

S. A. El-Nawawi and Y. A. Heikel, "Factors Affecting Gelations of High Ester Citrus Pectin," *Process-Biochemistry* **32**, 381–385 (1997).

B. R. Thakur and A. K. Handa, "Chemistry and Uses of Pectin—A Review", *Crit. Rev. Food Sci. Nutr.* **37**, 47–73, 229 (1997).

R. Whistler and J. Be Miller, *Carbohydrate Chemistry for Food Scientists*, Eagen Press, St. Paul, Minn., 1997, pp. 171–210, 217–231.

Marnie L. DeGregorio
Kraft Foods U.S.A.
Tarrytown, New York

FRUITS, SEMI-TROPICAL

ACEROLA

Acerola (*Malpighia glabra*, Barbadas cherry, or West Indian cherry, of the family Malpighiaceae) is native to the West Indies and South America. There are also large plantations in Puerto Rico. The fruit is grown on a large shrub, 10 to 15 ft in height, and resembles cherries. Each bright-red fruit contains three seeds and several vertical furrows. The fruit is the richest known natural source of vitamin C, containing 1000 to 4000 mg/100 g, over 20 times as much as oranges. The juice is often used to enrich the vitamin C content of other fruit products. The tart, fruity fruit and juice are used to make jams, jellies, preserves, and the like.

BAEL

Bael (*Aegle marmelos*, bel, or Bengal quince, of the family Rutaceae) is native to India and is also grown in Southeast Asia. The fruit is grown on a tree up to 40 ft tall and resembles a citrus fruit with a thick rind. The pulp is yellow and mucilaginous when fresh and becomes reddish when dried. The aromatic, pleasant-tasting fruit is eaten fresh or dried and in juice preparations. It is also used in the treatment of diarrhea and dysentary (1).

CALAMONDIN

Calamondin (*Citrus madurensis*, of the family Rutaceae) looks like a small orange. It is grown on a small evergreen shrub often planted as an ornamental. *Hortus III* (2) lists calamondin (*Citrofortunella mitis*) as a hybrid of *Citrus* and *Fortunella*. The fruit is very acidic and rarely consumed fresh, but it makes a desirable, flavorful ingredient for marmalades, jams, jellies, and the like.

CAROB

Carob (*Ceritonia siliqua*, John's bread, locust, or algaroba bean, of the family Leguminosae) is native to the eastern part of the Mediterranean area. The tree bears pods up to 1 ft in length that have a thick, juicy pulp containing up to 50% dry weight of sugar. The large carob seeds are contained in the pulp. The fruit is eaten raw, discarding the seeds, or the seeds can be dried and ground to make a flour for baking. Carob seeds also serve as a source of gum for the food industry.

CHIRONJA

Chironjas are a citrus fruit of the family Rutaceae. Chironja is believed to be a natural hybrid of the orange, *C. sinensis*, and the grapefruit, *C. paradisi*. It was found growing wild in the mountains of Puerto Rico in 1969. It is an excellent fresh fruit and can also be preserved in a manner similar to oranges and grapefruit (3).

FIG

Figs (*Ficus carica*, of the very large family Moraceae) are native to Asia Minor and spread to the Mediterranean area more than 4000 years ago. The fruit is a multiple fruit developing from a whole inflorescence inside a protective coating. The best-known cultivated varieties are the common, or Adriatic, fig and the Smyrna fig. The former produces fruit by parthenocarpy and does not need to be fertilized. The Smyrna fig is fertilized by a small wasp that enters the fruit through a small hole in the end. In the United States, the Smyrna fig is known as the Kalimyrna, and the Italian Dottato type is known as the Kadota. Figs may be consumed fresh or dried, canned, or frozen. They are used in conserves, jams, confections, and baked goods.

GRAPEFRUIT

The origin of the grapefruit (*C. paradisi*, of the family Rutaceae) is uncertain, but it is believed to have developed in the West Indies from a cross between the sweet orange, *C. sinensis*, and the pummelo or shaddock, *C. grandis*. Shaddock is native to Thailand and Malaysia and was brought to the Barbados by a ship captain named Shaddock. Grapefruits became an important import to Europe between the two world wars.

Grapefruit is an important crop, with world production estimated at about 3.5 million metric tons in 1992; the United States produced about half (4). Florida grows more than 80% of the U.S. crop and more than 90% of the export volume. About half of the crop is processed into a variety of products. Grapefruits are divided into two major groups, the white grapefruit, with a pale yellow flesh, and the pink, or pigmented, group. The red color is due to the presence of the carotenoid lycopene, which has a very appealing pink color in fresh fruit or juice but tends to turn brownish on heat processing. Juice is the major processed product from grapefruit. Grapefruit juice sometimes contains high concentrations of limonin, which produces a bitter taste. The content of limonin is often reduced to a desirable level by a solvent-extraction debittering step, but some limonin is retained because at low levels it imparts a desirable flavor. Oil recovered from the outer layer of the skin is an important product. Grapefruit is classified along with lemons and limes as yellow citrus, as opposed to the orange citrus group. Oil from all three is used as a flavoring additive in juice products. Canned grapefruit sections are a minor product compared with juice, but they are still very popular on the consumer market. Pectin may be produced from the peels. The peels are also dried and sold as cattle feed. The peels are usually treated with lime to break down the pectin before pressing and drying. The press juice may be concentrated into citrus molasses and sold as cattle feed.

KUMQUAT

Kumquats (of the family Rutinaceae) are considered by some to be a citrus fruit even though they belong to the genus *Fortunella* not *Citrus*. The two best-known species, *F. japonica* (the marumi kumquat) and *F. margarita* (the nagami kumquat) are native to China and cultivated in Japan and China. They are grown in the United States mainly as an ornamental plant. The small fruits (about 1 in. in diameter) have a fruity, acidic flavor and are often used in marmalades, jams, jellies, and preserves.

LEMON

The lemon (*C. limon*, of the family Rutaceae) is the most diversified and most widely grown of the citrus species. Worldwide production in 1992 was estimated at about 3.4 million metric tons (4), and the United States produced about 680,000 tons. The major product from lemons is juice, which is produced in a manner similar to orange and grapefruit juice. Lemon oil from the peels is considered to be superior as a flavoring agent to the other citrus oils because of its high content of aldehydes. The prominent aldehyde is citral, which is a mixture of nerol and geraniol, as compared with the decanol of oranges and the nootkatone of grapefruit. Lemon oil is highly prized as a flavorant for many juice drinks and other food products as well a fragrance for cosmetics.

LITCHI

Litchis (leechee, lichi, or lychee) belong to several species of the family Sapindaceae; they are native to China and also grown in the Philippines and India. The litchi, *Nephelium litchi*, is a true nut, with the inner nut (about 1 in. in diameter) surrounded by an edible white, fleshy aril layer and a red spiny outer coat. The term *litchi nut* usually refers to the whole nut with the dried aril inside; it tolerates shipment very well and is often found in European markets. The edible aril layer is usually eaten fresh, dried, or canned. The inner nut is not eaten (1).

LONGAN

Longans (*Nephelian longan*, of the family Sapindaceae), native to China, are also grown in India and the Malay Archipelago. The fruit resembles the litchi in size, structure, and appearance, except that the outer coat is brown. The edible white, fleshy aril layer is usually eaten fresh, dried, or canned. The inner nut has no value as a food.

LOQUAT

The loquat (*Eriobotrya japonica*, Japanese plum, or Japanese medlar, of the family Rosaceae) is native to China. It is widely grown in Japan and many other parts of the world. The fruit is a pear-shaped yellow pome, up to 3 in. long, with a five-lobed calyx and two to four black stony seeds. The fruit is eaten fresh or in the form of jams, jellies, sauces, or alcoholic beverages. The seeds are also used in cooking.

MANDARIN

Mandarins (*C. reticulata* or *C. nobilis*, mandarin orange, or satsuma orange or tangerine, of the family Rutaceae), native to southern China, is now grown in many parts of the world. The many varieties may be divided into five groups: *C. reticulata* Blanco (common tangerines), *C. unshiu* Marc (satsuma tangerines), *C. deliciosa* Tenore (Mediterranean tangerines), *C. nobilis* Lourerio (King tangerines), and *C. madurensis* Lourerio (calamondin tangerines). The satsumas, with yellow-orange flesh, are the largest and hardiest and are common in Japan and Europe. The mandarins are similar but have orange flesh. The tangerines are smaller, with red-orange flesh. One cultivar that originated in Algeria is being sold under the name *Clementine*. *Mandarin orange* is the preferred name for all groups, and the names *mandarin* and *tangerine* are often used interchangeably. Some important hybrids associated with tangerines are the tangelo (tangerine × pummelo) and the tangor (tangerine × orange). Tangerines are a major crop, with worldwide production in 1992 estimated at 6 million metric tons and U.S. production at 300,000 tons (5). The fruit is mainly eaten fresh or canned or processed into juice.

MEDLAR

Medlar (*Mespilus germanica*, of the family Rosaceae), native to Persia, is a very old fruit and was well-known by the Romans. The fruit is less appealing than other pomes and is usually used in jams (1).

ORANGE

Citrus fruits, of the family Rutaceae, date back over 4000 years and are believed to have developed on the slopes of the Himalayas in northeastern India. They soon spread throughout Europe and were introduced to the Americas by Columbus. Citrus juices constitute a major portion of the worldwide fruit products and are second only to grapes in volume. Worldwide production of oranges was estimated in 1992 at 36 million metric tons. Japan was the leading producer, at 1.9 million tons, with U.S. production at 310,000 tons (5).

Commercial oranges are divided into two groups: *C. sinensis*, the sweet orange, and *C. aurantium*, the sour, or Seville, orange. The sweet oranges can be subdivided into four groups: common, navel, blood, and acidless, or sugar, oranges. The dominant variety in the common group is the Valencia cultivar, and it is the main citrus variety grown in all the world. The navel orange dominates the second group, and it is usually grown for the fresh trade. Blood oranges are grown primarily in the Mediterranean area and are claimed to be the most delicious of all the citrus fruits. The red color is due to the presence of anthocyanin pigments in addition to the yellow-orange carotenoid pigments found in all the other citrus fruits. The acidless oranges produce fruit that is very low in acid and less desirable for the juice trade. The sour orange group is dominated by the Seville cultivar, which is grown primarily for its peel and used in the manufacture of marmalade. The juice by-product from the manufacture of marmalade can be debittered and added to 100% juice products at the 5% level in the United States.

Oranges for the fresh market are a major outlet, but most growers combine a fresh fruit operation with a processing capability to utilize the fruit that does not have outer skin of the desirable appearance for marketing as fresh fruit. For juice production, the fruit is washed, sorted, and conveyed to a juice extractor. The fruit is conveyed to upper and lower cups that have sharp edges that cut a 1-in. hole in the bottom of the fruit; the inner contents of the fruit are then pushed through. In one pass the fruit is separated into an oil emulsion, juice, peel, and core material. The oil emulsion is washed and centrifuged to collect the oil. The remaining liquid is then recycled through the process. The juice is filtered to remove some of the pulp and sent to the packaging units. The pulp is washed and filtered, and the pulp wash is added back to the juice. In many areas that grow citrus, varieties more suitable for the fresh trade are grown, and they sometimes produce a bitter juice due to the presence of limonin. The limonin content can be lowered by passing the juice through an ion exchange column. In 1990 the U.S. Food and Drug Administration (FDA) approved the debittering process for frozen concentrated orange juice and concentrated juice for manufacturing, thus permitting more efficient use of navel oranges. Citrus oil is an important by-product of citrus processing. The major component (90%) of orange oil is *d*-limonene, which contributes little to the flavor but is a carrier of other flavor components. The most prominent flavor components are the aldehydes, primarily decanol and ethyl butyrate. The excess pulp, core, peels, seeds, and so forth can be dried and sold as cattle feed. Frozen concentrated orange juice is the major processed product, but specialty products such as molasses, alcoholic beverages, and candied peel are also produced.

POMEGRANATE

Pomegranates (*Punica granatum*, of the family Punicaceae) are native to Iran and are grown in large quantities in India and the Far East. The pomegranate was held in great esteem in ancient times but is less popular today. The fruit is round, about 2 to 3 in. in diameter, and has a leathery, red outer skin. The seeds are surrounded individually by a reddish-purple, highly flavorful pulp. The fruit is eaten fresh or, more usually, as a flavorant in ice cream, sherbet, juice drinks, and the like.

PUMMELO

Pummelos (*C. grandis*, pomelo, or shaddock, of the family Rutaceae) are large citrus fruits up to 10 in. in diameter native to Thailand and Malaysia. Grapefruit is believed to be a cross between the pummelo and the sweet orange.

SOUR ORANGE

See *Orange*.

SUGAR APPLE

Sugar apples (*Annona squamosa*, custard apple, or sweetsop, of the family Annonaceae) are grown in the lowland areas of South America. The yellowish-green fruits, up to 3 in. in diameter, are consumed fresh or in juice drinks.

TANGERINE

See *Mandarin*.

UGLI

Ugli fruit, of the family Rutaceae, is a large citrus hybrid with a thick rind produced by a cross between *C. reticulata* and *C. paradisi*. It is cultivated in Jamaica.

BIBLIOGRAPHY

1. B. Brouk, *Plants Consumed by Man*, Academic Press, New York, 1975.
2. The L. H. Bailey Hortorium, *Hortus III*, Cornell University, Ithaca, N.Y., 1976.
3. C. E. Bueso, Soursop, Tamarind, and Chironja, in S. Nagy and P. E. Shaw, eds., *Tropical and Subtropical Fruits*, AVI, Westport, Conn., 1980, pp. 375–406.
4. D. Kimball, "Grapefruits, Lemons, and Limes," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 305–336.
5. D. Kimball, "Oranges and Tangerines," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 265–304.

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amherst, Massachusetts

FRUITS, TEMPERATE

APPLE

Apples have been known since the beginning of recorded history. The fruit referred to in the Bible by Adam and Eve is thought to be an apple. Apples were very popular in ancient Rome and Greece. Modern apples developed in southwestern Asia in the area from the Caspian to the Black Sea. The Stone Age lake dwellers of central Europe learned to preserve apples by drying them in the sun. The apple was brought to America by the colonists and soon spread across the new continent. The life of John Chapman, better known as Johnny Appleseed, born in Leominster, Massachusetts, in June 1776, is an American legend. He carried apple seeds with him on his travels west and planted them wherever he went. Apples are the most widely planted of all fruits and are found in nearly all temperate zones around the world (1).

The genus *Malus*, of the family Rosaceae, contains a number of species and literally hundreds of cultivars of both the edible and ornamental varieties. Large apples are decendants of the species *M. pumila*, which originated in southwestern Asia, and hybrids with *M. sylvestris* of Europe. Edible crab apples are derived from *M. baccata*. Ornamental apples are hybrids of the edible species. The taxonomy of modern cultivated apples is so obscured by centuries of breeding and selection by humans that it is very difficult to assign modern apples to any one species of *Malus* (2). Botanically, the apple is a pome fruit developed from an inferior ovary and is derived from the ovary wall and the floral tube. The fleshy mesocarp constitutes the main edible portion. The five cavities each contain two seeds.

World production of apples in 1993 was about 40 million metric tons, with the United States contributing about 5 million tons (3). Production is increasing because production in China, Russia, Korea, Poland, and Romania is believed to be underreported. In the United States, about 55% of the apple crop was marketed fresh and 45% processed. Of the processed portion, about 44% was utilized in juice, 26% was canned, 6% was dried, 4% was frozen, and 3% was used in miscellaneous products such as jelly, wine, and vinegar. All of the current cultivars are used to some extent for processing, and some cultivars are grown exclusively for processing; however, most apples used for processing are salvaged from the fresh market.

Apple juice is sold in many forms. Fresh apple juice, or sweet cider, is produced from sound, ripe fruit that has been pressed and bottled. No form of preservation is used other than refrigeration. In the United States, apple cider refers to the fresh juice, but worldwide, it usually means apple juice that has been fermented. Shelf-stable apple juice has been treated with some form of preservation, usually heat treatment. The processed juice can be in several forms: crushed, with a high pulp content; unfiltered, with a lower pulp content; or clarified. The most popular product in the United States has been treated with ascorbic or erythorbic acid to produce a lighter color, depectinized with a pectinase enzyme, and filtered before being pasteurized to produce a clear juice. The manufacture of applesauce is a relatively simple procedure. Apples are washed, sorted, and chopped. Sugar is added, the mixture is cooked, and the skins and seeds are removed with a screen extractor. The puree is then canned or bottled. For sliced apples the fruit is washed, graded, peeled, cored, and sliced. The slices are placed in a container, and a vacuum is applied to remove the air from the slices. The vacuum is broken by injecting a solution of water, salt, ascorbic acid, and/or sugar. The slices are then put into containers, sugar syrup is added, and the containers are steam vacuumed, closed, and thermally processed. For frozen slices, the apple slices are vacuum treated, blanched, put into 35-lb containers, and frozen. For dried apple slices, the slices are prepared as noted previously and treated with sulfur dioxide, or one of its salts, to maintain a light color and to minimize enzymatic activity. Two types are recognized in the United States: Evaporated apple slices have not more than 24% moisture, and dehydrated slices contain not more than 3.5% moisture. A number of specialty products are produced from apples, such as glazed apples, spiced crab apples, apple butter, apple jelly, apple vinegar, and baked apples. Apple butter is similar to applesauce except that it is produced with slower heating and the final product is darker in color, more caramelized, and thicker. Apple jelly is made from concentrated apple juice.

APRICOT

Apricots are native to China and have been cultivated for more than 4000 years. They were well-known in ancient Greece and Rome, having followed the trade routes westward. From Europe, the apricot was introduced to America on the ships of the early explorers and settlers. Worldwide production in 1990 was estimated at nearly 700,000 metric tons (4). U.S. production was estimated at about 123,000 metric tons, with 97% produced in California.

The apricot belongs to the genus *Prunus* of the family Rosaceae. It is a stone fruit, in which the seed is encased within a hard, lignified endocarp referred to as the stone. Most commercial apricots belong to *P. armeniaca*, but several other varieties (*P. siberica* and *P. mandshurica*) are known. *P. mume*, known as the winter-flowering plum in Asia, has several cultivars. There is considerable genetic diversity in the apricot, but nearly all commercially important cultivars in the United States are derived from *P. Armeniaca*.

Before World War II, most apricots in the United States were sun dried, but production of canned apricots currently exceeds that of dried products. About 16% of apricots are marketed fresh and about 10% frozen. For canned apricots, Patterson and Tilton are the varieties of choice. The apricots are either picked by hand or shaken off the trees mechanically and transported to the cannery. The fruits are washed in chlorinated water; extraneous matter such as leaves, sticks, immature fruit, and the like are removed; and the fruit is conveyed to the cutters. The fruit is cut along the sutures, the pits are removed, and the fruit is placed in containers. Syrup consisting of light or heavy sugar syrup, apricot juice, or pear juice is added, and the containers are thermally processed. For sun-dried apricots, Blenheim is the cultivar of choice because of its superior flavor, but Patterson and Tilton are also used. Sun drying is a method of preservation limited to climates with hot sun and dry atmospheres. There are six basic steps: (*1*) select and sort fresh fruits, (*2*) wash, (*3*) cut into halves and remove pits, (*4*) place fruit cut side up on drying trays, (*5*) treat with burning sulfur or gaseous sulfur dioxide, and (*6*) place trays in the drying yard in full sun. The sulfur dioxide preserves the color of the dried fruit by minimizing enzymatic browning and reduces degradation of carotene and ascorbic acid. The fruits usually take 5 to 10 days to dry to a moisture content of 15 to 20%. The trays are then removed from direct sunlight and stacked to allow the moisture content to rise to about 27%. The fruits are then placed in boxes for 2 to 3 weeks and allowed to equilibrate to the desired moisture content. Once cured, the apricots are graded and packaged for sale. Frozen apricot slices are produced by washing, grading, cutting, slicing, and freezing the fresh fruit.

BILBERRY

Bilberries (*Vaccinium myrtillus*, of the family Ericaceae) are native to northern parts of Eurasia and are also called whortleberries or blaeberries. The small (8 mm in diameter), dark blue fruits are closely related to blueberries and are eaten raw or as jams, jellies, and preserves. They are also used in bakery goods.

BLACKBERRY

Blackberries, of the family Rosaceae, belong to the genus *Rubus*, which, particularly its subgenus *Eubatis*, is highly genetically heterogeneous. A series of species and subspecies makes it difficult to identify the heritage of many of the current cultivars. The cultivated blackberries of North America are divided into five groups: (*1*) the erect or nearly erect types of the eastern United States, (*2*) the eastern trailing types, (*3*) the southeastern trailing types found along the Atlantic and Gulf Coasts, (*4*) the trailing types of the Pacific Coast, and (*5*) the semitrailing types of the Pacific Coast. Trailing blackberries are also called dewberries, running blackberries, or ground blackberries. A number of thornless blackberry cultivars have been introduced and are sometimes called boysenberries, loganberries, or youngberries. All blackberries consist of a collection of drupelets that are partly fleshy and partly hard (the seed). Blackberries can be distinguished from raspberries because when a raspberry is picked the core stays on the bush. When a blackberry is picked, the core remains with the berry and becomes part of the edible fruit. A tayberry is a cross between a blackberry and a red raspberry

Approximately 90% of the blackberry crop is processed, perhaps because the fresh fruit has a storage life of only 4 to 5 days. In earlier years, blackberries were more popular as fresh fruit rather than being processed because of the difficulty in handpicking canes with abundant thorns. The introduction of mechanical picking machines changed the economics of harvesting, and blackberry products became much more popular. Canning and freezing are the main methods of preservation. Juice production is increasing along with the national increase in consumption of fruit juices.

BLUEBERRY

Blueberries belong to the family Ericaceae, subfamily Vacciniaceae. True blueberries belong to the ancient genus *Vaccinium*, subgenus *Cyanococcus*. Blueberries are divided into two major groups, lowbush and highbush. The lowbush type generally grows wild over large areas and is the source of a large quantity of fruit on the commercial market. Northern lowbush blueberries consist of several species: *V. myrtilloides*, *V. angustifolium*, *V. lamarkii*, and *V. vacillans*. *V. angustifolium* is gradually replacing the others in areas where rotational burning is used for weed control and pruning. The northern highbush blueberry is *V. corymbosum*, and the southern type is *V. australe*. The rabbit-eye blueberry is *V. ashei*, so named because when the fruit begins to ripen the pink color resembles a rabbit's eye. It is grown mainly in the southern states.

Blueberry production in the United States was about 85,000 tons in 1993 and is increasing rapidly. Production increased 50% between 1992 and 1993 alone. About 60% of the blueberries were processed and 40% sold as fresh berries. The rapid increase was due to the addition of blueberry products to a wide variety of foods, including breakfast cereals, dessert toppings and fillings, pies, cakes, cheesecakes, salads, muffins, fruit cocktails, yogurts, ice creams, breads, bagels, pastries, frozen muffin and cookie doughs, and the like. The forms of blueberries used in these products are fresh, frozen, puree, juice, dehydrated, and concentrate.

CHERRY

Cherries, of the family Rosaceae, are believed to have originated in the Caspian and Black Sea area, and wild trees inhabit all of Europe (5). They were domesticated in Greece as early as 300 B.C. The colonists brought them to America. Cherries are drupe fruits with a stone center. The two main types of cherries are sweet (*P. avium*) and pie, tart, or sour (*P. cerasus*). U.S. production in 1993 was estimated at 170,000 tons for sweet cherries and 160,000 tons for tart cherries. Several preservation methods are available. Traditionally cherries have been canned. Canning involves soaking the cherries in cold water to firm them for the pitting operation. After pitting, the cherries are put into cans or jars, syrup is added, and the containers are thermally processed. Tart cherries are usually pitted, but most sweet cherries are not. Frozen cherries are usually pitted and blanched before filling and freezing. Brining involves soaking the cherries in a 1% solution of sulfur dioxide, or one of its salts, which inhibits microbial growth and enzyme activity. It also bleaches the cherries to a pale yellow color. Brined cherries can be stored for a long time and used for jam and jelly production. The red color returns after removal of the sulfur dioxide. In the past, this was a very popular method of preservation, but it has been replaced by freezing because the frozen product offers a superior flavor in the final product.

Maraschino cherries are produced from brined cherries by soaking the cherries in a 0.5% solution of calcium chloride to firm the fruit, soaking in a sodium sulfite solution for further bleaching, and neutralizing with a sodium bicarbonate solution. After addition of a red colorant, usually erythrosine, the cherries are soaked in a solution of citric acid to fix the color. The cherries are then put into containers, a syrup flavored with benzaldehyde is added, and the containers are thermally processed. Cherries are also pressed to produce single-strength or concentrated juice, which has been well received as a colorful and flavorful food ingredient. Cherries are also dehydrated to make a desirable component of bakery products. Cherries lend themselves to many specialty products such as pie fillings, glazed products, wines, sauces, juice powders, spreads, candies, and flavors (5).

CHOKEBERRY

The black chokeberry, also known as chokecherry (*Aronia melanocarpa*), is a member of the family Rosaceae. It is a native North American plant introduced into Europe in the late eighteenth century. It produces clusters of small stone berries that, particularly in Poland, have been used to make jams, jellies, juices, and wines. Chokeberry juice is very astringent and has been used to improve the taste of apple products. Its astringency is not unlike that of cranberries, and it may find a place as an adjunct for flavoring products that now use cranberries. Chokeberries have also been suggested as a source of anthocyanin pigments for applications as natural colorants (6). Chokeberries are a very minor crop in the United States.

CRANBERRY

The cultivated cranberry (*V. macrocarpon*, of the family Ericaceae) is native to peat bogs in many northern states from Massachusetts to Minnesota and some Canadian provinces. The wild cranberry, *V. oxycoccus*, is native to the Pacific states but not the eastern states. It is not grown commercially because of its small berry size. Another species, *V. vitis-idea*, lingonberry, is grown on the upland areas of Scandinavia and Alaska. It is sometimes called mountain cranberry, European cranberry, partridgeberry, or foxberry and is used locally for jams and jellies. These are not to be confused with the highbush cranberry, *Viburnum trilobum* or *V. opulus*; it is primarily grown as an ornamental shrub, but the berries are sometimes used for jellies. North America produces nearly all of the world crop of cranberries, and production in 1998 was estimated at 300,000 tons, of which 95% was processed (M.S. Starr, personal communication, 1999).

Cranberries are one of the three fruits native to North America, the others being blueberries and the Concord grape. Cranberries were part of the American diet long before recorded history in the United States. Native Americans made pemmican from dried meat, fat, and cranberries. The cranberries probably contributed a pleasant taste and appearance to a food that must not have been very palatable. They also probably acted as a preservative by raising the acidity of the product, and the benzoic acid content may have had some antimicrobial action. Pemmican may have been the original trail food.

The cranberry is a low-growing, trailing, woody, broadleaf, evergreen plant that is grown in bogs and swamp areas generally unsuited for other types of agriculture. A plentiful supply of water is required for irrigation and flooding to prevent frost damage before harvest. In earlier years, harvesting was accomplished by hand rakes to collect the berries. Today, mechanical harvesters are used on both dry and flooded bogs. Flooding the bogs before shaking the berries off the vines produces a bigger yield because the berries float and can be skimmed by harvesters. Dry harvesting is usually reserved for the fresh trade, and wet berries are usually sent to freezers for later use. North America currently supplies nearly all of the world's cranberries, but test plantings are being developed in Chile, the Ukraine, Poland, and central Europe.

Cranberries are used to produce three major products. Cranberry sauce is a mixture of cranberries, sugar, and water. The mixture is cooked, passed through a strainer to remove the skins and seeds, poured into cans, and thermally processed. Cranberry whole sauce is a similar product, but it contains the skins and seeds. Both products form a firm gel because of the high pectin content. For cranberry juice, frozen cranberries are thawed and pressed to collect the juice. Commercial juice, as marketed for consumption, contains about 20 to 30% juice, sugar, and water. The press juice has to be diluted because full-strength juice is too astringent and does not make a palatable product. Cranberry juice and a number of combinations with other juices such as raspberry, orange, apple, blueberry, and prune have proved to be very popular in the recent rapid increase in fruit juice consumption by the American public. The popularity of the fruit juices has led to increased demand for cranberry juice concentrate, which is made by filtering the press run juice and passing it through an evaporator. Cranberry juice concentrate also provides a very concentrated source of red anthocyanin pigments, which make a very attractive red color (7). Fresh and dried cranberries have been added to muffins, cakes, cookies, breads, fillings, toppings, and a variety of specialty products. One cranberry product, Craisins, is similar to raisins.

CURRANT

Cultivated currants, of the family Saxifragaceae, are currant-bearing shrubs grown in the temperate and cold regions of both North and South America. Currants are extremely cold hardy, and their culture extends nearly to the Arctic Circle. Red and white currants are *Ribes sativum*, and black currants are *R. nigrum*. The flowering currant is *R. odoratum*. The black currant produces a more vigorous plant with a higher yield and stronger flavor than the other types and is probably more important commercially; however, it is a minor crop. A hybrid of black currant and gooseberry named Jostaberry is even more vigorous and produces fruit that looks like a black currant except larger Jostaberries are attracting the interest of home gardeners. Currants are used to make jams and jellies and as a very flavorful juice concentrate for addition to other juices. Dried currants are not from the *Ribes* genus but are dried grapes from the Black Corinth cultivar of *V. vinifera*. They are sometimes called Zante currants.

GOOSEBERRY

Gooseberries, of the family Saxifragaceae, are closely related to currants and require essentially the same cultural conditions. Gooseberries of American origin are *R. hirtellum*, and those of European origin are *R. uva-crispa*. The fruit may be white, yellow, green, or red and may have a prickly, hairy, or smooth surface. Gooseberries have an acidic and astringent flavor and are used to make jams and jellies. They are a minor crop in the United States.

GRAPE

Grapes, of the family Vitaceae, are the largest fruit crop in the world. The Food and Agriculture Organization of the United Nations estimated the grape crop in 1989 to be about 60 million metric tons, of which about 80% was used in wine production. Grape growing in the United States is divided into four areas: (*1*) the European type *(Vitis vinifera)*, grown in California and Arizona; (*2*) the American bunch type *(V. labrusca)*, grown in the Great Lakes area (New York, Pennsylvania, Michigan, Ohio, and Ontario, Canada), the Pacific Northwest (Washington, Oregon, and British Columbia), and the Midwest (Arkansas, Missouri, Iowa, Illinois, Indiana, Kansas, and Nebraska); (*3*) French hybrids *(V. vinifera)*, grown in Ontario, New York, and British Columbia, and (*4*) muscadine *(V. rotundifolia)*, grown in the South Atlantic and Gulf states. Actually, with the rise in popularity of wines, nearly every U.S. state has a grape and wine production area. The French hybrids have an interesting background, particularly with regard to wine. They originated in France as a group of cultivars produced by crossing *V. vinifera* with certain wild American grapes, mainly *V. lincecumii* (post-oak or turkey grape) and *V. rupestris* (sand, sugar, or rock grape). The French breeders hoped to obtain cultivars that combined the desirable qualities of the European cultivars with the hardiness and disease resistance of the American species. The breeding program has been going on for more than 100 years, and a number of cultivars are available, mainly for the wine trade (8). There are literally thousands of grape cultivars available for the grape-growing regions around the world.

Grapes for fresh consumption are grown in many geographical areas, but most are in California. Cultivars of *V. vinifera* dominate the production of table grapes. The most prominent cultivar for juice production is the Concord *(V. labrusca)*, New York and Washington led the United States in production of Concord grapes in 1993 with 300,000 metric tons (9). Grapes for table consumption are usually picked by hand to preserve the integrity of the grape bunches. Grapes for juice production are usually mechanically harvested; a number of different machine designs are available, but all of them use beaters or rods to shake the grapes from the vines. The grapes are collected, transferred to bulk containers, and transported immediately to the factory. The grapes are dumped into a holding tank and then transferred by auger to a stemmer-crusher that separates the fruit from the stems. The mixture is transferred to a holding tank, heated to 140°F, and a pectolytic enzyme is added to break down the pectin. After 30 min, a filter aid, such as rice hulls or paper pulp, is added, and the mixture is pressed to remove the juice. The filtered juice is allowed to stand to precipitate the argols (crude tartar). The juice is then racked off, filtered, poured into containers, and thermally processed. Grape juice concentrate can be made from the juice. Cold pressing is similar to the method just mentioned except that the process is conducted at ambient temperature. Grape juice, prior to the tartar removal step, can be pasteurized and stored in large tanks of up to 100,000 gal for future manufacture into jams jellies, toppings, fillings, and the like.

Production of grape juice and grape juice concentrate has recently increased markedly as a replacement for sugar in many drinks on the market. Some of the concentrates are bland in flavor and mix very well with some of the more prominent flavors inherent in other fruits. A large industry has developed for grape concentrate with up to eight times the normal red pigment content of grape juice; this product provides a colorant as well as a desirable mild, fruity flavor. The colorant concentrate is made from selected grape cultivars with a very high pigment content. Both red and yellow concentrates are available (10). Grape colorants (under the generic name enocyanin), prepared from the press cake left over from the wine-making process, have been marketed for more than 120 years (11). The preparation and use of colorants from grapes were described by Francis (12).

HUCKLEBERRY

Huckleberries (*Gaylussacia baccata*, of the family Ericaceae) grow wild on shrubs in North America. The small black fruits grow in clusters and resemble blueberries; they are not true berries but drupes that contain 10 stones. The fruit is used raw or frozen.

KIWI

Kiwi, also called kiwifruit (*Actinidia chinensis*, of the family Actinidiaceae), was developed in New Zealand, where it was known as the Chinese gooseberry. This name did not lend itself to marketing, so the name was changed to Kiwi; it began to appear in U.S. stores in the 1980s. The fruit is oval, about 2 in. in length, with brown fuzzy skin, green flesh, and a refreshing, tart flavor. It is consumed fresh or made into jam, jelly, or wine. It is sometimes dried and used as a meat tenderizer. Kiwi grows on a vigorous, deciduous vine, and hardier types are finding favor in home gardens in the United States. The hardy kiwi, *A. arguta*, produces fruit about 1 in. in length, with a pleasant, tart, slightly phenolic flavor. The hardy kiwi requires both a male and a female plant for fruit production, but another cultivar, Issae, is self-fertile.

LOGANBERRY

Loganberries (*R. loganobaccus*, of the family Rosaceae) are believed to be a cross between an American variety of blackberry and a raspberry. The fruit is eaten raw or in jams and preserves.

MULBERRY

Mulberry trees are well-known in Asia because silkworms feed on their leaves. The mulberry trees (*Morus alba*, of the family Moraceae) grown in the northern United States are large and grown for ornamental purposes, as a windbreak, and also for their fruit, which is very attractive to birds. The trees are prolific in fruit production. The more tender black mulberry (*M. nigra* and *M. rubra*) is grown in the south. The fruit resembles a blackberry and has a fruity but rather insipid flavor. The fruit is used for juice, jam, jelly, toppings, and the like.

PEACH

The peach is considered to be the queen of temperate-zone fruits and is second only to the apple as the world's most widely grown tree fruit. Peaches are native to China and have been grown for over 4000 years. They were well-known by the ancient Greeks and Romans and came to America on the ships of the colonists. Peaches are grown on all continents, but most of the peaches in world commerce are grown in the United States. World production in 1990 was estimated at about 7 million metric tons (4).

All commercial peaches are *P. persica*, of the family Rosaceae. Breeding and selection down through the ages have resulted in numerous cultivars designed for fruit quality, size, yield, and horticulturally desirable characteristics. Canning is the most popular method of preservation for both freestone and clingstone types of peaches. Sound fruit is washed, peeled, pitted, cut, and put into cans or jars. Syrup is added, and the containers are thermally processed. Frozen peaches are prepared the same way except that the containers are frozen. Single-strength juice and concentrate can be prepared by pressing the fruit. Peach nectar is juice and sweetener with enough fruit pulp to produce a more viscous liquid. Dried peaches are prepared by sun-drying the peach halves or by dehydration, in the same manner as apricots.

PEAR

Pears, of the family Rosaceae, are native to Europe and Asia and were well-known by the ancient Greeks and Romans. Breeders in France 400 years ago developed a number of cultivars, and France is still the largest grower of pears in the world. The pear was introduced to England and then to America and soon became an important crop in many countries (13). The largest producers are France, Germany, the United States, Australia, New Zealand, South Africa, Argentina, and Japan. In North America, pears are of three botanical groups: (*1*) the European pear, *Pyrus communis*, which includes all the old standard cultivars; (*2*) the Asian, Oriental, or sand pear, *P. pyrifolia* or *P. serotind*; and (*3*) the Eurasian pear, *P. lecontei*, a hybrid between the previous two. The pear is a pome fruit closely related to the apple, but it never quite attained the popularity of apples, possibly because it does not store as well.

The major processed product is canned pears prepared in a manner similar to canned peaches and apples. Dried pears are prepared as described for peaches and apples. Pear juice, pear juice concentrate, and nectar are prepared by crushing and pressing the ripe pears.

PERSIMMON

Persimmons (*Diospyros kaki*, of the family Ebenaceae) are native to the Far East and were originally cultivated in China and Japan. Today persimmons are cultivated in Europe, the Mediterranean, the United States, and many other warm parts of the world. The fruit resembles a tomato in size and shape and has a fruity, tart, astringent flavor. Another variety, *D. lotus*, cultivated in Asia, is also known as the Chinese date plum. In the United States, another native species, *D. virginiana*, occurs, but its fruit is much smaller and inferior to *D. kaki*. All commercial plantings in the United States are based on *D. kaki* and are known as the Oriental or Japanese persimmon. They are usually eaten raw or processed into juice or nectar.

PLUM

Plums are fleshy stone fruits of the family Rosaceae. The large-fruited European type, *P. domestica*, is the most important type. It originated in the Caucacus Mountains, probably from a doubling of the chromosomes of a cross between *P. cerasifera* and *P. spinosa*. The small-fruited European type, *P. insititia*, includes the purple damsons and the yellow mirabelles. The Japanese plum, *P. salicina*, is next in importance. The Simon plum, *P. simonii*, was introduced from China. A number of plums are native to America, including *P. americana*, *P. americana nigra* (from Canada), *P. munsoniana* (the wild goose plum), and *P. hortulana*. *P. angustifolia*, the Chicasaw or sand plum, is native to the southern United States. *P. maritima*, the beach plum, is abundant in the Cape Cod area. *P. subcordata* grows wild in the western United States. Plums have been extensively hybridized; more than 2000 varieties exist, but only a few are commercially important. The large European plums are largely consumed fresh, but many of the other types are canned, frozen, dried, or processed into jams, jellies, preserves, nectars, and so forth. Yugoslavia is the largest producer, with 90% of its crop processed into slivovitz brandy. Germany is second and the United States third, with about 300 tons. California produces 90% of the U.S. crop.

PRUNE

Prunes are dried plums. All prunes are plums, but not all plums are prunes. One group of plums, *P. domestica*, is known as the prune type; it is a major source of prunes, but prune plums are also eaten fresh. Plum designates a variety primarily for uses other than drying such as for fresh consumption, canning, freezing, jams, and jellies. Most plum varieties ferment if dried with the pit; however, if they are dried after removal of the pit, they are called dried plums rather than prunes (14). World production of prunes in 1994 was about 800,000 tons; California produced 70% of the world crop and 98% of the U.S. crop in 1994.

In previous years, prunes were produced by sun drying, but today nearly all prunes are produced by dehydration. Prune juice can be produced by simply leaching prunes with hot water, but recently the disintegration process has become the method of choice. Prunes are cooked in a pressure cooker with agitation to break up the fruit. The juice is then separated by centrifugation or pressing and filtered. The clear juice is then concentrated under vacuum to 19 to 20°Brix and poured into cans or bottles. Prune juice concentrate is made by treating the prune juice with a pectolytic enzyme to reduce the viscosity and concentrating to the desired solids level. At 70°Brix for domestic use and 72°Brix for export, the product is shelf stable and does not require freezing or a chemical preservative. The bulk concentrate in 5-gal pails, 55-gal drums, or tank cars is often used to reconstitute single-strength juice.

QUINCE

Quinces (*Cydonia oblongata*, of the family Rosaceae), native to western Asia, were well-known by the ancient Romans. The fruit is closely related to the genus *Pyrus* and resembles an apple. The fruit is very firm, orange, and up to 5 in. in diameter. Six varieties are known: *C. sinensis, C. lusitanica, C. malformis, C. marmorata, C. pyriformis,* and *C. pyramidalis*. The pomological quinces also include four species of the Asiatic genus *Chaenomeles* such as *C. speciosa*. Most of these are grown as ornamentals because the fruit is unpalatable. The fruit of *C. oblongata* is the most popular and is often added to other fruit because of its high pectin content or used to make quince jam and jelly.

RAISIN

Raisins are a very old product and have been known since biblical times. Raisin production spread from the Middle East to many other parts of the world. The United States and Turkey produce two-thirds of the world production of about 1 million metric tons. Raisins are produced from varieties of grapes (*Vitis vinifera*) that have a high sugar content. The sultanas produced from the Thompson seedless variety of grapes are the most well-known. Grapes to be dried into raisins are picked by hand and spread on paper trays between the rows of grapevines. The grapes on the trays have to be turned to promote even drying. They are then transported to the packing shed for cleaning, grading, adjustment of moisture content, and packaging. Raisins are usually added to bakery products, breakfast cereals, confections, chocolate bars, and the like. Raisins can also be used to make brandy, such as the aniseed-flavored ouzo in Greece or raki in Turkey.

ROSE HIP

Rose hip is the name given to the fruit of a number of *Rosa* spp. in the family Rosaceae, a very large family with more than 100 genera and 2000 species. Nearly all rose hips are very high in vitamin C, with some species (eg, *R. laxa*) containing up to 10% dry weight of vitamin C. This is 200 times that found in orange juice, so rose hips have been promoted as a source of vitamin C. The hips are seldom eaten raw and usually made into juice extracts, jams, jellies, and preserves.

STRAWBERRIES

Strawberries are a major crop with worldwide production estimated at 1,260,000 tons (15). The U.S. produces about 700,000 tons with about 80% of the production in California. Strawberries are not true berries in the botanical sense but are aggregate or multiple fruits. Strawberries belong to the family Rosaceae and the genus *Fragaria*. Many domesticated cultivars are crosses between *F. chiloensis* and *F. virginiana*, also known as *F. ananassa*. The plants are planted one year, bear fruit the second year, and sometimes are allowed to produce for a third year before replanting.

Strawberries are picked by hand and transported in flats for the fresh trade or for processing. The major processed products are frozen purees and puree concentrates for use in jams, preserves, and nectars. Strawberries are also frozen as whole or sliced fruit individually or in blocks, with or without sugar. Strawberries are also canned or made into juice concentrate. Dehydrated berries, fruit preparations, fillings, syrups, toppings, beverages, and wines are other products. Combinations of strawberry juice with many other fruit juices are increasing in popularity due to the trend for increased consumption of fruit juices.

BIBLIOGRAPHY

1. B. J. E. Teskey, and J. S. Shoemaker, *Tree Fruit Production*, AVI, Westport, Conn., 1972.
2. The L. H. Bailey Hortorium, *Hortus III*, Cornell University, Ithaca, N.Y., 1976.
3. W. H. Root, "Apples and Apple Processing," in L. P. Somogyi, D. M Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa, 1996, pp. 1–36.
4. R. Scorza and Y. H. Hui, "Apricots and Peaches," in L. P. Somogyi, D. M Barrett and Y. H. Hui, eds , *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa, 1996, pp. 37–76.
5. M. R. McLellan, "Cherry and Sour Cherry Processing," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*. Technomic, Lancaster, Pa., 1996, pp. 77–94.
6. G. Mazza and E. Miniati, *Anthocyanins in Fruits, Vegetables and Grains*, CRC Press, Boca Raton, Fla., 1993.
7. F. J. Francis, "Cranberries. Effect of Production and Processing on Sensory Properties," in H. E. Pattee, ed., *Evaluation of Quality of Fruits and Vegetables*, AVI, Westport, Conn., 1986, pp. 199–216.
8. J. S. Shoemaker, *Small Fruit Culture*, AVI, Westport, Conn., 1975.
9. J. R. Morris and K. Striegler, "Grape Juice: Factors that Influence Quality, Processing Technology and Economics," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds. *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 197–234.
10. F. J. Francis, "Concentrates as Colorants," *World of Ingredients*, 26–27 (1995).
11. P. Markakis, *Anthocyanins as Food Colors*, Academic Press, New York, 1992.
12. F. J. Francis, *Handbook of Food Colorants*, Eagen Press, St. Paul, Minn., 1999.
13. B. Brouk, *Plants Consumed by Man*, Academic Press, New York, 1975.
14. L. P. Somogyi, "Plums and Prunes," in L. P. Somogyi, D. M. Barrett and Y. H. Hui, eds., *Processing Fruits: Science and Technology, Major Processed Products*, Lancaster, Pa., 1996, pp. 95–116.
15. C. L. Duell, "Strawberries and Raspberries," in L. P Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology, Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 117–158.

F. J. Francis
Editor-in-Chief
University of Massachusetts
Amherst, Massachusetts

FRUITS, TROPICAL

AVOCADO

Avocados (*Persea americana*, avocado pears, alligator pears, of the family Lauraceae) are native to Central America and are grown worldwide, but they became an important commercial crop in the United States and Mexico in the twentieth century. Worldwide production in 1992 was estimated to be about 1.4 million metric tons, of which Mexico produced about 785,000 tons and the United States 155,000 tons (1).

Avocados are classified into three types:

1. The West Indies type (*P. americana* var. *americana*) is grown in tropical regions. The fruit is large and round, with a thick skin and a yellowish-green pulp color. The oil content is less than 8%.
2. The Mexican type (*P. americana*, var. *drymifolia*) is grown in both tropical and subtropical regions. The small fruit has a thin green skin, and the green pulp has an oil content up to 30% by weight.
3. The Guatemalan type (*P. americana* var. *guatemalensis*) is grown in both tropical and subtropical regions. The green fruit has a thick skin, a high oil content, and a nutty flavor.

Avocados have traditionally been marketed fresh, but a large processing industry developed in the 1960s primarily to produce a spiced puree known as guacamole. The avocados are washed, sorted, sanitized in a hypochlorite solution, and cut to remove the large seed; the flesh is then passed through an extractor to remove the skin. The resultant puree is packed, frozen, and sold to both the consumer and institutional trade for the manufacture of guacamole. Oil can be extracted from the fruit by solvent extraction of the dried fruit, by hydraulic pressing of the dried slices, or by centrifugation of the fruit puree.

Avocados as purchased are usually very firm and have to be allowed to ripen before consumption. They do not ripen on the tree. After harvesting the rate of ripening depends on the maturity of the fruit, the temperature, the levels of oxygen and carbon dioxide, and the humidity. The processors require a uniform supply of ripe fruit, so the fruit is usually treated with ethylene gas in ripening rooms to ensure uniform ripening. For the fresh fruit trade, the fruit may be preconditioned with a short ethylene treatment to ensure more rapid ripening in supermarkets and restaurants. Discoloration after cutting is a major problem for the processors, but it has been minimized by rapid handling and proper selection of cultivars. Avocados are unique in having the highest protein and oil content of any fruit. The U.S. processed market has been estimated at 23,000 tons and will probably increase as Mexican foods become more popular (1).

BANANA

Bananas (*Musa paradisiaca* var. *sapientum*, of the family Musaceae) are native to India and Malaysia and are grown in every wet tropical area in the world. The cultivated varieties are derived from the wild species *M. acuminata* and *M. balbisiana*, and a number of other *Musa* spp. are grown as ornamental plants. The only edible species that thrives outside the tropics, where the temperature never falls below 18°C, is *M. cavendishii*, which despite its delicious flavor is not popular because its small brown fruit is less desirable in appearance. Bananas are the most widely consumed and most highly consumed (in terms of volume) of any fresh fruit in the world, partially due to their ease of shipping and year-round availability.

Musa spp. are large herbaceous plants that grow from an underground rhizome. The shoots, which may be up to 30 ft tall, produce an inflorescence at the top that develops into a stem of bananas. The weight of the bananas, up to 120 lb, causes the stem to bend over. After harvesting, the stem is cut off and left in the field. A new planting will produce a stem in about 18 months, and a daughter stem from the same rhizome will produce a stem in about 12 months. A third stem from the same rhizome may produce a stem in about 8 months, after which the field is usually replanted. The stem is cut while the fruit is immature and green in color, and the fruit ripens during and after shipping. This provides a 2- to 3-week period for shipping, which is very convenient for worldwide delivery. Most of the banana production is centered in Central America, South America, and the Caribbean.

The large fresh fruit industry provides the raw material for the processed industry because fruit that is unsuitable for the fresh trade or is in oversupply is usually processed. The green fruit is transported to the holding sheds, treated with ethylene gas to ensure uniform ripening, and processed. The major product is banana puree, which is made by mechanically peeling the fruit, pureeing the flesh, and deseeding the puree if desired. Actually, bananas have no seeds; the black specks are atrophied seed bags, but they are commonly called seeds. The seeds can be removed with a screen. The puree is then deaerated, which also removes some of the flavor essence. The flavor components are collected and added back to the puree. The puree can be packed as single strength or (more often) concentrated and thermally treated or frozen before packaging. Both single-strength and concentrated puree are used extensively in the baby food industry, as juice components, and in many baking and confectionery products. Banana flakes are prepared by drum-drying the puree. Banana powder is made by grinding banana flakes. Banana "figs" are made by drying slices of ripe bananas. Banana chips are made by slicing green cooking bananas and deep-frying them in oil. A number of other minor products are also made.

BREADFRUIT

Breadfruit (*Artocarpus altilis*, of the family Moraceae) is native to Polynesia. The breadfruit tree was discovered by Captain Cook in Tahiti, and it was thought that the fruit would be a good source of food for slaves in the West Indies. Accordingly, Captain Bligh, on the Bounty, was commissioned to bring seedlings to the West Indies. A mutiny thwarted his first try, but he succeeded on his second trip. He even planted one himself in the botanic garden of St. Vincent in 1793, and apparently it is still there today (2).

The fruits are large, up to 10 in. in diameter, and borne on a large tree up to 60 ft tall The edible portion is a thick, fleshy layer between the rind and the core. It is usually eaten immature when the flesh is still white and is boiled, baked, roasted, or fried but never eaten raw. Because of its high starch content, the fruit can be dried, ground into flour, and baked into bread, cakes, and a fermented dough product known as mahe. The usual breadfruit has no seeds and is propagated by cuttings, but some cultivars produce seeds, which are called bread nuts. The seeds are eaten boiled or roasted. Breadfruit has been introduced into all of the wet tropical countries, but it never attained the importance envisioned by Captain Cook.

CARAMBOLA

Carambolas (*Averrhoa carambola*, caramba, bliming, or country gooseberry, of the family Oxalidaceae) are native to Indonesia but are grown in all tropical countries. The yellow fruit is up to 5 in. long and 3 in. wide, with five pronounced ribs and a star-shaped cross section. The flesh is translucent, yellow, tart, and juicy and contains large brown seeds. The fruit is eaten raw or made into juices, jams, jellies, and tarts. A related species, *A. belimbi*, known as Belimbi, produces green acidic fruit that is used to make pickles, curries, and preserves.

CASHEW APPLE

The cashew tree (*Anacardium occidentale*, of the family Anacardiaceae) is native to tropical America, but today it is grown chiefly in India, Tanzania, and Mozambique. The tree produces three products of commercial interest. The cashew apple is a pear-shaped fruit about 4 in. long with a very acidic taste. The fruit is eaten raw in the local markets or made into fresh and fermented juices. A protuberance on the end of the apple contains the cashew nut, which is highly prized on the worldwide market. The outer coating of the cashew nut, which is removed before shipping, contains a very corrosive, black oil that is recovered and sold on the industrial market. Cashew nuts can be pressed to produce cashew nut oil, which is a prized flavorant and should not be confused with the oil from the seed coat.

CERIMAN

Ceriman (*Monstera deliciosa*, or monster plant) is native to Mexico and Guatemala and belongs to the family Aracea. This is a very large family with about 15 genera and 2000 species. Several aroids are grown in the tropics for their edible tubers (taro, dasheen, tannia, malonga, and ocumo) and are an important starch staple of the diet. *M. deliciosa* is a robust climbing vine that produces tiny fruit on a spadix up to 10 in. long. The spadix is eaten raw and is reputed to have a delicious flavor resembling that of pineapple.

CHERIMOYA

Cherimoyas (*Annona cherimola*, of the family Annonaceae) are native to South America. The fruit is heart shaped or conical and can weigh up to 1 lb. The soft white flesh contains many beanlike seeds, and the flavor somewhat resembles that of a pineapple. The fruit is eaten raw or processed into juice.

CUSTARD APPLE

Custard apples (*A. squamosa*, of the family Annonaceae), also known as sweetsops or sugar apples, are native to South America. The yellowish-green fruits, up to 4 in. in diameter, have a scalelike covering that resembles a coniferous cone. The edible custardlike, granular pulp is white, and the seeds are embedded in it. The pulp is very sweet, with up to 18% sugar. The fruit is very perishable and hence little known outside the producing regions. The fruit is eaten raw.

DATE

Dates (*Phoenix dactilifera*, of the family Palmae) probably are native to India despite the fact that they have been grown in the Arab countries for many thousands of years. The date palm is grown in all hot, dry areas of the world but primarily in the desert areas of the Middle East and the Sahara. Dates are also grown in California, Arizona, and Mexico. The date palm is a dioecious plant, meaning that both male and female plants must be grown to ensure pollination. The fruit is borne at the top of a very tall (up to 90 ft) tree and is pollinated by suspending a small branch of the male flowers over the female inflorescence. Each individual fruit contains a hard seed called a stone. The seed is surrounded by an edible fleshy endocarp that when dried contains up to 70% sugar. The high sugar content ensures that the fruit will not spoil during storage or shipping. Dry dates are consumed fresh or incorporated into a wide variety of bakery products, confections, dairy products, preserves, spreads, beverages, and other products. The Arabs are believed to have over 800 different uses for the date, perhaps because it is almost the only plant available in the desert areas (2).

DURIAN

Durians (*Durio zibethicus*, of the family Bombacaceae) are native to Malaysia. The fruits, weighing up to 10 lb, are borne on a very large tree up to 90 ft tall. The fruit has a spiny outer covering surrounding cream-colored flesh with a large seed. When ripe, the fruit drops to the ground and soon turns rancid. The durian is noted for the foul odor of the ripe fruit, and only people who can get past the odor can enjoy the delicious taste of the fruit.

FEIJOA

Feijoa (*Feijoa sellowiana*, or pineapple guava, of the family Myrtaceae) grows on a small tree native to South America. The green fruit tinged with red is 2 to 3 in. long. The white flesh is sweet and consumed fresh or made into juices, jams, jellies, and preserves.

GUAVA

Guavas (*Psidium guajava*, of the family Myrtaceae) are native to tropical America and are grown in nearly all tropical areas in the world. The fruit of the best-known guava, *P. guajava* var. *pyriferum*, varies greatly in size, up to 0.5 lb, and color, from white to yellow or red. The fruit from *P. guajava* var. *pomiferum* is smaller and redder. *P. cattleianum* or *P. littorale*, native to Brazil, has small, round, purple fruit. The small purple strawberry guava, *P. littorale* var. *longipes*, is also widely cultivated. The fruit may be consumed raw, canned, frozen, or in the form of juice. It is widely used for jams, jellies, preserves, and confections. The fruit contains about 240 mg/100 g of vitamin C and has been promoted as a good source of vitamin C.

ILAMA

Ilamas (*A. diversifolia*, of the family Annonaceae) are native to South America. The fruit is a medium-sized berry formed by the union of the pistils and the receptacle. The fruit is consumed fresh or in juices, jellies, sherbets, or fruit desserts.

JABOTICA

Jaboticas (*Myrciaria cauliflora*, of the family Myrtaceae) are native to South America and grow on small evergreen shrubs. The grapelike berries may be consumed fresh or as juice, jam, jelly, or preserves.

JACKFRUIT

Jackfruit (*Artocarpus heterophyllus* [Syn. *A. integrifolia* or *A. integra*]) belongs to the same family (Moraceae) as breadfruit. It is a native of India and is grown in almost every tropical area. The trees are very large, up to 70 ft tall, and bear the largest known fruit. The fruit is up to 4 ft long, 1 ft in diameter, and weighs up to 100 lb. It is a compound fruit developed from the entire female inflorescence, with a green coating covered with hexagonal spines. The pulp is composed of many fruits, each surrounding a large seed. The edible part is the red pulp surrounding the seeds, and it has a very sweet but rather insipid flavor. The pulp is consumed raw or as juice, jam, or jelly. The seeds are also edible after boiling or roasting. The immature fruit is consumed as a vegetable, either boiled or roasted.

JAVA PLUM

Java plums (*Syzygium cumini*, of the family Myrtaceae) are native to Asia. The small purplish-red fruit is eaten fresh.

JUJUBE

Jujubes (*Ziziphus jujuba* or *Z. mauritana*, Chinese jujube, or Chinese date, of the family Rhamnaceae) are native to the Mediterranean area. The fruit is small, mealy, deep yellow, and borne in clusters. It is used in sweet pickles, stews, preserves, and confections. It is sometimes called lotus in Greek mythology, not to be confused with the sacred lotus, an aquatic plant, *Nelumbo nucifera* or *Nelumbian nuciferum*, grown in India and China. In Homer's *Odyssey*, lotus probably refers to *Z. jujuba*, but it should be called a pseudocereal or pseudobeverage rather than a fruit (2).

LIMES

Limes (of the family Rutaceae), native to the East Indian Archipelago, are the least cold hardy of the citrus fruits and are grown in tropical areas throughout the world. Three lime species are recognized: the small-fruited *Citrus aurantifolia*, the large-fruited *C. latifolia*, and the sweet lime, *C. limettoides*. Limes are often described by their area of production. The most popular small-fruited type is called the West Indian, Mexican, or Key lime. The large-fruited types are called Tahitian or Bearss and are grown in Australia, Brazil, California, and Florida. The Indian type is the favorite sweet lime and is grown in India and Pakistan. U.S. production of limes in 1992 was estimated at 1.6 million boxes (3).

Limes are very acidic and are seldom eaten fresh. They are much in demand as a flavorant for other products such as juices, jams, jellies, baked goods, and alcoholic beverages. British sailors became known as limeys because they were fed limes for the prevention of scurvy. Despite this folklore, limes, at 37 mg/100 g, have less vitamin C than lemons (2).

MAMEY

Mameys (*Mammea americana*, or Mammey apple) belong to the family Guttiferae and are native to Central America and the West Indies. The fruit is a drupe the size and shape of an orange and with a brown russeted skin. The yellow, edible flesh surrounds a large seed. The fruit is consumed fresh or as jams and preserves.

MANGO

Mangos (*Mangifera indica*, of the family Anacardiaceae) are native to southern and Southeast Asia. It is one of the most important tropical and subtropical crops in terms of production and popularity. The country with the largest production is India, where it has been cultivated for over 4000 years, but it is now grown in almost all tropical areas. India produces 60% of the worldwide crop of about 17 million tons (4).

Mangos grow very large, handsome trees up to 90 ft tall with a spread of 125 ft. The fruit is ovoid or heart shaped, about 3 to 5 in. long, with a skin color varying from green to yellow to red. When ripe, the flesh is yellow-orange and fibrous, with a large central seed. The flavor is fruity with a terpene flavor. One irreverent British visitor described the wild mango as having a flavor resembling that of a piece of cotton batting dipped in turpentine. But centuries of breeding have resulted in hundreds of commercial cultivars with truly delicious flavors. The major processed product is mango puree, which is used as an ingredient for many other products such as juices, nectars, jams, preserves, confections, and baked goods. Mango slices, canned or frozen, are available. Immature, green frozen slices are also popular. Mango leather is an ethnic food in India. It is prepared from mango puree by removing some of the fiber and drying in the sun. The unripe fruit is used in chutneys and pickles.

MANGOSTEEN

Mangosteens (*Garcinia mangostana*, of the family Guttiferae) are native to Malaysia and are found in Java, Sumatra, the Philippines and Sri Lanka. The fruit is a reddish-purple berry about the size of a small apple with segments similar to citrus fruits. The edible portion is the white flesh in the arils surrounding the seeds.

MIRACLE FRUIT

Miracle fruit (*Synsepalum dulcificum*, of the family Sapotaceae) is native to tropical West Africa. The fruit is a small red berry with white flesh. It is consumed fresh with other foods. Its major quality is the ability to make sour foods taste sweet. This effect on the taste buds received much research attention, but the fruit was not commercialized because of lack of U.S. Food and Drug Administration (FDA) approval.

PAPAYA

Papayas (*Carica papaya*, or pawpaw, of the family Caricaceae) are native to tropical America. Brazil is the major producer, with worldwide production in 1992 estimated at 4 million metric tons (4). The papaya plant is a giant herb, rather than a tree, because it lacks woody tissue. The stem grows 20 to 30 ft tall and bears fruit near the stem. The fruit may be oblong or elliptical, up to 20 lb, and orange. The flesh may be orange to red and surrounds a large seed cavity containing many seeds. The fruit is consumed fresh or as puree, which is used to produce juices, nectars, jams, jellies, sherbets, dairy products, and confections Papaya leather is made by drying the puree. Papaya slices or chunks are canned or frozen and used in fruit salads, ice creams, sherbets, and confections.

Another plant is also called PaPaw. This is *Asimina triloba* (Michigan banana, of the family Annonaceae), a small ornamental tree that grows in temperate zones. The fleshy, oblong fruit is about 3 to 5 in. long and has a banana-like flavor. It is eaten fresh or as an ingredient in fruit salads, baked goods, and confections.

PASSION FRUIT

Passion fruit (*Passiflora edulis*, of the family Passifloraceae) is native to Brazil and is grown in all tropical areas around the world. A number of species are grown for their edible fruit: purple granadilla (*P. edulis*), yellow granadilla (*P. laurifolia*), sweet granadilla (*P. ligularis*), sweet calabash (*P. maliformis*), curaba (*P. mollissima*), and giant granadilla (*P. quadrangularis*). The round berry, grown on a woody vine, is about 2 in. in diameter and contains a juicy pulp with many seeds. Single-strength or concentrated juice, frozen or canned, is the major processed product. The flavor is very susceptible to damage by heat; therefore, the frozen product is preferred over the canned product, but recent advances in aseptic packaging have produced superior products. Passion fruit juice is very popular for blending with other juices. The fruit is also used in fruit salads, jams, jellies, and confections.

PINEAPPLE

Pineapples (*Ananas comosus*, of the family Bromeliaceae) are native to tropical South America and are grown in nearly all tropical areas. The plant is a rosette of long, stiff, fleshy leaves that produces a short flower stem. The individual fruitlets fuse to produce a compound fruit. Despite its economic importance, only one cultivar is of commercial importance. The cultivar Smooth Cayenne comprises 95% of the worldwide crop. The major processed products are solid pack (slices, chunks, and tidbits), crushed (dices), and juice. The fruits are handled by a machine called a ginaca, which processes about 100 pineapples a minute into eight fractions: the cylinder, core, skin, eradicator meat from the skin, crown end, eradicator meat from the crown end, butt end, and eradicator meat from the butt end. The cylinder is used to make the high-value products: slices, chunks, tidbits, and crushed pineapple. The eradicator fractions and the core are used to make juice. The skin, crown end, and butt end are used to make by-products. The mixture may be pressed to make mill juice, which can be added to the solid pack or used to dissolve sugar to make a syrup for addition to the solid pack. The residue from the pressing operation is sold as cattle feed. The mill juice can also be used as a sugar source for the production of alcohol or vinegar. Recent research indicates that mill juice can be purified sufficiently to allow the product to compete with grape juice as a sugar base for fruit beverages. Solid pack pineapple, crushed pineapple, and pineapple juice are usually preserved by thermal treatment (5). Pineapples are often consumed raw; many people consider the pineapple to be the most palatable fruit of all (2). They are often used as flavorful ingredients for juice products, jams, jellies, preserves, dairy products, and confections. The pineapple plant is also the source of the proteolytic enzyme bromelin.

PLANTAIN

Plantains (*M. paradisiaca*, or cooking bananas, of the family Musaceae) are closely related to bananas. They are grown in most tropical countries. They have a higher starch content than bananas and are eaten raw or as a cooked vegetable. A flour is often prepared from plantains and added, as a thickener, to a number of foods. The flour itself can be used to prepare baked goods. The fruit can be french fried to make chips or fermented to make alcoholic beverages.

PRICKLY PEAR

Prickly pears (*Opuntia ficus-indica*, tuna, or Indian fig), native to the dry areas of North and South America, belong to the Cactaceae or cactus family. Six other species of *Opuntia* also produce edible fruit. The red to purple fruit, 3 to 4 in. long, is covered with small prickles and is consumed fresh or dried. The fruit is also used in jams, jellies, preserves, and candy.

PULASAN

Pulasans (*Nephelium mutabile*, of the family Sapindaceae), native to Southeast Asia, are another arillate fruit similar to litchis and rambutans.

RAMBUTAN

Rambutans (*N. lappaceum*, of the family Sapindaceae) are native to Malaysia. The rambutan is similar to the litchi in appearance and structure except that rambutans are covered with soft spines. The fruit is eaten raw or canned, primarily for export from Thailand.

ROSE APPLE

Rose apples (*S. jambos*, of the family Myrtaceae) are native to Asia. The small yellow fruits are eaten fresh, dried, or in confections. The species *S. malaccense* is closely related, with larger fruit up to 2 in. in diameter. The fruit is also called Malay apple and is eaten raw, cooked, or in jams, preserves, and wine.

SANTOL

Santols (*Sandoricum koetjape*) are native to Asia. The medium-sized, spherical, yellow fruit has a fruity, sour taste and is consumed fresh or as jams, jellies, and preserves.

SAPODILLA

Sapodillas (*Manikara achras* [Syn. *M. zapotilla* or *Achras zapota*], sapotilla, sapota, zapotle, chiku, or naseberry, of the family Sapotaceae) are native to Mexico and South America and are grown in nearly all tropical areas. The fruit is a fleshy berry, up to 3 in. in diameter, with a rusty brown skin. The pulp is yellowish-brown to red in color, with a mild aroma and a slightly astringent, pleasant taste. The fruit is usually eaten fresh because processing degrades the delicate flavor (6).

SOURSOP

Soursops (*A. muricata*, guanabana, or sapote agrio, of the family Annonaceae) are native to North and South America. The oval fruit weighs up to 3 lb and has a dark green, leathery skin covered with many short, curved, fleshy spines. The white cottony pulp contains many shiny dark brown seeds and has a distinctive flavor and aroma. The fruit can be eaten fresh, but more often the pulp is extracted and used in beverages, sherbets, ice creams, and syrups. The pulp and the puree can be canned or frozen (6).

STAR APPLE

Star apples (*Chrysophyllum cainito*, or caimito, of the family Sapotaceae) are native to North and South America. The fruits are globular, up to 4 in. in diameter, and star shaped in cross section, with a light green to purple skin. The white, translucent pulp contains three to eight seeds, and the fruit is usually eaten fresh.

TAMARIND

Tamarinds (*Tamarindus indica*, tamarin, tamarindo, or imli, of the family Leguminosae) are probably native to East Africa but were introduced into India long ago. The tamarind was well-known by the Egyptians and Greeks of the fourth century. The tamarind grows on a large evergreen tree of the family Leguminosae. The fruit is an irregularly shaped pod about 8 in. long. As the pod matures it turns into a brown, brittle shell containing a dry sticky pulp that surrounds red-brown seeds. Tender shoots and flowers are consumed as vegetables in salads, soups, and curries. Immature pods are used as seasoning for cooked rice, fish, and meats. Pulp from the mature pods is pressed into cakes and is valued as highly as dates and figs. Tamarind pulp is an important ingredient in barbecue sauces and as a fruit base in juices, jams, jellies, preserves, sherbets, dairy products, and alcoholic beverages (6).

TREE TOMATO

Tree tomatoes (*Cyphomandra betaceae*, or tamarillo, of the family Solanaceae) are native to tropical America. The round, juicy, orange-red fruit, about 2 in. in diameter, grows on a 10-ft shrub and has a mild sour taste. The fruit can be eaten fresh but is usually made into jams, jellies, and preserves.

WAX APPLE

Wax apples (*S. samarangense*, java apple, or jambosa, of the family Myrtaceae) are native to Asia. The fruit is a whitish-red berry about 1 in. long with a mild insipid flavor. It is usually consumed fresh.

BIBLIOGRAPHY

1. A. Kurlaender, "Avocadoes," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 445–458.
2. B. Brouk, *Plants Consumed by Man*, Academic Press, New York, 1975.

3. D. Kimball, "Grapefruits, Lemons and Limes," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 305–336.
4. J. S. Wu and M. J. Scheu, "Tropical Fruits," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 387–418.
5. C. E Mumaw, "Pineapples," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology, Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 337–360
6. S. Lakshminarayana, "Sapodilla and Prickly Pear," in S. Nagy and P. E. Shaw, *Tropical and Subtropical Fruits*, AVI, Westport, Conn., 1980, pp. 415–441.

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amherst, Massachusetts

FRYING TECHNOLOGY

Frying is a process of dehydrating food from the surface inward. The process typically uses triglyceride-based oil (lipid) from an animal or vegetable origin to transfer thermal energy from a heat source to food immersed in the oil. The efficiency of heat transfer is mediated by surfactant chemical species (wetting agents) included or formed in the oil that control the contact time between hydrophobic oils and aqueous foods. Heat capacity of the oil is primarily determined by the ratio of triglyceride to polymer formed in the oil.

A dynamic balance occurs between water movement from and oil movement into the frying food at a given temperature (typically 150–190°C) and food immersion time. The dynamic balance and the kinetics of the process are further mediated by the state of degradation of the oil as influenced by its exposure to primarily heat, oxygen, water, and chemicals and particles from the frying food. Fatty acid and smaller organic molecules formed in the oil generally increase thermal conductivity of the oil at the food surface.

Three surveys of frying topics covering chemical, physical, engineering, sensory, and nutritional aspects of frying oils and fried food are currently available (1–3).

PROCESS ANALYSIS

Frying is a unit operation composed of multiple subsystems. A fryer is a process reactor wherein chemical and physical interactions are induced to occur between oil and food. Thermal energy is supplied to the reactor by heating the oil in the fryer vat through direct or indirect means, and waste energy escapes to the environment by conduction through the reactor walls and by water evaporation. Raw food containing an excess of water (and sometimes solids) enters the reactor in immersed batches (basketloads) or immersed or submerged continuous (conveyor) streams, and the food emerges in a cooked state separated from waste materials: water (liquid and vapor), organic volatiles, and particulate solids.

Oil in the reactor vat is replenished to make up for losses incurred by adsorption and absorption by the food. Oil is also filtered (cleaned) to remove particulates, some oil degradation chemicals, and interaction by-products formed between oil and food degradation products such as emulsified water. Oil filtration and/or treatment materials are discarded from the oil cleaning and/or filtering apparatus, and the oil is recycled into the fryer until the oil is out of specification for food manufacture; it is then discarded from the process. The process reactor when drained of oil also requires cleaning and sanitation to prevent fouling and to provide a contaminant-free process environment; this step also generates a waste stream of chemicals, particulates, and water.

Process monitoring and control involve thermal and other measurements of the reactor's input and output streams, sampling oil and food to judge product quality and potential shelf life, and sampling waste streams to suggest the extent of needed treatment.

ENGINEERING PRINCIPLES

It is possible to calculate the many aspects of heat and mass transfer most important to the frying process based on the expected loss of water and the mass of food to be dehydrated. Unfortunately, these calculations do not take into account the constant changes occurring in a degrading heat transfer medium (the oil) and the accumulation of surfactant species in the oil due to both food and process influences. Numerical modeling has not yet advanced beyond examining fresh oil model systems.

There are five stages in the life of frying oil that produce, in sequence, raw, cooked, and overcooked food. The following is a description of how frying proceeds and why surfactant chemicals control the kinetics and dynamics of the frying process. From the perspective of physical chemists and process specialists, the cooking of food in an oil can be reduced to simple engineering principles with parallel simple measurement and control procedures. This is a new paradigm of frying and is different from that of the paradigm of organic chemists and food scientists, who initially studied the complexities of frying oil and food chemistry to develop databases of results and observations.

The model of understanding frying in terms of physical chemistry and engineering leads to the belief that the foremost way to judge frying and frying oils is by evaluating the physical properties of fried foods. Only the process variables affecting the physical properties of fried foods can be controlled in the engineering sense. Temperature profiles, water loss from food, and oil absorption into food are amenable to process control. On rare occasions, the pressure over the frying oil is also controlled.

Factors such as flavor development and typical finished food color are not primarily controlled by the process. Rather, they are dependent on the source of the oil, the content and type of surfactants, the type and composition of food fried, and a range of organic reactions, only some of which depend directly on process variables.

3. D. Kimball, "Grapefruits, Lemons and Limes," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 305–336.
4. J. S. Wu and M. J. Scheu, "Tropical Fruits," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology. Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 387–418.
5. C. E Mumaw, "Pineapples," in L. P. Somogyi, D. M. Barrett, and Y. H. Hui, eds., *Processing Fruits: Science and Technology, Major Processed Products*, Technomic, Lancaster, Pa., 1996, pp. 337–360
6. S. Lakshminarayana, "Sapodilla and Prickly Pear," in S. Nagy and P. E. Shaw, *Tropical and Subtropical Fruits*, AVI, Westport, Conn., 1980, pp. 415–441.

F. J. FRANCIS
Editor-in-Chief
University of Massachusetts
Amherst, Massachusetts

FRYING TECHNOLOGY

Frying is a process of dehydrating food from the surface inward. The process typically uses triglyceride-based oil (lipid) from an animal or vegetable origin to transfer thermal energy from a heat source to food immersed in the oil. The efficiency of heat transfer is mediated by surfactant chemical species (wetting agents) included or formed in the oil that control the contact time between hydrophobic oils and aqueous foods. Heat capacity of the oil is primarily determined by the ratio of triglyceride to polymer formed in the oil.

A dynamic balance occurs between water movement from and oil movement into the frying food at a given temperature (typically 150–190°C) and food immersion time. The dynamic balance and the kinetics of the process are further mediated by the state of degradation of the oil as influenced by its exposure to primarily heat, oxygen, water, and chemicals and particles from the frying food. Fatty acid and smaller organic molecules formed in the oil generally increase thermal conductivity of the oil at the food surface.

Three surveys of frying topics covering chemical, physical, engineering, sensory, and nutritional aspects of frying oils and fried food are currently available (1–3).

PROCESS ANALYSIS

Frying is a unit operation composed of multiple subsystems. A fryer is a process reactor wherein chemical and physical interactions are induced to occur between oil and food. Thermal energy is supplied to the reactor by heating the oil in the fryer vat through direct or indirect means, and waste energy escapes to the environment by conduction through the reactor walls and by water evaporation. Raw food containing an excess of water (and sometimes solids) enters the reactor in immersed batches (basketloads) or immersed or submerged continuous (conveyor) streams, and the food emerges in a cooked state separated from waste materials: water (liquid and vapor), organic volatiles, and particulate solids.

Oil in the reactor vat is replenished to make up for losses incurred by adsorption and absorption by the food. Oil is also filtered (cleaned) to remove particulates, some oil degradation chemicals, and interaction by-products formed between oil and food degradation products such as emulsified water. Oil filtration and/or treatment materials are discarded from the oil cleaning and/or filtering apparatus, and the oil is recycled into the fryer until the oil is out of specification for food manufacture; it is then discarded from the process. The process reactor when drained of oil also requires cleaning and sanitation to prevent fouling and to provide a contaminant-free process environment; this step also generates a waste stream of chemicals, particulates, and water.

Process monitoring and control involve thermal and other measurements of the reactor's input and output streams, sampling oil and food to judge product quality and potential shelf life, and sampling waste streams to suggest the extent of needed treatment.

ENGINEERING PRINCIPLES

It is possible to calculate the many aspects of heat and mass transfer most important to the frying process based on the expected loss of water and the mass of food to be dehydrated. Unfortunately, these calculations do not take into account the constant changes occurring in a degrading heat transfer medium (the oil) and the accumulation of surfactant species in the oil due to both food and process influences. Numerical modeling has not yet advanced beyond examining fresh oil model systems.

There are five stages in the life of frying oil that produce, in sequence, raw, cooked, and overcooked food. The following is a description of how frying proceeds and why surfactant chemicals control the kinetics and dynamics of the frying process. From the perspective of physical chemists and process specialists, the cooking of food in an oil can be reduced to simple engineering principles with parallel simple measurement and control procedures. This is a new paradigm of frying and is different from that of the paradigm of organic chemists and food scientists, who initially studied the complexities of frying oil and food chemistry to develop databases of results and observations.

The model of understanding frying in terms of physical chemistry and engineering leads to the belief that the foremost way to judge frying and frying oils is by evaluating the physical properties of fried foods. Only the process variables affecting the physical properties of fried foods can be controlled in the engineering sense. Temperature profiles, water loss from food, and oil absorption into food are amenable to process control. On rare occasions, the pressure over the frying oil is also controlled.

Factors such as flavor development and typical finished food color are not primarily controlled by the process. Rather, they are dependent on the source of the oil, the content and type of surfactants, the type and composition of food fried, and a range of organic reactions, only some of which depend directly on process variables.

Two traditional means of controlling the transfer of thermal energy to frying food are heater temperature control (at designed energy flux) and residence time of the food in the heated oil bath. Overheated oil at the surface of the heaters is reduced to carbon deposits (coke) on the heater surfaces. As the carbon layer builds and becomes an insulating jacket, the heater cycle on times and temperatures are increased to keep the oil at frying temperature. A new frying oil somewhat resists this process, and heat transfer rates and ratios are essentially a static system. As the oil degrades, however, and products such as thermally formed and food-formed surfactants increase, the dynamics of the heat transfer system accelerates. Oil makes successively better contact with the food exterior and thus excessively dehydrates that layer. Thermal energy is expended to convert more and more surface water to steam. This ever-deepening dehydration of the crust phenomenon robs energy that otherwise would go to heat and cook the interior of the food.

The time food spends in heated oil can be varied to achieve a particular degree of cooking. Times vary from blanching for 30 s to cooking a chicken for 20 min. Manual placement and removal of basketloads of food control residence time in smaller fryers. Larger, or continuous, fryers often use conveyor systems to control frying food residence time. Unfortunately, heater designs and residence time variations leave out considerations of the chemical changes that take place in the oil with time. Such changes have been described in depth by analytical and organic chemists, but physicochemical changes that affect heat and mass transfer of the oil with use have been largely, although not completely, ignored. Although electromechanical devices for controlling fryer heaters and residence time are well developed (based on the incomplete engineering concept that the thermal properties of oils are constant), further analysis of frying systems has ignored available information related to chemical changes taking place in the frying oil.

FRYERS

Fryers are basically oil vats with heaters. The heaters transfer thermal energy into frying oil. The frying oil then transfers heat energy to the food's surface. Heat not used up at the surface is conducted into the interior of frying food. Heaters are turned on and then off as predetermined low and high set points of temperature (energy) in the oil are reached.

The heaters operate at temperatures considerably higher than the maximum set point temperature of the oil. The energy density (flux) of the heater's surface is high to heat the oil quickly to operating temperature regardless of energy losses to the environment. Cycle rates are adjusted by a set point thermocouple to counter overall heat loss at the heater surface, which includes energy dissipated to oil, fryer machinery, and also to further heat loss in the oil due to food loading.

Engineering of heater configuration is usually based on thermal calculations supposing that frying is really a food dehydration process. Heater design calculations entail determining the specific heat of the dry mass of the food, the percentage of water, the change of state of water from ice to water and water to steam, and the specific heat of the frying oil (figured as a constant representing new oil). For production fryers, heater placement and energy density in the oil vat are designed to compensate for food-loading points (high density) and take-away points (low density) and to compensate for heat sinks such as conveyers, crumb collectors, and filtering systems.

FOOD AND OIL QUALITY INTERACTIONS

A broad model describing all the qualities of frying oils and foods prepared in these oils is a continuum of frying oil degradation changes that could be associated with fried food qualities. Five stages of frying oil degradation are identified.

Figure 1 suggests that all frying oils behave similarly in the frying environment and that their bulk effects change slowly, even though the minor products of degradation may have a strong relationship to consumer acceptance of foods. Furthermore, each oil degradation stage can have different associated analytical values depending on the food being cooked. For instance, the degraded oil stage end points for potato chips, breaded vegetables, and fast food are found at 13%, 17%, and 25% total polar materials (TPM) content, respectively.

Within narrow windows, these TPM chemical index values seem relatively invariant across the food industry and are closely linked with the heating history of the oils. However, specific chemical markers used as process measurement end point specification values such as percentage of free fatty acids (FFAs) and other relatively minor degradation products vary with the mix of foods being fried. The percentage of FFAs end point values for the degraded stage are 0.5%, 2.0%, and 5.0%, respectively, for snack food, processed meat food, and fast-food industry segments. A generalized table for fast foods is shown in Table 1.

FRYING OIL DEGRADATION

The simple hydrolysis of oils (triglycerides) due to heat and moisture at ordinary frying temperatures forms quantities of FFAs. The FFAs then react with oxygen, each other, food juices, and a variety of intermediate degradation products

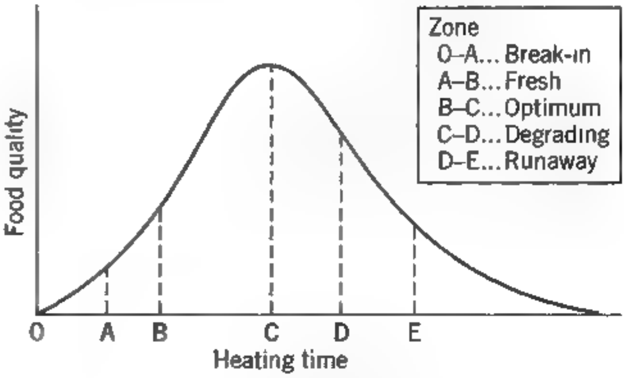


Figure 1. Frying oil quality curve.

Table 1. Values for Fast-Food Degradation by Stages

Parameter	0	Break-in	Fresh	Optimum	Degraded	Runaway
TPM (%)	<4	10	15	20	25	35
FFAs (%)	0.02	0.5	1.0	3.0	5.0	8.0
Mono- and diglycerides (%)	2	6	7	3	1	0.5
Surfactants	0–7	10	35	65	>150	>200
Dimer and polymer (%)	0.5	2	5	12	17	25
Oxidized fatty acids (OFA) (%)	0.01	0.08	0.2	0.7	1.0	2.0
Metals (ppm)	1	5	10	15	25	50

in the oil. These new chemicals in frying oil are either volatile (and leave) or are soluble (and some are even suspended colloids) in the triglycerides that comprise the oil. Monoglycerides and diglycerides formed after hydrolytic splitting off of fatty acids are generally more reactive than the original triglycerides. Acrolein, formed by the dehydration reaction of glycerol, may be an important toxicant or irritant in the oil and frying vapor, but it is rarely investigated.

The degradation of frying oil is also accelerated by the soluble chemicals that are either left in the oil as residues, additives, and artifacts of refining or that are introduced into the oil by frying food. As plant and animal cells (whole food) explode during cooking, the watery (plasma) juices of the cells introduce foreign chemicals into the oil. A similar process occurs when watery systems in formed and fabricated foods are introduced into hot frying oil.

Exposure to heat, light, crumbs, reactive metals, and food juices causes frying oil to react more and more rapidly with its own degradation products, with oxygen from the air, and with food-introduced contaminants. The ongoing combination of chemical changes finally causes a fry chef or line operator to dispose of a batch of oil because poor oil makes poor-quality fried foods.

Frying oils cook poorly when degraded by physical (thermal) stress and chemical changes. With use, oil changes its ability to hold heat. Heat capacity of oil increases with an increasing load of polymer (it takes more energy to attain a given temperature), and oil increases its rate of transferring heat (contact time increases with increasing levels of surfactants). Fresh oil, by comparison, holds heat well and acts like a thermal reservoir. Fresh oil also slowly releases heat to the surface of frying food.

Following are the most common chemical tests performed by inspectors in food production facilities, restaurants, and laboratories to indicate the approach of the specified discard point of a used frying oil.

Total Polar Materials (Chemical Index)

As oil breaks down, a group of polar materials includes most of the nontriglycerides. In the laboratory, a column chromatography method allows the determination of the percentage of polar materials in frying oils. The determination may also be made with a colorimetric quick test onsite; the quick test is then correlated to the standard method.

Polymer in Oil (Chemical Marker)

The formation of polymer in frying oils is directly related to the time oil is exposed to frying temperatures. At elevated temperatures, unsaturated fatty acids in triglycerides and also fatty acids free in solution combine with oxygen from the air. Some of the oxidized fatty acids crosslink to form high molecular weight polar compounds known as dimers. These dimeric triglycerides and fatty acids again cross-link during further exposure to frying conditions and form polar trimers, tetramers, and oligomers. Observation supports the hypothesis that no later than at about 50 h of heating and frying the oil becomes saturated with polymer, and any excess polymer plates out onto the walls of the fryer and onto the fry basket.

Free Fatty Acids

The measurement of FFAs as an indicator of oil quality is a widespread practice because it is easy to do and the percentage of FFAs rises with increasing use of a single batch of frying oil. However, the measurement is nearly meaningless because the rate of formation is not uniform, and many of the FFAs are continually being changed to volatile and nonacidic, nonvolatile decomposition products. The measured percentage of FFAs (titratable acidity) just documents what is left over from other degradation processes. In addition, when fresh oil is used to top up a fryer, the FFAs in the original batch are diluted to further confuse the actual relationship between oil quality and oil use.

An accepted reason to measure percentage of FFAs in the laboratory is to be able to predict the approximate resistance to oxidation of the oil on a potato chip distributed in a plastic bag. The FFAs are readily oxidized to yield a rancid odor. Colorimetric quick tests for percentage of FFAs are available for laboratory and for at-process control of frying oils.

Surfactants

Surfactants (wetting agents) in frying oil control the rate and amount of water removed from the surface of cooking food, overall oil pickup, and, by indirect (conductive and perhaps convective) forces, the degree of cooking of the interior. Although fatty acids are not surfactants, degradation products of fatty acids can be surfactants. For example, the soaps formed by combination of fatty acids with metals leached from food cells and food ingredients are surfactants. These parts per million of soaps along with other surfactants, such as monoglycerides, diglycerides, phospholipids, oxidized fatty acids, and oxypolymer fractions, control how (and even if) a food will fry in oils. The efficiency of heat transfer from heater to oil to food, the oil soakage into food, and the foaming tendency of the oil (oxygen incorporation route) are mediated by traces of surfactants. A colorimetric quick test for surfactants in frying oil is available.

Color in Oil

The often intense color of an oil is unrelated to the production of lightly colored, delicious food. The color bodies in oil do not seem to enter into the chemistry and physics of frying. They are inert with respect to the cooking process. The color of oil is not related to the color of food fried because the layer of oil on a food's surface is so thin it does not contribute significant color to the food. Removing color from oil is not equivalent to restoring cooking quality to the oil. Regardless of applicability, color matching and spectrophotometric techniques are available.

FOOD QUALITY

Very fresh oil does not cook well (food remains relatively raw) until it is broken in by frying a few portions of food or by adding a small amount of old oil to the new. Food fried in moderately used oil develops a well-cooked interior, a crisp exterior with a minimum of oil soakage, and typical golden colors and tempting flavors. Food fried in degraded oil does not cook completely in the interior, develops an excessively hard exterior, and suffers from oil soakage, dark coloration, and off-flavor.

Food fried in fresher oil is crisp, tasty, and well received. Food fried in degraded oil is limp and rejected. The quality of fried food from differently aged oil is often adjusted for by varying heater temperature and food residence time. These latter adjustments, however, never fully compensate for changes in the cooking properties of frying oil. Attainment of a crisp crust differentiated from a well-cooked interior is the goal of many groups that use frying to process food. The structure and chemical composition of fried crust is not yet understood. Dehydration produces hard structures, and oil incorporation (and perhaps interaction) produces an elastic component. This lack of knowledge keeps product development focused more on ingredient technology than on scientific principles.

Oil in Prefried Foods

The coatings of battered and breaded foods introduce soluble contaminants into the oil. When prefried foods are refried, the aged oil in the food comingles with the new frying oil and again introduces foreign chemicals. This latter case is especially important to food service operators and is difficult to guard against. Some prefried food vendors even tout the fact that their products are heavy with oil and will provide the food service fryer with extra free oil. This free oil is often loaded with soluble contaminant chemicals such as surfactants. Often a food service operation complains to its frying oil vendor that a new batch of oil was no good because it failed early in use. Actually, the prefried food was the culprit and the wrong vendor was blamed. An opportunity always exists to better control the production and increase the quality of fried foods by identifying the causative agent of variation in oil and its relationship to the process of frying.

NUTRITION AND TOXICOLOGY

The volatile and nonvolatile (possibly toxic) organic decomposition products of frying oils that have been extensively studied and commented on amount to, at most, a small percentage of the total mass of heated frying oil. Other, less studied components can be present in used oils at large percentages (20% or more for polymer) and have severe operating and economic consequences for fryer operators and consumers. It seems appropriate at this time to study the larger chemical variables and their consequences in frying technology. Fried food may be sterile on the surface as it emerges from the hot frying oil, but the interior bulk may still contain viable bacteria due to energy balance deficiencies in the process. This is especially worrisome in products such as coated meats, poultry, fish, and cheese and vegetable roll-ups.

REGULATORY

Recently, regulatory agencies and fast-food chains have shown an interest in having a simple means to determine when oil should be discarded. Many regulatory officials and nutritionists believe that it would be a wise practice to discard frying oils from food production before their TPM content has risen to 30% by weight of the oil. This attitude is nearly universal even though abused cooking oil consumed with fried food has not been shown to cause tumorigenesis or chronic toxicity in humans.

When a frying oil contains about 25% polar materials, numerous inspectors and restaurant operators believe the oil is abused and that such oil incorporated into food causes the food to be out of quality specification. The TPM content of oil included in food can then be regarded as a chemical index of fried-food quality. A food can be considered adulterated with respect to label declaration when the listed oil is only about 70 to 75% pure.

FRYING RESEARCH

Frying research has traditionally focused on the volatile and nonvolatile degradation chemicals formed in oils as a consequence of heating the oils in air. Summaries of important areas of study at an earlier time have been published (4,5). The degradation pathways and products of heated frying oils have been described (6). Important areas of frying oil analysis have been suggested (7). Diverse tools have been used to attempt to find relationships between different breakdown products in frying oils (8). Facets of oil use influencing fried-food quality have been studied (9,10). A simple surfactant formed in frying oils is related to oil soakage into food (11). Oil flavor chemistry has been described (12). Early evidence of a toxic material formed in abused frying oils has been presented (13). A summary of the nutrition and toxicology of frying oils has been published (14).

No researcher, however, has been able to specifically assign some element or elements in the complex mix of degradation chemicals in oils to the overall physical and sensory quality of food produced in aging frying oils. There is no textbook statement that if this element is examined in oil and controlled, the physical and sensory quality of fried food can be controlled. Some control of texture may be attainable if the chemistry causing crispness can be elucidated. The fractal nature of crust may be controlled by understanding the relationship between oil and starch interactions in potatoes, for example. Only general texts are available in this discipline (15). Flavor and taste components and rates and origins of organic reactions have been intensely studied, but the public as often as not still eats poor-quality fried food.

PROCESS OPTIMIZATION

The dynamics of heat and mass transfer while oil is undergoing chemical changes have not been well studied. Selective absorption of deleterious materials from frying oil solution has been proposed by the industry as a way to lengthen the service life of oils. Both filtration and infiltration of oil into frying food can be improved by studying aspects of fluid mechanics. Again, only general texts are available (16). Optimization of the frying process needs to be reduced to principles and rules for computerization in expert systems.

FRYING OIL SUBSTITUTES

To obtain either formulation compatibilities or reduced calories per serving, the food industry has frequently funded research on synthetic oils to be used either as replacers, extenders, or sensory enhancers. The group of discovered oil mimetics copy some sensory properties but cannot replace the functionality of frying oils in resistance to heat and oxidation. Oil replacers are created to substitute for natural triglyceride oils functionally but are not bioavailable and so do not contribute to nutrition. The lack of bioavailability is often touted as yielding low-calorie oil, but a consequence of replacers to date has been to remove fat-soluble materials, such as some vitamins, from the gut as the replacer passes through unchanged. Fortification of the replacers with fat-soluble vitamins is now suggested. The similarity between synthetics and the nonabsorbable polymer formed in abused oil is striking.

FRYING OIL CONTROVERSIES

Frying research has generally been conducted in ways that illustrate the dynamics and kinetics of oil degradation in nearly fresh oils or nearly abused oils. The middle ground, which is of most concern to industry and commerce, has been scarcely touched. Thus the emphasis has been on the onset of degradation or on thoroughly degraded systems that are not typical of food service or food processing by frying. Problems with processed oils such as flavor and color reversion, hydrogenation flavor, color associated with bitterness, high solids content, *trans*-fatty acids, unusual oxidation products, and processing residues and additives are rarely discussed. The importance of saturates and cholesterol and the potentially negative aspects of highly polyunsaturated oils, animal-source oils, saturated tropical oils, and heavily hydrogenated oils are not yet resolved.

BIBLIOGRAPHY

1. G. Varela, A. E. Bender, and I. D Morton, eds., *Frying of Food*, VCH, New York, 1988.
2. E. G. Perkins and M. D. Erikson, eds., *Deep Frying: Chemistry, Nutrition and Practical Applications*, AOCS Press, Champaign, Ill., 1996.
3. M. M. Blumenthal, "Frying Technology," in Y H Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., Vol. 3, John Wiley & Sons, New York, 1996, pp. 429–481.
4. H. Roth and S. P. Rock, *Bakers Digest* **46**(5), 38 (1972).
5. H. Roth and S. P. Rock, *Bakers Digest* **46**(6), 38 (1972).
6. C. W. Fritsch, "Measurements of Frying Fat Deterioration: A Brief Review," *J. Am. Oil Chem. Soc* **58**, 272 (1981).
7. S. G. Stevenson, M. Vaisey-Genser, and N. A. M. Eskin, "Quality Control in the Use of Deep Frying Oils," *J. Am. Oil Chem. Soc.* **61**, 1102 (1984).
8. P. Wu and W. W. Nawar, "A Technique for Monitoring the Quality of Used Frying Oils," *J. Am. Oil Chem. Soc.* **63**, 1363 (1986).
9. L. M. Smith et al., "Changes in Physical and Chemical Properties of Shortenings Used for Commercial Deep-fat Frying," *J. Am. Oil Chem. Soc.* **63**, 1017 (1986).
10. J. Mancini-Filbo et al., "Effects of Selected Chemical Treatments on Quality of Fats Used for Deep Frying" *J. Am. Oil Chem. Soc.* **63**, 1452 (1986).
11. M. M. Blumenthal and J. R. Stockler, "Isolation and Detection of Alkaline Contaminant Materials (ACM) in Used Frying Oils," *J. Am. Oil Chem. Soc.* **63**, 687 (1986).
12. S. S. Chang, R. J. Peterson, and C. T. Ho, "Chemical Reactions Involved in the Deep-Fat Frying of Foods," *J. Am. Oil Chem. Soc.* **55**, 718 (1978).
13. D. Firestone et al., "Heated Fats. I. Studies of the Effects of Heating on the Chemical Nature of Cottonseed Oil," *J. Am. Oil Chem. Soc.* **38**, 253 (1961).
14. E. G. Perkins and W. J. Visek, eds., *Dietary Fats and Health*, American Oil Chemists' Society, Champaign, Ill., 1983.
15. A. Harrison, *Fractals in Chemistry*, Oxford University Press, Oxford, United Kingdom, 1995.
16. E. L. Cussler *Diffusion, Mass Transfer in Fluid Systems*, 2nd ed., Cambridge University Press, Cambridge, United Kingdom, 1997.

Michael M. Blumenthal
Libra Technologies, Inc
Metuchen, New Jersey

FUMIGANTS

Fumigants have been used for centuries as a method of controlling stored insect pests. Because some insects deposit their eggs inside the grain kernel, fumigants must possess certain properties so that they exist in a gaseous state to penetrate the kernel to effectively control the infestation. In recent years the number of fumigants used worldwide has decreased to only two, phosphide and methyl bromide (1). The industry must take greater care in the use of the available products and exercise greater caution so that misuse cannot occur. Improper sealing of an area to be fumigated, poor application techniques coupled with wrong dosages, and inadequate fumigation time always result in failure. One of the principal concerns with poor fumigations is the development of resistant insects.

Newer government regulations require that applicators become more knowledgeable in regard to fumigant chemicals and their application. Safety of the applicator is a prime consideration for any fumigation, both during application of the chemical and the subsequent aeration of the fumigated commodity. The total procedure will only be successful if the space to be fumigated has been properly sealed so that the gas concentration can be maintained for the required time period to ensure total control. Improper sealing will result in loss of gas during the fumigation, so it is recommended that the applicator take gas readings during the fumigation to determine gas release and concentration. It is always surprising to hear how much time was taken for a fumigation that failed only to hear that some area was not sealed and that gas readings were not taken. Applications of this nature can only result in the development of resistance because generations of insects become exposed to nonlethal concentrations. The industry still has sufficient means to control stored product pests, but these methods must be used effectively and with proper techniques.

Each fumigation is different and, therefore, the selection of the appropriate fumigant to be used is crucial. It is especially significant that the properties of each chemical is considered for both success and to determine how best to apply the product so that all areas of the space to be fumigated have sufficient gas concentration during the treatment period. Fumigant products vary in mode of action depending on many factors including temperature, relative humidity, sorption, diffusion, and penetration.

A good fumigation will require prefumigation planning. All aspects of the fumigation must be decided so that success will be achieved. Among the factors to be considered are safety of the applicator, proper sealing of the structure, properties of the fumigant to be used, application equipment or techniques, gas readings, placement of warning signs, posting of guards, and notification of emergency personnel in the area in the event of an episode. The proper method of aeration of the treated commodity must also be analyzed and planned so that the release of the fumigant will not result in exposure or risk to anyone. Once all aspects of the fumigation have been established, it then is necessary to choose which fumigant will be used from among those available.

AVAILABLE FUMIGANTS

Metal Phosphides

There are two metal phosphide products that are presently used as a source of hydrogen phosphide: aluminum phosphide and magnesium phosphide.

Aluminum Phosphide. This product is manufactured as a tablet or pellet and is packaged in a formulation in a bag or sachet or as a tablet or pellet prepac. The paraffin-coated, 3-g tablets will produce 1 g of hydrogen phosphide gas when exposed to certain heat and humidity conditions. The pellets are one-fifth the size of the tablets and produce 200 mg of hydrogen phosphide gas. This gas has a density of 1.18 as compared to air and, as a result, has excellent diffusing and penetrating properties. The tableted product usually contains ammonium carbamate, which will break down into ammonia and carbon dioxide when exposed to heat. The gases escape from the tablet or pellet by causing ruptures in the paraffin coating. The ammonia also acts as a warning gas to the applicator. Atmospheric moisture then penetrates the ruptured coating to react with the aluminum phosphide resulting in the release of hydrogen phosphide gas, which is the resultant fumigant. Tablet and pellet prepacs release hydrogen phosphide in an identical manner. These formulations cause a delayed release until appreciable gas concentrations are reached and act as a safeguard to the applicator. Bag or sachet formulations also have a delayed release; atmospheric moisture must penetrate the paper or cloth packaging material before any toxic gas is released. It is important to follow the manufacturer's applicator's manual for use of aluminum phosphide because low temperature and low relative humidity can result in only partial reaction and a minimum of gas release. The result will be low concentration of gas, a good chance at fumigation failure, and possible problems on deactivation of the unreacted metal phosphide. When temperatures are lower or relative humidity is lower, then it is wise to consider the use of a magnesium phosphide product.

Magnesium Phosphide. These products are produced and marketed as either tablets or pellets or as magnesium phosphide impregnated in a plastic matrix. No bag or sachet formulation of this chemical is available. There is a magnesium phosphide pellet prepac product that is used only as a spot fumigant for plant processing equipment. The reactivity of magnesium phosphide with atmospheric moisture is so much faster than aluminum phosphide, it can be used in conditions of high temperatures, low relative humidity or low temperatures and low to medium relative humidity.

PROPERTIES OF METAL PHOSPHIDES

There are several properties of metal phosphide fumigant products that must be considered in any application.

1. Its density is such that any leakages will result in loss of gas.
2. Hydrogen phosphide gas can self-ignite at a concentration of 17,900 ppm if there is an ignition source available. The reaction between a metal phosphide and atmospheric moisture, which yields hydrogen phosphide, is exothermic and, therefore, the piling of tablets during fumigation is to be avoided as the heat generated from this exothermic reaction can be the ignition source.
3. Hydrogen phosphide reacts with certain metals, especially with copper, so care must be taken during fumigation to prevent corrosion of electrical and electronic equipment and instruments.
4. Note that 1 g of hydrogen phosphide gas per 1,000 ft^3 will result in a concentration of 25 ppm. There-

fore, if a dose of 20 tablets/1,000 ft^3 is applied, the theoretical maximum concentration would be 500 ppm (20 g × 25 = 500 ppm), which is equivalent to a dose of 0.7 g/m^3.

5. Aluminum or magnesium phosphide tablets and pellets that contain ammonium carbamate also release ammonia and, therefore, can be phytotoxic to living plants if sufficient concentration is present as well as darkening some nut meats when direct contact is made with the product. The darkening of nut meat is usually reversible. It would be better to use the magnesium phosphide impregnated in a plastic matrix (Fumi-Cel/Fumi-Strip) for some of these fumigations because it contains no ammonium salts.
6. Hydrogen phosphide has no effect on germination and, therefore, can safely be used to fumigate various seeds.
7. Hydrogen phosphide has no real CT product and fumigations must be no less than three days for complete control.
8. Do not fumigate if commodity temperature is below 5°C (40°F).
9. A fumigated commodity or space must be aerated until there is 0.3 ppm or less of hydrogen phosphide before reentry is allowed without respiratory protection.
10. Repeated fumigation with hydrogen phosphide will not result in a buildup of residues if the product is properly aerated following fumigation.

Finally, it must also be noted that hydrogen phosphide produced from metal phosphide product has a garlic or carbidelike odor. One word of caution in this regard is to emphasize that pure hydrogen phosphide has no odor, and the odor noted during a fumigation is an impurity and can be sorbed on a commodity, therefore, the lack of odor cannot be considered as a key for the lack of hydrogen phosphide being present. A measuring device must be used to determine concentration.

Metal phosphide products can be applied directly to raw agricultural commodity and animal feeds and have a tolerance of 0.1 ppm for this purpose. No direct contact can be made with these products on processed food so packaged metal phosphide products such as prepacs, bags, sachets, or Fumi-Cel/Fumi-Strip are used for these applications, and the approved tolerance is 0.01 ppm. Care should always be taken when deactivating residual materials as fumigation conditions could have resulted in having some unreacted phosphides in the residuals. The manufacturer's applicator's manual should be consulted prior to initiating this activity.

The half-life of hydrogen phosphide in the atmosphere is short and depends on climatic condition; it is in the range of 5–28 h. Hydrogen phosphide is known to be oxidized to phosphate both in the atmosphere and during fumigation.

Toxicology

Hydrogen phosphide has been shown to be an acute toxic material and has no chronic effects. Effects shown by experimental animals as a result of subchronic exposure were shown to be reversible. This substance also had no teratological or mutagenic action when these studies were completed.

Aluminum phosphide, a fumigant used extensively in India, is extremely toxic to humans with a fatal dose reported to be 1.5 g (2). Gupta and Ahlawat (3) attributed aluminum phosphide poisoning to the release of a cytotoxic phosphine gas to the major organs of the body including heart, lungs, and kidneys.

Respiratory Protection

If the application is made inside a structure, then gas readings must be taken and a gas mask with approved canister must be worn if the levels exceed 0.3 ppm. An air pack must be worn when the levels exceed 15 ppm. These same requirements also apply to aeration.

METHYL BROMIDE

Methyl bromide is packaged as a liquid under pressure in either small cans or in various sizes of steel cylinders. It has a density of approximately 3.3 times that of air and, as a result, should be used with fans to prevent stratification, resulting in fumigation failures in the higher portion of the area. To prevent some of this phenomenon from occurring, it is usually recommended that the product be released near top of the site to be fumigated whether it is a silo or mill. If fans are not employed, there is a possibility that the upper portion of the site will not be successfully fumigated. If bagged commodities are to be fumigated under a tarpaulin or in a truck or container, the entry tube should be leakproof and a pan should be placed under the end of the tube so that no liquid methyl bromide is allowed to come into contact with the bagged commodity, which can result in staining.

The properties of methyl bromide that must be considered prior to fumigation are as follows.

1. Its density is such that fans should be used to recirculate the gas for even distribution.
2. A heat exchanger may be necessary to properly volatize the methyl bromide.
3. Methyl bromide is nonflammable but can result in the formulation of hydrobromic acid when open flames are present. Hydrobromic acid can be corrosive.
4. Methyl bromide can be sorbed under certain condition and can react chemically with certain compounds resulting in damage or bad odors. These materials include certain foodstuffs, rubber goods, furs, leather goods, woolens, rayons, various paper products, photographic chemicals, cinder blocks, charcoal or any material that contains active sulfur compounds.
5. Methyl bromide can effect germination of seeds and should be used for this purpose with caution.
6. Methyl bromide does have a true CT product, and fumigation usually can be completed in 12–24 h depending on dosage.

7. Normally fumigation with methyl bromide is not recommended when the commodity temperature is lower than 15.5°C (60°F).
8. Following fumigation, aeration must continue until the level of methyl bromide is below 5 ppm. If the level exceeds this amount, then proper respiratory equipment must be worn.
9. Repeated fumigations with methyl bromide can result in residues exceeding tolerances established by federal agencies for various commodities.

Finally, it should be noted that methyl bromide is odorless and proper measuring instrumentation is required to measure concentration. The halide detector is not sensitive enough to be used to determine levels suitable for reentry into an aerated facility. Aeration may have to be prolonged due to sorption of methyl bromide to commodities or materials in the structure. Fans will help in the aeration process. The half-life of methyl bromide can be 15–18 months depending on climatic conditions.

Toxicology

Methyl bromide is known to be a chronic toxic chemical, that is, the effects due to exposure are cumulative and are not reversible. Major studies such as chronic toxicity, teratology, pharmacokinetics, and mutagenicity are being conducted to fill data gaps for regulatory purposes.

Methyl bromide is known to be a chronic toxic chemical, as the effects due to exposure are cumulative and not reversible. Consumption of methyl bromide from fumigated food has been estimated at 0.00125 mg/kg/day in the average diet. Wilson et al. (4) fed dogs a diet containing 0.27–0.28 mg/kg/day methyl bromide over a period of a year. No adverse effects were observed, indicating it did not present a significant health hazard to humans. Another recent feeding trial on rats and rabbits conducted by Kaneda et al. (5) concluded that methyl bromide was not fetotoxic or teratogenic to rat and rabbit fetuses up to dose levels of 30 and 10 mg/kg/day, respectively. Nevertheless, the use of methyl bromide as a fumigant was banned by the Montreal Protocol (an international treaty) as of 2001 because it is an ozone depleter. This could have a significant effect on Florida's horticultural industry unless alternative methods are found (6,7).

Respiratory Protection

If application is made inside a structure, then an air pack must be worn for any level above 5 ppm. This same restriction applies to aeration.

Alternatives to Methyl Bromide

A number of alternative methods are being explored for possible implementation prior to the ban on methyl bromide in 2001. Warner (8) suggested a combination of moist heat and controlled atmosphere as an alternative quarantine treatment for codling moth in fruit. Subjecting apples, pears, and cherries to 113–117°F (45–47°C) would be adequate to destroy the codling moth larvae. Storing the fruit in a controlled atmosphere of 0.5 % oxygen and 15% carbon dioxide shortened the heating time at 117°F needed to kill the larvae by as much as one half or two-thirds compared to just heating alone. Warner (9) also recommended irradiation as an alternative quarantine treatment to fumigation with methyl bromide for cherries. Irradiation up to 50 krad did not have a detrimental effect on the quality of the fruit. A recent study by Sholberg (10) used short chain organic acids (acetic, formic, and proprionic) as fumigants against postharvest decay by fungal pathogens. All three acids reduced decay in citrus by *Penicillium digitatum* from 86% to 11% and decay in pome fruit by *P. expansum* from 98% to 14%, 4%, and 8%, respectively. It is clear that alternative methods to fumigants will soon be implemented before the ban on methyl bromide comes into force.

CONTROLLED ATMOSPHERES

The gases primarily used for controlled-atmosphere fumigations are carbon dioxide, nitrogen, or a mixture of these gases. These gases, in effect, are applied to reduce the oxygen content in a storage.

Carbon Dioxide

The addition of this gas not only reduces the oxygen content but also acts directly on stored pests by acting as a dessicant on the insect body fluids. Carbon dioxide is usually applied from trucks or tanks that contain liquid carbon dioxide. Appropriate vaporizers and regulators are then used to supply gaseous carbon dioxide to the structure to be fumigated. The gas is usually applied at the top of a silo and because it is denser than air the gas will move down through the grain mass. The silo or warehouse to be fumigated must be tightly sealed, and carbon dioxide must be added daily to maintain a 60–70% concentration to be efficient. A system must also be used in the structure that will not allow the pressure inside to build up during application. Usually a vent is placed in the roof or a hatch or portion of polyethylene used to seal roof vents is opened during application.

Nitrogen

Nitrogen can also be used as an inert gas to control oxygen concentrations. A nitrogen generator is required, and normally the oxygen concentration must be reduced to approximately 1%. Fumigation time can vary but usually requires approximately 10 days for good control.

Inert Atmosphere Generators

There are several inert atmosphere generators that result in a mixture of nitrogen and carbon dioxide being applied to the structure to be fumigated.

To use a controlled atmosphere for fumigation, the following factors must be considered.

1. Temperature of the commodity and within the storage area should be 21°C (70°F) or higher to have an effective fumigation. Lower temperature will effect the mortality of the insect present in the structure or commodity.

2. A vent or pressure relief system must be present in the warehouse or silo to prevent structural damage due to pressure build up.
3. Application time can take up to 12 h with daily recharging, which will normally take 3–4 h.
4. Total fumigation time can be from 4 to 10 days depending on temperature and gas tightness of the structure.
5. Even though these gases are considered to be nontoxic, an air pack must be used when entering a facility that has low oxygen content during fumigation.

MIXTURES OF GASES

Research is being conducted by several groups to try to determine if mixtures of gases can result in more favorable fumigation conditions with a shorter duration. Mixtures such as hydrogen phosphide and carbon dioxide, methyl bromide and carbon dioxide, or increased temperature and carbon dioxide are being tested. Early studies show that some mixtures may be promising, but additional studies will have to be performed. Elevated temperature and carbon dioxide combinations have not been successful to date.

BIBLIOGRAPHY

1. R. W. D. Taylor, "The Future of Postharvest Fumigants," *Postharvest News and Information* **8**, 26N–31N (1997).
2. S. Singh et al., "Aluminum Phosphide Ingestion—A Clinico-Pathologic Study," *Journal of Toxicology and Clinical Toxicology* **54**, 703–706 (1996).
3. A. Gupta and S. K. Ahlawat, "Aluminum Phosphide Poisoning—A Review," *Journal of Toxicology and Clinical Toxicology* **33**, 19–24 (1995).
4. N. H. Wilson et al., "Methyl Bromide: 1-Year Dietary Study in Dogs," *Food and Chemical Toxicology* **36**, 575–584 (1998).
5. M. Kaneda et al., "Oral Teratogenicity Studies of Methyl Bromide in Rats and Rabbits," *Food and Chemical Toxicology* **36**, 421–427 (1998).
6. J. Noling and J. Gilreath, "USDA Evaluates Alternatives to Methyl Bromide in Florida," *Citrus and Vegetable Magazine* **62**, 21, 24 (1998).
7. J. J. VanSickle and T. Spreen, "Florida Without Methyl Bromide: The Potential Result," *Citrus and Vegetable Magazine* **58**, 26–28 (1994).
8. G. Warner, "Heat and CA Treatment May Replace Fumigation," *Good Fruit Grower* **46**, 8–9 (1995).
9. G. Warner, "Irradiation is Kinder to Fruit than Fumigation," *Good Fruit Grower* **47**, 9 (1996).
10. P. L. Sholberg, "Fumigation of Fruit with Short Chain Organic Acids to Reduce the Potential of Postharvest Decay," *Plant Disease* **82**, 689–693 (1998).

GENERAL REFERENCES

E. J. Bond, *Manual of Fumigation for Insect Control*, Food and Agriculture Organization of the United Nations, Rome, 1984.

Truman's Scientific Guide to Pest Control Operations, Purdue University/Edgell Communications.

FUNCTIONAL FOODS

Functional foods usually refer to foods containing significant levels of biologically active components that impart health benefits beyond basic nutrition. These components are often known as phytochemicals—meaning plant chemicals. Several terms are used to describe the many natural products currently being developed for health benefit. These include nutraceutical, functional food, pharmafood, designer food, vitafood, phytochemical, and foodaceutical. Functional food may be the most internationally recognized term for the category of enhanced foods with potentially strong preventive or therapeutic properties and is used widely in Japan and Europe. In North America, the terms functional food and nutraceutical have been used interchangeably, and a number of definitions of what are included under these all-encompassing headings have been advanced. According to Health Canada, a functional food is similar in appearance to conventional food, is consumed as part of the usual diet, and has demonstrated physiological benefits and/or reduces the risk of chronic disease beyond basic nutritional functions. A nutraceutical is a product produced from foods but sold in pills, powders (potions), and other medicinal forms not generally associated with food and demonstrated to have a physiological benefit or provides protection against chronic disease (1).

The following statement provides the definition for functional food endorsed by the European expert committee organized by the International Life Science Institute (LSI): "A food can be regarded as functional if it is adequately demonstrated to beneficially affect one or more target functions in the body, beyond normal nutritional effects, in a way which is relevant to either the state of well being and health and/or the reduction of the risk of a disease." Definitions are also based on, and relevant for, target groups. In Germany, for example, functional foods are targeted for the general population, while the term *nutraceuticals* is used for products aimed at specific age categories, and *medical foods* is the term for products designed for patients (2). Currently in Japan, the functional foods approved as Foods for Specified Health Use (FOSHU) regulation established in 1991 by the Japanese Ministry of Health and Welfare are defined as everyday foodstuffs expected to have a specific effect on health due to relevant constituent(s). This regulation allows claims regarding the foods' beneficial effect on health.

In the United States, the movement toward functional foods began during the late 1970s when Lee Wattenberg of the University of Minnesota extolled the health benefits of components of crucifers and cabbage. As the next step, the National Academy of Sciences' report on "Diet and Cancer" in 1982 not only made a connection between fat and fiber in the diet but also nonnutritive components that might be important in the diet. The term designer foods coined by Herbert F. Pierson, Jr., in 1989, was publicized in the popular press in the United States several years ago when the National Cancer Institute (NCI) announced a $20 million five-year research program to study the anticarcinogenic properties of components of citrus, flax, aged garlic extract, licorice extract, soybean meal, and umbelliferous vegetable juice beverage. In 1989, because of the regulatory confusion concerning foods versus drugs, Stephen L. DeFelice, chair of the Foundation for Innovation in Medicine, introduced the term nutraceuticals to include isolated nutrients, dietary supplements, genetically engineered foods, and herbal products. The term nutraceuticals has received considerable press and carries a high-tech image that fits comfortably with pharmaceutical companies.

The concept of functional foods is based on evidence that certain vegetables, herbs, fruit, and grains naturally rich in various phytochemicals have a protective effect against diseases such as cancer. There are at least 14 classes of phytochemicals known or believed to possess cancer-preventive properties (3). These include sulfides, phytates, flavonoids, glucarates, carotenoids, coumarins, monoterpenes, triterpenes, lignans, phenolic acids, indoles, isothiocyanates, phthalides, and polyacetylenes. A variety of these phytochemicals are abundantly present in garlic, green tea, soybeans, cereal grains, licorice roots, flaxseed, and plants from the cruciferous, umbelliferous, citrus, solanaceous, and cucurbitaceous family (Table 1).

Increased intakes of nonnutrient phytochemicals with potential health benefits can be improved by increased intake of a food rich in products such as garlic extract; by conventional plant breeding to increase the concentration in a crop; through biotechnology; by selective processing, such as milling and extraction, to enhance the concentration of one or more chemicals in a food; or by adding a phytochemical to a food. Food processing and preparation procedures also affect the physiological consequences of food. Fermented products, including dairy products, have long been recognized to alter gastrointestinal flora and even reduce circulating cholesterol. While heating tomatoes may improve lycopene availability and thus improve its antioxidant potential, heating of unpeeled garlic reduces its anticancer potential. Ingestion of functional foods represents an effective strategy to maximize health and reduce the risk of diseases. Interest in the health benefits of foods is propelled by rising health care costs, legislative changes (ie, the Nutritional Labelling and Education Act [NLEA] and Dietary Supplement Health and Education Act [DSHEA]) that permit claims for foods and associated components, and by new and exciting scientific discoveries. Functional food is one of today's most prevalent trends for food product development. According to Datamonitor, Inc., a global market research firm, the U.S. market for nutraceuticals has grown from $11 billion to $16.7 billion, representing a growth of 10.9% over the past four years. The firm's survey, "U.S. Nutraceuticals 1997," found that a general trend toward healthy eating (represented by low-fat, low-calorie foods) has shifted toward more active prevention of disease and maintenance of health.

FUNCTIONAL FOODS FROM PLANT SOURCES

Epidemiological studies have shown that plant-based diet can reduce the risk of chronic disease, particularly cancer and cardiovascular disease. This is supported by the low incidence of cardiovascular disease in vegetarians and reduced cancer risk in people consuming diets high in fruits and vegetables. Biologically active components of plants

Table 1. Phytochemicals from Major Plant Food

	Phenolic acids	Flavonoids	Coumarins	Carotenoids	Triterpenes	Monoterpenes	Glucarates	Lignans	Sulfides	Phytates	Isothiocyanates
Cereals	✓	✓	✓	✓	✓		✓			✓	
Soybeans	✓	✓	✓	✓	✓			✓		✓	
Flaxseed	✓	✓	✓					✓			
Solanaceous	✓	✓	✓	✓	✓	✓	✓				
Garlic	✓				✓	✓			✓		
Cruciferous	✓	✓	✓	✓	✓	✓	✓		✓		✓
Citrus	✓	✓	✓	✓	✓	✓	✓				
Umbelliferous	✓	✓	✓	✓	✓	✓					
Green tea	✓	✓	✓				✓				

Source Ref. 3

with demonstrated physiological benefits that may enhance health by reducing or alleviating the risk and/or incidence of diseases have been identified in plants commonly grown in commercial agriculture.

Cereals

Cereals such as wheat and rice are major constituents of the diet of a vast number of the world's population and therefore can play an important role in the nutritional quality of the diet and human health. The role of cereal grains in gastrointestinal health has long been recognized. Dietary fiber, particularly from cereals, has been postulated to be the major preventive factor against a number of cancers including colorectal carcinogenesis. However, a recent report (4) has challenged the connection between fiber consumption and colorectal cancer. Increased dietary fiber intake, especially soluble fiber, has also been linked to reduced risk of coronary heart disease. Oat is one of the few foods that have been rigorously tested as a dietary source of the cholesterol-lowering soluble fiber β-glucan in clinical settings among free-living individuals. Consumption of diets high in soluble fiber β-glucan has been associated with reduction in total and low-density lipoprotein (LDL) cholesterol, thereby reducing the risk of coronary heart disease (CHD). For this, the Food and Drug Administration (FDA) awarded the first food-specific health claim under the NLEA in January 1997 (5), in response to a petition submitted by the Quaker Oats Company. A food bearing the health claim must contain 13 g of oat bran or 20 g oatmeal and provide without fortification at least 1.0 g of β-glucan per serving. In February of 1998, the soluble fiber health claim was extended to include psyllium fiber. There is great expectation that the health claim for the soluble fiber β-glucan of oats will expand to other grains, including barley. In this regard, the prowash barley variety from Montana State University is said to contain twice the level of soluble cholesterol-lowering β-glucan fiber found in ordinary barley. Recently, stabilized full-fat rice bran performed similarly to oat bran in reducing hypercholesterolemia in humans (6). Wheat foods, the major source of dietary fiber in the North American diet, generally accepted in promoting regularity, is also being touted as having protective effects against colon and breast cancers. Wheat bran, even in processed forms, can offer protection against the development of colon adenocarcinoma even when the carcinogenic process is promoted by high-fat low-calcium high-risk Western-style diets (7). In this regard, the FDA has under consideration a petition from the Kellogg Company seeking approval for the health claim that eating products containing insoluble fiber from wheat bran can help reduce the risk of colon cancer.

Legumes

The Leguminosae plant family, of which soy is of the utmost economic importance, is most abundant in phytoestrogens. The low mortality due to breast, colon, and prostate cancer of Asians, especially Japanese and Chinese, compared with Westerners has been attributed mainly to extensive use of soy foods. Several classes of anticarcinogens have been identified in soybeans, including protease inhibitors; phytosterols; saponins; phenolic acids; phytic acid; the precursors of mammalian lignans, especially seicoisolariciresinol; and isoflavones, a class of phytoestrogens. The two primary isoflavones, genistein, reported to be the most biologically active phytoestrogen, and diadzein, present in high concentration in soybeans, are known to modulate hormone-related conditions, such as heart disease, osteoporosis, breast and prostate cancers, and menopausal symptoms. Since isoflavones are structurally similar to the estrogenic steroids, they may act as antiestrogens by competing with the more potent, naturally occurring endogenous estrogens for binding to the estrogen receptor. In this regard, an isoflavone extracted from soybeans, 17β-estradiol (Estrace®, Roberts Pharmaceuticals Canada Inc.), is being marketed as a prescriptive oral estrogen for effective management of menopause. The cholesterol-lowering effect of soy is the most well documented physiological effect and is attributed mainly to soy isoflavones. However, very recent studies suggest that the combination of both soy protein and isoflavones are necessary to produce the greatest cholesterol-lowering effect. In addition to being a high-quality protein, soybean protein is now thought to play a preventive and therapeutic role in cardiovascular disease. In this regard, the FDA has proposed to authorize a health claim regarding the association between soy protein and reduced risk of coronary heart disease as petitioned by Protein Technologies International, Inc. Soy protein in the amount of 6.25 g with a minimum of 12.5 mg of total isoflavones (equivalent to 25 g of soy) per day is required to qualify an individual food to bear the health claim (8). Recently, the hypocholesterolemic effects of protein concentrates from lesser known legume seeds have been demonstrated (9).

Oilseeds

Flaxseed is the most prominent oilseed studied to date as functional food since it is a leading source of the omega-3 fatty acid, α-linolenic acid (ALA) (52% of total fatty acids), and phenolic compounds known as lignans ($>500\ \mu/g$, as is basis). Dietary omega-3-rich oil moderates the recognized current American excess of omega-6 intake that leads to eicosanoid-mediated disorders (eg, thrombotic heart attack and stroke, cardiac arrhythmia, atherogenesis, arthritis, asthma, osteoporosis, tumor metastases, etc). In this context, flaxseed has been fed to poultry, cattle, and pigs to increase omega-3 fatty acids in eggs, milk, and meat. Omega-3 fatty acids, precursors of long-chain polyunsaturated fatty acids, have been shown to be important for visual and brain development, especially for termed infants. The lignan secoisolariciresinol diglycoside (SDG), extracted from flaxseed, is the precursor of the mammalian lignan enterodiol and its oxidation product, enterolactone. Because enterodiol and enterolactone are structurally similar to estrogens, they may play a role in the prevention of estrogen-dependent cancers. Flaxseed has a protective effect against both mammary and colon cancer. Long-term studies confirm the protective effect of SDG on tumor growth due to the biological properties of mammalian lignans derived from SDG. Dietary flaxseed also provides a significant benefit in preventing the decline in renal function and has been found to be a suitable adjunct to therapy in patients with lupus nephritis and other forms of progressive renal disease. Flaxseed is also a good source of dietary fiber known to improve glucose metabolism as well as other phytochemicals associated with the decreased risk of developing colorectal cancer (10). Consumption of flaxseed has been shown to reduce total and LDL cholesterol and platelet aggregation. Other oilseed crops are predominantly linoleic-acid-rich oils and have the advantage of being the richest natural dietary sources of vitamin E, in addition to providing the essential fatty acids. Recent trends for commercial vegetable oil have been the reduction of polyunsaturates, as in the case of sunflower (Nusun), to overcome the presence of undesirable *trans* fatty acids due to hydrogenation (11).

Horticultural Crops

Tomatoes. Lycopene, the primary carotenoid found in tomatoes, has been implicated in risk reduction of prostate cancer as well as other cancers, including breast, digestive tract, cervix, bladder, skin, and lung, whose risks have been inversely associated with serum or tissue levels of lycopene. Recently, intake of lycopene has also been inversely associated with risk of myocardial infarction, probably due to modulation of cholesterol metabolism (12).

Garlic. The purported health benefits of garlic include cancer chemopreventive, antibiotic, antihypertensive, and cholesterol-lowering effects. Allicin, produced when garlic cloves are crushed, and responsible for the characteristic odor of fresh garlic, spontaneously decomposes to form sulfur-containing compounds with chemopreventive activity.

Garlic has been historically used as a spice and remedy for common ailments. The reported medicinal properties of garlic such as antibacterial activity, antifungal activity, inhibition of platelet aggregation, and reduction of serum cholesterol levels are due to biologically active sulfur compounds. Attention has been directed to garlic and garlic extracts as potential cardiovascular and anticancer agents. Several epidemiological studies demonstrate the effectiveness of garlic in reducing human cancer risk, especially cancer of the stomach, colon, and gastrointestinal tract. Garlic has also been advocated for the prevention of cardiovascular diseases, possibly through antihypersensitive and cholesterol-lowering effects. Plants of the genus *Allium*—including garlic, onion, and leek—are also good sources of sapogenins, bioactive compounds that are relatively stable within the food during and after processing.

Cruciferous vegetables. Epidemiological studies indicate reduced risk of breast, colon, gastric, and prostate cancers on frequent consumption of cruciferous vegetables. The anticarcinogenic properties of cruciferous vegetables are attributed to their relatively high content of glucosinolates and their derivatives, of which the allyl isothiocynate and indoles are most potent. Indole-3 carbinol (I3C) is currently under investigation for its cancer chemopreventive properties, particularly of the mammary gland, and may be a novel approach for reducing the risk of breast cancer. Besides glucosinolates and its derivatives, several groups of compounds including dithiolthiones, sulfonates, and vitamins from cruciferous vegetables have been reported to alleviate diseases and promote health.

Fruits. Flavonoids are the main constituents in most fruits known to protect against a variety of human degenerative diseases. These and other fruit components are major sources of dietary antioxidants that increase the plasma antioxidant capacity resulting in inhibition of atherosclerosis-related diseases in humans with increased consumption of fruits (13). Flavonoid intake from various sources including fruits and vegetables has been inversely related to coronary heart mortality (14). The limonoids, a group of chemically related triterpene derivatives, present in large quantities in citrus fruits has been suggested as the preventive agent against a variety of human cancers. Cranberry juice has been recognized for a long time as efficacious in the treatment of urinary tract infections. The inhibition of the adherence of *Escherichia coli* to uroepithelial cells has also been attributed to fructose and a nondialyzable polymeric compound from cranberry juice (15). Flavonoids and other phenolics present in grape and grape products have been shown to possess anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, antiallergic, antithrombic, and antioxidative effects. The ability of phenolic substances in prevention of atherosclerosis and risk reduction of cardiovascular diseases has been aptly demonstrated by the benefits of red wine consumption, often referred to as the French Paradox. The health benefits of grape phenolics in inhibiting the oxidation of LDL can also be obtained from commercial grape juice (16). Red wine is also a significant source of *trans*-resveratrol, a phytoalexin with estrogenic and anticancer properties (17).

Polyphenols comprising up to 30% of the total dry weight of fresh tea leaves have been associated with the cancer chemopreventive effects particularly from green tea. Decrease recurrence of breast cancer in Japanese women and substantial reduction in risk of cardiovascular diseases have been associated with tea consumption (18). Phenol antioxidants from tea with lipoprotein-bound antioxidant activity constitute a major source of antioxidants in the North American diet.

Blueberry fruits, particularly *Vaccinium myrtillus* L., also known as bilberries are a rich source of anthocyanins and other flavonoids, which have demonstrated a wide range of biochemical and pharmacological effects, including anticarcinogenic, antiatherogenic, anti-inflammatory, antimicrobial, and antioxidant activities. Studies carried out in small animals or on a small number of humans, indicate that concentrated extracts of anthocyanins obtained from bilberries may benefit visual acuity, as well as provide protection against macular degeneration, glaucoma, and cataracts.

FUNCTIONAL FOODS FROM ANIMAL SOURCES

Dairy Products

Prebiotic- and probiotic-containing dairy products lead the functional food market in most of Europe and Japan. The term prebiotics has been applied to products such as oligosaccharides, used as bifidogenic factors in fermented dairy products to promote the growth of beneficial organisms. Natural sources of these bifidogenic oligosaccharides include chicory, Jerusalem artichokes, and other plants. Besides prebiotic effect, the major fructo-oligosaccharide inulin has physiological benefits in absorption of minerals, carbohydrate and lipid metabolism, immune functions, modulation of serum lipids, and reduction of cancer risks (19). The generally accepted definition of probiotics is that they are live microbial food or feed supplements that benefit humans and animals by improving the microbial balance in the intestine. Several health benefits have been attributed to probiotics, including anticarcinogenic, hypocholesterolemic, and antagonistic effects against enteric pathogens and other intestinal organisms. The role of probiotics in cancer risk reduction has been evident, particularly in colon cancer (20). Products that contain both prebiotics and probiotics, referred to as synbiotics, are being successful marketed in Europe. Traditional dairy products are also good sources of calcium and other milk-based bioactive compounds, such as casein-based bioactive peptides, whey proteins, and conjugated linoleic acid (CLA), with anticarcinogenic and immunogenic properties (21).

Beef

The mixture of positional and geometric isomers of linoleic acid with conjugated double bonds referred to as conjugated linoleic acid is most abundant in fat from ruminant animals. CLA, first isolated from grilled beef (22), has been shown to be effective in suppressing forestomach tumors and mammary carcinogenesis. Beef fat contains 3 to 8.5 mg CLA/g fat, and diets containing only 0.1 to 1% CLA have been shown to have anticarcinogenic effect in a mammary tumor model (23). CLA has also been suggested as a weight reduction agent since it can change body composition. Recent studies indicate that CLA has protective effects in cases of human breast cancer, chronic renal failures, and reduction in the severity of preexisting aortic atherosclerosis (23). It has been suggested that supplementation of CLA in diets alters the incorporation of n-6 fatty acids and CLA isomers into adipose tissue and enhances certain anticancer immune defenses.

Fish

Seafood lipids are rich in long-chain omega-3 polyunsaturated fatty acids (PUFA). The beneficial health effects of

Table 2. Selected Functional Foods and Bioactive Food Ingredients and Their Health Benefits

Functional food	Benefit
β-glucan derived from oats	Reduces risk of coronary heart disease
Canola oil with reduced saturated fats	Reduces risk of coronary heart disease
Dairy products with probiotics and prebiotics	Maintains a healthy digestive tract
Flaxseed lignans	Reduces risk of breast cancer and improves glucose metabolism
Flaxseed oil—high in omega-3 fatty acids	Decreases triglycerides and blood cholesterol
Fructo-oligosaccharides (especially for people with irritable bowel syndrome)	Restores colonic and intestinal health and reduces colonic precancerous lesions
Green tea extracts and beverages	Aids relaxation and reduces risk of cardiovascular disease and certain cancers
Herbal extracts	
Ginseng	Antistress, immunostimulant
Gingko biloba	Improves cognitive performance
St. John's wort	Controls mild to moderate depression
Echinaceae	Boosts immune system
Milk thistle	Supports healthy liver function
Valerian	Promotes restful sleep
High laurate canola oil	Lowers cholesterol
Insoluble fiber from cereals	Reduces risk of colon cancer
Oil rich in decosahexanoic acid	Enhances neurological and visual developments in infants
Oils engineered without *trans* fatty acids	Lowers heart disease risk
Omega-3 enriched eggs	Reduces serum triglycerides
Products made from blueberries, cranberries, bilberries and lingonberries (*Vaccinium genus*)	Reduces risk of inflammation and peripheral vascular disease, controls diabetes and improves vision
Resistant starch from grains	Promotes gastrointestinal health and lowers risk of colon disease
Shiitake mushrooms	Enhances immune activity, stimulates immune function and inhibits tumor growth
Soluble fiber from cereals	Aids in control of blood glucose
Soluble fiber from konjac glucomannan	Maintains intestinal health and suppresses appetite
Soy isoflavones	Reduces the negative effects of estrogen and lowers risk of several cancers
Soy protein	Reduces risk of coronary heart disease
Sunflower oil enriched with conjugated linoleic acid	Enhances anticancer immune defenses
Tomato with enhanced lycopene content	Reduces risk of specific types of cancer (eg, prostate)

omega-3 PUFA have been attributed to their ability to lower serum triglycerol and cholesterol. Consumption of 35 g or more of fish daily has been shown to reduce the risk of death from nonsudden myocardial infarction (24), and as little as one serving of fish per week was associated with a significantly reduced risk of total cardiovascular mortality (25). In addition, omega-3 PUFA are essential for normal growth and development and may also play a role in prevention and treatment of hypertension, arthritis, other inflammatory and autoimmune disorders, and cancer (26). In this context, several countries, including Canada, the United Kingdom, and Denmark, have dietary guidelines for omega-3 PUFA and requirements for its addition in infant formula.

CURRENT TRENDS

Consumer demand for foods capable of promoting good health and preventing or alleviating diseases is growing. This demand is likely to increase as baby boomers strive to manage the chronic health problems associated with aging and seek cost-effective alternatives to drugs. Also, with the ongoing legislative changes that permit claims for foods and associated components, and with the new and exciting scientific discoveries, many more companies, including multinationals, are and will continue to put more resources into the development and marketing of functional foods (27). Table 2 lists selected functional foods and bioactive food ingredients currently under active development in North America and around the world. In the process of developing new and improved functional products, a better understanding of food, nutrition, and health will likely emerge. Nonetheless, to gain wide acceptance, functional foods must offer health benefits that consumers can understand. Although terminology used in the functional food area can be confusing, it is generally agreed that the primary goal is to develop foods with added health benefits, rather than to rely on supplements. Any claim about the benefits of foods, however, must be based on sound and accurate scientific information. The development of functional foods also requires careful attention to safety, labeling, and claims as well as to nutritional and physiological rationale, cost, and sensory quality and should depend heavily on scientific substantiation.

BIBLIOGRAPHY

1. F. W. Scott et al., *Recommendations for Defining and Dealing With Functional Foods. A Discussion Paper*, Bureau of Nutritional Sciences, Food Directorate, Health Canada, Ottawa, 1996
2. B. F. Haumann, "Health Benefits May Be Key for Foods of the Future," *Int. News Fats, Oils Rel. Mat.* (INFORM) **10**, 18–22 (1999).
3. A. B. Caragay, "Cancer-Preventive Foods and Ingredients," *Food Technol.* **46**, 65–68 (1992).
4. C. S. Fuchs et al., "Dietary Fiber and the Risk of Colorectal Cancer and Adenoma in Women," *N. Engl. J. Med.* **340**, 169–176 (1999).
5. U.S. Dept. Health and Human Services/Food and Drug Administration, "Food Labelling: Health Claims; Oats and Coronary Heart Disease," *Federal Register* **62**, 3584–3601 (1997).
6. A. L. Gerhardt and N. B. Gallo, "Full-Fat Rice Bran and Oat Bran Similarly Reduce Hypercholesterolemia in Humans," *J. Nutr.* **128**, 865–869 (1998).
7. O. Alabaster, Z. Tang, and N. Shivapurkar, "Inhibition by Wheat Bran Cereals of the Development of Aberrant Crypt Foci and Colon Tumors," *Food Chem. Toxicol.* **35**, 517–522 (1997).
8. C. M. Hasler, "Functional Foods: Their Role in Disease Prevention and Health Promotion," *Food Technol.* **52**, 63–70 (1998).
9. C-F. Chan, P. C. K. Cheung, and Y-S. Wong, "Hypocholesterolemic Effects of Protein Concentrates From Three Chinese Indigenous Legume Seeds," *J. Agric. Food Chem.* **46**, 3698–3701 (1998).
10. B. D. Oomah and G. Mazza, "Flaxseed Products for Disease Prevention," in G. Mazza, ed., *Functional Foods Biochemical and Processing Aspects*, Technomic, Lancaster, Pa., 1998, pp. 91–138.
11. M. K. Gupta, "NuSun—The Future Generation of Oils," *Int. News Fats, Oils Rel. Mat.* (INFORM) **9**, 1150–1154 (1998).
12. S. K. Clinton, "Lycopene: Chemistry, Biology, and Implications for Human Health and Disease," *Nutr. Rev.* **56**, 35–51 (1998).
13. G. Cao et al., "Increases in Human Plasma Antioxidant Capacity After Consumption of Controlled Diets High in Fruit and Vegetables," *Am. J. Clin. Nutr.* **68**, 1081–1087 (1998).
14. H. M. Meltzer and K. E. Malterud, "Can Dietary Flavonoids Influence the Development of Coronary Heart Disease?" *Scand. J. Nutr.* **41**, 50–57 (1997).
15. D. R. Schmidt and A. E. Sobota, "An Examination of the Anti-adherence Activity of Cranberry Juice on Urinary and Nonurinary Bacterial Isolates," *Microbios.* **55**, 171–181 (1998).
16. A. P. Day et al., "Effects of Concentrated Red Grape Juice Consumption on Serum Antioxidant Capacity and Low-Density Lipoprotein Oxidation," *Ann. Nutr. Metab.* **41**, 353–357 (1998).
17. M. Jang et al., "Cancer Chemopreventive Activity of Resveratrol, A Natural Product Derived From Grapes," *Science* **275**, 218–220 (1997).
18. K. Nakachi et al., "Influence of Drinking Green Tea on Breast Cancer Malignancy Among Japanese Patients," *J. Jap. Cancer Res.* **89**, 254–261 (1998).
19. R. Klont and P. Mannion, "Inulin and oligofructose," *World of Ingredients*, 34–37 (Sept. 1998).
20. B. K. Mital and S. K. Garg, "Anticarcinogenic, Hypocholesterolemic, and Antagonistic Activities of *Lactobacilus acidophilus*," *Crit. Rev. Microbiol.* **21**, 175–214 (1995).
21. P. Jelen and S. Lutz, "Functional Milk and Dairy Products," in G. Mazza, ed., *Functional Foods Biochemical and Processing Aspects*, Technomic, Lancaster, Pa., 1998, pp. 357–380.
22. Y. L. Ha, N. K. Grimm, and M. W. Pariza, "Anticarcinogens From Fried Ground Beef: Health-Altered Derivatives of Linoleic Acid," *Carcinogenesis* **8**, 1881–1887 (1987).
23. E. Doyle, "Scientific Forum Explores CLA Knowledge," *Int. News Fats, Oils Rel. Mat.* (INFORM) **9**, 69–72 (1998).

24. M. L. Daviglus et al., "Fish Consumption and 30-Year Risk of Fatal Myocardial Infarction," *N. Eng. J. Med.* **336**, 1046–1053 (1997).
25. C. M. Albert et al., "Fish Consumption and Risk of Sudden Cardiac Death," *JAMA, J. Am. Med. Assoc.* **279**, 23–28 (1998).
26. F. Shahidi, "Functional Seafood Lipids and Proteins," in G. Mazza, ed., *Functional Foods Biochemical and Processing Aspects*, Technomic, Lancaster, Pa., 1998, pp. 381–401.
27. V. Brower, "Nutraceuticals: Poised for a Healthy Slice of the Healthcare Market?" *Nature Biotech.* **16**, 728–731 (1998).

B. DAVE OOMAH
G. MAZZA
Pacific Agri-Food Research Centre
Summerland, British Columbia
Canada

ENCYCLOPEDIA OF

FOOD SCIENCE AND TECHNOLOGY
Second Edition

VOLUME 1

Frederick J. Francis
University of Massachusetts
Amherst, Massachusetts

A Wiley-Interscience Publication
John Wiley & Sons, Inc.
New York / Chichester / Weinheim / Brisbane / Singapore / Toronto

This book is printed on acid-free paper. ♾

Copyright © 2000 by John Wiley & Sons, Inc. All rights reserved.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning or otherwise, except as permitted under Sections 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4744. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 605 Third Avenue, New York, NY 10158-0012, (212) 850-6011, fax (212) 850-6008, E-Mail: PERMREQ @ WILEY.COM.

For ordering and customer service, call 1-800-CALL-WILEY.

Library of Congress Cataloging-in-Publication Data:

Wiley encyclopedia of food science and technology.—2nd ed. / [edited by] Frederick J. Francis.
p. cm.
Rev. ed. of: Encyclopedia of food science and technology / Y.H. Hui, editor-in-chief. c1992.
Includes bibliographical references.
ISBN 0-471-19285-6 (set : cloth : alk. paper).—ISBN 0-471-19255-4 (v. 1 : cloth : alk. paper).—ISBN 0-471-19256-2 (v. 2 : cloth : alk. paper).—ISBN 0-471-19257-0 (v. 3 : cloth : alk. paper).—ISBN 0-471-19258-9 (v. 4 : cloth : alk. paper)
1. Food industry and trade Encyclopedias. I. Francis, F. J. (Frederick John), 1921- . II. Encyclopedia of food science and technology.
TP368.2.E62 2000
664'.003—dc21 99-29003
CIP

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

PREFACE

The preface to the First Edition of this encyclopedia provided a detailed account of its breadth and aims. This breadth is maintained in the Second Edition and enlarged to include more of the associated areas of food science. A number of these areas are of current prime importance, including food safety, functional foods, and neutraceuticals. Seventy years ago, when the discipline of food science and technology was first introduced, the accepted definition was the science concerned with food "from the farm gate to the consumer." It later became obvious that many of the conditions associated with conventional agricultural production practices had a definite impact on the quality and quantity of the resultant food, so the lines between agricultural production and food science and technology became blurred.

The above definition of food science and technology was appropriate for food production techniques in the early part of the twentieth century. We were primarily dependent on fresh fruits and vegetables in season and meats, augmented by commodities which could be easily stored such as grains, root crops, dried fruits and fermented foods, and beverages. The "New England boiled dinner," composed of corned beef, potatoes, carrots, cabbage, turnips, onions, and parsnips, was a good example. A major change from a seasonal concept of food supply to a processed system occurred in the 1920s with the development of food preservation techniques such as canning, freezing, dehydration, and chemical preservation. When this was combined with developments in packaging, storage, transportation, and consumer marketing, the rate of change to a processed-food society was speeded up. Today we have the advantages of a stable processed food supply combined with fresh fruits and vegetables produced locally and also shipped in from other countries. This has produced the best of both systems, but it has some problems. The concentration of production, packaging, storage, and transportation into large units has increased the possibility of contamination with chemicals and microorganisms, a major concern of regulatory officials; it may also be a source of concern for readers of this encyclopedia. Hopefully, the sections on food safety, estimation of risk, interpretation of risk, and management of risk will allay the fears for the safety of the global food supply.

Another change in the concept of food science and technology occurred in the 1950s with increased concern over adequate nutrition in food and the nutrition delivery system. The science of nutrition at that point had developed to the point where meaningful predictions could be made on the effect of components in the food supply on the health and well-being of the populace. This led to increased emphasis on food processors to maintain the nutritive value of their products in order to supply the "Acceptable Daily Intake" recommended by governmental and other agencies. However, maintenance of nutrients already present in food was not enough, and the science of fortification was developed primarily to prevent the development of conditions which could lead to a deterioration in the overall health of the individual. This concept was enlarged to encompass components of the diet which were not necessarily related to deficiencies and disease but were related to overall well-being. This led to the concept of "Neutraceuticals," components which combine nutritive and pharmaceutical properties. The term "functional foods" was also introduced to describe components which have a technological function in foods as well as a nutritive or health benefit. Both areas are rapidly expanding and may be of interest to readers of this encyclopedia.

ACKNOWLEDGMENTS

There are three groups of people who make possible the completion of a work of this size: the Editorial Board, the contributors, and the production personnel. The members of the Editorial Board were chosen for their understanding of particular areas of food science and technology that I thought should be given more consideration in the Second Edition. I believed that the First Edition had an excellent set of papers, primarily in technology, and I wanted to build on that base. All of the editors enjoy a superb reputation in their respective fields and I am grateful to them for their willingness to share opinions and expertise. The contributors supplied the real foundation for the encyclopedia and I am most grateful for their willingness to contribute their time and energy to write the papers. This expression of professionalism is reassuring for the future of the discipline. Finally, I would like to thank the group of professionals at Wiley for their administrative support in what otherwise would be an overwhelming assignment.

FREDERICK J. FRANCIS
Amherst, Massachusetts

PREFACE TO THE FIRST EDITION

Although this encyclopedia is designed for food scientists and technologists, it also contains information useful to food engineers, chemists, biologists, ingredient suppliers, and other professionals involved in the food chain.

The topics selected are based on the following frame of reference:

A. Basic and Applied Sciences
   1. Biology: botany, bacteriology, microbiology, mycology, eg, photosynthesis, food microbiology, mycotoxins.
   2. Chemistry: biochemistry, physical chemistry, analytical chemistry, organic chemistry, radiochemistry, forensic chemistry, eg, food chemistry, food analysis, interesterification, emulsification.
   3. Physics: rheology, thermodynamics, cryogenics, radiophysics, ultrasonics, eg, food rheology, thermodynamics, microwave, irradiation.
   4. Nutrition: basic, applied, and clinical nutrition, eg, nutrition, protein, energy, dietetics, nutritional quality and food processing.
   5. Psychology: sensory behaviors, eg, food and color, sensory sciences, taste and odor.
   6. Medicine: metabolisms, toxicology, heart diseases, eg, food utilization, cholesterol, foodborne diseases.
   7. Economics: marketing, development, eg, food marketing, food industry economic development.
   8. Integrated sciences: history of foods, food structure.

B. Processing Technology, Engineering, and the Twenty-three Unit Operations
   1. Raw material preparation, eg, apricots and apricot processing, milk and milk products.
   2. Size reduction, eg, potato and potato processing, crabs and crab processing.
   3. Mixing and forming, eg, confectionery, biscuits and cracker technology.
   4. Mechanical separations, eg, meat processing technology, cheeses, poultry product and technology.
   5. Membrane concentration, eg, milk and membrane technology
   6. Biotechnology, eg, genetic engineering
   7. Irradiation, eg, irradiation
   8. Blanching, eg, blanching, vegetables processing
   9. Pasteurization, eg, yogurt
   10. Heat sterilization, eg, aseptic processing
   11. Evaporation, eg, evaporation, distillation, heat transfer, heat exchangers
   12. Extrusion, eg, extrusion and extrusion cooking, snack foods
   13. Dehydration, eg, fruit dehydration, vegetable dehydration, drier technology and engineering
   14. Baking and roasting, eg, baking science and technology, breakfast cereals
   15. Frying, eg, frying technology, fats and oils
   16. Microwave and infrared radiation, eg, microwave science and technology
   17. Chilling and controlled- (modified-) atmosphere storage, eg, chilled foods, controlled atmospheres for fresh fruits and vegetables, packaging and modified atmospheres.
   18. Freezing, eg, food freezing, freezing systems, refrigerated foods
   19. Freeze drying and freeze concentration, eg, freeze drying, driers technology and engineering.
   20. Coating or enrobing, eg, fish and shellfish products, poultry processing technology,
   21. Packaging, eg, packaging materials, packaging and labeling
   22. Filling and sealing of containers, eg, aseptic processing and packaging
   23. Materials handling and process control, eg, computer and food processing, artificial intelligence, expert systems

C. Food Laws and Regulations
   1. Basic principles, eg, food laws and regulations
   2. Food identity, eg, standards and grades
   3. Food chemicals, eg, food additives
   4. Health and safety, eg, sanitation
   5. Food processing plants, eg, food plant design and construction, food processing: standard industrial classifications.
   6. Food plant inspection, eg, food laws and regulations.

Apart from members of the editorial advisory board, hundreds of professionals in the academic, industry, and government have personally counseled me on many aspects of the work. Their combined effort is reflected in the articles in this encyclopedia: appropriateness of the selection of topics; diverse expertise and background of each contributor; the excellent treatment of each article. The users are the best judge of these claims.

A small portion of the text is devoted to cover nontechnical data:

1. Descriptions of selected scientific institutions, eg, Institute of Food Technologists.
2. Food science and technology in selected countries in the world, eg, International Development: South Africa.

3. Research and development in major U.S. government agencies, eg, U.S. Department of Agriculture.

ACKNOWLEDGMENTS

Basically, there are three groups of people who make the completion of this large work possible: the advisors, the contributors, and the production personnel.

The advisors include the members of the editorial board, my friends, colleagues, and other experts. They helped me to formulate the subject matters, select contributors and reviewers, and solve scientific, technical, and engineering matters. Although it is not possible to name them all here, they are the foundation of the project. And I am grateful to them.

The contributors are the components and parts and give substance to the encyclopedia. Their contribution is most appreciated.

The production team puts the parts together and you are the best judge of their professionalism. Personally, I am specially grateful to Michalina Bickford, the managing editor for the encyclopedia.

The support of my family will always be appreciated.

Y. H. Hui
Cutten, California

CONTRIBUTORS

An entry that appears without a contributor's signature is based on the corresponding entry taken from the First Edition of this Encyclopedia and updated by the Editors of this Second Edition.

APV Crepaco, Inc., *Lake Mills, Wisconsin,* Corrosion and Food Processing; Distillation: Technology and Engineering; Dryers: Technology and Engineering; Evaporators: Technology and Engineering; Heat Exchangers: Fouling; Heat Exchangers: Paraflow; Heat Exchangers: Plate versus Tubular; Heat Exchangers: Scraped Surface

Judith A. Abbott, *USDA/ARS, Beltsville, Maryland,* Food Crops: Nondestructive Quality Evaluation

G. Abraham, *USDA, Southern Regional Research Center,* Oilseeds and Vegetable Oils

Raija Ahvenainen, *VTT Biotechnology and Food Research, VTT, Finland,* International Development: Finland

Robert E. Altomare, *General Foods USA, Tarrytown, New York,* Snack Food Technology

S. M. Alzamora, *Washington State University, Pullman, Washington,* Water Activity: Microbiology

Burton A. Amernick, *Pollock, Vande Sande, and Amernick, RLLP, Washington, D.C.,* Patents

Elizabeth Andress, *University of Georgia, Athens, Georgia,* Canning: Regulatory and Safety Considerations

Cathy Y. W. Ang, *North Carolina State University, Raleigh, North Carolina,* Poultry Flavors

Frank H. Arthur, *USDA/ARS, Manhattan, Kansas,* Grains and Protectants

Bonnie Atwood, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Alex A. Avery, *Hudson Institute, Churchville, Virginia,* Food Regulations: International, Codex Alimentarius

Dennis T. Avery, *Hudson Institute, Churchville, Virginia,* Food Regulations: International, Codex Alimentarius

Jerry K. Babbitt, *National Marine Fisheries Service, Kodiak, Alaska,* Crabs and Crab Processing

T. Sudhakar Babu, *University of Waterloo, Waterloo, Ontario, Canada,* Photosynthesis

Robert J. Baer, *South Dakota State University, Brookings, South Dakota,* Milk and Milk Products

M. O. Balaban, *State University of Campinas—UNICAMP, São Paulo, Brazil,* Supercritical Fluid Technology

G. V. Barbosa-Cánovas, *Washington State University, Pullman, Washington,* Water Activity: Microbiology

Shai Barbut, *University of Guelph, Guelph, Ontario, Canada,* Poultry Meat Processing and Product Technology

Steven A. Barker, *Louisiana State University, Baton Rouge, Louisiana,* Antibiotics in Foods of Animal Origin

Harold J. Barnett, *National Oceanic and Atmospheric Administration, Seattle, Washington,* Seafood: Flavors and Quality

Roger G. Bates, *University of Florida, Gainesville, Florida,* Hydrogen-Ion Activity (pH)

J. N. BeMiller, *Purdue University, West Lafayette, Indiana,* Carbohydrates: Classification, Chemistry, and Labeling; Gums; Lactose; Sweeteners: Nutritive; Starch; Sugar: Sucrose

Richard C. Berberet, *Oklahoma State University, Stillwater, Oklahoma,* Postharvest Integrated Pest Management

Carolyn Berdanier, *University of Georgia, Athens, Georgia,* Minerals: Micronutrients

R. G. Berger, *Institut für Lebensmittelchemie der Universität Hannover, Hannover, Germany,* Genetic Engineering: Food Flavors

Daniel Berkowitz, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Mrinal Bhattacharva, *University of Minnesota, St. Paul, Minnesota,* Extrusion Processing: Texture and Rheology

Darlena K. Blucher, *Humboldt State University, Arcata, California,* History of Foods

Michael M. Blumenthal, *Libra Technologies, Inc., Metuchen, New Jersey,* Frying Technology

Joseph Borsa, *MDS Nordion, Inc., Kanata, Ontario, Canada,* Irradiation of Foods

J. R. Botta, *Moncton Food Quality Investigations, Inc., Moncton, New Brunswick, Canada,* Lobster: Biology and Technology; Seafood: Sensory Evaluation and Freshness

Malcolm C. Bourne, *Cornell University, Geneva, New York,* Water Activity: Food Texture

Elizabeth A. E. Boyle, *Kansas State University, Manhattan, Kansas,* Meat Products

Francine Bradley, *University of California, Davis, California,* Poultry: Meat From Avian Species

J. W. Brady, *Cornell University, Ithaca, New York,* Molecular Modeling: Computer Modeling of Functional Properties

D. E. Briggs, *University of Birmingham, Birmingham, England,* Malts and Malting

Aaron L. Brody, *Rubbright•Brody, Inc., Duluth, Georgia,* Packaging: Part II—Labeling; Packaging: Part IV—Controlled/Modified Atmosphere/Vacuum Food Packaging

B. Brückner, *Institute of Vegetable and Ornamental Crops, Grossbeeren/Erfurt, Germany,* International Development: Germany

Christine M. Bruhn, *University of California, Davis, California,* Food Safety Education: Consumer

John Bumbalough, *Land O'Lakes, Inc., Arden Hills, Minnesota,* Margarine

David Burdick, *Hoffman-La Roche, Inc.,* Vitamins: Pyridoxine (B$_6$); Vitamins: Thiamine (B$_1$)

Charles J. Cante, *General Foods USA, White Plains, New York,* Breakfast Cereals; Snack Food Technology

Armand Cardello, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Marijana Caric, *Novi Sad University, Novi Sad, Yugoslavia,* Processed Cheese

Robert Casani, *Hoffman-La Roche, Inc.,* Vitamins: Vitamin E

Robert G. Cassens, *University of Wisconsin, Madison, Wisconsin,* Muscle as Food

Domenico R. Cassone, *Nabisco, Inc., East Hanover, New Jersey,* Biscuit and Cracker Technology

S. Cenkowski, *University of Manitoba, Winnipeg, Manitoba, Canada,* Cleaning-in-Place (CIP); Energy Usage in Food Processing Plants

Tuu-jyi Chai, *University of Maryland, Cambridge, Maryland,* Fish and Shellfish Microbiology

James V. Chambers, *Purdue University, West Lafayette, Indiana,* Waste Management: Animal Processing

Roberto S. Chamul, *Mississippi State University, Mississippi State, Mississippi,* Machine Vision Systems: Process and Quality Control

D. Chandarana, *National Food Processors Association, Dublin, California,* Aseptic Processing and Packaging Systems

Ping Yang Chang, *Food Industry Research and Development Institute, Hsinchu, Taiwan,* Sulfites and Food

Muhammad Munir Chaudry, *Cornell University, Ithaca, New York,* Muslim Dietary Laws: Food Processing and Marketing

Chee-Jen Chen, *Food Industry Research and Development Institute, Hsinchu, Taiwan,* Mushrooms: Cultivation

T. C. Chen, *University of Arkansas, Fayetteville, Arkansas,* Poultry Meat Microbiology

Wen-Lian Chen, *Food Industry Research and Development Institute, Hsinchu, Taiwan,* Soy Foods, Fermented

Munir Cheryan, *University of Illinois, Urbana, Illinois,* Membrane Processing

Young-Meng Chiang, *National Taiwan University, Taipei, Taiwan,* Seaweed Aquaculture: Taiwan

H. Chiba, *Kobe Women's University, Kobe, Japan,* Peptides

Yoon Hin Chong, *Palm Oil Research Institute of Malaysia, Selangor, Malaysia,* Palm Oil

B. B. Chrystall, *Massey University–Albany, Auckland, New Zealand,* International Development: New Zealand; Meat and Electrical Stimulation; Meat Science

J. Peter Clark, *Oak Park, Illinois,* Food Plant Design and Construction

Warren S. Clark, Jr., *American Dairy Products Institute, Chicago, Illinois,* Dry Milk; Evaporated Milk

J. T. Clayton, *University of Massachusetts, Amherst, Massachusetts,* Food Engineering

John Clemmings, *Häagen-Dazs Research and Development, Edina, Minnesota,* Ice Cream and Frozen Dessert

Dean Cliver, *University of California, Davis, California,* Bovine Spongiform Encephalopathy (BSE)

G. Coccodrilli, *General Foods USA, White Plains, New York,* Breakfast Cereals

Bernard Cole, *Leiner Davis Gelatin, West Krugersdorp, South Africa,* Gelatin

David Collins-Thompson, *Nestlé Research and Development Center, Inc., New Milford, Connecticut,* Chilled Foods

Chris Combs, *Dow Corning Corporation, Midland, Michigan,* Foams and Silicones in Food Processing

Richard Cotter, *Whitehall-Robins, Madison, New Jersey,* Osteoporosis

Roger A. Coulombe, Jr., *Utah State University, Logan, Utah,* Toxicants, Natural

Thomas Crosby, *Bunge Foods Corporation, Bradley, Illinois,* Fats and Oils: Properties, Processing Technology, and Commercial Shortenings

Gerrit W. Cuperus, *Oklahoma State University, Stillwater, Oklahoma,* Postharvest Integrated Pest Management

Ashim K. Datta, *Cornell University, Ithaca, New York,* Thermal Sterilization of Canned Liquid Foods

P. Michael Davidson, *University of Tennessee, Knoxville, Tennessee,* Antimicrobial Compounds

Robert V. Decareau, *Microwave Consulting Services, Amherst, New Hampshire,* Microwave Science and Technology

Eric A. Decker, *University of Massachusetts, Amherst, Massachusetts,* Antioxidants; Phytochemicals: Antioxidants

Marnie L. DeGregorio, *Kraft Foods USA, Tarrytown, New York,* Fruit Preserves and Jellies

David Deis, *Abbott Laboratories, Columbus, Ohio,* Enteral Formulas and Feeding Systems

J. M. de Man, *University of Guelph, Guelph, Ontario, Canada,* Fats and Oils: Chemistry, Physics, and Applications

C. E. Devine, *Technology Development Group, HortResearch, Hamilton, New Zealand,* Meat and Electrical Stimulation; Meat Science

D. E. Dixon-Holland, *Neogen Corporation, Lansing, Michigan,* Immunological Methodology

Cindy Dominguez, *Whitehall-Robins, Madison, New Jersey,* Osteoporosis

Mark L. Dreher, *Mead Johnson Nutritionals, Bristol-Myers Squibb, Evansville, Indiana,* Carbohydrates: Functionality and Physiological Significance

Grant E. DuBois, *The Coca-Cola Company, Atlanta, Georgia,* Sweeteners: Nonnutritive

Muri Dueppen, *AOAC INTERNATIONAL, Gaithersburg, Maryland,* AOAC INTERNATIONAL

C. Patrick Dunne, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Richard A. Durst, *University of Florida, Gainesville, Florida,* Hydrogen-Ion Activity (pH)

Phyllis Entis, *QA Life Sciences, San Diego, California,* Membrane Filtration Systems

Marilyn Erickson, *University of Georgia, Griffin, Georgia,* Food Spoilage

Ralph Ernst, *University of California, Davis, California,* Poultry: Meat From Avian Species

Felix E. Escher, *Swiss Federal Institute of Technology (ETH), Zurich, Switzerland,* International Development: Switzerland

N. A. M. Eskin, *University of Manitoba, Winnipeg, Manitoba, Canada,* Canola Oil

Terry D. Etherton, *Penn State University, University Park, Pennsylvania,* Lipids: Nutrition

David A. Evans, *University of Massachusetts, Amherst, Massachusetts,* Disinfectants

James Faller, *University of Illinois, Urbana, Illinois,* Food Processing

Daniel F. Farkas, *Oregon State University, Corvallis, Oregon,* High-Pressure Processing

Paul Fazio, *Haagen-Dazs Research and Development, Edina, Minnesota,* Ice Cream and Frozen Dessert

P. J. Fellers, *State of Florida Department of Citrus, Lake Alfred, Florida,* Limonin

P. Fellows, *Intermediate Technology Development Group, Rugby, United Kingdom,* Dehydration

Robin M. Fenwick, *New Zealand Dairy Board, Wellington, New Zealand,* Dairy Ingredients for Foods

Mario P. de Figueiredo, *Consultant, Chesterfield, Missouri,* Quality Assurance

Marshall Fishman, *USDA/ARS, Wyndmoor, Pennsylvania,* Pectic Substances

Dick Fizzell, *General Foods USA, White Plains, New York,* Breakfast Cereals

Henry Fleming, *USDA/ARS, Raleigh, North Carolina,* Vegetables, Pickling

John D. Floros, *Purdue University, West Lafayette, Indiana,* Mass Transfer and Diffusion in Foods; Optimization Methods in Food Processing and Engineering

F. J. Francis, *Editor-in-Chief, University of Massachusetts, Amherst, Massachusetts,* Aromatherapy; Biologically Stable Intermediates; Color and Food; Colorants Section; Fruits, Semi-Tropical; Fruits, Temperate; Fruits, Tropical

Daniel Y. C. Fung, *Kansas State University, Manhattan, Kansas,* Food Fermentation; Foodborne Diseases; Microbiology of Foods; Rapid Methods of Microbiological Analysis

Carol A. Gallagher, *Saint Joseph's University, Philadelphia, Pennsylvania,* Food Marketing

Elisabeth L. Garcia, *University of California, Davis, California,* Food Safety and Risk Communication; Food Safety and Risk Management; Pesticide Residues in Food; Toxicology and Risk Assessment

David R. Gard, *Solutia, Inc., St. Louis, Missouri,* Phosphates and Food Processing

Marianne H. Gellette, *McCormick & Co., Inc., Hunt Valley, Maryland,* Vanilla Extract

Aristippos Gennadios, *Banner Pharmacaps, Inc., High Point, North Carolina,* Edible Films and Coatings

Richard J. George, *Saint Joseph's University, Philadelphia, Pennsylvania,* Food Marketing

Shibani A. Ghosh, *University of Massachusetts, Amherst, Massachusetts,* Affluence, Food Excess, and Nutritional Disorders; Hunger, Food Deprivation, and Nutritional Disorders

Linda Gilbert, *HealthFocus, Inc., Des Moines, Iowa,* Vegetarian and Vegetarian-Aware Eating Trends

H. Douglas Goff, *University of Guelph, Guelph, Ontario, Canada,* Food Preservation

M. M. Góngora-Nieto, *Washington State University, Pullman, Washington,* Water Activity: Microbiology

J. Richard Gorham, *Uniformed Services University of the Health Sciences, Bethesda, Maryland,* Filth and Extraneous Matter in Food

Harold N. Graham, *New York City, New York,* Caffeine; Tannins; Tea

Bob Graves, *Balchem Corporation, Slate Hill, New York,* Encapsulation Techniques

Bruce Greenberg, *University of Waterloo, Waterloo, Ontario, Canada,* Photosynthesis

Scott M. Griffith, *Admix, Inc., Manchester, New Hampshire,* Beverages: Carbonated; Beverages: Noncarbonated

James A. Guzinski, *Kalsec, Inc., Kalamazoo, Michigan,* Spices and Herbs: Natural Extractives

Norman F. Haard, *University of California, Davis, California,* Marine Enzymes

Leigh Hadden White, *Häagen-Dazs Research and Development, Edina, Minnesota,* Ice Cream and Frozen Dessert

R. L. Hall, *Baltimore, Maryland,* International Union of Food Science and Technology

Yong D. Hang, *Cornell University, Geneva, New York,* Waste Management: Fruits and Vegetables

Milford A. Hanna, *University of Nebraska, Lincoln, Nebraska,* Edible Films and Coatings

Robert Harkins, *North Brunswick, New Jersey,* Sugar: Substitutes, Bulk, Reduced Calorie

Ronald D. Harris, *Kraft General Foods, Inc., Glenview, Illinois,* Cheese

Robert C. Hastert, *Hastech Corporation, Omaha, Nebraska,* Hydrogenation

James G. Hawkes, *Continental Colloids, Inc., Chicago, Illinois,* Food Processing: Effect on Nutritional Quality

Jim Heber, *Helsingborg, Sweden,* Freezing Systems for the Food Industry

Susan L. Hefle, *University of Nebraska, Lincoln, Nebraska,* Food Allergy

Barbara B. Heidolph, *Solutia, Inc., St. Louis, Missouri,* Phosphates and Food Processing

C. P. Herman, *University of Toronto, Toronto, Ontario, Canada,* Appetite

David Hettinga, *Land O' Lakes, Arden Hills, Minnesota,* Butter and Butter Products

S. Hill, *International Food Information Service, Reading, United Kingdom,* International Food Information Service (IFIS)

John T. Hines, *Kraft General Foods, Inc., Glenview, Illinois,* Cheese

Alan R. Hirsch, *Smell and Taste Treatment and Research Foundation, Chicago, Illinois,* Aphrodisiacs and Stimulants

Arnold L. Hirsch, *Alpharma, Inc.,* Vitamins: Vitamin D

Edward Hirsch, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Y. C. Ho, *Rutgers University, New Brunswick, New Jersey,* Packaging: Part I—General Considerations; Packaging: Part III—Materials

Patrick G. Hoffman, *McCormick & Co., Inc., Hunt Valley, Maryland,* Vanilla Extract

Stefan Hogekamp, *Universität Karlsruhe, Karlsruhe, Germany,* Agglomeration and Agglomerator Systems

David N. Holcomb, *Technical University of Nova Scotia, Halifax, Nova Scotia, Canada,* Rheology

Stanley Holgate, *U.S. Army Natick Research, Development and Engineering Center, Natick, Massachusetts,* United States: Armed Forces Food Research and Development

D. E. Hood, *Baltimore, Maryland,* International Union of Food Science and Technology

R. Carl Hoseney, *R & R Research Services, Manhattan, Kansas,* Cereals Science and Technology

R. J. Hron, *USDA, Southern Regional Research Center,* Oilseeds and Vegetable Oils

Fu-hung Hsieh, *University of Missouri, Columbia, Missouri,* Extrusion; Extrusion Cooking; Ultrafiltration and Reverse Osmosis

Victor Huang, *Haagen-Dazs Research and Development, Edina, Minnesota,* Ice Cream and Frozen Dessert

Y. H. Hui, *American Food and Nutrition Center, Cutten, California,* Alkaloids; Cultural Nutrition; Food Processing: Standard Industrial Classification; Food Utilization; Shellfish; Soybeans and Soybean Processing

W. Jeffrey Hurst, *Hershey Foods Technical Center, Hershey, Pennsylvania,* Laboratory Robotics and Automation

Robert L. Jackman, *University of Guelph, Guelph, Ontario, Canada,* Proteins: Denaturation and Food Processing

Lauren Jackson, *FDA-NCFST, Argo, Illinois,* Mycotoxin Analysis

Claude Janelle, *Agriculture and AgriFood Canada, Ottawa, Ontario, Canada,* International Development: Canada

D. S. Jayas, *University of Manitoba, Winnipeg, Manitoba, Canada,* Cleaning-in-Place (CIP); Energy Usage in Food Processing Plants

P. Jelen, *University of Alberta, Edmonton, Alberta, Canada,* Whey: Composition, Properties, Processing, and Uses

B. Jessen, *Danish Meat Research Institute, Roskilde, Denmark,* Meat Starter Cultures and Meat Product Manufacturing

Mark Kantor, *University of Maryland, College Park, Maryland,* Fresh-Cut Fruits and Vegetables: Modified Atmosphere Packaging

George Katsushi Iwama, *University of British Columbia, Vancouver, British Columbia, Canada,* Fishes: Anatomy and Physiology; Fishes: Species of Economic Importance

Frances Katz, *Institute of Food Technologists, Chicago, Illinois,* Institute of Food Technologists (IFT)

Tetsuya Kawakita, *Ajinomoto Company, Inc., Kawasaki, Japan,* Nucleotides; Proteins: Amino Acids

Michael Keane, *University College Cork, Cork, Ireland,* International Development: Ireland

Patricia Kearney, *PMK Associates, Arlington, Virginia,* Nuts

J. F. Kefford, *North Ryde, New South Wales, Australia,* International Development: Australia

D. Mark Kettunen, *General Foods USA, Tarrytown, New York,* Snack Food Technology

George G. Khachatourians, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* Genetic Engineering: Principles and Applications

J. W. Kiceniuk, *Fernleigh House, Halifax, Nova Scotia, Canada,* Lobster: Biology and Technology

Seok Joong Kim, *The University of Texas Health Science Center, San Antonio, Texas,* Phytochemicals: Melatonin

K. J. Kirkpatrick, *New Zealand Dairy Board, Wellington, New Zealand,* Dairy Ingredients for Foods

Donald Klingborg, *University of California, Davis, California,* Bovine Spongiform Encephalopathy (BSE)

William H. Knightly, *Emulsion Technology, Inc., Wilmington, Delaware,* Emulsifier Technology in Foods

Hannu Korkeala, *VTT Biotechnology and Food Research, VTT, Finland,* International Development: Finland

Penny M. Kris-Etherton, *Penn State University, University Park, Pennsylvania,* Lipids: Nutrition

David Kritchevsky, *The Wistar Institute, Philadelphia, Pennsylvania,* Cancer Risk and Diet

Donald H. Kropf, *Kansas State University, Manhattan, Kansas,* Meat, Modified Atmosphere Packaging

Volker Kuellmer, *Hoffman-La Roche, Inc.,* Vitamins: Ascorbic Acid

Rauno Lampi, *Westboro, Massachusetts,* Retort Pouch

Duane K. Larick, *North Carolina State University, Raleigh, North Carolina,* Poultry Flavors

A. K. Lau, *University of British Columbia, Vancouver, British Columbia, Canada,* Evaporation

Chang Y. Lee, *Cornell University, Geneva, New York,* Browning Reaction, Enzymatic; Phenolic Compounds

Chong M. Lee, *University of Rhode Island, Kingston, Rhode Island,* Surimi: Science and Technology

Lothar Leistner, *Federal Centre for Meat Research, Kulmbach, Germany,* Hurdle Technology

D. Paul Leitch, *U.S. Army Natick Research, Development and Engineering Center, Natick, Massachusetts,* United States: Armed Forces Food Research and Development

Eunice C. Y. Li-Chan, *University of British Columbia, Vancouver, British Columbia, Canada,* Hydrophobicity in Food Protein Systems

Hanhua Liang, *Purdue University, West Lafayette, Indiana,* Mass Transfer and Diffusion in Foods

I Chiu Liao, *Taiwan Fisheries Research Institute, Keelung, Taiwan,* Eel

Rong C. Lin, *U.S. Food and Drug Administration, Washington, D.C.,* Water Activity: Good Manufacturing Practice

Rudy R. Lin, *Swift-Eckrich, Inc., Downers Grove, Illinois,* Elastins and Meat Ligaments

Miia Lindström, *VTT Biotechnology and Food Research, VTT, Finland,* International Development: Finland

Ming-Sai Liu, *Food Industry Research and Development Institute, Hsinchu, Taiwan,* Bamboo Shoots

John K. Lodge, *University of California, Berkeley, California,* Phytochemicals: Lipoic Acid

Goran Löndahl, *Helsingborg, Sweden,* Freezing Systems for the Food Industry

Austin R. Long, *Louisiana State University, Baton Rouge, Louisiana,* Antibiotics in Foods of Animal Origin

G. R. Longdill, *Meat Industry Research Institute of New Zealand, Hamilton, New Zealand,* Meat Slaughtering and Processing Equipment

A. López-Malo, *Washington State University, Pullman, Washington,* Water Activity: Microbiology

B. S. Luh, *University of California, Davis, California,* Vegetable Processing

Ted M. Lupina, *Kalsec, Inc., Kalamazoo, Michigan,* Spices and Herbs: Natural Extractives

Bernadene Magnuson, *University of Idaho, Moscow, Idaho,* Food Toxicology

Raymond R. Mahoney, *University of Massachusetts, Amherst, Massachusetts,* Enzymology; Immobilized Enzymes

Charles H. Manley, *Takasago International Corporation, USA, Inc., Teterboro, New Jersey,* Dairy Flavors

Robert A. Martin, *Hershey Foods Technical Center, Hershey, Pennsylvania,* Laboratory Robotics and Automation

Paul R. Mathewson, *Food Technology Resource Group, Park City, Utah,* Enzymes in Food Production

Donald N. Maynard, *University of Florida, Bradenton, Florida,* Vegetable Production

G. Mazza, *Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada,* Functional Foods

Terry L. McAninch, *Birko Corporation, Westminster, Colorado,* Detergents

Julian McClements, *University of Massachusetts, Amherst, Massachusetts,* Food Analysis

B. E. McDonald, *University of Manitoba, Winnipeg, Manitoba, Canada,* Canola Oil

Richard R. McFeaters, *Nabisco, Inc., East Hanover, New Jersey,* Biscuit and Cracker Technology

Martina McGloughlin, *University of California, Davis, California,* Genetic Engineering: Animals

John U. McGregor, *Louisiana State University Agricultural Center, Baton Rouge, Louisiana,* Cultured Milk Products

Kristen McNutt, *Consumer Choices, Inc., Winfield, Illinois,* Food and Nutrition Science Alliance Organizations

M. Medina, *National Institute for Agricultural and Food Research and Technology (INIA), Madrid, Spain,* International Development: Spain

M. A. A. Meireles, *State University of Campinas—UNICAMP, São Paulo, Brazil,* Supercritical Fluid Technology

H. H. Meissner, *ARC—Animal Nutrition and Animal Products Institute, Irene, South Africa,* International Development: South Africa

Alice Meyer, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Suzette Middleton, *Procter & Gamble Co., Cincinnati, Ohio,* Fats and Oils: Substitutes

A. J. Miller, *United States Department of Agriculture, Philadelphia, Pennsylvania,* Sausages

J. David Miller, *Carleton University, Ottawa, Ontario, Canada,* Mycotoxins

David B. Min, *The Ohio State University, Columbus, Ohio,* Fats and Oils: Flavors

Enrico Miniati, *Perugia University, Perugia, Italy,* Phytochemicals: *Vaccinium*

Vikram V. Mistry, *South Dakota State University, Brookings, South Dakota,* Milk and Milk Products

Peter L. Montagne, *Sharples, Inc., Warminster, Pennsylvania,* Centrifuges: Principles and Applications

Alanna J. Moshfegh, *USDA/ARS, Beltsville, Maryland,* Food Consumption Surveys in the U.S. Department of Agriculture

Howard R. Moskowitz, *Moskowitz Jacobs Inc., White Plains, New York,* Sensory Science: Principles and Applications

K. Darwin Murrell, *USDA/ARS/NRRC, Peoria, Illinois,* Parasitic Organisms

Eric C. Mussen, *University of California Entomology Extension, Davis, California,* Beekeeping

S. Nakai, *University of British Columbia, Vancouver, British Columbia, Canada,* Proteins: Structure and Functionality

Ananth Narayan, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Jennifer Near, *University of California, Davis, California,* Poultry: Meat From Avian Species

A. C. Noble, *University of California, Davis, California,* Wine

John Norback, *University of Wisconsin, Madison, Wisconsin,* Food Processing: Technology, Engineering, and Management

M. Nuñez, *National Institute for Agricultural and Food Research and Technology (INIA), Madrid, Spain,* International Development: Spain

K. Rajinder Nuath, *Kraft, General Foods, Inc., Glenview, Illinois,* Cheese

John O'Brien, *University of Surrey, Guildford, United Kingdom,* International Development: United Kingdom

Robert Olsen, *Schreiber Foods, Inc., Tempe, Arizona,* Artificial Intelligence; Computer Applications in the Food Industry

B. Dave Oomah, *Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada,* Functional Foods

Jose M. Ordovas, *Tufts University, Boston, Massachusetts,* Olives and Olive Oil

Thomas M. Ott, *Purepulse Technologies, Westwood, New Jersey,* Pulsed Light Processing

Robert A. Outten, *Hoffmann-La Roche, Inc.,* Vitamins: Biotin

Joseph L. Owades, *Consultant, Sonoma, California,* Alcoholic Beverages and Human Responses; Beer; Distilled Beverage Spirits

Lester Packer, *University of California, Berkeley, California,* Phytochemicals: Lipoic Acid

Mahesh Padmanabhan, *University of Minnesota, St. Paul, Minnesota,* Extrusion Processing: Texture and Rheology

Patti Pagliuco, *Institute of Food Technologists, Chicago, Illinois,* Institute of Food Technologists: Awards

William D. Pandolfe, *APV Americas-Homogenizers, Wilmington, Massachusetts,* Colloid Mills; Homogenizers

Robert Parker, *Cornell University, Ithaca, New York,* Phytochemicals: Carotenoids

John G. Parsons, *South Dakota State University, Brookings, South Dakota,* Milk and Milk Products

Kenneth G. Payie, *University of Guelph, Guelph, Ontario, Canada,* Proteins: Denaturation and Food Processing

Peter L. Pellett, *University of Massachusetts, Amherst, Massachusetts,* Affluence, Food Excess, and Nutritional Disorders; Hunger, Food Deprivation, and Nutritional Disorders

Per Oskar Persson, *Helsingborg, Sweden,* Freezing Systems for the Food Industry

J. F. Pfeiffer, *University of California, Davis, California,* Wine

Thomas W. Phillips, *Oklahoma State University, Stillwater, Oklahoma,* Postharvest Integrated Pest Management

Raul H. Piedrahita, *University of California, Davis, California,* Aquaculture: Engineering and Construction

George M. Pigott, *Sea Resources Engineering, Inc., Kirkland, Washington,* Extraction; Fish and Shellfish Products; Heat; Heat Exchangers; Heat Transfer; Thermodynamics

J. Terry Pitts, *USDA/ARS, Manhattan, Kansas,* Grains and Protectants

Y. Pomeranz, *Washington State University, Pullman, Washington,* Wheat Science and Technology

Dorothy Pond-Smith, *Washington State University, Pullman, Washington,* Foodservice Systems

William Porter, *U.S. Army Natick, Natick, Massachusetts,* Military Food

John J. Powers, *University of Georgia, Athens, Georgia,* Food Science and Technology. Definition and Development; Food Science and Technology: The Profession; Institute of Food Technologists: History and Perspectives; Sensory Science: Standardization and Instrumentation

J. H. Prentice, *Axminster, Devon, United Kingdom,* Cheese Rheology

R. L. Preston, *Texas Tech University, Lubbock, Texas,* Livestock Feeds

George Purvis, *Purvis Consulting, Inc., Fremont, Michigan,* Infant Foods

Jeff Rattray, *Purdue University, West Lafayette, Indiana,* Mass Transfer and Diffusion in Foods; Optimization Methods in Food Processing and Engineering

Thimma R. Rawalpally, *Hoffmann-La Roche, Inc.,* Vitamins: Folic Acid; Vitamins: Pantothenic Acid

Carrie E. Regenstein, *University of Rochester, Rochester, New York,* Kosher Foods and Food Processing

Joe M. Regenstein, *Cornell Information Technologies, Ithaca, New York,* Kosher Foods and Food Processing; Muslim Dietary Laws: Food Processing and Marketing

D. S. Reid, *University of California, Davis, California,* Food Freezing

Russel J. Reiter, *The University of Texas Health Science Center, San Antonio, Texas,* Phytochemicals: Melatonin

Mikelle Roeder, *University of Idaho, Moscow, Idaho,* Animal Science and Livestock Production

Richard A. Roeder, *University of Idaho, Moscow, Idaho,* Animal Science and Livestock Production

Debra Rooney, *Abbott Laboratories, Columbus, Ohio,* Enteral Formulas and Feeding Systems

Diane Rosenwald, *Häagen-Dazs Research and Development, Edina, Minnesota,* Ice Cream and Frozen Dessert

Leif Rynnel, *Helsingborg, Sweden,* Freezing Systems for the Food Industry

Raymond G. Saba, *Rutgers University, New Brunswick, New Jersey,* Packaging: Part I—General Considerations; Packaging: Part III—Materials

Fred W. Schenck, *Sun City, Arizona,* Syrups (Starch Sweeteners and Other Syrups)

Donald Schlimme, *University of Maryland, College Park, Maryland,* Fresh-Cut Fruits and Vegetables: Modified Atmosphere Packaging

Glenn R. Schmidt, *Colorado State University, Fort Collins, Colorado,* Meat Products

Helmar Schubert, *Universität Karlsruhe, Karlsruhe, Germany,* Agglomeration and Agglomerator Systems

Gerald L. Schulz, *U.S. Army Natick Research, Development and Engineering Center, Natick, Massachusetts,* Military Food; United States: Armed Forces Food Research and Development

Henry G. Schwartzberg, *University of Massachusetts, Amherst, Massachusetts,* Coffee; Freeze Concentration; Freeze Drying

John W. Scott, *Hoffmann-La Roche, Inc.,* Vitamins: Survey; Vitamins: Vitamin B_{12}

V. N. Scott, *National Food Processors Association, Washington, D.C.,* Low-Acid and Acidified Foods

Jeremy D. Selman, *Campden Food & Drink Research Association, Campden, Gloucestershire, United Kingdom,* Blanching

Amaral Sequeira-Munoz, *University of California, Davis, California,* Marine Enzymes

P. Shatadal, *University of Manitoba, Winnipeg, Manitoba, Canada,* Cleaning-in-Place (CIP)

Kalidas Shetty, *University of Massachusetts, Amherst, Massachusetts,* Phytochemicals: Biotechnology of Phenolic Phytochemicals for Food Preservatives and Functional Food Applications; Solid-State Fermentation and Value–Added Utilization of Fruit and Vegetable Processing By-Products

Robert L. Shewfelt, *University of Georgia, Athens, Georgia,* Food Crops: Postharvest Deterioration; Food Crops: Varietal Differences, Maturation, Ripening, and Senescence

John M. Siddle, *Nestlé Research and Development Center, Inc., New Milford, Connecticut,* Chilled Foods

Joseph B. Sieczka, *Cornell University, Riverhead, New York,* Potatoes and Potato Processing

Juan L. Silva, *Mississippi State University, Mississippi State, Mississippi,* Machine Vision Systems: Process and Quality Control

Gerald Silverman, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Benjamin K. Simpson, *University of California, Davis, California,* Marine Enzymes

Kenneth L. Simpson, *University of Rhode Island, Kingston, Rhode Island,* Carotenoid Pigments

Durand Smith, *Cyclopss Corporation, Salt Lake City, Utah,* Ozone and Food Processing

Jeff L. Smith, *University of Guelph, Guelph, Ontario, Canada,* Proteins: Denaturation and Food Processing

J. F. Smullen, *Hershey Foods Corporation, Hershey Park, Pennsylvania,* Chocolate and Cocoa; Licorice Confectionery

Laszlo P. Somogyi, *Consulting Food Scientist, Kensington, California,* Food Additives; Fruit Dehydration; Vegetable Dehydration

Peter Sporns, *University of Alberta, Edmonton, Alberta, Canada,* Food Chemistry and Biochemistry

William J. Stadelman, *Purdue University, West Lafayette, Indiana,* Eggs and Egg Products

John L. Stanton, *Saint Joseph's University, Philadelphia, Pennsylvania,* Food Marketing

Aliza Stark, *Hebrew University of Jerusalem, Rehovot, Israel,* Fiber, Dietary

Clyde E. Stauffer, *Technical Food Consultants, Cincinnati, Ohio,* Emulsifiers, Stabilizers, and Thickeners; Enzyme Assays for Food Scientists, Bakery Leavening Agents; Bakery Specialty Products

Donald F. Steenson, *The Ohio State University, Columbus, Ohio,* Fats and Oils: Flavors

Keith H. Steinkraus, *Food Industry Research and Development Institute, Hsinchu, Taiwan,* Soy Foods, Fermented

K. Stevenson, *National Food Processors Association, Dublin, California,* Aseptic Processing and Packaging Systems

Robert R. Stickney, *Texas Sea Grant College Program, Bryan, Texas,* Aquaculture

William Stoddard, *Cyclopss Corporation, Salt Lake City, Utah,* Ozone and Food Processing

G. S. Stoewsand, *Cornell University, Geneva, New York,* Phytochemicals: Wine

Jean Storlie, *Nutritional Labeling Solutions, Maple Grove, Minnesota,* Packaging: Part II—Labeling

Jong-Ching Su, *National Taiwan University, Taipei, Taiwan,* International Development: Taiwan

Ellen J. Sullivan, *Institute of Food Technologists, Chicago, Illinois,* Food and Nutrition Science Alliance: FANSA

Taneko Suzuki, *Nihon University, Kanagawa, Japan,* Krill Protein Processing

J. E. Swan, *University of Waikato, Hamilton, New Zealand,* Animal By-Product Processing

Hugh Symons, *Kingston Upon Thames, England,* Refrigerated Foods: Food Freezing and Processing; Refrigerated Foods: Food Freezing and World Food Supply; Refrigerated Foods: Handling and Inventory

Bernard F. Szuhal, *Central Soya Company, Inc., Fort Wayne, Indiana,* Lecithins

Donna R. Tainter, *Tone Brothers, Inc., Ankeny, Iowa,* Spices and Seasonings

Takuji Tanaka, *University of Guelph, Guelph, Ontario, Canada,* Proteins: Denaturation and Food Processing

M. S. Tapia, *Washington State University, Pullman, Washington,* Water Activity: Microbiology

Irwin Taub, *U.S. Army Natick, Natick, Massachusetts,* Military Food

Steve L. Taylor, *University of Nebraska, Lincoln, Nebraska,* Food Allergy

A. A. Teixeira, *University of Florida, Gainesville, Florida,* Thermal Processing of Food

A. R. Thicker, *Institute for Toxicology and Chemotherapy, Heidelberg, Germany,* Nitrosamines

Gordon Timbers, *Agriculture and AgriFood Canada, Ottawa, Ontario, Canada,* International Development: Canada

Katherine S. Tippett, *USDA/ARS, Beltsville, Maryland,* Food Consumption Surveys in the U.S. Department of Agriculture

Ross Tomaino, *University of Massachusetts, Amherst, Massachusetts,* Phytochemicals: Antioxidants

Cindy B. S. Tong, *USDA/ARS, Beltsville, Maryland,* Food Crops: Storage

Susan Trimbo, *Whitehall-Robins, Madison, New Jersey,* Osteoporosis

M. Tsoubeli, *Campbell Soup Co., Camden, New Jersey,* Food Microstructure

Marvin A. Tung, *Technical University of Nova Scotia, Halifax, Nova Scotia, Canada,* Rheology

F. J. Vaccarino, *University of Toronto, Toronto, Ontario, Canada,* Appetite

Susan D. Van Arnum, *Westfield, New Jersey,* Vitamins: Niacin, Nicotinamide, and Nicotinic Acid; Vitamins: Vitamin A; Vitamins: Vitamin K

Martien van den Hoven, *Creamy Creations, Rijkevoort, The Netherlands,* Dairy Ingredients: Applications in Meat, Poultry, and Seafoods

Y. Vodovotz, *NASA Johnson Space Center, Houston, Texas,* Food Microstructure

A. L. Waldroup, *University of Arkansas, Fayetteville, Arkansas,* Poultry Meat Microbiology

Michael Sanderson Walker, *P&O Nedlloyd, London, United Kingdom,* Refrigerated Foods: Transportation

Chien Yi Wang, *USDA/ARS, Beltsville, Maryland,* Controlled Atmospheres for Fresh Fruits and Vegetables

Pie Yi Wang, *Armour Swift-Eckrich, Downers Grove, Illinois,* Meat Processing: Technology and Engineering

Shaw Wang, *Rutgers University, Piscatawav, New Jersey,* Kinetics; Surface (Interfacial) Tension

Alley E. Watada, *USDA/ARS, Beltsville, Maryland,* Controlled Atmospheres for Fresh Fruits and Vegetables; Food Crops: Storage

L. M. Weddig, *National Food Processors Association, Washington, D.C.,* Low-Acid and Acidified Foods

Herb Weiss, *Balchem Corporation, Slate Hill, New York,* Encapsulation Techniques

John C. Wekell, *National Oceanic and Atmospheric Administration, Seattle, Washington,* Seafood: Flavors and Quality

Curtis L. Weller, *University of Nebraska, Lincoln, Nebraska,* Edible Films and Coatings

Phillip Wells, *Bestfoods North America, Somerset, New Jersey,* Nutritional Labeling

J. Welti-Chanes, *Washington State University, Pullman, Washington,* Water Activity Microbiology

R. C. Whiting, *United States Department of Agriculture, Philadelphia, Pennsylvania,* Sausages

W. W. Widmer, *State of Florida Department of Citrus, Lake Alfred, Florida,* Limonin

Cecilia Wilkinson Enns, *USDA/ARS, Beltsville, Maryland,* Food Consumption Surveys in the U.S. Department of Agriculture

Carl K. Winter, *University of California, Davis, California,* Food Safety and Risk Communication; Food Safety and Risk Management; Pesticide Residues in Food; Toxicology and Risk Assessment

Dominic W. S. Wong, *United States Department of Agriculture, Albany, California,* Food Chemistry: Mechanism and Theory; Oxidation

Adrienne E. Woytowich, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* Genetic Engineering: Principles and Applications

Bih Keng Wu, *Food Industry Research and Development Institute, Hsinchu, Taiwan,* Mushrooms: Processing

Y. Victor Wu, *USDA/ARS, Peoria, Illinois,* Cereals, Nutrients, and Agricultural Practices

Rickey Y. Yada, *University of Guelph, Guelph, Ontario, Canada,* Proteins: Denaturation and Food Processing

Kit L. Yam, *Rutgers University, New Brunswick, New Jersey,* Packaging: Part I—General Considerations; Packaging: Part III—Materials

T. C. S. Yang, *U.S. Army Soldier and Biological Chemical Command, Natick, Massachusetts,* Aseptic Processing: Ohmic Heating

V. A. Yaylayan, *McGill University, St. Anne de Bellevue, Quebec, Canada,* Flavor Chemistry

Fumio Yoneda, *Fujimoto Pharmaceutical Corporation,* Vitamins: Riboflavin (B_2)

M. Yoshikawa, *Kobe Women's University, Kobe, Japan,* Peptides

Gideon Zeidler, *University of California, Davis, California,* Poultry: Meat From Avian Species

Zuoxing Zheng, *University of Massachusetts, Amherst, Massachusetts,* Solid-State Fermentation and Value–Added Utilization of Fruit and Vegetable Processing By-Products

B. L. Zoumas, *Hershey Foods Corporation, Hershey, Pennsylvania,* Chocolate and Cocoa

CONVERSION FACTORS, ABBREVIATIONS, AND UNIT SYMBOLS

SI Units (Adopted 1960)

The International System of Units (abbreviated SI), is being implemented throughout the world. This measurement system is a modernized version of the MKSA (meter, kilogram, second, ampere) system, and its details are published and controlled by an international treaty organization (The International Bureau of Weights and Measures) (1).

SI units are divided into three classes:

BASE UNITS	
length	meter† (m)
mass	kilogram (kg)
time	second (s)
electric current	ampere (A)
thermodynamic temperature‡	kelvin (K)
amount of substance	mole (mol)
luminous intensity	candela (cd)

SUPPLEMENTARY UNITS	
plane angle	radian (rad)
solid angle	steradian (sr)

DERIVED UNITS AND OTHER ACCEPTABLE UNITS

These units are formed by combining base units, supplementary units, and other derived units (2–4). Those derived units having special names and symbols are marked with an asterisk in the list below.

Quantity	Unit	Symbol	Acceptable equivalent
*absorbed dose	gray	Gy	J/kg
acceleration	meter per second squared	m/s^2	
*activity (of a radionuclide)	becquerel	Bq	1/s
area	square kilometer	km^2	
	square hectometer	hm^2	ha (hectare)
	square meter	m^2	
concentration (of amount of substance)	mole per cubic meter	mol/m^3	
current density	ampere per square meter	A/m^2	
density, mass density	kilogram per cubic meter	kg/m^3	g/L; mg/cm^3
dipole moment (quantity)	coulomb meter	$C\cdot m$	
*dose equivalent	sievert	Sv	J/kg
*electric capacitance	farad	F	C/V
*electric charge, quantity of electricity	coulomb	C	$A\cdot s$
electric charge density	coulomb per cubic meter	C/m^3	
*electric conductance	siemens	S	A/V
electric field strength	volt per meter	V/m	
electric flux density	coulomb per square meter	C/m^2	
*electric potential, potential difference, electromotive force	volt	V	W/A
*electric resistance	ohm	Ω	V/A

---

†The spellings "metre" and "litre" are preferred by ASTM; however, "-er" is used in the encyclopedia.

‡Wide use is made of Celsius temperature (t) defined by

$$t = T - T_0$$

where T is the thermodynamic temperature, expressed in kelvin, and $T_0 = 273.15$ K by definition. A temperature interval may be expressed in degrees Celsius as well as in kelvin.

Quantity	Unit	Symbol	Acceptable equivalent
*energy, work, quantity of heat	megajoule	MJ	
	kilojoule	kJ	
	joule	J	N · m
	electronvolt†	eV†	
	kilowatt-hour†	kW · h†	
energy density	joule per cubic meter	J/m^3	
*force	kilonewton	kN	
	newton	N	$kg \cdot m/s^2$
*frequency	megahertz	MHz	
	hertz	Hz	1/s
heat capacity, entropy	joule per kelvin	J/K	
heat capacity (specific), specific entropy	joule per kilogram kelvin	J/(kg · K)	
heat-transfer coefficient	watt per square meter kelvin	$W/(m^2 \cdot K)$	
*illuminance	lux	lx	lm/m^2
*inductance	henry	H	Wb/A
linear density	kilogram per meter	kg/m	
luminance	candela per square meter	cd/m^2	
*luminous flux	lumen	lm	cd · sr
magnetic field strength	ampere per meter	A/m	
*magnetic flux	weber	Wb	V · s
*magnetic flux density	tesla	T	Wb/m^2
molar energy	joule per mole	J/mol	
molar entropy, molar heat capacity	joule per mole kelvin	J/(mol · K)	
moment of force, torque	newton meter	N · m	
momentum	kilogram meter per second	kg · m/s	
permeability	henry per meter	H/m	
permittivity	farad per meter	F/m	
*power, heat flow rate, radiant flux	kilowatt	kW	
	watt	W	J/s
power density, heat flux density, irradiance	watt per square meter	W/m^2	
*pressure, stress	megapascal	MPa	
	kilopascal	kPa	
	pascal	Pa	N/m^2
sound level	decibel	dB	
specific energy	joule per kilogram	J/kg	
specific volume	cubic meter per kilogram	m^3/kg	
surface tension	newton per meter	N/m	
thermal conductivity	watt per meter kelvin	W/(m · K)	
velocity	meter per second	m/s	
	kilometer per hour	km/h	
viscosity, dynamic	pascal second	Pa · s	
	millipascal second	mPa · s	
viscosity, kinematic	square meter per second	m^2/s	
	square millimeter per second	mm^2/s	
volume	cubic meter	m^3	
	cubic diameter	dm^3	L (liter) (5)
	cubic centimeter	cm^3	mL
wave number	1 per meter	m^{-1}	
	1 per centimeter	cm^{-1}	

---

†This non-SI unit is recognized by the CIPM as having to be retained because of practical importance or use in specialized fields (1).

In addition, there are 16 prefixes used to indicate order of magnitude, as follows:

Multiplication factor	Prefix	Symbol
10^{18}	exa	E
10^{15}	peta	P
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{2}	hecto	h[a]
10	deka	da[a]
10^{-1}	deci	d[a]
10^{-2}	centi	c[a]
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a

[a] Although hecto, deka, deci, and centi are SI prefixes, their use should be avoided except for SI unit-multiples for area and volume and nontechnical use of centimeter, as for body and clothing measurement.

For a complete description of SI and its use the reader is referred to ASTM E380 (4).

A representative list of conversion factors from non-SI to SI units is presented herewith. Factors are given to four significant figures. Exact relationships are followed by a dagger. A more complete list is given in the latest editions of ASTM E380 (4) and ANSI Z210.1 (6).

CONVERSION FACTORS TO SI UNITS

To convert from	To	Multiply by
acre	square meter (m^2)	4.047×10^{3}
angstrom	meter (m)	1.0×10^{-10}†
are	square meter (m^2)	1.0×10^{2}†
astronomical unit	meter (m)	1.496×10^{11}
atmosphere, standard	pascal (Pa)	1.013×10^{5}
bar	pascal (Pa)	1.0×10^{5}†
barn	square meter (m^2)	1.0×10^{-28}†
barrel (42 U.S. liquid gallons)	cubic meter (m^3)	0.1590
Bohr magneton (μ_B)	J/T	9.274×10^{-24}
Btu (International Table)	joule (J)	1.055×10^{3}
Btu (mean)	joule (J)	1.056×10^{3}
Btu (thermochemical)	joule (J)	1.054×10^{3}
bushel	cubic meter (m^3)	3.524×10^{-2}
calorie (International Table)	joule (J)	4.187
calorie (mean)	joule (J)	4.190
calorie (thermochemical)	joule (J)	4.184†
centipoise	pascal second (Pa·s)	1.0×10^{-3}†
centistokes	square millimeter per second (mm^2/s)	1.0†
cfm (cubic foot per minute)	cubic meter per second (m^3/s)	4.72×10^{-4}
cubic inch	cubic meter (m^3)	1.639×10^{-5}
cubic foot	cubic meter (m^3)	2.832×10^{-2}
cubic yard	cubic meter (m^3)	0.7646
curie	becquerel (Bq)	3.70×10^{10}†
debye	coulomb meter (C·m)	3.336×10^{-30}
degree (angle)	radian (rad)	1.745×10^{-2}

---

†Exact.

CONVERSION FACTORS TO SI UNITS

To convert from	To	Multiply by
denier (international)	kilogram per meter (kg/m)	1.111×10^{-7}
	tex‡	0.1111
dram (apothecaries')	kilogram (kg)	3.888×10^{-3}
dram (avoirdupois)	kilogram (kg)	1.772×10^{-3}
dram (U.S. fluid)	cubic meter (m^3)	3.697×10^{-6}
dyne	newton (N)	1.0×10^{-5}†
dyne/cm	newton per meter (N/m)	1.0×10^{-3}†
electronvolt	joule (J)	1.602×10^{-19}
erg	joule (J)	1.0×10^{-7}†
fathom	meter (m)	1.829
fluid ounce (U.S.)	cubic meter (m^3)	2.957×10^{-5}
foot	meter (m)	0.3048†
footcandle	lux (lx)	10.76
furlong	meter (m)	2.012×10^{-2}
gal	meter per second squared (m/s^2)	1.0×10^{-2}†
gallon (U.S. dry)	cubic meter (m^3)	4.405×10^{-3}
gallon (U.S. liquid)	cubic meter (m^3)	3.785×10^{-3}
gallon per minute (gpm)	cubic meter per second (m^3/s)	6.309×10^{-5}
	cubic meter per hour (m^3/h)	0.2271
gauss	tesla (T)	1.0×10^{-4}
gilbert	ampere (A)	0.7958
gill (U.S.)	cubic meter (m^3)	1.183×10^{-4}
grade	radian	1.571×10^{-2}
grain	kilogram (kg)	6.480×10^{-5}
gram force per denier	newton per tex (N/tex)	8.826×10^{-2}
hectare	square meter (m^2)	1.0×10^{4}†
horsepower (550 ft · lbf/s)	watt (W)	7.457×10^{2}
horsepower (boiler)	watt (W)	9.810×10^{3}
horsepower (electric)	watt (W)	7.46×10^{2}†
hundredweight (long)	kilogram (kg)	50.80
hundredweight (short)	kilogram (kg)	45.36
inch	meter (m)	2.54×10^{-2}†
inch of mercury (32°F)	pascal (Pa)	3.386×10^{3}
inch of water (39.2°F)	pascal (Pa)	2.491×10^{2}
kilogram-force	newton (N)	9.807
kilowatt hour	megajoule (MJ)	3.6†
kip	newton (N)	4.448×10^{3}
knot (international)	meter per second (m/S)	0.5144
lambert	candela per square meter (cd/m^3)	3.183×10^{3}
league (British nautical)	meter (m)	5.559×10^{3}
league (statute)	meter (m)	4.828×10^{3}
light year	meter (m)	9.461×10^{15}
liter (for fluids only)	cubic meter (m^3)	1.0×10^{-3}†
maxwell	weber (Wb)	1.0×10^{-8}†
micron	meter (m)	1.0×10^{-6}†
mil	meter (m)	2.54×10^{-5}†
mile (statute)	meter (m)	1.609×10^{3}
mile (U.S. nautical)	meter (m)	1.852×10^{3}†
mile per hour	meter per second (m/s)	0.4470
millibar	pascal (Pa)	1.0×10^{2}
millimeter of mercury (0°C)	pascal (Pa)	1.333×10^{2}†
minute (angular)	radian	2.909×10^{-4}
myriagram	kilogram (kg)	10

---

†Exact.

‡See footnote on p. xviii.

CONVERSION FACTORS TO SI UNITS

To convert from	To	Multiply by
myriameter	kilometer (km)	10
oersted	ampere per meter (A/m)	79.58
ounce (avoirdupois)	kilogram (kg)	2.835×10^{2}
ounce (troy)	kilogram (kg)	3.110×10^{2}
ounce (U.S. fluid)	cubic meter (m^3)	2.957×10^{-5}
ounce-force	newton (N)	0.2780
peck (U.S.)	cubic meter (m^3)	8.810×10^{-3}
pennyweight	kilogram (kg)	1.555×10^{-3}
pint (U.S. dry)	cubic meter (m^3)	5.506×10^{-4}
pint (U.S. liquid)	cubic meter (m^3)	4.732×10^{-4}
poise (absolute viscosity)	pascal second (Pa · s)	0.10†
pound (avoirdupois)	kilogram (kg)	0.4536
pound (troy)	kilogram (kg)	0.3732
poundal	newton (N)	0.1383
pound-force	newton (N)	4.448
pound force per square inch (psi)	pascal (Pa)	6.895×10^{3}
quart (U.S. dry)	cubic meter (m^3)	1.101×10^{-3}
quart (U.S. liquid)	cubic meter (m^3)	9.464×10^{-4}
quintal	kilogram (kg)	1.0×10^{2}†
rad	gray (Gy)	1.0×10^{-2}†
rod	meter (m)	5.029
roentgen	coulomb per kilogram (C/kg)	2.58×10^{-4}
second (angle)	radian (rad)	4.848×10^{-6}†
section	square meter (m^2)	2.590×10^{6}
slug	kilogram (kg)	14.59
spherical candle power	lumen (lm)	12.57
square inch	square meter (m^2)	6.452×10^{-4}
square foot	square meter (m^2)	9.290×10^{-2}
square mile	square meter (m^2)	2.590×10^{6}
square yard	square meter (m^2)	0.8361
stere	cubic meter (m^3)	1.0†
stokes (kinematic viscosity)	square meter per second (m^2/s)	1.0×10^{-4}†
tex	kilogram per meter (kg/m)	1.0×10^{-6}†
ton (long, 2240 pounds)	kilogram (kg)	1.016×10^{3}
ton (metric) (tonnee)	kilogram (kg)	1.0×10^{3}†
ton (short, 2000 pounds)	kilogram (kg)	9.072×10^{2}
torr	pascal (Pa)	1.333×10^{2}
unit pole	weber (Wb)	1.257×10^{-7}
yard	meter (m)	0.9144†

---

†Exact.

BIBLIOGRAPHY

1. The International Bureau of Weights and Measures, BIPM (Parc de Saint-Cloud, France) is described in Appendix X2 of Ref. 4. This bureau operates under the exclusive supervision of the International Committee for Weights and Measures (CIPM).
2. *Metric Editorial Guide (ANMC-78-1),* latest ed., American National Metric Council, 5410 Grosvenor Lane, Bethesda, Md. 20814, 1981.
3. *SI Units and Recommendations for the Use of Their Multiples and of Certain Other Units (ISO 1000-1981),* American National Standards Institute, 1430 Broadway, New York, 10018, 1981.
4. Based on *ASTM E380-89a (Standard Practice for Use of the International System of Units (SI)),* American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103, 1989.
5. *Fed. Reg.,* Dec. 10, 1976 (41 FR 36414).
6. For ANSI address, see Ref. 3.

R. P. Lukens
ASTM Committee E-43 on SI Practice

G

GELATIN

Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen (1), which is the structural mainstay and most common protein in the animal kingdom. Today, gelatin is usually available in granular powder form, although in Europe sheet gelatin is still available.

There are two main types of gelatin. Type A, with isoionic point of 7 to 9, is derived from collagen with exclusively acid pretreatment. Type B, with isoionic point of 4.8 to 5.2, is the result of an alkaline pretreatment of the collagen. However, gelatin is sold with a wide range of special properties, such as gel strength, to suit particular applications.

Gelatin (2) forms thermally reversible gels with water, and the gel melting temperature (<35°C) is below body temperature, which gives gelatin products unique organoleptic properties and flavor release. The disadvantage of gelatin is that it is derived from animal hide or bone (not from horses as is a common perception), so there are problems with regard to kosher and Halal status. Vegetarians also have objections to its use. Competitive gelling agents, such as starch, alginate, pectin, agar, carrageenan, are all carbohydrates from vegetable sources, but their gels lack the melt-in-the-mouth and elastic properties of gelatin gels.

CHEMISTRY AND BIOCHEMISTRY

Gelatin is an amphoteric protein with isoionic point between 5 and 9 depending on raw material and method of manufacture. Like its parent protein, collagen (3), it is unique in that it contains 14% hydroxyproline, 16% proline, and 26% glycine. The only other animal product containing hydroxyproline is elastin, at a very much lower concentration, so hydroxyproline is used to determine the collagen or gelatin content of foods. The protein is made up of peptide triplets, glycine-X-Y, where X and Y can be any one of the amino acids but proline has a preference for the X position and hydroxyproline the Y position (3). Approximately 1,050 amino acids produce an α-chain with the left-handed proline helix conformation. Collagen exists in many different forms, but gelatin is only derived from sources rich in Type I collagen, which contains no cystine. However, hide or skin contains some Type III collagen, which can be the source of traces of cystine found in some gelatins. Although Type I collagen contains no cystine, the α-procollagen chains excreted by the cell do contain cystine at the C-terminal end of the protein that is thought to be the site of assembly of three α-chains. The three chains then spontaneously (4) coil together, zipper fashion, to form a right-handed helix. After spontaneous helix formation, cross-links between chains are formed in the region of the N-terminal telopeptides (globular tail portion of the chains), and then the telopeptides (containing the cystine and tyrosine of procollagen) are shed, leaving the rodlike, about 3,150-amino-acid-containing triple helix. These collagen rods assemble together with a quarter-stagger to form the collagen fiber, and the fibers are stabilized by further cross-links.

Gelatin is the product of denaturation or disintegration of collagen. Initially, the α-chains of collagen are held together with several different but easily reducible cross-links. As the collagen matures, the cross-links become stabilized (3). As time progresses, the eta-amino groups of lysine become linked to arginine by glucose molecules (Maillard reaction) to form the pentosidine type cross-links that are extremely stable (5). Hence, when the alkaline processing is used on young animal skin, the alkali breaks one of the initial (pyridinoline) cross-links and as a result, on heating, the collagen releases mainly denatured α-chains into solution (5). Once the pentosidine cross-links of the mature animal have formed in the collagen, the main process of denaturation has to be thermal hydrolysis of peptide bonds, resulting in protein fragments of various molecular weights (polydisperse protein fragments). With the acid process, the collagen denaturation is limited to the thermal hydrolysis of peptide bonds with a small amount of α-chain material from acid-soluble collagen in evidence (6).

Nutritionally, gelatin is not a complete protein food because the essential amino acid tryptophan is missing, and methionine is present at a low level.

Type A gelatin (dry and ash-free) contains 18.5% nitrogen, but because of the loss of amide groups, Type B gelatin contains only about 18% nitrogen (7). Gelatin is abnormally stable, and a special catalyst has to be used to obtain the correct Kjeldahl nitrogen content.

The amino acid analysis of gelatin (8) is variable, particularly for the minor constituents, depending on raw material and process used, but proximate values by weight are glycine, 21%; proline, 12%; hydroxyproline, 12%; glutamic acid, 10%; alanine 9%; arginine, 8%; aspartic acid, 6%; lysine, 4%; serine, 4%; leucine, 3%; valine, 2%; phenylalanine, 2%; threonine, 2%; isoleucine, 1%; hydroxylysine, 1%; methionine and histidine, <1%; and tyrosine, <0.5%. The peptide bond has considerable aromatic character, so gelatin shows an absorption maximum at about 230 nm.

Collagen is resistant to most proteases and requires special collagenases for its enzymic hydrolysis. Gelatin, however, is susceptible to most proteases, but they do not break gelatin down into peptides containing much less than 20 amino acids.

The cross-linking of gelatin with aldehydes is being used to extend the uses of gelatin. In particular, treatment of gelatin films with glutaraldehyde is receiving considerable study to improve thermal resistance, decrease solubility in water, and improve mechanical properties. In Japan and Brazil, the cross-linking of gelatin using the enzyme *trans*-glutaminase and its use in joining gelatin to other proteins is approved for food use. An occasional phenomenon is the loss of gelatin solubility after storage in a new kitchen cupboard where the residual formaldehyde vapor from the adhesives used causes cross-linking of the gelatin. This reaction has been used to make gelatin adhesives water resistant. Furthermore, the smokes used in food preservation are rich in aldehydes and thus can have unwanted reactions with gelatin.

GELATIN MANUFACTURE

There are a large number of unit processes used in the manufacture of gelatin. The raw materials from which it is derived are demineralized bone (called ossein), pigskin, cow hide, and fish skin. In China, donkey hide is used quite extensively. In theory, there is no reason for excluding any collagen source from the manufacture of gelatin, but the raw materials listed are currently commercially available. Interestingly, in countries where pork is sold with its skin intact, there is no pigskin available for gelatin manufacture.

As mentioned earlier, there are two processes by which collagen is processed to gelatin: the acid process and the alkali process. The acid process (studied in detail by Reich et al. [9]) is mainly used with pigskin and fish skin and sometimes bone raw materials. It is basically one in which the collagen is acidified to about pH 4 and then heated stepwise from 50°C to boiling to denature and solubilize the collagen. Thereafter, the denatured collagen or gelatin solution has to be defatted, filtered to high clarity, concentrated by vacuum evaporation or membrane ultrafiltration treatment to a reasonably high concentration, and then dried by passing dry air over the gel. The final process is one of grinding and blending to customer requirements and packaging. The resulting gelatin has an isoionic point of 7 to 9 based on the severity and duration of the acid processing of the collagen, which causes limited hydrolysis of the asparagine and glutamine amino acid side chains.

The alkali process (studied in detail by Cole [10]) is used on bovine hide and collagen sources where the animals are relatively old at slaughter. The process is one in which collagen is submitted to a caustic soda or lengthy liming process before extraction. The alkali hydrolyzes the asparagine and glutamine side chains to glutamic and aspartic acid relatively quickly (11), with the result that the gelatin has a traditional isoionic point of 4.8 to 5.2. However, with shortened (7 days or less) alkali treatment, isoionic points as high as 6 are produced. After the alkali processing, the collagen is washed free of alkali and treated with acid to the desired extraction pH (which has a marked effect on the gel strength to viscosity ratio of the final product). The collagen is then denatured and converted to gelatin by heating, as with the acid process. Because of the alkali treatment, it is often necessary to demineralize the gelatin solution to remove excessive amounts of salts using ion–exchange or ultrafiltration. Thereafter, the process is the same as for the acid process—vacuum evaporation, filtration, gelation, drying, grinding, and blending.

Although gelatin is often considered a commodity like sugar, the descriptions of the processes and raw materials above should indicate that gelatin has the potential for being a variable product. Users must ensure that they are using the best product for each particular application. In the past, little emphasis has been placed on the animal age of the raw material, particularly in the case of gelatins from bovines; however, it is now known that this factor plays a significant role in the molecular structure of the derived gelatin. The role of liming in the alkali process used to be considered one of progressive alkali hydrolysis of the collagen, which made it possible to denature the collagen at lower temperatures and thus maximize the yield of top-quality gelatin. Recently, however, it has been shown that the role of liming is limited to the hydrolysis of one collagen cross-link that fluoresces at 290 to 380 nm and that liming has increasing less effect on extractability in older animals. The result is that alkali treatment times have been greatly reduced. One of the less well-recognized effects of alkali treatment is the "opening up" of the hide collagen, as it is called in leather manufacture, or the destruction of the proteoglycans associated with the collagen fibrils. This probably results in a more pure gelatin via the alkali process, as is indicated by electrophoresis of the gelatin proteins (6).

At present, enormous developments are being made in the understanding of the structure of collagen and the changes occurring with senescence, and these developments are bound to have an impact on the appreciation of the variables in gelatin, particularly at the molecular level.

GELATIN SAFETY

Gelatin is regarded as a food ingredient rather than an additive, and it is generally regarded as safe (GRAS). In 1993, the Food and Drug Administration (FDA) reiterated the GRAS status of gelatin and stated that there was no objection to the use of gelatin from any source and any country, provided that the hide from animals showing signs of neurological disease were excluded and that specified raw materials were excluded from the manufacturing process. At the beginning of the bovine spongiform encephalopathy (BSE) scare in Europe, the popular media brought suspicion on all products of bovine origin as being possible transmitters of the disease to humans as Creutzfeldt-Jakob disease. This was a thoroughly unscientific assessment of the dangers of spreading infection. It is now recognized that BSE is a neurological and brain problem that is not associated with the hide of the animal. It is also recognized that the processes of manufacturing gelatin make it virtually impossible for the survival of a defective prion, if it were present in the first place.

Detailed and unbiased information on BSE is available from the Institute of Food Science and Technology website. Gelatin retains its GRAS status, and the Joint Expert Commission on Food Additives (JECFA) placed no limit on the use of gelatin in 1970.

Gelatin is an excellent growth medium for most bacteria, so considerable care needs to be taken during manufacture to avoid contamination. This care is evidenced by the use of documented hazard analysis of critical control points (HACCP) programs by manufacturers. In the same way, to ensure product reproducibility, most companies are implementing ISO 9000 quality management systems.

GELATIN PROPERTIES AND USES

Solubility in Water

Gelatin is only partially soluble in cold water, but dry gelatin swells or hydrates when stirred into water. Such mixtures should generally not exceed 34% gelatin. On warming to about 40°C, gelatin that has been allowed to hydrate for about 30 minutes melts to give a uniform solution. Alternatively, dry gelatin can be dissolved by stirring into hot water, but stirring must be continued until solution is complete. This method is normally only used for dilute solutions of gelatin.

If gelatin solutions are spray-dried or drum-dried from the sol state, the resulting gelatin is cold-water soluble, and such gelatins gel quickly when stirred into cold water. These gels are generally not clear, so the use of this form of gelatin is limited to milk puddings and other products where solution clarity is not required.

The compatibility of gelatin in aqueous solution with polyhydric alcohols, such as glycerol, propylene glycol, and sorbitol, is virtually unlimited, and they are used to modify the hardness of gelatin films.

Adhesive Properties

Possibly the oldest use of gelatin was as animal glue. For adhesion to take place, a warm gelatin solution must be used, and the gelatin must not have gelled before the surfaces to be glued are brought together. An example of this use of gelatin is in pharmaceutical or confectionery tableting and in licorice allsorts where it can be used to join the layers.

Gelling Properties

The most common use of gelatin is for its thermally reversible gelling properties with water, such as in the production of table jellies. Gelatin is also used in aspic to add flavor to meat products, and after gelling it also provides a pleasing shiny appearance to the product. In some cases, gelling is known as its water-absorbing property. For example, in canned hams, gelatin can be added to the can before cooking. After cooking, the exudate from the meat is absorbed by the gelatin and appears as a gel when the can is opened.

In confectionery, gelatin is used as the gelling binder in gummy products and wine gums. In the manufacture of these products, gelatin is combined with sugar and glucose syrups. Incompatibility between gelatin and glucose syrup can occur (12) and is a function of the concentration of glucose polymers containing more than 2 glucose units in the syrup. Competition between gelatin and glucose polymers for water in low-water content products can result in, at worst, precipitation of the gelatin and, at best, a marked loss in gelling properties or hardness of the product. It is also known that different gelatins with similar properties in water can have very different properties in confectionery formulations.

Some raw fruits, such as pineapple and papaya, contain proteolytic enzymes (bromelain) that hydrolyse gelatin and destroy its gelling ability. In such cases, it is essential that the fruit is cooked to destroy the protease before the fruit is added to gelatin solutions.

In general, one can say that the lower the mean molecular weight (MW) of a gelatin, the lower the gel strength and viscosity of its solution. However, the collagen chain (MW 100 kD; gel strength, 364 g Bloom) is the main contributor of gel strength (13), and higher MW components (β-chain, MW 200 kD; γ-chain, MW 300 kD; and microgel, MW > 300 kD) make a relatively low contribution to gel strength but a high contribution to viscosity.

Foaming Properties

Gelatin is a very efficient foam stabilizer, and this property is exploited in the manufacture of marshmallows. Different gelatins have different foam stabilizing properties, and gelatin for this use needs to be carefully selected. However, the foaming properties can be standardized by the use of sodium lauryl sulfate (14), if this is permitted by local food additive regulations. In marshmallows, the gelatin's film-forming properties are also used to stabilize the foam on cooling, and because the product is normally not acidified, it has to have a much lower moisture content (>85% solids) than gummy products (76% solids) to avoid mold growth in storage.

Protective Colloid and Crystal Habit-Modifying Properties

If a gelled jelly is frozen, the product will suffer from syneresis, and after thawing, the clear jelly will disintegrate with much exuded water. However, if water containing 0.5% gelatin is frozen, the water will freeze as millions of small discrete crystals, instead of forming a single solid block of ice. This effect is most desirable in frozen ice treats (popsicles or ice lollies). Gelatin is also used in ice cream manufacturing to obtain a smooth product with small ice crystals and to ensure that any lactose precipitates as fine crystals, which avoids the development of graininess with time.

Film-Forming Properties

Gelatin's film-forming properties are used in the manufacture of hard and soft (pharmaceutical) capsules. Gelatin films shrink with great force on drying; hence, such uses usually involve the addition of polyhydric alcohols to modify the adhesion and flexibility of the dry film. Also, for film forming, a gelatin with a high viscosity is preferred to one with a low viscosity. For hard capsules and in photography, ossein gelatin is preferred and commands a premium price.

Emulsifying Properties

The amphoteric character and the hydrophobic areas on the peptide chain give gelatin limited emulsifying and emulsion-stabilizing properties used in the manufacture of toffees and water-in-oil emulsions, such as lowfat margarine.

Stability

Dry gelatin has an almost infinite shelf life as long as the moisture content is such as to ensure that the product is stored below the glass-transition temperature.

The stability of gelatin in solution depends on temperature and pH. Generally, to minimize loss of gel strength and viscosity with time, the pH of the solution should be in the range 5 to 7, and the temperature should be kept as low as possible, consistent with the avoidance of gelation and the suitability of the solution viscosity to the particular application. Often, the cause of degradation or hydrolysis of gelatin in solution is microbial proliferation, so gelatin solutions should not be stored for longer than is absolutely necessary. After addition of the acid to confectionery formulations, the solution should be used and cooled or gelled with minimal delays.

Microencapsulation and Mixed Film-Forming Properties

Besides being precipitated by polymers competing for water, gelatin is amphoteric—it has positive and negative charges on the molecule (and no net charge at the isoionic point). Hence, at a pH where the basic side chains do not carry a charge, acid groups, for example, from gum arabic, can react with the basic groups of gelatin to form an insoluble gelatin–arabate complex that can be precipitated around emulsified oil droplets, forming microencapsulated oil. The microcapsules are hardened with formaldehyde or glutaraldehyde before harvesting and drying. In this application, the pI of the gelatin is critical. This process has been used in the food industry for encapsulating flavors.

Milk and Food Stabilizing Properties

Gelatin is used as a stabilizer, particularly in yogurt, where the addition of 0.3 to 0.5% acts to prevent syneresis, thus allowing the production of stirred and fruit-containing products. In this application, the gelatin reacts with the casein in the milk to reduce its tendency to separate water from the curd. Gelatin can also be used in cheese manufacturing to improve yield and in the stabilization of thickened cream.

Fruit Juice Clarifying Properties

In fining applications, gelatin reacts with polyphenols (tannins) and proteins in fruit juices, forming a precipitate that settles and leaving a supernatant that is stable to further cloud formation with storage time. In wine, usage levels are about 1 to 3 g/hL, and excess usage, which would lead to protein instability, needs to be avoided. Traditionally, low-Bloom-strength gelatins have been used, but high-Bloom-strength gelatins are equally effective (15). However, from the practical point of view, the use of low-Bloom-strength gelatin is cheaper and makes it easier to mix the gelatin into the bulk of the cold juice before gelation can occur. In this regard, it has become common practice to treat cold grapes, during the initial crushing process, with gelatin that has been hydrolyzed to the extent that it can no longer gel.

Texturizing Properties

Gelatin is used in dried soups to provide the appropriate mouth feel (viscosity) to the final product.

Nutritional Properties

As stated earlier, gelatin is not a complete protein source because it is deficient in tryptophan and low in methionine. However, the digestibility is excellent, and it is often used in feeding invalids. The high level of lysine (4%) is noteworthy. Although controversial, studies have shown that the consumption of 7 to 10 g/day can significantly improve nail growth rate and strength (16), and it also promotes hair growth (17). Gelatin has also been shown to benefit arthritis patients in a large proportion of cases (18).

Corrosive Properties

Although 304 stainless steel (s/s) can be used with milk, gelatin attacks 304 s/s, and tubing can be perforated after a few months of continuous usage. With gelatin, it is essential to use 316 s/s. If heat exchanger plates are involved, the use of 316 s/s with the minimum specified molybdenum content of only 2% can be unacceptable.

Fish Skin Gelatin

Fish skin gelatin is available commercially and can be produced for kosher use provided that the appropriate conditions are met (such as the use of fish with scales). Fish gelatin with normal gel strength has a normal hydroxyproline content (19) and is made from fish from warmer waters and not necessarily from fresh water, although this is normally the case. Fish gelatin with low or no gel strength (20) has a low hydroxyproline content (7) and is produced from cold water species that are obtained typically from the sea.

The low-gel-strength gelatin has been used to emulsify vitamin A before spray drying to give another type of microencapsulated product using gelatin.

GELATIN TESTING METHODS

The best published sources of gelatin testing methods are *British Standard 757* (21) or *Standard Methods for Sampling and Testing Gelatin*, published by the Gelatin Manufacturers of America (22) or the *Pharmacopoeias*. Many of the methods used in laboratories need to be modified to suit the peculiarities of gelatin.

Identification

Gelatin gives the normal positive trichloroacetic acid, biuret, and ninhydrin tests for protein. The precipitate with 5% tannic acid is a particularly sensitive test for very dilute solutions of gelatin. In addition, the thermally reversible gelation of a 6% solution in water between 10° and 60°C is unique for this protein.

Gel Strength

The most important attribute of gelatin is its gel strength, and when determined by the standard method (21), this is called the Bloom strength. Bloom strength is the force in grams required to press a 12.5-mm diameter plunger 4 mm into 112 g of a standard 6 2/3% w/v gelatin gel at 10°C.

Several penetrometer-type instruments have been adapted to determine Bloom strength.

A frequent question is how to substitute gelatin of one Bloom strength for a gelatin of another. As a guide one can say

$$C \times \sqrt{B} = k$$

or

$$C_1(\sqrt{B_1})/(\sqrt{B_2}) = C_2$$

where C = concentration, B = Bloom strength, and k = constant (11). However, there are other considerations besides gel strength that can invalidate such a substitution calculation. For example, in a gummy formulation, the texture using 250-Bloom gelatin is far less elastic and sticky than when 180-Bloom gelatin is used.

Viscosity

From the point of view of functionality, the solution viscosity of gelatin is probably the second-most important parameter. The standard method calls for the viscosity of a 6-2/3% solution at 60°C. Low viscosity (and a high gel strength) is required for poured confectionery, and high viscosity is required for film-forming applications.

In viscosity calculations, usually $C \log V = k$, but the model is not as good as the mathematical model for Bloom calculations.

Color and Clarity

Solution color and turbidity or clarity are attributes that may or may not be important depending on the application. Poor clarity markedly affects the ability to measure color (23), and at this stage, there are no internationally accepted methods for determining these attributes. However, if clarity is good, then gelatin color obeys Beer's law.

pH

Solution pH (1%) is usually about pH 5 but can vary considerably. At this pH, the viscosity of type B gelatin is minimal and the gel strength is maximal; hence, from the manufacturer's point of view, it is advantageous to manufacture gelatin at this pH. However, because of the strong buffering capacity of gelatin, this pH may not be the most advantageous for the customer.

Moisture

The moisture content of gelatin may be as high as 16%, but normally it is about 10 to 13%. At 13.0% moisture content, the glass-transition temperature (24) of gelatin is about 64°C, which allows particle size reduction to be a simple operation. In addition, at 13% moisture content and 25°C gelatin is close to equilibrium with ambient air moisture contents of about 46% relative humidity. Gelatin with a moisture content of 6 to 8% is very hygroscopic, and it becomes difficult to determine the physical attributes with accuracy.

Because of the variable granule size of gelatin, the rate of moisture loss at 105°C can be variable. Hence, it is normal to add water to the gelatin powder before placing the sample in the drying oven. This means that the gelatin melts and water is lost from a uniform thin film of protein. Metal dishes must be used because after drying, the film of gelatin shrinks and breaks glass or ceramic containers.

Finally, the drying of gelatin to very low moisture contents results in cross-linking and loss of solubility. It is thus difficult to distinguish between free and bound water in gelatin.

Ash

The gelatin ash content is determined by pyrolysis at 550°C. Ash contents up to 2.5% can usually be accepted in food applications. However, the nature of the ash can be important. For example, 2% $CaSO_4$ in gelatin can have excellent clarity in spite of the solubility product of the ash being exceeded (because of the crystal-habit modifying effect of gelatin). However, after dilution of the gelatin in a confectionery formulation, the ash can precipitate. Furthermore, ammonia is often used as a pH modifier in gelatin preparation, and salts such as NH_4Cl are not determinable by pyrolysis.

Sulfur Dioxide Content

Sulfur dioxide is used as a biocide and bleach in gelatin manufacture. The nationally permitted level of residual SO_2 in gelatin is variable, and the methods for its determination can give a great variation in results. It is known that gelatin promotes oscillating redox reactions (25,26), and the control of this contaminant is not easy. Hydrogen peroxide is often used to control the SO_2 content of gelatin, and sometimes the permitted level of this contaminant is also specified. It is interesting to note that both H_2O_2 and SO_2 can be shown to coexist in gelatin.

Heavy Metal Content

The determination of heavy metals in gelatin can be a problem. It is difficult to completely degrade gelatin, and the main component of the ash in gelatin can be of low solubility, such as calcium sulfate, and have a variable ability to absorb traces of heavy metals. Internal standards must be used wherever possible.

Isoionic Point

The isoionic point of gelatin (11) is best determined by passing a 1% solution of the gelatin at 40°C through a mixed-bed column of ion–exchange resin (Rohm & Haas MB3) at a flow rate of not more than 10 bed volumes per hour and measuring the pH of the eluate. After cooling, isoionic gelatin has poor clarity, and the conductivity should be between 1 and 5 μs/cm for type B gelatin.

Microbiological Properties

Gelatin is an excellent nutrient for most bacteria, so the manufacturing processes have to carefully avoid contamination. Most countries have microbiological specifications for gelatin, but generally they are not very onerous. Total mesophyllic plate counts of 1,000 are generally accepted with various countries limiting the presence of coliforms, *Escherichia coli*, *Salmonella*, clostridial spores, staphylococci, and sometimes even pseudomonads.

BIBLIOGRAPHY

1. A. J Bailey and R. G Paul, *Journal of the Society of Leather Technologists and Chemists* **82**, 104–110 (1998).
2. M Glicksman, *Gum Technology in the Food Industry*, Academic Press, New York, 1969, pp. 359–397.
3. A. J. Bailey and N. D. Light, "Genes, Biosynthesis and Degradation of Collagen," in A. J. Bailey and N. D. Light, eds., *Connective Tissue in Meat and Meat Products*, Elsevier Applied Science, London, United Kingdom, 1989, pp. 225–247.
4. D. J. Prockop, *Matrix Biol.* **16**, 519–528 (1998).
5. C. G. B. Cole and J. J. Roberts, *Proceedings of the International Union of Leather Technologists and Chemists Societies Congress*, 57–64 (1997).
6. C. G. B. Cole and J. J. Roberts, *Journal of the Society of Leather Technologists and Chemists* **80**, 136–141 (1996).
7. J. E. Eastoe and A. A. Leach, A Survey of Recent Work on the Amino Acid Composition of Vertebrate Collagen and Gelatin, in G. Stainsby, ed., *Recent Advances in Gelatin and Glue Research*, Pergamon Press, New York, 1958, pp. 173–178.
8. P. V. Stevens, *Food Australia* **44**, 320–324 (1992).
9. G. Reich, S. Walther, and F. Stather, *Deutsche Lederinstitut* **18**, 15–23 (1962).
10. C. G. B. Cole, "The Occurrence of Dark Coloured Gelatin," in *Occurrence, Measurement and Origins of Gelatin Colour as Determined by Fluorescence and Electrophoresis*, University of Pretoria, Pretoria, South Africa, 1995, pp. 19–155.
11. A. Veis, *The Macromolecular Chemistry of Gelatin*, Academic Press, New York, 1964, pp. 107–113, 196, 392–396.
12. W. M. Marrs, "Gelatin/Carbohydrate Interactions and Their Effect on the Structure and Texture of Confectionery Gels," in G. O. Phillips, P A. Williams, and D. J. Wedlok, eds., *Progress in Food Science and Nutrition 6*, Pergamon Press, New York, 1982, pp. 259–268.
13. E. Heidemann et al., *Proceedings of the 5th IAG Conference: Photographic Gelatin*, International Arbeitsgem. Photogelatine, Fribourg, Switzerland, 1989.
14. *Federal Register*, May 15, 1964, p. 6383.
15. W. Bestbier, *Wynboer* **621**, 6–62 (1983).
16. M. Schwimmer and M. G. Mulinos, *Antibiotic Medicine and Clinical Therapy* **IV**, 403–407 (1957).
17. United States Patent 4,749,684 (June 7, 1988), B. Silvestrini (to Bruno Silvestrini).
18. M. Adam, *Therapiewoche* **38**, 2456–2461 (1991).
19. European Patent 0 436 266 A1 (Published 10.07.91), S. Grossman (to Bar Ilan University).
20. A. G. Ward, "Conversion of Collagen to Gelatin, and Chemical Composition," in G. Stainsby, ed., *Recent Advances in Gelatin and Glue Research*, Pergamon Press, New York, 1958, pp. 137–139.
21. *Methods for sampling and testing gelatine. BS 757 : 1975. Gr8*, British Standards Institution, London, United Kingdom, 1975.
22. *Standard Methods for Sampling and Testing of Gelatin*, Gelatin Manufacturers of America, New York, 1986.
23. C. G. B. Cole and J. J. Roberts, *Meat Science* **45**, 23–31 (1997).
24. M. H. McCormick-Goodhart, "Research Techniques in Photographic Conservation," *Proceedings of the Copenhagen Conference* 65–70 (May 1995).
25. C. R. Chinake and R. H. Simoyi, *S. Afr. J Chem.* **48**, 1–7 (1995).
26. Z. Melichova, A. Olexova, and L. Treindel, *Z. Phys. Chem.* (Munich) **191**, 259–264 (1995).

GENERAL REFERENCES

A. J. Bailey and N. D. Light, *Connective Tissue in Meat and Meat Products*, Elsevier Applied Science, London, United Kingdom, 1989.

M. Glicksman, *Gum Technology in the Food Industry*, Academic Press, New York 1969.

G. Stainsby, *Recent Advances in Gelatin and Glue Research*, Pergamon Press, New York, 1958.

A. Veis, *The Macromolecular Chemistry of Gelatin*, Academic Press, New York, 1964.

A. G. Ward and A. Courts, *The Science and Technology of Gelatin*, Academic Press, New York, 1977.

BERNARD COLE
Leiner David Gelatin
Krugersdorp, South Africa

GENETIC ENGINEERING: ANIMALS. See GENETIC ENGINEERING: ANIMALS in the Supplement section.

GENETIC ENGINEERING: FOOD FLAVORS

Traditional food biotechnologies are frequently characterized by the formation of ethanol, acetic acid, propanoic acid, and lactic acid. The resulting products, though different from the original fruit must, flour paste, milk, or meat in many ways, were experienced not only as having no adverse effects on well-being (if consumed in moderation), but they even possessed new, attractive sensory properties. Based on these very roots of biotechnology (1), a great number of nonvolatile flavor compounds, such as acidulants, amino acids, 5′-nucleotides, and certain carbohydrates are now manufactured on an industrial scale (2). Although the enzymatic origins of the volatile flavor fraction of many foods are clearly recognized (3), aroma-producing microorganisms and enzymes thereof have, for a long time, been rather regarded as laboratory oddities (4). Today, the increasing number of papers and patents on the biotechnology of aromas indicates a strong trend in both academic and industrial flavor research (5–26). Increasing confidence in the future of biotechnology (27) and in the so-called soft chemistry approach has merged together with "all-natural" marketing claims into the recent development of pilot and production scale bioprocesses for aroma chemicals (Table 1).

Table 1. Industry Driving Forces

Technical Push

- High selectivities or specificity (chemo-, regio-, stereo-)
- High reaction rate at low molar fractions
- Environmentally compatible, renewable substrates and mild reaction conditions
- Multistep syntheses including cofactor regeneration (whole cell)

Business Pull

- Health- and nutrition-conscious lifestyles demand "natural" products
- Classical plant sources suffer from microbial or insect infestation, sociopolitical instability and depend on seasonal variation, fertilizers, etc
- Character impact components may possess additional bioactivities and, therefore, often occur in traces only in their plant sources

Source: Ref. 28.

Volatile flavors and fragrances command a growing (currently ca $10 billion) market (29). The market of food flavors in the United States alone is estimated at over $1 billion and is continuously increasing (21). The resulting demand of the industry for natural ingredients suggests that the agricultural sources with their inherent instabilities will no longer satisfy the need. The physiologically most active constituents of natural flavors, the so-called character impact components, are often present in plants in traces and in bound forms, an additional obstacle for an economic isolation. Recent advances in cell biology and in bioengineering—and particularly the ability to alter the biocatalyst's properties genetically—possess great potential for the flavor industry, as the legal definitions of the Food and Drug Administration and of the Council of the European Communities classify fermentation flavors (with few restrictions) as "natural" compounds. From a chemical viewpoint, the use of a chiral (bio)catalyst would offer the most adequate synthetic approach anyway: The sensory properties of many flavors strongly depend on the chiral structure of the molecules, and the enantiomeric forms of many terpenes, lactones, and so on may exhibit different, sometimes even opposite, sensory characteristics (30).

An estimated number of less than 100 flavor compounds and building blocks are now available on the market, among them carboxylic fatty acids, esters and lactones derived thereof, cheese and yeast flavors, methyl ketones, benzaldehyde, vanillin, cinnamates, and some other character impact components. Particular progress has been made using lipases and certain other hydrolases, whereas the improvement of cell based processes appears more difficult. According to the increasing structural complexity of the biocatalyst, this article highlights some recent achievements, but also current problems of the biotechnology of volatile flavors using enzymes, microbial and fungal cells, and plant cells.

ENZYME MIXTURES AND PURIFIED ENZYMES

Microbial enzymes, products of bioprocesses themselves, are an integrated part of modern food technology. Their long-established use by the food industry leaves no doubt that biocatalysts are particularly advantageous for the safe production of food and pure food ingredients. In view of regiospecifity and enantioselectivity, substrate specifity, and ability to function at statistically cold temperatures, it would seem obvious to use enzymes for the generation of chemically often sensitive, volatile molecules.

Indirect Action on Food Flavor

Enzymes of main group three (hydrolases) possess a share of about 80% of the industrial enzyme market. The application of pure or combined lipases, amylases, glucoamylases, cellulases, invertases, proteases, or ribonucleases may affect the overall flavor of food in various ways: (*1*) by direct accumulation of monomer flavor compounds from polymer or conjugated precursors; (*2*) by increasing the pool of precursors for a subsequent thermal flavor generation (reaction flavors); and, (*3*) by liberating previously adsorbed compounds from the hydrolyzed polymer carrier molecules.

Phosphatases and glycosidases were applied to liberate volatile flavors directly from their odorless transport or storage forms (Table 2). These cofactor independent enzymes are, like all hydrolases, conveniently applied to practical processes, as the cosubstrate water is abundant in most food systems. Acyl sugar esters represent a novel class of non-volatile precursors of volatiles. 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose and related ester compounds may be the natural progenitors of methyl and ethyl cinnamate, widely occurring volatiles in various fruits (32). Comparable to CoA-dependent activation of acyl moieties, 1-*O*-sugar esters are thought to be activated conjugates. In the intact plant cell specific transferases may convert them into new esters, while a spontaneous chemical alkylation was observed under slightly alkaline conditions in the presence of methanol or ethanol. As these hydrolases and transferases may not be easily separated from their producer cells, the plant gene may be isolated, linked to a suitable construct, and transferred into a microbial host, to finally overexpress the gene coding for the target enzyme. The rDNA approach has been suggested for endo-β-

Table 2. Gycoconjugated Flavors Liberated by Hydrolases

Source	Organ	Flavor products
Camellia sinensis (tea)	Leaf and aerial part	(3*Z*)-Hexenol, 2-phenylethanol
Carica papaya	Fruit	Benzaldehyde, benzyl isothiocyanate
Cydonia oblonga (quince)	Fruit	Norisoprenoids
Fragaria ananassa (strawberry)	Fruit	Furaneol
Malus domestica (apple)	Fruit and leaf	Alkanols, eugenol, octadiols, norisoprenoids
Vanilla planifolia	Fruit	Vanillin
Vitis vinifera (grape)	Fruit	Geraniol, nerol, linalool, norisoprenoids

Source: Ref 31.

glucosidases from *Aspergillus niger* or *A. oryzae*. Some of these fungal hydrolases are distinguished by a superior activity at elevated processing temperature and at high concentrations of ethanol; their application to wine should enhance the flavor.

Looking at the second field of application, rhamnosidase could be the target of a genetic approach. This enzyme is present as an impurity in commercial pectinases. Its action on *Citrus* wastes containing bitter glycosides results in the liberation of a glucose/rhamnose mixture. Glucose oxidase from *Gluconobacter suboxydans* is then used subsequently to remove any remaining glucose. The residual pure rhamnose is an important precursor of the widely used flavor, 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol) by reacting the methylpentose with an amino acid. The product resulting from the oxidation of glucose, 5-ketogluconic acid, may serve as a precursor of the savory flavor 4-hydroxy-5-methyl-3(2*H*)-furanone (26).

The third approach is exemplified by the treatment of extracted vanilla pods with an exo-enzyme mixture of *A. niger* that led to the additional formation of flavor (33). This idea has been transferred to other flavor-bearing plant sources with a rigid morphology, such as *Iris* rhizom. The addition of enzymes for the removal or prevention of off-flavors, for example, the enzymatic debittering of *Citrus* products, may also be interpreted as an indirect action of enzymes on the final sensory quality of the food product.

Classical Fermented Food

Numerous enzymes of various microorganisms must act together in a well-balanced mode to bring about the typical sensory characteristics of the complex substrates of fermented food. In an approach to rationalize the traditional technology, enzyme and substrate mixtures, or purified lipases and proteases together with defined substrates, such as butter oil, are used. Various microbial lipases preferably cleave the ester bond between glycerol and short- to medium-chain fatty acids. The liberated free fatty acids are the sensory backbone of many dairy flavors. Butter and cheese flavor are now generated by the action of lipases or combinations of enzymes on curd or milk fractions, and some of the flavor-enriched products are traded as “Enzyme Modified Cheese.”

Some meat products, such as salami-type sausages, were also described to benefit from added enzymes: lipase, protease, and collagenase enzymes improved taste and accelerated ripening (34–36). Proteolytic, lipolytic, and further biopolymer degrading activities can be isolated from yeast cultures and combined with the inactivated producer cells to obtain food flavors with savory notes. The claimed gain in flavor intensity is seldomly quantified, which makes the success of certain bioprocesses difficult to assess. If, in a food-type substrate, the addition of a single enzyme caused a fortified flavor, the traditionally used starter culture strain should benefit from a transmission of the corresponding gene or from the use of gene dosage effects. Several recent reviews deal with rDNA techniques for the elucidation and improvement of relevant properties of starter cultures (eg, 37,38), but their application to improve the flavor formation properties is still somewhat neglected (39).

Concerted Enzymatic Production of Flavor

Lipase and esterase preparations catalyze reverse hydrolysis (esterification, lactonization) and transesterification reactions in one-phase, two-phase, microemulsion, and even in supercritical fluid systems (40). Elegant methods perform the reaction in one of the substrates as the solvent and apply chemical or physical sinks to trap the reaction water. Although essential for the hydration of the enzyme, water is detrimental in excess and will lead to incomplete conversion. Various techniques for water limitation such as the use of molecular sieve, distillation, condensation on a cold surface, and reduced pressure were described. Flavor applications included bacterial, fungal, and mammalian sources of enzyme for the production of chiral aliphatic esters, terpenol esters, and lactones with fruity, flowery, nutty, and creamy odors (Table 3). Similarly, proteases were used for the production of aspartam, a dipeptide sweetener, and for peptides possessing *umami* or sodium glutamate-like flavors (44). Carbohydrate esters may find an industrial use as surfactants to stabilize emulsion-type composed *Citrus* flavors, for example, in nonalcoholic beverages (45).

Vice versa, the selective hydrolysis of racemic amides or esters yields chiral amines, alcohols, or acids. Since the early seventies important industrial applications of hydrolases are the enzymatic resolution of racemic amino acids, and the asymmetric hydrolysis of menthyl esters to produce optically pure L-menthol (candies, sweets, toothpaste, tobacco, ointments, etc), one of the bulk compounds of the flavor industry. More than 50 lipases of different origin have been characterized with respect to their efficiency and selectivity. Only tertiary alcohols and branched-chain and benzoic acids were usually excluded from the wide range of substrates. Subjects of recent work were the effects of solvent, pH, temperature, surfactants, water content, immobilization of the enzyme, pretreatment of the solid support, mass transfer-reaction interactions, isozymes, and chemical modification of the enzyme. Few researchers have been interested in the unambiguous identification of the catalytically active constituent of the often crude industrial enzyme mixtures. A closer look at commercial porcine pancreas extract showed that the N-terminal end of the active protein was homologous to a known cholesterol esterase sequence (47). This enzyme

Table 3. Compounds from Enzymatic Hydrolysis or Reverse Hydrolysis

Substrates	Products	Ref.
Racemic carboxylic acids	Chiral alcohols	41
Acetocarboxylic acid esters	Chiral hydroxy esters	42
Acid plus alcohol/esters	Geranyl butanoate etc.	43
Amino acids	Oligopeptides	44
Carbohydrate and fatty acid	Fructose stearate, linoleate, etc	45
Racemic lactones	Jasmolactone, massoia lactone, tuberolactone	46

catalyzed the selective esterification of 2-pentanol with butanoic acid in *n*-heptane. Proofed protocols for flavor ester synthesis can be obtained from some enzyme suppliers.

The step from hydrolytic/reverse hydrolytic reactions toward serial enzymatic reactions is bound to require cofactor-dependent enzymes (Table 4). With an ADH/NAD^+/CH_3CHO-system for the production of geranial (lemon flavor constituent), 1500 cycles of regeneration of the cofactor were achieved by coimmobilization of NAD. The bienzymatic method for cofactor regeneration instead of the coupled substrate method was suggested to convert ethanol to acetaldehyde, cinnamic alcohol to cinnamaldehyde, and leucine to 3-methylbutanal (malty odor) and 3-methylbutanol (fusel oil odor). In the latter case (50), *Streptococcus* enzymes were conencapsulated with substrate, NAD, and enzymes of *Gluconobacter oxidans*, which oxidize ethanol to acetic acid in a milk fat coat. Cheese that contained the enzyme capsules exhibited a stronger taste than controls. Such coimmobilizates of biocatalysts of different origin or of biocatalyst and substrate belong to a second generation of immobilized biocatalysts; they seem to be especially promising in the area of flavor production with its often poorly water soluble substrates and products.

An oxidoreductase from *Candida parapsilopsis* reduced the carbonyl function of keto esters; aliphatic, aromatic, and alicyclic ketones; aldehydes; and ketoacetals with high conversion rates (51). The products may be flavors or useful for flavor ester syntheses. To demonstrate its preparative value, methyl (*S*)-(+)-3-hydroxybutanoate, a versatile chiral building block, was synthesized with coupled coenzyme regeneration. Prenylation of small, polar compounds helps to increase their volatility and, thus, their odor activity. Prenylated odorants were found in roasted coffee and believed to contribute to the overall flavor (52). A transfer of the prenyl moiety onto benzoic acid derivatives was achieved using a membrane bound transferase from overproducing strains of *Escherichia coli* (53). This example documented particularly well the inherent advantages of enzyme catalysis: The substitution proceeded regioselectively, and no isomerization of the substituent's double bonds occurred.

MICROBIAL CELLS

Microbial cells may be regarded as a very natural form of immobilized enzymes that provide self-regeneration of catalytic activities and of all cofactors, active transport systems for substrates, and an optimal spatial arrangement of enzyme chains. Empirical biotechnologies rely on *Schizophyta* and *Protoascomycotina* to improve keeping quality, digestibility, and, as a welcome side effect, flavor of foods and beverages.

Table 4. Cofactor Coupled Reactions Generating Volatile Flavor

System components	Product	Ref.
Geraniol/Acetaldehyde/NAD^+/ADH	Geranial	48
CinnamylOH/Octanal/NAD^+/ADH/Lip-DH/DCIP	Cinnamaldehyde	49
L-Leucine/EtOH/NAD^+/Enzyme mixture	3-Methylbutanal/ol	50
Oxo compound/NADH	Chiral alcohol	51

Food Strains

Fatty acids and simple carbonyl compounds are the predominant volatile compounds produced by *Streptococci* and *Lactobacilli* (54). *Penicillium* strains used in the manufacture of surface-molded cheese and raw sausages add methylketones and, if the cells were injured by mechanical stress, C_8-compounds to the spectrum. Typical yeast volatiles are esters, thio-compounds, and reduced carbonyls. The fermentation of food strains on classical or on chemically defined substrates usually resulted in the production of these common flavor molecules. More interesting compounds were rarely formed or formed in low amounts ($<$mg L^{-1}) only.

However, some recent applications demonstrate that industry is willing to exploit the capabilities and the operational advantages of microbial cells, despite their obvious genetic limitations. The examples selected (Table 5) reflect some of the actual trends: new substrates and old strains, for example, for the malolactic fermentation of fruit and vegetable juices; better defined, often continuously operated systems for better understanding of biochemical regulation phenomena; cell recycle and immobilized systems for reuse of the biocatalyst (55,56). It has been observed that both quality and quantity of volatiles produced by mutants or mixed cultures with enhanced or complementary metabolic abilities were superior to products fermented with a pure standard strain. The cell counts in mixed cultures often run through transient maxima for the single strains resulting in fluctuations of formation of volatiles and their precursors. Sometimes the success of the entire process is jeopardized. Genetic engineers are now in the position to step forward from combining strains to recombinant strains with a more stable and complex physiology.

The reported generation of Furaneol (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone; "Enhansol" in the United States) in an aerated culture of *Lactobacillus helveticus* deserves special attention (57). This heterocyclic character impact compound with an intense fruity/pineapple to caramel/burnt note (depending on its actual concentration) belongs to the most potent flavor compounds, but it is rather unstable in aqueous fermentation media. Special precautions

Table 5. Food Strains and Flavor Formation

Substrate	Catalyst	Flavor	Ref.
Citrate	*Streptococcus lactis*	Diacetyl, etc	55
Whey plus amino acids	*Lactobacillus helveticus*	Furaneol, etc	57
Milk fat plus methionine	*Brevibacterium linens*	Methanethiol	56
Koji	*Pediococcus plus Yeast*	"Soy sauce"	58
Octanoic acid	*Penicillium roquefortii*	2-Heptanone	59
Citronellol	*Botrytis cinerea*	Terpenols, etc	60

were taken for its quantitative recovery from a submerged culture by ion-exchange resins. This should sharpen the view for a possibly concurrent or even predominant chemical formation of volatiles in the often complex fermentation broths. Furaneol was also detected in a casein peptone medium supplemented with L-rhamnose and grown with *Pichia capsulata* (61). However, Furaneol was formed from a hypothetical intermediate of rhamnose generated during heat sterilization of the medium, as Furaneol was neither detected in sterile filtered medium nor after separate heat sterilization of rhamnose and the peptone. Capillary gas chromatography coupled to combustion/isotope-ratio mass spectrometry confirmed that the carbons of Furaneol were actually derived from the rhamnose skeleton.

The closely related sotolone (4,5-dimethyl-3-hydroxy-2(5*H*)-furanone), another powerful odorant (lovage odor) was prepared using an amino acid oxidase of resting cells of the bacterium *Morganella morganii* and subsequent lactonization (62). The same compound is known to be developed during the aging of wine (63). Under experimental conditions, however, sotolone was formed by a purely chemical mechanism from α-ketobutanoic acid and acetaldehyde by an aldol addition followed by cyclization. Another strong odorant among this group of chemicals is 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone. The efficiency of numerous sugars and sugar phosphates to act as a precursor in the formation of this heterocycle by the shoyu yeast *Zygosaccharomyces rouxii* was investigated (64). Biosynthesis proceeded under conditions of an operating pentose-phosphate cycle in the yeast. The same potent flavor enhancer has also been found in Swiss cheese, an indication that another yeast-independent way of formation may exist (65).

A fermentation of soy sauce from koji with immobilized whole cells of lactic acid bacteria of the genus *Pediococcus* and yeast cells to produce a soy sauce flavor was reported by a Japanese group. The calcium alginate entrapped cells were kept in three column reactors with a working volume of 280 L. Thereby, the ecological situation of the fermenting microorganisms was simulated (58). Substrates of *Penicillia* in blue cheese flavor formation were pure fatty acids, ripened curd, or lipolyzed milk fat. Experiments to stabilize the active fungal spores in continuous culture by entrapment into alginate gel were successful (59). The reproducible manufacture of a high-quality fermented sausage is still a challenge to the producer, because choice of ingredients and processing conditions may severely alter product characteristics (66). An earlier drawback, the involvement of an incidental indigenous or contaminating microflora, has been overcome by the use of defined starter cultures; comprehensive investigations have also been devoted to the surface molds of salami (67,68). The key role of microorganisms in flavor formation is now widely recognized, but problems of isolating the lipophilic flavor from the fatty matrix have impeded a sound correlation of volatile compounds and the underlying microbial pathways. Because mixed populations are no longer regarded as the antithesis of good experimental work, new perspectives have opened up. The biodegradation of complex polyester, polyglycosidic, polyamidic, or polyaromatic structures usually leads to the formation of flavor intermediates. In all of these cases a stimulating effect of the various forms of mutualism or commensalism within microbial communities may be expected.

Nonfood Organisms

The range of accessible volatile flavors is considerably expanded by using nonfood species. The need to provide all those pathways with energy and substrates that are not directly required for an intended conversion reaction may be compensated by the cell's self-regeneration of active biocatalyst. Just when cofactor-dependent steps are aimed at, intact cells still appear to be the catalysts of choice.

A good example is the oxidation of fusel oil alkanols to carboxylic acids such as 2-methylbutanoic acid by *Enterobacteria*. A following conversion of this acid to its ethyl ester by a reverse hydrolysis step will yield one of the most potent fruit flavor compounds (13). Proliferating nonfood yeasts substituted purified, immobilized lipase or alcohol dehydrogenase in the production of carboxylic acid esters or aliphatic aldehydes including acetaldehyde, an impact component of citrus and yogurt flavors (69); and strains of *Penicillium*, *Pseudomonas*, and *A. niger* substituted the action of plant enzymes in degrading terpenoid structures such as carotenoids, resulting in, for example, tea and tobacco flavor constituents. Genetically altered *Pseudomonas* strains with special plasmids were described for effecting this catabolism (20). The formation of geosmin (4,8-dimethyl-decahydro-4-naphthalinol) with its strong, earthy smell needs to be controlled in food and drinking water. Bacteria, fungi, and some plants are able to synthesize the compound. Parameters that affects its accumulation in *Streptomyces tendae* and in *Penicillium expansum* were studied (70), and a hypothetical pathway of formation was suggested based on structural properties of some byproducts (71). Soil bacteria with their sometimes extensive secondary metabolism have also proven useful for the rapid degradation of ferulic acid to yield 11.5 g of vanillin per liter (72). The patent describes the use of *Amycolatopsis* species of the genus *Pseudonocardia* and adsorbent resins such as Amberlite, XAD-types, or Lewatites for product accumulation. Another character impact component, 2-acetyl-1-pyrroline (bread crust, popcorn), was isolated from cocoa fermentation boxes naturally contaminated with *Bacillus cereus*. A pathway of formation was made likely using ^{13}C- and ^{15}N-labeled precursors fed to various *Bacillus* species and an atomic emission detector coupled with the chromatographic separation of volatiles (73).

Protoplast fusion, another prominent novel technique to recombine genetic information, was used to overcome unstable cocultivations and to produce single compounds such as 4-ethylguaiacol, a seasoning agent, and to improve wine flavor (74). Problems encountered with hybrid cells are the sometimes difficult selection and insufficient genome stability of fusant strains. In a strict sense, the first transfer of flavor-related genes from one procaryotic species into another one was reported in 1995 only (75): An expression of genes coding for the transformation of limonene to α-terpineol (lilac odor) of *B. stearothermophilus* in *E. coli* was achieved. Compared with strains of *Ps. gladioli* and *P. digitatum* that perform the same reaction, the yields were still too low for a biotechnological application.

Higher Fungi

Eumycetes, taxonomically located between less-organized microorganisms with limited potential for flavor formation and the complex seed plants, appear to be a kind of golden mean for the biogeneration of flavors. They grow well in certain synthetic liquid media and offer a tremendous metabolic diversity. Some producer strains are related to edible species that should facilitate approval of the products by the authorities.

Not only mushroom-like compounds such as 1-octene-3-ol, but also fruity, floral, spicy, and even chocolate-like (due to pyrazines) odors emanated from fungal cell cultures (Table 6). Volatiles of almost every chemical class were synthesized de novo or via bioconversions by submerged cultured mycelia. Depending on strain and composition of the nutrient medium, frequently found volatiles were phenolics, terpenes, and lactones. With respect to the vast number of strains, their metabolic flexibility under changing conditions, and the broad bioengineering experience, the potential of fungal cells to produce commercial food flavors can hardly be overestimated. Previously reported low yields are not an inevitable feature of fungal cells but were rather due to suboptimal culture conditions.

The example of *Polyporus durus* demonstrated that these saprophytic organisms are very adaptable to changing culture conditions; thus, they are amenable to a stepwise optimization of product yields (Table 7; 76). The presence of a reesterified coconut oil fraction strongly stimulated the formation of volatiles. More than 60 constituents, among them almost 20 lactones, were accumulated in the culture broth. A total concentration of volatiles of about 800 mg L^{-1} was reached. Capillary gas chromatography on chiral and achiral phases showed a high enantiomeric purity of some of the chiral lactones (>98% ee). After the pioneering work using castor oil as a substrate for the fungal production of 4-decanolide (fruity-fatty odor) had ended up with yields of less than 5 g L^{-1} (77), the race for the best process was opened. During the past 10 years, every major flavor house started to develop its own lactone process. When, for example, species of the *Mucor* fungi were fed under aerated conditions with fatty acids or fatty acid esters, 4- and 5-alkanolides with the respective carbon numbers were produced in good yields (78). Similar effects of triacylglycerol substrates were reported for many other microorganisms.

Table 7. Stepwise Improvement of 4-Octanolide Production by ***Polyporus durus***

Parameter	Productivity [mg L^{-1} d^{-1}]
Standard medium	Traces
N-source	0.2
Transfer to submerged cultivation	0.6
C-Source, pO_2	3
Addition of precursor and surfactant	16
Preparation and size of inoculum	31
Semicontinuous cultivation	46

Source: Ref 74.

Table 6. Volatiles from Cultured Fungi

Species	Product(s)
Agaricus bisporus, etc	Benzaldehyde, phenyl acetaldehyde
Ascoidea hylecoeti	2-Phenyl ethanol
Bjerkandera adusta	Anisaldehyde, veratraldehyde
Hebeloma sacchariolens	2-Amino benzaldehyde
Hyanellum suaveolens	Coumarins
Inocybe sp.	Methyl cinnamate
Ischnoderma benzoinum	Benzaldehyde, anisaldehyde
Lentinus sp.	Benzyl acetate, methyl anisate, methyl cinnamate
Hycoacia uda	Methyl acetophenone, methyl benzylalcohol, *p*-tolualdehyde
Nidula sp.	Raspberry ketone, cinnamic acid derivatives
Phanerochaete chrysosporium	Veratraldehyde
Phellinus sp.	Methyl benzoate, salicylate
Pycnoporus cinnabarius	Methyl anthranilate, vanillin
Sirodesmium diversum	*p*-Hydroxybenzaldehyde
Sparassis ramosa	Oak moss impacts (orcine derivatives)
Trametes sp.	Anisaldehyde, methyl phenylacetate
Tyromyces sambuceus	Alkanolides, benzaldehyde, ethyl benzoate

Source. Ref 24.

Based on the universality of biochemistry, microbial models for studying flavor synthesis have widely replaced the inconvenient plant cell systems. One should, however, be aware that nature frequently provides more than just one pathway for a certain flavor molecule. This obviously restricts an unseen extrapolation of results. Genetic engineering should therefore be considered as a second step, after a biogenetic route has been fully elucidated and selected for gene transfer. Microbial versus plant formation of 4-dodecanolide (peachy-fatty odor; odor threshold in the $\mu g\ L^{-1}$ range) may illustrate the situation: A chiral 10-hydroxystearic acid was obtained by bacterial conversion of oleic acid, and a baker's yeast concluded the sequence by β-oxidation and lactonization (79). Data obtained with fruits of strawberry and peach, however, support a sequence starting with an epoxy fatty acid and proceeding through a β-oxidative chain shortening of the ω-3,4-dihydroxy fatty acid. Elimination of water from the hydroxylactone followed by reduction of the enlactone concluded the biosynthesis (80). Both routes led to the same optically pure (*R*)-form of the lactone.

Only about 0.2% (equivalent to 20 to 30 tons) of the vanillin consumed worldwide is isolated from the botanical source, whereas the remainder is synthetic. This disproportion has stimulated research for a biotechnological substitution, despite some arguing about the details of its biosynthesis (20). Until now, neither plant cell cultures of *Vanilla* nor bacteria or fungi yielded more than trace amounts of the target compound Again, as mentioned for the preceding bacterium (72), feeding of precursors (here lignin, eugenol, ferulic acid, curcumin, or benzoe siam resin) presented a solution. Different ^{13}C-isotope ratios of the vanillins allowed to assign the flavor compound to a specific geographical or biochemical source (81).

Benzaldehyde, the second most important flavor molecule after vanillin, is obtained from amygdalin in peach or apricot kernels and is a character impact ingredient in stone fruit flavors, such as cherry coke. Equimolar amounts of hydrocyanic acid are formed concurrently and cause major safety problems. Among the biotechnological alternatives is the microbial degradation of natural L-phenylalanine. This amino acid has become easily available (from biotechnology), as it is an intermediate of the synthesis of the high intensity sweetener, aspartame (26). Strains of the basidiomycete *Ischnoderma benzoinum* afforded significant yields of benzaldehyde upon phenylalanine feeding (82). The metabolic pathways were elucidated using submerged cultures of this fungus and ring-labeled deutero-L-phenylalanine as a precursor (83). Depending on the phase of the cell cycle operating, phenylalanine was almost completely converted to benzaldehyde or to 3-phenylpropanol (flowery, roselike odor).

Generally, the supplementation of cell cultures with suitable (natural) precursor substrates, customary in numerous bioprocesses for pharmaceuticals, is an efficient tool to further increase the yields of volatiles. While high yields will continue to be key to the economy of a bioprocess, a more critical evaluation of terms such as "optimization" or "best performance" has started: Optimization should not be regarded equivalent to the maximization of any one single parameter, but depends on the definition of a frame of reference to which the developmental activities are directed. A first step into this direction is the replacement of the term "yield" by time- and biomass-related productivity data.

PLANT CATALYSTS

Most of the volatile flavors processed by the food industry are directly or indirectly derived from plant metabolism. Pressing, distillation, extraction, and chromatography are the common techniques for obtaining raw materials of natural flavors from intact plants.

Crude Enzymes and Homogenates

Crude enzyme preparations from fruits and vegetables were able to partly restore the fresh odor impression in processed food by converting nonvolatile odorless precursors to volatile flavors. Developed in the late fifties, this so-called flavorese-enzyme concept failed from economic and biogenetic reasons. Successors used by-products of food processing as a less expensive source of enzymes. Following up earlier work with apple peel, a Hungarian group developed a preparative scale generation of apple flavor (84). A vigorous formation of the volatile C_6-compounds typical of plant lipoxygenase/hydroperoxide lyase systems occurred in homogenates of some apple cultivars and grass species. The yield of the main volatile compound, (2*E*)-hexenal (pleasant fresh-green note), reached some 100 mg $kg^{-1} h^{-1}$ (85,86).

Progress toward understanding how fatty acid peroxides are obtained from lipoxygenase action (87), how the products can be analyzed (88), and how these precursors are further transformed by a cytochrome-type lyase (89) has culminated in the development of immobilized biocatalysts (90,91). Patents describe, for example, the generation of (3*Z*)-hexenol by subjecting linolenic acid and watermelon foliage to mechanical shearing in the presence of a yeast (92), or the generation of several green note compounds using a lyase from guava (93). This biosynthetic principle has been extended to the cooxidation of carrot oleoresin (rich in carotenoids) by fatty acid hydroperoxides to obtain oxidative breakdown products, such as ionones (fruity-violet odors) or dihydroactinidiolide (fragrant-woody notes) (94). The genetic engineering approach holds a lot of promise for these bioprocesses, because the single enzymes involved are well known. The operational instability of plant lyases may be improved by enzyme engineering in the future.

Callus and Suspension Culture

Derived from a wounding-induced tissue (primary callus), a continuously growing sterile culture of plant cells can be established by mechanical separation and further subculturing on a synthetic medium (callus culture). After transfer to a liquid medium, cells of a macroorganism are finally reduced to the single-cell level (suspension culture). According to its origin, the somatic cell is provided with a complete genom and able to develop into a whole plant again. This totipotency was translated into the idea that all genetic functions, including, for example, essential oil and aroma formation, would be expressed in such cells (95). However, numerous disappointing experiments have caused the industry to almost completely refrain from further investments into this kind of plant biotechnology. The absence of flavor compounds in cell cultures of flavor-yielding plants has been attributed to a lack of morphologically differentiated structures (eg, oil glands, resin ducts, etc), or to a rapid catabolism of the presumed intermediates. Detailed studies on the synthesis of lower terpenes in callus cultures of essential oil bearing plants showed all species to possess high prenyltransferase and pyrophosphate isomerase activities, and most of the cultures to possess further activities of the mevalonate pathway, although a significant accumulation of products did not occur.

A physical stimulus to secondary metabolism in plant cells has been neglected for a long time: light. Although not always sufficient, the cytodifferentiation from heterotrophic to phototrophic cells induced the formation of leaf-typical constituents, such as pigments, quinones, and essential oils. Chlorophyll formation in the presence of light is tied to a preceding formation of phytol; this diterpenoid alcohol in turn can only be formed if the initial steps of the mevalonate pathway are operating. The accumulation of the intermediate lower terpenoids along this route is then to be expected. In addition to light a fine-tuning of the phytoeffector concentrations in the growth medium is mandatory to support both growth and aroma formation, as was shown with illuminated callus cultures of *Citrus* (96). These cells accumulated a full spectrum of volatile terpenes, and the best yields were about 5% of the volatiles isolated from a mature tissue of the mother explant (grapefruit peel; Table 8). Addition of precursors resulted in con-

Table 8. Some Volatile Constituents of *in vitro* cells of *Citrus* paradisi

Compound, mg kg^{-1} fresh wt	Low phytoeffector conc	High phytoeffector conc
Chlorophyll	193	310
(+)-Limonene	48	177
α-Pinene	0.09	0.58
β-Pinene	0.22	0.67
n-Octanal	1.17	0.41
Citronellal	0.95	0.59

Source: Ref 96.
24-h photoperiod and 3,000 lux

version to and stable accumulation of volatiles, especially in oxyfunctionalization reactions. One example of economical relevance could be the regioselective biotransformation of valencene into the sesquiterpene ketone nootkatone. After six hours of incubation, almost 70% of the substrate was transformed with no other detectable volatile by-products. Natural nootkatone with its grapefruit-like odor and bitter taste is a sought-after flavor compound with limited availability. As was found repeatedly in earlier work with plant cell cultures, flavor formation was favored by slow growth, an indication that flavor syntheses may be a general characteristic of a heterostatic physiology of the cell (26). The preceding findings disagree with early statements that either the cytotoxicity of the once formed volatiles or their instability in the surrounding medium compartment would prevent flavors from being accumulated in elevated concentration.

The addition of heat-inactivated microbial homogenates to growing plant cell cultures was discussed as a means of inducing metabolic cascades involved in chemical defense. As some flavor compounds interfere with bacterial or fungal growth, their synthesis was thought to be triggered by simulating a respective microbial attack. This so-called elicitation was demonstrated by the addition of autoclaved fungal mycelium to *Petroselinum* cells that responded with the formation of volatile phthalides, coumarins, and phenolics (97). A phototrophic state of the cells was required, because only the plastid differentiated cells tolerated the higher cytokinin concentrations and the fungal homogenate. This kind of ecological stress typically leads to the secretion of the newly formed compounds into the surrounding nutrient medium, where they unfold their bioactivity, and from where they can be easily recovered. The use of lipophilic traps (solvent or adsorbent) simulating a natural morpholicical accumulation site can be combined with the elicitor approach.

Cell immobilization is an expanding area of biotechnology. The characteristics of continuous operation, reuse of the biocatalyst, ease of process control, and improved biocatalyst stability should prove especially beneficial for cultured plant cells, as immobilized cells are similar in many respects to the tissues of intact plants. Aggregated cells face heterogeneous microenvironments, release intracellular products, and divide slower. These and other factors may redirect plant secondary metabolism. The use of immobilized plant cells in column or membrane reactors may offer better perspectives for a future application on an industrial scale. If single enzymes were rate limiting, as particularly in biotransformations, it should be possible to select for higher yields by making use of the somaclonal variation that builds up in plant cell cultures, or by enhanced mutagenesis. Increased levels of essential oils were reported for mutant plants of *Pelargonium* and other species regenerated from UV or ethyl methane sulfonate treated callus cultures; this type of approach is somewhat restricted by the laborious procedures and assays required (98).

In spite of the achievements it should not be overlooked that de novo syntheses as well as biotransformations using plant cells will always have to compete not only with field plants, but also with microbial and enzyme technologies. Plant cells are not very adaptable to extreme conditions of cultivation, and the logical consequence would rather be to use *in vitro* plant cells of suitable cultivars as a convenient source of genes and to link these plant genes to microbial operators in future rDNA experiments.

Specialized Plant Cells

The biotechnological ideal of an immobilized plant cell with active flavor metabolism, available in any amount, was created by nature: mature fruits. It has been known for a long time that aged fruit tissues are able to take up exogenous substrates, metabolizing them to flavor compounds. The same holds true for intact fruits and volatile substrates. A procedure was developed to expose fruits during storage to vapors of precursors of volatile flavors. By analogy with controlled atmosphere (CA) storage, this biotechnological concept was termed PA (for precursor atmosphere) storage. One example refers to the drying of banana tissue (99). Although many compounds of banana flavor showed a good retention in the starchy matrix of the tissue, the concentrations of some highly volatile carboxylic esters decreased by 50 to 80% during freeze drying. When bananas were submitted to PA-storage in 3-methylbutanol vapors before dehydration, the fruits rapidly accumulated volatile impact components. By managing endogenous organized enzymes and their substrates, a dried fruit with an amount of 3-methylbutyl esters comparable to that of the genuine fresh fruit was finally obtained (Table 9). As a result, a preceding PA storage can compensate for flavor deficiencies caused by one-sided breeding, by improper transport and storage, or by physical losses during thermal processing operations.

Table 9. Effect of a Preceding Precursor Atmosphere (PA) Storage on Impact Volatiles of Freeze-Dried Banana Slices

	Fruit		
		Freeze-dried	
3-Methylbutyl ester, μg 100 g^{-1} fresh wt	Fresh	Without	With PA-storage
Acetate	7460	2860	6990
2-Methylpropanoate	70	27	60
Butanoate	600	250	960

Source: Ref. 99.

COMMERCIAL BIOFLAVORS

Novel bioprocesses are now an accepted alternative source of a wide range of high-prized volatile flavors. A couple of companies advertise 100% natural compounds. Whether these were obtained by conventional physical treatments of plant (or other natural) sources, or whether they in fact originated from a bioreactor, is sometimes difficult to assess. True products of concerted bioprocesses may be diacetyl (butter note, from starter cultures), cheese flavors, yeast products (meaty and savory notes), fatty acids and related alcohols and esters (from lipase technology or intact microorganisms), C_6- and C_8-alcohols and related carbonyls (green and mushroom notes, from plant and fungal homogenates), and some specialties such as lactones, vanillin, and nootkatone from complex biosystems.

The legal definitions (and the average consumer) clearly discriminate artificial flavors against natural ones; this fact has undoubtedly contributed to the recent surge of activity in bioflavor research. The legal discrimination itself, however, must be regarded artificial, because "no compounds are made on earth other than those permitted by the laws of nature" (100). The existing legal restrictions might be challenged less by the public with its unfounded reservations than by problems to analytically distinguish natural from synthetic. Sophisticated analytical tools comprise multidimensional chiral gas chromatography, high resolution NMR-spectroscopy, and isotope ratio mass spectroscopy (30,81). Despite this selection of analytical options, some volatile compounds are still difficult to differentiate.

Regardless of the actual legal situation in a country, the superiority of biocatalysts to generate complex, sensitive, and chiral molecules is more and more recognized in organic synthesis. The classical field of food biotechnology will continue to profit from modern developments in gene recombination and delivery, bioreactor and sensor design, on-line control, and related techniques. The improvement of fungal strains and plant cultivars is still restricted by several fundamental scientific and applied hurdles. The use of eucaryotic and mitochondrial genome fragments and the chromosomal integration of rDNA characterize lines of current research. Special emphasis should be put on safety testing of food generated by genetically manipulated strains, particularly if these are to remain in the consumed products (101).

BIBLIOGRAPHY

1. R. H. Michel, P. E. McGovern, and V. R. Badler, "Chemical Evidence for Ancient Beer," *Nature* **360**, 24 (1992).
2. B. H. Lee, *Fundamentals of Food Biotechnology*, VCH, New York, 1996.
3. P. Schreier, *Chromatographic Studies of Biogenesis of Plant Volatiles*, Hüthig, Heidelberg, 1984.
4. E. Sprecher and H.-P. Hansen, "Recent Trends in the Research on Flavours Produced by Fungi," in R. G. Berger, S. Nitz, and P. Schreier, eds., *Topics in Flavour Research*, Eichhorn, Hangenham, 1985, pp. 387–403.
5. F. H. Sharpell Jr., "Microbial Flavors and Fragrances", in M. M. Young, ed., *Comprehensive Biotechnology*, vol. 3, Pergamon Press, Oxford, United Kingdom, 1985, pp. 965–981.
6. T. H. Parliment and R. Croteau, eds., *Biogeneration of Aromas*, ACS Symposium Series 317, American Chemical Society, Washington, D.C., 1986.
7. P. Schreier, ed., *Bioflavour '87*, deGruyter, Berlin, 1988.
8. P. Schreier, "Aspects of the Biotechnological Production of Food Flavours," *Food Rev. Int.* **5**, 289–315 (1989).
9. F. W. Welsh, W. D. Murray, and R. E. Williams, "Microbiological and Enzymic Production of Flavor and Fragrance Chemicals," *Crit. Rev. Biotechnol.* **9**, 105–169 (1989).
10. E. W. Seitz, "Flavor Building Blocks," in I. Goldberg and R. Williams, eds., *Biotechnology and Food Ingredients*, Van Nostrand Reinhold, New York, 1991, pp. 375–391.
11. D. A. Romero, "Bacteria as Potential Sources of Flavor Metabolites," *Food Technol.* **46**, 122–126 (1992).
12. M. I. Farbood, "Micro-organisms as a Novel Source of Flavour Compounds," *Biochem. Soc. Trans.* **19**, 690–694 (1992).
13. L. Janssens et al., "Production of Flavours by Microorganisms," *Proc. Biochem.* **27**, 195–215 (1992).
14. R. L. S. Patterson et al., eds., *Bioformation of Flavours*, Royal Society of Chemistry, Cambridge, United Kingdom, 1992.
15. J. M. Belin, M. Bensoussan, and L. Serrano-Carreon, "Microbial Biosynthesis for the Production of Food Flavours," *Trends Food Sci. Technol.* **3**, 11–14 (1992).
16. P. Winterhalter and P. Schreier, "Biotechnology: Challenge for the Flavor Industry," in T. E. Acree and R. Teranishi, eds., *Flavor Science*, American Chemical Society, Washington, D.C., 1993, pp. 225–258.
17. P. Schreier and P. Winterhalter, eds., *Progress in Flavour Precursor Studies*, Allured, Carol Stream, Ill., 1993.
18. S. Hagedorn and B. Kaphammer, "Biocatalysis in the Generation of Flavors and Fragrances," *Annu. Rev. Microbiol.* **48**, 773–800 (1994).
19. A. Gabelman, ed., *Bioprocess Production of Flavor, Fragrance, and Color Ingredients*, John Wiley & Sons, New York, 1994.
20. R. G. Berger, *Aroma Biotechnology*, Springer, Berlin, 1995.
21. K. Kevin, "A Brave New World: Capturing the Flavor Bug," *Food Proc.* 66–70 (1995).
22. M. Tyrell, "Advances in Natural Flavors and Materials," *Perf. Flavorist* **20**, 13–21 (1995).
23. P. Étiévant and P. Schreier, eds., *Bioflavour 95*, INRA, Paris, 1995.
24. U. Krings, B. Abraham, and R. G. Berger, "Plant Impact Volatiles—A Biotechnological Perspective," *Perf. Flavorist* **20**, 79–86 (1995).
25. G. Feron, P. Bonnarme, and A. Durand, "Prospects for the Microbial Production of Food Flavours," *Trends Food Sci. Technol.* **7**, 285–293 (1996).
26. R. G. Berger, ed., *Biotechnology of Aroma Compounds*, Adv. Biochem. Engin. Biotechnol. 55, Springer, Berlin, 1996.
27. G. R. Takeoka et al., eds., *Biotechnology for Improved Foods and Flavors*, ACS Symposium Series 637, American Chemical Society, Washington, D.C., 1996.
28. P. J. S. Cheetham, "The Use of Biotransformations for the Production of Flavours and Fragrances," *Trends Biotechnol.* **35**, 478–488 (1993).
29. L. Somogyi, "The Flavour and Fragrance Industry: Serving a Global Market," *Chem. Ind.* (London) **4**, 170–173 (1996).

30. A. Mosandl, "Enantioselective Capillary Gas Chromatography and Stable Isotope Ratio Mass Spectrometry in the Authenticity Control of Flavors and Essential Oils," *Food Rev. Int.* **11**, 597–664 (1995).
31. P. Winterhalter and G. K. Skouroumounis, "Glycoconjugated Aroma Compounds: Occurrence, Role and Biotechnological Transformation," in R. G. Berger, ed., *Biotechnology of Aroma Compounds*, Adv. Biochem. Engin. Biotechnol. 55, Springer, Berlin, 1996, pp. 73–105.
32. S. Latza, D. Ganßer, and R. G. Berger, "Carbohydrate esters of cinnamic acid from fruits of *Physalis peruviana, Psidium guajava and Vaccinium vitis-idaea*," *Phytochemistry* **43**, 481–485 (1996).
33. M. P. Pouget, A. Pourrat, and H. Pourrat, "Recovery of Flavor Enhancers From Vanilla Pod Residues by Fermentation," *Food Sci. Technol.* **23**, 1–3 (1990).
34. I. Zalacain et al., "Dry Fermented Sausages Elaborated With Lipase From *Candida cylindracea*," *Meat Science* **40**, 55–61 (1995).
35. B. F. Hagen et al., "Bacterial Proteinase Reduces Maturation Time of Dry Fermented Sausages," *J. Food Sci.* **61**, 1024–1029 (1996).
36. K. Marggrander, "Collageneous Proteins as Excipients for the Improvement of the Technological and Sensory Properties of Meat Products and Ready Meals," *Fleischwirtschaft* **76**, 725–726 (1996).
37. H. Teuber, "The Use of Genetically Manipulated Microorganisms in Food: Opportunities and Limitations," *Eur. Congr. Biotechnol.* **4**, 383–391 (1987).
38. D. R. Berry, "Manipulation of Flavour Production by Yeast: Physiological and Genetic Approaches," in J. R. Piggott and A. Paterson, eds., *Distilled Beverage Flavour*, Ellis Horwood, Chichester, United Kingdom, 1989, pp. 299–307.
39. P. Christen and A. López-Munguía, "Enzymes and Food Flavor—A Review," *Food Biotechnol.* **8**, 167–190 (1995).
40. A. Ballesteros et al., "Enzymes in Non-conventional Phases," *Biocatalysis and Biotransformation* **13**, 1–42 (1995).
41. K. Laumen, D. Breitgoff, and M. P. Schneider, "Enzymic Preparation of Enantiomerically Pure Secondary Alcohols," *J. Chem. Soc. Chem. Commun.* **22**, 1459–1461 (1988).
42. I. Glänzer et al., "Enantioselective Hydrolysis of Esters of Secondary Alcohols Using Lyophylized Baker's Yeast," *Enzyme Microb. Technol.* **10**, 744–749 (1988).
43. G. Langrand, C. Triantaphylides, and J. Baratti, "Lipase Catalyzed Formation of Flavor Esters," *Biotechnol. Lett.* **10**, 549–554 (1988).
44. K. Aso, T. Uemura, and Y. Shiokawa, "Protease Catalyzed Synthesis of Oligo-L-Glutamic Acid from L-Glutamic Acid Diethyl Ester," *Agric. Biol. Chem.* **52**, 2443–2449 (1988).
45. M. A. Ku and Y. D. Hang, "Enzymatic Synthesis of Esters in Organic Medium With Lipase From *Byssochlamys fulva*," *Biotechnol. Lett.* **17**, 1081–1084 (1995).
46. WO 94/07887 (Sept. 21, 1993), J.-P. Bourdineaud, C. Ehret, and M. Petrzilka (to Givaudan-Roure Intl.).
47. K. Sostmann and P. Schreier, "Esterification in Non-aqueous Solvents: Cholesterol Esterase as a Selective Biocatalysator From Porcine Pancreas," *Z. Lebensm. Unters. Forsch.* **200**, 428–431 (1995).
48. M. D. Legoy, H. S. Kim, and D. Thomas, "Use of ADH for Flavor Aldehyde Production," *Process Biochem.* **20**, 145–148 (1985).
49. J. S. Deetz and J. D. Rozzell, "Enzyme-Catalyzed Reactions in Non-aqueous Media," *Trends Biotechnol.* **6**, 15–19 (1988).
50. S. D. Braun and N. F. Olsen, "Microencapsulation of Cell-free Extracts to Demonstrate the Feasibility of Heterogeneous Enzyme Systems and Cofactor Recycling for Development of Flavor in Cheese," *J. Dairy Sci.* **69**, 1202–1208 (1986).
51. J. Peters, T. Minuth, and M.-R. Kula, "A Novel NADH-dependent Carbonyl Reductase With an Extremely Broad Substrate Range From *Candida parapsilopsis*: Purification and Characterization," *Enzyme Microb. Technol.* **15**, 950–958 (1993).
52. W. Holscher, O. G. Vitzthum, and H. Steinhart, "Prenyl Alcohol, Source for Odorants in Roasted Coffee," *J. Agric. Food Chem.* **40**, 655–658 (1992).
53. L. A. Wessjohann and B. Sontag, "Prenylierung von Benzoesäurederivaten katalysiert durch eine Transferase aus *E. coli* Überproduzenten," *Angew. Chemie Intl. Ed. Engl.* **35**, 1697–1699 (1996).
54. R. Imhof, H. Glättli and J. O. Bosset, "Volatile Organic Aroma Compounds Produced by Thermophilic and Mesophilic Mixed Strain Dairy Cultures," *Food Sci. Technol.* **27**, 442–449 (1994).
55. P. Schmitt et al., "Citrate Utilization by Free and Immobilized *Streptococcus lactis* ssp. *diacetylactis* in Continuous Culture," *Appl. Microbiol. Biotechnol.* **29**, 430–436 (1988).
56. S. C. Kim and N. F. Olsen, "Production of Methanethiol in Milk Fat-Coated Microcapsules Containing *Brevibacterium linens* and Methionine," *J. Dairy Sci.* **56**, 799–811 (1989).
57. J. Kowalewska et al., "Isolation of Aroma Bearing Material From *Lactobacillus helveticus* Culture and Cheese," *J. Dairy Sci.* **68**, 2165–2171 (1985).
58. K. Osaki et al., "Fermentation of Soy Sauce With Immobilized Whole Cells," *J. Food Sci.* **50**, 1289–1292 (1985).
59. C. Larroche and J.-B. Gros, "Special Transformation Processes Using Fungal Spores and Immobilized Cells," in R. G. Berger, ed., *Biotechnology of Aroma Compounds*, Adv. Biochem. Engin. Biotechnol. 55, Springer, Berlin, 1996, pp. 179–220.
60. P. Brunerie et al., "Bioconversion of Citronellol by *Botrytis cinerea*," *Appl. Microbiol. Biotechnol.* **27**, 6–10 (1988).
61. R. Roscher et al., "L-Rhamnose: Progenitor of 2,5-dimethyl-4-hydroxy-3(2H)-furanone Formation by *Pichia capsulata*?" *Z. Lebensm. Unters. Forsch.* **204**, 198–201 (1997).
62. K. Lerch and M. Ambühl, "Biotechnological Production of 4,5-dimethyl-3-hydroxy-2(5H)-furanone," in P. Étiévant and P. Schreier, eds., *Bioflavour 95*, INRA, Paris, 1995, pp. 381–384.
63. T. T. Pham et al., "Optimal Conditions for the Formation of Sotolone from α-Ketobutyric Acid in the French *'Vin Jaune'*", *J. Agric. Food Chem.* **43**, 2616–2619 (1995).
64. M. Sasaki, N. Nunomura, and T. Matsudo, "Biosynthesis of 4-hydroxy-2(*or* 5)-ethyl-5(*or* 2)-methyl-3(2H)-furanone by Yeasts," *J. Agric. Food Chem.* **39**, 934–938 (1991).
65. M. Preininger, M. Rychlik, and W. Grosch, "Potent Odorants of the Neutral Volatile Fraction of Swiss Cheese (Emmentaler)," in H. Maarse and D. G. van der Heij, eds., *Trends in Flavour Research*, Elsevier, Amsterdam, The Netherlands, 1994, pp. 267–270.
66. W. M. Park et al., "Effects of Lactic Acid Bacteria Isolated From Fermented Foods on the Microbiological Properties of Fermented Sausages," *Food Biotechnol.* **6**, 145–148 (1997).
67. W. P. Hammes, "Fermentation of Non-dairy Food," *Food Biotechnol.* **5**, 293–303 (1991).

68. H. J. Hwang, R. F. Vogel, and W. P. Hammes, "Entwicklung von Schimmelpilzkulturen für die Rohwurstherstellung," *Fleischwirtschaft* **73**, 327–332 (1993).
69. L. Janssens et al., "Fusel Oil as a Precursor for the Microbiological Production of Fruity Flavours," *Med. Fac. Landbouww. Rijksuniv. Gent* **54**, 1387–1392 (1989).
70. C. P. Dionigi and D. A. Ingram, "Effects of Temperature and Oxygen Concentration on Geosmin Production by *Streptomyces tendae* and *Penicillium expansum*," *J. Agric. Food Chem.* **42**, 143–145 (1994).
71. F. Pollak and R. G. Berger, "Geosmin and Related Volatiles in Bioreactor Cultured *Streptomyces citreus* CBS 109.60," *Appl. Environ. Microbiol.* **62**, 1295–1299 (1996).
72. EP 761817 (March 12, 1997), J. Rabenhorst and R. Hopp (to Haarmann & Reimer GmbH).
73. L. J. Romanczyk, Jr. et al., "Formation of 2-Acetyl-1-pyrroline by Several *Bacillus cereus* Strains Isolated From Cocoa Fermentation Boxes," *J. Agric. Food Chem.* **43**, 469–545 (1995).
74. S. Lino and M. Watanabe, "Wine-making by Killer Wine Yeast" [in Japanese], *After CA* **113**, 13754 (1989).
75. H. C. Chang, D. A. Gage, and P. J. Oriel, "Cloning and Expression of a Limonene Degradation Pathway From *Bacillus stearothermophilus* in *Escherichia coli*," *J. Food Sci.* **60**, 551–553 (1995).
76. R. G. Berger, K. Neuhäuser, and F. Drawert, "Odorous Constituents of *Polyporus durus* (Basidiomycetes)," *Z. Naturforsch.* **41c**, 963–970 (1986).
77. U.S. Patent 4,560,656 (Dec. 24, 1985), M. I. Farbood and B. J. Willis (to Fritzsche Dodge & Olcott, Inc.).
78. WO 89/12104 (Dec. 14, 1989), G. V. Page and G. R. Eilerman (to BASF Corp.).
79. S. Gocho et al., "Biotransformation of Oleic Acid to Optically Active γ-Dodecalactone," *Biosci. Biotech. Biochem.* **59**, 1571–1572 (1995).
80. M. Schöttler and W. Boland, "Über die Biosynthese von γ-Dodecanolacton in reifenden Früchten: Aroma-Komponenten der Erdbeere (*Fragaria ananassa*) und des Pfirsichs (*Prunus persica*)," *Helv. Chim. Acta* **78**, 847–856 (1995).
81. I. Benz and A. Muheim, "Biotechnological Production of Vanillin," in A. J. Taylor and D. S. Mottram, eds., *Flavour Science—Recent Developments*, Royal Society of Chemistry, Cambridge, United Kingdom, 1996, pp. 111–117.
82. C. E. Fabre, P. J. Blanc, and G. Goma, "Production of Benzaldehyde by Several Strains of *Ischnoderma benzoinum*," *Sci. Aliments* **16**, 61–68 (1996).
83. U Krings, M. Hinz, and R. G. Berger, "Degradation of $[^2\mathrm{H}]$ Phenylalanine by the Basidiomycete *Ischnoderma benzoinum*," *J. Biotechnol.* **51**, 123–129 (1996).
84. L. Vámos-Vigyázó, N. Kiss-Kutz, and A. Hersiczky, "Preparative Scale Generation of Apple Flavour from By-Products of Processing," in *3rd International Flavour Symposium*, Keksemet, 1984, pp. 34–35
85. R. G. Berger, A. Kler, and F. Drawert, "The C_6-Aldehyde Forming System in *Agropyron repens*," *Biochim. Biophys. Acta* **883**, 523–530 (1986).
86. F. Drawert, A. Kler, and R. G. Berger, "Optimierung der Ausbeuten von (2*E*)-Hexenal bei pflanzlichen Gewebehomogenater," *Lebensm.-Wiss Technol.* **19**, 426–431 (1986).
87. H W. Gardner, "Lipoxygenase as a Versatile Biocatalyst," *J. Am. Oil Chem. Soc* **73**, 1347–1357 (1996).
88. C. Schneider, P. Schreier, and M. Herderich, "Analysis of Lipoxygenase-derived Fatty Acid Hydroperoxides by Electrospray Ionization Tandem Mass Spectrometry," *Lipids* **32**, 331–336 (1997).
89. K. Matsui et al., "Bell Pepper Fruit Fatty Acid Hydroperoxide Lyase Is a Cytochrome P450 (CYP74B)," *FEBS Lett.* **394**, 21–24 (1996).
90. A.-F. Hsu, T. A. Foglia, and G. J. Piazza, "Immobilization of Lipoxygenase in an Alginate-Silicate Solgel Matrix: Formation of Fatty Acid Hydroperoxides," *Biotechnol. Lett.* **19**, 71–74 (1997).
91. A. Nunez et al., "Immobilization of Hydroperoxide Lyase From *Chlorella*," *Biotechnol. Appl. Biochem.* **25**, 75–80 (1997).
92. WO 95/26413 (March 8, 1994), R. B. Holtz, M. J. McCulloch, and S. J. Gargar (to Reynolds Technologies, Inc.).
93. WO 93/24644 (May 3, 1993), B. Muller, A. Gautier, C. Dean, and J.-C. Kuhn (to Firmenich, S.A.).
94. WO 94/08028 (Sept. 27, 1993), J.-M. Belin, B. Dumont, and F. Ropert (to BFA Laboratories).
95. T. Mulder-Krieger et al., "Production of Essential Oils and Flavors in Plant Cell and Tissue Culture," *Plant Cell Tissue Organ Cult.* **13**, 85–154 (1988).
96. G. Reil and R. G. Berger, "Accumulation of Chlorophyll and Essential Oil in Photomixotrophic Cell Cultures of *Citrus* sp.," *Z. Naturforsch.* **51c**, 657–666 (1996).
97. G. Reil and R. G. Berger, "Elicitation of Volatile Compounds in Photomixotrophic Cell Cultures of *Petroselinum crispum*," *Plant Cell Tissue Organ Cult.* **46**, 131–136 (1996).
98. A. H. Scragg, "The Production of Aromas by Plant Cell Cultures," in R. G. Berger, ed., *Biotechnology of Aroma Compounds*, Adv. Biochem. Engin. Biotechnol. 55, Springer, Berlin, 1996, pp. 239–263.
99. R. G. Berger, F. Drawert, and H. Kollmannsberger, "PA-Lagerung zur Kompensation von Aromaverlusten bei der Gefriertrocknung von Bananenscheiben," *Z. Lebensm. Unters.-Forsch.* **183**, 169–171 (1986).
100. R. Teranishi, "New Trends and Developments in Flavor Chemistry," in R. Teranishi, R. G. Buttery, and F. Shahidi, eds., *Flavor Chemistry*, ACS Symposium Series 388, American Chemical Society, Washington, D.C., 1989, p. 2.
101. D. Anderson and W. F. J. Cuthbertson, "Safety Testing of Novel Food Products Generated by Biotechnology and Genetic Manipulation," *Biotechnol. Genet. Eng Rev.* **5**, 369–395 (1987).

R. G. Berger
Institut für Lebensmittelchemie der Universität Hannover
Hannover, Germany

GENETIC ENGINEERING: PRINCIPLES AND APPLICATIONS

Genetic engineering is an important and widespread enabling technology in the development of the discipline of food science and technology and our notions of food, from production to processing. A decade ago, Douglas McCormick, the editor of *Bio/Technology*, wrote: "By most standards, all of this [biotechnology] is still brand new. Yet biotechnology is the product of intellectual earthquakes that change things so completely that it is difficult, after the event, to remember that once the landscape was different. It is easy to forget that the change is recent, and more

68. H. J. Hwang, R. F. Vogel, and W. P. Hammes, "Entwicklung von Schimmelpilzkulturen für die Rohwurstherstellung," *Fleischwirtschaft* **73**, 327–332 (1993).
69. L. Janssens et al., "Fusel Oil as a Precursor for the Microbiological Production of Fruity Flavours," *Med. Fac. Landbouww. Rijksuniv. Gent* **54**, 1387–1392 (1989).
70. C. P. Dionigi and D. A. Ingram, "Effects of Temperature and Oxygen Concentration on Geosmin Production by *Streptomyces tendae* and *Penicillium expansum*," *J. Agric. Food Chem.* **42**, 143–145 (1994).
71. F. Pollak and R. G. Berger, "Geosmin and Related Volatiles in Bioreactor Cultured *Streptomyces citreus* CBS 109.60," *Appl. Environ. Microbiol.* **62**, 1295–1299 (1996).
72. EP 761817 (March 12, 1997), J. Rabenhorst and R. Hopp (to Haarmann & Reimer GmbH).
73. L. J. Romanczyk, Jr. et al., "Formation of 2-Acetyl-1-pyrroline by Several *Bacillus cereus* Strains Isolated From Cocoa Fermentation Boxes," *J. Agric. Food Chem.* **43**, 469–545 (1995).
74. S. Lino and M. Watanabe, "Wine-making by Killer Wine Yeast" [in Japanese], *After CA* **113**, 13754 (1989).
75. H. C. Chang, D. A. Gage, and P. J. Oriel, "Cloning and Expression of a Limonene Degradation Pathway From *Bacillus stearothermophilus* in *Escherichia coli*," *J. Food Sci.* **60**, 551–553 (1995).
76. R. G. Berger, K. Neuhäuser, and F. Drawert, "Odorous Constituents of *Polyporus durus* (Basidiomycetes)," *Z. Naturforsch.* **41c**, 963–970 (1986).
77. U.S. Patent 4,560,656 (Dec. 24, 1985), M. I. Farbood and B. J. Willis (to Fritzsche Dodge & Olcott, Inc.).
78. WO 89/12104 (Dec. 14, 1989), G. V. Page and G. R. Eilerman (to BASF Corp.).
79. S. Gocho et al., "Biotransformation of Oleic Acid to Optically Active γ-Dodecalactone," *Biosci. Biotech. Biochem.* **59**, 1571–1572 (1995).
80. M. Schöttler and W. Boland, "Über die Biosynthese von γ-Dodecanolacton in reifenden Früchten: Aroma-Komponenten der Erdbeere (*Fragaria ananassa*) und des Pfirsichs (*Prunus persica*)," *Helv. Chim. Acta* **78**, 847–856 (1995).
81. I. Benz and A. Muheim, "Biotechnological Production of Vanillin," in A. J. Taylor and D. S. Mottram, eds., *Flavour Science—Recent Developments*, Royal Society of Chemistry, Cambridge, United Kingdom, 1996, pp. 111–117.
82. C. E. Fabre, P. J. Blanc, and G. Goma, "Production of Benzaldehyde by Several Strains of *Ischnoderma benzoinum*," *Sci. Aliments* **16**, 61–68 (1996).
83. U Krings, M. Hinz, and R. G. Berger, "Degradation of [^{2}H] Phenylalanine by the Basidiomycete *Ischnoderma benzoinum*," *J. Biotechnol.* **51**, 123–129 (1996).
84. L. Vámos-Vigyázó, N. Kiss-Kutz, and A. Hersiczky, "Preparative Scale Generation of Apple Flavour from By-Products of Processing," in *3rd International Flavour Symposium*, Keksemet, 1984, pp. 34–35
85. R. G. Berger, A. Kler, and F. Drawert, "The C_6-Aldehyde Forming System in *Agropyron repens*," *Biochim. Biophys. Acta* **883**, 523–530 (1986).
86. F. Drawert, A. Kler, and R. G. Berger, "Optimierung der Ausbeuten von (2*E*)-Hexenal bei pflanzlichen Gewebehomogenater," *Lebensm.-Wiss Technol.* **19**, 426–431 (1986).
87. H W. Gardner, "Lipoxygenase as a Versatile Biocatalyst," *J. Am. Oil Chem. Soc* **73**, 1347–1357 (1996).
88. C. Schneider, P. Schreier, and M. Herderich, "Analysis of Lipoxygenase-derived Fatty Acid Hydroperoxides by Electrospray Ionization Tandem Mass Spectrometry," *Lipids* **32**, 331–336 (1997).
89. K. Matsui et al., "Bell Pepper Fruit Fatty Acid Hydroperoxide Lyase Is a Cytochrome P450 (CYP74B)," *FEBS Lett.* **394**, 21–24 (1996).
90. A.-F. Hsu, T. A. Foglia, and G. J. Piazza, "Immobilization of Lipoxygenase in an Alginate-Silicate Solgel Matrix: Formation of Fatty Acid Hydroperoxides," *Biotechnol. Lett.* **19**, 71–74 (1997).
91. A. Nunez et al., "Immobilization of Hydroperoxide Lyase From *Chlorella*," *Biotechnol. Appl. Biochem.* **25**, 75–80 (1997).
92. WO 95/26413 (March 8, 1994), R. B. Holtz, M. J. McCulloch, and S. J. Gargar (to Reynolds Technologies, Inc.).
93. WO 93/24644 (May 3, 1993), B. Muller, A. Gautier, C. Dean, and J.-C. Kuhn (to Firmenich, S.A.).
94. WO 94/08028 (Sept. 27, 1993), J.-M. Belin, B. Dumont, and F. Ropert (to BFA Laboratories).
95. T. Mulder-Krieger et al., "Production of Essential Oils and Flavors in Plant Cell and Tissue Culture," *Plant Cell Tissue Organ Cult.* **13**, 85–154 (1988).
96. G. Reil and R. G. Berger, "Accumulation of Chlorophyll and Essential Oil in Photomixotrophic Cell Cultures of *Citrus* sp.," *Z. Naturforsch.* **51c**, 657–666 (1996).
97. G. Reil and R. G. Berger, "Elicitation of Volatile Compounds in Photomixotrophic Cell Cultures of *Petroselinum crispum*," *Plant Cell Tissue Organ Cult.* **46**, 131–136 (1996).
98. A. H. Scragg, "The Production of Aromas by Plant Cell Cultures," in R. G. Berger, ed., *Biotechnology of Aroma Compounds*, Adv. Biochem. Engin. Biotechnol. 55, Springer, Berlin, 1996, pp. 239–263.
99. R. G. Berger, F. Drawert, and H. Kollmannsberger, "PA-Lagerung zur Kompensation von Aromaverlusten bei der Gefriertrocknung von Bananenscheiben," *Z. Lebensm. Unters.-Forsch.* **183**, 169–171 (1986).
100. R. Teranishi, "New Trends and Developments in Flavor Chemistry," in R. Teranishi, R. G. Buttery, and F. Shahidi, eds., *Flavor Chemistry*, ACS Symposium Series 388, American Chemical Society, Washington, D.C., 1989, p. 2.
101. D. Anderson and W. F. J. Cuthbertson, "Safety Testing of Novel Food Products Generated by Biotechnology and Genetic Manipulation," *Biotechnol. Genet. Eng Rev.* **5**, 369–395 (1987).

R. G. Berger
Institut für Lebensmittelchemie der Universität Hannover
Hannover, Germany

GENETIC ENGINEERING: PRINCIPLES AND APPLICATIONS

Genetic engineering is an important and widespread enabling technology in the development of the discipline of food science and technology and our notions of food, from production to processing. A decade ago, Douglas McCormick, the editor of *Bio/Technology*, wrote: "By most standards, all of this [biotechnology] is still brand new. Yet biotechnology is the product of intellectual earthquakes that change things so completely that it is difficult, after the event, to remember that once the landscape was different. It is easy to forget that the change is recent, and more change is just around the corner" (1). McCormick's statement has been true so far and will hold true for some time. In this article we describe the vocabulary, concepts, tools, techniques, and applications of genetic engineering to food science and technology.

HISTORY

The discovery of DNA ligase in 1967 followed by the DNA restriction endonucleases and RNA reverse transcriptase in 1970 was the beginning of era of genetic engineering. Also, in 1970 the first record for total synthesis of a gene *in vitro* was produced. In 1972 the first *in vitro* recombinant DNA molecule was generated, and a year later the first plasmid vector-mediated transformation was demonstrated. In 1975 the methodology for Southern blot was published. The blotting system (Fig. 1) allows for isolation and identification of DNA fragments by gel electrophoresis and their transfer (or blotting) onto a membrane filter for direct hybridization to a single-stranded radioactively labeled or biotin-linked probe. Four years later, the discovery of introns and exons in eukaryotic genes changed our concepts of gene transcripts, and the same year rapid DNA sequencing methods became available. In the decades following these outstanding achievements we have seen a rise in the commercial application of genetic engineering and production of human, plant, and microbial processes or products (2). It is amazing how rapidly genetic engineering has grown and what in the future it holds.

PRINCIPLES OF GENETICS

The hereditary material of most cellular organisms is DNA, although in many bacteriophages and plant or animal viruses it could be either DNA or RNA. DNA is largely the basis for the preservation of the instructions for the organization, structure, and functioning of living cells. Historically, it was realized that the passage of hereditary traits to one's offspring depended on those that were found in the parents. In the early 1860s the Austrian monk Gregor Mendel not only experimentally confirmed this in pea plants, but also established the principal rules governing the appearance of specific traits, that is, the parental factors (subsequently these became known as genes) and their assortment into individual progeny. He also made the distinction between the external appearance of such traits (later named phenotypes) and those that are reflections of the composition (later named genotypes). Mendel also advanced the idea of the expression of traits when they were in single copies from those that required two copies (later named dominant and recessive genes). The advent of microscopy and the discovery of dyes that could differentially stain cellular parts permitted the examination of the anatomical features of cells including the nucleus. The hereditary material, housed in distinct and organized structures, were named chromosomes. The number of chromosomes per cell are constant in any species. In most cells, the chromosomes are found in pairs of homologous structures, varying in number from a few two to three dozen. The concept of ploidy was developed to reflect the presence of haploid (N) and diploid (2N) cells, such as those found in bacteria and gametes or sex cells and in nonsomatic or fertilized cells, respectively. The N was defined to be the number of nonhomologous chromosomes found in a cell.

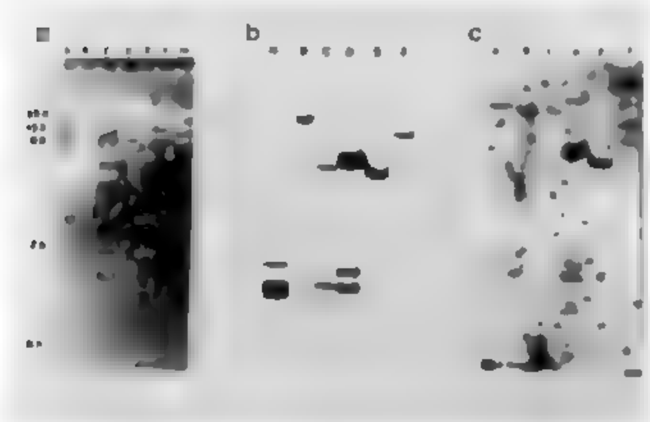


Figure 1. DNA obtained from human genome (a) has been hybridized by either (b) DNA or (c) RNA by Southern blotting method. *Source:* Photograph courtesy of J. M. Mac Pherson.

Analysis of Hereditary Materials and Exchanges

In the 1940 to 1950s the nature of DNA was elucidated by the use of quantitative genetic experiments, application of radioisotopes to analytical biochemistry, and the discovery of the electron microscope. These tools permitted the visualization of DNA and demonstrated its many activities: replication, repair, exchange, and recombination.

The structures of prokaryotic (eg, bacterial) and eukaryotic (eg, yeasts, fungi, plant, and animal) cells, which are both of significance in food science, and their genetic apparati have been investigated in great detail (3,4). The best-studied prokaryotic cell, the workhorse of many genetic engineering technologies and a causative of certain foodborne illnesses, is that of the bacterium *Escherichia coli*. This bacterial cell is cylindrical in shape, $1 \times 0.5\ \mu m$ in size (Fig. 2), and can grow and double in number every 20 min. The *E. coli* chromosome is in a covalently closed circular (ccc) form about 1 mm long and is made of 4,600,000 base pairs (4600 kilobase pairs, kb). The chromosome of this bacterium is mapped and completely sequenced.

We are beginning to realize that the structure of the genome in bacteria is more dynamic and diverse than once thought and accepted. Analysis of more than 150 bacterial species and isolates for genome size indicates a range, 580 kbp for *Mycoplasma genitalium* and 9200 kbp for *Myxococcus xanthus*, the smallest and the largest known genomes, respectively (3). These two organisms carry a gene number of 470 and more than 10,000, respectively, representing the lifestyles of an obligate parasite within living hosts and a metabolic generalist, undergoing sporulation, mycelial growth, and differentiation. The geometry of chromosomal DNA is found in a double-stranded circular, lin-

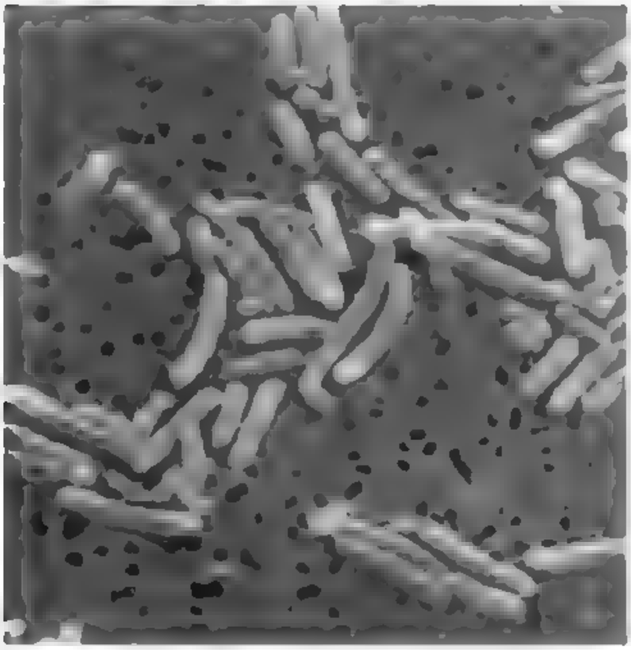


Figure 2. The simple binary fission of *Escherichia coli* cells along with the production of DNA-less minicells. *Source:* Ref. 30.

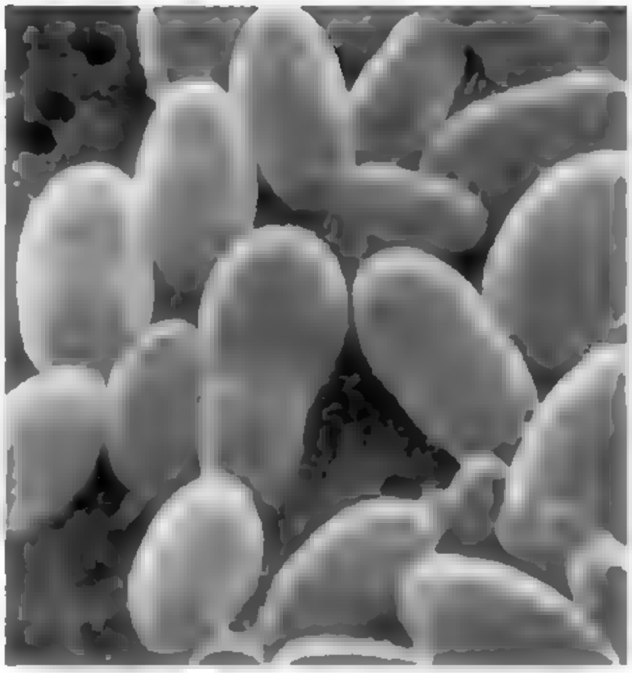


Figure 3. The yeast *Kluyveromyces marxianus* showing budding of a daughter cell and the location of previous bud scars.

ear, and folded configuration called the nucleoid or folded chromosome. Some bacteria have more than one set of chromosomes; for example, *Deinococcus radiodurans* has four chromosomes per cell in its stationary phase, suggesting that the classification of bacteria as haploid is an oversimplification (3). The current view of bacterial chromosome replication is that the DNA replication machinery is held at a relatively fixed position (at inner cell membrane) through which chromosomal DNA is threaded to produce two daughter chromosomes (5).

Often DNA of extra chromosomal origin, called plasmids, which are autonomously replicating pieces of DNA about 1/100 of the chromosome size, are found in the cytoplasm. These plasmids frequently contain genes for antibiotic resistance, conjugation, and production of proteins generally deemed nonessential to normal cell functioning. Some bacterial plasmids are nonconjugative, yet others are conjugative and permit their and/or other plasmids' transfer within the species or a broad range of hosts from different genera. Certain conjugative plasmids can transfer DNA between kingdoms; for example, the Ti plasmids of the bacterium *Agrobacterium tumefaciens* can be transferred to dicotyledonous and monocotyledenous plants or to the yeast *Saccharomyces cerevisiae*. In all cases, transfer of plasmids requires cell-to-cell contact and presence of transfer and mobilizing genes.

Cells of the yeasts *S. cerevisiae* and *Kluyveromyces marxianus* (Fig. 3), which are used in fermentation, bread making, and food-grade enzyme production, are quasi-spherical, 3 to 5 μm in diameter, and can double hourly. Yeasts may be haploid or diploid, depending on their life-cycle stage. All eukaryotic cell chromosomes contain some basic proteins or histones, which wrap around the DNA to form nucleosomes. *S. cerevisiae* contains 17 linear chromosomes ranging from 150 to 2500 kb. The collaboration of more than 600 laboratories in the United States, Canada, Europe, and Japan to sequence 12 billion bases and arrange 6000 genes of this yeast has been one of the largest decentralized experiments in molecular biology to date. A whole issue of the magazine *Nature* was devoted in 1997 to the yeast genome. DNA duplication in eukaryotic cells occurs during the S-phase of the cell cycle. Here the nuclear DNA will contain more than 100 putative replication factories, each with 300 or so replication points (forks) (6). Individual chromosomes can be separated by pulsed-field gel electrophoresis technique. Eukaryotic cells may also contain membrane-bound intracellular organelles such as mitochondria (Mtc), endoplasmic reticulum (Er), or chloroplasts (Chl). In Chl and Mtc (Fig. 4) a ccc DNA is compartmentalized and expressed. mtDNA are believed to have been derived from some prokaryotic cell genome. During cell division, all DNA, whether organellic, plasmid borne, or chromosomal, are divided equally and partitioned between the two daughter cells.

Bacterial cells are able to accept DNA from another parent or donor and undergo *in vivo* recombination. This was studied initially in the 1950s through the 1970s. These studies used whole cells and relied on natural exchange such as mating, transformation, and recombination occurring *in vivo* between donor DNA and recipient cells. During the 1960 to 1980s, after many of the requirements of DNA metabolism were discovered, it became possible to perform *in vitro* the synthesis, breakage, and joining the DNA from homologous or heterologous origins. These discoveries gave birth to the science and production of *in vitro* recombinant DNA (rDNA) molecules (7), which when transferred into living cells could express new traits.

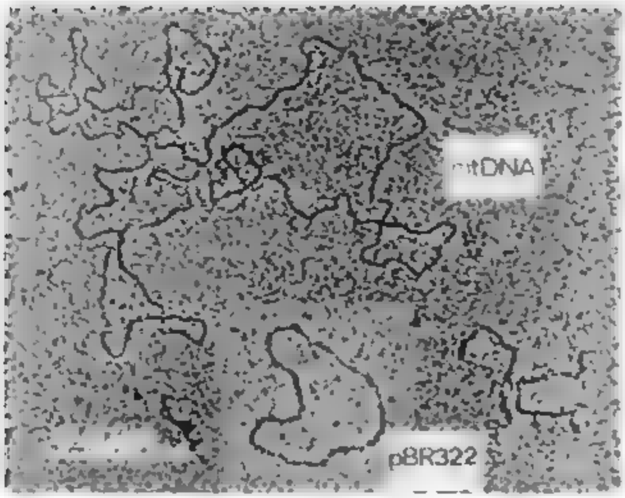


Figure 4. Covalently closed circular DNA molecules from the mitochondrion of a fungus and *E. coli* plasmid pBR322.

The terms transformation, transduction, transfection, and conjugation are used to indicate "natural" gene transfer into a cell (4). Transformation and transfection involve transfer of donor DNA into a recipient cell, whereas conjugation and transduction require the presence of a donor cell and a bacteriophage, respectively. Both natural and $CaCl_{2+}$ heat-induced transformation of many microorganisms by rDNA are now possible. Natural transformation of certain bacteria (eg, *Bacillus subtilis*) by linear duplex chromosomal DNA, and induced transformation of others (eg, *E. coli*) using ccc-plasmid DNA, has become common practice in genetic engineering (4). DNA can also be introduced into a cell through one of many laboratory-devised techniques of gene transfer, such as electroporation, particle gun or biolistics, microinjection, microlaser technique, and liposome fusion (see later). Transformation of many animal and plant cells is also possible, although the underlying mechanisms are quite different from those in bacteria. Transformation of cells can occur by intergeneric fusion of two individual cells from, in the order of their discovery, plant, microbial, and animal origins (8). Microbial and plant-cell fusion requires the production of protoplasts or cells without external surface layer(s). Protoplasts are osmotically unstable; in hypotonic environments they lyse, but in the presence of stabilizers (sugars, salts) they remain intact. When two protoplasts are brought into contact in the presence of a fusogenic substance (eg, Polyethylene glycol 6000) their membranes fuse, causing cytoplasmic and nuclear mixing events to occur. A transient fusant contains chromosomes from both parents; subsequently, karyogamy and recombination of nuclear materials and chromosomes can take place. With animal cells the fusogen could be an animal virus (8).

Analysis of the DNA as Genetic Code and Its Functions

The flow of genetic information is in general from DNA through transcription to RNA (messenger, transfer, and ribosomal RNA) and through translation of mRNA to proteins (Fig. 5). This was known as the central dogma of molecular biology until the discovery of the enzyme reverse transcriptase, which could synthesize a complementary DNA (cDNA) from an mRNA molecule. DNA and RNA molecules contain four bases: two purines, adenine (A) and guanine (G); and two pyrimidines, cytosine (C) and thymine (T) in DNA and uracil (U) in RNA. These bases are connected to the sugar deoxyribose (DNA) or ribose (RNA) to form a deoxynucleoside or nucleoside and are phosphorylated to form deoxyribonucleotides (DNA) or ribonucleotides (RNA), respectively (Fig. 6). The double helical or Watson-Crick form of base pairing for A:T and G:C in DNA was the first to be discovered (Fig. 7). Other types of Watson-Crick base pairing called k and π with a hydrogen bonding pattern (9) have increased the genetic alphabet from four to six letters. Many of the natural bases can be modified by addition of organic groups, for example, methylcytosine. Nucleotides are linked through phosphodiester linkage, and each polymer of DNA has a 5′ to 3′ polarity. The ratio of $(G + C)/(A + T)$ is known by the designation $(G + C)$ content or % $(G + C)$ and reflects taxonomic relatedness and molecular characteristics. The $(G + C)$ content of two different DNA molecules determines their separation during buoyant density-gradient centrifugation. Additionally, double-stranded (ds) DNA of higher %$(G + C)$ will have a higher melting temperature (Tm) than that with a higher %$(A + T)$. Through melting, dsDNA can be dissociated into two single-stranded (ss)DNAs, and through cooling down, they will regain the complementary ds structure. This process forms the basis for several DNA:DNA or DNA:RNA hybridization techniques. *In vitro*, every DNA molecule has a topological feature. The open-ended DNA molecule is either in ss or ds form and can be in rod shape. DNA molecules can bend; for example, a small polymer of 242 base pairs (bp) or larger can go from a linear (open-ended) to a circular (ccc) form by the joining of its open ends with ligase. More complex forms of dsDNA are also found. DNA molecules can assume many forms, including winding or unwinding, depending on their physical and enzymatic environments, for example, presence of enzymes called topoisomerases (for twisting) and endonucleases (for nicking and relaxing the twisted DNA). In addition, the sequence of bases within a ssDNA could create structures of their own. When a sequence of -AAAAAAAGCTTTTTTT- from a DNA duplex is allowed to separate into two ss polymers, each can generate a hairpinlike structure through base pairing of all A and T residues. Such sequences are thought to be recognition segments within DNA for its interaction with regulatory proteins. RNA structures also have unique organizations such as folding on itself and forming of a hairpinlike structure (eg, tRNA).

All DNA synthesis is enzymatic and utilizes the four deoxyribonucleotides. The pathways for the biosynthesis of deoxyribonucleotide triphosphates and degradation of DNA are well known. The enzyme responsible for DNA biosynthesis, DNA polymerase, appears in the forms I, II, and III in prokaryotic and α, β, and γ in eukaryotic cells and, along with several ancillary proteins, comprise the DNA synthesis machinery (6,10–12). DNA polymerase is used either for repair, in a 3′ to 5′ direction (which could also be due to exonuclease activity) or replication in a 5′ to

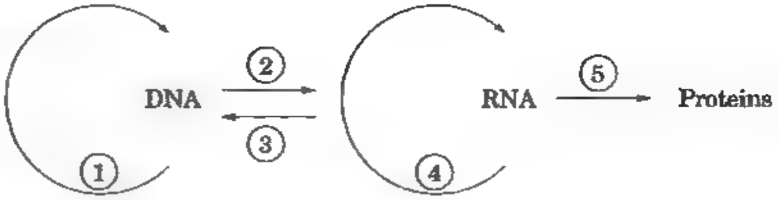


Figure 5. Flow of genetic information as perceived in molecular biology: (1) DNA replicates through DNA polymerase system; (2) transcription of DNA into RNA occurs via RNA polymerase system; (3) RNA is copied into DNA via reverse transcriptase action; (4) RNA as an enzyme can act on itself; (5) RNA being translated into proteins by the translational system.

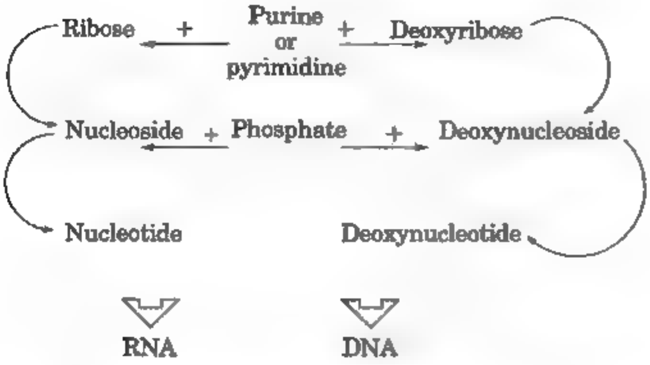


Figure 6. The biosynthesis of nucleosides and nucleotides for their use in DNA or RNA.

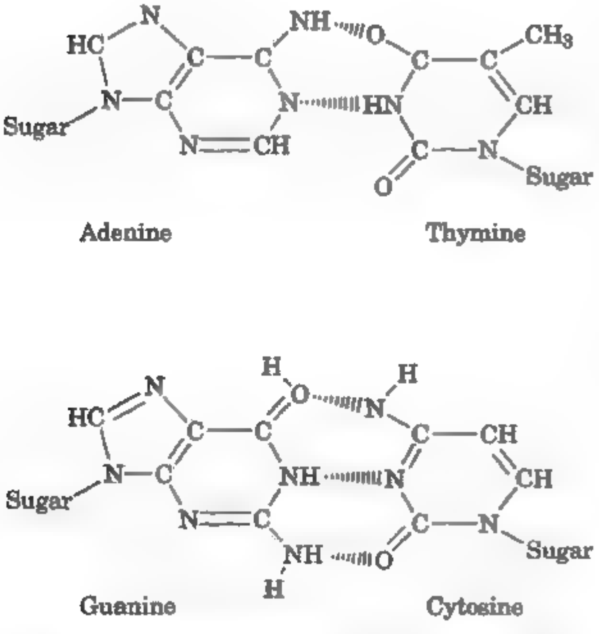


Figure 7. The base pairing between A:T and G:C in DNA.

3' direction. DNA helicases are essential motor proteins that function to unwind double-stranded (duplex) DNA by an active, rolling mechanism to yield the transient ssDNA intermediates for replication, recombination and repair (11). During the synthesis of DNA each deoxynucleotide is joined to the previous one through a phosphodiester bond. DNA polymerization occurs at a maximum speed of 100 and 1000 nucleotides per second for bacterial and animal cells, respectively. The open ends of the DNA at 3'-hydroxyl and 5'-phosphoryl position could be joined by DNA ligase (12). In certain mammalian cells, there are polynucleotide kinases that possess both 5-phosphotransferase and a 3-phosphatase activity that can restore DNA strand breaks, with 5-hydroxyl termini or 3-phosphate termini, or both, to a form that supports the subsequent action of DNA repair polymerases and DNA ligases, that is, 5'-phosphate and 3-hydroxyl termini (13).

A gene is a segment of DNA or, as in certain viruses, RNA made of a stretch of bases that, respectively, code for an RNA and a polypeptide molecule. Synthesis of a polypeptide from the transcript of a gene has been visualized (Fig. 8). It requires translational machinery to synthesize proteins from N-terminal to C-terminal by joining amino acids through their carboxy-terminals. In some viruses and eukaryotic organisms some genes and hence their RNAs are made of translatable and intervening sequences (known as exons and introns, respectively). The genetic code is read as a codon of three bases at a time. There are 64 codons, of which 61 code for amino acids and 3 for termination. There is some degeneracy in certain codons; for example, the amino acid glycine is coded for by the triplets GGA, GGG, GGU, and GGC. Different organisms have a bias in codon usage; for example, they may prefer to use one set of these over the other for the synthesis of polypeptides. Polypeptides in their nascent form are self-assembled into functional structures through the physicochemical properties of the constituent amino acids. Other polypeptides require a special class of proteins called nucleoplasmins, heat-shock proteins, and chaperon-

Figure 8. Transcription of bacterial DNA into an mRNA and its translation by polysomes into polypeptide. *Source:* Photograph courtesy of B. A. Hamakalo.

ins for assembly. Chaperonins are multifunctional proteins that catalyze the correct folding of other proteins by preventing side reactions such as aggregation (14). They are not themselves components of the final functional proteins. Different members of the chaperonin family assist in folding in a concerted manner. Some, but not all, molecular chaperonins are heat-shock or -stress proteins. Chaperonins act in the assembly of other proteins where they are not components of the final structure (14). Protein structure, folding, and enzymatic stability are dictated by covalent, disulfide linkages, and by noncovalent forces and interactions (15). Mutational replacement of amino acids within certain regions of a protein could affect its conformation and, hence, function, for example, thermostability and reaction rates (15).

Mutations and Selections

Most heritable variation of phenotypes is explained by mutations in genotypes. Improved mutant organisms have been used in industrial microbiology and food and fermentation technology. The initial attempts at strain improvements for food technology were through selection for spontaneously occurring variants from the original strain (16). Subsequently, fundamental studies on the occurrence and induction of mutations, enrichment and isolation of mutants, site-specific mutagenesis, and use of genetic recombination have made this process more manageable (15,16). *In vivo* mutations in microorganisms can occur spontaneously or with the mediation of mutagens at frequencies in the $10^{-(7-9)}$ or $10^{-(4-6)}$ range, respectively. Mutations alter DNA base sequences, by adding, deleting, or substituting base(s) and often affect the structure, and hence the function, of proteins. Mutagenic agents fall into three classes: physical (UV and X-ray irradiation), chemical (nitrous acid or nitrosoguanidine), and biological (transposons and mutagenic bacteriophages) (16).

A special class of genetic elements that are capable of insertion into any DNA are known as insertion sequences (ISs) and transposons (TNs). Insertion sequences constitute an important component of most genomes. More than 500 individual ISs have been described (17). The organization of a typical IS includes discrete DNA sequences of 0.8 to 1.4 kbp with a short DNA sequence at each terminus or 30 to 40 bp inverted repeats (IRs). Transposons are a class of genetic elements that contain at least one gene such as gene(s) for metabolism, antibiotic resistance, or synthesis of toxins (class I TNs), and in addition the transposase (class II TNs) (18). Insertion of a TN into the genome of a host requires the presence in the recipient DNA of ISs. Two functions are associated with a TN DNA once inserted into DNA molecules in a host cell. First, TN through its own insertion causes disruption of the sequence of a gene leading to a deletion-type mutation (18,19). Second, TN can affect the regulation of the expression of genes in the neighborhood of their integration site; for example, by inactivation of one gene and increased transcription of another adjacent gene (20). Another example is the deactivation of a regulatory phenotype by TN insertions in the *lac*A gene, encoding an endogenous *β*-galactosidase of *B. subtilis*, leading to inactivation of its negative regulator, *lac*R (21). The mechanism of such phenotypic change is due to effects involving one or several elements internal to the TN. On the other hand, the loss and restoration of mutator TN activity in maize has shown evidence against dominant-negative regulator associated with loss of activity (22).

As expected the exact removal of a TN DNA should restore the gene's function. This form of mutagenesis is called transposon mutagenesis and has wide application in genetic engineering (23). The end result of *in vivo* mutagenesis is alterations in DNA sequence(s), which must be fixed, replicated, and segregated. The rare mutant clone is then screened, enriched, purified, and characterized. One problem with *in vivo* mutagenesis is the difficulty in targeting specific genes. Certain industrially useful mutants are hard to grow or maintain because of the multiple or deleterious mutations they acquire during induced, but random mutagenesis. These problems can be eliminated by using *in vitro* mutagenesis. Here, a gene sequence is isolated, cloned into a suitable vector, and usually treated with a chemical mutagen, to specifically alter a base. More commonly, an oligonucleotide with a modified sequence is synthesized and then inserted into a gene (15,23). Through these approaches it is now possible to generate gene- or site-specific mutations. Recombination between two mutants with different characteristics can generate new organisms for industrial exploitation (6).

Elements and Regulation of Gene Expression

Gene expression requires template DNA, which is divided into regulatory and structural regions, and RNA transcription to produce the intermediate RNA (mRNA, rRNA, and tRNA). Although the primary function of mRNA is to serve as a blueprint for translation of a genetic message into a polypeptide (23), certain RNA molecules, called ribozymes, can function as self-splicing catalysts (24). The discovery of ribozymes has changed our dogmatic views of catalysis being solely the domain of protein enzymes.

To regulate their growth and metabolism, organisms must determine when and how much cellular constituents are needed. This task is achieved by controlling gene expression (3,23). The following elements regulate and control prokaryotic gene expression (Fig. 9). The operator is a DNA sequence where a repressor protein binds and pre-

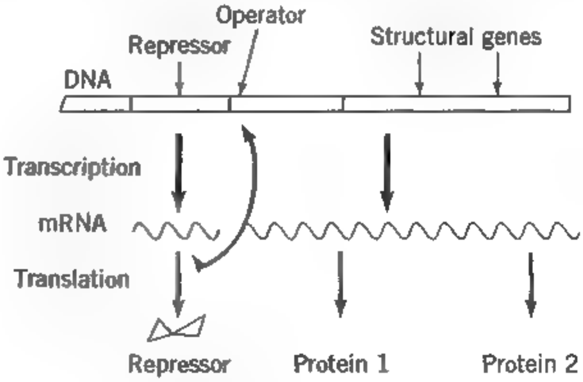


Figure 9. Gene expression in prokaryotic organisms.

vents transcriptional occurrence. Repressor-operator recognition involves a complex code of DNA base-pair sequences. Induction refers to expression of a gene subsequent to the removal of the repressor, usually by the addition of specific compound(s) called inducers. The promoter is located immediately in front of the gene and is the RNA polymerase recognition and transcriptional initiation site. Natural promoter efficiency varies, some are stronger than others. During the transcription of prokaryotic genes, RNA polymerase identifies in the 5′ to 3′ direction of the sense strand of the promoter before the initiation codon, a recognition sequence of bases, TTGACA or -35bps sequence, and a TATAAT (the Pribnow box) sequence at -10bps for its subsequent binding. Mutationally induced base changes in these two sequences can have mild or severe effects on transcription. Through its specificity for DNA transcriptional initiation sites, binding of RNA polymerase generates a localized melting and separation of the two strands of a small segment of the DNA leading to the initiation of RNA synthesis usually starting with GTP or ATP. DNA topoisomerases contribute to this process by relaxing DNA tension (25). There is continuous polymerization of ribonucleotides into an RNA transcript until the terminus of the gene is reached. At the terminus, either a sequence of six uridine residues and a hairpinlike structure or a sequence lacking such bases but requiring a termination factor, rho, to signal the stoppage of transcription. Some structural genes contain a leader and a trailer sequence, respectively, immediately after the promoter and before the terminator regions. At the end of transcription, RNA polymerase is freed to repeat the cycle.

The control of gene expression occur through transcriptional and translational control. One mechanism of control of transcription of genes is through induction of specific mRNA synthesis when an inducing substance is present (to turn on gene expression) and its repression (or turning off) once such inducers are removed. For example, when lactose is present in the growth medium of *E. coli*, the enzyme β-galactosidase is synthesized and, conversely, when it is absent, the repression of the enzyme synthesis occurs through a repressor protein. This modality of regulation is called negative control. In addition, several positive regulators of RNA polymerase are involved in control. For example, intracellular regulator cyclic AMP (cAMP) through its binding to cAMP binding protein (CAP) creates a complex, cAMP-CAP, which binds to specific promoters to activate certain operons. Finally, through a process called attenuation, the biosynthesis of the amino acid tryptophan is controlled. In this case, the transcription of the biosynthetic genes begins in the usual manner. However, instead of a complete transcript of the genes, an incomplete transcript of relatively short mRNA (162 bases) responsible for the tryptophan leader protein is made. The leader protein is tryptophan rich. When intracellular tryptophan supply is high, the mRNA makes a structure to block further continuation of transcription, by stalling (attenuating) the translation. With low tryptophan levels, a different structure in the stem-loop region of mRNA develops, which allows the continuation with transcription to the end of the operon. Therefore, in the case of tryptophan operon, the removal of repressor and attenuation generates a 70-fold (derepression) and another 8- to 10-fold (attenuation) or an overall of 600- to 700-fold increase in the expression (23).

In certain eukaryotic genes and hence their mRNAs (Fig. 10) there are some sequences of about 1000 bp inserted (introns) between the coding sequences (exons). Transcription begins at the promoter (5′) of a gene and proceeds through the exons and introns (if present) to the 3′ terminus, producing a primary mRNA transcript or heterogeneous nuclear RNA. In eukaryotic cells, several processing steps must occur to change the primary mRNA transcript into functional mRNA and allow its translation into proteins. Any introns present are subsequently removed or spliced out. The actual ending of the transcription in some cases could be several hundred base pairs beyond the polyadenylation site of the 3′ end of a mRNA. The third step required to change a primary mRNA transcript to a functional mRNA is the addition of a 5′ cap.

Eukaryotic promoters are recognized by specific DNA-binding proteins of less than 100 amino acid residues in size that bind to DNA and activate or repress gene transcription. Most bacterial RNA polymerases are large multimeric proteins; for example, *E. coli* enzyme contains two α, one β, and one β' subunits. This holoenzyme has additional subunits, the ∂ 70, ω, and σ subunits, which are integral to its functioning. In sporulating bacteria (eg, *B. subtilis*), RNA polymerase subunits are responsible for the selection of sporulation promoters. The RNA polymerases of eukaryotic organisms differ from the preceding both in molecular size and number of enzyme species. As many as two to four different types of RNA polymerases can be found in eukaryotic cells, and in several cases their β and β' subunits have conserved sequences. In higher eukaryotes, RNA polymerase I initiates transcription for rRNA genes from nucleolus, and RNA polymerases II and III transcribe mRNA, tRNA, and 5S rRNA from nuclear matrix (26). The transcriptional machinery of eukaryotic—as compared with the prokaryotic—cells is much more com-

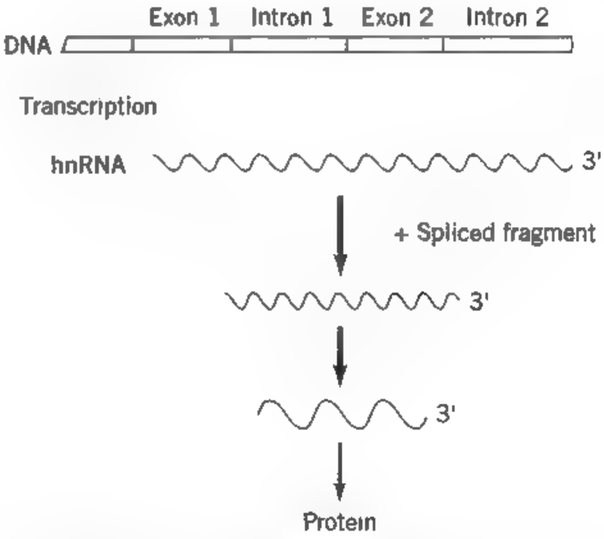


Figure 10. Transcription of an eukaryotic gene containing introns and exons.

plex (27) and can involve allosteric control by DNA in the case of selective gene transcription (28). Functions of RNA can be regulated by antisense RNA, which is an RNA complementary to the sequence in mRNA that through complementary, stable base pairing, prevents its translation (29). Antisense RNA also regulates or even prevents gene expression in prokaryotic, plant, and animal cells.

Whether prokaryotic or eukaryotic, the mRNAs are translated into proteins using ribosomes, charged tRNA molecules, and auxiliary proteins, beginning with codons ATG or GTG (23). The ribosome is a large multifunctional complex composed of both RNA and proteins. Accumulating evidence suggests that rRNAs play a central role in the critical ribosomal functions of tRNA selection, and binding, translocation, and peptidyl transferase (30). Usually several ribosomes (or a polysome) are present on a mRNA. In prokaryotic cells transcription and translation occur concurrently within the cytoplasm; but in eukaryotic cells, translation occurs outside the nucleus. Translational control includes the ribosome-binding sequence (Shine-Delgarno sequences) and attenuation, which is prevention of translation of mRNA because of its folding back on itself. After its transcription, the mRNA is degraded. Messenger stability, among other things, depends on the presence of AU-rich sequences at 3′-end, resulting in shorter half-lives than those lacking such sequences. If a nonsense codon is reached either at the end or through mutations within a gene, the translation is halted and a partial polypeptide results. Finally, protein stability and half-life depends on proteases that degrade not only normal but also for certain heterologous and abnormal (mutant) proteins. In eukaryotic cells protein stability depends on the small protein ubiquitin, which when conjugated to proteins leads to their rapid degradation.

Genetic Exchange and Recombination in Vivo

Prior to the discovery of genetic engineering *in vitro*, it was known that genetic information can be exchanged *in vivo* among various organisms (3,4). The simplest forms of genetic exchange—transformation, transduction, and conjugation—are those found among bacteria. Only conjugation requires cell-to-cell contact. Conjugation was initially discovered in the common enteric bacterium *E. coli*, but it occurs in many genera of bacteria and between members of two kingdoms, for example, *A. tumefaciens* and many plants species (31), as well as *E. coli* and *S. cerevisiae* (32). In certain bacteria such as streptococci, mating depends on the presence of special mating pheromones, which are small polypeptides promoting mating aggregation. In fungi, mating occurs when two haploid cells of different mating types fuse to allow genetic exchange to occur. In yeasts this requires special mating control locus (MAT) and switching loci (HML or HMR), which are silent cassettes that introduce the *α* and *α* mating-cell types through direct transposition of required genetic cassettes. In the absence of sexual cycle, certain fungi use parasexual mating events. In transformation, whether natural or induced, naked DNA or DNA contained in membranous vesicles called transformosomes are transferred into a recipient cell (4). In generalized and specialized transduction, DNA packaged into a bacteriophage (Fig. 11) is injected into a bacterial cell. The latter two modes of genetic exchange are used in recombinant DNA technology for packaging of rDNA (eg, bacteriophage λ) or transformation of commercially available competent *E. coli* cells. Once inside a cell, homologous DNA can undergo recombination using recombinational enzymes to generate hybrid DNA molecules. All mechanisms of genetic exchange are currently used in food research.

GENETIC ENGINEERING TECHNOLOGIES

Instrumentation and Tools

Central to and fundamental in the process of genetic engineering is the isolation, manipulation, insertion into, and expression of DNA in a host cell (33). The instrumentation and tools needed for the technical tasks are shown in Table 1.

Organisms

Microorganisms, plants, and animals are used organisms in food production and processing technologies. While the production of microbial cells through fermentation technology has been a long-established art, that of plant cells in cultures is rather new. In the 1970s several groups studied the ability of plant cells and tissues to grow in liquid nutritive media. Cultures from diverse origins such as (*1*) organs (roots, flowers, anthers), (*2*) meristems (shoot, leaf), (*3*) callus (undifferentiated cell mass), (*4*) cells (homogenized tissues), and (*5*) protoplasts were found to grow on defined salts media containing a carbon source, vitamins, plant hormones, and various other nitrogenous substances. Plant cell biotechnology and economic value-added or value-derived products have matured more rapidly with advances in plant cell culture systems, construction of transgenic plants, and other applications of biotechnology (see later).

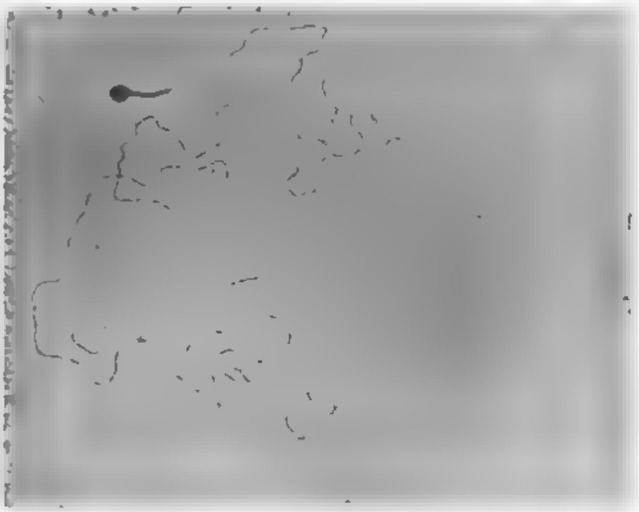


Figure 11. The bacteriophage lambda of *E. coli* ejecting its DNA.

Table 1. Basic Steps in a Simple Genetic Cloning

Step	Tools or systems needed
1. Propagation of organisms	Growth chambers, incubators, fermenters, greenhouses
2. DNA extraction and purification	French pressure cell, cell lytic enzymes, spectrophotometer, preparative centrifugation, cesium chloride density ultra centrifugation
3. DNA library preparation	Restriction enzymes
4. DNA size separation	Ultracentrifugation or electrophoresis
5. Vector and probe DNA	Polymerase chain reaction or cDNA, preparation technology, DNA manipulative enzymes, electrophoresis
6. DNA sequencing	Maxam-Gilbert, Sanger's and/automated sequencing methods
7. Introduction of rDNA into a host cell	Electroporation, bio-bolistics host transformation
8. Identification of transformed cells	Selective media, reporter genes, Southern blots, Northern blots, Western blots
9. Gene location	OFAGE, CHEF, various blots

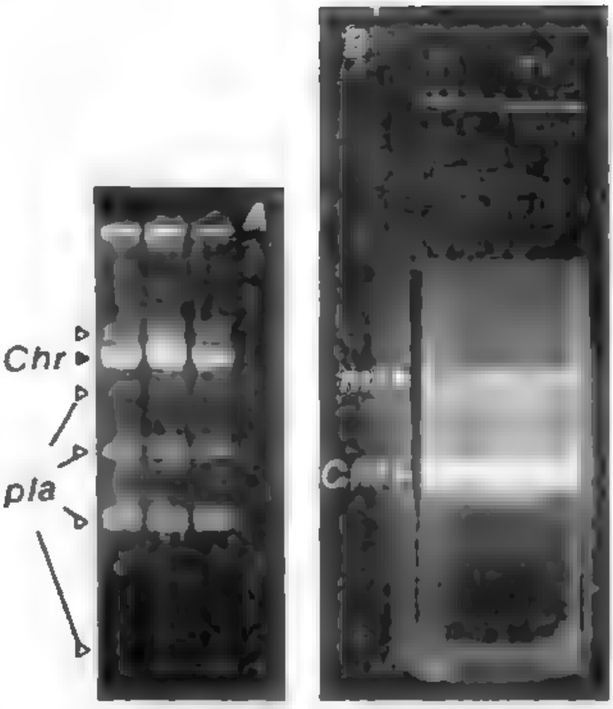


Figure 12. Separation of chromosomal and plasmid DNA from the bacterium *Bacillus thuringiensis* HD-1 by agarose gel electrophoresis (a) and chromosomal and mitochondrial DNA from the fungus *Beauveria bassiana* by cesium chloride bisbenzimide gradient centrifugation (b).

Isolation and Preparation of DNA

DNA from most cellular organisms can be isolated through the disruption of cell membranes and/or walls by using lytic enzymes or other physicochemical forces (osmotic pressure, shear forces, and ultrasound). DNA of high (chromosomal) and low molecular weights (from plasmids, mitochondria, and chloroplasts) can be extracted. Because cellular lysates are rich in DNA-degrading enzymes, such reactions are performed in the presence of chelating agents or cooler temperature. DNA is then purified from proteins and other cellular constituents by extraction with a mixture of phenol-chloroform and is recovered by ethyl alcohol or isopropanol precipitation. To purify further, high-speed dye (ethydium bromide or bisbenzimide) buoyant density ultracentrifugation is performed and to characterize the plasmid or other types of DNA molecules, agarose gel electrophoresis is employed (Fig. 12). Although DNA isolated by this method is of high quality, the procedure is a lengthy one requiring expensive equipment. Nowadays, commercial DNA purification kits that utilize silica-based resins and anion exchange have become available. These kits allow for the isolation of DNA in purity equivalent or superior to that obtained by two successive rounds of cesium chloride gradient centrifugation (34). Such plasmid DNA is now routinely used in such applications as transfection, microinjection, automated and manual sequencing, restriction analysis and *in vitro* transcription.

In electrophoresis, DNA molecules migrate according to their molecular weight from the negative to the positive electrode; that is, the smaller molecules move further from the origin. If such DNAs are cut by any of some 600 restriction endonucleases, enzymes that cleave phosphodiester linkages at specific sequences within DNA, the resultant fragments can be separated in an electrophoretic separation gel, according to their sizes (Fig. 13). DNA fragments after electrophoresis can be transferred permanently by blotting onto specific membranes. Often the electrophoretic separation of DNA followed by the ethidium bromide staining is used for visualization of DNA molecules, although the same can be achieved by electron microscopic analysis for size (Figs. 4 and 11) or heteroduplex analysis. Table 2 lists most commonly used enzymes for cell lysis, DNA extraction, and subsequent rDNA construction. While all DNA-sequencing protocols are based on the Sanger's chemical method (Fig. 14), other subsets of methodology by enzymatic (Klenow polymerase, Taq polymerase, and polymerase chain reaction, or PCR) systems are also used. Sequencing tasks are now automated and commercially available both for synthesis and sequencing (33).

Polymerase Chain Reaction

The PCR developed by Cetus (Emeryville, California) scientists in 1985 (35) is a powerful *in vitro* method for amplifying a segment of DNA that lies between two regions of known sequence, defined by a set of primers. The basic steps of PCR are denaturation of the DNA, annealing the primers to complementary sequences, and extension of the annealed primer with a thermostable DNA (eg, *taq*) polymerase. Together these steps represent one cycle of amplification.

As illustrated in Figure 15 the double-stranded DNA template is first denatured by heating in the presence of a

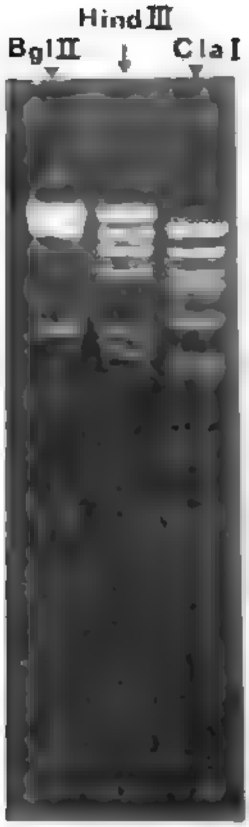


Figure 13. Restriction endonuclease digestion of bacteriophage lambda DNA into fragments. The names of enzymes are indicated on the top of each track.

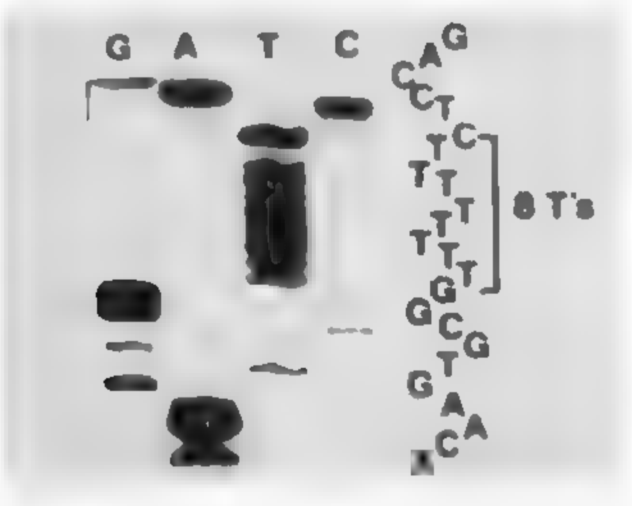


Figure 14. Sample autoradiogram of DNA sequencing reaction pattern by the Sanger method. *Source:* Photography courtesy of J. M. MacPherson.

large molar excess of two specific oligonucleotides and four dNTPs. The reaction is then allowed to cool to a temperature that allows for the annealing of pairs of oligonucleotides to their target sequences. Once annealing has occurred DNA polymerase mediates the 5′ to 3′ extension of the primer-template complex. These three steps are repeated several times and the major product of this reaction is a significant amplification of the desired segment of double-stranded DNA whose termini are defined by the 5′ termini of the oligonucleotide primers.

The first experiments in PCR used the Klenow fragment of *E. coli* DNA polymerase I, which has a temperature optimum of 37°C. However, since the Klenow fragment is inactivated at temperatures required to denature DNA, it was necessary to add a fresh aliquot of the enzyme to the reaction repeatedly. Unfortunately, these reactions tended to only work well for the amplification of DNA fragments less than 200 bp. For larger fragments it was found that the yields were poorer and the products were often heterogeneous in size (36). These problems were solved with the discovery of the thermostable *taq* DNA polymerase, isolated from the cells of thermophilic bacterium *Thermus aquaticus*. *Taq* polymerase can survive extended incubation at 95°C; therefore, all the components can be added at the start of the reaction without any further replenishment. Also, since annealing and extension can be carried

Table 2. Enzymological Aspects of rDNA Work

Purpose	Enzyme	Function
Cell lysis	Lysozyme, cellulase, mutanolysin	Removes cell walls
Proteolysis	Pronase, protease K	Removes proteins
RNA degradation	RNase H	Elimination of RNA
Nick translation	*E. coli* DNA Polymerase I	Synthesize DNA
	Klenow fragment	
	T4 DNA polymerase	
cDNA synthesis	Reverse transcriptase	(cDNA) from mRNA
Process DNA	Nuclease Bal31	
	Exonuclease digestion	
	Mung-bean nuclease,	
	Restriction endonucleases	Endonuclease cuts
	DNA methylases	Methylated bases
	Phosphatases	Remove 5′ phosphate
Joining of polynucleotides	Polynucleotide ligase	Join ends of DNA or RNA
Amplifying DNA	Taq polymerase	Produce oligonucleotides by polymerase chain reaction

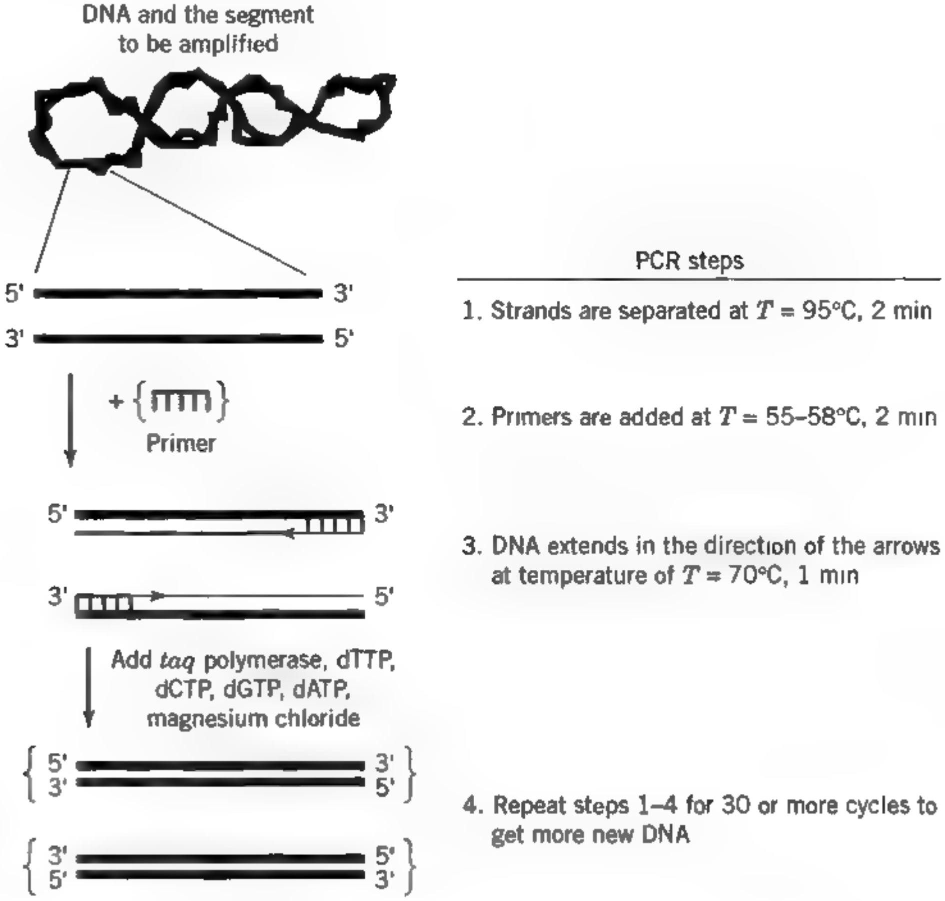

DNA and the segment to be amplified
5'
3'
3'
5'
+ Primer
5'
3'
3'
5'
Add *taq* polymerase, dTTP, dCTP, dGTP, dATP, magnesium chloride
5'
3'
3'
5'
3'
5'
5'
3'
PCR steps
1. Strands are separated at $T = 95°C$, 2 min
2. Primers are added at $T = 55–58°C$, 2 min
3. DNA extends in the direction of the arrows at temperature of $T = 70°C$, 1 min
4. Repeat steps 1–4 for 30 or more cycles to get more new DNA

Figure 15. PCR the double-stranded DNA template is first denatured by heating in the presence of a large molar excess of two specific oligonucleotides and four dNTPs.

out at elevated temperatures, mispriming is reduced, thus resulting in improvements in the specificity and yield of the amplification reaction.

PCR amplification is currently used in a variety of needs in molecular cloning and analysis of DNA, for example, probes, generation of large amounts of DNA for sequencing, chromosome crawling, creation of mutant sequences, and the generation of cDNA from small amounts of mRNA. PCR is also extensively being used for (*1*) the specific amplification of cellular protein coding genes by differential or global expression, (*2*) detection of nucleic acid sequences of genetic modified plants or plant products, (*3*) pathogenic organisms in food and clinical samples, (*4*) the diagnosis of genetic disorders, and (*5*) in forensic cases.

Several modified versions of the PCR method have been developed. Compared with the original use of PCR, that is, to amplify segments of DNA located between two specific primer hybridization sites, a single-sided PCR method has been developed that initially requires specification of only one primer hybridization side. The second site is then defined by the ligation-based addition of a unique DNA linker. This method, referred to as ligase chain reaction (LCR), allows for exponential amplification of any fragment of DNA. Another modification of the basic PCR has led to the development of anchored PCR. By using anchored PCR it is possible to amplify full-length mRNA when only a small amount of the sequence information is available. PCR-based techniques such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), DNA Amplification Fingerprinting (DAF), and microsatellite/PCR have been developed and used for identifying DNA markers.

In many cases it becomes essential to have a PCR detection system that can identify desired gene sequences quickly, with high specificity and in large volumes. The best analytical tool available to do this is mass spectrometry (37). A mass spectrometer works by vaporizing DNA and then accelerating the molecules through a vacuum chamber with the help of an electric field. Tiny differences in the time that it takes the fragments to reach the detector reveal small differences in their mass and hence their sequence. This technique is known as matrix-assisted laser desorption ionization time-of-flight mass spectrometry, or MALDI-TOF MS, and has the capability to analyze hundreds of DNA samples for a variety of assays for point mutations and polymorphism analysis (38). Although originally used over a decade ago for protein analysis, it was not available for DNA analysis until 1993, when various matrices were developed that would work with DNA fragments as long as 100 base pairs. However, for practical sequencing MALDI-TOF would have to work with DNA fragments much longer than the current 100 base pair capacity. Currently, new matrices are being studied that could extend MALDI-TOF reach to 1000 bases, and if this works, then this technique would be a major breakthrough for high-throughput sequencing.

Cloned Genes in Vitro

Gene cloning is the use of experimental techniques that generate rDNA molecules *in vitro* with the desire for its incorporation and expression in a cell. With the use of rDNA technology one can produce a genomic DNA or cDNA library and hence a fragment containing any gene(s) from any source (39). Cloned genes are essential foundations of biotechnology. To clone a gene (Fig. 16), the genetic information, whether DNA or RNA, is processed by restriction endonucleases or reverse transcriptase or amplified by PCR, as the case may be. The steps involved in cloning foreign DNA into host cells include: (*1*) isolating RNA or DNA to be cloned; (*2*) choosing a suitable vector; (*3*) processing the DNA or RNA by restriction endonucleases or reverse transcriptase, respectively; (*4*) inserting the DNA fragments into the vector; (*5*) transferring of DNA into the desired host cell; (*6*) identifying those cells which have taken up the DNA, and (*7*) confirming that the clones are carrying the desired DNA fragment. These are the general steps within each of which several options are available. Figure 16 depicts the strategies involved in a gene cloning.

Several criteria are needed in deciding on a choice for cloning vector. Depending on fragment size to be cloned, specific vectors must be employed. Thus, for fragments of DNA with sizes of <4 kb, <10 kb, <23 kb, and <46 kb, the recommended vectors are phage M13, bacterial plasmids (pEMBL, pBR, etc), λ phage (Fig. 11), and cosmids (33). Cosmids, which are a combination of a plasmid and a bacteriophage (lambda) based vector, can be made to contain large DNA fragments from the genomic library of an organism. The number of clones needed for fragments of 35 kb from *E. coli* genome would be 340; from *S. cerevisiae*, 6000; and from tomato plant cell, 60,000. Often the cloning strategies, for example, choice of available restriction sites, reporter genes (to indicate the expression of cloned gene), presence of one or more selectable phenotypes, autonomous replication in two or more hosts (shuttle vectors), nature of the experiment (eg, sequencing, preparation of probes, study of gene regulation), and exploitation and safety consideration (biohazard consideration, placement of nonconjugability functions, suicide vector system) decide the particular details of the system. Finally, in deciding about the choice for the vector, the question of cloned gene copy number (low or high) and ssDNA (used for heteroduplexing and sequence analysis) versus dsDNA phages should be made in advance. In recent years more advanced vectors have been produced, for example, expression vectors and cassette vectors. They contain a promoter, a terminator, and ribosomal-binding sites, with the additional feature of a restriction site where a desired structural gene will be inserted. Presence of strong regulatory sequences aid significantly in the expression of the inserted gene in such a tailor-made expression vector system. This system offers great advantages for the production of rDNA-derived foreign products from cells. Additional uses of cassette vectors occur in those cases where partial removal of the reading frame from a structural gene has been made. After the start, the chimeric DNA, containing fragments of two different organisms, is inserted. The foreign gene product is recovered as a single (stand-alone) or fused (chimeric) polypeptides.

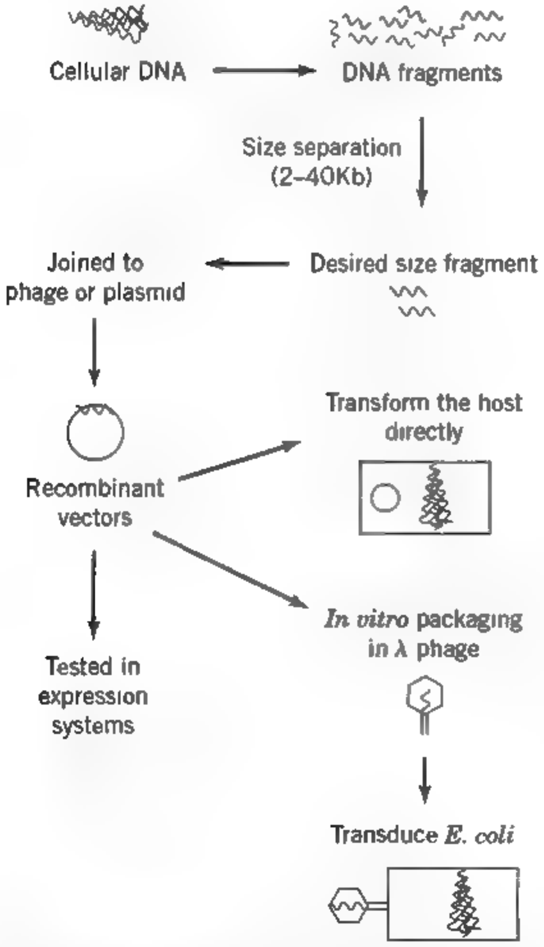

Cellular DNA
DNA fragments
Size separation (2–40Kb)
Desired size fragment
Joined to phage or plasmid
Recombinant vectors
Transform the host directly
In vitro packaging in λ phage
Tested in expression systems
Transduce *E. coli*

Figure 16. A summary of the basic gene cloning experiment using *E. coli* as the ultimate host.

To examine the polypeptide products from rDNA, several *in vitro* (various transcription and translation systems) and *in vivo* (whole bacterial cell, minicell, and maxicell systems) can be used (40,41). Minicells are unique in that they are polar buds from rod-shaped bacteria (Fig. 2) lacking chromosomal DNA and therefore permit the screening of only those proteins that have been derived from the rDNA. After a recombinant DNA is generated, it is introduced into the new host through one of many techniques of gene transfer, such as electroporation, particle gun or biolistics, microinjection, microlaser technique, and liposome fusion (33,41,42). The initial choice of a bacterial host for rDNA work reflects the specific usage, for example, laboratory versus industrial; however, for food usage the GRAS (generally regarded as safe) status is important and strains of microorganisms with particular genetic markings are available. The transformed cell is then identified by the expression of the recombinant gene product under some selection or growth condition or by using variety of DNA, RNA, enzyme function related, fluorescent (eg, the green fluorescent protein), and immunological probes for colony or plaque hybridization (Fig. 17) and detection (33).

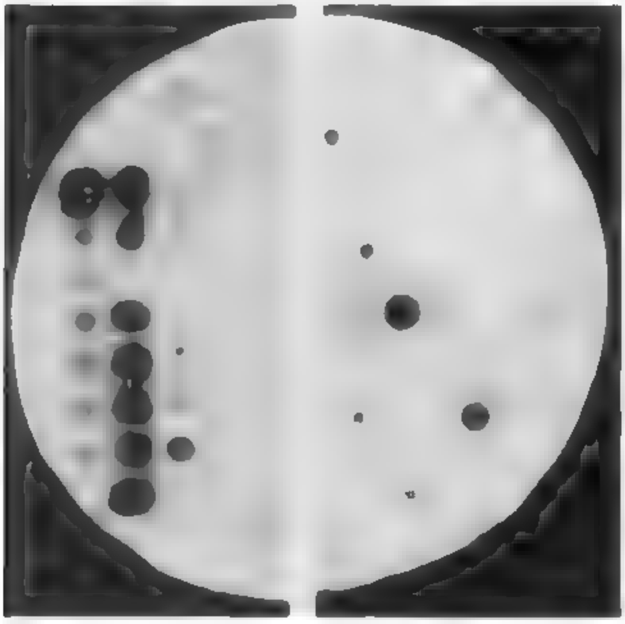


Figure 17. Autoradiogram of two halves of plates showing colony lift (left half) and plaque lift (right half). *Source:* Photograph courtesy of J. M. MacPherson.

APPLICATIONS OF GENETIC ENGINEERING IN FOOD SCIENCE

There are numerous applications of genetic engineering in food science and technology. The obvious use of this technology is in aiding the detection food contaminants or pathogens and safe foods (43). Additionally, however, we can change the starting materials in a food and add valuable attributes to the ingredients that are or get into the food system. In the following paragraphs, selected examples are given, more from a generic rather than commodity-specific points of view.

Genetic engineering tools have deciphered the total genomic sequences of more than a dozen microorganisms and tens of thousands of genes. Many molecular biology database systems have been established to deposit and acquire sequences, for example, EMBL (European Molecular Biology Laboratory), GeneBank (Genetic Sequence Data Bank), and NBRF (National Biomedical Research Foundation). These libraries can be accessed and searched through software tools and programs such as BLAST and FASTA (Lipman-Pearson search algorithms). The availability of many gene sequences has permitted inspection and identification of gene regulatory element-binding sites. One key element is the open reading frame (ORF) and the other is unidentified reading frame (URF). ORFs can locate ribosome attachment sites and, hence, through *in vitro* mutagenesis, base sequence changes within ORFs can be made without much need for formal genetic analysis (ie, mutants). Naturally occurring and engineered genes could be utilized from such gene banks.

The main source for the enzymes used in general, and the food industry in particular, are those enzymes that in terms of quantity of enzymes used come from microbial, plant, and animal sources, respectively. Enzymes not only transform food ingredients but also play a crucial role in restoring the lost flavor and aroma that can occur in certain instances of food processing. Various lipolytic, proteolytic, and multicomponent hydrolytic enzymes are being used for the addition of flavors to food. Thus, single-flavor compounds such as fatty acids, ketones, diacetyl, acetaldehyde, lactones, esters, pyrazines, and mustard oils and flavor enhancers such as nucleotides, amino acids, and terpens can all be produced by microbial enzymes for aroma and flavoring needs. In addition to the desirable flavors, enzymes such as lipoxygenases contribute to the off-flavoring of corn oil, while exerting a positive effect on the flavor of tomatoes (44,45). Genetic engineering, without creating a dilemma for the internal functions of a cell, could control the undesirable effects of many enzymatic reactions that affect postharvest or postprocessed foods. Through the knowledge of the DNA and protein sequence for many enzymes, the genetic analysis for the physical requirements of an enzyme (eg, active, catalytic, and ligand binding sites), and use of computer modeling the production of ideal enzymes should be possible (15). Production of a functional enzyme of minimal size or with improved thermotolerance, whether through solid-state peptide synthesis or synthetic oligonucleotides and rDNA technology, should be possible (15).

Recent production of transgenic animals and plants by such diverse methods as microinjection (plants and animal cells), conjugal transfer (microbial and plant cells), electroporation (microbial and plant cells), biolistics or microprojectile, and other less commonly known techniques has confirmed genetic engineering to be a strong and viable technology. Currently there are many fruits, vegetables, and seeds constructed through the tools of genetic engineering, which have produced transgenic plants containing viral, bacterial, and other eukaryotic genes presenting desirable traits such as resistance to disease, insects, herbicides, and tolerance to certain environmental factors (Table 3). Through an extension of this technology it should be possible for a majority of edible crops and other commercial plants to contain various nutritional and health-related qualities (45). Through combined knowledge of flowering-plant pigment and hormone biosynthesis, it is now possible to isolate, manipulate, and transfer genes for floral shape, and color. The extension of this knowledge toward derivation of food coloring and flavoring substances should be an exciting possibility. Use of antisense RNA in the area of genetic engineering of flowering plants and ripening of climacteric fruit and vegetables has been achieved.

Animal embryo transfer technology and production of transgenic animals by microinjection of foreign genes into eggs (transgenesis) to increase animal productivity and product value has become possible. Biotechnologists have produced transgenic sheep ("Dolly") and injected about 1 million copies of rat growth hormone gene into fish eggs to show about 5% of fish hatched were transgenic (with rat growth hormone). In sheep, goats, pigs, and cattle, presence of growth hormone produces leaner meat. In Edinburgh, Scotland, researchers have had success in creating transgenic sheep that produced milk with human anti-

Table 3. Examples of Transgenic Food Plants

Genetic modification	Plant
Amino acids	Canola/rapeseed
Antioxidant enzyme	Tomato
Carbohydrate metabolism	Soybean
Development	Soybean
Disease resistance	Apple, grapes, pear, potato, rice, wheat
Fertility	Corn
Fiber strength	Cotton
Flowering time	Apple
Herbicide tolerance	Beet, canola/rapeseed, corn, rice, soybean
Insect resistance	Alfalfa, corn, potato, rice, soybean, sunflower
Lysine content	Soybean
Male sterility	Corn
Maturity	Corn
Oil profile/quality	Canola/rapeseed, corn, soybean
Pectin esterase	Tomato
Protein quality	Barley, corn, soybean,
Reduced disulfides	Barley
Ripening	Melon, cantaloupe
Seed composition	Soybean
Shelf life	Mango, papaya
Size	Pear
Stalk strength	Corn
Vaccines	Bananas
Virus resistance	Grapes, papaya, plums, potato, soybean, tomato
Yield	Corn

hemophilic substance, factor IX, and have obtained sheep β-lactoglobulin from transgenic mice. In Australia through an ingenious injection of epidermal growth factor (Bioclip) in sheep and fitting of the animal with a net, the fleece falls within a week of injection, which is caught in a net. The commercial impact of the latter example is in saving spring shearing of some 150 million animals every year in Australia alone.

Genetic engineering, as described here, is a subject about which courses are given and textbooks are written. What is clear from this article is that which was said at the beginning: these changes are recent and indeed more change is just around the corner. Needless to say, the consumer education and buy-in position can ultimately determine how far this technology can go or stop: the bench top, the field, the factor, and the food menu.

BIBLIOGRAPHY

1. D. McCormick, "Genome Know-How," *Bio/Technology* **8**, 5 (1990).
2. Congress of the United States: Office of Technology Assessment, *Impact of Applied Genetics: Microorganisms, Plants and Animals*, U.S. Government Printing Office, Washington, D.C., 1981.
3. S. Casjens, "The Diverse and Dynamic Structure of Bacterial Genomes," *Annual Reviews of Genetics* **32**, 339–377 (1998).
4. E. A. Birge, *Bacterial and Bacteriophage Genetics*, Springer-Verlag, New York, 1994.
5. K. R. Lemon and A. D. Grossman, "Localization of Bacterial DNA Polymerase: Evidence for a Factory Model of Replication," *Science* **282**, 1516–1519 (1998)
6. S. Waga and B. Stillman, "The DNA Replication Fork in Eukaryotic Cells," *Annu. Rev. Biochem.* **67**, 721–751 (1998).
7. S. Cohen et al., "Construction of Biologically Functional Bacterial Plasmids *in vitro*," *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240–3244 (1973).
8. G. Poste and G. L. Nicholson, *Membrane Fusion*, Elsevier/North Holland, Amsterdam, The Netherlands, 1978.
9. J. A. Piccirilli et al., "Enzymatic Incorporation of a New Base Pair into DNA and RNA Extends the Genetic Alphabet," *Nature* **343**, 33–37 (1990).
10. Z. Kelman and M. O'Donnell, "DNA Polymerase III Holoenzyme. Structure and Function of a Chromosomal Replicating Machinery," *Annu. Rev. Biochem.* **64**, 171–200 (1995).
11. T. M. Lohman and K. P. Bjornson, "Mechanism of Helicase Catalyzed DNA Unwinding," *Annu. Rev. Biochem.* **65**, 169–214 (1996).
12. A. Kornberg, *DNA Replication*, W. H. Freeman, San Francisco, Calif., 1995.
13. U. K. Busheri-Feridoun et al., "Repair of DNA Strand Gaps and Nicks Containing 3′-Phosphate and 5′hydroxyl Termini by Purified Mammalian Enzymes," *Nucleic Acids Res.* **6**, 4395–4400 (1998).
14. P. B. Sigler et al., "Structure Function in Gro EL-Mediated Protein Folding," *Annu. Rev. Biochem.* **67**, 581–608 (1998).
15. R. L. Jackman, T. J. Cottrell, and L. J. Harris, "Protein Engineering," in Y. H. Hui and G. G. Khachatourians, eds., *Food Biotechnology: Microorganisms*, VCH, New York, 1995, pp. 181–236.
16. A. L. Demain and N. A. Solomon, eds., *Manual of Industrial Microbiology and Biotechnology*, American Society for Microbiology, Washington, D.C., 1986.
17. J. Mahillon and M. Chandler, "Insertion Sequences," *Microbiology and Molecular Biology Reviews* **62**, 725–774 (1998).
18. D. Berg and M. M. Howe, eds., *Mobile DNA*, American Society for Microbiology, Washington D.C., 1989.
19. N. L. Craig, "Target Site Selection in Transposition," *Annu. Rev. Biochem.* **65**, 427–474 (1996).
20. D. leCoq, S. Aymerich, and M. Steinmetz, "Dual effect of a transposon Tn917 insertion into the *Bacillus subtilis sacX gene*," *J. Gen. Microbiol.* **137**, 101–106 (1991).
21. R. A. Daniel et al., "Isolation and Characterization of the *lac*A Gene Encoding Beta-Galactosidase in *Bacillus subtilis* and a Regulator Gene, *lac*R," *J. Bacteriol.* **179**, 5636–5638 (1997).
22. J. Brown and V. Sundaresan, "Genetic Study of the Loss and Restoration of Mutator Transposon Activity in Maize: Evidence Against Dominant-Negative Regulator Associated With Loss of Activity," *Genetics* **130**, 889–898 (1992).
23. J. D. Watson, J. Tooze, and D. T. Kurtz, *Recombinant DNA: A Short Course*, W. H. Freeman, New York, 1992
24. T. R. Cech, "The Chemistry of Self-Splicing RNA and RNA Enzymes," *Science* **236**, 1532–1539 (1987).
25. J. C. Wang, "DNA Topoisomerase," *Annu. Rev. Biochem.* **65**, 635–692 (1996).
26. N. J. Proudfoot, "How RNA Polymerase II Terminates Transcription in Higher Eukaryotes," *Trends in Biochemistry* **14**, 105–109 (1989).
27. J. A. Lefstin and K. R. Yamamoto, "Allosteric Effects of DNA on Transcriptional Regulators," *Nature* **392**, 885–888 (1998).

28. J. M. MacPherson and G. G. Khachatourians, "Biotechnology and Genetics: Concepts and Applications," in P. N. Cheremisinoff and L. M. Ferrante, eds., *Biotechnology Current Progress*, Technomic, Lancaster, Pa., 1991, pp. 21–37.
29. P. J. Green, O. Pines, and M. Inouye, "The Role of Antisense RNA in Gene Regulation," *Annu. Rev. Biochem.* **55**, 569–597 (1986).
30. R. Green, and H. F. Noller, "Ribosomes and Translation," *Annu. Rev. Biochem.* **66**, 5679–5716 (1997).
31. D. M. Raineri et al., "*Agrobacterium*-mediated Transformation of Rice (*Oryza sativa* L.)," *Bio/Technology* **8**, 33–37 (1990).
32. J. A. Heinemann and G. F. Sprague, Jr., "Bacterial Conjugative Plasmid Mobilize DNA Transfer Between Bacteria and Yeast," *Nature* **340**, 205–209 (1989).
33. T. Manniatis, E. F. Fritsch, and J. Sambrook, *Molecular Cloning: Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989
34. M. Schleef and P. Heimann, "Cesium Chloride or Column Preparation? An Electron Microscopical View of Plasmid Preparations," *BioTechniques* **14**, 544 (1993).
35. K. B. Mullis et al., "Specific Enzymatic Amplification of DNA *in vitro*: The Polymerase Chain Reaction," *Cold Spring Harbor Symposium Quantitative Biology* **51**, 263–273 (1986).
36. S. J. Scharf, G. T. Horn, and H. A. Erlich, "Direct Cloning and Sequence Analysis of Enzymatically Amplified Genomic Sequences," *Science* **233**, 1076–1080 (1986).
37. J. Alper, "Weighing DNA for Fast Genetic Diagnosis," *Science* **279**, 2044–2045 (1998).
38. P. Ross et al., "High Level Multiplex Genotyping by MALDI-TOF Mass Spectrometry," *Nature Biotechnology* **16**, 1347–1351 (1998).
39. E. Jay, J. Rommens, and G. Jay, "Synthesis of Mammalian Proteins in Bacteria," in P. N. Cheremisinoff and R. P. Ouellete, eds., *Biotechnology Handbook*, Technomic, Lancaster, Pa., 1985, pp. 388–400.
40. G. G. Khachatourians and C. M. S. Berezowsky, "Expression of Recombinant DNA Functional Products in *Escherichia coli* Anucleate Minicells," *Biotechnology Advances* **4**, 75–93 (1986).
41. G. G. Khachatourians, "The Use of Anucleated Minicells in Biotechnology: An Overview," in P. N. Cheremisinoff and R. P. Ouellette, eds., *Biotechnology Handbook*, Technomic Publishing, Lancaster, Pa., 1985, pp. 308–318.
42. G. G. Khachatourians and A. R. McCurdy, "Biotechnology: Applications of Genetics to Food Production," in D. Knorr, ed., *Impact of Biotechnology on Food Production and Processing*," Marcel Dekker, New York, 1987, pp. 1–19.
43. G. G. Khachatourians and D. K. Arora, "Biochemical Identification Techniques—Modern Techniques," in R. K. Robinson, C. A. Batt, and P. Patel, eds., *Encyclopedia of Food Microbiology*, Academic Press, San Diego, Calif., 1999.
44. Y. H. Hui and G. G. Khachatourians, eds., *Food Biotechnology: Microorganisms*, VCH, New York, 1995.
45. Y. H. Hui et al., eds., *The Handbook of Transgenic Food Plants*, Marcel Dekker, New York, 2000.

George G. Khachatourians
Adrienne E. Woytowich
University of Saskatchewan
Saskatoon, Saskatchewan
Canada

GRAINS AND PROTECTANTS

Several insecticides are available for postharvest application to grains in the United States: malathion, chlorpyrifos-methyl (Reldan), pirimiphos-methyl (Actellic), methoprene (Diacon), *Bacillus thuringiensis* (Dipel), synergized pyrethrins, and diatomaceous earth (Table 1). Malathion, Reldan, and Actellic are most the commonly used insecticides; however, Reldan and Actellic can be applied only to specific grains.

Malathion, an organophosphate (OP) insecticide, has been labeled for application to grain since 1957. It can be applied to all major grain commodities in addition to some minor crops (1–4). The labeled rate for application to stored grains is 10.4 ppm and the established Environmental Protection Agency (EPA) tolerance is 8.0 ppm. In addition to its use as a grain protectant, malathion has been widely used in control programs for production agriculture and disease vector control. However, many companies have voluntarily withdrawn their formulations of malathion that were registered on stored grains, and there are doubts as to whether this product will be reregistered. Many insect pests of stored grain have developed resistance to malathion (5–13), which is also affecting decisions regarding registration.

In 1985, almost 30 years after the introduction of OPs as protectants, Reldan (chlorpyrifos-methyl) received a registration for direct application to grain (14). Reldan can be applied to barley, oats, sorghum, rice, and wheat, but not to corn. In addition, Reldan can be used as a prebin spray only if wheat, sorghum, rice, barley, and oats will be stored in the bin. Pirimiphos-methyl (Actellic) was registered in 1986 (1–4). It is labeled for corn and sorghum only and does not have a label for use as a prebin spray.

Acceptable daily intake (ADI) calculations are used by the EPA to regulate nononcogenic compounds. The ADI is an estimate of the daily exposure dose that can be consumed without potential for any harmful effects, and the intake of commodities treated with a particular pesticide is represented as a percentage of the ADI. Although in future actions by the EPA, ADI will be referred to as reference dose (rfd), it was not used at this writing because no EPA documents have used this terminology for pesticide registrations. The total ADI for all registered uses of an active ingredient should not exceed 100%. The calculated ADI for malathion is about 507% for the general population (1–4). In addition, in the most susceptible age group (nonnursing infants aged one to six years) the ADI exceeds 1100%. Malathion has broad use patterns and was registered prior to the EPA's use of ADI as a regulatory tool. Neither Reldan nor Actellic exceed the 100% calculated ADI value.

Synergized pyrethrins have been registered as a protectant for many years but have not been used extensively due to the limited availability and high cost. Synergized pyrethrins have a high acute toxicity to insects but low toxicity to mammals and are generally used as preventative surface applications instead of treatments to the entire grain mass. The base pyrethrum compounds are extracted from chrysanthemum flowers, and while the natural products give quick knockdown, insects often re-

28. J. M. MacPherson and G. G. Khachatourians, "Biotechnology and Genetics: Concepts and Applications," in P. N. Cheremisinoff and L. M. Ferrante, eds., *Biotechnology Current Progress*, Technomic, Lancaster, Pa., 1991, pp. 21–37.
29. P. J. Green, O. Pines, and M. Inouye, "The Role of Antisense RNA in Gene Regulation," *Annu. Rev. Biochem.* **55**, 569–597 (1986).
30. R. Green, and H. F. Noller, "Ribosomes and Translation," *Annu. Rev. Biochem.* **66**, 5679–5716 (1997).
31. D. M. Raineri et al., "*Agrobacterium*-mediated Transformation of Rice (*Oryza sativa* L.)," *Bio/Technology* **8**, 33–37 (1990).
32. J. A. Heinemann and G. F. Sprague, Jr., "Bacterial Conjugative Plasmid Mobilize DNA Transfer Between Bacteria and Yeast," *Nature* **340**, 205–209 (1989).
33. T. Manniatis, E. F. Fritsch, and J. Sambrook, *Molecular Cloning: Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989
34. M. Schleef and P. Heimann, "Cesium Chloride or Column Preparation? An Electron Microscopical View of Plasmid Preparations," *BioTechniques* **14**, 544 (1993).
35. K. B. Mullis et al., "Specific Enzymatic Amplification of DNA *in vitro*: The Polymerase Chain Reaction," *Cold Spring Harbor Symposium Quantitative Biology* **51**, 263–273 (1986).
36. S. J. Scharf, G. T. Horn, and H. A. Erlich, "Direct Cloning and Sequence Analysis of Enzymatically Amplified Genomic Sequences," *Science* **233**, 1076–1080 (1986).
37. J. Alper, "Weighing DNA for Fast Genetic Diagnosis," *Science* **279**, 2044–2045 (1998).
38. P. Ross et al., "High Level Multiplex Genotyping by MALDI-TOF Mass Spectrometry," *Nature Biotechnology* **16**, 1347–1351 (1998).
39. E. Jay, J. Rommens, and G. Jay, "Synthesis of Mammalian Proteins in Bacteria," in P. N. Cheremisinoff and R. P. Ouellete, eds., *Biotechnology Handbook*, Technomic, Lancaster, Pa., 1985, pp. 388–400.
40. G. G. Khachatourians and C. M. S. Berezowsky, "Expression of Recombinant DNA Functional Products in *Escherichia coli* Anucleate Minicells," *Biotechnology Advances* **4**, 75–93 (1986).
41. G. G. Khachatourians, "The Use of Anucleated Minicells in Biotechnology: An Overview," in P. N. Cheremisinoff and R. P. Ouellette, eds., *Biotechnology Handbook*, Technomic Publishing, Lancaster, Pa., 1985, pp. 308–318.
42. G. G. Khachatourians and A. R. McCurdy, "Biotechnology: Applications of Genetics to Food Production," in D. Knorr, ed., *Impact of Biotechnology on Food Production and Processing*," Marcel Dekker, New York, 1987, pp. 1–19.
43. G. G. Khachatourians and D. K. Arora, "Biochemical Identification Techniques—Modern Techniques," in R. K. Robinson, C. A. Batt, and P. Patel, eds., *Encyclopedia of Food Microbiology*, Academic Press, San Diego, Calif., 1999.
44. Y. H. Hui and G. G. Khachatourians, eds., *Food Biotechnology: Microorganisms*, VCH, New York, 1995.
45. Y. H. Hui et al., eds., *The Handbook of Transgenic Food Plants*, Marcel Dekker, New York, 2000.

George G. Khachatourians
Adrienne E. Woytowich
University of Saskatchewan
Saskatoon, Saskatchewan
Canada

GRAINS AND PROTECTANTS

Several insecticides are available for postharvest application to grains in the United States: malathion, chlorpyrifos-methyl (Reldan), pirimiphos-methyl (Actellic), methoprene (Diacon), *Bacillus thuringiensis* (Dipel), synergized pyrethrins, and diatomaceous earth (Table 1). Malathion, Reldan, and Actellic are most the commonly used insecticides; however, Reldan and Actellic can be applied only to specific grains.

Malathion, an organophosphate (OP) insecticide, has been labeled for application to grain since 1957. It can be applied to all major grain commodities in addition to some minor crops (1–4). The labeled rate for application to stored grains is 10.4 ppm and the established Environmental Protection Agency (EPA) tolerance is 8.0 ppm. In addition to its use as a grain protectant, malathion has been widely used in control programs for production agriculture and disease vector control. However, many companies have voluntarily withdrawn their formulations of malathion that were registered on stored grains, and there are doubts as to whether this product will be reregistered. Many insect pests of stored grain have developed resistance to malathion (5–13), which is also affecting decisions regarding registration.

In 1985, almost 30 years after the introduction of OPs as protectants, Reldan (chlorpyrifos-methyl) received a registration for direct application to grain (14). Reldan can be applied to barley, oats, sorghum, rice, and wheat, but not to corn. In addition, Reldan can be used as a prebin spray only if wheat, sorghum, rice, barley, and oats will be stored in the bin. Pirimiphos-methyl (Actellic) was registered in 1986 (1–4). It is labeled for corn and sorghum only and does not have a label for use as a prebin spray.

Acceptable daily intake (ADI) calculations are used by the EPA to regulate nononcogenic compounds. The ADI is an estimate of the daily exposure dose that can be consumed without potential for any harmful effects, and the intake of commodities treated with a particular pesticide is represented as a percentage of the ADI. Although in future actions by the EPA, ADI will be referred to as reference dose (rfd), it was not used at this writing because no EPA documents have used this terminology for pesticide registrations. The total ADI for all registered uses of an active ingredient should not exceed 100%. The calculated ADI for malathion is about 507% for the general population (1–4). In addition, in the most susceptible age group (nonnursing infants aged one to six years) the ADI exceeds 1100%. Malathion has broad use patterns and was registered prior to the EPA's use of ADI as a regulatory tool. Neither Reldan nor Actellic exceed the 100% calculated ADI value.

Synergized pyrethrins have been registered as a protectant for many years but have not been used extensively due to the limited availability and high cost. Synergized pyrethrins have a high acute toxicity to insects but low toxicity to mammals and are generally used as preventative surface applications instead of treatments to the entire grain mass. The base pyrethrum compounds are extracted from chrysanthemum flowers, and while the natural products give quick knockdown, insects often re-

Table 1. Protectants and Grain Surface Treatments, United States, Rar Agricultural Commodities

Product	Crops	Label rate (ppm)	Tolerance (ppm)	% ADI[a]
Malathion	Barley, corn, oats, wheat, sorghum, rice, rye, sunflowers, and almonds	10.4	8.0	507
Chlorpyriros-methyl (Reldan)	Wheat, milo (sorghum), rice, barley, and oats	6.0	6.0	<100
Pirimiphos-methyl (Actellic)	Corn and sorghum (milo)	6.0–8.0	8.0	<100
Methoprene (Diacon)	Corn, wheat, sorghum (milo), barley, rice, oats, and peanuts	5.0	5.0	<100
Bacillus thuringiensis (Dipel)	Grain, soybean, seed, popcorn, birdseed, herbs and spices	3.17	Exempt	N/A
Synergized pyrethrins (pyrethrins + piperonyl butoxide)	Wheat, corn, rye, and sorghum (milo)	3–20	3–20	<100
Diatomaceous earth	Grains	N/A	N/A	N/A

[a] ADI, the average daily intake, is an estimate of the daily exposure dose to a pesticide, and the intake of commodities treated with a pesticide is represented as a percentage of the ADI.

cover from this initial knockdown (15). Addition of the synergist piperonyl butoxide increases the toxicity of the compounds.

Diatomaceous earth is a silicaceous earth composed of the cell walls of diatoms. This insecticide kills insects by removing the waterproof layer, or exoskeleton, of the insect, causing a continuous loss of lipids from the exoskeleton (16). It is available in dust formulations, and many older products were physically irritating to exposed workers and were suspected of causing pulmonary fibrosis. Many new formulations have been registered in recent years that are not as irritating to humans as the older compounds.

A biological compound, Dipel (*B. thuringiensis*), was registered for use on grain and other stored commodities in 1977. *B. thuringiensis* is a naturally occurring pathogen isolated from insects and is exempt from an EPA tolerance and ADI consideration (1–4). The current application rate calculates to 3.17 ppm with respect to traditional pesticide tolerances, which equals 46.02 billion International Units of *B. theringiensis* per million pounds of grain. The grain surface application is for Lepidopteran pests (eg, Indianmeal moth, *Plodia interpunctella*, Angoumois grain moth, *Sitotroga cerealla*, Mediterranean flour moth, *Ephestia kuehiniella*), and almond moth (Cuda cautella) because this is the only group of grain pests affected by *B. thuringinensis*. Indianmeal moths can develop resistance to *B. thuringiensis* (17).

Diacon (methoprene) is an insect growth regulator (IGR) that was registered in 1988 (1–4). It affects insects by interrupting the molting process. Diacon has a low mammalian toxicity and a tolerance of 5 ppm on all of the major grain crops as well as uses in vector and household insect control. As indicated, Diacon affects insect molting and egg hatch in some species (Fig. 1). This product does not eliminate existing adults, but has a secondary effect on the F_1 generation by limiting population explosions of most stored grain insects. The rice weevil, *Sitophilus oryzae*, granary weevil, *Sitophilus granarius*, and lesser grain borer, *Rhyzopertha dominica*, are difficult to control with methoprene because the larvae develop inside the grain kernel. In countries other than the United States, additional compounds labeled as grain protectants include resmethrin, bromophos, fenitrothion, bioresmethrin, deltamethrin, and permethrin (18).

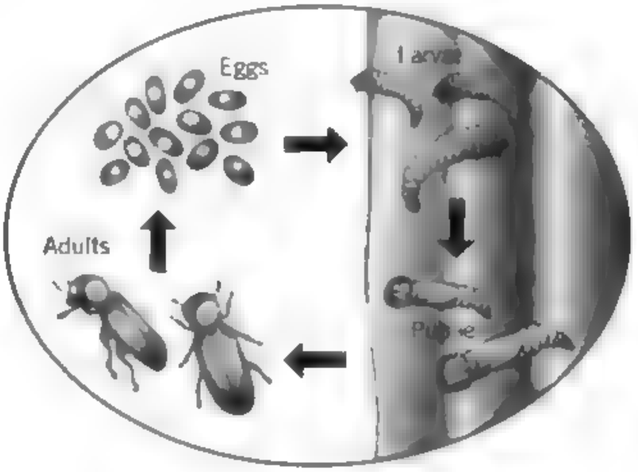


Figure 1. Effects of insect growth regulators (IGRs) on stored product insects (effected stages shown in shaded areas).

In addition to those protectants and grain surface treatments labeled in the United States, several chemicals are labeled for application to grain storage structures (19). Methoxychlor is a chlorinated hydrocarbon that has been registered for more than 30 years. As mentioned earlier, Reldan can be used as a prebinning treatment if wheat, oats, sorghum, rice and barley will be stored in the bin. Actellic is not labeled as a prebinning spray. Cyfluthrin (Tempo) is a pyrethroid insecticide that can be used as a residual spray treatment for all storage structures, including grain bins.

Currently, protectants formulated for direct application to grain are emulsifiable concentrates (EC) or ready to apply as dry material. The ECs are diluted in water or FDA-approved mineral or soybean oil. Liquid solutions are applied through gravity-flow or pressurized systems. In commercial facilities pressurized pump systems are used to treat from 30 t/h to 60,000 t/h (1,000 to 200,000 bu/h) (Fig. 2). The dry formulations are metered into the auger with mechanical applicators or dispersed on the grain surface, or cut into the grain with a scoop before unloading from a truck. As the grain is augured to the bin, distribution throughout the grain is adequate for intended protection. Only one application of a protectant is recommended

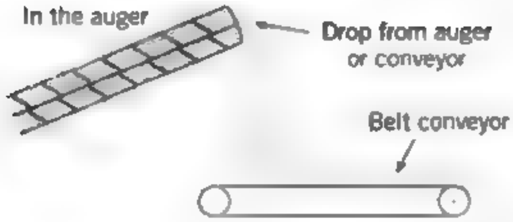
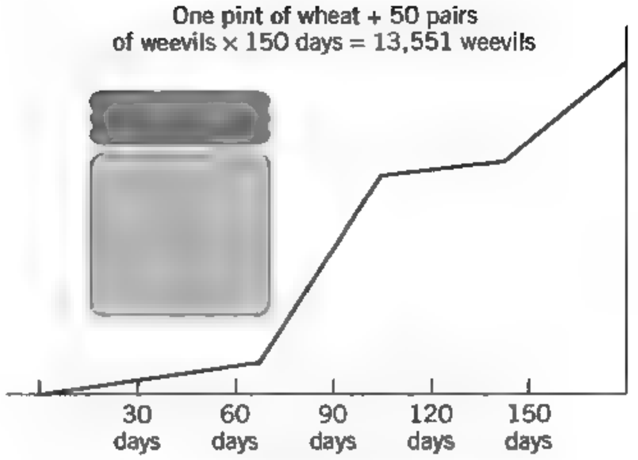


Figure 2. Suggested points at which chemical should be applied.



Number of offspring 5 months after
50 pairs of rice weevils were placed in wheat.

Grain moisture	60°	80°
10%	0	326
12%	58	9,661
14%	951	13,551

Figure 3. Number of offspring five months after 50 pairs of rice weevils were placed in wheat. *Source:* Ref. 21.

to ensure that residues remain below allowable tolerances. Protectants should be applied as the grain is loaded into the bin (Fig. 2). Organophosphate insecticides degrade more rapidly as temperature and moisture content increase (20), but evaluations for protectant registrations are made at the full application rate, which helps minimize the risks of exposure associated with protectant insecticides.

To illustrate the challenge of maintaining grain quality, a study on rice weevils (Fig. 3) shows how 50 adults can multiply to more than 13,551 adults in five months (21). Low moisture has a negative effect on weevil populations. The reproductive potential of several important species is shown in Table 2 (22). Studies conducted at the Gustafson Seed and Grain Technology Laboratory (Fig. 4) show that the percentage of insect-damaged kernels (IDK) is directly related to the insect population-to-grain ratio. The percentage of IDK was 18 and 97% with 2 and 50 insects per 200 g of wheat, respectively.

In May 1988, revised Federal Grain Inspection Service (FGIS) grading standards went into effect (23). These standards were more stringent; in wheat, rye, and triticale, one live weevil or lesser grain borer and one or more other live insects (OLI) injurious to stored grain in a 1-kg sample can cause that load of grain to be graded infested (Table 3). In corn, barley, oats, sorghum, soybeans, sunflower seed, or mixed grain, the infested grade is assigned when two or more live weevils or lesser grain borers, or one live weevil or lesser grain borer and five or more OLI injurious to stored grain, or ten or more OLI injurious to stored grain (Table 3).

An additional FGIS revision established an IDK limit of 32 damaged kernels per 100 g of wheat. Prior to the FGIS revisions when IDK was not included as part of the grade, a load of wheat could have significant damage and still have graded U.S. #1. Under the new standards, if a sample (100 g) reveals 32 or more IDK, the load is graded U.S. Sample Grade wheat. The grading agency then notifies the FDA and the load or lot is declared unfit for human consumption, forcing it to be marketed as feed grain or for alcohol production. This market can be from $0.20 to $1.50 per bushel less than food-grade wheat.

Given the reproductive potential of insects, the complex life cycles, and the fact that several different species of insects are capable of damaging stored grain, management plans that utilize multiple control strategies are used for stored grains. Many programs include but are not limited to the following:

1. Emphasis on maintaining high grain quality standards (recent FGIS changes have increased the awareness of the need for better management)
2. Sanitation and cleaning around storage facilities to prevent insects from establishing resident populations

Table 2. Stored-Grain Insects—Life Cycles and Characteristics

Insect	Life cycle (days), egg to egg	Adult life (days)	Number of eggs	Penetrate packages	Fly
Confused flour beetle	40	365	450	Yes (weak)	No
Flat grain beetle	45	365	240	Yes (weak)	Yes
Lesser grain borer	30	180	300–500	Yes	Yes
Rice weevil	30	90–185	300–400	Yes (weak)	Yes
Granary weevil	30–50	210–240	30–50	Yes (weak)	No
Sawtoothed grain beetle	20–80	135–300	280	Yes (weak)	No

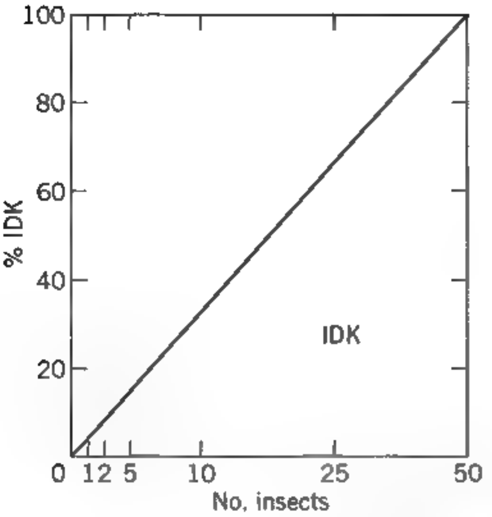


Figure 4. Relationship between numbers of rice weevils and percentage of insect-damaged kernels (IDK).

Table 3. Insect Infestation Tolerance Comparison

Grain	Insect standard
Wheat, rye, triticale	One live weevil[a] and one or more other live insects[b]
Corn, sorghum, soybeans, barley, oats, sunflowers, mixed grain	Two or more live weevils,[a] or one weevil and five or more other live insects,[b] or ten or more other live insects

Note: This table illustrates the number of live insects that, if detected in a 1-kg sample, will result in an infested grade under the FGIS insect-infestation standards.

[a] Weevils are rice, granary, maize, and cowpea weevils, as well as the lesser grain borer.

[b] Other live insects include the grain and flour beetles, moths, and vetch bruchids.

3. Application of grain protectants
4. The use of aeration to cool the grain mass at selected times during storage
5. Regular inspection and monitoring of the grain
6. Control of insects with fumigants if populations become excessive

Insect attractants (pheromones) and new methods of trapping insects have been developed to detect insects in stored grain (24). Traps are more efficient than traditional grain trier and sieve monitoring methods because they often can detect insects that would otherwise go undetected. There has been increased interest in recent years in new programs to sample insects with traps and to use this information in control programs. The grain storage industry is becoming increasingly more involved in Integrated Pest Management (IPM) programs by taking steps to manage insect populations, instead of reacting after large populations are detected and IDK are present.

BIBLIOGRAPHY

1. Environmental Protection Agency, "*Bacillus thuringiensis Berliner*, Viable Spores for Residues," *U.S. Code of Federal Regulations*, section 40, Part 180.1011, 1988, p. 456.
2. Environmental Protection Agency, "Established Tolerances for Residue of the Insecticide Methoprene," *Federal Register* **53**, 27391–27392 (1988).
3. Environmental Protection Agency, "Guidance for the Reregistration of Pesticide Products Containing Malathion as the Active Ingredient," Case-248, p. 315; "Malathion Tolerances for Residues," *U.S. Code of Federal Regulations*, Section 40, Part 180.111, 1988 pp. 33–335.
4. Environmental Protection Agency, "Pirimiphos-methyl, Final Rule," *Federal Register* **51**, 28223–28229 (1987).
5. P. C. Bansode and W. V. Campbell, "Evaluation of North Carolina Field Strains of the Red Flour Beetle for Resistance to Malathion and Other Organophosphorus Compounds," *Journal of Economic Entomology* **72**, 331–333 (1979).
6. R. W. Beeman, W. E. Speirs, and B. A. Schmidt, "Malathion Resistance in Indianmeal Moths Infesting Stored Corn and Wheat in the Northcentral United States," *Journal of Economic Entomology* **75**, 950–954 (1982).
7. J. L. Zettler, "Pesticide Resistance in *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae) From Flour Mills in the United States," *Journal of Economic Entomology* **84**, 763–767 (1991).
8. J. L. Zettler and G. W. Cuperus, "Pesticide Resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Tenebrionidae) in Wheat," *Journal of Economic Entomology* **83**, 1677–1681 (1990).
9. F. H. Arthur, J. L. Zettler, and W. R. Halliday, "Insecticide Resistance Among Populations of Almond Moth and Indianmeal Moth in Stored Peanuts," *Journal of Economic Entomology* **81**, 1283–1287 (1988).
10. C. L. Storey, D. B. Sauer, and D. Walker, "Insects and Fungi in Wheat, Corn and Oats Stored on the Farm," *Journal of Economic Entomology* **77**, 784–788 (1984).
11. B. H. Subramanyam and P. K. Harein, "Status of Malathion and Pirimiphosmethyl Resistance in Adults of Red Flour Beetle and Saw-toothed Grain Beetle Infesting Farm-Stored Corn in Minnesota," *Journal of Agricultural Entomology* **7**, 127–136 (1990).
12. B. H. Subranamyam and D. W. Hagstrum, "Resistance Measurement and Management," in B. H. Subramanyam and D. W. Hagstrum, eds., *Integrated Management of Insects in Stored Products*, Marcel Decker, New York, 1996, pp. 331–398.
13. J. L. Zettler, "Insecticide Resistance in Selected Stored-Product Insects Infesting Peanuts in the Southeastern United States," *Journal of Economic Entomology* **75**, 359–362 (1982).
14. Environmental Protection Agency, "Chlorpyrifos-Methyl Tolerances for Residues," *U.S. Code of Federal Regulations*, Section 40, Part 180.419, pp. 426–427.
15. R. D. Obrien, *Insecticide Actions and Metabolism; Pyrethroids*, Academic Press, Orlando, Fla., 1967, pp. 168–171.
16. P. Golub, "Current Status and Future Perspectives for Inert Dusts for Control of Stored Product Insects," *Journal of Stored Product Research* **33**, 69–79 (1997).
17. W. H. McGaughey and D. E. Johnson, "Indianmeal moth (*Lepidoptera: Pyralidae*) Resistance to Different Strains and Mixtures of *Bacillus thringiensis*," *Journal of Economic Entomology* **85**, 1594–1600 (1992).

18. N. D. G. White and J. G. Leesch, "Chemical Control," "Resistance Measurement and Management," in B. H. Subramanyam and D. W. Hagstrum, eds., *Integrated Management of Insects in Stored Products*, Marcel Decker, New York, 1996, pp. 287–330.

19. J. G. Touhey, "Future Development and Availability of Pesticides for Grain: How Pesticides are Registered by EPA," *UDAGIS Grain Insect Interagency Task Force Meeting*, Washington, D.C., July 21, 1988.

20. F. H. Arthur, J. E. Throne, and R. A. Simonaitis, "Degradation and Biological Efficacy of Chlorpyrifos-Methyl on Wheat Stored at Five Temperatures and Three Moisture Contents," *Journal of Economic Entomology* **85**, 1994–2002 (1982).

21. R. A. Higgins, *Management of Stored Grain Insects, Part 1*, Kansas State University MF-726, Manhattan, Kans., 1984.

22. F. J. Bauer, *Insect Management for Food Storage and Processing*, American Association of Cereal Chemists, Minneapolis, Minn., 1984, pp. 54–67.

23. U.S. Department of Agriculture, Federal Grain Inspection Service, "Grain Standards: Official U.S. Standards, Handling Practices and Insect Infestation, Final Rules," *Federal Register* **52**, 2424–2442 (1988).

24. T. W. Phillips, "Semiochemicals of Stored-Product Insects: Research and Applications," *Journal of Stored Product Research* **33**, 17–30 (1997).

J. TERRY PITTS
Gustafson, Inc.
Plano, Texas
FRANK H. ARTHUR
Agricultural Research Service, United States Department of Agriculture
Manhattan, Kansas

GRAPEFRUIT. See FRUITS: SEMI-TROPICAL.

GUMS

Food gums are water-soluble or water-dispersable polysaccharides (glycans) and their derivatives (1–6) and gelatin (3) (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). In general, they thicken or gel aqueous systems at low concentration. Although starches, flours, and modified food starches are polysaccharide materials and have similar properties, they are usually considered separately as they are in this encyclopedia (see the article STARCH). Polysaccharide food gums can be classified by source (Table 1) or by structure (Table 2).

The usefulness of gums is based on their physical properties, in particular their capacity to thicken and/or gel aqueous systems and otherwise to control water. Because all gums modify the flow of aqueous solutions, dispersions, and suspensions, the choice of which gum to use for a particular application often depends on its other characteristics, characteristics that are responsible for their utilization as binders, bodying agents, bulking agents, crystallization inhibitors, clarifying agents, cloud agents, emulsifying agents, emulsion stabilizers, encapsulating agents, film formers, flocculating agents, foam stabilizers, gelling materials, mold release agents, protective colloids, suspending agents, suspension stabilizers, swelling agents, syneresis inhibitors, texturing agents, and whipping agents, in coatings, and for water absorption and binding. Most gums are not true emulsification agents but stabilize emulsions and suspensions by increasing the viscosity and the yield value of the aqueous phase. Some also exhibit protective colloid action.

Table 1. Classification of Polysaccharide Food Gums by Source

Class	Examples
Seed gums	Guar gum, locust bean gum, tara gum
Tuber and root gums	Konjac mannan
Seawood extracts	Algins, carrageenans, agar, furcellaran
Plant extracts	Pectins
Exudate gums	Gum arabics, gum tragacanth
Fermentation/microbial gums	Xanthan gums, gellan gums, curdlan
Derived gums	CMCs, hydroxypropylcelluloses, HPMCs, MCs

Food gums are tasteless, odorless, colorless, and nontoxic. All (except the starches, starch derivatives, and gelatin) are essentially noncaloric and are classified as soluble dietary fiber (see the article FIBER, DIETARY).

Gums, in general, do not form true solutions because of their large molecular size and intermolecular interactions. Hence, the term hydrocolloid is often used interchangeably with gum. The rheology (flow characteristics and gel properties) of gum solutions is a function of the size, shape, flexibility, solvation, and ease of deformation of particles, and the presence and magnitude of charges on them. Particles may be hydrated molecules and/or molecular aggregates. Many gum solutions exhibit shear thinning; that is, they are pseudoplastic, occasionally thixotropic. The variables that affect their rheology are polymer structure, molecular weight, concentration, shear rate, temperature, pH, and the concentration of salts, other solutes, and sequestrants. Factors that affect dispersion and dissolution are pH, presence of salts, presence of other solutes, gum type, particle size, physical form of particles, shear rate, and method of dispersion (mixing efficiency).

Most gums are available in a range of viscosity grades, which are produced by depolymerization (reducing the molecular weight) of the native polysaccharide. If viscosity is the goal, a high-viscosity grade at low solids concentration is used; if binding or protective colloid action, for example, is the goal, a low-viscosity grade at high solids concentration is used.

In general, gels made with food gums are composed of 95.0 to 99.5% water and 0.5 to 5.0% gum. Important characteristics of gels are means of gelation (chemical gelation, thermal gelation), reversibility, texture (brittle, elastic, plastic), rigidity (rigid or firm, soft or mushy), tendency for syneresis, and whether they are cutable or spreadable.

Gums, excluding native and modified starches, that are used in foods in the United States are described later. Not included is curdlan, which was only recently introduced in the United States, inulin, or the seldom used gum ghatti and gum karaya. Uses of curdlan and inulin are being ex-

Table 2. Classification of Polysaccharidic Food Gums by Structure

Classification schemes	Examples
By shape	
Linear	Algins, carrageenans, cellulosics, furcellaran, konjac mannan, pectins
Branched	
Short branches on a linear backbone	Galactomannans (guar gum, locust bean gum, tara gum, enzymically modified guar gum), xanthan gums
Branch-on-branch structures	Gum arabics, gum tragacanth (tragacathin)
By monomeric units[a]	
Homoglycans	Cellulosics, curdlan
Diheteroglycans	Agarose, algins, carrageenans, furcellaran, galactomannans, konjac mannan, pectins
Triheteroglucans	Gellan gums, xanthan gums
Tetraheteroglycans	Gum arabics
Pentaheteroglycans	Gum tragacanth (tragacanthin)
By charge	
Neutral	Agarose, HPMCs, MCs, galactomannans, konjac mannan
Anionic (acidic)[b]	Algins, CMCs, carrageenans, furcellaran, gellan gums, gum arabics, gum tragacanth (tragacanthin), pectins, xanthan gum

[a] Considers only the basic monosaccharide units. A derivatized monosaccharide unit, such as D-galactopyranosyl 6-sulfate unit, is not considered as a unit separate from a D-galactopyranosyl unit, for example.

[b] From the presence of uronic acid, sulfate half-ester, or pyruvyl cyclic acetal groups.

plored. Also not included are the polysaccharides that occur naturally in many foodstuffs, that may be extracted during processing, and that impart the same functionalities as gums added as ingredients; examples of such polysaccharides are the arabinoxylans (pentosans) of cereal flours, xyloglucans of many cell walls, arabinogalactans and arabinogalactan-proteins of legumes, and psyllium seed gum. For β-glucan, which has the properties of a gum, but which is not isolated for commercial use, see the article FIBER, DIETARY.

ALGINS

Algins (alginates) (2,4–6) are extracted from brown algae (*Phaeophyceae*). Alginates are salts (generally sodium) and the propylene glycol ester of alginic acid. Alginic acid is a generic term for polymers of D-mannuronic acid and L-guluronic acid (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). In alginic acid molecules, at least three different types of polymer segments exist: poly(β-D-mannopyranosyluronic acid) segments, poly(α-L-gulopyranosyluronic acid) segments, and segments with alternating sugar units. Ratios of the constituent monomers and ratios of chain segments vary with the source. Alginates are linear polymers. The degree of polymerization (molecular weight) is controlled and varied in commercial products.

Specific properties of algins depend on the percentage of each type of building block. An important and useful property of sodium, potassium, and ammonium alginates is their ability to form gels on reaction with calcium ions. Different types of gels are formed with alginates from different sources. Alginates with a higher percentage of poly(guluronic acid) segments form gels more readily and form the more rigid, more brittle gels that tend to undergo syneresis. Alginates with a higher percentage of poly(mannuronic acid) segments form the more elastic, more deformable gels that have a lesser tendency to undergo syneresis.

Algins are most often used as thickeners and are available in a range of viscosity grades. Algin solutions are pseudoplastic, that is, exhibit shear thinning. Solutions of propylene glycol alginate (PGA, a partial propylene glycol ester) are somewhat thixotropic and much less sensitive to pH and polyvalent cations. The specific properties exhibited by an algin solution depend on the ratio of monomeric units, the concentration and type of cations in solution, the temperature, and the degree of polymerization.

Primary products using sodium alginate are breakfast and cereal bars, fruit fillings, dry mixes for reconstitution with water or milk, and frozen products. Calcium alginate gels are found in structured foods, such as fruit pieces, onion rings, and pimiento strips for Spanish olives. Primary products in which PGA (which can be labeled algin derivative) is used are pourable salad dressings, buttered pancake syrups, sauces, and beverages. It is also used as a beer foam stabilizer. Alginic acid, which swells in water, is used as a tablet disintegrant.

CARBOXYMETHYLCELLULOSES

Carboxymethylcellulose (CMC) (2,4–6) is the sodium salt of the carboxymethyl ether of cellulose (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). In an ingredient label, it can be designated cellulose gum, CMC, sodium CMC, sodium carboxymethyl cellulose, or carboxymethyl cellulose.

Modified polysaccharides, such as CMC, have a degree of substitution (DS). There are, on average, three hydroxyl groups per polysaccharide hexopyranosyl (six-carbon-atom sugar) unit. In the case of cellulose, each β-D-glucopyranosyl unit has three hydroxyl groups (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). The average number of hydroxyl groups derivatized (with a carboxymethyl ether group in the case of CMCs) per monomeric unit is the DS; the maximum DS of a cellulose derivative is 3.0.

Table 3. Some Properties of Food Gums

	Solubility						
Gum	RT[a] water or milk	Hot water or milk	Thickener[b]	Gel former[c]	Surface tension reduction	Emulsion/ suspension stabilizer	Interactions and synergisms
Agar		+		+			LBG, konjac mannan
Algins							
Na$^+$ alginate	+	+	+	Ca^{2+}			Proteins
PGA	+	+	+		+	+	
Cellulosics							
CMCs	+	+	+			+ (certain types)	Proteins, guar gum (weak)
MCs	+		+	Heat	+	+	Xanthan gums
HPMCs	+		+	Heat	+	+	Xanthan gums
Carrageenans							
κ-type	Na$^+$ only	+		K$^+$, Ca^{2+}, LBG			Milk proteins, LBG, konjac mannan, xanthan gums, guar gum (weak)
ι-type	Na$^+$ only	+		Ca^{2+}			Milk proteins, LBG, starches
λ-type	+	+					Milk proteins, starches (weak)
Furcellaran	+		+				LBG, guar gum (slight)
Galactomannans							
Guar gum	+	+	+				Xanthan, κ-carrageenan, furcellaran, CMC (all weak)
LBG	Partial	+	+	Xanthan gums, κ-carrageenan			Xanthan gums, κ-carrageenan, ι-carrageenan, agar, furcellaran
Gelatin		+		+	+	+	Agar, algins, carrageenans, gum arabics, LM pectins
Gellan gums		+		Cations			
Gum arabics	+	+		+	+		
Gum tragacanth	+	+	+		+	+	
Konjac mannan	Partial	+	+	OH$^-$, κ-carrageenan			Agar, κ-carrageenan, xanthan gums, starches
Pectins							
HM		+		H$^+$, sugar			
LM		+		Ca^{2+}			
Amidated LM		+		Ca^{2+}			
Xanthan gum	+	+	+	LBG		+	LBG, konjac mannan, κ-carrageenan, MCs, HPMCs, guar gum (weak)

Note: Blanks are negative indicators.
[a]RT = room temperature
[b]Commonly used to thicken.
[c]Used to form gels. Listing of another ingredient or ion signifies a requirement for gelation.

Solution characteristics of CMCs are primarily determined by the DS, the average chain length/degree of polymerization (DP), and the uniformity of substitution. The most widely used types have a DS of 0.7 or an average of 7 carboxymethyl ether groups per 10 β-D-glucopyranosyl units (Fig. 1). Many viscosity grades of CMC are manufactured.

CMCs hydrate rapidly and form clear, stable solutions. Viscosity building is their most important property. Solutions can be either pseudoplastic or thixotropic, depending on the type of product used, but are most often pseudoplastic. Like solutions of hydroxypropylcelluloses, meth-

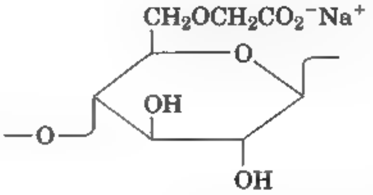


Figure 1. A representative unit of sodium carboxymethylcellulose, that is, a representative β-D-glucopyranosyl unit containing a carboxymethyl ether group.

ylcelluloses, and hydroxypropylmethylcelluloses, CMC solutions are Newtonian at low shear rates and become pseudoplastic as the shear rate is increased. The shear rate at which solutions change from being Newtonian to being pseudoplastic increases with increasing molecular weight and decreases with increasing concentration. Also, the higher-molecular-weight products are more affected by shear. Solutions are stable over a wide pH range (pH 4–10).

CMCs help to solubilize various proteins and to stabilize their solutions. They have a synergistic viscosity building effect when used with casein and soy protein.

Examples of CMC use are as a retarder of ice crystal growth in ice creams and related products; as a physiologically inert and noncaloric thickening and bodying agent in dietetic foods; in cake and related mixes to hold moisture, enhance organoleptic properties, and extend shelf life; as an extrusion aid; in batters for viscosity control, adhesion, and suspension stabilization; in icings, frostings, and glazes to hold moisture and retard sugar crystallization; as a thickener in pancake syrup; as a suspending agent and suspension stabilizer in hot cocoa and fruit drink mixes; as a water binder in slightly moist pet foods; as a casein stabilizer in dips and sour cream; and in frozen poultry sticks and nuggets.

CARRAGEENANS, AGAR, AND FURCELLARAN

Carrageenan is a generic term applied to polysaccharides extracted from a number of closely related species of red seaweeds (2,4–6). Agar and furcellaran are also red seaweed extracts and members of the same larger family. All polysaccharides in this family are derivatives of linear galactans, and all have alternating monosaccharide units and linkages. In agar, the monosaccharide units are D-galactopyranosyl and 3,6-anhydro-L-galactopyranosyl units. In κ-carrageenan and ι-carrageenan, they are D-galactopyranosyl and 3,6-anhydro-D-galactopyranosyl units. λ-Carrageenan contains only D-galactopyranosyl units (Fig. 2). Agar contains little or no sulfate half-ester groups. Other members of the family are sulfated: in increasing order, κ-carrageenan (~25%), ι-carrageenan (~30%), and λ-carrageenan (~35%). Each polymer type is heterogeneous; that is, none contains an exact repeating unit structure. Agar is the least soluble of this family of polysaccharides and λ-carrageenan the most soluble. Agar forms the strongest gels. λ-Carrageenan does not gel.

Commercial carrageenans are composed primarily of three types of polymer: κ-, ι-, and λ-carrageenans. The composition of an extract of a carrageenophyte with respect to each of the three types of polymers and its properties depends on the species collected, growth conditions, and treatment during production. Products are blended and standardized with respect to any of several properties.

As pH values decrease below 6, carrageenan solutions become increasingly unstable when heated. The loss of viscosity is due to polymer chain cleavage and, hence, is irreversible.

Carrageenans form complexes with proteins. Carrageenans are used extensively as gelling, thickening, and suspending agents in milk-based products. Preparations are blended to provide products that will form a variety of gels: clear gels and turbid gels, rigid gels and elastic gels, tough gels and tender gels, heat-stable gels and thermally reversible gels, gels that undergo syneresis and gels that do not. Carrageenan gels do not require refrigeration because they do not melt at room temperature.

Carrageenans react with milk proteins. The thickening effect of a κ-type carrageenan in milk is 5 to 10 times greater than it is in water; at a concentration of 0.025% in milk, a weak thixotropic gel is formed via interaction of κ-carrageenan with κ-casein micelles. This property finds use in the preparation of chocolate milk (to keep the cocoa suspended) and ice cream (to prevent whey-off). Other (stabilizing) interactions are important in producing evaporated milk, infant formulas, and whipped cream required to be freeze–thaw stable.

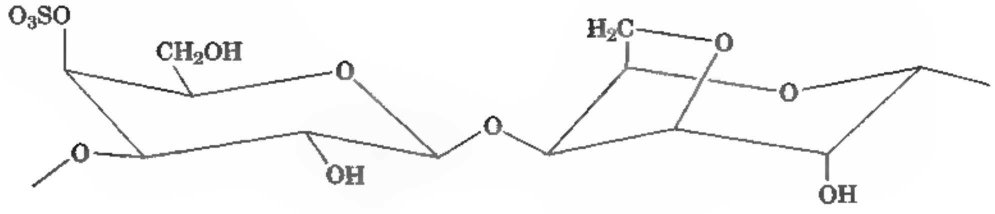
There is a synergistic effect between κ-carrageenan and locust bean gum; the two gums together produce a much more elastic gel with markedly greater gel strength and less syneresis. Such gels find use in canned pet foods, fruit gels, and processed meats and seafoods. The κ-carrageenan–locust bean gum mixture is also used to provide body and fruit suspension in yogurt, to stabilize low-fat yogurt, and in other dairy products such as cheese spreads, cottage and cream cheese, dips, ice creams, other frozen desserts and novelties, and whipped toppings.

ι-Type carrageenans form elastic, syneresis-free, thermally reversible gels that are stable to repeated freeze–thaw cycling. They are used in ready-to-eat milk gels. Blending is a common practice, and blends of κ- and ι-type carrageenans are used to prepare water dessert gels (where refrigeration is unavailable), whipped toppings, instant whipped desserts, and eggless custards and flans. ι-Type carrageenans exhibit a synergistic interaction with starches.

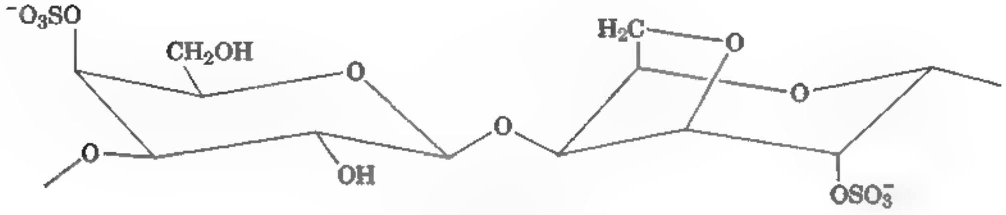
λ-Type carrageenans are nongelling and are used as emulsion stabilizers in such products as whipped cream, instant breakfast drinks, milkshakes, nondairy coffee creamers, and dry mix sauce systems.

Agar (agar–agar) is the least soluble of this class of polysaccharides. Agar is composed of a gelling polysaccharide, agarose, and a nongelling polysaccharide, agaropectin. Agar can be dispersed only at temperatures above 100°C (212°F). When its dispersions are cooled, strong, brittle, turbid gels form. Agar gels remelt when heated, undergo syneresis, and are unstable to freeze–thaw cycles. Agar is used primarily in bakery icings, glazes, and (in Asia) dessert gels. A form of agar that dissolves at lower temperature (about 65°C/150°F) is made by drying agar with sugars/or maltodextrins, producing a so-called agglomerated form.

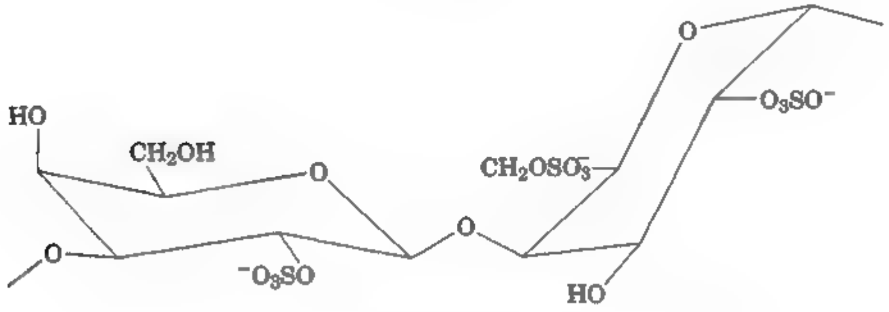
Furcellaran (Danish agar) is a less-sulfated κ-type carrageenan, placing it between agar and κ-carrageenan in properties. It is not always considered as a separate entity, often being included in the carrageenan family. Furcellaran is used to prepare milk-based puddings, flan and related jellies, and low-sugar jams and other fruit preserves and elsewhere where κ-type carrageenans might also be used.



κ-Carrageenan



ι-Carrageenan



λ-Carrageenan

Figure 2. Idealized structures of the three types of carrageenan.

GELATIN

Gelatin is a protein rather than a polysaccharide. It is produced by acid (type A) or alkali (type B) treatment of collagen obtained from pig skin, cattle hides, and bones. While gelatin contains 18 different amino acids, about 95% of the structure is composed of 7. The amino acid sequence of most of the chain is a tripeptide repeating unit of (glycine)–(proline or hydroxyproline)–(alanine, glutamic acid, aspartic acid, or arginine). Gelatin contains no tryptophan.

The important functional properties in food applications of gelatin (3) are gel strength, gelling temperature, setting rate, pH, and isoelectric pH. Typical values for some of these properties are as follows:

Type	Gel strength, g Bloom	pH	Isoelectric pH
A	75–300	4.5–6.0	8.0–9.5
B	75–275	5.0–7.5	4.7–5.4

The presence of ash, sulfur dioxide, and peroxides can affect product appearance, that is, can cause gel cloudiness and/or discoloration, but do not affect functional properties. The gelling temperature of gelatin solutions is related to concentration. Below pH 5, types A and B gelatins have a net positive charge and will interact with negatively charged polymers such as agar, carrageenans, algins, low-methoxyl (LM) pectins, and gum arabic.

Although gelatin could be used in a variety of food products, its use is essentially limited to preparation of meltable-in-the-mouth water-dessert gels (gelatin desserts), meat products, and marshmallows and other confectioneries. It is also used to stabilize emulsions; as a stabilizer for ice cream and other dairy products; as a clarifying agent for wines, vinegar, and juices; and in bakery fillings and icings. It is not normally used as a thickener but is used to thicken low-fat spreads where the gel structure is broken down by processing and tempered by hydrophobic ingredients such as oils and emulsifiers.

GELLAN GUM

Gellan, known commercially as gellan gum, is an extracellular microbial polysaccharide produced by the organism *Sphingomonas elodea* (5). It is composed of a linear tetrasaccharide repeating unit of D-glucose–D-glucuronic acid–D-glucose–L-rhamnose. Two forms of gellan gum are available—the native acylated form and a deacylated form. Cooling of hot solutions of the low-acyl gellan (gum) forms firm and brittle gels of texture similar to that of agar and κ-carrageenan gels.

Gellan gum requires cations for gelation; but unlike other ion-sensitive gelling polysaccharides, it gels with a wide variety of cations, including hydrogen ions. Divalent ions such as Ca^{2+} are, however, much more efficient at forming gels than are monovalent ions such as Na^+ and K^+. Since divalent cations decrease solubility, sequestering agents are required if such cations are present initially. Like agar gels, gellan gum gels exhibit marked setting and melting temperature hysteresis; and by selection of the ion and its concentration, both temperatures can be controlled.

Gellan gum is functional in milk, although a calcium ion-sequestering agent is normally required. It can be used in confectioneries; structured foods; pie and bakery fillings; bakery icings, frostings, and glazes; dairy products; water-based gels; and films and coatings. Generally, gellan gum is blended with other gums or ingredients to modify its performance.

GUAR AND LOCUST BEAN (CAROB) GUMS

Commercial guar gum (2,4–6) is the ground endosperm of seeds of the guar plant. The guar plant resembles the soy plant, and guar seeds are produced much as are soy beans. Commercial guar gum usually contains 80 to 85% polysaccharide, 10 to 14% moisture, 3 to 5% protein, 1 to 2% fiber, 0.5 to 1.0% ash, and 0.4 to 1.0% lipid.

Guaran is the purified polysaccharide from guar gum. It is composed of D-galactose and D-mannose and is, therefore, a galactomannan. It has a linear backbone chain of β-D-mannopyranosyl units, approximately 56% of which (on average) are substituted with an α-D-galactopyranosyl unit, giving a comblike structure (Fig. 3). The mannan chain is rather evenly substituted with D-galactopyranosyl units, but in a somewhat random manner.

Like guaran and the endosperm polysaccharides of other legumes, locust bean (carob) gum (LBG) is also a galactomannan. Like guaran, it has a linear mannan backbone. However, in locust bean gum, on average only approximately 20% of the β-D-mannopyranosyl units are substituted with an α-D-galactopyranosyl unit and the branched units are clustered. The locust bean gum molecule contains "smooth" regions that contain no α-D-galactopyranosyl side-chain units and "hairy" regions in which most main-chain units contain single-unit α-D-galactopyranosyl branches.

Commercial locust bean gum is the ground endosperm of the seeds of the locust (carob) tree. The tree, which grows primarily in the Mediterranean region, is slow to mature and does not begin to bear until it is about 15 years old. Hence, the supply of locust bean seeds is limited and is not expected to increase. Locust bean gum usually has about the same composition (polysaccharide, moisture, protein, fiber, ash, lipid content) as does guar gum.

Guar gum forms very high viscosity, slightly pseudoplastic solutions at low concentrations. Because commercial guar gum contains protein, fiber, and lipids, its solutions are cloudy. Particle size is important in the use of guar gums. Coarse granulations are used for rapid, easy dispersion; fine granulations give rapid hydration. All forms will develop some additional viscosity after their dispersions have been heated. Guar gum is nonionic, so the viscosity of its solutions is not greatly affected by pH. It hydrates most rapidly at pH 6 to 9.

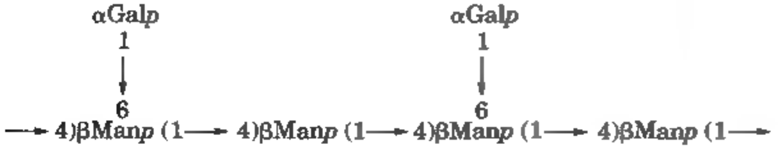
Locust bean gum has low cold-water solubility and is generally used when delayed viscosity development is desired. Only when dispersions of locust bean gum are heated (eg, to 85°C [185°F]) and cooled is high viscosity obtained. The general properties of locust bean gum are similar to those of guar gum. A difference is its synergism with κ-carrageenan, agar, and xanthan gum, with which it forms gels. Guar gum has only a weak synergistic effect on the viscosity of xanthan gum solutions and an even weaker effect on carrageenan and CMC solutions.

Guar gum is used in foods as a thickener and a binder of water. It is often used in combination with other gums, for example, with carrageenan and/or xanthan plus locust bean gum, particularly in dairy products such as ice creams, frozen novelties, whipped toppings, sour cream, cottage cheese, and low-fat yogurts. It is used with xanthan gum in pickle relish and sauces such as pizza and Sloppy Joe sauce to bind and control water. Both guar gum and locust bean (carob) gum are used (often in combination with carrageenan or xanthan gum) in stabilizers for ice cream. Other applications of these blends are in the manufacture of canned frostings, dips, cream cheese, cottage cheese dressings, and other processed cheese products. Guar gum is used in instant hot cereals and dry soup and other mixes, egg substitutes, dipping batters, pimiento strips for Spanish olives, sauces, condiments, and pet foods.

Two forms of enzymically modified guar gum are produced. One is a depolymerized guar gum designed to be a noncaloric bulking agent and a less viscous source of dietary fiber. The other is a guar gum that has had some of the D-galactosyl units removed to give it properties more like those of the more expensive locust bean gum.

Locust bean gum is almost always used in combination with one or more other gums in a variety of dairy products. In combination with xanthan gum and starch, it is used to make chewy fruit confections. Locust bean gum–carrageenan blends are used in pet food gels.

Tara gum is a galactomannan obtained from seeds of the tara shrub, which grows in northern regions of South America and Africa. It has a D-galactosyl unit:D-mannosyl unit ratio between those of guar gum and locust bean gum,



$$\longrightarrow 4)\overset{\begin{array}{c}\alpha\mathrm{Gal}p\\1\\\downarrow\\6\end{array}}{\beta\mathrm{Man}p}\,(1\longrightarrow 4)\beta\mathrm{Man}p\,(1\longrightarrow 4)\overset{\begin{array}{c}\alpha\mathrm{Gal}p\\1\\\downarrow\\6\end{array}}{\beta\mathrm{Man}p}\,(1\longrightarrow 4)\beta\mathrm{Man}p\,(1\longrightarrow$$

Figure 3. Idealized structure (shorthand) of guaran.

giving it properties most like those of a locust bean gum with a high D-galactose content. Only relatively small amounts of tara gum are currently used.

GUM ARABICS

Of the several gums that are dried, gummy exudations collected by hand from various trees and shrubs, only gum arabic, also called gum acacia and acacia gum, is still in significant use (2,4–6).

Gum arabics are collected by hand from various species of *Acacia*, a small tree. Tears of gum exude from wounds in trunks and branches. Wounds can be natural, but trees often are cut deliberately. Dried tears are sorted into lots based on clarity, color, and gross impurities. Representative samples of each lot are analyzed for ash content, water-insoluble impurities, and viscosity. The lots are then cleaned, converted into grains or powders of varying mesh sizes, and blended. The best gum comes from the region just south of the Sahara Desert. The most highly purified grade is produced by spray drying a clarified solution.

Gum arabic preparations are mixtures of highly branched, branch-on-branch, acidic polysaccharides, and protein-polysaccharides that occur as mixed salts. Their specific compositions and structures can vary with species, season, and climate.

Gum arabic is unique among gums because of its high solubility and the low viscosity and Newtonian flow of its solutions. While most other gums form highly viscous solutions at 1 to 2% concentration, 20% solutions of gum arabic resemble a thin sugar syrup in body and flow properties.

Gum arabic also has the unique property of being an effective emulsifier of flavor oils plus an effective stabilizer of the resulting emulsions. It is often used in the preparation of baker's emulsions of citrus and other essential oils. Another major application is in the preparation of dry fixed-flavor powders, which are prepared by adding citrus oils and other fruit or imitation flavors to gum arabic solutions and spray drying the resulting emulsions. Some gum arabic is also used in the preparation of confectioneries and lozenges.

GUM TRAGACANTH

Gum tragacanth (2,4–6), is the dried exudate of *Astragalus* species. It is produced from natural or deliberately made wounds in the roots, trunks, and/or branches of the small bushes, comes for the most part from Iran and Turkey, and is relatively expensive. Its use, therefore, is quite limited.

Gum tragacanth contains two polysaccharides. One (60–70%), termed both tragacanthic acid and bassorin, only swells in water, forming a gel. Tragacanthic acid contains a highly branched, acidic galactan covalently bound to protein. The minor polysaccharide is a neutral arabinogalactan in which L-arabinose is the predominant monosaccharide; it most probably consists of a core galactan chain to which highly branched arabinan chains are attached.

The most important physical properties of gum tragacanth are its relative acid stability, ability to lower surface and interfacial tensions, and hydration to a gel. The primary use of gum tragacanth is as an emulsifying agent and water controller in low pH foods. Gum tragacanth is found in some pourable salad dressings and pickle relishes and in certain bakery products.

HYDROXYPROPYLCELLULOSES

Hydroxypropylcelluloses (HP) (2,4–6) are cellulose ethers prepared by reacting cellulose with propylene oxide. The products are characterized in terms of moles of substitution (MS) rather than DS (see "Carboxymethylcelluloses"). MS is used because the reaction of a propylene oxide molecule with cellulose leads to the formation of a new hydroxyl group with which another alkylene oxide molecule can react to form an oligomeric side chain. Therefore, there is no limit to the moles of substituent that can be added to each D-glucopyranosyl unit. MS denotes the average number of moles of alkylene oxide that has reacted per D-glucopyranosyl unit (Fig. 4.)

In general, the MS controls the solubility of a hydroxypropylcellulose. Commercially available hydroxypropylcelluloses are insoluble in hot water, soluble in cold water, and compatible with several oils. HP is produced in a wide range of viscosity grades and forms clear, smooth, uniform solutions with pseudoplastic rheology similar to that of other cellulosics (see "Carboxymethylcelluloses").

Because they are nonionic gums, hydroxypropylcelluloses are unaffected by pH. Because of their ability to reduce surface and interfacial tension, they are used as emulsifying agents, emulsion stabilizers, and whipping aids. Flexible, nontacky, heat-sealable packaging films and sheets can be produced from hydroxypropylcelluloses by conventional extrusion techniques. Because hydroxypropylcelluloses form strong, edible films that provide a barrier to oxygen and water vapor, they are used to coat nuts and confections.

KONJAC MANNAN

Konjac mannan is the principal component of konjac flour, the product of commerce. Konjac flour, the powdered tuber of *Amorphophallus konjac*, is used primarily in Japan to make a gelled food product, in noodles, and as a fat replacer.

Konjac mannan is a glucomannan. It is a linear polymer of β-D-mannopyranosyl and β-D-glucopyranosyl (ratio 1.6:1.0) units all linked (1→4). Removal of some of the naturally occurring acetate ester groups with alkali is required for gelation.

Konjac flour is cold-water swelling but hydrates only slowly in room-temperature water, so unlike many other gum products, it disperses easily in water. As the particle size is reduced, the time required for hydration and dissolution is reduced. When fully hydrated, it forms solutions of high viscosity and pseudoplasticity.

Konjac mannan, by itself, when deacetylated, forms elastic gels that are stable in boiling water. In fact, gel strength increases reversibly on heating. This accounts for its traditional use in the stabilization of noodles that can

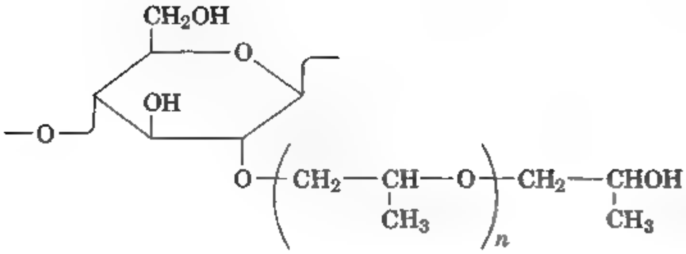


Figure 4. A representative β-D-glucopyranosyl unit of cellulose containing a hydroxypropyl group or a poly(propylene oxide) chain (n = 0, 1, 2, 3, etc).

be cooked in an autoclave. In addition, konjac mannan exhibits a strong synergism with agar and κ-carrageenan, forming elastic, thermally reversible gels after heating and cooling. It also will form a gel via synergistic interaction with xanthan and interacts synergistically with starches to increase viscosity and form heat-stable gels. Strong edible films can be made with konjac mannan as the base.

METHYLCELLULOSES AND HYDROXYPROPYLMETHYLCELLULOSES

Methylcelluloses (MCs) contain methoxyl groups in place of some of the hydroxyl groups along the cellulose molecule; hydroxypropylmethylcelluloses (HPMCs) contain, in addition to methyl ether groups, hydroxypropyl ether groups along the cellulose chain (2,4–6). The properties of MCs and HPMCs are primarily a function of the amount of each type of substituent group and the molecular-weight distribution.

MCs are made by reacting cellulose with methyl chloride until the DS (see "Carboxymethylcelluloses") reaches 1.1 to 2.2. HPMCs are made by using propylene oxide in addition to methyl chloride in the reaction; hydroxypropyl group MS (see "Hydroxypropylcelluloses") levels in commercial products are 0.02 to 0.3.

Members of this family of gums are cold-water soluble. Conversion of some of the hydroxyl groups of cellulose molecules into methyl ether groups increases the water solubility of the polymer and reduces its ability to aggregate (ie, reduces intermolecular interactions). Solubility and solution stability is increased even more when hydroxypropyl groups are added to MC.

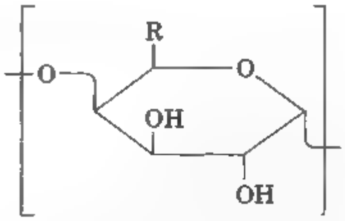
The most interesting property of these nonionic products is thermal gelation. Solutions of members of this family of gums decrease in viscosity when heated, as do solutions of most other polysaccharides. However, unlike solutions of other gums, when a certain temperature is reached (depending on the specific product), the viscosity will increase rapidly and the solution will gel. Gelation can occur at various temperatures from 45 to 90°C (115 to 195°F), depending on the viscosity type, DS/MS, and proportions of methyl and hydroxypropyl substituent groups. The thermal gelation is reversible; that is, the gel will revert to a fluid upon cooling.

These gums reduce surface and interfacial tensions and can, therefore, be used to stabilize emulsions and make foams. They will form high-strength films that are clear, water-soluble, and oil- and grease-resistant and have low oxygen and moisture vapor transmission rates. They are used in the preparation of dietetic foods, in baked goods for their glutenlike properties, in dipping batters (where film formation, low oil migration, moisture retention, thermal gelation, and tackiness are important properties), in whipped toppings, in frozen desserts and novelties, in canned fruit juice and fruit drink mixes (as a bodying agent, drying aid, emulsion stabilizer, and/or cloud agent), and as a binder and lubricator in extrusion processes. Their solutions exhibit pseudoplastic rheology similar to that of other cellulosics (see "Carboxymethylcellulose").

PECTINS

Pectins are mixtures of polysaccharides that originate from plants, contain pectinic acids as major components, are water soluble, and whose solutions gel under suitable conditions (2,4–7). Pectinic acids are galacturonoglycans [poly(α-D-galactopyranosyluronic acids)] with various, but greater than negligible, contents of methyl ester groups. Pectinic acids may have varying degrees of neutralization. Salts of pectinic acids are pectinates. Pectic acids are galacturonoglycans without, or with only a negligible content of, methyl ester groups. Pectic acids may have varying degrees of neutralization. Salts of pectic acids are pectates. The principal and key feature of all these molecules is a linear chain of α-D-galactopyranosyluronic acid units. In all commercial pectins, some of the carboxyl groups are in the methyl ester form; some or all of the remaining carboxylic acid groups may be in a carboxylate salt form (Fig. 5).

Pectins are subdivided according to their degree of esterification (DE), a designation of the percentage of carboxyl groups esterified with methanol. Pectins with DE values >50% are high-methoxyl (HM) pectins; those with DE values <50% are low-methoxyl (LM) pectins. The DE strongly influences the solubility, gel-forming ability, con-



R = $-CO_2CH_3$, $-CO_2^-$, or $-CO_2H$

Figure 5. Monomer units of pectins.

ditions required for gelation, gelling temperature, and gel properties of the preparation. In some LM pectins, termed amidated pectins, some carboxyl groups have been converted into carboxamide groups. The degree of amidation (DA) indicates the percentage of carboxyl groups in the amide form.

Pectins are soluble in hot water. The importance of pectin is predominately the result of its unique ability to form spreadable gels when a hot solution is cooled. HM-pectin gels are formed by the addition of sugar (at least 55%, but normally ~65%, soluble solids) to a hot, acidic (pH ~3) solution of pectin in a fruit juice. LM pectins will gel only in the presence of calcium ions and do not require soluble solids to gel. Increasing the concentration of calcium ions increases the gelling temperature and gel strength.

Any system containing pectin at potential gelling conditions (ie, necessary concentration of an appropriate pectin, pH, concentration of cosolutes, and concentration of divalent cations) must be prepared at a temperature above the gelling temperature. The temperature at which structure is formed upon cooling is the gelling temperature. LM pectins, particularly amidated pectins, are sensitive to divalent cations, such as calcium ions, and require use of a sequestering agent for dissolution if they are present.

The primary use of pectins is in the preparation of spreadable gels (jams, jellies, marmalades, and preserves, including the preserves in fruit yogurt and fillings for chocolates) from fruit juices or whole fruits, with or without added sugar. Pectins are also used in preparation of chewable fruit candies, fruit roll ups, canned fruit juices, cheese spreads, and icings and frostings.

XANTHAN GUMS

Xanthan (2,4–6) (also known as xanthan gum) is widely and extensively used as a food gum. It is an extracellular microbial polysaccharide produced by fermentation. Its characteristics vary somewhat with variations in the strain of the organism and the fermentation conditions used.

Xanthan gum has a linear main chain that has the same structure as cellulose (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). In xanthan gum, every second β-D-glucopyranosyl unit of the cellulosic backbone is substituted with a trisaccharide unit. About half of the trisaccharide side-chain units carry a terminal pyruvic acid cyclic acetal group.

Xanthan gum solutions are extremely pseudoplastic and have a high at-rest viscosity. These properties make xanthan gum almost ideal for the stabilization of aqueous dispersions, suspensions, and emulsions. Whereas other polysaccharide solutions decrease in viscosity when they are heated, xanthan solutions change little in viscosity over the temperature range 0 to 95°C (32 to 205°F). Although xanthan gum is anionic, pH has almost no effect on the viscosity of its solutions. A synergistic viscosity increase results from the interaction of xanthan gum with κ-type carrageenans, MCs, locust bean gum, and konjac mannan. The latter two combinations form thermally reversible gels when hot solutions of these polysaccharides are cooled.

The wide range of properties that make xanthan gum so widely and extensively used in foods includes its temperature stability, pH stability, ability to improve gloss (sheen), ability to hold moisture, and bland flavor; the high at-rest viscosity, stability to salts, high pseudoplasticity, and stable viscosity of its solutions from the freezing temperature to the boiling temperature of water; the extraordinary increase in solution viscosity it imparts with increases in concentration; and its synergistic interactions. Xanthan gum is sometimes used in combination with modified food starch. It is used in almost all pourable salad dressings, very frequently in combination with propylene glycol alginate. Among other products in which it is found are spoonable dressings, including reduced-calorie mayonnaise; bakery products; cereal bars; condiments; dairy products; egg substitutes; frozen foods; meat products; drink mixes; sauces; spreads; syrups; and toppings.

Blends of xanthan and locust bean gum and/or guar gum are excellent ice cream stabilizers and are often used in combination with a CMC and a carrageenan. Other applications of these blends are in the preparation of canned frostings, dips, cream cheese, cottage cheese dressings, other processed cheese products, low or nonfat dairy-based systems, sauces such as pizza and Sloppy Joe sauce, and starch-based gum candies.

BIBLIOGRAPHY

1. A.-C. Eliasson, ed., *Carbohydrates in Food*, Marcel Dekker, New York, 1996.
2. M. Glicksman, ed., *Food Hydrocolloids*, CRC Press, Boca Raton, Fla., 1982 (Vol. 1), 1983 (Vol. 2), 1986 (Vol. 3).
3. J. R. Mitchell and D. A. Ledward, eds., *Functional Properties of Food Macromolecules*, Elsevier Applied Science Publishers, London, 1986.
4. A. M. Stephen, ed., *Food Polysaccharides and Their Applications*, Marcel Dekker, New York, 1995.
5. R. L. Whistler and J. N. BeMiller, eds., *Industrial Gums*, 3rd ed., Academic Press, New York, 1993.
6. R. L. Whistler and J. N. BeMiller, *Carbohydrate Chemistry for Food Scientists*, Eagen Press, St. Paul, Minn., 1997.
7. M. L. Fishman and J. Jen, eds., *Chemistry and Function of Pectins*, American Chemical Society Symposium Series, Vol. 310, 1986

J. N. BEMILLER
Purdue University
West Lafayette, Indiana

H

HAZARD ANALYSIS AND CRITICAL CONTROL POINTS (HACCP).

See POSTHARVEST INTEGRATED PEST MANAGEMENT in the Supplement section.

HEAT

When two bodies are at different temperatures, there is a transfer of heat energy from the body having the higher temperature t_1 to the body having the lower temperature t_2. Hence, the state of energy of the colder body is increased and that of the warmer body decreased. This situation of unsteady-state heat transfer continues with the driving force $t_1 - t_2$, or δt, decreasing until both bodies are at the same temperature and there is no unbalanced state to give a driving force. Such is the case when a hot or warm food is placed in a container of cold water to decrease the temperature of the food.

Steady state heat transfer occurs when the temperature driving force Δt remains constant and the rate of heat transfer between two bodies is constant. An example of this type of steady-state transfer is boiling of water on a hot element of a stove. The element temperature is kept constant by a continuous flow of electrical energy being converted to heat and the boiling water stays constant at the boiling point of water (100°C at standard atmospheric pressure). On the other hand, if the heating element is kept constant and a food is being heated below the boiling point, there is an unsteady-state heat transfer, which results in a temperature rise of the food.

The last two examples also illustrate that there are different types of heat within a substance. Sensible heat is the amount of heat that can be added or removed from a given mass of product between two temperatures of the product (t_1 to t_2) without changing the state of the body (eg, heating or cooling a food by cooking or refrigerating). Latent heat refers to the amount of heat necessary to change a given mass from one state to another (eg, boiling water, freezing food products). Sensible heat results in a temperature rise within a body, whereas latent heat is the heat (at constant temperature) necessary to change the state.

There are numerous systems used in the world to quantify mass and energy, including the English (Imperial) system and several systems using metric units. This can be well demonstrated by the measurement of sensible heat. Each food or material requires a different amount of heat, called the specific heat (heat capacity), to raise or lower a given mass to a given temperature. Through laboratory research, specific heats have been determined and given in different unit systems. A British thermal unit (English system) is defined as the amount of heat required to raise the temperature of 1 lb of water 1°F. A kilocalorie in the metric system is the amount of heat required to raise 1 kg of water 1°C.

Several international organizations have attempted to standardize the unit systems, symbols, and quantities to prevent the confusion that often exists (1,2). The result is the Systeme International d'Unites, or the SI system, in which base metric units have been defined. Table 1 gives the SI system unit definitions along with factors for converting from the Imperial system to SI units.

In the SI system, the basic unit of force is the newton (N), the force that gives a mass of 1 kg an acceleration of 1 m/s. The basic unit of energy is the joule (J), the work done when the point of application of 1 N is displaced by a distance of 1 m. Hence specific heat c is expressed in the three principal systems as:

$$c = 1\ \text{Btu/lb°F} = 1\ \text{cal/g°C} = 4.1865\ \text{J/g K}$$

The mathematical relationship between heat, temperature, and specific heat in a food can be expressed in SI units as

$$Q = M \int_{t_1}^{t_2} c\,dt$$

where Q = heat (heat gained or lost in KJ), M = mass (kg), c = specific heat (kJ/kg°C), and dt = temperature change (°C).

If the specific heat is at constant pressure it is designated as c_p. If the process is carried out at constant volume (eg, a container of compressed gas), it is designated as c_v. For liquid and solid food, the difference between c_p and c_v is negligible. Because over the range of temperatures for food processes the c_p is essentially constant, heat in a system is normally calculated as:

$$Q = (M)(c_p)(t_1 - t_2) = Mc_p\Delta t$$

Thus if 100 kg of apples with a specific heat of 3.6 kJ/kg/K) are cooled from tree temperature of 18°C to 5°C, the amount of heat removed would be

$$Q = (100\ \text{kg})(3.6\ \text{kJ/kg} \cdot \text{K})(18\text{–}5\ \text{K}) = 4{,}680\ \text{kJ}$$

As a point of reference, 1 Btu equals 1.055 kJ; therefore, this would be equal to 4,436 Btu.

When learning the SI system, which will eventually be the world standard, it is useful to remember some basic approximate conversion factors to visualize the relationships between values. This is especially true for the United States where the English system has become so well entrenched. It is helpful to remember that 1 ft is approximately 0.3 m, 1 Btu is approximately 1 kJ, 1 lbf is approximately 4.5 N, 1 Btu/h is approximately equal to 0.3 W, and 1 Btu/lb is approximately 2.33 kJ/kg. If the equivalent English and SI values are visualized when working a problem in SI units, it will become easier to think in both systems.

Table 1. Base Units of SI System (Metric) and Conversion from Imperial to SI units

Measurable quantity	SI base unit	SI symbol	Imperial base unit	Imperial base symbol	Conversion factor (imperial × factor = SI)
Length	Meter	m	Foot	ft	0.30480
Mass	Kilogram	kg	Pound mass	lb	0.453592
Time	Second	s	Second	s	1 0
Temperature	Degree Kelvin	K	Degree Rankine	R	0.55556
Electric current	Ampere	A	Ampere	A	1.0
Amount of substance	Mole	mol	Mole	mol	1.0

PROCESSING FOODS BY ADDING OR REMOVING HEAT

The addition or removal of heat from a food increases shelf life and ensures the safety of the product. Heat is added to make a food more acceptable (improving sensory characteristics), to reduce microorganism populations, to lessen enzyme activity (pasteurization or cooking), or to kill microorganism and completely inactivate the enzymes (sterilizing). Heat is also added during dehydration processes to remove water through vaporization and thus lower the water activity a. Heat is removed to slow the growth of microorganisms and the action of enzymes (cooling or refrigerating) or to change the state (freezing), which prevents growth of microorganisms and further reduces enzyme action.

CHANGING THE STATE OF A FOOD PRODUCT

There are three states in which a food can exist, namely solid, liquid, or vapor. Because most natural foods are in some state of equilibrium with water, most of the significant changes of state in food products involve water. Food processes involving a change in state include freezing, heating to the point of evaporating a component (eg, vaporization or dehydration), condensing vapors to liquids (eg, solvent extraction), and subliming water directly from the frozen state to vapor.

Freezing Food

Freezing foods involves removing heat, resulting in the changing of liquid water in the food to ice. This has been demonstrated in the frozen fish industry where sensible heat is removed until the temperature reaches approximately −1°C (30°F). It then takes about 45 min during the so-called critical period to remove the latent heat of fusion from the water. After this period, the temperature drops rapidly as sensible heat is again removed (see FISH AND SHELLFISH PRODUCTS). It should be noted that foods are not pure substances so do not have precise temperatures at which there is a change in phase. This is because water has dissolved materials that increase or decrease in amount as a food is being processed and some of the water is bound to components of the food rather than being free to flow and act like pure water.

Vaporization Processes

The three vaporization processes involve the change in basic characteristics of a food or material by applying heat or other forms of energy that is converted to heat within the product.

Evaporation. Evaporization is the concentration of a liquid by supplying sufficient energy to vaporize the more volatile component or components. The process is used to concentrate solutions for stability or prior to further processing and to recover solvent from an extraction system.

Drying or Dehydrating. Drying or dehydration involves adding energy, usually heat, to vaporize a liquid, usually water, from a solid food. Foods are dried by supplying sufficient heat energy to vaporize water. This (1) preserves the product by reducing water activity; (2) reduces the cost or difficulty of packaging, handling, storing and shipment; and (3) produces convenience items (eg, instant coffee). In certain cases energy is supplied through other energy forms and then converted to heat in the product. For example, in processing by microwave heating, the energy is supplied to the system in the form of hertzian waves that are absorbed by the food, the resulting friction between vibrating molecules converts the wave energy to heat energy.

There are two distinct periods of drying in which different mechanisms control the rate of drying. During the first period, the constant rate period, the heat transfer predominates; all of the heat added is directly used in evaporating water and the rate of drying is independent of the nature of the food. In this case the moisture movement near the surface is rapid enough to maintain a saturated condition at the surface and the temperature of the food remains constant.

Each food has a critical moisture content at which the moisture can not migrate to the surface by diffusion rapidly enough to utilize all of the heat for evaporation. At this point the second phase, or falling rate period, begins and the food begins to absorb heat and rise in temperature.

A special form of drying involves sublimation of water directly from a frozen product to vapor, that is without passing through the liquid state. This is accomplished by placing a frozen food in a chamber under high vacuum, if the partial pressure of water vapor in the chamber is maintained below that of the ice at 0°C or 4.58 mm Hg, the water does not thaw prior to becoming a vapor.

Distillation. Distillation is the separation of two or more liquids through vaporizing the more volatile component or components. A principal use of this process in the food industry is in the recovery of a product after solvent extraction. In the case of organic solvent extraction of oilseeds, the solvent is recovered for reuse and the oil is retained as a pure food product.

Heat energy is certainly the most important factor involved in the processing of food products. In dealing with the science and engineering aspects of the food industry it is necessary to be constantly aware of the units involved in measuring the amount of energy being added to or removed from a food product. Unit equations (those showing the units as well as the numerical values) ensure the consistency of units and dimensions.

BIBLIOGRAPHY

1. R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, Inc., Orlando, Fla., 1984.
2. H. Wolf, *Heat Transfer*, Harper & Row, New York, 1983.

GEORGE M. PIGOTT
University of Washington
Seattle, Washington

HEAT EXCHANGERS

Heat Exchangers 1227
Heat Exchangers: Fouling 1231
Heat Exchangers: Paraflow 1237
Heat Exchangers: Plate Versus Tubular 1246
Heat Exchangers: Scraped Surface 1254

HEAT EXCHANGERS

Previous discussions on heat and the mechanisms for heating or cooling foods have emphasized that virtually every food-processing operation depends on transferring heat. The operations involving heat transfer use a wide variety of heat exchangers for heating or cooling products and for operational aspects of auxiliary equipment. Although there are many types of heat-exchanger equipment used in the food industry, there are relatively few principles that govern the heat transfer and operation of the equipment.

There are two basic classifications of heat exchangers. One is the contact type in which there is direct physical contact between the food product and the heating or cooling medium. The other is the noncontact type in which the heat is transferred through a body that separates the product from the heating or cooling source. Within these two categories there are many proprietary designs and models of heat exchangers depending on the specific requirement for transferring heat to or from a given type of food product.

Capital investment, safety, and economics of processing are important operational factors to consider when purchasing or installing exchanger equipment. Of equal value in determining the type or model of equipment to install is the effect on the physical and chemical properties of the end products. Maintaining or improving nutritional value, aesthetics, safety of the products, and sensory attributes affect the marketability.

Paramount to the successful long-term operation of heat exchangers in meeting the above goals is the efficiency and ease of sanitizing the entire equipment. This is especially important for closed systems that can not be dismantled while cleaning. The operation of cleaning in place (CEP) requires sanitary design that ensures complete sanitation when cleaning liquids are circulated through the exchangers. Hence, engineering design is the controlling factor in insuring successful heat-transfer operations involving food processing.

FOOD-PROCESSING OPERATIONS REQUIRING HEAT EXCHANGERS

It is common to think of all heat exchangers as being composed of two adjoining compartments through which fluids are flowing, each cooling or heating the other. This concept is natural because the basic engineering approach to studying heat transfer is to discuss each type of heat exchange operation as a specific process (eg, product freezing, refrigeration systems, batch heating, nonsteady-state operations, etc) with steady-state, liquid-liquid heat exchangers being studied under the subject of heat exchangers. However, when processing food, it is important to think of heat exchangers as being the total range of equipment that is used for cooking, blanching, pasteurizing, sterilizing, cooling, freezing, and cold-storage holding. In addition, there are many processes, such as drying and extraction, in which the transfer of heat is important to accomplish the basic goal of the process. Food products that are heated or cooled during processing, including the heat transfer medium, cover a wide range of vapors, liquids, and solids. Table 1 indicates how machinery and equipment must be used for the transfer of heat in the many different food-processing operations.

In addition to the type of heat transfer taking place during processing, the physical and chemical condition of the food before, during, and after heating or cooling must be considered. Products requiring different approaches to heat transfer during the food-processing operation include

1. Dense, hard, solid foods (eg, potatoes) that can stand considerable mechanical abuse during processing but become somewhat fragile during the final phase of heating.
2. Soft, solid foods (eg, tomatoes) that cannot stand any mechanical abuse during or after cooking.
3. Purées or soft items that flow like a liquid but congeal or gel to a solid when heated (eg, surimi being processed into seafood analogues and extruded cereals).
4. Liquids that range from heat stable to extremely heat labile.
5. Foods, particularly those high in protein, that congeal with heat and cause baked-on deposits on the heat-transfer surfaces.

Table 1. The State of a Food Product as Related to Classification of Heat Exchanger

Food form	Heat transfer media	Classification	Example
Vapor	Liquid	Noncontact	Condensing vapors
Liquid	Vapor	Contact	Steam infusion, steam injection
		Noncontact	Heating by steam (condensing), refrigerant cooling
	Liquid	Noncontact	Transfering heat between liquids
	Solid	Contact	Melting ice
		Noncontact	Heating on metal hot plate in a container
Solid	Vapor	Noncontact	Extrusion (heating by steam jacket)
		Contact	Drying, cooking
	Liquid	Contact	Blanching, immersion freezing, deep-fat frying, poaching, steeping
		Noncontact	Extrusion (heating by liquid jacket)
	Solid	Contact	Dry ice cooling, freezing on plates
		Noncontact	Cooking on stove
Any food	None	Noncontact	Radiant heating, irradiation

Processing requirements for these types of food range from individual batch cooking to large-scale continuous sterilizing. Many products must also be heated and then cooled to ensure maximum retention of nutrients and desired product form.

HEAT-EXCHANGER SYSTEMS AND PRINCIPLES OF OPERATION

Heat is transferred to or from a food in batch and continuous systems. Batch systems involve unsteady-state heat transfer whereby the food being heated or cooled begins at a given temperature and increases or decreases until the desired temperature is reached. The heat-transfer medium can vary in temperature (eg, a hot surface or liquid that changes temperature as it gives or receives heat) or can be at steady state (eg, condensing steam).

Batch steady-state systems involve a series of batch systems that give the overall effect of steady state processing. For example, liquid-filled tanks in series can be stepwise heated by batch heating but can be connected so that the end result of the overall heating is a steady-state emission of constant-temperature liquid continuously flowing at the same rate as the cold liquid entering the first tank.

A true steady-state system is found in flowing liquids or viscous solids whereby mass flow rate, temperature, pressure, and physical properties of the food and the heat transfer medium are constant at any given cross section.

Heating and Cooling Liquids

Batch Heating. Typical batch heating of a liquid food takes place in a steam-jacketed kettle. While the food is being heated, the system is under unsteady-state conditions. After the food reaches the desired processing temperature and is heated at a constant temperature, steady-state conditions are reached. Assuming that the final temperature of the food is to be maintained somewhat below that of the condensing steam, steady-state conditions prevail as the steam flow rate is adjusted to maintain the processing conditions. As is the practice for all commercial heat-exchanger equipment, the outside of the kettle is lagged to minimize heat loss to the surroundings and isolate the environment of the unit operation.

Large kettles (tanks) being used for heating a liquid often have coils in the tank to transfer heat. This greatly improves the heat-transfer efficiency by increasing the heat-transfer surface above that of the outside wall receiving heat from the jacketed heating (or cooling) source. An additional improvement of the heat transfer in tanks can be realized by installing mechanical stirring equipment, which increases heat transfer above that of natural convection.

Tubular Heat Exchangers. The simplest continuous heat exchange occurs when two fluids of different temperature are flowing through concentric pipes or tubes. The flow in steady-state heat exchangers can be either cocurrent or cocurrent (parallel) flow. During countercurrent flow, one stream (liquid or vapor) is introduced at the opposite end of the unit. By controlling the flow rates, it is possible to heat the cold liquid above the outlet temperature of the entering hot stream. Conversely, when the two liquids are introduced at the same point, the stream being heated can never leave at a temperature above that of the stream being cooled. This cocurrent system is normally less efficient than a countercurrent system because the temperature difference driving force can become quite small as the temperatures of the two streams meet. However, there are circumstances whereby cocurrent flow can be used to ensure that a heat-sensitive material does not rise above a certain temperature during processing. In the case of using steam to heat a flowing liquid, the food is heated while the condensing steam is maintained at the saturation temperature of the steam. As in the case of a steam kettle cooker, the most common and efficient heating medium is condensing steam. Shell-and-tube heat exchangers are essentially improved tubular heat exchangers where a few to many tubes replace the single concentric inner tube.

Whereas the heat transfer coefficient is constant under these steady-state conditions, the temperature driving force (ΔT) is calculated as the log mean temperature difference

$$\Delta T_M = \frac{(T_S - T_{f_1}) - (T_S - T_{f_2})}{\ln \dfrac{(T_S - T_{f_1})}{(T_S - T_{f_2})}} \tag{1}$$

and the steady-state heat transfer is calculated as

$$Q = UA\Delta T_M \qquad (2)$$

where T_S = temperature of condensing steam, T_f = temperature of liquid food being heated, U = overall heat transfer coefficient, and ΔT_M = log mean temperature driving force.

Plate Heat Exchangers. Plate heat exchangers solve one of the principal processing problems encountered with tubular and shell-and-tube heat exchangers, that of sanitation. It is virtually impossible to thoroughly clean and sanitize closed system exchangers when a food liquid or slurry is passed through the larger diameter or the shell side where velocity is low. The basic units of plate exchangers are stainless steel (for sanitation and corrosion resistance) plates that are pressed, machined, or formed to accomplish several special design features. The contour of a stack of the plates is such that, when a formed gasket is placed between each plate, a heat exchanger allowing two liquid streams to flow between plates is formed. In practical operation, the plates are suspended from horizontal rails or pipes that allow them to be brought together and tightly compressed during operation or separated for cleaning and maintenance.

These types of heat exchanger are used extensively in the dairy industry. The exchanger is highly efficient due to the high turbulence and minimum volume flowing between the channels. Capacity can be increased to any flow rate desired (eg, 10,000 kg/h) by increasing the number of plates in the frame. When operated at the proper flow rate, the velocity of liquid through the small channels decreases the tenacious baked-on deposits that are caused when colloidal suspended components (eg, proteins in milk) contact hot surfaces. Also, the plates can be separated for thorough cleaning and sanitizing during the maintenance periods.

The disadvantages of plate heat exchangers is the high initial cost compared to tubular type exchangers and the high cleaning and maintenance cost of taking the exchanger apart for each cleaning period. Also, the maximum velocity is limited by the small cross-sectional area and the pressure limitations of the gasketed plates. The minimum velocity is determined by the varying cross section, which allows dead spots of low velocity and subsequent bake-on of the suspended or dissolved solids.

Direct Steam Heating. Direct contact between steam and a food is the most efficient means of heating by directly transferring the latent heat of vaporization to the food. However, the product must be able to sustain the dilution effect caused by the added water resulting from the condensed steam that remains in the heated product. Furthermore, special consideration must be given to producing steam that is safe for human consumption.

When steam is added to a product, the process is known as steam injection. When the product is sprayed into a chamber of steam, thus adding the product to the steam, the process is called steam infusion.

Falling Film Evaporators. When a film of liquid is allowed to flow down a heated wall, the transfer of heat is extremely rapid. This type of unit is used for rapid heating of a fluid that is being evaporated. However, due to the limitation of flow rate as compared to other types of heat exchanger, this method is not often used solely for heating a product.

Heating and Cooling Solids

Batch Heating. Batch heating of solids is carried out in the type of heat-exchange facilities normally associated with cooking foods. Broiling, roasting, or baking operations are accomplished by a combination of radiant heat, convection, and conduction, the predominant mechanism of heat transfer depending on the specific commercial equipment. Ovens are heated by elements emitting radiation at a wavelength of 5×10^{-6} m at a temperature of 250–400°C. A more effective radiant heating occurs in chambers heated by an electric bulb infrared source. In this case the air is not heated by the source so that little heating of the food is due to convection or conduction heating.

Microwave heating is a specialized form of dielectric radiant heating that has many advantages over other dielectric methods because there is no requirement for critical spacing between the food and the capacitor plates. Heating is accomplished by the friction of excited molecules rubbing against each other as the strong alternating energy reverses the polarization of the molecules in the food many millions of times per second. The advantages of microwave heating include extremely rapid heating of the food, uniform distribution of the heat throughout the entire food mass, high efficiency, and good control of the energy being added to the food.

Many products are cooked and heated by convection heating when immersed in hot vegetable oil (deep frying) or poached in hot or boiling water. Batches of products are often heated by placing a container of the product on a hot surface (eg, hot plate or element of a stove) or in a steam environment. Steam retort canning of foods is a good example of batch heating by steam. Hermetically sealed cans are placed in baskets and placed in a steam chamber that can be closed and pressurized, normally at about 10 psi (117°C or 242°F). Condensing steam transfers energy to the outside of the can by conduction and convection, whereby the heat is conducted through the can by conduction and then to the food. Solid packs with no free liquid are heated in the can by conduction while a pack with free water transfers heat by both conduction and convection. After being held at the required temperature and time to accomplish sterilization, the cans are removed and air cooled. Sensitive products that tend to scorch or decrease in nutritional value during long air cooling periods are often pressure cooled with water prior to being removed from the retort.

Continuous Heating. More efficient production control, increased product throughput, and improved processed food quality can be accomplished when processes are upgraded from batch to continuous. Modern processing of solid foods involves large continuous production lines utilizing continuous baking ovens (eg, bakery products); deep-frying tanks (eg, french-fried potatoes), microwave ovens, radiant heat chambers, and steam chambers.

Batch Cooling or Freezing. Removing heat from foods, with the exception of cooling in tubular type exchangers, requires considerably different types of facilities than heating. This is due to the nature of the recycling refrigerant or the cryogenic liquids used to remove heat from a food. An additional factor is the psychrometric properties of air that are often recycled near the humidity saturation point in refrigeration facilities in which natural or forced convection is involved in the process.

The basic unit involved with refrigeration cooling of foods is a heat exchanger in which a refrigerant is introduced through an expansion valve into the coils that are located in the cooling or freezing chamber. Thus the cooling or freezing heat exchanger has a cold gaseous refrigerant on one side of the coil wall and the food or heat-transfer medium on the other. Air or liquid brines are the normal mediums used for transferring heat by convection from the food to the refrigeration coils. When the food product is in direct contact with the refrigeration coils, heat is transferred directly by conduction.

Blast Cooling and Freezing Facilities. Forced-air convection is used in blast refrigeration to transfer heat from refrigerated coils to the product. A blast freezer or cooler is actually a double heat exchanger. One exchange takes place between the product and direct contact with air flowing past, and the second is the cooling of the recirculating air as it passes over the freezing coils. During the first phase of this cycle, cold air is circulated over the product and through the freezing chamber, which results in an increase in humidity due to the humidity driving force H between the product environment and the colder refrigerant coil. Thus the air removes water from the freezing chamber and deposits it as ice on the refrigeration coils during the second phase when the air is cooled below the saturation point. If the product being frozen is not completely protected from the air, desiccation will occur in the product. Also, if the doors to the chamber are not completely sealed when closed, moist warm air will enter and further complicate the moisture transfer problem. Of course, as the moisture, in the form of ice, builds up on the coil it acts as a heat-transfer barrier and greatly reduces the efficiency of the system. Hence, the advantage of blast refrigeration is the relatively simple facility required while the main disadvantage is desiccation of the food and buildup of ice deposits that must be removed from the refrigeration coils. The ice greatly reduces the efficiency of heat transfer and increases the cost of operation.

Contact Plate Freezers. Many solid food products are frozen by conduction on freezer shelves, called plates, that contain circulating refrigerant. Thus plate freezing involves heat exchange between a solid food and a vapor refrigerant. Efficient plate freezing is limited to foods and food packages that have flat surfaces (eg, rectangular packages of vegetable) because irregular geometries (eg, turkeys) cannot contact the flat freezer plate. The efficiency of freezing suitable packages is further increased by plate freezer systems in which the plates can be adjusted after loading to contact both the top and the bottom of the package.

Immersion Freezing in Brine. Saturated brine solutions have freezing points well below the freezing point of water and can be used efficiently to freeze products, particularly irregularly shaped items such as turkeys and fish. The product is immersed in a cold brine solution that is maintained at the low temperature by freezing coils. As in the case of blast freezing, there is a two-phase heat transfer. The refrigerant takes heat from the brine and the brine removes heat from the food by conduction and convection. This heat exchange is between a solid food and a liquid.

Cryogenic Freezing. Immersion of a solid food in a liquid refrigerant is similar to freezing in a brine except that there is a much higher temperature driving force between the liquid (eg, liquid ammonia or freon) and the product. Thus the freezing is rapid. Batch freezing by this method is not ordinarily carried out commercially because the cost is prohibitive. Due to the extremely fast freezing, a cryogen immersion frozen product must be carefully tempered before further handling and processing or the internal stresses produced will cause cracking of the frozen item.

Continuous Cooling or Freezing. As in the case of heating a food, continuous freezing is much more efficient than batch freezing. Many modern freezing operations involve continuous lines whereby conveyors carry a food through a freezing apparatus. This includes blast freezing and cryogenic freezing. One improvement over immersion cryogenic freezing is the continuous freezing in tunnels in which a liquid cryogenic is flowed over the product. In this operation the liquid expands to a vapor through nozzles directed toward the moving product line. This freezing of a solid by direct contact with a vapor greatly reduces the amount of refrigerant used during processing. However, the cost of the liquid cryogens used (ammonia, freon, carbon dioxide, and nitrogen) are such that only continuous processing lines operating long hours can be justified economically.

There are many different types of heat exchanger and auxiliary equipment available to the food processor. The length of the processing season, the type of food or food product, the value of the raw materials, the cost of utilities at the processing location, environmental factors, and common sense are all factors that must enter into the plans for food-processing operations. Judicial selection of the equipment and facilities for a given food and a given process are necessary to insure that the highest quality product is produced efficiently and economically.

George M. Pigott
University of Washington
Seattle, Washington

HEAT EXCHANGERS: FOULING

THE FOULING FACTOR

In view of its complexity and variability and the need to carry out experimental work on a long-term basis under actual operating conditions, fouling remains a somewhat neglected issue among the technical aspects of heat transfer. Still, the importance of carefully predicting fouling resistance in both tubular and plate heat-exchanger calculations cannot be overstressed. This is well illustrated in Tables 1 and 2.

Note that for a typical water–water duty in a plate heat exchanger, it would be necessary to double the size of the unit if a fouling factor of 0.0005 were used on each side of the plate (ie, a total fouling of 0.001).

Although fouling is of great importance, there are relatively little accurate data available and the rather conservative figures quoted in Kern (*Process Heat Transfer*) are used all too frequently. It also may be said that many of the high fouling resistances quoted have been obtained from poorly operated plants. If a clean exchanger, for example, is started and run at the designed inlet water temperature, it will exceed its duty. To overcome this, plant personnel tends to turn down the cooling-water flow rate and thereby reduce turbulence in the exchanger. This encourages fouling and even though the water flow rate eventually is turned up to design, the damage will have been done. It is probable that if the design flow rate had been maintained from the onset, the ultimate fouling resistance would have been lower. A similar effect can happen if the cooling-water inlet temperature falls below the design figure and the flow rate is again turned down.

SIX TYPES OF FOULING

Generally speaking, the types of fouling experienced in most CPI (cleaning in place) operations can be divided into six fairly distinct categories. First is crystallization—the most common type of fouling that occurs in many process streams, particularly cooling-tower water. Frequently superimposed with crystallization is sedimentation, which usually is caused by deposits of particulate matter such as clay, sand, or rust. From chemical reaction and polymerization often comes a buildup of organic products and polymers. The surface temperature and presence of reactants, particularly oxygen, can have a very significant effect. Coking occurs on high-temperature surfaces and is the result of hydrocarbon deposits. Organic material growth usually is superimposed with crystallization and sedimentation and is common to sea water systems. And corrosion of the heat-transfer surface itself produces an added thermal resistance as well as a surface roughness.

In the design of the plate heat exchanger, fouling due to coking is of no significance since the unit cannot be used at such high temperatures. Corrosion also is irrelevant since the metals used in these units are noncorrosive. The other four types of fouling, however, are most important. With certain fluids such as cooling-tower water, fouling can result from a combination of crystallization, sedimentation, and organic material growth.

Table 1. Typical Water/Water Tubular Design. Clean Overall Coefficient 500 Btu/h · ft² · °F

Fouling resistance (h · ft² · °F/Btu)	Dirty coefficient (Btu/h · ft² · °F)	Extra surface, required, %
0.0002	455	10
0.0005	400	25
0.001	333	50
0.002	250	100

Table 2. R405 Paraflow, Water/Water Duty, Overall Coefficient 1000 Btu/h · ft² · °F; Single Pass-Pressure Loss 9 psig

Fouling Resistance (h · ft² · °F/Btu)	Dirty coefficient (Btu/h · ft² · °F)	Extra surface, required, %
0.0002	833	20
0.0005	666	50
0.001	500	100
0.002	333	200

A FUNCTION OF TIME

From Figure 1, it is apparent that the fouling process is time-dependant with zero fouling initially. The fouling then builds up quite rapidly and in most cases and levels off at a certain time to an asymptomatic value as represented by curve A. At this point, the rate of deposition is equal to that of removal. Not all fouling levels off, however, and curve B shows that at a certain time the exchanger would have to be taken off line for cleaning. It should be noted that a Paraflow is a particularly useful exchanger for this type of duty because of the ease of access to the plates and the simplicity of cleaning.

In the case of crystallization and suspended solid fouling, the process usually is of the type A. However, when the fouling is of the crystallization type with a pure compound crystallizing out, the fouling approaches type B and

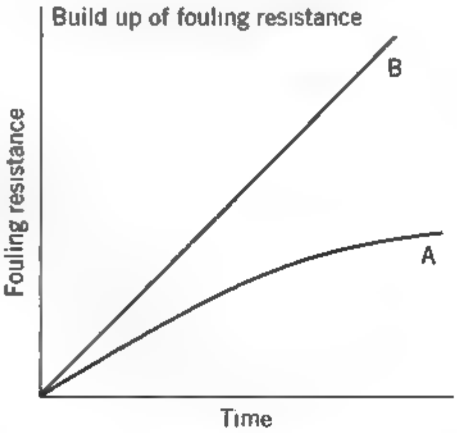


Figure 1. Buildup of fouling resistance.

the equipment must be cleaned at frequent intervals. In one particularly severe fouling application, three Series HMB Paraflows are on a 4-1/2 hour cycle and the units are cleaned in place for 1-1/2 h in each cycle.

Biological growth can present a potentially hazardous fouling since it can provide a more sticky surface with which to bond other foulants. In many cases, however, treatment of the fluid can reduce the amount of biological growth. The use of germicides or poisons to kill bacteria can help.

LOWER RESISTANCE

It generally is considered that resistance due to fouling is lower with Paraflow plate heat exchangers than with tubular units. This is the result of five Paraflow advantages:

1. There is a high degree of turbulence, which increases the rate of foulant removal and results in a lower asymptotic value of fouling resistance.
2. The velocity profile across a plate is good. There are no zones of low velocity compared with certain areas on the shell side of tubular exchangers.
3. Corrosion is maintained at an absolute minimum.
4. A smooth heat-transfer surface can be obtained.
5. In certain cooling duties using water to cool organics, the very high water film coefficient maintains a moderately low metal surface temperature, which helps prevent crystallization growth of the inversely soluble compounds in the cooling water.

The most important of these is turbulence. HTRI (Heat Transfer Research Incorporated) has shown that for tubular heat exchangers, fouling is a function of flow velocity and friction factor. Although flow velocities are low with the plate heat exchanger, friction factors are very high and this results in lower fouling resistance. The effect of velocity and turbulence is plotted in Figure 2.

Marriot of Alfa Laval has produced a table showing values of fouling for a number of plate heat-exchanger duties.

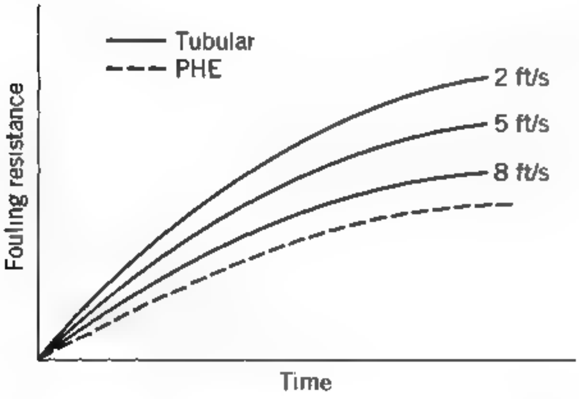


Figure 2. Effect of velocity and turbulence.

Fluid	Fouling resistance (hr · ft^2 · °F/Btu)
Water	
Demineralized or distilled	0.00005
Soft	0.00010
Hard	0.00025
Cooling tower (treated)	0.00020
Sea (coastal) or estuary	0.00025
Sea (ocean)	0.00015
River, canal	0.00025
Engine jacket	0.00030
Oils, lubricating	0.00010–0.00025
Oils, vegetable	0.00010–0.00030
Solvents, organic	0.00005–0.00015
Steam	0.00005
Process fluid, general	0.00005–0.00030

These probably represent about one half to one-fifth of the figures used for tubulars as quoted in Kern, but it must be noted that the Kern figures probably are conservative, even for tubular exchangers.

APV, meanwhile, has carried out test work that tends to confirm that fouling varies for different plates with the more turbulent type of plate providing the lower fouling resistances. In testing an R405 heating a multicomponent aqueous solution containing inverse solubility salts (ie, salts whose water-solubility decreases with increasing temperature), it was learned that the rate of fouling in the Paraflow was substantially less than that inside the tubes of a tubular exchanger. The tubular unit had to be cleaned every 3 or 4 days while the Paraflow required cleaning about once a month.

Additional tests on cooling water fouling were sponsored by APV at Heat Transfer Research, Inc. (HTRI) with the test fluid being a typical treated cooling tower water. Results of these experiments follow.

OBJECTIVES

In many streams, fouling is an unavoidable by-product of the heat-transfer process. Fouling deposits can assume numerous types such as crystallization, sedimentation, corrosion, and polymerization. Systematic research on fouling is relatively recent and extremely limited (1–3) and almost exclusively concentrated on water—the most common fluid with fouling tendencies. The few research data that exist usually are proprietary or obtained from qualitative observations in plants. However, it is generally recognized that the prime variables affecting fouling buildup are flow velocity, surface temperature, and surface material. In the case of water, the water quality and treatment must also be considered.

The importance of fouling on the design of heat exchangers can be seen from the rate equation

$$\frac{1}{U} = \frac{1}{h_1} + \frac{1}{h_2} + R_f$$

where U is the overall heat transfer coefficient, Btu/h · ft^2 · °F, h_1, h_2 is the film coefficients of the two heat-transferring fluids, Btu/h · ft^2 · °F, and R_f is the fouling resistance, h · ft^2 · °F/Btu. It is obvious from equation 1 that the higher the film coefficients, the greater effect the fouling resistance will have on the overall coefficient and therefore on the size of the exchanger.

In tubular heat exchangers, water-side heat-transfer coefficients in the order of magnitude of 1000 Btu/h · ft^2 · °F are quite common. In plate exchangers, the coefficients are substantially higher, typically around 2000 Btu/h · ft^2 · °F. Assuming that both types of equipment operate with water—water systems, overall clean coefficients of 500 and 1000 Btu/h · ft^2 · °F respectively are obtained. Using a typical fouling resistance of 0.001 h · ft^2 · °F/Btu (equal to a coefficient of 1000 Btu/h · ft^2 · °F), the inclusion of the fouling will cause the tubular exchanger size to increase by a factor of 4, while for the plate exchanger, the corresponding factor is 7.

This example clearly demonstrates the crucial importance of fouling, especially in plate exchangers. Yet, fouling resistances that are unrealistically high often are specified and invariably have been based on experiences derived from tubular equipment. The common source of water fouling resistances in TEMA which recommends R_f values spanning a tremendous range between 0.0015 and 0.005 h · ft^2 · °F/Btu.

Flow velocity, as mentioned earlier, is a crucial operating parameter that influences the fouling behavior. For flow inside the tubes, the definition of flow velocity and the velocity profile is straightforward. But in plate exchangers, flow velocity is characterized by constant fluctuations as the fluid passes over the corrugations. It is postulated that this induces turbulence that is superimposed on the flow velocity as a factor that diminishes fouling tendencies. This has been observed qualitatively in practical applications and confirmed by unpublished APV research.

TEST APPARATUS AND CONDITIONS

The plate heat exchanger (PHE) tested was an APV Model 405 using APV Type R40 stainless-steel plates 45 in. high and 18 in. wide. The plate heat-transfer area was 4 ft^2 with a nominal gap between plates of 0.12 in. with the plate corrugations, the maximum gap was 0.24 in. Seven plates were used, creating three countercurrent passages each of the cooling water and the heating medium. A schematic diagram of the installation is shown in Figure 3.

The PHE was mounted on the HTRI Shellside Fouling Research Unit (SFRU) together with two small stainless-steel shell-and-tube exchangers. The PHE was heated using hot steam condensate, and the fouling was determined from the degradation of the overall heat-transfer coefficient. Simultaneously, tests also were run on an HTRI Portable Fouling Research Unit (PFRU), which uses electrically heated rods with the cooling water flowing in an annulus. The SFRU and PFRU are described (5) elsewhere.

The fouling tests were conducted at a major petrochemical plant in the Houston, Texas area. The test units were installed near the cooling-tower basin on the plant. The cooling-tower operating characteristics are summarized in Table 3. The 140,000-gpm system had two 1600-gpm sidestream filters. Filter backwash accounted for most of the blowdown. The makeup water for the cooling-water system came from several sources and was clarified with alum outside the plant. The water treatment used is as follows:

- Chromate-zinc-based inhibitor for corrosion control (20–25 ppm chromate)
- Organic phosphonate and polymer combination as dispersant (2 ppm organic phosphonate)
- Polyphosphate as anodic passivator (5–6 ppm total inorganic phosphate)
- Chlorine for biological control (continuous feeding of 386 lb/day)
- Biocide for biological control (15 ppm biocide once per week)
- Sulfuric acid for pH control (pH 6–6.5)

TEST PROCEDURE

The velocity for the PHE is defined by

$$V = \frac{w}{\rho A_c}$$

where V is the velocity, w is the mass flow rate, ρ is the fluid density, and A_c is the cross-sectional flow area. The cross-sectional flow area is based on the compressed gasket spacing. In other words, the flow are is computed as if there were no corrugations but smooth plates instead. The surface temperature is defined as the fluid–deposit interface temperature. Since the PHE was heated by constant temperature steam condensate, the metal temperature remained constant and the surface temperature decreased as fouling built up. In addition, because of the counterflow nature of the PHE, the surface temperature was not constant from inlet to outlet. The surface temperature reported here is the initial surface temperature at the midpoint of the plate.

Conditions of operation were selected so that all the SFRU test exchangers operated roughly at the same nominal flow velocity and surface temperature. During the operation, weekly water samples were taken for chemical analysis. At the end of each test, deposits were photographed and sampled for chemical analysis. The exchanger plates then were cleaned and the unit reassembled and new test conditions established.

TEST AND RESULT DESCRIPTION

Five test series were performed testing several velocities and surface temperatures (eg, see test 2 in Fig. 4). Typical fouling time histories are shown in Figure 5 along with the operating conditions. Notice that the PHE fouling resistances establish a stable asymptotic value after about 600–900 h of operation. The results of the other tests are shown in Figure 6 only as the values of the asymptotic

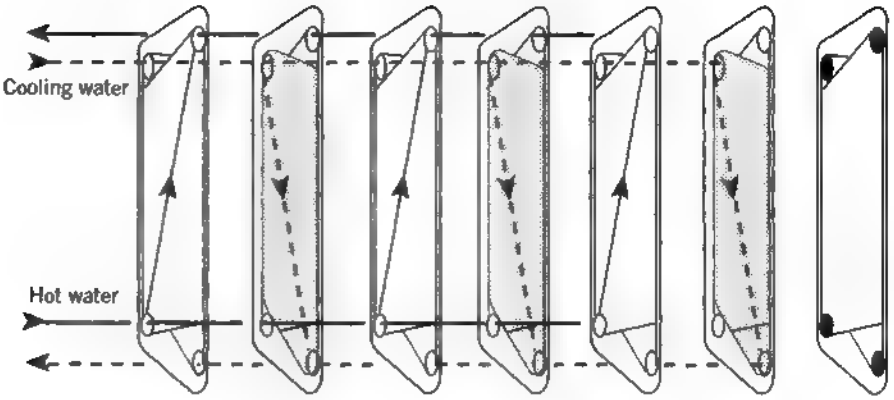


Figure 3. APV plate heat exchanger with three parallel countercurrent parallel passages for both the cooling water and the hot water.

Table 3. Cooling-Water System Characteristics

Tower and circulation system	
Circulation rate	140,000 gpm
Temperature difference across tower	23.4°F
Number of sidestream filters	2
Corrosion rate from carbon steel coupon tests	
	<1 mil/yr

Water description	Cooling-tower water	Makeup water
Treatment	10 cycle concentration Blowdown from coolers and sidestream filters	Unclarified San Jacinto River 1000 gpm Clarified San Jacinto River 2400 gpm Lissie Sand Well 475 gpm
Typical composition		
Total hardness as $CaCO_3$, ppm	520	48
Calcium as $CaCO_3$, ppm	420	40
Magnesium as $CaCO_3$, ppm	120	8
Methyl Orange alkalinity as $CaCO_3$, ppm	20	34
Sulfate as SO_4, ppm	1600	34
Chloride as Cl, ppm	800	48
Silica as SiO_2, ppm	150	18
Total inorganic phosphate as PO_4, ppm	10	
Orthophosphate as PO_4, ppm	7	
pH	6	7.5
Specific conductance, micromhos, 18C	5000	300
Chromate as CrO_4, ppm	25	
Chromium as Cr, ppm	0.5	
Soluble zinc as Zn, ppm	2.5	
Total iron as Fe, ppm		0.8
Suspended solids, ppm	100	10

fouling resistances, as these are the data required for design.

The chemical analysis of the fouling deposit was made after each test and the results are summarized in Table 4. The primary elements found were phosphorus, zinc, and chrome from the water treatment and silicon from suspended solids in the water.

The photograph of the plates of the PHE shown in Figure 4 at the termination of test 2 indicates that fouling occurred only in the upper third of the plates. This is the region near the hot-water inlet and cold-water outlet, region of high surface temperature. Figure 5 shows a surface temperature profile for test 2 conditions of 2.8 ft/s and an inlet bulk temperature of 90°F. From the water chemistry parameters, the saturation temperature for calcium phosphate above which precipitation is expected was calculated to be 163°F (6). The shaded region on Figure 7 indicates the expected precipitation region and corresponds closely to the fouled region seen in Figure 4.

The surface temperatures during some of the tests were higher than those experienced in normal PHE operation. This is the result of the setup condition criteria that the

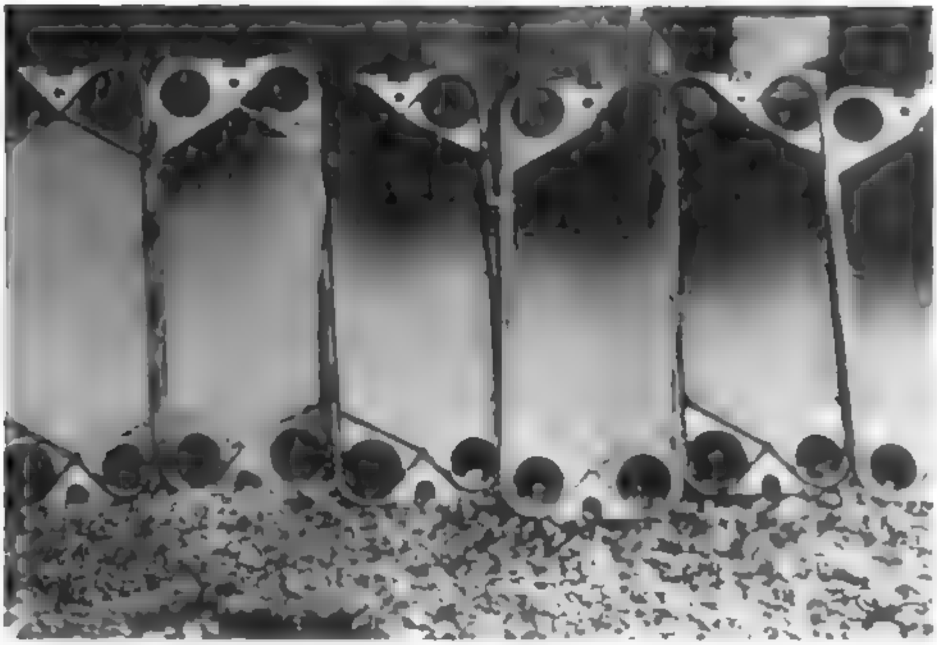


Figure 4. Fouled plates, test 2.

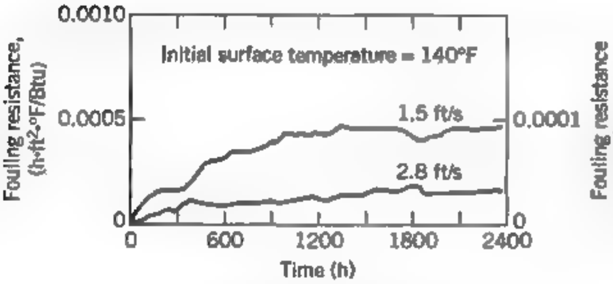


Figure 5. Typical PHE fouling curves.

Table 4. Fouling Deposit Analysis, %

	Series 2	Series 3	Series 4	Series 5	Series 6
Loss of ignition	—	25	25	17	21
Phosphorus	19	24	13	12	22
Aluminum	2	2	8	6	4
Silicon	1	4	16	16	13
Calcium	4	8	3	15	9
Chrome	5	13	15	15	9
Iron	2	7	4	5	18
Zinc	2	20	12	13	7
Sodium	—	1	1	—	1
Magnesium	—	—	1	—	1

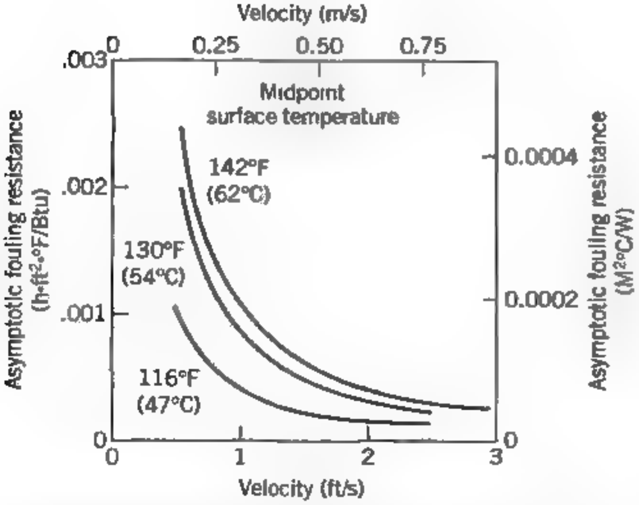


Figure 6. Asymptotic fouling resistance versus velocity with surface temperature as a parameter.

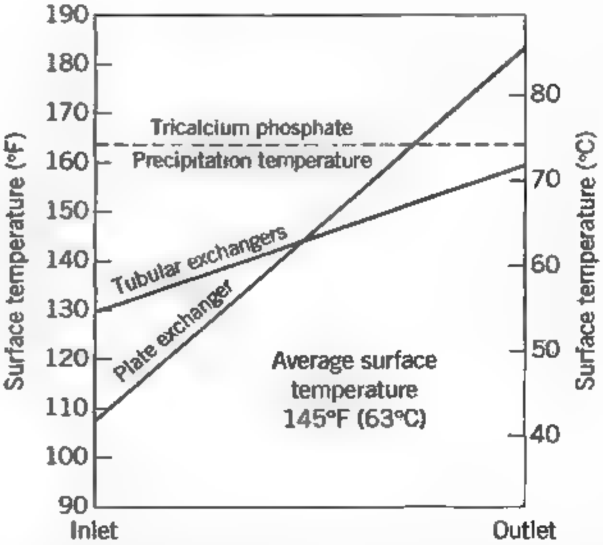


Figure 7. Surface temperature profile with indicated calcium phosphate precipitation.

three exchangers on the SFRU operate at the same midpoint surface temperature. Since the ratio of flow rate to surface area for the shell side of the shell and tube exchangers is twice that for the PHE, the cooling water had a longer residence time in the PHE than in the tubular exchanger. As a result, the lower flow rate to surface area ratio yields a larger temperature rise of the cooling water. The result is the steeper surface temperature profile shown in Figure 7. Although cocurrent operation was investigated as a correction for this problem, the required higher hot-water flow rate was not available. Consequently, the PHE data in some of these tests was slightly penalized because part of the surface is above the critical temperature for tricalcium phosphate precipitation, a condition that normally would not be encountered in industrial operations.

1. The loss of ignition (LOI) is performed by drying the sample to constant weight at 220°F (105°C) and then combusting at 1470°F (800°C). LOI represents bound water and organics present in the deposit. An organic test on samples of test series 5 showed that about 1/3 of the LOI was organic.
2. Aluminum, silicon, and iron come from the suspended solids. Aluminum comes from operation of the clarifier. The silicon is not from magnesium silicate since little magnesium is in the analyses.
3. Chrome, zinc, and phosphorus are derived from the water treatment.
4. Low values of calcium indicate little or no tricalcium phosphate. Most of the calcium comes from association with zinc and polyphosphates in the treatment.

It is difficult to make meaningful comparisons between the fouling tendencies of different types of heat exchangers. Unless carefully done, the results are misleading. This is particularly true when attempting to compare the fouling experience in the PHE to that of tubeside operation as in the PFRU. The geometries, surface area, and operational characteristics are very different even though the cooling water may be the same. Such comparisons often are attempted using TEMA recommended fouling resistances.

TEMA recommends a fouling resistance of 0.002 h · ft^2 · °F/Btu for the water system used. However, the maximum fouling resistance measured in the PHE was less than 0.0005 h · ft^2 · °F/Btu-only 25% of the TEMA recommendation. This confirms the earlier assumption that applying TEMA-recommended fouling resistances for a shell and tube exchangers to the PHE seriously handicaps the performance ratings. Because of the inherently high heat-transfer coefficients, the effects of fouling resistances are more pronounced. Consider a PHE operating at 1.5 ft/s. From Table 5, the fouled overall coefficient using a TEMA fouling resistance of 0.002 h · ft^2 · °F/Btu is about one half of the overall coefficient using the measured fouling resistance of this investigation. This example illustrates the need for caution in using TEMA fouling recommendations for equipment other than shell-and-tube exchangers.

Figure 8 compares the performance of the PHE with typical tube-side data and with the TEMA-recommended fouling resistance.

A direct comparison of the PHE and the shell-side exchangers can be made through the deposit analyses. A sample analysis is given in Table 6. The amounts of phosphates, calcium, chrome, and zinc are somewhat higher for

Table 5. Effects of Fouling Resistance on PHE Performance

	Overall heat-transfer coefficient		
		Fouling coefficient	
Operating velocity (both sides)	Clean	TEMA 0.002 h · ft^2 · °F/Btu (0.00035 m^2°C/W)	Present study 0.0005 h · ft^2 · °F/Btu (0.00009 m^2°C/W)
1.5 ft/s 0.45 m/s	1078 Btu/h · ft^2 · °F 6121 W/m^2 · °C	341 Btu/h · ft^2 · °F 1936 W/m^2 · °C	700 Btu/h · ft^2 · °F 3975 W/m^2 · °C

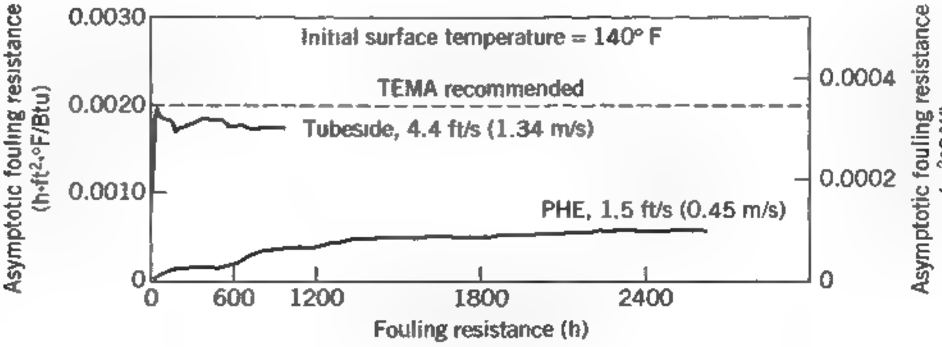


Figure 8. Comparison of tube-side and PHE fouling.

Table 6. Comparison of Deposit Analyses from Plate Exchanger and Shell-Side Test Exchanger for Series 5

	Fouling deposit analysis, %	
	Plate	Shell-side
Loss on ignition	17	24
Phosphorus	12	9
Aluminum	6	7
Silicon	16	33
Calcium	15	7
Chrome	15	9
Iron	5	4
Zinc	13	8

the PHE. The operation of the PHE at a higher surface temperature than the shell-side units resulted in more crystallization fouling. However, the amount of silicon for the shell-side unit is much higher than for the PHE. The high silicon concentration is due to sedimentation in the baffle-shell corners of the bundle, while the high turbulence promoted by the corrugated plates in the PHE minimizes sedimentation fouling. In heavily sediment-laden waters, the PHE would be especially superior.

CONCLUSIONS

Although the tests were performed on one water system only experience with tubular data indicates that the overall trends generally are valid; ie, plate exchangers should be designed to substantially lower values of fouling than would be used on tubular equipment for the same stream. However, the comparison is not always that straightforward, as flow velocity itself is not a valid criterion. The wall shear stress in a PHE operating at 2.8 ft/s is equivalent to that in a tube-side exchanger operating at 8.2 ft/s. On the basis of typical industrial velocities, it was found that the PHE at 1.5 ft/s fouls about one-half as much as a tube-side exchanger operating at 5.9 ft/s, Furthermore, because of the high-heat-transfer coefficients typical to the PHE, surface temperature may differ from those in tubular equipment and should be carefully watched.

The turbulence inducing corrugation pattern prevalent in heat-exchanger plates produces very high local velocities. This results in high friction factors and therefore high shearing. This high shear, in turn, results in less fouling in plate exchangers than in tubular units. For cooling-water duties, Heat Transfer Research Incorporated (HTRI) has shown that the plate exchanger fouls at a much lower rate than either the tube side or the shell side of tubulars. TEMA recommendations for fouling therefore should never be used for plate units since they probably are five times the value found in practice. In particular, the plate exchanger is far less susceptible to fouling with heavy sedimented waters. This is contrary to popular belief. It must be noted, however, that all particular matter must be significantly smaller than the plate gap and generally particles over 0.1 in. in diameter cannot be handled in any plate heat exchanger.

It has been shown that quoting a high fouling resistance can negate a plate heat exchanger design by adding large amounts of surface and thereby overriding the benefits of the high coefficients. Fouling design resistance, therefore, should be chosen with care, keeping in mind that with a Paraflow unit, it always is possible to add or subtract surface to meet exact fouling conditions.

DISCLOSURE STATEMENT

As a general policy, HTRI as the project operator disclaims responsibility for any calculations or designs resulting from the use of the date presented.

BIBLIOGRAPHY

Adapted from:
J. D. Usher and A. Cooper, "Paraflow Seminar, Part Four," *CPI Digest* 3, 6–9 (1974).
J. D. Usher and A. Cooper, "Cooling Water Fouling in Plate Heat Exchangers," *CPI Digest* 5, 2–9 (1979).

1. J. Taborek, T. Aoki, R. B. Ritter, J. W. Palen, and J. G. Knudsen, "Fouling—The Major Unresolved Problem in Heat Transfer, Parts I and II," *CEP* 68, (1972).
2. J. W. Suitor, W. J. Marner, and R. B. Ritter, "The History and Status of Research in Fouling of Heat Exchangers in Cooling Water Service," Paper No. 76-CSME/CSChE—19 presented at the 16th National Heat Transfer Conference, St. Louis, Missouri, August 8–11, 1976.
3. R. W. Morse and J. G. Knudsen, "Effect of Alkalinity of the Scaling of Simulated Cooling Tower Water," Paper No. 76-CSME/CSChE—24 presented at the 16th National Heat Transfer Conference, St. Louis, Missouri, August 8–11, 1976.
4. *Standards of Tubular Exchanger Manufacturers Association*, 5th Ed., New York, 1968.
5. P. Fisher, J. W. Suitor, and R. B. Ritter, "Fouling Measurement Techniques and Apparatus," *CEP* 71, 66 (1975).
6. J. Green and J. A. Holmes, "Calculation of the pH of Saturation of Tricalcium Phosphate," *Journal of the American Water Works Association* 39, (1947).

APV Crepaco, Inc.
Lake Mills, Wisconsin

HEAT EXCHANGERS: PARAFLOW

PRINCIPLES AND APPLICATIONS

The Paraflow is the original plate-type heat exchanger designed by APV to provide maximum efficiency and cost-effectiveness in handling thermal duties while minimizing maintenance downtime and floor space requirements.

Frame, Plates, and Gaskets

The Paraflow plate heat exchanger as shown in Figure 1 consists of a stationary head and end support connected

Table 6. Comparison of Deposit Analyses from Plate Exchanger and Shell-Side Test Exchanger for Series 5

	Fouling deposit analysis, %	
	Plate	Shell-side
Loss on ignition	17	24
Phosphorus	12	9
Aluminum	6	7
Silicon	16	33
Calcium	15	7
Chrome	15	9
Iron	5	4
Zinc	13	8

the PHE. The operation of the PHE at a higher surface temperature than the shell-side units resulted in more crystallization fouling. However, the amount of silicon for the shell-side unit is much higher than for the PHE. The high silicon concentration is due to sedimentation in the baffle-shell corners of the bundle, while the high turbulence promoted by the corrugated plates in the PHE minimizes sedimentation fouling. In heavily sediment-laden waters, the PHE would be especially superior.

CONCLUSIONS

Although the tests were performed on one water system only experience with tubular data indicates that the overall trends generally are valid; ie, plate exchangers should be designed to substantially lower values of fouling than would be used on tubular equipment for the same stream. However, the comparison is not always that straightforward, as flow velocity itself is not a valid criterion. The wall shear stress in a PHE operating at 2.8 ft/s is equivalent to that in a tube-side exchanger operating at 8.2 ft/s. On the basis of typical industrial velocities, it was found that the PHE at 1.5 ft/s fouls about one-half as much as a tube-side exchanger operating at 5.9 ft/s, Furthermore, because of the high-heat-transfer coefficients typical to the PHE, surface temperature may differ from those in tubular equipment and should be carefully watched.

The turbulence inducing corrugation pattern prevalent in heat-exchanger plates produces very high local velocities. This results in high friction factors and therefore high shearing. This high shear, in turn, results in less fouling in plate exchangers than in tubular units. For cooling-water duties, Heat Transfer Research Incorporated (HTRI) has shown that the plate exchanger fouls at a much lower rate than either the tube side or the shell side of tubulars. TEMA recommendations for fouling therefore should never be used for plate units since they probably are five times the value found in practice. In particular, the plate exchanger is far less susceptible to fouling with heavy sedimented waters. This is contrary to popular belief. It must be noted, however, that all particular matter must be significantly smaller than the plate gap and generally particles over 0.1 in. in diameter cannot be handled in any plate heat exchanger.

It has been shown that quoting a high fouling resistance can negate a plate heat exchanger design by adding large amounts of surface and thereby overriding the benefits of the high coefficients. Fouling design resistance, therefore, should be chosen with care, keeping in mind that with a Paraflow unit, it always is possible to add or subtract surface to meet exact fouling conditions.

DISCLOSURE STATEMENT

As a general policy, HTRI as the project operator disclaims responsibility for any calculations or designs resulting from the use of the date presented.

BIBLIOGRAPHY

Adapted from:

J. D. Usher and A. Cooper, "Paraflow Seminar, Part Four," *CPI Digest* 3, 6–9 (1974).

J. D. Usher and A. Cooper, "Cooling Water Fouling in Plate Heat Exchangers," *CPI Digest* 5, 2–9 (1979).

1. J. Taborek, T. Aoki, R. B. Ritter, J. W. Palen, and J. G. Knudsen, "Fouling—The Major Unresolved Problem in Heat Transfer, Parts I and II," *CEP* 68, (1972).
2. J. W. Suitor, W. J. Marner, and R. B. Ritter, "The History and Status of Research in Fouling of Heat Exchangers in Cooling Water Service," Paper No. 76-CSME/CSChE—19 presented at the 16th National Heat Transfer Conference, St. Louis, Missouri, August 8–11, 1976.
3. R. W. Morse and J. G. Knudsen, "Effect of Alkalinity of the Scaling of Simulated Cooling Tower Water," Paper No. 76-CSME/CSChE—24 presented at the 16th National Heat Transfer Conference, St. Louis, Missouri, August 8–11, 1976.
4. *Standards of Tubular Exchanger Manufacturers Association*, 5th Ed., New York, 1968.
5. P. Fisher, J. W. Suitor, and R. B. Ritter, "Fouling Measurement Techniques and Apparatus," *CEP* 71, 66 (1975).
6. J. Green and J. A. Holmes, "Calculation of the pH of Saturation of Tricalcium Phosphate," *Journal of the American Water Works Association* 39, (1947).

APV Crepaco, Inc.
Lake Mills, Wisconsin

HEAT EXCHANGERS: PARAFLOW

PRINCIPLES AND APPLICATIONS

The Paraflow is the original plate-type heat exchanger designed by APV to provide maximum efficiency and cost-effectiveness in handling thermal duties while minimizing maintenance downtime and floor space requirements.

Frame, Plates, and Gaskets

The Paraflow plate heat exchanger as shown in Figure 1 consists of a stationary head and end support connected

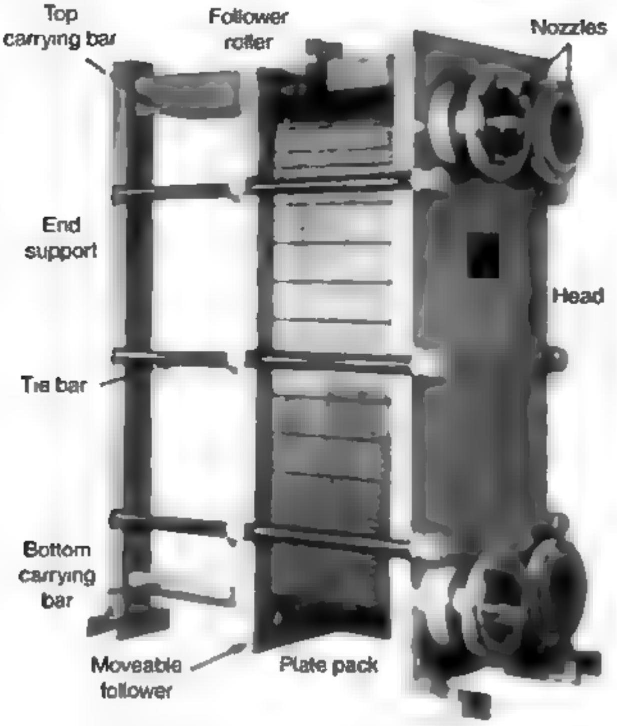


Figure 1. The APV CREPACO Paraflow plate heat exchanger.

by a top carrying bar and bottom guide rail. These form a rigid frame that supports the plates and moveable follower. In most units, plates are securely compressed between the head the follower by means of tie bars on either side of the exchanger. In a few models, central tightening spindles working against a reinforced end support are used for compression. When Paraflows are opened, the follower moves easily along the top bar with the aid of a bearing supported roller to allow full access to each individual plate.

With the exception of some sanitary models that are clad with stainless steel, Paraflow frames are fabricated of carbon steel and are finished in chemical-resistant epoxy paint. Frame ports accept bushings of stainless steel or alternative metals which, with various types of flanged or sanitary connections, form the inlet and outlet nozzles. By using intermediate connector plates as shown in Figure 2, units can be divided into separate sections to accommodate multiple duties within a single frame.

The closely spaced metal heat-transfer plates have troughs or corrugations that induce turbulence to the liquids flowing as a thin stream between the plates (Fig. 3).

The plates have corner ports which in the complete plate pack form a manifold for even fluid distribution to the individual plate passages (Fig. 4).

The seal between the plates is established by a peripheral gasket that also separates the thruport and flow areas with a double barrier. The interspace is vented to atmosphere to prevent cross-contamination in the rare event of leakage (Fig. 5).

As an exclusive feature, Paraflow heat-exchanger plates have interlocking gaskets in which upstanding lugs and scallops are sited intermittently around the outside edges. These scallops ensure that there are no unsupported portions of the gaskets and, in combination with the patented form of pressed groove, provide mechanical plate-to-plate support for the sealing system. The upstanding lugs (Fig. 6) maintain plate alignment in the Paraflow during pack closure and operation. The groove form provides 100% peripheral support of the gasket, leaving none of the material exposed to the outside. In addition, the gasket-groove design minimizes gasket exposure to the process liquid.

Plate Arrangement

Comparison of Paraflow plate arrangement to the tube and shell-side arrangement in a shell and tube exchanger is charted in Fig. 7. Essentially, the number of passes on the tube side of a tubular unit can be compared with the number of passes on a plate heat exchanger. The number of tubes per pass also can be equated with the number of passages per pass for the Paraflow. However, the comparison with the shell side usually is more difficult since with a Paraflow, the total number of passages available for the flow of one fluid must equal those available for the other fluid to within ±1. The number of cross passes

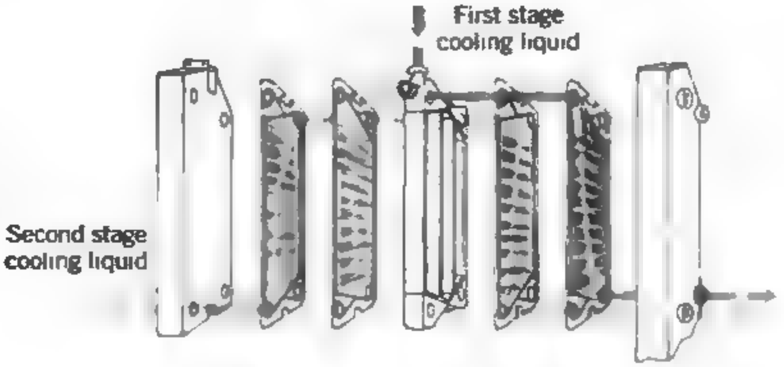


Figure 2. Two-section Paraflow with connector plate.

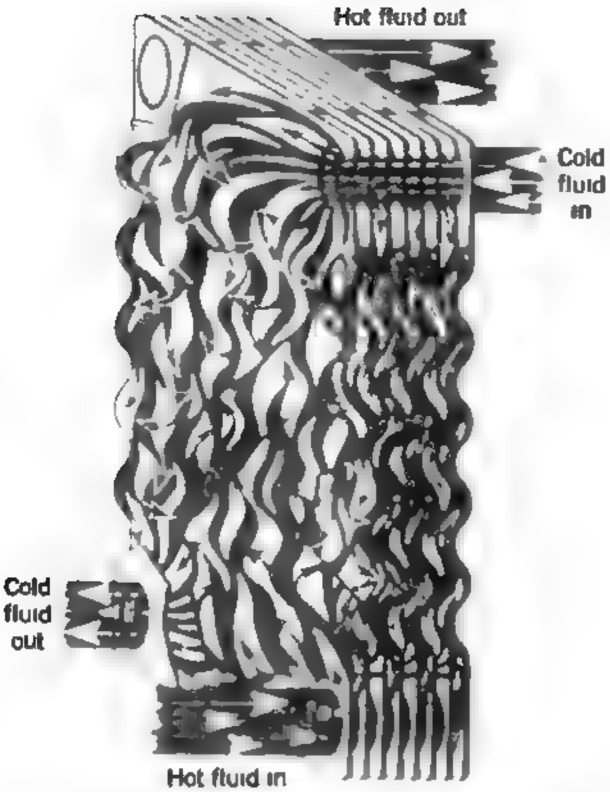


Figure 3. Cutaway of Paraflow plate shows turbulence during passage of product and service liquids.

on a shell, however, can be related to the number of plate passes and since the number of passages/pass for a plate is an indication of the flow area, this can be equated to the shell diameter. This is not a perfect comparison but it does show the relative parameters for each exchanger.

With regard to flow patterns, the Paraflow advantage over shell and tube designs is the ability to have equal passes on each side in full countercurrent flow, thus obtaining maximum utilization of the temperature difference between the two fluids. This feature is particularly important in heat-recovery processes with close temperature approaches and even in cases with temperature crossovers.

Whenever the thermal duty permits, it is desirable to use single-pass, countercurrent flow for an extremely efficient performance. Since the flow is pure counterflow, correction factors required on the LMTD approach unity. Furthermore, with all connections located at the head, the follower is easily moved and plates are more readily accessible.

Plate Construction

Depending on type, some plates employ diagonal flow while others are designed for vertical flow (Fig. 8). Plates are pressed in thicknesses between 0.020 and 0.036 in. (0.5–0.9 mm) and the degree of mechanical loading is important. The most severe case occurs when one process liquid is operating at the highest working pressure and the other at zero pressure. The maximum pressure differential is applied across the plate and results in a considerable unbalanced load that tends to close the typical 0.1–0.2-in. gap. It is essential, therefore, that some form of interplate support be provided to maintain the gap and this is done by two different plate forms.

One method is to press pips into a plate with deep washboard corrugations to provide contact points for about every 1–3 in.^{2} of heat-transfer surface (Fig. 9). Another is the chevron plate of relatively shallow corrugations with support maintained by the peak-peak contact (Fig. 10). Alternate plates are arranged so that corrugations cross to provide a contact point for every 0.2–1 in.^{2} of area. The plate then can handle a large differential pressure, and the cross pattern forms a tortuous path that promotes substantial liquid turbulence and thus a very high heat transfer coefficient.

Mixing and Variable-Length Plates

To obtain optimum thermal and pressure drop performance while using a minimum number of heat-exchanger plates, mixing and variable-length plates are available for several APV Paraflow plate heat-exchanger models. These

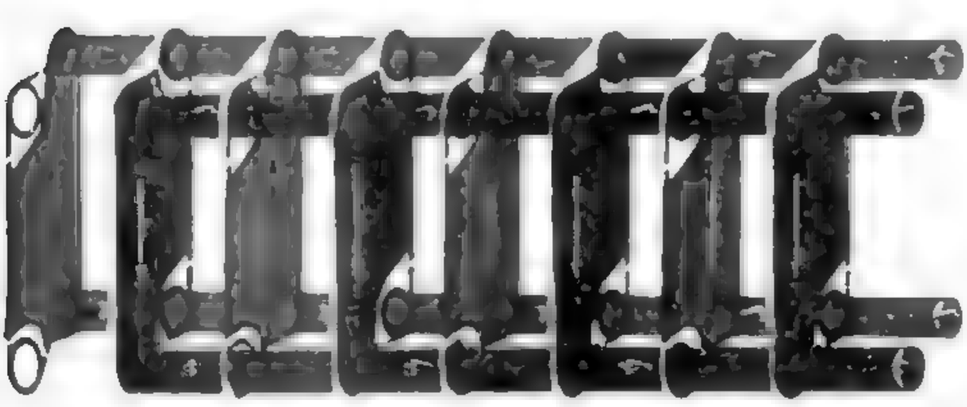


Figure 4. Single-pass countercurrent flow.

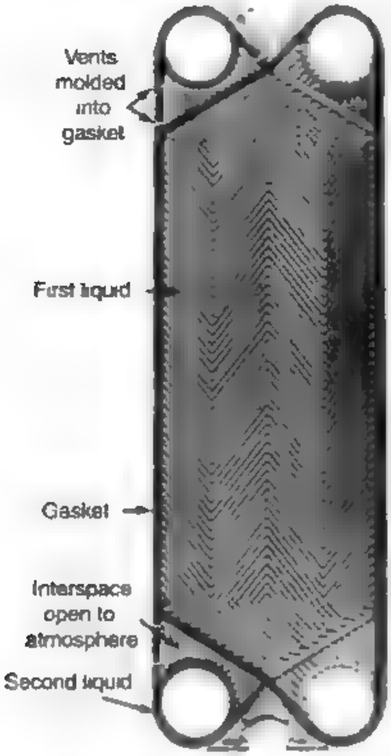


Figure 5. Gasket showing separation of throughport and flow areas.

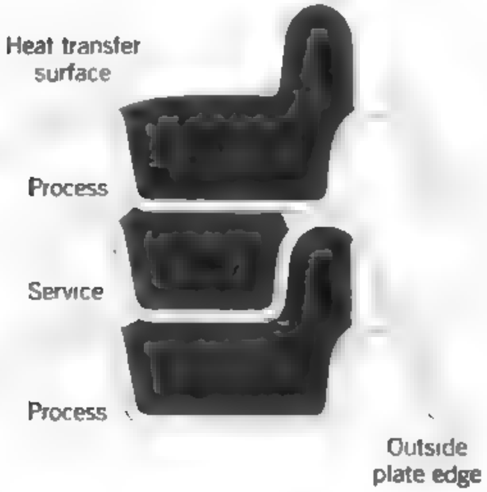


Figure 6. Exclusive interlocking gasket.

	Shell and Tube	Plate Equivalent	
Tube side	No. of passes	No. of passes	Side 1
Shell side	No. of tubes/pass	No. of passages/pass	Side 2
	No. of cross passes (No. of baffles + 1)	No. of passes	
	Shell diameter	No. of passages/pass	

Figure 7. Pass arrangement comparison: plate versus tubular.

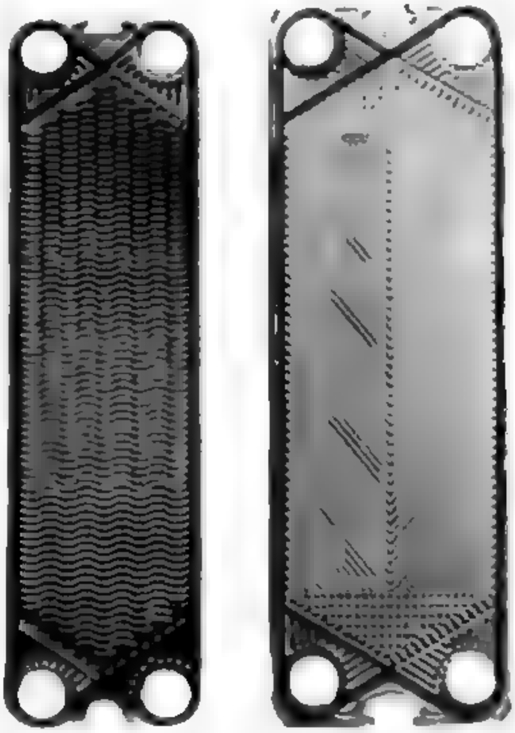


Figure 8. Diagonal and vertical flow patterns.

plates are manufactured to the standard widths specified for the particular heat exchanger involved but are offered in different corrugation patterns and plate lengths.

Since each type of plate has its own predictable performance characteristics, it is possible to calculate heat-transfer surface that more precisely matches the required thermal duty without oversizing the exchanger. This results in the use of fewer plates and a smaller, less expensive exchanger frame.

To achieve mixing, plates that have been pressed with different corrugation angles are combined within a single heat-exchanger frame. This results in flow passages that differ significantly in their flow characteristics and thus heat-transfer capability from passages created by using plates that have the same corrugation pattern.

For example, a plate pack (Fig. 11) of standard plates that have a typical 50° corrugation angle (to horizontal) develops a fixed level of thermal performance (HTU) per unit length. As plates of 0° angle (Fig. 12) are substituted into the plate pack up to a maximum of 50% of the total number of plates, the thermal performance progressively increases to a level that typically is twice that of a pack containing only 50° angle plates.

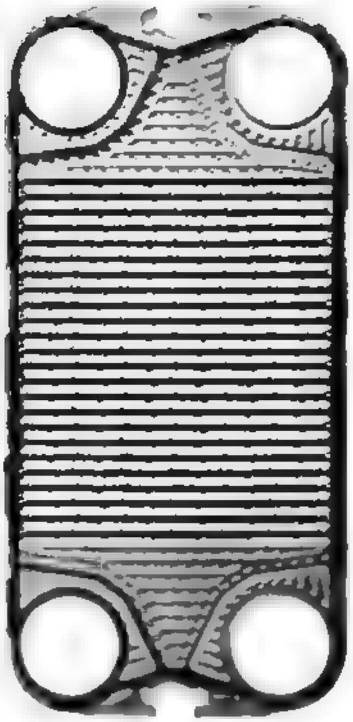


Figure 9. Corrugations pressed into plates are perpendicular to the liquid flow.

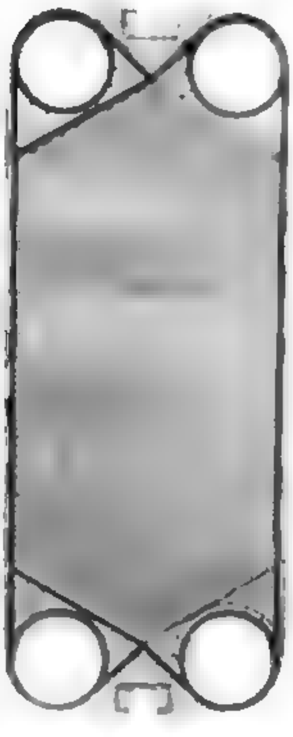


Figure 10. Troughs are formed at opposite angles to the centerline in adjacent plates.

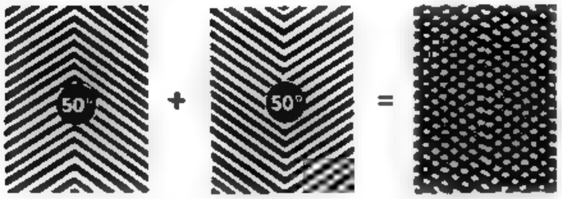


Figure 11. Low HTU passage.

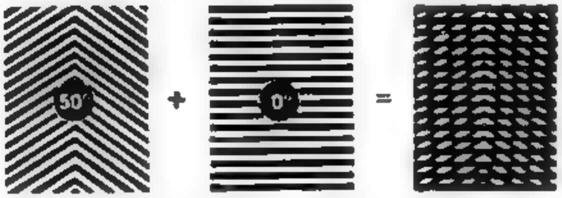


Figure 12. High HTU passage.

Thus, it is possible for a given plate length to fine-tune the Paraflow design in a single or even multiple-pass arrangement exactly to the thermal and pressure drop requirements of the application.

Of more recent development are plates of fixed width with variable lengths that extend the range of heat-transfer performance in terms of HTU. This is proportional to the effective length of the plate and typically, provides a range of 3–1 from the longest to the shortest plate in the series. As shown in Figure 13, mixing also is available in plates of varied lengths and further increases the performance range of the variable-length plate by a factor of approximately 2.

This extreme flexibility of combining mixing and variable-length plates allows more duties to be handled by a single-pass design, maintaining all connections on the stationary head of the exchanger to simplify piping and unit maintenance.

Plate Size and Frame Capacity

Paraflow plates are available with effective heat-transfer area from 0.28 to 50 ft^2, and up to 600 of any one size can be contained in a single standard frame. The largest Paraflow can provide in excess of 30,000 ft^2 of surface area. Flow ports are sized in proportion to the plate area and control the maximum permissible liquid throughput Figure 14. Flow capacity of the individual Paraflow, based on a maximum port velocity of 20-ft/s ranges from 15 gpm in the "junior" to 11,000 gpm in the Model SR235. This velocity is at first sight somewhat high compared to conventional pipework practice. However, the high fluid velocity is very localized in the exchanger and progressively is reduced as distribution into the flow passages occurs from the port manifold. If pipe runs are long, it is not uncommon to see reducers fitted in the piping at the inlet and exit connections of high-throughput machines.

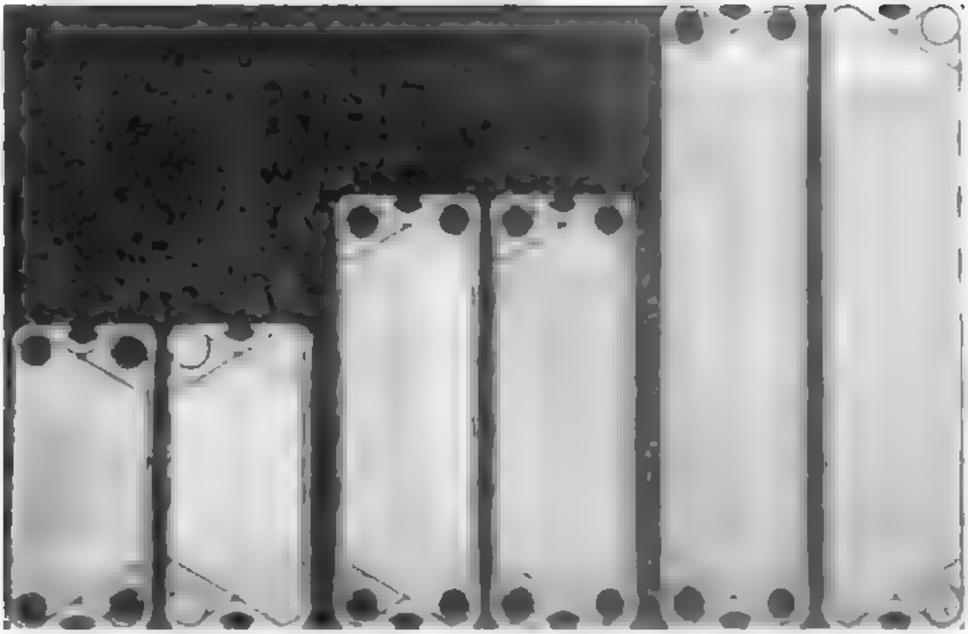


Figure 13. Variable-length plates with mixing options.

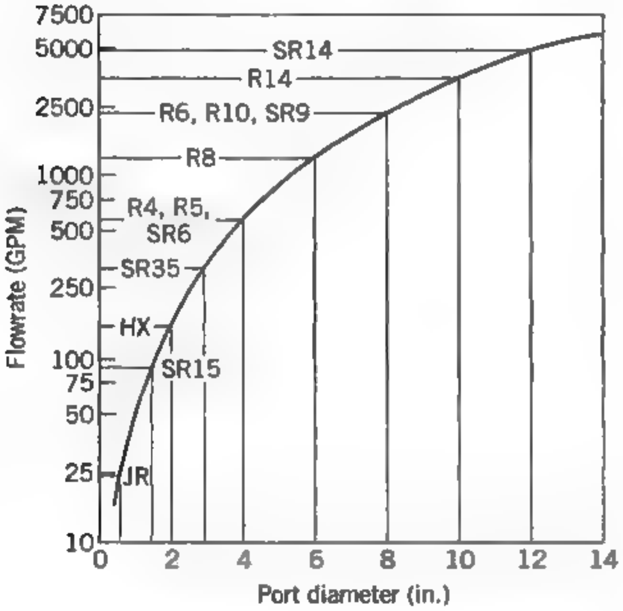


Figure 14. Throughput versus port diameter at 14 ft/s.

Plate Materials

Paraflow plates may be pressed from 304 or 316 stainless steels, Avesta 254SMO or 254SLX, nickel 200, Hastelloy B-2, C-276 or G-3, Incoloy 825, Inconel 625, Monel 400, titanium or titanium-palladium as required to provide suitable corrosion resistance to the streams being handled.

Gasket Materials

As detailed in Figure 15, various gasket materials are available as standard that have chemical and temperature resistance coupled with excellent sealing properties. These qualities are achieved by specifically compounding and molding the elastomers for long-term performance in the APV Paraflow.

Since the temperatures shown are not absolute, gasket material selection must take into consideration the chemical composition of the streams involved as well as the operating cycles.

THERMAL PERFORMANCE

The Paraflow plate heat exchanger is used most extensively in liquid—liquid duties under turbulent flow conditions. In addition, it is particularly effective for laminar flow heat transfer and is used in condensing, gas cooling, and evaporating applications.

Turbulent Flow

For plate heat transfer in turbulent flow, thermal performance can best be exemplified by a Dittus—Boelter-type equation:

$$\mathrm{Nu} = (C)(\mathrm{Re})^n(\mathrm{Pr})^m\left(\frac{\mu}{\mu_w}\right)^m$$

where Nu is the Nusselt number hD_e/k, Re is the Reynolds number vD_e/μ, Pr is the Prandtl number $C_p\mu/k$, D_e is the equivalent diameter (2× average plate gap), (μ/μ_w) is the Sieder—Tate correction factor, and reported values of the constant and exponents are

$$C = 0.15\text{–}0.40, \quad m = 0.30\text{–}0.45$$
$$n = 0.65\text{–}0.85, \quad x = 0.05\text{–}0.20$$

Typical velocities in plate heat exchangers for waterlike fluids in turbulent flow are 1–3 ft/s (0.3–0.9 m/s), but true

Gasket material	Approximate maximum operating temperature	Application
Paracril (medium nitrile)	275°F (135°C)	General aqueous service, aliphatic hydrocarbons
Paratemp (EPDM)	300°F (150°F)	High temperature resistance for a wide range of chemicals and steam
Paradur (fluoroelastomer)	400°F (205°C)	Mineral oils, fuels, vegetable and animal oils
Paraflor (fluoroelastomer)	400°F (205°C)	Steam, sulfuric acids

Figure 15. Paraflow gasket guide.

velocities in certain regions will be higher by a factor of ≧4 due to the effect of the corrugations. All heat-transfer and pressure drop relationships are, however, based on either a velocity calculated from the average plate gap or on the flow rate per passage.

Figure 16 illustrates the effect of velocity for water at 60°F on heat-transfer coefficients. This graph also plots pressure drop against velocity under the same conditions. The film coefficients are very high and can be obtained for a moderate pressure drop.

One particularly important feature of the Paraflow is that the turbulence induced by the troughs reduces the Reynolds number at which the flow becomes laminar. If the characteristic length dimension in the Reynolds number is taken at twice the average gap between plates, the Re number at which the flow becomes laminar varies from about 100 to 400 according to the type of plate.

To achieve these high coefficients, it is necessary to expend energy. With the plate unit, the friction factors normally encountered are in the range of 10–400 times those inside a tube for the same Reynolds number. However, nominal velocities are low and plate lengths do not exceed 7.5 ft so that the term $(V^2)L/(2g)$ in the pressure drop equation is much smaller than one normally would encounter in tubulars. In addition, single-pass operation will achieve many duties so that the pressure drop is efficiently used and not wasted on losses due to flow direction changes.

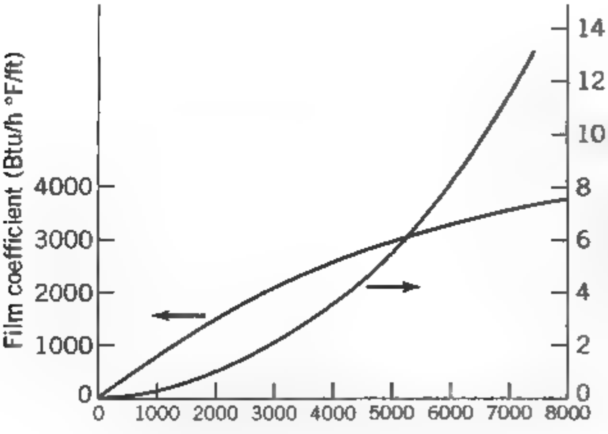


Figure 16. Performance details: Series HX Paraflow.

The friction factor is correlated with the equations:

$$f = \frac{B}{(\mathrm{Re})^{y}}, \quad \Delta p = \frac{fL_pV^2}{2gd}$$

where y varies from 0.1 to 0.4 according to the plate and B is a constant characteristic of the plate.

If the overall heat-transfer equation $Q = UA\Delta T$ is used to calculate the heat duty, it is necessary to know the overall coefficient U, the surface area A, and the mean temperature difference ΔT.

The overall coefficient U can be calculated from

$$\frac{1}{U} = r_{fh} + r_{fc} + r_w + r_{dh} + r_{dc}$$

The values of r_{fh} and r_{fc} (the film resistances for the hot and cold fluids, respectively) can be calculated from the Dittus—Boelter equations described previously and the wall metal resistance r_w can be calculated from the average metal thickness and thermal conductivity. The fouling resistances of the hot and cold fluids r_{dh} and r_{dc} often are based on experience.

The value taken for A is the developed area after pressing. That is the total area available for heat transfer and, because of the corrugations, will be greater than the projected area of the plate, ie, 1.81 ft^2 versus 1.45 ft^2 for an HX plate.

The value of ΔT is calculated from the logarithmic mean temperature difference multiplied by a correction factor. With single pass operation, this factor is about 1 except for plate packs of less than 20 when the end effect has a significant bearing on the calculation. This is due to the fact that the passage at either end of the plate pack only transfers heat from one side and therefore the heat load is reduced.

When the plate unit is arranged for multiple-pass use, a further correction factor must be applied. Even when two passes are countercurrent to two other passes, at least one of them must experience cocurrent flow. This correction factor is shown in Figure 17 against a number of heat

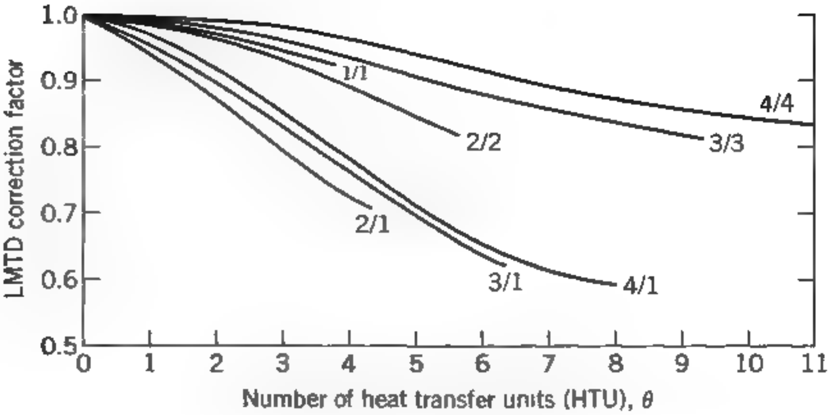


Figure 17. LMTD correction factor.

transfer units (HTU = temperature rise of the process fluid divided by the mean temperature difference). As indicated, whenever unequal passes are used, the correction factor calls for a considerable increase in area. This is particularly important when unequal flow conditions are handled. If high and low flow rates are to be handled, the necessary velocities must be maintained with the low fluid flow rate by using an increased number of passes. Although the plate unit is most efficient when the flow ratio between two fluids is in the range of 0.7–1.4, other ratios can be handled with unequal passes. This is done, however, at the expense of the LMTD factor.

The issue of how to specify a fouling resistance for a plate heat exchanger is difficult to resolve. Manufacturers generally specify 5% excess HTU for low fouling duties, 10% for moderate fouling, and 15–20% excess for high fouling. The allowed excess surface almost always is sufficient even though in many cases, it represents a low absolute value of fouling in terms of $(\text{Btu/h} \cdot \text{ft}^2 \cdot {}^\circ\text{F})^{-1}$. If a high fouling resistance is specified, extra plates have to be added, usually in parallel. This results in lower velocities, more extreme temperatures during startup, and the probability of higher fouling rates. Because of the narrow plate gaps and, in particular, because of the small entrance and exit flow areas, fouling in a plate usually causes more problems by the increase in pressure drop and/or the lowering of flow rates than by causing large reductions in heat-transfer performance. Increasing the number of plates does not increase the gap or throat area, and a plate unit sometimes can foul more quickly when oversurfaced.

The customer who does not have considerable experience with both the process and the plate heat exchanger should allow the equipment manufacturer to advise on fouling and the minimum velocity at which the exchanger should operate.

Laminar Flow

The other area suitable for the plate heat exchanger is that of laminar flow heat transfer. It has been pointed out already that the Paraflow can save surface by handling fairly viscous fluids in turbulent flow because the critical Reynolds number is low. Once the viscosity exceeds 20–50 cP, however, most plate heat exchanger designs fall into the viscous flow range. Considering only Newtonian fluids since most chemical duties fall into this category, in laminar ducted flow the flow can be said to be one of three types: (1) fully developed velocity and temperature profiles (ie, the limiting Nusselt case), (2) fully developed velocity profile with developing temperature profile (ie, the thermal entrance region), or (3) the simultaneous development of the velocity and temperature profiles.

The first type is of interest only when considering fluids of low Prandtl number, and this seldom exists with normal plate heat-exchanger applications. The third is relevant only for fluids such as gases that have a Prandtl number of about 1. Therefore, consider type 2.

As a rough guide for plate heat exchangers, the ratio of the hydrodynamic entrance length to the corresponding thermal entrance length is given by

$$\frac{L_{\text{th}}}{L_{\text{hyd}}} = 1.7 \text{ Pr}$$

Plate the heat transfer for laminar flow follows the Dittus—Boelter equation in this form:

$$\text{Nu} = c\left(\frac{\text{Re} \cdot \text{Pr} \cdot \text{De}}{L}\right)^{1/3}\left(\frac{\mu}{\mu_w}\right)^x$$

where L is the nominal plate length, c is the constant for each plate (usually in the range 1.86–4.50), and x is the exponent varying from 0.1 to 0.2 depending on plate type. For pressure loss, the friction factor can be taken as $f = a/\text{Re}$ where a is a constant characteristic of the plate.

It can be seen that for heat transfer, the plate heat exchanger is ideal because the value of d is small and the film coefficients are proportional to $d^{-2/3}$. Unfortunately, however, the pressure loss is proportional to d^{-4} and the pressure drop is sacrificed to achieve the heat transfer.

From these correlations, it is possible to calculate the film heat-transfer coefficient and the pressure loss for laminar flow. This coefficient combined with the metal coefficient and the calculated coefficient for the service fluid together with the fouling resistance then are used to produce the overall coefficient. As with turbulent flow, an allowance has to be made to use the LMTD to allow for either end effect correction for small plate packs and/or concurrency caused by having concurrent flow in some passages. This is particularly important for laminar flow since these exchangers usually have more than one pass.

Beyond Liquid–Liquid

Over many years, APV has built up considerable experience in the design and use of Paraflow plate heat exchangers for process applications that fall outside the normal turbulent flow that is common in chemical operations. The Paraflow, for example, can be used in laminar flow duties, for the evaporation of fluids with relatively high viscosities, for cooling various gases, and for condensing applications where pressure drop parameters are not overly restrictive.

Condensing

One of the most important heat-transfer processes if possible, is the condensation of vapors—a duty that often is carried out on the shell side of a tubular exchanger but is entirely feasible in the plate-type unit. Generally speaking, the determining factor is pressure drop.

For those condensing duties where permissible pressure loss is less than one pound per square inch, there is no doubt but that the tubular unit is most efficient. Under such pressure drop conditions, only a portion of the length of a Paraflow plate would be used and substantial surface area would be wasted. However, when less restrictive pressure drops are available the plate heat exchanger becomes an excellent condenser since very high heat-transfer coefficients are obtained and the condensation can be carried out in a single pass across the plate.

The pressure drop of condensing steam in the passages of plate heat exchangers has been investigated experimentally for a series of different Paraflow plates. As indicated in Figure 18, which provides data for a typical unit, the drop obtained is plotted against steam flow rate per passage for a number of inlet steam pressures.

It is interesting to note that for a set steam flow rate and a given duty, the steam pressure drop is higher when the liquid and steam are in countercurrent rather than cocurrent flow. This is due to differences in temperature profile.

Figure 19 shows that for equal duties and flows, the temperature difference for countercurrent flow is lower at the steam inlet than at the outlet with most of the steam condensation taking place in the lower half of the plate. The reverse holds true for cocurrent flow. In this case, most of the steam condenses in the top half of the plate, the mean vapor velocity is lower, and a reduction in pressure drop of 10–40% occurs. This difference in pressure drop becomes lower for duties where the final approach temperature between the steam and process fluid becomes larger.

The pressure drop of condensing steam therefore is a function of steam flow rate, pressure, and temperature difference. Since the steam pressure drop affects the saturation temperature of the steam, the mean temperature difference in turn becomes a function of steam pressure drop. This is particularly important when vacuum steam is being used since small changes in steam pressure can give significant changes in the temperature at which the steam condenses.

By using an APV computer program and a Martinelli—Lockhart-type approach to the problem, it has been possible to correlate the pressure loss to a high degree of accuracy. Figure 20 cites a typical performance of a steam heated Series R4 Paraflow. From this experimental run during which the exchanger was equipped with only a small number of plates, it can be seen that for a 4–5-psi pressure drop and above, the plate is completely used. Below that figure, however, there is insufficient pressure drop available to fully use the entire plate and part of the surface therefore is flooded to reduce the pressure loss. At only one psi allowable pressure drop, only 60% of the plate is used for heat transfer, which is not particularly economic.

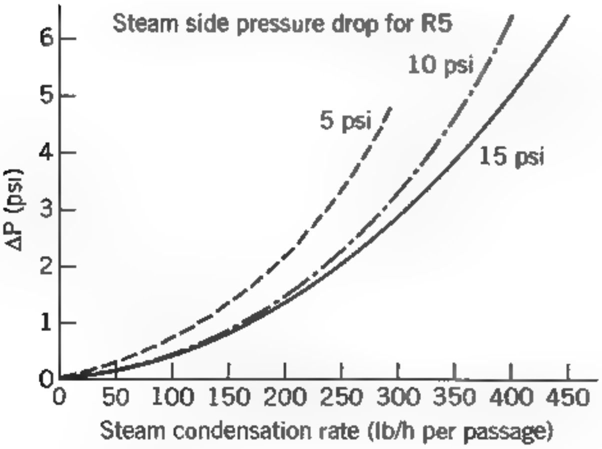


Figure 18. Steam-side pressure drop for R5.

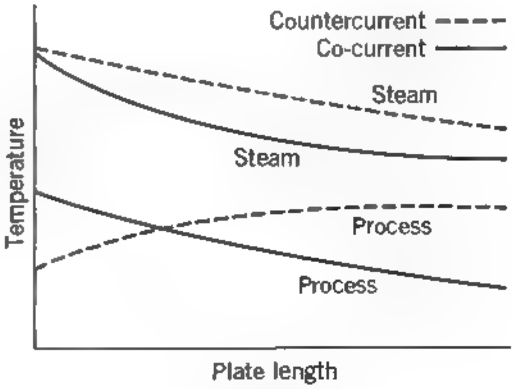


Figure 19. Temperature profile during condensation of steam.

Available pressure loss, psi	1	2	3	4
Total duty, Btu/h	207,000	256,000	320,000	333,000
Fraction of plate flooded	40	30	4	0
Effective overall heat-transfer coefficient, clean	445	520	725	770
Pressure loss, psi	1	2	4	4.5

Figure 20. Steam heating in an R4 Paraflow (water flow rate 16,000 lb/h, inlet water temperature 216°F, inlet steam temperature 250°F, total number of plates 7).

This example, however, well illustrates the application of a plate heat exchanger to condensing duties. If sufficient pressure loss is available, then the plate type unit is a good condenser. The overall coefficient of 770 Btu/h · ft^2 · °F for 4–5-psi pressure loss is much higher than a coefficient of 450–500 Btu/h · ft^2 · °F, which could be expected in a tubular exchanger for this type of duty. However, the tubular design would, for shell-side condensation, be less dependent on available pressure loss and for a 1-psi drop, a 450–500 Btu overall coefficient still could be obtained. With the plate, the calculated coefficient at this pressure is 746 Btu but the effective coefficient based on total area is only 60% of that figure or 445 Btu/h · ft^2 · °F.

Gas Cooling

Plate heat exchangers also are used for gas cooling with units in service for cooling moist air, hydrogen, and chlorine. The problems are similar to those of steam heating since the gas velocity changes along the length of the plate due either to condensation or pressure fluctuations. Designs usually are restricted by pressure drop, so machines with low pressure drop plates are recommended. A typical allowable pressure loss would be 0.5 psi with rather low gas velocities giving overall heat transfer coefficients in the region of 50 Btu/h · ft^2 · °F.

Evaporation

The plate heat exchanger also can be used for evaporation of highly viscous fluids when as a Paravap the evaporation occurs in the plate or as a Paraflash the liquid flashes after leaving the plate. Applications generally have been restricted to the soap and food industries. The advantage of these units is their ability to concentrate viscous fluids of up to 5000 cP.

CONCLUSION

It has been shown that the plate heat exchanger is a relatively simple machine on which to carry out a thermal design. Unlike the shell side of a tubular exchanger where predicting performance depends on baffle—shell leakage, baffle—tube leakage, and leakage around the bundle, it is not possible to have bypass streams on a Paraflow. The only major problem is that the pressure loss through the ports can cause unequal distribution in the plate pack. This is overcome by limiting the port velocity and by using a port pressure loss correlation in the design to allow for the effect of unequal distribution.

The flow in a plate also is far more uniform than on the shell side. Furthermore, there are no problems in calculation of heat transfer in the window, across the bundle, or allowing for dead spots as is the case with tubular exchangers. As a result, the prediction of performance is simple and very reliable once the initial correlations have been established.

BIBLIOGRAPHY

Adapted from:

J. D. Usher and A. Cooper, "Paraflow Seminar, Part One," *CPI Digest* **2**, 2–7 (1973). Copyrighted APV Crepaco, Inc. Used with permission.

APV Crepaco, Inc.
Lake Mills, Wisconsin

HEAT EXCHANGERS: PLATE VERSUS TUBULAR

SELECTION

In forming a comparison between plate and tubular heat exchangers, there are a number of guidelines that will generally assist in the selection of the optimum exchanger for any application. In summary, these are

1. For liquid—liquid duties, the plate heat exchanger usually has a higher overall heat-transfer coefficient and often the required pressure loss will be no higher.
2. The effective mean temperature difference will usually be higher with the plate heat exchanger.
3. Although the tube is the best shape of flow conduit for withstanding pressure, it is entirely the wrong shape for optimum heat-transfer performance since it has the smallest surface area per unit of cross sectional flow area.
4. Because of the restrictions in the flow area of the ports on plate units, it is usually difficult, unless a moderate pressure loss is available, to produce economic designs when it is necessary to handle large quantities of low density fluids such as vapors and gases.
5. A plate heat exchanger will usually occupy considerably less floor space than a tubular for the same duty.

6. From a mechanical viewpoint, the plate passage is not the optimum and gasketed plate units are not made for operating pressures much in excess of 300 psig.
7. For most materials of construction, sheet metal for plates is less expensive per unit area than tube of the same thickness.
8. When materials other than mild steel are required, the plate will usually be more economical than the tube for the application.
9. When mild steel construction is acceptable and when a close temperature approach is not required, the tubular heat exchanger will often be the most economic solution since the plate heat exchanger is rarely made in mild steel.
10. Plate heat exchangers are limited by the necessity that the gasket be elastomeric. Even compressed asbestos fiber gaskets contain about 6% rubber. The maximum operating temperature therefore is usually limited to 500°F.

Heat-Transfer Coefficients

Higher overall heat-transfer coefficients are obtained with the plate heat exchanger compared with a tubular for a similar loss of pressure because the shell side of a tubular exchanger is basically a poor design from a thermal point of view. Considerable pressure drop is used without much benefit in heat transfer because of the turbulence in the separated region at the rear of the tube. Additionally, large areas of tubes even in a well designed tubular unit are partially bypassed by liquid and low heat-transfer areas thus are created.

Bypassing in a plate-type exchanger is less of a problem, and more use is made of the flow separation that occurs over the plate troughs since the reattachment point on the plate gives rise to an area of very high heat transfer.

For most duties, the fluids have to make fewer passes across the plates than would be required through tubes or in passes across the shell. Since a plate unit can carry out the duty with one pass for both fluids in many cases, the reduction in the number of required passes means less pressure lost due to entrance and exit losses and consequently, more effective use of the pressure.

Mean Temperature Difference

A further advantage of the plate heat exchanger is that the effective mean temperature difference is usually higher than with the tubular unit. Since the tubular is always a mixture of cross and contraflow in multipass arrangements, substantial correction factors have to be applied to the log mean temperature difference. In the plate heat exchanger where both fluids take the same number of passes through the unit the LMTD correction factor is usually in excess of 0.95. As is illustrated in Figure 1, this factor is particularly important when a close or relatively close temperature approach is required.

In practice, it is probable that the sea water flow rate would have been increased to reduce the number of shells in series if a tubular had to be designed for this duty. While this would reduce the cost of the tubular unit, it would result in increased operating costs.

Design Case Studies

Figure 2 covering a number of case studies on plate versus tubular design demonstrates the remarkable heat-transfer performance that can be obtained from Paraflow units. Even for low to moderate available pressure loss, the plate heat exchanger usually will be smaller than a corresponding tubular.

Because of the high heat-transfer rates, the controlling resistance usually is fouling, so an allowance of 20–50% extra surface has been made based on APV experience.

One limitation of the plate heat exchanger is that it is rarely made in mild steel; the most inexpensive material of construction is stainless. Therefore, even when the plate surface requirement is much lower than the tubular on some heat-transfer duties, the tubular will be less expensive when mild steel construction is acceptable. If a close temperature approach is required, however, the plate unit always will cost less and where stainless steel or more exotic materials are required for process reasons, the plate unit usually will cost less.

Physical Size

One important advantage of the plate over a tubular unit is that for a particular duty, the plate heat exchanger will be physically smaller and require far less floor space. This

	Tubular	Plate
Size	3 shells in series: 20-ft tubes, 1 shell-side pass, 4 tube passes, 2238 tubes, 12,100-ft^2 area	R10 Paraflow with 313 plates arranged for 3 process passes and 3 service passes, 3630 ft^2 of area
Overall coefficient	270 Btu/h · ft^2 · °F	700 Btu/h · ft^2 · °F
MTD	7.72°F	9.0°F
Pressure loss	10–19 psi	10.8–8.8 psi

Figure 1. Duty: demineralized water/seawater. To cool 864,000 lb/h of water from 107°F to 82.4°F using 773,000 lb/h of 72°F sea water.

Case study	Fluid A (lb/h)	Fluid B (lb/h)	Duty (°F)	Tubular design pressure loss			Plate design pressure loss			
				A	B	ft²	A	B	ft²	type
A	13,380 hydrocarbon	53,383 water	320 A 120 120 B 92	0.42	3.05	301	0.3	2.2	92	HX
B	864,000 water	77,300 sea water	107 A 82.4 99.1 B 71.6	19	19	12,100	10.8	8.8	3,630	R10
C	17,500 solvent	194,000 water	140 A 104 95 B 79	2.9	4.5	1,830	3.0	3.7	445	R5
D	148,650 desalter effluent	472,500 salt water	222 A 100 106 B 68	10.8	8.0	1,500	4.5	10	380	R4

Figure 2. Case studies of plate heat exchangers and tubular designs. All tubular designs were carried out with the aid of the HTRI program ST3.

is shown most graphically in Figure 3, where a Series R405 Paraflow is being installed next to tubular units that need twice the amount of space for the identical duty. It is further illustrated in the volumetric comparisons of Figure 4.

If the tightened plate pack of a Paraflow is regarded as a rectangular box, each cubic foot contains 50–100 ft² of heat-transfer area according to the type of plate used. Allowing for metal thickness, the contained liquid is some 80% of this volume. Thus, the total of both heating and cooling media is about 5 gal. Expressed in another way, the liquid hold up per square foot for each stream varies according to plate type from about 0.06 gal down to 0.03 gal.

By comparison, one cubic foot of tubular exchanger of equilateral triangular pitch with a tube pitch-tube diameter ratio of 1.5 has a surface area of 10 ft² for 2-in.-OD tubes or 40 ft² for 1/2-in.-OD tubes. The average contained liquid is proportionately 0.27 gal/ft² down to 0.07 gal/ft² of heat-exchanger area with no allowance for the headers. If the heat-transfer coefficient ratio between plate and tubular is conservatively taken as 2, the plate exchanger volume to meet a given duty varies from 1/10 to 1/3 of that of the tubular. For a lower tube pitch-tube diameter ratio of 1.25, the comparison becomes 1/7 to 1/5. These facts demonstrate why the Paraflow plate heat exchanger is referred to as "compact."

Figure 3. At the Glidden Durkee Baltimore plant, a Series R405 Paraflow is being readied to cool 1000 gpm of deionized water. This unit requires only half the space of a tubular exchanger for the same duty.

Thermal Limitations

While it would appear offhand that the plate heat exchanger always provides a better performance at usually a lower price than the tubular exchanger, consideration must be given to the thermal as well as mechanical limitations of the plate-type machine. These usually are based on allowable pressure loss.

For single-phase liquid—liquid duties, the plate heat exchanger can be designed for moderately low pressure loss. However, if the pressure loss across any plate passage that has liquid flowing downward is lower than the available liquid static head, the plate will not run full and performance therefore will be reduced. This is termed low plate rate. Use of a plate below the minimum plate rate is inadvisable since it causes a wastage of surface area and results in unreliable operation. It is, however, possible to function below the minimum plate rate in a single-pass arrangement by making sure that the low plate rate is operated with a climbing liquid flow.

These problems are not quite so severe with a tubular exchanger and therefore, operation at a moderately lower available pressure loss is possible.

Conclusion

To summarize, the gasketed plate heat exchanger generally will be the most economical heat exchanger for liquid—liquid duties provided the material of construction is not mild steel and provided the operating temperatures and pressures are below 500°F and 300 psig respectively. For other types of duty such as gas cooling, condensation or boiling, the plate heat exchanger can be a very econom-

Plate			Tube			
Plate pitch (in.)	Heat-transfer area per ft^3 of exchanger (ft^2)	Liquid contained per ft^2 of heat-transfer area (gal)	Tube diameter (in.)	Ratio tube pitch tube diameter	Heat-transfer area per ft^3 of exchanger (ft^2)	Liquid containment per ft^2 of heat-transfer area (average of both sides) (gal)
1/4	50	0.06	2	1.5	10	0.32
1/8	100	0.03	1/2	1.5	40	0.085

Figure 4. Volumetric comparison, plate versus tube.

ical type of unit if the pressure loss allowed is sufficient to utilize the very high heat-transfer performance characteristics of the plate.

RECOVERY OF PROCESS HEAT

Since it is quite clear that never again will energy be as inexpensive as in the past, it therefore is necessary to conserve this natural resource—to recover more of the process heat that currently is dissipated to waterways and atmosphere. Some of this heat can be recovered with the aid of high-efficiency heat exchangers, which can operate economically with a close temperature approach at relatively low pumping powers. One type of unit that is particularly suited for this duty is the APV Paraflow plate heat exchanger. For many applications, this equipment can transfer heat with almost true countercurrent flow coupled with high coefficients to provide efficient and inexpensive heat transfer.

Unfortunately, the plate heat exchanger has been considered by many chemical engineers to be suitable only for hygienic heat-transfer duties. This, of course, is not so. Nowadays, many more plate type units are sold for chemical and industrial use than are sold for hygienic applications. A further mistake is the claim that the plate heat exchanger can be used only for duties when the volumetric flows of the two fluids are similar. Again, this is not true, although it must be stated that the plate heat exchanger is at its most efficient when flows are similar.

Basic Considerations

Since plate and tubular heat exchangers are the most widely used types of heat-transfer equipment, it is well to draw a brief comparison of their respective heat-recovery capabilities for the energy-conscious plant manager.

While the plate heat exchanger does have mechanical limitations with regard to withstanding high operating pressures above 300 psig, it is more efficient thermally than shell and tube units, especially for liquid—liquid duties. In many waste heat recovery applications, however, both pressure and temperature generally are moderate and the plate-type unit is an excellent choice since its thermal performance advantage becomes very significant for low-temperature approach duties. Higher overall-heat transfer coefficients are obtained with the plate unit for a similar loss of pressure because the shell side of a tubular basically is a poor design from a thermal point of view. Pressure drop is used without much benefit in heat transfer on the shell side due to the flow reversing direction after each cross pass. In addition, even in a well-designed tubular heat exchanger, large areas of tubes are partially bypassed by liquid and areas of low heat transfer thus are created. Conversely, bypassing of the heat transfer area is far less of a problem in a plate unit. The pressure loss is used more efficiently in producing heat transfer since the fluid flows at low velocity but with high turbulence in thin streams between the plates.

For most duties, the fluids also have to make fewer passes across the plates than would be required either through tubes or in passes across the shell. In many cases, the plate heat exchanger can carry out the duty with one pass for both fluids. Since there are fewer passes, less pressure is lost as a result of entrance and exit losses and the pressure is used more effectively.

A further advantage of the plate heat exchanger is that the effective mean temperature difference usually is higher than with the tubular. Since in multipass arrangements the tubular is always a mixture of cross and contraflow, substantial correction factors have to be applied to the log mean temperature difference. In the plate unit for applications where both fluids take the same number of passes through the exchanger, the LMTD correction factor approaches unity. This is particularly important when a close or even relatively close temperature approach is required.

Thermal Performance Data

Although the plate heat exchanger now is widely used throughout industry, precise thermal performance characteristics are proprietary and thus unavailable. It is possible, however, to size a unit approximately for turbulent flow liquid—liquid duties by use of generalized correlations that apply to a typical plate heat exchanger. The basis of this method is to calculate the heat-exchanger area required for a given duty by assuming that all the available pressure loss is consumed and that any size unit is available to provide this surface area.

For a typical plate heat exchanger, the heat transfer can be predicted in turbulent flow by the following equation

$$\frac{hD_e}{k} = 0.28\left(\frac{GD_e}{\mu}\right)^{0.65}\left(\frac{Cp\mu}{k}\right)^{0.4} \tag{1}$$

$$f = 2.5\left(\frac{GD_e}{\mu}\right)^{0.3} \tag{2}$$

$$\Delta P = \frac{2fG^2L}{g\rho D_e} \tag{3}$$

The pressure loss can be predicted from equations 2 and 3.

Obviously, equation 1 cannot represent accurately the performance of the many different types of plate heat exchangers that are manufactured. However, plates that have higher or lower heat-transfer performance than given in equation 1 usually will give correspondingly higher or lower friction factors in equation 2. Experience indicates that the relationship for pressure loss and heat transfer is reasonably consistent for well-designed plates. In the Appendix, equations A1–A3 are further developed to show that it is possible for a given duty and allowable pressure loss to predict the required surface area. This technique has been used for a number of years to provide an approximate starting point for design purposes and has given answers to ±20%. For accurate designs, however, it is necessary to consult the manufacturer.

Heat-Recovery Duties

In any heat-recovery application, it always is necessary to consider the savings in the cost of heat against the cost of the heat exchanger and the pumping of fluids. Each case must be treated individually since costs for heat, electricity, pumps, etc will vary from location to location.

One point is obvious. Any increase in heat recovery, and thus heat load, results in a decrease in LMTD and considering a constant heat-transfer coefficient, subsequently in the cost of the exchanger. This effect is tabulated in Figure 5. Because the cost of an exchanger increases considerably for relatively small gains in recovered heat above the 90% level, such applications, even with the plate heat exchanger, must be studied closely to verify economic gain. The economic break-even point is far lower for a tubular exchanger. Situations where it is advantageous to go above 90% recovery usually involve duties where higher heat recovery reduces subsequent heating or cooling of the process stream. High steam or refrigeration costs therefore justify these higher heat recoveries.

As shown in Figure 5, the cost of increasing heat recovery from 85 to 90% at a constant pressure loss of 12 lb/in.^{2} is $2600. From a practical standpoint, going from 90 to 95% requires a significantly higher pressure loss and nearly doubles the exchanger cost. However, even with this 95% heat recovery and assuming steam costs at $6.00/1000#, payback on the plate heat exchanger would take 530 hs.

The plate heat exchanger thus provides a most ecoXnomic solution for recovering heat (Fig. 6). This degree of heat recovery cannot be achieved economically in a tubular exchanger since the presence of cross flow and multipass on the tube side causes the LMTD correction factors to become very small or, alternately, requires more than one shell in series. This is shown in the Figure 7 comparison.

As detailed, this example illustrates that the plate heat exchanger has considerable thermal and therefore price advantage over the tubular exchanger for a heat recovery of 70%. Since the overall heat-transfer coefficient and the effective mean temperature difference both are much higher for the plate unit, reduced surface area is needed. Furthermore, because of cross-flow temperature difference problems in the tubular, three shells in series were needed to handle the duty within the surface area quoted. Using only two shells would have resulted in a further 40% increase in surface area.

The small size of the plate heat exchanger also results in a saving of space and a lower liquid holdup. For this type of heat recovery duty, a stainless-steel plate heat exchanger almost always will be less expensive than a mild steel tubular unit. Although the tubular exchanger physically will be capable of withstanding higher temperatures and pressures, there is a considerable and for the most part

Heat recovered (Btu/h)	%	Temperature (°F)	12 lb/in.^{2} Pressure loss					25 lb/in.^{2} Pressure loss				
			LMTD	Factor	Actual MTD	Area (ft^2)	Relative price ($)	LMTD	Factor	Actual MTD	Area, (ft^2)	Relative price ($)
3,600,000	60	200 → 140 160 ← 100	40	0.985	39.4	85	5,500	40	0.985	39.4	74	5,400
4,500,000	75	200 → 125 175 ← 100	25	0.975	24.4	197	6,450	25	0.980	24.5	172	6,250
5,100,000	85	200 → 115 185 ← 100	15	0.965	14.4	425	8,400	15	0.965	14.4	362	7,850
5,400,000	90	200 → 110 190 ← 100	10	0.95	9.5	739	11,000	10	0.92	9.2	629	10,100
5,700,000	95	200 → 105 195 ← 100	5					5	0.83	4.16	1580	18,100

Hot liquid 60,000 lb/h of water at 200°F; cold liquid 60,000 lb/h of water at 60°F.

Figure 5. Effect of percentage heat recovery on PHE cost.

Figure 6. Two shrouded Paraflow units provide an optimum return on investment with energy and cost saving regeneration of 88 and 81%.

Duty: To heat 1,300,000 lb/h of water from 73 to 97°F using 1,300,000 lb/h of water at 107°F. Available pressure loss 15 lb/in.^{2} for both streams.

Heat recovery = 70.5%
Number of heat-transfer units (HTU) = 2.4

	Plate	**Tubular**	
Heat-transfer area	4820	13,100	ft^2
Heat-transfer coefficient (clean)	734	386	Btu/h · ft^2 · °F
Heat-transfer coefficient (dirty)	641	271	Btu/h · ft^2 · °R
Effective mean temperature difference	10.0	8.7	°F
Pressure drop: hot fluid/cold fluid	4.6/4.7	15/15	lb/in^2
Fouling resistance: hot fluid/cold fluid	0.0001/0.0001	0.0005/0.0005	(Btu/h · ft^2 · °F)$^{-1}$
Pass arrangement	1/1	4 tube side 3 shells in series	baffled
Approximate price	$95,000 Stainless steel	$135,000 Mild steel	

This example demonstrates that quite high heat-transfer coefficients can be obtained from a PHE with only a moderate pressure loss.

Figure 7. Heat recovery—comparison between plate and tubular heat exchangers.

unnecessary penalty to pay for these features both in price and size.

For heat recovery duties in excess of 70%, the plate heat exchanger will become increasingly more economical than the tubular.

Typical Applications

One of the more common uses of regeneration is found in many of the nation's breweries, where it is necessary to cool huge amounts of hot wort before it is discharged to fermentation tanks. Typical in scope is an operation where 850 barrels per hour of wort (220,000 lb/h) are cooled from 200 to 50°F by means of 242,000 lb/h (R = 1.1:1) of water entering at 35°F and being heated to 165°F. As a result; approximately 33,000,000 Btu/h are saved, there is an excellent water balance for use throughout the brewery, and no water is discharged to the sewer.

For a dairy, regeneration usually involves the transfer of heat from pasteurized milk to cooler raw milk entering the system. After initially heating 100,000 lb/hr of milk from 40 to 170°F by high temperature, short time pasteurization, 90% regeneration permits cooling of the milk back down to 40°F with a savings of 11,700,000 Btu/h. Only 10%

of the total heat or cooling must be supplied, and no cooling medium such as city water is used and discarded.

Chemically, there are many and varied regenerative applications. For desalination, APV has supplied a number of Paraflows, which are virtually perpetual motion machines. These units achieve 95% regeneration in heating 87,000 lb/h (175 gpm) from 70 to 197°F while cooling a secondary stream flowing at 78,000 lb/h from 214 to 73°F. Savings in the Btu load in this case are 11,050,000 per hour.

While flow rates in hot oil applications are quite low in comparison, temperatures are very high. It is possible to cool 400 gph of vegetable oil from 446 to 226°F while heating 400 GPH of oil from 200 to 400°F with 80% regeneration.

In the production of caustic soda, where very corrosive product streams are encountered, Paraflows with nickel plates are being used to cool 10,000 lb/h of 72% NaOH from 292 to 210°F while heating 14,000 lb/h of 50% NaOH from 120 to 169°F.

Cost and Efficiency

To examine a hypothetical case of Paraflow regeneration from the viewpoint of efficiency and dollar savings, consider the following process duty:

Duty Heat 100 GPM of fluid 1 from 50°F to 200°F while cooling 100 gpm of fluid 2 from 200 to 50°F (see Tables 1–3)

Under ordinary conditions, steam required to heat fluid 1 would be in the nature of 7500 lb/h with an equivalent cooling requirement for the second stream of 625 tons of refrigeration. Using 85% regeneration, however, the energy needs are drastically reduced.

At this point in the process, fluid 2 in a conventional system has been cooled to only 90°F by means of 85°F city water and will require supplemental refrigeration for final cooling to 50°F. At the same time, fluid 2 in an APV regenerative system has been cooled to 72.5°F by means of 85% regeneration and must be cooled further to 50°F.

Duty	Cool from 90 to 50°F	Cool from 72.5 to 50°F
Supplemental refrigeration required	$\frac{50,000 \times 40}{12,000}$ = 166.5 tons	93.5 tons
Assuming average electrical cost of 4¢/kWh	166.5 × 3.5 = 583.8 kWh	93.5 × 3.5 = 327.25 kWh
Annual supplemental refrigeration cost	$\frac{4}{100} \times 583.8 \times 24 \times 365$ = \$204,571	$\frac{4}{100} \times 327.25 \times 24 \times 365$ = \$114,669

Definition

The number of heat-transfer units is defined as the temperature rise of fluid one divided by the mean temperature difference. A further term for the HTU is the temperature ratio (TR), ie,

$$\text{HTU} = \frac{t_1 - t_2}{\text{MTD}}$$

where MTD = mean temperature difference.

Table 1. Energy Needs, Fluid 1

	Conventional	85% Regeneration
	Heat 100 GPM 50 → 200°F	Heat 100 GPM 50 → 177.5°F by cooling fluid 2 from 200 to 72.5°F
Steam required	$\frac{50,000 \times 150}{10_3}$ = 7500 lb/h to heat to 200°F	$\frac{50,000 \times 22.5}{10^3}$ = 1125 lb/h suppemental heat to raise temperature to 200°F
Assuming average steam cost: \$6.00 per 1000#		
Steam cost/h	7.5 × \$6.00 = \$45.00/h	1.125 × \$6.00 = \$6.75/h
Annual steam cost	\$45.00 × 24 × 365 = \$394,200	\$6.75 × 24 × 365 = \$59,130

Table 2. Energy Needs, Fluid 2

	Conventional	85% Regeneration
	Cool 100 GPM 200 → 50°F	Cool 100 GPM 200 → 72.5°F by heating fluid 1 from 50 to 177.5°F
Equivalent refrigeration requred	$\frac{7,500,000}{12,000}$ = 625 tons to cool to 50°F	.15 × 625 = 93.5 tons supplemental cooling to lower temperature to 50°F
Using available 85°F cooling-tower water	200 gpm to cool 200 → 90°F	None
	200 × 60 = 12,000 GPH	None
Assuming average water cost of \$0.05 per 1000 gal		
Water cost/h	12 × .05 = \$.60/h	None
Annual water cost	\$.60 × 24 × 365 = \$5250	None

Table 3. Cost Comparison

	Original Cost, $	Regenerative System Cost, $	Regenerative Savings, $
Steam	394,200	59,130	335,070 annually
Tower cooling water	5,250	0	5,250 annually
Refrigeration	204,571	114,669	89,902 annually
	604,021	173,799	430,222

Note. Capital cost of a Paraflow plate heat exchanger for the above duty would be approximately $17,000.

APPENDIX

For heat transfer in a typical PHE with turbulent flow one can write the heat transfer performance in terms of a dimensionless Dittus—Boelter equation

$$\frac{hD_e}{k} = 0.28\left(\frac{GD_e}{\mu}\right)^{0.65}\left(\frac{Cp\mu}{k}\right)^{0.4}\left(\frac{\mu}{\mu_w}\right)^{0.14} \qquad \text{(A1)}$$

For applications in turbulent flow it is usually sufficiently accurate to omit the Sieder—Tate viscosity ratio and therefore the equation reduces to

$$\frac{hD_e}{k} = 0.28\left(\frac{GD_e}{\mu}\right)^{0.65}\left(\frac{Cp\mu}{k}\right)^{0.4} \qquad \text{(A2)}$$

The pressure drop can be predicted in a similar exchanger by equations A3 and A4.

$$f = 2.5\left(\frac{GD_e}{\mu}\right)^{-0.3} \qquad \text{(A3)}$$

$$\Delta P = \frac{2fG^2L}{g\rho D_e} \qquad \text{(A4)}$$

To solve these equations for a particular duty it is necessary to know G, L, and D_e, and it is shown below how these can be eliminated to produce a general equation.

For any plate,

$$G = \frac{m}{A_f} \qquad \text{(A5)}$$

where m is the total mass flow rate and A_f the total flow area.

D_e is defined by APV as four times the flow area in a plate divided by the wetted perimeter. Since the plate gap is small compared with the width, then:

$$D_e = \frac{4 \times A_f}{\text{wetted perimeter}} \qquad \text{(A6)}$$

but since $A_s = L \times$ wetted perimeter where A_s is the total surface area:

$$L = \frac{A_s}{4A_f} D_e \qquad \text{(A7)}$$

and it is possible to eliminate L from equation A4.

Similarly, by substituting G using equation A5 in equations A2, A3, and A4 and then by rearranging the equations to eliminate A_f, it is possible to arrive at equation A8 for the film heat-transfer coefficient.

$$h = \frac{J_2}{J_1^{0.241}}\left(\frac{m}{A_s}\right)^{0.241} D_e^{-0.28} \qquad \text{(A8)}$$

where

$$J_1 = \frac{2.5\ \mu^{0.3}}{2g\rho\Delta P}$$

and

$$J_2 = 0.28\left(\frac{Cp\mu}{k}\right)^{0.4} k\mu^{-0.65}$$

That is, the film heat-transfer coefficient is expressed only in terms of the surface area and equivalent diameter. A computer program solves equation A8 using a constant value of D_e and using an empirical factor Z to account for port pressure loss and other deviations from equations A2–A4. The equation is solved using the HTU approach where

$$\text{HTU} = \frac{(t_1 - t_2)}{\text{MTD}} = \frac{UA_s}{m_1c_1}$$

That is, the number of heat-transfer units is the temperature rise of fluid 1 divided by the mean temperature difference.

U is defined as

$$\frac{1}{U} = \frac{1}{h_1} + \frac{1}{h_2} + \text{metal resistance}$$

where h_1 and h_2 are calculated from an empirical modification of the constants in equation A8. The powers in equation A8 are not modified.

BIBLIOGRAPHY

Adapted from:
- J. D. Usher and A. Cooper, "Paraflow Seminar, Part Two," *CPI Digest* 2, 6–10 (1974).
- J. D. Usher and A. Cooper, "Thermal Performance," *CPI Digest* 2, 10–14 (1974).
- J. D. Usher and A. Cooper, "Recovery of Process Heat," *CPI Digest* 3, 2–7 (1974).

APV Crepaco, Inc.
Lake Mills, Wisconsin

HEAT EXCHANGERS: SCRAPED SURFACE

BASIC CONSIDERATIONS

The scraped-surface heat exchanger (SSHE) is a specialized piece of heat-transfer equipment that was patented around 1926 by Clarence Vogt in an effort to develop a more efficient freezer for making ice cream. The design incorporated a scraping action to prevent buildup of frozen ice cream on heat-transfer surfaces. The concept was successful. Thermal efficiency was improved and production capacity greatly increased.

Since that time, the SSHE has become essential for numerous processes in the dairy and food industries. Many of these applications are similar to the ice cream problem in that, as the product is heated or cooled, it fouls heat-transfer surfaces and reduces efficiency. Such applications, for example, include cooling peanut butter or plasticizing shortening and margarine. In each instance, fat crystals that form are like the ice crystals found in ice cream. Without the scraping of the heat exchanger sweeping the solidified fats away from the surface, heat-exchange efficiency would fall off very rapidly. Conversely, in heating applications where a product tends to "burn on," the scraping action removes product from the surface before fouling can occur. Typical heating examples are aseptic cheese sauces and aseptic puddings where processing temperatures approach 300°F (150°C).

The modern SSHE also is capable of processing products containing particulates. Currently, standard units can handle 1/2-in. (13-mm) particles without excessive breakage while special designs are available to accommodate 1-in. (26-mm) particulates.

While more expensive than other types of heat exchanger, the SSHE is the best and only thermal approach in hundreds of applications where high viscosity, large particulates, crystallization, and burn-on are problems that must be considered.

A scraped-surface heat exchanger (Fig. 1) basically consists of a jacketed cylinder fitted with a rotating shaft on which scraper blades are mounted. Product is pumped through the cylinder while a heating or cooling medium is circulated in the annular space between the cylinder and the jacket. The blades are fixed to pins that allow them to swing freely. No springs are necessary since centrifugal force holds the blades in position against the inside of the cylinder wall as product constantly is swept away from the heat-transfer surface and new product exposed to treatment.

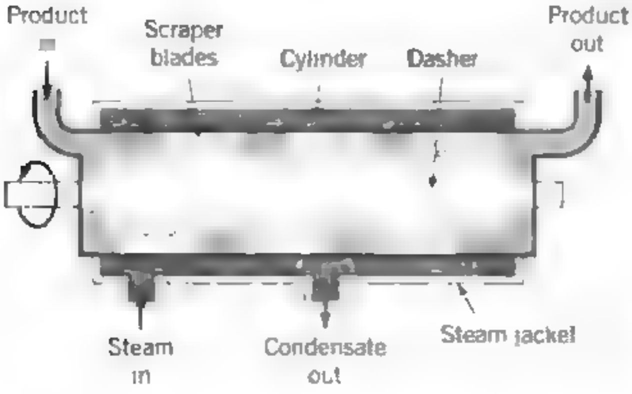


Figure 1. Cutaway of horizontal SSHE.

The standard horizontal exchanger generally has from one to three independently functioning jacketed cylinders mounted on a heavy steel base (Fig. 2, 3). A stainless-steel casing covers the cylinders, base and drives to form a completely enclosed system. Vertical units also are available for use when floor space is at a premium (Fig. 4).

Many processes require more than one unit, and in such cases where multiple cylinders are used, product always should be piped in series. Heat transfer will be higher because of the higher flow through each cylinder. Parallel

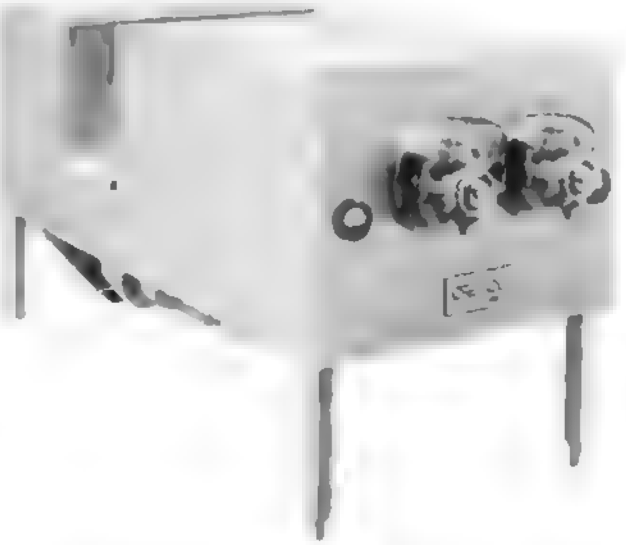


Figure 2. Model 2HD-648 SSHE with water flush seals.

Figure 3. A pair of scraped—surface heat exchangers provide an added dimension to an aseptic system by permitting the processing of fluids containing particulates.

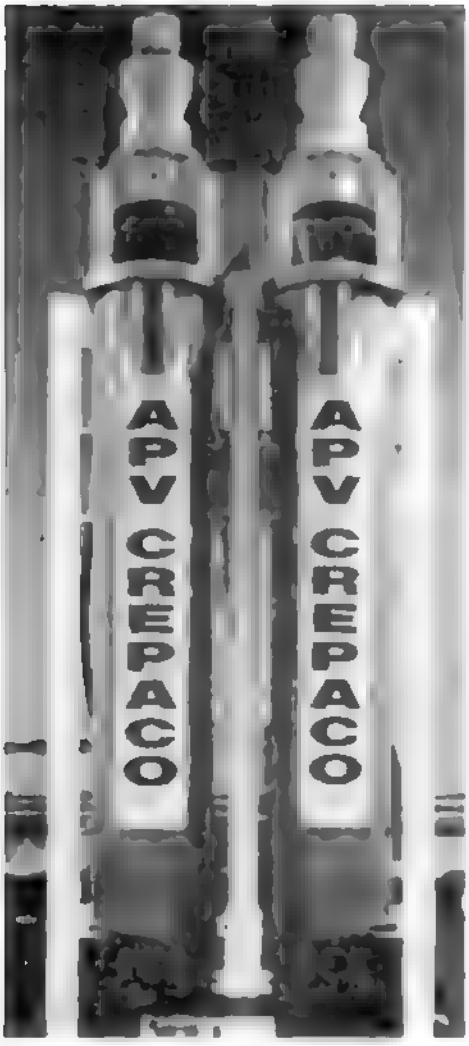


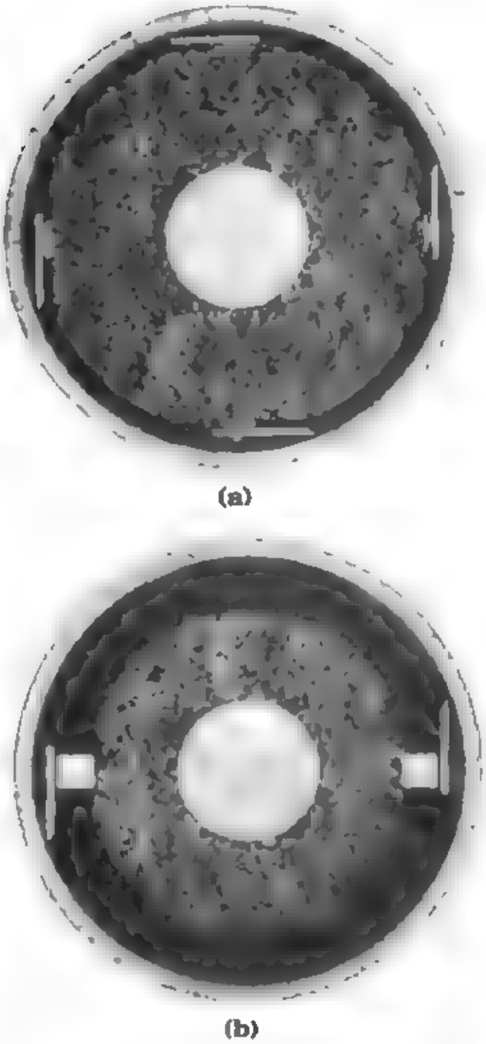
Figure 4. Vertical Model VExHD-884 SSHE.

flow arrangements fail because there is no way to ensure equal flow to each cylinder unless individual pumps are used for each circuit. It should be noted that a scraped surface heat exchanger does not do any pumping. A pump is required to move product through the unit.

Dashers

The shaft which carries the scraper blades is called a dasher by some manufacturers and a mutator by others—the "dasher" terminology being derived from early days when the exchanger initially was used as an ice cream freezer.

Dashers are engineered to achieve high heat-transfer coefficients with minimum power consumption and are supported by heavy-duty bearings located outside the product contact zone. Three standard designs (Fig. 5a, b, 6) provide product flow spaces of different sizes to accommodate different product viscosities, dwell time, level of blending, and size of particulates. For margarine or plasticizing applications, dashers with internal water circulation reduce adhesion of product to the dasher surface.


(a)

(b)

Figure 5. Dasher—blade assemblies cutaway. (a) Series 55; (b) Series 45.

Typical dasher speeds vary from 60 to 420 rpm with standard motors providing a choice of three drive methods—direct-driven hydraulic, belt-driven electric, or direct-driven gearhead.

Scraper Blades

Designed to promote the rapid removal of product from cylinder walls while enhancing product agitation and mixing, scraper blades are available in a selection of materials and configurations. Most common materials are stainless steel and plastics since the blade is the wearing part and as such, must be softer than the cylinder wall or lining.

Blade selection generally is determined by product temperature, pressure, and formulation as well as by the cylinder material and service media being used.

Heat-Exchange Cylinders

To provide optimum performance and economy of operation, heat-exchange cylinders are available in a selection of sizes and materials of construction.

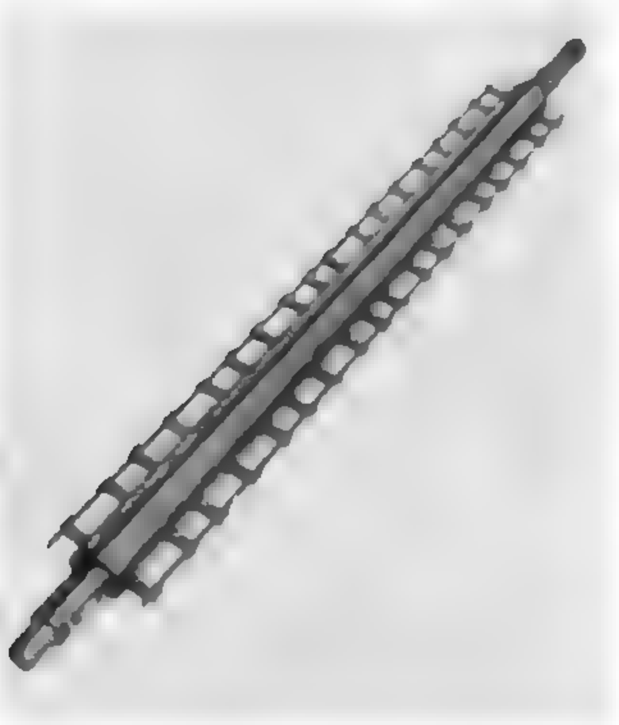


Figure 6. Series 30 dasher for viscous products or particulates.

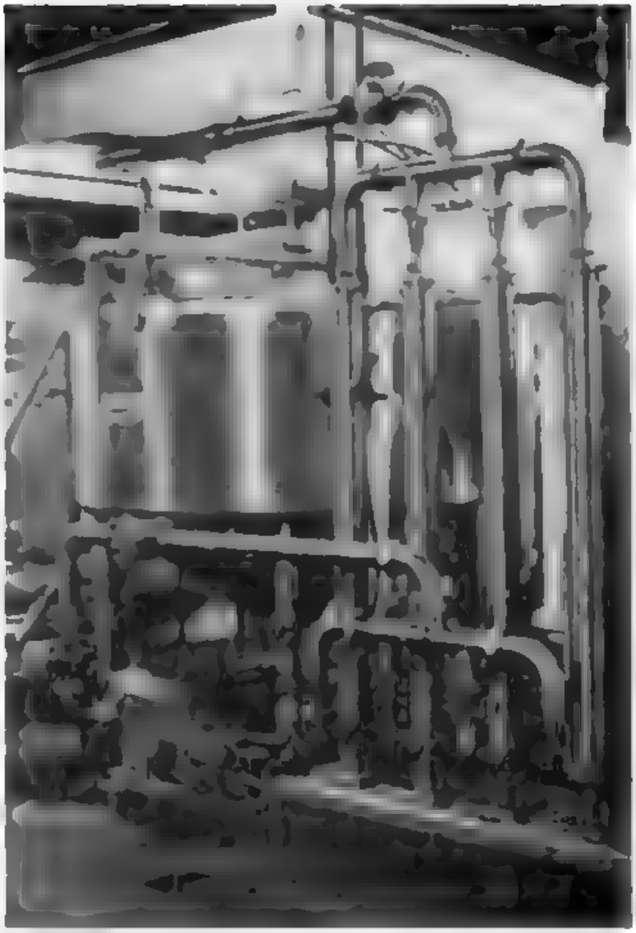


Figure 7. Four-barrel vertical scraped-surface heat exchanger is designed to cool meat gravies from 190 to 70°F. Unit capacity is 5000 lb/h.

The most common diameter for the scraped SSHE cylinder is 6 in. (152 mm) with lengths established at 48 in. (1220 mm) and 72 in. (1830 mm). There are, however, other sizes available. Some are 4 in. (102 mm) in diameter with lengths of 60 in. (1520 mm) (Fig. 7) and 120 in. (305 mm) while others are of 8 in. (203-mm) diameter and lengths to 84 in. (2130 mm).

The most common materials of construction are stainless steel, nickel with or without chrome plating, and, more recently, a bimetallic combination.

Since the cost per square foot of heat-transfer surface is higher for a SSHE than other types of heat exchangers, it is essential that cylinder material with the highest feasible heat-transfer coefficient be selected. This selection, however, must be tempered by consideration of the compatibility between cylinder and scraper blade materials and the susceptibility of these materials to acid attack and corrosion.

As charted in Table 1, nickel exhibits the best thermal conductivity while stainless steel is the least conductive. Even so, nickel is not suitable for all applications. It is relatively soft when compared to typical metal scraper blade materials and would wear rapidly if this combination were to be used. Since SSHE always are designed to make the blades rather than the more expensive cylinders the wearing part, plastic blades must be used with nickel cylinders if only for reasons of economics and reduced downtime. For the same reasons, only plastic blades are run on stainless-steel cylinders.

To retain the superior heat-transfer characteristics of nickel while benefiting from the extended durability of steel blades, nickel cylinders may be chrome-plated. The scraping surface then becomes the hardest material in use and can accommodate any of the common blade materials. The sole drawback is that the chrome is sensitive to salt and acid attack.

Table 1. Heat Transfer Coefficients of SSHE Cylinder Metals

Material	K, Btu/h · °F · in.
Nickel	52
Chrome nickel	52
Bimetallic	31
Stainless steel	9.4

Bimetallic cylinders offer some advantages over those of any other material, whether nickel, chromed nickel, or stainless. Produced from two different materials, the bimetallic cylinder usually is made by centrifugally casting a hard, corrosion-resistant alloy inside a tube that has high tensile strength and thermal conductivity approaching nickel. While the lining is not quite as hard as chrome, it is hard enough to withstand abrasion from both stainless-steel and plastic blades. One disadvantage other than a slight loss in heat transfer is that the inner alloy coating is susceptible to strong acid corrosion.

The selection of a cylinder with a compatible blade material is compounded by the products to be processed and the cleaning procedures used. For example, on slush freezing applications or where media temperature is below freezing, plastic blades can be abraded within hours as hard ice is scraped away. And as already noted, despite being the hardest cylinder material in use, chrome plated nickel is subject to attack by common acid CIP solutions and does not stand up well to salty products over extended periods of time. Table 2 shows the difference in corrosion for various materials and acids, and it is worth noting that it is the cleaning regimen rather than the product that causes problems in some cases. A change in CIP procedures may well extend the life or allow the use of nickel or chrome nickel cylinder material. In effect, much thought and experience must go into selecting the best cylinder and blade combinations—a selection best left up to the equipment manufacturer.

Cylinder Jackets—Media

The three common media used in a SSHE are water, steam, and refrigerants such as ammonia and Freon. Each requires a slightly different cylinder jacket design for optimum performance.

The water jacket is designed with a small spacing between the jacket and heat exchange cylinder. This induces high liquid media velocities that cause turbulence, which in turn, improves heat-transfer efficiency. It also minimizes fouling on the media side. Normally, countercurrent flow is recommended between product and media. In many cases, however, because of the high flow rate of media over product and the high temperature differences between media and product, the difference in performance between countercurrent and cocurrent flow is small.

On steam units, media enters the jacket via a header that distributes the steam over the entire length of the cylinder. Condensate runs to the bottom of the jacket, where it is collected and removed by a steam trap. Direction of product flow is immaterial.

When ammonia or Freon is used, the refrigerant is handled differentially by various manufacturers. Most exchanger designs are of the flooded type with media being fed at the bottom of the jacket and boiling occurring within the jacket. Since the key to efficient heat transfer is to maintain a wetted surface on the cylinder, not all of the refrigerant is allowed to evaporate. The combination of gas and liquid is carried over into a surge tank, where the phases are separated and the liquid recycled to the bottom of the jacket. Liquid level is maintained by a valve that allows more liquid to enter the surge tank to replace that which has been evaporated.

Table 2. Corrosion of SSHE Cylinder Metals

Acid	Common use/source	Material corrosion at 140°F (maximum in./yr)		
		Nickel	Chrome	316 SS
Nitric	Cleaning	0.050	nr[a]	0.002
Citric	Product	0.020	nr	0.002
Acetic	Product	0.020	nr	0.002
Phosphoric	Cleaning	0.050	nr	0.002
Malic	Product	0.020	nr	0.020
NaCl	Product	0.020	nr	0.020

[a] nr = not recommended.

One advantage of the flooded arrangement as illustrated in Figure 8 is the incorporation of a quick shutoff valve for instant stop/instant start. Although the valve is open during normal operation, it may be closed when a freeze-up is imminent or the operator want to stop cooling. Since the vapor cannot go up and out, it expands and rapidly pushes the liquid refrigerant down and away from the cylinder. When liquid no longer touches the cylinder and the surface no longer is wetted, all freezing stops. To restart the process, the valve is opened and with liquid quickly filling the jacket area, freezing begins again.

The primary advantage of the SSHE over other types of heat-transfer equipment is its ability to accommodate product viscosities of 500,000 cP and higher and to handle particulates up to 1 inch (26 mm) in size provided that they are not shear-sensitive. Where the product undergoes a major change of state, ie, liquid to gel, or of viscosity as occurs during cooling, the SSHE often is used as a finisher for either the heating or cooling mode in conjunction with other methods of heat transfer.

THEORY AND CALCULATIONS

The operating principle of scraped-surface heat exchange is based on the constant movement of a product away from the heat-exchange surface in order to minimize the formation of films that resist heat transfer.

In considering the scraped-surface heat exchanger, four types of thermal exchange are taken into account:

- *Sensible Heat*. The heat produced by the increase or decrease in temperature of a product (without change of state).
- *Latent Heat*. Heat exchange associated with a physical change in the material being processed.
- *Heat of Reaction*. The heat given off (exothermic) or taken up (endothermic) when two or more chemicals react.
- *Mechanical Heat*. Power is consumed in turning the dasher of a SSHE. Most of this energy is absorbed as heat energy into the product within the heat exchanger.

The ideal form of heat transfer occurs when one product at an elevated temperature is brought into direct contact with another material at a lower temperature. The warmer product gives up its heat without loss of energy and at a rate equivalent to the ability to mix or disperse the two materials.

In practice, however, it is rare that two materials may be brought into direct contact. As a general rule, there is an intervening heat-transfer surface such as a tank or tube

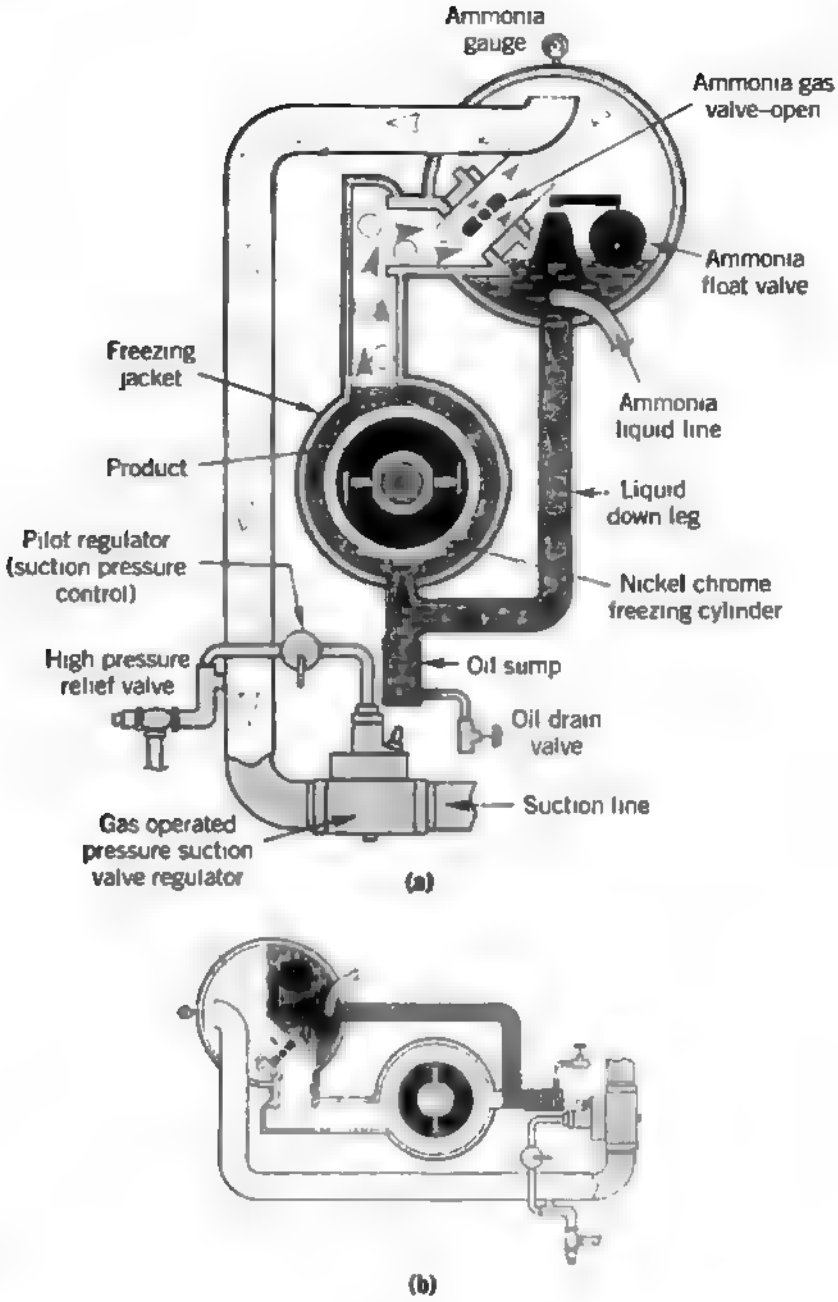

Ammonia gauge
Ammonia gas valve–open
Ammonia float valve
Freezing jacket
Ammonia liquid line
Product
Liquid down leg
Pilot regulator (suction pressure control)
Nickel chrome freezing cylinder
High pressure relief valve
Oil sump
Oil drain valve
Suction line
Gas operated pressure suction valve regulator
(a)
(b)

Figure 8. Full flooded SSHE refrigeration system (**a**) shown in operation, (**b**) shown shut down.

wall. This surface presents problems to heat transfer because it resists the passage of heat. It further induces resistances related to hydraulic drag and the buildup of deposits or other films that further retard the passage of heat. Figure 9 illustrates the various resistances that may be encountered.

Since these resistances are in series, they are additive. The adverse effect, however, may be reduced by agitating the fluids on both sides of the wall. Since the SSHE eliminates buildup of deposits on the product side of the tube wall, the resistance to heat transfer is minimized.

The total resistance to heat transfer then is

$$R = R_1 + R_2 + R_3$$

The difference in temperatures between the product and the media is the driving force to push across this resistance. Therefore, the equation for heat transfer is

$$\text{heat-transfer rate, Btu/h} \cdot \text{ft}^2 = \frac{\text{temperature, °F}}{\text{resistance}}$$

This equation can be developed into a useful form by letting the temperature difference be ΔT and resistance be R. Therefore

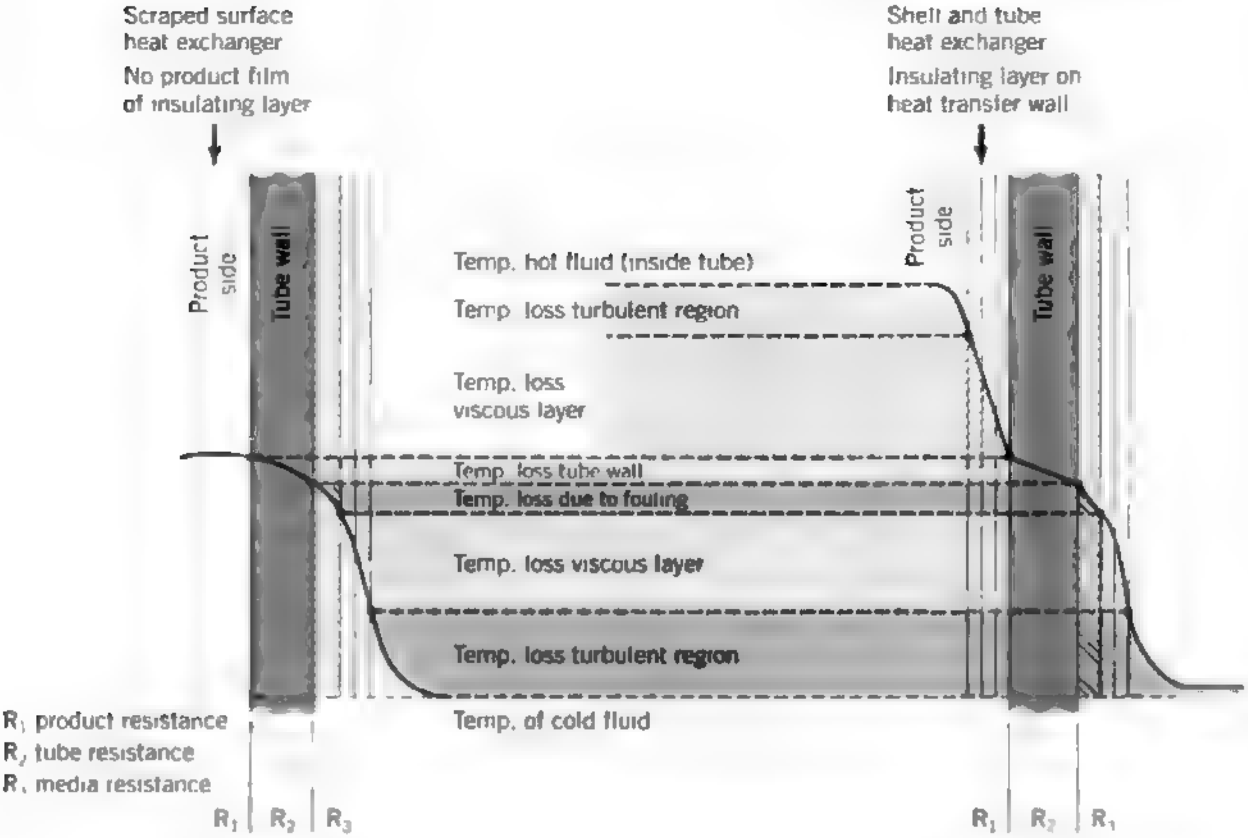

Scraped surface heat exchanger
No product film of insulating layer
Shell and tube heat exchanger
Insulating layer on heat transfer wall
Product side
Tube wall
Product side
Tube wall
Temp. hot fluid (inside tube)
Temp. loss turbulent region
Temp. loss viscous layer
Temp. loss tube wall
Temp. loss due to fouling
Temp. loss viscous layer
Temp. loss turbulent region
Temp. of cold fluid
R₁ product resistance
R₂ tube resistance
R₃ media resistance
R₁
R₂
R₃
R₁
R₂
R₁

Figure 9. Comparison of scraped surface and shell and tube heat transfer profiles.

$$\text{heat-transfer rate} = \Delta T/R$$

Furthermore, by taking the inverse of resistance, which is conductance, $1/R = U$. The heat-transfer equation now is heat-transfer rate $= U\Delta T$.

Heat-exchange U values in a scraped-surface heat exchanger consist of three parts as shown in this formula:

$$U = \frac{1}{R_1 + R_2 + R_3} = \frac{1}{1/h_p + t/k + 1/h_m}$$

where h_p is the heat-transfer coefficient for the product and ranges from 200 to 800 Btu/h · ft^2 · °F, t/k is the heat-transfer coefficient through the cylinder wall (t usually is between 1/4 and 1/8 in. and k varies depending on the material, and h_m is the heat-transfer coefficient for the media and ranges from 800 (water media) to 2000 (steam or ammonia) Btu/h · ft^2 · °F. The overall U value then varies from 150 to 420 Btu/h · ft^2 · °F. Unfortunately, the h_p is difficult to determine theoretically and, because h_p tends to influence the overall U value much more than h_m and t/k, manufacturers size the heat exchangers using a U that is determined from lab test data or from production runs. Attempts have been made to predetermine the expected U value, but many factors influence the heat-transfer rate:

SSHE geometry: an SSHE can be equipped with different diameter dashers. The larger the dasher diameter, the smaller the annular space in which the product travels. A small annular spacing will improve heat transfer.

Viscosity: SSHE units normally can handle viscosities of up to 1,000,000 cP. The greater the viscosity, the lower the U value. Products with viscosities that vary greatly at different temperatures; eg, peanut butter and cooking—cooling starch for salad dressings, usually produce lower U values. This is due to mass rotation. It normally is encountered in cooling applications where the low-viscosity warm product short-circuits through the heat-exchange cylinder while the cooler, thicker product rotates with the dasher.

Dasher speed: high dasher speeds improve heat transfer but heat generated by high rpm is counter-productive in cooling applications.

Number of scraper blades: dashers typically are equipped with two or four rows of scraper blades. Units with four rows of blades provide higher heat-transfer rates at any given dasher rpm. When supplying two rows of blades, many manufacturers compensate by increasing the dasher speed.

Specific heat: the lower the specific heat, the lower the U values.

Thermal conductivity: the lower the thermal conductivity, the lower the U value.

Flow rate: the higher the product flow rate, the higher the U value. Flow rates below 5 gpm are considered low. See Figure 10.

Applications: heating applications produce higher U values than do cooling applications.

Cylinder materials: the cylinder materials, as mentioned previously, can be nickel, stainless steel or bimetallic. Figure 11 charts the differences in overall U values when cylinder thickness or materials are changed. Nickel is the most efficient and is used as the basis to compare the other materials commonly available.

Once a U value for an application has been determined, a calculation to size the heat exchanger can be made.

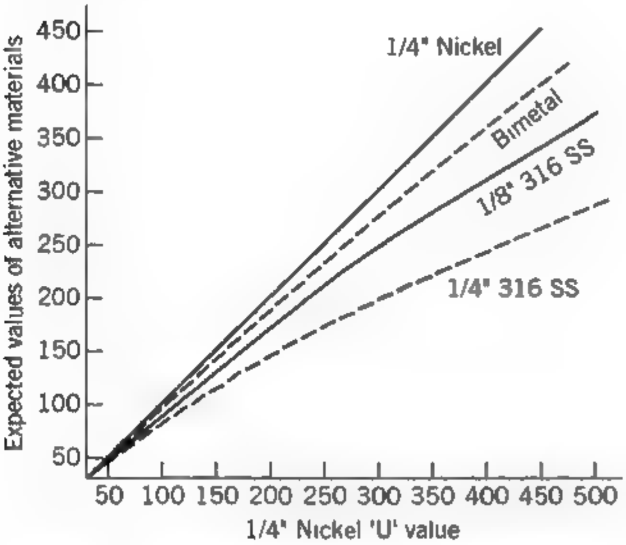


Figure 10. Comparison of nickel and other materials.

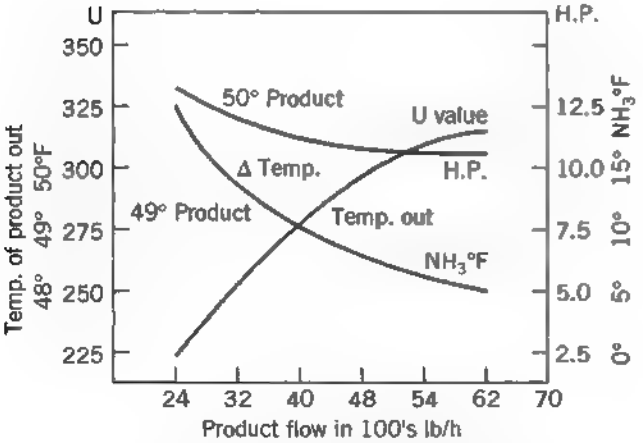


Figure 11. Curves represent a 6-ft^2 SSHE margarine cooler with 4-1/2-in.-diameter dasher. Discharge temperature 49–50°F flow rate increased from 2400 to 6200 lb/h; U value increases with flow rate and eventually levels off. Ammonia temperature is lowered to maintain proper exit temperature.

A Practical Formula

Deriving the practical scraped-surface heat exchange formula is achieved as follows:

$$\text{heat-transfer rate} = U\Delta T$$

Product heat load Q (heat load being transferred from the product to the media) will be equal to the heat-transfer rate times the heat transfer area A. Thus

$$Q = UA\Delta T$$

where A is the area, ft^2. The product heat load is equated

$$\begin{aligned} Q = {} & \text{(product) lb/h} \times \text{temperature change} \times \text{SpHt} \\ & + \text{latent heat} + \text{heat of reaction} \\ & + \text{mechanical heat} \end{aligned}$$

In the majority of applications, heat of reaction does not occur. Therefore, by eliminating this factor and combining equations, the generally accepted formula for calculating scraped-surface heat exchangers is

$$\begin{aligned} \text{area} = {} & [\text{product flow} \times (T_{\text{in}} - T_{\text{out}}) \times \text{SpHt} \\ & + \text{latent heat} + \text{hp} \times 2545\ \text{Btu/h/hp}] \\ & \div U \times \text{LMTD} \end{aligned}$$

$$\text{LMTD} = \frac{(T_{\text{in}} - T_{\text{media out}}) - (T_{\text{out}} - T_{\text{media in}})}{\ln\left[\dfrac{(T_{\text{in}} - T_{\text{media out}})}{(T_{\text{out}} - T_{\text{media in}})}\right]}$$

For steam, ammonia, and Freon units,

$$T_{\text{media in}} = T_{\text{media out}}$$

Area: square feet of surface required

Flow: pounds per hour of product

T_{in}: temperature of product in (°F)

T_{out}: temperature of product out (°F)

$T_{\text{media in}}$: temperature of media in (°F)

$T_{\text{media out}}$: temperature of media out (°F)

Latent heat: heat removed from ice formation or fat crystallization Btu/h

hp: dasher drive horsepower. Use only on cooling applications; disregard on heating. Note that horsepower is converted to Btu by the conversion factor 2545 Btu/h/HP

U: U value Btu/h · ft^2 × °F

LMTD: log mean temperature difference

Sample Calculation

Product: gravy

Specific heat: 0.8

Application: cool from 200 to 60°F using 5°F ammonia

U value: 280

Flow rate: 5000 lb/h

Assume 15 hp is required.

$$\text{LMTD} = \frac{(200 - 5) - (60 - 5)}{\ln[(200 - 5)/(60 - 5)]} = \frac{140}{1.266} - 110$$

$$\text{area} = \frac{5000\ \text{lb/h} \times (200 - 60^\circ) \times 0.8 + 15\ \text{hp} \times 2545}{110 \times 280}$$

$$= \frac{598{,}175}{30{,}800} = 19.4\ \text{ft}^2\ \text{required}$$

SELECTION OF COMPONENTS

The scraped-surface heat exchanger is used on a wide range of diverse applications, many requiring different materials of construction and component design. Choices are based on a number of criteria involving both the compatibility of materials with the product to be processed and compatibility between various heat-exchanger components and materials. To better understand the reasoning behind component and material selection, it is necessary to know the equipment options available, the properties of the product, and the process temperatures. The four major areas of concern are cylinder materials, blade materials, seal selection, and dasher sizing.

Selecting Cylinder Materials

Since the per square foot cost of SSHE heat-transfer surface is relatively expensive, it is desirable to select heat-transfer cylinder material that provides the highest heat transfer coefficient. This will maximize the overall thermal performance for any given application. The commonly used materials are stainless steel, nickel with or without chrome plating, and bimetallic combinations. Nickel offers the highest HTC with a coefficient five times that of stainless steel.

To contain internal product pressures and prevent implosion by media pressure, cylinders generally are designed with a quarter inch wall thickness. For lower-pressure applications, however, an 1/8-in. wall occasionally is used to improve the performance of stainless-steel cylinders.

Since nickel has such a high heat-transfer coefficient, it might be expected that this material would have universal application. Unfortunately, however, nickel is susceptible to acid corrosion, so stainless-steel cylinders must be used with high acid type products. Furthermore, nickel is relatively soft and wears rapidly if subjected to constant abrasion by metal blades. Since simple economics dictate that the blades rather than the more expensive cylinders accept the wear, plastic blades must be used with nickel cylinders. Also, because most metal blades are of a stainless alloy and there is not enough difference in hardness between blade and cylinder, plastic blades are used with stainless steel cylinders.

To benefit from both the heat transfer characteristics of nickel and the durability of steel blades, nickel cylinders may be chrome-plated. This provides a scraping surface harder than any of the common blade materials but still cannot be used for all applications since the chrome also is subject to salt and acid attack.

An alternative to nickel, chromed nickel or stainless is the bimetallic cylinder (Fig. 12). With a hard corrosion-resistant alloy centrifugally cast within a tube having high tensile strength and thermal conductivity higher than stainless and approaching that of nickel, the bimetallic cylinder provides an acceptable compromise. While slightly softer than chrome, the inner liner is hard enough for use with any blade material. One limitation on a bimetallic cylinder is that it can be damaged by strong CIP acids, especially nitric acid.

Selecting Blade Materials

While scraper blades are available in plastic or metal (Fig. 13), the determination of which blade will provide optimum service is influenced by the cylinder material, by the media being used, and by product temperature and formulation.

Generally speaking, plastic scraper blades can be used in conjunction with all types of cylinder materials while metal blades are run only against chrome plated surfaces

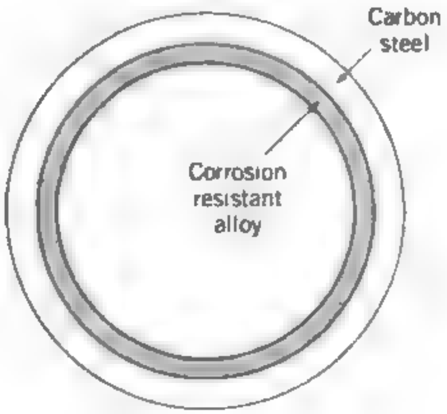


Figure 12. Bimetal cylinder cutaway.

Figure 13. Available scraper blades.

or bimetallic cylinders. Limitations on plastic blades are wear life, temperature, and certain solvents. Metal blades, on the other hand, provide a longer wear life, can be sharpened to extend their use, and are not affected by temperature and solvents.

Plastic Blades

While the most common material for plastic blades is fiber reinforced phenolic resin, new proprietary injected molded plastic materials also are available.

As may be seen from the curves plotted in Fig. 14, injected molded plastic blades can withstand higher temperatures than can those of phenolic resin. Note, however, that there is a break point at about 180°F where temperature becomes the important factor to wear. Below 180°F, abrasion caused by the rubbing of plastic on metal is the prime wear related factor. Since the plastic blade is a wearing part regardless of the application, it must be replaced periodically to preclude breakage and possible product contamination by plastic fragments.

As stated previously, metal blades are not affected by processing temperatures. They are made of any number of tempered stainless alloys such as 17-4 PH or 410 stainless steel, and this hardening improves the wear life of the blades.

This is especially important for slush freezing applications where there is an added abrasive action caused by the scraping of ice from the heat-transfer surface. Since ice impacts the scraping edge as the blade sweeps the surface, it is important that the blade be maintained with a sharp edge (Fig. 15). For ice cream freezing applications, resharpening normally is done on a monthly basis to ensure optimum performance and the blades are replaced every one or two years. In other applications, metal blades may be sharpened every 3–12 months and replaced every 1–3 yr.

Selecting Seals and Materials

Shaft seals tend to be the most troublesome maintenance area on a scraped-surface heat exchanger. SSHE applications differ widely, and no one seal can handle the variety of temperatures, pressures, and product characteristics constantly encountered.

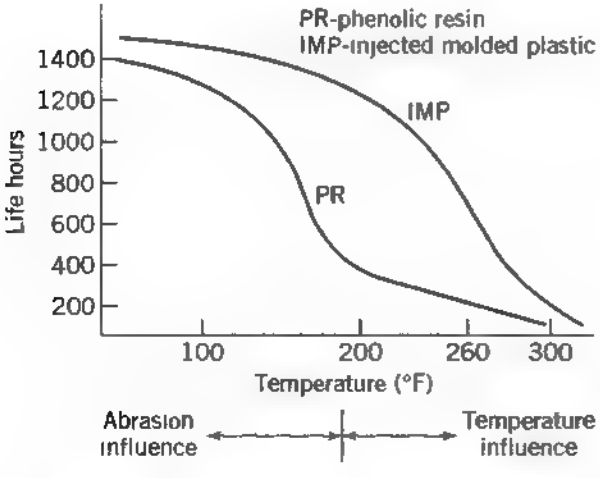


Figure 14. Temperature—wear relationship.

Most manufacturers offer a number of shaft seals, each designed to satisfy the demands of certain applications. The simplest and least expensive seal to maintain is the O-ring seal (Fig. 16a). This type of seal is recommended for use with products having high lubricity, eg, margarine, lard, shortening, ice cream mix, certain meat products, oils, and fats. Normally these products are not processed at temperatures above 140°F. High temperatures above 180°F shorten the effective life of an O-ring by causing it to lose elasticity. The sealing action of an O-ring seal depends on its ability to be sufficiently flexible to fill the gap between the rotating shafts and the static O-ring groove.

While the advantage of the O-ring is that it is inexpensive to replace when worn, the disadvantage is that it wears rapidly. Although daily replacement is not uncommon, this is not a problem when frequent equipment inspection is scheduled. The material of the O-ring depends on the fats or oils in the product to be processed. Typical materials are Buna N, Viton, and EPDM.

Rotary face seals are for all other applications (Fig. 16b). These can handle high temperatures and abrasive products, and offer longer wear life than O-rings. Rotary seals can be broken down into two groups—those with and without a seal water chamber.

The seal water chamber allows water or steam to flow around the edges of the seal faces and is recommended for use when handling products that tend to crystallize or dry out in the sealing area. Examples would be liquid sugars, tomato products, candies, fondants, and processed cheese. Water flushing increases the useful life of the seal. Steam is injected into the chamber for aseptic applications since FDA standards require a sterile barrier media on all rotating shaft seals in low acid aseptic processes. The steam, a sterial medium, prevents sterilized products from being contaminated should there be a seal leak. Many manufacturers build an integral water chamber into their equipment with the chamber to be used only as required.

Critical to the life of a seal is the materials used for the seal faces. The typical seal material is hardened stainless steel on carbon. Alternates for the carbon can be phenolic resins, plastics, silica carbide, or ceramics. Other more exotic seal materials to replace the hardened stainless include tungsten carbide and chrome oxide. Each has advantages and disadvantages, such as wear life, and cost. Normally, the manufacturer recommends the seal type and seal materials based on test work and field experience.

Selecting Dashers

Dashers or shafts that carry the scraper blades are available in diameters from 1-1/2 to 6-1/2 in. and are mounted within heat-exchanger cylinders that, in turn, are manufactured in diameters from 4 to 8 in. Since the most widely used cylinder has an ID of 6 in., the following is based on that model.

The best heat-transfer efficiency is provided when the annular space between a dasher and a cylinder is small. For example (Fig. 17), a 5-1/2-in. dasher within a 6-in. cyl-

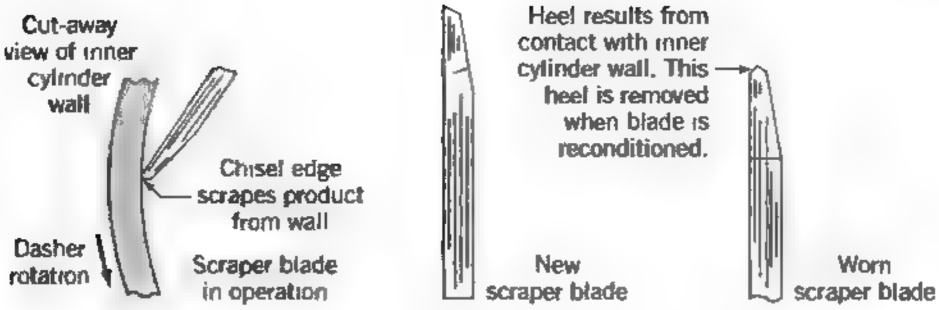

Cut-away view of inner cylinder wall
Chisel edge scrapes product from wall
Dasher rotation
Scraper blade in operation
Heel results from contact with inner cylinder wall. This heel is removed when blade is reconditioned.
New scraper blade
Worn scraper blade

Figure 15. Wear pattern on metal scraper blades.

inder provides a quarter inch annular product flow space, which causes a high axial flow velocity, induces turbulence, and offers a short product residence time. This is particularly useful in crystallizing applications such as for processing shortening and margarine since these products essentially are subcooled and allowed to crystallize after leaving the heat exchanger. Dashers for these products are water circulated.

The disadvantages of the 5-1/2-in. dasher are that it cannot handle viscous products because of high product pressure drop and cannot accommodate products containing particulates over a 1/4 in. in size. In such cases, the 4-1/2-in. dasher with its 3/4-in. annular product flow space is recommended. The smaller the dasher diameter, the larger the annular space, and the larger the particulates that can be processed. As the dasher diameter decreases, so does the effective *U* value for a given dasher speed.

Dasher speed is another design consideration. Typical dasher speeds vary from 60 to 420 rpm, and, in general, the higher the dasher speed, the higher the *U* value (Fig. 18). While this is desirable, increased dasher speed also increases the motor load, which can cause problems in cooling applications. Recalling the heat-transfer formula given previously, heat removed when cooling comes from both the product and the motor load required to turn the dasher. In some applications, the motor load actually can equal the product heat load. Therefore, in some cases a slower dasher speed nets a more beneficial end result.

The graph in Figure 19 compares horsepower and rpm involving various products. All are cooling applications performed on a 6-in. diameter by 72-in.-length unit. Note how small changes in dasher speed on marshmallow change the horsepower requirement significantly while the power requirements vary little while cooling cheese sauces at different speeds. It should be pointed out that horsepower requirements do not necessarily follow viscosity of the product. Rather, the term tenacity would best describe the measurement that would equate to the horsepower—rpm relationship. A good example is corn syrup versus a cooked starch slurry base for salad dressings. At the same viscosity, the corn syrup has much more tenacity in retarding dasher rotation than does the cooked starch.

To reduce motor loads while processing tenacious products, slow dasher speeds are required but the dasher cannot be run so slowly that the *U* value is reduced significantly. The properties of such products normally generate a low *U* value at any dasher speed, usually in the range of 120–180 Btu/h · ft^{2}°F. At very slow speeds, the *U* value is

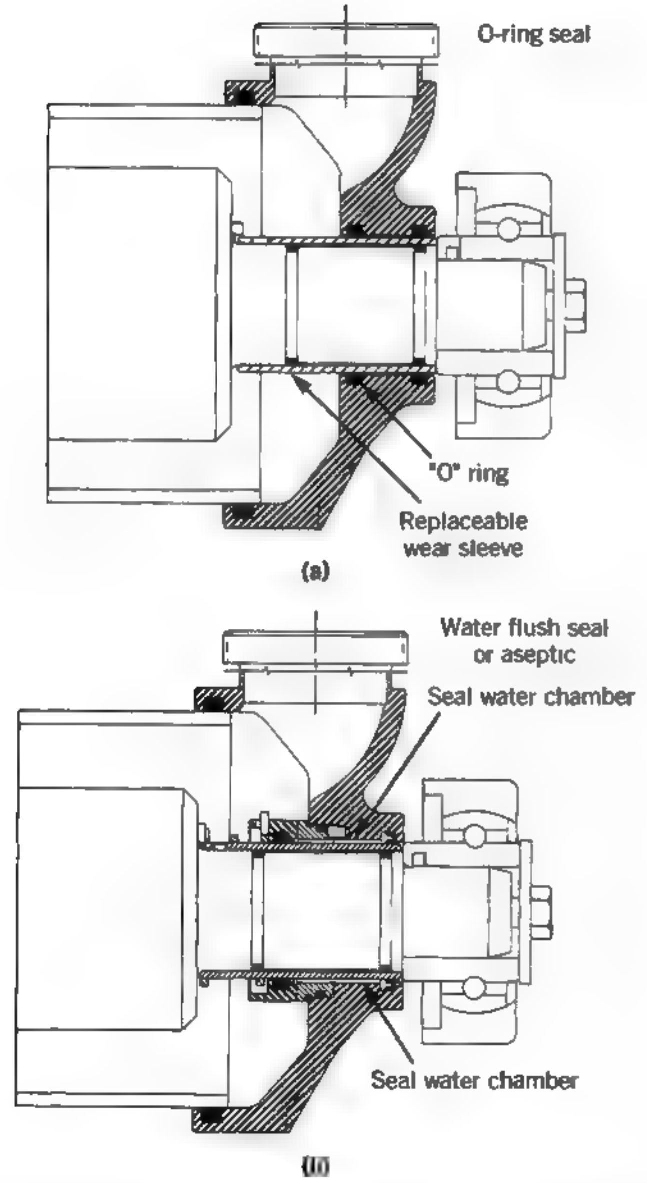

O-ring seal
"O" ring
Replaceable wear sleeve
(a)
Water flush seal or aseptic
Seal water chamber
Seal water chamber
(b)

Figure 16. Shaft seals: (a) simple O-ring seal; (b) rotary mechanical seal with water flush or stream aseptic outer chamber.

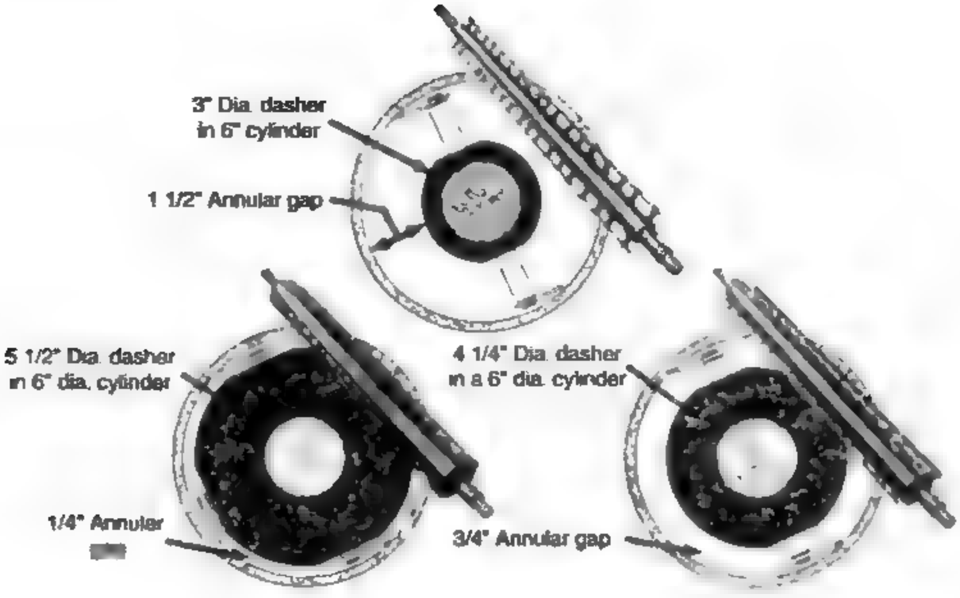

3" Dia. dasher
in 6" cylinder
1 1/2" Annular gap
5 1/2" Dia. dasher
in 6" dia. cylinder
1/4" Annular
4 1/4" Dia. dasher
in a 6" dia. cylinder
3/4" Annular gap

Figure 17. Dasher comparisons.

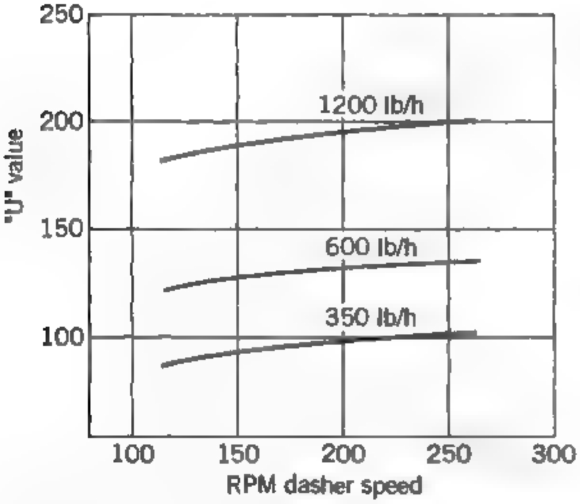


Figure 18. *U* value versus dasher speed and product flow.

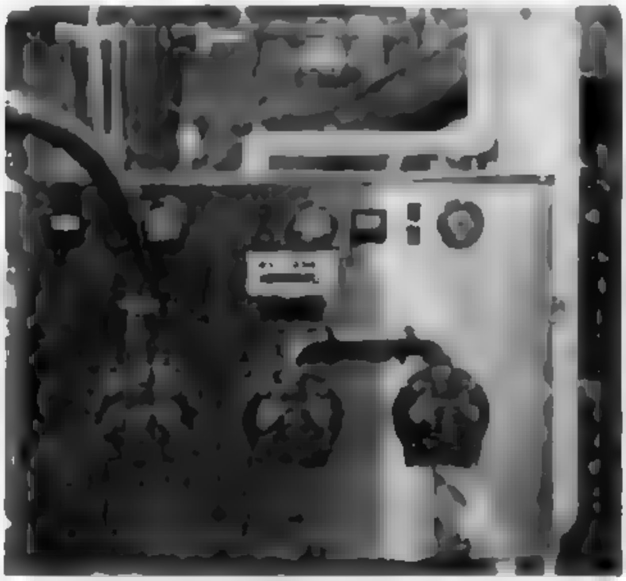


Figure 20. Three-cylinder SSHE uses ammonia to cool 6000 lb/h of MDM turkey from 50 to 35°F.

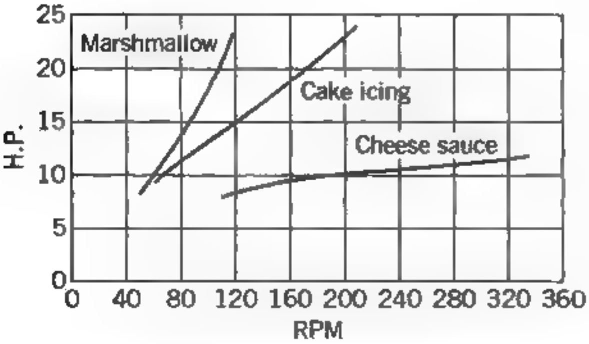


Figure 19. Dasher horsepower versus rpm.

further reduced from these already low levels and an economic sizing is not possible. It also should be noted that a certain minimum dasher speed is required to centrifugally "throw" and hold the scraper blades against the heat transfer cylinder. Therefore, there is a definite optimum dasher rpm that maximizes the net capacity of the heat exchanger for tenacious-type products in cooling applications.

Another reason for using low dasher speeds is retention of product identity. When large particulates are being processed with a sauce or gravy, fast-turning dashers tend to cause breakage of the particulates. The dasher speed

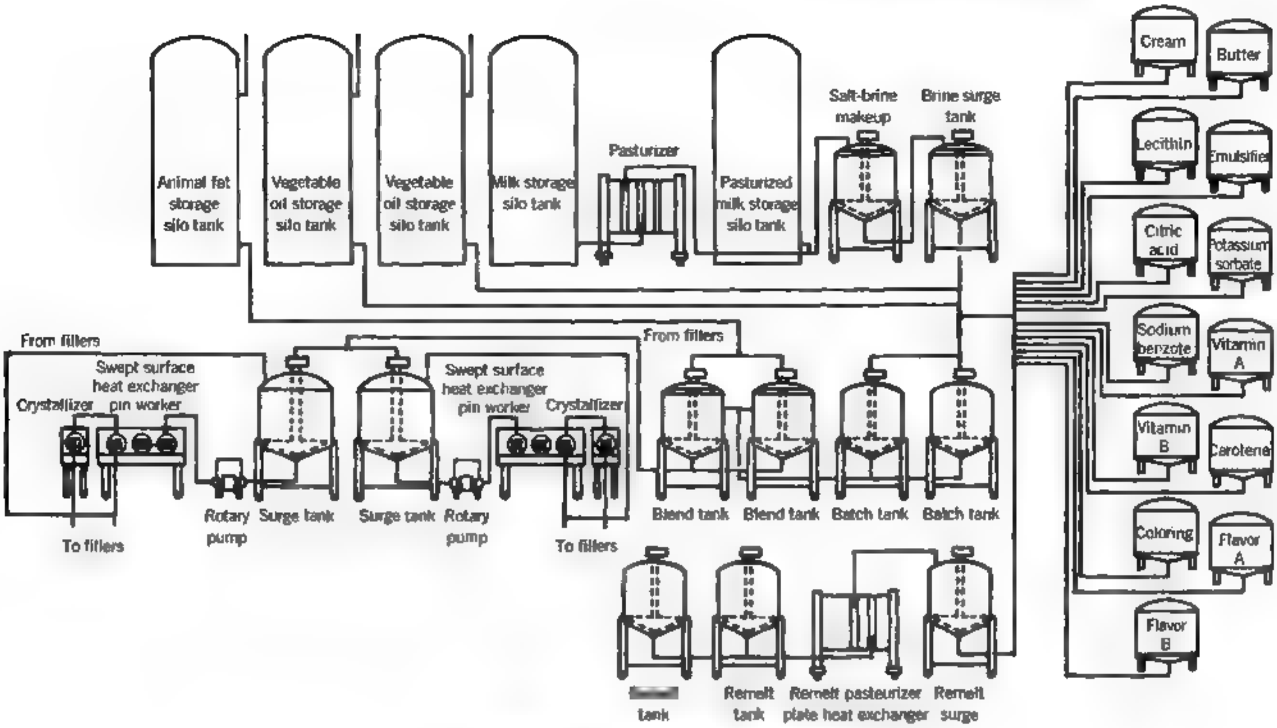

Animal fat storage silo tank
Vegetable oil storage silo tank
Vegetable oil storage silo tank
Milk storage silo tank
Pasturizer
Pasturized milk storage silo tank
Salt-brine makeup
Brine surge tank
Cream
Butter
Lecithin
Emulsifier
Citric acid
Potassium sorbate
Sodium benzoate
Vitamin A
Vitamin B
Carotene
Coloring
Flavor A
Flavor B
From fillers
Swept surface heat exchanger pin worker
Crystallizer
To fillers
Rotary pump
Surge tank
Surge tank
Rotary pump
Swept surface heat exchanger pin worker
Crystallizer
To fillers
From fillers
Blend tank
Blend tank
Batch tank
Batch tank
[redacted] tank
Remelt tank
Remelt pasteurizer plate heat exchanger
Remelt surge

Figure 21. Margarine manufacture.

Table 3. SFI Values for Typical Shortenings

Shortening	Plastic range	Melting point		SFI value				
		°C	°F	10°C 50°F	21.1°C 70°F	26.7°C 80°F	33.3°C 92°F	37.8°C 100°F
High stability[a]	Narrow	43	109	44	28	22	11	5
All-purpose[a]	Wide	51	124	28	23	22	18	13
14% hardfat in cottonseed oil[b]	Wide	51	124	16	14	14	12	11

Source: [a]Ref. 1 and [b]Ref. 2.

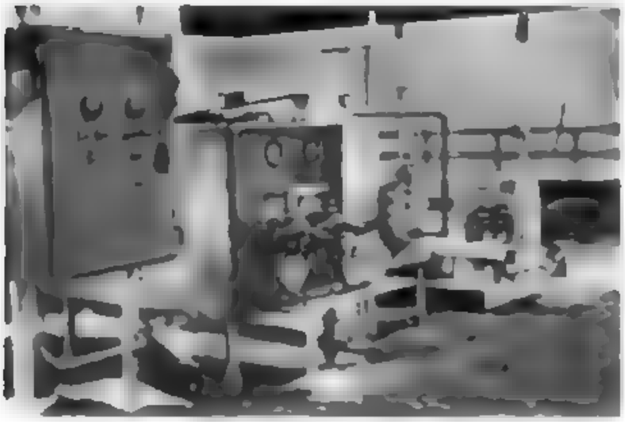


Figure 22. 10,000 lb/h system. Control panel, SSHE with water circulated dashers, pinworker, pumping unit with surge tank.

therefore is not always determined by the desired net heat-transfer efficiency but by limitations of the product.

In both the tenacious and particulate-type products, field measurements and/or lab test data are required to determine optimum dasher speeds.

SYSTEMS APPLICATIONS

As new products emerge from development labs across the country, many processors have determined that the scraped-surface heat exchanger is essential to effective, cost-efficient operations. In some cases, this type of heat exchanger is used for continuous and uniform heating and cooling of highly viscous pastes, fillings that contain up to 80% total solids, or products carrying discrete particles of up to 1 in. in size. In others, the SSHE has been selected for texturizing, gelling, whipping, plasticizing, and crys-

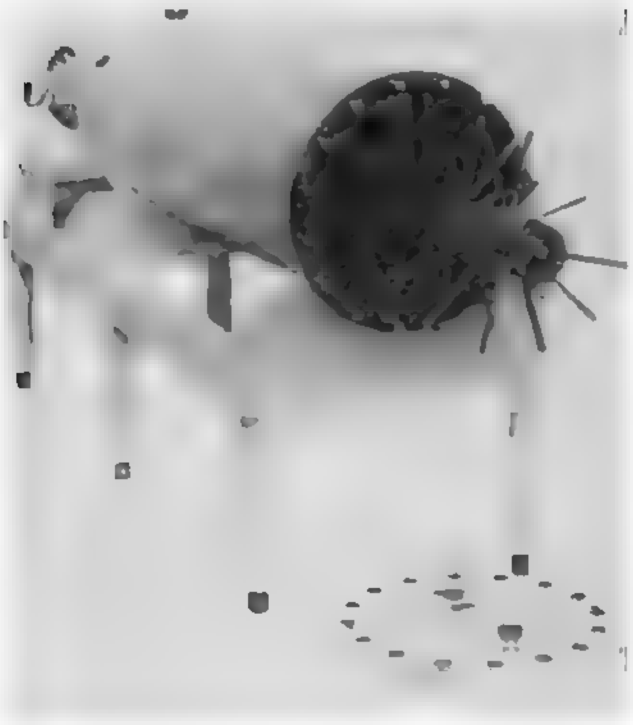


Figure 23. Pinworker with cover removed to expose shaft and pins.

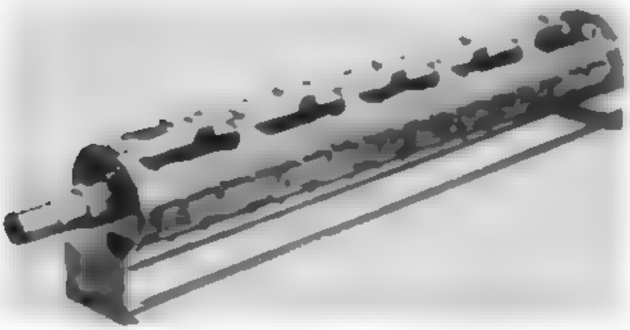


Figure 24. Plasticizer with combination scraped-surface heat exchanger and pinworker.

tallizing duties. A few of the more interesting applications are detailed on the following pages.

Mechanical Deboning

In the mechanical deboning of turkey, chicken, and red meats, the heat that is generated in the deboning process must be dissipated to prevent product spoilage. This is done best by means of a scraped-surface heat exchanger.

The mechanical deboning machine (MDM) works on the principle of separating hard material such as bone, gristle, sinew, and cartilage from soft, whole meat. Following prebaking or grinding to reduce large bones to 3/8-in. size, the meat and bones are compressed by an auger inside the deboner and are forced under high pressure into a perforated tube. The soft meat passes through the many small holes, thus separating it from the bones. The meat is emulsionlike in consistency and drops into a hoppered pump. This deboning action requires considerable mechanical working of the meat and, in doing so, increases product temperature by 10–20°F. A scraped-surface heat exchanger (Fig. 20) is used to cool the meat from 55 to 35°F since a temperature of 40°F or less is required to prevent rapid bacterial growth and subsequent spoilage.

When used as a meat emulsion chiller, the SSHE operates on ammonia and is equipped with chrome nickel cylinders and a dasher and blade assembly specifically designed for viscous or particulate products. The typical flow rate of product through the chiller ranges from 2500 to 6000 lb/h. Since turkey and red meat are lower in moisture than MDM chicken, they are more viscous. A chiller for these products, in general, operates at two-thirds the capacity of a unit processing chicken and requires a larger dasher drive motor.

Margarine

While a typical system generally consists of a series of storage and blending tanks combined with a crystallizer, high-pressure pumps, and both plate and scraped-surface heat exchangers, the latter is the key to the production of high-quality margarine.

As indicated by the schematic, (Fig. 21) basic oils and fats are drawn from storage and blended with water, salts,

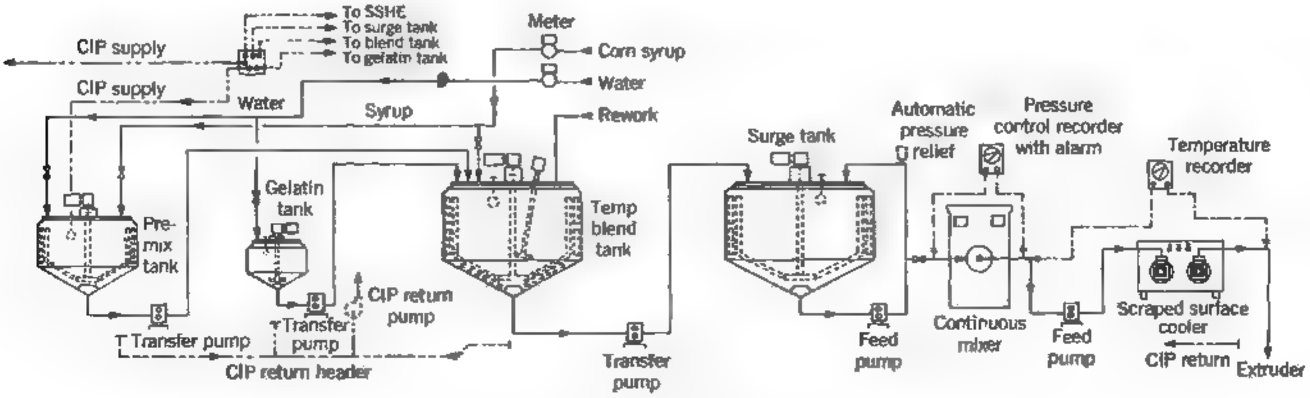

To SSHE
To surge tank
To blend tank
To gelatin tank
CIP supply
CIP supply
Meter
Corn syrup
Water
Water
Syrup
Rework
Gelatin tank
Pre-mix tank
Temp blend tank
Surge tank
Automatic pressure relief
Pressure control recorder with alarm
Temperature recorder
CIP return pump
Transfer pump
Transfer pump
CIP return header
Transfer pump
Feed pump
Continuous mixer
Feed pump
Scraped surface cooler
CIP return
Extruder

Figure 25. Marshmallow production system.

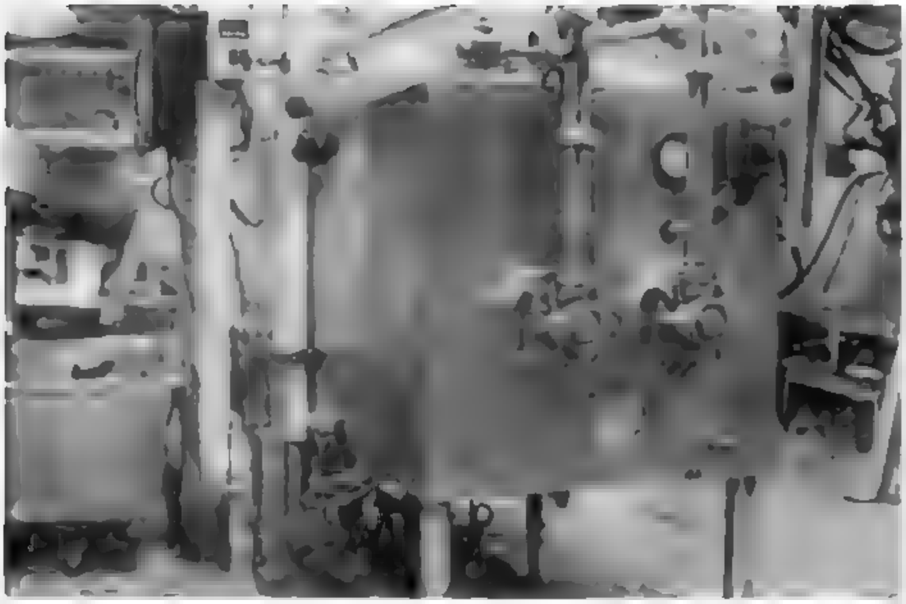
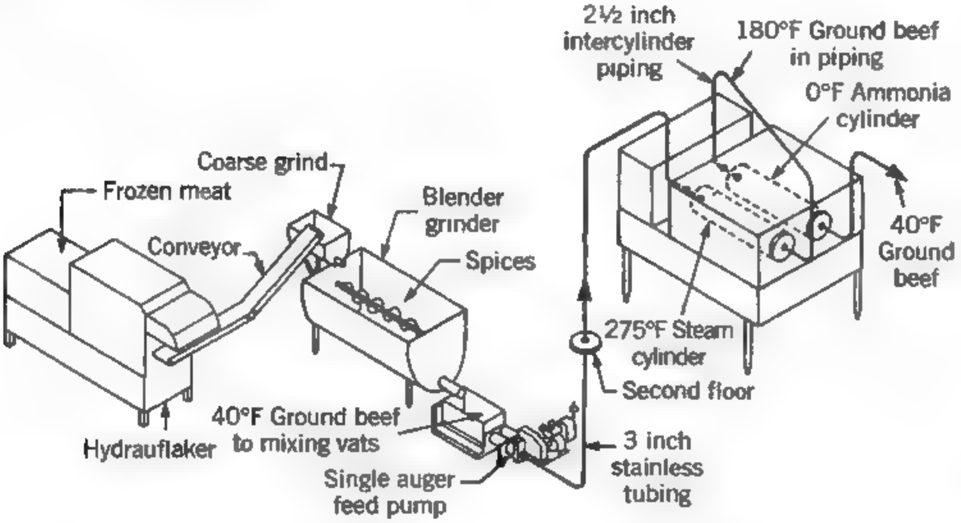


Figure 26. Scraped-surface heat exchanger cooks and cools ground beef for frozen food entrees. Ground beef is pumped into the rear of the left hand cylinder. Steam is used to head from 45 to 185°F. Cooked product flows out the front, through a holder tube, and into the rear of the right hand cylinder where ammonia at −40°F cools it to below 50°F.



2½ inch intercylinder piping
180°F Ground beef in piping
0°F Ammonia cylinder
Coarse grind
Frozen meat
Blender grinder
Conveyor
Spices
40°F Ground beef
275°F Steam cylinder
Second floor
40°F Ground beef to mixing vats
Hydrauflaker
Single auger feed pump
3 inch stainless tubing

Figure 27. Ground beef cooking and cooling.

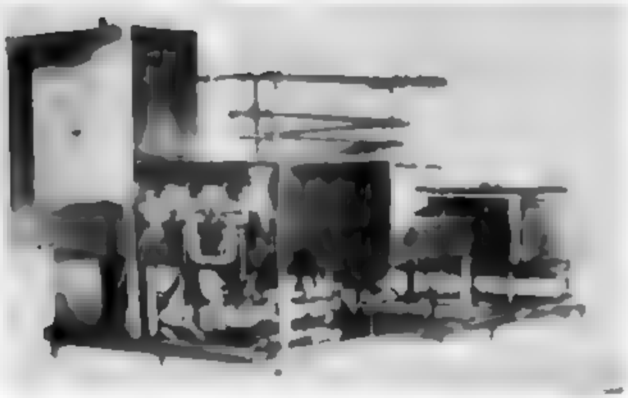


Figure 28. Three-cylinder SSHE aseptically heats and cools filling with 1/4–1/2 pieces of fruit.

emulsifiers, coloring, preservatives, and flavorings for containment in surge tanks at temperatures ranging from 110 to 130°F. If milk ingredients are involved, a Paraflow plate heat exchanger is used for legal pasteurization and cooling before the blending phase. The final mixture then is pumped through an SSHE unit for cooling to approximately 45°F.

While the scraped surface exchanger generally operates on ammonia and is equipped with chromed nickel cylinders and metal blades, the special feature for margarine production is the water-circulated dasher. Since margarine is a crystallizing application, product crystals would tend to stick and collect on the surface of a standard dasher. Such a buildup would increase not only product pressure drop through the exchanger but also the motor load and accelerate overall wear on seals and bearing components. By

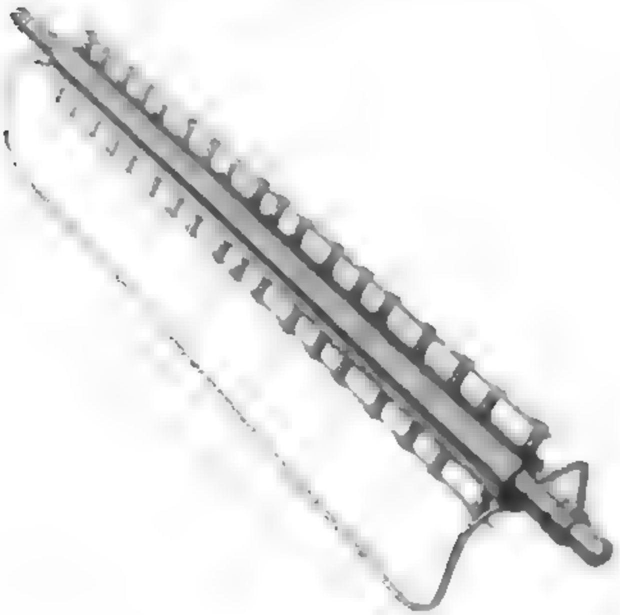


Figure 29. To minimize product damage, clearance between the blade and the dasher shaft should be equal or greater than the size of the particle that is being processed.

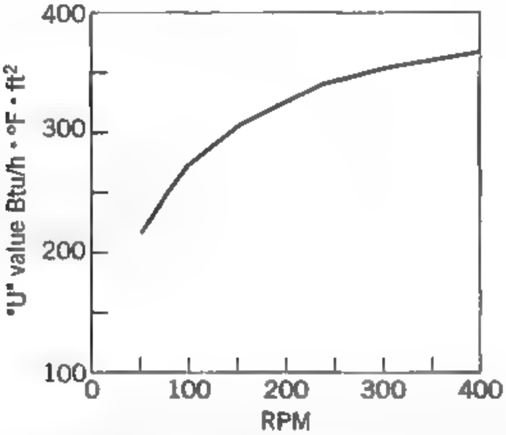


Figure 30. Dasher speed versus heat transfer (meat gravy).

passing water at about 100°F through the hollow dasher, enough heat is provided to prevent crystal formation while still not contributing significantly to the overall heat load.

Following the cooling cycle, the viscous product is pumped from the scraped-surface chiller through a static crystallizer for table-grade, stick-type margarine or to a pinworker crystallizer for tub, soft table, or bakery product. When an acceptable crystal structure has been achieved, the product is formed and packaged and allowed to gain its final viscosity during a quiescent period.

Plasticizing Shortenings

In the manufacture of solid shortening, one primary objective is to make a product that remains plastic and work-

Table 4. Partial List of Products Processed

Product	Heat	Cool
Candies, frostings, and fillings		
Cake frosting		X
Cookie filling		X
Fondant		X
Marshmallow		X
Dairy products		
Concentrated milk	X	
Cottage cheese curd		X
Cream cheese		X
Ice cream mix	X	
Melting butter	X	
Processed cheese	X	
Quark	X	X
Whey concentrate	X	X
Whipped butter		X
Yogurt	X	X
Fats and oils		
Margarine	X	X
Shortening		
Plasticizing Bulk		X
In-house		X
Vegetable oil	X	X
Fruit: Concentrates, juices, and fillings		
Aseptic applesauce	X	X
Aseptic bananas	X	X
Aseptic fruit fillings	X	X
Aseptic fruit purees	X	X
Citrus pulp		X
Jelly and jam preserves	X	X
Juice concentrate		X
Pumpkin and squash	X	X
Pumpkin pie filling		X
Meat products		
Cooking bacon	X	
Gelatin concentration		X
Lard		X
Low-temperature rendered meat		X
Meat pie fillings	X	X
Mechanically deboned		
Chicken		X
Red meat		X
Turkey		X
Sausage and hamburger	X	X
Sauces, starches, gravies and puddings		
Aseptic cheese sauce	X	X
Aseptic puddings	X	X
Chili	X	X
Enchilada filling		X
Gravies and sauces		X
Salad dressing starch	X	X

Table 4. Partial List of Products Processed *(continued)*

Product	Heat	Cool
Slush freezing for freeze concentration		
Coffee or tea extract		X
Grape juice		X
Orange juice		X
Various aroma extracts		X
Vinegar		X
Wine		X
Tomato products		
Barbecue sauce	X	X
Tomato paste	X	X
Tomato sauces	X	X
1/4-in. diced tomatoes	X	
Miscellaneous products		
Acetone—deep cooling		X
Adhesives and glues		X
Aseptic diced peppers	X	X
Autolyzed yeast	X	
Baby foods	X	X
Candle wax		X
Hand cream		X
Honey		X
Ink		X
Lipstick	X	X
Liquid antacid	X	X
Lithium grease		X
Paints	X	X
Peanut butter		X
Photographic gelatin		X
Pickle relish	X	X
Poi	X	
Resins	X	X
Shampoo		X
Shaving cream		X
Soup	X	X
Synthetic polymers	X	X
Toothpaste		X
Vanilla wafer batter		X
Wallpaper paste	X	X

able over a wide temperature range, specifically between 60 and 90°F. To do this, hydrogenated or hard fats are added to formulations to extend the plastic range. Solid shortening actually is a suspension of high-melting-point fats in liquid fat or oil. Different fats will melt or solidify as is shown in the solid fats index (SFI) charted in Table 3. At room temperatures of 68°F (20°C), the typical shortening is only about 30% solid.

Two methods commonly are used to achieve desired crystal structure, size, and dispersion when producing plasticized shortening. One is for full-scale production (Fig. 22); the other, for limited "in house" applications.

For normal production, an A or scraped-surface heat exchanger is used to cool oil from 140°F (60°C) (no solids) to 60–70°F (16–21°C). This subcooling is done rapidly, and only about 5% of the fats are crystallized. The shortening then enters a B or pinworker unit (Fig. 23), which acts as an agitated holding tube of about 3-min duration. During this time, the remaining crystals form with the latent heat of crystallization causing the shortening to heat up. Typically, if the shortening enters at 65°F (18°C), it will exit at 80°F (27°C). The now plasticized shortening is ready for filling into 50-lb cubes. Most plasticizing systems usually include a shell-and-tube exchanger to precool product from 140°F (60°C) to 120°F (49°C) and reduce the heat load on the SSHE. Nitrogen also is usually added before the SSHE to give the final product a white color.

For bakeries that plasticize shortening for in-house use, a single unit combining the features of A and B machines commonly is used. As shown in Figure 24, this involves an open type dasher shaft. The dasher is hollow and has wide slots at regular intervals. A set of beater bars acts as the pinworker, holding and working the shortening to a high degree in order to produce a soft, readily flowable texture that can be handled in pump lines and tanks downstream.

Marshmallow

Marshmallow is an aerated food product typically formulated from 45% sucrose, 27% corn syrup, 23% water, gelatin, invert sugar, and flavoring. (See Fig. 25.)

As shown in the flow schematic, a production system generally employs multiple tanks, an aerating mixer, and a scraped-surface heat exchanger. A premix tank first is used to blend and heat sugars to 245°F before the solution is transferred to another blending tank for cooling to 155°F. Gelatin and 150°F tempered water, meanwhile, are thoroughly mixed by means of a high shear agitator and blended with the sugar solution. The resulting syrup is charged to a surge tank and pumped as required to a high shear aerating mixer where it is beaten to a weight of between 35 and 48 oz/gal (specific gravity of 0.26–0.36). The 155°F marshmallow mix then flows to an SSHE for chilled water cooling to 90°F.

Marshmallow is a difficult duty for a scraped-surface heat exchanger because of the extremely high viscosity and the low heat-transfer properties of the product. Operating pressures in the mixer and heat-exchanger range from 150 to 300 psi to handle product viscosity and to force the marshmallow through an extrusion head to a belt where it is shaped and dusted with starch. The low product density results in a low *U* value and because of viscosity, a high torque is required to turn the dasher. Note in the Figure 19 dasher horsepower—rpm chart how dramatically the dasher hp changes relative speed. Too high an rpm results in excessive motor load that makes cooling more difficult because the motor heat has to be removed by the heat exchanger. Since even the optimum dasher speed results in about one half of the cooling load coming from the motor input, selecting the proper dasher speed for this application is very important. Care also must be given to the selection of shaft seals. Since marshmallow is mostly sugar, seals with abrasive resistant, high hardness qualities should be used and the seals should be water flushed to prevent crystals from building up on the seal faces.

Cooking–Cooling Ground Meat

While ground beef generally is cooked in steam-jacketed kettles for moderate size production runs, the tendency is to install scraped-surface heat exchangers for both cooking and cooling duties when production requirements exceed 10,000 lb/day (Fig. 26). Not only does the SSHE provide faster cooking; it also improves product yield since moisture and fat loss is far less than the 15–20% decline in product volume that occurs with the open-kettle method (See Fig. 27.)

The typical meat cooking system consists of a single auger feed hopper with a leveling ribbon followed in line by a two-cylinder SSHE, one cylinder with 275°F steam and the other with 0°F ammonia. Both the auger feeder and SSHE are hydraulically driven from a central power source. After meat is ground, spiced, and blended, it is fed to the heat exchanger by means of a single-discharge auger designed specifically to move viscous, sticky products directly into a rotary pump. The auger maintains a constant stuffing pressure at the rotary pump inlet by varying the operating speed while a leveling ribbon maintains a uniform meat level in the feed hopper. The scraped surface blade and dasher design promote rapid removal of product from the cylinder walls and enhance agitation and mixing during both cooking and cooling phases. Assembly and disassembly for blade inspection and/or replacement is quick and easy. Meat remaining in the auger feed pump, pipelines, or the scraped surface heat exchanger is easily recovered and, once clear of product, the closed system and piping are easily cleaned with a pump circulation loop.

Products with Particulates

When processing sauces, gravies, or juices containing pieces of meat, vegetables, or fruit, one of the key elements is maintaining product identity by reducing or eliminating particulate breakage. This becomes more of a concern if the particulates exceed 1/4 in. in size and the carrier is a thin liquid such as broth or soup. Thick sauces, on the other hand, seem to have a cushioning effect with less product damage occurring during passage through the system.

While the dominant type of heat exchanger used for particulate-type products is the scraped-surface unit (Fig. 28), each case still must be considered individually. Many processes require multiple SSHE cylinders that should be piped in series rather than in parallel. This eliminates the need for individual product pumps while ensuring higher heat-transfer rates and an equal flow to each cylinder.

To reduce particle breakage, three design areas must be considered—the gap between the dasher and scraper blade, the sizing of SSHE inlets and outlets, and the dasher operating speed.

The distance between the underside of the blade and the dasher shaft (Fig. 29) should be equal or greater than the largest particle to be processed. This allows passage without particle damage as the blade sweeps by. The disadvantage of a wide annular space between the dasher and the cylinder wall is a longer product retention period and a reduction in exchanger efficiency. The faster the product travels through the cylinder, the higher the *U* value attained. This results in improved exchanger performance with lower residence time and less product damage.

The sizing of inlets and outlets is directly related to product quality. Using the largest possible ports reduces not only pressure drop through the system but also shearing effect. Furthermore, passage of product is eased and the possibility of contact with inlet walls is minimized.

Finally, there is the matter of dasher speed. As shown by the curve in Figure 30, while higher dasher speed results in a higher *U* value, it also can cause increased particulate damage. Therefore, higher dasher rpm and the corresponding high heat-transfer efficiency will apply only to products with small particulates. When products contain delicate particulates of 1/2–3/4 in., a variable-speed drive is used to reduce dasher revolutions to about 120 rpm. While this protects product quality, it also lowers exchanger efficiency. Consequently, striking a balance between performance and quality should be determined by lab runs so that optimum operation at full scale becomes a matter of a proper dasher speed setting.

Peanut Butter

For the production of creamy or chunky peanut butter, a process system will consist of surge tanks, a deaerator, scraped-surface heat exchanger, ingredient feeder with in-line blender, and transfer pumps.

After mixing roasted nuts with a stabilizer, salt, and sugar to the desired formula, the product is ground and discharged at a temperature of about 150–200°F. Deaeration follows to eliminate air pockets, which initiate oil separation. During cooling from an SSHE inlet temperature of 140–190°F to a discharge temperature of 85–95° · F, the stabilizer is solidified in finely divided crystalline form and uniformly distributed throughout the mixture. At the proper temperature, the peanut butter becomes a viscous, extrudable mass. Crystal change continues and further solidification occurs after filling. For chunky style, a chunk feeder is located between the SSHE and filler or the transfer pump and deaerator.

Table 4 gives a listing of other products routinely processed using SSHEs.

BIBLIOGRAPHY

Adapted from: SSHE Handbook, SSH-1087. APV Crepaco, Inc. Copyrighted APV Crepaco, Inc. Used with permission.

APV Crepaco, Inc.
Lake Mills, Wisconsin

HEAT TRANSFER

BACKGROUND

Almost all food processes depend on or are affected by heat being added or removed at some stage in the operation. The efficient and effective utilization of heat results in economic savings, minimum adverse affects on nutrient components, higher quality consumer-ready products, and minimum effect on the many environmental factors associated with food processing. This efficient and effective use of heat depends on knowledge and subsequent application to install scraped-surface heat exchangers for both cooking and cooling duties when production requirements exceed 10,000 lb/day (Fig. 26). Not only does the SSHE provide faster cooking; it also improves product yield since moisture and fat loss is far less than the 15–20% decline in product volume that occurs with the open-kettle method (See Fig. 27.)

The typical meat cooking system consists of a single auger feed hopper with a leveling ribbon followed in line by a two-cylinder SSHE, one cylinder with 275°F steam and the other with 0°F ammonia. Both the auger feeder and SSHE are hydraulically driven from a central power source. After meat is ground, spiced, and blended, it is fed to the heat exchanger by means of a single-discharge auger designed specifically to move viscous, sticky products directly into a rotary pump. The auger maintains a constant stuffing pressure at the rotary pump inlet by varying the operating speed while a leveling ribbon maintains a uniform meat level in the feed hopper. The scraped surface blade and dasher design promote rapid removal of product from the cylinder walls and enhance agitation and mixing during both cooking and cooling phases. Assembly and disassembly for blade inspection and/or replacement is quick and easy. Meat remaining in the auger feed pump, pipelines, or the scraped surface heat exchanger is easily recovered and, once clear of product, the closed system and piping are easily cleaned with a pump circulation loop.

Products with Particulates

When processing sauces, gravies, or juices containing pieces of meat, vegetables, or fruit, one of the key elements is maintaining product identity by reducing or eliminating particulate breakage. This becomes more of a concern if the particulates exceed 1/4 in. in size and the carrier is a thin liquid such as broth or soup. Thick sauces, on the other hand, seem to have a cushioning effect with less product damage occurring during passage through the system.

While the dominant type of heat exchanger used for particulate-type products is the scraped-surface unit (Fig. 28), each case still must be considered individually. Many processes require multiple SSHE cylinders that should be piped in series rather than in parallel. This eliminates the need for individual product pumps while ensuring higher heat-transfer rates and an equal flow to each cylinder.

To reduce particle breakage, three design areas must be considered—the gap between the dasher and scraper blade, the sizing of SSHE inlets and outlets, and the dasher operating speed.

The distance between the underside of the blade and the dasher shaft (Fig. 29) should be equal or greater than the largest particle to be processed. This allows passage without particle damage as the blade sweeps by. The disadvantage of a wide annular space between the dasher and the cylinder wall is a longer product retention period and a reduction in exchanger efficiency. The faster the product travels through the cylinder, the higher the U value attained. This results in improved exchanger performance with lower residence time and less product damage.

The sizing of inlets and outlets is directly related to product quality. Using the largest possible ports reduces not only pressure drop through the system but also shearing effect. Furthermore, passage of product is eased and the possibility of contact with inlet walls is minimized.

Finally, there is the matter of dasher speed. As shown by the curve in Figure 30, while higher dasher speed results in a higher U value, it also can cause increased particulate damage. Therefore, higher dasher rpm and the corresponding high heat-transfer efficiency will apply only to products with small particulates. When products contain delicate particulates of 1/2–3/4 in., a variable-speed drive is used to reduce dasher revolutions to about 120 rpm. While this protects product quality, it also lowers exchanger efficiency. Consequently, striking a balance between performance and quality should be determined by lab runs so that optimum operation at full scale becomes a matter of a proper dasher speed setting.

Peanut Butter

For the production of creamy or chunky peanut butter, a process system will consist of surge tanks, a deaerator, scraped-surface heat exchanger, ingredient feeder with inline blender, and transfer pumps.

After mixing roasted nuts with a stabilizer, salt, and sugar to the desired formula, the product is ground and discharged at a temperature of about 150–200°F. Deaeration follows to eliminate air pockets, which initiate oil separation. During cooling from an SSHE inlet temperature of 140–190°F to a discharge temperature of 85–95° · F, the stabilizer is solidified in finely divided crystalline form and uniformly distributed throughout the mixture. At the proper temperature, the peanut butter becomes a viscous, extrudable mass. Crystal change continues and further solidification occurs after filling. For chunky style, a chunk feeder is located between the SSHE and filler or the transfer pump and deaerator.

Table 4 gives a listing of other products routinely processed using SSHEs.

BIBLIOGRAPHY

Adapted from: SSHE Handbook, SSH-1087. APV Crepaco, Inc. Copyrighted APV Crepaco, Inc. Used with permission.

APV Crepaco, Inc.
Lake Mills, Wisconsin

HEAT TRANSFER

BACKGROUND

Almost all food processes depend on or are affected by heat being added or removed at some stage in the operation. The efficient and effective utilization of heat results in economic savings, minimum adverse affects on nutrient components, higher quality consumer-ready products, and minimum effect on the many environmental factors associated with food processing. This efficient and effective use of heat depends on knowledge and subsequent application of the heat transfer mechanisms involved in heating, cooling, and changing the state of foods and food products. The rate at which heat is transferred depends on the type of product, the condition of the product, and the type of heat transfer by which heat enters or is removed from the product.

Other than maintaining a food under handling and storage conditions that ensure the highest quality product, the only option involving heat transfer is the control of processing conditions to give the desired rate of heat transfer. This means considering the three basic mechanisms that control the rate of heat transfer and selecting processing conditions and facilities that optimize the process. Hence a thorough knowledge of heat transfer mechanisms and the relationship between heat transfer rates and other physical and chemical factors is probably the most important consideration involved in the processing of foods. This is further emphasized by the fact that the control of mass and energy balances and fluid flow mechanics, the basics for designing, constructing, and operating the machinery, equipment, and facilities involved in food handling, storing, and processing, is dependent on considerations involving the mechanisms and rate of heat transfer.

Heat is transferred from one body to another by three different mechanisms, namely conduction, convection, and radiation. In fact, most processing facilities utilize two or all three of these means of transferring heat from or to a product. Before considering the mathematical relationships that describe heat transfer and allow the control of the amount and rate of transfer, it is well to have a visual understanding of how each functions.

Conduction heat transfer takes place when two bodies at different temperatures are in contact with each other. Through this direct contact, heat energy is transferred from particle to particle between the solid bodies with no bulk movement of material. When one places a hand on an object having a temperature different from body temperature there is a flow of heat. Hence, when the object is a blackboard in the classroom at room temperature, there is a sensation of the blackboard being cold. Conversely, if one touches a warm element on a stove, there is an immediate sensation of heat being transferred to the hand. The difference in temperatures between the two objects, as well as the characteristics of the conducting body, determines the rate of transfer. In the case of the blackboard, the temperature driving force was probably not more than 20°F (11°C), while the driving force between a warm stove-element and the hand is considerably higher. Hence the sensation of heat will be detected and create considerably more reaction than placing a hand on the blackboard. Other things to consider include the nature of the material to which heat is being transferred. This determines how fast the heat will penetrate as well as the temperature gradient within the material. In general, conduction is highly desired for food being frozen, especially those forms having flat surfaces that can insure maximum contact with plate freezers. In most other cases of freezing irregularly shaped items and heating, cooking, or sterilizing, a combination of conduction and other transfer mechanisms is more efficient and controllable.

Convection heat transfer depends on the bulk movement and mixing of liquids that are initially at different mass temperatures, or on the contact of a solid with a moving liquid stream of a different temperature. These two basic types of convection must be considered together in most heat transfer involved with processing food products. Regardless of whether hot liquids are being mixed in a batch or continuous basis, the temperature of the final combined liquid mixture reaches an equilibrium temperature somewhere between the original temperatures of the two liquids. If a solid is involved, the temperature equilibrium occurs between the bulk of the liquid and the surface of the solid. The velocity of a liquid flowing past a solid affects the rate of transfer between the two materials. Natural or free convection is caused by density gradients (thermal expansion) formed when a liquid is changing in temperature. These can not be controlled and are often a detriment to processing since the rate of transfer is at the mercy of the naturally rising or mixing streams. Forced convection, the pumping or blowing of a liquid or gas over a surface, can be controlled and the heat transfer rates can be predicted. Hence most food processes utilizing convection heat transfer depend on facilities and equipment that use forced convection. This can be demonstrated by considering the wind chill factor, by which a wind causes a person to feel colder than expected at a given outside temperature. For example, in the winter one might hear a weather report in which the outside temperature is given as 0°C and the wind chill factor makes it feel as if the temperature were −15°C. This is because the wind is a forced convection whereas the 0°C temperature is measured under shielded or still conditions.

Many food processing operations include a combination of conduction and convection heat transfer. This is demonstrated in cooling and freezing curves in which blast freezing or convection is the method of heat transfer (see Figure 9 in FISH AND SHELLFISH PRODUCTS) and a plate or conduction heat transfer is combined with blast freezing or convection (see Figure 9 in FISH AND SHELLFISH PRODUCTS). The critical period during which heat of fusion is being removed between about 30 to 22°F (−2 to −6°C) is 220 min in Figure 10 of FISH AND SHELLFISH PRODUCTS. The total time for cooling, freezing, and dropping from 50 to 0°F (10 to −18°C) is 472 min in Figure 10 and 93 min in Figure 9. As has been discussed in FISH AND SHELLFISH PRODUCTS, the more rapid freezing as shown in Figure 9 resulted in high quality frozen fish, similar to the fresh product. Conversely, the slower freezing in Figure 10 resulted in considerable cell degradation that greatly reduced the quality of the end product.

The third type of heat transfer used for heat processing of foods is radiation, the transmission of electromagnetic energy through space. Whereas conduction and convection are dependent on a physical medium through which to transfer heat energy, radiation requires no carrier to transfer wave energy from a surface of one body to another. The amount of energy transmitted is dependent on area and the nature of the exposed surface and the temperature of the body. The amount of radiation absorbed or deflected also depends on the nature of the absorbing body and the body temperature. Hence each body receives radiation from every other body, the amount depending on how much the bodies can "see" of each other and the ability of the body to radiate or deflect and absorb radiation (emissivity or absorptivity).

There are several different types of radiation used in the food industry, each used for a specific reason. The radiation heating from a warm or hot body to a food is akin to that of a person standing in the shade or the sun on a hot day. The ambient air temperature may be the same in both positions; however, a person becomes much warmer in the sun where the direct radiation is being added to the body in addition to the convection heating occurring from the surrounding air. The radiating body for heating a food by radiation can be the hot element in an oven or grill (roasting, baking, broiling). In this case the surface receives energy so rapidly that it can not be conducted into the food fast enough to prevent the desired browning (actually scorching and/or Maillard browning reaction) of the surface.

Other types of radiant energy are used in processing food. Some of these are becoming as prevalent as the conventional concept of cooking or processing by exposure to a hot element or heat source. When an alternating current is passed through a conductor, energy is emitted in the form of waves having a specific wavelength and frequency. Microwave heating is a good example of this process, whereby the energy absorbed by a food is converted into heat due to the friction of moving molecules or atoms.

Ionizing radiation from x rays and gamma rays, while not heating a food during a normal exposure time, can destroy microorganisms and thus accomplish the aim of pasteurizing or sterilizing a food.

PROCESSING FOODS BY CONDUCTION HEAT TRANSFER

The relationship between the factors involving heat transfer by conduction can be intuitively realized by considering the flow of heat energy. One would reason that heat flow (q) perpendicular to the direction of flow would be proportional to the area (A) of contact or flow and the temperature difference (dt) along the path of flow. Likewise, the thickness of the food or path through which the heat is flowing (dx) would be inversely proportional to this flow. Although modern mathematics allow one to derive such relationships, the basic conductive heat transfer relationship, known as Fourier's law, was derived empirically and confirmed by experimentation.

In addition to the factors, discussed above, the physical and chemical properties of a material have a significant effect on heat flow and can be represented by an experimentally determined proportionality constant called the thermal conductivity (k). The thermal conductivity is related to the number of free electrons in a material, varying from being high in metals to low in gases. It can be considered the flow of heat per unit time (watts or Btu per hour) through a given area (square meters or square feet) per unit thickness (meters or feet). The mathematical relationships involving conduction heat transfer can be represented by Fourier's law:

$$q = -kA\,dt/dx \tag{1}$$

The units of each item in SI and English units are shown in Table 1. It is conventional to place a negative sign in front of the equation to signify a positive heat flow from the higher to the lower temperature.

Although the complete mathematical analysis of heat transfer can become extremely complicated when it comes to considering complex three-dimensional flow and integrations over total volumes, most problems involving conduction in food processing systems can be greatly simplified. In most steady-state cases, considering the relatively short range of temperature changes, the uniformity in area over the distance of heat conduction, and the uniformity in thermal conductivity over these temperature ranges, equation 1 can be simplified to

$$q = -kA(t_1 - t_2)/\Delta x - kA\,\frac{\Delta t}{\Delta x} \tag{2}$$

Equation 2 is typical of many problems in nature that involve a driving force analogy. In the case of heat transfer, the rate of transferring some discrete quantity of energy q is equal to the driving force DF that makes the movement happen divided by the resistance R to this movement, or

$$q = \mathrm{DF}/R \tag{3}$$

A common use of this relationship is in the transfer of electrical energy, where the current flowing (I, amperes) is equal to the driving force (E, volts) divided by the resistance (R, ohms), or

$$I = E/R \tag{4}$$

The resistance in the heat transfer equation (2) equals the thickness (Δx), which is directly proportional to resistance to heat transfer divided by the area and thermal conductivity, both of which decrease the resistance,

$$R = \Delta x/kA \tag{5}$$

The reciprocal of the resistance is the conductance (C), where

$$C = 1/R = kA/\Delta x \tag{6}$$

Since the total heat being transferred through each of a series of materials in contact is the same for each material,

Table 1. Selected Coefficients of Thermal Conductivity

Property	SI units	English units
q is the heat flow	watts (W)	Btu heat flow Btu/h
h is the thermal conductivity	(W)(m)/(K)(m^2)	Btu/h of ft^2/ft
A is the area	m^2	ft^2
dt is the temperature difference	°C	°F
dx is the thickness	m	ft

$$q = q_1 = q_2 = q_x = -\frac{k_1 A_1 \Delta t}{x_1} = \frac{k_2 A_2 \Delta t_2}{x_2} = -\frac{k_x A_x \Delta t_x}{x_x} \qquad (7)$$

and considering that

$$q = -\frac{\Delta t}{R} = -\frac{\Delta t}{\Delta x / kA} \qquad (8)$$

then

$$q = q_1 = q_2 = q_x = -\frac{t_1 - t_2}{x_1/k_1A_1} = -\frac{t_2 - t_3}{x_2/k_2A_2} = -\frac{t_3 - t_x}{x_x/k_xA_x} \qquad (9)$$

Combining to obtain the overall resistance

$$R = (x_1/k_1A_1) + (x_2/k_2A_2) + (x_x/k_xA_x) \qquad (10)$$

and the overall driving force

$$\Delta t = t_1 - t_x$$

the overall heat transfer equation becomes

$$q = -\frac{\Delta t}{R} = -\frac{t_1 - t_x}{[(x_1/k_1A_1) + (x_2/k_2A_2) + (x_x/k_xA_x)]} \qquad (11)$$

When, as is often the case, $A_1 = A_2 = A_x$, the equation reduces to

$$q = -\frac{\Delta t}{R} = -\frac{t_1 - t_x}{(1/A)[(x_1/k_1) + (x_2/k_2) + (x_x/k_x)]} \qquad (12)$$

EXAMPLE 1

Using the conductance heat transfer equation, calculate the amount of heat lost through 2 m² (21 528 ft²) of a cold storage wall that is composed of three layers of material. The wall consists of 4-in. (0.1016-m) thick concrete block (x_c) on the outside, 6-in. (0.1524-m) thick polystyrene insulation (x_p), and 2-in. (0.0508-m) thick fir wood (x_f) on the inside. The inside wall of the cold room is −40°F (−40°C) and the outside wall is 68°F 20°C). From Table 2, the thermal conductivities are

$$k_c = 0.76\ \mathrm{W \cdot m/m^2 \cdot K} = 5.27\ \mathrm{(Btu)(in.)/ft^2)(h)(°F)}$$

$$k_p = 0.038\ \mathrm{W \cdot m/m^2 \cdot K} = 0.263\ \mathrm{(Btu)(in.)/(ft^2)(h)(°F)}$$

$$k_f = 0.11\ \mathrm{W \cdot m/m^2 \cdot K} = 0.76\ \mathrm{(Btu)(in.)/(ft^2)(h)(°F)}$$

Using equation 12 where the area is constant (Fig. 1),

$$q = -\frac{t_i - t_o}{(1/A)[(x_c/k_c) + (x_p/k_p) + (x_f/k_f)]}$$

In SI units:

Table 2. Thermal Conductivity of Selected Foods and Processing Facility Materials

Product	Temperature, °C	Thermal conductivity, k^a (W)(m)/(m²)(K)	(Btu)(in.)/(ft²)(h)(°F)
Apple	2–36	0.393	2.72
Aluminum	0	230	1594
Beef, lean	7	0.476	3.20
perpendicular to fiber	62	0.485	3.36
parallel to	8	0.431	2.99
fiber	61	0.447	3.10
Butter	46	0.197	1.37
Concrete, cinder	23	0.78	5.27
Fish muscle	0–10	0.557	3.86
Ice	0	2.3	15.9
Milk	37	0.530	3.67
Olive oil	15	0.189	1.31
	100	0.163	1.13
Polystyrene	24	0.038	0.263
Potato, raw	1–32	0.554	3.84
White fir	23	0.11	0.76
White pine	15	0.15	1.04

Source: Refs. 1 and 2.

[a] 1 (Btu)(in.)(ft.²)(h)(°F) = 0.144228 (W)(m)(m²)(K).

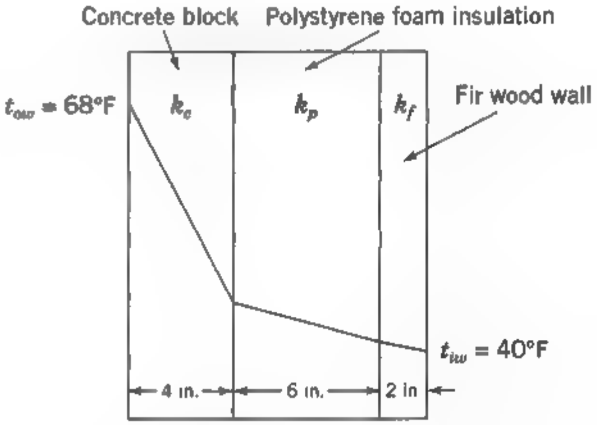


Figure 1. Temperature profile of wall in cold storage room.

$$q = -\frac{(-40) - (20)}{(1/2)[0.1016/0.76 + 0\ 1524/0.038 + 0.0508/0.11]}$$

carrying the unit with the numerical equation

$$q = \frac{\mathrm{°C\ or\ K}}{(1/\mathrm{m})[(\mathrm{m/Wm/m^2K}) + (\mathrm{m/Wm/m^2K}) + (\mathrm{m/Wm/m^2K})} = \text{watts}$$

$$q = 26.05\ \text{W lost through 2 m}^2\ \text{of the wall}$$

Likewise, in English units:

$$q = \frac{(-40) - (68)}{(1/21.528)[4/5.27 + 6/0.263 + 2/0.76]}$$

carrying the unit with the numerical equation

$$q = \frac{{}^\circ\mathrm{F}}{1/\mathrm{ft}[\Sigma(\mathrm{in./Btu})(\mathrm{in.})/\mathrm{ft}^2(\mathrm{h})(^\circ\mathrm{F})} = \mathrm{Btu/h}$$

$$q = 88.74 \text{ Btu/h lost through 2 m}^2 \text{ of the wall}$$

Checking units by converting English to SI:

$$q = (88.74 \text{ Btu/h})(0.29307 \text{ W-h/Btu}) = 26 \text{ W}$$

PROCESSING FOODS BY CONVECTION HEAT TRANSFER

Convection heat transfer is concerned with the mixing of fluids or the transfer of heat from a fluid to a surface. Therefore there is no solid-body thickness through which heat must be transferred, as in conduction. In normal food processing operations the concern is usually to transfer heat between a liquid and the surface of a solid or a fluid. Newton's law of cooling, which also applies to heating, defines the driving force as the temperature difference between the surface temperature (t_s) and the bulk temperature (t_f) of the fluid medium:

$$q = -hA(t_s - t_f) \qquad (13)$$

In this case the temperature of the surface is higher than the bulk temperature of the liquid and h is the convective heat transfer coefficient (commonly called the film coefficient), the experimentally determined proportionality constant that takes into consideration the flowing characteristics and liquid—solid interface effects. The units are $\mathrm{W/m^2 \cdot K}$ or $\mathrm{Btu/(h)(ft^2)(^\circ F)}$. Note that there is no dx or thickness factor so that the resistance R corresponding to conduction heat transfer is

$$R = 1/hA \qquad (14)$$

Figure 2 depicts the type of temperature profile that occurs when a hot fluid is transferring heat to a solid (or liquid) surface. The equilibrium is between the bulk temperature of the liquid (t_f) and the surface of the solid (t_s). Note that the temperature drops in a nonlinear manner. This is caused by the very thin layer of fluid that is almost stagnant because of the friction of the moving fluid, often referred to as the edge effect. This layer is flowing in the streamlined region and, since there is essentially no mixing with the main liquid stream, the heat transfer through this layer is actually by conduction but without a thickness (dx) to consider. The magnitude of the edge or fictitious film effect varies widely depending on the solid surface and explains why this coefficient must be experimentally determined for any given condition. Some select convection film coefficients are given in Table 3. The wide range of film coefficient values is due to the surface and geometry over which the fluid is passing and the turbulence (Reynolds number) of the flowing fluid.

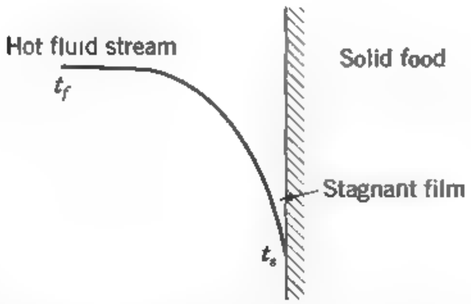


Figure 2. Temperature profile of hot flowing liquid near the surface of solid being heated

Over a period of time, scale deposits build up on the walls of vessels and pipes. The effects of these films or deposits are determined experimentally and expressed in units of the film coefficient h. Therefore films and scale-deposit film coefficients can be handled in the same manner as the thermal conductance of a flowing liquid.

Steady-state heat transfer processes occur in the operations that involve continuous heating or cooling of foods. These include continuous freezing tunnels, deep frying, and pasteurizing and sterilizing liquids. Many processing procedures in which a solid is being heated in a continuous moving system (eg, freezing or cooking a product on a moving belt) can be treated as a total steady-state system by using specific locations on the belt as base points for heat balances. In most of these cases steam, hot water or oil, or a refrigerant is the source for adding or removing heat, so that convection heat transfer is an important factor in these processes.

The only procedures that must be handled as batch, unsteady-state systems are those in which a product is being heated or cooled in place by a steady-state or non-steady-state source (eg, cooking in an oven, retorting canned foods, and freezing a product on a freezer plate). Normally the information required to calculate heat transfer in these situations is collected experimentally and then applied. For example, the thermal death time to ensure the sterilization of canned food is experimentally determined for each size of container and product and then applied to the commercial facilities. The actual retorting time is then increased by a sufficient time to insure that there is no error in obtaining sterilization. In the case of sterilizing products, the National Food Processors Association (in coordination with the Food and Drug Administration) provides

Table 3. Approximate Range of Convection Heat Transfer Coefficients

	Conduction heat transfer film coefficient, h	
Medium	$\mathrm{W/m^2 \cdot K}$	$\mathrm{Btu/(ft^2)(h)(^\circ F)}$[a]
Gases		
natural convection	2.8–28	0.9–9
forced convection	11–110	3.5–35
Viscous liquids,		
forced convection	56–560	17–178
Water, forced convection	560–5600	180–1,800
Boiling water	1,700–28,400	540–9,000
Condensing steam	5,600–1.1 × 10	1,800–3.5 × 10

Source: Refs. 1 and 2.

[a] $1\ \mathrm{Btu/(Ft^2)(h)(^\circ F)} = 3.15459\ \mathrm{W/m^2 \cdot K}$.

sterilizing time requirements to the processors. The processors must then record and maintain the records of retort time and temperature for each batch processed to insure compliance with the retorting requirements. These records are available in legal cases involving undercooking.

COMBINED CONVECTION AND CONDUCTION HEAT TRANSFER

Conduction and convection have been discussed separately in order to emphasize the different mechanisms that control the heating and cooling of food products. However, seldom is a food processing operation carried out in which the heat transfer is not a combination of conduction and convection. Although radiation is also present in most of these operations, the effect is usually quite insignificant unless the process is specifically a radiation process.

Figure 3 extends the transfer of heat from a liquid to a solid surface, through the solid, and then to another liquid. This could be the situation occurring in a heat exchanger where a hot liquid is being used to warm another liquid, with the solid being the wall of the heat exchanger. The resistances to heat flow through the hot fluid (R_h), solid pipe wall (R_w), and fluid being heated (R_c) can be summed to give a total resistance

$$R = R_h + R_w + R_c \qquad (15)$$

Although only three media are used in the example, there is no limit to the number of resistances that can be summed for a given problem.

An overall heat transfer coefficient U combining conduction and convection heat transfer coefficients now can be defined from equation 15, where, although only three media are used in the example, there is no limit to the summing of as many resistances as are necessary in a given situation

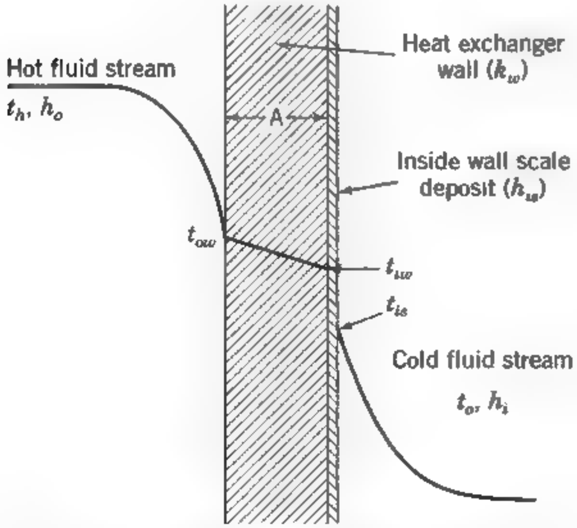


Figure 3. Temperature profile of hot liquid (t_h) transferring heat through heat exchanger wall to another colder liquid (t_c).

$$1/UA = 1/h_hA_h + x_w/k_wA_w + 1/h_cA_c \qquad (16)$$

or

$$UA = \frac{1}{1/h_hA_h + x_w/k_wA_w + 1/h_cA_c} \qquad (17)$$

Thus the overall heat transfer equation becomes

$$q = -UA\Delta t \qquad (18)$$

As noted, U and A are not independent since, if the areas of the heat transfer surfaces vary (eg, a pipe heat exchanger), A must be based on a mean area (A_m). For example, in the process of transferring heat through a pipe that has an inside scale deposit with a determined h_{is}, the overall conductance can be calculated as

$$UA = \frac{1}{1/h_oA_{ow} + x_w/k_wA_m + 1/h_{is}A_{is} + 1/h_iA_{iw}} \qquad (19)$$

where

$$A_{iw} = A_{is} = \pi DL$$
$$A_{ow} = A_{os} = \pi DL$$
$$A_m = (A_{ow} - A_{iw})/\ln(A_{ow}/A_{iw})$$

multiplying the denominator by A_m/A_m gives

$$UA = \frac{1}{(1/A_m)[A_m/h_oA_{ow} + x_w/k_w + A_m/h_{is}A_{io} + A_m/h_iA_{iw}]} \qquad (20)$$

and rearranging gives an expression for UA in a circular cross section where heat is being transferred:

$$UA = \frac{A_m}{A_m/h_oA_{ow} + x_w/k_w + A_m/A_{iw}h_{is} + A_m/A_{iw}h_{iw}} \qquad (21)$$

In this example, the effect of the scale deposit, h_{iw}, is on the inside wall of the pipe. There are commonly five resistances to heat transfer caused by the (1) heat transfer wall and (2) inside (i) and (3) outside (o) of the heat transfer wall, (4) 2 fluids (h_o and h_i), and (5) 2 fouling scales (h_{is} and h_{os}). Often there is a fouling scale on the surface of a transfer surface caused by oxidation or burn-on during operation. Protein foods are particularly bad in that the heat causes denaturation and subsequent adherence to the surface. When gas comes into contact with heat transfer surfaces (eg, a fire tube boiler) heavy scales can be formed. In fact, the maintenance and cleaning of heat exchanger surfaces is a continuous chore, usually accomplished by chemical cleaners. However, in extreme cases (especially in boiler tubes), a periodic rodding out is necessary to maintain the efficiency of the heat transfer.

Boilers are particularly important to the food processing industry since they are the source of steam for many heat transfer systems. The boiler is a heat exchanger that converts a liquid to a vapor. The vapor, after being condensed in a food processing heat exchanger, is normally collected and recycled. Often there are fouling scales or film deposits on both sides of a pipe or plate heat exchanger. This must be recognized in the calculation of the overall heat transfer coefficient.

EXAMPLE 2

In determining the heat transfer characteristics of a heat exchanger, it is important to have a clear picture of the physical exchanger prior to calculations. For example, in a pipe heat exchanger, it is common to determine the *UA* for a unit length of pipe. An exchanger can then be sized to meet the heat transfer requirements. Consider a heat exchanger of stainless steel tubes with the cross section shown in Figure 4. Each tube is 1-in. OD with the following dimensions:

D_i = inside diameter = 0.782 in. (0.0199 m)

D_o = outside diameter = 1.000 in. (0.0254 m)

x_w = wall thickness = 0.109 in. (0.00277)

The heat transfer coefficients are

inside: $h_i = 120$ Btu/(h)(ft^2)(°F) or 681 W/m^2 · K
inside fouling: $h_{is} = 660$ Btu/(h)(ft^2)(°F) or 3748 W/m^2 · K
outside fouling: $h_{os} = 1000$ Btu/(h)(ft^2)(°F) or 5678 W/m^2 · K
outside: $h_o = 1500$ Btu/(h)(ft^2)(°F) or 8517 W/m^2 · K

and the conductivity of the stainless steel tube is

$k = 9.4$ (Btu)(ft)/(h)(ft^2)(°F) or 16.3 W-m/m^2-K

Determine *UA* for the tubes in this heat exchanger. Basis:

1. Consider a 1-ft length (L) of tube.
2. Consider a 1-m length (L) of tube.

Since the area of the tube cross section is expanding perpendicularly to the heat transfer, a mean area (A_m) value must be sued in determining *UA*, and the basic equation becomes

$$UA = \frac{A_m}{(A_m/h_oA_{ow}) + (A_m/h_{os}A_{ow}) + (x_w/k_w) + (A_m/h_{is}A_{iw}) + (A_m/h_iA_{iw})}$$

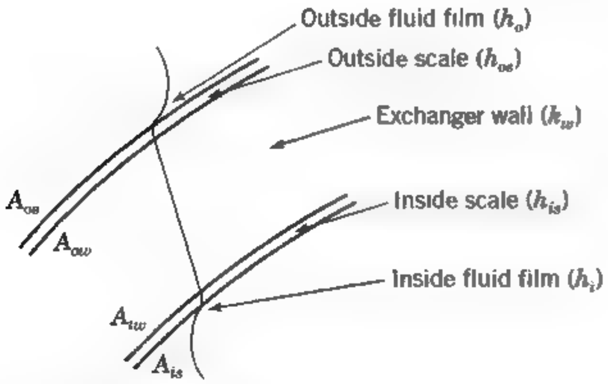


Figure 4. Temperature profile for heat exchanger in which heat transfer area is not constant

Calculation of areas in English units:

$$A_{ow} = A_{os} = \pi DL = \pi(1)(1)/12 = 0.262 \text{ ft}^2$$

$$A_{iw} = A_{is} = \pi \text{DL} = \pi(0.782)(1)/12 = 0.205 \text{ ft}^2$$

$$A = \frac{A_{ow} - A_{iw}}{\ln(A_{ow}/A_{iw})} = \frac{0.262 - 0.205}{\ln(0.262/0.205)} = 0.232 \text{ ft}^2$$

Calculation of areas in SI units:

$$A_{ow} = A_{os} = \pi DL = \pi(0.0254)(1) = 0.0798 \text{ m}^2$$

$$A_{iw} = A_{is} = \pi DL = \pi(0.0199)(1) = 0.0625 \text{ m}^2$$

$$A = \frac{A_{ow} - A_{iw}}{\ln(A_{ow}/A_{iw})} = \frac{0.0798 - 0.0625}{\ln(0.0798/0.0625)} = 0.0708 \text{ m}^2$$

UA in English units:

$$\begin{aligned} UA &= \frac{A_m}{[\Sigma A/hA + x/k]} \\ &= 0.232\Big/\left(\frac{0.232}{(1500)(0.262)} + \frac{0.232}{(1000)(0.262)} + \frac{0.109}{(12)(9.4)} \right. \\ &\quad \left. + \frac{0.232}{(660)(0.205)} + \frac{0.232}{(120)(0.205)}\right) \\ &= 17.1 \text{ Btu/(h)(°F)(ft length)} \end{aligned}$$

UA in SI units:

$$\begin{aligned} UA &= 0.0708\Big/\left(\frac{0.0708}{(8517)(0.0798)} + \frac{0.0708}{(5678)(0.0798)} + \frac{0.00277}{(16.3)} \right. \\ &\quad \left. + \frac{0.0708}{(3748)(0.0625)} + \frac{0.0708}{(681)(0.0625)}\right) \\ &= 29.55 \text{ W/(K)(m length)} \end{aligned}$$

RADIATION HEAT TRANSFER

Since all bodies continuously emit thermal radiation and absorb radiation from other bodies around them, both the received and emitted energy (combining emitted, received, and reflected energies) must be considered when determining the temperature state of a body. This total energy interchange between bodies is significantly affected by the nature of the bodies and the conditions in which these bodies exist. The ability of a body to absorb and emit energy is measured by its emissivity, the fraction absorbed or emitted compared to an ideal perfect radiator or so-called blackbody that has an emissivity (ϵ) of 1.0. It has been determined that the amount of energy emission from a body is proportional to the fourth power of the absolute temperature. A heat transfer equation can be balanced by the experimentally determined proportionality constant known as the Stefan-Boltzmann constant (σ), hence, for a blackbody

$$q = \sigma T^4 \tag{22}$$

where

$$q = W/m^2 \text{ or Btu/(h)(ft}^2\text{)} = 5.6697 \times 10^{-8}\ \text{W/m}^2\text{-K}^4$$
$$= 0.173 \times 10^{-8}\ \text{Btu/(h)(ft}^2\text{)(°R)}^4$$

$$T = K \text{ or °R}$$

In reality, there is no such thing as a blackbody that has an emissivity of 1.0, so the practical equation must take into account the experimentally determined emissivity (fraction of 1.0), resulting in the usable equation for radiation

$$q = \sigma \in T^4 \tag{23}$$

EXAMPLE 3

A person wearing a white shirt and pants ($\epsilon = 0.9$) at room temperature will radiate energy.

In SI units:

$$q = (5.6697)(10^{-8})(0.9)(\text{W/m}^2\text{K}^4)(293)^4(\text{K})^4$$
$$= 376\ \text{W/m}^2$$

In English units:

$$q = (0.1713)(10^{-8})(0.9)[\text{Btu/(h)(ft}^2\text{)(°R)}^4](528)^4(\text{°R})^4$$
$$= 119.8\ \text{Btu/(h)(ft}^2\text{)}$$

Checking units:

$$q = (119.8)(3.1546) = 377\ \text{W/m}^2$$

where

$$1\ \text{Btu/(h)(ft}^2\text{)} = 3.1546\ \text{W/m}^2$$

Since there is an interchange between two bodies, the net amount of radiation received from a source is the radiation received minus the radiation returned or emitted. The combination of the heat being absorbed by a food and the amount being emitted gives an equation or the net heat transfer to a food from a radiation source:

$$q = \sigma\{[(\varepsilon_S\alpha_R F_{S\text{-}R})(T_S)^4] - [(\varepsilon_R\alpha_S F_{R\text{-}S})(T_R)^4]\} \tag{24}$$

where σ is the Stefan-Botzmann constant, ϵ_S is the source emissivity, α_R is the receiver absorptivity, $F_{S\text{-}R}$ is the view factor, source to receiver, T_S is the temperature of source, ϵ_R is the receiver emissivity, α_S is the source absorptivity, $F_{R\text{-}S}$ is the view factor, receiver to source, and T_R is the temperature of receiver.

The amount of energy absorbed from a body depends on how much of the radiating body can be "seen" by the receiver. In most situations involving the processing of food by radiant heat transfer, the view factor is equal to unity since the source is directly below or adjacent to the receiver. Also, the emissivity is essentially the same as the absorptivity in a food processing operation. The emissivities for many surfaces have been determined experimentally and are available in standard data tables. The emissivity of many foods is approximately 0.9, while hot metal (as in the case of oven heating units) has a wide range emissivity values, from as low as 0.1 to 0.6 or higher.

EXAMPLE 4

Bread is being baked on a stainless steel sheet. The top is cooking nicely but the bottom is not getting done The oven (ceiling, wall, and floor) temperature is 400°F (204.4°C) and the emissivity and absorptivity are 0.90

1. The bottom of the stainless steel sheet has emissivity absorptivity of 0.25. Assuming a view factor of 1.0, what is the net rate of radiant heat transferred to the bottom of the sheet? Assume that the surface of the bread while cooking and vaporizing water is 212°F (100°C) and that the conduction heat transfer to the stainless steel sheet is to a surface at the same temperature.
2. What is the net rate of heat transfer to the top of the bread, which has emissivity—absorptivity equal to 0.90?

Solution. (1) By substituting the values in equation 22, the net heat that is being transferred to the bottom of the sheet is:

In English units:

$$q = (0.1713)(10)^{-8}[(0.9)(0.25)(1.0)(860)^4 - (0.25)(0.9)(1.0)(672)^4]$$
$$= 132\ \text{Btu/(h)(ft}^2\text{)}$$

In SI units:

$$q = (5.6697)(10)^{-8}[(0.9)(0.25)(1.0)(478)^4 - (0.25)(0.9)(1.0)(373)^4]$$
$$= 417\ \text{W/m}^2$$

Checking units:

$$(132) \times (3.1546) = 417\ \text{W/m}^2$$
$$\frac{\text{Btu}}{\text{(h)(ft}^2\text{)}}\,\frac{\text{(W)(hr)(ft}^2\text{)}}{\text{(m}^2\text{)Btu}} = \text{W/m}^2$$

(2) By substituting the values in equation 22, the net heat that is being transferred to the top of the bread is:

In English units:

$$q = (0.1713)(10)^{-8}[(0.9)(0.9)(1.0)(860)^4 - (0.9)(0.9)(1.0)(672)^4]$$
$$= 476\ \text{Btu/(h)(ft}^2\text{)}$$

In SI units:

$$q = (5.6697)(10)^{-8}[(0.9)(0.9)(1.0)(478)^4 - (0.9)(0.9)(1.0)(373)^4]$$
$$= 1508\ \text{W/m}^2$$

It is clear that the top portion of the bread is receiving more than 3.5 times the amount of heat being received on the bottom where it is in contact with the stainless steel sheet. This explains why the top, the portion receiving the most heat, is baking at a faster rate than the bottom. It should be noted that often cookies or items being baked in an oven burn on the bottom and are obviously being heated at a faster rate than the top of the item. This is due to the fact that often the metal container is in contact with a hot shelf and is thus receiving heat by conduction.

TRANSFERRING HEAT IN FOODS

Heat transfer was introduced as being an important unit operation in almost every food process. In fact, the transfer of heat to and from a food is certainly the most important factor to consider during processing, whether it occurs in a food processing operation or through natural temperature changes during holding and storage. The type of heat transfer and the rate at which the transfer occurs affect the nutritional value, product safety, and sensory properties of every food that is consumed by humans and animals.

The processes of canning, pasteurizing, and cooking depend on transferring heat into a product to elevate the temperature to a level that will destroy microorganisms. Heat sterilization of a product (eg, pouch packaging and canning) under anaerobic conditions requires closely controlled time and temperature process conditions to ensure that spores resistant to heat (eg, *Clostridium botulinum*) do not survive. Pasteurization temperature and time are just as important to ensure that food-borne diseases are not spread by the food. Cooking a food not only improves sensory acceptance but reduces the microbial content.

Combined heating and cooling operations are commonly carried out during processing or handling of foods. The cooling normally follows the required heating time and temperature period to minimize damage to nutritional and sensory attributes after the heat has accomplished the intended purpose. For example, some fruits and vegetables are blanched prior to freezing to inactivate enzymes that cause degradation and greatly reduced shelf life. Pasteurization to kill microorganisms that might cause public health problems leaves many other microorganisms that will continue to grow. Therefore, pasteurized products (eg, milk) must be held at refrigeration temperatures to ensure a reasonable shelf life. Cooked foods that are not consumed immediately after cooking must be stored in the refrigeration for the same reason.

Many food processes require that heat be added to a food for a purpose other than reducing microorganism contamination or enzyme activity. Vaporization processes such as evaporation, dehydration, and distillation utilize heat energy to vaporize a component, usually water, to change the characteristics of the food. Hence a concentrated juice or a dried cereal product may be subjected to an elevated temperature during the processing.

Often during food processing mechanical energy is converted to heat energy by friction from the particles of food as they rub together. The resulting energy release from such operations as cutting, grinding, screening, and extruding can significantly increase the temperature of a product.

Nature is responsible for adding the heat that is necessary for drying grains in the field to the extent that they can be harvested and stored for long periods of time before being finally processed and consumed. During extremely hot harvest times many fruits and vegetables can be severely damaged during the time it takes to harvest and transport them to a processing plant.

In all cases, whether the heat be intentionally added for safety and to improve texture or unavoidably added as a result of handling or processing conditions, a certain amount of nutritional damage occurs during the heat processing of a food. Depending on the food water content, pH, and oxygen exposure, heat can severely degrade many vitamins, denature proteins, cause free radicals to form, and adversely affect quality attributes such as texture and flavor.

In summary, the transfer of heat directly or indirectly effects the acceptance, quality, and safety of a food. A major responsibility of the food scientist and engineer working with food processes is to optimize the time and temperature conditions of processing and minimize the adverse side affects of heat and other processing conditions to insure that the consumer receives the highest quality food at the minimum cost.

BIBLIOGRAPHY

1. E. L. Watson and J. C. Harper, *Elements of Food Engineering*, 2nd ed., Van Nostrand Reinhold Company, New York, 1988.
2. R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, New York, 1984.

GENERAL REFERENCE

Heat Transfer, H. Wolf, Harper & Row, Publishers, New York, 1983.

GEORGE M. PIGOTT
University of Washington
Seattle, Washington

HIGH-PRESSURE PROCESSING

Consumers are shifting their food purchases from heat-processed to fresh-tasting, minimally processed foods since excessive heat treatment can reduce the perceived freshness of foods. Salads, fruits, vegetables, nuts, spices, oysters, and selected cheeses are traditionally consumed without heat treatment. However, the incidence of pathogenic microbes is increasing in minimally processed and in raw foods previously considered safe. *Escherichia coli* 0157:H7 has been found in fresh apple and orange juice. This has caused the U.S. Food and Drug Administration (FDA) to recommend pasteurization. High-pressure processing (HPP) is one alternative to heat to achieve the pasteurization or commercial sterility of many fresh and freshlike processed foods. An advantage of HPP is that the treatment does not break covalent bonds. As a result, HPP does not change the flavor, color, or nutrient content of the food. Heat and ionizing radiation break covalent bonds as a function of dose and thus have the potential for changing the chemistry of a treated food. An additional advantage of HPP over other food preservation methods is that the effect of HPP is instantaneous and uniform throughout the mass of food being treated. The process is completely independent of the volume, geometry, chemical composition, and physical structure of the food. The capabilities of HPP to yield a pathogen-free, minimally processed food, with an extended shelf life due to inactivation of vegetative forms of spoilage microbes (but not spores or viruses), have attracted the attention of food processors. HPP-treated foods are appearing in the marketplace in Japan, North America, and Europe.

TECHNOLOGY OF FOOD PRESERVATION BY HIGH PRESSURE

Mechanism of Action

Research during the past 100 years has demonstrated that pressures above 100 MPa (15,000 psi) can affect the activity of enzymes and the structure of proteins. The unfolding of a protein polymer and subsequent irreversible denaturation of a protein at pressures above 300 to 400 MPa (45,000 to 60,000 psi) can affect the ability of living cells to control the flux of water and ions across their cellular membranes. Electron micrographs of pressure-treated microbes indicate a loss of cell turgor and collapse of cell structures, indicating damage to the cell membranes. It is believed that this is the mechanism that allows high pressure to inactivate pathogenic and spoilage vegetative forms of microbes in foods. Bacteria, yeast, and molds are primarily single-cell plants. Cell membrane damage can be fatal to their survival. Similarly, parasites, insects, and insect eggs can be inactivated by high pressures in the range of 200 to 680 MPa (30,000 to 100,000 psi). Food products generally consist of large integrated masses of cells. Although high pressure does denature cell membranes and can cause the gelation of starches, many foods—including cooked meats, raw and cooked vegetables, firm fruits such as peaches, and cooked pasta and rice—show little change in structure and texture after treatment at pressures in the range of 680 MPa (100,000 psi). High-pressure inactivation rates of vegetative microbes have been observed to be a function of pressure and time at any given temperature. Experimental data have shown that the rate of inactivation and the shape of the inactivation curve may be influenced by the media in which the microbes are suspended. Generally a lowering of the pH will decrease pressure resistance of a given strain of microbe. By contrast, as the water activity of the suspending media is reduced, the vegetative microbes in the media become more pressure resistant. Also, the strains of any given species of microbe can display a wide range of pressure resistance, and food materials can increase the pressure resistance of a microbe when compared with its rate of inactivation in a phosphate buffer of the same pH. Classic inactivation kinetics based on a first-order reaction may not be followed. The rate of inactivation of a microbe as a function of pressure and time may not be proportional to the remaining concentration of viable microbes. An induction period may be observed, and tailing may also be observed as low numbers of survivors are reached. These findings require that any proposed HPP food-preservation treatment must be challenged with pathogens and spoilage microbes normally associated with the product. Recommended levels of challenge are 10^6 pathogenic microbes and up to 10^8 spoilage microbes per gram of product.

Shelf-stable (commercially sterile) acidic foods may be produced by high-pressure treatment. A pH below 4.5 will block the germination of spore-forming pathogens such as proteolytic and nonproteolytic *Clostridium botulinum*. High pressure may be used to produce pressure-pasteurized, extended-shelf-life, low-acid, refrigerated foods. A water activity above about 0.98 is essential for high-pressure treatment to be effective in the inactivation of vegetative microbial cells. High-pressure treatments have limited applications in the pasteurization of dry foods such as spices, dry fruits, vegetables, and meat products. Because parasites in animal tissue can be inactivated at pressures (200 MPa, 30,000 psi) that have little effect on fresh food quality factors (such as protein denaturation), pressure may be useful in treating fresh animal foods normally infected with parasites. Insect and insect egg inactivation is another possible application of HPP.

Processing Systems for Food Preservation by High Pressure

High-pressure food treatment systems are available for semicontinuous processing of liquid foods and batch processing of packaged foods. Liquid foods processed in semicontinuous systems may be packaged in aseptic or clean-fill packaging equipment after pressure treatment. The semicontinuous treatment of liquid foods requires the following steps. A conventional pump is used to transfer liquid food from a hold tank through an inlet port into a stainless steel high-pressure vessel treatment chamber. The chamber is fitted with a free piston that is displaced from the filling port end toward the opposite end of the chamber where high-pressure water is introduced to pressurize the chamber when the filling step is complete. The filling port is closed at the end of the filling step, and high-pressure water is pumped into the chamber to drive the piston against the food. Because water is compressed to 85% of its original volume at 680 MPa (100,000 psi), the piston is displaced up to 15% of the distance down the chamber. After an appropriate hold time at the desired process pressure, usually in the range of 30 s to 5 min, the pressure is released, and a sterile discharge port is opened. The pressure treated liquid is pushed out of the pressure vessel into a sterile hold tank by low-pressure water moving the free piston to the product inlet end of the vessel. The cycle is repeated under the direction of an automated control system that can monitor and record pressures, times, piston positions, product levels in tanks, process temperatures, and the status of critical seals and valves. A single high-pressure water pump may be used to pressurize several pressure vessels under the direction of the automated control system. A semicontinuous system is manufactured by Flow International (Kent, Washington).

The high-pressure treatment of packaged food is similar to a batch steam retort process. The food is vacuum packed in packages capable of withstanding compression of up to 15% of their volume. The packaged products may be loaded into a carrier that is placed in the pressure vessel. The vessel is sealed, and water is pumped into the vessel until the desired treatment pressure is reached. After the appropriate hold time, the pressure is released, the vessel opened, and the carrier removed. The treated product is then ready for distribution. The batch process may be automated using a control system similar to that used in a semicontinuous system. A single high-pressure pump may be used to pressurize several pressure vessels.

Research on the effect of temperature on the rate of microbial inactivation has demonstrated that temperatures in the range of 50°C (122°F) can increase the rate of microbial inactivation during pressure treatment. The use of controlled temperature conditions during semicontinuous or batch pressure treatment requires that all product must be at the desired process temperature for the time and pressure of the treatment. High-pressure treatment raises the temperature of high-water-content foods about 3°C (5.4°F) per 100 MPa (15,000 psi) of imposed pressure. This temperature increase is lost upon decompression. The specification of a food process temperature in conjunction with a high-pressure treatment could subject the proposed process to FDA regulation.

Systems for the high-pressure treatment of foods require four components. These are a pressure vessel and closure system, a low-pressure pump, an intensifier to deliver high-pressure water, and a control system. Safe, reliable, pressure vessels and closures are now available commercially with capacities of 25 to 250 L (0.88 to 8.8 cubic ft). These vessels are capable of operating at pressures of 680 MPa (100,000 psi) for more than 100,000 cycles. The extreme operating pressures and rapid cycling required for food processing applications has favored wire wound pressure vessels (Flow International, Kent, Washington) and other approaches to ensure safe, "leak before break," designs. Each time a pressure vessel is cycled, the inner wall is subjected to potential crack formation. Successful pressure vessel designs require that the inner wall be under some compression even when the vessel is at full pressure so that potential cracks cannot propagate. Pressure vessels are made from high-tensile-strength steels with several concentric cylinders assembled to ensure compression of the inner wall of the vessel. Wire winding, in which miles of steel wire are wound around a cylindrical liner to ensure compression, can be used as an alternative technology to obtain cycle lives well in excess of 100,000 cycles. The reader is referred to the general references for detailed descriptions of vessel construction technologies. High pressures are achieved by pumping oil, at pressures up to 34 MPa (5000 psi), to an intensifier. The oil works against a piston in the intensifier with an area perhaps 20 times larger than the piston delivering the high-pressure water. Thus the water pressure in this case is exactly 20 times greater than the oil pressure. Pressure vessels are available with built-in intensifiers, and systems can be built with external single-stroke intensifiers. The advantage of a single-stroke intensifier is reduction in system wear and ease of decompression. The control system use in the HPP treatment of foods must be able to deliver a record of the pressure and time of each cycle. A record of the food temperature is needed if temperature is a specified component of the process.

PACKAGING FOODS FOR HIGH-PRESSURE TREATMENT

A major consideration in the packaging of foods for pressure treatment is the effect of compression on the structure and barrier properties of the package. If rigid structures are used, means must be incorporated to allow for up to 15% reduction in volume of the product during pressure treatment at 680 MPa (100,000 psi). Otherwise, flexible packages such as pouches or semiridged structures, such as trays or bowls with easy open peel-seals, can be used. Foods packaged for high-pressure treatment must be vacuum treated to remove as much dissolved and occluded gas as possible. They must be packaged without a headspace because any gas will be forced into the food during compression. Compression of gas represents extra compression work and the presence of dissolved oxygen in the food may lead to accelerated development of oxidized flavors. Ultimately the product-package system must be treated, stored, and evaluated for product quality after the desired storage time at expected storage temperatures. As with all packaging, the food must be held in the proposed package to determine if undesirable flavors transfer from the package to the food.

PRODUCT SAFETY AND REGULATORY ISSUES

Regulations covering the manufacturing and sale of pressure-treated foods are under development at this date. Pressure-treated foods distributed as products labeled "requires refrigeration" are on the market in the United States. These types of products must be free of pathogens, must be manufactured under good manufacturing practices (GMP), and must remain free of spoilage microbes until their "use by" date. Naturally acidic and directly acidified products are covered by existing regulations for these products. Their safety requirements are the same as for refrigerated foods. Currently product safety and regulatory requirements for shelf-stable, low-acid foods, preserved by high pressure, and packed in hermetically sealed containers have not been developed in the United States. Good manufacturing practices (GMP) can be expected to be similar to those for heat-processed foods. It is expected that an HPP process will be required to provide the equivalent of a 12-fold decimal reduction of a pressure-resistant strain of *C. botulinum*.

Because spores are pressure resistant, all commercially sterile high-pressure preserved foods to date have been acidic products with a water activity very close to 1.0. Examples are yogurt, fruit preserves, fruit juices, and pourable salad dressings. Viable microbial counts may be obtained when commercially sterile pressure-treated acidic foods are plated on neutral pH media. For this reason all pressure-preserved products should be stored at an appropriate storage temperature for twice their proposed shelf life to assay for possible regeneration of microbes.

ECONOMIC CONSIDERATIONS

Although high-pressure treatment systems are inherently expensive to build and maintain, construction and maintenance costs are expected to decrease as commercial use expands. For any installed high-pressure treatment system, the cost per unit of food treated is a function of the amount of product treated per cycle (batch or semicontinuous operation) and the number of cycles run per year. The shape of the package is an important factor in determining the amount of product that can be treated each cycle. Cylindrical containers may occupy 30% of a pressure vessel while product packed in bags for food service may occupy up to 90% of the available vessel volume. Cycle time is a function of the required treatment time at pressure and the time needed to move the food in and out of the pressure vessel, pressurize, and decompress the vessel. Semicontinuous systems processing liquids with a 3-min hold time at 680 MPa (100,000 psi) can cycle up to 15 times per hour and utilize 100% of the available process vessel volume. A batch system treating packaged foods may require 5 min to load, close and seal, pressurize, decompress, open and unload, in addition to the 3-min process time. This will result in seven to eight cycles per hour. Another major cost factor is system availability; that is, the percentage of time the unit is not down for maintenance and repairs and is available for production. System availability is related to the reliability of the pressure vessel closures, valves, piping, pumps, intensifier, control systems, and material handling equipment. The food industry will need a system reliability in excess of 95% exclusive of programmed maintenance.

GENERAL REFERENCES

D. A. Ledward et al., eds., *High Pressure Processing of Foods*, Nottingham University Press, Loughborough, United Kingdom, 1995.

T. Ohlsson, ed., *High Pressure Processing of Foods and Food Components—A Literature Survey and Bibliography*, Swedish Institute for Food and Biotechnology, Göteborg, Sweden, 1996.

M. W. Peck, "*Clostridium botulinum* and the Safety of Refrigerated Processed Foods of Extended Durability" *Trends Food Sci. Tech.* **8**, 186–192 (1997).

Daniel F. Farkas
Oregon State University
Corvallis, Oregon

HISTORY OF FOODS

Writing a history of food is a formidable task that involves identifying, describing, and discussing what people ate where and at what time, where it came from, and how it was obtained. This article attempts to sketch a broad picture of the human quest for food through time and, primarily, will be an archaeological odyssey for reasons discussed below. It treats the history of food as the general history of the human subsistence economy.

Food is an integral part of the human experience, and because the human experience is long, so must be the story of food. The greater part of the human experience occurred long before the domestication of plants and animals. The invention of writing came considerably after the development of agriculture. For some two million years humanity wrote nothing that would aid us in reconstructing its subsistence. Even after writing was invented some 5500 yrs ago, very little was written about food for a long time. Therefore the history of food is for the most part based on archaeological materials.

A history of food must have a beginning. An anthropologist would choose to begin the story in the dim, distant ages of human prehistory some two million years ago with the earliest fossils human paleontologists place in the genus *Homo*. From the two-million-year point, our evolving ancestors and their food quests and habits will be traced to the present time. Important aspects of their lifestyles that bear on the evolution of their diets and general subsistence economies will also be briefly sketched. This approach takes us first, into the realms of physical anthropology and archaeology (paleoanthropology); second, into ancient history; and third, into history. We move from the earliest foraging and scavanging subsistence economies through gathering and hunting to the invention of agriculture and beyond. Finally, some modern cuisines will be briefly addressed in order to demonstrate the varied world origins of ingredients in given dishes and meals.

The earliest known writing appeared in the Near East about 5500 yr ago. All human experience before that, there and in the rest of the world, falls under prehistory. Prehistory is the province of the prehistorian. Most prehistorians are archaeologists. All research is archaeological and based on the few imperishable remains left to us. For example, for early times we must rely on stone, bone and antler tools, bones from animals that were consumed, charcoal and ash from campfires, traces of camps, and so on. Much of what people did and ate was not recorded or preserved (see below). Because our data are limited, there are massive holes in our story. Consequently, caution is necessary when presenting it.

For roughly two million years, humans foraged, gathered, and hunted for wild food. As time passed, we grew more efficient in exploiting our planet's wild resources. This was accomplished through the gradual development of more efficient and sophisticated tool technology based on stone, bone, wood, antler, and such and through developments in social and political organization and probably ideology, which allowed greater cooperation within social groups. These developments were accompanied by increasing brain size and intelligence. This long episode in human history is called the Paleolithic or Old Stone Age. The chronological subdivisions of the Paleolithic are used below for ease of presentation of the history of food (Fig. 1).

About 10,000 or 11,000 years ago in various areas of the Old World, strides were taken that resulted in the domestication of some plants and animals. The "agricultural revolution" had begun. By 9000 yr ago, the revolution was in full swing. There was still no writing. Neither were there cities and states, only small farming villages. Some groups in many world areas still hunted and gathered for their livelihood. The so-called invention of agriculture is prehistoric. In the Near East (sometimes called the Fertile Crescent and including southern Turkey, western Iran, Iraq, Syria, Jordan, Lebanon, and Israel), this earliest agricultural period is called the Neolithic (Fig. 1), and was truly revolutionary for much of humanity. While the invention of agriculture was not simultaneous in all Old World areas, developments in India and the Far East (Southeast Asia and China) were roughly concordant. For none of these the amount of product that can be treated each cycle. Cylindrical containers may occupy 30% of a pressure vessel while product packed in bags for food service may occupy up to 90% of the available vessel volume. Cycle time is a function of the required treatment time at pressure and the time needed to move the food in and out of the pressure vessel, pressurize, and decompress the vessel. Semicontinuous systems processing liquids with a 3-min hold time at 680 MPa (100,000 psi) can cycle up to 15 times per hour and utilize 100% of the available process vessel volume. A batch system treating packaged foods may require 5 min to load, close and seal, pressurize, decompress, open and unload, in addition to the 3-min process time. This will result in seven to eight cycles per hour. Another major cost factor is system availability; that is, the percentage of time the unit is not down for maintenance and repairs and is available for production. System availability is related to the reliability of the pressure vessel closures, valves, piping, pumps, intensifier, control systems, and material handling equipment. The food industry will need a system reliability in excess of 95% exclusive of programmed maintenance.

GENERAL REFERENCES

D A. Ledward et al., eds., *High Pressure Processing of Foods*, Nottingham University Press, Loughborough, United Kingdom, 1995.

T. Ohlsson, ed., *High Pressure Processing of Foods and Food Components—A Literature Survey and Bibliography*, Swedish Institute for Food and Biotechnology, Göteborg, Sweden, 1996.

M. W. Peck, "*Clostridium botulinum* and the Safety of Refrigerated Processed Foods of Extended Durability" *Trends Food Sci. Tech.* **8**, 186–192 (1997).

Daniel F. Farkas
Oregon State University
Corvallis, Oregon

HISTORY OF FOODS

Writing a history of food is a formidable task that involves identifying, describing, and discussing what people ate where and at what time, where it came from, and how it was obtained. This article attempts to sketch a broad picture of the human quest for food through time and, primarily, will be an archaeological odyssey for reasons discussed below. It treats the history of food as the general history of the human subsistence economy.

Food is an integral part of the human experience, and because the human experience is long, so must be the story of food. The greater part of the human experience occurred long before the domestication of plants and animals. The invention of writing came considerably after the development of agriculture. For some two million years humanity wrote nothing that would aid us in reconstructing its subsistence. Even after writing was invented some 5500 yrs ago, very little was written about food for a long time. Therefore the history of food is for the most part based on archaeological materials.

A history of food must have a beginning. An anthropologist would choose to begin the story in the dim, distant ages of human prehistory some two million years ago with the earliest fossils human paleontologists place in the genus *Homo*. From the two-million-year point, our evolving ancestors and their food quests and habits will be traced to the present time. Important aspects of their lifestyles that bear on the evolution of their diets and general subsistence economies will also be briefly sketched. This approach takes us first, into the realms of physical anthropology and archaeology (paleoanthropology); second, into ancient history; and third, into history. We move from the earliest foraging and scavanging subsistence economies through gathering and hunting to the invention of agriculture and beyond. Finally, some modern cuisines will be briefly addressed in order to demonstrate the varied world origins of ingredients in given dishes and meals.

The earliest known writing appeared in the Near East about 5500 yr ago. All human experience before that, there and in the rest of the world, falls under prehistory. Prehistory is the province of the prehistorian. Most prehistorians are archaeologists. All research is archaeological and based on the few imperishable remains left to us. For example, for early times we must rely on stone, bone and antler tools, bones from animals that were consumed, charcoal and ash from campfires, traces of camps, and so on. Much of what people did and ate was not recorded or preserved (see below). Because our data are limited, there are massive holes in our story. Consequently, caution is necessary when presenting it.

For roughly two million years, humans foraged, gathered, and hunted for wild food. As time passed, we grew more efficient in exploiting our planet's wild resources. This was accomplished through the gradual development of more efficient and sophisticated tool technology based on stone, bone, wood, antler, and such and through developments in social and political organization and probably ideology, which allowed greater cooperation within social groups. These developments were accompanied by increasing brain size and intelligence. This long episode in human history is called the Paleolithic or Old Stone Age. The chronological subdivisions of the Paleolithic are used below for ease of presentation of the history of food (Fig. 1).

About 10,000 or 11,000 years ago in various areas of the Old World, strides were taken that resulted in the domestication of some plants and animals. The "agricultural revolution" had begun. By 9000 yr ago, the revolution was in full swing. There was still no writing. Neither were there cities and states, only small farming villages. Some groups in many world areas still hunted and gathered for their livelihood. The so-called invention of agriculture is prehistoric. In the Near East (sometimes called the Fertile Crescent and including southern Turkey, western Iran, Iraq, Syria, Jordan, Lebanon, and Israel), this earliest agricultural period is called the Neolithic (Fig. 1), and was truly revolutionary for much of humanity. While the invention of agriculture was not simultaneous in all Old World areas, developments in India and the Far East (Southeast Asia and China) were roughly concordant. For none of these

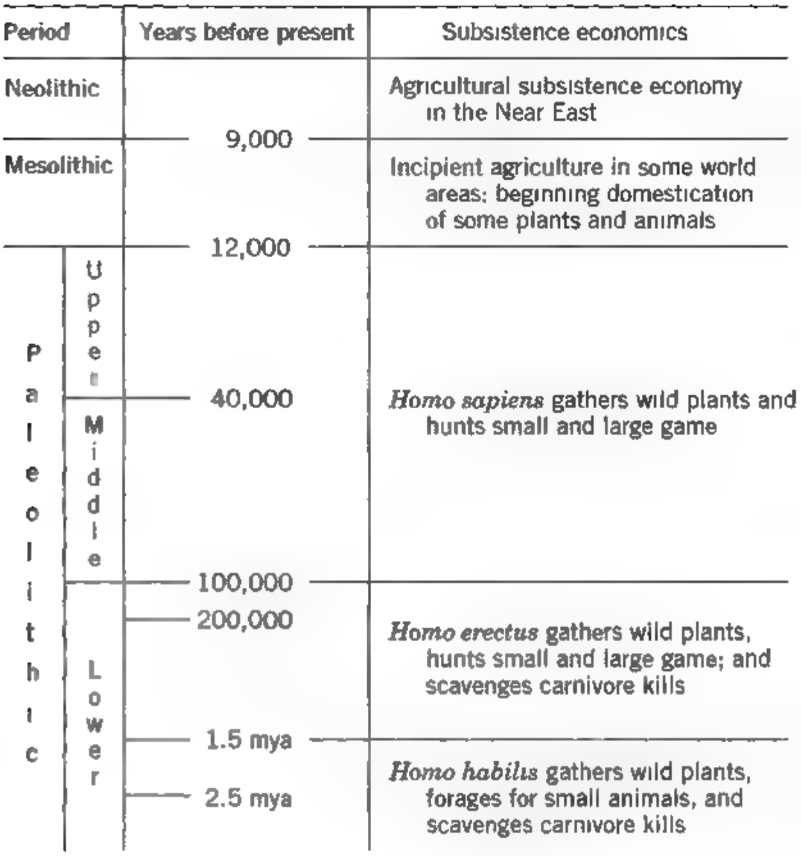

Period
Years before present
Subsistence economics
Neolithic
Agricultural subsistence economy in the Near East
9,000
Mesolithic
Incipient agriculture in some world areas: beginning domestication of some plants and animals
12,000
Paleolithic
Upper
40,000
Homo sapiens gathers wild plants and hunts small and large game
Middle
100,000
200,000
Homo erectus gathers wild plants, hunts small and large game; and scavenges carnivore kills
Lower
1.5 mya
Homo habilis gathers wild plants, forages for small animals, and scavenges carnivore kills
2.5 mya

Figure 1. Simplified chronological chart not to scale.

areas do we have a complete picture of the development of agriculture.

In the New World, agriculture began independently a few thousand years later than it did in the Near East. There were dramatic developments in Mexico, tropical Central and South America, and in the South American Andes Mountain region. Like those of the Old World, New World domesticates were to have worldwide consequences. Also like that of the Old World, the picture is frustratingly incomplete.

The development of agriculture was revolutionary in several senses: (1) it eliminated dependence on wild resources; (2) in its areas of origin and in areas that ultimately adopted it, it permitted population growth well beyond what wild resources could support; and (3) it laid the economic foundation for the eventual development of civilization. Without agriculture, there would be no cities and nation-states with their centralized government bureaucracies, monumental public works, social class systems and inequities, exact and predictive sciences, and all other civilizational characteristics such as taxes, welfare systems, and terrorists. Humanity could not afford them.

As indicated earlier, the major part of this presentation is based on archaeological research. It is essentially an archaeological odyssey through time and around the globe; thus, and because the reader is most likely not an archaeologist, it is necessary to present a brief discussion of the problems and methods involved in reconstructing the prehistory and history of food. A glossary of technical terms may be found at the end of the article.

BACKGROUND

Reconstructing the history of food is fraught with dangers and difficulties. Much of what is written, especially that for prehistoric times, is based on a number of assumptions, some warranted, and some not. Those based on human biological necessities probably have some validity. Those based on archaeological research are subject to two major problems. First, not everything preserves. Second, archaeological interpretation of what does remain is difficult and has itself been based on assumptions. We are all familiar with the image of "man the hunter" and the portrayal of Stone Age humans as mighty, intrepid hunters of massive, dangerous, and cunning game like mammoths and cave bears. This image comes to us through the magnificent Late Stone Age paintings of western Europe, from food bones found in caves and other living sites and from kill and butchery sites found throughout the Stone Age world. Because plant foods rarely preserve and are even more rarely portrayed, incautious interpretation by some has left the impression that prehistoric humans ate mostly meat. Except for environments like the arctic and the subarctic, where edible plants are rare, this is unlikely to be the case for a variety of reasons.

Humans are primates. Most primates are herbivorous and only accidentally omnivorous, and some are omnivorous (1,2). Higher primates tend to be plant eaters, although a few, such as chimpanzees, will deliberately hunt and eat meat (2). Humans are omnivorous today, and it is likely that our hominid ancestors, as primates, were also omnivorous. Early hominid teeth, like those of modern humans, are those of omnivores (2). The greater majority of modern humans cannot extract sufficient vitamins and minerals from meat alone to survive. Eskimos are an exception, but even they eat the stomach contents of the herbivorous animals they kill and take advantage of plant foods during the short growing season (1). Nor can we live on plants alone without bringing together the right combination of plants to supply us with complete proteins. Nutritionists tell us that humans need a diet balanced so that we receive all the vitamins and minerals the body demands for efficient functioning. This means a combination of plant and animal foods with heavy emphasis on plants as opposed to animal protein.

Anthropological study of historic and modern gatherer-hunters shows that, with the exception of the Siberian and North American Eskimos of the subarctic, the ratio of wild plant food gathered is almost always greater than that of hunted or fished animal protein. The actual ratio depends on both available environmental resources and cultural factors and differs between groups. An additional factor affecting the ratio is the seasonality of resources. In some seasons plant foods are more available than in others, such as winter in the temperate northern hemisphere, when, in the absence of plant preservation techniques or even with them, more meat will be eaten—or perhaps, less food will be consumed in general.

In a given environment various small animals are foraged, such as rodents, birds, reptiles, and insects. While larger game may be preferred, it is not always present or present in sufficient quantity to appease human appetites for animal protein.

In short, gatherer-hunters adapt to the environment in which they live and the ratio of plant to animal foods will reflect that adaptation. It will also reflect cultural factors such as religious taboos and the cultural definition of what is and is not food. We also recognize that hunting is high risk and not always successful. Gathering provides an ensured food supply.

Archaeologically, little trace of the plants consumed by recent gatherer-hunters will remain. Without the ethnographic documentation of these living people, we would never have complete knowledge of their subsistence economies. This is because plant material is notoriously perishable and subject to selective preservation. In some dry desert areas, plant remains do preserve. So do human coprolites. Ancient coprolites contain seeds, pollens, and husks or shell parts that aid in reconstructing paleodiet. But human coprolites are rarely found. In wet areas, such as bogs, plants often preserve, as do human bodies. Perhaps one of the most famous and scientifically valuable examples of preservation is Tollund Man, in whose preserved stomach were found the remains of his last meal, a porridge prepared from a wide variety of seeds of domesticated and wild plants (3). A very recent technique of extracting collagen from human bone and conducting trace-element and isotopic analysis enables us to determine whether and what kinds of cereal grasses were being consumed, for example, maize. But, of course, the bone itself must be preserved and unfossilized (4). Fossilized bone is completely mineralized and contains no organic material.

Animal bone is also subject to selective preservation. Large bones survive better than do small bones, and not all bone fossilizes. Acid soil destroys bone. Insect parts rarely preserve. In sum, little of what was originally deposited at a site by human subsistence activity preserves. There is no real way of reconstructing the ratio of plant to animal food consumed by our remote ancestors or even those closest to us in time.

We are left on shaky ground. Our assumptions are: (*1*) both presapiens and sapiens hominids were omnivorous; (*2*) they adapted to the varied environments in which they lived and exploited their edible resources using the then current technology and sociopolitical organization; (*3*) gathering plants and foraging small animals provided them with a more reliable food supply than did hunting large game; and (*4*) by the Late Stone Age, fully human cultural factors surfaced that led to food selection among the edibles when people could afford it.

Figure 1 is provided as a convenient guide through the time periods discussed below. The chart is highly simplified and applies only to Europe, northern Africa, and the Near East. Although data from other world areas are discussed within the time divisions, it is fully acknowledged that the terms Paleolithic (Old Stone Age), Mesolithic (Middle Stone Age), and Neolithic (New Stone Age) do not apply well to Subsaharan Africa, the Far East and North and South America. With this in mind, the periods used and their finer subdivisions are briefly defined below.

The Paleolithic or Old Stone Age began about 2,500,000 years ago (2.5 mya) and is subdivided into three major chronological stages on the basis of advances in stone tool technology: Lower or Early Paleolithic (2.5 mya–100,000 yr ago), Middle Paleolithic (100,000–40,000 yr ago), and Upper or Late Paleolithic (40,000–12,000 yr ago) (Fig. 1). The Paleolithic ended with the end of the last ice age, called the Wurm or Weichsel in the Old World and the Wisconsin in the New World.

The Mesolithic or Middle Stone Age was a short period that began with the final retreat of the last glacier about 12,000 years ago (Fig. 1). No definitive end date that applies to the planet can be given because the proper end of the period is with the beginning of agriculture. Agriculture began at different times in different parts of the globe. In the Near East, the period fades into what is sometimes called the Proto-Neolithic. The Proto-Neolithic (about 11,000–9000 yr ago) is called such because it was the era of incipient agriculture. Various animals and plants were brought under domestication, but wild animals and plants still constituted an important part of subsistence. There appear to have been some similar developments in Thailand dating to this period.

The Neolithic began in the Near East about 9000 years ago, when human subsistence was based fully on agriculture (Fig. 1). Fully agricultural economies were later in Europe, the Far East, and the New World.

PALEOLITHIC

Lower Paleolithic (Early Stone Age): 2.5 mya–100,000 Years Ago

East Africa; Olduval Gorge, Tanzania between 1.85 and 1.5 mya; Hominid Form: *Homo habilis.* A fully bipedal form with a manipulative hand and a complex brain, the upper range of which was more than half the size of the present human brain, *H. habilis* was probably an omnivore. Although no primate is a carnivore, *H. habilis* looks like one because of the nature of what has been found on its habitation sites (living floors). Isolated and fragmentary remains of almost every conceivable available animal from mice and turtles to gazelles and saber-toothed tigers have been found. Although some paleoanthropologists argue about just how these bones got there, the consensus is that *H. habilis* was a scavanger of carnivore kills who brought pieces back to eat raw. In some cases, as at Olduvai Gorge, Tanzania, and Koobi Fora, Kenya, it camped at large animal carcasses such as elephant and hippopotamus and ate them on the spot, bringing fragments of other scavanged animals to the same place (5). Although there is no way of knowing, it is possible that the meat was not always fresh. The smaller animals could have been hunted and/or foraged, but given the hominid's size (about 4 ft) and rudimentary stone tool technology, it is doubtful the large and more dangerous animals were hunted (2,5). Like historic Australian aborigines and Amazonian Tukanoan Indians, they may also have consumed grubs and insects (6,7). One assumes they would have eaten any eggs they found.

It seems obvious that *H. habilis* sought meat. Did it also eat plants? There are no remains, but the likelihood is that it did and probably in a higher proportion than meat. Its primate physiology would have demanded this. Seeds, nuts, edible leaves and twigs, and fruits were available.

Europe, Africa, China, and the General Far East; 1.5 mya–300,000–200,000 years ago. *Homo erectus*, the presumed descendant of *Homo habilis*, took the stage around 1.5 million years ago. Although the size of the *H. erectus* brain was not yet equal to that of the *H. sapiens sapiens* brain, *H. erectus* was otherwise fully human physically. Taller, heavier, more muscular, and with a brain the upper range of which was within the lower range of ours, *H. erectus* was more intrepid than *H. habilis* and accomplished more, such as controlling fire. *H. erectus* preferred warm to temperate cold climates. The latter would be characterized by seasonality of resources and in some cases, seasonal low availability of plant foods. It is likely that in the colder climates a high degree of nomadism was required in pursuit of plant foods. Of course, in the colder environments, more meat could and would have been eaten.

True to form, *H. erectus* ate everything that was available and edible, including it seems, itself (8). *Homo erectus* more than likely continued the practice of scavanging carnivore kills and foraging for small animals. However, we know that large game was hunted. Abundant evidence from Spain (elephant and horse) (9), France (10), and the Zhoukoudien Caves in northern China indicate this (5,8). No stone spear points are yet known, but wooden spears with fire-hardened tips would have served well. Evidence from China indicates that at least some meat was cooked, including *H. erectus* (8).

Evidence from France (10), East Africa (11), and China (8), although scarce, indicates that plants were eaten.

Middle Paleolithic (Middle Stone Age): 100,000–40,000 Years Ago

When this period began depends on what is being emphasized. For present purposes the discussion here begins at 100,000 yr ago and ends 40,000 yr ago. This period is characterized by the emergence of *Homo sapiens*. The form most commonly known is Neanderthal, but there were other varieties who are given other taxonomic names.

Homo sapiens lived in a variety of environments from the Ice Age northern European subarctic tundra to tropical forests and therefore evolved a variety of environmental adaptations specific to each. The ratio of plant to animal consumption probably reflected differential environmental adaptations. In cold and temperate climates mobility was the main pattern owing to the seasonality of resources. Hunting emerged as an important pattern around the then inhabited world and stone spear points are known. Massive game such as woolly mammoth and woolly rhinoceros were taken in cold climates, but smaller game including such large cold-climate animals as reindeer and warm-climate camel and gazelle and small game such as birds and rabbits were also hunted. Harpoon heads and fish bones indicate that some fishing was done on the tundra of northern Europe (9). Opportunistic scavanging probably continued. The diet appears to have been broad-spectrum and unspecialized. Meat was probably roasted.

While no plant food remains have been found except for Kalambo Falls, Zambia (11), digging stick tips from northern Europe (9) and tooth wear patterns indicate plant food consumption. How plant foods were prepared and whether they were cooked remains a mystery, as does the existence of methods of storing them for winter. The proportion of plant to animal foods would have depended on the environment.

Upper Paleolithic (Late Stone Age): 40,000–12,000 Years Ago

The modern human form called *Homo sapiens sapiens* characterizes this period. During these 30,000 years of the latter part of the last Ice Age, humans expanded into all inhabitable environments from the tropical forests of Africa to the subarctic tundra of Siberia and into Australia and North and South America. A wide variety of macroenvironmental and microenvironmental adaptations were necessary. Humanity—through its flexible mode of adaptation anthropology refers to as culture and society—adapted. The result was the emergence of a great deal of cultural diversity. If we were to emphasize the diversity, a discussion of the history of food would become immediately unmanageable. Therefore, only the broadest picture will be drawn.

Northern peoples made cold climate adaptations that led them to specialize in hunting certain large game. That is not to say they did not take smaller game. It seems, however, that the habits of the animals on which they depended dictated their basic lifestyle. The degree of nomadism a people pursued depended on the migratory patterns of the main animal. Large game ranged from woolly mammoth and woolly rhinoceros to bison, horse, and reindeer (9). The decimation and extinction of horse and bison herds in Europe suggest that meat was stored for the winter. Fishing was carried out, especially of salmon. In southern coastal areas people not only fished for salmon but also gathered large quantities of mollusks and shellfish (12). There are no plant food remains or indisputable evidence for the storage of plants for winter (13). It is possible that more meat was consumed during the ferocious winters. The ratio of plant to animal food is impossible to determine, but at least in some areas, a wide variety of berries and other fruits, roots, tubers, and nuts would have been available in summer and autumn. Given the intelligence, knowledge, and experience of these people, one would assume they had a variety of preservation and storage techniques for at least some plant foods.

Of course, one can never say anything about the consumption of eggs and honey, or even insects, since these have no way of preserving, but their consumption is likely throughout these time periods.

During the last Ice Age, northern Africa and the Middle East enjoyed more temperate climates. The massive cold climate game did not exist. Although temperate cold, temperatures were warmer and seasons more varied than in northern Europe. As a consequence, the ratios of plant to animal food probably reflected that difference. But little can be stated with certainty. Further south, one would expect a high ratio of plant to animal food and in desert regions, the highest. Today, large game is almost absent in most desert regions and, when present, is sparse. Small game such as rodents, birds, and reptiles and a variety of insects are characteristic fauna. The ratio of plant to animal consumption is very heavily in favor of plants.

For late Ice Age North and South America, depending on latitude and altitude, the same picture may be painted. There is a dearth of information on plant food consumption and a great deal bearing on the hunting of massive, large and small game.

Mesolithic: 12,000 Yr Ago to a Regionally Variable End Date

The Mesolithic or Middle Stone Age (sometimes called the Epipaleolithic) began with the final retreat of the last glacier about 12,000 yr ago. The period's end date is variable depending on when agriculture came into being in a given area.

With the end of the last glaciation, the climatic and vegetational picture characteristic of the modern world took over. Consequently, and especially in northern latitudes, there was considerable environmental change. Humanity had to respond and did. The emerging cultural adaptations were more complex than before and an even greater amount of cultural diversity based on, among other things, microenvironmental adaptations came into being. Again, only the broadest picture will be given.

Throughout the world, the Ice Age megafauna disappeared. Mammoths, cave bears, giant sloths, gigantic deer, and their like became extinct. Smaller game, fish and other seafood became the focus of humanity's search for protein. People adapted to forest, grassland, desert, lake, riverine, and seacoast niches (9). Most people exploited several adjacent niches, such as forest and lake, and in most areas, because of the seasonality of resources, were nomadic in their lifestyles. The ratio of plant to animal food would have varied considerably depending on the adaptive strategy in a given set of niches.

One of the consequences of environmental change was the establishment of vast stands of wild wheat and barley extending in appropriate niches from east of the Caspian Sea west and south into the Near East (14). Concurrent with this was the spread of herds of wild sheep and goats in the same region (15).

During what is sometimes called the Proto-Neolithic of the Near East, gatherer-hunters took full advantage of these abundant wild resources. Some even settled down into small, permanent communities and built permanent houses. They were able to gather sufficient wild wheat to do so. Jack Harlan conducted an experiment in the same area (the ancestral wild wheat still grows there) and estimates that in 3 weeks a quantity sufficient to last a full year could be gathered (16). It is likely that it is this that permitted sedentary living. People supplemented these plant resources with hunting sheep, goats, deer, pigs, cattle, and so forth. At some point, perhaps 11000 yr ago, they began managing herds of wild sheep and/or goats and animal husbandry was born (17,18). Somewhere around 9000 yr ago, wheat and barley were domesticated. Shortly thereafter, peas and lentils, the wild varieties of which grow in the same area, were added to the list of domesticates. So were pigs. Agriculture was born in the Near East around 9000 yr ago (7000 BC) (see Tables 1 and 2).

Neolithic (New Stone Age): 7000 BC to an End-Date Variable Depending on Other Cultural Developments

In the Near East, the plant/animal food ratio appears to have been greatly in favor of plants. Sickles, grinding stones, storage pits, and ovens are highly characteristic artifacts of the period. Impressions of wheat and barley kernels appear in mud bricks, hearth materials, and pottery. Bread and porridge probably formed the main part of meals. Sheep and goat herds seem to have been husbanded mainly for wool, hides, and milk. Cheese may have been invented and added to the diet. Animal slaughter was selective by age and sex. Meat, therefore, was not consumed as a daily ration. As in ancient historic times, domesticated sheep and perhaps goats may have been religious feast foods.

True to form, humans remained omnivorous, but in the Near East, at least, dairy foods in the form of milk and perhaps, cheese took the place of meat as a daily food. For a while, some hunting was done that would have placed meat on at least some tables. People may also have traded

Table 1. Origins of Major Domesticated Food Animals

Animal	Region of Original Domestication
Cattle	Near East
Chicken	India
Goat	Near East
Pig	Near East
Sheep	Near East
Turkey	Mexico

Table 2. Origins of Selected Domesticated Food Plants

Plant	Region of Original Domestication
	Grains
Amaranth	Mexico
Barley	Near East
Maize (corn)	Mexico
Millet	Asia
Oats	Europe
Quinoa	South American Andes
Rice	China and Southeast Asia
Rye	Turkey
Sorghum	India
Wheat	Near East
	Legumes
Common beans	Mexico and South America depending on species
Lentils	Near East
Peas	Near East
Peanuts	Bolivia
Soybeans	Far East
	Sugar
Sugarcane	Indonesia or New Guinea
Sugarbeet	Europe
	Root crops
Beet	Europe
Potato	South America
Sweet Potato	South America
	Vegetables[a]
Avocado	Mexico
Cabbage (cole) family	Mediterranean
Chives	Near East
Eggplant	India
Garlic	Near East
Leeks	Near East
Olive	Mediterranean
Onion	Near East
Peppers	Mexico
Squash	Mexico
Tomato	Mexico
	Fruits[a]
Apple	Western Europe and Asia depending on variety
Banana	Southeast Asia
Grape	Near East
Kiwi	China
Lemon	Southeast Asia
Lime	Southeast Asia
Mango	India
Orange	Southeast Asia
Pear	Western Europe and Asia depending on species
Pineapple	Brazil
	Nuts and edible seeds
Almond	Mediterranean
Brazil nut	Brazil
Cashew	Brazil
Hazelnut	Europe
Pecan	United States
Pistachio	Mediterranean
Sunflower	United States
Walnut	Iran
	Spices
Black pepper	India
Chili pepper	Mexico
Mustard seed	Mediterranean
Nutmeg	Malay Peninsula
Vanilla	Tropical America
	Beverages
Chocolate	Mexico
Coffee	Ethiopia
Tea	India and China

[a] Common, not botanical classificaton.

for meat with nonagriculturalists such as hunters and pastoralists, but it is likely, as mentioned above, that it was primarily a feast-day item.

Since meat would have been scarce, salt would have become necessary. Without the salt naturally present in meat, humans cannot survive. A substitution is necessary. Salt production and trade became important.

Ultimately, certain fruits such as figs, dates, and grapes came out of the Near East (see Table 2).

Let us look at the development of agriculture elsewhere. Many Near Eastern crops and animals made their ways into Europe and together with native plants (eg, perhaps cherry and plums) ended gathering and hunting there as the major subsistence mode about 6000 BC (19). Northern China domesticated millet, which eventually was adopted by eastern Africa. Rice was domesticated in Southeast Asia and has made its way all over the world. South America gave the world the potato, peanuts, manioc for tapioca, and the sweet potato, among other crops. Regarding animals, southern Europe and/or Turkey gave us cattle, the jungles of India the chicken, and Mexico the turkey (20) (see Tables 1 and 2). Charles Heiser's book, *Seed to Civilization: The Story of Food* (20), is a valuable source for the geographic origins of domesticated plants and animals.

Mexico has given much to the world. It was one of the planet's primary centers of domestication. It took approximately four thousand years for Mexico to domesticate the wealth of foods it has given (21). Some 21 domesticated plants have their origins in Mexico (22). Maize—corn, as we call it—is the result of 4000 yr of painfully slow selection and hybridization that began around 6000 BC. Corn is a staple food in parts of Africa and South America as well as in Middle America. Mexico has also given us a large variety of beans, chili peppers, and squashes, to say nothing of a variety of tree and cactus fruits. It also gave us the tomato, avocado, and chocolate. With the limitations of space here, it is impossible to list its valuable and varied contributions to the world's diet (see Table 2).

The development of agriculture in Mexico lasted from at least 6000 to 2000 BC, and there were several centers of domestication, some humid tropical, and some semiarid desert. Throughout most of its prehistory and history, plant foods have superseded animal foods in dietary importance. In the desert areas of the southern part of the state of Tamaulipas and in the Tehuacan Valley we have the plant preservation to prove this (21). We also have the historical records from conquest times. Some people rarely, if ever, saw meat prior to the arrival of the Spaniards, and this is true even now in the most poverty-stricken rural areas. Traditionally in Mexico and over much of Middle America, meat is a fiesta food, a special-occasion food eaten only a few times a year. (This has changed among the more affluent populace.) How, then, eating plant foods almost exclusively, did these people survive?

The fortuitous congruence of maize and beans, provided they are eaten together, provides complete protein. Therefore, a meal of tortillas and beans, in terms of protein, is healthful. The lime water in which the dried maize is soaked prior to grinding into flour for tortillas provides calcium. A few chilis and other vegetables or fruits added to the meal provides additional vitamins and minerals. The use of salt provides sodium. Adults do well on such a diet. Small children, however, are often malnourished because they need more animal protein. Infants who are weaned too soon die. Infant mortality is still very high in rural areas of Mexico and Guatemala.

Of course, before the arrival of the Spaniards, the population was not completely without sources of animal protein. Turkey eggs, ant larvae, other insects, and grubs were eaten. Eggs of other birds were probably gathered. In the coastal areas marine resources, including iguana, turtle and turtle eggs, fish, crustaceans, and mollusks were consumed, although perhaps some were not allowed to everyone in these socially stratified societies. In the tropical rainforests reptiles, monkeys, deer, wild pig, and other animals existed and were hunted. But again, the consumption of some of these was probably strictly controlled. Deer, wild pig, and small game such as badger, squirrels of various kinds, rabbit, and hare abounded in the central Mexican highlands, and it is probable that the consumption of the small game was not regulated. Throughout the Middle American region, the common people probably made do with beans and corn as their main source of protein and supplemented these with small game, insects, and eggs and in the region around modern Mexico City, with fish and larvae from lakes now drained.

It is of interest to note that the beginnings of agriculture in the Near East, Far East, and Middle and South America appear to have been roughly concurrent. This is not likely to have been due to any communication of ideas. Why agriculture began is subject to much debate, and there are no clear answers. Environmental change, population pressure, religion, and the human propensity to experiment have all been invoked (1,16,17,20). It is beyond the scope of this article to discuss these debates.

Another interesting fact is that in the probably unintentional search for complete protein in the virtual absence of meat consumption, humanity in various parts of the globe domesticated plants that, when consumed together, provide complete protein The case for Middle America has been briefly discussed. Rice and beans and other combinations also provide complete proteins (1).

A final fact that should be added is that in all world areas, the development of agriculture was a long, slow process. In a given area, not all plants were domesticated and brought under cultivation at the same time. Nor were all animals domesticated simultaneously. It took centuries and, in some cases, millennia to bring together the agricultural complexes that are prehistorically and historically known.

CONCLUSION

It would appear that for most of humanity's history, we were omnivores like many other primates and that, like other primates, we consumed more plant food than animal. Under special circumstances the ratio might swing in favor of animal over plant, but this is rare and due to special environmental circumstances. Even then, plant food is consumed, even if this means consuming the stomach contents of dead herbivores. Also rare is the society that does not seek some form of animal protein even if it means eating insects, grubs, or raw bird eggs.

The ratio of plant to animal food consumption is probably still in favor of plants around the planet, even for those of us who like our meat so much that we jokingly call ourselves carnivores. If we look at the modern Western diet, we find that even fast foods like hamburgers and pizza are heavy on the plant end. The hamburger comes on a bun made from flour and is accompanied by french fried potatoes, potato chips, or potato salad, is relished by plant products such as tomato catsup, mustard (seed, vinegar), pickles or pickle relish, onions, tomatoes, lettuce, and sometimes mushrooms. The basic pizza is, of course, made from flour and tomato sauce, regardless of the topping on the pizza. If we look at the Far Eastern diets of China, Southeast Asia, and Japan, we find that while meat or seafood may form part of the meal, the major constituent is vegetables and other plan foods like rice or noodles.

We have reached a point in the world where the history of any given dish or meal is utterly fascinating. The ancient northern Chinese somehow received wheat and the idea of flour from the Near East and ultimately invented the noodle. In the thirteenth century, Marco Polo went to China and allegedly, inter alia, encountered the noodle and found it to taste good. He took it back to Italy, where, ultimately, a variety of pastas were developed, including spaghetti. A moderately well-off Mexican sits down to a several-course meal. The first dish is *arroz à la mexicana* (Mexican-style rice). It consists of rice (Southeast Asia) toasted raw in lard (a pork product originating from the Near East) or vegetable oil (an eastern Mediterranean idea) seasoned with a sauce made from tomatoes (Mexico), onion, and garlic (both Near East) and cooked in chicken broth (ultimately India). It is served with corn tortillas (Mexico) or perhaps, flour tortillas (made from wheat flour which has its ultimate origins in the Near East). The next course is *carne de cerdo con salsa verde* (pork in green sauce). The pork is ultimately Near Eastern. The green sauce is made from *tomates* (*tomatillos* or green husk tomatoes) and chilis (Mexico), onion (Near East), and *cilantro* (Near East). The final course is *frijoles*, which, if done right, are cooked with lard or oil and also, perhaps, a little onion. Without the fats and onion, the *frijoles* are thoroughly Mexican. All may be accompanied by beer (a thoroughly European idea incorporating many Near Eastern ingredients) or carbonated soft drinks (a U.S. invention). Many of the "foreign" ingredients in the Mexican cuisine can, of course, be attributed to the sixteenth-century Spanish conquest, one of the results of which was a revolution in cooking. A glance at the history of Spain would illuminate how some of these ingredients got into its cuisine!

The cross-fertilization in our various cuisines is enormous and universal. A simple plate of bacon and hen's eggs served with orange juice, coffee, toast, and jam has its ultimate origins in the Near East, India, Southeast Asia, Ethiopia, and Europe or North America (if the jam is raspberry). If you add hashbrowns or grits, you must acknowledge South America or Mexico. A bowl of vegetable beef soup or chicken and vegetable soup contains ingredients from around the world. A vegan's vegetarian plate is a veritable travelog of time and space.

The history of food and cuisine is, among other needs and things, very much a product of trial and error experimentation and the human propensity to travel, trade, and even conquer and to bring home new and delightful products and ideas and to modify and invent new products, new combinations, and new dishes. Humans are indeed omnivores in every sense of the word.

GLOSSARY

Anthropology. The field that studies humans as biological and cultural beings. Such study includes human culture, biology, language, and history. See also *Culture*.

Archaeology. The subfield of anthropology that studies and reconstructs past cultures and societies.

Coprolites. Naturally dried fecal matter.

Culture. The patterned thought and behavior—such as social, political, economic, and religious—that individuals learn and are taught as members of social groups and that is transmitted from one generation to the next.

Ethnography. The anthropological study and description of the cultures of living groups: their social, political, economic, and ideological systems and all the behavior that relates to these.

Food bones. The bones of animals consumed by humans.

Foraging. The collection of edible wild plants and small animals, such as birds, rodents, reptiles, and insects.

Hominid. The common name for those primates referred to in the taxonomic family Hominidae: modern humans and their nearest evolutionary ancestors.

Homo. The genus to which modern humans and their nearest evolutionary ancestors belong: *Homo habilis* (handy human), *Homo erectus* (upright human), *Homo sapiens* (knowing human), and *Homo sapiens sapiens* (modern human).

Kill and butchery sites. Locations where an animal or animals were killed by humans and butchered on the same spot.

Living floors. Locations where concentrations of living debris are found. Such debris may be tools, debris from tool manufacture, food bones, and other evidence for human occupation.

Lower Paleolithic. Dating from 2.5 mya to about 100,000 yr ago. The beginning of the period is marked by the presence of the earliest known stone tools and probably, the first appearance of the *Homo* genus. The period is characterized by increasing human brain size, and capacity, intelligence, and the evolution toward greater complexity of human technology and culture.

Middle America. Mexico and Central America.

Mesolithic. A term that designates immediately preagricultural societies in the Old World. A diagnostic technological characteristic is the presence of microliths, small stone blades set into bone or wood. In the Near East, sickels used for the harvesting of wild grains were made using this technique.

Middle Paleolithic. Dating from about 100,000 to 40,000 yr ago. The period is marked by increased sophistication in stone tool technology, such as the making of tools from prepared cores, and by the presence of *Homo sapiens*. Human culture became more complex, particularly with regard to the ideological system. Religious ritual, as manifested archaeologically in the burial of the dead with grave goods, came into being.

mya. Million years ago.

Neolithic. A stage in cultural evolution generally marked by the appearance of ground stone tool technology (bowls, adzes, axes, etc) and frequently by domesticated plants and animals and permanent villages.

Paleoanthropology. The multidisciplinary approach to the study of human biological and cultural evolution. It includes physical anthropology, archaeology, geology, ecology, and many other fields.

Paleolithic. Dating from about 2.5 mya to 10,000 BC. The period during which stone tools were produced by percussion flaking (chipping). This period is characterized by the origin and evolution of modern humans and culture.

Physical anthropology. The subfield of anthropology that studies human biology and evolution.

Presapiens. Members of the genus *Homo* who lived before the appearance of the *sapiens* species: *Homo habilis* and *Homo erectus*.

Primates. The order of mammals that includes prosimians, Old and New World monkeys, apes, and humans.

Sapiens. The species to which Middle and Upper Paleolithic and modern humans belong.

Scavanging. The procurement of meat from animals killed by carnivores.

Site. A confined geographic area or location of interest to archaeologists in which the remains of earlier human activity are concentrated.

Subsistence economy. A term referring to food resources and their modes of procurement: foraging, gathering, and hunting for wild plants and animals, agriculture, marketing, etc.

Upper Paleolithic. Dating from about 40,000 to 12,000 yr ago. The final stage of the Paleolithic. It is characterized by the prevalence of modern humans with more sophisticated culture. Characteristic remains in western Europe are polychrome cave paintings, sculpture, engraving, and stone tools made from blades.

BIBLIOGRAPHY

1. P. Farb and G. Armelagos, *Consuming Passions: The Anthropology of Eating*, Houghton-Mifflin, Boston, 1980.
2. R. Jurmain, H. Nelson, and W. A. Turnbaugh, *Understanding Physical Anthropology and Archaeology*, 4th ed., West Publishing Co., St. Paul, Minn., 1990.
3. P. V. Glob, *The Bog People: Iron Age Man Preserved*, Ballentine Books, New York, 1969.
4. A. Sillen, J. C. Sealy, and N. J. Van der Merwe, "Chemistry and Paleodietary Research: No More Easy Answers," *American Antiquity* **54**, 504–512 (1989).
5. B. G. Campbell, *Humankind Emerging*, Scott, Foresman and Co., Boston, 1988.
6. N. Tindale, *Aboriginal Tribes of Australia*, University of California Press, Berkeley, 1974.
7. D. L. Dufour, "Insects as Food: A Case Study from the Northwest Amazon," *American Anthropologist* **89**, 383–387 (1987).
8. K. C. Chang, *The Archaeology of Ancient China*, 4th ed., Yale University Press, New Haven, Conn., 1986.
9. K. W. Butzer, *Environment and Archaeology: An Ecological Approach to Prehistory*, 2nd ed., Aldine-Atherton, Chicago, 1971.
10. H. de Lumley, "A Paleolithic Camp at Nice," *Scientific American* **220**, 42–50 (1969).
11. D. J. Clark, *The Prehistory of Africa*, Praeger, New York, 1970.
12. L. G. Straus, G. A. Clark, J. Altuna, and J. A. Ortega, "Ice Age Subsistence in Northern Spain," *Scientific American* **242**, 142–152 (1980).
13. R. Dennell, *European Economic Prehistory: A New Approach*, Academic Press, Orlando, Fla., 1985
14. J. R. Harlan and D. Zohary, "Distribution of Wild Wheats and Barley," *Science* **153**, 1074–1080 (1966).
15. J. R. Harlan, "Plant and Animal Distribution in Relation to Domestication," *Philosophical Transactions of the Royal Society of London* **275**, 13–25 (1976).
16. J. R. Harlan, "A Wild Wheat Harvest in Turkey," *Archaeology* **20**, 197–201 (1967).
17. C. Redman, *The Rise of Civilization: From Early Farmers to Urban Society in the Ancient Near East*, Freeman, San Francisco, 1978.
18. H. J. Nissen, *The Early History of the Ancient Near East 9000–2000 B.C.*, University of Chicago Press, Chicago, 1988.
19. A. Whittle, *Neolithic Europe: A Survey*, Cambridge University Press, Cambridge, 1985.
20. C. B. Heiser, Jr., *Seed to Civilization: The Story of Food*, Harvard University Press, Cambridge, 1990.
21. R. S. MacNeish and D. S. Byers, *The Prehistory of the Tehuacan Valley*, Vol. 1, *Environment and Subsistence*, University of Texas Press, Austin, 1967.
22. R. C. West and J. P. Augeili, *Middle America: Its Lands and Peoples*, Prentice Hall, Englewood Cliffs, N.J., 1989.

DARLENA K. BLUCHER
Humboldt State University
Arcata, California

See also BIOLOGICALLY STABLE INTERMEDIATES.

HOMOGENIZERS

The homogenizer, which is used today in many food and dairy applications, was invented in the 1890s. Although many changes and modifications have been made to the machine over the years, the basic components of the homogenizer are the same as those early machines. Before describing the homogenizer in detail, it is important to distinguish the type of homogenizer used in food and dairy processing from the more generic term homogenizer. Today, the term homogenizer is often applied to any piece of equipment that disperses or emulsifies. This equipment may include a turbine blade mixer, an ultrasonic probe, a high shear mixer, a colloid mill, a blender, or even a mortar and pestle. The more precise definition of a homogenizer is a machine consisting of a positive displacement pump and a homogenizing valve that forms a restricted orifice through which product flows.

EARLY HISTORY

The first homogenizers were invented at the turn of the century and were used for making artificial butter (1). Gaulin invented and patented his homogenizer for the processing of milk and first showed his machine to the public at the 1900 World's Fair in Paris (2,3). The early literature attributes the term homogenizing, or homogenization, to Gaulin (1). The homogenization of milk, that is, reducing the milk fat globules in size to retard separation and the resulting cream layer, was at least 26 years ahead of its time, because the pasteurization of milk had not been perfected and public acceptance of this product was yet to be realized. Although very little homogenized milk was produced in the early years, homogenizers were sold for ice cream and evaporated milk. Few changes were made to the homogenizer design from 1900 to 1930, but after that, improvements were made to make machines more cleanable and sanitary (1). Homogenized milk became more popular in the 1940s. Some of the benefits of homogenized milk that helped to sell it were reduction of curd tension (which made milk more digestible, especially for infants), uniformity of fat throughout the product, and improvement in the appearance and palatability of the milk (4,5). Today, of course, homogenized milk is universally accepted. The dairy industry is one of the largest users of homogenizers, but homogenizers are also used for other food products.

DESCRIPTION

Figure 1 shows a modern dairy homogenizer. As previously mentioned, the homogenizer consists of a pump and homogenizing valve. The pump is usually a reciprocating positive-displacement pump, which delivers a relatively constant flow rate despite the pressure or restriction to flow made by the homogenizing valve. The positive displacement pump has a power end and a liquid end. The power, or drive, end converts rotating motion to reciprocating motion. In most machines, an electric motor is connected to a drive shaft by V-belts and sheaves. The drive shaft turns an eccentric shaft by means of gears. In other cases, the motor is connected directly to the eccentric shaft by V-belts and sheaves.

The eccentric shaft has cams that drive the plungers or pistons by means of connecting rods and crossheads. The crosshead couples the connecting rod to the plungers or pistons. A piston and a plunger are both solid cylinders that displace fluid; however, for a piston the sealing elements are moving with the piston, and for a plunger the sealing elements are stationary. The sealing elements are called plunger packing (6).

The liquid end includes the pump chamber, or block, and all its components. Figure 2 shows a typical pumping chamber. The plungers move back and forth in the chamber. The eccentric shaft cams that drive the plungers are offset to provide a steady flow. If the pump has three plungers, they would be offset by 120 degrees. This means that the flow profile is represented by the summation of three overlapping sine curves producing some peaks and valleys, but, in general, a continuous flow will be produced. Some homogenizers have five, six, or seven plungers depending on the model.

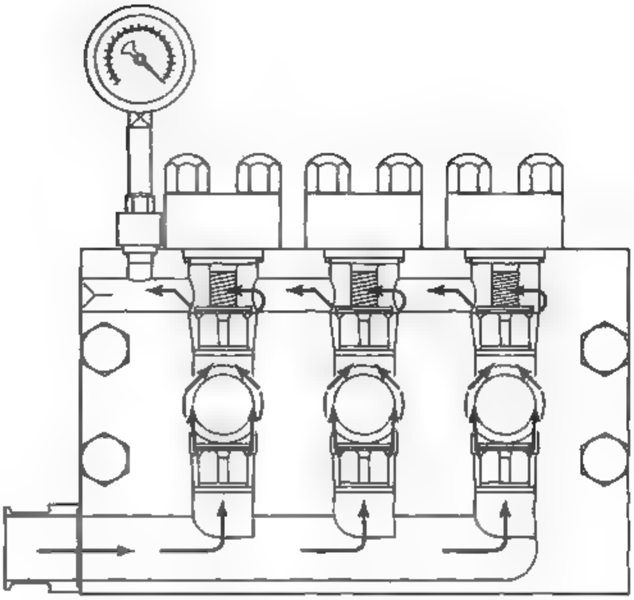


Figure 2. Pumping chamber of a dairy homogenizer.

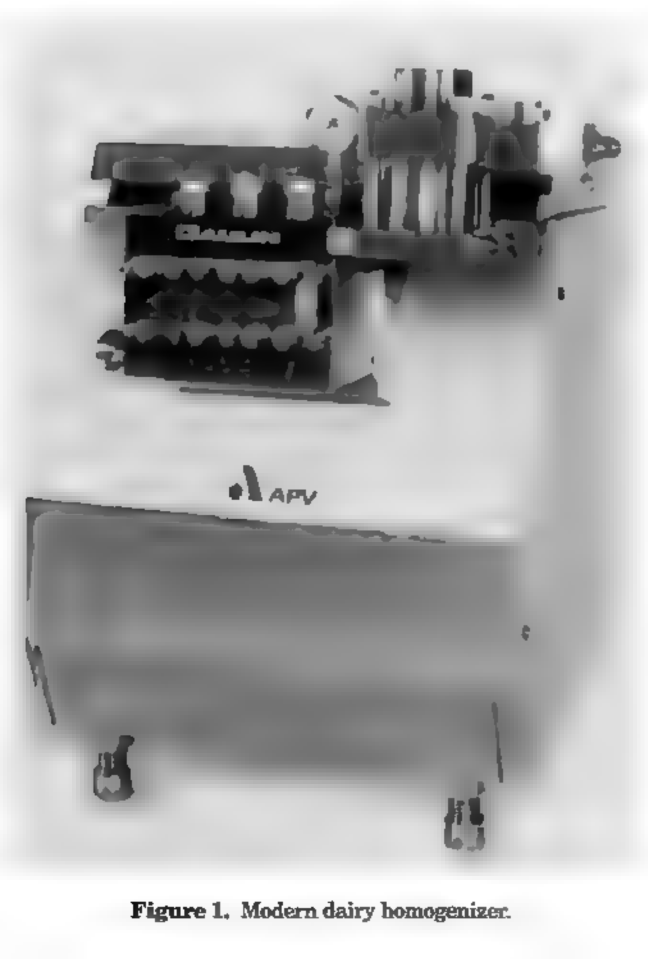


Figure 1. Modern dairy homogenizer.

In addition to the reciprocating plungers, the pumping chamber has suction and discharge valves. On the rearward motion of the plunger, the suction valve opens and liquid is drawn in while the discharge valve closes. On the forward discharge stroke the suction valve closes and the discharge valve opens. The plunger then displaces or pushes the liquid out of the chamber and through the discharge valve. These pump valves can be simply a ball sitting on a seat or a guided valve with a pilot aligning the valve to the seat. The piloted valve is called a poppet valve and the other a ball valve. The liquid end of the pump, especially components in contact with the liquid, is usually made of ceramics, stainless steel, or special alloys. These materials include 17-4, 15-5, 304, and 316.

Homogenizers can cover a wide range of flow rates and pressures. The range of flow rates for different machine sizes can go from 7.6 L (2 gal) per hour for a laboratory machine up to 52,996 L (14,000 gal) per hour for a large production machine. The maximum operating pressure can be 6.90 MPa (1,000 psi) up to 150 MPa (21,756 psi), but the higher the pressure rating, the lower the capacity for a given size machine. This is due to the limitation of force allowed on the drive end of the machine. For a given size machine, increasing capacity usually requires larger diameter plungers or pistons with a corresponding decrease in operating pressure due to the limiting thrust loading. Because the operating pressure is exerting force against the face of the plunger or piston, the larger diameter means that the power stroke requires greater force. Therefore, the size of the plunger or piston is limited by the maximum tolerable force on the drive end. Of course, as the pressure and capacity increase, the motor power needed also increases because the required motor power is directly proportional to the flow rate times the operating pressure.

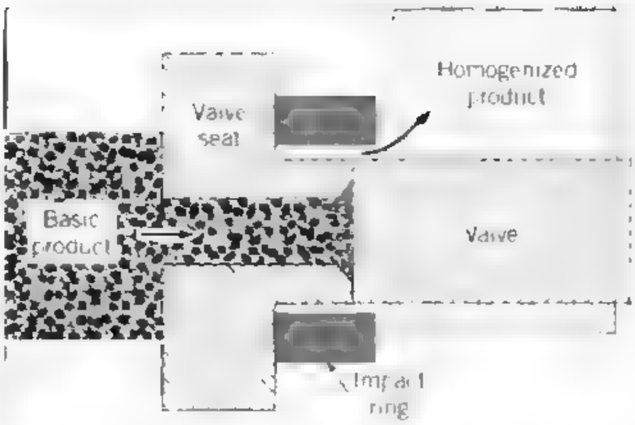


Figure 3. Simple homogenizing valve assembly.

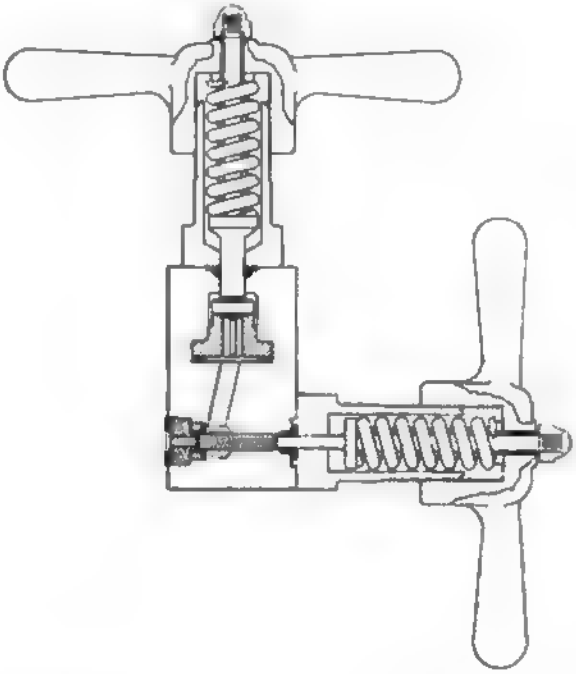


Figure 4. Two-stage manually actuated homogenizing valve assembly.

HOMOGENIZING VALVE

The positive-displacement pump delivers fluid to the homogenizing valve that is contained in a valve body attached to the pumping chamber of the machine. Figure 3 shows a simple homogenizing valve assembly and its designated parts. The fluid to be processed flows into the homogenizing valve seat and pushes against the face of the homogenizing valve. After flowing out through the restricted orifice between the valve and seat, the liquid impinges on the impact or wear ring. Without the impact ring the fluid would cut into the body of the homogenizing valve assembly, eventually requiring replacement of this expensive part. When an impact ring is used, the worn ring can be replaced at a low cost. Figure 4 shows a two-stage homogenizing valve assembly. The actuation of the valve requires applying force on the valve to counteract the force pushing the valve open due to the pressure of the fluid. This pressure is caused by the reduction of flow area when the valve is pushed toward the seat, while the pump is delivering a constant flow rate. The force exerted by the liquid is equal to the area of the valve in contact with the liquid times the pressure generated. For example, a valve with a contact diameter of 6.35 mm (0.25 in) at 68.95 MPa (10,000 psi) needs a counteracting force of 2184 N (491 lb). A valve with a contact diameter of 25.4 mm (1 in) at 68.95 MPa requires 34,936 N (7,854 lb) of counteracting force.

If the counteracting forces are large, then a spring-loaded handwheel (manual operation) will not deliver enough mechanical advantage to achieve high pressures and some other means of actuation such as a hydraulic or pneumatic system must be used. For some products, a two-stage homogenizing valve consisting of two valves in series is used, and the valves can be manual, hydraulic, or pneumatic depending on the valve size and pressure. The homogenizing valve, seat, and impact ring are usually made of special wear-resistant materials because of the high fluid velocities in the valve assembly and because some products contain suspended solids.

As previously described, Figure 3 shows the flow through the homogenizing valve. Although this flow profile may look simple, the fluid dynamics occurring are profound. Intense energy changes occur in the homogenizing valve as the liquid goes from high pressure and low velocity to low pressure and high velocity. The best way to understand these changes is to consider an example. A homogenizer operating at 13.8 MPa (2,000 psi) and 11,356 L (3,000 gal) per hour with a conventional homogenizing valve would, typically, have a calculated gap of about 0.152 mm (0.006 in) between the valve and seat. The pressure before the homogenizing valve seat is 13.8 MPa (2,000 psi) and the velocity is about 6.1 m (20 ft) per second. As the fluid enters the gap, the pressure drops and the velocity increases to 122 m (400 ft) per second. In this case 2.1 L (0.56 gal) per second are flowing through an opening of 25.2 mm^2 (0.039 in^2) in one second. These hydrodynamic changes occur over distances less than 0.254 mm (0.1 in) in less than 0.000002 s (7–9). Homogenization is completed shortly after the liquid leaves the gap area between the valve and seat. Therefore, the potential energy of the liquid stored in the pump chamber is rapidly converted to kinetic energy producing the homogenization effect. Walstra suggests that the energy density in the fluid during homogenization is as high as 10^{12} W/m^3 (10). Only a portion of this energy is used for emulsification, but these intense flow conditions are necessary to trigger homogenization.

THEORIES OF HOMOGENIZATION

Because of the dynamic forces occurring in the homogenizing valve, there has been some interest in determining the mechanism of homogenization. Many researchers have suggested theories to account for homogenization by relating the presumed flow condition in the homogenizing valve to known concepts of fluid dynamics. Homogenization includes the formation of emulsions (one immiscible liquid uniformly distributed into another) or dispersions (solid particles dispersed throughout a liquid), but most theories of homogenization only consider the formation of emulsions. Therefore, these theories usually relate to the mechanism by which dispersed oil droplets are disrupted into smaller droplets and distributed throughout a continuous water phase. This approach is a consequence of the fact that most researchers were investigating the homogenization of milk when they developed their theories.

Theories of homogenization include shear, impact, wire drawing, acceleration and deceleration, homogenizing valve vibration, turbulence, and cavitation (1,11–13). Many of these theories have been discounted over the years. For example, impact of the dispersed droplets on the impact ring is not the cause of homogenization because the required velocities at impact are not large enough to disrupt the droplet, and increasing the distance from the valve seat to the impact ring does not significantly affect homogenization of an emulsion. Wire drawing is the elongation and subsequent disruption of the thinned droplet, but this is unlikely to occur due to the flow conditions in the valve. Shear is commonly mentioned as a mechanism, but the velocity gradients in the valve gap boundary layer do not appear to be adequate for emulsification. Also, the successful homogenization of oils with viscosities greater than that allowed for shearing action to occur indicate a mechanism other than shear (14).

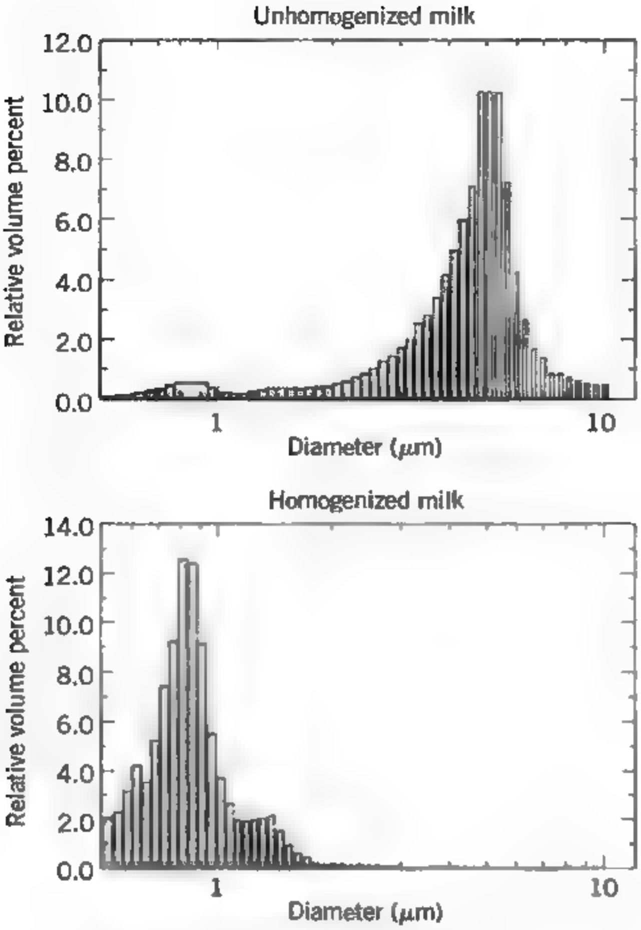
Two other prominent mechanisms of homogenization involve turbulence and cavitation (15,16). The intense turbulent eddies generated in the liquid at the instant of energy conversion (potential to kinetic energy) produce significant local velocity gradients that disrupt the droplets. Cavitation theory suggests that the extreme pressure drop in the homogenizing valve generates cavitation bubbles, and when these bubbles collapse, the shock waves in the fluid cause the droplets to break apart. Actual measurements of cavitation noise in the homogenizing valve have suggested that the greater the intensity of cavitation, the greater is the homogenizing effect (16). However, the fact that cavitation is present does not prove that it is the actual mechanism for homogenization. Published research has shown that if cavitation is dampened or suppressed, homogenization still occurs, suggesting that turbulence may be the predominant mechanism (17). Actual visualization of the emulsification process in a homogenizing valve using a Nd:YAG laser with a pulse duration of 10 ns has shown that homogenization occurs after discharge from the gap of the valve and that emulsification is produced by the intense turbulent mixing zone in the exit region. No homogenization occurs in the region between the valve and the seat (18).

HOMOGENIZING VALVE DESIGN

One consequence of understanding the mechanism of homogenization is the ability to improve the efficiency of homogenization. The geometry of the homogenizing valve is important in regard to the quality of the finished product. Gaulin realized this, and he experimented with different valve designs at the turn of the century (1). The objective of changing valve designs is to find one that gives the best product possible at the lowest pressure (19). Some basic designs include the plug valve (Fig. 3), the piloted valve, and a grooved valve. The piloted valve has a guide on the valve that fits into the seat to align the two. The grooved valve has concentric grooves on the valve and seat. These grooves are machined so that the peaks on the valve fit into the grooves on the seat. Different configurations of these grooves have been tried over the years. Variations of the plug and piloted valve include a knife edge on the seat having a short land or travel distance, replaceable screens on the faces of the valve and seat, and a cone-shaped valve fitting into a hollowed seat. There is even a valve consisting of a tightly compressed wire bundle through which the product flows. For large flow rates, the Micro-Gap® valve is used, and this valve consists of stacked valve plates that split the flow into equal parts for optimization of homogenization (19,20). These designs seek to make efficient use of the available homogenization energy. Along with the geometry of the valves it is important to consider the size of the valves, because as the flow rate increases, valve size also increases to maintain efficient homogenization. A laboratory homogenizer operating at 114 L (30 gal) per hour may have a valve diameter of 9.53 mm (0.375 in), while a production-size homogenizer operating at 15,142 L (4,000 gal) per hour might have a valve diameter of 76.2 mm (3 in). A homogenizer at 37,854 L (10,000 gal) per hour with a Micro-Gap® valve would have the equivalent diameter (by summing the stacked valves) of a valve 787.4 mm (31 in) across. Of course, as the size of the homogenizing valve increases, the required actuating force becomes larger and this must be considered when homogenizing valves are designed.

APPLICATIONS

The homogenizer is used in the processing of many food and dairy products. Figure 5 illustrates how homogenization of milk reduces the droplet size of fat globules. The particle size distribution was measured with an AccuSizer 770 (Particle Sizing Systems, Santa Barbara, California) on samples of unhomogenized and homogenized milk (equivalent to a Micro-Gap® valve at 12 MPa) demonstrating the change in mean diameter and size distribution achieved by homogenization. The reduction in size distribution produces an increase in total surface area for the emulsion illustrating the importance of surface stabilization for fine emulsions. For example, from the size distribution curves the unhomogenized sample has a calculated surface area of 1.73 m^2 per gram of fat and the homogenized sample has a surface area of 7.30 m^2 per gram of fat. Therefore, the surface area has increased by a factor of


Unhomogenized milk

Homogenized milk

Figure 5. Particle size distribution for unhomogenized and homogenized milk.

4.22 because of homogenization. For milk, the increased surface area means that the original milk fat globule membrane is insufficient to cover the newly formed fat surfaces, and plasma proteins are adsorbed from the milk serum to compensate for this extended surface area (21).

Figure 6 shows typical globule size reduction for milk homogenized with a standard homogenizing valve (curve 1) and a Micro-Gap® homogenizing valve (curve 2) illustrating the reduction in fat globule size with increasing homogenizing pressure.

Table 1 lists some common applications of the homogenizer. The pressure ranges given are for conventional homogenizing valves, but the actual pressures can vary depending on the product formulation, the required shelf life, and the product specifications of each processor. Also, some of these applications might require more than one pass through the homogenizer or the use of a two-stage homogenizing valve. When using the two-stage valve, it has been determined that the second stage pressure should be in the range of 10 to 15% of the total homogenizing pressure (12,19). The second stage valve eliminates cavitation in the first stage valve. This improves homogenization efficiency for an emulsion while maintaining the same total homog-

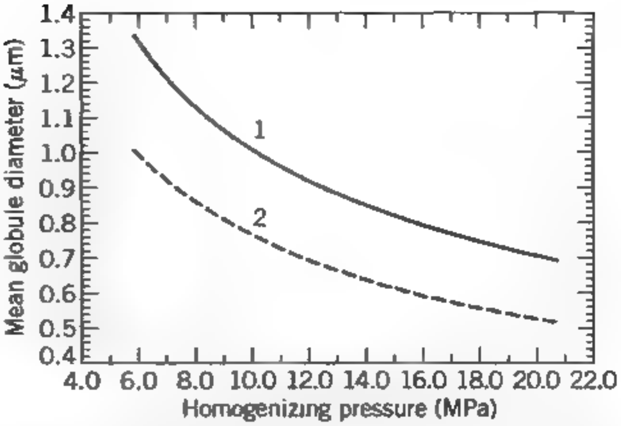


Figure 6. Mean fat globule diameter versus homogenizing pressure. Curve 1 is typical for a standard homogenizing valve at 23,000 liters per hour. Curve 2 is typical for a Micro-Gap® homogenizing valve at all flow rates. Mean diameters are measured using the spectroturbidity method. *Source:* Data from APV Americas-Homogenizers.

Table 1. Common Applications of the Homogenizer

Product	Pressure range (MPa)	Pressure range (psi)
	Dairy	
Evaporated milk	16.55–20.68	2400–3000
Half-and-half	12.41–13.79	1800–2000
Ice cream mix	13.79–17.24	2000–2500
Ice milk	12.41–15.17	1800–2200
Light cream	10.34–13.79	1500–2000
Pasteurized milk	12.41–15.17	1800–2200
Soft cheese	6.89–20.68	1000–3000
Sour cream	13.79–17.24	2000–2500
UHT milk	20.68–27.57	3000–4000
Yogurt base	17.24–20.68	2500–3000
	Food	
Baby foods	13.79–34.47	2000–5000
Coffee whiteners	13.79–34.47	2000–5000
Cream-base cocktails	20.68–27.58	3000–4000
Cream soup base	13.79–20.68	2000–3000
Flavor emulsions	20.68–34.47	3000–5000
Frozen whipped toppings	34.47–55.16	5000–8000
Fruit nectar	20.68–34.47	3000–5000
Infant formula	20.68–34.47	3000–5000
Liquid egg	6.89–20.68	1000–3000
Orange juice concentrate	20.68–34.47	3000–5000
Peanut butter	34.47–68.95	5000–10000
Puddings	13.79–34.47	2000–5000
Salad dressings	6.89–20.68	1000–3000
Soy beverages	20.68–34.47	3000–5000
Tomato ketchup	20.68–31.03	3000–4500
Tomato juice	3.45–6.89	500–1000
Tomato sauce	20.68–27.58	3000–4000
Toppings (hot fudge, etc)	20.68–34.47	3000–5000

enizing pressure of single stage operation. However, in some applications a single stage valve produces acceptable product, and two stages are not used.

OPERATION

Certain requirements must be met for successful operation of the homogenizer. The first of these is adequate in-feed pressure. A positive-displacement pump must have a positive in-feed pressure so the pump is not starved. If it is starved, transient high-pressure shock loading can occur, eventually resulting in severe damage to the pump (6). The required in-feed pressure will depend on the characteristics of the product and the size of the pump. Excessive amounts of entrained air in a product will cause a similar type of shock loading, and some products require deaeration to eliminate large amounts of entrained air. The correct type of pump valves should be used depending on the nature of the product. Viscous products may require ball valves. Abrasive products may require ball valves and special wear-resistant materials for the pump-valve seats and homogenizing valve. The homogenizer should be sanitary. Parts should be easily removable for cleaning or replacement, and the machine should have no flow areas where product can be trapped. Of course, the homogenizer must be rugged and reliable; for example, some dairies may require the homogenizer to operate more than 16 h a day, six days a week.

TESTING

Many methods are used to test a product for homogenization quality. These methods vary with the type of product and quality control requirements. A homogenized substance might be checked for viscosity change, either an increase or a decrease. The appearance of a product may be important; for example, texture, color, smoothness, graininess, or pulpiness. A dispersion might be checked microscopically for changes in the size of dispersed solids. An emulsion, such as milk, can be examined microscopically for the size of the milk fat globules, or more-sophisticated spectroturbidimetric methods may be used to determine the average diameter and size distribution of the fat globules (22).

SUMMARY

The homogenizer is a unique piece of equipment that was invented at the turn of the century for the processing of artificial butter and for milk but now is used for many applications in the food and dairy industries. The homogenizer consists of a pump and homogenizing valve. The pump is a positive displacement pump, and the homogenizing valve can be made in different sizes and configurations. Even after more than 90 years, the mechanism of homogenization is not completely understood, but homogenization has improved many consumer products.

BIBLIOGRAPHY

1. G. M. Trout, *Homogenized Milk*, Michigan State College Press, East Lansing, Mich., 1950
2. W. Clayton, *The Theory of Emulsions and Their Technical Treatment*, 2nd ed., J. & A. Churchill, London, United Kingdom, 1928.
3. U.S. Pat. 756,953 (April 12, 1904), Auguste Gaulin.
4. F. J. Doan, "Changes in the Physico-Chemical Characteristics of Milk as a Result of Homogenization," *American Milk Review* 54 (June 1954).
5. F. J. Doan and C. H Minster, "The Homogenization of Milk and Cream," *Pennsylvania State College Bull.* **287**, *School of Agriculture and Experiment Station*, 1933.
6. T. L. Henshaw, "Reciprocating Pumps," *Chemical Eng.* 104 (Sept. 1981).
7. C. C. Loo and W. M. Carleton, "Further Studies of Cavitation in the Homogenization of Milk Products," *J. Dairy Sci.* **36**, 64 (1953).
8. A. A. McKillop et al., "The Cavitation Theory of Homogenization," *J. Dairy Sci.* **38**, 273 (1955).
9. L. W. Phipps, "Action of the High Pressure Homogenizer," *Bienn. Rev. Natl. Inst. Res. Dairy*, 61 (1976).
10. P. Walstra, "Formation of Emulsions," in P. Becher, ed., *Encyclopedia of Emulsion Technology*, Vol. 1, Marcel Dekker, New York, 1983, p. 67.
11. H. Mulder and P. Walstra, *The Milk Fat Globule*, PUDOC (Centre for Agricultural Publishing and Documentation), Wageningen, The Netherlands, 1974.
12. H. G. Kessler, *Food Engineering and Dairy Technology*, Verlag A. Kessler, Freising, Federal Republic of Germany, 1981.
13. L. H. Rees, "The Theory and Practical Application of Homogenization," *J. Soc. Dairy Technol.* **21**, 172 (1968).
14. W. D. Pandolfe, "Effect of Dispersed and Continuous Phase Viscosity on Droplet Size of Emulsions Generated by Homogenization," *J. Disp. Sci. and Technol.* **2**, 459 (1981).
15. P. Walstra, "Preliminary Note on the Mechanism of Homogenization," *Neth. Milk Dairy J.* **23**, 290 (1969).
16. H. A. Kurzhals, "Untersuchungen Über die Physikalisch-Technischen Vorgänge Biem Homogenisieren von Milch in Hochdruck-Homogenisier-Maschinen," Ph.D. dissertation, Technical University Hannover, Federal Republic of Germany, 1977
17. L. W. Phipps, "Cavitation and Separated Flow in a Single Homogenizing Valve and Their Influence on the Break-up of Fat Globules in Milk," *J. Dairy Res.* **41**, 1–8 (1974).
18. U.S. Pat. 5,749,650 (May 12, 1998), R. R. Kinney, W. D. Pandolfe and R. D. Ferguson (to APV Homogenizer Group).
19. W. D. Pandolfe, "Development of the New Gaulin Micro-Gap Homogenizing Valve," *J. Dairy Sci.* **65**, 2035 (1982).
20. U.S. Pat. 4,383,769 (May 17, 1983), W. D. Pandolfe and H. Graglia (to APV Gaulin, Inc.).
21. M. E. Cano-Ruiz and R. L. Richter, "Effect of Homogenization Pressure on the Milk Fat Globule Membrane Proteins," *J. Dairy Sci.* **80**, 2732–2739 (1997)
22. W. D. Pandolfe and S. F. Masucci, "Rapid Spectroturbidimetric Analysis," *American Laboratory* 40 (Aug. 1984).

William D. Pandolfe
APV Americas-Homogenizers
Wilmington, Massachusetts

HUNGER, FOOD DEPRIVATION, AND NUTRITIONAL DISORDERS

Hunger, food deprivation, and nutritional disorders remain devastating problems facing many millions, especially the poor and disadvantaged (1,2). The word *hunger* has two customary meanings. The first is the "strong desire or need for food," and the second describes the "discomfort, weakness, or pain caused by a prolonged lack of food." However, to suffer hunger is not necessarily to be hungry. Estimates by various authorities over the last three decades for the numbers of the hungry or undernourished have ranged from about 12% to more than 50% of the populations of developing countries. More recent estimates approximate to 20% (1).

ASSESSMENT OF NUTRITIONAL STATUS

Major methods (Table 1) for nutritional assessment can be divided into two categories: those that are food related and those that are people related. For monitoring world food security, food supply data are used (3) in the form of food balance sheets (FBSs). FBSs show estimates of per capita food availability derived from national production, import, and export data. These data can then be aggregated into economic or geographic groups. Such data do not show actual consumption, nor do they consider uneven distribution of the available supplies.

GLOBAL NUTRITION SITUATION

Food Energy Availability

For developing countries, in general, there was good progress in food energy availability until the mid-1980s (Table 2). However, several countries, especially many in Africa, failed to make progress. The average food energy availability for developing countries as a group is projected to increase from the 1990–1992 value of 2520 kcal to 2770 kcal per person per day by the year 2010. Many of the poorest countries, however, are likely to show only minimal gains.

In addition to the quantity of food, the quality of the diet changes as countries become richer (Table 3). With increasing wealth (gross national product [GNP]), total protein and animal protein availability increase while the dependence on cereals as a source of protein declines. Cereals are a poor source of the essential amino acid lysine, whereas animal foods are a good source of lysine. An indicator, therefore, of the overall value of the diet can be mean daily lysine availability (4). Intake of lysine ranges from 2400 mg/day in countries with a GNP less than $500 per capita per year up to 6500 mg/day for those with a GNP greater than $10,000 per capita per year. At the same time, there are also large differences in average mortality rates for children less than 5 years of age. This mortality rate is only 9 per thousand in the wealthy countries but reaches 171 per thousand live births in the poorest.

Food Production

Many scientific innovations to increase food productivity have been proposed and practiced, most especially the green revolution. More attention is now paid to environmental issues and problems such as water distribution, energy photosynthesis, land limitations and degradation, salinity and drought resistance, reduced postharvest losses, and the role of biotechnology (5,6). Nevertheless, improved nutrition for many is probably more related to uneven distribution of people's rights to land and to water; unequal access to labor, credit, and tools; and inequalities in the control over the results of food production (7). Poverty remains the major determinant of food insecurity and poor health (2). Thus agriculture can have a twofold role for improving nutrition; the first is obvious and follows from the production of food of the desired quality and quantity. The second role, highly significant in low-income countries, is in providing employment and income for the poor.

Developed and Developing Country Profiles

International agencies have grouped countries by their economies into developing and developed (industrialized) nations. The majority of the world's population lives in the developing countries. The least developed countries (LDCs) are the poorest of the developing countries. The developing countries and especially the LDCs have high child mortality rates, but the situation improved between 1960 and 1996. The 1996 rate for the LDCs (Table 4) is, however, still twice the rate for the world as a whole and is nearly five times that occurring in the industrialized countries in 1960. The maternal mortality rate is also much higher in the LDCs, some 85 times that of the industrialized countries. This enormous difference reflects the lack of basic infrastructure and medical services. Safe drinking water and sanitation are also both almost universal in industrialized nations but are far less available in poorer countries.

The Undernourished

With the exception of sub-Saharan Africa, the percentages defined as undernourished in the developing countries

Table 1. Major Methods for Assessment of Nutritional Status

	Food-related data		Health and people-related data	
	Country	Individual	Country	Individual
Method	Food balance sheets	Diet surveys	Vital and health statistics	Anthropometry (biochemical, clinical)
Outcome	Average food and nutrient availability	Approximate nutrient intakes compared with requirements	Morbidity and mortality data (risk in community)	Effect of nutrition on development function, and clinical abnormalities

Table 2. Per Capita Food Supplies for Direct Human Consumption, Historical and Projected

	kcal/capita/day				
	1961–1963	1969–1971	1979–1981	1990–1992	2010[c]
Developing countries[a]	1960	2130	2320	2520	2770
Sub-Saharan Africa	2100	2140	2080	2040	2280
Near East and North Africa	2220	2380	2840	2960	3010
Eastern Asia	1750	2050	2360	2670	3030
Southern Asia	2030	2060	2070	2290	2520
Latin America and the Caribbean	2360	2510	2720	2740	3090
Developed countries	3020	3190	3280	3350	3390
Former CPEs[b]	3130	3330	3400	3230	3380
Others	2980	3120	3220	3410	3400
World	2300	2440	2580	2720	2900

Source: Ref. 1.

[a] Ninety three developing countries accounting for 98.5% of the total population of the developing countries

[b] CPEs, centrally planned economies

[c] Projections of aggregate food availabilities for direct human consumption divided by population projections

Table 3. Total Population, Nutrient Availability, Mortality Rate, and Life Expectancy for World Economic Classes (Data from 122 Countries)

GNP class (U.S. $/capita/year)	Countries (*N*)	Total population (millions)	Food energy (kcal/day)	Total protein (g/day)	Animal protein (%)	Cereal protein (%)	Lysine (mg/day)[a]	Under-5 MR[b]	Life expectancy (years)
<500	37	2990	2070	51	20	53	2405	171	52
500–2000	41	862	2570	65	31	50	3270	76	64
2000–10,000	21	548	2913	78	45	39	4484	39	69
>10,000	23	806	3335	101	61	24	6555	9	77

[a] Lysine values calculated from protein availability data.

[b] Under-5 mortality rate: deaths at less than 5 years of age per 1000 live births.

Source: Ref. 3.

declined between 1969–1971 and 1990–1992 (1). Because of population growth, however, there were increases in the absolute numbers of the undernourished in both sub-Saharan Africa and in southern Asia. For 1990–1992 these numbered 215 (43%) and 255 million (22%), respectively. Proportions are lower in eastern Asia (16%), Latin America and the Caribbean (15%), and the Middle East and North Africa (12%). When viewed on an agroecological basis, food deprivation has tended to be highest in the arid regions of the world. Poverty is a central characteristic of the food insecure. Although the numbers of undernourished are likely to decline, only slowly and insufficiently, from the current 839 million to possibly 680 million (1) by 2010, this would represent a significant decline from 21% to 12% of the total population of the developing countries.

MALNUTRITION AND NUTRITIONAL DISORDERS

Causation of Malnutrition

A complex set of factors determines the prevalence of hunger and malnutrition. The lack of food and/or nutrients acts in combination with other environmental factors, in particular with infectious diseases, to produce a spectrum of health problems with a whole range of intensities and outcomes. Determinants of malnutrition exist at three key levels of causation (8,9): the immediate, the underlying, and the basic (Table 5). Although many food and nutrition activities continue to operate at the immediate level, basic level changes are essential to any significant global progress in eliminating malnutrition.

Diet and disease interact in a mutually reinforcing way so that it is usually impossible to distinguish either as the primary cause of malnutrition (10). Infection can result in a loss of appetite and hence initiate food deficiency; it can also result in the depletion of body stores of specific nutrients and thus cause malnutrition. Through effects on the immune system, malnutrition can make infection more likely and increase its severity. Because poverty influences both the availability of food and the quality of public health, these interactions are of major worldwide significance.

Consequences of Malnutrition

Malnutrition results in a variety of conditions such as weight loss, growth failure, anemia, learning disabilities, lower activity levels and work capacity, increased susceptibility to other diseases, blindness, and various chronic conditions. These conditions translate into social and economic costs that no country can afford (1). Malnutrition is both a symptom of broader poverty and underdevelopment problems and a cause of these same problems (2). Recently the United Nations (UN) has explicitly stated that wide-

Table 4. Profile of the Industrialized, Developing, and Least Developed Countries (Data for 1995)

Variable	Industrialized countries	Developing countries[a]	LDCs[b]	World
Number of countries	23	110	37	150
Total population (millions)	830	4526	586	5696
Population growth (%)	0.6	2.0	2.6	1.7
Total fertility rate[c]	1.7	3.4	5.6	3.1
Population <18 years (%)	23	40	50	37
Population <5 years (%)	6	12	17	11
GNP per capital (U.S.$)	24,300	1023	233	4498
Health (%)[d]	12	4	5	10
Education (%)[d]	4	11	12	6
Defenses (%)[d]	10	13	19	10
GNP growth (%)	1.9	2.9	0.1	1.9
Life expectancy (years)[e]	77	62	52	64
U5MR[f]				
1960	37	216	283	191
1980	15	137	221	122
1995	8	99	173	90
IMR[g]	7	67	109	61
LBW (%)[h]	6	19	23	18
Maternal MR[i]	13	477	1052	428
Births attended[j]	99	53	29	57
Contraceptive prevalence[k]	72	54	18	57
Urban population (%)[l]	77	37	22	45
Safe water access (%)	—	71	55	71
Sanitation (%)	—	40	35	40
Primary school enrollment, males (%)	—	86	56	88
Primary school enrollment, females (%)	—	81	45	84
Adult literacy rate, males[m]	—	79	59	81
Adult literacy rate, females[m]	—	62	38	66

[a] Developing countries include the LDCs.

[b] LDCs, Least developed countries.

[c] Total fertility rate: The number of children that would be born per woman if she were to live to the end of her childbearing years and bear the children at each age in accordance with prevailing age-specific fertility rates.

[d] Percentages of government expenditure.

[e] Life expectancy at birth: The number of years newborns would live if subject to the mortality risks prevailing for the cross section of population at the time of their birth.

[f] Under-5 mortality rate: Probability of dying between birth and exactly 5 years expressed per 1000 live births.

[g] Infant mortality rate. Probability of dying between birth and exactly 1 year of age expressed per 1000 live births.

[h] Low birth weight: Weight of less than 2500 g.

[i] Maternal mortality rate: Annual number of deaths of women from pregnancy-related causes per 100,000 live births.

[j] Births attended Percentage of births attended by physicians, nurses, midwives, or primary health care workers trained in midwifery skills.

[k] Contraceptive prevalence Percentage of married women aged 15–49 years currently using contraception.

[l] Urban population. Percentage of population living in urban areas as defined according to the national definition used in the most recent population census.

[m] Adult literacy rate: Percentage of persons aged 15 and over who can read or write.

Source. Ref. 2.

Table 5. Levels of Determinants of Malnutrition

Immediate causes
Food intake and health
Underlying causes
Income, land, education, water, fuel, and health service availability
Basic causes
Resources, economics, politics

Source: Ref. 9.

spread malnutrition is a clear violation of human rights in general and children's rights in particular (2,11).

People-Related Nutritional Assessment

Health- and people-related assessment tools include clinical-biochemical determinations and anthropometry (Table 1). Clinical assessment involves direct observations on criteria such as changes in the body (eg, the skin, eyes, bones, and hair). Biochemical indicators are mainly measurements in blood and urine for the levels of components such as hemoglobin, specific enzymes, and metabolites. The values are compared with standards based on well-fed, healthy individuals.

Anthropometry uses objective measurements of body dimensions as a proxy indicator of nutritional status. The most commonly used measurements assess growth and development in children and body composition of adults (12). Child and adult nutritional status is presented in terms of ratios of weight and height (eg, weight for height and, in the case of children, weight for age and height for age), and these indicators are compared with age- and gender-specific points of reference (13). In this approach, deficits in the ratio of height to age measure chronic malnutrition (stunting), whereas deficits in weight to height indicate acute malnutrition (wasting). Finally, weight for age, a composite indicator, can signify the degree of undernutrition. Stunting may be a better cumulative indicator of well-being for children than underweight (2,14). For adults, body mass index (BMI; weight/height^2) is the most suitable anthropometric indicator of under- or overnutrition. Adults with low BMI (<18.5) generally have reduced work capacity and limited social activity (15). They also have lower incomes, more sickness, and, for females, more low-birth-weight (LBW) babies. In contrast, those with high BMIs (>30.0) are considered to be at greatly increased risk for several chronic diseases including diabetes and coronary vascular disease.

Malnourished Children. The numbers of wasted, stunted, and underweight children under 5 years of age in developing countries are shown in Table 6. Southern Asia and sub-Saharan Africa have the highest percentages of undernourished children in all three categories. By far the greatest numbers of undernourished children are in southern Asia because the total population there is so large. Globally some 180 million children are underweight, 215 million children are chronically malnourished (stunted), and nearly 50 million children are acutely malnourished (wasted). For all categories, not unexpectedly, the problem is far more severe in low-income countries than in middle- to high-income developing countries.

Low Birth Weight. LBW, a major health problem in developing countries, is defined internationally as birth weight less than 2500 g and includes both premature infants and those of small size at full term. Globally, about 18% of newborn babies are of low birth weight (see Table 4). In industrialized countries only some 6% are of low birth weight, and nearly all of these infants are premature. As many as 23% may be affected in the LDCs. In contrast, these are mostly full term. If LBW infants survive, they are likely to become malnourished children and underweight adults. Poor maternal health and inadequate nutrition, both associated with poverty, are key causative factors.

Protein-Energy Malnutrition

The term *protein-energy malnutrition (PEM)* has been used to describe a range of disorders, primarily characterized by growth failure or retardation of growth in children. Mild forms are assessed by anthropometry. Extreme clinical forms of PEM associated with large weight deficits are called marasmus and kwashiorkor. Marasmus describes a wasted and emaciated child, whereas kwashiorkor is characterized by water accumulation (edema) in the body, an enlarged belly, and often severe hair and skin changes together with reduced levels of serum protein. The major cause of nutritional marasmus is inadequate food intake often induced by infection. A common hypothesis for the causation of kwashiorkor combines a deficit of protein in the diet with an adequate intake of food energy. The disease is then precipitated by an infective episode. Infants and young children are the most severely affected by PEM because of their high energy and protein needs relative to body weight and their particular vulnerability to infection.

Adaptation to Undernutrition

When food energy intake is consistently below requirements, activity and/or body size is reduced. A new balance may be established because a body that is smaller and less active needs less food. The concept of adaptation has generated passionate dispute between different schools of thought in the field of nutrition, especially concerning children who are apparently healthy but are small for their age. Some have considered such adaptations to be benign (16), but others believe that they are of major long-term health significance (17,18).

Micronutrient Deficiency Disorders

Globally, the most widespread of the micronutrient deficiencies are lack of iron, with more than 2 billion affected;

Table 6. Estimated of the Number of Wasted, Stunted, and Underweight Children under 5 Years of Age in Developing Countries, 1990

	Wasted		Stunted		Underweight	
Region or economic class	(%)	(millions)	(%)	(millions)	(%)	(millions)
Region						
Sub-Saharan Africa	7.0	6.1	38.8	33.7	30.2	26.2
Near East and North Africa	8.8	4.4	32.4	16.0	25.3	12.5
Southern Asia	17.1	26.6	59.5	92.7	58.3	90.7
Eastern and Southeast Asia	5.2	9.4	33.3	59.8	23.6	42.5
Latin America	2.6	1.5	22.7	12.7	12.0	6.7
Economic class						
Low-income countries	10.3	45.2	45.2	174.4	38.2	147.6
Middle- to high-income countries	5.6	8.0	28.7	40.8	22.0	31.2
Total	9.1	47.9	40.7	215.2	33.9	178.8

Source. Ref. 1.

iodine, with more than 1 billion at risk; and vitamin A, with 40 million affected. Many millions are also affected by the dietary lack of zinc, selenium, and other trace elements (19,20). Zinc deficiency is most common in areas where the diet consists mainly of unprocessed cereals and must now be considered a potentially major public health problem (2,20).

Vitamin A. Vitamin A is present as retinol in animal foods and as carotenoids, especially beta-carotene, in plant foods. Beta-carotene is less efficiently absorbed than retinol but is the primary source of vitamin A in the diets of most in the developing world (20). The major dietary sources and the major features of vitamin A deficiency are listed in Table 7. Deficiency of vitamin A is the most common cause of preventable childhood blindness. It occurs primarily in areas where animal foods are rarely available and fruit and vegetable consumption is low (20,21). Deficiency is caused not only by the low content in the food supply but also by poor absorption and utilization (21). Vitamin A deficient children exhibit decreased resistance to infections, decreased physical growth (22), and increased morbidity and mortality due to diarrhea, respiratory conditions, and measles (23).

At least 190 million children live in areas where vitamin A intake is low. Of these, 13 million demonstrate clinical signs of deficiency, and each year 250,000 to 500,000 children become partially or totally blind as a result (12). Two-thirds of these children die within a few months of becoming blind. Programs of vitamin A supplementation have been highly successful in reducing blindness and overall mortality rates (2,20).

Table 7. Deficiency Disorders of Vitamin A

Dietary sources	Retinol or vitamin A: foods of animal origin, liver, butter, milk, and egg yolks; beta-carotene: dark green leafy vegetables, yellow and orange fruits and vegetables
Causation	Inadequate sources of retinol or beta-carotene in diet
Most at risk	Preschool children in developing areas with insufficient intake of fruits and vegetables
Major features	Inflammation of squamous cells; softening, desiccation, and ulceration of cornea; depressed immunity; poor night vision; severe eye lesions; Bitot's spots
Consequences	Night blindness or even permanent blindness
Areas of incidence	India, Bangladesh, Indonesia, and Brazil
Measures to eliminate	Provision of diet rich in fruits, vegetables, and animal foods and fortification of dairy products or providing supplements

Iron. Iron deficiency anemia is widespread worldwide, especially in women and children. Iron in the diet is present as either heme (ie, associated with the hemoglobin of blood) or nonheme. Heme iron absorption is high and ranges between 20 and 30%, whereas that for nonheme iron can be as low as 2% (24). In the diets of individuals in developing countries heme iron constitutes only a small proportion of intake because only animal foods, especially meat products, provide heme iron. The main dietary sources of iron are shown in Table 8. Additional factors such as the amount of vitamin C in the diet further influence iron status because such reducing agents can enhance the utilization of nonheme iron. Iron nutritional status is thus a function of the amount of dietary iron and the bioavailability of that iron (20,24). In adults the most common causes of iron deficiency are defective absorption of the mineral or blood losses from various causes including parasites.

Anemia in children and infants may retard physical and cognitive development (25). In adults it can cause fatigue, reduced work capacity, and impair reproductive functions (Table 8). In pregnancy anemia is associated with retarded

Table 8. Iron Deficiency Disorder

Dietary sources	Red meats, whole and enriched grain products, and dark green leafy vegetables
Deficiency name	Iron deficiency anemia
Causation	Inadequate diet, impaired absorption, blood loss, and repeated pregnancies
Most at risk	Infants, women of childbearing age, adolescent girls, and premature infants
Major features	Weakness, fatigue, pallor, difficulty in breathing on exertion, palpitation, and vague gastrointestinal complaints
Consequences	Impaired work tolerance, impaired body temperature regulation, growth retardation, impairment in behavioral and intellectual performance, immunity and resistance to infections, and adverse pregnancy outcomes
Areas of incidence	Worldwide, especially in areas with high risk of infections, poverty, and occurrence of multiparity and premature infants
Measures to eliminate	Oral iron supplementation therapy

fetal growth, LBW, and greater perinatal mortality, 20% of maternal deaths may be associated with anemia because it predisposes mothers to hemorrhage and infections (12).

Zinc. Human deficiency of zinc was described nearly three decades ago among the poor of the Middle East (25). Major features are shown in Table 9. Deficiency symptoms include severe growth retardation, with delayed sexual maturation, and pregnancy complications. Other manifestations include suppressed immunity, poor healing, dermatitis, and impairments in neuropsychological functions (20,25,26). Zinc deficiency is found to be associated with high-phytate, high-fiber, and low-protein diets, which are common in poorer regions of the Middle East. Conditions such as hookworm and schistosomiasis, which lead to blood loss, increase the incidence of both iron and zinc deficiency (26). Animal foods are major sources of zinc. Cereals are high in fiber and phytate, which makes much of the zinc poorly available. Dietary zinc supplementation often improves growth, especially for those recovering from malnutrition and diarrhea (20,22).

Table 9. Zinc Deficiency Disorder

Dietary sources

Shellfish, red meats, and whole grain cereals (low availability)

Deficiency name

No specific name

Causation

Insufficient intake with poor digestibility and absorption caused by the effects of malnutrition on gastrointestinal function

Most at risk

Growing infants and children, pregnant and lactating women in developing countries, patients fed total parenteral nutrition solutions lacking in zinc

Major features

Reduced growth; disturbance in nucleic acid metabolism and protein synthesis; reduction in circulating proteins, growth hormones, gonadotropins, sex hormones, prolactin, and thyroid hormones, corneal lesions; skin lesions; changes in hair; delayed wound and burn healing; and diarrhea in patients fed via total parenteral nutrition

Consequences

Growth retardation, delayed sexual maturation and impotence, hypogonadism and hypospermia, immune dysfunction, patchy loss of hair and decreased pigmentation, behavioral disturbances, night blindness, impaired taste, and lack of adaptation to darkness

Areas of incidence

Areas with high consumption of cereals in the diet, such as the Middle East

Measures to eliminate

Zinc supplementation or increased consumption of animal foods

Iodine. Iodine deficiency is endemic in regions of the world where the surface soil has been leached of iodine due to glaciation, snow, and rain (Table 10). It is the most common cause of preventable mental defects (2,20). More than 200 million people have goiters, 26 million have mental defects, and 6 million have the condition termed cretinism (characterized by mental retardation and stunted growth), all of which are associated with iodine deficiency. Fetal iodine deficiency is associated with increased incidence of stillbirths, spontaneous abortions, and congenital abnormalities (25). Iodine deficiency increases the risk of LBW and early death; in older children and adults it is characterized by goiter and its complications (27,28). Linear growth retardation is a common feature in the iodine deficient child. Fetal iodine deficiency has been hypothesized as exacerbating later growth retardation (22).

Other Micronutrient Deficiencies. The vitamin deficiency disease pellagra (niacin deficiency) occurred widely in the American South in the 1800s and beriberi (thiamine deficiency) was common until recently in Asia. These deficiencies are now rare (23,25), but they may still occur together with scurvy (vitamin C deficiency) in refugee camps and other deprived populations (12). Rickets, associated with lack of vitamin D and poor calcification of the

Table 10. Iodine Deficiency Disorders

Dietary sources

Seafood and plants grown in iodine-rich soil

Deficiency name

Iodine deficiency disorders

Causation

Low-iodine soils lead to lack of iodine in diet

Most at risk

Fetuses, neonates, children, adolescents, and adults with low-iodine diets

Major features

Reduced growth and development at all ages, especially in the fetal, neonatal, and infancy stages; impaired thyroid function; impaired mental function; goiter is the primary feature

Consequences

Spontaneous abortions, stillbirths and congenital anomalies; increased perinatal and infant mortality rates; increased neurological cretinism; and higher rates of neonatal, juvenile, and adult hypothyroidism

Areas of incidence

The Himalayas, the Andes, and the European Alps; elevated regions subject to glaciation, higher rainfall, and runoff into rivers; flooded river valleys such as the Ganges

Measures to eliminate

Administration of iodized salt; iodized oil (oral or injection) is used for moderate and severe deficiency cases bones, continues to affect many children (23,25). Scurvy has great historical significance not only because it influenced geographical exploration but also because its eventual cure and prevention followed one of the first controlled nutrition experiments (29). Deficiencies of folic acid and vitamin B_{12} are still important and affect the formation of red blood cells. Folic acid deficiency is of special significance in pregnancy (20). Pyridoxine, biotin, and pantothenic acid deficiencies have all been described but are rare.

Osteoporosis (bone mineral loss) is a major problem for postmenopausal women in developed countries. In developing countries, despite apparently lower calcium intakes, osteoporosis is much less common (20). High protein intakes and low levels of physical activity may increase risk. Copper, selenium, chromium, and fluoride deficiencies have all been described (23,25). Fluoride excess, causing fluorosis, may also be a problem in certain oasis areas. Although deficiencies of a number of other minerals such as boron, manganese, molybdenum, and vanadium can be demonstrated in specialized animal experimentation, they are either extremely rare or nonexistent in human populations.

Prevention of Micronutrient Deficiencies

Because of the severe social consequences of many micronutrient deficiencies, prevention is of major international significance (20). The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) (1,12) and United Nations Administrative Committee on Coordination, Sub Committee on Nutrition (ACC-SCN) (20) have outlined a number of approaches toward tackling these issues. Strategies include improving dietary diversity, food fortification, and nutrient supplementation.

Vitamin A deficiency can be treated or prevented by supplementing with high-dose capsules and also by injection (2,20). Iodine deficiency disorders can be prevented or treated by the administration of iodized oil (given orally or by injection). Oral iron supplementation programs are the most common public health intervention against iron deficiency. Food fortification involves the addition of micronutrients, particularly potassium iodate, vitamin A, and iron, to common foods (30).

The growth retardation seen in many children in developing countries may be a consequence of multiple deficiencies rather than the lack of a single nutrient (22; P. L. Pellett and S. Ghosh, unpublished data). When diets high in cereal products are consumed there may also be deficiencies of the key amino acid lysine. Dietary diversity can often improve micronutrient status. Animal foods contain high levels of both lysine and bioavailable micronutrients, and even small quantities can transform poor cereal-based diets (4). Finally, public health measures to address critical environmental factors such as water quality, sanitation, and food hygiene are also essential.

Other Nutritional Disorders

The problems of undernutrition are paralleled by extensive and growing chronic disease problems with obesity and the associated conditions of diabetes, coronary vascular diseases, and certain cancers. These conditions are occurring not only in rich industrialized countries but also in low- and middle-income countries, especially in urban areas. The nutritional relationships of these diseases are discussed in the article entitled AFFLUENCE, FOOD EXCESS, AND NUTRITIONAL DISORDERS. Anorexia nervosa, with persistent intentional weight loss, is generally classified along with bulimia among the psychogenic eating disorders (25).

THE FUTURE

The green revolution, which began in the 1960s, was a global technological achievement. The introduction of improved varieties, irrigation, pesticides, and mineral fertilizers for key commodity crops, accompanied by investment in institutional infrastructure and ongoing research programs, raised food production and productivity on a wide scale. The productivity gains in rice and wheat in Asia were especially significant. With population growth and a diminishing land area to produce food, further increases in productivity are still needed in the poorer, food-insecure countries (1,5).

Science and technology have provided tools for increasing food production. More varied crops and animals, plant breeding for nutritional enhancement, improved cropping systems, and greater emphasis on integrated pest management and plant nutrition are increasingly being used. In addition, ecoregional approaches to research are being adopted to reflect prevailing biological and physical constraints such as low labor productivity, dysfunctional markets, and limited access to mechanization and energy sources. The role of biotechnology remains the subject of intense international debate concerning ethics, safety, and intellectual property rights. Despite major developments in the technologically advanced countries, it may be another 10 to 20 years before significant benefits are realized by farmers in developing countries (1).

In many countries collaboration between governments, nongovernmental organizations, and UN agencies has produced highly significant gains in the field of child survival. The well-tried paths of the GOBI strategy (growth monitoring, oral rehydration, breast feeding, and immunization programs) have been mainly responsible. More widespread recognition that malnutrition is an abuse of children's rights (2,11) and greater awareness of the importance of nutritional care are other major signs of progress. A more equitable distribution of benefits and resources to the lower-income, food-insecure groups is urgently required. Experience has shown that science and technology are essential (1,5) but cannot by themselves solve the health and food security problems of developing countries. Appropriate political, social, economic, and institutional reforms are needed not only to make advances but also to maintain what has been accomplished so far.

BIBLIOGRAPHY

1. *World Food Summit. Technical Background Documents 1–5*, Food and Agriculture Organization of the United Nations, New York, 1996.

2. UNICEF, *The State of the World's Children 1998*, Oxford University Press, New York, 1998.
3. FAOSTAT, *Food Balance Sheets 1961-199, FAOSTAT-PC: FAO Computerized Information Series Version 3*, Food and Agricultural Organization of the United Nations, Rome, Italy, 1996.
4. P. L. Pellett, *Food Nutrition Bulletin* **17**, 204–234 (1996).
5. J. C Waterlow et al., eds , *Feeding a World Population of More Than Eight Billion People: A Challenge to Science*, Oxford University Press and Rank Prize Funds, New York, 1998.
6. United Nations Environment Programme, *Global Environmental Outlook, "For Life on Earth,"* Oxford University Press, New York, 1997.
7. B. Harriss-White, "Introduction," in B. Harriss and Sir R. Hoffenberg, eds., *Food: Multidisciplinary Perspectives*, Blackwell Publishers, Oxford, United Kingdom, 1994, pp. 1–26.
8. UNICEF, *Strategy for Improved Nutrition of Children and Women in Developing Countries*, UNICEF, New York, 1990.
9. U. Jonnson, *Food and Nutrition Bulletin* **16**, 102–111 (1995).
10. N. S. Scrimshaw, C. E. Taylor, and J E. Gordon, *Interactions of Nutrition and Infection*, WHO Monograph Series No. 57, World Health Organization, Geneva, Switzerland, 1968.
11. United Nations Administrative Committee on Coordination, Sub-Committee on Nutrition, "The Nutrition Challenge in the Twenty-first Century," *SCN News* **14**, 3–9 (1997).
12. FAO/WHO, *International Conference on Nutrition: Nutrition and Development—A Global Assessment*, Food and Agriculture Organization, Rome, Italy, 1992
13. *Physical Status: The Use and Interpretation of Anthropometry, Report of a WHO Expert Committee, Technical Series 854*, World Health Organization, Geneva, Switzerland, 1995.
14. United Nations Administrative Committee on Coordination, Sub Committee on Nutrition, "Stunting and Young Child Development," in *The Third Report on the World Nutrition Situation*, World Health Organization, Geneva, Switzerland, 1997, pp. 3–18.
15. FAO, Body Mass Index, A Measure of Chronic Energy Deficiency in Adults, FAO Food and Nutrition Paper No. 56, Rome, Italy, 1994.
16. D. Seckler, *Western Journal of Agricultural Economics* **5**, 219–227 (1980).
17. R. Martorell, *Human Organization* **48**, 15–20 (1989).
18. G. H. Pelto and P. J. Pelto, *Human Organization* **48**, 11–15 (1989)
19. United Nations Administrative Committee on Coordination, Sub Committee on Nutrition, "Focus on Micronutrients," *SCN News* **9**, 1993.
20 United Nations Administrative Committee on Coordination, Sub Committee on Nutrition, "Micronutrients" *The Third Report on the World Nutrition Situation*, World Health Organization, Geneva, Switzerland, 1997, pp. 19–52.
21. J. A. Olson, "Vitamin A, Retinoids & Carotenoids," in M. E. Shils, J A. Olson, and M Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., Vol. 1, Lea & Febiger, Philadelphia, Pa., 1994, pp. 287–307.
22. L. H Allen, *European Journal of Clinical Nutrition* **48**, S75–S89 (1994).
23. T. K. Basu and J. W. Dickerson, *Vitamins in Human Health and Disease*, CAB International, Wallingford, United Kingdom, 1996.
24. J. L. Beard and H. D. Dawson, "Iron," in B. L. O'Dell and R. A. Sunde, eds., *Handbook of Nutritionally Essential Mineral Elements*, Marcel Dekker, New York, 1997.
25. M. E. Shils, J. A. Olson, and M. Shike, *Modern Nutrition in Health and Disease*, 8th ed., Vol. 1, Lea & Febiger, Philadelphia, Pa., 1994.
26. H. H. Sanstead, *American Journal of Diseases of Childhood* **145**, 853–859 (1991).
27. B. S. Hetzel and M. L. Wellby, "Iodine," in B L. O'Dell and R. A. Sunde, eds., *Handbook of Nutritionally Essential Mineral Elements*, Marcel Dekker, New York, 1997, pp 557–582.
28. B. A. Hetzel and G. A. Clugston, "Iodine," in M. E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., Vol. 1, Lea & Febiger, Philadelphia, Pa., 1994, pp 264–268.
29. R. A. Jacob, "Vitamin C," in M E. Shils, J. A. Olson, and M. Shike, eds., *Modern Nutrition in Health and Disease*, 8th ed., Vol. 1, Lea & Febiger, Philadelphia, Pa., 1994, pp. 432–448.
30. A. Nilson and J. Piza, *Food and Nutrition Bulletin* **19**, 49–60 (1998).

PETER L. PELLETT
SHIBANI A. GHOSH
University of Massachusetts
Amherst, Massachusetts

HURDLE TECHNOLOGY

The microbial stability and safety as well as the sensory and nutritive quality of most preserved foods are based on a combination of several empirically applied preservative factors (called *hurdles*), and more recently on knowingly employed hurdle technology. Deliberate and intelligent application of hurdle technology allows a gentle but efficient preservation of foods and is advancing worldwide. Various expressions are used for the same concept in different languages: Hürden-Technologie in German, hurdle technology in English, *technologie des barrières* in French, *barjernaja technologija* in Russian, *technologia degli ostacoli* in Italian, *technologia de obstaculos* in Spanish, *shogai gijutsu* in Japanese, and *zanglangishu* in Chinese. The most important hurdles for the preservation of foods are temperature (high or low), water activity (a_w), acidity (pH), redox potential (Eh), preservatives (nitrite, sorbate, sulfite, etc), and competitive microorganisms (eg, lactic acid bacteria). However, more than 60 hurdles that influence the preservation and/or the quality of foods have been already described, and the list of possible hurdles is by no means complete. In this contribution the principles of hurdle technology, basic aspects, and the applications of hurdle technology for food preservation in industrialized as well as developing countries are discussed.

PRINCIPLES OF HURDLE TECHNOLOGY

The microorganisms present in a food should not be able to overcome (ie, leap over) the hurdles inherent in this food. This is illustrated by the so-called hurdle effect (1), which is of fundamental importance for the preservation of intermediate-moisture foods (2) and high-moisture foods (3), since the hurdles in a stable product control microbial spoilage and food poisoning as well as desired fermentation processes. Leistner and coworkers acknowledged that the hurdle effect illustrates only the well-known fact that complex interactions of temperature, water activity, acidity, redox potential, preservatives, and so on are significant for the microbial stability and safety of most foods (3). From an understanding of the hurdle effect, hurdle technology has been derived (4), which allows improvements of the microbial stability and safety of foods by deliberate and intentional combinations of hurdles. However, the sensory quality of a food also is determined by positive and negative hurdles. To secure the total quality of a food, the safety and the quality hurdles in a food must be kept in the optimal range (5). By an intelligent combination of hurdles, the microbial stability and safety as well as the sensory, nutritive, and economic properties of a food are secured. For the economy of a food item, it is for example, important how much water in a product is compatible with the microbial stability of this food.

Some examples will facilitate the understanding of the hurdle effect and the application of hurdle technology in food preservation. Figure 1 gives eight examples.

Example 1 represents a food that contains six hurdles: high temperature during processing (F value), low temperature during storage (t value), water activity (a_w), acidity (pH), redox potential (Eh), and preservatives (pres.). The microorganisms present cannot overcome these hurdles, and thus the food is microbiologically stable and safe. However, example 1 is only a theoretical case, because all hurdles are of the same height, that is, have the same intensity, and this rarely occurs.

A more likely situation is presented in example 2, since the microbial stability of this product is based on hurdles of different intensity. In this particular product the main hurdles are a_w and preservatives, whereas other less important hurdles are storage temperature, pH, and redox potential. These five hurdles are sufficient to inhibit the usual types and numbers of microorganisms associated with such a product.

If there are only a few microorganisms present ("at the start"), then a few or low hurdles are sufficient for the stability of the product (example 3). The superclean or aseptic packaging of perishable foods is based on this principle. On the other hand, as in example 4 if, due to bad hygienic conditions, too many undesirable microorganisms are initially present, even the usual hurdles inherent to a product may be unable to prevent spoilage or food poisoning. Example 5 is a food rich in nutrients and vitamins, which might foster the growth of microorganisms (this is called the booster effect or trampoline effect), and thus the hurdles in such a product must be enhanced, otherwise they will be overcome.

Example 6 illustrates the behavior of sublethally damaged organisms in food. If, for instance, bacterial spores in a food are damaged sublethally by heat, then vegetative cells derived from such spores lack "vitality" and therefore are inhibited by fewer or lower hurdles. In some foods the stability is achieved during processing by a sequence of hurdles, which are important in different stages of a fermentation or ripening process and lead to a stable final product. A sequence of hurdles operates in fermented sausages (example 7), and probably also in ripened cheeses or fermented vegetables, and so on. Finally, example 8 illustrates the possible synergistic effect of hurdles, which probably relates to a multitarget disturbance of the homeostasis of the microorganisms in foods, which will be discussed subsequently.

BASIC ASPECTS

Food preservation implies putting microorganisms in a hostile environment, to inhibit their growth, shorten their survival, or cause their death. The feasible response of microorganisms to such a hostile environment determines whether they may grow or die. In view of these responses more research is needed; however, recent advances have been made by considering the homeostasis, metabolic exhaustion, and stress reactions of microorganisms in relation to hurdle technology, as well as by introducing the concept of multitarget preservation for a gentle but effective preservation of foods (6,7).

Homeostasis

Homeostasis is the tendency to uniformity and stability in the internal status (internal environment) of organisms. For instance, the maintenance of a defined pH in narrow limits is a prerequisite and feature of all living cells, and this applies to higher organisms as well as microorganisms (8). In food preservation the homeostasis of microorganisms is a key phenomenon that deserves much attention, because if the homeostasis of these organisms is disturbed by preservative factors (hurdles) in foods, they will not multiply; that is, they remain in the lag phase or even die, before their homeostasis is reestablished ("repaired"). Thus, food preservation is achieved by disturbing the homeostasis of microorganisms in a food temporarily or permanently. Gentle food preservation means using an intelligent mix of hurdles that secures the safety and stability as well as the quality of foods (6). Gould drew attention to the interference by the food with the homeostasis of the microorganisms present in this food (9). During their evolution, a wide range of more or less rapidly acting mechanisms (eg, osmoregulation to counterbalance a hostile water activity of foods) has developed in microorganisms, that act to keep important physiological systems operating, in balance and unperturbed even when the environment around them is greatly perturbed (10). In most foods the microorganisms are operating homeostatically, in order to react to the environmental stresses imposed by the preservation procedures applied, and the most useful procedures employed to preserve foods are effective in overcoming the various homeostatic mechanisms that microorganisms have evolved to survive extreme environmental stresses (10). The repair of a disturbed homeostasis demands much energy of the microorganisms, and thus restriction of energy supply inhibits repair mechanisms of

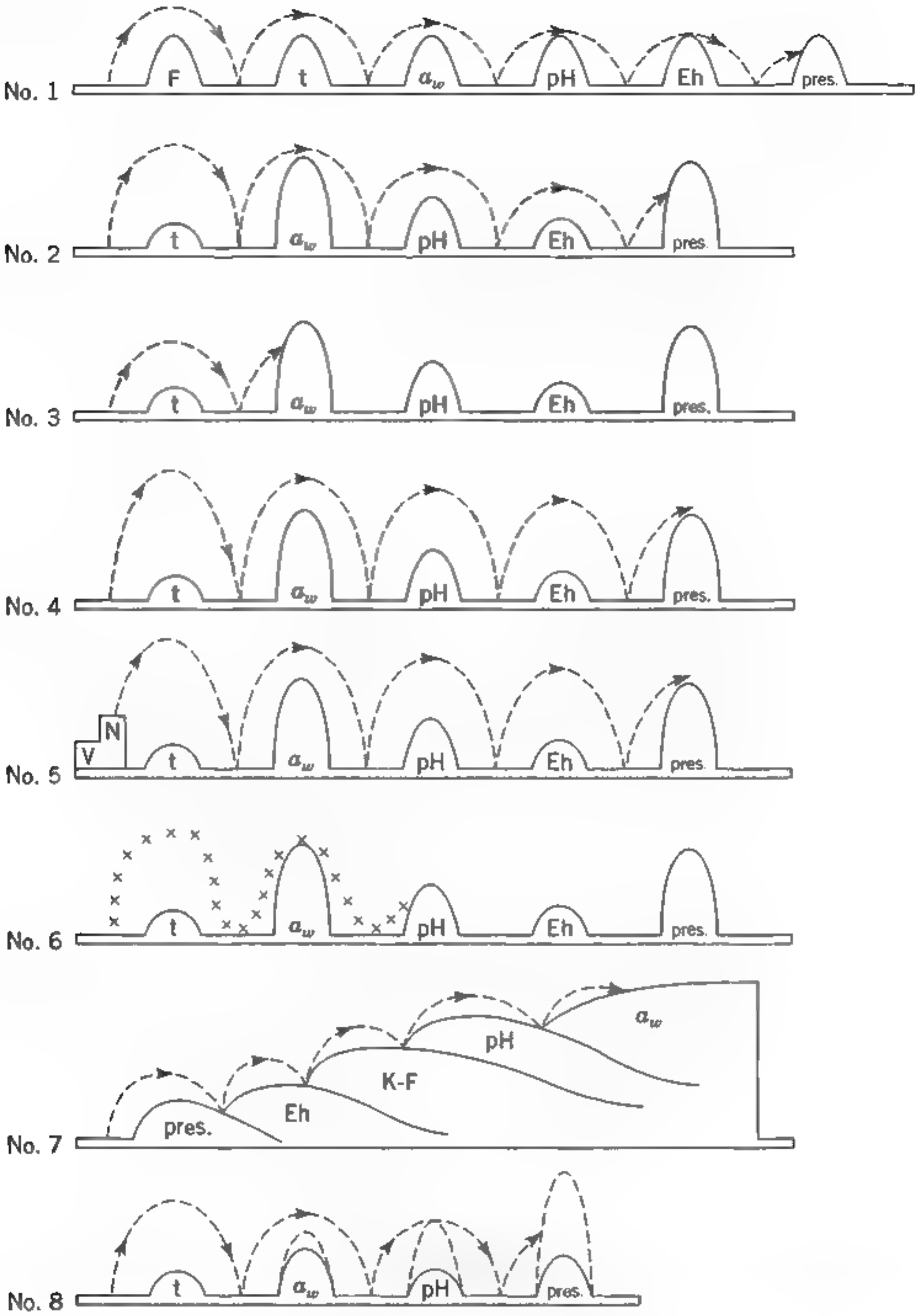

No. 1
F
t
a_w
pH
Eh
pres.
No. 2
t
a_w
pH
Eh
pres.
No. 3
t
a_w
pH
Eh
pres.
No. 4
t
a_w
pH
Eh
pres.
No. 5
V
N
t
a_w
pH
Eh
pres.
No. 6
t
a_w
pH
Eh
pres.
No. 7
pres.
Eh
K-F
pH
a_w
No. 8
t
a_w
pH
pres.

Figure 1. Eight examples of the hurdle effect that facilitate understanding of the application of hurdle technology in food preservation. See text for details. Symbols have the following meaning: F, heating; t, chilling; a_w, water activity; pH, acidification; Eh, redox potential, pres., preservatives; K-F, competitive flora; V, vitamins; N, nutrients.

the microbial cells and leads to a synergistic effect of preservative factors (hurdles). Energy restrictions for microorganisms are caused, for example, by anaerobic conditions, such as in modified atmosphere or vacuum packaging of foods (10). The interference with the homeostasis of microorganisms or entire microbial populations forms an attractive and logical focus for further improvements in food preservation techniques (10).

Metabolic Exhaustion

Another phenomenon of certainly practical importance is the metabolic exhaustion of microorganisms, which could lead to an "autosterilization" of foods. This was first observed in experiments with mildly heated (95°C core temperature) liver sausage adjusted to different water activities, inoculated with *Clostridium sporogenes*, and stored at

37°C. Clostridial spores that survived the heat treatment vanished in the product during storage (11). The most likely explanation is that bacterial spores that survive the heat treatment are able to germinate in these foods under less favorable conditions than those under which vegetative bacteria are able to multiply (12). Therefore, the spore counts in stable hurdle-technology foods actually decrease during storage, especially in unrefrigerated foods. A similar behavior has been observed with vegetative microorganisms in studies with high-moisture fruit products (HMFPs) (13,14). The counts of a variety of bacteria, yeasts, and molds that survived the mild heat treatment decreased quite fast in these products during unrefrigerated storage, since the hurdles applied (pH, a_w, sorbate, sulfite) did not allow growth. A general explanation for this behavior might be that vegetative microorganisms that cannot grow will die, and they die more quickly if the stability is close to the threshold for growth, storage temperature is elevated, antimicrobial substances are present, and the organisms are sublethally injured (eg, by heat). Apparently, microorganisms in stable hurdle-technology foods strain every possible repair mechanism to overcome the hostile environment. By doing this they completely use up their energy and die, if they become metabolically exhausted, which leads to an autosterilization of such foods. Thus, due to autosterilization the hurdle-technology foods, which are microbiologically stable, become even more safe during storage, especially at ambient temperatures (6).

Stress Reactions

A limitation to the success of hurdle technology could be stress reactions of microorganisms, because some bacteria become more resistant (eg, toward heat) or even more virulent under stress, since they generate stress shock proteins. Synthesis of these protective proteins is induced by heat, pH, a_w, ethanol, and so on, as well as by starvation. This response of microorganisms under stress might hamper food preservation and could turn out to be problematic for the application of hurdle technology. On the other hand, the switch on of genes for the synthesis of stress shock proteins, which help the organisms to cope with stress situations, should become more difficult if various stresses are received at the same time. This is because countering different stresses simultaneously will demand energy-consuming synthesis of several or at least much more protective stress shock proteins, which the microorganisms cannot deliver since they become metabolically exhausted. Therefore, multitarget preservation of foods could be the answer to avoid synthesis of stress shock proteins, which would make microorganisms in hurdle-technology foods more resistant (7).

Multitarget Preservation

The multitarget preservation of foods should be the ultimate goal for a gentle but most effective preservation of foods. For foods preserved by hurdle technology, it has been suspected for some time that different hurdles in a food could not just have an additive effect on microbial stability, but could act synergistically (1). Example 8 in Figure 1 illustrates this phenomenon. A synergistic effect could become true if the hurdles in a food hit, at the same time, different targets (eg, cell membrane, DNA, enzyme systems, pH, a_w, Eh) within the microbial cell, because then the repair of the homeostasis of the microorganisms as well as the switch on of stress shock proteins become more difficult. Therefore, employing different hurdles in the preservation of a particular food should have advantages, because optimal microbial stability could be achieved with an intelligent mix of gentle hurdles. In practical terms, this could mean that it is, for example, more effective to use different preservatives in small amounts in a food than only one preservative in larger amounts, because different preservatives might hit different targets within the microbial cell, and thus act synergistically (6). It is anticipated that the targets in microorganisms of different preservative factors (hurdles) for foods will be elucidated, and then the hurdles could be grouped in classes according to their targets within the microbial cells. A mild and effective preservation of foods, that is, a synergistic effect of hurdles, is likely if the preservation measures are based on an intelligent selection and combination of hurdles taken from different "target classes." The "multiple-drug attack" has proved successful in the medical field to fight bacterial infections (eg, tuberculosis) as well as viral infections (eg, AIDS), and thus a multitarget attack of microorganisms should be a promising approach in food microbiology, too (7).

APPLICATIONS OF HURDLE TECHNOLOGY

The intelligent application of hurdle technology for a mild but efficient food preservation is advancing worldwide in industrialized as well as in developing countries. Some examples and trends are presented next.

Industrialized Countries

Deliberate and intelligent hurdle technology for food preservation started about 20 years ago in Germany with meat products. First it was used for the gentle preservation of mildly heated, freshlike meats storable without refrigeration (4). In the meantime, several categories of these shelf-stable meat products have evolved, which are in large quantities on the German market and have caused no problems related to spoilage or food poisoning. In the manufacturing plants, processing these shelf-stable meats requires no microbiological tests to be carried out; however, other process parameters have to be strictly controlled; these are time, temperature, pH, and a_w (15). Furthermore, a better understanding of the sequence of hurdles that leads to microbial stability of fermented sausages (salami) has improved the safety and quality of these products (16). In fermented sausages, the microstructure of the products, which was studied by electron microscopy, also turned out to be an important hurdle related to the behavior of pathogens as well as starter cultures in salami (17).

More recent is the application of hurdle technology for microbial stabilization of novel healthful foods derived from meat, poultry, or fish, which contain less fat and/or salt and therefore are more prone to spoil or cause food poisoning. The reduction of salt and fat as well as the substitutes and replacers of these traditional ingredients for muscle foods diminish the microbial stability, since several hurdles (a_w, pH, preservatives, and possibly Eh and microstructure) will change. Compensation could be achieved by an intelligent application of hurdle technology (18). The advantages of hurdle technology are most obvious in high-moisture foods, which are shelf stable at ambient temperature due to an intelligent application of combined methods for preservation. However, the use of hurdle technology is appropriate for chilled foods, too, because in the case of temperature abuse, which can easily happen during food distribution, the stability and safety of chilled foods could break down, especially if low-temperature storage is the only hurdle. Therefore, it is advisable to incorporate into chilled foods (eg, sous vide dishes, salads, fresh-cut vegetables) some additional hurdles (eg, modified atmosphere packaging) that will act as a backup in case of temperature abuse. This type of safety precaution for chilled foods is called invisible technology, implying that additional hurdles act as safeguards in chilled foods, ensuring that they remain microbiologically stable and safe during storage in retail outlets as well as in the home (19).

Packaging is an important hurdle for most foods, since it supports the microbial stability and safety as well as the sensory quality of food products. Industrialized countries have the tendency to overpackage foods. This is especially true for Japan, where "active" packaging (using scavengers, absorbers, emmiters, antimicrobial or antioxidative packaging materials, etc.) has been developed to perfection. These "smart" packaging systems are very sophisticated, but wasteful, too. Therefore, Japanese experts are aiming now for less packaging of foods (20). Future packaging shall provide only necessary information and some convenience to the consumer; however, the required shelf life of the products should not come from the packaging but should be based (*1*) on superclean packaging combined with just-in-time delivery or (*2*) on the development of hurdle-technology foods that are stable and safe in spite of minimal packaging.

Finally, it also should be mentioned that novel emerging technologies for food preservation, that is, nonthermal preservation methods (high hydrostatic pressure, pulsed electric fields, oscillating magnetic fields, light pulses, etc), are often most efficient in combination with traditional food preservation methods. Thus, even for the application of futuristic food preservation methods, hurdle technology is essential (21).

Developing Countries

Most of the food in developing countries, which preferably must be storable without refrigeration since electricity is expensive and not continuously available, are based on empiric use of hurdle technology. However, recently such foods have been optimized by the intentional application of hurdles. Relevant examples are optimized meat products of China and Taiwan as well as dairy and meat products of India. Moreover, in several countries of Latin America (ie, Argentina, Mexico, Venezuela), by the application of hurdle technology, high-moisture fruit products (HMFPs) have been developed. In spite of a high water activity (a_w 0.98 to 0.93), HMFPs are storable in freshlike condition for several months at ambient temperatures and even become sterile during storage due to metabolic exhaustion of the bacteria, yeasts, and molds originally present in these products. There is a general trend in developing countries to move gradually away from the traditional intermediate-moisture foods because they are often too salty or too sweet and have a less appealing texture and appearance than high-moisture foods; this goal is achieved by the application of intentional hurdle technology. The progress made in the application of intelligent hurdle technology in developing countries of Latin America, China, India, and Africa has recently been reviewed (22).

FOOD DESIGN

Hurdle technology as a concept has proved useful in the optimization of traditional foods as well as in the development of novel products. However, it is beneficial to combine hurdle technology in food design with Hazard Analysis Critical Control Point (HACCP) and predictive microbiology. For the proper design of hurdle-technology foods a 10-step procedure has been suggested, which comprises hurdle technology, predictive microbiology, and HACCP (or GMP guidelines). This procedure has proved suitable for solving real development tasks in the food industry (15).

BIBLIOGRAPHY

1. L. Leistner, "Hurdle Effect and Energy Savings," in W. K. Downey, ed., *Food Quality and Nutrition*, Applied Science Publishers, London, United Kingdom, 1978, pp. 553–557.
2. L. Leistner and W. Rödel, "The Stability of Intermediate Moisture Foods With Respect to Micro-organisms," in R. Davies, G. G., Birch and K. J. Parker, eds., *Intermediate Moisture Foods*, Applied Science Publishers, London, United Kingdom, 1976, pp. 120–137.
3. L. Leistner, W. Rödel, and K. Krispien, "Microbiology of Meat and Meat Products in High- and Intermediate-Moisture Ranges," in L. B. Rockland and G. F. Stewart, eds., *Water Activity: Influences on Food Quality*, Academic Press, New York, 1981, pp. 855–916.
4. L. Leistner, "Hurdle Technology Applied to Meat Products of the Shelf Stable Product and Intermediate Moisture Food Types," in D. Simatos and J. L. Multon, eds., *Properties of Water in Foods in Relation to Quality and Stability*, Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1985, pp. 309–329
5. L. Leistner, *J. Food Eng.* **22**, 421–432 (1994).
6. L. Leistner, "Principles and Applications of Hurdle Technology," in G. W. Gould, ed., *New Methods of Food Preservation*, Blackie Academic and Professional, London, United Kingdom, 1995, pp. 1–21.
7. L. Leistner, *Emerging Concepts for Food Safety*, Proceedings 41st ICoMST, San Antonio, Tex., 1995.
8. D. Häussinger, *pH Homeostasis, Mechanisms and Control*, Academic Press, London, United Kingdom, 1988.

9. G. W. Gould, "Interference With Homeostasis—Food," in R. Wittenbury et al., eds., *Homeostatic Mechanisms in Microorganisms*, Bath University Press, Bath, United Kingdom, 1988, pp. 220–228.
10. G. W. Gould, "Homeostatic Mechanisms During Food Preservation by Combined Methods," in G. V. Barbosa-Cánovas and J. Welti-Chanes, eds., *Food Preservation by Moisture Control, Fundamentals and Applications*, Technomic, Lancaster, Pa., 1995, pp. 397–410.
11. L. Leistner and S. Karan-Djurdjić, *Fleischwirtschaft* **50**, 1547–1549 (1970)
12. L. Leistner, *Food Res. Int.* **25**, 151–158 (1992).
13. S. M. Alzamora et al., *Food Res. Int.* **26**, 125–130 (1993).
14. M. S. Tapia de Daza et al., "Microbial Stability Assessment in High and Intermediate Moisture Foods: Special Emphasis on Fruit Products," in G. V. Barbosa-Cánovas and J. Welti-Chanes, eds., *Food Preservation by Moisture Control, Fundamentals and Applications*, Technomic, Lancaster, Pa., 1995, pp. 575–601.
15. L. Leistner, *Food Design by Hurdle Technology and HACCP*, Adalbert Raps Foundation, Kulmbach, Germany, 1994.
16. L. Leistner, "Stable and Safe Fermented Sausages Worldwide," in G. Campbell-Platt and P. E. Cook, eds., *Fermented Meats*, Blackie Academic and Professional, London, United Kingdom, 1995, pp. 160–175.
17. K. Katsaras and L. Leistner, *Biofouling* **5**, 115–124 (1991).
18. L. Leistner, "Microbial Stability and Safety of Healthy Meat, Poultry and Fish Products," in A. M. Pearson and T. R. Dutson, eds., *Production and Processing of Healthy Meat, Poultry and Fish Products*, Blackie Academic and Professional, London, United Kingdom, 1997, pp. 347–360.
19. L. Leistner, "Combined Methods for Food Preservation," in M. Shafiur Rahman, ed., *Handbook of Food Preservation*, Marcel Dekker, New York, 1999, pp. 457–485.
20. K. Ono, *Packaging Design and Innovation*, Snow Brand, Tokyo, Japan, 1994.
21. G. V. Barbosa-Cánovas et al., *Nonthermal Preservation of Foods*, Marcel Dekker, New York, 1998.
22. L. Leistner, "Use of Combined Preservative Factors in Foods of Developing Countries," in B. M. Lund, A. C. Baird-Parker, and G. W. Gould, eds., *The Microbiological Safety of Foods*, Aspen Publishers, Gaithersburg, Md., 1999.

Lothar Leistner
Federal Centre for Meat Research
Kulmbach, Germany

HYDROGEN-ION ACTIVITY (pH)

The effective concentration of hydrogen ion in solution is expressed in terms of pH, which is the negative logarithm of the hydrogen-ion activity:

$$\mathrm{pH} = -\log_{10} a_{\mathrm{H}^+} \tag{1}$$

The relationship between activity and concentration is

$$a = \gamma c \tag{2}$$

where the activity coefficient γ is a function of the ionic strength of the solution and approaches unity as the ionic strength decreases; that is, the difference between the activity and the concentration of hydrogen ion diminishes as the solution becomes more dilute. The pH of a solution may have little relationship to the titratable acidity of a solution that contains weak acids or buffering substances; the pH of a solution indicates only the activity of unassociated hydrogen ions. If total acid concentration is to be determined, an acid–base titration must be performed.

Thermodynamically, the activity of a single ionic species is an inexact quantity, and a conventional pH scale has been adopted that is defined by reference solutions with assigned pH values. These reference solutions, in conjunction with equation 3, define the pH.

$$\mathrm{pH}(X) = \mathrm{pH}(S) - \frac{(E_x - E_s)\mathrm{F}}{2.303\mathrm{RT}} \tag{3}$$

E_S is the electromotive force (emf) of the cell:

$$\text{reference electrode} \mid \mathrm{KCl}(\geq 3.5M) \parallel \text{solution } S \mid \mathrm{H_2(g)},\ \mathrm{Pt}$$

and E_x is the emf of the same cell when the reference buffer solution S is replaced by the sample solution X. The quantities R, T, and F are the gas constant, the thermodynamic temperature, and the Faraday constant, respectively. For routine pH measurements, the hydrogen gas electrode [$\mathrm{H_2(g)}$, Pt] usually is replaced by a glass membrane electrode.

The availability of multiple pH reference solutions makes possible an alternative definition of pH:

$$\mathrm{pH}(X) = \mathrm{pH}(S_1) + [\mathrm{pH}(S_2) - \mathrm{pH}(S_1)]\,\frac{(E_{x2} - E_{s1})}{(E_{s2} - E_{s1})} \tag{4}$$

where E_{s1} and E_{s2} are the measured cell potentials when the sample solution X is replaced in the cell by the two reference solutions S_1 and S_2 such that the values E_{s1} and E_{s2} are on either side of, and as near as possible to, E_X. Equation 4 assumes linearity of the pH versus E response between the two reference solutions, whereas equation 3 assumes both linearity and ideal Nernstian response of the pH electrode. The two-point calibration procedure is recommended if a pH electrode, other than the hydrogen gas electrode, is used for the measurements.

pH DETERMINATION

Two methods are used to measure pH: electrometric and chemical indicator (1–6). The most common is electrometric and uses the commercial pH meter with a glass electrode. This procedure is based on the measurement of the difference between the pH of an unknown or test solution and that of a standard solution. The instrument measures the emf developed between the glass electrode and a reference electrode of constant potential. The difference in emf when the electrodes are removed from the standard solution and placed in the test solution is converted to a difference in pH. Electrodes based on metal–metal oxides (eg, antimony–antimony oxide) have also found use as pH sensors, especially for industrial applications where their superior mechanical stability is needed. However, because of the presence of the metallic element, these electrodes suffer from interferences by reduction-oxidation (redox) systems in the test solution. Nonglass pH electrodes have also been described in which synthetic organic ionophores, selective for hydrogen ions, entrapped in plasticized polymeric membranes have shown excellent pH-response behavior. More recently, the pH ion-sensitive field-effect transistor (ISFET) has shown utility for pH measurements, especially where solid-state ruggedness is desired (7). These devices are based on replacing the metal gate of an ordinary field-effect transistor by a pH-sensitive layer such as silicon nitride. These sensors exhibit ideal Nernstian response over pH ranges comparable to conventional glass pH electrodes.

The second method, which has more limited applications, is the indicator method. The success of this procedure depends on matching the color that is produced by the addition of a suitable pH-sensitive indicator dye to a portion of the unknown solution with the color produced by adding the same quantity of the same dye to a series of standard solutions of known pH. Alternatively, the color is matched against a color comparison chart for the particular dye. Because of the limited color resolution, the results obtained by the indicator method are less accurate relative to those obtained using a pH meter and also suffer from errors when used in highly colored solutions or those containing reactive substances such as bleaches. The indicator method, however, is simple to apply and inexpensive. In addition to being used by direct addition to the test solution, indicator dyes can be immobilized onto paper strips (eg, litmus paper) or, more recently, onto the distal end of fiber-optic probes that, when combined with spectrophotometric readout, provide more quantitative indicator-dye pH determinations.

Reference Buffer Solutions

The uncertainties introduced by the reference electrode liquid junction that exist in conventional electrochemical cells can be avoided by using a cell without transference, for example,

$$\mathrm{Ag, AgCl} \mid \mathrm{KCl}(m), \text{ solution } S \mid \mathrm{H_2(g), Pt}$$

Potassium chloride, KCl, of molality m is added to each reference solution. If the standard potential, $E°$, of the cell and the molality of the chloride ion, m_{Cl} are known, emf measurements yield values of the acidity function $p(a_H\gamma_{Cl})$, as shown by the following equation:

$$p(a_H\gamma_{Cl}) = -\log(m_H\gamma_H\gamma_{Cl}) = \frac{(E° - E)F}{2.303RT} + \log m_{Cl} \quad (5)$$

To eliminate the effect of the added KCl on the acidity of the buffer solution, $p(a_H\gamma_{Cl})$ is determined for three or more portions of the buffer solution that contain different amounts of added chloride. The limiting value of the acidity function is obtained by extrapolation to zero molality of chloride ion. If the single-ion activity coefficient of chloride ion in the buffer solution could be obtained, the activity of hydrogen ion would be readily accessible.

$$pa_H = -\log(m_H\gamma_H) = p(a_H\gamma_{Cl}) + \log\gamma_{Cl} \quad (6)$$

To establish a conventional scale of hydrogen-ion activity, it has been suggested (8) that the activity coefficient of chloride ion in selected reference buffer solutions having ionic strengths of ≦0.1 be defined as

$$-\log\gamma_{Cl} = \frac{A\sqrt{I}}{1 + 1.5\sqrt{I}} \quad (7)$$

where A is a constant of the Debye-Hückel theory and I is the ionic strength. The pa_H for these selected reference solutions is identified with pH(S) in the operational definition:

$$pa_H = \mathrm{pH}(S) \quad (8)$$

The pH(S) values at 25°C of the primary and secondary reference buffer solutions certified by the U.S. National Institute of Standards and Technology (NIST) are listed in Table 1 (2). Of particular note, the pH 6.86 and 7.41 phosphate buffers have long been accepted as the primary reference standards for blood pH measurements, with the knowledge that the ionic strength of these buffers is significantly different from that of blood, thus biasing (however reproducibly) the pH measurement in blood due to the residual liquid-junction potential. Recently, NIST certified two concentrations each of two zwitterionic buffer systems (HEPES/HEPESate and MOPSO/MOPSOate) as pH buffers. These secondary standards have been certified at ionic strengths comparable to that of blood and conse-

Table 1. pH Standards, Molality Scale

Solution composition	pH(S) at 25°C
Primary standards	
Potassium hydrogen tartrate (saturated at 25°C)	3.557
0.05 m Potassium dihydrogen citrate	3.776
0.05 m Potassium hydrogen phthalate	4.006
0.025 m KH_2PO_4 + 0.025 m Na_2HPO_4	6.863
0.008695 m KH_2PO_4 + 0.03043 m Na_2HPO_4	7.410
0.01 m $Na_2B_4O_7 \cdot 10H_2O$	9.180
0.025 m $NaHCO_3$ + 0.025 m Na_2CO_3	10.011
Secondary standards	
0.05 m Potassium tetroxalate · $2H_2O$	1.681
0.05 m HEPES[a] + 0.05 m NaHEPESate	7.503
0.08 m HEPES + 0.08 m NaHEPESate	7.516
0.05 m MOPSO[b] + 0.05 m NaMOPSOate	6.867
0.08 m MOPSO + 0.08 m NaMOPSOate	6.865
0.01667 m TRIS[c] + 0.05 m TRIS · HCl	7.699
$Ca(OH)_2$ (saturated at 25°C)	12.454

[a] HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

[b] MOPSO = 3-(N-morpholino)-2-hydroxypropanesulfonic acid.

[c] TRIS = tris(hydroxymethyl)aminomethane.

Source: Ref. 2, and NIST, private communication.

quently should minimize the residual liquid-junction potential. The International Union of Pure and Applied Chemistry recommends the NIST primary standards plus a series of operational standards, measured versus the phthalate reference value standard in a cell with a well-defined liquid junction, for the definition of the pH scale (9). However, this recommendation is currently under review, although significant change in the present scale is not anticipated other than its extension to higher ionic strength solutions.

Accuracy and Interpretation of Measured pH Values

The acidity function $p(a_H\gamma_{Cl})$, which is the experimental basis for the assignment of pH(S), is reproducible within about 0.003 pH unit from 10 to 40°C. If the ionic strength is known and ≤ 0.1, the assignment of numerical values to the activity coefficient of chloride ion does not add to the uncertainty. However, errors in the standard potential of the cell, in the composition of the buffer materials, and in the preparation of the solutions may raise the uncertainty to 0.005 pH unit.

The reproducibility of the practical scale that has been defined using the seven primary standards includes the possible inconsistencies introduced in the standardization of the instrument with seven different standards of different composition and concentration. These inconsistencies are the result of variations in the liquid-junction potential when one solution is replaced by another and are unavoidable. The accuracy of the practical scale from 10 to 40°C therefore appears to be in the range from 0.008 to 0.01 pH unit.

Variations in the liquid-junction potential may be increased when the standard solutions are replaced by test solutions that do not closely match the standards with respect to the composition and concentrations of solutes, or to the solvent composition, for example, nonaqueous and mixed solvents. Under these circumstances, the pH remains a reproducible number, but it may have little or no meaning in terms of the conventional hydrogen-ion activity of the medium. The use of experimental pH numbers as a measure of the extent of acid–base reactions or to obtain thermodynamic equilibrium constants is justified only when the pH of the medium is between 2.5 and 11.5 and when the mixture is an aqueous solution of simple solutes in total concentration of approximately $\leq 0.2\ M$.

Sources of Error

Although subject to fewer interferences and other types of error than most potentiometric ionic-activity sensors, that is, ion-selective electrodes (qv), pH electrodes must be used with an awareness of their particular response characteristics as well as the potential sources of error that may affect the other components of the measurement system, especially the reference electrode (see also "pH Measurement System Electrodes"). Several common causes of measurement problems are electrode interferences and/or fouling of the pH sensor, sample matrix effects, reference electrode instability, and improper calibration of the measurement system (10).

In general, the potential of an electrochemical cell, E_{cell}, is the sum of three potential terms:

$$E_{cell} = E_{pH} - E_{ref} + E_{lj} \tag{9}$$

where E_{pH} and E_{ref} are the potentials of the pH and reference electrodes, respectively, and E_{lj} is the ubiquitous liquid-junction potential. After substitution of the Nernst equation for the pH electrode potential term in equation 9,

$$E_{cell} = E^{\circ}_{pH} - \frac{RT}{F} \ln a_H - E_{ref} + E_{lj} \tag{10}$$

it can be calculated that a 1 mV error in any of the potential terms corresponds to an error of about 4% in the hydrogen-ion activity. Under carefully controlled experimental conditions, the potential of a pH cell can be measured with an uncertainty as small as 0.3 mV, which corresponds to a ±0.005 pH unit uncertainty.

The measurement of pH using the operational cell assumes that no residual liquid-junction potential is present when a standard buffer is compared with a solution of unknown pH. Although this may never strictly be true, especially with complex matrices, the residual liquid-junction potential can be minimized by the appropriate choice of a salt-bridge solution and calibration buffer solutions.

pH MEASUREMENT SYSTEM ELECTRODES

Glass Electrodes

The glass electrode is the hydrogen-ion sensor in most pH-measurement systems. The pH-responsive surface of the glass electrode consists of a thin membrane formed from a special glass that, after suitable conditioning, develops a surface potential that is an accurate index of the acidity of the solution in which the electrode is immersed. To permit changes in the potential of the active surface of the glass membrane to be measured, an inner reference electrode of constant potential is placed in the internal compartment of the glass membrane. The inner reference compartment contains a solution that has a stable hydrogen-ion concentration and counter ions to which the inner electrode is reversible. The choice of the inner cell components has a bearing on the temperature coefficient of the emf of the pH assembly. The inner cell commonly consists of a silver–silver chloride electrode or calomel electrode in a buffered chloride solution.

Immersion electrodes are the most common glass electrodes. These are roughly cylindrical and consist of a barrel or stem of inert glass that is sealed at the lower end to a tip, which is often hemispherical, of special pH-responsive glass. The tip is completely immersed in the solution during measurements. Miniature and microelectrodes are also commercially available and used widely, particularly in physiological studies. Capillary electrodes permit the use of small samples and provide protection from exposure to air during the measurements, for example, for the determination of blood pH. This type of electrode may be provided with a water jacket for temperature control.

The membrane of pH-responsive glass usually is made as thin as is consistent with adequate mechanical strength; nevertheless, its electrical resistance is high, for example, 10 to 250 MΩ. Therefore, an electronic amplifier must be used to obtain adequate accuracy in the measurement of the surface potential of a glass electrode. The versatility of the glass electrode results from its mechanism of operation, which is one of proton exchange rather than electron transfer; hence, oxidizing and reducing agents in the solution do not affect the pH response.

Most modern electrode glasses contain mixtures of silicon dioxide, either sodium or lithium oxide, and either calcium, barium, cesium, or lanthanum oxide. The latter oxides are added to reduce spurious response to alkali metal ions in high pH solutions. The composition of the glass has a profound effect on the electrical resistance, the chemical durability of the pH-sensitive surface, and the accuracy of the pH response in alkaline solutions. Both the electrical and the chemical resistance of the electrode glasses decrease rapidly with a rise in temperature. Therefore, it is difficult to design an electrode that is sufficiently durable for extended use at high temperatures and yet, when used at room temperature, free from the sluggish response often characteristic of pH cells of excessively high resistance. Most manufacturers use different glass compositions for electrodes, depending on their intended use.

The mechanism of the glass electrode response is not entirely understood. It is clear, however, that when a freshly blown membrane of pH-responsive glass is first conditioned in water, the sodium or lithium ions that occupy the interstices of the silicon–oxygen network in the glass surface are exchanged for protons from the water. The protons find stable sites in the conditioned gel layer of the glass surface. Exchange of the labile protons between these sites and the solution phase appears to be the mechanism by which the surface potential reflects changes in the hydrogen ion activity of the external solution. When the glass electrode and the hydrogen gas electrode are immersed in the same solution, their potentials usually differ by a constant amount, even though the pH of the medium is raised from 1 to 10 or greater. In this range, the potential E_g of a glass electrode may be written

$$E_g = E_g^\circ + \frac{RT}{F} \ln a_{H+} \qquad (11)$$

where E_g° is the "standard" (or formal) potential of that particular glass electrode on the hydrogen scale.

Departures from the ideal behavior expressed by equation 11 usually are found in alkaline solutions containing alkali metal ions in appreciable concentration, and often in solutions of strong acids. The supposition that the alkaline error is associated with the development of an imperfect response to alkali metal ions is substantiated by the successful design of cation-sensitive glass electrodes that are used to determine sodium, silver, and other monovalent cations (3).

The advantage of the lithium glasses over the sodium glasses in the reduction of alkaline error is attributed to the smaller size of the proton sites remaining after elution of the lithium ions from the glass surface. This interpretation is consistent with the relative magnitudes of the alkaline errors for various cations. These errors decrease rapidly as the diameter of the cation becomes larger. The error observed in concentrated solutions of the strong acids is characterized by a marked drift of potential with time, which is thought to result from the penetration of acid anions, as well as protons, into the glass surface (11).

The immersion of glass electrodes in strongly dehydrating media should be avoided. If the electrode is used in solvents of low water activity, frequent conditioning in water is advisable, as dehydration of the gel layer of the surface causes a progressive alteration in the electrode potential with a consequent drift of the measured pH. Slow dissolution of the pH-sensitive membrane is unavoidable, and it eventually leads to mechanical failure. Standardization of the electrode with two buffer solutions is the best means of early detection of incipient electrode failure.

Fouling of the pH sensor may occur in solutions containing surface-active constituents that coat the electrode surface and may result in sluggish response and drift of the pH reading. Prolonged measurements in blood, sludges, and various industrial process materials and wastes can cause such drift; therefore, it is necessary to clean the membrane mechanically or chemically at intervals that are consistent with the magnitude of the effect and the precision of the results required.

Reference Electrodes and Liquid Junctions

The electrical circuit of the pH cell is completed through a salt bridge that usually consists of a concentrated solution of potassium chloride. The solution makes contact at one end with the test solution and at the other with a reference electrode of constant potential. The liquid junction is formed at the area of contact between the salt bridge and test solutions. The mercury–mercurous chloride electrode—the calomel electrode—provides a highly reproducible potential in the potassium chloride bridge solution and is the most widely used reference electrode. However, mercurous chloride is converted readily into mercuric ion and mercury when in contact with concentrated potassium chloride solutions above 80°C. This disproportionation reaction causes an unstable potential with calomel electrodes Therefore, the silver–silver chloride electrode and the thallium amalgam–thallous chloride electrode often are preferred for measurements above 80°C. However, because silver chloride is relatively soluble in concentrated solutions of potassium chloride, the solution in the electrode chamber must be saturated with silver chloride to avoid dissolution of the electrode coating.

The commercially used reference electrode–salt bridge combination usually is of the immersion type. The salt-bridge chamber usually surrounds the electrode element. Some provision is made to allow a slow leakage of the bridge solution out of the tip of the electrode to establish a stable liquid junction with the standard solution or test solution in the pH cell. An opening is usually provided through which the electrode chamber may be refilled with the salt-bridge solution. Various devices are used to constrain the outflow of bridge solution, for example, fibers, porous ceramics, capillaries, ground-glass joints, and controlled cracks. Such commercial electrodes normally give very satisfactory results, but there is some evidence that the type and structure of the junction may affect the reference potential when measurements are made at very low pH and, possibly, at high alkalinities.

Combination electrodes have increased in use and are a consolidation of the glass and reference electrodes in a single probe, usually in a concentric arrangement, with the reference electrode compartment surrounding the pH sensor. The advantages of combination electrodes include the convenience of using a single probe and the ability to measure small volumes of sample solution or in restricted-access containers, for example, test tubes and narrow-neck flasks. A disadvantage of this arrangement is that if one of the electrodes becomes defective, the entire combination assembly must be discarded.

Theoretical considerations favor liquid junctions in which cylindrical symmetry and a steady state of ionic diffusion are achieved. Special cells in which a stable junction can be achieved are not difficult to construct and are available commercially.

A solution of potassium chloride that is saturated at room temperature usually is used for the salt bridge. It has been shown that the higher the concentration of the potassium chloride solution, the more effective the bridge solution is in reducing the liquid-junction potential (12). Also, the saturated potassium chloride calomel and silver–silver chloride reference electrodes are stable, reproducible, and easy to prepare. However, after long periods and with temperature lowering, the salt-bridge chamber may become filled with large crystals of potassium chloride that block the flow of bridge solution and thereby impair the reproducibility of the junction potential and raise the resistance of the cell. A slightly undersaturated (eg, 3.5 *M*) solution of potassium chloride is preferred. The calomel electrode has the added disadvantage that it shows a marked potential hysteresis with changes of temperature.

Samples that contain suspended matter are among the most difficult types from which to obtain accurate pH readings because of the so-called suspension effect, that is, the suspended particles produce abnormal liquid-junction potentials at the reference electrode (13). This effect is especially noticeable with soil slurries, pastes, and other types of colloidal suspensions. In the case of a slurry that separates into two layers, pH differences of several units may result, depending on the placement of the electrodes in the layers. Internal consistency is achieved by pH measurement using carefully prescribed measurement protocols, as has been used in the determination of soil pH (14).

Another problem that may result in spurious pH readings is caused by streaming potentials. Presumably, these are attributable to changes in the reference electrode liquid junction that are caused by variations in the flow rate of the sample solution. Factors that affect the observed pH include the magnitude of the flow-rate changes, the geometry of the electrode system, and the concentration of the salt-bridge electrolyte; therefore, this problem may be avoided by maintaining constant flow and geometry characteristics and calibrating the system under operating conditions that are identical to those of the sample measurement.

pH INSTRUMENTATION

The pH meter is an electronic voltmeter that provides a direct conversion of voltage differences to differences of pH at the measurement temperature (15). One class of instruments is the direct-reading analogue, which has a deflection meter with a large scale calibrated in mV and pH units. Most modern direct-reading meters have digital displays of the emf or pH. The types range from very inexpensive meters that read to the nearest 0.1 pH unit to the research models capable of measuring pH with a precision of 0.001 pH unit and drifting less than 0.003 pH unit over 24 h; however, it should be noted that the fundamental meaning of these measured values is considerably less certain than the precision of the measurement.

Because of the very large resistance of the glass membrane in a conventional pH electrode, an input amplifier of high impedance (usually 10^{12}–10^{14} Ω) is required to avoid errors in the pH (or mV) readings. Most pH meters have field-effect transistor amplifiers that typically exhibit bias currents of only a picoampere (10^{-12} ampere), which, for an electrode resistance of 100 MΩ, results in an emf error of only 0.1 mV (0.002 pH unit).

In addition, most of these devices provide operator control of settings for temperature and/or response slope, isopotential point, zero or standardization, and function (pH, mV, or monovalent/divalent cation/anion). Microprocessors are incorporated in advanced-design meters to facilitate calibration, calculation of measurement parameters, and automatic temperature compensation. Furthermore, pH meters are provided with output connectors for continuous readout via a strip-chart recorder and often with binary-coded decimal output for computer interconnections or connection to a printer. Although the accuracy of the measurement is not increased by the use of a recorder, the readability of the displayed pH (on analogue models) can be expanded, and recording provides a permanent record with information on response and equilibration times during measurement (5).

TEMPERATURE EFFECTS

The emf, E, of a pH cell may be written

$$E = E_g^{\circ\prime} - k\text{pH} \qquad (12)$$

where k is the Nernst factor $(2.303RT)/F$, and $E_g^{\circ\prime}$ includes the liquid-junction potential and the half-cell emf on the reference side of the glass membrane. Changes of temperature alter the scale slope because k is proportional to T. The scale position also is changed because the "standard" potential is temperature dependent: $E_g^{\circ\prime}$ is usually a quadratic function of the temperature.

The objective of temperature compensation in a pH meter is to nullify changes in emf from any source except changes in the true pH of the test solution. Nearly all pH meters provide automatic or manual adjustment for the change of k with T. If correction is not made for the change of standard potential, however, the instrument must always be standardized at the temperature at which the pH is to be determined. In industrial pH control, standardization of the assembly at the temperature of the measurements is not always possible, and compensation for shift of the scale position, though imperfect, is useful. If the value of $E_g^{\circ\prime}$ were a linear function of T, it would be easy to show that the straight lines representing the variation of E and pH at different temperatures would intersect at a point, the isopotential point or pH_i. Even though $E_g^{\circ\prime}$ does not usually vary linearly with T, these plots intersect at about pH_i when the range of temperatures is narrow. By providing a temperature-dependent bias potential of $k\mathrm{pH}_i$, an approximate correction for the change of the standard potential with temperature can be applied automatically (1,5).

NONAQUEOUS SOLVENTS

The activity of the hydrogen ion is affected by the properties of the solvent in which it is measured. Scales of pH only apply to the medium (single solvent or mixed solvents, eg, water-alcohol) for which they are developed. The comparison of the pH values of a buffer in aqueous solution to one in a nonaqueous solvent has neither direct quantitative nor thermodynamic significance. Consequently, operational pH scales must be developed for the individual solvent systems. In certain cases, correlation to the aqueous pH scale can be made but, in others, pH values are used only as relative indicators of the hydrogen-ion activity.

Other difficulties of measuring pH in nonaqueous solvents are the complications that result from dehydration of the glass pH membrane, increased sample resistance and large liquid-junction potentials. These effects are complex and are highly dependent on the type of solvent or mixture used (1,5).

INDICATOR pH MEASUREMENTS

The indicator method is especially convenient when the pH of a well-buffered colorless solution must be measured at room temperature with an accuracy no greater than 0.5 pH unit. Under optimum conditions an accuracy of 0.2 pH unit may be obtainable. A list of representative acid–base indicators is given in Table 2 with their corresponding transformation ranges. A more complete listing, including the theory of the indicator color change and of the salt effect, is given in Reference 1.

Because they are weak acids or bases, the indicators may affect the pH of the sample, especially in the case of a poorly buffered solution. Variations in the ionic strength or solvent composition, or both, also can produce large uncertainties in pH measurements, presumably caused by changes in the equilibria of the indicator species. Specific chemical reactions also may occur between solutes in the sample and the indicator species to produce appreciable pH measurement errors. Examples of such interferences include binding of the indicator species by proteins and colloidal substances and direct reaction with sample components, for example, oxidizing agents and heavy metal ions.

Table 2. Acid-Base Indicators

Indicator	pH range	Acid color	Base color
Acid cresol red	0.2–1.8	red	yellow
Methyl violet	0.5–1.5	yellow	blue
Acid thymol blue	1.2–2.8	red	yellow
Bromophenol blue	3.0–4.6	yellow	blue
Methyl orange	3.2–4.4	red	yellow
Bromocresol green	3.8–5.4	yellow	blue
Methyl red	4.4–6.2	red	yellow
Bromocresol purple	5.2–6.8	yellow	purple
Bromothymol blue	6.0–7.6	yellow	blue
Phenol red	6.6–8.2	yellow	red
Cresol red	7.2–8.8	yellow	red
Thymol blue	8.0–9.6	yellow	blue
Phenolphthalein	8.2–9.8	colorless	red
Tolyl red	10.0–11.6	red	yellow
Parazo orange	11.0–12.6	yellow	orange
Acyl blue	12.0–13.6	red	blue

INDUSTRIAL PROCESS CONTROL

Specialized equipment for industrial measurements and automatic control have been developed (16) (see "pH Instrumentation"). In general, the pH of an industrial process need not be controlled with great accuracy. Consequently, frequent standardization of the cell assembly may be unnecessary. On the other hand, the ambient conditions, for example, temperature and humidity, under which the industrial control measurements are made may be such that the pH meter must be much more robust than those intended for laboratory use. To avoid costly downtime for repairs, pH instruments may be constructed of modular units, permitting rapid removal and replacement of a defective subassembly.

The pH meter usually is coupled to a data recording device and often to a pneumatic or electric controller. The controller governs the addition of reagent so that the pH of the process stream is maintained at the desired level.

Immersion-cell assemblies are designed for continuous pH measurement in tanks, troughs, or other vessels containing process solutions at different levels under various conditions of agitation and pressure. The electrodes are protected from mechanical damage and sometimes are provided with devices to remove surface deposits as they accumulate. Process flow chambers are designed to introduce the pH electrodes directly into piped sample streams or into bypass sample loops that may be pressurized. Electrode chambers of both types usually contain a temperature-sensing element that controls the temperature-compensating circuits of the measuring instrument.

Glass electrodes for process control do not differ materially from those used for pH measurements in the laboratory, but the emphasis in industrial application is on rugged construction to withstand both mechanical stresses and high pressures. Pressurized salt bridges, which ensure slow leakage of bridge solution into the process stream even under very high ambient pressures, have been developed. For less-severe process-monitoring conditions, reference electrodes are available with no-flow polymeric or gel-filled junctions that can be used without external pressurization.

BIBLIOGRAPHY

1. R. G. Bates, *Determination of pH, Theory and Practice*, 2nd ed., Wiley-Interscience, New York, 1973.
2. Y. C. Wu, W. F. Koch, and R. A. Durst, *Standardization of pH Measurements*, National Bureau of Standards Special Publication 260-53, U.S. Government Printing Office, Washington, D.C., 1988.
3. G. Eisenman, ed., *Glass Electrodes for Hydrogen and Other Cations*, Marcel Dekker, New York, 1967.
4. G. Mattock, *pH Measurement and Titration*, Macmillan, New York, 1961.
5. C. C. Westcott, *pH Measurements*, Academic Press, New York, 1978.
6. "pH of Aqueous Solutions with the Glass Electrode," *ASTM Method E 70-77*, American Society for Testing and Materials, Philadelphia, Pa., 1977.
7. J. Janata, *Principles of Chemical Sensors*, Plenum Press, New York, 1989.
8. R. G. Bates and E. A. Guggenheim, *Pure Appl. Chem.* **1**, 163 (1960).
9. A. K. Covington, R. G. Bates, and R. A. Durst, *Pure Appl. Chem.* **57**, 531 (1985).
10. R. A. Durst, "Sources of Error in Ion-Selective Electrode Potentiometry" in H. Freiser, ed., *Ion-Selective Electrodes in Analytical Chemistry*, Plenum Press, New York, 1978, pp. 311–338.
11. K. Schwabe, "pH Measurements and Their Applications" in H. W. Nürnberg, ed., *Electroanalytical Chemistry*, John Wiley & Sons, New York, 1974, pp. 495–586.
12. E. A. Guggenheim, *J. Am. Chem. Soc.* **52**, 1315 (1930).
13. H. Jenny et al., *Science* **112**, 164 (1950).
14. A. M. Pommer, "Glass Electrodes for Soil Waters and Soil Suspensions" in G. Eisenman, ed., *Glass Electrodes for Hydrogen and Other Cations*, Marcel Dekker, New York, 1967, pp. 362–411.
15. A. Wilson, *pH Meters*, Barnes and Noble, New York, 1970.
16. F. G. Shinskey, *pH and pIon: Control in Process and Waste Streams*, John Wiley & Sons, New York, 1973.

GENERAL REFERENCES

Refs. 1–6 are also general references.

RICHARD A. DURST
Cornell University
Geneva, New York

ROGER G. BATES
University of Florida
Gainesville, Florida

HYDROGENATION

Hydrogenation is unique among the unit processes used in edible oils and fats processing in that it alters the molecular structure and composition of the glycerol esters comprising the naturally occurring triglycerides of vegetable, meat, and marine origin. It is a chemical reaction in which hydrogen is added to the ethylenic linkages (double bonds). It requires the presence of a catalyst—historically almost always nickel. It normally is performed batchwise in a hydrogen-pressurized stirred reactor. Agitation is necessary to suspend the catalyst and to effect solubilization of the hydrogen, thus enabling it to react on the surface of the catalyst with a double bond in the oil.

Hydrogenation is employed worldwide on a vast scale in the edible oils and fats industry. Its principal objectives are to convert liquid oils into plastic fats usable in a wide variety of cooking and baking applications, and to improve flavor stability by retarding deterioration through oxidation. Hydrogenation has also made a major contribution to the present high degree of interchangeability among a wide variety of oils and fats.

REACTION MECHANISM

The basic chemical equation for hydrogenation of an unsaturated carbon–carbon double bond is shown in equation 1(1).

$$\text{-CH=CH-} + H_2 \xrightarrow{\text{catalyst}} \text{-CH}_2 - \text{CH}_2\text{-} \quad (1)$$

As the equation indicates, and as alluded to earlier, hydrogenation takes place when the carbon to carbon double bonds in the liquid oil, the solid catalyst, and the gaseous hydrogen have been brought together—usually in a heated stirred reactor. The hydrogen must be dissolved in the oil since only dissolved hydrogen is available for reaction.

Although the hydrogenation reaction may appear straightforward, it is in actuality very complicated. The esters may contain one, two, three, or more unsaturated bonds, and each double bond may be hydrogenated at a different rate, depending on its position in the molecule. There may also be simultaneous isomerization of the unsaturated bonds. Isomerization is principally geometrical, but some positional isomerization can also occur. In addition, the position of the ester on the glycerol also has some effect in determining the physical properties of the molecule.

The complexity of the reaction is further illustrated by noting that the partial hydrogenation of soybean oil results in the production of a minimum of 30 different linolenic, linoleic, and oleic esters whose *cis* and *trans* forms could produce more than 4000 different triglycerides. The marvel is that producers have learned to not only control the reaction but actually utilize its many-faceted complexity to produce a great variety of oils, shortenings and margarines, each designed to have physical properties (functional characteristics) making them specifically desirable for a particular application. In recent years there has been an additional strong impetus to also make the final products nutritionally desirable.

SELECTIVITY

The term *selectivity*, as commonly used in the edible oils and fats industry, relates to equation 2 (2).

gel-filled junctions that can be used without external pressurization.

BIBLIOGRAPHY

1. R. G. Bates, *Determination of pH, Theory and Practice*, 2nd ed., Wiley-Interscience, New York, 1973.
2. Y. C. Wu, W. F. Koch, and R. A. Durst, *Standardization of pH Measurements*, National Bureau of Standards Special Publication 260-53, U.S. Government Printing Office, Washington, D.C., 1988.
3. G. Eisenman, ed., *Glass Electrodes for Hydrogen and Other Cations*, Marcel Dekker, New York, 1967.
4. G. Mattock, *pH Measurement and Titration*, Macmillan, New York, 1961.
5. C. C. Westcott, *pH Measurements*, Academic Press, New York, 1978.
6. "pH of Aqueous Solutions with the Glass Electrode," *ASTM Method E 70-77*, American Society for Testing and Materials, Philadelphia, Pa., 1977.
7. J. Janata, *Principles of Chemical Sensors*, Plenum Press, New York, 1989.
8. R. G. Bates and E. A. Guggenheim, *Pure Appl. Chem.* **1**, 163 (1960).
9. A. K. Covington, R. G. Bates, and R. A. Durst, *Pure Appl. Chem.* **57**, 531 (1985).
10. R. A. Durst, "Sources of Error in Ion-Selective Electrode Potentiometry" in H. Freiser, ed., *Ion-Selective Electrodes in Analytical Chemistry*, Plenum Press, New York, 1978, pp. 311–338.
11. K. Schwabe, "pH Measurements and Their Applications" in H. W. Nürnberg, ed., *Electroanalytical Chemistry*, John Wiley & Sons, New York, 1974, pp. 495–586.
12. E. A. Guggenheim, *J. Am. Chem. Soc.* **52**, 1315 (1930).
13. H. Jenny et al., *Science* **112**, 164 (1950).
14. A. M. Pommer, "Glass Electrodes for Soil Waters and Soil Suspensions" in G. Eisenman, ed., *Glass Electrodes for Hydrogen and Other Cations*, Marcel Dekker, New York, 1967, pp. 362–411.
15. A. Wilson, *pH Meters*, Barnes and Noble, New York, 1970.
16. F. G. Shinskey, *pH and pIon: Control in Process and Waste Streams*, John Wiley & Sons, New York, 1973.

GENERAL REFERENCES

Refs. 1–6 are also general references.

RICHARD A. DURST
Cornell University
Geneva, New York

ROGER G. BATES
University of Florida
Gainesville, Florida

HYDROGENATION

Hydrogenation is unique among the unit processes used in edible oils and fats processing in that it alters the molecular structure and composition of the glycerol esters comprising the naturally occurring triglycerides of vegetable, meat, and marine origin. It is a chemical reaction in which hydrogen is added to the ethylenic linkages (double bonds). It requires the presence of a catalyst—historically almost always nickel. It normally is performed batchwise in a hydrogen-pressurized stirred reactor. Agitation is necessary to suspend the catalyst and to effect solubilization of the hydrogen, thus enabling it to react on the surface of the catalyst with a double bond in the oil.

Hydrogenation is employed worldwide on a vast scale in the edible oils and fats industry. Its principal objectives are to convert liquid oils into plastic fats usable in a wide variety of cooking and baking applications, and to improve flavor stability by retarding deterioration through oxidation. Hydrogenation has also made a major contribution to the present high degree of interchangeability among a wide variety of oils and fats.

REACTION MECHANISM

The basic chemical equation for hydrogenation of an unsaturated carbon–carbon double bond is shown in equation 1(1).

$$\text{-CH=CH-} + H_2 \xrightarrow{\text{catalyst}} \text{-CH}_2 - \text{CH}_2\text{-} \qquad (1)$$

As the equation indicates, and as alluded to earlier, hydrogenation takes place when the carbon to carbon double bonds in the liquid oil, the solid catalyst, and the gaseous hydrogen have been brought together—usually in a heated stirred reactor. The hydrogen must be dissolved in the oil since only dissolved hydrogen is available for reaction.

Although the hydrogenation reaction may appear straightforward, it is in actuality very complicated. The esters may contain one, two, three, or more unsaturated bonds, and each double bond may be hydrogenated at a different rate, depending on its position in the molecule. There may also be simultaneous isomerization of the unsaturated bonds. Isomerization is principally geometrical, but some positional isomerization can also occur. In addition, the position of the ester on the glycerol also has some effect in determining the physical properties of the molecule.

The complexity of the reaction is further illustrated by noting that the partial hydrogenation of soybean oil results in the production of a minimum of 30 different linolenic, linoleic, and oleic esters whose *cis* and *trans* forms could produce more than 4000 different triglycerides. The marvel is that producers have learned to not only control the reaction but actually utilize its many-faceted complexity to produce a great variety of oils, shortenings and margarines, each designed to have physical properties (functional characteristics) making them specifically desirable for a particular application. In recent years there has been an additional strong impetus to also make the final products nutritionally desirable.

SELECTIVITY

The term *selectivity*, as commonly used in the edible oils and fats industry, relates to equation 2 (2).

$$\text{Linolenic} \xrightarrow{K_1} \text{linoleic} \xrightarrow{K_2} \text{oleic} \xrightarrow{K_3} \text{stearic} \qquad (2)$$

With some simplification, linolenic selectivity is defined as the ratio (Ln SR) of the rate of reaction of K_1 to K_2. With the same simplification, linoleic selectivity is defined as the ratio (Lo SR) of the rate of reaction of K_2 to K_3. Because of its much greater utility and its ability to be manipulated, when the term selectivity is used in the edible oil industry, the speaker or writer is referring to linoleic selectivity unless explicitly indicated otherwise. Another way of defining linoleic selectivity is as the degree of preferential conversion of dienes to monoenes compared with the conversion of monoenes to saturates.

Although few *trans* isomers occur in nature, during partial hydrogenation of oils the double bond may be either saturated or isomerized while it is being adsorbed on the catalyst surface. Some positional and many geometric isomers are formed, basically by way of the Horiute-Polanyi mechanism. Elaidic, the *trans* form of octadecanoic (oleic) acid, is the most common *trans* isomer. Its historic utility in margarine formulation is based on its having a significantly higher melting point (43.7°C) than the *cis* form (16.3°C), while still being considerably lower melting than the completely saturated stearic (69.6°C). Since the solids content of a fat at any given temperature depends on the distribution of all the esters of a triglyceride, the ratio of *trans* to *cis* is the principal determinant of the slope of the melting curve in the important refrigerator to body temperature range.

The preferential selectivity of nickel catalysts for the hydrogenation of a linolenic ester in a triglyceride compared with a linoleic (Ln SR) has been shown to be about 1.8 to 2.3. Since changes in process conditions do not significantly change this ratio, evidently the diene and the triene are hydrogenated by the same mechanism. Although it would seem the presence of the third double bond should double the chance of hydrogenation of one of the double bonds in the triene compared with the diene, this has not been shown to be the case. However, when copper catalysts are used to hydrogenate soybean oil, as will be discussed later, linolenic selectivity ratios of 8 to 12 are found. Copper catalyst obviously operates somewhat differently from nickel. It is surmised that copper catalyst causes conjugation of the linolenate triene on the catalyst surface. Since conjugated trienes have been shown to react about 200 times faster than nonconjugated ones, they do not accumulate in the product—evidently hydrogenating to a conjugated diene before desorbing from the catalyst surface. The conjugated dienes are then further reduced to monoenes. The monoenes are not reduced to saturates by the catalyst since hydrogenation with copper catalyst must involve two or more double bonds.

EFFECTS OF PROCESS CONDITIONS

When hydrogenating a specific oil with a chosen catalyst, the reaction parameters are temperature, pressure, catalyst concentration, and agitation (mixing). Because each parameter influences both reaction rate and selectivity, and because all are interrelated, it is not possible to specifically predict what their combined effect will be in a particular instance. Therefore, each of the parameters will be discussed separately, as they affect reaction rate and as they affect selectivity.

Effects of Temperature

Hydrogenation, like other chemical reactions, is accelerated by increasing temperature. Both preferential selectivity and isomerization are also greater with increasing temperature. Since there is an increasing tendency toward hydrolysis above 400°F (204°C), processors limit their upper temperature accordingly, with 450°F (232°C) being a maximum. Since some investigators claim that nutritionally undesirable positional isomers are also formed at high temperatures, a limitation as low as 400°F (204°C) is sometimes observed.

Effects of Pressure

Again, as with most chemical reactions, increasing pressure increases reaction rate. Commercial hydrogenation of edible triglyceride oils is usually performed under hydrogen pressure of 7 to 50 psig (0.5–3.5 atm)—at the lower end for partial hardening, which constitutes most of the commercial hydrogenation of edible oils such as soybean, canola, and so on. Within this lower range, even modest change in pressure has a significant effect on the inverse relationship of increasing reaction rate/decreasing selectivity—both preferential and *trans* isomer.

Effects of Catalyst Concentration

Increasing the catalyst concentration in a given system increases reaction rate up to the point where hydrogen availability becomes the limiting parameter. It has a minor effect on selectivity. The catalyst concentration employed in commercial partial hydrogenation of edible oils is chosen so that the converter reaction time fits into the other batch cycle steps of heat exchange and filtration for catalyst removal.

Effects of Agitation

Agitation (mixing) is of great importance in determining both the rate and selectivities (preferential and *trans*-isomeric) of edible oil hydrogenation. In general, better mixing increases activity and decreases selectivity. Agitation must accomplish the following: (*1*) distribute heat or cooling for temperature control; (*2*) keep the solid catalyst suspended; (*3*) solubilize and maintain the solution of hydrogen in the oil.

Whereas (*1*) and (*2*) are straightforward and quite easily achieved, (*3*) is complex. In conventional batch-operated tank-type hydrogenation reactors (Fig. 1), hydrogen is bubbled into the liquid through a spider-type gas distributor at the bottom of the converter. While the hydrogen to be solubilized comes principally from bubbles absorbed during their passage up through the oil from the spider, it partially comes from gas in the headspace that is continually being stirred back into the oil. Mixing in such a converter as shown in Figure 1 is provided by two or more turbine blades attached to a central shaft. Individual

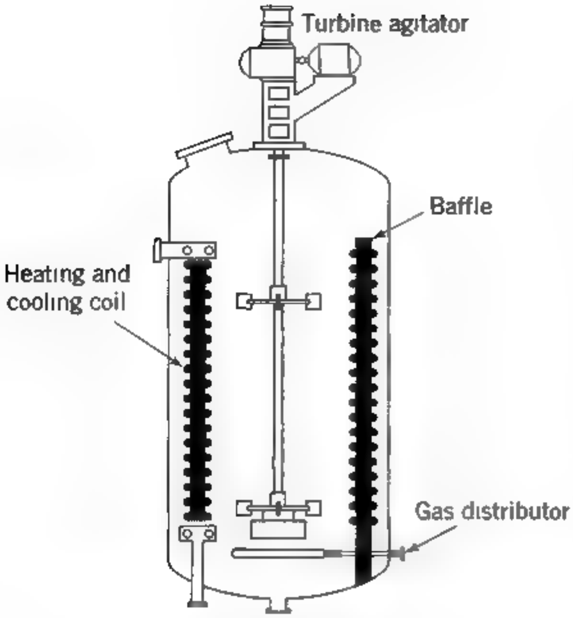


Figure 1. Conventional hydrogenation reactor. *Source:* Ref. 1.

blades may be flat/perpendicular, flat/canted, or foil shaped, depending on whether their purpose is to break the hydrogen into small bubbles, retard the flow of the bubbles upward, or create a vortex to reincorporate headspace hydrogen into the oil. Positioning of the blades on the shaft is very important. The rotation speed of the agitators, design and location of baffles on the side of the tank, and placement of heating/cooling coils also affect the flow of hydrogen bubbles passing through the oil and, consequently, the absorption of hydrogen into the oil. Mixers specially designed to better reincorporate hydrogen from the headspace into the body of the oil are gaining acceptance. They provide a more constant supply of hydrogen at the catalyst site, thus increasing the rate of reaction and also making it less variable. This is a significant aid in achieving uniform selectivity, which results in greater product uniformity. Three agitation systems designed to achieve this are depicted in Figures 2, 3, and 4.

The activity and selectivity effects of varying hydrogenation process conditions are summarized in Table 1.

CATALYSIS

According to the classic definition of Ostwald, a catalyst is a substance that alters the rate of a chemical reaction without affecting the energy factors of the reaction or being consumed in the reaction. Thus, the catalyst enters into the reaction over and over again, and a relatively small amount may be capable of transforming very large amounts of feedstock into reacted product. In the hydrogenation of vegetable oils, the concentration of nickel employed as a catalyst does not exceed a few hundredths of a percent. Furthermore, one catalyst may differ from another in its relative effect on alternate reaction rates. The hydrogenation of fats and oils furnishes examples of such specificity of catalyst action. Thus, the addition of 1 mol of hydrogen to a linoleic acid chain in a glyceride molecule may yield either a normal oleic ester or isomeric forms of it. Some nickel catalysts are more inclined than others to produce the isomeric forms.

Table 1. Activity and Selectivity Effects of Varying Hydrogenation Process Conditions

Increase in	Rate	Lo SR[a]	Isomerization
Temperature	+ + + +	+ + + +	+ + + +
Pressure	+ + +	− − −	− − −
Agitation	+ + + +	− − − −	− − − −
Catalyst concentration	+ +	−	−

[a] Lo SR, linoleic selectivity ratio.

Heterogeneous catalysts are the most important in industry generally. In edible oil hydrogenation they are used exclusively. In heterogeneous catalysis it is assumed the reaction proceeds through the formation of unstable intermediate compounds or adsorption complexes, in which the catalyst is temporarily combined with one or more of the reactants. If such compounds do exist, it is probable that in most cases they are not definite chemical combinations but consist merely of strongly bound molecules of the reactant held to the catalyst surface by secondary valence forces, or by complexing. In any event, it is essential that they be unstable. That is, capable of being either decomposed or desorbed, to permit reaction to proceed according to the scheme shown in equation 3.

$$\begin{aligned}\text{catalyst} + \text{reactants} \quad &\xrightarrow{\text{adsorption}} \quad \text{catalyst-reactant complex} \\ \xrightarrow{\text{desorption}} \quad &\text{reaction products} + \text{regenerated catalyst} \qquad (3)\end{aligned}$$

Although homogeneous catalysts for fats and oils hydrogenation have been studied, there is no current commercial interest in pursuing them. This is principally because of legal/environmental reasons. For homogeneous catalysts to be legally permitted in edible oil hydrogenation, they would need to achieve generally regarded as safe (GRAS) status. This would require either proof of their complete removal after use, or long-term human feeding tests demonstrating their harmlessness. In either case, the cost is too high and the potential liability too great to be considered seriously by either oil processors or catalyst manufacturers. Since enzyme catalysts might have the possibility of more easily being recognized as GRAS, they could be a future possibility.

Commercial hydrogenation catalysts are made by first combining nickel with other elements, such as in nickel oxide, nickel hydroxide, nickel carbonate, nickel formate, or nickel-aluminum alloy, and then reducing the compound to regain a portion of the nickel (now in a catalytic state) in metallic form. Considerable evidence indicates that the hydrogenation of an ethylenic compound must be preceded by two-point adsorption of the carbon atoms on either side of the double bond. This requirement imposes certain dimensional limitations on the space lattice of any catalytically active metal. Actually, the metals that are at all effective in the hydrogenation of double bonds, such as

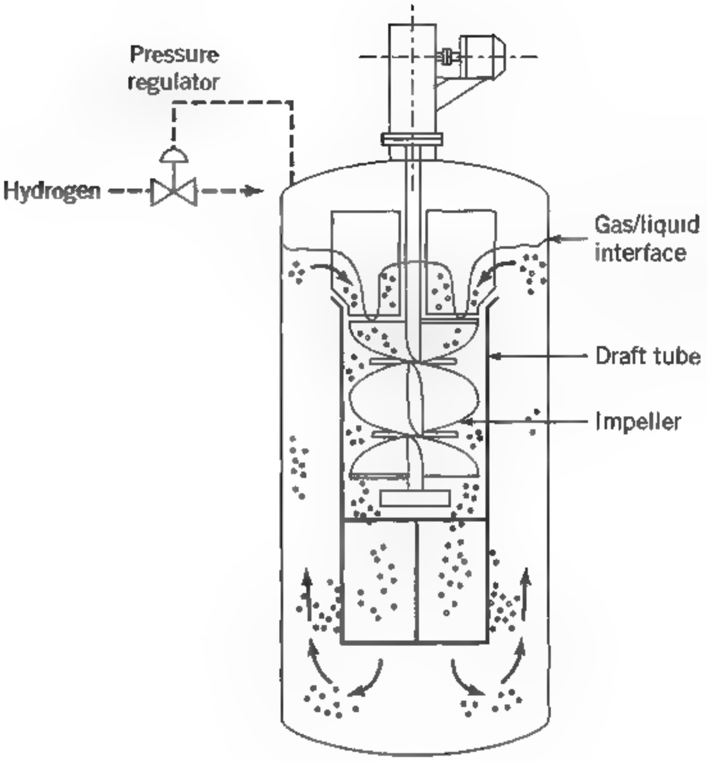

Pressure regulator
Hydrogen
Gas/liquid interface
Draft tube
Impeller

Figure 2. Helical screw mixer. *Source:* Ref. 1.

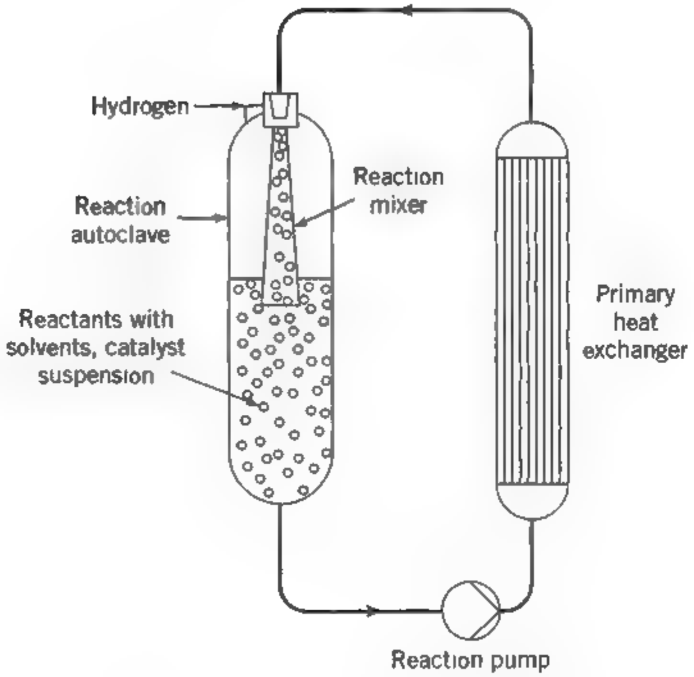

Hydrogen
Reaction mixer
Reaction autoclave
Reactants with solvents, catalyst suspension
Primary heat exchanger
Reaction pump

Figure 3. Loop Venturi reactor. *Source:* Ref. 1.

nickel, cobalt, iron, copper, platinum, and palladium, all have interatomic spacings close to that (2.73 Å) calculated as optimum for such two-point adsorption.

Catalysts are quite sensitive to heat and may be inactivated by temperatures considerably below the fusion point of massive nickel. This inactivation is apparently the result of a sintering process, which causes the active projecting nickel atoms to assume more stable positions on the catalyst surface. This sensitivity to heat makes their reduction to metallic form critical with respect to temperature since there is sometimes only a small interval between the temperature at which reduction becomes rapid and that at which sintering begins. Precipitated nickel catalysts are usually reduced at temperatures between about

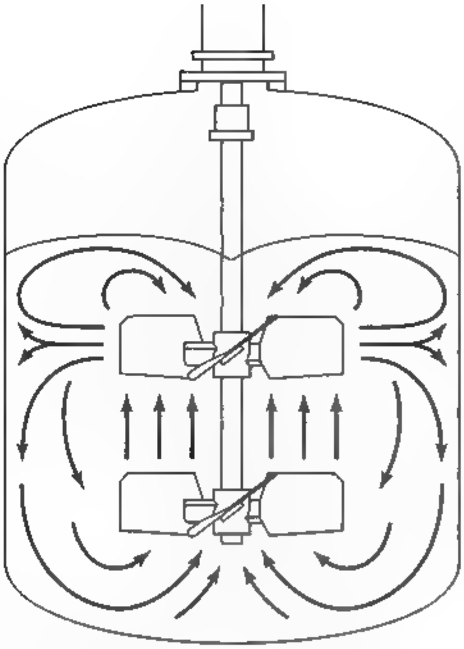


Figure 4. A340 Hydrofoil Mixer. *Source:* Courtesy of Lightnin.

800°F (425°C) and 900°F (480°C), well below nickel's sintering temperature, which begins at about 1000°F (540°C).

Freshly reduced catalysts are highly pyrophoric because of the reactive nature of their zero valence nickel atoms and their retention of hydrogen adsorbed during reduction. If exposed to air (oxygen) at this stage, they become inactivated as the atoms revert to their oxide form. Their pyrophoric properties and tendency to become inactivated through oxidation can be essentially eliminated by coating (protecting) the catalyst surface with an essentially inert material, usually a highly saturated fat. An alternate method of stabilization is by a slight back oxidation after reduction. Although this takes the "edge" off the catalyst's activity, it readily reactivates *in situ* under the reducing conditions of hydrogenation. It is a common and necessary practice to use reduced and stabilized catalysts for the hydrogenation of feedstocks to produce products in which even the small amount of protecting oil would be a serious contaminant. The conversion of fatty nitriles to amines is an example.

CATALYST POISONS

General

Since the activity of a catalyst depends on the presence of a relatively few metallic atoms of an unusually high degree of reactivity, it is to be expected that these atoms will display a marked avidity for many substances, in addition to hydrogen, if such substances are present as impurities in the reacting system. Substances that are thus able to cause catalysts to become inactive are generically termed catalyst *poisons*. They may be troublesome when present, even in traces, since the amount of catalyst is always small in relation to the amount of reactants, and the active portion of the catalyst is small in comparison with the total catalyst.

Impurities that have a negative effect on catalysts can be more specifically broken down into poisons, inhibitors, and deactivators, with each group acting according to a different mechanism. True poisons, such as sulfur and bromine, chemically react with the nickel in an irreversible manner. Inhibitors, such as phosphorous and alkali soaps, prevent hydrogenation from occurring by blocking the entrances to the catalyst pores. Deactivators, such as water and carbon dioxide, do not necessarily permanently affect the reaction since they can be removed under vacuum at elevated temperature.

Among the worst poisons for nickel catalysts are the gaseous sulfur compounds: hydrogen sulfide, carbon disulfide, sulfur dioxide, carbon oxysulfide, and so on. Historically, these compounds were of considerable concern because they may occur as impurities in hydrogen prepared by the steam–iron, water gas–catalytic, or hydrocarbon reforming processes. They are rapidly adsorbed by nickel catalysts and poison the catalyst irreversibly. The virtual disappearance of plants producing hydrogen by the steam–iron and water gas–catalytic processes, and the addition of purification steps to hydrocarbon reforming plants, have essentially eliminated gaseous sulfur compounds as being of significance in present-day edible oil hydrogenation.

Besides the sulfur compounds just mentioned, the gaseous catalyst poison that historically has been most likely to cause trouble in the hydrogenation of fats and oils is carbon monoxide. As with gaseous sulfur, the discontinuance of some methods of manufacture and the addition of purifiers, principally pressure swing adsorbers (PSAs), have also essentially eliminated carbon monoxide as a catalyst poison found in hydrogen.

Other gaseous impurities that can occur in hydrogen include carbon dioxide, nitrogen, and methane. Although these gases are not catalyst poisons per se, in a converter of the "dead-end" type they accumulate in the headspace as the reaction proceeds. By dilution, this reduces the hydrogen partial pressure and the reaction slows. Historically, this was a significant problem, particularly after the oil's iodine value had been lowered considerably. It was dealt with by venting, either occasionally or continuously. However, as with the other gaseous impurities, PSA systems have eliminated the problem. Small amounts of water vapor in the hydrogen (or the feedstock) have negligible poisoning effect on the catalyst but do cause formation of free fatty acids by hydrolysis of the triglyceride. These acids, in turn, react with the catalytic nickel to form noncatalytic nickel soaps. This phenomenon not only inactivates a portion of the catalyst but the resulting soaps slow posthydrogenation filtration.

Hydrogen produced by electrolysis contains a small amount of oxygen. Although oxygen is not a catalyst poison, its potential to cause oil oxidation makes it suspect as deleterious to product quality. It can also be readily reduced to a negligible level by a purification step.

Feedstock Poisons

All naturally occurring oils and fats contain constituents that can inhibit hydrogenation. They vary greatly, depending on the source—whether vegetable, meat, or marine. They also vary widely within those categories. For instance, in the case of vegetable oils, soybean and rapeseed contain considerable phosphorus, whereas palm has virtually none. Rapeseed oils contain sufficient sulfur to have a significant effect on hydrogenation; soybean has essentially none. Even a specific oil, such as rapeseed, can vary greatly in its content of sulfur, phosphorous, and chlorophyll. This variation may be caused by seed variety, weather, or handling. Whatever the cause or source of the hydrogenation-inhibiting substance, a principal function of the processing steps prior to hydrogenation is its reduction or elimination. This is successful to a greater or lesser degree depending on the crude material and on the effort expended.

Very significant progress has been made in oil purification. A principal impetus has been the realization by processors that the costs of purification are usually less than the downstream cost of larger catalyst usage, lower yield, and inferior product quality. The availability of improved processing controls and better analytical instruments have contributed substantially to this effort. For instance, instruments such as the inductively coupled plasma spectrophotometer have enabled processors to rapidly and accurately measure and control phosphorous and other metals.

CATALYSTS

Nickel Catalysts

Nickel alloy and wet-reduced nickel formate catalysts were historically used for edible oil hydrogenation, but they have been completely replaced by dry-reduced precipitated nickel catalysts. Catalysts of this type are prepared by precipitating a nickel salt, usually sulfate or chloride, on an inert support, such as silica or alumina or a combination thereof. The precipitate is filtered, washed, and dried. The resulting powder is reduced with hydrogen at 800 to 900°F (480–540°C). It is then coated (protected) with a triglyceride fat, chilled, and formed into a particle (pastule or flake) for packaging and shipment.

The performance characteristics (activity, selectivity, and filterability) of dry-reduced precipitated catalysts depend to a very great extent on the precipitation step. Such operating variables as the strengths of the solutions, the temperature at which precipitation takes place, the agitation of the solutions, their pH, the temperature to which the suspended precipitate is subjected, and so on, are all important and are rigidly controlled by the catalyst producer.

To a significant degree, the manufacture of dry-reduced precipitated nickel catalysts can be manipulated to produce products having various combinations of activity, selectivity, and filterability. This can be important and useful, depending on the feedstocks and the finished products to be manufactured.

Promoted Catalysts

A catalyst promoter is classically defined as a metal or other substance that enhances performance (usually activity) without actually being a catalyst for the reaction. The promotion of catalysts is much practiced in industries other than the hydrogenation of fats. In these cases, the employment of catalysts having two, three, four, or even more components is not unusual. In such complex systems the action of some of the components may more accurately be called synergistic rather than promotive, since more than one of the components may possess catalytic activity by itself.

A number of mechanisms have been proposed to explain the phenomenon of promotion. It has been supposed that the promoter acts as a secondary catalyst, accelerating the formation or decomposition of intermediate compounds, assisting in the adsorption of the reactants, or protecting the catalyst from poisons. With catalysts for the hydrogenation of fats, however, it appears more reasonable to assume that the function of a promoter would be simply structural, that is, to permit development of larger numbers of active centers on the catalyst surface.

Metals referred to in the patent literature as useful promoters for nickel catalysts used in the hydrogenation of edible oils, either as such or in the form of oxides, include chromium, cobalt, thorium, zirconium, copper, titanium, and silver. Practical use of some of them (eg, chromium) can be ruled out based on current environmental and/or nutritional laws and regulations. Most have not shown any cost-benefit performance advantage. Copper will be discussed separately, not as a promoter but as an alternate to nickel.

Nickel Sulfur Catalysts

The addition of sulfur to a nickel catalyst formulation has two effects. One is the diminishing of the nickel's hydrogenation activity and the second is an increase in *trans*-isomer formation. The increase in *trans*-isomer formation has spawned a widespread use of nickel sulfur catalysts to convert vegetable oils into products acceptable as cocoa butter substitutes useful in the manufacture of confectionery/coating fats.

Other Metal Catalysts

Although precious metals have not been used for commercial hydrogenation of fats and oils, they have been investigated quite extensively. They have several characteristics that make them attractive. These include satisfactory reactivity at about 60°C and an activity so powerful that only a few parts per million are required. However, because of the high price of the metal, to be cost-effective the metal in the spent catalyst would need to be at least 98% captured and reclaimed. Although that is feasible using current techniques, it would require considerable change in practice by both the hydrogenator and the catalyst manufacturer. Since neither has perceived any appreciable advantage in making such a change, it has not received serious attention. However, in the case of some unanticipated future development, such as the outlawing of nickel for edible oil hydrogenation, platinum or palladium would be viable substitutes.

Copper catalyst, because of its high linolenic selectivity (Ln SR), has been used to a limited extent for the hydrogenation of the linolenic ester in soybean oil. Soybean oil "brush" hydrogenated with copper catalyst, although much more linolenic preferential than nickel, still requires winterization to pass a cold test. The deleterious effect of even traces of residual copper on oxidative stability of the product is also a negative aspect. While copper is not currently used commercially, 1960s research on the possibility of doing so added considerably to understanding the selectivity of polyunsaturated ester hydrogenation.

HYDROGENATION IN PRACTICE

Batch Hydrogenation

Figure 5 is a flowsheet sketch depicting the unit operations of an industrial vegetable oil hydrogenation facility, commonly referred to as a hardening plant. The feedstock oil from upstream purification operations is brought to reaction temperature in a heat exchange with hydrogenated oil coming from the converter by way of the converter's drop tank. It is held in a hot oil measuring tank awaiting transfer to the converter. The cooled hydrogenated oil passes through a filter press where the catalyst is removed.

With state-of-the-art instrumentation and properly sized valves, pumps, and so on, the converter can easily be cycled 12 to 15 times per 24 h with even greater turnover if needed.

Hydrogen is supplied under pressure to the converter, either from on-site generation or as purchased from commercial suppliers. Catalyst is purchased from one of the three or four commercial manufacturers who supply it worldwide. It is added to the converter either as a liquid by pumping or by dry aspiration utilizing the converter's vacuum system.

A single-stage steam ejector system produces sufficient vacuum for the converter operations of evacuating air from the headspace, assisting in maintaining low-moisture feedstock, and aspirating catalyst. The converter is serviced by electricity for motors that drive the agitator and turn the centrifugal pumps that move the feedstock and hydrogenated product through the system. Although low-pressure heating steam is ordinarily available, except for startup, it is not needed in energy-efficient plants. Rather, most of the heat generated by the hydrogenation reaction exotherm is exchanged with the incoming feedstock, as already discussed, to bring it to reaction temperature. The excess calories, in the form of steam generated from the vaporization of converter cooling water, are used as a heat source for other plant operations.

Meter measurement of hydrogen usage can be a good method of determining how far the reaction has proceeded. It requires knowledge of the iodine value of a known quantity of the specific oil being hydrogenated in a reactor of known volume. Assuming no venting, the system, though indirect, is quite accurate.

An alternate method of determining the degree of hydrogenation is by measuring the refractive index of the oil and converting it to iodine value using the known relationship between them. Instruments are available to do this on-line and *in situ*. They utilize probes positioned closely together within the converter. One measures the oil's refractive index and the other measures its temperature.

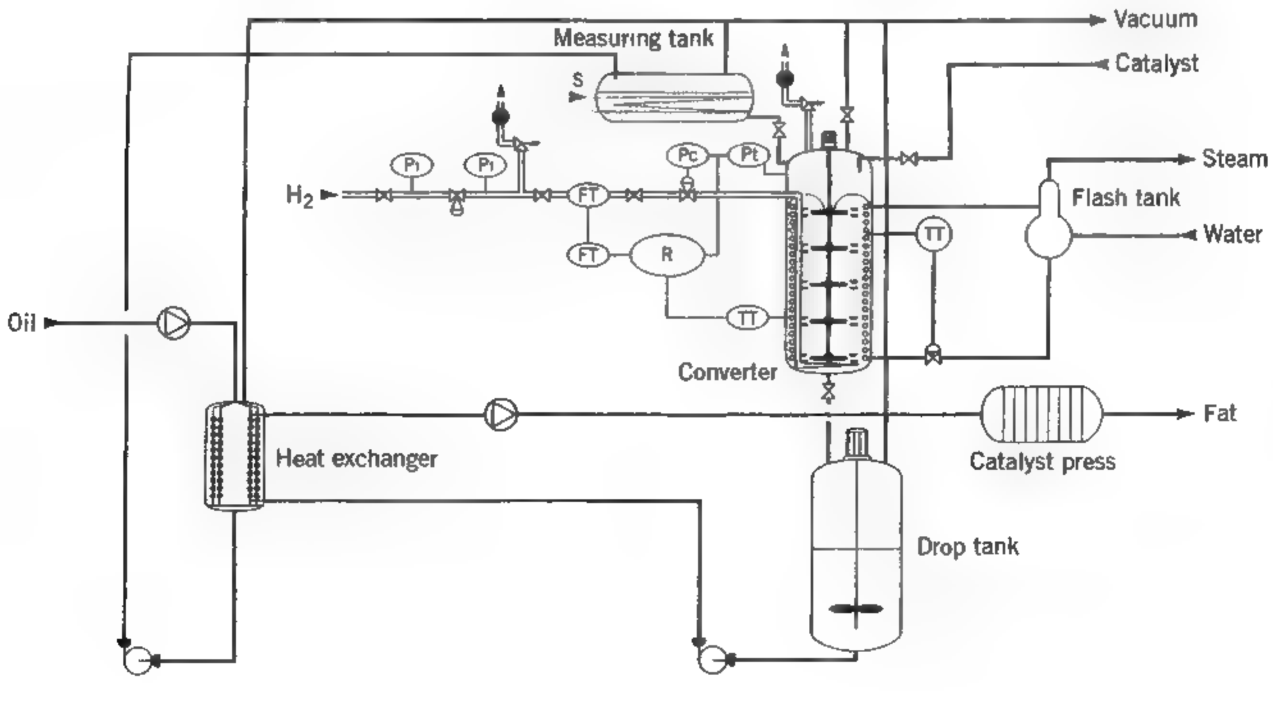


Figure 5. The hardening plant. *Source:* Ref. 1.

The probes are tied together in an external computer where software converts their measurements to iodine value at a specific reference temperature—usually 60°C.

Continuous Systems

Slurry. Employing a continuous system for hydrogenating edible oils has a number of operational advantages. A principal advantage is elimination of the numerous opportunities for equipment malfunction and human error that are inevitable in cycling operations. Since the temperature profile of the reaction is essentially constant in a continuous system, there is significantly more uniform selectivity of the hydrogenation reaction. In spite of these advantages, continuous slurry hydrogenation has achieved only very limited success in edible oil hydrogenation. The reason usually cited for its lack of acceptance is the contamination that inevitably occurs when switching from one product to another. Maintaining only a few base stocks can minimize the seriousness of this problem. Utilization of an on-line instrument to monitor refractive index (iodine value), as described previously, could also be helpful.

Fixed Bed. The fixed-bed mode may have a long-term future in continuous partial hydrogenation of triglycerides. With it, a relatively small amount of feedstock makes a single pass through a comparatively large quantity of formed catalyst. A filtration step is not needed to remove the catalyst. This simplifies the operation and enhances used catalyst disposition, since the spent will usually have a relatively high nickel content. The major disadvantage of fixed-bed systems is the gradual poisoning and/or inactivation of the catalyst caused by impurities in the feedstock accumulating on the catalyst. This affects the selectivity characteristics of the reaction and is reflected in a gradually changing melting profile of the hydrogenated product. Activity of the catalyst declines similarly. The catalyst is replaced when its activity and/or selectivity has declined to the point where either throughput or product quality becomes unacceptable. Some change in selectivity characteristics can be accommodated by manipulating the operational variables.

Impurity levels in feedstocks, and to some extent in hydrogen, have historically made the use of fixed-bed catalysts economically unattractive. More recently, perceiving the positive cost effect of lower catalyst usage, refiners have significantly reduced impurity levels in hydrogenation feedstocks. The availability of better and faster analytical techniques has been of help in this regard. Likewise, hydrogen purity has improved to the extent where it is no longer a factor in catalyst efficiency.

Based on significant lessening of the historical reasons for limited catalyst life in a fixed-bed mode, as just outlined, there may be a resurgence of interest in this approach. If nutritional advantages for the use of precious metal catalysts are verified, it would be a significant impetus for their use becoming attractive. If so, it would most likely be in a fixed-bed mode because of the much greater feasibility of precious metal reclamation from formed catalysts.

Catalyst Usage

The only correct manner for measuring catalyst usage efficiency is to relate it to quantity of catalyst consumed per quantity of feedstock hydrogenated. For accounting purposes it is translated into monetary units, for example, dollars per ton.

Although the price of catalyst has increased substantially over time, its usage efficiency cost has remained relatively constant. There have been two principal reasons for this: (*1*) a continual improvement in commercial catalyst performance, both in activity and uniformity, and (*2*) a continual improvement in feedstock purity. Purity improvements have resulted from better processing practices, from the farmers' field through oil separation, refining, and bleaching. Better and faster analytical techniques have enabled it to happen.

Some processors prefer to use catalyst once and then discard it. The principal reason they cite for this practice is catalytic performance predictability, both in activity and selectivity. Others have extensive reuse procedures. Generally, these break down into one of three alternate practices. The first is simple reuse; that is, employing the same catalyst over and over until its selectivity characteristics preclude making product specifications, or its activity extends reaction time to an unacceptable point. The second practice is to program catalyst reuse according to the type of hardening needed. This approach endeavors to take advantage of the phenomenon of preferential and *trans*-isomeric selectivities changing through use. These changes reflect the effect of feedstock poisons accumulating on the catalyst. Such a usage sequence might be a progression from brush hydrogenation for salad oil, through shortening base stock, through margarine base stock and finally, to complete hydrogenation to low iodine value stearine. A third reuse option of continually removing a portion of the catalyst and replacing it with an equal portion of fresh also has its advocates. While this option is uncomplicated, its downside is the acceptance of performance characteristics less than attainable with the other two approaches.

As referred to earlier, catalyst is often added to the converter by dissolving it in feedstock in a small side tank and then pumping into the reaction vessel. When adding new (dry) catalyst, a better way is to aspirate a weighed amount into the headspace of the converter, by utilizing the reactor's vacuum system.

Employing reused catalyst requires quite elaborate tank and measuring systems plus extensive record keeping and close monitoring. Over the years there has been a trend toward single use, particularly in the large new refineries where operations are computer controlled. A modified approach is to employ single use for all partial hardening and simple reuse for complete hydrogenation, where selectivity is not a consideration.

Filtration and Spent Catalyst Disposition

Utilizing plate and frame filter presses for removing catalyst from hydrogenated oil was standard for many years, and they are still sometimes used. They do an excellent job. Their shortcomings are high worker cost of operation and employee aversion to the hot and disagreeable job of cleaning them.

Metal screen pressure leaf filters have become standard. They are usually of the vertical plate type for ease of cleaning and used catalyst retrieval. Cleaning is by vibration. Filter aid is employed to precoat the screens to achieve rapid filtration with essentially complete removal of the catalyst. Through proper choice of filter aid and its minimal use, a level approaching 15% nickel can be achieved in the filter cake. This facilitates catalyst reuse, if desired. It also minimizes product loss from wetting of the filter aid. In addition, and most important, the high nickel content obtains the highest price for spent catalyst disposition. Because of potential liability, based on environmental concerns, less and less catalyst is being disposed of by landfill.

GENERAL REFERENCES

J. R. C. Hastert, "Hydrogenation," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., vol. 4, John Wiley & Sons, New York, 1996, pp. 213–300.

R. R. Allen, "Hydrogenation," in Daniel Swern, ed., *Bailey's Industrial Oil and Fat Products*, 4th ed., Vol. 2, John Wiley & Sons, New York, 1982, pp. 1–95.

D. R. Erickson and M. D. Erickson, "Hydrogenation and Base Stock Formulation Procedures," in D. R. Erickson, ed., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Ill. 1995, pp. 218–238.

H. B. W. Patterson, *Hydrogenation of Fats and Oils: Theory and Practice*, AOCS Press, Champaign, Ill., 1994.

ROBERT C. HASTERT
Hastech Corporation
Omaha, Nebraska

HYDROPHOBICITY IN FOOD PROTEIN SYSTEMS

BACKGROUND

Definition

The term "lyophobic" is used to describe a solute that has little or no affinity for the solvent medium in which it is placed. When the medium is composed of water or an aqueous solution, the more specific term "hydrophobic" is used as the descriptor. Similarly, the terms "lyophilic" and "hydrophilic" are used to describe affinity of a solute for solvent or water. In the case of proteins, the widely varying hydrophobicities of amino acid side chains has led to the use of a more neutral term, "hydropathy," to describe their relative preferences for aqueous and nonpolar environments (1).

The association of nonpolar solutes or moieties of a macromolecule is referred to as the hydrophobic effect, or hydrophobic interactions, rather than hydrophobic bonding. This association of nonpolar groups arises from enthalpically favorable interactions (mainly London forces between polarizable groups) in conjunction with the entropically driven tendency to minimize contact of the nonpolar groups with water. Furthermore, it is difficult to assign an absolute value of hydrophobicity to a solute or functional group. Commonly, hydrophobicity is described by an empirically measured or calculated parameter, which is related to the strength of hydrophobic interaction compared with a standard or with respect to an arbitrary scale.

Importance of Hydrophobic Interactions in Food Protein Systems

Almost all biological systems are composed of molecules that involve at least some nonpolar groups. Because water is a common constituent in these systems, it has been suggested that hydrophobic interactions must be ubiquitous in nature, playing a key role in both equilibrium and kinetic phenomena. Originally, interest in the hydrophobic effect was focused on its contribution to the stabilization of the structure of native protein molecules and on the mechanisms of protein folding. Although it was originally presumed that nonpolar or hydrophobic groups should be restricted to the interior of folded protein molecules, where they would be buried (ie, not exposed to the solvent water molecules), crystallographic analysis of the three-dimensional structures of many proteins has indicated that many hydrophobic groups are at least partly exposed on the surface of the protein molecules. The resulting hydrophobic patches play a key role in intermolecular interactions, such as the binding of small ligands or the association with other macromolecules, including protein–protein or protein–lipid interactions in biological membranes and micellar systems. A model has been proposed for elucidating quantitative structure–activity relationships by the correlation of biological properties with three structural parameters, namely, hydrophobic or lipophilic, electronic, and steric parameters (2).

In food systems, the role of the hydrophobic effect in stabilizing native protein molecules and structural networks is exemplified by the existence of stable casein micelles in milk. However, processing of foods leads to a variety of changes in both intramolecular and intermolecular interactions. The transformation of milk into cheese by enzymatic treatment and heating is one example of the dramatic effect of processing. Hydrophobic, electronic, and steric effects must all be taken into consideration to explain not only the biological properties of the native protein molecules in foods, such as enzymatic activity, but also the functional properties that give the food its characteristic texture and form. These properties include solubility, viscosity, gellability or coagulability, emulsifying, and foaming properties. For example, food products such as meringue and angel food cake rely on the unique properties of egg white proteins, including their excellent foaming properties and ability to form thermostable foams, as well as their capability to yield strong yet elastic gels on heating. The understanding of the basic molecular mechanisms responsible for these properties is essential to control the various aspects of food processing and ingredient selection.

THEORY AND MEASUREMENT OF PROTEIN HYDROPHOBICITY

Theory

The existence of hydrophobic interactions between solutes in an aqueous medium may be explained by two hypotheses. One approach is to explain the hydrophobic effect as simply arising from the attractive forces between nonpolar moieties and the phobia experienced by those groups when placed in a medium of water molecules (3). The other approach emphasizes the effect of nonpolar solutes on the structure of water molecules (4).

It is generally well recognized that water molecules exhibit strong attractive interactions with each other through hydrogen bonding to produce a continuous yet dynamic network. The entropy-driven hypothesis for hydrophobic interactions proposes that when nonpolar solutes are introduced into water, the hydrophobic effect results from disruption of the dynamic water structure, rather than an actual repulsion or phobia of the nonpolar groups by the water molecules. The ordering of water molecules around the nonpolar solutes is reflected in a large negative partial molar entropy change, that is, a decrease in entropy or randomness. To minimize this unfavorable change, nonpolar solutes or moieties associate to reduce their total surface area of contact with the water molecules. However, this hypothesis emphasizes only the disruption of water structure and does not explain the observation that the enthalpy and free energy of transfer of solutes such as phenol from an organic solvent to water differs considerably depending on the solvent, for example, octane versus toluene. Thus the contribution of favorable attractive forces between nonpolar groups to the net changes in enthalpy cannot be neglected.

In summary, hydrophobic interactions result from the tendency of nonpolar groups to interact with each other rather than with water. The hydrophobic effect is weakened by decreasing temperature, due to the loss of entropy resulting from increased ordering of water molecules into clathratelike structures around the nonpolar groups. Conversely at higher temperatures, the water-ordering effect is minimized and the hydrophobic interactions are maximized. The hydrophobic effect thus culminates from both the tendency to reduce the entropically unfavorable contact between nonpolar groups with water, as well as the need to form enthalpically favorable noncovalent associations, including those interactions broadly classified as van der Waals forces, which are the net effect of attractive London interactions, repulsive electron cloud overlap, and inducible dipole orientation and induction effects.

The attractive forces between nonpolar solutes can be calculated if the solutes are assumed to be approximately spherical bodies (5). Repulsion arising from the interaction of the diffuse double layers surrounding these molecules can also be explained by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (6). The overall attraction or repulsion potential resulting from the net effect of these forces can thus be assessed using these theories. The underlying assumptions, principles, and mathematics for this calculation have been clearly explained in standard textbooks and will not be elaborated here. However, it must be noted that in real food protein systems, much of the theory no longer holds true because ideal dilute solutions of spherical and uniform molecules are rarely encountered. Thus while the theory enables us to understand the phenomenon of attraction or repulsion between solute and solvent molecules, it is not useful for quantitative assessment of the strength of interaction of protein molecules in food.

Empirical Assessment of Hydrophobicity

Hydrophobicity Scales. Numerous scales have been proposed to assess the relative hydrophobicities of the amino acids that constitute proteins. Broadly speaking, these scales can be classified into two categories: (*1*) those that are based on the solubility behavior in solvents of different polarity, and (*2*) those that are calculated using crystallographic or other data showing the location of amino acid residues in the molecular structure, assuming that hydrophobic residues will locate in the interior of the molecule. The former scales include those based on the free energy of transfer ($\Delta G_{\text{transfer}}$) of the amino acid residues or their derivatives from water to an organic solvent or vapor, or the partition coefficients measured as a solubility ratio between water and a nonpolar immiscible organic solvent (1,7,8), as well as those based on relative retention times of amino acids and peptides during reverse-phase liquid chromatography (9). The latter scales include those based on the accessible or buried area of the residues (10,11), or on the location of amino acid residues in proteins assessed either in terms of the distance from the protein center of mass and average orientation of the side chain (12) or in terms of the average surroundings of residue types (13). A hydropathy scale has been reported based on both the average extent of buriedness and the $\Delta G_{\text{transfer}}$ from water to vapor (14). Calculation of hydrophobicity values based on the hydrophobic fragmental constant of constituent fragments has also been proposed (15). Table 1 shows some of the values of hydrophobicity for the 20 amino acids that have been reported in the literature; the different scales have been compared (16–18).

Using the hydrophobicity scales of the constituent amino acids, various approaches have been taken to calculate values for proteins. Based on Tanford's scale for $\Delta G_{\text{transfer}}$ of amino acid side chains from an organic to an aqueous environment, Bigelow's average hydrophobicity ($HØ_{\text{avg}}$) values are calculated using only information on the amino acid composition of the protein (19). In contrast, most other values require knowledge of the amino acid sequence or primary structure of the proteins as well. Computer programs are commonly used to calculate histograms or plots showing hydrophobicity profiles or hydropathy profiles (16,20) or amphipathic helix patterns (21).

A significant drawback to the use of hydrophobicity scales of amino acid residues to calculate the corresponding hydrophobicity values or profiles for proteins is the lack of consideration of the effect of the three-dimensional structure of each protein on the extent of exposure of the residues. The inadequacy of this approach is especially true for calculations using scales developed based on the

Table 1. Scales of Hydrophobicity for Amino Acid Residues in Proteins

	$\Delta G_{transfer}$[a] Kcal/mol								
	water → cyclohexane side-chain analogues	octanol → water calculated	Σf from RP-HPLC[b]	Molar fraction buried,[c] percent	Average surrounding hydrophobicity,[d] Kcal	Hydrophobicity index,[d] Kcal	Hydropathy index[e]	Rekker's fragmental constant[f]	Average area buried,[g] Å^2
Ala	−0.87	−0.39	−0.3	11.2	12.97	0.87	1.8	0.53	86.6
Arg	15.86	3.95	−1.1	0.5	11.72	0.85	−4.5	−0.82	162.2
Asn	7.58	1.91	−0.2	2.9	11.42	0.09	−3.5	−1.05	103.3
Asp	9.66	3.81	−1.4	2.9	10.85	0.66	−3.5	−0.02	97.8
Cys/2	−0.34	−0.25	—	—	—	—	2.5	0.93	—
Cys	—	—	6.3	4.1	14.63	1.52	—	1.11	132.3
Gln	6.48	1.30	−0.2	1.6	11.76	0.00	−3.5	−1.09	119.2
Glu	7.75	2.91	0.0	1.8	11.89	0.67	−3.5	−0.07	113.9
Gly	0	0	1.2	11.8	12.43	0.10	−0.4	0.00	62.9
His	5.60	0.64	−1.3	2.0	12.16	0.87	−3.2	−0.23	155.8
Ile	−3.98	−1.82	4.3	8.6	15.67	3.15	4.5	1.99	158.0
Leu	−3.98	−1.82	6.6	11.7	14.90	2.17	3.8	1.99	164.1
Lys	6.49	2.77	−3.6	0.5	11.36	1.64	−3.9	0.52	115.5
Met	−1.41	−0.96	2.5	1.9	14.39	1.67	1.9	1.08	172.9
Phe	−2.04	−2.27	7.5	5.1	14.00	2.87	2.8	2.24	194.1
Pro	—	−0.99	2.2	2.7	11.37	2.77	−1.6	1.01	92.9
Ser	4.34	1.24	−0.6	8.0	11.23	0.07	−0.8	−0.56	85.6
Thr	3.51	1.00	−2.2	4.9	11.69	0.07	−0.7	−0.26	106.5
Trp	−1.39	−2.13	7.9	2.2	13.93	3.77	−0.9	2.31	224.6
Tyr	1.08	−1.47	7.1	2.6	13.42	2.67	−1.3	1.70	177.7
Val	−3.10	−1.30	5.9	12.9	15.71	1.87	4.2	1.46	141.0

Source: [a] Ref. 1 (normalized to zero for glycine).

[b] Ref. 9.

[c] Ref. 10.

[d] Ref. 13.

[e] Ref. 14.

[f] Ref. 15.

[g] Ref. 16.

behavior of amino acids or small peptides. Scales that were formulated on the basis of location or buriedness of residues measured in different proteins attempt to address this problem but are still limited in universality of application, due to the need to extrapolate the behavior of residues to proteins other than those for which data are available. Some researchers (18) have proposed that different hydrophobicity coefficients should be computed for each of four structural classes of proteins ($\alpha\alpha$, $\beta\beta$, $\alpha + \beta$, and α/β). In the case of food systems, the problem is even more complex, due to the simultaneous occurrence of many different proteins. Determination of a net or average hydrophobicity value for complex systems requires not only the calculation of a hydrophobicity value for each protein, but also some knowledge of how these might be changed through possible interactions between the proteins. Furthermore, calculated values fail to take into account the effects of processing on buriedness or surface exposure of the residues. For these reasons, various methods of measuring parameters that may relate to the hydrophobicity of complex food protein systems are usually favored over calculation of average values or profiles based on the constituent amino acids. Examples of such methods are illustrated in the following sections.

Partition in Aqueous Two-Phase Systems. An indication of the relative hydrophobicity of proteins may be given by their partition coefficients measured as the solubility ratio between a polar and an immiscible nonpolar solvent. Due to the virtual insolubility of most proteins in organic solvents, two-phase aqueous systems containing dextran and poly(ethylene glycol), or PEG, are used (22,23). Esterification of PEG with a fatty acid such as palmitate is used to alter nonpolarity of the system. The difference in partition of proteins in phase systems with and without the fatty acyl hydrocarbon group bound to PEG is taken as a measure of hydrophobic interaction. Length of the hydrocarbon chain may be varied to investigate the effects on the partition behavior of different proteins. The method has been shown to yield useful data (22–24) but suffers from the tedious nature of the procedure and difficulties in solubilizing certain proteins.

Reverse-Phase and Hydrophobic Interaction Chromatography. The relative retention times of solutes during chromatography indicate their relative solubility in or affinity for a nonpolar stationary phase versus a mobile phase of differing polarity. According to the solvophobic effect theory (25), the retention time of peptides depends mainly on their nonpolar and polar surfaces, and thus may be a good measure of hydrophobicity. In fact, this hypothesis has proved true for small peptides (9). However, chromatographic behavior of proteins is not as straightforward. Possible denaturation of proteins under the harsh solvent conditions often required in reverse-phase chromatography has led to recommendations to use the milder conditions of hydrophobic-interaction chromatography, but the relevance of these retention data to hydrophobicity of native protein molecules has still come under question. The hydrophobicity of the stationary phase is an important parameter in maintaining native structure of the proteins upon elution (26). Nevertheless, differences in chromatographic retention between proteins have been demonstrated, and it has been suggested that the differences in binding to aliphatic versus aromatic types of adsorbent (27) may confirm the need to differentiate between these types of interactions in the hydrophobic effect (28–31).

Binding Methods. Various methods have been proposed for quantitating the binding of a nonpolar or hydrophobic ligand to proteins as a measure of the protein hydrophobicity. The ligands used have included aliphatic and aromatic hydrocarbons (32,33), sodium dodecylsulfate (34), Tween 80 (35), simple triglycerides (36), and corn oil (37). Commonly, the mixture of protein solution and ligand are incubated for a specified time to allow interaction, followed by removal of unbound or free ligand by dialysis, extraction, microfiltration, or other such techniques. The protein-bound ligand may then be quantitated by various methods, including gas chromatographic (32,33,36) or radioactive count (36) analysis, colorimetric reaction (34), or by a fluorescence probe method (37). Although the procedures are time-consuming, these binding methods can be useful in determining empirical parameters that may be relevant in assessing interaction of proteins with various nonpolar components of foods, such as flavor compounds, vitamins, pigments, fatty acids, or triglycerides.

Contact Angle Measurement. The quantitative determination of the Lifshitz–van der Waals (LW) and electron donor–acceptor or Lewis acid–base (AB) interactions that contribute to surface tension has been extended to the case of proteins. The method is based on Young's equation describing the relationship of the advancing contact angle of drops of a liquid on a flat solid surface (38). The solid surface in this case consists of protein sample prepared as flat layers. The advancing contact angles of droplets of three different well-characterized liquids (eg, water, glycerol, and α-bromonaphthalene) on the protein surface are measured. Based on Young's equation and the known surface tensions of the three liquids, the contact angles are then used to calculate the contributions of LW and AB interactions to the surface tension of the liquids on the layered protein. This approach has been used to obtain values for native hydrated proteins using only one liquid, namely drops of saline water. These values are correlated with the hydrophobicity of proteins measured as relative retention on hydrophobic chromatography (39,40). However, much higher surface tension values are obtained by contact angle measurement of a drop of protein solution on a solid polymer surface, compared with the values obtained by measuring the angle for the air-dried protein layer, or by other measurements for surface tension at an air–liquid interface such as platinum ring tensiometry, Wilhelmy plate, and pendant drop shape (39,40). This suggests that protein exposed at an interface may reorient to a much more hydrophobic configuration than found in its native hydrated state.

SPECTROSCOPIC METHODS: INTRINSIC FLUORESCENCE.

The intrinsic fluorescence spectra of proteins is primarily attributed to the aromatic amino acid residues of tryptophan, tyrosine, and phenylalanine. In practice, fluorescence from tryptophan is the most commonly studied aspect of the spectrum, because phenylalanine has a low quantum yield while tyrosine fluorescence is frequently weakened due to quenching by tryptophan residues and the protein backbone itself (41). The fluorescence of both tryptophan and tyrosine residues depends substantially on their environment, with the magnitude of fluorescence intensity as well as the wavelength of maximum fluorescence emission being sensitive to the polarity of the environment. Three spectral classes of protein tryptophan residues have been reported: the residues that are completely buried in nonpolar regions of the molecule, those that are completely exposed to the surrounding water, and those that have limited contact with water and are probably immobilized at the protein surface. The typical wavelengths of maximum emission for these three groups of tryptophan residues are 330 to 332 nm, 350 to 353 nm and 340 to 342 nm, respectively (42). More detailed information may be obtained by recording intrinsic emission spectra at different excitation wavelengths (43). Measurements of intrinsic fluorescence can give information on buriedness of aromatic residues and on the effect of interactions with other molecules (44). However, it is often difficult to directly relate this information to hydrophobic interactions as the fluorescence characteristics may also be altered by general changes in conformation.

Spectroscopic Methods: Derivative Spectroscopy. The ultraviolet absorption spectrum of proteins depends on the chromophoric properties of the constituent aromatic amino acids. Solvent perturbation and folded–unfolded difference spectra can be used to monitor the number of exposed tyrosine and tryptophan residues (41). Although the spectra of the individual chromophores are different, it is difficult to resolve quantitatively their contributions in the resulting broad absorption spectrum. Derivative spectroscopy, particularly of the second order ($d^2A/d\lambda^2$) and fourth order ($d^4A/d\lambda^4$), has the ability to resolve overlapping bands in the original spectrum (45–47). Moderately turbid samples can be analyzed by derivative spectroscopy since a horizontal baseline is obtained even with appreciable nonselective light scattering (45). In addition to quantitative determination of the aromatic amino acid contents, derivative spectroscopy has been proposed as a means to determine changes in polarity of the microenvironment around the chromophores. The maximum spectral shift observed in near ultraviolet second derivative spectra between solvent-exposed model compounds and side chains completely buried in an apolar protein core was found to be 5, 4, and 2 nm for tyrosine, tryptophan, and phenylalanine, respectively (46). However, for all three aromatic residues in proteins, there was no consistent correlation between absolute spectral band positions and average solvent accessibility, implying the influence of other local effects such as electrostatic interactions on the near ultraviolet spectra of proteins.

Spectroscopic Methods: Fluorescence Probes. Compounds whose quantum yields of fluorescence and wavelength of maximum emission depend on the polarity of their environment have been used to probe the hydrophobic or nonpolar nature of proteins. Figure 1 shows the chemical structures of several such fluorescence probes. The most popular types used include anionic probes of the aromatic sulfonic class, such as the amphiphilic 1-anilinonaphthalene-8-sulfonate (ANS), or its dimeric form, bis-ANS. These probes have high quantum yields of fluorescence in organic solvents but not in water; they thus fluoresce when bound to membranes or hydrophobic cavities in proteins (48,49). However, ANS fluorescence cannot always be directly correlated with hydrophobicity, as enhancement of fluorescence and a blue shift of the emission maximum have been observed in strong aqueous $MgCl_2$ solutions (50). It has been suggested that solvents or environments that are not necessarily nonpolar but that favor the rigid, planar configuration of the ANS molecule may influence fluorescence (51).

Another category of anionic fluorescence probes is the fatty acid analogue type, including *cis*-parinaric acid (CPA), which has been used as a probe for proteins and biological membranes (52–54). The parinaric acids are among the few nonaromatic fluorophores known. Their similarity to natural fatty acids, nonfluorescence in water, and good Stokes shift characteristics are among the advantages of their use as probes for hydrophobic regions that may be relevant to protein–lipid interactions in food systems. Good correlation was obtained between the relative hydrophobicity values of proteins determined by CPA fluorescence, and properties related to protein–lipid interactions such as interfacial tension and emulsifying activity (55).

Titration of protein solutions with increasing concentrations of the fluorescence probe can provide information on both the number and binding constants of the hydrophobic sites (56,57). Alternatively, proteins may be compared by calculation of an index of surface hydrophobicity S_o based on the initial slope of the measured relative fluorescence intensity of excess fluorescence probe as a function of increasing concentrations of protein (55). Transfer efficiency of the excitation energy from aromatic chromophores to bound probe at an adjacent hydrophobic site has also been proposed as an index of surface hydrophobicity (55).

Limitations in using anionic probes such as ANS and CPA include the possibility that electrostatic as well as hydrophobic interactions may contribute to the binding of the probes. The use of neutral or uncharged probes such as diphenylhexatriene (37), 6-propionyl-2-(dimethylamino)naphthalene or PRODAN (58) and Nile Red (59) may circumvent this problem.

Other Spectroscopic Methods. Most of the spectroscopic methods outlined previously provide information on the contribution to hydrophobic interactions from aromatic amino acids. Only a few methods have been proposed that can assess the involvement of aliphatic amino acids in hydrophobic interactions, including the CPA probe assay or ligand binding involving aliphatic hydrocarbons. A number of other spectroscopic methods can be used to investigate protein structure and environment of constituent amino acid residues in general. Each of these methods has particular advantages and limitations. However, consid-

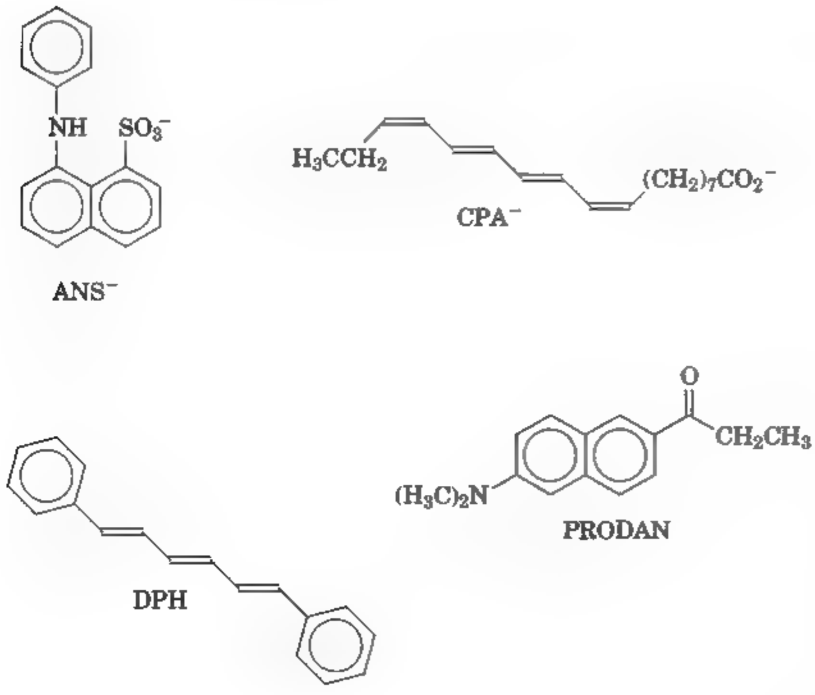


Figure 1. Chemical structures of some fluorescence probes that may be used for determination of protein hydrophobicity. ANS = 1-anilinonaphthalene-8-sulfonic acid; CPA = *cis*-parinaric acid; DPH = diphenylhexatriene; PRODAN 6-propionyl-2-(dimethylamino)naphthalene. The two anionic probes (ANS and CPA) are shown in the dissociated acid form. *Source:* Courtesy of Dr. C. A. Haskard.

ering the need to study proteins that are typically more than 20 kilodaltons in molecular weight and in a turbid solution or solid phase, the most promising techniques to study hydrophobicity in food protein systems are nuclear magnetic resonance (60), infrared (60), and Raman spectroscopy (60,61). The reader is referred to more specialized volumes on these techniques.

HYDROPHOBICITY OF FOOD PROTEINS

Table 2 is a compilation of the relative hydrophobicity values reported for some proteins of relevance in food systems. The hydrophobicity values were either calculated from the amino acid composition of the proteins or measured by some of the empirical methods outlined earlier. Unfortunately, there is still no consensus on a standard method for quantitative measurement of hydrophobicity. In comparing the values shown in Table 2, one should bear in mind that the methodologies differ in whether they are measuring average, total, or surface-exposed hydrophobicity. Furthermore, variability in published data may arise from differences in sources or purity of protein, as well as in the conditions during measurement, including the temperature, pH, ionic strength, or buffer composition.

Simple methods such as those based on fluorescence probes are the most widely used for the study of food protein systems, since these methods are based on monitoring the probe-accessible groups in nonpolar environments, which may correlate to the hydrophobic sites that are actually available for participation in functionality. The fluorescence probe methods are relatively simple and rapid to perform. Furthermore, empirical methods can be applied to study heterogeneous protein systems and to monitor the effects of processing. Typical hydrophobicity values measured using an aromatic (ANS) and an aliphatic (CPA) probe are listed in Table 3 for some food proteins, including complex systems composed of several molecular species.

IMPORTANCE OF HYDROPHOBICITY IN FUNCTIONALITY OF FOOD PROTEINS

Functional properties of food proteins have been defined as those characteristics, other than nutritional ones, that affect their utilization. This includes a diverse range of functionality, such as solubility, viscosity, water- and fat-binding properties, emulsification, foaming, film formation, gellability or coagulability, elasticity, and flavor. Functionality depends on the interaction of the protein molecules with other components in the food system, including water; macromolecules such as other proteins, complex carbohydrates, and lipids; and small molecules such as salts, simple sugars, and flavor compounds. These interactions may take place in the bulk phase, or at a surface or interface. The interplay of electrostatic, hydrophobic, and steric parameters, important in predicting biological activity of molecules (2), also holds true for the elucidation of the relationship between structure and functionality of food proteins (17,62). Computer-assisted studies for quantitative analysis of the structure–activity relationship (QSAR) of small molecules are widely applied (63), but progress in QSAR for functionality of food protein analyses has been much slower (62). Although the quali-

Table 2. Relative Hydrophobicity Values of Some Proteins Measured by Different Methods

		Fluorescence probes			DPH probe	Chromatography		Binding			Partition
Proteins	$H\O_{\text{one}}$[a]	S_oANS	S_oCPA	S_eCPA	TG binding	Rpc	Hic	Heptane	SDS	TG	ΔlogK
Albumin, bovine	1120	1000	100	100	100	100	100	100	100	100	100
Albumin, chicken	1110	7	2	96	15	0	9–86	2	14	3	1.25
Casein, α-	1200	57[b]	6–30	—	150	400	—	—	—	160	—
Casein, β-	1320	60[b]	50	107	—	315	—	—	—	—	—
Casein, κ-	1210	83	13–93	89	—	340	—	—	30	—	—
Chymotrypsin, α-	1030	0	3	—	41	—	57–139	9	—	3	9–33
Globulin, soy 7S	1090	47	9–77	—	50	165	—	—	21	—	—
Globulin, soy 11S	950	27	2–17	—	25	150	—	—	—	—	—
Lactalbumin, α-	1150	33	9–54	—	98	225	10	—	—	—	—
Lactoglobulin, β-	1230	13	54–146	80	49	0	67–102	100	62	130	41–78
Lysozyme	970	0.7	7	—	—	—	42–113	0	19	1	0
Ovomucoid, chicken	920	—	0	22	—	—	—	24	18	108	—
Pepsin	1063	0.7	2	57	32	—	92	—	—	95	—
Ribonuclease A	870	—	1	24	—	—	4–71	17	18	2	—
Transferrin, chicken	1080	—	4	108	—	—	16–31	29	20	118	9–20
Trypsin	940	8	3	34	—	—	—	—	13	—	9–27
Trypsin inhibitor	1040	0	0	—	1	0	—	—	—	—	—

Note. Data were compiled from Refs. 17, 18, 65, references cited therein, and unpublished data. With the exception of $H\O_{\text{one}}$ and S_o ANS, the data are expressed relative to 100 for bovine albumin to facilitate comparison of values. Where varying values were reported by investigators using essentially the same method, a range of values is presented.

[a] Abbreviations. $H\o_{\text{one}}$ = average hydrophobicity calculated by Bigelow's method (19), S_o = initial slope of relative fluorescence intensity versus protein concentration plot, using native protein; S_e = S_o measured for protein solutions after treatment with 1.5% SDS at 100°C for 10 min, ANS = 1-amino-8-naphthalene sulfonic acid, CPA = *cis*-parinaric acid, DPH = 1,6-diphenyl-1,3,5-hexatriene; TG = triglyceride; Rpc = reverse-phase chromatography, Hic = hydrophobic-interaction chromatography; SDS = sodium dodecyl sulfate.

[b] At the isoelectric pH, 530 and 800 for α- and β-casein, respectively.

Table 3. Hydrophobicity Values of Some Food Proteins Measured by Fluorescence Probe Method

Protein[a]	S_0ANS	S_0CPA
Albumin, bovine serum	1600	2750
Canola isolate	110	370
Casein, bovine	150	400
Egg albumen	40	135
Gelatin	0	0
Lactalbumen	145	1700
Lactalbumin, α-	90	280
Lactoglobulin, β-	40	7000
Lysozyme, hen	1	15
Muscle, chicken breast	75	350
Myosin, chicken breast	100	—
Ovalbumin, hen	10	40
Pea protein isolate	280	825
Soy protein isolate	250	1000
Sunflower protein isolate	155	910
Whey protein concentrate	70	3600
Zein	410	390

Note: See Table 2 for abbreviations.

[a] Measurements for S_0 determination were carried out for protein solutions in 0.01 M sodium phosphate buffer, pH 7.0–7.4; 0.6 M NaCl and 0.3 M NaCl were included in the buffer for salt extracts of chicken breast muscle and for isolated chicken myosin, respectively.

tative importance of the physicochemical properties in functionality is well recognized, quantitative prediction based on QSAR analysis of food proteins is still not a facile task, being limited by the need to measure relevant parameters, including hydrophobic interactions, which can take into account the effects of processing for incorporation into structure–functionality models. The following highlight the role of hydrophobic interactions in explaining some of the important functional properties of food proteins.

Solubility

Solubility has long been considered a critical property because of its effect on many other functional properties, such as emulsifying, foaming, and gelling properties. Charge frequency and hydrophobicity have been proposed to be the two major factors affecting protein solubility (17). Generally speaking, proteins are soluble in aqueous media when electrostatic repulsive forces between protein molecules are greater than the driving forces for hydrophobic interactions. Insolubility is more likely when the repulsive forces are at a minimum, near the isoelectric pH of the protein. However, proteins with relatively few exposed hydrophobic regions on the molecular surface may remain soluble even at pH near their isoelectric point. For example, the isoelectric precipitation of casein proteins during acidification of milk is attributed to their high surface hydrophobicity. On the other hand, the globular proteins in the whey fraction remain soluble. Denaturation of whey proteins, such as may occur by heat treatment, leads to exposure of previously buried hydrophobic groups and consequently to insolubilization upon acidification.

Aromatic amino acids have been suggested to play a greater role than aliphatic amino acids in contributing to the hydrophobic interactions affecting insolubilization (28). At pH values where charge effects are minimized (zero zeta potential), the insolubility of some food proteins has been correlated to hydrophobicity parameters measured using the aromatic fluorescence probe ANS or by reverse phase chromatographic behavior on phenyl Sepharose (PSC), but not to hydrophobicity measured using the aliphatic probe CPA. At other pH values, charge frequency measured as zeta potential and hydrophobicity measured by ANS or PSC are both significant parameters to explain the insolubility of proteins. Although calculations of hydrophobicity scale values based on free energy of transfer from organic solvent to water indicate that aromatic amino acids are more hydrophobic than aliphatic amino acids, statistical scales based on frequency of location of amino acid residues at the surface versus the interior of protein molecules indicate that aromatic amino acids are often considered less hydrophobic than aliphatic ones. While inherently more hydrophobic, the bulky nature of the aromatic residues may discourage their effective burial in the protein interior (29). These surface-exposed residues determine the strength of hydrophobic interactions that affect properties such as solubility.

The effects of various compounds on protein solubility can be explained on the basis of their opposite effects on electrostatic and hydrophobic interactions of the proteins. Salts may be ranked in terms of a lyotropic series describing their water structure-making or -breaking effects based on molal surface tension values (25). At high ionic strength, ammonium sulfate and sodium chloride increase the surface tension of water, whereas tetraethylammonium chloride and guanidinium chloride reduce it. The salting-in or salting-out of proteins by different salts can thus be explained theoretically by the lyotropic series. The salting-out constant of a protein is a function of its surface hydrophobicity, calculated as the hydrophobic contribution of each amino acid (7) and the fraction of exposed hydrophobic residues (10). Similarly, the stabilizing effects of sugars and polyols such as glycerol may be related to their water structure-enhancing effects, which intensifies the intramolecular hydrophobic interactions that stabilize protein structure. The effect of urea in solubilizing proteins has been explained by solvation of the hydrophobic moieties in urea, as well as by water structure-breaking effects. Precipitation by trichloroacetic or sulfosalicylic acid has been postulated to arise from an increase of accessible hydrophobicity of peptide moieties (64).

Emulsifying and Foaming Properties

Emulsion and foam-related properties depend on interactions of protein molecules at the oil–water interface and air–water surface, respectively. Although protein solubility is a key determinant in these functionalities, additional important considerations are molecular flexibility and hydrophobic interactions of the protein at the surface or interface, which result in formation and stabilization of the emulsion or foam. It has been reported that the decrease in interfacial tension and improvement in emulsifying activity are not related to hydrophobicity values calculated from the total content of hydrophobic amino acids, but rather to the effective or surface hydrophobicity of proteins measured by hydrophobic partition, hydrophobic interaction chromatography, or fluorescence probes (24,55). The extent of unfolding to expose areas for hydrophobic interactions is expected to be greater for foaming than emulsifying properties, which may be related to the higher tension at an air–water surface than an oil–water interface. The values of exposed hydrophobicity, measured by fluorescence probe assay after denaturation of protein molecules by heating in the presence of sodium dodecyl sulfate, were found to be correlated with foaming capacity (65). An example of the industrial relevance of hydrophobic interactions in surface properties is demonstrated in the positive correlation between the content of hydrophobic protein fractions separated from beer by hydrophobic interaction chromatography and beer foam stability (66).

In analogy to the hydrophile–lipophile balance (HLB) concept originally developed for nonprotein, synthetic emulsifiers, the amphiphilic nature of proteins is vital to their function as surface active agents. A balance in accessible hydrophobic and hydrophilic areas is required for optimal functionality. Solubility of the protein molecules facilitates diffusion to the surface or interface. However, once there, the ability of the protein to interact with oil or other protein molecules to form an interfacial layer depends on the flexibility and accessibility of surface groups, especially through hydrophobic interactions. The importance of hydrophobic interactions is illustrated in the observation that functionality can often be improved by mild denaturing treatments that increase surface hydrophobicity without impairing solubility (55,67,68). When solubility is a constant parameter, emulsifying properties are improved by increasing surface hydrophobicity, up to some critical point. However, as predicted by the HLB concept, excessive hydrophobicity is detrimental to functionality, and emulsifying properties are poor when proteins have excessively high values of hydrophobicity (17,62).

Thermal Functional Properties

The importance of intramolecular hydrophobic interactions in the stability of proteins to thermally induced denaturation has been assumed, due to the increasing strength of these interactions with increasing temperature up to 60 to 70°C. However, extensive comparison of hydrophobic indices in thermophilic versus mesophilic proteins has not demonstrated any definitive relationship between hydrophobicity and thermal stability. Stabilization is promoted by greater internal or intramolecular hydrophobic interactions and lower external or surface hydrophobicity. Because aliphatic residues have a greater tendency to be located in the protein interior than bulky aromatic side chains, it has been suggested that an aliphatic index may be more relevant to protein stability than a general hydrophobicity index (29).

Thermally induced gelation or coagulation are important in affecting textural characteristics of foods. Coagulation-type (concentration-dependent) and gelation-type (concentration-independent) proteins have been differentiated depending on molecular weight and relative content of hydrophobic amino acid residues. One hypothesis proposes that proteins having over 31.5 mol % of hydrophobic (Val, Pro, Leu, Ile, Phe, and Trp) residues would be of the coagulation type, whereas those with less mol % of those residues would form translucent-type gels (69). However, such generalizations assume that denaturing treatments prior to gelation result in total exposure of the hydrophobic residues. The balance of electrostatic repulsive forces and hydrophobic attractive forces on the molecular surface determine the formation of particulate, fine-stranded, or mixed structure gels, with distinctive textural characteristics and appearance (70,71). A striking example of the involvement of hydrophobic interactions is the setting phenomenon observed in particular fish species, wherein an elastic gel forms at relatively mild heating temperatures. Introduction of aromatic or aliphatic hydrophobic groups through chemical modification can induce nonsetting species to behave like easily setting fish species, exhibiting characteristics of increased viscosity and gelling on low temperature heating (72,73).

Hydrophobic interactions may be involved in the initial intramolecular stage of unfolding as well as the later stage of network or aggregate formation through intermolecular interactions. Both surface and exposed hydrophobicity may be important in the nature of the resulting product. It has been suggested that a low value of surface hydrophobicity of the native protein, coupled with a high value of exposed hydrophobicity after thermal denaturation, may lead to the formation of strong gels (74). The ability to unfold, or molecular flexibility, may be hindered by intramolecular stabilization through noncovalent interactions as well as covalent bonds, particularly of the disulfide type. The involvement of intermolecular sulfhydryl–disulfide reactions usually appears in later stages of these thermally induced phenomena and may be concentration dependent. At temperatures below 70°C, sulfhydryl reactivity is not typically involved and hydrophobic interactions may be the major driving force in network formation, with hydrogen bond formation during the cooling phase contributing to strengthening of the final gel structure (17).

Flavor

Although most proteins do not have a strong intrinsic flavor, they do influence flavor due to their ability to bind flavor compounds. This property can play an important role in the transmission of undesirable off-flavors, for example, in some foods containing soy proteins; conversely, binding of some flavor components may lead to a reduction in the perceived desirable flavor (75). The nonpolar nature of many flavor components such as aldehydes and ketones promotes hydrophobic interactions that may be critical in their binding to proteins. The binding affinity of aliphatic ketones with β-lactoglobulin increases proportionally with chain length of the ketone, suggesting that the association is primarily hydrophobic in nature (75). Influences of processing such as heat denaturation may be expected by consideration of their impact on the hydrophobicity of the protein and consequently, the flavor binding sites. Oddly, very little interest appears to have been devoted to the study of flavor retention and release, compared with the bulk of work on molecular interactions (76). Systematic study in this area should allow optimal design of flavors for new formulated foods, elimination of transmitted off-flavors and development of efficient flavor carrier systems (75).

Hydrolysis of proteins to form peptides also has a great influence on flavor. Formation of bitter peptides from casein and soy proteins, for example, is detrimental to the flavor acceptability of hydrolysates. Bitterness appears to be related to the average hydrophobicity (HQ) of the peptides, with those peptides having HQ values above 1400 cal/mol residue being bitter. However, the extent of exposure of hydrophobic residues and their incorporation into peptides in contrast to their existence as free amino acids also affects bitterness. Thus bitterness depends not only on the content of hydrophobic amino acids in the original protein, but also on the degree of hydrolysis and the location of the hydrophobic residue on the peptide sequence (77).

FUTURE TRENDS

Long-term, systematic research is required to establish quantitative, empirical rules to understand the role of hydrophobicity in functionality of food protein systems. This basic research is required to explain at the molecular basis, various well-known but not clearly understood phenomena such as the thermostability of egg white foams, elasticity of egg white gels, coagulation of casein to form cheese, binding and texture formation of muscle proteins, elasticity and extensibility of wheat gluten dough, and so on.

Although only one of the physicochemical parameters involved in elucidation of structure–function relationships, hydrophobic interactions often play a key role. Improvement of functionality including whipping and emulsifying properties by modifying the hydrophobic–hydrophilic balance of proteins is exemplified in the preparation of proteinaceous surfactants with different HLB values by attachment of hydrophobic amino acid alkyl esters to various food proteins (78). Development of novel functionality such as antifreeze or cryoprotectant properties has also been described using this approach (78). Another rapidly developing area is the enhancement of hydrophobic nature of enzymes through attachment of amphiphilic groups such as poly(ethylene glycol), coupled with selection of organic solvent media to alter the hydrophobic environment. This area has interesting applications such as the possible resolution of alcohols and acids by lipase-catalyzed esterification, protease-catalyzed synthesis of proteins, and lipase-catalyzed interesterification of fats and oils (79). The potential of "ultrahydrophobic sequences" and beta-sheet structures of corn, wheat, and other proteins in film-forming preparations and adhesives (80) may be of value in the development of edible films. The growing market for reduced- and low-fat foods, which often have high protein content, suggests that more research should be undertaken to study the role of hydrophobic interactions in the binding and release of flavor compounds by food proteins.

Site-specific modifications of proteins can now be realized through molecular biology and genetic engineering techniques. By establishing the relationship between food protein structure and function, systematic and predictable enzyme and protein engineering for tailoring of specific biological and functional properties should become a reality.

BIBLIOGRAPHY

1. T. E. Creighton, "Physical Interactions that Determine the Properties of Proteins," *Proteins. Structures and Molecular Properties*, 2nd ed., W. H. Freeman and Company, New York, 1993.
2. C. Hansch and A. J. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley & Sons, New York, 1979.
3. C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed., John Wiley & Sons, New York, 1980.
4. C. J. van Oss, "Hydrophilic and 'Hydrophobic' Interactions," in H. Visser, ed., *Protein Interactions*, VCH, New York, 1992, pp. 25–55.
5. H. C. Hamaker, "The London-van der Waals Attraction between Spherical Particles," *Physica* **4**, 1058–1072 (1937).
6. E. Dickinson, *An Introduction to Food Colloids*, Oxford University Press, Oxford, United Kingdom, 1992, pp. 94–97, 181–184.
7. Y. Nozaki and C. Tanford, "The Solubility of Amino Acids and Two Glycine Peptides in Aqueous Ethanol and Dioxane Solutions. Establishment of a Hydrophobicity Scale," *J. Biol. Chem.* **246**, 2211–2217 (1971).
8. L. M. Yunger and R. D. Cramer III, "Measurement and Correlation of Partition Coefficients of Polar Amino Acids," *Mol. Pharmacol.* **20**, 602–608 (1981).
9. K. J. Wilson et al., "The Behavior of Peptides on Reverse-Phase Supports During High Pressure Liquid Chromatography," *Biochem. J.* **199**, 31–41 (1981).
10. C. Chothia, "Principles That Determine the Structure of Proteins," *Annu. Rev. Biochem.* **54**, 537–572 (1984).
11. G. J. Lesser and G. D. Rose, "Hydrophobicity of Amino Acid Subgroups in Proteins," *Proteins: Struct., Funct., Genet.* **8**, 6–13 (1990).
12. H. Meirovitch, S. Rackovsky, and H. A. Scheraga, "Empirical Studies of Hydrophobicity. I. Effect of Protein Size on the Hydrophobic Behavior of Amino Acids," *Macromolecules* **13**, 1398–1405 (1980).
13. P. Manavalan and P. K. Ponnuswamy, "Hydrophobic Character of Amino Acid Residues in Globular Proteins," *Nature* **275**, 673–674 (1978).
14. J. Kyte and R. F. Doolittle, "A Simple Method for Displaying the Hydropathic Character of a Protein," *J. Mol. Biol.* **157**, 105–132 (1982).
15. R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier Science, New York, 1977.
16. G. D. Rose, L. M. Gierasch, and J. A. Smith, "Turns in Peptides and Proteins," *Adv. Protein Chem.* **37**, 1–109 (1985).
17. S. Nakai and E. Li-Chan, *Hydrophobic Interactions in Food Systems*, CRC Press, Boca Raton, Fla., 1988.
18. H. Cid et al., "Hydrophobicity and Structural Classes in Proteins," *Protein Eng* **5**, 373–375 (1992).
19. C. C. Bigelow, "On the Average Hydrophobicity of Proteins and the Relation Between It and Protein Structure," *J. Theor. Biol.* **16**, 187–211 (1967).
20. S. R. Krystek, Jr., and T. T. Anderson, "A Program for Hydropathy and Antigenicity Analysis of Protein Sequences," *Endocrinology* **117**, 1118–1124 (1985).

21. J. P. Segrest and R. J. Feldman, "Amphipathic Helices and Plasma Lipoproteins: A Computer Study," *Biopolymers* **16**, 2053–2065 (1977)
22. H. Walter and G. Johansson, eds., *Aqueous Two-Phase Systems*, Methods in Enzymology Vol. 228, Academic Press, New York, 1994.
23. P.-A. Albertsson, *Partition of Cell Particles and Macromolecules*, 3rd ed., Wiley Interscience, New York, 1986.
24. E. Keshavarz and S. Nakai, "The Relationship Between Hydrophobicity and Interfacial Tension of Proteins," *Biochim. Biophys. Acta* **576**, 269–279 (1979).
25. W Melander and C. Horvath, "Salt Effects on Hydrophobic Interactions in Precipitation and Chromatography of Proteins: An Interpretation of the Lyotropic Series," *Arch. Biochem. Biophys.* **183**, 200–215 (1977).
26. L. Szepesy and G. Rippel, "Comparison and Evaluation of HIC Columns of Different Hydrophobicity," *Chromatographia* **34**, 391–397 (1992).
27. B. H. Hofstee and N F. Otillio, "Modifying Factors in Hydrophobic Protein Binding by Substituted Agaroses," *J. Chromatogr.* **161**, 183–215 (1978).
28. S. Hayakawa and S. Nakai, "Relationships of Hydrophobicity and Net Charge to the Solubility of Milk and Soy Proteins," *J. Food Sci.* **50**, 486–491 (1985)
29. B. B. Mozhaev and K. Martinek, "Structure-Stability Relationships in Proteins: New Approaches to Stabilizing Enzymes," *Enzyme Microb. Technol.* **6**, 50–59 (1984).
30. S. K. Burley and G. A. Petsko, "Aromatic-Aromatic Interaction: A Mechanism of Protein Structure Stabilization," *Science* **229**, 23–28 (1985).
31. S. K. Burley and G. A. Putsko, "Weakly Polar Interactions in Proteins," *Adv. Protein Chem.* **39**, 125–189 (1988).
32. A. Mohammadzadeh-K., R. E Feeney, and L. M. Smith, "Hydrophobic Binding of Hydrocarbons by Proteins. I. Relationship of Hydrocarbon Structure," *Biochim. Biophys. Acta* **194**, 246–255 (1969).
33. A. Mohammadzadeh-K., R. E. Feeney, and L. M. Smith, "Hydrophobic Binding of Hydrocarbons by Proteins. II. Relationship of Protein Structure," *Biochim. Biophys. Acta* **194**, 256–264 (1969).
34. A. Kato et al., "Determination of Protein Hydrophobicity Using a Sodium Dodecyl Sulfate Binding Method," *J. Agric. Food Chem.* **32**, 284–288 (1984).
35. B. Lieske and G. A. Konrad, "A New Approach to Estimate Surface Hydrophobicity of Proteins," *Milchwissenschaft* **49**, 663–666 (1994).
36. L. M. Smith, P. Fantozzi, and R. K. Creveling, "Study of Triglyceride-Protein Interaction Using a Microemulsion-Filtration Method," *J. Am. Oil Chem. Soc.* **60**, 960–967 (1983).
37 T. Tsutsui, E. Li-Chan, and S. Nakai, "A Simple Fluorometric Method for Fat-Binding Capacity as an Index of Hydrophobicity of Proteins," *J. Food Sci.* **51**, 1268–1272 (1986).
38. C. J. van Oss, "Energetics of Cell-Cell and Cell-Biopolymer Interactions," *Cell Biophysics* **14**, 1–16 (1989).
39. C. J. van Oss et al., "Determination of the Surface Tension of Proteins. I. Surface Tension of Native Serum Proteins in Aqueous Media," *Biochim. Biophys. Acta* **670**, 64–73 (1981).
40. C. J. van Oss, et al., "Determination of the Surface Tension of Proteins. II. Surface Tension of Serum Albumin, Altered at the Protein-Air Interface," *Biochim. Biophys. Acta* **670**, 74–78 (1981).
41. R. A. Copeland, "Spectroscopic Probes of Protein Structure," *Methods for Protein Analysis*, Chapman & Hall, New York, 1994.
42. E. A. Burstein, N. S. Vedenkina, and M. N. Ivkova, "Fluorescence and the Location of Tryptophan Residues in Protein Molecules," *Photochem. Photobiol.* **18**, 263–279 (1973).
43. C. Myers, "Functional Attributes of Protein Isolates," in F. Felix, ed., *Characterization of Proteins*, Humana Press, Clifton, N.J., 1988, pp. 491–549.
44. M. R. Eftink, "Fluorescence Techniques for Studying Protein Structure," in C. H. Suelter, ed. *Methods of Biochemical Analysis*, Vol. 35, John Wiley & Sons, New York, 1991, pp. 127–205.
45. E. Padros et al., "Fourth-Derivative Spectrophotometry of Proteins," *Trends Biochem. Sci.* **9**, 58–510 (1984).
46. H. Mach and C. R. Middaugh, "Simultaneous Monitoring of the Environment of Tryptophan, Tyrosine and Phenylalanine Residues in Proteins by Near-Ultraviolet Second-Derivative Spectroscopy," *Anal. Biochem.* **222**, 323–331 (1994).
47. H. Mach et al., "Ultraviolet Absorption Spectroscopy," *Methods Mol. Biol.* **40**, 91–115 (1995).
48. L. Stryer, "The Interaction of a Naphthalene Dye With Apomyoglobin and Apohemoglobin. A Fluorescent Probe of Nonpolar Binding Sites," *J. Mol. Biol.* **13**, 482–495 (1965).
49. G. Weber and L. B. Young, "Fragmentation of Bovine Serum Albumin by Pepsin. 1. The Origin of the Acid Expansion of the Albumin Molecules," *J. Biol. Chem.* **239**, 1415–1423 (1964).
50. G. Penzer, "1-Anilinonapthalene-8-Sulfonate. The Dependence of Emission Spectra on Molecular Formation Studied by Fluorescence and Proton Magnetic Resonance," *Eur. J. Biochem.* **25**, 218–228 (1972).
51. S. Ainsworth and M. T. Flanagan, "The Effects That the Environment Exerts on the Spectroscopic Properties of Certain Dyes That Are Bound by Bovine Serum Albumin," *Biochim. Biophys. Acta* **194**, 213–221 (1969).
52. L. A. Sklar, B. S. Hudson, and R. D Simoni, "Conjugated Polyene Fatty Acids as Membrane Probes: Preliminary Characterization," *Proc. Nat. Acad. Sci. U.S.A.* **72**, 1649–1653 (1975).
53. L. A. Sklar, B. S. Hudson, and R. D. Simoni, "Model System Studies," *J. Supramolecular Structure* **4**, 449–465 (1976).
54. L. A. Sklar, B. S. Hudson, and R. D. Simoni, "Binding to Bovine Serum Albumin," *Biochemistry* **16**, 5100–5108 (1977).
55. A. Kato and S. Nakai, "Hydrophobicity Determined by a Fluorescence Probe Method and Its Correlation With Surface Properties of Proteins," *Biochim. Biophys. Acta* **624**, 13–20 (1980).
56. M. Cardamone and N. K. Puri, "Spectrofluorimetric Assessment of the Surface Hydrophobicity of Proteins," *Biochem. J.* **282**, 589–593 (1992).
57. J. Slavik, *Fluorescent Probes in Cellular and Molecular Biology*, CRC Press, Boca Raton, Fla., 1994, pp. 125–137.
58. G. Weber and F. J. Farris, "Synthesis and Spectral Properties of a Hydrophobic Fluorescent Probe: 6-Propionyl-2-(dimethylamino)naphthalene," *Biochemistry* **18**, 3075–3078 (1979).
59. D. L. Sackett and J. Wolff, "Nile Red as a Polarity-Sensitive Fluorescent Probe of Hydrophobic Protein Surfaces," *Anal. Biochem.* **167**, 228–234 (1987).
60. P. S. Belton, "New Methods for Monitoring Changes in Proteins," *Food Rev. Int.* **9**, 551–573 (1993).
61. E. Li-Chan, S. Nakai, and M. Hirotsuka, "Raman Spectroscopy as a Probe of Protein Structure in Food Systems," in R. Y. Yada, R. L. Jackman, and J. L. Smith, eds., *Protein Structure-Function Relationships in Foods*, Blackie Academic and Professional, London, United Kingdom, 1994, pp. 163–197.

62. S. Nakai and E. Li-Chan, "Recent Advances in Structure and Function of Food Proteins: QSAR Approach," *Crit. Rev. Food Sci. Nutr.* **33**, 477–499 (1993).
63. C Hansch and T. E. Klein, "Quantitative Structure-Activity Relationships and Molecular Graphics in Evaluation of Enzyme-Ligand Interactions," *Methods Enzymol.* **202**, 512–543 (1991).
64. M Yvon, C. Chabanet, and J.-P. Pelissier, "Solubility of Peptides in Trichloroacetic Acid (TCA) Solutions. Hypothesis on the Precipitation Mechanism," *Int. J. Pept. Protein Res.* **34**, 166–176 (1989).
65. A.-A. Townsend and S. Nakai, "Relationships Between Hydrophobicity and Foaming Characteristics of Food Proteins," *J. Food Sci.* **48**, 588–594 (1983).
66. S. Yokoi et al., "Hydrophobic Beer Proteins and Their Function in Beer Foam," *Journal of the American Society of Brewing Chemists* **52**, 123–126 (1994).
67. A. Kato et al., "Effect of Partial Denaturation on Surface Properties of Ovalbumin and Lysozyme," *Agric. Biol. Chem.* **45**, 2755–2760 (1981).
68. I. Nir et al., "Surface Properties and Emulsification Behavior of Denatured Soy Proteins," *J. Food Sci.* **59**, 606–610 (1994).
69. K. Shimada and S. Matsushita, "Relationship Between Thermocoagulation of Proteins and Amino Acid Compositions," *J. Agric. Food Chem.* **28**, 413–417 (1980).
70. S. Barbut, "Protein Gel Ultrastructure and Functionality," in N. S. Hettiarachchy and G. R. Ziegler, eds., *Protein Functionality in Food Systems*, Marcel Dekker, New York, 1994, pp. 383–433.
71. E. C. Y. Li-Chan, "Macromolecular Interactions of Food Proteins Studied by Raman Spectroscopy," in N. Parris et al., eds., *Macromolecular Interactions in Food Technology*, American Chemical Society, Washington, D.C., 1996, pp. 15–36.
72. E. Niwa, T. Hakayma, and I Hamada, "Arylsulfonyl Chloride Induced Setting of Dolphinfish Flesh Sol," *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 179–182 (1981).
73. E. Niwa et al., "Setting of Flesh Sol Induced by Ethylsulfonation," *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 915–919 (1981).
74. E. Li-Chan, S. Nakai, and D. F. Wood, " Muscle Protein Structure-Function Relationships and Discrimination of Functionality by Multivariate Analysis," *J. Food Sci.* **52**, 31–41 (1987).
75. T. E. O'Neill, "Flavor Binding by Food Proteins: An Overview," in R. J. McGorrin and J. V. Leland, eds., *Flavor-Food Interactions*, American Chemical Society, Washington, D.C., 1996, pp. 59–74.
76. N. Boudaud and J.-P. Doumont, "Interaction Between Flavor Components and β-Lactoglobulin," in R. J. McGorrin and J V. Leland, eds., *Flavor-Food Interactions*, American Chemical Society, Washington, D.C., 1996, pp. 90–97.
77. J. Adler-Nissen, *Enzymic Hydrolysis of Food Proteins*, Elsevier Science, New York, 1986.
78. S. Arai, M. Watanabe, and N. Hirao, "Modification to Change Physical and Functional Properties of Food Proteins," in R. E. Feeney and J. R. Whitaker, eds., *Protein Tailoring for Food and Medical Uses*, Marcel Dekker, New York, 1986, pp. 75–95.
79. H. W. Blanch and A. M. Klibanov, eds., *Enzyme Engineering 9*, Vol. 542, Annals of the New York Academy of Sciences, New York, 1988.
80. J. A. Rothfus, "Potential Beta-Sheets Surfaces of Corn and Wheat Proteins," *J. Agric. Food Chem.* **44**, 3143–3152 (1996).

GENERAL REFERENCES

References 1, 3, 15, and 17 are good sources of general references on protein hydrophobicity. The following are recommended for further reading on hydrophobic interactions in general, and on the relationship with food protein functionality.

A. Ben-Naim, *Hydrophobic Interactions*, Plenum Press, New York, 1980.

A. Ben-Naim, "Hydrophobic Interactions in Biological Systems," in A. S. V. Burgen and G. C. K. Roberts, eds., *Topics in Molecular Pharmacology*, Vol. 2, Elsevier Science, New York, 1983, pp. 1–52.

N. S. Hettiarachchy and G. R. Ziegler, eds., *Protein Functionality in Food Systems*, Marcel Dekker, New York, 1994.

S. Nakai and H. W. Modler, eds., *Food Proteins, Properties and Characterization*, VCH, New York, 1996.

EUNICE C. Y. LI-CHAN
University of British Columbia
Vancouver, British Columbia
Canada

ICE CREAM AND FROZEN DESSERT

BACKGROUND AND DEFINITIONS

Ice cream and frozen desserts, which include sherbets, ices (sorbets), frozen yogurt, dynamically and quiescently frozen novelties, and mellorine, play very important nutritional and social roles in our daily diet. Although debate exists as to whether ice cream originated in ancient China or Rome, there is little doubt that ice cream is America's favorite dessert. In 1996 the total retail sales of ice cream and other frozen related products reached a high of $10.8 billion (1). Ice cream eating occasions are frequently associated with fond memories of childhood, lazy summer days, and family get-togethers. In addition to its role in complementing various social occasions, ice cream provides important nutrients, particularly to infants and adolescents who consume large quantities of ice cream and frozen desserts. A 4-fl-oz (1/2 cup) serving of a typical ice cream provides 8% of U.S. RDA calcium and 5% of U.S. RDA protein as well as other key nutrients (eg, vitamin A, thiamine, riboflavin) in significant quantities.

Although overall ice cream consumption growth has decreased in recent years owing to changing demographic and dietary patterns, a substantial growth in dollar sales has been achieved through products that satisfy the consumers' craving for high-quality, indulgent, convenient, and healthy alternatives for different occasions. The premium and superpremium ice cream category, which satisfies consumers' craving for quality and indulgence, was pioneered in the early 1960s by Reuben Mattus who created Häagen-Dazs ice cream, using all top-quality natural ingredients. The wide popularity of this type of rich, clean-tasting ice cream with no artificial stabilizers or other additives is proven over the years by the success of the premium and superpremium ice creams in the United States as well as overseas. The success enjoyed by Häagen-Dazs and others in Japan and Europe demonstrates that people across different cultures have equal appreciation for ice cream.

Smooth, creamy ice cream as we know it today was developed in the United States during the early part of this century. Two significant technological breakthroughs contributed to this development (1). First, homogenization to reduce fat particle size and to create a smooth texture was developed by August Gaulin. Second, continuous freezing was made commercially feasible by Clarence Vogt to produce consistent ice crystal structure with adequate throughput. Following these initial technical breakthroughs, most of the advances in ice cream technology have consisted of refinements in formulation, stabilizer systems, and process systems.

Ice cream and frozen desserts are governed under Food and Drug Administration (FDA) and state health department regulations. Most states have adopted standards defined in the FDA *Code of Federal Regulations*, as presented in Ref. 2.

Ice cream is a food produced by dynamically freezing a pasteurized mixture of milk, cream, nonfat milk solids, sugars, and stabilizers combined with flavorings that may be added before or after pasteurization. Fruits, nuts, candies, syrups, and fudges are often added to semifrozen ice cream to create different flavors and taste impacts. Ice cream containing more than 1.4% egg yolk solids is called French ice cream or frozen custard. Ice cream contains at least 1.6 lb solids per gallon and weighs at least 4.5 lb per gallon. The minimum butterfat content for ice cream and French ice cream is 10% with a minimum of 20% total milk solids.

Sherbet is a frozen dessert containing small amounts of dairy solids (1–2% milkfat, 2–5% dairy solids) and not less than 0.35% titratable acidity (calculated as lactic acid) for fruit flavors. Sherbets may be flavored with fruit or other flavors, such as chocolate.

Water ices (sorbets) contain fruits, fruit flavors, or flavors other than fruit. Typically, these products are quiescently frozen and weigh at least 6 lb/gal. These products contain no dairy or egg ingredients, other than egg white.

Frozen yogurt is a frozen dessert with the same ingredients as ice cream that also contains yogurt bacteria of the species *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It contains at least 3.25% milkfat and 8.25% nonfat milk solids (NMS). Frozen yogurt weighs not less than 5 lb/gal. At this time, there are no federal standards for this product; however, states may regulate.

Quiescently frozen dairy confections (eg, stick novelties) are made much like water ices except that these products contain at least 13% by weight of milk solids and 33% by weight of total solids. *Quiescently frozen confections* may or may not contain milk solids.

Mellorine is made in much the same way as ice cream except that butterfat is partially or totally replaced with vegetable fat. The product should contain at least 6% vegetable fat and 2.7% protein. The addition of vitamin A must be such that 40 I.U. are available per gram of vegetable fat.

As of 1993, the National Labeling and Education Act (NLEA) created four new labels for ice cream and related products: reduced fat, light, lowfat, and nonfat ice creams (3). *Reduced fat* is made with 25% less fat than the reference product. *Light* or *Lite* products are made with 50% less fat or one-third fewer calories than the reference product (as long as <50% of the calories come from fat). *Lowfat* ice cream contains not more than 3 g milkfat/serving. *Nonfat* ice creams contain less than 0.5 g of fat per serving. With the creation of the NLEA, the FDA in 1995 revoked the standard of identity for *ice milk*.

ICE CREAM COMPOSITION AND PHYSICAL PROPERTIES

Ice cream mix contains a minimum of 10% milkfat and 20% total milk solids, except when fruit, nuts, chocolate, or other bulky flavors are added. If bulky flavors are added, the minimum milkfat is 8.8% and 16.6% milk solids. According to U.S. Federal Standards, ice cream must weigh at least 4.5 lb/gal. The minimum gallon weight permits the incorporation of air sufficient to roughly double the volume (or 100% overrun). Percent overrun is defined as:

$$\frac{\text{weight of 1 gal mix} - \text{weight of 1 gal ice cream}}{\text{weight of 1 gal ice cream}} \times 100$$

Superpremium ice creams typically have a range of 10 to 40% overrun (premium, 60–75%; economy, 75–90%).

Ice cream mix normally contains 10 to 15% sucrose, 5 to 7% corn sweetener, 0.2 to 0.3% stabilizer, 0.1% emulsifier, and natural or artificial flavors and colors. According to U.S. Federal Standards, ice cream may not contain more than 0.5% stabilizer or 0.3% emulsifier.

Physically, ice cream is an emulsion, a dispersion of ice crystals and a partly frozen foam. The ice cream mix is an emulsion in which the aqueous phases contain solutions of soluble proteins, lactose, mineral salts, and added sugars. The nonaqueous phases consist of dispersed solids such as stabilizers, proteins, and fat globules. During the freezing process and foam formation, the emulsion is partially destabilized. This emulsion destabilization allows the air cells to be stabilized by clusters of intact fat globules, which agglomerate by free oil or protein/protein interactions (4–7). The percentage of water changed into ice is dependent on the production temperature and concentrations of sugars and milk salts in solution.

Butterfat

Butterfat contributes to the rich and creamy flavor and texture of ice cream. Fat particles, which impart a smooth mouth-feel, are dispersed throughout the ice cream mix. Ice cream with high fat content tends to have smaller ice crystals and a slower rate of air incorporation. Butterfat can be obtained from a variety of sources, but fresh sweet cream is by far the most popular and desirable source. Sweet cream is about 40% butterfat. Because of its perishability, for best flavor, cream must be refrigerated and promptly used. Cream can be frozen to preserve the quality; however, extra care must be taken during handling to protect the flavors. Another source of butterfat is unsalted butter, which is about 82.5% fat and can replace 50 to 75% of the sweet cream fat. Other special fat sources sometimes used in ice cream are plastic cream (80% fat), anhydrous butter oil (99% fat), concentrated sweetened cream, and dried cream (65% fat) (3).

Milk Proteins

Milk proteins are important to the formation of ice cream. Their role in the overall structure is to help stabilize the foam or air cells and to contribute to emulsification of the fat. This is due to hydration during mixing, destabilization and complexing during heating, and attraction to the fat globules surface after homogenization. As a result, the finished ice cream is more stable and smooth.

Two main types of milk proteins are found in ice cream. These are whey proteins and caseins. Casein comprises approximately 80% of the proteins found in milk. Numerous sources of milk proteins are available for use in ice cream. Condensed skim milk at about 25 to 35% solids is widely used throughout the industry. However, it is perishable and must be used fresh. Dried skim milk, or nonfat dried milk, is also widely used and has a much longer storage life. Many types of powders (low, medium, and high heat-treated) are available, each with specific functional properties (3). Superheated condensed skim milk is sometimes used as a protein source. Heating the condensed skim milk denatures more protein, thereby imparting increased stability and whipping ability when used in ice cream. However, cooked off-flavors may result. Lactose reduced (LR) skim milk may be used to decrease the lactose concentration in ice cream to discourage the formation of lactose crystals that impart an undesirable grainy, gritty, or sandy ice cream texture.

Specialty products are also available as additional protein sources. These include sodium caseinate and dry whey, which both improve mix-whipping properties by increasing overrun and dryness. Dry whey contains high portions of both lactose and mineral salts and can replace up to 25% of the milk solids nonfat (MSNF). At higher levels, saltiness and sandiness may result. Milk protein concentrate (ultrafiltered skim milk, MPC) has potential use in the ice cream industry, having a higher protein concentration and lower lactose and soluble mineral content than NFDM. MPC is most beneficial due to its clean, milklike flavor (8).

Stabilizers and Emulsifiers

Although excellent ice cream products can be made with only the natural stabilizing and emulsifying materials present in milk (milk protein and phosphates), additional stabilizers and emulsifiers also have potential benefits.

Fluctuations in temperature during normal distribution cause ice crystals to melt and refreeze into larger ice crystals, resulting in negative textural changes in the ice cream. This phenomenon, exposure to either large temperature swings or high temperature for a period of time, is referred to as heat shock. Hydrocolloidal stabilizers function to physically bind the water formed by melting and, therefore, to help prevent the formation of large ice crystals. The amount and type of stabilizer needed is governed by the composition of the ice cream mix, processing condition, processing temperatures, storage times and conditions, and other factors (3,9–11). Most ice cream mixes are made with 0.2 to 0.5% added stabilizer. A stabilizer is capable of binding a large amount of water. A small amount is effective in producing a product with good texture and more resistance to temperature abuse (heat shock). Too much stabilizer will result in product that is gummy.

Many stabilizers used in ice cream are natural. Sodium alginates are widely used as ice cream stabilizers. Algins improve whipping qualities of the ice cream, help prevent heat shock, and give the product good eating qualities. Gelatins, which are derived from animal sources, are also used to stabilize ice cream products. Their structure and affinity for water help prevent the formation of large ice crystals and contribute to texture. Locust bean gum (carob) is normally used in conjunction with carrageenan (Irish moss) as a stabilizer. Locust bean gum has excellent water-binding properties and imparts superior heat shock resistance. Its tendency to cause wheying off, curdling of milk proteins, is prevented when it is used in combination with carrageenan. Guar gum is a complex carbohydrate that functions as a stabilizer and is usually used in conjunction with carrageenan. Guar has water-binding properties similar to that of locust bean gum and imparts similar heat-shock resistance. It solubilizes easily in cold mix, making it a good choice for mixes undergoing high-temperature short-time (HTST) pasteurization. A newer gum, similar to guar and locust bean, is tara gum, which is cold water soluble and protective to heat shock. Tara gum imparts a buttery mouthfeel and can be used at levels 20 to 25% less than locust bean gum. Sodium carboxymethyl cellulose (CMC) is also used as a stabilizer. It has excellent water-holding properties and dissolves easily in mix. It possesses some emulsifying properties. CMC provides the best results when used in conjunction with another stabilizer (4). Milk proteins also have stabilizing properties, depending on the heat treatment they have received.

The primary effect of emulsifiers in ice cream is related to their ability to de-emulsify the fat globule membrane formed during homogenization. This de-emulsification, arising from the disruption of the fat globule membranes during freezing, facilitates the agglomeration and coalescence of the fat globules, leading to partial churning out of the fat phase. The agglomerated fat globules stabilize air cells (3). Thus, emulsifiers are used to improve the whipping qualities of ice cream mix by producing smaller ice crystals and smaller air cells, resulting in a smoother ice cream texture and a drier, stiffer ice cream. Generally, a mixture of high and low hydrophile-lipophile balance (HLB) emulsifiers, such as mono- and diglycerides, and polysorbate 80 is used. Milk contains some naturally emulsifying compounds that aid in the manufacturing of ice cream. These include milk proteins, phosphates, and citrates. Egg yolks may also be used as an emulsifier as they are high in lecithin.

Fat Reduction

To achieve fat reduction in frozen desserts, one needs to replace both the functionality of the fat and the actual quantity of fat removed (formula percentage). Reducing fat in a frozen dairy product generally requires multiple approaches. Solids replacement is critical for stability (ice crystal growth) and the perception of warmth/coldness in the mouth. For example, in nonfat products there is proportionally more ice that must melt at least partially in the mouth so the product will seem "colder" than a high fat ice cream when both products are served at the same temperature. Various fat substitutes may be chosen to provide "body" or a mouth-feel that simulates the lubricity of fat. Flavor levels or flavor character may be changed to modify the flavor impact and release.

Dairy processors worldwide are continuing to adjust their fat-reduced ice cream formulas to duplicate the qualities of full-fat products. Ingredient suppliers are broadening the range and quality of fat substitutes, which significantly help ice cream manufacturers in their quest for the perfect low-fat and nonfat desserts. A recent market survey of low-fat and fat-free frozen desserts in the United States showed that 38% contained microcrystalline cellulose, 31% contained polydextrose, 31% contained maltodextrin, 25% contained modified food starch, and 50% contained added protein, for example, whey protein or egg white.

Fat substitutes can be classified as proteins, carbohydrates, or lipids. In addition to fat substitutes, a low-fat or nonfat frozen dessert may require flavor addition to compensate for the lack of fat and its effect on flavor release. When butterfat is reduced in a product with a standard of identity such as ice cream ("low-fat ice cream"), a lost nutrient, such as fat-soluble vitamin A, must also be replaced.

Protein-Based Fat Substitutes. Most of the protein-based fat mimetics function by emulating fat globules. Simplesse® is a microparticulated egg white and/or milk protein from The NutraSweet Kelco Company, which is said to mimic fat by simulating the particle size of fat globules. Dairy-Lo® or Dairy-Lite®, a heat-treated whey protein concentrate from Cultor Food Science, is another available protein-based fat substitute.

Carbohydrate-Based Fat Substitutes. Carbohydrates are generally used in ice creams to replace fat solids. Higher molecular weight carbohydrates with low sweetness are usually chosen since they will be used in addition to the carbohydrates (sweeteners) already included in the formula. One such carbohydrate is Oatrim®, a fat substitute that is produced by partially hydrolyzing the starch in oat bran or oat flour into maltodextrins. Modified starches, polydextrose, polyfructose, and pectin, such as Slendid from Hercules, are also commonly used as fat replacers. Microcrystalline cellulose is used to enhance the mouthfeel of frozen dairy products. Gelled carbohydrate solutions or suspensions provide some viscosity and lubricity but do not have the globular mouth-feel characteristics of fat. Carbohydrate usage levels may be limited by increased mix viscosity.

Lipid-Based Fat Substitutes. Lipid-based fat substitutes are the so-called designer fats that can fully replace butterfat functionality at a lower calorie level because they are only partially digested. They generally don't require other formulation changes, while protein and carbohydrates behave as fat mimetics and require formulation changes. These fat "substitutes" are chemically fats, but rendering the fat partially indigestible reduces the caloric content. Several lower-calorie fats are commercially available. Caprennin® from Procter & Gamble is an interesterified triglyceride with long-chain fatty acids, which contribute to its lower degree of digestibility. Salatrim®, a 5 calories per gram triglyceride from Cultor Food Science marketed as Benefat®, is another interesterification product between glycerol esters of acetic, propionic, and butyric acids and regular fats. FDA has approved Olestra®, a sucrose polyester with very low HLB from Procter & Gamble, for salty snacks, but not yet for frozen desserts. Emulsifiers can augment the functionality of fat.

Flavor Delivery Systems. There are two potential flavor issues associated with low-fat products: the fat replacer itself might have an off-flavor, and the loss of fat and replacement by the fat substitute might result in an unbalanced flavor release. Flavor delivery issues exist because fat replacers do not dissolve flavor compounds in the same way as fat. When fat melts, flavors are released in a characteristic sequence. In low-fat and fat-free formulations the flavor release can be faster, stronger, and less pleasurable. Some advances have been made by flavor encapsulation and controlled-release mechanisms. One example is Singer's (12) patented method for the delivery of fat-soluble flavor compounds into nonfat and low-fat food products in which fat components have been replaced by nonlipid fat substitutes. Hatchwell (13) discussed a flavor delivery system composed of fat globules into which elevated levels of fat-soluble flavor compounds have been loaded or incorporated into nonfat and low-fat food products, so that fat-soluble flavor compounds will be released in a more natural and familiar sequence.

Cholesterol Reduction. As consumer demand for low-fat cholesterol-free products has increased innovative technologies to remove cholesterol from butterfat or egg yolk have been developed to different scales of commercialization. The techniques include vacuum steam distillation, short path distillation, melt crystallization, supercritical fluid extraction, and chelation with β-cyclodextrin or quillaja saponins (14,15).

Sugars and Sweeteners

Sugars contribute to the sweet flavor of ice cream. The concentration of sugars in the mix will also determine the freezing point of ice cream. The greater the amount of sweetener contained in an ice cream mix, the lower the freezing point. Many kinds of sweeteners can be used in ice cream. They include cane and beet sugar, corn sweeteners, honey, invert sugar, lactose, fructose, and refiner's syrup.

Sucrose, the most widely used sugar, is processed from cane sugar and beet sugar. Most ice creams are made with a combination of corn syrup and sucrose. Corn syrup solids are made from a hydrolysis of cornstarch. Corn syrups are classified by dextrose equivalence (DE), which indicates its degree of hydrolysis. The higher the DE, the sweeter the corn syrup will be. The intermediate range (48–68 DE) and high conversion (58–68 DE) corn syrups are used to produce a product that will be very close in texture to that of a product made entirely with sucrose. Corn syrup imparts a denser body to the finished product and may improve its shelf life (3,9,11). Honey is used in ice cream primarily to make honey-flavored ice cream. Honey usually does not blend well with other ice cream flavors and therefore is rarely used as a sweetener.

A significant part of the MSNF is lactose, which also imparts sweetness. Lactose is a sugar found only in milk. It is less sweet and less soluble than sucrose. The concentration of lactose in mix is limited because it may separate out of solution in large crystals and produce an undesirable sandy feeling in the mouth. The maximum amount of lactose to avoid sandiness may vary depending on the freezing and storage conditions of the product.

Sucrose Reduction

For some diabetics, no-sugar-added products provide opportunities to consume frozen dairy desserts. However, it is the total amount of carbohydrate, not just the simple sugars, that affects the blood glucose response. The technologies for sucrose replacement have been reviewed by Deis (16). Traditionally, polyols such as glycerol, xylitol, sorbitol, lactitol, maltitol, palatinit, and hydrogenated starch hydrolysates are used. Polydextrose, with 1 calorie per gram, is another commonly used bulking agent with high intensity artificial sweeteners. Buzzanell et al. (17) reviewed the status and application of various high-intensity sweeteners including aspartame, saccharin, Acesulfame-K, cyclamate, sucralose, and Alitame®. Sucralose® has recently been approved in the United States in April of 1998. A current U.S. market survey of no-sugar-added products shows that the majority contain maltodextrin, polydextrose, sorbitol, and a combination of Acesulfame-K and aspartame.

A novel process for a no-sugar-added product uses lactase to hydrolyze the lactose in condensed skim to increase the mix sweetness and permit use of a higher concentration of milk without risking lactose recrystallization (sandiness) (18). This allows the complete replacement of sugar with a high potency sweetener (aspartame) without the use of bulking agents.

Flavorings

Hundreds of varieties of flavoring substances are used to flavor ice creams. There are two main characteristics to each flavoring, type and intensity. Both influence how well consumers like the finished products. Serving temperature and overrun also influence flavor.

The U.S. Federal standards of identity place flavors into the following three categories (2):

Category I. Pure extracts (no artificial flavor)

Category II. Pure extracts with synthetic or artificial components (natural flavor dominates)

Category III. Artificial flavors (or natural and artificial with artificial flavor predominate)

U.S. Federal laws also regulate how these various flavoring categories are to be labeled on the cartons. The majority of the ice creams are made with category II flavors, whereas most premium and superpremium brands use category I or pure flavors.

Vanilla is by far the most widely used flavor. Vanilla flavors are available from each of the three categories I, II, or III; pure vanilla flavor is a product of vanilla bean fermentation. Most vanilla flavors used are of the category II, pure vanilla plus some artificial components. Chocolate products are the second most popular flavorings. They come in many forms; cocoa powders, chocolate syrups, and chocolate liquor are the most predominant. Flavor intensity, color, and texture can be manipulated by using different types of cocoa products. Fruits and fruit extracts are also very popular as flavorings. They can be added fresh, dried, candied, as concentrated juices, or as fruit essences. Nuts, spices, candies, cookies, and sugars such as honey are also added to ice cream to provide a wide variety of flavors. Other substances are swirled throughout the ice cream to produce a rippled effect, or a variegated product. These include flavors such as fudge, butterscotch, marshmallow, caramel, and fruits. Liqueur flavorings such as fruit brandy distillates or fruit liqueurs are also used in some ice creams.

Other Ingredients

Any FDA-certified natural and artificial coloring may be added to ice cream. These must be declared on the label. Annatto is a very popular yellow vegetable color used to color ice cream and to add visual richness.

Egg yolk solids are an optional ingredient. They are added for their emulsifying properties. They also impart a characteristic subtle flavor. Custard-type ice creams, by definition, must include egg yolks.

ICE CREAM PROCESSING—MIX MAKING, FREEZING, AND HARDENING

After the processor has selected the formula, the ingredients are blended together to produce the ice cream mix. The basic steps inherent in almost all processing of this mix into a frozen product are blending ingredients, pasteurization, homogenization, cooling, mix storage, flavoring, freezing, packaging, hardening, and finished product storage and distribution.

Mix Blending

To begin mix processing, the fluid ingredients are pumped from their storage vessel into a blending vat. The amount of these ingredients, as determined by the formula, may be either metered or weighed into the blending vat with the use of load cells. Some blend systems contain both meters and load cells to use one to check against the other in an effort to improve the accuracy of ingredient addition.

Dry ingredients such as cocoa powders, nonfat dry milk, corn syrup solids, and stabilizers are added into a blend vat, using a high-shear mixer. A portion of the bulk fluid ingredients is circulated through by the mixer to aid incorporation into the blend vat.

Pasteurization

Pasteurization is the process of heating the ice cream mix to a required time and temperature for the primary purpose of destroying pathogenic microorganisms. Two primary methods are recognized for achieving the time and temperature pasteurization requirements, batch also known as low-temperature long-time (LTLT) or continuous. Continuous pasteurization can be accomplished at different combinations of temperature and time: high-temperature short-time (HTST), higher-heat shorter-time (HHST), and ultra high temperature (UHT) methods. The U.S. Public Health Service (USPHS) standards for the pasteurization of mix are (19).

LTLT: 155°F for 30 min
HTST: 175°F for 25 s
HHST: 191°F for 1.0 s or 212°F for 0.01 s
UHT: 280°F for a minimum of 2 s

To achieve a higher rate of microbe destruction and to impart the characteristic flavors, most processors will pasteurize at slightly higher temperatures and/or longer times.

Today, the most popular method is the continuous HTST procedure using plate heat exchangers. Using HTST, the processor benefits from (*1*) improved process control, (*2*) less stabilizer requirements, (*3*) savings of time and space, and (*4*) increased capacity. Other continuous heat exchanger designs include steam injection and infusion, tubular, and swept surface types.

Batch pasteurization may be accomplished by two methods. The classic method is when the batch pasteurizer vessel also acts as the blending vat. After the required ingredients per formulation have been added to the blending vat, hot water is circulated through the jacket of the vat. Depending on the temperature of the hot water and the flow rate through the jacket, the mix arrives at the required batch pasteurization temperature. After the legal hold period, cool water is circulated through the jacket and, when sufficiently cooled, is pumped to a cooling system similar to that for the HTST system. The second method used for batch pasteurization is a modified system employing the regeneration section and the heater section of the HTST system; however, the temperature is raised only to the required batch pasteurization temperatures. The mix is then held in holding tanks for 30 min and then consequently pumped to the regenerator and cooling system.

Homogenization

The purpose of homogenization is to reduce the size of the fat globules so that 90% of the globules are less than 2 μm. Decreasing the fat globule size to smaller globules increases the surface area. This allows for a more uniform and consistent product, smoother in texture, which resists churn out in the freezer.

Acceptable regulatory placements of homogenizers are (*1*) in front of the heater section, (*2*) after the heater section and in front of the holding tube, and (*3*) after the flow diversion device and before the regeneration section. The homogenizer is a positive displacement pump that may act as the timing pump in many HTST systems.

Cooling

After pasteurization and homogenization, the mix is passed through a cooling unit. The cooling medium may be a refrigerated glycol solution or brine solution (a concentrated food-grade salt solution). The method employed for cooling is a plate heat exchange system similar to the HTST or a surface cooler. The heat exchange system is sufficiently sized to decrease the temperature of the mix to 45°F or less. The cold mix then flows to mix holding tanks for storage.

Mix Storage

Although legal standards only require cooling mix to 45°F or less, it is advantageous for product safety and quality to cool the mix to 34 to 36°F. With respect to aging of the mix, it was believed that 24 h was necessary for the physical changes in protein structure and fat crystallization to create more consistent mix that can be processed. However, recent studies do indicate that as little as 4 h accomplish this goal.

Freezing

During the freezing process, refrigerated ice cream mix is introduced into the ice cream barrel. The barrel is a cylindrical scraped-surface heat exchanger. Ammonia or freon is used as the cooling medium. Part of the water content of the mix is frozen into small ice crystals. At the same time, air is incorporated into the emulsion with agitation. The mix is transformed into a 20 to 25°F flowable mixture that can be filled/shaped into its final form. In commercial freezers, stainless-steel blades attached to the dasher (or mutator) scrape the crystals as they are being formed on the wall surface. The dasher controls the flow dynamics of the mix. Dashers come in many designs that determine the texture of the product. Closed or partial open dashers are used for ice creams. Many freezer manufacturers have specially designed their own dashers to meet their customers needs (20–22).

There are two main types of freezers, batch freezers and continuous freezers. Originally all freezing was done with batch freezers. In the batch process, a certain volume of mix is placed into the freezing barrel. The mix is frozen as the dasher is whipping in air. Batch freezing relies heavily on operator experience.

Currently, most manufacturing plants use continuous freezers. Continuous freezers provide greater process efficiency and product consistency. In continuous freezing, ice cream mix is metered into the freezing barrel at a constant rate. As the mix passes through the cylinder, air is whipped in by the dasher. Particulates (fruits, nuts, cookies) are added through a fruit and ingredient feeder after freezing. Continuous freezers are now available fully automated. Computers check the main control points of the operation. These control points may include overrun, dasher speed, flow rates, viscosity, pressure, and temperature control. These freezers also include automatic start-up, CIP pumps, and freeze-up prevention.

Soft-serve freezers are a scaled-down version of the batch and continuous freezers (23). Mix is added to a refrigerated hopper and flows by gravity into the freezing barrel. Mix is frozen, and air is incorporated in the barrel. The finished product is drawn out at 18 to 20°F onto a cup or cone. Because the product may have to stay in the freezing barrel for hours, the compressor and dasher are cycled on/off to maintain a consistent texture (20–22).

Cleaning-in-Place (CIP)

Modern ice cream plants include as much CIP cleaning as possible. CIP refers to the cleaning and sanitizing of process equipment in its assembled condition. The detergent solutions and sanitizers are recirculated through the process pipelines (pasteurizing system, mix tanks, freezer, etc). For effective soil removal, the detergent solution must have sufficient concentration, temperature, velocity (5 ft/s), and time. Manual or clean-out-of-place (COP) cleaning is significantly more time-consuming, more expensive, and generally less effective.

The four primary steps of an effective cleaning and sanitizing program for the CIP circuit are:

1. Prerinse: Flushing with water until the water runs clear.
2. Recirculation of detergent solution at 150 to 180°F for 15 to 30 min, depending on the type of equipment to be cleaned.
3. Postrinse for 10 to 18 min to remove all traces of detergent and soil.
4. Recirculation of sanitizers using chlorine or acid sanitizing agents, or heat sanitization. Heat sanitization is the circulating of hot water, 180°F minimum, for 15 min.

Ice Cream Hardening and Storage

Most ice creams, with the exception of quiescently frozen novelties, must be passed through a final freezing process after packaging called hardening. Hardening completes the ice crystallization that began in the continuous or batch ice cream freezer, enables the ice cream to withstand storage and transportation, and aids in final textural development.

Ice cream hardening must be accomplished through the rapid extraction of heat to ensure that the small ice crystals previously formed in the ice cream freezers are kept intact, giving the ice cream its familiar cool and creamy mouth-feel. Formation of large ice crystals would lead to an undesirable defect of iciness or a coarse mouth-feel. Efficient heat extraction is achieved through the use of hardening tunnels (insulated cold boxes in which the product is continuously conveyed through an extremely cold environment).

The actual heat transfer occurs either by a cold air blast (−30°F or lower) circulated around the ice cream packages or through hollow stainless steel plates that act as both the conveyor and the heat transfer medium. Brine, ammonia, or glycol chilled to −20°F flows in the inside of the hollow plates, discharging the heat gained from the ice cream to the plant refrigeration system.

Hardening tunnels are designed to bring the center of a package down to 0°F. The tunnel conveyor system has adjustable speeds to accommodate the various packages that a normal plant will produce. Hardening times for a −40°F blast tunnel are typically 90 min for pint packages to 4 h or more for 2-1/2-ga bulk packages.

Automatic or manual unloading of the hardening tunnel into a palletizing area completes the plants responsibilities for the product The product will typically be warehoused at −20°F. It is then distributed to the retailers where the product should be held at 0°F or colder to survive its trip to the consumer's home.

ICE CREAM DEFECTS

High-quality ingredients are a requirement in the production of a high-quality ice cream product. Adherence to formulation, industry processing standards, and proper storage requirements also is critical. Incorporation of inferior dairy ingredients, sweeteners, or bulky ingredients always will result in a sub-standard product. Table 1 summarizes the characteristics and causes of predominant flavor and texture defects found in poor-quality ice cream (3,4).

Cryostabilization

Cryostabilization is a new approach to stabilizing a frozen product over its shelf life (23–26). It is an applied technology developed from the concept of food polymer science (FPS), the application to foods of the concept of polymer science. Since the late 1980s this field has been actively researched and applied to food product and technology development. Cryostabilization is based on fundamental understanding of the critical physicochemical and thermomechanical structure-property relationships that underlie the behavior of water in all nonequilibrium food systems at subzero temperatures (27,28).

A food structure can be in different states (liquid, rubber, or glass) depending on composition and temperature. The state of a material has enormous effects on the rate of change (eg, deformation, diffusion, or chemical reaction) within the material. Since ice cream defects such as iciness and sandiness are diffusion-controlled properties, cryostabilization can be an important tool for improving product quality. An ideally stable product will not change significantly during shelf life, so factors affecting the rate of change are critical to understanding and controlling product stability.

When ice cream is frozen, ice separates out from the system and the concentration of the unfrozen matrix (unfrozen water plus solutes such as sweeteners, electrolytes, proteins, and gums) between the ice crystals increases. As the temperature decreases further, more ice crystallizes until the viscosity of the unfrozen matrix is so high that there will be no more observable ice recrystallization in the time frame of the measurement. That temperature is called Tg′, the glass transition of the maximally concentrated unfrozen matrix (23–28). The Tg′ of a typical ice cream, as measured by DSC (differential scanning calorimetry), is approximately −32°C. It was agreed during the ISOPOW 6 meeting that "there was a lower temperature glass transition and a mobility transformation at Tg′ which coincided with the onset of ice dissolution into solution phase and correlated well with product stability" (29).

The Tg′ of a product will predict its stability. In distribution and in consumers' home freezers ice cream will not be held at temperatures below −32°C. However, the smaller the differential is between the temperature at which a product is stored and Tg′, the greater the stability

Table 1. Characteristics and Causes of Ice Cream Flavor and Texture Defects

Defect	Characteristics	Cause
Acid, sour	Tingly taste sensation	Uncontrolled bacterial activity
Cooked, eggy, custard	A cooked milk, condensed milk, caramel-like flavor	Excessive heat treatment Use of "cooked" cream
Lacks freshness, stale	Lacks clean, delicate, balanced flavor	General flavor deterioration of mix during storage Marginal quality dairy ingredients Absorption of odors from equipment
Oxidized, cardboardy, metallic	Astringent, metallic, papery, painty, fishy, tallowy	Old product Poor storage conditions Marginal quality dairy ingredients
Coarse, icy, grainy	Large ice particles distributed throughout the product or localized layerlike ice crystals	Slow, inadequate freezing; insufficient stabilizer Temperature fluctuations during storage
Sandy, gritty	Slow-melting crystals that remain on tongue or stick in throat after ice cream melts	High milk solids nonfat (lactose concentration) High total solids Temperature fluctuations during storage Added particulates such as nuts
Crumbly, fluffy, brittle	Tendency of ice cream to fall apart when scooped	Excessive overrun Low total solids Inadequate stabilization
Gummy, pasty, elastic	Opposite of crumbly Ice cream at consumption temperature is like taffy	Excessive stabilization Excessive corn syrup High sugar solids
Buttery, churned, greasy	Butter particles or greasy mouth-feel after ice cream has melted	Inadequate homogenization Excessive fat destabilization High milkfat content Overemulsification of mix

of the product as it is closer to the stable glassy state. This temperature differential can be reduced by increasing the Tg′ or lowering the storage temperature. One patented example of cryostabilization uses 20 to 33% 2–6DE maltodextrin to raise the Tg′ of frozen novelties to −13°C., reducing shrinkage and deformation (30). For the greatest product stability it is important to control the product's storage temperature as well as to formulate the product to create a higher Tg′, resulting in a reduced molecular diffusion rate.

NOVELTIES

Unlike packaged ice cream that normally is scooped or soft-served, novelties are unique ice cream products that are produced in a variety of shapes and sizes. Packaged in multi-pack or popular individual servings, early novelties included ice pops, Fudgesicles®, and ice cream sandwiches. Original marketing efforts were targeted toward children. Recently, new novelties have emerged and are capturing a more mature, sophisticated consumer niche. This resurgence has opened another exciting avenue for the ice cream industry.

Novelties can be made by two different processes, dynamically frozen and extruded or statically (quiescently frozen) hardened in brine. The dynamically frozen and extruded process involves freezing ice cream to as low a temperature as possible so that it retains its shape after extrusion through the nozzle. The product is wire cut, dropped onto a plate conveyor, and transferred into a hardening tunnel. For stick novelties, sticks are inserted before the hardening tunnel. After the hardener, the product is released from the plate and may be enrobed or dipped in coating and then packaged. Shapes are easy to alter by changing the nozzle configuration.

Quiescently frozen novelties are formed by hardening mix in metal molds. The molds travel through a two-section refrigerated brine tank. In the first section, mix is filled, sticks are inserted, and product is hardened. The molds then pass through a defrost section where the product is extracted from the molds, coated, and packaged.

SHERBETS AND SORBETS (ICES)

Sherbets, as defined by the *Code of Federal Regulations* (2), contain 1 to 2% butterfat and 2 to 5% total milk-derived solids by weight of the finished product. Because of their typically low total solid contents, sherbets rely on hydrocolloidal stabilizers to physically bind water and add viscosity. This aids in distribution stability and quality. Sherbets can be flavored by both fruit and nonfruit flavors. Fruit-flavored sherbets should have a titratable acidity of at least 0.85 (calculated as lactic acid). Formulas for sherbets are comparable to ice cream and ice milk.

Sorbets (ices) contain neither milk-derived ingredients nor egg ingredients other than egg white. Unlike ice creams and sherbets, ices can be made from unpasteurized mix owing to their typically high acidity formulation. Ices may be either dynamically or statically frozen. Water ices have enjoyed a tremendous resurgence in recent years as premium sorbets in single-serve novelties.

OTHER FROZEN DESSERTS

Frozen Yogurt

Frozen yogurt is a cultured dairy product produced using cultures of bacteria: *S. thermophilus* and/or *L. bulgaricus*. Generally accepted industry standards indicate that the finished product cannot be less than 8.25% nonfat milk solids and not less than 0.5% titratable acidity (expressed as lactic acid), and finished product has to weigh at least 5 lb/gal (3). Frozen yogurt butterfat content is similar to whole milk (3.25%). Frozen yogurt can be classified as low-fat or nonfat yogurt. Low fat contains no less than 0.5% and no more than 2% butterfat, whereas nonfat contains no more than 0.5% butterfat.

An acceptable frozen yogurt should be smooth in texture and have a pleasant flavor. It will handle and freeze much the same as sherbet or reduced or low-fat ice cream. The amounts and types of milk solids and sugars present will affect the body and texture of the finished product. Not all ice cream stabilizers can be used for frozen yogurt because stabilizers used in frozen yogurts must be acid stable. Gelatin-based stabilizers perform best with cultured products. Emulsifiers may be used to improve overrun, but can also react negatively in the culturing process. They may reduce the activity of the starter cultures, resulting in longer fermentation.

Fruit flavors will work well with the acid flavor profile of yogurt. Vanilla and chocolate have also been successful. When using chocolate as a flavoring ingredient, the yogurt should not be fully fermented to ensure a better flavor balance.

Mellorine

Mellorine products are similar in composition to ice cream except that the butterfat has been replaced by a combination of vegetable and animal fat. The types of vegetable fats used may include coconut, soybean, cottonseed, and other plant fats. Hydrogenated vegetable fats will improve flavor stability and texture because they closely resemble the melting properties of milk fat. Standards of identity require that all mellorine-type products must contain not less than 6% fat, and not less than 3.5% protein (the biological value of the protein in the product must be at least equivalent to that of whole milk protein). Finished product must weigh no less than 4.5 lb/gal and contain no less than 1.6 lb of total solids/gal (2). The processing, handling, and freezing of mellorine are similar to that of ice cream. More emulsifier may need to be used in mellorine products. The maximum stabilizer allowed is 1%.

Parevine

Parevine is a nondairy frozen dessert. It is consumed mainly by those who, for religious reasons, need to avoid mixing meat and dairy products. Parevine is made from a combination of fats, water, one or more protein or carbohydrate food ingredient (from other than milk or meat sources), and nutritive sweetening ingredients other than lactose. It may also contain eggs or egg products, flavoring, coloring, and added vegetable stabilizer or vegetable emulsifier. Standards of identity require that the finished product must contain at least 10% fat and total solids not less than 1.3 lb/gal of finished product. When bulky ingredients (such as nuts, cocoa, or chocolate) are added, the fat content can be reduced to, but to no less than 8% (2).

Parevine can only be sold in properly labeled factory-filled containers that cannot be larger than 1 gal. When parevine is sold on the premises, a sign must be conspicuously displayed stating "Parevine Sold Here" in letters that can be easily read by the consumer.

Other Nondairy Frozen Desserts

Other options exist for vegetarians, those who simply prefer to avoid dairy products, or lactose intolerant persons. These options include rice- and soy-based frozen desserts and novelties. These desserts rely on added stabilizers and emulsifiers to produce a creamy, smooth product similar to dairy counterparts.

FUTURE TRENDS

The frozen dessert segment is an approximately $10 billion per year industry. Nearly 100% of the U.S. population enjoys frozen desserts as part of their diet. Current trends suggest a modest industry growth rate.

The Nutrition Label Education Act (NLEA) significantly reshaped the landscape of frozen dessert standards. One result of the NLEA is that "ice milk" is no longer a legally recognized product in the United States. Along with the NLEA, consumer health concerns related to fat and calorie intake have driven the growth of new segments for *Reduced Fat, Low-Fat, and Fat-Free* ice creams, as well as *No Sugar Added* products. After initial explosive growth when good-quality low-fat products were no longer stigmatized with the "ice milk" label, these product segments shifted to more niche segments.

Historically, fat reduction has been a key driver in this segment's development. In response to increasing consumer awareness of health and wellness issues, efforts to reduce caloric content are expected to be a new motivator in the development of healthier products. Sorbet and water ices have enjoyed a significant growth in popularity. Other segments that will emerge include organic ice cream and nutritionally-fortified frozen desserts.

Fat replacers such as certain proteins or starches as well as fat mimetics (eg, Olestra) will create new opportunities for high-quality "Better for You" products.

Starch technology advances will continue to create greater opportunities for fat replacement and specific product textures.

Milk protein concentrates, lactose-reduced skim milk, and the lactase enzyme will be keys to developing ice creams that are free of the sandy texture caused by lactose crystal formation.

Antifreeze proteins (ice crystal growth-controlling proteins found in cold climate plants and animals) have the potential to revolutionize the frozen dessert industry. They can dramatically reduce the effects of heat-shock. Their future use is dependent on the development of a protein that is both economical and label-friendly.

In the 1980s *Listeria monocytogenes* was a major food safety concern in ice cream and frozen dessert manufacture. While contamination by pathogens such as *Listeria* continues to be an industry concern, Good Manufacturing Practices (GMPs), the application of hazard analysis and critical control points (HACCP), and improved equipment design have kept this potential issue in check. Control of food allergens in frozen desserts and in other foods has emerged as a critical food safety concern. The industry will again need to rely on HACCP in both ice cream manufacturing plants and suppliers' ingredient plants.

New forms of ice cream and ice cream novelties continue to be developed to meet specific consumer needs. The trend toward targeting specific consumer segments, as well as specific product use occasions, will be a key driver of future new products.

BIBLIOGRAPHY

1. *The Latest Scoop . . . Facts and Figures on Ice Cream and Related Products*, International Ice Cream Association, Washington, D.C., 1997.
2. *U.S. Code of Federal Regulations*, Part 21, Sections 135.110.
3. R. T. Marshall and W. S. Arbuckle, *Ice Cream*, 5th ed., International Thomson, New York, 1996.
4. K. G. Berger, "Ice Cream," in S. Friberg and K. Larsson, eds., *Food Emulsions*, 3rd ed., Marcel Dekker, New York, 1997, pp. 413–490.
5. K. B. Caldwell, H. D. Goff, and D. W. Stanley, "A Low-Temperature SEM Study of Ice Cream. I. Techniques and General Microstructure," *Food Structure* **11**, 1–9 (1992).
6. J. L. Gelin et al., "Structural Changes in Oil-in-Water Emulsions During the Manufacture of Ice Cream," *Food Hydrocolloids* **8**, 299–308 (1994).
7. S. Kokubo et al., "Agglomeration of Fat Globules During the Freezing Process of Ice Cream Manufacturing," *Milchwissenschaft* **53**, 206–209 (1998).
8. A. Kilara, "Formulating Frozen Desserts," *Dairy Foods* **94**, 69–70 (1993).
9. S. A. Wittinger and D. E Smith, "Effects of Sweeteners and Stabilizers on Selected Sensory Attributes and Shelf Life of Ice Cream," *J. Food Sci.* **51**, 1463–1466, 1470 (1986).
10. C. Sharma, "Gums and Hydrocolloids in Oil-Water Emulsions," *Food Technol.* **35**, 59 (1981).
11. T. Miller-Livney and R. Hartel, "Ice Recrystallization in Ice Cream: Interactions Between Sweeteners and Stabilizers," *J. Dairy Sci.* **80**, 447–456 (1997).
12. U.S. Patent 5,202,146 (April 13, 1993), N. S. Singer, S. Pookoter, L. C. Hatchwell, G. Anderson, A. G. Shazer, and B. J. Booth, (to The Nutrasweet Company).
13. L. C. Hatchwell, "Overcoming Flavor Challenges in Low-Fat Frozen Desserts," *Food Technol.* **2**, 98–102 (1994).
14. U.S. Patent 5,326,579 (July 5, 1994), T. Richardson and R. Jimenez-Flores, (to the Regents of the University of California).
15. E. Sunfeld, J. M. Krochta, and T. Richardon, "Separation of Cholesterol from Butteroil Using Quilaja Saponins. I. Effect of pH, Contact Time and Adsorbent," *Journal of Food Process Engineering* **16**, 191–205 (1993)
16. R. C. Dels, "Adding Bulk Without Adding Sucrose," *Cereal Foods World* **39**, 93–97 (1994).

17. P. Buzzanell and F. Gray, "Have High-Intensity Sweeteners Reached Their Peak?" *Food Review* **16**, 44–50 (1993).
18. S. F. Keller et al., "Formulation of Aspartame-Sweetened Frozen Dairy Without Bulking Agents," *Food Technol.* **45**, 102, 104, 106 (1991).
19 U.S. Public Health and Human Services, *Milk Pasteurization Controls and Tests*, 6th ed., U.S Government Printing Office, Washington, D.C., 1997.
20. *Food Equipment and Refrigeration*, Crepaco, Inc., Chicago, Ill.
21. *Ice Cream Plant Information Manual*, O. G. Hoyer, Inc., Lake Geneva, Wisc.
22. *Taylor Operator's Manual*, Taylor, Inc., Rockton, Ill.
23. H. Levine and L. Slade, "Thermomechanical Properties of Small Carbohydrate-Water Glasses and Rubbers: Kinetically-Metastable Systems at Subzero Temperatures," *J. Chem. Soc. Faraday Trans. I* **84**, 2619–2633 (1988).
24. H. Levine and L. Slade, "A Food Polymer Science Approach to the Practice of Cryostabilization Technology," *Comments Agric. Food Chem.* **1**, 315 (1989).
25. H. Levine and L. Slade, "Principles of Cryostabilization Technology from Structure/Property Relationships of Water-Soluble Food Carbohydrates—A Review," *Cryo-Letter* **9**, 21–63 (1988).
26. V. Huang and S. Platt, "The Latest Developments in Ice Cream Technology," *Chem. Ind. (London)* **2**, 37–72 (1995).
27. F. Franks, "The Properties of Aqueous Solutions at Subzero Temperatures," in F. Franks, ed., *Water: A Comprehensive Treatise*, Vol. 7, New York, Plenum Press, 1982, pp. 215–338.
28. F. Franks, "Complex Aqueous Systems at Subzero Temperatures," in D. Simatos and J. L. Multon, eds., *Properties of Water in Foods*, Dordrecht, The Netherlands, Martinus Nilhoff, 1985, pp. 497–509.
29. D. S. Reid, *The Properties of Water in Foods: ISOPOW 6*, Blackie Academic & Professional; New York, 1998, pp. xix–xx.
30. U.S. Patent 5,486,373 (January 23, 1996), C. B. Holt and J. H. Telford, (to Good Humor Corporation).

JOHN CLEMMINGS
PAUL FAZIO
VICTOR HUANG
DIANE ROSENWALD
LEIGH HADDEN WHITE
Häagen-Dazs Research and Development
Edina, Minnesota

IMMOBILIZED ENZYMES

In the processing of foods, enzymes have distinct advantages over chemical catalysts of which most notable are substrate specificity and activity under mild conditions of temperature and pH. However, the cost of using soluble enzymes is a drawback so there is much interest in the use of immobilized enzymes and cells. Immobilized enzymes have been defined as "enzymes that are physically confined or localized in a certain defined region of space with retention of their catalytic activities, and that can be used repeatedly and continuously" (1). Immobilization often results in the enzyme becoming water insoluble.

Compared with processing with soluble, unconstrained enzymes, immobilization offers several advantages, including:

- Reuse or continuous use of the catalyst, thereby reducing both capital and recurrent process costs
- Absence of the enzyme from the product, thus potentially allowing for a wider range of enzymes than those normally permitted in foods
- Ease of terminating the reaction without drastic measures such as heat denaturation or extreme pH
- Greater (sometimes) thermal and pH stability and prevention of self-digestion by proteases
- Less product inhibition, and more substrate depletion with continuous processes, giving faster conversion

The main disadvantages are the cost of producing the immobilized enzyme, including the cost of the support, and altered reaction kinetics, which often result from diffusional restrictions.

METHODS FOR IMMOBILIZATION

General methods for immobilization of enzymyes and cells can be classified as follows: (*1*) adsorption onto a solid carrier, (*2*) covalent attachment to a solid carrier, (*3*) cross-linking to form insoluble aggregates (*4*) adsorption followed by cross-linking, and (*5*) entrapment in gels or encapsulation by membranes.

Adsorption onto a support surface is the simplest method of immobilization. Binding of the enzyme to the substrate is typically through electrostatic binding, hydrogen bonding, hydrophobic interactions, or van der Waals forces. Biospecific interactions between enzyme and ligand, or enzyme and antibody, are also possible. Binding forces (except for biospecific interactions) are rather weak and usually readily reversible. This makes adsorption an attractive process commercially since loading, desorption following decay of activity, and reloading are relatively simple. The main problems are leakage of enzyme from the support and changed activity characteristics caused by intimate association of the enzyme with the support matrix.

Covalent attachment provides the most secure method of immobilizing enzymes. Since enzymes are proteins, the amino acid side chains contain several functional groups that can be used for coupling to a support. The amino group of lysine is widely used, but the sulfhydryl of cysteine, hydroxyl of tyrosine or serine, and carboxyl groups of glutamic and aspartic acids are also used for coupling. However, the attachment must be via some nonessential group in the protein surface or activity will be lost. Prior to coupling, the support matrix is treated with a reagent that activates some functional groups on its surface; then the enzyme is mixed with the activated support and coupling takes place. Although many chemical reagents can be used for coupling, safety restrictions on food processes mean that only glutaraldehyde is used in commercial applications at the present time.

Cross-linking involves formation of covalent bonds between enzyme molecules, by bifunctional reagents, to produce large insoluble aggregates. Glutaraldehyde is a typical cross-linking reagent. Cross-linking may lead to large increases in stability, however, the reaction is difficult to control and the gelatinous physical nature of the products is a major impediment in many applications. Cross-linking is a very useful technique to minimize leakage of enzymes already immobilized by adsorption.

Entrapment involves placing the enzyme within a polymer matrix (eg, a gel) or behind a membrane to allow penetration of substrate and products but to prevent release of the enzyme protein. This restricts the application to processes that involve small substrates and products. Specific techniques include: entrapment in gels such as polyacrylamide and calcium alginate, entrapment in hollow fibers or within the microcavities of synthetic fibers, and microencapsulation of the enzyme within tiny spheres having semipermeable membranes. Entrapment has the advantage of minimal alteration of enzyme properties since there is no significant interaction with the "support." Drawbacks of the method include: enzyme leakage, mass transfer/diffusional limitations due to passage of substrates and products across a barrier, and the lack of stabilizing effects often associated with a solid support.

An extraordinary variety of supports have been used for enzyme immobilization, ranging from feathers to stainless steel spheres. For most food processes, the best supports are physically strong, chemically inert except for those groups involved in linkage, and not subject to microbial attack. A good example is glass beads. Once immobilized, the enzyme can be used in a variety of batch or continuous reactor systems, including stirred-tank, plugged-flow, and fluidized-bed systems.

Immobilization can cause substantial alterations in the activity of enzymes. There are many reasons for this, but three major influences have been identified: mass transfer/diffusion, pH shifts, and partitioning.

When an enzyme is bound to a solid surface, mass-transfer limitations often occur due to the stagnant liquid film close to the surface through which the substrates and products diffuse. This can lead to concentration differences between the bulk phase and the microenvironment of the enzyme. Diffusional restrictions are also noticeable for enzymes entrapped in gels or particles.

Shifts in pH optimum are noted when enzymes are immobilized to charged supports. A negatively charged support abstracts protons leading to a more acidic microenvironment and a shift to a higher optimum pH in bulk solution. The opposite effect is observed with positively charged supports, leading to a lower pH optimum.

Partitioning of substrates and products between the bulk liquid and the immobilized enzyme system also leads to differences in concentrations. This partitioning can be caused by charge interactions or by hydrophobic/hydrophilic interactions between the substrate/product and the enzyme support system. The kinetic consequences of these influences have been carefully explored (2,3).

Immobilized Cells

An alternative to extracting enzymes is to use immobilized cells. These can be readily entrapped in gel media such as carrageenan or calcium alginate. The major advantages of such preparations compared with immobilized enzymes include: avoidance of expensive enzyme isolation procedures, enhanced catalyst stability due to protection by the cell membrane and the local microenvironment, and the ability to catalyze multistep enzymatic reactions, particularly those using cofactors that are otherwise difficult to regenerate. Disadvantages include the following: the possibility of side reactions and hence less pure products; and both substrate and product must be small enough to pass through the gel particles. Currently, immobilized cells are used either to catalyze simple conversions such as isomerization or as growing immobilized cells to produce primary metabolites, for example, amino acids. Immobilized living cells can also be used to detoxify waste materials, for example, to remove nitrates from drinking water and to remove phenols from industrial wastewater (4).

Limitations

Despite the advantages cited, the current use of immobilized enzymes in industrial-scale food processing is quite limited. In some cases the reasons are purely economic—the soluble enzyme is so cheap that there is no cost advantage in an immobilized enzyme process. Other limitations are directly related to processing in the reactor. A major constraint is that applications are restricted to liquid systems. Even then, in complex liquid foods such as milk, enzyme particles are subject to fouling by components such as proteins. Another problem with foods is to prevent microbial buildup in the reactor. Accordingly, reactors may need to be periodically sanitized by compounds such as H_2O_2 or quaternary ammonium compounds. Chlorine-based compounds and iodophors are effective sanitizers but can lead to rapid loss of activity with some enzymes (5).

CURRENT APPLICATIONS IN FOOD PROCESSING

Glucose Isomerization

In terms of throughput, isomerization of glucose to the sweeter sugar fructose represents the largest application of immobilized enzyme technology. The glucose substrate is generated from hydrolysis of starch, and the product—high-fructose corn syrup—contains about 42% fructose, where it is isosweet with sucrose on a solids basis. The product is used extensively in the soft drinks industry. The enzyme used—glucose isomerase—is actually a xylose isomerase. It can be obtained from several microbial sources (notably *Streptomyces* spp.) and immobilized by most of the methods already described. Some processes use enzyme adsorbed onto DEAE-cellulose. However, since the enzyme is intracellular and extraction is inefficient, several preparations use whole cells. These are usually heated prior to immobilization both to prevent enzyme release after cell lysis and to inactivate other enzymes that could cause undesirable side reactions. Generally, higher activity per unit volume is obtained using enzyme adsorbed onto supports, but this is offset by the cost of the support and, if necessary, its recovery.

Lactose Hydrolysis

Hydrolyzing lactose has two major benefits: (*1*) milk becomes easier to digest for those who are lactose intolerant and (2) sweet syrups can be produced from whey/whey permeate, thereby enhancing its value and increasing utilization. Treatment of milk requires a neutral pH enzyme. Immobilization of neutral lactases has been largely unsuccessful with one notable exception: yeast lactase has been entrapped in cellulose acetate fibers and used in Italy to process sterilized skim milk in a batch reactor at 5°C.

Immobilization of acid-pH lactase for processing acid whey is easier, and two preparations have been used on a major scale. The enzyme from *Aspergillus niger* has been covalently linked to glass beads and has been employed in pilot-scale plants by Corning Glass. Demineralized, deproteinized whey was used as a substrate at 35 to 50°C, the temperature slowly increasing to compensate for activity losses. In Finland, acid lactase has been immobilized by adsorption onto a phenol formaldehyde resin and used on a commercial scale by the Valio Dairy to process acid whey.

Sugar Refining

Raffinose, an α-galactoside present in sugar beet interferes with crystallization of sucrose from the molasses and depresses the yield. α-Galactosidase hydrolyzes raffinose into sucrose and galactose and is produced by several fungi—notably *Mortierella vinaceae*. Since extraction of the enzyme is costly, mycelial pellets of *M. vinaceae* containing α-galactosidase but lacking invertase are used in a continuous process. Molasses is pumped through chambers of suspended pellets and about 65% of the raffinose is converted to sucrose. The results of processing are better sucrose yields and a reduction in waste molasses.

Aspartic Acid Production

L-aspartic acid can be enzymically synthesized by aspartase-catalyzed addition of ammonia to fumaric acid. This has been done commercially in Japan since 1973 using the aspartase in *Escherichia coli* cells immobilized in acrylamide or carrageenan. Before entrapment, the cells are heat shocked to inactivate fumarase and other unwanted enzymes. About 95% conversion is obtained using 1*M* ammonium fumarate. The immobilized cells have better operational stability than either immobilized enzyme or free cells that were previously used in batch processes. Production costs are about half of those using the earlier methods.

Resolution of L and D Amino Acids

Chemical synthesis of amino acids results in a racemic mixture of D and L forms; however, only the L form is biologically available and used for amino acid supplementation of foods. Resolution of the mixture can be achieved by acetylation followed by deacetylation with amino acylase, which acts only on the L form. The L-amino acid can then be crystallized out of the mixture. A commercial process has been developed in Japan using amino acylase immobilized on DEAE-Sephadex in a packed column reactor. This process has been in operation since 1969 and represents the first commercial-scale use of immobilized enzyme technology. Operating cost savings are about 40% compared with using the soluble enzyme.

Glucose Syrup Production

In the conversion of starch to glucose, glucoamylase is used to hydrolyze dextrins formed by α-amylase action. Traditionally the enzyme is used in the soluble form. Although it is readily immobilized, it has proven difficult to achieve satisfactory yields of glucose due to formation of side products, such as isomaltose. However, Tate and Lyle (London) has developed a satisfactory preparation in which the enzyme is immobilized in a gel surrounding bone char as an inert support. This preparation is beginning to be used in commercial operations.

Production of Aspartame

Aspartame (α-L-aspartic acid-L-phenylalanine-methyl ester) is a dipeptide sweetner widely used in diet soft drinks. It is about 180 times as sweet as sucrose. It can be synthesized chemically from its constituent amino acids or enzymatically using the protease thermolysin working in reverse to synthesize the peptide bond. The enzyme can be immobilized by adsorption to Celite or cross-linked polyacrylic ester, followed by cross-linking with glutaraldehyde. The synthesis reaction is then carried out in buffer saturated with ethyl acetate. The reaction takes place in the aqueous phase, but the product is effectively partitioned into the organic phase, thereby disturbing the equilibrium to favor synthesis.

Isomaltulose Production

The disaccharide isomaltulose is a novel sugar with noncariogenic properties that make it useful in confectionery. It is produced by the enzyme isomaltulose synthetase, which is immobilized as a constituent of whole cells of organisms such as *Erwinia rhapontici, Protaminobacter rubrum*, or *Serratia plymuthica*.

FOOD ANALYSIS

Enzymes are ideal for quantitative analysis of foods because of their specificity and sensitivity. The advantages of using immobilized enzymes for analysis include: reuse of the catalyst, stability, and predictable decay rates. There are two major approaches. The enzyme can be used in a reactor that produces a product that can be readily detected, for example, colorimetrically. Alternatively, the enzyme can be immobilized onto a sensing device capable of giving a continuous output, such as an electrode. The resulting *enzyme electrode* can then be inserted into foods to measure substrate depletion or product accumulation or some associated change such as pH. Enzyme electrodes have been developed that are capable of detecting a wide range of food components, including sugars, alcohols, amino acids, organic acids, and even cholesterol. They are particularly useful in automatic closed-system flow analysis, for example, as in-line monitors.

Where the enthalpy change of the reaction is sufficient, substrates can be assayed calorimetrically with enzyme thermistors. The heat of the reaction in a small column of immobilized enzyme is measured as a temperature change by a thermistor in the column eluent and indicates the amount of substrate converted. Enzyme thermistors can be used for continuous monitoring and control of bioconversions, for example, to sense changes in glucose concentration and control the flow of whey pumped to an immobilized lactase reactor (6).

POTENTIAL APPLICATIONS AND FUTURE DEVELOPMENTS

Application of immobilized enzymes seems promising in several areas. These include the following: use of sulfhydryl oxidase to remove the cooked flavor in UHT milk, use of trypsin to control the development of an oxidized flavor in stored milk, and the dibittering of citrus juices by enzymes that alter the bitter component limonene. Immobilized proteases could be used for chill-proofing beer, for limited hydrolysis of soy proteins to improve functionality, and for continuous production of curd for cheese making. Immobilized yeasts could be used for brewing beer and immobilized bacteria for production of vinegar.

One area that is especially promising is the use of immobilized lipases for the chemical modification of fats. Interesterification with free fatty acids or with other triglycerides using regiospecific lipases allows upgrading of cheap fats into more valuable fats such as cocoa butter substitutes. The immobilized enzyme could also be used to replace conventional chemical catalysts used in random interesterification. These processes have been well developed on the pilot scale, but the extent of commercial production is uncertain.

Primary obstacles to implementation of these applications are process economics and unfavorable enzyme characteristics such as inadequate stability. However, developments in molecular biology coupled with protein engineering promise to provide a second generation of enzymes whose properties will be designed with a particular process in mind. Greater stability toward heat, pH, and chemical denaturants is likely, as is altered specificity. The ability of enzymes to work in organic solvents will undoubtedly be exploited for further transformation of lipids and synthesis of food additives. Use of multienzyme systems and of enzymes that require cofactor regeneration should also expand the list of potential applications. At the reactor level, a major breakthrough would result from the discovery of general methods for regenerating activity of immobilized enzymes.

BIBLIOGRAPHY

1. J. F. Kennedy and J. M. S. Cabral, "Enzyme Immobilization," in H. J. Rehm and G. Reed, eds., *Biotechnology*, Vol. 72, VCH, Weinheim, Germany, 1987.
2. M. Griffin, E. J. Hammonds, and C. K. Leach, "Enzyme Immobilization," *Technological Applications of Biocatalysts*, Butterworth-Heineman, Oxford, United Kingdom, 1993, pp. 75–117.
3. L. Goldstein, "Kinetic Behaviour of Immobilized Enzyme Systems," *Methods Enzymol.* **44**, 397–443 (1976).
4. I. Chibata and T. Tosa, "Immobilized Microbial Cells and their Applications," *Trends Biochem. Sci.* **5**, 88–90 (1980).
5. J. F. Rolands, "Requirements Unique to the Food and Beverage Industry," in W. H. Pitcher, Jr., ed., *Immobilized Enzymes for Food Processing*, CRC Press, Boca Raton, Fl., 1980, pp. 55–80.
6. B. Danielson et al., "The Use of an Enzyme Thermistor in Continuous Measurements and Enzyme Reactor Control," *Biotechnol. Bioeng.* **21**, 1749–1766 (1979).

GENERAL REFERENCES

R. J. D. Barker, ed., *Technological Application of Biocatalysts*, Butterworth-Heinerman, Oxford, United Kingdom, 1993.

R. D. King and P. S. J. Cheetham, eds., *Food Biotechnology–2*, Elsevier Applied Science, London, United Kingdom, 1988

T. Nagodawithana and G. Reed, eds., *Enzymes in Food Processing*, 3rd ed., Academic Press, San Diego, Calif., 1993.

H. E. Swaisgood, "Immobilized Enzymes: Applications to Bioprocessing of Food," in P. F. Fox, ed., *Food Enzymology*, Elsevier Applied Science, London, United Kingdom, 1991, pp. 309–341.

A. Tanaka, T. Tosa, and T. Kobayashi, eds., *Industrial Application of Immobilized Biocatalysts*, Marcel Dekker, New York, 1993.

RAYMOND R. MAHONEY
University of Massachusetts
Amherst, Massachusetts

IMMUNOLOGICAL METHODOLOGY

Since the late 1970s, application of immunoassays (IAs) has moved from the clinical field into the areas of agricultural residue and food analysis. Development of these immunoassays, and enzyme-linked immunosorbent assays (ELISAs) in particular, began when the attractive features of the assays used in the clinical diagnostic field were identified. These assays are capable of being more rapid, more sensitive, less expensive, and less laborious than many of the classical methods used for detecting compounds of importance in agriculture and the food industry. These compounds may be naturally occurring compounds such as mycotoxins, natural toxicants, or microbial contaminants. They may also be substances added to animal feeds (eg, antimicrobial agents) or applied to crops (eg, pesticides), which occur as potentially harmful residues in raw materials and finished food products. The ability to identify food constituents is also important to ensure that adulteration has not occurred.

Because of the explosion of research and development of ias in this area and the limited scope and length of this article, it is impossible to list and describe every compound of interest, whether it is a food component or a contaminant. This article will provide a fairly detailed summary of ias used in analysis of food proteins (meat and nonmeat proteins), beginning with the older classical methods (eg, agglutination and precipitin techniques), and then focus on the development of the newer, more innovative immunoassays (eg, ELISAs). The later sections, whether they describe ias designed for detecting naturally occurring substances or those designed for detecting residues resulting from application or administration of drugs and other chemicals, will focus on ELISAs.

amount of substrate converted. Enzyme thermistors can be used for continuous monitoring and control of bioconversions, for example, to sense changes in glucose concentration and control the flow of whey pumped to an immobilized lactase reactor (6).

POTENTIAL APPLICATIONS AND FUTURE DEVELOPMENTS

Application of immobilized enzymes seems promising in several areas. These include the following: use of sulfhydryl oxidase to remove the cooked flavor in UHT milk, use of trypsin to control the development of an oxidized flavor in stored milk, and the dibittering of citrus juices by enzymes that alter the bitter component limonene. Immobilized proteases could be used for chill-proofing beer, for limited hydrolysis of soy proteins to improve functionality, and for continuous production of curd for cheese making. Immobilized yeasts could be used for brewing beer and immobilized bacteria for production of vinegar.

One area that is especially promising is the use of immobilized lipases for the chemical modification of fats. Interesterification with free fatty acids or with other triglycerides using regiospecific lipases allows upgrading of cheap fats into more valuable fats such as cocoa butter substitutes. The immobilized enzyme could also be used to replace conventional chemical catalysts used in random interesterification. These processes have been well developed on the pilot scale, but the extent of commercial production is uncertain.

Primary obstacles to implementation of these applications are process economics and unfavorable enzyme characteristics such as inadequate stability. However, developments in molecular biology coupled with protein engineering promise to provide a second generation of enzymes whose properties will be designed with a particular process in mind. Greater stability toward heat, pH, and chemical denaturants is likely, as is altered specificity. The ability of enzymes to work in organic solvents will undoubtedly be exploited for further transformation of lipids and synthesis of food additives. Use of multienzyme systems and of enzymes that require cofactor regeneration should also expand the list of potential applications. At the reactor level, a major breakthrough would result from the discovery of general methods for regenerating activity of immobilized enzymes.

BIBLIOGRAPHY

1. J. F. Kennedy and J. M. S. Cabral, "Enzyme Immobilization," in H. J. Rehm and G. Reed, eds., *Biotechnology*, Vol. 72, VCH, Weinheim, Germany, 1987.
2. M. Griffin, E. J. Hammonds, and C. K. Leach, "Enzyme Immobilization," *Technological Applications of Biocatalysts*, Butterworth-Heineman, Oxford, United Kingdom, 1993, pp. 75–117.
3. L. Goldstein, "Kinetic Behaviour of Immobilized Enzyme Systems," *Methods Enzymol.* **44**, 397–443 (1976).
4. I. Chibata and T. Tosa, "Immobilized Microbial Cells and their Applications," *Trends Biochem. Sci.* **5**, 88–90 (1980).
5. J. F. Rolands, "Requirements Unique to the Food and Beverage Industry," in W. H. Pitcher, Jr., ed., *Immobilized Enzymes for Food Processing*, CRC Press, Boca Raton, Fl., 1980, pp. 55–80.
6. B. Danielson et al., "The Use of an Enzyme Thermistor in Continuous Measurements and Enzyme Reactor Control," *Biotechnol. Bioeng.* **21**, 1749–1766 (1979).

GENERAL REFERENCES

R. J. D. Barker, ed., *Technological Application of Biocatalysts*, Butterworth-Heinerman, Oxford, United Kingdom, 1993.

R. D. King and P. S. J. Cheetham, eds., *Food Biotechnology–2*, Elsevier Applied Science, London, United Kingdom, 1988

T. Nagodawithana and G. Reed, eds., *Enzymes in Food Processing*, 3rd ed., Academic Press, San Diego, Calif., 1993.

H. E. Swaisgood, "Immobilized Enzymes: Applications to Bioprocessing of Food," in P. F. Fox, ed., *Food Enzymology*, Elsevier Applied Science, London, United Kingdom, 1991, pp. 309–341.

A. Tanaka, T. Tosa, and T. Kobayashi, eds., *Industrial Application of Immobilized Biocatalysts*, Marcel Dekker, New York, 1993.

Raymond R. Mahoney
University of Massachusetts
Amherst, Massachusetts

IMMUNOLOGICAL METHODOLOGY

Since the late 1970s, application of immunoassays (IAs) has moved from the clinical field into the areas of agricultural residue and food analysis. Development of these immunoassays, and enzyme-linked immunosorbent assays (ELISAs) in particular, began when the attractive features of the assays used in the clinical diagnostic field were identified. These assays are capable of being more rapid, more sensitive, less expensive, and less laborious than many of the classical methods used for detecting compounds of importance in agriculture and the food industry. These compounds may be naturally occurring compounds such as mycotoxins, natural toxicants, or microbial contaminants. They may also be substances added to animal feeds (eg, antimicrobial agents) or applied to crops (eg, pesticides), which occur as potentially harmful residues in raw materials and finished food products. The ability to identify food constituents is also important to ensure that adulteration has not occurred.

Because of the explosion of research and development of ias in this area and the limited scope and length of this article, it is impossible to list and describe every compound of interest, whether it is a food component or a contaminant. This article will provide a fairly detailed summary of ias used in analysis of food proteins (meat and nonmeat proteins), beginning with the older classical methods (eg, agglutination and precipitin techniques), and then focus on the development of the newer, more innovative immunoassays (eg, ELISAs). The later sections, whether they describe ias designed for detecting naturally occurring substances or those designed for detecting residues resulting from application or administration of drugs and other chemicals, will focus on ELISAs.

BACKGROUND

Chromatographic methods such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC) are commonly used to determine levels of many agricultural residues (1–10). They are limited because chromatography is only a method of separation, not identification. Spectral identification is necessary if structural confirmation is desired (11).

Microbiological assay methods have traditionally been used to detect a wide variety of antibiotics, regardless of the structures and sample matrix involved. The basic protocols were established in 1945 (12) and serve as the basis for the analysis involved both for inhibition and diffusion methods (13). The overall drawback to either the turbidimetric or diffusion procedures is that they are nonspecific (14). The routine procedures only achieve purification by dilution before analysis. Second, the organisms used in the assays respond to more than one antibiotic family in addition to other nonspecific materials. Therefore, sources of analytical bias and inaccurate results are inherent in the microbiological assay systems. The sensitivity of the organisms to the antibiotic being detected is usually sufficient enough to minimize some of these problems. However, the lack of specificity remains the key flaw to these assays. Fairly elaborate separations are required to separate the various antibiotic families prior to analysis. When the formulation being analyzed is known this is not a problem. However, when unknown samples or materials of uncertain origin are to be analyzed, this can pose a serious problem (14).

Detection of food-borne pathogens in foods has relied on culture procedures that allow growth, selection, isolation, and identification of isolated organisms. The major disadvantages of these procedures is the time required to obtain a negative reading. The laborious, time-consuming cultural procedures hinder prompt therapy for patients suffering from food poisoning and prompt removal of any contaminated food product from the marketplace.

Due to the drawbacks mentioned with conventional chemical and microbiological methods attention turned to employing antibody-based assays for detection of residues and organisms. Immunobased assays use antibodies that are developed to recognize a particular compound (or lookalike). Antibodies are developed using a number of domestic animals including mice, rabbits, guinea pigs, sheep, and goats. Antibody production currently takes two forms; either polyclonal or monoclonal antibodies are produced for immunoassay development.

These different types of antibody have been used to develop isotopic immunoassays such as radioimmunoassay (RIA), nonisotopic immunoassays that use a labeled antigen or antibody such as fluoroimmunoassay (FIA) and enzyme immunoassay (EIA). Agglutination, immunodiffusion, and quantitative precipitin techniques have also been used as well as affinity columns for separation and detection of haptens (such as pesticides, drugs, mycotoxins, and other potentially harmful chemicals) and food-borne pathogens and food proteins (meat and nonmeat protein additives).

Definitions

Before proceeding with a further discussion, it is necessary to provide definitions of frequently used terms and procedures.

Antigen. Antigens are any substance that when introduced into an animal's body, are recognized as foreign and elicit an immune response. The ability of an antigen to stimulate an immune response is called its immunogenicity, whereas its ability to combine specifically with an antibody is called its antigenicity.

Hapten. Hapten is a small molecule with molecular weight of less than 1,000. It is nonimmunogenic in its own right and must be chemically linked to proteins (*in vivo* or *in vitro*) to produce antibodies. It reacts with antibodies of appropriate specificity. Many of the compounds that require testing in the food industry and agriculture are small molecules. Bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), and bovine thyroglobulin (BTG) are commonly used proteins. These antigens, composed of a large protein with attached haptenic groups, are called conjugate antigens.

Antibodies. Proteins that are formed in response to an antigen and react specifically with the antigen are antibodies. All antibodies belong to a family of proteins called immunoglobulins. Although the definition states that antibodies are formed only in response to an antigen, antisera may contain immunoglobulins that recognize and bind to certain antigens regardless of known exposure. These immunoglobulins are called natural antibodies (14).

Polyclonal Antibodies. To produce antibodies, a properly selected conjugate antigen, or large protein is injected into the host animal. The host's immune system will respond to the antigen because it is recognized as being a foreign substance. The resulting antibodies are a mixture. If the antigen is a large protein or a particulate antigen, with a number of repeating antigenic determinants or with one repeating determinant, some of the antibodies will bind to parts of the protein. If the antigen is a conjugate antigen, some antibodies will bind to parts of the carrier protein, while others will bind to the small molecule. Therefore, there are a number of different antigenic determinants to which the immune cells respond, resulting in a mixture of antibodies to those determinants. If a successful preparation is made, this mixed population will contain some antibodies that are very strong, that is their affinity and avidity for the foreign protein will be great.

Monoclonal Antibodies. These antibodies comprise a homogeneous population, all with the same specificity. They are produced and secreted by hybridomas, which are cells created by fusing hyperimmune spleen cells to myeloma cells. Mice are commonly used for the spleen cell donors as well as the species of origin of the myeloma cells. The spleen cells become immortalized (do not have a finite life span in culture) by fusion with the myeloma cells and can live forever if treated properly. (15) All of the cells in a population have originated from one cell separated and identified during cloning so that they all produce a homogeneous population of antibodies.

Immunoassay. A test format that employs antibodies that are specific for a certain antigen is called an immunoassay. They are used for detection of either antigens or antibodies. Where an assay is designed for detection of an antibody in a serum sample, another antibody is produced using the antibody of interest as the immunogen. In this case, the antibody that is to be detected is the antigen.

Isotopic Immunoassays. An assay in which hapten or antigen can be measured is an isotopic immunoassay. The assay is based on competition for antibody between a radioactive indicator antigen and its unlabeled counterpart in the test sample. As the amount of unlabeled antigen in the test sample increases, less labeled antigen is bound. The concentration of antigen in the test sample can be determined from comparison with a standard calibration curve prepared with known concentrations of the purified antigen (16).

The sensitivity of RIA assay is primarily limited by the amount of radioactivity that can be introduced into the radiolabeled antigen (17). Levels as low as 1 ng can be detected when carrier-free radioactive iodine ^{125}I is used as an extrinsic label. Precipitates are usually not evident, because extremely low concentrations are used. There are several procedures that can be used to separate free and bound indicator antigen. In the general method complexes of antibody bound to radiolabeled antigen are precipitated with antiserum prepared against the antibody moiety.

There are also a number of solid-phase assays that eliminate the requirement for the second antispecies antibody. In one method antibodies to the antigen are absorbed to the walls of plastic (eg, polystyrene) tubes. Radiolabeled antigen binds specifically to the adsorbed antigens and can be counted. When competing unlabeled antigen is also present, less radiolabeled antigen is proportionally bound. The remaining unbound fraction can be decanted. The method is inexpensive, rapid, and highly sensitive. For example, it is possible to detect less than 0.01 μg of antigen when a tube is coated with 1 μg of antibody (17).

Nonisotopic Immunoassays. These assays differ from isotopic immunoassays in terms of the type of label used, the means of end-point detection, and the possibility of bypassing a separation step. Fluoroimmunoassays and enzyme immunoassays are the two types of nonisotopic immunoassay.

Fluorescein and rhodamine are commonly used for labeling molecules. There are nonseparation fluoroimmunoassays that require no separation step of bound from unbound product. There are polarization fluoroimmunoassays that depend on antibody binding to enhance the signal, and quenching fluoroimmunoassays that depend on a decrease in signal from the bound fraction, which is attributed to the antibody's ability to impair the excitation, or the emission from the labeled hapten (18). A method has been developed for several aminoglycosides that enables the use of a simple fluorimeter (19).

Eias use enzyme labels and are grouped into two categories: homogeneous and heterogeneous. The difference between these two types of eias is that homogeneous assays requires no separation of unreacted reagents because the immune reaction affects the enzyme activity. Heterogeneous assays have separation steps; ELISA, a type of heterogeneous assay, requires washing between each step to remove unbound reagents (20). Commonly used enzyme labels include alkaline phosphatase (21), glucose oxidase (22), and horseradish peroxidase (23). These enzymes catalyze reactions that cause substrates to degrade and form a colored product that can be read spectrophotometrically or visually by eye. Depending on format, either antibody or antigen is adsorbed onto a solid phase, which can be polystyrene tubes, polystyrene microtiter wells, or membranes (ie, nitrocellulose and nylon).

Competitive Direct ELISA. This is an enzyme immunoassay where free hapten competes with an enzyme-labeled hapten for a number of limited antibody sites attached onto a solid phase. Unbound reactants are washed before substrate is added. Following substrate addition the color produced is indirectly related to the amount of hapten in the test sample.

Sandwich ELISA. This is an ELISA that is suitable for detecting large antigens (bacterial, viral, and other large proteins). Two preparations of antibodies are used, one to coat the solid phase and one onto which the enzyme is attached. These antibodies can have the same specificity or can be directed against separate antigenic determinants. The color development is directly related to the amount of antigen present. The assay derives its name from the position the antigen occupies in the test. It is sandwiched between the unlabeled antibody attached to the solid phase and the enzyme-labeled antibody, which is added following addition of the antigen. A washing is done between each step to remove unbound reactants. An indirect double sandwich elisa may also be developed. In this method the solid phase is coated with antibodies specific to the large protein produced in species A (eg, rabbit). Serial dilutions of standards and sample(s) are added and incubated for a specified time. Unbound antigen is removed by washing and a fixed amount of specific antibody from species (eg, mouse) is added. Following incubation and washing an antispecies antibody labeled with an enzyme (eg, goat antimouse IgG labeled with horseradish peroxidase) is added. The labeled antibody is incubated for a specified time and then the excess is removed by washing. Substrate is added and color development is directly related to the amount of antigen present (24).

Agglutination. The principle of the passive hemagglutination test is based on the least amount of soluble antigen required to inhibit agglutination of red blood cells. This is the amount of antigen in the last tube that will give a wide ring agglutination pattern (known as a mat). This concentration is the amount in the last tube that will still cause a mat to be formed (25).

Methods based on the agglutination are semiquantitative procedures. It is typical to use a twofold dilution scheme. Therefore, the procedures can only yield results that reflect the dilution sequence. If the interval between antigen concentration were greater, then the inherence error would be greater. On the other hand, the narrower the range, the more accurate the assay (14).

Hemagglutination assays are easy to use and possess the desired sensitivity. However, they require large quantities of antiserum and some antibody preparations do not react with red blood cells. The biggest potential drawback to their use is there may be nonspecific interactions due to other proteins that are capable of causing agglutination (24). Latex particles are also used for agglutination assays.

Affinity Column. Columns are packed with gel that has antibodies attached to the beads. These antibodies are often monoclonal antibodies. An extract is poured over the column and the substance that the antibodies are prepared against binds to the antibodies. The desired substance is then eluted from the column and analyzed. If the compound is fluorescent, such as the mycotoxin aflatoxin B_1, the solution can be placed into a cuvette and the fluorescence measured in a fluorometer. The sample eluted may also be analyzed by HPLC.

Immunodiffusion

Single Radial Immunodiffusion. The polyclonal antiserum prepared against the desired antigen is uniformly dispensed in an agar gel (26). Standards of known concentration of the antigen and sample extracts are placed in wells cut into the agar. Diffusion of the standards and samples into the agar cause formation of precipitin rings. At any given time the diameter of the rings is proportional to the initial antigen concentrations in the wells. Microgram quantities of antigen may be detected but the method requires large quantities of antiserum. The assay does not lend itself for analysis of sparingly soluble antigens. However, gels that contain urea do enable analysis of such proteins (eg, gluten).

Double Diffusion. Although double diffusion (27) methods are not quantitative, they provide the ability to gain information about the immunochemical relationships of several antigens. An extract may be screened against several different antisera by placing the extract in the center well and the antisera in peripheral wells.

Immunoelectrophoresis. In this procedure the antigen is electrophoretically separated, to provide resolution of different antigenic components in the mixture, prior to immunodiffusion against the antiserum (28). The antigen is electrophoresed in a gel, then a trough is cut parallel to the direction of the separation. The antiserum is placed in the trough and the separated antigens diffuse toward one another, which results in formation of precipitates.

Rocket Immunoelectrophoresis. In this method (29) the antiserum is dispersed into the agarose gel. The antigen standards and sample extracts are added to the small wells cut into one end of the gel. The antigens are then electrophoresed onto the antibody containing gel. The assay is set up so that the antigen migrates with little or no antibody migration. The rocket-shaped precipitates that form in the gel have heights that are dependent on antigen concentration.

Quantitative Precipitin Techniques. For quantitative precipitin techniques (30) an insoluble complex forms following interaction between a soluble antigen and specific antibodies in solution. The amount of precipitate is then analyzed by protein assay. The method is fairly sensitive but the assay requires long times to obtain maximal precipitate formation. Biphasic antigen concentration versus precipitate curves also may occur.

Nonimmunoassay

Bacterial Receptor Assay. Bacteria have surface receptors that will bind various antimicrobial agents. An assay can be designed in which a hapten (eg, sulfonamides or aminoglycosides) radiolabeled with ^{14}C or ^{3}H (tritiated thymidine) competes with the unlabeled counterpart in a sample for the bacterial receptors. The assay provides the desired sensitivity for detection of low levels of the haptens. However, the test lacks the ability to detect specific drugs within a particular family. For example, presence of any one or all of the family of β-lactams will be detected (penicillin G, ampicillin, cloxacillin, and cephalopsorins). A confirmatory test would have to be performed to identify which specific drug or which combination is involved in the reaction. Furthermore, the test employs radiolabeled reagents, which may present a health hazard. This is not an immunoassay format.

FOOD CONSTITUENTS

Nonmeat Protein Additives

A variety of nonmeat proteins can be added to processed meats and other foods. The most commonly used include soy, milk, and egg proteins. The proteins are called binders when they are added up to 3.5% of the total protein weight and extenders when they are added up to 10%. Fillers, emulsifiers, and stabilizers are other terms used in the literature.

Nonmeat proteins may be added to foods for any of the following reasons: (*1*) to reduce product costs by adding extenders; (*2*) to prevent water or fat loss thereby improving cooking yield, (*3*) to act as a binder and improve meat slicing, (*4*) to enhance flavor (egg and milk proteins are commonly added for this use); (*5*) to improve water or oil binding as well as to improve the stability of water soil emulsions, and (*6*) to increase or maintain the protein content of a meat product, while adding water or salt to it (31).

Since the late 1970s use of extenders has significantly increased, but the amount of meat substitutes that would be consumed is not nearly as high as the amount predicted. This is probably due to consumers' lack of desire to purchase artificial foods as well as a lack of cost advantage of product on these meat substitutes in numerous countries.

Development of immunoassays for detection and quantification of nonmeat proteins in foods is necessary both for routine surveillance of food products for adherence to government regulations and for control of diets of individuals suffering from food allergies and intolerance. For example, in the United States, vegetable and milk proteins are not permitted in traditionally named products. However, in foods with proper labeling they are allowed. For example, milk and vegetable proteins are permitted up to 3.5%. Products containing greater than this amount must be classified as imitation. Use of nonmeat proteins has gained acceptance in the U.S. diet with up to 30% hydrated vegetable protein being added to the meats for school lunch programs and the military. Soy ground beef patties are sold in supermarkets (31,32).

Rapid detection methods for nonmeat proteins can find widespread use for testing by individuals who suffer from food allergies and food intolerance who must avoid the offending food(s). Symptoms of food allergy may be manifested in the skin, gastrointestinal tract, and cardiovascular system. IgE antibodies that are specific to food proteins are found in the blood serum and on the surface of mast cells. Following binding of the protein allergen to the mast cells, allergic mediators are released that cause the allergy symptoms (33).

Grain and legumes such as soy, milk, and eggs can cause both food allergies and intolerance (34). Gastrointestinal disturbances are common to both food intolerance and hypersensitivity. Other symptoms include anemia, behavioral changes, and protein-losing enteropathy. Gluten-sensitive enteropathy is the symptom of food intolerance suffered by celiacs.

Nonimmunological methods for detection of nonmeat protein additives include microscopic and histological methods (32) and electrophoretic and chemical methods, based on amino acid analyses of peptides or analyses of nonprotein components characteristic of particular vegetable sources (35). These methods require expensive equipment and extensive sample preparation, so that immunological methods have been developed for detection of nonmeat protein additives.

Immunological methods other than RIA and EIA have been developed for detection of food proteins (Table 1). Agglutination assays exist for detection of food antigens. Hemagglutination inhibition assays are used for analysis of meat extracts and nonmeat proteins (36–38). These tests are quick and fairly sensitive but are only semiquantitative and may have nonspecific interactions due to nonprotein components in the food extracts.

Hemagglutination methods have been developed for detection of soy in meat products (39–42). The major problem with applying this methodology is that the need exists for establishing complex calibration curves to quantitate soy proteins in meat products. For example, good results were obtained with this method when meat products were heated only to 75°C but not when products were heated to temperatures exceeding 100°C (39). One group was able to quantify soy protein in meats cooked at 15°C (41) and another used indirect hemagglutination to detect <1% soy protein present in cooked sausages (42).

Hemagglutination methods have been used for quantifying milk protein in meat products. The method was only qualitative when autoclaved meats were tested (43). A similar method was developed with antigen-coated latex particles instead of red blood cells (44).

Milk proteins are considered easier to extract than other proteins (ie, gluten). However, some harsh solutions have been used for extraction prior to analysis using an immunochemical technique. Some of these extractants are 7 *m* urea (45) urea—mercaptoethanol (46,47) formic acid-methanol (48), and performic acid (49). Urea, TRIS, tricine, and performic acid treatment have been found to reduce the artifacts attributed to liver constituents in sausages

Table 1. Agglutination Gel Immunodiffusion Assays for Detection of Food Proteins

Assay	Food protein	References
	I. Agglutination	
Hemagglutination inhibition	Soy protein	40
	Soy protein in meat products	39,41
	Soy protein in cooked sausages	42
Hemagglutination	Milk protein in autoclaved meats	43
Latex agglutination	Milk protein in autoclaved meats	44
Hemagglutination inhibition	Milk protein in liver sausages	50
Indirect hemagglutination	Egg proteins in meats	43
	II. Quantitative	
Precipitin technique	Wheat seed protein	54
	Whey proteins in nonfat dry milk and buttermilk	55
	Casein and whey in foods	53
	III. Immunodiffusion	
Single radial immunodiffusion	Soy, casein egg white	56,57
	Soy in meat products	58,59
Double diffusion	Soy in	56,60–64
	Soy and wheat protein in meat	65
	Soy in meats, grits, flours, concentrates, isolates, and extrudates	66
	Milk proteins	59,64,67
	Whole egg or egg whites in meat	41,59,68
	Gliadins	68–71
Immunoelectrophoresis	Soy in meat products	63,72,73
	Milk protein in meat samples	74,75
	Origin of milk in	76

when hemagglutination is used for determining milk protein in liver sausages (50)

Another immunological method used for food analysis is the quantitative precipitin technique. An insoluble complex forms when soluble food antigens react with specific antibodies in solution. The precipitate is further analyzed by protein assay (30). Methods based on the turbidimetric or nephelometric analysis of suspended antigen–antibody complexes have become more popular (51–55). These methods are also automated for clinical use (31).

A large number of immunodiffusion assays have been developed for analysis of nonmeat proteins. A detailed list of references is provided in Table 1. It should be noted that although these methods have found widespread use for soy analysis there are a number of disadvantages encountered (77). (1) The diffusion in gel method is slow and is only suitable for limited numbers of samples. (2) Low recoveries of protein antigens are common because mild sample extraction conditions are used to decrease loss of protein antigenicity. (3) None of the antisera developed are able to recognize all of the soy protein derivatives. In addition, due to the low aqueous solubility and low molecular charge of gluten proteins, rocket immunoelectrophoretic methods have not found wide application in gluten detection. One qualitative method has been described (78). The classical methods based on antigen diffusion in gels are designed for optimal use with water-soluble proteins. Gluten and related cereal storage proteins are insoluble in aqueous buffers. Acidic buffer conditions have been used to analyze gluten extracts by double immunodiffusion (79) and 3 *M* urea containing gels have been used for gluten analysis by immunoelectrophoresis (80). Isotopic and nonisotopic ias have been described for nonmeat protein additives (Table 2).

An immunofluorescent method was described for detecting soy protein in meats. It was labor intensive with respect to sample preparation drying and fixing (81). The first RIA and EIA for soy protein determination were elucidated in the early 1980s (82,83). Another RIA was developed that detected native and formaldehyde-treated soy protein (83). There have been a number of EIAs developed for soy detection (Table 2). For example, a two-step antigen competition immunoassay was developed (83). The same investigators then analyzed a set of model beef burgers that contained known quantities of particular soy isolate (86). The effectiveness of the method has been tested in two collaborative studies (116,117). When compared to SDS–polyacrylamide gel electrophoresis (SDS–PAGE), neither method gave any false positives and similar interlaboratory variations were observed. More accurate determinations were obtained with the EIA with a range of flours, concentrates, isolates, and textured products, while the electrophoretic method showed less intralaboratory variation.

Table 2. Isotopic and Nonisotopic Immunoassays for Detection of Nonmeat Proteins

Assay	Sample	Ref.
Fluoroimmunoassay	Soy in cooked meats	81
Radioimmunoassay	Soy, gliadins	82–84
Enzymoimmunoassay	Soy in meat products	85–93
	Casein	94
	Hydrolyzed milk proteins in sausages	95,96
	Ovalbumin	97
	Gliadins	98–115

A great deal of information about the advantages of using ELISA for soy analysis can be gathered from reading the various references listed in Table 2. These articles describe the time saved when using ELISA over other methods, the extraction methods devised to overcome poor recoveries and immunoreactivity differences observed with different forms of soy proteins, attempts to develop a simpler ELISA than the two-step antigen competition immunoassay, assays designed on nitrocellulose solid phase, and the details of collaborative studies.

EIAs have been successfully developed for whole caseins (94) as well as those for detection of hydrolyzed milk protein in cooked meat products (95,96). Detection of ovalbumin in canned mushrooms was achieved by indirect EIA and SDS–PAGE immunoblotting techniques (97). The protein is widely used in canned mushrooms because it enhances water retention and drained weight of the product.

Wheat gluten is one of the least expensive sources of edible protein. It is also able to bind meat fragments in processed meats and is used as an extender in foods. Due to the large number of individuals who cannot tolerate wheat gluten and related proteins from rye and barley a great deal of effort has been spent on developing ELISA for rapid detection of gluten and related cereal proteins. Application of elisa to gluten analysis has become extensive. Gluten is the protein-rich mass left after a wheat flour dough is washed to remove starch, water, salt, and soluble proteins. It is composed of several dozen protein components that total over 80% of the flour protein. There are two major fractions, gliadin (mol wt 30,000–75,000) and glutenin (mol wt 20,000–100,000). The rhelogical properties in wheaten dough come from gluten (118). Much of the immunological work has been carried out on gliadin due to its greater extractability and solubility than gluten (Table 2).

Because gluten exhibits low aqueous solubility and low molecular charge, electroimmunodiffusion techniques have not found wide applicability. There have been numerous descriptions of RIAs and EIAs for gliadin detection (Table 2). A major problem with the EIA and immunodiffusion tests is their unsuitability for analysis of cooked or processed foods (31). The high-mobility gliadin components, α- and β-gliadin, are probably the most toxic gliadins in gluten intolerance, and their extractability and immunoreactivity is decreased with heating (105). However, cooked foods containing gluten retain their toxicity (31). Monoclonal antibodies have been produced to Ω-gliadins (101). These antibodies enabled detection of gliadin in foods after cooking (100°C for 100 min).

The need for improved extraction methods has been addressed. Seventy percent ethanol has been used to extract gluten from uncooked foods (106,109,110). This solvent is fairly selective for gliadin and may help to reduce matrix affects from other food proteins. Extraction of protein with urea has been found to provide more sensitive gliadin detection than aqueous alcohols (101). The appropriate solvent for each test format must be determined at the time of assay development.

With the trend toward governmental regulations and industry controls on food components and quality, it is possible that the amount of growth observed in antibody-based medical diagnostics that has taken place within the last decade will be equaled in the next decade in antibody-based food analysis. Food analysis has traditionally been viewed as a discipline derived from analytical chemistry. Now with the capabilities presented by EIA, food analysis will use commercially available kits for routine testing of large samples (31).

Meat Protein Additives

There have been IA developed for detection of meat proteins. Meat is the portion of animal flesh ingested as a food source. Biochemical and biophysical changes occur in the muscles following the death of the animal. These edible tissues are composed primarily of proteins that are also the most important constituents of meat. The major meat proteins are the myofibrillar, sarcoplasmic, and connective tissue proteins (24). There are similar proteins in all the muscles of all species with the interspecies differences being extremely small. Therefore, the ability to differentiate meat from different species by visual, histological, and empirical chemical analyses is nearly impossible to do (24).

At slaughterhouses and meat-cutting plants it is standard procedure to do hot deboning on the carcasses; which are then packaged as primal joints, chilled, aged, and possibly frozen prior to shipment to retail outlets. This process has been touted as a major cost-saving measure with respect to energy costs, chiller space, and weight loss resulting from the evaporation. These savings produce greater yields of salable meat (119). Using these practices the anatomical differences in the skeletons do not permit identification of the species of origin of the meat (24).

The need exists for reliable analytical methods to monitor meat speciation to insure fair trading and for consumer protection. It is a well-known fact that eating habits differ from one society to the next largely due to cultural and religious practices. A food item considered a delicacy in one culture may be viewed as a taboo by another. Therefore, it is possible that low-cost meats may be added as substitutes to frozen meat that are not considered food by some societies. The methods must be able to identify both fresh and heat-treated meat and meat products. Immunoassays satisfy these requirements (24).

A number of immunoassays have been developed for detection of meat proteins in fresh meats (Table 3). Immunoelectrophoresis has been used to identify the presence of beef and pork in fresh meat products (120). Different species of fish have also been identified using this electrophoretic method (121). This technique provides excellent resolution of antigenic components due to their differential migration in an electric field.

Immunodiffusion (139) methods have found wide application in analysis of fresh meat (122–128). Hemagglutination inhibition tests were developed for detection of adulterants in meat (129,130). This method was also used to test insect blood meats and identify the species of animals that these hematophagous insects had fed on (131).

Table 3. Immunoassays for Detection of Free-Meat Proteins

Assay	Sample	Ref.
I. Gel Immunodiffusion		
Immunoelectrophoresis	Beef, pork, fish	120,121
Double immunodiffusion	Meat (cow, pig, sheep, horse)	122–128
II. Agglutination		
Hemagglutination inhibition	Meats, insect blood meals	129–131
III. Labeled Immunoassays		
Radioimmunoassay	Cattle meat	132
Enzymoimmunoassay		
Indirect competitive	Horse meat	133
Indirect noncompetitive	Horse meat, beef	134–136
Sandwich elisa	Meat specification	137,138

Rias have not been extensively used for meat protein detection due to the short shelf life of the reagents, expensive equipment required for analysis, potential health hazards of exposure to radioisotopes, and governmental regulations pertaining to radioisotope use. A RIA was developed that could distinguish cattle meat from sheep, donkey, pig, horse, and kangaroo meats. Contamination as low as 5% could be determined (132).

A number of different eias have been developed for fresh meat speciation. Current slaughter methods leave residual blood, as much as 1.5–2.0% in the muscle tissue (140). Some of the blood proteins, namely the albumins and immunoglobulins, have served as immunogens for producing antibody-based meat species testing kits (133,141). An indirect competitive ELISA for meat speciation that enabled detection of 0–40% horse meat in beef has been developed (133).

Competitive ELISAs have been employed for quantitative determinations, whereas qualitative species identification of fresh meats have been obtained with indirect noncompetitive ELISAs (133–136,142,143). Horse meat was differentiated from beef and mutton with an indirect ELISA in which an antibody raised against horse serum albumin was used. Adulteration of beef with goat and kangaroo was also elucidated with an indirect ELISA.

The ELISA variants are not applied for quantification because they have relied on the presence of serum antigens that can vary greatly in meat and are not indicative of the meat content (24). Stunning and bleeding methods greatly affect the content of serum proteins in the muscles (142). The stability of the antigen with time is also a factor (143). Therefore, antigens that are genuine muscle components that are indicative of the meat content must be developed (124).

Sandwich ELISAs also permit speciation of meat (137,138). Adulteration of raw meat mixtures with pig meat was identified (129), and improved speciation of raw meat was achieved using this ELISA format. Levels as low as 0.5% (137) and 17% adulteration have been detected (138).

The task of identifying the species of origin of cooked or heat-processed meats becomes more difficult than speciation of fresh meat. Heating denatures or changes the species-specific epitopes that are used for identifying the species of fresh meat (24). When meat is heated at 45°C denaturation of myofibrillar and sarcoplasmic proteins occurs (144), and at 60°C collagen shortens and changes into a more soluble form (145). As the temperature is raised from 60 to 90°C, the percentage of collagen solubilized increases gradually (146). Therefore, to develop an immunoassay that permits identification of heat-treated meat, antigens must be isolated that will retain their species specificity following the heating process. They must not be lost in cooking juices and must be antigenic and immunogenic.

The first EIA developed (147) enabled detection of origin of heat-treated meats. Kangaroo meat was detected in frankfurter-type sausages and dry sausages. Antisera produced against thermostable muscle antigens (TMAs) were used to develop an indirect ELISA that enabled the detection of origin of heat-treated meats from closely related species (148). Immunodiffusion tests have demonstrated that TMAs are species, organ, and tissue specific (149–151).

Monoclonal antibodies were used to achieve speciation of heat-treated meats because cross-reactions could be eliminated. Monoclonal antibodies were produced against heat-treated kangaroo and cattle meats, which differentiated the two kinds of meat in an elisa (152). Use of monoclonal antibodies has the advantage of being specific without requiring absorptions needed when polyclonal antisera are used; therefore, the future trend would be to make use of monoclonal antibodies for speciation of meats from closely related species.

NATURAL CONTAMINANTS

Natural Toxicants

There are natural components of food that can potentially be toxic to consumers; however, attention has been largely focused on detecting food additives and contaminants and not these natural toxicants. It is true that many of the plant and animal portions that might cause toxic effects have over time been avoided by humans or are deemed safe for consumption when they are prepared in a certain manner. When normal practices are followed the naturally occurring toxins do not present acute problems to the consumer. It is when these normal practices are changed that problems can arise (153).

Until recently a dearth of information existed concerning toxin occurrence, toxicology, and metabolism following ingestion of foodstuffs containing natural toxins. One of the biggest problems has been the dilemma presented by analysis. Chemical methods (HPLC and mass spectroscopy) have helped improve toxin analysis. Immunoassays have greatly furthered this effort. They can be highly specific, which is desirable, because compounds with very similar structures will exhibit widely different toxicity potential. The high specificity will eliminate chances of misclassification of compounds. Immunoassays can also be very sensitive, which would permit detection of toxin metabolism following ingestion. The ability to quickly test large numbers of samples is also desirable. It is common for natural toxicants to exhibit nonhomogeneous patterns of distributions so that multiple subsamples must be tested to obtain data about overall concentrations. The multiple samples can also be tested at low cost (153).

A microtiter-well elisa for quinine detection in soft drinks has been developed (154). It is, of course, added as a bittering agent. Although the risk from consumption of soft drinks containing it is low, quality-control procedures for its analysis are important to the soft drink industry. The ability to test easily for the compound permits the industry to provide greater control on a potentially dangerous substance, which will boost consumer confidence in the product containing the substance.

A number of immunoassays have been described for caffeine which is found in a variety of food products, namely those containing teas, coffee, and chocolate. A radioimmunoassay and a substrate-labeled fluoroimmunoassay for caffeine detection have been described (155,156). Assays have been developed for analysis of naringin and limonin, which are bitter substances isolated from citrus fruits. The ria for naringin (157) and the elisa for limonin (158) permit the analysis of the distribution and metabolism of the compounds in the fruit. Assays of this nature may also be of use to citrus growers for quality-control testing of both raw materials and finished products.

Many toxic compounds are found in the potato, *Solanum tuberosum*. One group is collectively known as the potato glycoalkaloids. Ninety-five percent or more of the tuber is normally composed of α-chaconine, α-solanine, and derivatives of solanidine (159).

A ria, the first immunoassay described for glycoalkaloid analysis (160), employed an antiserum that did not recognize α-chaconine and α-solanine. A more useful elisa was developed (161) that enabled detection of all three compounds. It has been shown to have good correlation with conventional chemical methods (162,163). Immunoassays have been developed for testing glycoalkaloid concentrations in body fluids. Solanidine can be detected in serum (164,165) and saliva using a RIA.

Immunoassays that permit lycoalkaloid detection can be used by plant breeders to screen tubers of all potential new varieties. Testing can be performed on the retail level as well to prevent glycoalkaloid concentrations from exceeding 20 mg/1.0 g (166).

Mycotoxins

Mycotoxins are a chemically diverse group of secondary fungal metabolites that are toxic following ingestion or environmental exposure (167). Mycotoxins are haptens with molecular weights of 300–400. Many of these molecules are nonpolar and exhibit low solubility in water. The fungi that produce the mycotoxins are ubiquitous. They can grow and elaborate the toxins in numerous agriculture commodities either in the field or following harvest during storage and processing. The factors that influence toxin elaboration are temperature, relative humidity, and moisture content of the substrate. As a result of their toxicity, mycotoxins can cause severe economic losses to farmers and livestock producers as well as pose a threat to humans consuming contaminated foods. Affected agricultural commodities include corn, wheat, peanuts, cottonseed, rice, sorghum, almonds, walnuts, and milk (168).

The target tissues that can be affected by mycotoxins are the liver, kidney, spleen, gastrointestinal tract, lymphoid tissue, reproductive organs, skin, and nervous system. In the United States the most important toxins are the aflatoxins, zearalenone, the trichothecenes and to a lesser extent ochratoxin. These toxins are produced by species of *Aspergillus* and *Fusarium* and cause a variety of health problems to livestock and humans (Table 4).

Classical methods of analysis of mycotoxins include TLC, HPLC, GC, and mass spectroscopy (MS). TLC methods have found wide use because they are easy to perform, are inexpensive, and are relatively sensitive. Many of the mycotoxins naturally, fluoresce and absorb in the UV region, so that TLC methods (169–171) while TLC determination of mycotoxins are frequently only semiquantitative and require elaborate sample cleanup. GC and HPLC methods offer sensitive quantitative determinations of mycotoxins (172–174). Sample cleanup is also extensive and labor intensive prior to analysis by these methods. Due to these drawbacks, development of immunochemical methods for mycotoxin detection have become popular.

The Aflatoxins. The majority of work on mycotoxins has focused on the aflatoxins, the family of difurancoumarins, which act as hepatotoxins and hepatocarcinogens (175). Aflatoxin B_1 (AFB_1) is reported to be the most prevalent and most potent carcinogen. Results from the modified salmonella mutagenesis test show that AFB_1 is about 30 times more mutagenic than AFG_1 or AFM_1, which are more mutagenic than AFB_2, AFG_2, or AFM_2 (Fig. 1) (176). Similar patterns exist for hepatotoxicity and carcinogenicity. The biological effects of the toxin are attributed to the difuran ring with 8:9 vinyl ether, while the coumarin ring absorbs at 360 nm and fluoresces. The Food and Drug Administration (FDA) has set an action level of 20 ppb total aflatoxins in foods or feeds.

AFB_1 can be metabolized by hepatic enzymes (*in vivo* or *in vitro*) to different metabolite (173,174). The carcinogenicity results from the reaction of the 8,9 epoxide with cellular nucleophiles (RNA, DNA, and proteins). Other detoxification pathways involve reduction or hydroxylation, which form more polar compounds that are then removed from the animal. One hydroxylated metabolite, AFM_1, is also hepatocarcinogenic and will be found in milk of lactating animals that have ingested AFB_1 contaminated feed (177). The action set by the FDA for AFM_1 in dairy products is 0.5 ppb.

The Trichothecenes. Many of the mycotoxins produced by *Fusarium* species and other fungi (*Cephalosporium*, *Verticimonosporium*, *Myrothecium*, and *Stachybotrys*) are called trichothecenes (178). These mycotoxins are tetracyclic sesquiterpenoid molecules, all possessing an epoxy group. T-2 toxin and deoxynivalenol (DOH), also known as vomitoxin (Fig. 2), are frequently identified in *Fusarium* culture filtrates. The toxins cause a strong dermatitis reaction when applied to the skin of shaved guinea pigs, characterized by severe local irritation, inflammation, desquamation, and necrosis (175).

There have been more than 148 trichothecenes identified, but only four have been unambiguously identified in naturally contaminated food samples. Trichothecenes are difficult to analyze especially because, unlike the aflatoxins, they do not possess any useful spectroscopic characteristics. Analysis of the trichothecenes have been accomplished using TLC (179), HPLC (180,181), GC (182), and MS (183,184).

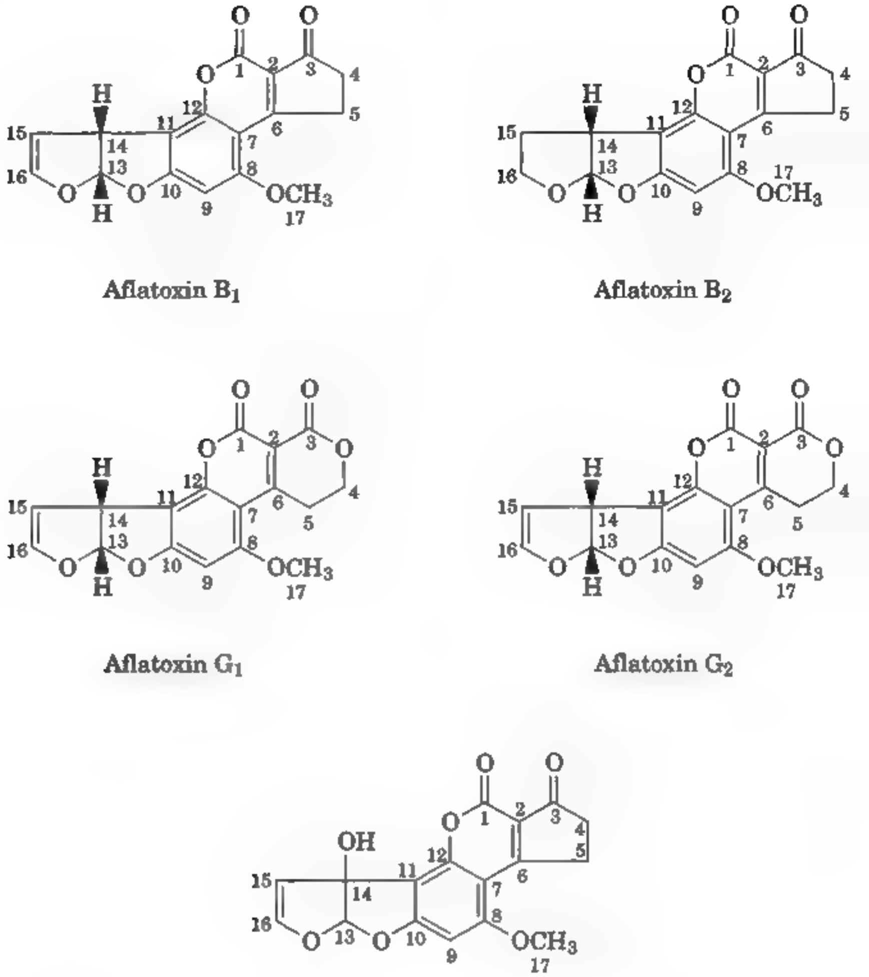
The trichothecenes are believed to function as protein synthesis inhibitors. The initiation or elongation termination steps of protein synthesis are believed to be affected.

The Zearalenones. *Fusarium* species, primarily *F. Sraminearium*, produce zearalenones which are resorcylic lactones (Fig. 3). The parent compound is zearalenone, which does not exhibit acute toxicity. However, high levels of zearalenone can interrupt normal estrus in sows and cause vulvar prolapse and enlargement of the uterus. In young males, the toxin causes testicular atrophy and mammary gland hyperplasia. Physiological response in swine occur when the zearalenone level in corn used for feeds exceeds about 1 ppm (185). Rats, mice, sheep, monkeys, and humans can be affected by zearalenone (168).

The Ochratoxins. Ochratoxin contamination of grains and food products is less of a concern in the United States than contamination of products with aflatoxins, zearalenones, and trichothecenes. It is frequently detected in Scandinavian and Balkan countries and occasionally in the United States in barley, corn, wheat, oats, rye, peanuts, hay, and green coffee beans (186). Ochratoxins are a group of structurally related metabolites produced by *Aspergillus ochraceus* and related species as well as *Penicillium viri-*

Table 4. Abbreviated List of Major Mycotoxins and the Potential Health Hazards Following Exposure

Mycotoxin	Fungi	Potential Health Risk
Aflatoxins	*Aspergillus flavus, A. parasiticus*	Mutagenic, teratogenic, hepatotoxic, and carcinogenic
Trichothecenes	*Fusarium* sp.	Feed refusal, emesis, immunotoxicity, gastrointestinal tract hemorrhaging, alimentary toxic aleukia
Zearalenones	*Fusarium roseum* and other *Fusarium* sp.	Estrogenic, infertility, and reproductive problems in cattle, swine, and poultry
Ochratoxins	*Aspergillus* sp. and *Penicillium* sp.	Nephrotoxic, teratogenic, and immunotoxic



Aflatoxin B_1

Aflatoxin B_2

Aflatoxin G_1

Aflatoxin G_2

Aflatoxin M_1

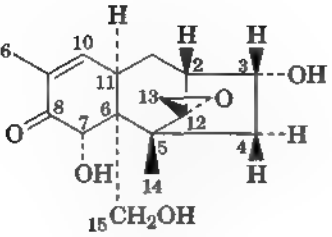
Figure 1. Structures of important aflatoxins.

dicatum and other *Penicillium* species. Ochratoxin A (OA) is the major mycotoxin in this group (Fig. 4). It has believed to cause endemic kidney disease in swine and poultry in Denmark and Sweden. When the hogs were fed diets containing 200 ppb OA, their kidneys became pale and swollen. The proximal tubules and interstitial cortical fibrils were observed to undergo atrophy (187). The toxin has been implicated in causing the human disease known as Balkan endemic nephropathy (187,188). Occurrence and toxicology of the toxin were recently reviewed (179,189).

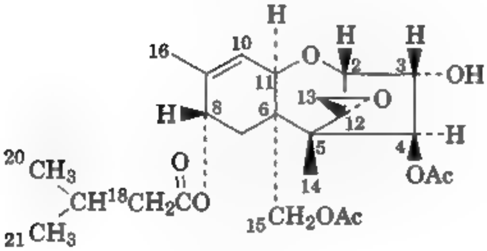
Mycotoxin Occurrence in the Food Supply. It is possible for feeds and foods to become contaminated with mycotoxins prior to harvest, during the interval between harvest and drying and during storage. There are some mycotoxins that are only produced in the field (ie, ergot). Mycotoxins can, therefore, be considered to occur naturally in raw agricultural products, processed foods and imported products (190).

There are a number of documented occurrences of mycotoxins in raw products (190–194), processed foods (195–202) and imported products (191,192,199). Most of the information available is for the aflatoxins with less information available for ochratoxin A, trichothecenes, and zearalenone. An extensive review was published that described the effects of food processing on selected toxins (200). The stability of the mycotoxins during food processing is affected by a number of factors such as the type of food, the type of processing used, the additives, moisture content, and the amount and type of contamination (189). The mycotoxins display different degrees of variability in foods under processing conditions. It has been demonstrated that the aflatoxins are stable to moderately stable in the majority of processes. However, they are unstable when alkaline conditions and oxidizing steps are used. DON is stable during bread baking, while ergot alkaloids have been shown to be partially destroyed (190).

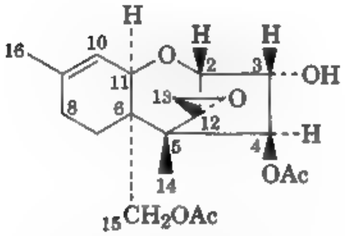
Although a number of mycotoxins could be identified in imported foods and feeds, the FDA only routinely tests for aflatoxins. Under the FDA compliance programs, the FDA tests about 300 samples of imported foods (eg, almonds, brazil nuts, peanuts and peanut products, sesame seeds, sunflower seeds, nutmeg, coffee beans, marzipan, cornmeal and flour, cocoa products) and about 200 feed samples (eg, corn, mixed feeds, cottonseed meal, copra pellets, and sorghum). Summaries of analyses from 1982 to 1996 are



Deoxynivalenol



T-2



Diacetoxyscirpenol

Figure 2. Structures of three trichothecanes.

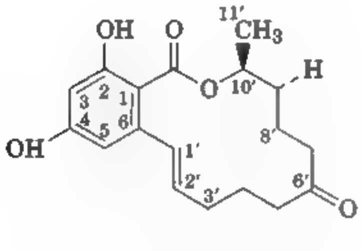


Figure 3. Zearalenone.

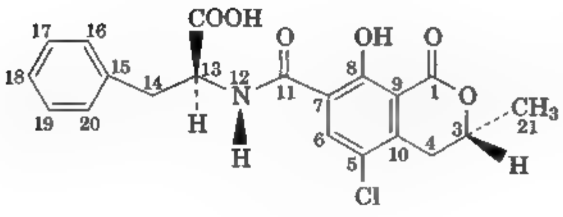


Figure 4. Ochratoxin.

provided (198), and information is also available on worldwide occurrence of mycotoxins. The data were collected from the FAO/WHO/UNMEP food contamination monitoring program in addition to other sources (198).

Regulatory Control of Mycotoxins. Although a wide range of mycotoxins have been found in the environment associated with human food and animal feeds, there is only formal regulation of aflatoxins by the FDA. The FDA does not regulate the other known mycotoxins based on the observed levels, incidence, estimated consumption, and toxicological profiles (190). The aflatoxins are regulated under the Food, Drug and Cosmetic Act Section 402(a)(1). In fact, in the United States when good manufacturing practices (GMPs) are followed, aflatoxins are regarded as unavoidable contaminants in food and feeds (191). When food is slated for human consumption, the action level of 20 ppb total aflatoxins is enforced, while for milk the level is much lower, 0.5 ppb AFM_1. The action level for aflatoxins in feeds is also 20 ppb. However, levels of 100 ppb are permitted in feeds slated for consumption by breeding cattle and swine and mature poultry. Levels of 200 ppb are allowed in food corn destined for finishing swine ($\leq$100 lb) and 300 ppb are allowed for aflatoxins in cottonseed meal incorporated in feeds and also for corn fed to feedlot beef cattle (190).

In Europe, regulatory control of mycotoxins is governed by the European Economic Communities (EEC) a body composed of 12 countries (Belgium, France, Germany, Italy, Luxembourg, the Netherlands, Denmark, the UK, Ireland, Spain, Portugal, and Greece). The formation of the EEC began with the Treaty of Rome in 1957. In 1987 the treaty was modified due to the establishment of the Single European Act. It described the stepwise establishment of the Common European Market by the end of 1992. Harmonization of food laws must be accomplished by the EEC to achieve this objective. The control of contaminants is one issue that must be addressed (203–207).

There have been increasing numbers of publications of immunochemical methods for detection of the various mycotoxins. It is now common practice to use ELISA to screen commodities for presence of mycotoxins (208). RIAs are described in some of the earlier publications. The majority of tests have been developed for aflatoxin detection (Table 5). The most attention has been focused on the aflatoxins due to their strict regulation by governmental agencies in the U.S. and other countries. There is a wealth of information provided in the references listed in Table 5. There are papers that describe assays that employ monoclonal antibodies (211,216,221) as well as those that employ polyclonal antibodies (209,210,212,215,217–220). There are descriptions of the chemistry involved in conjugate antigen production (208–224). The way in which the aflatoxin was derivatized and coupled to the large carrier molecule influences the specificity of the antibody (the antibody's ability to recognize and bind to only aflatoxin B_1 if other metabolites are bound as well). There are papers that provide comparisons of ELISAs and chemical methods when certain commodities are analyzed for aflatoxins (228) as well as comparisons of various commercial kits (229). There are also reports from different collaborative studies (225–227).

Table 5. List of Selected Immunochemical Methods for Mycotoxin Detection

Toxin	Method	References
Aflatoxin B_1	RIA	209–212
	ELISA	212–233
	Affinity column	231,234
Aflatoxin M_1	RIA	235,236
	ELISA	221,237–240
	Affinity column	241,242
Zearalenone	RIA	243
	ELISA	219,244–249
	Affinity column	249
T-2 toxin	RIA	250,251
	ELISA	230,252–258
Deoxynivalenol	RIA	259
	ELISA	260,261
Ochratoxin A	RIA	262,263
	ELISA	264–268

Both monoclonal (211,237) and polyclonal antibodies were produced against aflatoxin M-1. RIAs (235,236), ELISAs (221,237–240), and an affinity column cleanup using monoclonal antibodies (234,242) followed by direct fluorescence measurement of aflatoxin M-1 in raw milk (240) have been described. In some cases milk could be tested without any cleanup (221,235), whereas, some methods required extraction of the samples prior to analysis (235,238–242).

Zearalenone can be detected by RIA and ELISA (243,249) (Table 5). An affinity column was developed with a monoclonal antibody (247,249) and used to clean up milk samples prior to analysis by ELISA. The monoclonal and polyclonal antibodies described in these references (243,249) exhibit varying degrees of cross-reactivity to α- and β-Zearalenol. Zearalenone is metabolized to these analogues and they have been detected in trace levels in bovine milk after cows were fed feed containing high levels of zearalenone (269,270). Detection of these analogue using the antibodies is an attractive feature.

There have been immunoassays published for detection of various trichothecenes (Table 5). Both RIA and ELISA have been described for T-2, some of which employ monoclonal antibodies (230,252–255) and some employ polyclonal antibodies (256–258). A recent report was made of an immunochromatographic method (271) for isolation of group A trichothecenes of which T-2 is a member. These various antibodies exhibit differing degrees of cross-reactivity to 3′-OHT-2 and other metabolites. Detection of these metabolites in biological systems would be useful, because they are found in significant amounts when T-2 toxicosis occurs (272–274).

To date there have been few reports of antibodies produced to DON and their application to RIA or ELISA (Table 5). Two investigators have demonstrated repeated success at developing IAs for mycotoxin detection. Chu (University of Wisconsin at Madison, Food Institute) is regarded as one pioneer in mycotoxin analysis with emphasis in the area of immunoassay development. Another is Pestka (Michigan State University, Department of Food Science and Human Nutrition) a former postdoctoral associate in Chu's laboratory. The successes in developing antibodies to DON have been reported from their laboratories. Successful immunogens were prepared for immunization of mice (261) and rabbits (259,260) which enabled detection of DON by RIA and ELISA. Table 6 also provides a list of IAs developed for ochratoxin A detection. Ochratoxin A can be detected in barley (265) and wheat (266).

A number of companies sell kits that enable detection of various mycotoxins. The majority of commercial kits sold are for aflatoxin testing. The different companies claim differing cross-reactivities of their antibodies employed in their aflatoxin B_1 detection kits. Aflatoxin G_1, G_2, and B_2 are also detected in many of the kits. Aflatoxin B_1 detection ELISA kits are sold by the following companies: Neogen Corp. (Lansing, Mich.), International Diagnostics (St. Joseph, Mich.), Idexx (Portland, Maine), Environmental Diagnostics Corp. (Burlington, N.C.), and Transia (Lyon, France). Affinity columns are sold by VICAM (Somerville, Mass.) and Oxoid (Columbia, Md.). Neogen and Idexx sell AFM-1 ELISAs. Aflatoxin M_1 affinity columns are sold by VICAM and Oxoid. Environmental Diagnostics Corp. and Ube (distributor for the Japanese company is Wako Chemicals, Dallas, Tex.) market ELISA kits for ochratoxin A detection. Neogen Corp. and Environmental Diagnostics market elisa for zearalenone and T-2 toxin testing. Neogen Corp. the only company that markets an elisa kit that enables the user to test for the presence of DON in samples.

MICROBIAL CONTAMINANTS

Consumer in recent years have expressed considerable concern over the safety of the food supply. Concern has been aimed at the presence of hormones and drugs in meats, preservatives found in food, pesticides, microbial contaminants, and food additives. Reports last year of cyanide in grapes and Alar in apples have fueled the level of concern. This issue of the public's concern over the quality of food was addressed at a conference (the International Conference on Issues in Food Safety and Toxicology) held in the spring of 1990 at Michigan State University (MSU) East Lansing, Mich. The conference was sponsored by the U.S. Department of Agriculture (USDA) and MSU's Center for Environmental Toxicology. From various presentations it became apparent that the consumers' perceptions of risks from chemical agents in food differs drastically from the experts' opinions. The experts rank the risk from chemicals below microbial risk or nutritional considerations. The general public perceives the risks to be greatest from the chemical agents. It would be a gross omission to prepare a publication of this nature without providing a list of references for those individuals interested in reading more about IAs developed for detection of pathogenic microbes, particularly bacterial pathogens found in food. Detection of *Staphylococcus aureus* enterotoxins, *Clostridium perfringens* type A toxin, *Clostridium botulinum* toxins, *Bacillus cereus* enterotoxins, *Yersinia enterocolitica* toxin, *Escherichia coli* toxins, *Campylbacter jejuni, Aeromonas* toxin, and some streptococcol toxins would be desirable with IAs. *Salmonella* and *Listeria monocytogenes* can also be detected in foods using IA. Table 6 provides a list of

Table 5. List of Selected Immunochemical Methods for Mycotoxin Detection

Toxin	Method	References
Aflatoxin B_1	RIA	209–212
	ELISA	212–233
	Affinity column	231,234
Aflatoxin M_1	RIA	235,236
	ELISA	221,237–240
	Affinity column	241,242
Zearalenone	RIA	243
	ELISA	219,244–249
	Affinity column	249
T-2 toxin	RIA	250,251
	ELISA	230,252–258
Deoxynivalenol	RIA	259
	ELISA	260,261
Ochratoxin A	RIA	262,263
	ELISA	264–268

Both monoclonal (211,237) and polyclonal antibodies were produced against aflatoxin M-1. RIAs (235,236), ELISAs (221,237–240), and an affinity column cleanup using monoclonal antibodies (234,242) followed by direct fluorescence measurement of aflatoxin M-1 in raw milk (240) have been described. In some cases milk could be tested without any cleanup (221,235), whereas, some methods required extraction of the samples prior to analysis (235,238–242).

Zearalenone can be detected by RIA and ELISA (243,249) (Table 5). An affinity column was developed with a monoclonal antibody (247,249) and used to clean up milk samples prior to analysis by ELISA. The monoclonal and polyclonal antibodies described in these references (243,249) exhibit varying degrees of cross-reactivity to α- and β-Zearalenol. Zearalenone is metabolized to these analogues and they have been detected in trace levels in bovine milk after cows were fed feed containing high levels of zearalenone (269,270). Detection of these analogue using the antibodies is an attractive feature.

There have been immunoassays published for detection of various trichothecenes (Table 5). Both RIA and ELISA have been described for T-2, some of which employ monoclonal antibodies (230,252–255) and some employ polyclonal antibodies (256–258). A recent report was made of an immunochromatographic method (271) for isolation of group A trichothecenes of which T-2 is a member. These various antibodies exhibit differing degrees of cross-reactivity to 3′-OHT-2 and other metabolites. Detection of these metabolites in biological systems would be useful, because they are found in significant amounts when T-2 toxicosis occurs (272–274).

To date there have been few reports of antibodies produced to DON and their application to RIA or ELISA (Table 5). Two investigators have demonstrated repeated success at developing IAs for mycotoxin detection. Chu (University of Wisconsin at Madison, Food Institute) is regarded as one pioneer in mycotoxin analysis with emphasis in the area of immunoassay development. Another is Pestka (Michigan State University, Department of Food Science and Human Nutrition) a former postdoctoral associate in Chu's laboratory. The successes in developing antibodies to DON have been reported from their laboratories. Successful immunogens were prepared for immunization of mice (261) and rabbits (259,260) which enabled detection of DON by RIA and ELISA. Table 6 also provides a list of IAs developed for ochratoxin A detection. Ochratoxin A can be detected in barley (265) and wheat (266).

A number of companies sell kits that enable detection of various mycotoxins. The majority of commercial kits sold are for aflatoxin testing. The different companies claim differing cross-reactivities of their antibodies employed in their aflatoxin B_1 detection kits. Aflatoxin G_1, G_2, and B_2 are also detected in many of the kits. Aflatoxin B_1 detection ELISA kits are sold by the following companies: Neogen Corp. (Lansing, Mich.), International Diagnostics (St. Joseph, Mich.), Idexx (Portland, Maine), Environmental Diagnostics Corp. (Burlington, N.C.), and Transia (Lyon, France). Affinity columns are sold by VICAM (Somerville, Mass.) and Oxoid (Columbia, Md.). Neogen and Idexx sell AFM-1 ELISAs. Aflatoxin M_1 affinity columns are sold by VICAM and Oxoid. Environmental Diagnostics Corp. and Ube (distributor for the Japanese company is Wako Chemicals, Dallas, Tex.) market ELISA kits for ochratoxin A detection. Neogen Corp. and Environmental Diagnostics market elisa for zearalenone and T-2 toxin testing. Neogen Corp. the only company that markets an elisa kit that enables the user to test for the presence of DON in samples.

MICROBIAL CONTAMINANTS

Consumer in recent years have expressed considerable concern over the safety of the food supply. Concern has been aimed at the presence of hormones and drugs in meats, preservatives found in food, pesticides, microbial contaminants, and food additives. Reports last year of cyanide in grapes and Alar in apples have fueled the level of concern. This issue of the public's concern over the quality of food was addressed at a conference (the International Conference on Issues in Food Safety and Toxicology) held in the spring of 1990 at Michigan State University (MSU) East Lansing, Mich. The conference was sponsored by the U.S. Department of Agriculture (USDA) and MSU's Center for Environmental Toxicology. From various presentations it became apparent that the consumers' perceptions of risks from chemical agents in food differs drastically from the experts' opinions. The experts rank the risk from chemicals below microbial risk or nutritional considerations. The general public perceives the risks to be greatest from the chemical agents. It would be a gross omission to prepare a publication of this nature without providing a list of references for those individuals interested in reading more about IAs developed for detection of pathogenic microbes, particularly bacterial pathogens found in food. Detection of *Staphylococcus aureus* enterotoxins, *Clostridium perfringens* type A toxin, *Clostridium botulinum* toxins, *Bacillus cereus* enterotoxins, *Yersinia enterocolitica* toxin, *Escherichia coli* toxins, *Campylbacter jejuni*, *Aeromonas* toxin, and some streptococcol toxins would be desirable with IAs. *Salmonella* and *Listeria monocytogenes* can also be detected in foods using IA. Table 6 provides a list of

Table 6. Selected Immunoassays for Toxin Detection

	Toxin	References
	Immunodiffusion	
Single-gel diffusion	*Staphylococcus aureus*	275,276
	Clostridium perfringens	277
	Clostridium botulinum	278
Single-radial immunodiffusion	*S. aureus*	279
Double-gel diffusion	*S. aureus*	275,277,280–283
	Electroimmuno diffusion	
	S. aureus	284
	Agglutination	
Hemagglutination inhibition	*S. aureus*	285–287
Reverse passive hemagglutination	*C. bolutinum*	288
Latex agglutination	*S. aureus*	289
	C. perfingens	290
	Radioimmunoassay	
	S. aureus	291–305
	Escherichia coli	306,307
	C. botulinum	308,309
	C. perfingens	310
	ELISA	
	S. aureus	311–327
	C. botulinum	328–338
	C. perfringen	339–344
	E. coli	345–350

selected publications of IAs for detection of bacterial toxins. The table lists the type of assay, the species of bacterium that the assay was developed to detect, and references. It does not describe which *S. aureus* toxin or toxins are detected in the assays. Some assays only detect enterotoxin B, while others detect A and B or A, B, C, and D. To provide information on other agents that are considered to be endangering the food supply only a general list of sources has been provided. Information on risks associated with vehicles of food-borne pathogens and toxins is available (353).

To detect *Salmonella* in foods, investigators have relied on culture procedures that enable growth, selection, isolation, and identification of isolated organisms (354). The length of time needed to obtain a negative reading has always posed a problem. To overcome this disadvantage, numerous rapid testing methods have been proposed. They have not found wide acceptance due to problems with sensitivity and specificity (355). Two reports indicated the potential for use of EIA for screening for *Salmonella* (356,357) in foods. Since then there have been other reports of *Salmonella* detection using EIAs (358–363).

Listeria detection using an immunobased test requires highly specific antibodies because *Listeria* species have been shown to be antigenically related to other gram-positive organisms, namely *Staphylococcus, Streptococcus, Erisipelothrix*, and *Bacillus* species (364–368). Several groups have been successful at producing monoclonal antibodies to a genus-specific antigen (369–371).

There are several commercial kits available for toxin and pathogen detection. Oxoid Ltd. (Hampshire, UK) sells revised passive latex agglutination assays for detection of *S. aureus* toxins A, B, C, and D; *Clostridium perfringens* enterotoxin; *Vibrio chlorae* and *E. coli* heat-labile enterotoxin; and *S. aureus* toxic shock syndrome toxin. Organon Teknika Corp. (Durham, N.C.) sells Salmonella-Tek and Listeria-Tek which are ELISAs.

CHEMICAL AGENTS

Pesticides

Pesticides are used to produce high-quality, inexpensive food. With the use of pesticides, agricultural residues can be expected to occur in foods. Regulation of pesticides is shared by three federal agencies. The U.S. Environmental protection Agency (EPA) is responsible for registering or approving the use of pesticides and to setting tolerance, if residues will occur in food. The USDA is responsible for pesticides used on meat and poultry, and the FDA is responsible for enforcing tolerances for foods shipped in interstate commerce (372). Many states, including California, Florida, and Michigan, have pesticide analytical programs.

The FDA carries out a large-scale monitoring program, the objective of which is to prevent foods that contain illegal residues from entering interstate commerce. Testing of raw agricultural commodities is the focus of the monitoring. To date about 15,000 samples, 8,000 imported and 7,000 domestic (372), are collected and analyzed by the 21 field offices.

There are 300 pesticides and active ingredients with tolerances in or on foods in addition to other pesticides and related chemicals that can exist as residues (373). The EPA has supplied data showing that 53 of these pesticides have active ingredients that have been shown to be oncogenic or potentially oncogenic (351).

Gas chromatography has been used for analysis of pesticides (352,374–376). These methods were developed by the early 1960s. The use of immunoassays has spread into the area of pesticide analysis. A brief summary of chemicals that control weeds or pests in agricultural crops will be provided. There have been IAs described in the literature since the mid-1970s (Table 7). Prior to 1982 the majority of IAs described for pesticide analysis were rias and since that date there have been reports of EIAs. Much less attention has been given to development of fluoroimmunoassays for pesticide analysis.

With a few exceptions, the majority of tests developed for pesticide analysis employ polyclonal antibodies raised in rabbits. There have been some monoclonal antibodies produced against pentachlorophenol (406), surflan (407), malic hydrazide (399), paraquat (403), and paraoxon (401).

Table 7. Immunochemical Methods for Detection of Pesticides

Assay	Pesticide	Ref.
Radioimmunoassay	Aldrin	377
	Dieldrin	377
	Benomyl	378
	S-Bioallethrin	379
	2,4-D	380
	2,4,5T	381
	Diflubenzuron	382
	Parathion	383
	Paraquat	384,385
Fluoroimmunoassay	Benomyl	386
	Diclofop-methyl	386
Enzyme immunoassay	3,4-D	387
	Alachlor	388
	Aldicarb	389
	Atrazine	390
	S-Bioallethrin	391
	Blasticidin S	392
	Chlorsulfuron	393
	Cyanazine	394
	Dichlofopmethyl	395
	Diflubenzuron	396
	Fenpropimorph	397
	Irpodione	398
	Malic hydrazide	399
	Metalaxyl	400
	Paraoxon	401
	Paraquat	402–405
	Pentachlorophenol	406
	Surflan	407
	Terbutryn	408
	Triadimefon	409
	Triazines	410
Affinity chromatography	Kepone	411

For the majority of tests listed in Table 7 the limit of detection is 1 ng of pesticide, which corresponds to 1–10 ppb in a sample solution. RIA and EIA offer comparable sensitivity. The detection limit of RIA is more readily varied because it depends largely on the specific activity of the radioisotope label (17). Use of RIA is restricted to sophisticated laboratories with substantial investment in the equipment. EIA, with ELISA in particular, offers more versatility in analysis than RIA.

There are a number of pesticide detection kits sold commercially. Immuno Systems, Inc. (Biddeford, Maine) sells ELISA kits for triazine (atrazine, simazine, etc) detection as well as one for the cyclodiene insecticides (chlordane, heptachlor, etc). ELISAs for parathion and paraquat are available from Environmental Diagnostics, Inc. (Burlington, N.C.). An assay for pentachlorophenol detection is available from Westinghouse Bio-Analytical Systems (Rockville, Md.). Ohmicron Corp. (Newtown, Pa.) markets pesticide detection kits for aldicarb, atrazine, benomyl, alachlor, captan–captofol, and carbafuran that employ magnetic particle technology.

There are factors that make applications of immunoassays to pesticide analysis complex. It is often difficult to obtain representative samples of agricultural commodities. Commodities are localized temporally and geographically. Local practices can vary leading to complicated research efforts toward assay development. Agricultural commodities are often composed of mixtures of the pesticide and its metabolites at harvest. The high specificity of antibodies used in assays may be disadvantageous because metabolites may not be detected. Also there is fragmentation within the potential pesticide market, potential customers or users could be farmers, consumers, pesticide manufacturers, regulatory agency personnel, food processors, wholesalers, and retailers. The requirement for assay features can vary drastically among these users. Therefore, it is not easy for companies to identify customer needs and project the economic potential of the kits (373).

Officials at regulatory agencies have expressed concern about adopting these qualitative or quantitative immunochemical assays for routine screening of samples. Concern stems first from the need for familiarization and experience with the technology involved in the test systems. Once the testers feel confident that public health protection is not jeopardized by implementing this technology, they must address other concerns. It must be demonstrated for each method, presented in kit form, that there is no appreciable variability from lot to lot production (373).

Many of these immunochemical methods are more sensitive than the confirmatory tests and traditional methods. Therefore, qualitative screening results using IAs cannot be confirmed without effort given to increase the sensitivity of the confirmatory methods (373). As the concerns are addressed, the technology will continue to develop and practical applications of IAs for routine pesticide analysis will occur.

Drug Residues

There is an increasing number of publications that describe the development of ELISAs for drug residue analysis. One antimicrobial agent, sulfamethazine, has received a great deal of attention in the scientific literature as well as the media. There have been numerous reports of the use, or perhaps the abuse, of sulfamethazine in the livestock industry. It will be used as an example of how ELISAs have been developed for detection of the drug in place of the classical chemical methods.

Sulfonamides, namely sulfamethazine, have been incorporated into animal feeds since the 1950s. They are added as growth promotants and for control of certain diseases (412,413). The drugs are retained in the tissues of the animals eating medicated feeds. Some individuals who ingest the contaminated tissues will experience hypersensitivity allergic reactions. Furthermore, there will be preferential selection of bacterial mutants that are resistant to the drugs, which are also used in treatment of human diseases (414).

The residues are cleared from the tissues if the proper 15 day withdrawal period is followed by the producer (415). If it is not adhered to there will be a good chance for the carcasses to contain violative levels of the drug at the time of slaughter (>0.1 ppm).

Sulfonamide residues can also occur in milk due to a number of reasons, including use of the drugs in mastitis therapy, deliberate feeding, inadvertent feeding, or use of sulfamethazine-containing boluses to prevent infection in cows that have calved. One study (416) showed the 64% of milk sampled in the New York City area, central New Jersey, and eastern Pennsylvania contained one or more antibiotic–antimicrobial residues. Sulfonamide residues appeared in 42% of the samples. Other studies showed similar frequencies of the residues in milk (417,418). The FDA maintains a zero-effect tolerance. However, because approved methods cannot accurately measure extremely low levels of the sulfonamides, the tolerance level is set at 10 ppb.

The concern over the presence of sulfamethazine in meat, namely pork, and dairy products heightened when the drug was found to cause neoplasms in mice and rats (419). The need for a rapid screening method became apparent. Current methods for sulfonamide analysis include gas chromatography (1,3,420,421), gas chromatography–mass spectrophotometric (419), liquid chromatography (1,421–423), colorimetry based on the Bratton–Marshall reaction, and thin-layer chromatography (421,425–427) as well as tandem mass spectroscopy–mass spectroscopy (428,429). These methods are labor intensive. They require extensive sample cleanup or preparation and they often measure at the tolerance level. They do not lend themselves to screening samples in the field. A bacterial receptor assay (430) has been developed for detection of numerous sulfonamides. It can be used for screening samples; however, it lacks the specificity that an antibody-based test provides. The Charm Assay is marketed by Penicillin Assays, Inc. (Malden, Mass.).

The Food Safety and Inspection Service (FSIS) branch of the USDA began a residue avoidance program (RAP) designed to check animals before they are slaughtered (431). Sulfamethazine has been shown to cause the most violations in swine tissue, approximately 95% of the sulfonamide tissue violations (432). A correlation has been established for the concentrations of sulfamethazine present in the liver and edible tissues as well as how much is present in the urine and serum (432–435). Noninvasive samples can be collected and tested prior to or at the time of slaughter and tested to determine the concentration of the drug in the liver and edible tissues.

The early ELISA developed for detection of sulfamethazine in swine blood (436) required extraction of the drug from the sample and required fairly long times to perform the assay. They were not suitable for use at slaughterhouses. More recent publications describe assays with shorter times and serum or plasma samples can be used directly without extraction (437,438). Assays have also been described for detection of sulfamethazine in milk (439), feed, and tissue (440). A recent paper describes the use of high-performance immunoaffinity chromatography for drug residue analysis (441).

A number of biotechnology companies market inmunodiagnostics for sulfamethazine detection in dairy products (Neogen Corp., Lansing, Mich.; Idetek, San Bruno, Calif.; and Idexx, Portland, Maine), feed (Neogen Corp., Idetek, and Environmental Diagnostics, Inc., Burlington, N.C.), and urine and tissue (Idetek and Environmental Diagnostics).

CONCLUSION

This article has only begun to scratch the surface of all the research concerning immunoassay development for topics of interest in agriculture and the food industry. This is a day and age where consumers express concern over the quality of the food. With an ever-increasing awareness of the importance of a balanced diet and perceived dangers from additives and contaminants, as well as increased legislation, the need for the ability to test large numbers of samples routinely for the presence of potentially dangerous or offending substances becomes highly apparent. The selectivity and sensitivity of immunoassays make them excellent candidates as tools for routine analysis. ELISAs are highly appropriate assays for analyzing food and agricultural residues because they can be developed to qualitatively screen for the desired substance or they may be semiquantitative or quantitative. These assays can be cost effective, require little or no expensive equipment, permit rapid throughput of many samples, and can be performed by relatively unskilled personnel. These tests provide excellent accuracy and reproducibility, as well as sensitivity and specificity.

Over the next decade advances in immunoassays will benefit food analysts. It appears that those test formats that are the most user friendly for the end user will gain a great deal of ground. The less complex a test is to perform the more attractive it will be to nonlaboratory personnel (farmer, grain inspector, etc). Over the next decade it is likely that ELISAs will be routinely used for screening foods and feeds at governmental agencies as well as by quality-control chemists in the private sector. It is truly an exciting time to work in an area where so much evolution in analytical technology is occurring.

BIBLIOGRAPHY

1. M. C Allred and D. L. Dunmire, *J. Chromatogr. Sci.* **16**, 553–557 (1978).
2. A. Fravolini and A. Begliomini, *J. Assoc. Off. Anal. Chem.* **52**, 767–769 (1969).
3. R. J Daun, *J. Assoc. Off. Anal. Chem.* **54**, 1277–1288 (1971).
4. "Section 976.22," *Official Methods of Analysis*, 15th ed., Association of Official Analytical Chemists, 1990, pp. 1211–1212.
5. "Sections 986.17 and 986.18," in Ref. 4, pp. 1205–1206.
6. "Sections 980 20 and 989.06," in Ref. 4, pp. 1192–1195.
7. R. W Beaver, *J. Assoc. Off. Anal. Chem.* **73**, 69–71 (1990).
8. M. Omura, K. Hashimoto, K. Ohta, T. Tio, S. Ueda, K. Ando, H. Hiraide, and N. Kinae, *J. Ass. Offic. Anal. Chem.* **73**, 300–305 (1990).
9. R. G. Nash, *J. Assoc. Off. Anal. Chem.* **73**, 438–442 (1990).
10. L. Q. Huang and J. J. Pignatello, *J. Assoc. Off. Anal. Chem.* **73**, 443–446 (1990).
11. M. F. Delaney, *Liquid Chromatogr* **2**, 85–86 (1984).
12. F. Kavanaugh, *Analytical Microbiology*, Vol. 2, Academic Press, Inc., Orlando, Fla., 1972.
13. L. F. Knudsen and W. A. Randall, *J. Bacteriol.* **50**, 187–200 (1945).
14. D. E. Dixon, S. J. Steiner, and S. E. Katz, in A. Azalos, ed., *Modern Analysis of Antibiotics*, Marcel Dekker, Inc., New York, 1986, pp. 415–431.
15. J. W Goding, *J. Immunol. Methods* **39**, 285–308 (1980).
16. R. Luft and R. S. Yalow, *Radioimmunoassay Methodology and Application in Physiology and in Clinical Studies*, George Thieme Verlag, Stuttgard, 1974.
17. H. Eisen, *Immunology: An Introduction to Molecular and Cellular Principles of the Immune Responses*, Harper & Row, New York, 1980.
18. A. J. Munro, J. Landon, and E. J. Shaw, *J. Antimicrob. Chemother.* **9**, 423–424 (1982).
19. E. J. Shaw, R. A. A. Watson, J. Landon, and D. S. Smith, *J. Clin. Pathol.* **30**, 526–531 (1977).
20. E. Engvall and P. Perlman, *Biochem. Biophys. Acta.* **251**, (1971).
21. S Avrameas, *Immunochemistry* **6**, 43–53 (1968).
22. R. Maiolin, B. Ferrua, and R. Masseyeff, *J. Immunol. Methods* **6**, 355–362 (1975).
23. E. Engvall and P. Perlmann *Immunochemistry* **8**, 871–874 (1971).
24. K. E. Kang'ethe, "Use of Immunoassays in Monitoring Meat Protein Additives," in J. H. Rittenburg, ed., *Development and Application of Immunoassay for Food Analysis*, Elsevier Applied Science, Publishers, Ltd., Barking, UK, 1990, pp. 127–139
25. S. J. Steiner, *The Development of a Model Immunological Assay System for the Detection of Antibiotic Residues, Ph.D. dissertation, Rutgers University New Brunswick, N.J., 1981.*
26. G. Mancini, A. O Carbonara, and J. F. Heremans, *Immunochemistry* **2**, 235–254 (1965).
27. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **26**, 507–515 (1949)
28. P. Grabar and C. A. Williams, *Biochim. Biophys. Acta* **10**, 193–194 (1953).
29. C B. Laurell, *Anal. Biochem.* **15**, 45–52 (1966).
30. M. Heidelberger and F. E. Kendall, *J. Exp. Med.* **55**, 555–561 (1932).
31. J. H. Skerrit, "Immunoassays of Non-Meat Protein Additives in Food," in Ref. 24, pp. 81–125.
32. S. K. Raghavan, C. T. Ho, and H. Daun, *J. Chromatogr.* **351**, 195–202 (1986).
33. E. Bleumink, *Proc. Nutr. Soc.* **42**, 219–231 (1983).
34. S. L. Taylor, *Food Allergies Food Technol.* **39**, 98–105 (1985).
35. I. Kloczko and A. Rutkowski, *Nahrung* **28**, 9–13 (1984).
36. E. Degenkolb and M. Hingerle, *Arch. Lebensm. Hyg.* **18**, 241–247 (1967).
37. L. Kotter and C. Hermann, *Arch. Lebensm. Hyg.* **19**, 267–270 (1968).
38. C. Hermann, C. Merkle, and L. Kotter, *Fleischwirtschaft* **51**, 249–251 (1973).
39. H. Kruger and D. Grossklaus, *Fleischwirtschaft* **51**, 315–320 (1971).
40. E. J. Menzel, *Z. Ernahrungswiss.* **20**, 55–68 (1981).
41. C. Hermann and G. Wagenstaller, *Arch. Lebensm. Hyg* **24**, 131–134 (1973).
42. C. Ring and F. Sacher, *Fleischwirtschaft* **64**, 355–357 (1984).
43. C. Hermann, C. Merkl, and L. Kotter, *Ann. Nutr. Aliment.* **31**, 153–155 (1977).
44. G. Fromm, *Fleisch. Konserven. Arch. Hyg. Bakkt.* **151**, 702–708 (1967).
45. H. J. Sinell, and I. Mentz, *Folio Vet. Lat.* **7**, 41–54 (1977).
46. J. H. Fernandez and I. S. Lopez, *Proc. Eur. Mtg. Meat Res. Workers* **28**, 434–435 (1982).
47. M. I. Santillana, E. Ruiz, E. Casado, J. Huescar, and R. Jimenez, *Bol. Cent. Nac. Aliment. Nutr.* **10**, 10–12 (1982).
48. H. Klostermeyer and S. Offt, *Z. Lebensm. Unters-Forsch.* **167**, 158–161 (1978).
49. M. Bellatti and G. Parolari, *Ind. Conserv.* **54**, 3–5 (1979).
50. H J. Sinell, *Proc. Eur. Mtg. Meat Res. Workers* **27**, 5590–5593 (1981).
51. E. Gombocz, E. Hellwig, and F. Petuely. *Proc. Eur. Mtg. Meat Res. Workers* **27**, 594–597 (1981).
52. E. Gombocz, E. Hellwig, and F. Petuely, *Z. Lebensm. Unters. Forsch.* **172**, 355–361 (1981).
53. E. Gombocz and F. Petuely, *Z. Lebensm. Unters.-Forsch.* **177**, 2–7 (1983).
54. G. N. Festenstein, F. C. Hay, B. J. Miflin, and P. R. Shewry, *Planta* **164**, 135–141 (1985).
55. S. P. Greiner, G. J. Keilen, and D. E. Carpenter, *J. Food Sci.* **50**, 1106–1109 (1985).
56. J. Kraack, *Fleischwirtschaft* **53**, 697–698 (1973); **53**, 701–702 (1973).
57. J. Daussant and D. Bureau, "Immunochemical Methods in Food Analysis," in R. D. King, ed., *Development in Food Analysis Techniques 3*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1984, pp. 175–210.
58. M. Peter, *Arch. Lebensm. Hyg* **21**, 220–222 (1970).
59. E. Hauser, J. Bicanova, and W. Kuenzler, *Mitt. Geb. Lebensm. Hyg.* **65**, 82–89 (1974).
60. L. A. Hanson, *Veterinaer* **16**, 201–205 (1964).
61. O. Wyler and J. J. Siegrist, *Mitt Geb. Lebensm. Hyg.* **56**, 299–303 (1965).
62. H. Guenther, *Bestimmung Fremdeweiss Arch. Lebensm. Hyg.* **20**, 97–106 (1969).
63. H. J. Guenther, *Bestimmung Fremdeiweiss Fleuschwaren. Arch. Lebensm. Hyg.* **20**, 128–131 (1969).

64. O. D. Wyler, *Fleischwirtschaft* **51**, 1743–1745 (1971).
65. H. O. Guenther, *Ann. Nutr. Aliment.* **31**, 179–181 (1977).
66. J. C. Hammont, I. C. Cohen, and B Flaherty, *J. Assoc. Publ. Analysts* **14**, 119–126 (1976).
67. E Hauser, J. Bicanova, and W. Kuenzler, *Fleischwirtschaft* **54**, 240–242 (1979).
68. M. J. Escrebiano and P. Grabar, *Ann. Inst. Pasteur Paris* **110** (Suppl. 3), 84–88 (1966).
69. C. C. Nummo and M. T. O'Sullivan, *Cereal Chem.* **44**, 584–591 (1967).
70. M R. Booth and J. A. D. Ewart, *J. Sci. Food Agr* **21**, 187-192 (1970).
71. J. Zareba, *Pol. Tyg. Lek.* **23**, 1962–1963 (1968).
72. H. J. Sinell, *Lebensm. Arch. Lebensm. Hyg.* **19**, 121–125 (1968).
73. L. A. Appelguist, *SIK Rapport* **371**, 1–23 (1975).
74. R. Kluge-Wilm and H. J. Sinell, *Milchwissenschaft* **27**, 160–165 (1972).
75. H. O. Guenther, *Lebensm. Unters.-Forsch.* **149**, 98–99 (1972).
76. H. Elbertzhagen and E. Wenzel, *Z. Lebensm. Unters.-Forsch.* **175**, 15–16 (1982).
77. J. W. Llewellyn and R. Sawyer, *Ann. Nutr. Aliment.* **31**, 157–159 (1977).
78. S. Baudner, *Getreide Mehl Brot.* **32**, 330–337 (1978).
79. G. A. H Elton and J. A. D. Ewart, *J. Sci. Food Agr.* **14**, 750–758 (1963).
80. M. Benhamou-Glynn, M. J. Escribano, and P. Grabar, *Bull. Soc. Chim. Biol* **47**, 141–156 (1965).
81. J. Heitmann, *Fleischwirtschaft* **67**, 621–622 (1987).
82. E. J. Menzel and F. Glatz, *Z. Lebensm. Unters.-Forsch.* **172**, 12–19 (1981).
83. J. E. Menzel and H. Hagemeister, *Z. Lebensm. Unters.-Forsch.* **175**, 211–214 (1982).
84. P. J. Ciclitira and E. S. Lennox, *Clin. Sci.* **64**, 655–659 (1983).
85. C. H. S. Hitchcock, F. J Bailey, A. A. Crimes, D. A. G. Dean, and P. J. Davis, *J. Sci. Food Agr.* **37**, 157–165 (1981).
86. N. M. Griffiths, M. J. Billington, A. A. Crimes, C. H. S. Hitchcock, *J. Sci. Food Agr.* **35**, 1255–1260 (1984).
87. J. H. Rittenburg, A. Adams, J. Palmer, and J. C. Allen, *J. Assoc. Off. Anal. Chem.* **70**, 582–587 (1987).
88. D. B. Berkowitz and D. W. Webert, *J. Assoc. Off. Anal. Chem.* **70**, 85–90 (1987).
89. F. W. Janssen, G. Voortman, and J. A. deBaaij, *Z. Lebensm. Unters.-Forsch.* **182**, 479–483 (1986).
90. P Ravestein and R. A. Driedonks, *J. Food Technol.* **21**, 19–32 (1986).
91. F. W. Janssen, G. Voortman, and J. A. deBaaij, *J. Agr. Food Chem.* **35**, 563–567 (1987).
92. C. C. Hall, C. H. S. Hitchcock, and R. Wood, *J. Assoc. Publ. Analysts* **25**, 1–27 (1987).
93. Eur. Pat. 111,762 (1984), P. J. Davis, and P. Poter
94 D. Lifier and J C. Callin, *LeLait* **62**, 541–548 (1982).
95. P. Teufel and V. Sacher, *Fleischwertschaft* **62**, 1474–1476 (1982).
96. C. Staak and U. Kaempe, *Fleischwertschaft* **62**, 1477–1478 (1982).
97. C. Breton, L. Phan Thanh, and A. Paraf, *J. Food Sci.* **53**, 226–230 (1988).
98. J. H. Skerritt and R. A. Smith, *J Sci. Food Agr.* **36**, 980–986 (1985).
99. J. H Skerritt, *Food Technol. Aust.* **37**, 570–572 (1985).
100. J. H. Skerritt, *J. Sci. Food Agric.* **36**, 987–994 (1985).
101. J. H. Skerritt, J. A. Diment, and C. W. Wrigley, *J. Sci. Food Agr.* **36**, 995–1003 (1985).
102. R. Troncone, M. Vitale, A. Donatello, E. Farris, G. Rossi, and S. Aurrichio, *J. Immunol. Methods* **92**, 21–23 (1986).
103. H. Windemann, F. Fritschy, and E. Baumgartner, *Biochim. Biophys. Acta* **709**, 110–121 (1982).
104. P. J. Ciclitira, H. J. Ellis, D. J. Evans, E. S. Lennox, and R. H. Dowling, *Gut* **24**, A487 (1983).
105. J. H. Skerritt, R. A. Smith, C. W Wrigley, and P. A. Underwood, *J. Cereal Sci.* **2**, 215–224 (1984).
106. F. Fitschy, H. Wendemann, and E. Baumgartner, *Z. Lebensm. Unters.-Forsch.* **181**, 379–385 (1985).
107. P. J. Gosling, D. F. McKellog, and P. F Fottrel, "The Measurement of Wheat Ghadin by Enzyme Immunoassay," in *Protein Evaluation in Cereals and Legumes. Proceedings of the EEC Workshop, Thessobonikı, Greece*, Oct. 1985.
108. J. H. Skerritt and O. Martinuzzi, *J. Immunol. Methods* **88**, 217–224 (1986)
109. A. R. Freedman, G. Galfre, E. Gal, H. J. Ellis, and P. J. Ciclitera, *J. Immunol. Methods* **98**, 123–127 (1987).
110. A. R. Freedman, G. Galfre, E. Gal, H. J. Ellis and P. J. Ciclitera, *Clin. Chim. Acta* **166**, 323–328 (1987).
111. J. H. Skerritt, K. L. Jenkins, and A. S. Hill, *Food Agr. Immunol.* **1**, 161–172 (1989).
112. A. S. Hill and J H. Skerritt, *Food Agr. Immunol.* **1**, 147–160 (1989).
113. J. H. Skerritt and A. S. Hill, *J. Cereal Sci.* **11**, 103–122 (1990).
114. J. H. Skerritt and P. Y. Lew, *J. Cereal Sci.* **11**, 103–122 (1990).
115. R. B. Johnson, J. T. Labrody, and J. H. Skerritt, *Clin. Exp. Immunol.* **79**, 135–140 (1990).
116. A. A. Crimes, C. H. S. Hitchcock, and R. Wood, *J. Assoc. Publ. Analysts* **22**, 59–78 (1984).
117. W. J. Olsman, S. Dobbelaere, and C. H. S. Hitchcock, *J. Sci. Food Agr* **36**, 499–507 (1985).
118. B. J. Miflin, J. M. Field, and P. R. Shewry, in J. Daussant, J. Vaughan, and J. Mosse, eds., *Cereal Storage Proteins and their Effects on Technological Properties in Seed Proteins*, Academic Press, Inc., Orlando, Fla., 1985, pp. 255–319.
119. A. Cuthbertson, "Hot Processing Meat. A Review of the Rationale and Economic Implications," in *Development in Meat Science*, Vol. 1, Allied Publishers, New Delhi, 1980, pp. 66–88.
120. C. Casas, J. Tormo, P. E. Hernandez, and B. Sanz, *J. Food Technol.* **19**, 283–287 (1984); *J. Sci. Food Agr.* **35**, 793–796 (1984).
121. J. G. Sutton, J. Goodwin, G. Horscroft, R. E Stockdale, and A. Frake, *J. Assoc. Off. Anal. Chem.* **66**, 1164–1174 (1983).
122. K. S. Swarts and C. R. Wilks, *Aust Vet J.* **59**, 21 (1982).
123. F. D. Shaw, E. M. Deane, and D. W. Cooper, *Aust. Vet. J.* **60**, 25 (1982).
124. L. J. Glesson, W. J. Slattery, and A. J. Sinclair, *Aust. Vet. J.* **60**, 127 (1983).
125. I. G. S. Furminger, *Nature* **202**, 1332 (1964).
126. A. R. Hayden, *J. Food Sci.* **42**, 1189–1192 (1977).
127. A. R. Hayden, *J. Food Sci.* **43**, 476–478 (1977).
128. R. P. Mageau, M. E. Cutrufelli, B. Schwab, and R. W. Johnston, *J. Assoc. Off. Anal. Chem.* **67**, 949–954 (1984).

129. T. Kamiyama, Y. Katsube, and K. Imaizumi, *Jpn. J. Vet. Sci.* **40**, 653–661 (1978).

130. T. Kamiyama, Y. Katsube, and K. Imaizumi, *Jpn. J. Vet. Sci.* **40**, 663–669 (1978).

131. K. J. Lindqvist, J. M. Gathuma, and H. F. A. Kaburia, "Analysis of Blood Meals of Haemotophagous Insects by Haemagglutination Inhibition and Enzyme Immunoassay," in P. M. Tukei and R. M. Njogu, eds., *Proceedings of 3rd Medical Scientific Conference Current Medical Research in Eastern Africa*, Africa-Science International Publishers, Ltd., Nairobi, Kenya, 1982, pp. 122–133.

132. L. A. Y. Johnston, P. Tracey-Patte, R. A. Donaldson, and B. Parkinson, *Aust. Vet. J.* **59**, 59 (1982).

133. E. K. Kang'ethe, S. J. Jones, and R. L. S. Patterson, *Meat Sci.* **7**, 229–240 (1982).

134. M. R. Patterson and T. L. Spenser, *Meat Sci.* **15**, 119–123 (1985).

135. R. G. Whittaker, T. L. Spencer, and J. W Copland, *Aust. Vet. J* **59**, 125 (1982).

136. R. G. Whittaker, T. L. Spencer, J. W. Copland, *J. Sci. Food Agr.* **34**, 1143–1148 (1983).

137. S. J. Jones and R. L. S. Patterson, *Meat Sci.* **15**, 1–13 (1985)

138. R. M. Patterson, R. F. Whittaker, and T. L. Spencer, *J. Sci. Food Agr.* **35**, 1018–1023 (1984).

139. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **25**, 186–191 (1948).

140. P. D. Warriss, *J. Sci. Food. Agr.* **28**, 457–462 (1977).

141. I. R. Tizard, N. A. Fish, and F. Cadi, *J. Food Prot.* **45**, 353–355 (1982).

142. E. K. Kang'ethe, *Master's of science thesis*, University of Bristol, UK, 1981.

143. N. M. Griffiths and M. J. Billington, *J. Sci. Food Agr.* **35**, 909–914 (1984).

144. R. A. Lawrie, *Meat Science* 3rd ed., Pergamon Press, Oxford, UK, p. 77.

145. S. M. Machlik and H. N. Daudt, *J. Food Sci.* **28**, 711–718 (1963).

146. J. M. C. K. Snowden and J. F. Weidemann, *Meat Sci.* **2**, 1–18 (1978).

147. J. Manz, *Fleischwirtschaft* **63**, 1767–1769 (1983).

148. E. K. Kang'ethe and J. M. Gathuma, *Meat Sci.* **19**, 265–270 (1987).

149. E. K. Kang'ethe, K. J. Lindgvist, and J. M. Gathuma, "Immunological Reactions of Thermostable Muscle Antigens and Then Possible Use in Speciation of Cooked and Fresh Meats," in R. L. S. Patterson, ed., *Biochemical Identification of Meat Species*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1985, pp. 128–144.

150. E. K. Kang'ethe, J. M. Gathuma, and K. J. Lindqvist, *J. Sci. Food Agric.* **39**, 179–184 (1986).

151. E. K. Kang'ethe and K. J. Lindquist, "Species Identification of Internal Organs Using Antisera to Thermostable Muscle Antigens," in E. Peteaja, ed., *Proceedings of 33rd International Congress of Meat Science and Technology*, Helsinki, Finland, pp. 377–378.

152. R. Goerlich and E. Greuel, *Arch. Lebensm.* **37**, 87–90 (1986).

153. M. R. A. Morgan, and H. A. Lee, "Mycotoxins and Natural Food Toxicants," in Ref. 24, pp. 143–170.

154. C. M Ward and M. R. A. Morgan, *Food Addit. Contam.* **5**, 555–561 (1988).

155. C. E. Cook, C. R. Tallent, E. W. Amerson, M. W. Myers, J. A. Kepler, G. F. Taylor, and H. D. Christensen, *J. Pharm. Exp. Ther.* **199**, 679–686 (1976).

156. S. Pearson, J. M. Smith, and V. Marks, *Ann. Clin. Biochem.* **21**, 208–212 (1984).

157. P. S. Jourdan, E. W. Weiler, and R. L. Mansell, *J. Agr. Food Chem.* **31**, 1249–1255 (1983).

158. P. S. Jourdan, R. L. Mansell, D G. Oliver, and E W. Weiber, *Anal. Biochem.* **138**, 19–24 (1984).

159. V. Paseshnickenko and A. R. Guseva, *Biochemistry (USSR)* **21**, 606–611 (1956).

160. R. P. Vallejo and C. D. Ercegovich, *JNBS (U.S.)* **519** (Spec. Publ), 330–340 (1979).

161. M. R. A. Morgan, R. M. C. Nerney, J. A. Matthew, D. T. Coxon, and H. W.-S. Chan, *J. Sci. Food Agr.* **34**, 593–598 (1982).

162. M. R. A. Morgan, D. T. Coxon, S. Bramham, H. W.-S. Chan, W M. H. VanGelder, and M J. Allison, *J. Sci. Food. Agr.* **36**, 282–288 (1985).

163. K-E. Hellenas, *J. Sci. Food Agr.* **37**, 776–782 (1986).

164. J. A. Matthew, M. R. A. Morgan, R. McNerney, H. W-S. Chan, and D. T. Coxon, *Food Chem. Toxicol.* **21**, 637–640 (1983).

165. M. H. Harvey, M. McMillan, M. R. A. Morgan, and H. W-S. Chan, *Hum. Toxicol.* **4**, 187–194 (1985).

166. S. L. Sinden and R. E. Webb, *Am. Potato J.* **49**, 334–338 (1972).

167. J. J. Pestka, "Fungi and Mycotoxins in Meats," in A. M Pearson and J. Dawson, eds., *Advances in Meat Research*, Vol. 2, AVI Publishing Co., Inc., Westport, Conn., 1986, pp 277–302.

168. J. J. Pestka and D. E. Dixon-Holland, "Mycotoxin Detection by Immunoassay and Application of Hybridoma Technology," in B. Swaminathan and G. Prakash, eds., *Nucleic Acid and Monoclonal Antibody Probes. Applications in Diagnostic Microbiology*, Marcel Dekker, Inc., New York, 1989, pp. 657–678.

169. H. P. Van Egmonnd, W. E. Paulsch, and P. L. Schuyller, *J. Assoc. Off Anal. Chem.* **61**, 809–812 (1978).

170. H P. Van Egmond, W. E. Paulsch, E. Deyll, and P. L. Schuller, *J. Assoc. Off Anal. Chem.* **63**, 110–114 (1980).

171. S. Nesheim, N. F. Hardin, O. J. Francis, and W. S. Langham, *J. Assoc. Off. Anal. Chem.* **56**, 817–821 (1973).

172. O. C. Hunt, A. T. Boudon, P. J. Wild, and N. T. Crosby, *J. Sci. Food Agr.* **29**, 234–238 (1978).

173. J. F. Gregory and D. Manley, *J. Assoc. Off. Anal. Chem.* **64**, 144–151 (1981).

174. P. M. Scott, *J. Assoc. Off. Anal. Chem.* **65**, 876–883 (1982).

175. W. F. Busby and G. F. Wogan, "Mycotoxins and Mycotoxicoses," in H. Remann and F. L. Bryan, eds., *Food-Borne Infections and Intoxications*, 2nd ed., Academic Press, Inc , Orlando, Fla., 1979, pp. 519–610.

176. J. J. Wong and D. D. Hsieh, *Proc. Natl. Acad. Sci. USA* **73**, 2211–2244 (1976).

177. L. Stoloff, *J. Prot.* **43**, 226–230 (1980).

178. J. F. Grove, *Nat. Prod. Rep.* **5**, 187–190 (1988)

179. R. R. Marquardt, A. Frohlich, and D. Abramson, *Can. J. Physiol. Pharmacol.* **68**, 991–999 (1990).

180. M. W. Trucksess, M. T. Flood, M. M. Mossoboa, and S. W. Page, *J. Agr. Food Chem.* **35**, 445–448 (1987).

181. K. C. Erhlich, L. S. Lee, and A. Ciegler, *J. Liquid Chromatogr.* **6**, 833–843 (1983).

182. U. S. Lee, H. S. Jang, T. Tanaka, Y. Joh, C. M. Cho, and Y. Ueno, *J. Agr. Food Chem.* **35**, 126–129 (1987).

183. C. J. Mirocha, S. V. Pathre, B. Schanerhamer, and C. M. Christensen, *Appl. Environ. Microbiol.* **32**, 553–556.

184. R. D. Platter and G. A. Bennett, *J. Assoc. Off. Anal. Chem.* **66**, 1470–1477 (1983).

185. H. J. Kurtz and C. J. Mirocha, "Zearalenone (F2) induced Estrogenic Syndrome in Swine," in T. D. Syelie and L. G. Morehouse, eds., *Mycotoxic Fungi, Mycotoxins and Mycotoxicoses*, Vol 2, Marcel Dekker, Inc., New York, pp. 1256–1264.

186. CAST, *Aflatoxins and Other Mycotoxins: An Agricultural Perspective*, Report No. 80, Council for Agricultural Science and Technology, Ames, Iowa 1979.

187 P. Krough, "Ochratoxins," in J. V. Rodricks, C. W. Hasseltine, and M. A. Mehlman, eds., *Mycotoxins in Human and Animal Health*, Patotox Publishers, Park Forest South, Ill., 1977, pp. 489–498.

188. J. E. Smith and M. O. Moss, *Mycotoxins: Formation, Analysis and Significance*, John Wiley & Sons, Inc., New York, 1985, p. 148.

189. S. Neshiem, L. Friedman, and A. E. Pohland, "Ochratoxin," *J. Am. Oil Chem. Soc.* **66**, 432 (1989).

190. CAST, *Mycotoxins Economic and Health Risks*, Report No. 116, Council for Agricultural Science and Technology, Ames, Iowa, 1989.

191. C. F. Jelinek, A. E. Pohland, and G. E. Wood, *J. Assoc. Off. Anal. Chem.* **72**, 223–230 (1989).

192. L. G. Morehouse, "Mycotoxins of Veterinary Importance in the United States," in J. Lacey, ed., *Trichothecenes and Other Mycotoxins. Proceedings of the International Mycotoxin Symposis, Sydney, Australia, 1984*, John Wiley & Sons, Inc., New York, 1985, pp. 383–410.

193. P. Krogh, B. Hald, and E. J. Pederson, *APMIS* **81** (Sect. B.), 681–683 (1973).

194. P. Krogh, B. Hald, L. England, L. Rutquist, and O. Swohn, *APMIS* **82** (Sect. B), 701–702 (1974)

195. P. Lafont and J. Lafont, *Experientia* **26**, 807–808 (1971).

196. U. L. Diener, "Unwanted Biological Substances in Foods: Aflatoxins," in J. C. Ayres and J. C. Kushman, eds., *Impact of Toxicology on Food Processing*, AVI Publishing Co., Inc., Westport, Conn., 1981, pp. 122–150.

197. L. B. Bullerman, *J. Dairy Sci.* **64**, 2439–2452 (1981).

198. C. F. Jelinek, *Distribution of Mycotoxin—An Analysis of World Wide Commodities Data, Including Data from FAO / WHO / UNEP Food Contamination Monitoring Programme*, Joint FAO/WHO/UNEP Second International Conference on Mycotoxins, Bangkok, Thailand, Sept. 28–Oct. 3, 1987.

199. G. E. Wood, *J. Assoc. Off. Anal. Chem.* **72**, 543–548 (1989).

200. P. M. Scott, "The Occurrence of Vomitoxin (Deoxynivalenol, DON) in Canadian Grains," in H. Kuratha and Y. Ueno, eds., *Toxic Fungi—Their Toxins and Health Hazards*, Elsevier, Science Publishing Co , Inc., New York, 1984, pp. 182–189.

201. T. Tanaka, A. Hasegawa, Y. Marsuki, and Y. Ueno, *Food Addit. Contam.* **2**, 259–265 (1985).

202. Leistner, "Toxigenic Penicillia Occurring in Feeds and Foods," in Ref. 199, pp. 162–171.

203. AIN Symposium of 10.4.1978, "Principal Hazards in Food Safety and Their Assessment," *Fed. Proceed.* **37**, 2575–2597.

204. "Completion of the Internal Market: Community Legislation Foodstuffs," EEC Commission Document COM (85)603 Final, 1985.

205. "White Paper on Completing the Internal Market," EEC Commission Document COM (85)310 Final, 1985.

206. "Communication on the Free Movement of Food Stuffs within the Community," EEC Commission Document 89/C271/03, 1989.

207. "On the Approximation of the Laws of Member States Concerning Food Additives Authorized For Use in Foodstuffs Intended for Human Consumption," Council Directive, 89/107/EEC, 1989, p. 27.

208. J. J. Pestka, *J. Assoc. Off Anal. Chem.* **71**, 1075–1081 (1988).

209. J. J. Langone and H. VanVunakis, *J. Natl. Cancer Inst.* **56**, 591–595 (1976).

210. F. S. Chu and I. Ueno, *Appl. Environ. Microbiol.* **33**, 1125–1128 (1977).

211. J. D. Groopman, P. R. Donahue, A. Marshak-Rothstein, and G. N. Wagan, *Proc. Natl. Acad. Sci. USA* **81**, 7728–7731 (1984).

212. O. El-Nakib, J. J. Pestka, and F. S. Chu, *J. Assoc. Off. Anal. Chem.* **64**, 1077–1082 (1981).

213. D. W. Lawellin, D. W. Grant, B. K. Joyce, *Appl. Environ. Microbiol.* **34**, 94–96 (1977).

214. J. J. Pestka, P. K. Gaur, and F. S. Chu, *Appl. Environ. Microbiol.* **40**, 1027–1031 (1980).

215. T. S. L. Fan and F. S. Chu, *J. Food Prof.* **47**, 964–968 (1984).

216. A. A. G. Candlish, W. H. Stimson, and J. E. Smith, *Lett. Appl. Microbiol.* **1**, 57–59 (1985).

217. B. P. Ram, L. P. Hart, O. L. Shotwell, and J. J. Pestka, *J. Assoc. Off. Anal. Chem.* **69**, 904–907 (1986).

218. B. P. Ram, L. P. Hart, R. J. Cole, and J. J. Pestka, *J. Food Prot.* **49**, 792–795 (1986).

219. D. N. Mortimer, M. J. Shepard, J. Gilbert, and M. R. A. Morgan, *Food Addit. Contam.* **5**, 127–132 (1987).

220. R. L. Warner and J. J. Pestka, *J. Food Prot.* **50**, 502–503 (1987).

221. D. E. Dixon-Holland, J. J. Pestka, B. A. Bidigare, W. L. Casale, R. L. Warner, B. P. Ram, and L. P. Hart, *J. Food Prot.* **51**, 201–204 (1988).

222. R. B. Sashidhar and B. S. N. Rao, *J. Exp. Biol.* **26**, 984–989 (1988).

223. D. N. Mortimer, M. J. Shepard, J. Gilbert, and C. Clark, *Food Addit. Contam.* **5**, 601–608.

224. N. Reichert, S. Steinmeyer, and R. Weber, *Z. Lebensm. Unters.-Frosch.* **186**, 505–508 (1988).

225. D. L. Park, B. M. Miller, L. P. Hart, G. Yang, J. McVey, S. W. Page, J. Pestka, and L. H. Brown, *J. Assoc. Off Anal. Chem.* **72**, 326–332 (1989).

226. D. L. Park, B. M. Miller, S. Nesheim, M. W. Trucksess, A. Vekich, B. Bidigare, J. L. McVey, and L. H. Brown, *J. Assoc Off. Anal. Chem.* **72**, 638–643 (1989).

227. M. W. Trucksess, M. E. Stack, S. Neshiem, D. L. Park, and A. E. Pohland, *J. Assoc. Off. Anal. Chem.* **72**, 957–962 (1989).

228. J. W. Dorner and R. J. Cole, *J Ass. Off. Anal. Chem.* **72**, 962–964 (1989).

229. A. L. Patey, M Sharman, R. Wood, and J. Gilbert, *J. Assoc. Off. Anal. Chem.* **72**, 965–969 (1989).

230. N. Ramakrishna, J. Lacy, A. A. G. Candlish, J. E. Smith, and I. A. Goodbrand, *J. Assoc Off Anal. Chem.* **73**, 71–76 (1990)

231. M. W. Trucksess, K. Young, K. F. Donahue, D. K. Morris, and E. Lewis, *J. Assoc. Anal. Chem.*, 425–428 (1990).

232. L. S. Lee, J. H. Wall, P. J. Cotly, and P. Bayman, *J. Assoc. Off. Anal. Chem.* **73**, 581–583 (1990).

233. Koeltzow and S. N. Tanner, *J. Assoc. Off. Anal. Chem.* **73**, 584–589 (1990).

234. J. D. Groopman and K. Donahue, *J. Assoc. Off. Anal. Chem.* **71**, 861–867 (1988).

235. W. O. Harder and F. S. Chu, *Experientia* **35**, 1104–1105 (1976).

236. J. J. Pestka and Y. K. Li, W. O. Harder, and F. S. Chu, *J. Assoc. Off. Anal. Chem.* **64**, 294–301 (1981).

237. N. A. Woychik, R. D. Hinsdill, and F S. Chu, *Appl. Environ. Microbiol.* **48**, 1096–1099 (1984)

238. J. M. Fremy and F. S. Chu, *J. Assoc. Off. Anal. Chem.* **67**, 1098–1101 (1984).

239. W. J. Hu, N. Woychik, and F. S. Chu, *J. Food Prot.* **47**, 126–127 (1984)

240. S. Wu, G. Yang, and T. Sun. *Chin Joncol.* **5**, 81–84 (1983).

241. T. J. Hansen, *J. Food Prot.* **53**, 75–77 (1990).

242. D. N. Mortimer, J. Gilbert, and M. J. Shepard, *Chromotogr.*, 393–398 (1987).

243 D. Thouvenot and R. F. Morfin. *Appl. Environ. Microbiol.* **45**, 16–23 (1983).

244. J J. Pestka, M-T. Liu, B. Knudson, and M. Hogberg, *J. Food Prot.* **48**, 953–957 (1985).

245. M-T. Liu, B. P. Ram, L. P. Hart, and J. J. Pestka, *Appl. Environ. Microbiol.* **50**, 332–336 (1985).

246. R. L. Warner, B. P. Ram, L. P. Hart and J. J. Pestka, *J. Agr. Food Chem.* **34**, 714–717 (1986).

247. D. E. Dixon, R. L. Warner, B. P. Ram, L. P. Hart, and J. J. Pestka, *J. Agr. Food Chem.* **353**, 122–126 (1987).

248. O. A. MacDouggald, A. J. Thulin, and J. J. Pestka, *J. Assoc. Off. Anal. Chem.* **73**, 65–68 (1990).

249. J. I. Azcona, M. M. Abouzied, and J. J. Pestka, *J Food Prot.* **53**, 577–580 (1990).

250. F. S. Chu, S. Grossman, R. D Wei, and C. J. Mirocha, *Appl. Environ. Microbiol.* **37**, 104–108 (1979).

251. R. D. Wei, W. Bischoff, and F. S. Chu, *J. Food Prot.* **49**, 267–271 (1986).

252. E. H. Gendloff, J. J. Pestka, D. E. Dixon, and L. P. Hart, *Phytopathology* **77**, 57–59 (1987).

253. K. W. Hunter, A. A. Brimfield, F. D Finkelman, and F. S. Chu, *Appl. Environ. Microbiol.* **49**, 168–172 (1985).

254. I. A. Goodbrand, W. H. Stimson, and J. E. Smith, "A Monoclonal Antibody Based ELISA for T-2 Toxin," in B. A. Morris, M. N. Clifford, and R. Jackman, eds., *Immunoassays for Veterinary and Food Analysis*, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1984, pp. 355–358

255. J. Chiba, O. Kawamura, H. Kayii, K. Ohtami, S. Nagayama, and Y. Ueno, *Food Addit. Contam.* **5**, 629–640 (1988).

256. J. J. Pestka, S. S. Lee, H P. Lau, and F. S. Chu, *J. Am. Oil Chem. Soc.* **58**, 940a–944a (1981).

257. E. H. Gendloff, J. J. Pestka, S. P. Swanson, and L. P. Hart, *Appl Environ. Microbiol.* **47**, 1161–1163 (1984).

258. T. S L. Fan, G. S Zhang, and F. S. Chu, *J. Food Prot* **47**, 964–967 (1984).

259. Y. C. Xu, G. S. Zhang, and F. S. Chu, *J. Assoc. Off. Anal. Chem.* **69**, 967–970 (1986).

260. Y. C. Xu, G. S. Zhang, and F. S. Chu, *J Assoc. Off. Anal. Chem.* **71**, 945–949 (1988).

261. W. L. Casale, J. J. Pestka, and L. P. Hart, *J. Agr. Food Chem.* **38**, 663–668 (1988).

262. O Aalund, K. Brundeldt, B. Hald, P. Kroglo, and K. Paulsen, *APMIS* **83** (Sect. C), 390–392 (1975)

263. F. S. Chu, F. C. C. Chang, and R. D. Hindsdill, *Appl. Environ. Microbiol.* **31**, 831–835 (1976).

264. J. J. Pestka, B. W Steinart, and F. S. Chu, *Appl. Environ. Microbiol.* **41**, 1472–1474 (1981).

265. M. R. A. Morgan, R. McNerney, and H. W. S. Chan, *J. Assoc. Off. Anal. Chem.* **66**, 1481–1484 (1983).

266. S. Lee and F. S. Chu, *J. Assoc. Off. Anal. Chem.* **67**, 45–49 (1984).

267. W. J. Sidwell, H. W-S. Chan, M. R. A. Morgan, *Food Agr. Immunol.* **1**, 111–118 (1989)

268. O. Kawamuna, S. Sato, H. Kajii, S. Nagayame, K. Ohtani, J. Chiba, and Y. Ueno, *Toxicon* **27**, 887–898 (1989).

269. W. M. Hagler, G. Y. Danko, L. Horvath, M. Palyusik, and C. J. Mirocha, *Acta Vet. Hung* **28**, 209–216 (1980)

270. C. J. Mirocha, S. V. Pathre, and T. S. Robison, *Food Cosmet. Toxicol.* **19**, 25–30 (1981).

271. F. S. Chu and R. C. Lee, *Food Agr. Immunol.* **1**, 127–136 (1989).

272. T. Yoshizawa, C. J. Mirocha, J. C. Behreno, and S. P Swanson, *Food Cosmet. Toxicol.* **19**, 31–39 (1981).

273. T. Yoshizawa, T. Sakamoto, Y. Ayano, and C. J. Mirocha, *Agr. Biol. Chem.* **46**, 2613–2615 (1982).

274. T. Yoshizawa, T. Sakamoto, and K. Okamoto, *Appl. Environ. Microbiol.* **47**, 130–134 (1984).

275. R. B. Read, W. L. Pritchard, J. Bradshaw and L. A. Black, *J. Dairy Sci.* **48**, 411–419 (1965).

276. D. Y. C. Fung and J. Wagner, *Appl. Microbiol.* **21**, 559–561 (1971).

277. C. Genigeoris, G. Sakaguchi, and H. Rieman, *Appl. Microbiol.* **26**, 111–115 (1973).

278. B. L. Vermilyea, H. M. Walker, and J. C. Ayres, *Appl. Microbiol.* **16**, 21–24 (1968)

279. R. F. Meyer and M. J. Palmieri, *Appl. Environ.* **40**, 1080–1085 (1980).

280. R. B. Read, J. Bradshaw, W. L. Pritchard, and L. A. Black, *J. Dairy Sci.* **48**, 420–424 (1965).

281. R. Robbins, S. Gould, and M. Bergdoll, *Appl. Microbiol.* **28**, 946–950 (1974).

282. M. S. Bergdoll and R. W Bennett, "Staphylococcal Enterotoxins," in M. L. Speck, ed., *Compendium of Methods for the Microbiological Examination of Foods*, 2nd ed., American Public Health Association, Washington, D.C., 1984, pp. 428–457.

283. V. L. Zehren and V. F. Zehren, *J. Dairy Sci.* **51**, 635–644 (1968).

284. J. L. Smith and M. M. Bencivengo, *J. Food Safety* **7**, 83–100 (1985).

285. S. A. Morse and R. A. Mah, *J. Appl Microbiol.* **15**, 58–61 (1967).

286. S. J. Silverman, A R. Knott, and M. Howard, *Appl. Microbiol.* **16**, 1019–1023 (1968).

287. S. Yameda, H. I. Garishi, and T. Teryama, *Microbiol. Immunol.* **21**, 675–682 (1977).

288. G. M. Evancho, D. H. Ashton, E. J. Briskey, and E. J. Schantz, *J. Food Sci.* **38**, 764–767 (1973).

289. L. L. Salomon and R. W. Tew, *Proc Soc Exp. Biol. Med.* **129**, 539–542 (1968).

290. P. R. Berry, M. F. Stringer, and T. Uemura, *Lett. Appl. Microbiol.* **2**, 101–102 (1986)

291. H. M. Johnson, J. A. Bukovic, P. E Kauffman, and J. T. Peller, *Appl. Microbiol.* **22**, 837–841 (1971).

292. W. S. Collins, A. D. Johnson, J. F. Metzger, and R. W. Bennett, *Appl. Microbiol.* **25**, 774–777 (1973).

293. H. M. Johnson, J. A. Bukovic, and P. E. Kauffman, *Appl. Microbiol.* **26**, 309–313 (1973)

294. J. A. Bukovic and H. M. Johnson, *Appl. Microbiol.* **30**, 700–701.

295. H. Robern, M. Dighton, Y. Yano, and N. Dickie, *Appl. Microbiol.* **30**, 525–529 (1975).

296. H Robern, T M Gleeson, and R. A. Szabo, *Can. J. Microbiol.* **24**, 436–439 (1978).

297. H. Robern and T. M Gleeson, *Can. J. Microbiol.* **4**, 137–143 (1978).

298. S. Lindroth, and A. Niskanen, *Eur. J. Appl. Microbiol.* **4**, 137–143 (1977).

299. N. Niyomvit, K. E. Stevenson, and R. F. McFeeters, *J. Food Sci.* **43**, 735–739 (1978).

300. J. F. Metzger, and A. D. Johnson, *Abst. Annu. Methods Am. Soc. Microbiol.*, 259 (1977).

301. B. A. Miller, R. F. Reiser, and M. S. Bergdoll, *Appl. Environ. Microbiol.* **36**, 421–426 (1978).

302. P. D. W. Areson, S. E. Charm, and B. L. Wong, *J. Food Sci.* **45**, 400–401 (1980).

303. G. F. Ibrahim, H. M. Radford, and J. R. Fell, *Appl. Environ. Microbiol.* **39**, 1134–1137 (1980).

304. D. S. Orth, *Appl. Environ. Microbiol.* **34**, 710–714 (1977).

305. N. Dickie and S. H. Akhtar, *J. Assoc. Off. Anal. Chem.* **65**, 180–184 (1982).

306. H. B. Greenberg, D. A. Sack, W. Rodriquez, R. B. Sack, R. G. Wyatt, A. R. Kalica, K. L. Horswood, R. M. Chanock, and A. Z. Kapikian, *Infect. Immun.* **17**, 541–545 (1977).

307. M. Ciska, "Methods," in J. J. Langone and H. van Vunakis, eds., *Enzymology*, Vol. 84, Academic Press, Inc., Orlando, Fla., 1982, pp. 238–253.

308. D. A. Boroff and G. Shu-Chen, *Fed. Proceed.* **32**, 1032 (1973).

309. A. C. Ashton, J. S. Crowther, and J. O. Dolly, *Toxicon.* **23**, 235–246 (1985).

310. G. N. Stelma, J. C. Wimsatt, P. E. Kauffman and D. Shah, *J. Food Prot.* **46**, 1069–1073 (1983).

311. G. Stiffler-Rosenberg, and H. Fey, *J. Clin. Microbiol.* **8**, 473–479 (1978).

312. P. E. Kauffman, *J. Assoc. Off. Anal. Chem.* **63**, 1138–1143 (1980).

313. J. K. S. Kuo and G. J. Silverman, *J. Food Prot.* **43**, 404–407 (1980)

314. W. Lenz, R. Thelen, P. Pickerhahn, and H. Brandis, *Zentrabl. Bakteriol. Infectionskr. Hyg. Abt. A.* **253**, 466–475 (1983).

315. H. Fey, H. Pfister, and O. Ruegg, *J. Clin. Microbiol.* **19**, 34–38 (1984).

316. P. D. Patel and P. A. Gibbs, *Biochem. Soc. Trans.* **12**, 264–265 (1984).

317. G. C. Saunders and M L. Bartlett, *Appl. Environ. Microbiol.* **34**, 518–522 (1977).

318. J. W Koper, A. M. Hagenaars, and S. Notermans, *J. Food Safety* **2**, 35–45 (1980)

319. H. Buning-Pfaue, P Timmermans, and S. Notermans, *Z. Lebensm. Unters.-Forsch.* **173**, 351–355 (1981).

320. B. Berdal, P. Olsvik, and T. Omland, *APMIS Microbiol. Scand.* **89** (Sect. B), 411–415 (1981).

321. R. C. Freed, M. L. Everson, R. F. Reiser, and M. S. Bergdoll, *Appl. Environ. Microbiol.* **44**, 1349–1355.

322. S. Notermans, R. Boot, P. D. Tips, and M. P. DeNooij, *J. Food Prot.* **46**, 238–241 (1983).

323. A. A. Wieneke and R. J. Gilbert, *Int. J. Food Microbiol.* **4**, 135–143 (1987).

324. I. F. Hahn, P. Pickenhahn, W Lenz, and H Brandis, *J. Immunol. Methods* **92**, 25–29 (1986).

325. G. Terplan, M. Lohneis, and K. H. Jaschke, "Immunoluminometric Assay (ILMA), a New Ion-Isotopic Assay for Detection of Staphylococcal Enterotoxins," in *Proceedings of the Second World Congress on Foodborne Infections and Intoxications*, Institute of Veterinarian Medicine/Robert Von Ostertag Institute, Berlin, 1986, pp. 448–451

326. P. Boerlin, H. Pfister, and H. Fey, "An Immuno-Dot Assay for the Detection of Staphylococcal Enterotoxins in Cultures," in Ref. 323, pp. 444–447.

327. H. Fey and H. Pfister, "A Diagnostic Kit for the Detection of Staphylococcal Enterotoxins (SET) A, B, C and D," in S. Avrameas, P. Druet, R. Masseyeff, and G. Feldman, eds., *Immunoenzymatic Techniques*, Elsevier, Amsterdam, The Netherlands, 1983, p. 345.

328. S. Notermans, J. Dufrenne, and M. Van Schothorst, *Jpn. J. Med. Sci. Biol.* **31**, 81–85 (1978).

329. S. Notermans, J. Dufrenne, and S. Kozaki, *Appl. Environ. Microbiol.* **37**, 1173–1175 (1979).

330. S. Notermans, A. M. Hagenaars, and S. Kozaki, "The Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection and Determination of *Clostridium botulinum* Toxins A, B and E," in Ref. 306, 1982. pp. 223–238.

331. S. Notermans, J. Dufrenne, and S. Kozaki, *Jpn. J. Med. Sci. Biol.* **35**, 203–211 (1982).

332. S. Notermans, S. Kozaki, Y. Kamata, and G. Sakaguchi, *Jpn. J. Med. Sci. Biol.* **37**, 137–140 (1984).

333. G. E. Lewis, S. S. Kulinski, D. W. Reischard, and J. F. Metzger, *Appl. Environ. Microbiol.* **42**, 1018–1022 (1981).

334. M. Dezfulian and J. G. Bartlett, *J. Clin. Microbiol.* **19**, 645–648 (1984).

335. M. Dezfulian and J. G. Bartlett, *Diagn. Microbiol. Infect. Dis.* **3**, 105–112 (1985).

336. C. Shone, P. Wilton-Smith, M. Appleton, P. Hambleton, N. K. Modi, S. Gatley, and J. Elling, *Appl. Environ. Microbiol.* **50**, 63–67 (1985).

337. N. K. Modi, C. G. Shone, P. Hambleton, and J. Melling, "Monoclonal Antibody Based Amplified Enzyme-Linked Immunosorbent Assays for *Clostridium botulinum* Toxin Types A and B," in Ref. 323, pp. 1184–1188.

338. J. Ligieza, M. Michalik, J. Reiss, and J. Grzybowski, *Arch. Immunol. Ther. Exp.* **34**, 189–195 (1986).

339. G. N. Stelma, C. H. Johnson, and D. B. Shah, *J. Food Prot.* **48**, 227–231 (1985).

340. B. A. McClane and R. J. Strouse, *J. Clin. Microbiol.* **19**, 112–115 (1984).

341. O. Olsvik, P. E. Granum, and B. P. Berdal, *APMIS* **90**, 445–447 (1982).

342. K. G. Narayan, C. Genigeorgis, and D. Behymer, *Int. J. Zoonoses.* **10**, 105–110 (1983)

343. S. Notermans, C. Heuvelman, H. Beckers, and T. Uemura, *Zentralbl. Bakteriol. Mikrobiol. Hyg.* **179**, 225–234 (1984).

344. B. A. Bartholomew, M F. Stringer, G. N. Watson, and R. J. Gilbert, *J. Clin. Pathol.* **38**, 222–228 (1985).

345. R. H. Yolken, H. B. Greenberg, M H. Merson, R. B. Sack, and A. Z. Kapikain, *J. Clin. Microbiol.* **6**, 439–444 (1977).

346. I. Ketyi and A. S. Pacsa, *Acta Microbiol. Hung.* **27**, 89–97 (1980).

347. E. Back, A.-M. Svennerholm, J. Holmgren, and R. Bollby, *J. Clin. Microbiol.* **10**, 791–805 (1979).

348. A.-M Svennerholm and G. Wirklund, *J. Clin. Microbiol.* **17**, 596–600 (1983).

349 L. Beutin, L. Bode, T. Richter, G. Pettre and R. Stephan, *J. Clin. Microbiol.* **19**, 371–375 (1984).

350. S. L. Mosely, I. Huq, M.S.O.M. Samadpour-Matalebi, and S. Falkow, *J. Infect. Dis.* **142**, 892–898 (1980).

351. National Academy of Sciences, *Regulatory Pesticides in Foods*, Committee on Scientific and Regulatory Issues Underlying Pesticide Use Patterns and Agricultural Innovation, Board of Agriculture, National Research Council, Washington, D.C., 1987.

352. J. E. Lovelock and S. R. Lipsky, *J. Am. Chem. Soc.* **82**, 431 (1960).

353. F. L. Bryan, *J. Food. Prot.* **51**, 498–508 (1988).

354. W. H. Andrews, *Food Technol.* **39**, 77–82 (1985).

355. J. H. Silliker, *Food Technol.* **36**, 65–70 (1982).

356. B. Swaminathan, J. Ag Alexio, and S. A. Minnich, *Food Technol.* **39**, 83–89 (1985).

357. J. A. Mattingly, B. J. Robinson, A. Boehm and W. D. Gehle, *Food Technol.* **39**, 90–94 (1985).

358. R. S. Flowers, K. Eckner, D. A. Gabis, B. J. Robinson, J. A. Mattingly and J. H. Silliker, *J. Assoc. of Anal. Chem.* **69**, 786–798 (1986).

359. R. S. Flowers, M. J. Klatt, B. J. Robinson, J. A. Mattingly, D. A. Gabis and J. H. Silliker, *J. Assoc. Off. Anal. Chem.* **70**, 530–535 (1987).

360. R. S. Flowers, M. J. Klatt, B. J. Robinson, J. A. Mattingly, *J. Assoc. Off. Anal. Chem.* **71**, 341–343 (1988).

361. C. Lindhardt, P Bielefeld and M. Wilken, *J. Appl. Bacteriol.* **67**, 1989–1993 (1989).

362. G. M. Wyatt, H. A. Lee and M. R. A. Morgan, *Br. Poult. Sci.* **30**, 979–981 (1989).

363. A. L. Gorelova, G. A. Levina and S. V. Prozorovskii, *Mikrobiol. Epidemiol. Immunobiol.* **8**, 60–64 (1989).

364. R. L. Hopfer, R. Pinzon, M. Wenglar, and K. V. I. Rolston, *J. Clin. Microbiol.* **22**, 677–679 (1985).

365. M. A. Khan, A. Seaman, and M. Woodbine, *Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1* **239**, 62–69 (1977).

366. E. Neter, H. Azai, and E. A. Gorynski, *Proc. Soc. Exp. Biol. Med.* **105**, 131–134 (1960)

367. P E. Pease, L. Nicholls, and M. R. Stuart, *J. Gen. Microbiol.* **73**, 567–569 (1972).

368. P. A. Wentworth and H. K. Ziegler, *J. Immunol.* **138**, 2671–2678 (1987).

369. B. T. Butman, M. C. Plank, R. J. Durham, and J. A. Mattingty, *Appl. Environ. Microbiol.* **54**, 1564–1569 (1988).

370. J. A. Mattingly, B. T. Butman, M. C. Plank, and R. J. Durham, *J. Assoc. Off. Anal. Chem.* **71**, 679–681 (1988).

371. R. R. Beumer and E. Brinkman, *Food Microbiol* **6**, 171–178 (1989).

372. P. Lombardo, *J. Assoc. Off. Anal. Chem.* **72**, 518–520 (1989).

373. R. L. Ellis, *J. Assoc. Off. Anal. Chem.* **72**, 521–524 (1989)

374. W. P. McKinley and J. H. Mahon, *J. Assoc. Off. Agric. Chem.* **42**, 725–734 (1959).

375. A. Major, Jr., and H. C. Barry, *J. Assoc. Off. Agric. Chem.* **44**, 202–207 (1961).

376. J. P. Minyard and E. R. Jackson, *J. Assoc. Off. Agric. Chem.* **46**, 843–859 (1963).

377. J. J. Langone and H. VanVunakis, *Res. Commun. Chem. Pathol. Pharmacol.* **10**, 163–171 (1975).

378. W. H. Newsome and J. B. Shields, *J. Agr. Food Chem.* **29**, 220–222 (1981).

379. K. D. Wing, B. D. Hammock, and D. A. Wustner, *J Agr. Food Chem.* **26**, 1328–1332 (1978).

380. D. Knopp, P. Nuhn, and H. J. Dobberkau, *Arch. Toxicol.* **58**, 27–32 (1985).

381. D. F. Rinder, and J. R. Fleeker, *Bull. Environ. Contam. Toxicol.* **26**, 375–380 (1981).

382. S. I. Wie, A. P. Sylwester, K. D. Wing, and B. D. Hammock, *J. Agr. Food Chem* **30**, 943–948 (1982)

383. C. D. Ercegovich, R. P. Vallejo, R. R. Gehig, L. Woods, E. R. Bogus, *J. Agr. Food Chem.* **29**, 559–563 (1981).

384. T. Levitt, *Lancet* **2**, 358 (1977).

385. D. Fatori and W. M. Hunber, *Clin. Chim. Acta* **100**, 81–90 (1980).

386. H. R. Lukens, C. B. Williams, S. A. Levison, W. B. Dendliker, D. Murayama, and R. L. Baron, *Environ. Sci. Technol.* **11**, 292–297 (1977).

387. M. Schwalbe, E. Dorn, and K. Beyerman, *J. Agr. Food Chem.* **32**, 734–741 (1984).

388. B. S. Ferguson, in *Abstracts of Papers, The Sixth International Congress of Pesticide Chemistry, Ottawa, Canada*, IUPAC, 1986, Abst. 5C-09.

389. S. J. Wratten and P. C. Feng "Pesticide Analysis by Immunoassay," in Ref. 24, pp. 201–220.

390. J. F. Brady, J. R. Fleeker, R. A. Wilson, and R. O. Mumma, "Enzyme Immunoassay for Aidicarb," in R. G. W. Wang, C. A. Franklin, R. C. Honeyalt, and J. C. Reinert, eds., *Biological Monitoring for Pesticide Exposure*, American Chemical Society, Washington, D.C., 1989, pp. 262–284.

391. S. J. Huber, *Chemosphere* **14**, 1795–1803 (1985).

392. J. M. Van Emon, in Ref. 386, Abst. 5C-01

393. T. Kitagawa, T. Kawasaki, and H. Munechika, *J. Biochem.* **92**, 585–590 (1982).

394. M. M Kelley, E. W. Zahnow, W. C. Petersen, and S. T. Toy, *J. Agr. Food Chem.* **33**, 962–965 (1985).

395. K. M. Robotti, in *Abstracts of Papers, 192nd ACS National Meeting, Anaheim, Calif*, American Chemical Society, Washington, D.C. 1986, Abst. 42.

396. S. I. Wie and B. D. Hammock, *J. Agr. Food Chem.* **30**, 949–957 (1982).

397. F. Gung, H. H D. Meyer, and R. T. Hamm, *J. Agr. Food Chem.* **37**, 1183–1187 (1989).

398. W. H. Newsome and P. G. Collins, in Ref. 386, Abst. 5C-13

399. R. O. Harrison, A. A. Brimfield, K. W. Hunter, and J. O. Nelson, in Ref. 386, Abst. 5C-12.

400. W H. Newsome, *J. Agr. Food Chem.* **33**, 528–530 (1985).

401. A. A. Brimfield, D. E. Lemz, C. Graham, and K. W. Hunter, *J. Agr. Food Chem.* **33**, 1237–1242 (1985).

402. Z. Niewola, S. T. Walsh, and G. E. Davies, *Int. J. Immunopharmacol.* **5**, 211–218.

403. Z. Niewola, C. Hayward, B. A. Symington, and R. T. Robson, *Clin. Chim. Acta* **148**, 149–156 (1985)

404. J. M. Van Emon, J. N. Seiber, and B. D. Hammock, in P. A. Hedin, ed., *Bioregulations for Pesticide Control*, ACS Symp. Ser. No. **276**, American Chemical Society, Washington, D C , 1985, pp. 307–316.

405. J. Van Emon, B. Hammock, and J. N. Seiber, *Anal. Chem.* **58**, 1866–1873 (1986).

406. A. A. Brimfield and K. W Hunter, in *Abstracts of Papers. The 100th Annual Meeting of AOAC, Scottsdale, Ariz.*, 1986, Abst. 110.

407. K. H. Kuniyaki and S McCarthy, in Ref. 386, Abst. 5C-11.
408. S. J. Huber and B. Hock, *J. Plant Dis. Prot.* **92**, 147–156 (1985).
409. W. H. Newsome, *Bull. Environ. Contam. Toxicol.* **36**, 9–14 (1986).
410. B. Dunbar, B. Riggle, and G. Niswender, *J. Agr. Food Chem.* **38**, 433–437 (1990).
411. R. B. Koch, T. N. Patil, B. Glick, R. S. Stinson, and E. A. Lewis, *Pest. Biochem. Physiol.* **12**, 130–140 (1979).
412. D. D. VanHouweling and F. J. Kingma, *Am. J. Vet. Res.* **155**, 2197–2202 (1969).
413. R. P. Lehman, *J. Anim. Sci.* **35**, 1340–1345 (1972).
414. D. H Mercer, *Antimicrobial Drugs in Food Producing Animals. Veterinary Clinics of North America*, Vol 5, W. D. Saunders, Philadelphia, 1975.
415. *Fed. Regist.* **42**, 62211 (1977).
416. M. S. Brady, and S. E. Katz, *J. Food Prot.* **51**, 8–11 (1988).
417. D. L. Thompson, D. Wood, and I. Thomson, *J. Food Prot.* **31**, 512–515 (1988).
418. H. W. Wehr, *The Incidence of Antibiotics Other Than Penicillin in Producer Raw and Finished Milk Products*, paper presented at the Annual Meeting of the IAMFES, Anaheim, Calif., 1987.
419. National Center for Toxicological Research, *Chronic Toxicology and Carcionenicity Studies of Sulfamethazine in B6C3F1 Mice*, tech. rep., NCTR, Jefferson, Ark., 1988.
420. D. Barnes, and O. H. Rigglemar, *J. Assoc. Off. Anal. Chem.* **54**, 1195–11999 (1971).
421. W. Horwitz, *J. Assoc. Off. Anal. Chem.* **64**, 104–130 (1981).
422. G. W. Peng, A. F. Gadalla, and W. L. Chori, *Res. Commun. Pathol. Pharmacol.* **18**, 233–246 (1977).
423. K. Lanbeck and B. Lindstrom, *J. Chromatogr.* **154**, 321–324 (1978).
424. A. J. Manvel and W. A. Steller, *J. Assoc. Off. Anal. Chem.* **64**, 794–799 (1988).
425. A. C. Bratton, E. K. Marshall, D. Babbitt, and A. R. Hendrickson, *J. Biol. Chem.* **28**, 537–550 (1939).
426. F. Fisler, J. L. Sutter, J. N. Bathish, and H. E. Hagma, *J. Agr. Food Chem.* **48**, 278–279 (1968).
427. J. Reider, *Chemotherapy* **17**, 1–21 (1972).
428. L. R. Alexander and E. R. Stanley, *J. Assoc. Off. Anal. Chem.* **48**, 278–279 (1965).
429. E. M. H. Finlay, D. E. Games, J. R. Startin, and J. Gilbert, *Biomed. Environ. Mass Spectrom.* **13**, 633–639 (1986).
430. S. E. Charm and R. Chi, *J. Assoc. Off. Anal. Chem.* **71**, 304–316 (1988).
431. Residues Avoidance Program, "FSIS 20," U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C., 1982.
432. R. B. Ashworth, R. L. Epstein, M. H. Thomas, and L. T. Frobish, *J. Vet. Res.* **47**, 2596–2603 (1985).
433. V. W. Randecker, J A. Reagan, R. E. Engel, D. L. Soderberg, and J. E. McNeal, *J. Food Prot.* **50**, 115–122 (1987).
434. D. W. A. Bourne, R. F. Beville, R. M. Sharma, R. F. Gural, and L. W. Dittert, *J Vet. Res* **38**, 967–972 (1977).
435. R. F. Beville, K. M. Schemske, H G. Luther, E. A. Ozierzak, M. Limpoka, and D. R. Felt, *J. Agr. Food Chem.* **26**, 1201–1203 (1978).
436. J. R. Fleeker, and L. J. Lovett, *J. Assoc. Off. Anal. Chem.* **68**, 172–174 (1985).
437. D. E. Dixon-Holland and S. E. Katz, *J. Assoc. Off. Anal. Chem.* **71**, 1137–1140 (1988).
438. P. Singh, B. P. Ram, and N. H. Sharkov, *J. Agr. Food Chem.* **37**, 109–114 (1989).
439. D. E. Dixon-Holland and S. E. Katz, *J. Assoc. Off. Anal. Chem.* **72**, 447–450.
440. D. E. Dixon-Holland and S. E. Katz, *J. Assoc. Off Anal. Chem.* (submitted).
441. S. E. Katz and M. S. Brady, *J. Assoc. Off Anal. Chem.* **73**, 557–560 (1990).

GENERAL REFERENCES

G. Angunta et al., "Detection of Bovine Casein in Ovine Cheese Using Digoxigenated Monoclonal Antibodies and a Sandwich-ELISA," *Milchwissenschaft* **52**, 511–513 (1997).

D. Atkins and J. Norman, "Mycotoxins and Food Safety," *Nutrition and Food Science* No. 4/5, 260–265 (1998).

H. L. Beasley et al., "Development of a Panel of Immunoassays for Monitoring DDT, Its Metabolites and Analogues in Food and Environmental Matrixes," *J. Agric. Food Chem.* **46**, 3339–3352 (1998).

G. A. Bennet, T. C. Nelsen, and B. M. Miller, "Enzyme-Linked Immunosorbent Assay for Detection of Zearalenone in Corn, Wheat, and Pig Feed: A Collaborative Study," *J. AOAC Int.* **77**, 1500–1509 (1994).

B. W. Blais et al., "Polymacron Enzyme Immunoassay System for Detection of Naturally Contaminating *Salmonella* in Foods, Feeds and Environmental Samples," *J. Food Prot.* **61**, 1187–1190 (1998).

K. Byongki, "Quantification of Castor Bean Allergen in Castor Meal by Rocket Immunoelectrophoresis," *Foods and Biotechnology* **4**, 93–97 (1995).

N. J. Cook et al., "Radioimmunoassay for Cortisol in Pig Saliva and Serum," *J. Agric. Food Chem.* **45**, 395–399 (1997).

A. Dankwardt and B. Hock, "Enzyme Immunoassays for Analysis of Pesticides in Water and Food," *Food Technology and Biotechnology* **35**, 165–174 (1997).

H. P. van Egmond, "Analytical Methodology and Regulations for Ochratoxin A," *Food Additives and Contaminants* **13** (Suppl.), 11–13 (1996).

S. A. Eremin et al., "Polarization Fluoroimmunoassay Determination of the Pesticide 2,4,5-trichlorophenoxyacetic Acid," *Zh. Anal. Khim.* **50**, 215–218 (1995).

M. Franeck et al., "Development of a Microcolumn Radioimmunoassay for Screening of Polychlorinated Biphenyls in Milk and in Animal Fats," *J. Agric. Food Chem.* **40**, 1559–1565 (1992).

A. A. Frolich, R. R. Marequardt, and J. R. Clarke, "Enzymatic and Immunological Approaches for the Quantitation and Confirmation of Ochratoxin A in Swine Kidneys," *J. Food Prot.* **60**, 172–176 (1997).

T. Holzhauser et al., "Rocket Immunoelectrophoresis (RIE) for Determination of Potentially Allergenic Peanut Proteins in Processed Foods as a Simple Means of Quality Assurance and Food Safety," *Z. Lebensm. Unters Forsch.* **206**, 1–8 (1998)

V. Issert, P. Grenier, and V. Maurel Bellon, "Emerging Analytical Rapid Methods for Pesticide Residues Monitoring in Foods: Immunoassays and Biosensors," *Sciences des Aliments* **17**, 131–144 (1997).

K. Jorgensen, "Survey of Pork, Poultry, Coffee, Beer and Pulses for Ochratoxin A," *Food Additives and Contaminants* **15**, 550–554 (1998).

J. P. Joung and Schu Fun, "Assessment of Immunochemical Methods for the Analysis of Tricothene Mycotoxins in Naturally Occurring Moldy Corn," *J. AOAC Int.* **79**, 465–471 (1996).

T. Kakui et al., "Development of Monoclonal Antibody Sandwich-ELISA for Determination of Beer Foam-Active Proteins," *Journal of the American Society of Brewing Chemists* **56**, 43–46 (1998).

K. E. Kerdahi and P. E. Istafanos, "Comparative Study of Colorimetric and Fully Automated Enzyme-linked Immunoassay System for Rapid Screening of *Listeria* spp. in Foods," *J. AOAC Int.* **80**, 1139–1142 (1997)

D. A. Kurtz, J. H. Skerritt, and Stanker, *New Frontiers in Agrochemical Immunoassay*, AOAC International, Arlington, Va. 1996.

R. Martin et al., "Monoclonal Antibody Sandwich-ELISA for the Potential Detection of Chicken Meat in Mixtures of Raw Beef and Pork," *Meat Science* **30**, 23–31 (1991).

E. P. Meulenberg, "Immunochemical Detection of Environmental and Food Contaminants: Development, Validation and Application," *Food Technology and Biotechnology* **35**, 153–163 (1997).

T. V. Milanez, M. B. Atui, and F. A. Lazzari, "Comparison Between Immunoassay and Thin-Layer Chromatography for Determination of Aflatoxins, Ochrotoxin A and Zearalenone in Corn and Corn Meal," *Revista do Istituto Adolfo Lutz* **57**, 65–71 (1998).

J. M. Mitchell et al., "Antimicrobial Drug Residues in Milk and Meat: Causes, Concerns, Prevalence, Regulations, Tests, and Test Performance," *J. Food Prot.* **61**, 742–756 (1998).

H. Pichler et al., "An Enzyme-Immunoassay for the Detection of the Mycotoxin Zearalenone by Use of Yolk Antibodies," *Fresenius J. Anal. Chem.* **362**, 176–177 (1998).

B. Roberts, B. A. Morris, and M. N. Clifford, "Comparison of Radioimmunoassay and Spectrophotometric Analysis for the Quantitation of Hypoxanthine in Fish Muscle," *Food Chem.* **42**, 1–17 (1991).

E. Rodrigues et al., "Sandwich-ELISA for Detection of Goat's Milk in Ewes' Milk and Cheese," *Food Agric. Immunol.* **6**, 195–111 (1994).

F. Sanchez Garcia et al., "Polarization Fluoroimmunoassay of the Herbicide Dichloroprop," *J. Agric. Food Chem.* **41**, 2215–2219 (1993).

R. Schuhmacher et al., "Interlaboratory Comparison Study for the Determination of the Fusarium Mycotoxins Deoxynivlenol in Wheat and Zearalenone in Maize Using Different Methods," *Fresenius J. Anal. Chem.* **359**, 510–515 (1997).

A. Szekacs, "Enzyme-Linked Immunosorbent Assay for Monitoring the *Fusarium* Toxin Zearalenone," *Food Technology and Biotechnology* **36**, 105–110 (1998).

L. S. Shiow and F. M. You, "Determination of Aflatoxins in Liquor Products by Immuno-Affinity Column With Fluorometry and HPLC," *Journal of Food and Drug Analysis* **5**, 161–170 (1997).

E. Simon et al., "Development of an Enzyme Immunoassay for Metsulfuron-Methyl," *Food Agric. Immunol.* **10**, 105–120 (1998).

T. G. Sokari and S. O. Anozie, "Modified Single Radial Immunodiffusion Method for Screening Staphylococcal Isolates for Enterotoxin," *Food Microbiology* **6**, 45–48 (1989).

H. Tian et al., "Rapid Detection of *Salmonella* sp. in Foods by Combination of a New Selective Enrichment and a Sandwich-ELISA Using Two Monoclonal Antibodies Against Dulcitol 1-Phosphate Dehydrogenase," *J. Food Prot.* **59**, 1158–1163 (1996).

I. A. Toscano, G. S. Nunes, and D. Barcelo, "Immunoassays for Pesticide Analysis in Environmental and Food Matrixes," *Food Technology and Biotechnology* **36**, 245–255 (1998).

M. W. Trucksess et al., "Survey of Deoxynivalenol in U.S. in 1993 Wheat and Barley Crops by Enzyme-Linked Immunosorbent Assay," *J. AOAC Int.* **78**, 631–636 (1995).

M. Tuomola et al., "Time-Resolved Fluoroimmunoassay for Measurement of Androsterone in Porcine Serum and Fat Samples," *J. Agric. Food Chem.* **45**, 3529–3534 (1997).

Y. Ueno et al., "A 4-year Study of Plasma Ochratoxin A in a Selected Population in Tokyo by Immunoassay and Immunoaffinity Column-Linked HPLC," *Food and Chemical Toxicology* **36**, 445–449 (1998).

Y. Wanjun and S. Fun, "Improved Direct Competitive Enzyme-Linked Immunosorbent Assay for Cyclopiazonic Acid in Corn, Peanuts and Mixed Feed," *J. Agric. Food Chem.* **46**, 1012–1017 (1998).

A. K. Wintero, P. D. Thomsen, and W. Davies, "A Comparison of DNA-Hybridization, Immunodiffusion, Countercurrent Immunoelectrophoresis and Isoelectric Focusing for Detecting the Admixture of Pork to Beef," *Meat Science* **27**, 75–85 (1990).

G. M. Wood et al., "Ochratoxin A in Wheat. A Second Intercomparison of Procedures," *Food Additives and Contaminants* **13**, 519–539 (1996).

J. M. Yeung and P. G. Collins, "Determination of Soy Proteins in Food Products by Enzyme Immunoassay," *Food Technology and Biotechnology* **35**, 209–214 (1997).

X. Zhao et al., "Radioimmunoassay for Insulin-like Growth Factor-I in Bovine Milk," *Canadian Journal of Animal Science* **71**, 669–674 (1991).

D. E. DIXON-HOLLAND
Neogen Corporation
Lansing, Michigan

INFANT FOODS

INFANT NUTRITION

Infants are defined, somewhat arbitrarily, as less than 1 year of age. Infant foods and feeding practices have been adapted to encompass infants and young children to allow for feeding during the first 2 to 3 years.

The infant has unique nutritional needs, substantially different from those of an adult. Energy, protein, iron, and other needs are greater than at any other period in life. Food must be relatively concentrated, and with a controlled consistency, because the volume capability, chewing, and manipulation of food are limited. At no other point in life is the growth rate of infancy duplicated. Infants typically double their birth weight in 3 to 4 months and triple it by 1 year, and the rapid growth rate places nutritional demands on the food supply. To accompany the rapid growth rate, infants have a very high basal metabolic rate. Complicating this is a limited stomach capacity, requiring an appropriate concentration of nutrients. The limited renal capability must be considered to ensure that electrolytes and protein are not present in deleterious quantities. In addition, the digestive system is immature, which requires that foods be selected that are relatively easy to digest. The most obvious of the characteristics is the limited ability to chew foods; therefore the foods must be appropriate in consistency and texture.

Breast feeding is recognized as the preferred method for feeding very young infants, and breast milk should be the exclusive food for the first 4 to 6 months unless circumstances (mother unable to breast-feed, breast milk inade-

T. Kakui et al., "Development of Monoclonal Antibody Sandwich-ELISA for Determination of Beer Foam-Active Proteins," *Journal of the American Society of Brewing Chemists* **56**, 43–46 (1998).

K. E. Kerdahi and P. E. Istafanos, "Comparative Study of Colorimetric and Fully Automated Enzyme-linked Immunoassay System for Rapid Screening of *Listeria* spp. in Foods," *J. AOAC Int.* **80**, 1139–1142 (1997)

D. A. Kurtz, J. H. Skerritt, and Stanker, *New Frontiers in Agrochemical Immunoassay*, AOAC International, Arlington, Va. 1996.

R. Martin et al., "Monoclonal Antibody Sandwich-ELISA for the Potential Detection of Chicken Meat in Mixtures of Raw Beef and Pork," *Meat Science* **30**, 23–31 (1991).

E. P. Meulenberg, "Immunochemical Detection of Environmental and Food Contaminants: Development, Validation and Application," *Food Technology and Biotechnology* **35**, 153–163 (1997).

T. V. Milanez, M. B. Atui, and F. A. Lazzari, "Comparison Between Immunoassay and Thin-Layer Chromatography for Determination of Aflatoxins, Ochrotoxin A and Zearalenone in Corn and Corn Meal," *Revista do Istituto Adolfo Lutz* **57**, 65–71 (1998).

J. M. Mitchell et al., "Antimicrobial Drug Residues in Milk and Meat: Causes, Concerns, Prevalence, Regulations, Tests, and Test Performance," *J. Food Prot.* **61**, 742–756 (1998).

H. Pichler et al., "An Enzyme-Immunoassay for the Detection of the Mycotoxin Zearalenone by Use of Yolk Antibodies," *Fresenius J. Anal. Chem.* **362**, 176–177 (1998).

B. Roberts, B. A. Morris, and M. N. Clifford, "Comparison of Radioimmunoassay and Spectrophotometric Analysis for the Quantitation of Hypoxanthine in Fish Muscle," *Food Chem.* **42**, 1–17 (1991).

E. Rodrigues et al., "Sandwich-ELISA for Detection of Goat's Milk in Ewes' Milk and Cheese," *Food Agric. Immunol.* **6**, 105–111 (1994).

F. Sanchez Garcia et al., "Polarization Fluoroimmunoassay of the Herbicide Dichloroprop," *J. Agric. Food Chem.* **41**, 2215–2219 (1993).

R. Schuhmacher et al., "Interlaboratory Comparison Study for the Determination of the Fusarium Mycotoxins Deoxynivlenol in Wheat and Zearalenone in Maize Using Different Methods," *Fresenius J. Anal. Chem.* **359**, 510–515 (1997).

A. Szekacs, "Enzyme-Linked Immunosorbent Assay for Monitoring the *Fusarium* Toxin Zearalenone," *Food Technology and Biotechnology* **36**, 105–110 (1998).

L. S. Shiow and F. M. You, "Determination of Aflatoxins in Liquor Products by Immuno-Affinity Column With Fluorometry and HPLC," *Journal of Food and Drug Analysis* **5**, 161–170 (1997).

E. Simon et al., "Development of an Enzyme Immunoassay for Metsulfuron-Methyl," *Food Agric. Immunol.* **10**, 105–120 (1998).

T. G. Sokari and S. O. Anozie, "Modified Single Radial Immunodiffusion Method for Screening Staphylococcal Isolates for Enterotoxin," *Food Microbiology* **6**, 45–48 (1989).

H. Tian et al., "Rapid Detection of *Salmonella* sp. in Foods by Combination of a New Selective Enrichment and a Sandwich-ELISA Using Two Monoclonal Antibodies Against Dulcitol 1-Phosphate Dehydrogenase," *J. Food Prot.* **59**, 1158–1163 (1996).

I. A. Toscano, G. S. Nunes, and D. Barcelo, "Immunoassays for Pesticide Analysis in Environmental and Food Matrixes," *Food Technology and Biotechnology* **36**, 245–255 (1998).

M. W. Trucksess et al., "Survey of Deoxynivalenol in U.S. in 1993 Wheat and Barley Crops by Enzyme-Linked Immunosorbent Assay," *J. AOAC Int.* **78**, 631–636 (1995).

M. Tuomola et al., "Time-Resolved Fluoroimmunoassay for Measurement of Androsterone in Porcine Serum and Fat Samples," *J. Agric. Food Chem.* **45**, 3529–3534 (1997).

Y. Ueno et al., "A 4-year Study of Plasma Ochratoxin A in a Selected Population in Tokyo by Immunoassay and Immunoaffinity Column-Linked HPLC," *Food and Chemical Toxicology* **36**, 445–449 (1998).

Y. Wanjun and S. Fun, "Improved Direct Competitive Enzyme-Linked Immunosorbent Assay for Cyclopiazonic Acid in Corn, Peanuts and Mixed Feed," *J. Agric. Food Chem.* **46**, 1012–1017 (1998).

A. K. Wintero, P. D. Thomsen, and W. Davies, "A Comparison of DNA-Hybridization, Immunodiffusion, Countercurrent Immunoelectrophoresis and Isoelectric Focusing for Detecting the Admixture of Pork to Beef," *Meat Science* **27**, 75–85 (1990).

G. M. Wood et al., "Ochratoxin A in Wheat. A Second Intercomparison of Procedures," *Food Additives and Contaminants* **13**, 519–539 (1996).

J. M. Yeung and P. G. Collins, "Determination of Soy Proteins in Food Products by Enzyme Immunoassay," *Food Technology and Biotechnology* **35**, 209–214 (1997).

X. Zhao et al., "Radioimmunoassay for Insulin-like Growth Factor-I in Bovine Milk," *Canadian Journal of Animal Science* **71**, 669–674 (1991).

D. E. DIXON-HOLLAND
Neogen Corporation
Lansing, Michigan

INFANT FOODS

INFANT NUTRITION

Infants are defined, somewhat arbitrarily, as less than 1 year of age. Infant foods and feeding practices have been adapted to encompass infants and young children to allow for feeding during the first 2 to 3 years.

The infant has unique nutritional needs, substantially different from those of an adult. Energy, protein, iron, and other needs are greater than at any other period in life. Food must be relatively concentrated, and with a controlled consistency, because the volume capability, chewing, and manipulation of food are limited. At no other point in life is the growth rate of infancy duplicated. Infants typically double their birth weight in 3 to 4 months and triple it by 1 year, and the rapid growth rate places nutritional demands on the food supply. To accompany the rapid growth rate, infants have a very high basal metabolic rate. Complicating this is a limited stomach capacity, requiring an appropriate concentration of nutrients. The limited renal capability must be considered to ensure that electrolytes and protein are not present in deleterious quantities. In addition, the digestive system is immature, which requires that foods be selected that are relatively easy to digest. The most obvious of the characteristics is the limited ability to chew foods; therefore the foods must be appropriate in consistency and texture.

Breast feeding is recognized as the preferred method for feeding very young infants, and breast milk should be the exclusive food for the first 4 to 6 months unless circumstances (mother unable to breast-feed, breast milk inadequate, etc) do not allow exclusive breast feeding. Any food other than breast milk that is introduced should supplement rather than replace breast feeding.

For these reasons, a specific and well-defined set of foods have been developed for infants and young children. Infant feeding practices vary considerably in different parts of the world depending on the availability of food and custom. Definitions and standards have been developed by Codex Alimentarius but are, for the most part, utilized as guidelines.

HISTORY OF FOODS AND FEEDING PRACTICE

Infant foods have evolved largely parallel to the development of food science and technology, and the implementation of food safety and food processing practices has dramatically influenced the availability of safe foods. Before 1800 about two-thirds of children died before 5 years of age, and that statistic has dramatically changed. Wet nurses (women hired to suckle others' children) were widely used when breast feeding was impossible. Otherwise, supplementary foods were of poor quality and unclean; as a result, food was the most frequent cause of serious illness. A first major step was heat treatment of milk; pasturization was first, followed by the development of sweetened condensed milk, which provided a much safer source of milk for infants than the raw or certified milk available previously. Early efforts to prepare foods specifically for infants and young children included meat juices and cereal gruels (Mellon's Food), malted milk (Horlick's Malted Milk), and sweetened condensed milk (Borden). The milk-based foods combined with hydrolyzed complex carbohydrates (dextrins and maltodextrins) evolved to what is now considered infant formula, and foods derived from other sources have been termed baby foods and infant cereal.

INFANT FORMULA

Human milk serves as the nutritional model for infant formula. The original infant formula was derived from cow milk; however, the concentration and nutrient distribution of cow milk are not appropriate for infants. The protein content is too high, the casein is not well digested, the electrolyte concentration is too great, the fat is largely saturated and poorly absorbed, and the carbohydrate concentration is too low. Adjustments have been made using cow milk as a base, and other components have been formulated to simulate or "humanize" milk. Later in the evolution of infant formula, alternate protein sources were used; initially meat and later, during the 1960s, soy-based formulas were refined. Currently, approximately one-fourth of infant formula utilized in the United States is soy protein based.

Regulatory Aspects

Infant formula is unique in food regulation because it is regulated by specific legislative mandate in the United States. The Infant Formula Act of 1980 and subsequent amendments in 1986 specify nutrient requirements, manufacturing practices, and quality control. This specificity is considered essential because infant formula may provide the sole source of nutrition for an infant for an extended period during critical rapid growth and development.

Functionality

Infant formula has evolved as a nutritional entity to provide all the requirements for an infant during the first 6 months. After 6 months infant formula, like human milk, continues to provide a significant contribution to the nutrition of infants. The adequacy of infant formula to support growth and development is established by clinically controlled feeding trials.

Technologies

Several technologies have been utilized to ensure the safety and nutritional quality of infant formula. Concentration and controlled processing techniques ensure the sterility, keeping quality, and nutrient retention for undiluted infant formula. Drying techniques have been adapted to provide powdered infant formula with quality equivalent to liquid forms. Alternate protein sources predominately soy protein, have been refined to provide adequate protein quality for the normal growth and development of infants. Cow milk protein has been modified to imitate the casein to whey protein distribution of human milk. The carbohydrate and fat components have been adjusted to effectively imitate those of human milk. Clinical trials are used to confirm the nutritional adequacy of infant formula. Formulas based on extensively hydrolyzed protein are effectively used for infants with food protein allergies.

BABY FOODS

Over 300 varieties of baby foods are available in North America to fulfill specific needs during the development of the infant. Recommendations by the American Academy of Pediatrics and other involved organizations are that baby foods be introduced at 4 to 6 months to supplement human milk or infant formula.

Several terms are used, somewhat interchangeably, to define baby foods: supplementary foods, biekost, and solid food. Other accurate classifications include strained fruit juices, infant cereal, fruits, vegetables, and numerous combination foods with fanciful names.

The first foods introduced are generally infant cereals. Infant cereal is a specific food: precooked, partially hydrolyzed, dried cereal-based food. In North America, infant cereal is fortified with iron, vitamins, and minerals. Infant cereal is fed in a diluted form with water, human milk, or infant formula used as a diluent. Ready-to-use cereal is also available, diluted and mixed with fruit for convenient and safe feeding.

Fruits and vegetables are essentially the pureed version of the food sterilized in a container to provide an appropriate serving size. Their container (serving) sizes range from approximately 70 to 135 g (2.5–4.5 oz) for subsequent forms. Fruit juices are strained, single-ingredient and combination juices provided in appropriate-sized containers, fortified with vitamin C at a uniform level.

Meat and poultry are provided in pureed form and sterilized in small containers. Numerous combination foods are prepared, largely for consumer convenience. The terminology used for combination foods is manufacturer specific and intended to be descriptive for the consumer.

Functionality

The functionality of infant foods generally refers to the consistency (as either a puree, liquid, or slurry). Consistency relates to the physical limitations of the infant and young child, but other characteristics are equally important to establish the appropriateness of baby foods for consumption. Among these are nutritional adequacy, particularly calories and protein, and concentration. If the concentration is too high, the food may stress the infant's developing renal system. If too dilute, the food may satisfy the volume requirements for the infant digestive system without provision of adequate nutrients.

Technologies

The technologies for baby foods have been adapted from food processing techniques, with specific modifications that allow the foods to fulfill the specific requirements for the intended purpose. The processes have evolved, and specific improvements in food science and food processing have been applied. Baby foods, as presently recognized, were developed between 1920 and 1930 by numerous manufacturers. Originally, the distribution for baby foods was entirely through pharmacies; after 1933 convenience was enhanced by distribution through food outlets, first grocery stores and then supermarkets. The technology originated in North America and did not expand to other parts of the world until after World War II.

Infant cereal was developed in 1933 as a partially hydrolyzed, sterilized food prepared from flour and dried in a flake form to facilitate rapid dispersion. Pablum was the first widespread product and was the generic reference for infant cereal for many years. Supplemented with iron and water-soluble vitamins, it is a well-tolerated food used to supplement milk-based foods.

Canned baby foods are based on fruits, vegetables, and meats finely divided, or pureed. Baby foods were originally sterilized in 4- to 4.5-oz metal cans; conversion to glass containers was made in the period between 1955 and 1960. Combinations of fruit, vegetables, cereal, and meat have been made to provide convenience to the consumer.

CONTROVERSIES

Numerous controversies surrounded preparation and use of baby foods, and many were paralleled by similar discussions concerning the safety and suitability of the entire food supply. Discussions were initiated during a White House conference on nutrition in 1969, with allegations and questions that had not been comprehensively treated in earlier scientific and safety testing. Since 1969 most of the controversies have been resolved. Thorough evaluation, more extensive knowledge, and effective communications have provided consumers and scientists with assurance of the safety and suitability of the foods and ingredients used.

Salt

The intake of salt by older infants has been of interest and concern to nutrition scientists for the past 25 years. The concern was initiated based on the possibility that consumption of salt in infancy could influence the salt intake in later life, possibly contributing to the development of hypertension. The subject has been reviewed by the National Academy of Sciences and the American Academy of Pediatrics, which concluded that no advantage could be served by addition of salt to infant foods. Salt has not been an ingredient in infant foods since 1977.

Sugar

Sugar has been criticized as an ingredient in baby foods for two basic reasons: it adds calories without other nutrients and it may, if improperly used, cause tooth decay (infant dental caries). Both of the allegations or hypotheses were only partially substantiated, but most baby foods are prepared without added sugar.

Monosodium Glutamate

Monosodium glutamate (MSG) was used as a flavor enhancer in some meat-containing foods before 1969. As a result of safety questions and lack of clinical safety information, MSG is not used as an ingredient in baby foods.

Modified Food Starch

Modified food starch (MFS) has been used extensively in many foods as a digestible stabilizing system. Baby foods are an ideal use, because small amounts of MFS (1–2%) effectively suspend the particles and prevent age-associated separation. The safety, at levels of use, has been established by the National Academy of Sciences and the American Academy of Pediatrics. The use remains a source of controversy because critics of MFS consider it an extender rather than a stabilizer.

Artificial Colors and Flavors

Artificial colors, flavors, and preservatives are not used in baby foods primarily because they are not required and many consumers have an aversion to their use.

CONSUMPTION PATTERNS

Changes in Usage

From the time of their development in the 1950s and 1960s until 1972, the use of infant formulas increased substantially to a point that approximately 70% of infants were fed formula. As a result of concerted efforts by feeding advisers, primarily pediatricians, the practice of breast feeding significantly increased in both incidence and duration.

By the late 1980s the ratio had changed to a point that more than 70% of infants were breast-fed, and that ratio prevails to the present.

The consumption of baby foods increased considerably from the time of initiation in the 1920s until a maximum level of usage was reached, almost 800 jars per baby, in 1972. The advantages and the role were recognized: to provide a supplementary quantity of energy, a variety of foods, and valuable nutrients, particularly protein. Several recommendations were made during this period by the American Academy of Pediatrics in 1958, 1976, 1980, and 1986. During the decade after 1972, the usage decreased by approximately 20% as a result of a number of factors. Consumption of supplementary foods was perceived to interfere with breast feeding, questions arose concerning overfeeding, which resulted in infant obesity, and alternatives became available. Consumption has been stable during the past decade.

ALTERNATIVES

Numerous alternative foods and feeding practices were involved in the reduced consumption of baby foods. Foods were often prepared from family foods—home preparation—or were specifically prepared from selected ingredients. The use of milk-based foods, sometimes referred to as follow-on foods, was more extensive. In some instances breast feeding or infant formula was continued for a more extensive period. Consumer concerns, economics, and availability of foods were all contributing factors to the changes and use of alternatives.

GENERAL REFERENCES

American Academy of Pediatrics, *Pediatric Nutrition Handbook*, 3rd ed., American Academy of Pediatrics, Elk Grove Village, Ill., 1993.

American Academy of Pediatrics Committee on Nutrition, "Sodium Intake by Infants in the United States," *Pediatrics*, **68**, 444–445 (1981).

J. T. Bond et al., eds., *Infant and Child Feeding*, Academic Press, New York, 1981.

National Research Council Food Protection Committee, Food and Nutrition Board, *Report of the Subcommittee on Safety and Suitability of MSG and Other Substances in Baby Foods*, National Academy of Sciences, Washington, D.C., 1970.

G. A. Purvis and S. J. Bartholmey, "Infant Feeding Practices: Commercially Prepared Baby Foods," in R. C. Tsang and B. L. Nichols, eds., *Nutrition During Infancy*, Henly & Belfus, Inc., Philadelphia, Pa., 1988.

GEORGE PURVIS
Purvis Consulting, Inc.
Fremont, Michigan

INSTITUTE OF FOOD TECHNOLOGISTS (IFT)

The Institute of Food Technologists (IFT) was founded July 1, 1939, by unanimous vote of attendees at the closing session at the Second Food Technology Conference held at the Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts. The first conference had been organized by MIT and was chaired by Samuel Cate Prescott, Dean of Science and head of the biology department at MIT. The concept of the Institute of Food Technologists had been described at meetings at the New York State Agricultural Experiment Station in Geneva, New York, and G. J. Hucker was asked to seek opinion of scientists working in the food field, regarding the need for an organization dedicated to the use of sound science in the business of food.

The purpose of that early form of IFT was "to facilitate interchange of ideas among its members; to stimulate scientific investigations into technical problems dealing with the manufacture and distribution of foods; to promulgate the results of research in food technology; to offer a medium for the discussion of these results; and to plan, organize, and administer such projects for the advancement and application of science insofar as it is fundamental to wider knowledge of foods."

An early suggestion regarding a name for the Institute was "the Society of Food Engineers." The name "Institute of Food Technologists" was favored because it permitted a broader base of professionals. Various other names have been suggested from time to time, but the name has not been changed since the inception of the organization.

Samuel Cate Prescott was IFT's first president. He predicted, in 1941, that IFT would eventually have 1000 members. He would have been surprised at the present membership—approaching 29,000. Currently, the average member is male, about 42, and working for a food or beverage manufacturer. About 20% of members are employed by academic institutions, and a significant number of governmental agency employees are members. About 8% of total members are from countries outside the United States.

IFT operates with a paid staff of about 50 professionals, from its headquarters at 221 N. LaSalle Street, Chicago, IL 60601. The Institute uses a comprehensive committee system to coordinate its 24 divisions (which serve as centers of excellence), 55-plus regional sections, affiliate organizations, and student associations. The Executive Committee, made up of the current president, past president, president elect, treasurer, assistant treasurer, and executive director plus membership representatives, councilor representatives, and a student representative, receives recommendations for action from various standing committees and acts on many of them. Certain recommendations are made to the Council, elected by members on a yearly ballot, for action. The "three presidents" model has been developed to provide for easy transition from year to year.

The Institute has an annual meeting, during which the business of the Institute is conducted at the council meeting, and subsequent executive committee meeting and some 3000 technical presentations are given. At the same time as the annual meeting, Food Expo, the largest exposition of new ingredients, instruments, equipment, and services in the world is held, with about 1800 exhibitors covering several thousand square feet of floor space. Recently, IFT has added a virtual trade show, called

WorldFoodNet, which includes all exhibitors in the exposition, available for those who didn't attend and those who forgot what they might have seen.

The Institute is a professional society, and as such, does not lobby. The emphasis on all interaction with government is the dissemination of science-based information. The Science Communication department of IFT is devoted to the development of solid science for use by the industry, to consumers, and to the media. A project that is relatively recent is the support of a congressional fellow, who acts as a science adviser to members of Congress. This activity is supported by the IFT Foundation, which raises money to be used in important new directions for the Institute, as well as to support a number of scholarships for young people at the university level.

There is a strong educational component to IFT, both with food science departments that meet standards that have been developed by IFT, and in continuing education. Each year, 20-plus programs are given under the guidance of the Continuing Education department, to help food technologists keep up to date with new science and changing regulatory and nutritional emphasis.

Publications from IFT include *Food Technology*, a monthly magazine that includes news about Institute members and the associated academic, industrial, and regulatory components. The *Journal of Food Science* publishes technical papers, six issues per year. There is a membership directory, and Expo-associated publications. The Institute hosts a popular website at www.ift.org where more information is available.

Frances Katz
Institute of Food Technologists
Chicago, Illinois

INSTITUTE OF FOOD TECHNOLOGISTS: AWARDS

The Institute of Food Technologists (IFT) Committee on Awards seeks your nominations for IFT Achievement Awards described in the following. A single nomination form is provided. Election as an IFT Fellow requires a different form, which may be requested from IFT headquarters, by using the IFT E-XPRESS fax-on-demand service by dialing 800-234-0270 in the United States and Canada (elsewhere by dialing 913-495-2551) and requesting document number 3510, or by visiting the IFT Web site at www.ift.org. The Marcel Loncin Research Prize is given in even-numbered years, and instructions for applying for it are available on the IFT Web site or by using IFT-Express (request document 3530).

Rules for All IFT Achievement Awards

1. The nominee must not have received another IFT Achievement Award or the Marcel Loncin Research Prize (but may have been elected an IFT Fellow) within the previous 5 years. A list of prior award recipients appears in the IFT Membership Directory.
2. Any nonstudent IFT member can make a nomination with four exceptions: (*1*) nominations or letters may not come from a juror serving on the same jury, (*2*) a person cannot self-nominate, (*3*) a person may nominate his or her own company for the Industrial Achievement Award, and (*4*) student members can nominate for the Cruess Award.
3. The nomination must be received by IFT by December 1.
4. The nomination is handled in confidence. The identity of the awards jury members is confidential.
5. Unsuccessful nominations will be returned and may be resubmitted in subsequent years.
6. All material in excess of specified page limits will not be given to the jury. Letters of support from sections and divisions are included in these limits and should be addressed to the Awards Committee.
7. In any year in which none of the nominees meet the criteria, an award may not be presented.
8. When two or more people nominate the same person for the same award, the nominators will be contacted and asked to combine their nominations and submit a single nomination to the jury. If there is not enough time to do this, the first nomination received will be sent to the jury for consideration.

NICHOLAS APPERT AWARD

Award. $5,000 honorarium from IFT and a bronze medal from the Chicago Section of IFT.

Purpose. To honor an IFT member or nonmember for preeminence in and contributions to the field of food technology.

Eligibility. The nominee must have made consistent—and essentially lifetime—contributions to food science and technology. Factors of personality or achievement outside the field of food technology cannot be considered. For a detailed description and history of the award and the accomplishments of past winners, see the article "The Nicholas Appert Medalists—A Reflection of the Growth of Food Science and Technology," by Alina Surmacka Szczesniak, *Food Technology*, September 1992, pp. 144–152.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

BABCOCK-HART AWARD

Award. $3,000 honorarium from the International Life Sciences Institute North America and a plaque from IFT.

Purpose. To honor an IFT member who has attained distinction by contributions to food technology that result in improved public health through nutrition or more nutritious food.

Eligibility. The nominee must have made practical technological contributions resulting in improved public health, as distinguished from contributions to clinical nutrition or purely scientific research, unless it has led directly to technological developments. The technological contributions, or the technological development resulting from scientific research, must be in actual, large-scale production leading to better consumer nutrition. Such contribution could be made in the area of food production, food processing, or food packaging, storage, or distribution. An example of a contribution in processing might be the development of equipment that would process food in a way to conserve nutrients or prevent wastage; another might be the development of a process that would inhibit or prevent bacterial spoilage, oxidation, desiccation, or enzymatic deterioration of food, providing such contribution eventually influences the nutritive well-being of the consumer. An example of a contribution in food production might be genetic studies to develop improved strains of cereal, fruits, or vegetables that would provide greater yield or increased nutritive content; another might be studies on animal nutrition that improve efficiency of production or studies on animal diseases that result in decreased losses. Contributions could also be made in packaging, storage, or distribution conditions that would eventually affect nutritive value.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

SAMUEL CATE PRESCOTT AWARD

Award. $3,000 honorarium and a plaque from IFT.

Purpose. To honor an IFT member who has shown outstanding ability in research in some area of food science and technology.

Eligibility. The nominee must, by July 1 of the year of the presentation, (*1*) be younger than 36 years of age or (*2*) have received his or her highest degree within the previous 10 years. Special attention is given to contributions in methodology, competence shown, and effects of the research on advances in food science.

Special instructions. The nomination statement must not be more than four typed pages. Attach a numbered list of nominee's publications and patents and, to save space, refer to them in the nomination statement using these numbers.

INTERNATIONAL AWARD

Award. $3,000 honorarium and a plaque from IFT.

Purpose. To honor an IFT member or an institution whose outstanding efforts result in one or more of the following: (*1*) international exchange of ideas in the field of food technology, (*2*) better international understanding in the field of food technology, and/or (*3*) practical successful transfer of food technology to an economically depressed area in a developing or developed nation.

Eligibility. A balance between technology transfer and international exchange of ideas is desirable. *International exchange of ideas* may be defined as one-on-one interactions, including sustained consulting or advising in several countries. This may include educating and training students, researchers, plant workers, and others who return to foreign lands. *Better international understanding* may be defined as continued interaction through international seminars, symposia, or working groups. Organizing responsibility is an important component. *Technology transfer* is a hands-on operation involving successful one-to-one transfer of food technology from those who have the knowledge to those who need and use it. The result must be a new, commercially viable process, packaging system, postharvest preservation system, or other application of food science and technology of direct benefit to the local economy. Although such work could start with joint assessment of the need and potential market and involve training in the donor's laboratories or pilot plant, emphasis is on on-site training and development of sufficient duration (probably at least 6 months) so the process continues to function and benefit consumers long after the donor has returned home.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

WILLIAM V. CRUESS AWARD

Award. $3,000 honorarium from IFT and a bronze medal from the Northern California Section of IFT.

Purpose. To honor an IFT member who has achieved excellence in teaching food science and technology.

Eligibility. Any IFT member who has at least 5 years of experience in university teaching in a department offering a bachelor's degree in food science, food technology, or the equivalent is eligible. Courses taught by the nominee must deal directly with some aspect of food science or food technology.

Special instructions. Members of the IFT Student Association may nominate individuals for this award. A nomination statement is not required but, if provided, must not be more than one typed page. The nomination should be accompanied by up to eight letters signed by students, former students, or groups of students who have taken courses taught by the nominee. These letters should discuss one or more of the following criteria: (*1*) mastery of subject matter, (*2*) ability to communicate effectively the principles and practices of food processing and preservation, (*3*) enthusiasm for the field of food science and technology as a profession, (*4*) ability to stimulate students to select a professional career in food science and technology, and (*5*) ability to counsel effectively. Letters from up to four professional colleagues and administrators may also be offered but will not be used as primary evidence.

CARL R. FELLERS AWARD

Award. $3,000 honorarium from Phi Tau Sigma and a plaque from IFT.

Purpose. To honor a member of IFT and Phi Tau Sigma who has brought honor and recognition to the profession of food science and technology through a distinguished career in that profession displaying exemplary leadership, service, and communication skills that enhance the effectiveness of all food scientists in serving society.

Eligibility. Nominee must have been a professional member of IFT for at least 15 years before the nomination deadline of December 1. Nominees who are not members of Phi Tau Sigma shall be simultaneously elected to Phi Tau Sigma membership by that organization upon selection as recipient of the Carl R. Fellers Award. In considering the further qualifications of nominees, priority shall be given to a record of achievement in communicating to the profession, to governmental and international agencies, and/or to the public scientific information relative to the quality, wholesomeness, safety, nutritive value, and other aspects of food and the food supply; in bringing individuals into the profession; and/or in propagating knowledge of the importance of the profession to the public, governmental agencies, and scientific and academic communities.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

FOOD TECHNOLOGY INDUSTRIAL ACHIEVEMENT AWARD

Award. A bronze plaque from IFT.

Purpose. To honor a company or organization for developing an outstanding food process and/or food product that represents a significant advance in the application of food science and technology to food production.

Eligibility. The process or product must have been successfully applied in actual commercial operation at least 6 months but no more than 7 years before December 1 of the year in which the nomination is submitted.

Special instructions. The nomination statement should describe, as much as possible, the development itself and its public health, scientific, technological, and economic importance and must not be more than four typed pages. To assist in judging the nomination, you may include 10 copies of each of the following: reprints describing the product or process, sample of the product, and videotape not to exceed 5 minutes. Reprints should be limited to a maximum of 4 different articles, or a total of 20 pages.

CALVERT L. WILLEY DISTINGUISHED SERVICE AWARD

Award. $3,000 honorarium a plaque from IFT.

Purpose. To honor an individual who has provided continuing, meritorious, and imaginative service to IFT. The award was first presented to Calvert L. Willey, then executive director of IFT, in 1987 and initiated on the fiftieth anniversary of IFT in 1989.

Eligibility. The nominee must have been a professional member of IFT for 15 years or more, a member of IFT for 25 years or more, or on the IFT staff for 25 years or more. The nominee must not have received any other IFT Achievement Award. This does not include election as an IFT Fellow or the Marcel Loncin Research Prize. The primary consideration is continuing service to the institute rather than specific contributions to research, education, or development. Qualification is based on dedication, unique service, and supreme effort on behalf of IFT.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

STEPHEN S. CHANG AWARD FOR LIPID OR FLAVOR SCIENCE

Award. $3,000 honorarium and a Steuben crystal sculpture.

Purpose. To honor an IFT member food scientist or technologist who has made significant contributions to lipid or flavor science.

Eligibility. The contributions can be on any aspect of lipid or flavor science, ranging from basic chemistry to applied technology, but must have had some impact on commercial operations. The award applies to all disciplines involved in lipid or flavor science. For example, the nominee (*1*) studies the basic chemistry and mechanism of autoxidation of lipids and the knowledge is used by one or more companies to improve the stability of their products; (*2*) publishes papers concerning the chemistry and processes for the removal, from fats and oils, of impurities that might be harmful to health, and the knowledge is used by one or more companies to improve the wholesomeness of their products; (*3*) studies basic chemistry and the process of deep-fat frying, and this information is used by one or more companies to improve their deep-fat-fried foods; (*4*) significantly contributes to the understanding of the chemical synthetic or biosynthetic process for the optimization of production of new, or nature-alike, flavor chemicals; (*5*) identifies the chemical profiles of potential flavor sources and pinpoints the importance of key flavor chemicals by correlating them with physiological flavor response; or (*6*) makes significant advances in the development of analytical methodology in flavor chemistry.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

INDUSTRIAL SCIENTIST AWARD

Award. $3,000 honorarium and a plaque from IFT (if a team wins, each member of the team will receive a plaque and an equal share of the $3,000).

Purpose. To honor an IFT member industrial scientist or team of industrial scientists who have made a major technical contribution to the advancement of the food industry.

Eligibility. All team members must be IFT members. Team nominations should identify those persons primarily responsible for the achievement. Team size is limited to a maximum of five persons. Team nominations should identify the role of each team member in accomplishment. Once a nomination is submitted, no additional team members may be added.

The nominees must have made one or more major technical contributions to the advancement of the food industry while a member of an industrial food organization. The nominees' contribution must have had a significant, measurable impact in areas such as production; development of new products, processes, or packaging systems; improvement of food safety and nutrition; and so forth. Food research in industry is generally a team approach, making it difficult to assign credit for an outstanding technical accomplishment to one person or a small group. Nevertheless, the nomination should identify the person(s) most responsible for making the project(s) a success. Part of this success is the direct benefit of the scientists' contribution to consumers and to the profession of food science and technology.

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

RESEARCH AND DEVELOPMENT AWARD

Award. $3,000 honorarium and a plaque from IFT (if a team wins, each member of the team will receive a plaque and an equal share of the $3,000).

Purpose. To honor an IFT member or team of members who have made a recent, significant research and development contribution to the understanding of food science, food technology, or nutrition.

Eligibility. All team members must be IFT members. Team nominations should identify those persons primarily responsible for the achievement. Team size is limited to a maximum of five persons. Team nominations should identify the role of each team member in accomplishment. Once a nomination is submitted, no additional team members may be added.

The nominees must be primarily responsible for an achievement within the past 5 years in a research and development program. The achievement must significantly advance the discipline of food science, food technology, or nutrition. The contribution may be basic or applied in nature and must advance science or improve the human condition. The nominees may have worked in any field (including academic, industrial, government, consulting, self-employment, or other endeavors).

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications but cite relevant papers, patents, or articles that show the significance of the work.

ELIZABETH FLEMING STIER AWARD

Award. $3,000 honorarium from the New York Section of IFT and a plaque from IFT.

Purpose. To honor an IFT member for pursuit of humanitarian ideals and unselfish dedication that have resulted in significant contributions to the well-being of the food industry, academia, students, or the general public.

Eligibility. The nominee must have been an IFT member for the past 10 years and active in IFT at both the section and national levels. In instances where there is no IFT section level, activity in other food industry–related organizations may be considered. The nominee must not have received any other IFT Achievement Award but may have been elected an IFT Fellow.

The nominee must have made measurable contributions to the well-being of the food industry, academia, students in food science or closely related disciplines, and/or the general public. Such contributions must have demonstrated compassion and unselfish caring for the world and its people, measured not necessarily in terms of published papers or grants received but rather in terms of the number of lives enhanced by contact with the nominee or by the achievements of individuals as a result of such contact.

Examples include but are not limited to outstanding activities in (*1*) academic advising or mentoring; (*2*) service to IFT or the industry, institutions, governments, or publics it serves; or (*3*) service to groups or individuals in need (such as work in hunger programs or in the delivery of education to persons without other means of acquiring it).

Special instructions. The nomination statement must not be more than four typed pages. Do not include a curriculum vitae or lists of publications and patents.

BERNARD L. OSER FOOD INGREDIENT SAFETY AWARD

Award. $3,000 honorarium and plaque from the Bernard L. Oser Endowment Fund.

Purpose. To honor an IFT member for his or her contribution to the scientific knowledge of food ingredient safety or for leadership in establishing principles for food ingredient safety evaluation or regulation.

Eligibility. The nominee must have been a professional in the area of food additive or GRAS ingredient safety in an academic, industrial, and/or government setting for at least 10 years. The area of professional involvement for this award can include toxicological research, analytical research, safety/regulatory involvement, and associated scientific work in support of food additive and GRAS ingredient safety evaluation and policy development. Publications and examples of leadership qualities in the area of safety evaluation will be the criteria for final selection.

Special Instructions. Must not be more than four typed pages. Attach a numbered list of nominee's publications, and, to save space, refer to them in the nomination statement using these numbers.

PATTI PAGLIUCO
Institute of Food Technologists
Chicago, Illinois

INSTITUTE OF FOOD TECHNOLOGISTS: HISTORY AND PERSPECTIVES

The Institute of Food Technologists (IFT) is one of the premier societies for food science and technology in the world. Indeed it may be *the* premier society. Founded in 1939, IFT has grown to a membership of approximately 29,000 (1).

It has 56 regional sections, some of which have subsections in adjoining geographical areas where a number of IFT members live but distance is too great for them to attend regularly meetings of the regional section. There are 24 discipline or commodity divisions such as Carbohydrate, Food Chemistry, Food Engineering, Fruit and Vegetable Products, and Sensory and a very strong student association. The latter has recently petitioned to be designated as a division. That change would be more a matter of title than of increased stature, for there are already provisions for a student to be a voting member of the IFT's Executive Committee, its Finance Subcommittee, and several other committees.

FORMATION OF THE INSTITUTE

The formation of the Institute in 1939 was the result of the efforts of several food technologists and engineers who felt that there should be a discipline for a subject as important as the production, nutrition, and safety of foods. In 1937 a food technology conference was held at the Massachusetts Institute of Technology (MIT). The conference was chaired by Dr. Samuel C. Prescott, Dean of Biology at MIT. At the conclusion of the conference, Dr. Bernard E. Proctor, also of the MIT faculty, expressed the hope that similar gatherings would be held to function as clearinghouses of information for the scientific and industrial communities. Concurrently at the New York Agricultural Experiment Station in Geneva, New York, George J. Hucker was fostering the idea of an organization of food-related societies.

BEYOND BROACHING AN IDEA

In January 1939 representatives of the two groups met in New York City to discuss the details of such an organization. Lawrence V. Burton, then the editor of *Food Industries*, came prepared with a proposed draft for such an organization, suggested a definition of food technology, and proposed that the organization be called the Society of Food Engineers. The group selected instead the name Institute of Food Technologists, because several individuals realized that the organization should not restrict itself to engineers.

The purpose of the organization was specified as follows:

> To facilitate interchange of ideas among its members
>
> To stimulate scientific investigations into technical problems dealing with the manufacture and distribution of foods
>
> To promulgate the results of research in food technology
>
> To offer a medium for the discussion of those results
>
> To plan, organize, and administer such projects for the advancement and application of science insofar as it is fundamental to wider knowledge of foods

The Second Food Technology Conference was held at MIT, June 29 to July 1, 1939, under the direction of Dr. Proctor. At that meeting it was formally decided to establish the IFT. Prescott was elected president, Roy C. Newton of Swift & Co. vice president, and Hucker, secretary-treasurer. (Eventually, Burton, Hucker, and Proctor all were elected presidents of the IFT.) The first team of officials is the only one that served for a second year. (However, today the president does serve one year as president-elect and one year as immediate past president, having several official duties as president-elect and some as immediate past president.) There were 600+ scientists and engineers in attendance at the meeting in 1939

ANNUAL MEETING THEREAFTER

In Pittsburgh in October 1940, the Council (ie, the governing body of the Institute) held its first meeting, elected members, and scheduled the first IFT conference to be held in Chicago the following June. There were 839 founder and charter members. (In 1997 there were 9031 professional members, 14,855 members, 1709 emeritus members, and 2642 student members.) The Institute has had an annual meeting and food exposition every year since 1941 with the exception of 1945 when the meeting was not convened in order to aid the war effort by avoiding unnecessary travel.

EARLY–CURRENT OFFICERS

The Institute was served for five years by Hucker as its voluntary secretary-treasurer, then for five years by Carl R. Fellers. In 1949 Charles S. Lawrence, former commandant of the Quartermaster Food and Container Institute for the armed services was appointed secretary-treasurer on a one-half time basis. Lawrence set the IFT office up in Chicago where it still is though the Food and Container Institute was later moved to Natick, Massachusetts, and is now a part of the U.S. Army Natick RD&E Center. Lawrence served as secretary-treasurer until 1961. Calvert L. Willey was hired as a full-time executive secretary; the title was later changed to executive director. He retired in 1987. That year Howard W. Mattson, who had been director of public affairs, became the executive director. He resigned for health reasons in 1991. Daniel E. Weber, who had been director of meetings and expositions for several years, became executive director upon the resignation of Howard Mattson. In 1999, his title was changed to executive vice president.

IFT ORGANIZATIONAL STRUCTURE

The Council of the IFT is the policymaking body of the organization. Aside from policy, any changes in the Constitution must be acted upon by the Council. The Council is composed of one or more councillors elected by each of the regional sections and one councillor from each of the divisions. The number of councillors a regional section has is determined by the number of IFT members within its region. In essence, the Council represents the members, for each councillor is elected by the members of his or her regional section.

The executive committee is the administrative body for the members. It is composed of six members elected from among the councillors; six elected from among the members at large; the president-elect, the president, and the immediate past president; plus, as ex officio members without a vote, the treasurer of the Institute, the assistant treasurer, and the executive vice president of the Institute. The Finance subcommittee of the executive committee prepares the budget, but approval or disapproval rests in the hands of the executive committee. The executive committee is the agency that provides direction to the staff and assignment of committee responsibilities, and other matters relating to running the organization. The executive committee answers to the Council on matters relating to policy; for matters such as establishing the budget, it merely informs the Council of its decision.

By far the bulk of members' input is by way of the committee structure. There are 25 committees, plus some of the committees have subcommittees. The executive committee has four subcommittees: finance, publications, scientific editor, and information systems. The IFT has representatives on five other organizations such as International Union of Food Science and Technology (IUFoST) and Codex Alimentarius, and it has liaisons with 11 other organizations such as the American Association for the Advancement of Science, American Oil Chemists' Society, Society of Toxicology, and the American Dietetic Association. Committee members are appointed by the president-elect for a three-year term with one-third of the committee members rotating off the committee each year.

The staff of the IFT consists of the executive vice president and six other directors of such functions as finance and administrative services, science communications, information services and field services, and continuing education. In all, the staff consists of approximately 58 employees.

MEMBERSHIP CLASSES

The four membership classes have already been referred to briefly. To become a professional member, one must first have been a member for five years. Members must have a bachelor's degree or higher degree in food science or a branch of science or engineering associated with food technology. Two-thirds of the members hold degrees in food science itself or a related basic or applied science such as nutrition. Approximately 35% of the members hold a doctorate. Those not qualified to become professional members are individuals who are active in the food field, have substantial experience in it, and, generally, have a degree, but in some field other than science or engineering.

PROGRAMS OF THE INSTITUTE

The IFT has had a strong, active awards program since 1942, when the first of the Nicholas Appert Awards was given, and a scholarship/fellowship program since 1954 (1). In 1946 the Marcel Loncin Research Price carrying an award of $10,000 was initiated (2). Aside from the Nicholas Appert Award, considered the most prestigious of the awards, there are 11 other major awards for such things as excellence in teaching, significant contributions made to improved public health through nutrition, significant contributions to lipid or flavor science, international award for transfer of knowledge, and an award for technical contributions toward advancement of the food industry. Carrying no monetary award but considered a signal honor is election as a Fellow of the Institute.

In 1997–1998, the IFT awarded 17 freshman scholarships varying from $1000 to $1500, 15 sophomore scholarships in the same award range, 63 scholarships to juniors or seniors with the range of $1000 to $2000 each, and 38 fellowships to graduate students with the range of $1000 to $2500.

SCIENCE COMMUNICATION PROGRAM

In 1985 the IFT established its Office of Scientific Public Affairs (OSPA) (1). Under the direction of the director of OSPA, regional communicators were appointed in nearly all the states in the United States. The responsibility of these regional communicators was to serve as resource personnel whenever a food editor or anyone else wanted objective information on some aspect of public health, food additives, nutrition, or any other query having some relation to foods and the food industry. A few years later the name of the function was changed to science communicators. That program still functions by way of science communicators, but their roles have been expanded. By E-mail, fax, and other means the science communicators are given advance information of impending changes or thought, especially that coming from federal agencies or Congress, so that they can be well prepared not only to answer questions but also to take the lead by disseminating information without having to wait for someone seeking information to approach them. The Science Communication office is also responsible for coordinating the writing of Scientific Status Summaries. The staff does not do all the writing—that is mostly done by an expert in the particular subject-matter being written about—but either the Science Communications Office or the editor of *Food Technology* provides input to make the Status Summary as readable as complex subject-matter permits. The idea is to provide objective, factual information for all types of people—those in related science fields, media specialists of various types, and consumers themselves.

THE IFT FOUNDATION

The newest of the IFT's endeavors was the establishment of a foundation to provide financial support for IFT programs such as scholarships and the Science Communications Program, to augment funds going into the Career Guidance Program, and, most recently, to underwrite the expenses of the IFT funding a Science Congressional Fellow. So far, the Congressional Science Fellows have spent one year each as an assistant to a senator or a representative. The foundation supports jointly with the IFT 129 scholarships/fellowships and recently provided most of the funding to distribute 5312 career videos to schools. During the past year the science communicators responded to more than 1000 inquiries from the media. More than 20 continuing education courses were given, and a food science course has been established on the World Wide Web.

The foundation was first chartered in 1985. In 1993 the foundation initiated a fund drive that brought in approximately $2.7 million, which has permitted it to expand its activities greatly. The foundation was not dormant from 1985 to 1993. It just took time to work out the means to be able to fund IFT programs while ensuring sufficient financial support to be able to pay for the costs of a fund drive.

The foundation was chartered to serve as a fund-raising arm for the IFT with the objective of acquiring resources to reach the highest level of achievement in the food science profession. The foundation board elects six individuals to serve on the board. All need not be IFT members. The IFT executive committee appoints six members. Members are appointed for three-year terms. The treasurer of the IFT, the IFT's executive vice president, and the president of the IFT are ex offico members of the Board. In 1997 the board hired a director of development for the foundation.

IFT PUBLICATIONS

The IFT publishes two journals. One is the *Journal of Food Science*. Approximately 50% of the articles it publishes come from outside the United States, which attests to the journal's high repute. In 1950 the IFT purchased the journal *Food Research* from Garrard Press and changed its name to the *Journal of Food Science (JFS)*. The first issue of *Food Technology* was published in January 1947. *JFS* of course is peer-reviewed; so too are many of the articles in *Food Technology*. The latter contributes to the reputation of the IFT, for approximately 17.6% of IFT members reside abroad, and all members and professional members receive *Food Technology* as a part of their dues. *Food Technology* features some of the outstanding papers given at the annual meeting, various didactic or expository articles, and each Scientific Status Summary. Illustration of the last are "Assessing, Managing, and Communicating Chemical Food Risks" (3), "Foodborne Disease Significance of *Escherichia coli* O157:H7" (4), "Irradiation of Food" (5), and "Glass Transition in Low Moisture and Frozen Foods" (6).

PERSPECTIVES

The preceding subjects demonstrate that the IFT is active on several fronts in bringing food science to a high state of development. For a long time it has had an international presence too. Reference was made above to the number of articles published in *JFS* by others in the global community. For many years, the IFT has had four regional sections outside the country: England, British Columbia (Canada), Japan, and Mexico. Some years ago the IFT decided not to charter any more regional sections abroad. It did so to avoid any possible conflict with existing food science societies in other countries or with the formation of societies in countries presenting lacking such an organization. The IFT has, however, maintained affiliation with several societies abroad. At present there are 22 "affiliates" of the IFT. Some countries, such as Spain, have more than one affiliate. There currently is a recommendation that the IFT change the name of the relation from "affiliate" to "allied" to be sure that the relation is understood to be between equals.

IFT AND IUFOST

Within the past three years the IFT has turned toward instituting a greater international presence. The number of international companies exhibiting products, equipment, and services at the annual meeting of the IFT as well as the number of attendees and papers given by those from outside the United States attests to its strong international presence. In 1998 the Canadian Institute of Food Science and Technology, the Mexican Association for Food Science and Technology, and the IFT held their annual meetings or conferences at the same time in Atlanta. Within the past five years, the IFT has stepped up its supporting role in IUFoST.

IUFoST is an organization made up of member countries, each of which has official delegates to IUFoST, the number being different according to the dues paid to IUFoST. No country, however, can have more than six delegates. The United States, for example, has six accredited delegates. At four-year intervals IUFoST hosts a meeting. The last one was held in 1995 in Hungary, and the next one is to be held in Australia in 1999. Currently, the United States, actually the IFT, pays its yearly dues of $6000, but it also underwrites some of the administrative expenses of IUFoST by providing services to it. During 1996–1997, the IFT and IUFoST developed a plan, called the International Action Plan, to strengthen formally each other's endeavors to facilitate cooperation among the member bodies. The IFT and IUFoST envision that there are many areas where exhibitions, short courses, other types of training courses, and various forms of cooperation could be carried on more effectively and expeditiously, by joint efforts, without compromising in any way the interests of national bodies in other countries, if the IFT and IUFoST were to take the lead in funding and supporting such endeavors. At the present time, the IFT will do most of the funding, for IUFoST has always been handicapped by the limited resources it has been able to generate. Many of its member countries are among the poorest in the world.

Based on its record to date, the perspectives for the IFT are that it will become even more the knowledgeable and influential organization that it is in the United States. Because of its current commitment to bring its organizational skill and willingness to share its knowledge and resources with food science organizations elsewhere, the prospects are that the IFT will assume in the years ahead international roles even more beneficial to food science and technology and thus to the world's food supply and the well-being of humans.

BIBLIOGRAPHY

1. N. H. Mermelstein, "History of the Institute of Food Technologists *Food Technol.* **43**, 14–52 (1989).
2. F. R. Katz, *The 1997–1998 IFT Membership Directory*, Institute of Food Technologists, Chicago, Ill., 1997, p. 14.
3. C. K. Winter and F. J. Francis, "Assessing, Managing, and Communicating Chemical Food Risks," *Food Technol.* **51**, 85–92 (1997).
4. R. L. Buchanan and M. P. Doyle, "Foodborne Diseases Significance of Escherichia coli 0157:H7." *Food Technol.* **51**, 69–76 (1997).
5. D. G. Olson, "Irradiation of Food," *Food Technol.* **52**, 56–62 (1998).
6. Y. H. Roos, M. Karel, and J. J. Kokini, "Glass Transition in Low Moisture and Frozen Foods," *Food Technol.* **50**, 95–108 (1996).

JOHN POWERS
University of Georgia
Athens, Georgia

INTEGRATED PEST MANAGEMENT. See

POSTHARVEST INTEGRATED PEST MANAGEMENT in the Supplement section.

INTERNATIONAL DEVELOPMENT

International Development: Australia 1379
International Development: Canada 1383
International Development: Finland 1388
International Development: Germany 1393
International Development: Ireland 1399
International Development: New Zealand 1402
International Development: South Africa 1406
International Development: Spain 1410
International Development: Switzerland 1413
International Development: Taiwan 1416
International Development: United Kingdom 1421

INTERNATIONAL DEVELOPMENT: AUSTRALIA

Australia is an island continent similar in area to mainland United States, but with a much smaller population (16.3 million). Because it lies between latitudes 10° and 43.5° south, spanning temperate and tropical zones and corresponding to a climatic range in the Americas from northern California to Costa Rica, Australia is able to produce a wide range of food crops.

However, food production in Australia is greatly constrained by the limits of arable land and rainfall. Intensive agriculture is possible only on the eastern seaboard, in the southwestern corner, and in some inland irrigation districts. Progressively inland from the coast there is a band of country suitable for dryland farming of cereal crops and then large areas fit only for open grazing of cattle and sheep; half of the continent is desert.

Nevertheless, Australia has exportable surpluses of many foods, notably meat, wheat, rice, cane sugar, dairy products, fruits, and seafoods. Australia is second in world ranking, after the European Community, in exports of beef and veal, second after New Zealand in exports of mutton and lamb, third after the United States and Canada in exports of wheat and third after Cuba and Europe in exports of sugar. The major export markets for Australian food commodities are Japan (25%), other Asian countries (23%) and the United States (12%) (1).

Although the Australian aborigines lived off the land, the only indigenous plant product that has achieved commercial production is the macadamia nut. Of the native animals, only the kangaroo is used for meat, mainly for pet food, but the introduced and now-feral rabbit and buffalo are also used for human food.

THE FOOD INDUSTRY

Among the manufacturing industries in Australia the food industry stands first and accounts for about 20% of employment, turnover, and value added. The larger enterprises are mostly owned completely or partially by multinational corporations. However only 7.5% of the turnover is exported and, as for the primary commodities, the major markets for Australian processed foods are Japan (30%), other Asian countries (25%), and the United States (12%) (2).

Meat

Meat is the most important food commodity produced in Australia in terms of value, with a total production mostly beef, around 1.5 million tons per year of which about one-half is exported. Australia has a large population of sheep (150 million), but production of sheep meat (0.5 million t) is secondary to wool production.

Because cattle are raised mainly on the open range, the beef produced tends to be low in fat and high in protein and is most suitable for manufactured meat products such as hamburgers and sausages. Thus Australia has developed valuable export markets, notably in the United States and Japan, for frozen boneless beef and mutton and chilled vacuum-packed boneless beef. Consumption of meat in Australia is around 236 lb per head per year, which may be the highest in the world, and is made up of 94 lb beef, 50 lb lamb and mutton, 40 lb pork, and 52 lb poultry.

Innovative techniques developed in the Australian meat industry for converting live cattle into beef save manpower and eliminate many of the physically demanding and unpleasant tasks on the slaughter floor. Electric stimulation of beef carcases is widely applied to increase tenderness especially in conjunction with hot boning. Spraying carcases with hot water around 80°C for 10s removes 90–99% of contaminating bacteria including food poisoning organisms. Packaging of meat cuts under vacuum or in modified atmospheres in plastic films of the right barrier properties is now widely practiced to extend the storage life in chilled storage and distribution (3).

The poultry industry has moved from frozen whole chickens to marketing 85% of its production (approaching 0.5 million tons) in the chilled fresh form. The range of cut-up chicken products in raw, semiprocessed, and cooked forms has greatly increased. Modern plants with computerized control typically process 8,000 birds per hour (4).

Cereals

Wheat is the major cereal crop in Australia with annual production ranging up to 13 million t of which 80% is exported. Domestic usage of wheat is about 1 million t mainly for bread. A few large companies dominate the cereals industry with integrated operations embracing flour milling, computerized feed formulation for stock and poultry, and bread manufacture (5).

Australia is pioneering a modern application of an ancient technique for control of insect infestation in stored grains by creating an atmosphere of carbon dioxide, which effectively asphyxiates the insects but leaves no chemical residue. This technique is being applied on a large scale to the storage and transport of wheat, rice, and cocoa beans in silos, storage sheds, and intermodal containers.

Most Australian bread is made by an activated dough process developed by the Bread Research Institute. Consumer interest in dietary fiber has encouraged production of whole meal and multigrain breads and breakfast foods of the muesli type and those containing wheat, oat, and rice brans. Small speciality bakeries produce croissants, French pastries, rye breads, and Arabic breads.

An Australian company is third in the world in yeast manufacture and pioneered distribution of bulk cream yeast in refrigerated tankers. Some 20% of the flour milled in Australia is used for the manufacture of starch and gluten. Australia is one of the few countries in the world with a starch industry based on wheat rather than on maize or potatoes.

Rice growing under irrigation is a highly developed industry, which claims to achieve the highest yields of rice anywhere in the world. Of the annual production of 0.5 million t, 85% is exported. One feature of technology in this industry is the drying of rice in silos by the circulation of large volumes of ambient air.

A new grain legume crop, developed and commercially pioneered in Australia, is the sweet lupin, *Lupinus angustifolius*, which is extremely well adapted to the infertile sandy soils of Western Australia. Annual production is now around 1 million t of lupin seed, of which half is exported. Seeds of the sweet lupin are low in alkaloids and contain about 30% protein but very little starch. They are particularly useful for stock feeding and are beginning to be exploited for human food as sprouts, in fermented oriental foods, and in bread (6,7).

Dairy Products

Milk production in Australia amounts to 6 million t per year of which 29% is consumed directly, 20% go to butter manufacture, 22% is used in cheese manufacture, and the remainder goes to production of dried milks and casein. Most of the dried milks produced are exported.

Pasteurized milk is distributed in 1- and 2-L cartons and plastic bottles, but pressure from the green movement is encouraging a return to returnable and recyclable glass bottles. A range of modified milks is available: low fat, calcium fortified, and low lactose produced by hydrolysis of lactose by immobilized enzyme technology. Long-life milks are produced by aseptic processing and the same lines are used for shelf-stable cream and custards. Cultured dairy products, especially yogurt, have become popular items (8).

Cheese manufacture is largely mechanized and the inauguration of a continuous curd-making plant for Cheddar cheese in 1987 was a world first for Australia. Many small companies are making specialty cheeses in French. Swiss, and Italian styles. With an ice cream consumption of 18 L per head per year, Australia is second only to the United States.

Australia has been a leader in the application of membrane processes in the dairy industry. Ultrafiltration is being used to produce protein concentrates from whey and cheese base concentrates for cheese manufacture. Milk concentrated by reverse osmosis to twice the solids content is being exported in frozen form to Southeast Asian countries (9).

Fruits and Vegetables

Because of its wide climatic range, Australia is able to produce a great variety of fruits and vegetables Grapes, apples, citrus fruits, peaches, pears, apricots, pineapples, and bananas are the principal fruit crops. Minor crops include other stone fruits, berry fruits, and tropical fruits such as mango, avocado, and passion fruit as well as coffee and tea. These fruits provide the raw materials for the fruit canning, fruit juice, sun drying, and wine industries all of which export substantial proportions of their production. A recent development is the canning of fruits with no added sugar, using a fruit juice, particularly pineapple or pear juice, as the packing medium.

The annual crop of grapes in Australia approaches 0.8 million t, of which 62% are used for wine making; 33%, for drying into raisins, sultanas, and currants; and 5%, for table fruit. The Granny Smith apple, which originated in Australia as a chance seedling, is reputed to be the best of all apple varieties in keeping quality, keeping for a year or longer in controlled-atmosphere storage with reduced oxygen and increased carbon dioxide contents.

Among vegetables, potatoes, tomatoes, and green peas are grown in greatest amounts and, with many other vegetables, are distributed in fresh, frozen, and canned forms. Australia led the world in the introduction of free-flowing frozen peas packed in polyethylene film bags (10).

Fats and Oils

For the manufacture of margarines and edible oils, Australia uses 100,000 t/yr of locally produced oils (mainly sunflower, safflower, cottonseed, groundnut and maize oils) together with a similar quantity of imported oils (mainly palm oil with some canola, coconut, and soybean oils) and 70,000 t of edible tallow. A recent achievement by plant breeders is a linseed variety yielding an edible oil that has been called linola. Polyunsaturated margarines are widely consumed with polyunsaturated to saturated ratios 2:1 or 3:1 (ratios of *cis*-methylene-interrupted polyunsaturated fatty acids to saturated fatty acids).

Seafoods

In relation to the length of the coastline of the continent, the Australian fishing industry is small, but there are significant exports of several high-value seafoods, notably rock lobsters, prawns, and abalone. Australia is the largest producer of abalone in the world. Commercial aquaculture of Atlantic salmon and rainbow trout is being successfully practiced in Tasmania (11).

Sugar

The cane sugar industry in Australia produces 3 million t/yr of which 2 million tons are exported. Australian consumption of sugar directly and in manufactured foods and beverages is consistently ca 112 lb per head per year. Confectionery consumption averages 20 lb per head per year, which is considerably less than Switzerland and the UK but similar to consumption in the United States.

Beverages

Beer is the most popular alcoholic beverage in Australia with an annual consumption of 115 L per head; wine consumption is 20 L. Two companies dominate the brewing industry but there are many boutique brewers producing specialty brews. The major products are of the lager type with ales as the minor segment. Low-alcohol beers claim a significant share of the market, typically with 2.2% alcohol by direct brewing or 0.9% alcohol with alcohol removed by vacuum distillation; excise is related to alcohol content (12).

There are more than 500 wineries in Australia, but 80% of the wine is produced by 12 companies. Recent advances in wine technology include partial or complete clarification of grape juice for white wine before fermentation, temperature control and oxidation control with inert gas blanketing in tank storage, more rational use of sulfur dioxide and other antioxidants, and centrifugation and membrane filtration technology. There has been a large increase in wine sales in 5-L collapsible bag-in-box packages (13).

A wide variety of fruit juices is available in several processed forms: chilled (49%) and aseptically processed (28%) in gable-top paperboard containers or high-density polyethylene bottles, pasteurized (14%) in 2-L plastic bottles, and canned (9%). The cost of metal cans is currently about six times that of equivalent packs in polyethylene.

An Australian company has pioneered the development of cross-flow microfiltration technology with self-cleaning by gas back-flushing. This technology finds application in the wine and fruit juice industries for sterilization of grape juice prior to fermentation, for sterile filtration of wines prior to bottling, and for production of sterile, bright fruit juices; extension to the beer industry to replace diatomaceous earth filtration is envisaged. A further application is for replacement of conventional water-treatment technology in the soft drinks industry (14). Other recent developments are a countercurrent extractor for fruit juices with intermittent reversal of the screw, which greatly improves efficiency, and a spinning cone distillation column for recovery of fruit flavors and removal of alcohol to produce nonalcoholic wines (15).

EDUCATION

Degree courses in food science and technology requiring three or four years of postsecondary study to attain the bachelor's degree and one or two years in addition to the master's degree, are offered in a number of tertiary educational institutions in Australia: University of New South Wales, Sydney; University of Western Sydney, Hawkesbury, New South Wales; University of Queensland, Gatton; Deakin University, Geelong, Victoria; Royal Melbourne Institute of Technology, Victoria; Western Institute, St. Albans, Victoria; Ballarat College of Advanced Education, Victoria; and Bendigo College of Advanced Education, Victoria. The University of New South Wales offers a master's course in food engineering, and the Curtin University of Technology, Perth, Western Australia, a course in nutrition and food science.

Degree courses are also offered in a number of areas peripheral to food science and technology. biotechnology at the universities of New South Wales and Western Sydney; food microbiology at the South Australian Institute of Technology, Adelaide; wine science at Charles Sturt University, Wagga Wagga, New South Wales, and Roseworthy Agricultural College, South Australia; home economics at the University of Western Sydney, Brisbane College of Advanced Education, Queensland and Western Australian College of Advanced Education, Perth; hospitality studies at the University of Queensland, Gatton, Footscray Institute of Technology, Victoria and Wodonga Institute of Tertiary Education, Victoria; and applied toxicology at Royal Melbourne Institute of Technology. Graduate diploma courses are offered in food and drug analysis at the University of New South Wales, in food packaging at the University of Queensland, Gatton, and in human nutrition at Deakin University. Three universities (University of New South Wales, University of Western Sydney, and University of Queensland) accept candidates for the doctor of philosophy degree in food science and technology, which requires at least three years of study and research beyond the bachelor's degree. The annual output of graduates from all the Australian courses is around 100, and the job opportunities in the food industry are such that these people are readily absorbed.

In all the Australian states, in the capital cities, and in many regional centers, there are colleges of technical and further education which offer diploma courses in most of the areas already listed and also certificate or trade courses in baking, bread making, butchery, small goods manufacture, meat inspection, dairy products manufacture, food processing, food laboratory techniques, packaging, hospitality, and catering and commercial cookery.

RESEARCH AND DEVELOPMENT

Expenditure on food research and development in the food industry amounts to only 0.1–0.2% of turnover (16). Most of this work is development work related directly to the production and marketing plans of the particular company. The companies that have overseas affiliations share the benefits of research and development in their parent companies but it is seldom possible for the results of overseas research and development to be applied directly in Australia. Additional development steps are generally necessary because Australian raw materials and food regulations are different and Australian consumers make different demands.

The Commonwealth (Federal) government makes a major contribution to food research and development through the Commonwealth Scientific and Industrial Research Organization (CSIRO). Among the 40 divisions of CSIRO, those that engage in some research related to food science and technology are the divisions of Food Processing, Plant Industry, Human Nutrition, Horticulture, and Entomology. The Division of Food Processing has a staff of 120 professional scientists and 150 support staff and an annual budget of $15 million (Australian). Its headquarters are at the CSIRO Food Research Laboratory, North Ryde, New South Wales, which conducts research on food microbiology, food engineering, food packaging and transport, food acceptance, and the functional properties of food constituents. The division also embraces the CSIRO Meat Research Laboratory, Cannon Hill, Queensland which conducts research on meat quality, storage, and processing and the CSIRO Dairy Research Laboratory, Highett, Victoria, which works on the separation and utilization of milk components and the microbiology and technology of dairy products.

The Division of Plant Industry maintains the CSIRO Wheat Research Unit at North Ryde, New South Wales, which has projects on the proteins of wheat and the utilization of gluten. The research program of the Division of Human Nutrition includes investigations on the role of dietary fiber. The Division of Horticulture works on the postharvest handling and storage of fruits and vegetables, and the Division of Entomology has a project on the control of insect pests in stored grains. The Commonwealth Department of Community Services and Health is responsible for the collection of data on the composition of Australian foods and has recently published revised composition tables and a related computer data base (17). The Department of Defence Materials Research Laboratory at Scottsdale, Tasmania, undertakes food research and development to meet specific needs of the armed forces.

Some state governments support food-related research, notably the Victorian Department of Agriculture and Rural Affairs, which maintains the Food Research Institute at Werribee, Victoria, and the Queensland Department of Primary Industries, which has the International Food Institute of Queensland at Hamilton, Queensland. Most of the universities with food science and technology programs undertake contract research and development for the food industry and the University of New South Wales has the Food Industry Development Centre.

Commodity research trust funds in which the federal government matches amounts raised by compulsory levies have been set up for the meat, pig meat, poultry, egg, dairy, wheat, grain legume, dried fruit, fisheries, honey, and oilseed industries. These funds are used in part to support competitive grants for food research and development projects.

Three branches of the food industry have created research associations for carrying out cooperative research and development the Bread Research Institute of Australia, North Ryde, New South Wales; the Australian Wine Research Institute, Adelaide, South Australia; and the Australian Sugar Research Institute, Mackay, Queensland.

ORGANIZATIONS

The professional organization representing food science and technology in Australia is the Australian Institute of Food Science and Technology (AIFST), which began in 1950 as a regional section of the Institute of Food Technologists, the first regional section to be commissioned outside of the United States. In 1967, AIFST became an independent organization and now has 1,850 members in several grades: Fellows, Associates, graduate members, student members, licentiates, and affiliates, according to defined requirements of professional qualification and food industry experience. AIFST is the Australian representative body in the International Union of Food Science and Technology. AIFST has six state branches which come together in an annual convention; there are also technical interest groups in the areas of food microbiology, food processing and engineering, food service, seafood technology, and nutrition. The technical journal *Food Australia* is the joint official organ of AIFST and the Council of Australian Food Technology Associations (CAFTA).

CAFTA is an organization of companies in the food industry and its membership of 233 companies embraces the majority of food and beverage manufacturers, food ingredient suppliers, and some retailers. Its principal function is to represent the food industry on government committees concerned with food standards and regulations. CAFTA also conducts, in conjunction with AIFST, the Food Information Service, which seeks to inform the media, educators, and consumers about food safety, quality, and nutrition.

Other technical organizations serving the general area of food science and technology are the Cereal Chemistry Group of the Royal Australian Chemical Institute, the Australian Society for Viticulture and Oenology, the Dairy Industry Association of Australia, the Australian Society of Sugar Cane Technologists, and the Australian and New Zealand Branch of the Institute of Brewing (UK).

REGULATION

Regulatory control of the food industry in Australia is in the hands of the six states and two territories, each of which has a pure food act or an equivalent and accompanying regulations. Substantial uniformity in these regulations has been achieved through the Australian Food Standards Committee administered by the Bureau of Consumer Affairs in the federal attorney-general's department (18). The Food Standards Committee recognizes a commitment to harmonize Australian standards as far as possible with the international standards of the Codex Alimentarius Commission.

BIBLIOGRAPHY

1. S. W. Gunner, "Food Technology for the Export Market," *Food Australia* 41, 946–951 (1989).
2. W. N. Goff, "Food Export Strategy," *Food Australia* 41, 827–828 (1989).
3. D. J. Walker, "Trends in the Australian Meat Industry," *Food Australia* 40, 418–421 (1988).
4. J. G Fairbrother, "The Poultry Industry," *Food Australia* 40, 456–462 (1988).
5. A. J. Meers, "Trends in the Cereal Industry," *Food Australia* 40, 356–358 (1988).
6. J. S. Gladstones, "Development of Lupins as a New Crop Legume," *Food Australia* 42, 270–272 (1990).
7. D. S. Petterson and G. B. Crosbie, "Potential of Lupins as Food for Humans," *Food Australia* 42, 266–268 (1990).

8. L. L. Muller, "Advances in Dairy Processing," *Food Australia* **40**, 414–416 (1988)
9. J. G. Zadow, "Extending the Shelf Life of Dairy Products," *Food Australia* **41**, 935–937 (1989).
10. P. C. Thompson, "Refrigerated Food Industry," *Food Australia* **40**, 361–367 (1988).
11. P. G. Taylor, "Australian Seafoods," *Food Australia* **40**, 505–507 (1988).
12. D. C. O'Donnell, "Brewing in Australia 1970–1988," *Food Australia* **41** (Suppl.), 1–5 (1989).
13. B. Rankine, "The Australian Wine Industry," *Food Australia* **40**, 449–451 (1988).
14. S. Paterson and R. Wale, "New Developments in Membranes for Beverage Processing," *Food Australia* **41**, 852 (1989).
15. D. J. Casimir, "Food Process Engineering Over the Last Twenty-One Years," *Food Australia* **40**, 521–523 (1988).
16. J. R. Vickery, *Food Science and Technology in Australia*, Commonwealth Scientific and Industrial Research Organisation, Canberra, 1990.
17. K. Cashel, J. Lewis, and R. English, *Composition of Foods, Australia*, Australian Government Publishing Service, Canberra, 1989; *NUTTAB 89, Nutrient Data Base*, Australian Government Publishing Service, Canberra, 1989.
18. R. A. Edwards, "Food Legislation in Australia," *Food Australia* **40**, 369–375 (1988).

J. F. KEFFORD
North Ryde, New South Wales
Australia

INTERNATIONAL DEVELOPMENT: CANADA

SIGNIFICANCE

The food and beverage processing sector is a major contributor to the Canadian economy as a supplier of food, a market for agricultural production, and a source of economic growth. Since the 1960s the sector experienced substantial growth due to rising population and income levels, and lifestyle changes that emphasized convenience. However, increasing global economic integration since the mid-1980s, particularly in North America, compelled the sector to shift its focus from the domestic market and develop a more international, export orientation. This sector has significantly contributed to the provision of reasonably priced food to consumers, a remarkable achievement given the geographic distances, the diversity of markets and the climatic hurdles in Canada. In fact, relative to other developed nations, Canadian spending on food as a share of personal disposable income ranked second lowest after the United States.

Canada's food and beverage processing sector, in terms of its relative size, trade, and processing intensity, is similar to those in other large industrial countries. Domestically, food and beverage processing is the third largest manufacturing sector, accounting for 11.8% of manufacturing gross domestic product (GDP) and about 2% of total GDP in 1996 (Figure 1). The sector produced shipments valued at $52 billion in 1996 and provided 234,000 jobs (Table 1). The statistics used to describe the size and significance of the Canadian food and beverage processing sector are derived from Statistics Canada, the only source of information that could provide consistent and comparable data across Canada. The data presented may vary from provincial sources of information primarily because of differences in how food and beverage processing firms are defined.

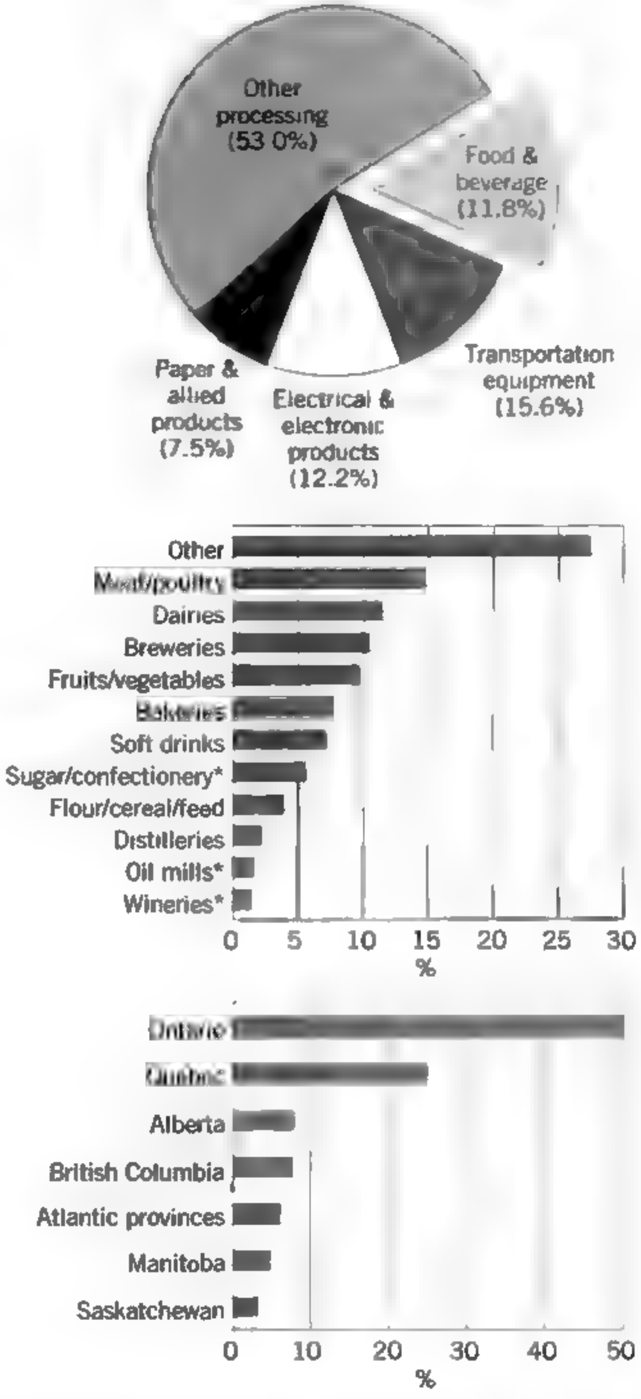


Figure 1. Share of GDP for manufacturing sectors and for the food and beverage processing sector by subsector and by region, 1996. *Source:* Ref. 1.

8. L. L. Muller, "Advances in Dairy Processing," *Food Australia* **40**, 414–416 (1988)
9. J. G. Zadow, "Extending the Shelf Life of Dairy Products," *Food Australia* **41**, 935–937 (1989).
10. P. C. Thompson, "Refrigerated Food Industry," *Food Australia* **40**, 361–367 (1988).
11. P. G. Taylor, "Australian Seafoods," *Food Australia* **40**, 505–507 (1988).
12. D. C. O'Donnell, "Brewing in Australia 1970–1988," *Food Australia* **41** (Suppl.), 1–5 (1989).
13. B. Rankine, "The Australian Wine Industry," *Food Australia* **40**, 449–451 (1988).
14. S. Paterson and R. Wale, "New Developments in Membranes for Beverage Processing," *Food Australia* **41**, 852 (1989).
15. D. J. Casimir, "Food Process Engineering Over the Last Twenty-One Years," *Food Australia* **40**, 521–523 (1988).
16. J. R. Vickery, *Food Science and Technology in Australia*, Commonwealth Scientific and Industrial Research Organisation, Canberra, 1990.
17. K. Cashel, J. Lewis, and R. English, *Composition of Foods, Australia*, Australian Government Publishing Service, Canberra, 1989; *NUTTAB 89, Nutrient Data Base*, Australian Government Publishing Service, Canberra, 1989.
18. R. A. Edwards, "Food Legislation in Australia," *Food Australia* **40**, 369–375 (1988).

J. F. KEFFORD
North Ryde, New South Wales
Australia

INTERNATIONAL DEVELOPMENT: CANADA

SIGNIFICANCE

The food and beverage processing sector is a major contributor to the Canadian economy as a supplier of food, a market for agricultural production, and a source of economic growth. Since the 1960s the sector experienced substantial growth due to rising population and income levels, and lifestyle changes that emphasized convenience. However, increasing global economic integration since the mid-1980s, particularly in North America, compelled the sector to shift its focus from the domestic market and develop a more international, export orientation. This sector has significantly contributed to the provision of reasonably priced food to consumers, a remarkable achievement given the geographic distances, the diversity of markets and the climatic hurdles in Canada. In fact, relative to other developed nations, Canadian spending on food as a share of personal disposable income ranked second lowest after the United States.

Canada's food and beverage processing sector, in terms of its relative size, trade, and processing intensity, is similar to those in other large industrial countries. Domestically, food and beverage processing is the third largest manufacturing sector, accounting for 11.8% of manufacturing gross domestic product (GDP) and about 2% of total GDP in 1996 (Figure 1). The sector produced shipments valued at $52 billion in 1996 and provided 234,000 jobs (Table 1). The statistics used to describe the size and significance of the Canadian food and beverage processing sector are derived from Statistics Canada, the only source of information that could provide consistent and comparable data across Canada. The data presented may vary from provincial sources of information primarily because of differences in how food and beverage processing firms are defined.

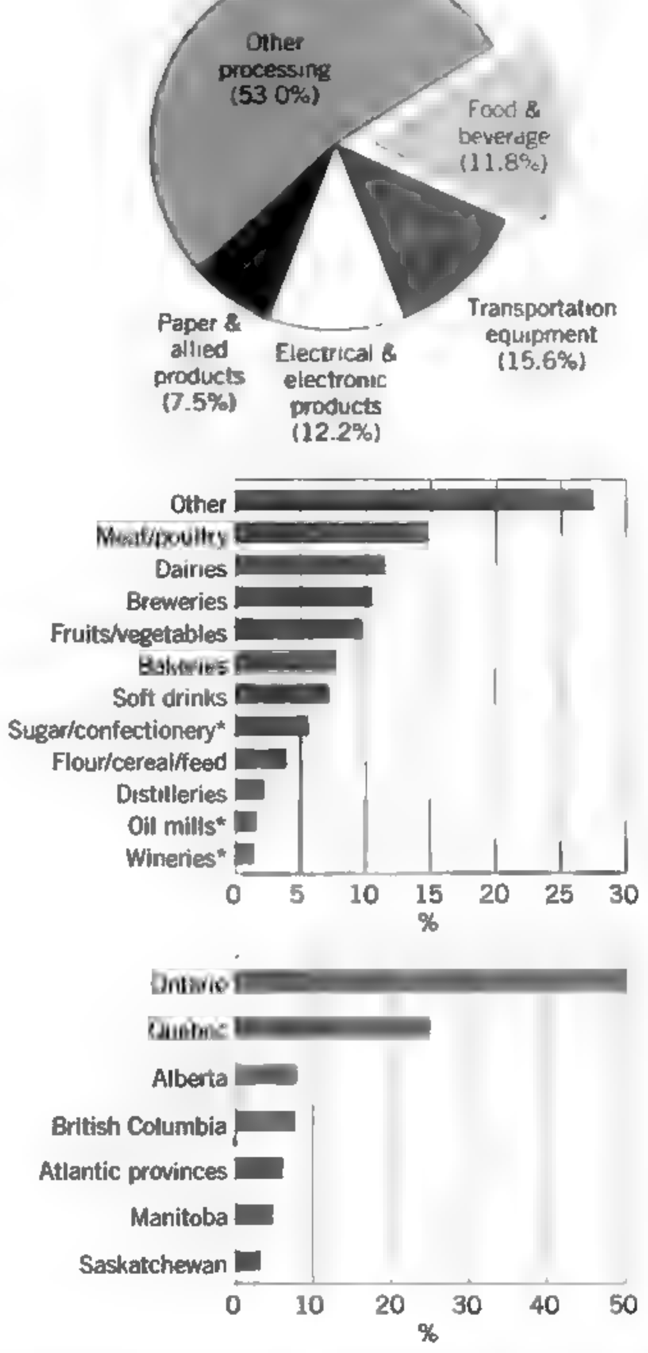


Figure 1. Share of GDP for manufacturing sectors and for the food and beverage processing sector by subsector and by region, 1996. *Source:* Ref. 1.

Table 1. Principal and Trade Statistics for the Food and Beverage Processing Sector (excluding fish), 1988–97

	1988	1991	1995	1996^d	1997^d
		Principal statistics			
GDP ($millions 1988)	11,108	10,924	11,952	12,225	—
Share of manufacturing GDP (%)	11.8	12.7	11.7	11.8	—
Establishments	3,147	2,976	2,790	—	—
Shipments ($millions)	40,196	41,418	48,791	51,793	—
Employment^a (000)	235	223	232	234	—
Investment ($millions)					—
Buildings and construction	260	285	196	371	—
Machinery and equipment	1,083	1,182	1,207	1,450	—
Value added^b ($millions)	14,309	16,301	17,698	—	—
Net profit margin^c (%)	3.93	2.45	2.26	2.76	—
Return on assets^c (%)	6.60	3.64	3.23	4.13	—
Return on equity^c (%)	14.42	8.45	8.20	11.07	—
		Trade statistics			
Exports ($million)	3,523	3,838	7,129	8,454	9,557
Imports ($million)	4,205	5,274	7,861	8,423	9,526
Balance of trade ($million)	(682)	(1,435)	(731)	31	31

^{a}Labour Force Survey.

^{b}Value added in the value of the product sold by a firm or industry less the value of the materials purchased and used by the firm or industry to manufacture the products

^{c}Includes fish

^{d}Preliminary for 1996 principal statistics and 1997 trade statistics.

Source Ref. 1.

About 40% of agricultural production is exported in raw form, and 12% is either sold directly to consumers or sold for nonfood uses. The remaining 45% is marketed as processed food through the food and beverage processing sector, which supplies more than 80% of the food consumed in Canada (Fig. 2). The sector accounted for 44% of agricultural and agrifood product exports in 1996, up from only 32% in 1988. Increasingly, international and domestic markets are characterized by trade in higher-valued products.

As a result of increased market globalization and freer trade, Canadian food and beverage processing firms, which were characterized as being big in a small market in the 1980s, are now rather small in a global market.

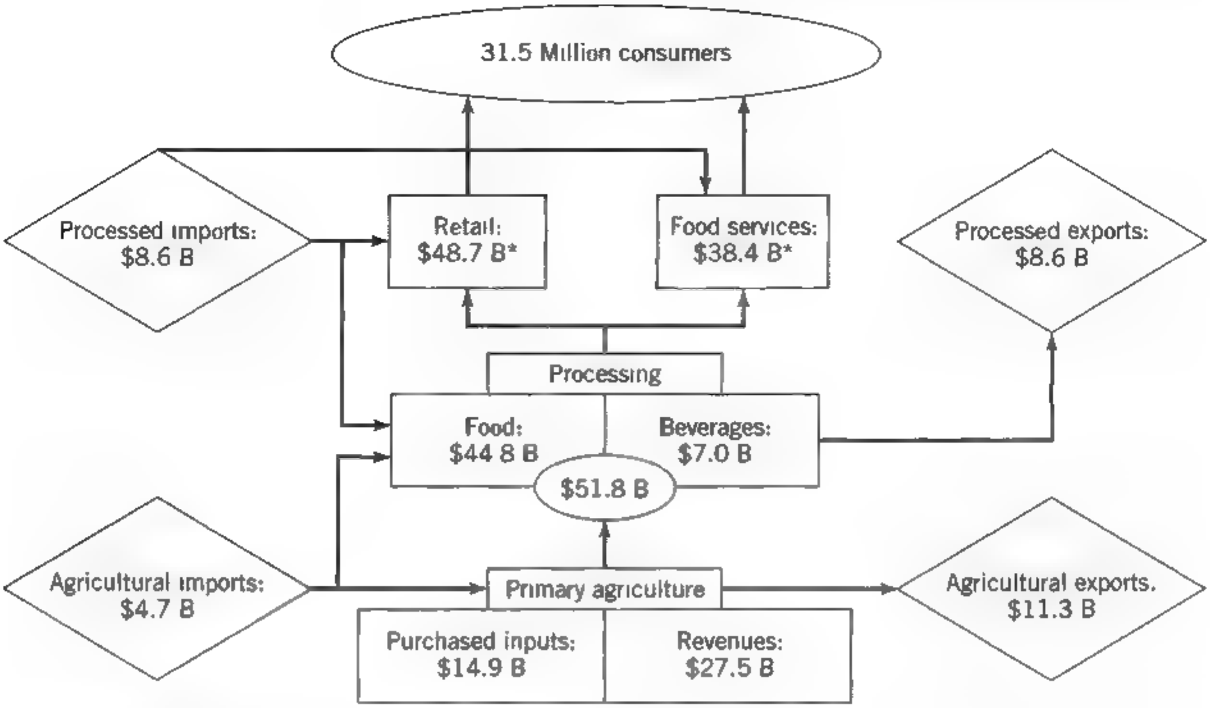
STRUCTURE

The food and beverage processing sector remains oriented to the domestic market. However, the share of production that Canada exports has increased from about 9% to about 16% between 1988 and 1996. Imports have also increased from about 10% of the domestic market in 1988 to about 16% in 1996. In 1997, based on preliminary data, Canadian processed food exports reached $9.6 billion, while imports increased to $9.5 billion. Because exports have increased at a faster rate than imports, Canada has enjoyed a positive trade balance of $31 million in both 1996 and 1997. The trade relationship with the United States continues to strengthen, accounting for about 73% of exports and 62% of imports in 1997, up from 60% and 47%, respectively, in 1988.

Although the bulk of food and beverage processing sector activity occurs in Ontario and Quebec, the economic significance in terms of the share of regional manufacturing activity is greatest in the Atlantic and Prairie provinces (Table 2). The influence of world trade in processed food and beverage products varies significantly by region. Export orientation is greatest for the Prairie provinces and lowest in Quebec. Plant locations also vary; red meat and fruit and vegetable processing plants tend to be located near sources of farm production, while fluid milk and bakery product plants tend to be clustered near large centers of population. Activity in the major subsectors occurs in every region. Meat processing is generally the most important activity in each region, particularly in the Prairie provinces. Dairy and meat products are the major products in Quebec, with the share of total shipments of dairy products being significantly higher than in most other regions.

The food and beverage processing sector is fragmented and diverse and is dominated by 67 large and very large firms. The two largest subsectors, meat and dairy products, account for about one-third of the total value of shipments in 1996. Import penetration and export orientation vary widely among the subsectors. For example, export orientation ranges from less than 10% in the supply-managed subsectors, canned and preserved fruits and vegetables, sugar, snack food, soft drink, and brewery subsectors, to more than 60% in the distillery products subsector.

The food and beverage processing sector is composed of relatively large Canadian-owned multinational enterprises (MNEs), foreign-owned MNEs, large and small cooperatives, and small and medium-sized enterprises


31.5 Million consumers
Processed imports: $8.6 B
Retail: $48.7 B*
Food services: $38.4 B*
Processed exports: $8.6 B
Processing
Food: $44 8 B
Beverages: $7.0 B
$51.8 B
Agricultural imports: $4.7 B
Primary agriculture
Agricultural exports. $11.3 B
Purchased inputs: $14.9 B
Revenues: $27.5 B

* Includes fish

Figure 2. Value of transactions between various components of the Agri-food system, 1996. *Source*· Ref. 1.

Table 2. Food and Beverage Processing Sector by Region, 1996

	Atlantic Provinces	Quebec	Ontario	Prairie Provinces	BC
Shipments ($million)	2,838	12,332	22,951	9,933	3,740
Share of Canadian shipments	5.5%	23.8%	44.3%	19.2%	7.2%
Share of provincial manufacturing	17.5%	12.8%	10.9%	22.1%	11.4%
Imports ($millions)	248	1,321	4,875	797	1,161
Exports ($millions)	415	1,488	3,720	2,316	515
Trade balance ($millions)	167	167	−1,155	1,519	−666
Export Orientation[a]	14.6%	12.1%	16.2%	23.3%	13.8%

[a]Exports divided by shipments
Source Ref 1

(SMEs), both Canadian and foreign owned. Although some Canadian food sector firms are relatively large, in general, Canadian firms are smaller and less diversified than many of their major U.S. competitors.

In 1996, the food and beverage processing sector was structured as follows:

- A core of 12 very large firms (annual sales of over $1 billion) represent about 35% of shipments; 8 of them are Canadian owned.
- Fifty-five large firms (annual sales between $100 million and $1 billion) produce about 25% of shipments; about 44% of them are foreign owned.
- Approximately 2000 SMEs (annual sales under $100 million) account for the remaining 40% of shipments—about three-quarters of these firms had sales of less than $10 million annually. Based on other sources of information, this number is underestimated and could reach an estimated 4000 SMEs.

A number of the large and medium-sized firms are publicly listed companies, while the small enterprises tend to be privately owned. Most foreign-owned firms are privately held by their parent corporations. Cooperatives, which account for about 20% of the value of shipments, play an important role in the Canadian food and beverage processing sector. Aside from their core role in marketing and providing inputs and services to farmers, cooperatives operate mostly in the dairy and poultry subsectors.

The market share of foreign-controlled firms has increased since 1989, to about 40%. These firms tend to be significantly larger and produce higher value-added products than domestic firms. MNE-dominated subsectors include beef, fruits and vegetables, prepared flour mixes and breakfast cereals, biscuits, confectionery, and breweries. Subsectors dominated largely by domestically controlled firms include pork, poultry, and bakery.

To strengthen and maintain competitiveness in the international market, industrialized nations have placed technological capacity high on their political and industrial agendas. Scientific research increasingly underpins the economic vitality of nations by providing the foundation for industrial renewal and growth.

FOOD RESEARCH AND DEVELOPMENT

Research and development is the ultimate source of technological improvements. Food research in Canada is conducted in several types of organizations and institutions distributed across the country; involves several hundred scientific/technical personnel; is highly variable in nature; is planned and coordinated to varying degrees; is funded federally, provincially, and privately; and is influenced in several ways by the characteristics of the Canadian economy, society, and infrastructure. Three basic kinds of organizations are responsible for food research in Canada: government establishments, universities, and private sector laboratories.

Private Sector

The private sector laboratories engage mainly in applied research because they are obliged to show results to shareholders and because they must be able to maintain a position in the marketplace where demands shift rapidly. The proprietary nature of most industrial research limits the information available in this area. In many cases industry implements the results of research and development conducted by the publicly funded organizations.

Canadian industry food research organizations range from a relatively few, fairly large and comprehensive programs consisting of several professionals, to a fairly large number of small programs with less than a full person-year committed to the activity. Many of the latter are integrated with the companies' quality assurance programs.

Canada's effort in private sector food research appears much smaller in comparison to other industrial nations. This may be partly due to Canada's branch-plant economy with its reliance on technology developed elsewhere. Research and development by multinational firms is centralized in the parent company and then the technology is adapted as seems necessary for branch-plant operations.

Government

Federal government food research is conducted by several departments. Agriculture and AgriFood Canada (AAFC) concentrates its research on agricultural products. Fisheries and Oceans Canada focuses on fish quality issues, and Health Canada is involved in the area of food safety and nutrition.

AAFC plays a major role of in Canadian agrifood research and development, maintaining a critical mass of fundamental, long-term, high-risk research activity, focusing on areas that the industry is unable to conduct on its own and to develop and maintain a communications/coordination/information infrastructure involving university, private sector, and provincial food research agencies. The Research Branch mission is "to improve the on-going competitiveness of the Canadian agrifood sector through the development and transfer of innovative technologies." The food research program contributes toward this by focusing on increasing the market value (quality and value-added processing) and the utilization (new uses and value-added processing) of agricultural products. In the Research Branch of Agriculture Canada, food research is conducted at six establishments across the country (Table 3).

The Health Protection Branch of Health and Welfare Canada also maintains a research program primarily at its central Ottawa laboratory, but with some regional activity. Its efforts relate to the Branch's mandate for ensuring food safety and preventing consumer fraud.

Several provinces have or are developing technology transfer facilities to work with food processing firms in their provinces (Table 4). Provincial food research endeavors are frequently oriented to enhancing industrial productivity in trade. They generally do not undertake fundamental research but work with industry in application and development work. The provincial facilities support the food processing sector through:

Development of food products and processing technologies incorporated the following:

- Value-added opportunities
- Unexploited or underused raw materials

Table 3. Agriculture and Agrifood Canada Food Research Establishments

Kentville Research Station, Kentville, Nova Scotia
Food Research and Development Centre, Saint Hyacinthe, Quebec
Southern Crop Protection and Food Research Program, London, Ontario
Saskatoon Research Centre, Saskatoon, Saskatchewan
Lacombe Research Centre, Lacombe, Alberta
Pacific Agri-Food Research Centre, Summerland, British Columbia

Table 4. Provincial Food R&D Establishments

Alberta Special Crops & Horticulture Research, Brooks, Alberta
Food Process Development Centre, Leduc, Alberta
POS Pilot Plant Corporation, Saskatoon, Saskatchewan
Canadian Food Products Development Centre, Portage La Prairie, Manitoba
Geulph Food Technology Centre, Guelph, Ontario
Service de Recherche sur les Aliments, MAPAQ St. Hyacinthe, Québec
Centre de Recherche sur les Aliments, Moncton, New Brunswick
New Brunswick Research and Productivity Council, Fredericton, New Brunswick
P.E.I. Food Technology Centre, Charlottetown, Prince Edward Island
The Marine Institute, St. John's, Newfoundland

- By-products of agriculture
- Provision of analytic and technical services for food products and food-processing systems
- Identification of domestic and foreign technologies appropriate to local regions
- Provision of technical information directed to improvement and development of food products and processes

One unique organization, representing a coalition of industry (49 companies) and government (federal and provincial) interests, is the Protein, Oilseeds and Starch (POS) Pilot Plant located in Saskatoon. This organization was established in 1976 to facilitate grain and oilseed technology transfer from the laboratory bench to commercial production.

Universities

Several universities across the country have food science programs (Table 5), some as freestanding departments, others in association with nutrition and home economics programs. The trend toward integration is increasing as universities seek to reduce administrative costs and minimize overhead.

The Canadian Council of University Food Science Administrators (CCUFSA) ensures that there is academic consistency in the food science programs across the country. The programs at the various universities follow guidelines established by the Education Committee of the Canadian Institute of Food Science and Technology.

Although the primary role of universities is education, a considerable amount of research is being conducted. As resources at universities become more scarce, future research will require close collaboration among disciplines and among sectors. Research collaboration between universities and the private sector, is an important facet of the Canadian food research and development scene. Scientific collaboration between the universities and the industry is encouraged through the AAFC/Natural Sciences and Engineering Research Council (NSERC) Partnerships Program.

Table 5. Universities with Food-Related Programs

University of British Columbia, Vancouver, British Columbia
University of Alberta, Edmonton, Alberta
University of Saskatchewan, Saskatoon, Saskatchewan
University of Manitoba, Winnipeg, Manitoba
University of Guelph, Guelph, Ontario
University of Toronto, Toronto, Ontario
Macdonald College of McGill University, Montreal, Quebec
Université Laval, Laval, Quebec
Acadia University (undergraduate program only), Wolfeville, Nova Scotia
Daltech Dalhousie University (graduate program only), Halifax, Nova Scotia

COORDINATION OF FOOD RESEARCH

Government, university, and private sector establishments have fundamentally different mandates and responsibilities. However, for at least the past two decades there has been a general realization of the value of coordination and cooperation in many areas of research. Linkages have been fostered in a number of ways.

In 1932 a major national provincial network of agricultural services personnel was established. This network included research scientists and other technical personnel from federal and provincial governments and universities. The organization, now known as the Canadian Agri-Food Research Council, has evolved into a complex, diversified organization with its membership drawn from industry, government, and academia as well as representation from the consumers' association. Within the network are provincial and federal food science committees, which provide a meeting ground for members to discuss food research needs and to develop and recommend strategies to meet these needs.

A second avenue that assists in achieving a coordinated and efficient approach to food research is the annual publication of the *Inventory of Canadian Agri-Food Research*. This provides a project-by-project compilation of food research being undertaken in the reporting establishments. This information is reasonably current and comprehensive, and therefore valuable for planning by research directors, coordinators, and individual scientists.

Perhaps the most effective mechanism for coordination is individual contact. An annual opportunity for this is provided by the national conference of the Canadian Institute of Food Science and Technology (CIFST), the membership of which includes people from all areas of food research and development. CIFST also publishes a bimonthly scientific journal as a means of communicating among scientists.

SCIENTIFIC AND TECHNICAL ACHIEVEMENTS

Many Canadian scientists in industry, universities, and government have made major contributions in the area of food science. The results of many of the early commercial successes are still having positive effects on the Canadian economy. William Heeney made a tremendous contribution with regard to the commercialization of frozen foods. His early work in frozen food technology is still as current today as he foresaw it before World War II. Joe Yarem, with the assistance of Percy Gittelman, commercialized the processing of mechanically deboned poultry meat in the late 1960s and early 1970s. This technical development currently generates in excess of $60 million in sales worldwide, in addition to several international joint ventures.

The development work done at Labatt's by Dr. Norman Singer with regard to synthetic low-calorie artificial fat substitutes during the 1970s was a major breakthrough in reduced-calorie foods. Dr. René Riel made a great contribution in the area of aseptic packaging, and Dr. Doug Emmons is credited for his work in acid coagulation for continuous production of cottage cheese. Dr. Wayne Modler developed a process and the equipment for continuous production of ricotta-type cheeses that is now a commercial reality. Subsequent development of this equipment has seen its application to continuous tofu manufacture.

The early engineering development work of Dr. Gordon Timbers and Dr. Moustafa Aref led to the freezing of liquid and semiliquid foods known as Cryogran in the late 1960s and early 1970s. This process as developed received several international awards and is now an international commercial success. In addition, Dr. Timbers, along with Dr. Robert Stark and Dr. Daniel Cumming, made a significant contribution during the 1970s and 1980s to the world's most energy-efficient ABCO vegetable-blanching system. These energy-efficient blanching systems are now installed and are used in commercial production worldwide.

Jim Squires and David Kennedy developed the first shelf-stable nonfrozen french fry, one of the first successful modified gas-packaged vegetable products. The evolution of this process took more than 10 years to bring to commercial reality in the early 1980s.

The commercial development and process engineering in Canada of the Sous Vide cooking method was undertaken by Ron White and Ray Mitchell. Although initially developed in France, it was their engineering and development work during the mid 1980s that made it commercially viable for large-scale products.

MDS Nordion has gained a worldwide reputation for its work in the development of food irradiation technology and manufactures and ships equipment meeting the highest standards for the application of this very important technology.

Percy Gitelman and his team comprised of Nora Martin, Dr. Paul Fedec, and Dr. John D. Jones made a commercial breakthrough in deheated ground mustard in the mid-1970s. This continues to be a commercial success with sales around the world. Donald Grier and Jeff Dyson during the mid- to late 1970s developed an energy-efficient impulse combustion-drying system that received worldwide patent protection and commercial installation. This system has one of the widest ranges of application in the drying of food, from liquids to solids.

Engineering developments applied to the food industry continue to make their mark. Recent installations of robotic systems for the separation of ribs from the meat of pork sides illustrate the high technology trends within the industry. This unit, and the associated computer algorithms, were developed by the Conseil de recherche Industrielle de Québec and have the capability to handle 1400 sides per hour. Also in the meat industry, a carcass pasteurizer developed by Dr. Colin Gill and associates provides an increased margin of safety for consumers. A process for the fractionation of oats developed by Drs. Burrows, Paton, Collins and Woods has renewed interest in this traditional crop with diverse applications in health (β-glucans), cosmetics, anti-irritants and other food and nonfood products. A primary focus is now toward the fractionation of various crops for the production of nutraceuticals and functional foods with their potential for impact on the health status of the population.

Biotechnology will play an increasing role in future of the Canadian agrifood industry as developments from the R&D community are adopted. Examples include enzymes for accelerated ripening of cheese (Dr. Byong Lee) and *bioingredients* (Dr. François Cormier). Biotechnology is taking its place beside conventional plant breeding as various plants, including the Canadian *Cinderella crop*, Canola, are being further tailored to meet food industry requirements. Of the highest priority in any national food supply is its safety. Canada is well regulated as to sanitary and safe food processing, handling, and distribution; new hazards arise from the evolution of knowledge, organisms, practices, and regulations. Examples include investigations concerning the effects of ionizing radiation on foods and microorganisms, as well as on food packaging materials and the development of handling and processing protocols for newly identified pathogens implicated in food-poisoning cases.

The demand for convenience features in foods continues to grow as discretionary family income grows, as an increasing proportion of families have more than a single breadwinner, and as society becomes more leisure-oriented. Much research is therefore focused on quality retention, sensory properties, product uniformity, and convenience features. This, in turn, has led to the need for a Sophisticated understanding of the molecular level changes in food undergoing various processes and the development of highly controlled processes.

BIBLIOGRAPHY

1. *Statistics Canada*, Agriculture and Agri-Food Canada, Food Bureau, Ottawa, Canada, 1997.

GORDON TIMBERS
CLAUDE JANELLE
Agriculture and AgriFood Canada
Ottawa, Ontario
Canada

INTERNATIONAL DEVELOPMENT: FINLAND

Finland became a member of the European Union (EU) in 1995, which has contributed to the national economy and food industry over the past few years. By the EU membership, the Finnish companies now operate in the Common Market. As a consequence, the share of food in the final consumption expenditure per household decreased from 16 to 13% during one and a half years. A significant reduction in prices was observed for pork, eggs, and oil plants. To hasten the adaptation to the new situation, large structural changes in the food industry have been undertaken. Food industry has grown slowly. Food companies from other EU countries have invaded the Finnish market by increasing their ownership of Finnish companies and extending their product selection Finland became a member of the European Monetary Union and among the first countries to accept European currency in 1999.

The predicted gross national product per capita has increased from $17,000 to $21,000 between 1991 and 1997 with the population growth from 5.0 to 5.1 million. The gross value of all Finnish industry in 1997 was $80 billion; the share of food industry was 10% (Fig. 1). Since 1992 the Finnish food industry has employed more than 40,000 people. The most significant branches in the Finnish food industry include slaughter and meat production, dairy production, and beverages. A proportion of 3.5% ($280 million) of gross value in food industry was allocated to research and development.

The export and import values of food industry were $1,020 million and $1,900 million, respectively, in 1997. Most export was directed to the Eastern European countries (Fig. 2). As a result of the difficult economic situation in Russia, the export from Finland to Russia has markedly decreased in 1997–1998, while export to other Eastern European countries has increased. The share of the Common Market was 30%. Most imports came from the Common Market (Fig. 3). The most important Finnish export and import products are presented in Table 1.

CHARACTERISTICS OF FINNISH FOOD CONSUMPTION

The food expenditure per household was $3,000 in 1997. Meat and meat products (26%), milk, cheese and eggs (19%), bread and cereals (17%), and fruits and vegetables (15%) formed the major food expenditures per capita. Con-

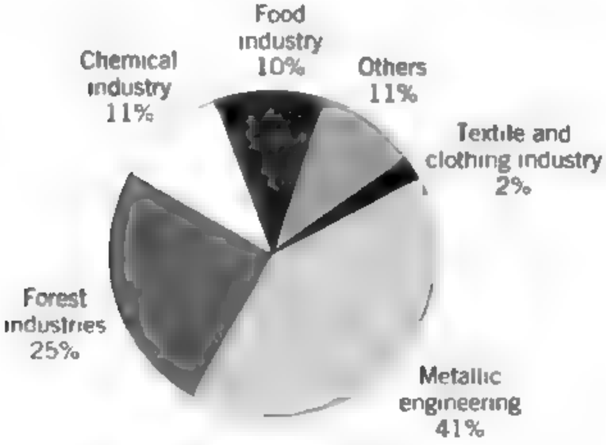


Figure 1. Gross value of production by branch of industry in 1997. Total gross value of production was $80 billion.

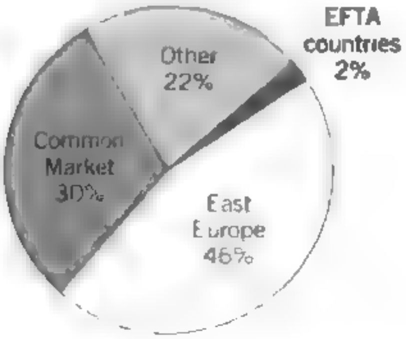


Figure 2. Food export by sales area in 1997.

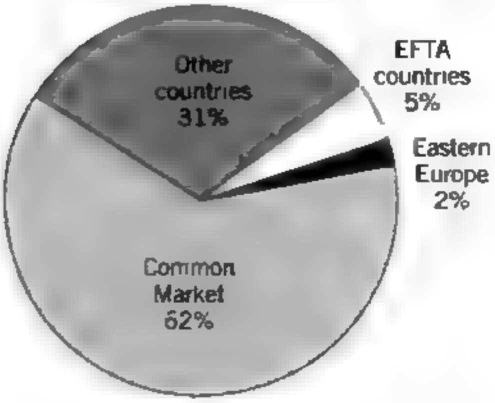


Figure 3. Food import by sales area in 1997.

Table 1. Finnish Export and Import in 1997

	Export value		Import value	
Product	$ million	%	$ million	%
	Industrial products			
Cheese	99	9.7	67	3.5
Chocolate	78	7.6	38	2.0
Butter and other milk fats	57	5.6	—	—
Alcoholic beverages	52	5.1	125	6.6
Margarine	50	4.9	—	—
Sucrochemical products	49	4.8	—	—
Pork	38	3.7	29	1.5
Coffee	36	3.5	—	—
Malt	31	3.0	—	—
Milk powder	30	2.9	—	—
Confectionery	29	2.8	—	—
Beer	21	2.1	—	—
Starch derivatives	—	—	70	3.7
Canned fish	—	—	46	2.4
Juices	—	—	40	2.1
Canned fruits	—	—	36	1.9
Other	304	29.8	547	28.8
Total	*872*	*85.5*	*1036*	*54.5*
	Primary products			
Oats	47	4.6	—	—
Barley	27	2.6	—	—
Edible fats and oils	13	1.3	42	2.2
Eggs	11	1.1	—	—
Raw coffee	—	—	220	11.6
Fruits	—	—	194	10.2
Vegetables	—	—	87	4.6
Oil seeds	—	—	72	3.8
Raw sugar	—	—	23	1.2
Other	27	2.6	108	5.7
Total	*124*	*12.2*	*747*	*39.3*
	Animal feed			
Total	*23*	*2.3*	*118*	*6.2*

sumption of milk and fermented milk (199 L/year/per capita) was highest, followed by fruits and vegetables (85 kg per capita), beer (84 L per capita), and vegetables (70 kg per capita). A total of 66 kg of meat (one-half of pork, one-third of beef, and one-sixth of poultry), and 60 kg of potatoes were consumed per capita. Consumption of coffee was among the highest in the world (9 kg per capita).

During the past few years the consumption of ready-to-eat and frozen foods has increased, whereas consumption of canned foods has slightly decreased. Organically farmed products have gained their own segment of consumption. Nearly 3% of cultivated area is under organic farming in Finland, while the European mean is 1%.

PUBLIC HEALTH AND FUNCTIONAL FOODS

Health aspect is considered one of the main selection criteria of foods. Cardiovascular diseases form a significant public health hazard in Finland. High sodium chloride level in the diet is considered one of the most important risk factors. Therefore, a marked reduction in salt intake has become a desirable trend. The level of dietary salt has been decreased by lowering the sodium chloride concentration in food products and by replacing sodium with magnesium and potassium. One such product is the Finnish innovation Pansalt, which is showing beneficial health effects (1,2). This product has been patented in 22 countries. A similar trend has been observed in consumption of fat. Food industry has reacted to the recommendations of health authorities by launching various low-fat and nonfat products into the market. Consumption of nonfat milk was one-fourth of total milk consumption in 1998.

In addition to lowering the salt and fat content of different foods, much research has been done on foods that promote human health or prevent diseases. Technological use of applications of medicine and biosciences has become a trend in food production. New products and processes are developed using enzymes and the natural bioactivity of plants. Probiotic lactic acid bacteria are studied as a means of influencing intestinal microecology, infection resistance, and gut barrier mechanisms.

FOOD SAFETY

High hygienic quality in primary production and effective food control guarantee the food safety in Finland. Significant infectious diseases in animals do not occur, and the rate of zoonoses is very low.

A lot of emphasis has been given to prevention of *Salmonella*. One reason for the low prevalence of *Salmonella* in poultry is competitive exclusion, a widely used method invented by Nurmi and coworkers in the 1970s (3). On the basis of the very low rate of salmonelloses in Finland, the EU commission accepted the National Salmonella Control Programme in Finland in 1994. The purpose of the programme is to ensure the safety of foods of animal origin by limiting the number of salmonelloses in Finnish food animals and in foods of animal origin to internationally low levels. Cattle, pigs, and poultry, as well as meat and eggs, are being controlled according to the program. The prevalence of *Salmonella* in food animals and foods of animal origin is expected to be below 5% in each slaughterhouse and cutting plant, whereas the total prevalence in Finland may not exceed 1%. Furthermore, imported beef, pork, and poultry meat, as well as living poultry and eggs, are expected to be completely free from *Salmonella*. A total of 0.1% of lymph node samples and surface swap samples in beef and pork were positive for *Salmonella*, while in living poultry the proportion of positive samples was 0.3% according to the results of the National Veterinary and Food Research Institute. The share of *Salmonella* positive cattle and pig herds was below 0.3% and 0.04%, respectively. Altogether 29,255 samples were investigated.

Finnish foodstuffs are generally considered pure. The use of drugs and pesticides is widely controlled in Finland. The National Veterinary and Food Research Institute is responsible for testing residues and contaminants in meat, milk, egg, and fish samples. According to the national residue-monitoring program, no prohibited growth promoters in meat production were detected. Residues of antimicrobial drugs over the maximum residue level were found from pork (0.05%) and milk (0.81%). The content of pesticides, heavy metal, and other contaminants was low. The number of investigated samples was 26,110. Cold winters and strict hygienic control obviously cause the relatively low rate of environmental contamination in foods compared with several other industrial countries. Therefore, Finnish food meets well the consumers' expectations of foods with low environmental contamination levels.

There is an extensive surveillance system for food poisoning outbreaks in Finland. The average rate of reported food poisoning outbreaks has been 0.7 outbreaks/100,000 inhabitants in the 1990s. The average number of cases in these outbreaks has been 32 cases/100,000 inhabitants. The surveillance system yields valuable information about the infectious agents and other factors leading to the epidemics. This information is used in the controlling of epidemics by the food control authorities that in cooperation with health authorities, veterinarians, and medical doctors are responsible for the investigations of food poisoning epidemics.

A lot of research on the entire production chain has been done in Finland to improve the safety of foods. Molecular biological methods have been applied in tracing the origin of epidemics (4) and outlining the controlling measures (5).

FOOD CONTROL

The organization of food control in Finland is presented in Figure 4. The division of labor at each administrative level is distinct, although the structure of central administration may seem complex. At the municipal level the food control authorities act under the direction of the pertinent ministry, which makes the municipal administration in Finland effective. In each of 452 municipalities in Finland an effective food control system is usually run by municipal veterinary officers. In the 1990s, the in-house control system based on Hazard Analysis of Critical Control Points (HACCP) has become a common practice in the food industry.

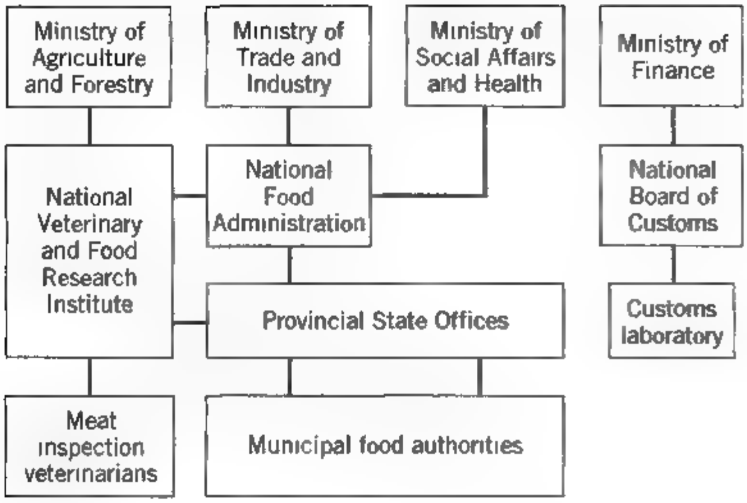

Ministry of Agriculture and Forestry
Ministry of Trade and Industry
Ministry of Social Affairs and Health
Ministry of Finance
National Veterinary and Food Research Institute
National Food Administration
National Board of Customs
Provincial State Offices
Customs laboratory
Meat inspection veterinarians
Municipal food authorities

Figure 4. Food control in Finland.

FOOD INDUSTRY IN FINLAND

Dairy Industry

The share of dairy industries in total value-added production was 16% in 1997. The dominating dairy processors are Valio Ltd. and Ingman Foods Oy Ab. Emmental cheese is the most important export product of the Finnish dairy industry. Due to the strict control and high hygienic quality of primary production, the quality of milk is high. Of raw milk, almost 91% was ranked as class E in 1998, which means that the geometric means of bacterial and cell counts were below 250,000/mL and 50,000/mL, respectively.

Milk and milk products are the main source of calcium in the diet of most Finnish people. In addition to normal milk, Valio Ltd. produces milk with 50% higher calcium content than in normal milk. HYLA products contain less than 1 g/100 g lactose.

The probiotic effect of lactic acid bacteria on the human gastrointestinal tract has been intensively studied in Finland. *Lactobacillus rhamnosus* GG is one of the most investigated probiotic strains in the world. The latest studies on *L. rhamnosus* GG have shown its stabilizing effect on gut permeability and suppression of allergic reactions in food hypersensitivity (6). Products containing *L. rhamnosus* GG include milk, sour milk, juices, yogurts, whey drinks, and capsules. Valio Ltd. has the worldwide commercial rights for the strain and is sublicensing the exclusive rights for its use to other countries. Products containing *L. rhamnosus* GG are on the market in 23 countries, and one of these products was officially accepted as the first probiotic food (Food for Specific Health Use) in Japan.

Meat Industry

The meat industry is the largest branch of the food industry in Finland. The gross value of the meat industry was $2,140 million in 1997, with the total value-added production being $440 million. A total of 31 kg of meat products were consumed per capita. The majority of these products were cooked sausages. The largest Finnish meat companies are Atria Oyj, HK Ruokatalo Oyj, and Saarioinen Oy. Finnish meat research includes food safety, development of process technology, and studies on product shelf lives. As a Finnish speciality, elk and reindeer meat are produced on a small scale. Elks are wild animals found in all parts of Finland, whereas semidomesticated reindeer are kept in North Finland.

Bakery

The baking industry is one of the largest branches of the food industry in Finland. A typical feature in the baking industry is a high demand of labor compared with other branches of the food industry; the bakery branch is the biggest employer in the food industry. The largest baking companies in Finland are Fazer Bakeries Ltd. and Vaasan & Vaasan Oy. In addition to these major bread producers, there are more than 1,000 small and medium-sized bakeries in Finland. Traditionally, freshness of the product has been considered the most important quality factor in bakery products. To fulfill consumer demand for fresh, tasteful, and easy-to-use bread products, production and use of frozen and prebaked products has increased heavily during the last years.

In addition, the nutritional value of whole-meal breads has obtained a growing interest. For example, commonly consumed rye bread is an excellent source of dietary fiber, vitamins, and minerals. Recent studies indicate the positive influences of rye on colon fermentation and suggest that the lignans located in the bran may play a role in preventing certain types of cancer.

Beverages

The inland sale of beers and soft drinks in 1997 was 725 million liters. More than half of the volume consisted of beer, and soft drinks and mineral water formed the rest. The volume of annual export was 53 million liters and import 11 million liters. Hartwall Plc and Oy Sinebrychoff Ab are the dominating beer and soft drink producers in Finland. Of the strong liquors, the most famous export article is Finlandia Vodka, whose production was initiated by Primalco Ltd. in 1970. By the late 1970s it had propagated worldwide.

The latest research on brewing and malting technology includes the entire production chain from barley to beer. Research in close cooperation between VTT Biotechnology and Research and industry has aimed at improvement of fermentation process of yeast, using immobilized yeast for fermentation, starter technology in malting, endogenous enzymes in malt, rapid microbiological control methods, and hygiene of dispensing systems. Novel investigations on transgenic barley producing β-glucanase have been initiated.

Margarine Industry

High concentration of LDL cholesterol in the diet has been considered as the main risk factor for atherosclerotic vascular disease. The incidence has been relatively high in Finnish people. Therefore, there is a tendency to introduce low-fat, low-salt, and low-cholesterol diets to consumers in order to minimize the risk of atherosclerotic vascular disease. According to recent research (7), margarine enriched with fat-soluble sitostanol, when substituting part of the daily fat intake in individuals with mild hypercholesteremia, was effective in lowering serum total cholesterol and LDL cholesterol. One of the most noteworthy innovations in the Finnish food industry has been the application of the aforementioned plant stanol ester into food products known as Benecol® (8). The first Benecol® product, a full-fat margarine, was launched into the market in 1995 by Raisio Group Plc, followed by a low-fat margarine. Both Benecol® products have been reported to effectively reduce the serum total cholesterol, especially the LDL cholesterol, as a part of a low-fat, low-cholesterol diet.

Candy Industry

The Finnish innovation xylitol, added to chewing gum, has been reported to prevent caries by inhibiting the growth of *Streptococcus mutans* (9,10). Moreover, the repairing effect of xylitol on primary damage in the dental enamel has been proved. The Finnish company Huhtamäki Leaf Oy launched a xylitol-containing chewing gum; thereafter, many other products with xylitol as a sweetener have come to the market. Since 1988 the Finnish Dental Association has thus recommended the use of xylitol to improve the dental quality of Finnish people. According to the recent research, daily use of xylitol has also been shown to inhibit adhesion of otopathogenic bacteria, therefore decreasing the incidence of acute otitis media in small children by 40% (11).

Oy Karl Fazer Ab is the market leader of confectionery in Scandinavia. The share of import of the whole turnover of Oy Karl Fazer Ab is 60%. Two-thirds of the sales of Fazer Confectionery Ltd. is generated abroad. The most important brand is Karl Fazer Milk Chocolate.

FOOD PROCESSING AND PACKAGING

New technologies, effectiveness, and preparedness to quickly react to consumers' demands are essential in the harsh competition of modern food processing. Purity and nutritive and sensory quality of foods, as well as lack of preservatives, have become important selection criteria for consumers. VTT Biotechnology and Food Research has intensively studied minimal processing techniques for improving the sensory quality and nutritional value of foods. Minimal processing, vacuum packaging, and extended chilled storage of such food products, however, possess certain microbiological risks that are currently being studied by VTT Biotechnology and Food Research and in the Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki.

New processing techniques and the yield of food products with extended shelf lives are reflected through the increasing need of vacuum packaging and modified atmosphere packaging (MAP). Research on smart and active packaging such as quality and leak indicators, edible films, oxygen removers, and biodegradable materials is currently being done by VTT Biotechnology and Food Research (12,13). Such packaging technologies seem promising with respect to sensory quality and product safety.

Of the traditional packaging material producers, Stora-Enso Oyj is one of the world's leading companies in the production of carton packages for liquid foods. Other significant cardboard and carton producers are Metsä-Serla Corporation and UPM Kymmene Corporation. The largest packaging film producers are Wihuri Wipak Oy and UPM Kymmene Corporation.

EDUCATION AND RESEARCH

Food sciences education is shared among four universities. The Faculty of Agriculture and Forestry at the University of Helsinki is concentrated on food technology, food chemistry, microbiology, and nutrition; the Department of Food and Environmental Hygiene in the Faculty of Veterinary Medicine, the University of Helsinki, focuses on food hygiene. Other branches are food chemistry in the University of Turku, nutrition in the University of Kuopio, and food technology and chemistry in the Helsinki University of Technology. Research on food science and technology is carried out in the preceding universities as well as in four governmental research institutes and two private institutes. The governmental institutes include the Agricultural Research Centre of Finland (MTT), National Public Health Institute (KTL), National Veterinary and Food Research Institute (EELA), and VTT Biotechnology and Food Research. The private institutes are the Meat Research Institute and Potato Research Institute. Universities and governmental institutes have organized postgraduate education, and in 1994 the Finnish Graduate School on Applied Bioscience: Bioengineering, Food & Nutrition, Environment (ABS) was founded. A total of 154 graduate students registered in the ABS Graduate School in 1998

BIBLIOGRAPHY

1. E. Mervaala et al., "Beneficial Effects of a Potassium- and Magnesium-Enriched Salt Alternative," *Hypertension* **19**, 535–540 (1992).
2. U.S. Pat. 4,931,305 (June 5, 1990), H. Karppanen and P. Karppanen (to Pharmaconsult Ltd.).

3. E. Nurmi and M. Rantala, "New Aspects of *Salmonella* Infection in Broiler Production." *Nature* **241**, 210–211 (1973).
4. M. K. Miettinen et al., "Molecular Epidemiology of an Outbreak of Febrile Gastroenteritis Caused by High Numbers of *Listeria monocytogenes* in Cold-Smoked Rainbow Trout," *J. Clin. Microbiol.* **37**, 2358–2360 (1999).
5. T. Autio et al., "Sources of *Listeria monocytogenes* Contamination in a Cold-Smoked Rainbow Trout Processing Plant Detected by Pulsed-Field Gel Electrophoresis Typing," *Appl. Environ. Microbiol.* **65**, 150–155 (1999).
6. M. Saxelin, "*Lactobacillus* GG—A Human Probiotic Strain With Thorough Clinical Documentation," *Food Rev. Int.* **13**, 293–313 (1997).
7. T. A. Miettinen et al., "Reduction of Serum Cholesterol With Sitostanol-Ester Margarine in a Mildly Hypercholesterolemic Population," *New Engl. J. Med.* **333**, 1308–1312 (1995).
8. U.S. Pat. 5,502,045 (March 26, 1996), T. Miettinen, H. Vanhanen, and I. Wester (to Raisio Ltd., Finland).
9. A. Scheinin, K. K. Mäkinen, and K. Ylitalo, "Turku Sugar Studies. V. Final Report on the Effect of Sucrose, Fructose and Xylitol Diets on the Caries Incidence in Man," *Acta Odontol. Scand.* (Suppl. 70) **33**, 337–343 (1975).
10. A. Scheinin et al., "Turku Sugar Studies. XVIII. Incidence of Dental Caries in Relation to 1-year Consumption of Xylitol Chewing Gum," *Acta Odontol. Scand.* (Suppl. 70) **33**, 307–316 (1975).
11. M. Uhari et al., "Xylitol Chewing Gum in Prevention of Acute Otitis Media: Double Blind Randomized Trial," *Br. Med. J.* **313**, 1180–1184 (1996).
12. M. Smolander, E. Hurme, and R. Ahvenainen, "Leak Indicators for Modified-Atmosphere Packages," *Trends Food Sci. Technol.* **8**, 101–106 (1997).
13. T. Mattila-Sandholm "Oxygen-Sensitive Colour Indicator for Detecting Leaks in Gas-Protected Food Packages," European Patent 0 666 977 B1.

MIIA LINDSTRÖM
HANNU KORKEALA
University of Helsinki
Helsinki, Finland
RAIJA AHVENAINEN
VTT Biotechnology and Food Research
VTT, Finland

INTERNATIONAL DEVELOPMENT: GERMANY

The Federal Republic of Germany (FRG), since the reunification on October 3, 1990, consists of 16 federal states (Bundesländer) covering an area of 357,030 square kilometers. The population density varies of almost 4000 persons/km^2 in the city state of Berlin and around 80 persons/km^2 in the northeastern states of Brandenburg and Mecklenburg-Vorpommern.

Germany is situated in the center of Europe at the point of intersection of many cultural regions. People in Germany—their culture, social behavior, and, last but not least, their eating habits (food production, processing, and preparation)—therefore have been formed by a great many influences. The broad spectrum of food and beverages was influenced in south Germany to some extent by southern Europe, but traces of influences of the adjoining countries also may be found in the west, north, and east of Germany. This can be exemplified by viticulture, which the Romans introduced into the regions north of the Alps 2000 years ago; by the pasta dishes, typical for parts of southern Germany, that came from Italy; and by many desserts from eastern Europe. Varieties of bread found in Scandinavia are also found in northern Germany. This development has been continuing; the food supply of today contains many products foreign workers brought into Germany.

In the many local regions of Germany different nutritional behaviors developed over the centuries. The great variety of sausages and meat dishes, the high number of bread varieties (mostly based on rye), and many kinds of beer have been known all over the world. Important cereal products have been unripe spelt grain and oat flakes. In the mountains, milk production and dairying have been the basis of income and nutrition. Fruit and vegetable processing also go back to the Middle Ages. Important products are sauerkraut, dried fruit, and cider.

In the Middle Ages, the qualities of food products such as bread, meat, and beer, produced in small craftsmen's establishments, were protected by guild laws and rules of the sovereign. One of these was the so-called pureness rule of 1516, governing the production of beer. Water, malt, and hops were the only ingredients allowed for beer. Similar, usually local, rules existed for other food items like sausages and flour. These rules and regulations may be regarded as the precursors of today's food laws. Due to the long tradition of crafts in Germany, food still is produced today mostly in small-sized plants.

The essential impetus for the industrialization of food production in central Europe, and thus in Germany, originated from the continental blockade Napoleon I imposed in 1806. Blocked off from the world market, Europe had to substitute for several essential foods or start food production by itself. Thus, industrial production of sugar was developed. Because coffee was no longer available, substitutes had to be developed, and industrial production soon started in Germany. At the end of the nineteenth century, the first industrially produced convenience food followed Liebig's invention of meat concentrate. Such production included dry soups and vegetable protein hydrolysates, which were processed into so-called seasonings for soups.

There was a need for preserved food in the French-German War of 1870/1871, which led to the development of condensed soups. Typical food manufacturing companies were founded in the late nineteenth century, and when surviving the intensified competition, today they partly exist as affiliates of large international food producing companies.

In the following sections, the demographic structure of the German population will be addressed, and determining data of the food industry in Germany will be presented. Subsequent sections will be devoted to the education of persons working in the food industry and to research institutions and developments in the field of food industry.

STRUCTURE OF THE POPULATION AND DEMOGRAPHIC DEVELOPMENT OF RELEVANCE FOR FOOD PRODUCTION AND PROCESSING

Important for determining the quantities of food production is the number of people to be supplied, whereas the age structure, size of private households, and kinds of jobs are essential for determining the kind of product spectrum. Another important factor influencing the product spectrum is available income. The population statistics of all European countries show a stagnation or a slight decrease of population numbers. This is true for the German population within the new Bundesländer and also for the former Federal Republic, but foreign people applying for the German citizenship exceed the decreasing number (Table 1). In 1995, 7.2 million foreigners in Germany accounted for 8.8% of the total population, the percentage in the new Bundesländer was 1.7% at the same time.

The low birth rate and the further extended life expectancy (1995: boys, 72.8; girls, 79.3) led to an increased proportion of older people, and to a lowered percentage of younger people (Table 2).

FOOD CONSUMPTION

The consumption behavior of people may, besides the distribution according to age and country of origin, also be influenced by the fact that the number of small households and households without children is continuously increasing (status of 1995 shown in Table 3). Despite the increasing number of unemployed (especially in the new Bundesländer, Table 4) the average consumption per household still increases (Table 5). The development after 1994 slowed down but kept the trend.

Total quantities of food sold in Germany will remain on an almost constant level. But demand will develop to more convenient products to serve older people or fulfill the needs of small households. Special food items are created to attract health-conscious consumers, and the exotic and ethnic products supply follows the needs of foreigners and experiences made on vacations all over the world. A new dimension arises, when extrinsic properties like environmentally friendly production and sustainable farming as well as social responsibility of companies become quality criteria. A consumer-oriented food development is growing in Germany, relatively new in the branches of fresh produce. Sensory science and consumer research assess the known and unknown desires of the consumer and replace the sole view on a product's properties (3–6).

Table 1. Development of the German Population from 1950 to 1995 in Millions of People

Year	Germany	Former Federal Republic	New Bundesländer and East Berlin
1950	68,724	50,336	18.388
1960	72,937	55,785	17.188
1970	78,069	61,001	17.068
1980	78,397	61.651	16,740
1990	79,753	63,726	16.028
1995	81,817	66,342	15.476

Source: Ref. 1.

Table 2. Development of Age Structure in Germany from 1955 to 1995

		Age		
Year	Population (millions)	Below 20 (%)	20–59 (%)	Above 60 (%)
1955	70.945	30.2	54.2	15.6
1965	75.591	28.6	52.7	18.6
1975	78.882	29.1	50.4	20.5
1985	77.709	24.2	55.9	19.9
1995	31.539	21.5	57.8	20.7

Source: Ref. 1.

AGRICULTURAL AND COMMERCIAL PRODUCTION

Agricultural and commercial food production are important economic factors in Germany. The net production value of agriculture and food industry was 34.3 and 48.8 billion DM respectively, compared with 2,759.6 billion of the German economy. General structural and economic data of Germany, the German food industry, and its comparison to other industry branches are presented in Tables 6 and 7.

Agricultural Production

In 1997, 522,000 enterprises cultivated 117,000 km^2 in the former Federal Republic, and only 33,145 enterprises produced on 56,000 km^2 within the new Bundesländer; together they covered 54.1% of the total area of Germany.

The number of employees and the total area is decreasing year by year, but production and turnover have been fairly stable within the last years. The most important produce, in terms of quantity, are cereals, sugar beets, potatoes, and meat (Table 8). Self-sufficiency depends on the crop (Table 8), only 18% in case of fruits and 41% in case of vegetables. Of the main agricultural crops, the self-supply is above 100%.

Commercial Food Production

Commercial food producing enterprises in Germany largely comprise small- to medium-sized companies. Of the companies in the German food industry, 72% employ less than 100 people, and only 3% of all enterprises employ more than 500 people (Tables 9 and 10). In addition to the producing food industry with a total number of 20 or more employees per enterprise, in 1995 there were 44,464 small craftsmen's shops with 1 to 19 employees per enterprise; 19,610 bakery shops; and 20,210 butcher shops producing and selling their products. In 1994 they employed 314,472 people (baker; 145,632; butcher: 138,019) and had a turnover of 31,204 million DM (baker: 11,241; butcher: 16,612).

In the food industry most of the employees work in the baking sector, slaughterhouse and meat processing industry, brewing industry, and dairy, and confectionery industries. These are the branches with the highest turnover and a low number of employees per enterprise, both indicating the traditional importance of the produced food items.

The total turnover, number of employees, and total payroll in the food industry is typical for the producing indus-

Table 3. Number of Households With and Without Children in 1995

Household, no. of members	Households (millions)	(%)	Without children (%)	With children (%)
		Former Federal Republic		
1	10.800	36.0	100	—
2	9.612	31.9	88.4	11.6
3	4.571	15.2	4.5	95.5
4	3.618	12.0	0.6	99.4
5 or more	1.518	5.0	0.3	99.7
Total	*30.119*	*100*	*64.8*	*35.2*
		New Bundesländer and East Berlin		
1	2.065	30.4	100	—
2	2.246	33.1	85.4	14.7
3	1.276	18.8	3.4	96.6
4	0.979	14.4	0.3	99.7
5 or more	0.227	3.3	0.0	100
Total	*6.793*	*100*	*59.3*	*40.7*

Source: Ref. 1

Table 4. Population, Potential Working and Unemployment

Year	Potentially working (millions)	Percentage of total population	Working (millions)	Unemployed (millions)
		Former Federal Republic		
1991	30.662	47.9	28.973	1.689
1994	30.872	46.9	28.316	2.556
1997	30.512	45.8	27.491	3.021
		New Bundesländer and East Berlin		
1991	8.503	53.4	7.590	0.913
1994	7.798	50.1	6.656	1.142
1997	7.748	50.4	6.385	1.363

Source: Ref. 2

Table 5. Structure of Consumption of Private Households in 1991, 1994, and 1997

	Former Federal Republic		New Bundesländer and East Berlin		Germany
Private consumption	1991	1994	1991	1994	1997
Million DM	1,446,940	1,647,070	183,390	255,790	2,086,200
Food, beverages, tobacco (%)	21.3	20.1	30.4	27.6	18.0
Clothing, shoes	7.7	7.1	7.3	5.5	6.5
Housing	15.9	18.3	4.2	12.7	19.9
Energy (no fuel)	4.1	3.6	5.0	4.4	3.6
Furniture, appliances	8.8	8.7	10.8	10.3	8.2
Health and body care	5.9	6.2	4.3	5.8	5.6
Mobility and communication	17.3	16.4	20.8	16.0	16.7
Education, entertainment, leisure	11.2	11.0	11.9	11.4	9.5
Personal goods	7.8	8.6	5.2	6.3	8.1

Source. Refs. 1 and 2.

try in Germany: slowly increasing turnover, slowly decreasing number of employees, and almost constant total payroll. The export share (foreign turnover divided by total turnover, Table 10) has steadily increased from 8.9% in 1993 to 11.1% in 1997. This is a small figure compared to 33.2% export share of the total producing industry, but there is considerable variation among the different sectors of food industry (Table 10). The value of imports was 71,534 million DM in 1996; the value of exports was in the same year 41,939 million DM. Over the last five years imports and exports slowly grew at the same rate.

The percentage of payroll in the total turnover of food industry (1996) was low (10.9%) compared with the producing industry of other branches (18.5%), whereas the

Table 6. General Structural and Economic Data of Germany, 1995 and 1997

	Germany 1995	Germany 1997
Population	81,700,000	82,100,000
Persons employed	34,800,000	33,900,000
agriculture and forestry	1,001,000	903,000
food industry	524,500	502,700
Private households	36,900,000	37,457,000
Gross national product (in present prices)	3,443 billion DM	3,624 billion DM
Private consumption	1,979 billion DM	2,096 billion DM
Expenditures for food, beverages, and tobacco	372 billion DM	376 billion DM
Net income per employed person and month	2,610 DM	2,710 DM
Total cost-of-living index (1993 = 100)	104.5	108.0
Food price index (1993 = 100)	102.4	104.7
Gross salary (wage) index (1993 = 100)	106.1	109.7
Net salary (wage) index (1993 = 100)	101.4	105.2
Foreign trade		
Total imports	664 billion DM	756 billion DM
Food imports	68 billion DM	72 billion DM
Total exports	750 billion DM	887 billion DM
Food exports	38 billion DM	42 billion DM

Source. Refs. 1 and 2.

Table 7. Comparative Structural Data, 1995

Industry	Number of enterprises	Number of employed	Turnover (billion DM)	Export (%)
Total producing industry	46,398	6,593,000	2,033	28.8
Food industry	5,085	525,000	221	9.8
Petrochemical industry	86	27,000	111	2.8
Chemical industry	1,717	536,000	219	41.6
Metallurgy	1,128	295,000	102	32.8
Metal products	6,498	1,044,000	251	42.7
Equipment for power plants	2,341	495,000	123	32.0
Vehicles and parts	1,047	689,000	262	47.6

Source. Ref. 1.

Table 8. Agricultural Production, 1997

Produce	Quantity (million tons)	Self-sufficiency (%)
Cereals (wheat, rye) feed and industry excluded	23.2	120
Sugar (as white sugar)	4.0	150
Potatoes	12.5	102
Fruit	0.948	18
Vegetables	2.561	41
Wine (million hL)	8.311	46
Meat (slaughter weight)	6.2	85
Beef	1.5	127
Pork	3.5	80
Cow's milk	28.7	
Eggs	13,890 million	72

Source. Ref. 2.

material value was high (50.8% compared with 39.2%). This indicates the high degree of automation that is supported by the above average investments made in the food industry. In 1994, 10,305 million DM were invested by the food industry, 86,321 million DM of the total producing industry of Germany. The energy consumption increased from 1996 to 1997 by 2.18% to 26,558 million kWh in the case of gas and by 1.24% to 12,378 million kWh in the case of electric current. Consumption of coals and fuel oils reduced in the same period by 9.8% to 17,424.2 million kWh.

EDUCATION AND RESEARCH

Education

Cultural and educational policies are within the competencies of the federal states of Germany. However, the federal government exerts coordinating functions providing for framework and financing laws. The German educational system therefore varies to some extent from state to state. The mandatory education period is 9 (in several states, 10) years of school attendance.

In 1999, 935,000 pupils finished school education, with the following breakdown (7):

With no qualification	8%
After successful elementary school	25%
Realschule (intermediate level)	40%
With admission to university or technical college (Ing.)	27%

Table 9. Branches of Food Industry, Number of Companies and Persons Employed, 1995

	Number of companies employing						Total number of companies	Total employed persons
	1 19 persons	20–49 persons	50–99 persons	100–499 persons	500–999 peresons	1000+ persons		
Commercial food production	505	1,988	1,148	1,281	120	35	5,077	534,000
Slaughterhouse and meat processing industry	85	520	248	275	18	5	1,151	105,900
Fish processing industry	28	32	16	28	4	1	109	12,100
Fruit and vegetable industry	56	95	65	70	5	2	293	28,600
Potato products	8	16	11	14	1	—	84	6,400
Fat and oil industries	2	5	10	19	2	1	39	8,500
Dairies, cheese producing plants (fresh and keeping)	34	85	83	109	12	4	324	49,600
Spices and sauces	9	26	10	24	4	3	76	15,400
Mills and starch	12	42	31	19	3	3	107	9,300
Baking products	71	650	334	325	24	3	1418	129,200
Sugar industry	1	2	5	37	1	—	46	8,800
Confectionery	8	28	45	59	14	7	161	37,900
Coffee and tea producing industry	3	9	15	20	3	—	50	7,900
Mineral water, soft drinks	31	73	50	86	4	—	244	24,900
Breweries and malting plants	46	206	117	107	11	4	491	49,300
Wineries	—	13	12	7	1	—	33	3,100
Spirits	29	51	21	16	2	—	119	7,600

Source Ref. 2

Table 10. Branches of Food Industry, Index of Net Production, Turnover, Total Payroll, and Export Share, 1997

	Index of net production (1995 = 100)						
Branch of industry	1993	1994	1996	1997	Turnover (million DM)[a]	Total payroll (% of turnover)	Export share (%)
Commercial food production	94.1	95.8	101.9	102.7	225,981	10.9	11.1
Slaughterhouse and meat processing industry	97.5	99.0	102.7	104.9	39,839	10.3	5.0
Fish processing industry	96.0	97.6	102.3	97.2	3,604	13.3	15.5
Fruit and vegetable industry	82.2	86.5	100.4	102.4	12,285	10.5	12.6
Potato products					1,691	15.6	9.1
Fat and oil industries	110.4	104.5	95.2	99.4	8,541	4.7	29.1
Dairies, cheese producing plants (fresh and keeping)	90.1	90.0	99.7	97.1	39,396	6.4	16.5
Spices and sauces					6,612	16.3	7.9
Mills and starch	82.8	80.4	106.4	112.4	6,345	9.6	22.4
Baking products	76.1	77.9	103.8	105.8	18,533	27.7	2.8
Sugar industry					6,383	7.5	15.9
Confectionary	105.5	108.3	103.1	99.3	14,893	11.0	18.2
Coffee and tea producing industry					9,472	4.8	12.8
Mineral water, soft drinks	91.4	97.7	100.5	103.1	11,770	12.1	1.8
Breweries and malting plants	101.8[b]	103.5	97.7	96.8	19,972	15.0[b]	6.0[b]
Wineries					2,675	5.8	
Spirits	110.6	107.7	97.2	89.4	6,668	5.0	3.3

[a] Without tax

[b] Only breweries.

Source· Ref. 2.

Professional qualification can be performed in multiple ways; the main levels of professional qualification are listed in Table 11.

After the successful attendance of 9 (or 10) years of the elementary school, a professional education of 2 to 3 years may follow. In addition to the training within the company, there is parttime school education. In some cases the complete education is conducted in specialized schools. It can be a choice between an industry and a smaller craftsman shop's environment education. Further qualification after several years of job experience can lead to a master or technician certificate. The first enables education of pupils within the industry's own branch.

Universities are institutions of the dual system of teaching and research. In 1998/99, 1,824,000 students were registered at German universities, 271,575 persons, 48.4% women, in the first semester. In 1996 the student numbers were lower, for the first time, than in the year before. In

Table 11. Levels of Professional Qualifications in Germany

School	Professional education	Course of qualification	Number of educated persons in 1996	Skills
12–13 years secondary school or gymnasium	4–5 years university academic		Agriculture 7,260[a] Food Sciences 4,870[b]	Work scientifically Self-responsible Management duties
12–13 years secondary school or gymnasium	4 years technical college Ing. graduate		Agriculture 2,692[a] Food Sciences 3,144[b]	Self-employed, transfer of scientific knowledge into practical problem solving, technical management
10 years Realschule	2 years school technical assistant			Implementation of technical work independently
9–10 years elementary school	3 years dual education within an enterprise and/or school education	Master additional school or courses experience		Performing specific work without instruction, instructing other workers, educating
9–10 years elementary school	3 years dual education within an enterprise and/or school education	2 years school technician experience		Planning of investment and operation of machinery, managing supply and labor requirements
9–10 years elementary school	3 years dual education within an enterprise and/or school education		Several professions thereof: Baker 17,642 Butcher 9,086	Technical skills, capable of performing difficult specific work
9–10 years elementary school	On-job training			Specific work

[a] Horticulture collage and university: 2871 students.

Source: Refs. 1, 2, and 8

the food sector, students specialize in food technology (Universities: Berlin, Bonn, Hohenheim, Munich), food chemistry (Berlin, Bonn, Braunschweig, Dresden, Erlangen-Nürnberg, Frankfurt/M, Hamburg, Hohenheim, Kaiserslautern, Karlsruhe, Munich, Münster, Stuttgart, Wuppertal, Würzburg), and nutrition science and nutrition economy (Bonn, Gießen, Hohenheim, Jena, Kiel, Munich, Potsdam). Horticultural universities are located in Berlin, Hannover, and Munich; agricultural branches are in Berlin, Bonn, Gießen, Göttingen, Halle-Wittenberg, Hohenheim, Kassel, Kiel, Munich, and Rostock. Additional disciplines taught at German universities are wine production, beverage technology, dairy economy, packaging technology, production technology, and automation and process technology. The German food industry also engages graduates from other disciplines, such as chemistry, physics, and pharmacy, thus providing transfer of knowledge and encouraging interdisciplinary concepts. It should be noted here that there are no restrictions or specific educational requirements to a position in the food industry. One exception is the official inspection of foods, where food chemists or veterinarians are required.

Research

Total expenditures for research and development in Germany amounted in 1996 to 88,600 million DM. Industry and trade spent 67% of research funds, 18% were spent by universities, and 15% by noncommercial governmental or private institutions.

Research at Universities. The dual system of research and teaching is traditionally practiced at German universities and technical universities. They are exclusively affiliated with the federal states. Scientists (professors) appointed to a chair for an unlimited period may conduct self-responsible research within their working field. The budget is financed by the federal government concerned. Additional money sources are industry, private foundations, the Deutsche Forschungsgemeinschaft (mainly fundamental research), or "Innovationskompetenz mittelständischer Unternehmen" by the Federal Ministry for Education and Research (applied research, development). Other sources of funding are the research projects supported by the EU in the FAIR, FLAIR-FLOW, and COST projects. More details can be found at the Web sites *www.exp.ie / flair.html* or *www.kowi.de*. Information on the fields of the German universities and addresses can be found at the Web sites *www.hochschulkompass.hrk.de*, *www.bmbf.de*, or *www.studienwahl.de*.

Federal Ministry for Nutrition and Agriculture. The Federal Ministry for Nutrition and Agriculture spends 500 million DM annually on research (9). The most important institutions are the 10 federal research centers, the Central Institution for Agricultural Documentation and Information (*www.dainet.de* (/zadi)) and 7 institutions (blue list), which are cofinanced with federal states. The topics of investigation are sustainable use of ecosystems and resources, protection of the natural environment and quality production, enhancement of food quality, further development of renewable resources, social economy, and information and documentation. The knowledge produced serves governmental decisions in the field of nutrition, agriculture and forestry, and consumer policy. It also is intended to support small- and medium-sized enterprises in answering technical questions and as an information source for the public.

Federal Ministry of Education and Research and the Commercial Environment. The Federal Ministry of Education and Research also funds a variety of projects within the food sector. As in the case of the Agricultural Ministry, the cooperation between university and other nonprofit research institutions and commercial R&D initiatives should be facilitated. Actual information can be obtained from *www.bmbf.de*. Searchers about actual developments within the commercial sector can be started from *www.behrs.de* or *www.fnii.ifis.org*.

MAIN FIELDS OF RESEARCH AND DEVELOPMENT

The developments in Germany are not independent of the developments in other countries. The aim of the substantial support by the European research programs is the transfer of ideas, knowledge, and techniques. Besides the traditional fields of technological and conceptional evolution, the following topics have emerged as fields of interest:

Preservation and modification of food by new technologies like high hydrostatic pressure, high-pressure freezing, high-intensity electric field pulses, and supercritical carbon dioxide.

Use of rapid and online measurements (ultrasound, NIR, NMR, electronic noses) to facilitate and document good manufacturing practice. Certification and accreditation have become increasingly necessary tools in quality concepts (quality assurance).

Assessment of allergic potential of food additives, novel food, or genetically engineered products.

Development of improved packaging, reduction of material. Recycling and elimination costs are extremely high in Germany and Austria compared with other countries.

Identification implementation, and promotion of food items with human health promoting functions (antioxidants, bioactive substances, secondary plant components, probiotic microorganisms, fiber). The claim of specific food effects on human health is illegal in Germany.

Use of sensory methodologies and other results of consumer research to implement consumer orientated product development, combining convenience, sustainable production, minimal processing, and new experiences.

BIBLIOGRAPHY

1. Statistisches Bundesamt, ed., *Datenreport*, Bundeszentrale für politische Bildung, Bonn, Germany, 1997.
2. Statistisches Bundesamt, ed., *Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten 1998*, Landwirtschaftsverlag, Münster-Hiltrup, Germany, 1999.
3. Z. Andani and H. J. H. MacFie, "Consumer Preference," in R. L. Shewfelt and B. Brückner, eds., *Fruit and Vegetable Quality: An Integrated View*, Technomic Publishing, Lancaster, Pa., 1999.
4. B. Brückner and H. Auerswald, "Instrumental Data—Consumer Acceptance," in R. L. Shewfelt and B. Brückner, eds., in *Fruit and Vegetable Quality: An Integrated View*, Technomic Publishing, Lancaster, Pa., 1999.
5. K. G. Grunert, "Food and Science. Wissenschaft im Dienst der Ernährung," *Proceedings of the Symposium 25th Anniversary "Euro Research and Development" CPC Europe, 35th Anniversary "Institute for Research and Development" CPC Deutschland*, 47–63, 1997.
6. H. R. Moskowitz, B. Krieger, and D. Cohen, "Meeting the Food Market Challenge: Creating Winning Food Concepts for Consumers in a 'Real Time' Mode," *Health and Pleasure at the Table. A Symposium held at the University of Montreal*, Montreal, Canada, May, 1994.
7. *Bundesanstalt für Arbeit. Beruf aktuell: Ausgabe 1998/99*, MEDIALOG Verlag, Mannheim, Germany, 1998.
8. *BLK für Bildungsplanung und Forschungsförderung, Studien- & Berufswahl*, BW Bildung und Wissen Verlag und Software, Nürnberg, Germany, 1998.
9. *Forschungsrahmenplan. Bundesministerium für Ernährung, Landwirtschaft und Forsten*, Referat 624, Bonn, Germany, 1998.

B. Brückner
Institute of Vegetable and Ornamental Crops
Grossbeeren/Erfurt, Germany

INTERNATIONAL DEVELOPMENT: IRELAND

The island of Ireland lies west of Britain off the northwest coast of Europe. It is compact, measuring about 220 km east to west, 480 km north to south. It lies in the north temperate zone but is warmed by the Gulf Stream. Mean 24-h temperature is 8 to 11°C, and rainfall is about 1080 mm/year.

Low (ca 1000-m maximum) mountain ranges lie behind the coastal plains, except on the central east coast where rivers draining the central limestone plain enter the Irish Sea. The Shannon River (384 km) drains the western region into the Atlantic. The climax vegetation is deciduous woodland, which covered about 12% of the land in A.D. 1600. Now about 8% is in forestry. Deep peat bogs have developed under the wet climate in some areas. Grass grows well everywhere, and the long grazing season, together with grass conservation for winter feed, is the foundation for agriculture.

The Republic of Ireland, with 3.6 million people, comprises 70,000 km^2 and achieved political independence in 1921. Northern Ireland, in the northeast of the country and part of the UK (qv), with 1.5 million people, comprises 14,000 km^2. Most of this article will deal with the Republic.

intended to support small- and medium-sized enterprises in answering technical questions and as an information source for the public.

Federal Ministry of Education and Research and the Commercial Environment. The Federal Ministry of Education and Research also funds a variety of projects within the food sector. As in the case of the Agricultural Ministry, the cooperation between university and other nonprofit research institutions and commercial R&D initiatives should be facilitated. Actual information can be obtained from *www.bmbf.de*. Searchers about actual developments within the commercial sector can be started from *www.behrs.de* or *www.fnii.ifis.org*.

MAIN FIELDS OF RESEARCH AND DEVELOPMENT

The developments in Germany are not independent of the developments in other countries. The aim of the substantial support by the European research programs is the transfer of ideas, knowledge, and techniques. Besides the traditional fields of technological and conceptional evolution, the following topics have emerged as fields of interest:

> Preservation and modification of food by new technologies like high hydrostatic pressure, high-pressure freezing, high-intensity electric field pulses, and supercritical carbon dioxide.
>
> Use of rapid and online measurements (ultrasound, NIR, NMR, electronic noses) to facilitate and document good manufacturing practice. Certification and accreditation have become increasingly necessary tools in quality concepts (quality assurance).
>
> Assessment of allergic potential of food additives, novel food, or genetically engineered products.
>
> Development of improved packaging, reduction of material. Recycling and elimination costs are extremely high in Germany and Austria compared with other countries.
>
> Identification implementation, and promotion of food items with human health promoting functions (antioxidants, bioactive substances, secondary plant components, probiotic microorganisms, fiber). The claim of specific food effects on human health is illegal in Germany.
>
> Use of sensory methodologies and other results of consumer research to implement consumer orientated product development, combining convenience, sustainable production, minimal processing, and new experiences.

BIBLIOGRAPHY

1. Statistisches Bundesamt, ed., *Datenreport*, Bundeszentrale für politische Bildung, Bonn, Germany, 1997.
2. Statistisches Bundesamt, ed., *Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten 1998*, Landwirtschaftsverlag, Münster-Hiltrup, Germany, 1999.
3. Z. Andani and H. J. H. MacFie, "Consumer Preference," in R. L. Shewfelt and B. Brückner, eds., *Fruit and Vegetable Quality: An Integrated View*, Technomic Publishing, Lancaster, Pa., 1999.
4. B. Brückner and H. Auerswald, "Instrumental Data—Consumer Acceptance," in R. L. Shewfelt and B. Brückner, eds., in *Fruit and Vegetable Quality: An Integrated View*, Technomic Publishing, Lancaster, Pa., 1999.
5. K. G. Grunert, "Food and Science. Wissenschaft im Dienst der Ernährung," *Proceedings of the Symposium 25th Anniversary "Euro Research and Development" CPC Europe, 35th Anniversary "Institute for Research and Development" CPC Deutschland*, 47–63, 1997.
6. H. R. Moskowitz, B. Krieger, and D. Cohen, "Meeting the Food Market Challenge: Creating Winning Food Concepts for Consumers in a 'Real Time' Mode," *Health and Pleasure at the Table. A Symposium held at the University of Montreal*, Montreal, Canada, May, 1994.
7. *Bundesanstalt für Arbeit. Beruf aktuell: Ausgabe 1998/99*, MEDIALOG Verlag, Mannheim, Germany, 1998.
8. *BLK für Bildungsplanung und Forschungsförderung, Studien- & Berufswahl*, BW Bildung und Wissen Verlag und Software, Nürnberg, Germany, 1998.
9. *Forschungsrahmenplan. Bundesministerium für Ernährung, Landwirtschaft und Forsten*, Referat 624, Bonn, Germany, 1998.

B. Brückner
Institute of Vegetable and Ornamental Crops
Grossbeeren/Erfurt, Germany

INTERNATIONAL DEVELOPMENT: IRELAND

The island of Ireland lies west of Britain off the northwest coast of Europe. It is compact, measuring about 220 km east to west, 480 km north to south. It lies in the north temperate zone but is warmed by the Gulf Stream. Mean 24-h temperature is 8 to 11°C, and rainfall is about 1080 mm/year.

Low (ca 1000-m maximum) mountain ranges lie behind the coastal plains, except on the central east coast where rivers draining the central limestone plain enter the Irish Sea. The Shannon River (384 km) drains the western region into the Atlantic. The climax vegetation is deciduous woodland, which covered about 12% of the land in A.D. 1600. Now about 8% is in forestry. Deep peat bogs have developed under the wet climate in some areas. Grass grows well everywhere, and the long grazing season, together with grass conservation for winter feed, is the foundation for agriculture.

The Republic of Ireland, with 3.6 million people, comprises 70,000 km^2 and achieved political independence in 1921. Northern Ireland, in the northeast of the country and part of the UK (qv), with 1.5 million people, comprises 14,000 km^2. Most of this article will deal with the Republic.

HISTORIC PERSPECTIVE

Given the large fertile land area relative to population, an abundance of temperate foodstuffs have been exported since the Viking age. Hides, wool, grain, butter, and fish were exported through the trading towns, such as Dublin, Cork, and Galway, to Britain and Europe (1).

In 1700 Ireland was similar to other western European countries; her population was about 4 million and most of them worked on the land. From the middle of the eighteenth century, the demand for agricultural products grew sharply. In a century, beef exports had quadrupled, pork increased eightfold, and the carefully supervised butter trade doubled. This supply of low-cost foods was critical in releasing British agricultural labor for factory work, thus contributing to the British industrial revolution. Ireland's population, supported by increased potato cultivation, reached 8 million by 1800, when the Act of Union joined Ireland's Parliament to that of the UK (2).

Much of this population lived in poverty as farm tenants and agricultural laborers, with most of the fertile land owned by the Anglo-Irish gentry in great estates. The Great Famine, 1845 to 1849, was a calamity in which about 1 million died and a further million emigrated (3). It arose from the devastation of the staple food, the potato, through blight. Land tenure was transformed in subsequent decades to family-owned and -operated farms, which now predominate (4).

MODERN ECONOMY

Ireland's joining of the European Union (EU) in 1973 accelerated economic development in both agriculture and industry. Economic prosperity has followed, with Ireland's gross national product (GNP) per head rising from 62% of the EU average in 1980 to 85% in 1998, with some projections suggesting equality by 2003 (5). Factors involved are external, such as trade access to the single EU market and EU structural fund transfers, and internal, such as consistent macroeconomic policy, major investment in education, and industrial strategy. The latter has involved concentration on selected industrial sectors, such as information technology, financial services, chemicals, pharmaceuticals, and food.

With economic development, primary agriculture has been a declining sector, with its share of gross domestic product (GDP) falling to 5.2% in 1998. The overall agrifood sector is still of major importance in the economy, however, accounting for nearly 13% of GDP in 1998 (6). Furthermore, the low import content of food exports means that the share of the agrifood sector in net foreign earnings is nearly 33% (ie, foreign earnings adjusted for imports of materials and repatriation of profits by foreign-owned companies). Agricultural employment has correspondingly fallen to about 9% of the total. Although the food industry is still dominated by meat and dairy products, advanced food ingredients and consumer products sectors have also emerged. The drinks sector is also very strong with global brands such as Guinness and Bailey's. The food industry is strongly export oriented with about half of all food manufacture being exported. Food industry structure has also changed rapidly, being dominated by about 10 leading companies that have expanded rapidly through merger and foreign direct investment, primarily in the UK, the EU, and the United States.

THE COOPERATIVE MOVEMENT

In 1889 Sir Horace Plunkett started the producer cooperative movement. The Co-op Societies grew rapidly in numbers and prospered, especially in the dairy and pigmeat sectors (7). Today the cooperative movement continues to flourish, with some of the larger cooperatives, particularly in dairying, being very successful international food business organizations. Since the mid-1980s a few of these larger cooperatives have adopted a rather unique cooperative–public company joint ownership structure. The linkage by the cooperatives to a public company structure has provided finance for international growth. Cooperative types involve both primary cooperatives, in which membership consists of farmer suppliers, and secondary cooperatives, in which particular functions are performed on behalf of the primary cooperatives that constitute the membership. The Irish Dairy Board, which markets dairy products internationally on behalf of primary dairy cooperatives under the brand name Kerrygold, is a good example of a successful secondary cooperative. The Irish Co-operative Organisation Society (I.C.O.S.) is the national representative and promotional agency for cooperatives in Ireland, while the Centre for Co-operative Studies in University College Cork conducts research and provides education on all aspects of cooperatives.

GOVERNMENT AND AGRIFOOD

The Department of Agriculture and Food is responsible for all aspects of supervision and development of the agrifood industry. For food, its goals include the further development of high-quality international markets and the fostering of added-value food exports rather than basic commodity trading. Related activities include the operation and implementation of EU schemes and regulations, the provision of grants for farm improvements, the operation of measures to improve livestock and horticultural production, the control and elimination of animal disease, ensuring quality control in food processing and marketing, and the formulation and operation of land policy (8).

The Department of the Marine and Natural Resources is a comparatively new government department, being first established as a Department of Fisheries, separate from Agriculture, in 1977, with subsequent functional and title changes. Ireland as an island has great scope for sea fisheries development and also is famous for freshwater fishing, both game and coarse. The Department has responsibility for all aspects of regulation, protection, and development of the marine resources. The Department is also responsible for all aspects of forest policy (8).

IMPORTANT STATE AGENCIES

Teagasc—the Agriculture and Food Development Authority—is the national body providing advisory, research, education, and training services to the agricultural and food industry. It was established in September 1988 and replaced ACOT, the agricultural advisory and training body, and An Foras Talúntais, the agriculture and food research organization. Of particular importance to Teagasc is the education and training for young farmers, research in the food industry, together with farm management, economics, marketing of agricultural products, and rural development (8). Teagasc has two major food research centers, the Dairy Products Research Center in Cork and the National Food Center in Dublin.

Bord Bia, the Irish Food Board, is the market development and promotional body established by the government to assist the Irish food and drink industry. Its role is to work in partnership with Irish companies, bringing them together with prospective buyers to build sales of Irish food and drink in export markets. It provides a range of services, including market information support, product promotion, inward buyer programs, quality assurance initiatives, and marketing enhancement programs for individual companies. It has a network of international offices, in Dusseldorf, London, Madrid, Milan, Moscow, New York, and Paris.

An Bord Glas, the Horticulture Development Board, was established in March 1990 as a statutory semistate body. The primary function of the board is to assist and encourage all aspects of horticulture so as to increase output, improve the domestic market, and thereby create employment and contribute more significantly to GNP. BIM/Irish Sea Fisheries Board is the state agency with primary responsibility for the sustainable development of the Irish seafish and aquaculture industry both at sea and ashore and the diversification of the coastal economy so as to enhance the employment, income, and welfare of people in coastal regions and their contribution to the national economy. It provides a wide range of financial, technical, educational, marketing, resource development, and ice supply services for the production sector through to the processing and marketing sectors.

UNIVERSITIES AND COLLEGES

Ireland has many universities, institutes of technology, and regional technical colleges that offer undergraduate and postgraduate food science and technology programs of relevance to the agrifood sector.

University College Cork has a faculty dedicated to food science and technology and is internationally recognized for the excellence of its education, research, and training programs. It is unique in Ireland for its commitment to food science and technology, food business, and food biotechnology. The Faculty of Food Science and Technology offers five undergraduate degree programs: B.S. in Food Business (offered jointly with the Commerce faculty), B.S. in Food Science, B.S. in Food Technology, B.S. in Nutritional Sciences, and B.E. in Food Process Engineering (offered jointly with the Engineering faculty). The faculty also offers a range of postgraduate opportunities, which include M.S. and Ph.D. by research, M.S. by a combination of research and coursework, and also higher diplomas. The Faculty occupies a dedicated Food Science and Technology Complex, including an extensive Food Processing Hall and specialized laboratory facilities where the National Food Biotechnology Center is incorporated. University College Dublin offers a B.S. in Food Science within the Faculty of Agriculture and provides both full-time and parttime taught master's programs in Food Science. In addition, graduates may be admitted for research programs leading either to master's or Ph.D. qualifications in Food Science.

The University of Limerick offers a B.S. in Food Technology associated with the Biological Sciences Department and also provides postgraduate programs. Trinity College Dublin offers undergraduate and postgraduate courses in nutrition and dietetics. The Dublin Institute of Technology offers a B.S. in Human Nutrition and Dietetics. In addition, a range of certificate and diploma courses is offered by the Institutes of Technology in Carlow, Cork, Dundalk, Letterkenny, Sligo, Tralee, and Waterford.

In Northern Ireland, the University of Ulster at Coleraine provides a B.S. in Human Nutrition, as well as postgraduate opportunities in the area. Loughry College also offers a B.S. in Food Technology, as well as postgraduate courses.

Extensive research programs in the food science and technology area are under way in most of the universities just listed. Funding comes from the European Commission, government agencies, and national and international industry.

BIBLIOGRAPHY

1. R. F. Foster, *Modern Ireland 1600–1972*, Allen Lane Penguin Press, London, United Kingdom, 1988.
2. L. M. Cullen, *The Formation of the Irish Economy*, Mercier Press, Cork, Ireland, 1969.
3. C Woodham-Smith, *The Great Hunger*, Hamish Hamilton, London, United Kingdom, 1962.
4. F. S. L. Lyons, *Ireland Since the Famine*, Collins/Fontana, London, United Kingdom, 1975.
5. *Agenda 2000, Implications for Ireland*, Institute for European Affairs, Dublin, Ireland, 1999.
6. Department of Agriculture and Food, *Annual Review and Outlook for Agriculture and the Food Industry*, Dublin, Ireland, 1998.
7. R. A. Anderson, *With Plunkett in Ireland: The Co-op Organiser's Story*, Irish Academy Press, Dublin, Ireland, 1983.
8. *I. P. A. Administration Year Book & Diary*, Institute of Public Administration, Dublin, Ireland, 1999.

MICHAEL KEANE
University College Cork
Cork, Ireland

INTERNATIONAL DEVELOPMENT: NEW ZEALAND

The characteristics of New Zealand and its economy, with special reference to food production and processing, will be discussed. The new science structures and funding are mentioned, and then some detail of food science and research providers is given.

New Zealand, with its nearest neighbor the continent of Australia, is located in the southwest Pacific Ocean. New Zealand consists of two main islands and is similar in size to Japan and to the UK. It is a diverse land, changing from subtropical in the far north to cool temperate in the south. The population of New Zealand is approximately 3.7 million (1996 census).

Seasons are opposite to the Northern Hemisphere, with January and February being the warmest months and July the coldest. The climate is temperate with averages from 8°C (46.4°F) in July to 17°C (62.6°F) in January, but summer temperatures occasionally reach the low 30s (mid 80s°F to low 90s°F) in many inland and eastern regions.

The mean average rainfall varies widely, from less than 400 mm (15.7 in.) in the lower central part of the South Island to over 12,000 mm (470 in.) in the Southern Alps—which form the backbone of the South Island. For most of the North Island and the northern South Island, the driest season is summer. However, for the west coast of the South Island and much of inland Canterbury, Otago, and Southland, winter is the driest season.

New Zealand is a food producing and exporting nation with food and beverage products comprising 48% of New Zealand's exports in 1998. Over the past 10 years there has been a decline in the earnings from unprocessed primary produce (particularly wool) and an increase in the earnings from processed products. The New Zealand economy is heavily dependent on overseas trade. Until the late 1980s New Zealand had an almost complete dependence on dairy, meat, and wool exports, but since that time increases in value of exports from forestry, fishing, horticulture, and the manufacturing industries have been very significant. Tourism has become a very important earner for the country.

The major trading partners that together account for about 70% of the country's exports are Australia, Japan, EC, the United States, Korea, and China (including Hong Kong). The EU has been the major market for food and beverage products, accounting for 28% of the value, but the patterns for different food items are very varied. North America is the primary market for beef, but Europe is the primary market for sheepmeat products. Milk powder is primarily targeted at Southeast Asia; butter and cheese at Europe, although North Asia is also a high importer of cheese; and more than half of the casein production is exported to North American markets. Fish and fish preparations markets tend to be in North Asia, although there are also significant markets in the North Americas.

Kiwifruit are the major earner in the horticultural sector, followed reasonably closely by apples and pears with vegetables third. The major markets for exported fruit are in Europe (58% of the value), whereas North Asia is more important for vegetables, accounting for some 35% of the export value. Australia is the largest market for fruit and vegetable preparations.

The value of totally processed primary produce is almost three times that of the unprocessed primary produce. Since the major contributors to the unprocessed primary produce are wood pulp and wool, it is obvious that most food products are in the processed primary products category.

NEW ZEALAND SCIENCE STRUCTURES

The government science agencies were completely reorganized in 1992. The activities of the Department of Scientific and Industrial Research (DSIR), the Ministry of Agriculture and Fisheries (MAF), the Forest Research Institute (FRI), and other government research organizations were reorganized into new business enterprises called Crown Research Institutes (CRIs). The CRI structure was established to provide an open and flexible framework for the management of science with the expectation that it would create better collaboration between the public and private sectors in areas of research and development (R&D) and technology transfer. Each institute is based around a productive sector of the economy or a grouping of natural resources and in general provides a vertical integration from production through to market.

The CRIs are as follows:

New Zealand Pastoral Agriculture Research Institute Ltd. (AgResearch)
The New Zealand Institute for Crop and Food Research Ltd. (Crop & Food)
Horticulture and Food Research Institute of NZ (HortResearch).
Industrial Research Ltd. (IRL)
Institute of Environmental Science & Research Limited (ESR)
Forest Research (FRI)
Institute of Geological & Nuclear Sciences Ltd. (IGNS)
Manaaki Whenua–Landcare Research (Landcare)
National Institute of Water & Atmospheric Research (NIWA)

The first three on the list have a significant focus on food; the fourth, IRL, contributes significantly to processing of biological materials and in equipment development for the food industry and the others can have some involvement providing specific skills for food projects.

RESEARCH FUNDING

The New Zealand government, via the Foundation for Research Science and Technology, makes available just under $NZ300 million for *public good science* each year Research organizations bid for these funds, which are distributed across 17 output areas that embrace everything from animal industries (production and processing) to research on Antarctica. There is no specific output for food and food science and technology; instead, it is an integral part of many of the outputs, especially those focused on animal industries, dairy industries, horticulture, arable and other food and beverage industries, manufacturing, and industrial technologies. Funding for these output classes collectively account for about half of the public good science funding. Since the funding is focused on the production aspects of the raw material as well as the food and other products related to the sector, it is difficult to get a measure of the government funding for food science and technology programs. Government funding provides the most obvious research funding source, but individual sector businesses and industries also fund universities and research institutes for R&D projects to adapt generic science or to undertake specific development projects. The dairy and meat industries support, to some degree, dedicated research institutes, but there is no overall food research institute.

FOOD SCIENCE

New Zealand has a number of significant providers of food science research: New Zealand Dairy Research Institute, MIRINZ Food Technology and Research Ltd., three of the CRIs (AgResearch, HortResearch, and Crop & Food), and two of the universities (Massey University and Otago University). Other CRIs and two other universities play some role in food science research.

New Zealand Dairy Research Institute

The New Zealand Dairy Research Institute (NZDRI) is the key provider of strategic product and process research and development to New Zealand's cooperative dairy industry. NZDRI has close links with AgResearch and the Dairy Research Corporation (a joint venture between the NZ Dairy Board and AgResearch) for production research and with Massey University for production, processing, and product research. Since R&D is integrated with marketing and manufacturing strategies, NZDRI works closely with the New Zealand Dairy Board's development laboratories around the world and with individual companies.

Research carried out by NZDRI primarily focuses on new milk products and processes for the export markets, with emphasis on recombined products and longer-life consumer products rather than fresh liquid milk products. Fundamental studies on the properties of milk components and how these can be influenced by production and manipulated during processing feeds research to provide products with specific attributes for defined-ingredient market niches. The research draws on specialized knowledge on separation and evaporation as well as basic protein and fat chemistry. The research on milk proteins has led to a wide range of milk powder products designed for specific bakery applications as well as some for meat products. The research on milkfats and how they can be manipulated has led to the development of butters that are spreadable at refrigeration temperatures. Considerable effort is also directed at cheese production so that the New Zealand industry can meet the demand for continuous supplies of high-quality cheese products with consistent flavor, the development of which is a slow, complex biological process. The two concerns are loss of starter strains (and of product) due to bacteriophage infection, and achievement of the correct balance of microflora for the development of required flavors. NZDRI aims to provide the strategic science to develop starter strains with improved resistance to bacteriophage infection. Knowledge of the physiological and enzymological systems of lactic acid bacteria that contribute to critical flavor compound development from the breakdown of protein and fat will provide the critical science necessary to predict and control flavor development in cheese.

Flavor is another critical component of milk-based products, and the research program aims to enable the industry to provide products with consistent and desired flavor attributes. The investigations focus on the relationships between the sensory characteristics of dairy products, and the concentrations of critical flavor compounds that differentiate the flavor of New Zealand products from that of competitors. Sensory characteristics are being defined using both consumer acceptability and descriptive analysis techniques and related to the concentrations of critical compounds accurately measured using stable isotope dilution assays. Concentrations of critical compounds in products will be related to processing procedures in the factory and to seasonal, dietary, and breed factors affecting milk characteristics on the farm. This information will be used to develop integrated flavor management strategies to meet market requirements for specific dairy flavors.

Much of NZDRI's R&D is confidential, providing products for specific end uses meeting specific applications requirements, but information can be obtained on research undertaken with public good science funding.

MIRINZ Food Technology and Research

Formerly known around the world as the Meat Industry Research Institute of New Zealand (Inc), (MIRINZ), institute has been regarded as the independent central R&D agency servicing the New Zealand meat processing industry. Established in 1955 to study all aspects of meat processing from farm gate to the consumer, the organization has been the primary driver for many of the technological innovations in the world meat industry. The organization was recently restructured with AgResearch taking a 30% shareholding in the new MIRINZ Food Technology and Research Limited while the other 70% remains with MIRINZ (Inc). At the same time the scope of activities was broadened so that MIRINZ Food Technology and Research can apply its skills to other foods.

MIRINZ has been at the forefront of research into meat science, methods, and systems to ensure eating quality; humane electrical stunning systems for sheep, cattle, and deer; packaging developments to maintain product safety over longer periods; and predictive microbiology techniques to alert processors to potential risks. Research continues in these areas to reduce costs, reduce variability, and integrate processes and procedures into pasture-to-plate management systems.

Modeling of biological processes responsible for the conversion of muscle to meat continues to play an important role in the improvement of methods to maintain quality of meat. As a consequence of the predictive models, new chilling and freezing processes have been developed to reduce energy wastage while improving potential product qualities. Mechanization of the dressing and boning processes have been significant areas of endeavor and continue to be important with machines and ergonomic tools being developed that simplify and improve processing of high value products.

Product development, especially of products with nutritional and health benefits, is a crucial area of research to increase the profitability of the industry. The minimization and treatment of wastes from animal and meat processing remains an important area of R&D, since the protection of the environment is crucial to maintenance of a sustainable industry.

MIRINZ Food Technology and Research provides a wide range of research and development activities—with its skills in meat science, physiology, biochemistry, microbiology, measurement technologies, electronics, environmental science, and engineering—to address the needs of the meat industry and, increasingly, the broader food industry (MIRINZ was absorbed into AgResearch in August 1999). The drive is to move the meat industry from being a commodity supplier to a supplier of specific tailored products.

New Zealand Pastoral Agriculture Research Institute Ltd. (AgResearch)

AgResearch rightly sees itself as providing "the bridge between the food, fiber and biotechnology products of New Zealand's pastoral industries and the consumer trends of the 21st century." Although largely focused on production there are three streams of food-related research at AgResearch. First, there is the research aimed at new foods or improving product quality; second, that aimed at allaying customer fears on food safety; and, finally, that aimed at ensuring food is grown in a healthy environment. Research in the first category includes attempts to tailor meat-based products to market specification; alter milkfat composition and casein production; enhance milk flavor; and provide velvet antler products with proven nutritional and health properties. Much of the product research is conducted by association with other groups such as NZDRI, MIRINZ, and Massey University so that the production-modified products can be processed to utilize the improved characteristics. Research to allay consumer fears of food safety has focused on animal disease control, residue elimination or reduction, and detection of natural toxins. The assurance that food is grown in a healthy environment is supported by the programs on animal welfare and development of sustainable production systems from soil management to the final product.

AgResearch has recently established the Agresearch Centre for Pastoral Food Research in Palmerston North as a joint venture with Massey University to develop innovative and differentiated food products with added value derived from forage and forage-fed animals. AgResearch has research capabilities in physiology, genetics, reproduction, nutrition, and management of grazed livestock, biotechnology, and environmental impacts on agriculture.

The New Zealand Institute for Crop and Food Research Ltd. (Crop & Food)

Crop & Food undertakes vertically integrated research and technology development programs to support the development of commercially successful products from the cereal, vegetable, flower, forestry, and seafood industries. For the vegetable and cereal products, there is a strong focus on safe and environmentally sustainable production, but there is also a link with the companies processing the products. Increasingly, Crop & Food supplies information on ways to grow, process, and use foods and their nutritional value for a better lifestyle. One area of nutritional research where Crop & Food has been involved is the relationship between dietary lipids and cardiovascular disease. Animal models are being developed that enable appropriate end points reflecting thrombosis and atherosclerosis to be developed.

Crop & Food is the primary provider of seafood product research. The group has considerable expertise in preservation of the quality of fish from catching to the marketplace, both as "live" fish and as processed product. Crop & Food seafood focus is development of technologies that enhance the consistency of product and product safety, thereby increasing its market value.

Crop & Food has recently established the Food Industry Science Centre (FISC) to link its activities with those of researchers at Massey University, NZDRI, and food companies operating in the North Island with the aim of developing a center of excellence for food, nutrition, and health. Research projects include increasing the storage life of vegetables, expanding the use of maize and oats in food products and the use of vegetables and fruits to promote healthy eating, as well as exploring consumer perceptions of food. The skills of the Institute's staff include expertise in plant breeding and biotechnology, food technology, nutrition, biochemistry, postharvest technology, agronomy, plant physiology, and pest and disease control.

Horticulture and Food Research Institute of NZ (HortResearch)

HortResearch is effectively the primary research arm of the horticulture industry working in partnership with horticulture and food operations. HortResearch is involved in research to assist growers of fruit, some vegetable crops, and flowers in developing new varieties, new environmentally friendly methods of crop production and protection, as well as novel harvesting and processing technologies underpinned by fundamental research. HortResearch has relaunched its food science capabilities under a Food + Innovation banner, to create food research solutions for business built on three key strengths: sensory and consumer science, food technology, and industry foresight with a strong commercial focus.

HortResearch provides research, development, and technology to help bring new and improved products and processes to the marketplace. New varieties of kiwifruit and apples tailored to meet the consumer requirements have resulted from HortResearch's efforts. Noninvasive measurement methods are being developed by HortResearch to enable consistent quality product to be delivered to the marketplace. Changing consumer trends toward convenience and "healthier" foods means that demand for ready-to-eat or use foods is increasing. Such items as peeled apples, sliced rock melon, and kiwifruit and prepared raw vegetables that stay fresh in the refrigerator for up to six months are the possibilities being contemplated by the latest research. Enzyme-peeled fruit technology does away with labor-intensive hand-peeling methods. HortResearch has already developed safe, high-quality clarified juice concentrates, with extended shelf life, that have the flavor and appearance of fresh juices. HortResearch pioneered the technology behind kiwifruit wine, clarified kiwifruit juices and concentrates, fruit nectars, and tamarillo juice products. It has also been involved with enzyme peeling of fruit, heat pump technology, and improved processing of kiwifruit. Processed kiwifruit is naturally tangy but no longer irritates the throat, thanks to new processing methods.

New preservation methods are being developed that reduce the use of artificial preservatives. The use of heat pump drying means manufacturers can reduce the use of preservatives. An example is a new vanilla drying process that improved product quality, increased vanilla content by up to four times, and reduced processing time from three months to a matter of weeks.

HortResearch's interests encompass forestry, environment, forage, animal & biomedical products, pipfruit, summerfruit, viticulture and ornamentals, kiwifruit, berryfruit, citrus, and exotic products as well as the food manufactured from them. Its skill base spans molecular biology, plant and tree breeding, crop production, food processing, fruit storage and transport, and the evaluation of consumer preferences. HortResearch also has a strong technology development unit supplying engineering solutions to assist the biological science developments.

Industrial Research Limited

Industrial Research Limited (IRL), although primarily involved with the manufacturing industries, services natural products processing industries and therefore has a significant role in food research in New Zealand. IRL is heavily involved in process equipment development and biotechnological processing. Key science disciplines are biochemistry, chemical and biological engineering, physics, organic and inorganic chemistry, mathematics, electrical and electronic engineering, mechanical engineering, and information science and technology.

New Zealand Universities

Two of New Zealand's universities have significant programs with a food focus and two others have small food science interests.

Massey University. At Massey University, food science, technology, and engineering can be part of undergraduate degrees in Applied Science, Science, Technology, and Veterinary Science and part of postgraduate degrees and diplomas. Although research into food is primarily conducted within the Institute for Food, Nutrition and Human Health (IFNHH), which is one of eight institutes within the College of Science, there is significant food product and food process research undertaken by other institutes within the College of Science. The IFNHH's scope includes food engineering, food science, food technology, agribusiness, and human and animal nutrition, as well as physiology and biochemistry, as they all relate to lifestyle science. Food science is an integral part of the degree in Food Technology, focusing on the application of sciences and engineering to food processing and food presentation. The emphasis is technological, with a major input in areas of food engineering, biochemistry, biophysics, food microbiology, product and process development, and quality assurance.

Research areas are as varied as the types of food and processes in the industry. Dairy technology research includes studies on casein, cheese manufacture, ultrafiltration and membrane processing, dairy fat characteristics, and uses of whey proteins. Separation technologies are key to many new food product developments. Food rheology is important research not only in terms of dairy products but also in terms of other products and the linkage of rheological characteristics with sensory attributes. Product and process development research involves the total process from product idea generation to product launching using a systematic method for product development, including linear programming, experimental designs, multidimensional scaling, and analysis and profile testing.

Research into food processing and packaging includes wine and fruit juice processing as well as investigation of the changes that occur during processing and storage. Research programs in food microbiology, nonenzymatic browning, and nutritional composition are also undertaken. There is also an increasing emphasis on nutrition and its role in sports and general health.

Within the IFNHH are two research centers with special roles in food research, the Milk and Health Research Centre and the Food Technology Research Centre. The former is a joint venture with the New Zealand Dairy Research Institute and the New Zealand Dairy Board undertaking research into the health-promoting properties of dairy products. The Food Technology Research Centre is a commercial center funded by grants and private research. Its research areas are new cereal and horticultural products, texture, shelf-life extension, consumer research, and product management.

Although there is food-related research undertaken by groups other than the IFNHH, generally it is undertaken by multidisciplinary teams. Examples are research on refrigeration processes from both an energetic efficiency view as well as from a product quality viewpoint and research on packaging technology, which encompasses the technology of the package and its component materials and performance and work on performance of packaging in relation to the product. The research on impacts of processing on quality will use food science and technology expertise often from the IFNHH. The multidisciplinary teams provide a strong resource to tackle almost any food processing problem: functional foods, supply chain management, food safety, food preservation, food formulation, consumer science, and sensory evaluation.

Otago University. At Otago University, a degree in food science is undertaken in the Faculty of Applied and Consumer Sciences. Food science is studied in relation to the chemistry of food, food processing sciences, food rheology, market acceptance, and sensory evaluation. Research is carried out on horticultural crops, especially concerning the composition, performance, and postharvest changes. Flavor research focuses on the impact of processing on flavor precursors. Some research has been conducted on venison. Some of the flavor studies are undertaken in a Plant Extracts Unit, which is a joint venture with Crop & Food. This group examines extracts from a wide range of natural products for uses as flavorings and as health products.

Otago University has two commercial research centers. The Food Product Development Research Centre undertakes development work of both a problem-solving and strategic nature. The center offers a complete service for the development and refinement of new and existing food products. It undertakes comprehensive applied research programs to improve the commercial profitability of food products and assists with in-house food product development programs.

The Sensory Science Research Centre undertakes fundamental and applied research on sensory perceptions and preferences. It investigates the senses that are important in the perception of consumer products such as foods and personal care products and provides training at both undergraduate and postgraduate levels; it also runs short courses on sensory science for industry.

Otago University has a strong nutritional science program that relates the chemistry department with health sciences in examining not only the food but also its impacts on human health and well-being.

Some food research is undertaken at Lincoln University through the Animal and Food Sciences Division and at Auckland University through the Chemistry Department.

FUTURE DIRECTIONS

The future role of food science and technology in New Zealand remains to deliver products that compete in terms of quality, service, and innovation against all others in the world market. Sustainable safe production supported by innovative processing to give long-life natural products that are free from residues and toxins but with characteristics demanded by the high-value customers and consumers is the key focus of the New Zealand food industry and its researchers. Because of the distance from markets, new and improved preservation methods will continually be sought so that New Zealand can satisfy the growing emphasis for convenience and health products. Innovative food products will be required to satisfy the potential demand for home meal replacements as well as the functional food interests.

Biotechnology will have an increasing influence on the direction of the New Zealand food industry leading to new products and new processes. Not only will researchers need to understand the molecular biological means of manipulating foods, they will also need to prove to a skeptical consumer that products are safe.

FURTHER INFORMATION

Further information on the food science and technology research providers and access to current information is available on the Internet from the following Web addresses or the E-mail address for NZDRI:

New Zealand Pastoral Agriculture Research Institute Ltd.: *http://www.agresearch.cri.nz*

The New Zealand Institute for Crop and Food Research Ltd.: *http://www.crop.cri.nz*

Horticulture and Food Research Institute of NZ Ltd.: *http://www.hort.cri.nz*

Industrial Research Ltd.: *http://www.irl.cri.nz*

New Zealand Dairy Research Institute: *Anne.Podd@nzdri.org.nz*

Massey University: *http://www.massey.ac.nz*

Otago University: *http://www.otago.ac.nz*

Lincoln University: *http://www.lincoln.ac.nz/ansc*

B. B. CHRYSTALL
Massey University–Albany
Auckland, New Zealand

INTERNATIONAL DEVELOPMENT: SOUTH AFRICA

The economy of South Africa has the distinct features of both a First World and a Third World sector. Since 1994 the annual growth rate has fallen short of the annual population increase; the population increased from 29 million in 1988 to 41 million in 1998. The result has been increasing unemployment, which has been aggravated by deregulation and globalization of the economy since 1994. From a food supply point of view South Africa nevertheless remains self-sufficient and a net exporter of agricultural produce.

The agricultural achievements may be regarded as remarkable, because the country is not endowed with high rainfall or good arable land. Annual precipitation is 465 mm, compared with the world average of 857 mm. Only 12% of the 86 million ha utilized by agriculture is arable, the vast majority being rangeland used for livestock production. Climatic regions vary from semidesert, with only 125 mm rainfall annually, on the Atlantic Coast to more than 1000 mm annually on the eastern escarpment. About 86% of the area receives almost exclusively summer rains, the exception being the southwestern Cape area, which has a Mediterranean climate and winter rains. The vegetation can be divided into five biomes: desert and semidesert, Mediterranean plants and shrubs, forests, savanna, and open grasslands.

The principal cropping and mixed farming regions are the 1500-m plateau of the Mpumalanga, Gauteng, and Free State area, as well as the northern and midlands of KwaZulu-Natal and the southwestern Cape. The summer rainfall regions constitute mixed farming, with production of corn, sorghum, groundnuts, and potatoes together with dairying and sheep, cow, pig, and poultry production. Sheep farming includes both wool and mutton production, although the major wool and also mohair production is found to the south in the vast expanse of the arid and semiarid Karoo and adjacent regions. The southwestern Cape, with its winter rainfall, is the primary region for the production of winter cereals, deciduous fruit, and wine. Since 1995, wine and grape production for the export market has increased dramatically, the bulk of the increase being produced in the Orange River irrigation scheme. The subtropical low-lying savanna area of Mpumalanga bordering the escarpment is an important region for citrus, subtropical fruit, and vegetables. These commodities also extend toward the frost-free coastal belt of KwaZulu-Natal and the eastern Cape, with additional crops being sugar and pineapples. The far northern and northwestern savanna, the open grasslands, and the area bordering the Kalahari Desert north of the Orange River are major beef producing areas.

AGRICULTURAL PRODUCTION AND EXPORT

The relative importance of agriculture is illustrated by the sectoral analysis of the gross domestic product (Table 1). Agriculture has declined since 1970 but has become more stable since 1990. Real growth rates in agricultural produce have increased by 1.7% per annum since 1960, with the respective sectors being livestock, 1.9%; field crops, 1.0%; and horticulture, 2.9%. In terms of gross value, animal products constitute 42%, field crops 33%, and horticultural products 25% of agricultural produce. As an export commodity, agricultural produce increased from 3.76 billion rand in 1988 to 11.983 billion rand in 1996, which represents a share of the total export of 7.6% and 9.5%, respectively. Animal products constitute a declining share of agricultural produce export, from 34% in 1988 to 11.4% in 1996. In contrast, field crops and horticulture, the major contribution being the latter, increased from 66% in 1988 to 89% in 1996.

Field and Horticultural Crops

Field crops are mostly produced without irrigation. Quantitatively, corn and wheat are the major crops, but significant quantities of sunflower seed, grain sorghum, groundnuts, and barley are also produced. South African corn is recognized worldwide for its quality and stringent control tests. Cultivars are established for yield under dryer conditions, disease resistance, persistence under poor climatic conditions, and lysine and methionine content. In the case of wheat, breeding lines are screened for baking qualities. Specific efforts to eliminate the Russian aphid have been successful. In total, 10 to 15 million t of field crops are produced annually.

The horticultural crop consists of some 30 types of deciduous and subtropical fruit, together with citrus and a number of vegetable species. Grapes are the most important fruit crop, with more than 80% finding their way into wine production. Wine is largely exported. Significant quantities of oranges, apples, pears, peaches, bananas, and other subtropical fruits are also exported. Fruit accounts for about 60% of the gross value of all horticultural products, vegetables and potatoes some 30%, and flowers, tea, and other products the remainder.

Animal Products

The gross value of white meat in 1997 was 5.651 billion rand compared with 4.907 billion rand for red meat and 4.699 billion rand for corn, the most important field crop. Due to the intensive nature and relative efficiency of poultry (white) meat production, the industry has made rapid strides in capturing a substantial share of the meat market. This share is further boosted by meat demand, because South Africa is a net importer of meat. The percentage composition of the different animal products produced is shown in Table 2.

A salient feature in Table 2 is the proportional decline in the market of red meat and fiber (mainly wool) production. Although South Africa still produces of the best-quality fine wool and mohair in the world, the decline in market share, as well as export, is noteworthy.

By far the majority of dairy produce is fresh milk, followed by ice cream, condensed milk, cheese, butter, and fermented products such as yogurt and fruit-based drinks. Dairy produce has lost share of the market (Table 2) since 1980, whereas egg production and ostrich products have shown a steady increase.

Food Consumption Patterns

The per capita consumption of food commodities comprises about 120 kg grain, 65 kg vegetables, 55 kg dairy produce, 35 kg sugar, and 30 kg red meat. Because of price, 30% of the food budget is spent on meat, 20% on bread and grain products, 18% on vegetables and fruit, 9% on dairy products, and the remainder on miscellaneous commodities including sugar, preserved products, and jam. The demographic character of South Africa has a major influence on food consumption patterns, as does urbanization. These

Table 1. Gross Domestic Product

Year	Agriculture (%)	Mining (%)	Manufacturing (%)	Commerce (%)	Other (%)	GDT[a] $(\times R10^6)$
1970	8.1	10.0	23.3	15.1	44	12,037
1980	7.0	22.0	21.6	11.6	38	58,056
1990	5.0	9.2	24.0	21.5	40	247,315
1997	5.2	8.0	23.2	21.8	42	268,142

[a] Values in nominal terms (average inflation 10%); R, rand, GDT, gross domestic total.

factors present exciting, innovative challenges to the food manufacturers.

MANUFACTURED FOOD PRODUCTS

The food and beverage industry is one of the largest industries in the country—in fact, the major manufacturing industry—and a major employment multiplier. The employment multiplier indicates the number of jobs generated in the economy for each new job generated in a specific sector. Table 3 shows the multiplier effect in the animal product and processing sectors. Generally, the multiplier for the primary livestock sectors is small, whereas the processing sectors have the highest multipliers. This indicates that the driving force behind employment creation in the livestock industry, as in the other agricultural sectors, lies within the processing sectors and not within the primary production sectors.

Foods and beverages are produced in fruit and vegetable factories, grain mills, bakeries, breakfast cereal plants, dairy factories, meat and fish plants, sweet and cold drink producers, food oil companies, and many others. The total production of manufactured food products in 1995 exceeded 40 million t, with the proportional contribution being about 81% grain and bakery products, 5.7% sugar and candy, 3.3% fat and oils, 0.6% fish products, 2.0% fruit and vegetable products, and 0.9% meat, dairy, coffee, and tea products (Table 4). The industry is modern, well organized, and growing to comply with the needs and demands of diverse local consumers as well as with those of countries that import manufactured foods and beverages.

Grain Products

The milling industry in South Africa processed 2.5 million t of wheat in 1995. Brown and whole wheat bread production amounted to 575,000 t and white bread 622,000 t. A unique feature of the grain products industry is the fact that corn products produced from white corn make up such a high proportion. This is because corn porridge is a popular food among the majority of the population. Yellow corn is primarily used in the animal feed industry, as is grain sorghum, although a substantial portion is also utilized to brew traditional sorghum beer.

Table 2. Gross Value of Animal Products Produced, %

Product	1980	1990	1997
Meat			
Red	46.0	39.9	29.0
White	15.5	22.3	33.4
Dairy products	18.7	16.6	16.6
Eggs	6.5	8.1	12.2
Fiber	11.9	9.8	4.1
Ostriches	0.5	0.9	1.8
Other	0.9	2.4	2.9

Table 3. Employment Multipliers of the Animal Industry for the Total Workforce, [illegible]

Sector	Multiplier
Meat products	11.7
Animal feeds	10.0
Dairy products	8.24
Meat animals	1.92
Milk	1.51
Poultry	1.48
Animal fiber	−1.17

Table 4. Manufactured Foods and Beverages, 1995

Product	Tons (thousands)	Percentage of total
Canned and prepared meats	121	0.3
Dairy products	206	0.5
Canned fruit and vegetables	804	2.0
Fish products and similar foods	234	0.6
Vegetable and animal oils and fats[a]	1,343	3.3
Grain mill products[a]	6,117	15
Bakery products	26,807	66
Sugar	2,183	5.4
Chocolates, sugar confectionery, and cocoa	136	0.3
Roasted peanuts and other nuts	351	0.9
Coffee roasting, tea blending, and packing	50	0.1
Other food products	113	0.3
Balanced animal feeds	4,261	
Alcoholic and nonalcoholic beverages	2,256 kiloliters	5.5

[a] Include by-products used in animal feeds.

Sugar Products

In 1995, 2.2 million t of sugar was produced, of which 60% was refined, 3.5% was golden brown, and 13.5% was exported as raw sugar. In the same year chocolates, sugar confectionery, and cocoa amounted to 136,000 t (Table 4).

Oils, Fats, and Fish Products

The production of oils and fats in 1995 amounted to 1.34 million t (Table 4), of which 10%was manufactured into margarine and 20% into edible oils and fats. Fish products for human consumption equaled 234,000 t, of which 25% were canned fish, 28% frozen fish, and 2.5% frozen crayfish. In contrast to many countries, the per capita consumption of fish compared with that of red and white meat is low (<15%).

Fruit and Vegetable Products

The total production of fruit and vegetable products in 1995 was 804,000 t (Table 4). Of this, jams amounted to 30,700 t, canned fruit and fruit salad 183,785 t, fruit juices and drinks 418,400 kL, and canned and frozen vegetables 150,185 t. The industry is expanding rapidly, with a growth rate of 5% per year.

Dairy and Meat Products

The combined production of manufactured products in 1995 amounted to 327,000 t (Table 4). Dairy products consisted of 9.5% butter, 24% cheeses, 25% condensed and powdered milk, and 36% ice cream and related products. Centralization in the dairy industry resulted in the virtual control of the market by four companies. Meat products consisted of 8% bacon, 4.5% ham, 8% patties, 29% polony, and 28% sausage types. As with the dairy industry, the largest proportion of the manufactured meat industry is controlled by three companies. From a consumption point of view, South Africans eat much more fresh meat than meat products.

QUALITY CONTROL AND GRADING

The Department of Agriculture is responsible for compiling specifications for the sorting and classification of most of the processed and unprocessed agricultural products. In the metropolitan areas, vegetables and fruit must comply with certain packaging, grading, and marketing conditions. Local and export regulations apply for canned vegetables and fruit, dehydrated vegetables, dried fruit, and frozen vegetables and fruit. Red meat carcasses are graded according to fatness, lean yield, and meat tenderness. Grading is compulsory for all carcasses auctioned in urban areas. The grading and classification of eggs in specific weight and quality groups apply to eggs for local consumption as well as for export. Poultry carcasses are graded in four classes according to their appearance, fleshing, market readiness, and moisture content in the case of frozen carcasses.

A parastate institution, the South African Bureau of Standards (SABS), operates an extensive testing service for a range of food products with regard to compulsory specifications for canned meat, frozen rock lobster, canned fish, other fish, mollusks, and frozen prawns and crab. It also voluntarily tests for certain canned meat products, carbonated soft drinks, grain sorghum beer, frozen fish, and edible gelatin. The SABS mark of approval guarantees the quality of the product, which is to the advantage of both the manufacturer and the consumer.

AGRICULTURE AND FOOD SCIENCE AND TECHNOLOGY RESEARCH

Research and development needs in a country with both First and Third World sectors are diversified and an enormous challenge. Research activities have to comply with the needs of both the resource poor rural communities, as well as the standards set by countries importing primary and secondary food products from South Africa.

Several universities, government institutions, semistate organizations, and private sector companies are involved in research and technology development in the agriculture and food and beverage industries. In agricultural research the Agricultural Research Council (ARC) of South Africa, a parastate organization, contributes about 65% of the agricultural research capacity, the remainder being from university departments and government stations in the provinces. The ARC, with 13 institutes appropriately situated across the country, caters to the needs of all agricultural commodity research, including in some instances food product and processing technology development. Other institutions with food and beverage research capacities include the Division of Food Science and Technology (Foodtek) of the Council for Scientific and Industrial Research (CSIR) and a number of food science and technology departments at universities and technicons. In addition, many multinational companies in the food and beverage industry operating in South Africa, as well as local meat, dairy, fruit, vegetable, bakery, and beverage manufacturers, have sufficient infrastructure to support product-oriented research, development, and quality control programs. These companies and the government-supported institutions employ food scientists and technologists with both generic and specific training.

To effectively operate as scientists and specialists, they found common ground in the South African Association for Food Science and Technology (SAAFoST). Different scientific societies also promote their sciences and professions and publish research results in disciplinary journals such as the *South African Food Review* and *Food Industries of South Africa*, to name only two of the major ones.

It would be impossible in a contribution of this scope to give an overview of research programs or achievements. Suffice it to mention that new cultivars of fruit with consumer-specific attributes and longer shelf life are introduced to the export market virtually every year, that the potentially problematic product cheese whey is reutilized to the extent of 70% in various dairy products, that South Africa is a world leader in irradiated food technology and nutritive enrichment, that an internationally recognized breakthrough was made with the isolation of fungi and mycotoxin on cereal grains and oilseeds, that a guava puree treated with pectolytic enzymes to remove stone cells has captured the world market, that South African wines compete successfully on the international market with leading wine producing countries, that a controlled-atmosphere packaging technique was perfected to facilitate marketing of deciduous fruit throughout the year, that tender lean beef with less than 15% fat is high in demand with tourists, and that various molecules and substances isolated from indigenous and other plants find their way into international pharmaceutical products. These examples show that the food and beverage industry and its research support are on par with those of the developed world.

GENERAL REFERENCES

D. J. Bosman, "The South African Livestock Industry," *Fundepec International Meeting*, Brazil, August 20–21, 1998

Manufacturing: Products Manufactured (Food, Beverages and Tobacco Products), Statistical Release p 3051 1, Central Statistical Service, Pretoria, South Africa, 1996.

M. R. Mokoena, *Impact Assessment of Livestock Production Research in South Africa*, ARC-Animal Improvement Institute, Irene, South Africa, 1998.

R. T. Naudé, "International Development. South Africa," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, 1st ed., 1988, John Wiley & Sons, New York, 1992.

H. H. Meissner
ARC—Animal Nutrition and Animal Products Institute
Irene, South Africa

INTERNATIONAL DEVELOPMENT: SPAIN

AGRICULTURE AND FISHERIES

Agriculture

Spain, with an area of 505,990 km^2 and a population of 39.8 million in 1999, is one of five large countries of the European Union (EU) Geographic and climatic differences between regions of continental Spain, with mean annual rainfall ranging from 200 to 1600 mm and annual mean temperatures ranging from 9 to 18°C, are responsible for a great variety in the agricultural production. The particular characteristics of the Balearic and, especially, the Canary Islands contribute to the diversity of Spanish agricultural systems.

Vegetables, with a production of 10.62 million t, citrus fruits, with 4.89 million t, and noncitrus fruits, with 3.08 million t, are among the most characteristic agricultural Spanish productions. Annual grape production raised to 3.35 million t, out of which 12% was table grapes. Olive production in 1996 reached 4.47 million t, table olives representing approximately 4% of total production. The production of grain cereals and pulses was 11.57 and 0.19 million t, respectively, and that of sugar beet and potatoes 7.63 and 4.18 million t, respectively (1).

Meat produced in Spain raised to 4.13 million t in 1996, with pork (56.1%), poultry (21.3%), and beef (13.7%) standing out as the major productions. Cows' milk production, mainly in northern Spain, was 5.56 million t, and significant amounts of ewes' milk and goats' milk (0.23 and 0.30 million t, respectively) were also produced, mainly in central and southern Spain. Egg production reached 0.75 million t per year (1).

The importance of agriculture and food industry for the Spanish economy is shown by the fact that agricultural exports contributed to 15.3% of total exported goods in 1997, for a value of 14,034 million euros (ME), out of which 50.5% were products from the food industry. The value of final agricultural production was 26,772 ME, and that of agricultural imports 11,704 ME. Other countries forming part of the EU constitute the major market (67.5% of the total value) for Spanish agricultural exports, and Spain, on its turn, receives 66.7% of total agricultural and food imports from its EU partners (1,2).

Population active in agriculture was 1.254 million in 1997, which added to 0.437 million active in the food industry sector accounted for 10.5% of total active population.

Fisheries

Fisheries and aquaculture are economic activities of a major importance for Spain, the second country in the world in per capita consumption of fish (more than 35 kg per year). Catches and production of fish and seafood raised to a total amount of 1.21 million t in 1995 (Secretaría de Pesca, unpublished data, 1999). Catches of fish species of marine origin accounted for 75.9% of the total tonnage and were marketed as fresh, frozen, or salted fish (77, 22, and 1%, respectively), with commercial values of 1203 ME, 230 ME, and 20 ME, respectively. Catches of crustaceans represented 2.1% of the total tonnage, with a commercial value of 191 ME, whereas catches of mollusks accounted for 10.9% of the total tonnage, with a commercial value of 218 ME per year.

Fish farming has increased exponentially during the present decade, both in marine and continental waters, to a production with an estimated value of 210 ME per year. Marine aquaculture yielded 0.11 million t in 1995, equivalent to 9.2% of the total tonnage of fish and seafood (Secretaría de Pesca, unpublished data, 1999). Mussels are the product with the highest tonnage. However, fish species such as sea bass, sole, bream, and turbot and seafood species such as oyster, clam, cockle, and prawn are gaining importance. Continental aquaculture produced 0.02 million t in 1995, equivalent to 1.8% of the total tonnage, with trout, tench, and eel as the main freshwater species.

THE FOOD INDUSTRY

The food industry is the most important manufacturing sector in Spain in terms of value of the final production (20% of total industrial production), contributing to 15% of added value and employing 17% of the workforce (3). The Spanish food sector ranks fifth within the EU, accounting for 9.9% of the total value. Total sales of the food industry in 1996 raised to 49,555 ME and total consumption of raw materials to 29,415 ME (2).

The high number of companies (37,857) and of manufacturing plants (42,264) is a characteristic of the Spanish food industry. The reduced volume of production of many small to medium companies results in 40% of the market being shared by a few large companies. Companies of a large size are especially found in the beer and sugar sectors. Bakery (39.9%), meat products (11.3%), and winery (9.6%) are the food industry sectors with the highest number of companies. Because of the considerable differences between company size of the various sectors, the highest total sales corresponded to meat products (19.8%), dairy products (10.8%), oils and fats (9.9%), and bakery (8.4%). Sales of the main Spanish food industry sectors in 1996 are shown in Table 1.

Andalusia (17.6%), Catalonia (13.9%), and Valencia (10.3%) are the Spanish autonomous regions (Comunidades Autónomas) with the highest number of food industry companies (2). Autonomous regions with the highest sales were Catalonia, Andalusia, Castilla-León, Madrid, and Valencia, with 22.0, 18.1, 9.1, 8.0, and 6.7%, respectively, of total sales of the Spanish food industry.

Sixty-four associations of different sectors of the food industry are grouped under the Spanish Federation of Food and Beverages Industries (FIAB). The FIAB is the representative organization of the food industry in committees for regulatory affairs and also offers different services to associates such as commercial and technical information, technology transfer, links between private companies with public research organisms, and so on.

FOOD CONSUMPTION

Food habits have changed considerably during the last three decades in Spain, with a higher proportion of meals

Table 1. Total Sales of the Main Sectors of the Spanish Food Industry, 1996

Food sector	Sales (ME)	%
Meat and meat products	9802	19.78
Milk and dairy products	5347	10.79
Oils and fats	4881	9.85
Bakery	4153	8.38
Canned vegetables	3469	7.00
Wines	3365	6.79
Nonalcoholic drinks	2879	5.81
Milling	1962	3.96
Chocolate and cocoa	1794	3.62
Beer and malt	1710	3.45
Fish and fish products	1630	3.29
Sugar	976	1.97
Spirits	738	1.49

Table 2. Expenditure per Capita for Selected Groups of Foods in Spain, 1998

Group of foods	Euros per capita	%
Meat and meat products	238.2	25.3
Fish and fish products	118.3	12.6
Bread	71.2	7.6
Fresh fruits	66.6	7.1
Dairy products	64.2	6.8
Fresh vegetables	55.1	5.9
Milk	50.1	5.3
Bakery	33.2	3.5
Oils	26.4	2.8
Processed fruits and vegetables	20.4	2.2
Nonalcoholic drinks	19.0	2.0
Eggs	15.0	1.6
Chocolate and cocoa	12.2	1.3
Total foods	939.2	100

out of homes and a decrease in the time employed for cooking at home. A significant increase in the consumption of products manufactured by the food industry with respect to unprocessed foods has occurred. The pattern of food consumption has also suffered from changes in lifestyle, although in general terms it still remains close to the Mediterranean diet. Consumption per capita of some selected groups of foods and beverages were 143.4 kg for fresh fruits and vegetables, 20.6 kg for processed fruits and vegetables, 116.8 L for milk, 28.5 kg for dairy products, 65.5 kg for meat and meat products, 28.4 kg for fish and fish products, 68.6 kg for bread and bakery products, 11.6 kg for rice and pasta, 34.8 L for wine, and 53.4 liters for beer (2).

A long-term tendency to the increase in the consumption of fresh and processed fruits, milk and dairy products, meat, and fish has been recorded during the last three decades, whereas the consumption of bread, pasta, potatoes, fresh and processed vegetables, eggs, and oil decreased during the same period. In the short term, the consumption of fish, olive oil, fruits, vegetables, and wine has increased; that of bread and eggs tends to keep constant; and that of sugar, potatoes, beer, and spirits decreases, indicating the good acceptance of the Mediterranean diet by the Spanish consumer.

Expenditure in foods by Spanish consumers in 1997 reached the total amount of 50,162 ME, out of which 73% was consumed at homes, 25% at public premises, and only 2% at collective facilities. Food consumption at homes was influenced by variables such as town size, age and working status of housewife, social class, and, very strongly, by the number of persons at home. Some data on per capita expenditure for selected groups of foods in 1998 (4) are presented in Table 2.

Food policy in Spain is oriented both to the safety and the quality of products. Food safety is under the competency of the Ministry of Health and the governments of the autonomous regions. Regulations concerning food safety are gradually being implemented at European level by EU directives, although some national regulations are still valid for particular products. Harmonization of national and international standards for different food products is under way.

Food quality programs, under the competency of the Ministry of Agriculture, Fisheries and Food and the governments of autonomous regions, have especially focused on the promotion and protection of high-quality traditional products under European regulations for Appellation d' Origine or Geographic Indication products. Agricultural and food industry products nowadays under these regulations are 111, with a total sales value of 2,799 ME in 1997 (2). The most important products are wines, with 53 Appellations d' Origine and 69% of total value, followed by spirits, with 10 Appellations d' Origine and 19% of total value, and cheeses, with 12 Appellations d' Origine and 2% of total value.

Products from ecological agriculture have considerably increased during the last decade, gaining market both in Spain and for export. Oppositely, the introduction of genetically modified foods in the marketplace is suffering from the rejection of a growing sector of Spanish consumers.

EDUCATION

University studies on food science and technology are relatively new in Spain. Those studies were comprised in other careers, mainly veterinary, pharmacy, and agricultural engineering, and have been redesigned as a second-cycle degree during the present decade. In the new plan of studies, the first cycle consists of the three first academic years of a disciplinary career such as chemistry, biology, biochemistry, and so on or of a more technical career such as veterinary, pharmacy, or agricultural engineering, after which the university graduate degree is attained. Afterward, food science and technology studies, the second cycle, consists of two academic years, although in some cases, depending on previous studies, some complementary subjects may be needed to obtain a five-university-years degree of *licenciado*. The third cycle to attain the philosophy doctor degree usually takes four years, one year of Ph.D. courses, and three years of research work. Twenty faculties all over Spain offer now the second cycle of food science and technology studies, with 50 to 75 new admissions per year in each faculty.

Technical and further education oriented to the different sectors of the food industry are also found in all autonomous regions of Spain, generally focused on the areas of the greatest importance for each particular region.

RESEARCH AND DEVELOPMENT

The Organization of Research

Spain has a complex public research and development system, under the competency of various ministries. Organisms funding research projects on food science and technology are the Inter-ministerial Commission for Science and Technology (CICYT); the Ministry of Agriculture, Fisheries and Food; the Ministry of Industry; and the governments of the autonomous regions. A Science and Technology Bureau (OCYT), reporting directly to the presidency of the government, was created in 1998 to plan, coordinate, and evaluate the research carried out in Spain under public funding.

The CICYT, working closely with the Ministry of Education, is the organism responsible for the National Plan of Science and Technology. Within the National Plan there is a National Program of Food Technology (which has recently changed its name to Agro-Food Resources and Technologies). During the period 1995 to 1998, the National Program of Food Technology distributed 61% of its budget for funding public research projects, 24% for projects in collaboration with private companies, 11% for scientific equipment, and 5% for special actions (CICYT, unpublished data, 1999).

Projects funded by the National Program of Food Technology, oriented both to basic knowledge on food science and to the development of products and technologies, were mostly carried out at university departments (53% of the National Program budget for projects) and at public research organisms such as the CSIC (National Council for Scientific Research), with several institutes actively working on food science and technology (38% of the National Program budget for projects).

The main objectives of the current National Program of Food Technology are (*1*) study of the modification of food components and functional properties of foods by physiological and industrial processes; (*2*) biotechnological processes occurring in or applied to fermented foods; (*3*) development of industrial equipment, processes, and products; (*4*) food safety, with particular reference to toxicological aspects and detection of alergenic compounds in foods; (*5*) nutrition, in particular methodology for the evaluation of the real nutritive value of foods; (*6*) evaluation of food quality, by means of the development of new analytical techniques for sensory grading and equipment for online determinations; and (*7*) improvement of raw materials for the food industry.

Three strategic actions within the area of food science and technology will be launched in the near future, on the following subjects: new species in aquaculture, control of food quality and safety, and improvement of wine quality and competitiveness. Strategic actions are oriented to increase the research effort carried out by private companies in dynamic sectors of the economy. Participation in a project of one or more private companies, generally together with a public research organism, is wished, with funding of the project from both public and private sources.

The Ministry of Agriculture, Fisheries and Food runs its own Sectorial Research Program, managed by the INIA (National Institute for Agricultural and Food Research and Technology), which funds projects on food science and technology, as well as on agriculture and forestry. These projects are mostly carried out at research centers dependent on the regional governments, where a more product-oriented research is done, although universities and other organisms may also compete for this type of funding. Current priorities of this Sectorial Research Program related to food science and technology are (*1*) development of raw materials for the agroindustry, (*2*) postharvest physiology and storage of raw materials, (*3*) applications of biotechnology to the improvement of agroindustry processes, (*4*) improvement of quality and safety of food, (*5*) improvement of artisanal food products, and (*6*) consumer acceptance and market tendency studies.

Particular types of research projects are integrated projects, such as the one already under way on olive oil. In these projects, with a vertical design, all steps from the production of the raw material, including genetics, irrigation, plant protection, and so on to technological processes, final product characteristics, consumer acceptance, marketing, and so on are covered. Multidisciplinary teams of a large size are thus intended to achieve a more global approach to the problems of a sector and to the most promising trends in research and innovation.

The Ministry of Industry, through its Center for Technological and Industrial Development (CDTI), funds concerted projects, jointly carried out by private companies and public research organisms, and development projects, carried out by private companies on themselves. Funding of projects by CDTI consists in no-interest or low-interest loans to private companies involved in the projects. There are no scientific priorities with regard to the subject of the projects funded by CDTI, technological innovation being the main criterion for the selection of projects. During the last three years, the meat, dairy, fruit, and wine industries have shown to be the most innovative sectors, taking into consideration the number of funded projects.

Participation of Spanish researchers in food science and technology projects funded by the EU has gradually increased throughout the successive European Commission Framework Programs, under ECLAIR, FLAIR, AAIR, and FAIR Programs. Research topics in which Spanish groups are involved in the area of Nutrition and Consumer Well-Being of FAIR projects are quality policy and consumer behavior, mealiness in fruits, impact of dietary fat reduction, foodborne glucosinolates, wine and cardiovascular disease, conjugated linoleic acid, food allergens of plant origin, and bioactive constituents in new food plant varieties. In the area of New and Optimized Food Materials, Spanish groups participate in projects on topics such as *N*-3 polyunsaturated fatty acids, molecular markers for olives, production of oral vaccines in plants, genetic manipulation of melon ripening, molecular improvement of strawberry quality, and genetic manipulation of vegetable shelf life. In the area of Advanced Technologies and Processes, current projects deal with the evaluation of fruits' and vegetables' internal quality, physical and biochemical markers of meat quality, high-pressure treatment of liquid foods, osmotic treatment of foods, sourdough starters, frozen food quality, onion wastes, dry sausages ripening, olives' texture, robotics technology, active and intelligent packaging concepts, and improvement of natural resistance of fruit. Finally, in the area of Generic Food Science the research of Spanish groups is focused on natural antimicrobial systems, quality of thermally processed foods, detection of pesticide residues in foods, whey proteins, risk assessment of pathogenic spore formers, and prevention of mold spoilage in bakery products (5).

The Development of New Products

As in any other European country, a considerable variety of new products appears in the Spanish food marketplace every year. Because of the increasing circulation of products within the EU, and also due to the fact that most large Spanish food production plants belong to multinational companies, part of these new products enter simultaneously in the global European market. Low fat content, high fiber content, vitamin enriched, calcium fortified, omega-3 fatty acids enriched, and so on form part of the labeling culture of the new products, which in most cases are compositional modifications of existing products oriented to a growing healthy-food market. Probiotics added to fluid milk, milk powder, fermented milks, and cheese have been extensively introduced in Spain in the last five years, being accepted as a nutritional benefit by consumers of these dairy products. Yogurt cannot be labeled as such if it has been heat treated, but dairy products based on heat-treated fermented milks are more and more common.

Prepared dishes, generally under frozen form, some of recent development but many others following traditional Spanish cuisine recipes, appear continuously in Spanish supermarkets. Traditional Spanish desserts under new commercial presentations are also gaining place in the supermarkets. More original is refrigerated pasteurized gazpacho, a new food industry product that eliminates time-consuming preparation at home of the cold vegetable soup abundantly consumed in hot summer months. The fish products industry has shown to be particularly innovative, with different fish block products, caviarlike products, and especially baby eel-like products (baby eel is a very appreciated, scarce, and expensive fish) developed by means of a sophisticated surimi technology.

The wine industry has considerably enlarged the scenario of Spanish wines, based on the conjunction of new wine-making technologies with the introduction of foreign grape varieties (Chardonnay, Cabernet Sauvignon, Merlot, Syrah) and the revalorization of local grape varieties. A strong promotional effort of moderate wine consumption on the basis of its beneficial health effects is being carried out, regaining market with respect to beer. Olive oil, another characteristic Spanish product, is also regaining market share against other oils and fats in spite of its higher price, on the basis of its nutritional advantages and favored by new attractive commercial presentations, especially of extra-virgin (cold-pressed) olive oil.

BIBLIOGRAPHY

1. *Agricultural Statistics 1997 Handbook*, Spanish Ministry of Agriculture, Fisheries, and Food, Madrid, Spain, 1998.
2. *Facts and Figures of the Spanish Agro-Food Sector in 1998*, Spanish Ministry of Agriculture, Fisheries, and Food, Madrid, Spain, 1999
3. *The Spanish Food Industry in 1998*, Spanish Federation of Foods and Beverages Industries, Madrid, Spain, 1999.
4. *Food Consumption Panel in 1998*, Spanish Ministry of Agriculture, Fisheries, and Food, Madrid, Spain, 1999.
5. *FAIR Agriculture and Fisheries, Food Projects Synopses*, Directorate General XII, European Commission, Office of Official Publications of the European Communities, Luxembourg, 1998.

M. NUÑEZ
M. MEDINA
National Institute for Agricultural and Food Research and Technology (INIA)
Madrid, Spain

INTERNATIONAL DEVELOPMENT: SWITZERLAND

COMMERCIAL FOOD PROCESSING

Environment and Structure

Switzerland is a country with an area of 41,293 km^2; its population was 7.08 million in 1996. It is entirely surrounded by land, has no natural resources, and, except for agricultural and forestry products, does not produce any raw material.

Currently, domestic agricultural production is providing 50 to 55% of the total energy that is consumed as food. Thus a considerable share of raw material for food processing and of processed foods is imported. The self-subsistence of Swiss agriculture varies from food group to food group. Consumption of Swiss products is reaching 100% for dairy products and is around 90% for potatoes; 80% for bread wheat, meat, and pome and stone fruits; 40% for eggs and crystal sugar; and 20% for vegetable oils.

In spite of the high dependence on food imports, the Swiss food and beverage industry has been able to develop into one of the most important industrial sectors of the national economy (see Table 1). Part of this success has been due to the strong presence of Switzerland on the export market (see Table 2) not only with traditional products such as chocolate and cheese but also with food additives (eg, flavors and vitamins) and other auxiliary

Table 1. Total Expenditure for Food and Beverages in Switzerland

Year	Swiss francs (billions)
1990	33.15
1995	36.33
1996	36.25

Table 2. Exports by Swiss Commercial Food Processing Companies (1997)

Product	Metric tons	Percentage of total production
Hard cheese	61,400	60
Chocolate and confectionery	66,782	49
Soups, sauces, and seasonings	39,036	65
Sweets	12,610	59
Process cheese	10,274	50
Canned foods	12,900	15
Pasta products	8,349	14
Baby foods	14,588	85

chemicals, food machinery, and licenses for individual food products and for whole food process operations. Several renowned food machinery companies are exporting more than 90% of their production. Today some Swiss food processing companies belong to the largest ones operating in Europe or the world.

Therefore, the ties of the Swiss food processing industry to other industrial sectors, particularly to the mechanical and chemical industries, have become as important as its traditional relation to agriculture. Nevertheless, agricultural cooperatives are still involved in some commercial food processing (eg, for dairy products, meat products, fruit juices, and wine). A substantial part of the food and beverage production lies in the hands of entrepreneurs with small tradelike operations. These entrepreneurs are professionally trained and federally licensed food specialists. A large proportion of cheese, bakery goods, and meat products are manufactured in this way.

In Switzerland, commercial food processing and retail markets are integrated to a considerable degree. This integration is particularly due to the existence of two large consumers' cooperative organizations that operate their own food processing plants or use plants as copackers. Their combined share on the retail food market is reaching almost 40%. Other parts of the tertiary food sector (eg, catering and restaurants) are mostly independent of the food processing companies.

On the retail market, a high living standard and a high purchasing power maintain the demand for quality and diversity of domestically produced and imported foods. Quality standards are met by an equally high standard of professional training on all levels of education. Diversity is enhanced by the cultural variety based on the four national languages of Switzerland (German, French, Italian, and Rhaeto-Romanic). In turn, the high standards for domestic food quality ensure a lead in quality on the export market, where Switzerland as a small nation would be unable to compete with low-priced bulk products.

Statistical Information

The number of persons involved in commercial food processing (estimated in 1995) is 10% of all persons involved in industrial production.

The number and size of commercial food processing operations was estimated in 1997. There are approximately 9000 small-scale tradelike operations, half of which have fewer than 10 employees. There are 1400 cheese plants, 4000 bakeries, and 2900 butcheries. Switzerland has 134 industrial food processing companies with approximately 190 production sites and approximately 22,000 employees. Of the 134 companies, 13 employ more than 500 people, 39 employ between 100 and 499, and 82 employ fewer than 100. There are 300 plants for beverage production (mineral water, soft drinks, beer, wine, and distilled beverages) with approximately 6800 employees. (Small wine farms with their own vinification and sales are not included in this estimate.)

EDUCATION IN FOOD SCIENCE AND TECHNOLOGY

University Level

University education is preceded by high school education and the Swiss Federal Maturity Examination (baccalaureate).

Swiss Federal Institute of Technology (Eidgenoessische Technische Hochschule, ETH), Zurich. Courses in food science and technology include food engineering, introductory nutrition, and biotechnology. The M.Sc. degree (diploma) is a nine-semester curriculum with 24 months of practical industrial training. It takes 3 to 4 years of graduate research work to earn the D.Sc. or Ph.D. degree. There is a 1-year postgraduate curriculum in nutrition as well.

Universities of Basel, Bern, Lausanne, and Zurich. Selected courses at the undergraduate and graduate levels in food chemistry (particularly food analysis), food microbiology, and food technology are part of the curricula in chemistry and veterinary medicine.

Swiss Federal Office of Public Health. The Swiss Federal Office of Public Health is responsible for licensing official state chemists after postgraduate study courses taught at the Swiss Federal Institute of Technology, Zurich, or the University of Basel, Bern, Lausanne, or Zurich.

College Level (*Fachhochschulen*)

Education on the college level is usually preceded by formal vocational training in the food industry (see "Vocational Training Level"). All colleges award a 4-year curriculum (1 year of general and 3 years of professional education) and a degree equivalent to a B.Eng. or a B.Sc. (Ingenieur FH).

Hochschule Waedenswil. There are two separate curricula in food technology and biotechnology.

Hochschule Sion. The curriculum in food technology includes courses in general biotechnology.

Education in Special Areas: Commodities. Additional college-level curricula are available in beverage technology (Changins), dairy technology (Zollikofen), and biotechnology (Winterthur).

Vocational Training Level

Education under the Swiss Federal Legislation on Professional Training. Programs include 3- and 4-year vocational training courses that are a combination of on-the-job learning at the production site and theoretical education in the classroom (1 to 2 days per week). The courses lead to federally recognized licenses for food technologists (in the areas of bakery technology, chocolate and confectionery technology, and soup and sauce manufacturing), bakers, baker-confiseurs, brewers, butchers, chemical and microbiological laboratory technicians, cheese processors, dairy technologists, fruit and wine processors, and milling technologists.

Further education of graduates from vocation training is possible at the Swiss School of Food Technology, Posieux (1-year course), and for milling technologists at the Swiss School of Milling, St. Gallen (2-year course). There are also advanced extension courses leading to the professional masters diploma in some of these fields. They are organized by the different Swiss associations of professional masters. For food technologists, there is also the possibility of advanced training in food technology and management.

RESEARCH AND DEVELOPMENT

Expenditure for Food Research and Development (1996)

Swiss Food Processing Industry. The Swiss food processing industry spends 0.5% of the total turnover, or approximately 150 million Swiss francs, for research and development. This amount is primarily used for in-company research (93%); the rest is split for contract research at universities (2.5%), joint ventures between industrial and public research (2.5%), and private research institutions (2%).

In addition, corporate research of several worldwide food companies is carried out in Switzerland. Expenditures by chemical industry for research on flavors and vitamins and by machinery industry on process and packaging equipment are also substantial.

Federal Government. The federal government spends 170 million Swiss francs for agricultural research and extension service, approximately 20% of which is allocated for food processing research. Therefore, public funding for food research amounts to approximately 20 million Swiss francs. Some aspects of the research projects are operated on a joint venture basis with industry.

Public International Research Projects. Switzerland is actively collaborating in public international research projects. Examples of these projects are the European Cooperation in Scientific and Technical Research (COST) projects and the Framework Programs of the European Union.

Public Establishments for Food Research

Public establishments for food research include the following:

Swiss Federal Institute of Technology (ETH) Zurich: Institute of Food Science (basic and applied research in food chemistry, technology, engineering, microbiology, biotechnology, and nutrition)

Hochschulen Waedenswil and Sion (applied research in food technology and biotechnology)

Swiss Agricultural Research Stations, Bern (dairy technology), Changins (plant products including wine), Posieux (meat products), Reckenhoz (plant products), and Waedenswil (fruits, vegetables, and wine)

Swiss Federal Office of Public Health, Bern

University of Berne (section for food chemistry), University of Lausanne (group for food chemistry), and University of Zurich (Institute of Veterinary Hygiene)

ORGANIZATIONS

Industrial Level

Industrial-level organizations include the following:

Federation of Swiss Food Industries (Federation des Industries Alimentaires Suisse, FIAL), Berne (comprising subgroups for various food products)

Federation of Swiss Machinery Industry (Verein Schweizerischer Maschinenindustrien, VSM), Zurich

Swiss Society of Chemical Industries (Schweizerische Gesellschaft der Chemischen Industrie, SGCI), Zurich

Several commodity-oriented organizations

Professional and Scientific Level

Professional- and scientific-level organizations include the following:

Swiss Society of Food and Environmental Chemistry

Swiss Society of Food Hygiene

Swiss Society of Food Science and Technology

Swiss Society of Nutrition Research

Swiss Association of Agricultural and Food Engineers and Scientists

The first four professional- and scientific-level organizations listed are represented in the Swiss National Committee to the International Union of Food Science and Technology (IUFoST) and to the European Federation of Food Science and Technology (EFFoST). Switzerland also has delegations in the International Union of Food Science and Technology (IUNS), the Division of Food Chemistry of the Federation of European Chemical Societies, in the Food Working Party of the European Federation of Chemical Engineering, in the International Association of Engineering and Food, and in several commodity-oriented food research organizations.

GENERAL REFERENCES

Lebensmittel und Getraenkeindustrie der Schweiz [Swiss Food and Beverage Industry], 17th ed., Orell Fuessli, Zurich, Switzerland, 1998.

J. Solms, "Directory of Swiss Educational and Research Establishments," [Compiled for the European Federation of Food Science and Technology, Department of Food Science, Swiss Federal Institute of Technology Zurich], *Lebensmittel-Technologie* **24**, 53–56 (1991).

Statistisches Jahrbuch der Schweiz [Statistical Yearbook of Switzerland], Neue Zuercher Zeitung, Zurich, Switzerland, 1998.

Swiss Association of Food Industries, *Jahresstatistik* [Annual Statistical Report], Bern, Switzerland, 1998.

Swiss Farmers Association, *Monatsstatistik* [Monthly Statistical Reports], Brugg, Switzerland, 1998.

FELIX E. ESCHER
Swiss Federal Institute of Technology (ETH)
Zurich, Switzerland

INTERNATIONAL DEVELOPMENT: TAIWAN

Taiwan is a tobacco-leaf-shaped island located due east of Fu-Jian Province of China across the Taiwan Strait. It has a surface area of 36,000 km^2, or about the size of the Netherlands, but almost three-fourths of the Island is covered by a steep mountain range running from the north to the south, in which more than 100 peaks are higher than 3000 m. The eastern end of the monsoon belt reaches the island, and the Tropic of Cancer dissects the middle. Thus the climate is subtropic in the plain area but cool temperate up in the hills, and the entire Island has abundance of rainfall. Rather broad and very narrow alluvial plain belts suitable for agricultural exploitation are found on the western and eastern coastal zones, respectively. Rivers originate at the central mountain range and run westward or eastward reaching the Taiwan Strait or the Pacific Ocean, respectively. They are short in length and rapid in flow, so the water is little retained before being discharged into the sea. Nevertheless, intensive and extensive tropical to subtropical agriculture is practiced on coastal plains. Some temporal agricultural activities are also found at the scattered mountain farms where mainly temporal fruits and vegetables are grown. Although forests are dense in mountains, lumbering is almost prohibited for land conservation reasons. Because of the prevalence of the wide range of tropical, subtropical, and temporal climatic conditions, a very rich fauna is found on the island. Similarly, in addition to the main cash crops such as rice, sugar cane, sweet potato, tea, pineapple, banana, and so on, diverse agricultural crops are produced in Taiwan.

Although Taiwan is rich in rainfall and favorable in temperature for agricultural operation, it often suffers from the visits of typhoons and the torrential rainfalls that accompany them. Damages caused by severe wind and excessive rainfall are not limited to agriculture but also extend to many social functions. The development and implementation of technologies for food processing, storage, transportation, and so on are needed for not only enhancing the usefulness and value of food materials but also meeting emergencies caused by natural disasters.

Among 21 million inhabitants, currently Taiwan's agricultural population is stabilized at about 18% of the total in about 800,000 households. The number fluctuates more or less within 5% according to changes in economic situations, because the agricultural economy serves as the buffering domain for accommodating fluctuation in the nonagricultural employment. The size of an average farm is 1.1 ha. The low economic efficiency of ordinary farming is attributed to the small farm size and has provided an incentive to establish containment facilities for the production of higher-value-added products such as medicinal plants, ornamental flowers, off-season fruits and vegetables, and so on, in recent years. The income of farming families is the lowest among all categories of households as seen from the fact that agricultural productions earn only 2.78% of total domestic productions in terms of the dollars.

Up to early sixties, the export of rice and bananas and some processed agricultural products such as refined cane sugar, canned pineapple, mushrooms and asparagus, and frozen fisheries products had played a significant role in earning foreign exchanges needed for industrial development. In 1968 rice and cane sugar alone still represented 11% of total national exports. Although the share of raw and processed food and feed products dropped from 31.6% of total export value in 1968 to 5.5% in 1988 and then 2.8% in 1996, the value increased from \$250 million to \$3.32 billion and then to \$4.08 billion in the same periods. Up to 1996, the total export value of food and feed-related products exceeded that of imports, meaning that the agricultural productivity and capacity of the food industry of Taiwan had been more than self-sustaining in feeding the population in terms of commodity exchange values until recently (Table 1). This situation is now reversed; Taiwan is a net importer of agricultural products by continuously expanding imports and diminishing exports simultaneously. The affluence of the society has caused significant changes in food consumption habits, such as shifting from the rice-based diet to the wheat-based one, and the consumption of more expensive formerly exported and now imported foods and beverages, such as fisheries products, dairy products, beef, and wines and liquors.

DOMESTIC FOOD CONSUMPTION AND ITS EFFECTS ON TRENDS OF THE FOOD INDUSTRY AND INTERNATIONAL TRADE

The per capita food intakes in recent years are shown in Table 2. Besides keeping a constant intake level of carbohydrate, intakes of the other two main dietary ingredients, protein and fat, are increasing steadily, contributing to increases in calorie intake. In particular, the consumption of animal protein has almost quadrupled in 40 years. Daily intakes of fat and carbohydrate were 40 g and 432 g, re-

Table 1. International Trades of Foods ($ million)

Year	Export	% of Total Export	Import	% of Total Import
1993	3484	4.0	2502	3.28
1994	3808	3.7	2906	3.37
1995	4284	3.4	3196	3.18
1996	4083	3.1	3372	3.29
1997	2551	1.8	2931	2.92

Table 2. Per Capita Food Intake

Year	1991	1992	1993	1994	1995	1996	1997
Calories	2857	2875	2930	2979	2971	2972	3045
Protein	91.45	93.64	96.89	96.06	97.72	97.85	101.14
Fat (g)	114.04	114.93	120.38	124.72	125.94	126.80	131.07
Carbohydrate (g)	373.80	373.24	372.51	375.23	369.47	367.45	372.97

spectively, in 1957. We may see a significant degree of substitution of carbohydrate-derived energy by that derived from fat during the past 40 years.

The overintake of food calories is now making obesity a national epidemic. The data in Table 3 illustrate the population's current trend of food habits. These data again illustrate the increase of protein and fat intake in the forms of meat, dairy products, eggs, and cooking oils and also the need for very active international trade of food commodities to satisfy the national appetite.

Among food ingredients, about one-half of cereals and almost all of cooking oils are derived from imported wheat and soybean, respectively. The little availability of arable land, the diversion of land for industrial use, and the growth of the population from about 6 million in 1945 to 21 million in 1997 have made it impossible to produce even a self-sustainable amount of staple food. In 1997 the imports of cereals and their products, mainly wheat and maize, and those of oil seeds and their flours, mainly soybean, were 7.202 million t (metric tons) and 2.818 million t, respectively. Wheat supplements rice as the staple food. Besides being used as the source of cooking oil, soybean is used for the manufacturing of various forms of soy protein foods, such as tofu, various forms of vegetarian meat, and seasoning agents such as miso paste, soy sauce, and so on. Maize, wheat bran, and oil seed flours are used as feeds in swine and poultry productions.

Summarizing the preceding section, we may say that agricultural production in Taiwan is aimed at a self-sustainable supply of foods, yet the former trend of exporting some specialized items for earning foreign exchanges still remains. Formerly such items needed rather low technology for production. Now the trend shifts to the development and manufacturing of high-value-added processed foods. They are for domestic use as well as for export to exchange raw materials that need large land areas for production, and also high-quality and even exotic foods for satisfying increasingly sophisticated social demands.

Food Industry

Most food industrial operations in Taiwan are located in rural villages for the convenience of collection and transportation of raw materials from farms. Production of canned, frozen, and salted foods is closely related to the rural economy and farmers' income. The total number of registered food processing plants in Taiwan has diminished from 6805 in 1987 to 6023 in 1997. The total number of employees is more than 110,000. Several large incorporated companies that have overseas manufacturing branches in mainland China and southeast Asia produce a full range of products such as cooking oils, seasonings, frozen foods, pickles, instant foods, candies, crackers, biscuits and other types of snacks, vegetable and fruit juices, soft drinks, and health foods. However, most factories are small or family businesses specializing in the production of one type of food, and 95% of them have a combined capital of less than $200,000 per operation. The frozen food industry is the largest, with a capital of $0.3 to 2 million and a workforce of 100 to 200 people for each operation. The canned food industry comes to the second, with a capital around $0.3 to $1 million and 50 to 150 employees per plant; the salted food industry is the third, with an approximate capital of $60,000 to $300,000 and 20 to 50 employees per plant. The other industries are worth $30,000 to $60,000 each, and each one employs only 5 to 20 people. The total production value of the food industry was $1.57 billion in 1988, which grew to $14.15 billion in 1997, showing a formidable ninefold increase in nine years. During the same period, per capita national income doubled from $5,829 to $12,019 at current prices, and the percentage of the consumption of food, beverages, and tobacco to the

Table 3. Per Capita Intake of Food Items (g)

Year	1991	1992	1993	1994	1995	1996	1997
Cereals	273.42	275.05	272.91	279.50	275.78	266.18	263.56
Tubers	58.03	60.08	59.42	58.00	49.49	53.93	64.11
Sugar and honey	72.03	71.17	69.42	68.83	67.18	68.14	66.98
Kernels and oil seeds	80.41	70.41	80.09	82.87	6.89	86.99	86.71
Vegetables	259.53	266.28	268.73	255.70	278.08	297.41	296.63
Fruits	379.97	354.70	396.11	374.08	378.98	379.32	411.17
Meats	176.73	183.51	192.68	198.33	199.34	205.03	211.80
Eggs	36.58	37.49	38.93	40.79	44.47	48.01	53.23
Fisheries products	108.82	120.08	130.02	105.12	105.08	104.91	116.03
Dairy products	46.91	50.04	55.29	58.25	63.10	62.92	64.20
Cooking oil	64.97	64.35	68.25	71.02	71.32	71.34	73.27

household expenditure decreased from about 35 to 25%. These trends show clearly that the demands for processed and prepared foods, although at prices much higher than the raw food materials available at markets, have increased in recent years. The main reasons for the shift in food habits may be listed as follows: More women are moving into the job market; more modern home appliances are available; the younger generation has become less interested to learn cooking; more outdoor activities are accepted as the lifestyle, and so on. Instant noodles (made from wheat or rice) are one of the fastest-growing products in this category. A wide range of baked goods, frozen foods, packaged dairy products and fruit and vegetable juice drinks, soft drinks, snack foods, and so on have a large share of food consumed in Taiwan. Besides using much of imported cereals, oil seeds, starches, fruits, and so on, the food industry depends largely on the domestic agricultural industry for raw materials. Thus, the food industry is considered to be a great booster to the rural economy.

The government policy of market liberalization and import tariff reduction, together with appreciation of the New Taiwan dollar in recent years, has also boosted dramatically the increase of importation of processed foods. The value increased from $679 million in 1987 to $2.40 billion in 1997. The flourishing economy and rising national income have led to a big change in consumer habits for foods and drinks. The trend of consumer taste is toward high-quality, semiprepared and flavored, or prepared and flavored foods because such products are much more convenient. The partial lifting of the monopoly policy over production and trade of alcoholic beverage has witnessed a sharp increase in the import of beers, grape wines, cereal and fruit liquors, and so on.

Cereal and Soybean Products

The import of wheat from the United States to Taiwan started in the fifties. Wheat products such as bread and noodles have replaced a large part of the traditional staple food rice, and bread and noodles in various forms have a big share of the domestic staple food market now. The instant noodle alone had a sales value of $26 million in 1977, which quadrupled to $110 million in 1987, and further increased to $346 million in 1997, all along with a proportionate increase in volume. As wheat products have increasingly supplanted the traditional staple food rice, although the brown rice production decreased from the peak value of 2.713 million t in 1976 to 1.663 million t in 1997, and the population increased from 16.508 millions to 21.683 millions in the same period, the domestic rice supply has been suffering from a surplus. The development and manufacturing of rice products, such as rice crackers and instant rice foods, are encouraged by the government to alleviate this situation. Rice crackers are popular; some are imported from Japan, and some are produced locally in collaboration with Japanese firms. However, they use mainly glutinous rice, which is not common for daily use, as the raw material. Imports of breakfast cereals and biscuits from Western countries are increasing. These facts also contribute to the deepening of the grave situation of rice surplus.

Besides producing some vegetable soybean that is consumed domestically and also exported to Japan, the supply of soybean in Taiwan depends almost exclusively on the import from the United States. As mentioned before, imported soybean is used mainly for oil extraction and the residual flour for feed formulation, and for manufacturing of various forms of vegetarian meats, including tofu and tofu products; it is also important in the soy sauce industry. In 1997 the production of soy sauce amounted to 1.034 million t and was valued at $95 million. Most soy sauce is consumed domestically, because it is not generally used in Western cuisine.

Meat and Poultry

Pork is the major meat consumed in Taiwan. Annual per capita pork consumption increased from 20.6 kg in 1967 to 33.86 kg in 1987 and then stabilized at around 39 to 40 kg since 1993. However, the ratio of pork consumption to total meat consumption has declined in recent years due to the rise in poultry and beef consumption.

Poultry ranks second as the preferred meat. The poultry industry, similar to the swine industry, has shown a tremendous growth, largely because of technological improvements, a high degree of specialization of the industry, and an ample supply of feeds through the very active international trade and development of the food industry, which yielded many by-products suitable for feed use. The per capita poultry consumption increased steadily from 5.12 kg in 1967 to 20.12 kg in 1988 and 33.49 kg in 1997, with its share in total meat consumption increased from 19.5 to 35.4 and 43.3%. Poultry consumption is expected to grow more.

Meat imports, including beef and mutton, rapidly grew with the quick development of the fast-food industry in recent years. Domestic production of cattle is limited, and the number raised is decreasing. The population of sheep is almost nonexisting. Frozen meats are imported from Australia, New Zealand, and the United States. There was 38,349 t of beef imported in 1988, which increased to 56,785 t in 1997, valued at $129 million and $169 million, respectively. The imported beef satisfied nearly 80% of the consumption. The import volume of mutton is about one-third that of beef. The per capita consumption of mutton was 0.86 kg in 1993 and 1.30 kg in 1997, or equivalent to 17,200 t and 27,300 t of annual import, respectively. We may expect that more imported beef and mutton will supplement the consumption of domestic pork.

Pork is the main item of meat Taiwan exports. The market is Japan. Annually nearly $500 million worth of pork had been exported, but, due to the outbreak of the foot and mouth disease in 1997, it has been halted since then.

Dairy Products

Taiwan launched the Dairy Farmer Support Program in 1958. Dairy farmers got reasonable profits from their milk production, and the program was further extended with the result that dairy farms increased from 804 in 1978 to 1,322 in 1988. Milk production increased from 44,615 to 144,390 t during the same period. Most milk produced is consumed fresh (eg, 105,703 t of the 1988 production was consumed fresh), but due to the variations in milk supply and market demand, surplus milk in winter is processed into dry milk and sweetened condensed milk. Fresh milk consumption and milk powder production reached the peak values of 215,799 t in 1995 and 9,603 t in 1992, respectively, but both declined to 179,567 t and 6,907 t in 1997. Although the self-supply ratio of dairy products increased and imports decreased, dairy products are still leading in imports. The main forms of imported dairy products are milk solids and butter with cheese and curd sharing about 10% of the total. The import value increased from $144 million in 1983 to $363 million in 1997. The average yearly milk consumption per capita comprises both locally produced milk and imported products. When converting their food values into full-cream milk equivalent, the total milk consumption per capita was 1.50 kg in 1957, which increased to about 50 kg in 1987. Because local production of fresh milk now meets only about 20% of the market demand, there is much room for the domestic dairy industry to develop.

Beverages

Besides the local brands of various types of soft drinks, either carbonated or noncarbonated, various kinds of soft drinks bearing international brands, such as Coca-Cola and Pepsi-Cola, are bottled in Taiwan.

Fruit juices and vegetable juices, either single or mixed, produced from local raw materials are abundant. But fruit juices, such as orange, grapefruit, peach, and so on, reconstituted from imported concentrates are also having a large market share. A very interesting feature of Taiwan's fruit and vegetable juice industry is its highly diversified products. You may find juices and juice drinks bearing the names of asparagus, guava, carambora, coconut, winter melon, plum, sugar cane, mango, pomelo, tangerine, litchi, water melon, passion fruit, honey dew, lotus root, and kiwifruit in addition to the names ordinary found in Western markets. Sport drinks aimed at replenishing blood salt balance and providing a quick energy supply grew fast at the initial phase of their introduction from 1985 to 1990 but are now stabilized. Because most people consume soft drinks and juices for quenching thirst, the seasonal variation in consumption is large.

Owing to the monopoly policy over alcoholic beverages, local products dominated the market until recently. Now, imports are taking a share of about 25% of the total consumption of $1.92 billion.

Locally produced beverages consumed in Taiwan in 1997 are (in kiloliters and $ million, respectively) alcoholic, excluding beer, 241,365 and 791; beer, 365,406 and 637; fruit and vegetable juices, 260,194 and 195; carbonated soft drinks, 456,620 and 311; sports drinks, 171 and 773. Other types of nonalcoholic beverages, including coffee and tea drinks, mineral water, traditional beverages, and so on, had a total sales value of $593 million.

Fruits and Vegetables

The consumption of fruits and vegetables is still increasing, although at a slow pace, as shown in Table 3. Most are consumed fresh. Canned, frozen, dehydrated, or preserved fruits and vegetables are not as important in the domestic market as in the foreign market. But manufacturers are paying more attention to them in the domestic market, because these items are now less competitive in the international market, and changes in the social situation have enhanced their domestic consumption.

The frozen food items for domestic consumption, except ice cream, include frozen vegetables (mainly mixed vegetables), pork dumplings, hamburger patties, pork balls, and fish balls. Frozen poultry and seafood have not yet received a welcome from consumers in Taiwan, but the government had launched a sales promotion campaign. To help consumers identify excellent frozen food, and to help producers enhance quality and sanitation in their products, the Council of Agriculture implemented an "Excellent Frozen Food Mark" system in 1988. Establishment of wholesale-type chain stores and supermarket facilities islandwide has overcome the difficulties experienced in the early stage of the promotion campaign, which were due mainly to the lack of cold chain facilities.

Canned pineapple, mushroom, and asparagus played an important role as the foreign exchange earner in the development of the Taiwan economy. The canned pineapple industry was initiated during the colonial era. It had a systematic operation from cultivation, harvesting, and processing to exports handled by a big company. The other two items, the cultivation of which was started in the fifties on small farms, were processed by small-scale factories by using raw materials collected locally. Exports of pineapple and asparagus have declined to almost nil, but supplies such as fresh fruit and vegetables are still available in the domestic market. Various kinds of canned mushrooms still have foreign markets, the export of which amounted to $283 million in 1996. Bamboo shoots have found a niche in the international market in the meantime, and the export of canned products amount to $531 million in 1996. Tomatoes have been grown in Taiwan as far back as the nineteenth century. Originally, they were treated as fruit, to be eaten raw. It was not until 1967 that local farmers began growing tomatoes specially for canning. At the peak time, Taiwan's canned tomato products, ranging from 2 million to 3 million standard cases a year, accounted for 2% of the world's canned tomato production. It is now rapidly declining, with the annual export values of $71.4 million in 1995, $12.9 million in 1996, and $3.5 million in 1997.

Frozen Food

The frozen food industry has been rapidly developing during the past two decades. Frozen food became a leading export item among the processed products in the early 1980s. Export value reached more than $2.25 billion in 1996. In spite of the appreciation of the New Taiwan dollar and keen competitions in the international market, frozen food processors in Taiwan have enjoyed a steady growth of exports in recent years. And yet they have been able to maintain outstanding quality, achieve significant reduction in production costs, and enhance production efficiency. Among the factors contributing to such an excellent performance are the advancement in quality control technology, the installation of modern production facilities, and the assistance provided by the related government organizations. The major frozen food items and their production values in 1996 (in metric tons and $ million) are vegetables, 32,326 and 59.05; fruits, 1461 and 2.52; seafood, 359,303 and 838.93; meat and offal, 163,227 and 4016.4; prepared foods, 16,541 and 199.2; and eggs and egg products, 13 and 0.70. The majority of frozen food factories are located close to their raw material supplying areas in central and southern Taiwan. However, frozen seafood producers choose their plant sites in areas close to the two main seaports, Keelung and Kaohsiung, located on the northern and southern tips of Taiwan, respectively. About 98% of Taiwan's frozen foods are exported to Japan, the Federal Republic of Germany, the United States, Hong Kong, and Canada. The three biggest items are pork, grass shrimp, and eel. The most important frozen vegetables are soybeans, pea pods, and string beans. Prepared foods in frozen form, including roasted eel and Chinese-style convenience foods, now enjoy a booming market in Japan.

Dehydrated and Preserved Foods

The dehydrated and preserved food industries are important sources of income for farmers who grow fruits and vegetables on slope land. The main items are ginger, prunes, eggplant, mustard, cucumber, mushrooms, and bulbous onions. The total export values for dehydrated and salted foods in 1996 were $60.6 million and $74.4 million, respectively; Japan imports 80% of Taiwan's salted foods. Because most products are in semifinished form, the profit is limited.

Others

Among the great changes in international trade that Taiwan has witnessed during the past half-century, we should mention the disappearance of cane sugar from the list of top export items, and the emergence of seasoning agents, mainly monosodium glutamate, a more-advanced product of fermentation technology, which enjoy a significant position continuously in the list of total productions and exports. In 1996 sugar and seasoning agents had total export values of $14.8 million and 144.9 million, respectively. These figures again signify that the era of agricultural development to support industrial takeoff has already become a part of Taiwan's history.

RESEARCH AND DEVELOPMENT AND FUTURE TRENDS

Taiwan has identified food science and technology as one of the major fields of national research and development (R&D) programs in the midseventies. The policy was formulated primarily not to enhance the international trade values of agricultural products, but to improve the nutrition and safety of foods as well as to enhance the economic values of agricultural resources so as to improve the rural economy. Since then the R&D capabilities at academic and commercial institutions have been strengthened along with the establishment of the Institute of Food Science and Technology (IFoST), an academic society supported by all related institutions and private scholars and technologists, to promote and coordinate all food-related activities. The number of students enrolled at both undergraduate and graduate levels in teaching institutions, the number of teaching and research staffs, the investment in R&D facilities and research program funding, and the number of R&D personnel holding advanced academic degrees have greatly expanded. We can see the outcomes of these efforts, first, in the continually growing food industry and, second, from the fact that the annual meeting of the IFoST is attended by more than 1000 participants. At the annual meeting, several hundred original research papers are presented and a rather large-scale food show is staged.

For practical on-the-job training, the Food Industry Research and Development Institute (FIRDI) located at Hsinchu (northern Taiwan), which was originally established under the sponsorship of the Canned Food Exporters' Association in the early sixties but is now a government-funded foundation, plays a major role. In addition, FIRDI provides contractual services on research and training for satisfying both government and industry needs. The Food and Drug Inspection Bureau of the Ministry of Health located in Taipei mainly researches food safety inspection technology. One of the major material bases for biotechnological R&D, the Type Culture Collection (microbial, animal, and plant cell lines), is also operated by FIRDI. Gene banks for supporting agricultural research are located at the Provincial Agricultural Research Institute in Taichung (central Taiwan) and the Asian Vegetable Research Institute in Shanhua, Tainan (southern Taiwan). These establishments started as rather modest operations but now have been greatly expanded to serve the international community as well.

The era of the development of specific type of foods at academic institutions is almost gone. Their research is now more concentrated on the basic aspects of food science and the development of modern biotechnology. So besides the Council of Agriculture–supported, mission-oriented practical aspects of technology development at government as well as private institutions, funding from the National Science Council is approved on a competitive basis and based on the scientific merits for future advancement rather than the prospect of immediate benefits.

We may see changes in the trends by citing the policy promulgated by the relevant government agencies as follows. The policy on the development of food technology includes:

1. Securing the sources of food supply and strengthening the production and marketing systems
2. Leveling-up the technology and improving the infrastructure of the industry
3. Leveling-up the food quality and safety
4. Enhancing competitiveness to promote domestic and international sales
5. Improving the environment for investment
6. Promoting an international cooperation mechanism in which the root remains in Taiwan
7. Streamlining of policy formulation and implementation

The strategy for development includes:

1. Streamlining a collaboration mechanism among government agencies related to food research, production, marketing, safety regulation, environmental concerns, and so on
2. Totally upgrading products
3. Strengthening research activities aimed at new products development
4. Rationalizing and enhancing productivity
5. Strengthening training
6. Modernizing production management and marketing systems
7. Promoting the interchange and cooperation between food industries on both sides of the Taiwan Strait

GENERAL REFERENCES

FIRDI Statistics Database, Food Industry Research and Development Institute, Taiwan, ROC, 1999.

Food Industry Statistics of the Republic of China, Food Industry Research and Development Institute, Taiwan, ROC, 1998.

Monthly Reports on Industry Statistics, Taiwan, ROC, Council for Economic Planning and Development, Taiwan, ROC, 1999.

Taiwan Statistical Data Book, Council for Economic Planning and Development, Taiwan, ROC, 1998.

JONG-CHING SU
National Taiwan University
Taipei, Taiwan

INTERNATIONAL DEVELOPMENT: UNITED KINGDOM

Services and industry are the major employers in the UK, and agriculture employs a relatively small workforce of half a million. This is equivalent to ~2% of the total civilian workforce, which is low by comparison with all other European countries (1). The food industry, on the other hand, employs more than 2.5 million. Agriculture and the food industry make a considerable contribution to UK exports. There is a high level of trade in food commodities: in 1997, food, beverage, and tobacco accounted for 6.5% of gross domestic product (GDP) as exports and 8.8% of GDP as imports (1).

THE REGULATORY ENVIRONMENT

The main piece of primary food legislation in Britain is the Food Safety Act (1990). Northern Ireland has equivalent legislation: the Food Safety (Northern Ireland) Order (1991). A feature of the Food Safety Act is that in addition to enabling legal rule-making, it also provides for defenses in law, including due diligence defense. Thus, manufacturers, importers, retailers, or individuals can use due diligence as a defense, if it can be shown that all reasonable precautions and due diligence were used to avoid the commission of an offense.

Central responsibility for the food supply in the UK is shared between the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health (DoH). Some responsibility in Wales, Northern Ireland, and Scotland is delegated to, for example, the Welsh Office, the Scottish Office Agriculture and Fisheries Department, the Department of Health and Social Services for Northern Ireland, and the Department of Agriculture for Northern Ireland. MAFF also maintains an executive agency, the Central Science Laboratory (CSL), at sites in Norwich and York. The CSL is responsible for the protection of consumers, food supplies, and the environment by means of food surveillance and research programs. Other executive agencies of MAFF include the Pesticides Safety Directorate (PSD; responsible for licensing and monitoring pesticides with respect to safety, effectiveness, and use) and the Veterinary Medicines Directorate (VMD; responsible for licensing and control of veterinary medicines and surveillance of residues in foods). The executive agency responsible for advising MAFF on land management, food production, waste disposal, and some aspects of food processing and retail, the Agricultural Development Advisory Service (ADAS), was privatized in 1997.

A number of independent advisory committees are established to advise government departments on aspects of food policy and safety. The main task of the Food Advisory Committee (FAC) is to advise on the safety of chemicals in foods, food additives, and food surveillance. However, policy on food labeling, including nutritional and health claims, is also determined to a large extent by the work of FAC. The assessment of novel foods, including genetically modified foods, is conducted by the Advisory Committee on Novel Foods and Processes (ACNFP). More specific toxicological expertise is available on the Committee on Toxicity of Chemicals in Foods, Consumer Products and the Environment (COT), to which specific questions may be referred by the FAC or the ACNFP. Similarly, the government has access to expert nutrition advice on the Committee on Medical Aspects of Food and Nutrition Policy (COMA). In the case of genetically modified organisms, two additional committees are involved in the research and development stages: the Advisory Committee on Genetic Modification (ACGM) and the Advisory Committee on Releases to the Environment (ACRE). The Health and Safety Executive, as advised by ACRE, is responsible for enforcing legislation concerned with occupational safety and the safety of members of the public exposed to agents in the environment. The Advisory Committee on the Microbiological Safety of Foods (ACMSF) advises the government on the risks to humans of microorganisms used or occurring as contaminants in foods. Other advisory committees that deal with the food supply include the Advisory Committee on Pesticides (ACP), the Consumer Panel, the Spongiform Encephalopathy Advisory Committee (SEAC), and the Veterinary Products Committee (VPC).

Enforcement of food legislation in the UK is largely decentralized. At a local level, food control is the responsibility of environmental health officers (EHOs) and trading standards officers (TSOs) employed by local authorities (of which there are 589 in the UK). The former deal with issues of safety and hygiene, the latter with food quality, The strategy for development includes:

1. Streamlining a collaboration mechanism among government agencies related to food research, production, marketing, safety regulation, environmental concerns, and so on
2. Totally upgrading products
3. Strengthening research activities aimed at new products development
4. Rationalizing and enhancing productivity
5. Strengthening training
6. Modernizing production management and marketing systems
7. Promoting the interchange and cooperation between food industries on both sides of the Taiwan Strait

GENERAL REFERENCES

FIRDI Statistics Database, Food Industry Research and Development Institute, Taiwan, ROC, 1999.

Food Industry Statistics of the Republic of China, Food Industry Research and Development Institute, Taiwan, ROC, 1998.

Monthly Reports on Industry Statistics, Taiwan, ROC, Council for Economic Planning and Development, Taiwan, ROC, 1999.

Taiwan Statistical Data Book, Council for Economic Planning and Development, Taiwan, ROC, 1998.

JONG-CHING SU
National Taiwan University
Taipei, Taiwan

INTERNATIONAL DEVELOPMENT: UNITED KINGDOM

Services and industry are the major employers in the UK, and agriculture employs a relatively small workforce of half a million. This is equivalent to ~2% of the total civilian workforce, which is low by comparison with all other European countries (1). The food industry, on the other hand, employs more than 2.5 million. Agriculture and the food industry make a considerable contribution to UK exports. There is a high level of trade in food commodities: in 1997, food, beverage, and tobacco accounted for 6.5% of gross domestic product (GDP) as exports and 8.8% of GDP as imports (1).

THE REGULATORY ENVIRONMENT

The main piece of primary food legislation in Britain is the Food Safety Act (1990). Northern Ireland has equivalent legislation: the Food Safety (Northern Ireland) Order (1991). A feature of the Food Safety Act is that in addition to enabling legal rule-making, it also provides for defenses in law, including due diligence defense. Thus, manufacturers, importers, retailers, or individuals can use due diligence as a defense, if it can be shown that all reasonable precautions and due diligence were used to avoid the commission of an offense.

Central responsibility for the food supply in the UK is shared between the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health (DoH). Some responsibility in Wales, Northern Ireland, and Scotland is delegated to, for example, the Welsh Office, the Scottish Office Agriculture and Fisheries Department, the Department of Health and Social Services for Northern Ireland, and the Department of Agriculture for Northern Ireland. MAFF also maintains an executive agency, the Central Science Laboratory (CSL), at sites in Norwich and York. The CSL is responsible for the protection of consumers, food supplies, and the environment by means of food surveillance and research programs. Other executive agencies of MAFF include the Pesticides Safety Directorate (PSD; responsible for licensing and monitoring pesticides with respect to safety, effectiveness, and use) and the Veterinary Medicines Directorate (VMD; responsible for licensing and control of veterinary medicines and surveillance of residues in foods). The executive agency responsible for advising MAFF on land management, food production, waste disposal, and some aspects of food processing and retail, the Agricultural Development Advisory Service (ADAS), was privatized in 1997.

A number of independent advisory committees are established to advise government departments on aspects of food policy and safety. The main task of the Food Advisory Committee (FAC) is to advise on the safety of chemicals in foods, food additives, and food surveillance. However, policy on food labeling, including nutritional and health claims, is also determined to a large extent by the work of FAC. The assessment of novel foods, including genetically modified foods, is conducted by the Advisory Committee on Novel Foods and Processes (ACNFP). More specific toxicological expertise is available on the Committee on Toxicity of Chemicals in Foods, Consumer Products and the Environment (COT), to which specific questions may be referred by the FAC or the ACNFP. Similarly, the government has access to expert nutrition advice on the Committee on Medical Aspects of Food and Nutrition Policy (COMA). In the case of genetically modified organisms, two additional committees are involved in the research and development stages: the Advisory Committee on Genetic Modification (ACGM) and the Advisory Committee on Releases to the Environment (ACRE). The Health and Safety Executive, as advised by ACRE, is responsible for enforcing legislation concerned with occupational safety and the safety of members of the public exposed to agents in the environment. The Advisory Committee on the Microbiological Safety of Foods (ACMSF) advises the government on the risks to humans of microorganisms used or occurring as contaminants in foods. Other advisory committees that deal with the food supply include the Advisory Committee on Pesticides (ACP), the Consumer Panel, the Spongiform Encephalopathy Advisory Committee (SEAC), and the Veterinary Products Committee (VPC).

Enforcement of food legislation in the UK is largely decentralized. At a local level, food control is the responsibility of environmental health officers (EHOs) and trading standards officers (TSOs) employed by local authorities (of which there are 589 in the UK). The former deal with issues of safety and hygiene, the latter with food quality, composition, labeling, and claims. There is some overlap between the activities of EHOs and TSOs and, in some cases (eg, contaminants), either or both may be involved in an action. Local authority food inspectors are responsible for enforcing the Food Safety Act. Public analysts support the activities of Environmental Health and Trading Standards departments. In addition, samples may be referred to other laboratories, such as the Public Health Laboratory Service (PHLS) or the Central Science Laboratory. Coordination of the activities of individual EHOs and TSOs is provided by the Local Authorities Coordinating Body on Food and Trading Standards (LACOTS), the PHLS, DoH, and MAFF.

Medicinal claims are only permitted for products licensed under the Medicines Act (1968). The FAC recently published draft guidelines on health claims on foodstuffs, which is the basis for a code of practice proposed by a working group drawn from industry, local authority representatives, and consumer representatives: the Joint Health Claims Initiative. The voluntary code of practice proposes two main types of health claims associated with all foods marketed (including supplements): generic health claims based on well-established knowledge, and innovative health claims where substantiation is required in accordance with proposed criteria. The market for organic foods in the UK is small compared with Germany, Austria, or Denmark but is growing rapidly. Most certification of organic foods in the UK is conducted by the Soil Association, which was established in 1946 and contributed to the formulation of the first EC organic food regulations in 1974.

The government published a white paper in 1998 containing proposals for the establishment of an independent Food Standards Agency for the UK (2). This has been the subject of much discussion among the interested parties, including food industry, professional societies, and the relevant government departments. The starting point for such an agency would no doubt be the current Joint Food Safety and Standards Group set up by MAFF and DoH. Various templates have been proposed for such an agency, including the current Health and Safety Commission and Health and Safety Executive (HSE). The overall mission of the proposed agency would be to provide objective advice to government on the food supply and to protect public health. More specifically, the following activities have been proposed (2): policy formulation; drafting secondary legislation; representing the UK internationally (eg, at EU, WTO, and Codex Alimentarius Commission); providing information and education, food research, and surveillance, and food law enforcement; and issuing licenses and approvals. It is proposed that some of the funding for the agency will come from a levy imposed on all food premises in the UK (~600,000 manufacturing plants, shops, and catering facilities). This controversial proposal has provoked much debate.

THE INDUSTRIAL ENVIRONMENT

Mergers and acquisitions have had an impact on UK food companies as elsewhere. The most notable of such mergers was between Grand Metropolitan (parent of Pillsbury and Burger King) and Guinness at the end of 1997, creating Diageo, the world's biggest alcoholic drinks company and the UK's ninth biggest company. Following Diageo, the other major UK food companies are Albert Fisher Group Plc, Associated British Foods, Booker Plc, CPC (UK) Ltd, Cadbury Schweppes Plc, Nestle Plc, Northern Foods Plc, RHM Ltd, Unigate Plc, Unilever Plc, and United Biscuits (Holdings) Ltd.

RECENT ISSUES AFFECTING THE UK FOOD CHAIN

In recent years, the UK food system has been characterized by a series of high-profile events concerned with food safety and the acceptability of new products by consumers: the bovine spongiform encephalopathy (BSE) crisis, outbreaks of food poisoning, and the introduction of genetically modified plant foods.

BSE was first recognized in cattle in the UK in 1986, although there are suggestions that it existed earlier. The disease resembles other transmissible spongiform encephalopathies (TSEs) such as Creutzfeldt-Jakob disease (CJD) and scrapie. The disease was attributed to the use of infected meat and bonemeal in cattle feed, although maternal transmission was suspected in some cases (3). The ban on such feeding practices led to a dramatic reduction in the incidence of the disease. However, the discovery of a variant form of CJD in British patients in 1996 (4) led to the suspicion that eating infected beef was responsible. The ensuing crisis resulted in a ban on exports of British beef and the slaughter and destruction of thousands of cattle. The immunological detection of the prion protein in the tonsils and appendices of patients with variant CJD may offer the potential to better assess the number of people likely to develop the disease (5,6). To date, all cases of the variant form of CJD have been in the UK, with only one exception, and all those affected have been homozygous for methionine at codon 129 of the PrP gene, which opens the possibility that there may be a genetic predisposition to the disease (4,6).

The incidence of the variant form of CJD is now being monitored by the National Creutzfeld-Jacob Disease Surveillance Unit in Edinburgh. Some evidence, based on postmortem data collected since 1995, indicates that the incidence may be increasing (7). However, an unusually high number of deaths from the disease in the last quarter of 1998 is currently complicating statistical analysis. Continued data collection over several years will be necessary before any firm conclusions are drawn about the etiology of the disease.

Several outbreaks of microbiological food poisoning have led to illness and death in the UK in the past 10 years. These events and the publicity surrounding them, in addition to the BSE crisis, have been major driving forces in the UK government's plans to establish a Food Standards Agency with executive responsibility for the food system. An infectious disease surveillance system is operated by the Communicable Disease Surveillance Centre (CDSC) of the Public Health Laboratory Service (PHLS). The most notorious outbreak occurred in Lanarkshire, Scotland, in November and December 1996 due to cooked meat contaminated with *Escherichia coli* 0157. Almost 500 people were affected and 18 died, the second highest number of deaths associated with an outbreak of *E. coli* 0157 in the world: The age range of those who died was 69 to 93 years. The outbreak was traced to a single butcher's shop, the owner of which was subsequently successfully prosecuted in a criminal trial. The butcher's shop was closed at the peak of the outbreak, but efforts to prevent infection were complicated by the diverse distribution chain from the shop at the source of the outbreak: a total of 85 outlets throughout central Scotland were eventually identified. An expert group was set up to investigate the outbreak under the chairmanship of Professor Hugh Pennington, University of Aberdeen. Some of the recommendations of the Pennington Group can be summarized as follows (8):

1. Better education and training of farm workers, slaughterhouse staff, and food handlers.
2. Hazard Analysis and Critical Control Point (HACCP) principles should be enshrined in legislation governing slaughter houses and meat transportation; accelerated implementation and enforcement of HACCP contained in existing legislation.
3. Licensing should make provision for HACCP implementation and record keeping, including temperature control and monitoring.
4. Inclusion of food hygiene on school curricula.

Although the consequences of the Lanarkshire outbreak were serious, verocytotoxin-producing *E. coli* is a relatively minor cause of food poisoning outbreaks in the UK. In 1997 and 1998, the main contributor to foodborne disease outbreaks was *Salmonella enteritidis* (9), which is closely associated with eggs and poultry. *Camplyobacter* infections are the major cause of laboratory-confirmed enteric infections (outbreaks plus sporadic) (9). The parasitic protozoan *Cryptosporidium* has been associated with waterborne enteric infections in the south of England. The most notable outbreak had 345 confirmed cases in the North Thames area in 1997 (10). The multi-drug-resistant strain, *S. typhimurium* DT 104 is increasing in importance as a cause of food poisoning (more than 4000 cases in 1996). Most outbreaks of enteric infections in the UK in recent years have been associated with hospitals and residential institutions.

Some preliminary evidence indicates that some cases of Crohn's disease may be linked to infection with *Mycobacterium paratuberculosis*, which is present in some dairy cows (11). The organism has been the subject of several studies in the UK because of the possibility of some cells surviving conventional pasteurization when levels of contamination are high. MAFF has commissioned a survey of raw and pasteurized cow's milk to determine the incidence of contamination with viable *M. paratuberculosis* cells.

One of the most significant scientific events in the UK in recent years was the world's first cloning of a sheep at the Roslin Institute near Edinburgh. The lamb, named Dolly, was cloned from an udder cell derived from an adult sheep (12,13). The same group has reported success in the use of transgenic sheep for the manufacture of human proteins such as the clotting Factor IX (14), which opens up the possibility of so-called pharming for the manufacture of pharmaceutical products.

RESEARCH INFRASTRUCTURE

Much UK research is supported through a series of autonomous research councils, funded by government through the Office of Science and Technology/Department of Trade and Industry. Those principally involved in food and nutrition research include the Biotechnology and Biological Sciences Research Council (BBSRC) and the Medical Research Council (MRC); the Engineering and Physical Sciences Research Council (EPSRC) and the Natural Environment Research Council (NERC) have a smaller impact on the food and agricultural sciences. The past decade has seen a major restructuring of the research infrastructure in the UK, changing the way in which food research is conducted and funded. The former Agricultural and Food Research Council (AFRC) was merged with the biological sciences program of the former Science and Engineering Research Council (SERC) in 1994 to establish the BBSRC. The BBSRC supports several institutes that contribute to food research, the most important of which is the Institute of Food Research (IFR) based at Norwich. Other BBSRC institutes that contribute to food research include the Institute of Arable Crops Research (Rothamsted, Long Ashton, and Broom's Barn), Institute of Animal Health (Newbury and Pirbright), John Innes Centre (Norwich), Roslin Institute (Edinburgh), and the Silsoe Research Institute (Bedford). On average, about half of the income of such institutes is derived from the BBSRC, usually a significant amount from MAFF, with the balance coming from research grants and industrial contracts.

Research associations are a major source of support for industry in the UK and also contribute to training and research output. In recent years, all of the UK associations concerned with food research have had considerable success in developing international markets. The three main associations are Leatherhead Food Research Association (Leatherhead, Surrey), Campden and Chorleywood Food Research Association (Chipping Campden, Gloucestershire), and the British Industrial Biological Research Association (Carshalton, Surrey; now more commonly known as BIBRA International).

GOVERNMENT-FUNDED RESEARCH PROGRAMS

MAFF funds food research to support its mission that is largely concerned with protecting the public and stimulating improved economic performance. The 1997/1998 budget was ~£130 million. Collaborative research is encouraged. The LINK program is a collaborative research program, encouraging applied research initiatives in a variety of areas in which 50% of the funding is provided by government and 50% by industry. The MAFF-led programs in the LINK scheme are concerned with food processing sciences, agrofood quality, and advanced and hygienic food manufacturing. As mentioned, the UK research councils (in particular the BBSRC) are major sources of funding for food research in the UK, through a variety of schemes.

The Foresight Programme was launched in 1994 by the Office of Science and Technology of the Department of Trade and Industry. The activities of the Programme are broad and are concerned with the identification of future challenges and opportunities for UK companies. For example, thematic panels address, *inter alia*, food chain and crops for industry; health care; education, and energy. Panels draw together relevant contributions to the knowledge pool from industry, government, academe, and professional societies. The output of the Foresight Programme influences government policy, spending decisions, and research programs and initiatives.

EDUCATION

The number of universities in the UK has grown considerably in recent years as a consequence of the award of charters to many former polytechnics, which were intermediate between universities and colleges of further education. Historically, the major centers of food science education in the UK were the University of Reading (also home to the National College of Food Technology, formerly based at Weybridge, Surrey), University of Nottingham, University of Leeds, and The Queen's University, Belfast. Currently, these universities offer both BSc and thought postgraduate courses (certificate, diploma, or MSc level) in food-related subjects. Primary degrees are also offered by Bournemouth University; Glasgow Caledonian University; Loughborough University of Technology; Manchester Metropolitan University; Oxford Brookes University; Queen Margaret University, Edinburgh; Robert Gordon University, Aberdeen; Scottish Agricultural College; Sheffield Hallam University; South Bank University; University of Huddersfield; University of Lincolnshire and Humberside; University of Newcastle; University of North London; University of Plymouth; University of Strathclyde; University of Surrey; University of Teeside; University of Ulster; and University of Wales Institute Cardiff. Postgraduate courses in food-related subjects are offered by the Glasgow College of Food Technology; Manchester Metropolitan University; Oxford Brooks University; Queen's University Belfast; Robert Gordon University; South Bank University; and the Universities of Birmingham, Bristol, Huddersfield, Lincolnshire and Humberside, Leeds, London (Wye College), Reading, Strathclyde, and Wales Institute Cardiff. Many of the preceding institutions also award Ph.D. degrees in food research. Nutrition degrees are offered by the Universities of London, Newcastle, Sheffield, Southampton, Surrey, and Ulster. A number of UK universities, such as Surrey, now offer modular degrees that enable students to chose from a selection of courses shared with other degree programs, such as toxicology, nutrition, physiology, and molecular biology. A number of UK colleges of further education offer courses in food science to Higher National Certificate (HNC) and Higher National Diploma (HND) level. Some of the UK professional societies are also involved in educational initiatives. For example, the Institute of Food Science and Technology (IFST) is an examining body for the Higher Certificate in Food Premises Inspection. The European Commission is considering plans that may lead to the accreditation of courses in food science throughout Europe.

PROFESSIONAL SOCIETIES

Most UK food scientists are members of the Institute of Food Science and Technology. The London-based society was established in 1959 and currently has more than 3000 members of which almost 600 are fellows of the institute. The IFST also acts as a professional qualifying body for food science in the UK, the grade of membership reflecting the professional competence of the individual. The Institute publishes a newsletter for members (*Keynote*) and two journals for members and subscribers (*Food Science & Technology Today* and the *International Journal of Food Science & Technology*). The activities of the institute broadly reflect the activities of members and the needs of UK consumers, regulators, and industry. A number of subject interest groups address areas of specific interest including food control, food law enforcement, and the Internet. The IFST publishes regular position statements on topical issues and in 1994 formed the Professional Food Microbiology Group reflecting interest in this area.

The Nutrition Society was established in 1941 and maintains registers of accredited nutritionists and public health nutritionists. In addition to a newsletter for members, the society publishes four journals: *Proceedings of the Nutrition Society, Nutrition Research Reviews, Public Health Nutrition*, and the *British Journal of Nutrition*. Special interest groups meet to discuss specific aspects of nutrition such as clinical nutrition and metabolism, micronutrients, animal nutrition and metabolism, and nutrition and behavior. The society hosts several conferences each year; the proceedings are published in paper or in the form of abstracts in the *Proceedings of the Nutrition Society*.

The Royal Society of Chemistry (RSC) is the professional body for chemists in the UK with 46,000 members, including food chemists and technologists. The RSC serves the needs of food scientists mainly through the Food Chemistry Group, which has more than 800 members. The Society is a major publisher and publishes many books in the field of food chemistry and nutrition. Most of the society's journals are devoted to chemistry, although the monthly magazine, *Chemistry in Britain*, covers general developments including news of developments in food science. The Society of Chemical Industry serves members in the chemical, pharmaceutical, and food industries. The Society has 20 subject groups, including Agriculture and Environment, Biotechnology, Crop Protection, Food Commodities and Ingredients, Food Engineering, Oils and Fats, and Consumer and Sensory Research. The Society publishes the *Journal of the Science of Food and Agriculture*, the fortnightly magazine *Chemistry and Industry*, and a range of books on food science.

Some UK food scientists are also members of several other professional societies such as the Biochemical Society, Institute of Biology, British Dietetic Association, Institute of Physics, Society of Dairy Technology, Society for General Microbiology, Association of Public Analysts, Institution of Chemical Engineers, Royal Society of Health, and the British Toxicology Society.

The Royal Society (London) is perhaps the most prestigious scientific academy in the UK, promoting both the basic and applied sciences. Founded in 1660, the Society comprises more than 1000 elected fellows. Previous fellows include Sir Isaac Newton, Sir Humphry Davy, Lord Lister, and Lord Kelvin. The activities of the Society are broad, including a program to support the public understanding of science, awarding of small research grants, hosting of conferences and meetings (including subjects relevant to food research), publishing reports, and acting as a forum for independent expert discussions on issues affecting British society.

BIBLIOGRAPHY

1. *OECD Economic Surveys 1997–1998: United Kingdom*, Organisation for Economic Cooperation and Development, Paris, France, 1998.
2. *The Food Standards Agency—A Force for Change*, The Stationery Office, London, United Kingdom, 1998.
3. R. M. Anderson et al., "Transmission Dynamics and Epidemiology of BSE in British Cattle," *Nature* **382**, 779–788 (1996).
4. J. Collinge et al., "Molecular Analysis of Prion Strain Variation and the Aetiology of 'New Variant' CJD," *Nature* **383**, 685–690 (1996).
5. D. A. Hilton et al., "Prion Immunoreactivity in Appendix Before Clinical Onset of Variant Creutzfeldt-Jakob Disease," *Lancet* **352**, 703–704 (1998).
6. R. Smith, "Early Identification of Variant Creutzfeldt-Jakob Disease," *Br. Med. J.* **316**, 563–564 (1998).
7. R. G. Will et al., "Deaths From Variant Creutzfeldt-Jakob Disease," *Lancet* **353**, 979 (1999).
8. The Pennington Group, *Report on the Circumstances Leading to the 1996 Outbreak of Infection With* E. coli *O157 in Central Scotland, the Implications for Food Safety and the Lessons to be Learned*, The Stationery Office, Edinburgh, Scotland, 1997.
9. H. S. Evans et al., "General Outbreaks of Infectious Gastrointestinal Diseases in Scotland and Wales: 1995 and 1996," *Comm. Dis. Pub. Health* **1**, 165–171 (1998).
10. L. Willcocks et al., "A Large Outbreak of Cryptosporidosis Associated With a Public Water Supply From a Deep Chalk Borehole," *Comm. Dis. Pub. Health* **1**, 239–243 (1998).
11. R. J. Chiodini, "Crohn's Disease and the Mycobacterioses: A Review and Comparison of Two Disease Entities," *Clin. Microbiol. Rev.* **2**, 90–117 (1989).
12. K. H. Campbell et al., "Sheep Cloned by Nuclear Transfer From a Cultured Cell Line," *Nature* **380**, 64–66 (1996).
13. I. Wilmut et al., "Viable Offspring Derived From Fetal and Adult Mammalian Cells," *Nature* **385**, 810–813 (1997).
14. A. E. Schneike et al., "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei From Transfected Fetal Fibroblasts," *Science* **278**, 2130–2133 (1997).

JOHN O'BRIEN
University of Surrey
Guildford, United Kingdom

INTERNATIONAL FOOD INFORMATION SERVICE (IFIS)

STRUCTURE AND AIMS

The International Food Information Service (IFIS) is a not-for-profit organization that provides information products and services and commissions research and provides education in information science for the international food science, food technology, and human nutrition community. It comprises two companies, IFIS Publishing in the UK and IFIS GmbH in Germany. Specifically, IFIS Publishing (Charity No. 1068176, Limited Company No. 3507902) is concerned with providing information products and services; IFIS GmbH is concerned with commissioning basic and applied research and providing education and training programs in information science. IFIS Publishing houses the headquarters of the organization at Shinfield near Reading (some 40 mi west of London). IFIS Publishing has 36 staff members (three are based at the British Library at Boston Spa in West Yorkshire) and its primarily concerned with producing and marketing the database *Food Science & Technology Abstracts (FSTA)*. IFIS GmbH is based in Frankfurt and has one part-time staff member who holds the title of managing director of IFIS GmbH. IFIS also has a Japanese representative based in Tokyo. Funding for IFIS is derived solely from product sales; the organization is fully self-supporting.

IFIS is governed by a board of governors, comprising two members each from CAB International in the UK; the Bundesministerium für Landwirtschaft, Ernährung, und Forsten (BML) (represented by Deutsche Landwirtschaft-Gesellschaft e.V. [DLG]) in Germany; the Institute for Food Technologists (IFT) in the United States; and PUDOC (Centrum voor Landbouwpublikaties en Landbouwdocumentatie) in the Netherlands. The governing organizations constitute a unique consortium of one intergovernmental organization, two government ministries, and one professional association; they represent the four organizations (or their direct descendants) that were responsible for establishing IFIS in 1968. The board of governors meets twice yearly. Day-to-day responsibility is delegated to the general manager, who is based at Shinfield; the general manager also has the title managing director to describe his role within IFIS Publishing.

DATABASES AND COVERAGE

Information in *FSTA* is provided in the form of abstracts, irrespective of the mechanism of access. Abstracts are faithful descriptions of the original work. All abstracts are written in English but are prepared from source material written in more than 40 languages. Detailed author and subject indexes are provided to facilitate searching the database. *FSTA* is available in printed form, on-line, on magnetic tape, on compact disk (CD-ROM) and via the Internet (*Food Science Alerts, fsa*); it has been available in both electronic and printed formats since its launch in 1969. *FSTA* has always been available in the state-of-the-art formats, and the appearance of the database on the Internet further demonstrates the commitment of IFIS in this direction. The database can be accessed via seven hosts (DataStar, Dialog, DIMDI, EINS, Orbit, Questel, and STN). The database contains approximately 530,000 records covering the period 1969–1999 inclusive and is updated monthly (except for the CD-ROM, which has quarterly updates). *FSTA* is growing at a rate of approximately 20,000 records per year. Approximately 1800 different primary journals are scanned regularly for articles of relevance to *FSTA*. Several hundred other journals and relevant literature are also scanned on an intermittent basis. *FSTA* contains records on basic sciences relevant to food (eg, chemistry, biochemistry, physics, microbiology, biotechnology, hygiene, and toxicology) as well as food processing, food products, packaging, economics, and legislation. Of the 18 different sections into which the database is subdivided, 12 contain records on specific foods or food groups as follows: speciality and multicomponent foods; alcoholic and nonalcoholic beverages; fruits, vegetables, and nuts; cocoa, chocolate, and sugar confectionery products; sugars, syrups, and starches; cereals and bakery products; fats, oils, and margarine; milk and dairy products (including butter); eggs and egg products; fish and marine products; meat, poultry, and game; and additives, spices, and condiments. There is an ongoing policy to review coverage to reflect developments in food science, food technology, and human nutrition as they occur. This policy is evident following increased coverage of biotechnology and related disciplines since the beginning of the 1989 volume of *FSTA*.

ADDITIONAL PRODUCTS AND SERVICES

A compact disk (POLTOX) has been produced in association with Cambridge Scientific Abstracts (CSA) and the U.S. National Library of Medicine. POLTOX contains relevant abstracts taken from *FSTA* relating to food safety. A second compact disk (Human Nutrition on CD-ROM) has also been produced in association with *CSA* and the U.S. National Library of Medicine; this disk gives one source access to 14 international databases including *FSTA*. The disk gives comprehensive coverage of research on human nutrition and its direct impact on human health.

An additional service to *FSTA* users is provided by food science profiles (FSP); these consist of sets of records specially selected from *FSTA*'s current input and cover any food-related subject from aseptic packaging to viscosity. FSPs give monthly updates in print or electronic format; 30 standard titles covering "hot topics" are routinely produced, but personalized searches are available on request. Current awareness services and retrospective database searches are also available to complement the FSP service.

In June 1998 a new service, the Food and Nutrition Internet Index (FNII), was launched by IFIS. FNII is a fully searchable Web site describing and indexing food and nutrition resources available on the Internet. The main focus is on food science, food technology, and human nutrition, although there is also coverage of food business and company information.

The 2000 edition of the *FSTA* companion thesaurus will soon be available; it is an invaluable user aid and is available in conjunction with the CD-ROM, with *fsa*, and from some on-line hosts. It is also available separately as a product in its own right in both print and electronic formats.

S. Hill
International Food Information Service
Reading, United Kingdom

INTERNATIONAL UNION OF FOOD SCIENCE AND TECHNOLOGY

The International Union of Food Science and Technology (IUFoST) is a voluntary, nonprofit organization that promotes worldwide exchange of ideas and experience in those scientific disciplines and technologies relating to the expansion, improvement, distribution, and conservation of the world's food supply. Its members, "adhering bodies" in the terminology of international scientific organizations, consist of one representative food science and technology organization or committee from each country. IUFoST also has associate members, who are individuals, and who receive the Union newsletter, but have no vote. IUFoST is a scientific member of the International Council of Scientific Unions (ICSU).

OBJECTIVES

The chief aims of IUFoST are the encouragement and fostering of

- international cooperation and exchange of knowledge and ideas among food scientists and technologists;
- further development of and support for food research;
- progress in the fields of theoretical and applied food science for improvements in the processing, manufacturing, preservation, and distribution of food;
- the education and training of food scientists and technologists; and
- development of both individual professionalism and professional organization among food scientists and technologists.

Increasingly, IUFoST sees its role as that of providing services that implement the preceding objectives to its various constituencies, including its adhering bodies, international organizations, national governments, and individual food scientists. Currently, IUFoST pursues its objectives primarily through stimulation and sponsorship of international and regional congresses, conferences, short courses, and symposia.

In these activities IUFoST subscribes to the principles of ICSU, which promote the participation by all bona fide scientists without regard to race, religion, political philosophy, ethnic origin, citizenship, language, or sex.

The Union establishes such committees, commissions, and working parties as are required to cope with international issues concerning the food supply. Its activities are coordinated to supplement the efforts of other similarly interested international scientific and technological groups. A number of these activities are carried out jointly with the International Union of Nutritional Sciences (IUNS), a closely related organization. It develops projects with the World Health Organization (WHO), and has official Non-Governmental Organization (NGO) status with the Food and Agriculture Organization (FAO).

ADHERING BODIES TO IUFOST

There are currently 54 adhering bodies in the Union; several countries have membership on a nonfee-paying basis. The complete current list is as follows: Albania, Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China (Beijing), China (Taipei), Czech Republic, Denmark, Egypt, Finland, France, Germany, Greece, Hungary, Indonesia, Ireland, Italy, Japan, Kazakhstan, Korea, Kuwait, Lesotho, Lithuania, Macedonia, Malawi, Malaysia, Mexico, Mozambique, Netherlands, New Zealand, Nigeria, Norway, Philippines, Poland, Portugal, Qatar, Russia, Saudi Arabia, Singapore, Slovenia, South Africa, Spain, Sweden, Switzerland, Thailand, Uganda, United Kingdom, USA, Zambia, Zimbabwe.

HISTORY

Discussion of the need to establish an international organization of food science and technology to advance food availability, food safety, and nutrition began in Britain and North America in 1959–1960. These early conversations resulted in the First International Congress of Food Science and Technology held in London, England, in 1962. At that time an International Committee of Food Science and Technology was formed to plan further congresses and a more formal international union. Four years later, in 1966, the Second Congress was held in Warsaw.

The IUFoST was formally inaugurated during the Third Congress held in Washington, D.C., USA, in 1970. Subsequent congresses, their dates, and venues were Madrid, Spain, 1974; Kyoto, Japan, 1978; Dublin, Ireland, 1983; Singapore, 1987; Toronto, Canada, 1991; and Budapest, Hungary, 1995. The Tenth International Congress will be held in Sidney, Australia, in 1999. IUFoST decided that, beginning with the Eleventh Congress in Seoul, Korea, in 2001, it will hold International Congresses every two years. The Twelfth Congress will be held in Chicago, Illinois, USA, in 2003.

ORGANIZATION

General Assembly

The Union is governed by a General Assembly, which meets at the international congresses of the Union, and by an Executive Committee, which exercises the authority of the General Assembly between congresses.

The General Assembly develops and controls IUFoST policy and action. Its responsibilities include: the consideration of proposals involving international cooperative scientific programs and activities, the approval of methods to finance Union activities and reports of expenditures, the development of rules and procedures governing Union activities and the election of officers, and the Executive and Finance Committees.

Each member nation is allocated a number of voting delegates to the General Assembly according to its annual fee assessment; the maximum number of delegates that a country can have is five. The Executive Committee has the power to admit temporarily an adhering body to nonvoting membership without fee.

At the Ninth Congress in Budapest, the General Assembly of the Union unanimously adopted the Budapest Declaration in response to the 1992 International Conference on Nutrition in Rome. The Declaration gave special attention to those areas in which food science and technology can make a major contribution to the quality and availability of food, including reduction in postharvest losses, improvements in food quality and safety, and adaptation and improvement of traditional foods and food processing.

Fees

There is an initial entrance fee of US$100, payable on acceptance into membership. An annual assessment is made on each adhering body according to the number of votes it has in the General Assembly, with one vote per delegate, as shown in Table 1.

Union Officers

The president, secretary general, and treasurer, and three vice-presidents are elected to four-year terms by the General Assembly. Not more than two officers may be citizens of the same country. The mailing address of the secretary general is the official headquarters of the Union. The president is the principal executive officer, and the secretary general/treasurer the principal administrative officer of the Union. The vice-presidents act for the president in his absence, lead major activities of the Union as determined by the Executive Committee, and represent the Union as determined by the president.

The Union plans to establish a permanent secretariat in order to expand its activities and further develop the services it can offer. The Swiss journal *Lebensmittel Wissenschaft und Technologie* (*LWT*, or *Food Science and Technology*) is the official journal of the Union. The Union publishes its own newsletter, *Newsline*, circulated three times yearly to adhering bodies, associate members, and subscribing libraries.

Table 1. Schedule of Assessment

	U.S. dollars (US$)	
No. of Delegates	1998	1999
1	310	310
2	1,240	1,280
3	2,480	2,560
4	3,720	3,840
5	6,200	6,400

Regional Groupings

IUFoST has several regional groupings whose constitutions and purposes are consistent with those of the Union, and whose programs are specifically oriented to the interests of their regions. These include: The European Federation of Food Science and Technology (EFFoST); The Federation of Food Science and Technology in ASEAN (FIFSTA—southeast Asia); the Eastern, Central, and Southern Africa Association of Food Science and Technology (ECSAFoST); and the Latin American and Caribbean Association of Food Science and Technology (ALACCTA).

Union Committees

The Executive Committee is comprised of the IUFoST officers, including the immediate past president, as ex officio members, and seven members elected by delegates to the General Assembly from among their number. The Executive Committee is empowered to execute the policies of the General Assembly, and to act for it in conducting the affairs of the Union between Assembly meetings. It approves budgets and major activities, selects the time and place of international congresses and meetings, and acts on applications from new adhering bodies. Insofar as possible, the Executive Committee meets in developing countries timed to coincide with locally organized scientific events. This allows for a broader international participation and provides speakers without incurring additional costs to the conference organizers.

The Finance Committee provides advice on financial matters and appropriate control procedures and recommends the selection of outside auditors.

The Congress Advisory Committee serves as consultant to the organizing committee of the country sponsoring an international congress.

The Scientific Activities Committee plans, organizes, and sponsors symposia, conferences, and other scientific programs in keeping with IUFoST objectives, often in association with other interested organizations. IUFoST symposia, typically three or four per year, have been held on a wide variety of topics including food safety, sensory evaluation, food biotechnology, food composition, nutritional quality, and preservation technology.

The International Liaison Committee works for cooperation between IUFoST and other international organizations. It has been active in helping to meet the needs of developing countries, especially in Africa where it has successfully promoted the establishment of ECSAFoST, mentioned earlier.

In 1995 the General Assembly established the International Academy of Food Science and Technology. Election as a Fellow of the Academy honors those throughout the world who have made outstanding contributions to the field.

D. E. Hood
Dublin, Ireland
R. L. Hall
Baltimore, Maryland

IRRADIATION OF FOODS

THE EVOLVING NATURE OF FOOD PROCESSING TECHNOLOGY

Food is one of the basic needs of humanity. Its production and processing constitute one of the major economic pillars of all societies. And the ways in which that production and processing are done reflect the general level of technological advancement within society at any given point in time. Current food processing techniques constitute a snapshot of a dynamic system in continuous, expansive change. Driven by ever more demanding requirements of consumers, food processors continually seek ways to improve their way of doing things. Even casual reflection allows identification of several relatively recent additions to the arsenal of food processing technologies. Familiar examples of such additions include microwave cooking, ultrafilteration, ohmic heating, modified atmosphere packaging, freeze drying, high pressure processing, blast freezing, supercritical fluid extraction, reduced-fat and -calorie formulations, myriad flavor extracts and enhancers, enzymic processing, and a host of functionality modifiers. Thus, technological change and innovation is no stranger to the food industry. It is in this context of continuous change, driven by changing needs, that radiation processing of food is emerging to join the food processors' arsenal.

THE CONCEPT OF FOOD IRRADIATION HAS A LONG HISTORY

The concept of using ionizing energy to process food has a long history. Soon after the discovery of roentgen rays in 1895, a number of visionaries suggested that this new form of energy could be of benefit to food preservation. Indeed, one of the first patents based on this idea dates back to the early years of this century (1). However, as history subsequently showed, practical limitations precluded early industrial and commercial development and application of these concepts. It is only much more recently that progress has advanced to the stage where significant industrial utilization of food irradiation is becoming widespread. This follows several decades of research and development, in government, university, military, and industrial laboratories around the world, which put in place the technical and scientific foundation for the safe and effective utilization of radiation for the processing of food.

CURRENT APPLICATIONS OF RADIATION TECHNOLOGY IN AGRICULTURE

There are a number of ways, other than food processing, in which radiation and related technology are utilized in the service of food and agriculture. Examples include the following: sterile male technique for insect pest control, mutation induction for plant breeding purposes, radiotracers for studies on agrichemical pathways in the environment, studies on utilization of fertilizers and other plant nutrients by plants cultivated as human food crops, radio-immunoassays for animal physiology studies, animal husbandry research, and vaccine production to protect animals against parasites. Collectively, these techniques constitute an important component of the toolbox available to agricultural scientists in their never-ending quest for improvements to the world's food production system(s).

GROWING INTEREST IN FOOD IRRADIATION

Acceptance of any new technology by society depends on there being a genuine need for the benefits offered by that technology. In recent years there has been a veritable explosion of consumer interest in, and concern with, foodborne illness. There is a growing realization that such illness constitutes a very real problem around the world, with significant costs inflicted on the personal as well as national levels. Food irradiation is increasingly viewed as a technology that has much to offer in the ongoing battle against foodborne illness. Responsible public health agencies are urging adoption of irradiation as an additional means of protecting the safety of our food supply. The net effect of all these developments is that there now is an unprecedented level of interest in food irradiation.

TECHNICAL ASPECTS

Definition of Food Irradiation

Food irradiation is a process involving the exposure of food to a field of ionizing energy (radiation) for the purpose of effecting some desired benefit. Hence, in respect to purpose, radiation processing of food is similar to the processing of food by more conventional methods.

Energy Considerations. Food processing of any type involves energy transfer into, or out of, the food substance being treated. The form of energy involved can differ and can include thermal, chemical, mechanical, microwave, pressure, or ionizing energy (synonymous with radiation). Thus, at this basic level, food irradiation differs from more conventional forms of food processing in the form of energy involved. However, it should be noted that at this level, the conventional forms of food processing differ from one another as well.

Types of Ionizing Energy. Ionizing energy is any form of energy whose individual quanta (discrete packets of energy) are energetic enough to create ions by ejecting electrons from the atoms within a material absorbing that energy. Such energy can be photonic (pure electromagnetic energy, with no physical particles involved) or particulate (real particles involved). Examples of the former type include γ-rays, spontaneously given off by certain radioactive elements (like cobalt-60 or cesium-137) and X rays (produced by X-ray generating machines). Beams of high-energy electrons, generated by special machines called electron accelerators, constitute the most common form of particulate radiation. Any of these types of ionizing energy can be used for processing materials. However, to avoid potential induction of radioactivity in the treated material, electron energies must be kept below 10 MeV, while photons must not exceed 5 MeV.

Radiation as Processing Energy. Ionizing energy, in its various forms, possesses several characteristics that make it extremely useful as a form of processing energy (2). These include its versatility in effecting a variety of technical end points in a wide variety of materials, its ability to penetrate through the bulk of the product being treated, its ability to effect treatment without significantly increasing the temperature of the material being treated, its controllability, its flexibility, its convenience, its low cost, its environmental friendliness and its lack of residues in the treated product. Figure 1 illustrates the general concept of radiation processing. As shown in Figure 1, radiation processing of any material gives rise to change(s) in the physical, chemical, and biological properties of the treated substance. One or more of these changes in material properties constitute the purpose of the treatment. It is worth noting that radiation processing is no stranger to our daily lives. Although most consumers are generally not aware of it, there already is widespread use of consumer products whose manufacture is associated with radiation processing in one way or another, reflecting the great utility and versatility of this type of industrial processing. Some familiar examples of such products include electrical insulation, automobile tires, personal hygiene products, bandages, cosmetics, plastic films and coatings, baby soothers, packaging materials for juices and other liquids, advanced composites used in aircraft manufacture, cellulosic products (eg, fabrics) derived from plant biomass, medical devices, and even jewelry.

Process of Food Irradiation

Conceptually, the process of food irradiation is very simple. The essential elements include a shielded chamber containing a field of ionizing energy, and appropriate equipment for conveying foodstuffs through the facility. In operation, the food material is transported into the irradiation chamber, kept there for an appropriate length of time during which the desired amount of ionizing energy is absorbed, and then removed from the chamber. Upon removal from the chamber, the food material is ready for immediate utilization, which could involve consumption, further processing, or storage, as appropriate. In actual practice, the irradiation facility operator must pay attention to a lot of detail to ensure that the process is carried out properly, but that detail is invisible to both the facility client and the end user of the treated product.

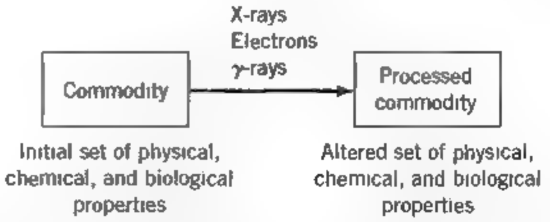

X-rays
Electrons
γ-rays
Commodity
Processed commodity
Initial set of physical, chemical, and biological properties
Altered set of physical, chemical, and biological properties

Figure 1. Illustration of principle of radiation processing. Prior to irradiation, the commodity has an initial set of properties. Following treatment, the processed commodity has an altered set of properties.

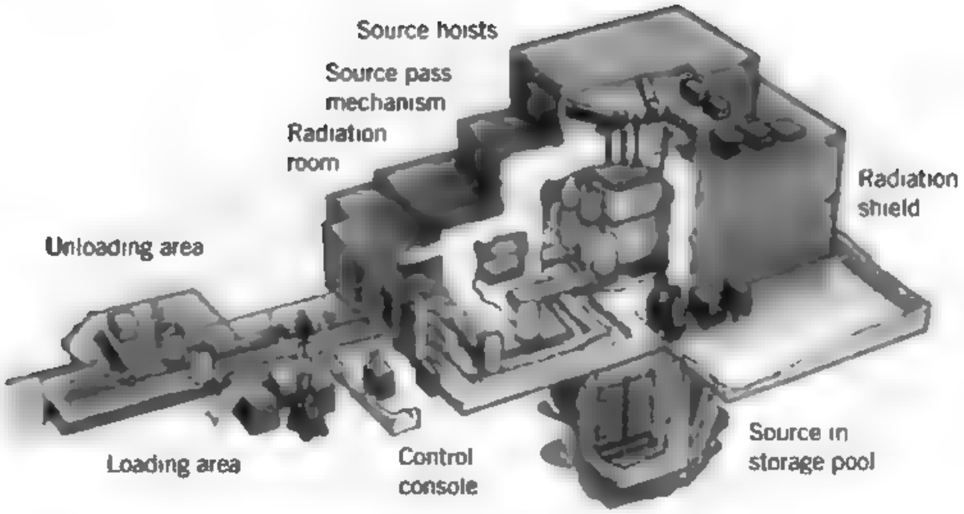


Figure 2. Cutaway view of typical γ-radiation facility, such as could be used for processing food.

Figure 2 illustrates a typical industrial irradiation plant, which can be used for treating food. The illustration shows the essential elements of such a facility, including the warehouse area, the biological shield, the maze (required to allow transfer of product through the shield while not permitting escape of radiation to the outside), the product carriers, the cobalt-60 source assembly, and the storage pool (needed for safe storage of the cobalt-60 when product is not being treated). It should be noted that, unlike the case with microwave cooking, food irradiation is an industrial process and cannot be miniaturized for use in home kitchens.

Mechanism of Action

Exposure of the food product to the field of ionizing energy within the radiation chamber results in a specified, desired amount of energy being absorbed by the food material. The amount of energy absorbed per unit mass of the absorbing material is termed the absorbed *dose* of radiation and is measured in units called *grays*. The practical unit for measuring radiation dose used in food processing is called the *kilogray*. It is the *absorbed* energy that gives rise to the effects ascribed to the treatment. Energy that passes through the product without being absorbed does not affect the exposed material. A central question is how the absorbed energy leads to the observed effects, which constitute the benefits and detriments of the process.

Thermal Effects Are Negligible. Table 1 presents data on the theoretical maximum temperature rise associated with irradiation of food products treated to effect the specified technical end points. This illustrates that, for all practical purposes, the maximum temperature rise is generally negligible. From this it follows that the benefits of the treatment are effected by nonthermal mechanisms.

Action Cascade as Mechanism. Food is a biological system, and the effects of irradiation on food constitute a particular subset of the more general effects of irradiation on any such system. Biological effects of irradiation result from a complex sequence of reactions (3) that are well described by the term *action cascade*. Figure 3 illustrates the essential features of this sequence. The action cascade consists of a series of stages, beginning with energy absorption (a physical, discrete process that occurs at random throughout the irradiated material). Through ionization and excitation pathways the absorbed energy generates a variety of primary reactive species (free radicals, ions, excited molecules) that serve to propagate the effects through the subsequent chemical, biochemical, physiological, and biological stages. The final result consists of physiological and biological effects that constitute the observable benefits and detriments deriving from the treatment. Note that each successive stage operates on a longer time scale than the preceding one.

Basis for Beneficial Effects. Given that the initiating event (energy absorption) of the action cascade is unavoidably random in its occurrence within the treated material, it is instructive to examine the basis for beneficial effects of food irradiation. Intuitively, it is not obvious how random acts of molecular damage can give rise to a net benefit. The secret lies in the fact that different functional entities within biological systems exhibit large intrinsic differences with respect to their sensitivity to inactivation by ionizing radiation. Empirical characterization of the individual dose response curves for inactivation of specific biological functions of interest allows exploitation of these differential dose responses for our benefit, in favorable cases. Thus, *differential dose response underlies the benefits of the process*. This is illustrated schematically in Figure 4. With reference to Figure 4, it can be seen that, because the individual dose response curves are separated along the dose axis, it is generally possible to select a treatment dose suitable for effecting a desired technical end point (such as insect killing) without at the same time inducing significant detriment to the nutritional value, taste, or texture of the treated food.

Benefits

Technical End Points of Irradiation. Empirical observation has demonstrated a variety of possible technical

Table 1. Temperature Rise Associated with Food Irradiation

Dose (kGy)	Typical application	Energy Absorbed (J/kg)	Temperature rise (°C., water)
0.1	Sprout inhibition	1×10^2	0.024
0.3	Insect disinfection	3×10^2	0.072
1	Ripening delay	1×10^3	0.239
3	Meat and poultry pasteurization	3×10^3	0.716
10	JECFI limit[a]	1×10^4	2.390
50	Sterilization	5×10^4	11.94

[a] The Joint Expert Committee on Food Irradiation (JECFI) in 1981 set an upper limit of 10 kGy on the permitted dose for food irradiation, pending further evaluation of the data on safety and wholesomeness at higher doses. That limit has since been removed.
Source. Ref. 8.

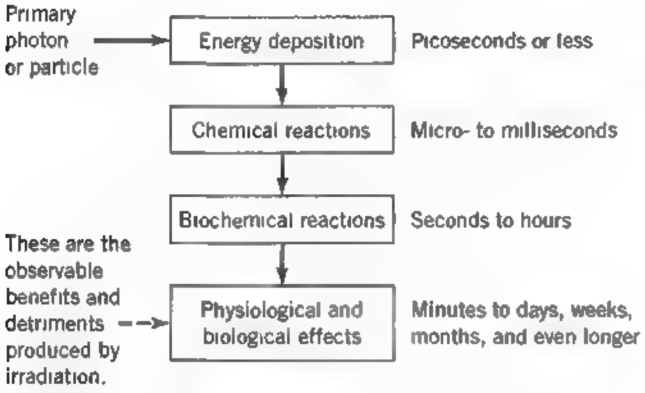

Primary photon or particle
Energy deposition
Picoseconds or less
Chemical reactions
Micro- to milliseconds
Biochemical reactions
Seconds to hours
These are the observable benefits and detriments produced by irradiation.
Physiological and biological effects
Minutes to days, weeks, months, and even longer

Figure 3. Schematic representation of the action cascade that underlies the biological effects of irradiation.

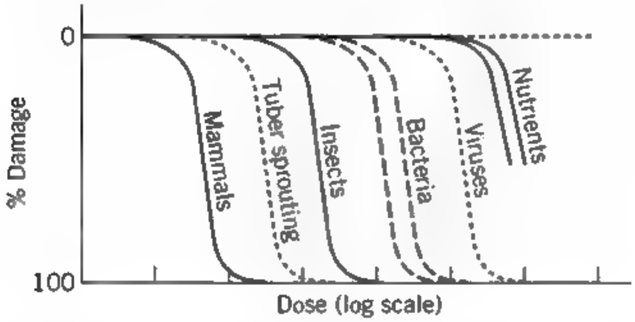


Figure 4. Schematic representation of dose response curves for inactivation of viability or other functionality in various biological systems. Differential dose response underlies the benefits of food irradiation.

benefits (4). These include microbial inactivation, insect disinfestation, sprout inhibition, maturation delay, and functional modification (eg, viscosity reduction, accelerated rehydration of dried preparations, reduced cooking time). Particular beneficial effects are associated with particular foods, and their achievement requires that an appropriate treatment protocol be followed. Only certain foods can be treated beneficially for any particular desired technical end point. It is generally not possible to elicit arbitrarily specified benefits in arbitrarily chosen foods, although some technical end points (eg, microbial inactivation) are less restrictive in this regard than others (eg, maturation delay).

Tangible Benefits for the Consumer. To the consumer, irradiation offers the benefits of food that is safer, fresher, more convenient, and therefore of higher value than the unirradiated counterpart. Of course these tangible consumer benefits derive from the specific technical end points just listed.

Limitations

As is the case with any food processing method, there can be detriments as well as benefits arising from irradiation. Negative effects of the radiation processing of food can include undesirable alteration of sensory properties (color, odor, taste, texture), loss of desired functional properties, and loss of some nutrient value. These negative effects tend to increase with increasing dose. To have a successful treatment, it is essential that unacceptable levels of detriment be avoided.

Avoidance of Detrimental Effects. To ensure a favorable result, irradiation must be carried out according to a proper treatment protocol, which has been validated by empirical testing. As with any processing method, improper application of the process will generally lead to unacceptable results. A treatment protocol is simply a 'recipe' wherein the process variables affecting the outcome are controlled within an appropriate range. Every product requires its own treatment protocol. In this sense irradiation is no different than conventional baking or cooking, which is done according to a proven recipe. There are many proven treatment protocols available, but there is ample opportunity for further development in this area.

SAFETY AND WHOLESOMENESS

Confidence in the safety and wholesomeness of irradiated food is critical for consumer acceptance of such products. It is instructive to examine the scientific approaches that have led to the current understanding in this vital area.

Approaches Used to Evaluate Safety and Wholesomeness

Two principal approaches are used to determine the safety and wholesomeness of food treated in any manner, including by irradiation. The first is based on chemical analysis and involves the identification and toxicological characterization of any new chemicals generated by the treatment in question. The second approach is one based on biological function and involves feeding the treated food to test animals, which are subsequently carefully observed for any manifestations of harm resulting from ingestion of that food. These two approaches are complementary, and together they constitute an extremely powerful paradigm for evaluating the overall safety and wholesomeness of irradiated food.

Summary of Findings from Chemical Approach

Chemical analysis of irradiated foods has led to the detection and identification of something in excess of 100 compounds referred to as radiolytic products (5). Evaluation of the toxicological significance of these identified compounds can be done by reference to available toxicological data for the compounds in question, or by comparisons with the same and similar compounds present in various foods either naturally or as the result of conventional processing. For the vast majority of identified radiolytic products the data reveal that the same compounds are present in various foods, either naturally or after conventional processing, and generally at much higher concentrations than are generated by irradiation. The results of these evaluations overwhelmingly support the conclusion that there is no indication of harm from consumption of irradiated foods (6).

Summary of Findings from Feeding Tests

There is a very large body of published literature describing toxicological studies on various animals fed a variety of irradiated foods (7). These tests have ranged from short term to long term, multigenerational in duration. In the various studies, the state of health of the test animals has been evaluated by a multiplicity of tests, examining many critical aspects of their function, including life span, reproductive ability, cancer incidence, birth defects, blood chemistry, physiology, chromosome aberrations, and even feed conversion efficiency. The vast body of literature describing these studies has been examined by several groups of experts (8,9,10). Results of these expert evaluations indicate that there are no reproducible findings of harm to humans and other mammalian species resulting from consumption of irradiated foods, in respect to dose, biological function monitored, test species, or variety of test foods.

Public Health Institutions Endorse the Safety and Wholesomeness of Irradiated Food

The available information on safety and wholesomeness of irradiated foods has been examined by many of the most prestigious and respected public health institutions around the world. Without exception these evaluations have led to unconditional reaffirmation of the absence of hazard attributable to irradiation. Such endorsements have been extended by the World Health Organization, American Medical Association, U.S. Food and Drug Administration, American Dietetic Association, American Gastroenterological Association, Mayo Clinic Health Letter, the UK Advisory Commission on Irradiated and Novel Foods, plus many others.

OVERVIEW OF IMPLEMENTATION PROGRESS

The Needs Driving Implementation

Several unmet needs are driving implementation of food irradiation. The two major ones include food safety concerns arising from foodborne pathogens and parasites, and the need for effective quarantine security treatment of agricultural commodities in interregional trade. Of lesser impact are the desire for extended shelf life of foods, reduction of food losses due to spoilage, and particular functional modifications.

Food Safety Concerns

Foodborne Illness Is Increasing. Most experts agree that foodborne illness is on a long-term upward trend in countries around the globe. This is in contrast to the dramatic decline of infectious disease overall (not foodborne), resulting from improvements in health care and in particular the advent of modern antibiotics and vaccines. The rise in foodborne illness has occurred in parallel to widespread, extensive changes in the structure of our food system, suggesting a cause-and-effect relationship. Over the last several decades, this system has evolved into its present form, markedly different from that which existed previously. In particular it has acquired new attributes that are essential for provision of a plentiful supply of affordable food to a largely urban population. These essential features include mass production of farm animals reared for food, central processing, widespread distribution of products, a diversity of sources of both raw and finished products, increasing trade between previously isolated regions, and recycling of nonedible portions of animals. These features make the system very efficient, but unfortunately they also make it very vulnerable to dissemination of infectious agents. Given that the social changes (principally urbanization) driving this evolution are effectively irreversible, it follows that the essential features of our current food system will not change. Thus, the negative effects will also persist, unless some intervention can be introduced to reduce or eliminate them without affecting the desired features.

Social Costs of Foodborne Illness. Estimates of the impact of foodborne illness on society are indirect and are based on incomplete data and imperfect methodology. Nevertheless, available data indicate that annually the financial cost in the United States alone is in the billions of dollars, with millions of cases of illness, and some thousands of deaths (11). There may be some disagreement on the exact values for these three categories of cost, but there is agreement that the magnitudes are reasonably accurate. From this it is evident that foodborne illness constitutes a serious burden on society, even in the developed countries. It can be expected that the problem is much worse in developing countries that lack the developed food system infrastructure central to the provision of safe and affordable food for large concentrations of people.

Irradiation and HACCP. Recent times have seen the introduction of new and more effective measures to protect the safety of our food supply. The Hazard Analysis and Critical Control Point (HACCP) approach represents the most advanced and comprehensive attempt in this regard. HACCP constitutes a rational plan for ensuring food safety by first identifying all possible hazards (biological, chemical, physical) associated with the production of any given food, and then introducing appropriate barriers or critical control points (CCPs) to prevent those hazards from reaching the consumer. For foods derived from animals (red meat, poultry, seafood) the microbial hazards (salmonella, *Escherichia coli* 0157:H7, campylobacter, listeria, vibrio, toxoplasma, other) are of greatest concern. HACCP plans for such foods require one or more CCPs of proven effectiveness against microbial pathogens to reduce these hazards. Irradiation is a true kill step and can reliably achieve several logs of kill (12,13) of all the common microbial pathogens responsible for the majority of foodborne illness in the United States and other countries. Inclusion of irradiation as a CCP against microbial hazards greatly enhances the effectiveness of any HACCP plan. Conversely, irradiation works best as part of an overall HACCP program. In this sense, irradiation and HACCP are natural partners in protecting the safety of our food.

Quarantine Security The need for effective quarantine security treatment constitutes the second major driver behind implemention of food irradiation.

Role in International Trade. An extremely important consideration in the trading of agricultural commodities between different regions involves protecting the importing region against the introduction of pests from the exporting region. Unwitting introduction of pests into a region where they are not yet present poses substantial risk of damage to the ecology and economy of the receiving region. For this reason such trade is contingent on the availability and use of effective quarantine treatment against pests of concern. In general, every importing jurisdiction has a system in place to enforce this requirement and prevent entry of products that have not been adequately treated. Historically, such treatment has involved the use of poison gas, such as methyl bromide, to fumigate the shipments of product. The need for treatment is pervasive, and essentially every traded product of agricultural origin is fumigated at least once, and in some cases several times, before reaching its final destination. Without availability of an approved and effective quarantine security treatment, international trade in agricultural products would be dramatically curtailed.

Loss of Methyl Bromide. For decades methyl bromide has served as the universal fumigant for quarantine security treatment of agricultural products. Unfortunately, methyl bromide is an ozone-depleting chemical and as such has been designated by international agreement (14) to be removed from service. The current phaseout schedule calls for this fumigant to be taken out of use in the United States early in the first decade of the twenty-first century. Loss of methyl bromide is expected to create a major disruption in trade of the affected commodities. Irradiation is a good alternative to methyl bromide for disinfestation of agricultural commodities for quarantine security purposes and may well be the only treatment available for many products when fumigation is no longer an option.

Costs of Food Irradiation

Cost considerations are critical in the determination of whether a particular process will be adopted by industry. Radiation processing is a capital-intensive technology and as such requires outlay of considerable funds before there can be any incoming flow of revenue.

Capital Costs. Capital costs for irradiation facilities depend on a number of variables, which will have different values for different enterprises. Main components common to all facilities include the biological shield, material conveyance system, source of ionizing energy, ventilation system, computer and operating software for the facility, and ancillary equipment (water purification and cooling). Depending on the required throughput capacity, type of plant, and degree of plant sophistication desired, capital cost for the irradiator could range between a low of perhaps $2 million (US) to possibly as high as $10 million with a median value of about $5 million. In addition, there will be a cost for land and warehouse requirements, which are site specific.

Operating Costs. The cost model for operating a food irradiation facility involves a large number of financial variables, including capital amortization and depreciation, labor, utilities, administration, taxes, insurance, radiation safety officer, license and permits, supplies, radioisotope replenishment (for a gamma plant), repair and maintenance, as well as process-related ones like dose, product density, radiation utilization efficiency, actual annual throughput, and plant utilization (% of maximum design capacity). Values for each of these variables will differ between individual operations, but in general the unit processing cost for a typical plant will be a few cents a pound, ranging from perhaps 2 cents to possibly 10 cents a pound, depending on the particular situation. The lower end of the cost range would apply to large capacity plants operated near maximum capacity, processing in excess of about 100 million lb per year.

Service Options

Radiation processing of food represents a fledgling industry, where the commercial infrastructure is not yet well developed. Thus, the delivery of service to potential users of this technology faces obstacles such as availability and accessibility of processing capacity, and additional costs arising from transport to and from existing facilities, which may be at some distance from the food manufacturing plant. It can be expected that as significant demand for service develops, the marketplace will implement solutions to these shortcomings. The ultimate structure of the industry will undoubtedly include a variety of irradiator business configurations, tailored to respond to different business needs.

Type of Facility. Irradiators can differ in regard to the type of source providing the required processing energy. This can either be radioisotope based, using cobalt-60 or, to a lesser extent, Cs-137 (both of which emit γ-rays), or it could be machine source based, utilizing electrons or X-rays. Each source option has particular advantages and disadvantages, and selection of the best choice for a particular application requires careful technical and economic evaluation in relation to the particular needs of that application.

Facilities can also differ in respect to their processing flexibility and relationship to the primary manufacturing location for the product requiring irradiation. Dedicated plants can be optimized for the particular product to be treated, providing both economic and product quality advantages. This type of plant would be integrated into the overall manufacturing process, both physically and operationally. It would be suitable for large processors who produce enough product to utilize the entire capacity of the irradiator. This option would not be suitable for the many smaller manufacturers whose requirements do not justify a dedicated facility. To service these smaller users, contract service providers using multipurpose facilities are the logical answer. Contract service facilities require greater processing flexibility than is present in the dedicated type and would be stand-alone enterprises, separate from the manufacturing site. Business considerations will ensure that such contract service facilities are optimally located and configured to provide access to efficient and economical radiation processing on a regional basis.

Since the existing irradiation facilities were mostly designed to treat nonfood items, their suitability for treating food, which has some very stringent processing requirements, is less than optimal. Several companies have initiated new designs intended to rectify the shortcomings of the existing facilities.

Business Arrangements. Needs relating to facility ownership, financing, location, and operation will be met by particular arrangements that reflect the unique circumstances of each business enterprise. The marketplace will ultimately decide on the most advantageous arrangements.

Regulations

Food irradiation can only be carried out in accordance with appropriate regulations in the country of use.

Philosophy of Regulatory Approach. In the majority of countries around the world, food irradiation is regulated by means of a blanket prohibition, with provision for exemptions, or clearances, which are granted for specific food items, for a specified purpose. Thus, only those food items that are explicitly named within the law as being exempt from the prohibition can legally be processed by irradiation. Origins of this regulatory approach date back to the early days of development of food irradiation technology when information about the safety and wholesomeness of irradiated foods was lacking. This prudent approach reflected the novelty of the process at that time, and the desire to ensure to the maximum extent possible the safety of a nation's food supply while leaving an avenue for industry to introduce useful new technology.

Clearances. The first clearance for irradiation of a food item was that for grain, granted in the former USSR in 1959 for insect disinfestation purposes. Currently there are some 227 specific clearances, in 39 countries (15), and the list continues to grow. South Africa leads the way, with 82 specific clearances. The effective list of clearances is much larger than the 227 items just noted, because many of the clearances are for categories, such as cereal grains, fish, seafood, fruits and vegetables, dehydrated mixtures and condiments, which are composed of a large number of individual food items within the category. At present there is a clear need to harmonize clearances internationally, especially between countries that are trading partners, to avoid development of nontariff trading barriers. This latter requirement is a special concern of the International Consultative Group on Food Irradiation (ICGFI), which is a United Nations–based organization mandated to coordinate food irradiation initiatives around the world.

Labeling Requirements. Current regulations governing food irradiation specify that irradiated product must be clearly labeled as having been treated in this manner. The exact wording of the label can vary somewhat from country to country, but in most cases it must include both the radura logo (shown in Figure 5) and text explicitly stating that the product has been irradiated. Most jurisdictions permit the inclusion of an additional message of an educational nature, conveying to the consumer the benefit effected by the process. Acceptable text on labels could be "Treated by irradiation to control harmful bacteria" or "Treated with radiation to control harmful bacteria." The phrase describing the benefit can be adjusted to accurately describe the purpose and benefit, as long as it is not misleading. The intent of these labeling requirements is to ensure that the individual consumer has the opportunity to make an informed choice in regard to selecting irradiated product. In the United States the prominence of the label text must be similar to that of the ingredient list.

Logistical Considerations

Implementation of food irradiation poses some difficult logistical challenges, stemming from the requirement to match scarce processing resources with the emerging needs in a regionally disseminated food industry. These

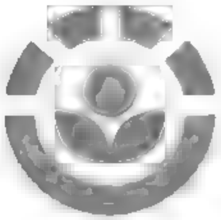


Figure 5. Radura symbol. This is the internationally designated symbol to identify food that has been treated by irradiation.

difficulties are most pronounced during the early stages of the implementation process and will diminish as new facilities are brought into service.

Limited Availability of Processing Capacity. At present few irradiation facilities are dedicated to food irradiation, anywhere in the world. Where food processing is being done, it generally utilizes surplus capacity in plants designed primarily to serve the sterilization needs of the medical device industry. Thus, aside from a few exceptions, little unused capacity is available for treating food. In the United States at present, one commercial irradiator, the Food Technology Services Incorporated plant in Florida, is dedicated exclusively to processing food, and at least two facilities have the capability for treating food along with other products. There is some spare capacity in existing contract irradiation plants, but far short of what would be required to handle even a small fraction of the country's potential food processing requirements. The same situation holds for most other regions of the world.

Geographic Distribution and Access to Facilities. The general lack of availability of radiation processing capacity for food is compounded by the fact that the location of the available capacity does not match with the location of the meat and poultry processors, which constitute the main base of potential clients for the technology in the United States. This reflects the fact that the existing irradiation facilities were located to serve needs unrelated to the needs of the food industry, and this lack of correspondence can only be resolved by building new facilities in more suitable locations. Such new facilities will be located by reference to both food manufacturing plants and existing geographic flow patterns of product. In general, irradiation plants are most efficient if located at node points (such as warehouses, distribution centers, manufacturing plants) in the product flow patterns, since such points are natural sites for product concentration. High throughput of product is essential for achieving favorable processing economics.

Time to Build. Experience has shown that from concept to commissioning of a new plant requires approximately 15 to 24 months. This time frame could be significantly shortened if a standardized design were adopted. In addition, several projects could be developed concurrently, so that after an initial lag period, new capacity could be coming on stream very rapidly.

Seasonality of Some Products. An additional complication arises from the fact that many agricultural products requiring treatment are seasonal. This is especially true for produce (fruits and vegetables). Thus, for part of the year there would be unused capacity, unless some secondary product(s) could fill in the low usage periods. This seasonal variation in availability of product for treatment impacts negatively on the processing economics.

CURRENT STATUS

State of Public Readiness to Accept Food Irradiation

Indications are that in North America there is an unprecedented willingness to accept irradiated food. This positive attitude cuts across a broad cross section of society, including regulators, politicians, public health professionals, academics, food processors, food industry associations, and the majority of consumers. Also, mass media has become more supportive. This favorable environment has developed relatively recently, and in a rather short time. The driving force behind these latest developments has been the explosive growth in public awareness of foodborne illness. This was unleashed by an outbreak of foodborne illness in the Pacific Northwest region of the United States in 1993, when several children died as a result of eating contaminated hamburgers in a fast-food chain outlet. Since that time there have been a continuing series of additional, highly publicized recalls of contaminated products, in some cases accompanied by plant closures and business failures. The net result is that consumers have become highly sensitized to the issue of foodborne pathogens like *E. coli* 0157:H7, listeria, salmonella, campylobacter, vibrio, and the like. The heightened level of consumer awareness and demand for safer food has led to implementation of new, more stringent food safety regulations in the United States, with specific pathogen reduction standards, increased testing and mandatory HACCP plans.

Along with the consumer awareness factor, there is a growing realization among food manufacturers of the limitations of end-product testing to combat foodborne disease, and of the need for an effective kill step to eliminate pathogens from their product. Radiation pasteurization of those foods that are at high risk of being contaminated is increasingly recognized as a safe and effective intervention technology to help solve this problem (16,17). The 1998 consumer survey conducted by the Food Marketing Institute in the United States clearly reveals that the majority of consumers are willing to purchase irradiated foods (18). This survey finding confirms the positive results obtained with real, albeit limited (but growing), market experience in the United States in the last few years. In addition, it is becoming more evident that as consumers learn more about the process, their willingness to accept it increases.

FUTURE OUTLOOK

From numerous indications, it is clear that there is a rising ground swell of support for implementation of food irradiation in the United States. It appears increasingly likely that a major breakthrough in terms of significant commercial and industrial use may happen relatively soon. When that comes to pass consumers will at long last have the opportunity to choose and pass judgment on the promise of this technology.

In summary, the current status is perhaps best described by a quotation from the late Adlai Stevenson:

> That which seems the height of absurdity in one generation . . . often becomes the height of wisdom in another.

Perhaps the age of wisdom in respect to food irradiation has finally arrived. The next few months and years will be critical.

BIBLIOGRAPHY

1. J. Appleby and A. J Banks, British Patent No. 1609 (Jan. 26, 1905), cited in J. F. Diehl, *Safety of Irradiated Foods*, 2nd ed., Marcel Dekker, New York, 1995.
2. *Proceedings of the 10th International Meeting on Radiation Processing, Radiation Physics and Chemistry* **52**, Numbers 1–6, June 1998.
3. C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor and Francis, London, United Kingdom, 1987.
4. "Practical Applications of Food Irradiation," in *Food Irradiation. A Technique for Preserving and Improving the Safety of Food*, World Health Organization, Geneva, Switzerland, 1988, pp. 33–43.
5. C. Merritt, Jr., "Qualitative and Quantitative Aspects of Trace Volatile Components in Irradiated Foods and Food Substances," *Rad. Res. Rev.* **3**, 353–368 (1972).
6. *Evaluation of the Health Aspects of Certain Compounds Found in Irradiated Beef*, Report AD-A045716, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md., 1977 (Supplement in 1979)
7. J. Barna, "Compilation of Bioassay Data on the Wholesomeness of Irradiated Food Items," *Acta Alimentaria* **8**, 205–315 (1979).
8. Joint Expert Committee on Food Irradiation, *Report of a Joint FAO/IAEA/WHO Expert Committee*, Technical Report No. 659. World Health Organization, Geneva, Switzerland, 1981.
9. *Ionizing Energy in Food Processing and Pest Control: I. Wholesomeness of Food Treated With Ionizing Energy*, Report No. 109, Council for Agricultural Science and Technology, Ames, Iowa, July 1986.
10. U.S. Food and Drug Administration, "Irradiation in the Production, Processing and Handling of Food. Final Rule," *Federal Register*, Friday April 18, 1986.
11. J. C. Buzby et al., *Bacterial Foodborne Disease: Medical Costs and Productivity Losses*, Agric. Econ. Report No. 741, U.S. Dept. of Agriculture, Washington, D C., 1996
12. T. Radomyski et al., "Elimination of Pathogens of Significance in Food by Low-Dose Irradiation· A Review," *J. Food Protect.* **57**, 73–86 (1994).
13. D. W. Thayer, "Use of Irradiation to Kill Enteric Pathogens on Meat and Poultry," *Journal of Food Safety* **16**, 181–192 (1995).
14. United Nations Environment Program, *Proc. 4th Meeting of the Parties to the Montreal Protocol on Substances that Deplete the Ozone Layer*, Copenhagen, Denmark, November 23–25, 1992.
15. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, *Clearance of Item by Country*, Food Irradiation Newsletter Supplement 1, International Atomic Energy Agency, Vienna, Austria, December 1996.
16. C. Bruhn, "Consumer Attitudes and Market Response to Irradiated Food," *J. Food Protect.* **58**, 175–181 (1995)
17. L. M. Crawford, "Food Irradiation's Advantages Will Not Escape Public Attention," *Food Technol.* **52**, 55 (1998).
18. *Consumers' Views on Food Irradiation*, Food Marketing Institute and Grocery Manufacturers of America, Washington, D.C., 1998.

GENERAL REFERENCES

J. F. Diehl, *Safety of Irradiated Foods*, 2nd ed., Marcel Dekker, New York, 1995.

J. Farkas, *Irradiation of Dry Food Ingredients*, CRC Press, Boca Raton, Fla., 1988.

Ionizing Energy in Food Processing and Pest Control: II. Applications, Task Force Report No. 115, Council for Agricultural Science and Technology, Ames, Iowa, 1989.

E. S. Josephson and M. S. Peterson, eds., *Preservation of Food by Ionizing Radiation*, Vol. 1–3, CRC Press, Boca Raton, Fla., 1982.

E. A. Murano, ed., *Food Irradiation: A Sourcebook*, Iowa State University Press, Ames, Iowa, 1995.

M. Satin, *Food Irradiation: A Guidebook*, 2nd ed., Technomic, Lancaster, Pa., 1996.

W. M. Urbain, *Food Irradiation*, Academic Press, Orlando, Fla., 1986.

World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations, *Food Irradiation: A Technique for Preserving and Improving the Safety of Food*, World Health Organization, Geneva, Switzerland, 1988.

JOSEPH BORSA
MDS Nordion, Inc
Kanata, Ontario
Canada

Index

Index terms **Links**

A

Abomasum 47
Absorption 509
Absorption refrigeration cycle 927
Absorption spectrophotometry 868
Absorptive phase, of metabolism 88
Acacia gum 1222
Acceptable daily intake (ADI) 1212
Acerola 1154
Acesulfame-K 859 2245 2248 2252 2255
Acetates, as antimicrobial in foods 64
Acetic acid, as antimicrobial in foods 64
Acetobacter 1632
Acetylated glycerides, films of 581
Acetylgeniposide 397
Acid cheeses 299
Acidic detergents, corrosion by 432
Acidification 1505 1615
Acidified foods 1501
Acid-modified starches 2207
Acidophilus milk 916
Acid phosphatase, in marine animals 1528
Acids, food surface sanitation 1039
Acidulants 1 134 172 624
Actellic 1212
Actinidine 30
Active modified atmosphere packaging 1140 1563
Additives *See* Food additives
Adenosine triphosphate (ATP), carbohydrate metabolism 1059 1062
Adenosylcobalamin 2553
Adhumulone 72 158
Adlupulone 72

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Adsorption	509			
Advanced Systems Concepts Directorate (ASCD)	2364			
Adverse reaction monitoring system (ARMS)	1053			
Advertising	937			
Aeration, aquaculture	116			
Aerobic plate count	1624			
Aeromonas	1958			
Aeromonas hydrophia	1087			
Aerosol containers	969			
Affinity column	1348			
Affluence, nutrition-related diseases	6			
Aflatoxins	1080	1353	1354	1700
	2344			
Africanized honeybees (AHBs)	151			
African saffron	390			
Agar	1218	1219		
Agaricus mushrooms	2338			
Agglomerated cereals	205			
Agglomerated powders	16			
Agglomeration	13			
caking	13			
coffee	360			
equipment	569			
fluid-bed agglomeration	567			
mechanism	568			
methods	14			
spray-bed dryer agglomeration	571			
spray dryers	567			
undesired	13			
Agglutination assay	1347	1357		
Aging, meat	1589			
Agitated batch (in-container) heating	2324			
Agitated thin film evaporator	678			
Agitation, hydrogenation and	1314			
Aglycones	375			
AgResearch (New Zealand Pastoral Agriculture Research Institute Ltd.)	1404			
Agriculture	902			
See also Crops; Food crops				
fumigants	1172			

Index terms	**Links**	
Agriculture *(Continued)*		
functional foods	1179	
Germany	1394	
grain protectants	1212	
grapes	2662	
integrated pest management (IPM)	2690	
organic farming	2425	
peanuts	2679	
radiation technology	1428	
South Africa	1407	
Spain	1410	
tea	2292	
ultrafiltration and reverse osmosis	2361	
U.S. Department of Agriculture	2365	
vegetables	2421	
Agriculture and AgriFood Canada (AAFC)	1386	
Agroclavine	29	
Agronomic crops	902	
Air, as leavening agent	133	
Air-blast freezers	1118	1119
Air-oxygen decarburization (AOD)	424	
Air suspension coating	622	
Air-to-air heat recuperators	565	2725
Air-to-liquid heat recuperators	566	
Ajmaline	29	
Alaskan snow crab	439	
Albacore tuna	831	
Albumen, films from	580	
Alcohol		
absorption	21	
fetal alcohol syndrome	25	
hangover	24	
as medicine	24	
metabolism	19	
Muslim dietary laws	1683	
physiological actions of	22	
sexual function and	81	
Alcohol dehydrogenase (ADH)	19	

Index terms **Links**

Alcoholic beverages 7 19
  See also Beer
  alcohol absorption 21
  alcoholism 25
  alcohol metabolism 19 20
  Australia 1381
  beer. *See* Beer
  champagne 919
  distilled beverage spirits. *See* Distilled beverage spirits
  enzymes and 654
  fermentation 918
  flavor 837
  hangover 24
  history 19
  nitrosamines in 1713
  physiological effects of alcohol 22
  sake 920
  Taiwan 1419
  types of 19
  wine. *See* Wine
Alcoholism 25
Aldehyde dehydrogenase (ALDH) 19
Ale 154
Algae 403
Alginates 580 1217
Alginic acid 1217
Algins 1217 1218
Alimentary system, food utilization 1058
Alimentary toxic aleukia 2347
Aliphatic alcohols, in olive oil 1765
Alitame 860 2245 2248 2254 2255
Alkaline detergents, corrosion by 432
Alkaline proteases in marine animals 1528
Alkaloids 26
  in chocolate 350
  classification 26 33
  ergot alkaloids 29 31 1703 2349
  food alkaloids 31 1703

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links	
Alkaloids *(Continued)*		
properties	26	
pyrrolizidine alkaloids	32	2340
solanum alkaloids	30	2341
Alkanes, in olive oil	1765	
Alkannet	379	380
Allergenic mites	2031	
Allergy. *See* Food allergy		
Allicin	71	
Allophycocyanins	399	
All-purpose flour	2649	
Allspice	2199	
Allyl isothiocyanate	2336	
Almonds	2681	
Alpha bitter acids	72	
Aluminum, corrosion	416	
Aluminum cans	1825	
Aluminum foils	1825	
Aluminum phosphide	1173	1174
Amadori and Heyns rearrangement products (AHRP)	840	
Amadori products	877	878
Amanita toxins	34	
Amaryllidaceae alkaloids	28	
Amatoxins	31	
American Bottlers of Carbonated Beverages (ABCB)	175	
American Bottlers Protective Association (ABPA)	175	
American cheese	917	
American Council on Science and Health (ACSH)	1015	
American Dietetic Association (ADA)	872	1015
American eel	586	
American foulbrood disease	150	
American lobster	1496	
American plaice	820	
American saffron	390	
American Society for Clinical Nutrition (ASCN)	873	
American Society for Nutritional Sciences (ASNS)	873	
Amino acids	1988	2015
analysis	2014	

Index terms	**Links**			
Amino acids *(Continued)*				
chemical properties	1992			
industrial production	1996			
in livestock feed	1492			
physical properties	1993			
safety	1998			
in tea	2294			
toxicity	1998			
uses	1993	1998		
Aminopeptidase	1061			
Ammonia, aquaculture	103	116		
Ammonia injury, fruits and vegetables	906			
Ammonium bicarbonate, as leavening agent	133	185		
Amnesic shellfish poisoning	1534			
Amperometric biosensors	2108			
Amphoteric emulsifiers	618			
Amylase	658	2170		
assay	648	649		
baked goods	653			
digestion	1060			
Amyloglucosidase	658			
Amylopectin	880	2203		
Amylose, structure	2203			
Anabasine	27			
Anaerobic fermentation, baked goods	137			
Anatase	402			
Ancho pepper	2404			
Anchovies	814	1537		
Androstenediol	83			
Androstenol	83			
Anemia	1300			
Anhydrous milk fat (AMF)	227	471	472	1659
Animal assay, mycotoxins	1691			
Animal breeding. *See also* Livestock production				
crossbreeding	35			
Animal by-product processing	35			
blood	36			
fine chemicals	42			
meals	41			

Index terms **Links**

Animal by-product processing *(Continued)*
  pet food 42
  rendering 36
  sausage casings 36 42
  waste management 2601
Animal fats, animal by-product processing 39
Animal nutrition 46
Animals
  aquaculture 95
  as bioreactors 2675
  genetic engineering 2671
  history of foods 1282
  livestock feeds 1485
  livestock production 43
  marine enzymes 1525
  marine toxins 1534
  parasitic organisms 1844
  pet food 42
  reproduction 44
  transgenic 53 2672
  transmissible spongiform encephalopathies 198 199
  U.S. Department of Agriculture 2366 2368
Animal science 43 44
  See also Animals; Livestock production
Animal slaughter 47 1586 1604 1683 1965
Anionic emulsifiers 617
Anise seed 2199
Ankaflavin 398
Annato 385 1524
Anorexia nervosa 9
Ansamacrolides 34
Anthocyanidins 1873
Anthocyanin-3-glucoside adducts 377
Anthocyanins 273 375 884 1923 1928
Anthranilic acid-derived alkaloids 27
Anthraquinones 378
Antibiotics 54

Index terms **Links**

Antibodies 1346
Anticaking agents 14 847 852 2403
Antifoaming agents 845 852
Antifreeze protein 1341
Antigen 1346
Antimicrobial compounds 848
  carbon dioxide 1833
  dimethyl carbonate 67
  in food animals 54
  in foods 63
  glucose oxidase 72
  naturally occurring 68
  nitrites 66
  onions and garlic 71
  organic acids and esters 64
  packaging 1833
  parabens 66
  phenolic antioxidants 67
  phenolics 72
  phosphates 68
  plant extracts 71
  pulsed light processing 2025
  sodium chloride 64
  sulfites 67 2219
  thyme 1906
"Antioxidant paradox" 76
Antioxidants 73 848 857
  acidulants as 5
  as antimicrobials 67
  in biscuits and crackers 186
  in fats 729
  lipoic acid 1917
  in military food 1648
  phenolic 67
  phytochemicals 1895
  sulfites as 2219
AOAC International 79
Aphrodisiacs 81
Apocrine glands 82

Index terms	**Links**		
Aporphine-benzylisoquinoline dimers	28		
Aporphines	28		
Apparent extract	169		
Appetite	87		
alcohol, effect on	23		
cognitive factors	93		
conditioned hunger	90		
control of	83	89	
emotional factors	92		
flavor and	90		
metabolic influences	88		
nutrient depletion	90		
perceptual salience	91		
physiological influences	88		
satiety	88	89	91
sensory factors and	91		
social factors	93		
stimulation of	92		
Apple brandy	527		
Apple-Britex	582		
Applejack	1103		
Apple pomace	2168	2169	2170
Apples	1146	1156	2618
Apricots	1146	1157	
Aquacultural engineering	110		
Aquacultural Engineering Society (AES)	110		
Aquaculture	95		
culture systems	100	112	
defined	95		
disease control	109		
engineering and construction	110		
genetics	108		
harvesting	110		
holding systems	100	112	
lobster	1498		
marketing	110		
nutrition and feeding	106		
processing	110		
production statistics	96		

Index terms	**Links**		
Aquaculture *(Continued)*			
regulation	99		
reproduction	108		
seaweed aquaculture	2100		
solids removal	117		
Spain	1410		
species under cultivation	96		
system design	111		
tanks	101	113	
U.S. Department of Agriculture	2369		
water sources and quality	104	115	
Arabica coffee	354	355	
Arabustas	354		
Areca nut	27		
Arecoline	27	31	
Armagnac	527		
Aroma			
coffee	356		
drying	495		
evaporation and	674		
Shiitake mushrooms	1680		
wine	2664		
Aroma chemicals	856		
Aromatherapy	118		
Artificial insemination, livestock production	53		
Artificial intelligence	119		
Artificial sweeteners	185	1336	2245
Asarone	2338		
Ascorbate peroxidase	78		
Ascorbic acid	77	216	2449
See also Vitamin C			
cancer and	1897		
cardiovascular disease and	1899		
oxidation	1800		
Ascorbic acid oxidase (AAO), enzyme assay	650		
Aseptic beverages, noncarbonated beverages	179		
Aseptic packaging	122	126	
Aseptic processing	122	126	2310
acidified food	1508		

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Aseptic processing *(Continued)*				
military food	1645			
nonthermal processing	126			
ohmic heating	128			
vegetables	2411			
Aseptic surge tanks	126			
Aseptic zones	126			
Ash, gelatin	1187			
Asian ethnic foods	446			
Aspartame	836	850	860	1344
	2245	2248	2255	
Aspartate transcarbamylase, in marine animals	1527			
Aspartic acid, production	1344			
Aspergillus	1080	2344		
Aspergillus flavus	1700			
Aspergillus ochraceous	1702			
Aspergillus parasiticus	1700			
Association of Official Agricultural Chemists	79			
Astaxanthin	277	280	389	
Astringency	835	2287		
Atlantic cod	816			
Atlantic halibut	818			
Atlantic herring	821	822		
Atlantic menhaden	823			
Atlantic redfishes	825			
Atlantic salmon	829			
Atomic emission spectroscopy (AES)	868			
Atomic force microscopy (AMF)	942			
Atomic spectroscopy	868			
Atomization, spray drying	559			
ATP assay	1093			
Atropine	27			
Austenitic stainless steel	414	423		
Australia, food industry	1379			
Australian Institute of Food Science and Technology (AIFST)	1382			
Austria, licorice	1468			
Automatic flow diversion, aseptic processing	126			

Index terms	Links		
Automation			
food plant design	947		
robotics	119	1457	
AUToPLATE	1123		
Autoxidation	1796		
fats and oils	728	731	
in military food	1648		
Avicel	750		
Avidin	70		
Avocado	1163		
Azeotropes	510		
Azeotropic distillation	510		
B			
Babcock-Hart Award (IFT)	1372		
Baby foods	1369		
Bacillus	1631		
Bacillus cereus	344	1083	1631
Back-slopping	921	1614	
Bacteria			
defined	1630		
in food	1630		
foodborne disease	1844		
Bacterial infection. *See* Foodborne disease			
Bacterial receptor assay	1348		
Bacteriocins	69		
Bactofugation, cheese making	302		
Bactometer	2040		
Bael	1154		
Baffle tray	513		
Bagels	140		
Bag-in-the-box (BNB) packaging	208		
Baked cereals	205		
Baked goods			
acidulants and	4	134	
additives	853		
antioxidants in	65		
biscuits and crackers	183		
dairy ingredients in	477		

Index terms	Links		
Baked goods *(Continued)*			
dough-conditioning agents	625		
encapsulation in	625		
energy usage for	631		
enzymes and	653		
fats and oils in	741		
Finland	1391		
leavening agents	133	625	
lecithin in	1464		
modified atmosphere packaging (MAP)	1837		
phosphates	1883		
proofing temperature	138		
specialty products	139		
Standard Industrial Classification	993		
sweeteners	2240		
texture	2622		
Baking chamber, cookie and cracker dough	188		
Baking powder	136	185	
Baking soda, as leavening agent	134		
Ball compressor	319		
Balling	539		
Bamboo shoots	145		
Bananas	179	1163	
Band freezers	1124		
Barbadas cherry	1154		
Barley	153	155	291
classification	297		
composition	287		
milling	288		
nutritional analysis	296		
physical properties	292		
production statistics	290	291	
structure	293		
Barley malt	153	155	170
Bar-press machine, cookie and cracker dough	188		
Basil	1906	1907	2199
Basket centrifuge	283		
Bastard saffron	390		
Batch cooling	1230		

Index terms	**Links**		
Batch distillation	511	516	
Batch dryers	542		
Batch freeze driers	1110		
Batch freezing	1230		
Batch heating			
liquids	1228		
solids	1229		
Batch hydrogenation	1318		
Batch pan evaporator	676		
Batch pasteurization, ice cream and frozen desserts	1337		
BAX screening system	2041		
Bay leaves	2199		
Bay scallops	2152		
Beef. *See also* Meat			
cuts of	47	49	
electrical stimulation of meat	1549		
functional foods	1180		
ground beef, cooking-cooling	1267	1269	
slaughtering process	47	1614	
texture	2618		
Beef cattle			
livestock feed	1490		
livestock production	43		
red meat production	47		
Beef sticks	2164		
Beef tallow	41		
Bee glue	151		
Beekeeping	148		
Bee milk	151		
Beer	153		
alcohol content	19		
Australia	1381		
blood alcohol level	21		
brewing	153	154	159
defined	153		
dry beer	920		
fermentation	154	163	920
Finland	1391		
glossary	169		

Index terms **Links**

Beer *(Continued)*
  history 153
  ice beer 920
  krausen 171 920
  light beer 167 920
  nitrosamines in 1713
  nutritional analysis 167 168
  packaging 166
  problems 166
  production statistics 169
  quality control 168
  raw materials 154
  spoilage 1027 1028
  types of 167
  wort 159 161 171 539 920

Bees. *See* Honeybees

Beeswax 152

Beet canning 2411

Beetroot 380

Beets, colorants from 380 381

Bee venom 151

Behavior, carbohydrates and 271

Behenic acid 258

Bel Paese cheese 306 332

Belt dryers 488

Belt freezer 1121

Belt-trough dryers 489 492 2401

Benefat 750 1335

Bengal quince 1154

Benzaldehyde, food flavor and 1194

Benzoates, as antimicrobial in foods 65

Benzoic acid 65 216

Benzophenanthridines 28

Benzylisoquinolines 28

Berberine 28

Beriberi 1301

Bernard L. Oser Food Ingredient Safety Award (IFT) 1375

Berries, dehydration 1147

Index terms	**Links**		
Beta bitter acids	72		
Betalains	273	380	
Betanidin	381	382	
Betanin	382		
Beta-Trim	750		
Betel nut	27		
BET monolayer value	482		
Beverage blends, agglomeration	17		
Beverages			
acidulants	3		
Australia	1381		
carbonated. *See* Carbonated beverages			
decaffeinated coffee	237	360	
defined	177		
egg-rich drinks	598		
enzymes and	654		
Finland	1391		
lecithin in	1465		
noncarbonated	177		
Standard Industrial Classification	996		
Taiwan	1419		
types	177		
whey beverages	2656		
Bidding, food plant design	951		
Bigeye tuna	831		
Bilberries	1158	1179	1922
Bile salts	1073		
Bin dryers	488	489	2402
Binge eating	91		
Bioassay			
antibiotics in foods	56		
antibiotics in foods of animal origin	56		
mycotoxins	1691		
Biochemical analysis	870		
Biocide	501		
Biofiltration, aquaculture	103		
Bioflavors	1196		
Biofuels	2611		
Biogas	2169		

Index terms	**Links**	
Biogenic amines, in fermented meat products	1619	
Biological amines	26	
Biological leavening	137	
Biologically stable intermediates	179	
dried codfish	180	
frozen foods	182	
grape juice	182	
sulfited fruits	180	
tomato products	181	
Biological preservation	957	
Biological reactor, kinetics	1445	
Bioluminescence	2109	
Biomass, estimation	2039	
Biopolymers, computer modeling	1667	
Bioreactors		
domestic animals as	2675	
kinetics	1445	
Biosensors	2104	2106
Biostat	501	
Biotechnology		
kosher foods and	1452	
Muslim dietary laws and	1683	
Biotin	2443	2467
Biotin deficiency	2468	
Biotoxins, marine toxins	1534	
Bisbenzylisoquinoline	28	
Biscuits and crackers		
equipment	187	
formulas and processes	186	
ingredients	183	
packaging	189	
storage	189	
Bisflavanols	2295	
Bis-phenols, as disinfectants	505	
Bisphosphonates	1793	
Bitterness	835	885
Bitterness unit	171	
Bivalves		
microbiology	765	

Index terms	**Links**			
Bivalves *(Continued)*				
processing	787			
raw consumption	769			
Bixin	385	387		
Black Americans, ethnic foods of	443			
Blackberries	1158			
Black chokeberries	1159			
Blackfin longtail tuna	831			
Black olives	1766	1767		
Black pepper	2200			
Blackstrap molasses	527			
Black tea	2295	2303		
beverage	2299			
manufacture	2297			
Blanching	190	215	246	965
energy usage	629			
mushrooms	1679	1680		
vegetables	190	215	656	2408
	2409			
Blast cooling, heat exchangers	1230			
Blast freezing	791			
heat exchangers	1230			
Blast room	1119			
Bleaching				
canola oil	256			
flour	2648			
starch	2208			
Bleaching agents	852			
Blended applejack	527			
Blended whisky	526			
Bleu cheese	917			
See also Blue cheese				
Bliming	1164			
Blood, animal by-product processing	36			
Blood alcohol level	21	22		
Blood char	36			
Blood glucose	88	271	1066	
Blood lipids, carbohydrates and	271			
Blood pudding	397			

Index terms	**Links**			
Blueberries	1158	1179	1922	
Blue cheese	300	302	305	307
	309	458	917	
Blue crab	440	765	767	
Bluefin tuna	831			
Blue king crab	438			
Blue mezcal	528			
Blue whiting	818			
Bluing, of crab	441			
Bobwhite quail	1955			
Body and texture, in butter	223			
Boiler water additive	848			
Bologna, poultry bologna	466	1972		
Bond dryers	550			
Bones				
composition	1791			
development	1789			
osteoporosis	1789			
Boning				
hot boning	1557			
mechanical deboning	1265			
part carcass boning	1609			
sheep and lamb	1608			
Bonito	831			
Borneol	71			
Boston bluefish	817			
Bottled water	178	179		
Botulism	1079			
Bourbon whisky	525	529	531	536
Bovine somatotropin (bST)	52			
Bovine spongiform encephalopathy (BSE)	42	198		
Box dryers	560			
Bracken fern	2341			
Brain, and eating, control of	83	89		
Brandy	523	526	538	920
Brassylic acid, alkyl esters of	258			
Brazil nuts	2682			
Bread crumbs, agglomeration	17			

Index terms	**Links**			
Breadfruit	1163			
Breadings, extrusion	705			
Breads				
Australia	1380			
fermentation	921			
leavening agents	133			
lecithin in	1464			
shortening in	742			
specialty products	139			
spoilage	1027			
Breakfast cereals	204			
additives	853			
extrusion cooking	204	704		
Standard Industrial Classification	992			
Breast of turkey	467			
Breeding, aquaculture	108			
Brevetoxins	1534			
Brevibacterium	1731			
Brewing (of beer)				
fermentation	154	163		
filtration	161			
finishing	163	165		
history	164			
kettle	159	161		
lautering	161			
light beer	167			
mashing	159			
milling	159			
packaging	166			
plant operations	168			
problems	166			
quality control	168			
raw materials	154			
yeast	159	160	162	163
	171			
Brewing (of coffee)	358			
Brewing (tea)	2299			
Brick cheese	299	300	302	303
	306	307	308	309
Brick margarine	1521			

Index terms	Links			
Brie cheese	301	302	333	
Brines, corrosion by	430			
Broadbill	832			
Broad fish tapeworm	1850			
Broken heating	2316			
Bromine, as disinfectant	502			
2-Bromo-α-ergocryptine	30			
Brown extract algae	403			
Brownies	186			
Browning reaction	208	657	660	1878
	2219			
Brown king crab	438			
Brown sugar	184			
Brunauer-Emmett-Teller (BET) equation	482			
Bubble-cap tray	513			
Buffer solutions	1309			
Bulgaricus milk	916			
Bulimia nervosa	9			
Bullet tuna	831			
Bullocks	43			
Bushel	539			
Butanol, production	2170			
Butter	218	1659		
composition	220	471		
consumption	218			
decholesterolized butter	228			
as emulsion	602			
energy usage	627			
flavor	456	458		
ghee	228	453		
grades	219			
history	218			
modification of milk fat	221			
processing	223			
rancidity	456			
reduced-fat butter	227			
spoilage	1026			
spreadable butter	225			
standards for	219			

Index terms	Links			
Butter *(Continued)*				
whipped butter	225			
Butterfat	743	1334		
Butter flavors	228			
Butterfly shrimp	2154			
Buttermilk	542	916		
Butter oil	1659			
"Butter oil case"	757			
Butter products	225			
Butter-vegetable oil blends	225			
Butylated hydroxyanisole (BHA)	67			
Butylated hydroxytoluene (BHT)	67			
Butylhydroquinone	77			

C

Index terms	Links			
Cabbage				
pickling	2428	2430		
sauerkraut	920	2610		
Cabinet drying	488	489	1144	2401
Cacao, colorants from	400			
Cachexia, enteral feeding for	635			
Caciocavallo cheese	304	309		
Cadmium, corrosion resistance	417			
Caffeic acid	72	2342	2344	
Caffeine	31	33	231	
analysis	232			
biogenesis	232	233		
carcinogenicity	237			
in chocolate and cocoa	350			
as drug	236			
in human diet	232			
metabolism	234	235		
mutagenicity	237			
occurrence	232			
pharmacology	234	236		
physical dependence on	236			
production	233			
properties	231			
in tea	2293			

Index terms	**Links**			
Caffeine *(Continued)*				
teratogenicity	237			
toxicity	236			
Cages, aquaculture	114			
Caimito	1167			
Cake doughnuts	142			
Cake flour	2649	2651		
Cakes				
emulsifiers in	607			
leavening agents	133			
lecithin in	1464			
shortening in	741			
Caking	13			
Calamondin	1154			
Calandria vacuum pans	666	668		
Calcitonin	1793			
Calcium, osteoporosis and	1792			
Calcium alginate	580	1217		
Calcium carbonate, as colorant	402	403		
Calico scallops	2152			
California buckeye, honeybees and	150			
California olives	1759	1768	1771	1773
Calorie-dense rations	1650			
Calvert L. Willey Distinguished Service Award (IFT)	1374			
Camelliin B	2289			
Camembert cheese	301	305	917	
Camphene	71			
Camphor	71			
Camptothecine	30			
Campylobacter	344	1958		
Campylobacter jejuni	1084			
Canada, food industry	508	1383		
Canada Dry pale dry ginger ale	174			
Canadian whisky	522	526	538	
Canadine	28			
Canavanine	2336			
Cancer	9			
ascorbic acid	1897			

Index terms	**Links**			
Cancer *(Continued)*				
carbohydrates and	271			
carotenoids and	1897			
dietary fats and oils	744			
enteral feeding for	635			
nitrosamines and	1715			
phenolic compounds and	1898			
phytochemicals and	1896			
risk and diet	239			
tocopherols and	1898			
wine and	1931			
Candy				
additives	853			
Finland	1391			
freezing	1130			
lecithin in	1464			
licorice	1466			
Canned foods				
beets	2411			
Clostridium botulinum	244			
corn	2413			
fish	467	766		
green beans	2412			
green peas	2414			
low-acid and acidified foods	1503	1507		
meat products	1580			
oysters	2151			
shrimp	2153			
spoilage	1027	1508		
Canning	243	955	970	1633
energy use	630			
level of carotenes	278			
microbiology	244			
mushrooms	1678			
process	245			
process design	245			
recalls	248			
regulatory controls	243			
safeguards	247			
safety concerns	244			

Canning *(Continued)*
  Standard Industrial Classification 990
  vegetables 2409
Canola 248 259
  breeding 251
  comparison with other oil seeds 253
  cultivars 249
  edible by-products 257
  harvest and storage 250
  non-edible by-products 258
  origin 249
  processing 254
Canola meal 253 257
Canola oil 251 253 1747 1752
  production 253 258
  rapeseed 248
Canonical analysis, sensory analysis 2144
Canonical discriminant analysis, sensory analysis 2145
Canthaxanin 280
Canthaxanthin 277 281 389
Cantleyine 30
Cape jasmine 883
Capillary electrophoresis (CE) 1689
Caprenin 750 1335
Capsaicin 31 2339
Capsanthin 280 385 387
Capsicum peppers 2199
Capsorubin 280 385 387
Carambolas 1164
Caramel 273
Caramel color 184 383
Caramel malt 156
Carapine 31
Caraway seed 2199
Carbanilides, as disinfectant 506
Carbohydrases, enzyme assay 648
Carbohydrate-based fat substitutes, in ice cream and frozen dessert 1335
Carbohydrates 262 268
  analysis for 870

This page has been reformatted by Knovel to provide easier navigation.

Carbohydrates *(Continued)*
  cancer risk and 240
  citric acid cycle 19 20 1063
  dietary 269
  digestion and absorption 1059 1061
  energy formation and storage 1059 1062
  food functionality 269
  gluconeogenesis 1064 1066 1069
  glycogenesis 1064 1066
  glycogenolysis 1064 1066
  glycolysis 1062
  health maintenance 270
  in human diet 1059
  labeling 267
  livestock nutrition 46
  Maillard reaction 454 657 839 877
  metabolism 1062 1068 1069
  monosaccharides 262
  oligosaccharides 265 268 269
  physiological effects 269
  polysaccharides 267 878
  respiratory chain 1064 1066
Carbon adsorption 509
Carbonated beverages 171
  acidulants 3
  additives 853
  bottler associations 175
  defined 173
  history 174
  ingredients 172
  marketing 176
  nutritional value 173
  production 175
Carbon black, as colorants 402
Carbon dioxide
  antimicrobial effects 1833
  as fumigant 1175
  photosynthesis 1886
  short-term controlled atmosphere 408
  in soft drink processing 172

Carbon dioxide freezer 1126
Carboxymethylcelluloses (CMC) 581 582 1217
Carboxypeptidase 648 1060 1061
Carcass 47
Carcinogens, risk assessment 1055
Cardamom 2199
Cardiovascular disease 8
  ascorbic acid and 1899
  carotenoids and 1899
  omega oils and 744
  phenolics and 1899
  phytochemicals and 1898
  tocopherols and 1899
Cardiovascular system
  alcohol, effect on 23
  offish 804
Carinates 1952
Carl R. Fellers Award (IFT) 1373
Carmine 379
Carminic acid 379
Carnosoic acid 77
Carob 1154
Carotenes 272 276 277 278 280 281 1300 1523
Carotenoid extracts 389
Carotenoids 272 883 1909 2546
  agricultural practices and 277
  analysis 277 1910
  annato 385
  bioavailability 1912
  biosynthesis 274
  cancer and 1897
  cardiovascular disease and 1899
  chemical synthesis 280
  as colorants 279 1913
  conversion to vitamin A 277 279
  as food colors 279 1913
  food processing and 278
  in foods 1912

Index terms **Links**

Carotenoids *(Continued)*
  function 280
  history 272 274
  lycopene 9 274 276 279 385 386
  nomenclature 272
  nutrition 1910 1912
  occurrence in nature 275
  paprika 385 388
  saffron 388
  singlet oxygen and 78
  structure 1910 1911
  synthetic 388
  tagetes 388
  in tea 2294
Carpaine 34
Carrageenan 581 750 879 1218 1219 2213
Carrier freezer 1120
Carrots, dehydration 2405
Carthamic acid 390
Carthamin 390 391
Carthemone 390
Carvacrol 71
Caryophyllene 158 159
CASBA II/III 2038
Casein 580 1060 1659
Casemates 461 473
Casein protein 476
Cashew apple 1164
Cashew nut 1164 2681
Cashew nut oil 1164
Casing, frozen foods 2049
Casings, sausage 36 42 579 2070
Castings, corrosion 427
Catalase 78 650 659
Catalasemeter 1091
Catalase test 2040
Catalysis, hydrogenation 1315

This page has been reformatted by Knovel to provide easier navigation.

Index terms **Links**

Catalyst poisons, hydrogenation 1317
Catalysts, hydrogenation and 1314 1318 1320
Catechins 1873 2295
Catechol 71
Catechol oxidase 210
Catfish, aquaculture 108
Catharanthus alkaloids 30
Cationic emulsifiers 619
Cattle
  animal nutrition and feeding 46
  Australia 1379
  bovine spongiform encephalopathy (BSE) 42 198
  breeds 45
  digestive process in 47
  drug residues 1359
  genetic engineering 2674
  livestock feed 1490
  livestock production 43
  milk production 48 52
  red meat production 47
  reproduction 45
  slaughtering process 47 1614
Caulerpicin 31
Cayenne pepper 385 2199
Celery, dehydration 2405
Celery seed 2199
Celiac disease 287 865
Cell-mediated food allergies 865
Cellobiase 659
Cellulase 659
Cellulase assay 649
Cellulose 754
Cellulose derivatives 581
Centers for Disease Control and Prevention (CDC) 2030
Central nervous system
  alcohol and 23
  caffeine and 234 236
Centrifugal atomization 559
Centrifugal extrusion 622

This page has been reformatted by Knovel to provide easier navigation.

Centrifugal fluidized-bed drying (CFBD), military food 1645
Centrifuges 282
Centri-term evaporator 670
Cephaeline 28
Cephalotaxine 29
Cephalotaxus alkaloids 29
Cereal-based foods, texture 2622
Cereal fortification 206
Cereal grains 179 286 290
  barley 288 291 293 296 297
  classification 297
  composition 287 296
  corn 287 291 293 296 297
  for distilled beverage spirits 529 531
  functional foods 1178
  insecticides, postharvest application 1212 2690
  in livestock feed 1491 1493
  malt 1517
  millet 292 296
  mycotoxins 1699
  oats 288 291 293 296
  physical properties 292
  postharvest deterioration 902
  production statistics 286
  rice 288 291 293 296 297
  rye 288 292 296
  sorghum 288 291 293 296
  South Africa 1408
  spoilage 1026 1028
  standards 297
  structure 292
  texture 2620
  wheat 287 290 292 296 297
Cereal proteins, as edible films 580
Cereals
  additives 853

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Cereals *(Continued)*				
Australia	1379			
beer brewing	153	156		
breakfast. *See* Breakfast cereals				
extrusion cooking	704			
fermented	921			
functional foods	1178			
lipoxygenase in	654			
nitrosamines in	1713			
Taiwan	1418			
ultrafiltration and reverse osmosis	2361			
Ceriman	1164			
Cetyltrimethylammonium bromide (CTAB)	619			
Chaconine	2341			
Chaffy parts	292			
Chain-breaking antioxidants	76			
Chaksine	30			
Chalkbrood disease	150			
Champagne	919			
Chanoclavine-1	29			
Chardonnay	2666			
Charm test	56			
Chavicine	31			
Cheddar cheese	299	302	303	304
	307	308	309	324
	328	329	332	333
	457	917	1657	
Cheese	453	1657		
Australia	1380			
characteristics of specific cheeses	303			
cheese analogues	311			
classification	299			
composition	471			
cottage cheese	301	302	307	308
	309	917		
cultures for	302			
defects	307			
enzymes and	655			
fermentation	916			
Finland	1391			

Index terms	Links		
Cheese *(Continued)*			
flavor	456		
foodborne disease	1087		
history	298		
as ingredient in foods	474	476	
nitrosamines in	1713		
process cheese	308		
production	299		
rheology	315		
ripening	303		
sales statistics	310		
salt content	457		
spoilage	1026		
types	457	917	
variation with age	333		
whey cheese	2655		
Cheese analogues	311		
Cheesemaking	299	457	
Chelates	858		
Chelators, as antioxidants	76	78	
Chemical agents, immunological analysis	1357		
Chemical insecticides	2693		
Chemical intoxication	1078	1079	
Chemical leavening	133	185	
Chemically sensitive field effect transistor (CHEMFET)	2105		
Chemical preservation	857	957	967
Chemical reactions, kinetics	980	1437	
Chemical residues	1052		
Chemical sensors	2104		
Cherimoya	1164		
Cherries	1158		
Cherry-Burrel Gold'n Flow and Creamery process	224		
Cheshire cheese	324	329	
Chewing gum, lecithin in	1464		
Chicken	1943	1964	
Chicken nuggets	467	1972	
Chicken patties	467		
Chick-Martin test	507		
Chicory	358		

Index terms	Links	
Chiku	1167	
Child Nutrition Act	1100	
Children		
enteral feeding for	636	
malnourished	1299	
Chili peppers	2198	2199
Chilled foods	337	
distribution	342	
European market	337	338
Japanese market	337	338
legislation	346	
microbiology	344	
packaging	341	
processing	339	
quality control	345	
shelf life	346	
storage temperature	343	
in United States	337	
Chilled Foods Association	347	
Chilling, techniques	341	
Chilling injury		
food spoilage	1030	
fruits and vegetables	902	906
China, ethnic foods of	446	447
Chinook salmon	826	
Chironjas	1154	
Chitin, films of	591	
Chitosan, films of	581	
Chitosan-lauric acid films	582	
Chloramines, as disinfectant	502	
Chlorhexidine, as disinfectant	405	
Chlorine, as disinfectant	502	1040
Chlorine dioxide, as disinfectant	502	
Chlorogenic acid	2342	2344
Chlorophyll	391	883
Chlorophyllide	391	
Chloroplasts	1886	
Chocolate	348	
alkaloids in	350	

Index terms **Links**

Chocolate *(Continued)*
  economic aspects 350
  emulsifiers in 609
  lecithin in 1464
  nutritional properties 350
  spoilage 1027
Chocolate liquor 348
Chocolate malt 156
Chokeberries 1158
Cholecystokinin (CCK), satiety agent 89
Cholera 770 1082
Cholesterol 737
  cardiovascular disease and 8
  fats 1600
  in fats and oils 744
  ice cream and frozen deserts 1336
  meat 1600
  metabolism 1077
Cholesterol esterase 1061
Choline 31
Chopping, meat 1967
Chromatography 869 1687
Chronic bee paralysis 150
Chronic obstructive pulmonary disease (COPD), enteral feeding for 634
Chronic wasting disease 199
Chum salmon 827
Chymotrypsin 1060 1527
Ciguatera 1088 1534
Ciguatoxin 1051 1534
Cilantro 2199
Cinchona alkaloids 30
Cinnamaldehyde 834
Cinnamic acid 72 216
Cinnamic aldehyde, antimicrobial activity 71
Cinnamon 71 2199
Circular dichroism (CD), protein structure 2018
Circular raceways 101
Citrates 1 66 309 310

Index terms **Links**

Citric acid 2611
  as acidulant 1
  as antimicrobial in foods 66
  production 2170
  properties 2
Citric acid cycle 19 20 1063
Citrus fruits 1155
Citrus juice
  limonin 1470
  production plant 666 667
Citrus oils 2340
Clams, food borne disease 1534
Claviceps purpurea 2349
Clavine alkaloids 29
Cleaning 499
  detergents 498
  freezing machinery 1132
  sanitation program 501
Cleaning-in-place (CIP) 351
  disinfection 507
  food surface sanitation 1043
  ice cream plants 1338
  milk processing plants 628
Climbing-film evaporator 666 668
Cloning 1209
Closed-cycle drying 575
Clostridium, in food 1631
Clostridium botulinum 1079 1631
  canned foods 244
  chilled foods 344
  low-acid and acidified foods 1503
  seafood 771 789
  toxins 886
Clostridium perfringens 1081 1631 1958
Cloves 71 2199
Club mosses 27
CLUES (software) 121
Clupeotoxin 1537
Cluster analysis (CA), sensory analysis 2136

Index terms	**Links**			
Coacervation	623			
Coalfish	817			
Coated papers	968			
Coatings, edible. *See* Edible films and coatings				
Cobnuts	2682			
Coca-Cola	174			
Coca-Cola Co.	175	2261		
Cocaine	27			
Cochineal	378			
Cocoa beans	348			
Cocoa butter	349	723		
Cocoa powder	349			
Coconut oil	1746	1747		
Codeine	28			
Codex Alimentarius	1006			
Cod (fish)	815			
Codfish, dried	180			
Coenzymes, food utilization	1059	1062		
Coffee	178	354		
brewing	358			
caffeine	31	33	231	
decaffeinated	237	360	699	
economic statistics	354			
flavor	837			
history	354			
instant coffee	359			
packaging	358			
processing	354			
roasting	355	357	357	
Standard Industrial Classification	997			
Coffee substitutes	358			
Coffi	579			
Cognac	528			
Cohesiveness, powders	2624			
Coho salmon	827			
Cohumulone	72	158		
Colby cheese	299	300	303	304
	308	309	917	
Colchicine	28			

Index terms	**Links**			
Cold shortening	1549			
Cold smoking	957			
Cold storage				
fish and seafood	764	791		
fruits and vegetables	906			
Cold water-swellable starches	2206			
Coliform bacteria. *See Escherichia coli*				
Collagen	1183	1591		
Collagenase	1183	1528		
Collagen casings	579			
Colley still	519	520		
Colloid mills	361			
Colony hybridization	1625			
Color				
blanching and	194			
butter	223			
drying	496			
fermented sausage	1616			
of food	363			
gelatin	1187			
licorice	1467			
marketing implications	371			
measurement of	365			
meat	369	652	1596	
in oil	1171			
poultry meat	1966			
preserves and jellies	1152			
sensory perceptions, effect on	370			
theory of	364			
Color Additive Amendment of 1960 (U.S.)	393			
Colorants	369	848	853	883
anthocyanins	375			
anthraquinones	378			
betalains	380			
caramel	383			
carotenoids	279	385		
carthamin	390			
chlorophylls	391			
food, drug and cosmetic colorants	393			

Colorants *(Continued)*
  haems 396
  history 372
  inorganic 402
  iridoids 397
  margarine 1524
  monascus 398
  organic 403
  phycobilins 399
  poly phenols 400
  regulation 373
  safety 393 395
  turmeric 401
Coloring adjunct 848
Coltsfoot 2340
Column chromatography 869
Column shells 511
Colupulone 72
Comfrey 2341
Comminuting, sausage 2070
Common swordfish 832
Commonwealth Scientific and Industrial Research Organization (CSIRO) 1381
Composite films 582
Compressors, in freezers 1135
Computer modeling, functional properties 1666
Computers. *See also* Automation
  artificial intelligence 119
  food industry applications 404
  livestock feeds and 1488 1494
Computer simulation, thermal processing 2317
Conalbumin 70
Concentrated juice 628 2048
Conchocelis, cultivation 2103
Condensation, freeze drying 1110
Condensed milk products 1659
Condensers
  distillation system 515
  evaporators 673
Conditioned hunger 90

Index terms **Links**

Conduction freezing 793
Conduction heating 2316
Conduction heat transfer 1273
Cone-bottom dryer 561
Confectionery
  lecithin in 1464
  licorice 1466
  peanuts in 2680
  texture 2626
Confectionery fats 742
Congeners 539
Coniine 33
Conjugated linoleic acid (CLA) 240 1180 1483
Conserves 1149
Consistency, butter 222
Constitutive immunity 2674
Consumer education, food safety 1014
Consumer promotion 936
Contact freezers 1118 1123
Containers. *See also* Packaging
  low-acid and acidified foods 1507
Contaminants
  blanching and 193
  marine toxins 1534
  microbial 1356
  mites 2031
  natural, immunological analysis 1352
  pesticide residues 1868
  toxicology 1051
Contamination 757
  freezers 1131 1132
  marine toxins 1536
  of poultry 1957
Continuous conveyor dryers 2401
Continuous cooling 1230
Continuous distillation 511 518
Continuous dryers 544 546 2401
Continuous freeze driers 1110
Continuous freezing 1230

Index terms	**Links**		
Continuous heating	1229		
Continuous hydrogenation	1320		
Continuous retort systems	2308		
Continuous rotary cookers	2308		
Controlled-atmosphere (CA) storage	408	906	
Controlled-atmosphere fumigation	1175		
Controlled atmosphere packaging (CAP)	1561		
Control point principles	798		
Control systems			
cleaning-in-place (CIP)	353		
distillation	514		
Convection blast freezing	791		
Convection freezing	792	793	
Convection heating	2316		
Convection heat transfer	1275		
Conveyor band dryer	552		
Conveyor dryers	488	1144	
Convicine	31		
Cookies	183		
additives	853		
lecithin in	1464		
synthetic sweeteners	185		
Cooking oil	740		
See also Fats and oils			
Cook room	246		
Coolers, distillation system	515		
Cooling			
aseptic processing	125		
canning	247		
ice cream and frozen desserts	1337		
potatoes	1935		
vegetables	2410		
Cooling agent	848		
Cooling brines, corrosion by	430		
Cooling effect	836		
Copper, corrosion	417		
Cordials	523	528	538
Coriander seed	2199		

Index terms	Links		
Corn	291		
canning	2413		
classification	297		
composition	287		
hot breakfast cereals	206		
mashing	531		
milling	287		
mycotoxins	1700		
nutrient composition	287		
nutritional analysis	296		
physical properties	292		
production statistics	290	291	1942
structure	293	295	
texture	2622		
Corn chips	2160		
Corn endosperm oil, as colorant	403		
Corn oil	1746	1747	
Cornstarch	2208		
Corn syrup	265	2242	2277
analysis	2278		
biscuits and crackers	184		
economic aspects	2278		
manufacture	2277		
properties	2277		
in soft drinks	172		
uses	2278		
Corn syrup solids	2243		
Corn whisky	525		
Correspondence analysis, sensory analysis	2145		
Corrosion			
by detergents	431		
by gelatin	1186		
by insulating materials	433		
by mildly corrosive foodstuffs	429		
by noncorrosive foodstuffs	428		
by rubber	436		
by sanitizing agents	432		
by service fluids	430		
food processing and	414		
of rubbers	435		

Corrosion *(Continued)*
  types 417
Corrosion fatigue 426
Corrosion resistance 414
Corydaline 28
Corynebacterium 1631
Corynebacterium diphtheriae 1631
Corynebacterium poinsettiae 281
Cosmetic industry, colloid mill 363
Cottage cheese 301 302 307 308 309 917 1657
Cottonseed oil 734 1746 1747 1749
Cottonseed products 403
Coulometric methods 869
p-Coumaric acid 72
Coumarin 2342 2344
Council for Agriculture and Science Technology (CAST) 2031
Council of Australian Food Technology Associations (CAFTA) 1382
Country gooseberry 1164
Cows. *See* Cattle
Coxsackievirus 1088
Crabs and crab processing 437 765 767 787
Crackers 183 853
Cranberries 1159 1922
Cranberry juice 1159 1179
Cranberry sauce 1159
Crateless retorts 2308
Crawfish, microbiology 765
Crayfish 1498
Cream 220 221 456 458 471 1658
Cream cheese 301 302 303 307 308 309 917
Cream of tartar 2
Creatine, metabolism 1072 1073
Creatine phosphate 1062
Creatinine, metabolism 1072 1073
Crepes, prepared 598

Index terms **Links**

Cretinism 1301

Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy (BSE) and 199 200

Crevice corrosion 420

Critical control point systems 2032

Crocetin 387

Crocin 277 387 883

Croissants 143

Crop & Food (New Zealand Institute for Crop and Food Research Ltd) 1404

Crops
  fumigants 1172
  grain protectants 1212
  grapes 2662
  integrated pest management (IPM) 2690
  maturation 909
  mycotoxins 1699
  nondestructive quality evaluation 897
  organic farming 2425
  peanuts 2679
  postharvest deterioration 902
  storage 179 905
  vanilla 2383
  varietal differences 908
  vegetables 2421

Crossbreeding, livestock production 46

Cross-linked stabilized starches 2207

Cross-linked starch 2206

Cross-linking agents, in surimi 2238

Croutons, extrusion 705

Cruciferous vegetables 1179

Crullers 143

Crushability index 2619 2626

Crustaceans, microbiology 765

Cryogenic freezers 793 1118 1124 1136 1230

Cryoprotectants 2045 2233

Cryostabilization 1339

Cryptoxanthin 277

Crystal Lean 750

Crystallization, butter 223

Index terms **Links**

Crystal malt 156

Cucumbers
  fermentation 921
  pickling 2428 2429

Cultural nutrition 442

Cultured dairy products 449
  buttermilk 542 916
  fermentation 916
  flavor 455 458
  as ingredient in foods 474 476
  kefir 916
  sour cream 916
  yogurt 455 458 627 916

Cultured milk, therapeutic value 916 1391

Cultures
  cheese making 302
  cultured milk products 450
  dry-cured sausages 1617
  fermented meat products 1614 1620
  pickling vegetables 2433

Culture systems, aquaculture 100 112

Cumin 2199

Curdled milk products. *See* Cultured dairy products

Cured fish 794 796 997

Curing 957
  accelerators 5
  acidulants and 6
  coffee 354
  fish 794
  meat 1578
  nitrites 66
  sausage 2071
  vanilla 2386

Curing agent 848

Curing salts, in sausage 2070

Currants 1159

Curry alkaloids 31

Custard apple 1156 1164

Cutting machines, in biscuits and crackers 186

Index terms	Links			
Cyanobilins	399			
Cyanogenic glycosides	2337			
Cyclamates	860	2245	2248	2250
	2255			
Cycloamyloses	266			
Cyclodextrins	266			
Cysteins, oxidation	1797			
Cysticercosis	1848			
Cystisine	31			
Cytisine	27			
Cytotoxicity, mycotoxins	1692			
D				
Dairy cattle				
livestock feed	1489			
livestock production	43			
milk production	48	52		
Dairy flavors	453			
Dairy ingredients	469			
in meat products	460			
Dairy-Lite	1335			
Dairy-Lo	750	1335		
Dairy products	453	1657		
See also Milk				
acidophilus milk	916			
acidulants	3			
additives	853			
Australia	1380			
bulgaricus milk	916			
butter and butter products	218			
buttermilk	542	916		
cheese	298	453		
composition	471			
cultured milk products	449			
dry milk	540			
enzymes and	655			
evaporated milk	661			
fermentations	916			
Finland	1391			

Index terms **Links**

Dairy products *(Continued)*
  flavor 453 838 841
  food borne disease 1087
  freezing 1129
  functional foods 1179
  heated-milk defects 345
  kefir 916
  koumiss 916
  membrane technology 1628
  milk 48 185 303 449 453
  nitrosamines in 1713
  packaging 1656
  phosphates 1883
  sour cream 916
  South Africa 1408
  Standard Industrial Classification 988
  sweet acidophilus milk 916
  Taiwan 1418
  ultrafiltration and reverse osmosis 2361
  waste management 2601
  whey 2652
  yogurt 455 458 627 916
Danish agar 1219
Danish pastry 144 742
Dark beer 156
Dasheen 1164
Dates 1147 1164
Dawadawa 2182
Death camas, honeybees and 150
5-Deazariboflavin 2524
Decaffeinated coffee 360 699
Decaffeinated tea 238 699
Decaffeination 237 2225
Decanter centrifuge 285
Decarbomethoxydihydrovobasine 29
2-Decarboxybetanidin 381 382
Decholesterolized butter 228
Decision making, food processing 1001

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Decoction brewing	160			
Deep-bed dryers	488			
Deficiency disorders	1299			
Defoaming agents	852			
Deformatin, nondestructive testing	899			
DEFT (direct epifluorescent filter technique)	1092	1622		
Degumming, canola	255			
Dehydrated food, Taiwan	1420			
Dehydration	480	956	966	976
	1633			
See also Drying				
defined	480	1226		
effects on foods	493			
equipment	488			
fish	766			
fish and shellfish	794			
food spoilage	1032			
freezing	1138			
fruits	1142			
meat	1580			
methods	1143			
military food	1644			
mushrooms	1680			
packaging	1146			
postdrying	1146			
potatoes	1935			
predrying	1145			
principles	1142			
rehydration	497			
theory	480			
vegetables	2399	2407		
Dehydrofreezing	1143			
Dehydrogenase, in marine animals	1526			
Delaney Clause	395			
Delivery systems, tube feeding	636			
Delvo test	56			
Denaturation, proteins	2003			
Dental caries, carbohydrates and	271			
Deodorization	257	739		

Index terms	Links		
Deoxynivalenol (DEN)	1699	2347	
Deoxyribonuclease	1061		
Deposit machines, cookie and cracker dough	188		
Dermatex	581		
Desaturated milk fat	228		
Desiccants, dehydrated vegetables	2403		
Desiccation, freezing	1115		
Design			
aquaculture systems	110		
distillation column	516		
food plants	946		
Dessert mixes, additives	853		
Detergents	498		
components	499		
corrosion by	431		
disposal	500		
food safety and	500		
properties	499		
types	498		
worker safety and	500		
Developed countries, nutritional status	1296		
Developing countries			
hurdle technology	1307		
nutritional status	1296		
Deviled eggs	597		
Dew point hygrometer	2639		
Dextrinase	1061		
Dextrose	2242	2265	2267
Dextrose equivalence (DE)	265		
Dextrose equivalent (DE)	184		
Diabetes, enteral feeding for	635		
Diabetes management, carbohydrates and	271		
Diabetes mellitus	8		
2,3:4,6-Diacetone-L-sorbose (DAS)	2455		
Diacetyltartrate ester of monoglyceride (DATEM)	617		
Diacon	1213		
Diarrhetic shellfish poisoning	1534		
Diatomaceous earth, as grain protectant	1213		
Diced egg products	597		

Diet
  calcium intake 1792
  cancer risk and 9 239
  cardiovascular disease and 8
  fats and oils in 744
  guidelines for Americans 7
  high-fiber diets 756 1178
  Mediterranean Diet Plan 10 2688
  nitrosamines 1714
  nutrition-related diseases 6
  vegetarian 2434
Dietary fats 1484
Dietary fiber 241 754 1178
Dietary laws
  kosher 1449
  Muslim 1682
Dietary proteins 1066
Dietary Supplement Health and Education Act (DSHEA) 1053
Dietary supplements, toxicology 1053
Dieting 92 756 1178
Diffusion, in foods 1538
Diffusivity 1543
Digester tankage 41
Digestion
  carbohydrates 1059 1061
  in cattle 47
  fats 1073
  proteins 1060 1066
  starch 1060 2208
  triglycerides 1074
Digestive system
  alcohol, effect on 23
  fish 800
  humans 1058
Dihydrochalcones 1873
Dihydroergotoxine 30
3,4-Dihydroxy-L-phenylalanine (DOPA) 31
Dill 2199
Dimagnesium diphosphate 135 136

Index terms	**Links**	
Dimethylallylpyrophosphate (DMAPP)	274	
Dimethyl dicarbonate (DMDC), as antimicrobial in foods	67	
Dimethyl trisulfide	2078	
Dioscorine	31	
Dipel	1213	
Dipeptidase	1061	
Diphenylamine (DPA)	281	
Direct epifluorescent filter technique (DEFT)	1092	1622
Direct heating, aseptic processing	124	
Direct microscopic count (DMC)	1092	
Direct steam heating	1229	
Disaccharidases	269	
Discriminant analysis (DA), sensory analysis	2138	
Disc stack centrifuge	284	
Disease. *See* Foodborne disease		
Disease control, aquaculture	109	
Diseases, of honeybees	150	
Disinfectants	501	
chemical agents	502	
evaluation	506	
food surface sanitation	1040	
legislation	507	
modular systems	507	
physical agents	506	
Disinfection		
effectiveness of	1044	
food surface sanitation	1040	
Disk away procedure	56	
Dispersions, colloid mills	361	
Distillation	509	
batch distribution	511	516
column design	516	
components	511	
defined	1226	
distilled beverage spirits	532	
history	509	
packaged systems	515	
terminology	509	
Distillation trays	511	

Index terms	Links		
Distilled beverage spirits	12	20	519
analysis	535		
export of	539		
fermentation	920		
glossary	539		
history	519		
manufacturing process	529		
maturation	534		
packaging	538		
production and consumption	522		
regulation	521		
standards of identity	522		
Diterpene alkaloids	30		
Dittus-Boelter equation	1244		
DNA			
genetic engineering	1199		
isolation and preparation	1206		
structure	1720	1723	
DNA probes	1094	1846	2041
Docosahexaenoic acid (DHA)	723		
Dog salmon	827		
"Dolly"	1210	2672	
Domoic acid	1534		
Dopamine	31		
Dose-response assessment	1054	2353	
Double-cone vacuum dryer	544		
Double diffusion	1348		
Double drains, aquaculture	113		
Double Gloucester cheese	324		
Dough-conditioning agents	625	852	
Doughnuts	142		
Dough strengthener	848		
Dover sole	821		
Dressings, additives	853		
Dried algal meal, as colorant	403		
Dried codfish	180		
Dried fish	766	794	
Drones (bees)	149		
Dr. Pepper formula	174		

Index terms	Links			
Drug residues, immunological analysis	1358			
Drugs, caffeine as additive	233	236		
Drum dryers	489	493	545	546
	1145	2402		
Dry beer	920			
Dry buttermilk	542			
Dry-cured ham	1619			
Dry-cured sausages	1617	1618		
Dryers	488	542		
batch dryers	542			
bond dryers	550			
box dryers	560			
cone-bottom dryer	561			
continuous dryers	544	546	2401	
energy utilization	553			
feed rate	557			
flat-bottom dryer	561			
fluidized-bed dryers	489	543	2401	
heat-recovery equipment	563	565		
multiple-stage dryers	561			
pneumatic dryers	489	490	550	555
	2401			
pollution-control devices	563			
rotary dryers	489	491	543	548
selecting	553			
single-stage dryers	560			
spin flash dryers	571			
spray-bed dryer	563	571		
spray dryers	489	491	545	547
	558	568	571	1144
tall-form dryers	560			
three-stage dryers	562			
tunnel dryers	489	492	1144	2401
two-stage dryers	561			
wide-body dryers	560			
Dry film	1090			
Drying	484	555	1633	
See also Dehydration				
closed-cycle drying	575			
coffee	359			

Index terms	**Links**	
Drying *(Continued)*		
defined	1491	
energy utilization	553	
krill	1455	
methods	1143	
microwave processing	1639	
nitrosamines	1710	
operation economics	556	
sausage	2070	
Shiitake mushrooms	1681	
vegetables	2399	
Drying agent	848	
Drying processes, for agglomeration	15	
Drying rate, calculation	485	
Drying time	1109	
Dry milk	540	1659
Dry rendering	37	
Dry wine	919	
Dual emulsions	602	
Dual-flow tray	513	
Dublin Bay prawn	1496	
Ducks	1949	1964
Dungeness crab	440	765
Dur-Em	750	
Durians	1164	
Dur-Lo	750	
Durum semolina	287	
Durum wheat	2642	
Dye reductions	1090	
Dyer's saffron	390	
Dynamic light scattering (DLS)	943	

E

Eating. *See also* Appetite; Diet; Hunger		
appetizer effects	92	
binge eating	91	
brain systems controlling	83	89
cognitive factors	93	
emotional factors	92	

Index terms	Links			
Eating *(Continued)*				
obesity and	91			
perceptual salience	91			
satiety	88	91	749	
social factors	93			
vegetarian diet	2434			
ECHO virus	1088			
Eclairs	143			
Edam cheese	303	306	307	308
	332	917		
Edible by-products	35			
Edible films and coatings	579			
Edible tallow	41			
Eels	584			
Eel skin	591	592		
Egg albumin proteins, denaturation	2009			
Egg processing, waste management	2603			
Egg products	596	598		
Eggs	593			
biscuits and crackers	185			
composition	594			
egg industry	593			
egg-rich convenience food	597			
fertile eggs	599			
nutrition	596			
pharmaceutical products from	599			
production	594			
production statistics	593			
shell egg processing	595			
spoilage	763	1026		
structure	593			
Egg white				
films from	580			
processing	596			
Egg white meringue, biscuits and crackers	185			
EIA (enzyme immunoassay)	57	1094	1347	
Eicosapentaenoic acid (EPA)	723			
Elastase	1060			
Elastins	599			

Index terms	Links			
Electrical conductivity, of particulate food products	129			
Electrical stimulation, meat	1549			
Electric hygrometer	2639			
Electroconductive blanching	192			
Electrodialysis (ED)	1627			
Electroimmunodiffusion	1357			
Electromagnetic radiation, nondestructive quality evaluation with	897			
Electron microscopy	941			
Electrophoresis	869			
ELFA (enzyme-linked fluorescent immunoassay)	2042			
ELISA (enzyme-linked immunoabsorbent assay)	57	1094	1347	1689
	1690	2042		
Elizabeth Fleming Stier Award (IFT)	1375			
Ellipticine	29			
Elvers	586			
Elymoclavine	29			
Embryo transfer	1210			
Embryo transplantation, livestock production	53			
Emetine	28			
Emmentaler cheese	303	305	309	329
	333	457		
Employee safety. *See* Worker safety				
Emu	1952			
Emulsifiable concentrates (EC)	1213			
Emulsifiers	602	729	843	848
amphoteric	618			
anionic	617			
cationic	619			
gelatin	1185			
government regulation	618			
ice cream	1334			
lecithin	1463			
Muslim dietary laws	1683			
nonionic	614			
process cheese	1980			
quality defects	1982			
uses	619			
Emulsifier salt	848			
Emulsifying properties, hydrophobicity and	1327			

Index terms **Links**

Emulsions 361 729
Encapsulation 620
Endemic 1079
Endocellulases 659
Endocrine system, offish 808
Endogenous enzymatic degradation 1031
Endothelium-derived relaxation factor (EDRF) 1898
Endotoxins 1081
Energy, cancer risk and 240
ENFET (enzymatically sensitive field effect transistor) 2107
Engineering
  aquaculture 110
  artificial intelligence 119
English muffins 141
English sole 821
Enhansol 1191
Enteral formulas 633
Enteral nutrition
  condition-specific 634
  delivery systems 636
  nutrient solution 633
  processing technology 638
  pumps 637
  regulation 639
Enteroaggregative E. *coli* (EAggEC) 1085
Enterococcus 1631
Enterohemorrhagic E. *coli* (EHEC) 1084 1085
Enteroinvasive E. *coli* (EIEC) 1085
Enterotoxigenic E. *coli* (EPEC) 1085
Enteroviruses 1088
Environmental contaminants 1051
Environmental Protection Agency (EPA)
  cancer risk assessment 1056
  chemical residues 1052
  pesticides 1357
Enzymatically sensitive field effect transistor (ENFET) 2107
Enzymatic browning reaction 208 657 660 1798
Enzyme assays 643

Index terms **Links**

Enzyme-catalyzed oxidation 1798
Enzyme electrode 1344
Enzyme immunoassay (EIA) 57 1094 1347
Enzyme labeled antibody (ELA) method 1625
Enzyme-linked fluorescent immunoassay (ELFA) 2042
Enzyme-linked immunoabsorbent assay (ELISA) 57 1094 1347 1689 1690 2042
Enzymes 658 848 2612
  blanching and 194
  browning reaction 208 657 660 1798
  defined 171
  endogenous enzymes 656
  flavor and 733 840 1189
  as food additives 854
  food deterioration and 954
  in food production 652
  food utilization 1059
  immobilized 1342
  inhibitors 645
  marine enzymes 1525
  Muslim dietary laws 1683
  production 2170
  specificity 643
  spoilage 1028 1031
  in tea 2295
Enzymology 658
Ephedrine 27
Ephemeron 28
Epicatechin 77
Epidemic 1079
Epidemiology 1079
Eptatretin 31
Equine leucoencephalomalacia (ELEM) 1701 2348
Ergonovine 29
Ergosterol 2564
Ergot alkaloids 29 31 1703 2349
Ergotamine 29 2350
Ergotoxine 30
Erucamide 258

Index terms	**Links**			
Erucic acid derivatives	258			
Erythorbic acid	216			
Erythritol	2212	2244	2260	
Erythrodiol, in olive oil	1765			
Escherichia	1632			
Escherichia coli				
in chilled foods	344			
detection	1623			
in fermented meat products	1618			
foodborne disease	1084	1086	1632	
Eskimos, ethnic foods of	448			
Essential oils	835	856	2191	2201
Esterase, enzyme assay	640			
Ester hydrolases, assay for	649			
Esters, as antimicrobial in foods	64			
Estimate of daily intake (EDI)	1054			
Estragole	2338			
Estrogen replacement therapy (ERT), osteoporosis	1792			
Estrous cycle, livestock	45			
Ethanol	19	2169		
See also Alcohol				
Ethnic foods	442			
cultured milk	916			
cultured milk products	450			
eels	590			
fermented	920			
kefir	916			
kimchi	921	2430		
koumiss	916			
lempuk	2628			
peanuts and nuts	2688			
roe	821			
sauerkraut	920	2610		
seasonings	2202			
seaweed	2102			
soy-based. *See* Soy foods				
surimi. *See* Surimi				
Ethoxylated monoglyceride (EMG)	614			
Ethyl carbamate	1932			

Ethyl cellulose (EC) 581
Ethylenediaminetetraacetic acid (EDTA) 858
Ethylene vinyl alcohol copolymer (EVOH), as packaging material 1829
Etiologic agent 1079
Eugenol, antimicrobial activity 71
Eulachon 830
Europe
  chilled food regulation 347
  chilled foods market 338
  ethnic foods of 442
  food legislation 508
  mycotoxin control 1355
European eel 585
European Suspension Test (EST) 1045
European Union
  bovine spongiform encephalopathy (BSE) 202
  food additives 851
Evaporated milk 661 1659
Evaporation 663 1142
  defined 1226
  equipment 665
  foods, effect on 674
  theory 663
Evaporators 628 665 676
  batch pan evaporator 676
  energy efficiency 670 683
  engineering conversion 686
  film evaporation 686
  forced circulation evaporators 668
  heat-transfer coefficient 680
  multiple-effect system 670 672 682
  natural circulation evaporators 666
  plate evaporators 679 688
  refrigerant cycle evaporators 672
  selecting 680
  theory 663
  tubular evaporators 676
  vapor recompression 671 691
Excel 750

Index terms	Links			
Exocellulases	659			
Exotoxins	1081			
Expert systems	120	121		
Exposure assessment	1054	2355		
Externality theory of obesity	91			
Extractable color of paprika	2198			
Extraction	696			
crude palm oil	1840			
mycotoxins	1685			
olive oil	1760			
Extractive distillation	510			
Extracts of tea	401			
Extraneous matter, in foods	757			
Extra virgin olive oil	1763			
Extruded snacks	2162			
Extruders	187	700	706	
Extrusion	699	706		
breakfast cereals	204	704		
centrifugal extrusion	622			
encapsulation by	622			
rheology	710			
texture and	707	715		
Extrusion cooking	702			

F

Factor analysis (FA), sensory analysis	2145			
Fagaronine	28			
Falling film evaporators	668	669	677	679
	686	689		
False saffron	390			
Fantail shrimp	2154			
Fasting phase, of metabolism	88			
Fatal familial insomnia	199			
Fat-coated salt	626			
Fatigue (of metals)	426			
Fat replacers	730	746	1335	1341
Fats. *See also* Fats and oils; Lipids				
animal by-product processing	39			
cancer risk and	240			

Index terms	**Links**			
Fats *(Continued)*				
cholesterol	1600			
degradation	1074			
dietary	1484			
digestion and absorption	1060	1061	1073	
health affected by intake of	9			
metabolism	1074			
milk fat	220	228	472	476
	722	1653		
synthesis	1075			
Fats and oils	721			
additives	853			
applications	740			
Australia	1380			
autoxidation	728	731		
biscuits and crackers	183			
color	1171			
consumption statistics	743			
cooking oil	740			
emulsions	729			
fat replacers	730	746		
fatty acids	721	737		
flavor	731			
frying oil	1169			
functionality	740			
glycerides	723	737	738	
hydrogenation	721	725	726	739
lard	41	1683		
in margarine	754	1522		
novel fats and oils	729			
nutrition	744			
oxidation	728	731		
phospholipids	724	737		
physical processing	727			
processing	724	734	738	
South Africa	1408			
spoilage	1027			
Standard Industrial Classification	995			
substitutes for	730	746		
supercritical fluid technology (SCF)	2225			

Index terms	**Links**			
Fats and oils *(Continued)*				
unsaponifiable components	724			
vegetable oils	722	734	747	1745
Fat substitutes. See Fat replacers				
Fatty acid esters, as antimicrobial in foods	66			
Fatty acids	721	737	744	
dietary	1484			
in olive oil	1764			
oxidation	1076			
supercritical fluid technology (SCF)	2225			
FD&C colorants	393			
Feasibility study, food plant design	946			
Feather meal	41			
Febrifugine	27			
Fecal coliform. *See Escherichia coli*				
Federal Grain Inspection Service (FGIS)	1214			
Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)	850			
Federal Republic of Germany (FG). *See* Germany				
Feeder cattle	43			
Feeding				
aquaculture	106			
livestock feeds	1485	1913	1998	2169
livestock production	46			
Feeding tankage	41			
Feeding tubes	636			
Feedlots	43			
Feijoa	1165			
Feints	539			
Fennel seed	2199			
Fenugreek	2200			
Fermentation	913			
See also Yeast				
alcoholic beverages	918			
amino acid production	1997			
baked goods	137			
beer	154	163	920	
black tea	2298			
breadmaking	921			
cheese	916			

Index terms	Links	
Fermentation *(Continued)*		
chemical reactions	915	
dairy products	916	
distilled beverage spirits	531	920
genetic engineering	922	1190
history	914	
of meats	921	
microbial interactions	914	
microorganisms for	914	
mixed-culture	914	
mold-modified foods	922	
nucleosides and nucleotides	1729	
pickling vegetables	2430	
as preservative	957	
processed meat products	1614	
sausage	2070	
single-culture	914	
solid-state fermentation	2165	
soy foods	2175	
spoilage	1028	
of vegetables	920	
vinegar	921	
whey	2658	
wine	918	2663
Fermented meat products	1614	
Fermented milk products. *See* Cultured dairy products		
Fermented sausage, color	1616	
Fermenting aid	848	
Fertile eggs	599	
Fertilizer, by solid-state fermentation	2168	
Ferulic acid	72	77
Fetal alcohol syndrome	25	
Fiber		
cancer and	241	
dietary	754	
Fiber-optic biosensors	2108	
Fibruline	751	
Fick's first law	1539	
Field crops	902	1407

Index terms	**Links**	
Figs	1147	1154
Filament hygrometer	2639	
Filberts	2682	
Filipinos, ethnic foods of	448	
Filled cheese	311	
Filling, food plant design	947	
Filling operations, canning	246	
Film evaporators	686	
Films		
edible. *See* Edible films and coatings		
gelatin	1185	
metallized	1825	
Filtermat spray drier	16	
Filth		
in foods	757	
in spices	2197	
Filtration		
beer brewing	161	
membrane filtration	1622	
Fine chemicals, animal by-product processing	42	
Finely comminuted meat products	460	461
Finland		
food industry	1388	
licorice	1468	
Firming agents	848	855
First law of thermodynamics	2331	
First-order rate processes	981	1438
Fish. *See also* Fish and shellfish products		
anatomy and physiology	799	
aquaculture	95	
canned	766	
cured	794	796
dried	766	794
dried codfish	180	
economically important species	813	
foodborne disease	1534	
freezing	1128	
functional foods	1180	
genetic engineering	2674	

Index terms	**Links**			
Fish *(Continued)*				
handling	787			
international fish block	2047			
livestock feed	1490			
marine enzymes	1525			
marine toxins	1534			
microbiology	763			
modified atmosphere packaging (MAP)	1735			
muscle	1724	2078		
nitrosamines in	1713			
parasites	1847			
processing	630			
protein denaturation	2008			
rancidity	2077	2079		
smoked	766	794		
Spain	1410			
species of	813			
spoilage	763	1026	1030	1031
Standard Industrial Classification	997			
surimi	1455	2229	2405	
total utilization	788			
Fishing vessels	783			
Fish meal	41			
Fish oils	722	723	788	
Fish poison	1537			
Fish and shellfish products	776			
antioxidants in	5			
consumption statistics	777			
fishery landings	778			
flavor	2071			
foodborne disease	771	1087	1534	1535
harvesting gear	778	780	783	
imports and exports	778	781	782	
irradiation	768	795		
microbiology	763			
processing	786			
quality of the catch	783			
refrigeration and freezing	790			
safety	797			
South Africa	1408			

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Fish and shellfish products *(Continued)*				
spoilage	763			
Fish skin gelatin	1186			
Fixed-bed hydrogenation	1320			
Fixed-tube rotary dryer	550			
Flaked cereals	205			
Flaking, meat	1967			
Flameless ration heater (FHR)	1651			
Flash dryers	551			
Flat-bottom dryer	561			
Flat breads	139			
Flatfishes	818			
Flavanol, biosynthesis	2285			
Flavanones	1873			
Flavones	1874			
Flavonoids	72	273	1179	1878
	1928			
Flavonols	72	1874	2292	
Flavor	834	884		
acidulants	3			
Amadori and Heyns rearrangement products (AHRP)	840			
appetite and	90			
bioflavors	1196			
blanching and	194			
of breakfast cereals	207			
butter	222			
butter and cream	456	458		
cheeses	305	456		
coffee and tea	837			
cultured dairy products	455	458		
dairy flavors	453			
dairy products	453	838	841	
defined	884	2071		
drying	495			
encapsulation	624			
enzymes and	733	840	1189	
fats and oils	731			
fermented sausage	1616			
flavor compounds	836	842		

Index terms **Links**

Flavor *(Continued)*
  fruits 838 841
  genetic engineering 1188
  hydrophobicity and 1328
  ice cream 456 1336
  licorice 1467
  Maillard reaction 839
  meat 837 1598
  milk 454 458 838 841
  nucleotides 1717 1720
  poultry 1937
  seafood 2071
  sensory basis 835
  sensory evaluation 734
  Shiitake mushrooms 1680
  shrimp 2155
  of soft drinks 172
  umami 1717 1720
  vanilla 2390
  vegetables 838 840
  wine 837 2664
  yogurt 455 458
Flavor enhancers 2 835 848 1990
Flavoring agent 848 855
Flavor profile method (FPM) 2133
Flavor-Tex 580
Flaxseed, functional foods 1178
Flounder 820
Flour 653
  biscuits and crackers 183
  bleaching 2648
  gums in 2213
  licorice 1467
  milling 2645
  treatment agents 2650
  types 2648
Flour treating agent 848
Flow, liquid foods 2063
Flow control, aseptic processing system 123

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links		
Flow diversion, aseptic processing	126		
Fluid balance, alcohol, effect on	22		
Fluid-bed agglomeration	567		
Fluid-bed encapsulation	622		
Fluid foods, rheology	2063		
Fluidized-bed agglomerators	14		
Fluidized-bed dryers	489	543	2401
Fluidized-bed freezers	1121	1123	
Fluorescein	1347		
Fluorescence polarization immunoassay (FPIA)	57		
Fluorescence probes	1325		
Fluorescent antibody	1092		
Fluoride, bone and	1793		
Fluoride excess	1302		
Fluorine, as disinfectant	502		
Fluoroimmunoassay	1347		
Fluorosis	1302		
Foamed products, freeze drying	1110		
Foaming agents, in soft drinks	173		
Foaming properties, hydrophobicity and	1327		
Foams, in food processing	844		
Foam stabilizers	613	1185	
Folk acid	2443	2478	
Folic acid deficiency	1302	2482	
Folicanthine	29		
Food Act of 1990 (United Kingdom)	347		
Food additives	846		
antifoaming agents	845		
antimicrobial compounds	63		
caffeine	232		
colorants	369		
emulsifiers	602		
foam stabilizers	613		
food preservation	957		
functionality	847		
government regulations	849		
livestock feed	1492		
nitrate and nitrite	1710		
in sausage	2070		

Index terms	**Links**			
Food additives *(Continued)*				
stabilizers	612			
thickeners	613			
toxicology	1053	1055		
types	848	852		
Food Additives Amendment (1958)	961			
Food allergy	863			
kosher food	1453			
nuts	2689			
peptides	1866			
Food analysis	866	1344		
Food animals, antibiotics in	54			
Foodborne disease	1051	1078		
anchovies	1537			
bacterial infection	1081			
bacterial and microbial intoxication	1079			
chemical intoxication	1078	1079		
clams	1534			
consumer education	1016			
defined	1079			
definitions	1078			
emerging	2031			
fish	1534			
food irradiation	1432			
herring	1537			
marine toxins	1534			
microbiology	1089	1346	1631	
mollusks	787			
mussels	1534			
oysters	1083	1088	1534	
parasitic organisms	1844			
pathogens	1346	1958		
poultry	1958			
scallops	1534			
seafood	769	1083	1087	
shellfish	771	1087	1534	1535
statistics	2030			
U.S. Department of Agriculture	2367			
viruses	771	1088	1844	
Foodborne Diseases Active Surveillance Network (FoodNet)	2031			

Index terms	**Links**			
Foodborne outbreak	1079			
Food chemistry	874	877		
books	874	875		
carbohydrates	877			
colorants	883			
education	874			
flavors	884			
lipids	880			
proteins	881			
toxicants, natural	885			
Food colorants. *See* Colorants				
Food consumption surveys, USDA	889			
Food crops				
fumigants	1172			
Germany	1394			
grain protectants	1212			
integrated pest management (IPM)	2690			
maturation	909			
mycotoxins	1699			
nondestructive quality evaluation	897			
organic farming	2425			
postharvest deterioration	902			
South Africa	1407			
Spain	1410			
storage	179	905		
varietal differences	908			
vegetables	2421			
Food and Drug Acts (U.S.)	373	374		
Food and Drug Administration (FDA)	1023	2352		
adverse reaction monitoring system (ARMS)	1053			
enteral nutrition	639			
labeling	1736	1741	1811	1815
livestock production	48			
low-acid and acidified foods	1505			
pesticides	1357			
Food, Drug, and Cosmetic Act (FDCA)	961	1355		
Food, drug, and cosmetic colorants	393			
Food engineering	911			
Food engineers	911			

Index terms	Links			
Food excess, nutrition-related diseases	6			
Food fermentation. See Fermentation				
Food freezing. See Freezing				
Food gums. See Gums				
Food handling, consumer education	1016			
Food industry				
Australia	1379			
Canada	1383			
Finland	1388			
Germany	1393			
Ireland	1399			
New Zealand	1402			
seasonings	2201			
South Africa	1406			
Spain	1410			
Switzerland	1413			
Taiwan	1416			
United Kingdom	1421			
United States Food Marketing System	2701			
Food infection	1078			
Food intoxication	1078	1079		
Food irradiation	956	964	965	974
	1428	1633		
benefits	1430			
costs	1433			
defined	1429			
disinfection with	506			
fish and shellfish	768	795		
food safety	1431			
fruits and vegetables	904	907		
meat products	1581			
mode of action	1430			
process	1429			
of proteins	2007			
regulation	964	1434		
seafood	768			
spices	2198			
Food labeling. *See* Labeling				
Food marketing. *See* Marketing				
Food and Nutrition Science Alliance (FANSA)	872			

Food plant. *See* Processing plants
Food poisoning 770 1079
Food powders, agglomeration 13
Food preservation 953 1633
  biological preservation 957
  chemical preservation 957 967
  high-pressure processing (HPP) 126 978 1034 1279
  history 971
  hurdle technology 1303
  of living tissue 961
  meat products 1580
  military food 1644
  multitarget preservation 1306
  nitrosamines 1710
  of nonliving tissue 962
  physical preservation 955
Food processing 950
  acidified food 1508
  Australia 1379
  Canada 1383
  chemical food preservation 967
  corrosion and 414
  decision making 1001
  dehydration. *See* Dehydration
  electrical stimulation of meat 1549
  filth and extraneous matter 758
  Finland 1388
  flour 2645
  foams 844
  food composition 959 961
  food laws 961
  food quality 999
  freeze concentration 1103
  freeze drying 15 360 1106
  freezing. *See* Freezing
  Germany 1393
  heat exchangers. *See* Heat exchangers
  heat transfer 1271
  heat treatment 963 970
  high-pressure processing (HPP) 126 978 1034 1279

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**		
Food processing *(Continued)*			
immobilized enzymes	1342		
Ireland	1399		
irradiation	964	965	974
kosher foods	1449		
low-acid canned foods	1507		
management	1001		
mechanical food preservation	966		
membrane technology	1628		
microbial deactivation	964		
military food	1644		
milk	1654		
mushrooms	1678		
Muslim dietary laws	1682		
New Zealand	1402		
nutritional quality, effect on	969		
olives	1768		
ozone and	1801		
packaging. *See* Packaging			
palm oil	1840		
particulate food products	1270		
phenolic compounds	1876	1878	
phosphates	1881		
pickling vegetables	2428		
poultry	629	1963	
preservation. *See* Food preservation			
preserves and jellies	1151		
processing plants	968		
protein reactions in	881		
protein structure, changes during processing	2021		
pulsed light processing	2025		
retorting	2059		
scallops	2152		
seafood	786		
sensors	2104		
shrimp	2155	2158	
silicone antifoams	845		
South Africa	1406		
soybeans	2187		
Spain	1410		

Index terms	**Links**		
Food processing *(Continued)*			
Standard Industrial Classification (SIC)	986		
surimi	2229	2238	
Switzerland	1413		
Taiwan	1416		
thermal processing	964	970	
thermodynamics in	2331		
toxicology	967		
United Kingdom	1421		
vegetables	2406		
wheat	2645		
Food protein, immunological analysis	1348		
Food quality	999	2029	
See also Quality assurance			
aseptic processing	130		
meat	1588		
potatoes	1935		
Food Quality Protection Act (FQPA)	1052		
Food regulation			
Australia	1382		
Canada	508		
carotenoids	1914		
chilled food regulation	347		
Codex Alimentarius	1006		
colorants	373		
disinfection	507		
distilled beverage spirits	521	525	
emulsifiers	611		
enteral nutrition	639		
Europe	508		
European Union	851		
fat replacers	751		
filth and extraneous matter	757		
Finland	1390		
food additives	850		
food irradiation	964	1434	1434
frying oil	1171		
Germany	1398		
high-pressure processing (HPP)	1281		
infant food	1369		

Food regulation *(Continued)*
  international 1014
  Ireland 1400
  Japan 851
  kosher foods 1452
  licorice 1468
  livestock feed 1493
  low-acid and acidified foods 1504
  margarine 1522
  meat products 1582
  mycotoxin control 1355
  nutritional labeling 1732 1812
  packaging 1810
  patents 1852
  peanuts 2678
  pesticide residues 1357 1869
  phosphates 1884
  postharvest integrated pest management 2692
  risk management 1013
  spices 2194
  Standard Industrial Classification (OMB) 986
  United Kingdom 347 508 1421
  United States 347 521 525 961
  vanilla extract 2388
  wine 2662
Foods
  acidified foods 1501
  allergies 863
  amino acids used in 1993
  analysis 866
  antibiotics in 54
  antimicrobial compounds in 63
  antioxidants in 73
  bovine spongiform encephalopathy (BSE) 202
  browning reaction 208 657 660
  carotenoids in 278
  changing state of 1226
  chemical composition 959 961
  chilled. *See* Chilled foods
  color of 363

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**		
Foods *(Continued)*			
corrosion by	428		
cultural nutrition	442		
dairy ingredients for	469		
dehydration	480		
deterioration	953		
diffusion in	1538		
drying	484	553	
emulsifiers	602		
encapsulation	620		
enteral formulas and feeding systems	633		
enzyme assays	643		
enzymes in	658		
ethnic foods	442		
evaporation	662		
extrusion	699		
fermentation. *See* Fermentation			
fiber sources	756		
filth and extraneous matter in	757		
food surface sanitation	1035		
functional foods	1176		
fungal contamination of	1711		
by highly corrosive foodstuffs	429		
history of	1282		
infant foods	1368		
labeling. *See* Labeling			
livestock feeds	1485		
low-acid foods	1501	1503	1506
marketing	934		
mass transfer and diffusion	1538		
microbiology. *See* Microbiology			
microorganisms in	1629		
microstructure	939		
microwave properties	1636		
military foods	1641		
moisture content	482		
natural foods	2439		
nitrosamines in	1708		
organic farming	2425		
organic foods	2439		

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Foods *(Continued)*				
pH	1501			
quality assurance	405	1102	1152	1524
	1649	2029		
sensory components	81			
soils	1036			
sulfites in	2218			
thermal processing. *See* Thermal processing				
toxicology	1050			
transferring heat in	1278			
utilization	1057			
vegetarian diet	2434			
water activity in	482			
Food safety				
Codex Alimentarius	1006			
consumer education	1014			
detergents	500			
dry-cured sausages	1618			
filth and extraneous matter	757			
Finland	1390			
fish and shellfish products	797			
foodborne disease. *See* Foodborne disease				
freezers	1131			
gelatin	1184			
high-pressure processing (HPP)	1281			
irradiation	1431			
low-acid and acidified foods	1503			
microbiological contamination	763			
military food	1649			
nonnutritive sweeteners	2246			
pickling vegetables	2432			
postharvest integrated pest management	2692			
processed food	967			
risk communication	1009			
risk management	1012			
soy foods	2178	2179	2181	2183
taste	2246			
U.S. food safety program	1016			
Food Safety and Inspection Service (FSIS)	47			
Food Science & Technology Abstracts (FSTA)	1425			

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links			
Food science and technology	1017			
Australia	1381			
Canada	1387			
Finland	1392			
genetic engineering	1210			
Germany	1398			
Ireland	1401			
modeling of functional properties	1666			
New Zealand	1402	1405		
optimization methods	1779	2319		
South Africa	1409			
Spain	1411			
Switzerland	1414			
United Kingdom	1423			
Foodservice systems	1098			
Food spoilage. *See* Spoilage				
Food storage. *See* Storage				
Food surface sanitation	1035			
cleaning-in-place (CIP)	1043			
evaluation	1043			
methods	1041			
Food systems	1465			
Food Technology Industrial Achievement Award (IFT)	1374			
Food texture. *See* Texture				
Food toxicology. *See* Toxicology				
Food utilization				
carbohydrates	1059	1062	1068	1069
digestive system	1058			
enzymes	1059	1060		
fats	1073			
food path	1058			
minerals	1077			
nucleic acids	1077			
proteins	1066			
vitamins	1077			
water	1077			
Forced circulation evaporator	668	677		
Foreshots	539			
Forming, meat processing	1968			

Index terms	**Links**			
Forming equipment, cookie and cracker dough	187			
Formulation aid	848			
Fortified wine	919			
FO sweetener	2258	2259		
Fractionation, butterfat	223			
France, licorice	1468			
Frankfurters, from poultry	1972			
Free fatty acids (FFAs)				
absorption	1074			
frying oil	1170			
Free flow agent	847			
Free-machining stainless steels	428			
Freeze concentration	628	1103	2048	
Freeze-dried foods, texture	2626			
Freeze driers	1110			
Freeze drying	15	1106	1142	1145
	1633			
advantages and disadvantages	1107			
condensation	1110			
drying time	1109			
foamed products	1110			
heat transfer	1108			
instant coffee	360			
military food	1644			
mushrooms	1680			
particulate products	1109			
process	1108			
sublimation temperature	1108			
system components	1107			
vegetables	2402	2408		
Freezers	1115	1117	1118	1130
Freezing	923	955	964	1034
	1112	2045	2047	
See also Frozen foods				
changes during	1113			
defined	1116			
desiccation during	1115			
economics of	1137			
equipment	1118			

Freezing *(Continued)*
  fish and shellfish products 790
  heat exchangers 1230
  holding time 1126
  ice cream and frozen desserts 1338
  ice crystallization 1113
  meat 1129 1580 1598
  microbiology 1114
  mushrooms 1680
  precooling 1118
  process 929
  refrigeration basics 923
  refrigeration history 1112
  refrigeration systems 1134
  specific foods (chart) 1127
  surimi 2233
  thermodynamics 1126 1226
  vegetables 2407
Freezing agent 848
Freezing injury, fruits and vegetables 906
Freezing point depression 1103 2639
Freezing rate 1117
Freezing stage 1117
Freezing time 1117
French crullers 143
French olives 1759
Fresh cheeses 299 301
Fresh Wax 625 582
Fried pies 144
Frigate tuna 831
FRIGoBELT 1121
Fritz continuous butter-making process 224 225
Frozen concentrated orange juice (FCOJ) 628 2048
Frozen desserts 1333 1657
  See also Ice cream
Frozen foods 182
  See also Freezing
  fish 766
  fruit and fruit juice 2048

This page has been reformatted by Knovel to provide easier navigation.

Index terms **Links**

- Frozen foods *(Continued)*
  - green peas 2415
  - handling 2049
  - history 924 2045
  - in-home storage 2050
  - meat 2047
  - packaging 2046
  - potatoes 2048
  - scallops 2152
  - seafood 2047 2152 2154
  - shrimp 2154
  - *Standard Industrial Classification* 990
  - surimi 1455 2045 2229
  - Taiwan 1419
  - thawing 2050
  - transport 2051
  - vegetables 2048 2415
- Frozen yogurt 1333 1340 1658
- Fructofuranosidase 2171
- Fructooligosaccharide sweetener (FO sweetener) 2258 2259
- Fructose 263 2241 2275
  - *See also* High-fructose corn syrup
- Fructose-glucose syrup 2242
- Fruitafit 751
- Fruit bars 2628
- Fruit butter 1149
- Fruit flavors, acidulants 3
- Fruit juices 177
  - Australia 1381
  - clarifying 1186
  - enzymes and 655
  - freeze concentration 1103
  - freezing 2048
  - Taiwan 1419
- Fruit processing, waste management 2609
- Fruits
  - ammonia injury 906
  - Australia 1380
  - browning reactions 208 657 660 1878

Index terms	**Links**		
Fruits *(Continued)*			
cancer risk and	242		
carotenoids in	276		
chilling injury	902	906	1030
controlled atmospheres	408		
dehydration	1142		
enzymes and	652	654	
flavor	838	841	
freezing	1128	2048	
freezing injury	906		
functional foods	1179		
maturation	909		
modified atmosphere packaging (MAP)	1139		
ozone and	1803		
polyphenols in	1875	1877	
postharvest deterioration	902		
preserves and jellies	3	1149	
processing wastes	2168		
ripening	652	909	
scald	409		
semi-tropical	1154		
senescence	910	1031	
South Africa	1408		
spoilage	1026	1028	
storage	905		
sulfited	180		
Taiwan	1419		
temperate	1156		
transport	2051		
tropical	1162		
varietal differences	908		
washing	966		
wax coating	582		
Fryers	1168	1169	
Frying	1168	1969	
Frying oil	741	1169	1172
See also Fats and oils			
Frying oil substitutes	1172		
Fucoxanthin	275	386	
Fumagillin	150		

Index terms	**Links**		
Fumarates, as antimicrobial in foods	66		
Fumaric acid	1	2	66
Fumigant	848		
Fumigants	1172		
Fumonisins	1701	2348	
Functional foods	1176	1390	
Functional properties, molecular modeling	1666		
Fungal diseases, of honeybees	150		
Fungi. *See also* Molds; Mycotoxins			
genetic engineering	1193		
mushrooms	1673		
Furaneol	1191		
Furans, in foods	838		
Furcellaran	1218	1219	
Furocoumarin	72	2342	
Fusarium, cereal grain mycotoxins	1699	2347	2348
Fusel oil	539		
Fuzzy logic	121	2144	

G

Galactose, carbohydrate digestion	269	2458	
Galanthamine	28		
Gallic acid	76		
Galvanic corrosion	418		
Game birds	1954		
Garden crops	902		
Gardenoside	397		
Gardoside	397		
Garlic	71	1179	2404
Gas barrier protection	1807		
Gas chromatography			
antibiotics in foods	59		
flavor evaluation	734		
Gas chromatography (GC), mycotoxins	1688		
Gas chromatography, pesticide detection	1358		
Gas chromatography-mass spectrometry			
antibiotics in foods	60		
flavor chemistry	843		

Index terms **Links**

Gas chromatography-olfactometry (GC), flavor chemistry 843
Gases, diffusion in 1547
Gas packaging. *See* Controlled atmosphere packaging (CAP), Modified atmosphere packaging (MAP)
Gastric lipase 1060
Gastrointestinal infection, foodborne 1084
Gastrointestinal system, anatomy 1058
Gatorade 178
Geese 1950 1964
Gelatin 579 1183 1218 1220 2213
  chemistry 1183
  gums 1218 1220
  in licorice 1467
  manufacture 1184
  Muslim dietary laws 1683
  properties 1185
  safety 1184
  testing methods 1186
  types 1183
  uses 1185
Gelatin desserts
  acidulants 3
  agglomeration of 18
Gelation 880
  preserves and jellies 1149
  surimi 2233
Gellan gum 1218 1220
Gels, with food gums 1216
Gelsemine 31
Gene cloning 1209
Gene expression 1203 1665
General Agreement of Tariffs and Trade (GATT) 1006
General attack 419
General Foods 204
General Foods Corp., nonnutritive sweeteners 2260
Generalized Procrustes analysis (GPA), sensory analysis 2142
Genes, PPO genes 212
Genetic engineering
  amino acid production 1997

Index terms **Links**

Genetic engineering *(Continued)*
  animals 2671
  applications 1205
  food fermentation 922
  food flavors 1188
  food science and 1210
  modeling of functional properties 1666
  oilseed plants 730
  principles 1198
  proteins 2023
Genetic exchange, in vivo 1205
Genetics
  aquaculture 108
  principles 1198
Genetic tests, microbiological analysis, rapid 2041
Genipingentiobioside 397
Geniposide 397
Geniposidic acid 397
Gentianine 30
Geosmin 1192
Germany
  food industry 1393
  licorice 1470
Gerstmann-Straussler-Scheinker syndrome 199
Ghee 228 453
Gibberella ear rot 1699
Gin 523 526 538
Ginger 2200
Ginger ale 174
Glass, as packaging material 1824
Glass electrodes 1310 1313
Glaucine 28
Glazing agents 856
Glaziovine 28
GLAZoFREEZE 1116
Glucagon, satiety agent 89
β-Glucanase 659
Glucitol 263
Gluconeogenesis 1064 1066 1069

Index terms	**Links**			
Glucono-delta-lactone (GDL)	3	263	625	
Glucose	263	265	2242	2258
	2266			
digestion	269	1059		
isomerization	1343			
Glucose isomerase	659			
Glucose oxidase	72	659		
Glucose syrup	1344	2242		
β-Glucosidase	659	2171		
Glucosinolates	886			
Glutamate dehydrogenase, in marine animals	1526			
Glutathione	77			
Glutathione peroxidase	78			
Glutathione-S-transferases, in marine animals	1527			
Gluten-sensitive enteropathy	865			
Glycerides	723			
Glyceryl monostearate, as emulsifier	608			
Glycinin	2185			
Glycoalkaloids	2341			
Glycogen, digestion	1059			
Glycogenesis	1064	1066		
Glycogenolysis	1064	1066		
Glycogen phosphorylase, in marine animals	1527			
Glycolysis	1062			
Glycosidase assay	649			
Glycosidases, flavors and	1189			
Glycosides, cyanogenic	2337			
Glycyrrhyzin	2245	2256	2340	
Goats, livestock feed	1490			
Goiter	1301			
Goitrin	32			
Gold, as colorant	403			
Golden king crab	438			
Goose	1950	1964		
Gooseberries	1159			
Gorgonzola cheese	303	305	308	309

Index terms **Links**

Gouda cheese 302 303 306 307 309 329 332 333 457 917
Gracilaria, cultivation 2100 2101
Grading
  livestock feed 1492
  mushrooms 1679
  poultry meat 1967
  South Africa 1409
Grain protectants 1212
Grains. *See* Cereal grains
Grain whisky 539
Gramine 32
Grapefruit 1154
Grapefruit juice, limonin 1470
Grape juice 182
Grapes 375 1159
  Australia 1380
  dehydration 1149
  growing 2662
Gravimetric Diluter 2037
Greek olives 1759 1771 1774
Green beans, canning 2412
Green cod 817
Green coffee 354 355
Green crab 439
Greenland halibut 819
Green olives 1766 1767
Green peas
  canning 2414
  frozen 2415
Green tea 2300 2303
Grewe diamine 2532
Grinding
  coffee 358
  meat 1568
  poultry meat 1967
  sausage 2070
Ground beef, cooking-cooling 1267 1269

Index terms **Links**

Gruyere cheese 303 305 309
Guanabana 1167
5'-Guanylic acid 1728 2080
Guaran 1221
Guar gum 1218 1221
Guavas 1165
Guinea fowl 1955 1964
Gum acacia 1222
Gum arabic 1218 1222 2213
Gums 860 1216 2213
  agar 1218 1219
  algins 1217 1218
  carboxymethylcelluloses (CMC) 581 582 1217
  carrageenans 581 750 879 1218 1219 2213
  furcellaran 1218 1219
  gelatin 1218 1220 2213
  gellan gum 1218 1220
  guar gum 1218 1221
  gum arabic 1218 1222 2213
  gum tragacanth 1218 1222
  hydroxypropylcellulose 581 1222
  hydroxypropylmethyl cellulose 581 1223
  konjac mannan 1218 1222
  locust bean gum (LEG) 1218 1221
  methylcellulose 581 1223
  pectin 581 879 1149 1218 2213
  pectins 1218 1223
  properties 1218
  rheology 1216
  in surimi 2238
  tara gum 1221
  xanthan gum 1218 1224 2213
Gum tragacanth 1218 1222
Gun puffing, cereals 204
GYROCOMPACT 1122

Index terms	Links			
H				
Haddock	816			
Haemoglobin	396			
Haems	396			
Hake	817			
Halibut	818			
Hallucinogenic mullet	1537			
Halogens, as disinfectants	502			
Ham, dry-cured	1619			
Hamanatto	2183	2184		
Hammer mill	361			
Handling				
chilled foods	339			
food plant design	948			
fresh fish and seafood	786			
postharvest	2426			
vegetables	2408			
Hangover	24			
Hatpin	1346			
Hard cheese	917			
Hard-cooked eggs	597			
Hardening, ice cream	1338			
Hard red wheat	2642			
Hard wheat flour	287	2648		
Harmala alkaloids	29			
Harmaline	29			
Harmine	29			
Harringtonine	29			
Harvey W. Wiley Award (AOAC)	79			
Hazard analysis	346	2032		
Hazard Analysis and Critical Control Points (HACCP)	759	772	798	1433
	2032			
Hazard identification, toxicology	1053	2353		
Hazelnuts	2682			
Heads	539			
Heart disease	8			
Heat	1225			
Heated-surface dryers	493			

Index terms	Links	
Heat exchangers	1227	2310
aseptic processing	124	
fouling	1231	
paraflow	1237	
plate vs. tubular	1246	
principles	1228	
scraped surface	1254	
Heath trays	108	109
Heating. *See also* Thermal processing		
aseptic processing system	123	
ohmic heating	128	
Heat penetration, in thermal processing	2316	
Heat processing. *See* Thermal processing		
Heat-recovery equipment, dryers	563	565
Heat resistance, thermal processing	2314	
Heat transfer	1271	
blanching	195	
freeze drying	1108	
in thermal processing	2315	
in thermal sterilization	2323	
Heat-transfer coefficient, evaporators	680	
Heat treatment. *See* Thermal processing		
Heavy filth	758	
Helminths, foodborne disease	1844	
Hemagglutination assay	1347	
Hemanatto	922	
Hemicelluloses	754	
Hemlock	34	
Hemolytic uremic syndrome (HUS)	1085	
Hemorrhagic colitis (HC)	1084	
Hepatitis, shellfish consumption	771	1088
Herbal teas	2303	
Herbs. *See* Spices		
Hermodactyl	28	
Herrings	821	1537
Heterocyclic amines	2349	
Hexachlorophene, as disinfectant	505	
HGMF (hydrophobic grid membrane filter)	1091	1622
High blood pressure	7	

Index terms **Links**

High erucic acid oil 257 258
High-fiber diets 756
High-fructose corn syrup (HFCS) 172 184 2273
High-intensity sweeteners 859
Highly corrosive foodstuffs 429
High-methoxyl (HM) pectins 581
High-performance liquid chromatography (HPLC)
  amino acid analysis 2014
  mycotoxins 1688
High-potency sweeteners 2245
High-pressure processing (HPP) 126 978 1034 1279
High-temperature preservation 955 1633
High wines 539
Hiproly 288
Hires root beer 174
Histamine 32
Histamine poisoning 771 1051 1088
Histidine-derived alkaloids 30
HM pectin 1150
Hogs, livestock production 44
Holding time 1126
Hold tube, aseptic processing 124
Homoaporphines 28
Homogenization
  ice cream and frozen desserts 1337
  milk 1654
  theories of 1293
Homogenizers 1290
  applications 1293
  description 1291
  history 1290
  homogenizing valve 1292 1293
  operation 1295
  testing 1295
Homogenizing valve 1292 1293
Homoharringtonine 29
Honey 152 184 2243
Honeybees 148 151

Index terms **Links**

Hop extraction, supercritical fluid technology (SCF) 2225
Hops 156 160 171
Hordenine 32
Hormone replacement therapy (HRT), osteoporosis 1792
Horticulture and Food Research Institute of New Zealand (HortResearch) 1404
Hot-air dryers 488 489
Hot boning, meat 1557
Hot breakfast cereals 205
Hot-gas blanching 192
HPLC heat units 2199
HTST extrusion cookers 703 2310
Huckleberries 1160
Humectant 848
Humidity, product storage and 906
Humulene 158 159
Humulones 72 157 158
Hunger 87 88
  See also Appetite
  conditioned 90
  global nutritional status 1296
  malnutrition 1297
  satiety 88 91 749
Hurdle technology 1303
Hydrastine 28
Hydrazines 2338
Hydrocolloids 860 2212 2238
Hydrogenated starch hydrolysates (HSHs) 2209
Hydrogenation 1313
  applications 1319
  canola oil 256
  catalysis 1315 1318 1320
  catalyst poisons 1317
  mechanism 1313
  of oils 721 725 726 739
  process conditions 1314
  selectivity 1313
Hydrogen-ion activity (pH) 1308
Hydrogen peroxide
  control of 78

Index terms	**Links**		
Hydrogen peroxide *(Continued)*			
as disinfectants	504		
Hydrogen phosphide	1174		
Hydrolases			
flavor and	1189		
in marine animals	1527		
Hydrophobic grid membrane filter (HGMF)	1091	1622	
Hydrophobicity, in food protein systems	1321		
Hydrostatic sterilizers	2309		
Hydroxyanisole, butylated	77		
Hydroxycinnamic acids	72	1872	
β-Hydroxy-β-methyl-glutaryl CoA	274		
Hydroxy-polycarboxylic acids	6		
Hydroxypropylcellulose (HPC)	581	1222	
Hydroxypropylmethyl cellulose (HPMC)	581	582	1223
6-Hydroxyriboflavin	2524		
Hydroxytoluene, butylated	77		
Hygiene, freezers	1132		
Hygrometer	2639		
Hyoscyamine	27		
Hyperfiltration, milk	1655		
Hypermetabolic stress, enteral feeding for	634		
Hypertension	7		
Hypobaric storage	408		
Hypochloride, as disinfectant	506		
Hypochlorous acid, as disinfectant	502		
Hypothalamus, eating and	89		
I			
IBCV method	225		
Ibotenic acid	34		
Ice beer	920		
Ice cream	1333	1657	
composition	1333		
defects	1339		
denned	1333		
emulsifier in	608		
flavor	456		

Index terms	**Links**	
Ice cream *(Continued)*		
processing	1337	
transport	2051	
Ice crystallization, during freezing	1113	
Iced tea	178	
Ices	1333	1340
Icing	741	1464
IgE-mediated food allergies	863	
llama	1165	
Illness. See Foodborne disease		
Image analysis, microstructure	944	
Imaging	899	
Imli	1167	
Immersion electrodes	1310	
Immersion freezing	1123	1230
Immobilized enzymes	1342	
Immune system, offish	812	
Immunoaffinity column (IAC), mycotoxins	1690	
Immunoassay	57	1347
Immunodiffusion	1348	1357
Immunoelectrophoresis	1348	
Immunological methods	1345	
agglutination	1347	1357
bacterial receptor assay	1348	
chemical agents	1357	
immunoassay	57	1347
immunodiffusion	1348	1357
immunomagnetic capture	2042	
meat protein additives	1351	
microbial contaminants	1356	
microbiological analysis, rapid	2041	
mycotoxins	1689	
natural contaminants	1352	
nonmeat protein additives	1348	
quantitative precipitin technique	1348	
Immunomagnetic capture	2042	
Impact response	899	
In-can sterilization	2307	
Inclusion complexation	623	

Index terms	**Links**	
Indicators	1313	
Indicaxanthin	382	
Indirect-contact freezers	1123	
Indirect heating, aseptic processing	124	
Individually quick-frozen (IQF) foods		
fruits	2048	
krill	1454	
Individual Quick Blanch (IQB) system	191	
Indole-3-acetic acid	29	
Indole alkaloids	29	
Indoles	1708	
Industrialized countries		
hurdle technology	1306	
nutritional status	1296	
Industrial Research Limited (IRL)	1405	
Industrial Scientist Award (IFT)	1374	
Inert atmosphere generators	1175	
Infant foods	1368	
Infant formula	1369	
Infection. *See* Foodborne disease		
Information technology, packaging and	1810	
Infrared heating	1969	
Infrared spectroscopy	868	2018
Inhibitors, enzymes	645	
Injection, meat processing	1567	1968
Inosine 5'-monophosphoric acid (IMP)	1727	2080
Inosinic acid	1727	
Insecticides		
fumigants	1172	
grains, postharvest application	1212	2690
grain protectants	1212	
integrated pest management (IPM)	2690	
pesticides	885	
Insect pests, mites	2031	
Inside Laboratory Management	81	
Inspection		
machine vision systems (MVS)	1511	
seafood	2151	2156

Index terms	Links		
Instant beverage powders, agglomeration	17		
Instant coffee	359		
Instant farina cereal	206		
Instant foods	13		
"Instantized"	13		
Instant powders, spray drying	567		
Instant tea	2301		
Institute of Food Technologists (IFT)	1015	1019	1371
Insulating materials, corrosion by	433		
Integrated pest management (IPM)	2690		
Interesterification	272	729	740
Interfacial tension	2226		
Intergranular corrosion	423		
Intermediate moisture food (IMF)	957	1143	
International Award (IFT)	1373		
International Committee on Microbiological Specifications for Foods (ICMSF)	771		
International development			
Australia	1379		
Canada	1383		
Finland	1388		
Germany	1393		
Ireland	1399		
New Zealand	1402		
South Africa	1406		
Spain	1410		
Switzerland	1413		
Taiwan	1416		
United Kingdom	1421		
International fish block	2047		
International Food Information Council (IFIC) Foundation	1015		
International Food Information Service (IFIS)	1425		
International Office of Epizootics (OIE)	1007	1008	
International Olive Oil Council (IOOC)	1775		
International Plant Protection Convention (IPPC)	1007	1009	
International Society of Soft Drink Technologists	175		
International Union of Food Science and Technology (IUFoST)	1378	1427	
Intrinsic fluorescence	1324		
Inulin-based fructose	2265	2282	

Index terms	**Links**			
Inventory control, frozen foods	2049			
Invertase	659			
Invert sugar	2243			
Iodine, as disinfectant	502			
Iodine deficiency	1301	1302		
Iodophors, as disinfectants	503	506		
β-Ionone	280			
Ion-selective electrodes (ISEs)	2104	2107		
Ion-selective field effect transistor (ISFET)	2105	2107		
Ipecac	28			
Ireland				
food industry	1399			
licorice	1470			
Iridoids	397			
Irish whisky	522	523	524	526
	538			
Iron-binding proteins	70			
Iron deficiency	1300			
Iron oxides, as colorants	402			
Iron supplementation programs	1302			
Irradiation of food. *See* Food irradiation				
ISFET (ion-selective field effect transistor)	2105	2107		
Islanditoxin	32			
Isoamylase	660			
Isobetanidin	381	382		
ISOGRID system	2038			
Isohumulones	157	158	171	
Isomalt	2210	2258	2259	
Isomaltase	1061			
Isomaltulose, production	1344			
Isopentenyl pyrophosphate (IPP)	274			
Isoquinoline	28			
Isosafrole	2338			
Isothiocyanates, as antimicrobial	71			
Isotopic immunoassays	1347			
Italian olives	1759			
Italy				
ethnic foods of	442			

Index terms	**Links**	
Italy *(Continued)*		
licorice	1470	
IUFoST (International Union of Food Science and Technology)	1378	1427

J

Jaboticas	1165	
Jackfruit	1165	
Jamaican rum	528	
Jams and jellies	3	1149
Japan		
chilled foods market	338	
ethnic foods of	447	
food additives	851	
Japanese eel	586	
Japanese medlar	1155	
Japanese plum	1155	
Japanese quail	1954	
Java plums	1165	
Jellied eels	590	
Jelly fungus	1677	
Jerky	2163	
Jet agglomeration	14	
Jewish dietary laws	1449	
Jews		
ethnic food of	444	
kosher foods	1449	
Journal of AOAC International	80	
Juice blends	178	
Juices	177	
concentrated juice	628	
frozen concentrated	628	2048
labeling	1821	
production plant	666	667
Jujubes	1165	

K

Kamaboko	1119	
Kamloops trout	828	
"Kazunoko"	821	

Index terms	Links			
K-Blazer	750			
Kecap	2177			
Kefir	916			
Kelcogel	750			
Kellogg Co.	204			
Keltrol	750			
Kenima	2182			
Kermes	379			
Kernel Extension Rapid New Evolution Level (KERNEL)	1514			
Ketone bodies	1076			
Ketopantolatone	2499			
Kettle chips	2160	2164		
Kidney disease, enteral feeding for	635			
Kidneys, fish	802			
Kiln drying	489	490	1144	
Kimchi	921	2430		
Kinetics	1437			
biological reactors	1445			
bioreactors	1445			
energy inputs	1443			
enzyme action	658			
enzyme- and cell-catalyzed reactions	1439			
first-order rate processes	981	1438		
food systems	979			
mass transfer and diffusion	1538			
principles	980			
shelf life	1447			
starch conversion	1442			
King crab	438			
Kirschwasser brandy	527			
Kiwi	1160			
Knock-out mice	2672			
Kocuria spp , fermented meat product starter culture	1617			
Kofi	920			
Koji	1192	2166	2176	2177
Konjac mannan	1218	1222		
Kosher foods	444	1449		
allergies	1453			
biotechnology and	1452			

Index terms	**Links**	
Kosher foods *(Continued)*		
equipment koshering	1450	
gelatin	1452	
ice cream	1340	
industrial cooking	1451	
Jewish dietary laws	1449	
kosher supervision	1451	
meat	47	
Passover	1450	
regulation	1452	
Koumiss	916	
Krausen	171	920
Krill	1453	
Krill protein processing	1453	
Kubelka-Munk equation	369	
Kumquats	1154	
Kuru	199	

L

Labeling	1811	
claims-related statements	1739	1817
history	1733	
information panel	1820	
ingredient list	1735	1820
manufacturer identity	1735	1821
net quantity of contents	1734	1815
nutritional labeling	1732	1812
nutrition facts	1735	1820
optional information	1736	
other requirements	1822	
percent-juice declaration	1821	
principal display panel	1813	
product identity statement	1734	1814
vegetables	2411	
vegetarian products	2438	
warning statements	1735	
wine	2666	
Lab-lab	106	
Laboratory automation systems (LAS)	405	

Index terms	**Links**			
Laboratory information management systems (LIMS)	405			
Laboratory robotics and automation	119	1457		
Lac	380			
Laccaic acid	380			
Laccase	209	210		
Lactase	659	1061		
Lactates, as antimicrobial in foods	65			
Lactic acid, as antimicrobial in foods	65			
Lactic acid bacteria	1391	1624	1631	
Lactitol	1461	2210	2244	2258
Lactobacillus, in food	1631			
Lactococcus	1631			
Lactoferricin B	70			
Lactoferrin	70			
Lactones, butter and cream	456			
Lactoperoxidase system (LPS)	70			
Lactose	269	475	476	1460
	1659	2244		
digestion	1460			
hydrolysis	1343			
in meat products	460	464		
products from	1461	2658		
properties	1460	1461		
sources	1460			
uses	1461			
Lactose intolerance	1460			
Lactulose	1461			
Lager beer	154			
Lake colorants	396			
Lamb				
cuts of	48	51		
freezing	1129			
slaughtering process	47			
Laminators, cookie and cracker dough	187			
Laminine	32			
Lancashire cheese	332			
Langouste	1496			
Lard	41	1683		
Laser Raman spectroscopy, protein structure	2018			

Lateral hypothalamus, eating and 89
Latex-agglutination inhibition immunoassay (LAIA) 57
Laurel leaves 2199
Lautering, beer brewing 161
Laws of thermodynamics 2331
Layered dough products 143
LD50 1049
Leaching 697
Lead, corrosion resistance 417
Least developed countries (LDG), nutritional status 1296
Leavening acids 134
Leavening agents 4 133 185 625 848
Lecithin 737 1462
  in chocolate 607
  as emulsifier 618
  in margarine 1463 1523
Lecithinase 1061
Leechee 1155
Legumes
  Australia 1380
  functional foods 1178
  spoilage 1026
Leicester cheese 324 332
Lemma 292
Lemons 155
Lempuk 2628
Leoconostoc 1631
Leurocristine 30
Leurosine 30
Levulose 2241
Licorice 1466
Light
  color, theory of 364
  food deterioration and 954
  nondestructive quality evaluation with 897
  pulsed light processing 2025
  PureBright light 2025
Light beer 167 920

Light Butter 227

Light filth 758

Light or Life products 1333

Light microscopy 939

Light whisky 526

Limburger cheese 301 303 306 308 309 917

Limes 1165

Limonene 2340

Limonin 1470

Limulus amoebocyte lysate (LAL) test 1093

Linear discriminant analysis (LDA), sensory analysis 2139

Linear raceways 101

Linoleic acid, cancer and 240

Lipase 659 1060 1061 1073
  enzyme assay 649
  food spoilage 1029
  solid-state fermentation 2171

Lipemia 1074

Lipid-based fat substitutes, in ice cream and frozen dessert 1335

Lipid films 581

Lipid oxidation catalysts, inhibiting 76 78

Lipids. *See also* Fats and oils
  analysis for 871
  livestock nutrition 46
  in military food 1648
  in milk 220
  nutrition 1479
  oxidation 880 1030 1598 1796
  physical chemistry 880
  in wheat flour 2644

Lipoic acid 1916

Lipoxygenase 78 1031
  in cereals 654
  enzyme assay 650 2409
  in marine animals 1526

Lipoxygenase-catalyzed peroxidation 1798

Liqueurs 528

Liquid chromatography, antibiotics in foods 58

Index terms	**Links**		
Liquid chromatography-mass spectroscopy, antibiotics in foods	60		
Liquid food concentration	628		
Liquid foods			
rheology	2063		
thermal sterilization	2321		
Liquid junctions	1311		
Liquid-liquid extraction	698		
Liquids			
agitated batch (in-container) heating	2324		
diffusion in	1547		
difrusivity in	1545		
nonagitated batch (in-container) heating	2327		
rheology	2324		
Liquid-solid extraction	697		
Liquid sweeteners	2240		
Listeria	344	1357	1958
Listeria monocytogenes	1087	1619	
Listeriosis	1087		
Litchis	1155		
Litesse	750		
Livestock feeds	1485		
amino acids in	1998		
by solid-state fermentation	2169		
carotenoids in	1913		
computers and	1488	1494	
ingredients	1491		
manufacture	1488		
nutrient requirements	1487		
processing	1493		
regulation	1493		
soy protein in	2189		
Livestock production			
animal nutrition and feeding	46		
animal reproduction	44		
antibiotics in	54		
artificial insemination	53		
breeds	45		
business aspects of	43		
crossbreeding	46		

Index terms	**Links**			
Livestock production *(Continued)*				
embryo transplantation	53			
feeds	1485			
recombinant DNA technology	52			
transgenic animals	53			
LM pectin	1150			
Lobeline	27			
Lobster	1496			
Locoweed, honeybees and	150			
Locust bean gum (LBG)	1218	1221		
Loganberries	1160			
Lo Han Kuo sweetener	2245	2257		
Longans	1155			
Lonza synthesis	2472	2473		
Loquats	1155			
Lorelite	750			
Low-acid foods	1501	1503	1506	
Low birth weight infants	1299			
Low-erucic acid rapeseed oil (LEAR)	729			
Low-ethylene controlled atmosphere (CA)	408			
Low-fat ice cream	1333			
Low-methoxyl (LM) pectins	581			
Low-oxygen controlled atmosphere (CA)	408			
Low-pressure storage	408			
Low-temperature preservation	1633			
Low-temperature-rendering (LTR) systems	37	39		
Low wines	539			
Lubricant	848			
Lumac	2039			
Lupinine	27			
Lupulone	72			
Lutein	275	276	386	2294
Lycadex	750			
Lychee	1155			
Lycopene	9	274	276	279
	385	386		
Lycopodine	27			
Lysergic acid	29			

Lysine 1998
Lysine-derived alkaloids 27
Lysozyme 69 599 650

M

Macadamia nuts 2683
Mace 2200
Machine vision 119
Machine vision systems (MVS) 1511
Mackerel 831
Mad cow disease 198
Magnesium phosphide 1173 1174
Magnetic resonance, nondestructive quality evaluation with 899
Mahe 1163
Ma Huang 27
Maillard reaction 454 657 839 877 1648 1709
Maitotoxin 1534
Maize. *See* Corn
Malathion 1212
Malaxation 1761
Malic acid 1
Malnutrition 1297
Malonga 1164
Malt 153 155 170 1517
Maltase 1061
Malted barley 524
Malthus system 2040
Malting 1518
Malting aid 848
Maltitol 2257
Malt liquors, alcohol content 19
Maltodextrins 2243 2277
Maltooligosaccharides 265
Maltose 2265
Maltrin 750
Malt whisky 525 539
Malvidin 1923

Index terms	Links		
Mamey	1165		
Management issues, decision making	1001		
Mandarin oranges	1155		
Mandarins	1155		
Mangos	1165		
Mangosteens	1166		
Mannitol	2211	2244	
Manufacturing execution systems (MES)	406		
Maple syrup	2265	2278	
Maraschino cherries	181	1158	
Margarine	456	1521	
additives in	853		
as emulsion	602		
fats in	754	1522	
Finland	1391		
history	1521		
ingredients	1522		
lecithin in	1463	1523	
manufacture	1265	1524	
monodiglycerides in	607		
quality assurance	1524		
regulation	1522		
Margetine	28		
Marine enzymes	1525		
Marine oils	722	723	
Marjoram	2200		
Marketing			
carbonated beverages	176		
Muslim dietary laws and	1683		
United States Food Marketing System	2701		
Marketing research	938		
Marmalade	1149		
Marshmallow, manufacture	1266	1268	
Mash filter	161		
Mashing			
beer brewing	159		
for distilled beverage spirits	529		
Massaging, meat processing	1567		
Mass transfer	195	1538	1543

Masticatory substance 848
Materials handling, food plant design 948
Mating. *See* Reproduction
Matrix solid-phase dispersion (mspd) techniques, antibiotics in foods 56
Maturing agents 852 2648
Matzoh 133
Maximum tolerated dose (MTD) 1056
Mayonnaise 362 853
Maytansine 34
Mead 19 20
Meals, animal by-product processing 41
Meat. *See also* Meat processing, Meat products, Meat science, Processed meat products
  aging 1589
  Australia 1379
  canning of 244
  cholesterol in 1600
  color 369 652 1596
  connective tissue 1585 1591
  cooking and tenderness 1592
  curing 6 66 1576
  electrical stimulation 1549
  fermented products from 921
  Finland 1391
  flavor 837 1598
  freezing 1129 1580 1598 2047
  functional foods 1180
  hot boning 1557
  mechanical deboning 1265
  modified atmosphere packaging (MAP) 1561 1835
  muscle 1585 1671 1725
  nutrition 1599 1600
  odor 1598
  phosphates 1883
  protein denaturation 2008
  quality 1588
  sausages 2069
  slaughtering process 47 1586 1604
  smoking 1570 1579
  South Africa 1408

This page has been reformatted by Knovel to provide easier navigation.

Index terms **Links**

Meat *(Continued)*
  Spain 1410
  spoilage 1026 1028
  storage 1598
  structure 462
  Taiwan 1418
  tenderness 1551 1590
  texture 2618
  USDA quality grades 48
Meat analogues, agglomeration of 17
Meat-based snacks 2163
Meat emulsions 462
Meat Industry Research Institute of New Zealand (MIRINZ) 1403 1605
Meat ligaments 599
Meat-meal tankage 41
Meat processing 1567
  chopping and emulsifying 1569
  grinding 1568
  injecting 1567
  massaging and tumbling 1567
  mixing 1569
  packaging 1573
  sausage 2070
  smoking 1570
  stuffing 1570
  waste management 2606
Meat products. *See also* Processed meat products
  acidification 1579
  antioxidants in 5
  by-products 1581
  cooking-cooling 1267 1269
  curing 1578
  dairy ingredients in 460
  home meal replacement 1581
  nitrosamines in 1712
  preservation 1580
  restructured meat products 1577
  seasonings 2202
  smoking 1570 1579
  Standard Industrial Classification 987

Index terms	**Links**			
Meat protein				
classification	465			
immunological analysis	1351			
interaction with milk proteins	464			
Meat science	1584			
See also Meat processing; Meat products; Processed meat products				
connective tissue	1585	1591		
cooking of meat	1592			
defined	1584			
meat aging	1589			
meat color	1596			
meat quality	1588	1594		
meat storage	1598	1600		
meat tenderness	1551	1590		
muscle structure and function	1584			
muscle to meat	1586	1671		
principles	1584			
Mechanical deboning	1265			
Mechanical refrigeration, in freezers	1134			
Mechanical vapor recompression (MVR) evaporators	671	674	684	691
Mechanized freezing tunnels	1119			
"Mechanofusion"	16			
Medications. *See* Drugs				
Mediterranean Diet Plan	10	2688		
Mediterranean-type diet	10			
Medium-chain triglycerides (MCTs)	730			
Medlars	1155			
Meitauza	2175			
Meju	2175			
Melanins	273			
Melatonin	29	1918		
Meleshin process	225			
Mellorine	1333	1340	1658	
Membrane filtration	1622			
Membrane separation, milk	1655			
Membrane spread technique	1622			
Membrane technology	1627			
membrane filtration	1622			
reverse osmosis (RO)	1627	1628	1655	2357

Index terms	Links		
Membrane technology *(Continued)*			
ultrafiltration	301	1655	2357
Menhaden	823	2077	
Mescaline	28		
Metabolic exhaustion, microorganisms	1305		
Metabolism			
carbohydrates	1062	1068	1069
cholesterol	1077		
creatine	1072	1073	
creatinine	1072	1073	
fats	1074		
nucleic acids	1077		
proteins	1070		
Metal catalysts, hydrogenation	1318		
Metallized films	1825		
Metalloporphyrins	883		
Metal oxide gas sensors	2106		
Metal phosphides	1173		
Metals, as packaging material	1825		
Methionine	1998		
Methocel	750		
Methoprene	1213		
10-Methoxyibogamine	29		
Methoxypsoralen	2344		
Methoxysalen	2343		
Methyl bromide	1174	1433	2693
Methyl cellulose (MC)	581	582	1223
Methylcobalamin	2553		
Methyldeacetylasperuloside	397		
2-Methylfuran	837		
Methyl pyrazine	32		
Methyl pyrroline	32		
Methylxanthines	231		
Mevalonic acid	272	274	
Mexican-Americans, ethnic food of	445		
Mexican oregano	2200		
Mezcal	519		
Mezcal azul	528		

Index terms	**Links**			
Microbacterium	1361			
Microbial contaminants	1356			
Microbial inactivation, nonthermal processes	964			
Microbial populations, estimation	2039			
Microbial receptor assay (mra)	56			
Microbiological assay, mycotoxins	1691			
Microbiology				
chilled foods	344			
dairy products	1656			
dry-cured ham	1619			
of fermentation	914			
fermented meat products	1617	1618		
fish and shellfish	763			
foodborne disease	1089			
of foods	1629			
freezing	1114			
gelatin	1187			
hurdle technology	1303			
low-acid and acidified foods	1501	1503		
military food	1649			
modified atmosphere package (MAP)	1563	1838		
pickling vegetables	2431			
poultry	1957			
pulsed light processing	2025			
rapid methods of analysis	2037			
spices	2198			
spoilage	1027			
thermal processing	2314			
Microcalorimetry	1092			
Micrococcus	1631			
Microcolony direct epifluorescent filter technique (Microcolony-DEFT)	1091			
Microcrystalline cellulose	1335			
Microencapsulation, gelatin	1186			
Microfiltration (MF)	302	1627	1628	1656
Micromineral deficiency	1662			
Microminerals	1661	1664		
Micronutrient deficiency disorders	1299			
Micronutrients, minerals	1660			

Index terms	Links			
Microorganisms				
in fermentation	914			
foodborne disease	1079	1089		
food deterioration	953			
in foods	1629			
genetic engineering	1205			
heat resistance	2314			
homeostasis	1304			
metabolic exhaustion	1305			
osmotic stress and osmoregulation in	2637			
solid-state fermentation	2166			
soy food manufacture	2178	2179	2181	2183
stress reactions	1306			
sulfites and	2219			
water activity and	2637			
wine	2663			
Microscopy, food microstructure	939			
Microstructure				
atomic force microscopy (AMF)	942			
dynamic light scattering (DLS)	943			
electron microscopy	941			
image analysis	944			
light microscopy	939			
Microwave blanching	192			
Microwave freeze drying (MWFD), military food	1645			
Microwave heating	1229	1635	1969	
Microwave popcorn	2162			
Microwave science	1635			
Middle Eastern ethnic foods	446			
Mildly corrosive foodstuffs	429			
Military food	1641			
retort pouch	2055	2061		
U.S. Armed Forces food R&D	2363			
Milk	48	453	469	1653
See also Dairy products; Milk products				
acidophilus milk	916			
analysis and testing	1656			
Australia	1380			
biscuits and crackers	185			

Index terms **Links**

Milk *(Continued)*
  bulgaricus milk 916
  buttermilk 542 916
  by-products from 1659 2652
  casein digestion 1060
  cheese making 303
  composition 471 1653 1657
  cultured milk, therapeutic value 916 1391
  cultured milk products 449
  drug residues 1359
  dry milk 540 1659
  as emulsion 602
  evaporated milk 661 1659
  fat. *See* Milk fat
  Finland 1391
  flavor 454 458 838 841
  flavor defects 454
  foodborne disease 1087
  genetic engineering for 2673
  heated-milk defects 345
  homogenization 1654
  kefir 916
  koumiss 916
  Maillard reaction 454 657 839 877
  modified 471
  packaging 1656
  pasteurization 454 656 1655
  powder 477
  processing 1654
  production 48 52 627
  properties 1653
  protein. *See* Milk protein
  spoilage 1026
  sweet acidophilus milk 916
Milk chocolate 350
Milk fat 472 1653
  in butter 220
  composition 722
  desaturated 228
  functional properties 476

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links		
Milk fat globule membrane (MFGM)	227		
Milking machine	48	52	
Milking parlor	48		
Milk powder	477		
Milk products	453		
See also Dairy products			
agglomeration	17		
Finland	1391		
flavor	454	458	
whey	2652		
Milk protein	472	655	1653
denaturation	2008		
films from	580		
functionality	467		
ice cream	1334		
immunological analysis	1349		
interaction with meat proteins	464		
in meat products	461	463	477
Millet	292	296	
Milling			
barley	288		
beer brewing	159		
corn	287		
for distilled beverage spirits	529		
rye	288		
sorghum	288		
Standard Industrial Classification	992		
wheat	287		
Mimosine	32		
Minerals			
absorption	1663		
analysis for	871		
bioavailability	1663		
cancer risk and	242		
encapsulated	625		
food utilization and	1077		
functions	1664		
gene expression and	1665		
in livestock feed	1491		
micromineral deficiency	1662		

Index terms **Links**

Minerals *(Continued)*
  as micronutrients 1660
  in tea 2295
  toxicity 1661
  uptake and transport 1663
Miniaturization, microbiological techniques 2040
Miracle fruit 1166
MIRINZ low-temperature-rendering (MLTR) system 39 40
Miso 922 2175 2178 2190
Mites, allergenic 2031
MIU value 40 41
Mixed natural culture fermentation 914
Mixed pure culture fermentation 914
Mixers, cookie and cracker dough 187
Mixing
  hydrogenation and 1314
  meat processing 1569 1968
Modeling
  functional properties 1666
  thermal processing 2317
Modified atmosphere (MA) storage 408
Modified atmosphere packaging (MAP) 342 1139 1830
  advantages and disadvantages 1838
  baked goods 1837
  fish 1735
  meat 1561 1835
  microbiological safety of foods 1838
  passive MAP 1140
  pasta 1837
  plant products 1837
  poultry 1735
Modified food starch 1370 2206
Modified milk 471
Mogroside 2245
Moist agglomeration 14
Moisture
  analysis for 870
  food deterioration and 954
Moisture content 870

Index terms	**Links**			
Molasses	184	2243	2279	
Mold-modified foods	922			
Mold-ripened cheeses	305			
Molds				
aflatoxins	1080			
defined	1630			
detection	1624			
fermented meat products	1618			
in food	1632			
foodborne disease	1080			
mycotoxins. *See* Mycotoxins				
preserves and jellies	1152			
spoilage	1028			
Molecular dynamics simulations, biopolymers	1668			
Molecular kinetics, mass transfer and diffusion	1538			
Molecular modeling, functional properties	1666			
Mollusks, foodborne diseases	787			
Monascin	399			
Monascorubramine	398			
Monascus	398			
Monellin	884			
Monitoring				
chilled foods	343			
pesticide residues	1870	2698		
Monoammonium glycyrrhizinate (MAG)	2256			
Monoclonal antibodies	1346			
Monoglycerides	614	1523		
Monosaccharides	262	1059		
Monosodium glutamate (MSG)	1370	1864	2080	
Monoterpenoid alkaloids	30			
Monounsaturated fatty acids	9	737	744	1481
Monster plant	1164			
Monterey Jack cheese	299	308	309	
Moroccan olives	1759			
Morphine	28			
Most probable numbers (MPN)	1090			
Mozzarella cheese	299	300	301	303
	306	308	309	332
	333			

Index terms	Links			
Muenster cheese	301	302	306	307
	309	332	333	
Mulberries	1160			
Mullet	823	1537		
Multidimensional scaling (MDS), sensory analysis	2138			
Multiple regression analysis (MRA), sensory analysis	2141			
Multiple-stage dryers	561			
Multistage band dryer	552			
Multitier freezer	1122			
Multivariate analysis (MVA)	2133			
Multivariate analysis of variance (MANOVA), sensory analysis	2143			
Murexine	32			
Muscaridine	32			
Muscarine	32			
Muscazone	34			
Muscimol	34			
Muscle	1584			
biochemical character of	1724			
cell structure	1585			
energy supply	1587			
fish	1724	2078		
glycolysis in	1588			
meat	1585	1671	1725	
modified atmosphere packaging (MAP)	1564			
scavenging of oxygen	1562			
Mushrooms				
cultivation	1673			
dehydration	2404			
nucleotides in	1725	1726		
processing	1678			
solid-state fermentation and	2169			
toxicants in	2338			
Muslim dietary laws	1682			
Mussels, foodborne disease	1534			
Must	2663			
Mustard oil	723			
Mustard seed	2200			

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Mycotoxins	887	1028	1051	1352
	1356	1698	2343	
analysis	1684			
in food supply	1354			
natural	1352			
regulatory control	1355			
Myoglobin	883	1616		
Myrcene	158	159		
Myristicin	2338			
Mysost	2655			
N				
Nanofiltration (NF)	1627	1628	1656	
Narciclasine	28			
Narciprimine	28			
Naringin	836			
Naseberry	1167			
Nasogastric tube feeding, enteral formulas and feeding systems	633			
Natamycin	69			
National Research Council (NRC)	1010			
National Shellfish Sanitation Program (NSSP)	797			
National Soft Drink Association (NSDA)	175			
Native Americans, ethnic foods of	443			
Native flat breads	139			
Natto	922	2175	2181	
Natural casings	579			
Natural circulation evaporators	666			
Natural contaminants, immunological analysis	1352			
Natural extractives, spices	2191			
Natural foods	2439			
Natural language processing	120			
Natural sweetener	859			
Natural toxicants	885	1051	1352	2336
See also Mycotoxins				
Nectarines, dehydration	1147			
Nematodes	1088			
Neobetanidin	381			
Neohesperidin dihydrochalcone (DHC)	860	2245	2254	2256

Index terms	Links		
Neoxanthin	386		
Nervous system, offish	806		
Net-pens, aquaculture	102		
Neural networks	120	121	2144
Neurosporene	274	276	
Neurotoxic shellfish poisoning	1534		
Neurotransmitters, eating and	90		
New Age products	178		
New England boiled dinner	179		
New Zealand			
electrical stimulation of meat	1550		
food industry	1402		
New Zealand Dairy Research Institute (NZDRI)	1403		
New Zealand Institute for Crop and Food Research Ltd. (Crop & Food)	1404		
New Zealand Pastoral Agriculture Research Institute Ltd. (AgResearch)	1404		
Niacin	2442	2444	2487
Niacin deficiency	1301	2493	
Nicholas Appert Award (IFT)	1372		
Nickel alloys	414	416	
Nickel catalysts, hydrogenation	1318		
Nickel sulfur catalysts, hydrogenation	1318		
Nicotinamide	2487		
Nicotine	27	32	
Nicotinic acid	2487		
Nicotinyl alcohol	2494		
Nisin	68		
Nitidine	28		
Nitrites, as antimicrobial in foods	66		
Nitrogen, as fumigant	1175		
Nitrosamines	887	1619	1707
Nitrosation	1707		
NIZO method	224		
Nonagitated batch (in-container) heating	2327		
Noncarbonated beverages	177		
Noncorrosive foodstuffs	428		
Nondairy frozen desserts	1341	1658	
Nondestructive quality evaluation, food crops	897		
Nonfat ice cream	1333		

Index terms	Links			
Non-insulin-dependent diabetes (NIDDM)	8			
Nonionic emulsifiers	614			
Nonmeat protein additives, immunological analysis	1348			
Nonnutritive sweeteners	848	859	2245	
acesulfame-K	859	2245	2248	2252
	2255			
alitame	860	2245	2248	2254
	2255			
aspartame	836	850	860	1344
	2245	2248	2251	2255
cost	2247			
cyclamates	860	2245	2248	2250
	2255			
erythritol	2212	2244	2260	
fructooligosaccharide sweetener (FO sweetener)	2258	2259		
glycyrrhizin	2245	2256		
isomalt	2210	2258	2259	
lactitol	1461	2210	2244	2258
Lo Han Kuo sweetener	2245	2257		
maltitol	2257			
neohesperidin dihydrochalcone (DHC)	860	2245	2254	2256
new developments in	2260			
saccharin	860	2245	2248	2249
	2255			
safety	2246			
solubility	2247			
stability	2247			
stevia sweeteners	860	2245	2255	
sucralose	860	2245	2248	2253
	2255			
taste	2245			
thaumatin	860	884	2245	2256
	2257			
Nonthermal preservation	126			
No observed adverse effect level (NOAEL)	1049			
No observed effect level (NOEL)	1049			
Norbixin	385	387		
Norepinephrine	32			
8-Nor-8-hydroxyriboflavin	2524			
Nori	2102			

Index terms	**Links**		
Northern anchovy	814		
Northern Ireland	1399		
Norwalk virus	1088		
Norway lobster	1496		
Nosema disease	150		
Nuclear magnetic resonance (NMR)	869	899	2018
Nucleic acid polymers, structure	1720		
Nucleic acids			
metabolism	1077		
optical properties	1722		
properties	1726		
structure	1720		
Nucleosidase	1061		
Nucleotidase	1061		
Nucleotides	1717	2081	
Numerical models, thermal processing	2317		
Nutmeg	2200		
Nutraceuticals	178	1023	
Nutra-Sweet®	2251		
Nutrient depletion, appetite and	90		
Nutrients			
aquaculture	106		
blanching and	193		
of breakfast cereals	207		
cancer risk and	240		
cereal grains	287	296	
livestock production	46		
Nutrient supplement	848		
Nutrition			
alcohol, effect on	23		
breakfast cereals	206		
butter	221		
carotenoids	1910	1912	
cultured milk products	451		
dietary fiber	755		
drying	496		
ethnic foods	442		
fat replacers	748	751	
fats and oils	744		

Nutrition *(Continued)*
  food processing and 969
  frying oil 1171
  gelatin 1186
  hazelnuts 2683
  infant foods 1368
  lecithin 1465
  lipids 1479
  livestock feeds 1485
  meat 1599 1600
  military food 1647
  milk 1653
  nuts and peanuts 2678
  olives 1757
  palm oil 1843
  peptides 1864
  pickling vegetables 2431
  quality assurance 2031
  spices 2194
  surimi 2237
  U.S. Department of Agriculture 2369
  Vaccinium spp., berries 1924
  wheat 2642
  whey 2659
Nutritional labeling 1732 1812
Nutritional status, global 1296
Nutrition Labeling and Education Act of 1990 (NLEA) 850 1733 1811
Nutrition-related diseases 6 10 1302
Nutritive sweeteners 848 859 2240
Nuts 2676
  almonds 2681
  Brazil nuts 2682
  cashews 1164 2681
  diet 2677 2688
  freezing 1130
  hazelnuts 2682
  health and 2676
  macadamia nuts 2683
  nutrition 2678
  peanuts 2677

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Nuts *(Continued)*				
pecans	2685			
pine nuts	2687			
pistachio nuts	2687			
as snack foods	2163			
spoilage	1027			
walnuts	2626	2688		

O

Oatrim	750	1335		
Oats	291			
composition	287			
hot breakfast cereals	205			
nutrient composition	288			
nutritional analysis	296			
physical properties	292			
processing	288			
production statistics	290	291		
rancidity	656			
structure	293			
Obesity	6	91	271	
Ocean perch	824			
Ochratoxins	1353	1702	2349	2350
Octopus ink, as colorant	404			
Ocumo	1164			
Odors				
aromatherapy	118			
blanching and	194			
meat	1598			
sexual arousal and	82			
Offals	35			
Official Methods of Analysis of AOAC International	80			
Ohmic heating	128	979		
Oil extraction, canola	254			
Oil-in-water emulsion	602			
Oils. See Fats and oils				
Oil of sassafras	2338			
Oilseed proteins, films from	580			
Oilseeds	1178	1745	1746	2361

Index terms	**Links**			
Okadaic acids	1534			
Olean	750			
Oleomargarine	1522			
See also Margarine				
Oleoresin paprika	280			
Oleoresins	2192	2201		
Oleo stock	41			
Olestra	730	850	1335	1341
Oleuropein	72			
Olfactory function, sexual arousal and	81			
Olfactory synesthesia	81			
Oligosaccharides	265	269		
Olive oil	10	734	1746	1747
	1751	1759		
Olives	1755	1757	1765	
Olive tree	1756			
Omega-3 fatty acids	744	1483		
Omega-6 fatty acids	1482			
Omelets, prepared	598			
Omnispec	2040			
Onions	71	2404		
On-line computer control	2319			
Oolong tea	2300			
Opium	28			
Opium alkaloids	28			
Optical rotatory dispersion (ORD), protein structure	2018			
Optimization methods	1779	2319		
Optimizing decision maker	1001			
Orange juice, color	369			
Oranges	1155			
Order of reaction	980			
Oregano	71	2200		
Organic acids, as antimicrobial in foods	64			
Organic farming	2425			
Organic foods	2439			
Organophosphate insecticides	1212	2693		
Original gravity	171			
Ornithine-derived alkaloids	26			

Index terms	Links			
Orthophosphates, cheese processing with	309			
Osmoregulation, microorganisms	2637			
Osmotic stress, microorganisms	2637			
Osteoporosis	1789			
Ostrich	1952	1953		
Ovens, cookie and cracker dough	188			
Oven spring	137			
Ovotransferrin	70			
Oxidases, enzyme assay	640			
Oxidation	1796			
catechin	2295			
dairy products	222			
enzyme-catalyzed	1798			
fats and oils	5	728	731	
fatty acids	1076			
fruits and vegetables	212			
lipids	880	1030	1598	1796
photosensitized	1796			
in proteins	1797			
vitamins	1799			
Oxidizing agent	848			
Oxidoreductase, in marine animals	1526			
Oxoaporphines	28			
Oxygen				
aquaculture	105	115	116	
food deterioration and	954	956		
Oxyrase	2042			
Oysters				
foodborne disease	1083	1088	1534	
food processing	2150			
Ozone	103	1801		

P

Pacific cod	816	
Pacific halibut	819	
Pacific herring	822	
Pacific ocean perch	824	
Pacific salmon	826	
Pacific tomcod	818	

Index terms	Links			
Packaged distillation systems	515			
Packaging	966	968	1807	
acidified food	1508			
almonds	2682			
aseptic	126			
beer	166			
biscuits and crackers	189			
of breakfast cereals	207			
chilled foods	341			
coffee	358			
controlled atmosphere packaging (CAP)	1561	1830		
dairy products	1656			
dehydrated vegetables	2402			
dehydration	1146			
distilled beverage spirits	519			
enteral products	639			
Finland	1392			
fish and shellfish products	797			
food-package interactions	1809			
food plant design	947			
frozen foods	2046			
functions	1807			
for high-pressure treatment	1281			
information technology and	1810			
instant coffee	360			
labeling. See Labeling				
low-acid and acidified foods	1507			
materials. See Packaging materials				
meat	1573			
milk	1656			
modified atmosphere packaging (MAP)	342	1139	1561	1830
mushrooms	1680			
Muslim dietary laws and	1683			
noncarbonated beverages	179			
potatoes	1935			
poultry	1960			
pulsed light processing	2026			
regulations	1810			
retort pouch	2055			
sausage	2071			

Index terms **Links**

Packaging *(Continued)*
  snack foods 2164
  vegetables 2411
  vitamin C and 2456
  waste disposal and 1810
Packaging materials 1824
  dehydrated vegetables 2402
  modified atmosphere packaging (MAP) 1561
  retort pouch 2056
Packings, distillation column 514
Paddy straw mushrooms 1677
Pahutoxin 32
Palatibility, appetite and 91
Palea 292
Palletizing, frozen foods 2040
Palm kernel oil 1746 1747 1751
Palm oil 1746 1747 1751 1839
Pancakes, prepared 598
Pandemic 1079
Pantolatone 2498
Pantothenic acid 2443 2496
Pantothenic acid deficiency 2502
Papaverine 28
Papayas 1166
Paper, as packaging material 1825
Paperboard, as packaging material 1825
Paprika 385 388 2198 2200
Paprika oleoresin 385 388
Paraflash 680 691
Paraflow 1237
Parahydroxybenzoic acid esters (parabens), as antimicrobial in foods 66
Paralytic shellfish poisoning 1051 1534
Parasitic organisms 150 1844
Paravap 679 690
Paraventricular nucleus, eating and 89
Parevine 1340
Parmesan cheese 300 303 306 309 332 333 917

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links			
Parsley	2200			
Partial least squares regression (PLS), sensory analysis	2142			
Particle-concentration immunoassay (PCIA)	57			
Particulate food products	129	1109	1270	
Partridge	1955			
Paselli D-Lite	750			
Passion fruit	1166			
Passive modified atmosphere packaging	1140			
Passover, kosher foods	1450	1453		
Pasta	705	998	1837	
Pasteurization	955	970	1633	2305
butter	627			
canned meat	1580			
chilled foods	339			
energy usage	627			
ice cream and frozen desserts	1337			
liquid eggs	596			
low-acid and acidified foods	1506			
microwave processing	1640			
milk	454	656	1655	
processes	2312			
soy foods	2177			
Pasteurized blended cheese	308			
Pasteurized in-pack (IPP)	339			
Pasteurized process cheese	308			
Pasteurized process cheese food	308			
Pasteurized process cheese spread	308			
Pastries	143			
Paté de choux	143			
Patents	1852			
Pathogens. *See* Foodborne disease				
Peaches	1147	1160		
Peanut butter	1271	2680		
Peanut flour	2680			
Peanut oil	1746	1747	1751	2680
Peanuts	2677			
Pears	1147	1161		
Pecans	2685			

Pectate 1861
Pectic acid 1861
Pectic substances 1858
Pectin 879 1218 1223 2213
  binding properties 1860
  films of 581
  food applications 1861
  preserves and jellies 1149
  structure 1859
Pectinase 660
Pectinate 1861
Pectin gel, pH 3
Pectinic acid 1861
Pediatrics, enteral feeding 636
Pediococcus 1631
Pehtze 2181
Pellagra 1301
Pelletierine 27
Pemmican 385 1922
Penetrometer, for cheese studies 319
Penicillium expansum 1702
Penicillium verrucosum 1702
2-Pentanone 837
Pepper 2200
Pepperoni, fermented 921
Peppers
  dehydration 2404
  Scoville heat units 2198
Pepsi-Cola 174
Pepsi-Cola Co. 175
Pepsin, in marine animals 1527
Peptidase, enzyme assay 648
Peptides 1862
Perceptual salience 91
Peroxidase 78
  blanching and 194 215
  enzyme assay 650 2409
  in marine animals 1526

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**		
Peroxidation, lipoxygenase-catalyzed	1798		
Peroxides	78	504	
Peroxyacetic acid, as disinfectant	505	506	
Persimmons	1161		
Pervaporation	1627		
Pesticides	885		
classification	1868		
in foods	1868		
immunological analysis	1357		
postharvest integrated pest management	2690		
regulation	1869		
residues	1870	2693	
use	1868		
Pests			
food deterioration and	954		
in foods	757		
food spoilage	1032		
fumigants	1172		
grain protectants	1212		
integrated pest management (IPM)	2690		
mites	2031		
Petasitenine	2340		
Pet food	42	992	
Petrale sole	820		
Petrifilm system	2038		
Peyote	28		
Pfizer Pharmaceutical Co., nonnutritive sweeteners	2260		
pH	1308		
control	34	848	856
determination	1308		
food deterioration	957		
of foods	1501		
gelatin	1187		
indicators	1313		
in nonaqueous solvents	1313		
process control and	1313		
temperature effects	1312		
Phallotoxins	32		
Pharmaceuticals, amino acids	1991		

Index terms	**Links**		
Pheasant	1955		
Phenolic compounds	1872	1923	
analysis	1874		
as antimicrobial	71		
as antioxidants	67	76	
biological and chemical activities	1878		
biosynthesis	1874		
cancer and	1898		
cardiovascular disease and	1899		
commercial applications	1879		
food processing	1876	1878	
in fruits and vegetables	1875	1877	
oligomeric	1923		
phytochemicals	1901		
structure	1872		
in wine	1927		
Phenols, as disinfectants	502	505	1929
Phenylalanine-derived alkaloids	27		
β-Phenylethylamine	32		
Phenylphenols, as disinfectants	505		
Pheophorbide	391	392	
Pheophytin	391	392	
Pheromones	82		
Philippines, ethnic foods of	448		
pH meter	1312	1313	
Phosphatase	640	1061	1189
Phosphates	68	1881	
Phosphatides	737		
Phosphocreatine	1062		
Phosphofructokinase (PFK), in marine animals	1527		
Phospholipids	724	1462	
Phosphostigmine	29		
Photobacterium	1632		
Photosensitized oxidation	1796		
Photosynthesis	1886		
Photosynthetic bacteria, carotenoids and	280		
Phototaxis	281		
Phototropism	281		
Phthalideisoquinolines	28		

Index terms	Links		
Phycobilins	399		
Phycocyanins	399		
Phycocyanobilin	399		
Phycoerythrins	399		
Phycoerythrobilin	399		
Physical preservation	955		
Phytic acid	2187		
Phytochemicals			
antioxidants	1895		
cancer and	1896		
cardiovascular disease and	1898		
carotenoids	272	883	1909
lipoic acid	1916		
melatonin	1918		
phenolic	1901		
plant toxins in foods	2236		
Vaccinium spp.	1922		
in wine	1927		
Phytoene	276		
Phytofluene	276		
Phytol	392		
Phytolaccatoxin	380		
Phytoplankton, oxygen production by	115		
Pickling agent	848		
Pickling vegetables	2407	2427	
cabbage	920	2428	2430
cucumbers	2428	2429	
fermentation	2430		
kimchi	921	2430	
microbiology	2431		
nitrosamines	1710		
nutrition	2431		
safety	2432		
sauerkraut	920	2610	
shelf life	2432		
starter culture	2433		
storage	2432		
Pie crust	144	742	
Pies	144		

Index terms	**Links**		
Pigeons	1951	1964	
Pigments, carotenoids	272		
Pigs			
breeds	46		
livestock production	44		
reproduction	45		
slaughtering process	47	1609	
Pilocarpine	30		
Pimaricin	69		
Pineapple	1166		
Pineapple guava	1164		
Pinene	71		
Pine nuts	2687		
Pink salmon	827		
Pipeline	32	2338	2339
Pisco brandy	527		
Pistachio nuts	2687		
Pita bread	139		
Pitting corrosion	422		
Pizza dough	140		
Plantains	1166		
Plant assays, mycotoxins	1692		
Plants			
anthocyanins in	375		
aquaculture	95		
chlorophylls	391		
flavor catalysts	1194		
functional foods	1177		
history of foods	1282		
International Plant Protection Convention (IPPC)	1007	1009	
photosynthesis	1886		
phytochemicals. *See* Phytochemicals			
plant toxins in foods	2236		
protein denaturation	2009		
toxic compounds	1051		
transgenic	1211		
Plasma lipids	270		
Plastic, as packaging material	1826		
Plate evaporators	679	686	

Index terms	**Links**			
Plate film evaporators	686			
Plate freezers	791	1123		
Plate heat exchangers	1229	1246		
aseptic processing	124			
Paraflow	1237			
Plauson mill	361			
Plesiomonas shigelloides	771	1087		
Plums	1148	1161		
Pneumatic dryers	489	490	550	555
	2401			
Pneumatic nozzles	560			
Pocket bread	139			
Pokeberries	381			
Pokeweed	380			
Polio virus	1088			
Polishing agents	856			
Pollen	151			
Pollen trap	151			
Pollination, honeybees	151			
Pollock	817			
Pollution-control devices, dryers	563			
Polstyrene, as packaging material	1828			
POLTOX	1426			
Polyelonal antibodies	1346			
Polycyclic alkaloids	27			
Polycyclic aromatic hydrocarbons	887			
Polydextrose	1336	2212		
Polyesters	2287			
Polyethylene, as packaging material	1826			
Polyethylene terephthalate (PET), as packaging material	1828			
Polyglycerol esters	616			
Polyhydric compounds, esters of	616			
Polymerase chain reaction (PCR)	1206	2041		
Polymeric phenols	1923			
Polyols	2209	2244		
Polyphenol oxidase (PPO)	660	2295		
blanching and	194			
browning of fruits and vegetables	208	1798		

Polyphenol oxidase (PPO) *(Continued)*
  enzyme assay 640
Polyphenols 400 1179
  fruits and vegetables 1875 1877
  in tea 2293
Polyphosphates, as antimicrobials 68
Polypropylene, as packaging material 1828
Polysaccharide films 580
Polysaccharide gums 269 1216
Polysaccharides 267 878 1858
Polysorbate 614
Polyunsaturated fatty acids 9 737 738 1481
Polyvinyl chloride (PVC), as packaging material 1828
Polyvinylidene chloride (PVDC), as packaging material 1829
Pomegranates 1156
Pomelos 1154 1156
Ponds
  aquaculture 100 112 115
  eel culture 588
  seaweed aquaculture 2101
Pop 174
  See also Carbonated beverages
Popcorn 2162 2164
Popped pork skins 2163
Porcinesomatotropin (PST) 52
Pork
  cuts of 48 50
  freezing 1129
  hot boning 1558
  slaughtering process 47
  Taiwan 1418
Porphyra, aquaculture 2100 2102
Porphyria, vitamin A and 281
Portuguese brandy 528
Postdrying 1146
Postharvest integrated pest management 2690
Postum Cereal Company 204
Potassium acid tartrate 2
Potassium bicarbonate, as leavening agent 134

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links	
Potassium bisulfite, as antimicrobial in foods	67	
Potassium metabisulfite, as antimicrobial in foods	67	
Potassium nitrite, as antimicrobial in foods	66	
Potassium sulfite, as antimicrobial in foods	67	
Potato chips	2159	2164
Potatoes	1933	
dehydration	2403	
elite basil for postharvest preservation	1907	
freezing	2048	
poisoning by	2342	
processing	1934	
spoilage	1027	
Potato granules, agglomeration	17	
Potentiometric biosensors	2107	
Pouches	968	
Poultry	1941	
Australia	1379	
chicken	1943	1964
consumption	466	
consumption statistics	1942	
contamination	1957	
ducks	1949	1964
flavor	1937	
freezing	1130	
game birds	1955	1964
genetic engineering	2674	
goose	1950	
history	1941	
livestock feed	1488	
microbiology	1957	
modified atmosphere packaging (MAP)	1735	
packaging	1960	
pathogens in	1958	
processing	629	1963
ratites	1952	
slaughtering process	1613	1965
squab	1951	
Taiwan	1418	
turkey	1946	1964
Poultry bologna	466	

Index terms	**Links**	
Poultry meal	41	
Poultry processing, waste management	2606	
Poultry products	1959	1970
Poultry rolls	466	1971
Powdered cellulose	2214	
Powders		
cohesiveness	2624	
enteral feeding formula	638	
Powdery scale disease	150	
PPP factors	2045	
Practical freezing time	1117	
Prawns	2154	
See also Shrimp		
Precious metal catalysts, hydrogenation	1318	
Precipitin techniques	1348	
Precooling, fruits and vegetables	905	
Predrying	1145	
Prefreezing	1116	1125
Pregelatinized starch	2206	
Preheaters	515	672
Prehumulone	158	
Prepared foods, egg products	597	
Preservatives	857	957
See also Food preservation		
in poultry	1959	
in soft drinks	173	
Preserved food, Taiwan	1420	
Preserves and jellies	3	1149
Pressure, hydrogenation and	1314	
Pressure agglomeration	16	
Pressure nozzles	559	
Presterilization	123	129
Pretzels	141	2163
Prickly pears	1167	
Primafresh	31	582
Principal component analysis (PCA)	2133	
Printing ink, canola oil-based	258	
Prions	200	

Index terms	**Links**			
Proanthocyanidins	72	2285		
Proaporphines	28			
Process cheese	308	332	917	1973
Process cheese analogue	311	1985		
Process cheese food	308	918		
Process cheese spread	308	918		
Process control				
computers in	405			
expert system for	121			
machine vision systems (MVS)	1511			
pH and	1313			
Process design, canning	245			
Process development, computers in	404			
Processed cheese. *See* Process cheese				
Processed cheese analogues	1981			
Processed food, food safety	967			
Processed meat products	460			
See also Meat products				
additives	853			
fermentation	1614			
restructured meat products	1577			
sausage	921	2069		
technology	1567			
Process engineering, optimization methods	1779	2319		
Processing aid	848			
Processing plants	968			
cleaning-in-place (CIP)	351			
design and construction	946			
energy usage in	627			
Processing waste				
solid-state fermentation	2168			
waste management	2601			
Process lethality	2315			
Process optimization	1779	2319		
Process piping, food plant design	949			
Procrustes analysis, sensory analysis	2142			
Proctor & Gamble Co., nonnutritive sweeteners	2260			
Procyanidin B	1923			
Produce. *See* Fruits; Vegetables				

Index terms	Links	
Product development	935	
Product-to-product heat exchangers, aseptic processing	124	
Pro-long	582	
Promoted catalysts, hydrogenation	1318	
Promotion	936	
Prontosil red	54	
Proof gallon	539	
Propellant	848	
Propionates, as antimicrobial in foods	65	
Propionibacterium	1631	
Propionibacterium shermanii	1631	
Propionic acid, as antimicrobial in foods	65	
ProPlus	750	
Propolis	151	
Propylene glycol alginate (PGA)	1217	
Propyl gallate	67	77
Proteases	646	660
Protectants, grain	1212	
Protein-based fat substitutes, in ice cream and frozen dessert	1335	
Protein-energy malnutrition (PEM)	1299	
Protein feed, in livestock feed	1491	
Protein films	579	
Protein paste, krill	1455	
Proteins		
absorption	1067	1070
amino acids	1988	2014
analysis for	870	
cancer risk and	240	
degradation	1070	1071
denaturation	2003	
dietary	1066	
digestion	1060	1066
folding	2001	
fortification of	1989	
functionality	882	
genetic engineering	2023	
hydrophobicity in food protein systems	1321	
lipids, interaction with	2021	
livestock nutrition	46	

Index terms	**Links**			
Proteins *(Continued)*				
metabolism	1070			
in military food	1647			
in milk	461	464	472	655
	1653			
oxidation in	1797			
in processing	881			
stabilization	2010			
structure	882	2000	2014	
structure-function relationship	2022			
in surimi	2237			
synthesis	1070			
tannin, interaction with	2288			
texturization	708			
water, interaction with	2021			
in wheat flour	2643			
Protoalkaloids	26			
Protoberberines	28			
Protocatechuic acid	71			
Protopectin	1861			
Protopines	28			
Protoplast fusion	1192			
Protoveratrine	30			
Protozoa, foodborne disease	1088	1844		
Provitamin	2564			
Provolone cheese	301	303	306	308
	309	332	333	
Prunes	1148	1161		
Pseudoalkaloids	26			
Pseudomonas	345	1631		
Pseudomonas putrefaciens	764			
Pseudopelletierine	27			
Psilocybine	29			
Psoralens	72	2343		
Psychrometrics	480	2639		
Ptyalin	1059	1060		
Puerto Rican rum	528			
Puerto Rico, ethnic food of	445			
Puffed cereals	204			

Index terms	Links	
Pufferfish poisoning	1051	1534
Puff pastry	143	742
Pulasans	1167	
Pullulanase	660	
Pulmonary disease, enteral feeding for	634	
Pulque	519	
Pulsed electric field (PEF)	126	979
Pulsed light processing	2025	
Pummelos	1154	1156
Pumps		
distillation system	515	
enteral feeding	637	
Puncture test, for cheese studies	320	
Pungency	836	
PureBright light	2025	
Purge and trap apparatus, flavor chemistry	843	
Purine alkaloids	33	
Purines	26	
Purple larve	2102	
Purpurinidin fructo-glucoside	377	
Pyacyanase	54	
Pyrazines, in foods	838	
Pyrethrins	1212	
Pyridoxine	2505	
See also Vitamin B6		
Pyrroles, in foods	838	
Pyrrolizidine alkaloids	32	2340

Q

Quail	1954	
Quality assurance	405	2029
foodservice systems	1102	
food transport	2055	
margarine	1524	
military food	1649	
preserves and jellies	1152	
Quality control		
beer brewing	168	
chilled foods	345	

Quality control *(Continued)*
  computers in 405
  dehydrated vegetables 2403
  machine vision systems (MVS) 1511
  South Africa 1409
Quality management 2033
Quantitative descriptive analysis (QDA) 2133
Quantitative precipitin techniques 1348
Quantitative sensory profiling (QSP) 2133
Quarg cheese 302
Quaternary ammonium compounds (QACs)
  as disinfectants 503 506
  as emulsifiers 619
Queen bees 149
Quenching fluoroimmunoassay (FIA) 57
Quercetin 1928
Queso fresco 301
Quiescently frozen dairy confections 1333 1340
Quik Pik 767
Quinces 1154 1161
Quinidine 30
Quinine 30
Quinones 209

R

RABIT (Rapid Automated Bacterial Impedance Technique) 2040
Raceways, aquaculture 101 113
Radiation. *See* Food irradiation
Radiation heat transfer 1277
Radioimmunoassay (RIA) 57 1093 1347 1357 1689 1690
Radiometry 1091
Raffinose 1344
Raftiline 751
Rainbow smelt 830
Rainbow trout 828
Raisins 1148 1162
Raloxifene 1793

Index terms	**Links**			
Rambutans	1167			
Rancidity	728			
butter	456			
fish	2077	2079		
oats	656			
Rapeseed	248			
Rapeseed oil	723	729	1746	1747
	1752			
Rapid controlled atmosphere (CA)	408			
Rapid methods of microbiological analysis	1692	2037		
Ratites	1952			
Rauwolfia alkaloids	30			
Reaction mechanism	980			
Reaction rate	980			
Reactive oxygen species, control of	78			
Ready-to-drink (RTD) tea	178	2302		
Ready-to-eat (RTE) breakfast cereals	204	206		
Real extract	171			
Rebaudioside A	2245	2256		
Reboiler, distillation system	515			
Recombinant DNA technology	52	1205		
Reconditioning	757			
Rectification	511			
Red claw crayfish	1498			
Red extract algae	403			
Redfish	824			
Red hake	817			
Redigel system	2038			
Red king crab	438			
Red mold poisoning	1699			
Red mullet	823			
Red pepper	2199			
Red surimi	1455			
Red swamp crayfish	1498			
Reduced-fat butter	227			
Reduced fat products	1333			
Reducing agents	848	2219		
Redundancy, aquaculture	99			

Index terms	**Links**		
Red wine	919		
Reference buffer solutions	1309		
Reference dose (RfD)	1049		
Reference electrodes	1311		
Refined olive oil	1763		
Refined sugar	2215		
Refining, canola	255		
Refrigerant cycle evaporators	672		
Refrigerated transport	2051		
Refrigeration	955	1034	633
absorption refrigeration cycle	927		
fish and shellfish products	766	790	
in freezers	1134		
history	923	1112	
meat	1580		
principles	924		
vapor compression cycle	924		
Rehydration	497		
Relaxation, in cheese	325		
Reldan	1212		
Release agents	848	858	
Renal disease, enteral feeding for	635		
Renal system, fish	802		
Rendering	36	725	
Rennet	660		
Rennet cheeses	299		
Rennin	1060	1452	
Reproduction			
aquaculture	108		
fish	809		
honeybees	149		
livestock production	44		
Republic of Ireland	1399		
Research and Development (IFT)	1375		
Reserpine	30		
Resin films	582		
Resins	835		
Respiratory chain	1064	1066	

Index terms	Links			
Respiratory system, of fish	805			
Response surface methodology (RSM), sensory analysis	2142			
Restructured meat products	1577			
Retinal	279			
Retinol	277	279		
Retort pouch	2055			
Retort processing	2307			
Retrogradation	2206			
Reuse systems, aquaculture	102			
Reverse osmosis (RO)	1627	1628	1655	2357
Reversible compression, military food	1644			
Rhamnosidase	1190			
Rhea	1952			
Rheology	2062			
of cheese	315			
extrusion	710			
fluid foods	2063			
gums	1216			
liquids	2324			
solid foods	2065			
Rhodamine	1347			
Riboflavin	403	2515		
See also Vitamin B2				
Riboflavin-5'-adenosine diphosphate	2524			
Riboflavin-5'-phosphate	2522			
Ribonuclease	1061			
RiboPrinter characterization system	2041			
Rice	291			
Australia	1380			
classification	297			
composition	287			
milling	288			
nutritional analysis	296			
physical properties	292			
production statistics	290	291		
structure	293			
texture	2620			
Rice wine	920			
Rickets	1301			

Index terms **Links**

Ricotta cheese 2655

Rideal-Walker method 506

Ripened cheeses 299

Ripening

  cheeses 303

  fruits and vegetables 652 909

Rising-falling film plate evaporator 679 680 688

Rising-falling film tube evaporator 677 678

Rising film evaporators 676 686

Risk analysis, quality assurance 2032

Risk assessment

  chilled foods 346

  toxicology 1053 2353

Risk characterization 1055 2356

Risk management, food safety 1012

RNA, structure 1721 1723

Roasted eels 591

Roasting, coffee 355 357 357

Robotics 119 1457

Robusta coffee 354 355

Roche synthesis 280

Rocket immunoelectrophoresis 1348

Rockfish 824

Rock sole 821

Roe 821

Roller dryers 493

Romano cheese 303 306 308 309

Root beer 173 174

Root cellars 179

Roquefort cheese 305 309 458 917 918

Rosanthin 280

Rose apples 1167

Rosehips 1162

Rosemary 2200

Roseoflavin 2524

Rosmarinic acid (RA) 1903

Rotary dryers 489 491 543 548

Index terms	**Links**			
Rotary molders, cookie and cracker dough	187			
Rout presses, cookie and cracker dough	188			
Royal jelly	151			
Rubber, corrosion	435			
Rubropunctamine	398			
Rubropunctatin	398			
Ruh	171			
Rum	523	527	538	920
Rumen	47			
Runner peanuts	2678			
Russian cheese	329			
Rye	292			
composition	287			
mashing	529	531		
milling	288			
nutritional analysis	296			
physical properties	292			
production statistics	290			
Rye whisky	525			

S

SA2	750			
Saccharin	860	2245	2248	2249
	2255			
Saccharomyces carlsbergensis	159	163		
Saccharomyces cerevisiae	159	163	531	
Safety. *See* Food safety; Worker safety				
Safflor red	390			
Safflor Yellow	391			
Safflower oil, flavor	734			
Saffron	388	2200		
Safrole	2338			
Sage	71	2200		
Saito effect	390			
Sake	920			
Salad dressing	363	602		
Salad dressings, additives	853			

Index terms	Links		
Salad oil	740		
See also Fats and oils			
Salami	921	1971	
Salatrim	730	1335	
Salicilanilides, as disinfectant	506		
Salivary amylase	1060		
Salmon	101	825	
Salmonella			
chilled foods	344		
detection	1357	1624	
in fermented meat products	1618		
in food	1632		
foodborne disease	1081	1632	
in poultry	1958		
Salmonella 1-2 test	2042		
Salmonella enteritidis	595	1081	
Salmonella typhimurium	1082		
Salt			
biscuits and crackers	186		
in infant food	1370		
as preservative	957		
in sausage	2069		
Salted fish	766		
Saltines	186		
Salt-preserved vegetables, nitrosamines	1710		
Salty taste	835		
Sample preparation	2037		
Sampling	867	1684	2037
Samuel Gate Prescott Award (IFT)	1373		
Sandwich ELISA	1347		
Sanguinarine	32		
Sanitary Code for Bottling Plants	175		
Sanitation	501		
See also Disinfection			
food surfaces	1035		
in poultry processing plant	1969		
product storage and	906		
scallops	2152		
shrimp	2157		

Index terms	Links			
Sanitizing agents. *See also* Disinfectants				
corrosion by	432			
Santols	1167			
Sapodillas	1167			
Sapote agrio	1167			
Sarcocystis	1846			
Sardines	821			
Satiety	88	89	91	749
Satsuma orange	1155			
Saturated fats, in meat	1600			
Saturated fatty acids	9	722	737	744
	1481			
Sauerkraut	920	2610		
Sausage	921	1578	1579	1614
	2069			
Sausage casings	36	42	579	2070
Savory	2200			
Saxitoxin	30	33	1051	1534
	1536			
Scald	409			
Scallops	1534	2152		
Scampi	1496			
Scandoside methylester	397			
School lunch	1100			
"SCHUGI" mixer	14	15		
Scombroid poisoning	1051	1088	1534	
Scombrotoxin	1534			
Scopolamine	27			
Scotch eggs	597			
Scotch whisky	522	526		
Scoville heat units	2198			
Scrambled eggs, prepared foods	597			
Scraped-surface heat exchangers (SSHE)	124	1254		
Scrapie	199			
Scurvy	1301	2463		
Seafood	2150			
See also Crabs and crab processing; Lobster; Scallops; Shellfish; Shrimp				
Australia	1380			

Index terms	**Links**			
Seafood *(Continued)*				
classifying by flavor and texture	2083			
cured and dried	794			
dairy ingredients in	467			
flavors	2071			
foodborne disease	769	1083	1087	
food poisoning	770			
freezing	1129	2047		
freshness	2095			
irradiation	768	795		
microbiology	763			
nitrosamines in	1713			
processing	786			
sensory analysis	786	2072	2083	2095
spoilage	763			
Standard Industrial Classification	997			
total utilization	788			
Sea scallops	2152			
Seasonings	2201			
Seaweed aquaculture	2100			
Secologanin	30			
Second law of thermodynamics	2331			
Second-order reaction	982			
Sectioned and formed meat products	464			
Securinine	29			
Seed oils	723			
Seerfish	831			
Selective corrosion	427			
Selectivity, hydrogenation	1313			
Self-heating rations	1651			
Self-pollution	99			
Self-rising flour	2649			
Semirigid containers	968			
Semisoft ripened cheese	917			
Semi-tropical fruits	1154			
Semolina	287			
Semolina flour	2651			
Semperfresh	582			
Senecio alkaloids	27			

Index terms	**Links**			
Senecionine	2340			
Senescence, fruits and vegetables	910	1031		
Sensor fusion	900			
Sensory analysis	734	1032		
evaluation	2131			
experimental design	2119			
hedonics	2119			
of military food	1646			
perceived intensity, measurement	2116			
principles	2111			
product optimization	2119			
seafood	786	2072	2083	2095
standardization and instrumentation	2132			
statistics	2119	2132		
Separation, milk	1654			
Separators, evaporators	673			
Sequesterant	848			
Sequestering agents	5	858	1039	
Serotinine	33			
Serotonin	29			
Sexual arousal	81			
Shaddock	1154	1156		
Sham-feeding	91			
Shanghai Institute of Organic Chemistry, nonnutritive sweeteners	2260			
Shanzhiside	397			
Shark liver poison	1537			
Sharp freezer	1119			
Shear (rheology)	316			
Shear viscosity, extrusion	711			
Sheep				
breeds	46			
"Dolly"	1210	2672		
electrical stimulation of meat	1550	1556		
livestock feed	1490			
livestock production	44			
reproduction	45			
slaughtering process	47	1604		
transgenic	1210	2672		

Index terms	**Links**			
Shelf life				
enteral products	639			
freezing and	1116			
kinetics	1447			
MAP fresh-cut produce	1141			
military food	1649			
moisture-sensitive packaged foods	1809			
oxygen-sensitive foods	1808			
pickling vegetables	2432			
water activity as indicator	2635			
Shelf-life dating, frozen foods	2049			
Shelf-life testing, chilled foods	346			
Shellac, as colorant	403			
Shellac coatings	582			
Shellac resin	582			
Shellfish	764	2150		
See also Fish and shellfish products; Seafood				
foodborne disease	771	1087	1534	1535
freezing	1129			
marine enzymes	1525			
marine toxins	1534			
microbiology	763			
spoilage	763			
Standard Industrial Classification	997			
viral diseases and	771			
Sherbet	1333	1340	1658	
Sherry wine	528			
Shi	2184			
Shigella	1082			
Shiitake mushrooms				
cultivation	1676			
drying	1681			
flavor and aroma	1680			
toxicants in	2338			
Shionogi process	2533			
Shortenings. *See also* Fats and oils				
biscuits and crackers	183			
plasticizing	1265			
Short-term controlled atmosphere	408			

Index terms	**Links**	
Shredded breakfast cereals	205	
Shrimp	2153	
flavor	2155	
microbiology	765	
processing	787	
Shucked oysters	2150	
Sieve tray	513	
Signal crayfish	1498	
Silicon dioxide, as colorant	403	
Silicone antifoams	845	
Silver, as colorant	403	
Simplesse	750	
Simple sugars	262	
Simulation, thermal processing	2317	
Sinapic acids	72	
Single-cell protein	2612	
Single-culture fermentation	915	
Single radial immunodiffusion	1348	
Single-stage dryers	560	
Singlet oxygen	78	
Single whisky	539	
Skipjack tuna	831	
β-Skytanthine	30	
Slaughter. *See* Animal slaughter		
Slender tuna	831	
Slendid	750	
Slivovitz	527	
Smell		
aromatherapy	118	
sexual arousal and	81	
Smelt	829	
Smoked eels	590	
Smoked fish	766	794
Smoked turkey breast	1971	
Smoking (of foods)	72	957
meat	1570	1579
poultry	1969	
sausage	2071	

Index terms	**Links**	
Snack foods	2159	
additives	853	
extrusion cooking	704	
from egg	598	
packaging	2164	
peanuts	2680	
seasonings	2202	
Snow crab	439	
Society of Soft Drink Technologists	185	
Sockeye salmon	828	
Soda	173	
See also Carbonated beverages		
Sodium acid pyrophosphate (SAPP), as antimicrobial	68	
Sodium alginate	1217	
Sodium aluminum sulfate	135	
Sodium benzoate, as antimicrobial in foods	65	
Sodium bicarbonate	134	185
Sodium bisulfite, as antimicrobial in foods	67	
Sodium chloride		
as antimicrobial in foods	64	
encapsulated	626	
Sodium dodecyl sulfate (SDS)	617	
Sodium hypochlorite, as disinfectant	502	
Sodium lactate, as antimicrobial in foods	65	
Sodium metabisulfite, as antimicrobial in foods	67	
Sodium nitrite, as antimicrobial in foods	66	
Sodium sulfite, as antimicrobial in foods	67	
Sodium tripolyphosphate (SHMP), as antimicrobial	68	
Soft cheese	917	
Soft drink industry	174	
See also Carbonated beverages		
Soft drinks	173	1391
See also Carbonated beverages		
Soft margarine	1522	
Soft pretzels	141	
Soft red winter wheat	2642	
Soft ripened cheese	917	
Soft wheat flour	287	2648

Index terms	Links		
Solanidine	30		
Solanine	33	2341	
Solanum alkaloids	30	2341	
Solar drying	489	492	1144
Soldier Science Directorate	2364		
Sole (fish)	820		
Solid-phase microextraction (SPME)	734	843	
Solids			
diffusion in	1547		
diffusivity in	1545		
rheology	2065		
Solid-state fermentation	2165		
Solka-Floc	750		
Solubility, hydrophobicity	1327		
Solvent	848		
Solvent dehydration	622		
Solvent extraction	509		
Solvent recovery	509		
Sorbates, as antimicrobial in foods	65		
Sorbets	1333	1340	
Sorbic acid, as antimicrobial in foods	65		
Sorbitan derivatives	614		
Sorbitan monostearate	607	615	
Sorbitol	263	2210	2244
Sorghum	291		
composition	287		
milling	288		
nutrient composition	288		
nutritional analysis	296		
physical properties	292		
production statistics	291		
structure	293	296	
Sorghum syrup	2280		
Sorgo	2265	2280	
Sorption isotherms	2615		
Sotolone	1192		
Soul food	443		
Sour cream	916		

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links			
Sour mash whisky	532			
Soursops	1167			
Sour taste	835			
South Africa, food industry	1406			
Soybean flakes	2189			
Soybean meal	2187			
Soybean oil	733	1746	1747	1752
	2187			
Soybean oil margarine	1522			
Soybean protein isolates	2189			
Soybeans	2185			
composition	2185			
enzyme treatment	657			
harvesting and storage	2187			
processing	2187			
production statistics	1942			
properties	2185			
Taiwan	1418			
Soy foods				
fermented	922	2175		
koji	1192	2166	2176	
meitauza	2175			
meju	2175			
miso	922	2175	2178	2190
natto	922	2175	2181	
pehtze	2181			
soybean flakes	2189			
soybean meal	2187			
soybean protein isolates	2189			
soy milk	2189			
soy protein	708	1350		
soy sauce	922	1192	2166	2175
	2190			
sufu	922	2175	2180	
taosi	2175	2183		
tempeh	922	2166	2175	2182
	2190			
tofu	2180	2190		
Soy milk	2189			
Soy protein	708	1350		

Soy protein-lipid films 580
Soy sauce 922 1192 2166 2175 2190
Spain
  food industry 1410
  licorice 1470
Spanish brandy 527
Spanish olives 1758 1768 1770
Spanish peanuts 2679
Spanish saffron 390
Sparging 161
Sparkling wine 919 2664
Sparteine 27
Specialty baked goods 139
Spectrophotometry, color measurement 366
Spectroscopy 868 1324
Spices
  analysis 2194
  as antimicrobials 71
  denned 2195
  economic factors 2196
  essential oils 835 856 2191 2201
  forms and applications 2193
  manufacture 2191 2192 2196
  natural extractives 2191 2201
  nutrition 2194
  oleoresins 2192 2201
  quality 2196
  regulation 2194
  whole spices 2199
Spin flash dryers 571
Spiny lobster 1496
Spiral freezer 1122
Spiral plating method 1090 2038
Spirits 539
Splendid 751
Spoilage 1025
  biochemical degradation 1030
  canned foods 1027 1508

Index terms **Links**

Spoilage *(Continued)*
  chilled foods 345
  chilling injury 1030
  description 1025 1027
  detecting 1032
  endogenous enzymatic degradation 1031
  enzymes and 1028 1031
  fish and shellfish 763
  high-pressure processing (HPP) 1281
  indicators 1026
  lipid oxidation 1030
  mechanisms 1027
  microbiological degradation 1027 2406
  modified atmosphere packaging (MAP) 1563
  physical degradation 1032
  predicting 1033
  preventing 1034
  sensory evaluation 1032
  vegetables 2406
  water activity as indicator 2635
  wines 919
Sponge-and-dough 186
Sports drinks 178
Spray-bed dryer 563 571
Spray chilling 623
Spray dryers 489 491 545 547 558 568 571 1144
Spray drying 16 558 628
  atomization 559
  coffee 359
  encapsulation by 621
  heat-recovery equipment 563 565
  multiple-stage drying 561
  pollution-control devices 563
  principles 558
  single-stage drying 560
  spray-bed dryer 563 571
  vegetables 2402
Spreadable butter 225
Squalene, in olive oil 1764

This page has been reformatted by Knovel to provide easier navigation.

Index terms	**Links**			
Stability, breakfast cereals	207			
Stabilized starch	2207			
Stabilizers	612	848	860	
gelatin	1186			
ice cream	1334			
Stainless steels	423			
corrosion	414	420	422	430
free-machining stainless steels	428			
Sta-Lite	750			
Stamping machines, cookie and cracker dough	188			
Standard freezing time (SFT)	1117			
Standard Industrial Classification (OMB)	986			
Staphylococcus	1617	1618	1631	
Staphylococcus aureus	1080	1618	1958	
Star apples	1167			
Starch	269	880	2203	
acid-modified starches	2207			
beer brewing	156	157		
bleaching	2208			
conversion, kinetics	1442			
cross-linked starch	2206			
digestion	1060	2208		
extrusion cooking	709			
films of	581			
metabolism	1059			
modified food starch	1370	2206		
molecular components	2203			
properties	2205			
retrogradation	2206			
stabilized starch	2207			
in surimi	2237			
uses	2208			
in wheat flour	2643			
Starch derivatives, films of	581			
Starch-related products	2209			
Star-Dri	750			
Starter cultures, cheese making	302			
Statistical quality control (SQC), charts	405			
Statistics, sensory analysis	2119	2132		

Index terms	Links		
Steam			
corrosion by	430		
as leavening agent	133		
Steam blanching	191	192	194
Steamed bone meal	41		
Steam fusion steam jet agglomeration	17		
Steam injection	559		
Steapsin	659	1060	
Stearic acid	730		
Steatorrhea	1073		
Steel cans	1725		
Steelhead trout	828		
Stephen S. Chang Award for Lipid or Flavor Science (IFT)	1374		
Sterility, aseptic processing	125	127	
Sterilization	2306		
aseptic packaging	126		
equipment	2306		
in-can sterilization	2307		
ohmic heater	129		
preproduction	123		
quantitative measure of	2232		
thermal sterilization of liquid foods	2321		
Sterilization agents, aseptic packaging	126		
Sterilizers	2306		
Sternback-Goldberg synthesis	2469		
Steroidal alkaloids	26	30	
Steroidal glycoalkaloids	2341		
Sterols	737	1764	
Stevia sweeteners	860	2245	2255
Stevioside	2245	2255	2256
Stick margarine	1521		
Stigmastadiene, in olive oil	1765		
Still wines	919	2663	
Stilton cheese	305		
Stimulants, sexual	81		
Stocker cattle	43		
Stocking, of ponds	100		
Stokes' law, centrifuges	286		

Index terms **Links**

Stomacher 2037
Storage *See also* Freezing; Refrigeration
  canning 247
  chilled foods 343
  controlled-atmosphere (CA) storage 408
  filth and extraneous matter in food 758
  fish and shellfish products 791
  food crops 179 905
  kinetics 1447
  meat 1598
  modified-atmosphere (MA) storage 408
  nuts 2683 2686 2687
  ozone and 1803
  pickling vegetables 2432
  temperature of 932
  vegetables 179 905 2408
Storage life
  fruits and vegetables 179 905
  for living-tissue foods 962
Straight whisky 525
Strain (rheology) 316
Straining, beer brewing 161
Strainmaster 161
Strawberries 1162
Streptococcus 1631
Stress (rheology) 316
Stress corrosion cracking (SCC) 424
Striped bass 814
Striped mullet 823
Stripping 510
Strychnine 30
Stuffing, meat processing 1968 2070
Stunning, animal slaughter 1604 1610
Sublimation temperature 1108
Succinic acid 2
Succinyl monoglyceride (SMG) 617
Sucralose 860 2245 2248 2253 2255
Sucrase 1061

This page has been reformatted by Knovel to provide easier navigation.

Index terms	Links			
Sucrose	267	859	2214	2240
	2241	2245	2258	
biscuits and crackers	184			
forms	2215			
properties	2216			
sources	2214			
structure	836	2214		
sweetness	2215			
uses	2216			
Sufu	922	2175	2180	
Sugar	269			
See also Sucrose; Sweeteners				
Australia	1380			
forms	2215			
in ice cream and frozen deserts	1336			
in infant food	1370			
invert sugar	2243			
in licorice	1467			
moisture content	2625			
polyols	2209	2244		
in preserves and jellies	1150			
refined sugar	2215			
in sausage	2070			
in soft drinks	172			
South Africa	1408			
sweetness	2215			
Sugar alcohols	2244			
Sugar apple	1156	1164		
Sugar cane	2214			
Sugar refining	1344			
Sugar wafers	188			
Sulfamethazine, as contaminant	1359			
Sulfa on site (SOS) TLC	58			
Sulfited fruits	180			
Sulfites	67	2218		
Sulfonamides	1359			
Sulforaphane, cancer and	9			
Sulfur dioxide	56	215		
Sumitomo synthesis	2472			

Index terms	**Links**		
Summer sausage	1971		
Sun drying	492	1144	
Sunette	859		
Sunflower oil	1746	1747	1753
Supercritical extraction	699		
Supercritical fluid chromatography (SFC), mycotoxins	1689		
Supercritical fluid extraction (SFE)	56		
Supercritical fluid technology (SCF)	2220		
Superoxide anion, control of	78		
Superoxide dismutase	78		
Super stainless steel	414	416	
Supro	750		
Supro Plus	750		
Surface-active agents	848	1038	
Surface finishing agents	848		
Surface removal agent	849		
Surface tension	2226		
Surfactants			
as disinfectants	503		
as emulsifiers in foods	603		
in frying oil	1170		
Surfactive active agents, as disinfectants	503		
Surge tanks, aseptic	126		
Surimi	1455	2045	2229
defined	2229		
manufacture	2229	2238	
texture	2237		
Surimi-based products	767	2235	
Surinjan	28		
Surugatoxin	34		
Swarming, honeybees	149		
Swat test on premises (stop)	56		
Sweet acidophilus milk	916		
Sweet bell pepper	2199		
Sweet chocolate	350		
Sweeteners	858	884	
See also Sugar			
biscuits and crackers	184		

Index terms	**Links**			
Sweeteners *(Continued)*				
ice cream and frozen deserts	1336			
liquid	2240			
nonnutritive	848	859	2245	
nutritive	848	859	2240	
polyols	2209	2244		
preserves and jellies	1150			
in soft drinks	172			
syrups	2265			
Sweet lupin	1380			
Sweetness	859	2215		
Sweetsop	1156	1164		
Sweet taste	835			
Sweet wine	919			
Swine				
livestock feed	1489			
livestock production	44			
Swiss cheese	300	302	303	305
	307	308	309	331
	457	917	918	
Switzerland				
food industry	1413			
licorice	1470			
Swordfish	832			
Synergist	5	848		
Synesthesia	81			
Synthetic sweeteners	185	859	1336	
Syrups	2265			
biscuits and crackers	184			
corn syrup	184	265	2242	2277
dextrose	2242	2265	2267	
high-fructose corn syrups (HFCS)	172	184	2273	
inulin-based fructose	2265	2282		
maltodextrins	2243	2277		
maple syrup	2265	2278		
molasses	184	2243	2279	
sorghum syrup	2280			

Index terms	Links	
T		
T-2 toxin	1702	
Tabasco sauce	385	
Taenia saginata	1848	
Tagetes	388	
Tails	539	
Tainting flavors	2077	
Taiwan		
food industry	1416	
seaweed aquaculture	2100	
Takasago Corp., nonnutritive sweeteners	2260	
Talc, as colorants	402	
Talin®	2257	
Tall-form dryers	560	
Tallow	41	
Tamari miso	2178	
Tamarinds	1167	
Tang	1537	
Tangerines	1155	
Tankage	41	
Tanks, aquaculture	101	113
Tanner crab	440	765
Tannia	1164	
Tannine	72	
Tannins	72	2285
Taosi	2175	2183
Taotjo	2183	
Tapeworms	1848	
Tara gum	1221	
Taro	1164	
Tarragon	2201	
Tartaric acid	1	
Tartness, acidulants and	2	
Taste		
nonnutritive sweeteners	2245	
peptides and	1863	
Tea	2292	
agriculture	2292	

Index terms **Links**

Tea *(Continued)*
  black tea 2295 2297 2303
  colorants from 401
  decaffeination 238 699
  flavonols 2292
  flavor 837
  fresh leaf composition 2292
  green tea 2300 2303
  herbal teas 2303
  instant tea 2301
  manufacture 2295
  physiological effects 2302
  ready-to-drink (RTD) tea 178 2302
  world production 2301
"Tear-down" examinations, cans 247
Tempeh 922 2166 2175 2182 2190
Temperate fruits 1156
Temperature
  chemical reaction rate and 982
  flow and 2065
  food deterioration and 954
  hydrogenation and 1314
  storage 932
Temperature control, chilled foods 343
Tempering, microwave processing 1638
Tenderness, meat 1551 1590
Tenderometer 1592
Tequila 528 538 920
Terpenes 158 159
Terpenoid alkaloids 26
Terpenoids 274
Tertiary butylhydroxyquinone (TBHQ), as antimicrobial 67
Tetrahydroisoquinolines 28
Tetramine 33
Tetrandrine 28
Tetrasodium pyrophosphate (TSPP), as antimicrobial 68
Tetrodotoxin 33 1051 1534 1535

Index terms **Links**

Texture
  apples 2618
  beef 2618
  blanching and 194
  breakfast cereals 207
  butter 223
  cereal grains 2620
  confectionery 2626
  drying, effect on 494
  enzymes and 652
  extrusion and 707 715
  freeze-dried foods 2626
  gelatin 1186
  surimi 2237
  water activity and 2614
Texture profile analysis 2066
Texturization, of proteins 708
Texturized protein products, extrusion cooking 705
Texturizer 848
Thalicarpine 28
Thaumatin 860 884 2245 2256 2257
Theaflavins, in tea 2295
Thearubigins 2295
Thebaine 28
Theflavic acids 2295 2296
Theobromine 33 231 350
Theophylline 33 231
Thermal death time 1504
Thermal functional properties, hydrophobicity and 1328
Thermal oxidation, of fats and oils 732
Thermal processing 2305 2313
  aseptic. *See* Aseptic processing
  blanching. *See* Blanching
  canning. *See* Canning
  computer simulation 2317
  heat penetration 2316
  low-acid and acidified foods 1506
  microbiology 2314

Index terms	**Links**			
Thermal processing *(Continued)*				
mushrooms	1679			
pasteurization. See Pasteurization				
retort processing	2307			
seafood	788			
sterilization. *See* Sterilization				
to disinfect	506			
to preserve	963			
vegetables	2410			
Thermal sterilization, liquid foods	2321			
Thermobacteriology	1503			
Thermocouple psychrometer	2639			
Thermodynamics	2331			
freezing	1126	1226		
heat	1225			
heat transfer	1271			
supercritical fluid technology (SCF)	2220			
Thermostable nuclease (TNase)	1094			
Thiamine	2526			
See also Vitamin B1				
Thiamine deficiency	1301	2532		
Thiazoles, in foods	838			
Thickeners	613	848	860	
Thin-film evaporator	669			
Thin-layer chromatography (TLC)	57	58	869	1687
Thiophenes, in foods	838			
Thiopropanal-*S*-oxide	71			
Third law of thermodynamics	2331			
Thirst	177			
Thistle saffron	390			
Three-stage dryers	562			
Threobromine	33			
Thrombotic thrombocytogenesis purpura (TTP)	1084			
Thruflo dryer	543			
Thua-mao	2182			
Thujone	71			
Thylakoid complexes	1889			
Thyme	71	1906	2201	
Thymol	71			

Index terms	**Links**			
Tiger puffer	1535			
Tilsiter cheese	306			
Time-temperature indicators (TTIs), military food	150			
Time-temperature integrators (TTIs)	2040			
Tin, corrosion resistance	417			
Tinned copper, corrosion	417			
Tissue culture, mycotoxins	1692			
Titanium, corrosion	417			
Titanium dioxide, as colorants	402			
Tocopherols	76	77	2585	2682
cancer and	1898			
cardiovascular disease and	1899			
in olive oil	1765			
oxidation	1800			
Tofu	2180	2190		
Tomatidine	30			
Tomatine	33			
Tomato, flavor	838			
Tomato products, as biologically stable intermediates	181			
Tonic	174			
See also Carbonated beverages				
Tortilla chips	2160	2164		
Tortillas	139			
Torularhodin	277			
Total freezing time (TFT)	1117			
Toushih	2183			
Toxicants, natural. *See* Natural toxicants				
Toxicity	2352			
See also Toxicology				
Toxicology				
chemical residues	1052			
dietary supplements	1053			
dose-response assessment	1054			
environmental contaminants	1051			
exposure assessment	1054			
food additives	1053	1055		
foodborne disease	769	787	1016	1051
foods	1050			
fumigants	1174	1175		

Index terms **Links**

Toxicology *(Continued)*
  hazard identification 1053
  methyl bromide 1175
  natural toxicants 885 1051
  pesticides 1869
  phosphide fumigants 1174
  principles 1048
  processed food 967
  reaction products 1052
  risk assessment 1053 2353
  risk characterization 1055
  toxicity 2352
Toxins, marine toxins 1534
Toxoflavin 33
Toxoplasma gondii 1845
Toxoplasmosis 1845
Tracer 849
Trade promotion 937
Traditional medicine 2341
Transcobalamin II 2554
Transesterification 740
Trans fatty acids 9 738 1481
Transfection 1201
Transferases, in marine animals 1526
Transformation 1201
Transgenesis 1210
Transgenic animals 53 2672
Transition metals, antioxidants to control 76 78
Transmissible mink encephalopathy 199
Transmissible spongiform encephalopathies 198 199
Transport, frozen foods 2051
Trappist cheese 306
Tray dryers 488
Tree tomatoes 1167
Trehalose 266 2244
Trematodes 1088
Triacylglycerols, in olive oil 1764
Trichinellosis 1847

Index terms	**Links**			
3,4,4′-Trichlorocarbanilide (TTC), as disinfectant	506			
Trichothecenes	1353	1356	1699	2347
Triglycerides	220	736	1074	
digestion	1074			
of milk fat	221			
physical properties	738			
Trigonelline	33			
TrimChoice	750			
Trimethylamine (TMA)	2076			
Trimethylamine oxide (TMAO)	1030	1031	2076	
TRIoFREEZE	1122			
Trisodium phosphate (TSP), as antimicrobial	68			
Tristimulus colorimetry	365			
Triterpene aklaloids	30			
Trolox	76			
Tropane alkaloids	26			
Tropical fruits	1163			
Trough dryers	489	492		
Trout, aquaculture	101			
Trypsin	1060	1527		
Tryptamine	33			
Tryptophan-derived alkaloids	29			
Tsu-tsai	2102	2103		
T-TT program	2045			
Tube feeding, enteral formulas and feeding systems	633			
Tubular centrifuge	285			
Tubular evaporators	676			
Tubular film evaporators	686			
Tubular heat exchangers	124	1228	1246	
Tumbling, meat processing	1567			
Tuna	467	766	831	
Tunnel dryers	489	492	1144	2401
Tunnel freezers	1119			
Turkey	466	467	1946	1964
	1971	1972		
Turkey bologna	466	1972		
Turkey breast	467	1971		
Turkey ham	1971			

Index terms	**Links**			
Turmeric	401	2201		
Turmeric oleoresins	401			
Turndown ratio	511			
Turrón, texture	2626			
Two-fluid nozzles	560			
Two-stage dryers	561			
Type A gelatin	1183			
TVramine	33			
Tyrosine-derived alkaloids	27			
U				
Ultrafiltration (UF)	2357			
cheesemaking	301			
milk	1655			
Ultrahigh hydrostatic pressure (UHP)	964	978		
Ultramarine blue, as colorants	402			
Ultrasound	899			
Ultraviolet light				
aquaculture	104			
to disinfect	506			
Ultraviolet processing, aseptic processing	126			
Umami taste	1717	1720	1727	1864
	1989	2080		
Undernutrition	1299			
Unfortified wine	919			
Uniform attack	419			
UNIQUE system	2042			
United Kingdom				
bovine spongiform encephalopathy (BSE)	202			
chilled food regulation	347			
food industry	1421			
food legislation	508			
food regulation	347	508	1421	
licorice	1470			
United States. *See also* under U.S.				
bovine spongiform encephalopathy (BSE)	202			
chilled food regulation	347			
chilled foods market	337			
fat consumption	743			

Index terms	**Links**			
United States *(Continued)*				
fish consumption	777			
food additive regulations	850			
food legislation. See Food regulation				
food safety program	1016			
licorice	1468			
U.S. Armed Forces food R&D	2363			
United States Food Marketing System	2701			
Université Claude Bernard, nonnutritive sweeteners	2261			
University of California at San Diego, nonnutritive sweeteners	2261			
Unsaponifiable matter	40	724		
Unsaturated fatty acids	722	731	1481	
Unsteady-state mass transfer	1543			
Urea, in livestock feed	1492			
Urease, in marine animals	1528			
Urethane	1932			
U.S. Armed Forces, food research and development	2363			
U.S. Bottlers Protective Association	175			
U.S. Department of Agriculture	347	2365		
consumer education	1015			
food consumption surveys	889			
grades and grading	961			
hot line	1015			
labeling	1811	1817	1819	1821
meat quality grades	48			
pesticides	1052			
U.S. Food and Drug Administration. See Food and Drug Administration				
Uvaol, in olive oil	1765			

V

Vaccinium spp.	1922			
Vacuum band dryers	489	493		
Vacuum drying	1142	1145		
Vacuum shelf dryers	489	493		
Valencia peanuts	2679			
Valve tray	513			
Vanilla				
curing	2386			
economics	2396			

Index terms	Links			
Vanilla *(Continued)*				
flavor	2390			
history	2392			
horticulture	2383			
ice cream and frozen deserts	1336			
Vanilla extract	2393			
food regulation	2388			
manufacture	2389			
Vanillin	71	834	2390	2395
Vapor compression refrigeration cycle	924			
Vaporization	1226			
Vapor pressure, measurement	2639			
Vapor recompression	671			
"Variety meats"	35			
Varroa mite, of honeybees	150			
Veal, slaughtering process	47			
Vegetable fats, for cheese analogues	312			
Vegetable oils	722	734	737	1745
See also Fats and oils				
Vegetable proteins, for cheese analogues	312			
Vegetables				
acidulants in processing	4			
ammonia injury	906			
aseptic processing	2411			
Australia	1380			
blanching	190	215	656	2408
	2409			
browning reactions	208	657	660	
cancer risk and	242			
carotenoids in	275	277		
chilling injury	902	906	1030	
classification	2416			
consumption statistics	2420			
controlled atmospheres	408			
defined	2416			
dehydration	2399	2407		
enzymes and	654			
fermented products from	920			
flavor	838	840		

Vegetables *(Continued)*
  freeze drying 2402 2408
  freezing 1127 2048
  freezing injury 906
  functional foods 1179
  growth requirements 2422
  handling 2408
  hybridization 2421
  maturation 909
  modified atmosphere packaging (MAP) 1139
  nitrosamines in 1713
  nucleotides in 1725
  ozone and 1803 1804
  packaging 2411
  pickled 1710 2407 2427
  polyphenols in 1875
  postharvest deterioration 902
  processing 2406
  processing wastes 2168 2609
  production statistics 2417
  propagation 2423
  ripening 909
  senescence 910 1031
  South Africa 1408
  Spain 1410
  spoilage 1026 1028
  storage 179 905 2408
  Taiwan 1419
  transport 2051
  varietal differences 908
  washing 966
  wax coating 582
Vegetarian eating 2434
Vehicle 848
Venison 1922
Vent condenser, distillation system 515
Ventromedial hypothalamic nucleus, eating and 89
Veratrum alkaloids 30
Vernors ginger ale 174
Verotoxin 1084

Index terms	**Links**			
Verrucarin	2347			
Viable cell count procedure	2037			
Viandas	445			
Vibration, nondestructive testing	899			
Vibrio cholerae	770	1082		
Vibrio fetus	1084			
Vibrio parahemolyticus	770	1083	1624	
Vibrio vulnificus	770	1083		
Vicine	33			
Vinca alkaloids	30			
Vincaleukoblastine	30			
Vincamine	30			
Vincaminol	30			
Vinegar, production	921			
Violaxanthin	386	2294		
Viral diseases, of honeybees	150			
Virginia peanuts	2679			
Virgin olive oil	1763			
Virus, defined	1630			
Viruses				
foodborne disease	1088	1844		
in seafood	771			
Viscoelasticity, solid foods	2065			
Viscosity, gelatin	1187			
Vitamers	2441	2444		
Vitamin A	279	1300	1654	2442
	2444	2538		
Vitamin A deficiency	272	1300	1302	2545
	2547			
Vitamin A palmitate	1523			
Vitamin A precursors	1910	1912		
Vitamin A toxicosis	1537			
Vitamin B1	2442	2444	2527	
Vitamin B2	2442	2444	2515	
Vitamin B3. *See* Nicotinamide				
Vitamin B6	2442	2444	2505	
Vitamin B12	2443	2551		
Vitamin B12 deficiency	1302	2553		

Index terms	**Links**			
Vitamin C	1800	1897	2443	2449
See also Ascorbic acid				
Vitamin C deficiency	1301	2463		
Vitamin D	1523	2442	2444	2562
Vitamin D deficiency	1302	2577		
Vitamin deficiency	1300			
Vitamin E	1800	1899	2442	2444
	2585	2682		
Vitamin K	2442	2444	2592	
Vitamins, *See also* individual vitamins				
analysis	2444			
in beer	167			
butter	221			
cardiovascular disease and	9			
drying, effect on	497			
economic aspects	2447			
encapsulated	625			
as food additives	861			
food utilization and	1077			
history	2440			
in livestock feed	1492			
livestock nutrition	46			
in military food	1648			
oxidation of	1799			
physiological function	2444			
production	2447			
properties	2441			
stability	2444			
structure	2440	2442		
in wheat flour	2645			
Vitek ImmunoDiagnostic Assay System (VIDAS)	2042			
Vitek system	2041			
Viticulture	2662			
Vodka	523	525	538	
Voice recognition systems	120			
Volatile oils	835			
Vomeronasal organ (VNO)	82			
Vomitoxin	2347			
Vulgaxanthin	381			

Index terms **Links**

W

Waffles, prepared 598
Walleye pollock 817
Walnuts 2626 2688
Warehouse and distribution 406 935
Warfarin 2343 2344
Wash 539
Washing, to preserve 966
Washing agent 849
Waste management
  animal processing 2601
  dairy products 2601
  egg processing 2603
  fruits and vegetables 2609
  meat processing 2606
Water
  for aquaculture 104 115
  biscuits and crackers 185
  bottled water 178 179
  for brewing beer 154
  corrosion by 430
  food surface sanitation 1038
  food utilization and 1077
  nitrosamines in 1714
  pulsed light processing 2026
  in sausage 2070
  in soft drink processing 172
Water activity 795 1143
  defined 2614 2630 2635
  food texture and 2614
  good manufacturing practice 2630
  measurement 2633 2638
  microbiology 2631 2635
  prediction 2640
  sorption isotherms 2615
Water blanchers 192
Water blanching 190
Water content, reduction of 956
Water-correcting agents 862

Index terms	**Links**	
Water ices	1333	
Water-in-oil emulsion	602	
Water-soluble polymers	860	
Wax apples	1167	
Wax coatings	582	
Waxes, in olive oil	1765	
Waxy corn	297	
West Indian cherry	1154	
Wet-rendered tankage	41	
Wet rendering	37	39
Wheat	290	2641
Australia	1379	
classification	297	
composition	287	
enzymes and	653	
gross composition	2642	
history	2641	
hot breakfast cereals	206	
malting	156	
milling	287	2645
nutrient composition	287	
nutrition	2642	
physical properties	292	
production	2641	
production statistics	290	
structure	292	294
texture	2621	
varieties	2641	2642
Wheat bran	1178	
Wheat gluten films	580	
Whey	2652	
by-products	2652	
composition	2652	
disposal	2659	
fermentation products	2658	
industrial processing	2655	
on-farm utilization	2659	
properties	2654	
types	2652	

Whey *(Continued)*
  uses 2659
Whey beverages 2656
Whey cheese 2655
Whey products, industrial 2657
Whey protein products 2657
Whey proteins, films from 580
Whipped butter 225
Whisky 522 523 538
White chocolate 348
Whitefish, volatile compounds 2074
White hake 817
White pepper 2200
White surimi 1455 2045
White wheat flour 287
White wine 919
Whither sensory-instrumental correlation 2146
Whiting 818
Whole wheat flour 2648
Whole whey products 2658
Wide-body dryers 560
William V. Cruess Award (IFT) 1373
Wine 539 2662
  appellations 2662
  aroma 2664
  cancer and 1931
  classification 919
  consumption 2669
  fermentation 918 2663
  flavonoids in 1928
  flavor 837 2664
  grape growing 2662
  health and 1930
  history 2662
  labeling 2666
  phenolic compounds in 1927
  phytochemicals in 1927
  production statistics 2669
  regulations 2662

Index terms	**Links**			
Wine *(Continued)*				
sensory properties	2664			
spoilage	919	1028		
vinification	1929			
viticulture	2662			
wine making	2663			
Wine aroma wheel	2664			
Wine gallon	539			
Wine making	2663			
Wiped film evaporator	678	679		
Wire-cut machines, cookie and cracker dough	188			
Worker bees	149			
Worker safety				
detergents	500			
freezers	1130			
Wort	159	161	171	539
	920			

X

Xanthan gum	1218	1224	2213
Xanthines, in tea	2293		
Xanthohumol	72		
Xanthophylls	272	273	277
Xanthosine monophosphate (XMP)	1731		
Xixona turrón, texture	2626		
X-rays, nondestructive quality evaluation with	898		
Xylanase assay	649		
Xylitol	1392	2211	2244
Xylopinine	28		

Y

Yeast. *See also* Fermentation				
baked goods	137			
beer brewing	159	160	163	164
	171			
biscuits and crackers	185			
defined	1630			
detection	1624			
distilled beverage spirits	532			

Yeast (Continued)
  fermented meat products 1618
  in food 1632
  food spoilage 1028
  genetics of 1200
  mash 532
Yeast-raised doughnuts 142
Yellow chili peppers 2339
Yelloweye rockfish 824
Yellowfin tuna 831
Yellowtail flounder 820
Yersinia enterocolitica 344 1085 1087 1619 1958
Ylang-ylang 2338
Yogurt 455 458 627 916 1658
Yohimbine 29

Z

Zapotle 1167
Zeacarotene 277
Zearalenone (ZEN) 1353 1356 1699 2347
Zeaxanthin 275 276 386 2294
Zein, coatings from 580
Zero-order reaction 980
Zeroth law of thermodynamics 2331
Zetacarotene 276
Zinc deficiency 1301
Zinc oxide, as colorants 402
Z-Trim 751

This page has been reformatted by Knovel to provide easier navigation.

K

KINETICS

The engineering of food materials, that is, the industrial production and processing of food materials, requires the knowledge of an engineer and a good understanding of the food system in terms of its chemical, physical, and biological properties. In other words, it requires the knowledge of an engineering and scientific nature. Among the said knowledge, one subject stands out that most closely links a scientist and an engineer; that is the topic of kinetics. Generally speaking, kinetics is the study of motion. Because motions are time dependent, kinetics is the study of rates of changes. Traditionally, scientists have been interested in knowing the nature or the mechanism of reaction, and engineers have been interested in the efficiency of the reactions and that of reactors. These two subject matters can be rephrased as molecular (chemical) kinetics and reactor (chemical engineering) kinetics, respectively. Both subjects are important in enabling a food engineer to arrive at an optimal design for a food process.

MATHEMATIZATION OF KINETIC BEHAVIOR

The ultimate goal of science is the formulation of laws, principles, or models, through which the behavior of a system can be deduced without the necessity of experimenting with and constructing the system. The extent to which this goal is reached is an objective measure of the scientific success achieved by the scientist or engineer. Economically, such achievement can help cut a great deal of the cost due to system construction from trial-and-error experimentation. Practically, such achievement is reflected in the predictability of the system derived from the use of the model. Several prerequisites are important in striving for predictability. The first is mathematization. This involves more than just fitting numbers into an equation. With the advent of computers, mathematical modeling has become a more powerful tool than in the past. A successful mathematization of a system, and predictability exerted by the model, depends on the understanding of the mechanistic aspect of the system involved and the identification of the pertinent variables of the system. Experimental design and statistical analysis are two powerful tools for gathering such information in the analytical approach to the system.

The mathematician in the scientist or engineer can say whether a given model is adequate or not, and if such and such conditions are met, a certain behavior will follow. However, it is the scientist or engineer in the mathematician who must judge the adequacy or appraise the predictability of the behavior. Mathematization is a necessity in forming models and making use of their predictability; sound experiments and good judgment are equally necessary in the formulation of foul proof models. Not to take any credit from the usefulness of a pure stochastic, statistical, or synthetic approach to rather unanalyzable problems, the subject matter in this article calls for the analytical, deterministic approach to problem solving.

Chemical kinetics is the study of rate, changes occurred per unit time, and mechanism by which one chemical species is converted to another. Mechanism is the elemental process of motion, that is, collisions among the molecules, atoms, radicals, or ions that take place simultaneously or consecutively in producing the observed overall rate. Overall rate depends on the nature of the participants and the environment surrounding them. The overall rate, in some cases, is controlled by the slowest step of the simultaneous or consecutive reactions. If the overall reaction involves a heterogeneous environment, where, for example, concentration is not uniform, the rate of the transport of the materials involved in the reaction can also control the overall rate of the reaction. In such a case, it is usually considered to be an overall rate controlled by transport (mass transfer), a physical change, not a chemical reaction. Similar arguments can be said for reaction systems with temperature variations. Overall rates can be controlled by heat-transfer rate in some situations.

The changes food materials undergo during processing, preparation, or storage are the results of complex physical changes, chemical reactions, and biological activities. Microscopically or macroscopically biological materials represent systems of heterogeneous natures in terms of the chemicals and biochemicals involved and in terms of the physical separations of these molecules into compartments in the system. Again, the overall rate of a change can be controlled either by the slowest step of a chemical reaction or by a physical change such as mass transfer of an essential substrate. Such a step is called the rate-limiting step or the rate-controlling step. The identification of the said step is necessary for the formulation of the correct mathematical model, which is useful for the design of efficient processes.

BASIC CHEMICAL KINETICS

General Considerations

The kinetics of chemical reactions are usually studied within a deterministic framework; that is, the considerations of reacting chemical species are assumed to be single-valued, continuous functions of time and position, uniquely determined by their initial values and a complete knowledge of the temperature, pressure, and so on throughout the system. The rate of homogeneous simple reaction (one corresponding to the actual molecular event) is assumed to follow the law of mass action (ie, to be proportional to the product of the concentrations of the reacting species).

For example, the rate of the unimolecular reactions A to P and bimolecular reaction A + B to P are expressed as

$$\frac{dp}{dt} = k_1a - k_{-1}p \tag{1}$$

$$\frac{dp}{dt} = k_2ab - k_{-2}p \tag{2}$$

respectively, where k_1 and k_2 are the rate constants for forward reaction and k_{-1} and k_{-2} are those for backward reactions; a, b, and p are the molar concentrations of chemical species A, B, and P, respectively. Most chemical reactions are reversible, to a certain extent. It is customary to write a chemical reaction left to right with the forward reaction rate constants (k_1 and k_2) larger than those of backward reactions (k_{-1} and k_{-2}). In measuring the initial rate of reaction $(dp/dt)_{\text{initial}}$, that is, in the early stage of a reaction, because product concentration p is rather small, equations 1 and 2 are reduced to equations 3 and 4:

$$\left(\frac{dp}{dt}\right)_{\text{initial}} = k_1a \tag{3}$$

$$\left(\frac{dp}{dt}\right)_{\text{initial}} = k_2ab \tag{4}$$

Order of Reaction

Equation 3 states that the initial rate of product formation, $(dp/dt)_{\text{initial}}$ is proportional to the concentration of the substrate A, namely a, to the first power. Hence, this reaction is called a first-order reaction. By the same token, equation 4 represents a second-order reaction. Experimentally, the order of a reaction can be determined by fitting experimental data to the following general equation:

$$\frac{dp}{dt} = ka^n \tag{5}$$

The value of n found could be fractions or whole numbers. Order is strictly an empirical concept. When the stoichiometric equation truly represents the mechanism of the reaction, the order and the molecularity both have the same value. In other words, on a molecular scale the reaction occurs exactly as written. These reactions are called elementary reactions. Many problems in food processing involve the studies of nonelementary reactions. Overall rates are usually measured without knowing the mechanism of the changes, including the steps of chemical reactions and physical changes such as mass and heat transfer.

FIRST-ORDER RATE PROCESSES

Macroscopic changes in the concentration (or other extensive quantity) of a substance tend to be exponential kinetically; in other words, first order in rate expression. This is exemplified by the phenomena of bacterial growth, radioactive decay, enzyme decay, pharmacokinetics (ie, kinetics of drug, absorption, etc), and many other chemical reactions and food processes. This is simply because the behavior of any one bacterium (atom, pair of reactive molecules) is governed by the law of chance. For any one bacterium and such there is a well-defined probability that it will change during a given period of observation. Or more simply, not one changing atom knows whether any one of the other unstable atoms in the sample population has decayed or not. The following example will illustrate how this condition leads to exponential decay. Consider a classroom containing 100 students; each one of them tosses a coin once a minute. Suppose that anyone who obtains a head is asked to leave the room. After the first toss, there will be approximately 50 people leaving; after two tosses, about 25 people leaving; and the number of people remaining in the room will decrease exponentially with time. Mathematically, the number of people, N, in the room, varies with time, according to the following equation (the negative sign recognizes the number is decreasing):

$$-\frac{dN}{dt} = kN \tag{6}$$

Separating the variables and integrating:

$$-\int_{N_0}^{N} \frac{dN}{N} = \int_0^t k\,dt;\ \ln N_0 = kt + \ln N \tag{7}$$

The rate of decrease of people in the room (decay of radioactivity, enzyme activity, or food quality, and microbial death rate in sterilization process), that is, $-dN/dt$, is dependent on the number of people at that time. The rate constant k, though, is independent of both time and the number of people (or other quantity such as enzyme concentration, radioactivity, etc). The half-life ($t_{1/2}$) of an exponential decay function is the time required for the population to become half of its original number; namely,

$$\ln\frac{N_0}{N} = \ln 2 = kt_{1/2};\ t_{1/2} = 0.693/k$$

Instead of decay, for growth such as cell culture after the lag phase, the rate is increasing (positive instead of negative in equation 6), and so the population doubling time is

$$\ln\frac{N}{N_0} = \ln 2 = kt_{1/2};\ t_{1/2} = 0.693/k$$

the same expression as that for half-life. It is worth noting that k, the first-order rate constant, or the exponential growth (or decay) constant, has a dimension of reciprocal of time.

Microbial cell growth rate at exponential phase and cell death rate in a thermal sterilization process are both first order. The specific growth rate and death rate are customarily denoted by μ and D (the decimal reduction time), respectively. They are shown in the following equations. For cell growth rate, x being cell concentration:

$$\frac{dx}{dt} = \mu x \text{ or } \frac{d\ln x}{dt} = \mu \tag{8}$$

For cell death rate:

$$\frac{dx}{dt} = -kx, \frac{d \ln x}{dt} = k \text{ or } \frac{d \log x}{dt} = -1/D \quad (9)$$

ENZYME- AND CELL-CATALYZED REACTIONS

Mechanistic mathematization of a biological phenomenon is the first step toward quantifying, solving, and controlling (quantitatively) it. The difficulty faced is not that it cannot be solved, but rather that the result is of such algebraic complexity as to render little conceptual understanding of the process involved. Not to condemn the total empirical approach (which is usually limited in its applicability) to the analysis of data (such as curve fitting), a rigorous mechanistic approach yielding monumental amounts of mathematical expressions fail, usually, just as badly in terms of its usefulness in actual process operation. In such cases, a midway approach seems to be beneficial. The development of simplified kinetics of enzyme-catalyzed reactions provides one of the best examples of the judicious use of approximations to render a complicated system-equation intuitively understandable and practically useful. As discussed in the following, the steady-state and equilibrium assumptions in enzyme kinetics achieve exactly that.

Most biological reactions, those catalyzed by enzymes, follow Michaelis-Menten kinetics (equation 10), which represents mixed-order reactions. Enzyme-catalyzed reactions follow a first-order kinetic at low value of substrate to enzyme concentration ratio, and they become zero order when values of the said ratios are high (in that case, enzyme molecules are saturated with substrates). The reaction rate in this region (zero order) is then independent of substrate concentration. Enzymes play important roles in the growth and maintenance of cells. The design of such enzyme-catalyzed reactions as the functioning unit of the metabolic machinery in biological systems is of primary importance to the economy of the cell. The growth and maintenance of the cell require a highly integrated interplay of anabolism and catabolism. The subtle and precise metabolic controls are achieved through the regulations of the rate of enzyme formation (induction) and of enzyme activities (activation or inhibition).

Cell growth involves concurrent and serial reactions using enzyme as catalysts. The overall specific growth rate of cells, in either microbial, animal, or plant cell culture, has been modeled by using Monod equation (equation 11), where S denotes the limiting substrate concentration.

$$\begin{aligned} \frac{dp}{dt} &= v = V_{\max}S/(K_M + S) \text{ where} \\ V_{\max} &= k_3e_0 \\ K_M &= (k_2 + k_3)/k_1 \end{aligned} \quad (10)$$

$$\left(\frac{1}{x}\right)\frac{dx}{dt} = \mu = \frac{\mu_{\max}S}{K_S + S} \quad (11)$$

K_M and K_S are saturation constants. When S/E is small, or $K_M \gg S$, equation 10 can be reduced to

$$v = \frac{V_{\max}S}{K_M} = kS; \text{ where } \frac{V_{\max}}{K_M} = k$$

(a first-order reaction)

At the other extreme, $S \ll K_M$, equation 10 becomes

$$v = \frac{V_{\max}S}{S} = V_{\max}$$

where the reaction is going at maximum velocity and is independent of substrate concentration (a zero-order reaction). In measuring the total activity of an enzyme preparation, the substrate concentration used should be large enough to saturate all the active sites of the enzyme molecules during the course of measurements (to follow the zero-order kinetics); whereas if a standard enzyme preparation is used to titrate the concentration of a substrate, substrate concentration should be diluted to make sure that first-order kinetic is followed ($v = kS$, velocity is a measure of substrate concentration).

Many food materials are composed of live cells (fresh food), many others are made by using the activities of live cells (such as fermentation processes) or by using enzymes (such as glucose from starch), and all food materials are affected by foreign live cells (food spoilage). From a food processing application point of view, two types of enzyme are currently important. These are hydrolytic enzymes (no cofactor required) and oxidoreductases (cofactors required).

The kinetic behavior of an enzyme system is not unique to the system. Other chemical kinetic analyses have found similar phenomena. For example, the concept of stationarity or a steady state is common in enzyme kinetics, in kinetic studies involving free-radical intermediates, and in those involving a stationary activated complex concentration.

The first satisfactory mathematical analysis of the diphasic activity curve was carried out by Michaelis and Menten in 1913. They assumed that the intermediate complex ES was reversibly formed according to the mass action law:

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P} \quad (12)$$

and that the rate of breakdown of ES to form product P was small in relation to the rate of establishment of the equilibrium described by k_1 and k_2. The constant that they derived is, therefore, the dissociation constant of ES complex. Twelve years later, the concept of steady-state approximation to enzyme kinetics was introduced (1). It was believed that the catalyzed reaction may deplete ES complex at a substantial rate and that the Michaelis constant K_M measured experimentally from kinetic curves is in fact $(k_2 + k_3)/k_1$, which can be derived from the steady-state solution (equation 10) to the rate equations for mechanisms describable by equation 12. In addition to the steady-state assumption, it should be noted that equation 10 has been derived for negligible P, the product concentration. In other words, in using equation 10, initial rate of reaction should be used. A practical definition of the Michaelis constant is that it is the substrate concentration at half maximum velocity. Under carefully defined conditions of temperature, pH, ionic strength of buffer, and so on, for an enzyme-substrate pair, K_M is a constant. It approximates the affinity of an enzyme for its substrate. In general, the affinities of respiratory enzymes (oxidoreductases) for their substrates are higher than those of hydrolytic enzymes for their substrates. As shown in Table 1, the K_M values for respiratory enzymes are lower than that of hydrolases. There are a few reports on the K_M values of immobilized enzymes (3,4). Because of the possible complications of external and internal diffusional resistance, the true values of K_M for immobilized enzymes are difficult to obtain. Experimentally, a high substrate-to-enzyme concentration ratio (zero-order region of Michaelis-Menten kinetics) in a batch reactor with high velocity of flow (high agitation rate in stirred reactor) would minimize the external mass-transfer effect; the use of ultrafine particles (or ultrathin membrane) as enzyme carriers would minimize the internal mass-transfer effect.

The activities of enzymes are affected by temperature and pH. Because of the protein nature of an enzyme, thermal denaturation of the enzyme protein is evident in the high end of the temperature range. Generally speaking, up to perhaps 45°C, the predominant effect will be an increase in reaction rate and have a temperature dependence of the Arrhenius form. Above 45°C, the opposing factor, namely thermal denaturation, becomes increasingly important. Around 55°C, rapid denaturation usually destroys the catalytic function of the enzyme protein.

The effect of pH, as that of temperature, on enzymatic activity is typified by a bell-shaped curve with a relatively narrow plateau. The plateau is usually called the optimal pH, or optimal temperature point. These optimal points are to be maintained if maximum enzymatic activities are desired. After an enzyme is immobilized on a carrier, the pH and temperature optimal points may be different from that of the free-enzyme counterpart. In addition to pH and temperature stabilities, operational stability and storage stability of immobilized enzymes are also important from practical points of view. Storage stability of 50 immobilized enzyme systems were reviewed (5). Of these, 30 exhibited greater storage stability, 8 exhibited less than the free-enzyme counterparts. Operational stability of immobilized enzymes depends on the enzyme itself, method of immobilization, operational conditions, and so on. Table 2 illustrates the half-life of various immobilized-enzyme systems, which range from a few days to more than a year.

The kinetic behavior of enzymes may be modified by the following factors when they are immobilized: (*1*) change in enzyme conformation, (*2*) steric effects, (*3*) microenvironmental effects, and (*4*) external and internal diffusional effects. Theoretically, the change in enzyme conformation would cause a change in innate activity of the enzyme, that is, the K_M value. Factors 2 to 4 are ineffective in modifying the true catalytic reaction per se, but nevertheless, obscure the kinetics of the reaction. If enzymes are bound to nonporous systems, the reaction rate can be controlled by one of the following steps: (*1*) diffusion of substrate from the bulk solution to the surface of the immobilized enzyme, (*2*) enzymatic reaction at the surface, or (*3*) diffusion of the reaction products back into the bulk solution. Assuming a linear gradient of substrate concentration across a Nernst diffusion layer, a near-stagnant layer of fluid around the immobilized enzyme particle surface, it has been suggested that the approximate behavior of such systems can be represented by:

$$N_S = -\frac{dS}{dt} = \frac{V_M S_B}{S_B + K_M + \left(\frac{V_M}{D_S}\right)\Delta x} \tag{13}$$

where N_S is the molar flux of substrate at the boundary layer, V_M is the maximum activity of the enzyme, S_B is the bulk concentration of substrate, Δx is the thickness of the Nernst layer, and D_S is the substrate diffusivity (12).

Table 1. Michaelis-Menten Constants K_M for Various Enzymes

Enzyme	Substrate	K_M, *M*
Maltase	Maltose	2.1×10^{-1}
Invertase	Sucrose	2.8×10^{-2}
Lactase (yeast)	Lactose	4.0×10^{-2}
Lactic dehydrogenase	Pyruvate	3.5×10^{-2}

Source Ref. 2.

The values of K_M and V_M from a Lineweaver-Burk plot ($1/v$ vs $1/s$) of data obtained using immobilized enzymes are usually dependent on flow conditions and particle size (or membrane thickness) of the immobilized enzyme used. If existing mass-transfer resistances are neglected in immobilized enzymes experiments, the apparent K_M obtained is usually higher in value than that of the free enzyme counterpart, unless the enzyme carriers have high affinity for the substrate. Because the thickness of the diffusion layer is inversely proportional to agitation rate, the apparent K_M value can be expected to decrease with increased substrate flow over the solid catalysts, provided bulk diffusional resistance is the principal factor affecting the kinetics of the process. As indicated before, an apparent K_M value close to the true K_M of the immobilized enzyme can be obtained by experiments where possible bulk diffusional effect is limited by using a high substrate-to-enzyme concentration ratio and by maintaining high substrate flow over the catalyst surface, and internal diffusional effect is minimized by using fine particles or thin membranes. True K_M values of immobilized enzymes are important parameters for the purpose of reactor scale-up and control for commercial purposes. On the other hand, because apparent K_M is dependent on mass-transfer effects and thus on reactor size, throughput and configuration, it is of little use in this connection. Thus the determination of true K_M is an important task. Basically there are three groups of experimental methods for evaluating the intrinsic kinetic parameters, such as K_M and V_M, in heterogeneous catalysis. These are as follows (13,14):

1. A certain type of immobilized-enzyme reactor configuration of a single size and a single loading of enzymes are used; the variable is substrate-surface concentration.

Table 2. Operational Stability of Some Immobilized Enzymes

Enzyme-carrier	Operation temperature (°C)	Operation time (days)	Estimated half-life (days)[a]	Reference
Glucoamylase–porous glass (zirconium-coated)	45	21	645	6
Glucoamylase–porous glass	60	15	4.2	6
Invertase–porous glass	23	28	42.5	7
Invertase–cellulose	23	16	3.7	7
Invertase–collagen	30	15	200	8
Glucose isomerase–porous glass	60	46	14.4	9
Glucose isomerase in cells–collagen	70	55	50	10
Acylase–DEAE-sephadex	50	35	65	11

[a] Half-lives are estimated as the time required for the loss of 50% of the initial activity during operation, assuming an unimolecular decay kinetics.

2. The variable is the loading of the enzyme or different-size enzyme particles.
3. An enzymatic membrane is inserted between two reservoirs with different substrate concentrations.

To understand the effects and the control of the live cells, kinetics studies of the enzyme-catalyzed reaction, namely, the functioning unit of metabolic machinery, are necessary as starters. Such studies are usually performed *in vitro*. The extrapolations of the results of *in vitro* studies to the *in vivo* situations are sometimes useful, yet are limited in most cases. Structural factors bearing on the relation of the organization of the cell to enzymatic action should be considered if such extrapolations are to be on the safe side. Most enzymes are quite soluble in water. The *in vitro* studies on such enzymes can be considered to be in a homogeneous catalytic environment. However, within cells, enzymes are not totally free to move, especially the so-called membrane-bound enzymes. Due to such considerations, sometimes the actual effect of an enzyme-catalyzed reaction in a cell cannot be explained by the chemical reaction studied *in vitro* alone. The coupling of mass-transfer effect to chemical reaction is necessary to explain the global effect of a reaction on a system with heterogeneous nature. While the advantages of immobilized enzymes in food manufacturing (eg, high fructose syrup production) are evident from an economical point of view, the research on immobilized enzymes will also help the food manufacturers in general, through better understanding of the regulatory machinery in the cells.

A typical microbial growth curve involves a lag phase, an exponential phase, a stationary phase, and a decline phase. In most industrial microbial processes, cells or products accumulated by cells are harvested in the late exponential phase or early stationary phase. During the exponential phase of growth for unicellular organisms undergoing binary fission, the growth rate dx/dt can be expressed in terms of a growth rate constant (or specific growth rate) (and the cell concentration x [equation 11]).

Most microorganisms, if grown properly, follow this first-order, exponential growth. There are exceptions. A linear growth model, namely, dx/dt = constant, was found in some hydrocarbon cultures where limitation is caused by the rate of diffusion of substrate from the surface of oil droplets that have essentially a constant surface area during cultivation. The growth of yeast and filamentous organisms do not follow the first-order kinetics of growth in some cases. In these organisms, growth occurs from the tip, but nutrients diffuse throughout the cellular tissue; sometimes fractional reaction orders are obtained in kinetic analyses.

The specific growth rate μ is essentially a first-order reaction rate constant with a dimension of reciprocal of time, that is, h^{-1}. The constancy of μ is limited. It is a constant under specified conditions of temperature, pH, type of substrate, substrate concentration, and so on. When different μ values are obtained by changing substrate concentration in the experiments, an expression for μ, the so-called Monod equation, is obtained (equation 11).

The form of this equation is exactly the same as that of the Michaelis-Menten equation for enzyme-catalyzed reactions. However, this equation is entirely empirical, whereas that of Michaelis-Menten is mechanistic. This equation usually fits experimental data when cell growth is limited only by a single limiting substrate in a pure culture situation. When there are inhibitors or mixed substrates in the medium, a modified Monod equation can be used to fit the data.

In addition to cell tissue, there are three types of product that can be produced by industrial fermentation processes. These are primary metabolites (eg, monosodium glutamate and food acidulants such as citric acid), secondary metabolites (eg, antibiotics), and enzymes. For primary metabolites and some enzymes, the rate of product formation is proportional to the rate of cell growth. The following equation has been proposed for organic acid fermentations (15):

$$\frac{dp}{dt} = \alpha\left(\frac{dx}{dt}\right) + \beta x$$

or

$$\left(\frac{1}{x}\right)\frac{dp}{dt} = \nu_p = \alpha\mu + \beta \qquad (14)$$

The production model expressed by equation 14 is called a growth-associated model. The kinetics for the production of secondary metabolites and enzymes (when gratuitous inducer is used) are such that they are accumulated or induced after the cells have advanced into late exponential phase or early stationary phase of growth where cell growth rate has started to decline. A nongrowth-associated model is appropriate in these cases:

$$\frac{dp}{dt} = \alpha x$$
$$\left(\frac{1}{x}\right)\frac{dp}{dt} \equiv \nu_p = \alpha \qquad (15)$$

The following growth-associated model has been proposed for the kinetics of enzyme induction (16):

$$\left(\frac{1}{x}\right)\frac{dE}{dt} = \alpha\mu - kE \qquad (16)$$

where the term kE on the right-hand side of equation 16 represents the monomolecular (first-order) decay of the enzyme synthesized *in vivo*. A more complete model on the kinetics of enzyme induction includes the effects of inducer and repressor concentrations on the kinetics, and the mechanism of protein synthesis can be used as basis for the construction of the model.

STARCH CONVERSION

When the starch granule is heated in the presence of an adequate or excess amount of water, gelatinization of starch takes place. Several steps are associated with gelatinization, such as hydration and swelling of granules, heat absorption, and the loss of crystallinity as judged by the disappearance of both optical birefringence and X-ray diffraction pattern. In the extrusion cooking of starch containing materials, starch is heated under high pressure, shear force, and a limited amount of water, to say less than 40%. At a high enough temperature and low moisture content (17,18), starch granules undergo both gelatinization and melting processes. Thus, starch conversion during food processing is not an elementary reaction as discussed before. The conversion itself is a complex phenomenon that is further complicated by heat, mass, and momentum transfer in a reactor like an extruder. The kinetics studies on starch conversion, and other similar processes, take the advantage of an overall rate approach and use the parameter percent conversion instead of concentration for the formulation of kinetics equations. Starch conversion is an endothermic reaction. The degree of conversion of starch during processing due to heat or shear can be evaluated by the decrease of the differential scanning calorimeter (DSC) endotherm area. This area is directly proportional to the mass of unconverted starch in the sample. The degree of conversion X can be defined as follows:

$$X = 1 - \frac{\Delta H_i}{\Delta H_0} \qquad (17)$$

where ΔH_0 is the enthalpy change of the DSC endotherm without conversion, and ΔH_t that with conversion. Thus X is equivalent to the degree of conversion and is defined as

$$X = \frac{M_0 \quad M_t}{M_0 - M_f} \qquad (18)$$

where M_0 is the initial amount of unconverted starch; M_f, the final amount of unconverted starch; and M_t, the amount of unconverted starch at time t. Thus the rate of the disappearance of native (unconverted) starch during a process can be written in the following form:

$$\frac{d(1 - X)}{dt} = -k(1 - X)^n \qquad (19)$$

There are two general approaches to analyzing kinetics data, namely, the differential and integral methods In the differential method of test, the differential at each specific time is calculated, and after taking the logarithm, the data are plotted. The reaction order is the slope of a plot of log $(d[1 - X]/dt)$ versus log $(1 - X)$, and the reaction-rate constant can be obtained from the intercept. In the integral method, a particular form of rate expression is assumed. After appropriate integration and mathematical manipulation, a certain concentration function versus time is plotted. If a reasonably good fit is obtained, then the rate equation is adopted to describe the reaction under study. There are advantages and disadvantages to each method. The advantages of the differential method are the ease of obtaining the appropriate reaction order and reaction rate, and the disadvantage is the magnification of experimental errors. The advantage of the integral method is that experimental data can be used directly, while the disadvantage is that a trial-and-error procedure must be followed. In general, it is suggested that the differential analysis be attempted first, then the integral analysis is performed to check if the obtained rate expression is appropriate or not. Most investigators apply the integral approach to the studies of starch gelatinization with an excess amount of water. The kinetics of starch gelatinization in the cooking of rice grains with 94% moisture content has been studied (19). The kinetics of the water diffusion and starch gelatinization at 91.4% moisture content has been reported (20). The kinetics of cooking of rice starch also in an excess amount of water has been studied (21). The gelation kinetics of barley starch in a diluted system (1% solid) has been investigated (22). A first-order kinetics equation has been employed by these investigators to describe the gelatinization of starch in an excess amount of water. The kinetics of starch conversion in a limited amount of water (40% or less) has not been as well studied, and that reaction order has been reported to be zero order (18,23).

In the presence of an excess amount of water (ie, >60% water for Amioca), different sources of starch gelatinize at different temperatures. The range of temperature is between 50 and 80°C. If a limited amount of water is used, the temperature at which gelatinization and melting (as detected in DSC thermogram) happen is elevated. Figure 1 shows the dependence of peak temperature T_p of the DSC thermogram on the water content of the Amioca samples. At water content higher than 60%, T_p stays constant, whereas at water content lower than 60%, there are two peaks in the thermogram. The second peak temperature, T_p, increases with decreasing water content according to

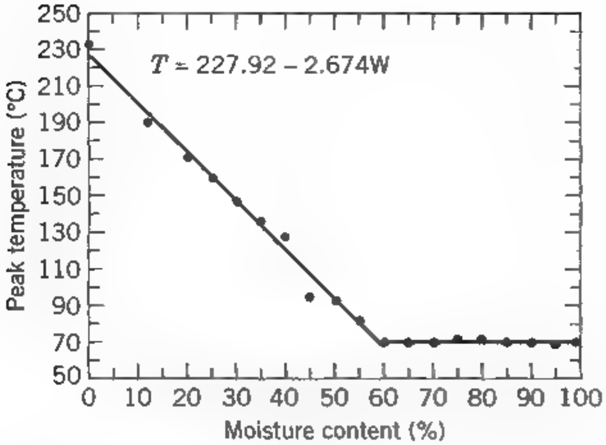


Figure 1. Peak temperatures from DSC endotherms for amioca with various moisture contents.

the linear equation $T_p = 227.92 - 2.674$ W, where W is the water content in wet wt %. To consider the combined effects of heat, moisture content, and the source of starch on the conversion of starch, a dimensionless temperature parameter T/T_p was developed (18). This parameter correlated well with the rates of conversion of starch. Figure 2 shows the plot of k, the zero-order rate of starch conversion versus T/T_p, T is the operating temperature and T_p, the second endotherm peak temperature, is a constant for a specific starch at a specified water content. In Figure 2, data from different studies are presented (18,19,24). Excluding data from Reference 19, which show obvious mass-transfer resistance (diffusion controlling), the rest of the data can be represented by the following equation:

$$k = \frac{x}{74.97x^2 - 167.21x + 93.38} \tag{20}$$

where $x = T/T_p$ and the limit for x is $0.63 < x < 1.06$, for the equation to be meaningful.

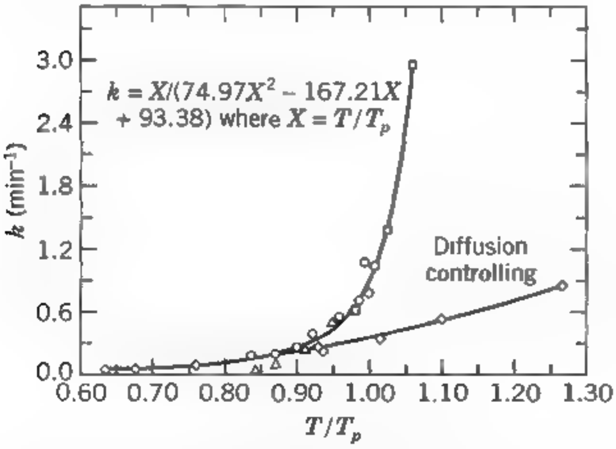


Figure 2. A general correlation between starch conversion rate constant k, and T/T_p, a dimensionless temperature parameter. T_p is peak temperature of DSC thermogram. *Source:* ○, Ref. 18; □, Ref. 24, △, Ref. 21; ◊, Ref. 19.

This equation is useful for calculating reaction rates of various starch conversion at various water contents and various temperatures. The source of starch and water content determines the value of T_p, which is measured by using DSC endotherms.

EFFECTS OF ENERGY INPUTS

It is generally correct to say that some form of energy input is required to cause a reaction to happen. A classic way of supplying the said energy is by heating up the system. As a rule of thumb, for every increment of 10°C, the reaction rate will double. That is the Q_{10} principle. The temperature effect can be visualized by looking at the increased kinetic energy of the molecules that makes the molecules move faster and so increases the probability of colliding with other molecules in the system. Such reasoning is good for explaining temperature effects in gaseous and solution chemistry. For food systems that are solid or semisolid, the resistances of heat and mass transfer and the fact that the mobility of macromolecules are relatively small cause the temperature effect to deviate from the simple Q_{10} principle. Another idiosyncrasy of biological reactions is that many of them are catalyzed by enzymes that are unstable at high temperatures. This causes nonlinearity of Arrhenius plot for biological and biochemical reactions. Arrhenius derived the following equation to fit experimental data obtained from studying the effect of temperature on the kinetics of sucrose inversion:

$$k = \alpha e^{-\Delta E/RT} \tag{21}$$

The logarithmic form of the preceding equation is shown as follows:

$$\ln k = \ln \alpha - \frac{\Delta E}{RT} \tag{22}$$

Equation 22 states that the natural log of reaction rate constant ln k varies linearly with the reciprocal of absolute temperature $1/T$ with a constant negative slope, $-\Delta E/R$. The evaluated value of the said slope provides a way to calculate ΔE, the activation energy of the reaction for the temperature range that the said slope was evaluated on. If 1.987 is used for the value of R, the gas constant in calculating ΔE, the dimension for the calculated value of ΔE is cal/g mol. For some reactions involved in food processing, different temperature ranges may result in different calculated activation energy. As shown in Table 3, the thermal activation energy for the conversion of several starches at different temperature ranges varies from about 10 *to* 230 kcal/mol. These differences can be rationalized by looking at the different granular sizes of the native starches from rice, corn, and potato; the different moisture contents in the samples; and possibly different mechanisms of conversion at different temperature ranges. In dealing with biological or biochemical reactions where activities of the catalysts (enzyme or cells) involved are also affected by temperature, a composite Arrhenius plot can be obtained. The study of the effect of temperature on the

Table 3. Activation Energy for Starch Conversion Due to Thermal Effect

Materials	Temperature (°C)	E_a (kcal/mol)	Reference
Amoica, 75%	145–160	44.25	18
Amoica, 80%	150–170	33.78	18
White rice	75–100	19.8	19
(65% water)	100–150	8.8	
Brown rice	50–85	18.5	28
(>45% water)	85–120	10.5	
Rough rice	50–85	24.7	28
(>45% water)	85–120	9.6	
Rice starch (90% water)	70–85	24.7	29
Potato starch (90% water)	<65	230	29
Potato starch	<67.5	196	24
(82% water)	<67.5	58.2	

catalytic breakdown of hydrogen peroxide by beef liver catalase uncovered the nonlinearity in an Arrhenium plot (ln k vs $1/T$) as shown in Figure 3 (25). The effect of temperature on the rate of production of penicillin with *Penicillium chrysogenum* was reported to show a similar effect (Fig. 4). (26). The data points on these two figures fall into two groups, one of which is related to the activation of the substrate(s) and the enzyme molecules (catalase, or other enzymes in the cell of *P. chrysogenum*), while the other is indicative of the inactivation of the enzyme or the cell at higher temperature range. The maximum of the roughly bell-shaped curve of Figure 3 and the intercept of the two lines in Figure 4 are corresponding to the critical temperatures causing the denaturation of the enzyme (53°C for catalase) and the inactivation of the cell activity. In studying activation energies for biological or biochemical reactions, care must be taken to avoid such high temperatures. It has been shown that the activation energy for cell growth is sufficiently different from that for penicillin production. This difference suggests that the enzymes involved in each sequence are different. (Penicillin, being a secondary metabolite, is produced by mature culture of the *Penicillium*.) Studies such as these are helpful in elucidating the kinetic mechanisms of reactions. To determine whether a biological or a biochemical reaction is kinetically controlled or mass-transfer controlled, the Arrhenius plot and the calculation of activation energy can be used. The activation energy of a catalyzed biological or biochemical conversion is in the range of 8 to 10 kcal/mol or higher, whereas that of diffusion is smaller than that (27).

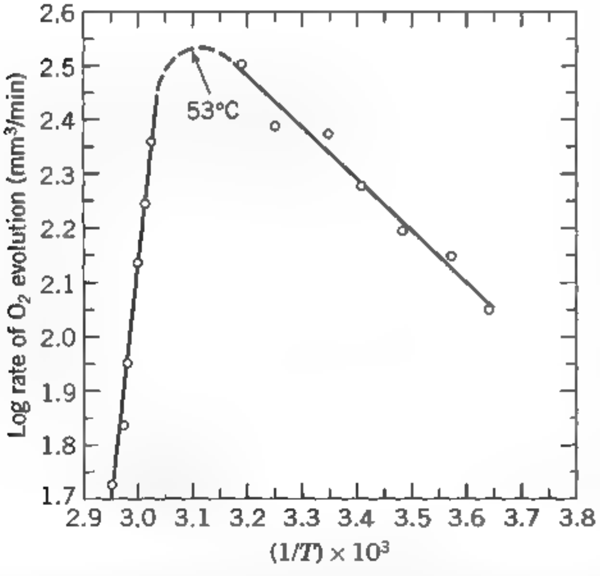


Figure 3. Nonlinearity of Arrhenius plot for breakdown rate of hydrogen peroxide in the presence of catalase. *Source:* Ref. 25.

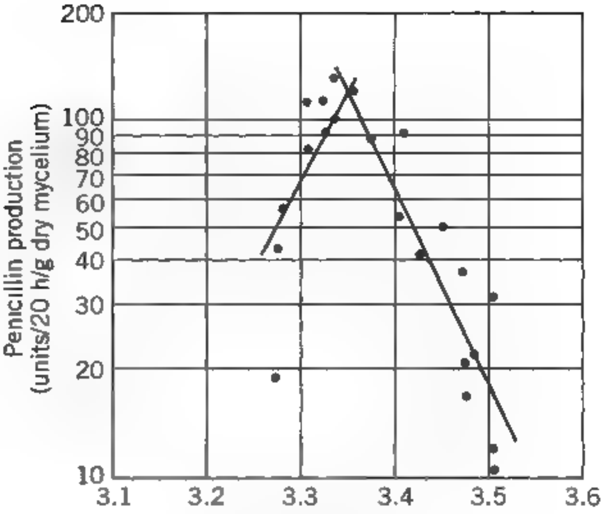


Figure 4. Nonlinearity of Arrhenius plot for penicillin production rate with *Penicillium chrysogenum*. *Source:* Ref. 26.

The activation energy calculation according to Arrhenius only deals with one type of energy source: heat or thermal. It is known that there are reactions that can be activated by other types of energy source, such as photoenergy, electrical energy, shear energy, and pressure-related energy. The process of photosynthesis is initiated by photoenergy. The mechanism of the transduction of light energy into chemical energy has been a challenge to the scientific community. Electrochemical reactions are initiated by electrical energy. Manufacturing of chlorine and sodium hydroxide are examples. Shear energy has been shown to initiate free-radical formation. What is the efficiency of these different energy source in causing the reactions to happen? Activation energy can be looked on as the energy barrier to the initiation of a reaction. What are the activation energy requirements for photochemical, electrical, or shear energy induced reactions? It is possible to look at the activation energy as an index for the efficiency of a particular energy source. The smaller the value, the higher the efficiency. Using starch conversion as an example, starch can be converted (based on DSC thermogram) by supplying thermal energy or shear energy in an extruder. It is known that starch can also be converted (based on DSC thermogram) by shear energy alone (30–32) that is without external heating or dissipated heat. The efficiency of shear energy in causing this conversion is two to three orders of magnitude higher than that by thermal energy. The thermal activation energies shown in Table 3 are in the range of kilocalories per mole, whereas those of shear, as shown in Table 4, are in the range of calories per mole. This is an important reason why extruders, due to their capability of providing high shear, are efficient reactors in which to carry out starch conversion for food processors.

BIOREACTOR

This section deals with the selection of the best types of reactor and operation for a particular biochemical or biological process. When faced with a design problem such as this, there are two previously fixed factors that must be considered to make a proper selection. These are, first, the scale of operation (production rate) and, second, the kinetics of the reaction involved. Invariably the criterion that is used to make the selection is a monetary one. Design and economics are two sides of the same coin. To be responsible to society as a whole, industries must also pay attention to safety and environmental and ethical problems in making decisions on the overall design of a process. This is much easier said than done. Engineering design and economics are quantitative, whereas social benefits and values are difficult to access. At the present time, the subject matter dealt here is limited to the scientific aspect of the problem that can be expressed clearly in quantities.

BIOLOGICAL REACTOR

In general, reactors have been classified in two ways, one according to the type of operation and the other according to design features. Based on the types of operation, there are batch, continuous, and semicontinuous reactors. Due to the relatively high productivity of a continuous process as compared with a batch one, when a process is reaction cost intensive, it is advisable to use the former rather than the latter. On the other hand, if the process is recovery cost intensive, high concentration of product instead of high productivity is desirable, and a batch process is usually preferred. Whether the process is a batch or a continuous one, the reactor is the heart of the process. The efficiency of the reactor frequently, if not always, determines the economics and, hence, the commercial feasibility of the process. To achieve high efficiency of the reactor, it must be designed according to the specific reaction system concerned, namely, the kinetics of the reaction.

Table 4. Activation Energy for Shear Effect on 65% Amioca at Different Temperatures

Temperature (°C)	Activation Energy (cal/mol)	Preexponent (k_{so})
21	108.01	6.99×10^7
25	91.77	8.60×10^3
35	71.60	4.29×10^4
40	37.32	4.79×10^3
50	24.42	7.45×10^2
70	20.52	9.77
80	12.97	8.10
90	10.44	9.48

Source: Ref 30.

Chemical reactors have also been classified according to the design features. There are tank reactors, tubular reactors, tower reactors, and fluidized reactors. Most of these reactors have found their uses in biological and biochemical processes. The most popular reactor used in biological and biochemical processes has been the tank reactor. The majority of the industrial fermentation process, waste products–treatment processes, and food-cooking processes use tank reactors. For factories making a repertoire of hundreds of products each at a small scale, the tanks are versatile and flexible, and batch operation is the one of choice. However, a batch process is inherently harder to control than a continuous one, even with the advent of computer-coupled control systems. The right decision between batch and continuous operation depends on the relative magnitude of labor and capital costs. What is best for a developed country is not necessarily good for a developing one.

In the following, the performances of distinctive reactors in continuous operation are discussed. For the sake of simplicity in mathematical analyses of the reactor performances, these reactors can be categorized into two ideal reactors: well-mixed (back-mixed or stirred tank) and plug-flow reactors, according to the flow patterns in the reactors. In a well-mixed reactor, it is assumed that there is no concentration distribution in the reactor; if the process is continuous, there is no distribution of residence time among the elements of fluid (or substrate) leaving the reactor tank. Under such a condition (complete mixing), the rate is constant throughout the reactor, and there is no need for a differential equation describing the change in concentration or conversion between the inlet and outlet streams. A simple material balance is sufficient. In a plug-flow reactor (wherein no longitudinal mixing but complete radial mixing is assumed), the concentration of the substrate varies longitudinally and so does the rate of the reaction (unless the reaction is of zero order with respect to the substrate concentration). In such a case, a differential equation is needed to express the performance of the reactor, and the performance of the reactor can be obtained by integrating the equation. Due to such differences in reactor features for well-mixed and plug-flow reactors, other things being equal, when a reaction is substrate inhibitory, it is desirable to use a well-mixed reactor. On the other hand, if the reaction is product inhibitory, it is desirable to use a plug-flow reactor. In most occasions, the selection of either a well-mixed or a plug-flow reactor depends mainly on the kinetics of the reaction concerned. In general, if a first-order enzymatic reaction is free of substrate and product inhibition, the amount of enzyme required for 99% conversion in a well-mixed reactor is 21 times that required for a plug-flow reactor. The ratio of the amount of the enzyme required (ECSTR/EPFR) becomes four to one when 90% conversion is desired. Consequently, other things being equal, for a first-order enzymatic reaction, it is advisable to use a plug-flow reactor rather than a well-mixed reactor.

The growth of cells is a self-catalytic process. The plot of cell growth rate versus cell concentration follows a bell-shaped curve with an optimum. In such processes, if the reaction rate in the reactor never reaches the maximum rate, theoretically the use of one single well-mixed reactor is superior to the use of a plug-flow reactor. This is based on the comparison of the efficiency of the reactors in carrying out a specific conversion under identical environmental conditions. If the cultivation of cell tissue is the purpose of a fermentation process, either in a batch or continuous process, the rate of cell growth in the fermenter usually does not exceed the maximum rate during the process. This is one of the reasons why a mechanically stirred-tank reactor with air dispersed into liquid medium by sparging is the most typical industrial fermenter, although the production of secondary metabolites and enzymes from fermentation processes usually includes the growth of microorganisms beyond the exponential phase. This type of process usually warrants a two-stage process, and theoretically the use of a stirred-tank reactor followed by a plug-flow reactor is most desirable (33). The most obvious difficulty of using a plug-flow reactor is the maintenance of the flow pattern, especially under aeration requirements.

Another aspect of the discussion of reactor kinetics is the use of immobilized enzymes in various reactor types for biocatalytic conversions. Free enzymes can be used in these reactors, except that a rather large amount of energy will have to be spent on the retention of enzymes usually through the use of ultrafilters. Basically, enzymes are expensive materials; because they are catalysts, their reusability is logical, provided it is economical to do so. Immobilization of enzymes seems to be the answer to this question. Although some of the immobilization processes are quite elaborate and potentially expensive, many are quite simple and effective (8,10).

The efficiency of a reactor used for immobilized enzymes depends on the following factors: mass-transfer resistances (external and internal), enzyme-loading factor, enzyme stability, substrate contact efficiency, and so on. In selecting reactors to house immobilized enzymes, these factors must be considered. As an example, the selection of a reactor for glucose isomerase is discussed. There are three types of immobilized glucose isomerase: immobilized cell-free glucose isomerase (8,9,34,35), immobilized cells with intracellular glucose isomerase (10), and free cells with intracellular glucose isomerase (36,37). As has been pointed out for immobilized glucose isomerase reactors (35) and as a general rule, for higher-order reactions or Michaelis-Menten kinetics with a high value of K_M, when high conversion is desired, much more enzyme is required in the continuous stirred-tank reactor than in the plug-flow reactor. The choice of a plug-flow reactor is also partly due to the fact that the intrinsic reaction kinetics there are equilibrium controlled. The Keg value of the isomerization reaction is close to the value of one at most operating temperatures. The concentration of product cannot be neglected in the rate expression. Applying reversible Michaelis-Menten kinetics to glucose-fructose conversion, the resulting rate expression is

$$v = \frac{V_M(S \quad P/K)}{K_M + S + (K_M P/K_P)} \tag{23}$$

where K is equilibrium constant (P/S at equilibrium), S is the glucose concentration, P is the fructose concentration, K_M and K_P are constants, v is the reaction rate, and V_M is equal to $(E)_{\text{total}}$ times k, the maximum reaction rate. This rate expression can be looked upon as one derived from the product-inhibition model, and, as discussed before, the use of a plug-flow reactor is more efficient for this type of reaction. In published data on immobilized glucose isomerase, mass-transfer resistances were not found to be limiting factors. Both internal and external resistances were found to be negligible. In accordance with this, it has been found that the activation energy of immobilized and soluble glucose isomerase are close, 15.7 and 14.5 kcal/g ~ mol, respectively (35). Due to the lack of mass-transfer resistances in this case, it is advisable to use an immobilized whole-cell system instead of an immobilized enzyme system, provided that the cells are fully induced and are high in glucose isomerase concentration (8).

In batch reactors, concentrations of reactant(s) and product(s) are a function of time. So batch processes are transient processes. Continuous processes, on the other hand, can be in either transient or steady state. There are two types of steady state in continuous operations: volumetric steady state and concentration steady state. Other things being equal, a volumetric steady state is a prerequisite for reaching a concentration steady state. Mathematically, steady state means that the variable (volume or concentration) is not changing with respect to time, dV/dt or $dC/dt = 0$. In operation, volume invariance can be achieved mechanically; for biological systems, concentration steady state, such as constant cell concentration, is difficult to achieve. In reality, $dC/dt = +$ or -10% of the mean is a good criterion for reaching the steady state of cell cultures (38).

Deviations from ideal reactors are common in practice. Many real reactors behave in between a well-mixed and a plug-flow reactor. A column reactor is closer to a plug-flow reactor, a tank reactor to a well-mixed reactor, and a fluidized-bed reactor is a hybrid of the two ideal reactors. A series of well-mixed reactors can be operated to perform like a plug-flow reactor. An extruder is closer to a plug-flow than a well-mixed reactor. These arguments can be evaluated by looking at the residence time distributions of various reactors.

An extruder is like a pump. It is a versatile reactor, capable of performing other operations in concert with its pumping function. For instance, extruders can be used as a mixer and as reactors to make pasta, pet foods, instants, confectioneries, and snacks and can be used to determine spices. Extrusion is a typical continuous process that has the potential of steady-state operation with high productivities. The engineering analysis of an extrusion process is a difficult task. Chemical and physical changes of materials that occur in extruders are the results of solid (powdery) or semisolid interactions under limited-moisture contents. The initiation, or activation, of such changes can be done by thermal energy input or mechanical energy input. In addition to changes at the molecular level, extruders are also most effective in rendering size reductions to affect changes at the particle level. All these changes are important in determining the product quality, especially in texture-related features. The kinetics of chemical and physical changes of starch and other food ingredients in extrusion process are difficult to study because of the highly nonideal environment, as opposed to gas or dilute solution, and the complication of transport processes (heat, mass, and momentum transfers) coupling with reaction kinetics. The idea of predicting the performance of an extruder by using transport coupled kinetic models is a desirable one. The task of correlating product qualities, such as specific texture, flavor, or color, to the performance of an extruder is a difficult one. Certainly innovative ideas are needed to achieve such a goal.

FOOD STORAGE AND PREDICTION OF SHELF LIFE

Quality deterioration in the form of decrease of nutritional value, color change, development of off-flavor, and textural change may occur in foods during storage. The purpose of food preservation is to minimize such quality deterioration. To provide adequate but not excess protection, the studies of environmental factors affecting quality deterioration are of prime importance. To predict quality changes, or to predict shelf life of foods, kinetics studies of essential parameters, such as product characteristics, storage environment, and container protection are necessary. Computer-aided simulation of models derived from such kinetics studies can provide information for adequate design of a preservation scheme for foods and also for prediction of the shelf life of foods (39–43). The success of such design and prediction depends mainly on the understanding of the nature of the chemical and physical changes of the food system involved and the subsequent derivation of a correct kinetics model from such understanding. A direct mathematization of an overall change without mechanistic understanding of such changes may result in an inadequate kinetics model that has limited application. Earlier studies on the quality deterioration of foods (44,45) mostly depend on such technique. The effects of storage temperature and time on the retention of thiamin and ascorbic acid in canned foods has been studied (44). Nomographs for ascorbic acid and thiamin retention based on first-order decay kinetics of these vitamins were constructed. By extrapolation, these homographs permit prediction of retention values beyond the conditions of the actual experiments. Similar techniques, were used in another study that went further to calculate activation energy and frequency factors (ΔE and α of equation 21) by studying the first-order decay reaction at different temperatures. By using these constants (ΔE and α), it would be also possible to calculate the effect of fluctuating temperature on the storage stability of the foods studied. Although both these studies found first-order decay of ascorbic acid in frozen spinach and various canned foods, respectively, another study (43) reported that the rate of oxidation of ascorbic acid is not only dependent on ascorbic acid concentration, but also on dissolved oxygen (DO) concentration if it is below a critical value, such as 8.71 ppm in that study. Unless there is a continuous supply of oxygen to the packaged food system, the DO value will decrease from an initial saturation value (solubility of oxygen in water is about 7–8 ppm at 25°C) to a limiting value during storage. The consideration of DO concentration in the rate expression of ascorbic acid degradation seems to be logical under such circumstances. As proposed, the following bimolecular kinetics equation is appropriate in describing the rates of ascorbic acid oxidation in infant foods (43).

$$-\frac{d(A)}{dt} = k(A)(B) \tag{24}$$

where (A) and (B) are concentrations of ascorbic acid and DO, respectively. It was also found that the rate constant k increases linearly with light intensity up to 1756 (transmitted) lux.

Shelf life, or storage stability of foods, depends on a large number of factors such as those of environmental origin—temperature, equilibrium relative humidity, oxygen partial pressure, light, intensity, and so on—and those of container characteristics—package permeabilities, package configurations, and so on. Studies of the effect of the critical factor or factors among those listed on the stability of food make it possible to derive kinetics equations suitable for modeling the changes and predicting the shelf life of foods. However, for the purpose of package design and optimization (overprotection is expensive), it is necessary to consider the deteriorative reactions that depend on the characteristics of the package. In other words, the kinetics model or the formulas derived from it will be useful for package design and optimization if the packing parameters are explicitly included in the formulas (42).

As an example of optimization of package design, the results of one study (40) are summarized here. Two critical factors affecting the shelf life of potato chips were identified as (*1*) transfer of moisture into the package, causing a textural change, and (*2*) the initial presence and continued transfer of oxygen, which causes oxidative rancidity. If shelf life is defined as the time to reach maximum allowable moisture content or the maximum allowable extent of oxidation, the optimized design of packaged potato chips is that in which these two limits are reached simultaneously. This requirement determines the selection of suitable film for packaging. A lamination of two films with one high in oxygen permeability but low in water permeability and the other high in water permeability but low in oxygen permeability would be the choice for such a case.

Kinetics models are useful in describing the rates of changes related to food quality, including spoilage. A more important aspect of kinetics model development is its ability to be used for predicting the spoilage before the foods are consumed. The traditional microbiological approach to determining the stability and safety of foods is retrospective. Information is obtained several days after the samples are collected and analyzed in the laboratories. The use of predictive microbiology, within which the growth rates of the microbes of concern in foods would be modeled with respect to critical environmental factors, such as temperature, pH, and water activity, has been proposed (46). Models relevant to broad categories of foods can be used to predict the spoilage rates of foods. More studies are needed to learn how much information is required to produce such a workable model. Nevertheless, modeling by using relevant kinetics equations offers the most cost-effective approach to the understanding and control of microbial growth in foods (46).

BIBLIOGRAPHY

1. G. E. Briggs and J. B. S. Haldane, "A Note on the Kinetics of Enzyme Action," *Biochem. J.* **19**, 338–339 (1925).
2. J. B. Neilands and P. K. Stumpf, *Outlines of Enzyme Chemistry*, John Wiley & Sons, New York, 1958.
3. G. B. Borglum and M. Z. Sternberg, "Properties of a Fungal Lactase," *J. Food Sci.* **37**, 619–623 (1972).
4. H. H. Weetall, "The Preparation of Immobilized Lactase and Its Use in the Enzymatic Hydrolysis of Acid Whey," *Biotechnol. Bioeng.* **16**, 295–313 (1974).
5. G. J. H. Melrose, "Insolubilized Enzymes: Biochemical Applications of Synthetic Polymers," *Rev. Pure Appl. Chem.* **21**, 83–119 (1971).
6. H. H. Weetal and N. B. Havewala, "Continuous Production of Dextrose From Corn Starch: Reactor Parameters Necessary for Commercial Application," *Biotechnol. Bioeng. Symposium* **3**, 241–256 (1972).
7. R. D. Mason and H. H. Weetall, "Invertase Covalently Coupled to Porous Glass: Preparation and Characterization," *Biotechnol. Bioeng.* **14**, 637–645 (1972).
8. S. S. Wang and W. R. Vieth, "Collagen-Enzyme Complex Membranes and Their Performance in Biocatalytic Modules," *Biotechnol. Bioeng.* **14**, 93–101 (1972).
9. G. W. Strandberg and K. L. Smiley, "Glucose Isomerase Covalently Bound to Porous Glass Beads," *Biotechnol. Bioeng.* **14**, 509–513 (1972).
10. S. S. Wang, W. R. Vieth, and A. Constantinedes, "Complexation of Enzymes or Whole Cells With Collagen," in E. K. Pye and L. B. J. Wingard, eds., *Enzyme Engineering*, Vol. 2, Plenum Press, New York, 1974, pp. 123–129.
11. I. Chibata et al., *Fermentation Technology Today*, Society of Fermentation Technology, Osaka, Japan, 1972.
12. M. D. Lilly and A. K. Sharp, *Chem. Eng.* (London) CE12 (Jan./Feb. 1968).
13. J. Engasser and C. Howath, "Effect of Internal Diffusion in Heterogeneous Enzyme Systems: Evaluation of True Kinetic Parameters and Substrate Diffusivity," *J. Theor. Biol.* **42**, 137–155 (1973).
14. Y. Kobayashi and K. J. Laidler, "Kinetic Analysis for Solid Supported Enzymes," *Biochim. Biophys. Acta* **302**, 1–12 (1973).
15. R. Leuedeking and E. L. Piret, *J. Biochem. Microbiol. Technol. Eng.* 1, 393–405 (1959).
16. A. Shinmyo, M. Okagaki, and G. Terui, "Kinetic Studies on Enzyme Production by Microbes. III: Process Kinetics of Glucoamylase Production by *Aspergillus niger*," in D. Perlman, ed., *Fermentation Advances*, Academic Press, Orlando, Fla., 1969, pp. 337–367.
17. J. W. Donovan, "Phase-Transition of the Starch-Water System," *Biopolymers* **18**, 263–275 (1979).
18. S. S. Wang, W. C. Chiang, A. I. Yeh, B. L. Zhao, and I. H. Kim, "Kinetics of Phase-Transition of Waxy Corn Starch under Extrusion Temperature and Moisture Content," *J. Food Sci.* **64**, 1298–1301 (1989).
19. K. Suruki et al., "Kinetic Studies on Cooking Rice," *J. Food Sci.* **41**, 1180–1183 (1976).
20. K. Kubota et al., "Studies of the Gelatinization Rate of Rice and Potato Starches," *J. Food Sci.* **44**, 1394–1397 (1979).
21. D. B. Lund and M. Wirakartakusumah, "A Model for Starch Gelatinization Phenomena," in B. M. McKenna, ed., *Engineering and Food, Vol. 1: Engineering Sciences in the Food Industry*, 1984, pp. 425–432.
22. C. Mok et al., "Kinetic Study on the Gelatinization of Barley Starch," *Korean Journal of Food Science Technology* **17**, 409–414 (1985).
23. M. Bhattacharya and M. A. Hannai, "Kinetics of Starch Gelatinization During Extrusion Cooking," *J. Food Sci.* **52**, 764–766 (1987).
24. C. I. Pravisani, A. N. Califano, and A. Calvilo, "Kinetics of Starch Gelatinization in Potato," *J. Food Sci.* **50**, 657–660 (1985).
25. I. W. Sizer, "Temperature Activation and Inactivation of the Crystalline Catalase-Hydrogen Peroxide System," *J. Biol. Chem.* **154**, 461–473 (1944).
26. C. T. Calam, N. Driver, and R. H. Bowers, "Studies in the Production of Penicillin, Respiration and Growth of *Penicillium chrysogenum* in Submerged Culture, in Relation to Agitation and Oxygen Transfer," *J. Appl. Chem.* **1**, 209–216 (1951).
27. P. Schneider and J. M. Smith, "Chromatographic Study of Surface Diffusion," *AIChE J.* **14**, 886 (1968).
28. A. S. Bakchi and R. P. Singh, "Kinetics of Water Diffusion and Starch Gelatinization During Rice Parboiling," *J. Food Sci.* **45**, 1387–1392 (1980).
29. Kubota et al., "Studies of the Gelatinization Rate of Rice and Potato Starches," *J. Food Sci.* **44**, 1394–1397 (1979).
30. S. S. Wang et al., "Development of an Energy-Equivalent Concept for the Formulation of Extrusion-Kinetic Equations," *Fiftieth IFT Annual Meeting*, Chicago, Ill., June, 1989.
31. X. Zheng and S. S. Wang, "Shear Induced Starch Conversion During Extrusion" *J. Food Sci.* **59**, 1137–1142 (1994).
32. S. S. Wang, and X. Zheng, "Tribological Shear Conversion of Starch," *J. Food Sci.* **60**, 520–522 (1995).
33. K. B. Bischoff, "Optimal Continuous Fermentation Reactor Design," *Can. J. Chem. Eng.* **44**, 281–284 (1966).
34. Ger. Pat. 2,303,872 (1973), D. Dinelli, F. Morisi, S. Giovenco, and P. Pansolli.
35. N. B. Havewala and W. H. Pitcher, "Immobilized Glucose Isomerase for the Production of High Fructose Syrup," in E. K. Pye and L. B. J. Wingard, eds., *Enzyme Engineering*, Vol. 2, Plenum Press, New York, 1974, pp. 315–328.
36. U.S. Pat. 3,623,953 (November 30, 1971), W. P. Cotter, N. E. Lloyd, and C. W. Hinman (to Standard Brands, Inc.).
37. U.S. Pat. 3,694,314 (September 26, 1972), N. E. Lloyd, L. T. Lewis, R. M. Logan, and D. N. Patel (to Standard Brands, Inc.).
38. C. K. Jin and S. S. Wang, "Steady State Analysis of the Enhancement in Productivity of a Continuous Fermentation Process Employing Protein Phospholipid Complex as a Protecting Agent," *Enzyme Microb. Technol.* **3**, 249–257 (1981).
39. T. P. Labuza, S. Mizrahi, and M. Karel, "Mathematical Models for Optimization of Flexible Film Packaging of Foods for Storage," *Trans. Am. Soc. Agricultural Eng.* **1G**, 150–155 (1972).

40. D. G. Quast and M. Karel, "Computer Simulation of Storage Life of Foods Undergoing Spoilage by Two Interacting Mechanisms," *J. Food Sci.* **37**, 679–683 (1972).
41. Y. S. Henig and S. G. Gilbert, "Computer Analysis of the Variables Affecting Respiration and Quality of Produce Packaged in Polymeric Films," *J. Food Sci.* **40**, 1033–1035 (1975).
42. K. I. Hayakaw, Y. S. Henig, and S. G. Gilbert, "Formulas for Predicting Gas Exchange of Fresh Produce in Polymeric Film Package," *J. Food Sci.* **40**, 186–191 (1975).
43. R. P. Singh, D. R. Heldman, and J. R. Kirk, "Kinetics of Quality Degradation: Ascorbic Acid Oxidation in Infant Formula During Storage," *J. Food Sci.* **41**, 304–308 (1976).
44. M. Freed, S. Brenner, and V. O. Wodicka, "Prediction of Thiamine and Ascorbic Acid Stability in Stored Canned Foods," *Food Technol.* **3**, 148–151 (1949).
45. A. L. Brody, K. Bedrosian, and C. O. Ball, "Low Temperature Handling of Sterilized Foods, V: Biochemical Changes in Storage," *Food Technol.* **14**, 552–556 (1960).
46. T. A. Roberts, "Combinations of Antimicrobials and Processing Methods," *Food Technol.* **43**, 156–163 (1989).

SHAW WANG
Rutgers University
Piscataway, New Jersey

KOSHER FOODS AND FOOD PROCESSING

The kosher dietary laws determine which foods are "fit or proper" for consumption by Jewish consumers who observe these laws. The laws are biblical in origin, coming mainly from the original five books of the Holy Scriptures. Over the years, the details have been interpreted and extended by rabbis around the world to protect the Jewish people from violating any of the fundamental laws and to address new issues and technologies. For example, the rabbis are currently dealing with issues related to biotechnology (see later section).

Why do Jews follow the kosher dietary laws? Many explanations have been given. The following by Rabbi Grunfeld is possibly the best written explanation and probably summarizes the most widely held ideas about the subject (1):

> "And ye shall be men of a holy calling unto Me, and ye shall not eat any meat that is torn in the field" (Exodus XXII:30). Holiness or self-sanctification is a moral term; it is identical with . . . moral freedom or moral autonomy. Its aim is the complete self-mastery of man.
>
> To the superficial observer it seems that men who do not obey the law are freer than law-abiding men, because they can follow their own inclinations. In reality, however, such men are subject to the most cruel bondage; they are slaves of their own instincts, impulses and desires. The first step towards emancipation from the tyranny of animal inclinations in man is, therefore, a voluntary submission to the moral law. The constraint of law is the beginning of human freedom. . . . Thus the fundamental idea of Jewish ethics, holiness, is inseparably connected with the idea of Law; and the dietary laws occupy a central position in that system of moral discipline which is the basis of all Jewish laws.
>
> The three strongest natural instincts in man are the impulses of food, sex, and acquisition. Judaism does not aim at the destruction of these impulses, but at their control and indeed their sanctification. It is the law which spiritualizes these instincts and transfigures them into legitimate joys of life.

These laws are not health laws. For a more complete discussion, see Ref. 2.

THE KOSHER MARKET

The kosher market covers more than 30,000 products in the United States. In dollar value, about $35 billion worth of products have a kosher marking on them. The actual consumers of kosher food, that is, those who specifically look for the kosher mark, are estimated to be about 6 to 8 million Americans, and they are purchasing almost $2 billion worth of kosher product each year. Only about one-third of the kosher consumers are Jewish; other consumers include Muslims, Seventh Day Adventists, vegetarians, people with various types of allergy—particularly dairy, grain, and legume—and other consumers who value the quality of kosher products. "We report to a higher authority" was the ad claim for Hebrew National hot dogs. *AdWeek Magazine* has called kosher "the Good Housekeeping Seal for the 90s." By undertaking kosher certification, food companies can incrementally expand their market by opening up new markets.

Although limited market data are available, the most dramatic data about the impact of kosher have been provided by Coors when they went kosher. According to their market analysis, their share of the Philadelphia market went up 18% on going kosher. Somewhat less dramatic increases were observed in other cities in the Northeast.

THE KOSHER DIETARY LAWS

The kosher dietary laws predominantly deal with three issues, all in the animal kingdom:

1. Allowed Animals
2. Prohibition of Blood
3. Prohibition of Mixing of Milk and Meat

However, for the week of Passover (in late March or early April), restrictions on "chometz," the prohibited grains and the rabbinical extensions of this prohibition, lead to a whole new set of regulations, focused in this case on the plant kingdom.

In addition, a separate set of laws deals with grape juice, wine, and alcohol derived from grape products, which must be handled by sabbath-observing Jews. However, if the juice is pasteurized (heated or "mevushal" in Hebrew), then this juice can be handled like any other kosher ingredient.

ALLOWED ANIMALS AND PROHIBITION OF BLOOD

Ruminants with a cloven hoof, most domestic birds, and fish with fins and removable scales are generally permitted. Pigs, wild birds, sharks, dogfish, catfish, monkfish and similar species along with all crustacean and molluscan

40. D. G. Quast and M. Karel, "Computer Simulation of Storage Life of Foods Undergoing Spoilage by Two Interacting Mechanisms," *J. Food Sci.* **37**, 679–683 (1972).
41. Y. S. Henig and S. G. Gilbert, "Computer Analysis of the Variables Affecting Respiration and Quality of Produce Packaged in Polymeric Films," *J. Food Sci.* **40**, 1033–1035 (1975).
42. K. I. Hayakaw, Y. S. Henig, and S. G. Gilbert, "Formulas for Predicting Gas Exchange of Fresh Produce in Polymeric Film Package," *J. Food Sci.* **40**, 186–191 (1975).
43. R. P. Singh, D. R. Heldman, and J. R. Kirk, "Kinetics of Quality Degradation: Ascorbic Acid Oxidation in Infant Formula During Storage," *J. Food Sci.* **41**, 304–308 (1976).
44. M. Freed, S. Brenner, and V. O. Wodicka, "Prediction of Thiamine and Ascorbic Acid Stability in Stored Canned Foods," *Food Technol.* **3**, 148–151 (1949).
45. A. L. Brody, K. Bedrosian, and C. O. Ball, "Low Temperature Handling of Sterilized Foods, V: Biochemical Changes in Storage," *Food Technol.* **14**, 552–556 (1960).
46. T. A. Roberts, "Combinations of Antimicrobials and Processing Methods," *Food Technol.* **43**, 156–163 (1989).

SHAW WANG
Rutgers University
Piscatawav, New Jersey

KOSHER FOODS AND FOOD PROCESSING

The kosher dietary laws determine which foods are "fit or proper" for consumption by Jewish consumers who observe these laws. The laws are biblical in origin, coming mainly from the original five books of the Holy Scriptures. Over the years, the details have been interpreted and extended by rabbis around the world to protect the Jewish people from violating any of the fundamental laws and to address new issues and technologies. For example, the rabbis are currently dealing with issues related to biotechnology (see later section).

Why do Jews follow the kosher dietary laws? Many explanations have been given. The following by Rabbi Grunfeld is possibly the best written explanation and probably summarizes the most widely held ideas about the subject (1):

> "And ye shall be men of a holy calling unto Me, and ye shall not eat any meat that is torn in the field" (Exodus XXII:30). Holiness or self-sanctification is a moral term; it is identical with . . . moral freedom or moral autonomy. Its aim is the complete self-mastery of man.
>
> To the superficial observer it seems that men who do not obey the law are freer than law-abiding men, because they can follow their own inclinations. In reality, however, such men are subject to the most cruel bondage; they are slaves of their own instincts, impulses and desires. The first step towards emancipation from the tyranny of animal inclinations in man is, therefore, a voluntary submission to the moral law. The constraint of law is the beginning of human freedom. . . . Thus the fundamental idea of Jewish ethics, holiness, is inseparably connected with the idea of Law; and the dietary laws occupy a central position in that system of moral discipline which is the basis of all Jewish laws.
>
> The three strongest natural instincts in man are the impulses of food, sex, and acquisition. Judaism does not aim at the destruction of these impulses, but at their control and indeed their sanctification. It is the law which spiritualizes these instincts and transfigures them into legitimate joys of life.

These laws are not health laws. For a more complete discussion, see Ref. 2.

THE KOSHER MARKET

The kosher market covers more than 30,000 products in the United States. In dollar value, about $35 billion worth of products have a kosher marking on them. The actual consumers of kosher food, that is, those who specifically look for the kosher mark, are estimated to be about 6 to 8 million Americans, and they are purchasing almost $2 billion worth of kosher product each year. Only about one-third of the kosher consumers are Jewish; other consumers include Muslims, Seventh Day Adventists, vegetarians, people with various types of allergy—particularly dairy, grain, and legume—and other consumers who value the quality of kosher products. "We report to a higher authority" was the ad claim for Hebrew National hot dogs. *AdWeek Magazine* has called kosher "the Good Housekeeping Seal for the 90s." By undertaking kosher certification, food companies can incrementally expand their market by opening up new markets.

Although limited market data are available, the most dramatic data about the impact of kosher have been provided by Coors when they went kosher. According to their market analysis, their share of the Philadelphia market went up 18% on going kosher. Somewhat less dramatic increases were observed in other cities in the Northeast.

THE KOSHER DIETARY LAWS

The kosher dietary laws predominantly deal with three issues, all in the animal kingdom:

1. Allowed Animals
2. Prohibition of Blood
3. Prohibition of Mixing of Milk and Meat

However, for the week of Passover (in late March or early April), restrictions on "chometz," the prohibited grains and the rabbinical extensions of this prohibition, lead to a whole new set of regulations, focused in this case on the plant kingdom.

In addition, a separate set of laws deals with grape juice, wine, and alcohol derived from grape products, which must be handled by sabbath-observing Jews. However, if the juice is pasteurized (heated or "mevushal" in Hebrew), then this juice can be handled like any other kosher ingredient.

ALLOWED ANIMALS AND PROHIBITION OF BLOOD

Ruminants with a cloven hoof, most domestic birds, and fish with fins and removable scales are generally permitted. Pigs, wild birds, sharks, dogfish, catfish, monkfish and similar species along with all crustacean and molluscan shellfish are prohibited. Insects are also prohibited so that carmine and cochineal (natural red pigments) are not used in kosher products. Civet, ambergesis, and castoreum are the major flavors that are automatically not kosher.

Furthermore, ruminants and fowl must be slaughtered according to Jewish law by a specially trained religious slaughterer. These animals are also subsequently inspected by the rabbis for various defects. In the United States, the desire for more stringent meat inspection requirements has led to the development of a kosher meat conforming to a stricter inspection requirement, referred to as "glatt kosher." The term "glatt," or smooth, refers to the condition of the lung. The highest standard requires that no adhesions be present; that is, the lung is totally smooth. Most glatt kosher meat is limited to a few such adhesions. For regular kosher meat, the religious meat inspectors remove all of the adhesions, and if the lung can still be inflated, the meat is kosher.

The meat and poultry must be further prepared by properly removing certain veins, arteries, prohibited fats, blood, and the sciatic nerve. In practical terms this means that only the front quarter cuts of meat are generally used because of the difficulty in removing the sciatic nerve. To remove the blood, the meat is soaked and salted within a specified time period. Furthermore, any materials that might be derived from animal sources are generally prohibited because they are difficult to obtain from strictly kosher animals. Thus, many products that might be used in the food industry, such as emulsifiers, stabilizers, and surfactants, particularly those that are fat-derived, need careful rabbinical supervision to ensure that no animal-derived ingredients are used. Almost all such materials are also available in a kosher form derived from plant oils.

PROHIBITION OF MIXING OF MILK AND MEAT

"Thou shalt not seeth the kid in its mother's milk." This passage appears three times in the Torah (the first five books of the Holy Scriptures) and is thus taken religiously as a very serious admonition. The meat side of the equation has been extended to include poultry. The dairy side includes all milk derivatives.

To keep meat and milk separate requires that the processing and handling of all products that are kosher will fall into one of three categories:

1. Meat products
2. Dairy products
3. Pareve (Parve) or neutral products

The last includes all products that are not classified as meat or dairy. All plant products as well as eggs, fish, honey, and lac resin (shellac)—which is used as a coating for some confections and fruits—are pareve. These pareve foods can be used with either meat products or dairy products, except that fish cannot be mixed directly with meat. Once a pareve product is mixed with either meat or dairy products, it takes on the status of meat or dairy, respectively

Some kosher-observant Jews are concerned about the possible adulteration of kosher milk with the milk of other animals (eg, mare's milk) and as such require that the milk be watched from the time of milking. This "Cholev Yisroel" milk and products derived from milk are required by some of the stricter kosher supervisory agencies for all dairy ingredients, so that dairy products would have to meet these requirements.

To ensure the complete separation of milk and meat, all equipment, utensils, and so on must be of the proper category. Thus, if plant materials (eg, a fruit juice) is run through a dairy plant, it would become a dairy product religiously. Some kosher supervisory agencies do permit such a product to be listed as "dairy equipment (D.E.)" rather than "dairy." The D.E. tells the consumer that it does not contain dairy but was made on dairy equipment (see allergy discussion). With the D.E. listing, the consumer can use the product immediately after a meat meal (on dairy dishes); ordinarily a significant wait would be required to consume a dairy product. In either case, the dishes would be switched from meat dishes to dairy dishes.

Kosher-observant Jews must wait a fixed time between meat and dairy consumption. Customs vary, but the wait after meat before consuming dairy is generally much longer (three to six hours) than the wait from dairy to meat (zero to one hour). However, when a hard cheese (defined as a cheese that has been aged for more than six months) is eaten, the wait is the same as that for meat. Thus, most companies producing cheese for the kosher market age their cheese for less than six months.

If one wants to make the product truly pareve, the dairy plant can usually be made pareve by the process of equipment kosherization (see later section).

PASSOVER

During this holiday, which occurs in the spring, all products made from the five prohibited grains (Hebrew: chometz)—wheat, rye, oats, barley, and spelt—cannot be used except for the specially supervised production of unleavened bread (Hebrew: matzos) that is prepared especially for the holiday. Special care is taken to ensure that the matzos do not have any time to "rise." In addition, products derived from corn, rice, legumes, sesame seeds, mustard seed, buckwheat, and some other plants (Hebrew: kitnyos) are prohibited for Jews whose origins are from central Europe. Thus, items like corn meal, corn syrup, corn starch, would be prohibited. Many rabbis permit kitnyos oils. Some rabbis permit liquid derivatives of kitnyos such as corn syrup. The major source of sweeteners and starches generally used for Passover production of "sweet" items is either real sugar or potato-derived products. Some potato syrup is also used. Passover is a time of large family gatherings. Overall, 40% of kosher sales for the traditional "kosher" companies occurs during the week of Passover.

EQUIPMENT KOSHERING

There are three ways to make equipment kosher and/or to change its status, depending on the equipment's prior production history. Note: After a plant (or a line) has been used to produce kosher pareve products, it can be switched to either kosher dairy or kosher meat without a special equipment kosherization step.

The simplest equipment kosherization occurs with equipment made of materials that can be koshered and that have only been handled cold. These require a good caustic/soap cleaning. However, materials such as ceramics, rubber, earthenware, and porcelain cannot be koshered. If these materials are found in a processing plant, new materials may be required for production; switching between different status conditions will be difficult.

Most food processing involves a heat treatment, generally above 115 to 120°F, which is defined rabbinically as "cooking." However, the exact temperature for "cooking" depends on the rabbi. To kosher these items, the equipment must be thoroughly cleaned with caustic/soap. The equipment must be left idle for 24 h and then the equipment must be flooded with boiling water (defined between 190 and 212°F) in the presence of a kosher supervisor.

In the case of ovens or equipment that use "fire," kosherization involves heating the metal until it glows. Again, the rabbi will generally be present while this process is taking place.

The procedures that must be followed for equipment kosherization can be quite extensive, so the fewer status conversions, the better. Careful formulating of products and good production planning can minimize the inconvenience and cost.

INDUSTRIAL JEWISH COOKING

Depending on what is being cooked, it may be necessary for the rabbi to "do" the cooking. In practical terms this is often accomplished by having a rabbi light the pilot light, which is then left on continuously.

In the case of cheese making, a similar concept usually requires the rabbi to add the coagulating agent into the vat. If the ingredients used during cheese making are all kosher but a rabbi has not added the coagulant, then the whey derived from such cheese would be considered kosher as long as the curds and whey have not been heated above about 120°F before the whey is drained off. As a result, there is much more kosher whey available than kosher cheese.

DEALING WITH KOSHER SUPERVISION AGENCIES

Kosher supervision is taken on by a company to expand its market opportunities. It is a business investment that, like any other investment, should be examined critically. It is appropriate for companies to look carefully at how they select a kosher supervisory agency. The agency's name recognition is only one important consideration. Other questions to be raised include: (*1*) How responsive is the agency? (*2*) How willing are the rabbis to work with the company on problem solving? (*3*) How willing are they to explain their kosher standards and their fee structure? (*4*) Is the "personal" chemistry right? and finally (*5*) What are the agency's religious standards and do they meet the company's needs in the marketplace?

One of the hardest issues for the food industry is the existence of so many different kosher supervisory agencies and standards. The trend in the mainstream kosher community today is toward a more stringent standard.

One can generally divide the kosher supervisory agencies into three broad categories. First there are the large organizations that dominate the supervision of larger food companies: the OU (Union of Orthodox Jewish Congregations), the OK (Organized Kashruth Laboratories), the Star-K, and the Kof-K—all of which are nationwide and "mainstream." Two of these, the OU and the Star-K are communal organizations: that is, they are part of a larger community religious organization. This provides them with a wide base of support but also means that the organizations are potentially subject to the other priorities and needs of the organization. The Kof-K and the OK are private companies whose only function is to provide kosher supervision. In addition to these national companies, there are smaller private organizations and many local community organizations that provide equivalent religious standards of supervision on a smaller scale. As such, products accepted by any of these mainstream organizations will generally be accepted by all other mainstream organizations. Local organizations may have a bigger stake in the local community; they may be more accessible and easier to work with. Although they often have less technical expertise, the smaller agencies are often backed up by one of the national organizations. For a company that nationally markets, a limitation of using a local kosher certifying agency may be name recognition of their kosher symbol elsewhere in the United States. With the advent of *KASHRUS* magazine, and its yearly review of symbols, this has become somewhat less of a problem. *KASHRUS* magazine (Box 204, Brooklyn, NY 11204) does not try to "evaluate" the standards of the various kosher supervisory agencies, but simply "reports" their existence. It is the responsibility of the local congregational rabbi to define his or her standards for the congregation. A remote and lesser-known certification organization may be difficult to recommend.

The second category of kosher supervision includes individual rabbis, generally associated with the "Hassidic" communities. These are often affiliated with the ultra-orthodox communities of Williamsburg and Borough Park in Brooklyn, Monsey, New York, and Lakewood, New Jersey. There are special food brands that cater to these communities, oftentimes providing continuous rabbinical supervision rather than the occasional supervision used by the mainstream organizations. The symbols of the kosher supervisory agencies representing these consumers are not as widely recognized beyond these communities as those of the major mainstream agencies in the kosher market. The rabbis will often do special supervisions of products using a facility that is normally under mainstream supervision—often without any changes, but sometimes with special needs for custom production.

The third level of supervision is done by individual rabbis who are more "lenient" than the mainstream standard. Many of these rabbis are Orthodox; some may be Conservative. Their standards are based on their interpretation of the kosher laws. More lenient standards may cut out some of the "mainstream" and stricter markets. Each company must weigh such issues when deciding about kosher supervision.

Ingredient companies are very much encouraged to use a "mainstream" kosher supervisory agency so that they can sell to as many customers as possible. Unless an ingredient is acceptable to the mainstream, it is almost impossible to gain the benefit of kosher certification.

With respect to interchangeability between kosher supervisory agencies: a system of certification letters is used to provide information among certifying rabbis about products they have approved. A supervising rabbi certifies that a particular plant produces kosher products, or that only products with certain labels or certain codes are kosher under his supervision. Such letters should be renewed each year and should be dated with both a starting and ending date. These letters are the mainstay of how companies establish the kosher status of ingredients as these ingredients move in commerce. Obviously a kosher supervisory agency will only "accept" letters from agencies they consider acceptable. Consumers may also ask to see such letters.

In addition, the kosher symbol of the certifying agency or rabbi usually appears on the packaging. (In some industrial situations, where kosher and nonkosher products are similar, some sort of color coding of products may also be used.) Most of these symbols are "trademarks" that are duly registered. In a few cases, the trademark is not registered, and more than one rabbi has been known to use the same kosher symbol.

With respect to kosher markings on products, two issues need to be highlighted:

1. It is the responsibility of the food company to show its labels to its kosher certifying agency prior to printing to ensure that the labels are marked correctly. This responsibility includes both the agency symbol and the documentation establishing its kosher status (eg, meat, dairy, or pareve). Many agencies currently do not require that "pareve" be marked on products; others do not use the "dairy" marking.
2. The labels for private label products marked with specific agency symbols cannot be moved easily between plants. This is why some companies—both private label and others—use the generic "K," which can continue to be used even if the kosher supervisory agency changes. Increasingly, sophisticated kosher consumers are questioning the use of this generic symbol.

GELATIN

Gelatin is probably the most controversial of all modern kosher ingredients. Gelatin can be derived from pork skin, beef bones, or beef skin. In recent years, some fish gelatins have also appeared. As a food ingredient, fish gelatin has many similarities to beef and pork gelatin. Depending on the species from which the fish, skins are obtained, its melting point can vary over a much wider range of melting points than beef or pork gelatin. This may offer some unique opportunities.

Currently available gelatins—even if called "kosher"—are not acceptable to the mainstream kosher supervisory organizations. A recent production of gelatin from the hides of kosher-slaughtered cattle has been available in limited supply at great expense and is accepted by the mainstream kosher supervisory agencies and some of the stricter ones.

Attitudes about gelatin certification vary greatly among the lenient kosher supervisory agencies. The most liberal view holds that gelatin, being made from bones and skin, is not being made from a food (flesh). Further, the process used to make the product goes through a stage where the product is so "unfit" for consumption that it is not edible by man or dog, thus becoming a new entity. (Rabbis holding this view may accept pork gelatin.) Most gelatin desserts with a generic "K" follow this ruling.

Other rabbis only permit gelatin from beef bones and hides but not pork. Other rabbis will only accept "India dry bones" as a source of beef gelatin. These bones, found naturally in India (because of the Hindu custom of not using cattle) are aged for over a year and are "dry as wood"; additional religious laws exist for permitting these materials. Again, none of these products is accepted by the "mainstream" kosher supervisions, rendering them unacceptable to a significant portion of the kosher community.

BIOTECHNOLOGY

Rabbis currently accept products like chymosin (rennin) that are made by simple genetic engineering. The production conditions in the fermentors must still be kosher; that is, the ingredients and the fermentors and any subsequent processing must use kosher equipment and ingredients of the appropriate status. A product produced in a dairy medium would be dairy; any product produced by cattle by excretion in the milk is defined as dairy. The rabbis continue to consider the status of more complex genetic manipulations. Note: A natural rennin extracted from the dried stomach of a kosher-killed and inspected calf is kosher and is pareve.

FEDERAL AND STATE REGULATIONS

Making a claim of kosher on a product is a "legal" claim. Federal regulation 21CFR101.29 has a paragraph indicating that such a claim must be appropriate. Approximately 20 states and a number of counties and cities have laws specifically regulating the claim of "kosher." Many of these laws refer to "Orthodox Hebrew Practice" or some variant of this term, and their legality in the 1990s is subject to further court interpretation.

New York State probably has the most extensive set of kosher laws, including a requirement to register kosher products with the Kosher Enforcement Bureau of the Department of Agriculture and Markets (55 Hanson Pl., Brooklyn, NY 11217). However, the laws in New Jersey—having been written after the state's original laws were declared unconstitutional by the state supreme court—probably have the clearest focus and, to date, no Constitutional issue. The regulations focus specifically on "consumer right to know" and "truth in labeling" issues. They avoid having the state of New Jersey define kosher. Rather, the rabbis providing supervision declare the information—adhered to by the food manufacturers—that consumers need to make an informed decision.

ALLERGIES

Many consumers use the kosher markings as a guideline for determining whether products might meet their special health needs. There are also limitations that the particularly sensitive consumer must consider.

With respect to all kosher products, two important limitations need to be recognized:

1. A process of equipment kosherization is used to convert equipment from one status to another. This is a well-defined religious procedure but may not lead to 100% removal of previous materials run on the equipment.
2. Religious law does permit certain ex post facto (after the fact) errors to be negated. Thus, trace amounts (less than 1/60 by volume under very specific conditions) can be nullified. In deference to a company's desire to minimize negative publicity, a kosher supervisory agencies may *not* announce when it has used this procedure to make a product acceptable.

Products that one might surmise to be made in a dairy plant—for example, pareve substitutes for dairy products and some other liquids like teas and fruit juices—may be produced in plants that have been kosherized, but they may not meet a very critical allergy standard. Another product that can be problematic is chocolate: many plants make both milk chocolate and pareve chocolate. Getting every last trace of dairy out of the pareve chocolate can be difficult.

Dairy and meat equipment: A pareve product may be produced on a dairy or meat line without any equipment kosherization. Again, religiously insignificant traces of dairy may remain that cannot be tolerated by a very allergic consumer. (The product can be used in a kosher home without the normal waiting period.)

Fish: In a few instances where pareve or dairy products contain small amounts of fish (eg, anchovies in Worcestershire sauce), this ingredient may be marked as part of the kosher supervision symbol. Many certifications, however, do not specifically mark this.

For Passover, there is some dispute about "derivatives" of both chometz and kitnyos materials. A few rabbis permit items like corn syrup, soybean oil, peanut oil, and similarly derived materials from these extensions. More generally, "proteinaeous" parts of these materials are not used. Thus, people with allergies to these items should purchase these special Passover products from supervisory agencies that do not permit kitnyos derivatives. With respect to "equipment kosherization", supervising rabbis tend to be very strict about the clean-up of the prohibited grains (wheat, rye, oats, barley, and spelt) but may not be as critical with respect to the extended prohibition of kitnyos.

Consumers should not assume that kosher markings ensure the absence of trace amounts of the ingredient to which they are allergic.

BIBLIOGRAPHY

1. I. Grunfeld, *The Jewish Dietary Laws*, The Soncino Press, London, United Kingdom, 1972.
2. J. M. Regenstein, "Health Aspects of Kosher Foods," *Activities Report and Minutes of Work Groups & Sub-Work Groups of the R & D Associates* **46**, 77–83 (1994).

GENERAL REFERENCES

M. M. Chaudry and J. M. Regenstein, "Implications of Biotechnology and Genetic Engineering for Kosher and Halal Foods," *Trends Food Sci. Technol.* **5**, 165–168 (1994).

J. M. Regenstein and C. E. Regenstein, "An Introduction to the Kosher (Dietary) Laws for Food Scientists and Food Processors," *Food Technol.* **33**, 89–99 (1979).

J. M. Regenstein and C. E. Regenstein, "The Kosher Dietary Laws and Their Implementation in the Food Industry," *Food Technol.* **42**, 86, 88–94 (1988)

JOE M. REGENSTEIN
Cornell University
Ithaca, New York

CARRIE E. REGENSTEIN
University of Rochester
Rochester, New York

KRILL PROTEIN PROCESSING

Krill belongs to the order Euphausiacea. There are 2 families, 11 genera, and 84 species. It is distributed in both shallow and deep seawaters. The appearance of krill is much like that of small shrimp. Among them the Antarctic krill (*Euphausia superba*) is most important and most abundant. It is distributed south of 60°S around the South Pole. The stock of krill is estimated at 360–1,400 million tons (1). Krill receives much attention because of its potential use as a protein resource for human foods. The use of Antarctic krill for human food is outlined here. More detailed reports concerning krill use are given in Refs. 2 and 3. Figure 1 shows Antarctic krill.

CHEMICAL COMPOSITION

The chemical composition of Antarctic krill varies slightly because of differences in the size, age, and sex of the sample and the fishing season. Average values of general composition of whole body of krill are as follows: moisture, 77.9–83.1%; crude protein, 11.9–15.4%; chitin and glucide, 2%; crude fat, 0.5–3.6%, and crude ash, as 3% (4,5). The composition of the muscle after removing the shell is more protein and less ash compared to the whole body of krill.

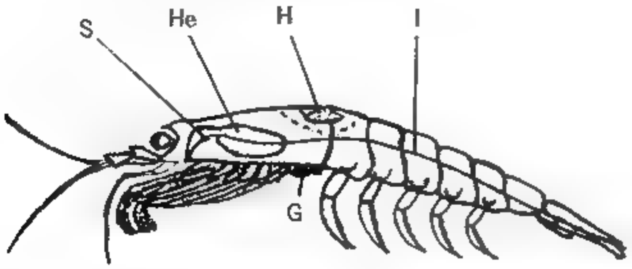


Figure 1. Antarctic krill. G, gill, S, stomach; He, hepatopancreas; H, heart; I, intestine

Table 1 shows the chemical composition of whole krill body taken in the period December through February. It can be seen that the chemical composition of krill is well balanced in foodstuff. The lipids of krill have a high iodine value of 110–190. About 70% of the whole lipids of krill is unsaturated fatty acid. Concentration of oleic acid, eicosapentaenoic acid, and docosahexaenoic acid are high. Krill lipids are, however, not easily oxidized, which may be due to their high concentration of vitamin E in the oil as antioxidant. Cholesterol levels of shellfish are higher than that of fish; krill is not an exception. Krill tissue contains 62.4–71.6 mg/100 g cholesterol. Krill contains large amounts of vitamin A, D, and the B group complex. Most of the vitamin A is astaxanthin in the whole body, which is highly concentrated in the eyes.

About 80% of the nitrogen compounds in krill meat is protein-nitrogen. Myofibrillar protein makes up 60–70% of the total protein (average 9.4% in muscle) (6). The value is similar to fish meats. Krill myofibrillar protein is mainly composed of myosin, actin, and paramyosin, making of similar to that of other invertebrate meats. The protein composition of krill meat is given in Table 2. Amino acid composition of krill protein is similar to that of common crustaceans, such as prawn or shrimp. Sulfur-containing amino acids are low compared to those in whole-egg protein; on the other hand, lysine and threonine are relatively abundant in krill (7). The amino acid score for krill protein ranges from 85 and 100. The discrepancy of the values obtained by different scientists are probably because of different analytical methods. Nutritive values of krill protein are lower than those of whole-egg protein but are similar to or higher than those of casein (8).

Table 1. Chemical Composition of Krill (% in Whole Body)

Date of fishing	Moisture	Crude protein	Crude fat	Crude ash	Chitine and glucide
Dec. 12	83.7	11.8	0.48	3.24	1.15
Dec. 26	81.8	13.8	0.60	3.19	1.19
Jan. 5	80.4	14.2	1.48	3.00	1.05
Jan. 15	80.8	13.5	1.90	3.07	1.08
Jan. 25	79.7	14.2	2.50	3.07	1.03
Feb. 5	78.8	14.2	3.61	3.08	1.09
Feb. 15	80.0	13.4	3.37	2.60	1.09

Table 2. Protein Contents in Krill Meat

Sample no.	Nonprotein-N (mg N/g)	Protein-N % in total protein-N			
		Sp	Mf	A-S	Stroma
1	4.72	14.2	70.0	14.1	1.6
2	4.58		84.0	14.7	1.5

Note. Sp. sarcoplasmic protein; Mf, myofibrillar protein; A-S, alkali-soluble protein.

PROCESSING

Raw Material Properties

Because of the characteristics of krill, processing into the final products must be accomplished as soon as possible after catch on board. The body of krill is so small (average body weight is 0.68 g) that its muscle is located close to its digestive organs containing protease, and muscle protein is easily affected by protease (9). Also the level of autoproteolysis varies considerably among different season catches from the same area (11). The speed of denaturation of myofibrillar protein of krill is much faster than it is for other fish and shellfish. Deterioration starts taking place immediately after death of krill. As krill fishing is not done continuously, harvested krill is stored in a pool until processing starts. The main proteases responsible for the autolysis are trypsinlike proteases at neutral pH and cathepsins (B, H, and L types) at acidic pH (10). Rolling and pitching of the ship causes damage to the bodies.

Fresh-Frozen Products

Fresh-frozen products of krill are made by quick freezing immediately after catch by means of a contact freezer at −40°C or individual quick freezing (IQF). The key point of this procedure is to freeze and store the material below −20°C while it is still fresh. Temperature control of krill on board is important; if the temperature is insufficient, blacking of krill bodies takes place. The cause of such deterioration has been found to be the enzyme (tyrosinase) contained in the hepatopancreas. Myofibrillar protein in krill muscle aggregates and its molecular size enlarges during freezing-storage. The freeze-denaturation of krill protein occurs more quickly than for other fish and shellfish (6). Frozen krill should be stored at −30°C; otherwise, unpalatable texture due to protein denaturation occurs. The specific odor of frozen krill is found to be caused by dimethylsulfide (DMS) (12). DMS evokes a flavor specific to crustacea when its concentration is low. If the concentration becomes over 1 ng/g tissue, the aroma becomes unpleasant. Storage at −30°C is desirable to prevent formation of DMS.

Peeled Meat

As krill has many unpalatable appendages, peeled meat is more palatable. There are two kinds of peeled krill products, boiled peeled meat and raw peeled meat.

To make boiled peeled meat, whole krill body is cooked in water at 90°C for 5 min, then excess water is removed by centrifugation, then the krill is quickly frozen. The frozen material is used for removing unpalatable parts, such as appendages and shells A shot-blast, vibration, screw-grinding, or air-blast method is employed for the removing process (2). The peeled meat is stored at −20°C.

To make raw peeled meat, the muscle of the tail of the krill is collected mechanically by a peeler (Fig. 2) (13,14). A pair of rollers, parallel to each other, rolls inward and then turns back. During this process, the shell of the krill is torn and the meat is pushed on the rollers and picked up by a jet stream of brine. This seawater is absorbed and makes the water content of the collected meat as high as 80–90%. The raw peeled meat is stored at −30°C. The yield of the raw peeled meat is very low, about 10–15% by weight of whole krill body Improvements of the peeler design are needed to increase productivity per hour and yield rate of meat from raw material. The raw peeled krill is produced in Japan to be used as an ingredient in many kinds of processed foods.

Dried Products

Drying is an easy way of processing krill on board. Half-dried and full-dried krill have been produced. The half-dried krill is better tasting than the full-dried krill because the fully dried krill are more fragile and have an unpalatable texture. The water content of full-dried krill is 5–25%; half-dried is 40–50%. Half-dried krill must be refrigerated.

Protein Paste

Krill paste has been developed and commercial produced mostly in the former USSR. Fresh krill is pressed and a juice containing protein is collected. The coagulated protein is obtained by heating and pressing. The obtained coagulated protein is kneeded to make paste and then frozen. A krill paste-manufacturing machine for use on board ship has been developed in the former USSR (15). The krill paste-manufacturing machine consists of parts for peeling, heating, protein-separating, and kneeding. The krill paste is pink in color and has the appearance of soft cheese. It is used as an ingredient in cheese and butter and various other foods, such as sausage, clroquette, and priozhki (15).

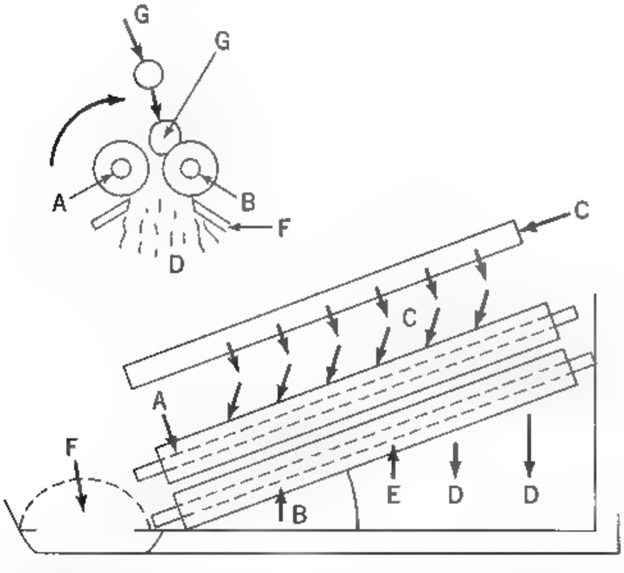


Figure 2. Roller-type peeling machine. A, B, roller; C, seawater; D, shell; E, krill meat in the getter of the peeler; F, krill meat; G, whole krill; H, knife.

Frozen Surimi

Frozen surimi from krill meat have been developed in processing on board. There are two types of krill surimi red surimi and white surimi. The method for preparing red surimi is to remove the internal organs by a centrifuge and collect the meat by a drum-type bone separater. The red surimi is contaminated red pigments from krill eyes. The starting material of white surimi is raw, peeled krill meat. Water-washing, which is always used in making surimi from other fish, is not used in either type of krill surimi processing because of difficulties in removing excess water after water-washing, owing to the strong water-holding ability of krill protein and also due to the fact that myofibrillar protein is quite soluble, and some is lost during washing (16). Five to 10% sorbitol and 0.3% polyphosphate are mixed to prevent protein denaturation of red and white surimi. Also, denaturated soybean protein, beef blood plasma, or egg white is added to diminish the effect of the residual protease; these nonmuscle proteins act as coenhancers of gelation. The gel-forming ability of red surimi is weaker than that of white surimi. Red surimi is rich in vitamin A and red pigment. White surimi is rich in taste compound and strong gel-forming ability similar to surimi made from fish meat. The Japan Fisheries Agency was engaged in a project beginning in 1977 to develop the use of Antarctic krill as food for humans. Krill surimi developed by this project is not yet commercially successful (17).

Various Processed Krill

Development of highly processed food with added value has received attention. For example, in Japan prepared

Table 3. Yield of Krill Products

Product	Yield (%^{a})	References
	On the market	
Frozen raw whole	100	2
Frozen raw peeled	10–28	3
Frozen boiled whole	105	4,18
Frozen boiled peeled	80–90	4,18
Frozen tail meat	20–30	4,18
Dried whole (15% M^b)	25	3
Dried whole (40% M^b)	35	3
Paste	10–40	2,15
	Being developed	
Red surimi	20–50	3
White surimi	10–20	3
Meat-textured fish protein concentrate	15	3,19
Imitation crabmeat	20	20
Isolated protein	10–15	2

[a] Yield, % = product weight/whole krill body
[b] M = moisture.

foods sold at delicatessens are made by mixing krill with vegetables and hamburger meat. Peeled, raw krill that has been boiled and canned has been officially adopted in the school lunch program in Japan. Because krill is rich with free amino acids and with shrimplike flavor, a method is being developed to produce a natural seasoning by liquefying krill with enzymes in a bioreactor, filtering the liquid to remove fragments of shell, and concentrating the result by vacuum or spray-drying. Table 3 lists the many kinds of krill products developed as human foods.

BIBLIOGRAPHY

1. I. Yamanaka, "Interaction Among Krill, Whales and Other Animals in the Antarctic Ecosystem," *Proceedings of the BIOMASS Colloquium in 1982, Memoir of the National Institute of Polar Research* Special issue No. 27, 220–232 (1983).
2. T. Suzuki and N. Shibata, "The Utilization of Antarctic Krill for Human Food" *Food Revue International* **6**, 119–147 (1990).
3. T. Suzuki, *Fish and Krill Protein: Processing Technology*, Applied Science Publishers, London, 1981.
4. G. J. Grantham, "The Utilization of Krill," Food and Agriculture Organization, Southern Ocean Fisheries Survey Programme, Rome, 1977.
5. M. Mori and S. Yasuda, *Bulletin Central Research Laboratory*, Nippon Suisan Co. LTD. **11**, 1–5 (1976).
6. N. Shibata, "Food Biochemical Study on Utilization of Muscular Proteins in Antarctic Krill for Human Consumption," *Bulletin of the Tokai Regional Fishery Research Laboratory* No. 11, 63–141 (1983).
7. T. Kinumaki, in *Comprehensive Studies on the Effective Utilization of Krill Resources in Antarctic Ocean*, The Science and Technology Agency, Japanese Government, 1980, pp. 135–140 (Text in Japanese).
8. Y. Obatake, Ref. 7, pp. 46–51.
9. S. Konagaya, "Protease Activity and Autolysis of Antarctic Krill," *Nippon Suisan Gakkai-shi* **46**, 175–183 (1980).
10. Y. Kawamura et al., "Effects of Protease Inhibitors on the Autolysis and Protease Activities of Antarctic Krill," *Agric. Biol. Chem.* **48**, 923–930 (1984).
11. K. Osnes and V. Mohr, "Peptide Hydrolases of Antarctic Krill *Euphasia superba*," *Comp. Biochem. Physiol.* **82B**, 559–606 (1985).
12. T. Tokunaga and co-workers, "Formation of Dimethyl Sulfide in Antarctic Krill, Euphausia superba," *Nippon Suisan Gakkai-shi* **43**, 1209–1217 (1977).
13. A. Kawakami and M. Tone, Jap. Pat. (Open No 55-31911).
14. M. Yanagimoto and T. Kobayashi, in Ref. 7, pp. 154, 174, 182–183.
15. T. Masuda and co-workers, "Utilization and Processing of Fish in USSR," Fisheries Agency, Japanese Government, 1980. (Text in Japanese).
16. P. Montero and M. C. Gomez-Guillen, "Recovery and Functionality of Wash Water Protein from Krill Processing," *J. Agric. Food Chem.* **46**, 3300–3304 (1998).
17. "Development of Technology of Antarctic Krill for Human Foods" Fisheries Agency, Japanese Government, 1980, 1981 (Text in Japanese).
18. H. Ozaki, "Peeling Technology of Krill and a Screw Grinder Method" *Shokuhin Kogyou* **24**, 34–44 (1981) (Text in Japanese).
19. T. Suzuki and co-workers, "Manufacturing of Meat-textured Krill Protein Concentrate," **48**, 105–111 (1982).
20. H. Takizawa and co-workers, "Manufacturing of Crab Meat-Textured Protein Fiber from Antarctic Krill," *Nippon Suisan Gakkai-shi* **52**, 1243–1248 (1986).

TANEKO SUZUKI
Nihon University
Kanagawa, Japan

LABORATORY ROBOTICS AND AUTOMATION

Robotics is not new to the general public but is a recent addition to the analytical laboratory. The history of robotics spans more than a half century, with the first use of the term in a 1921 play *Rossums Universal Robots*. The term robot is derived from the Czechoslovakian word "robota," which can be defined as one who performs compulsory labor much like an automaton (1). Science fiction literature and films are full of robots in various forms, ranging from humanoid such as Maria in the movie *Metropolis* to R2D2 and C3PO in *Star Wars* and a cerebral HAL in *2001, A Space Odyssey*. Probably the first exposure of many persons in the written literature came in the form of Robbie in *I, Robot* by Isaac Asimov (2). This volume also introduced the Three Laws of Robotics and the first robot psychometrician: (*1*) A robot may not injure a human being or through inaction allow a human being to come to harm; (*2*) A robot must obey orders given by human beings except where such would conflict with the First Law; (*3*) A robot must protect its own existence as long as the protection does not conflict with the First and Second Law.

Commercial laboratory robotics was introduced to the scientific community in 1982 in the form of Zymark Corporation's Zymate 1. Although there was a substantial number of organizations doing robotic research for the laboratory, this introduction allowed those who were interested in the technology to purchase it off the shelf. Laboratory automation before robotics was divided into two general categories: high performance liquid chromatography (HPLC)/gas chromatographic (GC) autosamplers and data capture and analysis units. The category of autosamplers is self-explanatory. The second category included units that ranged from instruments as simple as an integrator to as complex as in integrated laboratory information management system (LIMS). At that time, inefficient sample preparation was a substantial bottleneck to laboratory operations, so it was sensible and prudent that the early robots concentrated on sample preparation. Robotics was billed as filling the missing link in the laboratory. This article cannot hope to cover laboratory robotics in its entirety, but it provides an introduction to its uses in the laboratory and some additional references for further study.

ROBOT TYPES

Three general types of robots are used in the laboratory. They are the Cartesian or cylindrical, articulating arm or revolute, and X, Y, Z. Each has advantages and disadvantages. The most widely used and first to be commercially available is the Zymate series (Fig. 1), which is a cylindrical type. It has an established user base of about 1,500. *Forbes* dubbed this unit the "One Armed Chemist" (3). Other cylindrical robots are being marketed for the laboratory market, but they tend to resemble a reverse-engineered Zymate.

Figure 1. The Zymate series is a cylindrical type of robot and the first to be commercially available.

The articulating arm or revolute robot is exemplified by a number of units, including the second commercial additions to the technology from Perkin Elmer and Fisher Scientific. These units were based on the Mitsubishi RM501 Movemaster and were available for only a limited time. A number of additional ventures, based on the CRS-Plus/CRS-One series of robots, use this type of robot.

The final of the three types is the X, Y, Z robot, named so because it moves along three axes. Examples of this type include the MilliLab, the Biomek, and an offering by Source for Automation. Figures 2 through 4 illustrate several of these units. It is not within the scope of this article to discuss robotics in general or examine all the various robot types in detail, but these examples illustrate the diversity in the area of laboratory robotics. Finally, with the new and additional offerings from a variety of vendors, there will be a blurring of the distinctions among the robot types and probably a loss of distinction among robots and robotic and automated workstations.

ROBOT PROGRAMMING

Because robots are a computer-based system, they have a unique language that is used to program them and allows

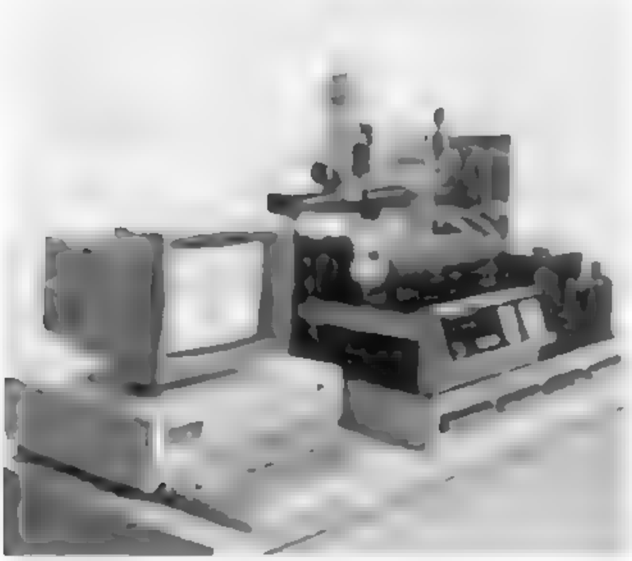


Figure 2. Window-based systems such as the MilliLab make X,Y,Z robots more user-friendly.

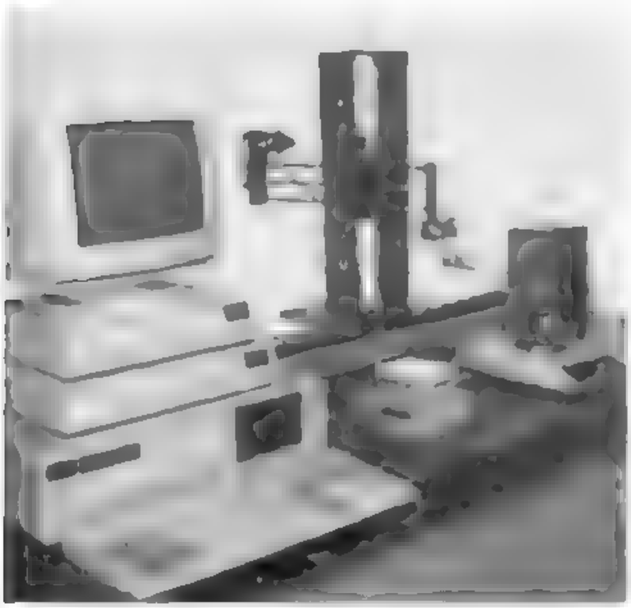


Figure 3. The Biomek, and X,Y,Z robot, communicates with peripheral devices.

them to communicate with other instruments or peripheral devices. The early generation of robots was difficult to program, not owing to a difficult robot language but largely to the fact that in some instances each robot movement had to be taught and then stored in the robot memory. The current generation of robots has eliminated this bottleneck with many systems being able to run samples within hours after their installation. This is due to a number of factors, including the introduction of PyTechnology by Zymark, where certain common laboratory operations had the necessary movements preprogrammed. Additional and also very important factors allowed for the use of standard MS-DOS computers with various window-based systems such as the MilliLab or icon-based system to make the units more user-friendly.

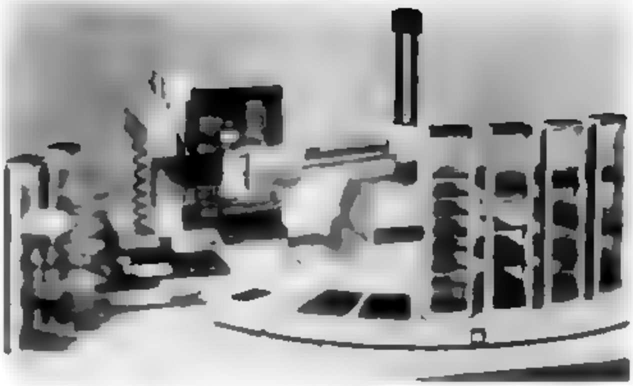


Figure 4. An X,Y,Z robot by Source for Automation.

APPLICATIONS

Early robots were purchased for a variety of reasons, including what could be called an evaluation of the technology, which was a very acceptable justification. This could be called the era of gee-whiz robotics. Because laboratory robotics is well established, the gee-whiz era has passed and users are more interested in an application-based system. These types of systems can be divided into two broad categories: (*1*) task based and (*2*) assay based. The task-based robot is configured to accomplish a particular laboratory operation or discrete series of laboratory operations. Laboratory unit operations are (4): weighing, grinding, manipulation, liquid handling, conditioning, measurement, control, data reduction, and documentation.

These various operations are common to laboratories and not particular to any specific segment. Examples of this might be liquid-liquid extraction, centrifugation, weighing, or solid-phase extraction. A laboratory might do enough of these operations to use a robot or robotic workstation. Additionally, a unit might be procured to accomplish a number of these operations, such as solid phase extraction (SPE) and HPLC injection. As one of the major uses of robotics is HPLC sample preparation, it is reasonable to assume that commercial units have been developed to concentrate in this area. The ASPEC and ASTED by Gilson and the Benchmate by Zymark are examples of these units that will accomplish several of the laboratory unit operations. Figures 5 and 6 illustrate two of these units. One could of course use a robotic system to accomplish operations, but it would not be used to the fullest capacity.

A second group of task-based units could be classified as robotic autosamplers for HPLC. A substantial number of vendors have this type of unit. In addition to acting as an HPLC autosampler, these types of units also have

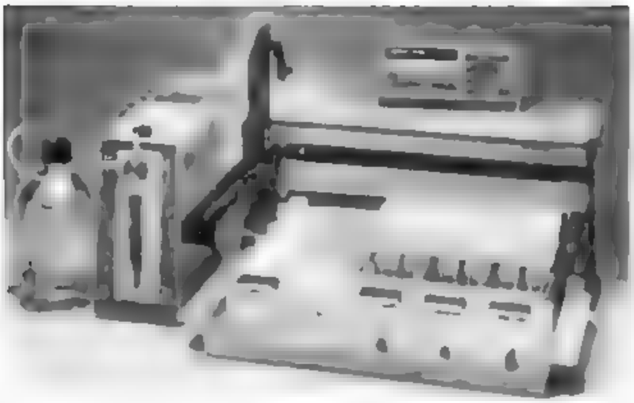


Figure 5. One of the major uses of robotics is HPLC sample preparation.

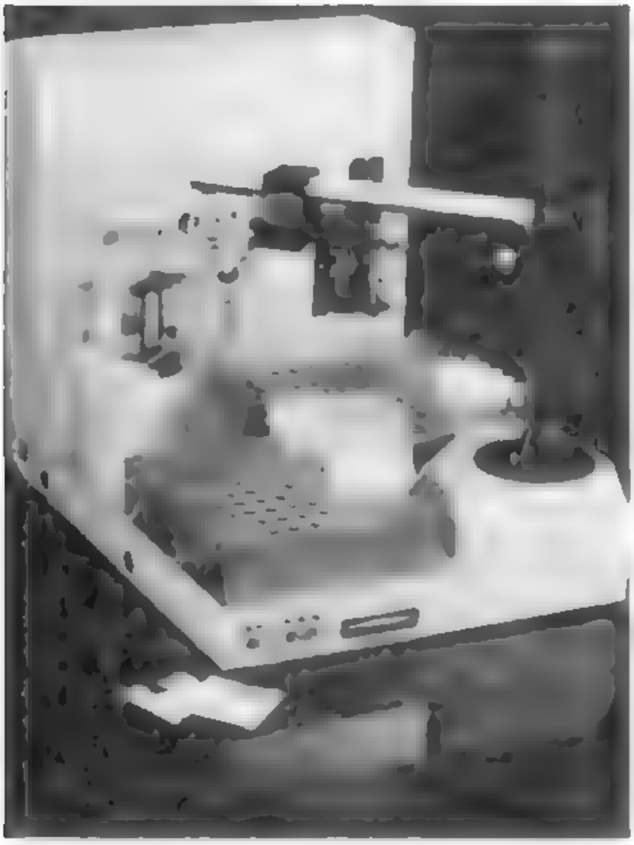


Figure 6. The Benchmate by Zymark accomplishes several of the laboratory unit operations.

the capability of making dilutions of samples and do automated derivative formation. One of the largest uses of this capability is the ability to form amino acid derivatives in a serial fashion. For example, the opa derivative for amino acids is subject to decay immediately after it is formed, so these cannot be made in a batch fashion. Using one of the robotic autosamplers, it is now possible to do the derivatization in a serial fashion so each derivative is injected onto the HPLC immediately after it is formed. This eliminates the data variability due to derivative formation and derivative decay. A final type of task-based instrument is typified by the AASP by Varian, which allows on-line solid-phase extraction and subsequent HPLC injection using a prepacked Solid Phase Extraction cassette. This unit has seen limited success in the food industry but has loyal following in a number of life sciences laboratories.

The other general category of robots that are in use could be classified as assay based. A prime example that is in use and likely one of the largest uses of robotics is in pharmaceutical organizations accomplishing drug dissolution testing. This particular assay is labor intensive and repetitive, making it very suitable for robotic implementation. A similar assay that is in use in the food industry is the Mojonnier assay. This assay is used for the determination of percentage fat content and is also labor intensive and repetitive. An organization can presently purchase a turnkey system through a partnership between a major robot vendor, Zymark, and a custom programming and device manufacturer, Forcoven Products. A number of these systems are in place throughout the world producing data that are equivalent or superior to that obtained by manual techniques (4).

Another type of assay-based system that is more generic in nature is a system capable of sample preparation for HPLC, injection onto the HPLC, and the transmission of the data to an external computer for subsequent analysis. Other examples of the use of robotics in the food industry include: Karl Fischer titration, particle size analysis, vitamin analysis, pesticide residue analysis, flavor compounding, pH analysis, and total dietary fiber.

This information provides some selected uses of the various forms of robotics in food industry laboratories. There is, of course, substantial overlap among many application areas, so it is not easy to segment them as an application used in the food industry, such as HPLC sample preparation, could easily apply to the petrochemical industry or a life sciences laboratory.

Sometimes a laboratory might have a custom application for robotics that does not fit easily into either the task-based or assay-based unit, so a custom robot is the obvious choice. When a custom application is needed, a user then has two ways to implement this procedure. This can be accomplished either through the use of internal resources or through the use of a system house. Each has its series of attributes, which will not be outlined in this article.

IMPLEMENTATION

When a robotic or other such automated system is chosen for a certain assay or procedure, the next logical phase involves the implementation. This phase starts with the installation and is completed when the system is integrated into the daily laboratory operations. In many of these components such an installation is straightforward. Likely an often encountered roadblock does not revolve around the hardware or the software; it involves a series of management issues such as personnel, management, and organizational support. Key questions to answer are

Do I have proven chemistry?
Do I have the correct person?
Do I have the correct application?
Do I have organizational commitment?
Do I have the right vendor?

Laboratory robotics presents an exciting opportunity to persons interested in laboratory automation. The technology is continuing to develop through evolution in laboratory and equipment design. Additionally, several organizations are actively involved in intelligent robots with feedback, knowledge engineering, and data-based systems. This article has provided an introduction to laboratory robotics and has overviewed the technology. Developments are occurring at a rapid pace; the adage "science is a moving target" certainly applies.

BIBLIOGRAPHY

1. I. Asimov and K. A. Frenkel, *Robots-Machines In Man's Image*, Harmony Books, New York, 1985.
2. I. Asimov, *I, Robot*, Ballantine Books, New York, 1950.
3. "The One-Armed Chemist," *Forbes* **135**, 116, 1985
4. Zymark Corporation, *Laboratory Unit Operations*, Hopkinton, Mass., 1984.
5. R. Bradley, *Laboratory Robotics and Automation* **1**, 17, 1989.

GENERAL REFERENCES

G. L. Hawk and J. Strimaitus, eds., *Advances in Laboratory Automation-Robotics 1984–1988*. Zymark Corp, Hopkinton, Mass.

These volumes are the Proceedings of The International Symposium on Laboratory Robotics and are an excellent compilation of applications and advances in the technology.

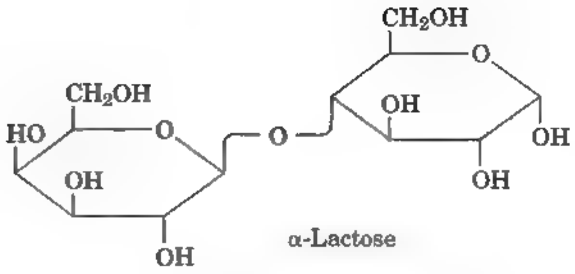
W. J. Hurst, and J. W. Mortimer, *Laboratory Robotics, A Guide to the Planning, Programming and Application*. VCH Publishers, New York, 1986.

This book provides an introduction in the technology.

W. Jeffrey Hurst
Robert A. Martin
Hershey Foods Technical Center
Hershey, Pennsylvania

LACTOSE

Lactose (4-*O*-β-D-galactopyranosyl-D-glucose), formerly called milk sugar, is a disaccharide in which a β-D-galactopyranosyl unit is attached to D-glucose by a (1 → 4) glycosidic linkage (1–6) (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING).



α-Lactose

SOURCE

Lactose is the major carbohydrate present in the milk of most mammals. The amount varies from species to species. Cow and goat milks contain approximately 4.5% lactose; human milk contains approximately 7%.

Lactose is produced commercially from cow's milk that has had the casein coagulated (the first step in the manufacture of cheese) by adjustment of the pH of the milk to the isoelectric pH of casein (4.5–4.7) and heating or by use of rennin. The resulting sweet whey, the solids of which are approximately 70% lactose, is subjected to ultrafiltration to remove remaining proteins (7). Then minerals are removed by ion exchange, and the solution is concentrated to 50 to 65% solids so that the lactose can be crystallized or precipitated. The recovered lactose is redissolved, decolorized with carbon, and recrystallized as α-lactose monohydrate (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING), the most commonly prepared form of lactose and the one that crystallizes from supersaturated solutions at temperatures below 93.5°C (1–6). For every kilogram (2.2 lb) of cheese produced, approximately 9 kg (19 lb) of whey is recovered (3). Because whey contains about 4.7% lactose, about 450 g (1 lb) is potentially available as a by-product of the manufacture of each kilogram of cheese. However, little commercial use is made of lactose.

Milk also contains 0.3 to 0.6% lactose-containing oligosaccharides, many of which are important as energy sources for growth of a specific variant of *Lactobacillus bifidus*, which as a result is the predominant microorganism of the intestinal flora of breast-fed infants.

PROPERTIES

α-Lactose monohydrate dissolves slowly, which means that it cannot be used in instant food products. Three forms of anhydrous α-lactose are known (1–6). A stable anhydrous form is prepared by heating α-lactose monohydrate in air at 130°C. β-Lactose is more soluble than α-lactose. It is prepared by heating α-lactose · H_2O in an alcohol in the presence of a base. α-Lactose is also sweeter than is α-lactose, but each anomer will form the same equilibrium anomeric mixture in solution. The equilibrated solution is 15 to 30% as sweet as a sucrose solution of the same concentration.

LACTOSE DIGESTION AND LACTOSE INTOLERANCE

The enzyme lactase, located in the small intestine, catalyzes the hydrolysis of lactose into its monosaccharide constituents, D-glucose and D-galactose. Both are rapidly absorbed and enter the bloodstream.

$$\text{lactose} \xrightarrow{\text{lactase}} \text{D-glucose} + \text{D-galactose}$$

Only monosaccharides can be absorbed from the small intestine. Therefore, if for any reason, ingested lactose is only partially hydrolyzed (digested) or is not hydrolyzed at all, a clinical syndrome results (8–10). Its symptoms are abdominal distention, cramps, flatulence (gas), and diarrhea. When there is an insufficient amount of lactase, some lactose is not absorbed from the small intestine and remains in the lumen. The presence of lactose draws fluid into the intestinal lumen by osmosis. It is this fluid that leads to the abdominal distention, cramps, and diarrhea. From the small intestine, the lactose passes into the large intestine (colon) where bacteria effect its anaerobic fermentation to short-chain fatty acids. Production of more molecules results in still greater retention of fluid. The acidic products of fermentation lower the pH and irritate the lining of the colon, leading to an increased movement of the contents. Diarrhea is caused by the retention of fluid and the increased movement of the intestinal contents. The gaseous products of fermentation (carbon dioxide, hydrogen, methane, and oxygen) cause bloating.

This syndrome resulting from an absence or deficiency of lactase is called lactose intolerance. Lactose intolerance is not usually seen in children until after about 6 years of age. At this point, the incidence of lactose-intolerant individuals begins to rise. There are varying degree of lactose intolerance. The difference in incidence among populations of different genetic backgrounds can be large. By 12 years of age, 45% of blacks develop the symptoms of lactose intolerance; among teenage blacks, the incidence climbs to 70% and by adulthood, 90% of the black population in the United States shows symptoms of lactose intolerance. Lactose intolerance is also high among Orientals. Among the whites of western European ancestry, the peak incidence in adulthood is about 15%.

Humans consume lactose in milk and other nonfermented dairy products. Cheese, cottage cheese, and most yogurts contain less lactose because some lactose is converted into lactic acid during fermentation and/or removed with the whey. Milk from which much of the lactose has been removed is available.

USES

Because of its low solubility and the common occurrence of lactose intolerance in adults, food uses of crystalline lactose are limited; but it must be kept in mind that lactose constitutes about 70% of the solids of sweet whey, so where whole liquid or dried whey is used as an ingredient, some functionality of lactose is realized. Crystalline lactose is used to a small extent in toppings, icings, pie fillings, confections, and ice creams to provide body and texture while contributing less sweetness than the same amount of sucrose would (3). It is used as a carrier for flavors, because enhancement of flavor and colors is one of its attributes (11–15). Lactose provides bulk and rapid disintegration and dissolution of pharmaceutical tablets. It is present in about 20% of tableted prescription drugs and in about 6% of over-the-counter drugs.

PRODUCTS FROM LACTOSE

Lactose is a reducing disaccharide with the aldose D-glucose at its reducing end. Reduction (hydrogenation) yields the disaccharide alditol (polyol), lactitol (1,4) (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). Lactitol can be crystallized as either a monohydrate or a dihydrate, both of which are nonhygroscopic. Therefore, it can be used in the manufacture of products such as chocolate that require that there be no moisture pickup during processing and bakery products that should remain crisp. Lactitol provides a clean, sweet taste (30–40% of that of sucrose) and provides foods with a bulk and texture similar to that provided by sucrose. Its solubility is slightly less than that of sucrose. Its heat of solution is slightly higher than that of sucrose and much below that of sorbitol. Lactitol is not acted on by human digestive enzymes, so it does not effect an increase in blood glucose or insulin levels and, thus, is safe for diabetics. Neither is it cariogenic. It is, however, fermented by colonic microorganisms, being converted into carbon dioxide and volatile fatty acids. There is, therefore, a limit to tolerance and daily consumption as with lactose in lactose-intolerant individuals.

Lactulose, 4-*O*-*β*-D-galactopyranosyl-D-fructose, is made by alkali isomerization of lactose, which converts the reducing end to the corresponding ketose, D-fructose (1,4) (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). Lactose is quite soluble; a 77% solution can be made at 30°C (86°F). Its sweetness is 48 to 62% that of sucrose, depending on concentration. Lactulose is not acted on by human digestive enzymes, but is readily fermented by colonic microorganisms, which accounts for its major use for and treatment of chronic constipation. Lactulose is a bifidus factor; in other words, it is effective in promoting the proliferation of bifidobacteria in the intestine, increasing the level of the organism in the feces of bottle-fed infants to that in breast-fed infants.

PROPERTIES

Many physical properties of lactose have been determined (6). These include solubility in water over a wide temperature range, solubility in salt solutions and organic solvents, heat of combustion, heat of solution, heat of dilution, heat capacity, and specific heat. Also determined have been the density, viscosity, refractive index, osmotic pressure, vapor pressure, freezing-point depression, and interfacial tension with organic liquids of lactose solutions and the rate of crystallization of lactose from solution.

Crystalline lactose has adsorptive properties and can be used as a carrier for aromas and flavors (11–15). The chemistry of lactose has been rather extensively examined (1,3), in part because of an interest in converting the rather abundantly available lactose into a more useful product.

ANALYSIS

Methods for the determination of lactose have been reviewed (4,16).

BIBLIOGRAPHY

1. J. R. Clamp et al., "Lactose," *Advances in Carbohydrate Chemistry* **16**, 159–206 (1961).
2. T. A. Nickerson, "Lactose Sources and Recovery," in G. G. Birch and R. S. Shallenberger, eds., *Developments in Food Carbohydrate-1*, Elsevier Applied Science, Barking, United Kingdom, 1977, pp. 77–90.
3. L. A. W. Thelwall, "Lactose," in C. K. Lee, ed., *Developments in Food Carbohydrate-2*, Elsevier Applied Science, Barking, United Kingdom, 1980, pp. 275–326.
4. L. W. Doner and K. B. Hicks, "Lactose and the Sugars of Honey and Maple: Reactions, Properties, and Analysis," in D. R. Lineback and G. E. Inglett, eds., *Food Carbohydrates*, AVI, Westport, Conn., 1982, pp. 74–112.
5. J. G. Zadow, "Lactose: Properties and Uses," *J. Dairy Sci.* **67**, 2654–2679 (1984).
6. V. H. Holsinger, "Lactose," in N. P. Wong, ed., *Fundamentals of Dairy Chemistry*, 3rd ed., Van Nostrand Reinhold, New York, 1988, pp. 279–342.
7. J. L. Short, "Prospects for the Utilization of Deproteinated Whey in New Zealand: A Review," *New Zealand J. Dairy Sci. Technol.* **13**, 181–194 (1978).
8. D. M. Paige and T. M. Bayless, *Lactose Digestion: Clinical and Nutritional Implications*, Johns Hopkins University Press, Baltimore, Md., 1981.
9. A. Lutkic and A. Votava, "Enzymic Deficiency and Malabsorption of Food Disaccharides," in C. K. Lee and M. G. Lindley, eds., *Developments in Food Carbohydrates-3*, Elsevier Applied Science, Barking, United Kingdom, 1982, pp. 183–211.
10. J. Delmont, *Milk Intolerances and Rejections*, S. Karger, Basel, Switzerland, 1983.
11. S. L. McMullin et al., "Heats of Adsorption of Small Molecules on Lactose," *J. Agric. Food Chem.* **23**, 452–458 (1975).
12. T. A. Nickerson, "Lactose Chemistry," *J. Agric. Food Chem.* **27**, 672–677 (1979).
13. K. F. Ehler et al., "Heats of Adsorption of Small Molecules on Various Forms of Lactose, Sucrose, and Glucose," *J. Agric. Food Chem.* **27**, 921–927 (1979).
14. F. W. Parish et al., "Demineralization of Cheddar Whey Ultrafiltrate with Thermally Regenerable Ion-Exchange Resins: Improved Yield of α-Lactose Monohydrate," *J. Food Sci.* **44**, 555–557 (1979).
15. J. W. Marvin et al., "Interactions of Low Molecular Weight Adsorbates on Lactose," *J. Dairy Sci.* **62**, 1546–1557 (1979).
16. W. J. Harper, "Analytical Procedures for Whey and Whey Products," *New Zealand J. Dairy Sci. Technol.* **14**, 156–171 (1979).

J. N. BeMiller
Purdue University
West Lafayette, Indiana

See also CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING; CARBOHYDRATES: FUNCTIONALITY AND PHYSIOLOGICAL SIGNIFICANCE.

LECITHINS

Lecithin is a tan to amber viscous liquid or solid primarily derived from the soybean (Fig. 1). It is a complex mixture of phospholipids combined with triglycerides, fatty acids, phytoglycolipids, sterols, and minor neutral lipid components (1). Fourcray presented evidence of its existence in 1793, and Gobley first identified it from egg yolk lipids in 1846 (2). The applications and uses have expanded for this natural surfactant since its discovery, with more than 300 million pounds now available for commercial application.

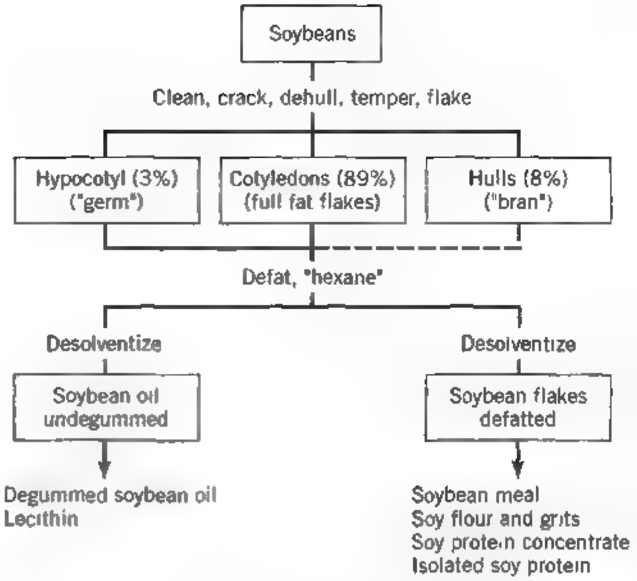


Figure 1. Processing of soybeans by solvent extraction. *Source:* Courtesy of Central Soya Company, Inc.

Lecithin is present from animal and vegetable sources. The classic definition of lecithin referred to phosphatidylcholine and not the complex mixture commercially available today. Chemical phosphatidylcholine is known as 1,2-diacyl-*sn*-glycero-3-phosphorylcholine according to IUPAC nomenclature (3). The archaic term Lecithin is no longer used for phosphatidylcholine.

COMMERCIAL LECITHIN

Commercial lecithin is a complex mixture of phospholipids, primarily phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and sometimes phosphatidylserine (Fig. 2). Various amounts of triglycerides and fatty acids are present, depending on consistency and end use.

Composition

Phospholipids are the major components in commercial lecithin. The phospholipids can range from 50 to 95%, depending on the neutral lipid content (Table 1). Triglycerides are the second major component and have fatty acids similar to the source oil. Fatty acids are natural components found in the lecithin manufacturing process, although additional fatty acids may be added as fluidizing agents. Again, the fatty acids resemble the source oil. Phospholipid fatty acids are peculiar to their source. Each vegetable or animal source can change the resulting fatty acid composition.

Phytoglycolipids are present in vegetable lecithins whereas animal lecithins may contain sphingomyelin, car-

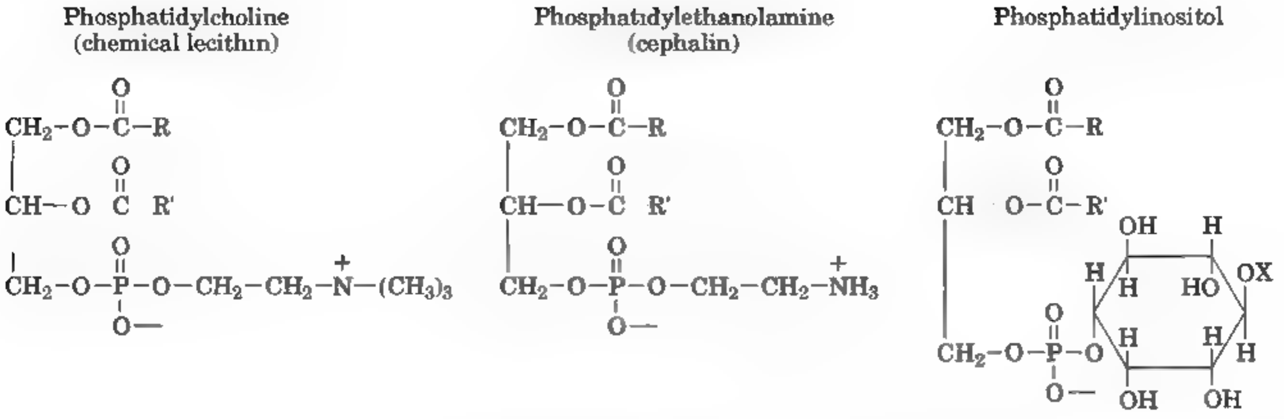

Phosphatidylcholine (chemical lecithin)
Phosphatidylethanolamine (cephalin)
Phosphatidylinositol

Figure 2. Three principal components of soybean lecithin. *Source:* Ref. 2.

diolipids, and other minor phospholipids. Minor amounts of sterols and sugars are carried through the manufacturing process. Sitosterol and stigmasterol are found in vegetable lecithin; cholesterol is found in animal sources.

Sources

Vegetable and Animal. The primary source of lecithin is from the plant kingdom, with the highest concentrations in seeds from soy, corn, rapeseed, and sunflower (4). Egg and brain are the major sources from the animal kingdom (5).

Microbial and Synthetic. Phospholipids are available from the extraction of microbes (6), and chemically derived and semisynthetic products (7) are available for medical research and cosmetics.

Manufacture

Production. Vegetable lecithins are prepared simply from the hydration of the crude oil source with centrifuging and drying (Fig. 3). Additives for viscosity and function may be added in the process (9). Animal products are usually prepared by solvent extraction of the tissue. Synthetic products usually are recovered from the reaction mixture by solvent extraction.

Analysis. Product composition and quality usually are determined by the methods established in the American Oil Chemists' Society Official Handbook of Methods, Section J (10). Tests include acetone insolubles (AI), acid value (AV), hexane insoluble matter (HIM), peroxide value (PV), phosphorus (P), composition by HPLC, viscosity, and moisture (Table 2).

Functional Uses

Lecithins are multifunctional products that have a wide range of applications at low use levels of 0.5–3.0% (2).

Emulsification. As surface-active agents they have wide usage in foods as emulsifiers. Their ability to bring two dissimilar liquids together make them important in water/oil (W/O) and oil/water (O/W) surfactants. These amphoteric surfactant properties make them ideal in food systems. The estimated HLB values for lecithin products range from 2–12 when compared to nonionic surfactants.

Release. Lecithins are good release or parting agents. Their polar groups tend to bind to metallic surfaces, and the nonpolar fatty acids act as slipping agents.

Instantizing. Most problems with dispersion deal with the rapid hydration of the dispersing item. Lecithin will reduce the solution rate and allow for easy dispersion. It also helps to disperse fat in high fat systems.

Anti-Spatter. In fat systems that contain water as an ingredient, lecithin keeps the moisture dispersed for slow evaporation under heat processing. Moisture will foam off rather than spatter.

Lubrication. The fatty acids present in the nonpolar part of lecithin give the added lubricity to prevent sticking in food-processing equipment. Because of its hydratability, the product is easier to wash off surfaces and it is completely edible.

Multifunctional. Lecithin users usually find that it is the combination of functional properties that make it a beneficial ingredient. Rarely do the functions counteract in a finished food system Emulsification and release go well in baking systems. Anti-spatter and release are perfect frying systems. Instantizing and emulsification work together in beverage mixes.

Applications

The following are from Ref. 11.

Margarine. One of the first uses for lecithin was in regular margarine around 1940. The emulsifying properties were combined with anti-spatter properties to make margarines more stable and better for sauteing. Diet margarines with the high water content use special lecithins to retain the tight emulsion and hold the moisture.

Table 1. Lecithin Nutritional Profile/Typical Analysis

	Granular lecithin	Typical liquid lecithin
Nutritional summary[a]		
Phosphatides, acetone insoluble %	95% min	60% min
Natural soybean oil	2–3%	39%
Moisture	1%	0.7%
Fat, per 100 g product	90 g	93 g
Monounsaturated (oleic)	9.2%	17.9%
Polyunsaturated (linoleic, linolenic)	65.9%	60.7%
Saturated (palmitic, stearic)	24.9%	20.3%
Calories, per 100 g product	700	790
Carbohydrates, per 100 g product	8 g	5 g
Cholesterol, per 100 g product	0 g	0 g
Approximate composition		
Fatty acid content, g/100 g product	60 g	66 g
Fatty acid content, relative composition		
Linoleic	58.9%	54.0%
Linolenic	7.0%	6.7%
Oleic	9.2%	17.9%
Palmitic	20.3%	15.6%
Stearic	4.6%	4.7%
Other fatty acids	0.0%	1.1%
Total	100.0%	100.0%
Primary acetone insolubles		
Phosphatidylcholine, g/100 g product	23 g	15 g
Phosphatidylethanolamine g/100 g product	20 g	12 g
Phosphatidylinositol, g/100 g product	14 g	9 g
Elemental analysis, mg / 100 g product		
Calcium		
Without flow agent	65 mg	40 mg
With flow agent (tricalcium phosphate)	745 mg	
Iron	2 mg	1 mg
Magnesium	90 mg	60 mg
Phosphorus		
Without flow agent	3,000 mg	2000 mg
With flow agent (tricalcium phosphate)	3,400 mg	
Potassium	800 mg	440 mg
Sodium	30 mg	10 mg

Source. Courtesy of Central Soya Company, Inc.

[a] The nutritional profile of lecithin depends, in part, on the amount and type of oil used. The breakdown shows a deoiled, granular lecithin and liquid lecithin containing a typical level of natural soybean oil

Confections. Chocolate is also one of the oldest uses for lecithin. It was found that lecithin could reduce the amount of cocoa butter needed for chocolate handling and also retarded the chocolate bloom from cocoa butter crystallization. Lecithin helps to reduce the viscosity of chocolate manufacturing and gives better flow-coating properties.

In chewing gum it prevents the drying out and cracking of the sticks on shelf storage. It also provides softness to the chicle gum base for better bite.

Caramel-coated products will not stick together when lecithin is added to the sugar. This gives the consumer a better-handling product with less staling.

Baking. Commercial lecithins use many of their functional properties in baking areas.

Breads. Lecithin distributes other emulsifiers in dough, gives less sticking in proofing, and retards bread staling on the shelf.

Cakes. The distribution of the cake and added moisture give quick hydrating and less lump formation in the batter for creamy smooth consistent cakes.

Cookies. Similar to cakes, lecithin provides better mixing of the batter and soft, smooth consistency. Lecithin can give-good chewiness for freshlike texture. It can also reduce dry-out.

Icings. Smooth creamy texture is needed for icing performance. Lecithin, when added to the fat system, will allow for better mouthfeel and good spreading qualities.

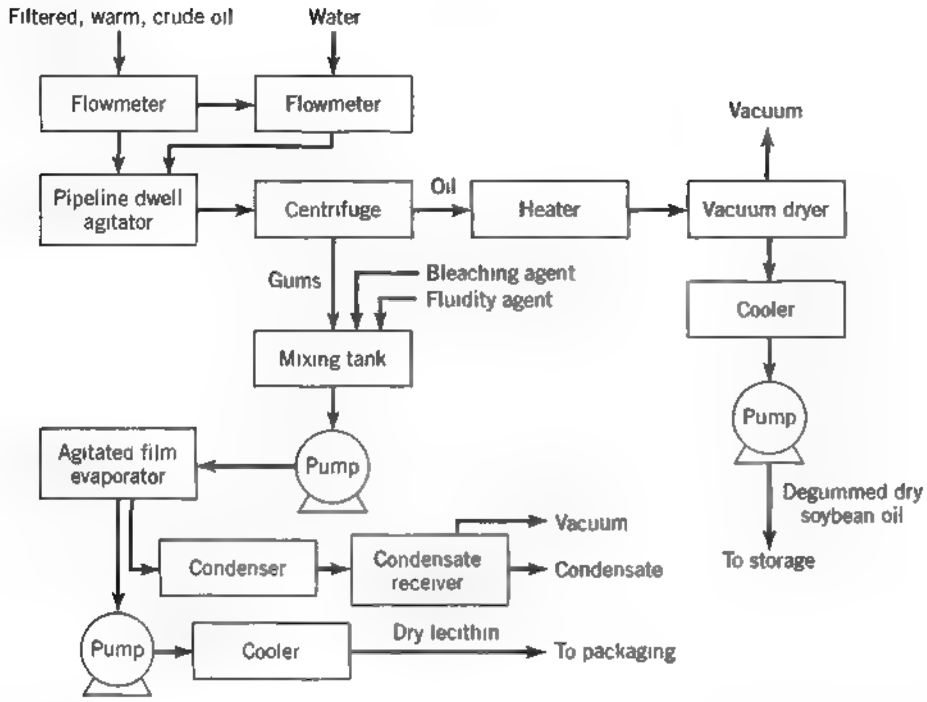

Filtered, warm, crude oil
Water
Flowmeter
Flowmeter
Vacuum
Pipeline dwell agitator
Centrifuge
Oil
Heater
Vacuum dryer
Gums
Bleaching agent
Fluidity agent
Cooler
Mixing tank
Pump
Agitated film evaporator
Pump
Degummed dry soybean oil
Vacuum
Condenser
Condensate receiver
Condensate
To storage
Dry lecithin
Pump
Cooler
To packaging

Figure 3. Flow sheet for degumming soybean oil and crude lecithin production. *Source:* Ref. 8.

Table 2. Food Grade Soybean Lecithin Specifications

Acetone-insoluble matter	Not less than 50.0%
Acid value	Not more than 36
Hexane-insoluble matter	Not more than 0.3%
Water	Not more than 1.5%
Peroxide value	Not more than 100
Limits of impurities	
Arsenic (as As)	Not more than 3 parts per million (0.0003%)
Heavy metals (as Pb)	Not more than 40 parts per million (0.004%)
Lead	Not more than 10 parts per million (0.001%)

Source: Ref. 10.

Beverages. Instant beverages are prepared by the careful application of lecithin to the powdered surface. Lecithin prevents the rapid hydration of the sugar or hydrophylic components and gives better dispersion.

Food Systems. Food systems are combinations of several ingredients that are used for covering food entrees such as meat or pastas or vegetable products in salads. Lecithin is a fine emulsifier for sauces and gravies that brings fat sources in contact with water. Lecithin keeps the oil-in-water systems intact even under heated conditions. In dressings, lecithin is a good emulsifier for high-stress systems where acidity and salt can be a problem. It provides a method of stabilizing the emulsion with a natural food amphoteric surfactant.

Health and Nutrition

Commercial lecithin and phosphatidylcholine have been extensively evaluated in health and disease areas (12). Lecithin may be effective in treating certain neurologic disorders such as tardive dyskinesia and some types of Alzheimer's disease. Most likely, lecithin plays an important role in health and nutrition as a precursor for choline (13) and may effect membrane fluidity as it effects membrane emulsion systems in food applications.

BIBLIOGRAPHY

1. B. F. Szuhaj and E. F. Sipos, "Food Emulsifiers from the Soybean," p. 115, in G. Charalambous and G. Doxastakis, G, eds., *Food Emulsifiers*, Elsevier, New York, 1989.
2. W. E. Prosise. "Commercial Lecithin Products: Food Use of Soybean Lecithin," in B. F. Szuhaj and G. R. List, eds., Lecithins, *American Oil Chemists' Society Monograph* **12**, 163–182, 1985.
3. C. R. Scholfield, "Occurrence, Structure, Composition, and Nomenclature," in Ref. 2, pp. 1–20.
4. J. P. Cherry, "Plant Sources of Lecithin," in B. F. Szuhaj, ed., *Lecithins: Sources, Manufacture and Uses*, American Oil Chemists' Society, 1989, pp. 16–31.
5. Kuksis, A., "Animal Sources of Lecithin," in Ref. 4, pp. 32–71
6. C. Ratledge, "Microbiological Sources of Phospholipids," in Ref. 4, pp. 72–96.
7. M. Ghyczy, "Synthesis and Modification of Phospholipids," in Ref. 4, pp. 131–144.
8. R. Brian, *J. AOCS* **53**, 27 (1976).
9. G. R. List, "Commercial Manufacture of Lecithin," in Ref. 4, pp. 145–161.
10. R. A. Lantz, "Industrial Methods of Analysis," in Ref. 4, pp. 162–173.
11. G. L. Dashiell, "Lecithin in Food Processing Applications," in Ref. 4, pp. 213–234.
12. S. H. Zeisel, "Lecithin in Health and Human Nutrition," in Ref. 4, pp. 225–236.

13. S. H. Zeisel, "Phosphatidylcholine: Endogenous Precursor of Choline," in *Lecithin: Technological, Biological, and Therapeutic Aspects*, vol. 33 in I. Hanin and G. B. Ansell, eds., *Advances in Behavioral Biology*, Plenum Press, New York, 1987, pp. 107–120.

Bernard F. Szuhal
Central Soya Company, Inc.
Fort Wayne, Indiana

See also Emulsifier technology in foods.

LICORICE CONFECTIONERY

Licorice (also liquorice) is the name given to both the flavor extracted from the root of the plant *Glycyrrhiza glabra* and to a popular confection whose flavor is derived from the extract. The word licorice comes from the Greek words "glykys" (meaning sweet) and "rhiz" (meaning root). In ancient times the root was valued not only for its sweet taste, but also for its perceived medicinal value, both as a treatment for illness and as a substance for preserving youth and beauty.

The licorice plant is native to an extensive region, which includes the Mediterranean, Asia Minor, and southern Asia (see Fig. 1). It has also been grown in Pontefract, England, and Bamberg, Germany. The prime sources of supply of licorice root currently are Iran, Turkey, China, Afghanistan, Syria, and Pakistan. Extracts from Spain and Italy are also available in limited quantities but at higher prices because they are less bitter in flavor.

The licorice plant takes about 4 years to grow to a height of 2–4 feet. At this age the root can be as thick as 1 inch but averages about a half inch. The lateral roots can be as long as 25 feet. The roots are harvested in autumn and spring and transported to baling stations where they are inspected, dried, and baled. The bales are shipped to extraction plants where the root is shredded and immersed in hot water. The extraction process can take place under pressure or atmospherically. The resulting liquor is then transferred to evaporators where it is concentrated into three basic forms:

1. *Syrup* containing up to 30% moisture
2. *Paste* or block licorice with moisture content between 14 and 22%
3. *Powder* with moisture content around 4.5%.

The different processes result in slightly different flavor profiles.

Licorice extract is used in the following industries:

Tobacco as flavor, to increase the mildness and as a moisture retainer.

Pharmaceutical, to mask the bitter flavor of some drugs and as a flavor enhancer.

Food and beverage as flavor, sweetness enhancer and foaming agent.

BY-PRODUCTS

As in most industries, the desire to maximize productivity and find uses for by-products has led to useful materials:

1. A foaming agent to put out fires, created via a second extraction process.
2. An insulation made from the fibers of the root results in a product with high structural strength.

CANDY

Commercial licorice candy as known today started in England in the second half of the 18th century and in the United States in the 19th century. Y&S Candies (the current market leader in sales of licorice candies) was founded

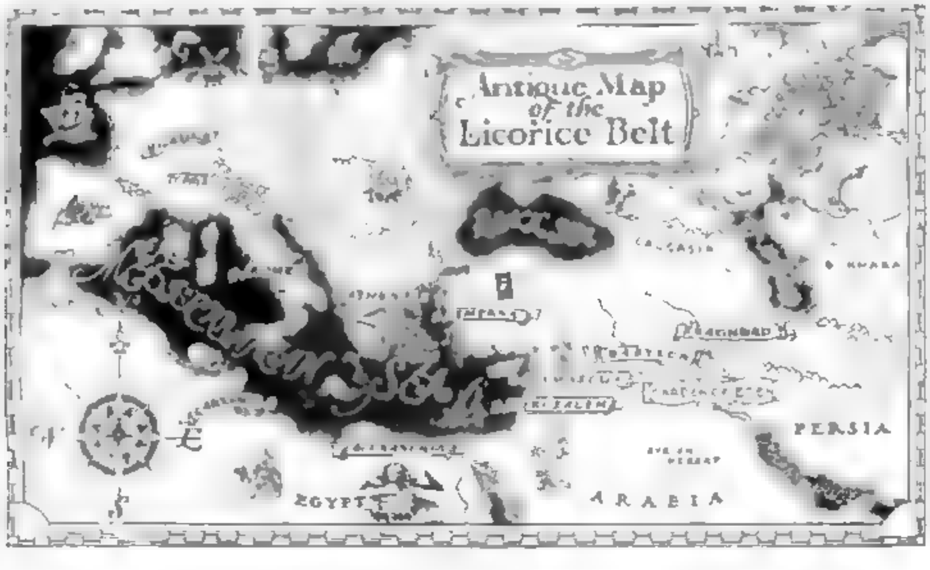


Figure 1. This curious old map shows the ancient lands in which licorice flourished thousands of years ago. This area, the Cradle of Civilization, is the principal source of licorice today.

13. S. H. Zeisel, "Phosphatidylcholine: Endogenous Precursor of Choline," in *Lecithin: Technological, Biological, and Therapeutic Aspects*, vol. 33 in I. Hanin and G. B. Ansell, eds., *Advances in Behavioral Biology*, Plenum Press, New York, 1987, pp. 107–120.

Bernard F. Szuhal
Central Soya Company, Inc.
Fort Wayne, Indiana

See also Emulsifier technology in foods.

LICORICE CONFECTIONERY

Licorice (also liquorice) is the name given to both the flavor extracted from the root of the plant *Glycyrrhiza glabra* and to a popular confection whose flavor is derived from the extract. The word licorice comes from the Greek words "glykys" (meaning sweet) and "rhiz" (meaning root). In ancient times the root was valued not only for its sweet taste, but also for its perceived medicinal value, both as a treatment for illness and as a substance for preserving youth and beauty.

The licorice plant is native to an extensive region, which includes the Mediterranean, Asia Minor, and southern Asia (see Fig. 1). It has also been grown in Pontefract, England, and Bamberg, Germany. The prime sources of supply of licorice root currently are Iran, Turkey, China, Afghanistan, Syria, and Pakistan. Extracts from Spain and Italy are also available in limited quantities but at higher prices because they are less bitter in flavor.

The licorice plant takes about 4 years to grow to a height of 2–4 feet. At this age the root can be as thick as 1 inch but averages about a half inch. The lateral roots can be as long as 25 feet. The roots are harvested in autumn and spring and transported to baling stations where they are inspected, dried, and baled. The bales are shipped to extraction plants where the root is shredded and immersed in hot water. The extraction process can take place under pressure or atmospherically. The resulting liquor is then transferred to evaporators where it is concentrated into three basic forms:

1. *Syrup* containing up to 30% moisture
2. *Paste* or block licorice with moisture content between 14 and 22%
3. *Powder* with moisture content around 4.5%.

The different processes result in slightly different flavor profiles.

Licorice extract is used in the following industries:

Tobacco as flavor, to increase the mildness and as a moisture retainer.
Pharmaceutical, to mask the bitter flavor of some drugs and as a flavor enhancer.
Food and beverage as flavor, sweetness enhancer and foaming agent.

BY-PRODUCTS

As in most industries, the desire to maximize productivity and find uses for by-products has led to useful materials:

1. A foaming agent to put out fires, created via a second extraction process.
2. An insulation made from the fibers of the root results in a product with high structural strength.

CANDY

Commercial licorice candy as known today started in England in the second half of the 18th century and in the United States in the 19th century. Y&S Candies (the current market leader in sales of licorice candies) was founded

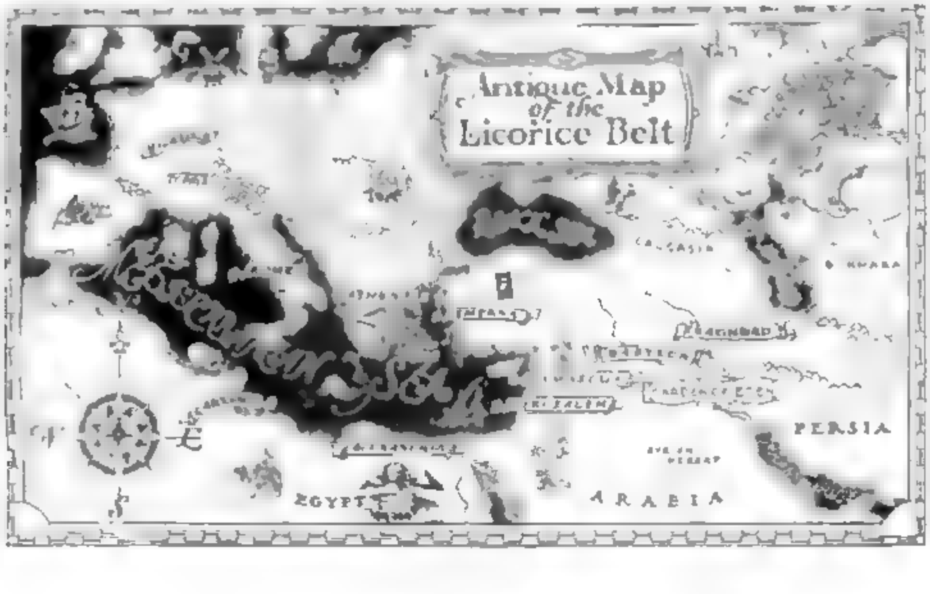


Figure 1. This curious old map shows the ancient lands in which licorice flourished thousands of years ago. This area, the Cradle of Civilization, is the principal source of licorice today.

in Brooklyn in 1845 by J. S. Young and C. A. Smylie. In 1902 they changed the name of the company to National Licorice and in 1968 changed the name back to Y&S Candies. Other well-known companies, such as Switzer and American Licorice, were founded early in the 20th century.

TYPES OF LICORICE

Licorice extract is added to gums, lozenges, and hard candies as a flavor and a demulcent for cough relief, but the most common form of candy is where the extract is added to a paste or dough and extruded into

1. Sheets, when combined with sheets of cream paste in sandwich form provide the main component pieces for licorice allsorts.
2. Strips of different shapes to form whips, braids, twists, shoelaces, reels, ribbons, etc.
3. Tubes, both hollow and filled.
4. Novelties, such as pipes and cigars.
5. Centers, for coating with a hard sugar shell and known as torpedoes, comfits, Good and Plenty®, and Goodies®.

INGREDIENTS

Licorice paste is made of wheat flour, crude sugars, molasses, gelatin, flavoring (which includes licorice extract), and color (see Table 1).

Flour

Wheat flour is normally used at levels between 25 and 40%. A strong flour, rich in elastic gluten, used to be recommended for licorice. Today, flour of low gluten content, which is less water absorbent, is preferred. More emphasis is placed on the starch content of the flour (approximately 69%), which contains 25% amylose and 75% amylopectin. A change in this percentage can regulate the gelatinization temperature and the degree of gelatinization (viscosity) before, during, and after cooking. Such changes in proportion can be obtained by the replacement of certain quantities of flour by native or modified maize, wheat, potato, rice, or other starches. The starch industry has made such advances in the last few years that it is possible to obtain a tailor-made product for each individual requirement. A hard wheat flour has a high protein content and is strong in gluten. Soft wheat flour is low in protein with little gluten. The gluten in soft flours is of a different character than that in hard flours and is more elastic. These variations in content and type of gluten give rise to differences in the texture of the licorice paste.

Table 1. Typical Formulas

Ingredient	Short texture	Long texture	Red color
Wheat flour	26.0	26.0	19.0
White sugar			18.0
Brown sugar	23.0	13.0	
Molasses	10.0		
Caramel color	4.0	13.0	
Dextrose	3.0		3.0
Corn syrup		7.0	12.0
Block licorice	3.0	4.0	
Salt	0.5		0.1
Gelatin (low bloom)	0.3	4.0	
Red color			0.3
Black color	0.1		
Glyceryl monostearate	0.1		
Fruit flavor			q.s.[a]
Anise oil	q.s.	q.s.	
Citric acid			0.6
Water	30.0	33.0	47.0

[a] q.s., quantity sufficient.

Sugar

Sugars used in licorice paste can be low grade provided that they meet food safety standards. Reducing sugars (present in low-grade sugars) are beneficial in retaining gloss and moisture of the paste. Low-grade or crude sugars also contribute to flavor. Molasses are used for the same reasons. Corn syrups are used in red licorice to replace the molasses; they have the same function, ie, that of inhibiting sugar crystallization, which in turn results in a loss of gloss. High dextrose or high fructose corn syrups are generally preferred.

Gelatin

Gelatin is sometimes necessary as a binder and also retards moisture loss. It contributes to gloss and inhibits cracking. Low-bloom quality is used at levels between 0 and 5%.

Flavor and Color

Flavors include (for black licorice) licorice extract, salt, molasses, and oil of anise, and (for red product) fruit flavors and acids. Color is contributed by licorice extract, molasses, caramel, and black (or red) dyes.

BATCH PROCESS MANUFACTURE

Premix

With the exception of flavors and acid, most of the ingredients mentioned in the above formulas are mixed in a slurry premixer. The practice of preparing a thick paste of flour with a little water in the cooker and then converting the paste into a creamlike slurry by gradually adding more water is not very satisfactory. It is slow, and small lumps of dry or damp flour often remain in the slurry. These not only spoil the finished paste, but also provide a lot of trouble in the extrusion process by blocking nozzles. An emulsifier or slurry mixer will prevent these shortcomings and produce a smooth, homogenous slurry. The correct quantity of water in relationship to flour and sugars is of paramount importance and is often neglected. Short textured licorice requires less water (30–32%) than long texture, which can go as high as 47%.

Sugars inhibit the swelling of starch in a water system and thus retard the gelatinization of starch. This factor can be used to advantage by adding all or part of the sugars at the premix stage and by adding the balance at some later stage of the cooking process.

Cook

Conventionally, licorice paste is manufactured in 1/2 to 1 ton batches in open steam-jacketed kettles. The mixing paddles must efficiently sweep the heated paste from the kettle walls as the paste has poor heat-transfer properties. Cooking times can vary from 60 to 220 minutes. Steam pressures around 60 psi are adequate.

Licorice paste has many unusual rheologic properties resulting in large variations in observed elasticity. These largely occur through variations in starch gelatinization. Factors that influence the degree of gelatinization are quantity of flour, quality of flour, batch boiling time, concentration of sugars, and available water.

The progress of gelatinization in the batch can be followed microscopically by alteration in appearance of starch granules. As boiling continues, the granules swell and eventually rupture. (This change can also be clearly seen under polarized light when the characteristic cross on the surface of the granules disappears when the granule rupture occurs.) Count lines, shoelaces, reels, etc (long textured product), will show starch that is fully swollen or ruptured whereas sandwich paste and centers for Good & Plenty® (short texture) should show only partial gelatinization of starch.

The gloss of licorice is dependent primarily on moisture content which typically in the final product is 16–20%. Too high moisture can lead to mold growth, so sometimes preservatives are added. At the end of the cooking process, moisture is reduced to 23–32%. After cooking, the licorice paste is partially cooled then extruded or stamped into its final shape and placed on trays in racks, after which it goes into a warm, dry room known as a stove where more moisture is removed down to its final moisture content. The time of stoving will vary depending on the thickness and shape of the final piece and the amount of moisture to be removed. If this process is rushed, a hard crust will form on the surface of the licorice, which inhibits the moisture loss from the center of the product. When the product is dried in this manner, the moisture from the center will ultimately migrate to the surface and cause it to be sticky. Good air circulation within the stove is essential. Stove temperatures should be 100–125°F, with relative humidities of 50–60%.

Figure 2 outlines the various steps in both the batch and continuous processes, along with an indication of the relative process times. A semicontinuous final moisture process is also included for comparative purposes.

CONTINUOUS PROCESS MANUFACTURE

Slurry to be cooked in a continuous mode, whether heated by steam injection or steam jacketing, must contain less flour and consequently more sugars than normally used for the batch process. This formula change offsets the higher cooking temperatures and tends to repress gelatinization. Because little or no evaporation takes place, the moisture content before and after cooking is substantially the same; therefore, the water used in the slurry must be reduced.

As is seen from Figure 2, the advantages of the semicontinuous and continuous processes are shorter process time, less floor space, reduced labor, and reduced amount of material in process. One negative is that it is difficult to get the same texture from a continuous process compared to the batch.

DOMESTIC AND FOREIGN STANDARDS AND REGULATIONS

- Standards and regulations given here are as of April 1989 as reported by the British Food Manufacturing Industries Research Association.
- *United States*. The standard for licorice as an ingredient for foodstuffs is covered by Title 21, part 184.1408, of the *Code of Federal Regulations*. Specific limitations (based on glycyrrhizin content) for the use of licorice in certain foodstuffs are laid down.
- *Argentina*. The Food Code lays down standards for licorice confectionery, namely licorice pastilles. These are pastilles (containing sugar, glucose, flavored distilled water, permitted natural or synthetic essences and colors, edible gums, and gelatin with or without maximum 5% starch or dextrin) to which at least 4% licorice extract or juice has been added. Modified or unmodified starches may be used in necessary proportions when gums or gelatin have not been used.
- *Austria*. Regulations on sugar products state that licorice is manufactured from a mixture of at least 5% sweet licorice extract or a corresponding quantity of dry extract with sugar, flour, and also the use of authorized gelling agents (as given in the 1988 Emulsifiers Regulations). The use of colors in licorice confectionery is prohibited.
- *Belgium, Denmark, Finland, Greece, Norway, and Sweden*. These countries have no standards of composition for licorice confectionery, although the use of additives in confectionery is controlled by the national regulations of each of the countries. Licorice confectionery should be of a safe and suitable composition such that it is not harmful to the consumer, and the label should not mislead the consumer as to the nature of the product.
- *Bolivia, and Dominican Republic*. Licorice is permitted as a color for foodstuffs unless otherwise restricted or prohibited by regulations for a specific type of food.
- *France*. The French standards are a little confusing, but appear to be broken down into three categories: pure licorice, licorice, and starched licorice. (1) The name licorice, with or without the name pure, is reserved for the product obtained by extraction of all or part of the soluble substances contained in the licorice root and containing no more than 15% water.

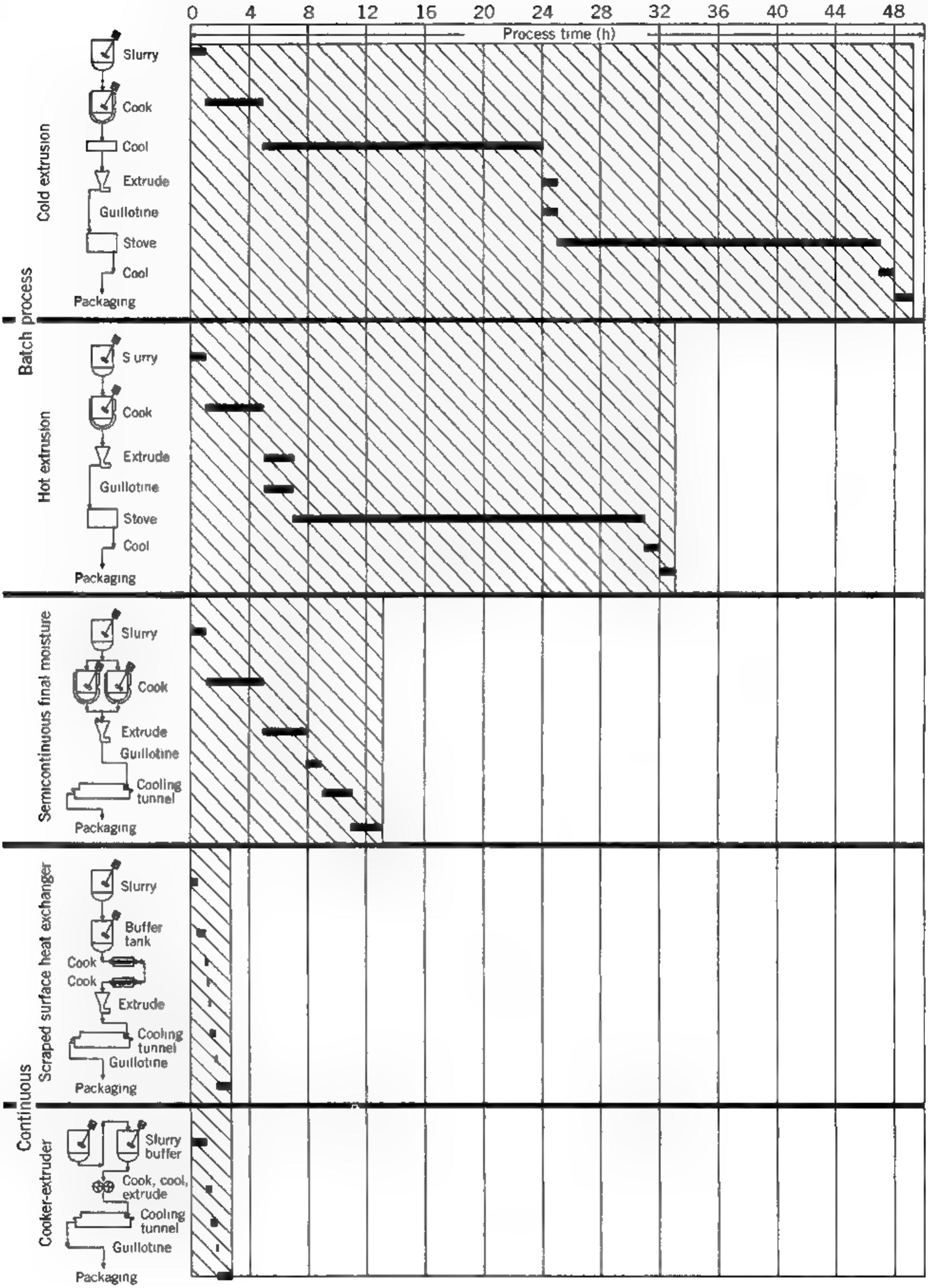


Figure 2. The various steps in both batch and continuous processes

This product can be qualified as pure. (2) It is permissible for the addition of edible sweetened matter or gum, as long as the product still contains 6% glycyrrhizin; however, this product cannot be labeled pure. (3) Starched licorice (the most common classification) permits the addition of edible sweetened matter, gum, starch matter, and dextrin. This category must contain at least 1.5% glycyrrhizin.

- *Italy*. There is no standard of composition laid down for licorice. However, the use of additives in confectionery products (which one assumes includes licorice) is covered by the additives list.
- *Netherlands, Ireland, and United Kingdom*. There are no standards of composition on licorice confectionery. Products that are made to a standard recipe and fulfill consumer expectation of licorice should be acceptable.
- *Peru*. Substances based on licorice root are permitted as foaming agents for food use.
- *Spain*. Spain has no specific regulation or compositional standards on licorice as such; however, the list of additives permitted for confectionery products covers licorice.
- *Switzerland*. There is no standard of composition laid down for licorice, but there are regulations on confectionery and bakery products. The use of additives in licorice and confectionery are covered by the additives list.
- *Germany*. The guidelines are quite lengthy but can be simply stated as any product labeled as licorice must contain a minimum of 5% licorice juice in the dried form as customarily traded. Interpretation of the dried form probably means the powder form; however, if one takes the as customarily traded statement into consideration it could include block licorice. Although the guidelines do not have the force of law, they are an indication of trade and consumer expectation and should be complied with.

Although this article has described the black-colored licorice-flavored candy product, a similar textured product containing fruit flavors and pastel colors is made and sold throughout the world. In the United States, this particular type of product (red-colored and strawberry or cherry flavored) outsells the black variety by about 10 to 1.

J. Frank Smullen
Hershey Foods Corporation
Hershey, Pennsylvania

LIMONIN

One of the most characteristic flavor attributes associated with grapefruit (*Citrus paradisi* Macf) juice is bitterness. This is a quality factor that, present in a moderate degree, is responsible for much of the bite or zestiness of the juice. This bite or zestiness is expected and appreciated by many grapefruit juice consumers. However, if present at too high a level, excess bitterness will result in low approval ratings from even people who like grapefruit juice. Bitterness is also one of the chief reasons why most consumers dislike the overall flavor of grapefruit juice (1–6), with only about 29% of all U.S. households purchasing grapefruit juice in 1983 (7). The two major constituents in grapefruit juice responsible for bitterness are limonin and naringin. Of the two, limonin is considered to be the more significant contributor. This may be because bitterness due to limonin is perceived sooner and lingers longer than bitterness from naringin (8).

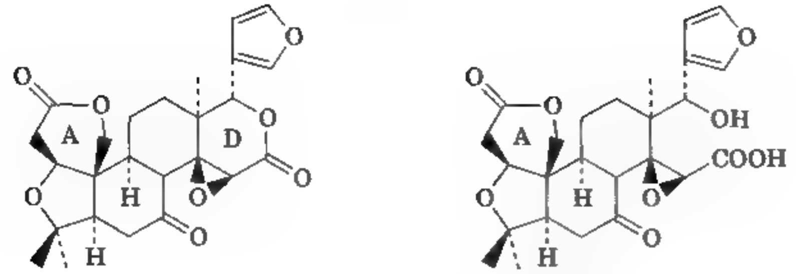
HISTORY OF LIMONIN IN CITRUS JUICES AND ITS DETERMINATION

Limonin was first reported (9) in 1938 as the cause of bitterness in Washington navel orange juice. It was not until 1961 that the complex chemical structure of limonin was finally worked out (10) and 1965 when Maier and Dryer (11) first reported the presence of limonin in commercial grapefruit juice. Limonin is a highly oxygenated triterpene derivative whose structural features include a furan ring, two lactone rings, a five-member ether ring, and an epoxide (Figure 1). Freshly squeezed citrus juices do not contain limonin. After extraction, limonin forms slowly over a period of several hours from a nonbitter precursor present in the fruit due to enzyme activity (12). Limonin forms rapidly if the juice is heated. The limonin precursor is limonoic acid A-ring lactone; being formed by closure of the D-ring as shown in Figure 1. An in-depth review of limonin and related structures has been presented by Maier et al. (13).

When Maier and Dryer (11) first discovered the presence of limonin in commercial grapefruit juice, thin-layer chromatography (TLC) was used. In 1970 Maier and Grant (14) developed a specific TLC assay for limonin. Scott (15), using an adaptation of their assay, reported limonin in many Florida citrus cultivars, including grapefruit. Tatum and Berry (16) in 1973 developed a quicker TLC method, which was adopted commercially.

Limonin today is generally assayed by high-performance liquid chromatography (HPLC), and a number of methods have been published. The real challenge with limonin determination by HPLC is one of detection. Although limonin is a major contributor to bitterness in citrus juices, it is only present at the low part per million level. Limonin does not contain a good UV absorbing chromophore, does not fluoresce, and is not easily reduced or oxidized using electrochemical detection. This limits LC methods to refractive index or UV detection at the lower wavelengths unless limonin is derivatized to enhance UV absorbance or fluorescence.

One of the first HPLC methods was developed by Fisher (17) and utilized refractive index detection. Subsequent HPLC methods (18–21) have utilized the more sensitive detection method of UV absorbance at lower wavelengths of 205 to 215 nm. Because limonin occurs in citrus juices in the low part per million range and most substances absorb in the UV below 220 nm, preparation of the sample



Limonin

Limonoic acid A-ring lactone

Figure 1. Structures of limonin and limonoic acid A-ring lactone, the precursor to limonin.

to obtain an extract free of interferences upon analysis is demanding. Rouseff and Fisher (18) developed an accurate method using chloroform extraction to isolate limonin. Analysis with a cyano column by normal phase chromatography with hexane:isopropanol as the mobile phase and UV detection at 214 nm produced excellent results. Analysis of the same chloroform extracts under reversed phase conditions with acetonitrile:water on a cyano column resulted in a good separation but short analytical column life.

A number of reversed phase chromotography methods that utilize the more robust octyl (C8) and octydecyl (C18) analytical columns have also been published. Sample cleanup to remove interfering components was accomplished by solid phase extraction (19–21). Solid phase extraction (SPE) offers several advantages over solvent extraction. It is less time and labor intensive. Less solvent is required for preparation of each sample. Reversed phase chromatography is also generally preferred over normal phase chromatography as water comprises most of the mobile phase solvent and can be produced relatively inexpensively. For limonin analysis, one problem with methods that utilize C8 and C18 reversed phase columns is that the mobile phase conditions must be changed depending on the type of citrus juice being analyzed. Some grapefruit juice samples require different analysis conditions than those that provide a good separation of orange juice extracts. The mobile phases required also generally consist of mixtures of methanol:acetonitrile:water or methanol:tetrahydrofuran:water. Mobile phases containing methanol:acetonitrile:water give low broad peaks reducing detectability. Tetrahydrofuran tends to form epoxides that absorb strongly in the low UV requiring diligent use of fresh solvents.

In 1991 Widmer (22) developed a general citrus juice analysis method with sample preparation by SPE, analysis on a cyano column under reversed phase conditions using acetonitrile:water, and detection by UV at 214 nm. Unlike chloroform extracts of citrus juices, the SPE extract preparation was optimized so integrity of the cyano analytical column under reverse phase conditions was maintained, resulting in less cost per sample analysis. Limonin recovery using SPE from spiked grapefruit and model juices ranged from 95 to 108%. Results from 25 samples analyzed by the new method and the method of Rouseff and Fisher (18) also showed good agreement between the two methods with differences in the range of 5 to 13%. The method has since been modified for analysis of citrus juices and citrus peel extracts by direct injection with an in-line automated sample cleanup (23). Both limonin and naringin are determined simultaneously with an analysis time of 20 min. Sample preparation for citrus juice requires only heating and (1:1) dilution with 40% aqueous acetonitrile. Preparation of peel extracts is accomplished by sonication of the peel in aqueous acidified acetonitrile.

Other novel methods for limonin analysis that have been developed include the rapid radioimmunoassay (RIA) developed by Mansell and Weiler (24) and the enzyme linked immunoassay (EIA) developed by Jourdan et al. (25). The EIA method was commercialized and available as a rapid test kit (26) for a number of years. Carter and coworkers (27) in 1985 compared the RIA and EIA methods to HPLC methods available at the time. Samples analyzed by EIA tended to have limonin values slightly higher than those analyzed by HPLC. Widmer and Rouseff (28) reported on a collaborative study done to assess the commercial EIA method. Problems with reproducibility in citrus quality assurance labs (private communications), sample analysis costs that were higher than anticipated, and subsequent development of rapid and reliable HPLC methods likely resulted in the loss of interest by the citrus industry for the commercial EIA method.

TASTE THRESHOLD LEVELS

Guadagni et al. (29) reported a threshold of 1 μg limonin/mL distilled water, while for naringin the threshold was 20 μg/mL. In orange juice they found the limonin threshold was dependent on juice pH. At a juice pH of 3.2 to 3.5 the limonin threshold was approximately 3.5 μg/g. The threshold increased to 6.5 μg/g at pH 3.8 and then decreased to about 4 to 4.5 μg/g at a juice pH greater than 4. This suggests natural orange juice components are effective in neutralizing or masking limonin bitterness to a certain degree and are pH dependent. Among the 27 tasters used in the study, the most sensitive was able to detect limonin at 0.5 μg/g in orange juice and the least sensitive required 32 μg/g. This range illustrates the wide variation in individual palates to limonin bitterness. Similar results were found by Widmer (unpublished data) in panel tests for limonin bitterness using model juice solutions containing 8% sucrose and 0.7% citric acid. Sensitivity to limonin ranged from 1 μg/g to greater than 16 μg/g limonin between individuals.

Limonin sensitivity in grapefruit juice is difficult to measure due to the presence of naringin and other bitter components. Work by Guadagni et al. (30), however, has shown that the relative bitterness contributions of limonin and naringin are additive.

REGULATORY ASPECTS FOR LIMONIN IN GRAPEFRUIT JUICE

Until recently there were no federal regulations for limonin content of grapefruit juice. Regulations issued by the state of Florida Department of Citrus (31) were in effect for many years to ensure the quality of juice produced in Florida. Recent changes in federal regulations, however, prohibit regulations at the state level more strict than federal regulations. Both grapefruit juice not from concentrate and grapefruit juice from concentrate products are covered under a federal standard of identity (32) with no mention of a maximum amount of limonin that may be present. Therefore, these may not be regulated at the state level for limonin content. Grapefruit juice concentrate does not have a federal standard of identity and therefore may still be regulated at the state level for limonin and naringin content. The Florida regulations (31) regarding limonin content in frozen concentrated grapefruit juice specify:

> For the period August 1 to December 1 of each season: (a) Grade "A" finished product shall meet at least one of the following requirements: (1) contains less than 5.0 ppm [5.0 μg/mL] limonin, measured by high pressure liquid chromatography, or (2) contains less than 600 ppm [600 μg/mL] naringin, measured by the Davis Test. (b) Grade "B" finished products shall meet at least one of the following requirements: (1) contains less than 7.0 ppm [7.0 μg/mL] limonin, measured by high pressure liquid chromatography, or (2) contains less than 750 ppm [750 μg/mL] naringin, measured by the Davis Test. (c) All products failing to meet the permissible limits of subsection (b) for Grade "B" product, shall be labeled Substandard Frozen Concentrated Grapefruit Juice, and may be held in appropriately marked bulk containers for subsequent blending into Frozen Concentrated Grapefruit Juice for Manufacturing only. (d) Maximum naringin and limonin requirements set forth in subsections (a) and (b) shall not apply: (1) to finished product produced solely from concentrate processed between December 2 and July 31, or (2) to finished product produced from concentrate or bulk single strength juice processed between December 2 and July 31, provided any raw juice blended therewith meets the permissible limits of naringin or limonin for Grade "A" product respectively.

LIMONIN CONTENT OF GRAPEFRUIT JUICE

Results of studies in which limonin levels in fresh grapefruit juice are reported follow. In an early study, Scott (15) found from 1 to 5 μg/mL limonin in juice from Marsh and Duncan grapefruit harvested in Florida at maturity during the 1967, 1969, and 1970 seasons; a TLC method was used. Mansell and McIntosh (33), using the RIA method, reported limonin contents of fresh grapefruit juice samples derived from Marsh, Duncan, and Ruby Red cultivars harvested from mid-December 1979 to early March 1980 and juiced at three state test houses in Florida. A mean limonin content of 6.76 μg/mL was found for 1058 samples analyzed, with a range of 1.35 to 13.8.

Ting and McAllister (4), using HPLC, determined the limonin contents for 156 samples of Florida-packed, canned grapefruit juice obtained from U.S. supermarkets. The overall mean was 5.6 μg/mL with a range of 1.5 to 10.9. Dougherty and Fisher (34) reported limonin contents (HPLC) for canned single-strength grapefruit juices obtained from surveys of Florida production during two seasons. For the 1975–1976 season the overall mean for 117 samples was 3.0 μg/mL, range 0.3 to 7.4. For the 1976–1977 season the overall mean for 99 samples was 4.7 μg/mL, range 0.6 to 12.8. Results by EIA for the 1985–1986 (87 samples) and 1986–1987 (61 samples) seasons showed means and ranges of 9.4 μg/mL, 2.8 to 18.9 and 9.4 μg/mL, 2.8 to 16.6, respectively (Barros, unpublished data). Grapefruit juice values in more recent years have shown this same type of variation for limonin content.

EFFECT OF SEVERAL FACTORS ON LIMONIN CONTENT OF GRAPEFRUIT JUICE

Mansell and McIntosh (33) reported limonin contents (EIA) for a large number of samples of the major Florida grapefruit cultivars. Samples from three Florida state test houses collected from mid-December 1979 through early March 1980 revealed the following limonin concentrations in the cultivars (mean and range in μg/mL): Duncan—394 samples, 6.03, 1.35 to 12.10; Marsh—537 samples, 7.15, 1.78 to 13.80; pink seedless—127 samples, 7.47, 2.41 to 12.00. The mean limonin content for Duncan was significantly lower ($P < 0.05$) than that for the Marsh or pink seedless.

In the same study, limonin contents were also reported for the three different test houses utilized (overall mean and range in μg/mL): test house 1—344 samples, 7.52, 2.38 to 12.30; test house 2—488 samples, 6.09, 1.35 to 12.30; test house 3—272 samples, 7.05, 1.78 to 13.80. The means for the three test houses differed significantly at the 95% confidence level.

Maturity

Generally, as grapefruit mature through the season the limonin content decreases. Tatum et al. (35) used a TLC method to analyze samples at about weekly intervals from two Florida plants from late November 1971, when the limonin level was 10 μg/mL, through May 1972, when the level had decreased to 2 μg/mL. The decline in limonin was gradual over the study period. Dougherty et al. (36), working with Marsh grapefruit from Florida packinghouse eliminations (fruit that was not suitable for marketing fresh, primarily because of skin blemishes, but was of processing quality), reported limonin contents by HPLC of juice from five separate batches of fruit harvested between September 12 and December 17, 1974, at irregular intervals. Using the mean limonin levels for the juice obtained with soft and hard extractor settings (to produce soft and hard squeezes) for each batch of fruit, the limonin content was found to decline gradually from 7.5 μg/mL in the first juice extracted to 2.5 μg/mL in the last. A gradual decrease in limonin content was again noted over the time period.

Until late 1989 it was generally believed that decreasing limonin as fruit matured was the result of transport of limonin from the fruit to developing seeds. Grapefruit seeds contain very high concentrations of limonin (37). However, Hasagawa et al. (38) discovered an enzyme that bound the limonin as a nonbitter glucoside as the fruit matured. Early season grapefruit contain few limonoid glucosides while mature grapefruit contain approximately 240 μg/g limonoid glucosides on average.

Extractor Pressure and Extractor Types

Dougherty et al. (36) also showed that there was an average 65% increase (range, 17–167%) in limonin content of juice obtained using a soft versus hard squeeze using an FMC Corp. (Lakeland, Fla.) extractor. This was based on values obtained from five batches of Marsh grapefruit harvested between September 12–17, 1974, and processed at both soft and hard squeeze settings. Similar results were obtained, but not presented, for the Duncan cultivar. In the 1975–1976 season, the same authors used FMC Corp. and Automatic Machinery Corp. (Winter Haven, Fla.) extractors to produce juice from three batches with each machine of Florida grapefruit at about monthly intervals beginning in October for both Marsh and Duncan cultivars. The data again showed more limonin extracted with the hard squeeze than with the soft squeeze and for both extractor types. In addition, one extractor produced an overall average of 34% (ranging from 6 to 63%) more limonin than did the other extractor for the same batches of fruit.

These results are not surprising as limonin is most prevalent in the seeds at levels in excess of 0.5% wet weight (37). Limonoic A-ring lactone, the nonbitter precursor to limonin occurs at highest concentrations in the fruit core, followed by the segment membranes, and peel (39). Any process that tends to crack the seeds or cause more peel and pulp constituents to be incorporated into the juice product will result in higher limonin values. Grapefruit core and segment membranes contain 500 to 1500 μg/g limonin; and the peel, 12 to 234 μg/g (39).

Season

Dougherty and Fisher (34) presented data showing season-to-season variation in limonin contents (HPLC) for Florida-canned, single-strength grapefruit juice. The mean of 117 samples from the 1975–1976 season with 3.9 μg/mL limonin while that for 99 samples from the 1976–1977 season was 4.7 μg/mL. During the latter season there was a freeze during mid-January, and the authors speculated that the freeze caused the peel of the fruit to break down, making limonin in the peel more available for extraction.

EFFECT OF LIMONIN IN GRAPEFRUIT JUICE ON SENSORY FLAVOR QUALITY

Studies with Small Experienced Taste Panels

Between September 12 and December 17, 1974, five batches of Florida Marsh grapefruit were harvested on an irregular basis (36). From each batch of fruit, packinghouse eliminations were obtained for quality evaluation. Juices were extracted using FMC equipment set for soft, medium, hard, and state test house pressure squeezes. Values for the four extraction pressures were averaged for limonin (HPLC) and flavor (nine-category hedonic scale as judged by 10–12 experienced panelists). During the period of the study, flavor increased from 5.0 (neither like nor dislike) to 5.9 (like slightly) while limonin decreased from 7.0 to 2.3 μg/mL. Naringin (by the Davis test) remained about the same, ranging from 600 to 700 μg/mL. The correlation between flavor and limonin was significant at the 99.9% confidence level ($r = -0.91$).

Flavor and several physical and chemical quality characteristics, including limonin content, have been determined in commercial Florida-produced canned and chilled single-strength grapefruit juices in a survey by the Citrus Research and Education Center, Lake Alfred, Florida, during each citrus season starting with 1973–1974. Samples were collected twice monthly from processing plants (14 initially; 7 during the 1986–1987 season). The number of samples collected annually varied between 61 and 168. Flavor was determined using 10 to 12 panelists judging samples on a nine-point hedonic scale. Details of the study and results covering two seasons' data were presented by Dougherty and Fisher (34). Over the first 10 seasons (1973–1974 to 1982–1983) in which the study was under way, limonin concentration (HPLC) in the juice was highly correlated with flavor at the 95% or better confidence level in 8 of the seasons (Barros and Dougherty, unpublished data). The best correlation coefficient was achieved for the 1978–1979 season for 113 samples from 11 plants ($r = -0.53$), explaining almost 28% of the variation in flavor due to limonin. Four individual plants that season showed significant correlations ($P < 0.05$) between flavor and limonin. The two seasons showing no significant correlations were the 1974–1975 and 1976–1977 seasons.

EIA and RIA procedures were used at the Citrus Research and Education Center to measure limonin concentration in juices for the 1983–1984 and 1984–1985 seasons (Barros, unpublished data). There were low but significant correlation coefficients ($P < 0.05$) for limonin content (both EIA and RIA) and flavor score for 1983–1984. For the 1984–1985 season, $r = -0.01$ for EIA limonin versus flavor was not significant ($P > 0.05$), while $r = -0.27$ for RIA limonin versus flavor was at the 99% level. It would have been interesting to have information to calculate r for HPLC limonin versus flavor. One plant showed significant correlation ($P < 0.05$) between limonin concentration and flavor for no fewer than 7 of the 12 study seasons (1973–1974 through 1984–1985); 5 seasons had limonin analysis by HPLC, 1 season with EIA and RIA, and 1 season with RIA methodology. On the other hand, another plant showed no significant correlation ($P > 0.05$) between limonin (HPLC) and flavor for the 10 years the plant was included in the study. Every other plant showed significant correlation ($P < 0.05$) between limonin (HPLC from 1973–1974 to 1982–1983; EIA and RIA the last 2 seasons) and flavor for 1 or more years. However, no significant correlations ($P > 0.05$) were noted between limonin (HPLC) and flavor for any of the 12 plants surveyed during the 1974–1975 season.

The two most recent years of the study, using only EIA methodology, showed highly significant correlations ($P < 0.01$) between limonin and flavor: for 135 samples during 1985–1986, $r = -0.28$; for 61 samples during 1986–1987, $r = -0.40$.

Fellers and Ting (unpublished data) surveyed the U.S. retail market to determine quality of three types of Florida grapefruit juice. There were 10 frozen concentrated grapefruit juices, 11 grapefruit juices from concentrates (the USDA term for grapefruit juices reconstituted from concentrates) and 14 canned (direct) grapefruit juices. Flavor was determined by 12-member experienced panels grading samples on a nine-point hedonic scale. Among the several chemical and physical analyses run on the juices was limonin by HPLC. No significant correlation ($P > 0.05$) was found between flavor score and limonin for any of the product types or for all samples combined ($r = -0.18$). The correlation coefficients were negative for the canned and reconstituted products, but positive for the concentrate. As has already been discussed, it is well established that one of the reasons grapefruit juice users like grapefruit juice is because of its zestiness due at least in part to bitterness. The limonin contents of the 10 concentrates were quite low with a mean of 3.0 μg/mL (range 1.3–7.5), apparently a concentration viewed more or less favorably by the taste panelists. The mean limonin contents for the canned and reconstituted products, respectively, were 4.2 μg/mL (range 1.3–7.5) and 5.2 μg/mL (range 2.5–10.0). The mean for the reconstituted products was significantly higher ($P < 0.05$) than the mean for the concentrates but not for the canned juices. It should also be pointed out that concentrate also has a higher Brix:acid ratio requirement than does the canned or reconstituted products. The degree Brix refers to the weight percent of soluble solids (which is mostly sugar) that are present in the juice. The Brix:acid ratio is a measure of the ratio of soluble solids to total acidity present and is an indicator of fruit maturity. Generally juice with a higher Brix:acid ratio will be perceived as sweeter and is produced from fruit that is more mature.

Study Utilizing a Small Consumer Panel

Ting and McAllister (4) reported on flavor and several other quality factors, including limonin content of 156 Florida-processed canned single-strength grapefruit juice samples obtained from the U.S. retail market. Juices were judged by 12 to 14 consumers (mostly housewives) from the Winter Haven, Florida area. Samples were graded on a nine-point hedonic scale. Limonin content (HPLC) correlated negatively and significantly with flavor ($r = -0.166$; $P < 0.05$). The mean limonin content for all samples was 5.6 μg/mL. Panelist's comments of bitterness were significantly correlated with limonin content ($r = 0.233$; $P < 0.01$) and with flavor ($r = -0.434$; $P < 0.01$). The average number of bitterness comments by the panel per sample was 2.19, the largest number for any unfavorable type of comment. Age of the sample from the date of packing also correlated significantly with limonin content ($r = -0.233$; $P < 0.01$).

Large Consumer Panel Studies

In a study by Fellers et al. (40), 30 Florida-processed grapefruit juices were obtained from the U.S. retail market during 1983 for flavor and chemical and physical analyses of quality factors, including limonin by the EIA method. Six juices of each of five types were used: frozen concentrated, grapefruit juice from concentrate packed in glass, cartons, and cans and grapefruit juice (canned directly from freshly harvested fruit). Ten products were evaluated by grapefruit juice users at each of three metropolitan test locations in the United States. Two of the five juice types were tested at each location. Each sample was tested by 108 consumers with each testing no more than two samples. Consumers evaluated samples on a nine-point hedonic scale (1 = dislike extremely, 9 = like extremely) for overall flavor and on a five-point scale (−2 = not at all enough; 0 = just right; +2 = much too much) for the attributes of sweetness, tartness, bitterness, aroma, and color.

Correlation analysis revealed no significant association ($P > 0.05$) between overall flavor and limonin content (EIA). However, the bitterness attributed, overall and for each product type, was somewhat too much ($P < 0.05$) and correlated significantly ($P < 0.01$) with limonin concentration.

A serious problem apparently arose from the inability of consumers to differentiate bitterness from tartness. There was a significant positive correlation between tartness and bitterness ($r = -0.66$; $P < 0.01$) as judged by the consumers and negative correlation between sweetness and bitterness ($r = 0.55$; $P < 0.01$); in addition, tartness correlated directly ($P < 0.05$) with both limonin and naringin content. However, bitterness, as measured by the consumer, also correlated directly at the 99% confidence level with limonin and naringin content.

In another study (6), the effect on flavor of limonin addition to grapefruit juice was determined. Using a single commercial Florida frozen concentrate, limonin levels (HPLC) of 2.3, 7.1, 8.8, 9.2, and 11.0 μg/mL were obtained in the respective reconstituted juices at 10.0° Brix with a Brix:acid ratio of 8.5. The target limonin levels were 2, 5, 7, 10, and 15 μg/mL. A stock solution of limonin in distilled water (obtained by slow addition of limonin to boiling water) was used to spike the juice. Both grapefruit juice users and nonusers at shopping malls in three large U.S. cities participated in the large consumer study. An overall flavor score was obtained on each product using a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely); a five-point scale (−2 = not at all enough; 0 = just right; +2 = much too much) was used to rate bitterness, sweetness, tartness, aroma, and color; finally, after each panelist had finished rating their two samples, they were asked which of the two samples was preferred. $P < 0.05$ was chosen as the level for significance.

Overall flavor means for all panelists (users and nonusers) varied within an acceptable range from the low end of the like moderately flavor category to the top end of the like slightly category (Table 1). The product having the highest limonin level (11.0 μg/mL) was assessed as having a flavor significantly inferior to the two products having

Table 1. Mean Flavor Score Values and Preference Ratings by Consumer Panels for Grapefruit Juice with Varying Limonin Contents

	Limonin Concentration (μg/mL by HPLC)				
Consumers	2.3	7.1	8.8	9.2	11.0
			Overall flavor score[a]		
Nonuser[b]	6.49[c]	6.33[c,d]	6.26[c,d]	6.21[c,d]	6.08[d]
Users[b]	7.07[c,d]	7.20[c]	6.97[c,d]	7.04[c,d]	6.91[d]
All Panelists[b]	6.77[c]	6.76[c]	6.62[c,d]	6.62[c,d]	6.49[d]
			Preference ration (%)		
Nonusers[b]	58[c]	50[c]	52[c]	44[d]	32[e]
Users[b]	51[c]	46[c]	51[c]	44[c]	36[d]
All Panelists[b]	55[c]	48[d]	51[c]	44[d]	34[e]

Source: Ref. 6.

[a] Based on a nine-point hedonic scale: 1 = dislike extremely, 9 = like extremely.

[b] Means in the same row sharing the same letter c, d, e, are not significantly different at the 95% confidence level.

the smallest amounts of limonin, but not inferior to the products with 9.2 or 8.8 μg/mL. There was no significant difference between the four products having the lowest limonin levels.

Significant flavor differences existed between users and nonusers for every product, the users in each case rating the product higher. Within the nonuser group there was a steady downward trend in flavor scores with increasing limonin concentration. Significant differences existed between the products having the lowest and highest limonin levels, with no significant difference noted between the products having from 7.1 to 9.2 μg/mL limonin. Among users, the highest rated product (7.2 flavor score) was the sample with the second lowest limonin levels (7.1 μg/mL). The only significant difference in flavor scores among users was between this highest rated product having 7.1 μg/mL limonin and the product having the most limonin (11.0 μg/mL) and the lowest score (6.91).

The data in Table 1 also show the panelists' preference measurements. For all panelists the sample with the least limonin (2.3 μg/mL) was significantly preferred over samples having 7.1, 9.2, and 11.0 μg/mL, but not the sample with 8.8 μg/mL limonin. The sample with the most limonin (11.0 μg/mL) was significantly least preferred of all.

When preference measurements by users were compared with those by nonusers, there was no difference in their preference for each product. Within the nonuser group the most highly preferred product had the least limonin and was significantly preferred to the products having the two highest limonin levels. The least preferred product had the highest limonin level and had only a 32% preference, 45% less than the most preferred product (Table 1). Among users, the product with the highest amount of limonin was again rated significantly lower than all other products. No significant difference in preference was noted among users between the samples having the lowest limonin contents. Apparently, a clear-cut consensus existed among both users and nonusers of grapefruit juice that grapefruit juice having ~11.0 μg/mL limonin (HPLC) was less preferred to grapefruit juice containing less limonin.

For those specific attributes of sweetness, tartness, and bitterness that were also rated (Table 2), there was a general tendency for the level of sweetness perceived by consumers to decrease with increasing limonin concentration in the juice. Simultaneously, the perception of tartness and bitterness intensity increased with increasing limonin content. Because the only differences between the five samples was limonin content, bitterness was perceived as tartness or reduced sweetness to a measurable degree, a phenomenon also noted in a study described earlier (40). All five samples were judged by consumers as being somewhat not sweet enough and somewhat too bitter and tart.

Correlation analysis revealed a significant negative correlation ($r = -0.973$; $P < 0.01$) between overall flavor score and limonin level. Differences in limonin levels explained over 94% of the variation in flavor. In addition, overall flavor score correlated significantly with two of the quality attributes judged by the consumers: directly with sweetness ($r = 0.852$; $P < 0.01$) and negatively with bitterness ($r = -0.962$; $P < 0.01$). Tartness did not quite make the 95% confidence level.

INTERACTION BETWEEN BRIX:ACID RATIO AND LIMONIN CONTENT IN AFFECTING SENSORY FLAVOR OF GRAPEFRUIT JUICE

There have been two studies showing that the Brix:acid ratio affects the perception of bitterness caused chiefly by limonin and naringin in grapefruit juice. Tatum et al. (35) studied seasonal effects on the flavor perception of limonin and naringin in grapefruit juice. Results from paired comparison taste panels led the authors to observe "when both naringin and limonin were higher and Brix:acid was higher, it was always judged least bitter." They concluded that the results suggest that "in many cases the Brix:acid ratio may be more important than naringin and limonin content in determining quality of grapefruit juice."

In the second study (Fellers and Carter, unpublished data), an experiment was done to determine the effect of

Table 2. Means and Comparison of Means for Selected Quality Attributes, as Evaluated by Consumers, of Grapefruit Juice Having Varying Limonin Contents

	Limonin Concentration (μg/mL by HPLC)				
Attributes	2.3	7.1	8.8	9.2	11.0
Sweetness	0.28^{a}	$-0.41^{a,b}$	$0.37^{a,b}$	$-0.36^{a,b}$	-0.47^{b}
Tartness	0.19^{a}	$0.30^{a,b}$	$0.29^{a,b}$	0.19^{a}	0.42^{b}
Bitterness	0.32^{a}	$0.38^{a,b}$	$0.39^{a,b}$	$0.41^{a,b}$	0.51^{b}

Source: Ref. 6.

Note: Each mean value based on about 300 judgments by consumers using a five-point scale: -2 = not at all enough of the attribute; 0 = just right; $+2$ = much too much of the attribute.

[a,b]Mean values in the same row sharing the same letter are not significantly different at the 95% confidence level

Brix:acid ratio on the flavor of grapefruit juice varying in the amount of added grapefruit pulpwash containing high levels of limonin and naringin. The three Brix:acid ratios were 8.0, 9.5, and 11.0. Approximate limonin (HPLC) and naringin (Davis test) contents were, respectively, for each of the pulpwash levels: 0% pulpwash, 2.5, 660 μg/mL; 7% pulpwash, 10.0, 1010 μg/mL; 14% pulpwash, 14.4, 1280 μg/mL. For each pulpwash level, 72 panelists drawn from USDA personnel in Washington, D.C., judged samples having each of the three ratios. The mean hedonic scores for the three Brix:acid ratios, when averaged across the three pulpwash levers, were, respectively, 6.04, 6.20, and 6.69. Analysis of variance indicated that the Brix:acid ratio had a significant effect on taste preferences. The effect of Brix:acid ratio at 11.0 was found to be significantly different from the effect at both 9.5 and 8.0 at the 99.9% confidence level; the effects at 8.0 and 9.5 did not significantly differ from one another ($P < 0.05$).

That the Brix:acid ratio had an effect on limonin (bitterness perception in grapefruit juice) was not totally unexpected. Guadagni et al. (29), using model systems containing sucrose, citric acid, and combinations of these constituents, increased the thresholds of limonin (and naringin) severalfold. In an orange juice, these workers were able to increase the limonin threshold from about 6.2 to 8.5 μg/g by increasing the Brix:acid ratio from 10 to 16, while keeping the pH at a constant 3.65. No tests were done using grapefruit juices.

METHODS FOR REDUCING THE LIMONIN CONTENT OF GRAPEFRUIT JUICE

To meet government regulations and customer quality specifications, juices are often blended. Orange juices are blended to produce the desired color, flavor, Brix, and Brix:acid ratio in the final product. Processed Florida grapefruit juice products are also blended to produce the desired aspects listed above and to control the level of bitterness (limonin and naringin) in the juice.

Although a great deal of research has been done in developing methods for bitterness reduction in citrus juices for more than 40 years, control of juice extraction conditions and juice blending were the only ways permissible for controlling bitterness until 1990. In response to a petition filed to allow commercial debittering of citrus juices, the Food and Drug Administration Department of Health and Human Services (FDA) sent a letter to the State of Florida Department of Citrus. In this letter the FDA withdrew previous objections to using treatments for reducing the levels of limonin and naringin in citrus juices using food-grade polymeric adsorption resins. This was provided the resin used conformed to food additive regulations, nutritional content of the juice was not altered, other organoleptic properties were not altered, and the limonin and naringin content were not lowered below those ordinarily found in a good-quality juice. There are now processors in the United States and throughout the world who utilize adsorption resins commercially to control the bitterness content in citrus juices that meet these requirements.

Early research was focused on reducing the limonin content of navel orange juice because delayed bitterness caused by excessive limonin was a real problem. Bitterness reduction really consists of two processes. Particulates must first be removed so adsorption materials are not fouled. Second is the process of bitter component adsorption or their conversion to nonbitter components. The debittering systems now commercially in use (41) for both orange and grapefruit consist of an ultrafiltration step to remove particulates, and a debittering chamber containing nonfunctionalized cross-linked hydrophobic styrene divinylbenzene polymer resin (SDVB) to adsorb limonin and naringin from the clarified serum. The oil content of the juice must also be low to prevent premature fouling of the system. After debittering, the clear serum and removed pulp and cloud particulates (or retentate) are recombined to obtain a juice with reduced bitterness. Some bitter components are still present in the final juice because the retentate portion, which comprises 10 to 20% of the whole juice, has not been treated. Natural flavor components may also be added to the treated juice, or it may then be blended with nontreated juice to obtain product with the desired levels of bitterness and other flavor characteristics.

The first commercial system in the United States was installed at Sunkist Growers, Inc., in California for the treatment of navel orange juice in 1990. Commercial systems for debittering citrus juices in Florida were available soon after (41). These systems were available through a partnership between Mitco Water Laboratories, Inc. (Winter Haven, FL), and Romicon (now owned by Koch Membrane Systems, Inc., Wilmington, MA). The two companies have since become independent. Most recently, Sepragen, Inc. (Haywood, CA), has introduced a commercial system for citrus juice debittering that utilizes a novel radial flow chamber for holding the debittering adsorption resin allowing for improved juice flow during the debittering step (42). How advantageous this novel design will be remains to be seen as the method for citrus juice processing is still being evaluated.

The ability of SDVB adsorbent resins for debittering citrus juices was first demonstrated by Puri who received a patent in 1984 for the process (43). Limonin and naringin levels could be reduced by 90 and 80%, respectively, in grapefruit juice. In navel orange juice, limonin reduction was 85%. Vitamin C losses were negligible. Norman (44) received a patent utilizing SDVB resins that were uniquely modified with hydrophillic amine groups. These resins acted as ion-exchange resins and were considered to not meet the restrictions specified by the FDA for adsorption resins and debittering. The most recent patent for debittering citrus juices awarded in 1998 to Sepragen Corp, Inc., utilizes SDVB resins with a smaller particle size for increased efficiency (42).

The earliest attempts at debittering were conducted in the late 1950s by Pritchett (45) and Swisher (46), who used lengthy selective extraction techniques. Years later in 1968, Chandler et al. (47) successfully used polyamides to adsorb significant quantities of limonin from Washington navel orange juice. Johnson and Chandler also tried cellulose acetate gels for limonin removal in orange juice (48), and cellulose acetate, various nylon-based materials, porous polymers, and ion-exchange resins to reduce bitterness and acidity in grapefruit juice (49). Variable success was achieved with each material and with certain combinations of materials. Cellulose acetate and two of the porous polymers were found to have powerful affinity for limonin. They were not as efficient as the styrene-divinyl benzene resins now being used, however. A comprehensive article by Johnson and Chandler (50) discusses the results of the Australian program from 1968 to 1988 in the field of adsorptive removal of bitter principals (limonin and naringin) from citrus juices.

Konno et al. (51,52) used a soluble β-cyclodextrin monomer to reduce the bitter taste of limonin and naringin in the juices of grapefruit, Iyo orange, and *Citrus natsudaidai*. Using this information, Shaw et al. (53) were able to utilize insoluble β-cyclodextrin polymer to remove limonin and naringin from aqueous solution and from filtered orange and grapefruit juices, employing both batch and continuous-flow column treatments. The commercial possibilities were enhanced with the successful use of β-cyclodextrin polymer in pilot-scale experiments (54).

Barmore et al. (55) showed that Florisil (activated magnesium silicate) added to grapefruit juice acts to significantly reduce limonin, naringin, narirutin, and total acid without reducing vitamin C, sugars, or flavor. In organoleptic testing, the flavor of Florisil-treated (30–60 g/L) juice was improved significantly ($P < 0.01$).

Utilizing a different approach, Hasegawa et al. (56) utilized an enzyme, limonoate dehydrogenase (no EC number assigned) of *Arthrobacter globiformis*, to prevent the development of, or removal of, limonin bitterness in citrus juice. They went on to develop a process using immobilized *A. globiformis* cells (57) and *Corynebacterium fascians* (58) where the enzyme system in the immobilized cells actively converted 80 to 85% of the limonin to nonbitter products. Maier et al. (59) used brief treatments of fruits for 3 h with 20 μg/mL ethylene to accelerate limonoid metabolism in navel oranges, lemons, and grapefruit to reduce bitterness in the juice expressed from the fruit.

Kimball (60) successfully removed an average of 25% of the limonin from California Washington navel orange juice using carbon dioxide at pressures between 21 and 41 MPa and temperatures between 30 and 60°C for 1 h. A 60% reduction in limonin was effected using 4-h runs. Unfortunately no mention was made of the effect of the process on flavor, and apparently no other citrus juices, such as grapefruit juices, were studied. Tamaki et al. (61) demonstrated that pressure treatments alone at 195 MPa for 10 m could reduce limonin content by 35 to 55% in orange and grapefruit juices. Acidity, brix, and vitamin C content were not affected. High-pressure treatments are expensive, however.

A recent process by Van Eikeren et al. (62) utilizes liquid/liquid/liquid extraction with a thin liquid membrane film immobilized and supported by a hydrophobic polymer membrane. Limonin and naringin in citrus juice are extracted by the thin immobilized liquid, diffuse across the liquid film and into a basic stripping liquid (pH 12–13) where the limonin and naringin are ionized and cannot diffuse back across the membrane into the citrus juice. Limonin reduction was demonstrated to be 85% in orange juice with no efficiency listed for naringin. Development of this new process on a commercial scale has not yet been accomplished.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Limonin has proved to be a consistent quality factor in grapefruit juice acceptance. Apparently, for grapefruit juice users its presence in moderate amounts contributes to the overall zesty flavor that characterizes this juice and is liked by this consumer group. However, even for users, excessive amounts of limonin results in reduced acceptance. For nonusers of grapefruit juice, limonin, with its perceived bitterness-tartness, is a key factor in grapefruit juice flavor leading to low acceptance scores. Research efforts should be continued to improve ways to modulate the negative effect of bitter limonin on consumer acceptance of grapefruit juice.

Limonin levels currently are only regulated in processed Florida grapefruit concentrate by the State of Florida Department of Citrus (21) for early season fruit processed harvested prior to December 1. Limonin levels can still be rather high in December and January, thus an extension of limonin caps for one or preferably two months would help ensure against products with excessive limonin (bitterness) being packed. There would also be another advantage in that juice having other flavor problems associated with maturity in the early season and high limonin values, such as green, immature flavor that are only measured subjectively, would also be reduced.

It is likely that the public would be very receptive to an alternative to current regular grapefruit juices; namely, a natural grapefruit juice low in limonin (<3 μg/mL by HPLC) and naringin (300–350 by Davis Test), and having a natural Brix:acid ratio > 9.5. This would likely increase the market to individuals who find moderate levels of bitterness in grapefruit unacceptable. With the availability of commercial debittering capability, there is no reason why such a product could not be offered along with regular grapefruit juice. The product could carry special identification and likely a premium price. But it should serve to satisfy the taste preferences of a significant number of people who currently are nonusers of grapefruit juice. It is also likely to satisfy some established grapefruit juice users who prefer lower bitterness levels in the grapefruit juice that is available later in the season when fruit is more mature, less tart, and less bitter.

ACKNOWLEDGMENTS

This article has been adapted and updated from P. J. Fellers, "A Review of Limonin in Grapefruit (*Citrus paradisi*) Juice, Its Relationship to Flavour, and Efforts to Reduce It," *Journal of the Science of Food and Agriculture* **49**, 389–404 (1989). © 1989 Society of Chemical Industry.

BIBLIOGRAPHY

1. United States Department of Agriculture, *Consumers' Use of and Opinions About Citrus Products*, Bureau of Agricultural Economics, Division of Special Surveys, USDA, Washington, D.C., 1950.
2. United States Department of Agriculture, Market Research Report No. **243**, Agricultural Marketing Service, Market Research Division, USDA, Washington, D.C., 1958.
3. R. E. Branson, C. Price, and H. V. Courtenay, *Consumer Panel Tests of Texas Fortified Red Grapefruit Juice*, Agric and Mech Coll Texas, Texas Agricultural Experimental Station, College Station, Tex., 1961.
4. S. V. Ting and J. W. McAllister, "Quality of Florida Canned Grapefruit Juice in Supermarket Stores of the United States," *Proceedings of the Florida State Horticultural Society* **90**, 170–172 (1977).
5. S. M. Barros et al., "Interrelationships of °Brix, Brix:Acid Ratio, Naringin and Limonin and Their Effect on Flavor of Commercial, Canned, Single-Strength Grapefruit Juice," *Proceedings of the Florida State Horticultural Society* **96**, 316–318 (1983)
6. P. J. Fellers, R. D. Carter, and G. de Jager, "Influence of Limonin on Consumer Preference of Processed Grapefruit Juice," *J. Food Sci.* **52**, 714–743, 746 (1987).
7. State of Florida Department of Citrus, *Orange and Grapefruit Consumer Profile Report Dec 1, 1982–Dec 30, 1983*, Market Research Report, State of Florida, Department of Citrus, Lakeland, Fla., 1984.
8. R. L. Rouseff, W. E. Lee III, and C. A. Huefner, "Time-Intensity Studies of Citrus Bitter Compounds," in G. Charalambous, ed., *Flavors and Off-Flavors '89: Proceedings of the 6th International Flavor Conference, Rethymnon, Crete, Greece 5 7 July 1989*, Elsevier, New York, 1990, pp. 213–223.
9. R. Higby, "Bitter Constituents of Navel and Valencia Oranges," *J. Am. Chem. Soc.* **60**, 3013–3018 (1938).
10. D. H. R. Barton et al., "Triterpenoids Part XXV. The Constitutions of Limonin and Related Bitter Principles," *J. Chem. Soc.* 255–275 (1961).
11. V. P. Maier and D. L. Dryer, "Citrus Bitter Principles IV Occurrence of Limonin in Grapefruit Juice," *J. Food Sci.* **30**, 874–875 (1965).
12. V. P. Maier and D. A. Margileth, "Limonoic Acid A-Ring Lactone, A New Limonin Derivative in *Citrus*," *Phytochemistry* **8**, 243–248 (1969).
13. V. P. Maier et al., "Limonin and Limonoids: Chemistry, Biochemistry, and Juice Bitterness," in S Nagy and J. Attaway, eds., *Citrus Nutrition and Quality*, American Chemical Society, Washington, D.C., 1980, pp. 63–82.
14. V. P. Maier and E R. Grant, "Specific Thin Layer Chromatography Assay of Limonin, A Citrus Bitter Principle," *J. Agric. Food Chem.* **18**, 250–252 (1970).
15. W. C. Scott, "Limonin in Florida Citrus Fruits," *Proceedings of the Florida State Horticultural Society* **83**, 270–277 (1970).
16. J. H. Tatum and R. E. Berry, "Method for Estimating Limonin Content of Citrus Juices," *J. Food Sci.* **38**, 1244–1246 (1973).
17. J. F. Fisher, "Quantitative Determination of Limonin in Grapefruit Juice by High-Pressure Liquid Chromatography," *J. Agric. Food Chem.* **23**, 1199–1200 (1975).
18. R. L. Rouseff and J. F. Fisher, "Determination of Limonin and Related Limonoids by High Performance Liquid Chromatography," *Anal. Chem.* **52**, 1228–1233 (1980).
19. P. E. Shaw and C. W Wilson, "Use of a Simple Solvent Optimization Program to Improve Separation of Limonin in Citrus Juices," *J. Chromatogr. Sci.* **24**, 364–366 (1986).
20. P. E. Shaw and C. W. Wilson, "Quantitative Determination of Limonin in Citrus Juices by HPLC Using Computerized Solvent Optimization," *J. Chromatogr. Sci.* **26**, 478–481 (1988).
21. Van Beek and A. Blaakmeer, "Determination of Limonin in Grapefruit and Other Citrus Juices By High Performance Liquid Chromatography," *J. Chromatogr.* **464**, 375–386 (1989).
22. W. W. Widmer, "Improvements in the Quantitation of Limonin in Citrus Juice by Reversed Phase High Performance Liquid Chromatography," *J. Agric. Food Chem.* **39**, 1472–1476 (1991).
23. W. W. Widmer and S. F. Martin, "Analysis of Limonin in Citrus Juices by Direct Injection and On-Line Sample Clean-Up," *Proceedings of the Forty-Fifth Annual Citrus Processors' Meeting* **45**, 21–24 (1994).
24. R. L. Mansell and E. W. Weiler, "Radioimmunoassay for the Determination of Limonin in Citrus," *Phytochemistry* **19**, 1403–1407 (1980).
25. P. S. Jourdan et al., "Competitive Solid Phase Enzyme-Linked Immunoassay for the Quantification of Limonin in Citrus," *Anal. Biochem.* **138**, 19–24 (1984).
26. *Limonin Standards*, Bitterdetek kit, Idetek, Inc., San Bruno, Calif., 1986.
27. B. A. Carter, D. G. Oliver, and L. Jang, "A Comparison of Methods for Determining the Limonin Content of Processed California Navel Orange Juice," *Food Technol* **39**, 82–86, 97 (1985).
28. W. W. Widmer and R. L. Rouseff, "Quantitative Analysis of Limonin in Grapefruit Juice Using an Enzyme-Linked Immunoassay: Interlaboratory Study," *J. Assoc. Off. Anal. Chem.* **74**, 513–515 (1991).
29. D. G. Guadagni, V. P. Maier, and J. G. Turnbaugh, "Effect of Some Citrus Juice Constituents on Taste Thresholds for Limonin and Naringin Bitterness," *J Sci. Food Agric.* **24**, 1277–1288 (1973).

30. D. G. Guadagni, V P. Maier, and J. G. Turnbaugh, "Effect of Subthreshold Concentrations of Limonin, Naringin and Sweeteners on Bitterness Perception", *J. Sci. Food Agric.* **25**, 1349–1354 (1974)
31. State of Florida Department of Citrus, *Official Rules Affecting the Florida Citrus Industry*, State of Florida Department of Citrus, Lakeland, Fla., 1982.
32. U.S. Food and Drug Administration, "Grapefruit Juice," *21 CFR 146.132*, Office of the Federal Register National Archives and Records Administration, Washington, D. C., 1992.
33. R. L. Mansell and C. A. McIntosh, "An Analysis of Limonin, Brix and Acid Content in Grapefruit Samples Collected from Three State Test Houses," *Proceedings of the Florida State Horticultural Society* **93**, 289–293 (1980).
34. M. H. Dougherty and J. F. Fisher, "Quality of Commercial, Canned, Single-Strength Grapefruit Juice Produced in Florida During the 1975–76 and 1976–77 Citrus Seasons," *Proceedings of the Florida State Horticultural Society* **90**, 168–170 (1977).
35. J. H. Tatum, J. C. Lastinger, Jr., and R. E. Berry, "Naringin Isomers and Limonin in Canned Florida Grapefruit Juice," *Proceedings of the Florida State Horticulture Society* **85**, 210–213 (1972).
36. M. H. Dougherty et al., "Grapefruit Juice Quality Improvement Studies, Introduction and Scope of the Study; and the Effect of Processing Variables, Temperature and Duration of Storage on the Quality of Grapefruit Juice," *Proceedings of the Florida State Horticultural Society* **90**, 165–167 (1977).
37. S. Hasagawa, R. D. Bennett, and C. P. Verdon, "Limonoids in Citrus Seeds: Origin and Relative Concentration," *J. Agric. Food Chem.* **28**, 922–925 (1980).
38. S. Hasagawa et al., "Limonoid Glucosides in Citrus," *Phytochemistry* **28**, 1717–1720 (1989).
39. R. L. Mansell and C. A. McIntosh, "Three Dimensional Distribution of Limonin, Limonoate A-Ring Lactone, and Naringin in Fruit From Three Grapefruit Cultivars," *Proceedings of the Thirty-Fifth Annual Citrus Processors' Meeting* **35**, 12–14 (1984)
40. P. J. Fellers et al., "Quality of Florida-Packed Retail Grapefruit Juices as Determined by Consumer Sensory Panels and Chemical and Physical Analyses," *J. Food Sci.* **51**, 417–420 (1986).
41. M. Wethern, "Citrus Debittering With Ultrafiltration/Adsorption Combined Technology," in *Transactions of the 1991 Citrus Engineering Conference*, American Society of Mechanical Engineers, 1991, Lakeland, Fla., pp. 48–65.
42. U S. Pat. 5,817,354 (October 6, 1998), Z. Mozaffar, Q. R. Miranda, and V. Saxena (to Sepragen Corp., Inc.).
43. U.S. Pat. 4,439,458 (March 27, 1984), A. Puri (to Coca Cola Co.)
44. U.S. Pat. 4,965,083 (October 23, 1990), S. I. Norman, R. T. Stringfield, and C. C. Gopsill (to Dow Chemical Co.).
45. U.S. Pat. 2,816,033 (Dec. 10, 1957), D. E. Pritchett.
46. U.S. Pat. 2,834,687 (May 13, 1958), H. E. Swisher.
47. B. V. Chandler, J. F. Kefford, and G. Ziemelis, "Removal of Limonin From Bitter Orange Juice," *J. Sci. Food Agric.* **19**, 83–86 (1968).
48. B. V. Chandler and R. L. Johnson, "Cellulose Acetate as a Selective Sorbent for Limonin in Orange Juice," *J. Sci. Food Agric.* **28**, 875–884 (1977).
49. R. L. Johnson and B. V. Chandler, "Reduction of Bitterness and Acidity in Grapefruit Juice by Adsorptive Processes," *J. Sci. Food Agric.* **33**, 198–293 (1982).
50. R. L. Johnson and B. V. Chandler, "Adsorptive Removal of Bitter Principles and Titratable Acid From Citrus Juices," *Food Technol.* **42**, 130–137 (1988).
51. A. Konno et al., "Bitterness Reduction of Citrus Fruits by β-Cyclodextrin." *Agric. Biol. Chem.* **45**, 2341–2342 (1981)
52. A. Konno et al., "Bitterness Reduction of Naringin and Limonin by β-Cyclodextrin," *Agric. Biol. Chem.* **46**, 2203–2208 (1982).
53. P. E. Shaw, J. H. Tatum, and C. W. Wilson III, "Improved Flavor of Navel Orange and Grapefruit Juices by Removal of Bitter Components with β-Cyclodextrin Polymer," *J. Agric. Food Chem.* **32**, 832–836 (1984).
54. C. J. Wagner, C. W. Wilson III, and P. E. Shaw, "Reduction of Grapefruit Bitter Components in a Fluidized β-Cyclodextrin Polymer Bed," *J. Food Sci.* **53**, 516–518 (1988).
55. C. R. Barmore et al., "Reduction of Bitterness and Tartness in Grapefruit Juice With Florisil," *J. Food Sci.* **51**, 415–416, 439 (1986).
56. S. Hasegawa, L. C. Brewster, and V. P Maier, "Use of Limonoate Dehydrogenase of *Arthrobacter globiformis* for the Prevention or Removal of Limonin Bitterness in Citrus Products," *J. Food Sci.* **38**, 1153–1155 (1973).
57. S. Hasegawa, V. A. Pelton, and R. D. Bennett, "Metabolism of Limonoids by *Arthrobacter globiformis II*: Basis for a Practical Means of Reducing the Limonin Content of Orange Juice by Immobilized Cells," *J. Agric. Food Chem.* **31**, 1002–1004 (1983).
58. U.S. Pat. 4,447,456 (May 8, 1984), S. Hasegawa.
59. V. P. Maier, L. C. Brewster, and A. C. Hsu, "Ethylene-Accelerated Limonoid Metabolism in Citrus Fruits: A Process for Reducing Juice Bitterness," *J. Agric. Food Chem.* **21**, 490–495 (1973).
60. D. A. Kimball, "Debittering of Citrus Juices Using Supercritical Carbon Dioxide," *J. Food Sci.* **52**, 481–582 (1987).
61. U.S. Pat. 5,049,402 (September 17, 1991), Y. Tamaki, O. Mutsushika, and H. Mieda (to Pokka Corp.).
62. U.S Pat. 5,263,409 (November 23, 1993), P. van Eikeren and D. J Brose (to Bend Research, Inc.).

W. W. WIDMER
P. J. FELLERS
State of Florida Department of Citrus
Lake Alfred, Florida

LIPIDS: NUTRITION

Fat, carbohydrate, and protein are the three macronutrients found in the diet that provide energy to sustain life. Each macronutrient class is comprised of many diverse types of constituents that have important biological functions. With respect to fat, there are many compounds that vary widely in structure and function. This chemical heterogeneity is important for numerous biological processes and contributes to sensory and functional characteristics of foods. Fats, commonly referred to as lipids, are found in animals or plants and are generally insoluble in water and soluble in organic solvents. There are three major classes of lipids: the neutral lipids, the glycerophosphatides, and the sphingolipids. The neutral lipids, referred to as triglycerides, are comprised of glycerol (a 3-carbon molecule) and three fatty acids. There are short-chain (≤6-carbon

30. D. G. Guadagni, V P. Maier, and J. G. Turnbaugh, "Effect of Subthreshold Concentrations of Limonin, Naringin and Sweeteners on Bitterness Perception", *J. Sci. Food Agric.* **25**, 1349–1354 (1974)
31. State of Florida Department of Citrus, *Official Rules Affecting the Florida Citrus Industry*, State of Florida Department of Citrus, Lakeland, Fla., 1982.
32. U.S. Food and Drug Administration, "Grapefruit Juice," *21 CFR 146.132*, Office of the Federal Register National Archives and Records Administration, Washington, D. C., 1992.
33 R. L. Mansell and C. A. McIntosh, "An Analysis of Limonin, Brix and Acid Content in Grapefruit Samples Collected from Three State Test Houses," *Proceedings of the Florida State Horticultural Society* **93**, 289–293 (1980).
34. M. H. Dougherty and J. F. Fisher, "Quality of Commercial, Canned, Single-Strength Grapefruit Juice Produced in Florida During the 1975–76 and 1976–77 Citrus Seasons," *Proceedings of the Florida State Horticultural Society* **90**, 168–170 (1977).
35. J. H. Tatum, J. C. Lastinger, Jr., and R. E. Berry, "Naringin Isomers and Limonin in Canned Florida Grapefruit Juice," *Proceedings of the Florida State Horticulture Society* **85**, 210–213 (1972).
36. M. H. Dougherty et al., "Grapefruit Juice Quality Improvement Studies, Introduction and Scope of the Study; and the Effect of Processing Variables, Temperature and Duration of Storage on the Quality of Grapefruit Juice," *Proceedings of the Florida State Horticultural Society* **90**, 165–167 (1977).
37. S. Hasagawa, R. D. Bennett, and C. P. Verdon, "Limonoids in Citrus Seeds: Origin and Relative Concentration," *J. Agric. Food Chem.* **28**, 922–925 (1980).
38. S. Hasagawa et al., "Limonoid Glucosides in Citrus," *Phytochemistry* **28**, 1717–1720 (1989).
39. R. L. Mansell and C. A. McIntosh, "Three Dimensional Distribution of Limonin, Limonoate A-Ring Lactone, and Naringin in Fruit From Three Grapefruit Cultivars," *Proceedings of the Thirty-Fifth Annual Citrus Processors' Meeting* **35**, 12–14 (1984)
40. P. J. Fellers et al., "Quality of Florida-Packed Retail Grapefruit Juices as Determined by Consumer Sensory Panels and Chemical and Physical Analyses," *J. Food Sci.* **51**, 417–420 (1986).
41. M. Wethern, "Citrus Debittering With Ultrafiltration/Adsorption Combined Technology," in *Transactions of the 1991 Citrus Engineering Conference*, American Society of Mechanical Engineers, 1991, Lakeland, Fla., pp. 48–65.
42 U S. Pat. 5,817,354 (October 6, 1998), Z. Mozaffar, Q. R. Miranda, and V. Saxena (to Sepragen Corp., Inc.).
43. U.S. Pat. 4,439,458 (March 27, 1984), A. Puri (to Coca Cola Co.)
44. U.S. Pat. 4,965,083 (October 23, 1990), S. I. Norman, R. T. Stringfield, and C. C. Gopsill (to Dow Chemical Co.).
45. U.S. Pat. 2,816,033 (Dec. 10, 1957), D. E. Pritchett.
46. U.S. Pat. 2,834,687 (May 13, 1958), H. E. Swisher.
47. B. V. Chandler, J. F. Kefford, and G. Ziemelis, "Removal of Limonin From Bitter Orange Juice," *J. Sci. Food Agric.* **19**, 83–86 (1968).
48. B. V. Chandler and R. L. Johnson, "Cellulose Acetate as a Selective Sorbent for Limonin in Orange Juice," *J. Sci. Food Agric.* **28**, 875–884 (1977).
49. R. L. Johnson and B. V. Chandler, "Reduction of Bitterness and Acidity in Grapefruit Juice by Adsorptive Processes," *J. Sci. Food Agric.* **33**, 198–293 (1982).
50. R. L. Johnson and B. V. Chandler, "Adsorptive Removal of Bitter Principles and Titratable Acid From Citrus Juices," *Food Technol.* **42**, 130–137 (1988).
51. A. Konno et al., "Bitterness Reduction of Citrus Fruits by β-Cyclodextrin." *Agric. Biol. Chem.* **45**, 2341–2342 (1981)
52. A. Konno et al., "Bitterness Reduction of Naringin and Limonin by β-Cyclodextrin," *Agric. Biol. Chem.* **46**, 2203–2208 (1982).
53. P. E. Shaw, J. H. Tatum, and C. W. Wilson III, "Improved Flavor of Navel Orange and Grapefruit Juices by Removal of Bitter Components with β-Cyclodextrin Polymer," *J. Agric. Food Chem.* **32**, 832–836 (1984).
54. C. J. Wagner, C. W. Wilson III, and P. E. Shaw, "Reduction of Grapefruit Bitter Components in a Fluidized β-Cyclodextrin Polymer Bed," *J. Food Sci.* **53**, 516–518 (1988).
55. C. R. Barmore et al., "Reduction of Bitterness and Tartness in Grapefruit Juice With Florisil," *J. Food Sci.* **51**, 415–416, 439 (1986).
56 S. Hasegawa, L. C. Brewster, and V. P Maier, "Use of Limonoate Dehydrogenase of *Arthrobacter globiformis* for the Prevention or Removal of Limonin Bitterness in Citrus Products," *J. Food Sci.* **38**, 1153–1155 (1973).
57. S. Hasegawa, V. A. Pelton, and R. D. Bennett, "Metabolism of Limonoids by *Arthrobacter globiformis II*: Basis for a Practical Means of Reducing the Limonin Content of Orange Juice by Immobilized Cells," *J. Agric. Food Chem.* **31**, 1002–1004 (1983).
58. U.S. Pat. 4,447,456 (May 8, 1984), S. Hasegawa.
59. V. P. Maier, L. C. Brewster, and A. C. Hsu, "Ethylene-Accelerated Limonoid Metabolism in Citrus Fruits: A Process for Reducing Juice Bitterness," *J. Agric. Food Chem.* **21**, 490–495 (1973).
60. D. A. Kimball, "Debittering of Citrus Juices Using Supercritical Carbon Dioxide," *J. Food Sci.* **52**, 481–582 (1987).
61. U.S. Pat. 5,049,402 (September 17, 1991), Y. Tamaki, O. Mutsushika, and H. Mieda (to Pokka Corp.).
62. U.S Pat. 5,263,409 (November 23, 1993), P. van Eikeren and D. J Brose (to Bend Research, Inc.).

W. W. Widmer
P. J. Fellers
State of Florida Department of Citrus
Lake Alfred, Florida

LIPIDS: NUTRITION

Fat, carbohydrate, and protein are the three macronutrients found in the diet that provide energy to sustain life. Each macronutrient class is comprised of many diverse types of constituents that have important biological functions. With respect to fat, there are many compounds that vary widely in structure and function. This chemical heterogeneity is important for numerous biological processes and contributes to sensory and functional characteristics of foods. Fats, commonly referred to as lipids, are found in animals or plants and are generally insoluble in water and soluble in organic solvents. There are three major classes of lipids: the neutral lipids, the glycerophosphatides, and the sphingolipids. The neutral lipids, referred to as triglycerides, are comprised of glycerol (a 3-carbon molecule) and three fatty acids. There are short-chain (≤6-carbon atoms), medium-chain (8- to 12-carbon atoms), and long-chain (>12- to 22-carbon atoms) fatty acids. The long-chain fatty acids predominate in the diet. Two or three different fatty acids are typically present in a triglyceride molecule. Because of the diversity of fatty acids found in nature, fats are a mixture of different triglycerides.

Chemically, fatty acids are categorized as either saturated or unsaturated. Saturated fatty acids (SFA) contain no double bonds, whereas the unsaturated fatty acids contain one or more double bonds varying in isomeric configuration. Fatty acids with one double bond are referred to as monounsaturated fatty acids (MUFA) and those with two or more double bonds are called polyunsaturated fatty acids (PUFA). With regard to MUFA and PUFA, further distinctions are made on the basis of the position of the first double bond from the CH_3 terminus (yielding the designations omega-9, omega-6, or omega-3) and the confirmation of the double bond (yielding the designations *cis* or *trans*). In *cis* fatty acids the hydrogen atoms are located on the same side of the double bond, whereas in *trans* fatty acids the hydrogen atoms are located on opposite sides of the double bond. The chain length of a fatty acid as well as the number and type (ie, *cis* or *trans*) of double bonds in the molecule determine its physical, chemical, and biochemical properties.

Fat is an essential nutrient in the diet as are protein and carbohydrate. A unique characteristic of fat is its energy density. Fat supplies more than twice as much energy as protein or carbohydrate on a mass basis (9 kcal/g versus 4 kcal/g) and provides approximately one-third of dietary energy. In addition, fat provides essential fatty acids that are required for normal growth and development. Fat also promotes absorption of important fat-soluble vitamins such as vitamins A, D, E, and K. Moreover, fat contributes to satiety after consumption of food and delays the onset of hunger after a meal due to slowing of gastric emptying. Fat also contributes importantly to sensory attributes of foods. For example, it contributes to the unique textures of certain foods (ie, flakiness of pastries, creamy mouthfeel of desserts and salad dressings, crunchy texture of snack foods and cookies) and imparts distinctive flavors to foods.

Fat has numerous biological, physical, and chemical properties. With respect to its biological effects, much work has been done to elucidate how they affect various physiological processes that affect risk of the major chronic diseases observed in developed countries. The ensuing discussion reviews our present understanding of the beneficial and adverse effects that total fat, fat classes, and individual fatty acids have on risk and incidence of obesity, cardiovascular disease (CVD), and cancer.

FAT EFFECTS ON HEALTH

Impressive strides have been made in increasing the understanding of how total fat, fat classes, and individual fatty acids influence risk of chronic diseases. The diversity of effects of total fat, fat class, and individual fatty acids on risk of chronic disease is remarkable. Because of this, when discussing the role of fat in chronic disease, both fat quantity and quality must be considered. Importantly, the type and amount of fat can affect risk of chronic diseases in different ways.

Total Fat

Several issues are being debated about the ideal quantity of total fat in the diet. The first relates to the amount of total fat that most favorably affects risk of CVD. Second, there is still uncertainty about the relationship between the quantity of fat in the diet and weight control In addition, there are questions about the relationship between fat intake and risk of certain cancers.

Inherent to the discussion about how much total fat should be in the diet is that saturated fat and cholesterol are low (ie, <10% of calories and <300 mg/day, respectively). The key question that follows is whether MUFA or carbohydrate should replace SFA calories. Scientists who favor a high MUFA diet (1) contend that replacing SFA with carbohydrate leads to a decrease in high-density lipoprotein (HDL) cholesterol and an increase in plasma triglyceride levels, both of which increase risk of CVD. In contrast, when MUFA replaces SFA, plasma triglyceride levels are not increased and HDL cholesterol is not decreased compared with a high-carbohydrate diet. Thus, advocates of a high-MUFA, low-SFA diet believe that it results in a more favorable overall CVD risk profile than a low-SFA, high-carbohydrate diet. Advocates of a high-carbohydrate, low SFA diet, however, argue that this diet will result in weight loss because fewer calories are consumed and, hence, elicit a beneficial effect on HDL cholesterol and plasma triglyceride levels (2). Intertwined in the debate is the question of whether a high-MUFA, low-SFA diet (a higher-fat diet) leads to an increase in energy consumption, thereby leading to weight gain resulting in overweight/obesity. Scientists who favor the position that increasing dietary fat leads to weight gain because of the higher caloric value of fat (3) maintain that calorie control is not regulated sufficiently, leading to overconsumption of energy. On the other hand, well-respected scientists oppose this conclusion and cite epidemiologic evidence that shows little association between total fat intake and the incidence of overweight/obesity (4). Furthermore, fat may play an important role in satiety and, thus, help control calorie intake and body weight. Irrespective of the ongoing discussion, the fact remains that control of energy intake, regardless of macronutrient source, will result in achieving and maintaining a healthy body weight (5).

Some epidemiologic evidence supports a relationship between dietary fat and the incidence of breast, prostate, colon, and lung cancers (6). However, because of the difficulties in evaluating this association due to limitations in assessment methodologies and confounding variables such as total energy and micronutrient intake as well as physiological and environmental factors (such as age at menarche and level of physical activity) the results are discrepant. Thus, with respect to total fat it is not possible to reach any meaningful conclusion about the relationship between total fat intake and risk of certain cancers. It is important to note, however, that there is a larger database for individual fatty acids and risk of certain cancers (discussed in the following).

As discussed, total fat is a mixture of individual fatty acids. Likewise, different food sources of fat differ markedly in their fatty acid profile. For example, canola oil, a fat that is very low in saturated fat, is comprised of more than 90% unsaturated fat, whereas coconut oil contains more than 95% saturated fat! The other fats in our food supply contain, in general, a prominent fatty acid class but also contain varying amounts of the other fatty acid classes. For instance, soybean oil contains about 60% PUFA and about 18% SFA and 22% MUFA. In contrast, lard contains approximately 42% SFA, 44% MUFA, and the remainder is other fatty acids including PUFA. Because of the considerable variation in fatty acid composition of fats found in the diet, scientists have begun to investigate how individual fatty acids affect risk factors for chronic diseases.

Saturated Fatty Acids

The Seven Countries Study (7) was a landmark epidemiologic investigation that played a seminal role in establishing a relationship between diet and the incidence of coronary heart disease (CHD). Moreover, this study also provided evidence that diet affected serum cholesterol levels and that an elevation in cholesterol increased risk of coronary disease. This marked the beginning of the diet-heart hypothesis era, a time during which numerous studies were conducted to examine the effects of different dietary factors on risk factors for coronary disease. Many of these studies evaluated the relationship between the type of fat in the diet and serum cholesterol levels.

An important finding of the Seven Countries Study was that saturated fat intake (as a percentage of calories) was significantly correlated with serum cholesterol levels; 80% of the variability was due to differences in dietary SFA among the populations. Moreover, SFA intake was also correlated with five-year incidence of CHD. This finding has been confirmed in numerous subsequent studies. This epidemiologic evidence provided the impetus for an era in which carefully controlled clinical studies were conducted initially to evaluate the effects of fat classes and then to assess the impact of individual fatty acids on plasma lipids and lipoproteins. The results from many of the well-controlled clinical studies were used to develop blood cholesterol predictive equations for estimating the changes in total cholesterol (TC) in response to changes in type of fat and amount of dietary cholesterol. The original equations developed by Keys et al. (8) and Hegsted et al. (9) demonstrated that saturated fat was markedly hypercholesterolemic, whereas PUFA lowered blood cholesterol levels. Saturated fat was found to be twice as potent in raising blood cholesterol levels as PUFA were in lowering them. Both groups of investigators reported that MUFA had a neutral effect and that dietary cholesterol raised the blood cholesterol level but less so than saturated fat. More recently, predictive equations have been developed for low-density lipoprotein (LDL) and HDL cholesterol. The LDL cholesterol response mimics that for total cholesterol. All fatty acid classes and dietary cholesterol increase HDL cholesterol.

These studies have been followed by investigations that evaluated the effects of individual fatty acids on plasma lipids and lipoproteins. A recent summary of the literature evaluating the effects of individual fatty acids is shown in Figure 1 (10). It is evident that the effects observed are quite divergent when comparisons are made among the different fatty acids and even within a fatty acid class. For example, when comparisons are made among the long-chain SFAs, it is apparent that there are pronounced differences in potency. Specifically, myristic acid (C14:0) is twice as potent as lauric acid (C12:0) in raising total and LDL cholesterol. However, stearic acid (C18:0) is uniquely different; it has either a neutral or slight cholesterol-lowering effect. The most potent cholesterol-lowering fatty acid is linoleic acid (C18:2). Oleic acid has effects that are intermediate to those of linoleic acid and the cholesterol-raising SFA. The *trans* isomer of oleic acid has effects that are quite different from the *cis* isomer; *trans* 18:1 raises serum total and LDL cholesterol and may decrease HDL cholesterol.

In contrast to the extensive literature on fat classes and individual fatty acids on blood lipid/lipoprotein responses, much less is known about the effects of dietary fat on thrombosis. Some epidemiologic evidence from the Atherosclerosis Risk in Communities (ARIC) Study indicates that a high intake of total fat, SFA, and cholesterol is associated with higher levels of Factor VII and fibrinogen, two hemostatic factors that play a role in blood clot formation. Likewise, in the Dietary Effects on Lipoproteins and Thrombogenic Activity (DELTA) Study, a well-controlled multicenter feeding study, a reduction in saturated fat decreased Factor VII. However, a reduction in total fat increased fibrinogen levels. It is important to note that the magnitude of response for both was modest (ie, 2–3%).

There has been great debate about the association between fat intake and breast cancer. However, a pooled analysis of seven major cohort studies from four countries did not find any association between total fat intake and incidence of breast cancer (11). Population studies have shown a relationship between SFA intake, especially from animal products, and risk of colorectal adenomas. In addition, there is some evidence that SFA increases risk of ovarian cancer (12) and prostate cancer (13).

Unsaturated Fatty Acids

Monounsaturated Fatty Acids. MUFAs are a unique class of fatty acids that provides great flexibility in diet planning. They can be used to replace SFAs or carbohydrate calories or both. Depending on the substitution made, there can be a variable change in the total fat content of the diet, varying from essentially no or little change to an approximate twofold increase. The impact of these scenarios has already been discussed. In brief, however, diets high in MUFA (that are low in SFA and cholesterol) will lower total and LDL cholesterol and plasma triglycerides and minimize any potential decrease in HDL cholesterol levels (14). In individuals with diabetes, MUFAs improve the glycemic profile (plasma glucose and insulin levels) (15). There is some evidence that MUFAs may decrease susceptibility of LDL particles to oxidative modification (which is an important initiating event in the development of atherosclerosis), thereby reducing their atherogenic potential (16).

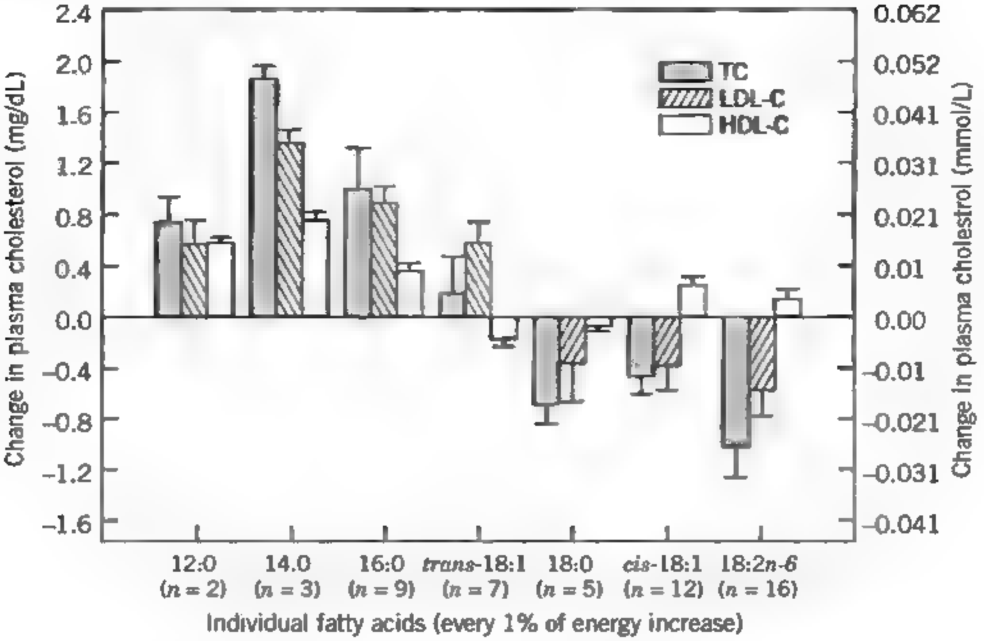


Figure 1. The effects of individual fatty acids compared with oleic acid. TC, plasma total cholesterol; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol. The results reported for oleic acid are based on comparisons with carbohydrate. The results reported for the other fatty acids are compared with oleic acid and carbohydrate.

***Trans* Fatty Acids.** Several epidemiologic studies have examined the relationship between *trans* fatty acid intake and both incidence of coronary disease and risk factors for CHD, notably lipids and lipoproteins (reviewed in Ref. 17). Evidence from some of these studies, but not all, has suggested a positive relationship. There are a number of limitations, however, of epidemiologic studies that prevent making cause-and-effect conclusions. These include the challenges associated with accurately assessing *trans* fatty acid intake due to limitations in diet assessment methodologies and an incomplete nutrient database for *trans* fatty acids. Moreover, there is a question of how accurately measures of recent food intake approximate long-term food intake, the latter of which is important relative to the chronic nature of the CHD. Despite the limitations of the epidemiologic studies conducted to date, enough have reported a positive association between *trans* fatty acid intake and CVD risk to warrant further evaluation in controlled clinical studies.

From a historical perspective, numerous early controlled feeding studies demonstrated that hydrogenated fats elicited a blood cholesterol response that was intermediate to that observed for unhydrogenated oils and saturated fats (reviewed in Ref. 18). More recently, studies have been conducted to evaluate the plasma lipid and lipoprotein effects of *trans* fatty acids. These studies have consistently shown that *trans* fatty acids increase plasma total and LDL cholesterol relative to unsaturated fatty acids (reviewed in Ref. 18). Compared with SFAs *trans* fatty acids elicit a similar or slightly lower cholesterol-raising effect (reviewed in Ref. 18). In addition, *trans* fatty acids lower HDL cholesterol, resulting in a worsening of the LDL:HDL ratio, which in turn increases CHD risk.

Polyunsaturated Fatty Acids ***Omega-6 Fatty Acids.*** The evidence that certain SFAs raise blood cholesterol levels whereas PUFAs lower them was a justification for several major diet trials designed to lower CHD risk by feeding a high-fat diet (about 40% of calories) that was low in SFA and very high in PUFA (16 to 20% of calories; Oslo Diet Heart Study, 19; VA Diet Heart Study, 20; Finnish Mental Hospital Study, 21). These studies all showed that this diet lowered CHD risk by 24 to 53% concomitant with a reduction in serum cholesterol of 13 to 15%. Because of the perception that these diets might be associated with a higher incidence of cancer and also because this diet is not consumed by population groups worldwide to meaningfully assess its safety, it has not widely been recommended for the prevention and treatment of CHD.

Recent studies have reported that while PUFA has a greater total and LDL cholesterol-lowering effect versus MUFA, the differences are not as great as once believed. Thus, for practical purposes, MUFA or PUFA will elicit effects that are quite similar when incorporated in a diet that meets current recommendations for total fat (≤30% of calories) and PUFA (<10% of calories). Some experts advocate, however, that PUFA not exceed approximately 7% of total energy (22). Collectively, the results of many studies indicate that while high levels of dietary PUFA seem to beneficially affect CHD, within the range of recommended intake, PUFA and MUFA have effects on plasma lipids and lipoproteins that are quite similar. Thus, either can be incorporated into the diet resulting in comparable effects.

Although some of the early studies suggested that high intakes of PUFA were associated with an increased risk of certain cancers, a recent meta-analysis of the literature concluded that a high intake of PUFA did not increase the risk of various cancers (23). This analysis did not rule out the possibility of a small increase in risk, however.

Omega-3 Fatty Acids. The early 1970s marked the beginning of a new era of investigation into the role of omega-3 fatty acids in the development of CVD. The seminal studies of Dyerberg et al. (24) showed that serum cholesterol and β-lipoprotein levels were lower in Greenland Eskimos compared with Danish subjects who consumed a high SFA diet. Interestingly, coronary atherosclerotic disease was rare in the Eskimos and prevalent in the Danes. These scientists attributed this difference in the incidence of CHD to the high intake of marine oils and in particular, two predominant constituent highly unsaturated fatty acids, eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). During the past 30 years numerous studies have demonstrated that these fatty acids have diverse biological effects. Collectively, they confer impressive cardioprotective effects via multiple mechanisms that involve antiarrhythmic actions, sudden death, thrombosis, growth of atherosclerotic plaques, lipids, and lipoproteins.

Restenosis is the disproportionate activation of one or several normal wound-healing responses that occur as the result of coronary artery bypass surgery or other invasive procedures. The clinical significance of this is that while angioplasty may initially increase lumen size by 20 to 40% and restore blood flow, a significant number of individuals (approximately 30 to 40%) undergoing the procedure will experience restenosis leading to luminal narrowing within 3 to 6 months, which requires another intervention procedure. Meta-analyses established that overall, restenosis was reduced by 14 to 29% by supplemental fish oils (25). Interestingly, when angiography was used to define coronary restenosis, there was a direct relationship between dose of omega-3 fatty acids and the reduction in restenosis. A recent large clinical trial, however, failed to find a beneficial effect of fish oil on restenosis after coronary angioplasty (26).

Impressive evidence indicates that omega-3 fatty acids reduce sudden death (27,28). In the initial report of the Lyon Diet Heart Trial (27), patients who had suffered a myocardial infarction (MI) followed an American Heart Association (AHA) Step 1 "Mediterranean-type" diet rich in linolenic acid versus a prudent Western-type diet. There was a striking reduction in coronary events and, in particular, no sudden deaths in the treatment group (versus 8 in the control group) despite no improvement in lipids, lipoproteins, and body mass index (BMI). These results have been corroborated in a population-based case-control study (28). In this study, as little as one fatty fish meal per week (ie, 1.4 g of omega-3 fatty acids per week) decreased the risk of primary cardiac arrest by 50%. More recently, the final report from the Lyon Diet Heart Trial (29) found that a Mediterranean dietary pattern (high in linolenic acid) reduced all cardiac death and nonfatal MI by approximately 70% and all coronary events measured by about 50%.

Fatty fish consumption has been shown to prevent cardiac arrest from ventricular fibrillation (28). In addition, omega-3 fatty acids have been shown to favorably affect susceptibility to premature ventricular contractions (30). These data are significant because they suggest that fish intake affects risk of CHD via events that are distinct from the effects of fish consumption on plasma lipids.

There is convincing evidence that a major beneficial effect of omega-3 fatty acids is on the prevention of thrombosis (31). Omega-3 fatty acids reduce platelet aggregation (including reactivity and adhesion) and vasoconstriction. In addition, omega-3 fatty acids favorably affect hemostasis. These effects are the result of enhanced fibrinolysis and reduced blood clot formation.

Fish oil has a marked hypotriglyceridemic effect in both normotriglyceridemic and hypertriglyceridemic ($\geq$2 mmol/L) individuals. The addition of approximately 9 to 13 g/day of fish oil (eg, 1.1 to 7 g/day of omega-3 fatty acids) resulted in a plasma triglyceride decrease of about 20 to 25% in normotriglyceridemic individuals and a decrease of about 26 to 33% in hypertriglyceridemic individuals. These levels of fish oil have a modest LDL cholesterol-raising effect (eg, 4 to 5%) in normotriglyceridemic individuals. In comparison, LDL cholesterol is elevated approximately 5 to 11% in hypertriglyceridemic individuals, and even more so (30%) in some individuals with familial hyperlipidemia (Type IV/V) (32). Thus, fish oil supplements can be an effective treatment for some patients with hypertriglyceridemia, although close monitoring by a physician is essential to ensure that there is not a concurrent significant increase in LDL cholesterol.

Conjugated Linoleic Acid. Conjugated linoleic acid (CLA) is a collective term given to a group of linoleic acid isomers in which the double bonds are conjugated instead of being in the typical methylene interrupted configuration. CLA levels are higher in animal products than in plant products and, in general, CLA levels are higher in ruminant than nonruminant tissues. CLA has attracted great interest recently because of the evidence that it favorably affects several major chronic diseases, most notably cancer, obesity/overweight, and CVD. The evidence about the biological effects of CLA to date has originated from cell culture and experimental animal studies. Providing 0.1 to 1% CLA in the diet has been reported to suppress tumor incidence in models using carcinogens that require or do not require metabolic activation, suggesting that more than one metabolic mechanism may account for the anticarcinogenic properties of CLAs. At least one study has shown that CLA supplementation to normal human mammary cells or to MCF-7 human mammary tumor cells did not lead to increased intracellular lipid peroxidation, whereas linoleic acid did (33). Thus, it is possible that the effect of CLA relates to differences in the ability to enhance free radical formation.

With respect to CVD there is limited but interesting evidence that CLA may beneficially affect plasma lipids and lipoproteins as well as atherosclerosis in rabbits (34) and hamsters (35). In both studies, the CLA treatment groups had markedly lower ($\approx$20%) total and LDL cholesterol levels and triglyceride levels. HDL cholesterol levels were unaffected by CLA. Based on the study of Nicolosi et al. (35), CLA had more potent effects than linoleic acid. Collectively, these studies are highly suggestive that CLA may have a more potent cardioprotective effect beyond that of the predominant fatty acids in the diet. In addition, while CLA has antioxidant effects, little is known about whether CLA protects against oxidative modification of LDL.

FAT AND FATTY ACID INTAKE

Data from the 1995 USDA Continuing Survey of Food Intake of Individuals (CSFII) indicates that total fat consumption is 32 to 33% of calories for males and females 19 years of age and greater. Saturated fat intake is approximately 11%, MUFA is 12 to 13% and PUFA is 6 to 7% of calories. As shown in Table 1, palmitic acid is the most predominant SFA in the diet, accounting for 5 to 6% of calories. Likewise, oleic acid is the major MUFA, and linoleic acid is the most abundant PUFA in the diet. *Trans* fatty acids comprise approximately 2.6% of total energy intake. The intake of omega-3 fatty acids in the United States is approximately 1.6 g/day (about 0.7% of energy intake). α-Linoleic acid is the predominant omega-3 fatty acid in the diet, accounting for 1.4 g/day. Intake of EPA and DHA is quite low (only 0.1 to 0.2 g/day). Otherwise, intake of CLA is estimated to be approximately 0.2 g/day in participants of the Nationwide Food Consumption Survey.

Total fat intake has declined from approximately 36 to 37% of calories in 1987–1988 to 32 to 33% of calories in 1995, which represents an approximate 10% reduction. Likewise, SFA intake has decreased from 13% of calories to 11% of calories during this same period of time (36). Present intake of total and saturated fat is approaching current dietary recommendations (30% of calories from total fat and <10% of calories from SFA).

Table 1. Fat and Fatty Acid Intake in Adult U.S. Population (≥19 Years of Age)

Fat/fatty acid[a]	Men	Women
Total fat	*33.3*	*32.4*
Total SFA	*11.2*	*10.7*
C12:0	0.3	0.3
C14:0	0.9	0.9
C16:0	5.2	5.9
C18:0	2.9	2.8
Total MUFA	*12.9*	*12.2*
C18:1 *cis*	11.9	11.4
Total trans	*2.6*	
Total PUFA	*6.6*	*6.8*
C18:2	5.9	6.0
C18:3	0.6	0.6
EPA + DHA	*	*

[a] % of energy

* Value of less than 0.05 but greater than 0

SUMMARY

Impressive progress has been made in understanding the role of the amount and type of fat in the diet on health. There is general agreement that certain SFAs in the diet be reduced to lower risk of CVD. However, debate continues about what nutrient source should replace SFA calories in the diet. Some scientists advocate that SFA be replaced with carbohydrate, resulting in a high-carbohydrate, low-SFA diet. Others believe that MUFA is the preferable replacement for SFA calories, resulting in a diet higher in total fat. There is an increasing consensus that *trans* fatty acids be reduced in the diet to decrease CVD risk. In contrast, compelling emerging evidence suggests that omega-3 fatty acids elicit cardioprotective effects and that CLA may confer a protective effect against certain cancers. There is a growing sense that intake of omega-3 fatty acids needs to be increased. The small database for CLAs is exciting; however, further studies are needed to define their health benefits.

The evidence we have about the effects of the amount and type of fats on chronic disease risk has enabled us to develop contemporary dietary recommendations that reduce risk of these diseases. As additional evidence is accumulated it is not unreasonable to speculate that further changes in the amount and type of fat in the diet can be made that will further enhance health.

BIBLIOGRAPHY

1. M. B. Katan, S. M. Grundy, and W. C. Willet, "Should a Low-Fat, High Carbohydrate Diet Be Recommended for Everyone? Beyond Low-Fat Diets," *N. Engl. J. Med.* **337**, 563–566 (1997).
2. W. E. Connor and S. Connor, "Should a Low-Fat, High Carbohydrate Diet Be Recommended or Everyone? The Case for a Low-Fat, High-Carbohydrate Diet," *N. Engl. J. Med.* **337**, 562–563 (1997).
3. G. A. Bray and B. M. Popkin, "Dietary Fat Intake Does Affect Obesity!" *Am. J. Clin. Nutr.* **68**, 1157–1173 (1998).
4. W. C. Willett, "Dietary Fat and Obesity: An Unconvincing Relation," *Am. J. Clin. Nutr.* **68**, 1149–1150 (1998).
5. R. L. Leibel et al., "Energy Intake Required To Maintain Body Weight Is Not Affected by Wide Variation in Diet Composition," *Am. J. Clin. Nutr* **55**, 350–355 (1992).
6. L. H. Kuller, "Dietary Fat and Chronic Diseases: Epidemiologic Overview," *Journal of the American Dietetic Association* **97** (Suppl), S9–S15 (1997).
7. A. Keys, "Coronary Heart Disease in Seven Countries," *American Heart Association Monograph*, No. 29, I-1–I-211 (1970).
8. A. Keys, J. T. Anderson, and F. Grande, "Serum Cholesterol Response to Changes in the Diet. IV. Particular Saturated Fatty Acids in the Diet," *Metabolism* **14**, 776–787 (1965).
9. D. M. Hegsted et al., "Quantitative Effects of Dietary Fat on Serum Cholesterol in Man," *Am. J. Clin. Nutr.* **17**, 281–295 (1965).
10. P. M. Kris-Etherton and S. Yu, "Individual Fatty Acid Effects on Plasma Lipids and Lipoproteins: Human Studies," *Am. J. Clin. Nutr.* **65** (Suppl.), 1628S–1644S (1997).

11. D. J. Hunter et al., "Cohort Studies of Fat Intake and the Risk of Breast Cancer—A Pooled Analysis," *N. Engl. J. Med.* **334**, 356–361 (1996).
12. H. A. Risch et al., "Dietary Fat Intake and Risk of Epithelial Ovarian Cancer," *J Nat. Cancer Inst.* **86**, 1409–1415 (1994).
13. J-R. Zhou and G. L. Blackburn, "Bridging Animal and Human Studies: What Are the Missing Segments in Dietary Fat and Prostate Cancer," *Am. J. Clin. Nutr.* **66** (Suppl.), 1572S–1580S (1997).
14. R. P. Mensink and M. B. Katan, "Effect of a Diet Enriched With Monounsaturated or Polyunsaturated Fatty Acids on Levels of Low-Density and High-Density Lipoprotein Cholesterol in Healthy Women and Men," *N. Engl. J. Med.* **321**, 436–441 (1989).
15. A. Garg et al., "Comparison of a High-Carbohydrate Diet With a High-Monounsaturated-Fat Diet in Patients With Non-Insulin-Dependent Diabetes Mellitus," *N Engl. J. Med.* **319**, 829–834 (1988).
16. P. D. Reaven et al., "Effects of Oleate-Enriched and Linoleate-Enriched Diets on the Susceptibility of Low Density Lipoprotein to Oxidative Modification in Hypercholesterolemic Subjects," *J. Clin. Invest.* **91**, 668–676 (1993).
17. A. H. Lichtenstein, "Trans Fatty Acids, Plasma Lipid Levels, and Risk of Developing Cardiovascular Disease: A Statement for Healthcare Professionals From the American Heart Association," *Circulation* **95**, 2588–2590 (1997).
18. M. A. Denke, "Serum Lipid Concentrations in Humans," *Am. J. Clin. Nutr.* **62**, 693S–700S (1995).
19. P. Lernen, "The Effect of Plasma Cholesterol Lowering Diet in Male Survivors of Myocardial Infarction. A Controlled Clinical Trial," *Acta Medica Scandinavica* (Suppl.) **466**, 1–92 (1966).
20. S. Dayton et al., "A Controlled Clinical Trial of a Diet High in Unsaturated Fat in Preventing Complications of Atherosclerosis," *Circulation* (Suppl.) **XL**, 1–63 (1969).
21. M. Miettinen et al., "Effect of Cholesterol-Lowering Diet on Mortality From Coronary Heart-disease and Other Causes. A Twelve Year Clinical Trial in Men and Women," *Lancet* **2**(7782), 835–838 (1972).
22. S. M. Grundy, "What Is the Desirable Ratio of Saturated, Polyunsaturated and Monounsaturated Fatty Acids in the Diet?" *Am. J. Clin. Nutr.* (Suppl.) **66**, 988S–990S (1997).
23. P. L. Zock and M. B. Katan, "Linoleic Acid Intake and Cancer Risk: A Review and Meta-Analysis," *Am. J. Clin. Nutr.* **68**, 142–153 (1998).
24. J. Dyerberg, H. O. Bang, and N. Hjørne, "Fatty Acid Composition of the Plasma Lipids in Greenland Eskimos," *Am. J. Clin. Nutr.* **28**, 958–966 (1975).
25. J. P. Gapinski et al., "Preventing Restenosis With Fish Oils Following a Coronary Angioplasty. A Meta-Analysis," *Archives of Internal Medicine* **153**, 1595–1601 (1993).
26. A. Leaf et al., "Do Fish Oils Prevent Restenosis After Coronary Angioplasty?" *Circulation* **90**, 2248–2257 (1994).
27. M. de Lorgeril et al., "Mediterranean Alpha-Linoleic Acid-Rich Diet in Secondary Prevention of Coronary Heart Disease," *Lancet* **343**, 1454–1459 (1994).
28. D. S. Siscovick et al., "Dietary Intake and Cell Membrane Levels of Long-Chain n-3 Polyunsaturated Fatty Acids and the Risk of Primary Cardiac Arrest," *JAMA—J. Am. Med. Assoc.* **274**, 1363–1367 (1995).
29. M. de Lorgeril et al., "Mediterranean Diet, Traditional Risk Factors, and the Rate of Cardiovascular Complications After Myocardial Infarction: Final Report of the Lyon Diet Heart Study," *Circulation* **99**, 779–785 (1999).
30. A. Sellmayer et al., "Effects of Dietary Fish Oil on Ventricular Premature Complexes," *American Journal of Cardiology* **76**, 974–977 (1995).
31. W. E. Connor, "The Beneficial Effects of Omega-3 Fatty Acids. Cardiovascular Disease and Neurodevelopment," *Current Opinion in Lipidology* **8**, 1–3 (1997).
32. W. W. Harris, "Fish Oils and Plasma Lipid and Lipoprotein Metabolism in Humans. A Critical Review," *J Lipid Res.* **30**, 785–807 (1989).
33. D. C. Cunningham, L. Y. Harrison, and T. D Shultz, "Proliferative Responses of Normal Human Mammary and MCF-7 Breast Cancer Cells to Linoleic Acid, Conjugated Linoleic Acid and Eicosanoid Synthesis Inhibitors in Culture," *Anticancer Res.* **17**, 197–203 (1997).
34. K. N. Lee, D. Kritchevsky, and M. W. Pariza, "Conjugated Linoleic Acid and Atherosclerosis in Rabbits," *Atherosclerosis* **108**, 19–25 (1994).
35. R. J. Nicolosi et al., "Dietary Conjugated Linoleic Acid Reduces Plasma Lipoproteins and Early Aortic Atherosclerosis in Hypercholesterolemic Hamsters," *Artery* **22**, 266–277 (1997).
36. A. H. Lichtenstein et al., "Dietary Fat Consumption and Health," *Nutrition Reviews* **56**, S3–19 (1998).

PENNY M. KRIS-ETHERTON
TERRY D. ETHERTON
Penn State University
University Park, Pennsylvania

LIVESTOCK FEEDS

Animals have been part of the human food chain since Neanderthals hunted them about 50,000 B.C. (1). Today, in those countries with a high standard of living, foods from animals (eg, meat, milk, and eggs) provide about 25 to 30% of the total caloric intake, whereas in developing countries it is a much lower proportion. High-quality protein is the primary nutrient supplied by animal products. In countries with adequate diets, half or more of the total protein intake comes from animal products, whereas in countries with inadequate diets, animal protein intake is 6 to 35% of the total protein intake. Protein in animal foods is high quality; the essential amino acids are present in high and well-balanced quantities. Thus, the consumption of animal foods supplements and complements the lower-quality protein found in many plant foods (eg, grains, vegetables, and fruits). A deficiency of animal protein in small children results in the disease kwashiorkor, a well-known problem in developing countries. Table 1 shows the edible protein consumed from different animal products on a worldwide basis (2).

Animal products are rich sources of required minerals (especially calcium, phosphorus, magnesium, potassium, iron, zinc) and vitamins (especially vitamin A, thiamin, riboflavin, niacin, vitamins B_6 and B_{12}) as shown in Table 2 (3). Because animal products contain protein, minerals, and vitamins in higher amounts relative to their caloric content in comparison to human dietary requirements, their consumption facilitates a balanced diet (4).

The efficiency with which animals convert feed resources into human food varies depending on the type of

Table 1. Edible Protein from Animal Sources Consumed Annually by the World Population

Source	Protein, ×1000 metric tons
Milk and milk products	18,550
Eggs	2,849
Meat	
Cattle	7,591
Buffalo	198
Sheep	979
Goat	282
Horse	70
Pork	5,859
Poultry	4,464
Other[a]	2,916
Edible offal	1,381
Fish	7,589
Total	*52,728*

[a] Includes camelidae, yak, deer, elk, reindeer, antelope, kangaroo, rabbit, and other small animals.

Table 2. Contribution of Animal-Derived Foods to the Nutrient Availability in the United States (1994)

	% of total nutrient intake			
Nutrient	Meat[a]	Dairy products[b]	Eggs	Total
Calories	14	9	1	25
Protein	39	19	4	62
Calcium	3	73	2	78
Phosphorus	25	33	4	62
Magnesium	13	16	1	30
Potassium	17	18	1	36
Iron	16	2	2	20
Zinc	42	19	3	64
Vitamin A	21	17	4	42
Thiamin	19	6	1	26
Riboflavin	18	31	6	55
Niacin	38	1		39
Folate	7	7	5	19
Vitamin B_6	36	10	2	48
Vitamin B_{12}	73	21	4	98

[a] Beef, pork, poultry, and fish.

[b] Excluding butter

Table 3. Gross Efficiency of Converting Animal Feed into Edible Products for Humans

	Percentage recovered	
Animal products	Calories	Protein
	Monogastric animals	
Broilers (meat)	11	23
Turkeys (meat)	9	22
Hens (eggs)	18	26
Swine (meat)	14	14
	Ruminant animals	
Cattle (milk)	17	25
Cattle (meat)	3	4
Sheep (meat)	3	4

Note: See text for discussion of net efficiency.

feed consumed by a particular class of livestock. Table 3 shows the percentage of energy and protein in the feed of animals that is recovered as edible animal products in the United States (5). These gross conversion efficiencies do not consider that livestock consume feeds of no value to humans (eg, range grass, pastures, hays, silage, straw, stover, stubble) or are not readily consumed by humans, such as feed grains (sorghum, barley, oats, millet, triticale) and by-products from the food industry (bran, gluten feeds, hulls, oilseed meals, tankage, meat and bone meal, feather meal, blood meal, whey, brewers and distillers grains, citrus and sugar-beet pulp, inedible fats). Table 4 shows the consumption of concentrates and forages, and the percentage of each used in the diets of U.S. livestock (5). China has the world's largest population of swine (33% of the total number of pigs), which is fed largely on forages, by-products, and wastes. Of the total land in the world, 11% is arable (suitable for crop production); 22% is range, pasture, and meadow; and 66% is not suitable for agricultural use. If animal efficiency is based on the calories that can be utilized directly by humans, their net efficiencies are nearly equal (6). Considering only the feed protein consumed by animals that could be utilized directly by humans, edible protein from animal products is produced with an efficiency of 60 to over 100% (7). Until all available land that can be used for photosynthesis yields food completely consumed directly by humans without any loss of plant material, including the supporting portions of the plant and by-products from the processing of plant materials for human consumption, animals will make a positive contribution to the supply of human food (8). Because the relationships between ruminants (cattle, sheep, goats) and human needs are complex and interdependent (Fig. 1), quantitative efficiency measurements are difficult (9).

The use of grains for food animals varies greatly throughout the world. In advanced countries, approximately 70% is used for animal feed and 30% for human food, whereas in developing countries, only about 8% is used as animal feed. Except for wheat, the principal end use of grains in the United States is for livestock feed. The current interest in oats as a source of dietary fiber in the human diet and corn for the production of fructose sweeteners and fuel alcohol has changed somewhat the usage for these two grains as well as the quantity of by-products available from these food industries.

Animals constitute a tremendous reservoir of human food, thereby reducing humankind's vulnerability to periods of crop shortages due to droughts, disease, and political events (2). Animals are found throughout the world, whereas grain reserves are found only in certain areas.

Animals also provide a host of other human needs. Wool is used for clothing and hides are used for shoes and other leather products. Many pharmaceutical products are isolated from animal glands. Fats and other lipids are used to manufacture soap and other products. Animals are used in many parts of the world for transportation and draft

Table 4. Consumption of Feed from Various Sources by Different Classes of Livestock in the United States

	Grains and high-protein concentrates		Forage	
Class of livestock	×1000 Metric tons	% of Diet	×1,000 Metric tons	% of Diet
Dairy cattle	25,602	37	43,739	63
Beef cattle	56,963	27	152,168	73
Sheep and goats	974	11	7,610	89
Hogs	51,396	86	8,178	14
Hens and pullets	20,499	97	734	3
Broilers	12,104	100		0
Turkeys	5,091	95	272	5
All livestock	185,105	46	218,879	54

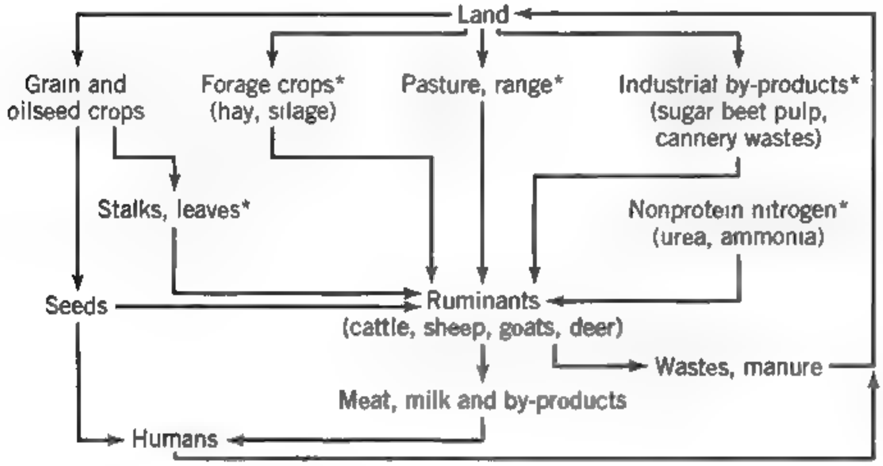

Land
Grain and oilseed crops
Forage crops* (hay, silage)
Pasture, range*
Industrial by-products* (sugar beet pulp, cannery wastes)
Stalks, leaves*
Nonprotein nitrogen* (urea, ammonia)
Seeds
Ruminants (cattle, sheep, goats, deer)
Wastes, manure
Meat, milk and by-products
Humans

Figure 1. The Role of ruminants in human nutrition. Items marked with an asterisk are converted by ruminants but are not consumed directly as food by humans.

power, especially in agricultural production. Animal wastes are valuable fertilizer, and animal manure is used as a source of heating fuel and building material.

FARMING UNCONVENTIONAL ANIMALS

Although small in magnitude, the commercial production of unconventional animals is increasing. Aquaculture for the production of fish and crustaceans is definitely on the increase. There is a small commercial rabbit industry. Farmers and ranchers are increasingly interested in the propagation of deer, other wild ruminants, and game birds for private hunting and for exotic meats. Proper nutrition and feeding of these new farm animals is of interest (10).

COMPANION ANIMALS

In addition to food-producing animals, feeds are used to support a large number and array of companion animals (horses, dogs, cats, and other pets). Horse owners in the United States use about 4.3 million metric tons (t) of mixed feed each year (11) to feed about 5.25 million horses (12). In addition, approximately this same amount is also fed as hay and pasture.

The dog and cat population in the United States is estimated to be 52 and 56 million, respectively (13); 60% of the households in the United States have pets. These pets consume about 5,133,500 t of food each year (about 73% by dogs and 26% by cats) resulting in $9.9 billion in pet food sales (14). Six companies account for 87% of the dog food, and five companies account for 87% of the cat food marketed through major food stores. Additionally, pet foods are sold as private label products through pet specialty stores, veterinarians, feed stores, and direct distribution. The trend has been toward more dry food products (88% of the total), less canned foods, and more specialty products (eg, high energy, high protein to reduce defecation and life-cycle products for growing and aging pets).

Other specialty feeds for rabbits, fur-bearing animals, birds, laboratory, and other animals total about 20% of that produced for dogs and cats. Feeding wild animals in captivity (zoos and game parks) is of increasing interest. Diet monotony is a problem in feeding zoo animals; adapting wild animals to diets and supplements formulated by humans is a challenge.

NUTRIENT REQUIREMENTS

Research continues on the nutrient requirements and interactions in all classes of livestock, companion animals, fish, and laboratory animals. These are summarized in a publication series *Nutrient Requirements of Domestic Animals* by the National Academy of Science–National Research Council (NAS–NRC) that are revised periodically (15). This series incorporates computer models to predict nutrient requirements based on genetic variance, environmental conditions, and the use of various production technologies. These are used by feed companies, animal practitioners, consultants, producers, and teachers as guides to formulating diets that will meet the nutritional requirements of animals, generally with some safety margin, from feeds typically consumed and therefore palatable to a particular animal. Many animal feeding guides are also available from state extension offices, animal associations, feed companies, and veterinarians.

COMPUTERS

During the past 35 years, the feed business has been revolutionized by computers. Diet formulation, least-cost diet formulas, process and inventory control, and accounting have been impacted by the introduction of computers. Nutritionists can not only formulate diets from palatable feeds that meet the nutritional requirements of animals, they can also formulate diets from ingredients that minimize the total cost of the formula. Data describing the physical characteristics of each ingredient, restrictions on the amount of a given ingredient, and current prices can now all be handled in great detail using linear programming and least-cost formulation (16).

Computers are also being used to simulate livestock production systems to study inputs and their impact on production output and net returns. These programs are more sophisticated than older measures of profit potential (17).

THE FEED MANUFACTURING INDUSTRY

Feed manufacturing is one of the 25 leading industries in the United States (18); it employs about 97,000 people (11). Feed purchases historically have been the largest U.S. farm expenditure, surpassing rent, interest, fertilizer, and energy; in 1984, feed purchases totaled $18.3 billion (11). Feed cost in the production of meat, milk, and eggs accounts for 50 to 75% of the total cost of production. The early development of this industry and the later application of science, engineering, and merchandising to feed manufacturing provides an interesting success story in the development of U.S. agriculture (19). The challenge facing the industry is to formulate, manufacture, and distribute feeds that will enable livestock, poultry, and fish farmers to produce quality products at the lowest possible cost (16).

Many feeds used in feed manufacturing were once considered waste materials from the food industry that were disposed of in ways that would be considered environmentally unacceptable by today's standards. In 1947, it was noted: "Sixty years or more ago, the flour mills in Minneapolis dumped wheat bran into the river because nobody wanted to buy it" (20). Feed mixing moved from the scoop shovel to a mechanized operation, and many companies entered the feed manufacturing business between 1895 and 1930. During this time, feeds were sold more for their mystical qualities than for demonstrated production and health responses in animals.

Many feed companies founded during this early period are still in business today. The Ralston Purina Company (now Purina Mills, Inc.) was founded in 1894; the Checkerboard trademark was copyrighted in 1900. A recent survey provides a listing of more than 800 existing feed companies, and poultry and livestock integrators (21). Of these companies, 95% produce complete feeds, 66% produce feed supplements, and 39% produce premixes. Most manufacture feed for hogs (76%) and beef cattle (73%), followed by feed for dairy cattle (68%) and laying hens (51%). Approximately 4% of the companies produce more than 227,000 t of feed annually, 30% produce between 18,000 and 227,000 t a year, and 66% produce less than 18,000 t. Farmer cooperatives account for about 22% of the formula feed tonnage. About 6700 feed mills were in operation in 1984 (11). In 1988, the U.S. feed manufacturing industry produced an estimated 94 million t of primary feed (feed mixed from individual ingredients). Secondary feed (feed mixed with one or more ingredients and a supplement) totaled 9 million t. Table 5 lists the livestock uses of the primary feed total (12).

Today's feed-manufacturing industry is a highly complex, scientifically based, quality-controlled, and heavily regulated industry (Fig. 2). It combines a variety of fields ranging from chemistry, nutrition, computer programming, engineering, materials handling, and transportation to personnel, inventory control, economic analysis, advertising, and law. Patents related to manufacturing processes, ingredients, nutrient forms, and drugs are many. It is a progressive and competitive industry that contributes advanced technology to the U.S. animal industry and enhances the supply, quality, and nutritional value of food for humans.

Several trade and related associations service the industry, including the American Feed Industry Association (AFIA; Arlington, VA), Association of American Feed Control Officials (AAFCO; West Lafayette, IN), Animal Health Institute (Alexandria, VA), American Association of Feed Microscopists (Sacramento, CA), National Grain and Feed Association (Washington, DC), several state grain and feed associations, and allied industry associations (22).

FEEDING SYSTEMS USED IN LIVESTOCK PRODUCTION

Poultry

For the most part, poultry (chickens, turkeys, ducks, geese) are fed in houses on complete mixed diets. Chickens are

Table 5. Livestock Use of Manufactured Feed in the United States (1997)

Livestock	Percentage of total
Starter, grower, layer, and breeder hens	12.7
Broilers	31.1
Turkeys	7.7
Dairy cattle	13.2
Beef cattle and sheep	15.4
Hogs	13.1
Other	6.8

Note: Primary feed mixed from individual ingredients as complete feed, supplement, or concentrate.

Figure 2. Feed Manufacturing plant with ingredient storage, feed elevators, warehouse, delivery trucks, and office. *Source:* Courtesy of Ezell-Key Grain Co., Inc., Snyder, Texas.

fed either for meat (broilers) or eggs (laying hens). Breeds and strains of chickens differ for these two purposes. Turkeys are also produced primarily in houses, although some range or pasture is still used. The poultry industry is very different from that of 40 years ago (23). Many broiler production units are vertically integrated (24) from hatching, production, and feed preparation through shipment of processed broilers to grocery stores (Fig. 3). Nearly all (98%) of the broiler feed is fed to the manufacturer's own chickens (11). The eight largest companies produce 55% of the total U.S. broiler production. Modern poultry production firms are well capitalized, take advantage of the economies of large-scale operations, and rapidly adopt new biological and technological advances. As a result, the relative price of poultry products has steadily decreased, which has greatly increased the demand and therefore the production of broilers and turkeys.

Swine

Swine are also largely produced in houses on complete mixed diets. The swine industry is becoming more vertically integrated (11). Many independent swine producers, large (>5000 head) and small (<100 head), purchase feeds and other inputs and sell finished pigs to meat-processing companies for final distribution of pork products. Many swine producers are farrow-to-finish, meaning they breed, produce, and market finished pigs. A few producers sell feeder pigs to others for finishing. Swine producers effectively use new technology in nutrition, feeding, and other management practices (Fig. 4).

Dairy Cattle

Milk production and the production of dairy cattle is still largely by individual producers, although many of these

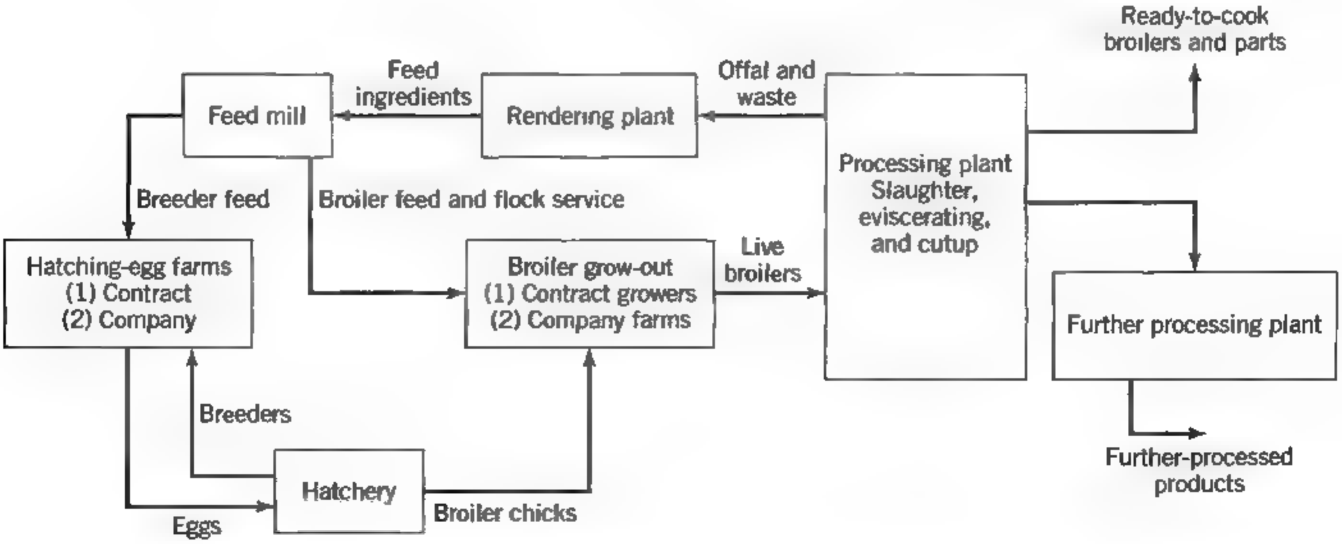

Ready-to-cook broilers and parts
Feed ingredients
Offal and waste
Feed mill
Rendering plant
Processing plant Slaughter, eviscerating, and cutup
Breeder feed
Broiler feed and flock service
Live broilers
Hatching-egg farms (1) Contract (2) Company
Broiler grow-out (1) Contract growers (2) Company farms
Further processing plant
Breeders
Hatchery
Further-processed products
Eggs
Broiler chicks

Figure 3. Many modern broiler firms control production from chick to consumer product.

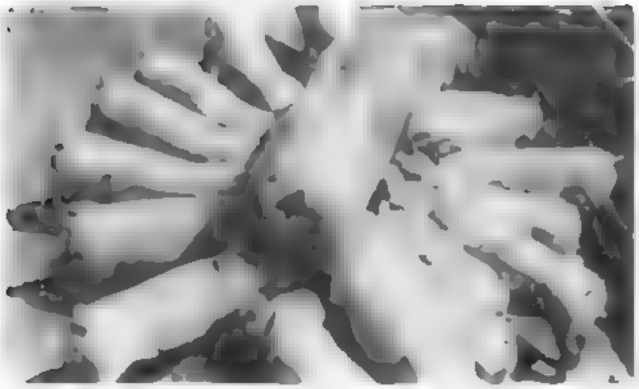


Figure 4. Swine production; sow and her baby pigs. *Source:* Courtesy of *National Food Review.*

producers are quite large (eg, 300 to 3500 cows). Some have integrated into producer-processor operations. Most producers also farm and produce a major portion of their own feed, especially roughage (eg, hay, silage, and pasture) and purchase a commercial concentrate feed. Large dairy units may purchase all of the required feed. With continuing increases in milk production per cow, due largely to genetic, feeding, and reproductive technology, the number of dairy producers continues to decrease but milk production per cow steadily increases (Fig. 5).

Beef Cattle

The beef cattle industry is made up of two distinct segments. The cow-calf industry is land based, largely in the western and southeastern areas of the United States, relying on range and pasture as the major feed input. Because it is land based, requiring a major capital investment, most cow-calf producers are small in size (eg, 50 cows), although a few are very large (eg, 20,000 cows). The feedlot industry, on the other hand, is made up of large feedlots (5000 to 200,000 head onetime capacity) located primarily in seven states (Texas, Nebraska, Kansas, Colorado, Iowa, California, and Arizona). These feedlots are often integrated with grain or feed companies (24). Some are integrated feedlot and meat-processing companies, and a few are integrated feed, feedlot, and meat-processing companies. Many feedlots custom-feed cattle on contract for absentee owners and investors. A third, less important, segment backgrounds cattle between weaning and a yearling age on pasture, crop stubble, and aftermath (eg, corn and sorghum stalks) and on various by-product feeds. Because of the diversified nature of the beef cattle industry and because many cow-calf producers derive a significant portion of their income from other farming enterprises or off-farm income, genetic and other new technologies are slow in becoming established. On the other hand, nutrition, health, and other technologies are rapidly applied by the feedlot industry, similar to the poultry industry (Fig. 6).

Sheep

Much of what characterizes the beef cattle industry is also true for the sheep industry, but on a smaller scale. Many of the producers are relatively small. Feedlot production of lambs is limited. In addition to meat, wool is an important source of income to a sheep producer (25). Sheep production in the United States has steadily declined over a long period of time.

Goats

The production of goats is similar to that described for beef cattle and sheep (25) but is a relatively small industry. Mohair from Angora goats and milk are the major products, although there is some consumption of goat meat. The Boer goat has been introduced into the United States primarily for meat production. Roughage (range, pasture, hay) is the primary feed input for goat production.

Fish

Fish and other aquatic animals are produced in commercial ponds and tanks or separated areas of lakes and seacoasts. For the most part, they are fed commercially prepared diets, although they also obtain food material from plankton in these waters. Catfish, trout, and salmon are the major fish, and shrimp and crawfish are the major crustaceans commercially produced in the United States

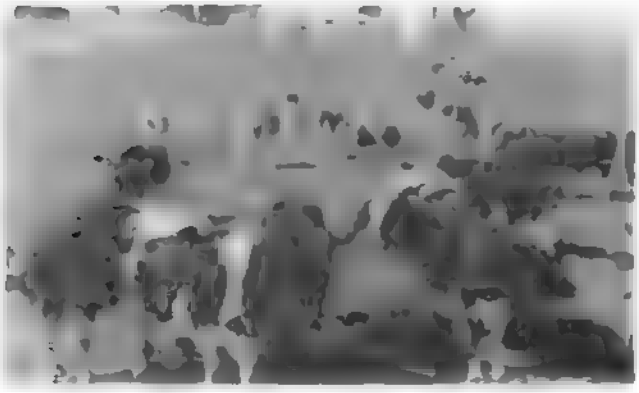


Figure 5. Dairy farm: dairy cows, milking parlor, barn, silo, and granary. *Source:* Courtesy of *National Food Review.*

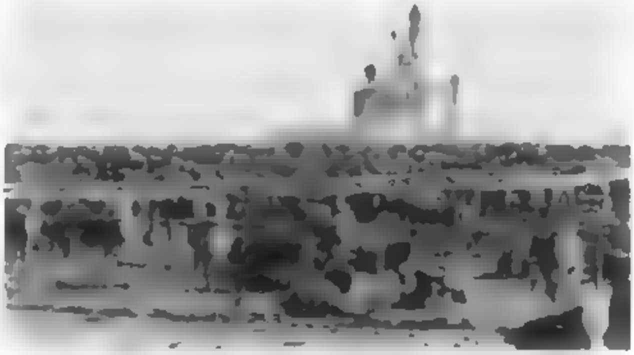


Figure 6. Cattle feedlot: cattle on feed and feed mill. *Source:* Courtesy of Texas Cattle Feeders Assoc., Amarillo, Texas.

(26). Catfish production increased 330% between 1980 and 1987. The catfish industry is largely vertically integrated. Because of its rapid growth, aquaculture will use increasing amounts of feed and improved technology to produce aquatic animals.

FEED INGREDIENTS

Basic Feed Ingredients

Roughages (range, pasture, hay, and silage) are characterized as high-fiber feeds (usually more than 18% crude, 30% acid detergent, or 45% neutral detergent fiber). They are important feeds for ruminant animals. In the rumen, anaerobic microorganisms degrade a large portion of the fiber and other organic constituents, thereby making the fiber available as an energy source. A minimum amount of roughage in the diet of ruminants is desirable for maintaining normal rumen function.

Range and Pasture. As mentioned earlier, 22% of the world's land surface is range, pasture, and meadow, two-thirds of which is not suitable for crop production. To utilize this land for the production of human food requires ruminant animals (cattle, sheep, goats, and wild ruminants). Rangeland is permanent pasture, in that it is not seeded periodically, although interseeding is sometimes practiced as an improvement method. Desirable native species of grasses and forbes are maintained and sustained largely through range management practices that include stocking rate, rotation of animals on range areas, complementarity between grazing animals, location of animal water supplies, fertilization, and the use of supplemental feeds. The major determinant of range production is the amount of precipitation.

Pasture is largely improved pasture because land is periodically reseeded to improve pasture varieties, often with the use of fertilizer and irrigation. Grazing of small grain crops, especially wheat, during the vegetative stage is an important source of pasture. Improved pastures are often part of a crop rotation system to improve soil fertility and humus and to enhance soil conservation practices. Genetic programs provide varieties and strains of plants for improved pastures. These pastures are used in the production of all ruminants but are especially important in dairy production.

Meadows are water drainage areas that are permanent grasslands between arable land areas and in stream valleys. These are grazed periodically, primarily by beef cattle and sheep, and are sometimes used for hay.

Harvested Roughages. There are many kinds of harvested roughages. Hays are forages that are cut and dried in the field and gathered relatively dry (85–90% dry matter) as baled or chopped hay. Both grass (eg, timothy, bromegrass, orchard grass) and legumes (alfalfa, clover) are used for hay. Approximately 136 million t of hay are fed to U.S. livestock each year, 57% of which is alfalfa (27). Alfalfa meal, often dehydrated, is an important specialty feed (1.76 million t; 11) in several diet formulas.

Crops can also be harvested as relatively high-moisture material (25–45% dry matter) and stored in oxygen-limiting structures (upright and bunker silos) where the bacteria present on the harvested material or added intentionally at the time of ensiling convert part of the carbohydrate in the crop to various organic acids (especially lactic acid), utilizing the oxygen present, thereby preserving the acidified product as silage. Corn, small grains (barley, oats), alfalfa, and other crops can be harvested as the entire plant for silage.

Grains. All grains can be used to feed all classes of livestock. The major feed grains in the United States are corn, sorghum grain (milo), barley, and oats. Small quantities of millet, rye, and triticale are also fed, and wheat is used when the price makes it competitive. Because grain is produced in the United States in excess of that required for human food, feeding grain to livestock is an important factor in maintaining the price of grain. About 114 million t of corn, 14 million t of sorghum grain, 7.6 million t of wheat and rye, 5.2 million t of barley, and 3.1 million t of oats are fed to U.S. livestock (12).

Protein Feeds. These feeds are largely by-products of the food-processing industry; most are the result of vegetable oil production. Soybean meal is the major protein feed (18.1 million t) followed by cottonseed meal (1.5 million t), canola meal (0.25 million t), sunflower meal (0.22 million t), peanut meal (0.15 million t), and linseed meal (0.10 million t). Corn gluten feed and meal (1.32 million t) are by-products of the corn starch and sweetener industries. Protein feeds derived from the processing of animals for food include tankage and meat meal (3.1 million t), fish meal and solubles (0.74 million t), milk products such as whey (0.36 million t), hydrolyzed feather meal (0.24 t), and smaller quantities of blood meal (11,12).

By-product Feeds. By-products used as livestock feeds are too numerous to list here; a table of by-product and unusual feed composition (28) lists 355 feeds. Those available in greatest quantities include wheat mill feeds (5.79 million t), molasses (1.66 million t), distillers grains (0.97 million t), fats and oils (0.86 million t), beet pulp (0.60 million t), rice mill feeds (0.58 million t), hominy feed (0.48 million t), brewers grains (0.34 million t), and miscellaneous by-product feeds (0.80 million t) (11,12). Other by-product feeds include almond hulls, apple pomace, bakery waste, citrus pulp, cannery waste, cottonseed hulls, cotton-gin trash, grape pomace, oat hulls, cull peas, potato wastes, soybean hulls, and many others.

Supplements

Minerals. Mineral additions to livestock diets are common practice, either to complete diets and supplements or as free-choice ingredients. Salt (NaCl) has been used for a long time; early pioneers sought out salt licks where horses and cattle obtained salt and other minerals. Commonly supplemented are calcium and phosphorus as limestone, calcium carbonate, bone meal, various calcium phosphates, sodium and ammonium phosphates, and phosphoric acid. Many of these sources are mined products from natural deposits, where fluorine is often present and must be reduced (defluorinated phosphate) to acceptable limits to prevent toxicity to livestock.

Other major minerals required by livestock include potassium, magnesium, and sulfur, which are supplemented as potassium chloride and potassium ammonium sulfate, magnesium oxide, flowers of sulfur, sulfate salts, and organic sulfur (methionine).

Livestock require eight trace minerals (cobalt, copper, iron, iodine, manganese, molybdenum, selenium, zinc) and perhaps several others (chromium, fluorine, silicon, nickel, vanadium). The required trace minerals are commonly added to complete diets, trace mineral supplements, and trace mineralized salt. Most are added as inorganic salts. There is increasing interest in the bioavailability of various organic forms of these trace minerals (eg, ethylenediamine dihydroiodide; zinc methionine, selenium methionine, and other mineral-amino acid complexes; iron and copper gluconate; and various chelated trace minerals). Some of these trace minerals have wide toxicity to requirement ratios (zinc) whereas others have very narrow ratios (selenium). As a result, trace minerals are seldom added as single ingredients to complete diets or supplements; they are formulated into multimineral premixes and then added as dry or liquid premixes.

Vitamins. All the required vitamins can be supplemented in livestock diets. These vitamins are chemically synthesized and are relatively inexpensive. Vitamin A is commonly added to all diets. The other fat-soluble vitamins—D, E, and K—are used in special situations (vitamin D for animals housed indoors, vitamin E to enhance the immunological competence, and vitamin K where bacterial synthesis in the intestine may be limited).

Water-soluble vitamins are commonly added to the diets of monogastric animals (swine, poultry, dogs, cats). Although these vitamins are synthesized by bacteria in the gastrointestinal tract, only in the case of ruminants where synthesis takes place at the beginning of the tract (rumen) is there opportunity for absorption eliminating the need for supplementation except in special situations. Thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, choline, and B_{12} are commonly added to diets for monogastric animals; biotin and folic acid (folate) are added in some cases. Vitamin C is required by fish but not required by livestock, although it may be useful in heat stress, especially in laying hens for maintaining egg shell strength.

Amino Acids. Methionine and methionine hydroxy analogue (MHA) have been used for S-amino acid supplementation for several years. Fermentive synthesis of lysine has made this often limiting amino acid an economical addition to many monogastric diets. Arginine, threonine, and tryptophan are also available.

Urea. Ruminant animals can make use of nonprotein nitrogen (NPN) through conversion to microbial protein in the rumen and subsequent digestion in the lower gastrointestinal tract. This conversion results in high-quality animal protein (milk and meat) for human consumption from an inexpensive, nonprotein source. Urea is the major source of NPN used in ruminant diets (355,500 t in 1984; 11). Biuret and various ammoniated feed products are also sources of NPN. Use of urea in ruminant diets is limited to about 1% of the total diet (one-third of the total required protein) for effective conversion to microbial protein. An excess of NPN can result in acute ammonia toxicity due to more rapid microbial conversion to ammonia in the rumen, compared with its incorporation into microbial protein.

Additives. Various other compounds are added to livestock diets for purposes other than nutritional supplements. Sales of animal health products totaled $3.6 billion in 1997 (29). Several antibiotics are added for growth promotion, improved feed utilization, and disease prevention and treatment. Chlortetracycline (aureomycin), oxytetracycline (terramycin), penicillin, virginiamycin, monensin, and lasalocid are the primary antibiotics used in livestock diets. Sulfa drugs are also used as bacteriostats. Coccidiostats, anthelmentics, and mold inhibitors are also used. These additives result in healthier animals. Use of these compounds is highly regulated by the U.S. Food and Drug Administration (FDA) both in terms of efficacy for a stated claim as well as safety for the target animal and for humans consuming foods from animals. Research costs can total $8 to $15 million to achieve final approval for one of these additives. The U.S. Food Safety and Inspection Service (FSIS) constantly monitors animal food products for the absence of residues. Use of additives constantly changes; the most current reference source on their approved use is the Feed Additive Compendium (30), which is updated annually plus monthly supplements.

Grading and Definitions

Many feeds, especially grains, are graded for quality factors (moisture, broken kernels, damage, foreign material) that are used in the trade as purchasing specifications (31). Many of these visual standards are defect-type factors that may or may not relate to the nutritional value of the feed. End-user factors such as moisture, protein, oil, starch, fiber, hardness, and amino acid ratios are important economically to the needs of specific grain users (32). Hay grading standards have also been proposed, but their use is limited.

Official processing and composition definitions for various feed ingredients have been established and are constantly updated by the Association of American Feed Control Officials (AAFCO) (33). These definitions are used by state and national feed control officials in regulatory aspects of the feed industry. Also listed are the names and addresses of state feed control officials, state feed registration requirements, the uniform state feed bill, related associations, and other useful information on livestock feeds

Analyses

The National Research Council (NRC) has published an atlas of feed composition (34) and a more recent table of feed composition (35) tabulating the composition of feeds. There are also many other tables of feed composition (28,36,37). Feed composition tables, along with actual feed analyses, are the basis for formulating nutritionally complete diets for livestock.

Feeds can be analyzed for many constituents. It is convenient to divide these into chemical and biological constituents (37). Chemical analyses for nutrients, antinutritional factors, toxicants, contaminants, and residues can be performed, but these results do not necessarily indicate the bioavailability of these constituents when consumed by animals. Thus, biological constituents are measured using a biological response such as digestibility, absorption, metabolic efficiency, or some biochemical response. Gross energy (GE or heat of combustion) indicates the total energy value of a feed but only a portion is digestible (digestible energy, DE), less is available for metabolism (ME), and the net energy (NE) value of a feed is the energy available for maintenance of body tissues and growth (meat), wool, milk, and egg production. Similar bioavailability considerations apply to protein, amino acids, minerals, vitamins, and other constituents. Feeds are commonly analyzed for moisture, crude protein, ash, fiber (crude, acid detergent, neutral detergent fiber), and crude fat. Additional analyses for individual amino acids, minerals, and vitamins can be performed.

Regulations

Many agencies and regulations impact the feeding of livestock and the manufacture of feeds, including state feed regulations, the FDA, the Environmental Protection Agency, and the Occupational Safety and Health Agency (19,38,39). In 1909, AAFCO was organized; its objective is ". . . to promote uniformity in legislation, definitions, and rulings, and the enforcement of laws relating to the manufacture, sale, and distribution of feeds and livestock remedies in the continent of North America." Good Manufacturing Practice Regulations for Medicated Feeds (30) were published by the FDA in 1976 and revised in 1986.

FEED PROCESSING

Most feeds must be processed before consumption by livestock. Only the free-grazing animal consumes feed that has not been processed.

Roughages

Hay is cut, field-dried, and generally baled into rectangular or round bales that weigh 25 to 300 kg each. The moisture content of baled material must be below 14% for proper storage. Hay can also be field chopped and hauled to storage structures or chopped and pressed into cubes (approximately 3 cm^3) either in the field or at hay storage sites. Dehydrated alfalfa is cut fresh, sometimes partially dried in the field, chopped, hauled to dehydrating facilities, and pelleted (0.6–1.3 cm). Forages can be cut and chopped in the field, hauled, and fed fresh to livestock (green chop). Forages and other plant materials can be chopped and ensiled in oxygen-limiting structures and fed as silage.

Roughages can also be treated with various chemicals to enhance their digestibility. Treatment with alkali (NaOH) has been practiced for a long time, especially in northern Europe. Use of anhydrous ammonia for enhancing the digestibility of straws and silage is a more recent practice. The use of alkaline peroxide appears especially promising for high-fiber roughages (40). Pressure treatment of acidified roughage material also enhances digestibility but is generally not cost effective. Treatment with ozone also increases the digestibility of roughages (41).

Roughages that are finely chopped (screen size less than 1.3 cm) and/or pelleted are generally consumed in greater amounts by ruminants and have a faster passage rate through the rumen, resulting in a lower digestibility of the fiber fraction. Because of greater intake, however, greater animal productivity generally occurs. Lactating dairy cows fed large amounts of concentrate feed should also be fed a minimum quantity of unchopped roughage (3 kg) to prevent abnormally low milkfat due to insufficient acetic acid production by rumen microorganisms.

Grains

Grains and other seeds can be dehulled, ground, cracked, rolled, steamed, steam-flaked, micronized, popped, extruded, roasted, and pelleted before feeding. In some cases, grains (corn) and other seeds (cotton and soybeans) are fed unprocessed (whole) to livestock. The need for processing depends on the grain, animal to be fed, feed mixture, potential improvement in utilization, and processing cost.

Swine, sheep, and goats chew their feed more thoroughly than do cattle; therefore, improvements in feed utilization through processing are comparatively small. Sorghum grain (milo), because of the small seed, must be processed for all livestock. Corn grain, on the other hand, can be fed whole to most classes of livestock except poultry, although improvements in utilization may be obtained with steam-flaking, owing to partial gelatinization of the starch granule. Concentrate ingredients may require processing to achieve a somewhat uniform particle size in complete mixed diets to prevent sorting and separation by animals. This is especially true for poultry and swine diets, and dairy and other concentrate mixes.

Grains can also be stored and fed as high-moisture or reconstituted grain. High moisture refers to grain that is harvested while the moisture content is still 20 to 30% and stored in oxygen-limiting structures. Reconstitution refers to grain that is relatively dry to which water is added to bring the moisture content to about 27% and then stored in oxygen-limiting structures. The stored grains are generally rolled when removed from the storage structures before feeding.

Complete Diets and Supplements

Common forms of formula feeds are meal or mash, pellets, crumbles and cubes, blocks, and liquids (11). Complete diets are sometimes pelleted before feeding to keep the diet from separating, to facilitate mechanical handling, and to decrease feed wastage by livestock. The pelleting process involves grinding the feed to a relatively small particle size; adding moisture, often as steam; and forcing the feed through metal dies with openings that vary from 0.3 to 1.6 cm in diameter, which generates considerable heat. The compressed feed is cut or broken into various lengths. As the compressed feed cools, it tends to set as pellets, with reasonable durability for mechanical handling. The starch content of grains enhances the pelleting process through partial gelatinization, and various pellet binders can be added (eg, lignosulfonates) to enhance the pelleting process and improve pellet durability.

Feeding pelleted diets to swine and poultry is relatively common. Larger-size pellets (cubes) are used to provide supplemental feed to grazing ruminants. Large blocks (ca 25–30 cm^3) of supplemental feeds are used for pasture and range livestock where the animals chew off portions of these blocks over time, enabling infrequent supplemental feeding of these animals. Tubs with solidified molasses plus supplemental nutrients are also convenient, allowing animals to consume the supplement over time by licking.

DIET FORMULATION AND EXAMPLE DIETS

Nutritionally complete diets for livestock can be formulated using calculators or elaborate computer programs. The NRC nutrient requirement series (15) give example methods for calculating complete diets. Because ruminant animals have fewer dietary requirements than monogastric animals, it is easier to formulate complete diets for these animals. A complex poultry diet may require balancing for 25 or more nutritional attributes.

The science of animal nutrition allows the formulation of complete diets from a multitude of feed ingredients. Therefore, there is not a best or ideal diet for any animal. Table 6 shows intakes per animal and example diets simply to illustrate what could be fed to these livestock.

FUTURE DEVELOPMENTS

The potential is great for improving our knowledge to optimize the nutrition of livestock, understanding the interactions between nutrients, quantitating the productive responses to varying nutrient inputs, and improving the economic return from the feeding of livestock. With genetic changes in livestock and the use of additives, the potential exists to produce livestock with improved efficiency that are better suited to consumer desires. Progress has already been made in increasing the leanness of animals with less waste fat (42). Through better understanding of metabolic processes, it may be possible to produce foods of animal origin with enhanced nutritional value. The potential impact of advanced technologies on animal productivity has been estimated (43).

Better and faster analysis of feed ingredients will enable more accurate formulation of livestock diets. Near-infrared reflectance spectroscopy is a promising technique in this regard. Enzyme assay may give rapid estimates of nutrient bioavailability in feeds. New and improved methods of processing feeds will improve their utilization, resulting in less animal waste. Through plant breeding, introduction of new strains, and biotechnology (44), feed materials will be improved to better meet the nutritional needs of livestock or lower the content of natural toxicants. High-lysine corn and canola meal are examples of what can be accomplished in this regard.

Table 6. Intakes per Animal and Example Diets

Ingredient	g/kg Diet
Growing chickens, 20–160 g / d	
Corn, ground	580
Soybean meal	350
Methionine, DL	2
Animal/vegetable fat	30
NaCl	5
$CaCO_3$	10
$CaHPO_4 \cdot 2H_2O$	20
Vitamin premix	1
Trace mineral premix	1
Antimicrobial premix	1
Growing swine, 1–3 kg / d	
Corn, ground	810
Soybean meal	140
Meat and bone meal	40
NaCl	3
$CaCO_3$	3
$CaHPO_4 \cdot 2H_2O$	1
Vitamin premix	1
Trace mineral premix	1
Antimicrobial premix	1
Lactating dairy cattle, 15–28 kg / d	
Alfalfa hay	180
Corn silage	500
Brewers grains, dried	100
Corn and cob meal	209
Urea	5
NaCl	3
$CaHPO_4 \cdot 2H_2O$	2
Trace mineral premix	1
Feedlot cattle, 5–14 kg / d	
Corn, whole or steam-flaked	637
Wheat middlings	80
Corn silage	120
Cottonseed hulls	70
Cottonseed meal	10
Corn gluten meal	10
Urea	5
Molasses, cane	40
Animal/vegetable fat	10
KCl	2
NaCl	3
$CaCO_3$	10
Trace mineral premix	1
Vitamin premix	1
Antimicrobial premix	1

New strains of livestock will have different nutritional requirements that must be determined to maximize genetic improvements. The use of production- and efficiency-enhancing compounds will also result in altered nutritional requirements. An increased lysine concentration in the diet of swine whose growth rate and leanness has been improved through the use of recombinant porcine growth hormone or other repartitioning agents is but one example.

Genetically engineered microorganisms that will predigest feed materials, better preserve ensiled crops, or enhance feed utilization in the rumen or large intestine of animals could have a major impact in livestock feeding. Genetically engineered organisms may also be effective in decreasing gastrointestinal problems and diseases.

The science of feeding livestock has an exciting future, but acceptance of scientific findings by producers and consumers will depend on a better understanding and appreciation of science in general. The current public backlash to the introduction of scientific improvements in general poses a major threat to the competitive position of the United States and to the competitive position of livestock in the human food chain

BIBLIOGRAPHY

1. S. M. Garn and W. R. Leonard, "What Did Our Ancestors Eat?" *Nutrition Reviews* **47**, 337–345 (1989).
2. T. J. Cunha, "The Animal as a Feed Resource for Man," *Feedstuffs* **64**, 18–32 (1982).
3. S. Gerrior and L. Bente, *Nutrient Content of the U.S. Food Supply, 1909–94*, Home Economics Res. Rpt. No. 53, USDA Center for Nutrition Policy and Promotion, Washington, D.C., 1987.
4. R. G. Hansen, "An Index of Food Quality," *Nutrition Reviews* **31**, 1–7 (1973).
5. W. F. Wedin, H. J. Hodgson, and N. L. Jacobson, "Utilizing Plant and Animal Resources in Producing Human Food," *Journal of Animal Science* **41**, 667–686 (1975).
6. K. L. Blaxter, "Efficiency of Food Conversion by Different Classes of Livestock in Relation to Food Production," *Federation Proceedings* **20**, 268–274 (1961).
7. R. L. Preston, "Let's Feed People and Animals," *Advance* **12**, 6–7, 11 (1975).
8. R. L. Preston, "Land Use—A Time for Reconsideration," *Journal of Animal Science* **46**, 1467–1468 (1977).
9. H. A. Fitzhugh et al., *The Role of Ruminants in Support of Man*, Winrock International, Petit Jean Mountain, Ark., 1978.
10. K. L. Blaxter, "Conventional and Unconventional Farmed Animals," *Proceedings of the Nutrition Society* **34**, 5156 (1975).
11. M. Ash, W. Lin, and M. D. Johnson, "The U.S. Feed Manufacturing Industry, 1984," *USDA-ERS Statistical Bulletin* No. 768 (1988).
12. "Feed Marketing and Distribution," *Feedstuffs* **70**, 6–20 (1998).
13. J. Corbin, "Pet Foods and Feeding," *Feedstuffs* **70**, 82–87 (1998).
14. R. A. Schoeff and P. Lobo, "Most Feed Potentials Up, Except—Surprise—for Broilers," *Feed Management* **49**, 7–16 (1998).
15. NAS-NRC, *Nutrient Requirements of Domestic Animals*, National Academy Press, Washington, D.C., ("Horses" [1989], "Dairy Cattle" [1989]; "Beef Cattle" [1996]; "Goats" [1981]; "Poultry" [1994]; "Sheep" [1985]; "Swine" [1998]; "Cats" [1986]; "Dogs" [1985]; "Laboratory Animals" [1995]; "Mink and Foxes" [1982]; "Rabbits" [1977]; "Fishes" [1993]; "Nonhuman Primates" [1978]).
16. R. W. Schoeff, "The Formula Feed Industry," in H. B. Pfost and D. Pickering, eds., *Feed Manufacturing Technology*, American Feed Manufacturing Association, Arlington, Va., 1976, pp. 1–17.
17. W. L. Alsmeyer, "A Multiple Regression Model for Net Income Estimates from 100-Sow Production Units," *Journal of Animal Science* **40**, 6–12 (1975).
18. W. Anderson, "The U.S. Formula Feed Industry Today," in R. R. McEllhiney, ed., *Feed Manufacturing Technology III*, American Feed Industry Association, Arlington, Va., 1985, pp. 9–12
19. R. W. Schoeff, "History of the Formula Feed Industry," in R. R. McEllhiney, ed., *Feed Manufacturing Technology III*, American Feed Industry Association, Arlington, Va., 1985, p. 28.
20. L. Wherry, *The Golden Anniversary of Scientific Feeding*, Business Press, Milwaukee, Wisc., 1947
21. "Survey Gives Updated View of Feed Industry," *Feedstuffs* **61**, 28–51 (1989).
22. "Feedstuffs 1998 Reference Issue," *Feedstuffs* **70**, 171–174 (1998).
23. "The Poultry Industry Today," *National Food Review* **12**, 9–12 (1989).
24. "The Integrators: The 'In-House' Feed Manufacturers," *Feedstuffs* **61**, 27 (1989).
25. C. S. Menzies et al., "The U.S. Sheep and Goat Industry: Products, Opportunities and Limitations," Council for Agriculture Science and Technology Report No. 94, CAST, Ames, Iowa.
26. D. Harvey, "Aquaculture: Meeting Fish and Seafood Demand," *National Food Review* **11**, 10–13 (1988).
27. "Hay: Production, Harvested Acreage, Yield, Prices Received by Farmers," *Feed Situation and Outlook Report* **305**, 53 (1988).
28. D. Bath et al., "Byproducts and Unusual Feedstuffs," *Feedstuffs* **70**, 32–38 (1998).
29. "Survey Shows Animal Health Product Sales Rose to $3.6 Billion in 1997," *Feedstuffs* **70**, 7 (1998).
30. S. Muirhead, ed., *1998 Feed Additive Compendium*, The Miller Publishing Co., Minnetonka, Minn., 1997.
31. *The Official United States Standards for Grain*, USDA Federal Grain Inspection Service, Washington, D.C., 1978.
32. C. R. Hurburgh, Jr., "Grain Quality, Defined by End-use Values, Moving into Market," *Feedstuffs* **61**, 1–56 (1989).
33. *Official Publication*, Association of American Feed Control Officials, Atlanta, Ga., 1989.
34. E. W. Crampton and L. E. Harris, *Atlas of Nutritional Data on United States and Canadian Feeds*, National Academy of Science, Washington, D.C., 1972.
35. NAS-NRC, *United States-Canadian Tables of Feed Composition*, 3rd rev., National Academy Press, Washington, D.C., 1982.
36. N. Dale, "Ingredient Analysis Table: 1998 Edition," *Feedstuffs* **70**, 24–31 (1998).
37. R. L. Preston, "Typical Composition of Feeds for Cattle, 1998," *Beef* **34**, FC1–FC15 (1998).
38. L. H. Boyd, "Feed Regulation," *Feedstuffs* **70**, 126–128 (1998).
39. L. H. Boyd, "Medicated Feed Regulation," *Feedstuffs* **70**, 129–132 (1998).
40. "Alkaline Hydrogen Peroxide Unlocks Energy in High-fiber Lignified By-products," *Nutrition Reviews* **44**, 251–252 (1986).
41. L. D. Bunting, C. R. Richardson, and R. W. Tock, "Digestibility of Ozone-Treated Sorghum Stover by Ruminants," *Journal of Agricultural Science (Camb)* **102**, 747–750 (1984).

42. V. W. Hays and R. L. Preston, "Nutrition and Feeding Management to Alter Carcass Composition of Pigs and Cattle," in H. D. Hafs and R. G. Zimbelman, eds., *Low-Fat Meats Design Strategies and Human Implications*, Academic Press, San Diego, Calif., 1994, pp. 13–33.
43. R. L. Preston, "Livestock Technologies," in B. C. English, R. L. White, and L. H. Chuang, eds., *Crop and Livestock Technologies, RCA III Symposium*, Iowa State University Press, Ames, Iowa, 1997, pp. 115–126.
44. B. K. Symonds and J. Orendorff, "Agriculture to be Changed by Emerging Biotechnology Age," *Feedstuffs* **70**, 12–18 (1998).

R. L. PRESTON
Texas Tech University
Lubbock, Texas

LOBSTER: BIOLOGY AND TECHNOLOGY

The animals referred to as lobsters are representative of a number of families of the decopod crustacea. The colors of lobsters are due to carotenoid pigments bound to proteins and deposited in the exoskeleton. These pigments are released from the proteins when the proteins are heat denatured during cooking, thus giving cooked lobster the characteristic reddish color (1).

There are more than 30 commercially exploited species of spiny lobsters alone. Description of distinguishing characteristics of species is beyond the scope of this article; refer to a review for guidance in lobster identification and world description (2). The families Galatheidae (squat lobster) and Scyllaridae (slipper or flat lobster) are minor contributors to world lobster catch (11 and 1%, respectively). On the other hand, the family Nephropinae (clawed lobsters) accounts for 71% of world commercial catch of lobster.

HOMARUS AMERICANUS

The American lobster has a carapace length of up to 210 mm (3) and is dark green with dark spotting above and yellowish color on the underside. These lobsters inhabit the shallow water of the Atlantic Coast of North America from shore to a depth of 480 m. Most commonly, they are found at depths of 4 to 5 m (4,5). These lobsters typically inhabit rocky areas but also make burrows under solid objects or in clay banks (4). They range from the Strait of Belle Isle, Newfoundland, to Cape Hatteras, North Carolina (4,6). References 3 and 7 give a thorough treatment of the biology of the species. This species accounts for 36% of the world lobster catch. *H. gammarus* (the European lobster) is biologically similar to *H. americanus* in all respects except distribution. It is a minor contributor in the world fishery, accounting for only 2% of the catch.

NEPHROPS NORVEGICUS

The Norway lobster (also known as scampi or Dublin Bay prawn) has an average carapace length of 30 mm for males and 20 mm for females at maturity; individuals grow as large as 70 mm (8). They live in burrows in muddy bottoms at depths of 15 to 820 m (9). Their range is from Iceland to the coast of Norway and from the Adriatic Sea south to the coast of Morocco. They are also found in the western Mediterranean (4). There are related species on the Brazilian coast and in the Indo-Pacific areas (10). In total, these species account for 34% of the world catch.

PALINURIDAE

The family Palinuridae (langouste or spiny lobster) is characterized by strong spines on the carapace and/or on the abdominal segments. Pincers, when present on the front legs, are small. European species belong to the genera *Panulirus* and *Jasus* (9,10). They are distributed throughout tropical and subtropical oceans (see Reference 1 for a map). For a discussion of geographic and vertical distribution of Palinurid lobsters, see Reference 11. Phyllosoma larvae hatch from eggs carried by females. These larvae are extremely flat, with long divided legs and delicate mouthparts. After about 12 molts while drifting in the ocean, the juveniles become puerile larvae; they swim actively and approach the coast. These larvae subsequently molt into the juvenile form of the adult at about 12 months of age (12). For biological details on one species (*P. argus*) see Reference 5. Palinuridae account for 17% of the world catch. The annual catch is about 80,000 t, mostly in Australia (12,000 t), Cuba (11,000 t), and Brazil (9000 t) (13).

CATCHING METHODS

American lobsters (*H. americanus*), European lobsters (*H. gammarus*), and spiny lobsters (*P. argus*) are primarily caught by traps (also called pots or creels) (14–17), but trawling of lobsters is legal in U.S. waters, except for in Maine (18). The recently observed practice of displaced New England cod fishermen turning to trawling for lobster is causing a great concern for the future state of the lobster resource (18,19). Some spiny lobsters are also caught by trawl or by diving (13). Norway lobsters (*N. norvegicus*) are primarily caught by trawl (20).

Traditionally, in both Europe and North America, half-round (semicylindrical) traps, made with wooden laths, were the common type of lobster traps used (15,17,21,22). Regardless of its shape, each trap contains one or more funneled entrances made of wood or netting (21,22), with the spacing of the laths set to allow undersized lobsters to escape (23). Because European lobstermen have learned that the traditional North American parlor pot is much more productive than their traditional single-chamber pots, they have introduced parlor pots into the European lobster fishery (13,24); some of these parlor pots are extremely large and made of galvanized metal (25). Similarly, in addition to the traditional wooden traps, metal traps are now also used by North American lobstermen (15,26).

A lobster trap made of wood with synthetic twine, metal and wire, or plastic does not biodegrade after the trap becomes lost on the fishing grounds. Therefore, these traps ghost fish much longer than the conventional wooden lob-

42. V. W. Hays and R. L. Preston, "Nutrition and Feeding Management to Alter Carcass Composition of Pigs and Cattle," in H D. Hafs and R. G. Zimbelman, eds., *Low-Fat Meats Design Strategies and Human Implications*, Academic Press, San Diego, Calif., 1994, pp. 13–33.
43. R. L. Preston, "Livestock Technologies," in B. C. English, R. L. White, and L. H. Chuang, eds., *Crop and Livestock Technologies, RCA III Symposium*, Iowa State University Press, Ames, Iowa, 1997, pp. 115–126.
44. B K. Symonds and J. Orendorff, "Agriculture to be Changed by Emerging Biotechnology Age," *Feedstuffs* **70**, 12–18 (1998).

R. L. Preston
Texas Tech University
Lubbock, Texas

LOBSTER: BIOLOGY AND TECHNOLOGY

The animals referred to as lobsters are representative of a number of families of the decopod crustacea. The colors of lobsters are due to carotenoid pigments bound to proteins and deposited in the exoskeleton. These pigments are released from the proteins when the proteins are heat denatured during cooking, thus giving cooked lobster the characteristic reddish color (1).

There are more than 30 commercially exploited species of spiny lobsters alone. Description of distinguishing characteristics of species is beyond the scope of this article; refer to a review for guidance in lobster identification and world description (2). The families Galatheidae (squat lobster) and Scyllaridae (slipper or flat lobster) are minor contributors to world lobster catch (11 and 1%, respectively). On the other hand, the family Nephropinae (clawed lobsters) accounts for 71% of world commercial catch of lobster.

HOMARUS AMERICANUS

The American lobster has a carapace length of up to 210 mm (3) and is dark green with dark spotting above and yellowish color on the underside. These lobsters inhabit the shallow water of the Atlantic Coast of North America from shore to a depth of 480 m. Most commonly, they are found at depths of 4 to 5 m (4,5). These lobsters typically inhabit rocky areas but also make burrows under solid objects or in clay banks (4). They range from the Strait of Belle Isle, Newfoundland, to Cape Hatteras, North Carolina (4,6). References 3 and 7 give a thorough treatment of the biology of the species. This species accounts for 36% of the world lobster catch. *H. gammarus* (the European lobster) is biologically similar to *H. americanus* in all respects except distribution. It is a minor contributor in the world fishery, accounting for only 2% of the catch.

NEPHROPS NORVEGICUS

The Norway lobster (also known as scampi or Dublin Bay prawn) has an average carapace length of 30 mm for males and 20 mm for females at maturity; individuals grow as large as 70 mm (8). They live in burrows in muddy bottoms at depths of 15 to 820 m (9). Their range is from Iceland to the coast of Norway and from the Adriatic Sea south to the coast of Morocco. They are also found in the western Mediterranean (4). There are related species on the Brazilian coast and in the Indo-Pacific areas (10). In total, these species account for 34% of the world catch.

PALINURIDAE

The family Palinuridae (langouste or spiny lobster) is characterized by strong spines on the carapace and/or on the abdominal segments. Pincers, when present on the front legs, are small. European species belong to the genera *Panulirus* and *Jasus* (9,10). They are distributed throughout tropical and subtropical oceans (see Reference 1 for a map). For a discussion of geographic and vertical distribution of Palinurid lobsters, see Reference 11. Phyllosoma larvae hatch from eggs carried by females. These larvae are extremely flat, with long divided legs and delicate mouthparts. After about 12 molts while drifting in the ocean, the juveniles become puerile larvae; they swim actively and approach the coast. These larvae subsequently molt into the juvenile form of the adult at about 12 months of age (12). For biological details on one species (*P. argus*) see Reference 5. Palinuridae account for 17% of the world catch. The annual catch is about 80,000 t, mostly in Australia (12,000 t), Cuba (11,000 t), and Brazil (9000 t) (13).

CATCHING METHODS

American lobsters (*H. americanus*), European lobsters (*H. gammarus*), and spiny lobsters (*P. argus*) are primarily caught by traps (also called pots or creels) (14–17), but trawling of lobsters is legal in U.S. waters, except for in Maine (18). The recently observed practice of displaced New England cod fishermen turning to trawling for lobster is causing a great concern for the future state of the lobster resource (18,19). Some spiny lobsters are also caught by trawl or by diving (13). Norway lobsters (*N. norvegicus*) are primarily caught by trawl (20).

Traditionally, in both Europe and North America, half-round (semicylindrical) traps, made with wooden laths, were the common type of lobster traps used (15,17,21,22). Regardless of its shape, each trap contains one or more funneled entrances made of wood or netting (21,22), with the spacing of the laths set to allow undersized lobsters to escape (23). Because European lobstermen have learned that the traditional North American parlor pot is much more productive than their traditional single-chamber pots, they have introduced parlor pots into the European lobster fishery (13,24); some of these parlor pots are extremely large and made of galvanized metal (25). Similarly, in addition to the traditional wooden traps, metal traps are now also used by North American lobstermen (15,26).

A lobster trap made of wood with synthetic twine, metal and wire, or plastic does not biodegrade after the trap becomes lost on the fishing grounds. Therefore, these traps ghost fish much longer than the conventional wooden lobster traps with cotton netting at the ends (15,27). Use of nonbiodegradable traps has led to state regulations regarding both biodegradable panels and escape vents (14,23).

Before being lowered to the bottom, each lobster trap is weighted with a flat stone or concrete and baited with fish such as herring, mackerel (28), or flatfish (29). At least with spiny lobsters, traps with live sublegal-sized lobsters used as decoys caught three times as many lobsters as did traps without lobster decoys (16). All lowered traps are attached to brightly painted wooden or plastic floats on the surface. The length of time the traps are left on the bottom depends on the location, the number of traps a fisherman has, the rate of deterioration of the bait, and weather (21). Maximum productivity is obtained from daily lifting of traps (14), and mechanical hauling is often a necessity (30).

In addition to being caught by standard baited wooden or plastic traps lowered to the bottom (31), by trawls, or by divers using spears (13), spiny lobsters are also caught commercially by Caribbean fishermen using concrete slabs kept a few inches off the bottom (32). Seeking shelter, the lobsters go underneath the concrete slabs. The fisherman dives to the bottom, taps the concrete slab with a gaff, and grabs the lobsters as fast as they crawl out from underneath the concrete (32).

With Norway lobsters, which live mainly in or on muddy bottoms (20), the spread of the trawl is a more important catching factor than the trawl's headline height (33). Consequently, the towing of two trawls simultaneously from a single stern trawler has proved to be a very efficient method of catching Norway lobsters (33).

As soon as lobsters are caught, the length of the carapace is measured (15,23,34) and the sublegal-sized lobsters are returned to the ocean. With lobsters that have large claws, the claws are immobilized using twine, string, or rubber bands, not wooden pegs (35,36).

On small boats on which live holding is not practical, North American (or European) lobsters are kept as cool and damp as possible so that the lobsters are all alive when the fishermen return to port (36) a few hours later. Larger North American lobster boats often have tanks of seawater that will keep the lobsters alive until they return to port (37). Similarly, a large proportion of spiny lobsters (*P. argus*) are also kept onboard in wells of seawater and landed alive when the vessels return to port (38). Large Norway lobsters (≦30 lobster/kg) are often landed alive and sold whole, whereas those that are too small or damaged to be sold whole are tailed onboard the vessel and then washed and stored in ice (39).

LIVE HOLDING

North American lobsters, European lobsters, spiny lobsters, and large Norway lobsters may be held live onboard commercial fishing vessels (37–39). Spiny lobsters and some Norway lobsters are usually frozen before distribution (13,19,40,41). However, the vast majority of North American and European lobsters are sold live (at the retail level) (13,41). Several systems may be used to hold live lobsters (19,41,42). For example, at the first tier of distribution, the systems range from inexpensive tidal ponds with little control over the conditions to Clearwater Fine Food's $13.5 million facility at Arachat, Nova Scotia, which holds 1.5 million lb of lobsters, with each lobster in its own environmentally controlled compartment (26). Individual compartments prevent disease and cannibalism, while controlled environmental systems promote hibernation and reduce the need for food (26). Apart from storing the animals, live holding also allows for the depuration of taint from lobster meat (43).

Regardless of the system used to hold North American and European lobsters live, one should always (*1*) check animals daily and remove weak or damaged lobsters; (*2*) turn over stock on a rotating system; (*3*) avoid toxic material in construction and operation; (*4*) band or tie claws securely; (*5*) check salinity and temperature regularly; (*6*) keep water temperature at 4.5 to 10°C; (*7*) change water frequently or use an efficient filter system; (*8*) keep tanks shaded and away from bright lights; (*9*) when preparing artificial seawater, use correct weight and chemical composition; and (*10*) where possible, keep lobsters in a single layer (41). In contrast, regardless of the system used to hold North American and European lobsters live, one should never (*1*) attempt to store soft, weak, or damaged lobsters; (*2*) handle lobsters roughly; (*3*) feed lobsters in tanks, which creates more problems than it solves; (*4*) cut or peg claws to prevent fighting; (*5*) cause sudden changes in temperature or salinity; (*6*) expose lobsters to extremes of temperatures; (*7*) overload storage systems; or (*8*) overfill tanks with water, because shallow water is adequate (41).

SHIPPING

Spiny lobsters and some Norway lobsters are usually shipped frozen (13,19,44) and should be kept at or below −18°C during primary (wholesale) distribution and at or below −15°C during final distribution to smaller retail stores (45). Norway lobsters shipped fresh should be adequately chilled before being shipped and surrounded with ice inside open plastic boxes or inside covered insulated boxes (45,46). The distribution of high-quality fresh or frozen lobsters requires an integrated system of chilled or cold storage distribution centers, refrigerated vehicles, and, at the retail level, refrigerated cabinets (47).

During the 1980s, the shipment of live lobsters (American, European, and some Norway) great distances increased dramatically (47–49). This shipment is accomplished either by consumers, at their point of departure, purchasing a live lobster that is packed with seaweed and dry ice in a styrofoam container, protected by a double corrugated box, and transported live by the consumer to its final destination (48), or commercially when live lobsters are bought and transported live via commercial passenger or freight airlines to their final destination, where they are sold live commercially (47,49). For live lobsters to be of good quality when sold at their final destination, it is necessary to get lobsters from a fisherman; keep the lobsters in a holding tank for at least 24 h; separate the weak and injured lobsters; shortly before shipment, store the strong and healthy lobsters in an insulated, leakproof, lightweight, but strong container along with gel ice (a frozen chemical solution in plastic bags) and Kem-Pak (a crepe cellulose fiber blanket) or wooden shavings; store the unsealed container in a cold storage room for at least 1 h; insulate, seal, and secure the container with double strapping; ship the container; and, on reaching the final destination, remove dead or weak lobsters and place the lobsters that are in good condition in tanks of recirculating seawater (47,49). Critical factors in the distribution of live lobsters are the condition of the lobsters before shipment, maintenance of a low storage temperature of 1 to 7°C, maintenance of at least 70% relative humidity, and prevention of injury to lobsters (49). The assurance of high quality standards within the commercial lobster industry involves the utilization of production plans based on hazard analysis critical control point (HACCP) (50,51,52).

PROCESSING

American and European lobsters are primarily sold live, with some sold frozen, a few sold fresh, and a very small number canned (19), but the production of lobster product frozen with or without brine has been increasing dramatically (18,53). The quality of frozen lobster has been reported to be good for at least 9 months of frozen storage (54). Spiny lobsters are often marketed in the United States as frozen raw tails and in Asia as live or fresh whole cooked spiny lobster (13,55). In the United Kingdom, Norway lobsters are almost always sold as frozen peeled tails, whereas in the rest of Europe, they are sold whole (live, freshly cooked, or cooked and frozen) (44).

Because live lobster is normally cooked well immediately before it is eaten, it does not cause any health problems. Canned lobster products that are thermally processed inside hermetically sealed containers are also safe. However, any lobster products that are not normally cooked before being consumed present a potential health problem because of postprocessing contamination with bacteria, particularly *Listeria monocytogenes* (56,57). Consequently, the Canadian Inspection Services Branch has published *Good Manufacturing Practices* (58) for frozen ready-to-eat lobster products, which will help ensure the acceptability of such products (59). Anyone who produces any ready-to-eat lobster products should follow these or similar good manufacturing practices. References 50 through 52 provide more detailed information concerning the safety of seafood and utilization of HACCP principles.

International codes of practice regarding the handling of lobsters at sea and onshore have been published (60), as have international standards for quick-frozen lobster (61) and general processing procedures for Norway lobster (62).

More than 12 alternatives to live lobster (such as fillets, medallions, tails, cocktail claws, whole frozen, and whole cooked) are offered by producers in Maine and Canada (19). In addition, lobster paté or bites have been developed from extracted lobster meat (63), lobster Mornay is being commercially produced in Australia (64), and a lobster bisque has been developed in Denmark (65).

AQUACULTURE

Juvenile and adult Palinuridae exhibit little aggression or cannibalism even under high densities. However, at the early stage (phyllosoma), they are difficult to rear. Scientists in New Zealand have recently succeeded in rearing one species of rock lobster (*J. edwardsii*) to the puerile stage in the laboratory (66), thus increasing hopes of aquaculture of the species. Western rock lobsters have been reared from puerile to commercial size, and optimum conditions for growth have been established (67). The pond culture of juvenile rock lobsters caught in the wild has been reported (68).

Homarid lobster larvae are easily cultured, have a short development time, and can be hatched at any time of the year. They are, however, cannibalistic and must be segregated, thereby raising the costs. Nutrition is also a major problem. Lobsters normally eat a varied diet of seaweed, small mollusks, and crustacea. Such a diet is too expensive and impractical to provide on a commercial basis (69). For techniques of homarid lobster culture, see References 12, 70, 71, and 72.

CRAYFISH

Members of the crayfish group are all freshwater species distributed worldwide but are most abundant in temperate climates (73,74). More than 500 species and subspecies are recognized to belong to three families (Ascidae, Parastacidae, and Cambaridae). They range in size from 2 to 50 cm total length (73). More than 300 species are found in North America. In Europe, native crayfish were decimated by a fungal disease introduced around 1880. Subsequently, disease-resistant North American species were successfully introduced into affected areas (74).

The signal crayfish (*Pacifastacus leniusculus*), which originated in California, is believed to have the most potential for farming in temperate climates (74), but other crayfish, such as the red claw crayfish (*Cherax quadricarinutus*), are reported to have good potential for aquaculture (75–77). However, presently, the single most commercially exploited species is the red swamp crayfish, *Procambarus clarkii*. This species accounts for about 85% of world production (78). Most commercial crayfish are produced in Louisiana in culture ponds and natural habitats (79). *Procrambarus* spp. burrow in the mud of pond or swamp banks during the summer as water levels recede. Females lay their eggs and attach them to their pleopods, where they remain until hatching in 2 to 3 weeks. In the fall, when water levels rise, adults and the young of the year emerge from the burrows and into the water. The young of the year grow rapidly and can attain marketable size (65 mm total length) in 2 to 3 months (80). More than 60,000 ha of culture ponds are estimated to be in production in the United States, mostly in Louisiana. The annual natural and cultivated production in the United States is about 66,000 t, and an additional 10 t are produced in Canada. This combined total is about 80% of total world production (78).

Both farmed crayfish (November to June) and wild crayfish (March to June) are caught (usually 4 to 6 days per week) using stand-up pillow, pyramid, or barrel traps baited with natural (fish) bait or formulated artificial bait (81–83). Once harvested, 40 to 50 lb of crayfish are tightly packed inside onion sacks, protected against both excess heat and dehydration, and then transported to the processing plant, where they are processed immediately or stored live in coolers (82). The main crayfish products are live crayfish (unwashed, washed, or purged by being starved for 12 to 48 h to rid them of waste), fresh whole boiled crayfish, fresh crayfish tail meat (fat-on or washed tail meat), frozen whole boiled crayfish, frozen fat-on or washed tail meat, and frozen soft-shell crayfish (84). Information on harvesting and processing crayfish, including soft-shell crayfish, is found in References 82 through 85.

BIBLIOGRAPHY

1. B. F. Phillips, J. S. Cobb, and R. W. George. "General Biology" in J. S. Cobb and B. F. Phillips, eds., *The Biology and Management of Lobsters*, Vol. 1, Academic Press, New York, 1980, pp. 1–82.
2. A. B. Williams, "Lobsters—Identification, World Distribution and the U.S. Trade," *Marine Fisheries Revue* **48**, 1–36.
3. F. H. Herick, "The American Lobster: A Study of Its Habits and Development," *Bulletin of the U.S. Fisheries Commission*, 15 (1895).
4. L. B. Holthius, "Lobsters of the Superfamily Nephrppidae of the Atlantic Ocean (Crustacacae: Decopoda)," *Bulletin of Marine Science* **24**, 732–884 (1974).
5. A. B. Williams, *Shrimps, Lobsters and Crab of the Atlantic Coast of the Eastern United States, Maine to Florida*, Smithsonian Institution Press, Washington, D.C., 1984.
6. A. B. Williams and D. M. Williams, "Carolinian Records for American Lobster *Homarus americanus* and Tropical Swimming Carb, *Callinectus bocourti*. Postulated Means of Dispersal," *Fishery Bulletin* **79**, 192–198 (1981).
7. F. H. Herrick, "Natural History of the American Lobster," *Bulletin of the U.S. Bureau of Fisheries* **29**, 149–408 (1911).
8. A. S. Farmer, "Synopsis of Biological Data on the Norway Lobster *Nephrops norvegicus*," *FAO Fisheries Synopsis* **112**, 1–97 (1975).
9. J. S. Cobb and D. Wang, "Fisheries Biology of Lobster and Crayfishes," in A. J. Provenzano, ed., *The Biology of Crustacea*, Vol. 10 Academic Press, New York, 1985, pp. 168–230.
10. M. Burton, *The New Larousse Encyclopedia of Animal Life*, Bonanza Books, New York, 1981.
11. R. W. George and A. R. Main, "The Evolution of Spiny Lobster (*Palinuridae*): A Study of Evolution in the Marine Environment." *Evolution* **21**, 803–821 (1967).
12. P. R. Walne, "The Potential for the Culture of Crustracea in Salt Water in the United Kingdom," *Laboratory Leaflet* **40**, Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Lowestoft, United Kingdom, 1977.
13. "Spiny Lobster. 1996 Buyer's Guide," *Seafood Leader* **16**, 197–204 (1996).
14. D. B Bennett and S. R. J. Lovewell, "The Effects of Pot Immersion Time on Catches of Lobsters (*Hommarus gammarus*) in the Welsh Coast Fishery," *Fisheries Research Technical Report No. 36*, Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Lowestoft, United Kingdom, 1977.
15. N. Griffen, "Closeup on Lobster," *National Fisherman* **69**, 18–21 (1988).
16. D. W. Heatwole, J. H. Hunt, and F S. Kennedy, Jr., "Catch Efficiencies of Live Lobster Decoys and Other Attractants in the Florida Spiny Lobster Fishery," *Florida Marine Research Publication No. 44*, Florida Department of Natural Resources, Bureau of Marine Research, St. Petersburg, Fla., 1988.
17. A. Spence, "Creels, Pots, Their Treatment and Materials Used in Construction," in A. Spence, ed., *Crab and Lobster Fishing*, Fishing News Books, London, United Kingdom, 1989, pp. 73–90.
18. "American Lobster. 1995 Buyer's Guide," *Seafood Leader* **15**, 155–166 (1995).
19. "American Lobster. 1997 Buyer's Guide," *Seafood Leader* **17**, 98–107 (1997).
20. A. Bjordal, "The Behaviour of Norway Lobster Towards Baited Creels and Size Selectivity of Creels and Trawl," *Fish. Dir. Skr. Ser Hav. Unders.* **18**, 131–137 (1986).
21. J. T. Everett, "Inshore Lobster Fishing," U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, *Fishery Facts* **4**, 1972.
22. R. Stewart, *A Living From Lobster*, Fishing News Books, London, United Kingdom, 1971.
23. *Atlantic Fisheries Regulations*, Canada Department of Fisheries and Oceans, Ottawa, Ontario, 1985.
24. S. R. Loveweil, A. E. Howard, and D B. Bennett, "The Effectiveness of Parlor Pots for Catching Lobsters (*Hommarus gammarus*) and Crabs (*Cancer pagurus*)," *J. Cons. Int. Explor. Mer.* **44**, 247–252 (1988).
25. H. Allen, "Giant Pots From West Coast Man . . . ," *Fishing News* 32–33 (Aug. 25, 1989).
26. J. Barnett, "Make it a Lobster Summer," *Seafood Business* 90–106 (July/August 1988).
27. P. Lazarus, "Resistance to Lobster Escape Panels Slows Down Legislation," *National Fisherman* **69**, 69–71 (1988).
28. *The Atlantic Lobster Story*, Clearwater Lobster Ltd., Halifax, Nova Scotia, Canada
29. A. Spence, "Bait, Different According to Season," in A. Spence, ed., *Crab and Lobster Fishing*, Fishing News Books, London, United Kingdom, 1989, pp. 116–121.
30. A. Spence, "Hauling Equipment and Other Auxiliary Controls," in A. Spence, ed., *Crab and Lobster Fishing*, Fishing News Books, London, United Kingdom, 1989, pp. 56–63.
31. C. Piatt, "Florida Fisherman Are Slow to Adopt to Plastic Traps," *National Fisherman* **69**, 66–68 (1988).
32. W. E. Garrett, "LaRuta Maya," *National Geographic* **176**, 424–479 (1989).
33. T. Wray, "Stern Trawlers Prove Multi-rigs," *Fishing News International*, 26 (June 1989).
34. *Guide to the Federal Management Regulations for Lobster*, The New England Fishery Management Council, Saugus, Mass., 1989.
35. H. D. DeBoer and J. D. Castell, "A Study of Various Devices Which Can Be Used to Immobilize Lobster Claws," *Canadian Industry Report of Fisheries and Aquatic Sciences* **129** (1981).
36. A. Spence, "Marketing, Care of the Catch," in A. Spence, ed., *Crab and Lobster Fishing*, Fishing News Books, London, United Kingdom, 1989, pp. 126–136.
37. M. Crowley, "Shafmaster's Log Low Lobster Boats Are Steady as a Rock," *National Fisherman* **67**, 34–36 (1986).
38. B. H. Lamadrid and A. W. Blanco, "Handling of Live Lobsters in Cuba," *Infofish Marketing Digest* **6**, 44–46 (1986).

39. "Nephrops Rising to Fame," *Seafood International* **3**, 50–55 (1988).

40. "Live Tanks Nourish Profits," *Seafood Leader* 171–181 (Fall 1987).

41. P. A. Ayres and P. C. Wood, "Live Storage of Lobsters," *Laboratory Leaflet No. 37*, Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Lowestoft, United Kingdom, 1977.

42. "Selling Seafood Live," *Seafood International* **3**, 37–43 (1988).

43. U P. Williams et al., "Tainting and Depuration of Taint by Lobsters (*Homarus americanus*) Exposed to Water Contaminated With a No. 2 Fuel Oil: Relationships with Aromatic Hydrocarbon Content in Tissue," *J. Food Sci.* **54**, 240–243, 257 (1989).

44. "Norway Lobster (*Nephrops norvegicus*), Buyer's Guide," *Seafood Leader* **7**, 214–220 (1987).

45. "Seafood on the Move," *Seafood International* **2**, 33–35 (1987).

46. "More Seafood Takes to the Road . . . as Specialist Distribution Steps Up," *Seafood Processing and Packaging* **2**, 19–25 (1988).

47. "Keep It Cool, Clean and Moving," *Infofish International* **6**, 24–28 (1988).

48. "Lobsters Take-off at U.S. Airports," *Seafood International* **2**, 17–18 (1987).

49. H. Lisac, "A Note on the Live Shipment of Live Lobsters," *Infofish Marketing Digest* **4**, 38–40 (1986).

50. S. Subasinghe, "Handling and Marketing Aquaculture Products," *Infofish International* **3**, 44–51 (1996).

51. E. S. Garrett, M. J. Jahncke, and J. M. Tennyson, "Microbiological Hazards and Emerging Food-Safety Issues Associated With Seafood," *J. Food Prot.* **60**, 1409–1415 (1997).

52. National Advisory Committee on Microbiological Criteria for Foods, "Hazard Analysis and Critical Control Point Principles and Application Guidelines," *J. Food Prot.* **61**, 762–775 (1998).

53. "Lobster Update," *Seafood Leader* **16**, 211 (1996).

54. "Freezing in a Flash," *Seafood Leader* **13**, 98–100 (1993).

55. "Spiny Lobster. 1993 Buyer's Guide," *Seafood Leader* **13**, 157–162 (1993).

56. R. Fitzgerald, "Listeria hysteria," *Seafood Leader* **6**, 232–233 (1988).

57. S. D Weagant et al., "The Incidence of *Listeria* Species in Frozen Seafood Products," *J. Food Prot.* **51**, 655–657 (1988).

58. Department of Fisheries and Oceans, Inspection Services Branch, *Good Manufacturing Practices (GMP)—Lobster Processing*, Document 890410-58-G-I-L, Ottawa, Ontario, Canada, 1988.

59. H. H. Huss, "Microbiological Quality Assurance in the Fish Industry," *Infofish International* **5**, 36–37 (1989).

60. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, *Thirteenth Session of the Codex Committee on Fish and Fishery Products, Bergen, Norway, May 7–11, 1979. Recommended International Code of Practice for Lobsters*, 1979.

61. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, "Codex Standard for Quick Frozen Lobsters," Codex Standard 95, in *Codex Alimentarius*, Vol. V, FAO/WHO, Rome, Italy, 1981.

62. J. C. Early, "Process Norway Lobsters," *Torry Advisory Note No. 29*, UK Ministry of Technology, Torry Research Station, 1965.

63. D. D. Duxbury, "Lobster Paté or Bites for Seafood Hors d'oeuvres," *Food Processing* **47**, 68–70 (1986).

64. "Constantia Food's Shellfish Microwave Appetisers," *Seafood International* **3**, 23 (1988).

65. P. Moustgaard, "Denmark's Changing Markets," *Seafood International* **4**, 24–29 (1989).

66. J. Illingworth et al., "Upwelling Tank for Culturing Rock Lobster (*Jasus edwardsii*) Phyllosomas," *Mar. Freshwater Res.* **48**, 911–914 (1997).

67. R. G. Chittleborough, "Review of Prospects for Rearing Rock Lobsters," *Australian Fish*, 4–8 (April 1974).

68. D. O. C. Lee and J. F. Wickens, *Crustacean Farming*, Blackwell Scientific Publications, Oxford, United Kingdom 1992.

69. E. Edwards, "The Future for Farmed Lobster," *Seafood International* **4**, 33–34 (1989).

70. T. W. Beard, P. R. Richards, and J. F. Wickins, "The Techniques and Practicability of Year-round Production of Lobsters, *Homarus gammarus* (L), in Laboratory Recirculation Systems," *Fisheries Research Technical Report 79*, Directorate of Fisheries Research, Lowestoft, United Kingdom, 1985.

71. A. J. Provenzano, Jr., "Commercial Culture of Decapod Crustacians," in A. J. Provenzano, ed., *The Biology of Crustacea*, Vol. 10, Academic Press, New York, 1985, pp. 269–323.

72. J. C. VanOlst, J. M. Carlberg, and J. T. Hughes, "Aquaculture," in J. S. Cobb and B. F. Phillips, eds., *The Biology and Management of Lobsters*, Vol. 2, Academic Press, New York, 1980, pp. 333–384.

73. H. H. Hobbs, Jr., "Crayfish Distribution, Adaptive Radiation and Evolution," in D. M. Holdich and R. S. Lowery, eds., *Freshwater Crawfish: Biology, Management, and Exploitation*, Croom Helm, London, United Kingdom, 1988, pp. 52–82.

74. R. E. Groves *The Crayfish: Its Nature and Nurture*, Fishing News Books Ltd, Surrey, United Kingdom, 1985.

75. M. C. Rubino, "Economics of Red Claw (*Cherax quadricarinatus*, von Martens, 1868) Aquaculture," *Journal of Shellfish Research* **11**, 157–162 (1992).

76. J. V. Huner, "An Overview of the Status of Freshwater Crawfish Culture," *Journal of Shellfish Research* **14**, 539–543 (1995).

77. D. B. Rouse, "Australian Crayfish Culture in the Americas," *Journal of Shellfish Research* **14**, 569–572 (1995).

78. J. V. Huner, "Overview of International and Domestic Freshwater Crawfish Production," *Journal of Shellfish Research* **8**, 259–265 (1989).

79. L. W. de la Bretonne, Jr., and R. P. Romaire, "Commercial Crawfish Cultivation Practices: A Review," *Journal of Shellfish Research* **8**, 267–275 (1989).

80. J. W. Avault, Jr. and J. V. Huner, "Crawfish Culture in the United States," in J. V. Huner and E. E. Brown, eds., *Crustacian and Mollusk Aquaculture in the United States*, AVI, Westport, Conn., 1985, pp. 1–54.

81. "Crawfish, *Cambaridae* and *Astaciadae*," *Seafood Leader* **9**, 262–270 (1989).

82. M. W Moody, "Processing of Freshwater Crawfish: A Review," *Journal of Shellfish Research* **8**, 293–301 (1989).

83. R. P. Romaire, "Overview of Technology Used in Commercial Crawfish Aquaculture," *Journal of Shellfish Research* **8**, 281–286 (1989).

84. "Crayfish. Seafood Buyer's Guide," *Seafood Leader* **14**, 213–221 (1994).

85. D. D. Cully and L. Duobins-Gray, "Soft-shell Crawfish Production Technology," *Journal of Shellfish Research* 8, 287–291 (1989).

J. R. BOTTA
Moncton Food Quality Investigation, Inc.
Moncton, New Brunswick
Canada

J. W. KICENIUK
Fernleigh House
Halifax, Nova Scotia
Canada

LOW-ACID AND ACIDIFIED FOODS

A low-acid food is a food that has a pH value greater than 4.6; an acid food is a food with a natural pH of 4.6 or below; and an acidified food is a low-acid food that has been treated to reduce the pH to 4.6 or below. The pH of a food is one of the most important parameters in determining the types of microorganisms that are capable of growing in the product. This in turn determines the types of processes that are appropriate to preserve that food. Although the terms *low-acid food* and *acidified food* do not in the strictest sense refer to canned foods, the terms are most frequently applied by the food industry to canned food products, and this will be the focus here. It should be noted that pH is not the only factor involved in preservation of canned foods. Reduced water activity, preservatives, and other factors may also be used in combination with heat to preserve foods; for some foods a heat treatment is not required because of the combinations of preservation. However, this article focuses on traditional low-acid and acidified canned foods.

PH OF VARIOUS FOODS

The pH of selected fresh, fermented, and commercially canned foods is shown in Table 1. In general, the pH of most fruits falls in the acid range (notable exceptions include bananas and cantaloupes), whereas the pH of meat, poultry, fish, and vegetables falls in the low-acid range. Fermentation may be used to reduce the pH of a low-acid food, as is done with sauerkraut, pickles, and yogurt. For other foods the pH is lowered by direct addition of acid (marinated vegetables such as mushrooms or three-bean salad). A variety of foods are formulated with low-acid and acid components. In such cases the acid component may be used to acidify the low-acid component. An example of this might be a spaghetti sauce in which the tomato component (tomatoes, tomato paste, and/or tomato sauce) acidifies the low-acid components (meat, onions, peppers).

MICROBIOLOGY OF LOW-ACID AND ACIDIFIED FOODS

All raw foods contain microorganisms. These organisms will grow and spoil foods unless the organisms are properly controlled. Even more important, among the microorganisms that can be found on raw foods are pathogens. Thus,

Table 1. pH Range of Selected Fresh, Fermented, and Commercially Canned Foods

Food	Approximate pH range
Apples	2.9–3.5
Apple juice	3.3–3.5
Apple sauce	3.4–3.5
Apricots	3.5–4.0
Asparagus	5.0–6.1
Bananas	4.5–5.2
Beans	
Baked	4.8–5.5
Green	4.9–5.5
Lima	5.4–6.5
Soy	6.0–6.6
Beans, with pork	5.1–5.8
Beef	5.3–6.2
Beef, corned, hash	5.5–6.0
Beers	4.0–5.0
Beets, whole	4.9–5.8
Blackberries	3.0–4.2
Blueberries	3.2–3.6
Boysenberries	3.0–3.3
Bread	
White	5.0–6.0
Date and nut	5.1–5.6
Broccoli	5.2–6.0
Brussel sprouts	6.3–6.6
Butter	6.1–6.4
Cabbage	5.2–6.3
Cantaloupe	6.2–6.5
Carrots	4.9–6.3
Catfish	6.6–7.0
Cauliflower	6.0–6.7
Celery	5.7–6.0
Cheese (most)	5.0–6.1
Camembert	6.1–7.0
Cottage	4.1–5.4
Parmesan	5.2–5.3
Roquefort	4.7–4.8
Cherries	3.2–4.7
Chicken	5.5–6.4
Chicken with noodles	6.2–6.7
Chop Suey	5.4–5.6
Cider	2.9–3.3
Clams	5.9–7.1
Codfish (Canned)	6.0–6.1
Corn	
On the cob	5.9–6.8
Cream style	5.9–6.5
Whole grain	
Brine packed	5.8–6.5
Vacuum packed	6.0–6.4
Crab	6.8–8.0
Crab apples—spiced	3.3–3.7
Cranberry	
Juice	2.5–2.7
Sauce	2.3
Currant juice	3.0
Dates	6.2–6.4
Duck	6.0–6.1
Dry sausages	4.4–5.6
Egg white	7.6–9.5
Egg yolk	6.0–6.3
Fermented vegetables	3.9–5.1
Figs	4.9–5.0
Frankfurters	6.2

Table 1. pH Range of Selected Fresh, Fermented, and Commercially Canned Foods *(continued)*

Food	Approximate pH range
Fruit cocktail	3.6–4.0
Ginger ale	2.0–4.0
Gooseberries	2.8–3.1
Grapefruit	
Juice	2.9–3.4
Pulp	3.4
Sections	3.0–3.5
Grapes	3.3–4.5
Haddock	6.2–6.7
Halibut	5.5–5.8
Ham, spiced	6.0–6.3
Hominey, lye	6.9–7.9
Honey	6.0–6.8
Huckleberries	2.8–2.9
Jam, fruit	3.5–4.0
Jellies, fruit	3.0–3.5
Lemons	2.2–2.4
Juice	2.2–2.6
Lettuce	6.0–6.4
Limes	1.8–2.0
Juice	2.2–2.4
Loganberries	2.7–3.5
Mackerel	5.9–6.2
Maple syrup	6.5–7.0
Mayonnaise	3.8–4.0
Milk	
Cow, whole	6.0–6.8
Evaporated	5.9–6.3
Molasses	5.0–5.4
Mushrooms	6.0–6.5
Olives, ripe	5.9–7.3
Onions	5.3–5.8
Oranges	2.8–4.0
Juice	3.0–4.0
Oysters	5.9–6.7
Peaches	3.1–4.2
Pears	3.4–4.7
Peas	5.6–6.8
Pickles	
Dill	2.6–3.8
Sour	3.0–3.5
Sweet	2.5–3.0
Pimentos	4.3–5.2
Pineapple	
Crushed	3.2–4.0
Sliced	3.5–4.1
Juice	3.4–3.7
Plums	2.8–4.6
Pork	5.3–6.4
Potatoes	
White, whole	5.4–6.3
Sweet	5.3–5.6
Potato salad	3.9–4.6
Prune juice	3.7–4.3
Pumpkin	5.2–5.5
Raspberries	2.9–3.7
Rhubarb	2.9–3.3
Salmon	6.1–6.5
Sardines	5.7–6.6
Sauerkraut	3.1–3.7
Juice	3.3–3.4
Scallops	6.8–7.1
Shrimp	6.8–8.2
Soda crackers	6.5–8.5
Soups	
Bean	5.7–5.8
Beef broth	6.0–6.2
Chicken noodle	5.5–6.5
Clam chowder	5.6–5.9
Duck	5.0–5.7
Mushroom	6.3–6.7
Noodle	5.6–5.8
Oyster	6.5–6.9
Pea	5.7–6.2
Tomato	4.2–5.2
Turtle	5.2–5.3
Vegetable	4.7–5.6
Spinach	4.8–6.8
Squash	5.0–5.3
Strawberries	3.0–4.2
Tomatoes	3.7–4.7
Juice	3.9–4.7
Tuna	5.9–6.1
Turkey	5.6–6.0
Turnips	5.2–5.6
Turnip greens	5.4–5.6
Vegetable	
Juice	3.9–4.3
Mixed	5.4–5.6
Vinegar	2.4–3.4
Walnuts	5.4–5.5
Water	
Distilled, CO_2	6.8–7.0
Mineral	6.2–9.4
Sea	8.0–8.4
Whiting	6.2–7.1
Wines	2.3–3.8
Yogurt	3.8–4.2

Source. Reprinted from Ref. 1 with permission from *Dairy, Food and Environmental Sanitation.* Copyright held by the International Association of Milk, Food and Environmental Sanitarians, Inc. Gravani is with Cornell University.

to ensure foods are safe, these pathogens must be destroyed or reduced to levels at which they pose no hazard to public health. In some instances, controlling the growth of pathogens that may be present will render the food safe to eat. The thermal processes designed for low-acid foods, and the pH along with a heat treatment for acid and acidified foods, destroy pathogens and spoilage organisms capable of growing in the product under normal distribution conditions. The can or jar prevents recontamination of the food after it has been heat treated. Thus these products are rendered shelf stable, and they are considered *commercially sterile*. They cannot be called *sterile* because some of these products may contain viable microorganisms that cannot grow under normal conditions. For example, low-acid canned vegetables may contain spores of thermophilic bacteria that cannot grow unless temperatures exceed 100°F for several days. Canned fruits may contain the spores of *Bacillus* or *Clostridium* species that cannot grow because the pH of the product is too low. Knowledge of the microorganisms that may be present on a product and their characteristics is necessary to establish an appropriate process to prevent health hazards and spoilage of canned foods.

It is not possible to cover the field of food microbiology here. Readers should consult additional texts for detailed information on this subject (2–4). This article briefly covers, in general terms, those organisms of concern in low-acid and acidified foods.

Low-Acid Canned Food Microbiology

While low-acid foods may be contaminated with viruses, yeast, molds, parasites, and bacteria, it is the spore-forming bacteria that are of greatest concern from the standpoint of sterilization. The bacterium most important for low-acid canned foods is *Clostridium botulinum*, which produces spores that survive boiling. When the vegetative cells of *C. botulinum* grow, they produce potent neurotoxins (Types A, B, and E are of greatest significance) that can cause death if consumed. *C. botulinum* Type A and proteolytic strains of Type B have decimal reduction (D) values at 250°F (121°C) of 0.10 to 0.20 minutes (5), the D value being the time in minutes at the specified temperature to destroy 1 log, or 90%, of the population. Thus, thermal processes for low-acid canned foods must be designed to destroy the spores of this organism. By convention, a process equivalent to 12D is considered adequate to protect the public health. However, there are other clostridia important in food spoilage that produce more heat-resistant spores. To produce shelf-stable products, processes are therefore designed to destroy the spores of organisms such as PA 3679, an organism similar to *Clostridium sporogenes*, that has a $D_{250°F}$ value of 0.50 to 1.50 minutes (5). The heat treatments required to render low-acid foods commercially sterile will destroy viruses, yeast, molds, parasites, and bacterial pathogens such as *Salmonella* and *Escherichia coli* O157:H7.

Acid and Acidified Food Microbiology

Since *C. botulinum* spores cannot germinate and grow in foods at pH values below approximately 4.8 (regulations setting pH 4.6 as the cutoff between low-acid and acid/acidified include a safety factor), heat treatments for acid and acidified foods need not destroy the spores of this organism. Heat treatments for products with pH values <4.0 are designed to inactivate yeast, molds, and lactic acid bacteria that can cause spoilage. The most resistant of these organisms have $D_{150°F}$ values on the order of 1.0 min. A notable exception are the ascospores of the heat-resistant molds such as *Byssochlamys, Neosartorya*, and *Talaromyces* (6–8). These organisms may have $D_{194°F}$ values of 1 to 12 min, which allow them to survive commercial processes; fortunately, such spoilage is rare.

For products with pH values between 4.0 and 4.6, there are spore-forming bacteria that can be important in spoilage. Of greatest importance are *Bacillus coagulans* and the butyric anaerobes such as *Clostridium pasteurianum* and *C. butyricum*. Products in this pH range are usually given more severe heat treatments to prevent spoilage from these organisms. Recently, spoilage in fruit juices has been caused by spore formers of the newly recognized genus *Alicyclobacillus* (9). These organisms can grow and spoil products at pH 3.5, and they can survive the commercial processes for juice products. Studies to characterize and control these organisms are under way; as with heat-resistant molds, spoilage of this type is not common.

Although the ability of pathogens such as *Salmonella* and *E. coli* O157:H7 to grow in acid and acidified foods depends on the food, the pH, the acidulent, and other factors, it is not always necessary for these pathogens to grow to make someone sick. Thus it is important that these organisms be destroyed by a heat treatment. The pasteurization treatments given acid and acidified foods are more than adequate to kill these pathogens, as well as organisms such as the enteric protozoan parasite *Cryptosporidium*. In recent years, all three of these pathogens have caused outbreaks of foodborne illness as the result of consumption of contaminated, unpasteurized fruit juices (10).

THERMOBACTERIOLOGY

To properly design a thermal process for a product, one must know the heat resistance of the organisms of concern; these organisms were just discussed. Procedures for determining the heat resistance of a microorganism are similar, regardless of whether the organism is a spore former or a vegetative cell.

Heating Medium

The heat resistance of a microorganism is frequently determined in a buffer such as phosphate buffer because it is a homogeneous, easily reproducible liquid. Moreover, when the heat resistances of microorganisms are determined in the same buffer, they can be compared. However, it is well known that foods may affect microbial heat resistance; heat resistance may be either higher or lower in a food compared with phosphate buffer. For example, fat may be protective of organisms, resulting in increases in resistance. Heat resistance can increase with reduced water activity, and it will be affected by the pH of the heating medium. Thus, determining the heat resistance of a microorganism in a food more accurately predicts the thermal process requirements. However, food products are more difficult to work with than buffer.

Heating Methods

A variety of methods have been used for determining the heat resistance of a microorganism:

1. For liquids being heated at 100°C or below, a three-neck flask can be used. A thermometer is included in one neck of the flask, a stirrer in another, and the third is used to add microorganisms and to remove samples. This is a convenient, rapid method for determining the heat resistance of vegetative cells. Heating lag times are minimized because small volumes of inoculum are added to a large volume of preheated liquid, but splashing or flocculation may give erroneous results.

2. One of the most common methods uses thermal death time (TDT) tubes to which a given volume (usually 1–2 mL) of product containing the microorganism is added, and the tube is sealed and heated in an oil or water bath (depending on the heating temperatures). The procedure is useful for liquids and liquefied food homogenates. There are heating and cooling lag times (since heat must penetrate into the tube and heat the product) that must be considered, but a thermocouple in a replicate sample can be used to measure the temperature during heating and cooling to account for these lags in the TDT calculations. It is easy to heat many replicates at one time. Usually 10 tubes per time interval and five or six time intervals are heated at the same time at a single temperature, and the procedure is repeated at additional temperatures. In general, the TDT tubes will need to be opened and the contents subcultured in an appropriate growth medium or the food product. The procedure is suitable for vegetative cells and spores.
3. A similar method uses capillary tubes, which are much smaller versions of TDT tubes. Because of the small diameter and thickness of capillary tubes, heating and cooling lags are negligible. This procedure is limited to liquids, and the contents must be subcultured. The external surface of the tube must be sterilized prior to subculturing the contents, because the tube is generally crushed to release its contents. The procedure is suitable for vegetative cells and spores.
4. A specialized piece of equipment known as a thermoresistometer has been used to determine the heat resistance of microorganisms at very high temperatures (121–138°C in saturated steam and 121–260°C in superheated steam) for very short time intervals (seconds). The procedure is suitable for liquids or diluted food homogenates. Heating and cooling lag times are negligible, and the system allows for very precise timing, which is critical with high temperature–short time studies. There is a high initial cost for the equipment, and product must be subcultured for survivors. This procedure is suitable for spores.
5. Another specialized piece of equipment for TDT studies is the thermal death time retort. These retorts are used to heat either TDT tubes (containing liquid or food homogenates) or TDT cans (containing ground or homogenized foods) at temperatures of 100 to 121°C. Use of TDT cans closely simulates canning practices, and the cans can be incubated directly. Spoilage by gas producers is detected by swelling of the can. Heating and cooling lag times are substantial, and it is essential to account for the lethality that occurs by measuring the temperatures with a thermocouple inserted in replicate cans. The incubation time can be lengthy—several months to ensure all injured organisms are recovered. This procedure is suitable for spores.

Recovery of Survivors

The apparent heat resistance of an organism depends on the recovery medium, as well as the substrate in which it is heated. When testing the heat resistance of a microorganism in a particular food, recovery in the product is preferred, as this will reflect the environment the injured organisms would see after processing. This procedure measures whether or not there are organisms that can grow in the product after the heat treatment, not how many there are. Alternatively, the heated product can be subcultured to determine survivors. This may be either a qualitative procedure, where the heated product is transferred to a broth medium to determine if there are organisms that can grow, or a quantitative procedure, where the survivors in the heated product are enumerated. Use of a culture medium has several advantages. The media used generally allow better recovery of survivors, as the medium can be formulated to favor growth of the specific organism being tested, and components that enhance the recovery of injured organisms can be added. Also, organisms generally recover more quickly in the more favorable environment.

Thermal Death Time Calculations

An in-depth discussion of TDT calculations is not possible here. Readers are referred to works such as Ref. 5. The following formula is used to calculate the D value:

$$D_T = \frac{t}{\log A - \log B}$$

where t is the heating time (corrected for the come up time) at temperature T, A is the initial number of organisms, and B is the number of survivors. Alternatively, the *D* value can be determined by plotting the number of survivors at several time intervals against time on semilogarithm paper. (The *D* value is calculated from the slope of the best-fit line.) *D* values determined at several temperatures are plotted on semilogarithm paper against temperature (called a phantom TDT curve), and the *z* value (the change in the thermal death rate with temperature) is calculated from the slope of the line. Knowing the *D* and *z* values for an organism allows the calculation of the *D* value at any other temperature. The *F* value, or the number of minutes to destroy a given number of organisms at a specific temperature, can also be calculated. The minimum health sterilizing value for a low-acid food is generally a 12D value for *C. botulinum* (12 times the *D* value determined for *C. botulinum* spores in the specific food); the commercial sterility value is generally 5D for *C. sporogenes* (PA3679) spores.

REGULATIONS GOVERNING LOW-ACID AND ACIDIFIED FOODS

The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) has responsibility for meat, poultry, and egg products. The Food and Drug Administration (FDA) has responsibility for all other foods, including seafood and shell eggs. For details on the regulations, see the article titled CANNING: REGULATORY AND SAFETY CONSIDERATIONS.

FDA Regulations

The FDA's general regulatory requirements for foods ("Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food") are found in Title 21 of the *Code of Federal Regulations (CFR)* Part 110. Requirements for "Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers" are found in 21 *CFR* 113, and requirements for "Acidified Foods" in 21 *CFR* 114. Foods that have a water activity of 0.85 or less and those that are refrigerated or frozen are exempt from the low-acid and acidified food regulations, as are carbonated and alcoholic beverages, jams, jellies, preserves, dressings, and condiments. The FDA has recently mandated Hazard Analysis and Critical Control Point (HACCP) regulations for seafood and seafood products (21 *CFR* 123), although canned foods complying with 21 *CFR* 113 or 114 do not have to include critical control points for microbiological hazards. In addition, 21 *CFR* 108 describes the emergency permit system that can be imposed on processors who fail to comply with the regulations.

The regulations in 21 *CFR* 113 define low-acid foods and commercial sterility. They specify the proper design, controls, and instrumentation for all the common retorting systems and the practices necessary in the operation of these systems to ensure safety. The regulations require that records be kept of all coding, processing, and container closure inspections and that these be reviewed prior to shipment of product. The regulations also describe the actions to take when a process deviation has occurred. They require that the heat process be designed by qualified persons having expert knowledge of thermal processing requirements (processing authority). The term *processing authority* is not explicitly defined in the regulations, there are no specific criteria for qualifications, nor does FDA (or USDA) maintain a list of processing authorities. However, certain organizations, such as the National Food Processors Association (NFPA), are widely recognized by government agencies and the food industry as having the experience and expertise to serve in this capacity (11). Some food processing firms have individuals on staff who can serve as the processing authority, and a number of consultants are recognized as processing authorities. Another requirement is that all thermal processing operations be conducted under the supervision of an individual who has satisfactorily completed an FDA-approved course of instruction; these Better Process Control Schools, sponsored jointly by the FDA, the Food Processors Institute (FPI) (12), and key universities, are held around the country every year. Occasionally such courses are given to specific companies or overseas.

The regulations in 21 *CFR* 114 define acidified foods and describe procedures for acidification (described in the next section). The regulations require that the process be established by a processing authority, and they define the procedures to be followed in the event of a deviation from the scheduled process or the pH exceeds 4.6. They describe the methods to determine pH or acidity for acidified foods and the records that must be kept. Supervisors with responsibility for pH control and other critical factors must receive instruction through an FDA-approved course.

USDA FSIS Canning Regulations

In general, foods containing more than 2% cooked (3% raw) meat or poultry fall under the jurisdiction of the USDA. The regulations for canned meat products are found in 9 *CFR* 318.300 and for canned poultry products in 9 *CFR* 381.300; they are essentially identical. Requirements are very similar to those for FDA products. As with the FDA, the USDA has also mandated HACCP regulations for meat and poultry products (9 *CFR* 417), although canned foods complying with 9 *CFR* 318.300 or 381.300 do not have to include critical control points for microbiological hazards.

ESTABLISHING A PROCESS

Acidified Foods

Processes for acidified foods are based on a relationship between the pH of the product and temperature. Such processes are to be established by a qualified person having expert knowledge in acidification and processing of acidified foods.

Acidification Procedures. Five methods are used in the industry to acidify a low-acid product (21 *CFR* 114.80):

1. *Blanching of the food ingredients in acidified aqueous solutions.* An effective way to acidify large particles, this method will require control of the time and temperature of the blanch, as well as the type and concentration of the acid.
2. *Immersion of blanched food in acid solution.* This two-step acidification process requires blanching the low-acid product in a normal steam or water blancher, followed by dipping the blanched product into an acid solution. The product is removed from the acid solution prior to filling into the containers. The blanch procedure, concentration of the acid solution, and length of time the product is in the acid solution need to be controlled to ensure proper acidification.
3. *Direct batch acidification.* This method is used for liquid products or other products mixed in batch kettles. Acid is added to the batch in predetermined levels that will achieve the desired lowering of pH. The pH level is confirmed prior to releasing the batch for filling.
4. *Direct addition of predetermined amount of acid in individual containers.* Liquid acid solutions or acid pellets are added to each container of product. Diligent control is necessary to ensure that each individual container receives the proper addition of acid. Control is also necessary to ensure that the proper solid-to-liquid ratio is obtained to achieve adequate acidification of the low-acid components.
5. *Addition of acid foods to low-acid foods.* Proper attention to formulation is necessary for this final

method of acidification that relies on the acid from the acid food to acidify the low-acid component. The proportion of acid and low-acid components will be critical to ensure a proper final equilibrium pH.

These five methods of acidification are all acceptable; each requires a certain degree of control for proper acidification. Some products may require more than one method to guarantee consistent acidification.

Pasteurization Process. Pasteurization processes for acidified foods are designed to destroy any vegetative pathogens and spoilage organisms that could grow in the reduced pH environment. The processes are established to either deliver a final product temperature at the end of heating or a specific F value for the microorganism of concern. The heating rate of the product needs to be determined for processes established to deliver a specific F value. Product heating rates for acidified foods are determined with heat penetration studies. These studies will be discussed in detail in the following section on low-acid foods. Processes that rely on a final product temperature may be monitored with maximum reading thermometers or by actual temperature measurements from containers periodically sampled at the end of the final heating section.

Low-Acid Foods

Establishing a thermal process for low-acid foods requires knowledge of both the heat resistance of microorganisms, which has been discussed previously, and the heating rate of the products. The heating rate of a product is determined by conducting heat penetration studies, which measure the changes in product temperature during processing. In addition to establishing a thermal process, the thermal process system must be operated in an appropriate manner to ensure the production of a commercially sterile product. Temperature distribution studies are conducted to establish the operating procedures for the thermal processing systems. The regulations previously discussed require that thermal processes and thermal processing system operating procedures be established by a processing authority.

Heat Penetration. Heat penetration studies involve measuring the product temperature inside test containers by placing one temperature sensor in each container. A series of tests are conducted to locate the slowest heating zone within the container and then to confirm the heating rate in the slowest heating zone. A processing authority will analyze the data from all of the heat penetration tests to determine the heating rate of the product and calculate thermal processes. The heat penetration tests should be designed to collect data under the "worst case" conditions that could be reasonably encountered in commercial production. Test design should consider product preparation, temperature sensor location, container fill procedures, container geometry and orientation, product initial temperature, retort temperature, and other possible critical factors

Products typically heat by conduction, convection, or a combination of both. Conduction heating involves particle-to-particle heat transfer with no gross particle movement. Products that heat by conduction are typically viscous, with little free liquid—pumpkin, stews, corned beef hash, and condensed soups are examples. Convection heating involves particle-to-particle heat transfer with particle movement. Products that heat by convection are typically thin or contain free liquid—brine-packed products such as mushrooms and green beans are examples.

Copper-constantan (Type T) thermocouples are usually the temperature sensor of choice for heat penetration testing. The time- and temperature-recording equipment used for heat penetration tests normally consists of a multichannel recording potentiometer or computer modified for data collection. The thermocouples should be connected to the recording equipment with copper-constantan thermocouple wires. The test containers may be processed in a commercial retort or in a pilot retort simulator. The thermocouple wires are normally installed through the retort wall using a stuffing box or packing gland to seal the opening into the retort.

The data must be analyzed by a processing authority. The time-temperature data from all heat penetration containers should be analyzed unless there is a problem noted with a specific temperature sensor. The slowest heating thermocouple located at the cold zone is used to define the product heating rate for process establishment. The processing authority will use this information, along with the thermal resistance data, to calculate a thermal process. Several mathematical methods have been developed to calculate a thermal process, but the scope of this chapter does not allow further discussion.

Temperature Distribution. Thermal processing systems (retorts) must be operated in such a way that the process timing does not start until the temperature-indicating device (mercury-in-glass thermometer [MIG] or equivalent) reaches the process temperature and uniform temperatures are achieved throughout the retort. Processing authorities conduct temperature distribution tests to develop the operating procedures that are necessary to establish uniform temperatures throughout the retort. For steam retorts, the operating procedure is commonly referred to as the venting procedure or vent schedule. Venting procedures are designed to remove the air present in the retort and replace it with saturated steam. Vent schedules have at least two critical components: time and temperature.

The timing of the retort process cannot begin until the scheduled process temperature has been achieved and the prescribed operating procedures have been completed. Properly designed operating procedures will ensure that the retort temperature, as indicated by the temperature-indicating device, is uniform in the retort environment.

The processing authority needs to be aware of the many variables that may affect the development of an adequate operating procedure. A few examples of these variables include container loading configuration, percent open area of the baskets and divider plates, vent pipe sizes, or pump speed.

The distribution temperature sensors (typically a continuous Type T thermocouple wire with the copper and constantan wire tips connected to form the temperature sensor) are distributed among the containers to monitor the temperatures throughout the retort load. In addition, at least one sensor is typically located next to the temperature-indicating device to serve as a reference against which all the other sensors can be compared. The temperature-recording equipment used for distribution tests is similar to that used for heat penetration tests.

The temperature distribution data are analyzed by the processing authority to establish the operating procedure, which will provide a reproducible relationship between the temperature-indicating device and the thermocouples located throughout the load of containers. Once established, the operating procedure needs to be followed each time the retort is operated to ensure adequate delivery of the scheduled process.

TYPES OF CONTAINERS

The types of containers used for low-acid and acidified foods have expanded quite a bit in the past 20 years. The metal can has proven to be a versatile and well-established container, but the industry is also producing low-acid and acidified products in glass, semirigid, and flexible containers.

The metal can commonly used is either a three-piece steel can or two-piece steel or aluminum can. The can designation is determined by the number of metal pieces needed to form the can. Three-piece cans are formed from three pieces of metal: top, bottom, and body. The cylindrical body is formed from one piece of metal rolled and joined by welding a side seam. Two-piece cans are formed from two pieces of metal: a top-end and can body that includes the bottom. The body and bottom are formed from one flat, circular piece of metal by either a drawing and ironing process or a drawing and redrawing process. The can tops (and bottoms for three-piece cans) are attached to the can bodies in a complex, double-seaming operation. In addition to the familiar cylindrical can, the industry also uses oblong, rectangular, and half steam table tray metal cans.

Glass packages (jars or bottles) are another common container and are probably more readily used for acid and acidified food than the metal can. The glass container is sealed with a metal closure to ensure the integrity of the package. The closure may be one of several different designs. The lug or twist cap and the PT (Press-on Twist-off) cap are commonly used for low-acid food products and will withstand the retort pressure process. The plastisol-lined, continuous thread cap is often used for acidified foods.

Semirigid and flexible containers are gaining wider acceptance in the marketplace. Some semirigid and flexible containers, such as the retort pouch, plastic containers with double seamed metal ends, and some semirigid containers with heat-sealed lids, will withstand the pressures of a retort process and are suitable for low-acid foods. Other containers (paperboard, flexible pouches, and semirigid containers with heat-sealed lids) will not withstand retort processing, but low-acid foods can be packaged acceptably into them with aseptic processing and packaging techniques. All of these semirigid and flexible containers have been adapted for use with acidified and acid products that do not require a severe heat process.

DEVIATION MANAGEMENT

A thermal process deviation is defined as a thermal process that is less than scheduled and/or in which one or more of the critical factors has not been satisfied. The corrective action taken and the specific procedures required to handle the deviation will depend on the regulatory agency involved. The FDA regulations concerning the handling of low-acid deviations are located in 21 *CFR* part 113.89. The corresponding regulations for the USDA are found in 9 *CFR* 318.308 and 381.308.

Deviations that are discovered prior to or during the thermal process may be corrected, thus eliminating the need to handle the thermal process as a deviation. All deviations must be completely documented on the thermal processing records. If it is not possible to correct a problem on the spot, or if the deviation is discovered after the fact, all product involved with the deviation must be placed on hold with one of the following options undertaken:

1. The deviant product may be reprocessed if a suitable reprocess is available from a processing authority.
2. The deviant process could be evaluated by a processing authority.
3. The deviant product could be destroyed. Records must be kept that delineate the disposition of the destroyed product.

All thermal processing, product disposition, and deviation evaluation records pertaining to a specific deviation need to be kept in a separate file. Alternatively a log may be kept that identifies all deviations.

The best way to manage deviations is to prevent them from occurring. This may be accomplished by training the retort operators and employees assigned to monitor critical factors to recognize the potential for the occurrence of a deviation and be familiar with corrective action.

PROCESSING SYSTEMS FOR LOW-ACID CANNED FOODS

There are many systems for the thermal processing of low-acid canned foods. Traditional thermal processing methods utilize retorts (pressure vessels) for processing products The food is filled into containers, the containers are sealed, and then containers are placed into the retort for processing. Many styles of retorts have been developed for use in the low-acid canned foods industry. The retorts can be categorized by container handling methods, type of processing medium, the use of overpressure, and the use of agitation.

Container Handling Methods

Retorts may be designed to use discontinuous (batch) container handling or continuous container handling. Generally, batch container handling systems require the processor to place the containers into metal crates or baskets to facilitate loading the retort. In a few systems, referred to as *crateless*, the cans are loaded directly into the retort. Batch retorts require an operator to close and tighten a door or lid to seal the pressure vessel prior to introducing the heating medium and/or bringing the retort up to the required temperature and pressure.

Continuous container handling systems have two self-sealing openings, which allow the retort to be constantly at the required temperature and pressure and still allow the introduction and discharge of containers. The self-sealing openings are either mechanical valves or columns of water depending on the system. The containers travel through the retort during the thermal process by either a rotating reel or a carrier chain.

Type of Processing Medium

The first retorts used steam at high temperatures and pressures as the processing medium for processing low-acid canned foods. Circulating water, steam/air mixtures and steam/water/air mixtures have also been used successfully as the processing medium. All of the processing media mentioned provide an efficient means of heat transfer to the containers. Regardless of the type of processing medium utilized, a system must be in place to circulate the medium to allow for uniform temperatures within the retort.

Use of Overpressure

Overpressure is a term used to describe the additional pressure supplied to the retort that exceeds that supplied by steam at a given temperature. Depending on the retort system, overpressure may be supplied in the form of air or steam. Overpressure is used to maintain the integrity of containers that have limited resistance to internal pressures (ie, glass jars, semirigid plastic containers, and flexible pouches).

Use of Agitation

Agitating the contents of the containers during processing may allow for a faster heating rate, thus resulting in a shorter process time for some products. The agitation of the containers acts to "stir" the contents during heating, allowing for faster and more uniform heat transfer. Several systems provide agitation—either intermittent or continuous—of the containers during processing.

PROCESSING ACIDIFIED FOODS

Because high temperatures and pressures are not required for processing acidified foods, the methods for thermal processing may be less complex. Processing acidified foods is often referred to as pasteurization. Pasteurization temperatures are generally at or lower than boiling water temperatures at atmospheric pressures. Pasteurizers, in the form of water baths, atmospheric cookers, or retorts, may be used for processing acidified foods. When a pasteurizer is used, hot or cold product is filled into the containers that are then sealed. The sealed containers are heated in the pasteurizer for a given period of time or until a specific temperature is achieved. Pasteurizers may be batch systems (such as a water bath) or continuous systems. Continuous pasteurizers may use steam, hot water, or cascading hot water as the processing medium. A moving belt or other mechanism is used to move the containers through the processing medium for heating and through cold water or sprays for cooling. The speed of the belt will determine how long the containers are in the pasteurizer. It is essential to operate pasteurizers properly to ensure that all containers achieve the same degree of heating. Improper speed or temperature settings or overloaded belts may have a negative impact on product heating.

Hot-fill-hold procedures are another method of processing commonly used for acidified foods. Hot-fill-hold procedures involve heating the product to a specified temperature, filling hot product into the containers, and holding the filled and sealed container at a minimum temperature for a period of time prior to cooling. Generally the containers will be inverted or tipped after sealing and prior to cooling to allow the hot product to contact and sterilize the inside surface of the lid.

ASEPTIC PROCESSING AND PACKAGING

Low-acid and acidified foods can also be aseptically processed and packaged. For details on how products are processed aseptically, see the article titled ASEPTIC PROCESSING: OHMIC HEATING.

SPOILAGE OF CANNED FOODS—DETERMINATION OF CAUSE

There are two major causes of spoilage of canned foods: postprocess contamination (leakage) and insufficient thermal processing. Postprocessing contamination usually results in a mixed microbial flora of vegetative cells and spore formers, generally with little heat resistance. There is very little risk that such contamination will present a public health hazard. Underprocessing of low-acid foods usually is characterized by pure cultures of spore-forming bacteria; there is the risk that *C. botulinum* and its toxins may be present. In addition, foods may spoil if stored at temperatures above 43°C (109°F) because of growth of thermophilic spore formers, which present no health hazard. In acid and acidified foods, underprocessing results in the growth of organisms that are not a health hazard; however, improper acidification can result in the potential for spores of *C. botulinum* to germinate and grow.

Detailed procedures for evaluating the cause of spoilage can be found in Ref. 13, along with methods for the microbiological examination of a variety of other foods, including meat and poultry products, milk and milk products, eggs, seafood, and fruits and vegetables. There are also procedures for isolating and identifying indicator organisms and foodborne pathogens. Cultural methods are being supplemented or replaced by techniques to identify specific proteins or DNA sequences, such as ELISA (enzyme-linked immunosorbent assay) methods, PCR (polymerase chain reaction) technology, and fingerprinting methods such as ribotyping and pulsed field gel electrophoresis. Such techniques are being used to determine not only what organisms caused the problem but also where the organism came from.

CONCLUSION

The pH of foods is one of the key factors in determining what microorganisms can grow in the food, which in turn defines how the product must be processed to ensure safety and prevent spoilage. An understanding of food microbiology, coupled with knowledge of the parameters critical to delivery of a thermal process, is essential for establishing an adequate thermal process. This expertise forms the basis for a processing authority, who will also be knowledgeable in the applicable regulations that apply to these products. Assistance in assessing the microbial inactivation requirements and how to deliver a process can be found through trade associations and universities, as well as contract laboratories.

BIBLIOGRAPHY

1. Robert B. Gravani, "Food Science Facts for the Sanitarian The pH of Foods (continued)," *Dairy Food Sanit.* **6**, 112–114 (1986)
2. M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds., *Food Microbiology, Fundamentals and Frontiers*, ASM Press, Washington, D.C., 1997.
3. International Commission on Microbiological Specifications for Foods, *Microorganisms in Foods 5: Microbiological Specifications of Food Pathogens*, Blackie Academic & Professional, London, United Kingdom, 1996.
4. International Commission on Microbiological Specifications for Foods, *Microorganisms in Foods 6: Microbial Ecology of Food Commodities*, Blackie Academic & Professional, London, United Kingdom, 1998.
5. C. R. Stumbo, *Thermobacteriology in Food Processing*, 2nd ed., Academic Press, New York, 1973.
6. H. G. Bayne and H. D. Michener, "Heat Resistance of *Byssochlamys* Ascospores" *Appl. Env. Microbiol.* **37**, 449–453 (1979).
7. L. R. Beuchat, "Spoilage of Acid Products by Heat-Resistant Molds," *Dairy Food Env. Sanit.* **18**, 588–593 (1998).
8. V. N. Scott and D. T. Bernard, "Heat Resistance of *Talaromyces flavus* and *Neosartorya fischeri* Isolated From Commercial Fruit Juices," *J. Food Protect.* **50**, 18–20 (1987).
9. I. Walls and R. Chuyate, "*Alicyclobacillus*—Historical Perspective and Preliminary Characterization Study," *Dairy Food Env. Sanit.* **18**, 499–503 (1998).
10. M. E. Parish, "Public Health and Nonpasteurized Fruit Juices," *Crit. Rev. Microbiol.* **23**, 109–119 (1997).
11. *Thermal Processes for Low-Acid Foods in Metal Containers*, Bulletin 26-L, 13th ed., National Food Processors Association, Washington, D.C., 1996.
12. A. Gavin and L. M. Weddig, eds., *Canned Foods Principles of Thermal Process Control, Acidification and Container Closure Evaluation*, 6th ed., Food Processors Institute, Washington, D.C., 1995.
13. C. Vanderzant and D.F. Splittstoesser, eds., *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed., American Public Health Association, Washington, D.C., 1992.

V. N. Scott
L. M. Weddig
National Food Processors Association
Washington, D.C.

See also Food processing.

M

MACHINE VISION SYSTEMS: PROCESS AND QUALITY CONTROL

Many processes and inspection systems in manufacturing operations were done manually until the early 1980s, when the normalized grayscale correlation (NGC) was adapted to vision systems. With the introduction of new technologies, increased emphasis on improved production quotas, labor costs and shortages, and the introduction of new products such as computer chips, the need to develop the machine vision system (MVS) for processing and quality/inspection of components or whole products was necessary. Machine vision has an additional advantage in that it can provide 100% inspection.

This powerful system can be compared to the human vision system (Fig. 1) in that it uses a lens (eye) with proper illumination that sends a signal (optic nerve) to a computer (brain) that analyzes the image and sends information to a controller (arm) that then proceeds with a programmed task.

MVS offers many advantages over manual and mechanical systems:

1. Accuracy and precision
2. Automation
3. Better quality
4. Improved production
5. Higher throughput

Machine vision may be defined as "the mechanism of gathering information optically, quickly and nondestructively to control machines or processes" (1). Others define it as "the automatic acquisition of images by noncontact means and their automatic analysis to extract needed data for controlling a process or activity" (2).

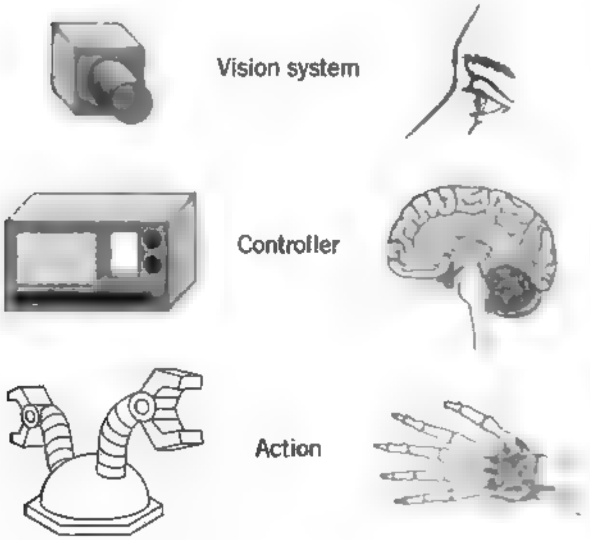


Figure 1. Analogy between human and machine vision.

To understand MVS, we have to look at its components and how they work; applications; and functions and types of MVS, including descriptions of hardware, software, and future developments.

THE SYSTEM AND ITS COMPONENTS

A machine vision system is designed to obtain, automatically, an electronic or digital image of an object through a camera lens. The object through a camera lens. The object is illuminated with a set light (light source) at a set angle such that the image can be recognized and processed by the system (image formation) (3). The camera extracts from the object the feature to analyze, and a board captures the image, digitizes it, and divides it into small sections called pixels or picture elements. A number is given to each pixel based on a binary scale, a gray scale, or a color scale. In binary images, the number represents either dark or light. Color processing uses information from the red, blue, and green color spectra to detect and differentiate shades of color (4). The gray scale number is proportional to the level of light intensity of the corresponding area represented by the pixel. The digitized image is placed into the vision-processor memory where a decision using vision algorithms is used to decide the output. The output is used to make a decision about the object in terms of processing or rejection (Fig. 2).

The MVS (Fig. 3) consists of an image-sensing device, an illuminator (not shown), an image-processing device, a control device, an operating interface, and an output device.

Image-Sensing Device

This part of the system is able to locate the object and positions itself so as to capture or acquire the measured feature in the orientation it was originally programmed to find (5). The image-sensing device captures a light intensity and changes this to an electrical signal, which is sent to the processor. Image-sensing devices are usually video

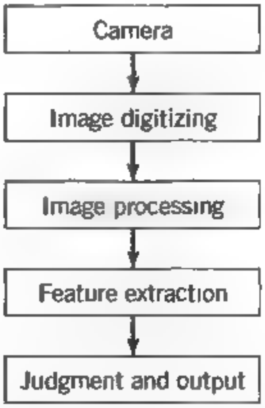


Figure 2. Flowchart for a machine vision system.

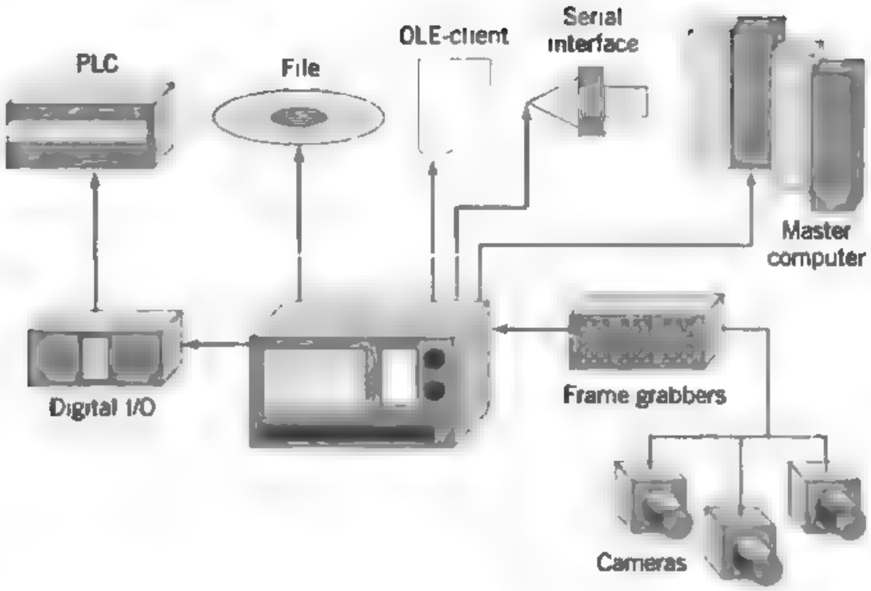


Figure 3. Components of a machine vision system.

cameras or solid-state cameras (charge-couple devices [CCD] or charge-injection devices [CID]); infrared [IR], ultraviolet (UV) or X rays; laser scanners, and ultrasonic sensing devices. These devices can be very small and sealed in housings, have low or high resolution, and be monochrome or color. The small, high-resolution and/or color cameras are suitable where detail is needed but are more costly and take longer to process the image.

Illuminator

Lighting is critical to an MVS in that it allows for accurate image capture. Broadband sources, fiberoptic illuminators, or lasers can be used as light sources. The wavelength can be from the IR to the UV to the X-ray regions. Light can be provided in the shape of a ring, a line, a point, or multiple points, or it can cover an area (1) and can be continuous or pulsed (flashes). The latter is used for rapidly moving objects. There are three basic lighting configurations, depending on the features to be highlighted (4,6):

1. Front lighting (front light), in which objects are lit from the top or front of the object but possibly at an angle. This is used for extracting surface features.
2. Back lighting (backlight), in which the light is placed below or behind the object, providing an outline of it. This is suitable for nontransparent objects where high contrast is needed and is usually coupled with a binary-image sensor device.
3. Structural or multidimensional lighting, in which light is directed at various angles so as to extract a two- or three-dimensional image. These may be used to measure a dimension or find shape defects in an object.

Image-Processing Device

This device processes the image depending on the application. An analogic-to-digital transform module changes the captured analogic image and transforms it to a digital image for processing in the computer or for display. The image-storage module stores the digitized image for further processing and analysis. An image pretreatment module filters the captured image for enhancement of it or its features. The image analysis module calculates the gray-level distribution of the image and counts the area for gray level.

Operating Device

This device simply translates the captured image to usable bits. Speed is necessary here so that a response is given quickly. The speed will depend on the capacity of the computer, the complexity of the image, and the type and quantity of information desired from it. The system and software should be optimized for the specific application so that the necessary frame grabber programs, algorithms, accelerators that interface with the CPU, and application software are adequate. Among the software available are NeuroCheck, a general-purpose image-processing system for industrial quality control; Eurosys, suitable for industrial machine vision; HACCON, which is flexible and gives high performance for research and education; and WiT, for image-processing development (2). WiT, along with VPE, ProtoPIPE, VPM, and Concept Vi, allow the user to pick the image-processing icon, drag it to the workspace canvas, connect I/Os using links, and define operator-specific parameters (7).

Choosing specifications for an MVS requires answers to the following questions (4):

1. How many parts must be processed or inspected per unit time?
2. Which and how many distinguishable features are there?
3. What environmental factors are present?
4. What level of technical knowledge is available on the floor?

5. Who will install, program, maintain, and integrate the system?
6. Will any other machines or components be interfaced with the vision system?
7. What are the upgrade and flexibility requirements?
8. What is the budget?

On the other hand, lack of need for precision of part appearance, poor visual access, or poor visibility due to the environment are key factors in not choosing an MVS (8).

Control Device

The control device, a board or, more commonly, a computer, is the heart of the system. It controls the imaging device and stores the digitized image, does image analysis and pattern recognition, and transmits the results or command to a controller. The size of this device will determine the speed of the process and the precision of the measurement or task(s).

Output Device

This device obtains the translated information and acts on it accordingly. In a manual system, the information (display) is passed to an operator who will make a decision and proceed accordingly. In an automated system, the processor makes a decision based on the data evaluated and sends a signal to some part of the process to act accordingly. This signal could trigger an alarm, stop a line, reject or accept the product, or send a signal to a robot to perform a task. In a closed-loop system, the feedback from the statistics process is provided to the controller to reduce process deviations.

Image recognition and inspection is done via image comparison (sample image vs. standard image) or feature extraction (sample image features vs. standard image features). These are named "something-somewhere" inspections, which are usually quantitative. A more flexible, qualitative image-inspection system based on the "anything-anywhere" principle works on the principle of example parts or illustrations. An example of the "anything-anywhere" principle is the system named Mentor®, which uses a filter generated from a neural network that creates and refines the filter during the set-up process and creates different algorithms to analyze different area parts (9).

APPLICATIONS FOR MVS

There are eight general categories for MVS applications (2):

1. Machine monitoring
2. Material handling
3. Process control
4. Quality control/assurance and inspection
5. Robot guidance
6. Safety
7. Sorting and grading
8. Test and calibration

Quality control/assurance and inspection has the function of removing defective products from the manufacturing process. This can be coupled with statistical process control (SPC) to increase information and efficiency. It is the single largest application because it requires repetitive tasks and constant attention. It may also be an important tool in the monitoring of critical control points (CCP) in hazard analysis and critical control points (HACCP) systems. One such application is the detection of metal particles. Bottling lines, poultry and fish processing lines, and confectionery lines are candidates for this system (Fig. 4).

Sorting or grading is a process whereby objects can be classified by size, dimension, color, or other physical attributes. Roasted nuts could be classified according to degree of browning and size for specific applications. Fresh shrimp are graded by size (count) to be able to be processed correctly and into the right category.

Food Industry Applications

Many repetitive, measured processes or quality/inspection-control functions could be performed by MVS. These applications range from sorting and inspection of raw product to product reduction or grading to inspection of final product, package, or both. Some of these applications are:

1. Process control
    a. Monitoring and controlling cooking operations
    b. Monitoring color of foods
    c. Detecting abnormal conditions
    d. Monitoring product fill
    e. Monitoring product size and shape for size reduction

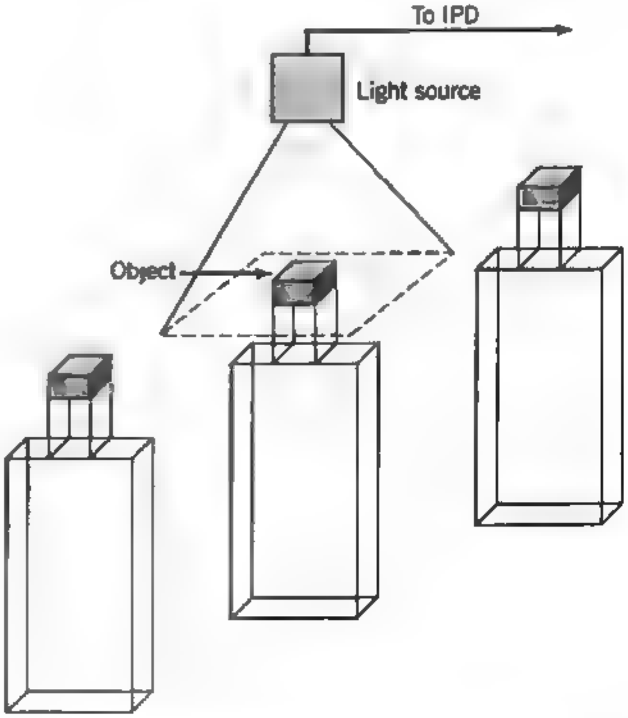


Figure 4. The frontlight (top) illumination method.

  f. Monitoring fermentation process through color development
2. Quality control/assurance
  a. Recognition of product size and variety
  b. Sorting of vegetables
  c. Egg inspection
  d. Inspection of food containers
  e. Inspection of packaging label and codes
  f. Detection of missing or different products placed in trays
  g. Detection of faulty or missing seal
  h. Detection and classification of defective product
  i. Detection of abnormal conditions

Process Control Applications

Color machine vision (CMV) can be used to monitor color of incoming and final products, extent of cooking process, and pigment concentration. Strong correlations were found between a colorimeter and CMV systems (CMVS) and colors as perceived by humans (10). Among the applications of a CMVS are the monitoring and measurement of product color, extent of retort, and other thermal processes through chemical markers. One such application is the monitoring of baked and roasted materials as an indication of quality or degree of cooking (11). Baked muffins (doneness), roasted peanuts, and pizzas (mapping) are some of the specific products (Fig. 5). Minimum description analysis is used when the quantity of irrelevant colors (e.g., blueberries in muffins, raisins with peanuts, etc.) vary between samples (12).

A system to predict crawfish molting based on color ratio was used to produce soft-shelled crawfish (13). The system used an IBM PC/AT to control a Versa Module Eurocard (VME) image-processing system. A Pulmix CCD color camera with a polarizing filter was used to acquire the image. A system consisting of an optical scanner, a computer and image-processing system, and a robot water jet cutter was designed to produce fat-free steaks (14). The system uses a 3D laser scanner to obtain the image profile with up to 0.1 pixel. A digital signal processor (DSP) program enables the image-processing system to receive the product's volume and thus its mass. Another similar system using rotating blades instead of water jets combines image processing with a scale to define the shape, orientation, and mass of product to be cut into portions (Fig. 6). This process allows for maximum efficiency and minimum waste. These systems not only save on labor and add efficiency but also are more effective in controlling hazards from handlers and cutting utensils.

Quality Control/Assurance Applications

The Kernel Extension Rapid New Evolution Level (KERNEL) was developed to grade morphological features and color defects in turkey carcasses (15). The turkey carcasses suspended on a chain are appraised for pin feathers, visceras, and filets/cuts yield. Different appearance (color) characteristics were extracted using color segmentation methods. A CMVS was developed to identify the reddish comb of each chicken and thus be able to count the chickens before placing them in the crate automatically. Another system was developed to measure count, uniformity ratio, color, and melanosis and to detect foreign objects in processing white shrimp (16). Shrimp area was used to estimate weight and calculate count and uniformity ratio. Color and melanosis were quantified by correlating with a trained inspector.

A CMVS was developed to inspect frozen dinners for proper food amount and ratio in each compartment (17). The image captured is analyzed by color-connectivity analysis where it is compared against predetermined tolerance limits and proximity of one food to another to check for

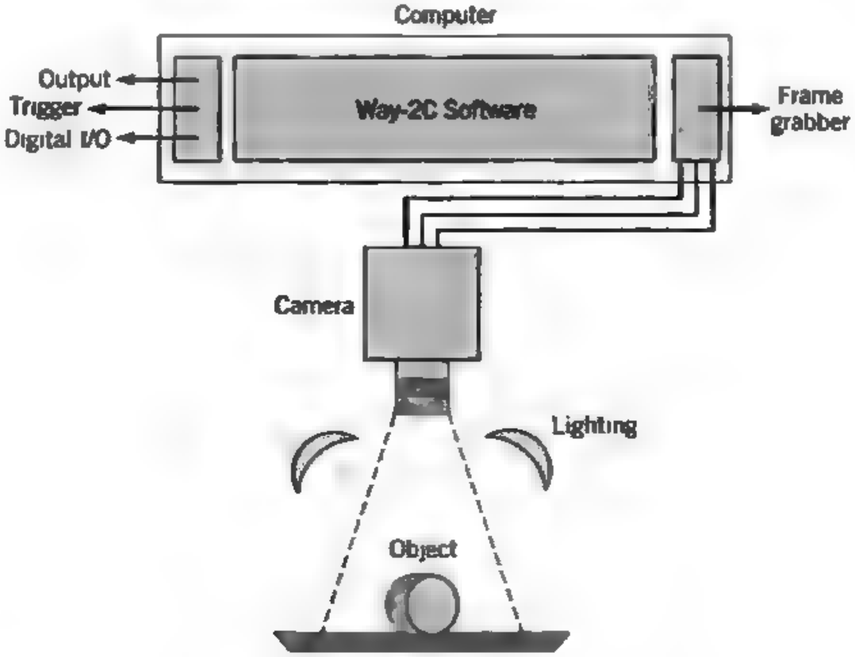


Figure 5. Color measurement using an MVS.

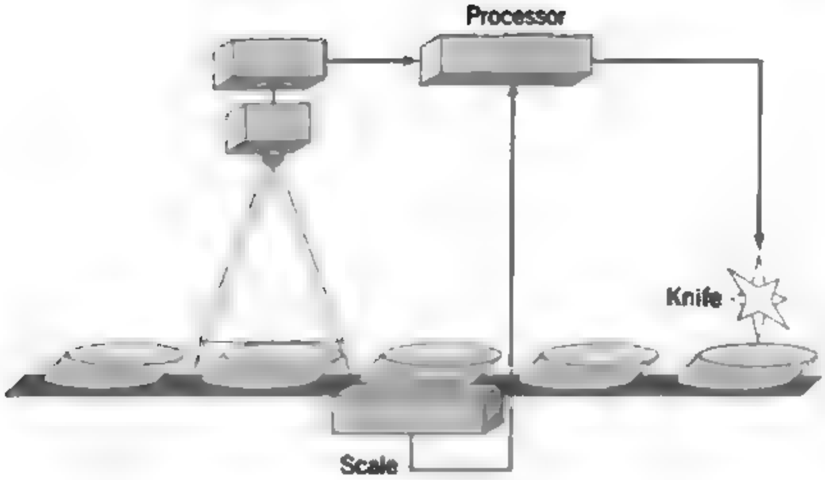


Figure 6. Product cutting/portioning using an MVS, a scale, and a cutting tool.

spills. A method to characterize the color of fruits and vegetables at different ripening states, describe color changes during storage of mushrooms, distinguish varieties by shape, and recognize shape defects was developed calculating the average red, green, and blue (RGB) color components of the pixels belonging to each product (18). Utilizing hue, saturation, and intensity, an (HSI) MVS was trained to distinguish between good and greened potatoes and yellow and green apples (19). The CMVS used multivariate discriminate techniques to hue histograms of representing features and increased accuracy by reducing the number of hue bins by selecting significant features or summing groups of hue bins. X-ray technology has been developed to detect metals, glass, minerals, stone, polyvinyl chloride (PVC), dense rubber, and other materials within a package or in a conveyor belt (20). The system consists of an X-ray generator, a detector, and a processor. Differences in density are detected, and the sensor converts the X-ray signal into light, then into an electrical signal before it is converted into a video signal. It can also be used for morphology processing and for detection of underfilled or broken packages.

FUNCTIONS OF MACHINE VISION SYSTEMS

MVS is capable of performing many functions, depending on the type of information extracted during image analysis. Functions are gauging, verification, flow detection, identification, recognition, finding position, and tracking (Fig. 7).

Gauging refers to the process of making measurements. These measurements can be made on-line and off-line. On-line inspects or sorts parts, provides adjustment to machines, calibrates, or gathers SPC information. An off-line system could be used to adjust or coordinate various machines or machine parts in a process.

Verification refers to the process whereby an activity is assured to happen correctly. This could be the correct positioning of a label or of an object prior to packaging. Flaw detection is the most common function of an MVS. It refers to the detection of any defect in an object, such as a cracked bottle, a defective can, an underfilled container, or a defective seal. Identification is usually accomplished by optical character recognition and bar coding. This is used where multiple products are packaged in the same container but the code reveals their identity, such as in warehouses to move the oldest manufactured product first (first-in, first-out [FIFO]), and for inventory (21).

Recognition, as compared to identification, utilizes characteristic features of the image to determine the object's identity. This is usually used in combination with robotics to identify an object or its position.

Finding position refers to the determination of the location and orientation of an object or objects. This allows for rapid and precise working of the object while reducing waste. Tracking usually precedes a position-finding function in an MVS, since the part position needs to be known before the tracking function begins (22).

CONFIGURATION AND TYPES OF MVS

Three configurations of MVS are available, depending on environment and requirements. Stand-alone systems are well suited for factory automation. They use application specific integrated circuits (AISCs) that deliver accelerated processing power. They are compatible with other factory automation devices [programmatic language controllers (PLCs), photoelectric sensors, and radio frequency identification (RF/ID) systems] and withstand adverse environments (temperature, vibration, electrical interference). The second type are PC-based systems, which consist of a computer and a motherboard or CPU with dedicated-vision ASICs. These are lower in price than stand-alone systems and take advantage of microprocessor technology, but they are not as resistant to environmental and other pressures as stand-alone systems. The third type are VME systems, which are robust yet flexible. The VME is an Institute of Electrical and Electronic Engineers (IEEE) bus standard, where system components are connected via pin-and-socket connections to the bus. These systems are more complex and costly than the others (23).

Machine vision systems can be classified into eight categories. Each has advantages and disadvantages (Table 1) and can be suitable to a particular operation. Turnkey

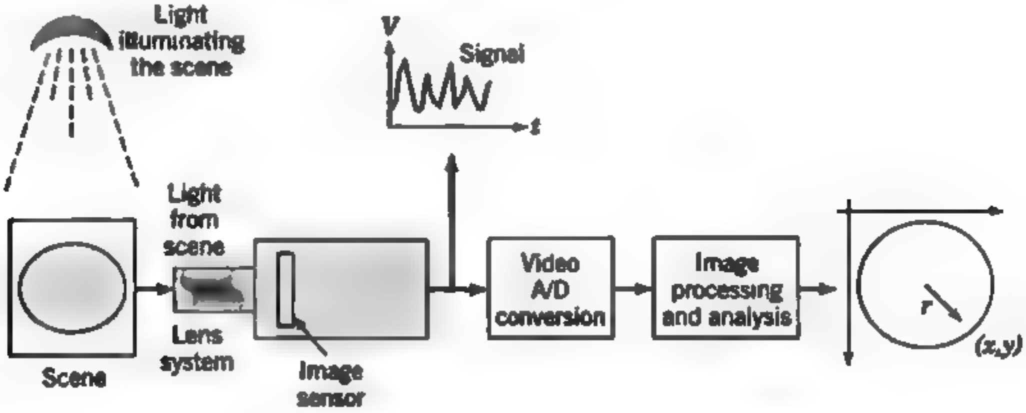


Figure 7. Typical machine vision system and its processes.

Table 1. Advantages$^{(+)}$ and Disadvantages$^{(-)}$ of Machine Vision System Types

	MVS type							
Characteristic	Turnkey standard	Embedded	Turnkey custom	General purpose	Vision engines	Board level	Modular	Custom
Low engineering cost	+	+	+/–	–	–	–	+	+
Long-term support	+	+	+/–	+	+	+/–	+	+/–
"Customer-friendly"	+	+	+/–	–	–	–	+/–	–
Low project risk	+	+	+/–	+/–	+/–	+/–	+	–
Lead time (short)	+/–	+/–	–	–	–	–	+	–
Low cost	–	+/–	+/–	+/–	+/–	+	+	–
Maximum flexibility	–	–	+	+	+	+	–	+

Source: Ref. 2.

standard MVS is designed as a complete package for a specific application but with a broad market basis. Embedded MVS is similar to the turnkey standard MVS except that the system is part of a larger machine. Turnkey custom integrated MVS is customized to the client's requirements. The general-purpose MVS is flexible in that it is designed for the end user as well as the system integrator. It is noted for its easy-to-use controls. Vision engines are designed for the original equipment manufacturer (OEM) or sophisticated system integrator and are noted for their high performance. Board-level MVS is designed as peripheral boards plugged onto a computer. Modular MVS integrates the camera and the processor into one piece. Custom MVS is characterized by uniquely engineered components, offering the highest flexibility but also the highest cost and risk.

BIBLIOGRAPHY

1. D. Zankowsky, *Laser Focus World* 4, 127–137 (1996).
2. R. Grosklaus, *Sensors* 14, 22–27 (1997).
3. E. Kubel, *Manuf. Eng.* 121, 42–44 (1998).
4. M. D. Wheeler, *Photonics Spectra* 32, 112–114 (1998).
5. J. R. Ggorki, *Mach. Des.* 2, 42–48 (1994).
6. C.-T. Yang, "Quality Control: Machine Vision System," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, 1st ed., John Wiley and Sons, New York, 1992, pp. 2220–2225.
7. D. LaCroix and B. Smyth, *Photonics Spectra* 28, 129–130 (1994).
8. A. Davis, *Advanced Imaging* 10, 52–56 (1995).
9. K. Harding, *Optics and Photonic News* 7, 29–33 (1996).
10. P. P. Ling et al., *ACS Symposium Series* 631, 253–256 (1997).
11. R. K. McConnell, Jr., and H. H. Blau, Jr., *Proc. FPAC IV Conf., ASAE*, Chicago, Ill., November 3–5, 1995.
12. R. K. McConnell, Jr., and H. H. Blau, Jr., *Proc. Electron. Imaging Int. Mtg.*, Boston, Mass., September 30–October 3, 1991.
13. T. Timmermans, F. E. Sistler, and T. B. Lawson, *J. World Aquac. Soc.* 26, 234–239 (1995).
14. J. Jacobs, *Advanced Imaging* 11, 21–23 (1996).
15. P. Marty-Mahé and P. Marchal, *C. R. Acad. Agric. Fr.* 83, 121–136 (1997).
16. D. A. Luzuriaga, M. O. Balaban, and S. Yeralan, *J. Food Sci.* 62, 113–118 (1997).
17. S. Montellese, *Photonics Spectra* 29, 107–108 (1995).
18. J. Felfoldi, F. Firtha, and E. Gyori, *Acta Alimentaria* 26, 289–292 (1997).
19. Y. Tao et al., *Trans. ASAE* 38, 1555–1561 (1995).
20. L. Mancini, *Food Eng.* 70, 89–96 (1998).
21. D. Braggins, *Sens. Rev.* 18, 152–153 (1998).
22. J. Testa, *Industrial Robot.* 22, 13–15 (1995).
23. T. Seitzler, *Advanced Imaging* 11, 56–58 (1996).

JUAN L. SILVA
ROBERTO S. CHAMUL
Mississippi State University
Mississippi State, Mississippi

MALTS AND MALTING

Malts are cereal grains that have been germinated to a limited extent to alter their physical and biochemical states. Usually they are dried and partially cooked (cured), and the rootlets are removed before use (1–10). There are many types of malt. Barley malts vary in color from a pale straw-yellow to nearly black. The darker malts have characteristic colors and aromas and they give particular flavors to products made from them, but in contrast to pale malts, they contain few or no enzymes. Most malts are prepared from barley, but smaller amounts are made from wheat, rye, possibly triticale, oats, sorghum, and millet. Barley is often preferred for malting because the adherent husk protects the grain during handling and malting whereas naked grains, like wheat and sorghum, are readily damaged and crushed. Barley can be induced to germinate evenly and generate useful levels of hydrolytic enzymes (including cytases, proteases, and amylases), and varieties are available for making many kinds of malts. Also, the relatively low gelatinization temperature of large barley starch granules (about 60–65°C [140–149°F]) renders them easily converted when the malt is mashed. Malts are used in making beers, African-style opaque beers, whiskeys, malt vinegars, and syrupy extracts and in some bakery products, confectionery, breakfast cereals, baby foods, malt coffee, and malted-milk beverages (1–10).

Small amounts of malt flours are used in baking. However, for most purposes malts must be mashed, sometimes after they are mixed with other starch-containing materials, to produce a sugary solution or extract (1,3,4). In mashing, ground malt is mixed in a chosen ratio with warm water and the mixture is heated, in a controlled way, to progressively higher temperatures. After a time the liquid or sweet wort, which contains the extract materials dissolved from the malt, is separated from the residual undissolved solids, known as spent grains or draff. The latter is usually used to feed animals, but other uses are being sought. The sweet wort is processed further to produce beer, whiskey, vinegar, extract, diastase, and so on (1–10). However, mashing to prepare opaque beers is somewhat different (1,3). If ground malt is extracted with cold, slightly alkaline water to prevent enzyme activity, then about 18 to 21% of the malt solids (the cold-water extract) dissolve. If the malt is mashed with hot water (e.g., 65°C; 149 °F) then the hot-water extract is about 77 to 83% of the dry matter of the malt, depending on the quality of the malt and the mashing procedure used. Mashing processes vary in the degree of grinding of the malt, the liquid-to-solids ratio, the temperatures used, and the durations of the different stages. The hot-water extract is much larger than the cold-water extract because during the hot mash, enzymes act to hydrolyze insoluble materials to give soluble products (1,2,4,9,10). Some degradation of proteins and cell-wall polysaccharides occurs, but the main change is the conversion of insoluble starch into a complex mixture of soluble sugars and dextrins. The increase in the density of wort above that of water is a measure of the amount of extract in solution (1,4). For most purposes the yield of hot-water extract and its quality (color, level of fermentable sugars, contents of amino acids and proteins, etc.) are the major measures of malt quality. In making beers, malt vinegars, and whiskeys it is the simple sugars that yeasts convert to ethanol and carbon dioxide (1,2,3,5,9). Malts are usually analyzed by one of three internationally recognized systems (1,4).

CHANGES OCCURRING IN MALTING GRAINS

Carefully selected grain, usually barley, is first hydrated, or steeped (1–4,6–11). Usually this is achieved by immersion in water, but sometimes water is sprayed onto the grain. At intervals the grain-water mixture is aerated by blowing compressed air in at the base. At the end of each immersion the water is drained to waste, and air (which may, to advantage, be cooled and saturated with water vapor) is sucked down through the grain. During each air-rest period the downward ventilation, or carbon dioxide extraction, cools the grain, provides oxygen, removes carbon dioxide, and accelerates germination. Typically the grain may be successively immersed in three batches of clean water. Later, water is sometimes sprayed on to adjust the moisture content.

After steeping, the grain, which has started to germinate, continues to grow surrounded by humid air (1–4,6–10). Rootlets (culms, sprouts) grow from one end of each grain and the embryonic coleoptile, also known as the shoot or acrospire, grows beneath the husk. The grain respires, using oxygen and producing carbon dioxide and heat. Hydrolytic enzymes are formed in the scutellum and later, partly in response to gibberellin hormones from the embryo, in the aleurone layer. These enzymes are released into the starchy endosperm, where they degrade the cell walls, some of the reserve proteins, and some of the starch. At the same time some sugars and simple nitrogenous substances accumulate. Collectively these changes are termed modification. When modification is sufficiently advanced, usually when the acrospire is about 0.8–0.9 the length of the grain (and certainly before it has grown out from beneath the husk) and endosperm cell-wall degradation is nearly complete, the malt is ready to be dried. This green malt (green means undried; it is not green in color) is now kilned or sometimes it is cooked and dried in a drum. In a very few cases the green malt is used directly (it cannot be stored), such as in grain distilleries where large amounts of starchy materials must be broken down in mashes and therefore maximum enzyme survival is needed (1,2). In kilning, the malt is dried and cooked, or cured, to a limited extent in a stream of heated air. This stops germination, produces a stable product, removes undesirable flavors, and gives the malt character (desirable color, flavor, and aroma). When pale malts are being made, the initial airflow is rapid and the air temperature is low to maximize enzyme survival and minimize color formation. As the malt dries, the air temperature is raised and the airflow is reduced without major effects on the enzymes, which are more stable in drier grain. When colored malts are being prepared, the temperatures used are higher and the airflows are less. At first the grain is held warm and moist, conditions that encourage first enzyme formation and then, as the temperature rises, enzyme inactivation, but that also favor the accumulation of sugars and amino acids (1,3,4,8). Later, as the temperature is raised, the sugars and amino acids interact to form colored melanoidins and flavorful, aromatic substances. Because darker malts contain lower levels of enzymes, they must be mashed with pale malts, which provide the enzymes to hydrolyze the starch.

After kilning the cooled malts are dressed, that is, sieved to remove the rootlets, which are sold for animal feed, sieved to remove malt and grain dust and thin grains (1–4,6–10). Barley malts lose only rootlets, but malts from naked (huskless) grains, such as wheat, rye, triticale, and sorghum, also lose shoots, because they are not protected by adherent husks (1). After kilning, pale malts are often stored for about 6 weeks when, for reasons that are not understood, their performance in mashing improves. In contrast, darker malts should be used soon after preparation, so that they do not lose their special flavors and aromas.

Roasted and caramel malts are prepared in roasting drums (1,3,4,8), which fall into two classes: (*1*) those in which kilned malts are roasted (brown, chocolate, and black malts, which are friable) and (*2*) those in which green, undried malts are processed (caramel malts in which the endosperms are replaced by hard, barley sugar–like masses).

Thus malting converts tough, raw-tasting, enzyme-poor grain into (usually) readily crushed, flavorful, and, at least in the case of pale malts, enzyme-rich materials that can easily be stored and transported and that when mashed give worts that are suitable for a range of uses, such as the manufacture of beer. However, matter is lost during this process. During the preparation of pale malts, made without additives, the total loss (dry weight) is about 10%, 1% in steeping, 4% as rootlets, and 5% as respiratory losses) (1–4,6–10). The losses incurred in making colored malts can be much greater. Losses may appear to be greater until it is realized that the initial moisture content of the barley going into steep may be 8 to 16%, and the moisture of the finished malt 1.5 to 6%.

MALTING TECHNOLOGIES

Barley or other grain may be delivered to a malting factory, or maltings, by road, rail, or barge (1–4,6–10). Before a load is accepted, each batch of grain will be checked, usually for viability, varietal purity, freedom from insect pests, moldy grains (which may lead to poorly flavored malt and carry mycotoxins and agents that cause beers to gush, or overfoam) (1,5), freedom from physical damage and premature sprouting, the appropriate protein ($N \times 6.25$) content, moisture content, and screenings (thin grains). If the load is substandard it may be rejected or accepted at a reduced price. The grain may be moved pneumatically, by drag-flight or helical screw conveyors, or by bucket elevators. The grain will quickly be precleaned, by sieving and aspiration, to remove most of the coarse and fine impurities and will be passed over magnets to remove fragments of iron and steel. If necessary the grain will later be dried to less than 12% moisture for prolonged storage. It will also be thoroughly cleaned to remove grains of other cereals, dust, weed seeds, small stones, and broken grains. The grain may also be graded, by sieving, to separate it into width classes, which may be malted separately. Usually the thinnest grains, or screenings, will be rejected and used for animal feed, but occasionally they are made into low-extract, enzyme-rich malt. The grain will be stored, at least until any dormancy has declined, under conditions that prevent its deterioration and the development of pest infestations.

The malting process itself may be carried out in many different sorts of plants. Old-fashioned floor maltings are still in limited use for making small quantities of specialist malts. Most malt is made in pneumatic maltings, which may operate in batch, continuous, or semicontinuous fashions. Most malting is carried out in batches, and steeping, germination, and kilning operations are carried out in different vessels. However, vessels are in use that combine steeping and germination (SGV), germination, kilning, and steeping (SGKV), and germination and kilning (GKV). Modern steeps are either cylindrical with conical bottoms or circular with flat bases. The latter have perforated false floors on which the grain rests, and they have mechanical leveling and unloading devices. Each is equipped to add and remove water, to blow compressed air into the base of the steep when the grain is underwater, and to suck air down through the vessel for CO_2-extraction between immersions. Sometimes provision is made to wash the grain as it is conveyed to the steep. When the grain is sufficiently moist (e.g. 44–46% fresh weight for many barley brewing malts and up to 50% for some distilling malts) it is drained and transferred to a germination compartment. Where their use is allowed, additives such as gibberellic acid and (though rarely) a bromate salt may be applied to the grain during the transfer by spraying on a solution (1,4). Gibberellic acid supplements the grains' own gibberellins and accelerates the formation of many enzymes, thus speeding the rate of modification. Bromates reduce rootlet growth, respiration, and therefore total malting losses (e.g., to 6% dry basis) and the levels of soluble protein released when the treated malt is mashed. Treated malts contain traces of bromide ions.

Traditionally, barley is germinated cool (10–14°C; 50–57°F) on smooth tiled or concrete floors. It used to be raked or turned by workers with shovels to control the temperature and prevent the rootlets from matting together. These processes are now partly mechanized (1,4). If the batch of barley (piece) becomes too warm, it is spread more thinly, for example, to 3 in., to allow it to cool. Conversely, if it is too cool, it is piled thicker, to 6 in. or more, to retain the heat. In modern plants grain temperatures and ventilation are achieved pneumatically, that is, by passing a variable flow of temperature-controlled and water-saturated air through a comparatively thick bed of grain (2 ft in the older plants, up to 8 ft in modern plants; about 4 ft is the present average). Maximum airflow rates may be 0.14 to 0.2 m^3/ton of barley steeped/s (11). Mechanical devices are used, about three times per day, to lighten the bed, to turn it, and to prevent the rootlets from matting. Various temperature regimes are used depending on the grain and the type of malt being produced, usually in the range of 13 to 18°C (54.4–64.0°F). In drums, horizontal metal cylinders, turning is achieved by intermittent rotation about the long axis. For most purposes drum batch sizes (average about 50 tons) are uneconomically small. Most malt is germinated in compartment vessels, usually of up to 300 tons capacity (of original barley). However, one Scottish combination steeping, germination, and kilning unit has a batch size of 520 tons of barley. Most compartments, like the early Saladin boxes, are rectangular, but newer plants are usually circular. In compartments grain is supported on a perforated deck through which the fan-driven stream of cooled and humidified air passes. Turning is achieved by a row of vertical, rotating helical screws, or augers, that move slowly through the grain mass, lifting it and separating the roots. In rectangular boxes the turners are supported from a carriage that moves slowly over the bed of grain. With circular compartments the set of turners, mounted below their support, may rotate around the center of the static bed of grain, or the bed of grain may be rotated and thus moved through a static row of turners.

When it is ready the green malt is usually transferred to a kiln, where it will be dried and slightly cooked in a stream of warm air. The temperatures used depend on the type of malt being made (1–4,6–11). For pale malts the initial air-on temperature may be 50 to 60°C (122–140°F), rising to 80 to 105°C (176–221°F). The temperatures of the grain and the air leaving the grain bed are less as the air is cooled by evaporating moisture from the malt. The grain is supported on a perforated deck, and the air moves through it from below. Kilns may be rectangular or circular in cross-section and may be of single-, double-, or, rarely, triple-deck construction. In modern kilns the grain beds may be 2 to 6 ft deep, and outside North America they are not turned. In double kilns the wetter batch, on the top deck, receives some air from the earlier, drier batch of malt below. This arrangement saves heat, and a similar effect is achieved by linking kilns with air ducts (1,4). Usually air-to-air heat exchangers or other devices are used to abstract heat from the warm and wet outgoing kiln air and convey it to the dry-air inlet, saving heat and reducing fuel usage. Sometimes, toward the end of the curing period when the malt is nearly dry, a large proportion of the hot air is recirculated, also to save heat. Kilns may be directly fired, that is, furnace gasses pass directly through the bed of malt. Alternatively, the furnace heats a heat exchanger that in turn heats the air. With such indirect heating the furnace gasses do not come into contact with the malt. Sometimes sulphur dioxide is added to the kiln airstream to reduce the color of the malt, to make the malt more acid so that the pH of the mashed malt is reduced, to increase the levels of soluble nitrogenous substances in the wort, and to prevent the formation of carcinogenic nitrosamines on the surfaces of the malt grains. The quantities of oxides of nitrogen that reach the malt during kilning are also limited, to minimize the formation of nitrosamines. Malt for making Scotch whiskey is flavored with peat smoke, applied during kilning, and *rauchmalz*, used in the making of some special German beers, is flavored with hardwood smoke (1–3,8,9). At the end of kilning the malt is cooled and dressed—that is, the sprouts or rootlets, which are now brittle, are broken by agitation, and they and dust are removed by sieving and aspiration. These, together with thin grains and grain dust, are used for animal feed, often after being pelletized. Some special malts are finished in roasting drums, commonly of 0.5–5 tons capacity (1,8). Brown, chocolate, amber, and black malts are made from plump, lightly kilned pale malts. In the United Kingdom roasted barley is regarded as a special malt. It imparts a sharper, drier flavor than true roasted malts. The malt is loaded into a horizontal metal cylinder that rotates around its long axis and, for this purpose, is heated from the outside. The temperature is carefully raised until the correct coloration is achieved For making black malts, when the highest temperatures are used, heating begins at about 75°C (167°F) and the temperature is raised to about 225°C (437°F). The products are quickly cooled, to fix their composition and reduce the risk of fire. They should not be charred. Any substantial amount of charcoal in these products makes them worthless. They deteriorate on storage and therefore are used as soon as possible. During roasting objectionable fumes are formed, and these must be destroyed, for example, by being consumed in an afterburner.

Crystal and caramel malts are prepared differently. Wet malt, either green malt or a pale malt that has been rewetted, is warmed in a closed cylinder, with no drying allowed, to 65 to 70°C (149–158°F). At this temperature the endosperms of the grains are mashed and the contents liquefy to a sugary mass. When all the grains have liquid contents, hot air is passed through the drums to dry the grains and to develop the desired color. At lower temperatures pale, luscious-flavored products are made, whereas at higher temperatures progressively darker and more strongly flavored materials are produced. When the malts are cooled the endosperm contents set solid and become hard, with a brittle, barley sugar–like texture. These are the only dry malts that should not be friable.

When malts are stored, care is taken to ensure that they are not mixed with unmalted grain; do not become damp; and do not become infested with insects, rodents, or other pests. Normally malt is transported in bulk, but for some purposes it is stored and moved in laminated, moisture-proof sacks of up to a 1-ton capacity.

MALT TYPES

The number of malt types in use is very large (1,4,7,8,10). Analyses of seven examples are given in Table 1. Malts vary with the cereal used, the quality and variety of the grain, the detail of the malting process, and the kilning and/or the drum heating process. It follows that the potential number of malt types is almost infinite. Undoubtedly the most common malts are pale with moderate enzyme levels (as in Pilsner and pale-ale brewing malts), with higher enzyme levels (as in North American brewing malts used with high levels of starch-rich adjuncts in mashes), or with exceptionally high enzyme levels (as in some distilling malts that must convert very large amounts of adjuncts during mashing). Usually the more enzyme-rich malts are made from protein-rich barleys that yield less extract than samples of the same variety that contain less protein. German kilned malts form a series of increasingly dark colors: (approximate; °EBC) Pilsner lager, 3.0; light lager, 3.5; Vienna-style malt, 7; and Munich-style malt, 17

Table 1. Analyses of Some Examples of Different Malts

Type of malt	Pale ale malt (UK)	Brewer's malt (N. America)	Grain distillers malt (Europe)	Lactic malt (Europe)[a]	Caramel malts (Europe)	Chocolate and black malts (UK)	Pale wheat malts (Europe)
Barley type (2- or 6-row)	2	2 or 6[b]	2 or 6[b]	2	2	2	n/a[c]
Hot-water Extract (% d.b)[d]	79–83	77–81	78–80	77	76–78	66–72	82–85
Protein (N × 6.25; % d.b)	8.8–10.3	10.5–13.5	12–13	11	11.0–12.5	9.4–10.6	11.6–13.6
Soluble/Total protein ratio (%)	38–42	38–48	50–55	50	31–34	—	36–50
Color							
(°EBC)	4–6	—	3–4	4–5	2–300	500–1,600	4–8
(°Lovibond)	—	1.5–2.0	—	—	—	—	—
Diastatic power							
(°Institute of Brewing)	30–45	80–130	180	65	0	0	100
(°Lintner)	35–50	90–145	200–250	70	0	0	110
(W.-K)	100–140	230–360	480–600	190	0	0	280
α-Amylase (DU)	30–55	33–49	105	25	0	0	47–55
Moisture (% fr. wt)	2–4	3.6–4.3	6.5	5–6	5–8	2	6–8

Note: Many types of malts are prepared to meet different specifications. Because malts are analyzed by different methods and often there are no true conversion factors, the example values given are approximate. Data mostly from reference 1. d.b-dry basis, fr. wt.-wet weight basis. I thank S. M. Sole of the Crisp Malting Group for much help.

[a] Lactic acid content 2–4%

[b] In general malts from 6-row barleys tend to have higher enzyme and protein contents and lower extracts than malts from 2-row barleys. Wheats, being husk-free, tend to give malts with higher extracts than barley, because the husk yields no extract.

[c] n/a = not applicable.

[d] Hot-water extracts determined on finely ground samples. Different laboratory mashing methods are used.

(1,8). There is also a series of caramel malts: carapils, 2–5; carahell, 20–33; and caramuench, 50–300. Common British malts usually have colors of about:- lager, 3; pale ale, 4–6; mild ale, 7–8, amber, 40–85; brown, 100–200; chocolate, 500–1,100; and black, 1,200–1,600 (1,4). A full range of caramel malts is also available. These color ranges are not legally defined, and malt may be made to any reasonable specification. In addition to color, these malts impart special characteristics to beers and some other products. They are of no value in making distilled products. Colored and special malts, lacking enzymes, are used in small proportions in mashes mixed with paler malts that do contain enzymes. Less usual barley malts include undergrown or chit, malts that have the advantages of raw grain adjuncts in mashes, are less costly to make than normal malts, and can be used legally where the use of unmalted grains is illegal. Proteolytic, enzymic, and lactic malts contain a proportion (e.g., 2–4%) of lactic acid. These malts are used to adjust mash pH values where other methods are not permitted (1,4,8). Mashes made with a proportion of these malts yield a good extract, and the wort is enriched with N-containing yeast nutrients. Wheat beers are made from mashes prepared mainly with wheat malts (1,8). These malts are sometimes used in small proportions in mashes making barley beers, to improve the beer foam. Smoked, caramel, and roasted wheat malts are also used to a small extent. Sorghum malts are increasingly being used in Africa to make lager-type beers as well as traditional opaque beers (1). Distillery malts are of two main classes. Those in which the malt is the main or only component of the mash must have high extract yields and need only moderate enzyme contents, but those used to convert large amounts of starchy adjuncts in the mash must be rich in enzymes, although their extract yield is not so critical. These may be green (undried) malts (1,2).

BIBLIOGRAPHY

1. D. E. Briggs, *Malts and Malting*, Blackie Academic and Professional, London, United Kingdom, 1998.
2. G. N. Bathgate and R. Cook, "Malting of Barleys for Scotch Whiskies," in J. R. Piggott, R. Sharp, and R. E. B. Duncan, eds., *The Science and Technology of Whiskies*, Longman Scientific and Technical/John Wiley and Sons, New York, 1989, pp. 19–63.
3. D. E. Briggs, *Barley*, Chapman and Hall, London, United Kingdom, 1978.
4. D. E. Briggs et al., *Malting and Brewing Science*, 2nd. ed., Vol. 1, Chapman and Hall, London, United Kingdom, 1981.
5. J. S. Hough et al., *Malting and Brewing Science* 2nd ed., Vol. 2, Chapman and Hall, London, United Kingdom, 1982.
6. W. C. Burger and D. E. La Berge, "Malting and Brewing Quality," in D. C. Rasmusson, ed., *Barley*, American Society of Agronomy, Madison, Wis., 1985, pp. 367–401.
7. A. H. Cook, ed., *Barley and Malt*, Academic Press, London, United Kingdom, 1962.
8. L. Narziss, *Die Bierbrauerei (6 Auflage), Band I, Die Technologie der Malzbereitung*, Ferdinand Enke Verlag, Stuttgart, Germany, 1976.
9. G. H. Palmer and G. N. Bathgate, "Malting and Brewing," in Y. Pomeranz, ed., *Advances in Cereal Science and Technology*, Vol. 1, American Association of Cereal Chemists, St. Paul, Minn., 1976, pp. 237–324.
10. R. E. Pyler and D. A. Thomas, "Malted Cereals. Production and Use," in K. J. Lorenz and K. Kulp, eds., *Handbook of Cereal: Science and Technology*, Marcel Dekker, New York, 1991, pp. 815–832.
11. G. Gibson, "Malting Plant Technology," in G. H. Palmer, ed., Aberdeen University Press, Aberdeen, Scotland, pp. 279–325

D. E. Briggs
University of Birmingham
Birmingham, England

MARGARINE

Margarine is a fatty food emulsion manufactured in semblance of butter. The appearance, color, texture, flavor, and nutritional content are equivalent to those of butter. The primary difference between margarines and butter is that the fat in margarine is not primarily milkfat. In turn, many lower-fat spread products have been invented and marketed in semblance of margarine. Margarines and lower-fat spreads share common attributes that are designed to resemble those of butter: product color is typically yellow; flavor is reminiscent of, although not identical to, that of butter; salt and acidity are comparable; functional utility is similar except for some cooking operations; and vitamin content is usually equivalent. The manufacturing processes are different, however, and shelf life length favors margarine over butter.

HISTORICAL MILESTONES

Margarine was invented in 1869 in France by Hippolyte Mege-Mouries in response to a need for a satisfactory butter substitute for military personnel. The original invention was prepared from beef fat, artificial gastric juice, salt, and milk. The resultant product was described as having a pearl-like luster, so Mege-Mouries named the material margarine, after the Greek *margarite*, or pearl-like. The inventor further described the product as a variety of true butter and later as artificial butter, butterine, or margarine.

The manufacture and sale of margarine was permitted by 12 April 1872 by action of the Council of Hygiene of Paris, which authorized its sale if not described as butter. Margarine manufacture expanded throughout Europe from 1872 to 1874. The first U.S. patent for margarine was granted in December 1873.

In the United States, the first Oleomargarine Act was passed in 1886. Amendments followed in 1902 and 1930. These actions placed a tax of $0.02/lb on margarine and required licensure of manufacturers and distributors to prevent adulteration of butter. The first definition of margarine was prescribed in 1941, and the 1950 Margarine Act removed the special taxation. The last state tax on margarine, in Minnesota, was abolished in 1975.

The history of margarine formulation in the United States is a series of incremental changes in processes and composition, all directed at attaining more favorable economics.

1890	Cultured milk used as a flavorant.
1917	Coconut oil used as a substitute for animal fat, most likely as a reaction to shortages of animal fat caused by World War I.
1923	Vitamin A fortification popularized. Butter contains about 15,000 IU/lb of this vitamin. Most margarines and spreads in the United States today are fortified to 16,000 IU/lb to permit labeling claims of 10% daily reference value intake per serving.
1934	Hydrogenated vegetable oils replaced coconut oil.
1941	U.S. federal Standard of Identity for margarine adopted.
1947	Beta-carotene (provitamin A) replaced coal tar colorants.
1950	Margarine Act passed by U.S. Congress, abolishing federal taxes on the product.
1952	First soft margarine in tub form appeared. One product was marketed as "Emde," at a price of over $3.00/lb and available only by prescription of a physician.
1956	Blended vegetable oil and butterfat products appeared.
1957	U.S. per capita consumption of margarine exceeded that of butter for the first time.
1957	Whipped sticks (6 per pound) first appeared.
1964	Soft tub margarine distributed nationally as mainstream grocery item.
1966	Diet margarine (40% fat) introduced.
1967	Fluid, pourable margarine developed and introduced.
1975	Lower-fat spreads (typically 60% fat) introduced.
1981	Butter/margarine blends introduced to national market.
1981–1997	Lower-fat spreads proliferated.
1991	U.S. Nutritional Labeling and Education Act passed by U.S. Congress.
1991	No-fat margarines appeared.

TYPES OF PRODUCTS

The products within the margarine and yellow fat spread category may be classified in any of several groups. Most relevant is grouping by physical form, which follows function.

Solid

Solid products are bulk cubes of 50 lb or 25 kg (55 lb), packed for institutional food processing or food service consumption. These products are typically formulated specifically for the intended use and generally are of firmer structure and higher solid fat content than retail margarine. Typical use is in general baking, with additional usage in layered pastries. Other types of solid margarine are brick and stick.

Brick margarine is typically of 1 lb net weight, wrapped in paper or foil laminate, and packed 24 to 35 lb per case. Customary uses are in general cooking, shallow pan frying, basting, and the like. The products are similar to retail formulations in texture, flavor, and consistency.

Stick margarines are made for household kitchen and table use and are typically packaged four sticks to the pound. These products are packaged first in paper or laminated foil, then in a carton of paperboard. Cases contain 12 to 36 lb. The lower-weight cases are recent developments to address inventory-management programs among grocers, who count case turnover in lieu of actual poundage. In the United States, two styles of stick margarine are found. The eastern stick is sold east of the Rocky Mountains and is packed either in flat units or in two-over-two cartons. The western-style product is shorter in length but larger in width and breadth. The two styles arose from availability of packaging equipment in the respective market areas. Solid margarine is also packaged in individual servings of 5 to 14 g, distributed via food service operators.

Soft

Soft products are generally intended for direct use, as for spreading on breads. Retail products are packed in twin cups of 8 oz net weight, sleeved two per pound, or in 16-oz tubs. Margarine that conforms to the U.S. Standard of Identity may not be sold in packages greater than 1 lb at retail. Many lower-fat spreads are packaged for retail sale in units of 2 or 3 lb or more. Soft products for food service use include portion-control cups of 5, 7, 10, or 14 g. Soft product is occasionally seen in single-serving pouches.

Whipped products are soft or stick products to which nitrogen is injected at manufacture, with the primary intent being expansion in volume for economy of serving. Typical volume expansion is about 50%, yielding 6 sticks per pound, or 24 fluid ounces at net weight of 16 oz.

Liquid margarines are typically formulated to be resistant to solidification at refrigerated temperatures while maintaining structure of the emulsion. Typical uses are as a topping for hot vegetables, waffles, pancakes, and popcorn.

THE REGULATORY IDENTITY OF MARGARINE

In the United States, margarine and butter are two foods that are defined by action of the U.S. Congress. Other foods are defined by regulations promulgated by the U.S. Food and Drug Administration, the U.S. Department of Agriculture, or other regulatory agencies.

Title 21, Section 166 of the Code of Federal Regulations (21 CFR 166) defines margarine labeling and composition. The labeling provision requires that the word "margarine" or "oleomargarine" appear on the principal display panel in type or size at least as large as any other type or lettering. Other regulations require a listing of ingredients in the product, with the sole exception that proprietary flavorant substances need only be identified as "artificial flavor."

The composition provision requires that the product contain at least 80% fat and be made from edible fats, oils, or mixtures thereof whose origins are vegetable, rendered animal carcass fats, or any form of oil from a marine species that has been affirmed as generally recognized as safe (GRAS) or listed as a food additive.

The product must also contain one or more of the following:

- Water and/or milk and/or milk products
- Suitable edible protein, including whey, lactose-reduced whey, non-lactose-containing whey components, albumin, casein, caseinate, vegetable proteins, or soy protein isolate, in amounts not greater than that reasonably required to accomplish the desired effect.
- Any mixture of 1 and 2. This must be pasteurized. Any mixture of this may be subjected to the action of harmless bacterial starters.
- Vitamin A of at least 15,000 IU/lb concentration.

Margarine may contain any of these optional ingredients:

- Vitamin D. If added, product must contain no less than 1,500 IU/lb.
- Salt, which typically ranges from about 1.25% to 2.0% by weight.
- Nutritive carbohydrate sweeteners
- Emulsifiers
- Preservatives, including but not limited to the following:
  - Sorbic acid, benzoic acid, and their sodium, potassium, and calcium salts, individually 0.1% or in combination, 0.2%
  - Calcium disodium EDTA, 0.0075%
  - Gallates, BHT, BHA, ascorbyl palmitate, and ascorbyl stearate, individually or in combination, 0.02%
  - Stearyl citrate, 0.15%; isopropyl citrate mixture, 0.02%
- Color additives (Beta-carotene or annatto or annatto/turmeric extracts)
- Flavoring substances
- Acidulants and/or alkalizers

MARGARINE FATS

Margarine is a water-in-oil emulsion, which is a semistable suspension of dispersed aqueous materials within a continuous matrix of fat. The fat typically is refined, mixed triglycerides of vegetable or animal carcass origin. The predominant vegetable oil in the United States is soybean oil, which is a by-product of soybeans. The soybean is grown to produce proteins for animal and human foodstuffs; soybean oil is about 18% of the soybean. Additional vegetable oils used in margarines are corn, safflower, sunflower, and palm. Lauric oils are seldom seen. Marine-origin oils that are GRAS are also permitted. Lower-priced margarines, especially in private-label segments, may contain lard, beef tallow, or combinations of these with soybean oil.

The structure of the fat or blend of fats is the most significant factor in margarine formulation. The triglycerides present in the oils are reaction products of 1 molecule of glycerol with 3 molecules of fatty acids, with mixed triglycerides predominant in nature. The triglycerides provide structure, lubricity, caloric density, and a satiety to the product.

Physical properties of margarines and spreads, especially texture, spreadability, color, appearance, and melting profile, are functions of the structure of the fat and the processing conditions used in manufacture. Margarines and spreads are composed of liquid oil, fat crystals, and the aqueous phase. The crystals give margarine the required consistency and stabilize the water droplets. The fat crystals are in effect frozen oil. Hydrogenation of the liquid fat is used to raise the melting point of the fat to provide the structure needed. Hydrogenation is the addition of hydrogen across the unsaturated carbon-carbon double bond.

Soybean oil margarine and/or spread is typically a blend of two or more partially hydrogenated components mixed in appropriate proportions for functional effect. The functional effect can be predicted from the measurement of solid fat index (SFI), which is the ratio of solid fat to liquid fat at specified temperatures. Measurement of SFI is done by dilatometry. A similar measurement, solid fat content, may be performed instrumentally via nuclear magnetic resonance. Table 1 demonstrates the differences in SFI among different types of margarines and spreads, with reference to butter.

The significance of the temperatures used in the test protocol are that 50°F is a typical temperature of processing, 70°F may describe the stability of the product outside the cold-storage area or refrigerator, 80°F is a typical temperature found in a commercial bakery or kitchen, 92°F is reflective of mouth temperatures, and 104°F is a typical bakery proofing chamber temperature. The relevance of the SFI temperatures is usage specific.

The process temperature of 50°F is typically the temperature at which product is packaged. For solids, bricks, and sticks, the product itself provides structure to the package. SFI content of about 25% is required for efficient packing of such product. For soft margarine, the solids content of only about 11% assures the product is still fluid and pourable when the containers are filled. The soft product will become dramatically firmer after resting in the package for as few as 5 min. Liquid margarine SFI of about 3% at 50°F indicates the product will remain fluid at refrigeration/cold-store temperatures.

At 70°F, the SFI indicates resistance to ambient temperature slump or partial melt. In the United States, stick margarines are occasionally offered for sale in supermarket aisles rather than from the refrigerated dairy case. This practice is caused in part by the economic phenomenon of slotting allowances that retailers require manufacturers to pay. Space in the dairy case is premium compared to dry shelf display, which is premium compared to aisle display. The practice of offering margarines for sale in nonrefrigerated areas is not condoned by the manufacturers, however.

The 92°F temperature in SFI is significant in that it is a quantitative measure of waxiness in the mouth. If the value is greater than about 3%, the product will melt slowly in the mouth and impart a slight coating or waxlike aftertaste. Significance of 80°F and 104°F were described previously.

Margarine fats are generally blends of oils. Hydrogenation is energy intensive and also produces *trans* fat isomers. Its use in margarine is generally minimized for economic as well as functional effect. For example, stick margarine fat may be a blend of 50% of partially hydrogenated soybean oil of iodine value 68 and 50% of refined, bleached, and deodorized liquid soy oil of iodine value 135. Tub margarine may be a blend of the same components, with the sole difference being the ratio of liquid oil to hard fat at 2:1 rather than 1:1 as for stick product. The liquid product may be a blend of about 98% of liquid soy oil and 2% of fully hydrogenated soybean oil. Bakery products typically combine partially hydrogenated oil and liquid oil with fully hydrogenated fat to maintain structure in warm bakeries.

Table 1. Typical Solid Fat Indices

	Margarine type				
Percent Solid fat at	Solid	Soft	Liquid	Bakery	Butter
50°F = 10°C	25–29	9–13	2–4	28–32	29–32
70°F 21.1°C	13–17	6–8	1–3	16–20	11–14
80°F = 26.7°C	8–11	4–6	1–3	11–15	6–8
92°F = 33.3°C	1–3	1–2.5	1–3	6–10	1–2.5
104°F = 40°C	0	0	1	2–6	0

OTHER FATTY TYPE INGREDIENTS

Fat-soluble margarine ingredients include soy lecithin, monoglycerides, derivatives of monoglycerides, vitamin A, beta-carotene, flavorants, and some preservatives.

Lecithin is phosphatidylcholine, a by-product of refining soybean oil. The addition of lecithin to margarine is to promote the frying performance of the margarine. Addition of about 0.2% of lecithin will dramatically reduce the tendency of margarines to "spatter" in frying. Lecithin prevents the coalescence of large droplets of water that would become steam at frying temperatures.

Monoglycerides and distilled monoglycerides are emulsifiers, or surface active agents that promote stability of the oil and water mixture by linking the naturally immiscible fats and water-laden ingredients at the fat-water interface. Use of 0.10 to 0.25% of monoglycerides also promotes creaminess in mouth-feel.

Derivatives of monoglycerides include polyglycerol esters, propylene glycol monoesters, and other such materials. Primary use of these materials is as whipping/creaming agents for expanding volume of the margarine or spread by incorporation of nitrogen, creating a three-phase foam of gas in water-in-oil.

Vitamin A palmitate is the palmitic acid ester of retinoic acid. Regulations require addition to total fortification of 15,000 IU/lb, or about 4,500 μg per pound, which equates to about 10 ppm. Vitamin A palmitate is the fat-soluble market form of vitamin A, as opposed to vitamin A citrate, a water-soluble form typically found in multivitamin tablets. Although no regulation requires spreads (nonstandard products) to be fortified, market forces have caused all nonmargarine spreads in the United States to be fortified with vitamin A at the same concentrations as those of margarine.

Vitamin D (calciferol) is an optional ingredient that may be added to margarine and spreads at 1,500 IU/lb.

Beta-carotene is a double molecule of retinoic acid and is used as the colorant of choice in the majority of margarines marketed in the United States. It is also called provitamin A because it is metabolized within the liver to become vitamin A. In practice, margarine is fortified with about 5 ppm of beta-carotene for optimal coloring. This permits the manufacturer to reduce the amount of vitamin A palmitate added, as both vitamin A palmitate and beta-carotene contribute to the total vitamin A activity in the product.

Annatto or annatto-turmeric extracts are also used as margarine colorants, typically at concentrations of about 10 to 20 ppm. These are vegetable extracts.

Preservatives may be added to protect the oil from oxidative rancidity or to retard the growth of microorganisms.

Flavorants typically added to margarine and/or spreads are generally mixtures of synthesized or extracted chemicals. Principal flavorant substances are 2,3 butadione (diacetyl); lactic acid; short-chain fatty acids such as butyric, caproic, or capryllic; lactones; keto acids; some aldehydes; indole; methyl indole; or similar materials. Typical usage is about 5.0 to about 300 ppm, depending on desired strength of flavor.

AQUEOUS COMPONENTS

The dispersed phase typically contains droplets of 1 to 20 μm in size. The primary aqueous material is water, to which may be added skim milk, salt, and water-soluble preservatives and/or flavorants. Lightly salted margarine is preferred in the United States. Typical salt concentration is about 1.5%.

Skim milk, whey, or other edible proteins are added to enhance the release of the flavor profiles and to permit the margarine or spread to perform in semblance of butter in shallow pan frying. The milk solids and protein will brown in the pan, leaving a golden color for frying. Water-based margarines that contain no milk products simply overheat and blacken in the pan. The flavor profiles are often described as deficient or not well rounded.

Lactic acid, if added, is both a flavorant and an acidulant. Addition of about 0.05% to 0.1% of lactic acid will reduce the typical serum phase pH to less then 5.5, which increases the effect of the preservatives.

The typical margarine and spread preservative system is a mix of potassium sorbate and sodium benzoate, each added at about 0.10% by weight. These preservatives are added to sustain the resistance to microbial spoilage after the package has been opened in the home.

MANUFACTURING PROCESS

The margarine manufacturing process is a series of unit operations that transform a blend of ingredients into the product. The five unit operations are emulsification, cooling, working, resting, and packaging.

Margarine is mixed either in batches or by continuous methods. In batching, discrete amounts of the ingredients are measured, either by weight or by volume; blended; and transferred to processing steps. Batching is the procedure of choice when the products are of a variety of recipes. Continuous mix simultaneously measures all ingredients and mixes them in a continuous output stream. One method of continuous mix uses a multiple-headed adjustable calibration pump operated from a common drive shaft. This method is useful if the type of product is constant.

Emulsification is achieved by measuring appropriate amounts of oils, emulsifiers, vitamin A, coloring, and oil-soluble flavorings. Aqueous-phase ingredients are blended separately, then combined with the oil phase at a temperature slightly above the melting point of the oil. Agitation of the mix is required to promote the formation of the emulsion.

Mixed emulsion at a temperature slightly higher than the melting point of the mixed fat (typically about 100–105°F) is pumped through a filter to a scraped surface heat exchanger cooling system such that the output temperature of the chilled margarine or spread is about 50 to 55°F, which is well below the melting point of the oils. This attribute of supercooling by rapid chilling is employed to reduce the size of fat crystals to a fine dispersion. If the emulsion were cooled via simple refrigeration, the fat crystals would be excessively large Large crystals promote grainy texture and instability of emulsion, which are both undesirable. Nitrogen gas is injected either before or after scraped surface heat exchange but prior to pinworker (see next paragraph). Chilling and crystallizing of molten product yields an increase in production line pressure to as much as 250 to 300 psig, depending on the specific composition of product and equipment configuration. The scraped surface heat exchange equipment is designed to tolerate pressure slightly in excess of this. Contact the manufacturer for specifics.

The chilled product is agitated by pinworker units that reduce the size of the fat crystals by mechanical working. This further softens the texture of the product and fully disperses the injected nitrogen gas. Working also lengthens the time for the product to fully set.

Supercooled fluid product is allowed to rest in crystallizing chambers before packaging. Stick and brick product can be packed by either of two processes: (*1*) form the stick, then apply wrap, collect, and carton; or (*2*) insert cut wrapping material into a mold, then inject the fluid product into the mold cavity, wrap, collect, and carton. Soft product is dispensed into preformed and printed cups or tubs, capped, then sleeved. Many soft products are packaged with a plastic shrink-wrap band for package surety.

Packed units are collected into cases, stacked on pallets, and then moved to cold storage temporarily until quality testing is completed.

QUALITY TESTING

Ingredients are tested for conformance to requirements. The increasing tendency is for suppliers to test and validate requirements by providing certified testing results with delivery of the ingredient. Primary testing of the oils is conducted at the plant. Significant attributes in routine testing include flavor, aroma, color, peroxide value, free fatty acids, moisture, refractive index or iodine value, and SFI, when applicable. Other ingredients are tested for microbiological quality as appropriate.

Manufacture processes are monitored by recording instruments, visual observation, and analysis of production efficiencies for conformance to requirements. Typical process temperatures are product specific, but general approximations are:

As mixed	ex SSHE	ex pinworker	As packed	10 min postpack
105°F	53°F	56°F	58°F	64°F

Product quality includes nominal chemical characteristics of moisture %, salt %, curd/milk solids %, with fat % determined by difference. Other characteristics of product that are determined at time of production include pH of the aqueous phase, color, flavor, appearance, and net weight. Several package characteristics, including completeness of wrap, presence of lids, and legibility of code dating are checked also. Microbiological assays for standard plate count (SPC), yeasts, molds, and coliforms are determined routinely.

Typical limits are SPC 3,000/g, yeasts, molds, and coliforms negative by analysis. Periodic sampling for additional quality characteristics is also required, including vitamin A assay and selected pathogens. Quality begins with clearly stated product requirements and is built into the product via instructions for formulation and manufacture.

GENERAL REFERENCES

A. J. C. Andersen and P. N. Williams, *Margarine*, 2nd ed., Pergamon Press, New York, 1965.

M. M. Chrysam, "Table Spreads and Shortenings," in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, 4th ed., Vol. 3, John Wiley & Sons, New York, 1965, pp. 41–126.

Code of Federal Regulations, Title 21 CFR Part 166, Secs. 401, 701, U.S. Government Printing Office, Washington, D.C., 1999.

D. Melnick, "Development of Organoleptically and Nutritionally Improved Margarine Products," *J. Home Economics* **6**, 793–798 (1968).

A. J. Haighton, "Blending, Chilling and Tempering of Margarines and Shortenings," *J. Am. Oil Chem. Soc.* **53**, 357–399 (1978).

Handbook of Soy Oil Processing and Utilization, American Soybean Association, St. Louis, Mo., and American Oil Chemists Society, Champaign, Ill., 1980.

Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., John Wiley & Sons, New York, 1996.

Margarine Statistics Report, National Association of Margarine Manufacturers, Washington, D.C., 1988.

S. F. Riepma, *The Story of Margarine*, Public Affairs Press, Washington, D.C., 1970.

L. H. Weiderman, "Margarine and Margarine Oil: Formulation and control," *J. Am. Oil Chem. Soc.* **55**, 823–829 (1978).

JOHN BUMBALOUGH
Land O'Lakes, Inc.
Arden Hills, Minnesota

See also FATS AND OILS: PROPERTIES, PROCESSING TECHNOLOGY, AND COMMERCIAL SHORTENINGS.

MARINE ENZYMES

Marine animals possess the same functional classes of enzymes as other living organisms, which enable them carry out virtually the same metabolic activities. These enzymes are present in the digestive glands and the muscle tissues of the animals and may be recovered in active and stable forms for commercial use. There is considerable demand for enzymes throughout the world for food, biomedical, and other commercial applications. Worldwide sales of commercial enzymes were estimated at $1.5 billion for 1997 (1). Traditional enzymes such as pepsins, rennets, trypsins, and lipases are derived from animal tissues, whereas bromelain, ficin, papain, lipoxygenase, and amylases are derived from plants. Plant and animal enzymes represent a small fraction of commercial enzymes, with the greatest diversity of commercial enzymes coming from microorganisms that have been stringently evaluated and certified as safe. Examples of microbial enzymes are glucose oxidase, pectinesterases, cellulase, and glucose isomerase. Microbial enzymes are used as replacements or substitutes for homologous enzymes from animals or plants because they are relatively easier and cheaper to produce. Other contributory factors to the decline in the use of plant and animal enzymes are related to political as well as agricultural and economic policies of various governments that regulate food and agricultural practices. The marine environment presents an excellent opportunity for supplying commercial enzymes to help meet the demands for these compounds. In several of the major fish-producing countries, the by-products of seafood harvesting comprise about 50% of the entire harvest. These materials are largely underutilized and discarded as waste. However, this abundant material also includes the enzyme-rich digestive organs, and the enzymes may be recovered in various forms to suit a range of commercial applications. It is estimated that less than half of the fish offal generated in fish plants is converted into value-added products such as pet food, fish meal, and compost, while the remainder poses problems related to disposal and environmental pollution. This discussion focuses on enzymes from fish and shellfish tissues that either have the potential for commercial application or constitute a source of concern for food-processing operations, especially extracellular enzymes produced by digestive organs and intracellular enzymes from fish muscle tissues. Enzymes from digestive organs have the greatest potential for commercial application because of their high tissue concentration. However, the recovery of enzymes present at low concentration, such as intracellular enzymes, is also of interest, because recent advances in biotechnology will facilitate the cloning of the genes for those enzymes and their subsequent production by fermentation. The use of immobilized forms of these enzymes will also allow use of expensive enzymes in clinical or biochemical applications.

ENZYME TYPES, DEFINITIONS, SOURCES, AND SOME PROPERTIES

Marine animals carry out essentially the same types of metabolism as land animals. Thus, it is to be expected that marine animals will contain all six functional classes of enzymes (i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases). The scope of this article will be limited to those enzymes from fish and shellfish that either have the potential for commercial application

As mixed	ex SSHE	ex pinworker	As packed	10 min postpack
105°F	53°F	56°F	58°F	64°F

Product quality includes nominal chemical characteristics of moisture %, salt %, curd/milk solids %, with fat % determined by difference. Other characteristics of product that are determined at time of production include pH of the aqueous phase, color, flavor, appearance, and net weight. Several package characteristics, including completeness of wrap, presence of lids, and legibility of code dating are checked also. Microbiological assays for standard plate count (SPC), yeasts, molds, and coliforms are determined routinely.

Typical limits are SPC 3,000/g, yeasts, molds, and coliforms negative by analysis. Periodic sampling for additional quality characteristics is also required, including vitamin A assay and selected pathogens. Quality begins with clearly stated product requirements and is built into the product via instructions for formulation and manufacture.

GENERAL REFERENCES

A. J. C. Andersen and P. N. Williams, *Margarine*, 2nd ed., Pergamon Press, New York, 1965.

M. M. Chrysam, "Table Spreads and Shortenings," in T. H. Applewhite, ed., *Bailey's Industrial Oil and Fat Products*, 4th ed., Vol. 3, John Wiley & Sons, New York, 1965, pp. 41–126.

Code of Federal Regulations, Title 21 CFR Part 166, Secs. 401, 701, U.S. Government Printing Office, Washington, D.C., 1999.

D. Melnick, "Development of Organoleptically and Nutritionally Improved Margarine Products," *J. Home Economics* **6**, 793–798 (1968).

A. J. Haighton, "Blending, Chilling and Tempering of Margarines and Shortenings," *J. Am. Oil Chem. Soc.* **53**, 357–399 (1978).

Handbook of Soy Oil Processing and Utilization, American Soybean Association, St. Louis, Mo., and American Oil Chemists Society, Champaign, Ill., 1980.

Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, 5th ed., John Wiley & Sons, New York, 1996.

Margarine Statistics Report, National Association of Margarine Manufacturers, Washington, D.C., 1988.

S. F. Riepma, *The Story of Margarine*, Public Affairs Press, Washington, D.C., 1970.

L. H. Weiderman, "Margarine and Margarine Oil: Formulation and control," *J. Am. Oil Chem. Soc.* **55**, 823–829 (1978).

JOHN BUMBALOUGH
Land O'Lakes, Inc.
Arden Hills, Minnesota

See also FATS AND OILS: PROPERTIES, PROCESSING TECHNOLOGY, AND COMMERCIAL SHORTENINGS.

MARINE ENZYMES

Marine animals possess the same functional classes of enzymes as other living organisms, which enable them carry out virtually the same metabolic activities. These enzymes are present in the digestive glands and the muscle tissues of the animals and may be recovered in active and stable forms for commercial use. There is considerable demand for enzymes throughout the world for food, biomedical, and other commercial applications. Worldwide sales of commercial enzymes were estimated at $1.5 billion for 1997 (1). Traditional enzymes such as pepsins, rennets, trypsins, and lipases are derived from animal tissues, whereas bromelain, ficin, papain, lipoxygenase, and amylases are derived from plants. Plant and animal enzymes represent a small fraction of commercial enzymes, with the greatest diversity of commercial enzymes coming from microorganisms that have been stringently evaluated and certified as safe. Examples of microbial enzymes are glucose oxidase, pectinesterases, cellulase, and glucose isomerase. Microbial enzymes are used as replacements or substitutes for homologous enzymes from animals or plants because they are relatively easier and cheaper to produce. Other contributory factors to the decline in the use of plant and animal enzymes are related to political as well as agricultural and economic policies of various governments that regulate food and agricultural practices. The marine environment presents an excellent opportunity for supplying commercial enzymes to help meet the demands for these compounds. In several of the major fish-producing countries, the by-products of seafood harvesting comprise about 50% of the entire harvest. These materials are largely underutilized and discarded as waste. However, this abundant material also includes the enzyme-rich digestive organs, and the enzymes may be recovered in various forms to suit a range of commercial applications. It is estimated that less than half of the fish offal generated in fish plants is converted into value-added products such as pet food, fish meal, and compost, while the remainder poses problems related to disposal and environmental pollution. This discussion focuses on enzymes from fish and shellfish tissues that either have the potential for commercial application or constitute a source of concern for food-processing operations, especially extracellular enzymes produced by digestive organs and intracellular enzymes from fish muscle tissues. Enzymes from digestive organs have the greatest potential for commercial application because of their high tissue concentration. However, the recovery of enzymes present at low concentration, such as intracellular enzymes, is also of interest, because recent advances in biotechnology will facilitate the cloning of the genes for those enzymes and their subsequent production by fermentation. The use of immobilized forms of these enzymes will also allow use of expensive enzymes in clinical or biochemical applications.

ENZYME TYPES, DEFINITIONS, SOURCES, AND SOME PROPERTIES

Marine animals carry out essentially the same types of metabolism as land animals. Thus, it is to be expected that marine animals will contain all six functional classes of enzymes (i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases). The scope of this article will be limited to those enzymes from fish and shellfish that either have the potential for commercial application or constitute a source of concern for food processing operations. They include representatives from the oxidoreductases, transferases, and hydrolases.

Oxidoreductases

Oxidoreductases are the group of enzymes that catalyze oxidation-reduction reactions. Examples include polyphenol oxidases, peroxidases, and the dehydrogenases. Polyphenol oxidases (PPO) catalyze the oxidation of phenolic compounds in the presence of molecular oxygen to form dark-colored melanins. These enzymes are found in the particulate and in the soluble fraction of skin homogenates of fish and shellfish. Examples of marine animals from which PPOs have been isolated are lobster (2,3), shrimp (4,5), crab (6), the common mussel (*Mytilus edulis*) (7), goldfish (8) and otopus (9). PPOs are metalloenzymes and have copper ions in their catalytic sites. They lose activity when the copper ions are removed or replaced by other metal ions. PPOs are active over a broad pH range from 5.0 to 8.5, and they are most stable within a weakly acidic to alkaline pH range of 6 to 10 (4,7,8). The enzymes also have different molecular weights such as 30 to 40 kDa for shrimp PPOs, 64 kDa for the lobster enzyme, 120 kDa for the enzyme from *Mytilus edulis*, and 125 kDa to 205 kDa for cephalopods (2–4,7,9). The enzymes are inhibited by p-amino benzoic acid, oxygen competitors like cyanide and N2, and metal chelators like diethyldithiocarbamate and EDTA.

Another group of oxidoreductases found in fish tissue is the lipoxygenases. These enzymes are of considerable economic importance to the fishing industry because of the adverse effects it causes with regard to fish texture, odor, and color during storage. These enzymes have been isolated from microsomal fractions of various fish species including herring (10), trout (11,12), flounder (13), Pacific rockfish (14), mackerel (14), gurnard, grouper, and tilefish (14). Lipoxygenases from marine animals catalyze the oxidation of polyunsaturated fatty acids, similar to plant lipoxygenases. These enzymes are most active between pH 6 and 7 and remain active at subzero temperatures (10). Microsomal lipid oxidation enzymes have been implicated in fish quality deterioration during iced or frozen storage. For example, lipoxygenases associated with fish skin promote postharvest bleaching of carotenoid pigments in fish skin and/or flesh (15).

Peroxidases are also oxidoreductases. Peroxidases with molecular weights of 35.5 kDa, 76.5 kDa, and 147 kDa have been isolated from the hepatopancreas of crayfish (16). These enzymes catalyze the incorporation of halogens into organic molecules, for example, halogenation of tyrosine to thyroxine, the thyroid hormone responsible for the prevention of goiter in humans.

The glycolytic pathway includes a number of dehydrogenases that participate in the removal of hydrogen atoms from various substrate molecules. The glycolytic enzymes remain active and catalyze their respective reactions in the postmortem animal. The degree to which glycolysis proceeds in the postmortem animal has important ramifications concerning the postharvest quality of fish and shellfish. Several of the glycolytic enzymes have been characterized from muscles of fish and shellfish such as lobster and cod (17), tuna and halibut (18), shrimp (19), and trout (20). Examples of the enzymes are lactate dehydrogenase (LDH), an oxidoreductase that catalyzes the interconversion between pyruvate and lactate under limited supply of oxygen. Lactate dehydrogenases from marine species exist as tetramers with subunits of 36 kDa and are inhibited in a noncompetitive fashion by 3-acetyl-pyridine NAD. The muscle of some invertebrates like squid does not contain lactate dehydrogenase, and pyruvate is metabolized by a different mechanism during anoxia.

Glutamate dehydrogenases have been isolated from various shellfish, for example, *Mytilus edulis* (21), mollusks (22), and *Modiolus demissus* (23). The enzymes catalyze the oxidative deamination of glutamate to α-ketoglutarate, thereby serving to increase concentration of TCA cycle intermediates (so-called anaplerotic reactions) to allow an increase in rate of oxidation of two-carbon units:

$$\text{glutamate} + \text{NAD}^+ + \text{H2O} \leftrightarrow \alpha\text{-ketoglutarate} + \text{NH}_3 + \text{NADH} + \text{H}^+$$

The same reaction may run in reverse, draining off TCA cycle intermediates for biosynthetic purposes. Glutamate dehydrogenases from marine animal tissues were shown to be most active from pH 7.4 to 9.5.

Another dehydrogenase of the glycolytic pathway that has been isolated from marine animal tissues is muscle-type glyceraldehyde-3-phosphate dehydrogenase. The enzyme catalyzes the substrate level oxidative phosphorylation of glyceraldehyde-3-phosphate (G3P) to the high-energy compound glyceraldehyde-1,3-diphosphate (1,3-DPG), whose high-energy group transfer potential is subsequently used to synthesize ATP. The same enzyme drives the reverse reaction, that is, the formation of glyceraldehyde-3-phosphate from 1,3-diphosphoglycerate during gluconeogenesis. Glyceraldehyde-3-phosphate dehydrogenase has been isolated from lobster and cod muscle and shown to exhibit maximum activity within pH range 8.5 to 9.0.

Uricase, also known as urate oxidase, is an oxidoreductase and catalyzes the oxidation of uric acid to allantoin prior to further degradation and excretion. The enzyme is usually located in peroxisomes and is used as a marker enzyme for this organelle and has a potential commercial value as a diagnostic enzyme. Uricase has been isolated from the livers of a number of fish, such as trout, mackerel, catfish, shark, and tilapia (24). Uricases from various marine animals are active over a broad pH range, from pH 7.0 to 9.5 with a maximum at 8.8. Uricase is an oligomeric enzyme containing subunits of 32.5 kDa. It is also a metalloenzyme, requires Cu^{2+} for activity, and is inhibited by chelating agents.

Transferases

The transferases are a group of enzymes that remove groups (other than H) from various substrates and then transfer them to acceptor molecules. Examples of the transferases are transglutaminases and the kinases. Transglutaminases (TGases) catalyze the Ca^{2+}-dependent acyl-transfer reactions between the γ-carboxyamide groups of protein-bound glutamine residues and the primary amino groups in a variety of compounds (including ϵ-NH_2 of lysine). TGases have the unique ability to modify the functional properties of protein molecules via covalent cross-linking. TGase activity has been described in the tissues of various marine animals such as carp (25), rainbow trout (26), mackerel (26), lobster (27), scallop, shrimp, and squid (26). The TGases have molecular weights ranging from 80 to 200 kDa and temperature optima ranging from 40 to 50°C. They are active over a broad pH range of 6.0 to 9.5. The kinases catalyze the phosphorylation of substrates in reactions that invariably require ATP as phosphate donor. Pyruvate kinase (PK) is an important regulatory enzyme in the glycolytic pathway. It catalyzes the reaction that prepares pyruvate for subsequent utilization in the TCA cycle, that is, the irreversible conversion of pyruvate to acetyl CoA. PK has been purified from various shellfish including *Mytilus edulis* (28), *Crassostrea gigas* (29), and *Cardium tuberculatum* (30). It is a tetrameric protein molecule with 70 kDa subunits (30) that requires monovalent and divalent cations for activity. It is activated by fructose-1,6-bisphosphate and inhibited by L-alanine, ATP, D-lactate, and citrate.

Phosphofructokinase (PFK) is another transferase, and it catalyzes the conversion of fructose-6-phosphate (F6P) to fructose-1,6-diphosphate (F1,6DP) in the glycolytic pathway. PFK is allosterically activated by F6P and AMP but is inhibited by ATP and citrate. The enzyme has been isolated from trout muscle (31).

Glycogen phosphorylase is a transferase and catalyzes the first step in the utilization of glycogen. The enzyme has been isolated from lobster muscle and shown to exist as a dimer with a molecular weight of 170 kDa. Lobster glycogen phosphorylase is similar to its mammalian counterpart in having two forms: the inactive dephosphorylated form known as phosphorylase b, and the phosphorylated active form known as phosphorylase a.

Glutathione-S-transferases are a group of multifunctional proteins that are involved in the detoxification of xenobiotics in animals. The enzyme has been isolated from the liver, kidney, and gills of rainbow trout and shown to exist as a dimer whose subunits have molecular weights ranging from 23.9 to 25 kDa (32). The enzyme is inhibited by 1-chloro-2,4-dinitrobenzene.

Aspartate transcarbamylase, also known as aspartate carbamoyltransferase, catalyzes the first committed reaction in the biosynthesis of pyrimidine bases. This involves the transfer of a carbamoyl group directly to L-aspartate without the formation of any intermediate products. The enzyme has been isolated from the mantle of *Mytilus edulis* (33) and is most active within pH range of 8.5 to 8.9. It is strongly inhibited by *p*-hydroxymercuribenzoate and Cu^{2+} but is strongly stimulated by organic solvents like dimethylsulfoxide and dimethylformamide.

Hydrolases

Hydrolases are the group of enzymes that catalyze the cleavage of covalent bonds in substrates with the participation of water molecules as reactants. The hydrolytic enzymes of importance in this regard include the proteases that catalyze hydrolytic cleavage of peptide bonds in proteins and polypeptides to produce low-molecular-weight peptides and/or amino acids, and lipolytic enzymes that catalyze the hydrolysis of ester bonds of triglycerides and phospholipids to produce fatty acids and other products. Proteolytic enzymes include those found in fish muscle as lysosomal proteases and those from digestive glands or fluids, namely, digestive proteases. Lysosomal proteases, known as cathepsins, have been detected in various species of fish such as carp (34), albacore (35), cod (36), salmon (37), Pacific sole (38), squid (39,40), and tilapia (41). Cathepsins from marine animals occur as single polypeptide chains with different molecular weights, for example, 13.6 kDa for squid cathepsin B (40) and 25 kDa for cathepsin C from squid hepatopancreas (39). Some cathepsins exhibit maximum activity within pH 3.5 to 7.0, whereas others are most active at alkaline pH (8.0). They are activated by Cl^- ions and usually require thiol reagents as well as chelating agents for activity and are believed to play an important role in postmortem deteriorative changes in fish texture prior to processing or storage (42). According to Siebert and Schmitt (43), fish muscle contains about 10 times more catheptic enzyme activity than mammalian muscle.

The most studied digestive proteases from marine animals are the pepsin(ogen)s, trypsin(ogen)s, and chymotrypsin(ogen)s. Pepsin(ogen)s are acidic proteases secreted by the gastric mucosal glands, whereas trypsin(ogen)s and chymotrypsin(ogen)s are secreted by pancreatic tissue and pyloric ceca. A number of exopeptidases are also secreted in the gut, where they act to degrade peptides into free amino acids. Pepsin(ogen)s have been isolated from several fish species, including tuna (44,45), salmon (46), dogfish (47), bonito (48), trout (49,50), sardine (51), hake (52), capelin (53), cod (54–56), and smelt (57). The carnivorous fish have been shown to have the highest levels of pepsins in their stomachs (58,59). Pepsin activity has not been detected in digestive tracts of stomachless fish (60,61). The pH optima of fish pepsins range from 2.5 to 3.0, which is slightly more alkaline than pH optima for mammalian pepsins (51). The molecular weights of pepsins isolated from various fish species range between 36 and 41 kDa. Gastricsin(ogen)s are acidic proteases present in the gastric mucosa of fish. Gastricsin(ogen)s have been purified from *Merluccius gayi* with molecular weights of 27 to 28 kDa (36). They exhibit maximum proteolytic activity at pH 3.0 and are generally stable up to pH 10.0, the point beyond which they rapidly lose activity. Marine gastricsins catalyze the clotting of milk similar to fish and mammalian pepsins but differ from pepsins by showing activation with sodium chloride (56). Trypsin(ogen)s and chymotrypsin(ogen)s have also been recovered from the digestive tracts of several fish species such as sardine (62), goldfish (61), capelin (63), catfish (64), rainbow trout (65), salmon (66), green chromide (67), cod (68–71), cunner (72), crayfish (73), shrimp (74), and starfish (75–78). They are generally most active within pH range 7 to 8.5 and are also most stable at alkaline pH, unlike trypsin(ogen)s from mammals (69). They are single polypeptide chains with relatively lower molecular weights, ranging from 21 kDa to 26 kDa.

Chymotrypsins have been isolated and characterized from marine species such as anchovy (79), Atlantic cod (79–80), capelin (81), herring (81), rainbow trout (82), and spiny dogfish (83,84). In general, these enzymes are single polypeptide molecules with molecular weights ranging between 25 and 28 kDa. They are most active within pH range 7.5 to 8.5 and are most stable at around pH 9.0 (82). Chymotrypsins from marine animals have a higher catalytic activity and hydrolyzed more peptide bonds in various protein substrates (casein, collagen, and bovine serum albumin) at subdenaturation temperatures than mammalian chymotrypsins. In general, the marine chymotrypsins were more heat-labile than mammalian chymotrypsins (80). Dogfish chymotrypsin was more active toward soy protein isolate from 5°C to 35°C than bovine chymotrypsin (84) and could also clot milk. Chymotrypsins from herring and capelin exhibited greater hydrolytic activities toward the synthetic substrate BTEE than the bovine enzyme (81).

Collagenases are capable of degrading the polypeptide backbone of native collagen under conditions that do not denature the protein. Collagenolytic enzymes have been isolated and characterized from the digestive glands of a variety of teleost fish (85) and crab (86–89). Marine collagenases resemble their mammalian counterparts in their mode of action, that is, they act on native collagen in solution and cleave the helical structure at loci 75%, 70%, and 67% from the NH_2-terminal of the molecule. However, they differ from mammalian collagenases in exhibiting trypsin- and chymotrypsin-like specificities toward synthetic substrates for these enzymes. They are also subject to inhibition by serine protease inhibitors such as PMSF and DIPF. In this regard they are described as serine proteases, unlike mammalian collagenases, which are mostly metalloproteinases. Marine collagenases show optimum activity within a pH range of 6.5 to 8.0 and are inactivated at pH values below 6.0. They are also inhibited by well-known serine protease inhibitors like diisopropylfluorophosphate, phenyl methyl sulfonyl fluoride, soybean trypsin inhibitor, and chicken ovomucoid. A collagenase purified from crab was shown to be a single polypeptide molecule with a molecular weight of ~25 kDa. Collagenases from hepatopancreas tissue have been implicated in postharvest texture deterioration in prawns during ice storage (90). Although true collagenases have not been isolated from the muscle of fish, there is evidence that they contribute to flesh softening in postharvest Pacific rockfish (91).

Alkaline proteases is the general term used to describe proteolytic enzymes present in fish muscle that show maximum activity as well as stability within the alkaline pH range. These enzymes have been isolated from muscles of various fish species such as filefish, croaker, anchovy, rockfish, yellowtail, shrimp, and garfish (92). They show optimum activity within pH range 7.9 to 8.1, are stable within a broad pH range (from 4.5 to 10.0), and generally are high-molecular proteins with molecular weights ranging from 780 kDa to 920 kDa (92). These enzymes require relatively high temperatures for activity; however, they show differences in their thermostabilities. For example, incubation of white croaker alkaline protease for 10 min at 60°C destroyed about 95% of original enzyme activity, whereas carp alkaline protease lost less than 5% of its original activity when subjected to the same time and temperature treatment (92). Alkaline proteases have been implicated in postmortem textural deteriorations in fish and fish products.

The enzyme urease catalyzes the breakdown of urea to ammonia and carbon dioxide during excretion in most fish species. The flesh of elasmobranchs contains much more nonprotein nitrogen of ureic origin than teleost fish, and this imparts a peculiar odor and sour-bitter taste to these fish species. During ice storage of meats from elasmobranchs like shark, a large amount of ammonia is generated due to the high content of urea. Some of the predominant microflora associated with skins of freshly caught sharks (i.e., *Pseudomonas, Micrococcaceae*, and *Moraxella*) produce urease that promotes ammonia formation from urea during storage, which is detrimental to the quality and acceptability of shark meats. This undesirable development in the product can be minimized by improved sanitary conditions during handling; soaking meat in water, lactic acid, salt, or urease solution prior to storage; blanching; or heat sterilization.

Lipolytic enzymes that have been characterized from fish include lipases from digestive organs or juices from both vertebrate and invertebrate fish, and phospholipases from microsomal fraction from muscle tissues of various fish such as skate (93), cod (94,95), trout (96,97), crayfish (98), pollock (99), and lobster (100). These lipolytic enzymes are single polypeptide chains with a broad pH-activity range (from 6 to 10) and are generally very heat labile. They are inhibited by thiol reagents, surface active agents, heavy metals, and oxygen. In general, the activities of lipolytic enzymes from marine species are lower than those from mammals. However, this is probably due to lower levels of enzymes present rather than the lower specific activities of the enzymic proteins.

Acid phosphatases are a group of heterogenous sialic acid–containing glycoenzymes that catalyze removal of phosphate groups from their substrates, and this reaction is important in the formation of triglycerides from phosphatidates. An acid phosphatase with molecular weight of 123 kDa has been isolated from liver of carp (101). The enzyme is stable over a broad pH range, between 3.5 and 5.5, and is most active between pH 4.5 and 5.0. It is strongly inhibited by L-(+) tartrate, molybdate, fluoride, urea, and mercuric ions.

In addition to the enzymes described previously, all the enzymes of the TCA cycle, with the exception of succinyl CoA synthetase, have been detected in the liver of rainbow trout (102). Citrate synthetase, α-ketoglutarate dehydrogenase, and succinate dehydrogenase were detected in the mitochondrial fraction, whereas the rest of the enzymes were detected in the cytosolic fraction. The intracellular distribution of TCA enzymes in trout was similar to that of mammalian species.

Comparative Biochemistry

Marine enzymes are similar to their mammalian counterparts in several of their characteristics. Generally, their pH-activity profiles, substrate specificities, molecular weights, and response to inhibitors or activators resemble those of their mammalian counterparts (44,56,70,84). However, they may differ from mammalian enzymes in a number of ways. For example, digestive proteases from marine organisms have been shown to be generally less stable under acid conditions, have higher catalytic activity, digest undenatured protein substrates more efficiently, and are heat labile but more stable at cold temperatures (44,69,70,84,103) Some of these distinctive properties of marine enzymes could be exploited successfully in various commercial operations.

APPLICATIONS, CURRENT USE, AND IMPACT OF BIOTECHNOLOGY

Biotechnology as it pertains to food production has been practiced for several years and has been or area of prolific growth in science and engineering within the past two decades. In the food and allied industries, major importance is now being attached to the use of enzymes for enhancing product quality, by-product utilization, increasing yields of extractive processes, product stabilization, and flavor. For example, fresh seafoods exhibit delicate aromas and flavors that are quite distinct from those usually evident in "commercially fresh" seafood. A group of enzymatically derived aldehydes, ketones, and alcohols has been found to be responsible for the characteristic aroma of very fresh seafood (104), and these compounds are similar to the C-6, C-8, and C-9 compounds produced by lipoxygenases. Chen and Li (105) isolated proteolytic enzymes from the cephalothorax of grass shrimp and demonstrated that enzymes like carboxypeptidase A and B, trypsin, chymotrypsin, cathepsin, and collagenase are important in seafood flavor production. They further demonstrated that these proteolytic enzymes from grass shrimp heads could tenderize meats just as well as papain or bromelain.

The use of enzymes in food processing and their potential for future applications have been extensively covered in publications by Godfrey and Reichelt (106), Schwimmer (107), and Reed (108). Studies carried out by various researchers have also demonstrated potential applications of certain marine enzymes as processing aids, based on some of their unique properties described earlier in this article. For example, pepsins from cold temperature–adapted fish have been used as a rennet substitute in preparation of cheddar cheese (55), while fish and shellfish trypsins and cathepsins have been applied to facilitate fermentation of capelin, squid, and herring (109–111). Cathepsins have also been shown to play an important role in patis fermentation (112). Enzyme preparations from digestive organs of herring, mackerel, sardine, and sprats have been used to refine the taste of mackerel preserves and to accelerate ripening of herring fillets (57). Similarly, trypsins from cod and cunner fish have been successfully applied to recover carotenoprotein from crustacean waste for incorporation into fish feeds and as flavorants, colorants, or both in surimi-based seafood analogs (72,113). Digestive proteolytic enzymes from stomachless marine species like cunner, crayfish, and mullet appear to be better suited to digestion and/or inactivation of native proteins than their counterparts derived from species with a functional stomach like mammals (73,114–116). These enzymes are currently being investigated with regard to their ability to inactivate PPOs, pectinesterases, or both in fruit juices. For example, apple juice develops a brown color very rapidly due to the presence of PPOs. To overcome the browning process, the substrates responsible for this phenomenon may be chemically altered to render them resistant to oxidation by PPOs. Another approach is to use a hot clarification process to inactivate the enzymes responsible for the discolorations, which adds to the energy cost as well as destroys some desirable heat-labile volatile components in fruit juices. An alternative approach that could offer an even greater appeal to consumers eager for "natural" food products may be to use digestive proteases from stomachless marine species described previously to inactivate both PPOs and pectinesterases in fruit juices. The successful application of such enzymes to inactive PPO could have a major impact on the shrimp-processing industry, which is facing the problem of finding effective alternative(s) to sulfiting agents used in some countries like the United States to control dark discolorations in these economically important marine species. Also, a potential interesting use of marine enzymes is the immobilization of lipolytic enzymes for catalyzing interesterification of fish oils to produce glycerides enriched with ω-3 polyunsaturated fatty acids (PUFAs). The consumption of ω-3 PUFAs from fish have been associated with a number of beneficial health effects (117). The use of marine enzymes for the interesterification reactions is suggested because the intended substrates are more natural to these enzymes than microbial or plant lipases. There are few instances where marine enzymes are applied on a commercial scale. For example, the Icelandic Fisheries Laboratory (IFL) has developed a process for utilizing trypsin-like enzymes from cod viscera for removal of skins, membranes, and scales from fish. The fish skins, membranes, and scales thus produced are used to prepare fish gelatin, which differs from mammalian gelatin in that it has a relatively low gelling temperature due to its lower proline and hydroxyproline content (118). At the present time, fish frames are hydrolyzed in Norway with cold-adapted pepsins from cod viscera to produce marine peptones for subsequent use as media for commercial cultivation of microorganisms for production of microbial proteins or fertilizer (119). Marine enzymes are also used in fish feed and for the production of immune stimulants for fish reared in captivity (118). For more information on the current and potential uses of marine enzymes in industry, there are excellent reviews on the subject by Haard (120) and Raa (118).

The major obstacles to using marine by-products as sources of industrial enzymes include their limited availability due to seasonal harvest; variability in levels and/or activity of enzymes due to nutritional status, seasonal variation, and spawning: and the highly perishable nature of the raw material. However, attitudes toward the use of marine enzymes in industry are slowly changing, and the potential profits to be derived from their commercial production may be enhanced by the use of recombinant DNA techniques for cloning enzyme protein genes from the marine animals into suitable bacteria or yeasts to facilitate production of the recombinant enzymes by fermentation.

Several intracellular enzymes from fish and shellfish have been studied, as shown earlier in the text. In general, large-scale production of intracellular enzymes from marine animals is limited to only a few enzymes, and sales from these enzymes represent only a small fraction of the total volume and value of commercial enzyme production and sales. However, with the rapid advances in enzyme technology in recent years, production of intracellular enzymes is very feasible for a number of reasons. For example, intracellular enzymes are widely used in clinical research, and their production is crucial to the furtherance of much biochemical research—for example, uricase is used as a diagnostic enzyme; however, the pH sensitivity of uricase presents a problem in clinical use for the quantification of serum uric acid, which is conducted around pH 7.1. The observation that trout uricase retains stability and activity at pH 7.0 may indicate its usefulness for this clinical application. Different kinds of fish enzymes have been cloned and expressed by microorganisms. Examples of such fish enzymes include tissue-type TGase from sea bream, which was successfully cloned and expressed in *E. coli* (121). In fish, TGases have been studied in relation to the elucidation of the molecular mechanisms for gel formation in fish mince products. It has been suggested that endogenous tissue-type TGases catalyze the setting reaction that involves the cross-linking of myosin heavy chains in fish paste during processing. This setting reaction is thought to be important with respect to the viscoelastic properties of fish paste (122). Another interesting fish enzyme that has been cloned is the cytochrome P450c17, which is a key enzyme in determining the synthesis of sex steroids (androgens and estrogens) and progestins. The enzyme is responsible for the sequential 17 α-hydroxylase and $C_{17,20}$-lyase reactions in the synthesis of the aforementioned compounds. The expression of an active form of shark P450 was achieved by Trant (123) using a *Pichia pastoris* GS115 yeast expression system and the expression vector pHiL-D2. Male et al. (124) described the nucleotide sequences and the structural models of five trypsins variants from Atlantic salmon, whereas Klein et al. (125) studied the trypsin gene expression in shrimp (*Penaeus vannamei*) and detected the cDNAs encoding five isoforms of trypsin. Gudmunsdottir et al. (126) also isolated cDNAs encoding two different forms of trypsinogen from Atlantic cod. LeBoulay et al. (127) reported the cloning and expression of cathepsin L from shrimp hepatopancreas, whereas Van Wormhoudt and Sellos (128) cloned and sequenced three cDNAs for amylase in *P. vannamei*, and Su et al. (131) constructed a cDNA library of the phospholipase from *Artemia* and expressed it in *E. coli* BL21(DE3). As more basic information on the distinctive properties of marine enzymes becomes available through research, it is expected that those marine enzymes with potential for industrial use would be produced in commercial quantities by gene cloning technology.

BIBLIOGRAPHY

1. Novo Nordisk, 1998.
2. O. J. Ferrer et al., "Phenoloxidase Levels in Florida Spiny Lobster (*Panulirus argus*): Relationship to Season and Molting Stage," *Comp. Biochem. Physiol.* **93B**, 595–599 (1989).
3. K. A. Savagaon and A. Sreenivasan, "Purification and Properties of Latent and Isoenzymes of Phenoloxidase in Lobster (*Panulirus homarus* Linn.)," *Ind. J. Biochem. Biophys.* **12**, 94–99 (1978).
4. B. K. Simpson, M. R. Marshall, and W. S. Otwell, "Phenoloxidases From Pink and White Shrimp: Kinetic and Other Properties," *J. Food Biochem.* **12**, 205–217 (1988).
5. M. E. Bailey, E. A. Fieger, and A. F Novak, "Physicochemical Properties of the Enzymes Involved in Shrimp Melanogenesis," *Food Res.* **25**, 557–563 (1959).
6. N. M. Summers, "Cuticle Sclerotization and Blood Phenol Oxidase in the Fiddler Crab, *Uca pugnax*," *Comp. Biochem. Physiol.* **23**, 129–138 (1967).
7. J. H. Waite, "Catechol Oxidase in the Byssus of the Common Mussel, *Mytilus edulis* L," *J. Mar. Biol. Ass. U.K.* **65**, 359–371 (1985).
8. K-H. Kim and T. T. Tchen, "Tyrosinase of the Goldfish *Carassius auratus* L. I. Radio-assay and Properties of the Enzyme," *Biochim. Biophys. Acta.* **59**, 569–576 (1962).
9. G. Prota et al., "Occurrence and Properties of Tyrosinase in the Ejected Ink of Cephalopods," *Comp. Biochem. Physiol.* **68B**, 415–419 (1981).
10. B. M. Slabyj and H. O. Hultin, "Lipid Peroxidation by Microsomal Fractions Isolated From Light and Dark Muscles of Herring (*Clupea harengus*)," *J. Food Sci.* **47**, 1395–1398 (1982).
11. R. J Hsieh and J. E. Kinsella, "Lipoxygenase-catalyzed Oxidation of N-6 and N-3 Polyunsaturated Fatty Acids. Relevance to Activity in Fish Tissue," *J. Food Sci.* **51**, 940–945, 996 (1986).
12. T.-J. Han and J. Liston, "Lipid Peroxidation and Phospholipid Hydrolysis in Fish Muscle Microsomes and Frozen Fish," *J Food Sci.* **52**, 294–296, 299 (1987).
13. R. E. McDonald and H. O. Hultin, "Some Characteristics of the Enzymic Lipid Peroxidation System in the Microsomal Fraction of Flounder Skeletal Muscle," *J Food Sci.* **52**, 15–21, 27 (1987).
14. N. Tsukuda, "Discoloration of Red Fishes," *Bull. Tokai Reg. Fish. Research Lab.* **70**, 103–174 (1972).
15. N. Tsukuda, "Studies on the Discoloration of Red Fishes. VI. Partial Purification and Specificity of Lipoxygenase-like Enzymes Responsible for Carotenoid Discoloration in Fish Skin During Refrigerated Storage," *Bull. Jap. Soc. Sci. Fish.* **36**, 725–733 (1970).
16. R. H. Ilgner and A. E. Woods, "Purification, Physical Properties and Kinetics of Peroxidases From Freshwater Crayfish (Genus *orconectes*)," *Comp. Biochem. Physiol.* **82B**, 433–440 (1985).
17. C. B. Cowey, "Comparative Studies on the Activity of D-Glyceraldehyde-3-Phosphate Dehydrogenase From Cold and Warm-blooded Animals With Reference to Temperature," *Comp. Biochem. Physiol.* **23**, 969–976 (1967).
18. P. S. Low, J. L. Bada, and G. N Somero, "Temperature Adaptation of Enzymes: Roles of the Free Energy, the Enthalpy, and the Entropy of Activation," *Proc. Nat. Acad. Sci.* **70**, 430–432 (1973).
19. M. T. Thebault, A. Bernicard, and J. F. Lennon, "Lactate Dehydrogenase From the Caudal Muscle of the Shrimp *Palaemon serratus*: Purification and Characterization," *Comp. Biochem. Physiol.* **68B**, 65–70 (1981).
20. T. Henry, and A. Ferguson, "Kinetic Studies on the Lactate Dehydrogenase (LDH-5) Isozymes of Brown Trout, *Salmo trutta L*," *Comp. Biochem. Physiol.* **82B**, 95–98 (1985).

21. A. R. Ruano et al., "Some Enzymatic Properties of NAD+-dependent Glutamate Dehydrogenase of Mussel Hepatopancreas (*Mytilus edulis* L.)—Requirement of ADP," *Comp. Biochem. Physiol.* **82B**, 197–202 (1985).
22. M. A. Wicker and R. P. Morgan II, "Effects of Salinity on Three Enzymes Involved in Amino Acid Metabolism From the American Oyster *Crassostrea virginia*," *Comp. Biochem. Physiol.* **53B**, 339–343 (1976).
23. P. M. Reiss, S. K. Pierce, and S. H. Bishop, "Glutamate Dehydrogenases From Tissues of the Ribbed Mussel, *Modiolus demissus*: ADP Activation and Possible Physiological Significance," *J. Exp. Zool.* **202**, 253–258 (1977).
24. J. E. Kinsella, B. German, and J. Shetty, "Uricase From Fish Liver: Isolation and Some Properties," *Comp. Biochem. Physiol.* **82B**, 621–624 (1985).
25. H. Kishi, H. Nozawa, and N. Seki, "Reactivity of Muscle Transglutaminase on Carp Myofibrils and Myosin B," *Nippon Suisan Gakkaishi* **57**, 1203–1210 (1991).
26. H. Nozawa, S. Mamagoshi, and N. Seki, "Partial Purification and Characterization of Six Transglutaminases From Ordinary Muscles of Various Fishes and Marine Invertebrates," *Comp. Biochem. Physiol.* **118B**, 313–317 (1997).
27. R. Myhrman and J. Bruner-Lorand, "Lobster Muscle Transpeptidase," *Meth. Enzymol.* **19**, 765–770 (1970).
28. D. A. Holwerda et al., "Regulation of Mussel Pyruvate Kinase During Anaerobiosis and in Temperature Acclimation by Covalent Modification," *Molec. Physiol.* **3**, 225–234 (1983).
29. T. Mustafa and P. W. Hochachka, "Catalytic and Regulatory Properties of Pyruvate Kinase in Tissues of a Marine Bivalve," *J. Biol. Chem.* **246**, 3196–3203 (1983).
30. J. Chrispeels and G. Gade, "Purification and Characterization of Pyruvate Kinase From the Foot Muscle of the Cockle, *Cardium tuberculatum*," *Comp. Biochem. Physiol.* **82B**, 163–172 (1985).
31. S. P. J. Brooks and K. B. Storey, "Revaluation of the Glycolytic Complex: A Multitechnique Approach Using Trout Muscle," *Arch. Biochem. Biophys.* **267**, 13–22 (1988).
32. I. A. Nimmo and C. M. Spalding, "The Glutathione S-transferase Activity in the Kidney of Rainbow Trout (*Salmo gairdneri*)," *Comp. Biochem. Physiol.* **82B**, 91–94 (1985).
33. M. Mathieu, "Partial Characterization of Aspartate Transcarbamylase From the Mantle of the Mussel *Mytilus edulis*," *Comp. Biochem. Physiol.* **82B**, 667–674 (1985).
34. K. Hara, A. Suzumatsu, and T. Ishihara, "Purification and Characterization of Cathepsin B From Carp Ordinary Muscle," *Nihon Suisan Gakkai-shi* **54**, 1243–1252 (1988).
35. H. S. Groninger, Jr., "Partial Purification of a Proteinase From Albacore Muscle," *Arch. Biochem.* **108**, 175–182 (1964).
36. A. Schmitt and G. Siebert, "Distinguishing Aliphatic Dipeptidases From Cod Muscle," *Z. Physiol. Chem.* **348**, 1009–1016 (1967).
37. C.-Y. Ting, M. Montgomery, and A. F. Anglemier, "Partial Purification of Salmon Muscle Cathepsins," *J. Food Sci.* **33**, 617–621 (1968).
38. G. M. Geist and D. L. Crawford, "Muscle Cathepsins in Three Species of Pacific Sole," *J. Food Sci.* **39**, 548–551 (1974).
39. K. S. Hameed and N. F. Haard, "Isolation and Characterization of Cathepsin C From Atlantic Short-finned Squid, *Illex illecebrosus*," *Comp. Biochem. Physiol.* **82B**, 241–246 (1985).
40. T. Inaba, N. Shindo, and M. Fuji, "Purification of Cathepsin B From Squid Liver," *Agric. Biol. Chem.* **40**, 1159–1165 (1976).
41. S. V. Sherekar, M. S. Gore, and V. Ninjoor, "Purification and Characterization of Cathepsin B From the Skeletal Muscle of Fresh Water Fish, *Tilapia mossambica*," *J. Food Sci.* **53**, 1018–1023 (1988).
42. S. B. K. Warrier, V. Ninjoor, and G. B. Nadkarni, "Involvement of Hydrolytic Enzymes in the Spoilage of Bombay Duck (*Harpodon nehereus*). Harvest and Postharvest Technology of Fish," *Soc. Fish. Technol. (India)*, pp. 470–472 (1985).
43. G. Siebert and A. Schmitt, "Fish Tissue Enzymes and Their Role in Determination Changes in Fish," in R. Kreuzer, ed., *The Technology of Fish Utilization*, Fishery News Books, Ltd., London, United Kingdom, pp. 47–52, 1965.
44. M. Tanji, T. Kageyama, and K. Takahashi "Tuna Pepsinogens and Pepsins. Purification, Characterization and Amino-terminal Sequences," *Eur. J. Biochem.* **177**, 251–259 (1988).
45. E. R. Norris and J. C. Mathies, "Preparation, Properties and Crystallization of Tuna Pepsin," *J. Biol. Chem.* **204**, 673–680 (1953).
46. J. S. Fruton and M. Bergmann, "The Specificity of Salmon Pepsin," *J. Biol. Chem.* **136**, 559–560 (1940).
47. T. G. Merrett, E. Bar-Eli, and H. Van Vunakis, "Pepsinogens A, C, and D From the Smooth Dogfish," *Biochemistry* **8**, 3696–3702 (1969).
48. M. Kubota, A. Ohnuma, and I. Karube, "Kinetics of Protease Inactivation by Gamma Irradiation," *J. Tokyo Univ. Fish.* **55**, 9–20 (1970).
49. T. G. Owen and A. J. Wiggs, "Thermal Compensation in the Stomach of the Brook Trout (*Salvelinus fantinalis* Mitchill)," *Comp. Biochem. Physiol.* **40B**, 465–473 (1971).
50. S. S. Twining et al., *Comp. Biochem. Physiol.* **75B**, 109–112 (1983).
51. M. Nora and K. Murakami, "Purification and Characterization of Two Acid Proteinases From the Stomach. Studies on Proteinases From the Digestive Organs of Sardine," *Biochim. Biophys. Acta.* **658**, 27–34 (1981).
52. L. Sanchez-Chiang, and O. Ponce, "Gastricsinogens and Gastricsins From *Merluccius gayi*—Purification and Properties. *Comp. Biochem. Physiol.* **68B**, 251–257 (1981).
53. A. Gildberg and J. Raa, "Purification and Characterization of Pepsins From the Arctic Fish Capelin (*Mallotus villosus*)," *Comp. Biochem. Physiol.* **75A**, 337–342 (1983).
54. K. Arunchalam and N. F. Haard, "Isolation and Characterization of Pepsin Isoenzymes From Polar Cod (*Boreogadus saida*)," *Comp. Biochem. Physiol.* **80B**, 467–473 (1985).
55. P. Brewer, N. Helbig, and N. F. Haard, "Atlantic Cod Pepsin—Characterization and Use as a Rennet Substitute," *Can. Inst. Food Sci. Technol. J.* **17**, 38–43 (1984).
56. E. J. Squires, N. F. Haard, and L. A. W. Feltham, "Gastric Proteases of the Greenland Cod *Gadus ogac*. II. Structural Properties," *Can. J. Biochem. Cell Biol.* **64**, 205–222 (1986).
57. N. F. Haard et al., "Modification of Proteins With Proteolytic Enzymes From the Marine Environment," in R. L. Feeney, J. R. Whitaker, eds., *Modification of Proteins*. Advances in Chemistry Series 198, American Chemical Society, Washington, D.C., pp. 223–224, 1982.
58. B. G. Kapoor, H. Smit, and I. A. Verighina, "The Alimentary Canal and Digestion in Teleost," *Adv. Mar. Biol.* **13**, 109–239 (1975).
59. Y-I. Hsu and J-L. Wu, "The Relationship Between Feeding Habits and Digestive Proteases of Some Fresh Water Fishes," *Bull. Inst. Zool. Acad. Sinica.* **18**, 45–53 (1979).

60. A. Nilsson and R. Fange, "Digestive Proteases in the Cyclostome *Myxina glutinosa* (L)," *Comp. Biochem. Physiol.* **32**, 237–250 (1970).

61. K. D. Jany, "Studies on the Digestive Enzymes of the Stomachless Bonefish *Carassius auratus gibelio* (bloch): Endopeptidases," *Comp. Biochem. Physiol.* **53B**, 31–38 (1976).

62. K. Murakami and M. Noda, "Studies on Proteinases From the Digestive Organs of Sardine—Purification and Characterization of Three Alkaline Proteinases From the Pyloric Ceca," *Biochem. Biophys. Acta.* **65B**, 17–26 (1981).

63. K. Hjelmeland and J. Raa, "Characteristics of Two Trypsin Type Isozymes Isolated From the Arctic Fish Capelin (*Mallotus villosus*)," *Comp. Biochem. Physiol.* **71B**, 557–562 (1982).

64. R. Yoshinaka et al., "Enzymatic Characterization of Anionic Trypsin of the Catfish *Parasilurus asotus*," *Comp. Biochem. Physiol.* **77B**, 1–6 (1984).

65. M. Kitamikado and S. Tachino, "Studies on the Digestive Enzymes of Rainbow Trout—II. Proteases," *Bull. Jap. Soc. Scient. Fish.* **26**, 685–694 (1960).

66. C. B. Croston, "Endopeptidases of Salmon Ceca: Chromatographic Separation and Some Properties," *Arch. Biochem. Biophys.* **112**, 218–223 (1965).

67. S. Sundaram and P. S. Sarma, "Purification and Properties of a Protease From the Gut of *Etroplus suratensis*," *Biochem. J.* **77**, 465–471 (1960).

68. B. K. Simpson, M. V. Simpson, and N. F. Haard, "On the Mechanism of Enzyme Action: Digestive Proteases From Selected Marine Organisms," *Biotechnol. Appl. Biochem.* **11**, 226–234 (1989).

69. B. K. Simpson and N F Haard, "Trypsin From Greenland Cod (*Gadus ogac*). Isolation and Comparative Properties," *Comp. Biochem. Physiol.* **79B**, 613–627 (1984).

70. B. K. Simpson and N. F. Haard, "Purification and Characterization of Trypsin From the Greenland Cod (*Gadus ogac*). I. Kinetic and Thermodynamic Characteristics. *Can. J. Biochem. Cell Biol.* **62**, 894–900 (1984).

71. J. Overnell "Digestive Enzymes of the Pyloric Ceca and the Associated Mesentery in the Cod (*Gadus morhua*)," *Comp. Biochem. Physiol.* **46B**, 519–531 (1973).

72 B. K. Simpson and N. F. Haard, "The Use of Proteolytic Enzymes to Extract Carotenoproteins From Shrimp Wastes," *J. Appl. Biochem.* **7**, 212–222 (1985).

73. G. Pfleiderer, R. Zwilling, and H Sonneborn, "Eine Protease von Molekulargewicht 11,000 und eine Trypsinahniche Fraktion aus *Astacus fluviatilis*," *Z. Physiol. Chem.* **348**, 1319–1331 (1967).

74. B. T. Gates and J. Travis, "Isolation and Comparative Properties of Shrimp Trypsin," *Biochemistry* (N Y.) **8**, 4483–4489 (1968).

75. C-S. Chen, T-R. Yan, and H-Y. Chen, "Purification and Properties of Trypsin-like Enzymes and a Carboxypeptidase A From *Euphasia superba*," *J. Food Biochem.* **21**, 349–366 (1978).

76. Z. Camacho, J R. Brown, and G. B. Kitto, "Purification and Properties of Trypsin-like Proteases From the Starfish *Dermasterias imbricata*," *J Biol. Chem.* **245**, 3964–3972 (1970).

77. H. F. Bundy and J. Gustafson, "Purification and Comparative Biochemistry of a Protease From the Starfish *Pisaster giganteus*," *Comp. Biochem. Physiol.* **44B**, 241–251 (1973).

78. E. P. Kozlovskaya and L. A. Elyakova, "Purification and Properties of Trypsin-like Enzymes from the Starfish *Lysastrosoma anthocticta*," *Biochim. Biophys. Acta.* **371**, 63–70 (1974).

79. M. S. Heu, H. R. Kim, and J. H. Pyeum, "Comparison of Trypsin and Chymotrypsin From the Viscera of Anchovy, *Eugraulis japonica*," *Comp. Biochem. Physiol.* **112B**, 557–568 (1995).

80. B. Asgiersson and J. B. Bjarnasson, "Structural and Kinetic Properties of Chymotrypsin From Atlantic Cod (*Gadus morhua*). Comparison With Bovine Chymotrypsin," *Comp. Biochem. Physiol.* **99B**, 327–335 (1991).

81. J. Kalac, "Studies on Herring (*Clupea harengus* L.) and Capelin (*Mallotus villosus* L.) Pyloric Ceca Proteases III. Characterization of Anionic Fractions of Chymotrypsins," *Biologia (Bratislava)* **33**, 939–945 (1978).

82. M. M. Kristjansson and H. H. Nielson, "Purification and Characterization of Two Chymotrypsin-like Proteases From the Pyloric Ceca of Rainbow Trout (*Oncorhynchus mykiss*)," *Comp. Biochem. Physiol.* **101B**, 247–253 (1992)

83. W. F. Racicot, *A Kinetic and Thermodynamic Comparison of Bovine and Dogfish Chymotrypsins*, Ph.D. thesis, Univ. of Massachusetts, Amherst, 1984

84. M. Ramakrishna, H. O. Hultin, and M. T. Atallah, "A Comparison of Dogfish and Bovine Chymotrypsins in Relation to Protein Hydrolysis," *J. Food Sci.* **52**, 1198–1202 (1987).

85. R. Yoshinaka, M. Sato, and A. Ikeda "Distribution of Collagenase in the Digestive Organs of Some Teleost," *Bull. Jap. Soc. Scient. Fish.* **44**, 263–268 (1978).

86. G. A. Grant, J. C. Sacchettini, and H. G. Welgus, "A Collagenolytic Serine Protease With Trypsin-like Specificity From the Fiddler Crab *Uca pugilator*," *Biochemistry* **22**, 354–358 (1983).

87. A. Z. Eisen and J. J. Jeffrey, "An Extractable Collagenase From Crustacean Hepatopancreas," *Biochim. Biophys. Acta* **191**, 517–520 (1969).

88. A. Z. Eisen et al., "A Collagenolytic Protease From the Hepatopancreas of the Fiddler Crab, *Uca pugilator*. Purification and Properties," *Biochemistry* **12**, 1814–1822 (1973).

89. H. Welgus et al., "Substrate Specificity of the Collagenolytic Serine Protease From *Uca pugilator*," *Biochemistry* **21**, 5183–5189 (1982).

90. W. K. Nip, C. Y. Len, and J. H. Moy, "Partial Characterization of a Collagenolytic Enzymes Fraction From the Hepatopancreas of the Freshwater Prawn, *Macrobranchium rosenbergii*," *J. Food Sci.* **50**, 1187–1188 (1985)

91 R. Cepeda et al., *An Immunological Method for Measuring Collagen Degradation in the Muscle of Fish. Seafood Biotechnology Workshop. August 31–September 1, 1989*, St. John's, Newfoundland, Canada, 1990

92. K. Iwata, K. Kabashi, and J. Hase, "Studies on Muscle Alkaline Protease—III Distribution of Alkaline Protease in Muscle of Freshwater Fish, Marine Fish and in Internal Organs of Carp," *Bull. Jap. Soc. Sci. Fish.* **40**, 201–209 (1974).

93. H. Brockerhoff and J. R. Hoyle, "Hydrolysis of Triglycerides by the Pancreatic Lipase of a Skate," *Biochim. Biophys. Acta* **98**, 435–436 (1965).

94 P. Chawla and R. F. Ablett, "Detection of Microsomal Phospholipase Activity in Myotomal Tissue of Atlantic Cod (*Gadus morhua*)," *J Food Sci.* **52**, 1194–1197 (1987).

95. H. Brockerhoff, "Digestion of Fat by Cod," *J. Fish. Res. Bd. Can.* **23**, 1835–1839 (1966).

96. J. E. Geromel, *Release of Lipase From Liposomes of Rainbow Trout* (Salmo gairdneri) *Muscle Subjected to Low Pressures*, Informativo Anual, Faculdade de Engenharia de Alimentos e Agricola, Universidad Estadual de Campinas, No. 8, 57–60, 1980.

97. C Leger, "Essai de Purification de la Lipase du Tissu Intercaecal de la Truite (*Salmo gairdneri* Rich)," *Ann. Biol. Anim. Biochim. Biophys.* **12**, 341–345 (1970).
98. D. L. Berner and E. G. Hammond, "Phylogeny of Lipase Specificity," *Lipids* **5**, 558–562 (1970).
99. M A. Audlrey, K. J Shetty, and J. E. Kinsella, "Isolation and Properties of Phospholipase A From Pollock Muscle," *J. Food Sci.* **43**, 1771–1775 (1978).
100. H. Brockerhoff, "On the Function of Bile Salts and Proteins as Cofactors of Lipase," *J. Biol. Chem.* **246**, 5828–5831 (1971).
101. A. Kubicz, E. Dratewka-Kos, and R. Zygmuntowicz, "Multiple Molecular Forms of the Acid Phosphatase From *Cyprinus carpio* liver: Isolation and Comparison With Those of *Rana esculenta*," *Comp. Biochem. Physiol.* **68B**, 437–443 (1981).
102. M. J. Walton, "Intracellular Distribution of Tricarboxylic Acid Cycle Enzymes in Liver of Rainbow Trout *Salmo gairdneri*," *Comp. Biochem. Physiol.* **82B**, 87–90 (1985).
103. A. Ooshiro, "Studies on a Proteinase in the Pyloric Ceca of Fish. II. Some Properties of Proteinase Purified From the Pyloric Ceca of Mackerel," *Bull. Jap. Soc. Scient. Fish.* **34**, 847–854 (1968).
104. D. B. Josephson, R. C. Lindsay, and D. A. Sturber, "Identification of Compounds Characterizing the Aroma of Fresh Whitefish (*Coregonus clupeaformis*)," *J. Agric. Food Chem.* **31**, 326–330 (1983).
105. H. Y. Chen and C F. Li, "Isolation, Partial Purification and Application of Proteinases From Grass Shrimp Heads," *Food Sci. (China)* **15**, 230–243 (1988).
106. T. Godfrey and J. Reichelt, *Industrial Enzymology*, Nature Press, New York, 1983.
107. S. Schwimmer, *Source Book of Enzymology*, AVI, Westport, Conn., 1981.
108. G. Reed, *Enzymes in Food Processing*, Academic Press, New York, 1975.
109. N. Raksakulthai, Y. Z. Lee, and N. F. Haard, "Effect of Enzyme Supplements on the Production of Fish Sauce From Male Capelin (*Mallotus villosus*)," *Can. Inst. Food Sci. Technol.* **19**, 28–33 (1986).
110. B. K. Simpson and N. F. Haard, "Trypsin From Greenland Cod as a Food-processing Aid," *J. App. Biochem.* **6**, 135–143 (1984).
111. Y. Z. Lee, B. K. Simpson, and N. F. Haard, "Supplementation of Squid Fermentation With Proteolytic Enzymes," *J. Food Biochem.* **6**, 127–134 (1982).
112. R. R. del Rosario and S. M. Maldo, "Biochemistry of Patis Formation. I. Activity of Cathepsins in Patis Hydrolysis," *Philippines Agriculturists* **67**, 167–175 (1984).
113. A. Cano-Lopez, B. K. Simpson, and N. F. Haard, "Extraction of Carotenoprotein From Shrimp Process Wastes With the Aid of Trypsin from Atlantic Cod," *J. Food Sci* **52**, 503–504, 506 (1987).
114. M. A. Kyei, *Trypsin and Trypsin Like Enzyme From the Stomachless Cunner—Preparation and Applications*, M.Sc. thesis, McGill University, Montreal, Canada, 1996.
115. B. K. Simpson and N F. Haard, "Trypsin and a Trypsin-like Enzyme From the Stomachless Cunner. Catalytic and Other Physical Characteristics," *J Agric. Food Chem.* **35**, 652–656 (1987).
116. N. Guizani et al., "Isolation and Characterization of a Trypsin From the Pyloric Ceca of Mullet (*Mugil cephalus*)," *Comp. Biochem. Physiol.* **98**, 517–521 (1991).
117. J. E. Kinsella, "Dietary Fats and Cardiovascular Disease," in *Seafoods and Fish Oils in Human Health and Disease*, Marcel Dekker, New York, pp. 1–24, 1987.
118. J. Raa, "Biotechnology in the Fish Processing Industry—A Success Story in Norway," in *Advances in Fisheries Technology for Increased Profitability, Proceedings of the Atlantic Fisheries Technological Conference, August 27–30, 1989*, St. John's, Newfoundland, Canada, 1990.
119. K. Almas, "Utilization of Marine Biomass for the Production of Microbial Growth Media and Biochemicals," in *Advances in Fisheries Technology for Increased Profitability, Proceedings of the Atlantic Fisheries Technological Conference, August 27–30, 1989*. St. John's, Newfoundland, Canada, 1990.
120. N. F. Haard, "Specialty Enzymes From Marine Organisms," *Food Technol* **52**, 64–67 (1998).
121. H. Yasueda et al., "Tissue-type Transglutaminase From Red Sea Bream, Sequence Analysis of the cDNA and Functional Expression in *Escherichia coli*," *Eur. J. Biochem.* **232**, 411–419 (1995).
122. G. G. Kamath et al., "Nondisulfide Covalent Cross-linking of Myosin Heavy Chain in Setting of Alaska Pollock and Atlantic Croaker Surimi," *J. Food Biochem.* **16**, 151–172 (1982).
123. J. M. Trant, "Functional Expression of Recombinant Spiny Dogfish Shark Cytochrome P450c17 in Yeast *Pichia pastoris*," *Arch. Biochem. Biophys.* **326**, 8–14 (1996).
124. R. Male et al., "Molecular Cloning and Characterization of Anionic and Cationic Variants of Trypsin From Atlantic Salmon." *Eur. J. Biochem.* **232**, 677–685 (1995).
125. B. Klein et al., "Molecular Cloning and Sequencing of Trypsin cDNAs From *P. vannamei*: Use in Assessing Gene Expression During the Moult Cycle," *Int. J. Biochem. Biol.* **28**, 551–563 (1996).
126. A. Gudmundsdottir et al., "Isolation and Characterization of cDNAs From Atlantic Cod Encoding Two Different Forms of Trypsinogen," *Eur. J Biochem.* **217**, 1091–1097 (1993).
127. C LeBoulay, A. Van Wormhoudt, and D Sellos, "Cloning and Expression of Cathepsin L-like Proteinases in the Hepatopancreas of Shrimp *Penaeus vannamei* During Intermolt Cycle," *J. Comp. Physiol.* **166B**, 310–318 (1996).
128. A. Van Wormhoudt and D. Sellos, "Cloning and Sequencing Analysis of Three Amylase cDNAs in the Shrimp *Penaeus vannamei*. Evolutionary Aspects," *J. Mol. Evol.* **42**, 43–551 (1996).
129. X. Su, F. Chen, and L. E. Hokin, "Cloning and Expression of a Novel, Highly Truncated Phosphoinositide-specific Phospholipase C cDNA From Embryos of the Brine Shrimp, *Artemia*," *J. Biol. Chem.* **269**, 12925–12931 (1994).

GENERAL REFERENCES

S. A. Assaf and D. J. Graves, "Structural and Catalytic Properties of Lobster Muscle Glycogen Phosphorylase," *J. Biol. Chem.* **244**, 5544–5555 (1969).

N. F. Haard, "A Review of Proteolytic Enzymes From Marine Organisms and Their Application in the Food Industry," *J. Aq. Food Product. Tech.* **1**, 17–35 (1992).

C. de Haen, K. A. Walsh, and H. Neurath, "Isolation and Amino Acid Terminal Sequence Analysis of a New Pancreatic Trypsinogen of the African Lungfish *Protopterus aethiopicus*," *Biochemistry* (N.Y.) **16**, 4421–4425 (1977).

K. A. Hansen, "Processing of Cod in Scandinavia 1990," in *Advances in Fisheries Technology for Increased Profitability, Proceedings of the Atlantic Fisheries Technological Conference, August 27–30, 1989*, St. John's, Newfoundland, Canada, 1989.

E. R. Norris and D. W. Elam, "Preparation and Properties of Crystalline Salmon Pepsin," *J. Biol. Chem.* **134**, 443–454 (1940).
R. L. Shewfelt, R. E. McDonald, and H. O. Hultin, "Effect of Phospholipid Hydrolysis on Lipid Oxidation in Flounder Muscle Microsomes," *J. Food Sci.* **46**, 1297–1301 (1981).

BENJAMIN K. SIMPSON
AMARAL SEQUEIRA-MUNOZ
McGill University
Ste. Anne de Bellevue, Quebec
Canada
NORMAN F. HAARD
University of California
Davis, California

MARINE TOXINS

A very wide variety of biotoxins occur with varying frequencies in fish, shellfish, mammals, reptiles, algae, and other animals and plants existing in the marine environment (1). Toxic organisms have been collected from most regions of the world, and many of them have caused illness in humans following ingestion. Ciguatera and scombroid poisoning together account for more than 80% of all seafood outbreaks and more than 50% of all outbreaks caused by the consumption of muscle protein with confirmed etiologies reported to the US Centers for Disease Control (2). This article emphasizes the similarities among the most frequently encountered or potentially encountered biotoxins in United States commercial fisheries, including imports. Significant differences between toxins or illnesses are also presented. Information is presented to assist in identifying major disorders and their circumstances. There are seven common and well characterized disorders—paralytic, neurotoxic, amnesic, and diarrhetic shellfish poisonings, ciguatera, scombroid poisoning, and pufferfish poisoning (poisonings or disorders refer to illnesses whereas toxins refer to specific chemical complexes) and these are summarized in Table 1. Three additional disorders that may increase in occurrence as international commerce of fishery products increases are briefly described at the end of the article: clupeotoxicity, hallucinogenic mullet poisoning, and shark liver poisoning.

BIOTOXINS

Intoxications are caused by a variety of biotoxins or families of biotoxins. Those common toxins that are chemically characterized are often grouped according to their chemical solubility. Brevetoxins (neurotoxic shellfish poisoning), okadaic acids (diarrhetic shellfish poisoning), and ciguatoxin (ciguatera) are lipid soluble, whereas, saxitoxins (paralytic shellfish poisoning), domoic acid (amnesic shellfish poisoning), maitotoxin (ciguatera), scombrotoxin (scombroid poisoning), and tetrodotoxin (pufferfish poisoning) are water soluble. All are low molecular weight (111–3425 D) containing no known sulfur or phosphorus groups. All but scombrotoxin and tetrodotoxin are produced by marine algae. Both scombrotoxin and tetrodotoxin are pro-

Table 1. Marine Biotoxins

Disorder	Toxin(s)	Agent(s)	Chemical structure	Sources	Assays	Controls	References
Paralytic shellfish poison	Saxitoxins (12)	*Protogonyaulax calenella, P. tamarensis*	$C_{10}H_{17}N_7O_3$, water soluble, 299 MW	Oysters, clams, scallops	Mouse, bioassay HPLC	Harvest restrictions	1,3,4,13,16, 17,18,19
Neurotoxic shellfish poison	Brevetoxins (5 or more)	*Ptychodiscus brevis*	$C_{50}H_{70}O_{14}$, ether soluble, 894 MW	Oysters, clams, coquina	Mouse bioassay, HPLC	Harvest restrictions	1,3,5,6,16,17, 18,19,20
Amnesic shellfish poison	Domoic acid	*Nitzchia pungens*	$C_{15}H_{21}O_6N$, water soluble, 311 MW	Mussels	Mouse, bioassay, HPLC	Harvest restrictions	1,3,7,16,17, 18,19
Diarrhetic shellfish poison	Okadaic acid and derivatives	*Dinophysis fortii, D. acuminata*	$C_{44}H_{70}O_{13}$, ether soluble, 804 MW	Mussels, scallops	Mouse and rat bioassays, HPLC, ELISA	Harvest restrictions, remove digestive gland	1,3,8,9,16,17, 18,19
Cigustera	Cigustoxin	*Gambierdiscus toxicus*	$C_{83}H_{77}O_{24}N$ or $C_{54}H_{78}O_{24}$, ether soluble, 1111 MW	Barracuda, grouper, snapper, amberjeck	Mouse, cat, and guinea pig bioassays, EIA, RIA	Avoid endemic areas, remove organs	1,3,10,16,17, 18,19
	Maltoxin	*G. toxicus*	?, water soluble, 3425 MW	Surgeonfish	Mouse bioassay	Avoid endemic areas, remove organs	1,3,10,16,17, 18,19
Scombroid poison	Histamine	Bacteria species	$C_8H_9N_3$, water soluble, 111 MW	Tuna, skipjack, mackerel, mahi-mahi	Guines pig bioassay fluorometry, HPLC	Refrigeration lot rejection	1,3,11,14,16, 17,18,19
Pufferfish poison	Tetrodotoxin	*Vibrio alginolyticus*	$C_{11}H_{17}O_8N_3$, water soluble, 319 MW	Pufferfish	Mouse bioassay, HPLC	Fugu dealers and chefs	1,3,12,15,16, 17,18,19

duced by bacteria. These groups of toxins cause very similar symptoms (neurologic and/or gastrointestinal) characterized by rapid onset. Four of these groups of toxins are found in molluscan shellfish consumed in the United States: saxitoxins, brevetoxins, domoic acid, and okadaic acids; and four in finfish: ciguatoxin, maitotoxin, scombrotoxin, and tetrodotoxin (3).

OCCURRENCE

Shellfish Poisoning

Shellfish toxins fall into three specific groups; paralytic, amnesic, and diarrhetic (20). None of these toxins are produced by shellfish themselves but are obtained from microalgae such as phytoplankton naturally present in the oceans.

Paralytic. Of the molluscan shellfish-associated disorders, three are found in temperate areas and one in semitropical. Paralytic shellfish poisoning (saxitoxins) is found in shellfish such as mussels, clams, oysters, and scallops that have fed on the toxic dinoflagellates (21), including several species of the genus *Alexandrium*, previously *Protogonylauax catenella* and *P. tamarensis* (4). The toxins produced by these dinoflagellates tend to persist in shellfish for varying periods of time, depending on shellfish species and tissues involved. Paralytic shellfish poisoning is reported from all temperate oceans of the world; *P. catenella* on the west coast of North America and *P. tamarensis* on the east coast. In most cases the water temperature must be 5–8°C. The three families of toxins involved (saxitoxins, neosaxitoxins, and gonyautoxins) are all water soluble and heat stable. The first European case was documented in Norway in 1962 (22). Between 1977 and 1981, the United States had 126 cases reported from nine outbreaks.

Amnesic. Amnesic shellfish poisoning (domoic acid) has been reported in blue mussels from Canada and the United States (Maine). The source of the toxin is the marine diatom *Pseudonitzschia* (formerly *Nitzschia*) (7,23). In 1987, more than 100 illnesses and three deaths were reported from the Prince Edwards Island area of Canada.

Diarrhetic. Diarrhetic shellfish poisoning (okadaic acid and derivatives) is found in shellfish, especially mussels, that have fed on toxic marine dinoflagellates and then concentrated the lipid soluble toxins in their hepatopancreas. It appears that the edible muscle tissues remain free of toxin. The causative dinoflagellates (*Dinophysis fortii* and *Dinophysis acuminata*) are widespread in occurrence (8). Europe (Netherlands, Spain, France, Sweden), Southeast Asia (Japan, Thailand), and South America (Chile) have all reported outbreaks of diarrhetic shellfish poisoning. As many as 1,300 cases were reported in Japan from 1976 to 1982; and during 1981, 5,000 cases were reported in Spain (9,24).

Neurotoxic. Neurotoxic shellfish poisoning (brevetoxins) is found throughout the Gulf of Mexico, the Atlantic coast of the southern United States, and the Carribbean Sea. The causative organism is the dinoflagellate *Ptychodiscus brevis* (5). It is most frequently found on the western coast of Florida but has been confirmed from Texas to North Carolina. Eleven people in three episodes were intoxicated in Florida in 1973–1974 (6). Cases continue to be reported. Oysters, clams, and coquina are primary transvectors for the toxins to humans.

Ciguatera Poisoning

About 50,000 cases of ciguatera poisoning are reported annually worldwide; more than 2,000 cases in the United States (mostly Hawaii, Puerto Rico, and Florida). The toxins occur throughout the tropical regions of the world (between 35°N and 34°S) in shallow reef areas, mostly around islands (3). The apparent causative organism is the dinoflagellate *Gambierdiscus toxicus* (10). Fish that cause human illnesses include mostly barracuda, grouper, snapper, and amberjack. Other affected fishes include mackerel and surgeonfish.

Scombroid Poisoning

Many species of time–temperature abused fresh, canned, salted, or dried fish have been associated with scombroid poisoning (11). Scombroid fish include tuna, skipjack, mackerel, bonito, albacore, bluefish, saury, butterfly kingfish, and seerfish. Nonscombroid fish include mahi-mahi, sardines, pilchards, anchovies, herring, black marlin, and kahawai. These fish normally contain high levels of free histidine that is converted to histamine by a variety of bacteria species. Optimum decarboxylation occurs at 20–25°C, pH 2.5–6.5. Chemical potentiators that are also present in flesh, ie, putrescine and cadaverine, enhance the oral toxicity. There were 78 outbreaks of scombroid poisoning reported to the US Centers for Disease Control between 1983 and 1987 (2).

Puffer Poisoning

Tetrodotoxin is found in the flesh of pufferfish (Tetraodontidae). Not all species of puffer are equally toxic. The most notoriously toxic species, *Fugu rubripes* (tiger puffer), is also said to be the tastiest. Toxin concentration, principally in the ovaries and to a lesser extent in the liver and intestines, is highest during the winter months before spawning. Representatives of the tetraodontids are found throughout a broad circumglobal belt extending from latitudes of 47°N to 47°S. Recently, the primary source of the toxin has been determined to be several marine bacteria including *Vibrio alginolyticus* (12). In Japan, the only major country where pufferfish (fugu) are a delicacy, tetrodotoxin caused an average of 84 deaths a year between 1886 and 1963; having a fatality rate of 59% of all reported intoxications (1).

HEALTH EFFECTS

All but one of these disorders (diarrhetic shellfish poisoning) are characterized by similar neurologic symptoms (particularly tingling of the extremities). Some also elicit gastrointestinal (nausea, diarrhea, abdominable pain) and/or cardiovascular (pulse rate and blood pressure changes) symptomology. All biotoxin disorders are characterized by rapid onset—within a few hours. The disorders associated with mulluscan shellfish—paralytic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning, and diarrhetic shellfish poisoning—all elicit nausea, diarrhea and abdominal pain. All but diarrhetic shellfish poisoning cause tingling in the extremities of consumers.

For paralytic shellfish poisoning, the toxins contain a tetrahydropurine skeleton with around 20 forms of basic, water-soluble saxitoxins identified (25) The saxitoxins act by binding to the sodium channels in nerve cell membranes and blocking nerve transmission. Symptoms occur within 1 hour, initially with a tingling sensation of the lips, gums, tongue, and face. Death occurs as a result of respiratory paralysis, usually within a period of 12 hours.

Symptoms of neurotoxic shellfish poisoning include reverse sensation of hot and cold temperatures, muscular incoordination and pain, and lowered pulse rate. Neurotoxic shellfish poisoning is caused by a family of about six chemically related toxins known as brevetoxins. Symptoms begin within about 3 hours and stop in 2–3 days. No deaths have been reported.

Amnesic shellfish poisoning is due to the potent neurotoxin domoic acid, which causes gastroentiritis and mental confusion (1,26). Diarrhetic shellfish poisoning symptoms occur from 30 minutes to 12 hours following ingestion of shellfish. The symptoms usually disappear within 1–3 days and produce no long-term effects. Diarrhetic shellfish poisoning can be distinguished from paralytic shellfish poisoning by the absence of neurologic symptoms.

The disorders associated with finfish: ciguatera, scombroid poisoning and pufferfish poisoning; all elicit nausea, diarrhea, abdominal pain, and tingling of the extremities (1). Other ciguatera symptoms include reversed sensation of hot and cold temperatures, headache, itching skin, muscle and joint pain, metallic taste, malais, anxiety, chills, convulsions, paralysis, hallucinations, lost equilibrium, changes in pulse rate, dilated eyes, and reduced blood pressure. Onset occurs in 2–12 hours. Severe itching, temperature reversal, and tingling of the extremities are the most distinctive symptoms, which may last many months. Morbidity in the Pacific region averages five per thousand (3).

Scombroid poisoning symptoms include rash, flushing, burning of the mouth and throat, and heart palpitation, in addition to nausea, diarrhea, abdominal pain, and tingling of the extremities. Onset occurs in a few minutes to a few hours and the duration is 4–12 hours (11,14,27).

Pufferfish poison is a potent vasodepressor that selectively blocks the sodium channel in excitable membranes (15). The symptoms of intoxication start with almost immediate tingling sensations (usually within minutes but up to 3 hours). In severe cases, respiratory failure and death occur. Because pufferfish poison is found in the highest concentration in the gonads, liver, and intestine, the fish are most dangerous to eat immediately before and during the reproductive season.

DETECTION

All of the more common biotoxins can be measured by bioassay, immunologic, or chemical techniques (16,17). It is generally more cost-effective to survey or monitor with relatively nonspecific bioassay methods and confirm with more specific methods. However, specific, rapid, low-cost, field methods are under development and will greatly improve controls of illness in the future. All the common toxins (except scombroid poison) can be detected by mouse bioassay, differing in sample preparation and extraction. Mouse bioassay is the most common regulatory method in use. Ciguatera is also measured by cat, mongoose, various aquatic invertebrates, and guinea pig ileum bioassay techniques. A method of measuring diarrhetic shellfish poison in common use in the Netherlands is the rat fecal consistency assay. The toxins of paralytic, neurotoxic, amnesic, and diarrhetic shellfish poisonings, scombroid poisoning and pufferfish poisoning are measured by relatively sophisticated analytic high-performance liquid chromatography (HPLC) methods. The toxins of ciguatera are not yet measured using HPLC. It is reported that ciguatoxin can be detected using a radioimmunoassay or an enzyme immunoassay.

Scombroid poison is detected by measurement of histamine using guinea pig ileum bioassay, fluorometry, colorimetry, enzyme isotopic assays, or thin-layer chromatography. Toxic samples contain 100–4,000 mg histamine per 100 g fish. The toxic threshold is affected by levels and types of potentiators and microflora present, spoilage conditions, and fish species.

Immunoassay methods have considerable potential for detecting marine toxins. The sensitivity and specificity of this method would quickly eliminate the negative samples so that only the positive sample will require further testing. A number of immunoassay-based kits are available for okadaic acid (28), saxitoxins (29), cigautoxins, brevetoxins, and diarrhetic shellfish toxins (30).

CONTROLS

Foods contaminated with marine toxins cannot be identified without exhaustive analyses. Cooking will not inactivate these toxins. Controls are largely dependent on restrictions on harvests and prompt recognition of illnesses (18,19). Harvests of all contaminated shellfish are usually restricted based on toxin levels in edible tissues. Systematic processes to screen fish and shellfish are not in place except for monitoring of paralytic and neurotoxic shellfish poisons in shellfish-growing waters under the National Shellfish Sanitation Program. The National Shellfish Sanitation Program is described in greater detail in another article. Harvests are prohibited when concentrations of saxitoxins exceed 80 μg/100 g tissue for paralytic shellfish poison and 20 mouse units of brevetoxins per 100 g tissue for neurotoxic shellfish poison. Because neurotoxic shellfish poison correlates with levels of causative dinoflagellates in the water column, many states prohibit harvesting shellfish when concentrations exceed 5,000 cells *P. brevis* per liter of seawater (31). These areas are kept closed until edible tissue levels of toxin fall below FDA tolerances specified above.

Harvests of amnesic shellfish poison-contaminated shellfish can be controlled by monitoring of the causative organism in the growing area, but this is not done routinely. Cell monitoring is not effective in controlling paralytic and diarrhetic shellfish poisonings. Ciguatera is controlled, in part, by avoiding harvests from reefs implicated in frequent intoxications. It is controlled by avoiding large specimens of frequently toxic species from suspect areas because the toxin is bioaccumulated as fish prey on small herbivores.

Scombroid poisoning can be completely controlled by rapid and constant refrigeration of susceptible species immediately following harvest and throughout all processing and handling steps. Lots suspected of time—temperature abuse can be analyzed for the presence of histamine and rejected when levels exceed 50 mg/100 g.

Some of the toxins can be controlled during processing, handling, and meal preparation. Removal of certain internal and reproductive organs of some fish and shellfish (scallops) will remove a majority of paralytic and diarrhetic shellfish poisons, ciguatera, and pufferfish poison. Pufferfish poisoning is largely controlled, however, by proper meal preparation by trained fugu dealers and cooks.

OTHER DISORDERS

Some other seafood toxins such as clupeotoxin, hallucinogenic mullet, or fish poison and shark liver poison occur infrequently but may have severe consequences (1,11). Clupeotoxin poisoning, which is caused by contaminated herrings (Clupeidae) and anchovies (Engraulidae), is found sporadically in the tropical Atlantic Ocean, Pacific Ocean, and Caribbean Sea. The ingestion of certain types of reef fish, five families including Acanthuridae (tang) and Mugilidae (mullet), that occur in the tropical Pacific and Indian oceans, sometimes cause hallucinogenic mullet poisoning. The causative agents for clupeotoxicity and hallucinogenic mullet poisoning are unknown but thought to be dinoflagellates or bluegreen algae.

The symptoms of clupeotoxicity are both gastrointestinal and neurologic, occur quickly, and are usually violent in nature. Mortality rates as high as 45% have been reported. Hallucinogenic mullet poisoning symptoms include those of the nervous system such as dizziness, loss of equilibrium, and hallucinations. The symptoms appear quickly (within 2 hours) and can persist for up to 24 hours. However, hallucinogenic mullet poisoning is relatively mild with no long-term effects.

Several species of fish have been reported to have toxic livers, especially some of the sharks. Shark liver poisoning is thought to be vitamin A toxicosis. Symptoms begin within 30 minutes of ingestion and include nausea, diarrhea, abdominable pain, headache, rapid pulse, cold sweats, and tingling and burning of the tongue, throat, and esophagus. Recovery occurs in 1 day to 2 weeks. Mortality is high.

Natural marine toxins are low molecular weight compounds usually produced by dinoflagellates that ascend the food chain. Fish and shellfish that contain the toxins cannot be identified by casual inspection. Generally, handling, processing, and cooking do not affect levels of toxins in tissues. The disorders are characterized by rapid onset and usually cause gastrointestinal and/or neurologic symptoms. Control of illnesses usually requires harvest restrictions and prompt recognition of symptoms. Bioassays are most often used to detect the presence of toxins in fish.

BIBLIOGRAPHY

1. B. W. Halstead, *Poisonous and Venomous Marine Animals of the World*, 2nd rev. ed , Darwin Press, Princeton, N.J., 1988.
2. United States Centers for Disease Control, *Foodborne Disease Surveillance Annual Summaries*, US Department of Health and Human Services, Atlanta, Geo.
3. E. P Ragelis, ed., *Seafood Toxins*, American Chemical Society, Washington, D.C., 1984.
4. E. J. Schautz, Ref. 3, pp. 99–111.
5. D. G. Baden, "Brevetoxins: Unique Polyether Dinoflagellate Toxins," *FASEB Journal* **3**, 1807–1817 (1989).
6. W H. Hemmert, "The Public Health Implications of *Gymnodinium breve* Red Tides, A Review of the Literature and Recent Events," in *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*, Boston, Mass., Nov. 4–6, 1974.
7. D. V. Subba Rao, M. A. Quillian, and R. Pocklington "Domoic Acid—A Neurotoxic Amino Acid Produced by the Marine Diatom *Nitzschia pungens* in Culture," *Canadian Journal of Fish. Aquatic Science* **45**, 2076–2079 (1988).
8. T. Yasumoto, T. Oshima, W. Sugaware, Y. Fukuyo, H. Oguri, T. Igarashi, and N. Fujita. "Identification of *Dinophysis fortii* as the Causative Organism of Diarrhetic Shellfish Poisoning," *Bulletin of the Japanese Society of Sci. Fish.* **46**, 1405–1411 (1980).
9. E. Stammon, D. A. Segar, and P. G. Davis, "A Preliminary Assessment of the Potential for Diarrhetic Shellfish Poisoning in the Northeast United States," *NOAA Technical Memorandum* NOS OMA 34, Rockville, Md., 1987.
10. T. Yasumoto, I. Nakajima, R. Bagnis, and R. Adachi. "Finding of a Dinoflagellate as a Likely Culprit of Ciguatera," *Bulletin of the Japanese Society Sci. Fish.* **43**, 1021–1026 (1977).
11. H. Dymsza et al., "Poisonous Marine Animals," in H. D. Graham, ed., *The Safety of Foods*, 2nd ed., Avi Publishing Co., Westport, Conn., 1980, pp. 625–651.
12. U. Simidu, T. Noguchi, D. F. Hwang, Y. Shida, and K. Hashimoto. "Marine Bacteria which Produce Tetrodotoxin," *Applied and Environmental Microbiology* **53**, 1714–1715 (1987).
13. J. J. Sullivan and W. T Iwaoka, "High Pressure Liquid Chromatographic Determination of Toxins Associated with Paralytic Shellfish Poisoning," *Journal of the Association of Official Analytical Chemists* **66**, 297–303 (1983).
14. S. L. Taylor, J. Y. Hui, and D E. Lyons, Ref 3, pp. 417–430
15. S. L. Hu and C Y. Kao, "The pH Dependence of the Tetrodotoxin-Blockade of the Sodium Channel and Implications for Toxin Binding," *Toxicon* **24**, 25–31 (1986).
16. E. F. McFarren, "Assay and Control of Marine Biotoxins," *Food Technology* **25**, 234–244 (1971).
17. S. Hall and Y. Shimizu, "Toxin Analysis and Assay Methods," in D. M. Anderson, A. W. White, and D. G Baden, eds., *Toxic Dinoflaggelates*, Elsevier, New York, pp. 545–548.

18. E. L. Bryan, "Epidemiology of Foodborne Diseases Transmitted by Fish, Shellfish and Marine Crustaceans in the United States, 1970–1978," *Journal of Food Protection* **43**, 859–876 (1980).
19. J. M. Hughes and M. H. Merson, "Current Concepts, Fish and Shellfish Poisoning," *Medical Intelligence* **93**, 1117–1120 (1976)
20. J. L. C. Wright, "Dealing with Seafood Toxins: Present Approaches and Future Options," *Food Research International* **28**, 347–358 (1995).
21. K. A. Steidinger, "Some Taxonomic and Biologic Aspects of Toxic Dinoflagellates," in I. R. Falconer, ed., *Algal Toxins in Seafood and Drinking Water*, Academic Press, San Diego, Cal., 1993, pp. 1–28.
22. H. P. van Egmond et al., "Paralytic and Diarrhoeic Shellfish Poisons: Occurrence in Europe, Toxicity, Analysis and Regulation," *Journal of Natural Toxins* **2**, 41–83 (1993).
23. S. S. Bates et al., "Pennate Diatom *Nitzchia pungens* as the Primary Source of Domoic Acid, A Toxin in Shellfish from Eastern Prince Edward Island, Canada," *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1203–1215 (1989).
24. T. Aune and M. Yndestad, "Diarrhetic Shellfish Poisoning," in I. R. Falconer, ed., *Algal Toxins in Seafood and Drinking Water*, Academic Press, San Diego, Cal., 1993, pp. 87–104.
25. Y. Oshima, S. Blackburn, and G. M. Hallegraff, "Comparative Study on Paralytic Shellfish Toxin Profile of the Dinoflagellate *Gymnodinium catenateum* from Three Different Countries," *Marine Biology* **95**, 217–220 (1993).
26. E. C. D. Todd, "Domoic Acid and Amnesic Shellfish Poisoning—A Review," *Journal of Food Protection* **56**, 69–83 (1993).
27. B. Bartholomew et al., "Scombrotoxic Fish Poisoning in Britain: Features of Over 250 Suspected Incidents Between 1976 and 1986," *Epidemiological Infections* **99**, 775–782 (1986).
28. J. D. Chin et al., Screening for Okadaic Acid, A New Probe for the Study of Cellular Regulation," *TIBS* **15**, 98–100 (1995)
29. E. Usleber, E. Schneider, and G. F. Terplan, "Direct Enzyme Immunoassay in Microtitration Plate and Test Strip Format for the Detection of Saxitoxin in Shellfish," *Letters in Applied Microbiology* **13**, 275–277 (1991).
30. U.S. Patent 4,816,392 (March 28, 1989), Y. Hokama.
31. Food and Drug Administration, *National Shellfish Sanitation Program Manual of Operations*, Part I, U.S. Department of Health and Human Services, Washington, D.C., 1988.

See also TOXICANTS, NATURAL.

MASS TRANSFER AND DIFFUSION IN FOODS

Mass transfer occurs in a number of food-processing operations such as dehydration, evaporation, concentration, distillation, solvent extraction, packaging, peeling, and leaching. Mass transfer is a common and important phenomenon in food processing and preservation. During osmotic drying of fruits, for example, sugar from a concentrated solution moves into the fruit while water leaves the fruit and migrates into the surrounding solution. By definition, mass transfer is the migration of matter from one location to another due to concentration or partial pressure gradient. According to this definition, the motion of air due to wind or the transport of water through a pipe by a pump is not mass transfer, because these motions occur due to either the presence of a total pressure difference (wind) or the application of mechanical work (pump).

Mass transfer subdivides into molecular mass transfer and convective mass transfer. For the former, the term diffusion is often used. Diffusion deals with the random molecular migration of matter through a medium, whereas convective mass transfer involves the migration of matter from a surface into a moving fluid or a stream of gas. Diffusion further subdivides into molecular diffusion, transitional diffusion, Knudsen diffusion, and Eddy diffusion. Among these, molecular diffusion is the most important phenomenon. It exists in most processes of mass transfer and often has a major influence when other kinds of diffusion are also present.

From the theory of molecular kinetics, it is known that when a randomly moving molecule diffuses through a capillary tube, it can move freely for some distance before a collision with another molecule or with the wall of the capillary tube occurs. If a molecule would collide with other molecules more often than it would with the wall, molecular diffusion occurs. If molecule-wall collisions are predominant, it is Knudsen diffusion. If both molecule-molecule and molecule-wall collisions are important, it is transition-type diffusion. The specific state can be determined by evaluating Knudsen's number (1). When bulk fluid motion is involved, the term Eddy diffusion applies.

Diffusion of a substance may occur in gases, liquids, or solids. Within the range of validity of the ideal gas law, the rate of diffusion in gases is affected primarily by temperature and pressure. When diffusion in liquids is considered, however, the effect of pressure is usually negligible because liquids are incompressible. Diffusion in solids is far more complex than diffusion in gases or liquids, because (1) the substance diffusing through a solid may actually be diffusing through a liquid or gas contained within the pores of the solid; and (2) many solids, such as crystals, polymeric films, and solids with capillaries, are anisotropic, in which case the molecules have a preferential direction of movement.

The general equation for all types of mass transfer is

$$\text{mass transfer rate} = \frac{\text{driving force}}{\text{resistance}}$$

where the driving force is the partial pressure difference or the concentration gradient (depending on the transferred matter); and the resistance is a function of the properties of the medium through which the matter is transferred.

Mass transfer can occur with steady-state or unsteady-state conditions. In a steady state the concentration or partial pressure, the resistance, and the transfer rate are constant over time. In an unsteady state, all these properties vary with time. Obviously, the mathematical treatment of steady-state problems is simpler than that involving

18. E. L. Bryan, "Epidemiology of Foodborne Diseases Transmitted by Fish, Shellfish and Marine Crustaceans in the United States, 1970–1978," *Journal of Food Protection* **43**, 859–876 (1980).
19. J. M. Hughes and M. H. Merson, "Current Concepts, Fish and Shellfish Poisoning," *Medical Intelligence* **93**, 1117–1120 (1976).
20. J. L. C. Wright, "Dealing with Seafood Toxins: Present Approaches and Future Options," *Food Research International* **28**, 347–358 (1995).
21. K. A. Steidinger, "Some Taxonomic and Biologic Aspects of Toxic Dinoflagellates," in I. R. Falconer, ed., *Algal Toxins in Seafood and Drinking Water*, Academic Press, San Diego, Cal., 1993, pp. 1–28.
22. H. P. van Egmond et al., "Paralytic and Diarrhoeic Shellfish Poisons: Occurrence in Europe, Toxicity, Analysis and Regulation," *Journal of Natural Toxins* **2**, 41–83 (1993).
23. S. S. Bates et al., "Pennate Diatom *Nitzchia pungens* as the Primary Source of Domoic Acid, A Toxin in Shellfish from Eastern Prince Edward Island, Canada," *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1203–1215 (1989).
24. T. Aune and M. Yndestad, "Diarrhetic Shellfish Poisoning," in I. R. Falconer, ed., *Algal Toxins in Seafood and Drinking Water*, Academic Press, San Diego, Cal., 1993, pp. 87–104.
25. Y. Oshima, S. Blackburn, and G. M. Hallegraff, "Comparative Study on Paralytic Shellfish Toxin Profile of the Dinoflagellate *Gymnodinium catenateum* from Three Different Countries," *Marine Biology* **95**, 217–220 (1993).
26. E. C. D. Todd, "Domoic Acid and Amnesic Shellfish Poisoning—A Review," *Journal of Food Protection* **56**, 69–83 (1993).
27. B. Bartholomew et al., "Scombrotoxic Fish Poisoning in Britain: Features of Over 250 Suspected Incidents Between 1976 and 1986," *Epidemiological Infections* **99**, 775–782 (1986).
28. J. D. Chin et al., Screening for Okadaic Acid, A New Probe for the Study of Cellular Regulation," *TIBS* **15**, 98–100 (1995)
29. E. Usleber, E. Schneider, and G. F. Terplan, "Direct Enzyme Immunoassay in Microtitration Plate and Test Strip Format for the Detection of Saxitoxin in Shellfish," *Letters in Applied Microbiology* **13**, 275–277 (1991).
30. U.S. Patent 4,816,392 (March 28, 1989), Y. Hokama.
31. Food and Drug Administration, *National Shellfish Sanitation Program Manual of Operations*, Part I, U.S. Department of Health and Human Services, Washington, D.C., 1988.

See also TOXICANTS, NATURAL.

MASS TRANSFER AND DIFFUSION IN FOODS

Mass transfer occurs in a number of food-processing operations such as dehydration, evaporation, concentration, distillation, solvent extraction, packaging, peeling, and leaching. Mass transfer is a common and important phenomenon in food processing and preservation. During osmotic drying of fruits, for example, sugar from a concentrated solution moves into the fruit while water leaves the fruit and migrates into the surrounding solution. By definition, mass transfer is the migration of matter from one location to another due to concentration or partial pressure gradient. According to this definition, the motion of air due to wind or the transport of water through a pipe by a pump is not mass transfer, because these motions occur due to either the presence of a total pressure difference (wind) or the application of mechanical work (pump).

Mass transfer subdivides into molecular mass transfer and convective mass transfer. For the former, the term diffusion is often used. Diffusion deals with the random molecular migration of matter through a medium, whereas convective mass transfer involves the migration of matter from a surface into a moving fluid or a stream of gas. Diffusion further subdivides into molecular diffusion, transitional diffusion, Knudsen diffusion, and Eddy diffusion. Among these, molecular diffusion is the most important phenomenon. It exists in most processes of mass transfer and often has a major influence when other kinds of diffusion are also present.

From the theory of molecular kinetics, it is known that when a randomly moving molecule diffuses through a capillary tube, it can move freely for some distance before a collision with another molecule or with the wall of the capillary tube occurs. If a molecule would collide with other molecules more often than it would with the wall, molecular diffusion occurs. If molecule-wall collisions are predominant, it is Knudsen diffusion. If both molecule-molecule and molecule-wall collisions are important, it is transition-type diffusion. The specific state can be determined by evaluating Knudsen's number (1). When bulk fluid motion is involved, the term Eddy diffusion applies.

Diffusion of a substance may occur in gases, liquids, or solids. Within the range of validity of the ideal gas law, the rate of diffusion in gases is affected primarily by temperature and pressure. When diffusion in liquids is considered, however, the effect of pressure is usually negligible because liquids are incompressible. Diffusion in solids is far more complex than diffusion in gases or liquids, because (1) the substance diffusing through a solid may actually be diffusing through a liquid or gas contained within the pores of the solid; and (2) many solids, such as crystals, polymeric films, and solids with capillaries, are anisotropic, in which case the molecules have a preferential direction of movement.

The general equation for all types of mass transfer is

$$\text{mass transfer rate} = \frac{\text{driving force}}{\text{resistance}}$$

where the driving force is the partial pressure difference or the concentration gradient (depending on the transferred matter); and the resistance is a function of the properties of the medium through which the matter is transferred.

Mass transfer can occur with steady-state or unsteady-state conditions. In a steady state the concentration or partial pressure, the resistance, and the transfer rate are constant over time. In an unsteady state, all these properties vary with time. Obviously, the mathematical treatment of steady-state problems is simpler than that involving unsteady-state conditions. For this reason, the following sections shall first deal with the simple steady-state cases. It is important to remember, however, that in reality every process begins with unsteady-state conditions, which over time change to steady state.

MOLECULAR DIFFUSION

Molecular diffusion of matter is analogous to the conductive transfer of heat. In heat conduction, energy moves from a region of high temperature to a region of low temperature due to the random motion of gas or liquid molecules or the vibration of solid molecules. Similarly, in molecular diffusion, matter moves from a region of high concentration to a region of low concentration due to the random motion of the molecules of that substance. Therefore, molecular diffusion can be defined as the net transport of matter on a molecular scale due to a concentration or partial pressure gradient through a medium, which is either stagnant or has a laminar flow with a direction perpendicular to that of the concentration gradient. For example, water will evaporate from an open surface into still surrounding air, and a piece of sugar will dissolve in a cup of coffee and spread without stirring.

Fick's First Law

Fick first recognized the analogy between heat conduction and mass diffusion in 1855. Following Fourier's equation for heat conduction, Fick quantitatively expressed the rate of diffusion of a substance, through an isotropic medium with a unit surface area, as being proportional to the concentration gradient measured as a vector normal to the surface:

$$j = -D\frac{dC}{dy} \tag{1}$$

where j is the diffusion rate, C is the concentration of the substance being transferred, y is the distance, and D is a measure of the resistance against the diffusion of matter, usually called the diffusivity or diffusion coefficient. The rate j is usually expressed in mol/m$^2\cdot$s; C, in mol/m^3; y, in m; and D, in m^2/s. The negative sign in Fick's law indicates that the flow of matter is in the direction of decreasing concentration. It should be noted that equation 1 applies equally well to diffusion in gases, liquids, and solids (assuming isotropic properties).

Diffusion in Gases

From the standpoint of molecular kinetics, gas molecules contain energy and are in a state of continuous random motion. During such movement, the molecules bounce against each other or against other surfaces, continuously changing their direction. Consequently, in a region of constant concentration, the probability of an individual molecule moving in any direction is the same. This phenomenon is called the random walk. When considering a homogeneous mixture of two gases, for example, and imaging a certain number of molecules traveling in one direction, there must be an equal number of molecules traveling in the opposite direction and no net mass transfer is taking place. If a concentration gradient present were, however, then the number of molecules traveling from the high to the low concentration region would be larger than that traveling in the opposite direction. Therefore, net mass transfer would take place from the high to the low concentration region.

Assuming that a gas A follows the ideal gas law, the concentration term in Fick's law can be expressed as:

$$C^A = \frac{n_A}{V} = \frac{P_A}{RT} \tag{2}$$

where n_A is the amount of gas A in moles, V is the total volume, P_A is the partial pressure of A, R is the gas constant, and T is the absolute temperature. Thus, Fick's law becomes

$$j_A = -\frac{D}{RT}\frac{dP_A}{dy} \tag{3}$$

In a more general case, the medium is moving in a laminar flow with a bulk speed v_b while the component is diffusing through the medium. N_A, the total flux of component A, is the sum of diffusion and bulk flow of the mixture

$$N_A = C_A v_d + C_A v_b \tag{4}$$

where v_d is the velocity of A due to true diffusion and $C_A v_d$ is the number of molecules being diffused. The term $C_A v_d$ can be expressed by Fick's law and is equivalent to j_A. Hence the total flux of A is

$$N_A = j_A + C_A v_b \tag{5}$$

For a binary mixture of gases A and B, the total mass flux is the sum of the fluxes of the two gases

$$N = N_A + N_B = C v_b \tag{6}$$

and solving for v_b gives

$$v_b = \frac{N_A + N_B}{C} \tag{7}$$

where C is the total concentration of the mixture ($A + B$). By substituting equation 7 into equation 5, and using $C_A = P_A/RT$, and $C = P/RT$, gives

$$N_A = j_A + \frac{C_A}{C}(N_A + N_B) \tag{8}$$

For ideal gases where $C_A/C = P_A/P$, Fick's law gives the following general equation for diffusion in gases

$$N_A = -\frac{D_{AB}}{RT}\frac{dP_A}{dy} + \frac{P_A}{P}(N_A + N_B) \tag{9}$$

Applications of equation 9 in two specific cases will be discussed in the following section.

Equimolecular Gas Counterdiffusion. Consider two gases A and B in two jars connected by a tube as shown in Figure 1. When the total pressure in each jar is the same, a molecular diffusion through a stationary medium will occur due to the presence of partial pressure difference between each individual component. Thus

$$N_A = j_A \text{ and } N_B = j_B$$

As long as the partial pressure $P_{A1} > P_{A2}$ and $P_{B2} > P_{B1}$, diffusion will continue to occur. The number of molecules of A diffusing from jar 1 to jar 2 is equal to the number of molecules of B diffusing in the opposite direction (to maintain the equal total pressure in both jars)

$$N_A = -N_B \text{ or } j_A = -j_B$$

Setting $N_A = -N_B$ in equation 9, we have

$$N_A = j_A = -\frac{D_{AB}}{RT}\frac{dP_A}{dy} = -j_B = -\left(-\frac{D_{BA}}{RT}\frac{dP_B}{dy}\right) \quad (10)$$

For steady-state conditions, integration of equation 10 gives

$$N_A = \frac{D_{AB}}{RTY}(P_{A1} - P_{A2} \quad (11)$$

and

$$N_B = \frac{D_{BA}}{RTY}(P_{B1} - P_{B2}) \quad (12)$$

where Y is the total distance of diffusion. Because $P = P_A + P_B$ = constant, it follows that $dP = dP_A + dP_B = 0$ and therefore $dP_A = -dP_B$. Substituting this expression into equation 10 gives

$$D_{AB} = D_{BA} \quad (13)$$

In other words, in the case of diffusion of ideal gases, the diffusivities of each individual component are identical.

Diffusion of Gas A Through a Stagnant Layer of Gas B. Consider water evaporating from an open flask into the air, as shown in Figure 2. In this case, water vapor A is diffusing through a stagnant layer of air B at the top of the water surface. We know that air is only slightly absorbed by water, whereas water vapor does escape from the water's surface through the still air layer and into the bulk stream outside the flask. Therefore

$$N_B = 0$$

and the general equation 9 becomes (by rearranging)

$$N_A dy = -\frac{D_{AB}}{RT}\frac{dP_A}{1 - \frac{P_A}{P}} \quad (14)$$

When dealing with a dilute system at constant temperature T and pressure P, D_{AB} can be considered constant, and thus integration of equation 14 gives

$$N_A = \frac{D_{AB}P}{RTY}\ln\left(\frac{P - P_{A2}}{P - P_{A1}}\right) \quad (15)$$

By introducing the concept of logarithmic mean value

$$P_{mln} = \frac{(P - P_{A2}) - (P - P_{A1})}{\ln\left(\frac{P - P_{A2}}{P - P_{A1}}\right)} = \frac{P_{A1} - P_{A2}}{\ln\left(\frac{P - P_{A2}}{P - P_{A1}}\right)} \quad (16)$$

we can rewrite equation 15 as

$$N_A = \frac{D_{AB}}{RTY}\frac{P}{P_{mln}}(P_{A1} - P_{A2}) \quad (17)$$

When the difference between P_{A1} and P_{A2} is small, say $P - P_{A1}/P - P_{A2}) < 1.5$, using an arithmetic mean P_m instead of a logarithmic mean introduces an error of less than 1.5%, and equation 16 becomes

$$P_{mln} = P_m = \frac{(P - P_{A1}) + (P - P_{A2})}{2} \quad (18)$$

Furthermore, when the partial pressure of the diffusing matter is low compared to the total pressure, then $P - P_{A1} \approx P - P_{A2} \approx P$ and $P_m \approx P$, and equation 17 becomes

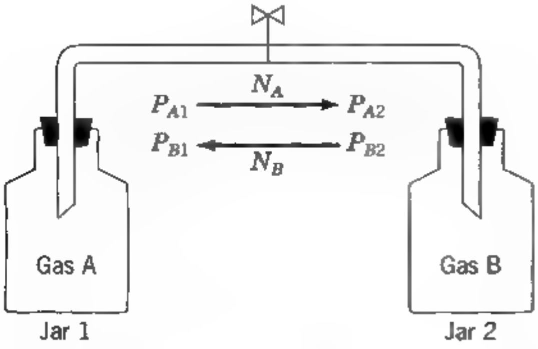


Figure 1. Equimolecular counterdiffusion.

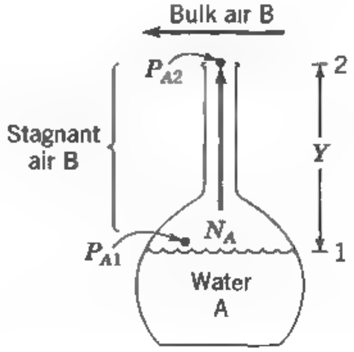


Figure 2. Diffusion of water vapor through stagnant air.

$$N_A = \frac{D_{AB}}{RTY} (P_{A1} - P_{A2}) \tag{19}$$

Diffusion in Liquids

Diffusion in liquids can be observed when a drop of food color, for example, is put into a cup of water. The color diffuses into the surrounding water due to the concentration difference until a homogeneous solution is formed. The mechanism of diffusion in liquids is similar to that of diffusion in gases. However, because liquids are denser than gases, the resistance to diffusion in a liquid is much greater and the diffusion rates are lower. Because liquids are incompressible, the total pressure has very little influence on the diffusion. In addition, because concentration in liquids is considerably higher and some chemical changes of the solute may occur, diffusion is often concentration dependent.

The general equation 8, derived for diffusion in gases, can be directly adopted here. Substituting j_A from equation 1 gives

$$N_A = -D_{AB} \frac{dC_A}{dy} + \frac{C_A}{C} (N_A + N_B) \tag{20}$$

This is the general equation for diffusion in liquids.

Equimolecular Liquid Counterdiffusion. Similar to the situation in gases, equimolecular diffusion in liquids has the feature of $N_A = -N_B$. Thus equation 20 can be simplified as

$$N_A = -D_{AB} \frac{dC_A}{dy} = D_{AB} \frac{(C_{A1} - C_{A2})}{Y} \tag{21}$$

When the difference in the concentration between two points is small, an average concentration ($\bar{C}_A$) can be defined as

$$\bar{C}_A = \frac{C_{A1} + C_{A2}}{2} \tag{22}$$

If we set $\bar{C}_{A1} \approx \bar{C}_A X_{A1}$ and $\bar{C}_{A2} \approx \bar{C}_A X_{A2}$, equation 22 becomes

$$N_A = \frac{D_{AB}\bar{C}_A}{Y} (X_{A1} - X_{A2}) \tag{23}$$

where X_{A1} and X_{A2} are the molecular fractions of A at point 1 and point 2, respectively. Similarly, for liquid B, we have

$$N_B = \frac{D_{BA}\bar{C}_B}{Y} (X_{B1} - X_{B2}) \tag{24}$$

Because the diffusivity D_{AB} is usually concentration dependent, an average concentration $\bar{C}_A$ should be used when calculating the value of D_{AB}.

Diffusion of Liquid A through a Stagnant Layer of Liquid B. In this case, $N_B = 0$, and equation 17 can be rewritten by substituting $C_{A1} = \bar{C}_A X_{A1} = P_{A1}/RT$, $C_{A2} = \bar{C}_A X_{A2} = P_{A2}/RT$, and $X_{mln} - P_{mln}/P$ so that

$$N_A = \frac{D_{AB}}{YX_{mln}} (C_{A1} - C_{A2}) = \frac{D_{AB}\bar{C}_A}{YX_{mln}} (X_{A1} - X_{A2}) \tag{25}$$

where X_{mln} is the logarithmic mean of the molecular fractions. For a small difference between X_{A1} and X_{A2}, the arithmetic mean (X_m) can be used. For a dilute solution, $1 - X_{A1} \approx 1 - X_{A2}$ and $X_m = 1.0$, giving

$$N_A = \frac{D_{AB}}{Y} (C_{A1} - C_{A2}) \tag{26}$$

Diffusion in Solids

Diffusion of gases and liquids in solids are phenomena occurring in many processes such as drying, packaging, catalytic reactions, leaching, and membrane separations. The two classifications of diffusion in solids are structure independent and structure dependent. In the former, the diffusing substance is dissolved in the solid to form a homogeneous solution, and thus Fick's law can be directly applied. In the latter, pores, capillaries, and other interconnected voids in the solid allow some flow of the diffusing substance. Because this porous solid is anisotropic, the diffusion path of molecules will be different than that described by Fick's law and an overall, apparent, or effective diffusivity coefficient may be used.

Structure-Independent Diffusion in Solids. A homogeneous solution in the solid is assumed here; therefore, diffusion is independent of the actual structure of the solid. The solute concentration gradient is the actual driving force, and the general equation 20 for binary diffusion can be applied directly. Usually the concentration C_A is small compared to the total concentration C, and C_A/C can be neglected. By further assuming that D_{AB} is constant, we can integrate to get

$$N_A = \frac{D_{AB}}{Y} (C_{A1} - C_{A2}) \tag{27}$$

When dealing with diffusion of gases in a solid, the solubility S of a gas solute A is usually expressed as (vol of A at 0°C and 1 atm)/(vol of solid · pressure of A). To convert this into concentration C_A (kmol of A/vol of solid), the following equation can be used.

$$C_A = \frac{SP_A}{22.414} \tag{28}$$

where 22.414 is the volume (in m^3) of 1 kmol of an ideal gas at 0°C and 1 atm.

In the case of diffusion through a solid cylinder in the radial direction, the total diffusing area is not constant. For a cylinder with an inner radius r_1, outer radius r_2, and length l, we have

$$N_A = \frac{\dot{N}_A}{A} = -D_{AB}\frac{dC_A}{dr} \tag{29}$$

Because $A = 2\pi rl$ for the cylinder, substituting this expression into equation 29, rearranging, and integrating gives

$$\dot{N}_A = D_{AB} \cdot 2\pi l \frac{C_{A1} - C_{A2}}{\ln\left(\frac{r_2}{r_1}\right)} \tag{30}$$

where $\dot{N}_A$ is the total flux in mol/s. It should be noted that in diffusion in solids, $D_{AB} \neq D_{BA}$ and both D_{AB} and D_{BA} are independent of the partial pressure of the gas or liquid outside the solid. However, the solubility in the solid is directly proportional to the partial pressure.

Structure-Dependent Diffusion in Solids. The presence of interconnected pores and capillaries in porous solids does not allow direct application of Fick's law, because the diffusing substance follows a tortuous path, which is not equal to the distance between two points. To correct for that tortuous path, it is necessary to define a factor τ, called the tortuosity, and rewrite the diffusion equation 27 as

$$N_A = \frac{\epsilon D_{AB}}{\tau Y}(C_{A1} - C_{A2}) \tag{31}$$

where ϵ is the porous fraction of the solid. Usually the porous fraction and tortuosity factor are combined into an effective diffusivity as

$$D_{AB_{\text{eff}}} = \frac{\epsilon}{\tau} D_{AB} \tag{32}$$

When equation 31 is used to describe diffusion of gases in solids, and because $C_A = P_A/RT$,

$$N_A = \frac{\epsilon D_{AB}}{\tau YRT}(P_{A1} - P_{A2}) \tag{33}$$

If the pores of the solid are very small compared to the mean free path of the gas, other types of diffusion may occur (2,3)

Diffusion in Nonideal Systems

Nonideal systems play an important role in food, biological, and life processes of humans, animals, plants, and microorganisms. In the presence of macromolecules in the system, interactions may occur between them and smaller molecules. Such phenomena cause the system to deviate from its ideal behavior, even though a true solution (e.g., colloids) might be formed. As a result, the mobility of the diffusing molecule changes unpredictably, even in dilute solutions. Diffusion in nonideal systems is complex and difficult to describe by theoretical models. Generally, it is assumed that the differential form of Fick's law applies

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial y}\left(D\frac{\partial C}{\partial y}\right) \tag{34}$$

where D is concentration dependent. Solution of this equation requires knowledge of the actual function of D and the corresponding boundary conditions. Later, D will be expressed as a function of concentration, temperature, viscosity of solution, and the properties of the medium.

DIFFUSION WITH BULK FLOW AND CHEMICAL REACTIONS

In some practical diffusion problems involving the presence of catalysts, enzymes, or other reagents, the diffusing substance A may undergo a chemical reaction. In such a case, the general equation 8 is still valid. To solve this equation, the relationship between N_A and N_B, which depends on the type of chemical reaction, must be known. In the following sections it is demonstrated how equation 8 can be solved for some chemical reactions commonly observed in food systems.

Reactions $A + Z \rightarrow B$

In this case, if 1 mol of A is diffusing toward the reacting surface, there must be 1 mol of B diffusing away in the opposite direction (as long as Z is adequate on that surface). Substituting $N_A = -N_B$ into equation 8 and observing that

$$j_A = -D_{AB}\frac{dC_A}{dy} = -CD_{AB}\frac{dX_A}{dy}$$

the following equation is obtained.

$$N_A = -CD_{AB}\frac{dX_A}{dy} \tag{35}$$

Equation 35 is identical to equation 21 for equimolecular counterdiffusion. Solving equation 35 gives

$$N_A = \frac{CD_{AB}}{Y}(X_{A1} - X_{A2}) \tag{36}$$

If the reaction is instantaneous, no A exists on the reacting surface and $X_{A2} = 0$. Equation 36 then becomes

$$N_A = \frac{CX_{A1}}{(Y/D_{AB})} \tag{37}$$

If the reaction is first order, N_A in the reacting surface can be expressed as

$$N_{A|_{y=Y}} = \mu C_{A2} = \mu C X_{A2} \tag{38}$$

where μ is the first-order reaction rate constant in mol/m^2 s. Solving equation 38 for X_{A2} gives

$$X_{A2} = \frac{N_{A|_{y=Y}}}{\mu C} \tag{39}$$

By substituting equation 39 into equation 36 and assuming steady-state conditions ($N_A = N_{A|_{y=Y}}$), the following is finally obtained.

$$N_A = \frac{CX_{A1}}{\left(\frac{Y}{D_{AB}}\right) + \frac{1}{\mu}} \tag{40}$$

In equations 37 and 40, the terms Y/D_{AB} and $Y/D_{AB} + 1/\mu$ provide a measurement of the resistance to diffusion in systems with instantaneous or first-order chemical reaction of the form $A + Z \rightarrow B$, respectively.

Reaction $A + Z \rightarrow 2B$

In this case, 1 mol of A produces 2 mol of B if Z is adequate on the reacting surface. Substituting $N_B = -2N_A$ into equation 8, noting that $C_A/C = X_A$ and rearranging, gives

$$N_A dy = -CD_{AB}\frac{dX_A}{1 + X_A} \tag{41}$$

For constant C, D_{AB}, and N_A, it is possible to integrate both sides of equation 41 so that

$$N_A \int_0^Y dy = -CD_{AB}\int_{X_{A1}}^{X_{A2}} \frac{dX_A}{1 + X_A} \tag{42}$$

which, after solving for N_A, gives

$$N_A = \frac{CD_{AB}}{Y}\ln\left(\frac{1 + X_{A1}}{1 + X_{A2}}\right) \tag{43}$$

For an instantaneous reaction with $X_{A2} = 0$ equation 43 can be simplified to

$$N_A = \frac{CD_{AB}}{Y}\ln(1 + X_{A1}) \tag{44}$$

For a first-order reaction, substituting equation 39 into equation 43 gives

$$N_A = \frac{CD_{AB}}{Y}\ln\left(\frac{1 + X_{A1}}{1 + \frac{N_A}{\mu C}}\right) \tag{45}$$

Equation 45 can be solved by trial and error, but it is appropriate to point out that the reaction rate in equation 45 is lower than that in equation 44, because $(1 + N_A/\mu C) > 1$.

UNSTEADY-STATE MASS TRANSFER

In the previous sections, only steady-state mass transfer phenomena were dealt with. In practice, mass transfer always begins with an unsteady-state condition. Steady-state conditions may be established only after some time has elapsed. In unsteady-state mass transfer, the concentrations or partial pressures and consequently the mass transfer rate are functions of time and position. Fick's second law can be used to describe the concentration changes with time and position. For one dimension, Fick's second law can be written as

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial y^2} \tag{46}$$

To solve equation 46, a set of initial and boundary conditions must be given. A simple example is the diffusion through a membrane with known initial and boundary conditions (4,5), stated as

$$\begin{aligned} &\text{at } t = 0,\ C = C_0, \text{ for } 0 < y < Y \\ &\text{at } y = 0,\ C = C_1, \text{ for } t > 0 \\ &\text{at } y = Y,\ C = 0, \text{ for } t > 0 \end{aligned} \tag{47}$$

The solution of equation 46 is then

$$C = C_1\left(1 - \frac{y}{Y}\right) + \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{C_1}{n}\sin\frac{n\pi y}{Y}\exp\left(-n^2\pi^2\frac{Dt}{Y^2}\right) \tag{48}$$

The total amount of substance diffused through the membrane in time t, Q_t, is

$$Q_t = \frac{DC_1t}{Y} - \frac{YC_1}{6} - \frac{2YC_1}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-n^2\pi^2\frac{Dt}{Y^2}\right) \tag{49}$$

When t approaches infinity, the exponential terms vanish and equation 49 simplifies to a linear form

$$Q_t = \frac{DC_1t}{Y}\left(t - \frac{Y^2}{6D}\right) \tag{50}$$

A plot of Q_t versus t gives an intercept L on the t-axis (where $Q_t = 0$) as

$$L = \frac{Y^2}{6D} \tag{51}$$

where L is the so-called time lag. Equation 50 can be used to determine D from experimental data. With more complex initial and boundary conditions, analytical solutions of equation 46 become more difficult and sometimes even impossible. In practice, numerical methods are often used (1,6).

MASS TRANSFER IN TWO-PHASE SYSTEMS

In the previous discussion, the mass transfer of one phase was considered. The concentration gradient of the other phase was assumed to be zero (the system was assumed either well stirred or very dilute). In most practical problems, however, concentration gradients may exist on both sides of the interface of the system. For example, consider that solute A is being transferred from a gas to a liquid phase. In such a case, A must travel from the bulk volume into the boundary layer of the gas phase, through the interface, into the boundary layer, and finally into the bulk volume of the liquid phase. The concentration profile of A is shown in Figure 3. Defining P_A as the partial pressure of A in the gas phase, C_A as the concentration in the liquid phase, P_{Ai} as the partial pressure of A in the gas boundary layer, and C_{Ai} as the concentration in the liquid boundary layer, the rate of transfer of A from the gas phase to the interface is

$$N_A = k_g(P_A - P_{Ai}) \tag{52}$$

The rate of transfer of A away from the interface and into the liquid phase is

$$N_A = k_l(C_{Ai} - C_A) \tag{53}$$

where k_g and k_l are transfer coefficients for the gas and liquid boundary layer, respectively. At steady state, the rate of transfer to the interface must be equal to the rate of transfer away, so that

$$\frac{k_l}{k_g} = \frac{(P_A - P_{Ai})}{(C_{Ai} - C_A)} \tag{54}$$

If $k_g \gg k_l$, then $(C_{Ai} - C_A) \gg (P_A - P_{Ai})$ and the mass transfer is controlled by the liquid boundary layer. Increasing k_l increases the overall transfer rate. If $k_l \gg k_g$, the process is controlled by the gas boundary layer, and the overall rate is greatly affected by k_g.

For equimolecular counterdiffusion, comparing equation 11 with equation 52, and equation 21 with equation 53 obtains the following.

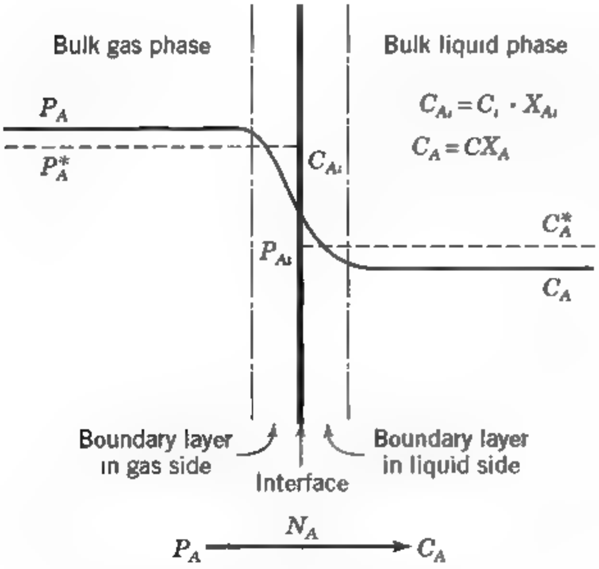

Bulk gas phase
Bulk liquid phase
P_A
P_A^*
$C_{Ai} = C_t \cdot X_{Ai}$
$C_A = CX_A$
C_{Ai}
C_A^*
P_{Ai}
C_A
Boundary layer in gas side
Interface
Boundary layer in liquid side
N_A
$P_A \longrightarrow C_A$

Figure 3. Concentration profile of a solute in a two-phase system.

$$k_g = \frac{D_{AB}}{RTY_g} \tag{55}$$

$$k_l = \frac{D_{AB}}{Y_l} \tag{56}$$

For a substance A diffusing through a stagnant layer of B, comparing equation 17 with equation 52, and equation 25 with equation 53 gives

$$k_g = \frac{D_{AB}}{RTY_g}\frac{P}{P_{m\ln}} \tag{57}$$

and

$$k_l = \frac{D_{AB}}{Y_l}\frac{1}{X_{m\ln}} \tag{58}$$

where Y_g and Y_l are the thicknesses of the gas and liquid boundary layers, respectively, and $P_{m\ln}$ and $X_{m\ln}$ are given by

$$P_{m\ln} = \frac{P_A - P_{Ai}}{\ln\left(\frac{P - P_{Ai}}{P - P_A}\right)} \tag{59}$$

and

$$X_{m\ln} = \frac{X_{Ai} - X_A}{\ln\left(\frac{1 - X_A}{1 - X_{Ai}}\right)} \tag{60}$$

The measurement of the boundary layer thicknesses, Y_g and Y_l, is difficult. For that reason, it is convenient at times to use the boundary layer coefficients for calculations.

DETERMINATION OF DIFFUSIVITY AND OVERALL MASS TRANSFER COEFFICIENT

So far, it has been assumed that the transfer coefficients are known constants. In reality transfer coefficients in food systems are unknown and not constant. Constant transfer coefficients can be used only in special situations and under restricted conditions. A number of experimental methods (7) and several empirical equations (1) have been developed to determine and predict the mass transfer coefficients. In the following subsections the theory that underlines some of the simpler experimental methods used is presented.

Diffusivity in Gases

One of the simplest methods for determining the diffusivity of a vapor or gas into another gas is to evaporate pure liquid A using a graduated cylinder with small diameter, as shown in Figure 4. The vapor A diffuses through an inert gas B, which is gently passed over the opening of the cylinder. The gas B moves slowly so that the layer at the top of liquid A (in the cylinder) can be assumed stagnant. The whole apparatus should be maintained at constant

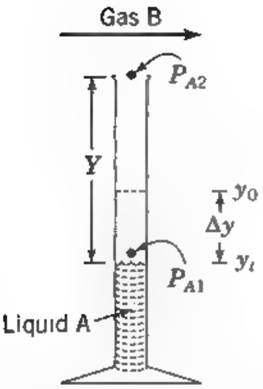


Figure 4. Evaporation of a liquid

temperature and pressure. The drop of the liquid's level is measured over time and used to determine the diffusivity. The amount of liquid A evaporated in a small time interval is

$$N_A = \frac{\rho_A dy}{M_A dt} \tag{61}$$

Substitution of equation 61 into equation 17 gives

$$\frac{\rho_A dy}{M_A dt} = \frac{D_{AB}}{RTY}\frac{P}{P_{mln}}(P_{A1} - P_{A2}) \tag{62}$$

Rearranging and observing that only y and t are variables under steady-state conditions gives

$$D_{AB}\int_0^t dt = \frac{RTP_{mln}\rho_A}{PM_A(P_{A1} - P_{A2})}\int_{y_0}^{y_t} y dy \tag{63}$$

and solving for D_{AB}

$$D_{AB} = \frac{RTP_{mln}\rho_A(y_t^2 - y_0^2)}{2PM_At(P_{A1} - P_{A2})} \tag{64}$$

where P_{A1} is the partial pressure of A at the liquid surface, P_{A2} is the partial pressure of A in the gas B, P_A is the density, and M_A is the molecular weight of the liquid.

Diffusivity in Liquids

When measuring diffusivity in liquids, it is important to be aware that D_{AB} depends on the concentration of the diffusing substance in solution. Constant D_{AB} is only an approximation and may be used in cases of very dilute solutions where only small changes in the concentration occur. It should also be noted that D_{AB} indicates the diffusivity of A through B, and it is different from D_{BA} (diffusivity of B through A). For gases, $D_{AB} = D_{BA}$. A simple method to determine D_{AB} when A diffuses through B is shown in Figure 5. Two large containers, 1 and 2, are connected by a long capillary tube with diameter $2r$ and length Y. A dilute solution (of A in B) in container 2 has an initial concentration C_{A2_0} and is slightly more concentrated than C_{A1_0} in container 1. Both ends of the tube are gently stirred to gain uniform concentration. Diffusion takes place in the capillary tube and after a certain time, quasi-steady state is established. The total mass diffused can be calculated as

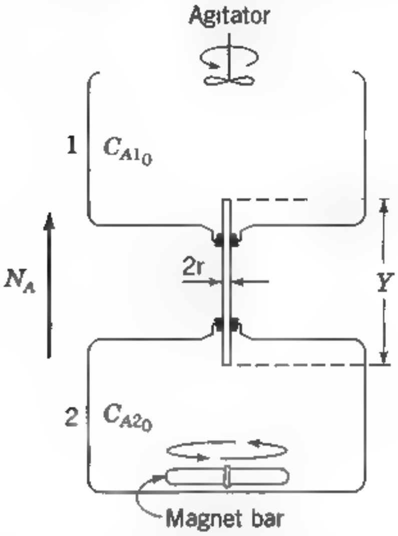


Figure 5. Arrangement for determination of diffusivity in liquids.

$$N_A = \frac{1}{\pi r^2}\frac{\Delta C_A}{\Delta t} = \frac{1}{\pi r^2}\frac{\bar{C}_A(X_{A2} - X_{A1})}{\Delta t} \tag{65}$$

where $\Delta C_A = \bar{C}_A(X_{A2} - X_{A1})$ is the concentration increase of the diffusing substance A in the upper container 1 during time Δt. Substituting equation 65 into equation 25 gives

$$\frac{1}{\pi r^2}\frac{(X_{A2} - X_{A1})}{\Delta t} = \frac{D_{AB}}{Y}\ln\left(\frac{1 - X_{A2}}{1 - X_{A1}}\right) \tag{66}$$

Solving for D_{AB} and accounting for the logarithmic mean of the molecular fractions gives

$$D_{AB} = \frac{Y(X_{A2} - X_{A1})}{\pi r^2 \Delta t}\ln\left(\frac{1 - X_{A1}}{1 - X_{A2}}\right) = -\frac{YX_{mln}}{\pi r^2 \Delta t} \tag{67}$$

Thus the diffusivity D_{AB} depends on the diffusion tube constant $Y/\pi r^2$ and the logarithmic mean X_{mln} of the molecular fraction in solution.

Diffusivity in Solids

When a solid slab with some initial moisture content is brought into an airstream, the moisture inside the solid slab will diffuse through the bulk of the solid and evaporate from its surfaces. Assuming that the slab has a thickness much smaller than its length and width, the one-dimensional Fick's second law (equation 46) can be applied. If the system is maintained at equilibrium and both surface moisture contents are equal, the initial and boundary conditions for the system can be written as

$$\begin{aligned} &\text{at } t = 0,\ C = C_0,\ \text{for } 0 < y < Y \\ &\text{at } y = 0,\ C = C_e,\ \text{for } t > 0 \\ &\text{at } y = Y,\ C = C_e,\ \text{for } t > 0 \end{aligned} \tag{68}$$

Equation 46 can be solved for the conditions of equation 68 (4) to give

$$\frac{C - C_e}{C_0 - C_e} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n + 1)^2} \exp\left[-(2n + 1)^2\pi^2 \frac{D_{\text{eff}}t}{Y^2}\right] \tag{69}$$

For approximation purposes, equation 69 can be simplified by keeping only the first term ($n = 0$) on the right-hand side. Taking the logarithm of both sides will give

$$\ln\left(\frac{C - C_e}{C_0 - C_e}\right) = \ln\left(\frac{8}{\pi^2}\right) - \frac{\pi^2}{Y^2} D_{\text{eff}}t \tag{70}$$

where C_0 is the initial concentration, C_e is the surface concentration at equilibrium, C is the average concentration in the solid at time t, Y is the thickness of the slab, and D_{eff} is the effective diffusivity of A in the solid. A plot of $\ln(C - C_e/C_0 - C_e)$ versus t will produce a line with $\lambda = -(\pi^2/Y^2)D_{\text{eff}}$. Thus

$$D_{\text{eff}} = -\frac{\lambda Y^2}{\pi^2} \tag{71}$$

For structure-dependent diffusion, it should also be recalled that $D_{\text{eff}} = f(D_{AB})$. Therefore, the diffusivity of a substance in solids is a function of concentration, structural parameters, and other system characteristics.

Diffusivity in Nonideal Systems

An experimental apparatus for measuring the diffusivity in nonideal systems is shown in Figure 6. Two chambers of equal volume, 1 and 2, are separated by a porous membrane and contain a true solution of a food system, whose concentration is slightly higher in 1. Both bulk concentrations are maintained uniform by agitation (rotating magnet), and diffusion takes place only through the membrane. Under quasi-steady-state conditions, the amount of A diffusing through the membrane in a small time interval can be found by taking the mass balance in chambers 1 and 2 separately. For chamber 1:

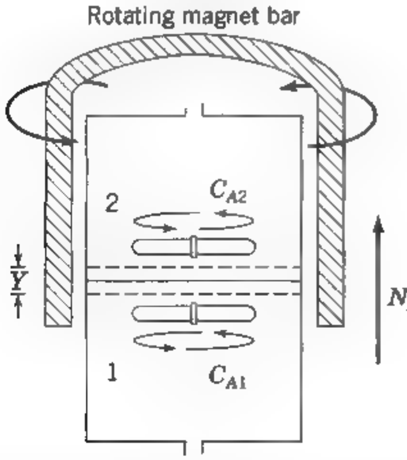


Figure 6. Diagram of a diffusion cell for determination of diffusivity in nonideal systems.

$$VdC_{A1} + \frac{\epsilon D_A}{Y}(C_{A1} - C_{A2})dt = 0 \tag{72}$$

For chamber 2:

$$VdC_{A2} + \frac{\epsilon D_A}{Y}(C_{A2} - C_{A1})dt = 0 \tag{73}$$

Combining equations 72 and 73, rearranging, and integrating gives

$$\ln\left(\frac{(C_{A1} - C_{A2})_t}{(C_{A1} - C_{A2})_0}\right) = -\frac{2\epsilon D_A}{YV}t = -\beta D_A t \tag{74}$$

where V is the volume of each chamber; $(C_{A1} - C_{A2})_0$ and $(C_{A1} - C_{A2})_t$ are the concentration differences between the two chambers at the initial and subsequent times, respectively; t is time; Y is the thickness of the membrane; ϵ is the effective porous fraction of the membrane; and β is a cell constant ($\beta = 2\epsilon/YV$) that can be determined by experimenting with a substance of known diffusivity.

The diffusivity of a nonideal system D_A is different from that of an ideal system D_{AB}. The relationship between D_A and D_{AB} is unpredictable and can be determined only by experiment. Furthermore, when binding reactions are present, the diffusivity (denoted by D_b) is different from D_A and D_{AB}. A semitheoretical equation has been presented (8) to estimate the diffusivity D_A of a globular-type protein in solution without binding action between the protein and the solute A as

$$D_A = D_{AB}(1 - 1.81 \times 10^{-3}C_p) \tag{75}$$

where C_p is the protein concentration in kg of protein/m^3. An equation that relates D_A, D_{AB}, and D_b during diffusion of sodium caprylate in bovine serum albumin solution was reported (9) as

$$D_A = D_{AB}(1 - \alpha\varphi_p)\left(1 - \frac{C_b}{C}\right) + D_b\left(\frac{C_b}{C}\right) \tag{76}$$

where α is the so-called diffusivity reduction shape factor for the protein, φ_p is the volume fraction of protein in solution, C_b is the concentration of protein-bound solute A in g/m^3, and C is the total concentration of solute A in g/m^3.

Overall Mass Transfer Coefficient

In a two-phase system, the mass transfer rate can be written in terms of the overall mass transfer coefficient and the bulk concentrations or partial pressures of both phases

$$N_A = K_g(P_A \quad P_A^*) \tag{77}$$

and

$$N_A = K_l(C_A^* \quad C_A) \tag{78}$$

where K_g and K_l are the overall mass transfer coefficients for the gas and liquid phases, respectively; P_A^* is the partial pressure of A at equilibrium with the bulk concentration of the liquid; and C_A^* is the concentration of A at equilibrium with the bulk partial pressure of the gas phase, as shown in Figure 3. For dilute solutions, Henry's law

$$P = HC \tag{79}$$

can be employed, where H is Henry's constant. It is also possible to write

$$(P_A - P_A^*) = P_A - P_{Ai} + (P_{Ai} - P_A^*) \tag{80}$$

Using equations 79 and 80, equation 77 becomes

$$N_A = K_g(P_A - P_{Ai}) + K_gH(C_{Ai} - C_A) \tag{81}$$

If these terms are rearranged, the following is obtained

$$\frac{1}{K_g} = \frac{P_A - P_{Ai}}{N_A} + \frac{H(C_{Ai} - C_A)}{N_A} \tag{82}$$

By combining equations 52, 53, and 82, the final expression is obtained

$$\frac{1}{K_g} = \frac{1}{k_g} + \frac{H}{k_l} \tag{83}$$

Hence, the overall mass transfer coefficient can be evaluated from the film coefficients. In a similar manner, an expression for K_l can be derived

$$\frac{1}{K_l} = \frac{1}{k_l} + \frac{1}{Hk_g} \tag{84}$$

Using equation 78 and noting that $P_A = H\overset{*}{C}_A$ and $P_A^* = H_{CA}$ gives

$$N_A = K_gH(C_A^* - C_A) \tag{85}$$

Comparison of equation 78 with equation 85 gives

$$K_l = K_gH \tag{86}$$

so that the overall mass transfer coefficient of the liquid phase can be related to that of the gas phase by Henry's constant.

APPLICATIONS OF DIFFUSION AND MASS TRANSFER IN FOOD PROCESSING

Absorption, distillation, evaporation, concentration, extraction, leaching, dehydration, and food packaging are some food-processing operations that involve diffusion or mass transfer. Among these, concentration deals with the removal of water from liquid foods and is normally dominated by heat transfer. Food packaging involves the diffusion of gases (O_2, CO_2, C_2H_4, etc.), water vapor, and volatile aroma components through one or more layers of polymeric films. The complex physical structure and chemical composition of foods, which may vary even within the same food and may change during processing or storage (10), makes the direct application of mass transfer and diffusion principles to food processing difficult. In this section some applications of mass transfer and diffusion principles are presented.

Diffusion in Gases

There are few cases of direct application of this principle to food processing, because convection effects are usually dominant. Corrections to the diffusivity are often necessary to account for such effects. The bulk flow of gases (convection) is caused by differences in total pressure and temperature created during processing. In many cases, mechanical equipment such as agitators, fans, or blowers are used to increase convection and, therefore, the transfer rate. Typical examples of diffusion in gases are (*1*) the evaporation of liquids from containers (e.g., benzene during cleaning of glass containers or hydrogen peroxide during sterilization of plastic containers for use in aseptic processing and packaging); (*2*) the escape of volatile-aroma food components from open storage tanks, mixing equipment, or concentration vessels; and (*3*) the loss of carbon dioxide from carbonated beverages.

Diffusion in Liquids

The phenomenon of diffusion in liquids is common in food processing, and its applications are extensive. A typical example is the extraction of sucrose from sugarcane or sugar beets (11), whereas other examples include the extraction of caffeine from coffee beans and edible oil from soybeans. Distillation, another application of diffusion in liquids, is used to remove or recover volatile components from foods (12), to produce ethanol and alcoholic beverages from fermentation liquids, and to purify solvents (10).

Diffusion in Solids and Nonideal Systems

The principle of diffusion in solids is used widely in food processing. Moisture diffusing through the porous skeleton of low-moisture foods during dehydration, gases or vapors diffusing through polymeric packaging films, small solutes diffusing through biomembranes during chemical peeling of fruits and vegetables (13), and oil leaching through the capillary pores of soybeans are some typical examples. The mechanism of diffusion in solids is more complicated than that in gases and liquids because solids usually have a heterogeneous structure. The concept of effective diffusivity is often employed to simplify the calculations. In some cases, interactions and binding actions of macromolecules affect the diffusion and movement of small molecules through food and other biological systems. Some examples include the diffusion of glucose in an agar gel and sucrose in gelatin (1), sorbic acid in agar (14), sodium chloride in meat muscle (15), and nitrite in beef (16).

NOMENCLATURE

A	Area
C	Concentration
C^*	Concentration at equilibrium with the bulk partial pressure
$\bar{C}$	Average concentration
D	Diffusivity
H	Henry's constant
j	Diffusion rate
k	Film coefficient
K	Overall mass transfer coefficient
l	Length of a solid cylinder
L	Time lag for unsteady-state mass transfer
M	Molecular weight
n	Mole number
N	Mass flux per unit area
$\dot{N}$	Total mass flux
P	Pressure
P^*	Pressure at equilibrium with the bulk concentration
Q	Total amount of substance being diffused in time t
r	Radius
$\bar{r}$	Mean capillary radius
R	Gas constant
S	Solubility
t	Time
T	Temperature
v	Velocity
V	Volume
X	Molecular fraction
y	Small distance of transfer
Y	Total distance of transfer

Subscripts

0	Properties at initial time
1,2	Properties at positions 1 and 2, respectively
A	Properties of matter A
AB	Properties of A when transferred through B
Ai	Properties of A at the boundary layer
b	Properties of bulk, or when binding effect is present
B	Properties of matter B
BA	Properties of B when transferred through A
d	Properties due to diffusion
e	Properties at equilibrium
g	Properties in the gas phase
i	Properties at the interface
l	Properties in the liquid phase
m	Arithmetic mean value
$m\ln$	Logarithmic mean value
p	Properties of protein
t	Properties at time t

Greek

α	Diffusivity reduction factor
β	Diffusion cell constant
δ	Thickness
ϵ	Porous fraction of solid or membrane
κ	Knudsen's constant
Λ	Molecular mean free path
λ	Slope of equation (70)
μ	First-order chemical reaction constant
ρ	Density
τ	Tortuosity of solid
φ	Volume fraction

BIBLIOGRAPHY

1. C. J. Geankoplis, *Transport Processes and Unit Operations*, 2nd ed., Allyn & Bacon, Boston, Mass., 1983.
2. R. M. Barrer, *Diffusion in and Through Solids*, Cambridge University Press, London, United Kingdom, 1951.
3. W. Just, *Diffusion in Solids, Liquids, Gases*, Academic Press, Orlando, Fla., 1960.
4. J. Crank, *The Mathematics of Diffusion*, 2nd ed , Clarendon Press, Oxford, United Kingdom, 1975.
5. J. D. Floros and M. S. Chinnan, "Determining the Diffusivity of Sodium Hydroxide through Tomato and Capsicum Skins," *J. Food Eng* **9**, 129–141 (1989).
6. H. S. Mickley, T. K. Sherwood, and C. E. Reed, *Applied Mathematics in Chemical Engineering*, 2nd ed., McGraw-Hill, New York, 1957.
7. J. Crank and G. S. Park, *Diffusion in Polymers*, Academic Press, Orlando, Fla., 1968.
8. C. K. Colton et al., "Diffusion of Organic Solutes in Stagnant Plasma and Red Cell Suspensions," *AIChE Symposium Series* **66**, 85–100 (1970).
9. C. J. Geankoplis, E. A. Grulke, and M. R. Okos, "Diffusion and Interaction of Sodium Caprylate in Bovine Serum Albumin Solutions," *Industrial and Engineering Chemistry, Fundamentals* **18**, 233–237 (1979).
10. G. D. Saravacos, "Mass Transfer Properties of Foods," in M. A. Rao and S. S. H. Rizvi, eds., *Engineering Properties of Foods*, Marcel Dekker, New York, 1986, pp. 89–132
11. D. R. Heldman and R. P. Singh, *Food Process Engineering*, 2nd ed., AVI, Westport, Conn., 1981.
12. J. L. Bomben et al., "Aroma Recovery and Retention in Concentration and Drying of Foods," in Chichester et al., eds., *Advances in Food Research*, Vol. 20, Academic Press, Orlando, Fla., 1973, pp. 2–111.
13. H. G. Schwartzberg and R. Y. Chao, "Solute Diffusivities in the Leaching Processes," *Food Technol* **36**, 73–86 (1982).
14. I. J. Pfrug et al., "Diffusion of Salt in the Desalting of Pickles," *Food Technol.* **21**, 1634–1638 (1975).

15 G. Dussap and J B Gros, "Diffusion-Sorption Model for the Penetration of Salt in Pork and Beef Muscle," in P. Linko et al., eds., *Food Process Engineering*, Elsevier Applied Science, Barking, United Kingdom, 1980.

16. J B Fox, "Diffusion of Chloride, Nitrite and Nitrate in Beef and Pork," *J. Food Science* **45**, 1740–1744 (1980).

GENERAL REFERENCES

J. D. Babbit, "On the Differential Equations of Diffusion," *Can. J. Res.* **28**, 449–474 (1950).

A. Leniger and W A. Beverloo, *Food Process Engineering*, D. Reidel, The Netherlands, 1975

J. Lewis, *Physical Properties of Foods and Food Processing Systems*, Ellis Horwood, Chichester, United Kingdom, 1987.

Loncin and R. L. Merson, *Food Engineering. Principles and Selected Applications*, Academic Press, Orlando, Fla., 1979.

A. Rao and S. S. H. Rizvi, *Engineering Properties of Foods*, Marcel Dekker, New York, 1986

T. Watson and J. C. Harper, *Elements of Food Engineering*, Van Nostrand Reinhold, New York, 1988.

JOHN D. FLOROS
JEFF RATTRAY
HANHUA LIANG
Purdue University
West Lafayette, Indiana

MEAT AND ELECTRICAL STIMULATION

Electrical stimulation of muscle from slaughtered animals hastens the process of rigor mortis. It does this through an initial pH fall that is then followed by a change in the rate of pH fall. The combined effect is that the muscles enter rigor mortis before the temperature falls sufficiently to result in *cold shortening* and toughening as a result of rapid chilling that may occur in present-day commercial situations. Electrical stimulation can be applied early postslaughter, in which cases relatively low voltages are effective as they operate via the nervous system. However, as the delay increases before stimulation, high voltages are then required. The applied electrical parameters generally used must consider the appropriate waveform and pulse frequency, duration, the prestimulation delay, the chilling rate, and the type of species involved. While electrical stimulation ensures that cold shortening is avoided, aging also starts at a high temperatures and is consequently more rapid. However, evidence suggests that other mechanism for tenderization are also taking place. Electrical stimulation must be considered as part of a total process from slaughter through chilling to final sale, considering electrical inputs and rates of pH fall and temperature fall to optimize tenderness and juiciness. Electrical stimulation has particular advantages for hot boning where the shortening and hence toughening conditions for unstimulated muscles are normally exacerbated by the improved chilling. When the stimulation conditions and chilling rates are tuned, hot boned meat is as tender as normal cold boned meat, and there can be significant improvements in other meat quality attributes such as drip and color.

HISTORY

The association between meat and electricity dates back to some of the earliest muscle physiology experiments (1). From Galvani's time, the use of electricity to study muscle function increased. The earliest reported use of electricity for meat improvement is its purported use by Benjamin Franklin in 1749 to electrocute turkeys with the result that they were "uncommonly tender" (2). In the 1950s the use of electrical stimulation as a means of improving tenderness of meat was investigated, but no commercial application of the process occurred then (3,4).

Electrical stimulation has been extensively used since the 1950s to hasten rigor mortis and to modify steps of the glycolytic pathway. Stimulation of horse muscle was used to facilitate a trial of microbial growth on prerigor and rigor muscles from the same animal (5). Others since have used it to modify and study steps of the glycolytic pathway (6). Landrace pigs were used to demonstrate that electrical stimulation accelerated the postmortem pH fall in normally slow glycolyzing muscles but produced no change in faster glycolyzing muscles (7). The principle was also used to predict the time course of rigor mortis (8).

The revived interest in electrical stimulation as an element of meat processing stems from the facts that it hastens development of rigor mortis (9–11), increases the rate of tenderization by postmortem aging (12,13), improves meat color (14), and provides an increased stiffness to muscles that can be of value for early boning.

DESCRIPTION

Electrical stimulation is a process that involves passing an electric current through the body or carcass of freshly slaughtered animals. This current causes the muscles to contract, increasing the utilization of muscle energy reserves, and leads to acceleration of glycolysis and subsequent rigor development. Electrical stimulation as a means of attempting to increase tenderness has been used experimentally on pigs, deer, goats, sheep, cattle, and various poultry species and, until recently, used commercially on all of these except pigs. This has changed as it has been shown that if stimulation is applied at 20 min postslaughter and the chilling rate is sufficiently fast (eg, deep leg to 10°C in 5–10 h), then the meat in those animals susceptible to pale, soft, and exudative (PSE) does not show it, and the remaining meat avoids cold shortening (15).

There are numerous physical methods by which electrical stimulation could be applied many different possible electrical specifications, and in reality many different perceptions of the response. Regardless of species, stimulation can be applied immediately after slaughter or at any point in time thereafter until the muscles become unresponsive. The time until muscles fail to respond is related to the natural rate of glycolysis and the voltage being applied, the duration of stimulation, and the type of response expected.

Most commercially used electrical stimulation systems employ the conveying rail as ground, and a live electrode contacts some other point of the body, carcass, or side. In the most basic systems, the live electrode contact is a clip

15. G. Dussap and J. B. Gros, "Diffusion-Sorption Model for the Penetration of Salt in Pork and Beef Muscle," in P. Linko et al., eds., *Food Process Engineering*, Elsevier Applied Science, Barking, United Kingdom, 1980.

16. J. B. Fox, "Diffusion of Chloride, Nitrite and Nitrate in Beef and Pork," *J. Food Science* **45**, 1740–1744 (1980).

GENERAL REFERENCES

J. D. Babbit, "On the Differential Equations of Diffusion," *Can. J. Res.* **28**, 449–474 (1950).

A. Leniger and W. A. Beverloo, *Food Process Engineering*, D. Reidel, The Netherlands, 1975.

J. Lewis, *Physical Properties of Foods and Food Processing Systems*, Ellis Horwood, Chichester, United Kingdom, 1987.

Loncin and R. L. Merson, *Food Engineering. Principles and Selected Applications*, Academic Press, Orlando, Fla., 1979.

A. Rao and S. S. H. Rizvi, *Engineering Properties of Foods*, Marcel Dekker, New York, 1986.

T. Watson and J. C. Harper, *Elements of Food Engineering*, Van Nostrand Reinhold, New York, 1988.

JOHN D. FLOROS
JEFF RATTRAY
HANHUA LIANG
Purdue University
West Lafayette, Indiana

MEAT AND ELECTRICAL STIMULATION

Electrical stimulation of muscle from slaughtered animals hastens the process of rigor mortis. It does this through an initial pH fall that is then followed by a change in the rate of pH fall. The combined effect is that the muscles enter rigor mortis before the temperature falls sufficiently to result in *cold shortening* and toughening as a result of rapid chilling that may occur in present-day commercial situations. Electrical stimulation can be applied early postslaughter, in which cases relatively low voltages are effective as they operate via the nervous system. However, as the delay increases before stimulation, high voltages are then required. The applied electrical parameters generally used must consider the appropriate waveform and pulse frequency, duration, the prestimulation delay, the chilling rate, and the type of species involved. While electrical stimulation ensures that cold shortening is avoided, aging also starts at a high temperatures and is consequently more rapid. However, evidence suggests that other mechanism for tenderization are also taking place. Electrical stimulation must be considered as part of a total process from slaughter through chilling to final sale, considering electrical inputs and rates of pH fall and temperature fall to optimize tenderness and juiciness. Electrical stimulation has particular advantages for hot boning where the shortening and hence toughening conditions for unstimulated muscles are normally exacerbated by the improved chilling. When the stimulation conditions and chilling rates are tuned, hot boned meat is as tender as normal cold boned meat, and there can be significant improvements in other meat quality attributes such as drip and color.

HISTORY

The association between meat and electricity dates back to some of the earliest muscle physiology experiments (1). From Galvani's time, the use of electricity to study muscle function increased. The earliest reported use of electricity for meat improvement is its purported use by Benjamin Franklin in 1749 to electrocute turkeys with the result that they were "uncommonly tender" (2). In the 1950s the use of electrical stimulation as a means of improving tenderness of meat was investigated, but no commercial application of the process occurred then (3,4).

Electrical stimulation has been extensively used since the 1950s to hasten rigor mortis and to modify steps of the glycolytic pathway. Stimulation of horse muscle was used to facilitate a trial of microbial growth on prerigor and rigor muscles from the same animal (5). Others since have used it to modify and study steps of the glycolytic pathway (6). Landrace pigs were used to demonstrate that electrical stimulation accelerated the postmortem pH fall in normally slow glycolyzing muscles but produced no change in faster glycolyzing muscles (7). The principle was also used to predict the time course of rigor mortis (8).

The revived interest in electrical stimulation as an element of meat processing stems from the facts that it hastens development of rigor mortis (9–11), increases the rate of tenderization by postmortem aging (12,13), improves meat color (14), and provides an increased stiffness to muscles that can be of value for early boning.

DESCRIPTION

Electrical stimulation is a process that involves passing an electric current through the body or carcass of freshly slaughtered animals. This current causes the muscles to contract, increasing the utilization of muscle energy reserves, and leads to acceleration of glycolysis and subsequent rigor development. Electrical stimulation as a means of attempting to increase tenderness has been used experimentally on pigs, deer, goats, sheep, cattle, and various poultry species and, until recently, used commercially on all of these except pigs. This has changed as it has been shown that if stimulation is applied at 20 min postslaughter and the chilling rate is sufficiently fast (eg, deep leg to 10°C in 5–10 h), then the meat in those animals susceptible to pale, soft, and exudative (PSE) does not show it, and the remaining meat avoids cold shortening (15).

There are numerous physical methods by which electrical stimulation could be applied many different possible electrical specifications, and in reality many different perceptions of the response. Regardless of species, stimulation can be applied immediately after slaughter or at any point in time thereafter until the muscles become unresponsive. The time until muscles fail to respond is related to the natural rate of glycolysis and the voltage being applied, the duration of stimulation, and the type of response expected.

Most commercially used electrical stimulation systems employ the conveying rail as ground, and a live electrode contacts some other point of the body, carcass, or side. In the most basic systems, the live electrode contact is a clip manually applied to the head end of the animal body that is suspended by one or both hind legs, resulting in a current flow to the grounded rail support. More sophistication and protection is required as voltages increase and as application of the electrode becomes automated.

Procedures range from stimulating stunned but not bled animals, whole bodies, skinned bodies, carcasses, or sides. The electrical characteristics of the waveforms used are often poorly described, defying anyone to reproduce the results. Voltages used vary from 32 to 3,600 V and may be described in various ways. The value specified might be that of the peak or the rms (root mean square) voltage or in some cases the average over the total time. The rms voltage is the effective value or heating capacity of a waveform. For a sine wave, the rms value is the peak voltage divided by $\sqrt{2}$ (ie, 1130 V peak 50 Hz is 800 V rms), but for derived waveforms it may be quite different. For the MIRINZ waveform, which uses every seventh half-sine wave of a 50 Hz sine wave, the rms voltage is the peak voltage divided by $\sqrt{14}$.

Figure 1 sets out to illustrate the meaning of the different terms used to describe voltages and waveforms. Defining a waveform with a frequency (Hz) is likely to lead to confusion unless the waveform is defined in terms of shape, duration, and pulse spacing. Figure 2 shows some different waveforms. The applied waveforms may be unipolar or bipolar and applied as discrete pulses or as pulse trains. Again, their description has often defied interpretation. Some of the waveforms and pulse shapes used have been well researched and described, whereas others seem to have been determined merely by extending the use of available equipment, for example, electric stunning tongs.

Safety has been of utmost importance during experimentation and implementation of electrical stimulation in New Zealand, Australia, the UK, and France, to the point that in some instances safety concerns have effectively prevented commercial adoption of the process. Although not considered of overriding importance in some countries, safety of personnel can always be assured if the normal electrical safeguards are applied.

In New Zealand, practical on-line sheep operations stimulating up to 56 carcasses/min are a common operation. Less than 30 min after slaughter, dressed carcasses are suspended by metal skids and gambrels from a grounded rail and moved through a stimulation tunnel to make contact, at shoulder level, with an electrode supplied with high voltage pulses (16,17).

Similar systems can be applied for stimulation of beef bodies and sides of carcasses, and a novel system has been developed whereby sides contact a moving chain electrode curtain that maintains contact as the side curls (18) (Fig. 3). A range of other systems has also been developed for beef stimulation (19) covering both batch and continuous operations. The batch systems may involve manually inserted electrodes or electrode bars that move out to make contact with the body or carcass. In these systems the carcass or carcasses are enclosed within a shielded cabinet during stimulation. Continuous systems consist of stationary rubbing electrodes, or, where the stimulation is applied to carcasses prior to inspection, the electrode system consists of a moving series of electrodes that are sterilized between carcasses. In most instances electrical stimulation is applied with the aim of ultimately improving tenderness, but in the United States considerable attention has been devoted to color changes that improve grading of carcasses (20).

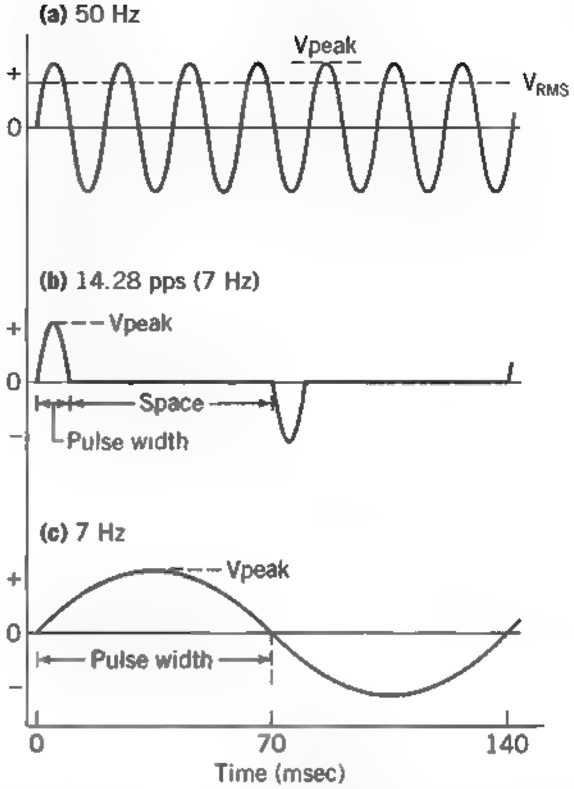


Figure 1. Terminology used to describe pulses and waveforms illustrated by sinusoidal (**a**) and (**c**) and derived waveforms (**b**). It is important to give the peak height in terms of either current or voltage while a carcass is being stimulated. The pulse shape must be specified either by description of the shape (eg, sinusoidal or square) or by specification of rise and fall times. The pulse width (mark) and space between pulses are also needed. The mark-to-space ratio helps specify a single cycle, which is the period from start to finish of the repetitive unit. The polarity of pulses is also necessary. The number of cycles per second then completes the description. The waveforms (**b**) and (**c**) both have the same period (inverse of wavelength) and peak but have different shape characteristics. The mark-to-space ratio for (**b**) is 1:7, whereas for (**c**) is 1:0. The time scale is given as an example only. The same-shaped waveforms could be given with different time scales.

High or low voltages can be used for stimulation. Low voltages (less than 80 V peak) are commonly used within 5 min of slaughter in small operations where electrodes are manually applied and removed. There is often an overlap in use of electrical currents to immobilize carcasses and the use of electrical currents to accelerate glycolysis—the name is different but the process is the same. Although, in general, plants stimulate with an electrode on the nose or stick wound and ground via the rail from which the carcass is suspended, the resistance of the hind leg can be very high, due to the high bone and tendon but low muscle content of the lower narrow portion of the leg. This high

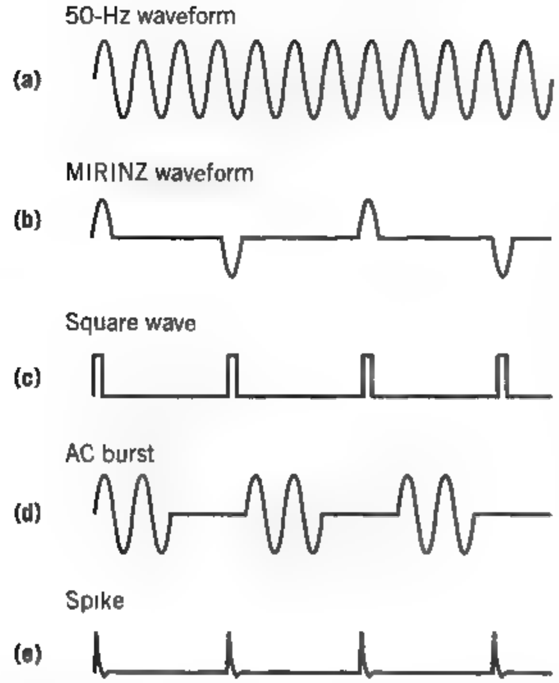


Figure 2. Common electrical waveforms that have been used for carcass electrical stimulation. All are shown at the same peak amplitude. (In the United States, a 60-Hz waveform is the basic supply.) (**a**) 50-Hz alternating current waveform (100 pulses/s). (**b**) MIRINZ waveform, derived by selecting every seventh pulse to give 14.28 pulses/s. (**c**) Square wave pulse train (14.28 pulses of 5-ms duration per second). (**d**) Bursts of the 50-Hz waveform. Common on-off times are 2 s on, 2 s off. (**e**) Spike pulses (commonly with base widths of 0.2–0.3 ms).

resistance can severely limit current flow. To solve this problem, there has been considerable effort to develop alternative procedures. The Australians (21) have used an anal electrode to provide good contact near the hind quarter and using this system have achieved effective low-voltage stimulation.

High voltages (greater than 200 V peak) are usually used for automated and high throughput systems to obtain maximum effect in the shortest period of time and to overcome variability of stimulation response that exists with low-voltage systems (22). Because high voltages are potentially lethal, considerable attention is paid to safety when they are being used.

MEAT TENDERNESS-TOUGHNESS

Meat tenderness has been regarded by many (23) as the prime determinant of consumer satisfaction with meat purchases. Although the ultimate measure of tenderness (or its converse, toughness) lies with the consumer, objective assessments can be made with a wide variety of mechanical devices, because these devices can reliably indicate differences attributable to animal and processing factors. Tenderness is the term used to describe the assessment by consumers (high scores being desirable), and the force to shear the meat, that is, shear force, gives an indication of the toughness (low values being desirable). The factors influencing meat quality, in particular tenderness, are covered the article MEAT SCIENCE.

Meat tenderness-toughness depends on both the myofibrillar strength and the connective tissue strength. Electrical stimulation seems mainly to modify the myofibrillar strength, although some work (24) suggests that electrical stimulation could also have an impact on the connective tissue component of meat tenderness. Most studies of electrical stimulation have been concerned with myofibrillar toughness as affected by cold shortening as muscle goes into rigor (termed conditioning). The basic tenets of much of meat science come from work that relates the degree of muscle shortening during the early postmortem period to the tenderness of cooked meat (25). The force required to shear a sample of cooked muscle across the grain rises steeply as the degree of muscle shortening exceeds 20%, reaching a peak at 40% shortening (see MEAT SCIENCE). The shear force of a sample shortened by 40% may be up to four times that of the nonshortened muscles.

If rigor (ie, conditioned) muscle is subsequently aged before cooking, it usually becomes appreciably more tender; however, the degree of tenderization is drastically reduced if the muscle has shortened during the conditioning (prerigor) phase (see MEAT SCIENCE). Even with extensive aging, there seems to be a limit beyond which any given muscle cannot be tenderized without loss of all structure. This background toughness is largely a result of the structure and composition of the connective tissues within the muscle. Aging reduces the myofibrillar strength (26,27) but is generally considered to have little effect on connective tissue, although some work questions this view (24).

Meat aging refers primarily to the tenderness improvement that takes place while postmortem muscle is held above freezing temperatures. Although there is no clear evidence as to when aging commences, much past evidence suggests that most of the aging commences with completion of rigor mortis (28). Other work (29) suggests that aging changes may commence prior to rigor completion, but they are relatively small. The changes in intramuscular connective tissue that have been noted (24) can be considered as aging changes.

The ultrastructural changes that occur during aging consist of a disappearance of Z disks and a disruption of the myofibrils at the junction of the I bands and Z disks. The ultrastructural changes are accompanied by parallel biochemical changes revealed by changes in the extractability of myofibrillar protein (30), changes to myofibrillar ATPase activity, and breakdown of myofibrillar proteins, for example, loss of troponin T (31,32). Proteinases termed calpains, with the inhibitor calpastatin, are considered to have a role (33). The ability of electrical stimulation to influence aging is disputed. However, it has been recently shown that rigor temperature affects tenderization with the optimum being 15°C (34), and this needs to be considered for optimum tenderization (Fig. 4) (36). Low level exercise affects nitric oxide (NO) in muscle (37), and recent work has shown that NO affects meat tenderizing, suggesting that electrical stimulation can have a similar role (38). Further work is still required to establish the effect of electrical stimulation on aging. The accelerated devel-

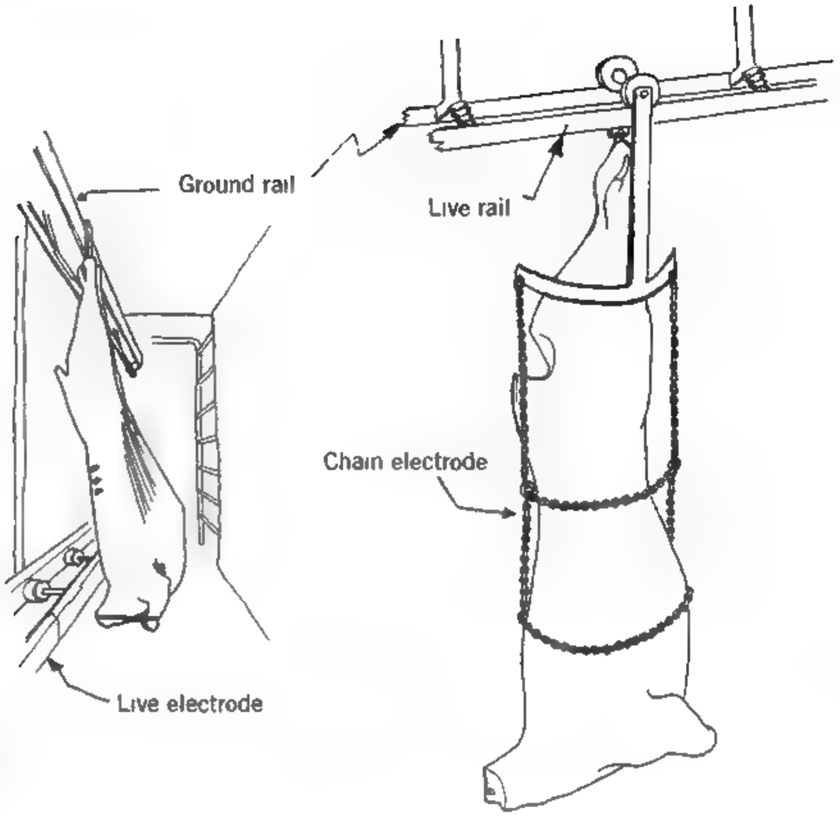


Figure 3. Diagrams showing electrode configuration used for high-voltage stimulation of sheep carcasses (only one is shown) and a novel chain electrode arrangement for beef sides. Stimulation area is enclosed in both situations and personnel entry controlled by floor switches and light beams. *Source:* Drawings by P. Hanara.

opment of rigor mortis means that even if aging does not start until after rigor is achieved, aging will commence at higher temperatures and, therefore, be more rapid (39).

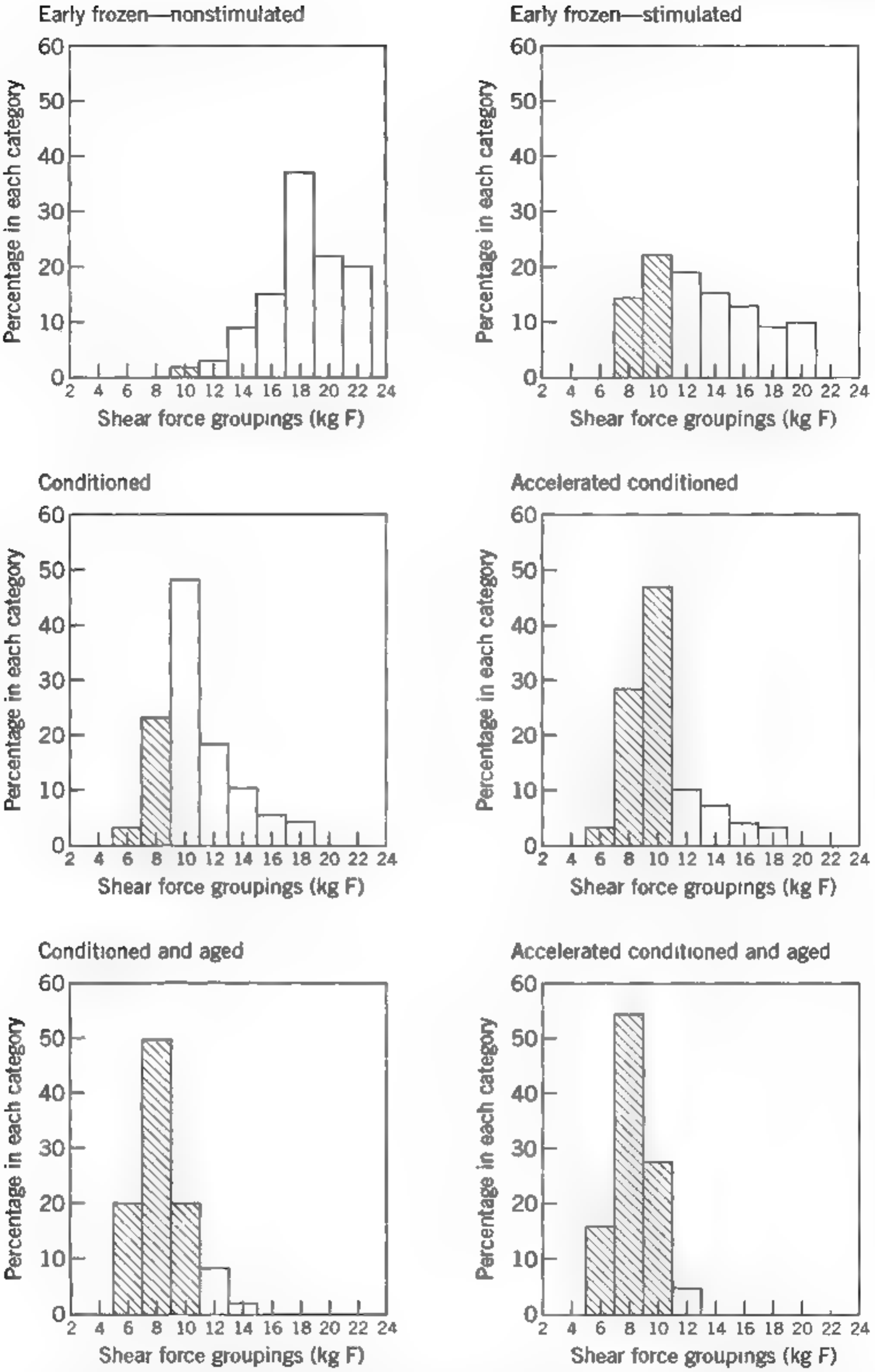
SCIENTIFIC BASIS

When muscles of freshly slaughtered animals are electrically stimulated, they contract. There is a concomitant increase in biochemical reactions in the muscle cells leading to an accumulation of lactate and resulting in an immediate drop in the muscle pH (ΔpH). After stimulation, the rate of pH fall (dpH/dt) as the muscles go into rigor is increased (Fig. 5) (40). If a muscle is free, it will contract during stimulation. When stimulation ceases, the muscle will partially relax but unless the muscle is subjected to some force, either restrained or tensioned, it will remain in a shortened state. Stimulation of suspended bodies or sides with a balanced arrangement of opposing muscle systems ensures that there is sufficient physical force to pull most muscles back to rest length. If muscles have been freed from their natural attachments soon after stimulation, however, as occurs with hot boning, there may be no restraining force to return stimulated muscles to their natural length. The same situation applies to stimulation of isolated muscles. Electrical stimulation of muscle influences the binding of many enzymes associated with the glycolytic pathway, and this is exhibited in the accelerated rate of pH fall and of ATP disappearance and the extremely rapid loss of creatine phosphate (41) after stimulation.

Although there are various theories as to the mechanism of how electrical stimulation promotes meat tenderness, most meat scientists tend to favor the view that stimulation accelerates glycolysis so that the time when muscles will contract on exposure to cold is reduced, thereby avoiding cold shortening.

There is also strong support for stimulation increasing meat tenderness by accelerating postmortem aging, because rigor is being achieved while temperatures are still high. It has been claimed (42) that much of the effect of electrical stimulation, under conditions where cold shortening is unlikely, results from mechanical damage and is not due to rapid aging at high temperatures. Interestingly, it has been found (43) that supercontracture bands occur in both stimulated and nonstimulated muscles.

Cold shortening occurs when muscles of freshly slaughtered beef and sheep are subjected to temperatures below 8°C while there is sufficient energy supply for contraction to occur. Cold shortening will occur in pigs and poultry, but the critical temperatures are lower. In normal beef and sheep muscles, this equates to a tissue pH of about 6.0. A rule of thumb in prevention of cold shortening is to maintain temperature above 10°C until muscle pH falls below 6.0 (44). For a discussion on cold shortening, see the article MEAT SCIENCE. Electrical stimulation, therefore, can have an immediate benefit under commercial conditions by causing the pH to fall and thereby shortening the time before carcasses can be chilled without causing toughness. Although many meat processors use electrical stimulation to reduce the time when muscles are susceptible to cold



Early frozen—nonstimulated

Early frozen—stimulated

Conditioned

Accelerated conditioned

Conditioned and aged

Accelerated conditioned and aged

Figure 4. The advantage of stimulation can be seen in the following series of commercial situations Distribution of force score values (kg F) from a MIRINZ pneumatic tenderometer with a wedge-shaped tooth, for loins from nonstimulated and electrically stimulated lamb carcasses. The early frozen product entered the freezer 40 min after slaughter, the conditioned carcasses were held at 10°C for 24 h before freezing, and the conditioned and aged product was held for 48 h at 10°C before freezing. The accelerated conditioned product was electrically stimulated at 30 min postmortem, then frozen 90 min later; the accelerated conditioned and aged product was electrically stimulated and held for 8 h at 7°C before freezing. All carcasses were frozen to a deep-leg temperature of − 4°C over a 12-h period, then reduced to − 12°C (66). *Source:* Ref. 35, used with permission.

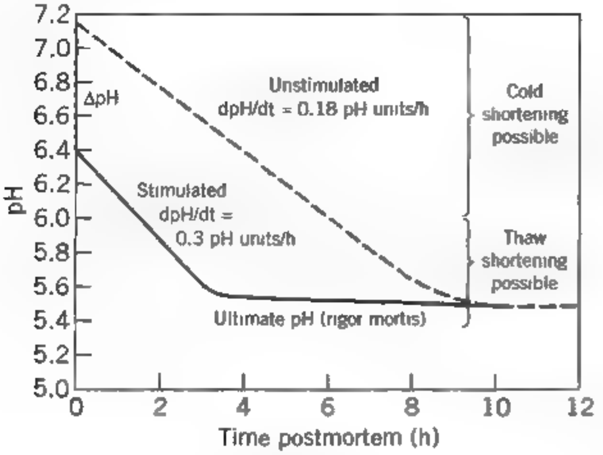


Figure 5. Postmortem pH fall in muscles after electrical stimulation. During stimulation the muscle pH falls (ΔpH). After stimulation the rate of pH fall (dpH/dt) is increased. At 35°C, unstimulated beef muscle dpH/dt = 0.18 pH units/h. With 120-s stimulation duration, the dpH/dt = 0.3 pH units/h.

shortening and get muscles into rigor so that aging commences while muscles are still warm, others have used electrical stimulation to produce color changes that can influence grading and even have used it for carcass immobilization. Electrical stimulation tends not to be used for pigs because early work (45) suggested that acceleration of rigor mortis through electrical stimulation increased the incidence of pale, soft, and exudative pork. However, no effect was found in the shoulders, but there was an increased paleness in the legs, unless rapid chilling was used (46), it is possible to use electrical stimulation if chilling is rapid enough (15).

Although remote from the actual changes produced by electrical stimulation, muscle pH has been used extensively as an indicator of progress into rigor. Muscle pH falls after stimulation provides a reasonable guide in normal muscles but is misleading in abnormal situations such as in the muscles from stressed animals. This is one case where muscle pH does not give a true indication of the stage of rigor development, because although the pH remains high, the ATP level may be zero and the muscle in rigor. The time to reach pH 6.0, or time to full rigor mortis, will be influenced by the magnitude of the ΔpH and the dpH/dt (Fig. 5) and provides a useful measure with which to evaluate the effectiveness of electrical stimulation systems in inducing early rigor. It does not provide an exact relationship with tenderness changes. Temperature will exert a substantial influence on both ΔpH and the dpH/dt.

FACTORS AFFECTING ΔPH

The magnitude of ΔpH is governed by the muscle fiber type, initial glycogen stores within the muscle, the electrical characteristics (current, frequency, pulse shape, and stimulation duration), the temperature of muscle, and the time after death that stimulation was applied.

Delay Postmortem

Postmortem delay before stimulation can have a threefold effect: muscle temperature can fall, greatly reducing the magnitude of ΔpH; glycolysis can have progressed so that muscle pH has fallen, reducing the ΔpH that can be achieved; and the nervous system can decay and become unresponsive so that its stimulation cannot elicit any muscle response. It has been shown that the ΔpH produced by any given stimulation decreases as prestimulation pH decreases (40,47). The ΔpH is 0 with a prestimulation pH of about 6.3.

The influence of temperature on the rate of pH fall has been considered in many reports (48–50) (Fig. 6). Since the advent of stimulation as a tool for meat science, the temperature dependence of the pH drop during stimulation and of the subsequent pH fall has been determined. When the muscle temperature falls, the magnitude of ΔpH is reduced. For example, in beef *m. sternomandibularis*, ΔpH ranges from 0.6 pH units at 35° to 0.018 units at 15°C. The energy of activation of ΔpH in stimulated beef *m. sternomandibularis* is calculated to be 97 kJ/mol (51), or very similar to that for calcium-activated actomyosin ATPase (44,50,52).

Muscle Type

Muscle type can influence ΔpH, for example, the fast-twitch beef *m. cutaneous trunci*, largely composed of white muscle fibers; and gives high values for ΔpH (and dpH/dt), whereas in the slow-twitch *m. masseter*, composed of red fibers, there is neither a distinct ΔpH nor an acceleration of dpH/dt, which is naturally rapid (0.4 pH units/h) (53). It has also been shown that ΔpH is minimal in predominantly slow-twitch muscles of beef (54). The greatest ΔpH

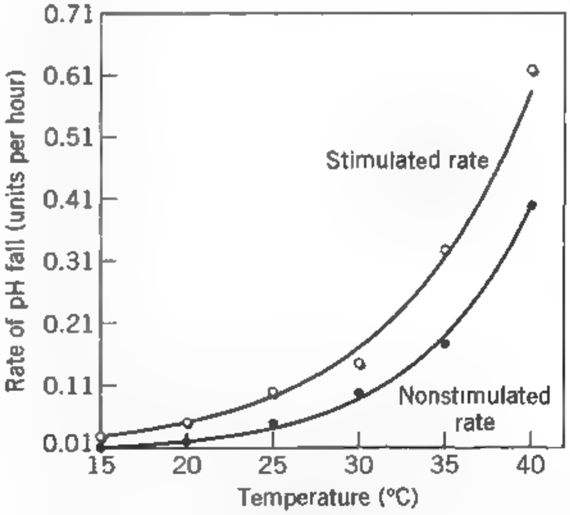


Figure 6. The various postmortem dpH/dt values for stimulated and nonstimulated muscle are plotted to show that the differences exist at all temperatures and illustrates how important it is to stimulate at high temperatures for full effectiveness. These results were obtained from beef *m. sternomandibularis* stimulated and held at the temperatures indicated

values have been shown in beef *triceps brachii* and the least in the *m. semimembranosus* (55). It was reported (56) that within the *m. sternomandibularis*, fast fibers with strong ATPase and weak succinic dehydrogenase (SDH) reactions showed most glycogenolysis in response to electrical stimulation, while slow fibers with weak ATPase and strong SDH reactions showed the least.

Muscles also differ in their optimum response frequency. Beef *m. longissimus* is more responsive to 14.28 pulses/s than to 40 pulses/s (40). The converse has been shown for the beef *m. semimembranous* (57). Rat muscles have a much shorter twitch time than beef muscles, with optimum responses to stimulation with a waveform of 33 pulses/s (58).

Electrical Characteristics

For any given muscle, the pH response will be governed by the electrical characteristics of voltage (current), and pulse frequency, shape, and polarity. In general, the higher the current (at a constant resistance, current increases with increased voltage), the greater will be the effect. This response will be asymptotic to some maximal value, however, so continually increasing the current will not lead to a continuing increase in effect (40,55). If the current level becomes too high, the muscle can cook due to resistive heating, this is especially true for high-frequency input. A practical consequence of this was the melting, within 45 s of the tendons of the *m. gastrocnemius* when used to suspend lamb carcasses during stimulation with 800 V rms at 50 Hz in some early studies.

For all of the species tested, the pulse shape does not seem to be a critical factor, with equivalent responses being achieved with 10 ms duration half-sine wave pulses and 5 ms duration square-wave pulses at the same frequency (40). The pulse frequency, however, can exert a profound effect, because of the interaction with muscle type and response frequency. If a single muscle is considered, for example, beef *m. sternomandibularis*, the magnitude of ΔpH is maximal with a pulse rate of between 9 and 16/s. At frequencies between 10 and 20 pulses/s, ΔpH values are, respectively, 40 and 75% greater than at 50 and 100 pulses/s (40). Pulse polarity has not received much attention, although it was indicated (35) that for low-voltage stimulation the polarity of pulses influenced the magnitude of ΔpH and the movement of the carcass during stimulation. The greatest responses were obtained when the cranial end of the animal was positive relative to the rest of the body. The physical response to low-voltage alternating polarity waveforms used for stimulation of beef cattle can be movement at half the waveform frequency, which disappears as the voltage is raised.

Many different voltages, frequencies, and pulse shapes have been used, and these are sometimes inaccurately described in scientific publications. In the United States, many groups have pulsed the 60 Hz AC waveform with 1- or 2-s periods of current flow, then a similar period of rest (19,46,59), whereas groups in Australia, the UK, Sweden, and New Zealand have used discrete pulses. An unusual, but apparently effective, combination of high- and low-voltage pulses has been used by the Dutch (60). In many instances the reasons for the chosen frequency do not appear to have a scientific basis.

FACTORS AFFECTING DPH/DT

Almost any stimulation can affect the dpH/dt. Rates of pH fall in curarized muscles are much slower than those in muscles of animals slaughtered without curarization (61). Vigorous muscle movement during slaughter of normal animals can induce the apparent maximal twofold increase in dpH/dt (61). Sometimes there can be unexpected electrical stimulation arising from the stunning (62) and immobilization of carcasses and even effects from current application from downward hide pulling (63).

Attempts have been made to relate increases in dpH/dt to phosphorylase a and myofibrillar ATPase stimulation (47). The muscle temperature has a major effect on dpH/dt (Fig. 6). In nonstimulated beef *m. sternomandibularis*, the energy of activation is 40–45 kJ/mol, whereas that of stimulated muscle approaches 70 kJ/mol (51). This high energy of activation means that any cooling of the muscles will have a marked effect on the time for rigor to be achieved. Because the achievement of ultimate pH is asymptotic, any reduction in maximum rate can greatly prolong the period when muscle is above the ultimate pH and still has a measurable level of ATP. The rate of pH fall and ATP loss is affected not only by the temperature during stimulation, but also by the temperature history of the muscle after stimulation during the period of study (64).

PRACTICAL RESULTS OF STIMULATION

The most dramatic practical result of stimulation is the tenderness difference between nonstimulated and stimulated early frozen lamb carcasses (10). Loins and legs from nonstimulated lamb carcasses put into a blast freezer (−25°C) within 2 h of slaughter and later cooked from the frozen state are exceedingly tough, with only about 1% of shear force determinations on loins being below a level considered to be the maximum for acceptably tender meat. In contrast, loins from similarly processed but high-voltage stimulated lamb carcasses give about 75% of shear force values in the acceptable range (65) (Fig. 4). Lamb carcasses, because of their small size, can be chilled or frozen rapidly and, therefore, the muscles are more liable to cold shortening than are those of larger nonstimulated animals. However, there is a considerable body of evidence that electrical stimulation of beef under conditions where the carcasses are rapidly chilled also results in protection of tenderness (11,66).

Under slower cooling conditions, the differences are much less pronounced, and, in fact, if the evaluation is made after three weeks of aging, nonstimulated product not subjected to rapid chilling may not differ in tenderness from rapidly chilled stimulated product. Under these conditions, stimulation merely facilitates rapid processing Rapidly chilled, nonstimulated product will, however, be significantly tougher than that from stimulated bodies (Table 1), as cold-shortened muscles have a decreased aging potential. Clearly it is more costly to hold nonstimulated

Table 1. Mean Shear Force Results (kg F) from Rapidly and Slowly Chilled Beef after Three Weeks of Holding Under Vacuum at 1°C

Stimulation	7°C chiller	2°C chiller
High-voltage stimulated	4.58	4.66
Low-voltage stimulated	5.99	8.2
Nonstimulated	8.1	12.2

Note: One side of each animal was chilled in 2°C air at 0.3 m/s, the other in 7°C air at 0.3 m/s. The lower the shear force values, the more tender the meat. Values above 11 kg F indicate unacceptable toughness. These results are for a MIRINZ tenderometer and correspond to a Warner Bratzler tenderometer if multiplied by 0.7 (67).

carcasses in a chiller for long durations to age to achieve the same desired tenderness of stimulated carcasses that occurs much earlier; thus, stimulation can be a cost-effective process.

The degree of tenderness improvement that researchers attribute to stimulation varies because of the widely different methods of assessing tenderness and the innumerable processing variations; comparison of results is, therefore, difficult.

Stimulation does not always result in tenderization, but neither does it in itself toughen meat. Studies showed that electrical stimulation of stressed animals caused a slight toughening of the *m. longissimus* in beef and sheep (68,69), although it was hypothesized (68,69) that the toughening was a consequence of muscles going into rigor during stimulation and remaining contracted. It has been suggested (70) that under the conditions present, the rapid reduction of the time that muscle pH is high reduces the aging that occurs, but additionally the lower tenderizing rate of intermediate pH meat may be important (71).

Electrical stimulation is also claimed to improve the color of lean and influence beef grading under the U.S. system (20,67,72). One study found no effect of low-voltage electrical stimulation on meat color as measured by reflectance but did find that postmortem aging significantly reduced reflectance values (73).

Comparisons of low- and high-voltage stimulation under comparable conditions have shown that if environmental conditions are such that cold shortening is unlikely, low- and high-voltage stimulation both result in an increase in tenderness (74), and both processes improve the youthful appearance and marbling scores of beef (75). Under conditions where chilling rates are more rapid, low-voltage stimulation does not give the same degree of protection against cold shortening as high-voltage stimulation (67). The interaction between three moderate chilling conditions and two electrical stimulation treatments were examined (76); it was shown that the temperature regime to which the muscles were exposed became more critical when low-voltage stimulation was used. When all things are considered, the use of electrical stimulation must be seen as part of a total process. The tuning of the amount of stimulation with chilling rate to reach rigor at 15°C results in optimum tenderization (Fig. 7) (36).

Not all muscles of a carcass are equally affected by stimulation. One study (11) showed that although the beef striploin and rump improved in tenderness, there was no change in the topside. Similarly a dramatic improvement in the tenderness of beef *m. longissimus* and *m. semimembranosus* was shown but not of the *m. triceps brachii* and *serrates ventralis* after high- or low-voltage stimulation. Some of the difference has been attributed to uneven distribution of the current during stimulation (59), but obviously some of the difference must be biochemically and physiologically based.

Because of the rapid fall in pH of pork and the tendency for PSE conditions of high temperatures and low pH values to be present, it was considered that there would be no advantage in stimulation. However, it has been shown that if electrical stimulation is applied at 20 min postslaughter and the chilling rate is sufficiently fast under ideal commercial conditions (eg, deep leg to 10°C in 5–10 h), then the meat in those animals susceptible to PSE does not show it and the remaining meat avoids cold shortening (15). There is an improvement in tenderness and drip loss with pelvic suspension (77). Further work has shown that there appears to be no advantages with electrical stimulation and slow chilling (77).

Cold shortening in poultry does not seem to cause the same toughness problems as in other species, as the maximum shortening occurs around 2°C (79), which is generally lower than the temperatures the birds reach following chilling by ice water and air chilling. However, rapid processing would be advantageous because the toughness that arises from portioning the birds early and removing the breast muscles in particular could be avoided. A stimulation system, part of the minimum time process system, was developed and patented by Campbell Institute for Research and Technology (80). The process proved ideal for further process applications with the meat at 3 h being the same as that of normal processing at 24 h, although meat was slightly tougher mainly because longer aging still would be beneficial. There are problems if the birds are either not chilled rapidly enough (81,82), or chilled rapidly and held at low temperatures (83), when it appears that there is no advantage in stimulation. If early boning is desirable, then stimulation is necessary. In a system developed and now in use in New Zealand with spin-chilling, when rigor is achieved at approximately 6°C, the breast muscles of such birds were able to be boned out in just over an hour poststun without any loss of tenderness (84). (D. J. C. Wild, C. E. Devine, and H. Reed, unpublished observations, 1988).

It is important to consider all aspects of stimulation and processing comparing results. It is almost impossible to compare results from one laboratory with those from another, because of the many variables introduced during processing. Not only are stimulation parameters different but also the processing conditions and the methods of evaluation can sometimes introduce artifacts; for example, freeing a normally restrained muscle can allow shortening, which can mask any other changes. Various stimulation treatments cause different degrees of internal heating and will produce different degrees of mechanical damage; for example, 2 Hz is claimed to cause no internal damage (42), whereas 60 Hz causes much more damage (12). Muscle

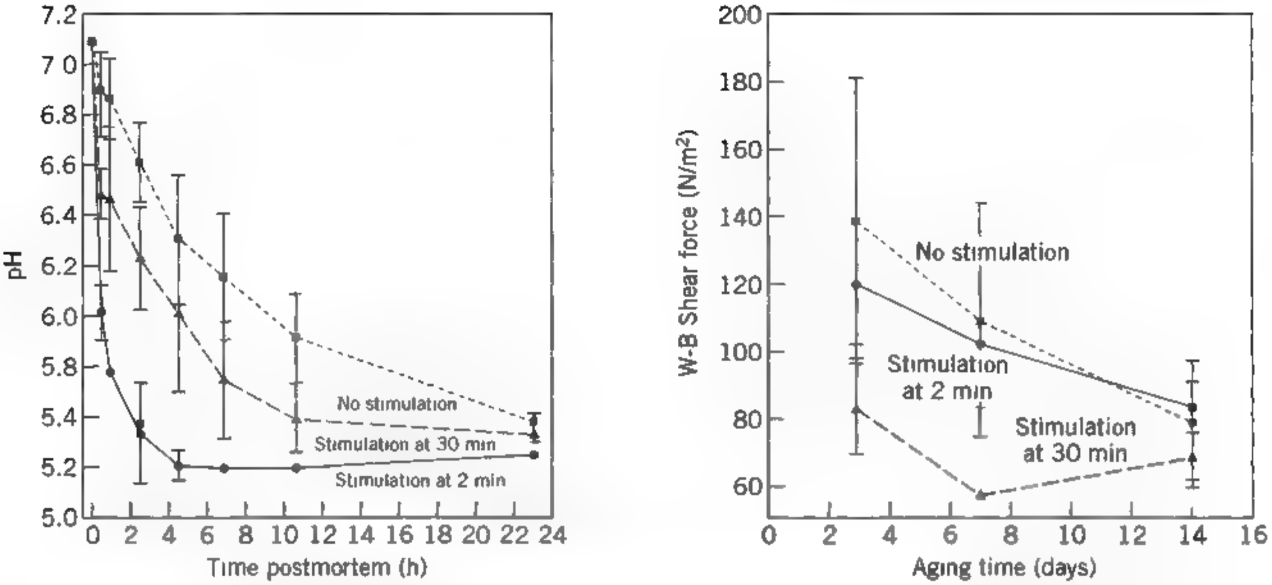


Figure 7. These two graphs show how the tenderness is affected by rigor temperature in a practical situation achieved through two different stimulation applications (37). The graph on the left-hand side shows the falls in pH The fastest fall in pH arises from stimulation within 2 min of slaughter, the next fastest fall in pH arises from stimulation at 30 min postslaughter, and the slowest rate of pH fall arises from no stimulation at all. When the tenderness is measured at three days, the meat with the medium rate of pH fall is the most tender, the meat stimulated within 2 min is next, and the unstimulated meat is the toughest. In time, after aging, the meat all becomes tender, but the meat stimulated at 30 min is still the most tender Temperature records showed that the meat stimulated at 30 min attained rigor close to 15°C. This study suggests that meat attaining rigor close to 15°C will be more tender than rigor at other temperatures, and future studies will determine the precise processing situations to achieve this.

damage seems unlikely to be the explanation for many of the effects of electrical stimulation.

ROLE OF ELECTRICAL STIMULATION IN HOT BONING

Hot boning, widely practiced in the past and common in many underdeveloped countries, is now experiencing revival in industrialized countries because of its economic advantages (savings in energy, space, labor, and time). The major constraints to the use of hot boning have been the contraction of prerigor excised muscles, the slippage of one muscle relative to another, and the extra shortening of excised muscles subjected to rapid chilling and in the interest of microbiological control. It has been suggested (84) that hot-boned beef should be chilled below 8°C within 4 h if boned at 37°C However, it has been noted (85) that a reduction to below 21°C within 9 h would be acceptable. When muscle shortens by approximately 20%, it can still age and be tender (86), so hot boning is feasible if temperatures are controlled (see the article MEAT SCIENCE).

The contraction and consequent toughening of most prerigor excised muscles can be minimized by adequate holding at above 10°C (conditioning). In an early study (87), conditioned muscles were boned at 2 h postmortem, and then the vacuum-packaged primals were aged at 15°C. This gave a product that was as tender as, or more tender, than control samples from normally chilled and boned sides. However, boning at 2 h postmortem does not readily fit with a continuous operation; therefore, commercial processors bone earlier. These processors must use controlled chilling and tight packaging to minimize contraction and toughening; thus, there is interest in being able to reduce processing time and restraints without influencing product quality.

One study considered the physics of rapid chilling and cold shortening and concluded that cold shortening in rapidly chilled beef cannot be prevented without electrical stimulation (88). The situation was even more critical with hot boning because of the more rapid chilling of isolated product.

Electrical stimulation has been used to minimize or eliminate potential tenderness problems in hot-boned beef. The tenderness of product boned from stimulated carcasses at 1 h postmortem has been compared with product boned after 24 h of chilling (89). It was found that the stimulated hot-boned meat, even though chilled rapidly to 7°C, was as tender as that from sides conventionally chilled and boned at 24 h. If the electrically stimulated sides were held for 5 h so that they had entered rigor before boning, the product was also tender (90). Vacuum-packaged primals were prepared for studies of hot boning with and without electrical stimulation (91). It was found that cold-shortening toughness was avoided by either a delayed and slow chilling (21 h to 7°C) or by electrical stimulation of the carcass before boning followed by rapid chilling (17 h to 7°C).

Some attempts have been made to use high-temperature conditioning (eg, holding for 3 h at 37°C) as a means of ensuring that hot-boned beef does not toughen. It was shown that this high-temperature conditioning of hot-boned cuts gave the same results as did prior electrical stimulation of the carcass (92). The effects differed between the three muscles tested, with improvement noted in the *m. longissimus*, no change in the *m. semimembranosus*, and a decrease in tenderness of the *m. semitendinosus* compared with nonstimulated controls. It was theorized that the high-temperature conditioning caused increased proteolysis in the *m. longissimus* but connective tissue shrinkage in the *m. semitendinosus*. This theory does not seem to be completely substantiated by the results.

In general, hot boning has resulted in an increase in the toughness of beef *m. psoas major* muscles, which are normally stretched when carcasses are supported by the Achilles tendon; but if the product was aged for a day at 20°C, the differences between hot and cold boning were removed (93). The sarcomere length of cold-boned product was 3.3 μm (stretched), whereas that of the hot-boned product was 1.95 μm, close to that of rest length of most muscles in the body. Other cuts showed a lesser difference. High-temperature conditioning is not necessary to ensure tenderness if an effective electrical stimulation system was employed (92,93). It should be noted that the effect of a small degree of passive shortening can be overcome by additional aging. The observation that rigor at 15°C produces the most tender meat whether hot boned or restrained (34), suggests that further temperature controls will result in significant tenderness improvements.

Hot boning of pork for whole-hog sausage gives lower cooking losses and increased juiciness scores than does postrigor processed pork (94). For primal cuts, hot boning is reputed to give poorer initial color and appearance, but the differences from cold-boned product disappear over the following 120 h (95). Some studies have considered the use of electrical stimulation to aid hot processing of pork, but the results are conflicting due to the PSE condition, which is increased in susceptible pigs (46,96). Electrically stimulated pork hot boned at 1 h postmortem and then rapidly chilled produces cuts that give less purge and are more juicy than slowly chilled product (15,97). If the chilling is slower in the case of hot-boned pork, stimulation resulted in no tenderness advantage and increased drip and was not be desirable (98).

The role of electrical stimulation in hot-boning applications is clearly to hasten the onset of rigor mortis, so that cold shortening is minimized in cuts that will cool more rapidly than the corresponding cuts on the carcass. Rapid cooling, with its greater control of microbial proliferation can then be used without irrevocably toughening the product. The sooner and more rapid the chilling, the more efficient the electrical stimulation must be to be effective. Very early boning and rapid chilling, even after electrical stimulation, will result in cold shortening and toughness. If a little stimulation is good, a lot is not necessarily better. Future work will refine the temperature conditions to produce the most tender meat enabled by precisely controlled stimulation for each carcass. Future work should be aimed at refining the whole process to increase the uniformity of tenderness across muscles and animals

BIBLIOGRAPHY

1. D. M. Needham, *Machina Carnis*, Cambridge Univ. Press, Cambridge, UK, 1971.
2. C. A. Lopez and E. W. Herbert, *The Private Franklin: The Man and His Family*, Norton, New York, 1975.
3. U.S. Pat. 2,544,681 (March 13, 1951), A. Harsham and F. E. Deatherage (to Kroger Co.).
4. U.S. Pat. 2,544,724 (March 13, 1951), H. C. Rentschler (to Westinghouse Electric Corp.).
5. M. Ingram and G. C. Ingram, "The Growth of Bacteria on Horse Muscle in Relation to the Changes after Death Leading to Rigor Mortis," *J. Sci. Food Agric.* **6**, 602–611 (1955).
6. G. T. Cori, "The Effect of Electrical Stimulation and Recovery on the Phosphorylase a Content of Muscle," *J. Biol. Chem.* **158**, 333–339 (1945).
7. O. Hallund and J. R. Bendall, "The Long Term Effect of Electrical Stimulation on the Postmortem Fall of pH in Muscles of Landrace Pigs," *J. Food Sci.* **30**, 296–299 (1965).
8. J. C. Forrest et al., "Prediction of the Time Course of Rigor Mortis through Response of Muscle Tissue to Electrical Stimulation," *J. Food Sci.* **31**, 13–21 (1966).
9. W. A. Carse, "Meat Quality and the Acceleration of Post-Mortem Glycolysis by Electrical Stimulation," *J. Food Technol.* **8**, 163–166 (1973).
10. B. B. Chrystall and C. J. Hagyard, "Electrical Stimulation and Lamb Tenderness," *New Zealand J. Agricultural Res.* **19**, 7–11 (1976).
11. C. L. Davey, K. V. Gilbert, and W. A. Carse, "Carcass Electrical Stimulation to Prevent Cold Shortening Toughness in Beef," *New Zealand Journal of Agricultural Research* **19**, 13–18 (1976).
12. J. W. Savell et al., "A Research Note: Structural Changes in Electrically Stimulated Beef Muscle," *J. Food Sci.* **43**, 1606–1607 (1978).
13. A. R. George, J. R. Bendall, and R. C. D. Jones, "The Tenderizing Effect of Electrical Stimulation of Beef Carcasses," *Meat Sci.* **4**, 51–68 (1980)
14. R. R. Riley et al., "Improving Appearance and Palatability of Meat from Ram Lambs by Electrical Stimulation," *J. Anim. Science* **52**, 522–529 (1981).
15. A. A. Taylor and M. Z. Tantikov, "Effect of Different Electrical Stimulation and Chilling Treatments on Pork Quality," *Meat Sci.* **31**, 381–395 (1992)
16. C. J. Hagyard, R. J. Hand, and K. V. Gilbert "Lamb Tenderness and Electrical Stimulation of Dressed Carcasses," *New Zealand J. Agricultural Res.* **23**, 27–33 (1980).
17. B. B. Chrystall and C. E. Devine, "Electrical Stimulation in New Zealand," in K. R. Franklin and H. R. Cross, eds., *Proc. Int. Symp. Meat Sci. Technol.*, National Livestock and Meat Board, Chicago, Ill., 1982, pp. 115–136.
18. Meat Industry Research Institute of New Zealand, *Annual Report*, MIRINZ, Hamilton, New Zealand, 1981.
19. J. W. Savell, "Industrial Applications of Electrical Simulation," in A. M. Pearson and T. R. Dutson, eds., *Electrical Stimulation*, Advances in Meat Research, Vol. 1, AVI, Westport, Conn., 1985.

20. G. C. Smith, "Effects of Electrical Stimulation on Meat Quality, Color, Grade, Heat Ring, and Palatability," in A. M. Pearson and T. R. Dutson, eds., *Electrical Stimulation*, Advances in Meat Research Vol. 1, AVI, Westport, Conn., 1985.
21. D. J. Walker, "Electrical Stimulation in Australia," in *Proc. Int. Symp. Meat Sci. Technol.*, National Livestock and Meat Board, Chicago, Ill., 1982, pp. 137–146
22. V Powell, "Quality of Beef Loin Steaks as Influenced by Animal Age, Electrical Stimulation, and Ageing," *Meat Sci.* **30**, 195–205 (1991)
23. P. V. Harris and W. R. Shorthose, "Meat Texture," in R. Lawrie, ed., *Developments in Meat Science—4*, Elsevier Applied Science, Barking, U.K., 1988, pp. 245–296.
24. E W. Mills, S. H Smith, and M. D. Judge, "Early Post-Mortem Degradation of Intramuscular Collagen," *Meat Sci.* **26**, 115–120 (1989).
25. R. H. Locker, "Degree of Muscular Contraction as a Factor in Tenderness of Beef," *Food Res.* **25**, 304–307 (1960).
26. R. H. Locker and D. J. C. Wild, "Yield Point in Raw Beef Muscle: The Effects of Ageing, Rigor Temperature, and Stretch," *Meat Sci.* **7**, 93–107 (1982).
27. R. H. Locker, D. J. C. Wild, and G. J. Daines, "Tensile Properties of Cooked Beef in Relation to Rigor Temperature and Tenderness," *Meat Sci.* **8**, 283–299 (1983).
28. C. E. Devine and A. E. Graafhuis, "The Basal Tenderness of Unaged Lamb" *Meat Sci.* **39**, 285–291 (1994).
29. D. J. Troy and P. V. Tarrant, "Changes in Myofibrillar Proteins from Electrically Stimulated Beef," *Biochem. Soc. Trans.* **15**, 297–298 (1987).
30. D. E. Goll and R. M. Robson, "Molecular Properties of Post-Mortem Muscle, 1: Myofibrillar Nucleosidetriphosphatase Activity in Bovine Muscle," *J Food Sci.* **32**, 323–329 (1968).
31. I. F. Penney and E. Dransfield, "Relationship between Toughness and Troponin T in Conditioned Muscle," *Meat Sci.* **3**, 135–141 (1979).
32. R. H. Locker and D. J. C. Wild, "The Fate of the Large Proteins of the Myofibril during Tenderising Treatments," *Meat Sci.* **11**, 89–108 (1984).
33. D. E. Goll et al., "Role of Muscle Proteinases in Maintenance of Muscle Integrity and Mass," *J. Food Biochem.* **7**, 137–177 (1983).
34. C. E. Devine, N. M. Wahlgren, and E. Tornberg, "The Effects of Rigor Temperatures on Shortening and Meat Tenderness," *Proc. 42nd Int. Congr Meat Sci. and Technol.*, Lillehammer, Norway, September 1–6, 1996.
35. B. B. Chrystall and C E. Devine, "Electrical Stimulation: Its Early Development in New Zealand," in A. M. Pearson and T R. Dutson, eds., *Electrical Stimulation* Advances in Meat Research, Vol. 1, AVI Publishing, Westport, Conn., 1985, pp 73–119.
36. N. M. Wahlgren, C. E. Devine, and E. Tornberg, "The Influence of Different pH-Courses during Rigor Development on Beef Tenderness," *Proc. 43rd Int. Congr. Meat Sci. and Technol.*, Auckland, New Zealand, July 27–August 1, 1997.
37 J. Jungersten et al., "Both Physical Fitness and Acute Exercise Regulate Nitric Oxide Formation in Healthy Humans," *J. Appl. Physiol.* **82**, 760–764 (1997).
38. C. J. Cook, S. M Scott, and C. E. Devine, "Measurements of Nitric Oxide and the Effect of Enhancing and Inhibiting It on the Tenderness of Meat," *Meat Sci.* **48**, 85–89 (1998).
39. C. L. Davey and K. V. Gilbert, "The Temperature Coefficient of Beef Aging," *J. Sci. Food Agric.* **27**, 244–250 (1976).
40. B. B. Chrystall and C. E. Devine, "Electrical Stimulation, Muscle Tension, and Glycolysis in Bovine Sternomandibularis," *Meat Sci.* **2**, 49–58 (1978).
41. D. J. Morton, J. F. Weidemann, and F M Clarke, "Enzyme Binding in Muscle," in R. Lawrie, ed., *Developments in Meat Science—4*, Elsevier Applied Science, Barking, UK, 1988, pp. 37–61.
42. B B. Marsh et al., "Effects of Early-Postmortem pH and Temperature on Beef Tenderness," *Meat Sci.* **5**, 479–483 (1980–1981).
43. S. Fabiansson and R. Libelius, "Structural Changes in Beef Longissimus Dorsi Induced by Postmortem Low Voltage Electrical Stimulation," *J. Food Sci.* **50**, 39–44 (1985).
44. J. R. Bendall, "Cold Contracture and ATP-Turnover in the Red and White Musculature of the Pig," *J. Sci. Food Agric.* **26**, 55–71 (1975).
45. J. C. Forrest and E. J. Briskey, "Response of Striated Muscle to Electrical Stimulation," *J. Food Sci.* **32**, 482–488 (1967).
46. D. D. Crenwelge et al., "Effect of Chilling Method and Electrical Stimulation on Pork Quality," *J. Anim. Sci.* **59**, 697–705 (1984).
47. R. P. Newbold and L. M. Small, "Electrical Stimulation of Post-Mortem Glycolysis in Semitendinosus Muscle of Sheep," *Meat Sci.* **12**, 1–16 (1985).
48. E. C. Bate-Smith and J. R. Bendall, "Factors Determining the Time Course of Rigor Mortis," *J. Physiol.* **110**, 47–65 (1949).
49. B. B. Marsh, "Rigor Mortis in Beef," *J. Sci. Food Agric.* **5**, 70–75 (1954).
50. R. E. Jeacocke, "The Temperature Dependence of Anaerobic Glycolysis in Beef Muscle Held in a Linear Temperature Gradient," *J. Sci. Food Agric.* **28**, 551–556 (1977).
51. B. B. Chrystall and C. E. Devine, "Electrical Stimulation Developments in New Zealand," *Proc 26th Eur. Meat Res. Workers Conf.*, Colorado Springs, Colo., August 31–September 5, 1980.
52 J. R. Bendall, *Muscles, Molecules, and Movement*, Heinemann, London, 1969
53. C. E. Devine, S. Ellery, and S Averill, "Responses of Different Types of Ox Muscle to Electrical Stimulation," *Meat Sci.* **10**, 35–51 (1984)
54. A. Houlier, C. Valin, and P. Sale, "Is Electrical Stimulation Muscle Dependent?" *Proc. 26th Eur. Meat Res. Workers Conf.*, Colorado Springs, Colo., 1980
55. H. Specht and J. Kunis, "Kalteverkurzung und elektrostimulation: Auswirkungen auf bei beschaffenheit von Schaf- und Rindfleisch," *Fleischwirkehaft* **69**, 1275–1280 (1989).
56. H. J. Swatland, "Cellular Heterogeneity in the Response of Beef to Electrical Stimulation," *Meat Sci.* **5**, 451–455 (1980–1981).
57. P E. Bouton, R. R. Wesle, and F D. Shaw, "A Research Note: Electrical Stimulation of Calf Carcasses: Response of Various Muscles to Different Waveforms," *J. Food Sci.* **45**, 148–149 (1980)
58. B. B. Chrystall and C. E. Devine, "Electrical Stimulation of Rats: A Model for Evaluating Low Voltage Stimulation Parameters," *Meat Sci.* **8**, 33–41 (1983).
59. M. B. Solomon, "Response of Bovine Muscles to Direct High Voltage Electrical Stimuli: A Research Note," *Meat Sci.* **22**, 229–235 (1988).
60. F. G. M. Smulders and G. Eikelenboom, "Electrical Stimulation of Carcasses: Development of Fully Automated Equipment," *Fleishwirtschaft* **65**, 1356–1358 (1985).

61. C. E. Devine, B. B. Chrystall, and C. L. Davey, "Studies in Electrical Stimulation: Effect of Neuromuscular Blocking Agents in Lamb," *J. Sci. Food Agric.* **30**, 1007–1011 (1979)
62. C. E. Devine et al., "Differential Effects of Electrical Stunning on the Early Post Mortem Glycolysis in Sheep," *Meat Sci.* **11**, 301–309 (1984).
63. P E. Petch and K. V. Gilbert, "Interaction of Electrical Processes Applied during Slaughter and Dressing with Stimulation Requirements," *Proc. 43rd Int. Congr Meat Sci. Technol.*, Auckland, New Zealand, July 27–August 1, 1997.
64. A. C. Kondos and D. G. Taylor, "Electrical Stimulation and Temperature on Biochemical Changes in Beef Muscle," *Meat Sci.* **19**, 207-216 (1985)
65. B. B. Chrystall, "Trends and Developments in Meat Processing," in R. Purchas and B. Hogg, eds., *The Production and Processing of Meat*, Animal Production Society, Hamilton, New Zealand, 1989.
66. J W. Savell, T R. Dutson, and Z. L. Carpenter, "Beef Quality and Palatability as Affected by Electrical Stimulation and Cooler Aging," *J. Food Sci.* **43**, 1666–1669 (1978).
67. A. E. Graafhuis et al., "Tenderness of Different Muscles Cooked to Different Temperatures and Assessed by Different Methods," *Proc. 37th Int. Congr. Meat Sci. Technol.*, Kulmbach, Germany, September 1–6, 1991.
68. T. R. Dutson, J. W. Savell, and G. C. Smith, "Electrical Stimulation of Antemortem Stressed Beef," *Meat Sci.* **6**, 159–161 (1982).
69. B. B. Chrystall et al., "Tenderness of Exercise-Stressed Lambs," *New Zealand J. Agricultural Res.* **25**, 331–336 (1982)
70. B. B. Marsh, "Effects of Early-Postmortem Muscle pH and Temperature on Tenderness," *Proceedings of the Reciprocal Meat Conference* **36**, 131–135 (1983).
71. A. Watanabe, C. C. Daly, and C. E. Devine, "The Effects of Ultimate pH of Meat on the Tenderness Changes during Ageing," *Meat Sci.* **42**, 67–78 (1995)
72. C. Calkins et al., "Quality-Indicating Characteristics of Beef as Affected by Electrical Stimulation and Postmortem Chilling Time," *J. Food Sci.* **45**, 1330–1340 (1980).
73. S. A. Pommier, L. M. Poste, and G. Butler, "Effect of Low Voltage Electrical Stimulation on the Distribution of Cathepsin D and the Palatability of Longissmus Dorsi from Holstein Veal Calves Fed a Corn or Barley Diet," *Meat Sci.* **21**, 203–218 (1987).
74. A. A. Taylor, N. F. Down, and E. Dransfield, "Effect of High and Low Voltage Stimulation on Tenderness of Muscles from Slowly Cooled Beef Sides," *Proc. 30th Eur Meat Res. Workers Conf.*, Bristol, UK, September 9–14, 1984.
75. D. M. Stiffler et al., "Comparison of the Effects of High and Low Voltage Electrical Stimulation on Quality-Indicating Characteristics of Beef Carcasses," *J. Food Sci* **49**, 863–866 (1984).
76. K. C. Koh et al., "Effects of Electrical Stimulation and Temperature on Beef Quality and Tenderness," *Meat Sci.* **21**, 189–201 (1987)
77. A. A. Taylor and A. M. Perry, "Improving Pork Quality by Electrical Stimulation or Pelvic Suspension of Carcasses," *Meat Sci.* **39**, 327–337 (1995).
78. P. D. Warriss et al., "Potential Interactions between the Effects of Preslaughter Stresses and Post-Mortem Electrical Stimulation of the Carcasses on Meat Quality in Pigs," *Meat Sci.* **41**, 55–68 (1995)
79. Yu B. Lee and D. A. Rickansrud, "Effect of Temperature on Shortening in Chicken Muscle," *J. Food Sci.* **43**, 1613–1615 (1978).
80. U.S. Patent 4,675,947 (June 30, 1987), K. A. Clatfelter and J. E. Webb (to Campbell Soup Company).
81. G. W. Froning and T. G. Uijttenboogaart, "Effect of Post-Mortem Electrical Stimulation on Colour, Texture, pH, and Cooking Losses of Hot and Cold Deboned Chicken Broiler Breast Meat," *Poult. Sci.* **67**, 1536–1544 (1988).
82. C. E. Lyon et al., "Effects of Electrical Stimulation on the Post-Mortem Biochemical Changes and Texture of Boiler Pectoralis Muscle," *Poult. Sci.* **68**, 249–257 (1989).
83. D. K. Wakefield et al., "Influence of Postmortem Treatments on Turkey and Chicken Meat Texture," *Int. J. Food Sci. Technol.* **24**, 81–92 (1989).
84. L. S. Herbert and M. G Smith, "Hot Boning of Meat: Refrigeration Requirements to Meet Microbiological Demands," *C S.I.R.O Food Research Quarterly* **40**, 65–70 (1980).
85. D. Y. C. Fung et al., "Initial Chilling Rate Effects on Bacterial Growth on Hot-Boned Beef," *J. Food Prot.* **44**, 539–544 (1981).
86. C. L. Davey, H. Kuttel, and K. V. Gilbert, "Shortening as a Factor in Meat Aging," *J. Food Technol.* **2**, 53–56 (1967).
87. G. R. Schmidt and K. V. Gilbert, "The Effect of Muscle Excision before the Onset of Rigor Mortis on the Palatability of Beef," *J. Food Technol.* **5**, 331–338 (1970).
88. J. Kunis and H. Specht, "Technologische kriterien fur da auftreten und die verhinderung von cold shortening in Rindfleisch," *Nahrung* **32**, 433–438 (1988).
89. K. V. Gilbert, C. L. Davey, and K. O. Newton, "Electrical Stimulation and Hot Boning of Beef," *New Zealand J. Agricultural Res.* **20**, 139–143 (1976).
90. K. V. Gilbert and C. L. Davey, "Carcass Electrical Stimulation and Hot Boning of Beef," *New Zealand J. Agricultural Res* **19**, 429–434 (1976).
91. A. A. Taylor, B. G. Shaw, and D. B. MacDougall, "Hot Deboning Beef with and without Electrical Stimulation," *Meat Sci.* **5**, 109–123 (1980–1981).
92. S. C. Seideman, J. D. Crouse, and H. R. Cross, "High-Temperature Conditioning of Hot-Boned Subprimals," *J. Food Quality* **12**, 145–153 (1989).
93. R. L. J. M. van Laack, *The Quality of Accelerated Processed Meats: An Integrated Approach*, Rijksuniversitat, Utrecht, The Netherlands, 1989.
94. H.-S. Lin, D. G. Topel, and H. W. Walker, "Influence of Prerigor and Postrigor Muscle on the Bacteriological and Quality Characteristics of Pork Sausage," *J. Food Sci.* **44**, 1055–1057 (1979).
95. N. G. Marriott et al., "Acceptability of Accelerated-Processed Pork," *J. Food Prot.* **43**, 756–759 (1980).
96. R. L. Swasdee et al., "Processing Properties of Pork as Affected by Electrical Stimulation, Post-Slaughter Chilling, and Muscle Group," *J. Food Sci.* **48**, 150–151, 162 (1983).
97. J. O. Reagan and K. O. Honikel, "Weight Loss and Sensory Attributes of Temperature Conditioned and Electrically Stimulated Hot Processed Pork," *J. Food Sci.* **50**, 1568–1570 (1985).
98. R. L. J. M. van Laack and F. J. M Smulders, "The Effect of Electrical Stimulation, Time of Boning, and High Temperature Conditioning on Sensory Quality Traits of Porcine *Longissimus dorsi* muscle," *Meat Sci.* **25**, 113–121 (1991).

B. B. CHRYSTALL
Massey University, Albany Campus
Auckland, New Zealand

C. E. DEVINE
Technology Development Group, HortResearch
Hamilton, New Zealand

MEAT, MODIFIED ATMOSPHERE PACKAGING

Chilled meat is packaged under modified atmospheres to preserve it against both microbial spoilage and nonmicrobial deterioration. Fresh meat is always contaminated by a variety of bacteria, which may include both spoilage and pathogenic strains. Meat is an ideal growth medium for many microorganisms, which have potential for growth to numbers that result in spoilage. Thus meat is a highly perishable food. However, altering the atmosphere can prevent the growth of some organisms and slow the growth of others, thus extending the time before microbial spoilage becomes evident or other microbial problems occur.

An important consideration for packaging and marketing is the retention of an attractive, fresh, salable appearance of meat in display. This includes most importantly muscle color, but also color of fat, bones, and purge in the package and includes prevention of excessive purge. During storage or display of meat, undesirable appearance gradually to rapidly occurs because of dulling, darkening, browning (even to tan or green) (1), and this color deterioration usually becomes a problem more rapidly than microbial spoilage. Dehydration of meat surfaces can also add to the appearance problem. Modified atmosphere packaging (MAP) packages are sealed and composed of films that are excellent barriers to water vapor transmission. MAP packages for red meats must maintain an atmosphere that prevents early deterioration of meat color. Nonmicrobial deterioration of meat flavor or texture will generally be significant only when microbial spoilage can be delayed for very long periods. These could include off-odors, flavors, or both caused by oxidative rancidity.

TYPES OF MAP PACKAGES

Three types of MAP packages include high oxygen, low oxygen, and ultra-low oxygen. Ultra-low oxygen is a more appropriate name for what some identify as controlled-atmosphere packaging (CAP). CAP implies control of in-package gas composition, which may not happen, but some systems definitely limit oxygen within the package and could fit into the ultra-low-oxygen category. Another relevant term is dynamic modified atmosphere packaging (2), sometimes called active packaging, and these systems may include a system to influence gas composition other than the influence of muscle metabolism.

Producing the initial gas atmosphere may involve an evacuation of air from the package followed by fill with the desired gas mix or, less likely, a displacement of air in the package with the gas mix. Gas mixes may be preformulated by the supplier or proper proportions from supply tanks of each desired gas mixed and controlled by a gas controller (mixer). Gas-mixture composition may be affected by the relative flow rates of the component gases during package filling. Given some variations in gas flows and variation in the amount of air removed by evacuation or displaced, the concentrations of the component gases of the modified atmosphere can be expected to vary by as much as 5% at the time packages are sealed.

High-oxygen MAP uses up to 80% O_2, 20 to 30% CO_2, and 0 to 20% N_2. Complete air evacuation is not as critical here as for low and ultra-low MAP High-oxygen packaging machines may be dangerous, and a spark can result in an explosion. Absolutely complete insulation of electrical wires and a choice of safe lubricants (not petroleum-based) is essential.

Low and ultra-low-oxygen MAP packages are gassed with CO_2 or a CO_2-N_2 mixture (3,4). The composition of the package atmosphere at the time of closure will depend on the extent to which air was removed from the package. With simple gas-displacement systems, the level of residual oxygen may be up to 10%. With chamber evacuation systems, oxygen levels of 0.1 to 1.0% could be achieved, depending on time of each cycle. The push in industry toward high production rate pushes this figure toward the higher percentage. If pouches are evacuated through snorkels while subject to atmospheric pressure, or to a system to put outside pressure on packages, this may result in a within-package oxygen level as low as 0.1% (1,000 ppm). However, package collapse closer to the seal end before complete package evacuation can trap pockets of air in the package. These distribute themselves into the in-package environment and result in higher-than-desired levels of oxygen.

CAP implies that an atmosphere of a known, desired composition is established within a pack and remains unchanged during the life of the package. The only type of packaging in current use that comes close to this description is a gas-impermeable pouch filled with an atmosphere of unmixed CO_2 (5). Attainment of reasonably stable gas composition depends on rapid placement of cuts in the package after cutting and low holding temperatures to minimize muscle and microbial metabolism. Packages of that type can be prepared using snorkel systems but without high assurance that residual oxygen is always reduced to very low concentrations at the time of package closure. CAP packages are preferably prepared using a system in which pouches are evacuated and gassed by snorkel within a chamber that is simultaneously evacuated, to prevent locking off of air-filled regions, then partially pressurized before the pouch is gassed to gently collapse the pouch and thus expel much of the remaining atmosphere. When such a system incorporates a high-volume pump to produce a high vacuum (≤2 torr, 29.90 in. of vacuum, a 99.7% vacuum), residual oxygen at package closure of less than 0.05% (500 ppm) can be attained providing the CO_2 gas contains 0.03% or less oxygen.

Packaging Materials

To adequately maintain modified atmospheres during storage, MAP packages must be constructed of materials that offer a high barrier to transmission of gases and water vapor. However, packages are constructed largely of materials that inevitably permit some degree of gas permeation. The amount of any gas actually transmitted by a film will depend on the film material, thickness of the materials, temperature of the film, and the difference between the partial pressure of the gas within and outside the pack. Most barrier packages are made of three layers of film. The outside layer must be scuff- and abrasive-resistant, the middle layer provides the gas barrier, and the inner layer is a sealant layer (6). Some barrier layers lose the barrier properties when wet and therefore must be protected from moisture on both sides (7,8). The rates at which different gases are transmitted through different films also vary widely, partially dependent on the chemical nature of the film polymer.

Gases that contribute to product life in MAP are CO_2 and O_2, and their relative rates of transmission depend on the film. As well as appropriately low gas transmission characteristics, films used for MAP packaging should have good sealing qualities. Although clipping or crimping is used in some systems for closing MAP packs, such closures are unreliable in that channels allowing excessive gas exchange may traverse the seals. To maintain a modified atmosphere, packaging materials that can be reliably sealed by heating are required. Tray and package design must consider film thickness at the thinnest points. An accordion-fold design meant to improve physical strength of a tray could result in thinning of gas barrier walls if the quantity of film material is limited.

Important physical characteristics needed include resistance to abrasion, as handling and transport are likely to impose abrasive stress; puncture resistance, particularly for bone-in products; appropriate thermoforming characteristics when tray forming is part of a packaging system; and, for display top-film layers particularly, good clarity and antifogging characteristics.

The critical gas-transmission characteristics are not well defined for many MAP purposes. In general, films with oxygen permeabilities of $<100\ cm^3/m^2/24\ h$ at standard atmosphere, measured for the film at a specified temperature and exposed to a specified humidity (commonly, 25°C and 75% RH), are considered possible materials for constructing MAP packages for low-oxygen MAP (9). However, such data give only an approximate indication of the oxygen transmission in actual use conditions and not a real indication of the CO_2 transmission that could occur. Consequently, trials are necessary to establish the utility of films for particular MAP purposes.

For high-oxygen MAP display, appearance of the package is a major commercial concern. That aspect of the package may therefore be optimized in practice, even at the cost of some reduction in the preservative capabilities of the packaging.

The atmosphere of a high-oxygen MAP display pack is dynamic. The contained meat respires, converting O_2 to CO_2; O_2 and CO_2 are transmitted at different rates through the film, at least through the lid, which must have good optical qualities; and the highly soluble CO_2 will dissolve in the meat to an amount determined by the meat mass, area of exposed meat surface, the muscle tissue pH, the ratio of fat to muscle tissue, the temperature, and the partial pressure of the gas in the within-package atmosphere (10). To maintain an atmosphere of adequate composition against the meat-related factors tending to change it requires a gas-volume-to-meat weight ratio of about 3. When free space within the package is inadequate, an excessive change in the O_2 concentration may occur during storage. Package collapse can also occur because a rising CO_2 concentration leads to a reduction in the atmosphere volume as a result of CO_2 dissolving in the meat. To guard against collapse upon initial or progressing dissolution of CO_2, it is common practice to include some nitrogen, which is physiologically inert, as ballast in the package atmosphere. Even when the package is properly constructed and filled to an appropriate gas-volume-to-meat weight ratio, changes in the atmosphere composition occur during storage.

The inevitable economic (cost) pressures lead to reducing the in-package headspace-to-meat ratio, especially when transportation for a long distance is involved. In the high-oxygen package, the concentrations of both O_2 and CO_2 will decline and that of N_2 will increase proportionally with decreasing concentrations of the other gases (11).

Pouches for low-oxygen MAP packages are usually composed of either a high or very high gas-barrier laminate (<100 or $<2\ cm^3\ O_2/m^2/24\ h/atm$). The pouch is evacuated and refilled with either CO_2 or a CO_2-N_2 mixture. Depending on the evacuation system used, residual oxygen may range from 10 to 0.1% (1,000 ppm) and sometimes as low as 300 ppm, although the latter, low level is not attained consistently.

After package closure, CO_2 will dissolve in the meat. When only CO_2 is added, if the quantity of CO_2 is insufficient to both saturate the meat at atmospheric pressure and fill the inevitable voids between meat pieces, the pack will collapse tightly on the product, compacting and deforming it. The partial pressure of CO_2 within the package will then be less than atmospheric, and the film may be prone to puncture by the contents, if bone-in cuts are used. Levels of CO_2 may also fall below the most effective antimicrobial levels. About 1.5 L of CO_2/kg of meat is generally required to avoid pack collapse (10).

Residual oxygen levels in low and ultra-low MAP packages may increase above initial levels for approximately 3 days, from an initial 2% to about 7% for a five–pork loin gas pack, then will decrease (12). The increase is partly due to oxygen that has diffused into the meat during exposure to air after cutting and before packaging. Some of this oxygen apparently diffuses out, increasing oxygen concentration of the in-package gas. Oxygen concentrations also rise because CO_2 dissolves into the meat. A shorter interval between cutting and packaging should decrease this short-term oxygen increase. After this initial increase, the concentration of residual oxygen within the MAP packages will tend to fall, because of reaction of the gas with the meat. However, the rate of such reactions will decline with the oxygen concentration and an equilibrium will ultimately be established between oxygen ingress through the film and scavenging of the gas from the package atmosphere. The equilibrium O_2 concentrations may reach about 1% in packs composed of high gas-barrier film but will be lower in packs composed of very high gas-barrier film (13). The subsequent lowering of oxygen concentration is affected by muscle metabolism (mitochondrial consumption and reaction with heme pigment), absorption by tissue fluids, and microbial metabolism.

MUSCLE SCAVENGING OF OXYGEN

Muscle metabolism can serve to scavenge oxygen within a MAP package, but such scavenging is slow at chilled meat temperatures and also slowed by lower concentrations of oxygen in the MAP headspace. Muscles differ in their ability to scavenge oxygen, with redder (more red fibers) muscle being more active. Scavenging rate may be influenced by pH.

Continued scavenging by muscle reduces its later ability to bloom (oxygenate) when exposed to air. Rapid reduction of in-package oxygen of ultra-low MAP packages seems important to retain substantial ability to bloom.

DYNAMIC MAP SYSTEMS

These systems (2) include active packaging, VSP (vacuum skin packaging) & MAP, CAPTECH, 2-phase MAP, static gas exchange, and dynamic gas exchange. VSP & MAP includes an inside vacuum skin of an oxygen-permeable film enclosed in a barrier bag into which a high-oxygen atmosphere (80% O_2, 20% CO_2) has been injected. The inner oxygen-permeable film allows oxygen at the meat surface while carbon dioxide controls microbial growth. The CAPTECH system has an inner oxygen-permeable film and the outer foil plus biaxially oriented nylon film that encloses a metered amount of carbon dioxide. The 2-phase MAP combines use of solid and gaseous carbon dioxide to avoid the package collapse that can result from CO_2 absorption by the meat.

Static gas exchange implies an inner package of oxygen-permeable film with an outer dome or master pack filled with a CO_2/N_2 mix. The outer dome or film is gas impermeable, and this system is dependent on a very good oxygen evacuation. This system may be vulnerable to oxygen that has diffused into meat after cutting and before packaging and later may partially diffuse out of the meat into the in-package atmosphere. Therefore the time interval between cutting and packaging should be very short.

Dynamic gas exchange implies mechanically replacing the original CO_2/N_2 gas mix with an 80% O_2/20% CO_2 or similar mix at the retail establishment. The original gas mix enables a longer storage and distribution product life of up to 3 weeks, and the gas exchange enables rapid bloom (oxygenation) of the myoglobin.

ACTIVE PACKAGING

Active packaging can use oxygen scavengers, carbon dioxide scavengers or emitters, chemically treated films, temperature-controlled packages, or time-and-temperature controlled packages (14). To insure early attainment and maintenance of continuous zero-oxygen concentrations in MAP, oxygen absorbers or scavengers can be added to the package to actively remove residual oxygen and oxygen that permeates the packaging film. The most common oxygen scavengers are reduced-iron powders mixed with acids, salts, or both to oxidize in the presence of oxygen. Most scavengers need to be activated by some wetted humectant (13). When a wetted humectant was added to the scavenger and placed in an aerobic atmosphere, oxygen scavenging occurred at near maximum rate (15). A scavenger used in an ultra-low-oxygen system can quickly lower oxygen levels below limits of detection.

Ageless® (Mitsubishi, Japan), a scavenger widely used by the food industry, has iron powder in a small packet that is highly permeable to oxygen. According to Nakamura and Hoshino (16), 1 g of iron can react with 0.0136 mol of oxygen, producing ferrous oxide. One gram or about 0.7 L of oxygen reacts with about 300 cm^3 of oxygen scavenger at standard pressure and temperature. Packets are available in sizes ranging from 20 to 2,000 cm^3 in oxygen-scavenging capacity. To remain within the scavenging ability of an oxygen scavenger, the barrier film permeability must be <20 $cm^3/cm^2/24$ hours, and the advantage of minimal oxygen to be scavenged is important.

An active packaging system (Cryovac OS 1000) released in 1998 (17) uses a polymeric oxygen-scavenging system that absorbs oxygen within the MAP package, and the polymer also serves as an oxygen barrier. The proprietary oxygen-scavenging layer consists of three primary components: an oxidizable polymer, a photoinitiator (PI), and a catalyst. The oxidizable polymer is the component responsible for binding the oxygen molecules. The PI absorbs the UV light and provides the energy to start the reaction. The catalyst helps to increase the rate of the scavenging reaction. The advantage of this system is that it is activated by UV light quickly after the package is sealed and does not require injection of an activating fluid.

An earlier stumbling block of sachet-type scavengers was a desire, especially by marketing people, not to have this "foreign material" in the final retail package. Some newer applications avoid this problem by attaching the iron-filled sachet to the outer barrier dome or bag, so it is disposed of and never appears with the final retail package. Most scavengers work more slowly at the low temperature required for chilled meat. This presents a problem as rapid scavenging of oxygen is a must for ultra-low-oxygen applications if rapid blooming is anticipated upon later exposure to air.

THE REACTIONS OF SPOILAGE FLORAS AND PATHOGENIC BACTERIA TO MODIFIED ATMOSPHERES

When lack of oxygen is not a factor, meat spoilage floras are inevitably dominated by strictly aerobic pseudomonads, at least at temperatures of 20°C or below. Those organisms will ultimately cause putrid spoilage of the meat. The presence of CO_2 in the atmosphere slows the rates of growth of both pseudomonads and competing species so that the pseudomonads retain the growth rate advantage that insures their predominance in the spoilage flora. Consequently, CO_2 in an aerobic atmosphere extends the time required for the spoilage flora to reach spoilage numbers but does not significantly alter the flora composition or the type of spoilage that finally occurs.

The inhibition of pseudomonads by CO_2 increases with decreasing temperature and with increasing CO_2 concentration. However, the increased inhibition is small for CO_2 levels $>20\%$. Thus, provided the CO_2 concentration does not fall below that level, changes in CO_2 concentration will have an insignificant effect on the storage life of meat packaged in a high-oxygen MAP pack (18). At chiller temperatures, the growth rate of pseudomonads is approximately halved by 20% CO_2 in the atmosphere. Therefore, high-oxygen MAP packaging will at best double the time before microbial spoilage of meat develops. Microaerobic or anaerobic conditions severely inhibit or suppress the growth of pseudomonads. Under essentially anaerobic conditions, low-pH (<5.8) meat will develop a flora composed solely of anaerobic but aerotolerant lactobacilli. Lactobacilli produce acid or dairy spoilage flavors in meat, but only substantially after the bacteria have achieved their maximum numbers (19).

Under the same conditions, high-pH muscle tissue and meat with extensive fat cover, which is of neutral pH, will develop a flora containing facultatively anaerobic organisms in addition to the lactobacilli. Species of *Enterobacteriaceae* will inevitably be present and cause putrid spoilage as they approach their maximum numbers. Two other species, *Brochothrix thermosphacta* and *Alteromonas putrefaciens*, may also be present when processing hygiene is poor. When those organisms are present in a spoilage flora, they can cause early spoilage when their numbers are relatively low; *B. thermosphacta* produces sour-aromatic odors and flavors, and *A. putrefaciens* causes putrid spoilage with much H_2S.

High concentrations of CO_2 impose significant lags on the lactobacilli and *B. thermosphacta* before they can commence growth and thus halve the growth rates of these organisms. In addition, the minimum temperature for growth of *B. thermosphacta* is increased from about -3°C to above 0°C. The effect of high CO_2 concentrations on the enterobacteria is even more pronounced, because their lag phase is extended to very long times, and they finally commence only very slow growth. The effect of high CO_2 concentrations of *A. putrefaciens* is less certain, but available evidence suggests that its growth is effectively suppressed (20).

Increasing concentrations of O_2 in a nominally anaerobic CO_2 atmosphere will tend to accelerate the weak growth of the facultative anaerobes. When the O_2 concentration is sufficiently high, slow growth of pseudomonads will occur. Thus low-oxygen MAP atmospheres, with a range of O_2 concentrations that usually increase during storage, tend to allow growth of mixed spoilage flora in which facultative anaerobes are a substantial fraction and in which even pseudomonads may occur. In contrast, ultra-low-oxygen-packaged meat will develop a flora of lactobacilli, irrespective of the meat pH. Poor microbiological condition before packaging has minimal effect on the type of flora that develops when the product is stored at 0°C or below. However, at higher storage temperatures, *B. thermosphacta* will grow on ultra-low-oxygen-packaged meat that was heavily contaminated with that organism during processing, at a rate similar to that of the lactobacilli. The presence of *B. thermosphacta* in the flora will cause early spoilage of the product. On ultra-low-oxygen-packaged meat, the ubiquitous enterobacteria will finally initiate growth to cause putrid spoilage when they approach the relatively low numbers of $10^6/cm^2$. Such putrid spoilage may, however, be preempted by acid-dairy spoilage due to the lactobacilli that attain maximum numbers of about $10^8/cm^2$ well before the enterobacteria start to grow.

In addition to spoilage organisms, cold-tolerant pathogenic species may grow on chilled meat, and mesophilic pathogens may grow when the chilled meat experiences temperature abuse. As with the spoilage organism, pathogen growth is generally slowed by substantial concentrations of CO_2. However, the response of bacteria to CO_2 is highly varied, and under some circumstances, a CO_2-containing atmosphere may confer a growth-rate advantage on an individual pathogen over competing spoilage types. In that situation, the pathogen might reach hazardous numbers before the meat is rejected because of spoilage. Such a condition can develop with vacuum-packaged meat and might be expected in high- and low-oxygen MAP packs. However, in ultra-low-oxygen-packs, the combination of anoxia and high CO_2 concentration tends to raise the minimum growth temperature and extend the lag phase of pathogens so that their growth is disadvantaged relative to that of spoilage organisms (21).

MUSCLE PIGMENT REACTIONS IN MODIFIED ATMOSPHERE

When purchasing fresh meat, consumers judge the acceptability of the product largely on the appearance, mostly color, of the exposed muscle tissue. Dull and/or discolored fat and darkened cut bone surfaces can detract from meat cut appearance when the muscle tissue appears attractive, but good appearances of fat and bone cannot compensate for a degraded appearance of the muscle tissue (22).

Muscle tissue appearance is determined by the chemical state of the muscle pigment, myoglobin, as shown in Figure 1. In the absence of oxygen, the pigment is in the deoxymyoglobin state, which has a dark, purplish-red color (23). On exposure to air, the pigment is rapidly oxygenated to form oxymyoglobin, the bright red that consumers have been taught to expect and find attractive. Deoxymyoglobin and oxymyoglobin, which are both in the reduced state, can oxidize to metmyoglobin, which has a dull brown color associated with deterioration of quality. Oxidation of deoxymyoglobin can be more rapid than oxidation of oxymyoglobin. That results in the faster oxidation of myoglobin at low (highest at 4 mm partial oxygen pressure) than at higher concentrations of oxygen (24).

Myoglobin oxygenation is rapid, and the fraction of the pigment in the oxygenated form increases with increasing oxygen concentration. Metmyoglobin is more stable and is

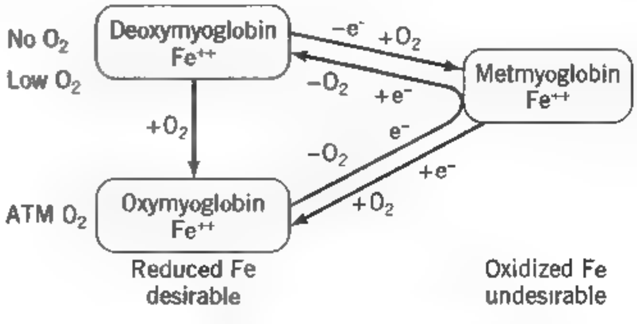


Figure 1. Fresh meat color triangle.

slowly converted to deoxymyoglobin by enzyme-mediated reactions termed metmyoglobin-reduction activity (25). Muscle tissue that is deficient in the enzymes that mediate metmyoglobin reduction or in the reduced cofactors necessary for the reduction reaction will be unable to reconvert metmyoglobin, which will persist once it is formed. Muscles vary widely in metmyoglobin-reduction activity. Those that tend to have a high activity, such as the longissimus dorsi, are more color-stable in air, their red color persisting for 3 or 4 times as long as color of unstable muscles of low metmyoglobin-reduction activity, such as the psoas. Metmyoglobin-reduction activity dissipates during storage of muscle. After lengthy storage, the color stability of initially color-stable muscles is similar to that of those muscles that were initially of relatively poor color stability (26).

Maintaining meat at as low a temperature as possible without freezing will slow pigment oxidation and increase the depth of the oxygenated surface layer. Two obvious means of preserving muscle color are to increase the proportion of oxidation-resistant oxymyoglobin by high-oxygen MAP or to largely exclude oxygen using ultra-low-oxygen MAP.

High-oxygen MAP is used mainly for retail product. If used for poultry, high-oxygen atmosphere may be inappropriate because of the meats' limited myoglobin level. The product may be packaged in gas barrier trays that contain the preservative atmosphere and are sealed with a lidding film of low gas permeability. Alternatively, several trays of product overwrapped with a film of high gas permeability may be master packaged in a bag of low gas permeability. The gas with which packs are filled may have a composition of about 65% O_2, 25% CO_2, and 10% N_2. The CO_2 is required to retard the growth of aerobic spoilage bacteria. Nitrogen has no preservative function for the meat and may be omitted, but it is often included to guard against the collapse of sealed trays that may result from the dissolution of CO_2 into the muscle.

The deoxymyoglobin chemical state predominates in normal muscle before cutting. Upon exposure to oxygen, usually in air, the oxygen diffuses further into the muscle with time, creating an oxymyoglobin layer on the meat surface. Diffusion into the muscle is more rapid in lower-pH muscle due to a more open muscle structure (27) and less scavenging of oxygen by muscle enzyme systems. Higher-oxygen tension at the meat surface, such as in high-oxygen MAP or by hyperbaric oxygen pressures, will drive the oxymyoglobin layer more deeply into the muscle, with the remaining muscle in the deoxymyoglobin state because of very low partial oxygen pressures in the meat-cut interior. At some variable time in display, metmyoglobin-reduction activity at the interface of the oxydeoxymyoglobin interface will be exhausted, so a layer of brown metmyoglobin begins to form, then becomes broader and begins to move toward the meat surface, diminishing the depth of the oxymyoglobin layer. At some point the brown metmyoglobin is close enough to the meat surface at some locations to contribute brown to the visual color as viewed on the meat surface, or as detected by a reflectance spectrophotometer. Because both the oxidative-reductive state and partial oxygen pressure may vary at different meat locations, the brownness is frequently spotty and not uniform, especially in ground meat where variable amounts of oxygen have been incorporated into the meat.

High-oxygen MAP systems often have atmospheres of 20% carbon dioxide and up to 80% oxygen and can produce and maintain a desirable red color in beef for up to 9 days and depress the formation of metmyoglobin by driving oxygen deep within the surface of the meat. Beef loin eye steaks in high-oxygen (70%) packages with 30% carbon dioxide had brighter, more desirable visual color scores and higher CIE a* (redness) values than vacuum controls (28) but with undesirable color beginning at 10 days of storage. Another study found that beef loin eye steaks were stable in high oxygen (70%) up to 14 days (29). Ground beef in a similar treatment was stable for only 6 days (30). Color life varies between species as well as muscle type. Pork loin chops in high oxygen had acceptable saturation indices and display color for 8 to 12 days with off-odor, not color, in beef steaks and pork the limiting factor to shelf life (31). High-oxygen-packaged beef loin eye steaks from loins in vacuum less than 7 days postmortem had much better display color stability than steaks packaged from loins vacuum aged for 14 or 28 days.

Carbon monoxide has been used in beef MAP systems to maintain a bright cherry-red color. Carbon monoxide binds strongly to myoglobin and hemoglobin, forming carboxymyoglobin and carboxyhemoglobin, respectively, pigments that are stable bright-red compounds (1). Round steak (pieces) stored at 0, 5, and 10°C in carbon monoxide from 0.5 to 1.0%, with the balance as nitrogen, had increased color and odor shelf life up to 20 days over that stored in air. Beef loin eye steaks at 4°C in a 0.4% carbon monoxide, 60% carbon dioxide, and 40% nitrogen system, compared with a high-oxygen (80%) system or vacuum packaging, were brighter red than the other two treatments and had better display color stability (28). Carbon monoxide at 0.4 to 0.5% is currently utilized safely in some domestic European fresh-meat markets. Low-oxygen packaging systems lack the benefit of high oxygen to cause oxymyoglobin to be formed more deeply into the meat. Low-oxygen systems often have too much oxygen for the meat to naturally scavenge and therefore may not discourage the formation of metmyoglobin.

In MAP systems with no oxygen in the gas mixture (ultra-low oxygen), a slight amount of residual oxygen frequently occurs due to small pockets of air not removed initially by the evacuation or flushing process. These small concentrations of oxygen frequently cause major discoloration, reduced color stability, or blooming ability problems. Initial oxygen concentrations >0.15% (1,500 ppm) seriously compromised the color stability of beef and lamb. Pork was affected by residual oxygen concentrations >1.0%. At low oxygen partial pressure, oxidation of oxymyoglobin to metmyoglobin is highly favored. Oxygen concentrations between 0.5% and 2% most rapidly revert myoglobin to metmyoglobin (24). Beef or lamb stored in such atmospheres may be initially discolored by the residual oxygen, but fresh red meat retains a limited reducing capacity by which it can recover from this initial discoloration. Compared to pork, beef and lamb were more sensitive to residual oxygen. Provided the amount of oxygen did not exceed 0.5%, fresh meat will naturally scavenge most residual oxygen, but more muscle scavenging will reduce later ability to bloom (oxygenate). Constant exposure to low concentrations of oxygen will have detrimental effects on reblooming ability and color stability. Therefore, if the residual oxygen could be removed earlier, the meat would have less discoloration, better rebloom, and improved color stability.

Rapid scavenging of oxygen in an ultra-low-oxygen package is important if the meat is expected to bloom with acceptable speed and completeness upon later exposure to oxygen. Muscle oxygen scavenging may not be fast enough, and added chemical scavengers may be necessary.

SUMMARY

Storage life of MAP-packaged meat may be limited by microbial deterioration; loss of salable appearance; or, in limited circumstances, by development of oxidized odors, tastes, or both.

The meat industry is rapidly moving toward preparation of case-ready retail cuts at the slaughter-fabrication plants, and MAP is essential to make such a system viable.

The essential keys to success are:

1. Clean, high-quality product into the package
2. Rapid completion of packaging after fabrication
3. Cold temperature and good temperature control
4. Complete evacuation and fill with MAP gases
5. Sound package, good seal, and minimum of leakers
6. Final retail package not very different from what customers are used to
7. Package remains sealed until consumer opens it

BIBLIOGRAPHY

1. D. H. Kropf, "Effects of Display Conditions on Meat Color," *Proc. 33rd Annu. Reciprocal Meat Conf.*, West Lafayette, Ind., June 22–25, 1980.
2. Y. Zhao, J. H. Wells, and K. W. McMillin, "Application of Dynamic Modified Atmosphere Packaging Systems for Fresh Red Meats Review," *J. Muscle Foods* **5**, 299–328 (1994).
3. E H. Sanders and H. M. Soo, "Increasing Shelf Life by Carbon Dioxide Treatment and Low Temperature Storage of Bulk Pack Fresh Chickens Packaged in Nylon/Surlyn Film," *J. Food Sci.* **43**, 1519–1527 (1978).
4. F. M. Christopher et al., "Effect of CO_2-N_2 Atmospheres on the microbial Flora of Pork," *J. Food Prot.* **43**, 268–271 (1980).
5. C. O. Gill, "Packaging Meat for Prolonged Chill Storage The CAPTECH Process," *Br. Food J.* **91**, 11–15 (1989).
6. W. H. Gehrke, "Film Properties Required for Thermo Formed and Thermal Processed Meat Packages," *Proc. 36th Annu. Reciprocal Meat Conf.*, Fargo, N.D., June 12–15, 1983.
7. M. Salame, "Barrier Polymers," in M. Bakker, ed., *The Wiley Encyclopedia of Packaging Technology*, Wiley, New York, 1986, pp. 48–54.
8. A. E. Lambden, D. Chadwick, and C. O. Gill, "The Oxygen Permeability at Sub-Zero Temperatures of Plastic Films Used for Vacuum Packaging of Meat," *J. Food Technol.* **20**, 781–783 (1985).
9. I. J. Eustace, "Some Factors Affecting Oxygen Transmission Rates of Plastic Films for Vacuum Packaging of Meat," *J. Food Technol.* **16**, 73–80 (1981).
10. C. O. Gill, "The Solubility of Carbon Dioxide in Meat," *Meat Sci.* **22**, 65–71 (1988).
11. G. C. Holland, "Modified Atmospheres for Fresh Meat Distribution," *Proc. Meat Industry Res. Conf.*, Chicago, Ill., March 27–28, 1980.
12. K. E. Warren et al., "Modified-Atmosphere Packaging of Bone-In Pork Loins," *J. Muscle Foods* **3**, 283–300 (1992).
13. D. J. Warburton, "The CAPTECH Process," *Proc. 25th Meat Industry Res. Conf.*, Hamilton, New Zealand, July 12–14, 1988.
14. T. P. Labuza and W. M. Breene, "Applications of 'Active Packaging' for Improvement of Shelf-Life and Nutritional Quality of Fresh and Extended Shelf-Life Foods," *J. Food Processing and Preservation* **13**, 1–69 (1989).
15. C. O. Gill and J. C. McGinnis, "The Use of Oxygen Scavengers to Prevent the Transient Discoloration of Ground Beef Packaged under Controlled, Oxygen-Depleted Atmospheres," *Meat Sci.* **41**, 19–27 (1995).
16. H. Nakamura and J. Hoshino, "Techniques for the Preservation of Food by Employment of an Oxygen Absorber," in *Sanitation Control for Food Sterilizing Techniques*, Sanyo, Tokyo, Japan, 1983
17. P. Cook, "Stealth Scavenging Systems: The Scavenger Is the Polymer," *Inst. Food Technologists Annu. Mtg.*, Atlanta, Ga., June 20–24, 1998.
18. C. O. Gill and K. H. Tan, "Effect of Carbon Dioxide on Growth of *Pseudomonas fluorescens*," *Appl. Environ. Microbiol.* **38**, 237–240 (1979).
19. K. G. Newton and C. O. Gill, "The Development of the Anaerobic Spoilage Flora of Meat Stored at Chill Temperatures," *J. Appl. Bacteriol.* **44**, 91–95 (1978).
20. C. O. Gill and J. C. L. Harrison, "The Storage Life of Chilled Pork Packaged under Carbon Dioxide," *Meat Sci.* **26**, 313–324 (1989).
21. C. O. Gill and M P Reichel, "The Growth of the Cold-Tolerant Pathogens *Yersinia enterocolitica, Aeromonas hydrophila*, and *Listeria monocytogenes* on High-pH Beef Packaged under Vacuum or Carbon Dioxide," *Food Microbiol.* **6**, 223–230 (1989).
22. C. O. Gill, "Extending the Storage Life of Raw Chilled Meats," *Meat Sci.* **43**, S99–S109, (1996)
23. D. B. MacDougall, in G. G. Birch, J. G. Brennan, and K. J. Parker, eds., *Sensory Properties of Foods*, Applied Science, London, 1977.
24. J. Brooks, "The Oxidation of Hemoglobin to Metmyoglobin by Oxygen, II: The Relationship between the Rate of Oxidation and the Partial Pressure of Oxygen," *Proc. Rl. Soc. London Ser. B* **118**, 560–577 (1935).
25. D. A. Ledward, "Post-Slaughter Influences on the Formation of Metmyoglobin in Beef Muscles," *Meat Sci.* **15**, 149–171 (1985).
26. V. J. Moore and C. O. Gill, "The pH and Display Life of Chilled Lamb after Prolonged Storage under Vacuum or under CO_2," *New Zealand J. Agricultural Res.* **30**, 449–452 (1987).
27. M. C. Hunt and D H Kropf, "Color and Appearance" in A. M. Pearson and T. R. Dutson, eds., *Restructured Meat and Poultry Products*, Advances in Meat Research, Vol. 3, 1987, pp 125–159.

28. O. Sörheim, H. Nissen, and T. Nesbakken, "Shelf Life and Color Stability of Beef Loin Steak Packaged in a Modified Atmosphere with Carbon Monoxide," *Proc. 43rd Int. Congr. Meat Sci. Technol.*, 1997.
29. G. L. Nortjé and B. G. Shaw, "The Effect of Aging Treatment on the Microbiology and Storage Characteristics of Beef in Modified Atmosphere Packs Containing 25% CO_2 plus 75% O_2," *Meat Sci.* **25**, 43–58 (1989).
30. C. O. Gill and T. Jones, "The Display of Retail Packs of Ground Beef after Their Storage in Master Packages under Various Atmospheres," *Meat Sci.* **37**, 281–295 (1994).
31. T. J. Clark, "Rebloom and Display Color Stability of Beef and Pork Packaged in an Ultra-Low Oxygen Modified Atmosphere Packaging System," M.S. Thesis, Kansas State University, Manhattan, Kans., 1998.

DONALD H. KROPF
Kansas State University
Manhattan, Kansas

MEAT PROCESSING: TECHNOLOGY AND ENGINEERING

The major processed meats consumed in the United States are made from beef, pork, turkey, or a combination of these. The term all-meat product usually refers to a mixture of beef and pork or beef, pork, and turkey. According to the type of process equipment used, the processed meats can be grouped into one of three categories: (1) muscle products, (2) coarse-ground products, and (3) emulsified products. Products in each group often share common process equipment. The process flowchart is illustrated in Figure 1. The goal of muscle product processing is to keep an original intact tissue appearance in the finished product. The products, such as ham, roast beef, and turkey breast, are produced from whole muscles or sectioned muscles. For coarse-ground products, the meat particles are substantially reduced and are then restructured back to a different physical form. The finished products, such as dry sausage and smoked sausage, still present the recognizable meat particles. Emulsified products are made from meats that are completely chopped and further reduced to a pastelike batter. The finished products are a smooth, homogeneous mass. The meat fibers are beyond recognition. Bologna and hot dogs belong to this category.

In the processed-meat industry, raw meats, either whole muscles or altered meat particles, are restructured to the predetermined product characteristics. The major processing equipment includes injectors and massagers for muscle products, grinders and mixers for coarse-ground products, and choppers and emulsifiers for emulsified products. Injecting and massaging processes facilitate brine distribution and protein extraction. Grinding reduces meat particle size, and mixing assures uniformity of chemical composition as well as protein extraction. The chopping process also reduces particle size and obtains salt-soluble protein. The emulsifying process forms a matrix in which the fat particle is encapsulated with the protein membrane (1).

Raw meats, after these preparation processes, are stuffed into casings or molds to form a defined geometric shape and size. During these processes, rheological properties are critical to equipment performance and final product quality (2–4). Regardless of the product category, the most important missions of process equipment are to extract salt-soluble protein for binding meats together and to hold moisture and fat inside the finished product.

INJECTING

Curing with a high concentration of salt was the sole method used for meat preservation in ancient times. With the availability of refrigeration today, mild curing is employed to improve eating quality more than for preservation. The common ingredients used in a pickle solution, or brine, are salt, nitrite, phosphates, sodium erythorbate, sugar, and starch. Because of the different solubility of each ingredient, the hard-to-dissolve ingredients should be mixed and dissolved first, and then the other ingredients should be added. The following preparation procedure is recommended (5).

1. Dissolve the sodium phosphates in cold water (40°F).
2. Add the sodium erythorbate and dissolve.
3. Add the remaining ingredients (salt, nitrite, sugar, etc).

Agitation in the brine tank is usually needed to prevent precipitation.

Accurate pumping and uniform brine distribution in meat muscle are two major process control points. The amount of pumping determines the profitability and legality of products. Uneven distribution of pickle can cause color, flavor, and texture problems. Small-diameter needles, closely spaced, provide more uniform injecting and less muscle damage. Because small-diameter needles are used for pickle injection, a filter system to prevent particles from plugging up the hole of the needles is vital for accuracy and uniformity. The pickle solution must be kept free of meat particles or other nondissolved ingredients before coming into the needle manifold. The injection needle can be single-hole or multihole. The former can create a large brine pouch inside the meat muscle and cause a leakage problem when the needle pulls out. The multihole needle is preferred for better distribution and less leakage. The amount of pumping is usually controlled by pressure, belt speed, and stitch sequence.

MASSAGING AND TUMBLING

After pickle injection, muscle products require a massaging or tumbling process to facilitate the salt-soluble protein extraction and pickle diffusion (6–8). The desired result is to produce a product with an original intact tissue appearance after heat processing. Before the massaging process, mechanical tenderization or maceration is often used to increase the surface available for protein extraction and to improve binding during cooking and pliability during stuffing. However, this step is not absolutely necessary. Tenderization or maceration has a tendency to disturb the

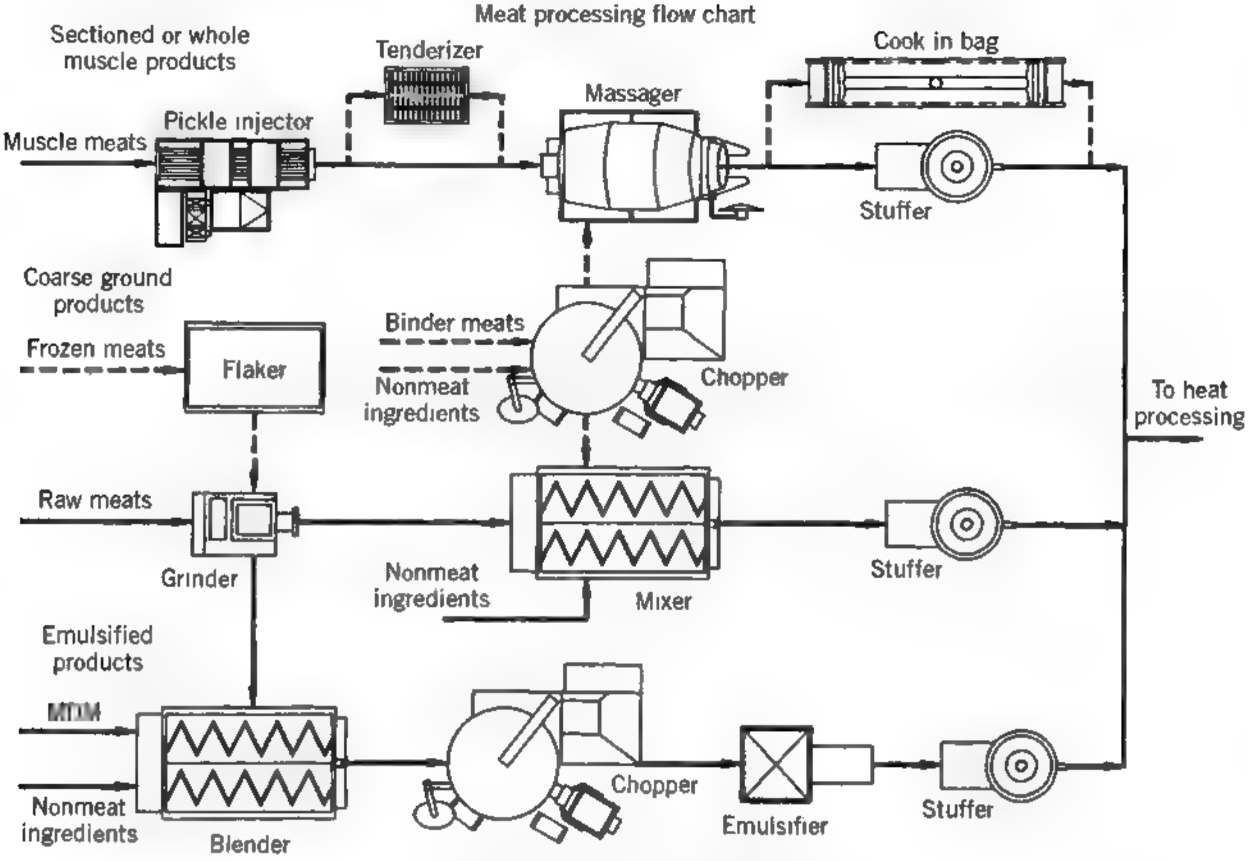

Meat processing flow chart
Sectioned or whole muscle products
Tenderizer
Cook in bag
Massager
Pickle injector
Muscle meats
Stuffer
Coarse ground products
Binder meats
Frozen meats
Flaker
Nonmeat ingredients
Chopper
To heat processing
Raw meats
Grinder
Nonmeat ingredients
Stuffer
Mixer
Emulsified products
MDM
Chopper
Emulsifier
Stuffer
Nonmeat ingredients
Blender

Figure 1. Meat processing flow chart.

original tissue appearance. The excessive pickle carried over from the injecting process is usually massaged into the product in order to attain a desirable brine level.

A massager is comprised of a stationary vat having one or two shafts extending therein with a plurality of paddles. The meats in the vat are kneaded by a slow-rotation mixing of paddles, which forces the meats to rub against one another. A tumbler is a rotating drum with blades built longitudinally on the inner surface. As the drum rotates, the meats inside the drum are lifted by the blades and fall on top of one another. If the blades have an angle with rotation direction, they force the meats to move against themselves.

A tumbler provides not only meat-on-meat friction but also falling-impact action. The most advanced tumbler has been improved to produce a gentle effect, similar to a massage machine. It consists of a rotating drum with two integral helical flights. The flights do not rotate independently from the drum and hence do not cause tearing and shearing effects as does a mixer. In addition, the flights are in a helical arrangement; they do not lift and drop meat like straight blades. There is no falling impact. As the drum rotates, the helical flights force the product into an elliptic motion and produce meat-on-meat massaging action. In industry, the massager and the tumbler are used equally because they provide the same function.

From a scanning electron micrograph study, fiber destruction was evident after a few hours of massaging (8). Further massaging resulted in longitudinal disruption. Intermittent tumbling resulted in more alteration in the cell structure than did continuous tumbling based on the constant tumbling time (9). Because the destruction of the cells on the surface and also deep inside the muscle provides more available salt-soluble protein, the massaging or tumbling process produces a better colored, more tender, and higher yield product. The application of a vacuum during massaging can further improve the product color, cohesion, and sliceability. Applying a vacuum during the massaging also gives a 2–3% higher yield. The optimum massaging temperature is suggested to be 43–46°F (10).

GRINDING

The grinder is the most commonly used piece of equipment in the meat industry for reducing particle size. The size of the meat particle is determined by the diameter of the hole in the plate and the revolutions per minute of the cutting knife. The grinding temperature is critical for obtaining clear particle definition and is found to be at or near the freezing point of meat, 28°F. The sharpness of cutting knives and the grinding plate is also vital to produce a product with a less smeared appearance. Products with high fat content can usually be ground at a colder temperature than can lean products. A double-plate grinding system can often be adapted to the regular grinder with adequate horsepower to reduce the capital and operation costs as well as to maximize use of space.

One quality concern of coarse-ground products is bone chips and gristle in the finished products. An automatic bone elimination system is often built into a grinder to remove bone, gristle, and any other hard-to-grind materials. There are two general types of removal systems: central removal and peripheral removal. The latter system passes the least amount of bone to the finished product and also has less of a plug-up problem for a prolonged period of use (11).

In poultry plants, a low-pressure grinding system has been used increasingly to separate meat from bone and sinew. Raw meat is fed into a precrusher, which consists of dual crushing rollers. The crushed meat is then carried and forced against a perforated drum by a flexible conveyor. Meat is pressed through the perforations into the inside of the drum. The deboned meat inside the drum is then discharged at the drum opening by a static product scraper. The bones, sinew, etc are carried around the outside surface of the drum and are then removed by a scraper knife. The low-pressure grinder functions very effectively for poultry products, but ineffectively for red meats, owing to the toughness of the muscle.

CHOPPING AND EMULSIFYING

A chopper is the other piece of equipment commonly used to reduce meat particle size. The major function of a grinder is solely to reduce particle size with clear particle definition. However, a chopper is employed to reduce particle size, to extract salt-soluble protein, to disperse fat, and to mix meats and spices uniformly. Products that go through a chopping process do not possess a particle definition as clear or as uniform as that for ground products. The chopper is usually used as the first phase of size reduction before the emulsifying process. Chopping is one of the most important processes to control emulsified product quality (12,13). The chopping process determines the amount of available protein and the size of fat particles. If the meats are not adequately chopped, there is a lack of soluble protein to coat the fat particles. If the meats are overchopped, the fat particle size is reduced and the surface area increases enormously. Eventually, the fat surface becomes so large that the protein solution cannot coat all the fat particles. In both cases, fat will render from emulsion during heat processing. To determine the optimum chopping condition, emulsion temperature is generally used as the process control criterion. An emulsion temperature of 55°F after chopping was found to be most stable (14). The variables that can effect emulsion stability in a chopper are chopping time, bowl speed, knife speed, bowl size, knife size, number of knives, and the mass of emulsion inside a chopper (2).

The emulsifier used in the meat industry is similar to a grinder. Because products that require an emulsifying process are usually prechopped or preground to a very fluid mass, an emulsifier normally does not have a feed screw to deliver product to the emulsifying plate. The cutting knives of the emulsifier function as impellers to suck meats from the hopper and to force them through the emulsifying plate simultaneously. The hole in the emulsifying plate ranges from 1 to 3 mm. The meat temperature out of the emulsifier should be kept below 70°F to assure emulsion stability.

In recent developments, one single piece of equipment combining chopping and emulsifying has been developed. The ground product is loaded in a bowl where a vacuum can be created and is chopped by two counter-rotating knives. Cutting time, temperature, and vacuum can be controlled by a microprocessor. A vertical augur turns clockwise gently while an agitator stirring arm lifts the product off the wall of the bowl. When the chopping cycle ends, the augur reverses and begins feeding the chopped meat into the emulsifier. The emulsifier consists of double plates. The product is forced through the plates by the impeller. The other new development is to eliminate chopping by combining chopping and emulsifying operations in one emulsion mill. The finely ground meat in a storage hopper is transferred to a deaerator. The deaerator plays dual functions; it removes air and accumulates meat temporarily. At the bottom of the deaerator, a positive pump forces meat through cutting knives and an emulsifying plate. The particle size and emulsion temperature are controlled by flow rate. Both systems can keep the whole process under vacuum conditions and also can save on unit operation cost.

Some physical and chemical properties of comminuted meats have been studied. The research on protein–protein interaction shows that the reaction among proteins results in molecular aggregation that is reversible between 39 and 86°F (15). A study on structural change during chopping and cooking has indicated that muscle fibers were reduced in size but some remain intact; fat particles were reduced in size with only a portion of them being surrounded by a protein interface, and collagen fibers were somewhat dispersed but otherwise unaltered (16). The application of a vacuum during the chopping process does promote cured color development (17).

MIXING

The mixing process is a necessary procedure for coarse-ground products. The only purpose of grinding is to reduce particle size. Lean and fat meat extruded through the grinder must be thoroughly mixed in order to assure the uniformity of chemical composition and to extract salt-soluble protein for fat-holding and water binding (18). A mixer consists of a hopper and a pair of agitators. Ribbon, paddle, or solid flight agitators are commonly used for mixing meat products. Agitators are designed to tumble and to circulate meat slowly inside the mixer. The agitators usually rotate in opposite directions to perform overlapping action. The optimum mixing temperature is suggested to be below 40°F to prevent smear problems.

Oxygen, which exists in meat products, accelerates the spoilage of finished product. A vacuum is often applied during the mixing process to evacuate air. To keep product temperature cold during the mixing operation, ice, chilled water, or liquid gas are added into the mixer. Meat is an excellent carbon dioxide (CO_2) absorbant. If dry ice is used for chilling, a vacuum should be applied to remove dissolved CO_2 at the end of the mixing cycle. Otherwise, the dissolved CO_2 will be released in heat processing or vacuum packaging and cause quality and shelf life problems.

STUFFING

Meat product is usually stuffed into natural casings, cellulose casings, collagen casings, cook-in-bags, or molds to form the final geometric shape. Several types of pumps are used in stuffers, such as piston, gear, vane, or worm gear types. For small link products, a continuous stuffing, linking, and looping machine is most commonly employed. The machine can produce 3,000–4,000 lb/h. For a large-diameter product, such as bologna, the meat dough is stuffed into the casing with a casing sizer to ensure a uniform diameter from one end to the other A metal mold is often used in producing large-diameter products to replace expensive casing.

Sausage manufacturers spend about 5 percent of the cost on casing to form product and then peel it off and throw it away. To save the cost, a highly automatic coextrusion system has been developed. The system consists of two meat ingredients; one is sausage meat and the other a collagen paste that is derived from hide. Two meat ingredients are fed through a co-extrusion nozzle simultaneously. The nozzle consists of a central tube surrounded by two cone-shaped elements between which there is a narrow gap ending in an orifice circling the tube. The cones spin in opposite directions. The collagen is forced through the gap and spun onto an endless rope of sausage meat. This rope is then passed through a brine bath to coagulate the collagen and then through a crimper that separates and forms the individual links. The diameter of sausage is determined by the size of the nozzle. The co-extruded products are then transferred onto a conveyor to the drying tower. The co-extruded product has bite characteristics similar to those of the natural-casing product.

HEAT PROCESSING

Meat products, after being restructured from raw materials, are stuffed into casings, bags, or molds and are subjected to heat processing—except for the fresh-product category. The basic functions of smoking and heating processes are to inhibit bacterial growth, to provide desirable color, and to impart specific flavor and texture. Heat processing in the meat industry covers smoking, drying, cooking, cooling, and freezing (19). The smokehouse is the major heat-processing equipment. When meat products are subjected to heat processing in a smokehouse, not only does simultaneous heat and mass transfer occur, but also microbial and biochemical reactions are induced. The theoretical approach to process and engineering design is limited According to material handling criteria, there are two types of smokehouses available. One is a batch-type smokehouse and the other is a continuous smokehouse. Batch-type smokehouses provide a cold-water shower for precooling. The final chilling is conducted at the separate blast-air chilling or brine chilling room For a continuous smokehouse, the product is loaded on a conveyor and automatically carried through smoking, drying, cooking, and chilling processes. Products, after leaving the continuous oven, are ready for peeling, slicing, and packaging. Detailed heat-processing flowcharts for both batch and continuous smokehouses are shown in Figure 2.

Smoking

The smoking of meat is an ancient practice to preserve meat products in addition to developing color and flavor. The role of smoke as a meat preservative has declined owing to the availability of refrigeration. Today, the smoking process is used to enhance flavor and surface color. Several hundred chemical compound have been isolated from wood smoke. The most important compounds are acids, phenols, and carbonyls. Phenols and carbonyls are major contributors of smoke flavor and color, respectively. The acidic substances accelerate the curing reaction and contribute to the pink color of cured meats. For processing convenience, natural smoke is, for some uses, condensed to liquid form. It can be added directly to a product or applied on a product surface by dipping, spraying, atomizing, or regenerating.

Natural Smoke Generation

Natural smoke is produced from thermal decomposition of wood and is known as pyrolysis. The major constituents of wood are cellulose, hemicellulose, and liqnin. During thermal decomposition, a temperature gradient exists between the outer and inner core of wood chip or sawdust. The outer surface is being oxidized, and the inner surface is being dehydrated before it can be oxidized. During the dehydration process, the outer surface is at about water boiling temperature. Carbon monoxide, carbon dioxide, and some volatile short-change organic acids are released during the dehydration process. When the internal moisture is dried out, the temperature rapidly rises to 300–400°C (570–750°F). As long as the internal temperature reaches this range, the decomposition occurs and smoke is given off. The hemicellulose fraction is the first to undergo degradation. Cellulose and liqnin degradation follow accordingly.

Under normal smoking conditions, the smoking temperature ranges from 100 to 400°C (212–750°F) or higher. This results in the generation of more than 400 smoke compounds. Smoke composition can vary substantially with smoke generation temperature as well as with different varieties of wood. The effective smoke compounds—phenols, acids, and carbonyls—change with generation temperature, as shown in Table 1 (20); wood used here is maple sawdust.

Thermal decomposition of wood is induced by high temperature. Heat sources can be hot plate, self-burning, or friction. Sawdust is normally used in a smoke generator with hot plates. Sawdust is loaded in a hopper, and the feeding rate is controlled by the rotation speed of the feed plate. Under the feed plate there are three hot plates with electric heaters under each plate. On top of each hot plate there is a wiper to sweep sawdust across the hot-plate sur-

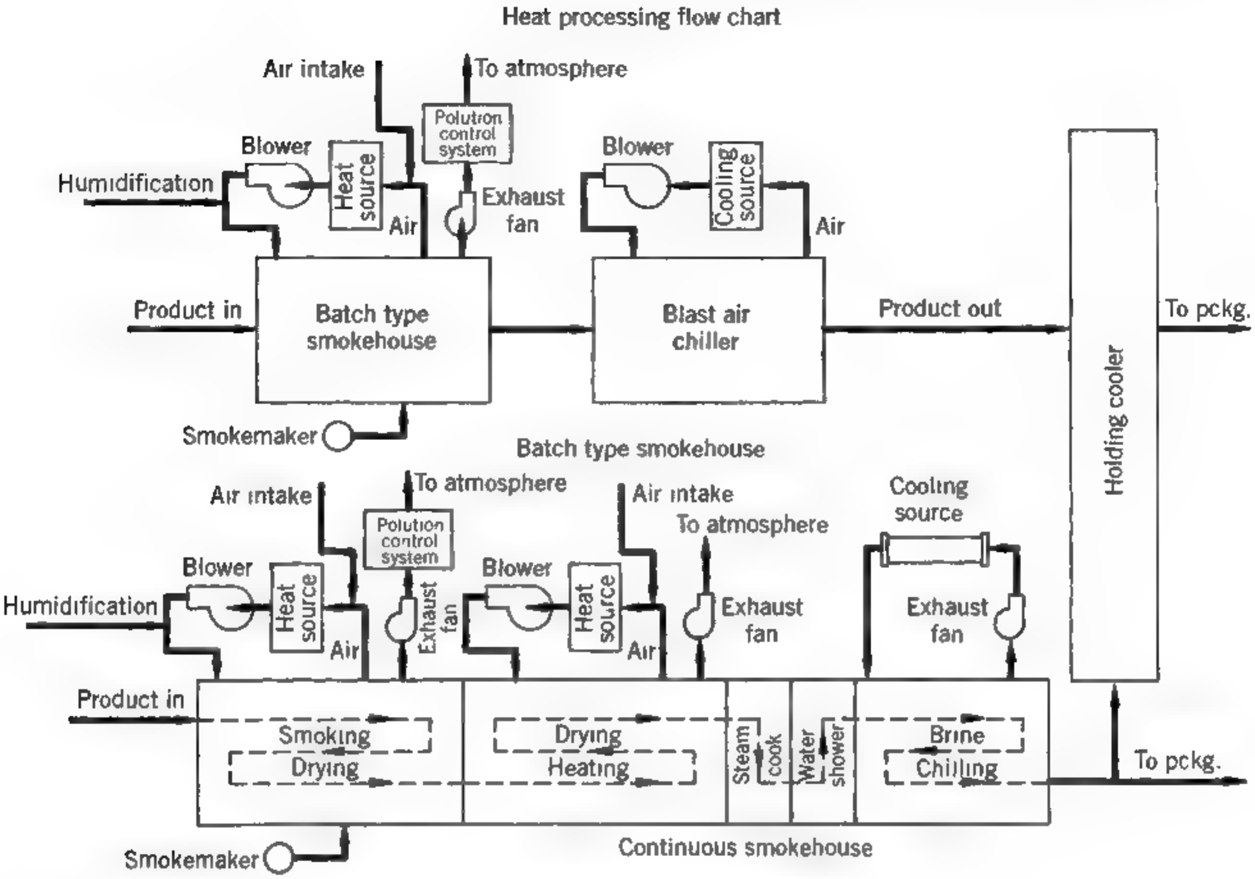

Heat processing flow chart
Air intake
To atmosphere
Polution control system
Blower
Heat source
Humidification
Air
Exhaust fan
Blower
Cooling source
Air
Product in
Batch type smokehouse
Blast air chiller
Product out
Holding cooler
To pckg.
Smokemaker
Batch type smokehouse
Air intake
To atmosphere
Polution control system
Air intake
To atmosphere
Cooling source
Blower
Heat source
Exhaust fan
Blower
Heat source
Exhaust fan
Exhaust fan
Humidification
Air
Air
Product in
Smoking
Drying
Drying
Heating
Steam cook
Water shower
Brine
Chilling
To pckg.
Smokemaker
Continuous smokehouse

Figure 2. Heat processing flow chart.

Table 1. Smoke Composition Change Subjected to Smoke Generator Temperature

Generator Temperature (°F)	Acids (wt%)	Phenols (wt%)	Carbonyls (wt%)
575	41	2.3	57
600	40	1.7	59
650	32	1.7	66
725	27	1.6	72
780	24	1.5	84

face. The final ash is discharged into a cold-water stream and carried to an ash collector.

In the smoldering smoke generator, smoking materials—sawdust or wood chips—are ignited by an electric heating element. The ignition time can be preset. When the set time is expired, the ignition automatically switches off. Air is injected at the bottom by a blower and is heated by the burning sawdusts or wood chips. The heated air is moving upward, and sawdust is supplied from the top, functioning as a countercurrent flow. Because of the heat of the burning sawdust beneath, smoke is generated as fresh sawdust travels from top to bottom. Smoke produced with the smoldering principle often has high air velocity, leading to excessively high smoldering temperature. The smoke-generation temperature can be lowered by reducing the air supply and slightly moistening the sawdust or wood chips to a moisture content of 30%.

The friction smoke generator produces smoke by pressing a piece of wood log tightly against a spinning disc. Heat is produced by fierce friction. This results in pyrolysis of the wood. Friction smoke generators produce less amounts of smoke than does the sawdust burning type of smoke generator. Another method used for smoke generation is the superheated steam method. The low-pressure steam is mixed with air and conducted to the superheater. The temperature of the steam/air mixture can be adjusted within the prescribed range of 243–400°C (470–750°F). The amount of air can be varied as required and can thus have a varying effect on smoke production. A screw conveyor brings sawdust and superheated steam/air mixture into a smoke-generating chamber to induce pyrolysis. Among these smoke-generation methods, the hot-plate smoke generator and the smoldering smoke generators are most popular in the United States.

Smoke Deposition

Smoke deposition on a product involves several physical and chemical processes linked closely with one another. An important chemical process is the carbonyl-amino reaction, which produces a desired golden brown color. As for the physical process, the dominant mechanism of the smoking process has been identified as vapor absorption. During the normal smoking process, vapor smoke and the liquified smoke particulates coexist in an equilibrium condition. The smoke particulates function as a reservoir of smoke constituents. When the equilibrium between smoke vapor and particulates is changed by dilution of air because of absorption from the product or as a result of increased smoke temperature, the reservoir releases a part of its contents to maintain the new equilibrium condition. Because the smoking process is an absorption, the major physical parameters effecting the rate of smoking deposition are smoke density, air velocity, relative humidity, and the surface condition of products. It is clear that a wet surface absorbs smoke faster than a dry surface and that smoke absorption should follow the first order of kinetics. The higher the concentration gradient between the ambient and surface, the faster the absorption rate. The rate of smoke deposition is also faster at high air velocities, although it is difficult to maintain high smoke density and high air velocity simultaneously. Smoke density and air velocity should be optimized according to smoke-house pressure as well as the exhaust system.

The smoking process based on air temperature can be classified as cold smoking, warm smoking, and hot smoking. The temperature ranges are below 27°C (80°F), between 22 and 54°C (80–130°F), and above 54°C (130°F), respectively. The relative humidity during cold smoking is not as critical as during hot smoking because of the low drying rate. In the hot-smoking process, the relative humidity should be precisely controlled to keep the product surface damp. The optimum relative humidity (RH) in a smokehouse for hot smoking is 60% RH and 71.1°C (160°F) (21).

Smoking of meat products results in altering the protein composition, such as lowering myofibrillar and sarcoplasmic protein nitrogens while increasing stromal protein nitrogen. These protein changes result in a cross-linking of surface protein or tough skin (22).

Liquid Smoke

To find a way of better controlling smoke reaction, decrease the chance of carcinogenesis, reduce smoke effluent, and make the application easier, liquid smoke has emerged. Liquid smoke is generally produced from natural smoke by condensation or water scrubbing. The most prevalent method used in the United States is the water-scrubbing method. This method includes the smoldering of sawdust, controlling oxidation condition, and absorption of smoke in water. In this process, the smoke solution is recycled until a given concentration is developed. The other method is the pyrolysis of hardwood sawdust, where the generated smoke is condensed to a liquid-form solution through a series of condensers. Liquid smoke can be applied directly into raw products, thereby producing smoke flavor and color simultaneously. Liquid smoke is often applied to a product surface. The application technique can be classified as dipping, spraying, atomization, and regeneration. Dipping or spraying is implemented immediately after stuffing or before thermal processing. Atomization is using high liquid pressure to form a cloud of liquid smoke to which products are then exposed. Atomization can be applied either before or during heat processing. Regeneration of liquid smoke is converting the liquid smoke from a liquid phase to a gas phase by heat. The regenerated smoke can have the same application as for natural smoke.

Liquid smoke has been available for a while. However, it cannot replace natural smoking because of an unsatisfactory flavor profile. In order to impact sufficient smoke color on the product surface, liquid-smoked products often develop bitter smoke flavor. Recently, liquid smoke flavor has been improved by adjusting the phenol and carbonyl ratio.

Pollution Control

The natural wood smoke that can cause irritation and odors is discharged to the atmosphere with the exhausted air that is necessary for humidity control inside the smokehouse. To eliminate or reduce air pollution, afterburners, closed-system smokehouses, water-scrubbing systems, or electrostatic precipitators have been employed. The afterburner uses heat, above 1,000°F, to burn the contaminants. It is a high-efficiency air-pollution control unit. High energy cost has prohibited its application. The basic principle of the closed-system smokehouse is to circulate humid air through a dehumidifier for humidity control without discharging the contaminated air into the atmosphere. However, a substantial amount of smoke must be carried into the smokehouse. Some air must be released in order to attain the material and pressure balance. Therefore, a complete closed-system house cannot be achieved for a natural smoke system (23). For a water-scrubbing system, air with smoke contaminants passes through a venturi. In the venturi, air with contaminants come in close contact with the scrubbing water. The collection of particulates on scrubbing is accomplished by inertia impaction, interception, and diffusion. The water droplets are collected from the stream by a baffle and a mist eliminator before the scrubbed air is discharged to the atmosphere. The water scrubber can achieve up to 80% efficiency. The electrostatic precipitator draws the contaminated air through prefilters that collect the larger particles and then the air passes through ionizers that electrically charge all the particles in the airstream. Ionized particles enter the collecting cells, where ground plates remove the particles. The electrostatic precipitator can effectively remove the particulate material in the air-stream but cannot eliminate smoke odors in the gas phase. To improve efficiency, a chemical absorption tower is installed after the electrostatic filter. The alkaline solution is circulated inside the tower to dissolve smoke odors as well as to keep the tower clean. The new system can provide up to 97% efficiency at a flow rate of 1,500 ft^3/min.

Drying and Heating

The smokehouse is the major heat-processing equipment for producing processed meats. The modern smokehouse is equipped with instruments for programming air temperature and humidity during the process cycle. The air can be heated with direct gas-firing, steam coil, or electric heating elements. The humidity of air in a smokehouse is controlled by the amount of air intake and exhaust and the injection of steam when necessary. The conditions of returned air (dry-bulb and wet-bulb temperatures) are usually used as the process control references. The conditioned air is then introduced into the smokehouse by a blower. The air circulation rate is normally greater than 10 air changes a minute. One air change is equivalent to one smokehouse volume in cubic feet. The airflow coming into the smokehouse is alternated from one side to the other to provide more uniform heat processing. Heat-processing schedules are specifically designed according to product quality requirement as well as microbial kill. Because there are many different products and quality specifications, heat-processing schedules also differ substantially. In designing a smokehouse, the production rate, product drying rate, and product heating rate with a specific set of heat-processing schedules should be predetermined. By setting up material and energy balance, the equipment capacity for air-handling, heating, and cooling can be determined.

The quality of processed meats is significantly affected by the heat-processing schedule (24–26). High humidity in a smokehouse can cause surface grease and poor color problems. These problems are common for batch-type smokehouses. The batch-type smokehouse usually starts at low temperatures because the cold-water shower of the previous lot. A wet-bulb temperature setting higher than the house temperature at the beginning of heat processing often calls an excessive amount of steam into the smokehouse. It is common practice to set the wet bulb at zero degrees in order to prevent the excessive humidity. Because the wet-bulb temperature is set at zero position, which is way below smokehouse temperature, the intake and exhaust dampers are controlled at a wide-open position, disregarding the humidity condition in the smokehouse. There is substantial variation in year-round weather conditions. If the exhaust capacity is determined under hot, humid summer conditions and a good quality product is produced, it is not necessary to run the exhaust at maximum capacity in cold, dry winter weather. Therefore, the exhaust dampers should be controlled according to the dryness of intake air (23). Microprocessors have increasingly been used to control the smokehouse system. The exhaust and intake air, steam injection, and exhaust fan can be controlled and operated independently without interlocking problem as are seen in current control systems. Microprocessor control provides much more flexibility for heat-processing optimization and energy conservation.

Cooling

From microbiological research, it has been illustrated that product shelf life strongly depends on product temperature. By lowering product temperature, the onset of microbial growth can be substantially delayed. To reduce product temperature after smoking and cooking, air cooling and liquid chilling are two of the more commonly used methods in the meat industry. Salt brine and propylene glycol are two common cooling mediums. However, glycol solution can only be used for products with impermeable casing. There are several disadvantages with air cooling such as slow cooling rate, nonuniform cooling, and moisture loss. Air cooling is normally used to chill products in impermeable casing or metal molds. Because a continuous-processing system requires more rapid cooling, liquid cooling is used in almost every system. In addition, to compensate for the disadvantages of air cooling, liquid cooling with high chemical concentration can also retard the microbial growth. In general practice, the economical way to apply liquid cooling can be achieved by two steps:

1. Precool products with regular water.
2. Further cool the product with a refrigerated cooling medium such as brine.

Qualities that can be affected by brine chilling are shrink, flavor, and color. When moisture is extracted by brine, product weight loss and darker color occur. As long as brine concentration is higher than the salt concentrations in the product, salt migrates into the product and develops a salty flavor. Therefore, salt concentrations is the most critical condition in the brine-chilling process. The optimum salt concentration should be determined for each product. The best operation conditions should be at the equilibrium conditions of salt migration.

For the last decade, poultry products have taken a significant market share of processed meats. According to USDA regulation, frozen whole turkey must be chilled down to 0°F core temperature before it can be shipped. Freezing whole-bird turkey is a time-consuming process. The poultry industry is looking for a rapid-chilling method to increase productivity. Cryogenic-freezing can provide a fast freezing rate. However, it is a high-cost operation. Blast-air chilling is an economical process but takes a long time to achieve 0°F core temperature. Liquid freezing is an effective and also economical method. The common chemicals used to depress the freezing point below 0°F are calcium chloride, ethanol, and propylene glycol. However, calcium chloride contributes to bitterness at as low as 1% of concentration and ethanol causes medicinal aftertaste (27). Propylene glycol usually does not develop objectionable flavor. The high concentration of glycol at low temperature causes excessive viscosity of fluid and develops a pumping problem. Hence, the glycol solution is limited in the immersion system. Because of flavor and viscosity problems, liquid freezing has not been widely accepted. The most common practice in the poultry industry is blast-air freezing or salt brine prechilling followed by blast-air freezing. It usually takes 24–48 hours to reach 0°F core temperature.

PACKAGING

Products after cooking and cooling are usually stored in a holding cooler before packaging. Sometimes holding or tempering is a necessary process for sliced or cellulose-casing products. Rapid cooling can cause slicing problems due to fat crystallization. Blast-air chilling enhances the cohesion of meat and casing. It is difficult to separate casing right after blast-air chilling. The tempering process can improve product sliceability as well as peelability.

The packaging area is normally under refrigeration and kept around 40°F. General packaging procedures are illustrated in Fig. 3.

Peeling and Slicing

Products must have the casing peeled off or be ejected from a mold; except for natural casing, edible collagen casing, or cook-in-bag products. The mold products are ejected with compressed air or mechanical plungers. For casing products, there are various types of machinery to remove cellulose casing. The most commonly used peeling machine for link products is that the casing is first moistened with steam and is then slit longitudinally by a sharp razor blade. When the slit casing is moved forward, an air jet aims at the knife cut location to blow casing off the product. Afterward, the casing is stripped away with a vacuum suction drum. The peeled products are randomly collected in a hopper. A collating machine is needed to rearrange the link products in an orderly form for automatically loading into the packaging packet. The sliced products can be arranged in shingled or stacked configuration. A slicer with a rotating blade is the most commonly used slicing machine. Emulsion products require slower rotation speeds for clean and smooth appearance. Muscle products need more spinning action to protect product integrity and produce uniform slices. The sliced products are often manually loaded into a packaging cavity because of stacking or shingling characteristics. The slicing temperature should be kept below 32°F.

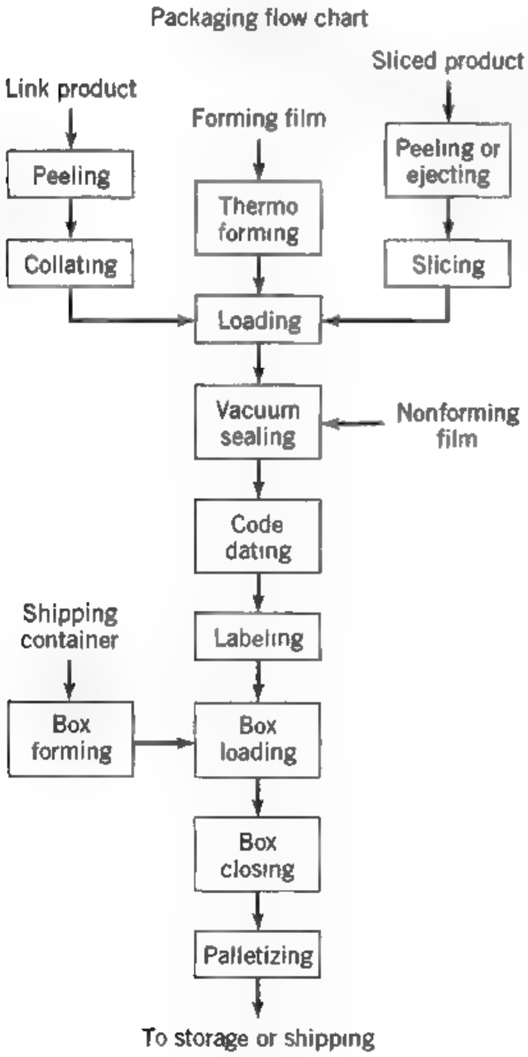


Figure 3. Packaging flow chart.

Packaging Machine

The most widely used packaging machine is a form-fill-seal machine. This machine requires two different types of film; a forming film and a nonforming film. The forming film is preheated and moved forward to the forming die where the desired shape and size can be formed by vacuum suction, forming plug, or both simultaneously. The formed cavity is indexed to the loading station. Products that come from a collator or slicer are automatically or manually placed into the formed cavity. After product loading, the nonforming film is brought in place over the formed film containing the product as the package moves to the final seal station. At the sealing station, air is evacuated from the cavity at a vacuum level higher than 27 in. and then both forming film and nonforming film are hermetically sealed together by heat-seal bars that melt the two sealant layers and weld them together under pressure. For some applications, such as a control atmosphere package, an inert gas or gas mixture is backflushed after the vacuumization before final sealing. The sealed packages are then trimmed and boxed. Oxygen causes processed meat to spoil and must be evacuated during mixing, chopping, emulsifying, and stuffing processes. Packaging film and a packaging machine alone cannot eliminate the residual oxygen inside the package. The performance of the packaging machine is extremely important. It can become out of adjustment easily, owing to its extreme high operating speed. A strict maintenance program is needed.

BIBLIOGRAPHY

1. D. M. Theno and G. R. Schmidt, "Microstructural Comparisons of Three Commercial Frankfurters," *Journal of Food Science* **43**, 845–848 (1978).
2. A. V. Gorbatov and V. M. Gorbatov, "Advances in Sausage Meat Rheology," *Journal of Texture Studies* **4**, 406–437 (1974).
3. P. Y. Wang, "Rheology of Sausage Dough," *American Society of Agricultural Engineering Paper* 79-6540 (1979)
4. R. Toledo, J. Cabot, and D. Brown, "Relationship Between Composition, Stability and Rheological Properties of Raw Comminuted Meat Batters," *Journal of Food Science* **42**, 725–727 (1977).
5. J. Cordray and D. Huffman, "The Art of Turkey Luncheon Meat," *Meat & Poultry* December, 16 (1987).
6. R. J. Krause et al., "Influence of Tumbling, Tumbling Time, Trim and Sodium Tripolyphosphate on Quality and Yield of Cured Hams," *Journal of Food Science* **43**, 853–855 (1978).

7. R. D. Cassidy et al., "Effect of Tumbling Method, Phosphate Level and Final Cook Temperature on Histological Characteristics of Tumbled Porcine Muscle Tissue," *Journal of Food Science* **43**, 1514–1518 (1978).
8. D. M. Theno, D. G. Siegel, and G. R. Schmidt, "Meat Massaging: Effect of Salt and Phosphate on Ultrastructure of Cured Porcine Muscle," *Journal of Food Science* **43**, 488–492 (1978).
9. R. R. Motycka and P. J. Bechtel, "Influence of Pre-Rigor Processing, Mechanical Tenderization, Tumbling Method and Processing Time on the Quality and Yield of Ham," *Journal of Food Science* **48**, 1532–1536 (1983)
10 D. Schied, "Cooked Ham Manufacturing," *Fleischwirtsch* **66**, 1022–1026 (1986)
11 P. H. Huebner, J. G. Sebranek, and D. G. Olson, "Effects of Meat Temperature, Particle Size, and Grinding Systems on Removal of Bone Chips from Ground Meat," *Journal of Food Science* **54**, 527–531 (1989).
12 K. W. Jones and R. W Mandigo, "Effects of Chopping Temperature on the Microstructure of Meat Emulsions," *Journal of Food Science* **47**, 1930–1935 (1982).
13 D. Brown and R. Toledo, "Relationship Between Chopping Temperatures and Fat and Water Binding in Comminuted Meat Batters," *Journal of Food Science* **40**, 1061–1064 (1975).
14. K. M. Ladwig, C. L. Knipe, and J. G. Sebranek, "Effects of Collagen and Alkaline Phosphate on Time of Chopping, Emulsion Stability and Protein Solubility of Fine-Cut Meat Systems," *Journal of Food Science* **54**, 541–544 (1989).
15. J. C. Deng, R. T. Toledo, and D. A. Lillard, "Protein-Protein Interaction and Fat and Water Binding in Comminuted Flesh Products," *Journal of Food Science* **46**, 1117–1121 (1981).
16. R. L. Swasdee, "Ultrastructural Changes During Chopping and Cooking of a Frankfurter Batter," *Journal of Food Science* **48**, 1039–1041 (1982).
17. K. Tantikarnjathep et al., "Use of Vacuum During Formulation of Meat Emulsion," *Journal of Food Science* **48**, 1039–1041 (1983).
18. L. W. Solomon and G. R. Schmidt, "Effect of Vacuum and Mixing Time on the Extractability and Functionality of Pre- and Post-Rigor Beef," *Journal of Food Science* **45**, 283–287 (1980).
19. P. Y. Wang, "Smoking and Heating Meat," in C. W. Hall, A. W. Farrall, and A. L. Rippen, eds., *Encyclopedia of Food Engineering*, 2nd ed., AVI Publishing Co., Westport, Conn, 1987.
20. S. Simon, A. A. Rydinski, and F. W. Tauber, "Water-Filled Cellulose Casing as Model Absorbents for Wood Smoke," *Food Technology* **20**, 1494–1498 (1966).
21. W. S. Chan, R. T. Toledo, and J. Deng, "Effect of Smokehouse Temperature, Humidity and Air Flow on Smoke Penetration into Fish Muscle," *Journal of Food Science* **40**, 204–243 (1975).
22. J. A. Maga, "Smoke in Food Processing," CRC Press, Boca Raton, Fla., 1988.
23. P Y Wang, "Reduction of Energy Consumption and Air Pollution from Smokehouse," *American Society of Agricultural Engineering Paper* 76-6518, 1976.
24. G S. Mittal, C. Y. Wang, and W. R. Usborne, "Smokehouse Process Conditions for Meat Emulsion Cooking," *Journal of Food Science* **52**, 1140–1144 (1987).
25. C W Monagle, R. T. Toledo, and R. L. Saffle, "Effect of Smokehouse Temperature, Humidity and Air Velocity on Rate of Heating and Quality of Frankfurters," *Journal of Food Science* **39**, 602–604 (1974).
26. I. Stech, W. R Osborne, and G. S. Mittal, "Influence of Smokehouse Air Flow Patterns, Air Changes and Cook Cycle on Texture and Shrinkage of Wieners," *Journal of Food Science* **53**, 421–424 (1988).
27. G. H. Cipolletti, Robertson, and D. F. Farkas, "Freezing of Vegetables by Direct Contact with Aqueous Solution of Ethanol and Sodium Chloride," *Journal of Food Science* **42**, 911–916 (1977).

GENERAL REFERENCES

S. W. Ahmed, K. Sushil, and P. K Mandal, "Tumbling and Massaging—An Emerging Technology for the Meat Industry," *Indian Food Industry* **14**, 37–41 (1995)

F M. Aramouni, E. A. E. Boyle, and L. R. Vogt, "Introduction to the Hazard Analysis Critical Control Point (HACCP) Concept in a Small Meat-processing Plant," *Dairy Food and Environmental Sanitation* **16**, 431–439 (1996).

T. Backers and B. Noll, "Dietary Fiber for Meat Processing," *Food Marketing & Technology* **11**, 4–6, 8 (1997).

S. Barbut, "Effects of Three Chopping Methods on Bologna Characteristics," *Canadian Institute of Food Science and Technology Journal* **23**, 149–153 (1990).

Z. Bem, B Zlender, and H. Hechelman, "Desirable and Undesirable Effects of Smoking Meat Products," *Fleischerei* **46**, III–VII (1995).

H. J. Buckenhueskes, "Functions of Sugars in Meat Processing," *Fleischwirtschaft* **78**, 1271–1275 (1998).

B. Carpentier, "Sanitary Quality of Meat Chopping Board Surfaces: A Bibliographical Study," *Food Microbiology* **14**, 31–37 (1997).

S. F. Chang, T. C. Huang, and A. M. Peason, "Control of the Dehydration Process in Production of Intermediate-Moisture Meat Products: A Review," *Advances in Food and Nutrition Research* **39**, 71–161 (1996).

K. J. Debrowski, S. Gwiazda, and A. Rutkowski, "Non-meat Proteins as Functional Ingredients in Meat Processing," *International Food Ingredients* No. 6, 26–30 (1991).

R. M. Estrada, E. A. E. Boyle, and J. L. Marsden, "Liquid Smoke Effects on *Escherichia coli* O157 H7 and Its Antioxidant Properties in Beef Products," *J Food Sci.* **63**, 150–153 (1998).

M. Fode et al., "First Results on Experimentation of Solar Dryers in Tropical Ambient," *Rivista di Ingegneria Agaria* **28**, 65–72 (1997).

U. Gerhardt, "Starch and Plant Proteins in Meat Processing," *Fleischerei* **48**, III–VIII (1997).

H. G. Glenk, "Technical Gases in Meat Processing," *Fleischerei* **45**, 20, 22, 25–26 (1994).

M. D. Guillen and M. L. Ibargoitia, "New Components With Potential Antioxidant and Organoleptic Properties, Detected for the First Time in Liquid Smoke Flavoring Preparations," *J. Agric. Food Chem.* **46**, 1276–1284 (1998).

M. D. Guillen et al., "Effects of Smoking on Meat Products," *Alimentacion Equipos y Tecnologia* **17**, 104–114 (1998).

B. Hermey and H. Patzelt, "Using Liquid Smoke," *Fleischwirtschaft* **75**, 445–447 (1995).

P. Iascone, "Packaging of Meat Products," *Italia Imballaggio* **2**, 18–25 (1995).

A. T. Kamdem Kenmegne and J. Hardy, "Influence of Various Conditions on Meat Grinding Characteristics," *J. Food Eng* **25**, 179–196 (1995).

E. Karmas, *Sausage Products Technology*, Noyes Data Group, Park Ridge, N.J., 1977.

C. Kuraishi, J. Sakamoto, and T. Soeda, "Application of Transglutaminase for Meat Processing," *Fleischwirtschaft* **78**, 657–658, 660, 702 (1998).

I. Lisse and A. L. Wack Raoult, "Drying of Meat Materials (Lean and Fat) by Deep-fat Frying in Animal Fat," *Sciences des Aliments* **18**, 423–435 (1998).

J. A. Maga, *Smoke in Food Processing*, CRC Press, Boca Raton, Fla., 1988.

D Marque et al., "Safety Evaluation of an Ionized Multilayer Plastic Film Used for Vacuum Cooking and Meat Preservation," *Food Additives and Contaminants* **15**, 831–841 (1998).

J. D Meyer, J. B. Luchansky, and J. G. Cerveny, "Inhibition of a Psychotrophic *Clostridium* Species by Sodium Diacetate and Sodium Lactate in a Cook-in-the-Bag, Refrigerated Turkey Breast Product," *J. Food Prot.* **58** (Suppl.), 9 (1995).

"Mincing, Chopping and Cutting: The Alpha and Omega of Meat Processing," *Fleischerei* **43**, 630–632 (1992).

S. A. Muller, "Packaging and Meat Quality," *Canadian Institute of Food Science and Technology Journal* **23**, 22–25 (1990).

A. M. Pearson and F. W. Tauber, *Processed Meats*, 2nd ed., AVI, Westport, Conn., 1987.

J. P. G. Piette et al., "Influence of Processing on Adherence of a Highly Extended Ham to its Cooking Bag," *Meat Science* **46**, 101–113 (1998)

"Processing and Packaging," *Food Review* **23**, 25, 27, 29, 31 (1996).

A. Rutkowski, *Advances in Smoking of Foods*, Pergamon Press, New York, 1976.

F. J. M. Smulders and R. L. J. M. van Laack, "Meat Quality. Ante Mortem Handling of Animals and Fresh Meat Processing. An Update," *Fleischwirtschaft* **72**, 1385–1389 (1992).

A. van der Leest, "Trends in the Modern Meat Industry," *Food Marketing & Technology* **8**, 64–66 (1994).

P. Y. Wang, "Meat Processing," in C. W. Hall, A. W. Farrall, and A. L. Rippen, eds., *Encyclopedia of Food Processing*, 2nd ed., AVI Publishing Co., Westport, Conn., 1987.

H. Weinberg, "Computer Controlled Manufacture of Meat and Sausage Products. Weight as a Factor in Manufacture of High-Quality Sausages," *Fleischerei* **44**, 200–203 (1993).

M. Weisenfels, "Low-Pollution Smoking in Batch Processes," *Fleischwirtschaft* **69**, 1111–1113, 1131 (1989).

M. Wilms, "Smoking of Meat Products. Development of Modern Smoking Processes From Economic and Ecological Viewpoints," *Fleischwirtschaft* **77**, 704–708 (1997).

R. Wittkowski, W Baltes, and W. G. Jenning, "Analyses of Liquid Smoke and Smoked Meat Volatiles by Headspace Gas Chromatography," *Food Chem.* **37**, 135–144 (1990).

Y. Zhao and J. G. Sebraneck, "Technology for Meat Grinding Systems to Improve Removal of Hard Particles From Ground Meat," *Meat Science* **45**, 389–403 (1997)

PIE YI WANG
Armour Swift-Eckrich
Downers Grove, Illinois

See also MEAT AND ELECTRICAL STIMULATION; MEAT SLAUGHTERING AND PROCESSING EQUIPMENT.

MEAT PRODUCTS

Meat has been processed into various products for thousands of years. The purposes of processing have included preservation for future use, increased palatability and variety, enhanced convenience and complete utilization of the carcass and organs. Generally, people in warmer parts of the world processed meat to preserve it by salting and drying, and populations in the colder parts of the world processed meat to increase the quantity and variety to eat by adding such nonmeat ingredients as oatmeal or potatoes. Obviously, all the historic reasons for processing meat still remain today, but flavor, convenience, safety, and variety are key to the continued growth of processed meat sales in modern societies.

In 1997, *Meat Marketing & Technology* trade magazine published a report of a study sponsored by the National Pork Producers Council and the National Cattlemens Beef Association entitled *Consumer Purchase Behavior of Processed Meats at Retail* (1). The material collected in 1995 reported that 4.25 billion pounds of processed meats were purchased for $9.83 billion at retail stores. The major types of processed meats purchased are shown in Table 1. Lunch meat refers to a wide variety of products that are usually sliced to be utilized in making sandwiches. The penetration of lunch-meat-type products is shown in Table 2. A growing category of processed meats is dinner sausages, which provide a variety of flavors and convenience of preparation. The leading dinner sausage types are shown in Table 3.

Table 1. Processed Meats Purchase at Retail

Product	$ Billion	Billion pounds
Lunch meat	5 32	1.73
Hot dogs	1.14	0.75
Bacon	1.23	0.73
Dinner sausage	1.09	0.56
Breakfast sausage	0.55	0 29

Table 2. Penetration of Lunch Meat Types

Product	Percent of households purchasing per month
Ham	30
Turkey	21
Bologna	18
Sliced beef	11
Dry salami	5
Loaves	5
Chicken breast	3
Pepperoni	3
All other salamis	3
Liver sausage	3
Variety packs	3
Cotto/cooked salami	2

Table 3. Top Dinner Sausage Types

Product	Percent of total dinner sausage pounds purchased
Smoked	28
Polish	23
Italian	19
Bratwurst	10
Hot	9
Mild	5

I. Lisse and A. L. Wack Raoult, "Drying of Meat Materials (Lean and Fat) by Deep-fat Frying in Animal Fat," *Sciences des Aliments* **18**, 423–435 (1998).

J. A. Maga, *Smoke in Food Processing*, CRC Press, Boca Raton, Fla., 1988.

D Marque et al., "Safety Evaluation of an Ionized Multilayer Plastic Film Used for Vacuum Cooking and Meat Preservation," *Food Additives and Contaminants* **15**, 831–841 (1998).

J. D Meyer, J. B. Luchansky, and J. G. Cerveny, "Inhibition of a Psychotrophic *Clostridium* Species by Sodium Diacetate and Sodium Lactate in a Cook-in-the-Bag, Refrigerated Turkey Breast Product," *J. Food Prot.* **58** (Suppl.), 9 (1995).

"Mincing, Chopping and Cutting: The Alpha and Omega of Meat Processing," *Fleischerei* **43**, 630–632 (1992).

S. A. Muller, "Packaging and Meat Quality," *Canadian Institute of Food Science and Technology Journal* **23**, 22–25 (1990).

A. M. Pearson and F. W. Tauber, *Processed Meats*, 2nd ed., AVI, Westport, Conn., 1987.

J. P. G. Piette et al., "Influence of Processing on Adherence of a Highly Extended Ham to its Cooking Bag," *Meat Science* **48**, 101–113 (1998)

"Processing and Packaging," *Food Review* **23**, 25, 27, 29, 31 (1996).

A. Rutkowski, *Advances in Smoking of Foods*, Pergamon Press, New York, 1976.

F. J. M. Smulders and R. L. J. M. van Laack, "Meat Quality. Ante Mortem Handling of Animals and Fresh Meat Processing. An Update," *Fleischwirtschaft* **72**, 1385–1389 (1992).

A. van der Leest, "Trends in the Modern Meat Industry," *Food Marketing & Technology* **8**, 64–66 (1994).

P. Y. Wang, "Meat Processing," in C. W. Hall, A. W. Farrall, and A. L. Rippen, eds., *Encyclopedia of Food Processing*, 2nd ed., AVI Publishing Co., Westport, Conn., 1987.

H. Weinberg, "Computer Controlled Manufacture of Meat and Sausage Products. Weight as a Factor in Manufacture of High-Quality Sausages," *Fleischerei* **44**, 200–203 (1993).

M. Weisenfels, "Low-Pollution Smoking in Batch Processes," *Fleischwirtschaft* **69**, 1111–1113, 1131 (1989).

M. Wilms, "Smoking of Meat Products. Development of Modern Smoking Processes From Economic and Ecological Viewpoints," *Fleischwirtschaft* **77**, 704–708 (1997).

R. Wittkowski, W Baltes, and W. G. Jenning, "Analyses of Liquid Smoke and Smoked Meat Volatiles by Headspace Gas Chromatography," *Food Chem.* **37**, 135–144 (1990).

Y. Zhao and J. G. Sebraneck, "Technology for Meat Grinding Systems to Improve Removal of Hard Particles From Ground Meat," *Meat Science* **45**, 389–403 (1997).

PIE YI WANG
Armour Swift-Eckrich
Downers Grove, Illinois

See also MEAT AND ELECTRICAL STIMULATION; MEAT SLAUGHTERING AND PROCESSING EQUIPMENT.

MEAT PRODUCTS

Meat has been processed into various products for thousands of years. The purposes of processing have included preservation for future use, increased palatability and variety, enhanced convenience and complete utilization of the carcass and organs. Generally, people in warmer parts of the world processed meat to preserve it by salting and drying, and populations in the colder parts of the world processed meat to increase the quantity and variety to eat by adding such nonmeat ingredients as oatmeal or potatoes. Obviously, all the historic reasons for processing meat still remain today, but flavor, convenience, safety, and variety are key to the continued growth of processed meat sales in modern societies.

In 1997, *Meat Marketing & Technology* trade magazine published a report of a study sponsored by the National Pork Producers Council and the National Cattlemens Beef Association entitled *Consumer Purchase Behavior of Processed Meats at Retail* (1). The material collected in 1995 reported that 4.25 billion pounds of processed meats were purchased for $9.83 billion at retail stores. The major types of processed meats purchased are shown in Table 1. Lunch meat refers to a wide variety of products that are usually sliced to be utilized in making sandwiches. The penetration of lunch-meat-type products is shown in Table 2. A growing category of processed meats is dinner sausages, which provide a variety of flavors and convenience of preparation. The leading dinner sausage types are shown in Table 3.

Table 1. Processed Meats Purchase at Retail

Product	$ Billion	Billion pounds
Lunch meat	5.32	1.73
Hot dogs	1.14	0.75
Bacon	1.23	0.73
Dinner sausage	1.09	0.56
Breakfast sausage	0.55	0.29

Table 2. Penetration of Lunch Meat Types

Product	Percent of households purchasing per month
Ham	30
Turkey	21
Bologna	18
Sliced beef	11
Dry salami	5
Loaves	5
Chicken breast	3
Pepperoni	3
All other salamis	3
Liver sausage	3
Variety packs	3
Cotto/cooked salami	2

Table 3. Top Dinner Sausage Types

Product	Percent of total dinner sausage pounds purchased
Smoked	28
Polish	23
Italian	19
Bratwurst	10
Hot	9
Mild	5

FORMULATIONS

The quality of meat products is a reflection of the materials that have gone into making those products. Meat, water, salt, spices, casings, packaging materials and other components all have an impact on the quality of the final product. Since meat is a biological material that varies naturally in composition, it is difficult to ensure that the composition of the meat source used for further processing is similar from batch to batch. Establishing a program to monitor the selection and quality of raw materials is important to obtain a consistently made, quality product. Jones (2) and Rust and Olson (3) described the significance of raw material quality for processed meat products.

It is important to define purchase specifications for raw materials. Specific criteria representing the type or kind of product needed from a supplier should be established. It is up to the processor to determine how detailed the purchase description should be. The amount of detail specified and the level of monitoring that is applied contribute to the quality of the finished product. For example, specifications could be as rigid as stipulating the acceptable range of volatile oil content of garlic in a spice blend or merely stating the amount of garlic a spice blend must contain.

It is generally not recommended to base raw material purchases solely on price. A working relationship with suppliers should be developed to become familiar with their capabilities and reliability. In addition, suppliers need to be able to provide materials that meet purchase specifications at an agreed-upon price. To control raw material quality, the following steps are recommended: (*1*) establish well-defined purchase specifications, (*2*) set up testing procedures to verify that purchase specifications are met, (*3*) determine sampling frequency for testing procedures, and (*4*) accept or reject raw materials based on meeting purchase specifications.

The characteristics of processed meat products depend not only on the quality of the raw material used to formulate the product but also on the type of meat used. Before purchase specifications are developed, the type of meat used in processed meat products should be selected. Meat cuts vary in moisture, fat, and protein content; in the amount of pigmentation (redness); and in the ability to bind fat and water. These values can be obtained from tables; however, to more accurately determine the composition of a meat source, an analytical method should be used.

Knowing the moisture/protein ratio of a meat source can provide a guide to predict a meat product's final composition. In general, meat with lower moisture protein ratios perform better in sausage formulations. Fat contributes to palatability, tenderness, and juiciness of a processed meat product. Knowledge of the fat and moisture content is important in order to comply with meat inspection regulations that place limitations on their use.

Meat color is related to the concentration of myoglobin, a pigment found in muscle. Meat becomes redder as myoglobin concentration increases. The concentration of myoglobin in meat is dependent on several factors. An older animal has more myoglobin than a younger animal. Beef contains more myoglobin than lamb, and lamb contains more myoglobin than pork. Muscles used for locomotion have more myoglobin than support muscles such as the ribeye (*longissimus dorsi*). Genetics, nutrition, and environment also play a role in influencing myoglobin concentration in meat. To help maintain a consistence appearance from batch to batch, a color value ranking should be used in the formulation of meat products. This is particularly useful if a least-cost formulation program is used.

In the meat processing industry, the term *bind* has several meanings. It generally refers to (*1*) the ability of contractile protein to entrap water and fat such as in a sausage batter or (*2*) the surface cohesion of meat chunks to each other. Raw meat materials vary in their binding ability. Meats that have a high contractile protein content and low collagen content have high binding ability and are used to form stable batters. Beef skeletal muscle is an example of meat with high binding ability. Meats that have a high collagen and fat content and low contractile protein content have low binding ability. Heart and pork jowls are examples of meats with low binding ability. Bind value tables are available for a variety of meat sources. Tables providing proximate composition, color, and bind values for many raw meat sources, as well as an excellent overview of least-cost formulations, can be found in Pearson and Gillett (4).

Given the natural variation of raw materials, producing a quality product is both an art and a science. To succeed, it is extremely important to use meat that has been handled properly. Meat provides an excellent environment for bacterial growth. Since the meat used for processed meat products undergoes considerable handling during processing procedures, there are many opportunities for meat to become contaminated with bacteria. It is essential that all equipment be clean and sanitized prior to processing, and the meat must be kept cold at all times.

The development of off-odors and off-flavors in fat is known as rancidity. Over time, meat becomes rancid even if stored in a freezer. To produce quality products, meat that has become rancid should not be used. The rancid flavor and odor cannot be diluted out by combining rancid meat with fresh meat. If raw materials have become rancid, they should not be used as a base for processed meat products.

Purchase specifications are written descriptions describing raw materials. Each raw material should have a purchase description. When purchasing meat, in addition to species and portion cut, the description may specify grade (quality and/or yield), state of refrigeration for delivery of product (chilled or frozen), fat thickness (maximum average thickness and maximum at any one point), weight and thickness tolerances, muscling, trimming, netting or tying, cutting, or other material requirements. *The Meat Buyers Guide* (5) is a good resource for developing purchase specifications for meat cuts.

RESTRUCTURED MEAT PRODUCTS

The term *restructured* has the general meaning of binding smaller pieces of meat together to generally give the impression that a larger meat cut has been created that can produce slices of desired size for sandwiches or steak for plating at dinner. Processors have used various technologies to restructure ham since the 1950s. Mixing pieces of meat with ingredients, but especially appropriate levels of salt and alkaline phosphates, results in the osmotic movement of the ingredients throughout the meat particles and the extraction of myofibrillar proteins. The extracted myofibrillar proteins within the particles and on the surfaces of the particles form a heat set gel when the products are cooked. This gel is a very effective binder of water and fat and results in the binding of the meat pieces together and the retention of water and fat during cooking. This basic reaction is the primary system for the production of the very popular lunch meat types such as ham, turkey, and sliced beef. These products may be prepared from whole muscles or pieces of muscles or combinations of the two. The essential ingredients of salt and alkaline phosphates are added in a pickle that is usually injected into the meat pieces with large multineedle injectors. Sometimes the particles are slashed at frequent intervals to increase the surface area for protein extraction. This is called maceration or tenderizing. To further enhance protein extraction, the injected meat pieces are subjected to mechanical action in mixers, blenders, massagers, or tumblers. Portions of the mixture are then placed in appropriate casings or forms to create the final product shape and size. Often the shape of the final product is devised to mimic the whole muscle counterpart such as a ham, a beef roast, or a turkey breast. Cooking sets the protein gel, and the product can be uniformly sliced to create slices free of visible fat and connective tissue. Key references in this area are Pearson and Dutson (6) and Franklin and Cross (7).

Although the salt/phosphate-based binding system has obtained very widespread application, it has several limitations. Products must be marketed either precooked or frozen as the material will not cohere in the uncooked refrigerated state. Salt discolors the raw meat and accelerates oxidative rancidity. In recent years, several systems have been developed to bind meat without cooking to make products that look like whole tissue meats. The products can be marketed raw and have enhanced flavor and color stability. The five cold binding systems to be discussed are based on alginate/calcium, fibrinogen/thrombin, surimi, transglutaminase, or egg/milk/soy proteins. Although restructuring systems make comminuted meat appear to be an intact cut of meat, the restructured steak or roast has bacteria distributed throughout the interior of the product that is different from an unprocessed intact steak or roast. Therefore, it is imperative the restructured product be stored and, especially, cooked properly. Otherwise, food safety concerns for restructured products are the same as for any other comminuted meat.

Alginate forms a chemical bond with calcium ions to form a cold-set but heat-stable gel. High guluronic acid alginate is mixed with meat, and then a moderately slow release calcium source such as calcium carbonate or encapsulated calcium lactate is added along with an acidulant such as glucono-δ-lactone. After the ingredients are mixed with the meat pieces, the product is formed and chilled for a day to permit the chemical binding to continue to form (8).

Fibrinogen/thrombin binding system is based on the mechanism used to clot blood (9). The materials are usually prepared from beef blood collected in a sanitary manner. Approximately 5% of a fibrinogen/thrombin mixture is added to meat pieces to be bound together, mixed, and formed into the final desired shape. Once all the materials are mixed, it is important to quickly form the product and let it set for about 12 h. The time to gel is fairly rapid, and the process must be done in relatively small batches to avoid forming delays. In addition, the materials are natural, biological materials obtained from beef cattle during the slaughtering process. Special care must be taken to minimize microbial contamination and growth during binder preparation, storage, and utilization.

Surimi is a wet concentrate of the proteins of fish muscle produced by water washing of minced fish. Cryoprotectants such as 4% sucrose and 4% sorbitol permit surimi to be stored frozen for more than a year with little loss of gel forming ability (10). Research has shown surimi can be used in red meats as a cold binding system. There has been no commercial application of this process, probably due to flavor effects.

Transglutaminase is an enzyme that catalyzes the formation of a chemical cross-link between peptides containing glutamine and lysine. Transglutaminase can be used to increase the gelation capacity, physical strength, viscosity, thermal stability, and binding capacity of protein foods. A commercial binder based on this enzyme is used at the 1% level to bind meat, poultry, and fish pieces. This is a fast gelling binder and must be used in a batch system to mix and form in 30 min or less and sit for at least 2 h to gel. It is used in conjunction with sodium caseinate, sugar fatty acid ester, and dextrin or with sodium polyphosphate, sodium pyrophosphate, sodium ascorbate, and lactose (Ajinomoto U.S.A., Inc. Teaneck, NJ).

A commercial blend of proteins is sold as a cold-setting meat binder. The blend called PEARL MEAT contains egg white, casein, lactalbumin, gelatin, hydrolytic egg white extractive, hydrolytic casein extractive, soybean protein, and hydrolytic flour protein extractive. The meat is coated with the powder and quickly pressed and formed as the binding will be completed in 30 min (Chiba Flour Milling Company, LTD. Japan).

All of the binding systems require that the particles of meat being bound together be naturally tender, lean, have good color and flavor, and react well during subsequent cooking. Also, as mentioned earlier, the surfaces of the meat pieces that are subsequently bound on the inside of a restructured roast or steak must be assumed to contain some pathogenic bacteria such as *Salmonella*. Thus, restructured meats must be cooked sufficiently to kill these bacteria present in the center of the restructured cut. Thus, many of the cold binding systems are limited by the quality and cost of the raw materials and the need for more extensive cooking than intact cuts.

CURING

Historically, meat was preserved using salt. Early sausage makers recognized that using certain salts produced a distinct color and flavor in meat products. It is believed that these salts contained an impurity called potassium nitrate, better known as saltpeter. It wasn't until the late 1800s that scientists began to understand the role that saltpeter played in meat curing. In 1891, it was identified that the nitrate in saltpeter was chemically changed to nitrite by the action of bacteria found normally on meat. At the turn of the century, it was established that the reddish-pink color of cured meat was due to nitrite and not nitrate. These and other discoveries led to the use of nitrite as a meat curing ingredient.

Typical meat curing ingredients include salt, sodium nitrite, ascorbates, phosphates, and seasonings. Salt is used as a seasoning and a preservative, and it functions by extracting myofibrillar proteins to bind a product together. Nitrite imparts several important qualities to cured meat. The most visible characteristic is the pink color of cured meat. This color results from nitrite reacting with myoglobin, which, when denatured by heat, forms a stable pink color. Nitrite also provides a characteristic flavor and acts as a potent antioxidant to prevent the development of off-flavors in cured meat during storage. One of the most important reasons nitrite is used in cured meats is to inhibit the growth of food poisoning and spoilage microorganisms, especially *Clostridium botulinum*, the bacteria that causes botulism. Ascorbates, including sodium ascorbate and erythorbic acid, are used to improve and maintain the color of processed meats. Phosphates are used to enhance juiciness and texture and to help prevent fat from becoming rancid in products such as ham, bacon, and cooked sausages. The amount of phosphate that can be used in meat products is limited to a maximum of 0.5%.

There has been concern over potential health risks from nitrosamines in cured meat products. Nitrosamines are compounds that can form when nitrites combine with amines, a natural component resulting from the breakdown of proteins. Most cured meat products contain approximately 10 ppm residual nitrite (11). This is an 80% reduction in residual nitrite content in cured meats compared to mid-1970s levels. As a result, a very limited amount of nitrite is available in cured meats to react with amines to form nitrosamines. Previously, traces of nitrosamines had been detected in bacon fried at high temperatures until it was crisp and very well done. To minimize the risk of nitrosamine formation in fried bacon, the amount of nitrite that may be used to cure bacon has since been reduced. Cassens (11) concluded that health risks from nitrosamine formation in cured meat should be reevaluated.

Meats can be cured by directly mixing cure ingredients into comminuted products, or by using a pickle or applying a dry rub to primal or subprimal cuts of meat. To make a pickle, cure ingredients are dissolved into water. The resulting pickle can be incorporated into meat by injection, tumbling or immersion, or a combination of these techniques. The most widely used method by the industry is injection curing (12). Multineedle injection machines, handheld stitch devices, and artery pumping are mechanisms for injection curing. Alternatively, meat may be immersed in a container or mechanically manipulated in a tumbler containing pickle, or meat may be injected, then immersed, tumbled, or massaged to enhance pickle pickup and promote myofibrillar extraction. Application of salt, sodium nitrate, and possibly sodium nitrite directly onto the surface of raw meat, then holding under controlled conditions for defined time periods is used for dry curing meats. This technique is most often used to produce regional specialty products.

ACIDIFICATION

Fermented sausages are acidified by the deliberate or natural inoculation with specific microorganisms, incubated to ferment a sugar source to lactic acid, and subjected to heating and/or drying to alter pH and the moisture protein ratio, and to prolong shelf life. An update of the technology of meat fermentation with a list of key references on the topic is provided by Prochaska, Ricke, and Keeton (13). Fermented sausages have been produced for centuries. Salamis, mettwurst, thuringer, cervelat, teewurst, Lebanon bologna, and some summer sausages are produced by fermentation and drying without any cooking step. Others such as cooked salami, cooked summer sausage, and mortadella are cooked. Traditionally, fermentation, drying, and smoking of these products are done at 12 to 25°C (54–129°F) during times ranging from weeks to several months. Fermentation is done at 20 to 25°C (68–129°F) with a relative humidity of 92 to 95%. The subsequent smoking and drying are done at less than 20°C (68°F) and gradually decreasing relative humidity to about 78% (14). In the United States, fermented sausages are often produced by the addition of *pediococcal* starter cultures, fermented at 24 to 43°C (75–108°F) and greater than 85% relative humidity to a pH of approximately 4.8 and then dried at about 13°C (55°F) and 65% relative humidity to a moisture protein ratio of 1.6:1. However, emerging pathogenic bacteria such as *Escherichia coli* O157:H7 are not destroyed by this treatment (15). Acidification can be accomplished in meat via direct addition of acids. Citric acid is used at 0.001 to 0.01% together with antioxidants to prevent oxidative rancidity in dry sausage, fresh pork sausage, and dried meats. Citric and lactic acids are also encapsulated in coatings of lipids for direct addition to sausage products to lower the pH and mimic the flavor and preservation effects of natural fermentation. Glucono-δ-lactone, a neutral ester of gluconic acid crystallized through dehydration, is a mild acidulant and is used for the same effect. Direct acidification is most widely used on the less expensive snack sticks, which are also cooked and are shelf stable.

SMOKING

Meat is smoked to produce a desirable color, flavor, and aroma. Of the more than 390 individual chemical compounds that have been detected in wood smoke, more than 70 of these compounds have been found in smoked foods. The type of wood used to generate smoke, the moisture content of the wood, and the temperature and method of producing smoke all influence what types of compounds will be generated from smoke.

Phenols, carbonyls, and acids form the three basic categories of compounds important to smoked meat. Smoked meat color is primarily formed by carbonyls in wood smoke reacting with components of meat proteins to produce the characteristic reddish-brown appearance. Phenols contribute to smoked color development and are largely responsible for the smoky flavor imparted to meat. On the surface of smoked meats, phenols have a preservative effect by retarding rancidity and inhibiting bacterial growth. Acids play an important role in coagulating proteins on the surface of smoked meats, which is essential to achieving good skin formation.

Smoke may be generated using a variety of fuels including cord wood, corn cobs, wood shavings, or sawdust or may be applied in the form of liquid smoke. Hardwoods such as oak and hickory are commonly used to generate smoke because of their low resin content. Although softwoods can be used, they can produce a strong, bitter flavor.

Commonly, smoke is applied during the initial phases of cooking or even before a product is cooked. The deposition of smoke on a meat product is affected by the smoke density, the humidity and air movement in a smoker, and the surface conditions of the meat to be smoked. Dampened fuel is heated to generate smoke. High humidities during smoking will increase smoke deposition but may cause a muddy brown appearance on the meat surface. If the meat surface is wet when smoke is applied, streaking can occur. Too little humidity during smoking can cause a meat surface to become very dry, leading to poor smoke adherence and a pale color. It is recommended to apply smoke while the meat surface is tacky to the touch.

Liquid smoke, derived from wood using a series of extraction and refinement procedures, contains the same functional compounds that are found in vaporous smoke. Meat products can be dipped or sprayed in liquid smoke, the smoke can be added directly into pickles or sausage batters, or liquid smoke can be atomized onto meat surfaces during thermal processing.

The theory of smoking and its application to meat products was described by Hanson (16). Recommendations for optimizing processing variables and reducing process variation in the cooking and smoking process also are discussed by Hanson (17).

PRESERVATION METHODS

Meat is preserved to extend product shelf life. This is accomplished by applying techniques to delay or prevent microbial, enzymatic, chemical, and/or physical changes from occurring that would render a product unacceptable. Historically, meat was preserved for future consumption by curing, smoking, and drying. Chilling, freezing, thermal processing, dehydration, and irradiation are preservation methods currently employed by the meat industry. van Laack (18) presented an overview of spoilage mechanisms and techniques for preservation of muscle foods.

Refrigeration and freezing are the most common methods used to prolong the shelf life of meat. Refrigerated meats are stored between -2 and 5°C (28–41°F). Chilling meat typically begins immediately after slaughter. Carcasses are placed into coolers with forced-air movement to facilitate carcass cooling, while poultry and fish are chilled by immersion in ice water. Following fabrication, refrigerated shelf life of meats is dependent on the initial microbial load, temperature, and humidity conditions during storage, packaging, species, and type of product stored. Although the freezing point of muscle is approximately -2°C (28°F), it is not until −30°C (−22°F) that nearly 100% of the water in meat is frozen. The quality of frozen meat depends on freezing rate conditions and subsequent frozen storage, length of frozen storage, packaging, lighting, and type of product frozen. The rate of freezing impacts the size of ice crystals formed within a product. If meat is rapidly frozen, many small ice crystals form resulting in minimal muscle fiber shrinkage and distortion, and less drip loss upon thawing. Improperly packaged meat is susceptible to freezer burn, which is caused by a loss of moisture on the surface of meat. Freezer-burned meat will have a dry, discolored surface that, when cooked, will be tough and taste bland or rancid.

Canned meat products may be pasteurized or commercially sterile. Pasteurized products are heated to 58 to 75°C (136–167°F), which results in the destruction of all pathogenic bacteria; however, spores and some thermoresistant spoilage organisms may survive. Canned, pasteurized products must be stored under refrigeration. Pasteurized canned hams and picnics are examples of this type of product. Commercially sterile products generally are heated to an internal temperature of at least 107°C (225°F) to render the product free of microorganisms capable of growing at nonrefrigerated conditions (above 10°C/50°F). Canned, commercially sterile products may contain spores of thermophilic bacteria that do not germinate below 43°C (109°F). Meats are canned using a steel tank, called a retort, in which metal crates or baskets containing filled, sealed cans are placed for cooking and subsequent cooling. The amount of heat, time, and temperature required for a given degree of sterility depends on the nature of the product, pH, ingredients such as salt and nitrite, shape and size of the can, and the type of retort used. Footitt and Lewis (19) and Pearson and Gillett (6) provide considerable information on canning meat products.

The preservative effects of dehydration are due to reduction of water activity (a_w). The lean portion of freshly cut meat has an a_w of 0.99. When meat is dehydrated, the a_w is reduced to a level that inhibits microbial growth, allowing products to be shelf stable without refrigeration. According to Leistner and Rodel (20), factors most important in influencing a_w in processed meat products are the addition or removal of water, the addition of salts, and the amount of fat. Nieto and Toledo (21) demonstrated that both soluble and insoluble components affected a_w in processed fish products. The fat content of meat products has an indirect effect on a_w due to its low water-binding properties. In general, a_w increases with increasing fat content in a meat product because fat has little effect in depressing a_w (22).

Meat is dehydrated by using hot air drying or freeze drying. Jerky is commonly dried using hot air drying. Freeze drying involves the removal of water from meat by sublimation where water, in the form of ice, is directly transformed into water vapor without going through a liquid phase. In a conventional freeze drying process, meat is frozen, then dried under vacuum with pressures of 1.0 to 1.5 mm of mercury while it is in a frozen state to a residual moisture content of less than 2%. Because freeze-dried meat products are susceptible to enzymatic changes, rancidity development, nonenzymatic browning, and protein denaturation, oxygen and moisture impermeable packaging is necessary.

Irradiation involves treating a food item with energy from electrons or γ-rays to prevent foodborne illnesses, spoilage, and insect infestations. Food does not become radioactive when treated with irradiation. This process is not a substitute for good sanitary practices, and it will not make a "dirty" food clean. Food irradiation makes meat and food of good quality safer, plus increases the length of time food can be stored before it becomes spoiled. Organizations such as the American Medical Association and World Health Organization have endorsed the safety of irradiation for food. The Food and Drug Administration (FDA) has approved irradiation of wheat and wheat flour to control insects; of white potatoes to control sprouting; of spices to kill insects and control bacteria; of pork to control trichinosis; of fruits, vegetables, and grains to control insects and growth and ripening; and of uncooked poultry to control bacteria, particularly *Salmonella*. In 1997, the FDA approved irradiation for fresh or frozen red meats. A list of materials approved by the FDA that can be used to package foods before irradiation is found in 21 *CFR* 179.45. A scientific status summary on irradiation, particularly muscle foods, was prepared by Olson (23).

HOME MEAL REPLACEMENT

Ready-to-cook, partially cooked, or ready-to-eat food products prepared and sold at a wide range of establishments, then consumed at home, are commonly referred to as home meal replacements (HMR), or meal solutions. Sociocultural and socioeconomic changes have driven the demand for convenient, fresh (preferably not frozen), appealing, high-quality food products that require minimal preparation by the consumer. This trend was described by Hoogenkamp (24) and Farquhar (25)

HMRs commonly include chilled or hot meals marketed through supermarkets, restaurants, and nontraditional retail outlets. These products are prepared and packaged at U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) or FDA inspected plants, by in-house supermarket or restaurant chefs, or prepared as made-to-order meals on-site at retail establishments. Johnson (26) summarized food safety hazards and risks associated with production and handling of HMRs.

BY-PRODUCTS

Two excellent references on the topics of edible and inedible meat by-products are Pearson and Dutson (27,28). Although values may vary considerably due to such factors as international trade and changing consumer patterns of preferences, by-products account for about 10% of the value of products from the slaughter of domestic animals (29).

Edible meat by-products that are consumed directly or used in processed products are parts such as tongues, livers, hearts, tails, and brains. Other materials that are classified as by-products are meat trimmings from the carcass, head, neck, and viscera. These materials include cheek meat, weasand meat, giblet meat, head meat, salivary glands, and diaphragm meat. A list of these by-products, their potential uses and world production figures are given by Goldstrand (29). More recent export values of meat and meat by-products are shown in Table 4.

Mechanically separated meat is a continuously changing edible meat by-product. Engineering developments and regulatory requirements greatly affect the production methods, quality, and utilization of these products. There are no up-to-date comprehensive reviews of these products, because much of the methodology of processing and engineering is proprietary. The USDA FSIS regulates the composition and labeling of meat produced by advanced meat/bone separation machinery and recovery systems (30). The development of meat/bone separators have advanced such that bones no longer need to be ground or crushed by the separating machine. Rather, the bones emerge from the process in a manner consistent with hand-deboning operations that use knives. The modern systems produce distinct whole pieces of skeletal muscle tissue with a well-defined particulate size consistent with ground meat. There is no powdered bone or bone marrow in the product. Systems for producing these products use compression, heavy-duty sieve cylinders, warming, and centrifugation to separate meat from bone and fat. These materials are widely used in comminuted meat products, with separated beef being used in ground beef patties and mechanically separated poultry being widely used in sausage products. The mechanically separated tissue is usually sold at a lower price than hand-deboned tissue. As it is often finely comminuted, it is especially prone to oxidation and must be kept frozen or refrigerated for a minimum time before being incorporated into processed products.

Edible tallow and lard are still produced in abundance as by-products of the slaughter, fabrication, and retail cutting and processing of cattle and hogs. The 1986 production of these two materials was 2.4 billion pounds in the United States alone (31).

Inedible by-products are produced in abundance by the meat industry. The market forces that cause changes in the demand for inedible by-products are the supply of live-

Table 4. U.S. Export Values for Selected Animal Products for Calendar Year 1997

Product	Export values ($ million)
Animal fats	531
Hides and skins	1618
Red meats, fresh, chilled, frozen	4090
Red meats, prepared or preserved	401
Pet foods, dog and cat food	735
Poultry meat	2423

Source: USDA Foreign Agricultural Service Statistics.

stock, feed supplies, substitute products, product safety and health concerns, trade barriers, and politics (32). The raw materials processed into inedible by-products are hides, skins, pelts, hair, feathers, hooves, horns, feet, heads, bones, toenails, blood organs, glands, intestines, and fatty tissues. By recycling these inedible products, not only are very useful materials created for human use and animal feeds, but the material from a meatpacking plant that is classified as waste is greatly reduced. Two major recent developments that affect by-product values were the outbreak of bovine spongiform encephalopathy (BSE) and the growth of the biotechnology industry for synthesizing substitutes for natural gland extracts. The BSE outbreak in the United Kingdom resulted in the prohibition of feeding ruminant meat and bone meal to other ruminants and may eliminate the feeding of much meat and bone meal to meat animals (33).

Pet food literature is limited, and much of the work is proprietary. As shown in Table 4, pet food is a large volume and value export from the United States. According to a 1998 survey by the American Pet Products Association, 59% of U.S. households own a pet and Americans spent more than $21 billion on their pets in 1997 (34). A complete discussion of the use of meat products in pet foods is presented by Corbin (35).

REGULATIONS AND STANDARDS

Meat inspection is regulated by the government to ensure that meat is wholesome and safe to eat Hale (36) reviewed major meat inspection laws and events that have impacted meat and poultry inspection. All meat that is sold must, by law, be inspected. Regulations for the meat industry are administered by the USDA FSIS. Currently, it is estimated that meat inspection costs each consumer 13 cents per month.

In 1906, the Federal Meat Inspection Act mandated inspection of all meat sold for interstate and foreign commerce. Prior to 1967, inspection of meat produced for intrastate commerce varied depending on the city and state where the plant was located. To standardize inspection programs, the Wholesome Meat Act of 1967 was passed. Individual states were given the opportunity to develop inspection programs that met or exceeded regulations established by the federal government. If a state chose not to set up an inspection program, the USDA FSIS assumed all meat inspection responsibilities within the state.

More recently, USDA FSIS (37) mandated implementation of Hazard Analysis and Critical Control Point (HACCP) programs for all inspected meat and poultry establishments. HACCP is a systematic, science-based process control system for food safety. This concept forms the basic structure for a preventative system for the safe production of meat products. Note that the key to this system is that it is a preventative approach to producing the safest possible meat products for human consumption. This means that potential biological, physical, or chemical food safety hazards—whether they naturally occur in food, are contributed by the environment, or are generated by a deviation in the production process—are prevented, eliminated, or reduced to produce safe meat products.

HACCP began in 1959 when the Pillsbury Company cooperated with the U.S. Army Natick Laboratories, the National Aeronautics and Space Administration (NASA), and the U.S. Air Force Space Laboratory Project Group to ensure the safety of food to be used for the space program. They used a system of analysis that had been developed by Natick called the "Modes of Failure," which was adapted and has since evolved into the concept now understood as HACCP. In 1971, HACCP was first presented to the public at the National Conference of Food Protection. Following the publication of a report in 1985 by the National Academy of Sciences, *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, HACCP received more recognition by the industry as a food safety concept. The seven principles of HACCP, as defined by the National Advisory Committee on Microbiological Criteria for Foods (38) are as follows:

1. Conduct a hazard analysis.
2. Determine the critical control points (CCPs).
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective actions
6. Establish verification procedures.
7. Establish record-keeping and documentation procedures.

The USDA FSIS Final Rule on pathogen reduction mandated requirements in efforts to reduce the occurrence and numbers of pathogens on meat and poultry products, reduce the incidence of foodborne illness associated with consuming these products, and provide a framework for modernization of the meat and poultry inspection system. The new regulations mandated establishment of four new programs. The first program required that each establishment develop and implement written sanitation standard operating procedures (Sanitation SOPs). Second, regular microbial testing was required for slaughter establishments to verify the adequacy of a plant's process controls for the prevention and removal of fecal contamination and associated bacteria. All slaughter plants and plants producing raw ground products were required to meet pathogen reduction performance standards for *Salmonella* for the third program. Last, all meat and poultry plants were required to develop and implement HACCP programs. Pearson and Dutson (39) is a resource describing the application of HACCP principles to meat, poultry, and fish processing.

Animals are inspected for signs of disease before and after slaughter. The inspector relies on a veterinarian's judgment in those instances where there is a health or wholesomeness question. Extreme attention is paid to produce a carcass free of contamination. To ensure the sanitary handling of meat and meat products, the sanitation of equipment, buildings, and grounds are also inspected. An inspector has the authority to have condemned products destroyed to prevent their use for human food.

An inspection stamp is put on large cuts of meat or meat products to assure consumers that the product was wholesome when it was shipped from the plant where the meat was inspected Every plant that is inspected has a unique, individual identification number. This number can be found as part of the round inspection stamp on meat labels. An edible ink is used to apply inspection stamps on fresh meat. USDA also regulates labeling of meat and poultry products.

Standards of identity have been established for some meat and poultry products that describe the use of certain meat and nonmeat ingredients and/or the amount of fat, moisture, and protein a product must contain. Standards of preparation have also been defined for some products. These regulations can be found in the *Code of Federal Regulations*. Additional regulatory information can be found in USDA FSIS policy memos and directives, and the *Standards and Labeling Policy Book*.

BIBLIOGRAPHY

1. B. Salvage, *Meat Marketing and Technology* **5**, special supplement 1–28 (1997).
2. K. W. Jones, "Quality Control of Ingredients," *1989 AMSA-AMI Intercollegiate Processed Meat Clinic*, Chicago, Ill., September 21–23, 1989.
3. R. Rust and D. Olson, "Processing Workshop, 1981–1986," *Meat and Poultry Magazine*, Oman Publ., Mill Valley, Calif., 1987
4. A. M. Pearson and T. A. Gillett, *Processed Meats*, 3rd ed., Chapman & Hall, New York, 1996.
5. *The Meat Buyers Guide*, North American Meat Processors Association, Reston, Va., 1997.
6. A. M. Pearson and T. R. Dutson, *Restructured Meat and Poultry Products*, Advances in Meat Research, Vol. 3, Van Nostrand–Reinhold, New York, 1987.
7. K. R. Franklin and H. R. Cross, *Meat Sci. and Technol. Int. Symp. Proc.*, National Live Stock and Meat Board, Chicago, Ill., November 1–4, 1982
8. U.S. Pat. 4,603,054 (July 29, 1986), G R. Schmidt and W. J. Means (to Colorado State University Research Foundation).
9. U.S. Pat. 4,741,906 (May 3, 1988), E. J. C. Paardekooper, S. Michielsgestel, and D. R. Gerrit Wijngaards (to Nederlandse Centrale Organisatie Voor Toegepast-Natuurwetenschappelijk Onderzoek).
10. D. D. Hamann, "Rheological Studies of Fish Proteins," in K. Nishinari and E. Doi, eds., *Food Hydrocolloids. Structures Properties and Functions*, Plenum, New York, 1993.
11. R. G. Cassens, "Residual Nitrite in Cured Meat," *Food Technol.* **51**, 53–55 (1997).
12. J. R. Claus, J.-W. Colby, and G. J. Flick, "Processed Meats/Poultry/Seafood," in D. M. Kinsman, A. W. Kotula, and B. C. Breidenstein, eds., *Muscle Foods*, Chapman & Hall, New York, 1994
13. J. F. Prochaska, S. C. Ricke, and J. T. Keeton, *Food Technol.* **52**, 52–57 (1998).
14. G. R. Schmidt, "Processing," in H. R. Cross and A. J. Overby, eds., *Meat Science, Milk Science, and Technology*, World Animal Science B3, Elsevier Science, New York, 1988.
15. N G. Faith et al., "Viability of *Escherichia coli* O157: H7 in Pepperoni during the Manufacture of Sticks and the Subsequent Storage of Slices at 21, 4, and −20°C under Air, Vacuum, and CO_2," *International Journal of Food Microbiology* **37**, 47–54 (1997).
16. R. E. Hanson, *Meat Business* **54**, 9, 20–21 (1993).
17. R. E. Hanson, *Reciprocal Meat Conference Proceedings* **50**, 33–42 (1997).
18. R. L. J. M. van Laack, "Spoilage and Preservation of Muscle Foods," in D. M Kinsman, A. W. Kotula, and B. C. Breidenstein, eds., *Muscle Foods*, Chapman & Hall, New York, 1994
19. R. J. Footitt and A. S. Lewis, eds., *The Canning of Fish and Meat*, Chapman & Hall, New York, 1995.
20. L. Leistner and W. Rodel, "The Significance of Water Activity for Micro-Organisms in Meats," in R. B. Duckworth, ed., *Water Relations of Foods*, Academic Press, New York, 1975
21. M. B Nieto and R. T. Toledo, *J. Food Sci.* **54**, 925–930 (1989).
22. H K. Leung, "Significance of Water Activity in Meat Products," *Proc. Meat Industry Res. Conf.*, American Meat Institute, Washington, D.C., 1984, pp 142–147.
23. D G. Olson, *Food Technol.* **52**, 56–62, 1998.
24. H Hoogenkamp, *National Provisioner* **212**, S40, S42 (1998).
25. J. W. Farquhar, *Reciprocal Meat Conference Proceedings* **50**, 19–20 (1997).
26. J. L. Johnson, *Reciprocal Meat Conference Proceedings* **50**, 20–24 (1997).
27. A. M. Pearson and T. R. Dutson, *Edible Meat By-Products*, Advances in Meat Research, Vol. 5, Elsevier Applied Science, New York, 1988.
28. A. M. Pearson and T. R. Dutson, *Inedible Meat By-Products*, Advances in Meat Research, Vol. 8, Elsevier Applied Science, New York, 1992.
29. R. E. Goldstrand, "Edible Meat Products: Their Production and Importance to the Meat Industry," in A. M. Pearson and T. R. Dutson, eds., *Edible Meat By-Products*, Advances in Meat Research, Vol. 5, Elsevier Applied Science, New York, 1988.
30. U.S. Department of Agriculture, Food Safety and Inspection Service (USDA FSIS), *Fed. Regist.* **63**, 17959–17966 (1998).
31. I. D. Morton, J. I. Gray, and P. T Tybor, "Edible Tallow, Lard, and Partially Defatted Tissues," in A. M. Pearson and T. R Dutson, eds., *Edible Meat By-Products*, Advances in Meat Research, Vol. 5, Elsevier Applied Science, New York, 1988
32. R. E. Goldstrand, "An Overview of Inedible Meat, Poultry, and Fish By-Products," in A. M. Pearson and T. R. Dutson, eds., *Inedible Meat By-Products*, Advances in Meat Research, Vol. 8, Elsevier Applied Science, New York, 1992.
33. R. M. Anderson et al., "Transmission Dynamics and Epidemiology of BSE in British Cattle," *Nature* **382**, 779–788 (1996).
34. D. E. Pszczola, "Poly Wants a Neutraceutical," *Food Technol.* **52**, 66–87 (1998).
35. J. E. Corbin, "Inedible Meat, Poultry, and Fish By-Products in Pet Foods," in A. M Pearson and T R. Dutson, eds., *Inedible Meat By-Products*, Advances in Meat Research, Vol. 8, Elsevier Applied Science, New York, 1992.
36. D. S Hale, "Inspection," in D. M. Kinsman, A. W. Kotula, and B. C. Breidenstein, eds., *Muscle Foods*, Chapman & Hall, New York, 1994.
37. U.S. Department of Agriculture, Food Safety and Inspection Service (USDA FSIS), *Fed. Regist.* **61**, 38806–38989 (1996).
38. National Advisory Committee on Microbiological Criteria for Food, *J. Food Prot.* **61**, 762–775 (1998).
39. A. M Pearson and T R. Dutson, eds., *HACCP in Meat, Poultry, and Fish Processing*, Advances in Meat Research, Vol 10, Blackie Academic and Professional, New York, 1995.

GENERAL REFERENCES

American Association of Meat Processors, URL: *http://www.aamp.com/*.

American Meat Institute, URL: *http://www.meatami.org/index.htm*.
American Meat Science Association, URL: *http://www.meatscience.org/*.
Code of Federal Regulations, URL: *http://www.access.gpo.gov/nara/cfr/cfr-table-search.html*
Institute of Food Technologists, URL: *http://www.ift.org*.
International HACCP Alliance, URL: *http://ifse.tamu.edu/haccpall.html*.
Meat and Poultry Association Links, URL: http://www.meatpoultry.com/assn.asp
Meat and Poultry Magazine, URL: *http://www.meatpoultry.com/*.
The Meating Place, URL: *http://www.mtgplace.com/*.
National Archives and Records Administration; Federal Register Online via GPO Access, URL: http://www.access.gpo.gov/su_docs/aces/aces140.html.
National Meat Association, URL: *http://www.nmaonline.org/*.
National Pork Producers Council, URL: *http://www.nppc.org/*.
North American Meat Processors Association, URL: *http://www.namp.com/*.
U.S. Department of Agriculture, URL: *http://www.usda.gov/*.
U.S. Department of Agriculture, Agricultural Research Services Nutrient Data Laboratory, URL: *http://www.nal.usda.gov/fnic/foodcomp/*.
U.S. Department of Agriculture/Food and Drug Administration Foodborne Illness Education Information Center, URL: *http://www.nal.usda.gov/fnic/foodborne/foodborn.htm*.
U.S. Department of Agriculture/Food and Drug Administration HACCP Training Programs and Resources Database, URL: *http://www.nalusda.gov/fnic/foodborne/haccp/index.shtml*.
U.S. Department of Agriculture Food Safety and Inspection Service, URL: *http://www.fsis.usda.gov/*.
United States Meat Animal Research Center, URL: *http://www.marc.usda.gov/*.
U.S. Meat Export Federation, URL: *http://usmef.org/*.
Worldmeat, URL: *http://www.worldmeat.com.au/*.

ELIZABETH A. E. BOYLE
Kansas State University
Manhattan, Kansas

GLENN R. SCHMIDT
Colorado State University
Fort Collins, Colorado

MEAT SCIENCE

WHAT IS MEAT SCIENCE?

Meat is a food prepared from skeletal muscle. The quality of the meat is affected by various live animal factors as well as the processing conditions. Meat science is a discipline that has evolved to understand the changes that occur from the live animal until the meat is ready for consumption. It should be distinguished from animal science, where meat yield and growth characteristics are considered to be the main aim rather than meat quality itself. This chapter considers all those factors that affect the main meat quality attributes—its tenderness, juiciness, flavor, color, and storage life—considering mainly meat from cattle, sheep, and pigs, with minor references to deer and poultry. Neither processed meats, in which meat and fat tissue are used as a raw material for derived foods, nor meat hygiene or meat packaging are considered. The freezing of meat will be considered, however, as this process effectively locks up the characteristics of the tissue that exist immediately before freezing. Therefore, the resultant product depends on the time postmortem when the meat was frozen, the rate of freezing, and frozen storage conditions.

PRINCIPLES OF MEAT SCIENCE

The major principle of meat science discussed in this chapter is that shortened muscle will be tough when cooked. Meat can age and become more tender, with the aging factors being affected by the amount of shortening and conditions at rigor. This gives rise to a second principle that, since shortening and degree of aging cannot be determined visually, the appearance of a piece of fresh meat cannot predict how tender or tasty it will be. The consequence of these two principles is that the consumer has to have faith that the meat has been processed properly. Such faith is often misplaced. The ultimate aim of meat science is to develop and encourage procedures in the production and processing of animals so that the full potential of tenderness, juiciness, and flavor, as well as good hygiene, can be achieved.

MUSCLE STRUCTURE AND FUNCTION

Definition of Meat and Muscle

Meat comes from the striated muscle of vertebrates. Some of the characteristics of the live muscle underlying meat quality, such as energy stores, connective tissue, and fat, are present at the time of death (see "Animal Factors Affecting Meat Quality," later in this article) and will affect the final meat quality. Other aspects of meat quality, particularly toughness, are affected by processing conditions that interact with some preslaughter attributes.

The muscles of a carcass, whatever the species, perform different functions while the animal is alive and are reflected in differences in physiology, structure, and biochemistry. The variation in these components underlies the differences between the various meat cuts, the use to which they are put, and the way they are cooked. The two major contributors of the muscle that have a bearing on meat quality and underpin meat science are the myofibrillar and the connective tissue proteins. To understand what happens when muscle changes into meat, including differences between the various meat cuts, it is necessary to consider the structure, biochemistry, and physiology of these proteins in detail.

Muscle tissue contracts in the living animal and can also contract after slaughter. The amount of shortening either before or after death is governed by skeletal restraint and the effect of antagonistic muscles. Figures 1 and 2 (1) show a muscle with its attachment to bones and a sequence of pictures of increasing magnification to show the structure of a single muscle fibril, 50 to 100 μm in diameter, at the electron microscope level.

American Meat Institute, URL: *http://www.meatami.org/index.htm.*
American Meat Science Association, URL: *http://www.meatscience.org/.*
Code of Federal Regulations, URL: *http://www.access.gpo.gov/nara/cfr/cfr-table-search.html*
Institute of Food Technologists, URL: *http://www.ift.org.*
International HACCP Alliance, URL: *http://ifse.tamu.edu/haccpall.html.*
Meat and Poultry Association Links, URL: http://www.meatpoultry.com/assn.asp
Meat and Poultry Magazine, URL: *http://www.meatpoultry.com/.*
The Meating Place, URL: *http://www.mtgplace.com/.*
National Archives and Records Administration; Federal Register Online via GPO Access, URL: http://www.access.gpo.gov/su_docs/aces/aces140.html.
National Meat Association, URL: *http://www.nmaonline.org/.*
National Pork Producers Council, URL: *http://www.nppc.org/.*
North American Meat Processors Association, URL: *http://www.namp.com/.*
U.S. Department of Agriculture, URL: *http://www.usda.gov/.*
U.S. Department of Agriculture, Agricultural Research Services Nutrient Data Laboratory, URL: *http://www.nal.usda.gov/fnic/foodcomp/.*
U.S. Department of Agriculture/Food and Drug Administration Foodborne Illness Education Information Center, URL: *http://www.nal.usda.gov/fnic/foodborne/foodborn.htm.*
U.S. Department of Agriculture/Food and Drug Administration HACCP Training Programs and Resources Database, URL: *http://www.nalusda.gov/fnic/foodborne/haccp/index.shtml.*
U.S. Department of Agriculture Food Safety and Inspection Service, URL: *http://www.fsis.usda.gov/.*
United States Meat Animal Research Center, URL: *http://www.marc.usda.gov/.*
U.S. Meat Export Federation, URL: *http://usmef.org/.*
Worldmeat, URL: *http://www.worldmeat.com.au/.*

Elizabeth A. E. Boyle
Kansas State University
Manhattan, Kansas
Glenn R. Schmidt
Colorado State University
Fort Collins, Colorado

MEAT SCIENCE

WHAT IS MEAT SCIENCE?

Meat is a food prepared from skeletal muscle. The quality of the meat is affected by various live animal factors as well as the processing conditions. Meat science is a discipline that has evolved to understand the changes that occur from the live animal until the meat is ready for consumption. It should be distinguished from animal science, where meat yield and growth characteristics are considered to be the main aim rather than meat quality itself. This chapter considers all those factors that affect the main meat quality attributes—its tenderness, juiciness, flavor, color, and storage life—considering mainly meat from cattle, sheep, and pigs, with minor references to deer and poultry. Neither processed meats, in which meat and fat tissue are used as a raw material for derived foods, nor meat hygiene or meat packaging are considered. The freezing of meat will be considered, however, as this process effectively locks up the characteristics of the tissue that exist immediately before freezing. Therefore, the resultant product depends on the time postmortem when the meat was frozen, the rate of freezing, and frozen storage conditions.

PRINCIPLES OF MEAT SCIENCE

The major principle of meat science discussed in this chapter is that shortened muscle will be tough when cooked. Meat can age and become more tender, with the aging factors being affected by the amount of shortening and conditions at rigor. This gives rise to a second principle that, since shortening and degree of aging cannot be determined visually, the appearance of a piece of fresh meat cannot predict how tender or tasty it will be. The consequence of these two principles is that the consumer has to have faith that the meat has been processed properly. Such faith is often misplaced. The ultimate aim of meat science is to develop and encourage procedures in the production and processing of animals so that the full potential of tenderness, juiciness, and flavor, as well as good hygiene, can be achieved.

MUSCLE STRUCTURE AND FUNCTION

Definition of Meat and Muscle

Meat comes from the striated muscle of vertebrates. Some of the characteristics of the live muscle underlying meat quality, such as energy stores, connective tissue, and fat, are present at the time of death (see "Animal Factors Affecting Meat Quality," later in this article) and will affect the final meat quality. Other aspects of meat quality, particularly toughness, are affected by processing conditions that interact with some preslaughter attributes.

The muscles of a carcass, whatever the species, perform different functions while the animal is alive and are reflected in differences in physiology, structure, and biochemistry. The variation in these components underlies the differences between the various meat cuts, the use to which they are put, and the way they are cooked. The two major contributors of the muscle that have a bearing on meat quality and underpin meat science are the myofibrillar and the connective tissue proteins. To understand what happens when muscle changes into meat, including differences between the various meat cuts, it is necessary to consider the structure, biochemistry, and physiology of these proteins in detail.

Muscle tissue contracts in the living animal and can also contract after slaughter. The amount of shortening either before or after death is governed by skeletal restraint and the effect of antagonistic muscles. Figures 1 and 2 (1) show a muscle with its attachment to bones and a sequence of pictures of increasing magnification to show the structure of a single muscle fibril, 50 to 100 μm in diameter, at the electron microscope level.

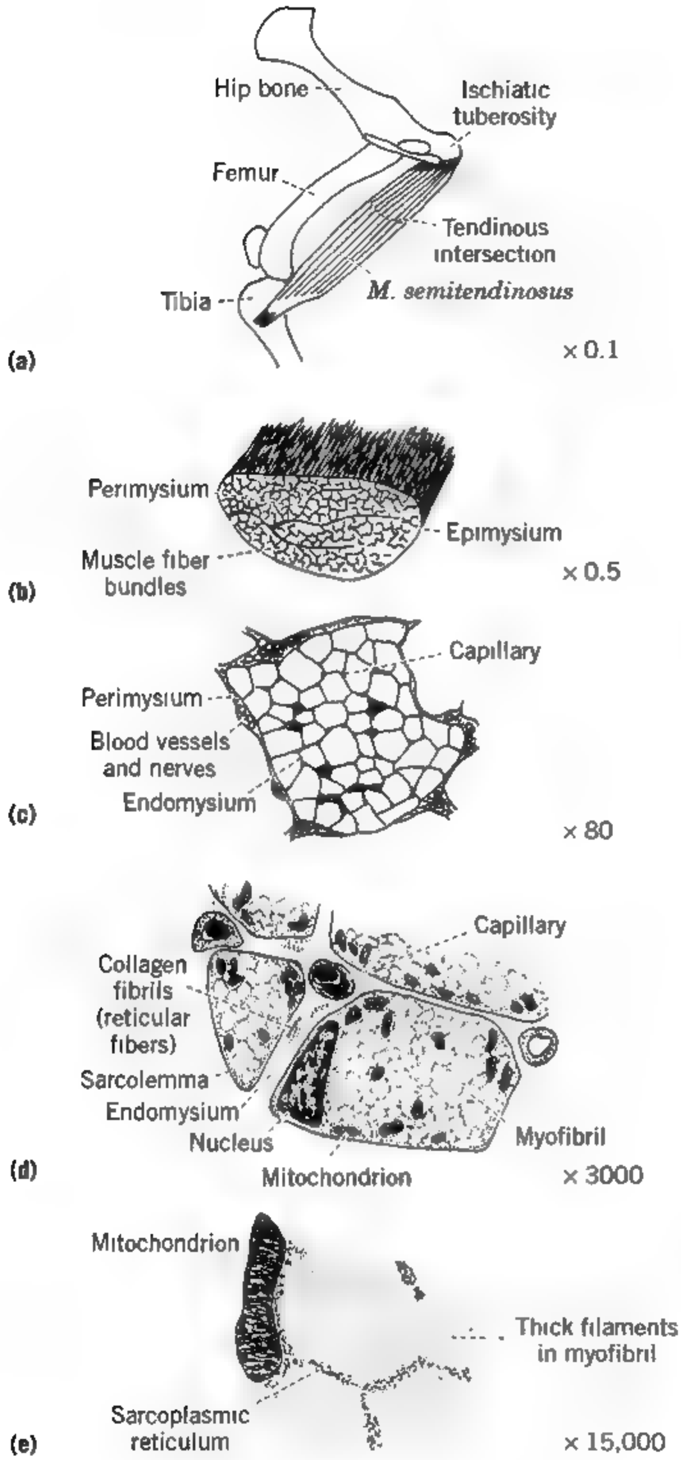

Hip bone
Ischiatic tuberosity
Femur
Tendinous intersection
M. semitendinosus
Tibia
(a)
× 0.1
Perimysium
Epimysium
Muscle fiber bundles
(b)
× 0.5
Capillary
Perimysium
Blood vessels and nerves
Endomysium
(c)
× 80
Capillary
Collagen fibrils (reticular fibers)
Sarcolemma
Endomysium
Nucleus
Myofibril
Mitochondrion
(d)
× 3000
Mitochondrion
Thick filaments in myofibril
Sarcoplasmic reticulum
(e)
× 15,000

Figure 1. Gross and ultrastructural anatomy of the sheep semitendinosus muscle. (**a**) The semitendinosus muscle lies caudally in the thigh region. (**b**) Seen grossly in transverse section, perimysial strands course through the muscle and enclose muscle fiber bundles. (**c**) Within a bundle, an endomysial connective tissue network is revealed by light microscopy to surround individual muscle fibers. (**d**) Electronmicroscopic examination shows a network of sarcoplasmic reticulum enclosing myofibrils within the fibers. The two complete fibers drawn sectioned here are smaller in scale than actual fibers in the sheep semitendinosus muscle. (**e**) At a still higher magnification, a pattern of thick filaments is visible within each myofibril. *Source:* Ref. 1, used with permission.

Muscle Connective Tissue and Collagen Types

Each bundle of muscle fibers is surrounded by a connective tissue sheath, the epimysium, which makes the body of the muscle look like it is made up of strands. Smaller units of connective tissue associated with blood vessels and nerves within the epimysium are termed perimysium, and the feltlike connective tissue sheath around a single fiber (the muscle cell) is termed the endomysium. The types of collagen and their arrangement around muscle fibers have been described (2), and the different arrangement underlies the differences in the various meat cuts. Collagen is discussed later in this article.

Muscle Cell Structure

The single muscle fiber consists of a sarcolemma (termed cell membrane or plasmalemma in nonmuscle cells), mi-

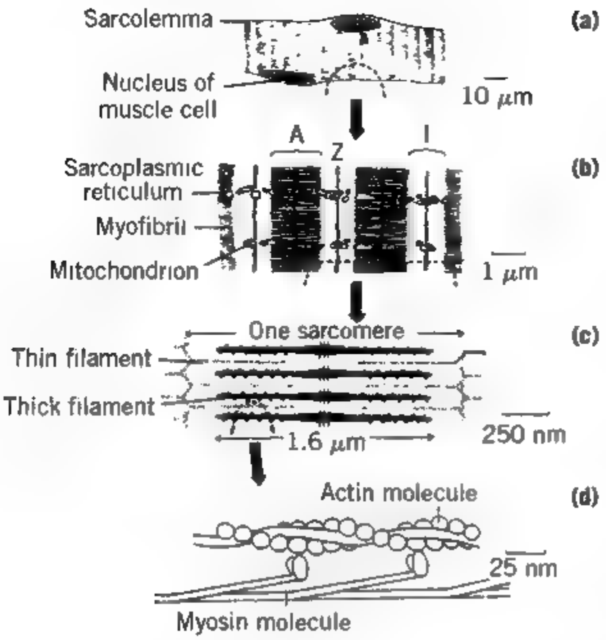


Figure 2. Fibrillar and molecular structure of the contractile elements of muscle. The skeletal muscle fiber in longitudinal section (**a**), as visualized by the light microscope; (**b**) and (**c**), by the electron microscope; and (**d**), as reconstructed from X-ray crystallographic evidence. *Source:* Ref. 1, used with permission.

tochondria, and the normal complement of cell organelles (Fig. 2). In particular, the cell is dominated by a unique arrangement of filamentous proteins, sarcoplasmic reticulum (termed endoplasmic reticulum in nonmuscle cells), and glycogen particles. In an electron micrograph, longitudinal sections show that the filaments consist of both thick (17 nm wide) and thin (8 nm wide) filaments corresponding mainly to the proteins myosin and actin, respectively. The thin filaments are attached to densely staining structures termed Z-lines, whereas the myosin filaments lie between the actin filaments and are joined to each other at the middle portion by an M-line. These structures, which together form a repeating unit from Z-line to Z-line termed a sarcomere, can be seen together with a further explanation in Figure 2. The part of the sarcomere containing myosin filaments is termed the A-band, and the part containing actin filaments is termed the I-band. Other filament structures, including filaments found in the gap of overstretched muscle (3,4), which have been now termed titin filaments, and proteins such as desmin and nebulin, are found with certain isolation, extraction, and staining procedures (see "Muscle to Meat" as follows). In transverse sections, the filaments appear as various size dots, and the Z-lines appear as smudges. When tissue is prepared from muscle at various stages of contraction, longitudinal sections show various degrees of interdigitation and overlap of thick and thin filaments. Transverse sections made at various levels of the sarcomere show corresponding patterns of dots associated with the overlap of the thick and thin filaments. Muscle and meat biochemistry have been reviewed (5).

Lying at the edge of the actin and myosin filament overlap, there is a system of tubules, termed T-tubules, derived from invaginations of the sarcolemma. The other tubular system, that is, the sarcoplasmic reticulum, lies within the cytoplasm of the muscle cell. The sarcoplasmic reticulum stores calcium ions. It is the controlled release of the calcium from the sarcoplasmic reticulum, raising the cytoplasmic calcium levels from 10^{-8} to 10^{-4} *M*, that causes a contraction (a shortening) in living muscle. The calcium ions are then retaken up into the sarcoplasmic reticulum and the muscle cell relaxes. The release of calcium from the sarcoplasmic reticulum is triggered by a wave of depolarisation via the T-tubules. Contraction occurs by the actin and myosin filaments sliding with respect to each other by attachment, release, and reattachment of portions of the myosin molecules (namely, myosin heads, also termed crossbridges), with specific regions of the actin filaments. This contractile activity, initiated via release of calcium from the sarcoplasmic reticulum, is activated and sustained by adenosine triphosphate (ATP). In the presence of calcium and absence of ATP (<1 μmol/g [6]), the muscles are in rigor, usually as a consequence of death. Eventually, ATP is completely transformed to inosine monophosphate, one of the flavor compounds of meat.

Muscle Fiber Types and Their Physiological Role

Although all striated muscles contract, the time course of contraction in the living animal varies with the muscle fiber type (fast twitch, slow twitch), substrate (ATP, creatine phosphate, protein, fatty acid, or glycogen), and the amount of oxygen available. Slow-twitch fibers (Type I fibers or red fibers) are mainly postural and are dominant in aerobic or endurance activity, whereas fast-twitch fibers (Type IIA and IIB fibers) are mainly fast acting and are involved in anaerobic activity. Type IIB muscles can take on a greater aerobic role than Type IIA muscles. Most body muscles consist of different proportions of all types, although instances of pure Type IIA (*m. cutaneous trunci*) or Type I (*m. masseter*) do exist in various parts of the body (7).

MUSCLE TO MEAT

The changes that occur when muscle in a living animal becomes meat are initiated from the moment the circulation stops, that is, slaughter. This stoppage of the circulation generally is preceded by preslaughter stunning and sticking. These events can affect meat quality in many different ways because they are extraordinarily variable. Slaughter by throat cutting can occur without a prior stun, can take place after a shooting with a penetrative captive bolt or nonpenetrative percussion head, or can take place after electrical stunning and be followed by electrical immobilization with important implications that are covered later. From the moment the blood supply stops, nutrients and oxygen are no longer available to the muscle from outside sources, so the energy stores present within the muscle start to be used up. Glycogen particles (see following) lying between the myofilaments and at various locations beneath the cell membrane are slowly depleted as the muscle's energy requirements are maintained, which may continue for a considerable period after slaughter. The depletion of muscle energy stores leads eventually to rigor mortis and a change in status; that is, the muscle is now meat. The pattern and extent of these changes are not the same for every muscle or species of animal and are influenced by a variety of physiological and physical interventions that have a major bearing on the ultimate quality of the meat.

The sliding of the myofilaments relative to each other can occur in muscle from living animals with the contraction initiated by the nervous system, as well as in muscle from animals that have been slaughtered, with the contraction initiated by physical and chemical changes. For example, if the carcasses of freshly slaughtered animals, particularly cattle and sheep, are exposed to cold, and as the normal metabolic arrangements fall down, the leakage of calcium into the cytoplasm (from mitochondria and sarcoplasmic reticulum) can cause irreversible cold contracture (a shortening with no relaxation). This ability to contract can last for many hours after death, depending on postslaughter conditions such as temperature and whether processes such as postmortem electrical stimulation were used.

The theory of muscle contraction based on a sliding of filaments with respect to each other is pivotal to the understanding of changes in muscle and the resulting effects on meat quality. Not only are the contractile filaments important, so too are a recently discovered set of filaments, originally termed gap filaments. These filaments, which contain a protein initially named connectin but now called titin, appear to contribute to the integrity of the myofibril (4,5,8–10) as well as play an important part in the postmortem changes that increase meat tenderness. Other minor proteins such as nebulin have an as-yet-unknown effect on meat tenderness, although desmin is susceptible to proteolysis and is likely to be involved in meat tenderness.

Muscle Energy Supply and Changes During Rigor

In a living muscle, or a muscle just at animal death, the immediate source of energy for contraction comes from ATP. Muscle ATP is quickly used up in two to five contractions, whereupon creatine phosphate immediately becomes dominant. Upon depletion of this source, glycogen takes over. Depending on the intensity and duration of muscle activity, and on the presence of blood circulation, either anaerobic or aerobic glycolysis occurs to sustain activity. Although protein or fat can be useful energy sources in sustained aerobic activity, glycogen is the only fuel that can be used for anaerobic activity. Therefore, glycogen is the only available energy source in postmortem muscle. It is metabolized when the muscle is triggered to contract via physical effects such as cold, and it is also metabolized slowly in noncontracting muscle. Without an intact circulation, lactic acid, a by-product of anaerobic glycolysis, accumulates in the tissue, causing the pH to fall (for reviews, see Ref. 6). At death, muscle tissue from rested, unstressed well-fed animals tends to contain 80 to 90 μmol of glycogen per gram of muscle tissue, but this is highly variable depending on muscle type and animal species. This glycogen allows the muscle to "survive" and contract until the pH falls from around 7.1 to below 6.0 to 6.2 (depending on animal species) and will still provide energy until the muscle goes into rigor at a pH of about 5.5 (Fig. 3) (11).

Immediately after slaughter, the muscle has an initial pH of 7.1 and is floppy and extensible. Upon attainment of rigor, the muscle is inextensible, and the pH has fallen to approximately 5.5. These changes have been explored in detail elsewhere (6,12). If the glycogen stores are less than optimum, the muscle pH cannot fall to the same degree. The consequences of this on meat quality are discussed in the section "Animal Factors Affecting Meat Quality."

Effect of pH on Myofibrillar Proteins and Water-Holding Capacity

Myosin, a highly charged molecule, undergoes some changes related to the pH of the muscle. At the pH of living tissue (approximately pH 7.1), the negative charges dominate, but at a pH of 5.5, which is closer to the muscle protein's isoelectric point, there is a similar number of negative and positive charges, and the capacity to bind water is least. When the muscle is in rigor, the bond between actin and myosin also causes the myofilament lattice to shrink, expelling water (13–15). Thus, for muscle in rigor that has a higher than normal ultimate pH, the lattice will not shrink so much, and the meat will bind water better than at a lower pH. Such meat with a pH greater than 6.0 is termed DFD (dark, firm, and dry). It has been suggested that changes involving the myosin heads by the temperature and pH conditions that exist (namely, rapid fall to pH 6.0 and below, when muscle temperature is still about 35°C) triggered by animal factors, result in pale, soft, exudative (PSE) meat that is caused by an even greater lattice contraction and greater water exudation than normal

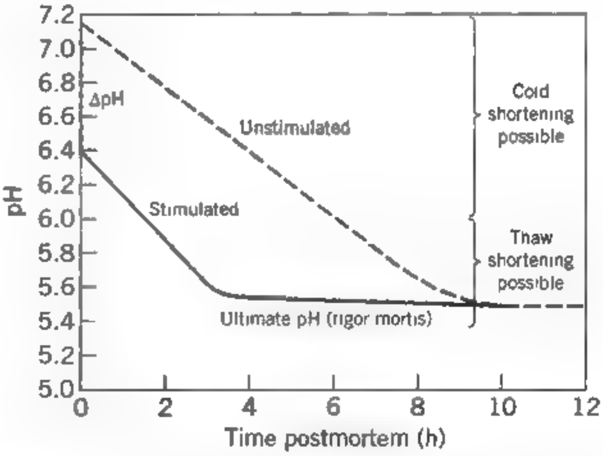


Figure 3. Fall in pH of unstimulated and stimulated beef sternomandibularis (neck muscle) held at 35°C. Unstimulated muscle reaches rigor and ultimate pH (approximately 5.5–5.6) in eight hours. Electrical stimulation for 120 s results in a concurrent fall in pH (ΔpH) to about 6.5. After stimulation, muscle pH falls at an increased rate. The combined effect of these pH falls is a decreased time for the muscle to enter rigor (approximately three to four hours). *Source:* Ref. 11, used with permission.

(15). Both DFD and PSE meat are discussed in more detail in the section "Animal Factors Affecting Meat Quality."

The water content of meat (approximately 70%) is important because it affects consumer acceptability as well as affecting the functional properties of meat and subsequent processed meat products (13). When meat pieces are treated with salt solutions, sometimes with added phosphate, weight gains of up to 40% may occur. Salting muscle before rigor can increase the water-holding capacity over that of meat salted postrigor (13). In salt solutions the fibers swell transversely, and with the addition of pyrophosphate, swelling occurs at lower salt concentrations. Recent work (16) has shown that the presence of the sarcolemma inhibits swelling, and this may cause considerable variation in the ability of different muscles to swell.

Muscle Glycolysis, pH Fall, and Temperature

The rate of production of lactic acid from glycogen in anaerobic muscle, which causes the postmortem fall in pH depends on muscle temperature (17). The rate in beef sternomandibularis muscle is 0.6 pH units per hour at 40°C and 0.15 pH units per hour at 15°C. As the temperature falls below 15°C, the rate increases; this can be attributed to the increased rate of glycogen used during cold shortening. The rate of muscle glycogen depletion can also be accelerated by preslaughter stunning (18), struggle (19), electrical stimulation by a factor of approximately two (20) (Fig. 3) (these effects are important and are covered by the article MEAT AND ELECTRICAL STIMULATION), and mincing (21). Under practical conditions, the rate of glycolysis slowly decreases due to the fall in muscle temperature in the cooling environments usually employed in chilling of carcasses. The rate of glycolysis also varies with muscle fiber type, being faster in beef *m. masseter* (0.4 pH units per hour at 35°C), which contains slow-twitch fibers, compared with the fast-twitch *m. cutaneous trunci* (0.2 pH units per hour at 35°C) (22). After electrical stimulation, the rates do not increase proportionately for particular muscles; the *m. cutaneous trunci* increases by a factor of two, but there is little change in the *m. masseter* (22). Differences in rates of glycolysis exist between species, with pigs having considerably faster rates than cattle. The rates of glycolysis are related to the basic metabolic rate of the animal, being very fast in small animals such as rats (23) and poultry. Chicken carcasses go into rigor in approximately four hours even when a fast chilling regime is used during processing (24). Cold shortening in poultry does not seem to cause the same toughness problems as in other species, as the maximum shortening occurs around 2°C (25), which is generally lower than the temperatures the birds reach following chilling by ice water and air chilling. However, rapid processing would be advantageous, because the toughness that arises from portioning the birds early and removing the breast muscles in particular could be eliminated. Electrical stimulation followed by rapid chilling of the birds to 2 to 6°C has been used to achieve rapid processing (see the article MEAT AND ELECTRICAL STIMULATION.)

The process whereby muscle goes into rigor is termed conditioning by New Zealand workers, and subsequent holding periods are termed aging. These terms are used throughout this article. Other countries use the term conditioning, or alternatively aging, for the whole process of going into rigor together with further postmortem holding. In general, the difference in terminology is unimportant, except when processes, such as electrical stimulation, are used that profoundly affect the conditioning stage. In this article, electrical stimulation is considered to produce its effects merely through acceleration of glycolysis to ensure that cold shortening is avoided and aging will start at high temperatures. This is not necessarily true in every instance, see the article MEAT AND ELECTRICAL STIMULATION.

MYOFIBRILLAR ASPECTS OF MEAT QUALITY

Muscle Shortening and Meat Quality

During normal conditioning procedures, as the muscle goes into rigor there is shortening, which depends on the temperature of the muscle, being greatest at high and low temperatures in unrestrained muscle, and differs with species. Early classical studies (26) showed that the minimum shortening for beef occurred at about 15°C (Fig. 4), and other studies showed that the greater the amount of shortening, the greater the toughening of the cooked meat (27) (Fig. 5). Increases in shortening at rigor temperatures above 15°C are as expected, as such contractures would increase with temperature in a normal way. Increases in shortening with temperature falls below 15° to 0°C are unexpected and are likely to arise from a combination of reduced calcium binding by cell organelles with the fall in temperature, causing an increase in intracellular calcium. The increased contracture by the muscle through increased calcium levels is more than offset by the concomitant falloff in muscle responses at the lowered temperature. A more quantitative analysis of the effect of

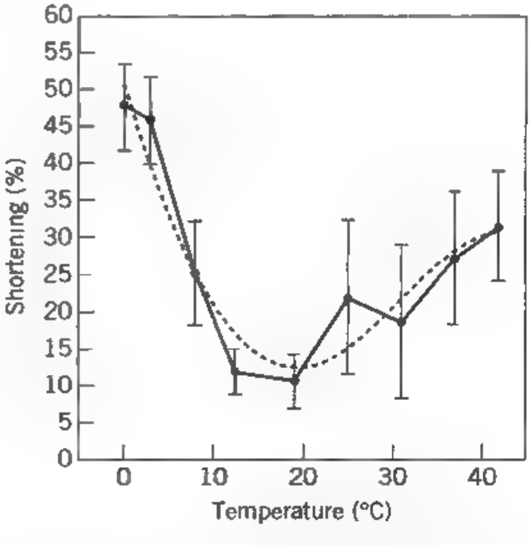


Figure 4. Mean ultimate shortenings of excised beef *m. sternomandibularis* stored at various temperatures during rigor development. A curve of best fit has been included *Source:* Ref. 26, used with permission.

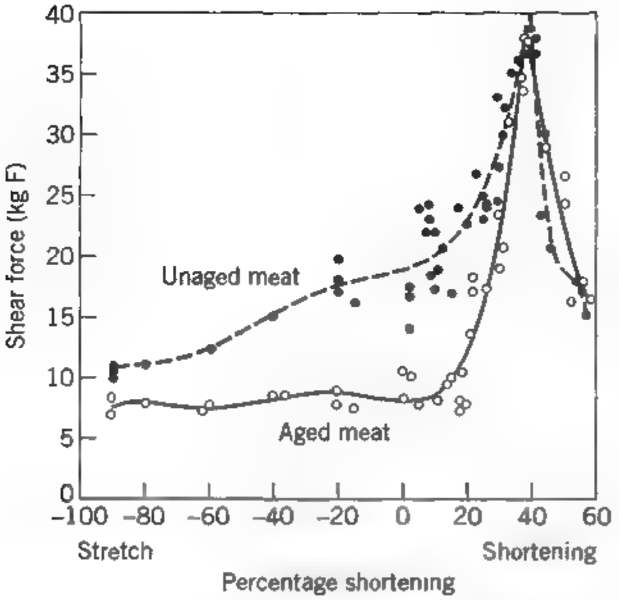


Figure 5. Influence of prerigor muscle shortening on the force score values of unaged and aged beef *m. sternomandibularis*. ●, rigor muscle; ○, muscle aged for three days at 15°C after setting in rigor at different degrees of shortening.[7] At shortenings close to rest, lengths the meat can tenderize, but as the shortenings increase, tenderization becomes less. The tenderization that occurs after the peak is as a result of shortenings being so large, the muscle tears itself apart. *Source:* Ref. 27, used with permission.

temperature on shortening and ATP utilization has been made (28).

Although shortening at temperatures higher than 15°C occurs, it is not strong and can be counteracted at these temperatures by postural alteration (29). With processing temperatures normally experienced, extreme toughness of the cooked muscle does not seem to be a problem except in poultry. Therefore, for most meat species, it seems likely that enzymes involved in postrigor proteolytic breakdown are more effective at higher temperatures and counteract any toughness due to shortening, although there are some problems with rigor at high temperatures where tenderizing enzymes are reduced (see "Variability in Tenderness"). The apparent anomalous shortening at temperatures below 15°C, which increases with colder temperatures, applies to most muscles and is termed cold shortening. Cold shortening is not a theoretical issue; it is a practical problem that results in tough meat, especially when carcasses are subjected to the rapid rates of chilling or freezing that currently take place in meat processing operations. When carcasses are treated in this way, and muscle temperatures fall below 10°C before the pH falls below pH 6.0, the muscles can shorten markedly. With extremes of shortening (arising from cold shortening, thaw shortening, or heat from prerigor cooking) the muscle fibers will supercontract in some regions with a consequent disruption in adjacent regions, leading to a tenderization (30) that corresponds to the tenderness beyond the peak of toughness in Figure 5.

Shortening can be partially prevented by hanging carcasses by the aitch bone (31), termed tenderstretch, or by other maneuvers of the carcass (29), or by electrical stimulation to accelerate rigor and to shorten possible exposure times to low temperatures while shortening can occur. If meat is frozen before rigor mortis is complete, the energy stores ensure that contraction will take place as the meat thaws, that is, thaw shortening (6,32). There are likely to be other as-yet-unknown effects that take place as muscles go into rigor. For example, even cold-shortened muscle becomes somewhat tender after being held at 37°C (33), and meat subjected to extremes of pressure will become tender (34). Even explosives will cause this (35).

The effects of cold shortening are greater when the low temperatures are attained soon after slaughter and seem to progressively disappear at a muscle pH between 6.2 and 6.0. Animals with a high ultimate pH generally reach rigor before shortening is manifest.

Cold shortening does not occur in all muscles of all species under the same conditions of temperature, and perhaps this is the underlying reason for its relatively late discovery. With the masseter, which consists of red (slow-twitch Type I) fibers, cold shortening readily occurs, yet in the cutaneous trunci (fast-twitch Type II A) cold shortening does not occur until muscle temperature reaches 0°C (22). Cold shortening is a problem with sheep and cattle carcasses, but not so much with pigs and poultry. With pig muscle, the rate of rigor is sufficiently fast and the tendency to shortening appears to be less in any event, so cold shortening does not appear to be a problem unless extremely high rates of cooling are involved. With poultry, cold shortening is also not a problem, as maximum shortening occurs at around 0°C and rigor occurs in approximately five hours. However, heat shortening at temperatures above 20°C can occur (25), and meat toughening occurs with stressed birds going into rigor early at high temperatures.

Prerigor muscle excised from the bone is free to shorten large amounts, the amount depending on the restraint of adjoining muscle and connective tissue and its temperature, and electrical stimulation is beneficial. If there is enough shortening, the meat will become tough. The practice of hot boning, therefore, has to be examined with respect to the end use of the meat. If the end use is for manufacturing processed meats, muscle shortening may not be a problem. Cold shortening does not cause changes in water-holding capacity (13), but thaw shortening changes the water-holding capacity, with some disadvantages in further processing. Muscle shortening, and hence toughening of the meat, cannot be predicted by meat appearance. Electrical stimulation can reduce the toughening of meat associated with hot boning for certain cuts (36).

Aging of Meat

Unshortened muscle at rigor has a basal level of tenderness that is enhanced by subsequent aging of the muscle. Severely cold-shortened meat does not age to any degree (Fig. 5) (27). Aging involves the breakdown of the muscle proteins (eg, troponin T, troponin I, tropomyosin, C-protein, and M-protein and cytoskeletal proteins, titin, nebulin, and desmin), by endogenous enzymes, termed calpains (37–39), although minimally involving the major proteins actin and myosin. Calpains are members of the cysteine class of proteases and are inhibited by calpastatins. They exert their effect by altering structural integrity at the junction of the A-I band, as well as fragmentation of the Z-lines. Calpains exist in two forms, μ- and m-calpains, that differ in their calcium requirements. μ-Calpain requires 1–30 μmol of calcium, and m-calpain requires 270 to 750 μmol of calcium for half-maximal activity. Binding of calcium also induces the autolysis of the calpains that results in loss of activity. In the presence of calcium sufficient to activate calpains, calpastatin complexes with calpains to inhibit activity; thus, the way calpain exerts its effects is still unclear. Even so, among the enzymes involved, the calpains seem to be the best candidates to tenderize meat, and the inhibitor calpastatin prevents their activity in normal tissues (40–43). Indeed there is more than enough calpastatin in any muscle to inactivate the calpains. A synergistic contribution by lysosomal (cathepsins) and calcium-dependent proteinases (calpains) has been proposed (44), with no direct relationship between aging rate and protease content of muscles found. The aging rate of meat increases dramatically as the temperature rises and has a temperature coefficient of 2.4, and aging even takes place during cooking but ceases dramatically at a temperature of 66°C (Fig. 6) (45).

As aging is temperature dependent and carcasses are in a cooling environment, processes such as electrical stimulation that produce an early rigor while the carcasses are still relatively warm ensure maximum aging in the shortest possible time (apart from also avoiding the effects of cold shortening). High temperature tends to suggest that conditions are present to allow increased bacterial growth as well as meat aging, but in practical terms, surface drying prevents microbial growth on the meat surfaces. Rapid aging is not required when there is sufficient time available between slaughter and retail for aging to occur. Vacuum packaging or modified atmosphere packaging can ensure that bacterial growth is low under these circumstances. Aging also occurs when frozen meat is thawed and continues during subsequent holding. In certain species such as venison and other game animals, extreme aging is often regarded as desirable.

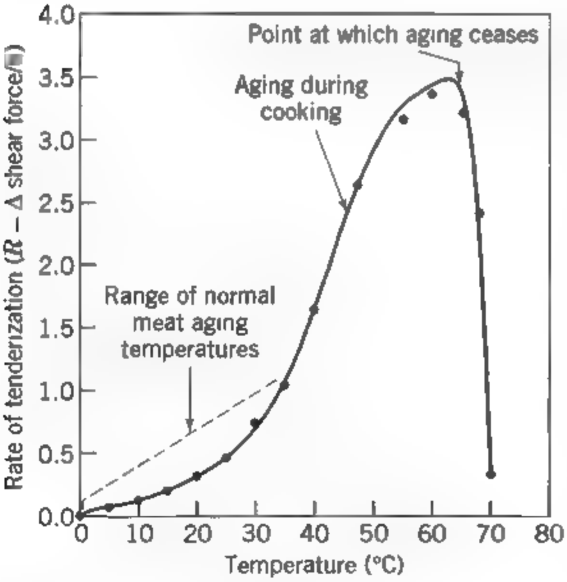


Figure 6. This diagram illustrates how the rate of tenderization of meat, through aging, increases with holding temperature. The rate of tenderization at temperatures of 0°C is relatively slow, and long holding periods are required at this temperature. As the temperature is raised during cooking, the rate of aging has an important part to play in the final tenderness. However, at temperatures above approximately 60°C, aging ceases. *Source:* Ref. 45, used with permission.

Variability in Tenderness

The initial tenderness of meat is not a constant and the tenderness of unaged meat and the extent of aging depends on muscle shortening (27). When one considers the variations of early tenderness of unaged meat, it is clear that there are large shortening-related changes in tenderness in the range close to rest length, and these changes will be influenced mainly by processing conditions, that effectively vary across a carcass. The sarcomere length is significantly changed when the carcass is hung from the pelvis rather than the Achilles tendon, and there are dramatic changes in tenderness with the increased sarcomere lengths (46–49).

Injections of various salts such as calcium (50) and phosphate (51) will increase tenderness and can overcome some effects of shortening. Marination of meat with acids does not dramatically improve tenderness (52), although the flavors added can enhance consumer appreciation. Making the meat alkaline (eg, sprinkling with bicarbonate) does increase tenderness, but with concomitant texture and small flavor changes that make it more useful in various ethnic dishes.

The endogenous enzymes responsible for tenderization are active throughout life in other roles, and by implication their action continues throughout the rigor process. With all inhibiting mechanisms in place effectively until rigor changes in tenderness are small; thus, aging effectively starts at rigor mortis (53).

Tenderization of meat is influenced by shortening, and in particular Locker and Wild (54) showed that proteolysis occurs in both cold-shortened and unshortened meat, but the cold-shortened meat was persistently tough. This suggests that myofibrillar fragmentation length, widely regarded as a measure of proteolysis, also must be considered with regard to processing conditions (53).

Dransfield et al. (56) suggested that there was some inhibition of aging at high rigor temperatures, which was shown by Devine et al. (53) to be greatest at 35°C and least at 15°C, and the mechanism was shown to involve an inactivation of calpains (56). Furthermore, it was found that by tuning electrical stimulation and obtaining the appropriate chilling rate, the greatest tenderness of meat could be achieved rapidly, when rigor occurs at at 15°C (57). Such mechanisms may also explain some aspects of tenderization of slow glycolyzing muscles.

Rapid chilling during hot-boning processing, when meat is removed prerigor, would exacerbate shortening; cold shortening in particular needs to be avoided. However, at 15°C, shortening is minimized and aging is maximized (58), thus making hot boning an effective option under such conditions (see the article MEAT AND ELECTRICAL STIMULATION). Because compounds such as endogenous nitric oxide affect the rate of tenderization (59), tenderness may be influenced by factors such as exercise that affect nitric oxide levels (60).

With so many factors affecting both the initial and ultimate tenderness of meat—animal factors such as breed, genetics, and sex—finishing regimes on meat tenderness must be considered only when rigorously controlling processing conditions (ie, rigor temperature and shortening).

ROLE OF CONNECTIVE TISSUE IN MEAT QUALITY

Closely associated with the contractile myofibrils in muscle tissue are connective tissue components, which form a three-dimensional network to support the muscle cells. The net of connective tissue encompasses complete muscle fiber bundles and even single muscle cells. Embedded in the connective tissue are also intramuscular fat, blood vessels, and nerves. The connective tissue elements contribute in a large way to meat texture as well as modifying meat tenderness.

Composition of Collagen

The major component of connective tissue is the protein collagen, with minor components elastin, glycoproteins, and proteoglycans (61). Connective tissue comprises less than 2% of most skeletal muscles (2). Such a small amount of protein, however, has a major impact on the tenderness of the meat (when other factors such as cold shortening are avoided).

Extensive studies have shown that it is not only the amount of collagen but also the degree to which cross-links in collagen are solubilized during cooking that determine tenderness. Collagen has a high mechanical strength, brought about through the formation within the collagen fibers of intramolecular cross-links (2,61), which are covalent in nature and result from a precise organization in the fiber. Two particular types of cross-links—an aldimine-type bond and a ketoimine-type bond—are replaced by more stable, nonreducible structures with increasing age. As the collagen molecules possess an extremely long biological half-life compared with most other proteins, changes in the numbers and type of cross-links tend to increase, causing the properties of the collagen to change with animal age. Thus, the fibers become progressively stronger and more rigid and less susceptible to enzymatic degradation and swelling by acids as the animals grow older. This maturation is achieved partly by further reactions to form multivalent cross-links that link several collagen molecules laterally, thus dramatically increasing the fiber stability.

Collagen Turnover

Collagen turnover varies considerably between tissues, and the relative growth rates of different muscles result in different levels of maturity of the collagen in a given animal (2). Similarly, differences in growth rates of bulls, steers, and double-muscled animals result in differences in both the amount of collagen and its maturity in terms of stable cross-links (2). Although the faster growth rate of bulls compared with steers would suggest that bull meat should be more tender, the rate of collagen turnover may well be different in bulls and allow earlier maturation of the bull collagen. On the other hand, the reported toughness of bull meat may arise from other factors, such as absence of intramuscular fat, which changes perceptions of tenderness during actual chewing, and sex effects such as an increased stress susceptibility and consequent elevation of muscle pH (see the section "Animal Factors Affecting Meat Quality"). Rapid finishing of animals appears to increase tenderness, which could be accounted for by a higher proportion of newly synthesized and consequently immature collagen being laid down. Possibly, larger amounts of intramuscular fat may also modify taste panel perceptions, by causing a smaller cross-sectional area of myofibrillar and collagen protein in any given bite.

Collagen Solubility

Animal age, due to a progressive increase in interfiber cross-links, has the most significant effect on meat texture, even with decreasing proportions of collagen, as the myofibrillar proteins increase in quantity during growth. The increase in toughness in cattle due to animal age was most clearly demonstrated (62) where some cuts had a fivefold increase in toughness in 5-year-old animals. The study also showed that the tenderness of grilling cuts was least influenced by animal age. Presumably this is because the amount of collagen is constant and low and any adverse effects of cross-linking cannot be expressed, as solubilization does not have time to occur during the brief cooking. This result has been confirmed in a study, using a sensory panel and instrumentally, which showed that toughness in *m. longissimus* did not increase and in *m. semitendinosus* increased in cows aged from 2 to 9 years (63).

For sheep *m. semimembranosus*, collagen concentration is highest in newborn animals, but decreases and becomes constant from 40 to 365 days. Collagen solubility was shown to be greatest in newborns, and as a consequence the meat is more tender (64). While the collagen solubility decreases with age, from approximately 50% in newborns to 31% at 42 days, down to 12% at one year (64), the decreases from then on clearly are not important in terms of tenderness. So although it is true that very young animals are tender from this component, it is not relevant in terms of the age at which animals are normally slaughtered. The studies also showed that high or low values for collagen levels and solubility in the *m. semimembranosus* were also true for the *m. biceps femoris* (64).

Studies on groups of steers and bulls slaughtered at various ages through to maturity also failed to show any major differences in terms of collagen solubility (65) and masculinity traits of bulls in meat quality (66).

Postmortem effects on collagen solubility are not fully explored. Researchers (67) showed that some proteolytic cleavage does occur but with little detectable change in solubility. More recently, it was shown that significant changes occur in the immediate postslaughter period (68), and it may be these early changes that are most important in any investigation of collagen solubility and its effect on tenderness. The effect of electrical stimulation on collagen solubility is unexplored, and the high rates of pH fall and consequent activation of various proteases that occur with electrical stimulation could profoundly affect the collagen molecules. Other connective tissue contributors to toughness may arise from changes in the collagen lattice, which alters significantly with muscle shortening and thus could affect texture (69).

COOKING AND MEAT TENDERNESS

Meat Tenderness/Texture

For consumers, the most important eating quality for meat is tenderness, which is generally rated on a consumer scale where high values are good. If the meat is evaluated by an instrument, the concept of tenderness is reflected in shear force values, where the lower it is, the better the meat. The word *tenderness*, however, does not completely describe the quality attribute, because various processed meats such as frankfurters are tender, yet do not have the texture associated with whole tissue meats. The tenderness/texture quality, a rather individual characteristic, is governed by a complex relationship between the myofibrillar structure and connective tissue structure, the response to which in turn is probably modified by associated juiciness parameters arising from fat and retained water. As a broad generalization, tenderness is more closely allied to myofibrillar factors, and texture is related to the connective tissue components. Texture is often noticed as the wad of tissue that remains after chewing. Ideally, to make a proper appraisal of meat, trained taste panels should always be used, but this is generally not feasible. An objective measuring device such as a tenderometer to measure the shear force is therefore desirable. It should be remembered, however, that many tenderometers measure only myofibrillar tenderness components to any degree of accuracy, although the measurements are influenced by extreme connective tissue variations. To some extent, texture issues are of lesser importance in cuts used for grillings, as only low connective tissue cuts are used.

Tenderometers

When meat is sheared in a tenderometer, the toothlike device or blade deforms the meat, essentially producing yield data for the cooked myofibrillar proteins with some contribution from the collagen. For all practical purposes, changes in the tenderness of meat from the same muscle correspond to changes in myofibrillar tenderness. A wide range of tenderometer devices have been developed and all have faults, but they yield useful information even though not accounting for all possible contributions to tenderness. The devices have been reviewed (70,71).

Only a few major basic designs of tenderometers are in current use (72). In the Warner Bratzler device, cylindrical or rectangular samples are placed in a triangular hole in a shear blade (some modifications use square-sectioned material in a square hole). There are a variety of tooth-shaped biting devices [Volodkevitch type toothed jaws, and a pneumatic tenderometer with toothlike jaws (MIRINZ tenderometer)] that are very similar in concept. In an Allo-Kramer shear press, there is a compression cell with an array of blades to penetrate the meat. Other measurements can also be made that indicate the contribution of connective tissue to toughness, such as the measurement of adhesion between fibers (72).

One difficulty of comparing tenderometer data is that no one has standardized the readings from the various types of tenderometers; thus, at present, each research group is unique in its presentation of data, although some attempt has been made to standardize them (73). All tenderometers work on cooked meat, and no group has yet devised a completely successful instrument for measuring raw meat characteristics that can be extrapolated to predict cooked meat values. There is potential in the use of near infrared spectroscopy where there is a correlation between measured tenderness values (74) and connective tissue solubility and concentration (75), from raw meat and predicted instrumental values, but such measurements only will estimate the tenderness at the time of measurement. From the preceding comments on meat conditioning, aging, and connective tissue changes during cooking, such correlations related to tenderness would need to consider time delays before consumption, as meat tenderness depends on various unpredictable aging treatments once product has left the meat plant. Any control of product is ultimately lost due to unpredictable handling by the consumer, but it should be acceptable at purchase. A noninvasive device applied to raw meat would be extremely valuable at this point.

Cooking of Meat

Tenderness/shear force and cooking have a complex interrelationship and are best discussed together. Cooking is the preferred method of preparing meat for serving. Cooking not only safeguards health by destroying bacteria and parasites, but the heat also coagulates the proteins, transforming a bland, chewy product to a juicy, flavorsome, and beautifully textured food

The final outcome of cooking depends on factors such as the rate of heat transfer. Grilled products have a flavor development on the surface and a variable texture from the seared surface to the softer juicy, bland interior, whereas casserolling gives a complex effect arising from browned surfaces, which cause even deepening of the flavor throughout the whole meat. Roasting allows a slow heat transfer and aging of the meat during cooking.

The juiciness of the product and the contribution of the connective tissue influence the final outcome. Thus, products to be grilled must have low amounts of connective tissue (however, readily hydrolyzable cross-links will not be affected rapidly enough), otherwise they will be tough. The long, slow cooking of a casserole (or a roast) allows aging to take place and also breaks the collagen cross-links. This allows consumption of certain cuts that would be relatively unsatisfactory for grilling. In essence, the tenderness of a piece of meat is a function of the postslaughter handling, including conditioning and aging procedures, the rate of heat transfer with the various denatured muscle cooking methods, and the amount and type of connective tissue present.

The choice of cooking procedure depends first on the amount of connective tissue present and the age of the animal and, second, on whether the meat will benefit from aging by prolonged cooking procedures. Clearly, microwave cooking of meat has few advantages, other than convenience, as the temperature rises too fast in an uncontrollable manner. There is not enough time to break down connective tissue, and the myofibrillar proteins also will not age significantly above 66°C (45) (Fig. 6). If meat has severely cold shortened prior to any cooking procedure, it will never become tender, even with slow cooking. Such meat often is fed to pets, after the consumer finds it almost inedibly tough.

Cooking and Tenderometer Measurements

For tenderness or shear force measurements, and many meat quality assessments, the meat must be cooked under a specified set of conditions, so that either only meat processing or animal-related factors are compared. Meat is cooked by subjecting it to heat before it is either eaten or assessed in other ways. Cooking denatures the myofibrillar proteins as well as the connective tissue proteins. There is a complex interplay between the myofibrillar and connective tissue proteins in the meat as the temperature is progressively raised, including aging of the meat, which is inhibited at temperatures above 66°C (45). Only meat that has not been cold shortened will be discussed.

Changes of Myofibrillar and Connective Tissue Proteins Due to Temperature

The following description (2) of the processes occurring during cooking emphasizes the relationship between the myofibrillar and connective tissue proteins (neglecting aging effects during cooking). As the temperature of a piece of meat is raised, the myofibrillar and connective tissue proteins denature at different rates, and it is the properties of the denatured proteins that determine the texture of the meat. At temperatures between 40 and 65°C there is an increase in toughness (Fig. 7) (76) as determined by shear value, which is caused by aggregation of the denatured myofibrillar proteins. At this stage, there is a loss of fluid and shrinkage of the muscle fibers within the endomysial sheath. The collagen in this sheath is not affected at this temperature. However, because the endomysial sheath was under tension in the raw meat, the shrinking of the myofibrillar proteins releases the tension, which forces fluid out of the meat. As the temperature continues to rise from 63 to 80°C, the shear values increase further due to additional shrinkage as the collagen in the endomysium and perimysium denatures and the water is squeezed out. Complete shrinkage of the sheath is prevented by the presence of the myofibrillar proteins. With further increases in temperature above 80°C, there is a reduction in shear values (increase in tenderness), possibly due to peptide bond cleavage and/or cross-link rupture of denatured collagen and breakdown of myofibrillar proteins. The increased tenderness on prolonged heating is almost certainly primarily due to degradation of the denatured collagen.

During cooking, meat collagen is denatured and, because of its partially crystalline nature, it shrinks at about 65°C (depending on animal age and species) to form insoluble gelatin. The thermally stable cross-links modify the shrinkage characteristics so that when some residual tension is maintained after denaturation, there is considerable remaining strength. The tension generated during cooking varies considerably depending on the heat stability of the perimysium, which in turn is determined by the nature and extent of the cross-linking. Thus, the older the animal, the higher the proportion of heat-stable cross-links and the greater the tension generated on shrinkage. The total amount of collagen in itself is no indicator of strength or texture in the cooked meat. Instead, the solubility of collagen is important, as that changes with animal age and thus affects the consequent cross-linking in a complex way. It is the heat stability of the cross-links that affects the strength/texture components of tenderness. The changes in collagen can cause noticeable effects during normal cooking, as they are responsible for the extensive curling of chops or steaks that occurs during grilling and the amount of cook loss.

The end point of cooking is of interest to the consumer. A rare steak is very pink and has an internal temperature less than 60°C, and a well-done steak is grey to brown at internal temperatures greater than 75°C. Dissatisfaction often arises if the color and "degree of doneness" do not match (77). Meat proteins, especially myoglobin, are more stable to temperature as the ultimate pH rises, and the degree of doneness at the same internal temperature often appears to be insufficient (ie, redder). For institutional cooking, variable ultimate pH values give a variable appearance to the steaks.

Moisture Loss in Cooking

The moisture loss upon cooking is predictable. Whereas frozen meat initially loses moisture on thawing, and drip exudes in vacuum-packaged meat, the total water loss is an inevitable consequence of the cooking process and depends on the degree of cooking (and amounts of connective tissue) as previously described, being least in a very rare steak.

Consumer Aspects of Meat Texture and Tenderness

The overall tenderness of meat to the palate involves three aspects: (*1*) the initial ease of penetration of meat by the teeth (tenderness), (*2*) the ease with which the meat breaks into fragments (tenderness and texture), and (*3*) the amount of residue remaining after chewing (texture). Clearly, some of these attributes are modified by the amount of fat and the temperature at which the meat is served as well as the cooking conditions used. Because consumers clearly reserve tougher cuts of meat for casseroles, there is a perception that certain levels of tenderness are desirable. The less desirable cuts increase dramatically in toughness with animal age due to connective tissue changes (62) leaving more mouth residue, but frying cuts

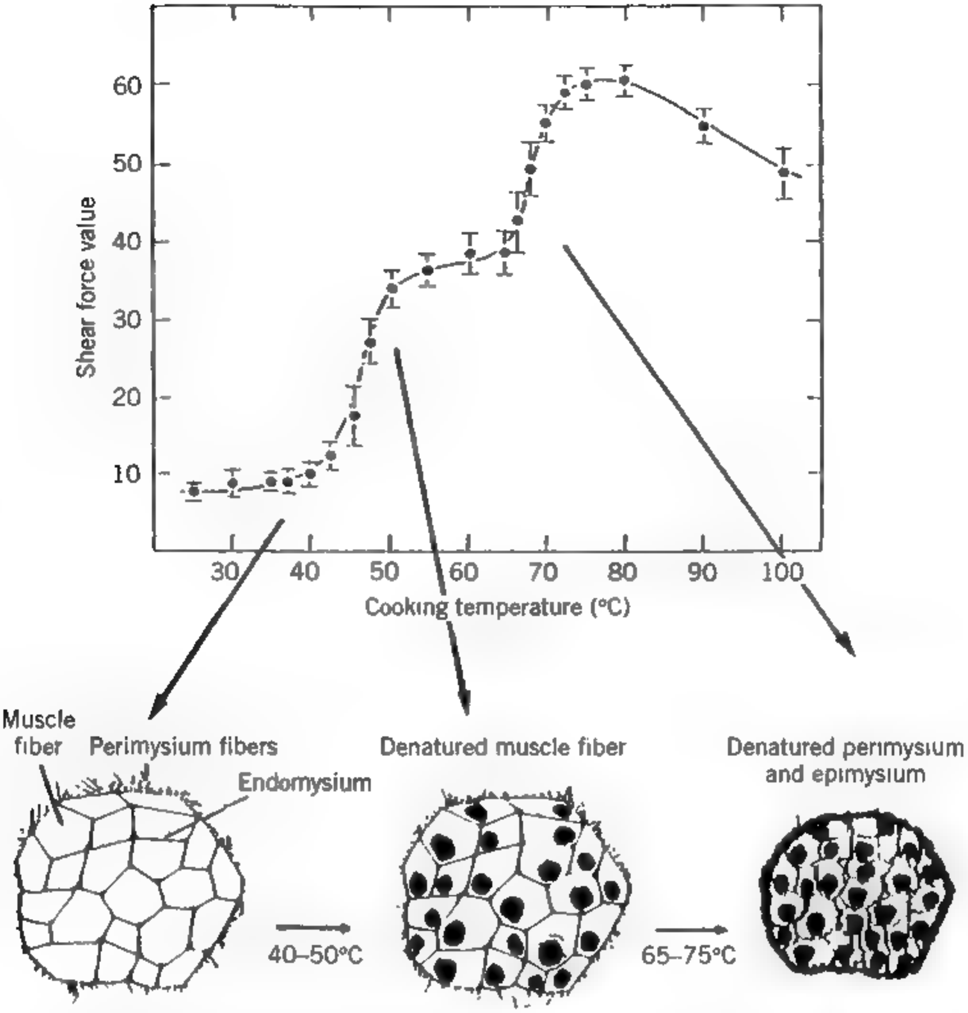


Figure 7. Diagrammatic representation of the changes in the muscle fibers and connective tissue during cooking in a cross section of muscle related to the change in shear value. The lower the shear values, the more tender the meat. The shear force units in this figure are on a different scale and are approximately four times those in other figures. The cooking temperatures for meat are very rare (55°C), rare (60°C), medium rare (65°C), medium (70°C), medium well done (75°C), well done (80°C) and very well done (85°C). *Source:* Ref 2, used with permission.

are virtually unaffected by animal age if cooked to a comparatively rare state. The amount of subcutaneous fat appears to affect tenderness mainly by reducing the chilling rate, and fat within the muscle (ie, marbling) is a poor predictor of tenderness and palatability once there is a minimum amount. It requires large increases in marbling to produce detectable changes in palatability (78,79). Tenderness increases (ie, shear force reduction) and palatability improvement of the cooked meat with excessive marbling are likely because there is less meat, but there is often consumer resistance if they see the meat raw first!

ANIMAL FACTORS AFFECTING MEAT QUALITY

Preslaughter and Slaughter Components

This section in general considers the meat quality changes from the live animal through slaughter, but only considers briefly factors such as breed (ie, genetics), growth, and sex. Apart from stress factors that may be breed or sex related (such as in the case of bulls where pH effects occur—see later) and animal age (which has an obvious influence only at extremes—discussed earlier) and fat cover, which affects chilling rates, the influence of these is minor. This is not to suggest that the preceding factors do not affect meat quality, but rather the effects are minor in comparison with processing effects and are perhaps overemphasized. Cold-shortening capacity appears to be greater in older animals, where the tension generated and shortenings are greater by a factor of three (80). There was shown to be a reduced tenderness in old bulls compared with young ox (81). The muscle used was beef *m. sternomandibularis*, which has a higher connective tissue content than other muscles. We may merely be considering the expected age-related changes in the connective tissue net in a relatively atypical muscle (see age-related toughness changes covered earlier

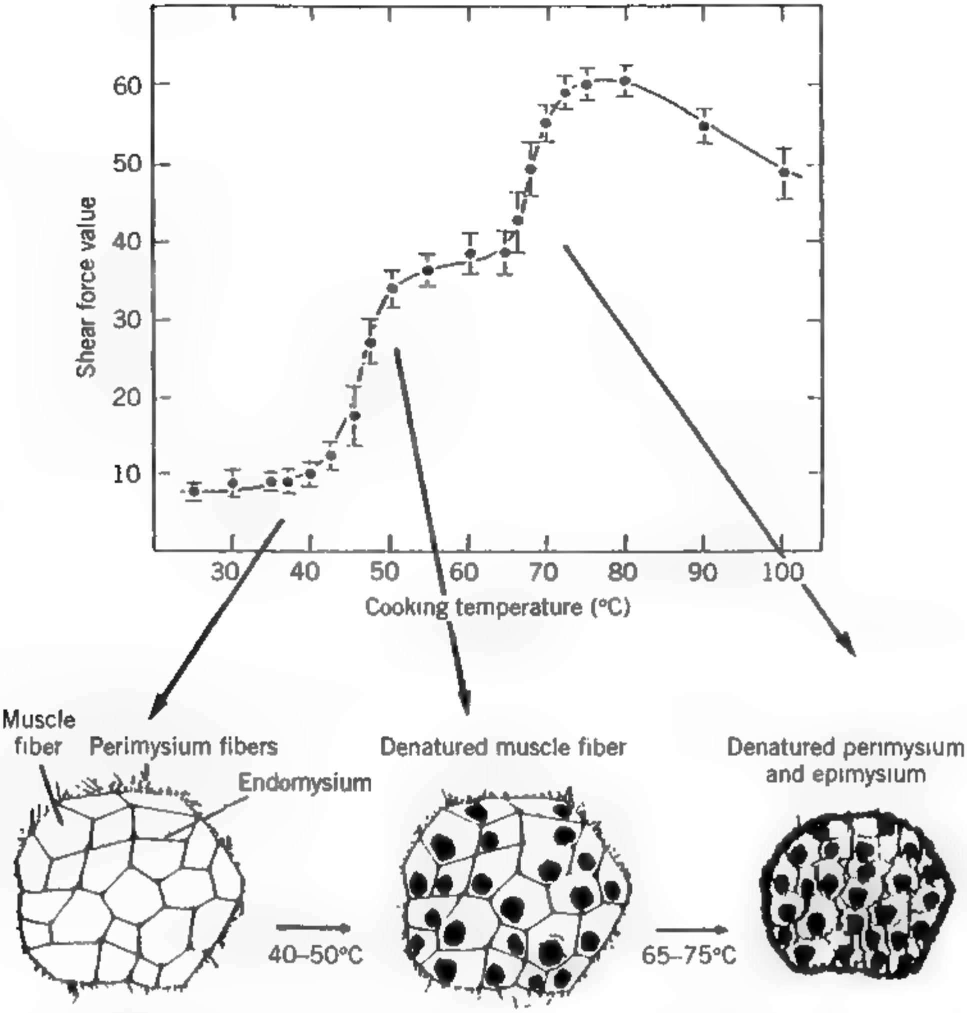


Figure 7. Diagrammatic representation of the changes in the muscle fibers and connective tissue during cooking in a cross section of muscle related to the change in shear value. The lower the shear values, the more tender the meat. The shear force units in this figure are on a different scale and are approximately four times those in other figures. The cooking temperatures for meat are very rare (55°C), rare (60°C), medium rare (65°C), medium (70°C), medium well done (75°C), well done (80°C) and very well done (85°C). *Source:* Ref 2, used with permission.

are virtually unaffected by animal age if cooked to a comparatively rare state. The amount of subcutaneous fat appears to affect tenderness mainly by reducing the chilling rate, and fat within the muscle (ie, marbling) is a poor predictor of tenderness and palatability once there is a minimum amount. It requires large increases in marbling to produce detectable changes in palatability (78,79). Tenderness increases (ie, shear force reduction) and palatability improvement of the cooked meat with excessive marbling are likely because there is less meat, but there is often consumer resistance if they see the meat raw first!

ANIMAL FACTORS AFFECTING MEAT QUALITY

Preslaughter and Slaughter Components

This section in general considers the meat quality changes from the live animal through slaughter, but only considers briefly factors such as breed (ie, genetics), growth, and sex. Apart from stress factors that may be breed or sex related (such as in the case of bulls where pH effects occur—see later) and animal age (which has an obvious influence only at extremes—discussed earlier) and fat cover, which affects chilling rates, the influence of these is minor. This is not to suggest that the preceding factors do not affect meat quality, but rather the effects are minor in comparison with processing effects and are perhaps overemphasized. Cold-shortening capacity appears to be greater in older animals, where the tension generated and shortenings are greater by a factor of three (80). There was shown to be a reduced tenderness in old bulls compared with young ox (81). The muscle used was beef *m. sternomandibularis*, which has a higher connective tissue content than other muscles. We may merely be considering the expected age-related changes in the connective tissue net in a relatively atypical muscle (see age-related toughness changes covered earlier

[62]) Similar studies have not been made on other muscles. Much work in other studies has merely revealed either no statistical difference or a minor difference between breeds and tends to focus on differences between *Bos taurus* and *B. indicus* (82). For pigs, genetic effects on meat quality exemplified by different breeds seem to be more important than for ruminants (83). In general, breed effects would pale into insignificance compared with the processing-related effects on meat quality.

Animal welfare issues for all meat species are not only consumer issues but also need to be addressed for optimum product quality and production efficiency. The effect on meat quality is subtle and is of particular important for pigs, fish, and poultry (84). Preslaughter effects commence on the farm and may arise from simple factors such as insufficient feed. From this point, mustering or rounding up, transport, and moving through races to the slaughter area contribute to bruising and stress, much of which is unnecessary. Different problems arise for pig raising facilities or feedlots for cattle than for range-fed animals. Careful design of pens, races, trucks, ramps, and other animal handling facilities can reduce many of the problems (85), but further research is needed. Washing may reduce stress in the case of pigs or can be stressful in the case of swim washing for sheep.

Slaughter procedures depend on the type of animal involved (84,86). For sheep and cattle, two types of electrical stunning can be used: head-only stunning, which allows the heart to continue beating; and head-back stunning, which causes cardiac arrest. Bleeding out and meat quality are the same with either stunning technique for lambs (87) or with delayed bleeding for beef (88). As head-only electrically stunned animals will recover within a few minutes if they are not slaughtered, the animals actually die through loss of blood initiated by the slaughterman. This type of preslaughter stunning is therefore acceptable for halal slaughter of sheep or cattle. Cattle are generally captive-bolt stunned, although percussion stunning and either type of electrical stunning can be used with no deleterious effects on bleeding out. Carbon dioxide stunning and electrical stunning are used for pigs. Carbon dioxide stunning appears to be associated with a reduced incidence of both blood splash (hemorrhages in muscle) and pale, soft, exudative meat (89), but it also seems likely that this relationship is partially a result of animal selection. Recent studies indicate that there are no welfare or issues of humaneness with current slaughter if properly applied (84,90).

High Ultimate pH (DFD Condition)

The process of anaerobic conversion of muscle glycogen to lactic acid after slaughter reaches an end point when the muscle pH falls to about pH 5.5, at which point complete inhibition of the enzymes responsible occurs even if glycogen is still present. The pH reached when glycolysis ceases is termed the ultimate pH. The ultimate pH will be higher if the initial (preslaughter) stores of muscle glycogen are low. In the worst instances of extreme stress, the muscle ultimate pH does not change from immediate postslaughter values. Such high pH values have important consequences for meat quality. The meat is darker in color, firmer in texture, and dry (DFD) and does not keep as well as normal pH meat in vacuum packaging (91).

Depletion of Muscle Glycogen

The levels of muscle glycogen at slaughter are a result of the cumulative stressors that occur from the farm or feedlot, through transport and lairage to the point of slaughter. Stressors can be classified as psychological if the levels of glycogen are affected by increased levels of adrenaline, producing a concomitant increase of corticosteroids, or physical if they involve physical exercise, sickness, or inadequate nutrition. In the case of physical exercise such as rounding up sheep and cattle, mounting behavior in cattle, or swim washing for sheep, the muscle glycogen is quickly converted to lactic acid, which is transported away from the muscles by the circulation. This lactic acid ultimately can be resynthesized to muscle glycogen, given sufficient time. Feeding immediately preslaughter helps some species, such as pigs, to replenish their muscle glycogen, as the ingested high energy carbohydrate can rapidly be reincorporated into muscle glycogen. In ruminants, however, the process is much slower, because the rumen organisms convert carbohydrates into short-chain acids such as propionic, butyric, and acetic acids, which in turn have to be synthesized into glycogen. Even in conditions of complete rest, for sheep it takes 17 h for muscle glycogen to be resynthesized (92), and in stressful slaughter conditions this is slowed (93). Long-term malnutrition also has the effect of reducing initial muscle glycogen, but this in itself is usually not enough to cause meat with an excessively high ultimate pH, unless it is combined with other factors (94). It is the cumulative effect of many stressors that is important, and malnutrition may merely be the first in the series (95). Psychological stressors, mediated through adrenaline and cortisol release, are related to factors such as transport, contact with dogs in the case of sheep, and interaction with unfamiliar peers in a strange environment for cattle and pigs.

There is probably never a single cause of high-pH meat. It is this multiplicity of causes—many of which are not recognized—that makes identification of causes of high-pH meat in most circumstances difficult. In addition, the response to individual stressors is also different for each species. For example, dogs are identified as a predator by sheep, but they are a mere annoyance to cattle; swim washing for sheep is inordinately stressful because it involves severe physical exercise (96), but washing of pigs and cattle before slaughter can relieve stress. Mixing of cattle (97,98) and mixing of pigs is stressful but does not appear to affect sheep. Mounting behavior in cattle is also stressful (but can be reduced by an overhead electrified grid). Thus, elimination of high-pH animals requires a coordinated effort from the producer right through to the processor.

PSE Condition

Pale, soft, and exudative (PSE) meat appears to be restricted to pigs for all practical purposes and is a consequence of a particular muscle response to animal stress.

The outcome is a very rapid fall in postmortem pH while the meat is hot, which denatures muscle proteins in such a way that water binding is reduced, and the muscle drips and is soft to the touch (14). The denatured proteins scatter light, giving a pale appearance. Rapid chilling postslaughter reduces the incidence of PSE; although the meat is initially tough, it tenderizes with aging (99). Animal agitation and electrical stimulation increase the occurrence of PSE in certain circumstances (100). However, if electrical stimulation is applied at 20 min postslaughter and the chilling rate is sufficiently fast (eg, deep leg to 10°C in 5–10 h), then the meat in those animals susceptible to PSE does not show it and the remaining meat avoids cold shortening (101). The situation is further improved by pelvic suspension (102). Hot boning of pork can allow sufficient cooling to prevent PSE (103). Pale, wet muscle can occur in muscles from very large cattle when chilling is suboptimal and is not a result of stress susceptibility as in the case of pork, but rather of high temperatures and low pH. It is never as severe as it is in pigs.

Interrelationship of DFD and PSE in Pigs

Not only do certain handling situations tend to trigger PSE, but the genetic makeup of a pig may predispose the animal to PSE, and some European breeds are especially susceptible (83,84). In some particularly stress-susceptible animals, there is sudden death, especially during mating. Animals may be tested using exposure to the gaseous anesthetic halothane. Susceptible animals respond by a rise in temperature, which, if not checked, can lead to death (malignant hyperthermia) (see Ref. 104).

In Europe, studies have shown that for pigs there is a complex relationship between PSE and DFD (89). These studies indicated that feeding prior to short transport gives high PSE and low DFD. Fighting lowers the PSE incidence but raises DFD. Short transport times gives high PSE and reduced DFD, but as the transport distance lengthens, the PSE becomes less and the DFD becomes greater. The contribution of the nervous system to PSE is very significant, and even though the effects can be controlled by drugs (105), such treatment is not accepted for food animals. Under ideal conditions, the two extremes in meat appearance, PSE and DFD, can be measured by various electronic grading probes.

Effect of Muscle Ultimate pH on Meat Tenderness

It was shown (106) that mutton with a very high ultimate pH—greater than 6.5—was more tender than mutton of a normal low ultimate pH, and that meat with a moderately elevated ultimate pH was tougher than normal. The latter observation has also been consistently found with bulls and steers (107). Studies using lamb that had been electrically stimulated showed a similar pattern of toughness in moderate-pH animals (Fig. 8) (108). In the range pH 5.5 to pH 5.9, toughness increases sharply. Therefore, small differences in ultimate pH can easily result in "rogue" tenderness results in any experimental situation.

As ultimate pH values are rarely measured routinely during meat processing, and then only with the aim of excluding those animals with pH values over 6.0, the significance of factors that increase the ultimate pH even slightly has not been generally considered. It was suggested (107) that short sarcomere lengths as a consequence of moderate-pH effects were related to increasing toughness. When aging of meat was prevented by injections of zinc salts, the initial toughness of high medium and low pH meat was the same (109). The rate of tenderization of (non-zinc-injected) intermediate pH meat was slowest, suggesting that aging rate rather than toughness is an intrinsic property of high-pH meat (108). This observation is supported by the reduced disappearance of nebulin and titin at these intermediate-pH values (110). This suggests that high-pH meat can tenderize if aging is long enough, but there were still some outliers. It appears that other factors still influence tenderness, and the enzymes responsible for tenderization may be affected by *rigor* temperature and pH in some way (53).

MEAT COLOR

The appearance of raw meat gives no indication of its tenderness and little indication of its flavor. Nevertheless, consumers often use meat color at point of purchase to assure themselves that the meat is not spoiled and will be of good eating quality. This approach raises color to a position far beyond its real value. It is true that color does indicate something about the meat, but only in terms of prior exposure to oxygen or drying conditions; and in extreme instances, it may be a guide to flavor changes. Consumers expect poultry to be white, veal and pork to be a pale pink, lamb and beef to be cherry red. The darkest meat comes from bulls, deer, and horses, even under normal ultimate pH conditions, since these meats have high concentrations of the pigment myoglobin, which gives rise to their dark color.

Color Changes During Processing

In cattle and sheep, the normal color of living muscle tissue is purplish. This is a consequence of both the pigment myoglobin being at a high pH and any oxygen present carried by the blood being rapidly used up by the living tissues, effectively ensuring an anoxic state. Immediately after slaughter, the situation continues, so that for prerigor meat in an unoxidized anoxic state, the myoglobin is dark purple. It changes to a paler, bright red when at rigor with oxygen present. From this point, the color is determined by the proteins myoglobin and hemoglobin, which are affected by muscle temperature and pH postslaughter (111–113). Changes in muscle proteins during rigor development change the light scattering properties of the muscle tissue, so that it becomes paler as the muscle pH falls to pH 5.5, that is, as the pH approaches the isoelectric point of the proteins (114). Translucent meat, which appears dark, occurs when the pH is greater than pH 6.0 and light scattering diminishes (111,114). Thus, antemortem depletion of muscle glycogen results in meat of high ultimate pH and hence darkening in color—so-called dark, firm, and dry (DFD) meat. With high-pH meat as well, there is a closer packing of the myofibrils, with a resultant greater translucency, so the meat is darker in color (14). The as-

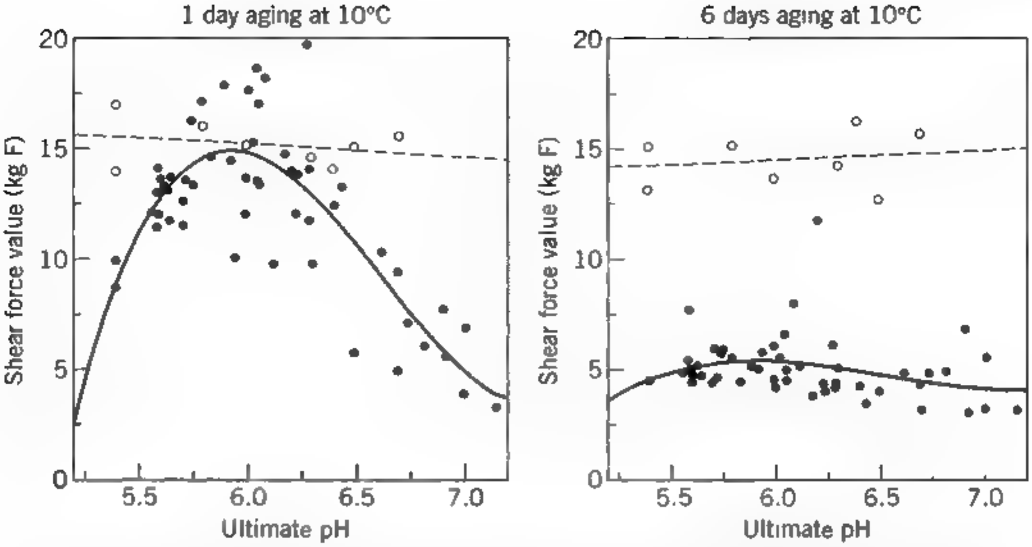


Figure 8. These results, at two aging times, were obtained from an experiment where the aging of some samples was inhibited by injection of zinc before rigor mortis. The shear forces of these muscles were high, were of similar values, and did not change with ultimate pH (○). There is a clear influence of ultimate pH on meat tenderness, where both high and low ultimate pH values result in tender meat, but meat with a moderate ultimate pH is tougher (●). After six days aging at 10°C, the meat with aging inhibited did not change, and all meat reached approximately the same levels of tenderness. The tenderness changes resulting from ultimate pH appear to relate to differences in aging rate, although there are still outliers arising from other factors. In the range of ultimate pH from 5.5 to 5.9, a small increase in pH results in large increases in toughness. *Source:* Ref. 108, used with permission.

sociated physical conditions of firmness and dryness are also associated with closer spacing of the myofibrillar lattice.

With normal ultimate pH values of around pH 5.5, the rate of cooling will also affect the muscle proteins. Portions cooled quickly scatter less light than those cooled slowly, such as regions in the muscle center (eg, beef hind quarter). In addition, aging of the meat also lightens meat color (111). The appearance of the meat is modified by electrical stimulation, as the rapid pH fall results in a more youthful appearance of the muscle, which improves the quality grade in the United States (115).

The most dramatic effect on meat color occurs in pork from stress-susceptible pigs (116). When these animals are stressed before slaughter, there is an extremely rapid fall in pH to pH 5.4 while the muscle is hot. The end result is extreme light scattering via denaturation of myofibrillar and sarcoplasmic proteins, to produce PSE meat.

Color Changes During Storage and Packaging

Red meats vacuum-wrapped in oxygen-impermeable films maintain a dark purple color. Consumer education has not reached the stage whereby this supposedly unattractive color is associated with the inherent advantages of either vacuum or anaerobic packaging for chilled meat, and gas-impermeable packaging for frozen meat. When vacuum or anaerobically packaged meats are removed from their package and exposed to air, the myoglobin oxygenates and the meat color changes from purple to bright red. When this color change occurs, the meat is said to *bloom*.

When fresh meat is exposed to high concentrations of oxygen—for example, as occurs in air, in a high oxygen modified atmosphere pack, or in a pack with an oxygen permeable wrapping—the myoglobin is converted to the bright red oxymyoglobin, which is resistant to further oxidation. However, with packaging in materials of low, but measurable, oxygen permeability, the low partial pressure of oxygen facilitates the formation of another more stable oxidized compound, metmyoglobin. Metmyoglobin is brown, giving the meat an unattractive color. In effect, the myoglobin of meat exposed to either high oxygen or no oxygen remains in a satisfactory state.

Even with exposure to high oxygen levels, the meat will finally turn brown. Metmyoglobin is slowly formed first in the deeper tissues where the critical low levels of oxygen occur. Eventually enough browning is produced that it can no longer be masked by the bright red oxymyoglobin lying above. Presumably, consumer experience with long storage of chilled meats, with eventual browning, has led to the association of dark meat color with "old meat" and spoilage.

Color Stability During Display

The important factors affecting the color stability of fresh meat are temperature, the packaging environment, and ultimate pH, the oxygen consumption and the reducing capacity of the meat. Thus, different muscles exhibit different rates of metmyoglobin formation, a change of temperature from 3 to 5°C in display will double the discoloration rate, and exposure to light also results in a decrease in display life (111–113). Exposure to light also accelerates rancidity development.

Aged meat can have a brighter color after blooming than unaged meat, not only because of increased light scatter and deeper penetration of the light, but also because of decreased catalytic oxidative capacity. However, aged meat has a poorer color stability (111–113).

Under certain conditions, microbial spoilage can affect meat color. Meat can turn green when sulfmyoglobin is formed. This occurs when certain spoilage organisms cause sulfur components to be released. Sulfmyoglobin formation is generally restricted to high-pH meat in vacuum packaging, where the environment is more conducive to sulfur-reducing bacteria (91). The addition of vitamin E to the diet improves the color stability at retail by reducing the metmyoglobin formation (117). This is more important in animals fed in a feedlot than for pasture-fed animals, which, in general, obtain sufficient from their daily food intake.

Frozen Meat Color

The freshly cut surfaces of frozen red meats are a dark purple but are lighter than the surfaces of chilled anaerobic meat because light transmission is inhibited and reflected light is scattered. In addition, only surface pigments can reoxygenate and affect frozen meat appearance, providing a redness overlying greyer myoglobin. Meat that has been frozen slowly is darker than meat that was rapidly frozen, for example, under liquid nitrogen; the difference being due to the smaller size of the ice crystals with rapid freezing.

Because of the low temperature of frozen meat, the deleterious effects of oxidation at the surface occur more slowly. However, various physical effects such as recrystallization occur at the meat surface. These reduce display life by affecting consumer acceptance. For this reason, it is much better to cut frozen meat into cuts immediately prior to display rather than to cut, package, and store the frozen cuts ready for retail display (118). As spoilage or flavor changes are minimal during frozen storage, cutting immediately prior to display is merely for consumer appeal and bears no relationship to actual product quality.

MEAT STORAGE AND FLAVOR CHANGES

Meat Odor and Flavor

Depending on species, meat has a characteristic odor/flavor, but this can be modified in various ways and significantly influences palatability (119). For nonruminants such as pigs and poultry, the type of food eaten (eg, fish meal) can influence the flavor of the meat more than in ruminants, where many flavor compounds are modified by the ruminant organisms. Even so, there are still some quality attributes that are different in pasture-finished and grain-finished animals that depend on the type of food eaten. For example, there are more desirable antioxidants and carotene in pasture-finished ruminant animals, but the flavor profile is different from the grain-finished animal, which has a higher glycogen content that could affect ultimate pH, a different fatty acid profile, and whiter fat. Preference of one over the other is mainly cultural. Animals with a high ultimate pH, via differences in the chemical reactions involving proteins and carbohydrates that take place during the cooking, may have a slightly different flavor than normal-pH animals.

Lipid Oxidation

When fresh meat is held in conditions free from bacterial spoilage, the meat will age and become more tender. In the presence of even small amounts of oxygen, oxidation will also take place at the double bond of the polyunsaturated fatty acids, leading to formation of peroxides that react chemically to produce secondary reaction products (120). These at first have a bland flavor but later result in a typical rancid flavor reminiscent of "old socks." The fats concerned are mainly the phospholipids associated with the cell membrane rather than the depot fatty tissues.

The lipids of raw meat are initially protected from oxidation by endogenous antioxidants, but eventually these antioxidants are inactivated and the rancidity process starts. Exposure to light and heavy metals promotes the onset of rancidity. Rancidity, being initiated by a free-radical reaction, continues even in the absence of additional oxygen, so conditions to minimize its initiation are most important. Vacuum packaging is one option; freezing is another. As all chemical reactions proceed more slowly at lower temperatures, meat should be stored at as low a temperature as practical.

One study on lamb (121) showed that at −5°C, rancidity was initiated, and it continued to progress even when the meat temperature was reduced to −35°C; rancidity did not appear to be initiated at the −35°C temperature, however. This study illustrates that, when considering rancidity development, the entire history of the meat should be considered, rather than mere storage duration at various temperatures.

Although oxidative rancidity changes occur slowly in frozen meat, they occur extremely rapidly in meat after cooking and produce a characteristic "warmed-over flavor." Although the development of warmed-over flavor is reduced if cooked meat is chilled rapidly, this flavor development makes commercial storage of cooked meat for more than a few days a practical impossibility, unless oxygen is prevented from reaching the meat by storing and cooking meat in its own oxygen-impermeable package. The stability of meat lipids to oxidation is lowest in pork and poultry, intermediate in beef, and highest in lamb.

Frozen Storage

Freezing slows down any deterioration of the meat, but changes still occur. Color change (see earlier) and the onset of rancidity are slowed by low temperatures, although rancidity will continue once initiated. When meat freezes, it does so in such a way that extracellular ice crystals first form so that water moves out of the cells. Over time in frozen storage, recrystallization of ice occurs, and the ice so formed can cause structural damage. Fast freezing results in smaller ice crystals and less damage (122). In frozen storage, meat surfaces will sublime water unless pro-

Table 1. Composition of Lean Cooked Meat

	Beef			Lamb		Mutton	Pork		Chicken		
Cut	Rump steak	Fillet steak	Topside roast	Loin chops	Leg	Loin	Loin chops	Leg shank	Whole bird	Legs	Breast
Cooking method											
	grill	grill	roast	grill	roast	roast	grill	roast	roast	broil	broil
Cooked lean (% raw cut)											
	60.3	64.0	61.7	36 0	45.4	25.2	28.9	42 6	37.9	31.0	39.2
Composition of cooked lean tissue											
Energy (kcal)	165.0	210 0	169.0	216.0	206.0	211 0	236.0	174.0	179.0	197.0	166.0
Proximates											
Fat (%)	5.9	10.7	4.9	12.7	11.1	10.8	11.7	7.1	9.4	11.1	4.6
Moisture (%)	64.0	59.0	61.9	60.7	60.7	59.8	52.5	63.6	65.9	63.1	61.8
Protein (%)	27.8	28.3	31.2	25.5	26.5	28.4	32.7	27.5	23.7	24.2	31.2
Minerals											
Calcium	6.0	5.6	4.2	9.8	5.4	10.4	22.6	8.5	6.9	25.0	13.6
Copper	0.12	0.10	0.06	0.04	0.10	0.12	0.06	0.08	0.02	0.04	0.02
Iron	3.6	4.4	3.6	3.6	4.6	4.0	2.7	2.2	3.0	2.6	1.9
Magnesium	20.5	23 0	23.5	20.5	20.5	21.8	22.5	19.5	18.0	15.0	22.5
Phospherous	230.0	260.0	260.0	220.0	240.0	190.0	270.0	240.0	210.0	200.0	260.0
Potassium	400.0	440 0	450 0	380.0	350.0	247.0	440.0	400.0	310.0	270.0	390.0
Sodium	60.0	66.0	59.0	109.0	78.0	50.0	89.0	82.0	71.0	93.0	65.0
Zinc	5.7	4.5	5.9	4.1	5.1	3.4	3.5	3.6	1.4	2.5	1.0
Vitamins											
B1	0.14	0.15	0.09	0.20	0.11	0.14	1.01	0.69	0.09	0.11	0.10
B2	0.14	0.14	0.06	0.08	0.13	0.07	0.06	0.10	0.09	0.17	0.04
Niacin	4.9	5.1	6.0	6.0	5.8	5.5	4.8	4.1	3.8	3.5	8.1
B6	1.04	0.73	0.93	0.52	1.63	0.9	0.41	0.45	0.61	0.70	0.59
B12	3.4	3.9	2.3	1.3	2.1	1.4	0.6	0.5	0.5	1.3	0.6
Cholesterol											
	84.0	106.0	81.0	115.0	109.0	101 0	118.0	100.0	109.0	148 0	112 0

Source: These results were obtained from unpublished studies by MIRINZ for the New Zealand Beef and Lamb Marketing Bureau, Inc., and from Ref. 125.
Note Units are mg/100 g lean tissue unless otherwise indicated.

tected, and the effect on such surfaces is termed *freezer burn*. Sites of freezer burn allow rancidity to penetrate deeper into the meat. When frozen meat is thawed, the reverse of freezing takes place so that extracellular water is produced, but this can be resorbed during postthawing storage if this is possible by the nature of its packaging. The complex aspects of freezing and thawing meat and its effect on quality have been reviewed (122,123).

NUTRITIONAL ASPECTS OF MEAT

The major nutritional components of the meat of all species from fish through poultry to pork and so-called red meats are protein and iron. Although the protein contribution is obvious, the iron contribution is often underrated. Iron deficiency is the most common nutrition disorder, and nonanemic iron deficiency is far more insidious in its effects than normally considered. Even contributes to an impairment of cognitive function (124). Meat is one of the main natural sources of iron (Table 1). The fat component of meat provides desirable energy in certain malnourished communities, but it is possibly erroneously becoming increasingly regarded as a useless by-product in our overfed Western societies. Meat should be regarded as a valuable component of a balanced diet, rather than as a food item providing unwanted calories. The fat component may be either nonseparable fat, as is usually found in the lean of meat, or separable fat that is visible and can be trimmed off. As separable fat can be removed, it comes into the same food category as the skin of vegetables like potatoes or carrots, and therefore the consumer has a choice as to whether to consume it. Diets of meat animals with a high energy content result in the animals having extensive fat deposits; this even extends to the lean in substantial amounts, to give marbling. The contribution of meat to health has been reviewed (126,127).

Nutrient Content of Typical Servings

The nutrient content of some typical cooked 100-g servings of lean from several meat species is shown in Table 1 (125) and would provide approximately 50% of the recommended adult daily requirement for protein, zinc, niacin, and vitamin B_{12} and 15% or more of the requirement for iron, riboflavin, and vitamin B_6. In particular, the iron in meat is important because it is heme iron, which is more bioavailable than inorganic iron, and meat itself enhances the absorption of iron from other sources. Some meats, such as fish and poultry, have less iron than others. Cooked meats lose water, resulting in a proportional increase in protein, fat, and other nutrients per unit weight. The presentation of cooked meat data, therefore, gives a much more realistic indication of the actual food value of meat.

Cholesterol and Fats

Of particular topical interest is the amount of cholesterol in meat, ranging from 80 to 150 mg/100 g of cooked lean meat (Table 1). Phospholipids containing cholesterol are a necessary and expected component of animal cell membranes, with the greatest amounts being found in the brain. In fatty tissues from ruminants, the amount of cholesterol increases above that of the lean by approximately 50%, but even so, values are less than that present in the lean of some fish and poultry. Even considerable detailed research does not show conclusively that cholesterol in meat has adverse effects on health, and the studies giving a bad image to cholesterol have been under attack from many quarters with considerable justification (128). The dietary intakes of cholesterol only supplement cholesterol produced naturally by the body, and extremes of reduction of dietary cholesterol are required to lower serum cholesterol. Hypercholestemic people are at risk, however, but it is unclear whether the high cholesterol levels arise only from diet or from endogenous cholesterol production. Low blood cholesterol levels are implicated in another set of disorders, including cancer. There is increasing evidence that lipid oxidation products rather than cholesterol per se is one of the initiators of arterial injury and the train of events leading to coronary heart disease (129). This would suggest that absolute levels of cholesterol are not as important as other factors.

The saturated fats in meat from ruminants are mainly straight-chain C16:0 and C18:0 compounds, but in poultry and pork, saturated fats contain an increased proportion of branched chains, depending on the diet. Very lean meats such as venison are very low in both depot fats and separable fat. In effect, the fats in venison are primarily those concerned with cell structure and integrity. The lean of most species contains substantial amounts of monounsaturated or monoenoic (C18:1) fatty acids, which are implicated in reductions of low-density lipoprotein cholesterol in the blood, thus having potentially favorable effects on controlling heart disease (130). Sheep and cattle lean also contain over 3% of polyunsaturated or polyenoic C18 and C24 fatty acids, which are predominantly $\omega3$ and $\omega6$ fatty acids. Fish are often promoted as beneficial due to their being a rich source of the $\omega3$ fatty acids (131), whereas meat is erroneously regarded as a poor source of these compounds, despite the fact that meat has been shown to have considerable proportions of both $\omega6$ and $\omega3$ fatty acids (132,133). Because of the relatively greater proportions of meat eaten than fish in many societies, the daily requirements of $\omega3$ fatty acids can easily be met, and, in addition, the nutritionally necessary $\omega6$ fatty acids, virtually absent in fish, can be supplied.

These comments on nutritional aspects are not exhaustive and clearly show a field in flux, as new information is sought to establish further the place of meat in the diet.

BIBLIOGRAPHY

1. A. S. Davies, "The Structure and Function of Meat Tissue in Relation to Meat Production" in R. W Purchas, B Butler-Hogg, and A. S. Davies, eds., *Meat Production and Processing*, Occasional Publication No. 11, New Zealand Society of Animal Production Hamilton, New Zealand, 1989, pp. 43–59.
2. A. Bailey, "Connective Tissue and Meat Quality," *Proc. Int. Congr. Meat Sci. and Technol.* **34**, 152–160 (1988).

3. R. H. Locker, "A New Theory of Tenderness in Meat, Based on Gap Filaments," in *Proceedings of the Reciprocal Meat Conference* **35**, 92–100 (1983).
4. R. H. Locker, "The Role of Gap Filaments in Muscle and in Meat," *Food Microstructure* **3**, 17–32 (1984).
5. A. M. Pearson and R. B. Young, *Muscle and Meat Biochemistry*, Academic Press, San Diego, Calif., 1989.
6. J. R. Bendall, "Post Mortem Changes in Muscle," in G. H. Bourne, ed., *The Structure and Function of Muscle*, Vol. 2, Academic Press, New York, 1973, pp. 243–309.
7. O. A. Young and C. L. Davey, "Electrophoretic Analysis of Proteins from Single Bovine Muscle Fibers," *Biochem. J.* **195**, 317–327 (1982).
8. K. Wang, R. Ramierez Mitchell, and D. Palter, "Titin Is an Extraordinary Long Flexible and Slender Myofibrillar Protein," *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3685–3689 (1984).
9. K. Maruyama, "Myofibrillar and Cytoskeletal Proteins of Vertebrate Striated Muscle," in R. A. Lawrie, ed., *Developments in Meat Science—3*, Applied Science, London, 1985, pp. 25–50.
10. F. Parrish, B. C. Paterson, and J. M. Paxhia, "Titin: A Myofibrillar/Cytoskeletal Protein of Gigantic Proportions," *Proc. Reciprocal Meat Conf.* **41**, 1520–1546 (1988).
11. C. E. Devine, "Electrical Stimulation of Meat: Long-Term Research Vindicated," *Chemistry in New Zealand* **44**, 89–93 (1980).
12. E. C. Bate-Smith and J. Bendall, "Factors Determining the Time Course of Rigor Mortis," *J. Physiol.* **110**, 47–65 (1949).
13. R. Hamm, "Post Mortem Changes in Muscle Affecting the Quality of Comminuted Meat Products," in R. A. Lawrie, ed., *Developments in Meat Science—2*, Applied Science, London, 1981, pp. 93–124.
14. G. Offer et al., "The Structural Basis of the Water Holding, Appearance, and Toughness of Meat and Meat Products," *Food Microstructure* **8**, 151–170 (1989).
15. G. Offer et al., "Myofibrils and Meat Quality," *Proc. Int. Congr. Meat Sci. and Technol.* **34**, 161–168 (1988).
16. P. Knight, J. Elsey, and N. Hedges, "The Role of the Endomysium in the Salt-Induced Swelling of Muscle Fibres," *Meat Sci.* **26**, 209–232 (1989).
17. R. E. Jeacocke, "The Temperature Dependence of Anaerobic Glycolysis in Beef Muscle Held in a Linear Temperature Gradient," *J. Sci. Food Agric.* **28**, 551–556 (1977).
18. C. E. Devine et al., "Differential Aspects of Electrical Stunning on the Early Post Mortem Glycolysis in Sheep," *Meat Sci.* **11**, 301–309 (1984).
19. C. E. Devine, B. B. Chrystall, and C. L. Davey, "Studies in Electrical Stimulation: Effect of Neuromuscular Blocking Agents in Lamb," *J. Sci. Food Agric.* **30**, 1007–1010 (1979).
20. B. B. Chrystall and C. E. Devine, "Electrical Stimulation, Muscle Tension, and Glycolysis in Bovine Sternomandibularis," *Meat Sci.* **2**, 49–58 (1978).
21. R. Hamm, "Post Mortem Breakdown of ATP and Glycogen in Ground Muscle: A Review," *Meat Sci.* **1**, 15–39 (1977).
22. C. E. Devine, S. Ellery, and S. Averill, "Responses of Different Types of Muscle to Electrical Stimulation," *Meat Sci.* **10**, 293–305 (1984).
23. B. B. Chrystall and C. E. Devine, "Electrical Stimulation of Rats: A Model for Evaluating Low Voltage Stimulation Parameters," *Meat Sci.* **9**, 33–41 (1983).
24. J. M. Jones and T. C. Grey, "Influence of Processing on Product Quality and Yield," in G. C. Mead, ed., *Processing of Poultry*, Elsevier Science, London, 1989, pp. 127–181.
25. Y. B. Lee and D. A. Rickansrud, "Effect of Temperature on Shortening in Chicken Muscle," *J. Food Sci.* **43**, 1613–1615 (1978).
26. R. H. Locker and C. J. Hagyard, "A Cold Shortening Effect in Beef Muscles," *J. Sci. Food Agric.* **14**, 787–793 (1963).
27. C. L. Davey, H. Kuttel, and K. V. Gilbert, "Shortening as a Factor in Meat Aging," *J. Food Technol.* **2**, 53–56 (1967).
28. K. O. Honikel, R. Roncales, and R. Hamm, "The Influence of Temperature on Shortening and Rigor Onset in Beef Muscle," *Meat Sci.* **8**, 221–241 (1983).
29. C. L. Davey and K. V. Gilbert, "Carcass Posture and Tenderness in Frozen Lamb," *J. Sci. Food Agric.* **25**, 923–930 (1974).
30. B. B. Marsh, N. G. Leet, and M. R. Dickson, "The Ultrastructure and Tenderness of Highly Cold Shortened Muscle," *J. Food Technol.* **9**, 141–147 (1974).
31. R. L. Hostetler and S. Cover, "Relationship of Extensibility of Muscle Fibres to Tenderness of Beef," *J. Food Sci.* **26**, 535–540 (1961).
32. B. B. Marsh, P. R. Woodhams, and N. G. Leet, "Studies in Meat Tenderness, 5: The Effects on Tenderness of Carcass Cooling and Freezing before Completion of Rigor Mortis," *J. Food Sci.* **33**, 12–18 (1968).
33. R. H. Locker and D. J. C. Wild, "Tensile Properties of Cooked Beef in Relation to Rigor Temperature and Tenderness," *Meat Sci.* **8**, 283–299 (1983).
34. P. E. Bouton et al., "Pressure Heat Treatment of Postrigor Muscle: Effects on Tenderness," *J. Food Sci.* **42**, 132–135 (1977).
35. M. B. Solomon et al., "Hydrodyne-Treated Beef: Tenderness and Muscle Ultrastructure," *Proc. 43rd Int. Congr. Meat Sci. Technol.*, Auckland, New Zealand, July 27–August 1, 1997.
36. B. B. Chrystall, "Hot Processing in New Zealand," in K. R. Franklin and H. R. Cross, eds., *Proc. Int. Symp. Meat Sci. and Technol.*, National Livestock and Meat Board, Chicago, Ill., 1983, pp. 211–221.
37. I. F. Penney, "The Enzymology of Conditioning," in R. A. Lawrie, ed., *Developments in Meat Science—1*, Applied Science, London, 1980, pp. 115–153.
38. I. F. Penney and E. Dransfield, "Relationship between Toughness and Troponin T in Conditioned Beef," *Meat Sci.* **3**, 135–141 (1979).
39. D. E. Croall and G. N. DeMartino, "Calcium Activated Neutral Protease (Calpain) System: Structure, Function, and Regulation," *Physiol. Rev.* **71**, 813–847 (1991).
40. D. E. Goll et al., "Role of Muscle Proteinases in Maintenance of Muscle Integrity and Mass," *J. Food Biochem.* **7**, 137–177 (1983).
41. M. Koohmarie, "The Role of Endogenous Proteases in Meat Tenderness," *Proc. Reciprocal Meat Conf.* **41**, 89–100 (1988).
42. D. E. Goll et al., "Role of Proteinases and Protein Turnover in Muscle Growth and Meat Quality," *Proc. Reciprocal Meat Conf.* **44**, 25–36 (1992).
43. D. E. Goll et al., "Properties and Biological Regulation of the Calpain System," in A. Ouali, D. I. Demeyer, and F. J. M. Smulders, eds., *Expression of Tissue Proteinases and Regulation of Protein Degradation as Related to Meat Quality*, ECCEAMST, Utrecht, The Netherlands, 1995, pp. 47–65.
44. A. Ouali, "Meat Tenderization: Possible Causes and Mechanisms, a Review," *J. Muscle Foods* **1**, 129–165 (1990).
45. C. L. Davey and K. V. Gilbert, "The Temperature Coefficient of Beef Aging," *J. Sci. Food Agric.* **27**, 244–250 (1976).

46. P. E. Bouton et al., "A Comparison of the Effects of Aging, Conditioning, and Skeletal Restraint on the Tenderness of Mutton," *J. Food Sci.* **38**, 932–937 (1973).
47. P. E. Bouton et al., "Changes in the Mechanical Properties of Veal Muscles Produced by Myofibrillar Contraction State, Cooking Temperature, and Cooking Time," *J. Food Sci.* **39**, 869–875 (1974).
48. H. K. Herring, R. G. Cassens, and E. J. Briskey, "Sarcomere Length of Free and Restrained Bovine Muscles at Low Temperatures," *J. Sci. Food Agric.* **16**, 379–384 (1965).
49. F. L. Hostetler, T. R. Dutson, and Z. L. Carpenter, "Effect of Varying Final Internal Temperature on Shear Values and Sensory Scores of Muscles from Carcasses Suspended by Two Methods," *J. Food Sci.* **41**, 421–423 (1976).
50. M. Koohmaraie, J. D. Crouse, and H. J. Mersmann, "Acceleration of Postmortem Tenderisation in Ovine Carcasses through Infusion of Calcium Chloride: Effect of Concentration and Ionic Strength," *J. Anim. Sci.* **67**, 934–942 (1989).
51. J. M. Stevenson-Barry and R. G. Kauffman, "Tenderisation of Beef Muscles by Injection of Salts," *Proc. 43rd Int. Congr Meat Sci. and Technol.*, Auckland, New Zealand, July 27–August 1, 1997.
52. N. Gault, "Marinaded Meat," in R. A. Lawrie, ed., *Developments in Meat Science—5*, Elsevier Applied Science, London, 1991, pp. 191–246.
53. C. E. Devine, N. M. Wahlgren, and E. Tornberg, "Effect of Rigor Temperature on Muscle Shortening and Tenderisation of Restrained and Unrestrained Beef *m. longissimus thoracicus et lumborum*," *Meat Sci.* **51**, 61–72 (1998).
54. R. H. Locker and D. J. C. Wild, "Aging of Cold Shortened Meat Depends on the Criterion," *Meat Sci.* **10**, 235–238 (1984)
55. E. Dransfield, D K. Wakefield, and I. D. Parkman, "Modelling Post Mortem: Texture of Electrically Stimulated and Non Stimulated Beef," *Meat Sci.* **31**, 57–74 (1992).
56. N. J. Simmons et al., "The Effect of Prerigor Holding Temperature on Calpain and Calpastatin Activity and Meat Tenderness," *Proc. 42nd Int. Congr. Meat Sci. Technol.*, Lillehammer, Norway, September 1–6, 1996.
57. N. M., Wahlgren, C. E. Devine, and E. Tornberg, "The Influence of Different pH-Courses During Rigor Development on Beef Tenderness," *Proc. 43rd Int. Congr. Meat Sci. Technol.*, Auckland, New Zealand, July 27–August 1, 1997.
58. C. E. Devine, N. M. Wahlgren, and E. Tornberg, "The Effects of Rigor Temperatures on Shortening and Meat Tenderness," *Proc. 42nd Int. Congr. Meat Sci. Technol.*, Lillehammer, Norway, 1996.
59. C. J. Cook, S. M. Scott, and C. E. Devine, "Measurements of Nitric Oxide and the Effect of Enhancing and Inhibiting It on the Tenderness of Meat," *Meat Sci.* **48**, 85–89 (1998)
60. J. Jungersten et al., "Both Physical Fitness and Acute Exercise Regulate Nitric Oxide Formation in Healthy Humans," *J. Appl. Physiol.* **82**, 760–764 (1997).
61. T. J Simms and A Bailey, "Connective Tissue," in R. A. Lawrie, ed., *Developments in Meat Science—2*, Elsevier Applied Science, London, 1981, pp. 29–59.
62. W. R. Shorthose and P. V. Harris, "Effect of Animal Age on the Tenderness of Selected Beef Muscles," *J. Food Sci.* **55**, 1–8 (1990).
63. N. Madsen, "Effect of Animal Age on Cull Cow Beef Tenderness," *Proc. 43rd Int. Congr. Meat Sci. Technol.*, Auckland, New Zealand, July 27–August 1, 1997.
64. O. A. Young et al., "Collagen in Two Muscles of Sheep Selected for Weight as Yearlings," *New Zealand J. Agricultural Res.* **36**, 143–150 (1993).
65. H. R. Cross, B. D. Schanbacher, and J. D. Grouse, "Sex, Age, and Breed Related Changes in Bovine Testosterone and Intramuscular Collagen," *Meat Sci.* **10**, 187–195 (1984).
66. T. Pietersen et al., "Secondary Sexual Development (Masculinity) of Bovine Males, 2: Influence on Certain Meat Quality Characteristics," *Meat Sci.* **31**, 451–462 (1992).
67. C. Stanton and N. Light, "Effects of Conditioning on Meat Collagen: Part 2—Direct Biochemical Evidence for Proteolytic Damage in Insoluble Perimysial Collagen after Conditioning," *Meat Sci.* **23**, 179–199 (1988).
68. E. W. Mills et al., "Early Post Mortem Degradation of Intramuscular Collagen," *Meat Sci.* **26**, 115–120 (1989).
69. R. Rowe, "Collagen Fibre Arrangement in Intramuscular Connective Tissue: Changes Associated with Muscle Shortening and Their Possible Relevance to Raw Meat Toughness Measurements," *J. Food Technol.* **9**, 501–508 (1974)
70. A. M. Pearson, "Objective and Subjective Measurements for Meat Tenderness" in *Proceedings of the Meat Tenderness Symposium*, Campbell Soup Company, Camden, N.J., 1963, pp. 135–160.
71. B. B. Chrystall, "Meat Texture Measurement," in A. M. Pearson and T. R. Dutson, eds., *Quality Attributes and Their Measurement in Meat, Poultry, and Fish Products*, Advances in Meat Research, Vol. 9, Blackie Academic and Professional, London, 1994, pp. 316–336.
72. P. V. Harris and W. R. Shorthose, "Meat Texture," in R. Lawrie, ed., *Developments in Meat Science—4*, Applied Science, London, 1989, pp. 245–296.
73. B. B. Chrystall et al., "Recommendation of Reference Methods for Assessment of Meat Tenderness," *Proc. 40th Int. Congr. Meat Sci. Technol.*, The Hague, The Netherlands, August 28–September 2, 1994.
74. K. I. Hildrum et al., "Prediction of Sensory Characteristics of Beef by Near-Infrared Spectroscopy," *Meat Sci.* **38**, 67–80 (1994).
75. O. A. Young, G. J. Barker, and D. A. Frost, "Determination of Collagen Solubility and Concentration in Meat by Near Infrared Spectroscopy," *J. Muscle Foods* **7**, 377–387 (1996).
76. C. L. Davey and K. V. Gilbert, "Temperature-Dependent Cooking Toughness in Beef," *J. Sci. Food Agric.* **25**, 931–938 (1974).
77. R. J. Cox et al., "The Effect of Degree of Doneness of Beef Sirloin Steaks on Consumer Acceptability of Meals in Restaurants," *Meat Sci.* **45**, 75–85 (1997).
78. M. E. Dikeman, "The Relationship of Animal Leanness to Meat Tenderness," *Proc. Reciprocal Meat Conf.* **49**, 87–101 (1996).
79. L. E. Jeremiah, "A Canadian/North American Overview of the Contribution of Fat to Beef Palatability and Consumer Acceptance," *Meat Focus Int.* **5**, 175–178 (1996).
80. C. L. Davey and K. V. Gilbert, "Cold Shortening Capacity and Beef Muscle Growth," *J. Sci. Food Agric.* **26**, 755–760 (1975).
81. C. L. Davey and K. V. Gilbert, "The Tenderness of Cooked and Raw Meat from Young and Old Beef Animals," *J. Sci Food Agric.* **26**, 953–960 (1975).
82 G. Whipple et al., "Evaluation of Attributes That Affect Longissimus Muscle Tenderness in *Bos taurus* and *Bos indicus* Cattle," *J. Anim. Sci.* **68**, 2716–2728 (1990)
83. P. D. Warriss et al., "The Quality of Pork from Traditional Pig Breeds," *Meat Focus Int.* **5**, 491–494 (1996)
84. N. G. Gregory, *Animal Welfare and Meat Science*, CABI Publishing, Wallingford, Oxon, UK, 1998.

85. T. Grandin, "Designs and Specifications for Livestock Handling Equipment in Slaughter Plants," *International Journal Studies on Animal Problems* **1**, 178–200 (1980).

86. T. M. Leach, "Preslaughter Stunning," in R. Lawrie, ed., *Developments in Meat Science—3*, Applied Science, London, 1985, pp. 51–87.

87 B B. Chrystall, C. E Devine, and K. G. Newton, "Residual Blood in Lamb Muscles," *Meat Sci.* **5**, 339–345 (1981).

88. J. C. Williams et al., "Influence of Delayed Bleeding on Sensory Characteristics of Beef," *Meat Sci.* **9**, 181–190 (1983).

89. P. A. Barton-Gade et al., "Slaughter Procedures for Pigs, Sheep, Cattle, and Poultry," in H R. Cross and A. J. Overby, eds., *Meat Science, Milk Science, and Technology*, World Animal Science B3, Elsevier Science, London, 1988, pp. 33–111

90. C. E. Devine et al., "The Humane Slaughter of Animals: A Realistic Goal," *Proc. 39th Int. Congr. Meat Sci. Technol.*, Calgary, Alberta, Canada, August 1–6, 1993.

91. K. G. Newton and C. O. Gill, "The Microbiology of DFD Fresh Meats: A Review," *Meat Sci.* **5**, 223–232 (1981).

92. B. B. Chrystall et al., "Animal Stress and Its Effect on Rigor Mortis Development in Lambs," in D. E. Hood and P. V. Tarrant, eds., *The Problem of Dark Cutting in Beef*, Vol. 10, Martinus Nijhoff, The Hague, The Netherlands, 1981, pp. 269–282.

93. G. Petersen, "The Effect of Swimming Lambs and Subsequent Resting Periods on the Ultimate pH of Meat," *Meat Sci.* **9**, 237–246 (1983).

94. A. Howard and R. A. Lawrie, "Studies on Beef Quality: Part II, Physiological and Biochemical Effects of Various Preslaughter Treatments," Department of Scientific and Industrial Research (DSIR) Special Report Food Investigation Board, No. 63, London, 1956.

95. A. Bray, A. E. Graafhuis, and B. B. Chrystall, "The Cumulative Effect of Nutritional, Shearing, and Preslaughter Washing Stresses on the Quality of Lamb Meat," *Meat Sci.* **25**, 59–67 (1988).

96. C. E. Devine and B. B. Chrystall, "High Ultimate pH in Sheep," in S. W. Fabiansson, W. R. Shorthose, and R. D Warner, eds., *Dark Cutting in Cattle and Sheep, Proceedings of an Australian Workshop*, Australian Meat and Livestock Research and Development Corporation, Sydney, Australia, 1989, pp. 55–65.

97. A. Lacourt and P. V. Tarrant, "Glycogen Depletion Patterns in Myofibres of Cattle during Stress," *Meat Sci.* **15**, 85–100 (1985).

98. P. V. Tarrant, "Animal Behaviour and Environment in the Dark Cutting Condition," in S. W. Fabiansson, W R. Shorthose, and R. D. Warner, eds., *Dark Cutting in Cattle and Sheep, Proceedings of an Australian Workshop*, Australian Meat and Livestock Research and Development Corporation, Sydney, Australia, 1989, pp 8–18.

99. F. Feldhusen and M. Kühne, "Effects of Ultrarapid Chilling and Ageing on Length of Sarcomeres and Tenderness of Pork," *Meat Sci.* **32**, 161–171 (1992).

100. O Hallund and J R. Bendall, "The Long Term Effect of Electrical Stimulation on the Post Mortem Fall of pH in the Muscles of Landrace Pigs," *J. Food Sci.* **30**, 296–299 (1965).

101. A. A. Taylor and M Z. Tantikov, "Effect of Different Electrical Stimulation and Chilling Treatments on Pork Quality," *Meat Sci.* **31**, 381–395 (1992).

102. A. A. Taylor and A. M. Perry, "Improving Pork Quality by Electrical Stimulation or Pelvic Suspension of Carcasses," *Meat Sci.* **39**, 327–337 (1995)

103. R. L. J. M. van Laack and F. J. M Smulders, "The Effect of Electrical Stimulation, Time of Boning, and High Temperature Conditioning on Sensory Quality Traits of Porcine *Longissimus dorsi* Muscle," *Meat Sci.* **25**, 113–121 (1991).

104. D. Lister, N. G. Gregory, and P. D. Warriss, "Stress in Meat Animals," in R. A. Lawrie, ed., *Developments in Meat Science—2*, Applied Science, London, 1981, pp. 61–92.

105. P. D. Warriss and D. Lister, "Improvement of Meat Quality in Pigs by Beta Adrenergic Blockage," *Meat Sci.* **7**, 183–187 (1982).

106. P. E. Bouton, P. V. Harris, and W. R. Shorthose, "Effect of Ultimate pH upon the Water Holding Capacity and Tenderness of Mutton," *J. Food Sci.* **36**, 435–439 (1971).

107. R. W. Purchas, "An Assessment of the Role of pH Differences in Determining the Relative Tenderness of Meat from Bulls and Steers," *Meat Sci.* **27**, 129–140 (1990)

108 C. E. Devine et al., "The Effect of Growth Rate and Ultimate pH on Meat Quality of Lambs," *Meat Sci.* **36**, 143–150 (1994).

109. A. Watanabe, C. C. Daly, and C. E. Devine, "The Effects of Ultimate pH of Meat on the Tenderness Changes during Ageing," *Meat Sci.* **42**, 67–78 (1995).

110. A. Watanabe and C. E. Devine, "The Effect of Meat Ultimate pH on Rate of Titin and Nebulin Degradation," *Meat Sci.* **42**, 407–413 (1996).

111 D. B. MacDougall, "Changes in the Colour and Opacity of Meat," *Food Chem.* **9**, 75–88 (1982).

112. D. A. Ledward, "Colour of Raw and Cooked Meat," in D. E. Johnston, M. K. Knight, and D. A. Ledward, eds., *The Chemistry of Muscle-Based Foods*, Royal Society of Chemistry, London, 1992, pp. 128–144.

113. C. Faustman and R. G. Cassens, "The Biochemical Basis for Discoloration in Fresh Meat: A Review," *J. Muscle Foods* **1**, 217–243 (1990).

114. B. B. Chrystall, "Detection of High pH Meat with an Optoelectronic Probe," *New Zealand J. Agriculture Res.* **30**, 443–448 (1987).

115. J. W. Savell, G. C. Smith, and Z. L. Carpenter, "Beef Quality and Palatability as Affected by Electrical Stimulation and Cooler Aging," *J. Food Sci.* **43**, 1666–1668 (1978).

116. J. R. Bendall and J. Wismer Pedersen, "Some Properties of the Myofibrillar Proteins of Normal and Watery Pork Muscle," *J. Food Sci.* **27**, 144–157 (1962).

117. R. N Arnold et al., "Tissue Equilibrium and Subcellular Distribution of Vitamin E Relative to Myoglobin and Lipid Oxidation in Displayed Beef," *J. Anim. Sci.* **71**, 105–118 (1993).

118. V. J. Moore, "Factors Influencing Frozen Display Life of Lamb Chops and Steaks: Effect of Packaging and Temperature," *Meat Sci.* **27**, 91–98 (1990).

119. O. A. Young, T. J. Braggins, and G A. Lane, "Animal Production Origins of Some Meat Colour and Flavour Attributes," in Y. L. Xiong, C. Ho, and F. Shahidi, eds, *Quality Attributes of Muscle Foods*, Plenum, New York (1999).

120. D Mottram, "Lipid Oxidation and Flavor in Meat and Meat Byproducts," *Food Sci. and Technol. Today* **1**, 159–162 (1987).

121. C. J. Hagyard et al., "Frozen Storage Conditions and Rancid Flavour Development in Lamb," *Meat Sci.* **35**, 305–312 (1993).

122. A. Calvelo, "Recent Studies on Meat Freezing," in R. A. Lawrie, ed., *Developments in Meat Science—2*, Applied Science, London, 1981, pp. 125–158.

123. C. E. Devine et al., "Red Meats," in L. E. Jeremiah, ed., *Freezing Effects on Food Quality*, Marcel Dekker, New York, 1996, pp. 51–84.

124. A. B. Brunner et al., "Randomized Study of Cognitive Effects of Iron Supplementation in Non-Anaemic Iron Deficient Adolescent Girls," *Lancet* **348**, 992–996 (1996).

125. J. West and B. B. Chrystall, "Composition of Cooked Tissues from Selected Lamb, Beef, Pork, and Chicken Cuts," *Food Technology in New Zealand* **24**, 23–39 (1989).

126. A. M. Pearson, "Meat and Health," in R. A. Lawrie, ed., *Developments in Meat Science—2*, Applied Science, London, 1981, pp. 241–292.

127. K. R. Franklin and P. N. Davis, eds., "Meat in Nutrition and Health," *Proc. Int. Symp. National Livestock and Meat Board*, Colorado Spring, Colo., September 2, 1981.

128. T. J. Moore, *Heart Failure*, Random House, New York, 1989.

129. P. B. Addis, "Coronary Heart Disease: An Update with Emphasis on Dietary Lipid Oxidation Products," *Food Nutrition News* **62**, 7–10 (1990).

130. S. M. Grundy, "Monounsaturated Fatty Acids and Cholesterol Metabolism: Implication for Dietary Recommendations," *J. Nutr.* **119**, 529–533 (1988).

131. M. S. Feder, "Fatty Acids of Current Interest, Omega 3," *Food Eng.* **59**, 64–66 (1987).

132. A. J. Sinclair and K. O'Dea, "The Lipid Levels and Fatty Acid Composition of the Lean Portions of Australian Beef and Lamb," *Food Technol. Australia* **39**, 228–231 (1987).

133. J. West and B. B. Chrystall, "Intramuscular Polyunsaturated Fatty Acids in New Zealand Meats," Technical Report No. 861, Meat Industry Research Institute of New Zealand, Hamilton, New Zealand, 1989.

C. E. Devine
Technology Development Group, HortResearch
Hamilton, New Zealand

B. B. Chrystall
Massey University, Albany Campus
Auckland, New Zealand

MEAT SLAUGHTERING AND PROCESSING EQUIPMENT

Mechanization and automation of animal stunning, slaughter, dressing, and boning operations can offer many benefits, including improved carcass hygiene through reduced hand-hide cross-contamination, better hide and pelt quality, better quality of the deboned product, improved worker safety, and reduced labor costs. The degree to which these operations have become mechanized or automated varies, depending on the species of animal.

Before animals can be appropriately dressed, they have to be presented to the processing slaughter equipment and personnel in a reproducible way. Thus the most effective method of stunning will be different for each species. Table 1 gives an overview of the stunning techniques currently being used.

OVINES

The Need for Mechanization

As a major producer of sheep meat for export, the New Zealand meat industry has over the last decade invested heavily in the development of slaughter, dressing, and boning equipment for sheep and lambs. This equipment was developed with inputs from meat processing companies, farmer producer boards, government research agencies, commercial engineering companies, and the Meat Industry Research Institute of New Zealand (MIRINZ). The program was initiated in response to increased labor costs and the need to meet more stringent hygiene regulations that had decreased slaughter and dressing productivity.

Table 2 illustrates the effect of carcass size and of mechanization on the labor requirements for processing various meat species from stunning to evisceration. The first column gives the number of worker-hours required to dress 10,000 kg of carcass weight. In the second column these data are adjusted to take into account the fact that mutton, lamb, and beef processing produces two salable products (the carcass and the skin or pelt), whereas for chicken and pork, the feathers and hair are of little economic value. With traditional manual dressing systems, even when the worker-hours are adjusted to take pelt value into account, the labor input for sheep and lambs is two to four times that for the other species. Therefore, for sheep meats to be competitive with other meats, the labor requirement of traditional sheep and lamb slaughter and dressing had to be reduced through mechanization.

Beef and pork have a high average carcass weight, which reduces the labor requirement to produce 10,000 kg of carcasses, because the manning for many operations (ie, hock removal, evisceration) is not affected by carcass size. On the other hand, although chickens have a very small carcass size, the labor requirement is relatively low primarily because chicken processing is heavily mechanized.

Stunning

Two types of electrical stunning, the preferred method for sheep, have been developed: head only and head to body. Head-only stunning, which meets halal slaughter requirements, results in an initially still animal that starts to produce a paddling or running movement even after the throat has been cut. Such movement can be reduced by passing an electric current through the carcass, preferably by using rubbing electrodes after shackling. A head-to-body stun results in cardiac arrest, and the current through the body reduces subsequent movement. Both types of stunning have been easily adapted for automation.

All automatic sheep stunners developed to date have used a "V" restrainer system for controlling the location of the animal throughout the operation. The first automatic stunner for sheep and lambs evolved from a unit developed in Europe for pigs. This unit was then modified for sheep by New Zealand researchers. MIRINZ developed an automatic stunner that used a single "V" restrainer. With this system, each sheep is brought to a position where its head is adjacent to two grids of nozzle electrodes. Once the animal is in place, the two grids move inward until they contact each side of the animal's head. The nozzle electrodes then simultaneously administer electrical current to the head and emit water, which assists passage of current (Fig. 1). The design of this stunning system was improved by New Zealand's Alliance Freezing Company to cope with

124. A. B. Brunner et al., "Randomized Study of Cognitive Effects of Iron Supplementation in Non-Anaemic Iron Deficient Adolescent Girls," *Lancet* **348**, 992–996 (1996).

125. J. West and B. B. Chrystall, "Composition of Cooked Tissues from Selected Lamb, Beef, Pork, and Chicken Cuts," *Food Technology in New Zealand* **24**, 23–39 (1989).

126. A. M. Pearson, "Meat and Health," in R. A. Lawrie, ed., *Developments in Meat Science—2*, Applied Science, London, 1981, pp. 241–292.

127. K. R. Franklin and P. N. Davis, eds., "Meat in Nutrition and Health," *Proc. Int. Symp. National Livestock and Meat Board*, Colorado Spring, Colo., September 2, 1981.

128. T. J. Moore, *Heart Failure*, Random House, New York, 1989.

129. P. B. Addis, "Coronary Heart Disease: An Update with Emphasis on Dietary Lipid Oxidation Products," *Food Nutrition News* **62**, 7–10 (1990).

130. S. M. Grundy, "Monounsaturated Fatty Acids and Cholesterol Metabolism: Implication for Dietary Recommendations," *J. Nutr.* **119**, 529–533 (1988).

131. M. S. Feder, "Fatty Acids of Current Interest, Omega 3," *Food Eng.* **59**, 64–66 (1987).

132. A. J. Sinclair and K. O'Dea, "The Lipid Levels and Fatty Acid Composition of the Lean Portions of Australian Beef and Lamb," *Food Technol. Australia* **39**, 228–231 (1987).

133. J. West and B. B. Chrystall, "Intramuscular Polyunsaturated Fatty Acids in New Zealand Meats," Technical Report No. 861, Meat Industry Research Institute of New Zealand, Hamilton, New Zealand, 1989.

C. E. Devine
Technology Development Group, HortResearch
Hamilton, New Zealand

B. B. Chrystall
Massey University, Albany Campus
Auckland, New Zealand

MEAT SLAUGHTERING AND PROCESSING EQUIPMENT

Mechanization and automation of animal stunning, slaughter, dressing, and boning operations can offer many benefits, including improved carcass hygiene through reduced hand-hide cross-contamination, better hide and pelt quality, better quality of the deboned product, improved worker safety, and reduced labor costs. The degree to which these operations have become mechanized or automated varies, depending on the species of animal.

Before animals can be appropriately dressed, they have to be presented to the processing slaughter equipment and personnel in a reproducible way. Thus the most effective method of stunning will be different for each species. Table 1 gives an overview of the stunning techniques currently being used.

OVINES

The Need for Mechanization

As a major producer of sheep meat for export, the New Zealand meat industry has over the last decade invested heavily in the development of slaughter, dressing, and boning equipment for sheep and lambs. This equipment was developed with inputs from meat processing companies, farmer producer boards, government research agencies, commercial engineering companies, and the Meat Industry Research Institute of New Zealand (MIRINZ). The program was initiated in response to increased labor costs and the need to meet more stringent hygiene regulations that had decreased slaughter and dressing productivity.

Table 2 illustrates the effect of carcass size and of mechanization on the labor requirements for processing various meat species from stunning to evisceration. The first column gives the number of worker-hours required to dress 10,000 kg of carcass weight. In the second column these data are adjusted to take into account the fact that mutton, lamb, and beef processing produces two salable products (the carcass and the skin or pelt), whereas for chicken and pork, the feathers and hair are of little economic value. With traditional manual dressing systems, even when the worker-hours are adjusted to take pelt value into account, the labor input for sheep and lambs is two to four times that for the other species. Therefore, for sheep meats to be competitive with other meats, the labor requirement of traditional sheep and lamb slaughter and dressing had to be reduced through mechanization.

Beef and pork have a high average carcass weight, which reduces the labor requirement to produce 10,000 kg of carcasses, because the manning for many operations (ie, hock removal, evisceration) is not affected by carcass size. On the other hand, although chickens have a very small carcass size, the labor requirement is relatively low primarily because chicken processing is heavily mechanized.

Stunning

Two types of electrical stunning, the preferred method for sheep, have been developed: head only and head to body. Head-only stunning, which meets halal slaughter requirements, results in an initially still animal that starts to produce a paddling or running movement even after the throat has been cut. Such movement can be reduced by passing an electric current through the carcass, preferably by using rubbing electrodes after shackling. A head-to-body stun results in cardiac arrest, and the current through the body reduces subsequent movement. Both types of stunning have been easily adapted for automation.

All automatic sheep stunners developed to date have used a "V" restrainer system for controlling the location of the animal throughout the operation. The first automatic stunner for sheep and lambs evolved from a unit developed in Europe for pigs. This unit was then modified for sheep by New Zealand researchers. MIRINZ developed an automatic stunner that used a single "V" restrainer. With this system, each sheep is brought to a position where its head is adjacent to two grids of nozzle electrodes. Once the animal is in place, the two grids move inward until they contact each side of the animal's head. The nozzle electrodes then simultaneously administer electrical current to the head and emit water, which assists passage of current (Fig. 1). The design of this stunning system was improved by New Zealand's Alliance Freezing Company to cope with

Table 1. Stunning Methods

Method	Uses and limitations
Captive Bolt	
Involves the controlled penetration of a steel bolt (8-mm dia.) into the brain. Energy is transmitted by a shockwave. Projection of the bolt is powered by blank cartridges or air.	Used for calves, cattle, deer, sheep; could be used for most other species; not reliable for pigs. There is a limited effect with animals having large bony skulls. The method is considered reliable and humane, but the bolt must be accurately shot into the head. Brains are not edible.
Percussion	
Percussion stunning is similar to captive bolt in principle but the projectile ends in a large flat or mushroom-shaped head. Energy is transmitted by a shockwave, but because the skull is not penetrated, there is variable dissipation of the energy, and stunning is therefore not controlled.	Used for calves, cattle, deer, sheep; could be used for most other species; not reliable for pigs. With percussion stunning there is a fine line between a reversible stun and irreversible brain damage, leading to doubts about humaneness in every situation. Brains can be recovered. Fulfills halal requirements.
Head-Only Electrical Stunning	
Electric current (50–60 Hz) is passed only through the brain, producing immediate unconsciousness. The animal will recover from the stun if left unslaughtered. Slaughter is by throat cutting or sticking. Electrodes can be in the form of two steel pins spread 5 cm apart with a pistol grip, or in the form of scissorlike tongs for small animals. Restraint and semiautomatic electrode placement is required for large animals such as adult cattle.	Used for sheep, calves, cattle, pigs, could be used for deer. Head-only stunning is humane if sticking quickly follows the stun. Poststun animal movement can be overcome by passing a current through the carcass shortly after slaughter (electrical immobilization). Automatic versions are available. Blood splash and petechial hemorrhages may occur. Brains can be recovered. Fulfills halal requirements.
Head-to-Body Electrical Stunning	
Electric current (50–60 Hz) is passed through the brain with electrodes as above, but current is also passed through the body to stop the heart (causing death) and to produce carcass stillness. Electrodes for the body can be sited on the back, forelegs, or brisket	Used for sheep, calves, cattle, pigs, and poultry; could be used for deer, rabbits. The method gives animal stillness, and humaneness is guaranteed by the heart being stopped. Sticking need not quickly follow the stun. Automatic versions are available Petechial hemorrhages may occur. Brains can be recovered. Nonhalal.
Carbon Dioxide Stunning	
CO_2 stunning involves anesthetizing the animal in an atmosphere of 60–70% CO_2 in air or oxygen. Animals are lowered into a chamber. Apparatuses for high or low throughputs are available.	Used only for pigs at present. CO_2 stunning reduces petechial hemorrhages and is claimed to reduce pale, soft, exudative meat.

horned stock. This improved stunning system uses a dual V restrainer system, in which one conveyor feeds the other. The use of two conveyors allows controlled spacing of the animals. The success rate of the machine was also improved by minor changes to the way the grids of electrodes operated. The machine has also been adapted for head-to-body stunning.

Pelting

Most of the effort devoted to mechanized sheep slaughter and dressing has been in the area of pelt removal. Many research organizations, engineering companies, and other groups have developed and refined various concepts designed to reduce the manpower needed for pelting.

In the late 1970s, the New Zealand meat industry initiated and funded a project to mechanize the pelt removal operation. People began to recognize the hygiene and labor benefits of depelting sheep from the shoulders to the hind legs, a process more easily done by suspending the animal from the front legs; traditionally sheep were depelted while hanging from their hind legs. Systems using this new carcass orientation became known as inverted dressing systems. A six-head rotary pelt removal machine, developed for the inverted orientation, removed the pelt from the belly, lower back, and hind legs by driving a ring between the pelt and the carcass, removing the pelt as a "sock" (Fig. 2). The machine was completed and released in 1982.

MIRINZ took over the project in 1983 and developed a simpler two-stage pelting system as an alternative. This system consists of a shoulder puller (Fig. 3), which removes the pelt from the shoulder and back regions, and a final puller (Fig. 4), which pulls the pelt off the rear legs. MIRINZ has refined the manual input of the inverted dressing system by introducing new butchering techniques to enhance the advantages that the inverted dressing system has over the traditional system, that is, less labor required, lower levels of microbial contamination particularly in the most valuable hind quarter area, reduced skills level required, and lower worker injury risk.

Table 2. Worker-Hours Needed for Producing 10,000 kg Dressed Carcass Weight

	Hours per 10,000 kg carcass weight	Data adjusted for pelt and hide value		Average carcass weight, kg
		Traditional systems	Mechanized sheep chain[a]	
Chicken	25	25		1.5
Lamb	83	66	32	14
Mutton	60	48	23	20
Pork	15	15		65
Beef	22	18		250

[a] Incorporating all of the automated operations listed in Table 3.

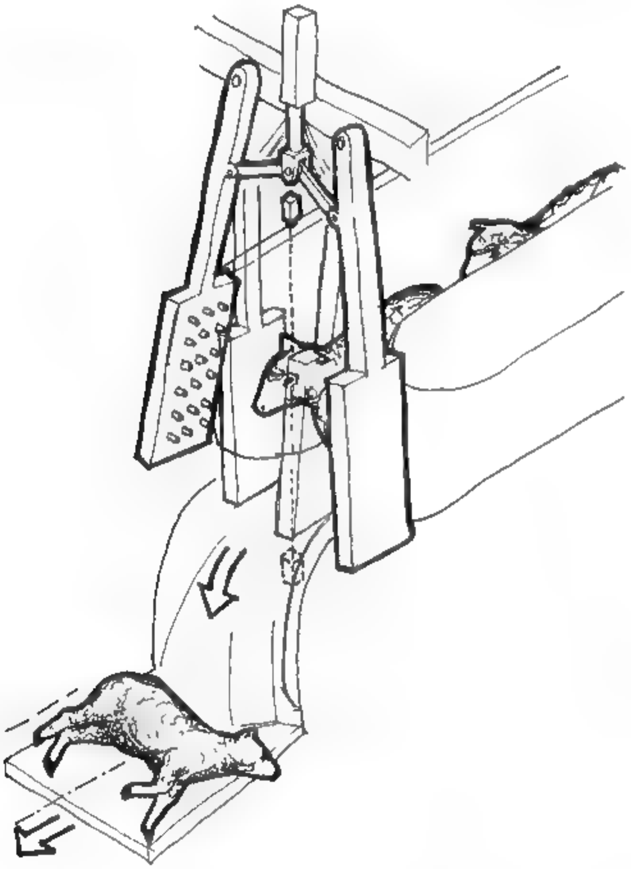


Figure 1. Automatic stunner for sheep.

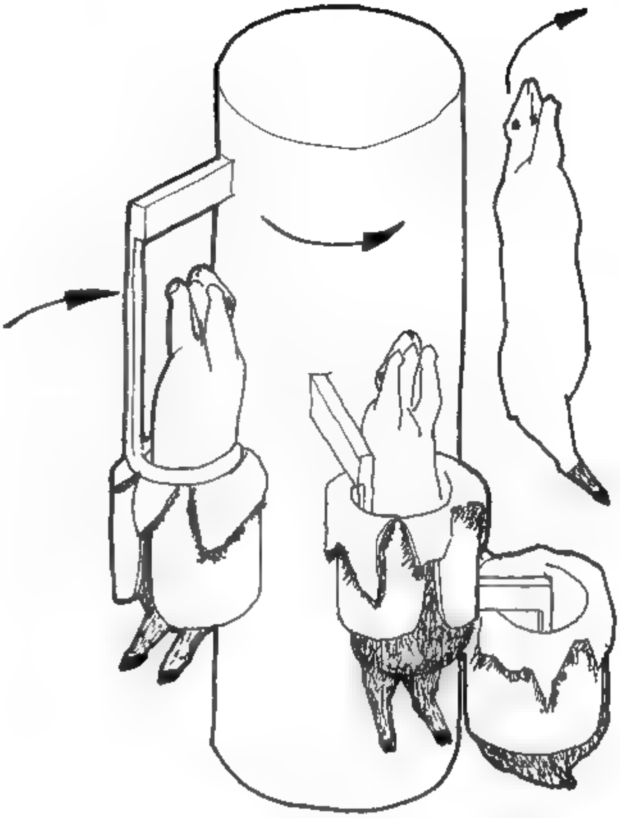


Figure 2. Rotary pelter.

The MIRINZ inverted dressing system, combined with the pelt removal machines developed by MIRINZ, yields pelts of a quality at least as good as those from traditional manual systems. Over the years, various types of hand tools have been developed to aid the removal of the pelt from the sternum or brisket. Recently, MIRINZ has developed a brisket clearing machine (Fig. 5) that simplifies brisket processing, particularly with inverted dressing systems.

MIRINZ has also developed machines to automatically remove the front and rear hocks from carcasses (Figs. 6 and 7). For transferring the forelegs from the wide spreader to the narrow hock holder, a special wide-to-narrow transfer machine was also produced (Fig. 8). The narrow hock holder grips both the radius and ulna bones of the forelegs. Therefore, the front hocks (or trotters) can be removed without losing carcass support. If the front trotters are removed before removing the pelt, the potential for the front trotters to contaminate the carcass is eliminated.

Evisceration

Evisceration and offal (viscera) handling has the next greatest manpower requirement, after pelt removal. The Mechanical Dressing Project has undertaken several developments to partially mechanize evisceration and offal handling. Brisket cutting and belly opening was the first area studied. An early machine mechanically cut the brisket of inverted carcasses. A new machine has since been developed that not only cuts the brisket but also opens up the belly area. This machine is now awaiting production trials in a meat plant. Work is continuing on improved and mechanized methods of ovine viscera removal and handling.

Head Processing

In 1975 head skinning became necessary for sheep meats destined for export to the EEC. As part of carcass inspec-

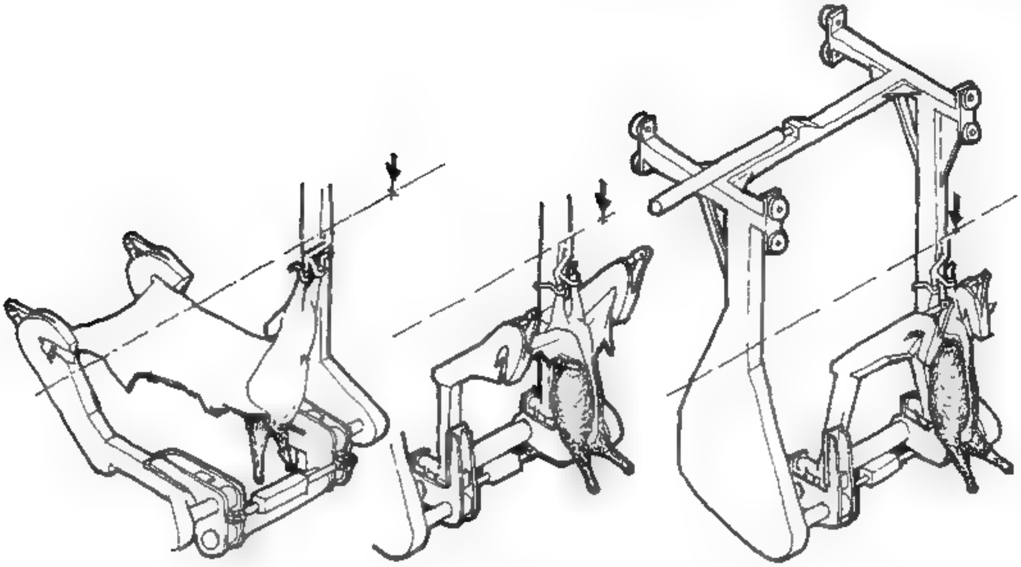


Figure 3. Shoulder puller.

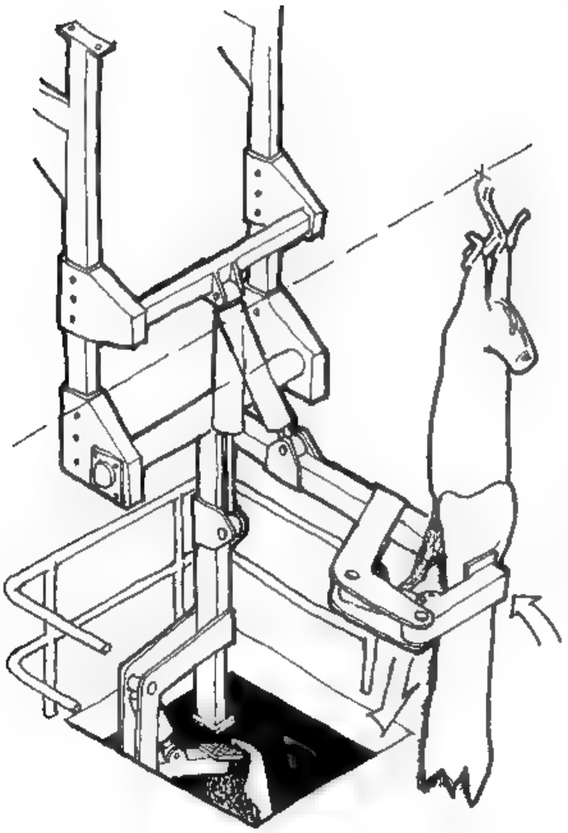


Figure 4. Final puller.

tion, the head, in a totally skinned state, had to be presented with the carcass for examination. In response to this requirement, several head-skinning machines were developed. The most successful was a machine that incorporated a small shaft that gripped a flap of skin near the nose and removed the skin by a rolling action. This machine was used in most meat plants throughout New Zealand and Australia.

The EEC regulation requiring heads to be presented with the carcass was partially relaxed in 1987, so that inspection was required only for those heads from which edible brains and tongues were to be saved. Therefore, the focus of developments in head processing (skinning and brain and tongue removal) has changed.

The Mechanical Dressing Project has recently developed several machines, including one that automatically severs the atlas joint at the base of the skull (Fig. 9), and one that automatically splits the head, followed by automatic extraction of the brain (Fig. 10).

Performance of a Mechanized Sheep-Dressing System

The potential manning levels for a mechanized sheep slaughter and dressing system in New Zealand, based on commercial developments so far and processing eight lambs per minute, are given in Table 3. This manning contrasts with that of the traditional manual system of 45 plus 15 assistants for the same throughput.

Carcass Hygiene

In 1988 a New Zealand meat plant replaced its traditional dressing system with an inverted system, along with four machines: wide-to-narrow transfer, front hock remover, shoulder puller, and final puller. This system processed 3500 lambs per day (one dressing chain). The plant, as part of its quality control program, regularly swabbed set places (flap, leg, brisket) on the surface of randomly selected carcasses, to give aerobic plate counts. These data reflect the hygienic condition of the carcasses processed in the old and new dressing systems.

With the new system, only 1% of the counts were above $10^4/\text{cm}^2$, whereas with the old system 11% were above that

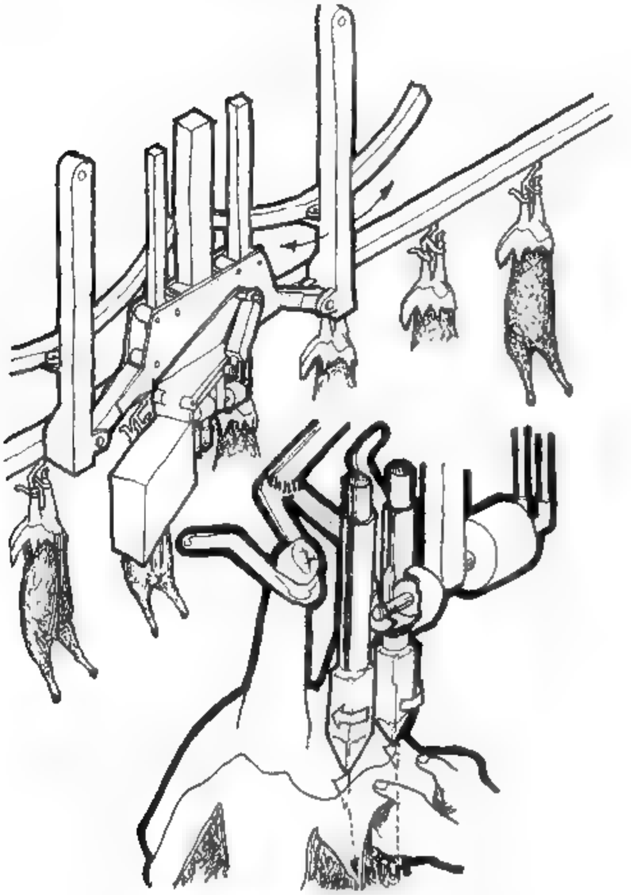


Figure 5. Brisket clearing machine.

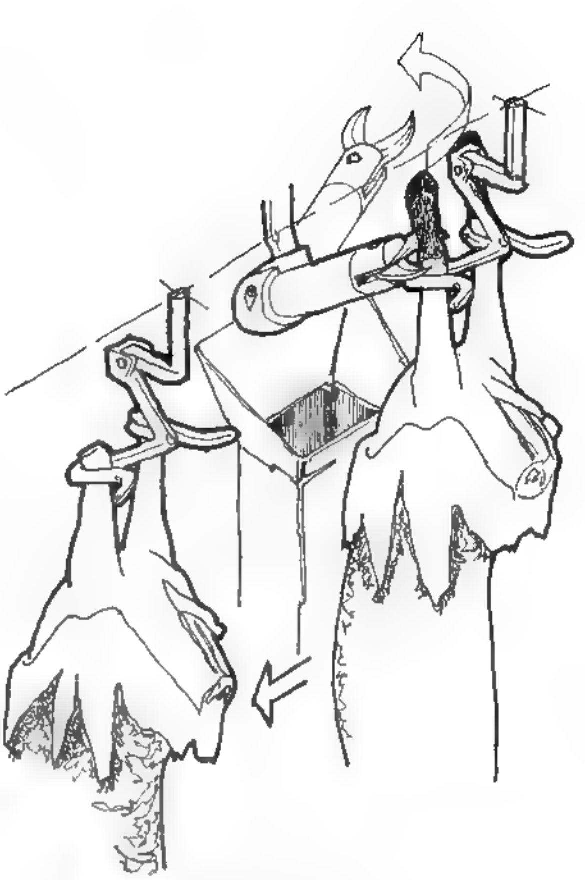


Figure 6. Front hock remover.

level. The new system also gave a lower mean count than did the old manual system (Fig. 11).

Sheep and Lamb Boning and Cutting

Recent developments at MIRINZ in the mechanization and automation of sheep and lamb boning and cutting are showing a number of benefits, including the production of structurally intact whole-tissue meat, consistent and improved meat quality, greater meat recovery (higher yield), greater operator productivity, and reduced labor costs. Two developments are described here; one for whole mutton carcass boning, the other for boning short loins on both sheep (mutton) and lamb.

Whole Carcass Boning—The Frame Boner. The frame boner removes the two soft sides from mutton carcasses. Each soft side includes an intact form: the shoulder and foreleg bones (scapula, humerus, ulna, and radius), the leg bones (femur, tibia, patella, and fibula), and the muscle, connective tissue, and fat on the shoulders and legs, on the top and both sides of the neck bones (cervical vertebrae), and external to the backbone (thoracic and lumbar vertebrae), rib bones and aitch bones. A fully automated machine configuration is used, to minimize labor input, maximize yields, maximize processing rate, produce a product of consistent high quality, and accommodate and adjust to a range of carcass sizes.

The frame boner consists of four main components: the load station, the pedestal and carcass support, the linear drive and boning head, and the control cabinet. The physical relationship of each component to the machine as a whole is shown in Figure 12. The boning process performed by the frame boner consists of a sequence of five operations:

1. Lifting a carcass off the rail, removing the gambrel, loading onto the carcass support, and making carcass length measurements; performed at the load station.
2. Rotating the carcass support about the horizontal axis to present the carcass to the boning head; performed on the pedestal.
3. Clearing the pelvis on the upward travel of the boning head by grasping and pulling the rear legs; performed at the linear drive.
4. Boning off the soft sides on the downward travel of the boning head using front leg tensioners together with a combination of rotating knives, flexible discs, ploughs, and moving wires to do the cutting; performed at the linear drive.
5. Ejecting the skeletal frame during carcass support rotation; performed on the pedestal.

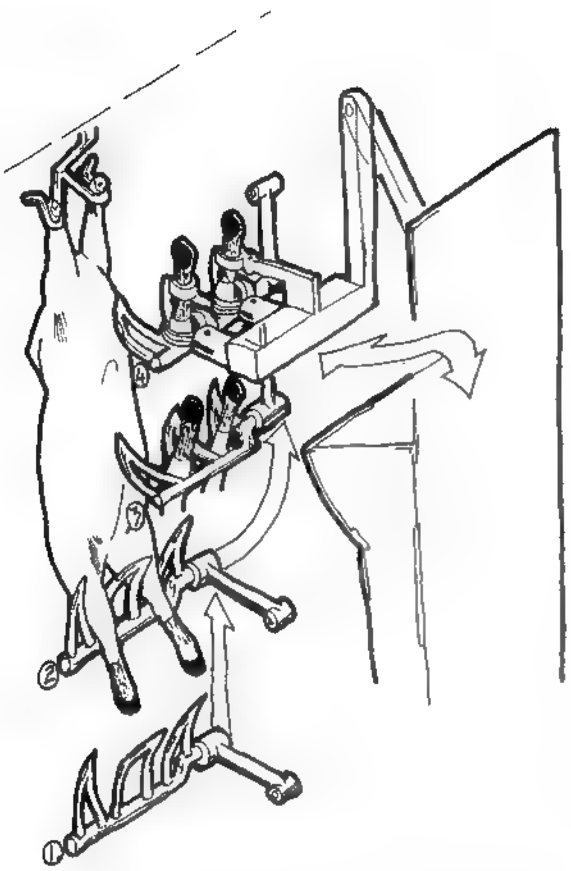


Figure 7. Automatic rear hock remover

Figure 12 shows the machine with a single carcass support. A production machine would have two carcass supports fitted. With twin carcass supports, operation 1 would take place simultaneously with operations 3 and 4.

The boning process is controlled by programmable logic controllers (PLCs) interfaced to the human operator through a control panel. In this PLC-based system, individual components can be either cycled independently for checking and adjustment purposes or cycled together to perform the automated boning process. The machine can process over 200 thawed or chilled carcasses per hour (with twin carcass supports), and extra-heavy carcasses do not slow down the boning process. The yield is between 71 and 75% of the carcass weight for bone-in, untrimmed soft sides and is greater than 63% of the carcass weight for boneless, untrimmed soft sides. (These yields are based on average carcass weights of between 19 and 23 kg and assume that the shoulder and leg bones are 8% of carcass weight.)

Product quality is consistent within carcass grades, and the surface of backstraps (longissimus dorsi muscle) is smooth and even, with a complete absence of knife cuts. The machine gives significant labor savings. A production rate of 200 carcasses per hour is approximately equivalent to the work of five boners performing manually the equivalent breaking-down operation. Some workup on the carcasses prior to boning on the machine is necessary; for example, fillet removal, a V cut over the tail, and neck and pelvis underside clearing. This, together with machine supervision duties, is expected to require two people; thus giving a worker saving of three people.

Part Carcass Boning—The Loin Boner. The loin boner is designed to remove the two loin eye muscles (longissimus dorsi) from lamb and mutton short loins (short saddles) that contain the lumbar vertebrae. This machine, which is designed to fit existing boning rooms, is compact and robust and allows either one or two person operation. The loin boner consists of a horizontally moving carriage on which the loin support and clamp are mounted, a vertically moving assembly on which the knives and ploughs are located, a control system consisting of a PLC, pneumatic valves, motor speed controller and operator's console, a frame, and covers to provide hygienic machine protection.

In the boning process, a short loin is placed on the support and clamped manually. When the boning cycle is initiated by the operator, the support with the short loin saddle proceeds along the machine. Simultaneously, the knives and ploughs move downward to land on the support just prior to the loin passing under them. Boning is achieved during the passing of the loin under the knives and ploughs, the knives cutting either side of the vertical bones closely followed by the ploughs, which clear from the chine and horizontal bones. Thus, the muscles are separated from the bones. The knives and ploughs are then raised to allow the support to return to the operator for muscle and bone removal.

The machine can process up to six loins per minute with one operator and eight loins per minute with two operators. Compared with existing manual boning methods, there is a greater than 10% increase in muscle removed. At a production rate of three loins per minute, two fewer people are required than for the equivalent manual boning process. Product quality is excellent, as the boned loin has a consistent size and shape, and a smooth surface finish with a complete absence of knife cuts. This boning process is being extended to long saddles (6–8 thoracic vertebrae plus the lumbar vertebrae). Figure 13 shows the loin boner machine layout with a dual-purpose loin and long saddle support.

PIGS

Although the basic steps of the pig slaughter process have not changed in the last 50 years, considerable development has taken place to improve and optimize each of these steps. These steps include lairage, stunning, scalding, dehairing, singeing, and evisceration. To maintain the best possible hygiene level within the slaughter process, much attention has also been given to product flow, to ensure that material for human consumption, material for pet food, and waste material are kept appropriately separated.

Lairage and Handling

The conditions existing for the animal prior to stunning have been a subject of concern for the last 10 to 15 years. Preslaughter holding conditions and animal handling have been recognized as areas that can greatly contribute to the

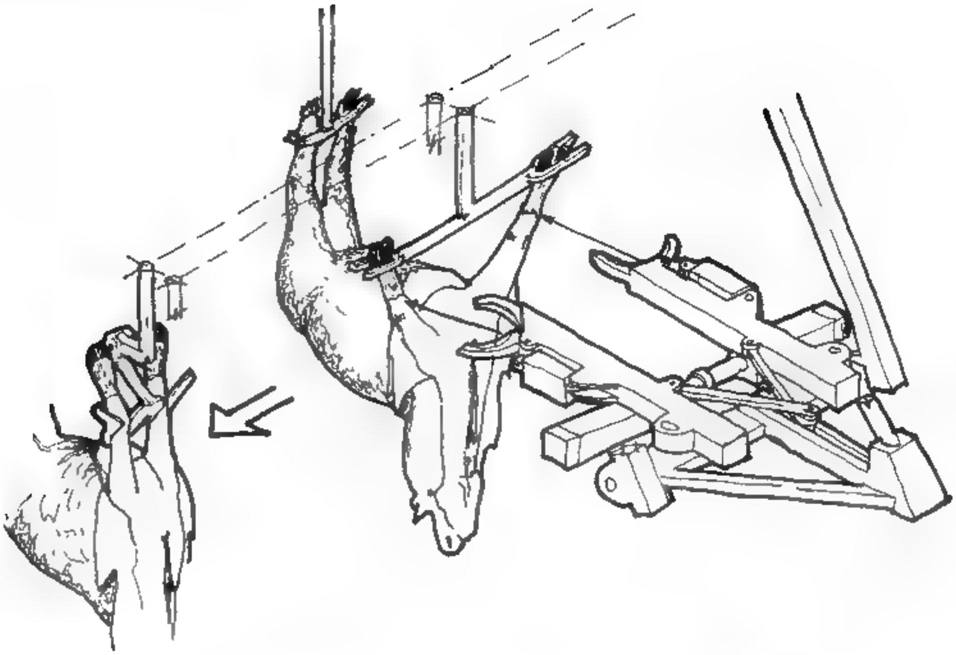


Figure 8. Wide-to-narrow transfer machine.

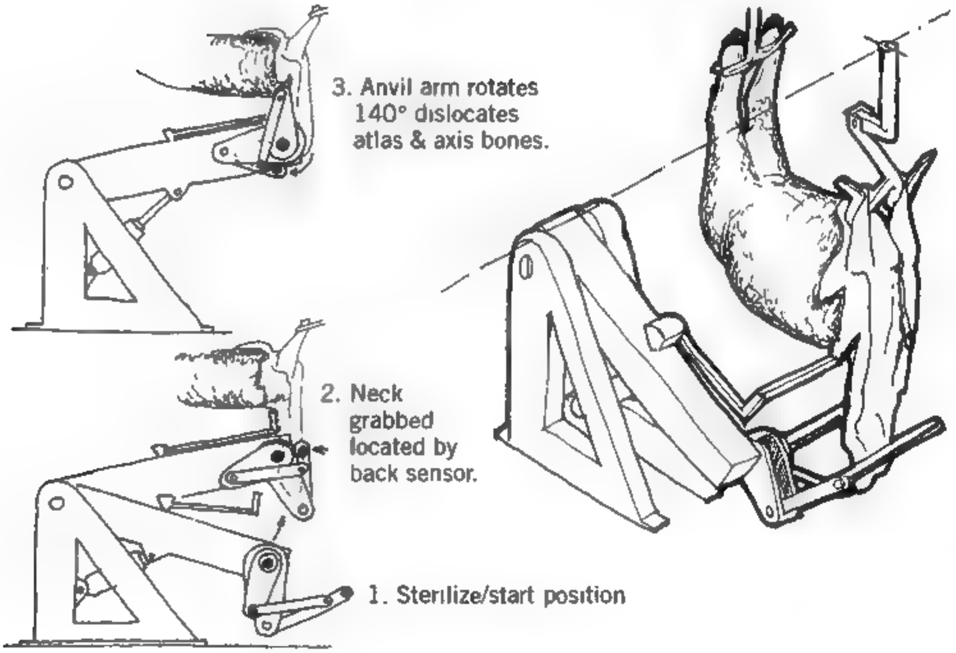

3. Anvil arm rotates 140° dislocates atlas & axis bones.
2. Neck grabbed located by back sensor.
1. Sterilize/start position

Figure 9. Automatic atlas joint severing machine.

stress level of pigs, which can affect meat quality. Here, traditionally, the animals have been forced into single file, a positioning that behavioral studies have shown is unnatural for these animals. Modern systems have been developed that use parallel single-file transport arrangements, which have greatly reduced the stress levels of the pig.

Stunning

The most common stunning method for pigs in the United States and Europe, particularly in larger plants, is electrical high-voltage stunning. The head-to-body version, causing cardiac arrest and reduction in poststunning movement, is preferred. More recently, studies in meat quality have shown that the occurrence of blood splash (small, visible hemorrhages within the muscle tissue) and breakage of the back bone and blade bone are attributable to electrical stunning methods. The incidence of these problems has been reduced by careful control of stun current and electrode position and by keeping the stun-to-stick time of the animal to a minimum.

CO_2 stunning is also very popular. Here the pig is lowered by a cradle into a pit containing a high concentration of CO_2 gas. The pigs are then raised out of the gas and

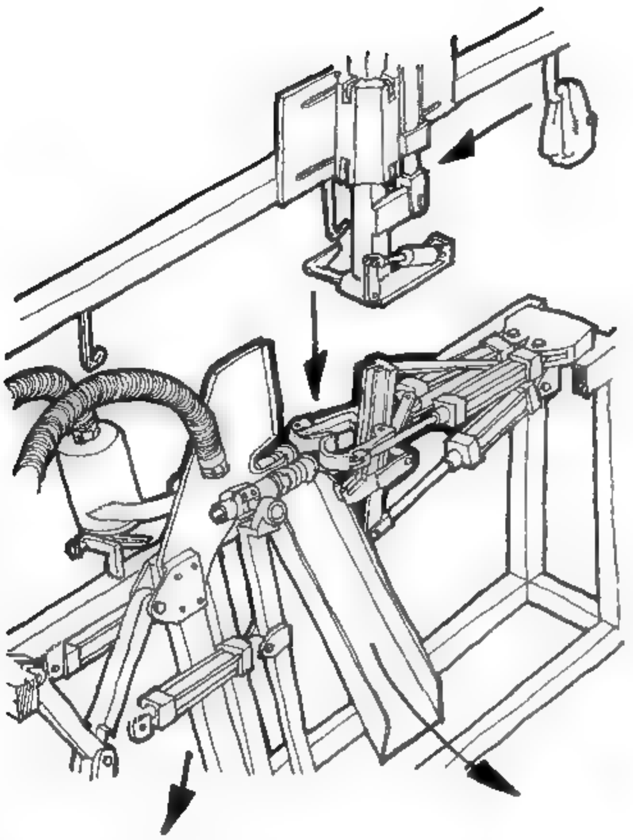


Figure 10. Automatic head splitting and brain extraction machine.

Table 3. Potential Chain Manning for Mechanized Lamb Slaughter and Dressing, Processing Eight Animals per Minute

Task	Workers
Auto stun	—
Shackle, open, bleed	4
Auto neck break	—
Head cheek, remove	3
Y cut, push flap	3
Clear neck, lift shanks	2
Auto wide to narrow transfer	—
Auto front hock remover	—
Load brisket clearer	1
Pull brisket piece, Y-cut rear legs	3
Load shoulder puller	1
Clear breaks, punch tunnels	1
Auto final puller	—
Trim anus	1
Auto rear hock remover	—
Auto wash	—
Auto brisket cut	—
Gambrel	1
Evisceration	5
Total	*25 + 11 assistants*

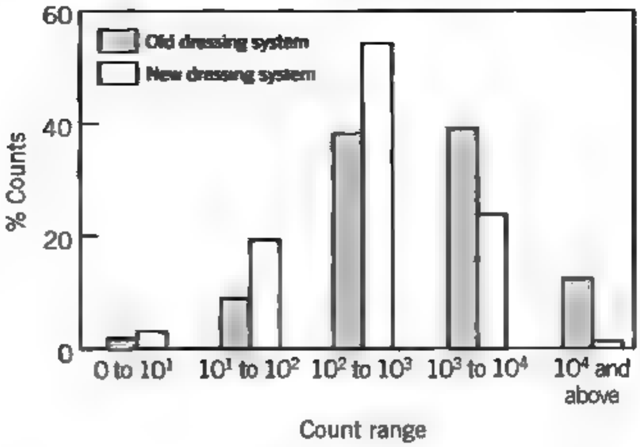


Figure 11. Aerobic plate counts (colony forming units/cm^2) for carcasses processed by a traditional dressing system and by an inverted dressing system in the same meat plant (n = 105 for each system).

discharged in a stunned state ready for sticking and further processing. There has been some criticism of CO_2 stunning of pigs, and this has been directed mainly toward the excitation phase that occurs after approximately 10 s exposure to the gas. In this phase, pigs tend to throw their heads and legs about with violent movements. It seems that no stunning method meets all the demands regarding meat quality, animal welfare, and worker safety. CO_2 stunning seems, however, to be one method that has potential for further development. Other stunning methods, such as the use of microwave energy, are also being assessed.

Scalding

Scalding is the most important part of the dehairing process. If the temperature of the scald is too high, there can be mechanical damage from the dehairing machinery due to skin softening. Also, too hot a scalding temperature and/or too long a time in the scalding tank has been associated with a higher level of PSE (pale, soft, exudative) meat.

Three type of scalding methods are used:

1. Scalding in a tank of water
2. Scalding by recirculating water sprinkled over the pigs
3. Individual scalding with temperate steam

There has been some debate as to whether water scalding is a clean operation or a contaminating one. That is, can water scalding cause contamination of the meat, or does meat contamination arise primarily by the sticking of the animal? Investigations have proved that neither shelf life nor flavor is affected negatively by the fact that the carcass has been scalded by either water or steam. The debate continues, however, and seems to suggest that scalding with steam will be the preferred option of the future. The problem with steam scalding is that heat transfer can be nonuniform. After scalding, the carcass is dehaired by means of a large number of rotating brushes.

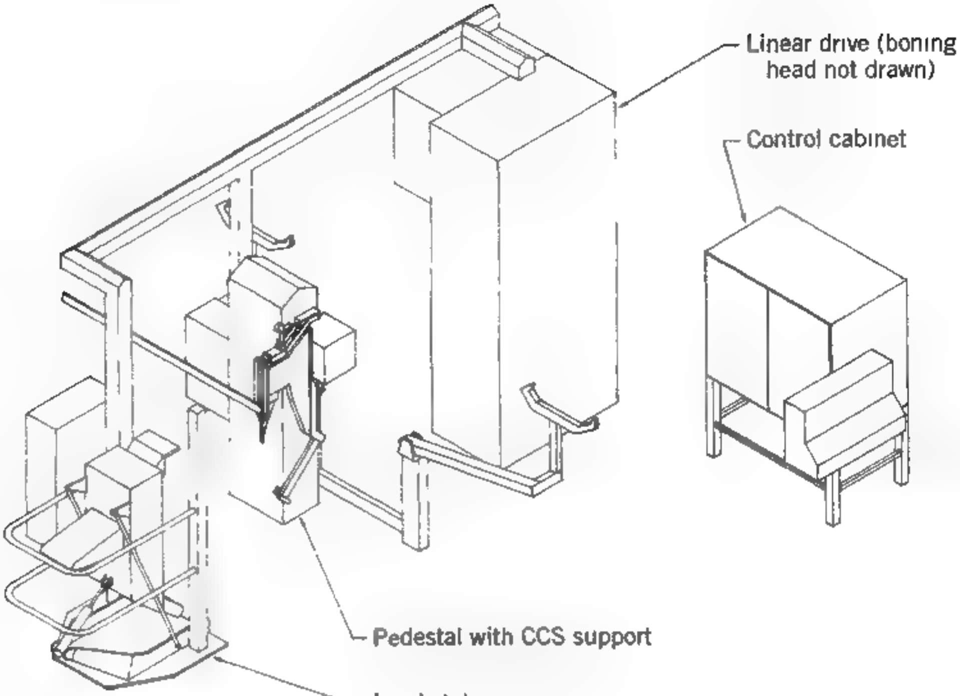

Linear drive (boning head not drawn)
Control cabinet
Pedestal with CCS support
Load station

Figure 12. Frame boner layout.

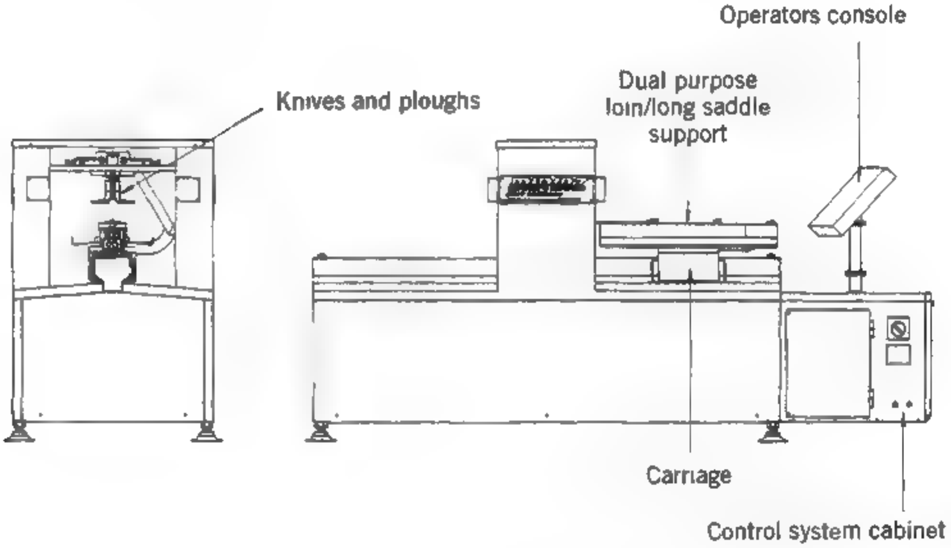

Operators console
Dual purpose loin/long saddle support
Knives and ploughs
Carriage
Control system cabinet

Figure 13. Loin boner machine.

Singeing

Carcasses are singed in cabinets, which are fired by either oil or gas, depending on the production setup. A newly developed ceramic material, coated on the inside of the furnace, reflects the energy of the flame and reduces the energy consumption. An exhaust gas heat exchanger retains half the energy that passes through the system. After singeing, the scraping treatment follows. Modern scraping machines use PLC technology. Scraper positions are hydraulically adjusted to an optimum position by sensing the height of the front legs. Hence, each pig receives optimal treatment from the scraper blades.

Dehiding

The method of dehairing just discussed consumes large quantities of energy and water. One alternative to dehairing is to dehide the pig. Dehiding pigs has the following advantages:

1. Longer shelf life for the meat (up to three days longer because of more hygienic slaughtering)
2. Less PSE because the hog carcass is not heated up by scalding and singeing
3. Lower energy and water costs

4. Reduced production area
5. An economic return from the hides

Disadvantages include:

1. Dehiding involves more work.
2. Grading circumstances are different.
3. Carcass stamping is more difficult.

Machinery Developments

A number of machines have been developed to assist the processing and slaughtering of pigs. One example is the fat end loosener, which drills out the fat end with the help of a vacuum. This machine was developed in Denmark and is now used in a large number of plants in that country. Prime quality fat ends have been increased from 75 to 95%. Another machine used in a number of abattoirs throughout Europe is an automatic pig carcass splitting machine. This machine replaces one operator and automatically splits the pig carcass into two halves. For hygienic operation, the cleaver mechanism that is used to split the carcass is sterilized between each pig. Considerable developmental resources are now being used in both Denmark and Holland to reduce the manpower involved in pig slaughtering. Some difficulties are being experienced with some of these machines when pigs are of an odd shape. Clearly, as robots become less expensive, there is a greater chance that these machines will succeed.

Automatic Grading

One of the great technical achievements in pig processing is the automatic carcass classification center, developed in Denmark. The center grades and stamps pigs automatically. These centers are now installed in a number of plants in Denmark. The system can cope with at least 400 pigs per hour. The main element of the center consists of an automatic measurement system. This system correctly positions 17 optical probes that measure the meat and fat thickness. The optical probes continuously measure the light reflectance value as they pierce the meat and fat.

Information from the probes is fed into a computer, which determines the fat and meat thickness. Subsequently, the carcasses are stamped automatically, with grading figures applied to each individual primal cut. The computer also transfers this information to a data sheet from which the farmer or supplier is paid.

POULTRY

The success of the poultry industry worldwide has been largely due to the automation of the slaughtering and dressing process. The development of this automation has taken place over the last 10 to 15 years.

Stunning, Slaughter, Dressing, Evisceration

The incorporation of mechanization into poultry processing lines has allowed the poultry meat industry to be cost competitive compared with other species. This mechanization started with the introduction of an automatic eviscerating machine. This machine started a revolution in methods of dressing chickens.

Today we have systems that involve overhead conveyors and a large number of machines—each capable of a line speed of around 4000 birds per hour. At present, the major manual effort into poultry processing lines occurs only where the birds are loaded into the processing conveyors. Once on these conveyors, the birds are transported through an electrical stunning area, a killing machine, a scalder, and a defeathering machine. Stunning generally occurs when the heads are immersed in a saline water bath, which causes cardiac arrest and also reduces post-stun movement. Problems reported to be associated with stunning can be eliminated. All machines on the killing line can be adjusted to suit the various sized birds being processed.

As with pig processing, the scalding process largely determines the defeathering result. Much work has been done to improve the temperature regulation of the water in the scalding tanks and to develop new systems for improved heat transfer from the water to the feathers.

The defeathering machine uses large rubber fingers which, when rotated in close proximity to the birds, remove the feathers. A number of other jobs are also carried out mechanically following this defeathering operation. The gullet is removed by machine, then a rotating knife removes the feet. In both cases, the design of the machine can cope satisfactorily with birds of various sizes.

After 10 years of effort, automation experts have succeeded in producing a fully automatic evisceration line for chicken. As with the killing lines, the principle of the evisceration line is to transport the birds on overhead conveyors, through a number of processing stations. These processing stations or machines include a vent cutter, an opener, an evisceration cut opener, and viscera removal. Machines are also being developed and manufactured for the automatic evisceration of other species, such as turkeys and ducks. Following evisceration, the birds are chilled, weighed, and packed. Much of this work has now been automated. For example, the birds are weighed on the conveyor and a trip system is used to grade the animals into certain precise weight ranges.

Cutting

During the last five years, the automated cutting of poultry into portions has attracted enormous interest, particularly in Western Europe. A number of manufacturers now produce automatic chicken portioning machines. Here again, these systems consist of an overhead conveyor, which transports the carcass through a number of cutting stations. Chickens can be cut into virtually any number of portions as desired, by switching cutting units off or on. Depending on the precision of the cutting, a throughput of around 2500 to a maximum of 3000 birds per hour is now achievable. The boning of chicken breasts is also now very popular. A number of machines are available to automatically fillet the breast. These machines operate at a rate of about 1500 birds per hour.

BEEF

Stunning and Slaughter

Cattle are normally stunned using a captive bolt or a percussion-type stunner and bled by a thoracic stick, but electrical stunning has been developed and used successfully. A head restraint system, which involves head capture and a chin lifter, is essential to allow reliable electrode placement. There are also advantages in using the same system for captive bolt or percussion stunning. If the current passes only through the head, the system is halal, although it is desirable to suppress poststun movement by electrical immobilization. If an additional set of electrodes makes contact with the brisket or other parts of the body of the animal, cardiac arrest occurs and poststun movement is reduced as well. A typical electrical stunning system is shown in Figure 14.

With electrical stunning systems, the stillness of the animal allows convenient typing off of the esophagus, preventing release of ingesta.

Dressing

After stunning, the carcasses are shackled and hoisted. The carcasses, suspended by their hind legs from overhead conveyors, pass along a number of platforms and units where the carcasses are dehided by means of hand tools and machines. On the first two platforms, both rear legs are dehided using knives and handheld dehiding tools. In this legging process, the hocks are removed by manually operated hock removers.

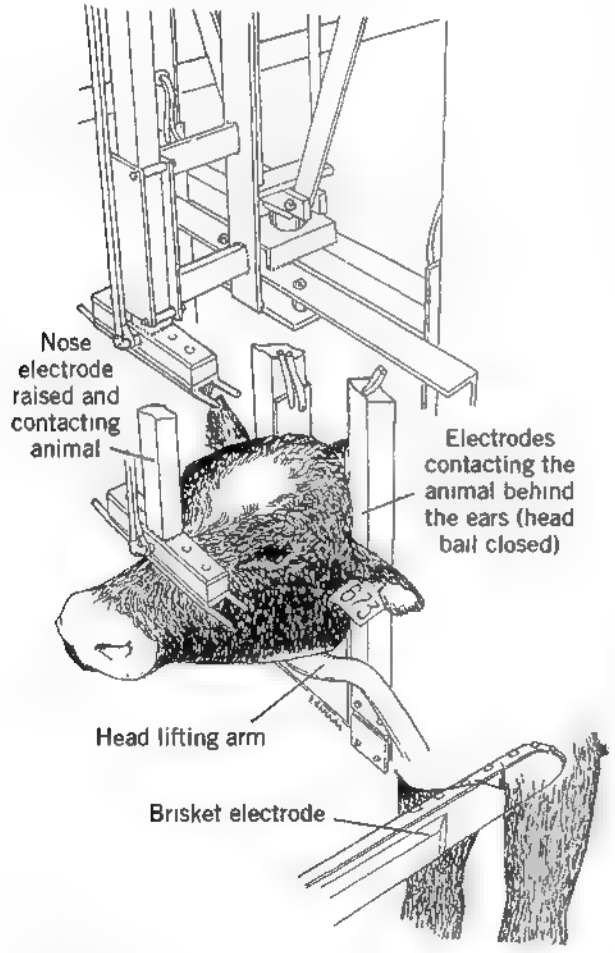

Nose electrode raised and contacting animal
Electrodes contacting the animal behind the ears (head bail closed)
673
Head lifting arm
Brisket electrode

Figure 14. Electrode and head restraint for electrical stunning of cattle

This preliminary dehiding is carried out in preparation for entry into the downward hide pulling machine. The dehiding machine consists of two operator platforms on either side of a powered rotating drum. The platforms and drum form an integrated assembly that moves up and down. Part of the hide that was removed during preliminary dehiding is fixed to the drum. As the platform-drum assembly is lowered, the hide is pulled off and rolled onto the drum. The two operators standing on each side of the carcass can assist the hide removal with handheld dehiding tools.

After complete removal of the hide, the drum is reversed and the pelt drops into a chute behind the machine. About 80 cattle per hour can be dehided with this system. After dehiding, the animal is eviscerated and all of the components of the viscera plus the carcass are inspected.

One machine development that has taken place in beef processing over the last 10 years has been automatic carcass splitting. This machine was developed in Europe and machines have been installed in the United States and Australia. The machine consists of a large circular saw that is controlled by a guiding system. The saw plus the guiding system is sterilized with hot water between each carcass. A large development program for automated beef dressing is nearing completion at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Meat Research Laboratory in Queensland, Australia. This technology could have a significant impact on beef dressing in the future.

G. R. Longdill
Meat Industry Research Institute of New Zealand
Hamilton, New Zealand

MEAT STARTER CULTURES AND MEAT PRODUCT MANUFACTURING

The origin of fermented meat products remains unknown, although the history of fermented sausages probably can be dated back more than 2000 years to the Mediterranean (1). Traditionally, the chopped meat was either presalted to promote development of lactic acid bacteria, or the "back-slopping" method was used. Today, large-scale industrial productions require consistent and accelerated processing, which has led to the present general use of meat starter cultures. Around 1920, pioneering work was done in this field, but it was not until the 1950s that starter cultures for sausage fermentation were introduced on a commercial scale. In the United States, pure cultures of pediococci were introduced in 1957 (2), while pure cultures of micrococci were introduced in 1961 in Europe (3). The usage of starter cultures in the manufacture of dry-cured sausages became common in the 1980s. Apart from the United States, where pure pediococci are still preferred due to manufacturing conditions, the trend is now increasing toward the use of mixed cultures composed of staphylococci strains and strains of lactic acid bacteria. Starter cultures contribute to appearance, texture, flavor, and aroma. The usage of starter cultures for fermented meat products is largely confined to dry-cured sausages. Only a minor portion is employed for dry-cured ham production.

FERMENTED SAUSAGE MANUFACTURE

Fermented sausages are manufactured by chopping together a mixture of raw meat (usually a beef-pork combination), pork back fat, salt, fermentable carbohydrate, fillers (eg, caseinate, starch, or other carbohydrates), spices, nitrate or nitrite, and an acidifying agent such as starter culture, glucono-δ-lactone (GdL), or fermented meat. Briefly, the procedure is as follows: The meat, including the pork back fat, is usually chopped frozen to avoid smearing during the cutting process. Pork back fat is used due to its high melting point and low content of unsaturated fatty acids as opposed to beef fat, which tends to impart a characteristic tallowlike taste. In some cases, sausage manufacturers use chilled meat, which has been pretreated by salting it to the required level prior to cutting the meat in the bowl chopper. This addition of salt brings about an accelerated acidification process as the resulting reduction in water activity promotes the growth of the indigenous lactic acid bacteria. Prior to cutting, the presalted meat is usually held for two to three days at a temperature not to exceed 5°C. To ensure proper mixing, all nonmeat ingredients, including the starter culture, are added during chopping of the lean meat. To achieve as low a water-binding effect as possible, salt and pork back fat are the last ingredients to be added. The mixing stops when the particle size of the mixture is reduced to the required size. Following these operations, the mixture is stuffed into casings, which can be either natural or of synthetic material. At the point of stuffing, the temperature of the mixture should not exceed -2 to 0°C so as to avoid smearing of the fat during the stuffing process. Appropriate steps must be taken to ensure that all air is removed from the mixture. This is most often achieved by using a vacuum stuffer. Once stuffed, the sausages undergo a conditioning period. During this period, the temperature of the sausage equilibrates to ambient and the sausage surface is allowed to dry in order to remove condensate before the product is placed in the climate chamber. If the condensate is not entirely removed prior to this step, the sausages may appear streaked as a result of uneven coloration arising from smoke components that have deposited on the surface.

In Europe, initial fermentation conditions are temperatures between 20 and 24°C and a relative humidity of 95%. The humidity is lowered along with the temperature, and this parameter should always be adjusted to a value 2 to 4% lower than in the sausages. This ensures a proper drying out and minimizes the risk of dry rim, which may block water diffusion from the sausage core. The total drying time will usually be determined by the time a required water loss is reached. Sausages manufactured in Northern and Central Europe are often smoked. As well as imparting smoke color and flavor, compounds in the smoke have inhibitory properties that retard mold growth on the casing surface during fermentation and drying periods. If considered undesirable, mold growth on the surface of salami casings can be inhibited by resmoking during the drying period. With some sausages, such as traditional Hungarian types and southern European types, it is considered desirable, and in some markets essential, that the product is covered with white mold, and in these cases the product is often inoculated with mold cultures. North American type of sausages are fermented at temperatures between 40 and 43°C and a relative humidity of 95%. The fermentation time is short, usually between 12 and 20 hours, and is followed by a heating process and/or a drying period. The heating process is dictated for unfrozen pork meat not guaranteed free of trichiniae. Final products can be grouped in dry sausages (eg, pepperoni) with a moisture to protein ratio in the range of 0.75 to 2.3:1.0 and semidry sausages (eg, summer sausage) with a moisture to protein ratio of 2.5 to 3.7:1.0 (4).

THE INFLUENCE OF STARTER CULTURES IN FERMENTED SAUSAGES

The purpose of using meat starter cultures for dry-cured sausages can be summarized by the terms *product quality product safety*, and *technological advantages* (Table 1).

Acidification

A sufficient reduction in pH can only be obtained if carbohydrate is included in the sausage recipe, as the concentration of glucose and phosphorylated glycolytic intermediates in raw meat is low, usually <0.1% (5). Fermentable carbohydrate, most often glucose, is added at a level of approximately 0.5% or higher. Sucrose might substitute glucose, and more slowly metabolized carbohydrates such as lactose or dextrin may be added as an extra carbohydrate source or as fillers. Efficient fermentation is achieved by homofermentation, where the carbohydrate added is metabolized via glycolysis, with lactic acid virtually being the only end product. Lactic acid bacteria for sausage fermentation are facultatively heterofermentative (6). This means that under certain conditions (eg, carbohydrate depletion), the lactic acid bacteria may switch to heterofermentation, resulting in formation of acetic acid and carbon dioxide in addition to lactic acid. Such compounds lead to defects in dry-cured sausages because acetic acid imparts a bitter, vinegarlike taste and excessive carbon dioxide may result in gas pockets. The speed of lactate formation determines the course of the fall in pH, which again controls gel formation and texture. Initial pH of the sausage mixture is typically within the range from 5.8 to 6.0, and drying will, in practice, be initiated around pH 5.3. The degree of drying out has a significant influence on the final texture, whereas the type and the degree of acid formation determines whether the taste of the sausage is mild, acidic, or tangy. A rapid acid formation will, in conjunction with the salt concentration, ensure that pathogenic as well as spoilage bacteria are significantly inhibited. Acidification can, as mentioned, also be obtained chemically by usage by

Table 1. The Influence of Meat Starter Cultures on Dry-cured Sausage Manufacturing

Mode of action	*Lactobacillus / Pediococcus*	*Staphylococcus / Kocuria*
Product quality		
Reduction in pH	■	
Texture	x	
Color formation	x	x
Color stabilization		x
Flavor formation	x	x
Product safety		
Inhibition of pathogenic bacteria	x	
Inhibition of spoilage bacteria	x	
Technological advantages		
Shortened processing time	x	
Consistent production	x	x

GdL, added at a concentration around 0.5%. In the presence of water, GdL is instantly hydrolyzed to gluconic acid, thereby lowering the pH. Once hydrolyzed to gluconic acid, GdL can be further metabolized by lactic acid bacteria to form acetic acid and carbon dioxide (7), which may have a negative influence on the sensory properties.

Color Formation

The color of meat is directly related to the content of myoglobin, and the meat will thus appear more red at high myoglobin levels. Myoglobin is unstable and reacts easily with oxygen either under formation of the cherry red, and also unstable, oxymyoglobin or under formation of the stable, but brown, metmyoglobin. The red color in dry-cured meat products comes from the pigment nitrosylmyoglobin, which is formed in reactions between nitric oxide and myoglobin or metmyoglobin. The main nitric oxide source is the very reactive nitrite. Quantitatively, the reaction of nitrite with ascorbate is the most important with respect to formation of nitric oxide (8). Ascorbate or erythorbate (isoascorbate) is for this reason a typical ingredient in dry-cured sausage recipes. Nitric oxide is also formed via nitrous acid, the conjugated acid of nitrite, with a pK_a of 3.22. Nitrous acid is unstable and dissociates into nitric oxide and nitrate. The more acidic the environment becomes, a condition promoted by the activity of lactic acid bacteria, the more the equilibrium is shifted in direction of nitric oxide and nitrate.

Nitrite is the most common curing agent and is added as nitrite salt, in the form of sodium chloride containing 0.4 to 0.5% sodium nitrite (9). Although 30 to 50 ppm nitrite is enough for proper color formation (9), EU regulation instructs addition of 150 ppm sodium nitrite and permits 50 ppm sodium nitrite in ready-to-sale dry-cured fermented meat products (10). However, nitrate can be added instead of nitrite. The corresponding values for nitrate are 300 ppm and 250 ppm sodium nitrate, respectively. U.S. regulation instructs 150 ppm sodium nitrite and 1700 ppm sodium nitrate and permits a maximum level of 200 ppm sodium nitrite in the finished product (11). Nitrate has no curing capability (9), and microbial reduction to nitrite is necessary to obtain curing effect. The reaction requires the nonendogenous enzyme nitrate reductase. The enzyme is produced by *Staphylococcus / Kocuria*, but even when nitrite is the only curing agent, *Staphylococcus / Kocuria* is reported to accelerate color formation (12). The reactions behind this formation are not known. It is hypothesized that because *Staphylococcus / Kocuria* utilizes nitrate as terminal electron acceptor for growth under anaerobic conditions, the reaction equilibria are shifted toward right, whereby the necessary nitric oxide for color formation is formed more rapidly.

Color Stabilization

Hydrogen peroxide is produced by the indigenous bacterial flora and can also be produced by lactic acid bacteria when oxygen is available. Even the more stable meat pigments are subject to oxidation and therefore also to reactions with hydrogen peroxide, which results in undesirable color pigments such as the green verdoheme and the yellow biliverdin (13). In combination with the red and brown meat pigments, the human eye will perceive this conglomerate of pigments as grey spots or even grey cores may be perceived to appear in the sausages (14). Both are typical defects associated with dry-cured sausages. Hydrogen peroxide is decomposed in presence of catalase, an enzyme produced by *Staphylococcus / Kocuria* (15).

Flavor Formation

The flavor and taste of cured meat products are to some extent defined by ingredients such as salt, nitrite/nitrate, and spices. The overall sensory quality is influenced by the choice of process (eg, smoking), as well as by microbial activity (16,17). The aroma of fermented meat products can be divided into the volatile compounds influencing flavor and the nonvolatile compounds determining the taste of the product. In both groups can be found a number of chemical compounds such as alkanes, alkenes, aldehydes, ketones, esters, organic acids, alcohols, phenols, furanes, terpenes, aromatic compounds, and compounds containing nitrogen, sulfur, or chloride (17,18). The aroma compounds derived from decomposition of carbohydrates, lipids, and proteins can be either of endogenous or microbial origin. Microbial activity transforms carbohydrate into lactic acid via glycolysis and provides dry-cured sausages with the characteristic tangy taste. If a part of the carbohydrate is metabolized via heterofermentation, the taste and the flavor is affected by the formation of compounds such as acetic acid, formic acid, and ethanol. The initial steps in protein breakdown are primarily facilitated by the endogenous enzymes (17). The degree of proteolysis is pH-dependent. Cathepsins as well as endopeptidases, active in protein degradation, have highest activity at low pH. As regional differences in processing technologies result in sausage types varying in final pH, the aroma will differ accordingly. Bacteria are involved in the later stages of protein degradation. Breakdown of peptides is partly caused by bacteria and they are also involved in amino acid catabolism (17,19). Aminopeptidase activity has been demonstrated in staphylococci and lactic acid bacteria. It is, however, remarkable that lactic acid bacteria apparently have not only a higher but also a more diverse aminopeptidase activity than staphylococci (20). The main bacterial impact on aroma lies probably in amino acid catabolism. The aldehydes 2-methylbutanal, 3-methylbutanal and 2-methylpropanal derived from branched-chained amino acids are important flavor components in dry-cured sausages (21). They can be of bacterial origin, and among starter culture strains, staphylococci seem to play a more important role than lactic acid bacteria (22). The lipids are decomposed via lipolysis and lipidoxidation. Lipolysis is caused by lipases and esterases, primarily of endogenous origin, and liberates free fatty acids of which particularly the short-chain acids affect taste. Staphylococcal lipases are reported but the activity may be limited at the conditions prevailing during fermentation and drying (23). Oxidation of fatty acids happens by chemical autoxidation or by microbial β-oxidation. There is evidence that the latter, resulting in methylketones and methylaldehydes as well as secondary alcohols, has a significant impact on the flavor generation in dry-cured sausages (19,24). Vice versa, synthesis catalyzed by lipases should also be considered. Fragrant esters, for example, are important flavor compounds in dry-cured sausages (25). The mechanism of their formation is not known; however, their presence and significance have been connected to sausages fermented with *Staphylococcus xylosus* (21).

Starter Cultures for Dry-Cured Sausages

Commercial starter cultures either consist of single-strain cultures or a mix of up to four or five strains. Single-strain cultures are most common in the United States with *Pediococcus acidilactici* being the typical species employed for high temperature fermentations (40–43°C). Under these conditions the decrease in pH will be so rapid that *Staphylococcus / Kocuria* are significantly inhibited. Mixed cultures consisting of lactic acid bacteria and *Staphylococcus / Kocuria* are normally chosen for fermentation at temperatures ranging from 18 to 24°C. To enhance aroma development, the cultures may also contain flavor-generating organisms such as yeast and *Streptomyces*. For surface treatment, single strain mold cultures or cultures composed of mold and yeast are available.

Lactic Acid Bacteria

The lactic acid bacteria, traditionally used for acidification of dry-cured sausages, are mesophilic species such as *Lactobacillus plantarum* and *Pediococcus pentosaceus*, and for American-type sausages, *P. acidilactici*. In recent years, psychrotrophic species such as *L. sakei* and *L. curvatus* have become more and more common in countries outside the United States. The lactic acid bacteria are added at a level of 5 to 10×10^6 cells/g meat mixture and reach approximately 10^8 cells/g in the final product. As described, lactic acid bacteria are responsible for acidification, for development of the acidic taste, and for development of texture. It has, however, also been demonstrated that some lactic acid bacteria possess enzymes normally attributed to *Staphylococcus / Kocuria*. It is reported that nitrate and nitrite reductases can be found in *L. plantarum* and *L. pentosus*, and a heme-dependent nitrite reductase has been demonstrated in *P. pentosaceus* (26). The heme-dependent nitrite reductase transforms nitrite into ammonia, whereas the end product from the heme-independent nitrite reductase is nitrous oxide, a precursor for the formation of nitric oxide. Results from practical experiments have, however, revealed that color formation, solely based on *Lactobacillus* strains possessing heme-independent nitrite reductase, was insufficient and caused color defects (26). Some lactic acid bacteria may have a color-stabilizing effect as they produce pseudocatalase, a peroxidase, which by nature is a flavoprotein, active under aerobic conditions (26).

Staphyloccous/Kocuria

A great part of meat starter culture preparations contain strains from the genera *Staphylococcus / Kocuria*. The genus *Staphylococcus* is more widely used than *Kocuria* due to their difference in oxygen requirement. The predominant species are *S. carnosus* and *S. xylosus*, whereas *Kocuria varians* is included only in a few preparations. *K. varians*, previously known as Micrococcus varians, was recently replaced with respect to genus (27). *Staphylococcus* is best suited for the sausage environment as they are facultatively anaerobic, whereas *Kocuria* by nature are aerobic. *Staphylococcus / Kocuria* are usually added at a level of approximately 5×10^6 cells/g meat mixture. They seldom increase in number during fermentation; however, they maintain a metabolic activity sufficient to ensure that the desired effects are achieved over a prolonged ripening period. *Staphylococcus / Kocuria* are, due to their nitrate and peroxidase reducing activity, involved in color development and color stabilization. Under anaerobic conditions, *Staphylococcus / Kocuria* utilize nitrate as an alternative electron acceptor in the respiration chain; that is, they gain metabolic energy via anaerobic respiration (28). *K. varians* does not reduce nitrite, but isolates among *S. carnosus* possess a dissimilatory nitrite reductase that results in increased growth under anaerobic conditions

(29,30). Acetate is the end product of anaerobic respiration, whereas lactate is the end product of fermentation. As described, *Staphylococcus / Kocuria* are also involved in flavor generation.

Molds

Raw ham and some fermented sausages are still being produced according to traditional methods, that is, inoculation with the *in-house mold flora*. However, by this inoculation method, toxinogenic strains may be among those colonizing the surface. Many of the mycotoxins that can be detected in culture media can also be detected when the same mold species grows on sausages or raw ham (31). Molded ham is generally regarded as more hazardous than molded sausages because the casing functions as a protecting barrier for the sausages. It is, however, not yet common to employ mold starter cultures for control of surface flora on dry-cured ham. In spite of this, there are multiple reasons to employ mold starter cultures. First, the starter culture provides the sausage surface with a white covering of a desirable shade, and, second, the starter culture dominates the flora and minimizes the risk for growth of mycotoxin-producing strains. In addition, the mold culture supplies the sausages with a characteristic flavor. Positive side effects such as reduced moisture loss due to mycelial covering, antioxidative effects because of catalase activity, oxygen consumption, and reduced oxygen penetration through the mycelium are factors mentioned among other advantages of a mold culture (32). Today, all mold strains used for surface treatment of dried sausages belong to the genus *Penicillium*. The most frequently used strain is *P. nalgiovense*, but also *P. chrysogenum* and *P. camembertii* are used (16,33,34). The preference of *P. nalgiovense* is linked to its growth ability on dry-cured meats as well as its toxicological safety. It should nonetheless be stressed that not all *P. nalgiovense* strains are nontoxic (35).

Yeasts

Commercial starter culture preparations contain only one type of yeast species, *Debaryomyces hansenii* or as the imperfect form *Candida famata*. Strains within this species are characterized by a high salt tolerance, no nitrate consumption, and high oxygen demands. The yeast is added directly to the meat at an inoculation level of 10^6 to 10^7 cells/g meat mixture. The activity is primarily in the periphery where the oxygen consumption accelerates the exterior color formation. The addition results in a characteristic flavor particularly desirable for Italian types of sausages (36).

PUBLIC HEALTH ASPECTS OF DRY-CURED SAUSAGES

Dry-cured sausages receive no heat-treatment prior to digestion, but they have a number of built-in microbiological hurdles such as low pH, low water activity, low redox potential, and nitrite. Despite these hurdles, there has been cases where severe illness could be associated to dry-cured sausages and outbreaks caused by bacteria have been reported. Mold-fermented sausages may encounter the risk of mycotoxin development, and chemical hazards comprise unacceptable levels of biogenic amines and nitrosamines.

Escherichia coli O157:H7

E. coli O157:H7 emerged as a food pathogen in 1982, and an outbreak was for the first time linked to dry-cured sausages in 1994. *E. coli* O157:H7 is more acid tolerant than other *E. coli*. It survives better at refrigeration temperature than at abuse temperature, and it has a low infectious dose, that is, <50 organisms (37). As a result of the outbreak, the U.S. Food Safety and Inspection Service issued guidelines for sausage manufacturers to validate processes to ensure a 5D-log unit reduction in counts of *E. coli* O157:H7 (38). In traditional processes for dry and semidry sausages, *E. coli* O157:H7 is reduced by 1 to 2 log units, and investigations have shown that the recommended 5D-log unit reduction can only be obtained by introducing a heating step following the fermentation (39,40). In European-type sausage manufacturing, similar results have been obtained. Thus, depending on inoculum, *E. coli* O157:H7 is reduced by 1 to 2 log units during normal processing (41). Subsequent storage results in further reduction, the reduction being more pronounced at ambient than at chill storage temperatures.

Salmonella

Nontyphoidal salmonellosis is described as a leading cause of foodborne illness in the United States (42), and in the mid-1990s, dry-cured sausages also caused outbreaks. In 1995, illness could be associated to consumption of Lebanon bologna, a semidry sausage, contaminated with *S. typhimurium*. In recent years, focus is more and more on phage type 104 strains of *Salmonella*, as they display a multiple resistance to antibiotics leading to increased hospitalization and increased mortality rate (43). To minimize the health hazard of *S. typhimurium* DT104 in dry-cured sausages in the United States, a more frequent sampling plan has been implemented for manufacturing plants not complying to the 5D-log reduction processes, described for *E. coli* O157:H7. *S. typhimurium* DT104 is more sensitive to manufacturing and storage conditions than *E. coli* O157:H7, but it is noteworthy that manufacturing of pepperoni is reported to result only in a 3D-log reduction (43). The survival is better in chill rooms than at ambient temperature.

Staphylococcus

Apathogenic strains of *Staphylococcus* are used as starter cultures, and, thus, the environmental conditions in dry and semidry sausages also allow *S. aureus* to compete successfully and grow. This organism is resistant to nitrite and salt and is capable of growing under anaerobic conditions. It has a minimum water activity for growth of 0.86, and the high fermentation temperatures used in the U.S. manufacturing process favor its rapid multiplication. At fermentation temperatures of 24 to 26°C, it has been demonstrated that the risk of food poisoning, resulting from the ingestion of enterotoxin produced by *S. aureus*, appears to be minor, provided the initial staphylococci count is low and provided an acidifying agent is used to ensure proper fermentation (44). Recent reports discuss pathogenicity of coagulase negative staphylococci, to which group the meat starter cultures belong (45). Hemolysins are demonstrated in strains of *S. carnosus* and *S. xylosus*, for example. In addition, enterotoxin production has been demonstrated in *S. xylosus*. Currently, there is no information available on the biological effect of hemolysins and enterotoxins. Neither is information available on parameters and factors affecting or controlling enterotoxin production (45).

Listeria monocytogenes

Studies have shown that *L. monocytogenes* may survive the combination of low pH, low water activity, and nitrite, which are the built-in hurdles in dry-cured sausages (46). Consequently, listeriosis should be regarded a potential health hazard in fermented meats, although no outbreaks so far have been associated with meat (47). *L. monocytogenes* has been shown to survive well in European as well as American-type sausages (41,48), the decrease in initial numbers being only one log unit. The survival of *L. monocytogenes* in stored, dry-cured sausages is better at chill than at ambient temperature. *In situ* produced pediocin, a bacteriocin effective against *L. monocytogenes* is, however, able to control the development of this pathogen in fermented sausages (48). Recently, a bacteriocin-producing starter culture, capable of controlling *L. monocytogenes*, has been launched for fermented sausages (49).

Yersinia enterocolitica

Y. enterocolitica is associated with pork and, as such should be regarded a potential health hazard in dry-cured meat products. No outbreaks or recalls have been associated with the organism. A reason for this could be that the nitrite level typically employed inhibits *Y. enterocolitica*, particularly in conjunction with a rapid decrease in pH. For example, *Y. enterocolitica* was eliminated in dry-cured sausages with added 80 ppm nitrite and fermented with a fast-acidifying starter culture (50).

Biogenic Amines

Biogenic amines in dry-cured sausages are of microbial origin. They are formed via decarboxylation of amines, by amination or transamination of aldehydes and ketones, or by hydrolysis of N-containing compounds. Due to their vasoactive properties, biogenic amines represent a health hazard. In retail dry-cured sausages, they are detected in varying levels and some of the biogenic amines are present in concentrations representing a risk for sensitive consumers (51). Many of the traditional starter cultures, such as *L. pentosus* and *L. plantarum*, are assumed not to form biogenic amines (52). Among the more recent meat starter cultures, *L. curvatus* has been shown to form biogenic amines, whereas the necessary decarboxylytic activity has yet to be demonstrated in *L. sakei* (53). High levels of biogenic amines in dry-cured sausages probably reflect usage of inferior raw materials, problems in recipe formulation or processing program, or choice of wrong starter culture (51)

N-Nitrosamines

The reaction of secondary or tertiary amines with nitric trioxide results in carcinogenic *N*-nitrosamines. The amount found in cured meat products is generally low (54), even though it has been demonstrated that GdL and starter cultures support the formation of *N*-nitrosamines, a phenomenon related to the fall in pH, which accelerates the reduction of nitrite to nitrous acid, the precursor for nitric trioxide. Ascorbate counteracts the reaction; however, it is claimed that more than 1000 ppm is needed to substantially limit the *N*-nitrosamine formation (55).

RAW HAM MANUFACTURE

Traditional dry-cured ham may be produced bone-in or boneless, the latter meaning that the aitchbone and shank is removed prior to salting. The next step is to rub a mixture of curing ingredients (sodium chloride and maybe nitrite and/or nitrate) onto the lean muscle surface of the ham. The ham is stored at temperatures below 5°C until the curing agents are evenly distributed throughout the ham. This period lasts from a few weeks to a few months. In between, the ham may be washed and resalted if necessary. The ham is then moved to the drying rooms and ripened at temperatures between 15 and 20°C and a relative humidity decreasing from 90 to 70%. In the last part of the ripening period, the ham may be covered with lard or pork fat to prevent excessive drying out. An in-house mold flora will usually grow on the ham surface. Some ham types, for example, country-style ham and German Westphalia ham, are smoked prior to the drying step. The raw material and the length of the ripening period characterize the type of ham. Thus the processing period of Serrano ham is 9 to 12 months, whereas it can be as long as 18 to 24 months for Iberian ham (56). Other important types of dry-cured ham are the Italian Parma ham and the French Bayonne ham.

Microbiology of Dry-Cured Ham

It can be debated whether dry-cured ham is a fermented product. The reason is that the microbial flora is seldom higher than approximately 10^4 cells/g meat, although the concentration can be as high as 10^8 cells/g on the surface (57,58). Growth is limited due to the initial low temperature and due to the low water activity throughout processing. The natural microflora, in the core as well as on the surface, consists of *Staphylococcus / Kocuria*. Initially, lactic acid bacteria, yeast, and the Gram-negative *Vibrio* may also be demonstrated, but the predominant organisms throughout processing are *Staphylococcus*, although their importance in the biochemistry of ham products is unknown (59). The characteristics of dry-cured ham are determined by type of raw material, processing conditions, and the biochemical changes taking place during manufacture. These are largely the result of muscle proteases and lipases (17,56). A recent study, however, suggests that microbial amino acid catabolism resulting in, among others, methyl-branched aldehydes, methylketones, and ethyl esters influences the flavor of dry-cured ham (58).

Particularly, staphylococci are capable of such reactions (22). Accelerated production processes have been developed, in which the dry-salting step is replaced by brine salting or brine injection pumping. The total processing time of such ham products is approximately one month as the drying and ripening period is significantly reduced, resulting in the final ham to deviate from the traditional type in texture and flavor. The application of starter cultures is an advantage for this production method. The starter cultures, most often pure cultures of staphylococci, but also occasionally mixed cultures of staphylococci (*S. carnosus, S. xylosus*) and lactic acid bacteria (*L. plantarum*), are added to the brine and support flavor development as well as color development and color stabilization.

MANUFACTURE OF MEAT STARTER CULTURES

Meat starter cultures are marketed as frozen or freeze-dried products, the latter being standardized with a carrier that is most often a carbohydrate. Meat starter cultures are produced as single-strain cultures that are mixed after fermentation and concentration (33). Starter cultures should be looked on as any other food additive; that is, they should be GRAS (Generally Recognized As Safe) approved. The legislation is not standardized and no official standard has been issued. However, requirements for meat starter cultures have been discussed in publications (33,60), and the following should be regarded as minimum requirements for a standard:

1. From a health perspective, only nontoxic and nonpathogenic strains should be used and the starter culture should be free of any impurity that could cause health risks.
2. Use of starter cultures should result in fermentations controlled with respect to acidification, texture, color, and aroma as well as microbiological stability of the meat product. Starter cultures should consist of phenotypically and genotypically stable as well as phage-resistant strains.

FUTURE PROSPECTS

In the future, lactic acid bacteria may play a more significant role as protective organisms in meat products, including those currently not treated with starter cultures. The mode of action comprises several activities, among which the production of bacteriocins is already in use for inhibition of *L. monocytogenes* in the manufacture of dry-cured sausages (49). The effect of a weak acid production combined with nutrient competition is utilized for control of the microflora of meat products packaged in vacuum or in modified atmosphere (61). The starter culture strain for this purpose is *L. alimentarius*. Probiotic bacteria such as *L. acidophilus, L. casei*, and *Bifidobacterium bifidum* are marketed for meat products to improve health (62). The idea is obviously adopted from dairy products but it is questionable whether the consumers will eat dry-cured sausages in such amounts that the probiotic effect is achieved. Finally, the use of genetically modified organisms (GMO) constructed by genetic engineering is another subject for the future. Legal approval may be difficult in certain regions, particularly in Europe, and consumer acceptance also remains to be obtained. It is, nonetheless, the most reliable way to develop strains contributing specific properties to the final products.

BIBLIOGRAPHY

1. C. S. Pederson, "Fermented Sausage," in *Microbiology of Food Fermentations*, 2nd ed., AVI, Westport, Conn. 1979, pp. 210–234.
2. U.S. Pat. 2,907, 661 (June 6, 1959), C. F Niven, R. H. Deibel, and G. D Wilson.
3. F. P. Niinivaara, M. S. Pohja, and S. E. Komulainen, "Some Aspects about Using Bacterial Pure Cultures in the Manufacture of Fermented Sausages," *Food Technol.* **18**, 147–153 (1964).
4. J Bacus, *Utilization of Microorganisms in Meat Processing: A Handbook for Meat Plant Operators*, Wiley, New York, 1984.
5. R. A. Lawrie, *Meat Science*, 5th ed., Pergamon, New York, 1991
6. O. Kandler and N. Weiss, "*Lactobacillus*," in P. H. A. Sneath et al., eds., *Bergey's Manual of Systematic Bacteriology*, Vol. 2, Williams & Wilkins, Baltimore, Md., 1986, pp. 1209–1234.
7. A. Kneissler et al., "Die wechselweise Beeinflussung von Glucono-delta-Lacton (GdL) und Starterkulturen bei der Rohwurstreifung," *Chem. Mikrobiol. Technol. Lebensm.* **10**, 82–85 (1986).
8. L. H. Skibsted, "Cured Meat Products and Their Oxidative Stability," in D. E. Johnston, M. K Knight, and D. A. Ledward, eds., *The Chemistry of Muscle-Based Foods*, Royal Society of Chemistry, Cambridge, UK, 1992, pp. 266–286.
9. F. Wirth, "Restricting and Dispensing with Curing Agents in Meat Products," *Fleischwirtsch.* **71**, 1051–1054 (1991).
10. European Parliament and Counsils, Directive 95/2/EC, (on food additives other than colours and sweeteners), Luxembourg, 1995.
11. U.S. Department of Agriculture, Food Safety and Inspection Service, *Meat and Poultry Inspection Regulations*, Washington D.C., 1986.
12. G. Mogensen, "Starter Cultures," in J. Smith, ed., *Technology of Reduced-Additive Foods*, Blackie Academic and Professional, London, 1995, pp. 1–25.
13. E. Slinde, "The Color of Meat" (in Norwegian), in B. Underdal, ed., *Kjøttteknologi*, Landbruksforlaget, Oslo, Norway, 1984, 71–78.
14. K. Coretti, *Rohwurstreifung und Fehlerzeugnisse bei der Rohwurstherstellung*, Rheinhessischen Druckwerkstätte, Alzey, Germany, 1971.
15. W. E. Kloos and K. H. Schleifer, "*Staphylococcus*," in P. H. A. Sneath et al., eds., *Bergey's Manual of Systematic Bacteriology*, Vol. 2, Williams & Wilkins, Baltimore, Md., 1986, pp. 1013–1035.
16. F.-K. Lücke, "Fermented Sausages," in B. J. B Wood, ed., *Microbiology of Fermented Foods*, Vol. 2, 2nd ed., Elsevier Applied Science, London, 1997, pp. 441–483.
17. A. Verplaetse, "Influence of Raw Meat Properties and Processing Technology on Aroma Quality of Raw Fermented Meat Products," *Proc. 40th Int. Congr. Meat Sci. Technol.*, The Hague, The Netherlands, August 28–September 2, 1994.
18. R. Dainty and H. Blom, "Flavour Chemistry of Fermented Sausages," in G Campbell-Platt and P. E. Cook, eds., *Fermented Meats*, Chapman & Hall, London, 1995, pp. 176–193.

19. M. C. Montel, F. Masson, and R. Talon, "Bacterial Role in Flavour Development," *Proc. 44th Int. Congr. Meat Sci. Technol.*, Barcelona, Spain, August 30–September 4, 1998.
20. M. C. Montel et al., "Peptidasic Activities of Starter Cultures," *Proc. 38th Int. Congr. Meat Sci. Technol.*, Clermont-Ferrand, France, August 23–28, 1992
21. L. Stahnke, "Dried Sausages Fermented with *Staphylococcus xylosus* at Different Temperatures and with Different Ingredient Levels. Part III, Sensory Evaluation," *Meat Science* **41**, 211–223 (1995).
22. J. L. Berdaguè et al., "Effects of Starter Cultures on the Formation of Flavour Compounds in Dry Sausage," *Meat Science* **35**, 275–287 (1993).
23. B. B. Sørensen and H. Samuelsen, "The Combined Effects of Environmental Conditions on Lipolysis of Pork Fat by Lipases of the Meat Starter Culture Organisms *Staphylococcus xylosus* and *Debaryomyces hansenii*," *International Journal of Food Microbiology* **32**, 59–71 (1996).
24. R. G. Berger et al., "Isolation and Identification of Dry Salami Volatiles," *J. Food Sci.* **55**, 1239–1242 (1990)
25. L. Stahnke, "Aroma Components from Dried Sausages Fermented with *Staphylococcus xylosus*," *Meat Science* **38**, 39–53 (1994).
26. W. P. Hammes, A. Bantleon, and S. Min, "Lactic Acid Bacteria in Meat Fermentation," *FEMS Microbiology Rev.* **87**, 165–174 (1990).
27. E. Stackebrandt et al., "Taxonomic Dissection of the Genus *Micrococcus: Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend.," *Int. J. Systematic Bacteriol.* **45**, 682–692 (1995).
28. C. Meisel, "Mikrobiologische Aspekte der Entwicklung von nitratreduzierende Starter-Kulturen für die Herstellung von Rohwurst und Rohschinken," Ph.D Dissertation, Hohenheim University, Stuttgart, Germany, 1988.
29. S. Hartmann, G. Wolf, and W P. Hammes, "Reduction of Nitrite by *Staphylococcus carnosus* and *Staphylococcus piscifermentans*," *System. Appl. Microbiol.* **18**, 323–328 (1995).
30. H. Neubauer and F. Götz, "Physiology and Interaction of Nitrate and Nitrite Reduction in *Staphylococcus carnosus*," *J. Bacteriol.* **178**, 2005–2009 (1996)
31. L. Leistner, "Toxinogenic Penicillia Occurring in Feeds and Foods: A Review," *Food Technol. Aust.* **36**, 404–406, 413 (1984).
32. F.-K. Lücke, "Microbiological Processes in the Manufacture of Dry Sausages and Raw Ham," *Fleischwirtsch.* **66**, 1505–1509 (1986).
33. B. Jessen, "Starter Cultures for Meat Fermentations," in G Campbell-Platt and P. E. Cook, eds., *Fermented Meats*, Chapman & Hall, London, 1995, pp. 130–159.
34. F.-K. Lücke and H. Hechelmann, "Starter Cultures for Dry Sausages and Raw Ham: Composition and Effect," *Fleischwirtsch.* **67**, 307–314 (1987).
35 J. Fink-Gremmels, A. A. El-Banna, and L. Leistner, "Developing Mould Starter Cultures for Meat Products," *Fleischwirtsch.* **68**, 1292–1294 (1988).
36. L. Leistner and Z. Bem, "Vorkommen und Bedeutung von Hefen bei Pökelfleischwaren," *Fleischwirtsch.* **50**, 350–351 (1970).
37. D. C. R Riordan et al., "Survival of *Escherichia coli* O157:H7 during the Manufacture of Pepperoni," *J. Food Prot.* **61**, 146–151 (1998).
38. C. A. Reed, "Approaches for Ensuring Safety of Dry and Semi-Dry Fermented Sausage Products," August 21, 1995, letter to plant managers, U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C., 1995.
39. J. C. Hinkens et al., "Validation of Pepperoni Processes for Control of *Escherichia coli* O157:H7," *J. Food Prot.* **59**, 1260–1266 (1996).
40. M. Calicioglu et al., "Viability of *Escherichia coli* O157:H7 in Fermented Semidry Low-Temperature-Cooked Beef Summer Sausage," *J. Food Prot.* **60**, 1158–1162 (1997).
41. H. Nissen and A. Holck, "Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella kentucky* in Norwegian Fermented, Dry Sausage," *Food Microbiology* **15**, 273–279 (1998).
42. C. J. Sauer et al., "Foodborne Illness Outbreak Associated with a Semi-Dry Fermented Sausage Product," *J. Food Prot.* **60**, 1612–1617 (1997).
43. A. M. Ihnot et al., "Behavior of *Salmonella typhimurium* DT104 during the Manufacture and Storage of Pepperoni," *International Journal of Food Microbiology* **40**, 117–121 (1998).
44. J. Metaxopoulos et al., "Production of Italian Dry Salami: Effect of Starter Culture and Chemical Acidulation on Staphylococcal Growth in Salami under Commercial Manufacturing Conditions," *Appl. Environ. Microbiol.* **42**, 863–871 (1981).
45. S. A. A. Jassim and S. P. Denyer, "Coagulase-Negative Staphylococci: Useful Organism or Potential Problem for Food Processing?" *Food Quality* **3**, 31–35 (1997).
46. J. Junttila et al., "Effect of Different Levels of Nitrite and Nitrate on the Survival of *Listeria monocytogenes* during the Manufacture of Fermented Sausage," *J. Food Prot.* **52**, 158–161 (1989).
47. P. M. Foegeding et al., "Enhanced Control of *Listeria monocytogenes* by In Situ–Produced Pediocin during Dry Fermented Sausage Production," *Appl. Environ. Microbiol.* **58**, 884–890 (1992).
48. J. L. Johnson et al., "Fate of *Listeria monocytogenes* in Tissues of Experimentally Infected Cattle and in Hard Salami," *Appl. Environ. Microbiol.* **54**, 497–501 (1988).
49. B. Jessen, L. Andersen, and B. Jelle, "Bioprotection of Meat and Dairy Products" (in Danish), *Alimenta* **19**, 9–11 (1996).
50. K. Asplund et al., "Survival of *Yersinia enterocolitica* in Fermented Sausages Manufactured with Different Levels of Nitrite and Different Starter Cultures," *J. Food Prot.* **56**, 710–712 (1993).
51 R Maijala, "Formation of Biogenic Amines in Dry Sausages with Special Reference to Raw Materials, Lactic Acid Bacteria, pH Decrease, Temperature, and Time," Ph.D. dissertation, National Veterinary and Food Research Institute, Helsinki, Finland 1994
52. R. Maijala and S. Eerola, "Contaminant Lactic Acid Bacteria of Dry Sausages Produce Histamine and Tyramine," *Meat Science* **35**, 387–395 (1993).
53 W. P. Hammes and H. Knauf, "Starters in the Processing of Meat Products," *Meat Science* **36**, 155–168 (1994).
54. H. Schmidt et al., "Einfluss von Bakterien sowie Natriumascorbat und Glucono-delta-Lacton auf den Ab-, Um-, und Aufbau von Nitrosaminen," *Fleischwirtsch.* **65**, 1487–1489 (1985).
55. M.-L. Liao and P. A. Seib, "Selected Reactions of L-Ascorbic Acid Related to Foods," *Food Technol.* **41**, 104–107, 111 (1987).
56. F Toldra, "The Enzymology of Dry-Curing of Meat Products," in F. J. M. Smulders et al., eds., *New Technologies for Meat and Meat Products*, ECCEAMST, Audet Tijdschriften bv, Nijmegen, The Netherlands 1992, pp. 209–231.
57. H. Silla et al., "A Study of the Microbial Flora of Dry-Cured Ham: 1, Isolation and Growth," *Fleischwirtsch.* **69**, 1128–1131 (1989).

58. L. Hinrichsen and S. B. Pedersen, "Relationship among Flavour, Volatile Compounds, Chemical Changes, and Microflora in Italian-Type Dry-Cured Ham during Processing," *J. Agric. Food Chem.* **43**, 2932–2940 (1995).
59. I. Molina et al., "Study of the Microbial Flora in Dry-Cured Ham: 2, *Micrococcaceae*," *Fleischwirtsch.* **69**, 1433–1434 (1989).
60. L. Leistner et al., *Anforderungen an Starterkulturen: Abschlussbericht zu einem Forschungsvorhaben*, Bundesanstalt Fleischforschung, Kulmbach, Germany, 1979.
61. L. Andersen, "Biopreservation with FloraCarn L-2," *Fleischwirtsch.* **75**, 1327–1329 (1995).
62. W. P. Hammes and C. Hertel, "New Developments in Meat Starter Cultures," *Proc. 44th Int. Congr. Meat Sci. Technol.*, Barcelona, Spain, August 30–September 4, 1998.

B. JESSEN
Danish Meat Research Institute
Roskilde, Denmark

MEMBRANE FILTRATION SYSTEMS

Membrane filtration has long been the method of choice for quantitative tests in water microbiology (1,2). Principally, this has been due to the ability to concentrate and detect low levels of indicator microorganisms by filtering large volumes of water. For this same reason, membrane filtration has been used to enumerate indicator organisms in beverages, including beer and wine. However, application of this tool in the beverage industry has been limited to those beverages that are low in particulate matter and that can easily pass through a membrane filter.

These same restrictions deterred food microbiologists from using membrane filtration as an analytical tool for many years. Early attempts to render food samples filterable met with limited success (3,4). In addition, the relatively narrow counting range of the standard 47-mm-diameter membrane filter (5) required a large number of sample dilutions to be filtered to ensure at least one countable filter. Despite these limitations, three very different approaches—membrane spread, direct epifluorescence, and hydrophobic grid membrane filter—have been used to adapt membrane filter techniques to food microbiology.

MEMBRANE SPREAD TECHNIQUE

A "membrane-spread" technique for enumerating *Escherichia coli*, first described in 1975, used a 90-mm-diameter membrane filter, laid on the surface of an agar plate (6). The sample aliquot was deposited on the membrane filter and spread over the entire surface with a bent glass rod. This 24-hour method, which used bile salts and a 44.5°C incubation temperature for selectivity and an indole reaction as the differential test, was highly specific for *E. coli* biotype 1. This method, later modified to include a nonselective repair step (7), is recognized as a valid method by the International Commission on Microbiological Specifications for Foods (8)

DIRECT EPIFLUORESCENT TECHNIQUE

A second membrane filter method, known as direct epifluorescent microscopy technique (DEFT), made use of fluorescent staining of cells trapped on the surface of a membrane filter followed by examination of the filter using a fluorescent microscope (9). This procedure overcame the limited counting range of standard membrane filtration by eliminating colony development. However, differentiation of viable and dead cells was not fully reliable, and reading the filters was tedious. Introduction of automated DEFT instruments addressed the latter problem (10,11). However, according to the developer of the technique, DEFT could not be applied to highly particulate food suspensions, nor could it be adapted to differential or selective enumerations (12).

Several researchers have improved on the original DEFT procedures by using prefiltration and enzyme digestion steps to reduce particle interference (13,14); adding short enrichment steps to enhance sensitivity (15); incubating the filters for a few hours after filtration to allow development of microcolonies, thus ensuring a true viable cell count and allowing for differential enumeration based on the culture media used (16,17); and using fluorescent antibody labeling in conjunction with DEFT to detect or enumerate specific target populations (18,19).

DEFT methods that do not incorporate enrichment or microcolony development steps offer the advantage of very fast results, with as little as 20 min required between analysis initiation and completion (14). Reliability of DEFT procedures has been evaluated for a variety of foods including raw minced meat (20), raw ham, ground beef and raw fish (21), raw lamb (22), and raw milk (14).

HYDROPHOBIC GRID MEMBRANE FILTRATION

The most widely adapted approach to membrane filtration of foods is the hydrophobic grid membrane filter (HGMF). The HGMF is a membrane filter on the surface of which a hydrophobic material is applied in a grid pattern. The hydrophobic material used to form the grid is a physical barrier to colony spread, largely restricting bacterial colonies to the confines of the individual grid squares in which they were first formed (see Fig. 1). Using this technique, the counting range of a single filter was extended to as high as 10^4 colony-forming units (23,24).

Initially, the HGMF was seen as a water microbiology and research tool. Beginning in 1978, its potential applicability to food microbiology was established when a series of studies on the filtering characteristics of a wide variety of food homogenates was published. It was reported that most food homogenates, if blended using a "Stomacher" and, where appropriate, if treated with a surfactant and/or an enzyme digestion, could be filtered (25). In a later study (26), a finely woven stainless steel cloth prefilter was used to remove particulate material from food homogenates immediately prior to filtration. The apparatus used to carry out this two-stage filtration is illustrated in Figure 2. This study established that neither prefiltration, nor surfactant treatment, nor enzyme digestions altered the bacterial counts obtained from food samples.

58. L. Hinrichsen and S. B. Pedersen, "Relationship among Flavour, Volatile Compounds, Chemical Changes, and Microflora in Italian-Type Dry-Cured Ham during Processing," *J. Agric. Food Chem.* **43**, 2932–2940 (1995).
59. I. Molina et al., "Study of the Microbial Flora in Dry-Cured Ham: 2, *Micrococcaceae*," *Fleischwirtsch.* **69**, 1433–1434 (1989).
60. L. Leistner et al., *Anforderungen an Starterkulturen: Abschlussbericht zu einem Forschungsvorhaben*, Bundesanstalt Fleischforschung, Kulmbach, Germany, 1979.
61. L. Andersen, "Biopreservation with FloraCarn L-2," *Fleischwirtsch.* **75**, 1327–1329 (1995).
62. W. P. Hammes and C. Hertel, "New Developments in Meat Starter Cultures," *Proc. 44th Int. Congr. Meat Sci. Technol.*, Barcelona, Spain, August 30–September 4, 1998.

B. JESSEN
Danish Meat Research Institute
Roskilde, Denmark

MEMBRANE FILTRATION SYSTEMS

Membrane filtration has long been the method of choice for quantitative tests in water microbiology (1,2). Principally, this has been due to the ability to concentrate and detect low levels of indicator microorganisms by filtering large volumes of water. For this same reason, membrane filtration has been used to enumerate indicator organisms in beverages, including beer and wine. However, application of this tool in the beverage industry has been limited to those beverages that are low in particulate matter and that can easily pass through a membrane filter.

These same restrictions deterred food microbiologists from using membrane filtration as an analytical tool for many years. Early attempts to render food samples filterable met with limited success (3,4). In addition, the relatively narrow counting range of the standard 47-mm-diameter membrane filter (5) required a large number of sample dilutions to be filtered to ensure at least one countable filter. Despite these limitations, three very different approaches—membrane spread, direct epifluorescence, and hydrophobic grid membrane filter—have been used to adapt membrane filter techniques to food microbiology.

MEMBRANE SPREAD TECHNIQUE

A "membrane-spread" technique for enumerating *Escherichia coli*, first described in 1975, used a 90-mm-diameter membrane filter, laid on the surface of an agar plate (6). The sample aliquot was deposited on the membrane filter and spread over the entire surface with a bent glass rod. This 24-hour method, which used bile salts and a 44.5°C incubation temperature for selectivity and an indole reaction as the differential test, was highly specific for *E. coli* biotype 1. This method, later modified to include a nonselective repair step (7), is recognized as a valid method by the International Commission on Microbiological Specifications for Foods (8)

DIRECT EPIFLUORESCENT TECHNIQUE

A second membrane filter method, known as direct epifluorescent microscopy technique (DEFT), made use of fluorescent staining of cells trapped on the surface of a membrane filter followed by examination of the filter using a fluorescent microscope (9). This procedure overcame the limited counting range of standard membrane filtration by eliminating colony development. However, differentiation of viable and dead cells was not fully reliable, and reading the filters was tedious. Introduction of automated DEFT instruments addressed the latter problem (10,11). However, according to the developer of the technique, DEFT could not be applied to highly particulate food suspensions, nor could it be adapted to differential or selective enumerations (12).

Several researchers have improved on the original DEFT procedures by using prefiltration and enzyme digestion steps to reduce particle interference (13,14); adding short enrichment steps to enhance sensitivity (15); incubating the filters for a few hours after filtration to allow development of microcolonies, thus ensuring a true viable cell count and allowing for differential enumeration based on the culture media used (16,17); and using fluorescent antibody labeling in conjunction with DEFT to detect or enumerate specific target populations (18,19).

DEFT methods that do not incorporate enrichment or microcolony development steps offer the advantage of very fast results, with as little as 20 min required between analysis initiation and completion (14). Reliability of DEFT procedures has been evaluated for a variety of foods including raw minced meat (20), raw ham, ground beef and raw fish (21), raw lamb (22), and raw milk (14).

HYDROPHOBIC GRID MEMBRANE FILTRATION

The most widely adapted approach to membrane filtration of foods is the hydrophobic grid membrane filter (HGMF). The HGMF is a membrane filter on the surface of which a hydrophobic material is applied in a grid pattern. The hydrophobic material used to form the grid is a physical barrier to colony spread, largely restricting bacterial colonies to the confines of the individual grid squares in which they were first formed (see Fig. 1). Using this technique, the counting range of a single filter was extended to as high as 10^4 colony-forming units (23,24).

Initially, the HGMF was seen as a water microbiology and research tool. Beginning in 1978, its potential applicability to food microbiology was established when a series of studies on the filtering characteristics of a wide variety of food homogenates was published. It was reported that most food homogenates, if blended using a "Stomacher" and, where appropriate, if treated with a surfactant and/or an enzyme digestion, could be filtered (25). In a later study (26), a finely woven stainless steel cloth prefilter was used to remove particulate material from food homogenates immediately prior to filtration. The apparatus used to carry out this two-stage filtration is illustrated in Figure 2. This study established that neither prefiltration, nor surfactant treatment, nor enzyme digestions altered the bacterial counts obtained from food samples.

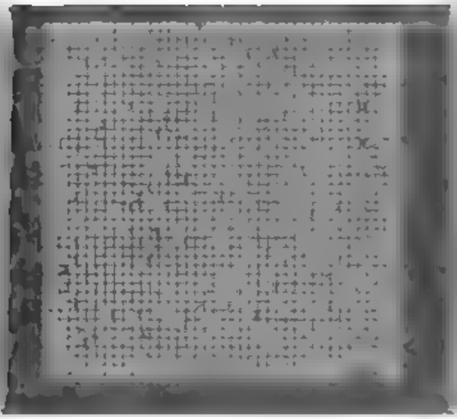


Figure 1. Coliform colonies growing on a hydrophobic grid membrane filter. The hydrophobic grid lines serve to confine colonies within the boundaries of the individual squares.

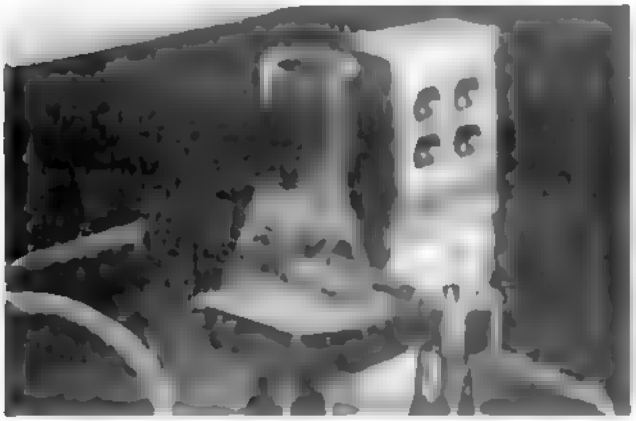


Figure 2. Filtration apparatus incorporating 5μ stainless steel prefilter used to inoculate hydrophobic grid membrane filters with food sample homogenates.

Since then numerous specific applications of the hydrophobic grid membrane filter to food microbiology have been introduced. These applications are listed in Table 1, and several of them are discussed in the following sections.

Table 1. Applications of Hydrophobic Grid Membrane Filter to Food Microbiology

Analysis	References
Aerobic plate count	27,41–43
Coliforms/*Escherichia coli*	28–31,54
E. coli O157·H7	32–36
Yeast and mold count	44–46,54
Staphylococcus aureus	54
Lactic acid bacteria	47,48,56
Fluorescent pseudomonads	57
Salmonella	37–40
Vibrio parahaemolyticus	49,50
Listeria monocytogenes	53

Coliforms, *Escherichia coli*, and *E. coli* O157:H7

The first HGMF method for total and fecal coliforms was an adaptation of the water microbiology fecal coliform test (27). Both counts were carried out using mFC Agar without rosolic acid. The total coliform test was incubated at 35°C; the fecal coliform test consisted of a preliminary resuscitation step followed by incubation of mFC Agar at 44.5°C. The companion test for *E. coli* was a direct adaptation of the Anderson and Baird-Parker membrane-spread method previously mentioned. These methods were subjected to validation studies in 1983 and were recognized as Official Methods by the Association of Official Analytical Chemists (now AOAC International) (28).

Developments in *E. coli* differential test procedures, notably the introduction of 4-methylumbelliferyl-β-D-glucuronide (MUG) as a differential reagent, prompted a redesign of the coliform and *E. coli* methods. In the newer method, a single HGMF was used for both the coliform and *E. coli* enumerations, eliminating the need for a 4-h resuscitation step. The entire analysis is complete in 24 h. This improved coliform/*E. coli* procedure was subjected to extensive in-house validation, followed by an AOAC-sponsored collaborative study (29,30). These studies demonstrated that the redesigned HGMF method produced quantitative total coliform and *E. coli* results that were not significantly different from the three-tube MPN method results over a wide range of food products. Furthermore, the confirmation rate of *E. coli* colonies on the HGMF method was in excess of 98% in the precollaborative study and more than 99% in the collaborative study. Based on combined results of both studies, the AOAC accorded "Official Action" status to the new procedure in 1990 (31).

The presence of *E. coli* O157:H7 in the food supply has become increasingly of concern. This *E. coli* serotype produces a negative MUG reaction and, therefore, is not detected by any *E. coli* enumeration procedures based on β-glucuronidase activity. It will also not grow in some culture media at elevated temperatures, making it more difficult to detect using the conventional three-tube MPN *E. coli* method. Several researchers have developed HGMF methods for *E. coli* O157:H7. The earliest of these described the development of HC Agar, a selective and differential culture medium for presumptive enumeration of *E. coli* O157:H7 (32). HC Agar relied on three differential reactions—MUG, indole, and sorbitol—to differentiate *E. coli* O157:H7 from other *Enterobacteriaceae*, including other *E. coli*. Two other methods employed enzyme-labeled antibody procedures to detect or enumerate *E. coli* O157:H7 (33,34).

Recently, SD-39 Agar was developed for use in conjunction with the HGMF to detect and enumerate presumptive *E. coli* O157:H7. The medium is based on three biochemical reactions that are read simultaneously: lysine decarboxylase, sorbitol fermentation, and β-glucuronidase production. Selectivity is provided by the presence of monensin and novobiocin and by incubation at 44 to 44.5°C. *E. coli* O157:H7 tolerates the elevated temperature because of the presence of NaCl in the culture medium. Confirmation is accomplished by subculturing presumptive positive colonies and carrying out O157 and H7 serological tests. This method was validated in a comprehensive precollaborative study and then in an AOAC-sponsored collaborative study (35,36). Results obtained by the HGMF method were either significantly higher than the reference method or not significantly different from the reference method, depending on the food product. The serological confirmation rates of presumptive positive *E. coli* O157:H7 were 99% in the precollaborative study and 94% in the collaborative study. Based on the combined results of both studies, the method was accorded First Action in 1997 (36).

Salmonella

In 1982 a rapid *Salmonella* detection procedure based on the HGMF was described (37). The method was subjected to collaborative study in 1984 and was granted official recognition by AOAC in that same year (38). Over time, it became evident that the plating media used in the original HGMF method—namely, Hektoen Enteric Agar and Selective Lysine Agar (SLA)—were not adequately specific to *Salmonella*. As a result, SLA was redesigned to increase its selective and differential properties. The improved medium, EF-18 Agar, relied on a dual biochemical differential reaction: lysine decarboxylation and sucrose fermentation. Alkaline colonies (ie, lysine positive and sucrose negative) were considered to be presumptive positive *Salmonella* and were subcultured for confirmation. Acid colonies (ie, lysine negative or sucrose positive organisms) were discarded.

EF-18 Agar was incorporated into the HGMF *Salmonella* method together with some relatively minor procedural changes. The improved method was subjected to extensive in-house validation against the conventional AOAC *Salmonella* method in a study comprising nearly 1000 naturally contaminated and inoculated food and feed samples (39). Overall, the HGMF method produced a false negative rate of 2.0% versus 1.9% for the reference method. The presumptive false-positive rate for the HGMF method was 0.3%, as compared with 8% for the reference method.

Following this successful validation, an AOAC-sponsored collaborative study of the method was organized. Thirty laboratories based in the United States and Canada took part, including both government and industry labs. The AOAC determined that the HGMF *Salmonella* method performance was statistically equivalent to that of the conventional AOAC *Salmonella* method, and awarded approval to the HGMF procedure (40).

Aerobic Plate Count

The HGMF aerobic plate count procedure has been an official method of the AOAC since 1985 (41) and is also a standard method of the American Public Health Association (42). The method consists of filtering a portion of sample homogenate through the HGMF and incubating on Tryptone Soy Agar supplemented with fast green dye at 35 to 37°C for 48 h. The method was subjected to extensive in-house validation (43), followed by an interlaboratory collaborative study (41). Results obtained by the HGMF method were at least statistically equivalent to the conventional pour plate method counts in both the in-house and collaborative validation studies while the colony-retaining properties of the hydrophobic grid allow the analyst to forgo duplicate plating and testing several serial dilutions without sacrificing accuracy or reproducibility.

Yeast and Mold Count

Historically, yeast and mold enumeration, with its requirement for a five-day incubation period, has been one of the slowest of the routine microbiology analyses to perform. Several attempts have been made over the years to shorten the incubation period by taking advantage of the ability of the HGMF to separate microorganisms from the food matrix and to confine the surface spread of colonies.

The first effort, reported in 1982, consisted of incubating the HGMF on Potato Dextrose Agar and staining the filter with safranin solution at the end of the incubation period to provide contrast between the colonies and the surface of the membrane filter (27). The method was refined by Lin et al. (44), who incorporated trypan blue dye into the Potato Dextrose Agar. The trypan blue was taken up by both yeasts and molds, producing blue colonies against the white background of the membrane filter. After determining that some molds would not develop visible colonies reliably within 48 h on Potato Dextrose Trypan Blue Agar, Entis and Lerner developed a new culture medium, YM-11 Agar, to optimize recovery of both yeasts and molds on the HGMF (45). YM-11 Agar enabled the enumeration of even slower growing yeasts and molds within a 50 ± 2 h incubation period at 25 ± 1°C. The performance of YM-11 Agar was evaluated in a precollaborative study and then further validated in an AOAC-sponsored collaborative study. The two-day HGMF method using YM-11 Agar performed equivalently to the five-day Potato Dextrose Agar pour plate method in both studies. As a result, AOAC recognized this HGMF method in 1995 (46).

Lactic Acid Bacteria

In 1988 a rapid method for differential enumeration of *Lactobacillus* and *Pediococcus* from meat starter cultures was described (47). An improved selective-differential culture medium was used together with the HGMF to achieve a differential count even when one genus was present in fairly large excess relative to the other. This method enabled confirmed enumeration of both genera in 48 h, as compared with a minimum of 72 h with the previous best method.

More recently, the development of an analogous procedure for enumerating *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yogurt was reported (48). Once again, the colony-separating properties of the grid, together with another newly designed culture medium, enabled direct differential enumeration of the two different bacterial species on a single filter even at cocci-to-rod ratios from 20:1 to 1:5.

Vibrio parahaemolyticus

An overnight HGMF enumeration method for *Vibrio parahaemolyticus* was first reported in 1983 (49) and later refined (50). The improved method consisted of first incubating the HGMF on a sucrose-containing selective differential agar for 16 to 18 h at 42°C. Sucrose-negative colonies (presumptive *V. parahaemolyticus*) were confirmed by removing the HGMF from the presumptive medium and placing it onto a secondary differential medium. Those squares producing typical reactions on both the primary and secondary media were counted as *V. parahaemolyticus*.

Enzyme Labeled Antibody and Colony Hybridization Methods

A number of researchers have taken advantage of the orderly positioning of colonies on the HGMF to develop either research or analytical tools based on either enzyme labeled antibody (ELA) or colony hybridization. ELA-based methods developed by Todd et al. (34) and by Doyle and Schoeni (33) for detecting or enumerating *E. coli* O157:H7 have already been mentioned. Peterkin et al. (51) developed a process for screening large numbers of DNA probes using HGMF. In addition, Kaysner et al. (52) reported on a DNA-DNA colony hybridization method to enumerate and differentiate *V. parahaemolyticus* and *V. vulnificus*, and Peterkin et al. (53) used a chromogen-labeled DNA probe to detect and confirm *Listeria monocytogenes* colonies on HGMF using a colony hybridization process.

Several characteristics of the HGMF contribute to its excellent performance. Principal among these are the colony-retaining and separating properties of the hydrophobic grid (23,27). The filter also serves as a passive support, enabling bacterial colonies to be transported undisturbed from one culture medium to another (32,54). Filtration often improves sensitivity of detection both by concentrating low levels of target organisms and by separating the organisms from any inhibitory materials (eg, preservatives, spice components) that might be present in the sample homogenate (27,54). The orderly grid array facilitates colony counting, thus reducing a significant source of between-analyst variation (23,27,55).

Numerous researchers have taken advantage of one or more of these HGMF characteristics to develop completely new procedures or to improve existing ones. It is inevitable that more uses will continue to be found for this highly versatile microbiological tool.

BIBLIOGRAPHY

1. A. Goetz and N Tsuneishi, "Application of Molecular Filter Membranes to the Bacteriological Analysis of Water," *Journal of the American Water Works Association* **43**, 943–969 (1951).
2. H. F Clark et al., "The Membrane Filter in Sanitary Bacteriology," *Public Health Reports* **66**, 951–977 (1951).
3. L. A. Nutting, P. C. Lomot, and F. W Barber, "Estimation of Coliform Bacteria in Ice Cream by Use of the Membrane Filter," *Appl. Microbiol.* **7**, 196–199 (1959).
4. W. K. Kirkham and P. A. Hartman, "Membrane Filter Methods for the Detection and Enumeration of *Salmonella* in Egg Albumen," *Poultry Science* **41**, 1082–1088 (1962).
5. American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 19th ed., American Public Health Association, Washington, D.C., 1995.
6. J. M. Anderson and A. C. Baird-Parker, "A Rapid and Direct Plate Method for Enumerating *Escherichia coli* Biotype I in Food," *J. Appl. Bacteriol.* **39**, 111–117 (1975).
7. R. Holbrook, J. M. Anderson, and A. C. Baird-Parker, "Modified Direct Plate Method for Counting *Escherichia coli* in Foods," *Food Technology in Australia* **32**, 78–83 (1980).
8. M. K. Rayman et al., "ICMSF Methods Studies. XIII. An International Comparative Study of the MPN procedure and the Anderson-Baird-Parker Direct Plating Method for the Enumeration of *Escherichia coli* Biotype I in Raw Meats," *Can. J Microbiol.* **25**, 1321–1327 (1979).
9. G. L. Pettipher et al., "Rapid Membrane Filtration-Epifluorescent Microscopy Technique for Direct Enumeration of Bacteria in Raw Milk," *Appl. Environ. Microbiol.* **39**, 423–429 (1980).
10. G. L. Pettipher and U. M. Rodriques, "Semi-Automated Counting of Bacteria and Somatic Cells in Milk Using Epifluorescent Microscopy and Television Image Analysis," *J. Appl. Bacteriol.* **53**, 323–329 (1980).
11. G. L. Pettipher et al., "Preliminary Evaluation of COBRA, an Automated DEFT Instrument, for the Rapid Enumeration of Micro-organisms in Cultures, Raw Milk, Meat and Fish," *Lett. Appl. Microbiol.* **14**, 206–209 (1992).
12. G. L. Pettipher, R. J. Fulford, and L. A. Mabbitt, "Collaborative Trial of the Direct Epifluorescent Filter Technique (DEFT), a Rapid Method for Counting Bacteria in Milk," *J. Appl. Bacteriol.* **54**, 177–182 (1983).
13. I. Walls et al., "Separation of Micro-organisms From Meat and Their Rapid Enumeration Using a Membrane Filtration-Epifluorescent Microscopy Technique," *Lett. Appl. Microbiol.* **10**, 23–26 (1990)
14. C. P. Champagne et al., "Determination of Viable Bacterial Populations in Raw Milk Within 20 Minutes by Using a Direct Epifluorescent Filter Technique," *J. Food Prot.* **60**, 874–876 (1997).
15. M. T. Rowe, and G. J. McCann, "A Modified Direct Epifluorescent Filter Technique for the Detection and Enumeration of Yeast in Yoghurt," *Lett. Appl. Microbiol.* **11**, 282–285 (1990).
16. U. M Rodrigues, and R. G. Kroll, "Rapid Selective Enumeration of Bacteria In Foods Using a Microcolony Epifluorescence Microscopy Technique," *J. Appl. Bacteriol.* **64**, 65–78 (1988).
17. U. M Rodrigues and R. G. Kroll, "Microcolony Epifluorescence Microscopy for Selective Enumeration of Injured Bacteria in Frozen and Heat-Treated Foods," *Appl. Environ. Microbiol.* **55**, 778–787 (1989)
18. M. L. Tortorello and S. M. Gendel, "Fluorescent Antibodies Applied to Direct Epifluorescent Filter Technique for Microscopic Enumeration of *Escherichia coli* O157:H7 in Milk and Juice," *J. Food Prot.* **56**, 672–677 (1993).
19. L. Restaino et al., "Antibody-Direct Epifluorescent Filter Technique and Immunomagnetic Separation for 10-h Screening and 24-h Confirmation of *Escherichia coli* O157:H7 in Beef," *J. Food Prot.* **59**, 1072–1075 (1996).
20. F. Boisen et al., "Quantitation of Microorganisms in Raw Minced Meat Using the Direct Epifluorescent Filter Technique: NMKL Collaborative Study," *J AOAC Int.* **75**, 465–473 (1992).
21. B. Abgrall and C. M Bourgeois, "Dénombrement de la Flore Totale de Produits Alimentaires par la Technique DEFT [Counting of Total Microflora of Food Products by Direct Epifluorescent Filter Technique (DEFT)]," *Sciences des Aliments* **9**, 713–724 (1989).

22. M.-L. Sierra, J. J. Sheridan, and L. McGuire, "Microbial Quality of Lamb Carcasses During Processing and the Acridine Orange Direct Count Technique (a Modified DEFT) for Rapid Enumeration of Total Viable Counts," *International Journal of Food Microbiology* **36**, 61–67 (1997).
23. A. N. Sharpe and G. L. Michaud, "Hydrophobic Grid-Membrane Filters: New Approach to Microbiological Enumeration," *Appl Microbiol.* **28**, 223–225 (1974).
24. A. N Sharpe and G. L. Michaud, "Enumeration of High Numbers of Bacteria Using Hydrophobic Grid-Membrane Filters," *Appl. Microbiol.* **30**, 519–524 (1975).
25. A. N. Sharpe, P. I. Peterkin, and I Dudas, "Membrane Filtration of Food Suspensions," *Appl. Environ. Microbiol.* **37**, 21–35 (1978).
26. P. Entis, M. H. Brodsky, and A. N. Sharpe, "Effect of Prefiltration and Enzyme Treatment on Membrane Filtration of Foods," *J. Food Prot.* **45**, 8–11 (1982).
27. M. H. Brodsky et al., "Enumeration of Indicator Organisms in Foods Using the Automated Hydrophobic Grid Membrane Filter Technique," *J. Food Prot.* **45**, 292–296 (1982).
28. P. Entis, "Enumeration of Total Coliforms, Fecal Coliforms and *Escherichia coli* in Foods by Hydrophobic Grid Membrane Filter: Collaborative Study," *J.—Assoc. Off. Anal. Chem.* **67**, 812–823 (1984).
29. P. Entis and P. Boleszczuk, "Direct Enumeration of Coliforms and *Escherichia coli* by Hydrophobic Grid Membrane Filter in 24 Hours Using MUG," *J. Food Protect.* **53**, 948–952 (1990).
30. P. Entis, "Hydrophobic Grid Membrane Filter/MUG Method for Total Coliform and *Escherichia coli* Enumeration in Foods: Collaborative Study," *J.—Assoc. Off. Anal. Chem.* **72**, 936–950 (1989).
31. "AOAC Official Method 990.11. Total Coliform and *Escherichia coli* Counts in Foods. Hydrophobic Grid Membrane Filter/MUG (ISO-GRID®) Method. First Action 1990. Final Action 1993," in *Official Methods of Analysis*, 16th ed., Vol. 1, AOAC International, Gaithersburg, Md., 1997.
32. R. A. Szabo, E. C. D. Todd, and A. Jean, "Method to Isolate *Escherichia coli* O157:H7 From Food," *J. Food Protect.* **49**, 768–772 (1986).
33. M. P. Doyle and J. L. Schoeni, "Isolation of *Escherichia coli* O157 H7 From Retail Fresh Meats and Poultry," *Appl. Environ. Microbiol.* **53**, 2394–2396 (1987).
34. E. C. D. Todd et al., "Rapid Hydrophobic Grid Membrane Filter-Enzyme-Labelled Antibody Procedure for Identification and Enumeration of *Escherichia coli* O157 in Foods," *Appl. Environ. Microbiol.* **54**, 2536–2540 (1988).
35. P Entis and I. Lerner, "24-Hour Presumptive Enumeration of *Escherichia coli* O157:H7 in Foods Using the ISO-GRID® Method with SD-39 Agar," *J. Food Protect.* **60**, 883–890 (1997).
36. P Entis, "Direct 24-Hour Presumptive Enumeration of *Escherichia coli* O157·H7 in Foods Using Hydrophobic Grid Membrane Filter, Followed by Serological Confirmation: Collaborative Study," *J. AOAC Int.* **81**, 403–418 (1998).
37. P. Entis et al., "Rapid Detection of *Salmonella* spp. in Food by Use of the ISO-GRID® Hydrophobic Grid Membrane Filter," *Appl. Environ. Microbiol.* **43**, 261–268 (1982).
38. P. Entis, "Rapid Hydrophobic Grid Membrane Filter Method for *Salmonella* Detection in Selected Foods: Collaborative Study," *J.—Assoc. Off. Anal. Chem.* **68**, 555–564 (1985).
39 P. Entis and P. Boleszczuk, "Rapid Detection of *Salmonella* in Food Using EF-18 Agar in Conjunction With the Hydrophobic Grid Membrane Filter," *J. Food Protect.* **54**, 930–934 (1991).
40. P. Entis, "Improved Hydrophobic Grid Membrane Filter Method, Using EF-18 Agar, for Detection of *Salmonella* in Foods: Collaborative Study," *J.—Assoc. Off. Anal. Chem.* **73**, 734–742 (1990).
41. P. Entis, "Hydrophobic Grid Membrane Filter Method for Aerobic Plate Count in Foods. Collaborative Study," *J. –Assoc. Off. Anal. Chem.* **69**, 671–676 (1986).
42. American Public Health Association, *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington, D C., 1992, pp. 237–239.
43. P. Entis and P. Boleszczuk, "Use of Fast Green FCF With Tryptic Soy Agar for Aerobic Plate Count by the Hydrophobic Grid Membrane Filter," *J. Food Protect.* **49**, 278–279 (1986)
44. C. C. S. Lin, D. Y. C. Fung, and P. Entis, "Growth of Yeast and Mold on Trypan Blue Agar in Conjunction With the ISO-GRID® System," *Can. J. Microbiol.* **30**, 1405–1407 (1984).
45. P. Entis and I. Lerner, "Two-Day Yeast and Mold Enumeration Using the ISO-GRID® Membrane Filtration System in Conjunction With YM-11 Agar," *J. Food Protect.* **59**, 416–419 (1996).
46. P. Entis, "Two-Day Hydrophobic Grid Membrane Filter Method for Yeast and Mold Enumeration in Foods Using YM-11 Agar: Collaborative Study," *J. AOAC Int.* **79**, 1069–1082 (1996).
47. R. A. Holley and G. E. Millard, "Use of MRSD Medium and the Hydrophobic Grid Membrane Filter Technique to Differentiate Between Pediococci and Lactobacilli in Fermented Meat and Starter Cultures," *International Journal of Food Microbiology* **7**, 87–102 (1988).
48. G. E. Millard, R. C. McKellar, and R. A. Holley, "Simultaneous Enumeration of the Characteristic Microorganisms in Yogurt Using the Hydrophobic Grid Membrane Filter System," *J. Food Protect.* **53**, 64–66 (1990).
49. P. Entis and P. Boleszczuk, "Overnight Enumeration of *Vibrio parahaemolyticus* in Seafood by Hydrophobic Grid Membrane Filtration," *J. Food Protect.* **46**, 783–786 (1983).
50. A. DePaola, L. H. Hopkins, and R. M. McPhearson, "Evaluation of Four Methods for Enumeration of *Vibrio parahaemolyticus*," *Appl. Environ. Microbiol.* **54**, 617–618 (1988).
51. P. I. Peterkin, E. S. Idziak, and A. N. Sharpe, "Screening DNA Probes Using the Hydrophobic Grid-Membrane Filter," *Food Microbiology* **6**, 281–284 (1989).
52. C. A. Kaysner et al., "A Research Note: Enumeration and Differentiation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* by DNA-DNA Colony Hybridization Using the Hydrophobic Grid Membrane Filtration Technique for Isolation," *J. Food Protect.* **57**, 163–165 (1994).
53. P. I. Peterkin, E. S. Idziak, and A. N. Sharpe, "Detection of *Listeria monocytogenes* by Direct Colony Hybridization on Hydrophobic Grid-Membrane Filters by Using a Chromogen-Labeled DNA Probe," *Appl. Environ. Microbiol* **57**, 586–591 (1991).
54. M. H. Brodsky et al., "Enumeration of Indicator Organisms in Foods Using the Automated Hydrophobic Grid-Membrane Filter Technique," *J. Food Protect.* **45**, 292–296 (1982)
55. A. N. Sharpe et al., "Colony Counting on Hydrophobic Grid-Membrane Filters," *Can. J Microbiol.* **29**, 797–802 (1983).
56. J. L. Chu et al., "La Quantification des Populations de Bactéries Lactiques par la Methode HGMF," *Belgian Journal of Food Chemistry and Biotechnology* **42**, 65–74 (1987).
57 S. J. Knabel, H. W. Walker, and A. A. Kraft, "Enumeration of Fluorescent Pseudomonads on Poultry by Using the Hydrophobic Grid Membrane Filter," *J. Food Sci.* **52**, 837–841, 845 (1987).

GENERAL REFERENCES

B. J. Dutka, ed., *Membrane Filtration—Applications, Techniques, and Problems*, Marcel Dekker, New York, 1981.

P. Entis, "Membrane Filtration Systems" in M. D. Pierson and N. J. Stern, eds., *Foodborne Microorganisms and Their Toxins. Developing Methodology*, Marcel Dekker, New York, 1986.

A. N. Sharpe and P. I. Peterkin, *Membrane Filter Food Microbiology*, Research Studies Press Ltd., Taunton, Somerset, United Kingdom, 1988.

PHYLLIS ENTIS
QA Life Sciences
San Diego, California

MEMBRANE PROCESSING

Membrane technology was originally developed in 1960 for production of potable water from seawater and brackish water. The food industry has especially benefited from this technology because it is a gentle and efficient way of fractionating, concentrating, and clarifying components in liquid and gaseous streams. It is based on the use of semipermeable membranes (membranes that are permeable to some components but not to others) to separate molecules primarily on the basis of size and, to a certain extent, on shape and chemical composition. For example, as shown in Figure 1, reverse osmosis (RO) can be used to concentrate the solids in a liquid food, whereas nanofiltration (NF) membranes are designed to separate salts (primarily monovalent ions) from multivalent salts, sugars, and larger compounds. NF can also separate organic compounds by its degree of dissociation. Ultrafiltration (UF) can be used to fractionate components in the feed solution, whereas microfiltration (MF) is used to clarify slurries or remove suspended matter.

In addition, pervaporation (PV) separates liquid mixtures by partial vaporization through a membrane (Fig. 2). It is an enrichment technique similar to distillation, but it uses a membrane that is permeable either to water or selected organic compounds in vapor form. The driving force is maximized by application of low pressure to the permeate side of the membrane (eg, a vacuum), combined with immediate condensation of permeated vapors.

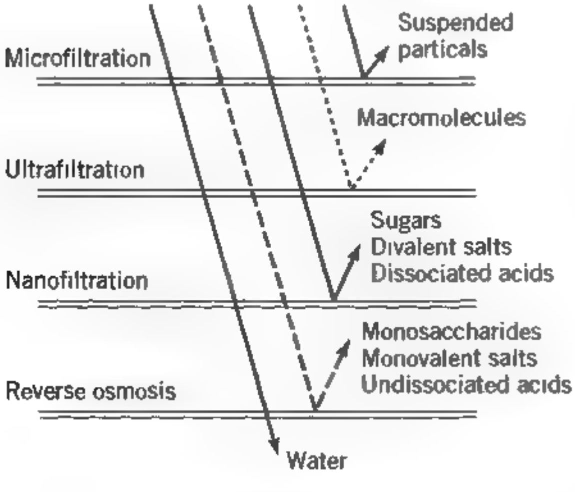


Figure 1. Definition of pressure-driven membrane processes.

Electrodialysis (ED) is the transport of ions through the membranes as a result of the application of direct electric current (Fig. 2). Only ionic species are transferred directly; thus ED can separate ionic species from nonionic components, so that both concentration and purification are possible. It is used mainly as a desalting technique.

Membrane technology requires less energy than many other dewatering techniques. Whereas open-pan evaporation may need over 600 kW h/ 1000 kg water removed and a five-effect evaporator requires 37–53 kW h/1000 kg, reverse osmosis for desalination requires 5–20 kW h/1000 kg water removed (1,2). In addition, no extremes of temperature are required as with evaporation and freeze concentration, thus preventing damage to heat-sensitive food components.

The technology is very simple. The membrane is assembled in a module, and the feed stream is pumped through the module over the membrane surface in a cross-flow mode (Fig. 3). The pressure gradient across the membrane forces solvent and solute molecules smaller than the pores on the membrane surface through the membrane into the permeate stream, while larger solutes are retained in the retentate stream.

IMPORTANT FACTORS

Several factors must be taken into account when considering a membrane process for a particular application: membrane material, membrane properties, module design, engineering factors, fouling and cleaning, and process design (1). Membranes have been made from more than 150 different polymers or inorganic materials, although fewer than 30 have achieved widespread commercial use. The majority of materials have been used for MF membranes and much fewer have worked successfully for RO, PV, and ED. Cellulose acetate and derivatives are still widely used for pressure-driven processes, despite their limitations of pH (generally usable only at pH 2–8) and temperature (not higher than 30°C). Thin-film composite membranes, containing a polyamide separating barrier on a polysulfone or polyethylene supporting layer, generally give better performance for RO and NF applications. Polysulfone, polyethersulfone, polyvinylidine fluoride, and polyacrylonitrile membranes are most common in UF and MF. MF membranes are also available in inorganic materials (alumina, zirconia/carbon composites, carbon/carbon composites, and stainless steel). Inorganic membranes have considerably widened the range of membrane applications, particularly in waste treatment, recovery and reuse of chemicals, and biotechnology applications where high temperature, acid and alkali stability, steam sterilizability, and cleanability are important.

Pore size is the most important property of a membrane. In reality, there is a distribution of pore sizes, which makes it difficult to get sharp or clean separations of molecules with membrane technology. Other factors affecting

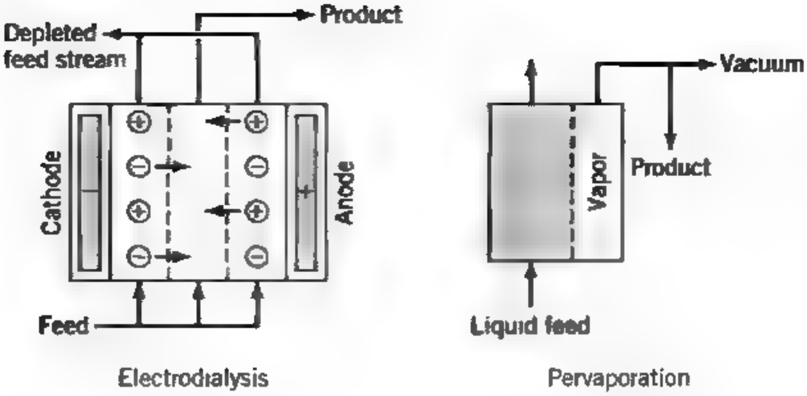


Figure 2. Schematic of electrodialysis and pervaporation.

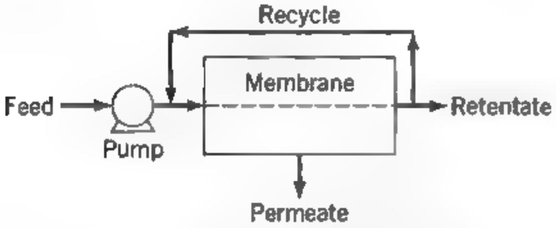


Figure 3. Typical flow diagram of a membrane process.

separation are the shape of the molecule; the presence of other solutes of similar size; operating parameters such as pressure, cross-flow rate, and temperature, and the microenvironment (1).

There are six different basic designs of membrane modules: tubular (with channel diameters >3 mm), hollow fiber or capillaries (self-supporting tubes, usually ≤2 mm i.d.), plates, spiral-wound, pleated sheets, and moving modules (1). The selection of a particular design depends on (1) the physical properties of the feed stream and retentate, especially viscosity and osmotic pressure; (2) particle size of suspended matter in the feed; (3) fouling potential of the feed stream, and (4) sanitary design, cleanability, and sterilizability.

FOOD APPLICATIONS

On a worldwide basis, the dairy industry is probably the largest user of membrane technology in food processing. RO is used as a preconcentration step prior to evaporation, NF is used for desalting cheese whey, ED is used for desalting milk and whey, and UF is used for purifying whey proteins from cheese whey to produce whey protein concentrates. UF is also used in the manufacture of cheese by preconcentrating the protein and fat to a level close to that normally found in the finished cheese. This precheese is then converted to cheese by conventional cheesemaking methods with appropriate modifications. The advantages of the UF method are that higher yields are obtained (10–30% in some cases) due to retention of whey proteins in the final cheese; the amount of enzyme required may be lower; and volume of milk to handle in the cheese vats is reduced and less whey is produced (although the milk permeate still needs to be disposed of). The UF method has been especially useful with soft cheeses.

MF is used for defatting and clarifying whey prior to ultrafiltration. MF is also used to reduce the microbial load of milk to extend the shelf life of pasteurized milk and the quality of cheeses.

The second-biggest use of membrane technology is for fruit juices, where UF and MF have been used in the manufacture of clear juices such as apple, pear, pineapple, kiwi, grape, lemon, lime, and cranberry. The goal is to replace the conventional holding, filtration, and decanting steps and perhaps the final pasteurization step. This has resulted in higher yields; improved juice quality due to removal of pectins, polyphenol oxidase, and tannin-protein complexes; elimination of filter-aid and fining agents; and much lower processing costs. Concentration of fruit juices by reverse osmosis to high levels (>42 Brix) using a combination of high-rejection and low-rejection RO membranes has been commercialized but is not widespread.

Other food applications include production of vegetable protein concentrates and isolates (eg, from soybeans), concentration of eggs prior to dehydration, processing of animal products such as blood and gelatin, refining of sugars and syrups, removal of yeast from beer and wine, and degumming vegetable oils. It is increasingly being used in waste treatment and recovery and reuse of chemicals such as acid and alkaline cleaners that are used for cleaning food equipment.

BIBLIOGRAPHY

1. M. Cheryan, *Ultrafiltration and Microfiltration Handbook*, Technomic, Lancaster, Pa., 1998.
2. M. Cheryan, "Concentration of Liquid Foods by Reverse Osmosis," in D. B. Lund and D. R. Heldman, eds. *Handbook of Food Engineering*, Marcel-Dekker, New York, 1992, pp. 393–436.

MUNIR CHERYAN
University of Illinois
Urbana, Illinois

MICROBIOLOGY OF FOODS

Food microbiology is the study of all aspects of microbial actions on food and food products, both directly and indirectly related to the welfare of mankind. Topics included in food microbiology are history of food microbiology, number and kinds of microbes in foods, intrinsic and extrinsic parameters of foods, methodologies, food spoilage, food preservation, and food-borne pathogens.

All living things smaller than 0.1 mm in diameter are classified as microorganisms because they are too small to be seen by the naked eye. Although human beings suspected the existence of these microbes for a long time, the real beginning of the field of study of microorganisms started when Antonie van Leeuwenhoek (1632–1723), a Dutch amateur lens grinder, reported the observation using his lens of many animalcules. He reported his observations to the Royal Society of London and described the microbes accurately, including bacteria (rod, sphere, and spiral), protozoa, and yeasts. Because he did not train any students to follow his work, no one really mastered his technique of lens grinding for the observation of microbes. His work was confirmed by Robert Hooke in 1678 using a compound microscope. No noticeable advancement in microbiology occurred until the middle of the 1800s.

The first major development in microbiology centered around the controversy over the theory of spontaneous generation. In Greek mythology humans were able to create life. People believed that animals could originate from the soil, maggots could be produced by exposing meat to warm air, and so on. Francesco Redi (1626–1697) showed that when gauze was placed over a jar containing meat, no maggots formed because flies were not allowed to contact the meat. Gradually the idea of life being generated from nonliving things was discarded by those performing experiments on the subject. However, this topic still lingered in the minds of many.

With the discovery of microbes the controversy rekindled. People questioned where these microbes came from. One camp believed that microbes occurred spontaneously, and the other camp believed that microbes were developed from other microbes and that life cannot come from nonliving matter. In the process of proving or disproving the theory of spontaneous generation, several important issues concerning microorganisms were raised and some experimental procedures were developed. John Needham in 1749 observed microorganisms in putrefying meat and explained this as evidence of spontaneous generation. Lazzaro Spallanzani boiled beef broth, sealed the flasks, and observed that no microorganisms developed in the broth. The pro-spontaneous generation camp proposed that exclusion of air prevented spontaneous generation. The con-spontaneous generation camp indicated that microorganisms in the air were the source of contamination in faulty experiments. By heating the air or trapping the microorganisms with heat-treated cotton, no microorganism would develop in heated broth even if the air was allowed to come in contact with the broth. The pro–spontaneous generation camp insisted that heat destroyed some vital force that prevented the development of life forms. Finally Louis Pasteur, using his famous goosenecked flask, showed that even unheated air, when allowed to contact heated broth, will not result in the development of microorganisms as long as the particles in air were allowed to settle at the bottom of the U-shaped neck of the flask. Disproving the theory of spontaneous generation was essential for the development of microbiology because this allowed scientists to observe microbiological events in sterile media with known inoculated cultures free from the uncertainties of some unknown living agent that might spontaneously develop in the media.

With his disproving of the theory of spontaneous generation, Pasteur introduced the field of fermentations and food microbiology. Since ancient times people have produced wine, beer, bread, and other fermented products without knowing the exact reason for the development of such foods. Pasteur was involved with fermentation because of the occurrence of spoilage in wine at that time in France. He started the project by first proving that alcoholic fermentation of grapes, fruits, and grains was the result of organisms he called ferments. He showed that good wine batches had certain types of ferment and bad batches had other types of ferment. By heating juice at 63°C for 30 min he could kill the bad ferments, and after cooling the juice he could consistently produce satisfactory wine by inoculating ferments from good wine batches into the juice. Not only did he solve the problem of wine disease, but he also developed the process of pasteurization of food and drink. In the process of these studies he discovered that some organisms can grow in the absence of air. His work resulted in the scientific understanding of food fermentation and thus greatly improved the quality of fermented food products. The value of his study was that he showed the direct relationship between activities of a specific microorganism in the development of a specific product (i.e., good yeasts acting on pasteurized grape juice under proper conditions will result in wine).

The precise time at which humans started to realize the role of microbes in food products cannot be determined. However, it is safe to assert that humans noticed the results of microbial action such as spoilage of food and food poisoning early in the history of food-gathering and food-producing periods. Humans experienced and noticed the changes occurring in foods without knowing the reasons behind such activities until the development of the science of microbiology. The real beginning of food microbiology coincided with the development of microbiology, especially Pasteur's work on food fermentation processes. Early studies in food microbiology centered on dairy bacteriology. Only about 20 years ago did the field of food microbiology become a recognized field. Today it has a great impact on issues such as food safety, genetic engineering of new food products, methodologies in applied microbiology, food technology, and preservation technologies.

NUMBERS AND KINDS OF MICROBES IN FOODS

The number and kinds of microbes in foods depend greatly on the food products and the conditions under which they are stored or processed. The microbial profiles of raw food products and processed products are completely different and pose different sets of problems in terms of food-spoilage potential and food-safety issues. In general, raw food products tend to have a heterogeneous population, whereas processed food usually contains those organisms that can survive the processes and subsequent storage conditions. Microorganisms are very small, and when they occur in foods they may be in the millions per gram, milliliter, or square centimeter. A convenient guide for categorizing microbial loads on meat surfaces is as follows: microbial load on meat surface is considered low when the count is 0–2 log colony-forming units (cfu)/cm^2; intermediate, 3–4 log cfu/cm^2; high, 5–6 log cfu/cm^2; and very high, 7 log cfu/cm^2 (1). Using this guide, samples with 0–4 log cfu/cm^2 would be considered as acceptable; samples with 5–6 log cfu/cm^2 would be considered questionable; and samples with 7 log cfu/cm^2 and above would be considered unacceptable from a food-spoilage standpoint. Generally, 7 log cfu/g, mL, or cm^2 is considered the index of spoilage, because food will exhibit odor, slime, or both at counts above 7.5 log cfu/g, mL, or cm^2. This guide does not include identification of potential pathogens in foods such as *Salmonella*, *Staphylococcus aureus*, or *Clostridium perfringens*.

The number of microorganisms in food is usually monitored by putting a known volume of liquid, weight of solid, or surface area sample into a sterile liquid diluent (usually a buffered liquid) and then appropriately diluting the sample such that when the sample is placed in a petri dish the number of organisms in the petri dish is in the range between 25 and 250. The agar medium used to grow commonly occurring microorganisms is called the standard plate count agar. A count is always converted to per milliliter, gram, or cubic centimeter for ease of comparison of data from laboratory to laboratory. The result provides only a count of all organisms that can grow in this agar under the time and incubation temperature (usually 48 h at 32°C). A differential count can also be made. By using different agars specifically designed for specific organisms, one can make a specific count, such as a coliform count by using a violet-red bile agar. This agar contains inhibitory compounds such that other organisms cannot grow, and when coliform organisms grow they will exhibit a characteristic color, shape, and size in the agar. By developing a variety of differential agars, scientists can monitor the occurrence of different types of organisms in food products and can make a *Staphylococcus aureus* count, a lactobacillus count, a *Clostridium perfringens* count, and so on. These types of differential counts give the food microbiologist a clearer picture of the specific population of microbes in certain food products. One of the concerns of food microbiologists is the recovery of injured cells by heat, cold, acid, base, radiation, and the like. On a nonselective agar the injured cells can grow due to rich nutrients. On a selective agar, however, many injured cells cannot grow due to added stress of the selective agents. Recently in the author's laboratory D. H. Kang and D. Y. C. Fung developed a procedure called thin agar layer method where a layer of nonselective agar was placed on top of a solidified selective agar. When injured cells are plated on top of the nonselective agar, they can repair and later will not be affected by the selective agents of the selective agar. With this method injured *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* were recovered efficiently using special selective agar, while other nontarget organisms, such as *Pseudomonas*, could be differentiated on the same plate.

To pinpoint the exact organism in a food product, a food microbiologist must take a colony and purify it, then perform a variety of morphologic, biochemical, and physiologic tests to ascertain the genus and species of the microorganism in question. The identification scheme would include morphology (rod, sphere, spiral, fruiting bodies), gram reaction (gram-positive or gram-negative), biochemical tests (carbohydrate fermentation, enzyme production), pigment production (yellow, red, blue, black, gray, green, etc.), nutritional requirements (organic, inorganic, complex, simple), temperature requirements (psychrophiles, 0–10°C; mesophiles, 10–45°C; thermophiles 45–75°C; and psychrotrophs, 0–30°C), pH requirements, fermentation products (acid, alcohols, etc.); antibiotic sensitivity, gas requirement (aerobic, anaerobic, facultative); pathogenicity, and serology (serotyping with specific antibodies).

After these tests are made, the food microbiologist can determine the exact nature of the organism in terms of genus and species. In many instances the number of specific pathogens in food is quite small. For example, there may be only 10 *Salmonella* among hundreds and thousands of other organisms in a food product, yet the food microbiologist must be able to detect them. To achieve this, food microbiologists have developed many elaborate preenrichment, enrichment, and selective-enrichment procedures so that target organisms (eg, *Salmonella*) will grow while other organisms are suppressed. For each target organism a separate scheme is developed. Consult the "General References" section at the end of this article for detailed information on methodologies in food microbiology.

The kind of microorganisms in food depend on the food and the conditions under which the food was made, processed, or stored. Microorganisms in food can be classified as:

> *Bacteria*. Bacteria are unicellular organisms ranging in size from 0.1 to 2.0 μm and occurring in rod, spherical, or curved shapes. They divide asexually by binary fission. Although no sexual stage occurs, bacteria can exchange genetic information from one to another.
>
> *Yeasts*. Yeasts are unicellular fungi occurring singularly with round or oval shape. Asexual reproduction is by budding, and sexual reproduction is by sexual spores.
>
> *Molds*. Molds are multicellular filamentous fungi, highly structured and organized in morphology. Reproduction is by sexual and asexual stages. They usually grow so profusely that humans can see them on foods (fuzzy masses) without magnification.
>
> *Viruses*. Viruses are submicroscopic entities that cannot reproduce without a living host. They occur with a protein coat enclosing a coil of DNA or RNA material. They can infect bacteria, plants, and animals. The shapes and sizes vary greatly among different groups of viruses.

BACTERIA

Bacteria of interest in food microbiology can be divided into the groupings discussed next.

Lactic Acid Bacteria

These are gram-positive, non-spore-forming bacteria producing lactic acid as the major or sole product of fermentation. As a group they are important in food spoilage because they cause souring and discoloration. However, they are also very important in pickling, cheese making, fermented dairy products, and silage technologies. The major genera of lactic acid bacteria include *Streptococcus*, *Lactococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, and *Lactobacillus*.

Streptococcus species such as *S. lactis*, *S. cremoris*, *S. thermophilus*, and *S. diacetilactis* are important starter cultures in the dairy industry. Some *Streptococcus* are pathogenic, such as *S. pyogenes* and *S. faecalis*. The dairy *Streptococcus* cultures are named *Lactococcus* and fecal *Streptococcus* cultures are named *Enterococcus*.

Pediococcus produces large quantities of lactic acid and is very important as a starter culture in the curing of meat.

Leuconostoc produces gas as well as slime in the presence of sugar. Although they are important spoilage organisms, they are also important producers of flavor compounds in dairy products.

Lactobacillus is a heterogeneous group of organisms consisting of slender, gram-positive rods. One group is homofermentative (producing large quantities of lactic acid) and another group is heterofermentative (producing acid and gas). *Lactobacillus* species are important in dairy, meat, and silage fermentation but are undesirable as spoilage organisms because of the production of large quantities of lactic acid in a variety of food products during storage.

Aerobic, Gram-Positive Catalase-Positive Cocci

Aerobic, gram-positive catalase-positive cocci occur in pairs, short chains, or clusters. They produce catalase (a very active enzyme) and form acid from carbohydrate. Some of them are quite heat resistant and salt tolerant and produce a variety of colors in food and culture media. Among them are important food pathogens and spoilage organisms.

Micrococcus species are aerobic, gram-positive cocci occurring widely in nature. Many species can grow under refrigeration and on inadequately sanitized equipment.

Staphylococcus is an important genus of gram-positive cocci. This is a facultative anaerobic organism that occurs widely in nature and on human skin. These bacteria produce a variety of extracellular enzymes and metabolites. The most important metabolite they produce is a group of heat-stable toxins called enterotoxins, which are the agents of staphylococcal intoxications. Once formed in food, these toxins are very difficult to destroy. Boiling for 1 h will not destroy the toxins but will kill the pathogens easily. The organism is salt tolerant and can spoil a variety of foods besides being an important food pathogen.

Spore-Forming Bacteria

The two important spore-forming bacteria in food microbiology are *Bacillus* and *Clostridium*.

Bacillus is a gram-positive, aerobic spore-forming bacteria. It occurs widely in nature and in soil. It forms large spores. Bacterial spores are highly resistant to all forms of food-processing techniques that usually kill vegetative cells. *Bacillus* possesses a wide range of physiologic activities, including fermentation of sugar, peptonization of protein, hydrolysis of starch, and rennin coagulation of milk. *Bacillus anthracis* is an important animal pathogen. Many *Bacillus* species are environmental contaminants and enter the food chain through air, water, and surface contacts. Some important *Bacillus* species are *B. subtilis*, *B. cereus*, and *B. stearothermophilus*. *B. cereus* has been implicated as a foodborne pathogen that produces an emetic toxin causing vomiting and a diarrheal toxin causing diarrhea in susceptible persons who consume contaminated food.

Clostridium species are gram-positive, anaerobic spore formers. Some are highly anaerobic and can be killed in the presence of molecular oxygen, whereas others are aerotolerant. Important species in food spoilage include *C. butyricum*, *C. putrefaciens*, and *C. sporogenes*. From the standpoint of food safety, *C. botulinum* is the most important because it produces a group of highly toxic protein toxins called botulin. These toxins are responsible for the often fatal disease called botulism. The canning industry has spent millions of dollars designing time and temperature treatments aimed specifically at killing the spores of this organism in canned goods. Fortunately, the toxin is heat sensitive. Boiling the toxin for 10 min will render it inactive.

Another species of *Clostridium* important in food microbiology is *C. perfringens*. Although this organism is less toxic than *C. botulinum*, *C. perfringens* accounts for about one-fifth of all food-poisoning cases in the United States annually. It produces a toxin that causes a mild diarrhea in human intestines after consumption of a large number (in the millions) of *C. perfringens* in foods such as meat and gravy.

Gram-Positive Irregularly Shaped Bacteria

Among the group of bacteria that have irregular shapes and are gram-positive is *Propionibacterium*, which is a small, anaerobic, pleomorphic rod (cell with irregular shapes). *P. shermanii* is the organism responsible in forming the eyes in Swiss-type cheeses. It also imparts desirable flavor in cheese fermentation. *Corynebacterium* is a pleomorphic rod arranged in Chinese letter morphology when observed under the microscope. Most of the species are environmental contaminants. *C. diphtheriae* is the agent responsible for diphtheria in humans. *Microbacterium* is a small, aerobic, heat-resistant rod. It can withstand 80°C for 10 min and is important in the spoilage of vacuum-packaged meat products

Gram-Negative Polarly Flagellated Bacteria

From the standpoint of food spoilage, *Pseudomonas* is considered one of the most important organisms. This gram-negative organism is a prolific metabolizer of organic compounds. The organism can grow in refrigerated temperatures (psychrotrophic) and form slime, fluorescent compounds, and pigments in cold-stored foods. It is responsible for the spoilage of chicken, meat, fish, vegetables, and all kinds of foods kept in cold storage. Because it is aerobic it usually is not responsible for the spoilage of canned or vacuum-packaged foods.

Acetobacter is also a member of this group of gram-negative bacteria. This organism oxidizes alcohol to acetic acid. In making vinegar it is desirable; however, in making wine it is the most important organism causing souring. *Photobacterium* can cause phosphorescence of meat and fish when incubated in suitable conditions. *Halobacterium* can grow in salt concentrations as high as 30%. It can produce pigments and spoil salty fish.

Gram-Negative Short Rods

The heterogeneous group of organisms consisting of gram-negative small rods, when motile, possess peritrichous (all-over-the-cell) flagella. They are facultative anaerobes that are found in water, soil, human and animal environments, and the food chain. Some of the organisms are exceedingly important in food microbiology. Many of them are food pathogens and food-spoilage organisms. The most important family in this group is the Enterobacteriaceae. According to the newest classification there are 14 genera in this family: *Escherichia*, *Shigella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Erwinia*, *Serratia*, *Hafnia*, *Edwardsiella*, *Proteus*, *Providencia*, *Morganella*, and *Yersinia*.

Escherichia is a true fecal coliform; the type species is the well-known *E. coli*. This organism has been used as an indicator of fecal contamination. Its presence indicates the potential presence of other, more pathogenic enteric organisms and is highly undesirable. Recently the serotype, *E. coli* O157:H7, has been the source of many major outbreaks involving hundreds of people and resulting in deaths. Major recalls of foods (up to 25 million lb of ground beef) were also made because of contamination by this organism. *E. coli* O157:H7 is now considered a food adulterant and is the cause of many food-safety regulations. *E. coli* is the most monitored organism in food and water microbiology.

Salmonella is an organism of great concern to the food industry. It is ubiquitous in the animal population and especially in poultry flocks. Some raw poultry products harbor *Salmonella*; thus all poultry products should be well cooked before consumption. The organism, when consumed in large number (1 million), can cause a disease called salmonellosis, which includes vomiting, nausea, diarrhea, chills, and fever. Mortality can occur in the very old, the very young, or the immunocompromised. More than 2,000 serotypes of *Salmonella* are reported. Many new rapid methods are now being developed to detect this organism in about 1 day. The conventional method takes about 5 to 7 days.

All members of the Enterobacteriaceae are potentially pathogenic. A more thorough discussion of bacterial pathogens is recorded in a different section of this volume (see the articles FOODBORNE DISEASES and RAPID METHODS OF MICROBIOLOGICAL ANALYSIS). It is important to note that occurrence of members of the Enterobacteriaceae in food is generally undesirable. Proper food handling will prevent or retard their growth and keep food safer for the consumers.

It should be noted that the naming of bacteria is a dynamic process in a constant state of revision and updating as new research demands. Consult *Bergey's Manual of Determinative Bacteriology*, 9th edition (2) for the current genus and species names of bacteria of interest.

YEASTS AND MOLDS

In general, yeasts and molds are considered spoilage organisms in food microbiology. When conditions are not favorable for the growth of bacteria, yeasts and molds will take over and spoil the food items. For example, in citrus foods the pH is too low for bacterial growth, so yeasts and molds are more active in spoiling those foods. Another example is dry goods. Bread is spoiled by mold more easily than by bacteria because molds can grow with much less water than can bacteria.

The only real food-poisoning concern of molds is the possible production of mycotoxins and aflatoxins by some molds such as *Aspergillus flavus* and *A. paraciticus*. Some of these compounds are carcinogenic and thus are of concern to regulatory agencies. In the area of food fermentation, yeasts and molds are of great importance because a large variety of fermented foods are produced by direct or indirect activities of yeasts and molds (see the article FOOD FERMENTATION).

INTRINSIC AND EXTRINSIC PARAMETERS OF FOODS

All foods possess a set of conditions called intrinsic parameters. These parameters can be influenced by another set of conditions called extrinsic parameters. Together, these two groups of parameters have great influence on the number and kinds of microorganisms occurring in and on a food and their physiologic activities. Intrinsic parameters of food include pH, moisture, oxidation-reduction potential (presence or absence of oxygen), nutrient content, occurrence of antimicrobial constituents, and biologic structures.

All microorganisms have a minimum, maximum, and optimal pH tolerance; a moisture requirement; an oxygen-tension requirement; and a nutrient requirement. By knowing these parameters, one can predict the presence and growth potential of specific microorganisms in certain types of foods. A pH of 4.5 is considered the demarcation line between acidic foods (<pH 4.5) and basic foods (>pH 4.5). Yeast and molds can grow down to pH 1 whereas bacteria cannot grow below pH 3. Thus acidic foods such as citrus fruits and carbonated soft drinks will be spoiled more by yeasts and molds than by bacteria. On the other hand, in a more basic food (>pH 4.5) bacteria will outgrow yeasts and molds owing to their higher metabolic rates in a favorable growth environment. Moisture content is another important parameter. This is usually expressed as water activity (A_w). Most moist foods are in the range of 0.95 to 1.00 A_w). When the A_w drops to 0.9, most spoilage bacteria reach their minimum level. Most spoilage yeasts have their minimum at 0.88, and molds have theirs at 0.80.

Thus in dry food products yeasts and molds grow much better than bacteria, and in moist food bacteria will outgrow yeasts and molds. The role of oxygen tension in and around food also has a great impact on the type of organisms growing there. Bacteria can be aerobic, anaerobic, or facultative anaerobic, so they can grow in a variety of oxygen levels (although different types will grow in different oxygen-tension environments). Yeast can grow both aerobically and anaerobically. Most molds, however, cannot grow anaerobically. In a properly vacuum-packaged food, for example, one should not find mold growing. The amount of oxygen measured in terms of oxidation-reduction potential also dictates the types of bacteria that can grow in the food. Disrupting the oxygen tension of a food (e.g., grinding a piece of meat to make ground beef from a steak) makes it easier for aerobic organisms to spoil the food.

Nutrient content (water; source of energy for metabolism; source of nitrogen, vitamins, and growth factors; and minerals) of different foods will support different types of microbes. In general, a food nutritious for human consumption is also a good source of nutrients for microbes. Some foods have natural antimicrobial compounds, such as eugenol in cloves, allicin in garlic, and lysozyme in egg, that can suppress the growth of some microbes. Biologic structures of some foods are also important for the prevention of microbial invasion. An example is the skin of an apple. When the apple is bruised, microbes can easily enter the fruit and spoil it.

Extrinsic parameters of food also play an important role in the activities of microbes. Temperature of storage greatly influences the growth of different classes of microbes. The amount of moisture in the environment (relative humidity) also influences the absorption of moisture or the dehydration of the food during storage and thus also influences the growth of different organisms. Varying the gaseous environment in storage will also change the types and growth rates of different organisms during storage of the food items. And last, the length of time of food storage also influences the spoilage potential by microbes in the food.

Thus intrinsic and extrinsic parameters of food are of great concern to food microbiologists. Skillful manipulation of these parameters by food microbiologists will result in more stable, more nutritious, fresher, and safer foods for the consumer.

FOOD-PRESERVATION TECHNIQUES

Food-preservation techniques can be grouped under drying, low-temperature freeze-drying, high-temperature, radiation, and chemical treatments. The technologies of these processes are recorded in other articles in this encyclopedia. In this article only their effects on microorganisms are discussed.

Drying is the most widely used method of food preservation in the world. Meat, fish, cereals, fruits, and vegetables are dried and preserved for a long time. Controlled dehydration and sun drying of foods removes water from foods so that microorganisms cannot grow. This process does not sterilize the food, so when water is reintroduced (rehydration), microbial growth may resume and may result in spoilage of food. Spores of bacteria and mold are known to survive for long periods (months and years) in dried foods.

Low-temperature preservation of food is based on retardation of enzymatic activity of microbes. As temperature decreases, the enzymatic activity of microbes also decreases and eventually stops at around freezing temperature and below. Freezing will kill approximately 10% of the initial microbial population, but the remaining population can survive for a long time (years). Refrigeration temperature (0–10°C) will retard growth of most organisms; however, a group of organisms called psychrotrophs will grow slowly under refrigeration temperature and may eventually spoil the cold-stored food. Psychrotrophic bacteria (*Pseudomonas*, *Micrococcus*, etc.), yeasts (*Candida*, *Debaryomyces*, etc.), and molds (*Penicillium*, *Mucor*, etc.) can spoil foods under prolonged storage. Although most psychrotrophs are nonpathogenic, some pathogens such as *C. botulinum* and *Listeria monocytogenes* have been found to grow at or around 4 to 6°C. Freeze drying or lyophilization of foods depends on the unique property of water—the triple point of water. Food is first frozen and then a vacuum is applied to sublime the water out of the food mass. Freeze-dried foods need not be refrigerated because microorganisms cannot grow without water. However, rehydrated food will have the same spoilage potential as the original food. Freeze drying does not kill microorganisms effectively. In fact, freeze drying is the best method to preserve bacterial cultures.

High-temperature preservation depends on heat coagulation of proteins and enzymes, thus killing microorganisms. Pasteurization, a form of high-temperature preservation, usually refers to treatment of food at 63°C for 30 min or 72°C for 15 s. These time and temperature combinations are designed to kill most vegetative cells in milk, especially *Mycobacterium tuberculosis* and *Coxiella burnetti*. Thermoduric organisms are those that can survive pasteurization and later grow and spoil the pasteurized food. To reach sterilization temperature, food must be cooked under pressure in sealed containers using 121°C for 1 min as 1 sterilization unit. This is the practice used in commercial canning. The purpose is to achieve time and temperature combinations such that the most heat-resistant spores of *C. botulinum* are destroyed in the specific food item being canned. Commercial canning has an excellent record of safety. Improper home canning is the major source of botulism due to toxins produced by *C. botulinum* that survive the improper heating and sealing procedures in canning.

Radiation treatment of food is of two types: ionizing radiation and nonionizing radiation. Ionizing radiation such as α, β, γ, and X ray kill microorganisms by breaking chemical bonds of essential macromolecules such as DNA (target theory) or by the ionization of water, which results in the formation of highly reactive free radicals such as HO^- or H_2O^- capable of splitting chemical bonds in the microorganisms. Ionizing radiation destroys microorganisms without generation of heat, and thus it is called cold sterilization. Interest in radiation preservation of food has been fluctuating in the past 30 years. Recently interest has resurged on an international scale in the use of radiation for food preservation. In 1999, U.S. approved levels of radiation in foods are 1.5 to 3.0 kGy for poultry (fresh or frozen) and 4.5 kGy max for fresh red meat and 7.0 kGy max for frozen red meat (1 Gy = 100 rad; 1 kGy = 1000 Gy).

Nonionizing radiation includes UV treatment and microwave treatment. UV has poor penetration and thus is used only for surface decontamination. Microwave treatment of food has gained great popularity. It is estimated that 75% of the homes in the United States have a microwave oven. The waves at 2540 MHz when applied to foods bound back and forth and create vibration of asymmetric, dielectric molecules (such as water), which generates heat. This heat cooks food as well as kills microorganisms. There may exist some as yet unexplained mechanism of microwave destruction of microorganisms besides purely the effect of heat (3). Microwave cooking will continue to be an important method for homes and institutions, and it is an effective means of destroying microorganisms in foods as long as the food is given enough microwave exposure time.

Chemicals are used to kill organisms (bactericidal) or to prevent them from growing (bacteriostatic). Many chemicals are used to treat equipment and the environment such as sanitizers, disinfectants, strong acids and bases, and halogens. In terms of food science, the subject of food additives is of great interest. Food additives are compounds added to foods for purposes such as improvement or modification of flavor, texture, rheology, color, pH change, water-holding capacity, and emulsification. Some of these compounds (lactic acid, acetic acid, propionic acid, sorbate, etc.) are used as preservatives because they can kill microorganisms in foods. Compounds approved for use in foods are controlled by the Food and Drug Administration and are listed on the generally recognized as safe (GRAS) list and periodically updated (4).

There are many recent developments in methodologies pertaining to food microbiology. Rapid methods and automation in microbiology have been the subject of many national and international symposia. The four major developments are miniaturization of conventional procedures and development of diagnostic kits, such as Fung's mini systems, API, Enterotube, Spectrum 10, IDS, Minitek, MicroID, and Biolog; modification of viable cell count procedures, such as 3M Petrifilm, Redigel, Isogrid, Spiral System, and DEFT test; development of alternative approaches for the estimation of microbial populations, such as the use of adenosine triphosphate, electrical impedance or conductance, microcalorimetry, or radiometry to indirectly measure biomass (bacteria, yeasts, and molds) in food products; and identification of microbes by novel and sophisticated instruments and procedures, such as Vitek system, DNA Probe (Genetrak), polymerase chain reaction (PCR), ribotyping, ELISA (Organon Teknika, Tecra), motility enrichment (BioControl), protein profiles, and fatty acid analysis.

The field of food microbiology is very important for food science and technology. It is one of the central disciplines in food science. Food microbiologists are called on to solve microbiologic problems related to other branches of food science and technology. The future of food microbiology is very bright indeed. See the article RAPID METHODS OF MICROBIOLOGICAL ANALYSIS for more details.

BIBLIOGRAPHY

1. D. Y. C. Fung et al., "Mesophilic and Psychrotrophic Bacterial Populations on Hot-Boned and Conventionally Processed Beef," *J. Food Prot.* **43**, 547–550 (1980).
2. J. Holt et al., *Bergey's Manual of Determinative Bacteriology*, 9th ed., Williams & Wilkins, Baltimore, Md., 1994.
3. D. Y. C. Fung and F. E. Cunningham, "Effects of Microwave Cooking on Microorganisms in Foods," *J. Food Prot.* **43**, 641–650 (1980).
4. B. L. Oser and B. K. Bernard, "Thirteen GRAS Substances," *Food Technol.* **38**(10), 66–84 (1984).

GENERAL REFERENCES

Associations of Official Analytical Chemists, *Official Methods of Analysis*, AOAC Arlington, Va., 1988.

J. C. Ayres, O. Mundt, and W. E. Sandine, *Microbiology of Foods*, W. H. Freeman, San Francisco, Calif., 1980.

G. J. Banwart, *Basic Food Microbiology*, AVI, Westport, Conn., 1979; 2nd ed., Chapman & Hall, New York, 1989.

C. M. Bourgeois, J. Y. Leveau, and D. Y. C. Fung, Microbiological Control of Food and Agricultural Products (English edition), VCH, New York, 1995.

J. E. L. Corry, D. Roberts, and F. A. Skinner, *Isolation and Identification Methods for Food Poisoning Organisms*, Academic Press, New York, 1982

M. P. Doyle, L. R. Beuchat, and T. J. Montville, Food Microbiology: Fundamentals and Frontiers. ASM, Washington D.C., 1997.

Food and Drug Administration, *Bacteriology Analytical Manual*, 6th ed., Association of Official Analytical Chemists, Arlington, Va., 1984; 18th ed., Association of Official Analytical Chemists International, Arlington, Va., 1995.

D. Y. C. Fung, "Microbiology of Batter and Breading," in D. R. Suderman and F. E. Cunningham, eds., *Batter and Breading Technology*, AVI, Westport, Conn., 1983, pp. 106–119.

D. Y. C. Fung, "Rapid Methods for Determining the Bacterial Quality of Red Meats," *J. Environ. Health* **46**, 226–228 (1984).

D. Y. C. Fung, "Types of Microorganisms," in F. E. Cunningham and N. A. Cox, eds., *Microbiology of Poultry Meat Products*, Academic Press, New York, 1987.

D. Y. C. Fung, M. A. Buono, and L. E. Erickson, "Mixed Culture Interactions in Anaerobic Fermentations," in L. E. Erickson and D. Y. C. Fung, eds., *Handbook on Anaerobic Fermentations*, Marcel Dekker, New York, 1988.

D. Y. C. Fung and C. L. Kastner, "Microwave Cooking and Meat Microbiology," *Proceedings Reciprocal Meat Conference* **35**, 81–85 (1983)

D. Y. C. Fung and K. Vicheinroj, *Introduction to Food Fermentation*, Kansas State Univ. Press, Manhattan, Ks., 1998.

W F. Harrigan and M E. McCance, *Laboratory Methods in Food and Dairy Microbiology*, Academic Press, New York, 1976.

P. A. Hartman, *Miniaturized Microbiological Methods*, Academic Press, New York, 1968.

C. G. Heden and T. Illeni, eds., *Automation in Microbiology and Immunology*, Wiley, New York, 1975.

C. G. Heden and T. Illeni, eds., *New Approaches to the Identification of Microorganisms*, Wiley New York, 1975.

B. C. Hobbs and J. H. B. Christian, *The Microbiological Safety of Food*, Academic Press, New York, 1973.

J. G. Holt et al., *Bergey's Manual of Determinative Bacteriology*, 9th ed., Williams & Wilkins, Baltimore, Md., 1994.

International Commission on Microbiological Specifications for Foods, *Microorganisms in Foods*, Vol. 2, University of Toronto Press, Toronto, Canada, 1974.

International Commission on Microbiological Specification for Foods, *Microorganisms in Foods, vol. 5· Microbiological Specifications of Food Pathogens*, Blackie Academics and Professionals, New York, 1996.

J M. Jay, *Modern Food Microbiology*, 3rd ed., Van Nostrand-Reinhold, New York, 1986; 5th ed., 1998.

F. V Kosikowski and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed. Vol. 1: *Origins and Principles*, Vol 2: *Procedures and Analysis*, Edwards Brothers, Ann Arbor, Mich., 1997.

R. T. Marshall, *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington, D.C., 1992.

E H. Marth, ed., *Standard Methods for the Examination of Dairy Products*, American Public Health Association, Washington, D.C., 1978.

B. M. Mitruka, *Methods of Detection and Identification of Bacteria*, CRC Press, Cleveland, Ohio, 1976

D. A. A. Mossel et al., *Essentials of the Microbiology of Foods*, Wiley, New York, 1995.

J T. Nickerson and A. J. Sinskey, *Microbiology of Foods and Food Processing*, American Elsevier, New York, 1972.

H. Rieman and F. L. Bryan, eds., *Food-Borne Infections and Intoxications*, Academic Press, New York, 1979.

T. A. Roberts and F. A. Skinner, *Food Microbiology Advances and Prospects*, Academic Press, New York, 1983.

A. N. Sharpe, *Food Microbiology: A Framework for the Future*, Charles C. Thomas, Springfield, Ill., 1980

J. H. Silliker et al., *Microbial Ecology of Foods*, 2 Vols., Academic Press, New York, 1980.

R. C. Tilton, *Rapid Methods and Automation in Microbiology*, American Society of Microbiology, Washington, D C., 1982.

C. Vanderzant and D. Splittstoesser, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, D.C., 1990

H. H. Weiser, G. J. Mountney, and W. A. Gould, *Practical Food Microbiology and Technology*, AVI, Westport, Conn., 1971.

DANIEL Y. C. FUNG
Kansas State University
Manhattan, Kansas

MICROWAVE SCIENCE AND TECHNOLOGY

Interest in microwave processing began shortly after World War II with the introduction of the first microwave oven by the Raytheon Co. (Waltham, Mass.) a manufacturer of magnetrons for radar. The first U.S. patent granted to Raytheon Co., which claimed the novelty of microwave cooking, was illustrated with a food product moving on a conveyor belt past a microwave source (1). Many other patents followed, research was carried out on the effects of microwave energy on food materials, microwave cooking technology was developed, and eventually a consumer microwave oven sales boom occurred so that today this appliance is commonplace in most homes in the United States and a similar trend is taking place in most of the developed countries of the world. Microwave energy use in food processing is showing a similar growth trend, although at a much more modest rate.

FUNDAMENTALS OF MICROWAVE HEATING

Microwaves are electromagnetic (EM) waves of very short wavelength. In the EM spectrum, microwaves lie between the television frequencies and infrared. In terms of wavelength, radio waves are measured in kilometers, television frequencies in meters, microwaves in centimeters, and infrared in microns. The wavelength and frequency are related by the expression:

$$\text{wavelength } (\lambda_0) = \frac{\text{speed of light } (c)}{\text{frequency } (f)}$$

Thus for a typical microwave oven frequency of 2,450 MHz, the wavelength (λ_0) is

$$\frac{3 \times 10^{10} \text{ cm/s}}{2.45 \times 10^{9} \text{ cycles/s}} = 12.25 \text{ cm/cycle}$$

Microwaves, as with other wave energy, radiate from a source in all directions. These waves carry energy and the amount of energy they carry depends on the amount of energy imparted to them. The energy of microwaves comes from electrical energy that is converted by a power supply to high voltages that in turn are applied to the microwave power tube or generator. The most common power tube used in microwave ovens is the magnetron and it broadcasts its energy into the applicator, which may be an oven, a waveguide, or some other device containing the material to be heated.

Microwaves, like infrared and visible light, are reflected, transmitted, and absorbed. They are reflected from metal surfaces: the microwave oven is basically a metal box in which the waves reflect from the walls and create a resonant system. Microwaves are transmitted, that is, they pass through many materials including glass, ceramics, plastics and paper. Some materials are only partially transparent to microwaves; that is, they absorb some energy. When microwaves are absorbed their energy is converted to heat.

How Microwaves Produce Heat

Microwaves in themselves are not heat. The materials that absorb microwaves convert the energy to heat. In foods, it is the polar molecules that for the most part interact with microwaves to produce heat. Water is the most common polar molecule and is a component of most foods. In the presence of a microwave electric field, water molecules attempt to line up with the field in much the same manner iron filings line up with the field of a magnet. Because the microwave field is reversing its polarity billions of times each second, the water molecule, because it is constrained by the nature of the food of which it is a part only begins to move in one direction when it must reverse itself and move in the opposite direction. In doing so, considerable kinetic energy is extracted from the microwave field and heating occurs. The phenomenon is similar to the heating of the human body when exposed to the sun or any other heat source. Energy in the form of infrared rays from the sun, is not heat until it is absorbed by the body and the

International Commission on Microbiological Specifications for Foods, *Microorganisms in Foods*, Vol. 2, University of Toronto Press, Toronto, Canada, 1974.

International Commission on Microbiological Specification for Foods, *Microorganisms in Foods, vol. 5: Microbiological Specifications of Food Pathogens*, Blackie Academics and Professionals, New York, 1996.

J. M. Jay, *Modern Food Microbiology*, 3rd ed., Van Nostrand–Reinhold, New York, 1986; 5th ed., 1998.

F. V. Kosikowski and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed. Vol. 1: *Origins and Principles*, Vol 2: *Procedures and Analysis*, Edwards Brothers, Ann Arbor, Mich., 1997.

R. T. Marshall, *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington, D.C., 1992.

E. H. Marth, ed., *Standard Methods for the Examination of Dairy Products*, American Public Health Association, Washington, D.C., 1978.

B. M. Mitruka, *Methods of Detection and Identification of Bacteria*, CRC Press, Cleveland, Ohio, 1976

D. A. A. Mossel et al., *Essentials of the Microbiology of Foods*, Wiley, New York, 1995.

J. T. Nickerson and A. J. Sinskey, *Microbiology of Foods and Food Processing*, American Elsevier, New York, 1972

H. Rieman and F. L. Bryan, eds., *Food-Borne Infections and Intoxications*, Academic Press, New York, 1979.

T. A. Roberts and F. A. Skinner, *Food Microbiology Advances and Prospects*, Academic Press, New York, 1983.

A. N. Sharpe, *Food Microbiology: A Framework for the Future*, Charles C. Thomas, Springfield, Ill., 1980

J. H. Silliker et al., *Microbial Ecology of Foods*, 2 Vols., Academic Press, New York, 1980.

R. C. Tilton, *Rapid Methods and Automation in Microbiology*, American Society of Microbiology, Washington, D.C., 1982.

C. Vanderzant and D. Splittstoesser, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, D.C., 1990

H. H. Weiser, G. J. Mountney, and W. A. Gould, *Practical Food Microbiology and Technology*, AVI, Westport, Conn., 1971.

DANIEL Y. C. FUNG
Kansas State University
Manhattan, Kansas

MICROWAVE SCIENCE AND TECHNOLOGY

Interest in microwave processing began shortly after World War II with the introduction of the first microwave oven by the Raytheon Co. (Waltham, Mass.) a manufacturer of magnetrons for radar. The first U.S. patent granted to Raytheon Co., which claimed the novelty of microwave cooking, was illustrated with a food product moving on a conveyor belt past a microwave source (1). Many other patents followed, research was carried out on the effects of microwave energy on food materials, microwave cooking technology was developed, and eventually a consumer microwave oven sales boom occurred so that today this appliance is commonplace in most homes in the United States and a similar trend is taking place in most of the developed countries of the world. Microwave energy use in food processing is showing a similar growth trend, although at a much more modest rate.

FUNDAMENTALS OF MICROWAVE HEATING

Microwaves are electromagnetic (EM) waves of very short wavelength. In the EM spectrum, microwaves lie between the television frequencies and infrared. In terms of wavelength, radio waves are measured in kilometers, television frequencies in meters, microwaves in centimeters, and infrared in microns. The wavelength and frequency are related by the expression:

$$\text{wavelength } (\lambda_0) = \frac{\text{speed of light } (c)}{\text{frequency } (f)}$$

Thus for a typical microwave oven frequency of 2,450 MHz, the wavelength (λ_0) is

$$\frac{3 \times 10^{10}\ \text{cm/s}}{2.45 \times 10^{9}\ \text{cycles/s}} = 12.25\ \text{cm/cycle}$$

Microwaves, as with other wave energy, radiate from a source in all directions. These waves carry energy and the amount of energy they carry depends on the amount of energy imparted to them. The energy of microwaves comes from electrical energy that is converted by a power supply to high voltages that in turn are applied to the microwave power tube or generator. The most common power tube used in microwave ovens is the magnetron and it broadcasts its energy into the applicator, which may be an oven, a waveguide, or some other device containing the material to be heated.

Microwaves, like infrared and visible light, are reflected, transmitted, and absorbed. They are reflected from metal surfaces: the microwave oven is basically a metal box in which the waves reflect from the walls and create a resonant system. Microwaves are transmitted, that is, they pass through many materials including glass, ceramics, plastics and paper. Some materials are only partially transparent to microwaves; that is, they absorb some energy. When microwaves are absorbed their energy is converted to heat.

How Microwaves Produce Heat

Microwaves in themselves are not heat. The materials that absorb microwaves convert the energy to heat. In foods, it is the polar molecules that for the most part interact with microwaves to produce heat. Water is the most common polar molecule and is a component of most foods. In the presence of a microwave electric field, water molecules attempt to line up with the field in much the same manner iron filings line up with the field of a magnet. Because the microwave field is reversing its polarity billions of times each second, the water molecule, because it is constrained by the nature of the food of which it is a part only begins to move in one direction when it must reverse itself and move in the opposite direction. In doing so, considerable kinetic energy is extracted from the microwave field and heating occurs. The phenomenon is similar to the heating of the human body when exposed to the sun or any other heat source. Energy in the form of infrared rays from the sun, is not heat until it is absorbed by the body and the polar molecules in the surface layers of the body convert it into heat.

Space charge polarization is an equally important microwave heating mechanism. Ions, are caused by the microwave field to flow first in one direction then in the opposite direction as the field is reversed. The effect of ionic conduction can be observed in the microwave heating of salted water in that higher temperatures are found at the surface. Ionic conduction has a negative effect on microwave energy penetration, thus foods with a high salt content show greater surface heating.

Microwave Properties of Foods

The amount of energy that can be absorbed by a substance is expressed by the relationship:

$$P = \sigma' E^2 \ (\mathrm{W/cm^3}) \qquad (1)$$

where P is the the power absorbed in watts/cm^3, σ' is the equivalent dielectric conductivity, and E is the voltage gradient in V/cm. The dielectric conductivity,

$$\sigma' = 2\pi \varepsilon_0 \varepsilon'' f \qquad (2)$$

where f is the frequency of the energy source, ε_0 is the dielectric constant of vacuum (8.85×10^{-14} F/cm), and ε'' is the dielectric loss factor of the substance. Substituting equation 2 into equation 1 gives

$$P = 55.61 \times 10^{-14} f \varepsilon'' E^2 \qquad (3)$$

Dielectric Loss Factor

Because the field strength (E) and the frequency are essentially constant for the microwave oven being used, the loss factor (ε'') is the only variable. The term loss originated from the discovery that energy loss occurred when the electrical energy through a capacitor was cycled on and off. The term today, in microwave heating technology, is representative of a desirable condition, whereas it once was considered undesirable. In the context being used, the loss factor represents a property of the material being processed, and a lossy material is one that heats well, while a low loss material is one that heats poorly and is, therefore, more transparent to microwave energy The loss factor is a measurable quantity and a considerable volume of dielectric loss data have been accumulated (2–6).

The loss factor is the product of two measurable properties: the dielectric constant (ε') and the loss tangent (tan δ). The loss factor varies with temperature and frequency as shown in Table 1 and indicates that the penetration of microwave energy decreases with increasing dielectric loss.

Table 1. Dielectric Loss of Raw Turkey Roll as a Function of Temperature and Microwave Frequency

	Frequency, MHz		
Temperature, °C	2,450	915	300
−40	0.13	0.17	0.29
−20	0.61	0.73	1.21
5	21.6	26.4	58.1
25	21.5	33.2	86.2
45	23.8	42.8	118
65	26.8	53.1	159
80	25.0	69.0	179
100	27.1	78.7	205
110	29.2	83.4	218
115	29.9	85.8	224
120	30.3	88.2	231

Source: Ref 7.

Penetration

The penetration depth is that depth in a material at which the energy level is 37% (or $1/e$) of the surface value. The term half-power depth is also used and is that depth in a material at which the power level is one-half that at the surface. The equation for converting dielectric property data into penetration depth (d) is

$$d = 1/2\alpha$$

for $1/e$, and for half-power depth it is

$$d = 1/2.886\alpha$$

where α is the attenuation constant, which is calculated as:

$$\alpha = \frac{2\pi}{\lambda_0}\left[\frac{\varepsilon'}{2}\left(\sqrt{1 + \tan^2\delta} - 1\right)\right]^{1/2}$$

Thus the main difference between microwave heating and other heating methods is that microwave energy penetrates deeply into food materials and is converted to heat as it penetrates. The temperature profile shown in Figure 1 illustrates the effect of microwave heating on a large food mass and the effect of time after the energy is no longer being applied. It is clear in this case that conduction heating will always play a role in any microwave heating process where a temperature gradient exists.

EFFECT OF VARIOUS PHYSICAL FACTORS

In addition to the dielectric properties of foods there are a number of other factors that affect microwave heating performance. Among these factors are geometry (shape), surface-to-volume ratio, specific heat, density, thermal conductivity, and evaporative cooling. These are important factors in food processing as well as cooking and heating in consumer and institutional microwave ovens.

Geometry

The shape of food items is critical to good microwave heating results. The sphere is the ideal shape as energy tends to be focused to give heating at the center of the sphere. Obviously as the diameter is increased it may be impossible for center heating to occur except by conduction.

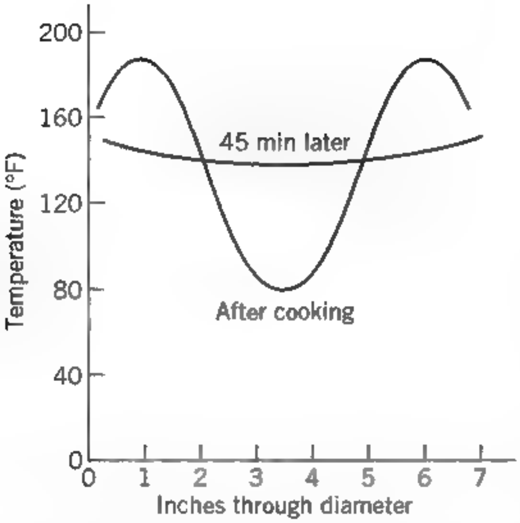


Figure 1. The effect of thermal conduction on the temperature profile of a large roast beef after 45 min standing out of a microwave oven.

Heating can be concentrated in the center of spheres with diameters (8) that measure between 20 and 60 mm. Such concentrated heating can be a disadvantage in that conduction heating may not be able to dissipate the temperature gradient and the mass would erupt, as in the heating of an egg in its shell. At lower microwave heating rates, more time is provided for conduction heating to take place. In the example of beef roasting the postmicrowave heating time could have been reduced if a lower heating rate were used, as the temperature gradients would not have been as great.

Computer simulation studies as well as actual heating of spheres and cylinders of various diameters have been carried out (8). A phantom food mixture with dielectric properties similar to food materials was used in these studies and temperature patterns were obtained by color thermography. For cylinders, thermography indicated maximum core heating occurred at diameters of 35 mm. At 50 mm, the core and surface heating occurred, while at 75 mm only surface heating was observed. Calculations support maximum center heating for diameters of 20 to 35 mm, while surface heating was more prominent at 40 to 50 mm and was dominant for larger cylinders.

While spherically shaped foods are commonplace among many natural foods eg, beets, onions, potatoes and some prepared foods (meatballs), the majority of foods are not spherical in shape. The cylinder is the next best shape in terms of heating performance.

For many foods it is the food container or dish that determines the shape and, therefore, affects the heating performance. A rectangular shape is common, but it is clear that food in the corners of a rectangular container will be overcooked before the remainder of the food. Overcooking in the corners also occurs when using a conventional oven, but because conduction cooking is much slower the temperature differences are not as pronounced. Where this shape is unavoidable, the corners can be shielded with aluminum foil to reduce the heating rate in these areas. Even when corners are unavoidable they should have generous radii to minimize the overheating effect. Also vertical or near vertical sides are preferable to sloping sides. Excessive sloping gives shallow areas where dehydration can occur.

Shielding and Shadowing

Shielding is the use of metal to reflect microwave energy and thereby reduce the heating rate in selected areas. Aluminum foil is usually used because of its availability, but also because it can be wrapped around the specific parts to be shielded. Shadowing can be described as a shielding effect by a food product that results in a reduced heating rate where the products are in contact. Thus it is a mutual effect with products shadowing each other. Food masses should always be spaced apart for best cooking or heating results.

Surface-to-Volume Ratio

As with conventional heating, the greater the surface area, the faster that cooking occurs. Thus potatoes and carrots can be cooked faster if they are diced. Vegetables such as corn and peas have a high surface-to-volume ratio and cook rapidly. This same condition also means that foods will cool more rapidly. This is an important consideration when developing new products, and means to reduce the cooling rate should be provided where possible, for example, by using insulated containers or preheated serving dishes.

Specific Heat

The specific heat of a material is the ratio of its thermal capacity to that of water. It is a measure of the energy required to raise product temperature by a specific amount. The specific heat of a food is closely related to its moisture content.

Density

Usually there is a clear relationship between density and moisture content. Thus bread, which has a low moisture content (about 35%) is not as dense as beef (65%); for example, 1 lb of bread requires about two-thirds as much energy as 1 lb of beef to heat to the same temperature. Thermal conductivity also comes into play in that the dimensions involved could mean substantial overheating of the surface of beef because of its density, while the more open structure of bread would not rely on thermal conductivity to the same extent.

Thermal Conductivity

Thermal conductivity is a measure of a material's ability to transfer heat in response to a temperature difference. Even in microwave cooking it plays an important role in spite of the penetrating nature of microwave energy. Without heat conduction unacceptable temperature differences would occur in most microwave heated or cooked foods. Such differences can be minimized by reducing the rate of microwave heating.

Starting and Final Temperature

Heating time depends not only on the power level but also on the temperature range that must be bridged to accomplish the desired result. Thus frozen foods take longer than refrigerated foods, which in turn take longer than shelf-stable food heated from room temperature assuming all were heated to the same final temperature. An additional quantity of heat is required to heat from the frozen state, ie, the heat of fusion.

Evaporative Cooling

This is a phenomenon that is much more evident when cooking with microwave energy. This effect is responsible for the lower surface temperature of some foods cooked in microwave ovens and, therefore, is responsible for the myth that microwaves cook from the inside out. It is not noticeable in a conventional oven because of heat radiation to the surface of foods.

FOOD-PROCESSING APPLICATIONS

A number of food-processing applications representative of current activity are listed in Table 2. Some are well-established commercial operations. Others have been demonstrated on a pilot scale. Still other have been discontinued for one reason or another.

ADVANTAGES OF MICROWAVE PROCESSING

Speed

Because microwave heating occurs within the material being processed and not just at the surface the process can be greatly accelerated. This is particularly true for materials that are poor thermal conductors and for drying processes where the high dielectric loss properties of water result in selective heating of moist areas and rapid diffusion of water vapor to the surface.

Energy Savings

Microwave energy is not expended in heating the walls of the equipment or the environment. Heat is developed where it is needed, ie, in the material being processed. In drying processes, microwave energy is applied after conventional drying has reached the critical point where the drying rate falls off, thus the bulk of the heat of vaporization can be supplied by cheaper forms of energy

Table 2. Microwave Food-Processing Applications

Process	Food products
Tempering	Frozen foods
Drying	Pasta, snack foods, onions, rice cakes, seaweed, fruit juices
Cooking	Bacon, meat patties, potatoes, poultry
Pasteurization	Fresh pasta, bread, meals
Sterilization	Meals
Rendering	Tallow
Roasting	Coffee, cocoa beans
Denaturation	Sausage emulsion
Baking	Bread

Space Savings

The increase in processing rate makes it possible to design more compact equipment. In many cases plant capacity can be increased severalfold without the need for additional building space.

Fast Start-Up and Shutdown

There is practically no thermal lag with microwave processing so that the system can be operational almost immediately, adding in some cases extra hours of productive operation. Cleanup after shutdown can begin sooner in most cases because the equipment is cooler and can be worked on sooner. In a hybrid system, where conventional heat is used processing can be carried out at lower temperatures.

Precise Process Control

Heating adjustments are immediately effective and the operation can be fine-tuned easily. Systems respond instantaneously to feedback from sensors.

Improved Working Conditions

Because only the material being processed is heated there are negligible heat losses to the working environment. Worker productivity is enhanced and the requirement for ventilation and air-conditioning is reduced.

EXAMPLES OF MICROWAVE PROCESSING APPLICATIONS

Tempering

Conventional frozen food thawing methods are extremely inefficient taking many hours to several days depending on the product. The reason for this is that the thermal conductivity of the frozen product is about three times that of the thawed product. Thus as the surface thaws, heat is more slowly conducted toward the center.

Complete thawing of frozen foods by means of microwave energy also has its problems It has been pointed out that as the frozen products approached 0°C, microwave penetration decreases rapidly and care has to be taken to prevent runaway heating (9). Furthermore, if frozen materials are held at ambient temperature too long before being exposed to microwave energy most of the energy will be absorbed at the surface and thawing to the core will not be possible.

Fortunately it is not necessary in most cases to completely thaw frozen materials. The tempering process stops short of thawing by several degrees; thus tempering has come to mean "raising the temperature of frozen food to a higher temperature that is still below the freezing point, but at which temperature the product is firm but no longer hard." Properly tempered frozen food is easily sliced, diced, separated, or otherwise handled for further processing.

Some data on the energy required to freeze foods are also pertinent to thawing foods and clearly illustrate the benefits of stopping the process prior to complete thawing (10). From Table 3 it can be seen that the energy required to raise the temperature of frozen beef from −17.7 to −4.4°C is about one-half that required to raise the temperature to −2.2°C. Thus, where possible, tempering should be terminated at the lowest acceptable temperature. It should also be noted that microwave tempering is uniform throughout the frozen mass being tempered.

Microwave tempering is one of the more economical processes in terms of energy consumption. The cost of tempering in terms of energy and magnetron replacement costs is less than $0.01/lb. Other factors that contribute to the return on investment include improved yield and quality, space and labor savings, simplified production scheduling, and increased plant productivity.

Most microwave tempering equipment today is operated at 915 MHz at power levels varying from 25 to at least 150 kW and are continuous. Batch units are available for smaller food-processing operations and usually operate with 40 kW of power at 915 MHz. Continuous equipment also is available at 2,450 MHz and varies in power from 33 to 132 kW. The microwave penetration at this frequency is less than at 915 MHz and requires the use of a refrigerated air circulation system to prevent surface thawing so that internal tempering can be accomplished. There are approximately three hundred microwave tempering systems in operation worldwide, with about one hundred eighty systems in the United States. These systems average 100 kW of microwave power. Annual tonnage of products tempered in such equipment is on the order of 5 billion lb.

Drying

Pasta drying is an excellent example of a process in which a relatively small amount of microwave energy is needed to generate maximum benefits. This process is accomplished in three stages: conventional hot air drying of the freshly extruded pasta followed by a microwave-hot air stage and a temperature equalization stage. The fresh pasta enters the first stage at about 30% moisture where it is exposed to hot air at 71–82°C. Rapid moisture loss to about 23% occurs in minutes and drying is continued for a total of about 35 min to reduce the moisture to about 18%. The product then falls by gravity into one end of the microwave thermal dryer where the combination of microwave energy at 915 MHz and hot air (82–93°C, 15–20% RH, 30.5 mpm) lowers the moisture level to 13–13.5% in about 12 min. The product finally falls once again by gravity to the final stage where it is conveyed slowly through a 70–80% RH zone to cool to temperatures low enough to prevent thermal stress cracking. About 1 h is required. An additional 0.5–1.0% moisture loss occurs in this stage. The major advantages of this process are time, space, and labor savings.

Table 3. Energy Requirement to Thaw Frozen Beef

J/g	°C
0	−40
50	17.7
112.8	−4.4
173.3	−2.2

Source. Ref. 10

Another drying process of interest is a system for the production of bread crumbs from bread dough. The fresh dough is extruded into one end of an 80 kW, 2450 MHz microwave-hot air tunnel where it is baked and dried so that the finished product can be cooled and ground into bread crumbs.

Some snack food items are being produced in a 240 kW, 915 MHz baking-drying unit. The dough is extruded as parallel strands onto a 5-ft-wide belt that passes through 12 independent sections, each with 25 kW of microwave power and an independent air system, where it is puffed, baked, and partially dried.

Microwave vacuum drying of fruit juice concentrates has been successfully demonstrated. In this process the concentrate is pumped into the vacuum chamber onto a conveyor belt where it foams under the effect of microwave energy and reduced pressure to a thickness of 80–100 mm. The product dries to a meringuelike condition, is crushed, scraped, and brushed from the belt into a receiver that is isolated from the vacuum chamber. A 48 kW, 2,450 MHz system will dry concentrate to 2% moisture in about 40 min at a rate of 50 kg/h.

Cooking Processes

A pair of microwave conveyor units has been used in the cooking of poultry parts. They operated at a frequency of 2,450 MHz: one with 80 kW of microwave power, the other with 50 kW of power. Cut-up breasts and thighs were processed in the 80 kW unit, while wings and legs were processed in the 50 kW unit. Saturated steam was introduced from a manifold of nozzles along the back walls of the microwave tunnels. As the cooked parts passed out of the ovens they were conveyed through several water chillers to lower their temperature. After the batter and breading operations the parts were blast frozen and packaged for the commercial food service trade. The processing rate was 2,500 lb/h.

Although in use for more than a decade in supplying precooked bacon to the institutional food service trade, there was a major escalation a few years ago in the requirement for precooked bacon by the fast-food business that now absorbs almost the entire production of this product. The major systems in use today are microwave-only systems, which operate at power levels up to 400 kW, and hybrid systems, which combine microwave energy (150 kW) and hot air.

An advantage of the microwave process is in the evenness of cooking such that the difference between microwave-cooked bacon 22–24 slices/lb and 16–18 slices/lb cannot be discerned. The microwave product is more tender and shrinks much less than the conventionally cooked product. To one fast-food firm, the greater number of slices per pound translates into substantial savings in shipping costs because 38% less raw bacon is required to meet their needs. Based on a monthly consumption of 600,000 lb of raw bacon, a 38% reduction amounts to a savings of almost $164,000 in raw bacon costs. In addition, the high quality of the rendered fat by-product commands a good price.

There are approximately fifty systems installed worldwide with an annual production of 8 billion slices of precooked bacon. Systems average 500 kW; however, some systems are 600 kW and at least one is 1,000 kW. Microwave cooking has the advantage over other methods in that nitrosamines are essentially absent.

A process was developed in Sweden for the cooking of meat patties; 30–45 kW, 2,450 MHz systems are typical. In practice, meat patties are formed by automated patty-making equipment at a rate of 1,000 kg/h (16,000 patties/h) and deposited on a conveyor belt. A few grams of margarine are deposited on each patty to improve heat transfer and surface browning as the patties are carried between Teflon belts that move between heated aluminum platens. The browned patties then pass through the microwave unit where in little more than a minute they are cooked to the proper degree. Typically, the temperature is raised from 40 to 70°C.

Pasteurization

Fresh pasta is a product that by its very nature is not easily pasteurized by conventional thermal methods. The loose condition of the product in the package makes conduction heat transfer impractical. Until recently the product was being marketed in modified atmosphere packaging that provides about 30 days of refrigerated shelf life. The combination of modified atmosphere packaging and microwave pasteurization extends the shelf life to 90–120 days.

The process is carried out by filling the fresh product into plastic trays, gas flushing, lidding, and passing the trays through a microwave tunnel oven to raise its temperature to 80°C, then cooling. Processing time is 3–5 min followed by a holding time at 80°C that varies with the product but is typically about 7 min. Cooling may take an additional 45 min. Thus far, this process has been carried out only at 2,450 MHz. A typical plant producing fresh gnocchi processes 3,000 lb/h. The microwave process is effective against molds, yeasts, and thermolabile microorganisms.

There are a number of full-scale systems in operation today for the microwave pasteurization of sliced packaged bread. The purpose of this process is twofold: (1) to increase the shelf life so that specialty breads, in particular, do not have to be baked as frequently and (2) to eliminate the need for chemical preservatives.

The most recently installed systems carry out pasteurization by conveying baskets of prepackaged bread through a microwave tunnel. The density of the product is sufficiently low that heating occurs deep within the product. As the baskets exit from the tunnel they are stacked to allow the temperatures to equilibrate. This may take 1–2 h. This practice appears to have no harmful effect on product quality and shelf life is substantially increased.

A wide variety of prepared foods are being pasteurized in package by two different microwave processes and sold at retail from refrigerated cabinets. One process involves hot filling the product in microwavable plastic dishes, lidding them, followed by a series of passes through a controlled-temperature tunnel and a microwave-hot air tunnel before being cooled. A refrigerated shelf life of six weeks at 4°C is claimed. The microwave system is operated at 2,450 MHz using 48–1.2 kW magnetrons. The other process involves microwave heating the products while they are immersed in a shallow, controlled-temperature water bath. This technique claims to provide more uniform control of product temperature.

FUTURE DEVELOPMENT

Food manufacturers are well aware of the acceptance of microwave cooking and heating by consumers because of the microwave oven sales boom over the last 20 years in the United States and in recent years in other nations throughout the world. There is already a large market for foods designed and packaged for microwave oven use. A substantial increase in this market can be expected if microwave pasteurization and sterilization of portion packed foods is successful. These two processes could account for a dominant share of the microwave equipment business and in the case of sterilization possibly cut deeply into the conventional equipment market for these processes.

Although practically all of the microwave sterilization of food has been carried out at 2,450 MHz and the first production installations have also been at this frequency, eventually major installations will shift to 915 MHz simply because of the power demand (i.e., hundreds of kilowatts of power). Experiments at this lower frequency indicate more uniform temperature distribution. All of the results are not yet in, but this has been the case with other major processes, including tempering and cooking bacon.

The first installations of microwave sterilization of ready-meals went on stream a few years ago. Total installed power is only a few hundred kilowatts, at 240 MHz. Process time is about 30 min, including cooling time. Interesting progress at the U.S. Army Natick Research and Development Laboratories (Natick, Mass.) has been made in development of a chemical method to assess process adequacy. Several chemical markers have been identified that are produced during normal processing of foods and correlate well with microbiological lethality. Thus, it is possible with a rapid analytical technique to determine the effectiveness of a process in minutes instead of the several days required for microbiological methods

Microwaves may also find use in aseptic processing of flowable materials containing particulates, a process that has proven elusive because of the difficulty in ensuring that the fastest-moving particulates have received an adequate sterilization.

The adoption of microwave-based processes could influence the design of food plants of the future Not only is space savings made possible by a reduced process time, but the reduced space requirements could have a powerful effect on process economics when one considers the cost of real estate. The labor requirement may also be reduced. A microwave tempering operation could be automated from freezer storage at least through the tempering stage. Pasta dryers already have reduced the space requirement three to four times with no reduction in production. Microwave bread baking could mean smaller bakeries located closer to their markets and have a significant impact on transportation costs. Microwave-assisted baking is another process that could find a niche in the supermarket bakeries to provide just-in-time baked products and thereby eliminate the daily loss of excess product to ensure latecoming shoppers of the same choice available to early shoppers.

Existing processes may be improved by including a microwave capability Depending on the process, microwave heating could be applied as a preheating step, be built into existing equipment for simultaneous application of two or more forms of heating, or added on at the end of conventional equipment to increase the production rate.

Tools are available to evaluate new microwave processes without resorting to empirical methods alone. Microwave-compatible temperature-measuring systems are being used today. Infrared detectors are being used to follow surface temperature changes that can be translated into meaningful feedback data to control subsequent microwave input. Microwave radiometry, which has been demonstrated in measurement of temperature in human muscle tissue undergoing diathermy, could conceivably be used to noninvasively monitor product temperature during microwave pasteurization and sterilization to provide data for FDA approval. Continuous weighing systems have been used to monitor weight changes during microwave processing. There also is a large body of dielectric property data in the literature that can be useful in mathematical modeling and computer simulation in process development. All in all microwave processing has a promising future.

BIBLIOGRAPHY

1. U.S. Pat. 2,495,429 (Jan. 24, 1950), P. L. Spencer (to Raytheon Co.).
2. N. E. Bengtsson and P. O. Risman, "Dielectric Properties of Foods at 3 GHz as Determined by a Cavity Perturbation Technique. I. Measurements on Food Materials," *Journal of Microwave Power* **6**, 107–123 (1971).
3. R. E. Mudgett et al., "Prediction of Dielectric Properties in Solid Food of High Moisture Content at Ultrahigh and Microwave Frequencies," *J. Food Proc. & Pres.* **1**, 119–151 (1977).
4. T. Ohlsson et al., "The Frequency and Temperature Dependence of Dielectric Food Data as Determined by a Cavity Perturbation Technique," *Journal of Microwave Power* **9**, 129–145 (1974).
5. T. Ohlsson and N. E Bengtsson, "Dielectric Food Data for Microwave Sterilization," *Journal of Microwave Power* **10**, 93–108 (1975).
6. E. C. H. To et al., "Dielectric Properties of Food Materials," *Journal of Microwave Power* **9**, 303–316 (1974).
7. D. I. C. Wang and S. A. Goldblith, "Dielectric Properties of Foods," Final Report, Contract DAAG17-72-C-0142, U.S. Army Natick R & D Command, Natick, Mass.
8. T. Ohlsson and P. O. Risman, "Temperature Distribution of Microwave Heating—Spheres and Cylinders," *Journal of Microwave Power* **13**, 303–310 (1978).
9. N. Meisel, "Tempering of Meat by Microwaves," *Microwave Energy Appl. Newsl.* **5**, 3–7 (1972)
10. L. Riedel, "Calorimetric Investigations of the Meat Freezing Process," *Kaltetechnik* **9**, 38–40 (1957).

GENERAL REFERENCES

D. A. Copson, *Microwave Heating*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1975.

R. V. Decareau, *Microwaves in the Food Processing Industry*, Academic Press, Inc., Orlando, Fla , 1985

A. C Metaxas, *Foundations of Electroheat*, John Wiley & Sons, Chichester, United Kingdom, 1996.

A. C. Metaxas and R. J Meredith, *Industrial Microwave Heating*, Peter Peregrinus Ltd., London, 1983.

R. V. Decareau and R. A. Peterson, *Microwave Processing and Engineering*, Ellis Horwood Ltd , Chichester, UK, 1986.

E. C. Okress, ed., *Microwave Power Engineering, Vol 2, Applications*, Academic Press, Inc., Orlando, Fla., 1968.

ROBERT V. DECAREAU
Microwave Consulting Services
Amherst, New Hampshire

MILITARY FOOD

MILITARY SUBSISTENCE PROGRAM

Historical Background

To sustain troops under the operational demands and logistical constraints of modern warfare, military subsistence must possess characteristics markedly distinct from those of food products successful in the commercial marketplace. No program of research and development to diagnose and meet peculiarly military subsistence problems existed, or was even envisioned, until the then Office of the Quartermaster General (OQMG) established the Subsistence Research Laboratory at the Chicago Quartermaster Depot on July 24, 1936—less than six years before Pearl Harbor.

The World War I experience, without people knowledgeable in subsistence and unaided by systematic scientific investigation of military requirements, left procurement officials often dependent on almost miraculous improvisations by individuals within the food industry and the army. The troops were fed and the war won but the food margin was frequently so narrow that OQMG feared it could easily go the other way in the future if not addressed in time. The OQMG established the QM Subsistence School in 1919 toward generating a cadre of military experts in subsistence procurement, inspection, processing, transportation, and storage. Partly because of budgetary problems, the school was deactivated on June 30, 1936.

The new laboratory became heir to the school's research equipment, primarily a reasonably adequate kitchen, and was authorized $300 for supplies and equipment. It began operations with a staff of three: an officer-in-charge and two civilians who had worked at the school. Its official objectives were "to test foods and new style packaging of dryers already have reduced the space requirement three to four times with no reduction in production. Microwave bread baking could mean smaller bakeries located closer to their markets and have a significant impact on transportation costs. Microwave-assisted baking is another process that could find a niche in the supermarket bakeries to provide just-in-time baked products and thereby eliminate the daily loss of excess product to ensure latecoming shoppers of the same choice available to early shoppers.

Existing processes may be improved by including a microwave capability Depending on the process, microwave heating could be applied as a preheating step, be built into existing equipment for simultaneous application of two or more forms of heating, or added on at the end of conventional equipment to increase the production rate.

Tools are available to evaluate new microwave processes without resorting to empirical methods alone. Microwave-compatible temperature-measuring systems are being used today. Infrared detectors are being used to follow surface temperature changes that can be translated into meaningful feedback data to control subsequent microwave input. Microwave radiometry, which has been demonstrated in measurement of temperature in human muscle tissue undergoing diathermy, could conceivably be used to noninvasively monitor product temperature during microwave pasteurization and sterilization to provide data for FDA approval. Continuous weighing systems have been used to monitor weight changes during microwave processing. There also is a large body of dielectric property data in the literature that can be useful in mathematical modeling and computer simulation in process development. All in all microwave processing has a promising future.

BIBLIOGRAPHY

1. U.S. Pat. 2,495,429 (Jan. 24, 1950), P. L. Spencer (to Raytheon Co.).
2. N. E. Bengtsson and P. O. Risman, "Dielectric Properties of Foods at 3 GHz as Determined by a Cavity Perturbation Technique. I. Measurements on Food Materials," *Journal of Microwave Power* **6**, 107–123 (1971).
3. R. E. Mudgett et al., "Prediction of Dielectric Properties in Solid Food of High Moisture Content at Ultrahigh and Microwave Frequencies," *J. Food Proc. & Pres.* **1**, 119–151 (1977).
4. T. Ohlsson et al., "The Frequency and Temperature Dependence of Dielectric Food Data as Determined by a Cavity Perturbation Technique," *Journal of Microwave Power* **9**, 129–145 (1974).
5. T Ohlsson and N. E Bengtsson, "Dielectric Food Data for Microwave Sterilization," *Journal of Microwave Power* **10**, 93–108 (1975).
6. E. C. H. To et al., "Dielectric Properties of Food Materials," *Journal of Microwave Power* **9**, 303–316 (1974).
7. D. I. C. Wang and S. A. Goldblith, "Dielectric Properties of Foods," Final Report, Contract DAAG17-72-C-0142, U.S. Army Natick R & D Command, Natick, Mass.
8. T. Ohlsson and P. O. Risman, "Temperature Distribution of Microwave Heating—Spheres and Cylinders," *Journal of Microwave Power* **13**, 303–310 (1978).
9. N. Meisel, "Tempering of Meat by Microwaves," *Microwave Energy Appl. Newsl.* **5**, 3–7 (1972)
10. L. Riedel, "Calorimetric Investigations of the Meat Freezing Process," *Kaltetechnik* **9**, 38–40 (1957).

GENERAL REFERENCES

D. A. Copson, *Microwave Heating*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1975.

R. V. Decareau, *Microwaves in the Food Processing Industry*, Academic Press, Inc., Orlando, Fla , 1985

A. C Metaxas, *Foundations of Electroheat*, John Wiley & Sons, Chichester, United Kingdom, 1996.

A. C. Metaxas and R. J Meredith, *Industrial Microwave Heating*, Peter Peregrinus Ltd., London, 1983.

R. V. Decareau and R. A. Peterson, *Microwave Processing and Engineering*, Ellis Horwood Ltd , Chichester, UK, 1986.

E. C. Okress, ed., *Microwave Power Engineering, Vol 2, Applications*, Academic Press, Inc., Orlando, Fla., 1968.

ROBERT V. DECAREAU
Microwave Consulting Services
Amherst, New Hampshire

MILITARY FOOD

MILITARY SUBSISTENCE PROGRAM

Historical Background

To sustain troops under the operational demands and logistical constraints of modern warfare, military subsistence must possess characteristics markedly distinct from those of food products successful in the commercial marketplace. No program of research and development to diagnose and meet peculiarly military subsistence problems existed, or was even envisioned, until the then Office of the Quartermaster General (OQMG) established the Subsistence Research Laboratory at the Chicago Quartermaster Depot on July 24, 1936—less than six years before Pearl Harbor.

The World War I experience, without people knowledgeable in subsistence and unaided by systematic scientific investigation of military requirements, left procurement officials often dependent on almost miraculous improvisations by individuals within the food industry and the army. The troops were fed and the war won but the food margin was frequently so narrow that OQMG feared it could easily go the other way in the future if not addressed in time. The OQMG established the QM Subsistence School in 1919 toward generating a cadre of military experts in subsistence procurement, inspection, processing, transportation, and storage. Partly because of budgetary problems, the school was deactivated on June 30, 1936.

The new laboratory became heir to the school's research equipment, primarily a reasonably adequate kitchen, and was authorized $300 for supplies and equipment. It began operations with a staff of three: an officer-in-charge and two civilians who had worked at the school. Its official objectives were "to test foods and new style packaging of foods; prepare drafts of proposed specifications or reedit those which have become obsolete; to conduct studies of various reserve and emergency rations or components thereof; to prepare information bulletins and maintain liaison with other Government agencies." Its resources during the prewar period increased less rapidly than did its activities. By late 1941 its staff numbered 13. It had developed, field tested, and standardized the original C Ration, completed development of the D Ration, started work on the K Ration, essentially completed development of three-way frozen boneless beef, resumed the school's instructional functions, revised existing and prepared new specifications, and examined a melange of commercial products for potential military usefulness. This was done with no real advance knowledge, except possibly intuition, of the characteristics required for military suitability in the kind of conflict World War II proved to be. The type of food analyses the laboratory could then perform—mainly cooking tests, taste tests, and visual examination—was at best rough. It made a beginning in storage testing; laboratory members believed rations should be sufficiently palatable, as well as nutritional, to be eaten after long and adverse storage, but the implications of that belief were yet to be realized.

When World War II began there was virtually no experience in designing food or food packages for the conditions soon to be encountered overseas. A few separate agencies and individuals had done some research but without any attempt to translate results into food products meeting still-unknown military requirements. The food industry was concentrating on inexpensive and palatable foods that were stable enough to meet domestic distribution conditions. The small, poorly equipped, understaffed Subsistence Research Laboratory began its task of converting the immense industry to producing foods of a completely different type, despite labor and material shortages, as quickly as possible. Having no means of knowing precisely what was needed overseas, the laboratory began to formulate functional requirements as specifically as possible in the light of the best information it could obtain and to develop standard tests. Improvisation and trial and error were frequent in the evolution of products and rations. In general, the laboratory began to act as the hitherto missing military link between research groups and production groups—with a high degree of cooperation from both. That its work became effective was due largely to the notable cooperation it received from the industry.

For nearly three and a half years of the war, the laboratory concentrated on incorporating the most modern production procedures into specifications, enabling them to be put into operation in plants throughout the country. In 1944 it began to emphasize and accomplish development of new and better products and more acceptable rations. With the experience it had already gained, some overseas reports, increased facilities and additional technical personnel, it had a far clearer idea of what was needed and the means of obtaining it. Industry had also acquired experience and cooperation became more effective. With ability to define the broad types of military operation in terms of the subsistence problems distinguishing them, the laboratory could give clearer direction to its work by recognizing its goal as the development of food products and rations possessing the nutrition, stability, acceptability and utility properties required under the different operational conditions. By the end of the war it was recognized that science and technology would have to be expanded to meet known requirements more efficiently and to effectively respond to new demands.

Besides dealing directly with hundreds of food and food-packaging concerns and academic research groups, the laboratory had both formal and informal relationships with many government agencies, among them the U.S. Department of Agriculture, the Federal Food and Drug Administration, the Fish and Wildlife Service of the Department of the Interior, the other military services, and the Office of the Army Surgeon General. In recognition of its actual accomplishments and operations, it was redesignated the Quartermaster Food and Container Institute for the Armed Forces in 1946, and its mission, previously conducted without a specific charter, formalized. The new mission statement expanded relationships with other government agencies and added other military, federal, national, and international technical organizations. In 1950, as a result of Department of Defense (DOD) assignment to the army of primary responsibility for research and development of food, the institute was designated as the implementing agency for all DOD components, Following relocation of the institute to Natick, 1962–1963, DOD updated and expanded this responsibility in 1968 and added food service equipment.

LOGISTICS

Our national posture is defensive, requiring readiness to counter aggression wherever and whenever it occurs. The logistical demands inherent in this policy include need to stockpile enough food to ensure initial readiness for contingencies on a worldwide basis, transportability by any available means to and within operational areas, even when supply lines are threatened or cut by hostile action, and an economical production base to achieve mobilization objectives and to resupply troops once initial reserves are exhausted.

These demands present major and continuing challenges to our national food and packaging technical capability. They also impact the principles of nutritional adequacy, acceptability, stability, and military utility that the laboratory had formulated for operational subsistence during World War II.

Nutritional Adequacy

The primary purpose of military subsistence is to maintain the health and effectiveness of the persons subsisted. Military nutritional standards are based on the National Academy of Sciences Recommended Dietary Allowances (RDAs) as modified by military experience. As some of the known essential nutrients suffer loss in processing and preparation, raw, perishable foods would appear to be the best nutritional option, but the need for stockpiling, conservation of space and weight in storage and transport, and potential for worldwide distribution of the stored items obviously precludes reliance on the freezer facilities this would entail. In addition, availability of freezer and food service facilities and personnel on a worldwide basis during early days of emergencies is unlikely. To compensate for nutrients lost during processing and storage, it is necessary to fortify selected ration components that are stable carriers for the respective nutrients.

Acceptability

Nutrients are of no value unless consumed, and military experience has shown that troops will not necessarily eat "enough of anything" if they are hungry enough. At the least, unpalatable food is not a morale booster and can add to combat stress. The properties that make food desirable to eat—flavor, texture, odor, color and appearance—are sensory. Preservation to retain food wholesomeness over extended periods cannot be expected to improve these characteristics. A goal of food processing and preservation technology is to provide efficient, practicable preservation techniques that do minimal damage to initial food properties.

Consumer attitudes complicate acceptability problems. The people in the military services have generally formed their food habits, including likes and dislikes, before induction. Individual differences, changing food preferences, need to appeal to persons under extreme physical and emotional stress, for whom food may be the only break from the unpleasantness, discomfort, and monotony of fighting, are also part of the problem. So is the inherent American habit of griping about institutional foods.

Acceptability presents a major challenge to operational ration design. Capability of achieving it continues to challenge food science and technology.

Military Utility

A ration that cannot be used for the purpose intended will at best hamper the operation and the persons trying to conduct it. The criterion of utility affects the other attributes and ultimately dictates the form in which they appear. In general, all rations should be economical of weight and space in transport and storage, of facilities and labor in unloading, handling, and preparation, easy for the consumer to carry and eat, and should retain their configurations despite rough handling throughout the supply and distribution system.

Stability

Past experience has shown that operational rations must travel long distances, be exposed to wide temperature and humidity ranges, be handled roughly, and be stored where facilities are inadequate or none exist. Unless they retain the properties making them suitable and desirable for consumption, they are useless. Past emergencies have demonstrated both the economic waste and threat to preparedness of deteriorated, unserviceable supplies. Fielding of a ration entails capability of predicting its keeping quality under given time and temperature conditions, including prediction of the first date after manufacture at which stored rations must be opened to determine whether they should be issued, destroyed, or extended to another inspection period, along with the criteria for making these serviceability judgments.

Thus operational subsistence presents a continuing and open-ended demand on the nation's technical resources, but its demand on economic resources clearly cannot be open ended. Scale-up to volume production and continuing procurement on a competitive basis, within economic resources and without sacrificing a ration's essential characteristics, are major recurring hurdles. Unless they are overcome, the ration will be at best an academic curiosity with, perhaps, some aspects of knowledge or art it reflects applied to another, more workable item. There is irony in the recognition that products developed initially for specific military exigencies generally cannot be obtained for these unless they also prove to have some application in the civilian marketplace. Operational rations thus reflect compromises throughout their life cycle, but their essential characteristics cannot be compromised if the nation is to remain prepared.

CURRENT RATIONS

Military personnel located in fixed facilities are fed in dining halls not unlike those used for institutional feeding in the civilian sector. Because these situations are not unique, our focus in this article will be on foods used in field feeding situations that have no direct civilian counterpart.

Group feeding, where semifixed food preparation and serving facilities are possible, rations may be A Rations, B Rations, or T Rations, depending on the degree of sophistication of facilities, combat situation, availability of refrigeration, etc. A Rations are meals prepared from scratch, using primarily fresh or frozen ingredients, as well as many canned components. Although generally regarded as the most desirable, A Ration preparation requires facilities frequently unavailable in the field. In addition, trained cooks are needed to prepare and serve the food.

B Rations, prepared from shelf-stable ingredients such as canned foods, dehydrated foods, and packaged mixes also require trained cooks and equipment for frying, baking, broiling, etc, which may not be available, particularly in early stages of a conflict. T Rations consist of prepared foods thermally processed in tray packs, rectangular metal cans approximately the size of half-size steam table trays (10-1/16 × 12-3/8 × 2 in.). Sufficient tray pack entrées, starches, vegetables, and desserts have been developed to provide good menu variety for lunch/dinner meals, and improvements in breakfast item variety is currently a priority area of interest at Natick RD&E Center. T Rations offer the major advantage of requiring only heating, minimizing the need for equipment and trained food service personnel. Additionally, the tray can configuration allows processing of many popular foods such as lasagna, stuffed peppers and chicken breasts, which could not be thermally processed in conventional cylindrical cans.

For field-feeding situations where no preparation facilities are available and individuals must be self-sufficient, the basic ration is the Meal, Ready-to-Eat, Individual

(MRE). In addition, a number of special-purpose rations are stocked to meet specific, unique military needs, some of which will be described in this section.

The Meal, Ready-to-Eat, Individual has been in use since the early 1980s and has undergone major revision since first introduced. Based on retort pouch technology described in detail elsewhere, the MRE is a flexibly packaged, shelf-stable ration consisting generally of a casserole-type entrée or a meat/starch combination; fruit; crackers and spread; a cookie, cake, or brownie dessert item; as well as accessory items, condiments, candy, and beverage mixes. All MRE menus meet or exceed the recommended daily levels of vitamins, minerals, and calories prescribed for operational rations by the Office of the Surgeon General. Recent development of a highly acceptable packaged bread having a projected shelf life in excess of three years has enhanced the MRE acceptability and the technology developed for the bread is currently being applied to numerous other highly popular items such as shelf-stable packaged pizza, burritos, and a variety of new cake and snack items. Addition of these items as MRE components will add variety to the menu and further improve acceptability as a result of incorporation of more contemporary food items desired by today's service personnel.

To meet the unique needs of military personnel operating in the field during extremely cold weather where conventional hot meals cannot be provided, the Ration, Cold Weather (RCW) has been developed and is currently being procured. This ration, the first ever designed specifically for arctic/extreme cold conditions, is composed of dehydrated items that will not freeze. Salt and protein levels are adjusted to minimize water requirements, and several soup and hot drink mixes are included to help encourage adequate water intake because dehydration is a major problem in cold weather. A 4,500-calorie RCW weighs 2.7 lb. and has a volume of 225 in.^{3} for a man-day supply of food. For comparison purposes, it would be necessary to carry four MREs to provide the 4,500 calories required for cold weather feeding. One RCW furnishes the total calorie requirement and saves 55% of the weight and approximately 40% of the cube when compared to the MRE.

The military inventory also contains survival subsistence items, generally in the form of small food packets. These are intended for use only for short periods of time in emergency situations. Since the space available for their storage aboard lifeboats and aircraft or in other survival gear is extremely limited, high-caloric-density foods are required. Also, because of the likelihood of little or no potable water available to personnel in such emergencies, foods low in protein and high in carbohydrate are required to provide the necessary calories while minimizing water requirements. Through the application of new technologies, a new survival ration is under development. This ration consists primarily of an assortment of high carbohydrate food bars including cornflake, shortbread, glucose, granola, and chocolate chip. The familiar flavor and texture of the new food bars will greatly increase acceptability, in comparison to current survival packet components.

TECHNOLOGY AND SCIENCE

Preservation Processes

The objective in processing or handling foods for military distribution is to retard or eliminate deteriorative processes that undermine food quality. Various biological and physicochemical processes, if uncontrolled, lead to spoiled and unsafe foods. Because microbiological contamination is the main concern, preservation technologies are used that retard microbial metabolism or destroy specific disease-causing or spoilage-inducing microbes.

One common strategy in retarding microbial growth is to limit the availability of water for utilization by the microorganism. It is particularly effective when combined with temperature lowering. Water can be made unavailable by freezing the food, by adding ingredients that tightly bind it, or by dehydrating the food either partially or completely.

The basic strategy in eliminating microbial growth is to destroy the microbes outright or to render them incapable of replication. When thermal energy or ionizing radiation energy is absorbed, cell rupture occurs, cellular processes are disrupted, or the DNA is irreversibly damaged. Thermoprocessing is very generally applied in stabilizing moist products for storage at ambient temperatures.

Dehydration

Freeze Drying. The primary dehydration technology used for certain ration components is freeze drying (1,2). By maintaining the product frozen well below 32°F and by removing the water through sublimation, highly desirable features are retained in the dried product. Freeze-dried components include fruits, vegetables, meat patties, and entrée items. All are blanched or precooked prior to processing.

Large-scale freeze drying by industry is done on a batch process basis following procedures that optimize the drying rate without compromising product quality. Pre-frozen products spread out on trays placed on radiant heating platens within a vacuum chamber are slowly processed to sublime away the moisture. Typically, the product temperature is maintained around 0– – 40°F and the chamber pressure is controlled to about 0.1–1.0 torr. As the process proceeds, the outer surface layer dries and the frozen ice core recedes. The energy for sublimation must transfer through the dried layer to the ice and the water vapor must migrate through the dry, porous layer to the surface. The transfer of this heat energy is driven by the difference between surface temperature and ice temperature and is affected by thermal conductivity of the porous layer. The migration of the moisture is driven by the difference between vapor pressure of the ice and the chamber pressure and is affected by the permeability of the porous layer. Typically, processors limit the slab thickness, adjust the platen temperature to maintain product surface temperature at or below 150°F, and try to achieve a suitable porosity in the product. Drying times are in the range of 10–15 h. The final product is packaged under vacuum or nitrogen in a water and oxygen impermeable material.

Reversible Compression. Because certain military rations must be compact, many freeze-dried components are reversibly compressed (3). A product that is "plastic" before compression recovers its original volume and textural properties upon rehydration. Such plasticity is achieved by spraying the freeze-dried material with enough moisture so that it has a uniform moisture content of about 12%. After compression, the product is redried and packaged in a protective container. Compression reduces the volume effectively, a ratio of 16:1 being achievable for green beans.

Microwave Freeze Drying. Microwave-assisted, freeze drying (MWFD) has been explored for producing products equivalent in quality to conventionally freeze dried products, but at lower cost. It has the advantage that the energy for sublimation is directly absorbed by the ice and that the remaining moisture can be continually redistributed throughout the sample. Control of microwave power and sample temperature is crucial, because the ice must not melt and the ice temperature must not be so low as to decrease the efficiency with which frozen water absorbs microwave radiation at 2,450 MHz. This advantage is evident in the decrease in drying time down to 5.5 h for green peas. Another advantage is the ability to dry the sample to a relatively uniform moisture level of 12%, which makes it directly compressible.

Centrifugal Fluidized-Bed Drying. Centrifugal fluidized-bed drying (CFBD) is another rapid drying technology. The fluidization is achieved by counterbalancing the centrifugal force exerted on diced or spherical samples contained in a rapidly rotating drum against the force of hot air streaming through small perforations in the drum wall. Air temperatures between 175–212°F are used. In 15 min, 50% of the moisture in green peas can be removed. Further drying would be inefficient and detrimental to quality. A combination of CFBD with MWFD could be used to optimize the dehydration process.

Thermoprocessing

Conventional Retorting. The primary thermoprocessing technology used in producing shelf stable tray-packed and pouch-packed ration components is retorting (4). By subjecting foods in these containers to high temperatures for specified times in steam-charged retort vessels, any contaminating disease-causing or spoilage-inducing microorganisms that might otherwise multiply during storage are destroyed. The minimum temperature–time requirement for the slowest heating point in a geometrically distinct container depends on the acidity of the product and on the heat resistance of the target microorganism. For most of the tray-packed and flexible pouch-packed components, the pH exceeds 4.5 and the targets are mesophilic spore-forming bacteria, which could grow between 50 and 105°F. It suffices then to ensure that the integrated temperature–time exposure is equivalent to 6 min at 250°F, which would reduce the population of the reference putrefying anaerobe, PA 3679, by 6 D (ie, six orders of magnitude) and which provides a large margin of safety for reducing *Clostridium botulinum* by about 24 D. For these products, the retorting temperature is set between 230 and 250°F, and the integrated lethality (ie, F_0) of the process takes into account the change in thermal resistance of PA 3679 as the product goes through the heat-up, cook, and cool-down cycles.

Industrial thermoprocessing of such different items as potatoes in butter sauce, frankfurters in brine, beef slices in barbecue sauce, lasagna with meat sauce, and spice cake is currently done in batch mode using either a still or rotating retort. The heating medium could be pressurized steam or water heated with steam. Tray packs or flexible pouches, after being either vacuum sealed or hot filled and purged with steam and then sealed, are loaded into the retort vessel. Once the process is begun, the heat is transferred from the surface of the container into the food. The flat shape of the 2-in.-deep tray-pack container and the 0.5-in.-thick profile of the pouch, in principle, allow the heat to penetrate quickly and uniformly, which should produce a high-quality product. If the food has a significant fluidity, the heat penetration is aided by natural convection; if it is solid, the penetration is exclusively conductive. In a rotating retort, the agitation introduced further facilitates convective heat transfer and would lead to a faster, more uniform heat treatment.

Before the process for a particular product in a specific retort is filed with the USDA or FDA, verification must be obtained that the cycle to be used ensures attainment of commercial sterility at the slowest heating point in the slowest heating container. For this purpose, thermocouples are used to obtain heat penetration data and calculations are made to specify the cycle time and temperatures needed to achieve the minimum F_0. Processors typically will add a safety factor to ensure that the processing is in excess of the requirement for microbial lethality. If an entirely new product or container has been developed, inoculation studies may be required.

Ration components so processed are not only safe, but show high retention of nutrients and score high in ratings of color, flavor, and textural attributes. However, some products might be overprocessed due to wide variation in treatment from container to container. Techniques are now being investigated to validate, noninvasively, that each container received a treatment that is greater than the minimum requirement and less than the maximum set as a high-quality limit. One promising technique uses a optically read, bar-coded label with a patch that becomes darker in color as the integrated temperature–time exposure increases.

Aseptic Processing. Another thermoprocessing technology that is receiving attention for possible future use is aseptic processing. It is a high-temperature, short-time exposure process that sterilizes the food product before it goes into a container. In this process, a fluid product with relatively large particulates, such as a stew with chicken cubes, potato dices, and carrot dices, is pumped through a scraped surface heat exchanger, then through a holding tube maintained at the specified high temperature, then through a rapid cool down system, and finally into a sterile filler unit utilizing surface-sterilized packaging material. Temperatures of 260–280°F can be used, and residence times of about 3 min in the holding tube are all that is needed to ensure that the center of the particulate has received an adequate lethal treatment. This technology has several inherent advantages including high quality and nutrient retention, lower overall energy input, continuous processing, and the option to package the product in any size container without the need for changing and verifying process parameters.

SENSORY ANALYSIS OF RATIONS

As described in preceeding sections, the development of today's rations is driven by the logistical requirements of light weight, low volume, minimal preparation, and long-term stability under climatic extremes. These requirements have forced dramatic changes in the ingredients and processing technologies for rations and have resulted in significant changes to the familiar appearance, flavor, and texture of the foods and beverages served to the soldier. These changes have the potential to dramatically affect the acceptability of the rations and, in turn, the soldier's nutritional status, because rations that are not acceptable will not be consumed.

To ensure that military rations retain high standards for quality and consumer acceptability, all rations are engineered to specific sensory criteria and levels of consumer acceptability. The process of evaluation used to insure these criteria includes a wide variety of sensory and psychophysical techniques. Many of these methods and their applications in military ration development have been published over the years (4–10).

Primary among the methods are those known as affective, ie, those that address the criteria of consumer liking for the product. In one of the largest in-house laboratory consumer-testing programs in the world, all military rations undergo extensive testing in the computerized sensory analytic laboratories at Natick, where they are evaluated by a volunteer panel of over 500 civilian and military personnel. The most commonly used rating scale in these tests is the nine-point hedonic scale developed in the 1950s at the Quartermaster Food and Container Institute in Chicago, Natick's predecessor laboratory. This simple scale with points labeled from "dislike extremely" to "like extremely," has been shown to be both a valid and reliable measure of consumer acceptability and is now employed throughout the food industry to assess likes and dislikes for foods and beverages.

In addition to hedonic testing, which can be conducted either in the laboratory or in the field, extensive attitudinal testing is conducted on rations and ration concepts. These tests frequently take the form of written surveys administered to subjects to assess their beliefs, feelings and attitudes toward foods, beverages, meal items/concepts, methods of packaging and preparation, food names, labels, condiment usage, etc. These data provide useful quantitative information for product and package developers to best meet the needs of the soldier in the field.

During the past few years greater emphasis has been placed on qualitative methods of consumer research, eg, focus group testing, for the assessment of consumer perceptions of ration and ration concepts. These qualitative procedures now supplement the quantitative survey and interview approaches. In addition, the concept of marketing military rations has been emphasized through closer examination of the importance of food names, packaging designs, and the role of consumer expectations in military feeding.

In contrast to the affective methods of ration assessment are the analytic methods, or those that use specialists with training in the lexicon and evaluation of the sensory characteristics of food. These methods include such approaches as flavor and/or texture profiling and food quality scoring. They are frequently used when (1) designing a ration item to meet an ideal or "target" sensory profile, (2) developing instrumental tests to correlate with specific sensory attributes, or (3) assessing the degradation of flavor or texture with time in storage.

Last, but no less important, are the wide variety of univariate and multivariate psychophysical methods that are applied to rations. These methods are most often used to analyze the combined effects on the soldier–consumer of the multiple sensory attributes of each ration item, and/or to determine the levels of each attribute required to achieve maximal acceptability of the item. These methods of analysis, coupled with the traditional methods described previously, have enabled military ration developers to more quickly achieve a desired target product than has previously been possible. Moreover, this sensory technology has been used to assist numerous other government agencies, such as the U.S. Departments of Commerce and Agriculture, who frequently engage Natick to help solve problems of national concern and attention. Such technology transfer from the military feeding program to the public sector is a spin-off of a military program designed to solve problems on the cutting edge of food technology.

In summary, the engineering of today's military rations is keyed on the criteria of sensory quality and soldier acceptability. Through detailed consumer and sensory analytic testing in the laboratory and subsequent field testing, it can be insured that fielded rations will be well liked and consumed with minimum waste, so that all of the available nutrient content will be used by the soldier to optimize performance and maintain readiness for combat.

RATION TESTING AND EVALUATION

The evaluation of military rations has traditionally focused on the sensory and hedonic responses to the food items that make up the ration (see above). In a typical sequence the food item is first tested under controlled conditions in a laboratory setting with civilian panelists. If the product is satisfactory, it is incorporated into the ration and subsequently tested for its acceptability to military consumers in a field-feeding context. If the ration is acceptable under field conditions, it is purchased and incorporated into the military feeding system.

Several converging lines of evidence have led to a reappraisal of this methodology. First a series of recent ration field tests that employed measures such as body weight, food consumption, water consumption, hydration status and consumer perceptions of the ration as well as the more traditional acceptance measures revealed that this more comprehensive profile is needed to adequately assess the effectiveness of operational rations for long-term use (11,12). These studies also revealed that, although the rations received high acceptance scores in the field, they were not consumed in sufficient quantity to meet the surgeon general's recommended daily allowance for calories. A decision to feed these rations over an extended time period based solely on acceptance measures would have been a mistake. A second shortcoming of relying solely on acceptance measures was revealed in a series of laboratory and field studies (13,14). This research showed that social and situational variables, can influence food acceptance and consumption as much as the intrinsic properties of the food. The third consideration that prompted a review of the way military rations are tested is that rations of the future are likely to emerge as unfamiliar and unconventional products in order to meet ever more stringent weight and volume requirements. Based on these factors, the U.S. Army Natick Research, Development and Engineering Center has developed a comprehensive seven-phase ration testing program.

Phase I—Consumer Marketing

The purpose of this initial step in the ration-development process is to identify potential items or ration concepts that match customer interests and requirements. It uses focus group testing, product profiling, marketing analyses, and food-preference surveys to define for the product developer what the military user would like in the ration. This phase of the testing program emphasizes that the military consumer is a customer who cannot be treated as a passive recipient if rations are to be successful products.

Phase II—Individual Item Sensory Testing

This phase is initiated when the product developer has fabricated prototype items and serves to insure that people find the food item acceptable.

Phase III—Consumer Meal Testing, Laboratory

Food items that passed through Phase II would be tested to determine their suitability as components of meals where item compatibility was evaluated. This work would be conducted in the laboratory in the context of a meal using civilian and military consumers where selection rate, item and meal acceptance, and consumption would be measured.

Phase IV—Meal Testing, Field

Phase IV would be identical to phase III except testing would occur with military consumers in a field environment.

Phase V—Prototype Ration Testing

When a sufficient number of food items have successfully passed through the preceding phases of testing, a new prototype ration would be assembled and compared to the ration it was meant to replace in a field test. The measures taken during this field test would include selection rate and consumption of all items, daily nutrient and fluid intake, body weight, hydration status, food acceptance, and consumer satisfaction with the utility of the ration. The testing would occur in all appropriate environments (hot, cold, temperate, and altitude) during field-training exercises. The data from these tests would serve as the basis for a precurement decision for rations that did not pose unusual nutritional problems or were designed for extended periods of use. Rations that fell into these latter categories would be more completely tested in Phase VI.

Phase VI—Extended Ration Use Validation

Some rations are designed to be used for extended time periods or are for special missions and do not provide adequate nutrition. Rations of this nature would be tested by the Military Nutrition Division of the U.S. Army Institute of Environmental Medicine to fully assess the ration's impact on health and performance. In addition to the measures employed in Phase V they would also monitor nutritional status, physical symptoms, as well as physical and mental performance.

Phase VII—Quality-Control Testing

This phase of the ration testing program is included to insure that the quality of rations that are part of the military feeding system are being maintained at their original, high level by the manufacturers. The sensory testing methods employed in Phase II would be used to answer this question.

At the present time this ration testing program is being introduced. Phases I, II and V and VI have been in operation for several years and have led to improvements in the operational rations (12). The program will be evaluated and modifications made as experience is obtained.

NUTRIENT STABILITY

As indicated above, nutritional adequacy of foods provided to military personnel is paramount. Because of the necessity to use processed foods with long storage lives, nutrient and microbiological stability are essential to insure that nutritional content is adequate and that foods are wholesome at time of consumption. An area of major emphasis, therefore, is focused on nutrient losses during processing and subsequent storage. Space constraints preclude a detailed discussion of all work ongoing in these areas. Following is a summary of several recent or current studies at Natick RD&E Center in the areas of nutrient and microbiological stability.

Proteins

Among the various degradative reactions that take place in foods, the most difficult to arrest are those between proteins and reducing carbohydrates. This situation is even more aggravated when foods, such as combat rations are stored for extended periods without refrigeration. Even when reducing carbohydrates are not part of product formulations, hydrolysis of the polysaccharides and oligosaccharides may occur during processing or during prolonged storage, and then protein quality loss will follow.

Maillard Reaction. Although it is generally recognized that protein quality losses occur during processing and storage through the Maillard reaction, the quantitative aspects of the protein-reducing sugar reaction, which leads to a decline in lysine availability, are subject to interpretation depending on the methodology employed (15). In the initial phase of the reaction, the free epsilon amino groups of lysine and *N*-terminal amino groups in the protein react with the reducing sugar to form a Schiff base, which cyclizes then rearranges to form a ketose sugar derivative, commonly referred to as the Amadori compound. This compound is not digested by mammalian proteolytic enzymes and, therefore, becomes biologically unavailable. Furthermore, the amino acids adjacent to the blocked lysine in food proteins may also be unavailable due to steric hindrance to enzymatic action. Acid hydrolysis of foods that have suffered quality loss due to Maillard reaction leads to the formation of a cyclic compound, furosine, in 20% yield, which may serve as an indicator of unavailable lysine (16). It has been used in Natick as a marker to determine protein quality losses in stored dairy bars.

Aminocarbonyl Interactions in Model Systems. Investigations focused on interactions in model systems between lysine, acetyllysine or albumin, and glucose at 40, 50, and 60°C and a low water activity, $a_w = 0.20$. The reaction was monitored by measurement of color, fluorescence, reducing capacity, furosine, glucose, and lysine. There was poor correlation of fluorescence and color increase with lysine loss. Both furosine and reducing capacity correlated highly with lysine loss (17).

Bioavailability of Essential Amino Acids in Food Products. The enzymatic availability of essential amino acids in beef stew processed using three technologies (retort pouch, retort can, and freeze-dehydration) were determined using three proteolytic enzymes (pronase, carboxypeptidase, and amino peptidase), followed by derivatization of amino acids with phenyl isothiocyanate and analysis by HPLC. As expected, because of less total heat input required to attain a comparable sterilization value in the retort pouch, lysine and methionine losses were slightly less in pouches than in # 21/2 cans. Accelerated storage at 125°F for 28 days did not further decrease available lysine.

Lipids

Autoxidation and Antioxidants. The central problem has been the radically different course of lipid autoxidation and its prevention by antioxidants in dispersed versus bulk systems. This was addressed by two systems: lipids coated as a monolayer on silica powder and lipids freeze dried onto carboxymethylcellulose. Oxygen uptake measurement was by gas partition chromatography. Among the findings from the monolayer work was the ordering of the relative effectiveness of antioxidants for lipids coated as monolayers on surfaces (18). The carboxymethylcellulose work revealed the relative effectiveness of many known and some previously unknown natural antioxidants. Their synergism with food-grade synthetic antioxidants was also proved (19).

Fluorescent Detection of Lipid Oxidation Products by Polyamide Surfaces. It was discovered that the volatiles from oxidizing lipids produce surface fluorescence on polyamide powder coated on glass or plastic (20). This enabled rapid vapor phase monitoring of oxidation either in tests of antioxidant effectiveness or in in-package quality monitoring.

Relative Effectiveness in Bulk Lipids vs Dispersed Lipids. Using cobalt-accelerated soy lecithin sonicated emulsions (liposomes) and dispersed and freeze-dried whole milk, a rationale to predict the effectiveness of polar versus nonpolar antioxidants in bulk versus dispersed systems has been formulated (21). In general, this rationale predicts that nonpolar antioxidants, eg, butylated hydroxytoluene (BHT), function best in polar lipid emulsions and membranes, while polar antioxidants, eg, gallic acid, are relatively more effective in nonpolar lipids and in bulk systems.

Rapid, Front-Face Fluorometric Methods of Quality Loss Assay on Comminuted Whole Tissue Foods. Experience with front-face, solid-surface fluorescence measurement extended the method to comminuted slurries of whole tissue foods. Rapid assays of the state of lipid oxidation, Maillard sugar-amine, and ascorbic acid-amine browning are possible with good discrimination between the types of quality loss. Dried whole milk and retorted wet-pack fruit and freeze-dried fruit have been studied (22).

Vitamins

Vitamins can be lost from foods during processing, storage, and preparation for consumption. Certain vitamins such as thiamin and ascorbic acid are more sensitive to processing conditions than are others such as riboflavin. However, riboflavin can be lost through exposure to light, so packaging becomes important in determining storage stability.

Ration Processing Effects. Studies on tray packs have concentrated on the effects of processing and preparation for serving on the vitamins thiamin, riboflavin, pyridoxine, and ascorbic acid (23–25). The products studied were beef stew (fortified at a level of two times the RDA per meal), sliced pork, and peas and carrots. It was found that riboflavin was stable to processing while thiamin content decreased by 53%, 68%, and 57%, respectively. Vitamin B_6 also showed losses due to processing. Ascorbic acid loss from peas and carrots due to processing was 72%, whereas the fortified beef stew showed only a 20% loss of this vitamin.

Ration Storage Studies. Both the Meal, Ready-to-Eat (MRE) (based on retort pouch technology) and the Long Range Patrol (LRP) (based on freeze drying technology) have been subjected to short- and long-term storage at 4°C, 21°C, and 38°C (26). The 1973 prototype MRE was nutritionally adequate after storage at 38°C for six months and at 4 and 21°C for four years. Losses of thiamin, riboflavin, pyridoxine, vitamin A, and ascorbic acid occurred at 21 and

38°C. Niacin and carotene were stable. In the 1980 procurement MRE storage study, the indicator vitamins thiamin and ascorbic acid were followed in individual meals and/or in composites of cases containing 12 meals. Case composites showed a loss of thiamin, but after 24 mo at 38°C this loss was only 12%. Ascorbic acid showed a 50% loss from cases after 24 mo at 38°C, but no loss after 60 mo at 4°C. As with the MRE, the LRP showed good stability of thiamin at 4°C. At 21°C losses did occur, being greatest for chicken stew (39%) and beef stew (46%) over 13 years of the study.

MICROBIOLOGICAL STABILITY

The role of microbiology in military subsistence systems is to ensure the safety and microbiological integrity of food-delivery systems and rations. This responsibility must respond to conventional feeding systems as well as those, present and future, that are considerably less conventional. These rations are prepared and delivered to their customers in a wide variety of facilities (dining halls, hospitals, submarines, ships, airplanes, in the field, etc) at widely scattered geographical locations and are either perishable or microbiologically stabilized rations produced commercially or under contract.

The methods currently used to stabilize military rations from microbiological deterioration and to render them safe are thermal processing, water activity control (dehydration, lowering osmotic pressure with humectant, salts, freezing, etc), low pH, microbial growth inhibitors (nitrites, benzoate, acids, etc), and refrigeration.

Inoculated pack studies on complex military rations that have been developed in-house are conducted to determine their thermal processing requirements. For these studies a simple, biphasic technique, which yields extremely heat resistant and clean spores of *Clostridium sporogenes* strain PA 3679, has been developed.

The problem of thermophiles, which are highly heat resistant organisms that grow above 45°C, is significant in military rations due to the increased chance of temperature abuse of rations during distribution and storage. Therefore, commercial sterility, based on minimum heat input necessary to provide stability and an acceptable safety margin for foods handled in a carefully controlled distribution environment, may be inadequate for military rations.

Experience has shown that to control or destroy microbial populations in a ration system of diverse foods, it is necessary to study each food group. For example, beef snacks, which are low-moisture foods designed for use in the field, must be processed so as to attain a water activity that will ensure that the organism of main concern for safety, in this case *Staphylococcus aureus*, will not grow. Water activity is the ratio of the water vapor pressure of the product to the vapor pressure of pure water equilibrated at a given temperature and is a measure of biological moisture available for microbial growth. Similarly, the minimum water activities for cakes, formulated for the tray pack container configuration, were also determined. The organism of concern in this case being *Clostridium botulinum*.

It is often necessary to use combinations of microbial inhibitory agents and environmental conditions to achieve microbial stability. The three-year shelf life required for canned bread is attainable by the combined use of a sufficiently low water activity to prevent the growth of *Clostridium botulinum* and the inclusion in the package of an oxygen absorber to prevent spoilage by fungal growth.

The military is also concerned with being able to predict and monitor the shelf life of perishable rations. This effort has resulted in the issuance of a guide designed to prevent the unnecessary discarding of temperature abused, although still safe and edible, perishable rations in commissaries.

To further ensure the safety of perishable foods a medium for quantitating *Listeria* organisms was developed which is particularly efficient in recovering heat-injured cells. This was accompanied by the development of a technique that can identify pathogenic *Listeria* in less than eight hours.

A program to evaluate microbial inactivation kinetics for the aseptic processing of particulates for application for future ration design is being actively pursued. For this purpose extremely thermoresistant *Bacillus stearothermophilus* spores have been produced. The application of modified atmospheres (carbon dioxide, nitrogen, oxygen removal, etc) to inhibit microbial growth and extend the shelf life of perishable foods is also being studied.

QUALITY MONITORING OF STORED FOODS

Rations formulated and processed to retain their nutritive value, to prevent microbial growth, and to appeal to the military man or women must be able to maintain such quality attributes throughout storage and distribution. Unfortunately, food cannot be preserved indefinitely and will slowly show some degradation. Several degradative processes take place, even in canned products, that are due to chemical, biochemical, or physical reactions. Browning in cream sauces and in fruits reflects the chemical reaction between sugars and proteins; decomposition of proteins or fats, which could affect texture or flavor, reflects an enzyme-catalyzed biochemical reaction; and the crystallization of starch in breads, which contributes to the perception of staling, reflects a physical process. All of these processes are influenced by the temperatures encountered in transportation to and from storage facilities and by the fluctuation in temperature during storage. Rapid, simple ways of accounting for or monitoring such degradation are needed to simplify the logistical burden of rotating products out of storage and into the hands of users before the quality of these products becomes significantly compromised.

Shelf Life Dating

Based on years of experience and extensive research by academia, industry, and government, the basic shelf lives of fresh or processed products stored at specified temperatures are known (27) and are used in establishing Pull Date, Sell by Date, or Best if Used by Date. These designations represent the dates at which a time that roughly corresponds to the product's shelf life has elapsed since the Date of Pack. One or another of these dates printed either explicitly or in coded form can be seen on many products intended for refrigerated, frozen, or room temperature storage. Although normally adequate for situations where temperature is relatively well controlled, this approach is inadequate to ensure quality if the storage equipment fails or if high average temperature is unavoidable.

Temperature Effects

The influence of the combined temperature–time effect can be illustrated with results on the acceptance of Meal, Ready-to-Eat components stored at different temperatures (28). Based on shelf life defined as the time it takes for an acceptance rating to decrease to 5 on a 1 to 9 hedonic scale, researchers found a wide variation in shelf lives among different components at any single temperature and in the sensitivity of shelf lives to temperature changes. At 80°F, the average shelf life for all entree items was found to be 140 mo, whereas pastry items last for only 40 mo. Most important, the pastry items are insensitive to changes in temperature, the shelf life dropping slightly to 32 mo as the temperature increases to 110°F, while in contrast, the fruits are extremely sensitive, their average shelf life dropping from 180 mo at 90°F to 20 mo at 100°F. The combined effect of temperature and time is such that, in terms of quality degradation, exposure for short times at high temperature is equivalent to long times at lower temperatures.

Time–Temperature Indicators (TTIs)

Research into several different time-temperature indicators that integrate this exposure and reflect the cumulative history of such stress has been undertaken. One promising indicator label made by I-Point Technologies is based on the enzymatic decomposition of fats, which produces an acid and causes a color change in the label from green to yellow to red. Another promising indicator made by LifeLines Technologies contains, in addition to the bar code, a patch with a substituted diacetylene monomer that is initially white and eventually becomes bluish and purplish as it polymerizes. Both can be made with different formulations to alter the sensitivity to temperature. The LifeLines TTI can be scanned with an optical reader to obtain product information and the percent reflectance of light from the patch. In studies of products with such labels and exposed purposely to different temperatures, data have been obtained indicating that the change in color reflectance correlates with changes in product quality. From such correlations, a scale can be developed from which an individual responsible for managing stock rotation could determine, from the percent reflectance of the patch, the time remaining at a given temperature before the shelf life limit is reached.

The suitability of such labels for stock management ultimately depends on how well the label temperature sensitivity matches the product's temperature sensitivity. If the label is more sensitive, it will overstate the loss in quality; conversely, if it is less sensitive, it will understate the loss. Moreover, the particular quality attribute in a product that most affects acceptance must be identified and the temperature sensitivity of this attribute should serve as the basis for selecting a TTI label. Current research indicates that sufficient flexibility exists in adjusting label temperature sensitivity to achieve the desired match.

Automated Stock Management

Other investigations are in process to establish an automated system for monitoring the integrated temperature stress on stored rations and for deciding on when to rotate the stock. In principle, it is possible to read such label with a handheld optical scanning wand, to store the data in a handheld computer system, and then to transmit the data over the phone lines to a central data collection and decision-making facility. Using the concept of Least Fresh—First Out, any product that experiences a high temperature stress would be stored for a shorter period than originally planned to ensure good acceptance when it is eventually delivered for use. The actual quality of the product becomes the determining factor instead of an assumed quality based on shelf life and date of pack.

FUTURE CONCEPTS

Calorie-Dense Rations

The need to reduce the size and weight of supplies soldiers must carry and to provide them with an eat-on-the-move capability has motivated research on calorie- and nutrient-dense rations. Provided that these rations are well accepted, their compactness and convenient shape offer significant benefits with respect to soldier performance and to ration storage and distribution. Consequently, some new concepts for ration design and tailoring are being explored.

Dehydration, Infusion, Compression, and Extrusion. Certain conventional and novel technologies, alone or in combination, can be considered for making calorie-dense ration components (29). Caloric density is defined as kilocalories per cubic centimeter (kcal/cc). One conventional approach involves dehydrating the product, which leads to a porous food, and then compressing it, which removes most of the air space. Caloric densities of about 1.1 kcal/cc can be obtained this way. One novel approach now being explored, even for civilian products, involves infusing a porous food with energy-rich ingredients, filling all of the air spaces with stable and nutritious constituents. Caloric densities between 5 and 6 kcal/cc are achievable. The technologies for producing the porous food could be either conventional dehydration or the more recently exploited cooking-extrusion process. Extrusion involves processing a flour and water mixture under high shear and high temperature within a confining extruder barrel so that, when the doughy mass is forced through a die opening, the superheated moisture rapidly vaporizes, blowing holes in the extrudate and forming a honey-combed structure. Commercial corn curls illustrate this type of product. Another way to achieve high density that does not require a structured, porous material is to directly compress dried powders.

Caloric Densities and Compactness. The volume reduction achievable through use of these technologies can be put into perspective by comparing some of the past and current rations. The Meal, Combat, Individual (MCI), with its moisture-laden food packed in metal cans, had a caloric density of 0.8 kcal/cc; the Meal, Ready-to-Eat (MRE), with similar foods packed in flexible pouches, weighs less but has a similar caloric density. The Long Range Patrol (LRP), with its dehydrated but noncompressed components, weighed less but had only a slightly higher density of 1.1 kcal/cc. The Food Packet Assault (FPA), with dry and compressed components, reached 1.5 kcal/cc, and the new Ration, Lightweight 30 Day (RLW30), with carefully selected and optimally processed dry and compressed components, reaches a high of 3.7 kcal/cc. Several experimental rations have reached as high as 7.1 kcal/cc. Assuming that the goals for acceptance, compactness, and cost are optimized, it should be possible to produce a ration with a caloric density of 4.8 kcal/cc. This sixfold greater density compared to the MRE means that, for an equivalent amount of calories, the proposed new ration would take up only one-sixth the space.

Current Thrusts. Research in this area is concentrating on refining the technologies so a ration can be produced with any prescribed nutritional or physical characteristics. The texture and infusibility of an extruded matrix are determined by the size and number of the air spaces as well as by the thickness and fragility of the walls defining those spaces. The nutritive value, the physical stability, and the textural attributes of the liquid with suspended particles that are infused into the extrudates are very much influenced by the size and shape of these particles and by their ability to penetrate the extrudate (30). Industry is currently infusing certain dried fruits with sugar solutions to increase their stability and appeal. Because one of the ultimate goals is to be able to tailor the ration composition to satisfy particular nutritional requirements for different climatic or tactical situations, the research should lead to an ability to produce the prescribed components by specifying the formulation and by adjusting the process parameters.

Self-Heating Rations

There has always been a need to heat certain foods to palate temperature to improve the taste, which in turn enhances the morale of the soldier in the field. Currently, the heating is done by burning a fuel tab under a canteen cup filled with water into which the soldier places a pouch-packed entrée item, such as beef in barbecue sauce. Heating food this way is relatively inefficient, limits the soldier to a fixed location, and, because of the open flame, sends out a visible signal. Newer developments aim for chemical methods of flamelessly heating the food within its packaging and while the soldier is on-the-move.

Chemical Heating Pad. A new product has been developed, through joint military—civilian cooperation, that uses a water-activated chemical system for efficiently generating and transferring heat (31). It contains a supercorrosive magnesium–iron alloy powder blended with sodium chloride and imbedded in a porous polymeric matrix. It is produced as a thin, rectangularly shaped pad enclosed in a perforated paperboard package and weighs 20 g. The exothermic reactions that take place with 2 oz of added water include the oxidation of magnesium, reduction of oxygen, and formation of gaseous hydrogen. Approximately 33 kcal of energy are released. The high ratio of energy released to the weight of the pad and the action of the streaming gas and steam in heating the packaged ration component make this product attractive.

Flameless Ration Heater (FRH). The initial application for this chemical heating pad will be as a flameless heater for MRE entrées. Whether packed with the MRE or issued separately, the FRH includes a heater pad in a polyethylene bag that is sized to accept the MRE pouch and is marked with a line to indicate a level of added water equivalent to 2 oz. It is capable of raising the temperature of an 8 oz entrée from 40 to 140°F. In practice, the entree is placed in the bag, the water added, the top of the bag folded over to avoid spillage, and the whole system placed back in the small carton originally used to protect the pouch. The carton is then set aside or carried (perhaps in the pocket of the soldier's uniform) for about 10 min while the heating takes place. If left in the carton, the entrée will stay warm for an hour.

Self-Heating Individual Meal Module. Another application of this chemical heating pad will be for use in an individual meal module designed as a laptop serving tray package containing all ration components, including utensils. The heater pad would be located in recessed sections of the tray just under the components to be heated. Water could be included in a puncturable reservoir or added when needed. In either case, channels would be included in the package to direct the water to the pads and to focus the hot gases around the compartments with the heatable components. Entrées, starchy items such as noodles, and vegetables such as carrots would be heated to the desired temperatures by judicious choice of the pad size and weight. This same general concept could be used to cool dessert items by choosing reagents whose reactions with water are endothermic.

Future Heating Concepts. Because this chemical heater concept is not practical for boiling water, other flameless heating concepts are being considered for supplementary use. A small device in which organic fuel could be catalytically combined with oxygen, without the propogation of a flame, would be especially attractive. Simple ways to initiate the catalytic combustion and to transfer the heat to a canteen would have to be included. Prospects for meeting these requrements are good.

BIBLIOGRAPHY

1. R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, Inc., Orlando, Fla., 1984.
2. M. Karel, O. R. Fennema, and D. B. Lund, *Physical Principles of Food Preservation. Part II.* Marcel Dekker, Inc., New York, 1975.
3. C. Andres, "New Class of Foods—Compressed Foods," *Food Processing* (Winter 1973).
4. A. V. Cardello and O. Maller, "Relationships Between Food Preferences and Food Acceptance Ratings," *Journal of Food Science* **47**, 1553–1561 (1982).
5. O. Maller and A. V. Cardello, "Ration Acceptance Methods: Measuring Likes and Their Consequences," *Nederlands Military Geneeskundig Tijshrift*, 91–96 (1984).
6. A. V. Cardello and O. Maller, "Psychophysical Bases for the Assessment of Food Quality," in J. Kapsalis, ed., *Objective Methods in Food Quality Assessment*, CRC Press, Inc., Boca Raton, Fla., 1987, pp. 61–125.
7. A. V. Cardello and O. Maller, "Sensory Texture Analysis: An Integrated Approach to Food Engineering," in H. R. Maskowitz, ed., *Food Texture*, Marcel-Dekker, Inc., New York, 1987, pp. 177–215.
8. A. V. Cardello, O. Maller, and M. V. Klicka, "Computer-aided Sensory Analysis and Evaluation: A Comprehensive System for the Assessment of Foods and Beverages," in H. R. Maskowitz, ed., *Applied Sensory Analysis of Foods*, CRC Press, Inc., Boca Raton, Fla., 1988, pp. 35–81.
9. H. L. Meiselman, R. Popper, and E. Hirsch, "Sensory, Hedonic and Situational Factors in Food Acceptance and Consumption," *Proceedings of the International Symposium on Food Acceptability.*
10. R. Popper, E. Risvik, H. Martens, and M. Martens, "A Comparison of Multivariate Approaches to Sensory Analysis and the Prediction of Acceptability," *Proceedings of the International Symposium on Food Acceptability.*
11. E. S. Hirsch et al., "The Effects of Prolonged Feeding of Meal-Ready-to-Eat (MRE) Operational Rations," *Technical Report NATICK/TR-85/035*, 1985.
12. R. D. Popper et al., "Field Evaluation of Improvement MRE, MRE VII, and MRE IV," *Technical Report NATICK/TR-87/027*, 1987.
13. H. L. Meiselman and E. S. Hirsch, "Situational and Sensory Factors Controlling Acceptance of Military Rations," *Proceedings of the 29th Conference of the Military Testing Association*, 1987, pp. 303–308.
14. H. L. Meiselman, E. S. Hirsch, and R. D. Popper, "Sensory, Hedonic and Situational Factors in Food Acceptance and Consumption," in D. M. H. Thompson, ed., *Food Acceptance*, Elsevier, 1988, pp. 77–87.
15. J. Mauron, "The Maillard Reaction in Food. A Critical Review from the Nutritional Standpoint," in C. Eriksson, ed., *Progress in Food and Nutrition Science. Maillard Reactions in Food*, Pergammon Press, Ltd., Oxford, UK, 1981.
16. R. F. Hurrel and K. J. Carpenter, "The Estimation of Available Lysine in Food Stuffs after Maillard Reaction," in Ref. 15.
17. K. A. Narayan and R. E. Andreotti, "Kinetics of Lysine loss in Compressed Model Systems Due to Maillard Reaction," *Journal of Food Biochemistry* **13**, 105–125 (1989)
18. W. L. Porter, L. A. Levasseur and A. S. Henick, *Journal of Food Science* **42**, 1533 (1977).
19. S. J. Bishov and A. S. Henick, *Journal of Food Science* **40**, 345 (1975).
20. W. L. Porter et al., "Analytical Use of Fluorescence-Producing Reactions of Lipid and Carbohydrate-Derived Carbonyl Groups with Amine End Groups of Polyamide Powder," in G. R. Waller and M. S. Feather, eds., *The Maillard Reaction in Foods and Nutrition*, ACS Symposium Series 215, American Chemical Society, Washington, D.C., 1983, p. 47.
21. W. L. Porter, E. D. Black and A. M. Drolet, *Journal of Agricultural and Food Chemistry* **37**, 615 (1989).
22. W. L. Porter et al., "Proceedings of the Second Natick Science Symposium," *Natick/TR-88/052*, June 1988, p. 175.
23. M. H. Thomas, B. M. Atwood, and K. A. Narayan, "Stability of Vitamins C, B$_1$, and B$_2$, and B$_6$ in Fortified Beef Stew," *Natick/TR-86/061*, Sept. 1986.
24. M. H. Thomas, B. M. Atwood, and K. A. Narayan, "Effect of Processing and Preparation for Serving on Vitamin Content in T, B, and A Ration Pork," *Natick/TR-86/022*, Mar. 1986.
25. Y. Kim, "Effect of Processing and Preparation for Serving on Vitamin Content in T, B, and A Ration Peas and Carrots," *Natick/TR-86/047*, May 1986.
26. Natick RD&E Center, unpublished data
27. T. P. Labuza, *Shelf-Life Dating of Foods*, Food and Nutrition Press, Inc., Westport, Conn., 1982.
28. E. W. Ross et al., "A Time-Temperature Model for Sensory Acceptance of a Military Ration," *Journal of Food Science* **52**, 1712 (1987).
29. J. L. Briggs et al., "A Calorically Dense Ration for the 21st Century," *Army Science Conference Proceedings*, Vol. 1, West Point, N.Y., 1986, p. 81.
30. A. H. Barrett et al., "Simulation of the Vacuum Infusion Process Using Idealized Components: Effects of Pore Size and Suspension Concentration," *Journal of Food Science* (in press).
31. U.S. Pat. 4,522,190 (June 11, 1985), W. E. Kuhn, K. H. Hu, and S. A. Black (to University of Cincinnati).

GERALD L. SCHULZ
ALICE MEYER
IRWIN TAUB
DANIEL BERKOWITZ
ARMAND CARDELLO
EDWARD HIRSCH
C. PATRICK DUNNE
ANANTH NARAYAN
BONNIE ATWOOD
WILLIAM PORTER
GERALD SILVERMAN
U.S. Army Natick
Natick, Massachusetts

MILK AND MILK PRODUCTS

By definition as mammals, humans are milk-drinking animals. Since at least from the beginning of recorded history, humans have used the milk of other mammals as a food source. Before urbanization, each family depended on its own animals for milk. Later, dairy farms were developed close to cities. The milk industry became a commercial enterprise when methods for the preservation of fluid milk were introduced. Like many other early industries—made up of many small producers and associated with a local production area—the dairy industry developed into large production units and large processing plants often located far from the production areas. The successful evolution of the dairy industry from small to large units of production, that is, from farm to dairy plant, depended on sanitation of product and equipment, cooking facilities, health standards for animals and workers, transportation systems, construction materials for process machinery and product containers, pasteurization methods, containers for distribution, and refrigeration for products in stores and in homes.

COMPOSITION AND PROPERTIES

Cow's milk consists of about 87% water and 13% total solids (Table 1). The latter are composed of solids-not-fat (SNF) and fat. Milk with higher fat content also has higher solids-not-fat content, with an increase of SNF of 0.4% for each 1% fat increase. The principal components of solids-not-fat are protein, lactose, and minerals (ash). Fat content and other constituents of milk vary with species, and, for the milk cow, vary with breed. Likewise, composition of milk may be influenced by many factors such as feed, stage of lactation, health and age of animal, and seasonal and environmental conditions. The SNF and moisture relationships are well established and can be used as a basis for detecting adulteration with water. The physical properties of milk are given in Table 2.

Fat

Milkfat is considered the most complex of all common fats. It is composed of triglycerides (96%) and contains more types of fatty acids than vegetable oils. Milkfat is mainly saturated (65%) but also contains monounsaturated (32%) and polyunsaturated (3%) fatty acids. Milkfat typically contains 7% short-chain fatty acids (C_4–C_8), 15–20% medium-chain fatty acids (C_{10}–C_{14}), and 73–78% long-chain fatty acids ($\geq C_{16}$). The fat is a carrier of numerous lipids (cerebrosides, sterols, and phospholipids) and the fat-soluble vitamins A, D, E, and K. Whole milk (3.3% fat) contains 14 mg cholesterol per 100 g milk, whereas milk of 0.18% fat contains 2 mg cholesterol per 100 g milk. Milk is an emulsion of fat in water (serum). The emulsion is stabilized by phospholipids that are absorbed on the fat globules. The emulsion is broken during treatments such as homogenization and churning. Current U.S. legal standards now allow skim milk (<0.5% fat) to use the terms fat free or nonfat, 1% milk can use light or lowfat, 2% milk can use reduced fat, and whole milk (3.25% minimum fat) remains unchanged.

Protein

Milk protein is composed of two major fractions, caseins and whey proteins. Milk proteins are important in human nutrition. They are also of more value today to dairy producers because they may be included in milk pricing structures, thus adding extra value to milk.

Nutritional Content

Milk is an excellent source of calcium, phosphorous, and riboflavin. Because raw milk is a poor source of vitamin D, vitamin D–fortified milk has been sold since the 1920s to prevent rickets in children. Originally milk was fortified with vitamin D by irradiation or by feeding irradiated yeast to the cows. Ergosterol is converted to vitamin D by ultraviolet irradiation. Currently, vitamin D is added directly to whole milk to provide 400 IU or 10 μg per quart.

Table 1. Gross Composition of Milks from Various Mammals

	Composition (g/100 g)						
Species	Water	Fat	Casein	Whey protein	Lactose	Ash	Energy (kcal/100 g)
Human	87.1	4.5	0.4	0.5	7.1	0.2	72
Rabbit	67.2	15.3	9.3	4.6	2.1	1.8	202
Rat	79.0	10.3	6.4	2.0	2.6	1.3	137
Guinea pig	83.6	3.9	6.6	1.5	3.0	0.8	80
Horse	88.8	1.9	1.3	1.2	6.2	0.5	52
Donkey	88.3	1.4	1.0	1.0	7.4	0.5	44
Pig	81.2	6.8	2.8	2.0	5.5	1.0	102
Camel	86.5	4.0	2.7	0.9	5.0	0.8	70
Reindeer	66.7	18.0	8.6	1.5	2.8	1.5	214
Cow	87.3	3.9	2.6	0.6	4.6	0.7	66
Zebu	86.5	4.7	2.6	0.6	4.7	0.7	74
Yak	82.7	6.5	5.8		4.6	0.9	100
Water buffalo	82.8	7.4	3.2	0.6	4.8	0.8	101
Goat	86.7	4.5	2.6	0.6	4.3	0.8	70
Sheep	82.0	7.2	3.9	0.7	4.8	0.9	102

Source. Ref 1

Table 2. Physical Properties of Milk

Property	Value
Density at 20°C of milk with 3–5% fat, average, g/cm^3	1.032
Weight at 20°C, kg/L[a]	1.03
Density at 20°C of milk serum, 0.025% fat, g/cm^3	1.035
Weight at 20°C of milk serum, 0.025% fat, kg/L[a]	1.03
Freezing point, °H[b]	−0.540
Boiling point, °C	100.17
Maximum density at °C	−5.2
Electrical conductivity, S ($=\Omega^{-1}$)	$(45–48) \times 10^{-3}$
Specific heat at 15°C, kJ/(kg · K)[c]	
Skim milk	3.94
Whole milk	3.92
40% cream	3.22
Fat	1.95
Relative volume 4% milk at 20°C = 1, volume at 25°C	1.002
40% cream 20°C = 1.0010 at 25°C	1.0065
Viscosity at 20°C, mPa · s (=cP)	
Skim milk	1.5
Whole milk	2.0
Whey	1.2
Surface tension of whole milk at 20°C, mN/m (= dyn/cm)	50
Acidity, pH	6.3–6.9
Titratable acid, %	0.12–0.15
Refractive index at 20°C	1.3440–1.3485

[a] To convert kg/L to lb/gal, multiply by 8.34.

[b] Degrees Hortvet.

[c] To convert kJ/(kg · K) to Btu/(lb · °F), divided by 4.183.

Vitamin A must be added to lower-fat milk to provide 2,000 IU per quart. Multivitamin, mineral-fortified milk is available to meet recommended dietary requirements. The daily nutritional needs for an adult and the constituents of milk are given in Table 3. Average composition of selected dairy products is given in Table 4.

PROCESSING

Commercial dairy farm operations usually consist of a milking machine, a pipeline to convey the milk directly to the tank, and a refrigerated bulk milk tank in which the milk is cooled and stored. Milk at approximately 34°C from the cow is cooled rapidly to 4.4°C or below to maintain quality.

Processing operations for fluid milk or manufactured milk products include centrifugal sediment removal; cream separation; pasteurization; sterilization; homogenization; membrane separation; and packaging, handling, and storing.

Separation

Continuous-flow centrifugal cream separators using cone disks in a bowl were introduced in 1890. Originally, the cream separators were the basic plant equipment, and dairy plants were then known as creameries. Today's separators are pressure- or forced-fed, sealed airtight units. Separators develop 5,000 to 10,000 times the force of gravity to separate the fat (cream) from the milk. Cold incoming raw milk is generally filtered, clarified, or both in the plant to remove sediment. A clarifier is a special type of separator in which sediment is continuously removed from milk before further processing. Bactofugation is a specialized process of clarification in which high-speed centrifugal devices are used to remove most of the bacteria from milk. This process is used for sterile milk or cheese.

In today's large, automated fluid milk plants, milk is standardized automatically. A continuous separator splits the milk stream into fat-free milk and cream, the latter of which is added back to the milk stream to yield the desired fat content. Automatic (on-line) sampling and testing equipment along with air valves control the final tests of the desired product.

Homogenization

Homogenization is the process by which a mixture of components is treated mechanically to give a uniform product

Table 3. Reference Daily Intake[a] and Nutritional Content of Whole Milk

Nutrient	Reference daily intake	Supplied by 100 g	Supplied by 1 quart
Energy, kJ (kcal)[b]		257 (61)	2,510 (600)
Protein, g (daily reference value)	50	3.29	32.11
Calcium, g	1.0	0.119	1.165
Phosphorus, g	1.0	0.93	0.912
Magnesium, mg	400	13	131
Zinc, mg	15	0.38	3.71
Vitamin A, IU	5,000	31	303
Thiamine, mg	1.5	0.038	0.371
Riboflavin, mg	1.7	0.162	1.581
Niacin, mg	20	0.084	0.82
Ascorbic acid, mg	60	0.94	9.17
Vitamin D, IU	400	41[c]	400[c]

Source Ref. 2.

[a] U.S. Food and Drug Regulations.

[b] To convert kJ to kcal, divide by 4.184; food calorie = 1 kcal.

[c] Fortified milk

Table 4. Average Composition of Selected Dairy Products, %

Product	Moisture	Protein	Total fat	Total carbohydrate	Ash	Calcium	Phosphorous	Sodium
Fat-free milk	90.8	3.4	0.2	4.9	0.8	0.12	0.10	0.05
Whole milk	88.0	3.3	3.3	4.7	0.7	0.12	0.09	0.05
Half-and-half	80.6	3.0	11.5	4.3	0.7	0.10	0.09	0.04
Heavy whipping cream	57.7	2.0	37.0	2.8	0.4	0.06	0.06	0.04
Evaporated whole milk	74.0	6.8	7.6	10.0	1.5	0.26	0.20	0.10
Plain yogurt	87.9	3.5	3.3	4.7	0.7	0.12	0.10	0.05
Cultured low-fat buttermilk	90.1	3.3	0.9	4.8	0.9	0.12	0.09	0.10
Ice cream (economy)	60.8	3.6	10.8	23.8	1.0	0.13	0.10	0.09
Ice cream (premium)	58.9	2.8	16.0	21.6	0.7	0.10	0.08	0.07
Sherbet (orange)	66.1	1.1	2.0	30.4	0.4	0.05	0.04	0.05
Dried whole milk	2.5	26.3	26.7	38.4	6.1	0.91	0.80	0.40
Nonfat dry milk	3.2	36.2	0.8	52.0	7.9	1.26	0.97	0.53
Dried sweet whey	3.2	12.9	1.1	74.5	8.3	0.80	0.93	1.08
Dried acid whey	3.5	11.7	0.5	73.4	10.8	2.05	1.35	0.97
Creamed cottage cheese	79.0	12.5	4.5	2.7	1.4	0.06	0.13	0.40
Cheddar cheese	36.7	24.9	33.1	1.3	3.9	0.72	0.51	0.62
Swiss cheese	37.2	28.4	27.4	3.4	3.5	0.96	0.60	0.26
Mozzarella	54.1	19.4	21.6	2.2	2.6	0.52	0.37	0.37
Butter	15.9	0.9	81.1	0.1	2.1			
Butter oil	0.2	0.3	99.5	0.0	0.0			

Source: Ref 1.

that does not separate. In milk, the fat globules are broken up into small particles that form a stable emulsion in the milk, that is, the fat globules do not rise by gravity to form a cream line. Today, most fluid milk products are homogenized.

A homogenizer is a high-pressure positive pump in which milk is forced through small passages under pressure of 14–17 MPa (2000–2500 psi) at velocities of approximately 183–244 m/s. The fat globules are broken up as a result of a combination of factors, namely shearing, impingement, distention, and cavitation. Fat globules in raw milk (1–15 μm in diameter) are reduced to 1 to 2 μm by homogenization.

Pasteurization

Pasteurization is the process of heating milk to kill yeasts, molds, and pathogenic and most other bacteria and to inactivate certain enzymes without greatly altering the flavor. The principles were developed by and named after Louis Pasteur and his work on wine in 1860 to 1864 in France. The basic regulations are included in the Pasteurized Milk Ordinance (3), which has been adapted by most local and state jurisdictions in the United States.

Pasteurization may be carried out by batch or continuous-flow processes. In the batch process, each particle of milk must be heated to at least 62.8°C and held continuously at or above this temperature for at least 30 min. In the continuous process, the milk is heated to at least 71.7°C for at least 15 s. This is known as high-temperature short-time (HTST) pasteurization.

Other continuous pasteurization processes using higher temperatures and shorter times called ultra-high-temperature (UHT) are commercially employed (Table 5). For milk products with a fat content above that of milk or with added sweeteners, additional time and temperature are required (see Table 5). Following pasteurization, the product should be cooled quickly to 7.2°C or less.

Membrane Separation

The separation of components of liquid milk products can be accomplished using semipermeable membranes by either ultrafiltration (UF), reverse osmosis or hyperfiltration (RO), microfiltration (MF), or nanofiltration (NF). The materials from which the membranes are made is similar for all processes and include cellulose acetate, polyvinyl chloride, nylon, polysulfone, and polyamide. Ceramic and mineral membranes are also widely used.

Ultrafiltration. Membranes capable of selectively passing molecules (molecular weights <10,000) are used. Pressures of 0.1 to 1.4 MPa (up to 200 psi) are exerted over the solution to overcome the osmotic pressure while still providing an adequate flow through the membrane for use UF separates protein from lactose and mineral salts, the protein being the concentrate. Also, UF is used with skim milk to obtain a protein-rich concentrate from which cheese is made. The whey protein obtained by UF can be spray dried. UF is also used for removing minerals from whey and buttermilk.

Reverse Osmosis (Hyperfiltration). Membranes are used for separation of smaller components (molecular weights <500). They have smaller pore size than those used for UF. High-pressure pumps, usually of the positive-piston type or multistage centrifugal type, provide pressures up to 4.14 MPa (600 psi).

RO is used to remove only water from milk or whey; hence a concentrate similar to condensed milk is produced. RO has numerous applications in the dairy and food in-

Table 5. Pasteurization Times and Temperatures for Dairy Products

	Vat		HTST		UHT	
Product/Process	Time	Temp.[a]	Time	Temp.[a]	Time	Temp.[a]
Fluid milk	30 min	145°F	15 s	161°F	2.0 s	280°F
Milk products	Increase viscosity and added sweetener, stabilizers, etc.		15 s	166°F	2.0 s	280°F
	30 min	150°F				
Eggnog	30 min	155°F	25 s	175°F	2.0 s	280°F
Ice cream mix	Strictly on viscosity		15 s	180°F	2.0 s	280°F

Source Ref 3.

[a] To convert °C to °F, °C = (°F − 32) × 5/9

dustries, among which are concentration of milk before cheese making and concentration of whey before drying.

Microfiltration. Membranes are used to selectively separate particles with molecular weights >200,000. This allows for the separation of proteins and bacteria. This technology has been used for the cold pasteurization of milk.

Nanofiltration. Nanofiltration falls between ultrafiltration and reverse osmosis. It removes particles with molecular weights of 300 to 1,000. It can therefore be used for demineralization as well as concentration of whey. It can also be used for the removal of salt from salty whey.

CLEANING SYSTEMS

In most plants today, the equipment surfaces are cleaned in place (CIP) by mechanical-chemical action. The results of CIP are influenced by equipment surfaces and time of exposure, mechanical action, and the temperature and concentration of the solution being circulated.

In the CIP procedure, a cold or tempered aqueous prerinse is followed by circulation of a cleaning solution for 10 min to 1 h at 54 to 82°C. A wide variety of cleaning solutions may be used, depending on the food product, the hardness of the water, and the equipment. Alkali or chlorinated alkaline cleaners are preferred. A chlorinated alkaline cleaner may be used separately or in combination with an acid detergent. The best combination of chemical, timing, and temperature is determined experimentally.

The CIP system is highly automated with valves, controls, and timers. The programmer controls the timing and airflow to the valves so that cleaning solutions are directed to the proper locations for a specific time, followed by a rinse and draining or air blow to clear the lines.

PACKAGING

Following pasteurization, milk and milk products are packaged for consumer use in a variety of package sizes and materials. The filling operation must be carried out without contamination of the product and as soon after pasteurization as possible. Following filling and closing, the packages are placed in a case. The containers are stacked on pallets and moved to a refrigerated area (2–4°C). These operations are mechanized, with continuous operator supervision.

Aseptic packaging has developed in conjunction with high-temperature processing and has continued to make sterile milk and milk products a commercial reality worldwide. In the United States, aseptic or UHT milk has been much slower to emerge, owing to well-developed refrigeration systems to handle dairy and other food products. As a result, most UHT systems are currently processing fruit juices and some cream and ice cream mixes.

ANALYSIS AND TESTING

Milk and milk products are subjected to a variety of tests to assure public safety and to meet composition standards (4).

Microbial Quality

The microbial quality of dairy products is related to the number of viable organisms present. A high number of microorganisms in raw milk suggests it was produced under unsanitary conditions or that it was not adequately cooled after removal from the cow. If noncultured dairy products contain excessive numbers of bacteria, in all likelihood postpasteurization contamination occurred, or the product was held at a temperature permitting substantial growth. Raw-milk and pasteurized-milk products are examined for the concentration of microorganisms by the agar plate method or the direct microscopic method.

The agar plate method consists of adding a known quantity of sample (usually ≤1.0 mL, depending on the concentration of bacteria) to a sterile petri plate and then mixing the sample with a sterile nutrient medium. After the agar medium solidifies, the petri plate is incubated at 32°C for 48 h, after which the bacterial colonies are counted and the number expressed in terms of standard plate count or colony-forming units per 1 mL or g of sample. This procedure measures the number of viable organisms present and able to grow under test conditions. An alternative method to the agar plate method is the Petrifilm® aerobic count (PAC) method. This method uses a cold-water soluble gelling agent instead of agar in the nutrient medium. The methodology is similar to the agar plate count, but the Petrifilm® plates require less labor in preparation and less incubator space.

The direct microscopic count determines the number of viable and dead microorganisms in a milk sample. The small amount (0.01 mL) of milk is spread over a 1.0 cm^2 area on a microscope slide and allowed to dry. After staining with an appropriate dye (usually methylene blue), the slide is examined with the aid of a microscope (oil-immersion lens). The number of bacteria cells and clumps of cells per microscopic field is determined and by proper calculations is expressed as the number of organisms per 1 mL of sample.

The keeping quality of milk and milk products is determined by several variations of the agar plate method. Coliform bacteria are detected by using the agar plate method and a selective culture media (violet-red bile agar). Because coliform bacteria do not survive pasteurization, they are an indication of recontamination or improper processing. Psychrotrophic bacteria are capable of growing at refrigerator temperatures, 2 to 10°C. They are responsible for fruity, putrid off-flavors in milk. These bacteria are measured by the agar plate method and incubation at 7°C for 10 days. Additional tests for milk quality can be found in Ref. 4.

Somatic Cells

Mastitis is an inflammation of the cow's udder that results in high levels of somatic cells in the milk. A level of somatic cells >750,000/mL is considered abnormal, and such milk must not be offered for human consumption. Treatment for mastitis with antibiotics may result in such compounds getting into the milk supply. When this occurs, starter culture growth and hence cheese making may be inhibited, or persons consuming such milk may have allergic reactions to these compounds. The presence of such chemicals is determined by a variety of very sensitive and rapid test procedures (4).

Other Tests

The phosphatase test (4) is a chemical method for measuring the efficiency of pasteurization. All raw milk contains phosphatase, and the thermal resistance of this enzyme is greater than that of pathogens over the range of time and temperature of heat treatments recognized for proper pasteurization. Underpasteurization as well as contamination of a properly pasteurized product with raw product can be detected by this test. Pesticides are often found in feed or water consumed by cows; subsequently, they may appear in the milk. At present, low-level residues of some of these chemicals are permitted in milk, and hence milk and its products must sometimes be tested for chlorinated hydrocarbon pesticides (4).

Milk Composition: Fat and Protein

Milk and milk products are purchased from the farmer and sold in stores based on content of fat, protein, or both for raw milk and according to federal and state standards. Milk was first analyzed for fat content by the Babcock test developed in 1890. Today, the Babcock test is used primarily to standardize newer, indirect fat-testing equipment such as the turbidimetric method, mid-infrared spectroscopic methods, and near infrared reflectance methods. Mid-infrared methods measure fat at specific wavelengths (3.48 and 5.723 μm) and are automated, capable of testing hundreds of samples per hour.

The protein content of milk can be determined by a variety of methods, including dye-binding, Kjeldahl, and colorimetric. Recently, procedures using infrared analyses have been adapted to the same equipment that measures fat, so that fat and protein can be measured at the same time using different wavelengths of light. Currently, much of the raw milk is being purchased based on fat and protein or fat and SNF contents. These fat and protein tests are described in detail in Ref. 5.

MANUFACTURED PRODUCTS

Cheese

Cheese making is based on the coagulation of casein from milk or, to a minor extent, of the proteins of whey. Casein is precipitated by acidification, which can be accomplished by adding bacteria that will produce lactic acid from lactose in milk. The procedures for making cheese vary greatly, and cheese products are countless.

Considerable art is involved in making the 400-plus existing cheese varieties. The composition and handling of the original milk, bacterial flora, and starter culture are the basic variables that along with heat treatments, flavoring, salting, and forming of the final product affect the final product. The composition of various cheeses is given in Table 4. Low-fat varieties can have higher moisture levels.

Cheddar Cheese. Pasteurized milk is inoculated with a lactic acid culture and rennet to coagulate casein. The coagulated milk is cut into cubes and cooked to remove whey. Whey is drained out, and the curd cubes are allowed to knit closely together by the cheddaring process. At the end of the cheddaring process, the curd is milled into smaller cubes and salted. The salted cheese is pressed overnight for further whey removal and aged up to a year for flavor development.

Cottage Cheese. Cottage cheese is made from skim milk. Compared to most other cheeses, cottage cheese has a short shelf life and must be refrigerated usually at 4.4°C or below to maintain quality and to provide a shelf life of 3 to 4 weeks. Cottage cheese is a soft, uncured cheese that contains not more than 80% moisture.

Cottage cheese is made by several procedures. In general, pasteurized skim milk is inoculated with lactic acid culture to coagulate the protein. A small amount of rennet may be added to firm the curd. The coagulated material is divided or cut, and the resulting curd is cooked to remove the whey. The whey is drained, and the curd is washed with water. Washed curd is mixed with cream. Mechanized operations are used for large-scale production.

Frozen Desserts

Ice cream is the principal frozen dessert produced in the United States. Known as the American dessert, it was first sold in New York City in 1777. The composition of various frozen desserts is given in Table 4.

Ice Cream. Ice cream is a frozen dessert prepared from a mixture of dairy ingredients (16–35%), sweeteners (13–20%), stabilizers, emulsifiers, flavoring, and fruits and nuts. Ice cream has 8 to 20% milkfat and 8 to 15% SNF with a total of 38.3% (36–43%) total solids. These ingredients can be varied. U.S. regulations also allow for reduced-fat ice cream (25% less fat than the reference ice cream), light ice cream (50% less fat or 1/3 fewer calories than the reference ice cream, as long as in the case of calorie reduction less than 50% of the calories are derived from fat), low-fat ice cream (not more than 3 g of fat per 1/2 cup serving), and nonfat ice cream (less than 0.5 g of fat per 1/2 cup serving). The dairy ingredients are milk or cream; milkfat, which is supplied by milk, cream, butter, or butter oil; as well as SNF, which is supplied by condensed whole, nonfat, or dry milk. The quantities of these products are specified by standards. Milkfat provides the characteristic texture and body in ice cream. Sweeteners are a blend of cane or beet sugar and corn syrup solids. Quantities of these vary, depending on the sweetness desired, cost, and effect on body and texture.

Stabilizers that improve the body of ice cream include gelatin, sodium alginate, sodium carboxymethyl cellulose, pectin, and guar gum. Emulsifiers such as lecithin, monoglycerides, diglycerides, and polysorbates assist in the incorporation of air and improve the whipping properties. Commercial ice cream stabilizers and emulsifiers are usually available as a blend. Use of stabilizers and emulsifiers is optional, and it is possible to produce good-quality ice cream without them. The liquid mixture of components for making ice cream is called ice cream mix.

Preceded by a blending operation, the ingredients are mixed in a freezer that whips the mix to incorporate air and freezes a portion of the water. Freezers may be of a batch type for a small operation or continuous type, in which most commercial ice cream is produced.

Incorporation of air decreases the density and improves the consistency of ice cream. If one-half of the final volume is occupied by air, the ice cream has 100% overrun and weighs 0.53 kg/L. The ice cream from the freezer is at about −5.5°C, with one-half of the water frozen, preferably in small crystals.

Packaged ice cream is hardened on plate-contact hardeners or by convection air blast as the product is carried on a conveyor or through a tunnel. Air temperatures for hardening are −40 to −50°C. The temperature at the center of the container as well as the storage temperature should be −23°C or below. Approximately one-half of the heat is removed in the freezer and the remainder in the hardening process. Although ice cream is by far the most important frozen dessert, other frozen desserts such as ice milk, frozen yogurt, sherbet, and mellorine-type products are also popular.

Frozen Yogurts. Frozen yogurts have become popular with health-conscious consumers. Product formulations vary widely. Frozen yogurt usually consists of 80% ice milk mix and 20% yogurt.

Sherbets. Sherbets have a low fat content (1–2%), low milk solids (2–5%), and a sweet (25–35% sugar) but tart flavor. Ice cream mix and water ice can be mixed to obtain a sherbet. The overrun in making sherbets is about 25 to 40%.

Mellorine. Mellorine is similar to ice cream except that the milkfat is replaced with a vegetable fat (6% minimum). It contains 10 to 12% milk solids.

Nondairy Frozen Dessert. Nondairy frozen dessert is a frozen product containing no dairy ingredients. It may contain vegetable fat, vegetable protein, sugar, eggs, and stabilizers.

Other Frozen Desserts. Other frozen desserts include parfaits, ice cream puddings, novelties, and water ice products. New low-fat and nonfat products and products containing high-intensity sweeteners and fat replacers are being introduced to the market.

Yogurt

Yogurt is a fermented milk product that is rapidly increasing in consumption in the United States. Milk is fermented with *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* organisms that produce lactic acid and the characteristic yogurt flavor. Usually some cream or nonfat dried milk is added to the milk to obtain a heavy-bodied yogurt.

Yogurt is manufactured similarly to cultured buttermilk. Milk with a fat content of 1 to 5% and SNF content of 11 to 14% is heated to about 82°C and held for 30 min. After homogenization, the milk is cooled to 43 to 46°C and inoculated with 2% culture. The product is incubated at 43°C for 3 h in a vat or in the final container. The yogurt is cooled and held at ≤4.4°C. The cooled product should have a titratable acidity of 0.9 to 1.2% and a pH of 4.3 to 4.4. The titratable acidity is expressed in terms of percentage of lactic acid, which is determined by the amount of 0.1 *N* NaOH/9 grams required to neutralize the substance. Thus, 10 mL of 0.1 *N* NaOH represent 1% acidity. Yogurts with ≤2% fat are popular. Fruit-flavored yogurts are also common; 30 to 50 g of fruit is placed in the bottom of the carton (sundae style) or mixed with the yogurt (swiss style).

Cream

Cream is a high-fat product that is obtained by mechanical separation through differential density of the fat and the skim. Fat content may range from 10 to 40%, depending on use and federal and state laws. The U.S. *Code of Federal Regulations* (6) defines creams as products that contain not less than 18% milkfat. Whipping cream has a fat content of 36 to 40%; table, coffee, or light cream has a fat content of 18 to 30%. Half-and-half, a mixture of cream and milk, has not less than 10.5% milkfat and in some states up to 12%.

The sale of fresh cream for home use has decreased greatly over the past 20 years, primarily as a result of changing customer demand based on dietary concerns. A variety of cream and fat substitutes are available for spreads, toppings, whiteners, and cooking.

Dry Milk

Drying generally follows concentration in an evaporator. Clarification and homogenization precede evaporating and drying. Homogenization of whole milk at 63 to 74°C with pressures of 17 to 24 MPa (2500–3500 psi) is particularly desirable for reconstitution and the preservation of quality.

Dry milk is generally made by the drum-drying or spray-drying method. The spray process uses less heat than the drum process; hence the powder produced by the spray process is more soluble in water. Solubility of the powder can be further improved by coupling the agglomeration (or instantization) process with the spray process. In the agglomeration process, powder particles are agglomerated into larger particles that dissolve more easily than smaller particles.

Dry whole milk should be vacuum- or gas-packed to maintain quality under storage. Products containing milkfats deteriorate in the presence of oxygen, giving oxidation off-flavor. Antioxidants of many kinds have been used with various degrees of success, but a universally acceptable antioxidant that meets the requirements for food additives has not yet been found.

Dry milk reduces transportation costs, provides long-term storage, and supplies a product that can be used for food-manufacturing operations. It has been used primarily for manufactured products but is now used to a much greater extent for beverage products. The moisture content for nonfat dry milk, the principal dry milk product, is ≤5.0% for standard grade and ≤4.0% for extra grade. Dry whole milk contains ≤3.0% moisture.

Butter

Butter is defined as a product that contains 80% milkfat with not more than 16% moisture. It is made from cream containing 25 to 40% milkfat. The process of making butter is primarily a mechanical one in which the cream, an emulsion of fat in serum, is changed to butter, an emulsion of serum in fat. The process is accomplished by churning or, in recent years, by a continuous operation with automatic controls. Per capita annual sales of butter have remained steady at approximately 4.5 to 5.5 lb for the past 20 years.

Anhydrous Milkfat; Butter Oil

Butter oil is a high-milkfat material that is 99.7% milkfat. It is called anhydrous milkfat or anhydrous butter oil if <0.2% moisture is present. Although the terms are used interchangeably, anhydrous butter oil is made from butter and anhydrous milkfat is made from whole milk. For milk and cream there is an emulsion of fat in serum. For butter oil and anhydrous milkfat there is an emulsion of serum in fat, such as with butter. It is easier to remove moisture in the final stages to make anhydrous milkfat with the serum-in-fat emulsion.

Condensed Milk Products

Evaporated milk is produced by condensing milk to half its volume in a vacuum evaporator. The final product must have at least 6.5% milkfat and 23% total milk solids including fat. The process for evaporated fat-free milk is similar to that for whole milk except that the final product contains much less fat and total solids. The final product is packaged in cans and sterilized at 116 to 118°C for 15 to 20 min. It is subsequently cooled to room temperature within 15 min. Vitamins A and D and stabilizing salts such as sodium citrate and disodium phosphate may be added.

Condensed product made without sterilization is called condensed milk. Additionally, sugar may be added to the final product so that the final product contains 43 to 45% sugar, at least 8.5% milkfat, and 28% total milk solids. This product is called sweetened condensed milk. Owing to the high sucrose content, defects such as age thinning and age thickening may occur. These defects can be prevented by careful control of the manufacturing procedure. Condensed milk products are widely used in the manufacture of ice cream as well as bakery, confectionery, and other food products.

By-Products from Milk

Milk is a source for numerous by-products resulting from separation or alteration of the components. These components may be used in other nondairy manufactured foods, dietary foods, pharmaceuticals, animal feeds, and industrial products.

Lactose. Lactose or milk sugar makes up about 5% of cow's milk. Compared to sucrose, lactose has about one-sixth the sweetening strength. Because of its low solubility, lactose is limited in its application. However, it is soluble in milk serums and can be removed from the whey. Lactose is used in processed foods and pharmaceutical products.

Casein. Casein is the principal protein of milk, accounting for 2.5 to 4% of the weight. It possesses unique functional properties that enable its use as an ingredient in many food products. It can be separated from milk by acidification and removing the whey that is formed. The precipitate left behind, casein, is dried into a powder.

Casein is used to fortify flour, bread, and cereals. Casein also is used for glues and microbiological media. Calcium caseinate is made from pressed casein by rinsing, treating with calcium hydroxide, heating, and mixing followed by spray drying. A product of 2.5 to 3% moisture is obtained. Casein hydrolyzates are produced from dried casein. With appropriate heat treatment and addition of alkalies and enzymes, digestion proceeds. Following pasteurization, evaporation, and spray drying a dried product of 2 to 4% moisture is obtained.

Many nondairy products such as coffee creamers, toppings, and icings utilize caseinates. In addition to fulfilling a nutritional role, the caseinates impart creaminess, firmness, smoothness, and consistency to products. Imitation meats, soups, and the like use caseinates as an extender and to improve moistness and smoothness.

BIBLIOGRAPHY

1. N. P. Wong et al., *Fundamentals of Dairy Chemistry*, 3rd ed., Van Nostrand Reinhold, New York, 1988, p. 21.

2. U.S. Department of Agriculture, *Composition of Foods—Dairy and Egg Products*, USDA Agricultural Handbook No. 8-1, U.S. Government Printing Office, Washington, D.C., 1976.
3. U.S. Food and Drug Administration, *Pasteurized Milk Ordinance*, Publication No. 229, U.S. Department of Health and Human Sciences, Washington, D.C., 1995 edition, 1997 Revision.
4. R. T. Marshall, ed., *Standard Methods for the Examination of Dairy Products*, 16th ed., American Public Health Association, Washington, D.C., 1992.
5. P. Cunniff, ed., *Official Methods of Analysis*, 16th ed., Association of Official Analytical Chemists, Arlington, Va., 1995.
6. *Code of Federal Regulations*, Parts 100–169, Office of the Federal Register, National Archives and Records Administration, Washington, D.C., 1998.

GENERAL REFERENCES

References 1, 4, and 5 are good general references. Also refer to issues of *Dairy Science Abstracts* and *Food Science and Technology Abstracts* (England).

F. W. Bodyfelt, J. Tobias, and G. M. Trout, *The Sensory Evaluation of Dairy Products*, 3rd ed., Van Nostrand Reinhold, New York, 1988.

J. G. Brennan et al., *Food Engineering Operations*, 2nd ed., Applied Science Publishers, London, United Kingdom, 1976.

G. Bylund, *Dairy Processing Handbook*, Lund, Sweden, 1995.

J. R. Campbell and R. T. Marshall, *The Science of Providing Milk for Man*, McGraw-Hill, New York, 1975.

A. W. Farrall, *Engineering for Dairy and Food Products*, John Wiley & Sons, New York, 1963.

P. F. Fox, ed., *Advanced Dairy Chemistry, 1. Proteins, 2. Lipids, 3. Lactose, Water, Salts and Vitamins*, Elsevier Applied Science, New York, 1992–1996.

C. W. Hall, A. W. Farrall, and A. L. Rippen, *Encyclopedia of Food Engineering*, AVI, Westport, Conn., 1971.

C. W. Hall and T. I. Hedrick, *Drying of Milk and Milk Products*, 2nd ed., AVI, Westport, Conn., 1971.

W. J. Harper and C. W. Hall, eds., *Dairy Technology and Engineering*. AVI, Westport, Conn., 1976.

J. L. Henderson, *The Fluid Milk Industry*, 3rd ed., AVI, Westport, Conn., 1971.

H. G. Kessler, *Food Engineering and Dairy Technology*, Verlag A. Kessler, Freising, West Germany, 1981.

F. V. Kosikowski and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed., Vol. I and II, F. V. Kosikowski, L.L.C., Westport, Conn., 1997.

M. Robinson, *Concentrated and Dried Milk Products (Evaporation) (Drying)*, Victorian College of Agriculture and Horticulture, Victoria, Australia, 1993.

P. Walstra and R. Jenness, *Dairy Chemistry and Physics*, John Wiley & Sons, New York, 1984.

JOHN G. PARSONS
ROBERT J. BAER
VIKRAM V. MISTRY
South Dakota State University
Brookings, South Dakota

See also CULTURED MILK PRODUCTS.

MINERALS: MICRONUTRIENTS

The minerals as nutrients are divided into two groups. Calcium, phosphorus, sodium, chloride, and magnesium comprise the macromineral group. The micromineral group is subdivided into a trace mineral group and an ultra trace mineral group. Iron, copper, and zinc comprise the former group and chromium, manganese, fluoride, iodide, cobalt, selenium, silicon, arsenic, boron, vanadium, nickel, cadmium, lithium, lead, and molybdenum comprise the latter. The basis for this subdivision lies with the magnitude of need. Whereas the human body needs the trace minerals in milligram amounts, the ultra trace minerals are needed in microgram (or less) amounts. For many of the microminerals the evidence of their essentiality is meager for humans, and yet reports exist on their essentiality to other species. Of the 18 microminerals thought to be essential, only four have been studied sufficiently such that a database exists to support a recommendation for a daily recommended dietary allowance (RDA). Table 1 gives the RDAs for these minerals (1). An additional five minerals have been studied sufficiently such that an intake recommendation that is generally recognized as safe and ade-

Table 1. Recommended Dietary Allowances (RDAs) for Iron, Zinc, Iodine, and Selenium

	RDA			
Age	Iron (mg)	Zinc (mg)	Iodine (mg)	Selenium (μg)
		Infants		
0–6 months	6	5	40	10
7–12 months	10	5	50	15
		Children		
1–3 years	10	10	70	20
4–7 years	10	10	90	20
8–11 years	10	10	120	30
		Males		
12–14 years	12	15	150	40
15–18 years	12	15	150	50
19–24 years	10	15	150	70
25–50 years	10	15	150	70
51+ years	10	15	150	70
		Females		
12–14 years	15	12	150	45
15–18 years	15	12	150	50
19–24 years	15	12	150	55
25–50 years	15	12	150	55
51+ years	10	12	150	55
		Pregnancy		
	30	15	175	65
		Lactation		
0–6 months	15	19	200	75
7–12 months	15	16	200	75

quate has been made. These are listed in Table 2. This figure is based on data extrapolated from animal studies and on data collected from human population studies. These studies of healthy humans may have included analysis of the food consumed as part of a regular diet and, where possible, tissue analysis. Blood values, especially those indicators of anemia; hair values; urine analysis; and fecal analysis may have been included as items in an epidemiological study. Although hair and blood analysis can reveal mineral nutrition status, these analyses are not without problems. The difficulty in using these analyses is that the mineral content can be transient—that is, blood levels of trace minerals represent the immediate mineral intake rather than long-term exposure, and hair mineral content can represent not only food or drink minerals but also airborne minerals. Hair minerals can be contaminated by shampoos and hair treatments such as coloring, bleaching, curling agents, and conditioning agents.

MICROMINERAL TOXICITY

Many of the microminerals are toxic when consumed in large amounts (2). Inadvertent exposure to a variety of minerals, whether via inhalation, absorption through the skin, or ingestion through food or drink, can elicit a toxic response. The gastrointestinal system offers the first line of defense against ingested excess mineral: vomiting and diarrhea. Through vomiting, contaminated food is expelled. Through diarrhea, excretion of excess consumed mineral is facilitated. This reduces the time that the intestinal tract is exposed to the mineral and thus reduces its subsequent uptake. The defense against excess exposure also involves the kidneys and the bone as well as the bilary tract. The kidney tubules will attempt to reduce the body load by increasing urinary excretion. Some minerals, however, namely, copper, iron, zinc, and lead, are not as subject to renal filtration as are other minerals, namely, magnesium, selenium, sodium, potassium, iodine, calcium, and molybdenum. Those minerals subject to renal filtration will appear in the urine; those that are not filtered out are excreted via the bile into the intestinal tract. When coupled with diarrhea, this provides a stimulated loss pathway. Last, reduction of the circulating toxic load of a micromineral is accomplished via deposit of the excess mineral in the bones. Bone mineral content has been used to document cases of suspected toxicity. Accidental or intentional poisoning can sometimes be masked by other nonspecific symptoms, but bone analysis can provide the documentation needed to support or deny a supposition of toxicity.

Table 2. Generally Recognized Safe and Adequate Intakes for Selected Minerals

Mineral	Intake
Copper	1.5–3.0 mg/day
Fluoride	1.4–4.0 mg/day
Manganese	2.0–5.0 mg/day
Chromium	50–200 μg/day
Molybdenum	75–250 μg/day

Note: These ranges are not age or gender specific.

The adverse effects of excess micromineral exposure are as diverse as the minerals themselves. Table 3 lists the characteristic signs and symptoms of micromineral toxicity as well as deficiency. Each mineral has its preferred target in the body. For some, the target is DNA (3). Certain minerals (copper, arsenic, nickel, chromium) bind to DNA in a cross-link fashion. The binding is covalent and is independent of a DNA binding protein. In this setting the excess mineral produces either a nonfunctional DNA or a DNA that cannot repair itself. Evidence of this cross-linking has been demonstrated *in vitro* using a variety of cell types. Chinese hamster cells have been used to show copper-induced, chromium-induced, and nickel-induced DNA cross-linking, and human fibroblasts and epithelial cells have been used to show arsenic-induced cross-linking.

The concept of chemically induced cross-linkage of DNA as a factor in carcinogenesis has been proposed to explain the role of asbestos and the development of mesothelioma (4). Mesothelioma is a malignant growth of the pleural and peritoneal cavities and develops as a response to the inhalation of asbestos fibers (pleural growth) or inadvertent ingestion (peritoneal growth) of the fibers. Asbestosis, another symptom of asbestos inhalation, results in impaired pulmonary function. This is due in part to iron-catalyzed free-radical damage to the lung tissue because asbestos contains iron as a contaminant. However, cytokines, growth factors, and proteases as well as effects of asbestos on gene expression are also involved. Malignant tumors are stimulated to grow by the presence of asbestos fibers that act as artificial linkers of DNA, resulting in mutations within the pleural and peritoneal cells (4). Changes in the CDKN2 gene that encodes protein 16 seem to be involved. This gene is either lost or mutated. Its gene product is a regulator of the phosphorylation of protein 105, a tumor suppressor. Unphosphorylated protein 105 can inhibit passage from the G1 to the S phase of the cell cycle, whereas phosphorylated protein 105 permits this passage. Passage inhibition is a common feature of cancer cell initiation. Any substance that interferes with this passage could be regarded as a carcinogen. Minerals in excess quantities can have this effect, and excess intakes of some have been linked with certain forms of cancer.

Excess exposure to certain minerals can invoke the stimulation of free-radical generation. Free radicals can damage membranes, altering their function, and can target numerous intracellular constituents (DNA, enzymes, transporters, signaling systems). Free-radical generation may be one of the responses to excess iron exposure and this in turn may link this mineral to cancer development (5). The mechanism whereby excess iron has its effect is far from clear. It may induce DNA cross-linking as described previously, but it may also act to stimulate free-radical formation. Free radicals can damage cell membranes and cell constituents as well as DNA. Free-radical attack of DNA could cause mutations and could explain an association between excess iron intake and cancer (6). At this time, more data are needed to support or deny such associations. Excess mineral intake either as a single mineral or as a mixture can have effects on metabolism as well

Table 3. Characteristic Signs of Micromineral Deficiency and Toxicity

Mineral	Deficiency	Toxicity
Iron	Anemia, ↓ amounts of hemoglobin, ferritin	Bloody diarrhea, vomiting occasionally, liver failure, hemorrhage, metabolic acidosis, shock
Zinc	Poor growth and sexual maturation, anemia, enlarged liver and spleen, rough skin, lethargy	Nausea, vomiting, epigastric pain, abdominal cramps, diarrhea; central nervous system deficits, copper deficiency
Fluoride	Excess tooth decay	Fluorosis; mottling of teeth
Copper	Rare; anemia, poor wound healing, muscle weakness; lethargy, depressed collagen synthesis	Rare
Iodine	Goiter (underproduction of thyroxine)	None
Manganese	Abnormal bone and connective tissue growth, decreased Mn superoxide dismutase	Rare
Molybdenum	No clear-cut symptoms	Rare; interferes with copper use
Selenium	Decreased glutathione peroxidase activity, fragile red cells; enlarged heart, skeletal muscle degeneration	Neuromuscular defects, trace mineral imbalance, liver and muscle damage

as on cellular respiration. Where the mineral is divalent (2^+), it can interfere with the actions of other similarly charged ions. Within the normal range of intakes, mineral–mineral interactions are common. However, when these ranges are exceeded, specific metabolic processes will be inhibited.

MICROMINERAL DEFICIENCY

Rarely are humans deficient in only one mineral. Usually the deficiency involves more than one, and these combined deficiencies have cumulative effects on health. As noted in Tables 3 and 4, micromineral interactions are common. Anemia usually due to iron deficiency can also be due to intake deficiency of zinc, copper, or both. Poor growth is typical of micromineral deficiency, either singly or collectively. Humans consuming a wide variety of foods therefore run scant risk of micronutrient deficiency. The inclusion of red meat, dairy foods, seafoods, and fresh fruits and vegetables should provide sufficient supplies of the microminerals to the average healthy adult. Of course, special health considerations, such as growth, pregnancy, excess blood loss, and the like, may call for adjustments in micromineral intake, and these adjustments might call for an oral supplement.

ANTAGONISMS AND INTERACTIONS AMONG TRACE MINERALS

No general discussion of trace minerals would be complete without mention of mineral interactions. Numerous antagonisms and synergisms have been reported. This should be expected, because many of the trace minerals have more than one charged state and living cells have preferences with respect to these states. For example, the uptake of iron is much greater when the iron is in the ferrous (2^+) state than when in the ferric (3^+) state. Minerals that keep iron in the ferric state will interfere with its absorption and use. Minerals that do the reverse will enhance iron uptake. Such is the beneficial action of copper on iron. The copper ion (Cu^{2+}) keeps the ferrous ion from losing electrons. Thus, copper prevents the conversion of the ferrous to ferric ion. The interactions of essential minerals are best illustrated in Figure 1 and listed in Table 4. Some of these interactions have important therapeutic value in

Table 4. Micronutrient Interactions

	Calcium	Phosphorus	Potassium	Sodium	Magnesium	Zinc	Iron	Copper	Iodine	Fluorine
Calcium	X	↑a	↓a	↓a↑m	↑m↓a					
Phosphorus	↑a	X	↑m	↑m	↓a					
Potassium	↑↓m	↑a	X	↑a	↓a, ↑m					
Sodium	↑↓m	↑a		X	↓a,↑m					
Magnesium	↓a	↑m		↓a,↑m	X				↑m	
Zinc						X		↓a, ↑m		
Iron	↑m	↓a				↓a	X	↓a, ↑m		
Copper						↓a	↓a,↑m	X		
Iodine									X	
Fluorine	↓a									X
Cobalt							↓a			
Chromium						↓a				
Manganese					↓a		↓a			
Molybdenum							↑m	↓a		
Selenium								↑m		

Note: ↑ = increase; ↓ = decrease; a = absroption; m = metabolism.

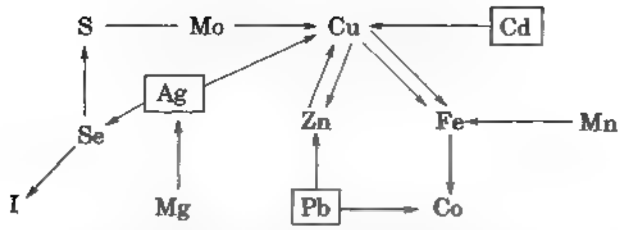


Figure 1. Toxicity and trace mineral interactions. Minerals that are important environmental toxins are in boxes. Abbreviations used: S, sulfur; Se, selenium; I, iodide; Ag, mercury; Mg, magnesium; Zn, zinc; Cu, copper; Pb, lead; Co, cobalt; Fe, iron; Mn, manganese; Cd, cadmium; Mo, molybdenum.

the treatment of inadvertent and toxic exposure to a single mineral. For example, lead toxicity can be minimized by consumption of a diet rich in calcium and phosphorus. The transport proteins of the enterocytes prefer calcium to lead. If the enterocytes are saturated with calcium, the lead will not be absorbed and will pass out of the body in the feces. A similar situation exists with iron and lead. Mineral interactions need to be recognized in the manufacture of special diets. Sometimes, so-called purified ingredients contain mineral contaminants. This is especially a problem in the protein portion of the diet. Proteins can bind minerals, and these minerals can be found in the food proteins. Soybean protein can contain phytate-bound phosphorus and magnesium; casein and lactalbumin, depending on origin, contain variable amounts of calcium, magnesium, and selenium. Unless the investigator determines the mineral content of the diet ingredients, an unexpected mineral imbalance could potentially occur, and this imbalance could affect the nutritional value of the product.

MINERAL ABSORPTION

Of all the elements mammals need, few enter the absorptive cell by passive diffusion. Several are carried into the body by proteins to which the minerals are loosely attached. Iron, zinc, and copper are imported via these transporters. Iodide and fluoride enter by way of an anion–cation exchange mechanism. Few of the minerals are 100% absorbable. For the others, the absorption process is dependent on a number of factors: the binding capacity of the transport protein (if needed); solubility; the composition of the diet; the mixture of elements present in the gut contents; and the presence of materials such as phytate and ethylenediaminetetraacetic acid (EDTA), which bind specific elements, thus changing the mineral mixture presented to the enterocyte. All of these factors contribute to the bioavailability of the essential micronutrients. In addition, physiological factors (eg, age, hormonal status, and health status) also influence absorption and subsequent use. Those minerals that are variable in terms of their absorption are those that are either divalent or multivalent ions (ie, ions with more than one charged state). Iron and chromium are multivalent ions whereas selenium, manganese, zinc, and molybdenum are divalent.

BIOAVAILABILITY

One concept that nutritionists have developed relates not only to absorption efficiency but also to mineral interactions at the site for absorption and the site of use. This concept is that of bioavailability (5). Bioavailability is defined as the percentage of the consumed mineral that enters via the intestinal absorptive cell, the enterocyte, and is used for its intended purpose. Thus, bioavailability includes not only how much of a consumed mineral enters the body but also how much is retained and available for use. An example might be the comparison of iron from red meat to the same amount of iron from spinach. Iron from red meat has a greater bioavailability than iron from spinach because it is an integral component of the protein heme. It is this form (heme iron) that is efficiently absorbed and used. The iron in the spinach is bound to an oxalate, and even though some of this iron can be released from the oxalate, it is in the ferric state and poorly absorbed.

APPARENT ABSORPTION

Another term referring to absorption is frequently used: apparent absorption. This term refers to the difference between the amount of mineral consumed and the amount that appears in the feces. Some minerals are recirculated via the bile whereas others are not. This recirculation, especially in a poorly absorbed mineral, can contribute to the mineral content of the feces, but there is no correction for the bilary contribution to the fecal mineral content. The term apparent absorption refers *only* to the difference between intake and fecal excretion.

MINERAL UPTAKE AND TRANSPORT

Because of their ionic nature, minerals can form electrovalent bonds to a variety of substances. Although ingested as salts, minerals ionize to their component parts, and it is the resultant ions that are absorbed, used, stored, or excreted. If absorbed, many of the microminerals are retained because there is little excretion. As ions, minerals react with charged amino acid residues of intact proteins and peptides. Table 5 provides a list of minerals and the amino acids with which they react. Depending on their valence state, these mineral–amino acid bonds can be very strong, moderate, or very weak associations.

Many minerals are carried in the blood by specific transport proteins. The reasons for this are not fully known. However, we do know that there are preferred ligand bonding groups as shown in Table 6. Other features of these

Table 5. Mineral–Amino Acid Interactions

Minerals	Amino acid
Copper	Histidine
Selenium	Methionine, cysteine
Zinc	Cysteine, histidine
Iron	Histidine

Table 6. Preferred Ligand Binding Groups for the Microminerals

Metal	Ligand groups
Mn^{2+}	Carboxylase; phosphate and nitrogen donors
Fe^{2+}	-SH, NH^2 > carboxylates
Fe^{3+}	Carboxylate, tyrosine, $-NH_2$, porphyrin (a nitrogen donor)
Co^{3+}	Similar to Fe^{3+} but is usually chelated by the NH groups as in vitamin B_{12}
Cu^+	-SH (cysteine)
Cu^{2+}	Amines ≫ carboxylates
Zn^{2+}	Imidazole, cysteine
Mo^{2+}	-SH
Cd^{2+}	-SH

transport proteins (eg, size, shape, amino acid sequence, tertiary and quatenary structures) may also determine specificity. As yet we lack this information for each of the specific proteins that bind the microminerals. In some instances a transport protein will carry more than one ion. An example is metallothionein, which will carry both zinc and copper. It will also carry some of the heavy metals, but its affinity for zinc and copper is greater than its affinity for heavy metals such as lead.

Many of the microminerals can be chelated by organic materials. Chelation is a common phenomenon in biological systems, and this process has many clinical applications. For example, EDTA is a potent chelator and is used to remove lead or other heavy metals from the body. It will also chelate calcium and magnesium, so the clinician using EDTA to treat lead overload will have to be aware of this feature as well. Penicillamine is another chelator of importance. It is used to remove excess copper, for example, in patients with genetically inherited Wilson's disease. 2,3-D-Dimercaptopropanol-1 is a chelator of lead, mercury, arsenic, copper, cadmium, tin, and other toxic metals. It solubilizes these metals and chelates them, allowing for their excretion in the urine. Chelation is important to the biological function of several minerals. Examples are the chelation of cobalt within the structure of vitamin B_{12} and the chelation of iron within the structure of hemoglobin, as shown in Figure 2. In the B_{12} structure, chelation is a function of the many nitrogen molecules within this very large molecule.

FUNCTIONS OF MICROMINERALS

The microminerals are widely dispersed in the living body. They serve a variety of functions, as summarized in Table 7, and most of these minerals can be found in trace amounts in the skeletal system. The bones and teeth are primarily calcium phosphate salts, yet within the bone matrix other minerals can be found as well. Some of the microminerals have only one function, for example, cobalt in vitamin B_{12} and iodide in thyroxine, but others serve at multiple levels, from influencing gene expression to serving as cofactors in enzymatic reactions to contributing to the stability and hardness of bone.

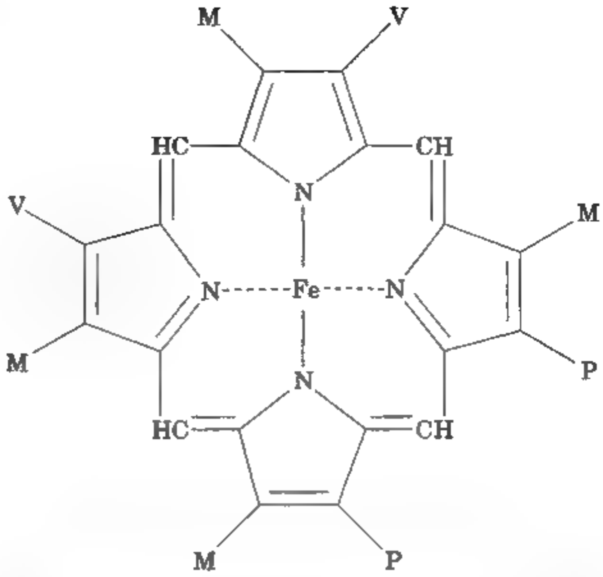


Figure 2. Heme as a chelator of iron. Abbreviations used: A, acetate (CH_2-COO_3H); M, methyl ($-CH_3$); P, proprionate ($-CH_2$-CH_2-COOH); V, vinyl ($-R-CH{=}CH_2$). These are the residues of the amino acids that project out from the heme protein structure.

Table 7. Microminerals and Their Functions

Mineral[a]	Function
Iron	Essential component of iron–sufur centers that function in redox reactions as well as in the cytochromes and hemoglobin; serves in the expression of the genes for the metallothioneins, ferritin and the transferrin receptor (7–9).
Zinc	Cofactor in more than 100 enzymatic reactions. Essential component of the zinc fingers that characterize many of the nuclear DNA-binding proteins. Serves in the expression of genes for metallothionein; has a vital role in apoptosis (7, 10–13).
Copper	Essential cofactor in several reactions concerning iron use, collagen synthesis, and free-radical suppression. Serves in the expression of the genes for metallothionein superoxide dismutase, and several mitochondrial enzymes (14–17).
Cobalt	Essential to the structure of vitamin B_{12}.
Iodine	Essential for thyroid hormone synthesis (18).
Manganese	Essential cofactor in many enzymatic reactions, especially those using ATP or UTP
Molybdenum	Activates adenylate cyclase; cofactor in sulfite oxidase and xanthine oxidase (19).
Selenium	Essential to glutathione peroxidase and thyroxine deiodinase. Serves in the expression of the gene for glutathione peroxidase (20,21)
Fluorine	Increases hardness of teeth

[a] Arsenic, chromium, nickel, silicon, and vanadium appear to be essential for optimal growth and metabolism of several animal species, but their essentiality and function in humans has yet to be documented.

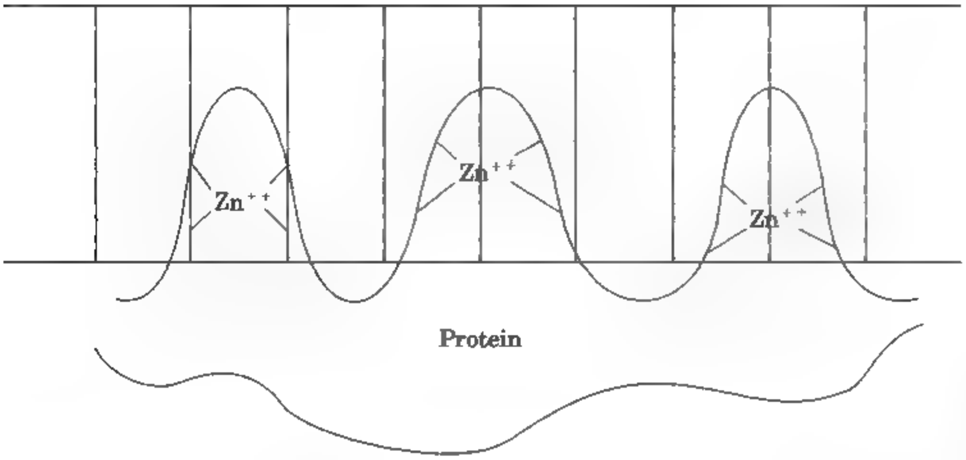


Figure 3. Zinc bound to cysteine and histidine residues from fingerlike projections that bind to DNA at specific regions.

MINERALS AND GENE EXPRESSION

As noted in Table 7, several of the microminerals influence the expression of genes encoding one or more important proteins (7–21). Doubtless we will discover many more of these gene products that require one or more of the microminerals as part of the regulation of the transcription and translation of their cognate genes. Perhaps the mineral that has been studied the most is zinc because it is part of a group of DNA-binding proteins called transcription factors. In this role, zinc is bound by the histidine and cysteine residues of these binding proteins, giving these regions fingerlike projections that in turn bind to DNA at specific regions. These are illustrated in Figure 3. These proteins are called zinc finger proteins and the zinc-bound regions, zinc fingers. A number of nutrients, namely, vitamin A and vitamin D, and hormones, namely, steroids, insulinlike growth factor I, growth hormone, and others, have their effects on the expression of specific genes because they can bind to very specific proteins that contain zinc fingers that in turn bind to very specific DNA sequences.

If there is a mutation in the genes that encode these DNA-binding proteins such that they lack the requisite two residues each of histidine and cysteine in the linear part of their structure, then the functional attributes of vitamins and hormones requiring these proteins at the genetic level will be ablated. Instances of such mutations have been published, as have instances where these zinc fingers have been purposefully modified as a therapeutic approach to disease control. As an example, a zinc-containing transcription factor, Zif268, has been modified such that its sequence-specific recognition by viral DNA results in an ablation of the viral invasion and takeover of specific target cells (22). Although this modification was done *in vitro* and not tested in whole animals, this approach has a potential use in battling viral infections.

Copper, zinc, and iron are all involved in the expression of genes for the metal-binding proteins, the metallothionines. These cysteine-rich proteins transport these divalent ions through the intestinal absorptive cells. Several genes are involved. Each has a metal response element that is specific for each of the minerals. In the case of copper, a copper-containing DNA-binding protein attaches to a specific DNA sequence called the CUP1 element. A zinc-containing DNA-binding protein is also required, and together these proteins stimulate the transcription of the metallothionine genes. The binding sites for these mineral-containing proteins are upstream from the promoter region of the genes, and the minerals by themselves (not incorporated into the DNA-binding proteins) do not directly stimulate transcription. In addition to the metallothionine genes, copper has been found to influence the expression of at least 10 other genes, all of which have a copper response element in their DNA sequence (17). Five of these are mitochondrial sequences, suggesting that copper has a function in mitochondrial gene expression as well as in nuclear gene expression.

Just as zinc, copper, and iron play multiple roles in gene expression, so too does selenium (20,21). Selenium is an essential cofactor to the activity of glutathione peroxidase in multiple forms. Selenium is also essential to the expression of the genes that encode these enzymes. In selenium-deficient animals, glutathione peroxidase messenger RNA falls to undetectable levels, indicating that this mineral functions in the transcription of this messenger RNA. How this occurs has not yet been elucidated, but likely the mechanism of action will be similar to that described earlier for copper.

Table 8. Genetic Diseases in Micromineral Metabolism

Disorder	Characteristics
Hemochromatosis	Excess iron absorption, resulting in damage to pancreas, liver, heart
Menkes disease	Defective intestinal copper absorption
Wilson's disease	Excessive copper accumulation due to impaired incorporation of copper into ceruloplasmin and decreased bilary excretion
Graves' disease	Autoimmune disease characterized by impaired thyroid gland function. Goiter (underproduction of thyroxine) or hyperthyroidism (overproduction of thyroxine) due to thyroid stimulating hormone receptor autoantibodies

Last, genetic errors or mutations in genes that encode specific proteins involved in micromineral metabolism have been described. These are listed in Table 8. These diseases are uncommon, yet their presence has allowed scientists to understand aspects of mineral metabolism previously unapproachable. Through the study of humans with Wilson's disease and Menkes disease, for example, an understanding of copper absorption and turnover was gained. Similar insights into the role of iron in the heme structure as well as iron–sulfur centers were gained through the study of hemochromatosis.

BIBLIOGRAPHY

1. Food and Nutrition Board, National Academy Press, Washington, DC, 1989.
2. D. Medeiros, R. Wildman, and R. Liebes, "Metal Metabolism and Toxicities," in *Handbook of Human Toxicology*, CRC Press, Boca Raton, Fla. 1997
3. D. J. Paustenbach, B. L. Finley, and S. Kacew, "Biological Relevance and Consequences of Chemical or Metal Induced Cross Linking," *Proc. Soc. Exp. Biol. Med.* **211**, 211–217 (1996).
4. D. W. Kamp and S. A. Weitzman, "Asbestosis: Clinical Spectrum and Pathogenic Mechanisms," *Proc. Soc. Exp. Biol. Med.* **214**, 12–26 (1997).
5. B. Fairweather-Tait, "Bioavailability of Trace Elements," *Food Chem.* **43**, 213–217 (1992).
6. R. Yip, D. F. Williamson, and T. Byer, "Is There an Association between Iron Nutrition Status and the Risk of Cancer?" *Am. J. Clin. Nutr.* **53**, 30–34 (1991)
7. C. D. Berdanier and J. L. Hargrove, *Nutrition and Gene Expression*, CRC Press, Boca Raton, Fla., 1993.
8. O. S. Chen, L. Schalinske, and R. S. Eisenstein, "Dietary Iron Intake Modulates the Activity of Iron Regulatory Proteins and the Abundance of Ferritin and Mitochondrial Aconitase in Rat Liver," *J. Nutr.* **127**, 238–248 (1997).
9. G. T. Gassner et al., "Structure and Mechanism of the Iron-Sulfur Flavoprotein Phthalate Dioxygenase Reductase," *FASEB J.* **9**, 1411–1418 (1995).
10. S. R. Davis, R. J. McMahon, and R. J. Cousins, "Metallothionein Knockout and Transgenic Mice Exhibit Altered Intestinal Processing of Zinc with Uniform Zinc Dependent Zinc Transporter-1 Expression," *J. Nutr.* **128**, 825–831 (1998).
11. P. J. Fraker and W. G. Telford, "A Reappraisal of the Role of Zinc in Life and Death Decisions of Cells," *Proc. Soc Exp. Biol. Med.* **215**, 229–236 (1997).
12. N. P. Koritschoner et al., "A Novel Human Zinc Finger Protein That Interacts with the Core Promoter Element of a TATA Box-less Gene," *J. Biol. Chem.* **272**, 9573–9580 (1997).
13. R. T. Nolte et al., "Differing Roles for Zinc Fingers in DNA Recognition: Structure of a Six-Finger Transcription Factor IIIA Complex," *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2938–2943 (1998).
14. V. C Culotta et al , "The Copper Chaperone for Superoxide Dismutase," *J. Biol. Chem.* **272**, 23469–23472 (1997).
15. P. Wang et al., "Overexpression of Human Copper, Zinc-Superoxide Dismutase (SOD1) Prevents Post Isechemic Injury," *Proc. Natl. Acad. Sci U.S.A.* **95**, 4556–4560 (1998).
16. Y. Chen, J. T. Saari, and Y. J. Kang, "Copper Deficiency Increases Metallothionein mRNA Content Selectively in Rat Liver," *J. Nutritional Biochem.* **6**, 572–576 (1995).
17. Y. R. Wang et al., "Enhanced Expression of Hepatic Genes in Copper Deficient Rats Detected by the Messenger RNA Differential Display Method," *J. Nutr.* **126**, 1772–1781 (1996)
18. S. Boyages, "Iodine Deficiency Disorders," *J. Clin. Endocrinol. Metab.* **77**, 587–591 (1993)
19. K. V. Rajagopalan, J. L. Johnson, and B. E Hainline, "The Pterin of the Molybdenum Co-Factor," *Fed. Proc.* **41**, 2608–2612 (1982).
20. S. Weiss and R. A. Sunde, "Selenium Regulation of Classical Glutathione Peroxidase Expression Requires the 3^1 Untranslated Region in Chinese Hampster Ovary Cells," *J Nutr.* **127**, 1304–1310 (1997).
21. W-H. Cheng et al., "Cellular Glutathione Peroxidase Knockout Mice Express Normal Levels of Selenium-Dependent Plasma and Phospholipid Hydroperoxide Glutathione Peroxidases in Various Tissues," *J. Nutr.* **127**, 1445–1450 (1997).
22. H. Wu, W-P. Yang, and C. F. Barbas, "Building Zinc Fingers by Selection: Toward a Therapeutic Application," *Proc. Natl. Acad. Sci. U S.A.* **92**, 344–348 (1995).

CAROLYN BERDANIER
University of Georgia
Athens, Georgia

MOLECULAR MODELING: COMPUTER MODELING OF FUNCTIONAL PROPERTIES

One of the principal goals of food science and technology is to be able to modify the measurable functional properties of a food by specific changes to the structures of the component molecules. Examples of this type of manipulation of molecular properties include direct chemical modification of polymers, as in modified starches, and the preparation of mutant proteins through site-directed mutagenesis (1). Although this goal of control of functional properties can be occasionally achieved as the serendipitous result of random experimentation, in principle, successful design of novel functional properties requires an understanding of the way in which these functional properties depend on molecular structure.

In general, such an understanding involves a detailed knowledge of the atomic and molecular-level interactions responsible for macroscopic bulk properties. Unfortunately, this knowledge is often unavailable, because few experimental techniques can directly probe chemical processes in the necessary detail. Even in those cases where experiment can directly examine molecular structure, such as X-ray diffraction experiments, it generally remains unclear how the molecular architecture functions or how it might be modified to successfully alter its workings. Fortunately, recent developments in theoretical chemistry now allow molecular events to be directly simulated on fast computers. These developments not only permit us to learn how biopolymer systems function but also present the possibility of modeling numerically the effects of changes in a polymer structure, such as a series of active site mutations in a protein, without the time and expense of actually producing each of these mutants in the laboratory. Experimental studies can thus be made more efficient by only making those mutants that the simulations suggest might

Last, genetic errors or mutations in genes that encode specific proteins involved in micromineral metabolism have been described. These are listed in Table 8. These diseases are uncommon, yet their presence has allowed scientists to understand aspects of mineral metabolism previously unapproachable. Through the study of humans with Wilson's disease and Menkes disease, for example, an understanding of copper absorption and turnover was gained. Similar insights into the role of iron in the heme structure as well as iron–sulfur centers were gained through the study of hemochromatosis.

BIBLIOGRAPHY

1. Food and Nutrition Board, National Academy Press, Washington, DC, 1989.
2. D. Medeiros, R. Wildman, and R. Liebes, "Metal Metabolism and Toxicities," in *Handbook of Human Toxicology*, CRC Press, Boca Raton, Fla. 1997
3. D. J. Paustenbach, B. L. Finley, and S. Kacew, "Biological Relevance and Consequences of Chemical or Metal Induced Cross Linking," *Proc. Soc. Exp. Biol. Med.* **211**, 211–217 (1996).
4. D. W. Kamp and S. A. Weitzman, "Asbestosis: Clinical Spectrum and Pathogenic Mechanisms," *Proc. Soc. Exp. Biol. Med.* **214**, 12–26 (1997).
5. B. Fairweather-Tait, "Bioavailability of Trace Elements," *Food Chem.* **43**, 213–217 (1992).
6. R. Yip, D. F. Williamson, and T. Byer, "Is There an Association between Iron Nutrition Status and the Risk of Cancer?" *Am. J. Clin. Nutr.* **53**, 30–34 (1991)
7. C. D. Berdanier and J. L. Hargrove, *Nutrition and Gene Expression*, CRC Press, Boca Raton, Fla., 1993.
8. O. S. Chen, L. Schalinske, and R. S. Eisenstein, "Dietary Iron Intake Modulates the Activity of Iron Regulatory Proteins and the Abundance of Ferritin and Mitochondrial Aconitase in Rat Liver," *J. Nutr.* **127**, 238–248 (1997).
9. G. T. Gassner et al., "Structure and Mechanism of the Iron-Sulfur Flavoprotein Phthalate Dioxygenase Reductase," *FASEB J.* **9**, 1411–1418 (1995).
10. S. R. Davis, R. J. McMahon, and R. J. Cousins, "Metallothionein Knockout and Transgenic Mice Exhibit Altered Intestinal Processing of Zinc with Uniform Zinc Dependent Zinc Transporter-1 Expression," *J. Nutr.* **128**, 825–831 (1998).
11. P. J. Fraker and W. G. Telford, "A Reappraisal of the Role of Zinc in Life and Death Decisions of Cells," *Proc. Soc Exp. Biol. Med.* **215**, 229–236 (1997).
12. N. P. Koritschoner et al., "A Novel Human Zinc Finger Protein That Interacts with the Core Promoter Element of a TATA Box-less Gene," *J. Biol. Chem.* **272**, 9573–9580 (1997).
13. R. T. Nolte et al., "Differing Roles for Zinc Fingers in DNA Recognition: Structure of a Six-Finger Transcription Factor IIIA Complex," *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2938–2943 (1998).
14. V. C Culotta et al , "The Copper Chaperone for Superoxide Dismutase," *J. Biol. Chem.* **272**, 23469–23472 (1997).
15. P. Wang et al., "Overexpression of Human Copper, Zinc-Superoxide Dismutase (SOD1) Prevents Post Isechemic Injury," *Proc. Natl. Acad. Sci U.S.A.* **95**, 4556–4560 (1998).
16. Y. Chen, J. T. Saari, and Y. J. Kang, "Copper Deficiency Increases Metallothionein mRNA Content Selectively in Rat Liver," *J. Nutritional Biochem.* **6**, 572–576 (1995).
17. Y. R. Wang et al., "Enhanced Expression of Hepatic Genes in Copper Deficient Rats Detected by the Messenger RNA Differential Display Method," *J. Nutr.* **126**, 1772–1781 (1996)
18. S. Boyages, "Iodine Deficiency Disorders," *J. Clin. Endocrinol. Metab.* **77**, 587–591 (1993)
19. K. V. Rajagopalan, J. L. Johnson, and B. E Hainline, "The Pterin of the Molybdenum Co-Factor," *Fed. Proc.* **41**, 2608–2612 (1982).
20. S. Weiss and R. A. Sunde, "Selenium Regulation of Classical Glutathione Peroxidase Expression Requires the 3^1 Untranslated Region in Chinese Hampster Ovary Cells," *J Nutr.* **127**, 1304–1310 (1997).
21. W-H. Cheng et al., "Cellular Glutathione Peroxidase Knockout Mice Express Normal Levels of Selenium-Dependent Plasma and Phospholipid Hydroperoxide Glutathione Peroxidases in Various Tissues," *J. Nutr.* **127**, 1445–1450 (1997).
22. H. Wu, W-P. Yang, and C. F. Barbas, "Building Zinc Fingers by Selection: Toward a Therapeutic Application," *Proc. Natl. Acad. Sci. U S.A.* **92**, 344–348 (1995).

CAROLYN BERDANIER
University of Georgia
Athens, Georgia

MOLECULAR MODELING: COMPUTER MODELING OF FUNCTIONAL PROPERTIES

One of the principal goals of food science and technology is to be able to modify the measurable functional properties of a food by specific changes to the structures of the component molecules. Examples of this type of manipulation of molecular properties include direct chemical modification of polymers, as in modified starches, and the preparation of mutant proteins through site-directed mutagenesis (1). Although this goal of control of functional properties can be occasionally achieved as the serendipitous result of random experimentation, in principle, successful design of novel functional properties requires an understanding of the way in which these functional properties depend on molecular structure.

In general, such an understanding involves a detailed knowledge of the atomic and molecular-level interactions responsible for macroscopic bulk properties. Unfortunately, this knowledge is often unavailable, because few experimental techniques can directly probe chemical processes in the necessary detail. Even in those cases where experiment can directly examine molecular structure, such as X-ray diffraction experiments, it generally remains unclear how the molecular architecture functions or how it might be modified to successfully alter its workings. Fortunately, recent developments in theoretical chemistry now allow molecular events to be directly simulated on fast computers. These developments not only permit us to learn how biopolymer systems function but also present the possibility of modeling numerically the effects of changes in a polymer structure, such as a series of active site mutations in a protein, without the time and expense of actually producing each of these mutants in the laboratory. Experimental studies can thus be made more efficient by only making those mutants that the simulations suggest might actually possess the desired functional properties. This article provides an introduction to these theoretical simulation methods and their application to biopolymers of the types important in the physical properties of foods.

COMPUTER MODELING OF BIOPOLYMERS

The observable physical properties of any molecule are ultimately the result of the physical laws that determine the behavior of individual molecules and atoms. At the most fundamental level, of course, the properties of molecules are governed by quantum mechanics, and modeling the behavior of molecules numerically requires the direct solution of Schrödinger's equation for all of the nuclear and electronic degrees of freedom for all molecules in the system. Unfortunately, it is not practical to solve this time-dependent equation at the necessary level of accuracy for molecules containing more than a few heavy (nonhydrogen) atoms. It is thus not possible to make a direct quantum mechanical simulation of large and complex systems such as the mixtures of proteins, fats, water, salts, and other components that make up a typical food. However, in many cases, part of the solution of the quantum mechanical problem is known by other means. Typically, important features of the molecular structure, such as equilibrium bond lengths and angles, are known approximately from studies of less complicated molecules, and the primary challenge is to understand the conformational behavior of polymers, how different molecules interact, and the connection between basic structure and macroscopic properties. The link between basic structure and bulk properties is statistical mechanics, and the most common theoretical studies of biopolymer physical properties are various attempts to model this statistical mechanical connection.

Theoretical calculations that exploit a knowledge of molecular energies as a function of nuclear coordinates are called molecular mechanics calculations (2–4). Because it is impossible to accurately solve Schrödinger's equation for large polymer systems, it is common in theoretical simulations to approximate the quantum mechanical energy with continuous energy functions that have approximately the same behavior. By invoking the Born-Oppenheimer approximation (3), which says that the electronic motions of a molecule are far faster than the nuclear motions, it is possible to separate the inherently quantal problem of the electronic motions from the much more classical problem of the motions of the nuclei, by treating the electronic energy for each value of the nuclear coordinates as a potential energy function that governs the motion of the nuclei. It is then possible to use continuous, analytic functions to approximate this nuclear potential energy function. These semiempirical energy equations have theoretically reasonable functional forms that are parameterized to the results of various experiments and theoretical calculations such that properties calculated using these functions are those found in the experiments. Energy functions of this type usually contain terms to represent bond stretching, bond angle bending, hindered torsional rotations about chemical bonds, and nonbonded and electrostatic interactions. A typical example of such a function of the internal coordinates $\mathbf{q}$ might be

$$V(\mathbf{q}) = \sum k_b(b - b_0)^2 + \sum k_\theta(\theta - \theta_0)^2 + \sum k_\varphi[1 + \cos(n\varphi - \delta)] + \sum_{i<j}\left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} + \frac{q_i q_j}{r_{ij}}\right)$$

where the b's are the various bond lengths, which have equilibrium values b_0, the θ's are the bond angles with equilibrium values θ_0, the φ's are the torsional angles about each bond, and q_i and q_j are the partial atomic charges of each atom, separated by a distance r_{ij}. This equation treats bond stretching and bending as harmonic oscillator terms with force constants k_b and k_θ, and also includes a periodic hindered torsional energy term in the angle φ with the force constant k_φ, periodicity n, and phase δ. van der Waals interactions are treated as a typical 10–12 Lennard-Jones-type interaction, and the electrostatic interactions are represented by Coulomb's law. The various adjustable constants that appear in such equations (k_b, k_θ, k_φ, A_{ij}, B_{ij}, q_i) must be selected with great care in order for the results to be physically reasonable. This parameterization is accomplished by matching experimental properties of small molecules (equlibrium bond lengths and angles, IR and Raman spectral frequencies, etc) to the values calculated using the energy equation as a function of the adjustable parameters (2–4).

Energy Minimization

The most common types of molecular mechanics calculation are conformational energy studies (2–4). Conformational energy calculations involve the search for the molecular geometry with the lowest potential energy, on the assumption that the structure with the lowest mechanical energy (as calculated from the semiempirical energy functions) will be the form primarily found in nature. This search is accomplished using automatic energy minimization algorithms, which minimize the gradient (derivative) of the potential energy. Predictions of the structure-dependent physical properties of the biopolymer system are then based on those calculated from the lowest energy conformation. Unfortunately, the major problem with this approach is that due to the enormous complexity of large systems, it is difficult to identify the lowest energy structure. The problem arises because for large systems, there will, in general, be many local minimum energy geometries that are locally stable, with a zero gradient of the potential energy, but that are higher in energy than the structure with the lowest energy, which is called the global energy minimum. There is no general mathematical procedure for finding this global energy minimum. Most minimization techniques will simply locate the nearest local stable geometry for which the gradient is zero and then be unable to proceed over any energy barriers that separate this local minimum from any lower energy structures. This multiple-minimum problem (5) is the reason that it is not yet possible to predict the tertiary conformation of a protein given its primary sequence, even though in principle the sequence for most proteins contains all of the information necessary for correct folding (see later). Even if the global minimum-energy structure is identified, there are other possible problems with this type of approach. Polymers such as proteins are not static, but actually fluctuate considerably, sampling a number of nearby thermally accessible conformations (6) (which are usually quite similar to the lowest energy structure), and the experimental, or physical, structure is actually an appropriately weighted average of all these structures. Energy minimization studies, however, generally cannot include such conformational averaging, or its entropic effects (7). Perhaps most important, energy minimization calculations cannot directly include the effects of solvation, which can be quite crucial in many biological systems.

In spite of their limitations, energy minimization calculations can be of great utility in studying certain limited questions, such as predicting the preferred binding geometries of substrates in enzyme active sites or the local effects of a single point mutation in a protein. This type of calculation can be especially useful when combined with computer graphics representations of molecular structures, as is now frequently done in the pharmaceutical (8) and food industries in binding studies. Visual inspection of computer-generated three-dimensional color images of interacting molecules such as a target protein and a drug molecule allow researchers to use their chemical intuition and experience to identify promising approximate binding configurations, which can subsequently be refined by the application of energy minimization calculations. In this way, the human-guided input can avoid calculations for many of the unpromising minima that blind energy minimizations would have wasted much computer time exploring needlessly.

Molecular Dynamics Simulations

More theoretically realistic models of the behavior of molecular systems can be generated by recognizing that macroscopic properties are averages over large numbers of fluctuations of the system. Statistical mechanical calculations that explicitly average over these different states will thus give a more physical picture of the behavior of such systems. Among these types of calculations are Monte Carlo calculations (9) and molecular dynamics simulations (3,4,10). Molecular dynamics studies are a type of molecular mechanics calculation in which atomic motions are simulated numerically using a large digital computer. In this type of calculation, the classical Newton's equations of motion are solved numerically for every atom in the system as they respond to the forces arising from the empirical force field. Because the negative gradient, or derivative, of the potential energy function V with respect to the atomic coordinates is equal to the force $\mathbf{F}_i$ along that coordinate acting on the particle,

$$\mathbf{F}_i = -\nabla_i V$$

the derivatives of this analytic function can be used to numerically integrate the coupled Newton's equations of motion (11)

$$\mathbf{F}_i = m_i \mathbf{a}_i$$

to give a complete description of the motion of every atom in the molecule as a function of time, given the initial conditions (initial positions and velocities for each atom). For those molecules for which some plausible structure is known, either from reasonable chemical conjecture or from experiments such as X-ray diffraction from crystals, a random selection of initial atomic velocities from a Boltzmann distribution allows the researcher to produce a theoretical trajectory describing the way in which this molecule would move if it were possible to view it at the atomic level in such detail. Within the limitations imposed by the approximations (such as the use of empirical energy functions), this trajectory contains everything that can be known about the system, and all of its properties can be calculated as the appropriate time average over the trajectory, if it is of sufficient length. This absolute knowledge is the source of the great power of molecular dynamics simulations.

Unfortunately, the main limitation of this method is contained in the caveat that the trajectory must be of sufficient length. The statistical nature of the macroscopic properties requires averaging over either a very large number of systems identical in all respects except starting conditions or averaging over a single trajectory of very great duration. The numerical integration techniques used to solve the classical equations of motion require very small time steps, on the order of a femtosecond, to maintain an acceptable level of accuracy. Each time step in the integration is costly, requiring the evaluation of the forces arising from the extremely large number of nonbonded interactions, which varies roughly as N^2, where N is the number of atoms in the system. As a result, practical considerations concerning the amount of computer time available limit the trajectory time that can actually be simulated. The calculation of thermodynamically converged properties can thus be frustrated by large statistical inaccuracies. Nonetheless, such simulations have become routine in recent years and have been of great value in understanding a variety of physical systems. For example, much knowledge of the details of aqueous solutions has been based on such simulations (12). Calculations have now been reported for a variety of small molecules; for water and aqueous solutions (12,13), including those containing ions (14); and for proteins (15–18), carbohydrates (19–23), lipids, and nucleic acids (24,25).

A major advantage of this type of calculation over simple energy minimization calculations is that the trajectories contain entropic effects automatically "built in" to their behavior. Furthermore, systems do not necessarily become trapped in higher-energy local minimum geometries, because the momentum of the moving atoms can carry them over energy barriers and down the opposite side into other local minima. Thus, in principle, a trajectory of sufficient length would not suffer from the multiple minimum problem. Another important advantage of molecular dynamics simulations is that this type of calculation easily allows the modeling of solutions or crystal systems through the explicit inclusion of solvent water molecules or crystal neighbors in the calculations. Because solvation can play a critical role in determining the conformational behavior of polymers, and particularly proteins, the inclusion of water molecules directly in theoretical simulations can significantly increase their ability to model nature. Quite recently, a successful simulation of the complete folding of a very small globular protein in aqueous solution was reported (26) This goal, the prediction of a protein tertiary conformation from sequence alone, is the "holy grail" of protein modeling, and although the present simulation, which required more than two CPU months on one of the world's largest computers, is not necessarily the full realization of that goal, it offers renewed hope that such predictions might actually be possible in the future.

APPLICATION OF MOLECULAR MECHANICS CALCULATIONS TO FOOD SYSTEMS

Although molecular mechanics calculations have been widely used to study all major types of biological polymers, such calculations have only recently been attempted for typical food molecules. However, the potential for future use is great. This section simply indicates a few of the types of problems where such simulations have been profitably employed. Many other types of problems exist that could be usefully studied with theoretical calculations, however, and calculations of this type on food systems will probably become commonplace in the near future.

Because techniques already exist to modify the sequence of a protein almost at will by site-directed mutagenesis, perhaps the area with the greatest potential for practical applications for molecular mechanics is in studying possible protein mutants with the objective of modifying or improving their functionality. Such studies are already common in the biotechnology industry (8). However, because of the extreme difficulty or practical impossibility of making a prediction of protein conformation based solely on sequence information (see earlier), it is currently necessary to study those proteins whose tertiary structures have been previously determined by X-ray diffraction experiments. Fortunately, such diffraction studies are becoming more common for important food proteins.

One food protein whose tertiary conformation is known is the whey protein β-lactoglobulin (27). The structure of this 162-residue protein has been reported at 2.8-Å resolution. From this work it was determined that β-lactoglobulin has approximately the same tertiary structure as its sequential homologue, retinol-binding protein (28). Both proteins exist as a β-barrel in a calyx-shaped structure that contains an inner binding pocket that strongly binds retinol (β-lactoglobulin in fact binds retinol even more tightly than does retinol-binding protein). β-Lactoglobulin has already been used in food formulations as a carrier for additives immiscible in water. Among the modifications to the β-lactoglobulin molecule that could potentially be useful would be to increase its thermal stability, because this molecule denatures at a relatively low temperature, exposing a buried free cysteine to cross-linking with other proteins, including the free cysteine of κ-casein. Conformational energy calculations are currently being used to identify which mutants are possible that would allow the formation of an additional, third disulfide bond by including the free Cys-121 residue and another residue mutated into cysteine. Several such mutants identified by this procedure have been modeled by molecular dynamics simulations to determine whether the third disulfide bond does in fact introduce greater stability (29). Conformational energy calculations and docking studies are being used to model alternate substrates into the binding pocket of various additional mutants with substitutions on the inner surface of this pocket in an attempt to identify mutants that would have a high binding constant for these alternate substrates and that would be specific for these molecules. In this way, novel commercial molecular carriers for various substances might be developed that could function in somewhat the same way as the cyclodextrins, but with greater specificity. Such proteins could also potentially be designed to function as molecular scavengers for undesirable substances such as off-flavor compounds.

Other food-related proteins that have been modeled include the collagen of gelatin (30–32); various caseins (33); the enzyme xylose (glucose) isomerase (34), which is potentially of great commercial value in the corn syrup industry; and various cellulases (35). Because these enzymes are slow acting, and their mechanisms are still a matter of debate (36,37), simulations are being used not only to investigate the actual enzymatic mechanisms but also to suggest mutants that might have increased activity or specificity for other substrates. Similar studies could in principle be performed for any protein of interest whose tertiary conformation is known. The rate at which protein conformational determinations are reported continues to increase, and the list of known food proteins is growing. Notable recent examples include turkey troponin and the intensely sweet proteins monellin and thaumatin (38,39).

Proteins, of course, are not the only molecules to which molecular modeling calculations can be applied. Simulations of food lipids have in the past been rare, but there have been a number of molecular dynamics studies of membranelike phospholipid bilayers (40), and of a few milkfat triglycerides (41,42). The example of the sweet proteins mentioned in the previous paragraph serves to introduce another broad area of potential application of theoretical techniques to food molecules, which is the development of sweeteners. Theoretical studies might help not only to elucidate what makes these proteins sweet but also to identify the important functional characteristics of other sweeteners (or any other flavorant) as well. In general, the interaction of a flavorant molecule with its receptor is the same problem as in drug binding. Currently such studies are hampered by a nearly complete lack of information about the receptor proteins. This situation also exists for many medical problems, and theoretical studies under such circumstances employ techniques called quantitative structure-activity relationship (QSAR) studies (43) to identify structural features in the drug or flavorant that might give rise to the desired functional property

Perhaps the most extensive use has been made of various types of modeling in the study of carbohydrates. Molecular mechanics conformational energy studies have long been used to assist in the interpretation of X-ray fiber diffraction patterns of polysaccharides (44,45). These polymers do not form a single crystal as do proteins, and the limited information provided by fiber diffraction experiments must be augmented by energy calculations to identify possible structures. Recently molecular dynamics studies of carbohydrates have begun to appear, including studies of carbohydrate solvation (20–23,46). Simulations have been used to understand the solution conformation of table sugar (47), and whether or not it is different from the known crystal structure, and to simulate the solution properties of the components of carrageenans (48–50). A recent simulation of the putative double-helical structure of carrageenan in water found the individual strands remained in the helical conformation proposed from fiber diffraction, but the double helix unraveled, indicating that the juncture zone in carrageenan gels may not be a double helix as previously thought (50). Simulations of water structuring around sugars (51) have not only explained the anomeric preference in sugars such as glucose and xylose (23,52), but have more generally revealed how these solutes affect their solvent water by imposing new structural arrangements on it and how this structuring depends on the stereochemistry of the sugars. Such information could be most useful in guiding the design of polymers with specific viscosities, gelation capacities, freezing points, and so on.

CONCLUSIONS

The growth of genetic engineering in the biotechnology industry offers the promise of the next industrial revolution in the coming decades as artificial proteins are designed for a host of applications and as the properties of wild-type proteins are altered through recombinant experiments. However, the ability to create improved protein products will require considerable guidance from chemical theory to select those mutants most likely to possess the desired functional properties. Molecular mechanics studies of possible mutant proteins will thus become increasingly common in biotechnology. Theoretical molecular design studies, particularly of molecular binding, have already become the principal methods of drug design in the pharmaceutical industry, and they will undoubtedly be widely used in the design of flavorants in the food industry. Other more complicated systems that might be susceptible to molecular design calculations as computers become larger and faster are gelation and textural properties. Computer simulations cannot currently be applied to every practical problem of interest, so care must be exercised in selecting for study those problems for which a well-defined objective might reasonably be accomplished with the resources available. Nevertheless, for those systems for which they are appropriate, theoretical calculations can provide information that is not available by any other technique and can be quite valuable in making the production of functional mutants more efficient.

BIBLIOGRAPHY

1. L. Perry and R. Wetzel, *Science* **226**, 555–557 (1984).
2. U. Burkert and N. L. Allinger, *Molecular Mechanics*, American Chemical Society, Washington, D.C., 1982, Vol. 177.
3. C. L. Brooks, M. Karplus and B. M. Pettitt, "Proteins: A Theoretical Perspective of Dynamics, Structure, and Thermodynamics," in I. Prigogine and S. A. Rice, eds., *Advances in Chemical Physics*, Wiley-Interscience, New York, 1988, Vol. 71.
4. J. A. McCammon and S. C. Harvey, *Dynamics of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, U.K., 1987.
5. H. A. Scheraga, *Biopolymers* **22**, 1–14 (1983).
6. R. Elber and M. Karplus, *Science* **235**, 318–321 (1987).
7. M. Karplus and J. N. Kushick, *Macromolecules* **14**, 325–332 (1981).
8. L. Regan and W. F. DeGrado, *Science* **241**, 976–978 (1988).
9. N. Metropolis et al., *J. Chem. Phys.* **21**, 1087–1092 (1953).
10. A. Rahman, *Phys. Rev.* **136**, A405–A411 (1964).
11. L. Verlet, *Phys. Rev.* **159**, 98–103 (1967).
12. F. H. Stillinger, A. Rahman, *J. Chem. Phys.* **60**, 1545–1557 (1974).
13. P. J. Rossky and M. Karplus, *J. Am. Chem. Soc.* **101**, 1913–1937 (1979).
14. G. Pálinkás, W. O. Riede, and K. Heinzinger, *Z. Naturforsch.* **32A**, 1137–1145 (1977).
15. J. A. McCammon, B. R. Gelin, and M. Karplus, *Nature* **267**, 585–590 (1977).
16. W. F. van Gunsteren et al., *Proc. Natl. Acad. Sci. USA* **80**, 4315–4319 (1983).
17. M. Levitt, *J. Mol. Biol.* **168**, 595–620 (1983).
18. M. Levitt, *J. Mol. Biol.* **168**, 621–657 (1983).
19. C. B. Post et al., *J. Mol. Biol.* **190**, 455–479 (1986).
20. J. W. Brady, *J. Am. Chem. Soc.* **108**, 8153–8160 (1986).
21. J. W. Brady, *J. Am. Chem. Soc.* **111**, 5155–5165 (1989).
22. J. W. Brady and R. K. Schmidt, *J. Phys. Chem.* **97**, 958–966 (1993).
23. R. K. Schmidt, M. Karplus, and J. W. Brady, *J. Am. Chem. Soc.* **118**, 541–546 (1996).
24. S. C. Harvey, M. Prabhakaran, and J. A. McCammon, *Biopolymers* **24**, 1169–1188 (1985).
25. U. C. Singh, S. J. Weiner, and P. Kollman, *Proc. Natl. Acad. Sci. USA* **82**, 755–759 (1985).
26. Y. Duan and P. A. Kollman, *Science* **282**, 740–744 (1998).
27. M. Z. Papiz et al., *Nature* **324**, 383–385 (1986).
28. M. E. Newcomer et al., *The EMBO Journal* **3**, 1451–1454 (1984).
29. Y. Cho et al., *Protein Eng.* **7**, 263–270 (1994).
30. H. A. Scheraga, *Biopolymers* **20**, 1877–1899 (1981).
31. G. Némethy and H. A. Scheraga, *Biochemistry* **25**, 3184–3188 (1986).
32. J. R. Grigera and H. J. C. Berendsen, *Biopolymers* **18**, 47–57 (1979).
33. C. Holt and L. Sawyer, *Protein Eng.* **2**, 251–259 (1988).
34. H. L. Carrell et al., *Proc. Natl. Acad. Sci. USA* **80, 4440–4444** (1989).
35. J. S. Taylor et al., *Protein Eng.* **8**, 1145–1152 (1995).
36. C. A. Collyer, K. Henrick, and D. M. Blow, *J. Mol. Biol.* **211**, 211–235 (1990).
37. G. K. Farber et al., *Biochemistry* **28**, 7289–7297 (1989).
38. A. M. de Vos et al., *Proc. Natl. Acad. Sci. USA* **82**, 1406–1409 (1985).
39. S.-H. Kim et al., *Protein Eng.* **2**, 571–575 (1989).

40. K. Tu, M. L. Klein, and D. J. Tobias, *Biophys. J.* **75**, 2147–2156 (1998).
41. S. B. Engelsen, J. W. Brady, and J. W. Sherbon, *J. Agric. Food Chem.* **42**, 2099–2107 (1994).
42. Z.-Y. Yan et al., *J. Agric. Food Chem.* **42**, 447–452 (1994).
43. C. Hansch et al., *J. Med. Chem.* **25**, 777–784 (1982).
44. V. S. R. Rao et al., "Conformational Studies of Amylose," in G. N. Ramachandran, ed., *Conformation in Biopolymers*, Vol. 2, Academic Press, London, 1967, pp. 721–737.
45. D. A. Brant, *Ann. Rev. Biophys. Bioeng.* **1**, 369–408 (1972).
46. L. M. J. Kroon-Batenburg and J. Kroon, *Biopolymers* **29**, 1243–1248 (1990)
47. S. B. Engelsen and C. Herve du Penhoat, S. Peréz, *J. Phys. Chem.* **99**, 13334–13351 (1995)
48. K. Ueda and J. W. Brady, *Biopolymers* **38**, 461–469 (1996).
49. K. Ueda and J. W. Brady, *Biopolymers* **41**, 323–330 (1997).
50. K. Ueda, A. Imamura, and J. W. Brady, *J. Phys. Chem. A* **102**, 2749–2758 (1998).
51 Q. Liu and J. W. Brady, *J. Am. Chem. Soc.* **118**, 12276–12286 (1996).
52. S. Ha et al., *J. Am. Chem. Soc.* **113**, 1553–1557 (1991).

J. W. BRADY
Cornell University
Ithaca, New York

MONOSODIUM GLUTAMATE (MSG). See FLAVOR CHEMISTRY.

MUSCLE AS FOOD

There are four types of biological tissue—epithelial, connective, muscular, and nervous. Muscle tissue is a major component of the animal and is valued as a food because of the nutritional value afforded as well as satiety. Skeletal muscle is voluntarily controlled and identified histologically because it is striated and multinucleated. In mature muscle the nuclei are located peripherally in the cell, and they do not divide. Cardiac muscle cells are also striated, but they are mononucleated and have, in addition, a distinguishing feature called the intercalated disc. The intercalated discs are found at opposing ends of the cardiac muscle cells and help to maintain cohesion of one cell with another. Smooth muscle cells are spindle shaped, involuntary, nonstriated, and mononucleated. Smooth muscle is located in such places as blood vessels, the digestive tract, and ducts of glands. Smooth muscle controls the internal environment of an organism by moving or controlling movement of substances through various routes by peristaltic movement. Cardiac muscle is responsible for pumping blood around, and striated muscle moves the organism or parts of it.

COMPOSITION OF MUSCLE TISSUE

Proteins are the important constituents that allow for the physiological functioning of meat animals. Meat provides a major source of high-quality proteins for the human diet. The proteins in muscle are categorized as sarcoplasmic, myofibrillar, and connective tissue proteins. Numerous other proteins are found in other parts of the animal, such as in the organs, bones, and skin.

Sarcoplasmic proteins, at a concentration of about 55 mg/mL, are found in the sarcoplasm or fluidlike substance that surrounds and bathes the contractile apparatus and other organelles of the muscle cell. The sarcoplasmic proteins are primarily metabolic enzymes, and the protein responsible for the color of meat is known as myoglobin. The function of myoglobin is to bind oxygen; it has a molecular weight of 17,500 and occurs in muscle at a concentration of 0.1 to 20 mg/g muscle, depending on function of the muscle and specie and age of the animal. The heme portion of myoglobin binds the oxygen, which is transported to the muscle cell via the circulatory system and bound in blood to a similar protein, hemoglobin. Other sarcoplasmic proteins include nucleoproteins, which are a complex of proteins and nucleic acids and function in protein synthesis, and lyosomal proteins, which are hydrolytic enzymes.

The contractile apparatus of muscle consists of thick and thin filaments that interdigitate and slide past each other as muscle shortens; these filaments are composed of myofibrillar proteins. The major protein of the thick filaments is myosin, and for thin filaments it is actin. Myosin has a molecular weight of 480,000 and consists of two heavy subunits and four light subunits. Two fragments are formed when myosin is subjected to digestion with trypsin. The larger fragment has a molecular weight of 350,000 and is called heavy meromyosin. It is soluble at low ionic strength and contains both actin binding and ATPase activity. The smaller fragment has a molecular weight of 150,000 and is called light meromyosin. Heavy meromyosin can be further digested into S-1 and S-2 fragments. The monomeric form of actin is called G actin and has a molecular weight of 42,000. G actin polymerizes to form the filament form of F actin. Tropomyosin and troponin are other muscle proteins that bind to actin and are responsible for regulation of contraction.

Connective tissue consists of a variety of cell types and proteins filaments that are secreted by them. It is the latter that are of interest in meat, since they not only hold the cells together and in place but are responsible for toughness. Muscle has three layers of connective tissue—one surrounding the entire muscle, known as the epimysium; another layer that segments the muscle into bundles of fibers, known as the perimysium; and a layer surrounding each individual cell or myofiber, known as the endomysium. Collagen and elastin are the two connective tissue proteins. Collagen is an abundant protein in muscle and is unique because it has a high complement of the amino acids hydroxyproline and hydroxylysine. It is converted to soluble gelatin at the usual cooking temperatures of meat. Elastin is, in fact, a minor component of connective tissue, contains the unique amino acids desmosine and isodesmosne, and does not solubilize upon heating, nor does it swell by acid or alkaline treatment.

Fats comprise 10 to 30% of the carcass weight of meat animals and are primarily triglyceride. The majority of fat is located in fat cells, but lipid also occurs in cellular membranes. The fat cells within muscle are located in the perimysial connective tissue, and their accumulation depends on genetics and nutrition. Also, species is important, as, for example, when beef or pork is compared with chicken or fish, with the latter having a much lower fat content. The composition of fat varies likewise. Softer or more unsaturated fat is more prone to autooxidation and the associated "off" or rancid odors or flavors. Other lipid components, although occurring in small amounts, are phospholipids, sterols, and free fatty acids.

Glycogen is the major carbohydrate in muscle and is normally present at a concentration of 0.5 to 1.0%. Ash is normally 1% or less and results from minerals such as iron, zinc, and calcium.

CONVERSION OF MUSCLE TO MEAT

When an animal is stunned and exsanguinated, oxygen supply to the muscle ceases and a series of biochemical changes commences. The end result depends on the condition of the animal and preslaughter handling. The main metabolic events are a depletion of the ATP energy store and a degradation of the glycogen, which is converted anaerobically to lactic acid. Accumulation of lactic acid results in a decline of pH from near 7.0 to the range of 5.5 to 5.9; this is a postmortem condition easily monitored to establish rate and status of changes in the muscle as it is converted to meat. Rigor mortis also occurs as the muscle is converted from a soft, pliable, and extensible condition to one of rigidity. This is explained by a locking together of the myosin to actin filaments as ATP depletes. The time for rigor mortis differs with species, with beef and lamb being longer than pork and poultry. Some shortening of muscle occurs during the onset of rigor mortis. There are also two other shortening conditions of interest to meat science. Cold shortening results when muscle is exposed to a low temperature prior to ordinary development of rigor mortis, and it may result in considerable toughening. If meat is frozen prior to the onset of rigor mortis, it may then shorten greatly when thawed. This is known as thaw rigor and results in severe toughening. The postmortem changes also influence color and water-holding capacity of the resulting meat. This is quite important in pork, where a rapid pH decline results in so-called pale, soft, and exudative (PSE) meat. In beef, a dark, firm, and dry (DFD) condition may result if the pH remains high. These undesirable conditions are due largely to the genetic predisposition of the animal. However, proper handling both pre- and postmortem is important. Preslaughter stress to the animal can greatly influence postmortem events. Likewise, postmortem handling, such as proper temperature control during the chilling process, influences the final outcome. Other procedures, such as electrical stimulation, employed during postmortem time can be used to control tenderness and quality of meat.

PROCESSING AND FABRICATION

Two types of meat are made available to consumers. These are fresh and processed, which are also known as not-ready-to-eat and ready-to-eat, respectively. With fresh meat, the carcass is normally broken down to smaller portions and then shipped to retailers for final cutting and packaging. Interest is strong in being able to send retail ready packages directly from the processing plants. Only a small amount of meat is sold in frozen form, although freezing is an excellent means of preservation. Vacuum packing of fresh meat during transfer to the retailer is routinely practiced, and more interest is being shown in other controlled atmosphere packaging. Numerous forms of processed meats are made, including canned products. The majority, however, are cured, which means they have been treated with nitrite, heated, and then vacuum packaged. The shelf life of these products is normally in weeks as compared with fresh meats, which last only a few days.

Nitrite and heating require special mention. Nitrite curing results in typical cured color and also imparts specific preservation qualities to the meat. The heating associated with curing not only gives desirable palatability changes but also essentially pasteurizes the product so that it is ready to eat and, if packaged properly, will have a very long shelf life.

Processed meat is often equated with sausage. Sausages have had a very long history and are often named after origins, as, for example, frankfurters from Frankfort. They are usually ground or chopped and made into specific shapes with characteristic flavor. They may be fresh or cured, and there are unique variations such as fermented sausages. Another variation of processed meats is restructured meat, in which smaller pieces of meat are formed into larger portions by bonding them together with extracted meat proteins. During the past 10 years, a new generation of processed meats has appeared that are low or no fat. In general, these are made by lowering fat content and adding water, which is held within the product by gums, carbohydrates, or other substances.

GENERAL REFERENCES

R. G. Cassens, *Meat Preservation, Preventing Losses and Assuring Safety*, Food & Nutrition Press, Inc., Trumbull, Conn., 1994

P. J. Bechtel, ed., *Muscle as Food*, Academic Press, New York, 1986.

J. F. Price and B. S. Schweigert, eds., *The Science of Meat and Meat Products*, W. H. Freeman and Company, San Francisco, Ca., 1987.

ROBERT G. CASSENS
University of Wisconsin
Madison, Wisconsin

MUSHROOMS: CULTIVATION

DESCRIPTION

The golden rule for testing edible mushrooms is never to experiment with fungi that you have not identified with certainty as harmless. It is only fair to point out that there are many fungi that are both edible and excellent, and in many countries they have formed part of the human diet for centuries (1,2). They are highly valued as a food source worldwide, and more than 30 species are sold commercially. Edible mushrooms contain more protein than any vegetable and are rich in vitamin B (3). The most delicious mushrooms include *Boletus edulis, Clitopilus prunulus, Macrolepiota procera, Cantharellus cibarius*, and *Tricholoma matsutake*. All edible fungi should be picked when young and fresh and cooked as soon as possible. The commercially grown products are much safer because growers have been aware over the years of the problems that exist. Also, the strains marketed today are those with a long history of reliability as edible species (3,4).

The observation that certain edible mushrooms grow naturally on certain decomposing organic matter and the desire to produce a tasty source of food have led to the development of various techniques for the cultivation of mushrooms, and in some cases they have been fairly profitable. The cultivated species, however, have never been the most prized by gourmets. The favorite types are still the boletes, specific species of *Amanita*, and truffles; all of them are mycorrhizal fungi, and their carpophores cannot be developed without the right symbiotic plant (1). To date, the only successes have been with the saprophytic species, that is, those that feed on dead organic matter. Among these the best known is undoubtedly the champion, *Agaricus bisporus*, which has given rise to a thriving agricultural industry. Each year millions of tons of these mushrooms are produced for direct consumption, for the canning industry, and for the preparation of soups and sauces.

In recent decades the microbiological industry has been enormously improved, and the production of mushrooms in a controlled environment has become an example of highly advanced technology. Today horse dung still constitutes the basis of the culture soil (5). With a given quantity of straw and droppings, the dung initially undergoes a process of natural though controlled fermentation, first out of doors and then in an enclosed area where it is enriched with nitrogenous sugar substances and vitamins required for the development of the field mushroom. After a variable period of time, depending on the composition of the substrate, now called compost, sowing takes place, using cereal seeds covered with *Agaricus bisporus* mold. Two main varieties of field mushrooms are used: white and brown. The white are better suited to canning, whereas the brown, with their hazel-colored cap and small brown scales, are more widely used in Europe for direct consumption because the flesh is firmer and tastier. The American preference for the white variety is an expression of the same prejudice reflected in the choice of white eggs, bread, and sugar (4).

The cultivation of the oyster mushroom, *Pleurotus ostreatus*, and closely related species is also highly developed, especially in the Orient. They are also found in western markets in various varieties. *Volvariella volvacea* (paddy straw mushroom) (6) and *Lentinula edodes* (shiitake mushroom) (5), common in Oriental cuisine, can now be obtained in speciality shops in North America. For other species, such as the morels, production techniques had been developed (5,7). Production lines are being launched for *Stropharia rugosoannulata* and *Agrocybe aegerita*.

All the saprophytic species, especially those growing on wood, can easily be cultivated, even though in many instances they do not command a large market because of their meager amount of flesh. However, they can be cultivated on a small scale or at home by infecting unhealthy plants or dead stumps with fragments of the cap. The species that lend themselves best to this practice are *Flammulina velutipes* and *Pleurotus ostreatus*.

The cultivation of mycorrhizal fungi, which need to live with a host plant in order to produce their fruiting bodies, is a demanding undertaking. This is known as indirect cultivation. The mycorrhizal symbiotic type that is cultivated quite extensively in France is the legendary French or Perigord truffle, the high quality and price of which amply justify the cost of installing artificial truffle beds and the long years of waiting before the carpophores can be picked. In centers of modern truffle cultivation, production is followed extremely closely and nothing is left to chance. The acorns are sterilized on the outside in a chemical bath or dip and are then put in direct contact exclusively with truffle spores. The seedings, which develop in small bags filled with sterilized earth, will have the truffle as the only symbiotic organism with which to associate. Once planted out in soil with the right characteristics and due care, they will start to produce their first truffles after about 10 years.

CONSERVATION OF CULTURE

Most mushroom growers have to rely entirely on commercial spawn producers and the spawn plants of larger mushroom farms to provide reliable products. Successful mushroom production depends on the proper maintenance of pure culture and spawn that is capable of producing fruiting bodies of high productivity, excellent flavor, texture, color, and resistance to pests, disease, or both (5). There is no satisfactory way to check and evaluate the qualities of spawn by any rapid on-the-spot examination. A method of preserving selected strains that have been thoroughly tested and proved desirable is of primary importance.

Stock cultures are often maintained in an actively growing state under optimum laboratory conditions on a suitable solid substrate, using a system of periodic transfers at reasonable intervals of time. Alternatively, spawn cultures are reestablished under field cultivation by using single or mass basidiospore isolation or tissue culture techniques from freshly harvested fruiting bodies. At present, the most effective methods for preservation of edible mushrooms are freezing and storing in liquid nitrogen (8–10). Both sporulating and nonsporulating fungi can be preserved by freezing with liquid nitrogen. If sporulation or production of aerial mycelia is abundant, fungi are cultured on agar slants or millet that mycelia had grown on.

If sporulation is sparse, or if the culture is nonsporulating and the production of aerial mycelium is sparse, cultures are grown in broth and fragmented in a sterile Waring blender (5). Some strains must be concentrated by centrifugation to obtain sufficient material for freezing. Strains such as the yeastlike *Tremella fuciformis* yield colonies in liquid culture small enough to be pipetted and are grown in flasks containing a suitable broth, either stationary or on a shaker. Survival of nonsporulating cultures or fragments of mycelium by freezing at subzero temperatures has been reported at −196°C (11). The cryogenic storage temperatures now commonly used are those of liquid nitrogen (−196°C) and liquid nitrogen vapor (−150°C and below). With the commercial availability of cryogenic materials and equipment, this process has been extensively used throughout the world on a routine basis by various laboratories with widely differing objectives. The advantages of liquid nitrogen for conservation of microorganisms have been emphasized.

The experimental results and experience with edible mushrooms of the Culture Collection and Research Center (CCRC) at the Food Industry Research Development Center in Taiwan have proved that storage of mushroom spawn cultures in the frozen state at liquid nitrogen temperatures has great value for the spawn-making and mushroom industries. In addition, the production performance of CCRC cultures of cultivated mushrooms of *Agaricus bitorquis* (Quélet) Saccardo and *Pleurotus cystidiosus* O.K. Miller, which have been frozen in liquid nitrogen at CCRC, has been tested in the Mushroom Research Laboratory at the Taiwan Agricultural Research Institute (12). *Pleurotus cystidiosus* has recently been brought into cultivation on a commercial scale in Taiwan for more than 20 years.

Three agar discs containing the hyphal tips or six to eight spawn grains are placed in polyvinyl vials of 1.5-mL capacity, and 0.8 mL of 10% (vol/vol) glycerol or 5% (vol/vol) dimethyl sulfoxide (DMSO)–water menstruum as a cryoprotective agent is added. The vials are precooled to 5°C to prevent overheating during the operation and attached to prelabeled canes and placed in the freezing chamber of a controlled-rate freezer. The initial cooling is carried out at a rate of 1°C/min from room temperature to −70°C; subsequent cooling to −120°C is rapid and uncontrolled. The vials are then immediately transferred to a liquid nitrogen refrigerator and stored in the liquid phase at −150°C and below

Freezing injury to biological systems is very complicated and may occur physically, chemically, or both, depending on the cryoprotective agents and the cooling rate (13). Changes include ice crystal formation, pressure, solute concentrations, availability of water temperature, pH, and other factors. Best results for the survival and recovery of fungi from the frozen state generally have been achieved by slow cooling at approximately 1°C/min. Good recoveries can be expected with most fungi following freezing by either rapid or slow cooling if the frozen material is thawed rapidly in a 37°C water bath until the last trace of ice is dissipated (14,15).

Spawn maintenance by cryogenic freezing rather than periodic transfers of cultures eliminates degeneration and mutation, prevents losses due to contamination, and reduces the necessity for repeated production tests. Mushroom growers should now be assured of the ready availability of reliable, high-quality spawn.

CULTIVATION

Cultivation of the Common White Mushroom

The methods used for the cultivation of *Agaricus bisporus* are essentially similar to those pioneered by French horticulturists (9), but the old practices have been gradually refined and modified to the point where mushroom production is a closely controlled and scientific process. Mushroom spawn for each cultivation was simply transferred to an existing mushroom bed. A problem with this straightforward approach was the tendency for the spawn to gradually lose its vigor with repeated transfers from one bed to the next. Other improvements in the process have mainly concerned the development of specialized mushroom houses in which ventilation, temperature, and humidity can be carefully controlled to provide the best conditions for fruiting. In fairly deep caves the temperature remains remarkably stable, and for certain stages of mushroom development this advantage is obvious. Specially built mushroom houses are, however, more efficient, especially when it comes to preparing the mushroom beds or harvesting the crop.

The first stage in cultivating *Agaricus bisporus* is the germination of spores (5). This is carried out by taking spores or fragments from a mature fruiting body selected for desirable qualities such as color, taste, and vigor. The spores or fragments are then germinated on sterile nutrient medium to give a master culture. The master culture is used to prepare the spawn, which is then distributed to growers. This spawn was produced by inoculating a suitable sterile medium, such as horse-manure medium, but today a variety of spawn types are available, including grain spawn. Grain spawn is now almost universally used in houses; the grain may be wheat, rye, sorghum, or millet, in decreasing order of size. Whatever the medium, spawn production is merely a process of bulking up the initial mycelium to the point where it can be conveniently introduced into the culture beds themselves (Fig. 1, Fig. 2).

The fermentation process removes most of the simple sugars from the medium and leaves insoluble substances such as cellulose and lignin, which are readily utilized by *Agaricus bisporus*. The point of the composting step is to create a medium that both favors the mushroom mycelium and discourages its natural rivals. The composting process is usually carried out in the open, with the piles being regularly turned to ensure an even fermentation that results in the whole pile becoming hot. The length of time required to get the compost into the right condition varies, depending on the age of the manure, but normally it is at least 2 weeks. Composts for growing in mushroom houses vary according to the nature of the locally available materials and the type of pasteurization employed. Most trays now in use have short legs, which require the addition of spacing blocks for cropping. However, some farms use longer legs, so that the trays are always stacked at the full dis-

Figure 1. Gram spawn of *Agaricus bisporus*, with the fungus growing on sterilized wheat grains.

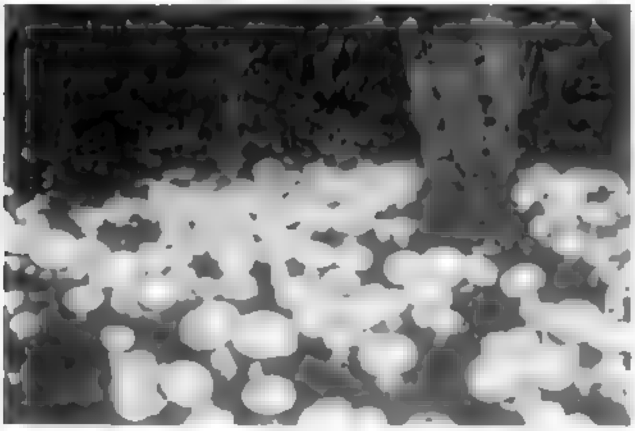


Figure 2. *Agaricus bisporus* growing in a commercial bed.

tance apart, as for cropping. Although the principle involved is the same in all cases, different growers often have their own minor modifications designed to improve the end product because their different ingredients and preparations require adjustments to the control of temperature and aeration. Correct timing is all-important. Undercomposted manure tends to overheat in the beds and leads to poor mycelial growth. On the other hand, overcomposting will remove many of the nutrients essential for production of a good crop. Once the compost has reached the correct stage of fermentation, the beds themselves can be prepared.

It has been known since mushrooms were first cultivated that the beds must be covered with a casting of soil or other suitable material to induce the change from vegetative growth of mycelium to fruiting. The virtues of different types of soil as casing materials are frequent subjects of argument among growers. Clay soils are reputedly better than sandy ones, but interest has also been extended to artificial substitutes, such as mixtures of peat and vermiculite (an artificial potting medium).

For many years the reason for casing was completely unknown. Recently research has revealed some of the effects produced by the soil. The change in growth conditions accompanying the addition of soil induces the formation of tiny primordia, which subsequently mature into mushrooms, but the exact action by which casing stimulates fruiting is still unknown.

The final stages in mushroom cultivation are cropping and packing. Following the first crop of mushrooms, several additional periods of fruiting usually occur at intervals of 2 or 3 weeks. The time at which the mushrooms are picked is important. It is agreed that commercial growers usually harvest their mushrooms before the veil has broken. The major consideration is the longer life of slightly immature mushrooms once they have reached the shop. In common with many other food items, the prevalent belief that a clean, uniform product is somehow superior to a more varied and possibly tattered product has no doubt contributed to the monopoly of the button mushroom. Once the beds are finished, the composting is removed and sold as manure for various horticultural activities.

Mushrooms are picked by gripping the cap and bending or twisting to break off the stem at the base. This is easily done with closed mushrooms; open ones are fragile, and more care is needed to ensure that the stem breaks at the bottom, not at the top.

The stage of growth at which mushrooms are picked is important. All open mushrooms, whatever their size, are picked. In many countries open mushrooms are considered extremely low grade, and in most they are cheaper in the market than closed cups or buttons. After opening, the stem grows but the weight of the cap does not increase appreciably, so that even disregarding quality and price, there is no further increase in yield. Up to the point of opening, the weight of the mushroom increases very rapidly, doubling in 24 h at its peak rate, so leaving closed mushrooms to grow as long as possible raises their picked weight. With tight picking, the more frequent flushes compensate for the loss of weight on the individual mushrooms. Closed mushrooms also have a better shelf life in the shop, particularly in warm weather.

Picking mushrooms has always been a major factor in wage costs. With closely packed trays or shelves, picking is carried out under cramped conditions, usually with poor lighting, so that selection and grading of mushrooms are difficult (5).

Two recent developments have promised to improve and accelerate the picking operation. One is development picking lines, in which the trays are transported to the pickers, where mushrooms can be picked under ideal conditions. The other is carrying of trays by forklift truck from the cropping room to a destacker at the input end of the line. They are automatically destacked and passed along by a conveyor system at a controlled speed that can be adjusted to run fast if there are not many mushrooms and slower for a heavy flush. Pickers stand on both sides of the line with baskets handy and pick as the trays pass in front of them. The speed of the conveyor is adjusted so that the trays are cleared just before they reach the end of the line. There may be conveyor belts for stumps and the like and another conveyor for filled baskets of mushrooms. There is also an overhead spray line at the end of the conveyor so that trays can be automatically watered as they pass along.

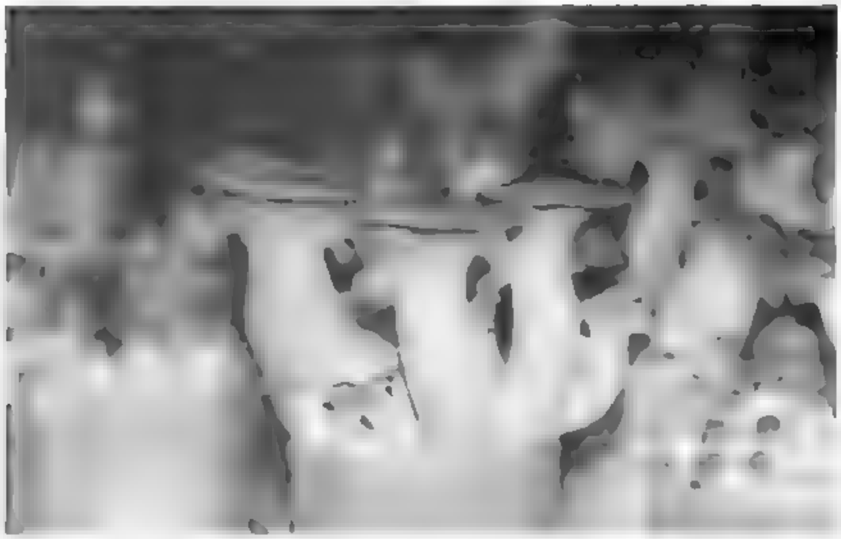


Figure 3. Cultivated *Pleurotus eryngii* growing in reused plastic bottles.

The other development is mechanical harvesting. With pickers' wages becoming an increasingly important factor in production costs, the possibility of harvesting mushrooms by machine has interested many growers. A machine that travels over the bed, cuts mushrooms with a reciprocating blade just over the casing, draws the cut mushrooms back over the blade, and delivers them to one side behind the machine has been described. Patents have also been filed for a machine that locates mushrooms by a number of sensor units, grips them on the top of the cap by suction, and plucks them off the bed (5). Mushrooms on a bed must all, or nearly all, be at the same stage of development, because they must all be cut at once. Any mushrooms that are higher or lower in the bed will be cut with too much stalk or through the cap,and any that are crooked will also suffer. Harvesting machines already in existence can pick from shelves or could be mounted over picking lines. It is necessary to provide for the machine to run on the beds or side boards or to be fitted with sensors that control its height above the casing. This may require the provision of bare strips of bed at each side of the strip to be harvested. A harvesting machine produces ungraded mushrooms. Postharvest grading for size is most easily done in the water flumes used in canneries so that the first application of these machines may be postharvest.

Cultivation of the Shiitake Mushroom

The shiitake grows naturally on logs of deciduous trees, in particular, beech, chestnut, hornbeam, and oak. Cultivation is therefore carried out on specially cut large branches from suitable trees, more substantial logs being in demand for construction purposes. Today a more scientific system is used (16). The logs are soaked in water and pounded to break the bark, or holes are made with a broad drill or a specially designed hammer. The logs are then inoculated with a spore emulsion prepared from mature fruiting bodies. Alternatively, a spawn consisting of mycelium grown on wood chips or sawdust is introduced into the holes. Then the infected logs are placed in a carefully selected site in

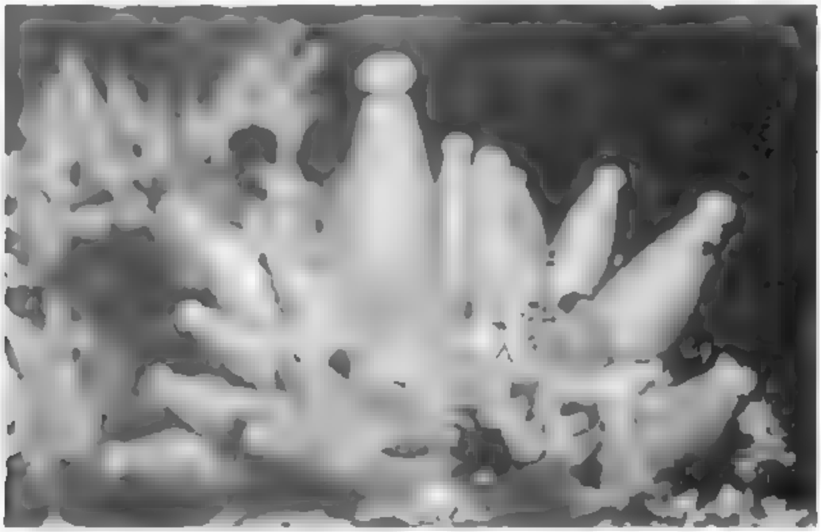


Figure 4. New developed and cultivated mushroom: *Tricholoma cystidiosus.*

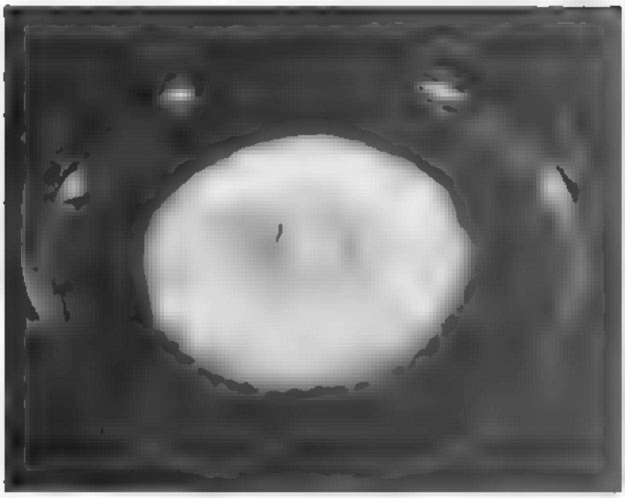


Figure 5. Mushroom primordium of fruiting body, *Fistulina hepatica*, of mycelia growing on potato dextrose agar (PDA) medium.

Figure 6. A fruiting body of cultivated *Fistulina hepatica*.

the forest, known as the laying yard. Choice of site is very important; if it is too moist, the natural wood-decaying competitors are favored and the crop is reduced or even lost altogether. The best laying yards are usually in well-ventilated clearings or positions at the edge of the forest. The logs themselves are placed crosswise at a slight angle to the ground.

Another method is cultivation in a polypropylene bag or space bag containing sterilized sawdust and rice bran (9:1 vol/vol) with a moisture of ca. 65%. The primordia are produced after one week when the plastic bags are opened and the humidity is kept at ca. 80 to 90%. This method is efficient for commercial cultivation and is now the major method in Taiwan and Japan (5).

Cultivation of Other Mushrooms

In addition to the aforementioned species, certain other fungi have been or still are subject to some form of primitive cultivation. The method used to grow cultivated paddy straw mushrooms (*Volvariella volvacea*) is similar to that used for the common white mushroom (17–21). Both methods are based on the preparation of special beds and the introduction of mushroom spawn, but the paddy straw mushroom is mainly cultivated on a small scale using traditional, much less scientifically refined methods.

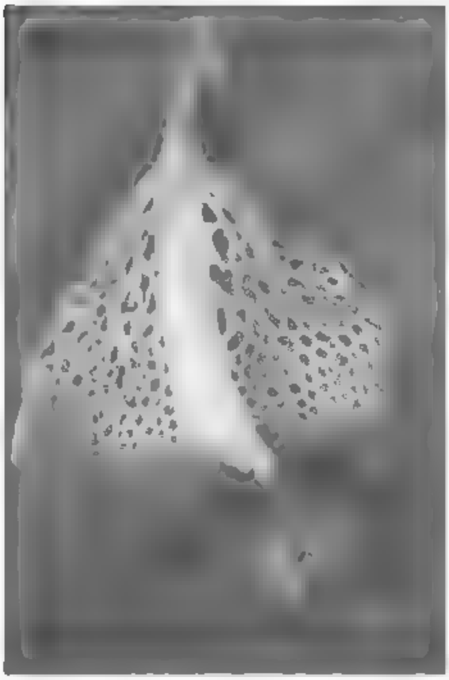


Figure 7. A longitudinal section of *Fistulina hepatica*. Its common names are beefsteak fungus, ox-tongue, and poor man's beefsteak.

The jelly fungus (*Auricularia polytricha*) is quite popular in the Far East and is produced in large quantities in China. A proportion of this crop is grown on logs or polypropylene bags set up with the specific intention of encouraging the fungus. A similarly haphazard process has been used to cultivate *Pleurotus ostreatus*, the oyster mushroom. Several other fungi have been grown deliberately on a small or experimental scale. These include *Pholiota nameko, Pleurotus eryngii* (Fig. 3), *Stropharia rugosoannulata, Auricularia* spp., *Tremella fuciformis, Tuber* spp., *Tricholoma matsutake, Tricholoma titans* (Fig. 4), *Flammulina velutipes, Morchella esculenta* (7), *Fistulina hepatica* (Fig. 5–7), *Agrocybe aegerita, Armillaria mellea*, and *Dictyophora indusiata*. Though some sort of systematic cultivation is probably feasible for many fungi, the major obstacle to large-scale production is the difficulty of obtaining economically worthwhile yields.

BIBLIOGRAPHY

1. G. H. Lincoff, *Simon & Schuster's Guide to Mushrooms*, Simon & Schuster, New York, 1981
2. R. Philips, *Mushrooms and Other Fungi of Great Britain and Europe*, Pan Books, London, 1981.
3. G. H. Lincoff, *The Audubon Society Field Guide to North American Mushrooms*, Alfred A. Knopf, New York, 1984.

4. C. Dickinson and J. Lucas, *The Encyclopedia of Mushrooms*, Orbis Publishing Limited, London, 1979.
5. S. T. Chang and W. A. Hayes, *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, 1978.
6. B. A. Oso, "Mushrooms and the Yorba People of Nigeria," *Mycologia* **67**, 311–319 (1975).
7. U.S. Pat. 4,594,809 (June 17, 1986), R. D. Ower et al. (to Neogen Corporation)
8. A. Dietz, "Nitrogen Presservation of Stock Cultures of Unicellular and Filamentous Microorganisms," in A. P. Rinfred and B. LaSalle, eds., *Round Table Conference on the Cryogenic Preservation of Cell Cultures*, National Academy of Sciences, Washington, D.C., 1975, pp. 22–36.
9. D. J. N. Hossack, "Liquid Nitrogen Frozen Inocula—A Year's Experience," *Proc. Soc. for Analytical Chem.* **9**, 36–38 (1972).
10. H. T. Meryman, "Review of Biological Freeying," in H. T. Merymann, ed., *Cryobiology*, Academic Press, New York, 1966, pp. 1–114.
11. S. W. Hwang, "Effects of Ultra-Low Temperature on the Viability of Selected Fungus Strains," *Mycologia* **52**, 527–529 (1960).
12. S. C. Jong and J. T. Peng, "Identity and Cultivation of a New Commercial Mushroom in Taiwan," *Mycologia* **67**, 1235–1238 (1975).
13. A. U. Smith, *Biological Effects of Freezing and Super-cooling*, Edward Arnold, Publishers Ltd., London, 1961.
14. R. D. Goos, E. E. Davis, and W. Butterfield, "Effect of Warming Rates on the Viability of Frozen Fungus Spores," *Mycologia* **59**, 58–66 (1967).
15. P Mazur, "Survival of Fungi after Freezing and Desiccation," in G. C Ainsworth and A. S Sussman, eds., *The Fungi*, Vol. 3, Academic Press, New York, 1968, pp. 325–395.
16. P. J. Wuest, D. J. Royse, and R. B. Beelman, *Cultivating Edible Fungi*, Elsevier, Amsterdam, The Netherlands, 1987.
17. S. T. Chang, *The Chinese Mushroom*, The Chinese University of Hong Kong, Hong Kong, 1972.
18. S. T. Chang, "Production of Straw Mushroom (*Volvariella volvacea*) from Cotton Wastes," *Mushroom J.* **21**, 248–354 (1974).
19. S. E. Chua and S. Y. Ho, "Fruiting on Sterile Agar and Cultivation of Straw Mushrooms (*Volvariella* species) on Padi Straw, Banana Leaves and Sawdust," *World Crops* **25**, 90–91 (1973).
20. K. Ramakrishan et al., "A Simple Technique for Increasing Yield of Straw Mushroom, *Volvariella diplasta* (Berk. and Br.) Sacc.," *Madras Agric. J.* **55**, 194–195 (1968).
21. D. J. Pegler, *Mushrooms and Toadstools*, Mitchell Beazley, London, 1983

CHEE-JEN CHEN
Food Industry Research and Development Institute
Hsinchu, Taiwan

MUSHROOMS: PROCESSING

PROCESSING

Canning

The mushroom processed in large quantities by canning, either in cans or in glass jars, is *Agaricus* spp. It is the common mushroom and called champignons in French. This is the most common edible mushroom cultivated and processed in the United States and in France. It is the only mushroom used for processing and more than two-thirds of the crop goes to processors in the United States (1).

Raw Material. Mushrooms which are destined to be sold canned in brine must be picked at the tight-cap stage of maturity, ie, the veil of the mushroom is closed and the gills are not visible, while more mature specimens may be utilized in other types of pack, such as chopped or sliced mushrooms, in soup, or in butter sauce. Mushrooms of large strain are cultivated and flat mushrooms with the cap fully opened are used for sliced mushrooms in butter sauce in Australia.

Mushrooms are sent to the cannery with the soil or bottom end of the stem either cut or uncut in the United States. It is believed that a mushroom with the soil end of its stem uncut is protected from dehydrating or losing its moisture. To obtain maximum quality, freshly harvested mushrooms to be packed in brine must be processed as quickly as possible. Otherwise, they must be cold stored until they are ready to be processed.

Washing and Sorting. Mushrooms with the soil end attached must be trimmed mechanically at the cannery. Workers place the mushrooms in the V-shaped grooves of the machine, which revolves and cuts the soil end from the stem and then cuts the stem from the cap if the mushrooms are to be packed as buttons or sliced buttons. For button mushrooms not more than 1/8 in. of the stem stub should be attached to the cap.

Generally, the trimmed mushrooms are dumped into a large vat filled with water and allowed to soak in water for a while to losen the adhering dirt. They are then moved through a spray washer. Cull mushrooms are removed either before or after spray washing. To permit more uniform blanching, size grading of the washed mushrooms before passing them to the blancher is recommended.

Post-Harvest Treatments. Mushrooms lose weight and shrink during blanching and thermal processing, mostly during blanching. This loss of canned product weight ranges between 30 and 40% (2). Since mushrooms are an expensive commodity and the canned product is sold on the drained weight basis, excessive shrinkage will result in considerable economic loss to the processor. A 5% reduction in the shrinkage could increase the revenue of mushroom processors by as much as 20% (3).

Postharvest storage and soaking of mushrooms in water prior to blanching could increase the yield of canned mushrooms. The PSU-3S process has been recommended (4). The process is a combined soaking and storage process, ie, fresh mushrooms are soaked in water [50–60°F (10–16°C)] for 20 min, followed by 18 h of cold storage at 35°F (2°C) and a 2-h second soaking prior to blanching. Soaking in water for 2 h prior to blanching could increase the yield of canned product by about 2%, and the increase could reach 2.9–4.4% when mushrooms are held at 35°F (2°C) for 24–48 h prior to processing (2). However, the PSU-3S process demonstrated yield increases as high as 9.4%. This process was made more feasible for the canning industry by replacement of the second soaking by vacuum soaking, in

4. C. Dickinson and J. Lucas, *The Encyclopedia of Mushrooms*, Orbis Publishing Limited, London, 1979.
5. S. T. Chang and W. A. Hayes, *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, 1978.
6. B. A. Oso, "Mushrooms and the Yorba People of Nigeria," *Mycologia* **67**, 311–319 (1975).
7. U.S. Pat. 4,594,809 (June 17, 1986), R. D. Ower et al. (to Neogen Corporation)
8. A. Dietz, "Nitrogen Presservation of Stock Cultures of Unicellular and Filamentous Microorganisms," in A. P. Rinfred and B. LaSalle, eds., *Round Table Conference on the Cryogenic Preservation of Cell Cultures*, National Academy of Sciences, Washington, D.C., 1975, pp. 22–36.
9. D. J. N. Hossack, "Liquid Nitrogen Frozen Inocula—A Year's Experience," *Proc. Soc. for Analytical Chem.* **9**, 36–38 (1972).
10. H. T. Meryman, "Review of Biological Freeying," in H. T. Merymann, ed., *Cryobiology*, Academic Press, New York, 1966, pp. 1–114.
11. S. W. Hwang, "Effects of Ultra-Low Temperature on the Viability of Selected Fungus Strains," *Mycologia* **52**, 527–529 (1960).
12. S. C. Jong and J. T. Peng, "Identity and Cultivation of a New Commercial Mushroom in Taiwan," *Mycologia* **67**, 1235–1238 (1975).
13. A. U. Smith, *Biological Effects of Freezing and Super-cooling*, Edward Arnold, Publishers Ltd., London, 1961.
14. R. D. Goos, E. E. Davis, and W. Butterfield, "Effect of Warming Rates on the Viability of Frozen Fungus Spores," *Mycologia* **59**, 58–66 (1967).
15. P Mazur, "Survival of Fungi after Freezing and Desiccation," in G. C Ainsworth and A. S Sussman, eds., *The Fungi*, Vol. 3, Academic Press, New York, 1968, pp. 325–395.
16. P. J. Wuest, D. J. Royse, and R. B. Beelman, *Cultivating Edible Fungi*, Elsevier, Amsterdam, The Netherlands, 1987.
17. S. T. Chang, *The Chinese Mushroom*, The Chinese University of Hong Kong, Hong Kong, 1972.
18. S. T. Chang, "Production of Straw Mushroom (*Volvariella volvacea*) from Cotton Wastes," *Mushroom J.* **21**, 248–354 (1974).
19. S. E. Chua and S. Y. Ho, "Fruiting on Sterile Agar and Cultivation of Straw Mushrooms (*Volvariella* species) on Padi Straw, Banana Leaves and Sawdust," *World Crops* **25**, 90–91 (1973).
20. K. Ramakrishan et al., "A Simple Technique for Increasing Yield of Straw Mushroom, *Volvariella diplasta* (Berk. and Br.) Sacc.," *Madras Agric. J.* **55**, 194–195 (1968).
21. D. J. Pegler, *Mushrooms and Toadstools*, Mitchell Beazley, London, 1983

CHEE-JEN CHEN
Food Industry Research and Development Institute
Hsinchu, Taiwan

MUSHROOMS: PROCESSING

PROCESSING

Canning

The mushroom processed in large quantities by canning, either in cans or in glass jars, is *Agaricus* spp. It is the common mushroom and called champignons in French. This is the most common edible mushroom cultivated and processed in the United States and in France. It is the only mushroom used for processing and more than two-thirds of the crop goes to processors in the United States (1).

Raw Material. Mushrooms which are destined to be sold canned in brine must be picked at the tight-cap stage of maturity, ie, the veil of the mushroom is closed and the gills are not visible, while more mature specimens may be utilized in other types of pack, such as chopped or sliced mushrooms, in soup, or in butter sauce. Mushrooms of large strain are cultivated and flat mushrooms with the cap fully opened are used for sliced mushrooms in butter sauce in Australia.

Mushrooms are sent to the cannery with the soil or bottom end of the stem either cut or uncut in the United States. It is believed that a mushroom with the soil end of its stem uncut is protected from dehydrating or losing its moisture. To obtain maximum quality, freshly harvested mushrooms to be packed in brine must be processed as quickly as possible. Otherwise, they must be cold stored until they are ready to be processed.

Washing and Sorting. Mushrooms with the soil end attached must be trimmed mechanically at the cannery. Workers place the mushrooms in the V-shaped grooves of the machine, which revolves and cuts the soil end from the stem and then cuts the stem from the cap if the mushrooms are to be packed as buttons or sliced buttons. For button mushrooms not more than 1/8 in. of the stem stub should be attached to the cap.

Generally, the trimmed mushrooms are dumped into a large vat filled with water and allowed to soak in water for a while to losen the adhering dirt. They are then moved through a spray washer. Cull mushrooms are removed either before or after spray washing. To permit more uniform blanching, size grading of the washed mushrooms before passing them to the blancher is recommended.

Post-Harvest Treatments. Mushrooms lose weight and shrink during blanching and thermal processing, mostly during blanching. This loss of canned product weight ranges between 30 and 40% (2). Since mushrooms are an expensive commodity and the canned product is sold on the drained weight basis, excessive shrinkage will result in considerable economic loss to the processor. A 5% reduction in the shrinkage could increase the revenue of mushroom processors by as much as 20% (3).

Postharvest storage and soaking of mushrooms in water prior to blanching could increase the yield of canned mushrooms. The PSU-3S process has been recommended (4). The process is a combined soaking and storage process, ie, fresh mushrooms are soaked in water [50–60°F (10–16°C)] for 20 min, followed by 18 h of cold storage at 35°F (2°C) and a 2-h second soaking prior to blanching. Soaking in water for 2 h prior to blanching could increase the yield of canned product by about 2%, and the increase could reach 2.9–4.4% when mushrooms are held at 35°F (2°C) for 24–48 h prior to processing (2). However, the PSU-3S process demonstrated yield increases as high as 9.4%. This process was made more feasible for the canning industry by replacement of the second soaking by vacuum soaking, in which mushrooms were held at 2-mmHg pressure for 5 min and kept in water for another 10 min after vacuum release. Vacuum soaking alone would result in a yield increase of 5%, but when it is combined with storage treatment the yields would be greater than those obtained with the PSU-3S process. The yield increases were the same (12%) regardless of the order in which the vacuum soaking and storage were carried out. The vacuum soaking also offers a significant advantage to commercial processors by reducing the time required for optimum water absorption by the product from 2 h to 10 min (5,6).

Mushrooms stored following harvest for 18 h at 35°F (2°C), 53°F (12°C), and 72°F (22°C) increased in yield, compared to the control, by as much as 3.6%, 7.5%, and 9.5%, respectively (5). Although the higher temperature and longer storage time resulted in a higher canned product yield, it also was accompanied by quality deterioration. The optimum process involved soaking mushrooms in water for 20 min, storing at 53°F (12°C) for 18 h, and applying vacuum soaking prior to blanching. The yield increase, compared to the control, was 15.3% and the quality was comparable to those processed after 18-h storage at 35°F (2°C) (6). When mushrooms were stored at 53°F (12°C) with 95% RH for 72 h, canned product yield increased up to 19% and the free amino acid content of the tissues increased proportionately. It has been confirmed that the increase of canned product yield caused by postharvest storage was due to qualitative protein changes that alter the water binding capacity and water holding capacity of the proteins (7).

The Food Industry Research and Development Institute of Hsinchu recommended an enzyme vacuum soaking process to the industry in Taiwan. In this process fresh mushrooms are soaked in water containing papain (0.02%) for 3 min at 50 mmHg pressure, followed by storage at room temperature for 2 h prior to blanching. The enzyme vacuum soaking process alone could result in an increase of the canned product yield by 5.2%, but when the process and 2-h storage at room temperature were combined, the yield increase was 10.1%. When an enzyme activator (such as L-cysteine) was added to the vacuum soaking water containing papain, the yield increase was even more significant (8).

Blanching. The precooking of fresh material is referred to as blanching in canning, freezing, or dehydration processes. Boiling water or steam may be used in the blanching of mushrooms, and the blanching times are adjusted accordingly to bring the center temperature of mushrooms to about 180°F (82°C). The blanching process removes the gases in the mushroom tissue, inactivates the polyphenoloxidase enzyme, improves the texture for slicing, and reduces the bacterial loads. Blanched mushrooms should be immediately cooled by potable water spray and soaking in running water unless the containers can be filled and thermal processed in a short time after blanching, as in the cases of chopped or sliced mushrooms in butter sauce or soup packs.

Size Grading and Filling. The blanched and cooled mushrooms next go through a size-grading machine, which is designed so that certain sizes of mushrooms will drop through holes of a particular size as the machine revolves. The mushrooms which do not drop through the holes will then be passed on to the next larger holes in succession. The machine sorts mushrooms so that uniformly sized mushrooms can be put into cans. Mushrooms may be placed in cans by hand or by machine. Before being put into cans, mushrooms are reinspected and defective ones removed. For sauce-type pack, mushrooms are chopped or sliced immediately after blanching and then cooked with other ingredients before being packed into cans. For mushrooms packed in brine, the cans filled with mushrooms are filled up with hot brine or hot water and a salt tablet. The salt concentration in the finished product is preferably about 1%. Ascorbic acid may be used up to 37.5 mg for each ounce of drained weight of mushrooms, and must be labeled. The filled cans, if not sufficiently hot, must be exhausted before being closed.

Thermal Processing. Since mushrooms are a low-acid food, they must be thermal-processed under pressure, either in a continuous processing machine or in a batch-type retort. In 1973 the Food and Drug Administration (FDA) published its findings on the presence of botulinum toxin in some cans of United States and foreign origin (but not from Taiwan). Many mushroom canneries in the United States were investigated and intensive studies on heat penetration rate were conducted. From the studies it is evident that a relatively wide variety of process times and temperatures were in use for canned mushroom products. This is a result of new styles of pack and new filling and processing methods used in the industry. The heat resistance of the spores from *Clostridium botulinum* isolates obtained from commercially canned mushrooms was determined and the *D* values for all of the *C. botulinum* spore crops overall were reported to be slightly higher in the buffer than in mushroom puree. The mean *D*-value (240°F) in buffer for the 10 spore crops was 0.24 min compared to a value of 0.19 min for spores in mushroom puree (9).

Good manufacturing practices for thermally processed low-acid foods packaged in hermetically sealed containers and an emergency permit control were promulgated in 1973 and in 1974. The processors must list their establishments and the scheduled processes required and used in their plants for their particular styles of pack with the FDA. Critical factors associated with each process must be specified in the scheduled process. Initial temperature is one of the critical factors for most of the processes. Other critical factors for still retort processing include slice thickness and the size of chopped or diced pieces, the proportion of small chips or fine pieces, the fill weight, the method of filling and brining, and the can position during the process (eg, horizontal or vertical). Other control factors determined for individual packing operations include the mushroom size and maturity as indicated by veil condition, the duration and temperature of storage before canning, the uniformity and degree of shrinkage during the blanching, and any condition or operation affecting the degree of compactness of the product within the can. Production and quality control records must also be kept on file to provide confirmation that the critical factors are under satisfactory control.

Sulfide spoilage of canned mushrooms was once a big problem encountered in the canneries located in the southern part of Taiwan. This has resulted in more severe thermal processing practices for the canned product since 1965. Later, however, the processors began to apply a higher temperature, shorter time, process to compensate for the deterioration in the quality of the product caused by the prolonged thermal processing (10,11). The yield, color, and texture decrease with increasing process time at all temperatures. Recently, activation energies and Z values during thermal processing were reported (12). The processed products must be cooled rapidly and not be allowed to stand at the higher temperatures.

Canning of Other Mushrooms. To a much lesser extent other mushrooms, such as *Volvaricella volvacea*, straw mushrooms; *Pleurotus ostreatus*, abalone mushrooms and *Flammulina velutipes*, called Enokitake in Japan are canned and consumed in the Orient. The canning processes for these are similar to those applied to Agaricus, described above. *Tremella fuciformis*, a white jelly fungus, is mainly utilized for cooking and for medical purposes by the Chinese. It is usually canned with sugar syrup and the net drained weight is always very light compared to the net weight. The consumption of *Tremella fuciformis* seems to be limited to the Chinese. The canned form is much as popular than the dried form.

Freezing and Freeze-Drying

Only a small portion of common (Agaricus) mushrooms produced are frozen or freeze-dried, and a large part of the frozen mushrooms are sold to reprocessors, such as freeze-dryers, or used in specialized frozen food products.

Washing and Blanching. Lower microbial loads are the basic requirements for these products, and vigorous washing and utilization of some disinfectants have been found effective for this purpose. High levels of chlorine, up to 50 ppm in the wash water, have been found beneficial for reducing the microbial load.

Mushrooms contain a highly potent polyphenoloxidase. It is this enzyme which causes the undesirable discoloration that appears in bruised or cut mushrooms. During the washing operation, soluble oxidized phenolic compounds resulting from the enzymatic action are washed off, and in some cases the washing water may turn a distinct red. Blanching adequate to destroy the enzyme also produces an undesirable gray color. Therefore, mushrooms for freezing are usually given a short blanching in hot water, primarily to help reduce microbial contamination rather than for enzyme inactivation. It is necessary to follow such a blanching immediately with a cold-water quench. A short-time dip in sodium bisulfite solution containing 200 ppm SO_2 or in 1% salt and 0.09% sodium bisulfite solution is an effective alternative for prevention of this enzyme discoloration.

Freezing and Freeze-Drying. A rapid freezing rate is essential for mushroom freezing. It is desirable to freeze mushrooms to a center temperature of −10°F (−23°C) in 3 to 5 min for diced or sliced mushrooms and in about 20 min for button or whole mushrooms. I.Q.F. (individually quick frozen) freezing and refrigerant dip freezing are recommended.

Freeze-drying is carried out in a high-vacuum chamber in which the ice on or in the mushroom tissue disappears by sublimation. Uniform loading is essential, otherwise burning or incomplete drying of a part of the product may occur. When the ice on the surface of the mushrooms disappears, application of heat can be started. The end point of the freeze-drying is the point at which the moisture content of the product reaches about 3%. From 12 kg of raw mushrooms, 1 kg of freeze-dried product is obtained. After reconstitution, 1 kg of the freeze-dried product will yield 8 kg of reconstituted product.

Packaging. Vacuum packaging inhibits oxidative discoloration (13) and a sauce coating may also serve the same purpose. The freeze-dried product is usually packed in airtight and moistureproof containers under nitrogen gas.

Dehydration

Among the cultivated mushrooms, the common mushroom (Agaricus) and Shiitake mushroom (*Lentinus edodes*) have enjoyed a good reputation internationally. While Agaricus is mainly canned and consumed by Western people, *Lentinus edodes*, of which the common name is Shiitake in Japanese, is mainly dried and consumed by Orientals. It is interesting to note that Japanese consumption of Shiitake per capita is about the same as that of Agaricus in European or North American countries. Japan is by far the world's largest producer of this mushroom. In Japan consumption of dried Shiitake is always higher than that of fresh Shiitake by about 20%.

Flavor and Aroma of Shiitake Mushrooms. The 5′-ribonucleotides have been shown to enhance the flavor of foods. As with L-glutamate (MSG), 5′-guanylate and 5′-inosinate are flavor potentiators, which are the compounds that amplify the effects of other flavor agents. While vegetables are generally low in 5′-nucleotides, ranging 1 to 10 μmol per 100 g fresh weight, mushrooms contain large amounts of these nucleotides. Common mushroom (Agaricus) contains about 50 μmol and fresh Shiitake mushroom from 182 to 235 μmol of 5′-nucleotides per 100 g fresh weight. Its content in the extract of dried Shiitake mushrooms is twice that in the extract from fresh ones. The process of drying not only makes it possible to preserve Shiitake for a long time but also enhances the flavor with a unique taste. Guanylic acid has been identified as the main constituent of the particular good taste of Shiitake extract. An extract containing 1–2 mg of guanylic acid can be obtained from 1 g of dried Shiitake, but a little free guanylic acid can be detected in fresh or dried Shiitake. The increase of guanylic acid content is due to the decomposition of ribonucleic acids by ribonuclease during cooking at 140°F (60°C)–158°F (70°C). Lenthionine ($C_2H_4S_5$) has been identified in the aroma from Shiitake mushrooms (14).

The Chinese believe that Shiitake is effective in the prevention of cerebral hemorrhage. Recently the medical effects of the Shiitake mushroom have been studied by many Japanese researchers, and its remarkable ability to remove serum cholesterol and its antiviral or antitumor activities have been shown through biological tests (15,16).

Drying of Shiitake. Nowadays harvested Shiitake are dried by artificial heating to maintain good flavor and the luster of the cap. Cabinet dryers are generally used for artificial heating. Revolving dryers are suitable for mass production. The mushrooms are dehydrated starting at 86°F (30°C) and the temperature is then increased 2°F (1°C)–4°F (2°C) per hour until a temperature of about 122°F (50°C) is reached (usually in 12–13 h). Finally they are heated to 140°F (60°C) and held there for 1 h to enhance the flavor and bring out the luster of the cap.

Drying of Other Mushrooms. In Europe and South America the most important dried mushroom is *Boletus edulis* (or *B. luteus*), a wild mushroom. In Chile, *B. luteus* is predried in the sun to about 15–20% moisture, held for a short time in temporary warehouses, and then taken to a central drying plant. Here the mushrooms are fumigated, cleaned, and sorted to remove dirt and foreign matter. The mushrooms are then sliced, spread on trays, and tunnel-dried. The drying cycle in Chile is reported to begin with a relatively low air temperature of about 120°F (49°C) which is gradually increased to a final drying temperature of 160°F (71°C). The final moisture content is approximately 10%.

Auricularia auricula-judae, which is referred to as Mu-Erh in Chinese and as Jew's-ear in English, is easy to grow and is preserved mostly in the dried form. The fruiting body can be dried under sunlight or with artificial heating systems. Since the water content is about 90% of a fresh fruiting body, but only 9–15% in the dried state, the weight of the dried Auricularia is only 10–12% of the fresh one. The duration of sun drying depends on the sunlight intensity and usually takes one to several days. If the fruiting bodies are dried in drying sheds with an artificial heating system, such as an electric heater or stove, the duration can be shortened to less than 24 h.

Tremella fusiformis, the white jelly fungus, is also called silver ear by the Chinese. They not only use this mushroom as a drug but consume it as a precious food as well. Because of its high cost it was considered a luxury only for the tables of the rich. The canned form is much less popular than the dried form. It is not necessary to avoid premature opening or discoloration of the fruiting bodies since they do not turn dark. The drying is usually done only by the sun or by an artificial heat source. Drying under the sun alone would not give good results in tropical and subtropical climates. Modern management calls for specially constructed drying sheds with a variety of arrangements, like those used for drying Shiitake. The drying process takes up to 8 h and the temperature is first held at 122°F (50°C), then gradually decreased until it reaches 104°F (40°C). During drying, a strong air flow should be maintained to supply hot air and to maintain an optimal temperature. Weak air circulation and high temperatures will affect product quality. Generally the dried white jelly mushrooms weigh only about 6–8% as much as fresh ones. Some instant-dried silver ear products have been produced recently. The fungus is cooked with sugar syrup prior to drying. This product can be rehydrated with hot water. The best of the dried fruiting bodies are packed in plastic bags and kept in airtight containers for storage. Otherwise there is always a danger of deterioration caused by insects or molds.

BIBLIOGRAPHY

1. W. B. Van Arsdel and M. J. Copley, *Food Dehydration, Vol II, Products and Technology*, AVI Publishing Co., Inc., Westport, Conn., 1964.
2. F. J. McArdle and D. Curwen, "Some Factors Influencing Shrinkage of Canned Mushrooms," *Mushroom Science* **5**, 547 (1962).
3. C. W. Coale and W. T. Butz, "Impact of Selected Economic Variables on the Profitability of Commercial Mushroom Processing Operations," *Mushroom Science* **8**, 231 (1972).
4. R. B. Beelman, G. D. Kuhn, and F. J. McArdle, "Influence of Post-Harvest Storage and Soaking Treatments on the Yield and Quality of Canned Mushrooms," *J. Food Sci.* **38**, 951–953 (1973)
5. F. J. McArdle, G. D. Kuhn, and R. B. Beelman, "Influence of Vacuum Soaking on Yield and Quality of Canned Mushrooms," *J. Food Sci.* **39**, 1026–1028 (1974).
6. R. B. Beelman and F. J. McArdle, "Influence of Post-Harvest Storage Temperatures and Soaking on Yield and Quality of Canned Mushrooms," *J. Food Sci.* **40**, 669–671 (1975).
7. D. L. Eby, F. J. McArdle, and R. B. Beelman, "Post-Harvest Storage of the Cultivated Mushrooms (*Agaricus bisporus*) and Its Influence on Quality Changes Related to Canned Product Yield," *J. Food Sci.* **42**, 22–24 (1977).
8. "Feasibility Study on Mushroom Pretreatment with Vacuumed Solution (VS) in Canning Industry," *1988 Annual Report*, Food Industry Research and Development Institute, Hsinchu, Taiwan, 1989, pp. 36–40.
9. T. E. Odlaug, I. J. Pflug, and D. A. Kautter, "Heat Resistance of *Clostridium botulinum* Type B Spores from Isolates from Commercial Canned Mushrooms," *J. Food Protection* **41**, 351–353 (1978).
10. B. K. Wu, K. C. Lin, and K. M. Chiou, "Studies on Color Improvement and Spoilage Control of Canned Mushrooms. I. Control of Sulfide Spoilage," *Food Industry Research and Development Institute Research Report* **69**, 1–20 (1974).
11. B. K. Wu, Y. H. Lee, K. M. Chiou, K. L. Chang, and T. Y. Liu, "Studies on Color Improvement and Spoilage Control of Canned Mushrooms, II. Improvement in Color of Canned Mushrooms," *Food Industry Research and Development Institute Research Report* **69**, 21–42 (1974).
12. R. C. Anantheswaran, S. K. Sastry, R. B. Beelman, A. Okereke, and M. Konanayakam, "Effect of Processing on Yield, Color and Texture of Canned Mushrooms," *J. Food Sci.* **51**, 1197–1200 (1986).
13. E. Steinbunch, "Quality Retention of Unblanched Frozen Vegetables by Vacuum Packing," *J. Food Technol.* **14**, 321–323 (1979).
14. S. Wada, H. Nakatani, and K. Morita, "A New Aroma-Bearing Substance from Shiitake, an Edible Mushroom," *J. Food Sci.* **32**, 559–561 (1967).

15. T. Mizuno, "Shiitake, *Lentinus edodes*: functional properties for medicinal and food purposes," *Food Reviews International* **11**, 111–128 (1995).

16 T. Kawazoe and K. Yuasa, "Producing vitamin D_2-fortified shiitake mushroom powder in ultraviolet irradiation device," *Journal of Japanese Society of Food Science and Technology* **44**, 442–446 (1997).

BIH KENG WU
Food Industry Research and Development Institute
Hsinchu, Taiwan

MUSLIM DIETARY LAWS: FOOD PROCESSING AND MARKETING

Muslim dietary laws are a set of rules and requirements governing the lives of Muslims, or followers of the Islam religion. The food industry, like any industry, responds to the needs and requirements of consumers. Today's consumers are not only concerned with health and nutrition, they are also conscious of what goes into their bodies as food. Some of these restrictions by consumers are self-imposed philosophical or nutritional choices, whereas others are mandated by their religious beliefs. Muslim dietary laws are based on the Muslim scriptures, the Quran and the Hadith.

Muslims constitute almost 20% of the world population (1). With 1.2 billion adherents, Islam is the world's second-largest religion, after Christianity (2). In a area extending from the Atlantic Coast of Africa to Pakistan and from Central Asia to the Sahara, Islam is the religion of 90% of the population (2), as it is also in Bangladesh and Indonesia. Although the Muslims are concentrated in Asia and North Africa, they are also present as minorities throughout Europe (32 million), North America (5.5 million), Latin America (1.4 million), and even Oceania (0.4 million) (1). The basic tenets of Islam or the primary duties of Muslims are the belief in the oneness of God (Allah) and His messenger (Muhammad); performing the prayers, five times a day; almsgiving, or contributing to the welfare of the poor; fasting (abstaining from eating, drinking, having sex) from dawn to dusk during the 30 days of the month of Ramadan; and a pilgrimage to Makka in Saudi Arabia once in a lifetime, if physically and financially capable (3).

MUSLIM DIETARY LAWS

In addition to the basic duties, a set of guidelines direct the daily life of a Muslim, and these include dietary laws. The dietary laws are composed of the concepts of Halal, Haram, Mashbooh, and Makrooh (4).

What Is Halal?

Halal means lawful or permitted for the Muslims. According to the Quran, all good and clean foods are Halal. Consequently almost all foods from the sea, plants, and animals are considered Halal except those that have been specifically prohibited.

What Is Haram?

Haram means unlawful or prohibited for consumption. The prohibited categories mentioned in the Quran include the following:

- Carrion, or the meat of dead (unslaughtered) animals
- Blood
- Swine
- Animals blessed to others than God (Allah) (ie, to idols)
- Intoxicants, including alcoholic drinks

Additionally, carnivorous animals, birds of prey, and land animals without external ears are prohibited to the Muslims (5). Any products contaminated with Haram items also become Haram.

What Is Mashbooh?

Mashbooh means suspect, in doubt, or questionable. If the origin of a certain food item is in doubt, or there is uncertainty about its permission or prohibition under Islamic laws, then the product is considered Mashbooh. A wide range of products in today's marketplace fall under this category, which forms the gray area between what is permitted and what is prohibited (5).

What Is Makrooh?

Makrooh means detested or discouraged. Products that are discouraged by God or His messenger, Muhammad; are offensive to one's psyche; or may be otherwise harmful fall under this category, including stimulants and smoking (4).

Although the range of Haram and Makrooh foods in Islam is quite narrow, emphasis on observing the prohibitions is very strong. Islam is not oblivious to the exigencies of life, however. Islam permits a Muslim, under the compulsion of necessity, to eat a prohibited food in sufficient quantities to survive (4).

HALAL FOOD PROCESSING

There are in excess of 8,500 grocery items on the shelves of even a small supermarket in North America and Europe, and many new products are being added daily (6). Due to the complexity of food-product development and food processing, an uncertainty exists about the nature and kinds of ingredients present in a particular food. Muslims may either abstain from purchasing such foods or may end up eating what is prohibited. Food processors would need to use the following key points in the production of Halal products (7):

- Products must be from naturally Halal animals, such as cattle, goats, chicken, and the like.
- The animals must have been slaughtered properly, as described later.
- Products must be processed and packed using utensils, equipment, and machinery that has been properly cleaned.

- Products must be free from ingredients that are Haram, Makrooh, or Mashbooh.
- All raw materials used must be Halal.
- Products must be free from cross-contamination and must not come in contact with Haram substances.

Proper Method of Slaughter

There are strict requirements for slaughtering animals: the animal must be a Halal species; the animal must be slaughtered by a Muslim of proper age; the name of Allah must be pronounced at the time of slaughter; and the slaughter must be done by cutting the throat of the animal in a manner that induces rapid, complete bleeding and results in the quickest death (5). Slaughtering should be done with a sharp knife in one swift cut. Certain other conditions should also be observed to ensure humane treatment of animals before and during slaughter. It is required that land animals be slaughtered according to the preceding method. There is no such requirement to slaughter fish or other sea creatures. The meat of animals thus slaughtered is called Zabiha. The term Zabiha, or Dhabiha, implies that the animal has been slaughtered by a Muslim.

Ingredients

For the formulation and manufacture of Halal products, special consideration should be given to the following ingredients (6):

Alcohol: Ethyl alcohol is an intoxicant and is prohibited as such. Many liquid flavors, such as natural vanilla, contain alcohol to satisfy U.S. legal requirements. Use of alcohol in the food and flavor industries is quite common for a number of technical reasons, such as flavor extraction and standardization, ingredient precipitation, or use as a solvent. Although Halal production permits the use of synthetic or grain alcohol in their manufacture, it would be prudent for manufacturers to minimize the levels of alcohol present in the final products through evaporation or other methods. Though there is a small allowance for industrial alcohol, the tolerance is zero for grape wine and other liquors, both in manufacturing and the food-service industries. Many Muslim countries have set up quite elaborate analytical laboratories to test for alcohol in products at their points of entry.

Gelatin: Gelatin is generally made from pork skins, cattle bones, calf or cattle skins, or fish skins. Generally product labels make no mention of the source of gelatin. For Halal products, gelatin must not be porcine. Fish gelatin is Halal, and gelatin from cattle and calves is considered Halal when the animals are slaughtered by Muslims.

Lard: Lard is pork fat and is prohibited to Muslims. Products for Muslim markets must not be formulated with lard.

Enzymes: Enzymes can be from animals, plants, or microbes or produced through biotechnology. The use of enzymes is common in the manufacture of cheese and other foods. Use of porcine enzymes is not permitted in the manufacture of Halal foods. The use of enzymes extracted from animals that are not slaughtered by Muslims is also discouraged by most Muslim countries. Enzymes from plants, microbial sources, and biotech sources are generally considered Halal (see "Ingredients from Biotechnology").

Emulsifiers: Emulsifiers such as mono- and di-glycerides are made from vegetable oils, beef fat, or lard. Since vegetable mono- and di-glycerides are readily available, emulsifiers from beef fat which are Mashbooh and from lard which are Haram can easily be avoided by the food manufacturers.

Ingredients from Biotechnology

Biotechnology covers a wide range of activities and therefore leads to a large number of different applications in the food industry. For Muslims these new technologies open up opportunities for expanding their food supply. At the same time, these new technologies may create some difficulties in making Halal determinations for food ingredients, food products, food materials, and even modified species of animal and plants. Information should be made available to Muslim leaders on concepts and practices in food biotechnology and genetic engineering so they can properly evaluate their Halal implication for foods. In general, biotechnology ingredients and enzyme cultures are accepted by Muslims, with some reservations about certain products (eg, a porcine-derived biotech-produced enzyme) that need to be reviewed on a case-by-case basis (8).

Packaging

The Halal status of packaging materials is also questionable. Though a plastic container may appear acceptable, the source of some of the ingredients used to create the plastic may be of concern. In many cases, stearates from animal sources are used in the production of plastic containers. The formation and cutting of metal cans may require the use of oils, which may be derived from animals (9). Steel drums could have been used to carry foods containing pork or pork fat, and despite rigorous cleaning, these could retain small amounts and contaminate otherwise Halal products.

Marketing

With the global emphasis on food marketing, both food service and retail, people are becoming more accustomed to buying prepared or partially prepared meal items. Presently, two of the strongest import markets for Halal food products are Southeast Asia and the Middle East, accounting for more than 400 million Muslim consumers. A strong emphasis on Halal certification of products in Malaysia, Singapore, Indonesia, Thailand, and the Middle East is shaping the ways in which the exporting countries of Europe, North America, Australia, and New Zealand are modifying their production and marketing practices.

BIBLIOGRAPHY

1. B. Brunner, ed., "Religious Population of the World, 1996," *The TIME Almanac*, Information Please LLC, Boston, Mass., 1998.

2. C. I. Waslien, "Muslim Dietary Laws, Nutrition, and Food Processing," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, Vol. 2, John Wiley and Sons, Inc., New York, 1992, pp. 1848–1850.
3. S. Twaigery and D. Spillman, "An Introduction to Moslem Dietary Law," *Food Technol.* **7**, 88–90 (1987).
4. M. M Hussaini, *The Islamic Dietary Concepts and Practices*, Islamic Food and Nutrition Council of America, Chicago, Ill., 1993.
5. M. M Chaudry, "Islamic Food Laws: Philosophical Bases and Practical Implications," *Food Technol.* **12**, 92–104 (1992).
6. M. N. Riaz, "Halal Food—An Insight into a Growing Food Industry Segment," *Int. Food Marketing and Technol.* **12**, 6–9 (1998).
7. M. M. Chaudry et al., *Halal Industrial Production Standards*, My Own Meals, Inc , Deerfield, Ill., 1996.
8. M. M. Chaudry and J. M. Regenstein, "Implications of Biotechnology and Genetic Engineering for Kosher and Halal Foods," *Trends Food Sci. Technol.* **5**, 165–168 (1994).
9. M. M. Chaudry, "Islamic Foods Move Slowly Into Marketplace," *Meat Processing* **36**, 34–38 (1997).

MUHAMMAD MUNIR CHAUDRY
Islamic Food and Nutrition Council of America
Chicago, Illinois
JOE M. REGENSTEIN
Cornell University
Ithaca, New York

MYCOTOXIN ANALYSIS

Mycotoxins are a heterogeneous class of fungal toxins that contaminate virtually all agricultural commodities. They play an important role in foodborne disease in humans and animals, with toxic effects as variable as their composition. Although it is difficult to prevent mycotoxin contamination in food and feed, it may be possible to reduce exposure through rigorous monitoring programs that require accurate and precise analytical methods. In addition to monitoring and surveying, analytical methods are needed to determine compliance with tolerances and guidelines and for research purposes. A great deal of research has been devoted to developing sensitive, specific, and accurate methods for quantifying mycotoxins in food and feed. The progress of such efforts can be seen in the numerous recent reviews on mycotoxin analysis (1–9) and in the General Referee Report on Mycotoxins that appeared in the January/February issues of the *Journal of the Association of Official Analytical Chemists* (AOAC) *International* (10–14). Several excellent reference books have been written on mycotoxin analysis (15,16).

GENERAL ANALYTICAL PROCEDURES

Analysis of mycotoxins in foods and feeds requires trace analytical techniques because the mycotoxins are typically present in agricultural commodities at levels ranging from ng/g to μg/g. Mycotoxins vary greatly in their structural properties, and thus also their physical properties. Consequently, it is impossible to develop methods that are applicable to all mycotoxins. In addition, analytical difficulties exist since mycotoxins are not evenly distributed in food, and food matrices contribute interferences to sample extracts.

The procedure used to estimate the concentration of mycotoxins in food and feed consists of three steps. First, a random sample is taken from the lot. Second, the entire test sample is ground in a mill or grinder, and a subsample is removed from the ground sample. Finally, mycotoxins in the subsample are extracted with solvent and quantified. An accurate and precise estimate of the true concentration of a mycotoxin in a food depends on all three parts of the analysis (17). Of the three steps, initial sampling is the most critical because it contributes the most to variability in the analytical result. The analytical aspects will usually contribute the least error while sample preparation will usually have an error in between the two (18).

SAMPLING AND SUBSAMPLING

Much literature has been published on procedures for sampling agricultural commodities for contaminants in general (19) and for mycotoxins specifically (20–27), since sampling is one of the most critical aspects of contaminant analysis. Van Egmond (28) tabulated official sampling plans that are used by regulatory agencies throughout the world for monitoring mycotoxin levels in food and feed. Special sampling procedures for aflatoxins have been worked out by the Food and Agriculture Organization (29) and are under preparation by the Codex Alimentarius Commission (30) for control purposes (7).

The major goal of sampling is to obtain a portion that accurately represents the concentrations of individual mycotoxins in a given lot of food or feed. Traditional sampling methods for agricultural crops are usually not adequate for mycotoxin analyses because mycotoxins are rarely evenly distributed within lots of food and feed. Pockets of contamination may be found in bulk storage of grains, oilseeds, oilseed cakes, flours, or ground mixed feeds. These "hot spots" may be due to mold contamination and proliferation in a small portion of the field where the commodity was grown. It also may occur when small containers of contaminated material are added to loads of uncontaminated food. Pockets of contamination can form in stored commodities, especially in areas of high moisture (18).

It is important to design a sampling procedure that will allow the lowest variability that resources will allow. In general, increasing the sample size will result in more reliable analytical results. However, the amount of material collected should be cost effective and manageable for sample analysis. Success at obtaining representative samples of food or feed for analysis depends on several factors: (*1*) the physical characteristics of the product sampled, (*2*) the distribution of the contaminant, and (*3*) the sampling procedure (17). In general, it is easier to obtain representative samples of commodities that are liquids (ie, milk) or can be made into pastes or powders by grinding (ie, peanut butter, flour) than particulate foods such as grains, whole nuts, and mixed feeds (17). Liquids and samples with small particle sizes require little sample preparation because they can be readily made homogeneous by

2. C. I. Waslien, "Muslim Dietary Laws, Nutrition, and Food Processing," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, Vol. 2, John Wiley and Sons, Inc., New York, 1992, pp. 1848–1850.
3. S. Twaigery and D. Spillman, "An Introduction to Moslem Dietary Law," *Food Technol.* **7**, 88–90 (1987).
4. M. M. Hussaini, *The Islamic Dietary Concepts and Practices*, Islamic Food and Nutrition Council of America, Chicago, Ill., 1993.
5. M. M. Chaudry, "Islamic Food Laws: Philosophical Bases and Practical Implications," *Food Technol.* **12**, 92–104 (1992).
6. M. N. Riaz, "Halal Food—An Insight into a Growing Food Industry Segment," *Int. Food Marketing and Technol.* **12**, 6–9 (1998).
7. M. M. Chaudry et al., *Halal Industrial Production Standards*, My Own Meals, Inc., Deerfield, Ill., 1996.
8. M. M. Chaudry and J. M. Regenstein, "Implications of Biotechnology and Genetic Engineering for Kosher and Halal Foods," *Trends Food Sci. Technol.* **5**, 165–168 (1994).
9. M. M. Chaudry, "Islamic Foods Move Slowly Into Marketplace," *Meat Processing* **36**, 34–38 (1997).

MUHAMMAD MUNIR CHAUDRY
Islamic Food and Nutrition Council of America
Chicago, Illinois
JOE M. REGENSTEIN
Cornell University
Ithaca, New York

MYCOTOXIN ANALYSIS

Mycotoxins are a heterogeneous class of fungal toxins that contaminate virtually all agricultural commodities. They play an important role in foodborne disease in humans and animals, with toxic effects as variable as their composition. Although it is difficult to prevent mycotoxin contamination in food and feed, it may be possible to reduce exposure through rigorous monitoring programs that require accurate and precise analytical methods. In addition to monitoring and surveying, analytical methods are needed to determine compliance with tolerances and guidelines and for research purposes. A great deal of research has been devoted to developing sensitive, specific, and accurate methods for quantifying mycotoxins in food and feed. The progress of such efforts can be seen in the numerous recent reviews on mycotoxin analysis (1–9) and in the General Referee Report on Mycotoxins that appeared in the January/February issues of the *Journal of the Association of Official Analytical Chemists* (AOAC) *International* (10–14). Several excellent reference books have been written on mycotoxin analysis (15,16).

GENERAL ANALYTICAL PROCEDURES

Analysis of mycotoxins in foods and feeds requires trace analytical techniques because the mycotoxins are typically present in agricultural commodities at levels ranging from ng/g to μg/g. Mycotoxins vary greatly in their structural properties, and thus also their physical properties. Consequently, it is impossible to develop methods that are applicable to all mycotoxins. In addition, analytical difficulties exist since mycotoxins are not evenly distributed in food, and food matrices contribute interferences to sample extracts.

The procedure used to estimate the concentration of mycotoxins in food and feed consists of three steps. First, a random sample is taken from the lot. Second, the entire test sample is ground in a mill or grinder, and a subsample is removed from the ground sample. Finally, mycotoxins in the subsample are extracted with solvent and quantified. An accurate and precise estimate of the true concentration of a mycotoxin in a food depends on all three parts of the analysis (17). Of the three steps, initial sampling is the most critical because it contributes the most to variability in the analytical result. The analytical aspects will usually contribute the least error while sample preparation will usually have an error in between the two (18).

SAMPLING AND SUBSAMPLING

Much literature has been published on procedures for sampling agricultural commodities for contaminants in general (19) and for mycotoxins specifically (20–27), since sampling is one of the most critical aspects of contaminant analysis. Van Egmond (28) tabulated official sampling plans that are used by regulatory agencies throughout the world for monitoring mycotoxin levels in food and feed. Special sampling procedures for aflatoxins have been worked out by the Food and Agriculture Organization (29) and are under preparation by the Codex Alimentarius Commission (30) for control purposes (7).

The major goal of sampling is to obtain a portion that accurately represents the concentrations of individual mycotoxins in a given lot of food or feed. Traditional sampling methods for agricultural crops are usually not adequate for mycotoxin analyses because mycotoxins are rarely evenly distributed within lots of food and feed. Pockets of contamination may be found in bulk storage of grains, oilseeds, oilseed cakes, flours, or ground mixed feeds. These "hot spots" may be due to mold contamination and proliferation in a small portion of the field where the commodity was grown. It also may occur when small containers of contaminated material are added to loads of uncontaminated food. Pockets of contamination can form in stored commodities, especially in areas of high moisture (18).

It is important to design a sampling procedure that will allow the lowest variability that resources will allow. In general, increasing the sample size will result in more reliable analytical results. However, the amount of material collected should be cost effective and manageable for sample analysis. Success at obtaining representative samples of food or feed for analysis depends on several factors: (*1*) the physical characteristics of the product sampled, (*2*) the distribution of the contaminant, and (*3*) the sampling procedure (17). In general, it is easier to obtain representative samples of commodities that are liquids (ie, milk) or can be made into pastes or powders by grinding (ie, peanut butter, flour) than particulate foods such as grains, whole nuts, and mixed feeds (17). Liquids and samples with small particle sizes require little sample preparation because they can be readily made homogeneous by stirring before the sample is removed for analysis. In general, more heterogeneous samples with large particle sizes require a larger sample size (eg, peanuts) than samples with small particle size (grains). The nature and distribution of the mycotoxin is another important factor in obtaining representative samples (17). Several mycotoxins are very heterogeneously distributed in foods, making sampling difficult. For example, aflatoxin has been shown to contaminate only several kernels in an ear of corn or in a batch of peanuts (21).

Samples may be taken from crops in the field, during handling, during storage, and at other points in the production and processing system (31). The most effective sampling plans involve gathering equal portions at random points throughout a lot of material (17). Of the two methods that exist for collecting samples of contaminated food, the best sampling method is continuous stream sampling. Portions are collected at specific time intervals from a continuous stream of material and combined into a single sample. Often it is necessary to sample commodities held in bins, sacks, rail cars, barges, trucks, or other large containers. Portions are taken from these static populations using hand-operated or mechanical probes (18), then combined to obtain a representative sample of the entire bin of material.

Once a sample has been drawn from a lot, it is often too large to be analyzed. A smaller subsample must be taken from which the mycotoxin is extracted. The same heterogeneity that existed in the lot also occurs in the sample. Therefore, the samples should be ground to proper size and blended before mycotoxins are extracted (22). Ideally, analyses should be completed immediately after sampling. If this is not possible, samples must be stored properly to prevent further fungal growth and formation of mycotoxin. Warm, moist conditions should be avoided. Samples should be frozen or refrigerated in a dry state.

EXTRACTION

After sampling, the next phase of mycotoxin detection involves extraction of the toxin from the food matrix. Officially recognized extraction methods for foods are prescribed by the AOAC INTERNATIONAL Official Methods of Analysis (32) and for feeds by the International Standards of Organization (ISO) publication or the European Economic Community (EEC) directives (5,33–35). The main purposes of extraction are to transfer toxin from the sample to a solvent and to remove unwanted contaminants and interferences (2). The efficiency of extraction of mycotoxins depends on the physiochemical properties of the food matrix as well as those of the toxin.

Mycotoxins are often extracted from food or agricultural products by blending or shaking the sample with appropriate amounts of solvent (6). High-speed blending has the advantage of reducing the sample particle size, which may lead to better extraction (5). The selection of solvent type and ratio of sample to solvent volume depends on the mycotoxin of interest and the source material. The solvent-to-sample ratio is usually 2–5 mL:1 g sample. Most mycotoxins are readily soluble in organic solvents but sparingly soluble in water (5). Typical extraction solvents include chloroform, ethyl acetate, methanol, acetone, acetonitrile, and mixtures of these organic solvents and water. The water wets the substrate and increases penetration of the solvent mixture into the hydrophilic matrix (16). The aqueous phase can be acidified to release the toxin from interactions with proteins in the food. Sodium chloride or other inorganic salts are often added to the aqueous phase to reduce emulsion formation. In addition to liquid solvents, supercritical fluids (eg, CO_2 with methanol or acetonitrile as modifiers) have been successfully used to extract aflatoxin (36) and trichothecenes (37,38) from grain and feed. Nonpolar solvents such as hexane or 2,2,4-trimethylpentane can be used to defat samples of high-lipid content before extraction.

After extraction, filtration is often necessary to remove unwanted solids. When using highly nonpolar solvents, a common practice is to add a diatomaceous earth filter aid to assist in filtration. Centrifuging extracts prior to cleanup is a common practice when filtration difficulties arise (5).

CLEANUP METHODS

Some analytical methodologies, that is, immunoassays, require minimum sample cleanup for quantitation of mycotoxins. However, in most cases, extensive cleanup of agricultural commodities is needed before the final analysis is feasible. Removal of interfering substances is a key element in obtaining accurate quantitative results and is normally the most time-consuming stage in the analysis of mycotoxins. The choice of sample cleanup method depends on the type of mycotoxin and matrix, the expected concentration of the mycotoxin, and the available final analytical method used for detection and quantitation (5,6). Four varieties of cleanup methods have been used: dialysis, anionic precipitation, liquid–liquid partitioning, and column chromatography. For some samples one cleanup step is enough, but others may require a combination of two or more cleanup procedures before quantitation.

Dialysis cleanup procedures in combination with liquid–liquid partitioning have been developed for a variety of mycotoxins (39,40). The aqueous anionic precipitation method of cleanup is used mainly for extracts in polar organic solvents containing more than 55% water (5). Anionic precipitation involves coprecipitating plant pigments and proteinaceous substances with various reagents; lead, zinc and ammonium salts, phosphotungstic acid, iron, and copper compounds. Liquid–liquid partitioning in conjunction with column cleanup has been used to separate complex mixtures of mycotoxins having different physiochemical properties. Acids or bases can be added to the aqueous phase to facilitate separation of mycotoxins possessing strong functional groups such as the phenolic zearalenone and zearalenol (41), or acidic mycotoxins penicillic acid and ochratoxin (5). Use of liquid–liquid partitioning is declining because it requires large sample sizes and large volumes of potentially hazardous solvents.

Column chromatographic methods are the most widely used cleanup procedure. These methods include classical open column chromatography and chromatography using small disposable prepacked columns, also known as solid-phase extraction columns. In both types of column chromatography, packing materials can be normal or reverse-phase silica, alumina, polyamide, XAD-2, ion exchange, Sephadex®, charcoal, Florisil® and immunoaffinity. Available bonded phases for reverse-phase columns include ethyl, octyl, octadecyl, cyclohexyl, and phenyl.

Classical column chromatography has been used for purifying mycotoxins present in food and feed. Different modes of chromatography such as adsorption, partition, ion exchange, size exclusion, and affinity chromatography have been used (42). Due to the large amounts of solvent needed and the cost of waste solvent disposal, classical column chromatography is not commonly used today. One of the most widely used screening methods for mycotoxins (aflatoxins, ochratoxin A, zearalenone) uses prepacked cartridges containing one or more layers of different adsorbents to purify toxins before analysis (32,43–46). Prepacked, disposable solid-phase extraction (SPE) columns (cartridges) are increasingly being used for sample cleanup. The use of these cartridges saves time, uses less solvent than traditional liquid–liquid extraction or classical column chromatography, and is ideally suited to the analysis of large numbers of samples. The prepacked cartridges are reported to be more consistent than laboratory-packed columns, although batch-to-batch variations can occur. Consequently, recovery trials should be carried out on all batches of columns (5). SPE cleanup procedures have been developed for ochratoxin (47), trichothecenes (48,49), aflatoxins (50,51), fumonisins (52,53), and other mycotoxins. Recently, SPE disks requiring even less solvent than SPE cartridges have been developed (54) and were used for isolating zearalenol and zearalenone from corn extracts (55).

An important new cleanup technique is the use of antibody-based immunoaffinity columns (IAC). Advantages of IAC over other cleanup methods include specificity, speed, low solvent use, possibility of automation, and possibility of column reuse (56). Specific antibodies to a number of mycotoxins (aflatoxins B_1, B_2, G_1, B_2, M_1; fumonisins B_1 and B_2; ochratoxin A; deoxynivalenol; and zearalenone) have been developed (56) and are the basis of commercially available IAC. Among the commercial IAC, the Aflatest P is finding widespread use in the analysis of corn, peanuts, and complex foods and feeds (56). The Aflatest P test kit, which includes a fluorometer for quantitation, has been certified by the AOAC as an official method (32,57). Mycotoxins are extracted from a sample with aqueous methanol or acetonitrile. The extract is diluted further with water or buffer, and a portion of the diluted sample is passed through the column. The column is then washed with water to remove interferences. The purified mycotoxin is then eluted using methanol or acetonitrile. Quantification of the toxin can be accomplished using fluorometry or a variety of chromatographic methods.

Several studies have shown that IAC could be reused without loss of efficiency (58–60). Currently, multiple antibody IAC are under development to allow simultaneous separation of chemically diverse toxins from the same extract (56). Scudamore et al. (61) linked IAC in series to determine aflatoxin and ochratoxin levels in a variety of dry cereal-based pet foods. Maragos et al. (62) developed an IAC that isolated fumonisins B_1, B_2 and hydrolyzed forms of these toxins.

After cleanup procedures are complete, mycotoxins are present at low concentrations in large volumes of solvent. Therefore, concentration is required before analysis. This is usually done by heating at low temperatures in a water bath, temperature-controlled heating block, or a rotary evaporator (5). Evaporation under nitrogen is recommended for six mycotoxins listed in the *AOAC International Official Methods of Analysis* book (32), and exposure to light should be minimized.

ANALYSIS: SEPARATION, DETECTION, QUANTITATION

General Considerations

Choice of Analytical Method. The choice of analytical method used to separate, detect, and quantify mycotoxins depends on the physiochemical properties of the mycotoxin, concentration of the mycotoxin in the food or feed, cost and time constraints, and availability of analytical methodology and instrumentation. Early studies used biological methods, for example, animal toxicity assays, to detect the presence of mycotoxins in food and feed. With the development of sophisticated instrumentation, chemical methods became the method of choice for mycotoxin analysis. For mycotoxins that contain a chromophore having high molar absorptivity and/or high fluorescent properties, such as aflatoxin, zearalenone, patulin and ochratoxin, sample extracts are usually subjected to either thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC). For both methods, the compound of interest is identified by comparing the R_f (a measure of migration distance) or retention time to that of an analytically pure standard of the compound. Quantitation is accomplished by determining the fluorescence intensity or UV absorbance of an elution spot or an HPLC peak. Mycotoxins that do not contain a distinctive chromophore, for example, fumonisin and some of the trichothecenes, can be derivatized and analyzed by HPLC or gas chromatography (GC). Alternatively, these compounds can be analyzed directly by gas chromatography–mass spectrometry (GC/MS) or liquid chromatography–mass spectrometry (LC/MS).

Analytical Standards. Essential to mycotoxin analysis is obtaining pure analytical standards for the compound of interest. While standards for the most common mycotoxins are commercially available, newly discovered or uncommon toxins are often available only from the researchers who originally purified and identified the compounds. Analytical criteria of important mycotoxins were published by the Food Chemistry Commission of the International Union of Pure and Applied Chemistry (63). In addition, several extensive tabulations of chemical, physical and spectra characteristics of mycotoxins have been published by Cole and Cox (64) and Savard and Blackwell (65). Purity of primary standards should be determined by at least two analytical methods. If possible, concentration of standards in solution should be determined spectrophotometrically rather than gravimetrically. Some mycotoxins can be chemically labile, consequently, stability of the pure standard in solvent should be determined. Proper storage of the standard is essential, and periodic check of stability during storage should be determined. Since all mycotoxins are potentially hazardous, care should be taken while working with the pure compounds, and all manipulations should be performed in a hood or glove box (5,32).

Confirmation Methods. Interfering compounds can be present in sample extract even after exhaustive cleanup procedures are employed. Consequently, confirmation methods are needed to establish the true identity of the analyte. There are two main techniques for confirmation of identity: chemical derivitization and mass spectrometric analysis. Pre- and postcolumn derivatization of mycotoxins can be used to confirm the identity of mycotoxins analyzed by HPLC. For example, fumonisin previously analyzed by derivitizing with σ-phthaldialdehyde (OPA) can be analyzed using another derivitizing agent, for example, naphthalene dicarboxaldehyde (NDA). Diode array detectors are commonly available HPLC detectors that can be used to confirm the identity of mycotoxins having characteristic UV spectra. Using several detectors in tandem is another excellent confirmatory method (5). Numerous chemical methods for confirming the identity of aflatoxins and other mycotoxins exist with TLC. Various reagents can be sprayed onto developed chromatograms, and the reactions can be compared to those with pure standards (66,67).

The most powerful method for confirming the identity of mycotoxins is by mass spectrometry (MS), and numerous methods exist for the identification of mycotoxins by GC/MS (68–71) or LC/MS (72–77). The mass spectra of compounds eluting from a GC or HPLC column can be used to aid in the structural determination of unknowns or provide unequivocal identification of peaks (6). Selectivity can be increased by using select ion monitoring (SIM) that is unique to the analyte of interest. The affordability of GC/MS and LC/MS systems allows most modern laboratories to be equipped with this powerful analytical tool.

CHEMICAL METHODS

Chromatography

Introduction. In the past ten years, chromatography has been the most studied technique for detection, analysis, and characterization of mycotoxins. Chromatographic methods employed for these purposes include TLC, GC, HPLC, and supercritical fluid chromatography (SFC). A book by Betina (16) and reviews by Shephard (78) and Dorner (6) are excellent references on the use of chromatography for mycotoxin analysis.

Thin-Layer Chromatography. Despite the fact that many other techniques have been increasingly applied in the determination of mycotoxins in food and feed, TLC is still one of the most popular because of its relative simplicity and low operating cost. Other advantages are that it allows simultaneous analysis of many extracts, offers great selectivity, and makes multitoxin analysis possible (6). As with other chromatographic techniques, TLC requires extensive sample cleanup to remove interferences. Several excellent reviews have been published on TLC methods used for mycotoxin screening and quantitation (67,79–83).

The choice of type of TLC plate adsorbent and mobile phase depends on the chemical characteristics of the analyte as well the matrix from which it was extracted. Adsorbents used for normal-phase TLC include silica gel, aluminum oxide, and cellulose, whereas reverse-phase TLC plates have a layer of silica gel bonded to various nonpolar functional groups including ethyl, octyl, octadecyl, and phenyl. The majority of TLC plates used for mycotoxin analyses are glass or plastic supports coated with silica gel. Other substances, such as oxalic acid, sulfuric acid, or glycolic acid, can be incorporated into the silica gel layer to aid in the separation of acidic mycotoxins (citrinin, cyclopiazonic acid) (8). TLC plates can be prepared in the laboratory, but most frequently, they are purchased as precoated plates since they are more uniform and reproducible.

Sample extracts are applied to the TLC plate with a micropipet or syringe, and the plates are then placed in a mobile phase for chromatographic development. During development, components of the extract partition between the stationary and mobile phases. Mobile phases depend on the mycotoxins to be separated, the type of adsorbent, and the presence of compounds in the extracts that interfere with the analyte (6). In normal-phase TLC, the major component of the mobile phase is usually a relatively nonpolar solvent (chloroform, ethyl ether, or ethyl acetate). Polar solvents (acetone, alcohols, water, or organic acids) are usually added to achieve the desired separation of the analytes. With reverse-phase TLC, the mobile phase is usually composed of water in combination with methanol or acetonitrile. Development of plates is usually carried out with one mobile phase. However, use of more than one solvent can be used to improve resolving power (6).

When sample extracts contain few interfering substances, one-dimensional TLC is sufficient for separation and quantitation of analytes. Using this method, many sample extracts can be simultaneously analyzed (81). When cleanup is insufficient to remove interferences, the use of two-dimensional TLC (2D-TLC) can improve resolving power. In 2D-TLC, the plate is dried after developing in the first mobile phase, then it is rotated 90° and developed in a different solvent. 2D-TLC has been applied to the analysis of aflatoxins, ochratoxin A, and citrinin (81).

To visualize the mycotoxin spots on TLC plates, the plates are either examined under UV light for fluorescent mycotoxins such as aflatoxins, zearalenone, sterigmatocystin, penicillic acid, citrinin, and ochratoxin A, or they are sprayed with a chemical reagent that reacts with the mycotoxins to produce a colored or fluorescent derivative (83). Under long-wave UV light (365 nm), aflatoxins appear as blue, while zearalenone, citrinin, sterigmatocystin, and penicillic acid appear as blue-green, yellow, red, and purple spots, respectively (81). Aluminum chloride has been used to make fluorescent derivatives of deoxynivalenol (84) and sterigmatocystin (85), while ammonia was used to increase the fluorescence of ochratoxins (86) and patulin (87). Nonfluorescent mycotoxins are detected by chemical derivatization. For example, T-2 toxin can be detected by spraying plates with a mixture of sulfuric acid and methanol, then heating the plate for several minutes at 110°C (81). Mycotoxins are quantified by comparing the intensity of the fluorescence of the sample spot with that of a series of standards. Visual comparison has been widely employed for mycotoxin screening. More accurate quantitation is achieved instrumentally with a densitometer. Detection limits for TLC are in the range of pg/g to ng/g depending on the analyte, source of contamination, and cleanup method (83).

The development of high-performance TLC (HPTLC) in recent years has kept TLC competitive with other more sophisticated techniques. Plates are smaller than conventional TLC plates, and they are uniformly coated with a 0.1- to 0.3-mm layer of small particle size (2–10 μm) adsorbents (82). The small particle size results in rapid separation of analytes. HPTLC uses automatic sample application equipment for spotting small volumes of test sample on plates and a densitometer to quantify analytes. Detection limits for aflatoxins using HPTLC have been reported in the low picogram range (82).

High-Performance Liquid Chromatography. One of the first publications describing the use of HPLC to analyze mycotoxins in food was written by Seiber and Hseih (88). Since then HPLC has become the method of choice for quantifying mycotoxins in food and feed. HPLC is similar to TLC in principle, but it offers the analyst greater resolution, sensitivity, accuracy, and precision (25). HPLC allows ultratrace analysis of mycotoxins; some analytes can be determined at the nanogram or even picogram level. Other advantages of HPLC are its ability to analyze thermally labile, poorly volatile, polar, and ionic compounds. The introduction of autosamplers, computerized data retrieval systems and a variety of sensitive detectors make HPLC very useful for large-scale analyses. Important limitations of HPLC include the high cost of instrumentation and the lack of a sensitive universal detector. Like most of the other chromatographic methods, rigorous cleanup is usually required to achieve accurate, reproducible results. Reviews on the use of HPLC to analyze mycotoxins have been written by Shephard (78), Coker and Jones (89), Frisvad and Thrane (90), Shepherd et al. (91) and Kuronen (92).

The versatility of HPLC makes it ideal to analyze chemically diverse compounds such as the mycotoxins. This is mainly due to the advances made in the past decade in the chemistry of adsorption materials for column packings. Although use of normal-phase silica columns was initially favored for analysis of some of the mycotoxins, reverse-phase chromatography is currently being used most often for aflatoxins (93–97), zearalenone (41,98,99), nivalenol and deoxynivalenol (100), citrinin (101), ochratoxin (102), fumonisins (52,53), *Alternaria* toxins (103–105), patulin (106,107), and sterigmatocystins (108). There have been some reported use of ion-exchange chromatography (IEC) in the determination of moniliformin (109).

Several detectors are available for use with HPLC, which provides for great selectivity and sensitivity. Unlike GC, there is no truly effective universal detector for HPLC. Refractive index (RI) detectors lack sensitivity, which limits their use in mycotoxin analysis. An evaporative light-scattering detector (ELSD) is another universal detector that can be used for the detection of low-volatility components in purified extracts. As with RI detectors, ELSDs lack the sensitivity needed for detection of most mycotoxins. A literature search found only one published method for fumonisins (110).

As most mycotoxins absorb UV light, UV detectors are commonly used detectors for mycotoxin analysis. The photodiode array (PDA) detector has proven to be a powerful HPLC detector for detecting and quantifying mycotoxins. The advantage of PDA detectors is that they provide both multiwavelength and spectra information in a single chromatographic run (42). In combination with computer searching capabilities, it is possible to monitor the spectra of eluting components, allowing further identification of mycotoxins.

The fluorescence detector is commonly used to detect mycotoxins that fluoresce or fluoresce when derivitized. Fumonisins, a recently discovered group of mycotoxins, contain an amino group and several carboxylic acid groups that can be derivitized with fluorogenic reagents such as *o*-phthaldialdehyde (OPA) (52), naphthalene dicarboxaldehyde (111), or *N*-hydroxysuccinimidylcarbamate (112). The trichothecenes and sterigmatocystin are other examples of mycotoxins that are derivatized with fluorescent compounds (113,114). Fluorescence detectors, which are highly selective and more sensitive than UV detectors, are the detectors of choice for aflatoxin and fumonisin analyses. In addition to UV and fluorometry, electrochemical detectors have been used for the detection of several mycotoxins (115–117).

Development of LC/MS techniques has been the subject of considerable study since its development in the late 1960s (118). Since that initial work LC/MS interfaces have undergone considerable development and improvement. First (direct liquid introduction, moving belt, particle beam) and second generation (thermospray, electrospray) interfaces have led the way to the generation of so-called atmospheric-pressure ionization (API) interfaces. The API techniques that comprise chemical ionization and other ionization techniques (electrospray and ionspray), have opened a new window in LC/MS, allowing analysis of either slightly polar analytes of low to medium molecular mass or more polar analytes and ions in solutions of medium to high molecular mass (75). Instruments have become smaller (benchtop size), less expensive, more rugged, and compatible with commonly used HPLC mobile phases and flow rates (75). Reports exist on the use of LC/MS for determination of such mycotoxins as aflatoxins (72), trichothecenes (73,74), zearalenone (75), ochratoxin (76), and fumonisins (77,119).

Gas Chromatography. GC is the method of choice for some mycotoxins that exhibit little or no UV absorption or fluorescence. GC methods have been published for virtually all mycotoxins, although their greatest utility lies in the analysis of the trichothecenes (6,119). With GC, extracts containing the analytes are first subjected to vigorous cleanup procedures. The next phase involves volatilizing the analytes. Most mycotoxins are not volatile at GC temperatures (30–350°C), and must be derivatized to a volatile form. The functional group in mycotoxins allowing derivatization is in most cases a hydroxyl group, and the derivatives formed are usually trimethylsilyl (TMS) ethers or heptafluorobutyryl (HFB), pentafluoropropionyl (PFP), or trifluoroacetyl (TFA) esters (120). Onji et al. (121) have described a method for analyzing several *Fusarium* mycotoxins including deoxynivalenol, 3-acetyldeoxynivalenol, fusarenon-X, diacetoxyscirpenol, 15-monoacetylscirpenol, T-2 toxin, scirpentriol and zearalenone without derivatization.

Packed and capillary GC columns are used for the separation of analytes. Packed columns are usually borosilicate glass tubes that are packed with an inert support that is coated with the polymer to be used as the stationary phase. With capillary columns, fused silica tubing is coated with thin films of stationary phase. Capillary columns offer great efficiency, which explains why the majority of methods published for the determination of trichothecenes used capillary columns (6).

Several types of detectors are available for GC, but for mycotoxin analysis, the flame ionization detector (FID), the electron capture detector (ECD), and the mass spectrometer (MS) are the most common. The FID detector is considered the universal GC detector because it detects all organic compounds. Although rugged and sensitive, FID detectors are not at all selective and require rigorous cleanup of extracts to obtain reliable results (6). The ECD is a more selective detector than the FID, but it is not necessarily more sensitive. ECD gives strong signals for halogenated compounds but not for hydrocarbons (6). MS is the ultimate detector for use with GC; it is sensitive, selective, and enables confirmation of the identity of eluting compounds. Selectivity can be enhanced by selecting SIM mode to monitor a single ion mass. GC/MS is a powerful tool especially for the analysis of trichothecene mycotoxins (69–71). Several recent publications (70,122) describe the use of GC/fourier transform infrared (FTIR) spectroscopy to aid in the characterization and identification of *Fusarium* mycotoxins. The use of GC/FTIR together with GC/MS provides a very powerful combination for the identification of a variety of mycotoxins that represent diverse structure types in food or feed extracts.

Supercritical Fluid Chromatography. Supercritical fluid chromatography (SFC) uses a mobile phase with the solvating power of a liquid and the diffusivity and viscosity of a gas to separate nonvolatile or thermally labile compounds (123). The combination of SFC with MS allows the analysis of compounds which require derivatization prior to GC/MS. Young and Games (124) used SFC on packed HPLC columns combined with UV and a moving belt MS to separate and identify some *Fusarium* mycotoxins (deoxynivalenol, isodeoxynivalenol, 3-acetyldeoxynivalenol, 3,15-diacetyldeoxynivalenol, calonectrin) in liquid culture extracts. The SFC separations were rapid, and detection limits for a variety of mycotoxin standards analyzed by MS were from 10 to 250 mg. Roach et al. (123) described a method for analyzing trichothecenes by SFC-negative ion chemical ionization MS. A disadvantage of SFC/MS is that the sensitivity is lower than GC/MS or LC/MS. In all, SFC is in its infancy due to high instrument costs compared with other analytical equipment.

CAPILLARY ELECTROPHORESIS

Capillary electrophoresis (CE) is capable of separating several charged and water-soluble molecules in a single analysis. The method often has similar cleanup requirements as available HPLC methods. An advantage of CE over HPLC is the elimination of organic solvents for the determinative step (125). Using a wide range of methods, CE has been used to separate and quantify aflatoxin, fumonisins, ochratoxin A and B, zearalenone, moniliformin, and α-cyclopiazonic acid with varying success (125–128). Maragos (127) and Maragos and Greer (125) described procedures for analyzing aflatoxin B_1 and fluorescein isothiocyanate-labeled fumonisin in corn using CE with laser-induced fluorescence detection. They reported that the limits of detection for these two toxins obtained by CE were as good as if not better than, the detection limits obtained by HPLC.

IMMUNOCHEMICAL METHODS

General Aspects

Within the past decade numerous reports have been published concerning the use of immunoassays (IAs) for mycotoxin analysis. IAs are rapid, simple, specific, sensitive, and portable and require little analyst training or skill (129). Because IAs are extremely sensitive, often in the picogram/milliliter range, simple dilution of the sample can often remove the need for extensive sample cleanup. However, with most food matrices, some form of extraction of the sample is needed. Three IAs that have been developed for mycotoxin analysis include the radioimmunoassay (RIA), the enzyme-linked immunosorbent assay (ELISA), and the IAC assay. The RIA and ELISA analyses are based on the competition of binding between unlabeled mycotoxin in the sample and labeled mycotoxin in the assay for the specific binding sites of antibody molecules (130). The IAC assay involves the use of antibody columns that specifically trap the mycotoxins in a sample. Trapped mycotoxin can then be eluted and quantified. Some IAs require specialized equipment, such as ELISA readers, tube readers, scanners, or fluorometers for quantitation, while others rely on visual quantitation (129).

Chu (131) listed four main criteria that need to be considered when choosing an IA for mycotoxin analysis. First, a well-labeled mycotoxin (as a marker) is needed in the assay system in addition to a specific antibody. Second, a good method for the separation of free and bound forms of mycotoxin is needed. Third, the degree of specificity of the antibody in the assay should be known. Finally, the sample matrix should be tested before the assay to determine if structurally related compounds in the sample react with the antibody.

Various commercial kits using immunochemical techniques such as ELISA and IAC assays are now available for several mycotoxins including aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, and zearalenone (129). Such commercial tests will find universal acceptance if officially listed to be at least comparable with present analytical methods. Such assessment is currently under way with the AOAC International in the United States and the Ministry of Agricultural, Food and Fisheries (MAFF) in the United Kingdom (132). Several quantitative IA methods have been adopted as AOAC International Official methods for mycotoxins (10,129,130,133).

The development of recombinant antibodies and the making of reusable IAC should reduce the cost of IA and make it one of the more important techniques in the analysis of mycotoxins. In addition, recently published methods (134,135) have indicated that the possibility exists for quantifying several mycotoxins in the same IA. Several excellent reviews have been written on IA in general (132,136) and on the use of IA in the analysis of mycotoxins (56,58,129,137–140).

Radioimmunoassay (RIA)

Although the RIA is not used routinely for mycotoxin analysis, it is currently used by some laboratories for research purposes. RIA has been used to detect aflatoxins (140), nivalenol (141) deoxynivalenol (142), ochratoxin A (143), and T-2 toxin (144,145) in food.

In RIA, sufficient radiolabeled analyte (^{125}I, ^{14}C or ^{3}H) is added to an antibody such that 50% of the label becomes antibody bound. A proportion of this label is then displaced when a known concentration of standard analyte or an unknown amount of sample analyte is added. Activated charcoal is then used to remove the antibody-free fraction. The amount of analyte can be calculated by comparing the concentration of isotope in either the supernant fluid or the residue to a standard curve (146). Detection limits for purified mycotoxin are 0.25 to 0.5 ng when tritiated mycotoxins are used as markers (131). The sensitivity of the method can be improved by using radioactive markers of high specific activity or using a simple cleanup step after extraction. RIA has several drawbacks, including the instability and expense of some radioisotopes as well as the hazards in handling isotopes (146).

Competitive Enzyme Linked Immunosorbent Assay (ELISA)

Of the several available IA techniques, the most popular is the ELISA. Two different ELISA formats have been used for the analysis of mycotoxins, both of which involve the separation of free toxin in one phase from the bound toxin in another phase (130). In direct competitive (DC) ELISA, the mycotoxin-specific antibody is immobilized in the wells of a microassay plate or on the wall of an assay tube. Sample extracts and mycotoxin standard solutions are pipetted into the antibody-coated wells or tubes with a fixed amount of enzyme-labeled mycotoxin. The plates or tubes are incubated between five minutes and more than an hour, which allows the labeled and unlabeled mycotoxin to compete for binding sites on the antibodies. The unreacted material is then washed away and the amount of labeled analyte bound by the immobilized antibody is quantified by addition of an enzyme substrate that forms a colored product. The amount of color formed is inversely proportional to the amount of unlabeled mycotoxin in the sample (136). To avoid sample matrix interferences that can occur with DC ELISA, the sample can either be diluted or subjected to a cleanup step.

In indirect competitive (IDC) ELISA the mycotoxin is coupled to a carrier protein such as bovine serum albumin and bound to the wells of a microtiter plate or assay tube (136). The sample extract is added to the microassay well followed by addition of a fixed amount of mycotoxin specific antibody. The antibody in solution partitions between the free mycotoxin in the sample and the toxin bound by the microassay plate. The antibody not bound to the microassay plate is washed away and quantified by addition of a second antibody that specifically binds the first antibody. An enzyme substrate is added, and the amount of color formed is proportional to the amount of analyte in the sample extract. A major advantage of IDC ELISA is that it requires much less antibody than DC ELISA (130). One drawback of IDC ELISA is that it requires a longer analysis time than DC ELISA (131).

Several studies have investigated the accuracy and precision of ELISA by comparing the technique with other analytical methods. In general, excellent correlations have been found between results obtained by ELISA and TLC, HPLC, or GC for aflatoxins (147,148), deoxynivalenol (149), and fumonisins (150,151). However, fumonisin results obtained by ELISA can be higher than those determined by chromatographic methods (151–153). This phenomenon is due to the presence of structurally related compounds that cross-react with the antibodies. Use of antibodies of higher affinity to the analyte may prevent this problem (131).

In recent years, ELISA kits have been made available from commercial sources for quantitative and semiquantitative analysis of aflatoxins, deoxynivalenol, fumonisins, ochratoxin, and zearalenone in food and feed (129). The kits usually are self-contained and include all the reagents: standards, controls, buffers, and substrates. Several commercial ELISA kits have been approved as AOAC official methods for aflatoxin and zearalenone (32,129). A membrane-based visual dipstick enzyme immunoassay was developed for analyzing up to five mycotoxins simultaneously (134).

Immunoaffinity Columns

IACs are prepared by adsorbing immunospecific antibodies onto a gel support contained in a plastic column or cartridge (131). The sample extract is loaded onto the column, and as the extract is forced through, the analyte is captured by the antibodies. Impurities not bound to the antibodies are washed from the cartridge. Mycotoxins are then desorbed with methanol and quantitated by HPLC or by fluorometry with derivitization, if needed. Advantages of IAC over ELISA methods include selectivity, the ability to trap toxins from large volumes of sample extract, and the ability to combine with different analytical techniques. The cost of columns can be high (>$10.00 each), which can make analysis of many samples expensive (129). However, several reports have indicated that IAC could be reused, in some cases more than 100 times (58–60,129). Several investigators used IAC for analyzing several toxins simultaneously. Maragos et al. (62) developed a single IAC that simultaneously isolated fumonisin B_1 and B_2 and hydrolyzed forms of these toxins. In contrast, Scudamore et al. (61) linked two IACs in series to purify aflatoxins and ochratoxin A.

IACs are available from commercial sources for cleanup of aflatoxins B_1, B_2, G_1, G_2 and M_1, deoxynivalenol, fumonisins B_1 and B_2, ochratoxin A, and zearalenone. Antibodies against other mycotoxins (citrinin, patulin, cyclopiazonic acid, kojic acid, sterigmatocystin, and several others) have been produced. However, no reports have indicated the use of IAC for purification of these mycotoxins (129). IAC purification and SPE gave generally similar results for aflatoxins (154) in milk, corn, and sorghum, but higher recoveries were obtained when IACs were used to isolate fumonisin B_1 from canned and frozen corn (155).

BIOLOGICAL METHODS

General Concepts

Historically, biological assays have played a vital role in the detection and isolation of unidentified fungal metabolites in food and feed. In the classic studies of the early 1960s (156), a duckling bioassay was an instrumental tool in the isolation and purification of aflatoxin from groundnut meal. Since then biological assays have aided in the characterization of most mycotoxins. In general, chemical assays are preferred over biological assays for mycotoxin analysis because they are more rapid, reproducible, and specific. However, chemical analysis cannot be used until the toxin has been chemically characterized, standards purified, and appropriate methodology developed (157).

Before using a bioassay as a routine procedure for monitoring mycotoxins, several factors should be considered (157). First, the bioassay should be able to respond to a diverse range of mycotoxins. Second, the sensitivity and repeatability of the assay must be acceptable. Third, the cost of the assay should be relatively low. Fourth, the results should be obtained in a short time (preferably less than 24 h). Fifth, blanks should be included to determine if toxic effects occur with solvents or carrier reagents. Finally, the occurrence and frequency of false positives should be minimal to none (157). Bioassays can be classified by the organism used—microorganisms, animals, and plants. Vertebrate animals are the most dependable for detection of vertebrate toxins and they include intact animals, skin tests, and chick embryo assays. Bioassays can be highly specific or general, and the choice of type depends on the application. Excellent reviews on the use of biological assays for screening mycotoxins were written by Yates (158) and Cole et al. (157).

Microbiological Assays

The discovery that mycotoxins have antimicrobial properties has led to the development of mycotoxin screen assays using microorganisms (bacteria, fungi, and yeast). Surveys were carried out on numerous microorganisms to demonstrate which were sensitive to mycotoxins. Of those microorganisms tested, *Bacillus* spp. have been shown to be well suited for the detection of aflatoxin B_1 (159,160), patulin (161), penicillic acid (162), ochratoxin (163), and roquefortin (164). One of the most extensive testings of mycotoxins on a bacterial species was carried out by Boutibonnes et al. (165) on *B. thuringiensis*. Virtually all of the mycotoxins tested induced some toxic effect, indicating that the organism may be a good candidate for mycotoxin screening. Mycotoxin screening methods for mycotoxins were developed using *B. megaterium* (166), *B. cereus* (163), *B. stearothermophilus* (164,167), and *B. subtilis* (161). Yeasts such as *Kluyveromyces fragilis* and *Rhodotorula rubra* have been used to assay trichothecene mycotoxins (168,169).

Many of the mycotoxins have been subjected to the Ames mutagenicity assay (170) of which the aflatoxins, fusarin C, and some *Alternaria* toxins are reported to be mutagenic to at least one strain of *Salmonella typhimurium* (171). Other organisms that have been used to screen mycotoxins for mutagenicity include *B. subtilis* mutants, *Saccharomyces cerevisiae*, DNA polymerase deficient *Escherichia coli* and *Neospora crassa* (158). Advantages of mutagenicity assays include their ability to yield semiquantitative data and their sensitivity and simplicity.

Animal Assays

Vertebrates. Laboratory animals, especially mice and rats, have been successfully used for the detection and isolation of mycotoxins including aflatoxins, zearalenone, rubratoxins, and aflatrem (157). Advantages of using mice are that they are small, commercially available, and relatively inexpensive. Ducklings and chickens have been used successfully to detect ochratoxin A (172), α-cyclopiazonic acid (173), tremorgenic mycotoxins (174–176), moniliformin (177), and some trichothecenes (178). One of the most sensitive bioassays for aflatoxin is the production of liver carcinoma in rainbow trout (179). However, the test is difficult to run and is impractical as a screening method because it takes more than four months to complete (157).

Several specific animal tests exist for the trichothecene mycotoxins. In the skin assay, a test solution is applied to the back skin of an experimental animal such as a rabbit, rat, or guinea pig, and the area is monitored for skin lesions. Sensitivities for this bioassay have been reported to be 0.1 μg using T-2 toxin (180). Another trichothecene bioassay involves the rejection or acceptance of drinking water by mice (181). The test sample is dissolved in distilled water that is presented to mice. The volume of the contaminated water consumed by the animals is compared with the volume of control water consumed. A positive test occurs when the mice refuse to drink, indicating the presence of trichothecene.

A specific bioassay for zearalenone is based on the hyperestrogenic effects of the mycotoxin (157). Swine are the most sensitive animals, although dairy cattle, lambs, chickens, turkeys, and laboratory animals (rats, mice, guinea pigs, and monkeys) are also affected. The rat uterotopic bioassay has been used as an assay for zearalenone if other analytical methods are unavailable (182).

The only vertebrate bioassay adopted by the AOAC is the chick embryo assay also known as the CHEST (chick embryo toxicity screening test) assay (157). The assay involves injecting test solutions into the air cell of fertile chicken eggs. Toxicity of the test solution is determined by comparing the mortality of the embryos injected with the test substance with that of the undosed controls. The CHEST assay has been used to determine toxic effects of individual mycotoxins (183,184) as well as combinations of several toxins (185). Overall, the method is simple, inexpensive, and sensitive, but it is nonspecific and prone to false positives (157). The reproducibility of the CHEST assay depends on the type and volume of carrier solvent, the site of injection, and the observation period (186). The sensitivity of the CHEST assay is in the 0.1- to 100-μg range (158).

Factors that must be considered when using whole animal bioassays include the method of administration and the vehicle for administration (157). Oral routes of administration are the most valid since they simulate the processes that occur during ingestion, digestion, and absorption of the mycotoxins. Ideally, the test material is administered by feeding ad libitum in an inert carrier. Because of solubility problems, some toxins cannot be readily formulated in inert carriers. In addition, carriers can be toxic themselves or affect the absorption process (157).

Invertebrates. Several invertebrates (brine shrimp, protozoa, planaria, mollusks, and insects) have been screened for their ability to detect mycotoxins. The brine shrimp (*Artemia salina*) bioassay is one of the first and most widely used biological screening methods for mycotoxins and has been used to screen aflatoxin (187), trichothecenes (188), ochratoxin A (189), and fumonsin (184). The sensitivity range of the assay for 10 trichothecenes ranged from 0.04 to 0.4 μg/mL, with T-2 toxin being the most toxic (188). Attractive features of the assay include its sensitivity, simplicity, rapidity, and low cost. A disadvantage of the brine shrimp assay is that this organism is sensitive to many compounds present in normal foods and feeds, resulting in a high percentage of false-positive bioassay results (157).

Tissue Cultures and Cytotoxicity. Numerous vertebrate tissue and organ cultures have been used for bioassays for mycotoxins, including calf kidney cells; embryonic lung cells; rabbit reticulocytes; HeLa cells; human fibroblasts; and rat kidney, liver, and muscle cells (157). Cytotoxicity studies involve incubating the test substance with cell cultures for various periods of time. Cytological, morphological, and biochemical methods are used to evaluate toxicity (157). A mutagenicity/carcinogenicity assay using primary rat hepatocytes has been useful for determining if mycotoxins and other compounds induce chromosomal aberrations and micronuclei (190). Advantages of tissue culture and cytotoxicity tests are that they are sensitive, relatively inexpensive, and readily available. Several drawbacks include a lack of specificity and the fact that they do not take into account ingestion, digestion, and absorption of the toxin (157).

Plant Assays

Use of plants (whole, tissues) to detect mycotoxins has had limited application. However, there are several cases where plants have been excellent sensors for mycotoxins. Plant bioassays have an advantage over whole animal studies in that they are easier and less expensive to conduct. Plant bioassays have been used in the past to detect and isolate plant growth regulators. Since some mycotoxins also affect plant growth, whole plants and plant tissues are also used for mycotoxin screening. Malformin A, moniliformin, diacetoxyscirpenol, fusaric acid, and *Alternaria alternata* toxins are examples of mycotoxins that have been screened using bean, corn, rice, wheat, jimsonweed, and tobacco plants (191–193). The wheat coleoptile bioassay has been a useful tool for detecting chaetoglobosin K (194) and is especially sensitive to the 12,13-epoxytrichothecenes (157).

RAPID SCREENING METHODS

One of the simplest and most rapid mycotoxin screening methods uses black or long-wave UV light (365 nm) to examine cracked or coarsely ground corn. The characteristic bright greenish yellow fluorescence (BGYF) observed against a dark background is used to identify specific lots of corn contaminated with aflatoxin (133). When one BGYF glower is observed in 1 kg of ground corn, the corn must be analyzed by more quantitative methods (ie, TLC or HPLC). The main disadvantage of this test is that it gives a high rate of both false-positive and -negative results (133). Visual tests are also used in the United States to inspect peanuts for *Aspergillus flavus* conidial heads, and if present, suspected lots are not allowed into commerce for human consumption (95).

TLC has been an effective and fairly rapid method for qualitative/quantitative analysis of agricultural commodities for aflatoxin. The only supplies that are needed include aflatoxin standards, solvent, silica-coated TLC plates, a developing tank, and UV light. Samples are ground in a mill, extracted with chloroform:water, then spotted on a silica gel–coated plate. Plates are developed in tanks containing anhydrous diethyl ether and then examined under long-wave UV light. Identity and quantitation are accomplished by comparing unknown spots to those made by pure standards. This method can detect as low as 20-ng aflatoxin/g (133).

Minicolumn chromatography is a rapid screening method for aflatoxin, ochratoxin A, and zearalenone in foods before examination by other analytical techniques. Minicolumn screening methods include the following steps: extraction, purification, concentration, and development on a minicolumn for detection under UV light. The methods are simple, require little experience and no sophisticated equipment, and are useful for field analyses. The minicolumn method of Romer (44) has been approved by the AOAC as an official method for the detection of aflatoxin in mixed feeds, corn, peanuts, and cottonseed. Similar methods have been developed for ochratoxin A (45) and zearalenone (46). Detection limits for these methods range from 5 to 20 ng/g.

Widely used rapid screening methods use commercially available IA kits. By shortening the incubation time and adjusting antibody and enzyme concentration, microtiter ELISA screening kits can rapidly detect aflatoxin, deoxynivalenol, and zearalenone levels as low as 20 ng/g, 1 μg/g and 100 ng/g, respectively (32). Rather than use microtiter plates, some commercial kits have antibody mounted to a paper disk or membrane, a plastic cup, or polystyrene beads (130). Generally, these tests indicate mycotoxin levels above or below certain target levels. When no color develops, the test sample is above the target level. These tests, which are well suited for field analysis, can provide results in less than 10 min. All samples giving a positive response by the ELISA screening test must be analyzed by another procedure to verify the ELISA results. The ELISA procedure can reduce the laborious chemical testing of the majority of samples, which are negative for mycotoxin, thus saving time and expense (3). Rapid screening methods have been developed using IAC to clean up sample extracts containing fluorescent mycotoxins (aflatoxin, ochratoxin) followed by detection and quantitation using a fluorometer (2). Thompson and Maragos (195) designed a fiber-optic immunosensor that has the potential for screening corn for fumonisins in 6 min.

FUTURE DEVELOPMENT

Much research has been devoted in recent years to developing sensitive, accurate, and precise methods for measuring mycotoxin levels in food and feed. The efforts are continuing and keeping in pace with progresses in analytical chemistry. Considerable work needs to be done in reducing the time and expense required for the analysis of mycotoxins. In addition, research is needed to develop methods for analyzing several mycotoxins in the same assay. With the rapid development of new immunochemical methodologies, these needs may be fulfilled in the near future.

BIBLIOGRAPHY

1. D. W. Wilson et al., "Mycotoxin Analytical Techniques," in K. K. Sinha and D. Bhatnagar, eds., *Mycotoxins in Agriculture and Food Safety*, Marcel Dekker, New York, 1995, pp. 135–182.
2. F. S. Chu, "Recent Progress on Analytical Techniques for Mycotoxins in Feedstuffs," *J. Anim. Sci.* **70**, 3950–3963 (1992).
3. J. L. Richard et al., "Analysis of Naturally Occurring Mycotoxins in Feedstuffs and Food," *J. Anim. Sci.* **71**, 2563–2574 (1993).
4. J. Gilbert, "Recent Advances in Analytical Methods for Mycotoxins," *Food Additives and Contaminants* **10**, 37 48 (1993).
5. P. S. Steyn, P. G. Thiel, and D. W. Trinder, "Detection and Quantitation of Mycotoxins by Chemical Analysis," in J. E Smith and R. S. Henderson, eds., *Mycotoxins and Animal Foods*, CRC Press, Boca Raton, Fla., 1991, pp. 165–221.
6. J. W. Dorner, "Chromatographic Analysis of Mycotoxins," in T. Shibamoto, ed., *"Chromatographic Analysis of Environmental and Food Toxicants,"* Marcel Dekker, New York, 1998, pp. 113-168.
7. W. Langseth and T. Rundberget, "Instrumental Methods for Determination of Nonmacrocyclic Trichothecenes in Cereals, Foodstuffs and Cultures," *J. Chromatogr A* **815**, 103–121 (1998).
8. D. J. Webley, K. L. Jackson, and J. D. Mullins, "Mycotoxins in Food: A Review of Recent Analyses," *Food Australia* **49**, 375–379 (1997).
9. P. M. Scott, "Mycotoxin Methodology," *Food Additives and Contaminants* **12**, 395–403 (1995).
10. M. W. Trucksess, "Mycotoxins, General Referee Reports, Committee on Natural Toxins," *J. AOAC INT.* **78**, 135–141 (1995).
11. M. W. Trucksess, "Mycotoxins, General Referee Reports, Committee on Natural Toxins," *J. AOAC INT* **79**, 200–205 (1996).
12. M. W. Trucksess, "Mycotoxins, General Referee Reports, Committee on Natural Toxins," *J. AOAC INT.* **70**, 119–125 (1997).
13. M. W. Trucksess, "Mycotoxins, General Referee Reports, Committee on Natural Toxins," *J. AOAC INT* **81**, 128–137 (1998).
14. M. W. Trucksess, "Mycotoxins, General Referee Reports, Committee on Natural Toxins," *J. AOAC INT.* **82**, 488–495 (1999).
15. R. J. Cole, ed., *Modern Methods in the Analysis and Structure Elucidation of Mycotoxins*, Academic Press, Orlando, Fla., 1986.
16. V. Betina, ed., *Chromatography of Mycotoxins*, Elsevier, Amsterdam, The Netherlands, 1993.
17. D. L. Park and A. E. Pohland, "Sampling and Sample Preparation for Detection and Quantitation of Natural Toxicants in Food and Feed," *J. AOAC* **72**, 399–404 (1989).
18. A. D. Campbell et al., "Sampling, Sample Preparation, and Sampling Plans for Food Stuffs and Mycotoxin Analysis," *Pure and Appl. Chem.* **58**, 305–314 (1986).
19. F. M. Garfield, "Sampling in the Analytical Scheme," *J. AOAC* **72**, 405–411 (1989).
20. T. B. Whitaker and J. W. Dickens, "Variability Associated with Testing Corn for Aflatoxins," *J. AOAC* **56**, 789–794 (1979).
21. J W. Dickens and T. B. Whitaker, "Sampling and Sample Preparation Methods for Mycotoxin Analysis," in R. J. Cole, ed., *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*, Academic Press, New York 1986, pp 29–49.
22. T. B. Whitaker, J. W. Dickens, and F. G. Giesbrecht, "Testing Animal Feedstuffs for Mycotoxins: Sampling, Subsampling, and Analysis," in J. E. Smith and R. S. Henderson, eds., *"Mycotoxins and Animal Foods,"* CRC Press, Baca Raton, Fla., 1991, pp. 153–164.
23. T. B. Whitaker et al., "Effects of Sample Size and Sample Acceptance Level on the Number of Aflatoxin-Contaminated Farmers' Stock Lots Accepted and Rejected at the Buying Point," *J. AOAC INT.* **77**, 1672–1680 (1994).
24. T. B. Whitaker et al., "Variability Associated with Sampling, Sample Preparation, and Chemical Testing for Aflatoxin in Farmers' Stock Peanuts," *J AOAC INT.* **77**, 107–116 (1994).
25. T. B. Whitaker et al., "Variability Associated with Analytical Methods Used to Measure Aflatoxin in Agricultural Commodities," *J. AOAC INT.* **79**, 476–485 (1996).
26. T. B. Whitaker et al., "Variability Associated with Testing Shelled Corn for Fumonisin," *J. AOAC INT.* **81**, 1162–1168 (1998).

27. T. B. Whitaker, W. M. Hagler, Jr., F. G. Giesbrecht, "Performance of Sampling Plans to Determine Aflatoxin in Farmer's Stock Peanut Lots by Measuring Aflatoxin in High-risk Grade Components," *J. AOAC Int.* **82**, 264–270 (1999).
28. H. P. Van Egmond, "Current Status on Regulations for Mycotoxins, Overview of Tolerances and Status of Standard Methods of Sampling and Analysis," *Food Additives and Contaminants* **6**, 139–188 (1989).
29. "Sampling Plans for Aflatoxin Analysis in Peanuts and Corn," Technical Report 55, Food and Agriculture Organization, Rome, 1993
30. Codex Alimentarius Commission, "General Guidelines in Sampling," World Health Organization, Geneva, Switzerland, 38-MAS, 1996.
31. N. D. Davis et al., "Protocols for Surveys, Sampling, Post-Collection Handling, and Analysis of Grain Samples Involved in Mycotoxin Problems," *J. AOAC* **63**, 95–102 (1980).
32. P. M. Scott, "Natural Toxins," in P. Cunniff, ed., *AOAC Official Methods of Analysis*, 16th ed., Vol. 2, AOAC INTERNATIONAL, Gaithersburg, Md., 1997, pp. 49-1 to 49-51.
33. Anonymous, "Determination of Aflatoxin B_1," *Official Journal of the European Communities* **L102/9** (1976).
34. Anonymous, "Animal Feedstuffs—Determination of Aflatoxin B_1 Content," *International Standard 6651*, 1st ed., International Organization for Standardization, Geneva, Switzerland, 1983.
35. Anonymous, "Animal Feedstuffs—Determination of Zearalenone Content," *International Standard 6870*, 1st ed., International Organization for Standardization, Geneva, Switzerland, 1985.
36. M. Holcomb et al., "Determination of Aflatoxins in Food Products by Chromatography," *J. Chromatogr* **624**, 341–352 (1992).
37. R. P. Huopalahti, J. Ebel, and J. D Henion, "Supercritical Fluid Extraction of Mycotoxins from Feeds with Analysis by LC/UV and LC/MS," *J. Liq. Chromatogr Rel. Technol.* **20**, 537–540 (1997).
38. E. P. Jarvenpaa et al., "The Use of Supercritical Fluid Extraction for the Determination of 4-Deoxynivalenol in Grains: The Effect of the Sample Clean-up and Analytical Methods on Quantitative Results," *Chromatographia* **46**, 33–38 (1997).
39. B. A. Roberts and D. S. P. Patterson, "Detection of Twelve Mycotoxins in Mixed Animal Feedstuffs Using a Novel Membrane Clean-up Procedure," *J. AOAC* **58**, 1178–1181 (1975).
40. D. S. P. Patterson and B. A. Roberts, "Mycotoxins in Animal Feedstuffs; Sensitive Thin Layer Chromatographic Detection of Aflatoxin, Ochratoxin A, Sterigmatocystin, Zearalenone and T-2 Toxin," *J. AOAC* **62**, 579–585 (1979).
41. G. A. Bennett, O. L. Shotwell, and W. F. Kwolek, "Liquid Chromatographic Determination of α-Zearalenol and Zearalenone in Corn: Collaborative Study," *J. AOAC* **68**, 958–961 (1985)
42. P. Kuronen, "High-Performance Liquid Chromatographic Screening Method for Mycotoxins Using New Retention Indexes and Diode Array Detection," *Arch. Environ. Contam. Toxicol.* **18**, 336–348 (1989).
43. C. E. Holaday, "Minicolumn Chromatography: State of the Art," *J. AOAC* **58**, 931A–934A (1981).
44. T. R. Romer, "Screening Method for the Detection of Aflatoxins in Mixed Feeds and Other Agricultural Commodities with Subsequent Confirmation and Quantitative Measurement of Aflatoxins in Positive Samples," *J. AOAC* **58**, 500–506 (1975).
45. T. R. Romer and A. D. Campbell, "Collaborative Study of a Screening Method for the Detection of Aflatoxin in Mixed Feeds, Other Agricultural Products and Foods," *J. AOAC* **59**, 110–117 (1976).
46. T. R. Romer, N. Ghouri, and T. M Boling. "Minicolumn Screening Methods for Determining Aflatoxins: State of the Art," *J. Am. Oil Chem. Soc.* **56**, 795–797 (1979).
47. A. Biancardi and A. Riberzni, "Determination of Ochratoxin A in Cereals and Feed by SAX-SPE Clean Up and LC Fluorimetric Detection," *J. Liq Chromatogr and Rel. Technol.* **19**, 2395–2407 (1996).
48. B. R. Malone et al., "One-Step Solid-Phase Extraction Clean-up and Fluorometric Analysis of Deoxynivalenol in Grains," *J. AOAC Int.* **81**, 448–452 (1998).
49. R. Krska, "Performance of Modern Sample Preparation Techniques in the Analysis of *Fusarium* Mycotoxins in Cereals," *J. Chromatog. A* **815**, 49–57 (1998).
50. H. Cohen and M R. Lapointe, "High Pressure Liquid Chromatographic Determination and Fluorescence Detection of Aflatoxins in Corn and Dairy Feeds," *J. AOAC* **64**, 1372–1376 (1981).
51. J. E. Thean et al., "Extraction, Cleanup and Quantitative Determination of Aflatoxins in Corn," *J. AOAC* **63**, 631–633 (1980).
52. G. S. Shephard et al., "Quantitative Determination of Fumonisins B_1 and B_2 by High-Performance Liquid Chromatography with Fluorescence Detection," *J. Liq. Chromatogr.* **13**, 2077–2087 (1990).
53. L. G. Rice et al., "Evaluation of a Liquid Chromatographic Method for the Determination of Fumonisins in Corn, Poultry Feed, and *Fusarium* Culture Material," *J AOAC Int.* **78**, 1002–1009 (1995).
54. S. Chiron and D. Barcel, "Identification of Trichothecenes by Thermospray, Plasmaspray and Dynamic Fast-Atom Bombardment Liquid Chromatography-Mass Spectrometry," *J. Chromatogr* **645**, 125–134 (1993).
55. G. M. Ware et al., "Preparative Method for Isolating α-Zearalenol and Zearalenone Using Extracting Disk," *J. AOAC Int.* **82**, 90–94 (1999).
56. P. M. Scott and M. W. Trucksess, "Application of Immunoaffinity Columns to Mycotoxin Analysis," *J. AOAC Int.* **80**, 941–949 (1997).
57. Anonymous, "Vicam's Aflatest®," *J. AOAC Int.* **78**, 17A–19A (1995).
58. J. D. Groopman and K. F. Donahue, "Aflatoxin, A Human Carcinogen: Determination in Foods and Biological Samples by Monoclonal Antibody Affinity Chromatography," *J. AOAC* **72**, 861–867 (1988).
59. J. I. Azcona, M. M. Abouzied, and J. J. Pestka, "Detection of Zearalenone by Tandem Immunoaffinity-Enzyme-Linked Immunosorbent Assay and Its Application to Milk," *J. Food Prot.* **53**, 577–580 (1990).
60. A. Farjam et al., "The Determination of Aflatoxin M_1 Using a Dialysis-Based Immunoaffinity Sample Pretreatment System Coupled On-Line to Liquid Chromatography Reusable Immunoaffinity Columns," *J. Chromatogr* **589**, 141–149 (1992).
61. K. A. Scudamore et al., "Determination of Mycotoxins in Pet Foods Sold for Domestic Pets and Wild Birds Using Linked-Column Immunoassay Clean-up and HPLC," *Food Additives and Contaminants* **14**, 175–186 (1997).
62. C. M. Maragos, G. A. Bennett, and J. L. Richard, "Affinity Column Clean-up for the Analysis of Fumonisins and Their

Hydrolysis Products in Corn," *Food and Agric. Immunol.* **9**, 3–12 (1997).

63 A. E. Pohland et al., "Physiochemical Data for Some Selected Mycotoxins," *Pure Appl. Chem.* **54**, 2219–2284 (1982).

64. R. J. Cole and R. H. Cox, *Handbook of Toxic Fungal Metabolites*, Academic Press, New York, 1981

65. M. E. Savard and B. A. Blackwell, "Spectral Characteristics of Secondary Metabolites from *Fusarium* Fungi," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain, Compounds Other Than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 59–257.

66 C. P. Gorst-Allman and P. S. Steyn, "Screening Methods for the Detection of Thirteen Common Mycotoxins," *J. Chromatogr.* **175**, 325–331 (1979).

67. L. S. Lee and D. B. Skau, "Thin Layer Chromatographic Analysis of Mycotoxins: A Review of Recent Literature," *J. Liq. Chromatogr* **4**, 43–62 (1981).

68. R. F. Vesconder and W. K. Rohwedder, "Gas Chromatographic-Mass Spectrometric Analysis of Mycotoxins," in R. J. Cole, ed., *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*, Academic Press, Orlando, Fla., 1986, pp. 335–357.

69. C. J. Mirocha et al., "Analysis of Deoxynivalenol and Its Derivatives (Batch and Single Kernel) Using Gas Chromatography/Mass Spectrometry," *J. Agric. Food Chem.* **46**, 1414–1418 (1998).

70. M. M. Mossoba, S. Adams, and J. A. G. Roach, "Analysis of Trichothecene Mycotoxins in Contaminated Grains by Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectroscopy and Gas Chromatography/Mass Spectrometry," *J. AOAC Int.* **79**, 1116–1123 (1996).

71. M. Schollenberger et al., "Determination of Eight Trichothecenes by Gas Chromatography-Mass Spectrometry after Sample Clean-up by a Two-Stage Solid-Phase Extraction," *J. Chromatogr.* **815**, 123–132 (1998).

72. W. J. Hurst, R. A. Martin, Jr., and C. H. Vestal, "The Use of HPLC/Thermospray MS for the Confirmation of Aflatoxins in Peanuts," *J. Liq. Chromatogr.* **14**, 2541–2545 (1991).

73. T. Krishnamurthy et al., "Mass Spectral Investigations on Trichothecene Mycotoxins, VII: Liquid Chromatographic-Thermospray Mass Spectrometric Analysis of Macrocyclic Trichothecenes," *J. Chromatogr* **469**, 209–222 (1989).

74. R. Kostiainen, N. Matsuura, and K. Njima, "Identification of Trichothecenes by Thermospray, Plasmaspray and Dynamic Fast-Atom Bombardment Liquid Chromatography-Mass Spectrometry," *J. Chromatogr.* **562**, 555–562 (1991).

75. E. Rosenberg et al., "High-Performance Liquid Chromatography-Atmospheric-Pressure Chemical Ionization Mass Spectrometry as a New Tool for the Determination of the Mycotoxin Zearalenone in Food and Feed," *J. Chromatogr. A* **819**, 277–288 (1998).

76. M Becker "Column Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry for the Analysis of Ochratoxin," *J. Chromatogr.* **818**, 260–264 (1998).

77. E. D Caldas et al., "Electrospray Ionization Mass Spectrometry of Sphinganine Analog Mycotoxins," *Anal. Chem.* **67**, 196–207 (1995).

78. G. S. Shephard, "Chromatographic Determination of the Fumonisin Mycotoxins," *J. Chromatogr. A* **815**, 31–39 (1998).

79 V. Betina, "Thin-Layer Chromatography of Mycotoxins," *J. Chromatogr.* **334**, 211–276 (1985).

80. D. Abramson, T. Thorsteinson, and D. Forest, "Chromatography of Mycotoxins on Precoated Reverse-Phase Thin-Layer Plates," *Arch. Environ. Contam. Toxicol.* **18**, 327–330 (1989).

81. R. D. Coker, A. E. John, and J A. Gibbs, "Techniques of Thin Layer Chromatography," in V. Betina, ed., *Chromatography of Mycotoxins*, Elsevier Press, Elsevier, Amsterdam, The Netherlands, 1993, pp 12–35.

82. S. Nawaz, R. D. Coker, and S. J. Haswell, "HPTLC—A Valuable Chromatographic Tool for the Analysis of Aflatoxins," *J. Planar Chromatogr.* **8**, 4–9 (1995).

83. L. Lin et al., "Thin-Layer Chromatography of Mycotoxins and Comparison with Other Chromatographic Methods," *J. Chromatogr. A* **815**, 3–20 (1998).

84. R. M. Eppley et al., "Thin Layer Chromatographic Method for Determination of Deoxynivalenol in Wheat: Collaborative Study," *J. AOAC* **69**, 37–40 (1986)

85. O. J. Francis, Jr., et al., "Thin Layer Chromatographic Determination of Sterigmatocystin in Cheese," *J. AOAC* **68**, 643–645 (1985).

86. F. S. Chu, "Studies on Ochratoxins," *CRC Crit. Rev. Toxicol.* **2**, 499–524 (1977).

87. T. F. Salem and B. G. Swanson, "Fluorodensitometric Assay of Patulin in Apple Products," *J. Food Sci.* **41**, 1237–1238 (1976).

88. J. W. Seiber and D. P. H. Hseih, "Application of High-Speed Liquid Chromatography to the Analysis of Aflatoxins," *J. AOAC* **56**, 827–830 (1973).

89. R. D. Coker and B. D. Jones, "Determination of Mycotoxins," in R. Macrae, ed., *HPLC in Food Analysis*, Academic Press, London, 1988, pp. 335–375.

90. J. C. Frisvad, and U. Thrane, "Liquid Column Chromatography of Mycotoxins," in V. Betina, ed., *Chromatography of Mycotoxins*, Elsevier, Amsterdam, The Netherlands, 1993, pp. 253–372.

91. M J. Shepherd "High-Performance Liquid Chromatography and its Application to the Analysis of Mycotoxins," in R. J. Cole, ed., *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*, Academic Press, Orlando, Fla., 1986, pp 293–315.

92. P Kuronen, "Techniques of Liquid Chromatography," in V. Betina, ed., *Chromatography of Mycotoxins*, Elsevier, Amsterdam, The Netherlands, 1993, pp. 36–77.

93. P. G. Thiel, S. Stockenstrom, and S. Gathercole. "Aflatoxin Analysis by Reverse Phase HPLC Using Post-Column Derivatization for Enhancement of Fluorescence," *J. Liq. Chromatogr.* **9**, 103–108 (1986).

94. D. M Wilson, "Analytical Methods for Aflatoxins in Corn and Peanuts," *Arch. Environ. Contam. Toxicol.* **18**, 308–314 (1989).

95. R. W. Beaver, "Determination of Aflatoxins in Corn and Peanuts Using High Performance Liquid Chromatography," *Arch. Environ. Contam. Toxicol.* **18**, 315–320 (1989).

96. M. W. Trucksess et al., "Immunoaffinity Column Coupled with Solution Fluorometry of Liquid Chromatography Post-column Derivatization for Determination of Aflatoxins in Corn, Peanuts and Peanut Butter: Collaborative Study," *J. AOAC* **74**, 81–88 (1991).

97. A. L. Patey, M. Sharman, and J. Gilbert, "Liquid Chromatographic Determination of Aflatoxin Levels in Peanut Butters Using an Immunoaffinity Column Clean-up Method: Internal Collaborative Trial," *J. AOAC* **74**, 76–81 (1991).

98. H. L. Chang and J. W. DeVries, "Short Liquid Chromatographic Method for Determination of Zearalenone and α-Zearalenol," *J. AOAC* **67**, 741–744 (1984).

99 T. Tanaka et al., "Sensitive Determination of Zearalenone and α-Zearalenol in Barley and Job's-Tears by Liquid Chromatography with Fluorescence Detection," *J. AOAC* **76**, 1006–1009 (1993).

100. P. J. Martin et al., "Chromatography of Trichothecene Mycotoxins," *J. Liq. Chrom.* **9**, 1591–1602 (1986).

101. C M. Franco et al., "Simple and Sensitive High-Performance Liquid Chromatography-Fluorescence Method for the Determination of Citrinin: Application to the Analysis of Fungal Cultures and Cheese Extracts," *J. Chromatogr. A* **723**, 69–72 (1996).

102. B. I. Vazquez et al., "Simultaneous High-Performance Liquid Chromatographic Determination of Ochratoxin A and Citrinin in Cheese by Time-Resolved Luminescence Using Terbium," *J. Chromatogr. A* **727**, 185 (1996).

103. E. G. Hiesler et al., "High-Performance Liquid Chromatographic Determination of Major Mycotoxins Produced by *Alternaria* Molds," *J. Chromatograph.* **194**, 89–92 (1980).

104. M. E. Stack et al., "Liquid Chromatographic Determination of Tenuazonic Acid and Alternariol Methyl Ether in Tomatoes and Tomato Products," *J. AOAC* **68**, 640–642 (1985)

105. T. Delgado, C. Gomez-Cordoves, and P. M. Scott, "Determination of Alternariol and Alternariol Methyl Ether in Apple Juice Using Solid-Phase Extraction and High-Performance Liquid Chromatography," *J. Chromatogr. A* **73**, 109–114 (1996).

106. J. Prieta et al., "Determination of Patulin by Reversed-Phase High-Performance Liquid Chromatography with Extraction by Diphasic Dialysis," *Analyst* **118**, 171–174 (1993).

107. A. R. Brause et al., "Determination of Patulin in Apple Juice by Liquid Chromatography: Collaborative Study," *J. AOAC INT.* **79**, 451–455 (1996).

108. D. Abramson and T. Thorsteinson, "Determination of Sterigmatocystin in Barley by Acetylation and Liquid Chromatography," *J. AOAC* **72**, 342–344 (1989).

109. M. J. Shepherd and J. Gilbert, "Method for the Analysis in Maize of the *Fusarium* Mycotoxin Moniliformin Employing Ion-Pairing Extraction and High-Performance Liquid Chromatography," *J. Chromatogr.* **358**, 415–422 (1986).

110. J. G. Wilkes et al., "Determination of Fumonisins B_1, B_2, B_3 and B_4 by High Performance Liquid Chromatography with Evaporative Light Scattering Detection," *J. Chromatogr.* **695**, 319–323 (1995).

111 G. A. Bennett and J. L. Richard, "Liquid Chromatographic Method for Analysis of the Naphthalene Dicarboxaldehyde Derivative of Fumonisins," *J. AOAC INT.* **77**, 501–506 (1994).

112. C. Velazquez et al., "Derivation of Fumonisins B_1 and B_2 with 6-Aminoquinolyl N-Hydroxylsuccinimidylcarbamate," *J. Agric. Food Chem.* **43**, 1535–1537 (1995).

113. D. R. Lauren and M P. Agnew, "Multitoxin Screening for *Fusarium* Mycotoxins in Grain," *J. Agric. Food Chem.* **39**, 502–507, 1991.

114 W L. Childress, I. S. Krull, and C. M. Selavka, "Determination of Deoxynivalenol (DON, Vomitoxin) in Wheat by High-Performance Liquid Chromatography with Photolysis and Electrochemical Detection (HPLC-hv-EC)," *J. Chromatogr. Sci.* **28**, 76–82 (1990).

115. G. M. Ware et al., "Determination of Zearalenol and Zearalenone Using Electrochemical Detection," *Anal. Lett.* **22**, 2335–2339 (1989).

116. B. T. Duhart et al., "Determination of Aflatoxins B_1, B_2, G_1 and G_2 by High-Performance Liquid Chromatography with Electrochemical Detection," *Anal. Chimica Acta* **208**, 343–346 (1988).

117. F. Palmisano "Determination of *Alternaria* Mycotoxins in Foodstuffs by Gradient Elution Liquid with Electrochemical Detection," *Chromatographia* **27**, 425–429 (1989).

118. M. Careri, A. Mangia, and M. Musci, "Applications of Liquid Chromatography-Mass Spectrometry Interfacing Systems in Food Analysis: Pesticide, Drug and Toxic Substance Residues," *J. Chromatogr.* **727**, 153–184 (1996).

119. R. D. Plattner, "Detection of Fumonisins Produced in *Fusarium moniliforme* Culture by HPLC with Electrospray MS and Evaporative Light Scattering Detector," *Natural Toxins* **3**, 294–300 (1995).

120. P. M. Scott, "Gas Chromatography of Mycotoxins," in V. Betina, ed., *Chromatography of Mycotoxins*, Elsevier, Amsterdam, The Netherlands, 1993, pp. 373–425.

121. Y. Onji et al., "Direct Analysis of Several *Fusarium* Mycotoxins in Cereals by Capillary Gas Chromatography-Mass Spectrometry," *J. Chromatogr. A* **815**, 59–65 (1998).

122. J. C. Young, and D. E. Games, "Analysis of *Fusarium* Mycotoxins by Gas Chromatography-Fourier Transform Infrared Spectroscopy," *J. Chromatogr A* **663**, 211–218 (1994).

123. J. A. G. Roach et al., "Capillary Supercritical Fluid Chromatography/Negative Ion Chemical Ionization Mass Spectrometry of Trichothecenes," *Biomed. Environ. Mass Spec.* **18**, 64–70 (1989).

124. J. C. Young and D. E. Games, "Analysis of *Fusarium* Mycotoxins by Supercritical Fluid Chromatography with Ultraviolet or Mass Spectometric Detection," *J. Chromatogr. A* **653**, 374–379 (1993).

125. C. M Maragos and J. I. Greer, "Analysis of Aflatoxin B_1 in Corn Using Capillary Electrophoresis with Laser-Induced Fluorescence Detection," *J. Agric. Food Chem.* **45**, 4337–4341 (1997).

126. B. C. Prasongsidh et al., "Analysis of Cyclopiazonic Acid in Milk by Capillary Electrophoresis," *Food Chem.* **61**, 515–519 (1998).

127. C. M. Maragos, "Capillary Zone Electrophoresis and HPLC for the Analysis of Fluorescein Isothiocyanate-Labeled Fumonisin B_1," *J. Agric. Food Chem.* **43**, 390–394 (1995).

128. B. Bohs, V. Seidel, and W. Lindner, "Analysis of Selected Mycotoxins by Capillary Electrophoresis," *Chromatographia* **41**, 631–637 (1995)

129. M. W. Trucksess and G. E Wood, "Immunochemical Methods for Mycotoxins in Foods," *Food Testing and Analysis* **3**, 24–27 (1997).

130. F. S. Chu, "Current Immunochemical Methods for Mycotoxin Analysis," in M. Vanderlaan, ed., *Immunoassays for Monitoring Human Exposure to Toxic Chemicals in Food and Environment*, ACS Symposium Series, American Chemical Society, Washington, D.C., 1990, pp. 140–157.

131. F. S. Chu, "Recent Studies on Immunoassays For Mycotoxins," in R. C. Beier and L. H. Stanker, eds., *Immunoassays for Residue Analysis, Food Safety*, American Chemical Society, Washington, D.C. 1996, pp. 294–313.

132. A. A. G. Candlish, "Immunological Methods in Food Microbiology," *Food Microbiol.* **8**, 1–14 (1991).

133. M. W. Trucksess, "Methods Used in Testing of Aflatoxins, Deoxynivalenol, Ochratoxin A, and Zearalenone in Grains and Grain Products," in J. W. DeVries et al., eds., *Food Safety from a Chemistry Perspective, Is There a Role for HACCP?*, Analytical Progress Press, Minneapolis, Minn., 1996, pp. 101–112.

134. E. Schneider et al., "Multimycotoxin Dipstick Enzyme Immunoassay Applied to Wheat," *Food Additives and Contaminants* **12**, 387–393 (1995).

135. M. M. Abouzied and J. J. Pestka, "Simultaneous Screening of Fumonisin B_1, Aflatoxin B_1, and Zearalenone by Line Immunoblot: A Computer-Assisted Multianalyte Assay System," *J. AOAC Int.* **77**, 495–501 (1994).

136 L. H. Stanker and R. C. Beier, "Introduction to Immunoassays for Residue Analysis," in R. C. Beier and L. H. Stanker, eds., *Immunoassays for Residue Analysis, Food Safety*, American Chemical Society, Washington, D.C., 1996, pp. 2–15.

137. F. S. Chu, "Immunoassay for Analysis of Mycotoxins," *J. Food Protect.* **47**, 562–569 (1984).

138. J. J. Pestka, "Enhanced Surveillance of Foodborne Mycotoxins by Immunochemical Assay," *J. AOAC* **71**, 1075–1081 (1988).

139. J. J. Pestka, "Application of Immunology to the Analysis and Toxicity Assessment of Mycotoxins," *Food and Agricultural Immunol.* **6**, 219–234 (1994).

140. F. S. Chu and I. Ueno, "Production of Antibody Against Aflatoxin B_1," *Appl. Environ. Microbiol.* **33**, 1125–1128 (1977).

141. R. Teshima "Radioimmunoassay of Nivalenol in Barley," *Appl. Environ. Microbiol.* **56**, 764–768 (1990).

142. Y.-C. Xu, G.-S. Zhang, and F. S. Chu, "Radioimmunoassay of Deoxynivalenol in Wheat and Corn," *J. AOAC* **69**, 967–969 (1986).

143. D. M. Rousseau et al., "Detection of Ochratoxin A in Porcine Kidneys by a Monoclonal Antibody-Based Radioimmunoassay," *Appl. Environ. Microbiol.* **53**, 514–518 (1987).

144. F. S. Chu et al., "Production and Characterization of Antibody Against Diacetoxyscirpenol," *Appl. Environ. Microbiol.* **48**, 777–780 (1979).

145. J. J. Park, and F. S. Chu, "Assessment of Immunochemical Methods for the Analysis of Trichothecene Mycotoxins in Naturally Occurring Moldy Corn," *J. AOAC Int.* **79**, 465–471 (1996).

146. A. P. Wilkinson, C. M. Ward, and M. R. A. Morgan, "Immunological Analysis of Mycotoxins," in H. F. Linskens and J. F. Jackson, eds., *Modern Methods of Plant Analysis*, Springer-Verlag, Berlin, 1992, pp. 185–225.

147. F. S. Chu et al., "Improved Enzyme-Linked Immunosorbent Assay for Aflatoxin B_1 in Agricultural Commodities," *J. AOAC* **70**, 854–857 (1987).

148 J. W. Dorner and R. J. Cole, "Comparison of Two ELISA Screening Tests with Liquid Chromatography for Determination of Aflatoxins in Raw Peanuts," *J. AOAC* **72**, 962–964 (1989).

149 R. C. Sinha and M. E. Savard, "Comparison of Immunoassay with Gas Chromatographic Methods for the Determination of the Mycotoxin Deoxynivalenol in Grain Samples," *Canad. J Plant Path.* **18**, 233–236 (1996).

150. E W. Sydenham et al., "Polyclonal Antibody-Based ELISA and HPLC Methods for the Determination of Fumonisins in Corn: A Comparative Study," *J. Food Prot* **59**, 893–897 (1996).

151. M M Sutikno et al., "Detection of Fumonisins in *Fusarium* Cultures, Corn, and Corn Products by Polyclonal Antibody-Based ELISA: Relation to Fumonisin B_1 Detection by Liquid Chromatography," *J. Food Protect.* **59**, 645–651 (1996).

152. F. S. Chu, "Immunochemical Methods for Fumonisins," in L. S. Jackson, J. W. DeVries and L. B. Bullerman, eds., *Fumonisins in Food*, Plenum Publishing, New York, 1996, pp 123–133.

153. M. W. Trucksess and M M Abouzied, "Evaluation and Application of Immunochemical Methods for Fumonisin B_1 in Corn," in R. C. Beier and L. H. Stanker, eds., *Immunoassays for Residue Analysis*, American Chemical Society, Washington, D.C., 1996, pp. 358–367

154. S. Dragacci et al., "Use of Immunoaffinity Chromatography as a Purification Step for the Determination of Aflatoxin M_1 in Cheeses," *Food Additives and Contaminants* **12**, 59–65 (1995).

155. M. W. Trucksess et al., "Immunoaffinity Column Coupled with Liquid Chromatography for Determination of Fumonisin B_1 in Canned and Frozen Sweet Corn," *J AOAC Int.* **78**, 705–710 (1995).

156. K. Sargeant et al., "The Assay of Toxic Principle in Certain Groundnut Meals," *Vet Res.* **73**, 1219–1223 (1961).

157. R. J. Cole, H. G. Cutler, and J. W. Dorner, "Biological Screening Methods for Mycotoxins and Toxigenic Fungi," in R. J. Cole, ed., *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*, Academic Press, New York, 1986, pp. 1–28

158. I. E. Yates, "Bioassay Systems and Their Use in the Diagnosis of Mycotoxicoses," in C. Richard and T. Thurston, eds., *Diagnosis of Mycotoxicoses*, Martinus Nijhoff Publishers, Boston, 1986, pp. 331–378.

159 N. L. Clements, "Note on a Microbiological Assay for Aflatoxin B_1: A Rapid Confirmatory Test by Effects on Growth of *Bacillus megaterium*," *J AOAC* **51**, 611–612 (1968).

160. N. L. Clements, "Rapid Confirmatory Test for Aflatoxin B_1 Using *Bacillus megaterium*," *J. AOAC* **51**, 1192–1194 (1968).

161. J. Reiss, "*Bacillus subtilis*: A Sensitive Bioassay for Patulin," *Bull. Environ. Contam. Toxicol.* **13**, 689–691 (1975).

162. F. J. Olivigni and L. B. Bullerman, "A Microbiological Assay for Penicillic Acid," *J. Food Prot.* **41**, 432–434 (1978).

163 D. Broce et al., "Ochratoxins A and B Confirmation by Microbiological Assay with *Bacillus cereus* Mycoides," *J. AOAC* **53**, 616–619 (1970).

164 G. Koppe, and H.-J. Rehm, "A Biological Assay for Quantitative Determination of Roquefortine," *Z. Lebesm.-Unters. Forsch.* **169**, 90–91 (1979).

165 P. Boutibonnes et al., "Mycotoxin Sensitivity of *Bacillus thuringiensis*," *IRCS Med. Sci. Biochem. Pharmacol.* **11**, 430–431 (1983).

166. L. Viitasalo and H. G. Gyllenberg, "Toxicity of Aflatoxins to *Bacillus megaterium*," *Lebesm.-Wiss. Technol.* **12**, 113–114 (1968).

167. J. Reiss, "Mycotoxin Bioassays Using *Bacillus stearothermophilus*," *J. AOAC* **58**, 624–625 (1975).

168. K. T. Schappert and G G Khachatourians, "A Yeast bioassay for T-2 Toxin," *J. Microbiol. Meth.* **3**, 43–46 (1984).

169. P. E. Stone, T. Rubidge, and K. D MacDonald, "*Rhodotorula rubra* Bioassay for T-2 Toxin: Increased Sensitivity and Wider Application," *J. Microbiol. Meth.* **5**, 59–64 (1986).

170. D. M. Maron and B. N. Ames, "Revised Methods for the Salmonella Mutagenicity Test," *Mutation Res.* **113**, 173–215 (1983)

171. J. W. ApSimon, "The Biosynthetic Diversity of Secondary Metabolites," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain, Compounds Other Than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 3–18.

172. K. J. Van der Merwe, P. S. Steyn, and L. Fourie, "Mycotoxins, Part II: The Constitution of Ochratoxins A, B, and C, Metabolites of *Aspergillus ochraceus* Wlh," *J. Chem. Soc.* **74**, 7083–7088 (1965).

173. C. W. Holzapfel, "The Isolation and Structure of Cyclopiazonic Acid, a Toxic Metabolite of *Penicillium cyclopium* Westling," *Tetrahedron* **24**, 2101-2119 (1968).

174. R. J. Cole, J. W. Kirksey, and J. M. Wells, "A New Tremorgenic Metabolite from *Penicillium paxilli*," *Can. J. Microbiol.* **20**, 1159–1162 (1974).

175. R. J. Cole et al., "Tremorgenic Toxin from *Penicillium verruculosum*," *Appl. Microbiol.* **24**, 248–257 (1972).

176. R. J. Cole et al., "Papalum Staggers: Isolation and Identification of Tremorgenic Metabolites from Sclerotia of *Claviceps paspali*," *J. Agric. Food Chem.* **25**, 1197–2011 (1977).

177. R. J. Cole et al., "Toxin from *Fusarium moniliforme*: Effects on Plants and Animals," *Science* **179**, 1324–1326 (1973).

178 J. A. Lansden et al., "A New Trichothecene Mycotoxin Isolated from *Fusarium tricinctum*," *J. Agric. Food Chem.* **26**, 246–249 (1978).

179. R. O. Sinnhuber "Trout Bioassay of Mycotoxins," in J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, eds., *Mycotoxins in Human and Animal Health*, Pathotox, Park Forest South, Ill., 1977, pp. 731–744.

180. R.-D. Wei, E. B. Smalley, and F. M. Strong, "Improved Skin Test for Detection of T-2 Toxin," *Appl. Microbiol.* **23**, 1029–1030 (1972).

181. H. R. Burgmeister, R. F. Vesconder, and W. F. Dwolek, "Mouse Bioassay for *Fusarium* Metabolites: Rejection or Acceptance when Dissolved in Drinking Water," *Appl. Environ. Microbiol.* **39**, 957–961 (1980).

182 C. J. Mirocha, S. V. Ptre, and C. M. Christensen, "Zearalenone," in J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, eds , *Mycotoxins in Human and Animal Health*, Pathotox, Park Forest South, Ill., 1977, pp. 345–364.

183. M. J. Verrett, J.-P. Marliac, and J. McLaughlin, Jr., "Use of the Chicken Embryo in the Assay of Aflatoxin Toxicity," *J. AOAC* **47**, 1003–1006 (1964).

184. J. J. Hlywka, M. M. Beck, and L. B. Bullerman, "The Use of the Chicken Embryo Screen Test and Brine Shrimp (*Artemia salina*) Bioassays to Assess the Toxicity of Fumonisin B_1 Mycotoxin," *Food Chem. Toxicol.* **35**, 991–999 (1997).

185. C. W. Bacon, J. K. Porter, and W. P. Norred, "Toxic Interaction of Fumonisin B_1 and Fusaric Acid Measured by Injection into Fertile Chicken Egg," *Mycopathologia* **129**, 29–35 (1995)

186. D. B. Prelusky et al., "Optimization of Chick Embryotoxicity Bioassay for Testing Toxicity Potential of Fungal Metabolites," *J AOAC* **70**, 1049–1055 (1987).

187. R. F. Brown, J. D. Wildman, and R. M. Eppley, "Temperature-Dose Relationships with Aflatoxin on the Brine Shrimp, *Artemia salina*," *J. AOAC* **51**, 905–906 (1968).

188 R. M. Eppley, "Sensitivity of Brine Shrimp (*Artemia salina*) to Trichothecenes," *J. AOAC* **57**, 618–620 (1974).

189. R. F. Brown, "The Effect of Some Mycotoxins on the Brine Shrimp *Artemia salina*," *J. Am. Oil Chem. Soc.* **46**, 119 (1969).

190. S. Knasmuller et al., "Genotoxic Effects of Three *Fusarium* Mycotoxins, Fumonisin B_1, Moniliformin and Vomitoxin in Bacteria and in Primary Cultures of Rat Hepatocytes," *Mutation Res.* **391**, 39–48 (1997).

191. H. K. Abbas, T. Tanaka, and W T. Shier, "Biological Activities of Synthetic Analogues of *Alternaria alternata* Toxin (AAL-Toxin) and Fumonisin in Plant and Mammalian Cell Cultures," *Phytochem.* **40**, 681–689 (1995).

192 H. K. Abbas, C. D Boyette, and R. E. Hoagland, "Phytotoxicity of *Fusarium*, Other Fungal Isolates, and of the Phytotoxins Fumonisin, Fusaric Acid, and Moniliformin to Jimsonweed," *Phytoprotection* **76**, 17–25 (1995).

193. C. W. Bacon and D. M. Hinton, "Fusaric Acid and Pathogenic Interactions of Corn and Non-Corn Isolates," in L. S. Jackson, J. W. DeVries, and L. B. Bullerman, eds., *Fumonisins in Food*, Plenum Publishing, New York, 1996, pp. 175–191.

194. H. G. Cutler et al., "Chaetoglobosin K: A New Plant Inhibitor and Toxin from *Diplodia macrospora*," *J. Agric. Food Chem.* **28**, 139–142 (1980).

195. V. S. Thompson and C. M. Maragos, "Fiber-Optic Immunosensor for the Detection of Fumonisin B_1," *J. Agric. Food Chem.* **44**, 1041–1046 (1996).

LAUREN JACKSON
FDA-NCFST
Argo, Illinois

MYCOTOXINS

Mycotoxins are chemicals that are produced by filamentous fungi that affect human or animal health. By convention, this excludes mushroom poisons. These fungi are called *toxigenic* fungi. All of these species are deuteromycetes (asexual) some of which have a known ascomycetous (sexual) stage. All of the mycotoxins discussed here are secondary metabolites of the fungi concerned, that is, compounds that are produced after one or more nutrients become limiting (1–3). The occurrence of mycotoxins is entirely governed by the existence of conditions that favor the growth of the fungi concerned. Under environmental conditions, different fungal species are favored as diseases of crop plants or as saprophytes on stored crops and sometimes other materials. When the conditions favor the growth of toxigenic species, it is an invariable and unfortunate rule that one or more of the compounds for which the fungus has the genetic potential are produced. Modern methods of agriculture appear to be selecting for increased prevalence of toxigenic strains (4).

With modern studies of toxigenic fungi, it has been appreciated that they can produce many compounds, often from different biosynthetic families. The ecological significance of the occurrence of these mixtures has been a fertile area of study in recent years. As it relates to human and animal toxicology, the potency of the contaminated material is due to the mixtures present (5,6). At the time of the discovery of penicillin and the first wave of fungal-derived antibiotics, the researchers believed that these compounds were active in nature. In the postwar period, secondary metabolites were characterized as everything from waste products to the consequences of "displacement activities" of the fungi. The current view is that these compounds are important as virulence factors and as mediators of interference competition; that is, they exclude competing microbes and animals from the food source (4,7,8)

Mycotoxins have affected human populations since the beginning of organized crop production. Ergotism is discussed in the Old Testament of the Bible. Some claim that the ancient Chinese used ergot for obstetrical purposes 5000 years ago. Many epidemics of ergotism were reported in western Europe from about A.D. 800. The screams of the

173. C. W. Holzapfel, "The Isolation and Structure of Cyclopiazonic Acid, a Toxic Metabolite of *Penicillium cyclopium* Westling," *Tetrahedron* **24**, 2101-2119 (1968).

174. R. J. Cole, J. W. Kirksey, and J. M. Wells, "A New Tremorgenic Metabolite from *Penicillium paxilli*," *Can. J. Microbiol.* **20**, 1159–1162 (1974).

175. R. J. Cole et al., "Tremorgenic Toxin from *Penicillium verruculosum*," *Appl. Microbiol.* **24**, 248–257 (1972).

176. R. J. Cole et al., "Papalum Staggers: Isolation and Identification of Tremorgenic Metabolites from Sclerotia of *Claviceps paspali*," *J. Agric. Food Chem.* **25**, 1197–2011 (1977).

177. R. J. Cole et al., "Toxin from *Fusarium moniliforme*: Effects on Plants and Animals," *Science* **179**, 1324–1326 (1973).

178 J. A. Lansden et al., "A New Trichothecene Mycotoxin Isolated from *Fusarium tricinctum*," *J. Agric. Food Chem.* **26**, 246–249 (1978).

179. R. O. Sinnhuber "Trout Bioassay of Mycotoxins," in J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, eds., *Mycotoxins in Human and Animal Health*, Pathotox, Park Forest South, Ill., 1977, pp. 731–744.

180. R.-D. Wei, E. B. Smalley, and F. M. Strong, "Improved Skin Test for Detection of T-2 Toxin," *Appl. Microbiol.* **23**, 1029–1030 (1972).

181. H. R. Burgmeister, R. F. Vesconder, and W F. Dwolek, "Mouse Bioassay for *Fusarium* Metabolites: Rejection or Acceptance when Dissolved in Drinking Water," *Appl. Environ. Microbiol.* **39**, 957–961 (1980).

182 C. J. Mirocha, S. V. Ptre, and C M. Christensen, "Zearalenone," in J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, eds , *Mycotoxins in Human and Animal Health*, Pathotox, Park Forest South, Ill., 1977, pp. 345–364.

183. M. J. Verrett, J.-P. Marliac, and J. McLaughlin, Jr., "Use of the Chicken Embryo in the Assay of Aflatoxin Toxicity," *J. AOAC* **47**, 1003–1006 (1964).

184. J. J. Hlywka, M. M. Beck, and L. B. Bullerman, "The Use of the Chicken Embryo Screen Test and Brine Shrimp (*Artemia salina*) Bioassays to Assess the Toxicity of Fumonisin B_1 Mycotoxin," *Food Chem. Toxicol.* **35**, 991–999 (1997).

185. C. W. Bacon, J. K. Porter, and W. P. Norred, "Toxic Interaction of Fumonisin B_1 and Fusaric Acid Measured by Injection into Fertile Chicken Egg," *Mycopathologia* **129**, 29–35 (1995)

186. D. B. Prelusky et al., "Optimization of Chick Embryotoxicity Bioassay for Testing Toxicity Potential of Fungal Metabolites," *J AOAC* **70**, 1049–1055 (1987).

187. R. F. Brown, J. D. Wildman, and R. M. Eppley, "Temperature-Dose Relationships with Aflatoxin on the Brine Shrimp, *Artemia salina*," *J. AOAC* **51**, 905–906 (1968).

188 R. M. Eppley, "Sensitivity of Brine Shrimp (*Artemia salina*) to Trichothecenes," *J. AOAC* **57**, 618–620 (1974).

189. R. F. Brown, "The Effect of Some Mycotoxins on the Brine Shrimp *Artemia salina*," *J. Am. Oil Chem. Soc.* **46**, 119 (1969).

190. S. Knasmuller et al., "Genotoxic Effects of Three *Fusarium* Mycotoxins, Fumonisin B_1, Moniliformin and Vomitoxin in Bacteria and in Primary Cultures of Rat Hepatocytes," *Mutation Res.* **391**, 39–48 (1997).

191. H K. Abbas, T. Tanaka, and W T. Shier, "Biological Activities of Synthetic Analogues of *Alternaria alternata* Toxin (AAL-Toxin) and Fumonisin in Plant and Mammalian Cell Cultures," *Phytochem.* **40**, 681–689 (1995).

192 H. K. Abbas, C. D Boyette, and R. E. Hoagland, "Phytotoxicity of *Fusarium*, Other Fungal Isolates, and of the Phytotoxins Fumonisin, Fusaric Acid, and Moniliformin to Jimsonweed," *Phytoprotection* **76**, 17–25 (1995).

193. C. W. Bacon and D. M. Hinton, "Fusaric Acid and Pathogenic Interactions of Corn and Non-Corn Isolates," in L. S. Jackson, J. W. DeVries, and L. B. Bullerman, eds., *Fumonisins in Food*, Plenum Publishing, New York, 1996, pp. 175–191.

194. H. G. Cutler et al., "Chaetoglobosin K: A New Plant Inhibitor and Toxin from *Diplodia macrospora*," *J. Agric. Food Chem.* **28**, 139–142 (1980).

195. V. S. Thompson and C. M. Maragos, "Fiber-Optic Immunosensor for the Detection of Fumonisin B_1," *J. Agric. Food Chem.* **44**, 1041–1046 (1996).

LAUREN JACKSON
FDA-NCFST
Argo, Illinois

MYCOTOXINS

Mycotoxins are chemicals that are produced by filamentous fungi that affect human or animal health. By convention, this excludes mushroom poisons. These fungi are called *toxigenic* fungi. All of these species are deuteromycetes (asexual) some of which have a known ascomycetous (sexual) stage. All of the mycotoxins discussed here are secondary metabolites of the fungi concerned, that is, compounds that are produced after one or more nutrients become limiting (1–3). The occurrence of mycotoxins is entirely governed by the existence of conditions that favor the growth of the fungi concerned. Under environmental conditions, different fungal species are favored as diseases of crop plants or as saprophytes on stored crops and sometimes other materials. When the conditions favor the growth of toxigenic species, it is an invariable and unfortunate rule that one or more of the compounds for which the fungus has the genetic potential are produced. Modern methods of agriculture appear to be selecting for increased prevalence of toxigenic strains (4).

With modern studies of toxigenic fungi, it has been appreciated that they can produce many compounds, often from different biosynthetic families. The ecological significance of the occurrence of these mixtures has been a fertile area of study in recent years. As it relates to human and animal toxicology, the potency of the contaminated material is due to the mixtures present (5,6). At the time of the discovery of penicillin and the first wave of fungal-derived antibiotics, the researchers believed that these compounds were active in nature. In the postwar period, secondary metabolites were characterized as everything from waste products to the consequences of "displacement activities" of the fungi. The current view is that these compounds are important as virulence factors and as mediators of interference competition; that is, they exclude competing microbes and animals from the food source (4,7,8)

Mycotoxins have affected human populations since the beginning of organized crop production. Ergotism is discussed in the Old Testament of the Bible. Some claim that the ancient Chinese used ergot for obstetrical purposes 5000 years ago. Many epidemics of ergotism were reported in western Europe from about A.D. 800. The screams of the victims, the stench of rotting flesh, extremities falling off, and death all feature in the descriptions of the disease. Large-scale mortalities persisted into the eighteenth century, when governments and the church promoted methods for the removal of sclerotia (9).

Outbreaks of ergotism have been reported in developing countries in modern times. However, because the cause of the problem—the consumption of ergot-infested grain—is something easily seen by the naked eye, ergotism is now uncommon. During the thirteenth century, rye was replaced by wheat in western Europe. The former species is resistant to *Fusarium* diseases and, as noted, the latter typically susceptible, leading to the accumulation of trichothecene mycotoxins. These compounds are prevalent in small grains in western Europe (10). In her book *Poisons of the Past* (11), the American historian Mary Matossian has analyzed the effect of weather and food consumption patterns during the plague epidemics in Europe. She found that plague epidemics took place when there were surpluses of small grains, the favourite food of rats. The occurrence of plague in the Middle Ages is very strongly associated with rainy and humid crop years. She found that the two years prior to the pandemic in Europe in 1378, the weather was extraordinarily rainy.

Although there are hundreds of fungal metabolites that are toxic in experimental systems, only five are of major agricultural importance: deoxynivalenol, aflatoxin, fumonisin, zearalenone, and ochratoxin (5). In grains, the toxins are concentrated into bran fractions during milling (12). All these toxins are stable in the processes typical of food and feed processing (12–15). Animal products can be a minor dietary source of ochratoxin and fumonisin; for the remaining three toxins, animal sources are not important under normal circumstances (16). Milk can contain aflatoxin M1 a derivative of aflatoxin B1 with much lower toxicity (17).

A recent report by the U.S. National Academy of Sciences notes that even with the high-quality food system in the United States, the carcinogenic mycotoxins in American diets may increase cancer rates. This is absolutely the case in many developing countries, where mycotoxins are a major population health problem (18,19). Information on regulations and guidelines for mycotoxins can be found in Van Egmond (20) and Kuiper-Goodman (21), and was discussed at the 1999 FAO Conference on Mycotoxins, in Tunis (*http://www.fao.org*).

A number of mycotoxins that occur from time to time in food in certain parts of the world will be considered. Human diseases associated with uncommon mycotoxins on rice and other crops are described in Pitt (22) and Beardall and Miller (23). There are also fungal toxins that occur in pastures that are not considered in this treatment. Inhalation exposure to mycotoxins is also not covered in this treatment but is a problem in farming and grain handling and in buildings with appreciable mold growth (24–27).

DEOXYNIVALENOL AND ZEARALENONE

These toxins occur when wheat, barley, corn, and sometimes oats and rye are infected by *Fusarium graminearum* and *F. culmorum*. These species cause *Fusarium* head blight in small grains, a major agricultural problem worldwide. These species cause a similar disease in corn called *Gibberella* ear rot. Disease incidence is most affected by moisture at flowering, and most cultivars and hybrids used today lack genetic resistance to the disease. *F. graminearum* is common in wheat from North America and China. *F. culmorum* is the dominant species in cooler wheat growing areas such as Finland, France, Poland, and The Netherlands (28). Oats, rye, and triticale have also been reported to contain deoxynivalenol (10,17,29–31). Wheat, corn, and barley comprise two-thirds of the world production of cereals; hence, deoxynivalenol is the mycotoxin to which the greatest number of humans are exposed.

A third species, *F. crookwellense* can also cause head blight or corn ear rot and produces nivalenol and zearalenone and many of the same families of minor metabolites as the related species (32). Nivalenol was first isolated from grains contaminated by *F. sporotrichioides* and misidentified as "*Fusarium nivale* Fn2B" (33,34). The toxic profile of nivalenol is thought to be similar to that of deoxynivalenol, although nivalenol is more potent in assays for some acute effects.

Different populations of *F. graminearum* and *F. culmorum* produce different toxins. In 1983, it was recognized that Japanese strains produce deoxynivalenol via 3-acetyldeoxynivalenol, but Canadian strains produced the 15-acetate. This has implications for consumers of grain contaminated with one or other of the populations because the toxicities of the two acetates are different (24), and there is always some of the acetate present. North and South American strains of these two species produce the 15-acetate, and most strains from Asia and Europe produce the 3-acetate (32,35,36). Authentic strains of *F. graminearum* have been shown to produce nivalenol instead of deoxynivalenol in Japan, New Zealand, Australia, and Italy (37–39). This has not been shown in North American isolates to date (the nivalenol in North American grains is presumed to come from *F. crookwellense*). *F. graminearum* and *F. culmorin* produce a wide variety of metabolites from several biosynthetic families (31). This includes trichothecenes, apotrichothecenes (40), zearalenone, calonectrins (41,42), sambucinol and sambucoin (42), culmorins (43), and butenolide.

Red mold poisoning was reported in rural Japan throughout the 1950s (44). Eventually, Japanese researchers discovered deoxynivalenol in grain that had made humans ill (45). The same chemical was subsequently rereported as *vomitoxin* from *F. graminearum*–contaminated corn in 1973 (46). Deoxynivalenol was a widespread contaminant of wheat in the northeast of the United States and in eastern Canada in 1979 to 1981 and then again in 1993 to 1996 in the Great Lakes area and Red River Valley. Much of wheat crop of Ontario and several states could not be used in 1996 because of deoxynivalenol contamination. Large-scale acute human toxicoses from deoxynivalenol have occurred in modern times in India (47), China, and Korea, among other countries (23,48).

Trichothecenes are potent low-molecular-weight inhibitors of protein synthesis (49). In addition, they cause physical damage to membranes resulting in cell lysis. Red blood cells are a compartment for trichothecene metabolism and these cells will lyse in the presence of excess circulating toxin. The amount of toxin required to lyse red blood cells varies according to animal species (50).

Swine are the domestic animal species most sensitive to the effects of trichothecenes (6). Experiments feeding pure deoxynivalenol to swine suggested that the diets containing <2 mg/kg deoxynivalenol would have little impact on growth. However, many experiments using naturally contaminated grains often demonstrated that such grain was more toxic than indicated from the deoxynivalenol content (51,52). Several of the minor toxins from *F. graminearum* were present in such grain, sometimes at concentrations similar to deoxynivalenol (53). These co-occurring toxins were shown to increase the toxicity of deoxynivalenol when fed in combination to insects (54). This might be also true in swine (55). Toxicological interactions between trichothecenes were also discovered in yeasts (56) and in animals (57,58). The metabolism of deoxynivalenol and other trichothecenes at subacute doses results in rapid elimination in swine, other domestic animals, and rodents (16,17,25).

The basis for the feed refusal is the impressive neurotoxicity of dexoynivalenol. Experiments involving the dosing of the toxin by a continuous exposure osmotic pump implanted intraperitoneally resolved that the effects could not be due to taste or learned responses (52). A single dose of 0.25 mg/kg (IV) changed neurotransmitter concentrations in the hypothalamus, frontal cortex, and cerebellum up to 8 days postdosing (59). A very low dose (10 μg/kg) IV resulted in changes in cerebral spinal fluid neurotransmitters (60). Based on acute human exposure-emesis data, humans are not less, and are probably more, sensitive to deoxynivalenol than swine (21). Feed refusal also occurs in mice and in lifetime studies; this reduced the incidence of spontaneous liver tumors due to calorie restriction (61).

Because deoxynivalenol is less acutely toxic than the potent trichothecenes, the immunotoxic and neurotoxic properties of trichothecenes were recognized. Changes in immune system function in male mice occur at dietary concentrations often encountered by humans (62). As with other trichothecenes, high exposures increase susceptibility to facultative pathogens such as *Listeria*. Deoxynivalenol exposure produces prolonged elevations in serum IgA and mesangial IgA leading to hematuria (63). The calonectrins produced by *F. graminearum* also affect immune functions *in vitro* (64). Human IgA disregulation (Berger's disease) is common, and the only agents so far demonstrated to reproduce this condition in experimental animals are trichothecenes (63).

Cattle and cows are tolerant to deoxynivalenol, and milk production is not affected at typical field concentrations of deoxynivalenol (ie, dietary concentrations less than 5 mg/kg). Trichothecenes are detoxified by rumen bacteria. Poultry species are also tolerant to typical field concentrations of deoxynivalenol (6). Deoxynivalenol is not a carcinogen (17). The occurrence of deoxynivalenol in diets affects uptake of sugars and minerals (65). A review of the toxicology of deoxynivalenol is found in Rotter et al. (66).

As noted, crops that are contaminated by deoxynivalenol can often contain zearalenone, albeit at a lower frequency. Zearalenone is more common in maize than small grains (17). Zearalenone is an estrogen analogue and causes hyperestrocism in female pigs at low levels; the dietary no-effect level is less than 1 mg/kg. Cows and sheep are also sensitive to the estrogenic effects of this toxin with depressed ovulation and lower lambing percentages. The no-effect dietary levels are not clearly known (6). Nonhuman primates are also very sensitive to the estrogenic effects of zearalenone (20). Zearalenone has been implicated in several incidents of precocious pubertal changes in girls in Europe and South America (67,68); thus, this is a true environmental estrogen (69). There is limited evidence for its rodent carcinogenicity (17). A detailed description of the toxicology of zearalenone is found in Kuiper-Goodman et al. (70).

AFLATOXIN

Aspergillus flavus and *A. parasiticus* (Aflatoxins)

Aflatoxin is a problem in many commodities; however, as far as grains are concerned, it is primarily a problem in maize. This is because maize is colonized in the field depending on environmental conditions, whereas other grains are not. Of the other grains, rice is an important dietary source of aflatoxin in circumstances of poor storage in tropical and subtropical areas. The character of the problem varies by region. In the United States, storage systems are very good (71) and the problem is preharvest contamination of maize and peanuts (4,72). In tropical countries, such as Thailand and the Philippines, crop storage is a substantial problem (73). Aflatoxin contamination is managed by the development of systems to detect and segregate contaminated kernels and better storage systems. For corn and peanuts, all manner of efforts have been made to prevent aflatoxin contamination, including plant breeding and biological control to little effect (74).

In the United States, Mexico, and South America, *A. flavus* and *A. parasiticus* infect corn, although *A. parasiticus* is relatively uncommon (72). In the environmental circumstances prevalent in the corn belt of the United States, *A. flavus* contamination of corn occurs in two basic ways: (*1*) airborne or insect-transmitted conidia contaminate the silks and grow into the ear when the maize is under high temperature stress, or (more commonly) (*2*) insect- or bird-damaged kernels become colonized with the fungus and accumulate aflatoxin. In either case, drought-, nutrient-, or temperature-stressed plants are more susceptible to colonization by *A. flavus* (75). This is also the case for peanuts, which are mainly colonized by *A. parasiticus*. Aflatoxin contamination can be limited by preventing late season drought by irrigation where this is possible (74).

Insects and arthropods readily become contaminated with *A. flavus*. Soil-inhabiting mites feed on the germinated sclerotia and hence acquire conidia. Nitidulid beetles feed on moldy ears of maize, perhaps preferentially. Nitidulids are attracted to damaged ears, including those caused by corn ear worms and the European corn borer spreading the fungus into damaged kernels (4,76). In drought years, insects are attracted to peanuts and both wound the plant and bring the fungus.

The ecology of *A. flavus* in corn in subtropical regions of Asia appears to be different from that already described. In subtropical Asia there is a rapid rise in aflatoxin concentrations immediately postharvest. American studies have shown that, typically, *A. flavus*–infested kernels are randomly distributed in ears after wound inoculation (77). The kernels that were the sites of initial infection have very high aflatoxin contents. In studies done in Thailand, approximately 19% of kernels from 130 samples of maize collected from farmers fields throughout Thailand contained *A. flavus* (73).

There are many detailed reviews of the toxicology of aflatoxin, including that of the International Agency for Research on Cancer (IARC) (17). Aflatoxin B1, the most toxic of the aflatoxins, causes a variety of adverse effects in different animal species, especially chickens. In poultry, these include liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior eggshell quality, inferior carcass quality, and increased susceptibility to disease (78). Swine are somewhat less sensitive than poultry species, with the LD_{50} being perhaps half of that of chickens. Aflatoxin is hepatotoxic, and its acute and chronic effects in swine are largely attributable to liver damage (79). In cattle, the primary symptom is reduced weight gain as well as liver and kidney damage. Milk production is reduced (80). Aflatoxin is also immunotoxic in domestic and laboratory animals with oral exposures in the ppm range. Cell-mediated immunity (lymphocytes, phagocytes, mast cells, and basophils) is more affected than humeral immunity (antibodies and complement) (63). The effects of aflatoxin on laboratory animals has been exhaustively reviewed by IARC (17).

Naturally occurring mixtures of aflatoxins were classified as class 1 human carcinogens, and aflatoxin B_1 is also a class 1 human carcinogen. There was inadequate evidence of the human carcinogenicity of aflatoxin M_1, the metabolite of aflatoxin B_1 found in human and animal milk (17). Many people in developing countries are seropositive for hepatitis B and C, which are also liver carcinogens. Although aflatoxin is a potent chemical carcinogen, its ability to alter response to the hepatocarcinogenic viruses is perhaps of greater importance. The relative rates of liver cancer in hepatitis B–positive populations are an order of magnitude greater (60X) when exposed to aflatoxin. This is because the toxin interferes with the processing of the virus (81,82).

The immunotoxicity of aflatoxin is also being increasingly studied; some think that it would have to be regulated for this toxicity regardless of its carcinogenicity. In one study, serum aflatoxin-lysine adducts were higher in protein energy malnourished (PEM) children compared with control children. Aflatoxin metabolism was affected, with relatively higher serum concentrations in PEM children. A second study compared PEM children with high and low serum aflatoxin concentrations. The serum aflatoxin positive group of PEM children showed a significantly lower hemoglobin level ($p = 0.02$), longer duration of edema ($p = 0.05$), an increased number of infections ($p = 0.03$), and a longer duration of hospital stay ($p = 0.008$). This finding was echoed in another study, which suggested that malaria infections in children were increased in children exposed to aflatoxin (18).

FUMONISINS

Fumonsisins are produced by *F. verticilloides* (formerly *moniliforme*), *F. proliferatum*, and several uncommon fusaria (83,84). Fumonisins were discovered in 1988 by two groups working independently. One was investigating the cause of human esophageal cancer in parts of southern Africa (85). The other was attempting to find the cause of a disease of horses known since 1850, equine leucoencephalomalacia (ELEM) (86). There are at least three naturally occurring fumonisins—B_1, B_2, and B_3; FB_1 occurs at highest concentration followed by B_2 and B_3. Fumonisins have been found as a very common contaminant of corn-based food and feed in the United States, China, Europe, southern Africa, South America, and Southeast Asia (5,17,87). In addition, there are a number of minor fumononisins. *F. verticilloides* also produces fusarin C, heat- and light-unstable compounds, and fusaric acid. *F. proliferatum* produces fumonisins, fusarins, fusaric acid, and moniliformin (88,89). Moniliformin is toxic to poultry species (6) and can occur in food (90).

F. verticilloides and *F. proliferatum* are the most common fungi associated with corn. For many years, *F. moniliforme* has been known to occur systematically in leaves, stems, roots, and kernels (91). These fungi can be recovered from virtually all corn kernels including those that are healthy, which suggests that it may be an endopyte that is, a mutualistic relationship (28). *F. verticillioides* and *F. proliferatum* cause a "disease" called fusarium kernel rot. In parts of the United States and lowland tropics, this is one of the most important ear diseases and is associated with warm, dry years and insect damage and fumonisin (92,93). Corn plant disease-stress also promotes the growth of *F. verticillioides* and fumonisin formation (94).

Fumonisins are toxic in all types of cells (yeast, plant, animal, human) due to their effects on sphinglolipid synthesis (95). Alteration in sphingolipid base ratios occurs almost immediately after exposure because fumonisin inhibits ceraminde synthetase. There are also many changes in the amounts and ratios of complex ceramides. In addition, fumonisins induce apoptosis leading to cell proliferation, which may explain their carcinogenic properties (96,97). Fumonisins B_2 and B_3 have similar toxic properties to B_1 but are less potent (98).

Pure fumonisin was demonstrated to cause equine leucocencephalomalia (ELEM) in 1988 (99). ELEM involves a massive liquefactive necrosis of the cerebral hemispheres; hence, the disease involves neurological manifestations including abnormal movements, aimless circling, lameness, and so on. At high exposures, death can occur within hours after the onset of visible symptoms. Damage to liver and kidneys in horses, features of fumonisins exposure in other animals, is poorly characterized in horses (6)

As in equine species, alterations of sphingolipid base ratios are indicative of fumononisin exposure in swine

(95). At high exposures, porcine pulmonary edema (PPE) has been shown to be caused by both pure fumonosin and *F. moniliforme* culture material and maize containing fumonisin (6). This is thought to be caused by fumonisin-induced heart failure (100). At lower exposures, both liver and kidney damage has been reported in swine (101). Fumonisin causes feed refusal and changes in carcass quality at dietary concentrations in the low mg/kg range (102,103). Feeder calves were reported to be unaffected by fumonisin (6).

Exposure to *F. verticilloides*–contaminated maize has been linked to the elevated rates of esophageal cancer in the Transkei for 25 years, and this has since been directly linked to fumonisin exposure (17,87,104). Fumonisin B_1 has been demonstrated to exhibit cancer-promoting activity in diethylnitrosamine-initiated rats (105). Fumonisin B_1 has been also shown to be hepatotoxic and hepatocarcinogenic in rats fed 50 mg/kg (90% purity) (106). Very pure fumonisin produced tumors in male and female mice and rats in the U.S. National Toxicology two-year bioassay (NTP TR 496; *http://www.ntp.gov*). In Fisher 344 rats, fumonisin B_1 exposure resulted in tumors at doses about 10 times less than aflatoxin. IARC (17) examined the human carcinogenicity of grain contaminated with *F. verticillioides* containing fumonisins and fusarin C and found them to be possible human carcinogens. There is an enormous amount known about the rodent toxicities of fumonisins from the just-noted NTP assay. An exhaustive treatment of the toxicology of fumonisins can be found in WHO (87).

OCHRATOXIN

Despite many contrary reports, ochratoxin is known to be produced by only one species of *Penicillium, verrucosum*. *Aspergillus ochraceous* and several related species also produce ochratoxin on grapes and coffee (17,107,108; M. Frank, unpublished data, 1999). A small percentage of surface-disinfected wheat and barley kernels collected at harvest in the UK and Denmark were contaminated by *P. aurantiogriseum* and *P. verrucosum*; this was similar in studies done in western Canada over many years. Infestation of some kernels by the ochratoxin-producing fungus *P. verrucosum* occurs from anthesis, and surface contamination is common at harvest. The absolute level of preharvest infestation varies according to site and season (5).

Ochratoxin is a potent nephratoxin in swine and causes kidney cancer in male Fisher 344 rats. Pigs are affected at low exposures in terms of kidney damage, but typically there are no overt signs of biochemical/hematological changes. At higher concentrations (>2 μg/g), decreased weight grains occur (6). Poultry are similarly affected, with reduced growth rate and egg production at low ochratoxin concentrations > 2 μg/g. Higher dietary ochratoxin concentrations are often fatal. Cattle are resistant to ochratoxin concentrations found in naturally contaminated grain (6). Ochratoxin is often found with other toxins such as citrinin and the naphthaquinone mycotoxins from *P. aurantiogriseum* (17,109). Citrinin mimics the effects of ochratoxin, although it is less potent (109). The naphthaquinones xanthomegnin and viomellein from *P. aurantiogriseum* are nephrotoxic (110). Interactions between ochratoxin and citrinin have been demonstrated in some experiments (6).

Ochratoxin is suspected as the cause of urinary tract cancers and kidney damage in areas of chronic exposure in parts of eastern Europe (17,111; M. Frank unpublished data, 1999). Human exposure to ochratoxin primarily occurs from whole-grain breads. Some exposure comes from the consumption of animal products, especially pork and pig-blood-based products (14). There is a great deal known about human serum ochratoxin concentrations in Europe (111,112). Despite considerable effort, no satisfactory conclusion has been reached regarding the linkage of ochratoxin with urinary tract cancers in humans (19,82,113). The last IARC evaluation of ochratoxin determined it to be a possible human carcinogen (17). As of 1999, The Joint Expert Committee for Food Additives & Contaminants of the WHO/FAO has a tolerable human daily intake for this toxin based on its renal toxicity. Ochratoxin exposure in the United States and Canada are considered to be low based on testing by Health Canada and the Food & Drug Administration.

OTHER MYCOTOXINS THAT CAN OCCUR IN FOOD

T-2 toxin was isolated from "strain T-2" of *F. sporotrichioides* misidentified as *F. tricinctum* isolated from corn associated with cow mortalities (33,114). This compound has been the subject of considerable toxicological study (17) because it is easy to isolate and purify. During World War II, there were large-scale poisoning of the rural population in the former Soviet Union caused by the consumption of grains left in the field over winter (estimates range to 1,000,000 victims). The disease was called alimentary toxic aleukia. Samples of extracts made at the time have been shown to contain the trichothecenes T-2 and HT-2 toxin. Shortly after consuming food prepared with contaminated grain, people reported a burning sensation in their mouths, vomiting, weakness, fatigue, and tachycardia. After a period of time, affected individuals felt better, but there was a progressive leucopenia, anemia, and decreased platelet count, lowering "the resistance of the body to bacterial infection." As consumption of toxic grains continued, petechial hemorrhages on the upper part of the body appeared together with necrotic lesions in the mouth and face. Bacterial infections were common. Patients who reached this stage almost always died (115). The toxicology of T-2 toxin is similar in character to that previously described for deoxynivalenol and is reviewed extensively in Ref. 17.

Patulin (116) is primarily found in apple and grape juices where is occurs from the growth of *Penicillium expansum*. Many other species of molds produce patulin. The presence of this toxin in juice is a sign that damaged fruit was present. The sparse toxicological data on this compound have been reviewed by the California Environmental Protection Agency in 1998 (*http://www.oehha.org*). This compound has low acute toxicity, and there is insufficient evidence to conclude that it is carcinogenic in rodents (IARC category 3). Human exposure in some countries has been high, and industry-government efforts have been made in North America and Europe to reduce the occurrence of patulin in juice.

Ergot alkaloids seldom appear in meaningful concentrations in food samples in the North America or Europe because the presence of *Claviceps* sclerotia in grains is a grading factor. However, infections by ergot alkaloid-producing fungi remain common in the sense that plants on the edges of rye, barley, and wheat fields are often infected. Thus the majority of products made from wheat or rye contain traces of ergot alkaloids (117). Grains downgraded to animal feed can contain ergot. Cattle are more sensitive than mice, sheep, or swine and sclerotial contents of about 0.1% appear to be tolerable. In cattle, the symptoms include lameness, and in other domestic animals reduced weight gain can be expected (6). Ergotism in humans has been reported in India and parts of Africa in modern times (29).

BIBLIOGRAPHY

1. J. D. Bu'Lock, "Secondary Metabolism," in J. E. Smith and D. R. Berry, eds., *The Filamentous Fungi*, Academic Press, New York, 1975, pp. 33–58.
2. J. D. Bu'Lock, "Mycotoxins as Secondary Metabolites," in P. S. Steyn, ed., *The Biosynthesis of Mycotoxins. A Study of Secondary Metabolism*, Academic Press, New York, 1980, pp. 1–16.
3. J. D. Miller, M. E. Savard, and S. Rapior, "Production and Purification of Fumonisins From a Stirred Jar Fermentor," *Natural Toxins* **2**, 354–359 (1994).
4. D. R. Wicklow, "Maize Cultivation Selects for Mycotoxin-Producing Ability Among Kernel Rotting Fungi," in C. H. Chou and G. R. Waller, eds., *Phytochemical Ecology: Allelochemicals, Mycotoxins and Insect Pheromones*, Academia Sinica Monograph Series 9, Taipei, China, 1989, pp. 263–274.
5. J. D. Miller, "Fungi and Mycotoxins in Grain: Implications for Stored Product Research," *J. Stored Product Res.* **31**, 1–6 (1995).
6. D. B. Prelusky, B. A. Rotter, and R. G. Rotter, "Toxicology of Mycotoxins," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain: Compounds Other than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 359–404.
7. B. B. Jarvis and J. D. Miller, "Natural Products, Complexity and Evolution," in J. T. Romeo, J. A. Saunders, and P. Barbossa, eds. *Phytochemical Diversity and Redundancy in Ecological Interactions*, Plenum Press, New York, 1996, pp. 265–294.
8. D. R. Wicklow, "Interference Competition and the Organization of Fungal Communities," in D. T. Wicklow and G. C. Carroll, eds., *The Fungal Community*, Marcel Dekker, New York, 1981, pp. 351–375.
9. S. J. Van Rensburg and B. Altenkirk, "*Claviceps purpurea*-Ergotism", in I. F. H. Purchase, ed., *Mycotoxins*, Elsevier, New York, 1974, pp. 69–96.
10. H.-M. Muller et al., "*Fusarium* Toxins in Wheat Harvested During Six Years in an Area of Southwestern Germany," *Natural Toxins* **5**, 24–30 (1997).
11. M. K. Matossian, *Poisons of the Past*, Yale University Press, New Haven, Conn., 1989
12. J. C. Young et al., "Effect of Milling and Baking on Deoxynivalenol (Vomitoxin) Content of Eastern Canadian Wheats," *J. Agric. Food. Chem.* **32**, 659–664 (1984).
13. L. A. Goldblatt, ed., *Aflatoxin, Scientific Background, Control and Implications*, Academic Press, New York, 1969
14. T. Kuiper-Goodman and P. M. Scott, "Risk Assessment of the Mycotoxin Ochratoxin A," *Biomedical and Environmental Sciences* **2**, 179–248 (1989).
15. M. M. Castelo, S. S. Sumner, and S. S. Bullerman, "Stability of Fumonisins in Thermally Processed Corn Products," *J. Food Protection* **61**, 1030–1033 (1998).
16. D. B. Prelusky, "Residues in Food Products of Animal Origin," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain: Compounds Other than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 405–420.
17. *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*, International Agency for Research on Cancer, Monograph 56, Lyon, France, 1993.
18. National Academy of Sciences, *Carcinogens and Anticarcinogens in the Human Diet*, National Academy Press, Washington, D.C., 1996.
19. J. D. Miller, "Global Significance of Mycotoxins," in M. Miraglia et al., eds., *Mycotoxins and Phycotoxins—Developments in Chemistry, Toxicology and Food Safety*, Alaken, Fort Collins, Colo., 1998, pp. 3–16.
20. H. P. Van Egmond, "Current Situation on Regulations for Mycotoxins, Overview of Tolerances and Status of Sampling Methods of Sampling and Analysis," *Food Additives and Contaminants* **6**, 139–188 (1989)
21. T. Kuiper-Goodman, "Prevention of Human Mycotoxicosis Through Risk Assessment and Risk Management," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain: Compounds Other than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 439–470.
22. J. I. Pitt, "*Penicillium* Toxins," *Australian Centre for International Agricultural Research Proc.* **36**, 99–103 (1991).
23. J. M. Beardall and J. D. Miller, "Diseases in Humans with Mycotoxins as Possible Causes," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain: Compounds Other than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 487–540.
24. J. L. Autrup et al., "Exposure to Aflatoxin B in Animal Feed Production Plant Workers," *Scand. Environ. Health. Perspect.* **99**, 195–197 (1993)
25. V. R. Beasley, ed., *Trichothecene Mycotoxins: Pathophysiologic Effects*, Vols. 1 and 2, CRC Press, Boca Raton, Fla., 1989.
26. B. Flannigan and J. D. Miller, "Health Implications of Fungi in Indoor Environments—An Overview," in R. Samson et al., eds., *Health Implication of Fungi in Indoor Environments*, Elsevier, Amsterdam, 1994, pp. 3–28.
27. E. L. Hintikka, "Human Stachybotrytoxicosis," in T. D. Wyllie and L. G. Morehouse, eds., *Mycotoxigenic Fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook*, Vol. 3, Marcel Dekker, New York, 1978, pp. 87–89.
28. J. D. Miller, "Epidemiology of *Fusarium* Ear Diseases," in J. D. Miller and H. L. Trenholm, eds., *Mycotoxins in Grain: Compounds Other than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 19–36.
29. J. M. Beardall and J. D. Miller, "Natural Occurrence of Mycotoxins Other than Aflatoxin in Africa, Asia and South America," *Mycotoxin Res.* **10**, 21–40 (1994).

30. P. M Scott, "The Natural Occurrence of Trichothecenes, in V. R. Beasley, ed., "*Trichothecene Toxicosis. Pathophysiological Effects,*" Vol. 1, CRC Press, Boca Raton, Fla., 1989, pp. 2–26.
31. M. W Trucksess et al., "Determination and Survey of Deoxynivalenol in White Flour, Whole Wheat Flour and Bran," *J AOAC Int.* **79**, 883–887 (1996).
32. J. D. Miller et al., "Trichothecene Chemotypes of Three *Fusarium* Species," *Mycologia* **83**, 121–130 (1991).
33. W. F O. Marasas, P. E. Nelson, and T. A. Toussoun, *Toxigenic Fusarium Species*, Pennsylvania State University Press, University Park, Pa., 1984.
34. T. Tatsuno et al., "Nivalenol, A Toxic Principle of Fusarium nivale", *Chem. Pharm. Bull.* **16**, 2519–2520 (1968).
35. C. J. Mirocha, "Variation in Deoxynivalenol, 15-Acetyl Deoxynivalenol, 3-Acetyl Deoxynivalenol and Zearlalenone Production by *Fusarium graminearum* Isolates," *Appl. Environ. Microbiol.* **55**, 1315–1316 (1989).
36. M. S. Piñeiro and G. E. Silva, "*Fusarium* Toxins in Uruguay. Head Blight, Toxin Levels and Grain Quality," *Cereal Res. Comm.* **25**, 805–806 (1997).
37. M. Ichinoe et al., "Chemotaxonomy of *Gibberella zea* with Special Reference to the Production of Trichothecenes and Zearalenone," *Appl. Environ. Microbiol.* **46**, 1364–1369 (1983).
38. D. R. Lauren, S. T. Sayer, and M. E. di Menna, "Trichothecene Production by *Fusarium* Species Isolated from Grain and Pasture Throughout New Zealand," *Mycopathologia* **120**, 167–176 (1992).
39. A. Logrieco, A. Bottalico, and C. Altomare, "Chemotaxonomic Observations on Zearalenone Production by *Gibberella zea* from Cereals in Southern Italy," *Mycologia* **80**, 892–895 (1988).
40. R. Greenhalgh et al., "Apotrichothecenes, Minor Metabolites of the *Fusarium* Species," in S. Natori, T. Hashimoto, and Y. Ueno, eds., *Proc. 7th Int. Symp. on Mycotoxins and phycotoxins*, Tokyo, 1989, pp 223–232.
41. R. Greenhalgh et al., "Production and Characterization of Deoxynivalenol and Other Secondary Metabolites of *Fusarium culmorum* (CMI 14764, HLX 1503)," *J. Agric. Food Chem.* **34**, 98–102 (1985).
42. R. Greenhalgh et al., "Isolation and Characterization by Mass Spectrometry and NMR Spectroscopy of Secondary Metabolites of Some *Fusarium* species," in P. S. Steyn, ed., *Proc. 6th IUPAC Int. Symp. on Mycotoxins and Phycotoxins*, August 16–19, 1988, pp. 137–152.
43 G. C. Kasitu et al., "Isolation and Characterization of Culmorin Derivatives Produced by *Fusarium culmorum* CMI 14764," *Can. J. Chem.* **70**, 1308–1316 (1992).
44 S. Udegawa, "Mycotoxicoses—The Present Problem and Prevention of Mycotoxins," *Asian Med. J.* **31**, 599–604 (1988).
45. N. Morooka et al., "Studies on the Toxic Substances in Barley Infected with *Fusarium* Species," *J. Food Hygiene Soc. Jap.* **13**, 368–375 (1972).
46. R. Vesonder, A. Ceigler, and A. H. Jensen, "Isolation of the Emetic Principle from *Fusarium graminearum*-infected Corn," *Appl. Microbiol.* **26**, 1008–1010 (1973).
47. R. V. Bhat et al., "Outbreak of Trichothecene Mycotoxicosis Associated with Consumption of Mould-Damaged Wheat in Kashmir Valley, India," *Lancet* **7**, 35–37 (1989).
48. J. D. Miller, "Contamination of Food by *Fusarium* Toxins: Studies from Austria–Asia," *Proc. Jap. Assoc. Mycotoxicol.* **32**, 17–24 (1990).
49. B. Feinberg and C. S. MacLaughlin, "Biochemical Mechanism of Action of Trichothecene Mycotoxins," in V. R. Beasley, ed. *Trichothecene Mycotoxins: Pathophysiologic Effects*, Vol. 1, CRC Press, Boca Raton, Fla., 1989, pp. 27–36.
50. G. C. Khachatourians, "Metabolic Effects of the Trichothecene T-2 Toxin," *Can. J. Physiol. Pharmacol* **68**, 1004–1008 (1990).
51. B. C. Foster et al., "Evaluation of Different Sources of Deoxynivalenol (Vomitoxin) Fed to Swine," *Can. J. Animal. Sci.* **66**, 1149–1154 (1986).
52. D. B. Prelusky, "Effect of Intraperitoneal Infusium of Deoxynivalenol on Feed Consumption and Weight Gain in the Pig," *Natural Toxins* **5**, 121–125 (1997).
53. B. C. Foster et al., "Fungal and Mycotoxin Content of Slashed Corn," *Microbiol. Aliment. Nutr.* **4**, 199–203 (1986)
54. P. F. Dowd, J. D. Miller, and R. Greenhalgh, "Toxicity and Interactions of Some *Fusarium graminearum* Metabolites to Caterpillars," *Mycologia* **81**, 646–650 (1989).
55. R. G Rotter et al., "A Preliminary Examination of Potential Interactions Between Deoxynivalenol and Other Selected *Fusarium* Metabolites in Growing Pigs," *Can. J. Animal Sci.* **72**, 107–116 (1992).
56. H. A. Koshinski and G. C. Khachatourians, "Trichothecene Synergism, Additivity and Antagonism: The Significance of the Maximally Quiescent Ratio," *Natural Toxins* **1**, 38–47 (1992).
57. H. B. Schiefer, D. S. Hancock, and A. R. Bhatti, "Systemic Effects of Topically Applied Trichothecenes. I. Comparative Study of Various Trichothecenes in Mice", *J. Vet. Med.* **33A**, 373–383 (1986).
58. T. N. Bhavanishankar, H. P. Ramesh, and T. Shantha, "Dermal Toxicity of *Fusarium* Toxins in Combinations," *Arch. Toxico.* **61**, 241–244 (1988).
59. D. B. Prelusky et al., "Effect of Deoxynivalenol on Neurotransmitters in Discrete Regions of Swine Brain," *Arch. Environ. Contam. Toxicol.* **22**, 36–40 (1992).
60. D. B. Prelusky, "The Effect of Low-Level Deoyxivalenol on Neurotransmitter Levels Measured in Pig Cerebral Spinal Fluid," *J. Environ. Sci. Health.* **B26**, 731–761 (1993).
61. F. C. Iverson et al., "Chronic Feeding Study of Deoxynivalenol in B6C3F1 Male and Female Mice," *Teratogenesis, Carcinogenesis and Mutagenesis* **15**, 283–306 (1995).
62. D. M. Greene et al., "Role of Gender and Strain in Vomitoxin-Induced Dysregulation of IgA Production and IgA Nephropathy in the Mouse," *J. Toxicol. Environmental Health* **43**, 37–50 (1994).
63. J. J. Pestka and G. S. Bondy, "Mycotoxin-Induced Immune Modulation," in J. H. Dean et al., eds., *Immunotoxicology and Immunopharmacology*, Raven Press, New York, 1994, pp. 163–182.
64. G S. Bondy et al., "Murine Lymphocyte Proliferation Impaired by Substituted Neosolaniols and Calonectrins, *Fusarium* Metabolites Associated with Trichothecene Biosynthesis," *Toxicon.* **29**, 1107–1113 (1991).
65. G. K. Hunder et al., "Influence of Subchronic Exposure to Low Dietary Deoxynivalenol, A Trichothecene Mycotoxin, On Intestinal Absorption of Nutrients in Mice," *Food Chem. Toxicol.* **29**, 809–814 (1991).
66. B. A. Rotter, D. B Prelusky, and J. J. Petska, "Toxicology of Deoxynivalenol (Vomitoxin)," *J. Toxicol. Environ. Health* **48**, 101–134 (1996).
67. G. Falkay et al., "Affinity of *Fusarium* Toxins on Human Myometrial Estradiol Receptors," in S. Gorog, ed., *Proc. 5th*

Symp. on the Analysis of Steroids, Szombathely, Hungary, 1993, pp. 25–32.

68. H. B. Schiefer, "Mycotoxins," in Y. H. Hui, ed., *Encyclopedia of Food Science & Technology*, John Wiley and Sons, New York, 1990, pp. 1862–1869.
69. K. R. Price and G. R. Fenwick, "Naturally Occurring Oestrogens in Foods, A Review," *Food Additives and Contaminants* **2**, 73–106 (1985).
70. T. Kuiper-Goodman, P. M. Scott, and H. Watanabe, "Risk Assessment of the Mycotoxin Zearalenone," *Regulatory Toxicol. and Pharmacol.* **7**, 253–306 (1987).
71. D. B. Sauer and J. F. Tuite, "Conditions That Affect Growth of *Aspergillus flavus* and Production of Aflatoxin in Stored Maize," in M. S. Zuber, E. B. Lillehoj, and B. L. Renfro, eds., *Aflatoxin in Maize*, CIMMYT, Mexico, 1987, pp. 41–50.
72. G. A. Payne, "Aflatoxin in Maize," *Curr. Rev. Plant Sci.* **10**, 423–440 (1992).
73. P. Siriacha, P. Tanboon-Ek, and D. Buangsuwon, "Aflatoxin in Maize in Thailand," *Australian Centre for International Agricultural Research Proc.* **36**, 187–193 (1991).
74. R. J. Cole, J. W. Dorner, and P. D. Blankenship, "Management Strategies for Prevention and Control of Mycotoxins," in M. Miraglia et al., eds., *Mycotoxins and Phycotoxins—Developments in Chemistry, Toxicology and Food Safety*, Alaken, Fort Collins, Colo., 1998, pp. 189–202.
75. U. L. Diener, R. J. Cole, and R. A. Hill, "Epidemiology of Aflatoxin Formation by *Aspergillus flavus*," *Ann. Rev. Phytopathol.* **25**, 249–270 (1987).
76. W. W. McMillian, "Maize Plant Resistance to Insect Damage and Associated Aflatoxin Development," in M. S. Zuber, E. B. Lillehoj, and B. L. Renfro, eds., *Aflatoxin in Maize*, CIMMYT, Mexico, 1987, pp. 250–253
77. M. G. Smart, D. T. Wicklow, and R. W. Caldwell, "Pathogenesis in *Aspergillus* Ear Rot of Maize: Light Microscopy of Fungal Spread from Wounds," *Phytopathol.* **80**, 1287–1294 (1990).
78. R. D. Wyatt, "Poultry," in J. E. Smith and R. S. Henderson, eds., *Mycotoxins and Animal Foods*, CRC Press, Boca Raton, Fla., 1991, pp. 553–606.
79. B. H. Armbrecht, "Aflatoxicosis in Swine," in T. D. Wyllie and L. G. Morehouse, eds., *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses*, Vol. 2, Marcel Dekker, New York, 1978, pp. 227–235.
80. A. C. Keyl, "Aflatoxicosis in Cattle," in T. D. Wyllie and L. G. Morehouse eds., *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses*, Vol. 2. Marcel Dekker, New York, 1978, pp. 9–27
81. G.-S. Quian et al., "A Follow-up Study of Urinary Biomarkers of Aflatoxin Exposure and Liver Cancer Risk in Shanghai, Peoples' Republic of China," *Cancer Epidemiol. Biomarkers and Prevention* **3**, 3–10 (1994).
82. C. P. Wild, A. W. Daudt, and M. Castegnaro, "The Molecular Epidemiology of Mycotoxin Related Disease," in M. Miraglia et al., eds., *Mycotoxins and Phycotoxins—Developments in Chemistry, Toxicology and Food Safety*, Alaken, Fort Collins, Colo., 1998, pp. 213–232.
83. K. O'Donnell, E. Cigelnik, and H. I. Nirenberg, "Molecular Systematics and Phylogeography of the *Gibberella fujikuroi* Species Complex," *Mycologia* **90**, 465–493 (1998).
84. P. E. Nelson, A. E. Desjardins, and R. D. Plattner, "Fumonisins, Mycotoxins Produced by *Fusarium* Species: Biology, Chemistry and Significance," *Ann. Rev. Phytopathol.* **31**, 233–252 (1993).
85. S. C. Bezuidenhout et al., "Structure Elucidation of the Fumonisin Mycotoxins from *Fusarium moniliforme*," *J. Chem. Soc. Chem. Commun.*
86. D. Laurent et al., "Etude en RMN ^{1}H et ^{13}C de la Macrofusin, Toxine Isolee de Mais Infeste par *Fusarium moniliforme*," *Sheld. Analusis* **18**, 172–179 (1990).
87. *International Program for Chemical Safety, Fumonisins*, World Health Organization, Geneva, 1999.
88. J. D. Miller et al., "Production of Fumonisins and Fusarins by *Fusarium moniliforme* from Southeast Asia," *Mycologia* **85**, 385–391 (1993).
89. J. D. Miller et al., "Mycotoxin Production by *Fusarium moniliforme* and *Fusarium proliferatum* from Ontario and Occurrence of Fumonisin in the 1993 Corn Crop," *Can. J. Plant Pathol.* **17**, 233–239 (1995).
90. M. Sharman, J. Gilbert, and J. Chelkowski, "A Survey of the Occurrence of the Mycotoxin Moniliformin in Cereal Samples from Sources Worldwide," *Food Additives and Contaminants* **8**, 459–466 (1991).
91. D. C. Foley, "Systemic Infection of Corn by *Fusarium moniliforme*," *Phytopathol.* **68**, 1331–1335 (1962).
92. C. De Leon and S. Pandey, "Improvement of Resistance to Ear and Stalk Rots and Agronomic Traits in Tropical Maize Gene Pools," *Crop Sci.* **29**, 12–17 (1989).
93. H. Lew, A. Adler, and W. Edinger, "Moniliformin and the European Corn Borer (*Ostrinia nubilalis*)," *Mycotoxin Res.* **7**, 71–76 (1991).
94. A. W. Schaafsma et al., "Ear Rot Development and Mycotoxin Production in Corn in Relation to Inoculation Method and Corn Hybrid for Three Species of *Fusarium*," *Can. J. Plant Pathol.* **15**, 185–192 (1993).
95. R. T. Riley et al., "Evidence for Disruption of Sphingolipid Metabolism as a Contributing Factor in the Toxicity and Carcinogenicity of Fumonisins," *Natural Toxins* **4**, 3–15 (1996)
96. W. H. Tolleson et al., "The Mycotoxin Fumonisin Induces Apoptosis in Cultured Human Cells and in Livers and Kidneys of Rats," *Adv. Exp. Med. Biol.* **392**, 237–250 (1996).
97. W. H. Tolleson et al., "Apoptotic and Antiproliferative Effects of Fumonisin B_1 in Human Keratinocytes, Fibroblasts, Esophageal Epithelial Cells, and Hepatoma Cells," *Carcinogenesis* **17**, 239–249 (1996).
98. K. A. Voss et al., "*In vivo* Effects of Fumonisin B_1-producing and Fumonisin B_1-nonproducing *Fusarium moniliforme* Isolates are Similar: Fumonisins B_2 and B_3 Cause Hepato- and Nephrotoxicity in Rats," *Mycopathologica* **141**, 45–58 (1998).
99. W. F. O. Marasas et al., "Leucoencephalomalacia in a Horse Induced by Fumonisin B_1 Isolated from *Fusarium moniliforme*," *Onderspoort J. Vet. Res.* **55**, 197–203 (1988)
100. G. V. Smith et al., "Effects of Fumonisins on Cardiovascular Function in Swine," *Fund. Appl. Toxicol.* **31**, 169–172 (1996).
101. Riley et al., "Alteration of Tissue and Serum Sphinganine to Sphinganine Ratio: An Early Biomarker of Exposure to Fumonisin-containing Feeds in Pigs," *Toxicol. Appl. Pharmacol.* **118**, 105–112 (1993).
102. B. A. Rotter et al., "Response of Growing Swine to Pure Fumonisin B_1 During an 8 Week Period: Growth and Clinical Parameters," *Natural Toxins* **4**, 42–50 (1996)
103. B. A. Rotter et al., "Impact of Pure Fumonisin B_1 on Various Metabolic Parameters and Carcass Quality of Growing–Finishing Swine, Preliminary Findings," *Can. J. Animal Sci.* **77**, 465–470 (1997).

104 P G. Thiel et al., "The Implications of Naturally Occurring Levels of Fumonisins in Corn for Human and Animal Health," *Mycopathologia* **117**, 3–9 (1992).

105 W. C. A. Gelderblom et al., "Fumonisins—Novel Mycotoxins with Cancer-Promoting Activity Produced by *Fusarium moniliforme*," *App. Environ. Microbiol.* **54**, 1806–1811 (1988).

106 W C. A. Gelderblom et al., "Toxicity and Carcinogenicity of the *Fusarium moniliforme* Metabloite, Fumonisin B_1 in Rats," *Carcinogenesis* **12**, 1247–1251 (1991).

107. T. Battaglia, T. Hotzold, and R. Kroes, "Occurrence and Significance of Ochratoxin A in Food," *Food Additives and Contaminants* **13**, 1–57 (1996)

108 K. Jorgenson, "Survey of Pork, Poultry, Coffee, Beer and Pulses for Ochratoxin A," *Food Additives and Contaminants* **5**, 550–554 (1998).

109. P. Krogh, "Porcine Nephropathy Associated with Ochratoxin A," in J. E Smith and R. S. Henderson, eds., *Mycotoxins and Animal Foods*, CRC Press, Boca Raton, Fla., 1991, pp. 627–646.

110 W. W. Carlton, M. E. Stack, and R. M Eppley, "Hepatic Alterations Produced in Mice by Xanthomegnin and Viomellein, Metabolites of *Penicillium viridicatum*," *Toxicol. Appl. Pharm.* **38**, 455–459 (1976).

111. Scientific Committee for Food Reports, URL: *http://www europa.com*

112. M. Castegnaro et al., eds., *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, IARC Scientific Publication 115, Lyon, France, 1991.

113. D. Cvoriscec et al., "Endemic Nephropathy in Croatia," *Clin. Chem. Lab. Medicine* **36**, 271–277 (1998).

114. Y. Ueno, "Trichothecenes—Chemical, Biological and Toxicological Aspects," in Y. Ueno, ed , *Developments in Food Science*, Vol. 4, Elsevier, Amsterdam, The Netherlands, 1983.

115. A. Z. Joffe, "Toxicity of *Fusarium poae* and *F. sporotrichioides* and Its Relation to Alimentary Toxic Aleukica," in I. F. H. Purchase, ed., *Mycotoxins*, Elsevier, New York, 1974, pp. 229–262.

116. P. M. Scott, "*Penicillium* and *Aspergillus* Toxins," in J. D Miller and H. L. Trenholm, eds., *Mycotoxins in Grain: Compounds Other than Aflatoxin*, Eagan Press, St. Paul, Minn., 1994, pp. 261–286.

117. P M. Scott et al., "Ergot Alkaloids in Grain Foods Sold in Canada," *J. AOAC Int.* **75**, 773–779 (1992).

J. DAVID MILLER
Carleton University
Ottawa, Ontario
Canada

See also TOXICANTS, NATURAL.

N

NITROSAMINES

The chemistry of nitrosation of amines in foods is a complex process in which oxides of nitrogen (in oxidation states +3 and +4) formed during food processing, preservation, and preparation can react with amino compounds and other nucleophiles to produce N-, C-, O-, and S-nitroso compounds. The two major sources of oxides of nitrogen (nitrosating agents) result from (*1*) the addition of nitrate and/or nitrite to foods and (*2*) the heating and/or drying of foods in combustion gases in which molecular nitrogen can be oxidized to oxides of nitrogen. The only common feature of all *N*-nitroso compounds is the presence of the N-N=O functional group. Consequently, a wide range of chemical and physical properties exist for *N*-nitroso compounds, depending on the substitution on the amine/amide nitrogen.

The occurrence of *N*-nitroso compounds in foodstuffs probably represents the most comprehensively researched exposure situation for any class of genotoxic carcinogenic compounds in the human diet due to the possible link between various human cancers and exposure to *N*-nitroso compounds (1). Almost 80% of all *N*-nitroso compounds tested induce cancer in experimental animals, and some representative compounds of this class induce cancer in at least 40 different animal species including higher primates (2). Tumors induced in experimental animals resemble their human counterparts with respect to both morphological and biochemical properties. Extensive experimental and some epidemiological data suggest that humans are susceptible to carcinogenesis by *N*-nitroso compounds (1,3).

CHEMISTRY OF NITROSATION

The classic method for producing a nitrosating agent is the reaction between nitrite ions (NO_2^-) with protons (H^+ or H_3O^+) to give nitrous acid (HONO). Neither nitrite or nitrous acid per se are nitrosating agents, but are intermediates in the formation of the nitrosating agents dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4) and the nitrous acidium ion (H_2O^+NO) as shown in Figure 1. The acidity of the aqueous medium determines the relative proportions of the nitrosating species. Under moderately acidic conditions (pH 2–5) all three nitrosating species are present. At moderate pH (ca 3.0), N_2O_3 is the predominant nitrosating agent active in the nitrosation of secondary amines. At low pH (<2.0), H_2O^+NO is the predominant agent for nitrosation reactions involving weakly basic amines and amides.

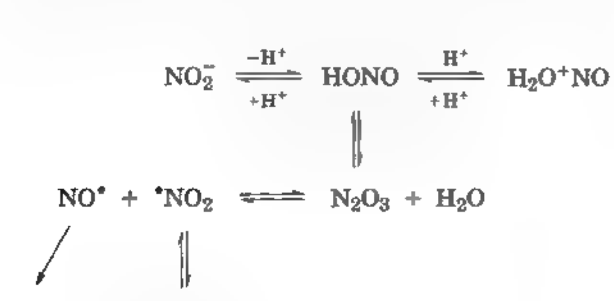


Figure 1. Equilibrium reactions of nitrite in aqueous media.

Primary amines are generally not considered to be precursors of *N*-nitrosamines, as nitrosation reactions generally proceed via diazotization and nucleophilic replacement of the amino group. However, nitrosation of the simplest aliphatic amine, methylamine, results in a complex mixture of products that include *N*-nitrosodimethylamine (NDMA). Nitrosation of higher primary aliphatic amines does not result in the formation of *N*-nitroso compounds.

Nitrosation of secondary amines is second order in terms of the concentration of nitrous acid and first order in terms of the amine concentration in which only the unprotonated amine reacts. Therefore, the reaction is strongly pH dependent. Strongly basic amines (eg, morpholine) are nitrosated to the full extent at ca pH 3.4. Introduction of ionizable groups near to the nitrosatable amino moiety reduces the basicity of the amine and the pH maxima for nitrosation is reduced to ca pH 2.5, as in the case of amino acids such as proline, sarcosine and hydroxyproline. For weakly basic amines (eg, *N*-alkylaromatic amines), nitrosation by N_2O_3 is very slow but proceeds more rapidly with the more reactive nitrosating species H_2O^+NO and NO^+ at low pH.

Tertiary amines present a rather complex situation in which simple trialkylamines react slowly with nitrous acid; more complex tertiary amines with substituents other than simple alkyl groups are readily nitrosated (eg, gramine and hordenine in malt). In most cases there appears to be no clear systematic approach in predicting which alkyl substituent will be replaced by the nitroso group.

In amides, the presence of the carbonyl group next to the nitrogen considerably reduces the basicity of the nitrogen. Nitrosation appears to involve an electrostatic interaction between the positively charged nitrosating species (eg, H_2O^+NO and NO^+) and the carbonyl oxygen, followed by rearrangement of the nitroso group onto nitrogen. The initial step (*O*-nitrosation) is readily reversible, but for rearrangement to the *N*-nitroso product, relatively strong acidic conditions are required, making this process unfavorable in food matrices.

Nitrosation reactions can be either catalyzed or inhibited by many factors. Nucleophilic anions such as I^-, Br^-, Cl^-, CNS^-, acetate, phthalate, weak acids, and some carbonyl compounds can exert a catalytic effect. The catalytic role of carbonyl compounds is related to their structure; acetone is inactive while formaldehyde and chloral are effective catalysts for the nitrosation of dialkylamines even under alkaline conditions. Other carbonyl catalysts include benzaldehyde and pyridoxal. Essentially any chemical that reacts with nitrite can inhibit the formation of N-nitroso compounds. Examples of such compounds include primary amines, sulfhydryl compounds, and certain aromatic compounds (eg, phenols). Several naturally occurring compounds in foods (eg, tannins in milk) can inhibit nitrosation via the formation of *C*-nitroso and *S*-nitroso compounds. In plant-based foods, phenolic compounds can catalyze and inhibit nitrosation, depending on their structure, the relative concentrations of the phenolic compound and the nitrosating agent, and the pH. High nitrite-to-phenol ratios favor catalysis while low ratios favor inhibition; 1,3-dihydroxyphenol (and related natural derivatives) are catalytic while 1,2- and 1,4-dihydroxyphenols inhibit nitrosation. Nitrosation can also result from the direct or indirect transfer of a nitric oxide radical (NO·) from N-, S-, O-, and C-nitroso compounds to a second nitrosatable precursor. The most important inhibitors of nitrosation reactions in food systems are redox compounds such as ascorbate and vitamin E. Ascorbate inhibits the formation of nitrosamines by competing with nitrosatable precursor compounds for the available nitrosating agent. On reaction with dinitrogen, tri- and tetraoxides nitric oxide is formed, which is not a nitrosating agent. However, this inhibition is not complete as oxidation of nitric oxide by molecular oxygen regenerates the nitrosating species. Complete inhibition is only effective in the presence of a large excess of ascorbate and/or under anaerobic conditions.

FORMATION OF NITROSAMINES IN FOODS

N-Nitroso compounds can be divided into either *N*-nitrosamines or *N*-nitrosamides. *N*-Nitrosamines can be subdivided into two groups depending on their physical properties: (*1*) Volatile *N*-nitrosamines (VNA), which are *N*-nitrosated analogues of simple low molecular weight dialkylamines and cyclic compounds such as NDMA, *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosothiazolidine (NTHZ), and *N*-nitrosomorpholine (NMOR), as shown in Figure 2; and (2) nonvolatile *N*-nitrosamines (NVNA), which include a wide range of *N*-nitrosated hydroxylated or polyfunctional compounds, as shown in Figure 3. The most commonly encountered NVNA are the *N*-nitrosated amino acids *N*-nitrosoproline (NPRO), *N*-nitrososarcosine (NSAR), *N*-nitrosohydroxyproline (NHPRO), and a wide range of *N*-nitrosated heterocyclic carboxylic acids formed by nitrosation of the condensation products of the amino acids cysteine, serine, threonine, and tryptophan with simple aldehydes such as formaldehyde and acetaldehyde. The condensation of cysteine and formaldehyde to yield thiazolidine-4-carboxylic acid produces the amine precursor to *N*-nitrosothiazolidine-4-carboxylic acid (NTCA), which is the most frequently encountered *N*-nitrosated heterocyclic carboxylic acid formed by this type of reaction. Other examples of *N*-nitrosated heterocyclic carboxylic acids are *N*-nitroso-2-(hydroxymethyl)thiazolidine-4-carboxylic acid (NHMTCA) formed by condensation of cysteine with glycolaldehyde and the oxazolidines *N*-nitrosooxazolidine-4-carboxylic acid (NOCA) and *N*-nitroso-5-methyloxazolidine-4-carboxylic acid (NMOCA) formed by the condensation of formaldehyde with serine and threonine, respectively.

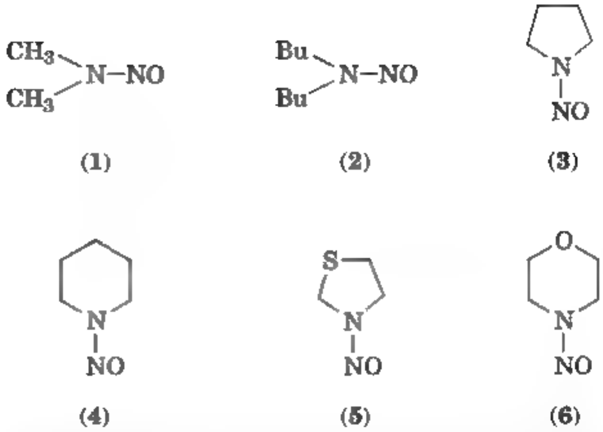


Figure 2. Chemical structures of volatile nitrosamines (VNA) occurring in foods: (1) NDMA, *N*-nitrosodimethylamine; (2) NDBA, *N*-nitrosodibutylamine; (3) NPYR, *N*-nitrosopyrrolidine; (4) NPIP, *N*-nitrosopiperidine; (5) NTHZ, *N*-nitrosothiazolidine; and (6) NMOR, *N*-nitrosomorpholine.

Several other nonvolatile *N*-nitroso compounds are probably present in certain foods and beverages; however, their presence remains uncertain (4). Examples of such compounds include *N*-nitrosated dipeptides or polypeptides with *N*-terminal proline or hydroxyproline residues, *N*-nitrosated 3-substituted indoles, *N*-nitrosated derivatives of certain pesticides (eg, *N*-nitrosocarbaryl, *N*-nitrosoatrazine, and *N*-nitrosoglyphosate), nonvolatile hydroxylated nitrosamines formed by the nitrosation of spermine and similar compounds, *N*-nitrosated glycosylamines and Amadori compounds.

Nitrosation of peptides and polypeptides to form stable *N*-nitroso compounds has been considered as a major potential source of NVNA in cured meats. The amide linkage is relatively unreactive towards nitrosation by nitrite in aqueous solution. However, nitrosation of dipeptides *N*-terminal in proline produces stable *N*-nitrosoprolyl dipeptides while higher peptides with *N*-terminal proline or hydroxyproline form stable derivatives under identical conditions.

Indoles occur in several plant species, particularly in the *Brassica* family, and are biosynthesized during the germination of grains. Naturally occurring indoles can be readily nitrosated under mild conditions; when carbon-3 is unsubstituted, C-3 nitrosation occurs in the first instance, where C-3 is already substituted, then nitrosation occurs preferentially at nitrogen unless the C-3 substituent is a powerful electron donor, in which case C-2 nitrosation results. Gramine, an indole alkaloid in barley malt acts as a precursor to NDMA found in beer during direct-drying of malt with hot flue gases containing nitrogen oxides. Nitrosation of gramine under aqueous acidic conditions yields NDMA and several nonvolatile reaction products, the major one of which is *N*-nitroso-3-nitromethylindole (5). Other indoles found in foods that

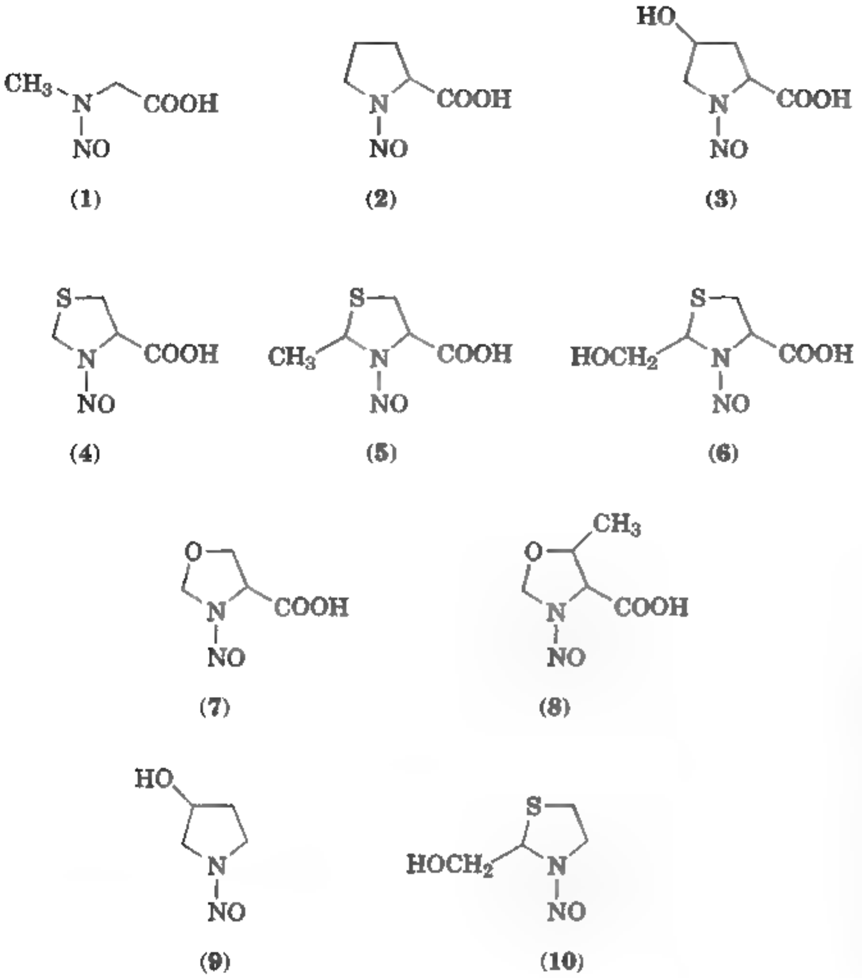


Figure 3. Chemical structures of nonvolatile nitrosamines (NVNA) occurring in foods: (**1**) NSAR, *N*-nitrososarcosine; (**2**) NPRO, *N*-nitrosopoline; (**3**) NHPRO, N-nitrosohydroxyproline; (**4**) NTCA. *N*-nitrosothiazolidine-4-carboxylic acid: (**5**) NMTCA, *N*-nitroso-2-methylthiazolidine-4-carboxylic acid: (**6**) NHMTCA, *N*-nitroso-2-hydroxymethylthiazolidine-4-carboxylic acid; (**7**) NOCA, *N*-nitrosooxazolidine-4-carboxylic acid; (**8**) NMOCA, *N*-nitroso-5-methyloxazolidine-4-carboxylic acid; (**9**) NHPYR, *N*-nitrosohydroxypyrrolidine; and (**10**) NHMTHZ. *N*-nitroso-2-hydroxymethylthiazolidine.

react with nitrite under acidic aqueous conditions include indole-3-acetonitrile, 4-methoxyindole-3-acetonitrile and 4-methoxyindole-3-acetaldehyde found in Chinese cabbage and 4-chloro-6-methoxyindole in fava beans (*Vicia faba*) All of which produce powerful direct-acting bacterial mutagens after nitrosation. The presence of nitrosated indoles have not yet been reported in foods or beverages.

During the cooking and baking of foods, nonenzymic browning (Maillard) reactions between aldoses or ketoses with amine compounds can result in low molecular weight volatile compounds (pyrazines, furans, etc) and high molecular weight compounds (melanoidines) such as glycosylamines and Amadori compounds. D-Fructose-L-amino acids formed by the Maillard reaction are readily nitrosatable under mild conditions, and a range of *N*-nitroso glycosylamines and Amadori compounds have been synthesized and shown to be direct-acting bacterial mutagens. Suitable methods for their analysis are still not available.

N-Nitrosamide precursors in foods and beverages include *N*-alkylureas, *N*-alkyl, carbamates, simple *N*-alkylamides, cyanamides, guanidines, amidines, hydroxylamines, hydrazones and hydrazides. At present, their is no direct evidence available to support the presence of *N*-nitrosamides in foodstuffs.

N-NITROSAMINES IN FOOD

Over the last decade, more than 500 publications on the occurrence of *N*-nitroso compounds in foodstuffs have been published, and several reviews have recently been published (4,6,7). The chemical stability of volatile nitrosamines, and the simplicity of their determination by distillation techniques followed by gas chromatography has resulted in the analysis of this group of *N*-nitroso compounds in almost all Western foods. In the first comprehensive dietary survey (8), 2,826 food samples on the German market were analyzed, revealing that only three volatile nitrosamines, namely NDMA, NPYR, and NPIP, were regularly present in foods; all other volatile nitrosamines were present only in rare cases. NDMA was detected in 30% of all samples, 6% of which contained concentrations above 5 μg/kg. NPYR and NPIP at concentrations above 0.5 μg/kg (ppb) were found in only 3 and 2% of all foods, respectively. Over the last decade, several major food surveys have been made in more than 10 different countries from which it is possible to evaluate basic trends in VNA contamination. The results from these surveys show that foodstuffs most commonly contaminated with *N*-nitroso compounds can be classified into several broad groups:

Foodstuffs preserved by either addition of nitrate and/or nitrite.

Foodstuffs preserved by smoking.

Foodstuffs subject to drying by combustion gases.

Pickled and salt-preserved vegetables.

Foodstuffs stored under humid conditions favoring fungal contamination, particularly the growth of *Fusarium moniliforme*.

Foodstuffs contaminated by food contact materials.

Foodstuffs with Added Nitrate and Nitrite

The addition of nitrite and/or nitrate salts to meat, poultry, fish, and to a lesser extent cheese at low concentrations (up to a few 100 ppm) has been a common method of preservation for centuries. Nitrite addition to meat products results in the formation of characteristic pink-red colorations in cured meats, prevents rancidity occurring under normal storage conditions, and inhibits *Clostridium botulinum* growth. As an alternative to direct nitrite addition, nitrate addition is used in several products as a continuous direct source of nitrite by reduction involving microbial enzymes.

The chemistry of interactions involving nitrite in food products is complex, owing to the highly reactive nature of nitrite. In cured meat products, a rapid decrease to about 50% of the initially added nitrite concentration occurs during processing, and a steady further decline occurs during storage. The decline in nitrite may result through several different mechanisms, of which reduction by ascorbic acid to nitric oxide and the formation of an equilibrium state between nitrite, nitrous acid, and oxides of nitrogen such as dinitrogen trioxide are the most important (refer to Fig. 1). Dinitrogen trioxide, which is a chemically active nitrosating species, reacts with several constituents present in meat. The fate of nitrite can be roughly summarized as follows: 1–5% is lost as nitric oxide gas or becomes bound to lipids, 1–10% is oxidized to nitrate, 5–10% remains as free nitrite, 5–15% reacts with sulfhydryl compounds or myoglobin, and 20–30% becomes bound to proteins. In addition, nitrosation of amino compounds (nitrosamine formation) may occur as well as the formation of organic nitrites and nitrates. To prevent the formation of *N*-nitroso compounds, nitrite cured meats are required to be formulated with 500 mg/kg sodium ascorbate in the United States and in most other countries

Foodstuffs Preserved by Smoking

Smoking under traditional smokehouse conditions results in prolonged direct contact of foodstuffs with elevated concentrations of oxides of nitrogen present in wood smoke. Consequently, smoked meat products, fish, and cheese normally contain *N*-nitroso compounds. In cured meat products, several nonvolatile heterocyclic *N*-nitroso compounds have been identified that are not usually present in unsmoked products (4,9). Wood smoke contains several carbonyl compounds including formaldehyde, acetaldehyde, propionaldehyde, glyoxal, methylglyoxal, and glycolaldehyde. Formaldehyde and acetaldehyde readily condense with bifunctional food components containing thiol, hydroxyl, and free amino groups to form heterocyclic compounds that are subject to rapid nitrosation by oxides of nitrogen present in wood smoke. In smoked meats, poultry, and fish it is generally accepted that the condensation of formaldehyde with cysteamine and cysteine to form thiazolidine and thiazolidine-4-carboxylic acid, followed by nitrosation, results in the formation of NTHZ and NTCA (10). The potential routes of NTHZ and NTCA formation are summarized in Figure 4. Other simple carbonyl compounds in wood smoke may also undergo condensation reactions with amino acids such as cysteine, lysine, serine, threonine, and tryptophan to yield a wide range of nitrosatable heterocyclic amino acid derivatives. The corresponding *N*-nitroso derivatives, of which several examples are known, are commonly found in smoked meats and to a lesser extent in poultry and fish (reviewed in Ref. 4).

Foodstuffs Dried by Combustion Gases

By an analogus process, food-processing operations involving the drying of foodstuffs in combustion gases (hot flue gases) containing oxides of nitrogen (NO_X) can result in the formation of N-nitroso compounds. The kilning (drying process) used in preparing malt for the brewing industry is directly responsible for the levels of NDMA in beer (11). Furthermore, specific types of malt produced by characteristic drying methods contain varying concentrations of NDMA as well as NPRO and NSAR. Amine precursors of NDMA in malt include dimethylamine, trimethylamine, and the alkaloids gramine and hordenine, which are biosynthesized in green malt during germination. In model experiments, both gramine and hordenine undergo nitrosation to yield 76 and 11% NDMA, respectively, at pH 4.4. In addition, gramine also yields the nonvolatile *N*-nitroso compound *N*-nitroso-3-nitromethylindole (5); the latter has not yet been detected in beer or malts. The identified nitrosation products from the alkaloid gramine are shown in Figure 5. Modification of kilning methods, in particular the introduction of indirect heating or low-temperature gas burners, produces lower NO_X concentrations and hence results in reduced levels of nitrosamines in malts. Malts treated with sulfur dioxide during the kilning process, either by burning sulfur or sulfur-containing compounds in the combustion flame, or by direct addition of sulfur dioxide gas into the combustion gas flow of direct drying kilns, generally contain lower VNA concentrations but have little or no effect on the reduction of NPRO in malt (12).

Foodstuffs produced by drying processes include dried soup mixes, tea, spices, coffee, soy protein isolates, cereal products, infant formulations, and dairy products such as cheese and nonfat dried milk powder. All these products have at one time been shown to be regularly contaminated with traces of VNA (mainly NDMA) whose formation was attributed to the drying process. However, with the exception of brewing malt, the occurrence of specific NVNA has not been demonstrated to occur in products dried with combustion gases.

Pickled and Salt-Preserved Vegetables

Nitrate, and to a lesser extent nitrite, are natural components of several plant species and vegetables. Fermen-

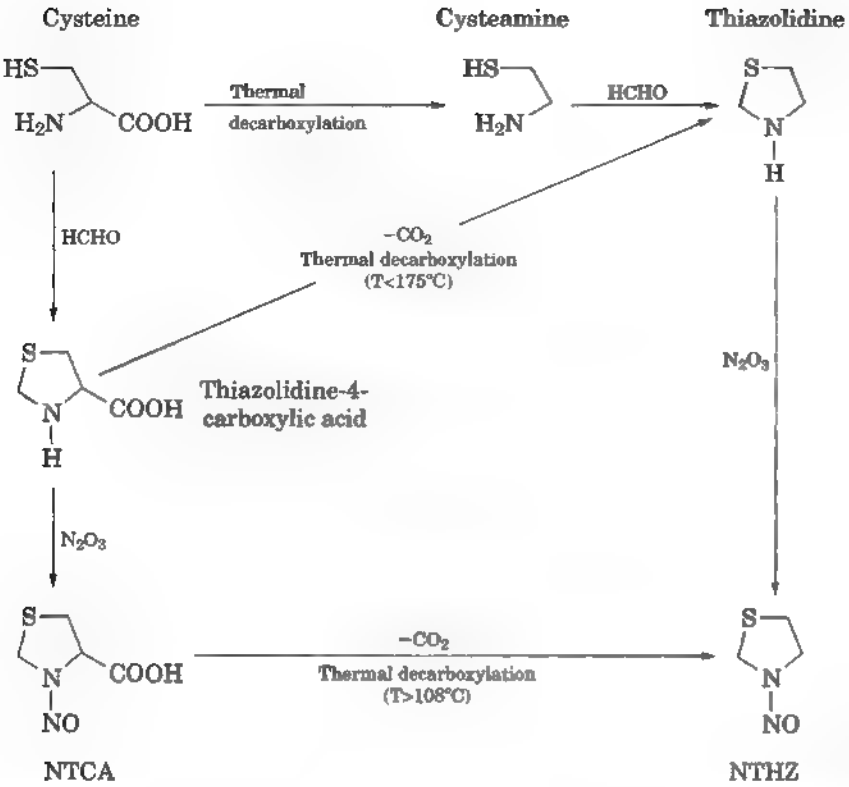

Cysteine
Cysteamine
Thiazolidine
HS
H2N
COOH
Thermal
decarboxylation
HCHO
HCHO
−CO2
Thermal decarboxylation
(T<175°C)
Thiazolidine-4-carboxylic acid
N2O3
N2O3
−CO2
Thermal decarboxylation
(T>108°C)
NTCA
NTHZ

Figure 4. Proposed mechanisms for the formation of *N*-nitrosothiazolidine-4-carboxylic (NTCA) acid and *N*-nitrosothiazolidine (NTHZ).

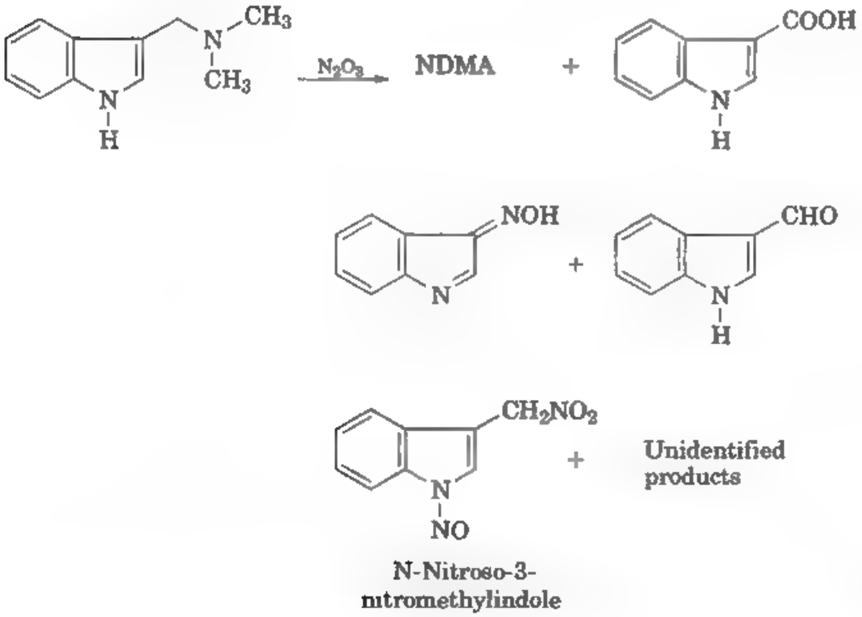

N2O3
NDMA
Unidentified products
N-Nitroso-3-nitromethylindole

Figure 5. Nitrosation products of the alkaloid gramine.

tation by microorganisms (and surface bacteria) with nitrate-reductase activity can result in a large increase in nitrite content. On prolonged storage, nitrosamine formation may occur.

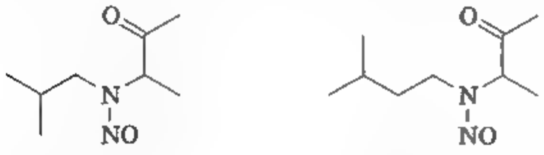
Fungal Contamination of Foods

The catalysis of nitrosamine synthesis from secondary amines and nitrate by several microorganisms and fungal biosynthesis of nitrosamines from primary amines and nitrite occurs under certain conditions in a limited range of foods. In certain areas in China where widespread contamination of foods by *Fusaria* occurs, low concentrations (<2 μg/kg) of *N*-nitroso-*N*-methylacetonyl-*N*-2-methylpropylamine (NMAMPA) and *N*-nitroso-*N*-methylacetonyl-*N*-3-methylbutylamine (NMAMBA) have been identified in moldy millet and wheat flour contaminated by *Fusaria* (13). A nonenzymic reductive condensation of isoamylamine, a decarboxylation product of leucine, and 3-hydroxy-2-butanone (acetoin) preset in cereal grains have been suggested as a possible route to the formation of the amine precursor to NMAMBA. The structures of NMAMBA and NMAMPA are shown in Figure 6.

Contact Materials

The contamination of amines by their corresponding *N*-nitroso analogues and the use of dialkylamine-derived accelerators and stabilizers in the production of rubber results in inadvertant *N*-nitrosamine contamination of rubber products. As a result, direct contact of foodstuffs with nitrosamine-contaminated food-packaging materials, in particular rubber, can result in nitrosamine contamination. Rubber nettings for the packaging of cured meats results in contamination of meat products with NDEA, *N*-nitrosodibutylamine (NDBA), and *N*-nitrosodibenzylamine (NDBzA), three *N*-nitroso compounds not generally encountered in foods (14), due to both active migration of the preformed nitrosamine into the food matrix and the interaction of nitrite in cured meats with amine additives (eg, zinc dialkyl or diaryldithiocarbamates) used as accelerators during rubber vulcanization. The use of rubber baby bottle nipples and pacifiers presents a more serious problem in which both nitrosatable amines and nitrosamines migrate from the rubber matrix into baby formulations. Government regulations in most countries have limited the permissible levels of VNA to 10 μg/kg and nitrosatable amines following extraction to 200 μg/kg (expressed in terms of nipple weight). Monitoring programs in most countries currently show negligible nitrosamine concentrations in rubber nipples and pacifiers produced after 1984. In most cases, nitrosamine free products are available.

Commercial wax preparations used for coating food-packaging materials, and liquid waxes used for coating apples and citrus fruit present a potential source of *N*-nitrosomorpholine (NMOR) contamination (15). Morpholine (which normally contains NMOR as an impurity) is used as a boiler additive for steam used in the manufacture of paper. Both morpholine and morpholineoleate are used as solvents for waxes used to treat paper for water resistance and to coat fresh fruits. NMOR contamination of milk packed in waxed cartons and dairy products in waxed paper wrappers was reported in several countries about a decade ago. However, this is not a current problem because waxed packaging has been replaced with plastic-coated surfaces.



NMAMPA NMAMBA

Figure 6. Nitrosamines produced from fungal metabolites in *Fusarium*-contaminated foods. Nonvolatile nitrosamines abbreviated as NMAMPA, *N*-nitroso-*N*-methylacetonyl-*N*-2-methylpropylamine, and NMAMBA, *N*-nitroso-*N*-methylacetonyl-*N*-3-methylbutylamine.

TRENDS

Over the last decade, nitrosamine contamination trends in most Western food products have drastically decreased as a result of improved production technology, reduction in the use of nitrates and/or nitrites for curing, and changing from direct to indirect drying methods. Theses changes have resulted in a mean ten-fold reduction in the levels of VNA in cured meats and almost a 100-fold reduction in the level of NDMA in beer. The following data are representative of the current nitrosamine situation in food commodities in which a nitrosamine problem still exists.

Meat Products

Only nitrite- and nitrate-preserved meat products are contaminated with *N*-nitrosamines, the concentration and range of different *N*-nitroso compounds is increased following smoking. Early studies in the late 1970s in several countries showed that almost a third of all cured meat products contain VNA. In Germany (8), 32% of all cured meat samples analyzed contained VNA: <5 μg/kg NDMA was detected in 30% of all samples, with concentrations above 5 μg/kg in 2.1% of the samples analyzed; 6.8% contained <5 μ/kg NPYR (6.8% containing above 5 μg/kg NPYR); and 4% contained up to 6.4 μg/kg NPIP. *N*-Nitroso-3-hydroxypyrrolidine (NHPYR), a carcinogenic NVNA, was reported at about the same concentration levels. Similar trends were reported in the United States, Canada, and the United Kingdom. Fried bacon was identified as consistently having the highest concentrations of nitrosamines. NTHZ, a new nitrosamine, was identified in 1983 at concentrations under 5 μg/kg in fried bacon and at concentrations between 1.6–31.9 μg/kg in other cured meat products (16). Since these earlier reports, changes in production technologies, reduction in the levels of nitrate and nitrite added during curing, and the inclusion of nitrosation inhibitors such as ascorbic acid and α-tocopherol to the curing mixture have resulted in significant reductions in the levels of volatile nitrosamines, particularly for NPYR in bacon. During cooking and frying of cured meat products, the concentration of NDMA usually remains constant while the concentration of NPYR increases considerably. The volatility of NDMA causes losses during heating that are partially balanced by thermally induced synthesis. The lower volatility of NPYR decreases its losses during heating while at the same time synthesis of NPYR occurs from both reactions of pyrrolidine with residual nitrite and the thermal decarboxylation of NPRO and *N*-terminal *N*-nitrosoproline containing peptides. NPYR formation is increased during high-temperature frying for short periods as compared to low-temperature frying for prolonged periods. VNA exposure from consumer bought and cooked at home fried bacon has been calculated (ng/person/d) to be 18 ng NDMA, 6.6 ng NPIP, 76 ng NPYR, and 40 ng/person/d NTHZ. Microwave cooking produces almost undetectable (<0.1 μg/kg) levels of VNA. The thermal decarboxylation of NTCA during cooking appears to be the most important factor involved in the formation of NTHZ in fried bacon. Thiazolidine, the amine precursor to NTHZ, has not been reported in foods but may be formed by the condensation of cysteamine and formaldehyde or in cysteamine/glucose browning systems. The use of elastic rubber nettings for cured meat packaging provides an additional source of mainly NDEA, NDBA, NDBzA, and, to a lesser extent, NMOR in cured meat products (14).

Considerably higher concentrations of NVNA occur in nitrite cured and/or smoked meat products: Concentration ranges of < 410 μg/kg NSAR, 20–360 μg/kg NPRO, and 10–560 μg/kg NHPRO occur in unsmoked meat products while condensation reactions between carbonyl compounds (aldehydes) and amino acids during smoking produces concentration ranges of up to 1620 μg/kg NTCA and 2,100 μg/kg NHMTCA (4,9). Some traditional smoked foods, eg, Icelandic smoked mutton (a Christmas delicacy that is first cured and then smoked over sheep dung) contain 40–70 μg/kg NOCA and 30–120 μg/kg of its 5-methyl derivative (NMOCA). Thermal decarboxylation of *N*-nitrosated amino acids during cooking yields the corresponding volatile *N*-nitrosamines; NPRO, NHPRO, NSAR and NTCA decarboxylate to NPYR, NHPYR, NDMA, and NTHZ, respectively. No evidence has been found for the presence of the decarboxylated analogues of NOCA and NMOCA in foods.

Fish and Sea Foods

Fresh fish and seafoods that are naturally rich in amines do not contain *N*-nitroso compounds; however, nitrosamines are occasionally found after processing. Early reports from England, the United States, and Canada showed 1–10 μg/kg NDMA concentrations in almost 80% of processed fish products. Today, NDMA is still present in very rare cases, but at levels seldom above 2 μg/kg. Processed products based on shrimp are nearly always contaminated with 5–30 μg/kg NDMA and in exceptional cases with several hundred μg/kg NDMA in shrimp sauces. The use of sea salt for fish drying can lead to nitrate contamination of fish and provides a potential source of nitrite for nitrosamine formation. Salt-dried fish in Japan contains about 1.0–34 μg/kg NDMA. Coalgas broiling, the traditional way of cooking fish in Japan, can increase the concentrations of NDMA in salted fish to 300 μg/kg and the concentration of NDMA in dried squid from 15–84 to 24–310 μg/kg. The formation of 2.4–13 μg/kg NPYR and up to 94 μg/kg NPRO on broiling squid has also been reported. Chinese and Cantonese salt-preserved fish often contains concentrations up to 130 μg/kg NDMA while dried fish from Greenland also contains up to 38 μg/kg NDMA.

Smoked fish and seafood contain high concentrations of NTHZ and NTCA: Smoked oyster has been reported to contain 109 μg/kg NTHZ and 167 μg/kg NTCA, while concentrations of 5–10 μg/kg NTHZ and 10–350 μg/kg NTCA in commercially smoked fish are not uncommon. Other *N*-nitrosamines including NPYR (<2 μg/kg) and NPRO (<10 μg/kg) are occasionally found in smoked fish.

Limited data show that most sun-dried fish, which forms a large part of the staple diet in several developing countries, is also subject to widespread NDMA contamination. The effect of traditional cooking methods such as smoking fish wrapped in grass or leaves on the concentration of *N*-nitroso compounds has not been investigated.

Cheese and Dairy Products

Current evidence shows that cheese is only a very minor dietary source of NDMA. No correlation exists between nitrate addition (used to prevent microbiological defects) and nitrosamine contamination. Concentrations of 5 μg/kg NDMA in cheese reported a decade ago are now reduced to <1 μg/kg in the limited number of samples still found to contain NDMA. A nationwide American survey (carried out in 1981) of non-fat dried milk powders produced by directly heated combustion gas driers showed 0.1–3.7 μg/kg (average 0.6 μg/kg) NDMA in 48 out of 57 dried-milk samples, with occasional traces of NPYR (0–0.8 μg/kg, average 0.1 μg/kg) and NPIP (0–0.5 μg/kg, average 0.1 μg/kg). Similar studies on dried-milk products produced in Europe and New Zealand using indirectly heated driers gave negative results, emphasizing the role of production technology on the formation of *N*-nitroso compounds. Changes in drying methods have since been introduced in the United States and contamination of dried-milk products is not a current problem.

Vegetables and Cereal Products

Volatile *N*-nitroso compounds have been detected rarely at trace levels in grain crops stored under humid conditions. The reported contamination of cereal products in New Zealand with levels of up to 1.2 μg/kg NDMA in grain, 2.8 μg/kg NDMA in maize meal, and 4.2 μg/kg NDMA in wheaten cornstarch is not representative of the general situation in cereal products where good agricultural practice (avoidance of wet harvesting and storage under humid conditions) does not favor mold growth and the subsequent possibility of nitrosamine contamination. Japanese fermented vegetables have been reported to contain <5 μg/kg NDMA and NPYR. Widespread contamination of homepickled vegetables with 1–15 μg/kg NDMA, 1–3 μg/kg *N*-nitrosoethylmethylamine (NEMA), 1–5 μg/kg NDEA, and <100 μg/kg NPYR have often been reported from China, Japan, and several Asian countries. Commercial products contain far lower concentrations. In some areas of China, contamination at levels of less than 2 μg/kg NMAMBA and NMAMPA in millet and wheat flour containing molds, particularly *Fusarium moniliforme*, is not uncommon (13). Indian and Tunisian foods containing red and black peppers, paprika, and spices contain various levels of VNA. Touklia, a stewing base that is the main ingredient of Tunisian food, contains relatively high concentrations of 12 μg/kg NDMA, 43 μg/kg NPIP, and 5.8 μg/kg NPYR. Contamination of most dried spices with NDMA and dried chillies with NPYR is a fairly widespread problem. However, the amounts of spices used in most Western cooking practices are not likely to lead to a significant exposure to the compounds.

Beer and Alcoholic Beverages

In the late 1970s, NDMA contamination of beer was reported in the former West Germany showing that almost

60% of bottled, canned, and draft beers contained a mean concentration of 2 μg/L NDMA with a maximum value of 68 μg/L NDMA. Similar concentrations were also reported in the United States, Canada, and most other European countries. Modifications in malt drying and kilning techniques, shown to be the stages involving the formation of NDMA, have resulted in considerable reductions in the levels of NDMA in beer. The current mean levels of NDMA reported in beer are 0.07 μg/L in the United States, 0.10 μg/L in Canada and West Germany, with most other European beers containing less than 0.3 μg/L. The mean concentration of NPRO, the only NVNA identified in beer, is less than 2 μg/L in most European, Australian, and American beers produced from indirectly kilned malt. Although NSAR and NPYR have been reported in malts, no evidence is available to show their presence in beer.

The only other commercially produced alcoholic beverage that contains nitrosamines is whiskey, in which current levels are generally below 0.3 μg/L. NDMA formation occurs during malt production, and no other *N*-nitroso compounds have been reported in whiskey. Contrary to earlier reports, *N*-nitrosamines are not present in wine, sherry, port, apple brandy, liqueurs, or fortified and distilled spirits such as rum, gin, and vodka. There is some evidence to support the presence of VNA, mainly NDMA and NDEA, in some African fermented beverages and both African and Indian illicitly distilled spirits.

Drinking Water

Early reports on the possible presence of nitrosamines in drinking water drawn from wells containing high nitrate concentrations have never been substantiated. Although industrial waste water and sewage sludge occasionally contain traces of NDMA, NDEA, and possibly other VNA at total concentrations of less than 5 μg/L, their incorporation into domestic water supplies has never been confirmed.

Other Food Products

In systematic studies carried out in several countries, only sporadic contamination of trace quantities of individual VNA have been found in other foods and beverages. In most cases, contamination occurred in single samples and could not be verified in other countries, or at a later date. Food contamination by direct contact materials has nearly always been assumed in unexplained nitrosamine contamination cases such as NMOR contamination of margarine packed in waxed paper wrappers.

DIETARY EXPOSURE TO *N*-NITROSO COMPOUNDS

The dietary exposure to NDMA (the most commonly occurring VNA in the diet) has been calculated in a number of food surveys and is summarized in Table 1. It should be taken into consideration that exposure estimates of this kind suffer from uncertainties in food consumption trends averaged over a population. Over the last decade, reductions in the use of nitrates and nitrites used for curing meats to the minimum amount required to inhibit bacterial growth, and modification of malting techniques in the brewing industry have resulted in significant reductions in the levels of NDMA. In most dietary surveys, cured meats and beer have been implicated as the major dietary sources of NDMA. As a direct consequence, NDMA exposure over the last decade has probably decreased from about 1 μg/d to ca 0.3 μg/d NDMA in most Western countries. An exposure estimate of between 10–100 μg/d for currently identified NVNA would not seem unreasonable. In developing countries, particularly China and other Asia countries, the occurrence and concentrations of VNA in common dietary items are considerably higher than in Western foods and a far higher dietary exposure to nitrosamines would be expected.

Table 1. Food Surveys and Daily NDMA Intake

Country	Year	NDMA intake (μg/d)	Major NDMA source
UK	1978	0.53	Cured meats (81%)
UK	1987	0.60	Beer, cured meats
Holland	1980	0.38	Beer (71%)
Holland[a]	1990	0.10	Not evaluated
The former FRG	1980	1.02 (men) 0.57 (women)	Beer (65%), cured meats (10%)
The former FRG	1983	0.53 (men) 0.35 (women)	Beer (40%), cured meats (18%)
Japan	1980	1.80	Dried fish (91%)
Japan	1984	0.50	Fish products (88%)
Sweden	1988	0.12	Cured meats (61%), beer (32%)
Finland[b]	1990	0.08 (adult) 0.02 (child)	Beer (75%), smoked fish (25%) for adults Smoked fish
China	1988	No data	Marine foods
Italy	1988	No data	Cured meats
The former USSR	1990	No data	Meat and fish

Source. Ref. 3.

Note: Beer not included in the survey.

[a] Determined by 24-h duplicate diet analysis.

[b] Based on limited data.

NITROSAMINE EXPOSURE AND HUMAN CANCER

Experimental studies provide evidence that the biological activity of *N*-nitroso compounds in humans is not substantially different from that in experimental animals (1). In contrast to animal experiments, in which exposure (normally at high concentrations) to single *N*-nitroso compounds may induce cancer (2), human exposure (3,6,7,17) results via several different sources (eg, diet, occupational exposure, and tobacco consumption) at a wide range of different concentrations. Dose-response studies using experimental animals show that NDEA, NMOR, and NPYR continuously administered in drinking water at exposure levels of 0.075 mg/L (0.075 ppm), 0.07 mg/L, and 0.01 mg/kg body weight/d, respectively, are sufficient to induce a significant incidence of tumors. In animal carcinogenicity experiments, the absence of a lower no-effect threshold and the syncarcinogenic activity of low concentration combinations of *N*-nitroso compounds at concentrations at which individual *N*-nitrosamine concentrations alone would not be expected to induce carcinogenesis suggest that multiexposure to several different *N*-nitroso compounds in the diet may present a potential carcinogenic risk to humans (1,3,18).

Furthermore, micronutrient deficiencies may modify nitrosamine-induced carcinogenesis with respect to both organotropism and cancer incidence (2). These confounding factors are partially responsible for the failure of epidemiologic studies in identifying exposures to individual compounds and foodstuffs as potential risk factors for human carcinogenesis despite the fact that there is sufficient evidence to support the hypothesis that humans are susceptible to *N*-nitroso compound induced carcinogenesis (1,3,18). Thus, continuous exposure to low concentrations of several different *N*-nitroso compounds in the diet would be expected to be an etiologic risk factor for certain human cancers. Epidemiologic case-control studies indicate that dietary nitrosamine exposure is an important risk factor for cancers of the esophagus, stomach, and nasopharynx (18).

BIBLIOGRAPHY

1. H. Bartsch and R. Montesano, "Relevance of Nitrosamines to Human Cancer," *Carcinogenesis* **5**, 1381–1393 (1984).
2. R. Preussmann and B. W. Stewart, "*N*-Nitroso Carcinogens," in E. Searle, ed., *Chemical Carcinogens. ACS Monograph 182*, American Chemical Society, Washington, D.C., 1984, pp. 643–828.
3. A. R. Tricker and R. Preussmann, "Carcinogenic *N*-Nitrosamines in the Diet: Occurrence, Formation, Mechanisms, and Carcinogenic Potential," *Mutations Research* **259**, 277–289 (1990).
4. A. R. Tricker and S. J. Kubacki, "Review of the Occurrence and Formation of Nonvolatile *N*-Nitroso Compounds in Foods," *Food Additives and Contaminants* (1991).
5. M. U. Ahmad, L. M. Libbey, J. F. Barbour, and R. A. Scanlan, "Isolation and Characterization of Products from the Nitrosation of the Alkaloid Gramine," *Food Chemistry and Toxicology* **23**, 841–847 (1985).
6. A. R. Tricker and R. Preussmann, "*N*-Nitroso Compounds and Their Precursors in the Human Environment," in M. J. Hill, ed., *Nitrosamines—Toxicology and Microbiology*, Ellis Harwood, Chichester, UK, 1988, pp. 88–116.
7. J. A. Hotchkiss, "A Review of Current Literature on *N*-Nitroso Compounds in Foods," *Advances in Food Research* **31**, 53–115 (1987).
8. B. Spiegelhalder, G. Eisenbrand, and R. Preussmann, "Volatile Nitrosamines in Food," *Oncology* **37**, 211–216 (1980).
9. A. R. Tricker et al., "Incidence of Some Nonvolatile *N*-Nitroso Compounds in Cured Meats," *Food Additives and Contaminants* **1**, 245–252 (1984).
10. J. W. Pensabene and W. Fiddler, "Effect of *N*-Nitrosothiazolidine-4-Carboxylic Acid on Formation of *N*-Nitrosothiazolidine in Uncooked Bacon," *Journal of the Association of Official Analytical Chemists* **68**, 1077–1080 (1985).
11. B. Spiegelhalder, G. Eisenbrand, and R. Preussmann, "Contamination of Beer with Trace Quantities of *N*-Nitrosodimethylamine," *Food and Cosmetics Toxicology* **17**, 29–31 (1979).
12. P. Johnson et al., "An Investigation into the Total Apparent *N*-Nitroso Compounds in Malt," *Journal of the Institute of Brewing*, **93**, 319–321 (1987).
13. C. Ji et al., "A new *N*-Nitroso Compound, *N*-(2-Methylpropyl)-*N*-(1-Methylacetonyl)Nitrosamine, in Moldy Millet and Wheat Flour," *Journal of the Agricultural and Food Chemistry* **34**, 628–632 (1986).
14. N. P. Sen, S. W. Seaman, P. A. Baddoo, and D. Weber, "Further Studies on the Formation of Nitrosamines in Cured Pork Products Packaged in Elastic Rubber Nettings," *Journal of Food Science* **53**, 731–734, 738 (1988).
15. N. P. Sen, "Migration and Formation of *N*-Nitrosamines from Food Contact Materials," in J. H. Hotchkiss, ed., *Food and Packaging Interactions. ACS Symposium Series No. 365*, American Chemical Society, Washington, D.C., 1988, pp. 146–158.
16. J. W. Pensabene and W. Fiddler, "*N*-Nitrosothiazolidine in Cured Meat Products," *Journal of Food Science* **48**, 1870–1874 (1983).
17. A. R. Trickler, B. Spiegelhalder, and R. Preussmann, "Environmental Exposure to Preformed Nitroso Compounds," *Cancer Surveys* **8**, 251–272 (1989).
18. P. N. Magee, "The Experimental Basis for the Role of Nitroso Compounds in Human Cancer," *Cancer Surveys* **8**, 207–239 (1989).

GENERAL REFERENCES

H. Biaudet, T. Malvelle, and G. Debry, "Mean Daily Intake of *N*-Nitrosodimethylamine from Foods and Beverages in France in 1987–1992," *Food Chemical Toxicol.* **32**, 417–421 (1994).

A. I. Burykin et al., "Effects of Drying Method on Carcinogen Content of Dairy Products," *Molochnaya Promyshlennost* **4**, 14–18 (1992).

R. G. Cassens, "Use of Sodium Nitrite in Cured Meats Today," *Food Technol.* **49**, 72–79, 115–116 (1995).

Lim Yung Choo et al., "The Formation of *N*-Nitrosamines During Storage of Salted Mackerel, *Scomber japonicus*," *J. Korean Soc. of Food Sci. and Nutrition* **26**, 45–53 (1997).

J. Cornee et al., "An Estimate of Nitrate, Nitrite and *N*-Nitrosodimethylamine Concentration in French Food Products of Food Groups," *Sciences des Aliments* **12**, 155–197 (1992).

T. P. Coulate, "Contaminants from Food Processing," *Food Sci. and Technol. Today* **9**, 44–49 (1995).

A. Dellisanti, G. Cerutti, and L. Airoldi, "Volatile *N*-Nitrosoamines in Selected Italian Cheeses," *Bull. Environ. Contamination and Toxicol.* **57**, 16–21 (1996).

W. Fiddler et al., "Nitrosamine Formation in Processed Hams as Related to Reformulated Elastic Rubber Netting," *J. Food Sci.* **63**, 276–278 (1998).

W. Fiddler et al., "*N*-Nitrosodibenzylamine in Boneless Hams Processed in Elastic Rubber Netting," *J. AOAC INTERNATIONAL* **80**, 353–358 (1997).

W. Fiddler et al., "Alaska Pollock (*Theragra chalcogramma*) Mince and Surimi as Partial Meat Substitutes in Frankfurters: *N*-Nitrosodimethylamine Formation," *J. Food Sci.* **58**, 62–65 (1993).

M. B. A. Gloria et al., "Influence of Nitrate Levels Added to Cheesemilk on Nitrate, Nitrite, and Volatile Nitrosamine Contents in Gruyere Cheese," *J. Agric. Food Chem.* **45**, 3577–3579 (1997).

M. B. A. Gloria, J. F. Barbour, and R. A. Scanlan, "*N*-Nitrosodimethylamine in Brazilian, U.S. Domestic and U.S. Imported Beers," *J. Agric. Food Chem.* **45**, 814–816 (1997).

M. B. A. Gloria, J. F. Barbour, and R. A. Scanlan, "Volatile Nitrosamines in Fried Bacon," *J. Agric. Food Chem.* **45**, 1816–1818 (1997).

R. Goutefongea, "Nitrosamines: Formation and Occurrence in Food Products," *Comptes Rendus de l'Academie d'Agriculture de France* **80**, 53–62 (1994).

J. S. Helmick and W. Fiddler, "Thermal Decomposition of the Rubber Vulcanization Agent, Zinc Dibenzyldithiocarbamate, and Its Potential Role in Nitrosamine Formation in Hams Processed in Elastic Nettings," *J. Agric. Food Chem.* **42**, 2541–2544 (1994).

M. Izquierdo-Pulido, J. E. Barbour, and R. A. Scanlan, "*N*-Nitrosodimethylamine in Spanish Beers," *Food Chem. and Toxicol.* **34**, 297–299 (1996).

H. Katoaka, M. Kurisu, and S. Shindoh, "Determination of Volatile *N*-Nitrosamines in Combustion Smoke Samples," *Bull. Environ. Contamination and Toxicol.* **59**, 570–576 (1997).

J. G. Kim, S. J. Lee, and N. J. Sung, "Influence of Nitrite and Ascorbic Acid on *N*-Nitrosamine Formation during Fermentation of Salted Anchovy," *J. Korean Soc. Food Sci. and Nutrition* **26**, 606–613 (1997).

D. Kuchne, "Nitrosamines in Meat Products—The Present Situation," *Mitteilungsblatt der Bundesanstalt fuer Fleischforschung Kulmbach* **34**, 220–225.

C. Lintas, G. Boccia Lombardi, and S. Nicoli, "Effect of Cooking on Availability and *In vitro* Nitrosation Precursors of Volatile *N*-Nitroso Compounds in Seafood," *Food Additives and Contaminants* **7**, 37–42 (1990).

R. N. Loeppky and C. J. Michejda, *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biochemistry*, American Chemical Society, Washington, D.C., 1994.

J. Marsden and R. Pesselman, "Nitrosamines in Food-Contact Netting: Regulatory and Analytical Challenges," *Food Technol.* **47**, 131–134 (1993).

R. C. Massey "Volatile, Nonvolatile and Total *N*-Nitroso Compounds in Bacon," *Food Additive Contamination* **8**, 585–598 (1991).

T. Mavelle, B. Bouchikhi, and G. Debry, "The Occurrence of Volatile *N*-Nitrosamines in French Foodstuffs," *Food Chem.* **42**, 321–338 (1991).

C. N. Mendoza et al., "Level and Occurrence of *N*-Nitrosodimethylamine, *N*-Nitrosodiethylamine and *N*-Nitrosopyrrolidine in Cured Meat Products," *Alimentos* **18**, 15–19 (1993).

J. W. Pensabene and W. Fiddler, "Effect of Carbohydrate Cryoprotecting Agents on the Formation of *N*-Nitrosodimethylamine in Surimi-Meat Frankfurters," *J. Food Safety* **13**, 125–131 (1993).

J. W. Pensabene, W. Fiddler, and R. A. Gates, "Nitrosamine Formation and Penetration in Hams Processed in Elastic Rubber Nettings," *J. Agric. Food Chem.* **43**, 1919–1922 (1995).

S. Raoul et al., "Rapid Solid-Phase Extraction Method for the Detection of Volatile Nitrosamines in Food," *J. Agric. Food Chem.* **45**, 4706–4713 (1997).

R. Rywotycki, "The Concentration of Nitrosamines in Pasteurized Beef Ham as Influenced by Heat Treatment and Functional Additives," *Medycyna Westerynaryina* **54**, 554–558 (1998).

R. A. Scanlan, "Volatile Nitrosamines in Foods—An Update, Food Flavors, Generation, Analysis and Process Influence," *Proc. 8th Int. Flavor Conf.: Developments in Food Science* **37**, 685–704 (1995).

R. A. Scanlan et al., "*N*-Nitrosodimethylamine in Nonfat Dry Milk," in R. N. Loeppky and C. J. Michejda, eds., *Nitrosamines and Related N-Nitroso Compounds, Chemistry and Biochemistry*, ACS Symposium Series, Washington, D.C., 1994, pp. 34–41.

R. A. Scanlan, J. E. Barbour, and C. I. Chappel, "A Survey of *N*-Nitrosodimethylamine in U.S. and Canadian Beers," *J. Agric. Food Chem.* **38**, 442–443 (1990).

N. P. Sen, P. A. Baddoo, and S. W. Seaman, "Nitrosamines in Cured Pork Products in Elastic Rubber Nettings: An Update," *Food Chem.* **47**, 387–390 (1993).

N. P. Sen et al., "Trends in the Levels of *N*-Nitrosodimethylamine in Canadian and Imported Beers," *J. Agric. Food Chem.* **44**, 1498–1501 (1996).

F. Shahidi, J. Synowiecki, and N. P. Sen, "*N*-Nitrosamines in Nitrite-Cured Chicken-Seal Salami," *J. Food Prot.* **58**, 446–448 (1995).

Nak Ju Sung, Jung Hye Shin, and Soo Jung Lee, "*N*-Nitrosamine in Korean Beer," *J. Food Sci. Nutrition* **1**, 6–9 (1996).

B. A. Tomkins and W. H. Griest, "Determinations of *N*-Nitrosodimethylamine at Part-Per-Trillion Concentrations in Contaminated Groundwaters and Drinking Waters Featuring Carbon-Based Extraction Disks," *Analytical Chem.* **68**, 2533–2540 (1996).

A. R. Tricker and S. J. Kubacki, "Review of the Occurrence and Formation on Nonvolatile *N*-Nitroso Compounds in Foods," *Food Additives and Contaminants* **9**, 39–69 (1992).

A. B. Tricker et al., "Mean Daily Intake of *N*-Nitrosoamines from Foods and Beverages in West Germany in 1989–1990," *Food and Chemical Toxicol.* **29**, 729–732 (1991).

C. Trova, G. Gandolfo, and G. Cossa, "*N*-Nitrosodimethylamine Levels in Beer," *Industrie delle Bevande* **22**, 528–530 (1993).

I. Vittozi, "Toxicology of Nitrates and Nitrites," *Food Additives and Contaminants* **9**, 579–585 (1992).

E. Wiegler, H. Kolb, and C. Ruehl, "Studies on the Nitrosamine Content of Pizza and Toast Products," *Fleischwirtschaft* **74**, 1296–1298 (1994).

C. P. Wild and R. Montesano, "Immunological Quantitation of Human Exposure to Aflatoxins and *N*-Nitrosamines," American Chemical Society Series 451, Washington, D.C., 1991, pp. 215–228.

A. R. THICKER
Institute for Toxicology and Chemotherapy
Heidelberg, Germany

See also TOXICANTS, NATURAL.

NUCLEOTIDES

Proteins and nucleic acids function as the key components to maintain life for plants and animals in terms of genetic heritage, self-duplication of genetic information, and regulation of metabolites for growth. It is, moreover, interesting that the components of these proteins and nucleic acids—such as L-glutamate in amino acids and 5′-ribonucleotides in nucleic acids—play a key role in making meals, which are indispensable for human life, very tasteful and joyful.

In the long history of humankind, umami was only first added to the restricted idea of four "basic" or primary tastes by Ikeda (1). He extracted monosodium L-glutamic acid from seaweed and identified the taste-active ingredient obtained as umami.

Not only must we consider the unique taste of L-glutamate, but also the remarkable effect of certain 5′-ribonucleotides in causing a synergistic taste effects in combination with L-glutamate. The culinary practices of Japanese cuisine have long taken advantage of the taste effects of certain combinations of natural products used as condiments, especially the seaweed sea tangle (*Laminaria*), in combination with either black mushroom (*shiitake*) or dried bonito (*katsuo-bushi*). The taste-active ingredients of black mushroom and bonito were identified as inosine 5′-monophosphate by Kodama (2) and guanosine 5′-monophosphate by Kuninaka (3), respectively. Kuninaka (4) discovered that monosodium glutamate (MSG) and certain 5′-ribonucleotides added together to foods have a synergistic effect in enhancing food flavor.

Nucleotides are now used not only as umami flavor enhances but also as the raw material of pharmaceuticals. Industrial production of nucleotides started in Japan in 1961, using the RNA degradation method. Nowadays, nucleotides are mostly produced by a fermentation method using microorganisms, which was developed mostly in Japan. The main purposes are to produce two umami flavor enhancers inosine 5′-monophosphate (5′-IMP) and guanosine 5′-monophosphate (5′-GMP).

SYNERGISM OF 5′-NUCLEOTIDES FOR UMAMI TASTE

When glutamic acid and 5′-nucleotides (free forms or salts) coexist, umami is dramatically enhanced. This phenomenon, well known as the synergistic effect of umami substances, has already been investigated and reported (5) mainly with regard to human taste sensations.

Ikeda (6) confirmed the synergistic effect in actual food. He conducted a discrimination test of MSG and IMP in a large variety of stocks or broth and compared the results with those of MSG and IMP in simple aqueous solutions. The thresholds of MSG and IMP were reported to be 0.03 and 0.025%, respectively. The nucleotide and glutamic acid contents in some of the stocks used are popular and commonly used in cooking to enhance the palatability of foods. Although the contents of umami substances are surprisingly low, these stocks are effective in foods apparently because of the synergism among the umami substances they contain.

The detection thresholds for five taste substances in single aqueous solutions and in 5 mM MSG (0.094%) or IMP (0.26%) solution are shown in Table 1. The presence of MSG or IMP did not lower the thresholds of the four basic tastes, although IMP raised that of quinine sulfate, and MSG and IMP raised that of tartaric acid. This means that umami does not improve sensitivity toward the four basic tastes. Only IMP remarkably lowered the threshold of MSG by more than 50-fold. This result suggests that umami neither enhances the four tastes nor is itself enhanced by the basic tastes. Only the synergistic effect between the two kinds of umami substances enhances the umami taste. Electrophysiological studies on the synergism have been carried out using the cat, rat, and hamster (7,8). In these studies, the synergism was examined under conditions in which both L-glutamate and 5′-ribonucleotides induced the taste responses. Hence, quantitative analysis of the data obtained was rather difficult, and it was not known by what mechanism the synergism occurs.

The recording in Figure 1 shows the responses of the rat chorda tympani nerve to 20 mM MSG in the absence and the presence of 0.1 mM 5′-GMP. As seen from the baseline of the recording, the response to 0.1 mM GMP itself is very small. The response to MSG is so greatly enhanced by the presence of 5′-nucleotides that it may derive mostly from the synergism between two substances.

The effect of various species of nucleotides on the response to L-glutamate is shown in Table 2. 5′-Nucleotides with a purine base, such as GMP, deoxy-GMP, guanosine diphosphate (GDP), guanosine triphosphate (GTP), adenosine 5′-monophosphate (AMP), and IMP, have the ability to enhance the response to L-glutamate, whereas prymidine nucleotides carrying the pyrimidine base, such as uridine 5′-monophosphate (UMP) and cytidine 5′-monophosphate (CMP), have only a small or no enhancing effect. Cyclic GMP, guanosine, and sodium phosphate also have only a small or no enhancing effect.

The preceding results demonstrate that only the nucleotides having a specific structure exhibit a synergistic effect on the responses to L-glutamate. Whether the monophosphate, diphosphate, or triphosphate is used does not affect the synergistic effect. Moreover, the nucleotides with a deoxy sugar have a small amount of synergistic effect.

Behavioral and electrophysiological studies on several mammalian species indicated that L-glutamate is a taste stimulus and that the synergism with 5′-ribonucleotides occurs in several species of mammals (9). Feeding studies have shown that weanling calves respond to L-glutamate positively, as indicated by their higher consumption of the diet with 0.2% MSG. Torii and Cagan (10) demonstrated that in the presence of low levels of certain 5′-ribonucleotides, the binding activity of the taste receptor prepared from bovine circumvallea papillae (containing approximately 90% of the tastebuds) for L-glutamate increased by severalfold, as shown in Figure 2. There was no effect on the low level of binding to the control epithelium. This was the first demonstration that the taste synergism of glutamate and 5′-ribonucleotide is a peripheral biochemical event, occurring at the level of the taste receptor membrane, and does not require neural integration. The specificity of the effect with respect to the nucleotide was similar, though not identical, with that for humans. In humans, the order of effectiveness is GMP > IMP > XMP, and AMP

Table 1. Detection Thresholds of Five Taste Substances

	Sucrose	NaCl	Tartaric acid	Quinine	MSG
Water	0.086	0.0037	0.00094	0.000049	0.012
5 mM MSG solution	0.086	0.0037	0.0019	0.000049	—
5 mM IMP solution	0.086	0.0037	0.03	0.0002	0.00019

Note. Expressed in percent (w/v).

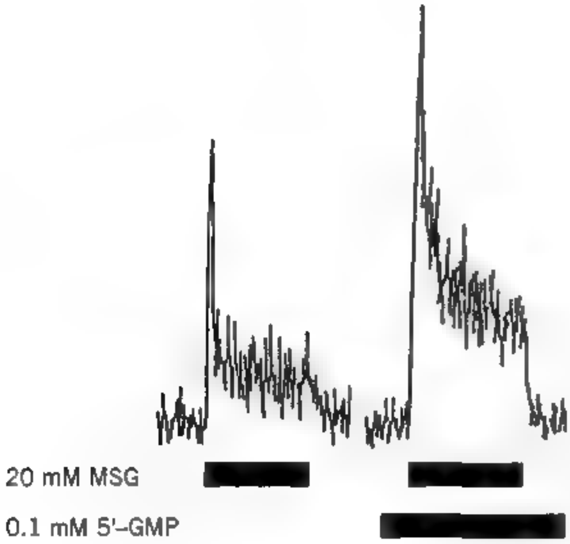


Figure 1. The summated responses of the rat chorda tympani nerve to 20 mM MSG in the absence and the presence of 0.1 mM 5′-GMP. The bars at the base of the recordings represent the duration of the application of the chemical stimuli.

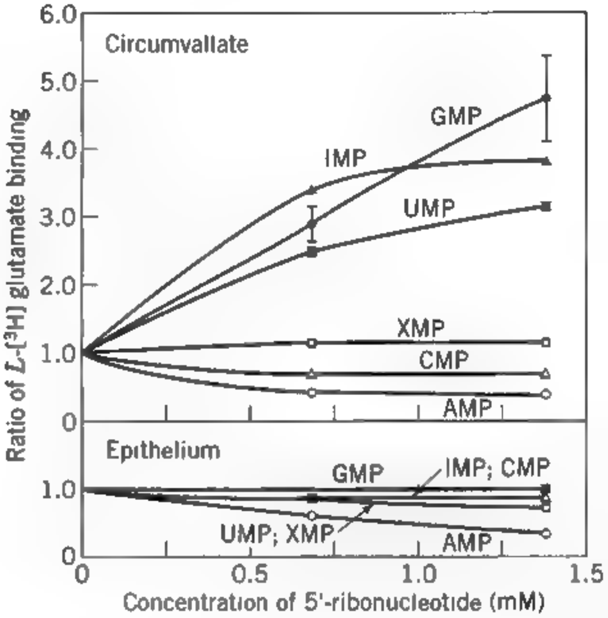


Figure 2. Effects of 5′-ribonucleotides on binding of L-glutamate to bovine taste receptors.

Table 2. Relative Effect of Various Nucleotides, Guanosine, and Sodium Phosphate on Rat Taste Nerve Response to 10 mM of Glutamate (taken as 100)

Nucleotide	Magnitude of responses
GMP	232 ± 13
dGMP	260 ± 24
GDP	192 ± 6
GTP	188 ± 22
cGMP	126 ± 31
Guanosine	109 ± 9
AMP	255 ± 27
IMP	171 ± 26
UMP	125 ± 25
CMP	124 ± 16
Sodium phosphate	108 ± 9

Note. Concentration, 10 mM; and pH 6.0.

is ineffective (11). The pyrimidine nucleotides are reportedly not effective (3). With the bovine system, the order of effectiveness was GMP ≥ IMP > UMP, and XMP, CMP, and AMP were effective (Fig. 2). Additional structure–activity considerations were studied by examining the effects of the free bases, the nucleoside, and the di- and triphosphates of GMP and AMP. The data in Figure 3 show that only 5′-GMP was effective in increasing binding of L-glutamate. The regions of the molecule that appear to be critical in the interaction with the regulatory site are shown in Figure 4. The keto function on the aromatic ring appears critical. With an amino group at this position, there is no synergistic activity. The data from human studies show that 5′-GMP and 5′-IMP are potent synergistically, 5′-XMP is less effective, but 5′-UMP is not effective. The binding studies agree with the earlier human psychophysical results showing the importance of the 4- or 6-position on the ring, the 2-position on the ring, and the 5′-monophosphate group on the ribose. There are two points of difference in the bovine and human data: (*1*) XMP did not enhance binding but is synergistic in humans, and (*2*) UMP increased binding but is not effective in humans.

The characteristics of the chemical structure of nucleotides attributed to the taste of umami for humans are summarized as follows: (*1*) a purine base on which the OH-bond is located at the 6-position carbon, and (*2*) a pentose on which the phosphate bond locates at 5′-position of the OH-bond.

When the total concentration of L-glutamate and IMP is kept constant (0.015 g/dL), the intensity of umami increases with the increasing ratio of IMP and goes to maximum when the ratio of IMP is 50%; it then decreases with the increasing ratio of IMP, which is shown in Figure 5.

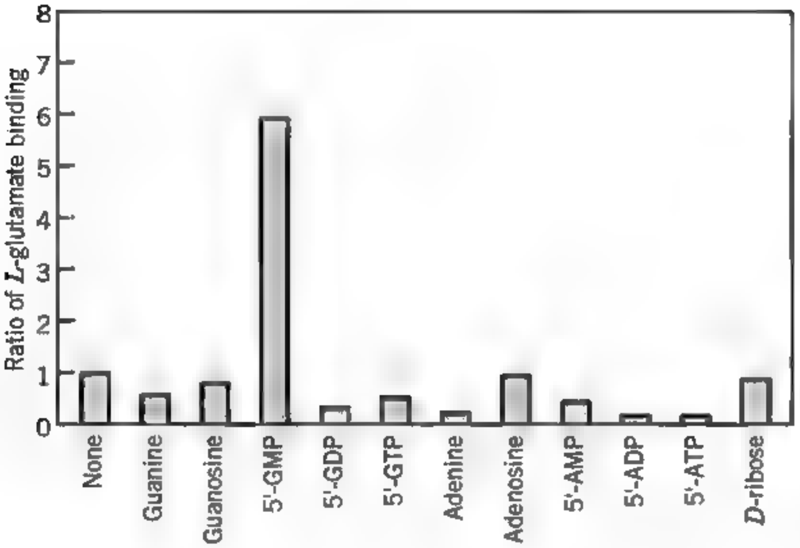


Figure 3. Effect of 5′-ribonucleotides, nucleosides, and free bases on binding of L-glutamate to bovine taste receptors.

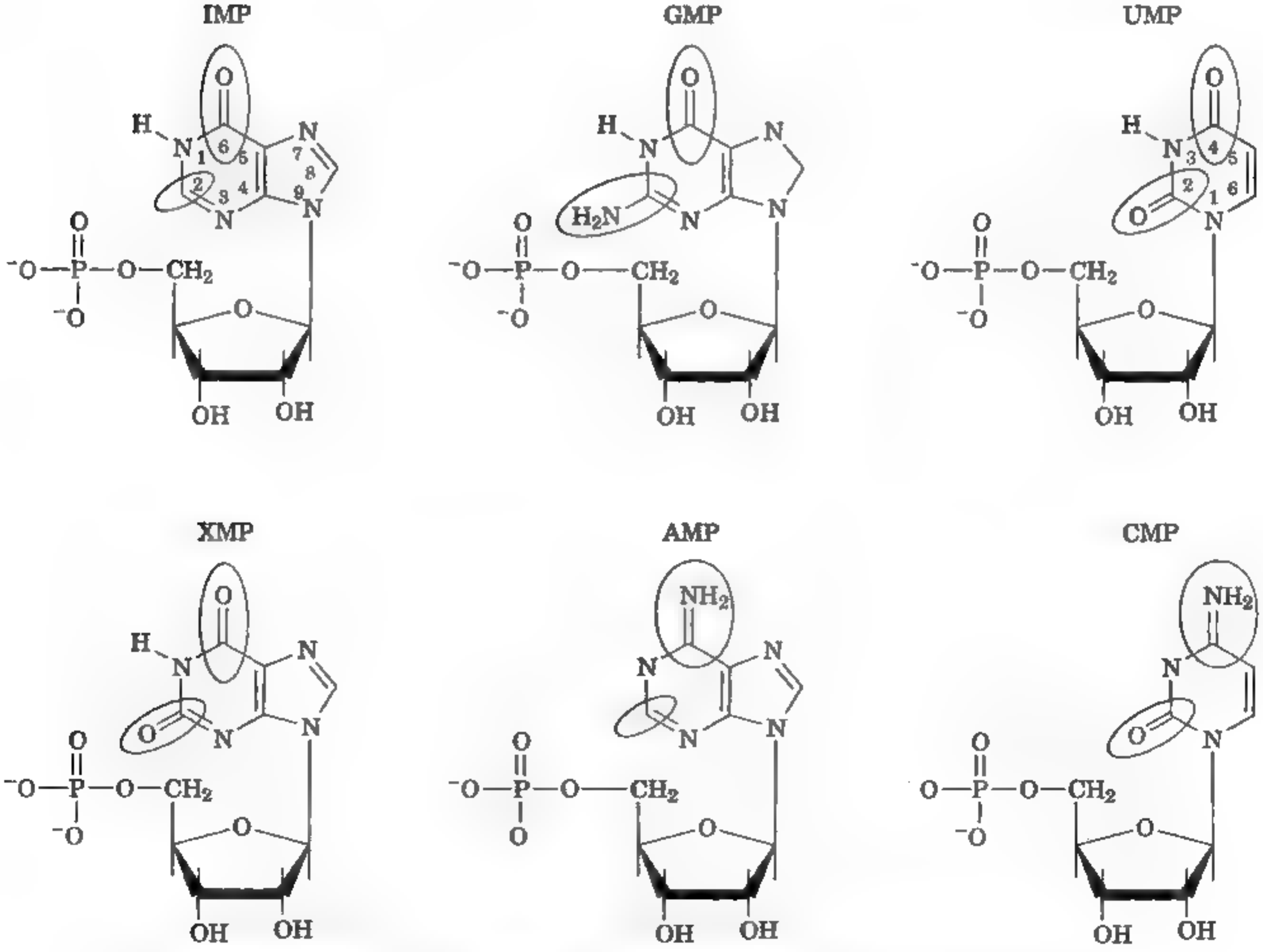


Figure 4. Structure of 5′-ribonucleotides investigated for synergistic biochemical effect with L-glutamate.

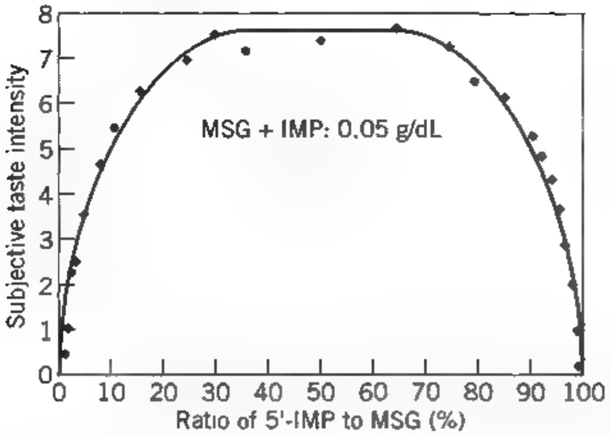


Figure 5. The synergistic effect shown as the relative intensity of umami taste to MSG, changing the ratio of 5′-IMP to MSG while the total concentration of MSG + 5′-IMP remained constant as 0.05 g/dL.

This intensity of umami of L-glutamate when IMP coexists is expressed by the following relation (5):

$$y = \mu + 1{,}200\mu\ v$$

where y is the concentration of L-glutamate (%) that shows the same intensity of umami as that shown by the mixture of L-glutamate and IMP; μ is the concentration of L-glutamate in mixture (%); and v is the concentration of IMP in mixture (%), respectively.

When using 5′-guanylic acid (GMP) in place of IMP in the mixture, the relation of intensity of umami was summarized as follows:

$$y = \mu + 2{,}800\mu\ v'$$

where v' is the concentration of GMP in mixture (%).

The concentration–response relationship for L-glutamate does not follow the simple taste equation obtained under the assumption that there exists only one type of receptor site for L-glutamate. Assuming that two types of receptor sites exist—with different dissociation constants for L-glutamate—in rat taste cell membrane we used the following equation (12):

$$R = R_H/K_H/S + 1 + R_L/K_L/S + 1$$

Here, R is the magnitude of the response; R_H and R_L are the magnitudes of the maximal responses brought about by occupation of high- and low-affinity sites, respectively; and S is the concentration. The best-fitted curves for the data were obtained with a computer utilizing a program for the linear square iterative fitting of the preceding equation. The parameters for the curves were determined as follows:

	L-glutamate alone	L-glutamate + 0.1 mM GMP
K_H (mole)	0.01	0.0015
K_L (mole)	0.32	0.17

The values of $R_H = 1.18$, and $R_L = 15.59$ were unchanged by the presence of 0.1 mM GMP.

There seem to be specific binding sites for purine-based 5′-nucleotides in the taste receptor membranes, because only the specific nucleotides have the ability to show the synergism. The binding sites for the nucleotides seem to be part of or closely associated with L-glutamate receptors.

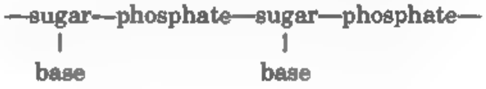
STRUCTURE OF NUCLEIC ACIDS

The intrinsic parts of nucleic acids are more complex, being threefold aggregates of purine and pyrimidine bases, sugar, and phosphoric acid. The number of "building blocks," however, is much smaller, being six altogether, and in the great majority of cases, only four. Depending on the characteristic of the sugar and on the presence of uracil in place of thymine, two kinds of nucleic acid—ribonucleic acid (RNA) and deoxynucleic acid (DNA)—are defined.

The component unit, which can be thought of as analogous to an amino acid in a peptide chain, is

base—sugar—phosphate

and the linkage is by the phosphate to two sugars in a stepwise pattern, as follows:



$$-\underset{\text{base}}{\underset{|}{\text{sugar}}}-\text{phosphate}-\underset{\text{base}}{\underset{|}{\text{sugar}}}-\text{phosphate}-$$

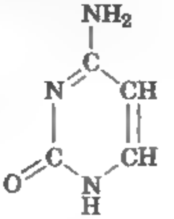
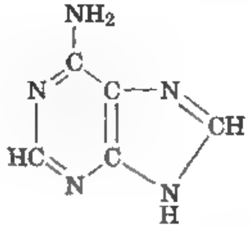
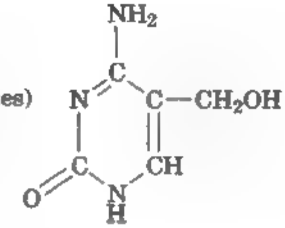
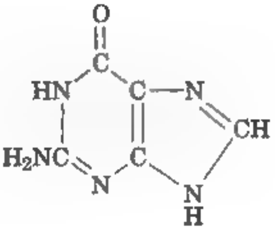
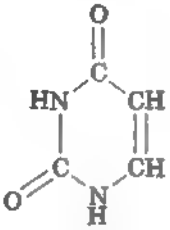
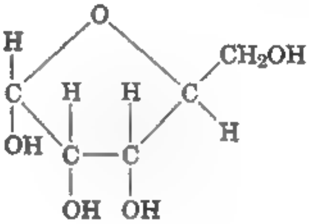
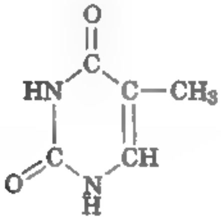
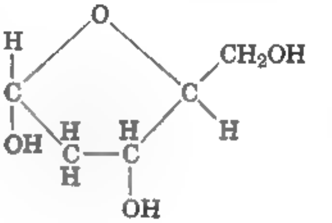
A combination between a base and a sugar (ribose or deoxyribose) is called a nucleoside. When this is combined with a phosphate, it becomes a nucleotide. The important separate bases, six in number, are listed in Table 3, with their structural formulas. These, together with their sugar and phosphate complements, are linked into enormous molecules having molecular weights in the millions. The structure of nucleic acids is becoming understood and bringing with it an advance in molecular biology. The actual coupling among base sugar, and phosphate is shown in terms of the structural formula in Figure 6. This coupling permits the formation of a simplified double helix.

THE STRUCTURE OF NUCLEIC ACID POLYMERS

DNA

A firm knowledge of the structure of DNA is of crucial importance. The gross dimensions of DNA were found by light-scattering or viscosity measurements, which indicate that DNA is a long, thin, relatively rigid structure whose molecular weight is ~6×10^6 and density is 1.6 to 1.75 g/cm^3. Chemical evidence indicates that the phosphate (PO_4) groups are very reactive, but the amino ($-NH_2$) and CO groups are not readily titratable. The analysis of Wyatt and Chargaff (13) indicated that the ratio of thymine to adenine is unity, and the ratio of cytosine or its derivatives to guanine is also about unity. The absorption of polarized infrared light indicates that the $-NH_2$ and $-C-O$ groups of the bases are oriented perpendicular to the molecular axis.

Table 3. Bases Involved in Nucleic Acids

Pyrimidines		*Purines*	
Cytosine		Adenine	
Alpha hydroxymethyl cytosine (found in virus DNA in some cases)		Guanine	
Uracil (in RNA only)		*Sugars* Ribose	
Thymine (in DNA)		Deoxyribose	

The P=O groups, on the other hand, are inclined an angle of ~55° to the axis. X-ray diagrams indicated that the planes of the bases were separated by a distance of 3.4 Å. Impressive advances in knowledge have come from the recognition by Watson and Crick (14,15) of the specific base pairing in DNA. Helical DNA has a pitch of 34 Å with 10 repeat units per turn, and its fundamental structure is not one helix but two right-handed helices of radius about 8.5 Å, spaced about one-half the pitch apart. The pairs of purines and pyrimidines that occur in equal amounts in DNA, namely, thymine–adenine and cytosine–guanine, have complementary structures that easily form hydrogen bonds. Watson and Crick found that the bases would be in a proper position for strong hydrogen bonds if the two helical chains had sequences of atoms in opposite directions. The helical chains are the phosphate–sugar backbones. The bases extend toward the center of the helix and hydrogen bond across the axis of the double helix. Thus the DNA spirals are held together by interchain bonding, whereas protein helices keep their configuration by means of intrachain hydrogen bonds.

The double spiral for the structure of DNA has very practical properties concerned with information storage and the duplication of genetic material.

RNA

The primary structure of RNA is similar to that of DNA, but with a few notable exceptions. First, in RNA, instead of thymine, the pyrimidine base uracil occurs, forming a complementary base pair with adenine in regions of double-stranded RNA. Also, a wide variety of ribonucleotides having modified or minor bases are found in naturally occurring RNA, one of the commonest of which is pseudouridine. In human tRNAs, as many as 25% of the bases are nonstandard. Although the role of base modification is not clear, it may be important for biological recognition. The other important feature of the primary structure of RNA is the presence of the 2′-hydroxyl group in ribose. Although this hydroxyl group is never involved in phosphodiester linkages, it does impose restriction on the helical conformations accessible to double-stranded RNA.

RNAs are usually single-stranded molecules that are stable allowing different regions of the ribonucleotide to form distinct secondary structural elements. When self-complementary regions of the RNA strand are aligned, duplex regions, which may have Watson–Crick base pairs, are formed.

RNA has a variety of functions within a cell; for each function, a specific type of RNA is required. Messenger

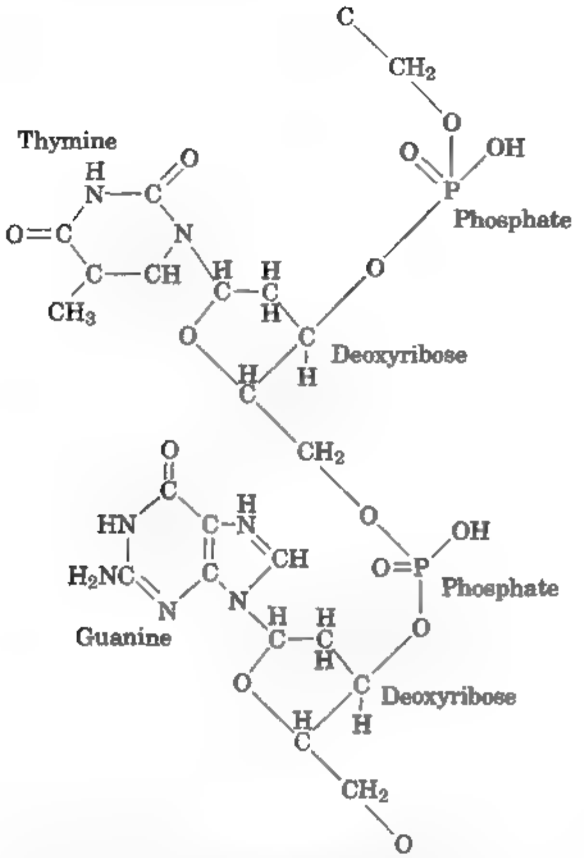


Figure 6. The detail of coupling between base, sugar, and phosphate in deoxyribonucleic acid.

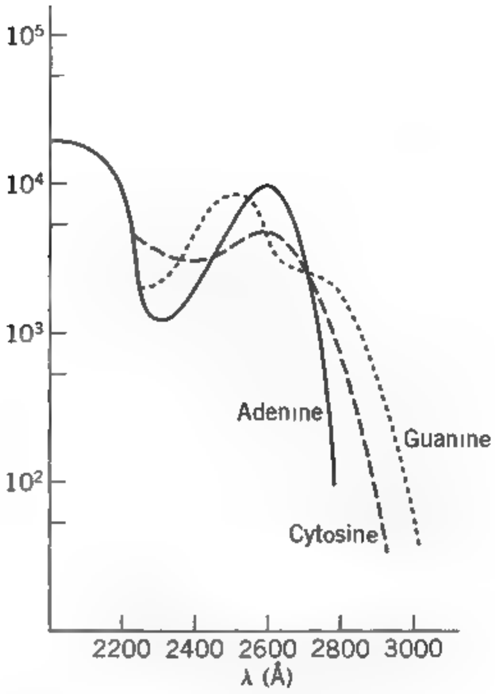


Figure 7. Absorption UV spectra of nucleoside that constitutes DNA at neutral pH in the dry state.

RNA (mRNA) serves as an intermediary for carrying genetic messengers from the DNA to the ribosome where protein synthesis takes place. Ribosomal RNA (rRNA) serves both structural and functional roles in the ribosome; it is diverse, both in terms of its size and structure. Transfer RNA (tRNA) is a group of small molecules that have a central role in protein synthesis. The multifunctional character of RNA, particularly the involvement of RNA in enzymatic processes, had led to the hypothesis that life on earth evolved from RNA, and that RNA had both the genetic and catalytic functions commonly associated with DNA and protein, respectively.

The functional diversity of RNA is directly related to its structural diversity. In contrast to DNA, RNA molecules are synthesized as single-stranded polynucleotides that fold to give complex tertiary structures. These structures, which incorporate hairpins, loops, bulges, and junctions between single-stranded and double-stranded regions, exhibit long-range interactions within the folded tertiary structure.

OPTICAL PROPERTIES OF NUCLEIC ACID

Ultraviolet Absorption

Figure 7 shows the absorption spectra of some bases of nucleic acids. From this figure one can see that the absorption spectra for nucleic acids would have a maximum at 260 μm. The optical densities shown in the figure were calculated from data obtained from thinner and more dilute solutions on the assumption that the Beer–Lambert law holds. At the longer ultraviolet wavelengths, nucleic acids will have about 20 times the optical density of an equal mass concentration of protein. Most of the amino acids are nonaromatic and contribute practically nothing to the protein absorption spectrum at wavelengths greater than 210 μm. The high and characteristic absorption by nucleic acids makes it relatively simple to detect them by ultraviolet spectroscopy and to localize them in cells by ultraviolet microscopes.

In Table 4, the wavelengths of maximum absorbency and the intensity (ϵ) for various bases, nucleosides, and nucleotides are summarized, which are tentative values compiled by the National Academy of Science.

Infrared Absorption Spectra

Infrared (IR) absorption methods are used mainly for two different purposes: one is as a “fingerprint” to identify a particular compound and to detect it in the mixture, the other is for structural studies. The logical approach to a spectroscopic study of the nucleic acids is to examine separately the base, sugar, and phosphate moieties; their combination in nucleosides and nucleotides; and their comparison with nucleic acids. The approximate vibrational frequency ranges for the components of functional groups related to nucleic acids are shown in Table 5.

Bases and Nucleosides

The fundamental modes associated with base residues occur throughout the IR spectrum and thus overlap bands

Table 4. The Maximum Absorbance Intensity (ϵ) for Various Nucleic Acids

Nucleic acid	The maximum absorbance			Ratio of observance between two wavelengths	
	Intensity ($\epsilon \times 10^3$)	Wavelength (m μ)		250/260	280/260
Adenine	13.1	262	0.1 N HCl	0.76	0 38
Adenosine	15.4	259	pH 7	0.98	0.15
5′-AMP	15.4	259	pH7	0.80	0.15
Cytosine	10.4	275	0.1 N HCl	0.48	1.50
Cytidine	13.0	280	0.1 N HCl	0.45	2.10
5′-CMP	13.0	280	pH2	0.45	2.10
Guanine	14.4	248	0.1 N HCl	1.37	0.84
Guanosine	13.6	252.5	pH 7	1.15	0.67
5′-GMP	13.7	252	pH 7	1.16	0.66
Hypoxanthine	10.6	250	pH 7	1.32	0.09
Inosine	12.2	248.5	pH 6	1.73	0.24
Uracil	8.2	260	pH 7	0.83	0.20
Uridine	10.1	262	pH 7	0.74	0.36
5′-UMP	10.0	262	pH 7	0.74	0.38
Thymine	7.89	264.5	pH 7	0.67	0.53
Xanthosine	11.4	248.5	pH 8	1.31	1.13

Table 5. Approximate Frequency Ranges for Characteristic Vibrations of Nucleic Acids and Related Compounds (infrared)

Frequency (cm^{-1})	Mode	Origin of vibration
2800–3500	ν OH	Water, sugar
	ν NH	Base residue
	ν CH	Sugar and base residue
1800–1500	ν C=O	Base residues, mixed bodies
	ν C=N	
	ν C=C	
	δ NH	
	δ HOH	Water
1250–950	νPO_2^-	Antisymmetric stretch
	νPO_2^-	Symmetric stretch
	ν CO	Sugar
1000–700	ν PO	Phosphate
	ν CO	Sugar
	τ NH	Base residue

Note· Modes: ν, stretching; δ, in-plane bending; τ, out-of-plane bending.

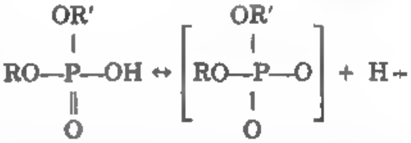
from the other entities in nucleic acids. Between 1500 and 1750 cm^{-1} several strong bands occur that are free from interference by phosphate and sugar groups and are related to the structure of the purine and pyrimidine rings in nucleosides and nucleotides, and nucleic acids. These bands also arise from strongly coupled vibration of the C=O (1725 cm^{-1} stretch), C=N (1600 cm^{-1} stretch), and C=C types with in-plane NH (1680 cm^{-1} bend) deformation.

On the basis of protonation studies of the base–sugar, the following are concluded (16):

1. The acidic form of adenosine has a proton attached to one of the ring nitrogens, not to $-NH_2$.
2. Guanosine exists in acidic, neutral, and alkali forms, with two possible neutral forms. Each form has a different IR spectrum. The alkali form is deprotonated in position 1, and the acidic form in position 7.
3. Deprotonation of inosine also causes π-electron delocalization with concomitant decrease in C=O stretching frequency from 1675 cm^{-1} to 1595 cm^{-1}.

Phosphate Group

Linking the sugars in the nucleotide backbone, the phosphate diester linkage can exist in two different states, protonated and charged:



$$RO{-}\overset{OR'}{\underset{O}{P}}{-}OH \leftrightarrow \left[RO{-}\overset{OR'}{\underset{O}{P}}{-}O\right] + H^+$$

In the IR spectrum the P-O modes are considerably stronger than those for the P-O-R linkage: 1230 cm^{-1} for PO_2^- asymmetric stretch, 1080 cm^{-1} for PO_2-symmetric stretch, and 980 cm^{-1} for PO_3^{2-}.

Sugar Moiety

The most characteristic group of bands occurs in a broad distribution in the range of 1000 to 1100 cm^{-1} and arises from C-O-C and C-C(OH)-C modes. The deoxy derivatives exhibit a sharp band at 975 cm^{-1}, which is absent in the ridonucleosides. There is some evidence that DNAs and RNAs may be distinguished in this manner

DNA and RNA

As expected, the spectra of DNA and RNA show features in common with their simpler analogs. In the 1500 to 1750 cm^{-1} region there are broad, poorly resolved bands corresponding to the complex ring modes. The strong phosphate (asymmetric) mode is in the 1240 cm^{-1} region, and the broad and complex band is in the 1000 to 1100 cm^{-1} region for the sugar vibrations. The strong band in the 3400 cm^{-1} region, which contains the NH stretching mode, is influenced by the presence of water. One of the major difficulties of studying even solid-state nucleotides is that water almost inevitably crystallizes with the nucleotides.

Dichroic Ratios

The measurement of dichroic ratios of biological systems to determine their structure requires polarized light and oriented structures. Polarized IR light may be produced by reflection from or transmission through films of selenium 4 μm thick, set at the polarizing angle to the incident light. This technique has been applied to the analysis of nucleic acid structure (DNA). The position of the N-H and O-H frequencies indicates that these groups participate in hydrogen bonds. The dichroism of the N-H, O-H, and C=O groups indicates that the hydrogen bonds are preferentially oriented perpendicular to the molecular axis, and so they probably represent intermolecular regions of attraction. The P-O-C groups also have a component perpendicular to the molecular axis, but the absence of any dichroism in the P=O absorption band at 1250 cm^{-1} implies that they are oriented at angles of 55° to the molecular axis, which is in good agreement with the structural model of DNA discussed previously.

CONTENTS OF NUCLEOTIDES IN THE INGREDIENTS OF FOODS

Biochemical Character of Muscles

The greater part of muscle fiber is made up of a set of specialized protein molecules that are in some way concerned with the process of muscular action. There is far less nucleic acid and very little ribonucleic acid in muscle tissue. The specialized molecules constitute about two-thirds of the protein, while the remaining one-third is concerned with the enzymatic use of the substances brought in by the blood capillaries that surround the fiber in the whole muscle.

In 1939 Engelhaldt and Ljubimowa discovered that myosin is an enzyme that catalyzes the conversion of ATP to the diphosphate ADP, with consequent release, or transfer, of configuration energy. More thorough studies by Banga and Szent-Gyorgyi (17) showed that two proteins were involved in myosin, namely, actin and myosin, which form a combination known as actomyosin, a combination capable of changing shape in a manner very reminiscent of the behavior of muscle itself. Additional studies have brought out that its molecular weight is 450,000, with an axial ratio of about 50 and a total length of 1600 Å. That of actin is 60,000, with an axial ratio of 12 and a length of 290 Å.

In the biochemistry of muscle, ATP, whose general structure is a triphosphate of adenine and ribose, is characterized by a phosphate grouping that, on hydrolysis, can yield a diphosphate and orthophosphoric acid, resulting in a more stable total configuration of the same atoms. This increase in stability means that energy is released when the process takes place.

Fish Muscle

In the muscle of fish, nucleotides such as adenosine ATP, ADP, AMP, and IMP have been detected. The changes in these nucleotides in muscle between the conditions of *at rest* and *in fatigue* are shown in Table 6, which summarizes the analysis of the codfish (18). In the condition of *at rest*, a large amount of ATP and quite a bit of adenosine and inosinic acid were found. When *in fatigue*, conversely, contents of inosinic acid increased. As already mentioned, the muscle released energy in the active state by the release of phosphate bonds from ATP, converting it into ADP, then AMP. This AMP is then changed to IMP by the aid of the enzyme deaminase. The procedure is as follows:

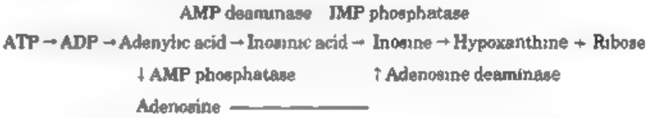
$$\text{ATP} \rightarrow \text{ADP} + \text{Pi by ATPase}$$

$$\text{ADP} \rightarrow \text{AMP} + \text{Pi by myokinase}$$

$$\text{AMP} \rightarrow \text{IMP} + \text{NH3 by deaminase}$$

The autodigestion process occurs even after the death of animals; the change of ATP to AMP proceeds, and finally IMP is accumulated in the muscles. Therefore, meats in fatigue contain a high level of ATP in muscles so that, even after death, no conversion to IMP, yet occurred the taste of this supposed to be short of umami.

Fish meat after death contains mainly IMP, followed by inosine, which is decomposed to inosinic acid by the release of phosphoric acid; quite a bit of ADP and AMP remain. But *Crustacea* and *Mollusca* such as robster, crab, octopus, oysters, and cuttlefish kept in good condition for use as fresh meat have, on the contrary, a large amount of ADP or AMP and a trace of IMP because of a lack of the enzyme adenylic acid deaminase. Hence, there are two pathways of decomposition of adenylic acid in fish, shown as follows:


AMP deaminase
IMP phosphatase
ATP → ADP → Adenylic acid → Inosinic acid → Inosine → Hypoxanthine + Ribose
↓ AMP phosphatase
↑ Adenosine deaminase
Adenosine

For *Crustacea* and *Mollusca*, adenylic acid is converted by AMP phosphatase in place of AMP daminase into adeno-

Table 6. Nucleotide Contents of Rested and Exhausted Codfish Muscle

	Content in muscle (μmoles/g)	
Nucleotide	Rested	Exhausted
DPN	0.107	0.338
TPN	0.0008	0.0005
AMP	0.69	0.57
ADP	0.576	0.426
ATP	5.32	0.26
IMP	1.26	5.86
GTP	0.0025	0
UTP	0.0036	0

Note: Values are mean determinations on six fish. DNP, diphosphopyridine nucleotoide; TPN, triphosphopyridine nucleotide; UTP, uridine 5′-triphosphate.

sine and then converted by adenosine deaminase into inosine. So far, in the *Mollusca*, AMP deaminase is not found; therefore, no inosinic acid is produced. Some kinds of *Crustacea* retain AMP deaminase in muscles; after killing, in a small amount of inosinic acid is detected in *Crustacea*.

As shown in Table 7, the IMP in fish meat is in the range of 100 to 200 mg%, and is regarded as the prime ingredient for taste of umami with L-glutamate. Base components constituted of nucleotides such as hypoxanthine, adenine, and guanine are found in the muscles of aquatic animals; in particular, a large amount of hypoxanthine is sometimes detected in many fish meals.

Meat

The contents of 5′-nucleotides in the meat of animals are summarized in Table 8. 5′-Inosinic acid is a prime ingredient in 5′-nucleotide for every animal and is found at a level of 100 mg% or so. The changes in the 5′-nucleotides during the maturing process have been investigated. Chicken (Fig. 8) has an active adenylic acid deaminase; in the case of storage in a refrigerator at 4°C, the concentration of inosinic acid in meat reached a maximum of 200 to 280 mg% in 4 to 10 h after killing, through the process of ATP → ADP → adenylic acid → inosinic acid conversion. Then after further storage in a refrigerator, inosinic acid began to decompose gradually to hypoxanthine, through inosine, and decreased to a half the original amount after 5 to 6 days. For pork, 3 days after slaughter the contents of inosinic acid reached a maximum, 100 to 170 mg%, and then gradually decomposed to hypoxanthine; after 10 days kept in a refrigerator, the contents of inosinic acid remained at 60 mg%, which proceeded through the conversion process inosinic acid → inosine → hypoxanthine, as well. In storage, no other 5′-nucleotides such as GMP and CMP were detected.

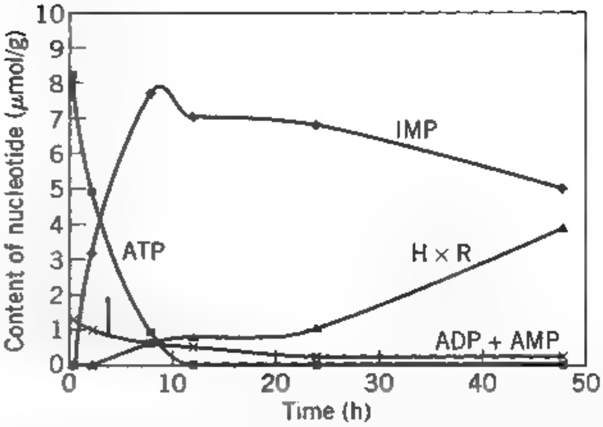


Figure 8. Change in the concentration of nucleic acid–related substances in chicken muscle after slaughter when kept at 4°C.

Table 7. Nucleotide Contents of Fish Meat

Fish	5′-AMP	5′-IMP
Saurel	6.4	212.6
Ayufish	7.2	189.0
Sea bass	8.4	124.9
Sardine	0.7	188.7
Sea bream	11.0	277.1
Saury	6.7	149.5
Mackerel	5.7	188.1
Salmon	6.9	154.5
Tuna	5.2	188.0
Swellfish	5.6	188.7
Eel	17.6	108.6
Sagittated calamari	163.2	0
Octopus	23.3	0
Lobster	72.5	0
Crab	10.1	0
Squilla	32.6	17.4
Ormer	71.7	0
Traugh shell	86.5	0
Scallop	102.3	0
Short-necked clam	10.8	0

Note: Content, mg %.

Table 8. Nucleotide Contents of Meat

Meat	5′-AMP	5′-IMP	5′-GMP	5′-UMP	5′-CMP
Beef	6.6	106.9	2.2	1.6	1.0
Pork	7.6	122.2	2.5	1.6	1.9
Chicken	11.5	75.6	1.5	1.3	2.6
Whale	2.1	214.5	3.6	1.9	—

Note: Content, mg %.

Vegetables and Mushrooms

In vegetables, a small amount of 5′-adenylic acid (10.4 mg% for tomato; 8.4 mg% for green soybeans; 6.5 mg% for corn), 5′-uridylic acid, and 5′-cytidylic acid were detected, while 5′-inosinic acid and 5′-guanylic acid were below the limit of measurement, as shown in Table 9. The taste of umami of vegetables mainly derives from the combination of L-glutamate and 5′-adenylic acid, which shows a weak synergistic effect with L-glutamate.

In mushrooms, as shown in Table 10, the order of qualitative amounts of nucleotides are as follows: 5′-adenylic acid > 5′-guanylic acid > 5′-uridylic acid, and 5′-inosinic acid was not detected. The 5′-guanylic acid with L-glutamate is regarded as the main umami component in mushrooms.

Table 9. Nucleotide Contents of Vegetables

Vegetable	5′-AMP	5′-IMP	5′-GMP	5′-UMP	5′-CMP
Asparagus	3.8	—	±	1.9	1.9
Welsh onion	0.9	—	—	0.4	—
Lettuce	0.9	±	±	0.5	—
Tomato	10.4	—	—	2.2	0.5
Field pea	1.9	—	—	1.3	±
Cucumber	0.5	—	—	0.6	±
Japanese radish	1.3	±	—	1.4	±
Onion	0.8	±	—	0.5	±
Bamboo shoot	1.1	—	—	1.3	0.5

Note: Content, mg %.

Table 10. Nucleotide Contents of Mushrooms

Mushroom	5′-AMP	5′-IMP	5′-GMP	5′-UMP	5′-CMP
Shiitake, *Cortinellus shutake*	154.9	0	70.1	37.6	29.4
Dried shutake	106.9	0	146.7	111.2	55.6
Mushuroom	11.3	0	—	6.4	—
Dried mushroom	167.7	0	—	59.7	—
Matsutake, *Armillarid matsutake*	99.3	0	64.6	65.2	35.6
Truffle	13.9	0	5.8	6.8	4.2
Hatsutake, *Latarius hatsutake*	51.4	0	58.1	41.5	7.4
Fly agaric, *Amanitamuscavia*	0.2	0	0	1.6	0.3

Note: Content: mg %.

PROPERTIES OF NUCLEIC ACIDS

Chemical Properties

Deamination Reaction. This reaction is the most important one with regard to production of the seasoning; it converts the amino bond to a hydroxyl bond on the purine base via nitrous acid, which may occur at any stage in nucleotides, nucleosides, and bases. Inosinic acid is produced by this process from adenylic acid (19).

Acylation Reaction. The hydroxyl bond on the sugar site of nucleosides has the ability of acylation. The order of the reaction ability is as follows: 5′-site > 3′-site > 2′-site.

When reacted with $POCl_3$ a product containing a mixture of three kinds of monophosphate esters was obtained; although the ratio of these products changes with the mole-ratio of water, no selective condition for 5′-phosphate was found.

Hydrolysis by Acid or Alkali. Both pyrimidine bases and purine bases are relatively resistant to the attack of acid or alkali under ordinary conditions, except that those with an amino group have a tendency to convert into a hydroxyl group when reacted with acid. Any *N*-glycoside bond of a nucleoside is relatively stable under alkali conditions, but not under acid conditions. The *N*-glycoside of pyrimidine-riboside is, contrarily, stable under acid conditions, which is related to the existence of the double bond between the 4- and 5-positions of the pyrimidine, for when this double bond is cleaved by an addition of hydrogen or bromide, *N*-glycoside is easily hydrolyzed with acid. The *N*-glycoside of a purine-riboside is easily hydrolyzed by acid as in the case of ordinary *N*-glycoside, but in the case of reaction of nitrous acid to adenosine or guanosine, no change in the site of the *N*-glycoside bond occurs, these two are converted to inosine or xanthosine, respectively. On the other hand, the *N*-glycoside of purine-deoxyriboside is extremely unstable under the reaction of nitrous acid.

In acid or alkali, the phosphate bond, conversely to the *N*-glycoside bond, is easily hydrolyzed by alkali and comparatively resistant to acid. Hence, when nucleotide was treated with alkali, both nucleoside and phosphoric acid were produced; with acid, on the other hand, base and pentose phosphoric acid ester were produced. But under conditions in the vicinity of pH 4, it is reported that the cleavage of a P=O bond occurred by a quite different mechanism.

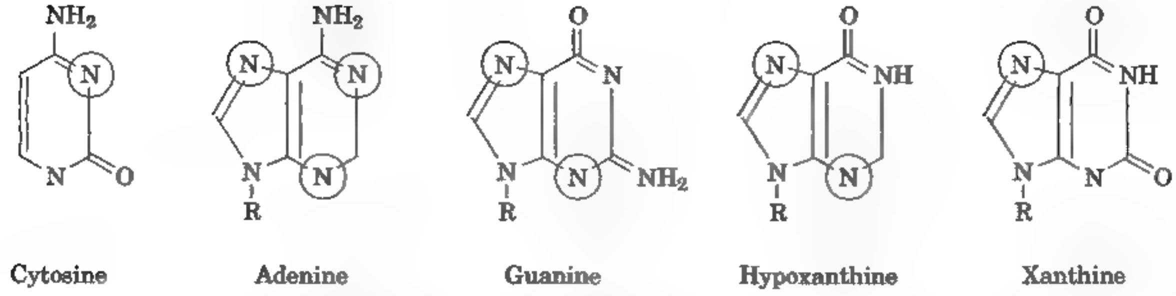
DNA is stable with alkali but is unstable with acid, as mentioned earlier; the purine base is released by a cleavage of the *N*-glycoside bond of purine-nucleotide sites. RNA is easily hydrolyzed both with alkali and with acid; when in 1 N alkali solution for one day at room temperature, RNA decomposed to the mixture of mononucleotide and 2′- and 3′-nucleotide. When in 1 N HCl for 1 h at 100°C, RNA decomposed qualitatively into base, phosphoric acid, and ribose. Nevertheless, it is impossible to obtain 5′-nucleotides by using a reaction of the acid or alkali. For this purpose, an enzymatic hydrolysis method is utilized to obtain 5′-nucleotide from RNA.

Physical Properties

Tautomerism of the Bases. The pyrimidine base has two OH bonds that exist tautomerically (20), in the enol and lactam forms, respectively. Both in the solid state and in solution, pyrimidine is found to exist in the lactam form, as found from the IR spectrum (solid state) and the UV absorption spectrum (in solution) compared with relative compounds. Hydrogen in the NH= bond, which is located at the 3-position of pyrimidine, reacted with pentose, which constitutes a nucleoside. The ionic form of these pyrimidine bases changes with changing pH or solvent as cationic form (NH^+ at the 3-position of the ring), anionic form (=O at the 4-position, changeable to an $-OH^-$ bond), which shows the same amphoteric substance as amino acids. A purine base has both $OH^- =$ and $NH_2^- =$ bonds in the ring and shows the same both tautomerism and amphoteric character that a pyrimidine base does—lactam form for oxypurine (inosine, guanine) and amino form for aminopurine (adenine).

Nucleoside is also an amphoteric substance. No substantial change of physical properties is observed, except for the optical rotation caused by the induction of pentose, which changes with changes in pH in solution.

Localization of the Attachment and Detachment of Protons in Nucleosides and Nucleotides. It is natural to expect that a proton would be attached to those atoms in the molecule on which the highest net negative charge due to π electrons is concentrated, to the nitrogen atoms forming the two σ bonds. The possible sites of protonation (shown inside the circle in Fig. 9) in the predominant tautomeric forms of the bases are N3 in cytosine; N1, N3, and N7 in adenine; N3 and N7 in guanine and hypoxanthine; and N7 in xanthine. From the theoretical calculation, which takes into account



Cytosine Adenine Guanine Hypoxanthine Xanthine

Figure 9. Chemical structure of various nucleosides; R denotes a hydrogen atom or various radicals

the lone pair electrons, the position of the attachment of the proton is predicted to be the N1 position of adenine and the N7 of guanine and hypoxanthine. The results of X-ray structural analysis confirm the correctness of these conclusions regarding the site of proton attachment to the basis moieties of crystalline protonated bases, nucleosides, and nucleotides. On the basis of the UV spectra of bases, the location of protons in the ionized compounds can be established precisely.

Arguments in favor of protonation of adenosine at N1 and of guanosine at N7, as well as evidence of the protonation of cytidine at N3, at least in nonaqueous solvents, are given by the results of NMR spectroscopy (21).

Ionization Constants of the Bases of Nucleic Acids. Ionization constants are expressed in the form $pK_a = -\log K_a$.

Some of the bases of nucleic acids possess strong basic properties and are protonated in a weakly acidic medium but are deprotonated only in a strongly alkaline medium; other bases are weak acids and, although they form anions in a weakly alkaline medium, they are protonated only in a strongly acid medium. The first group includes cytosine and adenine; the values of pK_a are associated with

$$\begin{aligned} &BH^+ \leftrightarrow B + H^+ \\ &K = (B)(H^+)/(BH^+) \end{aligned} \tag{1}$$

The second group includes thymine and uracil; the values of pK_a are

$$\begin{aligned} &BH \leftrightarrow B^- + H^+ \\ &K = (B^-)(H^+)/(BH) \end{aligned} \tag{2}$$

Hypoxanthine, xanthine, and guanine show an intermediate position; they are protonated at comparatively high pH values for these compounds in accordance with equation 1 and are deprotonated in accordance with equation 2 at fairly low pH values in the alkaline region. Accordingly, their acid–base properties are described by two values of pK_a. Phosphate groups in nucleotides exert an appreciable influence on the pK_a value of the base. Values of the corresponding ionization constants pK_a for base, nucleosides, and nucleotides are given in Table 11.

The reason for the increase in pK'_a in series observed—nucleoside < nucleoside-2′, and 3′-phosphates < nucleoside-5′-phosphate—is evidently interaction of the ionized base with the phosphate group of the nucleotide.

It can be concluded from the conformation of ribose and deoxyribose that the possibility of such interaction is particularly great in the case of nucleoside-5′-phosphates, the phosphates groups of which may be in spatial proximity to the ring of the base. On dissociation of uracil derivatives, interaction between the negative charge on the ionized base and the negative charges of the phosphate group must lead to a decrease in the stability of the ionized form of the base and the shift of equilibrium toward the neutral form; pK'_a must increase in this case (22). These pK'_a values are utilized for the separation of these bases by ion exchange chromatography or electrophoresis.

Characteristics of Inosinic Acid and Guanylic Acid

Regarding 5′-nucleotides, both inosinic acid and guanylic acid are used as umami seasoning. The characteristics of these substances are summarized here (23).

Inosinic Acid. The common forms are 5′-inosinic acid, inosine 5′-phosphate, and inosine 5′-monophosphoric acid (5′-IMP). Molecular formula: $C_{10}H_{13}O_8N_4P$; molecular weight 348.2; C: 34.9 (%), H: 3.76 (%), O: 36.76 (%), N: 16.09 (%), P: 8.90 (%).

5′-IMP, which was the first nucleotide discovered by von Liebig in 1847, was isolated from the broth extracted from muscle as a salt of barium. It is said that von Liebig recognized that this inosinic acid related to the taste of the meat extract broth. In 1913, 5′-inosinic acid histidine salt was isolated from the extract of dried bonito as a umami component by Kodama. Later, the contribution of histidine to the umami taste was denied.

Inosinic acid, which is produced by a conversion of 5′-AMP through enzymatic deamination reaction, is found in the muscle of any kind of animals. In the metabolic pathway of nucleotides of microorganisms, 5′-IMP is produced first and then is converted to 5′-AMP by an amination reaction, and then proceed to 5′-XMP by oxidation with the aid of NAD; it then goes to 5′-GMP by a second amination reaction. Hence, 5′-IMP in microorganisms was observed when any enzymatic reactions in the pathway were blocked.

Table 11. pK′ₐ Values for Bases, Deoxyribonucleosides, and Ribonucleosides

				Nucleotides				
Derivatives	Base	Deoxyribonucleosides	Ribonucleosides	2′-Phosphate	2′(3′)-Phosphate	3′-Phosphate	5′-Phosphate	2′,3′-Cyclic phosphate
Uracil	9.5	9.3	9.25		9.96		10.06	9.47
Cytosine	4.46	4.3	4.1		4.43		4.54	4.12
Thymime	9.9	9.8	9.68	—		—	10.47	—
Guanine	—	9.33	9.22	9.87		9.84	10.00	—
Hypoxanthine	8.94	—	8.75	—		—	9.62	—
Xanthine	7.53	—	5.50					
Adenine	4.1	3 8	3.6	3.81		3.70	3.88	—

Note Values determined spectrotometrically at 20°C.

For base and deoxyribonucleosides and ribonucleoside of guanine and adnine in solution of ionic strength = 1; in other cases in 0.1 M glysine or 0 025 M acetate buffers For nucleotides, at zero ionic strength when pK'_a for deoxyguanosine was 9.50, pK'_a for deoxyguanosine 5′-phosphate was 10.0.

5′-IMP Characteristics

When condensed to the 5′-IMP solution, transparent syrup was obtained; this syrup, dried with 98% H_2SO_4, changed to a glassy solid.

Easily soluble in water; amorphous sediment was obtained with alcohol; sparingly soluble in organic solvent.

$pK_1 = 1.54$, $pK_2 = 6.04$, $pK_3 = 8.88$, acidic in solution; several kinds of metallic salts are formed; metallic salts with NH_4, Na, and K are transparent crystals.

Specific rotation in neutral or acidic solution; $[\alpha] = 18.5$ (0.1 N HCl solution). UV absorption spectrum changes with pH in solution; maximum wavelength is 248.5 nm at pH 6.0; molar absorbency coefficient: $\epsilon = 12.2 \times 10^3$.

In acidic solution, when heated it is hydrolyzed to ribose-5′-phosphoric acid and hypoxanthine; in alkali solution, when heated, phosphoric acid is released and inosine is produced.

Sodium Salt of Inosinic Acid

5′-IMP, when neutralized with 2 moles of NaOH and then crystallized, produces Na_2-5′-IMP-7.5H_2O.

These crystals are usually used as umami seasoning with L-glutamate.

Crystal group: orthorhombic.

Chemical formula: $C_{10}H_{11}N_4O_8PNa_2 \cdot 7.5H_2O$; molecular weight 527.32; C = 22.4 (%), H = 2.07 (%), N = 10.45 (%), O = 23.87 (%), P = 5.79 (%), Na = 8.58 (%).

From X-ray diffraction analysis: orthorhombic; space group $P2_1\ 2_1\ 2_1$, a = 11.63 Å, b = 21.84 Å, and c = 8.704 Å; Crystal density: 1.6063 g/cm³; Z = 4.

Limited humidity at 20°C is more than 90% (less hygroscopy).

Easily soluble in water; about 24 g/p 100 mL in water; in acid (such as vinegar) about 30 g/100 mL.

Threshold value of umami is 0.025% in solution; thermal stability with a change of pH is summarized as follows and shows that Na_2 5′-IMP salts are stable for thermal and pH conditions in the range of ordinary cuisine conditions.

Thermal stability			Residual ratio (%)	
pH	Temp.	Initial con.(g/L)	After 1 h	2 h
2.0	100	2.5	55.5	43.2
5.6	100	2.5	94.9	92.8
7.0	110	2.1	97.0	92.8
9.3	100	2.5	96.3	94.3

K and NH_4 salts of Inosinic Acid

Two moles ion are combined with 5′-IMP, similar to Na salt.

5′-Guanylic Acid. Guanosine 5′-monophosphate (GMP); chemical formula $C_{10}H_{14}O_8N_5P$; molecular weight 362.5; C: 33.06 (%), H: 3.88 (%), O: 35.24 (%), N: 19.28 (%), P: 8.53 (%).

Contrary to 5′-IMP, almost no distribution of 5′-GMP is reported in meats. Shiitake mushrooms contain 0.1 to 0.2% of 5′-GMP (as in dry base). In 1961 Kuninaka found that 5′-GMP was the third umami ingredient extracted from shiitake mushrooms.

Characteristics

Fine transparent needle crystals and no hydrate attached, decomposed at 190 to 200°C.

Sparingly soluble in cold water; comparatively strong acidic compound. Dissociation constants as follows: $pK_1 = 0.8$ (phosphoric acid), $pK_2 = 2.4$ (amino bond), $pK_3 = 6.1$ (phosphoric acid), $pK_4 = 9.4$ (enol).

Specific rotation is levo in solution.

UV spectrum changes with pH; at pH 2, maximum absorbency wavelength is 256 nm ($\epsilon = 12.3 \times 103$), and minimum absorbency wavelength is 228 nm ($\epsilon = 2.5 \times 103$), 261 nm is an isobestic point ($\epsilon = 11.6 \times 103$).

Sodium Salt of Guanylic Acid

With 2 moles of Na, 5′-GMP results in Na_2-5′-GMP $\cdot$ 8H_2O. This salt is difficult to obtain as a crystal form from aqueous solution and tends to white powder.

From the X-ray analysis: orthorhombic; space group $P2_12_12_1$; a = 21.33 Å, b − 21.18 Å, and c − 9.05 Å; Crystal density: 1.643 g/cm³; Z = 8.

A little bit of hygroscopy (to the extent of 20 ~ 30% of water adsorbed), but no deliquescence observed.

Easily soluble in water (about 25 g/100 mL in water at 20°C); sparingly soluble in alcohol and acetone.

Threshold value for umami: 0.0125%, two- to threefold the synergistic effect of Na_2IMP with L-glutamate reported.

Industrial Production

The industrial production of IMP and GMP was started in 1961 by the enzymatic method; the cleavage of RNA by 5′-nuclease was produced by *Penicillium crysogenum* (24) and *Streptomyces aureus* (25).

Enzymatic Degradation of RNA. RNA distributed mainly in the cytoplasm of cells accounted for about 5 to 10% of the total in plants and animals, with only a limited amount in the nucleus. Yeast, especially, contains abundant, RNA—more than 10% on a dry basis—and a limited amount of DNA.

The separation of yeast is performed by centrifugation after the fermentation using cane molasses or pulp waste liquor as a carbon source. RNA can be extracted from yeast with hot sodium chloride solution and precipitated by acidifying the extract. The precipitate is neutralized and used for preparing 5′-nucleotides. An aqueous solution of yeast RNA is incubated with the heat-treated crude enzyme 5′-nuclease P1 (26). After incubation, the four 5′-nucleotides formed are separated by means of anion exchange resin column chromatography and purified. AMP is converted to IMP with *Aspergillus* adenyl deaminase.

Fermentation Method. Following the successful industrial production of amino acids by fermentation, extensive research was performed to establish the fermentation method for nucleosides and nucleotides. However, several different characteristics of amino acid fermentation are as follows: (*1*) in the metabolic pathway of nucleotides, there are both de novo synthesis and salvage synthesis, and moreover, mutual transformations are involved in purine nucleotide biosynthesis; (*2*) enzymes capable of degrading nucleotides to nucleosides, and to base and phosphoric acid, are widely distributed in microorganisms; and (*3*) nucleotides produced in a cell have less permeability through the cell membrane and do not easily secrete across the permeability barrier into broth.

For IMP, several methods are reported to overcome the preceding problems: (*1*) a direct fermentation method using a mutant; (*2*) a semifermentation method in which inosine, which accumulates more easily in broth, than IMP, was fermented by the fermentation method, to which phosphoric acid was added with a chemical or enzymatic method; (*3*) hypoxanthine added as a precursor was transformed into IMP by the ability of salvage synthesis of the microorganism; and (*4*) adenine (adenosine obtained by a chemical or fermentation method) was transformed by a chemical or enzymatic method into IMP. Methods (*1*) and (*2*) are regarded as more economical and were adopted in industrial production in line with enzymatic degradation of RNA.

Metabolic Pathway. The metabolic pathway of the nucleotide is shown in Figure 10 (27).

Regulation of Enzymatic Activity. PRPP-amidotransferase is an important initial enzyme for AMP, and GMP synthesis is specifically completely inhibited by AMP and ADP, but not by ATP, IMP, and GDP. SAMP (succino AMP)-lyase, which also takes part in the synthesis of AMP, and GMP are strongly inhibited by AMP and antagonistically inhibited by the substrate SAMP. IMP-dehydrogenase, the enzyme related to the IMP-to-GMP pathway, is strongly inhibited by the final product, GMP, and especially strongly by intermediate XMP as well. The enzyme SAMP-synthetase, related to the pathway from IMP to AMP, is strongly inhibited by AMP, followed by ADP and then GDP, but not by ATP and GMP (28,29).

Regulation of Enzyme Production. PRP-amidotransferase, IMP-trasformylase, and SAMP-lyase are released from repression under the conditions of deficiency of purinenucleotides and show high levels of enzyme activity but are repressed completely by addition of either adenine derivatives or guanine derivatives (see Fig. 11). IMP-dehydrogenase, which is important for the synthesis of GMP, is repressed completely by guanine derivatives, but not by adenine derivatives (30).

Bacillus subtilis (31) which has a characteristic high activity both of phosphatase and of 5′-nucleotidase, seems to be appropriate for the production of nucleoside; on the other hand, *Brevibacterium ammoniagenes* (32), having a phosphatase with low enzymatic activity is appropriate for the production of nucleotide.

Production of Nucleoside. For effective secretion of nucleoside through the cell membrane, the following properties seem to be necessary (33): (*1*) the activity of 5′-nucleotidase or phosphatase that catalyzes the cleavage of nucleotide should be absent or be at a very low level; (*2*) the regulation of enzyme involved in nucleotide formation, such as PRPP amidotransferase, by AMP and related nucleotides should be released, and (*3*) both SAMP and GMP reductase of IMP dehydrogenase should be lacking or present at a very low level.

Inosine. The appropriate strains for the accumulation of inosine are *B. subtilis* and *B. ammoniagenes*.

The adenine-requiring strain of *B. subtilis* was used as a parent strain and mutated by UV treatment to be deficient in the activity of both AMP deaminase and GMP reductase; moreover, from this strain an 8-azaguanine-resistant mutant was derived, one of the xanthine auxotrophic mutants that strengthens the ability of PRPP amidotransferase, and shows the result of accumulation of inosine at 16 to 18 g/L in broth (34)

Furuya et al. (35) derived 6-mercaptoguanine-resistant mutants from an adenine-leaky, IMP-producing strain of *B. ammoniagenes*. Furthermore, they induced the mutant that is devoid of IMP dehydrogenase and requires guanine for growth. Addition of the guanine-requiring character was most effective for accumulation of inosine. Under optimal conditions, this strain shows high accumulation of inosine: about 30 g/L from 17% of inverted cane molasses, which is equivalent to more than a 17% yield of inosine based on the consumed sugar.

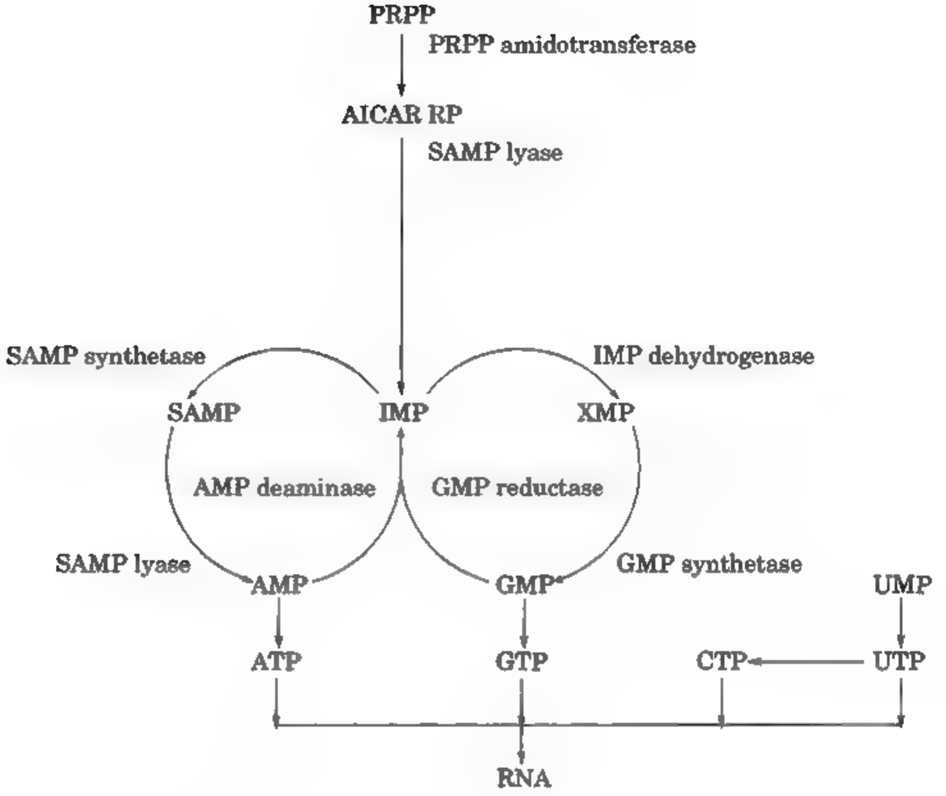

PRPP
PRPP amidotransferase
AICAR RP
SAMP lyase
SAMP synthetase
IMP dehydrogenase
SAMP
IMP
XMP
AMP deaminase
GMP reductase
SAMP lyase
GMP synthetase
AMP
GMP
UMP
ATP
GTP
CTP
UTP
RNA

Figure 10. Metabolic pathway of nucleotide by de novo synthesis.

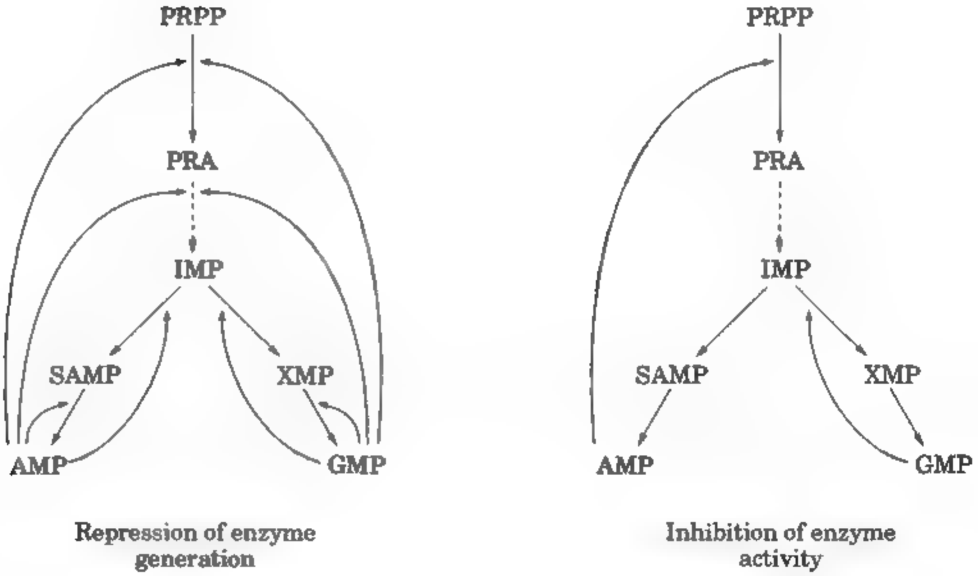

PRPP
PRA
IMP
SAMP
XMP
AMP
GMP
Repression of enzyme generation
PRPP
PRA
IMP
SAMP
XMP
AMP
GMP
Inhibition of enzyme activity

Figure 11. Regulation of metabolic pathway.

Guanosine. From *B. subtilis*, which is capable of producing inosine, in which PRPP amidotransferase IMP dehydrogenase (IMP → XMP), and GMP synthetase (XMP → GMP) are inhibited or repressed by GMP and guanine, methionine sulfoxide–, psicofuranine-, and decoyinine-resistant mutants were derived (36). The key to increasing guanosine is the loss of repression and feedback inhibition of IMP dehydrogenase, GMP synthetase, SAMP lyase, and PRPP amidotransferase. This strain showed the accumulation of 16 g/L of guanine and both hypoxanthine and inosine in the culture, glucose 80 g/L and L-histidine 0.3 g/L (72 h at 34°C).

Phosphorylation by the Chemical Method. For nucleosides, direct reaction with $POCl_3$ resulted in phosphorylation of not only a 5′-OH bond but also 2′- and 3′ OH bonds, simultaneously. So it is difficult to selectively obtain 5′-nucleotide. Obtaining 5′-nucleotide, 2′- and 3′-OH in the ribose of nucleosides was protected previously by acetone or benzaldehyde to form an isopropiridene bond or benziridene bond; the product was then reacted with $POCl_3$ to substitute the phosphate bond for 5′-OH on the riboside, at lower temperature (0 to 5 °C), and then a removal reaction of protected groups for the 2′- and 3′-bonds on the riboside was performed. The improved process is reported as follows: in the appropriate solvent with a small amount of water, $POCl_3$ was added directly to nucleoside; the protection of 2′- and 3′-OH bonds and 5′-phosphorylation occurred, simultaneously, and a highly pure 5′-nucleotide solution was obtained. To this solution, a large amount of water was added to stop the excess phosphorylation reaction, and then sodium hydroxide was added to crystallized the disodium 5′-nucleotide, producing, for example, Na_2GMP and Na_2IMP crystals.

Direct Fermentation

5′-Inosinic Acid. The adenine-leaky auxotrophic strain of *B. ammoniagenes* showed the ability to accumulate IMP; 12.8 g/L in broth was obtained (37), which is the first report exhibiting the possibility of direct fermentation of IMP. This strain showed a high sensitivity for Mn^{2+} ions and optimum concentration; an extremely low and narrow range of value (10 to 20 μg/L; and a change in cell shape and a decrease in vital cell number when it contained Mn^{2+} at a concentration suitable for IMP accumulation during cultivation. By adding the nitrosoguanizine-resistance ability, this strain mutated to Mn^{2+}-nonsensitive, and no change in cell shape was observed during cultivation, which accompanied the change in the mechanism of leakage of IMP, due to the improvement of the permeability of IMP through cell membrane associated with inhibition of the conjugated decomposition system (IMP → Hx) by glucose in the cell membrane.

These results lead to the conclusion that the leakage of IMP through the cell membrane attributes to the energy-dependent reaction.

5′-Guanylic Acid. So far GMP is not successfully produced by the direct fermentation method, mainly because of the intrinsic problem concerning the metabolic pathway; that is, no base corresponding to GMP for salvage synthesis exists, such as hypoxanthine, xanthine for IMP, or guanine for XMP (xanthosine monophosphate). Therefore, it is impossible to obtain the mutant-blocked pathway to GMP, and GMP itself is a cell-constituted component, different from IMP and XMP; then there exists a strict barrier in the cell membrane that inhibits GMP from passing through (38).

From the industrial viewpoint, the following three-step fermentation method is employed. First, XMP was produced by using the mutant of *B. ammoniagenes*, and then it was transformed into GMP by the enzymatic method using the other mutant of *B. ammoniagenes* (39).

1. *XMP fermentation*. In the case of XMP, *B. ammoniagenes* mutated to have both an adenine requirement (leakage type) and a guanine requirement. Nucleotide activity weakened, showing high productivity of XMP; also, no cell membrane barrier existed for XMP, which is different from IMP.
2. *Enzyme for conversion of XMP to GMP*. The nucleotide weakened *B. ammoniagenes* with docoynine resistance, which strengthens the activity of the GMP synthetase that was obtained. This strain showed an increased ability to convert XMP to GMP, with no cleavage of GMP and XMP. The cell membrane permeability of XMP and GMP was improved by adding a cationic surface-active agent, polyethylene stearylamine (40).
3. *Conjugated reaction with ATP energy system*. The XMP aminase reaction is a one-step reaction for conversion of XMP to GMP, conjugated with energy regenerated by the conversion of ATP to AMP and pyrophosphoric acid (PPi), which required an indispensable cofactor, the Mg^{2+} ion:

$$\mathrm{XMP + NH_3 + ATP \rightarrow GMP + AMP + Ppi\ XMP\ aminase\ (Mg^{2+})}$$

Brevibacterium sp. treated with an active surfactant have an increased ability to synthesize ATP through the Krebs cycle from glucose. By using this process, in place of ATP, glucose was used as an energy supply source; conjugated with this process, the conversion of XMP to GMP was achieved economically. The enzymatic process was as follows:

$$\mathrm{PPi \rightarrow 2Pi\ (pyrophosphatase)}$$

$$\mathrm{AMP + ATP \rightarrow 2ADP\ (AMP\ kinase)}$$

$$\mathrm{ADP + Pi + Glucose \rightarrow ATP\ (ATP\text{-}generating\ enzyme\ system)}$$

These enzymes, contained in a cell, are must be regulated appropriately to prevent the subreaction from occurring. Controlling the temperature to repress the activity of nucleotide monophosphate kinase and the concentration of phosphate ion ($PO^{3-}{}_4$) increases the ability of the ATP-generating system. If Mg^{2+} and PPi form insoluble magnesium pyrophosphate (Mg_2PPi), the preceding reactions cannot proceed smoothly. Phytic acid, a chelating agent, was able to prevent the formation of this Mg_2PPi.

When using purified XMP as raw material, overall conversion to GMP was more than 80% after 22 h (41).

Increasing the activity of XMP aminase is expected to reduce the number of cells required for conversion when whole cells are used as the enzyme sources, while reducing to a minimum the degradation of products and by-products GDP and GTP. The proliferation of the XMP aminase strain of *Escherichia coli* using genetic engineering techniques was attempted, which involved the subcloning of the *guaA* gene for XMP aminase into pBR322, followed by the construction of a plasmid utilizing the trp promoter. The amount of XMP aminase produced by the strain carrying the *gauA* gene accounted for 10% of total cellular protein, and the *E. coli* K294/XAR33 strain showed an increase in activity of about 80 times compared with *E. coli* K294. The conversion ratio from XMP to GMP was more than 90% after 7 h (42).

BIBLIOGRAPHY

1. K. Ikeda, *J. Tokyo Chem. Soc. Japan* **30**, 820 (1909).
2. S. Kodama, *J. Tokyo Chem. Soc. Japan* **34**, 751 (1913).

3. A. Kuninaka, M. Kibi, and K. Sakaguchi, *Food Technol.* **18**, 287 (1964).
4. A. Kuninaka, *J. Agric. Chem. Soc. Japan* **34**, 487 (1960).
5. S. Yamaguchi et al., *J. Food Sci.* **36**, 846 (1971).
6. S. Ikeda, *New Food Ind.* **7**, 41 (1965).
7. A. Adachi, M. Funakoshi, and Y. Kawamura, *Olfaction Taste* **2**, 411 (1967).
8. M. Sato, S. Yamashita, and H. Ogawa, *Olfaction Taste* **2**, 399 (1967).
9. R. H. Cagan, in M. R. Kare and O. Maller, eds., *The Chemical Sense and Nutrition*, Academic Press, New York, 1977, pp. 175–203.
10. K. Torii and R. H. Cagan, *Biochim. Biophys. Acta* **627**, 313 (1980).
11. A. Kuninaka, in W. H. Schluz, E. A. Day, and L. M. Libbey, eds., *The Chemistry and Physiology of Flavor*, AVI, Westport, Conn., 1967, pp. 515–535.
12. J. H. Hara, in J. H. Hara, ed., *Chemoreception in Fishes*, Elsevier, Amsterdam, The Netherlands, 1982, pp. 135–180.
13. E. Chargaff, *Essay on Nucleic Acids*, Elsevier, Amsterdam, The Netherlands, 1963.
14. J. D. Watson and F. H. C. Crick, *Proc. R. Soc. London, Ser. A* **223**, 80 (1954).
15. J. D. Watson and F. H. C. Crick, *Nature* **171**, 737 (1953).
16. T. Shimanouchi, M. Tsuboi, and T. Kyogoku, *Biochim. Biophys. Acta* **15**, 1 (1962).
17. A. Szent-Gyorgy, *Chemistry of Muscular and Contraction*, Academic Press, New York, 1951.
18. N. R. Jones and J. Murray, *Biochem. J.* **77**, 567 (1960).
19. Seasoning: Its Science and Production, in R. Takada, ed., Koseido, Tokyo, Japan, 1966, pp. 73–86.
20. N. K. Kochetkov and E. I. Budovskii, eds., *Organic Chemistry of Nucleic Acids*, Part A, L. Todd and D. M. Brown, trans., eds., Plenum, London and New York, 1971, pp. 134–000.
21. B. Pullman and A. Pullman, *Quantum Biochemistry*, Wiley, New York, 1963.
22. P. O. P. Ts'o, S. A. Rappaport, and F. J. Bollum, *Biochemistry* **5**, 4153 (1966).
23. A. Sumida, *New Food Ind.* **6**, 78–81 (1964).
24. A. Kuninaka et al., *Bull. Agric. Chem.* **23**, 239 (1959).
25. K. Ogata et al., *Agree. Biol. Chem.* **27**, 110 (1963).
26. A. Kuninaka, in K. Ogata et al., eds., *Microbial Production of Nucleic Acid Substances*, Kodansha, Tokyo, Japan, 1976, pp. 75–86.
27. J. M. Buchanan and S. C. Hartman, in F. F. Nord, ed., *Advances in Enzymology*, Vol. 21, Interscience, New York, 1959.
28. K. Ishii and I. Shiio, *J. Biochem.* (Tokyo) **63**, 661 (1968).
29. K. Ishii and I. Shiio, *J. Biochem.* (Tokyo) **68**, 171 (1970).
30. H. Nishikawa, H. Momose, and I. Shiio, *J. Biochem.* (Tokyo) **62**, 92 (1967).
31. I. Shiio and H. Ozaki, *J. Biochem.* (Tokyo) **83**, 409 (1978).
32. M. Misawa, T. Nara, and K. Nakayama, *J. Agric. Biol. Chem. Japan* **38**, 167 (1964).
33. H. Enei, in T. Tochikura, ed., *New Biotechnology*, Japan Bioindustry Association, Tokyo, Japan, 1988, pp. 135–147.
34. H. Matsui et al., *Agric. Biol. Chem.* **46**, 2347 (1982).
35. A. Furuya, S. Abe, and S. Kinoshita, *Appl. Microbiol.* **20**, 263 (1970).
36. H. Matsui, *J. Agric. Biol. Chem. Japan* **58**, 175 (1984).
37. A. Furuya, S. Abe, and S. Kinoshita, *Appl. Microbiol.* **16**, 981 (1968).
38. T. Nara et al., *Agric. Biol. Chem.* **33**, 739 (1969).
39. M. Misawa et al., *Agric. Biol. Chem.* **33**, 370 (1969).
40. A. Furuya, S. Abe, and S. Kinoshita, *Biotechnol. Bioeng.* **13**, 229 (1971).
41. T. Fujio, K. Kotani, and A. Furuya, *J. Ferment. Technol.* **62**, 131 (1984).
42. A. Maruyama, T. Fujio, and S. Teshiba, *Agric. Biol. Chem.* **50**, 1879 (1986).

GENERAL REFERENCES

E. Chargaff and J. N. Davidson, eds., *The Nucleic Acids, Chemistry and Biology*, Vol. 1, Academic Press, New York, 1955.

D. O. Jordan, ed., *The Chemistry of Nucleic Acids*, Butterworth, London, 1960.

A. Kuninaka, in H. Pape and H.-J. Rehm, eds., *Nucleic Acids, Nucleotides, and Related Compounds*, Biotechnology, Vol. 4, VCH Verlargsgesellschaft mbH, Weinheim, Germany, 1986, pp. 71–114.

J. Rehmann, in *Encyclopedia of Chemical Engineering*, Vol. 17, *Nucleic Acids*, Wiley, 1982, pp. 507–543.

R. B. Setlow and E. C. Pollard, eds., *Molecular Biophysics*, Addison-Wesley, London, 1962.

TETSUYA KAWAKITA
Ajinomoto Company, Inc.
Kawasaki, Japan

NUTRITIONAL LABELING

Americans love their food. Their interest in all aspects of food has been steadily growing since the 1950s. Public demand for more and more information on food-product labels has been growing right along with this interest. Today, labels on food packages provide a wealth of facts about the products inside. It wasn't always this way, however. This chapter summarizes the development of food labeling in the United States and covers key elements of current labeling requirements. Emphasis is on the requirements of the U.S. Food and Drug Administration (FDA), which regulates the labeling of most packaged foods, except for meat and poultry products. These are the responsibility of the U.S. Department of Agriculture (USDA). Differences between what FDA and USDA require on product labels are minor.

SOME KEY MILESTONES IN FOOD LABELING

Before 1906, there were no requirements to include any information at all on food labels. In that year, the Federal Food and Drugs Act and the Federal Meat Inspection Act were first enacted. These groundbreaking laws formed the base for regular additions and refinements, right up to today's detailed labeling requirements.

The two 1906 acts authorized the federal government to regulate the safety and quality of food and assigned the responsibility for doing so to the U.S. Department of Ag-

3. A. Kuninaka, M. Kibi, and K. Sakaguchi, *Food Technol.* **18**, 287 (1964).
4. A. Kuninaka, *J. Agric. Chem. Soc. Japan* **34**, 487 (1960).
5. S. Yamaguchi et al., *J. Food Sci.* **36**, 846 (1971).
6. S. Ikeda, *New Food Ind.* **7**, 41 (1965).
7. A. Adachi, M. Funakoshi, and Y. Kawamura, *Olfaction Taste* **2**, 411 (1967).
8. M. Sato, S. Yamashita, and H. Ogawa, *Olfaction Taste* **2**, 399 (1967).
9. R. H. Cagan, in M. R. Kare and O. Maller, eds., *The Chemical Sense and Nutrition*, Academic Press, New York, 1977, pp. 175–203.
10. K. Torii and R. H. Cagan, *Biochim. Biophys. Acta* **627**, 313 (1980).
11. A. Kuninaka, in W. H. Schluz, E. A. Day, and L. M. Libbey, eds., *The Chemistry and Physiology of Flavor*, AVI, Westport, Conn., 1967, pp. 515–535.
12. J. H. Hara, in J. H. Hara, ed., *Chemoreception in Fishes*, Elsevier, Amsterdam, The Netherlands, 1982, pp. 135–180.
13. E. Chargaff, *Essay on Nucleic Acids*, Elsevier, Amsterdam, The Netherlands, 1963.
14. J. D. Watson and F. H. C. Crick, *Proc. R. Soc. London, Ser. A* **223**, 80 (1954).
15. J. D. Watson and F. H. C. Crick, *Nature* **171**, 737 (1953).
16. T. Shimanouchi, M. Tsuboi, and T. Kyogoku, *Biochim. Biophys. Acta* **15**, 1 (1962).
17. A. Szent-Gyorgy, *Chemistry of Muscular and Contraction*, Academic Press, New York, 1951.
18. N. R. Jones and J. Murray, *Biochem. J.* **77**, 567 (1960).
19. Seasoning: Its Science and Production, in R. Takada, ed., Koseido, Tokyo, Japan, 1966, pp. 73–86.
20. N. K. Kochetkov and E. I. Budovskii, eds., *Organic Chemistry of Nucleic Acids*, Part A, L. Todd and D. M. Brown, trans., eds., Plenum, London and New York, 1971, pp. 134–000.
21. B. Pullman and A. Pullman, *Quantum Biochemistry*, Wiley, New York, 1963.
22. P. O. P. Ts'o, S. A. Rappaport, and F. J. Bollum, *Biochemistry* **5**, 4153 (1966).
23. A. Sumida, *New Food Ind.* **6**, 78–81 (1964).
24. A. Kuninaka et al., *Bull. Agrc. Chem.* **23**, 239 (1959).
25. K. Ogata et al., *Agree. Biol. Chem.* **27**, 110 (1963).
26. A. Kuninaka, in K. Ogata et al., eds., *Microbial Production of Nucleic Acid Substances*, Kodansha, Tokyo, Japan, 1976, pp. 75–86.
27. J. M. Buchanan and S. C. Hartman, in F. F. Nord, ed., *Advances in Enzymology*, Vol. 21, Interscience, New York, 1959.
28. K. Ishii and I. Shiio, *J. Biochem.* (Tokyo) **63**, 661 (1968).
29. K. Ishii and I. Shiio, *J. Biochem.* (Tokyo) **68**, 171 (1970).
30. H. Nishikawa, H. Momose, and I. Shiio, *J. Biochem.* (Tokyo) **62**, 92 (1967).
31. I. Shiio and H. Ozaki, *J. Biochem.* (Tokyo) **83**, 409 (1978).
32. M. Misawa, T. Nara, and K. Nakayama, *J. Agric. Biol. Chem. Japan* **38**, 167 (1964).
33. H. Enei, in T. Tochikura, ed., *New Biotechnology*, Japan Bioindustry Association, Tokyo, Japan, 1988, pp. 135–147.
34. H. Matsui et al., *Agric. Biol. Chem.* **46**, 2347 (1982).
35. A. Furuya, S. Abe, and S. Kinoshita, *Appl. Microbiol.* **20**, 263 (1970).
36. H. Matsui, *J. Agric. Biol. Chem. Japan* **58**, 175 (1984).
37. A. Furuya, S. Abe, and S. Kinoshita, *Appl. Microbiol.* **16**, 981 (1968).
38. T. Nara et al., *Agric. Biol. Chem.* **33**, 739 (1969).
39. M. Misawa et al., *Agric. Biol. Chem.* **33**, 370 (1969).
40. A. Furuya, S. Abe, and S. Kinoshita, *Biotechnol. Bioeng.* **13**, 229 (1971).
41. T. Fujio, K. Kotani, and A. Furuya, *J. Ferment. Technol.* **62**, 131 (1984).
42. A. Maruyama, T. Fujio, and S. Teshiba, *Agric. Biol. Chem.* **50**, 1879 (1986).

GENERAL REFERENCES

E. Chargaff and J. N. Davidson, eds., *The Nucleic Acids, Chemistry and Biology*, Vol. 1, Academic Press, New York, 1955.

D. O. Jordan, ed., *The Chemistry of Nucleic Acids*, Butterworth, London, 1960.

A. Kuninaka, in H. Pape and H.-J. Rehm, eds., *Nucleic Acids, Nucleotides, and Related Compounds*, Biotechnology, Vol. 4, VCH Verlagsgesellschaft mbH, Weinheim, Germany, 1986, pp. 71–114.

J. Rehmann, in *Encyclopedia of Chemical Engineering*, Vol. 17, *Nucleic Acids*, Wiley, 1982, pp. 507–543.

R. B. Setlow and E. C. Pollard, eds., *Molecular Biophysics*, Addison-Wesley, London, 1962.

TETSUYA KAWAKITA
Ajinomoto Company, Inc.
Kawasaki, Japan

NUTRITIONAL LABELING

Americans love their food. Their interest in all aspects of food has been steadily growing since the 1950s. Public demand for more and more information on food-product labels has been growing right along with this interest. Today, labels on food packages provide a wealth of facts about the products inside. It wasn't always this way, however. This chapter summarizes the development of food labeling in the United States and covers key elements of current labeling requirements. Emphasis is on the requirements of the U.S. Food and Drug Administration (FDA), which regulates the labeling of most packaged foods, except for meat and poultry products. These are the responsibility of the U.S. Department of Agriculture (USDA). Differences between what FDA and USDA require on product labels are minor.

SOME KEY MILESTONES IN FOOD LABELING

Before 1906, there were no requirements to include any information at all on food labels. In that year, the Federal Food and Drugs Act and the Federal Meat Inspection Act were first enacted. These groundbreaking laws formed the base for regular additions and refinements, right up to today's detailed labeling requirements.

The two 1906 acts authorized the federal government to regulate the safety and quality of food and assigned the responsibility for doing so to the U.S. Department of Agriculture and its Bureau of Chemistry. They became separate agencies when the Bureau of Chemistry was spun off in 1927, eventually to become the Food and Drug Administration. The USDA retained the responsibility for regulating meat and poultry products.

The 1906 acts set no detailed labeling requirements. At the time, it was expected these would be worked out based on enforcement actions taken against goods considered mislabeled. In 1913, an amendment to the 1906 acts established the first specifics by requiring labels to state the net quantity of contents in each food package. In 1938, the Federal Food, Drug and Cosmetic Act replaced the 1906 Food and Drugs Act and added the requirement that each package label state the name and address of the manufacturer or distributor. This act also required that labels list ingredients, including artificial colors, flavors, and preservatives, for most products and forbade false or misleading statements on the label. The amounts of vitamins and minerals were required only for products intended for special dietary uses.

The Fair Packaging and Labeling Act, passed in 1966, authorized and directed FDA to require more complete information on food product labels. Under this Act, FDA regulations specifically defined several key food label elements, for example, "label," "package," and "principal display panel." The regulations also established specific details as to the way the statement of identity of the food; the name and address of the manufacturer, packer, or distributor; and the net quantity of contents should be shown, such as location on the label and type style and size.

Another major milestone came in 1973 when FDA issued the first nutrition information labeling regulations, which became effective in 1975. These rules required nutrition information on the labels of only those products for which nutrition claims were made or to which nutrients were added. Many food companies took the opportunity to add nutrition information voluntarily to the labels of many products for which no claims were made, and as a result nutrition information became relatively common.

As interest in nutrition grew, especially in reducing calorie intake, FDA proposed regulations in 1978 that defined claims such as "low calorie" and "reduced calorie." They became final in 1980 after some debate. Low-calorie foods could contain only 40 cal per gram of food, whereas the calories in a serving of a reduced-calorie food had to be at least one-third lower than in a serving of the regular food. Products that met the qualifications for reduced-calorie were sold without being labeled as "imitation." Unfortunately, a loophole soon became obvious. Because the regulations did not define other terms that implied calorie reduction, a number of products that could not meet the requirements for reduced-calorie without a perceived sacrifice in taste were marketed as "light" or "lite," with relatively smaller reductions in calories. In 1984, a regulation was proposed to add sodium to the list of nutrients required to be included in the nutrition information. The rule was made final in 1985 and also defined claims such as "low sodium" (<140 mg per serving) and "sodium free" (<5 mg per serving).

A comparable regulation that defined claims about cholesterol in food products was proposed in November 1986. "Low cholesterol" was defined as <20 mg per serving, and "cholesterol free" was set at <2 mg per serving. Although this proposal was never made final, a number of food-product labels made claims based on the requirements, with FDA's official approval. Closely related claims for fat and fatty acid content were also discussed during this period but never formally proposed. Food-product labeling was about to undergo its next major revision.

THE NUTRITION LABELING AND EDUCATION ACT OF 1990

Several forces led Congress to pass the Nutrition Labeling and Education Act of 1990 (NLEA). As public interest in foods and nutrition, especially the relationship between diet and health, continued to grow, a number of food manufacturers had begun in the mid 1980s to make health and nutrition claims on certain product labels. Probably the best known of these was the claim that fiber in the diet was believed to reduce the risk of cancer. Because government regulations did not provide for this and other claims, questions and controversies arose that led to pressure on both Congress and FDA for labeling reform. In 1990, in what amounted to a race with Congress, FDA was the first to propose extensive labeling revisions. These were all superseded when, on its own, Congress passed the NLEA in October. The president signed NLEA in November 1990.

The key provisions of the NLEA, prescribing exactly what Congress wanted on food labels, are as follows:

Nutrition Labeling Required

All food products had to provide nutritional labeling, with very few exceptions. Exceptions included foods served in restaurants and food-service operations, foods containing insignificant levels of nutrients, and products from small manufacturers. Produce sold at retail, including fresh fruits, vegetables, and seafood, was expected to provide nutrition information voluntarily. Requirements would become mandatory if there was not "substantial compliance," as defined by FDA, by 30 months after the signing of the NLEA.

Government to Establish Serving Sizes

Serving sizes were to be established by government regulation, derived from amounts that were "commonly consumed" by the public. This provision was based on a perception that manufacturers often set unrealistically small serving sizes for high-fat/high-calorie products, or, conversely, unrealistically large serving sizes for foods with low levels of nutrients.

Specific Nutrient Requirements

The nutrition information had to include specific nutrients that were considered to represent major public concerns and interest. These were calories, calories from fat, total fat, saturated fat, cholesterol, sodium, total carbohydrates, sugar, dietary fiber, and protein. The door was left open for FDA to require additional nutrients on labels

Prescribed Label Format

The information was to be presented in a form the public could easily understand. In effect, this provision required the government to establish a clear and consistent format for the nutrition information.

Government to Define Which Health and Nutrition Claims Would Be Permitted

The government was required to set detailed and specific requirements for any health or nutrition claims on labels. No claim could be made that was not government-approved. NLEA further required FDA to define terms such as "free," "low," "light" or "lite," "reduced," "less," and "high."

Preemption of State Labeling Regulations

Federal labeling regulations preempted any state or local regulations with respect to nutritional labeling but not with respect to safety. This added to clarify and ease of understanding label information across the country. It also prevented individual states from setting different labeling requirements, which might result in manufacturers having a different label for each state. This provision did not apply to specific warning statements, which states could still require individually.

Expanded Ingredient Labeling

Labels had to provide more details on certain ingredients, such as listing all certified Food, Drug and Cosmetic (FD&C) artificial food colors by name. NLEA also required declaration of the amount of juice in products that contain fruit juice.

"Hammer" Deadline

NLEA set a deadline of November 8, 1992, for all regulations based on the requirements of the act to become final. Any proposals not formally finalized would automatically become final on that date. This hammer provision was designed to make sure that the responsible government agencies published the detailed rules that would be required without undue delay.

Action by FDA and USDA

NLEA officially amended the federal Food, Drug and Cosmetic Act. Technically this required only FDA to develop new regulations for packaged foods under its authority. USDA, however, also developed NLEA-based regulations for meat and poultry products that are essentially the same as those of FDA.

FDA and USDA began to harmonize the earlier proposals with NLEA requirements. Both agencies issued the first new proposals in late November 1991, a little over a year after the signing. In July 1992, FDA followed up by publishing several different format designs. USDA proposed its own slightly different formal in August. Allowing time for review of public comments, final rules were scheduled for publication early in November to meet the hammer deadline. This was delayed at the last minute due to differences of opinion between the two agencies over several issues. Agreement was reached in December, and the final rules were formally published in the *Federal Register* of January 6, 1993. But this was not quite the end. On April 2, 1993, FDA published corrections to the final rules. These were followed on August 18 by additional technical revisions based on comments from the public. At this point, the major labeling provisions based on NLEA were finally complete.

The original target date for compliance was May 1993. Because of the delays, however, FDA extended the deadline to apply to all foods labeled on or after May 8, 1994. Exceptions were meat and poultry products, for which USDA extended the compliance date to July 6, 1994.

CURRENT U.S. FOOD-PRODUCT LABELING REQUIREMENTS

Today's food-product labels in the United States incorporate the requirements of the NLEA along with those established by the earlier laws and regulations. The provisions are many and very detailed, so only key elements are covered here. The discussion focuses mainly on FDA requirements, but those of the USDA are essentially similar. There is one major difference between the two agencies. USDA must review and approve labels before they go on the package. FDA does not have the authority for prior approval.

Labeling Panels on Food Packages

Labeling regulations define two key panels that carry required information:

1. The front label panel, known as the principal display panel (PDP)
2. The information panel, usually the panel just to the right of the PDP

The location of the information panel may vary depending on the size and shape of the package, but the PDP must always be the panel that is seen by the consumer at the point of purchase, almost always the front of the package.

Required Label Information

All food-product labels are required to carry the following information:

Statement of Identity (Product Name) This is a "common or usual" name that accurately describes the product. If the product is one that is regulated by a standard of identity, such as ice cream, the name established by the standard is the common or usual name. A brand name by itself is usually not enough. Although percentage labeling is not a general requirement, certain foods that contain characterizing ingredients, for example, apples in applesauce, may be required to include the percentage of that ingredient as part of the name.

Net Quantity Statement (Net Contents) Usually, solid foods are declared by weight, whereas liquid foods are declared by volume, but there are many exceptions. The United States still uses the avoirdupois system—pounds, ounces, pints, quarts, and so on. Use of metric units is optional for the time being; however, most package labels do carry metric equivalents.

Both the statement of identity and the net quantity statement *must* appear on the PDP.

Name and Address of the Manufacturer, Packer, or Distributor If the product is not actually made by the company whose name is on the label, the company name must be qualified by terms such as "manufactured for" or "sold by."

List of Ingredients All ingredients in a food product must be listed by name in descending order of predominance by weight:

- Type of oil, such as corn, soy, or canola, must be stated.
- Flavor enhancers such as monosodium glutamate and disodium inosinate, yeast extract, and certain vegetables that are commonly used as seasonings—garlic and onion, for example—must be listed by name. They may not legally be included in a general term such as "natural flavors" or "flavorings."
- Sulfiting agents such as sulfur dioxide and potassium metabisulfite that may be used as preservatives must be listed.
- Ingredients that are contained in other ingredients must be listed individually. For example, if cheese is an ingredient in a food, it is not enough to simply declare "cheese." All the ingredients in the cheese must be listed, for example, "cheese (milk, cheese cultures, enzymes, salt)."
- It is especially important that the most common allergens be listed in the ingredient statement, whether they are added separately or as a component of another ingredient. These are peanuts, tree nuts, soy, milk and dairy products, eggs, wheat, fish, and shellfish (crustacea and mollusks).

The NLEA and associated new FDA regulations added several more specific ingredient-labeling requirements:

- All packaged food products must carry a complete list of ingredients. Exemptions from full ingredient labeling for a few products covered by standards of identity, such as macaroni products, were removed.
- All artificial colors subject to certification, FD&C colors must be listed by name, for example, Blue 2 or Red 3.
- Hydrolyzed proteins, often used to enhance savory flavors, must now be listed by source, for example, "hydrolyzed soy protein" rather than "hydrolyzed vegetable protein." This is required to inform those with allergies to specific foods. Like monosodium glutamate, hydrolyzed proteins may not be listed simply as "natural flavors."
- Labels of drinks that contain fruit juice must now state the percentage of juice.

A few types of ingredients may still be declared by general rather than specific terms:

- Spices, except those that add color such as paprika, turmeric, and saffron. They may be declared either by name or as "spice and coloring."
- Flavors may be declared simply as "natural flavor(s)" or "artificial flavor(s)" without naming each individual flavoring material. Some countries recognize a "nature-identical" category of flavorings, which are made synthetically but are chemically identical to the natural flavoring, but FDA considers these artificial.
- Colorings that do not require certification may be declared simply as "artificial color" or "color added." Many companies voluntarily declare these by name and function, for example, "beta-carotene for color," "caramel color," and "colored with beet juice."

Warning Statements Warnings are required in only a very limited number of instances, such as cautions against overheating aerosol containers, saccharine and cancer, and so on.

Nutrition Facts Nutrition labeling is required by the NLEA for nearly all packaged foods sold at retail in the United States. The Nutrition Facts box is the most noticeable addition to food labels. The format and contents of the Nutrition Facts box and related nutritional labeling requirements are discussed in detail in a separate section.

The NLEA regulations did provide for a few exemptions from the requirements for nutritional labeling:

- Foods sold by businesses with less than $500,000 in gross annual sales
- Foods served in restaurants and other establishments for immediate consumption; ready-to-eat foods
- Products sold exclusively for food service or other institutional use rather than directly to consumers. Any of these products that may end up in warehouse or club stores must carry nutrition labeling.
- Foods with insignificant amounts of nutrients, such as spices, coffee, and tea
- Fresh produce and fish, meat, and poultry
- Products in very small packages with <12 in.^{2} of total surface area available to bear labeling. These products must list an address or phone number where consumers can obtain nutrition information.
- It is very important to note the requirement that any products that make any nutrition or health claims lose their exemption and must carry nutritional labeling.
- Medical foods, infant formulas, and dietary supplements have specific labeling requirements that are different from those for foods and are not discussed here.

The company name and address, ingredient list, nutritional labeling, and any warning statements may appear on either the PDP or the information panel, but the information panel is the most common location. There must be no intervening material, such as directions, recipes, logos, or the like, between these requred items.

Figure 1, adapted from FDA's *A Food Labeling Guide*, shows a typical food label for a box of cereal, with the location of the required information on the PDP and the information panel.

Optional Label Information

Food-product labels carry much information in addition to that required by FDA or USDA. This can include pictures of the product; preparation directions; serving suggestions; recipes; sell-before, buy-before, or use-before dates; and claims for the product. Some of this optional information—health or nutrition claims, for example—may in fact trigger additional requirements.

The Universal Product Code (UPC) is an optional element that has become essential. The UPC is the familiar series of bars that stores scan at the checkout register. It is used to keep track of information such as prices and inventory. Although it is not officially required by any government agency, it is required for practical purposes on any product that is sold in today's market.

Food labels must contain all the required information in the correct places as well as all the optional information that makes a label attractive and helpful to consumers. Developing a new food label can thus present a real challenge.

NUTRITION INFORMATION BASED ON NLEA

NLEA focused on two key areas of food-product labels in the area of nutrition: how the nutrition information should be presented, and how claims should be regulated. Although nutrition information is required for most food labels, claims are optional, but making them may trigger additional requirements. Claims must be only those defined by NLEA regulations.

Nutrition Facts

According to the regulations, the nutritional values in the Nutrition Facts box are to be for the food "as packaged," that is, the product that is in the package. The regulations allow for providing nutritional values "as prepared," but this is optional.

FDA considered several approaches to how food-product labels should provide nutrition information. The final regulations followed NLEA requirements to establish what information must appear and prescribed the Nutrition Facts box as the exact format.

Figure 2 shows the Nutrition Facts box for the food label as it appears in FDA's regulations, with all the required details. This is known as the standard format.

Figure 3 is from a presentation by FDA and explains some of the considerations used to develop the Nutrition Facts box. The features were designed to help consumers apply the label information to their own diets.

Figures 2 and 3 show the conversion guide for calories per gram of fat, protein, and carbohydrate at the bottom of the Nutrition Facts box. The August 1993 amendments to the original rules made this optional, so few labels in the market actually carry it.

The serving size for each food product must be based on the established reference amounts using methods specified in the regulations. The purpose is to have consistent serving sizes for similar products, making it easier for consumers to make comparisons. Serving sizes must be expressed both in common household measures, such as teaspoons and cups, and in metric amounts, such as grams or milliliters. The exact amounts of each household measure are specified, for example, 1 teaspoon = 5 mL, 1 tablespoon = 15 mL, and 1 cup = 240 mL. The regulations list reference amounts for more than 120 foods and food categories, typically expressed both as common household measures and as metric amounts that are considered appropriate. It was hoped that providing the information in both forms would be another way to help make serving sizes easier for consumers to understand and use.

Servings per container seems straightforward at first, but it can get complicated in some situations. For example, the reference amount for carbonated beverages is 8 fluid ounces, but 12-oz cans are common. The rules require a container that contains up to twice the reference amount to be considered 1 serving. It can get even more complicated for products sold in large discrete units, such as pies or cakes of different sizes, in which the size of a common fraction of the unit—a slice, for example—may not match the official reference amount exactly. In such cases, serv-

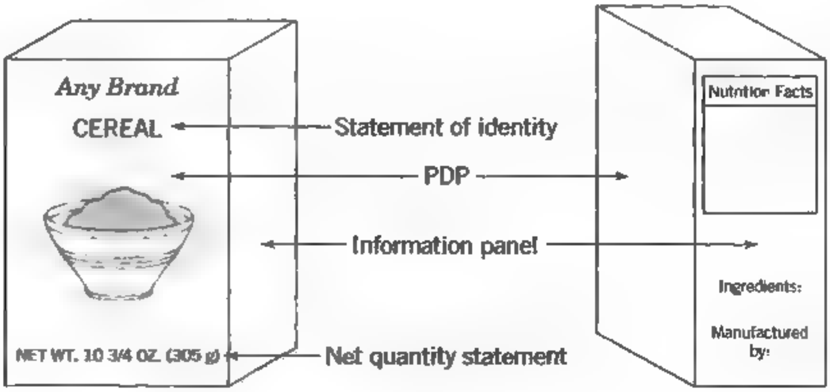
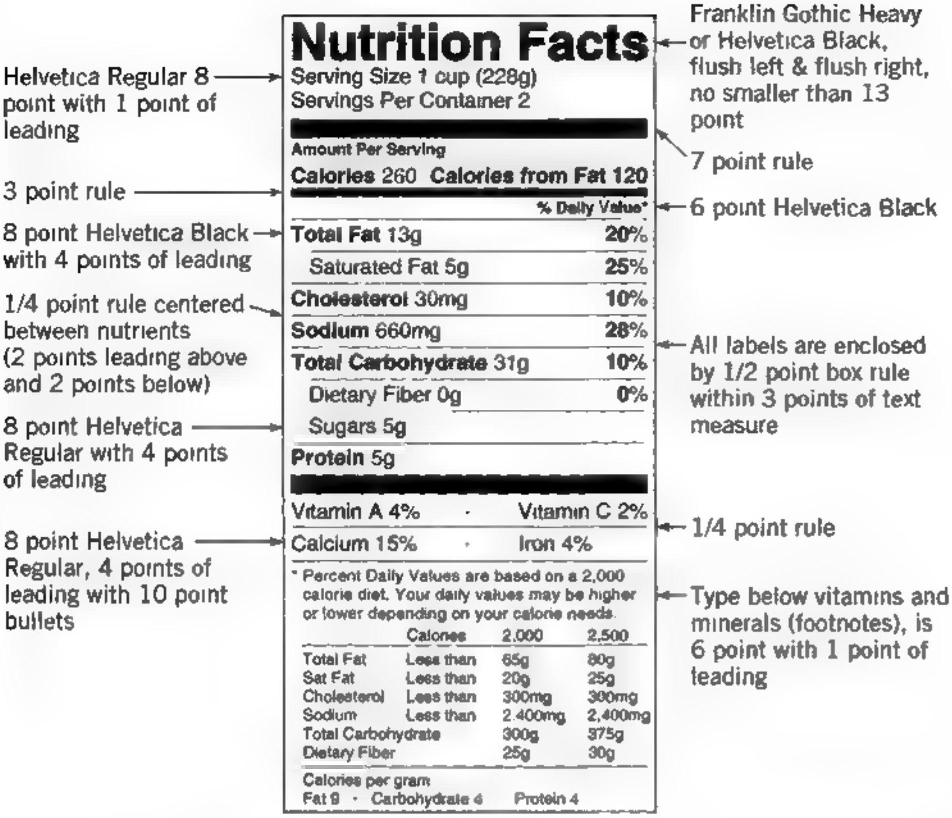


Figure 1. Typical placement of required label information on a package. *Source:* Ref. 1.



Nutrition Facts
Serving Size 1 cup (228g)
Servings Per Container 2

Amount Per Serving
Calories 260 **Calories from Fat** 120

	% Daily Value*
Total Fat 13g	20%
Saturated Fat 5g	25%
Cholesterol 30mg	10%
Sodium 660mg	28%
Total Carbohydrate 31g	10%
Dietary Fiber 0g	0%
Sugars 5g	
Protein 5g	

Vitamin A 4% • Vitamin C 2%
Calcium 15% • Iron 4%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram
Fat 9 • Carbohydrate 4 Protein 4

Helvetica Regular 8 point with 1 point of leading
3 point rule
8 point Helvetica Black with 4 points of leading
1/4 point rule centered between nutrients (2 points leading above and 2 points below)
8 point Helvetica Regular with 4 points of leading
8 point Helvetica Regular, 4 points of leading with 10 point bullets
Franklin Gothic Heavy or Helvetica Black, flush left & flush right, no smaller than 13 point
7 point rule
6 point Helvetica Black
All labels are enclosed by 1/2 point box rule within 3 points of text measure
1/4 point rule
Type below vitamins and minerals (footnotes), is 6 point with 1 point of leading

Figure 2. The new food label (graphic enhancements by FDA). *Source:* Ref. 1.

ings per container must be expressed in approximate terms—"about 6," for instance.

The nutrients shown in Figures 2 and 3 are those required by the regulations. For labeling purposes, they are divided into two groups: macronutrients, calories through protein (those between the two heaviest lines in the box), and micronutrients, the vitamins and minerals below the lower heavy line.

The regulations define each component and prescribe exactly how the numerical values shown in the nutrition facts box are to be expressed and rounded:

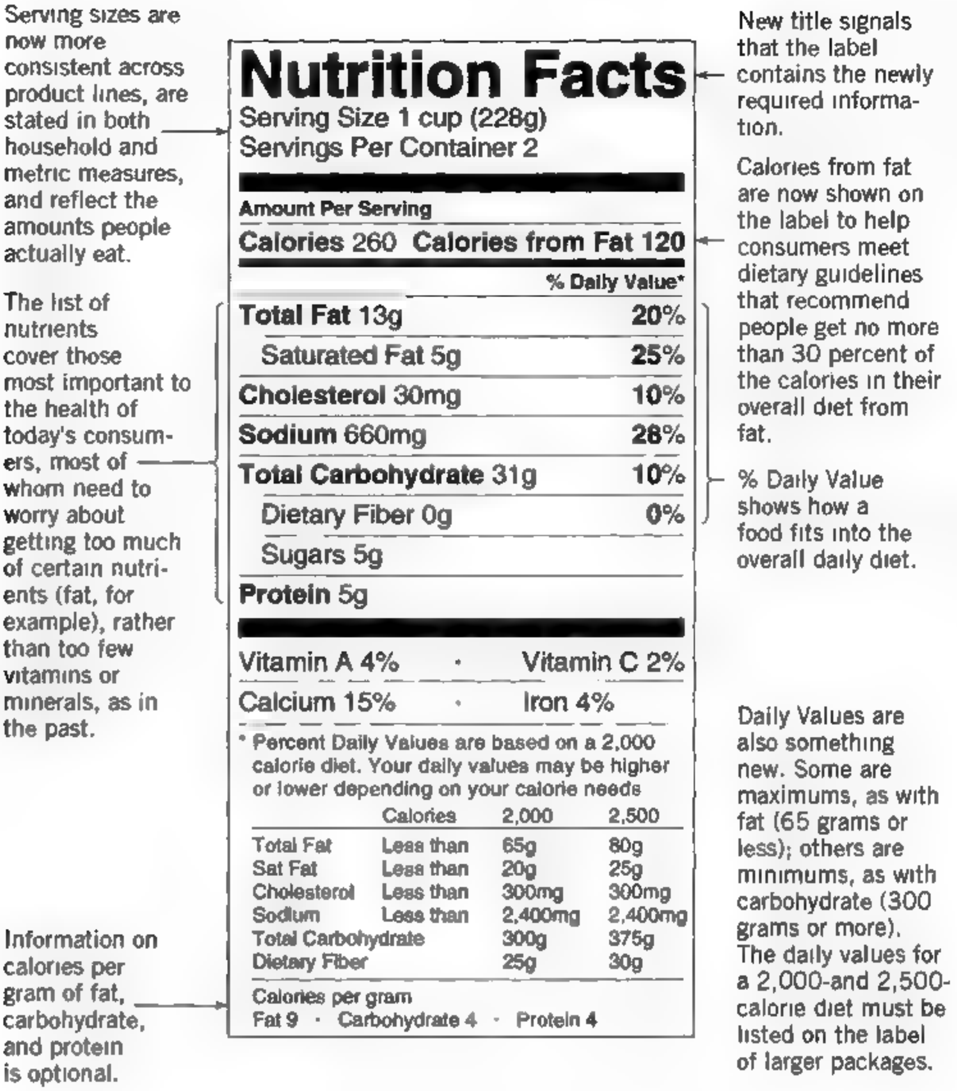
> Serving size (common household measure and metric units): Metric units (grams or milliliters) must be rounded to the nearest 0.2 up to 2.0, the nearest 0.5 up to 5.0, and to the nearest whole unit thereafter
>
> Calories and calories from fat: 5-cal increments up to 50 cal; 5-cal increments thereafter
>
> Total fat and saturated fat: 0.5-g increments up to 5 g; 1-g increments thereafter
>
> Cholesterol: 5-mg increments
>
> Sodium: 5-mg increments up to 140 mg; 10-mg increments thereafter
>
> Total carbohydrate, dietary fiber, sugars, and protein: nearest whole gram
>
> Sugars: For labeling purposes, sugars are defined as the sum of monosaccharides (e.g., dextrose and fructose) and disaccharides (e.g., sucrose, or table sugar).
>
> Percent daily value (DV) is rounded to the nearest 1% DV for total fat, saturated fat, cholesterol, sodium, total carbohydrate, and dietary fiber. There is no DV for sugars. The % DV for protein is optional and is usually not shown.
>
> Vitamins and minerals: The % DV are rounded to the nearest 2% DV up to 10% DV, the nearest 5% DV from 10 to 50% DV, and the nearest 10% DV thereafter.

FDA expects the label values to be correct within the limits specified in the regulations. Nutrients that occur naturally in foods may vary by as much as 20% from the label value and still be within compliance limits. On the other hand, the amounts of any nutrients that are added must be at least 100% of the value stated on the label.

For example, suppose the label for a flavored milk drink declares "Protein 7 g" for a serving. If the protein is derived only from the milk ingredient, it would be "naturally occurring." The actual amount could be slightly less than 6 g in some individual products, and they would still be in compliance. However, if the product contains any added protein, such as that from soy or sodium caseinate and the label declares "Protein 9 g," the product would be required to contain no less than 9 g per serving.

A food product typically contains significant amounts of a few or several nutrients and smaller or even insignificant amounts of others. The regulations established levels that are considered "nutritionally insignificant" for each nutrient. If a serving of a food product contains less than this nutritionally insignificant amount, it may be declared as 0.

The specific nutritionally insignificant amounts are:



Nutrition Facts
Serving Size 1 cup (228g)
Servings Per Container 2

Amount Per Serving
Calories 260 **Calories from Fat** 120

	% Daily Value*
Total Fat 13g	**20%**
Saturated Fat 5g	**25%**
Cholesterol 30mg	**10%**
Sodium 660mg	**28%**
Total Carbohydrate 31g	**10%**
Dietary Fiber 0g	**0%**
Sugars 5g	
Protein 5g	

Vitamin A 4% • Vitamin C 2%
Calcium 15% • Iron 4%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram
Fat 9 • Carbohydrate 4 • Protein 4

Serving sizes are now more consistent across product lines, are stated in both household and metric measures, and reflect the amounts people actually eat.

The list of nutrients cover those most important to the health of today's consumers, most of whom need to worry about getting too much of certain nutrients (fat, for example), rather than too few vitamins or minerals, as in the past.

Information on calories per gram of fat, carbohydrate, and protein is optional.

New title signals that the label contains the newly required information.

Calories from fat are now shown on the label to help consumers meet dietary guidelines that recommend people get no more than 30 percent of the calories in their overall diet from fat.

% Daily Value shows how a food fits into the overall daily diet.

Daily Values are also something new. Some are maximums, as with fat (65 grams or less); others are minimums, as with carbohydrate (300 grams or more). The daily values for a 2,000-and 2,500-calorie diet must be listed on the label of larger packages.

Figure 3. The new food label at a glance. *Source:* Ref. 2.

Calories and calories from fat: 5

Total fat, saturated fat: 0.5 g

Cholesterol: 2 mg (between 2 and 5 mg may be expressed as less than 5 mg)

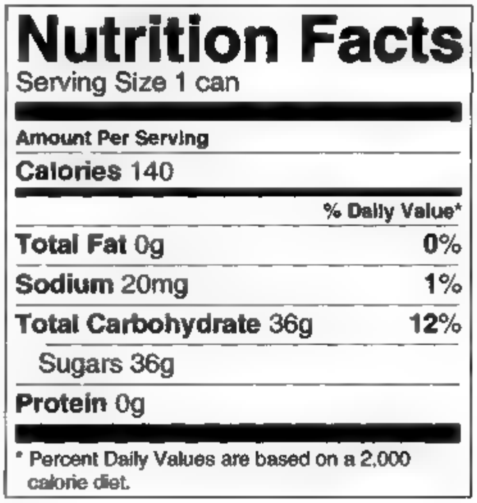
Sodium: 5 mg

Total carbohydrate, dietary fiber, sugars, and protein: 0.5 g (values between 0.5 and 1 g may be expressed as "less than 1 g")

Vitamins and minerals: less than 2% DV

If a serving of a food contains insignificant amounts of 7 or more of the required 13 (total fat and calories from fat count as 1), several of the nutrients may be left off the label, and the Nutrition Facts may be in a simplified format (Fig. 4). The regulations set five "core nutrients"—total calories, total fat, sodium, total carbohydrate, and protein—that must be shown even if the amount present is 0.

The regulations list a number of nutrients that are optional. These include polyunsaturated fat, monounsaturated fat, potassium, soluble fiber, insoluble fiber, sugar



Nutrition Facts
Serving Size 1 can

Amount Per Serving
Calories 140

	% Daily Value*
Total Fat 0g	0%
Sodium 20mg	1%
Total Carbohydrate 36g	12%
Sugars 36g	
Protein 0g	

* Percent Daily Values are based on a 2,000 calorie diet.

Figure 4. Simplified format (example: soft drink). *Source:* Ref. 1.

alcohols (such as xylitol or sorbitol), and other carbohydrates (including starches).

Claims may result in some of the optional nutrients being required. For example, a "cholesterol free" claim requires monounsaturates and polyunsaturates to be shown in the nutrition facts box in addition to saturates.

In addition to the four required micronutrients, the regulations list 21 more vitamins and minerals, for a total of 25. Any of those listed that are present at levels of 2% DV or more may also be included in the Nutrition Facts. Any that are added by the manufacturer to foods such as breakfast cereals or fortified drinks must be included. Also, claims related to a vitamin or mineral, such as "high in niacin," requires the particular nutrient to be included in the nutrition facts. No vitamin or mineral that is not on the list in the regulations may be shown.

The information below the vitamins and minerals that shows the DV is called the footnote. The DV are based on recommendations agreed to by several well-known health groups, such as the American Medical Association and the American Heart Association. The amounts for total fat, saturated fat, and total carbohydrate were set to approximate the amounts of these nutrients in an ideal diet with about 30% of calories from total fat, 10% of calories from saturated fat, and 50 to 60% of calories from total carbohydrate. The amounts for the 2,000-calorie diet are used to calculate the "% Daily Values" in the macronutrient portion of the Nutrition Facts box.

The % DV for the vitamins and minerals are based on the Recommended Daily Intakes (RDIs) set by FDA regulations. These in turn are derived from the Recommended Dietary Allowances (RDAs) established by the National Academy of Sciences and the National Research Council (NAS/NRC).

The agency clearly wants the Nutrition Facts box to have a consistent look on all products. A few variations in the format of the Nutrition Facts box may be permitted under certain conditions, especially for packages that are small or have a shape that might restrict the area for labeling. (The simplified format mentioned earlier is based on nutrient content, not labeling area.) For example, some packages that are horizontal rather than upright may display the footnote to the side of the nutritional values instead of directly under them. Packages that have an area available for labeling of less than 40 in.^{2} may leave off the footnote or use a tabular display. Very small packages with labeling areas of less than 12 in.^{2} may use a linear display. Figure 5 shows examples of the tabular and linear displays.

Other format variations that are allowed include one for bilingual labeling, large Nutrition Facts boxes that show nutritional values for each different food in a multiproduct pack, and a format for showing products "as prepared" as well as "as packaged." Details of all formats must be exactly as prescribed in the regulations.

Claims

There are two categories of nutrition-related claims: nutrient content claims and health claims. The regulations define the claims that are permitted and the required qualifications quite strictly; they also provide for the review and possible approval of new claims.

Nutrient Content Claims/Descriptors Nutrient content claims and descriptors refer to elements of the composition of a food. "Reduced in calories" is one example. The new regulations officially expanded the earlier rules for reduced-calorie foods. Nutrient content claims are now permitted in the areas of sodium, fat and cholesterol, and light/lite.

The regulations set very detailed and specific requirements for each claim. For example, a "fat free" food must contain <0.5 g of fat per serving. "Cholesterol-free" means <5 mg of cholesterol per serving. A "low fat" food may contain no more than 3 g of fat in a serving, or, if the serving <2 tablespoons, no more than 3 g of fat in 50 g. This, together with established serving sizes, is supposed to help prevent manufacturers from reducing serving sizes to unrealistically small amounts just to make a claim.

The requirement for a "reduced calorie" food was changed from the earlier 33% (1/3) minimum reduction in calories to a 25% (1/4) reduction compared to the regular food. This was to make it easier for manufacturers to provide foods with reductions in calorie content. Higher reductions were applied to "light" or "lite" products, which must be reduced in calories by at least 33%, or reduced in fat by at least 50% compared to the regular food.

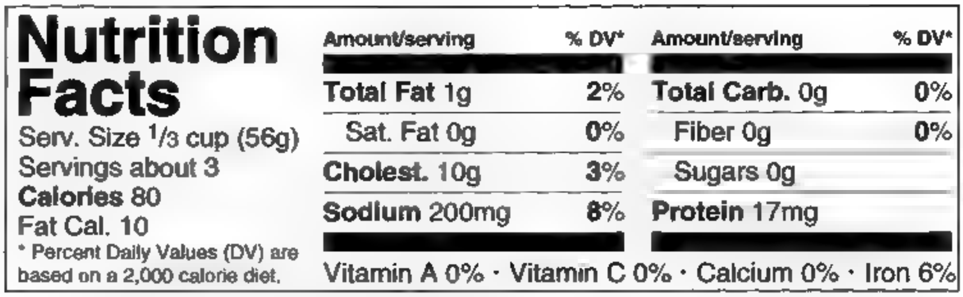
The new rules further extended the previous regulations for reduced-calorie foods to provide important incentives for manufacturers to develop foods with improved nutrition. Under old regulations, a food that was formulated to contain less fat or fewer calories might need to be labeled as "imitation." The specific provisions of the new rules make it clear that most foods that qualify for a "reduced calorie" claim, for example, but are nutritionally equivalent to the regular food in all other aspects, need not be called "imitation." Thus, we now see "reduced calorie" instead of "imitation" on products with at least 25% fewer calories.

The regulations also define the requirements for claims about vitamins and minerals. A food that is a "good source" of vitamin C, for example, must have at least 10% DV in a serving. To be "high" or "rich in" a nutrient, a serving of a food must have at least 20% DV. "More" means 10% or more of the DV, so that a drink that has "more calcium than milk" must contain at least 40% of the daily value of calcium compared to the 30% in a glass of whole milk.

Nutrient content claims are summarized in Table 1. The NLEA regulations list several synonyms for each claim. Examples are "zero," "no," and "without" for "free"; "little" or "contains a small amount of" for "low;" and "lower" or "fewer (calories)" for "reduced." No nutrient content claims or synonyms are allowed other than those that are specifically permitted, defined, and listed.

The regulations also define "healthy." A "healthy" food must at least meet the requirements for a food that is low in fat and saturated fat and must contain at least 10% DV, without fortification, of one or more of vitamin A, vitamin D, calcium, iron, dietary fiber, or protein. The 10% DV requirement in the original proposal might have prevented calling some fresh fruits and vegetables "healthy" because of their high moisture content. The final rules, however, specifically exempted fresh fruits and vegetables from this requirement, permitting them to be described as "healthy."

Tabular Display



Nutrition Facts

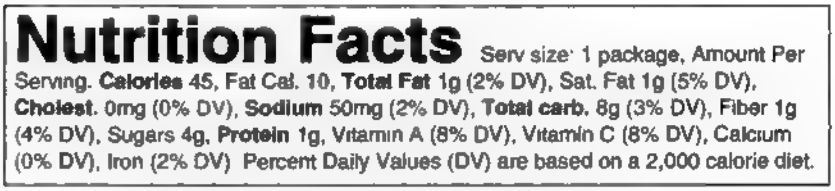
Serv. Size 1/3 cup (56g)
Servings about 3
Calories 80
Fat Cal. 10
* Percent Daily Values (DV) are based on a 2,000 calorie diet.

Amount/serving	% DV*	Amount/serving	% DV*
Total Fat 1g	2%	**Total Carb.** 0g	0%
Sat. Fat 0g	0%	Fiber 0g	0%
Cholest. 10g	3%	Sugars 0g	
Sodium 200mg	8%	**Protein** 17mg	

Vitamin A 0% · Vitamin C 0% · Calcium 0% · Iron 6%

May be used when the area available for labeling is less than 40 square inches.

Linear Display



Nutrition Facts Serv size: 1 package, Amount Per Serving. **Calories** 45, Fat Cal. 10, **Total Fat** 1g (2% DV), Sat. Fat 1g (5% DV), **Cholest.** 0mg (0% DV), **Sodium** 50mg (2% DV), **Total carb.** 8g (3% DV), Fiber 1g (4% DV), Sugars 4g, **Protein** 1g, Vitamin A (8% DV), Vitamin C (8% DV), Calcium (0% DV), Iron (2% DV) Percent Daily Values (DV) are based on a 2,000 calorie diet.

May be used when the area available for labeling is less than 12 square inches.

Figure 5. Examples of permitted format variations. *Source.* Ref. 1

Table 1. Summary of Nutrient Content Claims and Descriptors that May Be Used on Food Labels

Descriptor[a]	Definition[b]
Free	A serving contains no or a physiologically inconsequential amount: <5 cal, <5 mg of sodium, <0.5 g of fat, <0.5 g of saturated fat, <2 mg of cholesterol, or <0.5 g of sugar
Low	A serving (and 50 g of food if the serving size is small) contains no more than 40 calories, 140 mg of sodium, 3 g of fat, 1 g of saturated fat and 15% of calories from saturated fat, or 20 mg of cholesterol; not defined for sugar; for "very low sodium," no more than 35 mg of sodium
Lean	A serving (and 100 g) of meat, poultry, seafood, and game meats contains <10 g of fat, <4 g of saturated fat, and <95 mg of cholesterol
Extra lean	A serving (and 100 g) of meat, poultry, seafood, and game meats contains <5 g of fat, <2 g of saturated fat, and <95 mg of cholesterol
High	A serving contains 20% of more of the daily value (DV) for a particular nutrient
Good source	A serving contains 10–19% of the DV for the nutrient
Reduced	A nutritionally altered product contains 25% less of a nutrient or 25% fewer calories than a reference food; cannot be used if the reference food already meets the requirement for a "low" claim
Less	A food contains 25% less of a nutrient or 25% fewer calories than a reference food
Light	1. An altered product contains one-third fewer calories or 50% of the fat in a reference food; if 50% or more of the calories come from fat, the reduction must be 50% of the fat); or 2. The sodium content of a low-calorie, low-fat food has been reduced by 50% (the claim "light in sodium" may be used); or 3. The term describes such properties as texture and color, as long as the label explains the intent (e.g., "light brown sugar," "light and fluffy")
More	A serving contains at least 10% of the DV of a nutrient more than a reference food. Also applies to fortified, enriched, and added claims for altered foods
% Fat Free	A product must be low-fat or fat-free, and the percentage must accurately reflect the amount of fat in 100 g of food. Thus, 2.5 g of fat in 50 g of food results in a "95% fat-free" claim
Healthy	A food is low in fat and saturated fat, and a serving contains no more than 480 mg of sodium and no more than 60 mg of cholesterol
Fresh	1 A food is raw, has never been frozen or heated, and contains no preservatives (irradiation at low levels is allowed); or 2. The term accurately describes the product (e.g., "fresh milk" or "freshly baked bread")
Fresh frozen	The food has been quickly frozen while still fresh; blanching is allowed before freezing to prevent nutrient breakdown

[a] See the regulations for acceptable synonyms.
[b] These definitions have been simplified for this table; see the regulations for specific restrictions and additional requirements.
Source. Ref. 3.

Similar exemptions are expected for canned and frozen fruits and vegetables, as well as for bread and other grain products that have been fortified to meet the requirements of a standard of identity.

Health Claims Health claims refer to the possible effects of a food or the diet on health. The regulations as published in 1993 permitted health claims in only seven specific areas:

- Calcium and osteoporosis
- Dietary fats and cancer
- Sodium and high blood pressure
- Saturated fat, cholesterol, and heart disease
- Fruits, vegetables, and grain products that contain dietary fiber and cancer
- Fruits, vegetables, and grain products that contain dietary fiber and heart disease
- Fruits and vegetables (components other than fiber) and cancer)

Other permitted areas have since been added:

- Folic acid and neural tube defects in infants
- Sugar alcohols (such as xylitol) and dental caries (cavities)
- Soluble oat fiber in oats (oat bran) and heart disease

Direct claims for dietary fiber and cancer are not allowed at this time. This is ironic because the fiber versus cancer claims were among the first to appear on foods in the mid 1980s and helped reinforce the current interest in nutrition. Claims must be carefully worded to indicate that disease is the result of many factors and to position the food and its beneficial component within a healthy diet and lifestyle.

Several products on the market have taken advantage of the ability to make health claims. For example, consumers are familiar with product claims that link the fiber in oats with lessening the risk of heart disease, the calcium in dairy products with preventing osteoporosis, and the xylitol in chewing gum with cavity prevention.

Other Label Claims Labels may make other claims that are not considered related to nutrient content or health. "Fresh" and "fresh frozen" (see Table 1) are not nutrient content claims but were defined because of ongoing, existing prior differences in opinion as to their exact meaning as applied to specific foods.

FDA also considers statements such as "milk free" (related to allergy or religious concerns), "contains no preservatives" (no nutritive function), and "oat bran muffins" (statement of identity) not to be nutrient content claims. On the other hand, the statement "high in oat bran" is considered an implied nutrient content claim such that the product would have to meet all the necessary requirements, such as a minimum soluble fiber content of 0.75g/serving.

The Influence of Claims on Other Labeling Requirements Nutrient content or health claims typically lead to additional labeling requirements. For example, originally the label of any food that carried a nutrient content claim such as "low-fat" also had to carry a referral statement that directed consumers to the nutrition facts box. The line "See back panel for nutrition information" was typical. This requirement was made optional in 1998 in the belief that consumers are now familiar with the presence and location of the Nutrition Facts box.

The regulations also set disclosure levels, or levels that were considered high, for fat (13 g), saturated fat (4 g), cholesterol (60 mg), and sodium (480 mg). If a serving of a "reduced calorie" food, for example, contains more than the disclosure level of 480 mg of sodium, the label must carry a disclosure statement. The typical line "See nutrition information for sodium content" meets that requirement.

For some foods, the level of one nutrient may exclude a claim based on another. For example, peanut butter is naturally cholesterol free, but a serving also contains about 3 g of saturated fat. The regulations prohibit cholesterol-free claims for foods that contain more than 2 g of saturated fat in a serving, so the peanut butter label may not claim that the product is cholesterol free, although it may list "Cholesterol 0 mg" in the Nutrition Facts box.

Foods that exceed the same disclosure levels that trigger the disclosure statement for nutrient content claims may not make health claims at all.

FUTURE ISSUES AND CONCERNS

The public's interest in what is in their foods continues to be high, and the pressure to provide more information will surely continue. A few matters are left over from NLEA issues.

For the present, FDA considers the voluntary nutrition-labeling programs for fresh produce and fish to be adequate and has not proposed rules making nutrition labeling mandatory for these products.

Pressure continues from some quarters for the agency to require higher-profile nutrition labeling for all foods served in restaurants, not just those that make claims. This question is of particular concern to the operators of individual restaurants because of the difficulty involved in trying to make sure menu items that are prepared and served individually are consistent enough in composition for nutritional values to be meaningful.

Trans fats are unsaturated fats that form during partial hydrogenation of oils. They are different in structure from the *cis* forms that occur naturally. Products that contain significant amounts of partially hydrogenated fats or oils, such as shortenings and stick margarines, may be high in *trans* fats. Some recent research reports have suggested that *trans* fats may raise cholesterol levels in the body; however, whether the research findings have any significance in actual diets is not yet fully understood. The questions of whether and how to declare *trans* fats must eventually be decided.

The number of nutrient content claims and health claims that will be allowed will increase as more knowledge becomes available. The current interest in dietary supplements and functional foods will likely lead to the development of new products. Labeling will be complicated by questions of which products are foods and which are supplements.

In other areas, labels of foods that have been irradiated must disclose that fact. FDA has published guidelines for label statements on foods that require refrigeration and may eventually determine that detailed instructions for storage, preparation, and handling of certain foods should be required rather than optional. Labeling that calls specific attention to the presence of common allergens is beginning to appear. Standards for organic foods are being developed, and appropriate labeling will undoubtedly be required.

Within the public's interest in foods, a strong right-to-know element has been growing. Several consumer interest groups have asked the FDA to require percentage declaration of the major ingredients in foods. Specific groups have petitioned the agency to require the quantitative declaration of food components such as monosodium glutamate and caffeine. Groups have demanded that foods produced using biotechnology be labeled accordingly.

All this leads to the basic question of what kind of information should go on the label. Because label space is finite, a prevailing position has been that food-product labels should be required to carry only significant information about the composition and safety of the product in the package. It may be time for FDA and USDA to take positive steps to help avoid too much label clutter. It is worth remembering and realizing that there are many different ways to provide information to satisfy the public's right to know that do not necessarily involve the label itself.

ADDITIONAL INFORMATION

The FDA's *A Food Labeling Guide* provides detailed, practical information on food labeling in a question-and-answer format. It is available from the following:

Center for Safety and Applied Nutrition
Food and Drug Administration
5600 Fisher's Lane
Rockville, Maryland 20847

Several publications that describe the new food labels and are designed to help consumers use the information in planning their diets are available at this address as well.

The *Food Labeling Guide* and the other publications are also available on-line at *http://www.fda.gov/*. A wealth of additional information on labeling, nutrition, food safety, and related regulatory issues, including many of the materials used as references for this chapter, can be found at this site.

Nutrition and labeling information from the U.S. Department of Agriculture is available on-line at *http://www.usda.gov/*.

The magazine *FDA Consumer* is an excellent source of accurate and readable information on not only foods and labeling, but also other regulatory, health, and safety issues. FDA publishes it six times a year. Subscriptions are available from the following:

Superintendent of Documents
P.O. Box 371954
Pittsburgh, PA 15250-7954

BIBLIOGRAPHY

1. Food and Drug Administration, *A Food Labeling Guide*, Washington, D.C., 1994.
2. Food and Drug Administration, "21 CFR Part 1, et al.: Food Labeling; General Provisions; Nutrition Labeling; Label Format; Nutrient Content Claims; Health Claims, Ingredient Labeling; State and Local Requirements; and Exemptions; Final Rules," *Fed. Regist.* **58**, 2066–2941 (1993).
3. N. H. Marmelstein, "A New Era in Food Labeling," *Food Technol.* **47**, 81–96 (1993).

GENERAL REFERENCES

M. B. Browne *Label Facts for Healthful Eating: Educator's Resource Guide*, National Food Processors Association in cooperation with the Food and Drug Administration, U.S. Department of Health and Human Services, and the Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C., 1993.

Food and Drug Administration, "Focus on Food Labeling: Read the Label, Set a Healthy Table," *FDA Consumer* (Special Report) (1993).

Food and Drug Administration, "The New Food Label," *FDA Backgrounder, BG* 92–94 (1992).

Food and Drug Administration, *Notice to Manufacturers: Label Declaration of Allergenic Substances in Foods*, Washington, D.C., June 10, 1996.

Food and Drug Administration, "21 CFR Parts 1 and 101: Food Labeling; Mandatory Status of Nutrition Labeling and Nutrient Content Revision, Format for Nutrition Label; Correction," *Fed. Regist.* **58**, 17328–17340 (1993).

Food and Drug Administration, "21 CFR Parts 5 and 101: Food Labeling; Nutrient Content Claims; General Principles, Petitions, Definition of Terms; Definitions of Nutrient Content Claims for the Fat, Fatty Acid, and Cholesterol Content of Foods; Correction," *Fed. Regist.* **58**, 17341–17346 (1993).

Food and Drug Administration, "21 CFR Part 5, et al.: Food Labeling for Human Consumption; Rules, Proposed Rule, and Notice," *Fed. Regist.* **58**, 44019–44096 (1993).

Food and Drug Administration, "21 CFR Part 101. Food Labeling; Nutrient Content Claims, General Provisions," *Fed. Regist.* **63**, 26978–26980 (1998).

Food and Drug Administration and U.S Department of Agriculture, Food Safety and Inspection Service, *The New Food Label. Check It Out*, Washington, D.C., 1994.

M. S. Meskin, "Regulating Organic Foods," *Food Technol.* **52**(7), 144 (1998).

Nutrition Labeling and Education Act, 101st Congress, November 8, 1990.

U. S. Department of Agriculture, Food Safety and Inspection Service, "9 CFR Parts 317, 320, and 381: Labeling of Meat and Poultry Products; Final Rule," *Fed. Regist.* **58**, 631–691 (1993).

B. A. Watkins, "*Trans* Fatty Acids: A Health Paradox?" *Food Technol.* **52**(3), 120 (1998).

PHILLIP WELLS
Bestfoods North America
Somerset, New Jersey

NUTS. See NUTS in the Supplement section.

NUTRITIONAL QUALITY AND FOOD PROCESSING. See FOOD PROCESSING: EFFECT ON NUTRITIONAL QUALITY.

OILSEEDS AND VEGETABLE OILS

Most oils of vegetable origin are derived from plant seeds, hence the term oilseed. However, some vegetable oils, such as olive and palm oil, come from the plant's mesocarp, the fleshy pulp covering the seed. This article will discuss only the more economically significant vegetable oils. The terminology is reviewed and then the processing of oilseeds to make various products is discussed. Information about specific oils is also presented. Tables 1–3 show the makeup of the oilseeds, the fatty acid composition of the oils, and the amino acid composition of the proteins. Figure 1 shows the worldwide production for the last 10 yr of the most economically important vegetable oils. Although the relationship between various human diseases and fats and oils in the human diet is of great concern, space limitations prevent a discussion of this topic and a variety of sources are available (14).

A vegetable oil is made up of monoglycerides, diglycerides and triglycerides with the latter predominating. A triglyceride is composed of a glycerol molecule backbone with three fatty acid groups branched off it. Technically the term fatty acid applies to the series of compounds referred to as $Cn{:}n'$, where n is the number of carbon atoms and n' is the number of double bonds. The simplest fatty acid is formic acid (C1:0). However, fatty acids that occur in vegetable oils start with caproic (C6:0) and go up to behenic (C22:0) and erucic (C22:1). A saturated fatty acid has no double bonds between any of the carbon atoms. An unsaturated fatty acid has one (monounsaturated) or more (polyunsaturated) double bonds between the carbons. Oils having a higher percentage of saturated fatty acids have higher melting points. For example, shortening and margarine have a higher proportion of saturated fatty acids than salad or cooking oils. Occasionally the terms fat and oil are used interchangeably. However, in common usage, a fat refers to a triglyceride mixture that is solid at room temperature, whereas an oil is liquid at room temperature. Different degrees of solidification or plasticity can be achieved by mixing a saturated fat, such as palm or coconut, with an unsaturated oil such as soybean. An unsaturated oil can also be made solid through a reaction called hydrogenation. In this process, hydrogen gas, in the presence of a catalyst, attaches to the double bonds of the unsaturated fatty acids and transforms them into saturated fatty acids. This large-scale industrial process is used extensively to manufacture margarine and shortening.

When iodine monobromide or monochloride reacts with an unsaturated fatty acid, the bromine or chlorine add to the double bonds. This reaction forms the basis of an oil characteristic known as iodine number. This quantity, defined as the number of grams of iodine absorbed under standard conditions by 100 g of oil, is a measure of the unsaturation of the oil, ie, higher numbers denote greater unsaturation. Saponification is the reaction of a fat or oil and an alkali to yield glycerol and a salt of the alkali metal. This process is used in the manufacture of soap. The saponification number is defined as the number of milligrams of potassium hydroxide required to saponify 1 g of oil. Once the composition of a pure oil is known its saponification number can be calculated. This number can be compared with the experimentally determined saponification number of an oil of unknown purity to determine the amount of unsaponifiable, ie, nonoil, material in the latter oil. The refractive index is a measure of the degree that incidental light is bent in the oil. Because this index is a function of the oil's molecular structure and impurities, it provides an easy and quick method to identify an oil and determine its purity.

PROCESSING OILSEEDS

Once harvested, oilseeds must be transported to processing plants and may be stored before being processed. Preventing a reduction in oilseed quality during transportation and storage is a major problem. Although weather conditions throughout the growing season affect the quality of oilseeds, rain at harvesting causes particular problems. Wet seed must be dried and cooled before storage to prevent mold growth and free fatty acid formation; the latter is caused by enzymatic hydrolysis of the triglycerides. Wet seed is typically dried and cooled by passing air through the seed piles in the storage houses. Oil is removed in two ways: mechanically pressing it from the seeds and soaking the seeds in a solvent, usually hexane, that dissolves the oil. Because of its efficiency, this latter process, called solvent extraction, is used in almost all commercial operations (15). A combination of these two processes, prepress solvent extraction, is sometimes used for high oil content oilseeds.

Figure 2 is a simplified schematic of the oilseed solvent extraction operation. First the dried seeds are cleaned, to remove stones, metals, and other objects that would damage the processing equipment. They are then dehulled and flaked. The flakes, typically about 0.025 cm thick, provide a more efficient extraction medium. The flakes are either prepressed or sent directly to the extractor. Extracted flakes are heated in a desolventizer to vaporize the solvent, which is recycled to the extractor. The meal from the desolventizer can be used as is or processed further. The oil-solvent mixture coming from the extractor, called miscella, is sent to an evaporator, where the solvent is driven off and recycled. The crude oil is sent to refining to be treated with caustic to remove most of the nontriglyceride components. The oil is then bleached and deodorized to give it a light color and bland odor. If it is to be used as a salad oil, it is also winterized. In the winterization process the oil is cooled to a low temperature and any crystallized material is removed from the liquid oil, which is now a suitable salad oil.

Table 1. Composition of Various Oilseeds and Characteristics of Their Oils

	Coconut[a]	Corn[b]	Cottonseed[c]	Olive	Palm[d]	Palm Kernel	Peanut	Rape	Soybean	Sunflower
					Seed composition					
Oil, %	34	3.6	36	17	70	40	49	40	20	47
Protein, %	3.3	8.0	33	0.9		8.4	26	23	36	23
Crude fiber, %	4.3	2.5	2.0			5.8	4.9	6.7	5.0	4.2
Moisture, %	47	16	4.7	44		8.4	6.5	7.5	8.5	5.4
Ash, %	1.0	1.2	4.6	2.2		1.8	2.3	4.5	4.9	
Number of seeds g	0.0005	3	9	0.5	0.03	0.3	0.4	180	6	11
					Oil					
Saponification number	250–284	187–193	189–198	185–200	195–206	242–255	188–195	170–180 190[e]	189–195	188–194
Iodine number	7.5–10.5	103–128	99–115	77–94	51–58	10–23	82–106	97–108 112–131[e]	120–141	125–136
Refractive index	1.448–1 450	1.470–1.474	1.468–1.472	1.469–1.470	1.453–1.456	1.449–1.452	1.470–1.472	1 470–1.474	1.470–1.476	1.466–1.684
Specific gravity	0.91–0.919	0.915–0.920	0.916–0.918	0.912–0.913	0.857–0.860	0.856–0.874	0.910–0.915	0.906–0.914 0.916–0.917[e]	0.917–0.921	0.894–0.899
References	1,3	2,3	1,3	3,4	5	5	6,7	8	3,6	1,9

[a] Coprs.

[b] Corn contains more than 60% starch.

[c] Delinted seed.

[d] Does not include the kernel.

[e] The second value is for canola (low–erucic-acid rapeseed).

Table 2. Fatty Acid Content of Various Oilseed Oils in Weight Percent

	Canola	Coconut	Corn	Cottonseed	Olive	Palm	Palm kernel	Peanut	Rape	Soybean	Sunflower
					Saturates						
Caproic C6:0[a]							0.2				
Caprylic C8:0		7.1					4.0	0.1			
Capric C10.0		7.3		0.5			3.9	0.1			
Lauric C12:0		54.0		0.4			50.4	0.6		0.1	
Myristic C14:0	0.1	17.4		0.8		2.5	17.3	0.3	0.1	0.3	
Palmitic C16:0	5.7	6.1	11.0	19.9	11.0	40.8	7.9	13.3	2.9	10.8	6.0
Stearic C18:0	2.1	1.6	1.8	3.1	2.2	3.6	2.3	2.1	1.4	3.2	4.0
Arachidic C20:0	0.2		0.2					1.2		0.1	
Behenic C22:0	0.2							2.9	0.5	0.1	
Total saturates	*8.3*	*93.5*	*13.0*	*24.7*	*13.2*	*46.9*	*86.0*	*20.6*	*4.9*	*14.6*	*10.0*
					Unsaturates						
Oleic C18:1	57.7	5.0	25.3	25.7	72.5	45.2	11.8	47.8	33.0	24.0	18.0
Linoleic C18:2	24.6	1.3	60.1	48.5	7.9	7.9	2.1	29.2	15.4	54.4	70.0
Linolenic C18:3	7.9		1.1	0.1	0.6				6.2	6.8	
Gadoleic C20:1	1.0				0.3			1.2	12.2		
Erucic C22:1	0.2							0.1	25.5		
Total unsaturates	*91.4*	*6.3*	*86.5*	*74.3*	*81.3*	*53.1*	*13.9*	*78.3*	*92.3*	*85.2*	*88.0*
References	10	11	12	11	13	3	3	11	10	11	9

[a] C6:0 means fatty acid has 6 carbons and 0 double bonds, C18:3 means fatty acid has 18 carbons and 3 double bonds, etc.

SPECIFIC OILS

Coconut

Description. There is one species of coconut palm, *Cocos nucifera* L., but there are several geographical varieties. These are divided into two groups: the tall typica and the dwarf nana: The tall coconut palms can live longer than 80 yr. After an initial flowering period of 5–8 yr, they continuously produce from 80 to 150 nuts per year. The coconuts from this variety have high oil yields and good-quality fiber and copra (dried coconut meat). The dwarf varieties live for about 40 yr, producing fruit after their third year. These colorful palms produce coconuts with a tough copra unsuitable for commercial purposes and so are planted mainly as an ornamental. Coconuts consist of an outer husk covering a thin, hard shell that in turn covers the copra. The inner cavity of the copra contains a watery substance called milk. A ripe nut weighs 2–3 kg and is about 25 cm long.

Origin and Cultivation. Records indicate that the coconut palm has existed for more than 3,000 yr. Although some theories place its origin in Central or South America, it most likely originated in Southeast Asia (16). The coconut palm grows in tropical areas throughout the world; in the western hemisphere, it is found as far north as Florida and as far south as Brazil. India, Indonesia, and the Philippines are the main coconut-producing countries. Africa, Latin America, and Oceania also are significant producers.

Composition and Uses. The husk consists of fibers that have a variety of uses and comprise more than 50% of the nut's mass. The shell and the milk each make up about 12% of the mass and the copra accounts for about 18%. More than 60% of the copra is a highly saturated oil. Because a large percentage of the oil's fatty acids is lauric (C12:0), coconut oil is known as a lauric acid oil. Although the oil has the greatest economic value, all parts of the coconut are used (16). The fibers from the husk, called coir, are used to make mats, nets, bags, ropes, and similar items. The shells are used to make activated carbon. The oil, extracted from the copra by crushing, is used in baking and in a variety of prepared foods. Of all the edible oils, coconut oil has the most nonedible uses. It is used in cosmetics, toiletries, and in the manufacture of soap by saponification. The glycerol by-product from soap manufacture is used in pharmaceuticals and in the manufacture of explosives. Coconut oil is used in the production of plasticizers, resins, detergents, and as a lubricant and fuel.

Corn

Description. *Zea mays* L. is a tall annual plant belonging to the grass family Graminese. Although it is commonly called maize throughout most of the world, it is referred to as corn in the United States and Canada. The female inflorescence, the ear, is where corn kernels are produced. Typically an ear of corn has about 800 kernels attached to its inner cylinder, called the cob (17). Kernels at either end of the ear are rounded whereas those at the center are flattened by pressure from adjacent kernels. An average kernel from the center of the ear is about 0.4 × 0.8 cm thick and about 1.2 cm long. Dent and sweet are two types of corn grown extensively in the United States. When drying, the center part of the dent corn kernel collapses making a distinct indentation. The sweet variety has mutant genes that retard the conversion of sugar to starch in the kernel.

Table 3. Amino Acid Composition of the Protein in the Various Oilseed Meals in Weight Percent

	Coconut	Corn	Cottonseed	Peanut	Rape	Soybean	Sunflower
Ala	0.2	0.8	1.5	1.0	1.7	1.7	1.1
Arg	0.6	0.4	4.4	3.1	2.3	2.8	2.4
Asp	0.3	0.7	3.5	3.2	3.1	4.6	2.5
Cys	0.1	0.2	0.9	0.3	0.4	0.6	0.5
Glu	0 8	1.8	8.2	5.4	6.3	7.1	5 6
Gly	0.2	0.4	1.6	1.6	1.9	1.7	1.5
His[a]	0.1	0.3	1.0	0.7	1.1	1.0	0.6
Ile[a]	0.1		1.2	0.9	1.5	1.8	1.1
Leu[a]	0.3	1.1	2.2	1.7	2.7	3.0	1.7
Lys[a]	0.2	0.2	1.7	0.9	2.3	2.4	0.9
Met[a]	0.1	0.2	0.5	0.3	0.7	0.5	0.5
Phe	0.2	0.4	2.0	1.3	1.5	1.9	1 2
Pro	0.1	0.8	1.4	1.1	2.7	2.1	1.2
Ser	0.2	0.4	1.6	1.3	1.7	2.1	1.1
Thr[a]	0.1	0.3	1.2	0.9	1.7	1.6	0.9
Trp[a]	0.04	0.1	0.5	0.3	0.4	0.5	0.4
Tyr	0.1	0.3	1.2	1.1	0.9	1.4	0.7
Val[a]	0.2	0.4	1.7	1.1	1.9	1.8	1.3
References	1	2	1	6	8	6	1

[a] Essential amino acid.

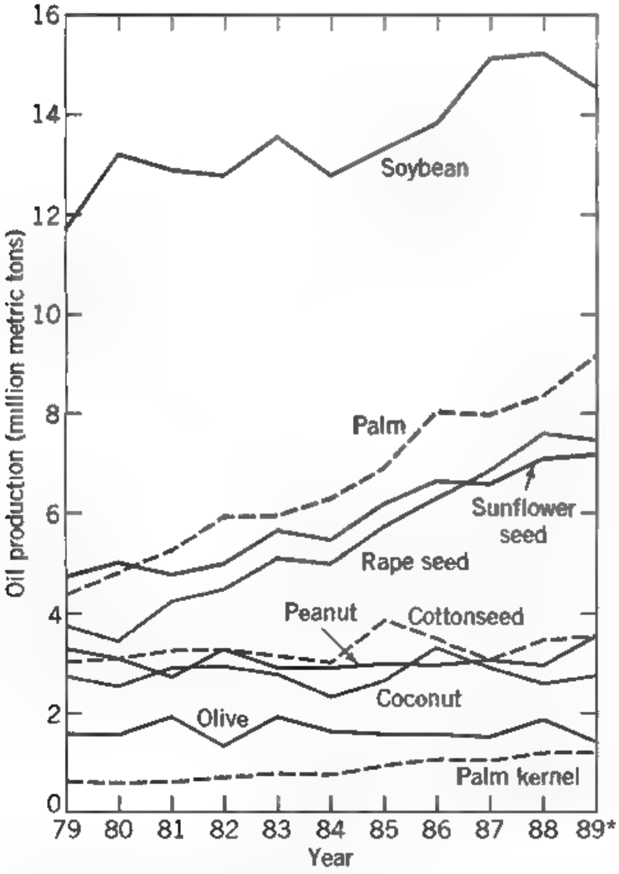


Figure 1. Graph of oil production for the last 10 yr for individual oils. Asterisk denotes projection.

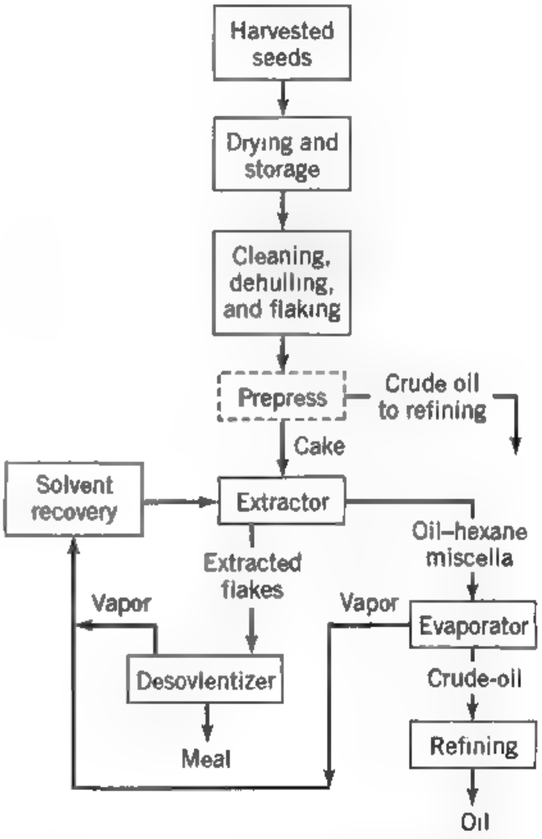

Harvested seeds
Drying and storage
Cleaning, dehulling, and flaking
Prepress
Crude oil to refining
Cake
Solvent recovery
Extractor
Oil–hexane miscella
Extracted flakes
Vapor
Vapor
Evaporator
Desovlentizer
Crude-oil
Refining
Meal
Oil

Figure 2. Oilseed solvent extraction flow sheet.

Origin and Cultivation. Archaeological evidence of earliest corn places its age at greater than 7,000 yr. The evidence further shows that this corn was domesticated, ie, natural seed dissemination was impossible. Corn probably originated in Mexico and spread north and south. Early

European explorers brought it to Europe, from where it spread to Africa and Asia (18). Every continent except Antarctica produces corn.

Today, the U.S. Corn Belt is the largest area of corn cultivation in the world. Through years of managed hybridization, Corn Belt corn is also the world's most productive variety. In fact, depending on climatic conditions, the per acre yield of corn is greater than any other crop. Over the last 60 yr, U.S. corn-growing area has decreased by half while production has doubled because of increased yields (18). Corn grows in the middle latitudes of both hemispheres. The growing season lasts through the freeze-free period. Corn grows from seed to a mature plant, 2–4 m tall, in about four months. Weather changes cause corn yield to vary across the Corn Belt by as much as 20% in a given year.

Composition and Uses. Grain makes up more than 40% of the dry matter of the corn plant. Stover, the nongrain parts of the plant, make up the rest of the dry matter. Because of its low oil content, corn is not generally considered an oilseed. More than 60% of the kernel is starch; oil comprises less than 4% (Table 1). In the United States, corn is primarily an animal feed crop, with less than 15% used for human consumption or various industrial purposes. For nonfeed purposes, corn is processed in one of two ways. Dry milling involves separating the germ from the whole kernel with an abrading action. The germ is then pressed or solvent extracted to remove the oil, which is used primarily as a salad and cooking oil. The rest of the kernel is then milled to produce grits, cornmeal, and flour. These products are used in a variety of applications, including snack products, breakfast cereals, brewing, pharmaceuticals, and building products. In wet milling, the corn is first softened by soaking it in a steeping liquor. The softened corn is then drained, coarsely ground, and combined with water to make a slurry. The slurry is then separated, in either hydroclones or floatation tanks, into an oil-bearing germ overflow and a starch underflow. The germ is further processed to extract oil. Most of the starch is processed into sweeteners and ethyl alcohol.

Cottonseed

Description. Four species of cotton are cultivated: *Gossypium arboreum*, L. *G. herbaceum* L., *G. hirsutum* L., and *G. barbadense* L. These are divided into two groups: the Old World (or Asiatic) and the New World. The Asiatic group, consisting of *G. arboreum* L. and *G. herbaceum* L., has short, harsh fibers that limit their use in fabrics. Their yield is comparatively low. The New World group consists of *G. hirsutum* L. and *G. barbadense* L. Their fibers are long, from 2 to 4.5 cm, and fine, making them well suited for a variety of uses. The seeds of the former are egg shaped and about 0.8–1.2 cm in length. They are densely covered with short cotton fibers, called linters, which remain after the fiber has been removed by ginning. The seed coat, or hull, is quite strong. The seeds of *G. barbadense* are similar in shape and size but have no adhering linters after ginning.

Origin and Cultivation. Archaeological evidence suggests that cotton was used for string and fabric in India around 3000 B.C. Although there have been claims that cotton fabric was used earlier than this in Egypt and Peru, the most reliable information suggests that this earlier material was actually linen (flax). The earliest reference to cotton in recorded literature appears in a Hindu hymn around 800 B.C. Evidence exists that the oil from cottonseed was also used by early Hindus and Chinese (19,20). However, throughout much of history, cultivation of the cotton plant was for fiber; the seeds were generally left to decay and yield fertilizer. In modern history, one of the earliest uses of cottonseed as a by-product of the cotton plant was in 1665, when inhabitants of the British West Indies made oil from the cotton flower to use for medicinal purposes. In 1769, a group of Pennsylvanians exhibited oil expressed from cottonseed and presented a sample of it to the American Philosophical Society to promote its use (21). The value of the residue of the seed after removal of the, oil, ie, cottonseed cake, soon began to generate interest. The Royal Society of Arts of London discussed the value of cottonseed cake as cattle feed and the potential of using sugar mill equipment for expression of the oil. In 1783, to spur the development of this technology, the Society offered a gold medal to any planter in the British West Indies who could produce 1 t of oil and 500 lb of cake within one year. Although this offer was renewed annually for six years no one ever succeeded; the large quantity required was, apparently, too great an obstacle. The difficulty of manually separating seed and fiber caused cotton, and consequently cottonseed, production to be relatively small. However, with the invention of the cotton gin in 1793, this manual operation became mechanized and production of cotton rapidly increased. The problem of disposing the large amount of seed being produced soon became apparent. Although methods did exist for crushing seeds to extract oil, cottonseed presented a particular problem. The kernel, which contains most of the oil, is surrounded by a tough hull making, it difficult to grind. Furthermore, this hull is covered with short fibers that absorb the oil being expressed, thus reducing yields. This changed in 1829 when the first cottonseed hulling machine was patented. That same year, the first cottonseed oil mill was put into production in Petersburg, Va. Because these early hullers applied a grinding action to the seed, screening did not completely separate the hulls, fibers, and meats. In 1857 an improved huller was patented that cut the seed open so that the kernels could fall cleanly out of the hulls. This huller, which uses the same principles as modern hullers, was instrumental in the rapid expansion of the cottonseed crushing industry following the Civil War

During the crushing industry's early years, oil was not much in demand. It was not well suited for illumination, lubrication, or drying purposes and was not particularly desired as an edible oil in the United States. However, a market for edible cottonseed oil did develop in Europe that led to a significant export trade with the United States. In the early 1870s, 9 million gal were being produced in the United States, mostly for export. Later, the discovery that an acceptable substitute for lard could be made by mixing cottonseed oil with certain animal fats greatly increased the domestic market for the oil. By 1883 yearly production of cottonseed oil had reached 15 million gal, most of which was consumed domestically. By the turn of the century, annual production was 115 million gal.

The Asiatic group is grown mainly in China, India, and Pakistan, although cultivars from the New World group are replacing them. *G. hirsutum*, commonly referred to as American Upland Cotton, probably originated in southern Mexico and Guatemala and eventually spread to the southeastern United States. American Upland accounts for most of the U.S. production and 75% of the cotton produced worldwide. Sea Island cotton, the common name for *G. barbadense*, originated in Peru and spread to the Caribbean Islands. It adapted to islands off the coast of Georgia and the Carolinas and to nearby coastal areas of the mainland. Because of its limited growing regions and eventual devastation from the boll weevil, its production in the United States has been abandoned. A variety of *G. barbadense*, called Pima or Egyptian, is still produced in the United States although it accounts for only a small percentage of the U.S. production, its demand has increased in recent years due to its long, silky fibers. This variety, probably developed by crossing *G. barbadense* with *G. hirsutum*, was transferred from the Americas to Egypt and is the only variety of cotton grown in that country. The Egyptian government is very protective of the seed's germplasm, prohibiting export of the seed or import of other varieties.

Composition and Use. The cottonseed kernel makes up about 55% of the seed's weight. The surrounding hull and linters comprise about 32% and 13%, respectively. After processing, the seed yields about 16% crude oil, 45% meal, 9% linters, and 26% hull by weight (22). Most of the oil is in the kernel, which contains 35% oil and 39% protein. The hull contains less than 1% oil and 4% protein. Figure 3 shows a cross section of seeds from two varieties of *G. hirsutum*. The dark specks distributed over the glanded cross section (Fig. 3a) are pigment glands, which contain various material that impart the characteristic yellow-red color to the oil and extracted meal. The major constituent of these glands is gossypol, a yellow polyphenolic pigment found in all parts of the cotton plant, although it is concentrated mostly in the seed. Gossypol is insoluble in water but is soluble in oil, which accounts for the dark color of extracted crude oil. The amount of gossypol in a moisture-free kernel varies for different varieties and growing conditions, generally ranging between 0.39 and 1.7% (23). The presence of gossypol is a major problem in the use of cottonseed as an animal feed. Although cattle, sheep, and other ruminating animals can consume large quantities of raw cottonseed with no ill effects, rabbits and swine are very sensitive and can die from the gossypol's interference with the oxygen-carrying capacity of the blood. Chickens are moderately sensitive; green egg yolks result when chickens are fed too much cottonseed.

The gossypol in ginned seed before any processing treatment is said to be free. Once moist heat is applied to the seed, as is done in expeller processing or in the cooking step before solvent extraction, the gossypol reacts with the protein and is largely inactivated. This form of gossypol is said to be bound. Once bound, the gossypol's toxicity to nonruminating animals and its ability to discolor hen eggs is, for the most part, negated. However, because gossypol binds to the lysine, the quality of the protein is reduced.

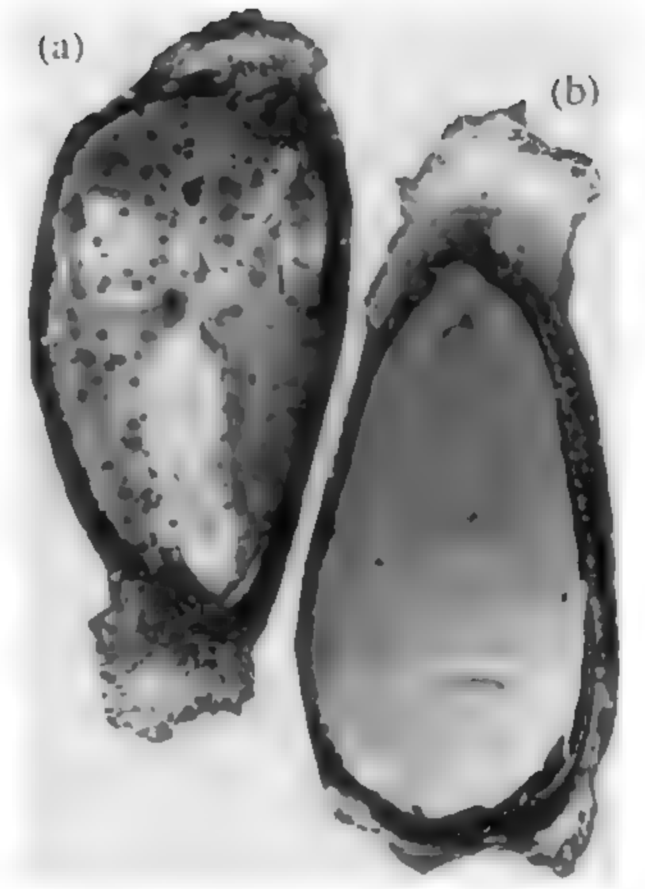


Figure 3. Photograph of a cross section of (a) glanded and (b) glandless cottonseed.

In 1953 a variety of *G. hirsutum* was discovered being grown by the Hopi Indians in Arizona that was free of gossypol glands on the leaves and bolls. The seeds, however, were still glanded. When these Hopi strains were crossed with cultivated Upland cotton, selections with glandless seed were found. After several generations of selecting for a reduced number of glands a true breeding glandless type was produced (24,25) (Fig. 3b). The glandless variety has a number of advantages. The crude oil is much lighter in color and consequently easier and less costly to refine. The meal is completely edible to both ruminants and monogastric animals. A variety of human food products, such as breads and snack items, can be produced from glandless cottonseed flour (26). However, despite the advantages, glandless varieties have not been a commercial success because the glandless varieties increase the potential value of only the seed, not the fiber. Because cottonseed represents less than 10% of the value of the cotton crop, farmers see no reason to shift to new cultivars (with all of the attendant uncertainties) for only a small potential increase in profits.

Of all the oilseeds, cottonseed probably has the most uses. The three major constituents of the seed, ie, kernel, hulls, and linters, all have economic value (27). The crude oil extracted from the kernel is refined and used in an assortment of food products. Snack and fast-food frying oil, salad and cooking oil, salad dressing, shortening, and margarine are major uses. One nonfood use for the refined oil has been as a carrier for agricultural sprays. By-products from the refining process are used in soap manufacture and as a source of fatty acids, which in turn have innumerable industrial uses. The meal is used as feed for beef and dairy cattle, swine, poultry, and fish and as fertilizer. The hulls are used in animal feed, as poultry litter, and as a mulch for soil conditioning. The linters are used in the preparation of high-quality bond paper and as felts for pads, cushions, comforters, and mattresses. They can be made into yarns or used as absorbent cotton for medical purposes. They can be processed so that the cellulose can be used in such diverse products as plastics, food casings, x-ray film, and many other industrial uses.

In recent years, the feeding of whole cottonseed to dairy cows has become increasing popular among dairy farmers (28). In 1981, 72% of the cottonseed production was crushed and 22% was fed as whole seed. In 1988, 64% was crushed and 35% was fed as whole seed (29). The whole seed is high in fat that, after digestion, increases the fat content, and hence the value, of the cow's milk.

Although gossypol has not yet been used commercially, much work has been done to find economically viable uses for it (30). Its antioxidant properties are well known. It also has significant antimicrobial properties. Possibly the area receiving the most attention is gossypol's potential use as a male contraceptive. In 1957 a Chinese researcher reported that a particular village had not had a single childbirth for a 10-year period between 1930s and 1940s. Before and after this time there did not seem to be a birthing problem. The researcher found that because of poor economic times, the villagers had switched to crude cottonseed oil for cooking. He postulated that its use might have caused female infertility. In the early 1970s researchers found it is the male who is actually affected. The gossypol initially reduces sperm motility and subsequently blocks sperm production (31). Much current research is being conducted on gossypol's potential contraceptive uses.

Olive

Description. The olive tree, *Olea europaea* L., is the only member of the Oleaceae family (which contains trees such as ash and shrubs such as lilacs) that is an important food source. The tree is a perennial evergreen, pyramidal in shape, that can grow to 20 m, but under cultivation is pruned to under 5 m. The fruit is fleshy, oblong or crescent in shape, and has a center stone. Olives change in color from green to red to black as they ripen.

Origin and Cultivation. The age of the olive tree is uncertain, but olives have been crushed for their oil for more than 6,000 years. The tree probably originated in Lebanon and Syria. It is believed to have been brought to Italy, through Greece, by Phoenician traders before the end of the fifth century B.C. (4). Olive trees grow in subtropical areas having dry summers and mild winters. For centuries, olives and olive oil have been among the most important agricultural products of Italy, Spain, Greece, and northern Africa.

Composition and Uses. The components of the olive are the edible pulp, which makes up 65–85% of the ripe fruit; the pit which consists of 13–23%; and the seed inside the pit, which is about 2–3% of the fruit. Olives have a high oil content but little protein. Green table olives are unripe and must be soaked in lye to remove oleuropein, a bitter principle, before pickling in brine. Ripe black olives do not need caustic treatment.

Oil is removed from olives by pressing. The oil obtained from the first pressing is called virgin. Virgin olive oil, which is used with no refining, is considered to be the highest quality salad and cooling oil. A second pressing of the olive yields an oil of lesser quality that must be refined, bleached, and deodorized. The residue from pressing can be solvent extracted to remove the remaining oil. The remaining olive cake has no feed use, because of its low protein content, and consequently is used as a soil conditioner.

Palm and Palm Kernel

Description. The oil palm, *Elaeis guineensis* Jacq., has a single stem that grows to 35 m in height and is topped by leaves typically 7 m in length. About six months after pollination, the fruit matures. It consists of a reddish orange oily pulp surrounding a kernel. The fruit, which is oval or pear shaped, about 3 × 5 cm, and weighs up to 30 g, grows in bunches in the axil of the leaves. These bunches can contain 1,500 fruits and weigh 20 kg. An adult palm tree is capable of producing 12 bunches per year. The trees are commercially useful for about 30 years.

Origin and Cultivation. *E. guineensis* Jacq. originated in west Africa along the Guinea coast. It was spread to other tropical regions by 15th-century Portuguese explorers. Palm oil, however, did not enter world trade until the end of the 18th century, making it one of the youngest of the major vegetable oils. Malaysia and Indonesia are the major producers of palm oil.

Composition and Uses. Both the pulp and kernel yield oil; each has a different fatty acid composition (Table 2). The pulp makes up 60–90% of the fruit's weight. On a dry weight basis, more than 70% of the pulp and 40% of the kernel consists of oil. A fruit bunch will yield about 20% palm oil and 2% palm kernel oil. Because palm oil is a relatively saturated oil, its food uses are mostly as shortening and frying oil. It is also used in the manufacture of soaps and fatty acids. Palm kernel oil is similar in composition and use to coconut oil.

Peanut

Description. *Arachis hypogaea* L., called peanut or groundnut, is a member of the family Leguminosae. Many varieties exist, but two general types are grown commercially. One is an upright plant with a single central stem reaching 30 cm in height and numerous upright branches. The second is a recombinant type, reaching 20 cm in height, with numerous creeping branches. The upright plant is better suited to mechanical harvesting. In all varieties of the peanut plant, small flowers form at the end of auxiliary branches called pegs. A few days after fertilization, the pegs push the flowers into the ground, where the peanut pod develops. At maturity, the pod contains one to three nuts or kernels.

Origin and Cultivation. The peanut originated in eastern South America. Evidence exists of its cultivation in Peru around 2000–3000 B.C.; it probably has a much longer history of domestication (9). By the time of the Columbus expedition in 1492 the peanut plant had spread to Central America and the Caribbean. Early European explorers probably brought the plant from Brazil to West Africa, from where it spread to India. Many varieties of peanuts now grown in the United States come from stocks developed in Africa. The plant requires sunshine and high temperatures. It can grow as far north as Canada and as far south as southern Argentina.

Composition and Uses. Most of the world production of peanuts is processed for recovery of the oil for edible use. Peanut oil, typically produced by solvent extraction, has a high smoke point, about 227°C, making it a good frying oil. However, peanuts are one of the few oil crops that can be used directly as food. They are among the highest protein content oilseeds (Table 1) and thus are nutritionally desirable. Eaten whole after shelling and oil- or dry-roasting (the terms used for deep-fat frying or hot-air cooking, respectively), they are one of the most popular snack foods in the United States. Peanuts are also used in a variety of confections, candy bars, and baked goods. About 25% of the U.S. supply of peanuts is processed into peanut butter, which is recognized as a nutritious, satisfying, and stable food and snack item for people in all age groups.

Rapeseed–Canola

Description. Rape refers to the oilseed forms of *Brasica napus* L. and *Brassica campestris* (32), which are in the same family as mustard seeds and are closely related to cabbage, cauliflower, broccoli, and other cole vegetables. The round seeds, about 0.1 cm in diameter, contain approximately 40% oil and yield a meal with more than 40% protein The meal is used primarily as a feed supplement for livestock and poultry

Origin and Cultivation. This family of oilseeds appears to have evolved separately in the Himalayan region of Asia and in the Mediterranean area of Europe. Sanskrit writings of 2000–1500 B.C. specifically mention the use of rapeseed oil for cooking and illumination; Greek, Roman, and Chinese writings between 500 and 200 B.C. ascribe medicinal value to them. It has been cultivated in Europe since about A.D. 1200. The Canadian rapeseed industry, the world's largest, started after World War II. By 1955 rapeseed oil was being used in Canada by major processors for salad oils (33).

Because rapeseed can survive at relatively low temperatures, they are one of the few vegetable oil sources that can be successfully cultivated in the colder temperate regions. For this reason they have become a major crop in Canada and throughout Europe. This characteristic also makes it possible for them to be cultivated as a winter crop in the subtropics. The winter form of rapeseed, typically found in Europe and Asia, is normally sown in August to September and is harvested in July. The summer form, found in Canada and Europe, is sown in April to May and harvested in September.

Composition and Use. Rapeseed oil differs from other vegetable oils because it contains significant quantities of eicosenoic and erucic fatty acids (Table 2). Studies since 1949 showed that erucic acid was poorly metabolized by rats and caused much physiological damage. In July 1956 the Canadian Department of National Health and Welfare ruled that rapeseed oil was not approved for edible purposes in Canada and all sales of oil for edible purposes were to cease immediately. In October of that same year the ban was rescinded, pending a thorough review, because of lack of evidence of harm to humans. Even though the ban was never implemented, the perception remained that health problems could arise from rapeseed oil consumption. Because of this, breeders began work to produce new varieties with reduced erucic acid content. In 1968 Oro, a variety of *B. napus*, was the first of several low-erucic-acid rapeseeds to be released. Industry began changing to these new rapeseed cultivars in the early 1970s. To differentiate the new oil composition, the terms low-erucic acid rapeseed (LEAR) and Canbra have been used. Sinola is the term used in the FGR for rapeseed containing less than 2% erucic acid. Most countries currently use the name canola for low-erucic acid rapeseed (Table 2).

Because rapeseed oil is increasing in importance for both edible and industrial uses in the United States, the U.S. Department of Agriculture is currently formulating standards for rapeseed. The proposed standards require that rapeseed oil with an erucic acid content of greater than 40% be used for industrial purposes only. Rapeseed oil with no greater than 2% erucic acid is edible and is called canola oil. Rapeseed oil containing between 2 and 40% erucic acid has little known commercial value. Although canola is used exclusively as an edible oil, rapeseed oil's high viscosity has traditionally made it a favorable lubricant for metal surfaces (34). However, where it was once used extensively for lubrication of steam locomotives and marine engines, synthetic derivatives are now used. Long-chain fatty amides of erucic acid are good plasticizers for vinyl chloride resins.

Soybean

Description. The cultivated soybean, *Glycine max* (L.) Merr., is an annual legume. It is usually erect, about 75 cm in height, and produces pods throughout its many branches. These pods, typically 2–10 cm in length, contain three yellow round seeds each about 0.5–1 cm in diameter.

Origin and Cultivation. *G. max* probably evolved from the wild species *Glycine soja* Sieb. and Zucc. Soy is sometimes referred to as one of the oldest cultivated crops. However, the earliest written record of it was 11th century B.C., long after the domestication of sesame in the Middle East. Although soybeans were first domesticated in northeastern China, the most recent evidence suggests that the plant originated in Australia (9). Seeds obtained from China were planted in Europe in the 1700s. In the early 1800s soybeans were introduced to the United States and later that century to Brazil. Not until the early 20th century was oil expressed from domestic soybeans in the United States, now the world's largest producer. Soybeans have only recently become important in Brazil; that country, the United States, and China are the world's major producers. Although soybean is a warm-temperature plant, it adapts well to temperate climates. Canada, the former USSR, South Africa, and Australia are some of the 35 countries that commercially produce soybeans.

Composition and Uses. Soybeans have the highest protein content of all the oilseeds. Processing the whole bean for food use is particularly popular in Asia. Soymilk, made by grinding beans that have been soaked in water, is consumed directly or used as a base for making tofu. Soy sauce is prepared by fermenting soybeans. The oil, obtained by solvent extraction, has many uses. Most of the production is consumed as salad oil, cooking oil, and margarine. It is also used in a variety of prepared foods such as frozen desserts, confections, and coffee whiteners (creamers). Because of its high linolenic acid content, soybean oil is considered a semidrying oil and has a variety of industrial uses. The major nonfood markets for the oil are in paints, resins, and plastics. Even though soybean oil is the dominant vegetable oil worldwide, the major economic value of soybean is in the protein. Defatted soybean meal is used mostly as animal feed; some, however, is also processed into flour, protein concentrate, and protein isolate, used in the manufacture of prepared foods.

Sunflower

Description. *Helianthus annuus* L. is the most common of the 67 species of sunflower and the only one grown commercially for oilseed production. A member of the family Compositae, the sunflower, is related to daisies, asters, marigolds, and dandelions. Although sunflower stems may reach heights of 5 m and have heads as large as 0.75 m in diameter (34), they typically are 1–3 m high with heads 0.3 m in diameter. The diameter of the stem is 3–6 cm. The head is actually a composite of 1,000–4,000 little flowers (florets) and will produce several hundred seeds. The head of the sunflower is heliotropic, ie, it turns to face the sun, until the majority of the flowers are fertilized, then it remains facing east. The easterly exposure minimizes the plant's temperature, which helps the seeds to set (9). The seed, more properly called an achene, is oblong and flattish, about 1.0–2.5 cm long, 0.75–1.5 cm wide, and 0.3–0.75 cm thick.

Origin and Cultivation. Sunflower seeds have been found at several archaeological sites in Mexico and southern and western United States. Although evidence exists of early precontact native Americans gathering wild sunflowers and making meal from the seeds, there is no evidence that they cultivated the plant. Even though corn, squash, and beans were cultivated, the prolific nature of wild sunflowers may have obviated the need to cultivate them. Around the 1500s, several native American nations were cultivating sunflower, although not nearly as much as they were cultivating corn. This is reasonable because corn is one of the best food plants known to humans.

The sunflower, like many other indigenous American plants, was taken back to Europe by early explorers. The first study of the sunflower, written by a Belgian herbalist (35), was published in 1568, and by 1616 sunflowers were common in England. Europeans did not at first know the uses for sunflower. In 1783 it was reported that not only are the seeds an excellent poultry feed but are also easily expressed to yield a good-quality oil. When sunflowers spread to Russia in the 1800s, the seeds became immensely popular there as a food source. This was because, during the forty days before Easter and the forty days before Christmas, the Russian Orthodox Church observed strict dietary guidelines. Almost all foods rich in oil were not to be eaten during these periods. Because the sunflower had only recently entered the country, it was not one of the prohibited foods. The people took advantage of this loophole and Russia became the foremost producer of sunflowers.

Although important in Europe, sunflowers were little cultivated by early American settlers. American seed companies did not offer sunflower seeds until Russian varieties were introduced in the 1880s. Even then, they never achieved great popularity. Only recently has the sunflower become an important oil crop in the United States. Ironically, even though the sunflower originated in the western hemisphere, USSR-cultivated varieties are the ones used almost exclusively throughout the world. Sunflower is commercially produced in warm to temperate regions. It grows well in the 20–28°C range. The former USSR is the major producer of sunflowers, although in recent years it has gained popularity in many countries. In the United States it is grown mainly in Minnesota and North Dakota.

Composition and Use. The oil content of the seed can vary from a low of 24%, caused by adverse growing conditions, to a high of 65% for experimental strains. The commercial seed typically contains about 40% oil. Its protein content is usually between 15 and 20% (Table 1). Probably the oldest use of sunflower seeds is in whole form as human food. In the former USSR they are eaten as commonly as peanuts are in the United States. In recent years, whole sunflower seeds have become increasingly popular in the United States because of the general increased interest of health food conscious consumers in whole grains and dietary fiber. Most of the commercial production of sunflower seeds goes into the manufacture of oil and animal feed. Because of its relatively high iodine number (ca 130) sunflower is considered a semidrying oil. As such, it can be used in the formulation of paints and for other industrial uses. It is, however, much more popular as a food and is considered by some as desirable a salad oil as olive. It is also used in cooking, frying, and in the manufacture of margarine and shortening. The meal left after oil removal is usually used as animal feed. In Canada and the former USSR the hulls are pressed into logs and used as fuel. The hulls are used in the manufacture of ethanol and furfural, as plywood filler, and in the production of yeast.

BIBLIOGRAPHY

1. U.S. Department of Agriculture, *Composition of Foods: Nut and Seed Products*, Agricultural Handbook No. 8–12, U.S. Government Printing Office, Washington, D.C., 1984.
2. K. N. Wright, "Nutritional Properties and Feeding Value of Corn and Its By-products," in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.
3. N. O. V. Sonntag, "Composition and Characteristics of Individual Fats and Oils," in D. Swern, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 1, 4th ed., Wiley-Interscience, New York, 1979.
4. J. M. Martinez Suarez and J. A. Mendoza, "Olive Oil Processing and Related Aspects. Extraction and Refining: General Aspects," in A. R. Baldwin, ed., *Proceedings World Conference on Emerging Technologies in the Fats and Oils Industry, Cannes, France, November 3–8, 1985*, American Oil Chemists' Society, Champaign, Ill., 1986.
5. A. L. Winton and K. B. Winton, *Structure and Composition of Foods*, John Wiley & Sons, Inc., New York, 1932.
6. U.S. Department of Agriculture, *Composition of Foods: Legumes and Legume Products*, Agricultural Handbook No. 8–16, U.S. Government Printing Office, Washington, D.C., 1986.
7. E. M. Ahmed and C. T. Young, "Composition, Quality, and Flavor of Peanuts," in H. E. Pattee and C. T. Young, eds., *Peanut Science and Technology*, American Peanut Research and Education Society, Inc., Yoakum, Tex., 1982.
8. W. J. Pigden, "World Production and Trade of Rapeseed and Rapeseed Products," in J. K. G. Kramer, F. D. Sauer, and W. J. Pigden, eds. *High and Low Erucic Acid Rapeseed Oils*, Academic Press, Inc., Orlando, Fla., 1983.
9. E. A. Weiss, *Oilseed Crops*, Longman Inc., New York, 1983.
10. J. K. G. Kramer and F. D. Sauer, "Cardiac Lipid Changes," in Ref. 8.
11. R. Sreenivasan, "Component Fatty Acids and Composition," *Journal of the American Oil Chemists' Society* **45**, 259–265 (Apr. 1968).
12. F. T. Orthoefer and R. D. Sinram, "Corn Oil: Composition, Processing, and Utilization," in Ref. 2.
13. U.S. Department of Agriculture, *Composition of Foods: Fats and Oils*, Agricultural Handbook No. 8-4, U.S. Government Printing Office, Washington, D.C., 1978.
14. C. Galli and E. Fedeli, eds., *Fat Production and Consumption*, NATO ASI Series, Plenum Press, New York, 1987.
15. F. A. Norris, "Extraction of Fats and Oils," in D. Swern, ed., *Bailey's Oil and Fat Products*, Vol. 2, 4th ed., Wiley-Interscience, New York, 1982.
16. K. Satyabalan, "Coconut," in G. Röbbelen, R. K. Downey, and A. Ashri eds., *Oil Crops of the World*, McGraw-Hill, Inc., New York, 1989.
17. S. A. Watson, "Structure and Composition," in Ref. 2.
18. G. O. Benson and R. B. Pearce, "Corn Perspective and Culture," in Ref. 2.
19. A. N. Gulati and A. J. Turner, *A Note on the Early History of Cotton*, Indian Central Cotton Committee, Bulletin No. 17, Bombay, India, 1928.
20. M. R. Cooper, "History of Cotton and U.S. Cottonseed Industry," in A. E. Bailey, ed., *Cottonseed and Cottonseed Products*, Interscience Publishers, Inc., New York, 1948.
21. R. C. Curtis, *Cottonseed Meal: Origin, History, Research*, Robert S. Curtis Co., Raleigh, N.C., 1938.
22. J. P. Cherry, "Cottonseed Oil," *Journal of the American Oil Chemist's Society* 60, 360–367 (Feb. 1983).
23. L. C. Berardi and L. A. Goldblatt, "Gossypol," in I. E. Liener, ed., *Toxic Constituents of Plant Foodstuffs*, 2nd ed., Academic Press, Inc., Orlando, Fla., 1980.
24. S. C. McMichael, "Hopi Cotton, A Source of Cottonseed Free of Gossypol Pigments," *Agronomy Journal* **51**, 630 (1959).
25. P. A. Miller, "Genetic Basis and Breeding Procedures for Glandless Seeded Cotton," in *Glandless Cotton: Its Significance, Status, and Prospects: Proceedings of a Conference, ARS-USDA and National Cottonseed Products Association, Dallas, Tex., Dec. 13–14, 1977*. Agricultural Research Service-Southern Region, USDA, New Orleans, La., 1978.
26. D. C. Blankenship and B. B. Alford, *Cottonseed. The New Staff of Life*, TWU Press, Denton, Tex., 1983.
27. National Cottonseed Products Association, *Cottonseed and Its Products*, 9th ed., NCPA, Inc., Memphis, Tenn., 1989.
28. A. J. Kutches, W. Chalupa, and J. Trei, "Delinted Cottonseed Improves Lactational Response," *Feedstuffs* 16–17 (Aug. 17, 1987).
29. U.S. Department of Agriculture, *Oil Crops Situation and Outlook Report*, Economic Research Service, USDA, OCS-21, 37 (Apr. 1989).
30. R. J. Hron, Sr., S. P. Koltun, J. Pominski, and G. Abraham, "The Potential Commercial Aspects of Gossypol," *Journal of the American Oil Chemists' Society* **64**, 1315–1319 (Sept. 1987).
31. S. Z. Qian and Z. G. Wang, "Gossypol: A Potential Antifertility Agent for Males," *Annual Reviews of Pharmacology and Toxicology* **24**, 329–360 (1984).
32. R. K. Downey, "The Origin and Description of the *Brassica* Oilseed Crops" in Ref. 8.
33. J. M. Bell, "From Rapeseed to Canola: A Brief History of Research for Superior Meal and Edible Oil," *Poultry Science* **61**, 613–622 (1982).
34. J. S. R. Ohlson, "Rapeseed Oil," *Journal of the American Oil Chemists' Society* **60**, 337A–338A (Feb. 1983).
35. C. B. Heiser, *The Sunflower*, University of Oklahoma Press, Norman, Okla., 1976.

GENERAL REFERENCES

B. Alford, G. U. Liepa, and A. D. VanBeber, "Cottonseed Protein: What Does the Future Hold?," *INFORM* **5**, 1151–1155 (1994).

Anonymous, "Modern Practices in Cottonseed Oil Extraction," *Oils and Fats Int.* **5**, 32–33 (1989).

Anonymous, "Soybean-Based Tofu Production Develops in U.S.," *Oil Mill Gazetter* **103**, 20 (1997).

S. Berot et al., "Cottonseed Protein-Rich Products from Glandless African Varieties, 1. Pilot-Plant Scale Production of Protein Concentrates," *Sciences des Aliments* **15**, 203–215 (1995).

J. L. Bourely, "Development of Glandless Cottonseed as a Food Plant, Technological and Nutritional Aspects," *Sciences des Aliments* **10**, 485–514 (1990).

E. C. Canapi et al., "Coconut Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 125–158.

Y. B. M. Che et al., "Aqueous Enzymatic Extraction of Coconut Oil," *J. American Oil Chemists' Society* **73**, 683–686 (1996).

G. Cole et al., "New Sunflower and Soybean Cultivars for Novel Vegetable Oil Types," *Fett/Lipid* **100**, 177–181 (1998).

M. G. Conception et al., "Chemical Characterization of Commercial Soybean Products," *Food Chem.* **62**, 325–331 (1998).

H. E. Davidson et al., "Sunflower Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 603–689.

D. D. Duxbury, "Genetic Engineering Alters Fat Composition of Canola Oil," *Food Processing* **53**, 45–46 (1992).

N. A. M. Eskin and B. E. McDonald, "Canola Oil," *Br. Nutrition Foundation Nutrition Bull.* **16**, 138–146 (1991).

N. A. M. Eskin et al., "Canola Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 1–95.

R. A. Ferrari, W. Esteves, and G. E. Plonis, "Triglyceride Composition of Corn Oil," *Ciencia e Tecnologia de Alimentos* **13**, 184–193 (1993).

L. Forman, S. Matthews, and C. C. King, "New Food, Feed Uses for Glandless Cottonseed," *INFORM* **2**, 737–739 (1991).

A. S. Guemueskesen and T. Cataloz, "Chemical and Physical Changes in Cottonseed Oil During Deodorization," *J. American Oil Chemists' Society* **69**, 392–393 (1992).

E. Hernandez and E. W. Lusas, "Trends in Transesterification of Cottonseed Oil," *Food Technol.* **51**, 72, 74–76 (1997).

J. Hounhouigan et al., "Revival of Coconut Oil Production Through the Hot Oil Immersion Drying Technique," *Plantations Recherche Development* **5**, 111–118 (1998).

L. A. Jones and C. C. King, "Cottonseed Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 159–240.

A. P. Kiritsakis, *Olive Oil: From the Tree to the Table*, Food and Nutrition Press, Trumbull, Conn., 1998.

D. M. Klockman, R. Toledo, and K. A. Sims, "Isolation and Characterization of Defatted Canola Meal Protein," *J. Ag. and Food Chem.* **45**, 3867–3870 (1997).

D. Kritchevsky, S. A. Tepper, and D. M. Klurfield, "Lectin May Contribute to the Athergenicity of Peanut Oil," *Lipids* **33**, 821–823 (1998).

M. Naczk et al., "Current Developments of Polyphenolics of Rapeseed/Canola: A Review," *Food Chem.* **52**, 489–502 (1998).

J. R. K. Niemela, I. Wester, and R. M. Lahtinen, "Industrial Frying Trials with High Oleic Sunflower Oil," *Grasas y Aceites* **47**, 1–4 (1996).

J. M. Rouanet et al., "Comparative Study of Nutritional Qualities of Defatted Cottonseed and Soybean Meals," *Food Chem.* **34**, 203–213 (1989).

E. E. Sipos and B. E. Szuhaj, "Soybean Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 497–601.

K. D. Tano and Y. Ohta, "Aqueous Extraction of Coconut Oil by an Enzyme-Assisted Process," *J. Sci. of Food and Ag.* **74**, 497–502 (1997).

M. Tsimidou, "Polyphenols and Quality of Virgin Olive Oil in Retrospect," *Ital. J. Food Sci.* **10**, 99–116 (1998).

R. E. Wilson, "New Commodity Products from Soybean Through Biotechnology," *Oil Mill Gazetter* **104**, 27–33 (1998).

C. T. Young, "Peanut Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 337–392.

G. ABRAHAM
R. J. HRON
U.S. Department of Agriculture
Southern Regional Research Center

OLESTRA. See FATS AND OILS: SUBSTITUTES.

OLIVES AND OLIVE OIL

The cultivated olive (*Olea europaea*, Oleaceae) is a long-lived evergreen tree native to the Mediterranean basin. It is valued for its fruit and oil. The world production of olives in 1998 was 12,780,055 t (see Table 1). The Mediterranean countries, with ~9,000,000 ha dedicated to this cultivar, accounted for 95% of the production. In the Mediterranean, 90% of the olive trees are grown for oil. World olive oil production is currently increasing; the commercial crop during 1998 was 2,527,929 t (see Table 2), with the European Union (EU) producing around 1,876,000 t (Spain 51%, Italy 24%, and Greece 23%), Tunisia 170,000 t, and Turkey 190,000 t of oil. World table-olive production in

Table 1. World Production of Olives by Country for the Year 1998

Country	Production (t)	% World production
Afghanistan	900	0.01
Albania	40,000	0.31
Algeria	124,060	0.97
Argentina	91,940	0.72
Australia	1,000	0.01
Azerbaijan	300	<0.01
Brazil	4	<0.01
Chile	12,500	0.10
China	2,890	0.02
Croatia	10,405	0.08
Cyprus	9,000	0.07
Egypt	210,000	1.64
El Salvador	3,500	0.03
France	13,500	0.11
Gaza Strip	3,000	0.02
Greece	1,879,430	14.71
Iran	24,782	0.19
Iraq	13,000	0.10
Israel	18,700	0.15
Italy	2,232,000	17.46
Jordan	75,000	0.59
Kuwait	18	<0.01
Lebanon	98,000	0.77
Libya	58,000	0.45
Malta	10	<0.01
Mexico	7,205	0.06
Morocco	450,000	3.52
Peru	31,138	0.24
Portugal	287,000	2.25
Slovenia	235	<0.01
Spain	3,800,000	29.73
Syria	763,186	5.97
Tunisia	1,000,000	7.82
Turkey	1,300,000	10.17
United States	86,180	0.67
Uruguay	3,000	0.02
West Bank	130,000	1.02
Yugoslavia	172	<0.01
World	12,780,055	100.00

Source: FAOSTATS, Statistical database, Food and Agriculture Organization of the United Nations, 1998, *http://apps.fao.org*.

Y. B. M. Che et al., "Aqueous Enzymatic Extraction of Coconut Oil," *J. American Oil Chemists' Society* **73**, 683–686 (1996).

G. Cole et al., "New Sunflower and Soybean Cultivars for Novel Vegetable Oil Types," *Fett / Lipid* **100**, 177–181 (1998).

M. G. Conception et al., "Chemical Characterization of Commercial Soybean Products," *Food Chem.* **62**, 325–331 (1998).

H E. Davidson et al., "Sunflower Oil," in Y. H Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp 603–689.

D. D. Duxbury, "Genetic Engineering Alters Fat Composition of Canola Oil," *Food Processing* **53**, 45–46 (1992).

N. A. M. Eskin and B. E. McDonald, "Canola Oil," *Br. Nutrition Foundation Nutrition Bull.* **16**, 138–146 (1991).

N A. M. Eskin et al., "Canola Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 1–95.

R. A. Ferrari, W. Esteves, and G. E. Plonis, "Triglyceride Composition of Corn Oil," *Ciencia e Tecnologia de Alimentos* **13**, 184–193 (1993).

L. Forman, S. Matthews, and C. C. King, "New Food, Feed Uses for Glandless Cottonseed," *INFORM* **2**, 737–739 (1991).

A. S. Guemueskesen and T. Cataloz, "Chemical and Physical Changes in Cottonseed Oil During Deodorization," *J. American Oil Chemists' Society* **69**, 392–393 (1992).

E. Hernandez and E. W. Lusas, "Trends in Transesterification of Cottonseed Oil," *Food Technol.* **51**, 72, 74–76 (1997).

J. Hounhouigan et al., "Revival of Coconut Oil Production Through the Hot Oil Immersion Drying Technique," *Plantations Recherche Development* **5**, 111–118 (1998).

L. A. Jones and C. C. King, "Cottonseed Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 159–240.

A. P. Kiritsakis, *Olive Oil: From the Tree to the Table*, Food and Nutrition Press, Trumbull, Conn., 1998.

D. M. Klockman, R. Toledo, and K. A. Sims, "Isolation and Characterization of Defatted Canola Meal Protein," *J. Ag. and Food Chem.* **45**, 3867–3870 (1997).

D. Kritchevsky, S. A. Tepper, and D. M. Klurfield, "Lectin May Contribute to the Athergenicity of Peanut Oil," *Lipids* **33**, 821–823 (1998).

M. Naczk et al., "Current Developments of Polyphenolics of Rapeseed/Canola: A Review," *Food Chem* **52**, 489–502 (1998).

J. R. K. Niemela, I. Wester, and R. M. Lahtinen, "Industrial Frying Trials with High Oleic Sunflower Oil," *Grasas y Aceites* **47**, 1–4 (1996).

J. M. Rouanet et al., "Comparative Study of Nutritional Qualities of Defatted Cottonseed and Soybean Meals," *Food Chem.* **34**, 203–213 (1989).

E. E. Sipos and B. E. Szuhaj, "Soybean Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 497–601.

K. D. Tano and Y. Ohta, "Aqueous Extraction of Coconut Oil by an Enzyme-Assisted Process," *J. Sci. of Food and Ag.* **74**, 497–502 (1997).

M. Tsimidou, "Polyphenols and Quality of Virgin Olive Oil in Retrospect," *Ital. J. Food Sci* **10**, 99–116 (1998).

R. E. Wilson, "New Commodity Products from Soybean Through Biotechnology," *Oil Mill Gazetter* **104**, 27–33 (1998).

C. T. Young, "Peanut Oil," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 2, 5th ed., John Wiley & Sons, New York, 1996, pp. 337–392.

G. Abraham
R. J. Hron
U.S. Department of Agriculture
Southern Regional Research Center

OLESTRA. See Fats and oils: substitutes.

OLIVES AND OLIVE OIL

The cultivated olive (*Olea europaea*, Oleaceae) is a long-lived evergreen tree native to the Mediterranean basin. It is valued for its fruit and oil. The world production of olives in 1998 was 12,780,055 t (see Table 1). The Mediterranean countries, with ~9,000,000 ha dedicated to this cultivar, accounted for 95% of the production. In the Mediterranean, 90% of the olive trees are grown for oil. World olive oil production is currently increasing; the commercial crop during 1998 was 2,527,929 t (see Table 2), with the European Union (EU) producing around 1,876,000 t (Spain 51%, Italy 24%, and Greece 23%), Tunisia 170,000 t, and Turkey 190,000 t of oil. World table-olive production in

Table 1. World Production of Olives by Country for the Year 1998

Country	Production (t)	% World production
Afghanistan	900	0.01
Albania	40,000	0 31
Algeria	124,060	0.97
Argentina	91,940	0.72
Australia	1,000	0.01
Azerbaijan	300	<0.01
Brazil	4	<0.01
Chile	12,500	0.10
China	2,890	0.02
Croatia	10,405	0.08
Cyprus	9,000	0.07
Egypt	210,000	1.64
El Salvador	3,500	0.03
France	13,500	0.11
Gaza Strip	3,000	0.02
Greece	1,879,430	14.71
Iran	24,782	0.19
Iraq	13,000	0.10
Israel	18,700	0.15
Italy	2,232,000	17.46
Jordan	75,000	0.59
Kuwait	18	<0.01
Lebanon	98,000	0.77
Libya	58,000	0.45
Malta	10	<0.01
Mexico	7,205	0.06
Morocco	450,000	3.52
Peru	31,138	0.24
Portugal	287,000	2.25
Slovenia	235	<0 01
Spain	3,800,000	29.73
Syria	763,186	5.97
Tunisia	1,000,000	7.82
Turkey	1,300,000	10.17
United States	86,180	0.67
Uruguay	3,000	0.02
West Bank	130,000	1.02
Yugoslavia	172	<0.01
World	12,780,055	100.00

Source: FAOSTATS, Statistical database, Food and Agriculture Organization of the United Nations, 1998, *http://apps.fao.org*.

Table 2. World Production of Olive Oil by Country for the Year 1998

Country	Production (t)	% World production
Afghanistan	73	<0.01
Albania	2,500	0.10
Algeria	46,000	1.82
Argentina	11,500	0.45
Australia	90	<0.01
Chile	1,500	0.06
Croatia	1,572	0.06
Cyprus	1,200	0.05
El Salvador	525	0.02
France	2,000	0.08
Greece	430,000	17.01
Iran	1,650	0.07
Israel	23	<0.01
Italy	450,000	17.80
Jordan	14,100	0.56
Lebanon	6,500	0.26
Libya	10,400	0.41
Malta	2	<0.01
Mexico	400	0.02
Morocco	70,000	2.77
Portugal	44,000	1.74
Slovenia	470	0.02
Spain	950,000	37.58
Syria	110,000	4.35
Tunisia	170,000	6.72
Turkey	190,000	7.52
United States	490	0.02
West Bank	12,904	0.51
Yugoslavia	30	<0.01
World	2,527,929.24	100.00

Source: FAOSTATS, Statistical database, Food and Agriculture Organization of the United Nations, 1998, *http://apps.fao.org*.

Table 3. World Production of Table Olives for 1991–1992

Country	Production (thousand t)	% World production
Spain	230.0	25.4
Italy	130.0	14.3
Turkey	110.0	12.1
Morocco	90.0	9.9
Greece	85.0	9.4
Syria	50.0	5.5
United States	50.0	5.5
Argentina	30.0	3.3
Portugal	16.0	1.8
Israel	15.0	1.7
Tunisia	14.0	1.5
Jordan	13.0	1.4
Algeria	11.0	1.2
Peru	10.0	1.1
Egypt	9.0	1.0
Chile	9.0	1.0
Mexico	8.0	0.9
Cyprus	7.0	0.8
Other	7.0	0.8
Lebanon	4.0	0.4
Libya	3.5	0.4
Australia	2.0	0.2
France	2.0	0.2
Brazil	1.0	0.1
Yugoslavia	0.5	0.1
Total	907.0	100.0

Source: FAOSTATS, Statistical database, Food and Agriculture Organization of the United Nations, 1998, *http://apps.fao.org*.

1992 was 907,000 t. According to 1992 statistics, Spain is the world's largest producer (25.4%) and exporter of table olives, followed by Italy (14.3%), Turkey (12.1%), Morocco (9.9%), Greece (9.4%), Syria (5.5%), and the United States (5.5%). In Australia, Chile, China, Mexico, New Zealand, and South Africa, olives are considered a new crop (see Table 3).

The olive oil and table-olive industries play an important role in the agricultural and processing sectors of the major olive-producing countries. Most olive oil is consumed within the Mediterranean countries; only 18% of production enters world trade. On average, the world olive oil market represents ~6% of the quantity and ~23% of the value of the world trade in fluid edible oils. To a large extent olive oil does not compete with other vegetable oils but occupies a specialty niche market.

THE OLIVE TREE

Origin and Historical Evolution

The olive tree has a wide range of adaptability. It requires a mild climate with warm summers and cold winters. The tree needs substantial chilling for good fruiting (1) but is injured when temperatures fall below −10°C. Olive is considered a drought-resistant species because it thrives in areas where water stress is frequent, such as Mediterranean climates. It has been postulated that the minimum water requirement for olive is 2,000 m^3/ha per year, mainly during flowering and fruit setting in late spring and again in the summer as the fruit increases in size (2). Olive trees will grow on poor soils and rocky hillsides, but deep soils produce the best-quality fruit. They tolerate saline or alkaline soils and those with a high lime content. Their root system is relatively shallow and will not tolerate waterlogged soils.

The first reference to the existence of the olive tree is found in the Old Testament Book of Genesis, where the flight of the dove with an olive branch announces the end of the Flood. The origin of the cultivation of the olive tree is known through legends and tradition. It can be situated within a wide strip of land in the Mediterranean area (3) and adjacent zones comprising Asia Minor and parts of India, Africa, and Europe. However, the botanical ancestor of the olive tree is not precisely known (4). The two possible candidates are the oleaster *Olea sylvestris* and *O. chrisolphylla*, both of which might have had a common ancestor that covered most of the Sahara desert before the last glaciation. There also different hypotheses regarding the spread of its cultivation in the Mediterranean basin. This might have involved originally the Phoenicians and later the Greeks and the Romans. At the end of the Roman Empire, the olive trees were cultivated throughout the Arab and Roman worlds (5).

During the fifteenth and sixteenth centuries, colonizers and Spanish Franciscan monks extended the planting of the olive tree to various parts of the New World, finally reaching California during the eighteenth century. In the same manner, Italian and Spanish emigrants and missionaries spread the olive tree to Australia, South Africa, and Japan, completing its extension in both hemispheres. It is now distributed approximately between lat. 25 and 45° N and 15 and 35° S.

The Fruit

Composition and Changes during Growth and Maturation. The fruit of the olive tree is an edible, fleshy drupe, more or less oblong according to the variety, of a green color that changes to purple or black when mature and reaches a weight range of 1.5 to 12 g. The length of the fruit is generally between 2 and 3 cm and its transverse diameter between 1 and 2 cm. The specific weight is close to unity. The percentage of flesh, intensely bitter mainly when still green, varies between 70 and 90% of the fruit. The pit or stone represents 10 to 30% of the fruit, according to the variety, extent of growth, and maturity. The seed it contains accounts for less than 10% of the weight of the stone.

All the physical characteristics mentioned herein as well as the chemical composition of the edible flesh depend on several factors, among which the predominant are variety and degree of growth and ripeness when the fruits are harvested (6,7). Table 4 gives an overview of the composition of the fruit.

The main quantitative constituents of the flesh are water and oil, which show an inverse relationship for the same degree of maturity. The moderate degree of saturation of their fatty acids, and specifically, the high content of oleic acid, contributes to the fact that olive oil and table olives are considered high in biological and nutritive value. Water represents the main constituent of the olive fruit, accounting for up to 70% of its weight. Water serves as a solvent for water-soluble substances, including organic acids, tannins, and oleuropein.

Table 4. Composition of the Fruit of the Olive Tree and of the Fresh Pulp

			Weight %
	Fruit		
	Epicarp		
Pericarp		Pulp	70–90
	Mesocarp		
		Stone	9–27
Endocarp			
		Seed	1–3
	Pulp		
Moisture			50–75
Lipids (oil)			6–30
Reducing sugars, soluble			2–6
Nonreducing sugars, soluble			0.1–0.3
Crude protein (N × 6.25)			1–3
Fiber			1–4
Ash			0.6–1
Organic acids and their salts			0.5–1
Phenolic compounds			1–3
Pectic substances			0.3–0.6
Other components			3–7

Oil is dispersed within the fruit cells in droplets that vary from 40 to 60 μm in diameter. The amount of oil increases through autumn and winter, reaching its maximum between late November and January when the fruit displays a reddish-bluish color, an indicator that it has reached the optimum-maturity stage. The oil content is lower in colder climates.

Soluble reducing and nonreducing sugars are the most important compounds for the fermentation and preservation stages of the process involved in the preparation of table olives. Glucose as a major component, followed by fructose and to a minor extent by sucrose, have been quantified; small amounts of xylose and rhamnose are also present, as well as mannitol (in the range of 0.5–1%), which is a poliol also important as fermentative matter. The evolution of all these components during the growth and maturation of the fruits has been studied for different varieties by gas-liquid chromatography (8).

Fiber is the fundamental support for the structure of the fruits. Its major components for fresh green fruits, in order of decreasing percentage, are cellulose, lignine, and hemicelluloses, with cellulose accounting for more than 50%, depending on the variety. However, when the degree of maturity advances, enzymatic degradation may produce changes in the relationship of the components. Thus, for Hojiblanca variety at different stages of ripeness, cellulose percentage goes from 40 to 45% down to 23%; lignine remains almost steady, between 33 and 38%; and hemicelluloses goes from 22 to 23% up to 41% when fruits are completely ripe (9).

Hemicellulose isolated from fruits of the Gordal and Manzanilla varieties have been studied (10,11). Cellulolytic enzymes were detected in the flesh of the Hojiblanca variety (12). These enzymes partially hydrolyze the cellulose fraction of the fiber to glucose and contribute to the softening of the fruits. Different factors influencing their action, such as incubation time, presence of sodium chloride, temperature, storage time, and degree of maturity when fruits are harvested, have been studied (13,14). Characterization and partial purification of the enzymatic complex cellulases and their inhibitors have been carried out (15,16).

Protein content is relatively low, between 1 and 3%, and remains almost constant during growth and ripening of the fruits. Hydrolysis shows that all essential amino acids are present (17). Ash percentage varies from 0.6 to 1, and the ash includes, in order of decreasing importance, K, Ca, P, Na, Mg, S, and, to a lesser extent, Fe, Zn, Cu, and Mn. The importance of the presence of organic acids and their salts in the juice of the fruits must be emphasized because of their buffering action during the fermentation stage. They range between 0.5 and 1%, based on the weight of the flesh

Phenolic compounds, ranging from 1 to 3%, are responsible for color changes; pectic substances (0.3–0.6%) and pectic enzymes are related to texture (18); oleuropein, the most abundant phenolic glycoside found in olives (up to 2% in immature olives), is responsible for the bitterness of the fruit. It diffuses into the aqueous phase during the processing of the olive fruit and is hydrolyzed by alkaline solutions. Finally, certain vitamins, such as carotene, thiamine, and riboflavin, complete the known picture of the composition of the fresh fruit (19,20).

The chlorophyll and carotenoid presence in fruits of Hojiblanca and Manzanilla olive varieties have been studied (20,21). The qualitative composition is the same for both and does not change with maturation time. However, during the growth and development of the fruit, a gradual, homogeneous decrease is observed in the individual concentration of both chlorophylls and carotenoids (22). Hojiblanca variety always shows a greater amount of pigments than does Manzanilla.

As a general rule, water content decreases. Conversely, oil percentage, weight, and volume of the fruits, and flesh-to-pit ratio increase during growth and maturation. Soluble reducing and nonreducing sugars also decrease in a continuous manner. On the contrary, protein, ash, and total fiber remain almost stable, although qualitative changes may be produced in the total fiber

Main Varieties The chemical composition and physical properties of the fruit that are closely related to variety and harvesting time are decisive factors in the quality of the final product. Varieties of olive trees in different countries may be either autochthonous or imported. Autochthonous varieties, because of their nature, and imported varieties, because of changes in climate, soil, and methods of cultivation, may yields products with diverse characteristics (23–27). Olive tree varieties are not well known in many cases, so national or local names rather than botanical classification are used to identify them.

Among available varieties, it is necessary to select the most suitable for the specific use (olive oil vs. table olives), the type of processing, and a definite style. For that selection, different factors must be carefully analyzed: (*1*) geographical situation of planting age and distribution on the soil; (*2*) agricultural characteristics, such as productivity of the trees, ripening cycle, and pest resistance; (*3*) type of culture (irrigated or nonirrigated system); (*4*) pruning methods; and (*5*) harvesting procedure (manual or mechanical). All these variables have an important influence on composition and hence on parameters to be considered in further processing.

Physical properties of fruits also have a relevant importance at the time a certain variety is selected for a definite type of processing. Among them, the most important are size, shape, flesh-to-pit ratio, ease of pitting, color, and texture.

Varieties used for oil production have a ratio of flesh to pit ranging from 4:1 to 8:1 and a ripe fruit that contains 15 to 40% of oil, whereas table oil varieties have a ratio of 7:1 to 10:1 and a lower upper range of oil content.

Spanish Varieties Among the 22 dominant Spanish varieties (Seed and Plant Genetic Resources Service B AGPS. Olive Germplasm: Cultivars and World-Wide Collections. Food and Agriculture Organization of the United Nations), the major cultivars used for oil production include Picual, Hojiblanca, and Lechin de Sevilla, whereas those for processing table olives are the Gordal (or Sevillana), Manzanilla, Morona, and Cacereña.

Picual. Picual has the widest spread of any single cultivar in Spain and is well suited to oil production. It is the main tree for the Jaen area (Andalucia), the largest olive-growing region in the world. It grows well in hilly to mountainous areas and is able to withstand cold winters. The fruit is elongated and nearly symmetrical, with a slight bend at the apex. At green maturation it has a somewhat dark shade. Blackening starts from the apex, and the ripe fruit is uniformly dark black. Fruit size varies from 3 to 5g according to yield and growing conditions. The mesocarp is light colored, smooth, and relatively firm. The oil content is medium-high, 21 to 25%, on a fresh-weight basis. The flesh-to-pit ratio oscillates between 3.8 and 5.9:1.

Hojiblanca (O. europaea arolensis). Hojiblanca ripens later than Gordal and Manzanilla and has a higher oil content, between 23 and 29%. It is the second most common Spanish variety with regard to market volume, being the most appreciated for processing either as natural black olives in brine or as pickled black olives in brine, although it is also used as pickled green olives in brine. The shape of the fruits is regular, the pit is straight and the olives vary greatly in size, from 230 to 700 fruits/kg. Smaller-size grades are used for oil extraction. The flesh-to-pit ratio oscillates between 4.9 and 6.6:1.

Lechin. Lechin has its origin between Cordoba and Seville and is cultivated in the area of Seville and Granada. The fruit is ellipsoidal and slightly bulged on the back. The size ranges from 3.58 to 3.80 g, with an oil content between 23.5 and 26.8%. The flesh-to-pit ratio ranges between 4.0 and 6.1:1.

Gordal or Sevillana (O. europaea Regalis Clemente). Gordal or Sevillana is a big, ellipsoid hearth-shaped fruit, with an average size of 100 to 120 fruits/kg and a relationship of flesh to pit of 7.5:1. The form is ellipsoidal with an incision in the area of the peduncle that gives it a shape resembling a heart. It has a fine epicarp, a mesocarp with good texture, and a right and regular endocarp. The color is deep green that changes to purple-black when the fruit is fully mature. Oil content is low, generally below 10%, and the sugar content is around 4 to 6%. Early ripeness is characteristic.

Manzanilla (O. europaea pomiformis). Different subvarieties, depending on the region, are known by different local names, including Fina, Serrana, and Carrasqueña. The fruits are the most appreciated variety in the international market, mainly because of their prominent organoleptic properties. It is the most important variety in Spain and together with Gordal and Morona is almost exclusively destined for the preparation of Spanish-style pickled green olives in brine. Average size is 200 to 280 fruits/kg; the flesh-to-pit ratio is 6:1. Manzanillas are apple-shaped, with fine skin and flesh with an excellent texture. The color is light green with mottled white spots, the fruit turns black-violet when fully mature. The pit is right and presents a very smooth surface. This variety reaches ripeness later than Gordal and shows a higher oil content, sometimes up to 15% by weight of the fruit, and a lower sugar content.

Morona. The Morona variety is very close to Manzanilla, it is perhaps the same variety. Its production is relativey small and is located around Moron de la Frontera (Sevilla province).

Cacereña. Although quite close to Manzanilla, Cacereña generally presents a lower average size and rougher texture. It is most suitable for processing pickled black olives in brine.

Greek Varieties. The major cultivars used for oil production include Koroneiki, Megaritiki, and Tsounati. Those for processing table olives include Conservolea, Kalamata, and Halkidiki.

Megaritiki (O. europaea argentata). Variety with fruits of small size (2–5 g) with a curved, cylinder-conic shape. About half of the production is dedicated to oil and half to dry salt black olives.

Conservolea (O. europaea media rotunda). Conservolea is the most important Greek variety, representing 80 to 85% of olive production in the country. Fruits, varying in shape between round and oval, show certain characteristics similar to those of Manzanilla and Hojiblanca, although closer to the latter. Average size is 180 to 200 fruits/kg; flesh-to-pit ratio is around 8:1. The flesh is fine and the texture consistent. Oil content varies from 22 to 25%.

Halkidiki. Some authors consider the not-well-defined Halkidiki variety a subvariety of Conservolea. However, its shape is more elongated, its pit is slightly curved, and the flesh does not present as good a texture as does Conservolea. Average size is 120 to 140 fruits/kg, and flesh-to-pit ratio is 10:1. Used mainly for pickled green olives in brine, it is the second-most-produced Greek variety on the market. The oil content ranges from 19 to 20%.

Kalamata (O. europaea ceraticarpa). This excellent variety, of limited culture, is third in production in Greece. Fruits are cylindric-conic shaped and curved, showing a prominent tip at the end. Average size is 180 to 360 fruits/kg; the flesh-to-pit ratio is analogous to that of Conservolea (8:1). It reaches a nice natural black color on late ripeness, and the oil content is high (25.5%). It is used mainly for special styles of natural black olives in brine.

Italian Varieties. Italy is the second-largest producer of olive oil, with cultivars such as Leccino and Frantoio, but lags behind in table olive varieties.

Nocellara di Belice. Considered the prime Italian table olive. The fruit is medium size, round or oval, and similar to Manzanilla or Conservolea. Flesh-to-pit ratio is 6.5:1 to 8:1.

Ascolana Tenera. This is the most common variety in Italy and is also cultivated in Argentina, California, Israel, and Mexico. The average size is 115 fruits/kg, with an ellipsoidal, slightly asymmetric shape. For the production of Spanish-style olives, the fruit is harvested when the skin color is yellowish green. The flesh is soft and the skin has little resistance to alkaline treatment (soaking the olives in a strong alkaline solution for several days). Oil content varies from 17 to 18%.

Cucco. A resistant variety exclusive to Italy. The fruit maintains the green color for a longer time than other varieties and changes to a violet black when it reaches full maturation. Oil content is about 17%.

Sant' Agostino. The fruits are clustered in groups of two or three with an oblong ellipsoidal form. The average size is 135 fruits/kg with an oil content of 14 to 15%.

Santa Caterina. Variety from central Italy with an average size of 120 fruits/kg with an asymmetric ellipsoidal shape. The oil content is about 17%.

Bella di Spagna or Cerignola. Variety from central Italy with fruit of large size (110 fruits/kg). The shape is oblong ellipsoidal and the mature color is black with white spots.

Moroccan Varieties. The main cultivated variety for pickling in Morocco is denominated Picholine. It comes from Argelia, although its origin is probably French. Average size is around 280 fruits/kg. It is oval shaped, with an elongated, slightly curved pit. The flesh-to-pit ratio is around 5.1:1. It is a resistant variety, well adapted to different kinds of soil. The flesh is flavorful.

Argentinian Varieties. Argentina prepares and exports mainly the Arauco variety, also called Criolla, of Spanish origin. This olive has flesh-to-pit ratio of 7:1 and high oil content (22–24%); the fruits are elongated with a tip at the end. Pits are slightly curved. It is used mainly as pickled green olives in brine.

Turkish Varieties. The most appreciated Turkish varieties are Domat, for pickled green olives in brine, and Gemlik, for natural black olives in brine or in dry salt. Domat averages 180 to 190 fruits/kg; Gemlik 270 to 280 fruits/kg. Oil content is high for both, oscillating between 22 and 24%.

Californian Varieties. The United States is the main importer of olives, but it also produces a significant amount of table olives (see Table 3): 82,000 tons in 1988–1989 and 86,500 tons in 1991–1992. Five varieties are cultivated in California: Manzanilla, Mission, and Gordal or Sevillana, of Spanish origin; Ascolana from Italy; and Barouni, from Tunisia, the last being the least important in production. The Mission variety is the oldest in California. The oil content is about 22%; average size, 240 to 260 units/kg; and the ratio of flesh to pit is 6.5:1 for processed fruits. This is a very tasty variety that shows a nice black color when ripes.

Ascolana variety averages 110 to 120 fruits/kg, with a flesh-to-pit ratio of 8.2:1. Oil yield is not very high (19%), and the flesh is very delicate. It may be used as pickled green or black olives in brine.

French Varieties. France is the smallest European producer, with nearly 40,000 ha planted with olive trees. French production during 1993–1994 was 2 million t of olive oil.

Table olives and olive oil from France are products of high quality. Olive growing represents an important economic and social factor in the Mediterranean region. The major varieties used in France for olive oil are Aglandau, Bouteillan, Deayet roux, Cayon, Salonenque, Brun, and Ribier. For table olives the varieties most commonly used are Belgentieroise, Picholine, and Lucque.

OLIVE OIL

Atherosclerosis and coronary heart disease (CHD) as its main clinical manifestation have a multifactorial origin.

An individual's susceptibility to CHD is determined by both genetic and environmental influences. Among the latter, diet plays a central role. Dietary factors exert their influence largely through their effects on blood lipids and lipoproteins, but also through their great influence on other established, modifiable risk factors.

The dietary factors most directly implicated in CHD are dietary fats. Numerous comparisons between populations have shown a strong correlation between the intake of saturated fatty acids (SFA) and CHD morbidity and mortality. A diet high in SFA, such as is customary in the U.S. and Northern European countries, is associated with high levels of CHD. On the other hand, the Mediterranean countries, where the traditional diet derives the majority of its fat calories from olive oil, display a low incidence of CHD.

The Seven Countries study (28) gave scientific proof of the association between a diet low in animal products and saturated fat and low mean population levels of serum cholesterol with low incidence and mortality from CHD. It also documented a negative correlation between the intake of monounsaturated fatty acids (MUFA) and the MUFA-to-SFA ratio as well as CHD. All-cause and CHD death rates were low in cohorts with MUFA-rich olive oil as the main fat, underscoring the favorable role of olive oil in the diet. A body of indirect evidence from interventional studies shows that the traditional Mediterranean diet with its abundance of plant foods, preferential and regular intake of olive oil, and low to moderate consumption of animal foods efficiently protects against CHD.

Recent findings indicate that olive oil and the Mediterranean diet yield their benefits not only through their effects on established CHD risk factors such as hyperlipemia, hypertension, diabetes, and obesity, but also through directly protective effects, particularly their antioxidative properties. In addition, it has been documented that a Mediterranean diet adapted from the traditional Cretan diet rich in monounsaturated fat, is efficient in the secondary prevention of coronary events and death (29).

Constituents of Olive Oil

Olive oil is a complex compound made of fatty acids, vitamins, volatile components, water-soluble components, and microscopic bits of olive (26). Primary fatty acids are oleic and linoleic acids. Oleic acid is monounsaturated and constitutes 55 to 85% of the fatty acids in olive oil. Linoleic acids is polyunsaturated and represents about 9%. The polyunsaturated linolenic acid is present in small amounts ranging from 0 to 1.5%. The major vitamins are vitamin E and carotene. The specific fatty acid composition, the vitamin content, and the presence of several other minor components such as phenolic compounds may all contribute to the multiple health benefits associated with the consumption of olive oil (30–32).

The unique organoleptic characteristics of olive oil are attributed to a number of volatile components (26,32–35). Aldehydes, alcohols, esters, hydrocarbons, ketones, furans, and other compounds have been quantitated and identified by gas chromatography-mass spectrometry, nuclear magnetic resonance (NMR), and other techniques (6,36). The presence of flavor compounds in olive oil is closely related to its sensory quality. Hexanal, trans-2-hexenal, 1-hexanol, and 3-methylbutan-1-ol are the major volatile compounds of olive oil. Volatile flavor compounds are formed in the olive fruit through an enzymatic process. Olive cultivar, origin, maturity stage, storage conditions, and processing (6,26,27,34,37,38) influence the flavor components of olive oil and therefore its taste and aroma. The components octanal, nonanal, and 2-hexenal, as well as the volatile alcohols propanol, amyl alcohols, 2-hexenol, 2-hexanol, and heptanol, characterize the olive cultivar. Some slight changes in the flavor components in olive oil are obtained from the same cultivar grown in different areas. The highest concentration of volatile components appears at the optimal maturity stage of the fruit. During storage of olive fruit, volatile flavor components, such as aldehydes and esters, decrease. Phenolic compounds also have a significant effect on olive oil flavor. There is a good correlation between aroma and flavor of olive oil and its polyphenol content (39). Hydroxytyrosol, tyrosol, caffeic acid, coumaric acid, and p-hydroxybenzoic acid influence mostly the sensory characteristics of olive oil. Hydroxytyrosol is present in good-quality olive oil, whereas tyrosol and some phenolic acids are found in olive oil of poor quality. Various off-flavor compounds are formed by oxidation, which may be initiated in the olive fruit. Pentanal, hexanal, octanal, and nonanal are the major compounds formed in oxidized olive oil, but 2-pentenal and 2-heptenal are mainly responsible for the off-flavor (26,40–42).

Extraction of Olive Oil

A good olive oil is obtained from healthy, ripe olives that are processed without delay because the fermentation processes begin right after harvest and deteriorate the quality of the product, elevating the acidity and affecting the aroma and flavor of the oil (7).

The steps involved in the extraction of olive oil involve cleaning, milling, mixing, and the actual extraction, and these are described in detail. An scheme showing a traditional pressing process is shown in Figure 1.

Cleaning. The first operations to which the olives are subjected before oil extraction are the removal of leaves and washing. Leaves are removed using an automated aspiration system, and washing takes places using forced water that removes leftover leaves, branches, dust, soil, pebbles, and pesticides. The removal of the leaves is important for the final organoleptic characteristic as leaves can give the oil a bitter taste.

Milling. This operation breaks the flesh cells to induce the release of the oil from the vacuoles and their coalescence into larger oil droplets that can be separated from the other components.

A traditional milling machine consists of a bowl in which several heavy wheels turn, crushing the olives. Traditionally, the bowl and the wheels were made of stone and the oil stones, between 2 and 6 per machine, were either conical or cylindrical. The average machine could handle up to 500 kg of olives, turning at speeds of 15 rpm and taking between 15 and 30 min. This procedure has been used for several centuries and has many advantages:

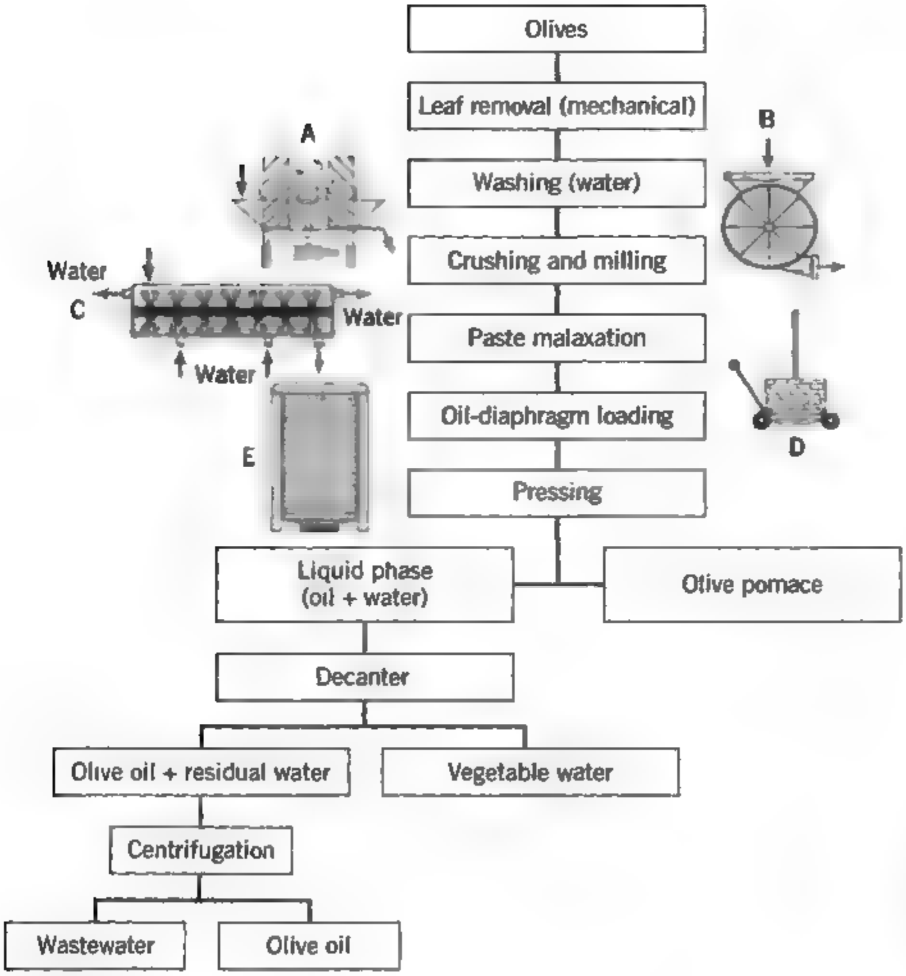

Olives
Leaf removal (mechanical)
Washing (water)
Crushing and milling
Paste malaxation
Oil-diaphragm loading
Pressing
Liquid phase (oil + water)
Olive pomace
Decanter
Olive oil + residual water
Vegetable water
Centrifugation
Wastewater
Olive oil
A
B
C
D
E
Water
Water
Water

Figure 1. Scheme showing a typical pressing process. The crushing and milling can be done with (**a**) a traditional mill with cylindrical stones, or with the most modern apparatus, (**b**) steel hammer, (**c**) mixer, (**d**) oil diaphragms on trolley, or (**e**) hydraulic press.

- It does not produce emulsions.
- It does not raise the temperature.
- There is no contamination from metals.
- It crushes the pits to the proper size and achieves malaxation.

However, it also has certain disadvantages:

- High cost
- Low efficiency
- Discontinuous processing
- Need for highly skilled workers
- Increased oxidation due to lengthy air exposure of the paste

More recently, metal olive-crushing equipment, either roller, cylindrical, disc, toothed, or hammer, has been used. These work at much higher speeds (3,000 rpm) within a chamber that moves in the opposite direction at about 80 rpm. The advantages of these mechanical systems are:

- Continuous operation
- Lower cost
- Higher capacity
- Smaller size

Some drawbacks include:

- The high operating speed may leave a paste that is not properly prepared because the crushing is carried out quickly and may not be complete.
- Emulsions are created that are difficult to break.
- Alteration of the organoleptic characteristics of the oil induces bitterness.
- Wear and tear of the metallic parts results in contamination of the product.

Mixing (Malaxation). The mixing or malaxation of the paste resulting from the crushing involves stirring the mash slowly (~20 rpm) and continuously for 15 to 60 min. This process takes place in hemicylindric or semispheric water-heated (<30°C) stainless-steel vats with double walls and rotating blades. The mixing time depends on the crushing technique. When the traditional stone cones are used, 15 min at room temperature are enough, but when hammers are used, the mixing needs to be extended up to 60 min and the temperature increased to ~30°C. This temperature acts as an antiemulsionant and facilitates the activity of enzymes in the olives, which disrupt the membranes that surround small oil droplets and prevent self-aggregation. Modern techniques include the addition of inert coadjuvants (talc powder) during the mixing step to facilitate the emulsion separation and of enzymes to disrupt the membranes.

The purpose of this operation is to break the oil–water emulsion and promote the fusion of the small oil droplets into droplets of a diameter greater than 30 μm, the minimal dimension required for oil separation in continuous phase. Even after optimal crushing, only 40 to 45% of oil droplets have a diameter >30 μm. This percentage increases to 80 to 85% following good malaxation. Therefore, this operation is essential to increase the yield of the extraction regardless of the techniques utilized. Droplets of smaller size (<30 μm) remain as emulsion, and they stay in the subproducts.

Temperatures >30°C, lengthy mixing times, or both will have a negative effect on the aroma of the oil (43), as well as its antioxidant and vitamin content.

Extraction. The olive oil is separated from the olive paste using selective filtration (partial extraction), pressure (traditional system), or centrifugation (continuous system).

Selective Filtration. Selective filtration is based on the lower superficial tension of the oil compared to that of the water. Therefore, when both liquids are put in contact with the pores of the filtering surface, oil will pass through whereas water will be retained. The objective of selective filtration is not the total extraction of the oil in the paste, but the extraction of the oil naturally separated during the mixing step. Therefore, the factors that influence the amount of oil extracted by this system depend on:

- The amount of "free" oil in the paste
- Duration of the process, with an optimal time of about 30 min
- The characteristics of the equipment (filtering surface, rpm of the extractors, oil/water and oil/solids ratios)

The oil obtained by this procedure has a humidity content of ~1% and has to be centrifuged immediately.

Up to 60% of the oil in the paste can be extracted by this method. The product obtained from selective filtration maintains excellent organoleptic characteristics, low levels of acidity, and greater resistance to rancidity. Moreover, it has some other operational advantages, including:

- Low installation cost and maintenance
- Possibility of inclusion in previously established production lines (traditional or continuous)
- Low requirements in terms of labor and energy
- Facilitation of subsequent processing of the paste, thus improving the final recoveries

To understand the high cost of this product, is important to consider that the production of 1 L of olive oil by this process requires 11 to 12 kg of highly select olives, whereas only 5 kg are required for the extraction of 1 L of olive oil by the pressure processes described next.

Pressure. Pressure extraction the oldest procedure used to obtain olive oil. Originally, oil separation was achieved with pressure applied by humans or animals. Today, traditional olive oil mills use hydraulic presses. The olive paste is placed in thin layers (2–3 cm) over disks of filtration material (oil diaphragms). These diaphragm–paste layers are piled on top of each other and placed over a cart or trolley fitted with a central shaft to provide even distribution and support when these turrets are subjected to hydraulic pressure. This combination of trolley, shaft, oil paste, and diaphragms is subjected to pressure (300–400 kg/cm^2), the oil is extracted, and the apparatus is disassembled. Therefore this is a discontinuous process. The factors affecting the pressure process include:

- The characteristics of the oil diaphragms, which depend in part on the olive kernels in the paste and the degree of dispersion and concentration of the colloid constituents of the olive paste
- The humidity of the paste, the size and shape of the particles, and the physical characteristics of the olive (type, variety, ripeness, and temperature)

It is common to include a second step. Once the maximum pressure has been achieved, the pressure is partially released, allowing sponging of the paste and the diaphragms and reopening of channels that after pressure is reapplied allows the extraction of additional oil.

This system produces oils of excellent quality due to the low temperatures used during the process; however, it has elevated costs due to labor, the discontinuity of the process, and the use of optimal filtering materials.

Centrifugation. The use of centrifugation for oil extraction is relatively recent and is based on the different densities of the oil, the water, and the solids (pomace). Some of the first practical experiences with this technique were carried out with the Corteggiani system, a centrifuge with the capacity to process about 100 kg of paste spinning at 900 rpm. Water was added to facilitate the oil separation, and the process had to be stopped for removal of the solids.

Currently the process is carried out with a horizontal centrifuge (decanter). This system allows continuous solid–liquid separation and consists of a cylindrical–conical bowl that spins at speeds of 3,000 to 4,000 rpm. The interior consists of a hollow component of the same shape containing helical blades. There is a small difference between the speeds at which the bowl spins and the inner screw gyrates. The faster speed of the latter results in the movement of the pomace to one end of the decanter and the olive and water to the other end. The oily must is then fed to a vertical centrifuge revolving at 6,000 to 7,000 rpm for the final separation of the oil.

The separation of the solid and liquid phases by centrifugation requires the addition of water to the olive paste. The amount of water and the temperature influence the oil yield, and it is necessary to adjust both factors for each type of equipment.

Some of the advantages of the centrifugation process are:

- Small size of the equipment
- Automated process with semicontinuous cycles
- Reduced need for highly skilled labor
- Lower acidity of the oil
- Similar yields to those obtained using more-traditional systems
- Stainless-steel materials decrease the risk of contamination by other metals
- No diaphragms are used, thus improving the hygiene of the process and lowering the risk of contamination

Some of the disadvantages are:

- The initial capital investment is high.
- The process requires large amounts of water, which pollutes the environment and removes a significant amount of natural antioxidants.
- The energy consumption is high.
- The organoleptic characteristics of the oil may be adversely affected.

A variant consisting of a two-phase centrifugal continuous system reduces the use of water and eliminates the production of waste water (alpechin) (see Fig. 2).

Classification of Olive Oil Grades

Virgin Olive Oil. Oil extracted from olives by mechanical or other methods that do not modify its basic properties is called virgin olive oil. It is a completely natural product that maintains the taste as well as the chemical and biological characteristics of the olive. Within the virgin grade are three recognized quality levels:

Extra. Oil with the best organoleptic characteristics and with an acidity level not exceeding 1% is classified as extra virgin. The highest-quality extra virgin olive oils have an acidity level of at least 0.4 to 0.5% to maintain the organoleptic characteristics and lower than 0.7 to 0.8% so as not to exceed the maximum legal level. Its production requires the greatest care and attention from the cultivation stage all the way through to final processing.

The olives, unaffected by parasites, need to be harvested at the ideal point of maturation. If the fruit is still unripe, the extracted oil will be too sour. If the fruit is too ripe, the oil will be too sweet. The olives need to be harvested by hand to avoid any damage from mechanical processes. They should not be piled but should be arranged in layers of 15 to 30 cm in rigid containers. They should not be stored; pressing should take place immediately after removal of leaves and other detritus. If storage is required, it should be done only for the briefest time at low relative humidity (50%) and temperature (10–15°C). The use of water needs to be limited, and any rise in temperature should be avoided during the extraction process to ensure the highest-quality oil Extra virgin oil is extracted during the first phase of pressing and therefore comes directly from the pulp of the fruit, whereas any oil that comes out later will also contain oil from the pit of the fruit. The oil extracted from the press needs to be separated immediately from the sludgy dregs and should be stored in a cool place away from direct light. Laboratory analysis is insufficient to detect the organoleptic qualities of extra virgin olive oil; taste tests are necessary. Olive oil tasters are highly qualified experts who use strict and sophisticated methods (26,44–55).

The cost of production is higher than that of other vegetable oils. This is due to the high amount of manual labor required for the cultivation and harvest of the olives. A person can pick from 60 to 100 kg of olives in a day, and from these about 13 to 20 L of oil can be extracted. The high cost is more than compensated, however, by the high yield and the oil's distinctly superior properties.

Average. Average oil has a good taste and acidity levels not exceeding 3.3%. This may be classified as:

- *Superfine virgin olive oil*: Obtained by mechanical extraction from olives and having undergone washing, sediment removal, and filtering with no chemical manipulations. It should not contain more than 1.5% acidity.
- Fine virgin olive oil: Obtained by mechanical extraction from olives and having undergone no chemical manipulations but only washing, sediment removal, and filtering. It should not contain more than 3.3% acidity.

Strong (Lampante). Strong virgin olive oil has inadequate taste, or acidity levels above 3.3%.

Refined Olive Oil. Refined olive oil is obtained by refining virgin oil whose taste, acidity levels, or both make it unsatisfactory for direct consumption. This is a healthy and perfectly acceptable food product, but it does not have the full taste of virgin olive oil.

Olive Oil. Olive oil is made by blending both refined and virgin olive oil. This type of oil is very much a standard in the marketplace; its properties fall somewhere between those of its two components.

Adulteration of Olive Oil

The high market price and the well-known health benefits attributed to olive oil make this product a target for different types of fraud, such as adulteration and mislabeling. This is especially true for extra virgin olive oil, which in the international trade market reaches a much higher price than any other vegetable oil (see Table 5).

Several physical, chemical, chromatographic, and spectroscopic tests are in use to detect adulteration of high-quality olive oil with low-grade olive oil (56,57), hazelnut oil (57), and seed oils (58,59). These have classically included determination of iodine value, saponification value, viscosity, density, refractive index, ultraviolet absorbance, fluorescence, and colorimetric reactions. More recently, FT-Raman spectra (60,61), mid-infrared spectroscopy (62), CO_2 laser infrared optothermal spectroscopy (58), reversed-phase liquid chromatography coupled to gas chromatography (63), and NMR (36) have been reported to detect the presence of <1% of other oils in extra virgin olive oil. These tests rely on the detection in the olive oil of unusual amounts of certain fatty acids or other compounds

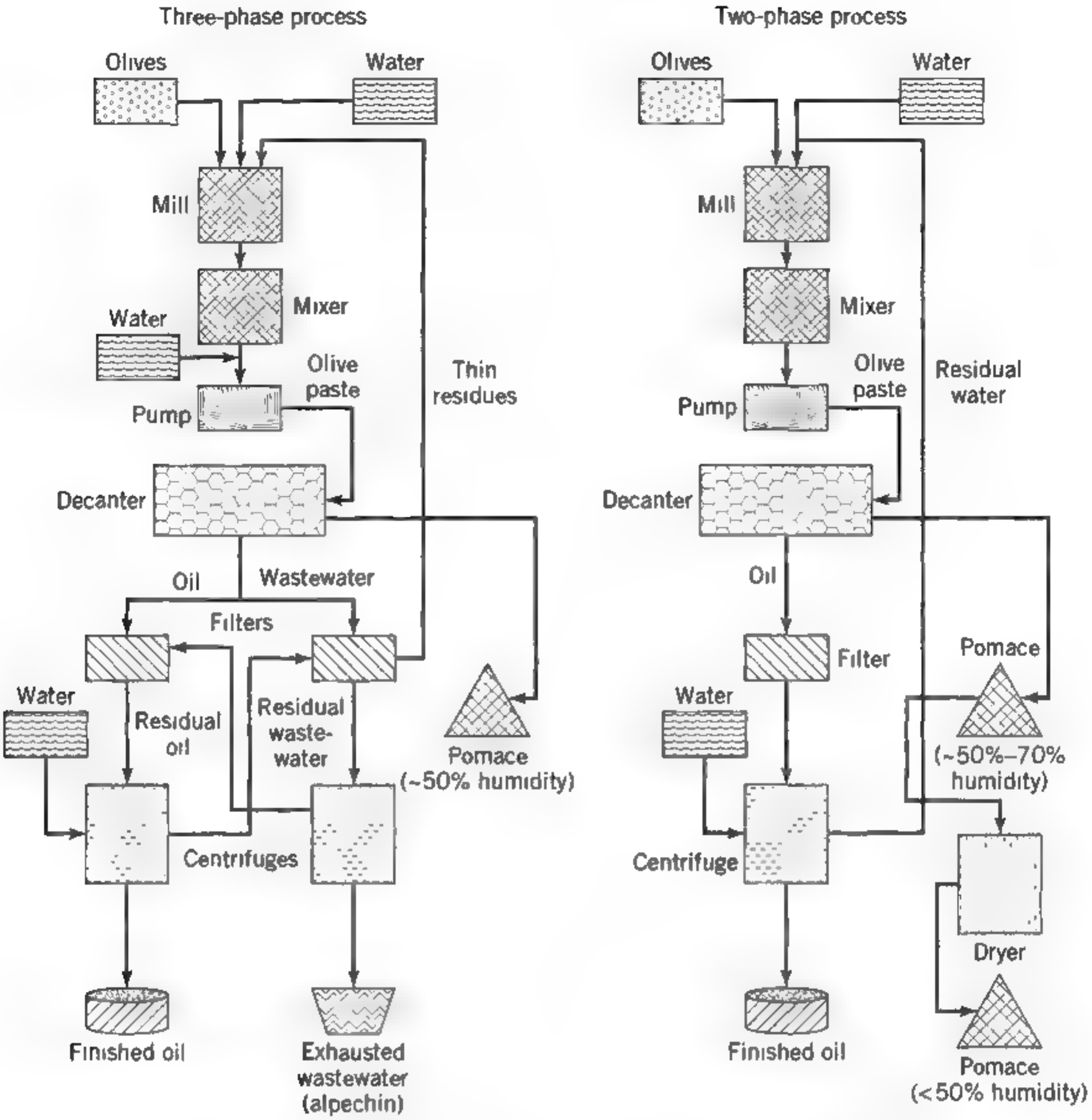

Three-phase process
Olives
Water
Mill
Mixer
Water
Olive paste
Thin residues
Pump
Decanter
Oil
Wastewater
Filters
Water
Residual oil
Residual waste-water
Pomace (~50% humidity)
Centrifuges
Finished oil
Exhausted wastewater (alpechin)
Two-phase process
Olives
Water
Mill
Mixer
Olive paste
Residual water
Pump
Decanter
Oil
Filter
Pomace
Water
(~50%–70% humidity)
Centrifuge
Dryer
Finished oil
Pomace (<50% humidity)

Figure 2. Scheme of three- and two-phase centrifugal processes for olive oil extraction.

such as fatty acids, triacylglycerols, squalene, sterols, tocopherols, alkanes, aliphatic alcohols, waxes, erythrodiol and uvaol, and stigmastadiene.

Fatty Acids. The International Olive Oil Council (IOOC) has established the normal ranges for the different fatty acids in olive oil. Gas chromatography has been used to measure these fatty acids, and this is the most common analytical technique for the assessment of the quality of olive oil (64). Among the different types of fatty acids, special attention should be paid to *trans* fatty acids. *Trans* fatty acids are not present in crude olive oils; however, they are formed during bleaching and deodorization. In addition to gas liquid chromatography, other techniques, such as infrared and nuclear magnetic resonance spectroscopy, high-performance liquid chromatography (HPLC), and supercritical fluid chromatography, have been applied to the detection of *trans* fatty acids (64).

Triacylglycerols. The triacylglycerol profile can provide information about the origin of the olive oil and its adulteration with other oils. Several methods are available for the determination of triacylglycerols, including high-temperature capillary gas chromatography and reversed-phase HPLC (65,66).

Squalene. Squalene content is very high in olive oil. Adulteration of olive oil decreases its content. Moreover, the refinement of olive oil induces squalene isomerization. Therefore, adulteration of olive oil with refined oil can be detected by the presence of these isomers using gas liquid chromatography (67) or HPLC (68).

Sterols. Large quantities of stigmasterol suggest adulteration with soybean oil. The ratio of beta-sitosterol (high in virgin olive oil) to campesterol + stigmasterol has been used for the detection of adulteration with seed oils.

Table 5. Price Ranges for Different Edible Vegetable Oils

Oil	Price ($/kg)
Extra virgin olive oil	2.98–3.25
Refined olive oil	2.10–2.17
Refined pomace olive oil	1.08–1.11
Peanut oil	1.08–1.09
Sunflower oil	0.79–0.80
Corn oil	1.01–1.02
Soybean oil	0.75–0.76
Canola oil	0.75–0.76

Note: Wholesale prices for edible oils quoted by the Roman Chamber of Commerce on September 17, 1998.

These compounds are analyzed using gas chromatography (69–71).

Tocopherols. Seed oils tend to contain higher quantities of beta, gamma, and delta tocopherol (soybean oil) and gamma tocopherol (cottonseed oil) than olive oil. These compounds can be detected using HPLC (72).

Alkanes. Olive oil contains primarily alkanes with chain lengths of C_{23}, C_{25}, and C_{27}, whereas other edible oils contain predominantly C_{27}, C_{29}, and C_{31} (73).

Aliphatic Alcohols. Olive oil contains lower levels of long chain alcohols and mono- and diunsaturated derivatives than does olive-pomace oil. This measurement could be used to assess contamination of virgin olive oil with low-grade olive oils (63,70).

Waxes. Extra virgin olive oil does not contain waxes; however, these are found in refined and olive-pomace oils. Therefore this determination could be used for detecting adulteration of extra virgin olive with olive-pomace oil (68,74,75).

Erythrodiol and Uvaol. Both erythrodiol and uvaol are increased in solvent-extracted olive oils (54); however, due to the variability of these compounds in olive oils obtained from different cultivars, these determinations are not commonly accepted as a measure to distinguish between pressed and solvent-extracted olive oils.

Stigmastadiene. Stigmasta-3,5-diene is formed by the removal of H_2O from beta-sitosterol during the refinement of olive oil. The determination of stigmastadiene has been proposed to detect refined oils in crude olive oil (68).

TABLE OLIVES

Pickled foods, which include table olives, are defined as those vegetables in which the preparation and preservation is carried out by fermentation, acidification, or both. This system of processing leads to end products with very special and well-defined sensorial characteristics, largely accepted by a wide consumer market; permits the preservation of perishable raw materials over a long time until they can be definitively processed; constitutes a manufacturing method that consumes little energy because the product is generally preserved under the final conditions of low pH value and relatively high acidity, thus not requiring any thermal treatment or requiring at most a simple pasteurization; and, finally, as a consequence of the previous considerations, retains more easily the natural nutrients, some of which are heat labile, and maintains important physical properties, such as color and texture.

In general, fermentation is carried out by homo- and heterofermentative lactic bacteria yeasts, or both. Both germ groups, in the natural sequence of events, are preceded by gram-negative aerobic bacteria and, if the ambient conditions are not adequate in certain moments of the sequence, can be accompanied by other genera, such as *Clostridium, Propionibacterium, Bacillus*, oxidative yeasts and molds, and other producers of diverse alterations.

The fundamental role that each microorganism is assigned in nature can be modified or substituted, partially, by technologically varying the related ambient conditions. These depend on a limited number of factors, including pH value, acidity, sodium chloride content, buffering capacity, temperature, degree of aerobicity or anaerobicity, and use of pure cultures.

To apply these modified technologies correctly, all investigators require the following: (*1*) knowledge, as wide-ranging as possible, of the chemical composition of the fruit or vegetable under study as well as the natural sequence, specific for each product, of the developing microorganisms; (*2*) selection of appropriate varieties as raw materials; and (*3*) determination of the optimum harvesting time.

These three points are evidently interdependent, but the natural germ sequence, whether maintained or modified, is perhaps what best determines the importance of the distinct components of the raw material and the physical and chemical modifications that are produced and must be produced in the composition. In other words, the aforementioned sequence frequently will mark the steps that must be the focus of basic research oriented toward technological advances. It is in this form that research on table olives has developed, initiated in the 1930s by Cruess (76) and continued mainly by the research of Vaughn et al. (77) de la Borbolla et al. (78), and Balatsouras (79).

Definition

According to the Unified Qualitative Standard Applying to Table Olives in International Trade, “Table olives are the sound fruit of specific varieties of the cultivated olive tree (*Olea europaea* sativa) harvested at the proper stage of ripeness and whose quality is such that, when they are suitably processed as specified in this standard, produce an edible product and ensure its good preservation as marketable goods. Such processing may include the addition of various products or spices of good table quality.” The main purpose of processing is the removal, at least partially, of the natural bitterness of the fruit, which makes it suitable as either a food or an appetizer.

Table 5. Price Ranges for Different Edible Vegetable Oils

Oil	Price ($/kg)
Extra virgin olive oil	2.98–3.25
Refined olive oil	2.10–2.17
Refined pomace olive oil	1.08–1.11
Peanut oil	1.08–1.09
Sunflower oil	0.79–0.80
Corn oil	1.01–1.02
Soybean oil	0.75–0.76
Canola oil	0.75–0.76

Note: Wholesale prices for edible oils quoted by the Roman Chamber of Commerce on September 17, 1998.

These compounds are analyzed using gas chromatography (69–71).

Tocopherols. Seed oils tend to contain higher quantities of beta, gamma, and delta tocopherol (soybean oil) and gamma tocopherol (cottonseed oil) than olive oil. These compounds can be detected using HPLC (72).

Alkanes. Olive oil contains primarily alkanes with chain lengths of C_{23}, C_{25}, and C_{27}, whereas other edible oils contain predominantly C_{27}, C_{29}, and C_{31} (73).

Aliphatic Alcohols. Olive oil contains lower levels of long chain alcohols and mono- and diunsaturated derivatives than does olive-pomace oil. This measurement could be used to assess contamination of virgin olive oil with low-grade olive oils (63,70).

Waxes. Extra virgin olive oil does not contain waxes; however, these are found in refined and olive-pomace oils. Therefore this determination could be used for detecting adulteration of extra virgin olive with olive-pomace oil (68,74,75).

Erythrodiol and Uvaol. Both erythrodiol and uvaol are increased in solvent-extracted olive oils (54); however, due to the variability of these compounds in olive oils obtained from different cultivars, these determinations are not commonly accepted as a measure to distinguish between pressed and solvent-extracted olive oils.

Stigmastadiene. Stigmasta-3,5-diene is formed by the removal of H_2O from beta-sitosterol during the refinement of olive oil. The determination of stigmastadiene has been proposed to detect refined oils in crude olive oil (68).

TABLE OLIVES

Pickled foods, which include table olives, are defined as those vegetables in which the preparation and preservation is carried out by fermentation, acidification, or both. This system of processing leads to end products with very special and well-defined sensorial characteristics, largely accepted by a wide consumer market; permits the preservation of perishable raw materials over a long time until they can be definitively processed; constitutes a manufacturing method that consumes little energy because the product is generally preserved under the final conditions of low pH value and relatively high acidity, thus not requiring any thermal treatment or requiring at most a simple pasteurization; and, finally, as a consequence of the previous considerations, retains more easily the natural nutrients, some of which are heat labile, and maintains important physical properties, such as color and texture.

In general, fermentation is carried out by homo- and heterofermentative lactic bacteria yeasts, or both. Both germ groups, in the natural sequence of events, are preceded by gram-negative aerobic bacteria and, if the ambient conditions are not adequate in certain moments of the sequence, can be accompanied by other genera, such as *Clostridium*, *Propionibacterium*, *Bacillus*, oxidative yeasts and molds, and other producers of diverse alterations.

The fundamental role that each microorganism is assigned in nature can be modified or substituted, partially, by technologically varying the related ambient conditions. These depend on a limited number of factors, including pH value, acidity, sodium chloride content, buffering capacity, temperature, degree of aerobicity or anaerobicity, and use of pure cultures.

To apply these modified technologies correctly, all investigators require the following: (*1*) knowledge, as wide-ranging as possible, of the chemical composition of the fruit or vegetable under study as well as the natural sequence, specific for each product, of the developing microorganisms; (*2*) selection of appropriate varieties as raw materials; and (*3*) determination of the optimum harvesting time.

These three points are evidently interdependent, but the natural germ sequence, whether maintained or modified, is perhaps what best determines the importance of the distinct components of the raw material and the physical and chemical modifications that are produced and must be produced in the composition. In other words, the aforementioned sequence frequently will mark the steps that must be the focus of basic research oriented toward technological advances. It is in this form that research on table olives has developed, initiated in the 1930s by Cruess (76) and continued mainly by the research of Vaughn et al. (77) de la Borbolla et al. (78), and Balatsouras (79).

Definition

According to the Unified Qualitative Standard Applying to Table Olives in International Trade, "Table olives are the sound fruit of specific varieties of the cultivated olive tree (*Olea europaea* sativa) harvested at the proper stage of ripeness and whose quality is such that, when they are suitably processed as specified in this standard, produce an edible product and ensure its good preservation as marketable goods. Such processing may include the addition of various products or spices of good table quality." The main purpose of processing is the removal, at least partially, of the natural bitterness of the fruit, which makes it suitable as either a food or an appetizer.

Harvesting, Transport, and Quality Evaluation

Harvest for table olives is fundamentally dependent on the type for which the fruits are destined and is influenced by distinct factors such as variety, climate, and cultivation systems.

Olives for green type are harvested when fruits have reached a green–straw yellow color and when the flesh is easily freed from the pit by finger torsion after the fruit is cut around the transverse diameter.

For turning-color type, fruits are harvested when the epicarp presents a purple color and the mesocarp is still white. For black-olive type, harvesting is at almost the same time; however, because fruits are further oxidized after lye treatment, the permissible range of color at harvest is wider than for the turning-color type.

Olives for natural black type must be harvested when ripeness is completed. Superficial color is not a good criterion in this case. It is necessary to cut along and close to the pit to obtain a representative sample of fruit and observe the flesh color. It is considered to be at a good stage of maturity if the purple color reaches at least 2 mm from the pit.

Generally, fruits are transported to the factory in holed plastic boxes of about 20- to 22-kg capacity. Parallelepipedic metallic bins with lateral plastic network walls of approximately 500-kg capacity are also used for this purpose. Bulk transportation in trucks is not used so as to avoid damaging the olives.

When the fruits reach the factory, different factors must be controlled, at arrival and before processing, to identify lots and sublots of the raw material. Among these the most important are variety, supplier, origin, cultivation system, date of harvesting, data of arrival at the factory, date of processing, stage of maturity, moisture content, and net weight.

To fix price and quality, it is necessary to determine, through a suitable sampling, the average weight and size distribution as well as the percentage of defects.

World Production and Distribution

According to recent data from the IOOC, the current world production of table olives is about 980,000 t. This figure, when compared to the 789,000-t figure for the 1987–1988 crop and the 450,000 t produced in 1967–1968, represents an increase of approximately 120% during the last 30 years. Spain is the major producer at 320,000 t, with 50% of this production consumed internally. Overall the European Economic Community (EEC) produces and takes to the market about 50% of world production.

About 277,000 t, 28% of world production, are exported yearly, with Spain the major exporter (125,000 t). The major importers are the United States and Canada (43.3%), the European Union (36.1%), and the Arab countries (7.2%). According to market trends during the last three decades, an average annual increment of at least 5% in production and consumption may be foreseen for the future; the industry could easily increase this figure by upgrading quality, preparing new derived items, and developing new presentation forms. This represents a challenge for food technologists.

Types of Table Olives

Differences among types of table olives are based on the degree of ripeness when they are harvested and the color of the fruit. According to Spanish standards, there are four established types.

Green Olives. Green olives are obtained from fruits harvested during the ripening period, before coloring and when they have reached normal size. Such olives must be firm, sound, resistant to a slight pressure between the fingers, and without marks other than the natural pigmentation, subjected to the tolerances set out in the standards The color of the fruit may vary from green to straw yellow.

Turning-Color Olives. Turning-color olives are obtained from rose, wine-rose, or brown fruits harvested before the stage of complete ripeness is attained. They may or may not have been subjected to alkaline treatment and are ready for consumption. Californian green ripe olives are included in this type.

Black Olives. Black olives are obtained from fruits harvested when not fully ripe that have been blackened by oxidation and have lost their bitterness through alkaline treatment. These olives must be canned in brine and preserved by heat sterilization. Californian ripe olives are included in this type.

Natural Black Olives. Natural black olives are obtained from fruits harvested when fully ripe or slightly before full ripeness is reached. According to production region and time of harvesting, they may be reddish black, violet black, deep violet, greenish black, or a deep chestnut color.

IOOC standards consider only three types: green, turning-color, and black olives. However, under the title of commercial preparation, both the Spanish and IOOC standards agree and differentiate clearly between alkali-treated (black) and untreated (natural black) olives.

Commercial Preparation

The denomination of commercial preparation, or its equivalent, trade preparation, is used for all products destined for consumers. These preparations are diverse and include not only traditional methods of processing but also those derived from them and improved by new technologies. To facilitate better understanding, it is useful to first explain some other concepts.

Removal of the fruit's natural bitterness due to the presence of oleuropein glucoside may be carried out completely by alkaline hydrolysis, treating olives with an aqueous solution of sodium hydroxide before fermentation (80). In those cases, denomination in IOOC standards includes the word treated. The removal of oleuropein may also be achieved, slowly and partially, during the acid fermentation of fruits put directly into the brine or kept in dry salt. In those cases, the word untreated appears in the name.

Generally, the complete name for a specific commercial preparation includes information on (*1*) the type of raw material (green, turning-color, or black olives); (*2*) the procedure used to eliminate bitterness (treated or untreated);

and (3) the method of preservation (brine or dry salt). Different preparations described in the standards mentioned previously are as follows.

Treated Green Olives in Brine. The olives are treated in an alkaline lye and then packed in brine in which they undergo natural lactic fermentation, that is, either complete fermentation (Spanish style) or partial fermentation. Should the olives not undergo complete natural fermentation, their subsequent preservation at a pH value within the limits specified in the standards may be ensured by sterilization or pasteurization, the addition of preserving agents, refrigeration, or inert gas without brine.

Untreated Green Olives in Brine. Untreated green olives are placed directly in brine and preserved by natural fermentation.

Turning-Color Olives Treated in Brine. Turning-color olives are obtained after alkaline treatment and preserved by natural fermentation in brine or by heat treatment.

Untreated Turning-Color Olives in Brine. Directly treated in brine, turning-color olives are preserved by natural fermentation and are ready for consumption.

Olives Darkened by Oxidation. Olives darkened by oxidation are obtained from fruits that, when not fully ripe, have been darkened by oxidation and whose bitterness has been removed by treatment in alkaline lye. They have to be packed in brine and preserved by heat sterilization. Californian-style ripe olives correspond to this group.

Treated Black Olives. Treated black olives are obtained after alkaline treatment and are preserved by natural fermentation through one or a combination of the following procedures: brine, sterilization or pasteurization, or addition of a preserving agent.

Untreated Black Olives. Untreated black olives are placed directly in brine. They have a fruity flavor that is more marked than that of the treated black olives, and they usually retain a slightly bitter taste. They are preserved by natural fermentation through one or a combination of the following methods: brine, sterilization or pasteurization, or addition of a preserving agent. Greek-style natural black olives in brine correspond to this group.

Shriveled Black Olives. Shriveled black olives are harvested just before they are fully ripe and, after a brief immersion in a weak alkaline solution, are preserved by sprinkling salt in wooden casks, which are rotated daily until the olives are prepared for sale.

Untreated Naturally Shriveled Black Olives. Untreated naturally shriveled black olives are harvested when fully ripe, after they have become shriveled on the tree, and are subsequently treated directly in brine.

Treated Black Olives in Dry Salt. Treated black olives in dry salt are obtained from firm, practically ripe fruits that, following slight alkaline treatment, are preserved in alternating layers of olives and dry salt or by sprinkling dry salt over the olives.

Untreated Black Olives in Dry Salt. Untreated black olives in dry salt are obtained from fruits harvested when fully ripe and treated immediately or after partial drying in alternating layers of olives and dry salt or by sprinkling dry salt over the olives. They retain a degree of bitterness and a fruity flavor more marked than those of treated olives in dry salt.

Untreated Naturally Shriveled Black Olives in Dry Salt. Untreated naturally shriveled black olives in dry salt are obtained from fruits harvested when fully ripe that, after they have become shriveled on the tree, are preserved in alternating layers of olives and dry salt or by sprinkling dry salt over the olives.

Untreated Pierced Black Olives in Dry Salt. Untreated pierced black olives in dry salt are obtained from fruits harvested when fully ripe that, after the skin has been pierced, are preserved in alternating layers of olives and dry salt or by sprinkling dry salt over the olives.

Dehydrated Black Olives. Dehydrated black olives are obtained from ripe fruit that, after having been blanched and partially dehydrated in salt, undergo very gentle heating.

Bruised Olives. Bruised olives are obtained from whole fruit, fresh or previously treated in brine, subjected to a process whereby the flesh is opened without breaking the stone, which remains whole and intact within the fruit. They may be treated in weak lye and are preserved in brine, possibly flavored, with or without the addition of vinegar. They are prepared from either green or turning-color type.

Split Olives. Split olives are green olives, turning-color olives, or black olives split lengthwise by cutting into the skin and part of the flesh and then placed in brine, with or without vinegar; olive oil and aromatic substances may be added. They may be untreated or treated previously with alkali.

Specialties. Olives may be prepared by means distinct from those specified in the standards. Such specialties retain the name "olive" as long as the fruit used complies with the general definitions of those standards. The names used for these specialties should be sufficiently explicit to prevent any confusion as to the origin and nature of the products and, in particular, with respect to the designations in the standards.

Presentation Forms

According to their type and commercial preparation, olives may be offered to the consumer in one of the following styles, also denominated presentation forms.

- Whole olives: Olives that have their natural shape and from which the stone or pit has not been removed
- Without stem: Whole olives from which the stem has been removed
- With stem: Whole olives retaining the stem
- Pitted olives: Olives from which the pit has been removed and that basically retain their natural shape
- Stuffed olives: Pitted olives stuffed either with one or more suitable products (pimiento, onion, almond, celery, anchovy, olive, orange, or lemon peel, caper, etc.) or with their prepared natural pastes
- Halved olives: Pitted or stuffed olives sliced into two approximately equal parts, perpendicular to the major axis of the fruit
- Quartered olives: Pitted olives split into four approximately equal parts along the major axis of the fruit and perpendicular to the same
- Divided olives: Pitted or stuffed olives cut lengthwise into more than four approximately equal parts
- Sliced olives: Pitted or stuffed olives sliced into segments of fairly uniform thickness
- Chopped or minced olives: Small pieces of pitted olives of no definite shape and practically devoid of identifiable stem-insertion units as well as of slice fragments
- Olive paste: Exclusively olive flesh, finely crushed. Ingredients or additives may be incorporated for its preservation.
- Broken olives: Olives accidentally broken while being pitted or stuffed. They normally contain pieces of the stuffing material.
- Salad olives: Broken or broken-and-pitted olives with or without capers, plus stuffing material. The olives predominate in the total product marketed in this style
- Olives with capers: Whole or pitted olives, usually small in size, with capers and with or without pimiento. The olives predominate in the total product marketed in this style

According to the manner in which they are placed in the container, the olives may be presented as follows:

- Place-packed olives: Fruits are placed in transparent, rigid packs either in orderly, symmetrical fashion or forming geometrical shapes. Whole, pitted, stuffed, and halved olives may be exported in this manner.
- Random (thrown) packed olives: Fruits are not placed in an orderly fashion in the packs. All the olive styles may be exported in this manner.

Main Processes

The three preparations of table olives that represent high economic and commercial importance in international trade are:

1. Spanish- (or Sevillan-) style pickled green olives in brine
2. Californian-style pickled black olives in brine
3. Greek-style natural black olives in brine

The three preparations are similar in that the final products are prepared and preserved in diluted water solutions of sodium chloride, at different concentrations. For each style, fruits are harvested at a different stage of ripeness; green, black, and natural black, respectively. In each case, the adjective in the style name points out the country or state in which the process was first developed and used. In the first two styles, bitterness is removed by an alkaline treatment; for that reason, the word pickled appears in the name. In the case of the Greek-style process, fruits are placed directly into the brine, and removal of oleuropein is slow and only partial.

Sensory properties for each preparation are very different, although this variation may also occur even within the same preparation when parameters of the final product are adapted by technology to consumer preferences, which depend on the region or country where the product is going to be marketed.

For Spanish-style green olives in brine, a lactic fermentation constitutes the fundamental process (81). For Greek-style natural black olives, the main process responsible for the spontaneous fermentation are yeasts; lactic cocci and bacteria coexist in a smaller proportion.

Californian-style pickled black olives in brine do not necessarily require a fermentation process; they may be directly treated with lye and oxidized, then washed, placed in brine, canned, and heat sterilized. However, a fraction of the crop may be placed directly into brine for a variable preservation period and further processed. That process produces a kind of fermentation quite similar to that of natural black olives in brine.

Processed Olives

Regulations. Because of its organoleptic properties and its composition, the table olive is identified in many countries with a high standard of living. It is widely used as an appetizer to accompany all kinds of beverages, and as a decorative or nutritional element of various dishes. It also constitutes a basic food for the majority of people in some countries, mainly those situated in the Mediterranean area.

Table olives, prepared by different processes, are traded internationally by both developed and less-developed countries. For those transactions the IOOC through a committee of experts has evolved the Unified Qualitative Standard Applying to Table Olives in International Trade, mentioned previously, which is revised periodically. In the same manner, the Joint Program Food and Agriculture Organization/World Health Organization (FAO/WHO), with the cooperation of the IOOC has developed nutritional standards for table olives. The two sets of standards agree quite well in all their content and constitute an important aid in commerce and in the protection of consumers.

Spain, the main producer of table olives, has developed its own standards, which are even more detailed and more restrictive in certain aspects than those mentioned previously, although they are in agreement with them. In the same way, other exporter countries have developed their own standards, too.

Although the IOOC recommends that international standards apply to all transactions, it is also necessary to consider special regulations of importer countries in each particular case. Therefore Spain, the main supplier for the United States, regulates its exports of table olives according to U.S. regulations, which are gradually revised and published in the *Code of Federal Regulations* and *Federal Register*, Title 7, Chapters IX (Agricultural Marketing Service) and XXVIII (Agricultural Food Safety and Quality Service); and Title 21, Chapter I (Food and Drug). The EEC is taking actions to adopt the IOOC standards as official for its members.

Composition and Nutritive Value. Among the different factors that may affect the composition of the final product, the most important are the treatment of the fruit before fermentation or conditioning; the fermentation or conservation in brine; the storage after processing, bottling, or canning; and final conservation until consumption (shelf life) (82).

Table 6 shows the main components for some Spanish varieties that were processed by the three methods studied in this article. For Spanish-style pickled green olives in brine, ranges indicated in the table include four varieties: Gordal, Manzanilla, Hojiblanca, and Verdial. For Greek-style natural black olives in brine and Californian-style pickled black olives in brine, typical values for Hojiblanca variety are presented.

The concentration of moisture and oil in the flesh shows an inverse relationship to the concentration in fresh fruit. The wide range in the values is influenced by variety, maturity of the raw material, and processing conditions. The concentration of proteins as well as of fiber is slightly reduced, probably due to solubilization during the lye treatment and fermentation. Fiber is well balanced, showing a good digestibility because the ratio of lignin to cellulose is generally less than 0.5.

The percentage of ash increases as a consequence of the alkaline treatment, fermentation, and storage in brine. The majority of essential amino acids as well as a noticeable amount of vitamins and mineral elements are also present.

The caloric value is quite variable, depending mostly on the lipid content of different varieties. The quality of the oil fraction is good because of its low content of saturated fatty acids (12–19%) and the presence of an important fraction of linoleic acid (5–8%). For all these reasons, it can be said that table olives provide good food value (83,84). For Spanish-style pickled green olives in brine, soluble sugars disappear during fermentation and are absent from the final product. The same happens to the primary source of bitterness, oleuropein, which is mostly hydrolyzed during the alkaline treatment (80). Organic acids and their salts represent 1.5% of the weight of the pulp; they consist partly of the organic acids present in the fresh fruit and partly of those formed during the lye treatment and further fermentation.

Table 6. Composition of Processed Table Olives: Concentration Ranges and Typical Values in the Pulp

Component	Spanish style	Californian style	Greek style
	Major component (weight %)		
Moisture	61.00–80.56	68.98	60.34
Lipids	9.05–28.19	21.24	23.43
Protein	1.00–1.45	1.12	1.15
Fiber	1.40–2.06	2.35	2.18
Ash	4.19–5.46	2.04	6.88
	Vitamins		
Carotene (mg/100 g)	0.02–0.23	0.09	
Vitamin C (mg/100 g)	1.44–2.87		
Thiamin (μg/100 g)	0.40–3.37	0.21	14.50
Riboflavin (μg/100 g)		96.30	91.00
	Essential Amino Acids (mg / 100 g)		
Valine	55–157	119	88
Isoleucine	43–121	118	37
Leucine	82–227	147	44
Threonine	5–64		
Methionine	13–79	32	10
Phenylalanine	39–111	89	47
Lysine	5–31		5
Tryptophan	13–18	15	11
	Minerals (mg / 100 g)		
Phosphorus	7–21	15	3
Potassium	34–109	12	29
Calcium	35–86	68	28
Magnesium	6–40	18	8
Sodium	1,313–1,753	634	3740
Sulfur	14–38	8	6
Iron	0.58–1.16	7	0.30
Manganese	0.06–0.12	0.14	0.02
Zinc	0.25–0.41	0.49	0.25
Copper	0.42–0.82	0.29	0.06
	Caloric value (cal / 100 g)		
	102–280	222	249

Chlorophyll degradation during the processing of Spanish-style pickled green olives in brine can take place by two different mechanisms, resulting in a mixture of pheophytins and pheophorbides in the final product (85). In relation to the carotenoid fraction, only β-carotene and lutein are resistant. Phytofluene and ζ-carotene disappear, and violaxanthin, luetoxanthin, and neoxanthin give rise to their isomers auroxanthin and neochrome. The degradation taking place, however, does not give rise to uncolored final products with pigmentation loss, and the total balance of pigments remains constant with time.

In the case of Greek-style natural black olives in brine, prepared without a previous lye treatment, the concentration of soluble sugars remains relatively high (≧0.3%) even after long storage in brine. The same happens to oleuropein and tannins, as a consequence of a very slow fermentation process. For that reason, sensory properties of this final product are quite different from those of the other two types and are also very appreciated by the consumer.

The main difference between Californian-style pickled black olives in brine and those previously described is due to the oxidation in an alkaline medium. If storage in brine before processing is used, the characteristics of the fruit at that stage are similar to those processed as natural black olives in brine, with only the difference in color due to the stage of maturity at which they are harvested. As a result of the processing conditions, tannin and oleuropein content, as well as total acidity of the final product, are very low, and it must be sterilized by heat as a consequence of the relatively high pH value.

In summary, the changes that occur during processing may be divided into three groups: (*1*) compounds that are simply lost during the different stages of the process; (*2*) compounds that are transformed by simple chemical reaction or by the action of enzymes originally present in the fruit or produced by microorganisms; and (*3*) compounds produced during fermentation by different microorganisms. Table 7, Table 8 and Table 9 show the main changes produced in the three types of processing mentioned in this article.

Main Industrial Processes

Spanish-Style Pickled Green Olives in Brine. Within the green-olive type, which accounts for ca. 42% of the world production of table olives, the most important commercial preparation is that of Spanish- or Sevillan-style, which in turn accounts for the highest percentage of the international trade. They generally may be preserved as is; however, if the fermentation is only partial, they keep the denomination pickled green olives in brine but do not enter within the Spanish style. In that case, their further preservation, at a pH value within the limits specified in that standard, may be ensured by (*1*) sterilization or pasteurization, (*2*) the addition of preserving agents, (*3*) refrigeration, or (*4*) inert gas, without brine.

Other green olives are prepared without a prior alkaline treatment and are placed directly into the brine, either to undergo a natural fermentation or to be preserved by any of the procedures mentioned earlier. In those cases, they keep the denomination green olives and the word pickled disappears. Their commercial importance is minor, although final products of excellent quality may be obtained by those procedures. Table 7 shows the different phases for the processing of Spanish-style pickled green olives in brine.

The fruits are picked when their color is still green or yellowish green and are harvested by hand, although numerous attempts have been made to harvest them mechanically. Tree shakers have not been well accepted yet because of the damage they cause to the trees and fruits.

Fresh fruits are then transported to the factory either in crates of perforated plastic material that allow access of air, with a 20 to 25-kg capacity, or in bins with perforated mesh walls of higher capacity. The fruits remain in their containers for a period varying from several hours to 3 or 4 days, depending on the variety. After sorting and an optional size grading, the fruits proceed to the lye treatment.

Fruits are treated with a diluted solution of sodium hydroxide to eliminate the greater part of the bitter glucoside oleuropein. In this treatment, the lye penetrates the pulp to a depth of two-thirds to three-fourths the distance between the skin and the stone. The lye concentration used varies generally between 1.3 and 2.6% (wt/vol), depending on temperature, variety, and stage of maturity of the fruits,

Table 7. Spanish-Style Pickled Green Olives in Brine: Scheme of the Process and Compositional Changes

Phases	Operations	Flow chart	Changes in composition
Prior phase	None	Fresh olives ⇓ Harvesting ⇓ Transport to the factory ⇓ Sorting (optional size grading) ⇓	None, under normal conditions
Fundamental phase	Alkaline treatment and washing with water	Lye treatment ⇓ Washing ⇓	Hydrolysis of oleuropein Loss of sugars and organic acids Formation of organic acids from sugars
	Fermentation in brine, mainly lactic acid bacteria Secondary action of other microorganisms	Brining ⇓ Lactic fermentation ⇓	Lactic acid formation from sugars and other fermentable compounds Formation of other organic acids Degradation of pigments
Final phase		Sorting and size grading ⇓ (optional pitting and stuffing) ⇓	
	Conservation in brine Bottling	Packing	None, under normal conditions

Table 8. Californian-Style Pickled Black Olives in Brine: Scheme of the Process and Compositional Changes

Phases	Operations	Flow chart	Changes in composition
Prior phase	None (optional fermentation in brine, by lactic acid bacteria and yeasts)	Fresh olives ⇓ Harvesting ⇓ Transport to the factory ⇓ Sorting (optional size grading) ⇓ (optional storage in brine) ⇓	None, under normal conditions (if optional fermentation is used: slow loss of sugars, tannins, and oleuropein. Formation of organic acids and possibly ethanol and aromatic compounds)
Fundamental phase	Alkaline treatment Washing with water Oxidation by air Fermentation in brine, mainly lactic acid bacteria Secondary action of other microorganisms Canning Heat sterilization	Lye treatment and air oxidation ⇓ Washing ⇓ Color fixing and brining ⇓ (Optional pasteurization) ⇓ Sorting and size grading ⇓ (Optional pitting, slicing, splitting, etc.) ⇓ Canning and heat sterilization ⇓	Hydrolysis of oleuropein Loss of sugars and organic acids Formation of organic acids from sugars Degradation of pigments
Final phase	Storage of the sealed and sterilized product	Storage	None, under normal conditions

Table 9. Greek-Style Pickled Black Olives in Brine: Scheme of the Process and Compositional Changes

Phases	Operations	Flow chart	Changes in composition
Prior phase	None	Fresh olives ⇓ Harvesting ⇓ Transport to the factory ⇓ Sorting (rough size grading) ⇓	None, under normal conditions
Fundamental phase	Washing with water Spontaneous fermentation in brine Yeast predominate; lactic acid bacteria sometimes present	Washing ⇓ Brining ⇓ Fermentation ⇓ Sorting and size grading (air exposure) ⇓	Slow loss of sugars, tannins, and oleuropein. Formation of organic acids, ethanol, acetaldehyde, and ethyl acetate.
Final phase	Storage in brine Canning	Packing and storage	None, under normal conditions

and it is regulated in such a way that the treatment takes a determined number of hours to reach the suitable penetration for each variety. As a general rule, time for the majority of the varieties is 5 to 7 h, except for Gordal and Ascolano, which require a slower treatment, around 9 to 10 h, with more diluted lyes because of the texture and composition of the skin and flesh. A good control of the factors mentioned previously (lye concentration, lye penetration, and time of the treatment) is essential for the quality of the final product. When the fruits are treated with too much of a low-concentration lye and hence over a longer time, the further developed color may be only fairly acceptable and the fermentation will be poor. Conversely, a high-lye concentration may produce texture deficiencies and a high loss of fermentable matter, which is important to further fermentation.

After the alkaline treatment, the fruits are washed with water to eliminate the major portion of the lye that remains in the flesh. The duration and number of necessary washings are also important factors. An excessive number of washings can deplete the fermentable matter and nutrients in such a way that it will be necessary to add more of those compounds to complete the fermentation process. An excessive amount of organic salts may also be lost, producing as a consequence a lack of buffer capacity of the medium. Finally, a long period of washing may lead to serious bacterial contamination before the fruits are placed into the brine. On the contrary, excessively short washings produce a high concentration of organic salts (frequently called residual lye) in the fruits, which further prevents the attainment of suitable pH values during fermentation (81).

In summary, for each variety, stage of maturity, and temperature, a compromise must be found between lye treatment and the washing system that permits the fruits, when placed in brine, to retain enough fermentable compounds to reach a suitable degree of free acidity during fermentation and a concentration of organic salts (normally around 0.1 N) that will maintain the right pH value for safe storage of the fruits in brine. If the lye treatment is correct, a quick rinse after the alkaline treatment, followed by a first washing of 2 to 3 h and a second washing of 10 to 12 h, is the schedule normally used in the factories.

After washing, fruits are placed into a brine of sodium chloride in which lactic acid fermentation takes place. The brine, by osmosis of the components of the fruit, is transformed in a rich culture medium for microorganisms that are responsible for fermentation. The speed of that transformation depends on the variety, lye treatment and washings, ratio of fruit to brine, salt concentration, temperature, and so on. The order of appearance of growth of different microorganisms is dependent on their nutritive requirements.

The concentration of initial brine is quite important. If too low, the also low osmotic pressure can lead to spoilage by sporulating microorganisms of the *Clostridium* type during the first stage of fermentation, if the pH value remains too high On the contrary, if the concentration of salt is too high, fruits may become irreversibly wrinkled (86). Depending on the variety and the stage of maturity of the fruit, initial concentration must be regulated between 9 and 11 Beaume degree units.

After the initial decrease in the salt concentration, it must be gradually increased to maintain a good texture in the fruit and allow good fermentation and storage (86). However, the increments of salt must be slow enough to permit the right growth of the lactic acid bacteria.

A good approach is to maintain the salt concentration between 5 and 6% during the greater part of the fermentation phase, rising to 7% at the end of that part of the process. Furthermore, it may be increased up to 8% or even more during the storage phase to avoid the growth of *Propionibacteria* during the last stage of fermentation, which may produce a depletion of lactic acid.

At present, the containers used for all the fundamental phases of the process (treatment with lye, washing, fermentation, and further storage of the fermented product) in the majority of the producer countries are fermentors made of polyester and fiberglass. They can be fully closed to create anaerobic conditions and exclude the growth of yeast films on the surface of the brine. The containers have a large opening on the top and valves at the top and the bottom that facilitate the unloading of the fruits and the circulation of the brine. The most common capacity for these is about 10 t of fruit, some even 15 t. The same material is used for underground fermentors. In those cases, the opening at the top is used for all operations—loading, unloading, and recirculation of the brine—by use of suitable pumps.

The four phases of spontaneous fermentation of pickled green olives in brine have been described in detail. Each of them is characterized by a different development of the microbial population as well as by changes in the physical chemical characteristics of the culture medium, which is constituted by the initial brine and the soluble compounds of the fruit that pass to it by osmosis.

The duration of each phase as well as the relative importance of the growth of certain microorganisms depend on a series of factors, among which the following must be emphasized.

- Prior treatments of the olives (lye treatment, washing operations, and initial concentration of the brine)
- Capacity of the fermentor and, as a consequence, the ratio of fruit weight to brine volume
- Climatic and environmental conditions in the factory
- Variety of the olives
- Sanitary conditions of the plant and equipment

In all cases, the natural sequence of appearance, growth, and disappearance of microorganisms may be established as follows:

- Gram-negative bacteria, the majority of which belong to the Enterobacteriaceae family. They are the first to appear and grow, at the relatively high pH values of the initial brine.
- Lactic-acid-producing cocci, mainly from the genus *Pediococcus* (homofermentative) and *Leuconostoe* (heterofermentative). Their major or minor growth may have, together with other factors and the action of gram-negative bacteria, an influence on *Lactobacilli*, mainly from the species *L. plantarunz*, which, under normal conditions, become predominant in the third phase and are responsible for the typical fermentation of Spanish-style pickled green olives in brine.
- *Propionibacterium* genus, which, as indicated earlier, may grow during the fourth phase with depletion of lactic acid if salt concentration in the brine is not suit-

ably controlled during the storage of the fermented fruits.

A variable and nonabundant fermentative yeast population coexists with *Lactobacilli* throughout the whole fermentation period and probably partially contributes to the sensory properties of the final product. However, film-forming oxidative yeasts must necessarily be avoided with a suitable anaerobic closing of the fermentors. Each genus and species gradually adapts the characteristics of the medium to the requirements of the microorganism that succeeds it within the natural sequence, until the last one becomes dominant.

The correct control of a reduced number of factors, such as pH value, free and combined acidity, salt concentration, temperature, degree of aerobiosis or anaerobiosis, and use of a pure culture as starter, permits food technologists to vary the duration and relative importance of each of the phases and avoid the microorganisms that can have negative effects on the quality of the final product.

Acidification or injection of CO_2 into the brine during the first stage to prevent the development of gram-negative bacteria, and a further inoculation with a pure culture of *L. plantarum* or a well-fermented brine with a good population of active *Lactobacilli*, is a very efficient method to reduce to a minimum the effect of the first and second phases and accelerate the start of the fundamental third phase. A well-controlled fermentation process produces a final product that, in general, shows pH values of 3.8 to 4.2 units and a free acidity between 0.8 and 1.2%, expressed as lactic acid and mainly composed of that acid. The figures for combined acidity, also improperly called residual lye, oscillate between 0.09 N and 0.11 N, approximately. The final salt concentration should be about 7%, if the storage period is expected to be relatively short and room temperature is not too high. However, for longer periods of preservation or high summer temperatures, it is better to raise the sodium chloride concentration to at least 8%, to avoid the growth of *Propionibacterium* species. Only *Lactobacilli* in their declining phase and some fermentative yeasts constitute the microbial population of the fermented product.

Green olives to be sold are bottled or canned in small, hermetically sealed containers, which generally present the characteristics summarized in Table 10. The right selection of those parameters, the most important of which are fixed by the international standard of the IOOC, permits the bottled or canned product to be kept safely without pasteurization, and no sediment is formed in the container (87). According to those standards, salt concentration may be reduced up to 2% and pH value raised up to 4.3 if the containers are pasteurized.

Table 10. Typical Ranges for the Main Physical Chemical Parameters of Pickled Olives in Brine

Parameter	Spanish-style green olives	Californian-style black olives	Greek-style black olives
pH value	3.6–4.2	5.8–8.0	4.3–4.5
Free acidity (as lactic acid)	1%		0.3–0.5%
Sodium chloride	2–8%	1–5%	6–10%

The conditions for bulk pasteurization of green olives and pasteurization of different sizes and shapes of cans and bottles filled with different sizes of pickled green olives have been studied for Gordal and Manzanilla varieties (88). An objective spectrophotometric method by measurements of reflectance has been developed to follow the color evolution of the fruits during fermentation and further treatments (89).

Californian-Style Pickled Black Olives in Brine. Within the turning-color type, which represents about 20 to 23% of the world production of table olives, the most important commercial preparation is that of Californian-style pickled black olives in brine (called ripe olives in the United States). The main producer countries are the United States (46–48% of the world production of these olives) and Spain (25–32%). Table 8 shows the different phases for the process of this style. The process may be carried out either directly with the fresh fruits or after a holding period of the olives in brine, which varies from 2 to 6 months. The reason for the optional storage in brine is that not all the fruit available at the time of the harvest can be directly processed, because of the capacity of the processing plants and because it is not practical to keep large stocks of canned product for a long time. In the first procedure, sorted fresh olives are successively treated with sodium hydroxide aqueous solutions for varying periods to get a gradual penetration of the lye into the flesh. After each lye treatment, the fruits are placed in plain water and oxidized by air injection under pressure to transform the polyphenolic compounds and so permit a complete blackening of the skin and a uniform color in the flesh.

The color of the skin of the fruit at the time of harvest varies greatly from yellowish green to purple, and the taste, color, and texture of the final product will be highly dependent on the maturity stage and the variety. Mission and Manzanilla in the United States and Hojiblanca and Cacerena in Spain are the best varieties for this type of processing.

The number of lye treatments has traditionally been between 3 and 5, although at the present time industry tends to reduce this number to a minimum. In general, a first treatment just to penetrate the skin, a second one to penetrate 1 mm of the flesh, and a third one that permits the lye to reach the pit constitute a typical industrial schedule. The concentration of the lye depends on the maturity stage of the fruit, the variety, the temperature, and the desired penetration. It generally varies between 1 and 2%, the highest being used to penetrate the skin. Air oxidation periods, between lyes, are variable, frequently around 12 h.

Tanks for lye treatment, air oxidation, and washings have different sizes and shapes and are made of concrete, stainless steel, or polyester and fiberglass and must be suitably arranged for uniform distribution of pressurized air throughout the whole mass of fruits and liquid.

The number and duration of washings must be regulated to get the washed olives to reach a final pH value around 7 units. Washing water must be changed frequently to avoid the growth of spoiling aerobic microorganisms.

Generally, 0.1% of ferrous gluconate is added to the last washing water to fix the color of the fruits, obtained by oxidation. The effects of ferrous gluconate and ferrous lactate, at the same level of iron content, have been compared, and similar results with the two compounds have been reported (90). In addition, olives are placed and equilibrated into a brine containing 3% sodium chloride, canned, and heat sterilized. Varnished tinplate is generally used for the inside of the containers. The use of a needle board to puncture the skins of certain varieties of olives before the brining to avoid shriveling and a pasteurization before the canning operation to minimize the action of some aerobic bacteria during brining are optional parts of the process.

The fruits that are not directly treated after harvesting and that remain for a variable period in holding brines may undergo a spontaneous fermentation process that is similar in almost all aspects to the one followed by natural black olives in brine (Greek style), which will be described next. At the end of this holding period, fruits are processed in the same way as indicated previously for fresh fruits. However, lye concentration and time for each treatment will be influenced by the conditions used during the previous storage and must be carefully controlled.

Although traditionally this previous storage in brine has been undertaken under relatively close to anaerobic conditions to avoid films of yeasts and molds, which may spoil olives, an alternative aerobic procedure has been developed, with excellent results in Manzanilla and Hojiblanca varieties (91,92).

The final product for Californian-style pickled black olives in brine shows typical pH values between 5.8 and 7.9 units and salt concentrations between 1 and 3% sodium chloride (wt/vol). However, for certain consumer preferences, salt concentrations close to 5% are also used.

Greek-Style Natural Black Olives in Brine. Within the black-olive type, which represents about 33 to 40% of the world production of table olives, the most significant commercial preparation is that of Greek style. The main producing countries are Turkey (24–27% of the world production of this type) and Greece (18–21%). Table 9 shows the different phases for the processing of this style.

Fruits are harvested when fully ripe or slightly before full ripeness is reached, and they may, according to production region, variety, and time of harvesting, be reddish black, violet black, deep violet, greenish black, or a deep chestnut color. The olives are transported to the factory as described previously for the Spanish-style pickled green olives in brine. After a sorting to separate damaged fruits, and occasionally after a rough grading to eliminate small sizes, the fruits are quickly washed with water to remove superficial dirt and then placed into a brine with a salt concentration between 8 and 10 Beaume degree units, or slightly lower.

At present, the polyester and fiberglass fermentors described for green olives are also used for this type of fruit. They are generally buried underground to avoid high temperatures, because of the high degree of ripeness of the olives. Traditionally, the processors have tried to maintain strict anaerobiosis in the tanks to avoid the growth of film-forming yeasts and molds, which affect the texture and flavor of the olives. The fermentation process is very slow, because diffusion of soluble components of the fruit to the brine is slow due to the fact that it has not been treated with lye. A complex microflora develops during fermentation: gram-negative bacteria, gram-positive cocci of lactic acid, yeasts, and occasionally *Lactobacilli*—the last in the case of relatively low concentrations of sodium chloride, below 7 to 8%. Yeasts constitute the dominant population, and the most representative species are *Saccharomyces oleaginosus* and *Hansenula anomala*.

Under those conditions, the final product has pH values between 4.5 and 4.8 units, and free acidity ranges from 0.1 to 0.6%, expressed as lactic acid. For these reasons, there is a tendency to gradually raise the salt concentration, which frequently is about 10% or even higher at the end of the fermentation process.

Afterward, fermented olives are exposed to air to improve the skin color, classified by size; and packed with a new brine. Containers are either wooden or plastic barrels of about 130 to 150 kg of fruit, cans of 10 to 15 kg, or smaller plastic bags. During this traditional process, the spoilage, called *alambrado* or fish eyes, frequently produces big losses in certain varieties of olives. This effect is attributed to different causes, such as the fruit's metabolism, influenced by the anaerobic conditions of the medium and the maturity stage of the fruit itself, and the action of the most representative yeasts previously mentioned.

A new controlled aerobic fermentative process, which avoids spoilage, has been developed (93,94). It injects air through the mass of fruits and brine during fermentation. The system may be applied in either a continuous or discontinuous manner. The best conditions are volume of air, 0.1 to 0.5 L/h/L capacity; sodium chloride concentration, 10%; and pH value of 4.5 units, controlled by the addition of food-grade acetic acid. Under these circumstances, a population of yeasts of oxidative and facultative metabolism is responsible for fermentation, with *Debaryomyces hansenii* and *Toritlopsis candida* the most representative species. The system for polyester and fiberglass tanks, which are most frequently used in the industry, have been further optimized (94). After finishing the phase of active fermentation and interrupting the airflow, the investigators indicate that the safest method to avoid further spoilage is the addition of the necessary amount of potassium sorbate to reach 0.05%, expressed as sorbic acid, in the equilibrium (95).

Further studies indicate that the use of a low concentration of sodium chloride, around 2% at the beginning of the aerobic fermentative process, and a gradual increase of salt up to the final value of 9% is satisfactory (96). Some other species of representative yeasts have been isolated, such as *Pichia membranaefaciens*, *Hansenula mrakii*, and *Candida boldinii*. The effect of different levels of aeration and its mode, initial concentration of salt, maturity stage of the olives, and control of the pH value have been studied using a factorial design experiment (97).

The aerobic procedure is being gradually and successfully adopted by the processors of this style of black olive. The best conditions for packing the olives in small containers destined for the consumer have been studied (98). Two methods are equally effective: a pasteurization at 80°C for 4 min, and the addition of potassium sorbate 0.05%, expressed as sorbic acid, at the equilibrium. Typical values for these products, after 11 months of storage, are pH values of 4.40 to 4.45 units and sodium chloride between 7.2 and 7.4%.

REDUCTION AND REUSE OF WASTES

Most food industries that transform natural products, of either animal or vegetable origin, contribute to ambient pollution. So is the case with olive oil mills and olive-pickling plants. This situation has constituted an important concern for scientists and technologists in the olive oil and table olives industries for the last 35 years (99).

By-products of Olive Oil Production

Olive pomace and wastewaters are the major by-products resulting from the elaboration of olive oil (100–102). Olive pomace is the material remaining after most of the oil is removed from the olive paste. It contains fragments of skin, pulp, fragments of kernels, and some oil. In chemical terms, the major constituents are cellulose, protein, and water. The minor components are represented by a complex mixture of polyphenols (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, and caffeic acid). Olive pomace has some commercial value depending upon its oil and water content. The olive pomace can be further processed to extract the remaining oil. The exhausted pomace (kernel wood) can be used for several applications: (*1*) Its combustion can generate heating water in the olive oil mills; (*2*) kernel wood, after the removal of the stones, can be used as livestock feed; and (*3*) its ashes can be used as fertilizers.

The wastewater represents the water content of the olive fruit plus the water added during the processing of the olives in the olive mill. Carbohydrates are the major organic substances in wastewater; these are followed by proteins, organic acids, phenols and polyphenols, and some emulsified oil. Among the inorganic components, the most abundant are phosphates, sulphates, and chlorides, followed by carbonates and silicates. Sodium, potassium, and calcium are also found in large quantities. Wastewater has been used to grow yeast, to produce butanol using microorganisms, to isolate anthocyanin compounds for use in the food industry, and to produce steam. Efforts are being made to reduce wastewater by recycling in the milling process and to decrease its environmental pollution by treatment with biological or physical processes prior to its discharge (103–105).

By-products of Olive Pickling

Some important progress in solving the problem of olive-pickling by-products has been made in two complementary ways (102,106):

1. Reducing the volume of industrial wastes and reusing them in processing operations
2. Regenerating the fermentation brines to be further used in packaging the final product

For the first point, a series of internal-control regulations have been adopted, such as reusing lyes for the treatment of olives; reducing the lye concentration up to a minimum effective value; and reducing, and even completely eliminating the washing operations by neutralizing the residual free lye after the alkaline treatment (99). With regard to the regeneration of the fermentation brines, to be used in bottling and canning operations, the most successful procedures include ultrafiltration and a previous treatment with active coal followed by a filtration (107,108).

At present, it can be said that this is a problem that is technically solved, although it requires an economic adjustment to be developed industrially and generally applied.

THE INTERNATIONAL OLIVE OIL COUNCIL

Establishment and Functions

The IOOC was established in 1959 to administer the International Agreement on Olive Oil of 1956. This agreement has evolved over the last 40 years through a series of amendments approved at several United Nations conferences on olive oil. The most comprehensive International Agreement on Olive Oil and Table Olives was negotiated at the U.N. Conference on Olive Oil held in Geneva from June 18 to July 1, 1986, and extended with amendments until December 31, 1998. The chief objectives of the 1986 agreement are to facilitate international cooperation on world problems affecting olive products; to promote research and development for the modernization of olive cultivation and the industries processing its products by setting up technical and scientific programs to improve the quality and reduce the prices of the products obtained; to facilitate the study and application of measures devised to expand international trade in olive products; to seek to balance production and consumption, especially by increasing the latter; to reduce handicaps brought about by fluctuating market supplies; to forestall and oppose unfair competition practices and ensure delivery of goods that comply with the contracts signed; to foster the coordination of policies covering olive products; to enlarge market accesses; to ensure reliability of supplies and devise trade structures, especially by improving consultations and the supply of information; and to continue to develop the tasks undertaken within the context of the previous olive oil agreements.

The council undertakes, in conjunction with United Nations Development Programme (UNDP), FAO, and other international organizations, to collect technical information and circulate it to all members; to promote action to coordinate technical improvement activities among members; to assist national planning relating to technical improvements, in particular in the developing olive-growing countries; and to encourage the transfer of technology to developing olive-growing countries; and to encourage the transfer of technology to developing olive-growing countries from countries highly advanced in olive cultivation and oil-extraction techniques. The council provides a special fund for this purpose and, as part of the development of international cooperation, it procures financial and technical assistance from competent international, regional, and national organizations.

The agreement provides for a joint promotion fund set up every year with compulsory contributions from the mainly producing members. Mainly importing members may contribute to the fund under special agreement. The council is entitled to receive, for promotional purposes, voluntary contributions from a governments or other sources. The council also gives special attention to any scientific research on the biological value of the products obtained from the olive that might prove to be of use to consumption throughout the world.

Composition

The contracting parties to the International Agreement on Olive Oil and Table Olives, 1986, as amended and extended, are as follows:

- Mainly producing and mainly importing members: European Union (Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Spain, Sweden, United Kingdom)
- Mainly producing members: Algeria, Cyprus, Israel, Lebanon, Morocco, Syrian Arab Republic, Tunisia, Turkey, Yugoslavia
- Mainly importing member: Egypt
- Observers: Argentina, Australia, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Cuba, Dominican Republic, Ecuador, India, Islamic Republic of Iran, Iraq, Japan, Jordan, Libya, Mexico, New Zealand, Norway, Pakistan, Panama, Peru, Poland, Romania, Russian Federation, Saudi Arabia, Slovak Republic, Slovenia, Thailand, United States, Uruguay, Venezuela, the Palestinian National Authority.

General Publications

International Olive Oil Council Information Sheet (twice monthly); *OLIVAE* (5 times per year); *National Policies for Olive Products* (annual); various leaflets giving information on the International Agreement on Olive Oil and Table Olives, the IOOC, and IOOC activities; market research, technical handbooks, recipe books

SUMMARY

According to Greek mythology, thousands of years ago, Poseidon, god of the sea, and Athena, goddess of wisdom, were arguing over who was to going to control the destiny of a newly built city. They decided that the one who would present the city with the most valuable gift would be the one honored. Poseidon struck a rock with his trident, and a spring appeared. As the water started to flow, a horse emerged, symbol of strength and power. Athena stuck her spear in the ground, and it turned into an olive tree, symbol of peace, wisdom, and prosperity. Athena's gift was deemed the most valuable, and the city was named Athens in her honor. Today, an olive tree stands where the legend says Athena placed her spear. It is said that all the olive trees in Athens were descended from that first olive tree offered by Athena. The reality is that the olive tree and its fruits have played an integral part in the history of the people of the Mediterranean. Olive oil has been considered the cornerstone of the so-called Mediterranean diet. This diet has been associated with multiple health benefits, and current researchers are investigating the molecular basis for these effects. This chapter describes briefly some of the more technical aspects of the production of olive oil and table olives. Several monographs go into more detail about these aspects of olive oil; among the most recent and comprehensive is one published by A. Kiritsakis (109).

BIBLIOGRAPHY

1. G. C. Martin, L. Ferguson, and V. S. Polito, "Flowering, Pollination, Fruiting, Alternate Bearing, and Abscission," in L. Ferguson, G. Steven Sibbett, and G. C. Martin, eds., *Olive Production Manual*, Univ. of California, Div. Agr. Natural Resources, Oakland, Calif., 1994, pp. 51–56
2. G. Bongi and A. Palliotti, "Olive," in B. Schaeffer and P. C. Andersen, eds., *Handbook of Environmental Physiology and Fruit Crops*, CRC Press, Boca Raton, Fl. 1994, pp. 165–188.
3. M. J. Fernandez-Diez, "Olives," in H. J. Rehm and G. Reed, eds., *Biotechnology*, Verlag Chemie, Weinheim, Germany, 1983, pp. 379–397.
4. N. Liphschitz et al., "The Beginning of Olive *Olea Europaea* Cultivation in the Old World: A Reassessment," *Journal of Archaeological Science* **18**, 441–454 (1991).
5. R. B. Hitchner and D. J. Mattingly, "Fruits of Empire: The Production of Olive Oil in Roman Africa," *Research and Exploration* **7**, 36, 38–48, 50–55 (1991).
6. R. Aparicio and M. T. Morales, "Characterization of Olive Ripeness by Green Aroma Compounds of Virgin Olive Oil," *J. Agric. Food Chem.* **46**, 1116–1122 (1998)
7. J. M. Garcia, S. Seller, and M. C. Perez Camino, "Influence of Fruit Ripening on Olive Oil Quality," *J. Agric. Food Chem.* **44**, 3516–3520 (1996).
8. J. Fernandez-Bolanos et al., "Sugars and Polyols in Green Olives: III, Quantitative Determination by Gas-Liquid Chromatography," *Grasas y Aceites* **34**, 168–171 (1983).
9. R. Guillen et al., "Dietary Fiber in Olives: Characterization of fractions," *European Journal of Clinical Nutrition* **49**, S224–S225 (1995).
10. A. Gil-Serrano, M. P. Tejero-Mateo, and J. Fernandez-Bolanos, "Polysaccharides in Olives: III, Partial Hydrolysis and Acetolysis of a Hemicellulose B Isolated in Olives of the Manzanilla Variety," *Grasas y Aceites* **35**, 224–227 (1984).
11. A. Jimenez et al., "Cell Wall Composition of Olives," *J. Food Sci.* **59**, 1192–1196 (1994).
12. A. Heredia-Moreno and J. Fernandez-Bolanos, "Cellulases in Olives and Its Possible Influence in the Changes of Texture: II, Activated Cellulolytic Activity in the Hojiblanca Variety," *Grasas y Aceites* **36**, 130–133 (1985).
13. A Heredia-Moreno and J. Fernandez-Bolanos, "Cellulases in Olives and Its Possible Influence in Texture Changes: III, Study of Some Factors That Could Modify Its Activity," *Grasas y Aceites* **36**, 171–176 (1985).

14. A. Heredia-Moreno, J. Fernandez-Bolanos, and R. Guillen-Bejarano, "Cellulolytic Activity in Olives during Maduration and Softening," *Alimentaria* **198**, 49–52 (1988).
15. A. Heredia-Moreno, J. Fernandez-Bolanos, and R. Guillen-Bejarano, "Characterization and Partial Purification of Cellulolytic Enzymes in Olives," *Grasas y Aceites* **40**, 190–193 (1989).
16. A. Heredia-Moreno, J. Fernandez-Bolanos, and R. Guillen-Bejarano, "Inhibitors of Cellulolytic Activity in Olive Fruits (*Olea europaea*, Hojiblance var.)," *Z. Lebensm. Unters.-Forsch.* **189**, 216–218 (1989).
17. M. Nosti-Vega, R. Castro-Ramos, and R. Vazquez-Ladron, "Composition and Nutritive Value of Spanish table olives: VI, Changes Due to Elaboration Processes," *Grasas y Aceites* **35**, 11–14 (1984).
18. M. I. Minguez-Mosquera, "Evolution of Pectic Constituents and Pectinolytic Enzymes During the Maturation and Storage of the Hojiblanca Olive Variety," *Grasas y Aceites* **33**, 327–333 (1982).
19. P. Manzi et al., "Natural Antioxidants in the Unsaponifiable Fraction of Virgin Olive Oils from Different Cultivars," *J. Sci. Food Agric.* **77**, 115–120 (1998).
20. B. Gandul Rojas and M. I. Minguez-Mosquera, "Chlorophyll and Carotenoid Composition in Virgin Olive Oils from Various Spanish Olive Varieties," *J. Sci. Food Agric.* **72**, 31–39 (1996).
21. M. I. Minguez-Mosquera and J. Garrido-Fernandez, "Chlorophyll and Carotenoid Presence in Olive Fruit (*Olea europaea*)," *J. Agric. Food Chem.* **37**, 1–7 (1989).
22. M. I. Minguez-Mosquera and L. Gallardo Guerrero, "Disappearance of Chlorophylis and Carotenoids during the Ripening of the Olive," *J. Sci. Food Agric.* **69**, 1–6 (1995).
23. L. Ferreiro and R. Aparicio, "Influence of Altitude on Chemical Composition of Andalusian Virgin Olive Oil Mathematical Classification Equations," *Grasas y Aceites* **43**, 149–156 (1992).
24. L. Cinquanta, M. Esti, and E. La Notte, "Evolution of Phenolic Compounds in Virgin Olive Oil during Storage," *J. Am. Oil Chem. Soc.* **74**, 1259–1264 (1997).
25. I. T. Agar et al., "Quality of Fruit and Oil of Black-Ripe Olives Is Influenced by Cultivar and Storage Period," *J. Agric. Food Chem.* **46**, 3415–3421 (1998).
26. A. K. Kiritsakis, "Flavor Components of Olive Oil: A Review," *J. Am. Oil Chem. Soc.* **75**, 673–681 (1998).
27. J. Tous et al., "Chemical and Sensory Characteristics of 'Arbequina' Olive Oil Obtained in Different Growing Areas of Spain" (in Spanish), *Grasas y Aceites* **48**, 415–424 (1997).
28. A. Keys et al., "The Diet and 15-Year Death Rate in the Seven Countries Study," *Am. J. Epidemiol.* **124**, 903–915 (1986).
29. M. de Lorgeril et al., "Mediterranean Diet, Traditional Risk Factors, and the Rate of Cardiovascular Complications After Myocardial Infarction: Final Report of the Lyon Diet Heart Study," *Circulation* **99**, 779–785 (1999).
30. F. Visioli and C. Galli, "The Effect of Minor Constituents of Olive Oil on Cardiovascular Disease: New Findings," *Nutrition Reviews* **56**, 142–147 (1998).
31. F. Visioli and C. Galli, "Oleuropein Protects Low Density Lipoprotein from Oxidation," *Life Sci.* **55**, 1965–1971 (1994).
32. F. Visioli, S. Bellosta, and C. Galli, "Oleuropein, the Bitter Principle of Olives, Enhances Nitric Oxide Production by Mouse Macrophages," *Life Sci.* **62**, 541–546 (1998).
33. F. Angerosa et al., "Characterization of Seven New Hydrocarbon Compounds Present in the Aroma of Virgin Olive Oils," *J. Agric. Food Chem.* **46**, 648–653 (1998).
34. F. Caponio, V. Alloggio, and T. Gomes, "Phenolic Compounds of Virgin Olive Oil: Influence of Paste Preparation Techniques," *Food Chem.* **64**, 203–209 (1999).
35. A. Bianco et al., "Microcomponents of Olive Oil: III, Glucosides of 2(3,4-dihydroxy-phenyl)ethanol," *Food Chem.* **63**, 461–464 (1998).
36. R. Sacchi et al., "Characterization of Italian Extra Virgin Olive Oils Using H-NMR Spectroscopy," *J. Agric. Food Chem.* **46**, 3947–3951 (1998).
37. A. Kiritsakis et al., "Effect of Fruit Storage Conditions on Olive Oil Quality," *J. Am. Oil Chem. Soc.* **75**, 721–724 (1998).
38. A. Ranalli, G. De Mattia, and M. L. Ferrante, "Comparative Evaluation of the Olive Oil Given by a New Processing System," *International Journal of Food Science and Technology* **32**, 289–297 (1997).
39. M. Esti, L. Cinquanta, and E. La Notte, "Phenolic Compounds in Different Olive Varieties," *J. Agric. Food Chem.* **46**, 32–35 (1998).
40. M. Rahmani and A. S. Csallany, "Role of Minor Constituents in the Photooxidation of Virgin Olive Oil," *J. Am. Oil Chem. Soc.* **75**, 837–843 (1998).
41. T. Gomes and F. Caponio, "Investigation on the Degree of Oxidation and Hydrolysis of Refined Olive Oils: An Approach for Better Product Characterisation," *Italian Journal of Food Science* **9**, 277–285 (1997).
42. M. T. Morales, J. J. Rios, and R. Aparicio, "Changes in the Volatile Composition of Virgin Olive Oil during Oxidation: Flavors and Off-Flavors," *J. Agric. Food Chem.* **45**, 2666–2673 (1997).
43. F. Angerosa et al., "Biogeneration of Volatile Compounds in Virgin Olive Oil: Their Evolution in Relation to Malaxation Time," *J. Agric. Food Chem.* **46**, 2940–2944 (1998).
44. R. Aparicio, J. J. Calvente, and M. T. Morales, "Sensory Authentication of European Extra-Virgin Olive Oil Varieties by Mathematical Procedures," *J. Sci. Food Agric.* **72**, 435–447 (1996).
45. N. Antoun and M. Tsimidou, "Gourmet Olive Oils: Stability and Consumer Acceptability Studies," *Food Research International* **30**, 131–136 (1997).
46. F. Angerosa et al., "Sensory Evaluation of Virgin Olive Oils by Artificial Neural Network Processing of Dynamic Head-Space Gas Chromatographic Data," *J. Sci. Food Agric.* **72**, 323–328 (1996).
47. R. Raoux, "Sensory Analysis," *Ocl-Oleagineux Corps Gras Lipides* **4**, 369–372 (1997).
48. R. Aparicio, M. T. Morales, and V. Alonso, "Authentication of European Virgin Olive Oils by Their Chemical Compounds, Sensory Attributes, and Consumers' Attitudes," *J. Agric. Food Chem.* **45**, 1076–1083 (1997).
49. P. C. Van Bruggen et al., "Robust Sensory Evaluation of Olive Oil by a Non-Parametric Scoring System," *J. Sci. Food Agric.* **67**, 53–59 (1995).
50. E. Pagliarini and C. Rastelli, "Sensory and Instrumental Assessment of Olive Oil Appearance," *Grasas y Aceites* **45**, 62–65 (1994).
51. M. Bertuccioli, "A Study of the Sensory and Nutritional Quality of Virgin Olive Oil in Relation to Variety, Ripeness, and Extraction Technology: Overview of the Three Year Study and Conclusion," *Grasas y Aceites* **45**, 55–59 (1994).
52. J. Mojet and S. De Jong, "The Sensory Wheel of Virgin Olive Oil," *Grasas y Aceites* **45**, 42–47 (1994).
53. R. Aparicio et al., "Relationship between the COI Test and Other Sensory Profiles by Statistical Procedures," *Grasas y Aceites* **45**, 26–41 (1994).

54. D. H. Lyon and M. P Watson, "Sensory Profiling: A Method for Describing the Sensory Characteristics of Virgin Olive Oil," *Grasas y Aceites* **45**, 20–25 (1994).
55. M. A. Albi and F. Gutierrez, "Study of the Precision of an Analytical Taste Panel for Sensory Evaluation of Virgin Olive Oil Establishment of Criteria for the Elimination of Abnormal Results," *J. Sci. Food Agric.* **54**, 255–268 (1991).
56. G. P. Blanch, J. Villen, and M. Herraiz, "Rapid Analysis of Free Erythrodiol and Uvaol in Olive Oils by Coupled Reserved Phase Liquid Chromatography–Gas Chromatography," *J. Agric. Food Chem.* **46**, 1027–1030 (1998).
57. M. L. R. del Castillo et al., "Rapid Recognition of Olive Oil Adulterated with Hazelnut Oil by Direct Analysis of the Enantiomeric Composition of Filbertone," *J. Agric. Food Chem.* **46**, 5128–5131 (1998)
58. J. P. Favier et al., "CO2, Laser Infrared Optothermal Spectroscopy for Quantitative Adulteration Studies in Binary Mixtures of Extra-Virgin Olive Oil," *J. Am. Oil Chem. Soc.* **75**, 359–362 (1998).
59. A. S. Amr and A. I. Abu Al Rub, "Evaluation of the Bellier Test in the Detection of Olive Oil Adulteration with Vegetable Oils," *J. Sci. Food Agric.* **61**, 435–437 (1993).
60. V. Baeten et al., "Detection of Virgin Olive Oil Adulteration by Fourier Transform Raman Spectroscopy," *J. Agric. Food Chem.* **44**, 2225–2230 (1996).
61. R. Aparicio and V. Baeten, "Fats and Oils Authentication by FT-Raman," *Ocl-Oleagineux Corps Gras Lipides* **5**, 293–295 (1998).
62. N. A. Marigheto et al., "A Comparison of Mid-Infrared and Raman Spectroscopies for the Authentication of Edible Oils," *J. Am. Oil Chem. Soc.* **75**, 987–992 (1998).
63. F. Angerosa et al., "Carbon Stable Isotopes and Olive Oil Adulteration with Pomace Oil," *J. Agric. Food Chem.* **45**, 3044–3048 (1997).
64. O. Jimenez De Blas and A. Del Valle Gonzalez, "Gas Chromatographic Differentiation of Virgin, Refined, and Solvent-Extracted Olive Oils," *J. AOAC Int.* **79**, 707–710 (1996).
65. F. Santinelli, P. Damiani, and W. W. Christie, "The Triacylglycerol Structure of Olive Oil Determined by Silver Ion High-Performance Liquid Chromatography in Combination with Stereospecific Analysis," *J. Am. Oil Chem. Soc.* **69**, 552–556 (1992).
66. E. Salivaras and A. R. Mccurdy, "Detection of Olive Oil Adulteration with Canola Oil from Triacylglycerol Analysis by Reversed-Phase High-Performance Liquid Chromatography," *J. Am. Oil Chem. Soc.* **69**, 935–938 (1992).
67. A. De Leonardis, V. Macciola, and M. De Felice, "Rapid Determination of Squalene in Virgin Olive Oils Using Gas-Liquid Chromatography," *Italian Journal of Food Science* **10**, 75–80 (1998).
68. M. Amelio, R. Rizzo, and F. Varazini, "Separation of Stigmasta-Diene, Squalene Isomers, and Wax Esters from Olive Oils by Single High-Performance Liquid Chromatography Run," *J. Am. Oil Chem. Soc.* **75**, 527–530 (1998).
69. O. Jimenez De Blas and A. Del Valle Gonzalez, "Determination of Sterols by Capillary Column Gas Chromatography: Differentiation among Different Types of Olive Oil: Virgin, Refined, and Solvent-Extracted," *J. Am. Oil Chem. Soc.* **73**, 1685–1689 (1996).
70. A. Cert, W Moreda, and J. Garcia-Moreno, "Determination of Sterols and Triterpenic Dialcohols in Olive Oils Using HPLC Separation and GC Analysis: Standardization of the Analytical Method," *Grasas y Aceites* **48**, 207–218 (1997).
71. F. Lanuzza, G. Micali, and G. Calabro, "On-Line HPLC-HRGC Coupling and Simultaneous Transfer of Two Different LC Fractions: Determination of Aliphatic Alcohols and Sterols in Olive Oil," *HRC-Journal of High Resolution Chromatography* **19**, 444–448 (1996).
72. E. Psomiadou and M. Tsimidou, "Simultaneous HPLC Determination of Tocopherols, Carotenoids, and Chlorophylls for Monitoring Their Effect on Virgin Olive Oil Oxidation," *J. Agric. Food Chem.* **46**, 5132–5138 (1998).
73. G. Bianchi and G. Vlahov, "Composition of Lipid Classes in the Morphologically Different Parts of the Olive Fruit, cv. Coratina (Olea europaea Linn.)," *Fett Wissenschaft Technologie* **96**, 72–77 (1994).
74. E. J. Birch et al., "Methods for Analysis of Esterified and Free Long-Chain Fatty Acids in High-Lipid Food Processing Wastes," *J. Agric. Food Chem.* **46**, 5332–5337 (1998).
75. W. Moreda, M. C. Perez-Camino, and A. Cert, "Determination of Some Purity Parameters in Olive Oils: Results from a Collaborative Study," *Grasas y Aceites* **46**, 279–284 (1995).
76. W. V. Cruess, "Pickling Green Olives," *California Agricultural Experimental Station Bulletin* **498**, 1–42 (1930).
77. R. H. Vaughn, H. C. Douglas, and J. R. Gililland, "Production of Spanish Type Green Olives," *California Agricultural Experimental Station Bulletin* **678**, 1–82 (1943).
78. J. M. R. de la Borbolla y Alcala and C. Gomez-Herrera, "La industria de aderezo de aceitunas verdes," *Revista de Ciencia Aplicada* **7**, 120–132 (1949).
79. G. D. Balatsouras, "The Chemical Composition of the Brine of Stored Greek Black Olives," *Grasas y Aceites* **17**, 83–88 (1966).
80. M. Brenes and A. de Castro, "Transformation of Oleuropein and Its Hydrolysis Products during Spanish-Style Green Olive Processing," *J. Sci. Food Agric.* **77**, 353–358 (1998).
81. J. E. De La Torre et al., "Physical, Chemical, and Microbiological Studies of the Fermentation of Arbequina Green Olives," *Grasas y Aceites* **44**, 274–278 (1993).
82 A. H. Sanchez, A. Montano, and L. Rejano, "Effect of Preservation Treatment, Light, and Storage Time on Quality Parameters of Spanish-Style Green Olives," *J. Agric. Food Chem.* **45**, 3881–3886 (1997).
83. V. Marsilio and B. Lanza, "Effects of Lye-Treatment on the Nutritional and Microstructural Characteristics of Table Olives (Olea europaea L.)," *Revista Espanola de Ciencia y Tecnologia de Alimentos* **35**, 178–190 (1995).
84. A. I. Yassa, A. B. S. S Emmam, and R. T. Ahmed, "Effect of Different Methods of Processing on the Nutritional Value of Azizi and Picual Table Olives," *Annals of Agricultural Science* **35**, 775–786 (1990).
85. M. I. Minguez-Mosquera, J. Garrido-Fernandez, and B. Gandul Rojas, "Pigment Changes in Olives during Fermentation and Brine Storage," *J. Agric. Food Chem.* **37**, 8–11 (1989).
86. A. Jimenez et al., "Changes in Texture and Cell Wall Polysaccharides of Olive Fruit during 'Spanish Green Olive' Processing," *J. Agric. Food Chem.* **43**, 2240–2246 (1995).
87. J. M. R. de la Borbolla y Alcala and F. Gonzalez-Pellisso, "Studies on the Spanish-Style Packed Olives: XI, Sediment Inhibition," *Grasas y Aceites* **23**, 107–117 (1972).
88. F. Gonzalez-Pellisso, L. Rejano Navarro, and F. Gonzalez-Cancho, "The Pasteurization of Olives Sevillan Style," *Grasas y Aceites* **33**, 201–207 (1982).
89. A. Montano Asquerino, A. H. Sanchez Gomez, and L. Rejano Navarro, "Method for the Color Determination of Green Table Olives in Brine," *Alimentaria* **193**, 79–83 (1988).
90. P. Garcia-Garcia, M. Brenes Balbuena, and A. Garrido Fernandez, "The Use of Ferrous Lactate in the Elaboration of Black Table Olives," *Grasas y Aceites* **37**, 33–38 (1986).

91. A. Garrido Fernandez, M. C. Duran Quintana, and P. Garcia Garcia, "Study of Different Conservation Forms of Turning Color Olives of the Variety Manzanilla," *Grasas y Aceites* **37**, 1–7 (1986).
92. M. Brenes Balbuena et al., "Comparative Studies of the Conservation Systems of Black Table Olives," *Grasas y Aceites* **37**, 123–128 (1986).
93. P Garcia Garcia, M C. Duran Quintana, and A Garrido Fernandez, "Aerobic Fermentation of Ripe Olives in Brine," *Grasas y Aceites* **36**, 14–20 (1985).
94. A Garrido Fernandez, P. Garcia Garcia, and F. Sanchez-Roldan, "New Fermentation Aerobic Process for Natural Black Olives," *Alimentacion* **4**, 73–81 (1985).
95. A. Garrido Fernandez, P. Garcia Garcia, and M. C. Duran Quintana, "Conservation of Natural Black Olives Proceeding from Aerobic Fermentation," *Grasas y Aceites* **36**, 313–316 (1985).
96. M. C. Duran Quintana, P. Garcia Garcia, and A. Garrido Fernandez, "Aerobic Fermentation of Ripe Olives in Brine with Alternating Air Injection," *Grasas y Aceites* **37**, 242–249 (1986).
97. A. Garrido Fernandez, M. C. Duran Quintana, and P Garcia Garcia, "Aerobic Fermentation of Natural Black Olives in Brine," *Grasas y Aceites* **38**, 27–32 (1987).
98. P. Garcia Garcia, M. C. Duran Quintana, and A. Garrido Fernandez, "Packing of Aerobically Fermented Natural Black Olives," *Grasas y Aceites* **37**, 92–96 (1986).
99. P. Garcia Garcia et al., "Physicochemical Depuration of the Waste Waters from the Green Table Olive Packing Industries," *Grasas y Aceites* **41**, 263–269 (1990).
100. S. Vitolo, L. Petarca, and B. Bresci, "Treatment of Olive Oil Industry Wastes," *Bioresour. Technol.* **67**, 129–137 (1999).
101. A. G. Vlyssides et al., "Olive Oil Processing Wastes Production and Their Characteristics in Relation to Olive Oil Extraction Methods," *Fresenius Environ. Bull.* **7**, 308–313 (1998).
102. G. C. Kopsidas, "Wastewater from the Table Olive Industry," *Water Res.* **28**, 201–205 (1994).
103. F. Cabrera et al., "Land Treatment of Olive Oil Mill Wastewater," *International Biodeterioration and Biodegradation* **38**, 215–225 (1996)
104. F. Flouri et al., "Decolorization of Olive Oil Mill Liquid Wastes by Chemical and Biological Means," *International Biodeterioration and Biodegradation* **38**, 189–192 (1996).
105. I. Chatjipavlidis et al., "Bio Fertilization of Olive Oil Mills Liquid Wastes: The Pilot Plant in Messinia, Greece," *International Biodeterioration and Biodegradation* **38**, 183–187 (1996)
106. B. Inigo Leal, "New Technological Trends in the Production and Preservation of Table Olives," *Alimentaria* **28**, 61–65 (1991).
107 A. Garrido Fernandez, M. Brenes Balbuena, and P. R. Garcia Garcia, "Treatment for Green Table Olive Fermentation Brines," *Grasas y Aceites* **43**, 291–298 (1992).
108. A. Garrido, P. Garcia, and M. R. Brenes, "The Recycling of Table Olive Brine Using Ultrafiltration and Activated Carbon Adsorption," *Journal of Food Engineering* **17**, 291–305 (1992).
109. A. K. Kiritsakis, *Olive Oil: From the Tree to the Table*, 2nd ed., Food and Nutrition Press, Trumbull, Conn., 1998.

JOSE M. ORDOVAS
Tufts University
Boston, Massachusetts

OMEGA-3 FATTY ACIDS. See FATS AND OILS: CHEMISTRY, PHYSICS AND APPLICATIONS.

OPTIMIZATION METHODS IN FOOD PROCESSING AND ENGINEERING

THE BEST

One primary goal of food process engineering is to find and understand the mechanisms and phenomena that underscore a given food manufacturing process. Such basic knowledge allows the food engineer to grasp how a process works and thus operate an existing or design a new processing system. In today's rapidly changing, highly sophisticated, and extremely competitive world, however, such basic knowledge is not always enough. Often, it is important not only to know how a process works but also how it works "best." The meaning of best changes from system to system and depends on the criteria used. For example, if the chosen criterion is process yield, then the highest value (maximum) is best. On the other hand, if cost is the criterion, then the lowest value (minimum) is best. The desired extreme value (maximum or minimum) is optimum, and the values of the adjustable processing variables that produce this optimum are called the optimum conditions. The theory, methods, and techniques used to attain an optimum and to locate the optimum operating conditions are the subject of optimization. *Optimization* has been defined simply as "getting the best you can out of a given situation" (1). In addition, "it [optimization] opens up the possibility of achieving the very best, of reaching perfection, of choosing the superior course of action among all possible alternatives" (2).

The scope of this article is to (*1*) introduce the subject of optimization in simple terms; (*2*) clarify some of the terminology, theory, and methods used; and (*3*) cover some of the areas in food science and engineering where optimization is being applied or potential applications are likely to occur. The references provide more explicit and in-depth coverage of the subject.

THE ELEMENTS OF OPTIMIZATION PROBLEMS

In every case of optimization, the problem must first be formulated and then the system to be optimized must be defined. This requires specifying the variables that affect the system's behavior. For example, in making a hot cup of tea, an obvious variable that affects the quality of the tea is the length of time the tea bag stays in the hot water. Performing experiments to measure the quality of the tea as a function of time generates a curve like the one shown in Figure 1. Choosing the maximum quality value and tracing it back to the corresponding optimum time optimizes this oversimplified and trivial system. Accounting for the effect of the water temperature on the tea quality complicates the system. In such a case a series of constant quality curves might be obtained (Fig. 2), which produce a set of optimum conditions for temperature and time.

91. A. Garrido Fernandez, M. C. Duran Quintana, and P. Garcia Garcia, "Study of Different Conservation Forms of Turning Color Olives of the Variety Manzanilla," *Grasas y Aceites* **37**, 1–7 (1986).
92. M. Brenes Balbuena et al., "Comparative Studies of the Conservation Systems of Black Table Olives," *Grasas y Aceites* **37**, 123–128 (1986).
93. P Garcia Garcia, M C. Duran Quintana, and A. Garrido Fernandez, "Aerobic Fermentation of Ripe Olives in Brine," *Grasas y Aceites* **36**, 14–20 (1985).
94. A Garrido Fernandez, P. Garcia Garcia, and F. Sanchez-Roldan, "New Fermentation Aerobic Process for Natural Black Olives," *Alimentacion* **4**, 73–81 (1985).
95. A. Garrido Fernandez, P. Garcia Garcia, and M. C. Duran Quintana, "Conservation of Natural Black Olives Proceeding from Aerobic Fermentation," *Grasas y Aceites* **36**, 313–316 (1985).
96. M. C. Duran Quintana, P. Garcia Garcia, and A. Garrido Fernandez, "Aerobic Fermentation of Ripe Olives in Brine with Alternating Air Injection," *Grasas y Aceites* **37**, 242–249 (1986).
97. A. Garrido Fernandez, M. C. Duran Quintana, and P Garcia Garcia, "Aerobic Fermentation of Natural Black Olives in Brine," *Grasas y Aceites* **38**, 27–32 (1987).
98. P. Garcia Garcia, M. C. Duran Quintana, and A. Garrido Fernandez, "Packing of Aerobically Fermented Natural Black Olives," *Grasas y Aceites* **37**, 92–96 (1986).
99. P. Garcia Garcia et al., "Physicochemical Depuration of the Waste Waters from the Green Table Olive Packing Industries," *Grasas y Aceites* **41**, 263–269 (1990).
100. S. Vitolo, L. Petarca, and B. Bresci, "Treatment of Olive Oil Industry Wastes," *Bioresour. Technol.* **67**, 129–137 (1999).
101. A. G. Vlyssides et al., "Olive Oil Processing Wastes Production and Their Characteristics in Relation to Olive Oil Extraction Methods," *Fresenius Environ. Bull.* **7**, 308–313 (1998).
102. G. C. Kopsidas, "Wastewater from the Table Olive Industry," *Water Res.* **28**, 201–205 (1994).
103. F. Cabrera et al., "Land Treatment of Olive Oil Mill Wastewater," *International Biodeterioration and Biodegradation* **38**, 215–225 (1996)
104. F. Flouri et al., "Decolorization of Olive Oil Mill Liquid Wastes by Chemical and Biological Means," *International Biodeterioration and Biodegradation* **38**, 189–192 (1996).
105. I. Chatjipavlidis et al., "Bio Fertilization of Olive Oil Mills Liquid Wastes: The Pilot Plant in Messinia, Greece," *International Biodeterioration and Biodegradation* **38**, 183–187 (1996)
106. B. Inigo Leal, "New Technological Trends in the Production and Preservation of Table Olives," *Alimentaria* **28**, 61–65 (1991).
107. A. Garrido Fernandez, M. Brenes Balbuena, and P. R. Garcia Garcia, "Treatment for Green Table Olive Fermentation Brines," *Grasas y Aceites* **43**, 291–298 (1992).
108. A. Garrido, P. Garcia, and M. R. Brenes, "The Recycling of Table Olive Brine Using Ultrafiltration and Activated Carbon Adsorption," *Journal of Food Engineering* **17**, 291–305 (1992).
109. A. K. Kiritsakis, *Olive Oil: From the Tree to the Table*, 2nd ed., Food and Nutrition Press, Trumbull, Conn., 1998.

JOSE M. ORDOVAS
Tufts University
Boston, Massachusetts

OMEGA-3 FATTY ACIDS. See FATS AND OILS: CHEMISTRY, PHYSICS AND APPLICATIONS.

OPTIMIZATION METHODS IN FOOD PROCESSING AND ENGINEERING

THE BEST

One primary goal of food process engineering is to find and understand the mechanisms and phenomena that underscore a given food manufacturing process. Such basic knowledge allows the food engineer to grasp how a process works and thus operate an existing or design a new processing system. In today's rapidly changing, highly sophisticated, and extremely competitive world, however, such basic knowledge is not always enough. Often, it is important not only to know how a process works but also how it works "best." The meaning of best changes from system to system and depends on the criteria used. For example, if the chosen criterion is process yield, then the highest value (maximum) is best. On the other hand, if cost is the criterion, then the lowest value (minimum) is best. The desired extreme value (maximum or minimum) is optimum, and the values of the adjustable processing variables that produce this optimum are called the optimum conditions. The theory, methods, and techniques used to attain an optimum and to locate the optimum operating conditions are the subject of optimization. *Optimization* has been defined simply as "getting the best you can out of a given situation" (1). In addition, "it [optimization] opens up the possibility of achieving the very best, of reaching perfection, of choosing the superior course of action among all possible alternatives" (2).

The scope of this article is to (*1*) introduce the subject of optimization in simple terms; (*2*) clarify some of the terminology, theory, and methods used; and (*3*) cover some of the areas in food science and engineering where optimization is being applied or potential applications are likely to occur. The references provide more explicit and in-depth coverage of the subject.

THE ELEMENTS OF OPTIMIZATION PROBLEMS

In every case of optimization, the problem must first be formulated and then the system to be optimized must be defined. This requires specifying the variables that affect the system's behavior. For example, in making a hot cup of tea, an obvious variable that affects the quality of the tea is the length of time the tea bag stays in the hot water. Performing experiments to measure the quality of the tea as a function of time generates a curve like the one shown in Figure 1. Choosing the maximum quality value and tracing it back to the corresponding optimum time optimizes this oversimplified and trivial system. Accounting for the effect of the water temperature on the tea quality complicates the system. In such a case a series of constant quality curves might be obtained (Fig. 2), which produce a set of optimum conditions for temperature and time.

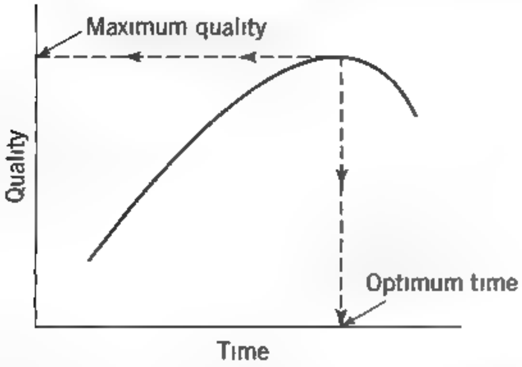


Figure 1. Quality of tea as a function of time the tea bag stays in hot water.

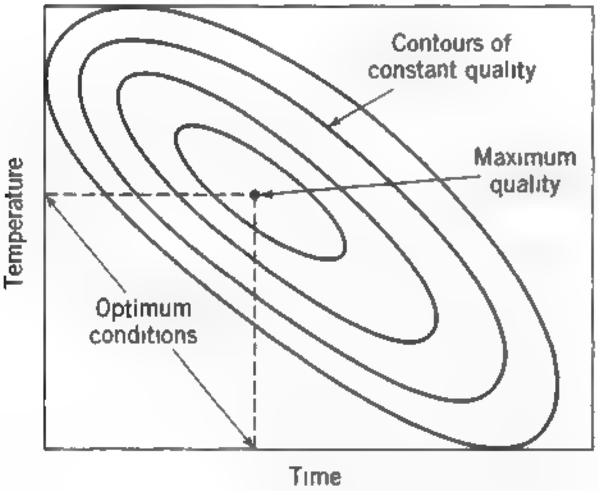


Figure 2. Quality of tea as a function of water temperature and processing time.

To make the preceding system more realistic, other variables could be included in the optimization problem: the amount of stirring, the amount of tea in the tea bag, the storage history of the tea leaves (temperature and humidity), and so on. All these factors will have some effect on the quality. Furthermore, it is difficult to measure and define quality, because it depends on the taste and preference of individuals, among other things. Despite the simplicity of the preceding example, it illustrates the basic problem of optimization and the need to formulate the problem, define the system, find a function (or mathematical model) that describes the system, and find the system's optimum.

More explicitly, all optimization problems must consider several important elements (2,3):

Selection of Decision Variables. The most critical element in optimization problems is to select all the appropriate variables that contribute to the decision-making process of attaining the optimum. These input, or independent variables affect the performance of the process or system and can be adjusted, changed, and controlled.

Definition of the Performance Function. Another important element of optimization problems is the process of defining which dependent variables require optimization (maximization or minimization). These measured variables, called performance or objective functions, determine and reflect the performance of the system or process.

Identification of Constraints. In most optimization problems, certain constraints may limit the values that dependent or independent variables can take, restricting, therefore, the region of allowable solutions. Such constraints may be imposed by physicochemical or other impossibilities (in the tea example, the temperature of the water cannot be more than 100°C). They may also represent restrictions endogenous to the system (eg, federal or state requirements on the composition of a food product, grading guidelines, and equipment restrictions). Whatever the case might be, such constraints must be taken into account, because they influence the solution of a given problem.

Development of a Mathematical Model to Describe the System. Attempts to solve a problem by trial and error require painstaking physical experimentation and application of statistical theory. Instead of this, a mathematical model is used for most cases of optimization. In some cases, mathematical models may already exist (eg, thermal processing and drying), but in other situations models must be developed based on available scientific and engineering knowledge. It is necessary to be aware of how to develop a model (4,5). Sometimes the complexity of the system might be such that a model cannot be developed or, if developed, cannot be solved. This is common in biochemical and biological systems such as foods, and usually an approximating (or graduating) function is developed, which represents the true nature of the system as closely as possible (6). Such functions are usually polynomials or transformations thereof, and a limited number of experiments are required to find their constant coefficients (7,8). Data collection methods (experimental design) and model fitting (linear, nonlinear, and other regression) techniques are important elements of finding the appropriate approximating function (3,7–11).

Attaining the Optimum. The final element of all optimization problems is to choose an appropriate method to optimize the system. It is imperative to realize that the scope of every optimization method is to adjust and readjust the values of the decision variables to locate the settings that maximize or minimize (optimize) the performance function(s), while ensuring that all variables (dependent or independent) satisfy the constraints of the system.

Figure 3 gives typical relationships between the important elements of an optimization problem and their role in its solution. The following section discusses the available optimization techniques and methods. This information

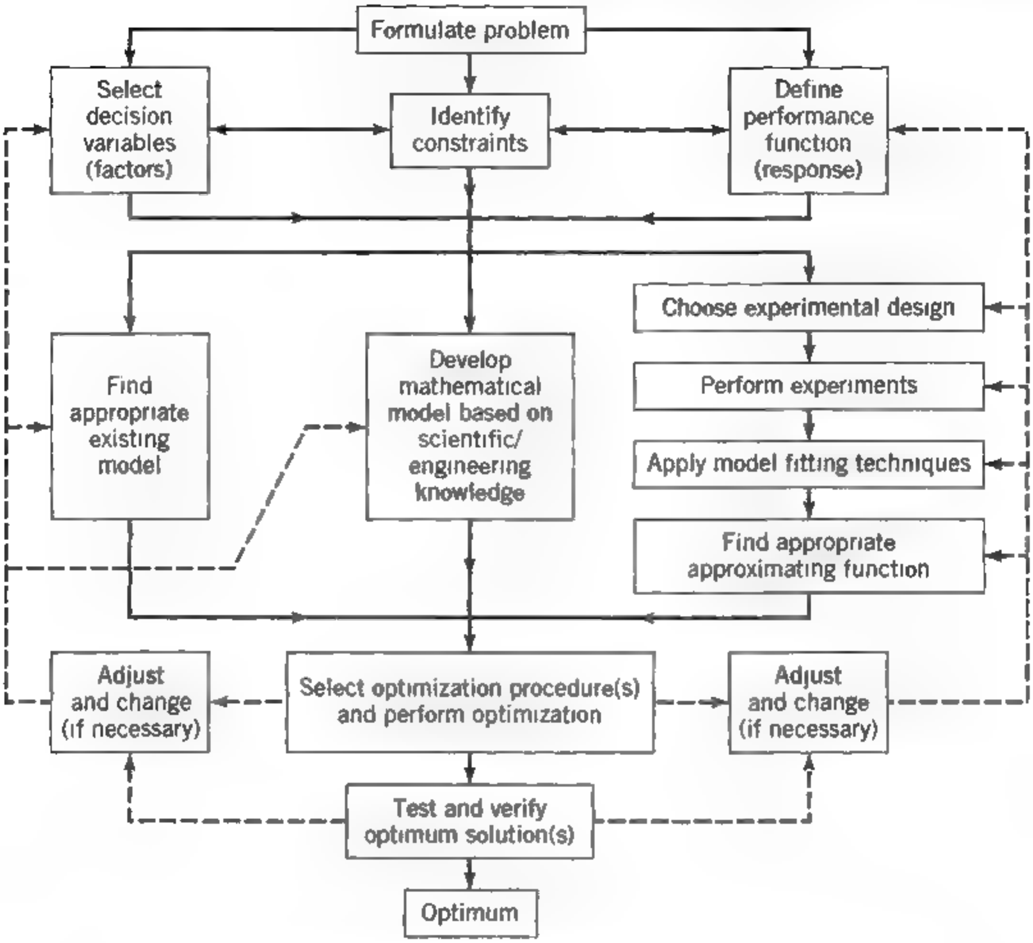

Formulate problem
Select decision variables (factors)
Identify constraints
Define performance function (response)
Choose experimental design
Perform experiments
Apply model fitting techniques
Find appropriate approximating function
Find appropriate existing model
Develop mathematical model based on scientific/ engineering knowledge
Adjust and change (if necessary)
Select optimization procedure(s) and perform optimization
Adjust and change (if necessary)
Test and verify optimum solution(s)
Optimum

Figure 3. The important elements of an optimization problem and their interactions.

should be complemented with the more in-depth discussion given in the references cited therein.

OPTIMIZATION METHODS

Basic Theoretical Background

Among the various optimization techniques, each is best suited to operate on a particular type of mathematical model. Consequently, when formulating a problem and developing a specific model, the strong and weak points of the various optimization techniques must be kept in mind.

The basis for all optimization methods is the classic theory of maxima and minima. Mathematically, the theory is concerned with finding the minimum or maximum (extreme points) of a function of n variables, $f(x_1, x_2, \ldots, x_n)$, where n is any integer greater than zero. To simplify the concept, assume that f is a function of only one variable x (Fig. 4). In this example, point A is located at the boundary and it is a local minimum. Point B, where the first derivative f' is discontinuous, is a global maximum. Point C, where $f' =$ zero, is a local minimum, D ($f' = 0$) and G are local maxima, and E is the global minimum. Points C, D, E, and F are stationary points, because their first derivative f' is zero. A stationary point is a maximum (local or

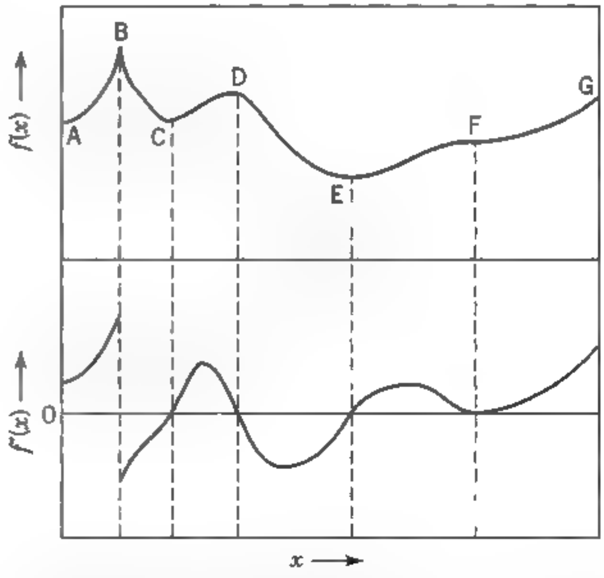


Figure 4. A schematic representation of a function $f(x)$ and its first derivative $f'(x)$.

global) if movement away from it causes the function $f(x)$ to decrease. A point is a minimum if movement away from it causes an increase. Mathematically, a point x^* is a local maximum of the function $f(x)$ if

$$f(x^*) \geq f(x) \tag{1}$$

and

$$|x^* - x| < \delta \tag{2}$$

where δ is a small positive number. The point x^* is termed a global maximum when the inequality (Equation 1) holds true for all values of x. Definitions of local and global minima are similar. Point F, however, whose first derivative is zero (a stationary point), is neither a maximum nor a minimum, but a mini-max, better known as a saddle point. In this case, movement away from point F will result in an increase or decrease in $f(x)$ depending on the direction of the movement.

The classic theory of maxima and minima is simply a search to find all local maxima or minima and then a comparison of the individual values to determine the global (absolute) maximum or minimum. The critical places to look for the extreme values are

1. at the stationary points (where $f'(x) = 0$),
2. on the boundaries of the domain of the decision variables, and
3. at points where the first derivatives are discontinuous.

When $f(x)$ and $f'(x)$ are continuous functions, the extreme points will most likely be at the stationary points (except for cases involving saddle points). Thus the problem becomes one of locating the points where the partial derivatives are zero. To accomplish this goal, the following n algebraic equations

$$\frac{\partial f}{\partial x_j}(x_1, x_2, \ldots, x_n) = 0 \quad (j = 1, \ldots, n) \tag{3}$$

must be simultaneously solved. Unfortunately, differential calculus does not always provide a method for solving such equations, particularly when real solutions do not exist. If some continuous and real functions $z_j(x)$ exist, an approximate solution to the equation

$$z_j(x) = 0 \quad (j = 1, \ldots, n) \tag{4}$$

can be obtained by minimizing the sum of squares of the residuals defined by

$$\sum_{j=1}^{n} (z_j(x))^2 \tag{5}$$

The calculus approach is useful when the equation 3 can be solved directly (when they are all linear, eg). It can also reduce the dimensionality, the number of variables required to solve the problem, in some cases.

These analytical methods may not always work. Optimization can still be performed, however, by iterative methods using a computer. Iterative methods are useful for solving simultaneous equations such as equation 4, but cannot guarantee optimization when used to solve equation 3, primarily because stationary points are not always optima (they may be saddle points). For that, it is usually better to apply the iterations directly to the function $f(x)$ and try to find the minimum (or maximum) by the following strategy (12):

1. Take a trial solution, x_k.
2. Find a direction from this trial solution in which $f(x)$ decreases (or increases).
3. Find a point x_{k+1} in this direction so that $f(x_{k+1}) < f(x_k)$ (or $f(x_{k+1}) > f(x_k)$).
4. Repeat the process from this new trial solution.

Even iterative methods might fail to produce an optimum, however. This might be due to a number of reasons, including the nature or structure of the system, slow convergence, rounding-off errors, and finding a local instead of a global extreme. With the preceding background, restrictions, and limitations in mind some basic optimization methods are presented next.

Unconstrained Optimization

Unconstrained optimization techniques are applicable when searching for a minimum (or maximum) of a function $f(x)$ that is not subject to any constraints. Sufficient conditions must be developed to allow evaluation of the nature of the stationary points and determine if they are minima, maxima, or other. Once all of the local maxima and minima of the function $f(x)$ are located, a comparison among them produces the global optimum (maximum or minimum).

If the function $f(x)$ has only one independent variable, a Taylor series expansion about the stationary point x^* can be performed.

$$f(x) = f(x^*) + f'(x^*)(x - x^*) + \frac{1}{2}f''(x^*)(x - x^*)^2 + \ldots + \text{higher order terms} \tag{6}$$

The first derivative vanishes at the stationary point ($f(x^*) = 0$). Also, if x is sufficiently close to x^*, the higher-order terms are negligible compared with the second-order terms, and equation 6 becomes

$$f(x) \approx f(x^*) + \frac{1}{2}f''(x^*)(x - x^*)^2 \approx \frac{1}{2}f''(x^*)(x - x^*)^2 \tag{7}$$

Because $(x - x^*)^2$ is always positive, the nature of $f(x^*)$ depends on the value of $f''(x^*)$, the second derivative of the function at the stationary point:

If $f''(x^*) > 0$, then $f(x^*)$ is a minimum.
If $f''(x^*) < 0$, then $f(x^*)$ is a maximum.

In the case of $f''(x^*)$ = zero, higher order derivatives must be examined. This method can be extended to systems with two or more variables by using multidimensional Taylor series expansions and Hessian matrices (5,13–15).

Another popular approach for finding local maxima (or minima) is an optimization algorithm known as Newton's method. It computes $f(x)$ and $f'(x)$ at an initial point x_i and then finds an improved estimate x_{i+1} by linear extrapolation (12). Algebraically, this is

$$x_{i+1} = x_i - \frac{f(x_i)}{f'(x_i)} \tag{8}$$

If the function $f(x)$ is either convex or concave, Newton's method should converge toward a local maximum (or minimum) very rapidly. If $f(x)$ is an S-shaped function, and the initial point x_i is poorly chosen, the method may oscillate with a continuously increasing amplitude. Newton's method can be extended and applied to multidimensional optimization (12). Other methods such as conjugate gradients, quasi-Newton methods, and various Newtonlike methods have also been successfully applied (12,16).

Finally, several other optimization methods work well with one-dimensional unconstrained systems. The bisection method and its variations, the method of false position and its modifications, the golden section search, the Fibonacci search, and so on (12,13) all have certain advantages and disadvantages.

When searching for a method to optimize a multidimensional system, the following questions deserve consideration (12):

1. Is it easy to calculate the values of the function?
2. Can the first derivatives be found easily?
3. Can the second derivatives be found easily?
4. Can the ($n \times n$) Hessian matrix and its inverse be stored affordably?
5. Are many of the Hessian's elements zero? Can that be capitalized on and the calculation time reduced?
6. Is there anything special about the particular problem that might make the solution easier?

Because there are no easy answers to most of the preceding questions, it is advisable to further study and understand the strengths and weaknesses of each optimization method before applying it to solve specific problems.

Constrained Optimization

In a number of practical situations, optimization of a function occurs over a restricted domain of the independent variables. For example, flow rates, concentration, shelflife, and so on may never take negative values. Optimization techniques still locate the stationary points of the function, but this time the solutions for optima must be subject to equality or inequality constraints.

Optimization of Systems with Equality Constraints. For a function $f(x_1, x_2, \ldots x_n)$ of n independent variables and subject to $m < n$ constraint equations

$$g_i(x_1, x_2, \ldots, x_n) = 0 \quad (i = 1, \ldots, m) \tag{9}$$

three basic analytical methods can be used to locate the extreme points: direct substitution, solution by constrained variation, and Lagrange multipliers. The first method involves a simple substitution of the m constraint equation 9 directly into the function to be optimized. This results in an equation with $(n - m)$ unknowns subject to no constraints. The methods of unconstrained optimization may then be applied. Equation 9 is usually complicated, and it is not always possible to perform the substitutions.

Figure 5 illustrates the second method, solution by constrained variation. In this case, there are two independent variables x_1 and x_2, and the function $f(x_1, x_2)$ is subject to the constraint $g(x_1, x_2) = 0$. It is obvious that the maximum of the constrained system is point B and the maximum of the unconstrained system is point A. At point B the curve $f(x_1, x_2)$ = constant and the curve $g(x_1, x_2) = 0$ have the same slope and are tangent. Therefore, infinitesimal changes dx_1 and dx_2 affect the dependent variables $f(x_1, x_2)$ and $g(x_1, x_2)$ in a similar way. In other words

$$\left(\frac{dx_2}{dx_1}\right)_f = \left(\frac{dx_2}{dx_1}\right)_g \tag{10}$$

Because f = constant and $g = 0$, the total derivatives of both functions at point B are

$$df = \frac{\partial f}{\partial x_1}\, dx_1 + \frac{\partial f}{\partial x_2}\, dx_2 = 0 \tag{11}$$

$$dg = \frac{\partial g}{\partial x_1}\, dx_1 + \frac{\partial g}{\partial x_2}\, dx_2 = 0 \tag{12}$$

It should be recalled that in this case (constraint optimization) $\partial f/\partial x_1$ and $\partial f/\partial x_2$ are not zero (as they would have been in an unconstraint case). Equations 10 to 12 can be

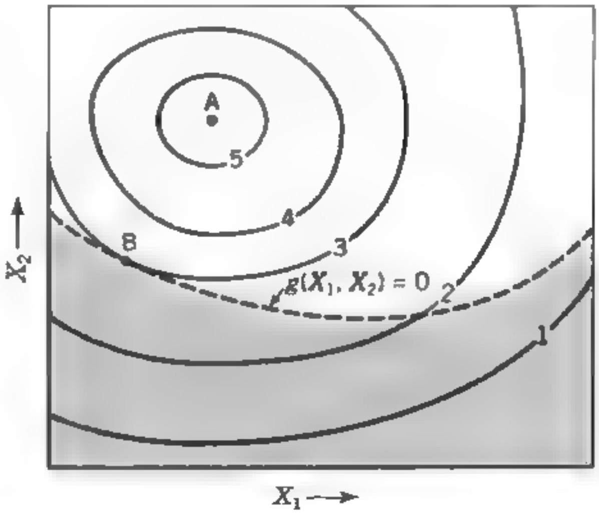


Figure 5. A schematic representation of a function $f(x_1, x_2)$ subject to constraint $g(x_1, x_2) = 0$, which restricts solutions to the shaded area only.

solved in combination with $g(x_1, x_2) = 0$ to locate the stationary point (5).

The preceding simple two-dimensional case generalizes to the case of n independent variables subject to $m < n$ constraints. Applying the same reasoning as before produces the so-called Jacobian determinants, which in turn can be used with the constraint equation 9 to obtain the stationary points (5).

The most commonly used of the three analytical methods mentioned is Lagrange multipliers. Its simplest form is the two-dimensional case. Multiplying equation 12 by a constant λ and adding the result to equation 11 yields

$$\left(\frac{\partial f}{\partial x_1} + \lambda \frac{\partial g}{\partial x_1}\right)dx_1 + \left(\frac{\partial f}{\partial x_2} + \lambda \frac{\partial g}{\partial x_2}\right)dx_2 = 0 \qquad (13)$$

Equation 13 transforms to

$$\frac{\partial}{\partial x_1}(f + \lambda g)dx_1 + \frac{\partial}{\partial x_2}(f + \lambda g)dx_2 = 0 \qquad (14)$$

The constant λ is the Lagrange multiplier of the system and is determined by equating the bracketed terms of equation 13 to zero. This produces (see equation 14) the augmented function Λ

$$\Lambda = f + \lambda g \qquad (15)$$

which guarantees that the stationary point(s) of the system will be located if λ is considered to be an independent variable and the following system of equations is solved.

$$\frac{\partial f}{\partial x_1} + \lambda \frac{\partial g}{\partial x_1} = 0 \qquad (16)$$

$$\frac{\partial f}{\partial x_2} + \lambda \frac{\partial g}{\partial x_2} = 0 \qquad (17)$$

$$g(x_1, x_2) = 0 \qquad (18)$$

In the general case of n independent variables with m constraint equations, the augmented function takes the form

$$\Lambda = f + \lambda_1 g_1 + \ldots + \lambda_m g_m \qquad (19)$$

and a system of $(n + m)$ equations must be solved to locate the stationary points. This is usually easier than a solution by direct substitution or constraint variation, unless the system is very simple.

Optimization of Systems with Inequality Constraints. Scientists and engineers understand that a profound difference exists between mathematical abstraction and physical reality. In the world of mathematics, an equality sign represents perfect equality, but in the real world equality requirements are physically impossible and unrealistic. For example, constraints such as net weight = 425 g or room temperature = 20°C are difficult to realize. However, targets such as contain no less than 425 g, or room temperature less than 20°C, are easier to achieve. Thus inequalities are an inherent part of the real world and require attention. With respect to optimization, several techniques exist to solve problems with inequality constraints.

If the system under consideration is linear, linear programming can be used to optimize it. The term *programming* here does not refer to computer programming, but rather to mathematical programming and scheduling. Several explanations of the mathematical relations involved in linear programming are available (12,16–21).

In some cases, it might be interesting to find out how a solution to a linear programming problem changes as the problem's data change in systematic and predetermined ways. Parametric programming can solve this problem. The algorithm for obtaining solutions of parametric programming problems has been published (12). In other cases, the objective function might not be linear, but quadratic instead. In such cases, quadratic programming can determine the optima (16). In more general terms, either the objective function or some (or all) of the constraints or both might be nonlinear in nature. Methods of nonlinear programming must then be applied (12,18). Other types of mathematical programming include (12,16) integer programming, which deals with optimization problems whose variables (some or all) are required to take integer values (ie, the number of machines or components required for some processing system). There is also geometric programming, which solves nonlinear programming problems containing special functions constructed from terms of the form

$$t_i = c_i \prod_{j=1}^{n} x_j^{a_{ij}} \qquad (20)$$

where t_i and c_i are positive coefficients and a_{ij} are constants (16).

Optimization of Dynamic Systems

A system is dynamic if it varies with time or distance. Optimization must account for such variation. An example of a dynamic system is the time-dependent behavior of batch and continuous fermentation vessels. The problem of optimizing dynamic systems can be stated as follows (19): given all the performance equations of a system (possibly a set of differential equations) and the initial-final values of some state variables, find the piecewise continuous decision control variable(s) that maximizes or minimizes the objective function.

Large dynamic systems with many decision variables can be broken down into stages and reduced to a series of interrelated systems, each containing only a few variables. The stages may be process components or equipment, units of time, or any other suitable entity. One of the methods to optimize such multistage dynamic systems is the so-called dynamic programming. Bellman (22) presented the basic theory and developed the principle of optimality. It states that an optimal policy has the property that whatever the initial state and initial decisions, the remaining decisions must constitute an optimal policy with regard to the state resulting from the first decision. The principle of optimality can be stated mathematically (5). In simple terms, the principle of optimality states that in a multistage serial system every component affects every downstream component. Figure 6 shows an example of a multistage process. Each stage has an input (I_n), an output (I_{n-1}), a decision (d_n), and a return (R_n). The output and return are dependent on the input and decisions. A dynamic programming analysis usually begins with the last stage, which affects no other stage in the system, and ends with the first stage, yielding an optimum for every input. For a multistage process, it is necessary first to find $f_1(I_1)$. With $f_1(I_1)$ known, then it is possible to find $f_2(I_2)$, then $f_3(I_3), \ldots, f_n(I_n)$.

Several other approaches can also solve dynamic optimization problems. Among the ones frequently applied are Pontyagrin's maximum principle (19,22–24), and the calculus of variations (25).

Experimental Optimization

Experimental optimization methods incorporate elements of statistical thinking into traditional scientific, engineering, and mathematical modeling and optimization. When the behavior of a system or process is unknown and a sufficiently simple deterministic (mathematical) model cannot be developed for further analysis, experimental optimization may be used to analyze the system and search for optimum conditions. The term *experimental* indicates that physical experimentation is involved. Well-designed experiments may produce statistically sound data, which in turn may result in reliable empirical models. Such models can predict or optimize, and often they provide the needed basis (information) for developing either more rugged or deterministic models. There are three well-known and widely used experimental optimization methods.

Response Surface Optimization. The term *response surface methodology* (RSM) refers to a group of mathematical and statistical techniques that, through limited physical experimentation, provide the means for attaining optimum operating conditions of complex systems. The theoretical basis of RSM (6) is a powerful tool for experimental optimization.

The basic idea of RSM is that for any given system, there must be a functional relationship φ that correlates the factors x_i (decision variables) to the response y (performance function)

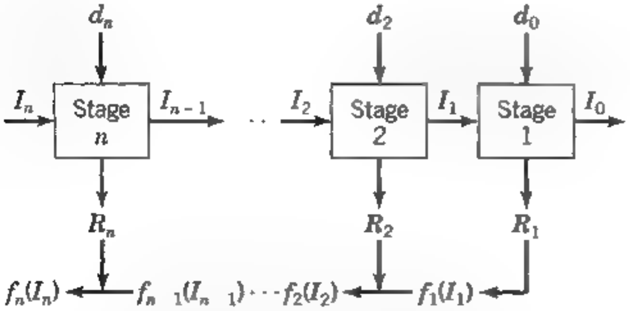


Figure 6. A multistage dynamic process.

$$y = \varphi(x_1, x_2, \ldots, x_n) \tag{21}$$

If the form of φ is explicitly known, then any of the methods discussed earlier can be used to optimize the system. If the function φ is unknown or very complex, another mathematically simpler function f must be found to approximate φ and describe the system. This new function

$$\hat{y} = f(x_1, x_2, \ldots, x_n) \tag{22}$$

estimates $\hat{y}$ rather than the true value y; it is called an approximating or graduating function and may take the form of practically any mathematical expression. The most commonly used expressions are polynomials of first or second order given by equations 23 and 24, respectively

$$\hat{y} = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \epsilon \tag{23}$$

$$\hat{y} = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j + \epsilon \tag{24}$$

where β_0, β_i, β_{ij} are constant coefficients usually determined by least-squares methods and ϵ is the error involved in estimating the coefficients β from experimental data. At times, polynomials of higher order are used.

Polynomials are popular approximating functions for several reasons. They provide simple curvilinear relationships that can approximate practically any true continuous function within a specified range, and they usually possess a clearly defined optimum. They can be expanded to include any number of decision variables (x_i). Finally, a number of transformations such as logarithmic, exponential, inverse, power, and trigonometric, may be applied to the independent (x_i) or dependent (y) variables, which add some asymmetry and flexibility desirable in many cases of biological and biochemical systems. The weakness associated with the use of polynomials is that they are "smooth" functions without any biological or biochemical justification. Therefore, extrapolation beyond the experimental space (the region where data were collected to estimate β's) is usually not allowed.

The first step in a RSM study is to select an appropriate experimental design with a limited number of experimental runs k (where $k > n$), which will allow estimation of the coefficients β by minimizing the sum of squares of errors E

$$E = \sum_{i=1}^{k} \epsilon_i^2 \tag{25}$$

Ready-made designs already exist in terms of coded variables, which are linear functions of the actual decision variables, and minimize the overall error ϵ

$$\epsilon = \epsilon_v + \epsilon_b \tag{26}$$

where ϵ_v is the variance error, sometimes referred to as the experimental error, and is a result of the sampling variation, and ϵ_b is the so-called bias error (26), which refers to the failure of the approximating function (ie, polynomials) to exactly represent the true function $\varphi(x)$. Among the most commonly used designs for RSM studies are central composite designs (6,7), three-level designs (27), mixture designs (28–30), and several other fractional factorials (9,31,32).

After the coefficients β's have been determined (to satisfy equation 25), the stationary point(s) are determined. Taking the first partial derivatives of equation 23 or 24, equating them to zero, and solving the system of n equations (see equation 3 and related discussion) solves this issue. Matrix algebra can accomplish the same objective (7,8).

A canonical transformation of equation 23 or 24 will indicate the nature of the stationary point (8). If complicated ridge systems exist (when the stationary point is faraway from the origin, outside the experimental region), they may require further ridge analysis (33).

If the system has only one response (one performance function), then following the methods and analyses discussed achieves optimization easily. Often more than one response is involved, and sometimes the objectives are competing with each other. Under these circumstances, methods of multiresponse optimization (MRO) will locate the optimum operating conditions. The simplest form of MRO is the graphical approach. Equation 23 or 24, depending on the system, creates predictive models and contour plots. Superimposing contour plots of several responses identifies critical (optimum) regions. Examples of such graphical optimization approach exist in the literature (34–36).

An improved graphical method has been presented (3) that allows three (instead of two) independent variables to be represented continuously and simultaneously in a contour plot for one or more discrete values of the response y. It facilitates the process of locating optimum regions in multivariable systems, and requires some computer programming. An extended discussion and an application example of the improved graphical method are available (3,36).

Several other MRO techniques exist, such as the generalized distance approach (36–39), the extended response surface procedure (39), the so-called overall desirability function (7,40), and the normalized function approach (41). The application of these techniques requires caution, because they may lead to unanticipated and practically undesirable conditions (7).

Evolutionary Operation (EVOP). This is an experimental strategy for improving industrial processes on the plant floor during operation. The theoretical basis, methodology, and some application examples of this method have been presented (42,43). The basic idea of EVOP is to change a current manufacturing process slowly and methodically during production until no further improvement is possible. For example, if a process is presently producing an output y_p at factor settings x_{1p} and x_{2p} (Fig. 7), then according to EVOP, the factor setting should be changed to measure y_a, y_b, y_c, and y_d (in that order). These values are statistically compared to y_p. If significant differences exist, the best value becomes the center (the new y_p), and the process repeats until no further improvement occurs. Because experimentation takes place during production, it is important to avoid large changes in the factor settings, which may result in significant product variation or monetary loss. If applied wisely, EVOP provides a simple and straightforward technique for continuous process improvement. It works best when applied to simple systems with up to three independent variables (factors); therefore, its use should be limited to such systems.

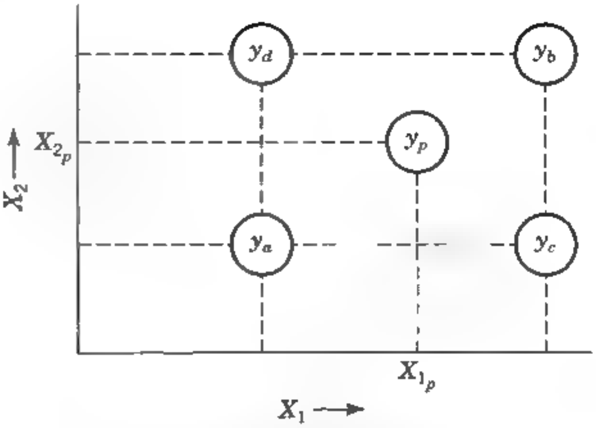


Figure 7. An evolutionary operation (EVOP) strategy.

Taguchi Methods. A broad spectrum of new ideas on applied statistics and engineering characterize this approach. Followers of these methods advocate a new philosophy on fundamental statistical theory, probability, experimental design, regression theory for modeling, quality and reliability, basic engineering design, and optimization. These ideas and philosophy are generally referred to as Taguchi methods (44,45).

To begin with, every quality characteristic (performance function or response) y relates to some loss L if the characteristic is not exactly on target (if it is not an optimum). Traditionally, the loss function $L(y)$ was thought of as being constant when y lay outside the specified limits (specifications, guidelines, or other quality-control requirements) and zero when y lay within those limits (Fig. 8). Expressed mathematically, this is

$$L(y) = \begin{cases} 0 & \text{for LL} \le y \le \text{UL} \\ c & \text{for } y < \text{LL or UL} < y \end{cases} \tag{27}$$

where c is a constant and LL and UL are the lower and upper specification limits, respectively. The Taguchi loss function is a quadratic expression (Fig. 8)

$$L(y) = k(y - a)^2 \tag{28}$$

where a is the target (optimum) value and k is a characteristic constant of the system. For a known loss value L corresponding to a given value of y, k is

$$k = L/(y - a)^2 \tag{29}$$

This loss function may be asymmetric, but it should approximate the true loss. By definition, this is the total loss

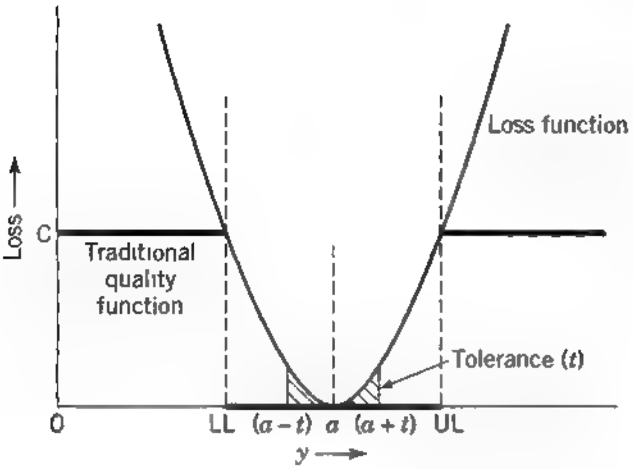


Figure 8. A comparison of Taguchi's loss function with the traditional quality function.

to society, not the loss to a company or an individual, when a product or process deviates from its target (optimum) value a. It has been further suggested that effort should be made to keep the process-product within a tolerance t from the target ($a - t \leqq y \leqq a + t$). For values of y outside the tolerance region (Fig. 8), the loss to society is greater than the amount of effort required to correct the process.

Another aspect of the Taguchi methods deals with the so-called factor space and states that all systems are affected (subject to changes) by some controllable and some uncontrollable factors. The controllable factors are the decision variables, which can be measured, controlled, and adjusted. The uncontrollable factors are either unknown variables or variables over which there is no real control. In either case the effect of uncontrollable factors is termed *noise* and should be minimized during optimization. Mathematically, this is

$$y = f(x_1, x_2, \ldots, x_n, z_1, z_2, \ldots, z_m) \qquad (30)$$

where y is the response (performance function), $x_1, x_2, \ldots, x_n$ are the controllable factors (the normal decision variables), and $z_1, z_2, \ldots, z_m$ are the uncontrollable factors (noise). To optimize this system, the optimum values $x_1^*, x_2^*, \ldots, x_n^*$ must be found such that

$$y^* = f(x_1^*, x_2^*, \ldots, x_n^*, z_1, z_2, \ldots, z_m) \qquad (31)$$

In this case, y^* is the value of y close to the ideal optimum, where the variability due to the effect of $z_1, z_2, \ldots, z_m$ is minimum. An example application in this area would be the formulation of a cake mix that produces consistently good cakes under uncontrollable variations in baking temperature and time.

The Taguchi methodology goes further and divides the uncontrollable factors into outer noise (ambient temperature, relative humidity, etc), inner noise (machine or equipment deterioration, component wear and tear, etc), and between product noise (product-to-product variation). Fractional factorial designs and orthogonal arrays are used to perform experiments and part of the analysis is the controversial signal to noise ratio.

Overall, the Taguchi methodology follows three basic design phases: (*1*) system design, the basic engineering design for plants, processes, and equipment; (*2*) parameter design, optimizing overall processing conditions; and (*3*) tolerance design, refining the system. This minimizes noise effects, reduces variation, and produces rugged and robust products, processes, and systems. Taguchi methods can also characterize and optimize multiresponse processes (46). Further information on this subject is available (43–46).

Artificial Intelligence. The increasing availability of low-cost computing power has made computationally intensive modeling methods more practical. These include neural network modeling (47), genetic algorithms, and fuzzy logic (48). These methods can provide models that conform more closely to the true response surface than traditional polynomials, but they require significantly more data to create such models.

APPLICATION AND IMPLEMENTATION

Several areas in food science and engineering research can benefit from the application of optimization methods. This is even more so in the broad area of food processing and food manufacturing. A general categorization of the types of problems that optimization methodology may assist solving has been attempted (2). Equipment design, which involves the design of individual components of a machine or a total system (eg, multiple effect evaporators), is a common area for application of optimization methods. Most equipment and food manufacturing companies use optimization techniques to design the best equipment possible.

Another area of application is plant design and layout, where the whole food manufacturing plant is considered as one system and may be optimized for overall efficiency, total operating cost, yield, total return, quality of output, and throughput. This is a far more difficult task than designing individual equipment, and thus its implementation has lagged behind. The optimization of food processing plants is more complex than other industrial plants because, except for the regular process optimization, changes in raw materials (input) and final product properties (output) must be accounted for (49). Thus, optimization of food processing plants is a subset of a much broader system called food chain optimization (49). This includes optimization of raw materials properties, process optimization, and product optimization. It is imperative to stress the importance of applying optimization methods in all of the previously mentioned areas.

Optimization also improves the management and control of processing plants (2). With the increased use of computers and computer control in food manufacturing, optimization should be an integral part of every sophisticated processing plant. However, routine application of optimization methods during operation (on- or off-line) exists in only a small fraction of industrial plants (2). This must change to increase efficiency, productivity, and quality and reduce energy use, product loss, and environmental pollution. The stiff competition in the world's markets will force an increased use of optimization on food manufacturers. Furthermore, population dynamics and changing consumer attitudes toward food and the environment will necessitate the adoption of optimization philosophy, ideas, and techniques throughout the food handling system. This will extend from the harvesting of raw materials to the transportation, processing, packaging, distribution, and consumption of the final products.

BIBLIOGRAPHY

1. R. Aris, *Discrete Dynamic Programming*, Blaisdell, New York, 1964.
2. L. B. Evans, "Optimization Theory and Its Application in Food Processing," *Food Technol.* **36**, 88–93 (1982).
3. J. D. Floros and M. S. Chinnan, "Computer Graphics-Assisted Optimization for Product and Process Development," *Food Technol.* **42**, 72–78, 84 (1988).
4. E. A. Bender, *An Introduction to Mathematical Modeling*, Wiley-Interscience, New York, 1978.
5. C. L. Smith, R. W. Pike, and P. W. Murrill, *Formulation and Optimization of Mathematical Models*, International Textbook, Scranton, Pa., 1970.
6. G E. P. Box and K. B. Wilson, "On the Experimental Attainment of Optimum Conditions," *Journal of the Royal Statistical Society Ser B* **13**, 1–45 (1951).
7. G. E. P. Box and N. R. Draper, *Empirical Model Building and Response Surface*, Wiley, New York, 1987.
8. R. H. Myers, *Response Surface Methodology*, Allyn and Bacon, Boston, Mass , 1971.
9. W. G. Cochran and G. M. Cox, *Experimental Designs*, Wiley, New York, 1957.
10. O. L. Davies, *Design and Analysis of Industrial Experiments*, Oliver & Boyd, Edinburgh, Scotland, 1954.
11. N. R. Draper and H Smith, *Applied Regression Analysis*, Wiley, New York, 1981.
12. E. M. L. Beale, *Introduction to Optimization*, Wiley, New York, 1988.
13. I. Saguy, "Optimization Methods and Applications," in I. Saguy, ed., *Computer-Aided Techniques in Food Technology*, Marcel Dekker, New York, 1983.
14. T. N Edelbaum, "Theory of Maxima and Minima," in G. Leitman, ed., *Optimization Techniques*, Academic Press, Orlando, Fla., 1962.
15. H. Hancock, *Theory of Maxima and Minima*, Dover, New York, 1960.
16. R. Fletcher, *Practical Methods of Optimization*, Wiley, New York, 1987.
17. V. Vemuri, *Modeling of Complex Systems*, Academic Press, Orlando, Fla., 1978
18. G. P. McCormick, *Nonlinear Programming: Theory, Algorithms, and Applications*, Wiley, New York, 1983.
19. F. E. Bender, A. Kramer, and G. Kahan, "Linear Programming and Its Applications in the Food Industry," *Food Technol.* **35**, 94–96 (1982).
20. F. E. Bender and A. Kramer, "Linear Programming and Its Implementation," in I. Saguy, ed., *Computer-Aided Techniques in Food Technology*, Marcel Dekker, New York, 1983.
21. S. Vajda, *Problems in Linear and Nonlinear Programming*, Oxford Univ. Press, New York, 1975.
22. R. Bellman, *Dynamic Programming*, Princeton Univ Press, Princeton, N.J., 1957.
23. I. Saguy, "Optimization of Dynamic Systems Utilizing the Maximum Principle," in I. Saguy, ed., *Computer-Aided Techniques in Food Technology*, Marcel Dekker, New York, 1983.
24. R. E. Kopp, "Pontyagrin Maximum Principle," in G. Leitman, ed., *Optimization Techniques*, Academic Press, Orlando, Fla., 1962.
25. G. Leitman, ed., *Optimization Techniques: With Applications to Aerospace Systems*, Academic Press, Orlando, Fla., 1962
26. G. E. P. Box and N. R. Draper, "A Basis for Selection of a Response Surface Design," *J. Am Stat. Assoc.* **54**, 622–654 (1959).
27. G. E. P. Box and D. W Behnken, "Some New Three Level Designs for the Study of Quantitative Variables," *Technometrics* **2**, 455–475 (1960)
28. J. A. Cornell, "Experiments with Mixtures: An Update and Bibliography," *Technometrics* **21**, 95–105 (1979).
29 D. R. Thompson, "Designing Mixture Experiments: A Review," *Trans. ASAE* **24**, 1077–1086 (1981).
30. L. B. Hare, "Mixture Designs Applied to Food Formulations," *Food Technol.* **28**, 50–56, 62 (1974).
31. K. Mullen and D. M. Ennis, "Rotatable Designs in Product Development," *Food Technol.* **33**, 74–80 (1979).
32. K. Mullen and D. Ennis, "Fractional Factorials in Product Development," *Food Technol.* **39**, 90–103 (1985)
33. N. R. Draper, "Ridge Analysis' of Response Surfaces," *Technometrics* **5**, 469–479 (1963).
34. J. D. Floros and M. S. Chinnan, "Optimization of Pimiento Pepper Lye-Peeling Process Using Response Surface Methodology," *Trans ASAE* **30**, 560–565 (1987).
35. J. D. Floros and M S. Chinnan, "Seven Factor Response Surface Optimization of a Double-Stage Lye (NaOH) Peeling Process for Pimiento Peppers," *J. Food Sci.* **53**, 631–638 (1988).
36. A. A. Guillou and J. D. Floros, "Multiresponse Optimization Minimizes Salt in Natural Cucumber Fermentation and Storage," *J. Food Sci.* **58**, 1381–1389 (1993).
37. A. I. Khuri and M. Conlon, "Simultaneous Optimization of Multiple Responses Represented by Polynomial Regression Functions," *Technometrics* **23**, 365–375 (1981).
38. A. I. Khuri and J. A. Cornell, *Response Surfaces, Designs, and Analysis*, Marcel Dekker, New York, 1987
39. J. Fichtali, F R. Van De Voort, and A. I. Khuri, "Multiresponse Optimization of Acid Casein Production," *J. Food Process Eng.* **12**, 247–258 (1990).
40. G. C. Derringer and R. Suich, "Simultaneous Optimization of Several Response Variables," *J. Qual. Technol* **12**, 214–219 (1980)
41. J. D. Floros and H. Liang, "Multiresponse Optimization by a Normalized Function Approach," *Dev. Food Sci.* **37**, 2139–2150 (1995).
42. G. E. P. Box and N. R. Draper, *Evolutionary Operation*, Wiley, New York, 1969.
43. T. P. Ryan, *Statistical Methods for Quality Improvement*, Wiley, New York, 1989.
44. P. J. Ross, *Taguchi Techniques for Quality Engineering*, McGraw-Hill, New York, 1988.
45. G. Taguchi, *Systems of Experimental Design*, Vols. 1 and 2, American Supplier Institute, Dearborn, Mich., 1987

46. N. Logothetis and A. Haigh, "Characterizing and Optimizing Multi-Response Processes by the Taguchi Method," *Qual. Reliab. Eng. Int.* 4, 159–169 (1988).
47. V. Gnanasekharan and J. D. Floros, "Back Propagation Neural Networks. Theory and Applications for Food Science and Technology," *Dev. Food Sci.* 37, 2151–2168 (1995)
48. I. G. Vradis and J D. Floros, "Genetic Algorithms and Fuzzy Theory for Optimization and Control of Food Processes," *Dev. Food Sci.* 37, 2169–2182 (1995).
49. P. Filka, "Optimization of Food Processing Plants," in M. Renard and J. J. Bimbenet, eds., *Automatic Control and Optimization of Food Processes*, Elsevier Applied Science, Barking, UK, 1986, pp. 405–415.

JOHN D. FLOROS
JEFF RATTRAY
Purdue University
West Lafayette, Indiana

ORANGES. See FRUITS, SEMI-TROPICAL.

OSTEOPOROSIS

Osteoporosis is a disease characterized by low bone mass and increased bone fragility and susceptibility to fracture. Adequate calcium, vitamin D, and other nutrients are critical to achieving optimal peak bone mass and modify the rate of bone loss with aging. After menopause, women experience rapid bone loss due to declining estrogen levels. Hormone replacement therapy, in conjunction with calcium, has been shown to be effective in reducing bone loss. Although less common in men, prolonged use of medications such as glucocorticoids as well as other factors may lead to osteoporosis in men.

DEFINITION

The National Institutes of Health (NIH) Consensus Development Conference defined osteoporosis as "A systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (1).

Osteoporosis affects more than 25 million people in the United States and is the major underlying cause of bone fractures in postmenopausal women and the elderly (2).

SYMPTOMS

Osteoporosis is often referred to as the silent disease because it can progress painlessly until a fracture occurs, typically in the hip, spine, or wrist. As the disease progresses, the vertebrae of the spine can become compressed leading to stooped posture and a loss of height. The abnormal curvature of the spine that results is referred to as kyphosis or a Dowager's hump.

The World Health Organization categorizes bone mass according to how it compares with a young adult reference range (see Table 1). The categories refer to the number of standard deviations that the patient's bone density differs from young, healthy women.

Table 1. World Health Organization Criteria for Osteoporosis in Women

Normal	BMD or BMC ≤ 1 SD below the young adult reference range.
Low bone mass	BMD or BMC 1–2.5 SD below the mean of young, healthy women.
Osteoporosis	BMD or BMC >2.5 SD below the mean of young, healthy women.
Severe osteoporosis	BMD or BMC >2.5 SD below the mean of young, healthy women and the presence of one or more fragility fractures.

Note. BMD = bone mineral density; BMC = bone mineral content.
Source: Ref. 3.

RISK FACTORS

The typical osteoporosis victim is a white or Asian postmenopausal woman with a thin body frame. Early menopause, either natural or surgical, combined with a diet low in calcium, family history of osteoporosis, sedentary lifestyle, smoking, and excessive alcohol intake further increase the risk. Numerous other factors increase the risk for osteoporosis. Table 2 provides a list of risk factors. Table 3 lists the types of osteoporosis.

BONE DEVELOPMENT OVER A LIFETIME

Bone is constantly in a state of flux. This state of flux is the result of two opposing forces: bone formation and resorption. Bone formation begins in utero, and the acquisition and development of bone mass continues throughout childhood. During growth and development of the skeleton, the cells lying on the surface of the developing bones, called *osteoblasts*, stimulate bone formation (Fig. 1). At the same time, a second group of surface cells, called *osteoclasts*, are involved in the process of resorption (breakdown) of bone. Both cell types are affected by physical and environmental factors (4,5).

At puberty, acquisition of bone mass is accelerated. This accelerated process is believed to be approximately 95% complete by age 18. Beyond this age, the process slows with the remaining 5% deposited in the bone matrix into the third decade of life (4,6,7). It is during these phases of growth and development that adequate intake of bone-related nutrients (calcium, vitamin D, magnesium, phosphorus, protein, vitamin K, boron, manganese, copper and zinc) is vital to a lifetime of strong and healthy bones (8–17).

During adulthood, from approximately the third decade of life to the onset of menopause in women at approximately 45 to 55 years of age, bone mass is considered stable and at its peak (18). With the onset of menopause, there occurs a rapid reversal of bone metabolism from acquisition to bone mass reduction. This is in response to declining estrogen levels, which initiate the menopausal process (4). This reduction process occurs in two phases. The first

Table 2. Osteoporosis Risk Factors

Genetic
Females
Asian or Caucasian individuals
Family history of osteoporosis
Small bone structure
Lifestyle
Inactive lifestyle
Prolonged bed rest
Never having been pregnant
Smoking
Nutritional
Excessive use of alcohol
Chronic low calcium intake
Vitamin D deficiency
High protein/phosphate ratio
High caffeine intake
Medical
Premenopausal oophorectomy
Premenopausal amenorrhea
Gastrointestinal dysfunction
Osteogenesis imperfecta
Parathyroid overactivity
Low testosterone levels in men
Medications
Prolonged glucocorticoid use
Prolonged use of some anticonvulsants
Chronic phosphate-binding antacid use
Diuretics producing calciuria
Miscellaneous
Late onset of menstruation
Early menopause, whether natural or surgical
Advanced age

stage lasts approximately 5 to 10 years and results in rapid reduction of bone mass, approximately 2 to 5% per year. In the second stage, bone loss is more gradual, approximately 0.2 to 0.5% per year. This loss continues unabated, in the absence of hormone replacement therapy, for the rest of the woman's life span, thereby increasing the risk of osteoporosis (4,19).

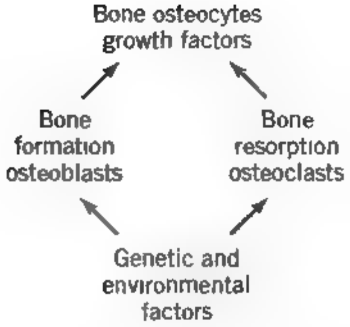

Bone osteocytes growth factors
Bone formation osteoblasts
Bone resorption osteoclasts
Genetic and environmental factors

Figure 1. Bone turnover and remodeling

As men age, the process of bone acquisition also converts to bone loss, but the process is much more gradual without the initial rapid losses created by the estrogen decline of menopause. The loss is consistent, however, and bone is continually lost as the male ages. Although this loss can lead to osteoporosis with age, over 75% of male osteoporosis is secondary to underlying chronic diseases, which accelerate bone loss similar to the effect of menopause in women (20). Other factors may also include age-related decline in testosterone, adrenal androgens, as well as other cellular factors (20–22).

As should be evident from the preceding, two critical factors determine the risk for osteoporosis. These are the peak bone mass attained and the level of bone loss after peak bone is attained (6). Peak bone mass is the total bone present in the final skeleton at maturity. Peak bone mass is affected by genetic factors, nutrition, hormonal factors, and exercise, among others (6,7).

The reduction in peak bone mass with aging is due to a number of interrelated factors. In women, estrogen decline after menopause is a major reason. In elderly men and women, other factors are important in age-related bone loss. With aging, there appears to be a general decline in osteoblast activity, as well as a reduction in calcium absorption from the GI tract due to a decrease in vitamin D intake and conversion to 1,25 $(OH)_2D$, the active form of vitamin D in the skin, kidney, and liver. In response to, and driven by this declining calcium uptake is hyperparathyroidism, which further drives the balance to resorption and bone loss.

Table 3. Types of Osteoporosis

	Type I, Postmenopausal	Type II, Senile	Type III, Secondary
Typical age	51–75	75–90	Any age
Gender	Female	Female: male ratio 2:1	Male or female
Risk factors	Estrogen deficiency	Advanced age	Certain medications, cancer, endocrine disorders, malnutrition or other conditions.
Bone involvement	Increased bone resorption without change in bone formation, particularly in trabecular bone	Bone formation and resorption are decreased, both trabecular and cortical bone are affected	Trabecular and cortical bone are affected equally
Fracture site	Vertebral, hip, wrist	Hip, spine, pelvis, and humerus	All types of fractures can occur

BONE COMPOSITION

Bone is composed primarily of calcium and phosphorus, in the form of hydroxyapatite crystals deposited in a collagen matrix (23). Adults have two types of bone: cortical and trabecular. Cortical bone provides rigidity and is the major component of the long bones of the skeleton. Cortical bone accounts for approximately 80 to 85% of the skeletal mass. Examples of cortical bone are the dense long bones of the hands, arms, and legs and bones of the feet. Trabecular bone is spongy in appearance and provides strength and elasticity. Trabecular bone makes up only about 15 to 20% of the skeletal mass. Examples of trabecular bone are the skull, the sternum, ribs, hip and shoulder girdle, and spinal column.

It would appear from the distribution of cortical and trabecular bone, that the cortical skeleton would be the most important area of bone turnover. However, bone turnover is a surface phenomenon, and trabecular bone has the greatest surface area. Therefore, 80% of bone turnover occurs in trabecular bone with only 20% occurring in cortical bone (4,5,19). This accounts for the greater fracture rate of trabecular bone versus cortical bone. A postmenopausal woman may lose 35% of her cortical bone and approximately 50% of her trabecular bone. A man of the same age, conversely, will lose about 12% of his cortical bone and approximately 17% of his trabecular bone (19).

GENETIC AND ENVIRONMENTAL FACTORS

Genetic and environmental factors are partners in the development of bone density and bone health over a lifetime (6). At present, the knowledge about genetic factors that are important in this process, and thus about the risk of osteoporosis, is meager at best. Genes, gene products, and nutrient–gene interactions are currently an evolving science (24,25). Ongoing research to understand the human genome, as well as many studies targeted at the environmental aspects of osteoporosis, will in time improve our knowledge. It is known now that hundreds of genes are involved in the growth, development, and maintenance of the skeletal system (6,26). A complete understanding of these genetic factors will help to clarify the underlying mechanisms of osteoporosis.

DIAGNOSIS

A number of techniques are available for measuring bone mass, each with its own advantages and disadvantages (see Table 4). Ideally, the technique should be rapid, reliable, accurate, inexpensive, and provide minimal radiation exposure. In addition, it should provide a prediction of the risk of subsequent fracture and have the ability to measure multiple sites (27).

Recommended Indications for Bone Density Studies

- Patients being treated for osteoporosis, to monitor bone mass
- Estrogen deficient women at risk for osteoporosis
- Patients with vertebral abnormalities, hip fractures, wrist fractures, or osteopenia

Table 4. Comparison of Bone Mass Measurement Techniques

Technique	Advantages	Disadvantages
Dual energy absorptiometry (DPA, DXA)	Best precision for measurement of spine or proximal femur Distinguishes between bone and soft tissue Multiple sites of measurement Low radiation exposure Moderate to low cost per test	Cannot distinguish between cortical and trabecular bone Limited mobility of equipment Moderate equipment cost
Peripheral DXA	Low equipment cost Low radiation exposure Equipment portable	Limited to study of wrist or heel Limited correlation to spine or hip
Quantitative Computed Tomography (QCT)	Distinguishes trabecular and cortical bone density Provides measure of volumetric BMD	More difficult to perform than DXA or DPA Higher radiation exposure than DXA Meticulous performance and calibration necessary Most costly of densitometric techniques Limited mobility of equipment Small body regions evaluated Accuracy can be severely affected by variations in fat content of marrow within the spine or femur
Radioabsorptiometry	Low equipment cost Equipment portable	Limited to study of phalanges Limited as a screening test Limited correlation to spine or hip
Single energy absorptiometry (SPA, SXA)	Useful for regions with low soft tissue (ie, forearm) Relatively inexpensive Equipment portable Low radiation exposure	Cannot measure bone mass near soft tissue (ie, vertebrae or femur) Limited correlation to spine or hip Limited as a screening test
Ultrasound	Low equipment cost Equipment portable No radiation exposure	Limited mesurement sites Limited as a screening test

- Patients receiving long-term glucocorticoid therapy
- Patients with primary hyperparathyroidism

PREVENTION OF OSTEOPOROSIS

Role of Calcium

Adequate calcium is critical to achieving optimal peak bone mass and modifies the rate of bone loss associated with aging (2). Numerous studies confirm the bone sparing effect of calcium. Reid et al. found that calcium supplementation significantly slowed axial and appendicular bone loss in normal postmenopausal women who received 1000 mg of calcium supplementation daily in addition to diet. The mean rate of loss of total body bone mineral density was reduced 43% in the calcium group compared with placebo (28). Recker et al. concluded that 1200 mg calcium per day reduced the incidence of spine fractures and halted measurable bone loss in elderly women with a history of spine fractures and self-selected calcium intakes of less than 1000 mg per day (29).

Recommended Calcium Intake

The NIH Consensus Development Panel on Optimal Calcium Intake defined optimal daily calcium intake as (2):

Infants birth to 6 months	400 mg
Infants 6–12 months	600 mg
Children 1–5 years	800 mg
Children 6–10 years	800–1200 mg
Adolescents and young adults 11–24 years	1200–1500 mg
Women 25–50 years	1000 mg
Pregnant or lactating women	1200 mg
Postmenopausal women on estrogen replacement therapy	1000 mg
Postmenopausal women not on estrogen replacement therapy	1500 mg
Men 25–65	1000 mg
Men and women over 65 years	1500 mg

The 1997 Dietary Reference Intakes (DRIs) for calcium recommend the following (30):

Males and females	9–13 year	1300 mg
	14–18 years	1300 mg
	19–50 years	1000 mg
	51–70 +	1200 mg
Pregnancy and lactation	<18 years	1300 mg
	19–30 years	1000 mg
	31–50 years	1000 mg

National Osteoporosis Foundation Recommendations

The National Osteoporosis Foundation (NOF) advises all patients to obtain an adequate intake of dietary calcium (at least 1200 mg per day, including supplements if necessary) and vitamin D (400 to 800 IU per day for individuals at risk of deficiency).

Average Calcium Intake by Individuals

A large percentage of Americans fail to meet currently recommended guidelines for optimal calcium intake (2). According to the Third National Health and Nutrition Examination Survey (NHANES III), the average intake of calcium by females is only 744 mg per day. For women 50 years and older, calcium intake is even lower (626–711 mg per day). The average intake for males is considerably higher at 976 mg per day, although it is lower for men 70 and older (721–808 mg) (31). Since adequate calcium intake is critical to achieving optimal peak bone mass and modifies the rate of bone loss associated with aging, calcium supplementation may be indicated in individuals who cannot meet their need by ingesting conventional foods (2).

Sources of Calcium

Dairy products are the most concentrated food sources of calcium. While green, leafy vegetables can help contribute to calcium intake, these vegetables also contain oxalic acid that can bind with calcium and interfere with absorption. The following chart lists the calcium content of some common foods. Values listed are approximate.

Food	Calcium content
Plain lowfat yogurt, 1 cup	400 mg
Canned sardines (with bones), 3 oz.	400 mg
Skim, lowfat and buttermilk, 8 oz.	300 mg
Cheese (most hard cheeses), 1 oz.	200 mg
Tofu (processed with calcium), 4 oz.	150 mg
Broccoli (cooked), 1/2 cup	75 mg
Almonds, 1 oz.	75 mg
Cottage cheese, 1/2 cup	50 mg
Whole wheat bread, 1 slice	20 mg

Pharmacologic Interventions

A number of pharmaceutical approaches are currently available for the management of osteoporosis. These interventions are sometimes classified as either influencing bone formation or bone resorption. Major interventions are described briefly.

Hormone Replacement Therapy (ERT and HRT). Of the therapies currently available, estrogen replacement therapy (ERT) is the only one approved for both prevention and treatment of osteoporosis. Estrogen acts to reduce bone resorption and increase bone density. Increases in bone density vary from site to site, that is, cortical (1–3%) and trabecular (2–5%). The greatest benefits of ERT and hormone replacement therapy (HRT) are observed in the years immediately following menopause, and rapid bone loss is observed immediately following discontinuation of estrogen replacement therapy. A daily dose of 0.3 to 1.25 mg unopposed estrogen is effective in protecting against bone loss (32).

Epidemiologic data suggest a significant reduction in the incidence of all types of osteoporotic fractures in ERT users. Retrospective and prospective studies report a 70 to 80% reduction in vertebral fractures (33,34).

Combination hormone therapy, estrogen and progestin, are commonly prescribed for women with a uterus. A three-year, multicenter trial examining the impact of combination therapy on bone mineral density showed significant increases in the spine and hip while the placebo group exhibited a reduction in BMD (35). The optimal duration of HRT therapy for bone maintenance is still undefined. The ongoing Women's Health Initiative is expected to clarify this and other open questions about use of HRT.

A meta-analysis of the effect of calcium supplementation on the efficacy of estrogen and calcitonin on bone mass change suggests that a high calcium intake potentiates the positive effect of estrogen on bone mass at all skeletal sites and perhaps that of calcitonin on bone mass of the spine. An open study included in the analysis found that 0.3 mg estrogen was equally as effective as the higher standard estrogen dose of 0.625 mg when given in conjunction with a high calcium intake (1700 mg per day) (36).

In an accompanying editorial to the meta-analysis, Bess Dawson-Hughes indicates the meta-analysis supports the notion that the calcium intake requirement of women treated with estrogen may not be lower than that of average postmenopausal women. In fact, the studies do not exclude the possibility that women receiving antiresorptive treatments may benefit from even higher calcium intake than average women.

Raloxifene. Raloxifene is the most recent drug therapy to gain approval in the United States for prevention of osteoporosis in postmenopausal women and is considered a first choice when ERT or HRT is not indicated. Raloxifene is classified as a selective estrogen receptor modulator (SERM), meaning that it has both estrogen agonistic and antagonistic activities that vary by tissue type. A number of recent reviews detail the properties of Raloxifene and its safety and efficacy. Three large-scale clinical trials were conducted to look at Raloxifene's effect on osteoporosis. Delmas et al. (1997) reported that bone mineral density increased significantly over baseline in lumbar spine, total hip, femoral neck, and total body following 24-month treatment with 30, 60, or 150 mg Raloxifene per day (37). The other trials showed similar results (38–40). A number of shorter-term clinical studies, which compared Raloxifene to ERT in postmenopausal women, found comparable decreased bone turnover and resorption. To date, no data have been gathered on Raloxifene's effect on fracture rate. Other related compounds, that is, Droloxifene, Idoxifene, and Levormeloxifene, are currently under development.

Bisphosphonates. Bisphosphonates are synthetic analogs of naturally occurring pyrophosphates that are known to improve bone mineralization. The mode of action is not fully defined. Rogers et al. (1997) have reviewed the recent evidence (41). These compounds are viewed as effective treatments for osteoporosis. Long-term usage has been shown to increase bone density. Although bisphosphonates are effective, poor absorption and esophageal irritation limit compliance and effectiveness. Improved forms of bisphosphonates are currently being investigated. Two forms, etidronate and alendronate, have been shown to reduce fracture rate (42–44).

Calcitonin. Calcitonin is described as an antiresorptive peptide that acts by inhibiting osteoclast activity. It must be administered parenterally or nasally to preserve its activity and may be particularly suitable for women who cannot take HRT. A number of reviews have been published recently (45–47) that detail the pharmacology and clinical effectiveness of calcitonin. In a two-year placebo controlled trial conducted in early postmenopausal women, spinal bone loss was prevented in the group receiving 100 IU calcitonin (48). Calcitonin appears to be effective in increasing bone mass for approximately one to two years, and then effectiveness plateaus. Intermittent administration may enhance effectiveness of the therapy. A maximum of about a 10% increase in bone mass can be achieved under conditions of optimal use. This agent has been shown to have a good safety profile. At present, however, it is approved for use in a relatively small number of countries due to the limited portfolio of clinical data available. Data are not yet available to establish the effect of calcitonin on fracture rate.

Fluoride. Fluoride, an essential element in bone formation, has been used for decades in Europe in the management of osteoporosis. Supplemental fluoride, given in milligram quantities (50–75 mg/day), significantly enhances bone mineral density, by stimulating osteoblast activity. Conflicting data are currently available on fluoride's impact on fracture rate. A number of side effects have been reported with flouride usage; these include pain in the lower extremities and gastric irritation. Administration without adequate calcium intake impaired mineralization. A number of investigations are under way to evaluate the efficacy of other forms of fluoride as well as preferred dosage regimens (49).

Other Pharmacologic Therapies

A number of hormones such as PTH, growth hormone, and anabolic steroids have been studied in the management of osteoporosis. These agents and their utility have been effectively reviewed in recent publications by O'Connell. Although bone formation may be enhanced by a number of these agents, various side effects, for example, glucose intolerance, hypertension, and virulization, diminish their potential (49).

Role of Exercise in Prevention and Treatment

Exercise plays a pivotal role in building and maintaining bone mass throughout life. Bone formation is enhanced by mechanical loading and results in modest increases in bone mass and improvements in bone structure. The type and frequency of exercise appear to significantly influence bone health. Strength training and weight-bearing exercise appear to offer the greatest benefit. The American Academy of Sports Medicine recommends 50 to 60 min of weight-bearing exercise three times per week (50).

In the prepubescent and pubescent years, the years of greatest bone building, physical activity enhances bone mineralization (51). Physical activity at a young age is a known determinant of bone mass (52). However, excessive exercise leading to amenorrhea is detrimental to building bone mass (53). Studies conducted in young and middle-aged women indicate that bone mass may be enhanced in those who have undertaken training programs that include weight-bearing exercise (54–56).

A small number of intervention trials have examined the benefits of exercise on osteoporosis (57). Collectively, these trials indicate that regular exercise is beneficial in preventing and treating osteoporosis. The benefits of weight-bearing exercise have been reviewed by Reid (1996) (58). Exercise conferred benefits such as improved balance, strength, coordination, and flexibility, which appear to diminish the risk of falls and fall-related injury (59). Low muscle strength and mobility are recognized independent risk factors for hip fracture (60). In a meta-analysis of seven exercise intervention trials in postmenopausal women, a 10% reduction in fall frequency was found among exercising subjects (59).

Several studies have also examined the value of exercise when combined with other treatments. Prince et al. (1995) investigated the combined effects of calcium supplementation and HRT with exercise and found that consumption of approximately 1.8 g calcium/day coupled with a mild increase in exercise significantly reduced bone loss in the hip (61). An earlier study by Prince et al. (1991) demonstrated that both exercise plus HRT and exercise plus calcium supplementation were effective in slowing or stopping bone loss (62).

RESOURCES FOR MORE INFORMATION

National Osteoporosis Foundation
1150 17th Street, NW, Suite 500
Washington, DC 20036-4603
Phone: 202-223-2226
Web site URL: www.nof.org.

Osteoporosis and Related Bone Diseases–National Resource Center
1150 17th Street, NW, Suite 500
Washington, DC 20036
Phone: 202-223-0344 or 800-624-BONE
TTY: (202)466-4315
E-mail: orbdnrc@nof.org.

National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892

U.S. National Library of Medicine
8600 Rockville Pike
Bethesda, MD 20894
Phone: 800-272-4787 or 301-496-6308.

BIBLIOGRAPHY

1. National Institutes of Health Consensus Development Conference, "Osteoporosis," *American Journal of Medicine* **94**, 646–650 (1993).
2. NIH Consensus Development Panel on Optimal Calcium Intake, "Optimal Calcium Intake," *JAMA* **272**, 1942–1948 (1994).
3. "Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis," WHO Technical Series 843, 1–129 (1994).
4. G. Birdwood, *Understanding Osteoporosis and Its Treatment*, Parthenon, New York, 1996.
5. J. Glowacki, "The Cellular and Biochemical Aspects of Bone Remodeling," in C. J. Rosen, ed., *Osteoporosis Diagnostic and Therapeutic Principles*, Humana Press, Totowa, N.J., 1996, pp. 3–14.
6. V. Matkovic et al., "Skeletal Development in Young Females: Endogenous versus Exogenous Factors," in P. Burckhardt, B. Dawson-Hughes, and R. Heaney, eds., *Nutritional Aspects of Osteoporosis*, Springer-Verlag, New York, 1998, pp. 26–41.
7. V. Matkovic, "Skeletal Development and Bone Turnover Revisited," *J. Clin. Endocrinol. Metab.* **81**, 2013–2016 (1996).
8. J. P. Bonjour et al., "Calcium Enriched Foods and Bone Mass Growth in Prepubertal Girls: A Randomized, Double Blind, Placebo-Controlled Trial," *J. Clin. Invest.* **99**, 1287–1294 (1997).
9. C. M. Weaver, G. P. McCabe, and M. Peacock, "Calcium Intake and Age Influence Calcium Retention," in P. Burckhardt, B. Dawson-Hughes, and R. P. Heaney, eds., *Nutritional Aspects of Osteoporosis*, Springer-Verlag, New York, 1998, pp. 3–10.
10. H. K. Kinyama et al., "Dietary Calcium and Vitamin D Intake in Elderly Women. Effect on Serum Parathyroid Hormone and Vitamin D Metabolites," *Am. J. Clin. Nutr.* **67**, 342–348 (1998).
11. B. Dawson-Hughes et al., "Rates of Bone Loss in Post Menopausal Women Randomly Assigned to One of Two Dosages of Vitamin D," *Am. J. Clin. Nutr.* **61**, 1140–1145 (1995).
12. R. P. Heaney, "Nutrition and Catch-Up Bone Augmentation in Young Women," *Am. J. Clin. Nutr.* **68**, 523–524 (1998).
13. D. Teegarden et al., "Dietary Calcium, Protein, and Phosphorus Are Related to Bone Mineral Density and Content in Young Women," *Am. J. Clin. Nutr.* **68**, 749–754 (1998).
14. D. Feskanich et al., "Vitamin K Intake and Hip Fractures in Women: A Prospective Study," *Am. J. Clin. Nutr.* **69**, 74–79 (1999)
15. H. P. Dimai et al., "Daily Oral Magnesium Supplementation Suppresses Bone Turnover in Young Adult Males," *J. Clin. Endocrinol. Metab.* **83**, 2742–2748 (1998).
16. C. D. Hunt, "Copper and Boron as Examples of Dietary Trace Elements Important in Bone Development and Disease," *Curr. Opin. in Orthopaedics* **9**, 28–36 (1998).
17. P. P. Saltman and L. G. Strause, "The Role of Trace Elements in Osteoporosis," *J. Am. College of Nutrition* **12**, 384–389 (1993).
18. V. Matkovic et al., "Timing of Peak Bone Mass in Caucasian Females and Its Implications for the Prevention of Osteoporosis," *J. Clin. Investigation*, 799–808 (1994).
19. G. R. Mundy, "Bone Remodeling and Mechanism of Bone Loss in Osteoporosis," in P. J. Meunier, ed., *Osteoporosis: Diagnosis and Management*, Mosby, New York, 1998, pp. 517–535.
20. P. Pilar and N. Guanabens, "Male Osteoporosis," *Curr. Opin. in Rheumatology* **8**, 357–364 (1996).
21. E. Seeman, "Osteoporosis in Men," *Bailliere's Clin. Rheumatology* **11**, 613–629 (1997)
22. M. E. Mussolino et al., "Risk Factors for Hip Fracture in White Men: The NHANES I Epidemiologic Follow-Up Study," *J. Bone Miner. Res.* **13**, 918–924 (1998).
23. A. Vies and B. Sabsay, "The Collagen of Mineralized Matrices," in W. A. Peck, ed., *Bone and Mineral Research*, Vol. 5, Elsevier, Amsterdam, The Netherlands, 1984.
24. E. Seeman et al., "Interaction between Genetic and Nutritional Factors," in P. Burckhardt, B. Dawson-Hughes, and R.

Heaney, eds., *Nutritional Aspects of Osteoporosis*, Springer-Verlag, New York, 1998, pp. 85–97.

25. S. Ferrari, R. Rizzoli, and J. P. Bonjour, "Genetics Dietary Calcium Interactions and Bone Mass," in P. Burckhardt, B. Dawson-Hughes, and R. Heaney, eds., *Nutritional Aspects of Osteoporosis*, Springer-Verlag, New York, 1998, pp. 99–106
26. V. Matkovic et al., "Factors Which Influence Peak Bone Mass Formation: A Study of Calcium Balance and the Inheritance of Bone Mass in Adolescent Females," *Am. J. Clin. Nutr.* **52**, 878–888 (1990).
27. H. K. Genant et al., "Measurement of Bone Mineral Density: Current Status," *American Journal of Medicine* **91**, 49S–53S (1991).
28. I. R. Reid et al., "Effect of Calcium Supplementation on Bone Loss in Postmenopausal Women," *New Engl. J. Med.* **328**, 460–464 (1993).
29. R. Recker et al., "Correcting Calcium Nutritional Deficiency Prevents Spine Fractures in Elderly Women," *J. Bone and Mineral Res.* **11**, 1961–1966 (1996).
30. Institute of Medicine, *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium Vitamin D, and Fluoride*, National Academy Press, Washington, D.C., 1997.
31. U.S. Department of Health and Human Services, *Advance Data: Dietary Intake of Vitamins, Minerals, and Fiber of Persons Ages Two Months and Over in the United States*, Third National Health and Nutrition Examination Survey, Phase 1, 1988–91, No. 258, 1994
32. H. K. Genant, et al., "Low-Dose Esterified Estrogen Therapy," *Archives of Internal Medicine* **157**, 2609–2615 (1997).
33. B. Ettinger, H. K. Genant, and C. E. Cann, "Long-Term Estrogen Replacement Therapy Prevents Bone Loss and Fractures," *Ann. Intern. Med.* **102**, 319–324 (1985).
34. R. Lindsay et al., "Prevention of Spinal Osteoporosis in Oophorectomized Women," *Lancet* **2**, 1151–1154 (1980).
35. The PEPI Writing Group, "Effects of Hormone Therapy on Bone Mineral Density," *JAMA* **276**, 1389–1396 (1985).
36. J. W. Nieves et al., "Calcium Potentiates the Effect of Estrogen and Calcitonin on Bone Mass: Review and Analysis," *Am. J. Clin. Nutr.* **67**, 18–24 (1998).
37. P. D. Delmas et al., "Effects of Raloxifene on Bone Mineral Density, Serum Cholesterol Concentrations, and Uterine Endometrium in Postmenopausal Women," *New Engl. J. Med.* **337**, 1641–1647 (1997).
38. Eli Lily, Evista (raloxifene hydrochloride) package insert, Indianapolis, Ind., December 1997.
39. W. Khovidkhunkit and D. M. Shoback, "Clinical Effects of Raloxifene Hydrochloride in Women," *Ann. Intern. Med.* **130**, 431–439 (1999).
40. J. A. Balfour and K. I. Goa, "Raloxifene," *Drugs Aging* **12**, 335–341 (1998)
41. M. J. Rogers, D. J. Watts and R. G. Russell, "Overview of Bisphosphonates," *Cancer* **80**, (Supplement), 1652–1660 (1997).
42. T. Storm et al., "Effect of Intermittent Cyclical Etidronate Therapy on Bone Mass and Fracture Rate in Women with Postmenopausal Osteoporosis," *New Engl. J Med.* **322**, 1265–1271 (1990).
43. N. B. Watts et al., "Intermittent Cyclical Etidronate Treatment of Postmenopausal Osteoprosis," *New Engl. J. Med.* **323**, 73–79 (1990).
44. D. M. Black et al., "Randomized Trial of Effect of Alendronate on Risk of Fracture in Women with Existing Vertebral Fractures: Fracture Intervention Trial Research Group," *Lancet* **348**, 1535–1541 (1996).
45. R. Eastell, "Treatment of Postmenopausal Osteoporosis," *New Engl. J. Med.* **338**, 736–746 (1998).
46. S. Silverman, "Calcitonin," *Am. J Medical Sci.* **313**, 13–16 (1997).
47. C. Gennari and D. Agnusdei, "Calcitonins and Osteoporosis," *Br. J. Clin. Practice* **48**, 196–200 (1994).
48. K. Overgaard et al., "Effect of Calcitonin Given Intranasally on Early Postmenopausal Bone Loss," *Br. Med. J* **299**, 477–479 (1989).
49. M. O'Connell, "Prevention and Treatment of Osteoporosis in the Elderly," *Pharmacotherapy* **19**, 7S–20S (1999).
50. American College of Sports Medicine, "ACSM Position Stand on Osteoporosis and Exercise," *Med. Sci. Sports Exer* **27**, i–vii (1995).
51. C. W. Slemenda, et al., "Influences on Skeletal Mineralization in Children and Adolescents: Evidence for Varying Effects of Sexual Maturation and Physical Activity," *J. Pediatrics* **125**, 201–207 (1994).
52. P. Kannus, et al., "Effect of Starting Age of Physical Activity on Bone Mass in the Dominant Arm of Tennis and Squash Players," *Ann. Intern. Med.* **123**, 27–31 (1995).
53. D. C. Cumming, "Exercise Induced Amenorrhea, Low Bone Density, and Extrogen Replacement Therapy," *Arch. Int. Med.* **156**, 2193–2195 (1996).
54. A. L. Friedlander et al., "A Two Year Program of Aerobic and Weight Training Enhances Bone Mineral Density of Young Women," *J. Bone Miner. Res.* **10**, 574–585 (1995).
55. T. Lohman et al., "Effects of Resistance Training on Regional and Total Bone Mineral Density in Premenopausal Women: A Randomized Study," *J. Bone Miner Res.* **10**, 1015–1024 (1995).
56. B. P. Conroy et al., "Bone Mineral Density in Elite Junior Olympic Weight Lifters," *Med. Sci. Sports Exer* **25**, 1103–1109 (1993)
57. E. Ernst, "Exercise for Female Osteoporosis: A Systematic Review of Randomized Clinical Trials," *Sports Medicine* **25**, 359–368 (1998).
58. I. R. Reid, "Therapy of Osteoporosis: Calcium, Vitamin D, and Exercise," *Am. J. Med. Sci.* **312**, 278–286 (1996).
59. M. A. Province, "The Effects of Exercise on Falls in Elderly Patients: A Preplanned Metaanalysis of FICsit Trials," *JAMA* **273**, 1341–1347 (1995).
60. S. R. Cummings, et al., "Risk Factors for Hip Fracture in White Women," *New Engl. J Med.* **332**, 767–773 (1995).
61. R. Prince et al., "The Effects of Calcium Supplementation (Milk Powder or Tablets) and Exercise on Bone Density in Postmenopausal Women," *J. Bone Miner Res.* **10**, 1068–1075 (1995).
62. R. Prince et al., "Prevention of Postmenopausal Osteoporosis: A Comparative Study of Exercise, Calcium Supplementation, and Hormone Replacement Therapy," *New Engl. J. Med.* **325**, 1189–1195 (1991).

RICHARD COTTER
CINDY DOMINGUEZ
SUSAN TRIMBO
Whitehall-Robins
Madison, New Jersey

See also MINERALS: MICRONUTRIENTS.

OXIDATION

Oxidation is one of the most important reactions occurring in food and food-related systems. The reaction affects not only the chemical nature but many interactions among food constituents, leading to both desirable and undesirable products.

Oxidation reactions can be categorized into chemical and enzymatic. Both processes can be naturally occurring or initiated and enhanced by food handling or processing. The former includes, for example, lipid oxidation and thermal oxidation, which are the frequent cause affecting the acceptability and qualities of many processed food products. Oxidation of proteins by radicals and disulfide exchange often results in cross-linking and subsequent changes in the physical and functional properties. Autoxidation of the heme iron in myoglobin is known to be the major cause of color change in meat products. Enzyme-catalyzed oxidations such as oxygenation of unsaturated fatty acids by lipoxygenase, the browning reaction catalyzed by polyphenol oxidase, and the conversion of glucose to lactone by glucose oxidase have been linked to various changes and interactions in food systems. The oxidative degradation of vitamins can lead to loss in nutrition, and similar types of reaction mechanisms are involved in the antioxidation effect of certain vitamins.

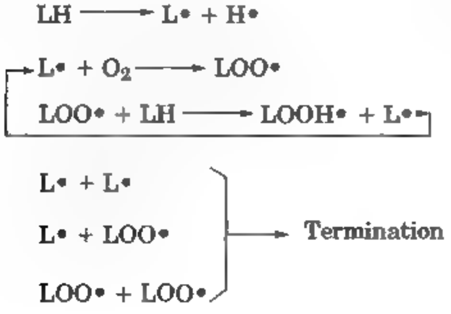
Lipid Oxidation

The reaction of oxygen with unsaturated lipids is one of the most extensively studied areas in food chemistry. Two different pathways have been identified- autoxidation and photosensitized oxidation (1).

Autoxidation

Autoxidation is a free-radical chain reaction involving initiation, propagation and termination steps (Scheme 1). Abstraction of the hydrogen from an unsaturated lipid is favored by resonance stabilization of the conjugated system. The initiation step is catalyzed by metal ion chelates or reducing agents present in biological systems, such as thiol groups in proteins, ascorbate, NADH, and $FADH_2$ (2,3). Both heme and nonheme irons present in muscle tissues are also implicated as initiator (4,5).

The peroxy radical formed by the reaction between the lipid alkyl radical and oxygen can react with another lipid molecule to form hydroperoxide and free radical. It is now well known that the breakdown of hydroperoxides constitutes the major source of rancid flavors in foods. The first step involves decomposition of the hydroperoxide to the alkoxy and hydroxy free radicals, followed by a carbon-carbon cleavage on either side of the alkoxy radical (Scheme 2). The alkoxy radical then reacts with other radicals. These reactions account for the formation of carbonyls, alcohols, esters, and hydrocarbons in peroxidizing lipid systems.



Scheme 1.

Photosensitized Oxidation

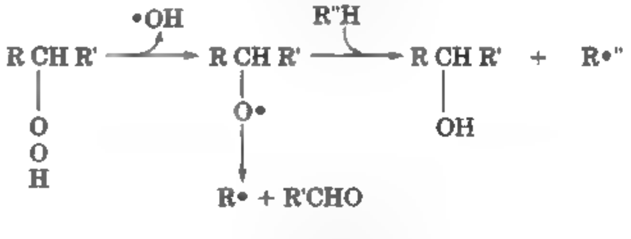
In systems containing a light sensitizer, such as chlorophyll in green vegetables, lipid oxidation proceeds via an "ene" reaction, in which the singlet oxygen molecule is added onto the double-bond (Scheme 3) (6). As free radicals are not involved in this reaction mechanism, the distribution of hydroperoxide products differs from that of autoxidation (1,7).

In peroxidized linolenic acid, cyclization of the hydroperoxide can lead to the formation of malonaldehyde, a dialdehyde that is known to cross-link proteins, enzymes, and nucleic acids. Malonaldehyde can form a Schiff base intermediate with the lysyl-ϵ-amino groups of proteins causing intermolecular cross-linking (7–9).

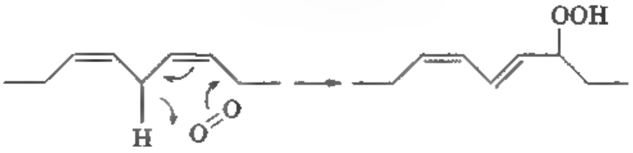
The radicals generated from oxidation of lipids can interact with some amino acid residues side chains in proteins to form (*1*) lipid-protein polymers, or (*2*) protein free radicals (P·), which may react further with other protein molecules to form cross-linked products (Scheme 4) (10,11). These subsequent polymerization reactions change the chemical structure and functionality of food proteins present in oxidized lipid systems. Cross-linked proteins are expected to possess very different nutritional properties, including reduced digestibility and loss of some of the most sensitive amino acids, such as histidine, methionine, lysine, and cysteine/cystine.

Oxidative Thermal Reactions

Thermal oxidation of saturated fatty acids generally occurs at the α, β, or γ position to form alkoxy radicals, followed



Scheme 2.



Scheme 3.

Formation of protein radical

$$L\bullet + P \longrightarrow LH + P\bullet$$
$$LO\bullet + P \longrightarrow LOH + P\bullet$$
$$LOO\bullet + P \longrightarrow LOOH + P\bullet$$

Liquid-protein cross-link

Termination

$$L\bullet + P\bullet \longrightarrow LP$$
$$LO\bullet + P\bullet \longrightarrow LOP$$
$$LOO\bullet + P\bullet \longrightarrow LOOP$$

Displacement

$$L\bullet + P \longrightarrow LP\bullet \xrightarrow{P} LPP\bullet \text{ etc}$$
$$LO\bullet + P \longrightarrow LOP\bullet \xrightarrow{P} LOPP\bullet \text{ etc}$$
$$LOO\bullet + P \longrightarrow LOOP\bullet \xrightarrow{P} LOOPP\bullet \text{ etc}$$

Protein-protein cross-link

$$P\bullet + P\bullet \longrightarrow PP$$
$$P\bullet + P \longrightarrow PP\bullet \xrightarrow{P} PPP\bullet \text{ etc}$$

Scheme 4.

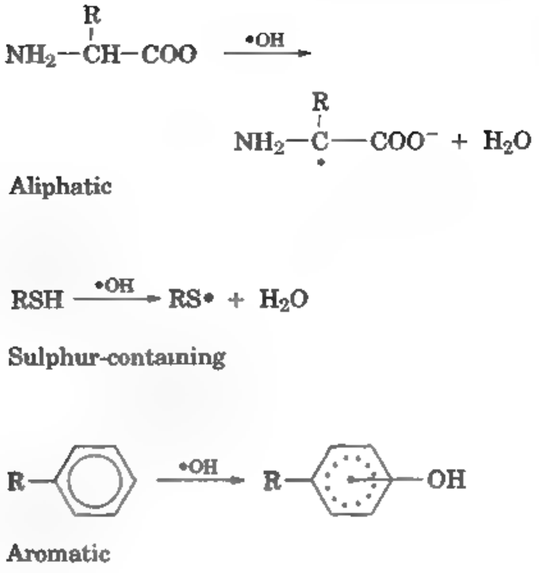
by carbon-carbon cleavage producing various carbonyl compounds (12). Oxidative decomposition of unsaturated fatty acids yields dimers, trimers, and tetramers with polar groups. Radicals generated in these processes can enter into very complex combination reactions. Polymer formation causes increasing viscosity of the frying oil.

OXIDATION IN PROTEINS

Proteins are subjected to chemical reactions due to many reactive side-chain groups of the amino acids. The reactivity of these amino acids depends on their chemical nature, and very much on the environment in which they are placed. The pH of the system has a strong influence on the reaction. Depending on the particular pK_a, the side-chain group may be protonated or nonprotonated, and in general, it is the least protonated form that is most nucleophilic and reactive. The accessibility of the various groups also determines their differences in reactivity under a particular set of conditions.

The Hydroxy Radical

Proteins are very reactive toward reactive oxygen species generated by radiolysis of water in biological and food systems (11) (Scheme 5). Aromatic amino acids react with hydroxy radical (OH·), forming substituted cyclohexadienyl radicals (13). All sulfur-containing amino acids react at diffusion-controlled rates. The radical intermediates have been demonstrated to form cross-linked products between proteins and DNA (9,14).



$$NH_2{-}CH(R){-}COO \xrightarrow{\bullet OH} NH_2{-}\dot{C}(R){-}COO^- + H_2O$$

Aliphatic

$$RSH \xrightarrow{\bullet OH} RS\bullet + H_2O$$

Sulphur-containing

Aromatic

Scheme 5.

Photosensitized Oxidation

In the presence of a suitable sensitizer such as flavin, cysteine is oxidized to cysteic acid and methionine to methionine sulfoxide (Scheme 6) (11,15). Interestingly, the same oxidation products are obtained using chemical agents such as hypochlorite or hydrogen peroxide. The treatment of soy proteins with 0.5% hydrogen peroxide oxidized half of the methionine to sulfoxide (16).

Oxidation of Cysteine

The oxidation reaction especially important to the functions of organized protein systems involves the reduction and reoxidation of disulfides. This type of sulfhydryl-disulfide reaction plays a major role in dough mixing (17–19). There is a direct correlation between the sulfhydryl-disulfide content and the rheological quality of doughs made from wheat flours of several varieties (20,21). However, the exact interpretation of disulfide exchange in the molecular model of dough forming remains unclear (22).

Another example of chemical and physical changes caused by reduction and reoxidation is exhibited by the whey protein, β-lactoglobulin. The protein is heat sensitive and starts to polymerize and aggregate when heated above 65°C. The primary reaction is believed to be the reduction of the two disulfides and reoxidation of the thiols forming intermolecular or intramolecular interchange with the free cysteine-121 (23). Sulfhydryl-disulfide interchange also occurs between β-lactoglobulin and κ-casein, producing a complex that is susceptible to heat-induced calcium phosphate precipitation and resistant to rennin action (24,25).

$$2RSH + H_2O \xrightarrow[2O_2,\ h\nu]{\text{Sensitizer}} RSO_3H + RS\bullet + H_2O_2$$

Scheme 6.

$$Fe^{2+} + O_2 \longrightarrow FeO_2^{2+}$$

$$FeO_2^{2+} + Fe^{++} \longrightarrow 2\,Fe^{3+} + O_2^{2-}$$

Scheme 7.

The loss in functionality limits the usefulness of this abundant whey protein as ingredients in food processing. The development of recombinant technology provides a novel procedure to clone the β-lactoglobulin gene, and specifically modify the free cysteine, with the ultimate aim of improving its thermal stability and functional properties (26–28).

Autoxidation of Heme Iron

Myoglobin, the protein that determines the color of meat products, is known to undergo autoxidation. The bright-red exterior color of fresh meat is derived from oxymyoglobin, which contains a heme iron in the +2 oxidation state with an oxygen molecule reversibly bound to the sixth coordination position. The Fe(II) is readily oxidized to Fe(III) to give ferrimyoglobin characteristic of the brown surface color of aged meat. The autoxidation of the heme iron has been shown not to occur by a simple one-electron transfer in which the iron is oxidized from +2 to +3 state and O_2 is reduced to $O^-{}_2$ (29,30). Instead, autoxidation in this case involves a two-electron reduction of the oxygen to peroxide via an iron-dioxygen complex (Scheme 7).

ENZYME-CATALYZED OXIDATION

Lipoxygenase-Catalyzed Peroxidation

The action of the enzyme lipoxygenase (EC1.13.11.12) in food and food-related systems leads to the formation of hydroperoxides in the aerobic pathway, and dimers and oxodienoic acids in the anaerobic reaction (31,32). The enzyme substrate must be unsaturated fatty acids containing *cis,cis*-1,4-pentadiene system, and the reaction is regiospecific and stereospecific in contrast to autoxidation. As the result of free-radical formation in the intermediate process, proteins present in the same system may be oxidized, and subsequent protein-protein polymerization yields many compounds similar to those outlined in autoxidation (33). Oxidized lipids also cause chemical changes in some sensitive amino acids in proteins, and cross-linking reaction between proteins and aldehydes generated from the breakdown of hydroperoxide.

Soybean lipoxygenase has been utilized to bleach flour by incorporating soy flour in wheat flour. The oxidative destruction of pigments in flour by the addition of lipoxygenase is believed to be a free-radical mediated reaction in which the alkyl or peroxy radicals are involved. Besides the bleaching effect, using soy flour in bread making often results in an improvement of the dough-forming properties (34–36). It has been proposed that in a peroxidizing system, the radicals enter into coupled oxidation of cysteine residues in the flour proteins and cause structural changes in the dough (Scheme 8).

The hydroperoxides formed from lipoxygenase-catalyzed oxidation of unsaturated lipids are converted to flavor compounds by the enzyme hydroperoxide lyase, which has been identified and purified from cucumber and tomato fruits, watermelon seedlings, pear, soybean seedlings and leaf tissues. The major flavor compound in cucumber, (*E,Z*)-2,6-nonadienal, is one of the products of this enzymatic rearrangement. Other aldehydes also originate from fatty acid through similar pathways.

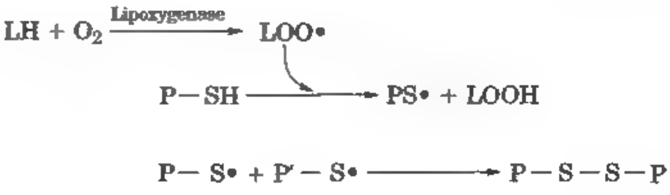
Enzymatic Browning Reactions

Polyphenol oxidase (EC1.10.3.1) is an enzyme of great importance in that it not only causes the browning of cut or bruised fruits and vegetables but also plays a major role in the curing of tea, coffee, and tobacco. The enzyme functions as a monooxygenase in the ortho hydroxylation of monophenols to dihydroxyphenols, and a two-electron oxidase in the oxidation of the *o*-diphenols to *o*-quinone (Scheme 9) (37–39). The enzyme has broad substrate specificity. Some of the common substrates include catechol, 4-methylcatechol, dopamine, pyrogallol, catechin, caffeic acid, chlorogenic acid, *p*-cresol, tyrosine, and *p*-hydroxycinnamic acid. The product of the oxidation of diphenols is o-quinone, which polymerizes to form colored pigments (40–42).

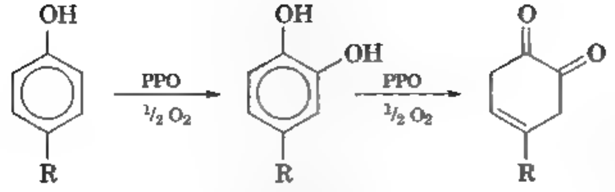
In the oxidation of dopamine, the primary substrate found in banana, the dopamine quinone undergoes nonenzymatic rearrangement to indole-5,6-quinone that in turn is polymerized to form melanin (43,44).

The development of flavor (and color as well) in the fermentation of tea is related to the oxidation of phenolic compounds. The most important reaction is the oxidation of flavanols by polyphenol oxidase. The oxidized flavanols condense to form theaflavin, which is one of the major constituents of tea flavor (45,46). Similar type of desired activity is also evidenced in coffee cocoa, prunes, dates, and dark raisin production.

The oxidized flavanols can also undergo Strecker degradation with amino acids to form various aldehydes. Flavor compounds, such as isobutanal, 2-methylbutanal, isovaleraldehyde, and phenyacetaldehyde present in fermented tea aroma have been suggested to be the products of degradation of valine, isoleucine, leucine, and phenylalanine. The oxidation of flavanols to the *o*-quinone form



Scheme 8.



Scheme 9.

also causes the oxidative degradation of carotene to volatile products, such as ionone and 5,6-epoxy-*β*-ionone.

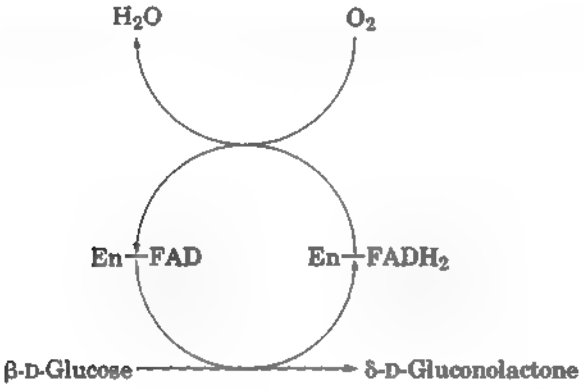
The polymerization of *o*-quinone to polyaromatic pigments requires water in the reaction components. The effort to stabilize the qunone for the purpose of regiospecific oxidation of the aromatic compounds has been attempted. Polyphenol oxidase has been found to function in chloroform whereby phenol substrates can be converted to stable quinone, which can be quantitatively reduced to catechols (47–49).

Glucose oxidase (EC1.1.3.4) is a food enzyme used for the removal of glucose to prevent the Maillard reaction in egg solids, dried meats, potatoes, and so on. When coupled with catalase, it is used for the removal of oxygen from the head space of packaged foods. The enzyme is also used in the production of gluconic acid, and for the quantitative determination of D-glucose in food, agricultural, and pharmaceutical products (50–52). The enzyme catalyzes the irreversible oxidation of a number of sugars to the corresponding lactones. Common substrates include glucose, deoxyglucose, mannose and galactose.

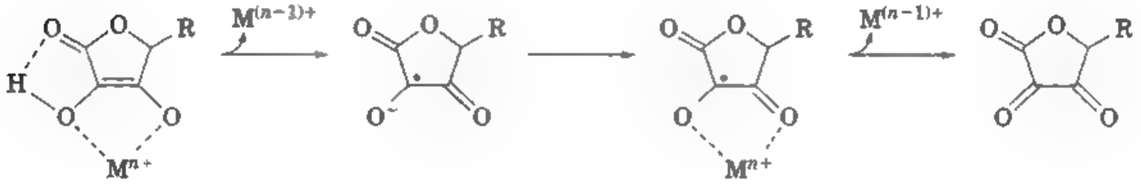
The enzyme is a dimer containing flavin adenine dinucleotide (FAD) as cofactor. In the reaction, the substrate is oxidized to the lactone while the FAD is reduced to $FADH_2$ (Scheme 10). In a subsequent step, the lactone is nonenzymatically hydrolyzed to gluconic acid, and the reduced enzyme is reoxidized (53).

OXIDATION OF VITAMINS

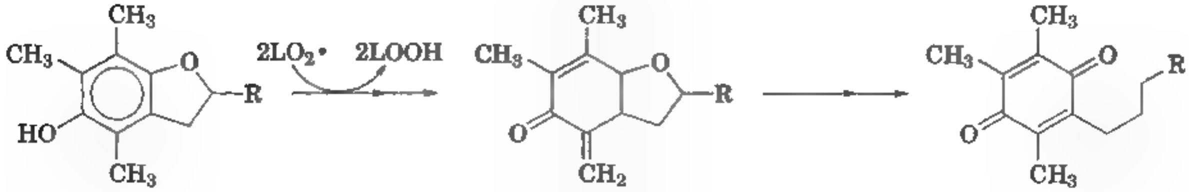
Loss of vitamins during food handling can be due to many types of degradations and one of these important reactions is oxidation.



Scheme 10.



Scheme 11.



Quinone methine

Scheme 12.

Ascorbic Acid

Vitamin C is one of the least stable vitamins mainly due to oxidative degradations (54). Ascorbic acid can be oxidized by metal ions such as Fe(III) and Cu(II) in a two-sequential one-electron transfer to yield dehydroascorbic acid. Alternatively, metal ions can also catalyze the oxidation of ascorbic acid via the formation of an ascorbate-metal-dioxygen complex immediate (Scheme 11). In this reaction, the two-electron transfer occurs between the ascorbic and the dioxygen leading to the formation of dehydroascorbic acid and hydrogen peroxide (55).

Dehydroascorbic acid undergoes further degradation in acid medium (56), or reacts with amino acids via Strecker degradation. The latter reaction leads to the formation of scorbamic acid and, subsequently, polymeric compounds (57,58).

Tocopherol

Vitamin E also undergoes oxidative degradation via a free-radical pathway that accounts for its high efficiency as a chain-breaking antioxidant. The first step of the free-radical reaction involves the abstraction of two hydrogen atoms, forming the quinone methine that undergoes successive rearrangements to the stable quinone (Scheme 12). In the reaction, the tocopherol molecule consumes two peroxy radicals (LOO·) to form a nonradical product (59,60).

From the subject matter covered in the discussion, it is obvious that oxidation is a widespread reaction affecting every constituent in food and food-related systems. The chemistry involves free radical, singlet oxygen, coupled reduction-oxidation, oxidizing chemical, metal ion, enzyme catalysis, cofactor, and so on. Product interaction often leads to new degradation reactions, cross-linking, polymerization, and subsequently alteration in physical and chemical properties as well as functionality of the constituents. These reactions and interactions are complex and certainly should deserve more attention from food scientists and researchers.

BIBLIOGRAPHY

1. E. N. Frankel, "Lipid Oxidation," *Prog. Lipid Res.* **19**, 1–22 (1980).
2. L. Ernster and K. Nordenbrand, "Microsomal Lipid Peroxidation: Mechanism and Some Biomedical Implications," in K. Yagi, ed., *Lipid Peroxides in Biology and Medicine*, Academic Press, New York, 1982, pp. 55–79.
3. J. M. C. Gutteridge and B. Halliwell, "The Measurement and Mechanism of Lipid Peroxidation in Biological Systems," *TIBS* **15**, 129–135 (1990).
4. A. L. Tappel, "Hematin Compounds and Lipoxidase as Biocatalysts," in H. W. Schultz, E. A. Day, and R. O. Sinnhuber, eds., *Lipids and Their Oxidation*, AVI, Westport, Conn., 1962, pp. 122–138.
5. J. Kanner, J. B. German, and J. E. Kinsella, "Initiation of Lipid Peroxidation in Biological Systems," *CRC Crit. Rev. Food Sci. & Nutr.* **25**, 317–364 (1987).
6. F. D. Gunstone, "Reaction of Oxygen and Unsaturated Fatty Acids," *J. AOCS* **61**, 441–444 (1984).
7. E. N. Frankel, "Lipid Oxidation: Mechanisms, Products and Biological Significance," *J. AOCS* **61**, 1908–1917 (1984).
8. K. S. Chio and A. L. Tappel, "Inactivation of Ribonuclease and Other Enzymes by Peroxidizing Lipids and by Malonaldehyde," *Biochem.* **8**, 2827–2832 (1969).
9. M. Dizdaroglu and M. G. Simic, "Radiation-Induced Crosslinks Between Thymine and Phenylalanine," *Int. J. Radiat. Biol.* **47**, 63–69 (1985).
10. K. M. Schaich, "Free Radical Initiation in Proteins and Amino Acids by Ionizing and Ultraviolet Radiation and Lipid Oxidation, Part III: Free Radical Transfer from Oxidizing Lipids," *CRC Crit. Rev. Food Sci. & Nutr.* **13**, 189–244 (1980).
11. B. S. Berlett and E. R. Stadtman, Protein Oxidation in Aging, Disease, and Oxidative Stress," *J. Biol. Chem.* **272**, 20313–20316 (1997).
12. E. D. Cmjar, A. Witchwoot, and W. W. Nawar, "Thermal Oxidation of a Series of Saturated Triacylglycerols," *J. Agric. Food Chem.* **29**, 39–42 (1981).
13. P. S. Rao and E. Hayon, "Reaction of Hydroxyl Radicals with Oligopeptides in Aqueous Solutions, A Pulse Radiolysis Study," *J. Phys. Chem.* **79**, 109–115 (1975).
14. O. Yamamoto, "Radiation-Induced Binding of Methionine with Serum Albumin, Tryptophan or Phenylalanine in Aqueous Solution," *Int. J. Radiat. Phys. Chem.* **4**, 335–345 (1972).
15. S. F. Yang, H. S. Ku, and H. K. Pratt, "Photochemical Production of Ethylene from Methionine and its Analogues in the Presence of Flavin Mononucleotide," *J. Biol. Chem.* **242**, 5274–5280 (1967).
16. K. C. Chang, H. F. Marshall, and L. D. Satterlee, "Sulfur Amino Acid Stability, Hydrogen Peroxide Treatment of Casein, Egg White, and Soy Flour," *J. Food Sci.* **47**, 1181–1183 (1982).
17. A. Graveland et al., "Superoxide Involvement in the Reduction of Disulfide Bonds of Wheat Gel Proteins," *Biochem. Biophys. Res. Comm.* **93**, 1189–1195 (1980).
18. A. Graveland et al., "A Model for the Molecular Structure of the Glutenins from Wheat Flour," *J. Cereal Sci.* **3**, 1–16 (1985).
19. P. R. Shewry and A. S. Tatham, "Recent Advances in Our Understanding of Cereal Seed Protein Structure and Functionality," *Comments Agric. and Food Chem.* **1**, 71–94 (1987).
20. C. C. Tsen and W. Bushuk, "Reactive and Total Sulfhydryl and Disulfide Contents of Flours of Different Mixing Properties," *Cereal Chem.* **45**, 58–62 (1968).
21. R. Lasztity, *The Chemistry of Cereal Proteins*, CRC Press, Boca Raton, Fla., 1984.
22. D. K. Mecham, "Wheat Proteins—Observations on Research Problems and Progress, Part 1," *Food Technol. in Australia* **32**, 540–587 (1980).
23. M. Z. Papiz et al., "The Structure of β-Lactoglobulin and its Similarity to Plasma Retinol-Binding Protein," *Nature* **324**, 383–385 (1986).
24. P. F. Fox and M. C. T. Hoynes, "Heat Stability of Milk: Influence of Colloid Calcium Phosphate and β-Lactoglobulin," *J. Dairy Res.* **42**, 427–435 (1975).
25. D. M. Mulvihill and M. Donovan, "Whey Proteins and Their Thermal Denaturation—A Review," *Irish J. Food Sci. Technol.* **11**, 43–75 (1987).
26. L. K. Creamer, D. A. D. Parry, and G. N. Malcolm, "Secondary Structure of Bovine β-Lactoglobulin B," *Arch. Biochem. Biophys.* **227**, 98–105 (1983).
27. A. C. Jamieson et al., "Cloning and Nucleotide Sequence of The Bovine β-Lactoglobulin Gene," *Gene* **61**, 85–88 (1987).
28. C. A. Batt et al., "Expression of Recombinant Bovine β-Lactoglobulin in *Escherichia coli*," *Agric. Biol. Chem.* **54**, 949–955 (1990).

29. C. E. Castro, R. S. Wade, and N. O. Belser, "Conversion of Oxyhemoglobulin to Methemoglobin by Organic and Inorganic Reductants," *Biochem.* **17**, 225–231 (1978).
30. D. J. Livingston and W. D. Brown, "The Chemistry of Myoglobin and Its Reactions," *Food Technol.* **35**, 244–252 (1981).
31. K. J. Nelsen and S. P. Seltz, "The Structure and Function of Lipoxygenase," *Curr. Op. in Structural Biol.* **4**, 878–884 (1994).
32. D. W. S. Wong, *Food Enzymes: Structure and Mechanism*, Chapman & Hall, New York, 1995, pp. 237–270.
33. G Matheis and J R. Whitaker, "A Review: Enzymatic Cross-Linking of Proteins Applicable to Foods," *J. Food Biochem.* **11**, 309–327 (1987).
34. P J. Frazier et al., "The Effect of Lipoxygenase Action on The Mechanical Development of Doughs From Fat-Extracted and Reconstituted Wheat Flour," *J. Sci. Food Agric.* **28**, 247–254 (1977).
35. P. J. Frazier, "Lipoxygenase Action and Lipid Binding in Breadmaking," *Baker's Digest* **53**, 8–20, 29 (1979).
36. R. Kieffer and W. Grosch, "Verbesserung der backeigenschaften von weizenmehlen durch die typ ii—lipoxygenase aus sojabohnen," *Z. Lebensm. Unters. Forseh* **170**, 258–261 (1980).
37. R. S. Himmelwright et al., "Chemical and Spectroscopic Studies of The Binuclear Copper Active Site of *Neurospora* Tyrosinase: Comparison to Hemocyanins," *J. Am. Chem. Soc.* **102**, 7339–7344 (1980).
38. D. E. Wilcox et al., "Substrate Analogue Binding to The Coupled Binuclear Copper Active Site in Tyrosinase," *J. Am. Chem. Soc.* **107**, 4015–4027 (1985).
39. M. A. Pavlosky and E. I. Solomon, "Near-IR CD/MCD Spectral Elucidation of Two Forms of the Non-Heme Active Site in Native Ferrous Soybean Lipoxygenase-1: Correlation to Crystal Structures and Reactivity," *J. Am. Chem. Soc.* **116**, 11610–11611 (1994).
40. C. R. Dawson and W. B. Tarpley, "On the Pathway of the Catechol-Tyrosinase Reaction," *Ann. New York Acad. Sci.* **100**, 937–948 (1963).
41. R. S. Phillips et al., "Oxygenation of Fluorinated Tyrosines by Mushroom Tyrosinase Releases Fluoride Iron," *Arch. Biochem. Biophys.* **276**, 65–69 (1990).
42. A. Nagai and H. Yamamoto, "Insolubilizing Studies by Water-Soluble Poly(LysTyr) by Tyrosinase," *Bull. Chem. Soc. Jpn.* **62**, 2410–2412 (1989).
43. J. K. Palmer, "Banana Polyphenoloxidase. Preparation and Properties," *Plant Physiol.* **38**, 508–513 (1963).
44 W. N. Vannesta and A. Zuberbuhler, "Copper-containing Oxygenases," in O. Hayaishi, ed., *Molecular Mechanisms of Oxygen Activation*, Academic Press, New York, 1974, pp. 371–404.
45. G. W Sanderson, H. Co, and J. G. Gonzalez, "Biochemistry of Tea Fermentation: The Role of Carotenes in Black Tea Aroma Formation," *J. Food Sci.* **36**, 231–236 (1971).
46. G. W. Sanderson and H. N. Graham, "On the Formation of Black Tea Aroma," *J. Agric. Food Chem.* **21**, 576–585 (1973).
47. R. Z. Kazandjian and A. M. Klibanov, "Regioselective Oxidation of Phenols Catalyzed by Polyphenol Oxidase in Chloroform," *J. Am. Chem. Soc* **107**, 5448–5450 (1985).
48. A. Zaks and A. M. Klibanov, "Enzymatic Catalysis in Nonaqueous Solvents," *J. Biol. Chem.* **263**, 3194–3201 (1988).
49. J. S. Deetz and J. D. Rozzell, "Enzyme-catalyzed Reaction in Non-Aqueous Media," *TIBTECH* **6**, 15–19 (1988).
50 M. Rohr, C. P. Kubicek, and J. Kominek, "Gluconic Acid," in H.-J. Rehm and G. Reed, eds., *Biotechnology*, Vol. 3, Verlag Chemie, Weinheim, Germany, 1983, pp. 455–465.
51. A. P. F. Turner, I. Karube, and G. S. Wilson, *Biosensors, Fundamentals and Applications*, Oxford University Press, Oxford, U.K. 1987.
52. F. Cioci and R. Lavecchia, "Effect of Polyols and Sugars on Heat-Induced Flavin Dissociation in Glucose Oxidase," *Biochem. Mol. Biol. Int.* **34**, 705–712 (1994).
53. D. W. S. Wong, *Food Enzymes. Structure and Mechanism*, Chapman & Hall, New York, 1995, pp 308–320.
54. M.-L. Liao and P. A. Seib, "Chemistry of L-Ascorbic Acid Related to Foods," *Food Chem.* **30**, 289–312 (1988).
55. A. E. Martell, "Chelates of Ascorbic Acids, Formation and Catalytic Properties," in P. A. Seib and B. M. Tolbert, eds., *Ascorbic Acid: Chemistry, Metabolism, and Uses*, American Chemical Society, Washington, D.C., 1982, pp. 153–178.
56. T. Kurata and M Fujimaki, "Formation of 3-Keto-4-Deoxypentosone and 3-Hydroxy-2-Pyrone by the Degradation of Dehydro-L-Ascorbic Acid," *Agric. Biol. Chem.* **40**, 1287–1291 (1976).
57. T. Hayashi, M. Namiki, and K. Tsuji, "Formation Mechanism of the Free Radical Products and Its Precursor by the Reaction of Dehydro-L-Ascorbic Acid with Amino Acid," *Agric. Biol. Chem.* **47**, 1955–1960 (1983)
58. T. Hayashi et al., "Red Pigment Formation by the Reaction of Oxidized Ascorbic Acid and Protein in a Food Model System of Low Moisture Content," *Agric. Food Chem.* **49**, 3139–3144 (1985).
59. E. H. Gruger, Jr., and A. L. Tappel, "Reactions of Biological Antioxidants: 1. Fe(III)—Catalyzed Reactions of Lipid Hydroperoxides with α-Tocopherol," *Lipids* **5**, 326–331 (1970).
60. G. W. Burton and K. U. Ingold, "Autoxidation of Biological Molecules. 1. The Antioxidant Activity of Vitamin E and Related Chain-Breaking Phenolic Antioxidants In Vitro," *J. Am. Chem. Soc.* **103**, 6472–6477 (1981).

DOMINIC W. S. WONG
USDA
Albany, California

OZONE AND FOOD PROCESSING

OZONE BACKGROUND

Mentioning ozone to most people in the 1990s evokes the thought of the ozone hole discovered by NASA's Landsat spacecraft over the South Pole or unhealthy air over some of our nation's cities. In both instances, the thoughts accompany a negative connotation of pollution by man-made chlorinated fluorocarbons destroying the ozone layer or a by-product of the photolysis of automobile exhaust. History has been much more favorable recognizing the positive attributes of ozone, and other countries have embraced the application of ozone for its environmentally safe biocidal and deodorizing properties for more than a century (1).

Ozone has been a natural part of our environment well before recorded history and indeed has enabled man to live today protected from dangerous ultraviolet rays from the sun. Ozone is created naturally by lightning and photochemically by ultraviolet (UV) light. Electrical discharge and UV light breaks the naturally occurring oxygen molecule, O_2, into two unstable atomic atoms, O, that can combine with other oxygen molecules and form ozone, O_3. Ozone produced by lightning is responsible for the fresh smell following a storm. If ozone were not formed in the upper atmosphere by O_2 absorption of the sun's UV rays, the radiation would be able to reach the surface of the earth where it can cause skin cancer in humans and other negative effects on plants and animals.

From the early writings of Homer, man has observed the smell that accompanied lightning as "full of sulphur" odor (2). In 1785 a Dutch physicist, van Marum (3), observed ozone's characteristic odor when electrical sparks were passed through air. From the electrolysis of water, another process to produce ozone, Cruickshank (3) observed in 1801 the same odor near the anode. However, it was Schöenbein who is credited with the discovery of ozone (4). In a memoir presented to the Academy of Munich in 1840, Schöenbein concluded that the odor during electrical discharge and electrolysis and after a flash of lightning were the same substance. He named the substance *ozone* after the Greek word *ozein* meaning "to smell."

Ozone exists naturally at ground level in low concentrations. It dissipates rapidly by releasing one of the three oxygen atoms and reverting to common molecular oxygen. Thus, ozone's unstable property necessitates that it be manufactured on-site where it will be used. Today, for most practical applications, electrical discharges, UV radiation, and electrolysis are used to produce ozone. Electrical discharges produce the highest ozone gas concentrations efficiently, up to 80 g O_3/kWh and up to 20% by weight in oxygen. UV radiation produces the lowest efficiencies, about 8 g O_3/kWh (5). Electrolysis can produce ozone directly in water, eliminating the need to dissolve the ozone gas water.

OZONE DISINFECTION PROPERTIES

Ozone is one of the strongest oxidizing reagents known. Its oxidation potential makes ozone effective as a biocide. Theories suggest that ozone attacks the lipid double bonds in the cell membrane and results in a change in cell permeability leading to lysis (6,7).

A demonstration of ozone's disinfection capability is its sterilization of medical instruments infected with the germicidal resistant microorganisms (8). Table 1 shows the spectrum of microorganisms encountered in the medical environment from the most resistant bacterial spores, *B. subtilis*, to the relatively sensitive lipid containing viruses, Herpes simplex 1. Ozone exposure conditions in the medical sterilizer were 12 to 14% ozone concentrations in oxygen, ambient temperature, and 85 to 95% relative humidity. Foodborne human pathogenic bacteria, molds, and viruses are represented by the spectrum of microorganisms listed in the table. In addition, ozone is effective in killing foodborne and waterborne parasites, such as *Giardia lamblia* and *Cyrptosporidum parvum*.

Table 1. Ozone is Effective in Killing Human Pathogens

Microorganism	20-min exposure	30-min exposure	90-min exposure
Bacillus subtilis	9/20[a]	16/20	0/20
Bacillus stearothermophilus	4/20	4/20	0/20
Clostridium sporogenes	3/20	1/20	0/20
Staphylococcus aureus	0/20	0/20	0/20
Salmonella choleraesuis	0/20	0/20	0/20
Pseudomonas aeruginosa	0/20	0/20	0/20
Mycobacterium phiel	0/20	0/20	0/20
Aspergillus niger	0/20	0/20	0/20
Polio 1	0/10	0/10	0/10
Herpes simplex 1	0/10	0/10	0/10

[a] The number of growers out of the number possible.

Reduction of the population of a specific microorganism by a given fraction depends uniquely on the product of the ozone concentration level, C, and exposure time, t. Different microorganisms' mortalities are easily compared by means the "$C \cdot t$" product for the same reductions (9). For example, the $C \cdot t$ for *B. subtilis* is much greater than for Herpes simplex 1 for the same population reduction. Furthermore, comparison of disinfection in air and water can be made on a media mass fraction basis, that is, normalization of $C \cdot t$ to the mass of the fluid (10). Using the ratio of the mass of water to air, for example, indicates that a $C \cdot t$ would have to be 500 times greater in air for the same disinfection capability in water. For disinfection applications to food compared to medical sterilization, not only are the specific microorganisms anticipated quite different, the reduction objectives are much lower. In food disinfection, the objective is to reduce the foodborne microorganism populations to level preventing sickness and not to achieve clinical sterilization or twice the $C \cdot t$ required to achieve a zero population.

Besides destroying microorganisms, like mycotoxin-producing molds, ozone has the potential to destroy the mycotoxin itself. For example, patulin found at high levels in some natural apple juice and produced by several *Penicillium* species can be oxidized and decomposed by ozone rendering it harmless. Research in the oxidation of herbicide and pesticide residue by ozone has also demonstrated promise (11).

GRAS APPROVAL OF OZONE

In July 1997, a panel of experts concluded that there is sufficient scientific evidence to certify that ozone and its use is Generally Recognized as Safe (GRAS) when used at levels and by methods consistent with good manufacturing practices (12). This announcement fulfilled the requirements of a U.S. Food and Drug Administration (FDA) process that permits the use of ozone in those products for which it has jurisdiction; namely, fruits, vegetables, and seafood. Since then the U.S. Department of Agriculture (USDA) has approved the use of ozone on a case-by-case basis with submittal of a description of the process using ozone for meat and poultry. While the number of ozone applications is increasing in the United States, ozone seems to be a solution looking for a problem. The status quo is the use of chlorine and chlorine dioxide in various forms and commercial sanitizers, such as quaternary ammonium. The main problem ozone has in gaining acceptance is a lack of a track record and the high up-front cost associated with the generating equipment. Fortunately, there is continuing improvement on ozone's track record and equipment cost.

FOOD PROCESSING

Ozone is an effective biocide in air and oxygen gas and in water for applications from food storage to food processing and from fruits and vegetables to meat, poultry and seafood. Table 2 summarizes effective points where ozone has been introduced in the food supply chain. Like chlorine, ozone in water is more effective in disinfecting the water and preventing contamination of uninfected product than disinfecting the products directly (13). Unlike chlorine, ozone decomposes to oxygen and simple by-products, leaving no residue or buildup of compounds that requires the water to be discharged. Furthermore, ozonized water will have lower biological and chemical oxygen demand and lower fats, oil, and grease characteristics, reducing the environmental burden.

From the field to the grocery store, ozone is playing a more significant role in providing safe and environmentally sound food supply system as part of producers' Hazard Analysis and Critical Control Point program (14). Ozone plays a role in disinfecting wash water for fruits and vegetables before packing and storage. Potable water is generally not available for field processing in some areas and countries. Ozone is capable of disinfecting the water without leaving a chemical residue on the product or contaminating water that will find its way into the environment.

Ozone concentrations of 0.5 to 15 ppm in air applied to food storage have the capability to extend shelf life by inhibiting growth to destroying molds, viruses, and bacteria on food surfaces. In food processing plants, ozone treatment of the product transport flumes reduces the bacterial content of the final product by controlling the bacteria levels and cross-contamination. Ozonized water in place of chemical sanitizers is used in clean-in-place systems to disinfect process lines in food, dairy, and other beverage processing lines.

Animal slaughter plants can use ozone to wash down carcasses on the kill floor prior to cooldown and storage (15). Ozonized water spray and ozone gas applied in beef storage facilities controls mold growth. In poultry processing plants, filtration and ozonation of poultry chill water returns the water quality to a state that it can replace potable processing water (16). Not only is the water saved, but the energy cost of lowering the temperature to near 32°F is saved as well. The fishing industry is using ozone to wash freshly caught fish and storing them on ozonized ice on board ship to extend the shelf life.

Table 2. Successful Points for Ozone Intervention in the Food Supply Chain

Food	Successful ozone applications
Fruits and vegetables	Storage, field processing, plant processing
Grains	Storage
Milk and dairy products	Clean-in place, storage
Meat, poultry, and seafood	Postslaughter, poultry chiller, storage
Beverages	Clean-in place

OZONE APPLICATIONS FOR FOOD STORAGE

Ozone has found extensive use in extending the storage times for fruits, vegetables, eggs, cheese, meats, poultry, and fish (17). Many European countries have developed the technology and applications. Research began in this application in the early part of this century, but today's technology is based on research since World War II. Ozone's application continues to grow as the costs of generators, sensors, and control systems have come down. Today in the United States, fruit and vegetable storage and ripening rooms and farm storage facilities are using ozone gas to control molds and bacteria as well as ripening.

Fruits

Ripening rooms first gained popularity in the storage and forced ripening of bananas. Today, ripening rooms maintain temperature within 1.5°C and relative humidity to 85 to 95% with computer-controlled systems (18). Ozone offers the potential to control fungal spoilage on fruits, control or destroy pathogens, destroy ethylene gas produced as the fruits ripen, and improve the overall hygiene of the ripening room and air-handling equipment. By reducing ethylene levels, ripening of the fruit can be delayed until the fruit is ready to be shipped to market; then, introducing ethylene gas in the room accelerates the ripening.

Vegetables

From the relatively small volumes of ripening rooms (180 m^3) to vegetable storage facilities (25,000 m^3), ozone controls fungal spoilage on vegetables, reduces product shrinkage, and improves product margins. Most of the beneficial evidence of ozone in vegetable storage is anecdotal and difficult to compare because of the differences in storage facilities and differences from season to season. The owner of an onion storage facility with a Cyclopss ozone system reports that while neighboring storage facilities have black mold, his facility is mold free.

OZONE APPLICATIONS FOR FOOD PROCESSING

There are several points in a food processing plant where ozone can be used effectively. Many processing plants use water as part of the product washing or transporting between process steps. Ozone can effectively replace chlorine disinfectants by dissolving ozone gas in the water and controlling levels by dissolved ozone sensors or standard oxidation-reduction potential (ORP) sensors used to monitor chlorine levels. Ozone can be used to disinfect the water subsequently used in the finished product, as in the beverage industry. The FDA gave GRAS approval for bottled water in 1991 and today is used in over 95% of bottle water. Products that are processed dry can be treated with ozone

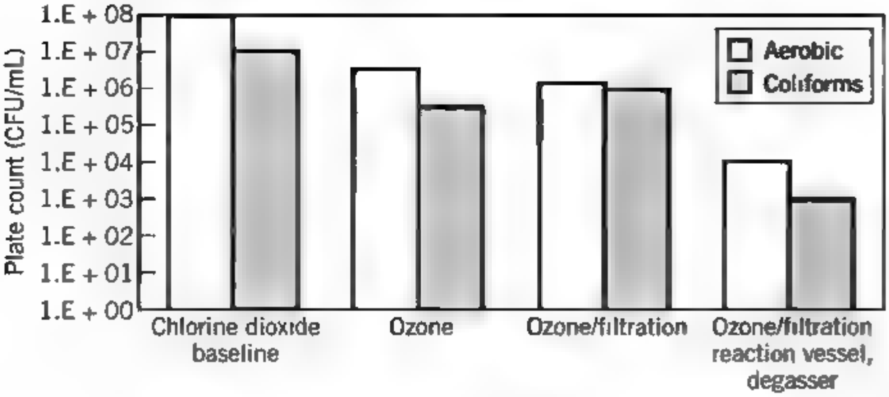


Figure 1. A comparison of ozone with chlorine dioxide shows ozone's disinfection capabilities with various application methods.

gas. For example, the dietary supplement ginger that has been partially dehydrated can be treated in ozone at 8% for 30 min to realize a 4-log reduction in total aerobic plate counts.

Vegetables

Ozone used in flume water for an onion processing line produced a 3.8-log reduction of total aerobic and coliform plate counts over chlorine dioxide. Ozone was used in the flume water between a peeler and a slicer on an onion line that processed an average of 6,000 kg/h. Figure 1 shows the comparison of chlorine dioxide and ozone with various water treatment steps. The first pair of columns represents the total aerobic and coliform plate counts with chlorine dioxide. The next three sets of columns shows ozone alone, ozone with a 100-μm filter prior to ozone injection, and ozone with the 100-μm filter, a 450-L reaction vessel, and a centrifugal gas separator. The quantity of ozone was adjusted to maintain ozone off-gassing below 0.05 ppm at the open water tanks for personnel safety. The amount of ozone used for each of the three conditions was 550, 330, and 500 g/h.

Figure 1 illustrates the effect of the organic load on the consumption of ozone. Comparison of ozone alone with ozone and filtration reduces the ozone required by over 20% for the same disinfection. To achieve the most efficient use of ozone for disinfection, filtration is required to reduce the organic load. Finally, one can also observed the benefit of increasing the contact time of the ozone with the flume water by the last pair of columns in the figure, where the plate counts were at their lowest levels. Similar results were obtained for potato processing lines where, again, ozone was used to replace chlorine dioxide. In the latter case, the organic load was from potato pulp, gratings, proteins, and starch content (as high as 15% by volume) in the process water. In both examples, ozone and filtration treatments lead to the extended reuse of the process water.

RECOMMENDATIONS

There are many successful applications of ozone to food processing as well as many disappointments. A user selecting knowledgeable ozone engineering companies is the main characteristic of the successes. Ozone engineering includes demonstrating the understanding of the $C \cdot t$ product to achieve a given objective, ozone safety, equipment specification, ozone compatible materials selection, installation, test, and support service. Ozone has great potential to address many of today's pressing food safety issues. Applications require significant investment in capital equipment up-front as opposed to pay as you go with chemicals. Be sure the application is done right by selecting a competent ozone engineering company so you will reap the rewards of a successful application.

BIBLIOGRAPHY

1. R. G. Rice and Aharon Netzer, eds., *Handbook of Ozone Technology and Applications*, Vol. 1, Ann Arbor Science Publishers, Ann Arbor, Mich., 1982.
2. Homer, *The Odyssey*, translated by W. H. D. Rouse, The New American Library, New York, 1937, p. 146.
3. E. K. Rideal, *Ozone*, Constable & Co., Ltd., London, 1920.
4. C. F. Schöenbein, "Recherches sur la Nature de l'Odeur Qui se Manifests dans Certaines Actions Chimiques," *Compte Rendus Hebd. Seances Acad. Sci.* **10**, 706–710 (1840).
5. R. Barker, *Improving the Efficiency of Ozone in Odor Treatment by Activation with UV Light*, Electricity Council Research Centre, Capenhurst, Chester, UK, 1979.
6. S. Farooq, E. S. K. Chian, and R. S. Engelbrecht, "Basic Concepts in Disinfection with Ozone," *J. Water Pollution Control Fed.* **49**, 1818–1831 (1977).
7. W. Riesser et al., "Possible Mechanisms of Poliovirus Inactivation by Ozone," in R. G. Rice and M. E. Browning, eds., *Forum on Ozone Disinfection*, International Ozone Association, Norwalk, Conn., 1977, pp. 186–192.
8. 510(k) Filing for Ster-O-Zone® Medical Sterilizer to the U.S. FDA, Cyclopps Corporation, Salt Lake City, Utah, January 1995.
9. G. B. Wickramanayake and Otis J. Sproul, "Kinetics of the Inactivation of Microorganisms," in S. S. Block, ed., *Disinfection, Sterilization, and Preservation*, 4th ed., Lea & Febiger, Malvern, Pa., 1991, pp. 72–84.
10. W. J. Kowalski, W. P. Bahnfleth, and T. S. Whittam, "Bactericidal Effects of High Airborne Ozone Concentrations on

Escherichia coli and *Staphylococcus aureus*," *Ozone Sci. and Eng.* **20**, 205–221 (1998).

11. G Crozes et al., "Evaluation of Ozone for Cryptosporidium Inactivation and Atrazine Oxidation in a Lime Softening Plant," *Ozone Sci. and Eng* **20**, 177–190 (1998).
12. Electric Power Research Institute, *Expert Panel Report Evaluation of the History and Safety of Ozone in Processing Food for Human Consumption*, Electrical Power Research Institute, Pleasant Hill, Calif., 1997.
13. R. E. Brackett, "Fruits Vegetables and Grains," in M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds., *Food Microbiology*, ASM Press, Washington, D.C., 1997.
14. U.S. Department of Health and Human Services, *1997 Food Code, Annex 5, HACCP Guidelines*, Government Printing Office, Washington, D C., 1997.
15. B. M. Gorman et al., "Evaluation of Hand-Trimming, Various Sanitizing Agents, and Hot Water Spray-Washing as Decontamination Interventions for Beef Brisket Adipose Tissue," *J. Food Protection* **58**, 899–907 (1995).
16. A. L. Waldroup, R. E. Hierholzer, and R. H. Forsythe, "Recycling of Poultry Chill Water Using Ozone," *J. Appl. Poultry Sci.* **2**, 330–336 (1993).
17. R. G. Rice, J. W Farquhar, and L. J. Bollyky, "Review of the Applications of Ozone for Increasing Storage Times of Perishable Foods," *Ozone Sci. and Eng* **4**, 147–163 (1982).
18. *Banana Handling Manual*, Dole Food Company, Inc., 1996.

DURAND SMITH
WILLIAM STODDARD
Cyclopss Corporation
Salt Lake City, Utah

PACKAGING: PART I—GENERAL CONSIDERATIONS

MARKETPLACE DEMANDS AND DRIVING FORCES

Packaging is critical in providing products that meet the consumer needs in a society. Packaging is even more critical in providing food products because they are more perishable and fundamental to the health and progress of the society.

Many driving forces result in increased marketplace demands for foods that offer quality, convenience, safety, and low cost. Some of these driving forces are lifestyle changes, demographic changes, and market globalization. The most noticeable lifestyle changes are influenced by smaller families, the increasing number of single-person households, and dual-income families. The major demographic change was the population spike that occurred after World War II ("Baby Boomers"), which resulted in an increased number of older consumers who demand more nutritious and healthier foods that are easy to prepare. Market globalization has resulted in increased importing and exporting of foods to and from many different and distant regions, creating strong consumer demands for regulations that ensure food safety and better product labeling. Some other driving forces are the macroeconomies in Europe and North America, the continued consolidation in industry, and the public concern over packaging disposal problems.

To meet the challenges presented by these driving forces, the packaging industry has made numerous technical innovations. Many innovations have come from the area of plastics packaging, because plastics are cost effective, lightweight, nonbreakable, heat sealable, microwavable, easily fabricated, and corrosion resistant. For example, significant advancements have been made in coextrusion, lamination, and coating technologies, which enable the design of plastic packaging materials with a wide range of properties (1). Another innovation is active packaging, including the technologies of modified atmosphere packaging for fresh produce, oxygen scavengers, time–temperature integrators, and antimicrobial films (2).

New concepts in food packaging technology are needed to meet the challenges of the Information Age. One of the concepts emerging is a greater emphasis on a systems approach in the delivery of better product value; one that addresses the integration of the product, the package, and the environment as a complete resource cycle, from raw material through use and recovery. Another concept is the use of information technology to enhance the communication role of packaging, allowing packaging to serve as a more intelligent messenger for information sharing in this resource cycle.

FUNCTIONS OF A FOOD PACKAGING SYSTEM

A major goal of food packaging is in the efficient delivery of products to the consumer. To accomplish this goal, the package must serve the important functions of containing the food, protecting the food, providing convenience, and conveying product information. The package protects the food against physical, chemical, and biological damages. It also acts as a physical barrier to oxygen, moisture, volatile chemical compounds, and microorganisms, which are detrimental to the food. The package provides the consumer with convenient features such as microwavability, resealability, and ease of use. The package conveys useful information such as product contents, nutritional values, and preparation instructions. These functions make food packaging an essential technology for maintaining the original food quality, with fewer chemical additives and less food waste.

The food package can function best when integrated into a food packaging system, which involves certain physical components and operations. The major physical components are the food, the package, and the environment (Fig. 1). It is useful to divide the environment into internal and external—the internal environment (referred to hereafter as the headspace) is inside the package and is in direct contact with the food, and the external environment is outside the package and depends on the storage and distribution conditions. The operations are the manufacturing, distribution, and disposal of the food package. In designing the food packaging system, these physical components and operations must be considered to prevent overpackaging and underpackaging, which result in higher costs, lower quality, and, in some cases, health risks.

GAS BARRIER PROTECTION AND SHELF LIFE

For foods that are sensitive to oxygen or moisture, gas barrier protection is the major function of the package in providing adequate shelf life, the time period during which the food maintains acceptable quality. For example, moisture can move from the external environment through the package into the headspace. This increase in relative humidity in the headspace can cause a moisture-sensitive food, such as potato chips, to have less crispness and a shorter shelf life. To ensure adequate shelf life, the package must help to reduce the moisture movement from the external environment to the headspace.

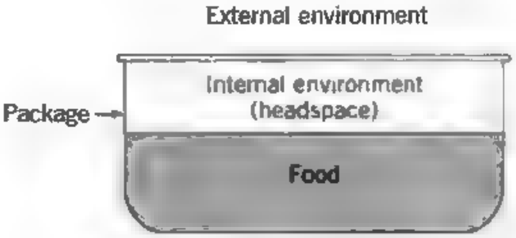


Figure 1. Physical components of packaging system.

Movement of gases between the external environment and the headspace through the package can occur by means of permeation and leakage. Gas permeation is an important consideration in packaging foods with plastics, because food packaging plastics are permeable to moisture, oxygen, carbon dioxide, nitrogen, and other gases. The gas-permeation rates of most interest are the oxygen transmission rate (OTR) and water vapor transmission (WVTR). Gas permeation is not an important consideration in packaging foods with metal or glass because these materials are not permeable.

Leakage can occur in packages made of plastics, metal, glass, or paper. Pinholes are sometimes found in thin metal foils and metallized films. Channel leaks are most commonly found in weak seals or contaminated seals. Pinholes and channel leaks can shorten the shelf life of a packaged food and, in some cases, allow microbial penetration, leading to a potential health risk

Shelf life is best determined by actual field studies, although accelerated laboratory studies and computer simulations can sometimes provide useful shelf life predictions (1,3). The variables that affect the shelf life of packaged food are discussed next.

Shelf Life of Oxygen-Sensitive Foods

Equation 3 predicts the shelf life of packaged food that is oxygen sensitive. The equation assumes the package is made of a plastic material with uniform thickness and has complete integrity (no pinholes and channel leaks); hence, permeation is the only means of oxygen transfer. The equation can assist in understanding the relationship between the shelf life and the physical components of the food packaging system—the food, package, headspace, and external environment. Two useful terms, permeability ($\bar{P}$) and maximum allowable oxygen ($O_{2\max}$), are introduced.

The OTR of a permeable package (eg, a plastic pouch) from the external environment to the headspace can be calculated using

$$\mathrm{OTR} = \frac{\bar{P}A}{L}(P_e - P_i) \tag{1}$$

where OTR is the oxygen transmission rate, cc O_2/day; A is the surface area of package, in.^{2}; L is the thickness of package, mil; $\bar{P}$ is the oxygen permeability, (cc $O_2 \cdot$ mil)/(100 in.$^2 \cdot$ day $\cdot$ atm); P_e is the partial oxygen pressure in external environment, atm; and P_i is the partial oxygen pressure in headspace, atm.

The shelf life (t_s) can be estimated using

$$t_s = \frac{O_{2\max}}{\mathrm{OTR}} \tag{2}$$

where t_s is the shelf life, day and $O_{2\max}$ is the maximum allowable oxygen, cc O_2. Equation 2 assumes that O_2 permeation through the package is the major factor in limiting shelf life. This is the case for food packages of good oxygen barrier, in which the oxidative reaction rate of the food is greater than the oxygen transmission rate of the package.

Substituting equation 1 into equation 2 yields

$$t_s = \frac{O_{2\max}L}{\bar{P}A(P_e - P_i)} \tag{3}$$

This equation is useful for evaluating many what-if scenarios. For example, if the thickness is decreased by 25% and the surface area is increased by 20%, then the equation predicts that the shelf life will be decreased by 37.5%. Equation 3 also shows that shelf life depends on the variables determined by the food, the package, and the environment.

The variable determined by the food is $O_{2\max}$. Oxidation (especially lipid oxidation) is an important mode of deterioration for many foods (4). The incentive for studying oxidation is that it provides an objective measure of the food stability in addition to the subjective measure of sensory evaluation. The sensory acceptability of an oxygen-sensitive food can then be correlated to the extent of oxidation reaction. However, measuring the extent of oxidation reaction requires a great effort because it depends on the concentration and diffusion of oxygen in the food.

$O_{2\max}$ is sometimes used as an alternative measure of oxygen stability by the food packaging industry. It is the maximum amount of additional oxygen the food can absorb before becoming unacceptable. It is a simpler but less accurate measure of oxygen stability than the extent of oxidation reaction because $O_{2\max}$ usually ignores the effects of concentration and diffusion of oxygen in the food. Reference values for $O_{2\max}$ are available in the literature for some foods (3); for example, the $O_{2\max}$ for instant coffee is reportedly between 1 and 5 ppm. If the upper limit of 5 ppm is assumed, $O_{2\max}$ for a package containing 454 g of instant coffee = 454 (5×10^{-6}) = 2.37×10^{-3} g. Nevertheless, it is often necessary to determine $O_{2\max}$ experimentally, which involves correlating the food quality with the amount of oxygen absorbed in the food. $O_{2\max}$ is the difference between the critical oxygen level (at which the food is no longer acceptable) and the initial oxygen level (which depends on the initial condition of the food).

The variables determined by the package are L, A, and $\bar{P}$. L and A are specified by the dimensions of the package. The permeability $\bar{P}$ is a measure of the permeation of the gas through the packaging material—the lower the $\bar{P}$, the better the gas barrier. The values of $\bar{P}$ depend on the packaging material, permeant gas, temperature, and sometimes relative humidity. For example, the O_2 permeability of polyethylene terephthalate (PET) at 23°C is between 3 and 6 (cc $\cdot$ mil)/(100 in.$^2 \cdot$ day $\cdot$ atm). The relative humidity is not specified because it has little effect on the permeability of PET. $\bar{P}$ and gas permeation are further discussed in PACKAGING: PART III—MATERIALS.

The variables determined by the environment are P_e and P_i. P_e is the partial O_2 pressure in the external environment, which is about 0.21 atm for a normal storage environment. P_i is the partial O_2 pressure in the headspace, which is sometimes deliberately reduced using the techniques of vacuum packaging or modified atmosphere packaging for shelf life extension. (In vacuum packaging, the air in the headspace is removed before sealing. In modified atmosphere packaging, the air in the headspace is replaced by an inert gas such as nitrogen before sealing.) An important environmental variable not explicitly stated in equation 3 is temperature. An increase in temperature causes increases in the oxidation rate of the food and the package permeability.

The term $P_e - P_i$ in equation 3 is often called the concentration driving force—the larger the driving force, the higher the permeation rate. If the food is packaged in air, the external environment and the headspace have the same oxygen concentration, the driving force is $P_e - P_i = 0$, and there is no net flow of oxygen. If the food is packaged in a reduced oxygen environment, the driving force is $P_e - P_i > 0$. This causes a net flow of oxygen into the package, but at a slower rate as time passes, because $P_e - P_i$ continues to decrease.

Shelf Life of Moisture-Sensitive Packaged Foods

A more elaborate approach to predict the shelf life of moisture-sensitive packaged foods is described elsewhere (5). The approach requires the definition of critical limits of a_w, above or below which (depending on whether the food gains or loses moisture) the food is no longer considered acceptable. The moisture gain or loss through the package depends on the H_2O permeability and the temperature as well as the relative humidities in the headspace and the external environment.

Some foods are both oxygen and moisture sensitive. For example, potato chips can lose crispness because of moisture gain and become rancid because of oxygen absorption. In packaging these foods, oxygen and moisture protection are required. In packaging foods that are less sensitive to oxygen or moisture, physical protection is more important than gas barrier protection.

PHYSICAL PROTECTION

During distribution, the packages are exposed to physical abuses caused by shock, vibration, compression, or handling. The food packaging system must provide physical protection for the food package as well as facilitate safe and cost-effective product distribution. The important considerations in designing for distribution protection are the distribution environment and product fragility.

The distribution environment consists of all the events (including handling, storage, and transportation) that the food package encounters before consumer usage. The most important considerations in defining the distribution environment are shock, vibration, and compression (6). Severe shocks occur most likely when the package is dropped. Shock protection is often defined in terms of the most severe drop height to protect against, and this drop height is often selected based on the size and weight of the package as well as the probability of being dropped. Vibration occurs most likely during transportation, and the transportation vibration environment is often complex and random in nature. To protect the package from vibrational damage, the resonant frequencies of the package must be identified and protected against. Compression occurs most likely during warehousing and shipping. Static compression is determined by applying a load very slowly, and dynamic compression is determined by applying the load rapidly.

Product fragility describes the susceptibility of the product (the food and the package) to physical abuses, and it can be evaluated by product performance tests and packaging material tests (7). Product performance tests (such as drop test, incline impact test, vibration test, compression test, and burst test) are performed with the final package to ensure it can survive during distribution. The final package may be a food tray or a corrugated box containing several food trays. Packaging material tests (such as tensile test, Izod impact test, and creep test) are performed with the packaging materials. The properties of the packaging materials (such as tensile strength and tensile modulus) obtained from these tests are used to extrapolate the performance of the final package.

In addition to distribution, the manufacturing operations (such as forming, heat sealing, and retorting) can also cause physical stresses to the food package. During forming, the packaging material may be stretched into another shape (such as from a plastic sheet into a multicompartment tray). During heat sealing, the package is exposed to the heat of the sealing bar. During retorting of the packaged food product, the package is exposed to high temperature and pressure. These physical stresses may cause the package to break, deform, or develop leaks.

FOOD–PACKAGE INTERACTIONS

Food–package interactions are chemical and physical interactions that occur between the food and the package. Corrosion of a metal food can is an example of a chemical interaction between the food and the inner surface of the can. Mass transport between the food and the package is an example of a physical interaction—volatile compounds can move from the package into the food, a phenomenon called migration; conversely, volatile compounds or flavor from the food can be absorbed by the package, a phenomenon called scalping.

The first phenomenon, migration of volatile compounds into the food, is a greater concern because of the potential health hazard resulting from exposure to toxic migrants. The volatile compounds may arise from many sources, such as residual monomers and oligomers in the plastic packaging materials that cannot be easily eliminated during the polymerization process; residual processing aids and additives (lubricants, plasticizers, slip agents, antioxidants, light stabilizers, antistatic agents, etc.); and residual printing ink solvents used on the package. These volatile compounds may cause the food to acquire undesirable flavors or, in some cases, toxic components.

The migration of volatile compounds from microwave susceptors has received considerable attention in the past decade (8). A microwave susceptor is a metallized plastic or paper laminate that can convert the microwave energy into heat to rapidly achieve a high surface temperature (>400°F). It allows the food in contact with the hot surface to brown and crisp. The high temperature can cause degradation of the plastic layer in the microwave susceptor and result in migration of undesirable volatile compounds into the food.

The second phenomenon, scalping, may lead to diminished quality of the food. Flavor scalping is a major concern for aseptic fruit juice packages (9). Research has shown that absorption of flavor compounds may also adversely affect the barrier characteristics of the packaging material.

PACKAGING AND WASTE DISPOSAL

The disposal of postconsumer packaging has been a significant issue in the past two decades. The public concern about the disposal of food packaging heightened with the introduction of PET plastic beverage bottles because of their high visibility. The concern broadened to include the nonplastics, postconsumer packaging materials, and other materials destined for disposal through the traditional channels of landfill or incineration. There were numerous efforts by public and private groups to establish regulations for the control of packaging by such means as deposit laws, mandatory recycling, bans, and taxation.

The federal government and various industrial groups began to develop a more rational strategy to address the packaging disposal concerns. The Environmental Protection Agency (EPA) recommended a plan of integrated solid waste management, which consists of a prioritized strategy: source reduction, recycling, incineration, and landfill. The strategy provides useful guidelines for designing the food package.

REGULATIONS

Nearly every package or label is subject to some type of legislation that affects packing, shipping, selling, advertising, grading, standardizing, or marking. The regulations and compliance problems have been growing in number and complexity. Therefore, one should seek guidance of a law counsel when information on specific legal aspects of packaging is needed.

In general, the packaging-related regulations fall into three broad categories: weight and measure, adulteration, and public safety. Regulations related to weight and measure are designed to ensure that the consumer is not misled or deceived by the printing or appearance of the package. Regulations related to adulteration deal primarily with the wholesomeness of the product. These regulations prevent the use of any packaging material that might seriously affect the product by direct or indirect addition of foreign components. Regulations related to public safety, such as tamper-resistant packaging, also exist.

OTHER CONSIDERATIONS

In developing a food package, it is necessary to select the packaging machinery that is most suitable for the food product. The selection depends on the nature of the food product to be handled (size, form, flow behavior, moisture content, or fragility). The selection also depends on the speed and versatility of the packaging machinery. Speed is essential for high-volume production, and versatility is essential for production operations that require frequent changes in the package size or product.

In most cases, economic considerations determine the success or failure of the food package. The total cost is derived from material, processing, distribution, waste disposal, promotion, and research and development. Marketing research is needed to identify product life cycle, establish pricing and distribution policy, and project the potential market volume of new products. Good packaging graphics are also needed to attract customers, communicative value, and create the desire to purchase.

INFORMATION TECHNOLOGY AND PACKAGING

In the future, the integration of information technology and packaging technology is expected to deliver greater product value by providing more efficient and reliable decision making (10). Microwave reheating or cooking of prepared food has certain technical limitations that can be overcome by enabling the food, the package, and the microwave oven to share vital information. In the application shown in Figure 2, the package carries the vital informa-

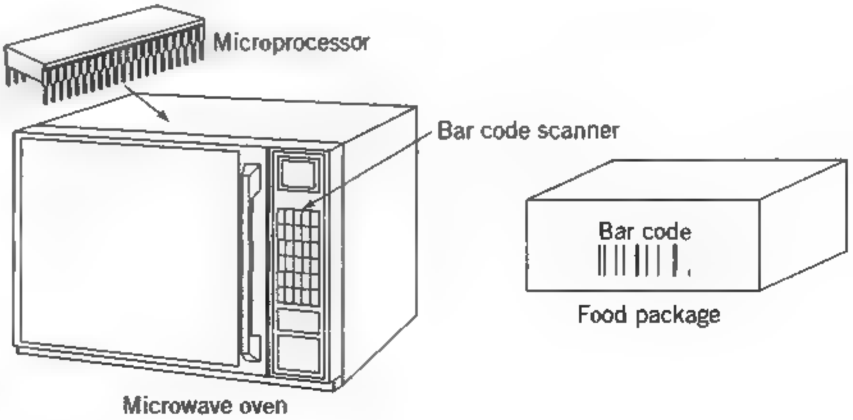


Figure 2. An application of information technology to packaging.

tion about the food and the package in a printed bar code, and the microwave oven is equipped with a bar code scanner and a microprocessor. The microprocessor, which is linked to the scanner, contains information about the microwave oven characteristics and also contains the logic to process the data from the scanner. Information sharing takes place when the bar code is scanned, and then the scanner transmits the information to the microprocessor, which in turn controls the magnetron and turntable (if applicable) in the microwave oven.

This application offers the advantages of convenience, accuracy, speed, and quality. The consumer has the convenience of automatic data entry that provides greater accuracy and speed, and it is particularly helpful with instructions involving multiple sequence cooking. The microprocessor allows the use of more sophisticated programming instructions to control the power and time of the microwave oven, which can improve the quality of the prepared food. The microprocessor can also be programmed to meet the food preferences of individual consumers.

The integrated technology can also help to facilitate handling, improve traceability, ensure safety, and reduce product loss. For example, other information devices can be incorporated into the package to enable the package to gather and share information on the time–temperature history, distribution pathway, and the quality or safety index of the product.

BIBLIOGRAPHY

1. W. E. Brown, *Plastics in Food Packaging*, Marcel Dekker, New York, 1992.
2. M. L. Rooney, *Active Food Packaging*, Blackie Academic & Professional, New York, 1995.
3. G. L. Robertson, *Food Packaging: Principles and Practice*, Marcel Dekker, New York, 1993.
4. O. R. Fennema, *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996.
5. T. P. Labuza, "Moisture Gain and Loss in Packaged Foods," *Food Technol.* **36**, 92–97 (1982).
6. W. Soroka, *Fundamentals of Packaging Technology*, Institute of Packaging Professionals, Herndon, Va., 1995.
7. A. L. Brody and K. S. Marsh, eds., *Encyclopedia of Packaging Technology*, John Wiley & Sons, New York, 1997.
8. S. J. Risch and J. H Hotchkiss, *Food and Packaging Interactions II*, American Chemical Society, Washington, D.C., 1991.
9. J. H. Hotchkiss, *Food and Packaging Interactions I*, American Chemical Society, Washington, DC, 1988.
10. K. L. Yam and R. G. Saba, "Intelligent Product Delivery: A Paradigm Shift in Packaging," *J. Technol. Eng.* **7**, 22–26 (1998)

KIT L. YAM
RAYMOND G. SABA
Y. C. HO
Rutgers University
New Brunswick, New Jersey

PACKAGING: PART II—LABELING

Regulation of food labeling in the United States falls mainly under the jurisdiction of two federal agencies: the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA). USDA's Food Safety and Inspection Service (FSIS) oversees food labeling of products containing meat or poultry. All other food products, including certain products containing only small amounts of meat or poultry and certain products (eg, traditional sandwiches) not associated by consumers with the meat and poultry industry, fall under the jurisdiction of FDA's Center for Food Safety and Applied Nutrition (CFSAN). Although the two regulatory agencies approach many aspects of food labeling in a similar manner, occasionally they differ.

Other agencies may also have requirements that affect food labeling. For example, the U.S. Bureau of Alcohol, Tobacco and Firearms of the Treasury Department regulates labeling of alcoholic beverages. The Agricultural Marketing Service (AMS) within USDA administers grading of agricultural products.

KEY DIFFERENCES BETWEEN FDA AND USDA

FDA regulations are governed by the Federal Food, Drug, and Cosmetic Act and the Fair Packaging and Labeling Act (FPLA). (The Nutrition Labeling and Education Act of 1990 [NLEA] is an amendment to the Food, Drug and Cosmetic Act.) The Federal Meat Inspection and Poultry Products Inspection Acts define USDA's regulatory role and responsibilities. Because the two agencies are governed by separate laws, they have different missions, philosophies, and approaches to food labeling, resulting in subtle differences throughout the regulations. USDA plays a more "hands-on" role (ie, through plant inspections and label preapproval) in monitoring food production and packaging practices (Table 1).

Mission and Approach

FDA's regulatory history began with the passage in 1906 of the Pure Food and Drugs Acts, which instituted federal oversight of food labeling. The 1906 act was replaced in 1938 by the current law. In 1973, FDA initiated a voluntary nutrition-labeling program that represented a regulatory turning point—the inclusion of meaningful health information on food labels.

Under the Federal Meat Inspection and Poultry Products Inspection Acts, USDA's primary regulatory role has been to prevent public health hazards resulting from improper handling of meat and poultry during production and packaging. USDA has an extensive network of field offices responsible for conducting plant inspections, and the national office administers a label preapproval program.

Regulatory Documents and Guidance

Both FDA and USDA release regulatory changes in the *Federal Register* (*FR*), published daily. Each year all fed-

Table 1. Key Differences Between FDA and USDA

FDA	USDA
Mission and approach	
Early emphasis on truth in labeling	Emphasis on prevention of public health hazards from improper handling
Broader span of regulatory oversight, limited resources	Extensive network of field offices available for consulting and monitoring
Regulatory documents and guidance	
Federal Register	*Federal Register*
Title 21 of the *Code of Federal Regulations*	Title 9 of the *Code of Federal Regulations*
Compliance policy guides	Policy manual and policy memos
Label preapproval	
No label preview or approval	Label designs reviewed and approved
Mistakes identified through market surveys, and warning letters issued	Plant inspections monitor application of approved labels
Nutrition labeling	
Mandated by NLEA	Exempted from NLEA
Spearheaded nutrition-labeling changes	Followed FDA regulations in general
Food categories regulated	
All packaged foods containing less than 2% cooked meat or poultry, or 3% or less raw meat or poultry Traditional sandwiches Pizzas not containing meat or poultry Dairy products Eggs Fish and seafood Fruits Vegetables Soups not containing meat or poultry Salads not containing meat or poultry Nuts and legumes	Food containing 2% or more cooked meat or poultry, or more than 3% raw meat or poultry Fresh meats Fresh poultry Processed meats and poultry Soups with meat or poultry Salads with meat or poultry Pizzas with meat or poultry Meat or poultry snacks Mixed dishes containing meat or poultry (eg, chili, lasagna, TV dinners, stews)

eral regulations are updated and compiled in the *Code of Federal Regulations* (*CFR*); FDA labeling regulations appear in Title 21 and USDA regulations in Title 9 of the *CFR*. Some USDA regulations cross-reference FDA regulations. FDA has issued Compliance Policy Guides, which reflect the agency's interpretations of the regulations and policies for enforcement. USDA publishes a standards and labeling policy manual and periodic labeling policy memos, which address similar issues. Both agencies provide consultation; however, manufacturers of USDA products obtain far more agency interaction and guidance through the label preapproval program and plant inspections.

Label Preapproval

USDA administers the label preapproval program in which food label designs traditionally have been reviewed and approved by the agency before they are printed. If manufacturers make mistakes, USDA tells them what is wrong so that manufacturers are able to learn the regulations and rely on the agency's guidance. Plant inspectors examine labels to make sure they correspond to the approved design.

FDA does not preview labels and offers no approval process. It places the responsibility on the manufacturer to understand the regulations and correctly apply the rules. The agency typically does not find mistakes until labels are printed and products are in the marketplace.

Nutrition Labeling

In 1990, Congress passed the NLEA, which made nutrition labeling mandatory on most packaged food products and mandated that FDA initiate extensive changes in the content and format of the nutrition label.

Nutrition labeling of USDA products was not included in the congressional mandate, but USDA decided to follow FDA to prevent consumer confusion arising from two different nutrition labels. In most significant aspects of the nutrition-labeling regulations, the two agencies have the same requirements; however, there are some differences.

USDA Special Requirements

USDA product labels are subject to additional requirements, some of which also pertain to select FDA products.

Handling Instructions. At times, FDA food products are required to carry warning statements or handling instructions. USDA product labels usually carry special instructions to ensure the safety and quality of the product (eg, keep refrigerated, keep frozen). In addition, raw meat and poultry products must carry safe-handling instructions with specified statements and graphics.

Inspection legends. All USDA products also must bear an official inspection legend in accordance with established inspection procedures.

Voluntary Grading Services

Voluntary grading services are available for certain products under both FDA and USDA jurisdiction. USDA's Agricultural Marketing Service (AMS) performs grading services for certain products (eg, dairy, egg, fruit, vegetable, meat, poultry) subject to FDA or USDA regulation. To use approved grade designations (eg, U.S. Grade A, U.S. No. 1, U.S.D.A. Prime, U.S. Fancy), a product must have been inspected and determined to comply with grade standards established by AMS.

Principal Display Panel

A principal display panel (PDP) must be included on all food sold in packaged form. The PDP is the part of the label that is most likely to be displayed for retail sale. The product identity statement and net quantity of contents declaration must be included on the PDP At times, other required statements must also be displayed on the PDP.

Product Identity Statement. The product identity statement is intended to communicate important information to the consumer about the type and form of food contained in the product package. It should be incorporated into the PDP with sufficient prominence that the consumer can readily view it on a retail shelf. The terminology, as well as the placement and type size, of the identity statement are dictated by the regulations.

Net Quantity of Contents. The purpose of the net quantity of contents declaration is to tell the consumer the amount of product the package contains.

Information Panel

The information panel typically is contiguous to and immediately to the right of the PDP as viewed by the consumer. It includes detailed information about a food product that helps the consumer make knowledgeable purchasing decisions. The mandatory information required on the information panel consists of (*1*) the ingredient list, (*2*) the nutrition facts, and (*3*) the manufacturer identity statement. At times, other information may be required, such as warning statements, a percent-juice declaration, and claims-related statements. Mandatory information may be displayed on the PDP instead of on the information panel.

Ingredient List. A list of the ingredients of a multi-ingredient product must be included on the food label, typically on the information panel.

Nutrition Facts Information. With the introduction of the NLEA, the format of nutrition information has become much more strictly defined. A number of nutrition facts layouts are available, and specific criteria dictate their use.

Manufacturer Identity Statement. The manufacturer identity statement tells the consumer the name and place of business of the manufacturer, packer, or distributor. This information usually is placed on the information panel or on the PDP if there is no information panel.

Other Information. At times, additional statements may be required on the information panel, the PDP, or both. For example, if a PDP bears a comparative claim, such as "light" or "reduced," quantitative information comparing relevant nutrients in the product to those in a reference food must be included on the information panel or the PDP. Some products may require a warning statement, percent-juice declaration, or special handling instructions.

Exemptions

The regulations apply to retail primary packages, and so certain containers and wrappings are exempt from the labeling requirements. The regulations define the term "package" as any container or wrapping in which a food is enclosed for retail sale. Packages used for shipping, display, or other functions are exempt. In addition, some very small packages are exempt.

Inner Wrappings. When multiunit packages of food are not intended to be sold individually, their inner wrappings that bear no information are exempt from labeling requirements.

Shipping Cartons. Shipping cases for distribution of commodities in bulk are exempt from labeling requirements. If the shipping cartons will be sold as retail units (eg, in a warehouse club), however, all mandatory requirements apply.

Small Confections. Small confections (eg, "penny candy") weighing less than 1/2 oz (or 15 g) are exempt from all labeling requirements when the shipping container or retail package meets the labeling requirements.

PRINCIPAL DISPLAY PANEL

All retail food packages must bear a principal display panel (PDP) that includes the following mandatory label information: a product identity statement and a declaration of net quantity of contents. If there is no information panel, an ingredient list, a nutrition facts panel, and a manufacturer identity statement must also appear on the PDP. On some products, additional information may be required, such as claims-related information, a warning statement, or special handling instructions.

Location

The PDP is considered the area of the package that is most likely to be displayed, presented, or examined by the consumer at the point of sale. The PDP may be a spot label on a package surface, or it may be the whole surface. On jars and bottles, a spot label on the lid may be used for the PDP. A header strip attached across the top of a transparent or opaque pouch that contains no other printed or graphic material may serve as the PDP.

It is acceptable to include more than one PDP on a package design, but when this approach is used, all of the mandatory information must be duplicated in full.

Size

The PDP must be large enough to accommodate all mandatory label information with clarity and conspicuousness, and without obscuring or crowding by designs or vignettes. The size of the PDP determines the minimum letter height for some of the mandatory information contained in the

PDP. The area of the PDP is the same in similar-size packages, regardless of the size of the label. It is not the size of the label but the size of the container that determines the area of the PDP. For example, if a product carries a spot label, it is not the size of the label that determines the area of the PDP, but rather the area of the surface displayed to the consumer.

Rectangular Packages. For rectangular packages, one entire surface of the package is considered the PDP. Typically the PDP appears on the largest surface of the package. Multiply the height times the width of that surface.

$$\text{Area of PDP} = \text{height} \times \text{width}$$

Cylindrical Containers. For cylindrical (or nearly cylindrical) containers, such as jars, bottles, and cans, the PDP is considered 40% of the height multiplied by the circumference of the container. If a spot label is used, the area of the PDP is still determined by this formula.

$$\text{PDp area} = (\text{height} \times \text{circumference}) \times 0.40$$

Cone-Shaped Containers. For cone-shaped containers, such as jars, bottles, cans, and tubs, the PDP is considered 40% of the height multiplied by the circumference of the container. To determine the circumference, measure both the widest and the narrowest circumferences and average the two numbers.

$$\text{PDP area} = \text{height} \times \left(\frac{\text{widest circumference} + \text{narrowest circumference}}{\text{[illegible]}}\right) \times 0.40$$

Irregularly Shaped Packages. For containers of other shapes, 40% of the total surface of the container is considered the PDP. If there is an obvious surface to serve as the PDP (eg, top of a triangular or circular package of cheese), this entire surface should be measured. For extremely irregular containers, substitute an easily measured container of the same capacity.

$$\text{PDP area} = \text{total surface area} \times 0.40$$

PRODUCT IDENTITY STATEMENT

The product identity statement communicates what the product is. It includes the name of the food as well as any other defining characteristics, such as the form of the food, if it is an imitation food, or if it contains certain ingredients

Terminology

The product name, typically a standard name or a common or usual name of the food, is used for the product identity statement (eg, orange juice). Sometimes an appropriately descriptive term (eg, taco seasoning) or a fanciful name (eg, Coca-Cola®) commonly used by the public for the food is allowed.

Form of Food. If a food is marketed in various forms (eg, whole, sliced, diced), the form of the food is a necessary part of the product identity statement. If the form of the food is visible through the container (eg, whole pickles in a clear jar) or it is depicted by an appropriate vignette, the particular form does not need to be stated.

Imitation Foods. If a food is an imitation food, the word "imitation" must be used as part of the product identity statement, immediately preceding the name of the food.

Juice-Containing Beverages. Special rules affect how beverages containing fruit or vegetable juices may be named. The following rules highlight some of the issues to consider:

- If a product contains less than 100% but more than 0% juice *and* the product name includes the word "juice," the name must also include a term such as "beverage," "cocktail," or "drink."
- If a product name specifically identifies a juice that has been reconstituted, the name must also include a qualifying term, such as "from concentrate" or "reconstituted."
- On a 100% juice *or* a diluted juice product, if specific juices are identified as part of the product name (or elsewhere outside of the ingredient list), either the juices must be listed in descending order of predominance by volume, or their relative predominance must be shown by other means. For example, a juice name could be combined with "flavored" to indicate a nonpredominant juice.
- When a label for a multiple-juice product represents that particular juices are present but does not identify all juices in the product, the label must reveal that other juices are present (eg, " . . . in a blend of two other juices").

In addition to the product name requirements, beverages that contain, or appear to contain, fruit or vegetable juice are subject to requirements for percent-juice labeling.

Characterizing Ingredients. Labels on some products may be required to include information about characterizing ingredients in the product identity statement. An ingredient is considered a characterizing ingredient when the proportion of an ingredient present in a food has an influence on the price or consumer acceptance of the product, or when labeling may create an erroneous perception (eg, pictorials depicting an ingredient).

The required information may be:

- The percentage of a characterizing ingredient
- The presence or absence of a characterizing ingredient
- The need for the consumer to add a characterizing ingredient

For example, foods packaged for use in preparing main dishes or dinners (eg, spaghetti dinner kit) require information about the need to add ingredients.

Characterizing Flavors. If the product labeling, advertising, or both make a direct or an indirect representation of any recognizable flavors through words or pictorial, the flavor is considered a characterizing flavor. A characterizing flavor must be incorporated into the product identity statement. For example, depiction of vanilla beans on an ice cream carton would trigger the inclusion of "vanilla" in the product name (eg, vanilla ice cream). The word "flavored" or "artificial" may have to accompany the name of the flavor in certain circumstances.

Design Elements

In addition to dictating the terminology, the regulations outline location, placement, and type-size requirements for the product identity statement. When other required information is incorporated into the product identity statement (eg, form of food, "imitation," "reconstituted," characterizing ingredients, characterizing flavors), additional design requirements apply.

Product Identity Statement. The product name must be in conspicuous type, located prominently on the PDP. The type size should be reasonably related to the largest printing on the PDP.

Form of Food. When language describing the form of the food is required, the letters should be in a type size bearing a reasonable relation to the type size of the other components of the product identity statement.

Imitation. If "imitation" is required, it must be in the same type size and prominence as the name of the food.

Reconstituted. When a term such as "reconstituted" or "from concentrate" is required as part of a juice beverage name, the letters may be no smaller than half the height of the letters in the name of the juice.

Characterizing Ingredients. Percent ingredient declarations must be in an easily legible, prominent type that distinctly contrasts with other printed or graphic material.

Characterizing Flavors. When a characterizing flavor must be included in the product name, the letters must be no smaller than half the height of the letters in the product name. If the word "natural," "artificial," or "flavored" is included, it must be no smaller than half the height of the letters in the characterizing flavor.

DECLARATION OF NET QUANTITY OF CONTENTS—FDA

A declaration of net quantity of contents, which generally must appear on all PDPs, provides information about the amount of product contained in the package. If there are alternate PDPs, this declaration must appear on each PDP.

Metric Labeling

The current regulations are based on the inch-pound system of measures (avoirdupois system) and also metric measures (International System of Units [SI]).

Selecting the Proper Unit. The net quantity of contents may be expressed as weight, a fluid or dry measure, or a numerical count. Units used in declaring the net quantity vary depending on the form of the food. If the food is sold in liquid form, a fluid measure must be used. If the food is solid, semisolid, viscous, or a mixture of solid and liquid, a weight measure must be used. Fresh fruits, vegetables, and other dry commodities may be labeled with a dry measure. If there is a firmly established consumer usage and trade practice for declaring the contents of a liquid product by weight—or solid, semisolid, or viscous product by fluid measure—it may be used. Similarly, if there is a firmly established consumer usage and trade practice of declaring net quantity by numerical count, linear measure, or area measure, it may be used and possibly augmented by a weight or fluid measure. It is *never* acceptable to use any adjective qualifying the unit (eg, jumbo quart, full gallon).

Using the Correct Term. When the net quantity is declared as a weight measure, the terms "net weight" and "net" are used. Net weight is used when the inch-pound declaration is first. If a fluid measure is used, then either net or net contents is used.

Converting to Metric. To assure uniformity and precision in net quantity declarations, the regulations provide conversion factors to use in declaring the inch-pound and metric amounts.

Largest Whole Unit. The net quantity of contents should be declared in the largest whole unit, with any remainder expressed as a decimal or a common fraction of the unit. Alternatively, the remainder may be expressed in terms of the next smaller whole unit and any decimal or common fraction of that unit. Common fractions are halves, quarters, eighths, sixteenths, or thirty-seconds. Common fractions must be reduced to the lowest (simplest) terms. Decimal fractions should be carried to no more than three places.

Dual Ounce-Pound Declaration. The current net quantity of contents regulations require a dual ounce-pound declaration on packages weighing between 1 and 4 lb or containing between 1 pt and 1 gal. This dual declaration requirement results in a statement of ounces followed by a parenthetical pound-ounce declaration (eg, 18 oz [1 lb 2 oz]) or a statement of fluid ounces followed by a parenthetical declaration in largest whole fluid unit with the remainder in fluid ounces (eg, 36 fl oz [1 qt 4 fl oz]). Under the metric labeling regulations, this dual ounce-pound declaration requirement is eliminated. Including a dual ounce-pound declaration would be optional; however, if used, it must appear on one line and may precede or follow the metric declaration.

Abbreviations. The regulations list the only abbreviations that may be used in the declaration of net quantity of contents (Table 2). Generally, periods and plural forms are optional.

Design Elements

The regulations specify a number of design requirements for the net quantity of contents declaration. It must appear as a distinct item in the lower portion of the PDP, be surrounded by white (ie, blank) space, and meet a minimum type-size requirement based on the area of the PDP.

Location. The net quantity of contents declaration must appear within the bottom 30% of the PDP. If the PDP is 5 square inches or less, any available space on the PDP may be used. The net quantity of Contents declaration must be placed in lines parallel to the base on which the package rests as it is displayed. It may appear on more than one line.

Copy-Free Area. The net quantity of contents declaration must be separated from other printed matter. Spacing requirements do not apply to pictorials or graphics, provided they do not render the declaration inconspicuous. The declaration may be placed closer to the extreme lower border than the space prescribed for below the statement.

Type Style. The net quantity of contents declaration must be in conspicuous type that is prominent and easily legible. The declaration must appear in distinct contrast (by typography, layout, color, embossing, or molding) to other matter on the package, *unless* it is blown, embossed, or molded on a glass or plastic surface, in which case the contrast is not mandatory.

Type Size. To ensure that all packages of the same size will have the same size net quantity of contents, the regulations define minimum type-size requirements based on the area of the PDP. To determine the minimum type size for the net quantity of contents, measure the area of the PDP (Table 3).

Table 2. Abbreviations for Net Quantity of Contents

Unit	Abbreviation
Weight	wt
Pint	pt
Ounce	oz
Quart	qt
Pound	lb
Fluid	fl
Gallon	gal
Kilogram	kg
Gram	g
Milligram	mg
Microgram	mg
Cubic Centimeter	cm^3
Liter	L or l
Milliliter	mL or ml

Special Labeling Provisions

The regulations allow for modification of the net quantity of contents declaration in certain circumstances. In some cases, a modified declaration is required (eg, multiunit packages). In many other cases, the requirements are simply relaxed to offer more flexibility in package design.

Multiunit Packages. Packages containing two or more individually packaged, identical units that may also be sold individually have special labeling requirements. The net quantity of contents declaration must be on the outside of the package if the individual labeling is obscured by the outer packaging. The net quantity declaration must include the number of individual units, the quantity of each individual unit, and, in parentheses, the total quantity of the multiunit package. The total quantity optionally may be preceded by "total" or "total contents."

Bulk Foods. Food sold in bulk containers at the retail level is exempt from declaring the net quantity of contents, provided it is accurately weighed, measured, or counted within the purchaser's view or in accordance with the purchaser's order.

Random-Weight Packages. Packages from one lot or shipment of the same commodity with no fixed weight pattern are random-weight packages (eg, cheese cut from a bulk block). If the product label contains the net weight, price per pound (or kilogram), and total price, the requirements for type size, metric declaration, location, and copy-free area are waived. In addition, the weight may be declared in decimal fractions of a pound even if the weight is less than 1 lb.

Individual Servings Not Intended for Retail Sale. Small, individual-serving containers for use in restaurants, institutions, and passenger carriers that are not intended for retail sale are exempt from declaring the net quantity. These packages must be less than 1/2 oz or 1/2 fl oz.

Shell Eggs. Cartons containing 12 shell eggs are exempt from placing the net quantity in the bottom 30% of the PDP.

Other Provisions. Special allowances are made for certain categories of food packaged in specific containers. These exceptions provide flexibility in package design and conformity to established trade practices.

Table 3. Type-Size Requirements for Net Quantity of Contents

Area of more than	The PDP less than or equal to	Minimum type size letter height not less than
	5 sq in	1/16 inch (1.6 mm)
5 sq in	25 sq in	1/8 inch (3.2 mm)
25 sq in	100 sq in	3/16 inch (4.8 mm)
100 sq in	400 sq in	1/4 inch (6.4 mm)
400 sq in		1/2 inch (12.7 mm)

Deviation from Declaration in Largest Whole Unit. Due to established trade practices that preceded the regulations, a variety of products may declare 8-fl-oz and 2-q volumes as 1/2 p and 1/2 gal, respectively, rather than following the rule for declaring the quantity in the largest whole unit. Products to which this deviation applies include bottled water, fruit juice beverages, ice cream and frozen desserts, and milk and fluid dairy products.

DECLARATION OF NET QUANTITY OF CONTENTS—USDA

Units and Terminology

As with FDA, units used in declaring the net quantity of USDA foods depend on the form of the food. Because most USDA foods are solid, semisolid, viscous, or a mixture of solid and liquid, a weight measure must be used. A food sold in liquid form requires a fluid measure (eg, chicken soup). However, firmly established consumer usage or trade practice for declaring the contents of a liquid product by weight—or solid, semisolid, or viscous product by fluid measure—may be used.

When the net quantity is declared in a weight measure, the term "net weight" or "net wt." is used. If a fluid unit is used, either "net contents" or "contents" should be used.

Dual Declaration

On certain-size packages, USDA requires that the net quantity be declared in ounces followed in parentheses by the quantity in pounds with any remainder expressed in ounces of a pound (similar requirements pertain to fluid measures). This is referred to as dual declaration. FDA eliminated its dual declaration requirement when it introduced metric labeling. USDA also requires metric declaration in addition to ounce-pound.

Abbreviations

USDA does not designate abbreviations that may be used in the net quantity declaration. Therefore, companies may use the FDA-defined abbreviations or other abbreviations.

Special Labeling Provisions

USDA allows for modification of the net quantity declaration in specific cases. USDA requires a modified declaration on multiunit packages. Some USDA requirements are relaxed to accommodate certain packaging situations and established trade practices.

Multiunit Packaging. USDA packages that contain two or more individually packaged, identical units that may also be sold individually have special labeling requirements. The net quantity declaration must be on the outside of the package if the individual labeling is obscured by the outer packaging. The declaration must include the number of individual units, the quantity of each individual unit, and, in parentheses, the total quantity of the multiunit package in ounces or fluid ounces. The dual declaration requirement is waived.

Random-Weight Packages. USDA packages from one lot or shipment of the same commodity with no fixed-weight pattern are considered random-weight packages (eg, ground beef). The net quantity declaration must be applied to random-weight consumer packages before retail sale; however, the declaration is exempt from type-size, dual declaration, and placement requirements, provided it appears conspicuously on the PDP.

Small Packages. If the shipping carton of individually wrapped, small packages (less than 1/2 oz) carries an accurate net quantity declaration, the USDA net quantity requirements are waived for the individual packages. When these small packages bear the net weight, price per pound, and total price, they are exempt from type-size, dual declaration, and placement requirements, provided the net quantity appears conspicuously on the PDP.

Margarine. Some margarine products contain animal fat and fall under USDA jurisdiction. Margarine in 1-lb rectangular packages (with the exception of whipped and soft margarine, or packages containing more than 4 sticks) are exempt from the requirements of placement in the bottom 30% of the PDP and the dual declaration. The net quantity declaration must appear as "1 pound" or "one pound" in a conspicuous manner on the PDP.

Sliced, Shingle-Packed Bacon. USDA exempts certain-size packages (ie, 8-oz, 1-lb, and 2-lb rectangular packages) of sliced, shingle-packed bacon from placement of the net quantity in the bottom 30% of the PDP and from dual declaration. These products are placed on boards that wrap around the top and serve as the PDP. Due to the space limitations of the PDP, these products have been granted more flexibility in incorporating the net quantity declaration. The declaration must appear conspicuously on the PDP.

In addition, sliced, shingle-packed bacon in any other size container (ie, other than 8-oz, 1-lb, and 2-lb) must show the net quantity declaration with the same prominence as the most conspicuous feature on the package.

CLAIMS-RELATED STATEMENTS

When a product includes a claim, a number of elements must be incorporated into the package design. The regulations do not specify where a claim must be placed; however, most claims appear on the PDP because it is the most conspicuous panel.

Types of Claims

The regulations define two categories of claims: nutrient content claims (sometimes referred to as "descriptors") and health claims. Nutrient content claims are statements about the level of a nutrient in a food (Table 4). Health claims, on the other hand, link the nutrient profile of a food to a health or disease condition (Table 5). Nutrient content claims are used widely by the food industry. Because the regulations governing health claims are more complicated and restrictive, health claims are not as common.

Nutrient Content Claims. Nutrient content claims characterize the level of a nutrient in a food. Only defined terms may be used on the label to describe a food's nutrient content. When these terms are used, a product must meet specific criteria. Nutrient content claims may be expressed or implied, comparative or absolute. These distinctions are important in package design because the type of claim dictates the information required.

Expressed Claim. Any direct statement about the level or range of a nutrient in a food is considered an expressed nutrient content claim. Specific terms are defined by regulation.

Implied Claim. An implied claim is any statement that leads the consumer to assume that a nutrient is absent or present in a certain amount or that the food may be useful in achieving dietary recommendations. Implied claims must follow the same requirements defined for expressed claims.

Comparative (or Relative) Claim. A claim comparing the level of a nutrient in one product to the level of that nutrient in another product or class of foods is considered a comparative claim (also called a relative claim). For example, "light," "reduced," "less," and "more" are comparative claims.

Absolute Claims. In contrast to comparative claims, absolute claims make a statement about the nutrient level in a food without stating or implying any comparison to another product. "Free," "low," "very low," "high," and "source of" are examples of absolute claims.

Table 4. Nutrient Content Claims

Comparative	Absolute
Light/Lite	Free
Reduced	Low
Less	Very Low
More	High
	Source of
	Healthy
	Lean
	Extra Lean

Table 5. Health Claims

Calcium and osteoporosis
Sodium and hypertension
Dietary saturated fat and cholesterol and risk of coronary heart disease
Dietary fat and cancer
Fiber-containing grain products, fruits, and vegetables and cancer
Fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease
Fruits and vegetables and cancer
Folic acid and neural tube defects
Sugar alcohols and dental caries

Health Claims. FDA has defined 9 health claims which may be used on labels (Table 5), and has created very strict criteria for using these claims. No other nutrient/disease associations may be made in food labeling. Written statements, third-party references, use of certain terminology in a brand name, symbols, and pictorials may be considered a health claim if the context in which they are presented either suggests or states a relationship between a nutrient and a disease. When a statement, symbol, pictorial, or other form of communication suggests a link between a nutrient and a disease, it is considered an implied health claim, and it is subject to all the requirements for health claims.

Nutrient Content Claims

When a product bears a nutrient content claim (whether it is expressed or implied), certain information must be incorporated into the design of the package. Because many claims appear on the PDP, this panel usually is affected. When a claim appears elsewhere on a package, however, certain information must appear on the panel with the claim.

Required Statement. Depending on the type of claim and whether the product is governed by FDA or USDA, the requirements for claims-related information vary. FDA mandates more extensive information than USDA (ie, the inclusion of either a referral or a disclosure statement). Both agencies require additional information on products bearing comparative claims.

Claims on FDA Products. All FDA products bearing nutrient content claims require either a referral or a disclosure statement to be included on each panel where a claim appears, except the nutrition facts panel. A referral statement, appearing next to the largest claim on each panel, directs the consumer to the nutrition facts statement. When a product contains excessive levels of key nutrients that are associated with health risks, the referral statement is replaced with a disclosure statement that flags the nutrient(s) of concern and directs the consumer to the nutrition facts panel.

Comparative Claims. Both FDA and USDA product labels that bear a comparative claim must include a nutrient claim clarification statement and quantitative information. On FDA products, this information is required in addition to a referral or a disclosure statement.

The nutrient claim clarification statement identifies the comparison food and states the percentage (or fractional) difference in the subject nutrient(s) between the product and its comparison food (eg, 50% less fat than [comparison food], 1/3 fewer calories than [comparison food]).

The quantitative information provides the absolute amounts of the subject nutrient(s) in the product and in the comparison food.

Design Elements. USDA and FDA differ slightly in their design requirements for claims-related information. USDA does not require referral or disclosure statements; FDA applies the type-size standards defined for these statements to other claims-related statements.

Claims Statements. Claims may not have undue prominence because of type style in comparison to the product identity statement. A claim may be no longer than two times the size of the product identity.

Referral or Disclosure Statement. On FDA products, a referral or a disclosure statement must be immediately adjacent to the claim. No intervening material may be placed between it and the claim, except for other claims-related statements, or a standard name that is modified by the claim, or both. It must appear on each panel where the claim is located, except for the panel that contains the nutrition facts (eg, if the nutrition facts and the claim are both on the PDP, the statement is not required). If multiple claims appear on a panel, the referral or disclosure statement must be adjacent to the largest claim.

It must be in easily legible, boldface type, in distinct contrast to other printed or graphic matter. It may be no smaller than the net quantity declaration, unless the claim is less than two times the size of the net quantity declaration.

Nutrient Claim Clarification Statement. The nutrient claim clarification statement must be placed in immediate proximity to the most prominent comparative claim. It must follow the same type-size requirements as the referral statement. If different comparative claims appear on the same label, the nutrient claim clarification statement must be placed in immediate proximity to the most prominent presentation of each claim.

Quantitative Information. Clear and concise quantitative information must appear adjacent to the most prominent claim or on the Information Panel.

Health Claims

When a product bears a health claim, very specific language must be used on the label. The regulations governing health claims are very complex; therefore, health claim situations should be handled on a case-by-case basis. The language associated with a health claim must conform to regulatory guidelines, which provide model health claim statements. Products bearing health claims must undergo careful review by legal experts, regulatory experts, or both to be certain the language is accurate and any graphics are acceptable.

Model Statements. The regulations pertaining to each health claim outline the assertions that may be made and any additional required statements. When a claim is implied through graphic representations, a complete claim statement must be included on the label.

Design Elements. All information required in a health claim must appear in one place, in the same type size, and without other intervening material. Because the required statements can be quite lengthy, the complete claim may appear on a back or side panel. When this approach is taken, a reference statement may be placed on the PDP, flagging the claim and directing the consumer to the location of the claim (eg, "See ______ for information about the relationship between ______ and ________"). The first blank contains the location of the health claim (eg, back panel, attached pamphlet), the second blank states the nutrient, and the third blank names the disease or health-related condition.

When any graphic material implying or expressing a health claim is used on the label or in accompanying labeling materials (eg, pamphlet), the entire claim statement or a reference statement must appear in immediate proximity to the graphics.

ADDITIONAL USDA LABELING REQUIREMENTS

The USDA has additional labeling requirements: official inspection legend and keep frozen/refrigerated statements.

Official Inspection Legend

The PDP of all USDA products must include an official inspection legend in accordance with established inspection procedures. The number of the official establishment (ie, the identity of the plant at which the product was manufactured) must also be included in one of the following manners:

- Within the official inspection legend
- Outside the official inspection legend, but elsewhere on the label (eg, on the lid of a can). When it is placed outside the inspection legend, the prefix "EST." must precede the establishment number. It must be shown in a prominent and legible manner in a size that ensures easy visibility and recognition.
- Off the exterior of the container (eg, on a metal clip used to close casings or bags) or on other packaging material in the container (eg, on aluminum pans and trays within the container). When it is placed on the exterior of the container or on other packaging material, a statement of its location must be printed continuous to the official inspection legend.
- On an insert label placed under a transparent covering, if it is clearly visible and legible and accompanied by the prefix "EST.".

Keep Frozen/Refrigerated Statements

Many USDA products require special instructions to ensure the safety and quality of the product. These products may include either of the following statements: "Keep Frozen" or "Keep Refrigerated." The statement must appear on the PDP in a conspicuous manner. There are no minimum-size requirements.

Other Required Information

On some products, additional information may be required on the PDP (Table 6). Products bearing claims have additional requirements that usually affect the design of the PDP. Other products require warning statements, some of which must be placed on the PDP. Certain products require special handling instructions to ensure the safety and

Table 6. Additional Information That May Be Required on the PDP

Information required on some products	FDA location	USDA location
Claims statements		
Disclosure or referral statement	Adjacent to largest claim on each panel	Not required
Nutrient claim clarification	Adjacent to most prominent claim	Adjacent to most prominent claim
Quantitative information	Adjacent to most prominent claim or on information panel	Adjacent to most prominent claim or on information panel
Safe-handling instructions for raw meat and poultry	Not required	Information panel or other panel
Percent-juice declaration	Information panel and/or PDP	Not required
Warning statements	Requirements vary	Requirements vary

quality of the product. When these statements are required, the regulations dictate the size and placement of the information.

INFORMATION PANEL

A package may be designed to include an information panel where some of the mandatory information may be placed (Table 7). If the ingredient list and manufacturer identity statement do not appear on the PDP, the package must have an information panel that includes these statements. Whenever practical, the nutrition facts statement should appear on the information panel with these statements. Other label statements required on some products (eg, a percent-juice declaration, some warning statements, special handling instructions) also may appear on the information panel.

Statements intended for the information panel may alternatively appear on the PDP if space on the information panel is insufficient. Also, it is permissible to omit an information panel, incorporating all mandatory statements on the PDP. Exemptions from labeling requirements for nonretail packages apply to the components of the information panel as well as to the PDP.

Location

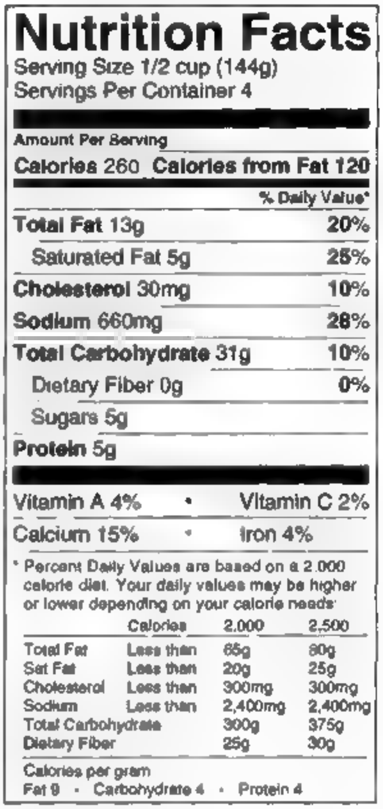
The information panel generally is considered the part of the package immediately contiguous to and to the right of the PDP as observed by an individual facing the PDP. For situations when this arrangement is not possible, alternative locations are specified.

NUTRITION FACTS

Only when the nutrition facts statement (Figure 1) cannot be accommodated on the information panel or the PDP along with other mandatory information may it be placed elsewhere on the package. Incorporating it into the information panel (or PDP) should always be explored first.

Table 7. Information Panel

Mandatory information	Additional required information
Nutrition facts	Percent-juice declaration
Ingredient list	USDA safe-handling instructions
Manufacturer identity	Special handling instructions
	Warning statements



Nutrition Facts
Serving Size 1/2 cup (144g)
Servings Per Container 4

Amount Per Serving
Calories 260 **Calories from Fat** 120

	% Daily Value*
Total Fat 13g	**20%**
Saturated Fat 5g	**25%**
Cholesterol 30mg	**10%**
Sodium 660mg	**28%**
Total Carbohydrate 31g	**10%**
Dietary Fiber 0g	**0%**
Sugars 5g	
Protein 5g	

Vitamin A 4% • Vitamin C 2%
Calcium 15% • Iron 4%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram
Fat 9 • Carbohydrate 4 • Protein 4

Figure 1. Typical nutrition facts label.

INGREDIENT LIST

All products fabricated from two or more ingredients must include an ingredient list on either the PDP or the information panel.

Terminology

Ingredients are listed by their common or usual name in descending order of predominance by weight. Ingredients present in amounts of 2% or less may be listed at the end of the ingredient list following an appropriate qualifying statement (eg, "Contains ____ percent or less of ____, less than ____ percent of ____").

Design Elements

The ingredient list must be conspicuous and in a type size no smaller than 1/16 in.

Standardized Foods

With the inception of NLEA, standardized foods now typically must declare all ingredients.

Special Provisions for Bulk Foods

A food received in bulk containers at retail may provide the ingredient list by either displaying it on the bulk container or posting a counter card or sign with the required information.

MANUFACTURER IDENTITY

The manufacturer identity states the name and place of business of the manufacturer, packer, or distributor. The information panel typically includes this information.

Terminology

If the manufacturer is a corporation, the corporation name must be used and may be preceded or followed by the name of the particular division. If the manufacturer is an individual, a partnership, or an association, the name under which the business is conducted must be used. If the food is not manufactured by the person whose name is on the label, the name must be qualified with "Manufactured for ____," "Distributed by ____," or other wording that expresses the relationship.

The statement must include the street address, city, state, and postal code. If the name is in a current telephone directory, the street address may be omitted. The principal place of business may replace the actual place where the food was manufactured, packed, or distributed, assuming this would not be misleading.

PERCENT-JUICE DECLARATION

With certain exceptions, any beverage that purports or appears to contain a fruit or vegetable juice must declare the percentage of juice in the product. Any of the following situations could trigger this requirement: using the name (or variation) of a fruit or vegetable in advertising, labels, or labeling; depicting a fruit or vegetable in a vignette or other pictorial representation; or formulating a beverage to contain the color and flavor of a fruit or vegetable juice.

Percent-juice labeling (Table 8) is generally required if a beverage has the appearance and flavor of containing a fruit or vegetable juice, even if the product in fact contains no juice. Certain exceptions are made for products containing minor amounts of juice (typically less than 2%) that are labeled with a fruit or vegetable name and a term such as "flavored."

Terminology

The percentage of juice must be declared, and the type of juice may be declared. If a product does not contain juice but the labeling or color and flavoring suggest that it does, it must be declared as 0% (or a similar phrase).

Table 8. Juice Content Information

If beverage contains juice	If beverage does not contain juice
Contains ___ percent (or %) juice	Contains 0% juice
Contains ___ percent (or %) (*type*) juice	Contains 0% (*type*) juice
___ percent (or %) juice	Does not contain (*type*) juice
___ percent (or %) (*type*) juice	Contains no (*type*) juice Contains no fruit/vegetable juice

USDA SAFE-HANDLING INSTRUCTIONS

USDA has regulations requiring safe-handling instructions (Table 9) on all raw meat and poultry products not intended for further processing at another USDA-inspected establishment. These safety instructions are intended to heighten consumer awareness of proper food-sanitation procedures and reduce the incidence and severity of foodborne illnesses.

Terminology

The regulations specify the exact wording that must be used.

Heading. The safe-handling information must be presented on the label under the heading "Safe Handling Instructions."

Rationale Statement. "This produce was prepared from inspected and passed meat and/or poultry. Some food products may contain bacteria that could cause illness if the product is mishandled or cooked improperly. For your protection, follow these safe handling instructions."

Safe Handling Statements. "Keep refrigerated or frozen. Thaw in refrigerator or microwave." Any portion of this statement that is in conflict with the product's specific handling instructions may be omitted (eg, instructions to cook without thawing).

"Keep raw meat and poultry separate from other foods. Wash working surfaces (including cutting boards), utensils, and hands after touching raw meat or poultry."

Table 9. Safe-Handling Instructions Model

Design elements
Set off by border
Prominent location, visible at point of purchase
Graphic illustrations next to safe-handling statements
Type no smaller than 1/16 in, except heading, which must be larger
One-color printing on single-color, contrasting background

"Cook thoroughly."

"Keep hot foods hot. Refrigerate leftovers immediately or discard.

OTHER LABELING REQUIREMENTS

In certain situations, other labeling requirements apply. The requirements may be triggered by voluntary declaration of related information (eg, stating number of servings, making a claim) or because a key substance is used in the product or packaging (eg, aspartame, saccharin, self-pressurized containers).

Labeling with Number of Servings

Although it is not required, some packages include a statement about the number of servings contained in the package. When this information is provided, the regulations require that the net quantity of each serving be stated adjacent to the number-of-servings declaration. The statement of net quantity must be in the same type size used in the declaration of number of servings. This net quantity information should be consistent with the serving size declaration in the nutrition facts statement, unless the product is exempt from nutrition labeling.

Quantitative Information for Comparative Claims

Products bearing a nutrient content claim that is considered a comparative claim must include quantitative information either adjacent to the most prominent claim or on the information panel.

Warning Statements

Certain products require warning statements that must be placed on the information panel (or the PDP). For example, products packaged in self-pressurized containers must warn consumers about health and environmental risks. Protein products in liquid, powder, tablet, capsule, or similar form must warn consumers about the risks of very-low-calorie diets. Products containing ingredients that have a health or safety concern (eg, aspartame, saccharin) are required to display warning statements or other notices.

Exemptions

Despite the goal of NLEA that virtually all packaged food products carry a nutrition label, some products are exempt (Table 10). Manufacturers of exempt products may voluntarily include nutrition labels on their products; however, all the requirements for nutrition labeling must be followed on such voluntary labels. Generally, if an otherwise exempt product carries a nutrition claim or other nutrition information on its label (or in labeling or advertising), nutrition labeling is required.

Foods Intended for Further Processing. Foods that are shipped to food manufacturers, distributors, or retailers for further processing, repacking, or labeling are generally exempt. The regulations detail the situations that qualify for exempt status. FDA and USDA take basically the same approach on this point; however, their criteria are outlined differently.

Foods Prepared in Restaurant, Food-Service, and Retail Settings. Restaurant and food-service foods are generally exempt from nutrition labeling requirements. Ready-to-eat foods from bakeries, delicatessens, and retail settings where the food is prepared on-site are also exempt.

Small Businesses. Both agencies exempt products of small businesses; however, each agency has its own criteria for defining a small business. Originally FDA used gross sales to define a small business, but due to a congressional mandate, FDA revised the small-business exemption, basing it on the number of full-time equivalent employees and units of product produced in a year. USDA's exemption criteria are based on the number of employees and pounds of product produced annually.

Other Exemptions. FDA exempts foods with no nutritional significance (eg, teas, coffee, spices); this provision is not applicable to USDA foods (ie, foods containing 2% or more cooked meat or poultry have significant nutrients). Foods intended for export and custom-slaughtered fish and game are exempt.

FDA oversees infant formula, medical foods, and dietary supplements. The labeling of these products is addressed under separate regulations; thus, they are exempt from the general nutrition-labeling regulations.

Special Labeling Provisions

Both FDA and USDA regulations have special labeling provisions for certain food categories. These products are not exempt from nutrition labeling, but some of the labeling requirements are more relaxed.

Small Packages. Both agencies allow food packed in small packages (less than 12 sq in of space available to bear labeling) that do not bear claims to omit the nutrition facts, but the label must include an address and/or a phone number where the consumer can obtain nutrition information. If nutrition labeling is required, there are special layout and design provisions. USDA exempts from nutrition labeling packages containing less than 0.5 oz, provided no nutrition-related claims are made or nutrition information is provided.

Gift Packs. For packages that contain a variety or assortment of foods intended to be used as a gift, FDA allows the nutrition labeling to be enclosed within the package. It may be provided on a composite basis for FDA-approved reasonable categories of foods that have similar dietary uses and nutritional characteristics. USDA also allows nutrition information to be provided by a label insert.

Raw Products. Raw fruits, vegetables, and fish are exempt from mandatory nutrition labeling, but FDA has outlined expectations for voluntary nutrition labeling by retailers at the point of sale. USDA has a similar program.

Foods for Infants and Children. Foods intended specifically for infants and children under 2 and under 4 years of

Table 10. Foods Exempt from Mandatory Nutrition Labeling

Exemption criteria[a]	FDA exempts	USDA exempts
Foods intended for further processing, labeling, or packaging, not for sale to consumers	Yes, even if bearing claims	Yes
Restaurant and food-service products	Yes	Yes
Food prepared at retail site	Yes	Yes
Food produced by small businesses[b]	Yes	Yes
Food with no nutritional significance (coffee, tea, spices)	Yes	N/A
Donated foods	Yes	No
Custom-slaughtered and processed products	Yes[c], even if bearing claims	Yes, even if bearing claims
Products intended for export	Yes, even if bearing claims	Yes, even if bearing claims
Infant formula, medical foods, and dietary supplements	Yes[d]	N/A

[a] Nutrition-related claims trigger mandatory nutrition labeling, except in a few situations in which the exemption is available regardless of the presence of claims.

[b] Both FDA and USDA provide for a small-business exemption; however, the agencies have different criteria for defining a small business.

[c] FDA oversees game meats, which have provisions for voluntary labeling.

[d] Addressed under separate regulations.

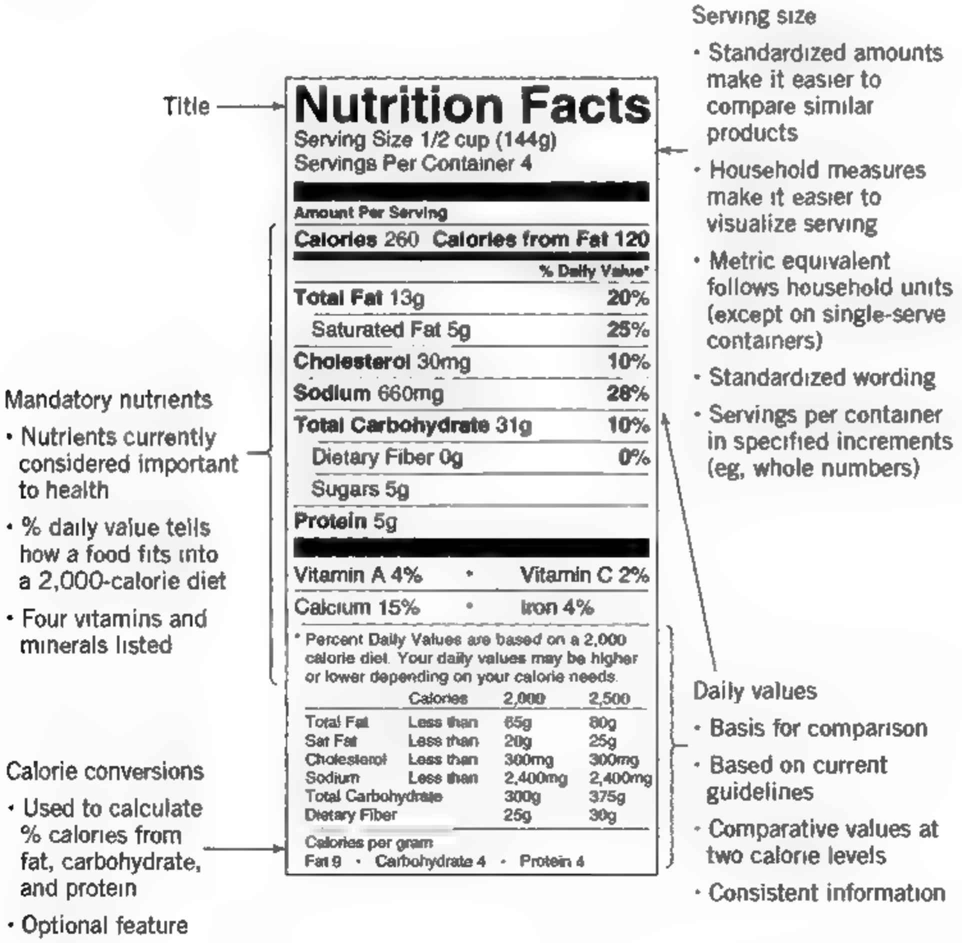
age have special format provisions, and certain nutrients are omitted.

Features of the Nutrition Label

Title. The label is entitled "Nutrition Facts" (Fig. 2).

Serving Size Information. Serving sizes are provided in common household measures (eg, cups), followed by the metric equivalent in parentheses. The regulations define standardized amounts based on the servings people typically consume (eg, 12 oz can of soda is 1 serving, not 2) and detail how to derive serving size declarations.

Nutrient List. Information on the following nutrients is mandatory: calories, total fat, saturated fat, cholesterol,



Nutrition Facts
Serving Size 1/2 cup (144g)
Servings Per Container 4

Amount Per Serving
Calories 260 **Calories from Fat** 120

	% Daily Value*
Total Fat 13g	20%
Saturated Fat 5g	25%
Cholesterol 30mg	10%
Sodium 660mg	28%
Total Carbohydrate 31g	10%
Dietary Fiber 0g	0%
Sugars 5g	
Protein 5g	

Vitamin A 4% • Vitamin C 2%
Calcium 15% • Iron 4%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram
Fat 9 • Carbohydrate 4 • Protein 4

Title

Serving size
- Standardized amounts make it easier to compare similar products
- Household measures make it easier to visualize serving
- Metric equivalent follows household units (except on single-serve containers)
- Standardized wording
- Servings per container in specified increments (eg, whole numbers)

Mandatory nutrients
- Nutrients currently considered important to health
- % daily value tells how a food fits into a 2,000-calorie diet
- Four vitamins and minerals listed

Daily values
- Basis for comparison
- Based on current guidelines
- Comparative values at two calorie levels
- Consistent information

Calorie conversions
- Used to calculate % calories from fat, carbohydrate, and protein
- Optional feature

Figure 2. Nutritional label.

sodium, total carbohydrate, dietary fiber, sugars, protein, and four vitamins and minerals. In addition, certain nonmandatory nutrients may be included. The actual amount of each nutrient present in a serving is listed beside the nutrient. In the right-hand column, the amount of the nutrient is expressed as "% Daily Value," which shows how a food fits into a 2,000-calorie reference diet.

Daily Values Footnote. The daily values table at the bottom of the nutrition label presents the reference numbers used to calculate the % daily value. These numbers reflect current recommendations for health maintenance and disease prevention. They are provided at two calories levels and remain the same for all labels. On smaller packages and packages qualifying for the simplified format, this information may be omitted.

Calorie Conversion Footnote. Calorie conversions assist in calculating the percentage of calories from carbohydrate, protein, and fat. This feature is now optional.

Mandatory Design Elements

The regulations dictate a number of mandatory design elements that must be followed for all the nutrition facts layouts.

Hairline Box. The nutrition facts information must be set off from other printed material by a hairline box. The regulations are very precise on this point. No other information may be enclosed within the box. The box must be in the same color ink used for the type.

Typesetting. The type must be set in a single, easy-to-read type style. Helvetica is the recommended font; however, any sans serif type is permissible. Upper- and lowercase letters must be used. Minimum type sizes are specified for each component of the label.

The nutrients must be separated by 4 points of leading. All other text must be separated by at least 1 point of leading.

ACKNOWLEDGMENTS

This text is derived from *Food Label Design: A Regulatory Resource Kit*, published by the Institute of Packaging Professionals. Used with permission.

GENERAL REFERENCES

F. Olsson, and P. C. Weeda, *U.S. Food Labeling Guide*, The Food Institute, Fair Lawn, N.J., 1998.
R. Shapiro, *Nutrition Labeling Handbook*, Marcel Dekker, New York, 1996.
J. Storlie, *Food Label Design: A Regulatory Resource Kit*, Institute of Packaging Professionals, Herndon, Va., 1996.

AARON L. BRODY
Rubbright•Brody, Inc.
Duluth, Georgia
JEAN STORLIE
Nutrition Labeling Solutions
Maple Grove, Minnesota

PACKAGING: PART III—MATERIALS

The basic food-packaging materials can be divided into glass, metal, paper, and plastic. Each of these materials has both advantages and disadvantages (Table 1). The proper selection of these materials should be based on the functional requirements (see PACKAGING: PART I—GENERAL CONSIDERATIONS) and the economics of specific applications. To optimize the performance and cost, most food packages use more than one type of packaging material. For example, the packaging for a typical box of cereal consists of an inner plastic bag for moisture protection and an outer paperboard box for strength and rigidity.

GLASS

The glass container (bottle or jar) is one of the oldest food packages. It is an excellent barrier for protecting food from oxygen and moisture. It can provide food with excellent visibility and an image of cleanliness. Physically, glass is a very high viscosity supercooled liquid. The major constituents of container glass are silica (SiO_2), soda (Na_2O), and calcia (CaO), along with small amounts of other inorganic oxides. The properties of glass (such as strength, transparency, and moldability) can be modified by minor changes in the composition of these constituents.

Some important considerations in designing the glass container are mechanical, thermal, and optical properties. Mechanical properties include vertical load strength, internal pressure strength, impact strength, scratch resis-

Table 1 Advantages and Disadvantages of Food-Packaging Materials

Advantages	Disadvantages
Glass	
Excellent barrier against oxygen and moisture Chemically inert Transparent	Easily breakable Relatively heavier
Metal	
Excellent barrier against oxygen, moisture, and light Good mechanical strength and durability Good thermal stability	Susceptible to corrosion Metal cans are generally more difficult to open and reseal
Paper	
Relatively inexpensive Excellent printability Lightweight	Poor gas and moisture barrier Greatly reduced mechanical strength when wet
Plastic	
Most versatile packaging material Can be formed easily into many shapes Lightweight	More susceptible to migration and flavor-scalping problems

sodium, total carbohydrate, dietary fiber, sugars, protein, and four vitamins and minerals. In addition, certain nonmandatory nutrients may be included. The actual amount of each nutrient present in a serving is listed beside the nutrient. In the right-hand column, the amount of the nutrient is expressed as "% Daily Value," which shows how a food fits into a 2,000-calorie reference diet.

Daily Values Footnote. The daily values table at the bottom of the nutrition label presents the reference numbers used to calculate the % daily value. These numbers reflect current recommendations for health maintenance and disease prevention. They are provided at two calories levels and remain the same for all labels. On smaller packages and packages qualifying for the simplified format, this information may be omitted.

Calorie Conversion Footnote. Calorie conversions assist in calculating the percentage of calories from carbohydrate, protein, and fat. This feature is now optional.

Mandatory Design Elements

The regulations dictate a number of mandatory design elements that must be followed for all the nutrition facts layouts.

Hairline Box. The nutrition facts information must be set off from other printed material by a hairline box. The regulations are very precise on this point. No other information may be enclosed within the box. The box must be in the same color ink used for the type.

Typesetting. The type must be set in a single, easy-to-read type style. Helvetica is the recommended font; however, any sans serif type is permissible. Upper- and lowercase letters must be used. Minimum type sizes are specified for each component of the label.

The nutrients must be separated by 4 points of leading. All other text must be separated by at least 1 point of leading.

ACKNOWLEDGMENTS

This text is derived from *Food Label Design: A Regulatory Resource Kit*, published by the Institute of Packaging Professionals. Used with permission.

GENERAL REFERENCES

F. Olsson, and P. C. Weeda, *U.S. Food Labeling Guide*, The Food Institute, Fair Lawn, N.J., 1998.

R. Shapiro, *Nutrition Labeling Handbook*, Marcel Dekker, New York, 1996.

J. Storlie, *Food Label Design: A Regulatory Resource Kit*, Institute of Packaging Professionals, Herndon, Va., 1996.

Aaron L. Brody
Rubbright•Brody, Inc.
Duluth, Georgia

Jean Storlie
Nutrition Labeling Solutions
Maple Grove, Minnesota

PACKAGING: PART III—MATERIALS

The basic food-packaging materials can be divided into glass, metal, paper, and plastic. Each of these materials has both advantages and disadvantages (Table 1). The proper selection of these materials should be based on the functional requirements (see PACKAGING: PART I—GENERAL CONSIDERATIONS) and the economics of specific applications. To optimize the performance and cost, most food packages use more than one type of packaging material. For example, the packaging for a typical box of cereal consists of an inner plastic bag for moisture protection and an outer paperboard box for strength and rigidity.

GLASS

The glass container (bottle or jar) is one of the oldest food packages. It is an excellent barrier for protecting food from oxygen and moisture. It can provide food with excellent visibility and an image of cleanliness. Physically, glass is a very high viscosity supercooled liquid. The major constituents of container glass are silica (SiO_2), soda (Na_2O), and calcia (CaO), along with small amounts of other inorganic oxides. The properties of glass (such as strength, transparency, and moldability) can be modified by minor changes in the composition of these constituents.

Some important considerations in designing the glass container are mechanical, thermal, and optical properties. Mechanical properties include vertical load strength, internal pressure strength, impact strength, scratch resis-

Table 1 Advantages and Disadvantages of Food-Packaging Materials

Advantages	Disadvantages
Glass	
Excellent barrier against oxygen and moisture Chemically inert Transparent	Easily breakable Relatively heavier
Metal	
Excellent barrier against oxygen, moisture, and light Good mechanical strength and durability Good thermal stability	Susceptible to corrosion Metal cans are generally more difficult to open and reseal
Paper	
Relatively inexpensive Excellent printability Lightweight	Poor gas and moisture barrier Greatly reduced mechanical strength when wet
Plastic	
Most versatile packaging material Can be formed easily into many shapes Lightweight	More susceptible to migration and flavor-scalping problems

tance, and abrasion resistance (1). The strength of the glass container depends greatly on the condition of its surface, and the strength can be greatly reduced by a slight surface scratch. To reduce scratches and impact breakage, various lubricating coatings, such as special waxes and silicones, are often applied to the outer surfaces. Fairly thick glass is often needed to provide sufficient strength to prevent breakage due to internal pressure (especially for carbonated beverage bottles), impact, or thermal shock. This thicker glass, however, adds considerable weight to the package.

Thermal properties include thermal strength, which is a measure of the ability to withstand sudden temperature change. Stresses from sudden cooling are more damaging than those from sudden heating. A simple experiment to determine thermal shock resistance is to observe the number of breakages that occur when glass containers at a high temperature are suddenly transferred to a cold-water bath. The temperatures used in the experiment depend on the actual process temperatures that the container will experience, such as in pasteurization, hot pack, or retorting (1).

Optical properties include the degree of transmission to light and its effects on the quality of the product. Glass can be colored for decoration or light protection. Flint is basic clear glass used for most food-packaging applications. Amber is the familiar brown glass used for beer that can filter out ultraviolet light (300–400 nm). Emerald is bright-green glass used mostly in the beverage industry. Other blue, green, and opaque glasses are also available (2).

In recent years, thin layers of silica (SiO_x) are deposited onto films such as polyethylene terephthalate (PET), oriented polypropylene (OPP), and low-density polyethylene (LDPE). These silica-coated (or glass-coated) films have good gas barrier (3), and they are clear, recyclable, and retortable; however, the commercial applications of these films are rather limited because of the high cost and technical difficulties in ensuring good silica adhesion to certain base polymers (4).

METAL

Metal packages provide excellent protection against the intrusion and negative effects of oxygen, moisture, and light. Steel (with tin or chromium in trace amounts) and aluminum (with magnesium and manganese in trace amounts) are the most commonly used metals for food packaging. Cans and trays are the most common forms of metal packages.

Steel Can

The steel can (also referred to as tin can) is made of a low-carbon steel coated with a very thin layer of tin on both the inner and outer surfaces. Typically, the steel can is made of three pieces: the can body, the top lid, and the bottom lid. The can body may be mechanically seamed, bonded with adhesive, welded, or soldered (2).

The tin coating protects the steel from corrosion through reactions with water, oxygen, acids, and a host of other chemicals or foods. Most often, the inner tin surface is also coated with a nonmetallic lacquer to prevent undesired reactions of the tin with the foods.

Aluminum Can

Most beer cans and soft-drink cans are made of aluminum. Compared to the steel can, the aluminum can is lighter, more corrosion resistant, more formable, lower in structural strength, and more costly.

To a large degree, the lower structural strength has limited the use of the aluminum can to the bottling of carbonated beverages, where the can gains structural support from the internal gas pressure. However, the lower strength and greater formability also allow the two-piece aluminum can (can body plus the top lid) to be formed more readily. This is accomplished by forging an aluminum disk into a cup so that the bottom and the sides are all in one piece. The two-piece can eliminates the side and bottom joints, and thus the chance of leakers is also reduced.

The popularity of the aluminum can is partly attributed to its recyclability. The economics incentive for recycling of aluminum cans is high, and the aluminum industry has established an effective collection and reprocessing system.

Aluminum Foils and Metallized Films

Aluminum foil (between 0.0003 and 0.0015 in thick) is used in such food-packaging applications as pouches and liddings. The foil is usually laminated to one or more layers of plastic or paper. An example is the military meal-ready-to-eat pouch that uses a laminated structure of PET/adhesive/aluminum/adhesive/polypropylene, from outside to inside. The aluminum foil layer provides an excellent barrier to moisture and gases, unless pinholes exist.

The metallized film consists of a very thin layer of aluminum (compared to the foil) that is vapor deposited onto the surface of a plastic film, such as polyester or nylon. The aluminum improves the gas- and light-barrier properties of the film as well as its aesthetic appeal. However, the gas-barrier properties of the metallized film are easily reduced due to handling that results in the formation of pinholes. The metallized film is used in such applications as bags for potato chips or snacks. It can also be laminated to paperboard for such applications as liquid-box juice containers.

Susceptor is a special type of metallized film used in microwavable food packages (1). A typical susceptor is a laminate consisting of the structure PET/adhesive/aluminum/adhesive/paperboard. The aluminum in the laminate is a very thin, discontinuous layer of aluminum particles that can convert microwave energy into heat, causing the temperature of the susceptor to rise above 200°C rapidly. The high temperature is important for applications such as microwavable popcorn and pizza. However, the high temperature also raises safety concerns about thermal degradation of the PET and the migration of undesirable compounds from the susceptor to the food.

PAPER AND PAPERBOARD

Paper and paperboard are made from wood fibers composed of cellulose, hemicellulose, and polymeric residues.

Although there is no strict distinction between paper and paperboard, structures less than 0.012 in thick are generally considered to be paper. Because of their good mechanical strength, paper and paperboard are used mostly to protect the product from physical damage. However, paper and paperboard have poor gas-barrier properties, and their mechanical strength can decrease greatly in wet or humid conditions.

Many types of paper are used for food-packaging applications (1,2). Kraft paper is typically a coarse paper used in applications such as bags and wrapping where strength is required. Kraft paper is available either in unbleached brown form or in bleached form. Greaseproof paper consists of densely packed fine fibers that provide good oil and grease resistance. Greaseproof paper may be further treated with fluorochemicals or coated with polyvinylidene chloride (PVDC) to improve gas- and moisture-barrier properties. Glassine paper is produced by further processing greaseproof paper through a super-calendering operation, making the paper smoother, denser, and translucent. Waxed paper consists of a continuous layer of wax on a base paper such as greaseproof or glassine paper. The wax serves as both a moisture barrier and a heat-sealable layer.

There are also many types of paperboard used for food-packaging applications (5). Corrugated boxes are used mostly for shipping food packages. Folding cartons are used as outer boxes for packaging cereal products, cookies, snacks, frozen foods, and the like. The appearance and printing quality of paperboard can be enhanced by coating with clay and other minerals.

Paperboard is often coated or laminated with plastics or aluminum for better performance. For example, polyethylene (PE) is coated onto gable-top paperboard containers, which are commonly used to contain milk or juices. The coating protects the paperboard against moisture absorption, and it also provides the paperboard with heat sealability. Another example is the aseptic Tetra Pak juice carton, which is made from a paperboard laminate having the structure PE/paper/aluminum/PE. In this laminate structure, the inner PE layer protects the aluminum from the juice, the aluminum layer provides oxygen and a moisture barrier, the paperboard layer provides strength and rigidity, and the outer PE layer provides moisture protection for the paperboard.

PLASTIC

Plastics, relatively new in packaging materials, are used in many of the food-packaging applications that were once dominated by the more traditional packaging materials—glass, metal, and paper (6). Plastics are polymers or long-chain macromolecules that can be molded, extruded, and cast into various shapes such as films, sheets, and containers. Polymers are derived from petroleum, natural gas, or coal by polymerization processes. Most polymers used in food-packaging plastics have molecular weights between 50,000 and 150,000. In addition, most plastics contain small amounts of additives (such as plasticizers, lubricants, antioxidants, antistats, heat stabilizers, and ultraviolet stabilizers) that are used to facilitate processing or to impart desirable properties to the plastics. These additives can sometimes migrate into the food, causing the food to acquire undesirable flavors (see PACKAGING: PART I—GENERAL CONSIDERATIONS). Most packaging polymers are thermoplastics, which are characterized by the absence of cross-linking between polymer chains and can be softened repeatedly by heat and pressure to form different shapes (7–9).

Polymers can be formulated to provide a wide range of performance properties that are dependent on chemical structure, crystallinity, side-chain branching, molecular weight, molecular weight distribution, orientation, and so on (1). The chemical structures of some food-packaging polymers are shown in Figure 1. As a general rule, higher crystallinity results in greater strength, better gas-barrier properties, higher soften point, and lower clarity. The type of polymer used in a retail container can often be identified by the recycling code and lettering on the bottom.

The important considerations in selecting plastics for food-packaging applications are gas-barrier properties, strength, stiffness, chemical resistance, sealability, printability, formability, appearance, and cost. Plastics are unique in that they have a broad range of gas-barrier properties (Fig. 2), whereas glass and metal provide a total gas barrier, and paper provides virtually none. Thus plastics offer the versatility for packaging many different foods, including those that require a high gas barrier (eg, potato chips) and those that require a low gas barrier (eg, fresh produce).

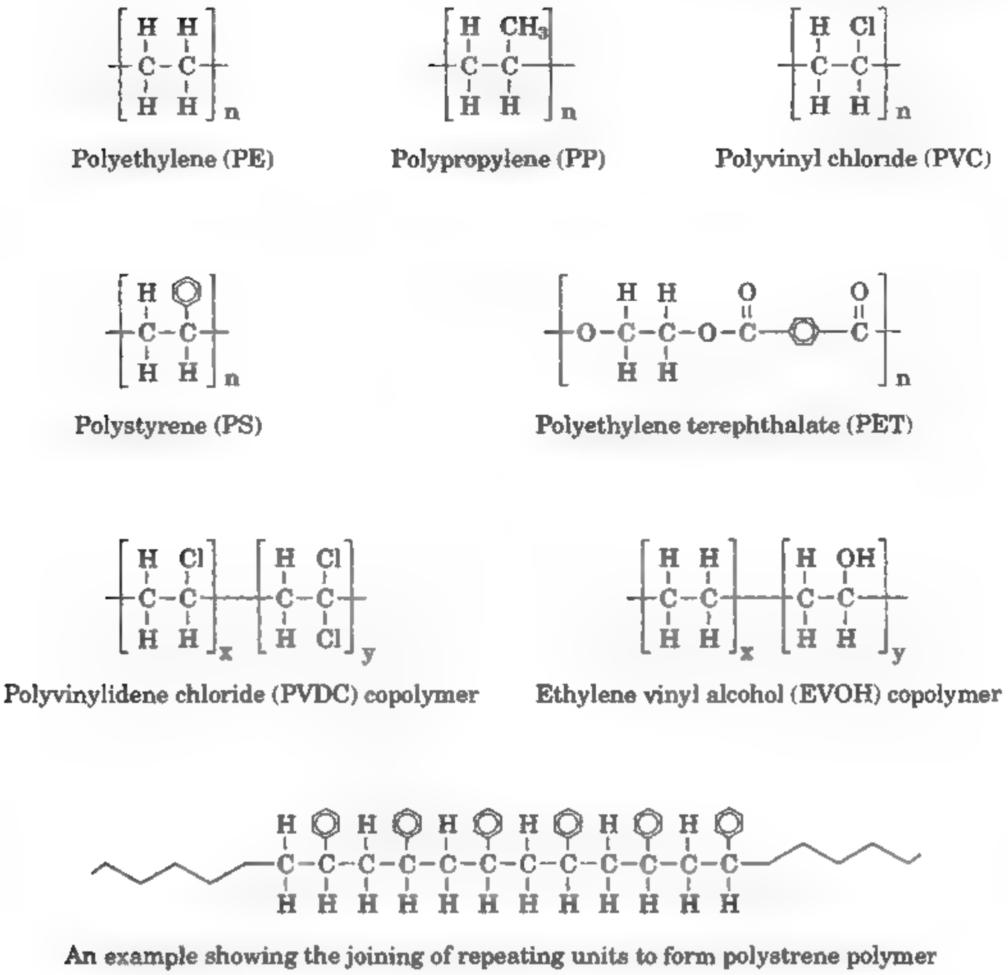
The gas-barrier properties of polymers are often expressed in terms of permeability: the lower the permeability, the better the gas barrier. Permeability depends on the plastic material; permeant gas; temperature; and, sometimes, relative humidity. The permeabilities of some commonly used food-packaging polymers are listed in Table 2.

Following are general discussions of some commonly used food-packaging polymers. These polymers are used mostly as bottles, containers, bags, films, and so on. Because these polymers have some overlapping properties, more than one polymer can often meet the performance requirements of a food-packaging application. The specific properties of a polymer film, sheeting, or container are usually provided by the manufacturer.

Polyethylene

PE is the most frequently used polymer in food-packaging applications because of its low cost, easy processing, and good mechanical properties. PE also has the simplest chemical composition of all polymers, being essentially a straight-chain hydrocarbon. The major classifications of PE are high-density polyethylene (HDPE), low-density polyethylene (LDPE), and linear low density polyethylene (LLDPE). These classifications differ in density, chain branching, crystallinity, and so on. Within each classification are numerous grades that have different additives, molecular weight distributions, and other properties.

HDPE has a density typically between 0.94 and 0.97 g/cm^3. It is a linear polymer with relatively few side-chain branches (Fig. 3), and hence the macromolecules can fold and pack into an opaque, highly crystalline structure.


Polyethylene (PE)

Polypropylene (PP)

Polyvinyl chloride (PVC)

Polystyrene (PS)

Polyethylene terephthalate (PET)

Polyvinylidene chloride (PVDC) copolymer

Ethylene vinyl alcohol (EVOH) copolymer

An example showing the joining of repeating units to form polystrene polymer

Figure 1. Chemical structures of some common food-packaging polymers.

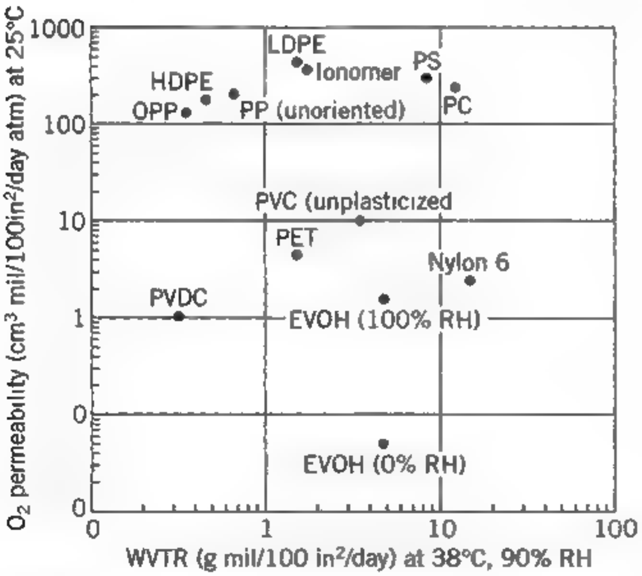


Figure 2. Gas barrier properties of some common food-packaging polymers.

Compared to LDPE, HPDE has a higher melting point (typically 135°C vs 110°C), greater tensile strength and hardness, and better chemical resistance. HDPE is used mostly to make blow-molded bottles for the packaging of products such as milk and water. It is also used for food containers, bags, films, extrusion coating, bottle closures, and the like.

LDPE has a density between 0.91 and 0.93 g/cm^3. It is a polymer with many long side-chain branches. LDPE is used mostly as film for packaging fresh produce and baked goods, as an adhesive in multilayer structures, and as waterproof and greaseproof coatings for paperboard packaging materials. The packaging film made from LDPE is soft, flexible, and stretchable. The film also has good clarity and heat sealability.

LLDPE has a density about the same as that of LDPE. LLDPE is a copolymer with many short side-chain branches. LLDPE has similar clarity and heat sealability to that of LDPE, as well as the strength and toughness of HDPE. With its superior properties, LLDPE has been replacing LDPE in many food-packaging applications. In recent years, many development efforts have focused on using single-site or metallocene catalyst to produce LLPDE films with superior heat seal and hot tack properties, clarity, abuse resistance, and strength (10,11).

Table 2 Barrier Properties of Some Common Packaging Plastics

Plastics	O_2 permeability[a]	CO_2 permeability[a]	WVTR[b]
PE			
Low density	300–600	1200–3000	1–2
High density	100–250	350–600	0.3–0.6
PP			
Unoriented	150–250	500–800	0.6–0.7
Oriented	100–160	300–540	0.2–0.5
PS	250–350	900–1050	7–10
PET	3–6	15–25	1–2
PVC			
Unplasticized	5–15	20–50	2–5
Plasticized[c]	50–1500	200–8000	15–40
PVDC	0.1–2	0.2–0.5	0.02–0.6
EVOH			
0% RH	0.007–0.1	0.01–0.5	—
100% RH	0.2–3	4–10	—
Ionomer	300–450	—	1.5–2
Nylon 6	2–3	10–12	10–20
PC	180–300	—	10–15

[a] Unit in (cm^3 mil)/(100 in^2/day atm) at 25°C.
[b] Unit in (g mil)/(100 in^2/day) at 38°C, 90% RH.
[c] Values depend greatly on plasticizer content.
Source: Data are taken from several sources.

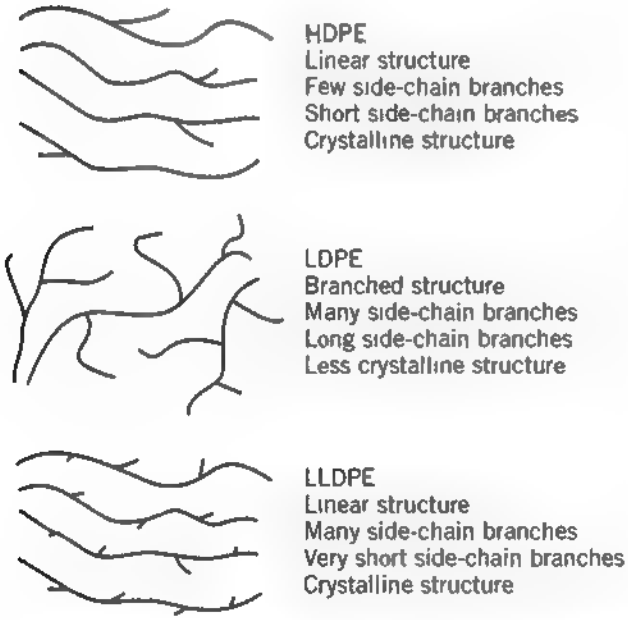


Figure 3. Molecular arrangements of HDPE, LDPE, and LLDPE.

Polypropylene

Polypropylene (PP) is a linear, crystalline polymer that has the lowest density (0.9) among all major plastics. Compared to PE, PP has higher tensile strength, stiffness, and hardness. It also has a higher melting temperature (165°C), and hence it is more suitable for hot filling and retorting applications.

A major application for PP is in packaging films. Commercial PP films are available in oriented and unoriented forms. Orientation can be achieved by stretching the film either uniaxially or biaxially during the film-forming process. Oriented PP film (OPP) has improved strength, stiffness, and gas-barrier properties; however, it is not heat sealable. Unoriented PP film has excellent clarity, good dimensional stability, and good heat-seal strength. Beside films, other major applications for PP are containers and closures.

Polystyrene

Polystyrene (PS) is an amorphous polymer that has excellent clarity. Solid PS is a clear, low-gas-barrier, hard, low-impact-strength (unless modified) material. It has a relatively low melting point (88°C) and can be readily thermoformed or injection molded into items such as food containers, cups, closures, and dishware. Because of its excellent clarity, PS film is often used as windows in paperboard boxes to display products (such as baked goods) that do not require a good gas barrier.

Expanded PS (EPS) of various bulk densities are manufactured by adding foaming agents in the extrusion process. EPS is used to make cups, bowls, plates, meat trays, clamshell containers, and egg cartons as well as protective packaging for shipping.

Polyethylene Terephthalate

PET (also abbreviated PETE) is the major polyester used for food packaging. The amorphous form of PET (APET) is used mostly as injection blow-molded bottles for carbonated soft drinks, water, edible oil, juices, and similar products. PET bottles are stronger and clearer and provide a better gas barrier than HDPE bottles, although they are more expensive.

APET is also used to produce films that have high strength, a high melting point (267°C), high scuff resistance, good clarity, good printing characteristics, and excellent dimensional stability. Because of their poor heat sealability, PET films are often laminated or extrusion coated with a heat-sealable layer such as PVDC and LDPE.

The crystallized form of PET (CPET) can withstand temperatures up to 220°C without deformation. Thus CPET food trays are used in microwave/conventional dual ovens. PETG is a copolymer of PET and 6% cyclohexane dimethanol that is used in thermoforming applications.

Polyvinyl Chloride

Polyvinyl chloride (PVC) is a clear, amorphous polymer used mostly for films and containers. Most often, plasticizers (organic liquids of low volatility) are added to the polymer to yield widely varying properties, depending on the type and amount of plasticizers used. Plasticized PVC films are limp, tacky, and stretchable, and the films are commonly used for packaging fresh meat and fresh produce. Unplasticized PVC sheets are rigid, and the sheets are often thermoformed to produce inserts for snacks such as chocolate and biscuits.

PVC bottles have better clarity, oil resistance, and barrier properties than those of HDPE. However, the use of PVC bottles in food packaging is relatively small due to poor thermal processing stability and environmental concerns with chlorine-containing plastics.

Polyvinylidene Chloride

PVDC commonly known under the trade name Saran, is a copolymer of vinylidene chlorine (85–90%) and vinyl chloride. The most notable advantages of PVDC are its excellent oxygen- and moisture-barrier properties. PVDC films have good clarity and grease and oil resistance.

PVDC is used in films, containers, and coatings. Monolayer PVDC films are used in household wraps. PVDC is often coextruded or laminated with other lower-cost polymers (such as OPP and PET) to form multilayer films or sheets. The multilayer films are used to package foods that require a good oxygen barrier, and the multilayer sheets are often thermoformed into semirigid containers. PVDC is also used in the form of latex for coating paper, film, and cellophane to achieve a better oxygen and moisture barrier, grease resistance, and heat sealability.

PVDC is more costly than most other commonly used food-packaging polymers. Similar to PVC, PVDC has poor thermal processing stability, and environmental concerns are associated with its use.

Ethylene Vinyl Alcohol Copolymer

Ethylene vinyl alcohol copolymer (EVOH) is a crystalline copolymer of ethylene (usually between 27 and 48%) and vinyl alcohol, and its properties are highly dependent on the relative concentrations of these comonomers. EVOH is best known for its exceptionally good oxygen-barrier properties, even compared to PVDC. The oxygen barrier increases with decreasing ethylene concentration and decreasing relative humidity. EVOH also has good resistance to hydrocarbons and organic solvents. EVOH is the most expensive of all commonly used food-packaging polymers (8).

EVOH is mostly used as an oxygen-barrier layer in multilayer structures, either laminated or coextruded. The multilayer structures containing EVOH are used as films or containers for packaging oxygen-sensitive foods. An example of a multilayer structure is PP/adhesive/EVOH/adhesive/PP, where the PP layers provide strength and a moisture barrier and the EVOH layer provides the oxygen barrier. This multilayer structure is suitable for hot filling and retorting applications.

Other Food-Packaging Polymers

Many other food-packaging polymers exist in addition to the major ones described previously. Ethylene vinyl acetate (EVA) is a low-cost copolymer used as an adhesive in coextrusion and as a heat-sealable layer. Compared to EVA, ionomers are more expensive adhesives that have better hot tack, adhesion to aluminum foil, and ability to heat seal through food contaminants (9). Nylon is a polyamide primarily used in applications that require good abrasion resistance and toughness; however, nylon has poor moisture-barrier properties and heat sealability. Polycarbonate (PC) is a relatively expensive polyester that has exceptional impact properties, and its food-packaging application is presently limited mostly to large returnable water bottles. Other packaging polymers include edible films (9), biodegradable films (9), oxygen-scavenging films (12), and temperature-compensating films (4).

BIBLIOGRAPHY

1. G. L. Robertson, *Food Packaging: Principles and Practice*, Marcel Dekker, New York, 1993.
2. W. Soroka, *Fundamentals of Packaging Technology*, Institute of Packaging Professionals, Herndon, Va., 1995.
3. E. Finson and R. J. Hill, "Glass-Coated Packaging Films Ready for Commercialization," *Packaging Technol. Eng.* 4, 36–43 (1995).
4. U. Stöllman, F. Johansson, and A. Leufvén, "Packaging and Food Quality," in C. M. D. Man and A. A. Jones, eds., *Shelf Life Evaluation of Foods*, Blackie Academic & Professional, New York, 1994, Chapter 4.
5. F A. Paine, *The Packaging User's Handbook*, Van Nostrand Reinhold, New York, 1991.
6. W. A. Jenkins and J. P. Harrington, *Packaging Foods with Plastics*, Technomic, Lancaster, Pa., 1991.
7. W. E. Brown, *Plastics in Food Packaging: Properties, Design, and Fabrication*, Marcel Dekker, New York, 1992.
8. K. R. Osborn and W. A. Jenkins, *Plastic Films: Technology and Packaging Applications*, Technomic, Lancaster, Pa., 1992.
9. A. L. Brody and K. S. Marsh, eds., *The Wiley Encyclopedia of Packaging Technology*, 2nd ed., John Wiley and Sons, New York, 1997.
10. T. D Stirling, "Solutions for Today's Food Packaging Challenges via Single-Site Catalyst Technology," in *Conf. Proc. of Future-Pak '97*, George O. Schroeder Associates, Appleton, Wisc., 1997.
11. J. deGroot et al., "ELITE Enhanced Polyethylene: A Performance and Cost Effective Alternative to EVA/LLDPE Blends," in *Conf. Proc. of Future-Pak '97*, George O Schroeder Associates, Appleton, Wisc., 1997.
12. M. L. Rooney, *Active Food Packaging*, Blackie Academic & Professional, New York, 1995.

KIT L. YAM
RAYMOND G. SABA
Y. C. HO
Rutgers University
New Brunswick, New Jersey

PACKAGING: PART IV—CONTROLLED/ MODIFIED ATMOSPHERE/VACUUM FOOD PACKAGING

The shelf life of foods such as fresh and processed meat, eggs, fish, poultry, fresh fruits, fresh vegetables, and soft bakery goods is limited in the presence of atmospheric oxygen due to three important factors: the biochemical effect of atmospheric oxygen, the activity of oxidative enzymes, and the growth of aerobic spoilage microorganisms. A fourth factor of no small importance is attack by insects. Each of these factors, alone or in conjunction with one another, can result in changes in color, flavor, odor, and overall deterioration in food quality, and the hazard of microbiological safety. Technologies employed by food processors to retard these deteriorative changes include chilled storage, freezing, thermal processing, water removal, osmotic adjustment, pH change, and the use of chemical additives and preservatives. However, increasing energy costs associated with freezing and drying, quality changes imposed by the processes themselves, and growing consumer concerns about chemical additives has compelled the food industry to look for alternative methods of food preservation.

Controlled/modified atmosphere/vacuum packaging is a relatively new preservation technology used extensively for quality retention. Though these technologies have been in commercial use only since the 1960s, the volume of food preserved under them exceeds that of canned or frozen foods in North America—and is perhaps even proportionately higher in Europe.

The normal composition of air is 20.9% oxygen (O_2), 78% nitrogen (N_2), 0.9% argon, and 0.03% CO_2. A controlled-atmosphere process involves alteration of the gaseous environment in and around a food and its maintenance at a specified level throughout the preservation period. A modified atmosphere, as the name implies, is one in which the normal composition of air is changed or modified within a package, but the change is not constant due to continued product respiration and permeation of gases through the package. Any modification usually, but not always, results in a reduction of the O_2 content of the air in the package headspace while increasing the level of CO_2. This results in the potential for enhanced quality retention for food products without the use of covert chemical or physical treatments such as preservatives, freezing, and drying. Controlled- and modified-atmosphere preservation and packaging are almost always enhancements of refrigeration as a preservation technology.

Vacuum processing and packaging means the removal of oxygen from the foods' environment by mechanical methods. Oxygen removal may also be accomplished by displacement with an inert or nearly inert gas such as nitrogen.

The concept of controlled/modified atmospheres for shelf-life extension of food is not new in food preservation. During the nineteenth century scientists discovered that the elevation of CO_2 and reduction of O_2 retarded catabolic reactions in respiring foods and slowed the growth of aerobic spoilage microorganisms. Basic research on the use of modified atmospheres for shelf-life extension of fruit, vegetables, fish, and meat was performed during the 1920s and 1930s. By 1938, 26% of chilled-carcass beef shipped from Australia and 60% of that shipped from New Zealand was being shipped under a CO_2-enriched atmosphere.

In the United States and Europe, successful application of controlled-atmosphere storage is extensive for apples and pears, which can be stored for up to 9 months using the correct $CO_2/O_2/H_2O$ vapor mixture in conjunction with temperature, ethylene, and relative humidity control. This technology has been commercial for more than 35 years.

Distribution of food in retail and hotel/restaurant/institutional (HRI) units packaged under modified atmospheres, that is, modified-atmosphere packaging (MAP), is a major application of technology for a variety of fresh and minimally processed food products. MAP is defined as the packaging of food products in which the gaseous environment has been changed to slow respiration rates, reduce microbiological growth, retard enzymatic spoilage, and slow biochemical changes with the intent of prolonging quality retention. The objectives of this article are to review the reasons for the market growth of MAP technology, the roles of gases used in MAP, the methods of atmosphere packaging, and the applications of MAP for quality retention of specific food groups. The advantages and disadvantages of MAP technology and the public health concerns of this technology are also addressed.

GROWTH OF MAP TECHNOLOGY

Despite the paucity of visibility, MAP technology has emerged as the premier packaging technology of the last years of this century. Currently, the United States leads the way in MAP technology, followed by the United Kingdom, France, and Germany. Thousands of food processors around the world use MAP technology for shelf-life extension and food distribution. It is estimated that the production of CAP/MAP/vacuum-packaged foods in North America is well in excess of 30 billion lb annually. The growth of MAP technology, for both medium- and long-term preservation of food, is due to a number of interrelated factors: consumer desire for higher-quality, more nearly fresh, and higher nutritional quality foods; the development of better but still imperfect distribution systems; improved packaging technologies; and energy costs.

Developments in New Polymeric Barrier Packaging/ Materials

The success of any food-packaging technology as a means of extending the shelf life of food is dependent on the characteristics of the package materials surrounding a product. Developments in polymer chemistry have resulted in production of low-gas-permeability package films, such as polyvinylidene chloride (PVDC) and ethylene vinyl alcohol (EVOH), and high-gas-permeability materials such as styrene block copolymers. PVDC and EVOH films have excellent water vapor–oxygen-, and carbon dioxide–barrier characteristics and can be laminated to structural and/or water vapor–barrier polymers to impart the desired strength, heat sealability, and permeability characteristics for quality retention of packaged food products. In addition, developments in high-speed, continuous, vertical, horizontal, and thermoforming packaging equipment compatible with the machinability characteristics of these films have also promoted the growth of new packaging technologies.

Market Needs and Consumer Demands for Convenience

Over the past 30 years, many changes have occurred in consumers' lifestyles and food preferences. The fundamental change is in relation to the traditional roles of women, one of which in the past was meal preparation. With more than 60% of women in industrialized countries in the workplace, the time previously available for shopping and food preparation has decreased very substantially. The result is that today, with the need for convenience, many consumers are prepared to pay with their disposable incomes to have shelf-stable convenience foods that require a minimum of preparation. In fact, the consumer is indirectly asking the food industry to take over a part of, or in some cases all, of the more time-consuming steps associated with food preparation. The food industry has responded to these consumer demands by providing a variety of high-quality, more nearly fresh, easy-to-prepare, preservative-free foods packaged under a modified atmosphere. One surprising result has been that the proportion of family disposable income spent on food has been decreasing.

Increasing Energy Costs

Increasing energy costs associated with traditional methods of food preservation/storage, such as freezing, has resulted in the growth of less energy-intensive and more economical methods of short- and long-term preservation, such MAP. It has been estimated that MAP is 18 to 20% less energy intensive compared to freezing for shelf-life extension of bakery products. Thermal processing to achieve ambient temperature shelf stability is energy intensive, but not nearly as much as freezing, which requires removal of heat of fusion as well as temperature reduction and maintenance of low temperature. Further, it has been demonstrated repeatedly that the 0°F (−18°C) traditionally employed for frozen storage is well above the optimum temperature. Frozen foods are best stored at below their glass transition temperatures, a more costly and energy-intensive process being overtly resisted by commercial frozen food–distribution interests. These groups have failed to recognize the quality and hence economic benefits to be derived from optimum temperatures.

These same distribution channel members have also refused to reduce chill temperatures to their optima and have thus generated what might be described as the development of private chilled-food chains operated by food processors themselves to maintain safety and quality.

As a result of these interrelated factors, MAP is rapidly emerging as the packaging technology of choice for the preservation of minimally processed food products.

METHODS OF ATMOSPHERE MODIFICATION

Methods of atmosphere modification within a packaged food product may be subdivided into two main categories: passive modification and active modification. In commodity-generated or passive modification, the product is packaged in a film with the correct gas permeability characteristics, and the atmosphere within the packaged product is modified as a result of the consumption of O_2 and generation of CO_2 through respiration of the product, plus the permeation of gases through the package materials and structure. Passive modification is commonly used to modify the gas atmosphere of fresh respiring fruits and vegetables. However, to maintain the correct gas mixture within the packaged product, the gas permeabilities of the packaging films must be selected to allow O_2 to enter the package at a rate similar to its consumption by the product. Similarly, CO_2 must be vented from the package to offset the production of CO_2 by the product. Failure to achieve this gas balance will result in a depletion of O_2 and a buildup of CO_2, resulting in adverse changes in products. Depletion of oxygen to near 0 leads to anaerobic respiration or fermentation and the production of adversely flavored compounds. Most fresh vegetables and fruits are vulnerable to this type of injury under anoxic conditions.

Several methods can be used to actively modify the gas atmosphere within the packaged product. These include vacuum packaging, vacuum followed by gas, and injection of or sweep by gas mixtures.

Vacuum packaging is used extensively by the meat industry to extend the shelf life and the keeping quality of primal cut or wholesale cuts of fresh red meat. The product is placed in a package structure fabricated from film of low oxygen permeability, air is removed under vacuum, and the package is heat sealed. Under conditions of a good vacuum, headspace O_2 is reduced to $<1\%$. CO_2, produced from tissue plus microbiological respiration, may eventually increase to 10 to 20% within the package headspace. These conditions, that is, low O_2 and/or elevated CO_2 levels, extend the shelf life of meat by inhibiting the growth of aerobic meat-spoilage microorganisms, particularly *Pseudomonas* and *Alternaria* species.

A novel method of active modification is through the use of oxygen absorbents after mechanical oxygen removal. These consist of sachets that are placed inside the packaged product. Alternately, the oxygen scavenger may be incorporated into the film. Another method of active modification is the use of ethanol vapor generators that modify the gas atmosphere by producing ethanol vapor within the package headspace to suppress mold growth. Both oxygen absorbents and ethanol vapor generators have been used for shelf-life extension of food.

Active modification may also be achieved by gas packaging. Gas packaging is simply an extension of vacuum packaging technology and involves the evacuation of air followed by the injection of the appropriate gas mixture.

GAS-PACKAGING EQUIPMENT

The gas-packaging technique involves removing air from the pack and replacing it with a mixture of gases; the pressure of gas inside the package usually reaches about 1 atm, that is, equal to the external pressure. This is usually achieved by one of three types of packaging equipment: horizontal or vertical flexible packaging equipment, thermoform/fill/gas flush/seal or preformed tray fill/vacuum gas flush/seal equipment, and snorkel or related bulk packaging equipment.

In the continuous flow wrap/gas flushing technique, the machine creates a tube of flexible material that encloses the product either by itself or on a carrier tray. The appropriate gas mixture is introduced in a continuous countercurrent flow into the package to force the air out, the ends of the package are heat sealed, and the packages are cut from each other (Fig. 1).

In either the preformed tray or thermoform/fill/gas flush/seal technique, a compensated vacuum method is used to introduce the gas mixture. In this method, product is placed into a thermoformed tray and a vacuum is drawn to remove most of the air. The vacuum is broken by the appropriate gas mixture and the package heat sealed with a top web of film (Fig. 2). An example of a thermoformed gas-packaged product is shown in Figure 3. One advantage of the in-line thermoform method of gas packaging is the high efficiency of removing oxygen to residual levels of <1%. The in-line thermoforming type of equipment may also be used for vacuum packaging or vacuum skin packaging. The advantage of gas flushing versus vacuum plus back gas flush is its high production rate, with up to 120 packages/min being gas packaged.

The third type of equipment is the snorkel or bulk package equipment. The product, whether in the packaged form or without a package, is placed inside a large flexible pouch. The machine holds the pouch and inserts probes or snorkels that remove the air from inside the bag. The vacuum is broken by the addition of the appropriate gas mixture, the probes are removed, and the package is sealed. This type of equipment is used for bulk packaging and retains the product in the gas-packaged bag throughout storage and distribution of the product. Obviously, this type of equipment may also be employed for vacuum packaging, such as for primal cuts of fresh red meat or fresh-cut vegetables.

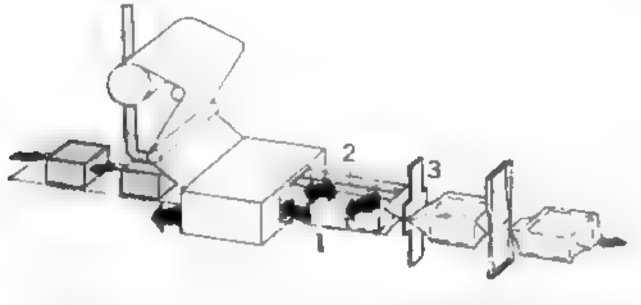


Figure 1. Horizontal flow wrap/fill/seal gas packaging equipment.

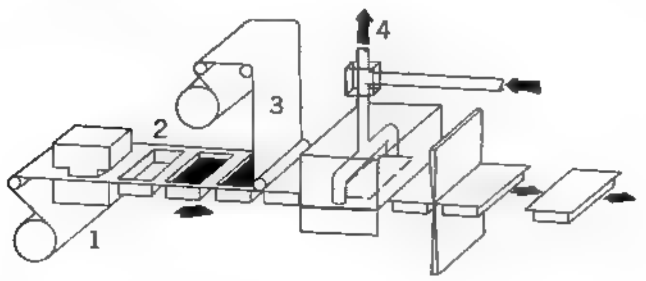


Figure 2. Thermoform/fill/vacuum/gas flush/seal packaging equipment. 1, Sheet; 2, formed cavity; 3, top closure web; 4, gas flush.

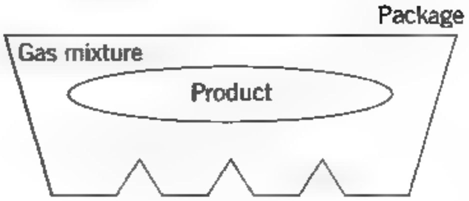


Figure 3. Example of a thermoformed gas-packaged meat product. The irregular base keeps the product above the package floor in order to have exposure to the modified atmosphere on all surfaces.

ROLES OF GASES USED IN MODIFIED ATMOSPHERE PACKAGING

Gases commonly used in gas packaging are nitrogen, oxygen, and carbon dioxide. Because the gases are those we breathe, the gases used in MAP are neither toxic nor dangerous, nor are they regarded as food additives.

Each gas plays a distinct and specific role in MAP foods. Nitrogen is an inert gas that has no effect on the food and has no antimicrobial properties. It is used mainly as a sweep gas to remove oxygen and as a filler gas to prevent package collapse in products that can absorb CO_2. It can also be used to replace oxygen in foods to retard biochemical spoilage of food, such as oxidations.

Oxygen is generally avoided in gas-packaging mixtures unless it is used to fulfill one of three functions: (*1*) it might be used with gas packaging of red meats to retain the desired oxymyoglobin red color or "bloom," apparently desired by many consumers; (*2*) it is used in low concentrations in packaging of products that respire, such as fresh-cut fruits and vegetables; (*3*) it may prevent anaerobic conditions and limit the growth of potentially harmful anaerobic microorganisms, specifically *Clostridium botulinum*, although this function has not been clearly demonstrated. Additionally, beneficial effects of high oxygen (above 40%) have been reported in retarding respiration rates of lettuce and on slowing microbiological growth on fresh nonfatty fish.

Carbon dioxide (CO_2) is both bacteriostatic and fungistatic, that is, it inhibits bacterial and mold growth. It can also be used to prevent insect growth in packaged and stored food products. Carbon dioxide is highly soluble in water and fats where it forms carbonic acid. Its high solubility may lower the pH, resulting in slight flavor changes in the food, and its absorption by the product may also cause package collapse. A summary of the salient properties of N_2, O_2, and CO_2 are shown in Table 1.

Table 1. Summary of Gas Properties Used in MAP

Oxygen	Maintains "bloom" (red color of fresh meat) Sustains basic metabolism of respiring foods Retards adverse effects of anaerobic respiration May prevent anaerobic microbiological growth
Nitrogen	Chemically inert Filler gas to occupy volume Retards oxidation by replacing oxygen Retards oxidative rancidity by replacing oxygen
Carbon dioxide	Inhibits bacterial and mold growth Fat and water soluble Prevents insect attack High concentrations can discolor products (meat) or injure produce (fresh fruits, vegetables)

Other gases that have antimicrobial or other functional properties include carbon monoxide, argon, nitrous oxide, ozone, sulfur dioxide, ethylene oxide, and ozone. Carbon monoxide, SO_2, ethylene oxide, and ozone generally are not included in MAP systems for a variety of reasons such as poor stability of the gas, limited approval for use in foods, or the formation of toxic or allergenic residues. Carbon monoxide (CO), however, is permitted in trace amounts (1–4%) in MAP of lettuce heads to prevent oxidative discoloration. Argon has been demonstrated to enhance MAP, perhaps by virtue of its molecular weight and size that may assist in removing oxygen more thoroughly than does nitrogen. Argon is being applied in fresh-cut vegetables and fish (and also in wine and salty snacks and chips that are not MAP applications).

ANTIMICROBIAL EFFECTS OF CO_2

Although the preservative action of CO_2 in foods has been known for many years, its mechanism of antimicrobial action has not been fully determined. Several theories have been postulated, however. One theory was that the displacement of O_2 was the main reason for the antimicrobial properties of CO_2. This theory was refuted by Coyne, who showed that aerobic spoilage organisms of fish grew well in 100% N_2 but not in 100% CO_2, indicating that displacement of O_2 was not the only reason for the antimicrobial effect of CO_2. Valley and Rettger (1) suggested that CO_2 acted by lowering extracellular pH as a result of the dissolution of CO_2 in the aqueous phase of the product. However, several studies have shown that when the pH is lowered by inorganic acid to values equivalent to those achieved under CO_2 atmospheres, bacterial and mold growth was less inhibited. Furthermore, CO_2 will inhibit microbial growth in buffered media and in naturally buffered foods such as meat. In response to these observations, Wolfe suggested that the inhibitory effect of CO_2 may be due to intracellular, rather than extracellular, pH changes that could interfere with enzymatic activities associated with cell metabolism. Several studies have shown that CO_2 inhibits oxaloacetate decarboxylase, succinate dehydrogenase, and cytochrome oxidase activity. However, King and Nagel (2) observed that CO_2 did not inhibit extracts of these enzymes. They observed that CO_2 specifically inhibited malic and isocitric dehydrogenase activity *in vitro* and concluded that the inhibitory effect of CO_2 may be due to its mass action effect on decarboxylases within the cell. Another theory suggests that CO_2 acts on the cell membrane, affecting the permeability characteristic of the membrane and its external environment by redistributing lipids at the surface. This has been demonstrated using a model system by Sears and Eisenberg (3) and has been proposed as the mechanism by which CO_2 may inhibit aerobic spore germination.

In conclusion, though many studies have been performed on the effect of CO_2 on microorganisms, there is little conclusive evidence of its mechanism of action. In a review on the effects of carbon dioxide on microbial growth and food quality by Daniels et al. (4) the following appear to be the salient points of investigations:

- The exclusion of oxygen by replacement with carbon dioxide may contribute to its overall antimicrobial effect by slowing the growth of aerobic spoilage microorganisms.
- The carbon dioxide/bicarbonate ion has an observed effect on the permeability of cell membranes.
- Carbon dioxide is able to produce a rapid acidification of the internal pH of the microbial cell with possible ramifications relating to metabolic activities.
- Carbon dioxide appears to exert an effect on certain enzyme systems.

Whatever the reason for its antimicrobial effect, CO_2 is effective in extending the shelf life of perishable foods by retarding microbiological growth. The overall effect of CO_2, in conjunction with refrigeration, is to increase both the lag phase and the generation time of spoilage microorganisms.

FACTORS INFLUENCING THE ANTIMICROBIAL EFFECT OF CO_2

Several factors influence the antimicrobial effect of CO_2, specifically, temperature, types and numbers of microorganisms, gas concentration, and package material properties. Each of these factors will be briefly reviewed.

Types of Microorganisms

The numbers and types of microorganisms present in a food product influence the antimicrobial effect of CO_2. Microorganisms differ considerably in their sensitivity to CO_2, and this sensitivity is related to the oxygen requirements of microorganisms (Table 2). It has been shown that CO_2 is most effective against aerobic spoilage microorganisms. Common aerobic spoilage organisms of meat, fish, and poultry (*Pseudomonads, Acinetobacter / Moraxella*) are inhibited by low concentrations of CO_2, a fact that is exploited in the gas packaging of muscle foods. Molds, most of which require oxygen for growth, are similarly inhibited by CO_2. Several studies have shown that low concentrations of CO_2 (10%) can be used to suppress mold growth. However, as with bacteria, mold species may vary in their sensitivity to the inhibitory effects of CO_2.

Table 2. Oxygen Requirements of Typical Food Spoilage Microorganisms

Aerobes	Require atmospheric oxygen for growth	Pseudomonads *Acinetobacter/Moraxella* *Micrococcus* Film yeasts Molds
Microaerophiles	Require low levels of oxygen	*Campylobacter* *Listeria* *Lactobacillus*
Facultative anaerobic microorganisms	Growing in the presence or absence of oxygen	*Staphylococcus* *Bacillus species* Enterobacteriaceae Vibrio Fermentative yeasts
Anaerobes	Inhibited (or killed) by oxygen	*Clostridium botulinum* *Clostridium perfringens*

Although CO_2 is effective against aerobic spoilage microorganisms, it has little or no antimicrobial effect against other spoilage microorganisms. Several studies have shown that CO_2 has little or no effect on the growth of facultative microorganisms in the Enterobacteriaceae or microaerophilic lactic acid bacteria. These organisms have been reported capable of growth in high concentrations of CO_2 (75–100%). Anaerobic bacteria, such as the food-poisoning organisms *Clostridium botulinum* and *C. perfringens*, are not affected by the presence of carbon dioxide, and the anaerobic conditions inside MAP foods may be conducive to their growth. There is concern that these organisms represent a potential public health hazard if present in gas-packaged foods, particularly if packaged under completely anaerobic conditions and stored under conditions of temperature abuse. Thus, although low concentrations of CO_2 (20–30%) may suppress the growth of aerobic microorganisms, they do little to inhibit the growth of facultative anaerobes, microaerophiles, and strict anaerobes. Under MAP conditions, the microbiological population shifts from a predominantly aerobic one to one comprising almost entirely CO_2-resistant anaerobic bacteria. In effect, the MAP suppresses competitive spoilage microorganisms and offers conditions conducive to pathogenic anaerobic microbiological growth. For this reason, prevention of contamination of MAP foods with pathogenic microorganisms is very important.

The age of the microbiological population also influences the inhibitory effect of CO_2. It has been shown that, as bacteria move from the lag phase to the log phase, the inhibitory effects of CO_2 are reduced. Thus, the earlier the product is gas packaged, the more effective CO_2 will be.

Concentration of CO_2

Early experiments clearly established that success in controlling aerobic spoilage deterioration of food was not simply dependent on the elimination of oxygen; rather, there was a definite requirement for CO_2 in the gas atmosphere. Coyne reported that the growth of *Achromobacter, Flavobacterium, Micrococcus, Bacillus*, and *Pseudomonas* was markedly inhibited by 25% CO_2 and completely inhibited by 50% CO_2 (5). In a later study, Coyne (6) reported that the optimal concentration for inhibition of aerobic spoilage microorganisms was 40 to 60% CO_2. No additional extension of shelf life was obtained by using higher concentrations of CO_2, and bacterial growth was less inhibited below these concentrations. Gill and Tan (7) examined the effect of various concentrations of CO_2, equivalent to pressures of 100 to 300 mm Hg, that is, 13 to 39% CO_2 in air, on the respiration rates of *Pseudomonas* species, *Acinetobacter, Alteromonas putrefaciens, Yersinia enterocolitica*, and *Enterobacter*. They reported that the respiration rates of most of the common spoilage organisms under investigation, with the exception of *Enterobacter* and *Brocothrix thermosphacta*, were affected by elevated levels of CO_2 in air. The level of CO_2 that resulted in maximum inhibition of the common spoilage organisms was approximately 200 mm Hg, or 26% CO_2 in air. However, the level of CO_2 investigated in this study had no antimicrobial effect on *B. thermosphacta*, which requires concentrations of 75% or more CO_2 for complete inhibition.

Several studies have shown that mold growth is also inhibited by low concentrations of CO_2. For example, many *Aspergillus, Rhizopus*, and *Cladosporium* species are completely inhibited by 5 to 10% CO_2 at 1°C. Other studies have shown that 20 to 30% CO_2 was sufficient to prevent the growth of meat-associated molds whereas 30 to 50% CO_2 was found to completely inhibit all mold species associated with the spoilage of bread and cakes. Again, this was not simply due to lower partial pressures of O_2 in the gas atmosphere, because it has been shown that many molds continue to grow normally when the O_2 concentration is maintained as low as 1%.

It is evident from these studies that the concentration of CO_2 in the gas mixture is very important in obtaining the desired extension of microbiological shelf life of the product. For most food products, with the exception of fruits and vegetables, a minimum of 20 to 30% CO_2 by volume is required to inhibit the growth of aerobic spoilage microorganisms and extend the shelf life of food, whereas for maximum shelf-life extension a concentration of 50 to 60% should be used. Though there is little or no increased antimicrobial effect or extension in shelf life at concentrations of CO_2 >50 to 60%, slightly higher concentrations are sometimes used to compensate for losses of headspace CO_2 through packaging films. However, too high a concentration of CO_2 may result in discoloration problems and drip loss in muscle foods. For fruits and vegetables, the maximum concentration of CO_2 is approximately 5 to 20% by volume because higher concentrations result in carbon dioxide damage.

Temperature

Temperature is a key variable in the antimicrobial activity of carbon dioxide. It has been shown that CO_2 is a very effective antimicrobial agent at low storage temperatures but less effective at higher temperatures. This increased inhibitory effect has been attributed to the greater dissolution of CO_2 in the aqueous phase of products at lower storage temperatures and resultant changes in intracellular pH and enzymatic activities of microorganisms. Therefore, any decrease in inhibition of spoilage and extension of shelf life at higher storage temperatures results from the lower solubility of CO_2 in the aqueous phase of the product. MAP should not be regarded as a substitute for proper storage temperature. Although MAP slows the deterioration of a food product, it never totally arrests deterioration. For respiring products, increasing the storage temperature also increases the rate of respiration, resulting in a decrease in shelf life.

The effects of temperature abuse are particularly important from the standpoint of safety. Temperature abuse of MAP muscle foods may result in the rapid growth of both spoilage and pathogenic bacteria. The minimum temperature for growth of *Salmonella* and *Escherichia coli* inoculated in ground meat and packaged in low- and high-permeability film is 0°C. Of major concern with respect to safety of MAP fish is the growth of and toxin production by *C. botulinum* type E, which has been demonstrated to be capable of growth at temperatures as low as 3.3°C. Proper refrigeration is therefore essential to assure the effectiveness of CO_2 as an antimicrobial agent and to prevent potential growth of pathogenic organisms that may not be suppressed by CO_2.

Package Material Permeability

The success or failure of MAP for respiring and nonrespiring foods depends on both the O_2 and CO_2 permeability of package materials to maintain the correct gas mixture in the package headspace. In addition, films used in gas packaging should also have low water vapor–transmission rates to prevent moisture loss or moisture gain, that is, to maintain the proper water vapor concentration because it enters into the MAP reaction. Polymers commonly used for MAP of food include polyester, polyamide (nylon), polypropylene (PP), polyvinylidene chloride (PVDC), ethylene vinyl alcohol (EVOH), ethylene vinyl acetate (EVA), and polyethylene (PE) (Table 3). Because all the desired characteristics of a package material, namely, structural strength, permeability, and heat sealability, are seldom found in one polymer, individual polymers may be coextruded or laminated to one another to produce films with the desired characteristics for MAP. Examples of laminated structures, some of which are shown in Table 4, for MAP of nonrespiring products include nylon/PE, nylon/PVDC/PE, EVA/EVOH/EVA, and nylon/EVOH/PE, all high-gas barrier structures. These composite structures have the desired characteristics for gas packaging of nonrespiring products, specifically strength, provided by the layer of gas and moisture-vapor impermeability (EVOH, PVDC, or nylon), and heat sealability, usually provided by PE.

Table 3. Examples of Food-Packaging Materials

Common name	Abbrev.	Subspecies
Polyethylene	PE	LDPE (low-density PE) LLDPE (linear LDPE) HDPE (high-density PE) Metallocene polyethylene
Polyvinyl chloride	PVC	UPVC (unplasticized PVC)
Polyvinylidene chloride	PVDC	
Polystyrene	PS	
Polyethylene terephthalate polyester	PET	
Ethyelene vinyl alcohol	EVOH	
Ethylene vinyl acetate	EVA	

Table 4. Examples of Laminated Films Used in MAP Foods

		Permeability ($cm^3/m^2/24h/1$ atm)		
Laminate	Gauge (μm)	O_2	CO_2	N_2
PET/PVDC/PE	12/3/50	8–10	30	8
UPVC/LDPE	400/75	15	30	4
Nylon/PVDC/PE	60/5/100	9	34	2.5
PVDC-coated PET/PE	15/60	2–4		
UPVC/PE	400/75	15		
Nylon/EVAL/Nylon/PE	25/10/25/100	5	20	1

For fruits and vegetables, the selection of the correct package material has been even more challenging for the packaging technologist because of the dynamic nature of the product. The ideal package material for gas packaging of fruits and vegetables must be able to keep a balance of low O_2 concentration (3–5%) within the package headspace and prevent buildup of high CO_2 concentrations (10–20%). Packaging films commonly used to achieve this balance include metallocene PE, EVA, and polypropylene.

APPLICATION OF GAS PACKAGING FOR SHELF-LIFE EXTENSION OF FOOD

The application of MAP using various gas mixtures has been used successfully by many food-processing and food-packaging companies around the world to extend the shelf life and retain quality of a variety of food products. Examples of food products currently gas packaged as well as the composition of gas mixtures used to extend the shelf life of each product are shown in Table 5. The optimum blend of gases for a specific product can be determined not simply by trial and error but only through a detailed, systematic study of the interdependent variables influencing product shelf life. These include the physical, chemical, and microbiological composition of the food product, the expected shelf life of the product under the normal storage conditions, and the choice of packaging film of correct gas- and moisture vapor–permeability characteristics. The application of MAP involving gas mixtures for shelf life extension of selected food groups is briefly reviewed.

Muscle Foods (Meat, Fish, and Poultry)

The shelf life of fresh beef, pork, fish, and poultry is limited in the presence of atmospheric oxygen by the growth and biochemical activities of gram-negative, psychrophilic (cold-loving) strains of microorganisms such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Moraxella* species. Several studies have indicated that these spoilage

Table 5. Examples of Gas Mixtures for Selected Food Products

Product	Temp (°C)	O_2 (%)	CO_2 (%)	N_2 (%)
		Meat products		
Fresh red meat	0–2	40–80	20	Balance
Cured meat	1–3	0	30	70
Pork	0–2	40–80	20	Balance
Offal	0–1	40	50	10
Poultry	0–2	0	20–100	Balance
		Fish		
White fish	0–2	30	40	30
Oily fish	0–2	0	60	40
Salmon	0–2	20	60	20
Scampi	0–2	30	40	30
Shrimp	0–2	30	40	30
		Plant products		
Apples	0–4	1–3	0–3	Balance
Broccoli	0–1	3–5	10–15	Balance
Celery	2–5	4–6	3–5	Balance
Lettuce	<5	2–3	5–6	Balance
Tomatoes	7–12	4	4	Balance
		Baked products		
Bread	RT[a]		60	40
Cakes	RT		60	40
Crumpets	RT		60	40
Crepes	RT		60	40
Fruit pies	RT		60	40
Pita bread	RT		99	1
		Pasta and ready meals		
Pasta	4		80	20
Lasagna	2–4		70	30
Pizza	5		52	50
Quiche	5		50	50
Sausage rolls	4		80	20

[a] Room temperature; staling is accelerated at refrigerated temperatures.

organisms can be inhibited by packaging the product under a CO_2-enriched atmosphere. Under these packaging conditions, the growth of common spoilage microorganisms is inhibited, and microaerophilic strains of lactic acid bacteria, which are more tolerant of high CO_2 concentrations, become the dominant spoilage microorganisms. As a result of bacterial growth being suppressed, levels of chemical compounds, such as trimethylamine in fish, and total volatile nitrogen, which are chemical indicators of microbial spoilage of food, are also reduced.

It is evident from Table 5 that a variety of gas mixtures can be used to extend the shelf life of muscle foods. Although microbial quality is usually the primary concern of the processor, biochemical changes can take place over time within the food itself that adversely affect color, flavor, and texture. For example, with red meat, oxygen is necessary for the bright red color or "bloom" that many consumers associate with good-quality meat. However, oxygen also promotes microbial growth and oxidative rancidity. Carbon dioxide is a bacteriostatic agent, that is, it inhibits microbial growth, but in excess quantities it will discolor fresh meat. The problem of balancing these two separate effects can be overcome by use of a gas mixture incorporating CO_2, O_2, and N_2. The N_2 is needed to prevent the package structure from collapsing around the product as CO_2 dissolves in the flesh, the fat, or both. When intact fresh beef and pork are packed in an atmosphere of 40 to 80% O_2, 20% CO_2, and the balance N_2 and kept under chilled conditions, a shelf life of 10 to 12 days can be expected, providing the meat was of good microbiological condition at the time of packaging. Ground meat displays shorter microbiological shelf life due to its microbiological dispersion. High concentrations of O_2 may be favored because they have been demonstrated to exert an antimicrobial effect and also ensure that the desired bright red color will be maintained.

For cured meat products containing color-fixing agents, where O_2 is not necessary and is even detrimental to product color, it is necessary to package in an O_2-free vacuum—a mixture of CO_2:N_2, or 100% of either gas.

With fish, enzymatic and/or autooxidative changes lead to the formation of low molecular weight aldehydes, ketones, alcohols, and carboxylic acids. Here, the gas mix employed depends on the product's fat content, which varies from 1% to a maximum of 20% for mackerel. Low-fat fish can be packaged in 60% CO_2:40% O_2 whereas high-fat fish, such as mackerel and herring, should be packaged in an oxygen-free environment to prevent rancidity problems. In the United States, low-oxygen fish packaging is discouraged because of fear of growth of nonproteolytic anaerobic *C. botulinum*, capable of growth and toxin production at temperatures as low as 3.3°C.

For meat, fish, and poultry products, the main areas affected by packaging of product under a modified gas atmosphere are summarized in Table 6. It is evident that the major impact of gas packaging is shelf-life extension of product, which, for meat products, can be doubled or tripled compared to air-packaged products (Table 7). Similar extensions in shelf life have been attained for fish and poultry. However, these shelf-life extensions can be obtained only if MAP products are stored under refrigerated-temperature distribution conditions. The effect of distribution temperature is therefore critical to ensure

Table 6. Changes in Meat, Fish, and Poultry as Brought About by Modified Atmospheres

Enzymatic aging process	Unaffected
Microbial spoilage	Increased CO_2 reduces growth of aerobic spoilage pyschrotrophs and psychrophiles
Fat oxiation	Reduced O_2 reduces oxidation of fats, although some oxidation can still occur at low O_2 tensions
Oxidation of myoglobin	Increased CO_2 promotes metmyoglobin formation and color darkening
Enzymatic oxidation	Can be reduced
Color	Elevated O_2 can retain oxymyoglobin cherry red color

Table 7. Shelf-Life Extension of MAP Meat at Chilled Temperatures

		Shelf life (days)	
Product	Temperature (°C)	Air-packaged products	MAP products
Beef cuts	4	4	10–12
Pork buts	4	4	6–9
Ground beef	4	2	4
Offals (eg, liver)	4	1–2	6

maximum benefits of CO_2-enriched atmospheres for shelf-life extension of muscle foods.

Plant Products

MAP, in conjunction with proper temperature control, can be used to reduce spoilage and quality losses in fresh fruit and vegetables. The objectives of MAP of fresh produce are to:

- Inhibit microbiological spoilage, such as mold growth on product surface
- Reduce the respiratory activities of products, thereby delaying ripening and senescence
- Reduce enzymatic browning of plants

As a result of the biological nature of the stored products, the packaging of fruits and vegetables under MAP conditions is one of the most challenging problems facing the packaging industry. The major difference between plant products and other fresh foods is that plant products continue to respire after being harvested. This results in a depletion of O_2 and a buildup of CO_2. However, when O_2 supplies are too low or CO_2 levels too high, anaerobic respiration (fermentation) occurs, resulting in the production of alcohols, aldehydes, and ketones or other volatiles that impart undesirable off-odors and off-flavors to the product. To avoid oxygen starvation in MAP products, headspace oxygen generally should not fall below 2%. Some varieties of apples can withstand up to 10% CO_2, whereas strawberries can withstand 25% CO_2 for prolonged periods. Examples of gas mixtures for shelf-life extension of fruits and vegetables are shown in Table 5. Although carbon monoxide is not permitted in MAP of food, 4% by volume is permitted by the FDA in modified atmospheres to retard browning of lettuce cores. Currently, this is the only use of CO for food preservation permitted by regulatory authorities, although it is used rarely, if ever.

The key to successful MAP distribution of fresh and fresh-cut fruits and vegetables is to ensure the correct balance of gases within the package headspace. The challenge for the packaging industry in MAP of plant products is to develop package materials that allow the transfer of selected gases and moisture in a controlled fashion to reduce biochemical activity without a major reduction in headspace oxygen or a buildup of carbon dioxide. This is a very complex task, requiring the control of significantly more variables than with other products. Common films used are PE, PP, EVA, and styrene block copolymer. Microperforated films (eg, P-Plus from Sidlaw), films diluted with minerals (eg, FreshHold), and films containing special polymers responsive to temperatures (eg, Landec) are all commercially available to control oxygen concentrations within packages of respiring foods. The use of PE bags with silicone rubber windows for controlled permeation of O_2 and CO_2 have been used in France. These bags were capable of maintaining 3 to 5% O_2 and CO_2 at 3°C.

Using MAP in conjunction with temperature control, shelf-life extensions of 15 to 30 days are possible for fruits, vegetables, and prepared salads. Excellent reviews on MAP of fruits and vegetables can be found in the text by Brody and the paper by Zagory and Kader (8).

Bakery Products

The major problems limiting the shelf life of many bakery products are mold spoilage and staling. The bakery industry can use several methods to control mold spoilage, the most common being the use of preservatives, such as sorbates and benzoates. However, preservatives can lead to off-odors and off-flavors in certain products. Furthermore, with increasing consumer concerns about preservatives, the bakery industry has been actively searching for alternative methods to extend the mold-free shelf life of bakery products. One such method is MAP. Research on the use of CO_2-enriched atmospheres for shelf-life extension of bakery products has been performed by Seiler (9) at the Flour Milling and Bakery Research Association in England. He reported shelf-life extensions of 300 to 400% for bread and cakes stored in concentrations of 60% CO_2 or more. The shelf-life extension obtained was dependent on the water activity (a_w) of the product, with the most significant increases in shelf life occurring in products of lower water activities, that is, $a_w \leq 0.85$. This effect is due to the types of mold present. In low-moisture products, xerophilic molds (low moisture tolerant), such as *Aspergillus* species, were the predominant spoilage molds, while *Penicillium* species were more common in products with higher a_w values, such as crumpets and fruit pies. Ooraikul reported a mold-free shelf-life extension of 1 month or more for English-style crumpets packaged under 60% CO_2. Though slightly longer extensions were possible using higher CO_2 concentrations, the gas was absorbed by the product, creating a vacuum-packed effect. Bakery companies in Europe commonly use gas packaging for shelf-life extension of bread and cakes. In addition to extending the mold-free shelf life of products, CO_2-enriched atmospheres have also been reported to prevent staling in many bakery products. Examples of bakery products commonly gas packaged are shown in Table 5. Packaging films commonly used with these products are barrier films such as nylon/PE and PVDC-coated polypropylene laminated or extrusion coated with polyethylene or ionomer.

Fresh Pasta and Other Products

MAP is widely employed to extend the shelf life and keeping quality of fresh pasta and snack-food products. Major spoilage problems associated with moist (30% H_2O) pasta products are growth associated with bacteria and molds and rancidity problems from fat oxidation. These spoilage problems can be retarded by eliminating headspace oxygen in the packaged products by packaging under an appropriate CO_2:N_2 gas mixture or under zero oxygen using an oxygen scavenger (Table 5). For other products, such as snack foods, which generally have a lower water activity, specifically, a_w, <0.6, packaging in as close to 100% N_2 as possible is sufficient to minimize oxidative rancidity problems because these products are not susceptible to microbial spoilage due to their low a_w and therefore do not need CO_2 in the gas mixture.

ADVANTAGES AND DISADVANTAGES OF MAP

The main benefits associated with MAP of food products are better quality retention, extended product shelf life and associated increase in market area, improved product presentation and consumer appeal, and a reduction in energy costs associated with freezing and freezer storage costs (Table 8).

Some of the disadvantages of the technique (Table 9) include:

- The initial higher cost of packaging equipment
- Higher cost of package materials
- Secondary fermentation problems caused by CO_2-resistant microorganisms
- Production of acidic-type odors due to dissolution of CO_2, in certain products such as fish
- A reduction in water binding capacity and an increase in drip loss of muscle foods due to change in product pH
- Slight discoloration problems in muscle foods such as meat
- Package collapse in products using a high CO_2 concentration (100%)
- The potential for generating conditions favorable for anaerobic pathogenic microbiological growth

The high CO_2 problems can be inhibited by reducing the proportion of CO_2 in the gas mixture and replacing it with a filler gas (N_2).

Table 8. Advantages of MAP of Food

Incrased shelf life
Superior quality retention
Incrased market area
Reduction in production and distribution costs
Improved presentation
Fresh appearance
Clear view of product when desired

Table 9. Disadvantages of Gas Packaging of Food

Initial high cost of packaging equipment, films, etc.
Discoloration of meat pigments
Leakage
Fermentation and swelling
Potential growth of anaerobic microorganisms of significance to public health

MICROBIOLOGICAL SAFETY OF MAP FOODS

A major concern about MAP foods is that they may be a public health risk, particularly if subjected to temperature abuse during distribution and retail storage. Further, a higher risk exists for consumer temperature abuse, mishandling, and overextending the product's shelf life.

The major microbiological concern with MAP foods is the growth of and toxin production by *C. botulinum* types A, B, and E. These spore-forming microorganisms pose the greatest threat to consumer safety due to:

- The complete or partial reduction of aerobic microorganisms and potential indicators of incipient spoilage by CO_2-enriched atmospheres, and
- The presence of elevated levels of CO_2 and anaerobic packaging conditions conductive to the growth of, and toxin production by, *C. botulinum*.

Several studies have shown that spores of *C. botulinum* can outgrow and produce toxin in MAP fish and in other products such as nitrogen-packed sandwiches and vacuum-packaged potatoes, especially if stored under temperature-abuse conditions. Though several challenge studies have been done with *C. botulinum* in MAP food, there is little conclusive evidence that MAP represents a significantly greater hazard than packaging in air, particularly under conditions of temperature abuse where CO_2 is less effective. Some believe that the inclusion of oxygen in the package headspace may prevent the growth of *C. botulinum* in products that may be susceptible to contamination by this pathogen; however, recent studies have refuted this claim and have shown that the inclusion of O_2 in the package headspace may offer no additional protection against *C. botulinum*.

Non-spore-forming pathogens in MAP foods include *Salmonella, Staphylococcus, Listeria*, and *Yersinia* species. Several studies have concluded that MAP storage does not increase the microbiological hazards from *Salmonella* species and *Staphylococcus aureus*. Hintlian and Hotchkiss (10) reported that the growth of both *S. aureus* and *Salmonella* species is inhibited by high CO_2 concentrations and that the level of inhibition increased as storage temperature decreased. Other pathogens, such as *Y. enterocolitica* and *L. monocytogenes* can grow slowly at refrigerated storage temperatures and so should be controlled by other means. Little is known about the ability of these organisms to grow in MAP foods, however, and further research is needed in this area.

CONCLUSION

MAP involving gas mixtures is not a panacea and should not be regarded as a substitute for good manufacturing practices, HACCP programs, and proper distribution temperature conditions for shelf-life extension of food. When used in conjunction with these factors, however, substantial extensions in shelf life and economies in production and distribution are possible. Although the concept of MAP may appear to be simple, its implementation requires the careful evaluation of the chemistry, physiology, and microbiology of the food system in relation to the dynamics of the microenvironment and the packaging material.

BIBLIOGRAPHY

1. G. Valley and L. F. Rettger, "The Influence of Carbon Dioxide on Bacteria," *J. Bacteriol.* **14**, 101–137 (1927).
2. A. D. King and C. W. Nagel, "Influence of Carbon Dioxide Upon the Metabolism of *Pseudomonas aeruginosa*," *J. Food Sci.* **40**, 362–366 (1975).
3. D. F. Sears and D. F. Eisenberg, "A Model Representing a Physiological Role of Carbon Dioxide at the Cell Membrane," *J. Gen. Physiol.* **44**, 869–877 (1961).
4. J. A. Daniels, R. Krishnamurthi, and S. H. Rizvi, "A Review of the Effects of Carbon Dioxide on Microbial Growth and Food Quality," *J. Food Prot.* **48**, 532–537 (1985)
5. F. P. Coyne, "The Effect of Carbon Dioxide on Bacterial Growth with Special Reference to the Preservation of Fish, Part I," *J. Soc. Chem. Ind. (London)* **51**, 119T–121T (1933).
6. F. P. Coyne, "The Effect of Carbon Dioxide on Bacterial Growth with Special Reference to the Preservation of Fish, Part II," *J. Soc. Chem. Ind. (London)* **52**, 19–24 (1933).
7. C. O. Gill and K. H. Tan, "Effect of Carbon Dioxide on Meat Spoilage Bacteria," *Appl. Environ. Microbiol.* **39**, 317–324 (1980).
8. D. Zagory and A. A. Kader, "Modified Atmosphere Packaging of Fresh Produce," *Food Technol.* **42**, 70–77 (1988)
9. D. A. L. Seiler, "The Microbiology of Cake and Its Ingredients," *Food Trade Rev.* **48**, 339–344 (1978).
10. C. B. Hintlian and J. H. Hotchkiss, "The Safety of Modified Atmosphere Packaging: A Review," *Food Technol.* **40**, 70–76 (1986).

GENERAL REFERENCES

N. Y. Aboagye et al., "Energy Costs in Modified Atmosphere Packaging and Freezing Processes as Applied to a Baked Product," in M. LeMaguer and P. Jelen, eds., *Food Engineering and Process Applications*, Vol. 2, Elsevier Applied Science, Amsterdam, The Netherlands, 1986, pp. 417–427.

B. Blakistone, *Principles and Applications of Modified Atmosphere Packaging of Food*, 2nd ed., Blackie, Glasgow, Scotland, 1998.

A. L. Brody, "Integrating Aseptic and Modified Atmosphere Packaging to Fulfill a Vision of Tomorrow," *Food Technol.* **50**, 56–66 (1996).

A. L. Brody, *Controlled / Modified Atmosphere / Vacuum Packaging of Foods*, Food and Nutrition Press, Trumbull, Conn., 1990

A. L. Brody, "Minimally Processed Foods Demand Maximum Research and Education," *Food Technol.* **52**, 62, 64, 66, 204, 206 (1998).

A. L. Brody, *Modified Atmosphere Food Packaging*, Institute of Packaging Professionals, Herndon, Va., 1994.

S.-O. Enfors and G. Molin, "The Influence of High Concentrations of Carbon Dioxide on the Germination of Bacterial Spores," *J. Appl. Bacteriol.* **4**, 279–285 (1978).

J. Farber and K. L. Dodds, *Principles of Modified Atmosphere and Sous Vide Product Packaging*, Technomic Lancaster, Pa., 1995.

G. Finne, "Modified- and Controlled-Atmosphere Storage of Muscle Food," *Food Technol.* **36**, 128–133 (1982).

C. A. Genigeorgis, "Microbial and Safety Implications of the Use of Modified Atmospheres to Extend the Storage Life of Fresh Meats and Fish," *Int. J. Food Microbiol.* **1**, 237–251 (1985).

K. E. Goodburn and A. C. Halligan, *Modified Atmosphere Packaging: A Technology Guide*, British Food Manufacturing Research Association, Leatherhead, England, 1988.

A. D. Lambert, J. P. Smith, and K. L. Dodds, "Combined Effects of Modified Atmosphere Packaging and Low-Dose Irradiation on Toxin Production by *Clostridium botulinum* in Fresh Pork," *J. Food Prot.* **54**, 94–101 (1991).

B. Ooraikul, "Gas Packaging for a Bakery Product," *Can. Inst. Food Sci. Technol. J.* **15**, 313–317 (1982).

S. A. Palumbo, "Is Refrigeration Enough to Restrain Food Borne Pathogens?," *J. Food Prot.* **49**, 1003–1009 (1986).

R. T. Parry, *Principles and Applications of Modified Atmosphere Packaging of Food*, Blackie, Glasgow, Scotland, 1993.

M. L. Rooney, *Active Food Packaging*, Blackie, Glasgow, Scotland, 1995.

J. P. Smith et al., Novel "Approach to Oxygen Control in Modified Atmosphere Packaging of Bakery Products," *Food Microbiol.* **3**, 315–320 (1986)

J. P. Smith et al., "Shelf Life Extension of a Bakery Product Using Ethanol Vapor," *Food Microbiol.* **4**, 329–337 (1987).

J. P. Smith, E. D. Jackson, and B. Ooraikul, "Microbiological Studies on Gas Packaged Crumpets," *J. Food Prot.* **46**, 279–283 (1983).

S. K. Wolfe, "Use of CO and CO_2 Enriched Atmospheres for Meats, Fish, and Produce," *Food Technol.* **34**, 55–58 (1980).

L. L. Young, R. D. Reviere, and A. B. Cole, "Fresh Red Meats: A Place to Apply Modified Atmospheres," *Food Technol.* **42**, 65–69 (1988).

AARON BRODY
Rubbright•Brody, Inc.
Duluth, Georgia

See also FRESH-CUT FRUITS AND VEGETABLES: MODIFIED ATMOSPHERE PACKAGING; CONTROLLED ATMOSPHERES FOR FRESH FRUITS AND VEGETABLES.

PALM OIL

Palm oil has now become the second most important vegetable oil after soybean oil in the world's oils and fats market. The dynamic growth of the palm-oil industry in Malaysia began around 1970. The pace of growth, in fact, has been so rapid that palm oil has now overtaken soybean oil and rapeseed oil as the growth leader. The oil palm is planted commercially in more than a dozen countries around the world, in West and Central Africa, Southeast Asia, Central and South America, China, and Papua New Guinea. However, the Southeast Asian nations of Malaysia and Indonesia are the major palm-oil-producing countries and between them account for about 80% of the world's palm-oil production. The overwhelming importance of Malaysia in world palm-oil exports can be seen from the fact that in 1989 it exported nearly 6 million tons and produced about 58% of the world palm-oil output (1,2).

used in conjunction with these factors, however, substantial extensions in shelf life and economies in production and distribution are possible. Although the concept of MAP may appear to be simple, its implementation requires the careful evaluation of the chemistry, physiology, and microbiology of the food system in relation to the dynamics of the microenvironment and the packaging material.

BIBLIOGRAPHY

1. G. Valley and L. F. Rettger, "The Influence of Carbon Dioxide on Bacteria," *J. Bacteriol.* **14**, 101–137 (1927).
2. A. D. King and C. W. Nagel, "Influence of Carbon Dioxide Upon the Metabolism of *Pseudomonas aeruginosa*," *J. Food Sci.* **40**, 362–366 (1975).
3. D. F. Sears and D. F. Eisenberg, "A Model Representing a Physiological Role of Carbon Dioxide at the Cell Membrane," *J. Gen. Physiol.* **44**, 869–877 (1961).
4. J. A. Daniels, R. Krishnamurthi, and S. H. Rizvi, "A Review of the Effects of Carbon Dioxide on Microbial Growth and Food Quality," *J. Food Prot.* **48**, 532–537 (1985)
5. F. P. Coyne, "The Effect of Carbon Dioxide on Bacterial Growth with Special Reference to the Preservation of Fish, Part I," *J. Soc. Chem. Ind. (London)* **51**, 119T–121T (1933).
6. F. P. Coyne, "The Effect of Carbon Dioxide on Bacterial Growth with Special Reference to the Preservation of Fish, Part II," *J. Soc. Chem. Ind. (London)* **52**, 19–24 (1933).
7. C. O. Gill and K. H. Tan, "Effect of Carbon Dioxide on Meat Spoilage Bacteria," *Appl. Environ. Microbiol.* **39**, 317–324 (1980).
8. D. Zagory and A. A. Kader, "Modified Atmosphere Packaging of Fresh Produce," *Food Technol.* **42**, 70–77 (1988)
9. D. A. L. Seiler, "The Microbiology of Cake and Its Ingredients," *Food Trade Rev.* **48**, 339–344 (1978).
10. C. B. Hintlian and J. H. Hotchkiss, "The Safety of Modified Atmosphere Packaging: A Review," *Food Technol.* **40**, 70–76 (1986).

GENERAL REFERENCES

N. Y. Aboagye et al., "Energy Costs in Modified Atmosphere Packaging and Freezing Processes as Applied to a Baked Product," in M. LeMaguer and P. Jelen, eds., *Food Engineering and Process Applications*, Vol. 2, Elsevier Applied Science, Amsterdam, The Netherlands, 1986, pp. 417–427.

B. Blakistone, *Principles and Applications of Modified Atmosphere Packaging of Food*, 2nd ed., Blackie, Glasgow, Scotland, 1998.

A. L. Brody, "Integrating Aseptic and Modified Atmosphere Packaging to Fulfill a Vision of Tomorrow," *Food Technol.* **50**, 56–66 (1996).

A. L. Brody, *Controlled / Modified Atmosphere / Vacuum Packaging of Foods*, Food and Nutrition Press, Trumbull, Conn., 1990

A. L. Brody, "Minimally Processed Foods Demand Maximum Research and Education," *Food Technol.* **52**, 62, 64, 66, 204, 206 (1998).

A. L. Brody, *Modified Atmosphere Food Packaging*, Institute of Packaging Professionals, Herndon, Va., 1994.

S.-O. Enfors and G. Molin, "The Influence of High Concentrations of Carbon Dioxide on the Germination of Bacterial Spores," *J. Appl. Bacteriol.* **4**, 279–285 (1978).

J. Farber and K. L. Dodds, *Principles of Modified Atmosphere and Sous Vide Product Packaging*, Technomic Lancaster, Pa., 1995.

G. Finne, "Modified- and Controlled-Atmosphere Storage of Muscle Food," *Food Technol.* **36**, 128–133 (1982).

C. A. Genigeorgis, "Microbial and Safety Implications of the Use of Modified Atmospheres to Extend the Storage Life of Fresh Meats and Fish," *Int. J. Food Microbiol.* **1**, 237–251 (1985).

K. E. Goodburn and A. C. Halligan, *Modified Atmosphere Packaging: A Technology Guide*, British Food Manufacturing Research Association, Leatherhead, England, 1988.

A. D. Lambert, J. P. Smith, and K. L. Dodds, "Combined Effects of Modified Atmosphere Packaging and Low-Dose Irradiation on Toxin Production by *Clostridium botulinum* in Fresh Pork," *J. Food Prot.* **54**, 94–101 (1991).

B. Ooraikul, "Gas Packaging for a Bakery Product," *Can. Inst. Food Sci. Technol. J.* **15**, 313–317 (1982).

S. A. Palumbo, "Is Refrigeration Enough to Restrain Food Borne Pathogens?," *J. Food Prot.* **49**, 1003–1009 (1986).

R. T. Parry, *Principles and Applications of Modified Atmosphere Packaging of Food*, Blackie, Glasgow, Scotland, 1993.

M. L. Rooney, *Active Food Packaging*, Blackie, Glasgow, Scotland, 1995.

J. P. Smith et al., Novel "Approach to Oxygen Control in Modified Atmosphere Packaging of Bakery Products," *Food Microbiol.* **3**, 315–320 (1986)

J. P. Smith et al., "Shelf Life Extension of a Bakery Product Using Ethanol Vapor," *Food Microbiol.* **4**, 329–337 (1987).

J. P. Smith, E. D. Jackson, and B. Ooraikul, "Microbiological Studies on Gas Packaged Crumpets," *J. Food Prot.* **46**, 279–283 (1983).

S. K. Wolfe, "Use of CO and CO_2 Enriched Atmospheres for Meats, Fish, and Produce," *Food Technol.* **34**, 55–58 (1980).

L. L. Young, R. D. Reviere, and A. B. Cole, "Fresh Red Meats: A Place to Apply Modified Atmospheres," *Food Technol.* **42**, 65–69 (1988).

AARON BRODY
Rubbright•Brody, Inc.
Duluth, Georgia

See also FRESH-CUT FRUITS AND VEGETABLES: MODIFIED ATMOSPHERE PACKAGING; CONTROLLED ATMOSPHERES FOR FRESH FRUITS AND VEGETABLES.

PALM OIL

Palm oil has now become the second most important vegetable oil after soybean oil in the world's oils and fats market. The dynamic growth of the palm-oil industry in Malaysia began around 1970. The pace of growth, in fact, has been so rapid that palm oil has now overtaken soybean oil and rapeseed oil as the growth leader. The oil palm is planted commercially in more than a dozen countries around the world, in West and Central Africa, Southeast Asia, Central and South America, China, and Papua New Guinea. However, the Southeast Asian nations of Malaysia and Indonesia are the major palm-oil-producing countries and between them account for about 80% of the world's palm-oil production. The overwhelming importance of Malaysia in world palm-oil exports can be seen from the fact that in 1989 it exported nearly 6 million tons and produced about 58% of the world palm-oil output (1,2).

THE OIL PALM AND ITS FRUITS

The oil palm *Elaeis guineensis* has its origin in West Africa. The world *Elaeis* is derived from the Greek word *elaion*, meaning oil, while *guineensis* refers to its origin in Guinea. *Elaeis guineensis* is the highest yielding oil-bearing plant and is now planted as a commercial crop throughout oil-palm growing countries of the world, thriving best within 10° of the equator (3,4).

The Central and South American oil palm, *Elaeis oleifera*, is another species in the genus, but it is of little economic importance owing to its poor oil yield. However, it is an important source of breeding material for oils of greater unsaturation.

Within the species *Elaeis guineensis*, several strains can be identified on the basis of variations of the endocarp of the fruits:

Dura: thick shell, comparatively less oil-bearing mesocarp.

Pisifera: very thin shell to shell-less, high proportion of oil-bearing mesocarp.

Tenera: hybrid of dura and pisifera, thin shell, more mesocarp, hence more oil.

Today, in Malaysia the commercially planted palms are mostly tenera.

The oil palm produces fruits by 2–3 years after planting. The mature palm produces 20–25 tons of fruit bunches per hectare annually, and the economic life span of the tree is about 25–30 years (see Fig. 1). The proportion of oil to bunch of tenera palms is around 25%, although the factory oil extraction ratio is about 20–22% owing to milling losses. The oil yield per hectare annually can average between 4–5 tons (3,4).

The palm fruits are borne on spikelets of the fruit bunch. The mature fruit bunch contains from a few hundred to a few thousand fruits and weighs as much as 40 kg. The loose or detached palm fruit is avoid in shape and varies from 2 to 5 cm in length, weighing 8–20 g. Unripe fruits of the tenera are mostly deep violet to black in color, ripening to a deep orange to bright red.

The palm fruit consists of the outer skin, the mesocarp (pulp), and the seed containing the endocarp (shell) and the kernel (see Fig. 2). Palm oil is extracted from the mesocarp and should be distinguished from palm kernel oil derived from the kernel.

EXTRACTION OF CRUDE PALM OIL

Several stages of processing can be identified in the extraction of palm oil (1,5) from fresh fruit bunches:

Sterilization

Freshly harvested fruit bunches are brought to the mill and sterilized by high-pressure steam (120–140°C at 40 psi) with minimal delay so as to inactivate the lipolytic enzymes that cause oil hydrolysis and fruit deterioration. Sterilization also loosens the fruits from the bunch stalk and coagulates the protein and mucilaginous materials present in the palm fruit to prevent emulsion formation during oil recovery.

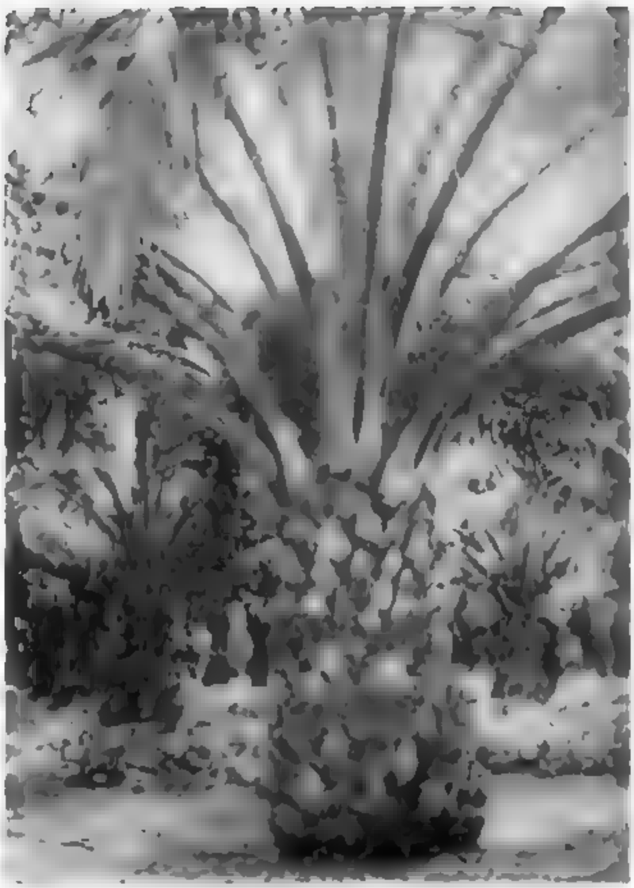


Figure 1. The oil palm bearing fruit bunches.

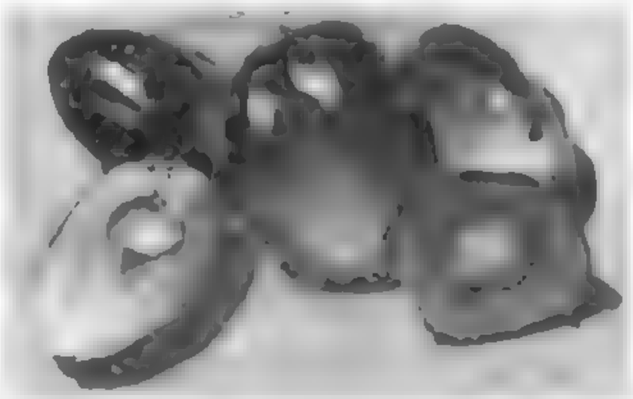


Figure 2. Oil palm fruits and sections showing the oil-bearing mesocarp and the endocarp (kernel).

Bunch Stripping

After sterilization, the fruits are separated from the bunch stalks by mechanical stripping. The sterilized fruits go to the digester while the empty fruit bunches are incinerated, yielding an ash rich in potash that is used as a fertilizer, particularly in acid fields.

Digestion

The fruits are reheated (digested) by steam to a temperature of 80–90°C. The digestion process prepares the fruits for oil extraction by rupturing the oil-bearing cells in the mesocarp and loosening the mesocarp from the nuts.

Oil Extraction

Crude palm oil is extracted from the digested fruit mash by the use of the screw press without nut (kernel) breakage. Crude palm-oil liquor and fiber and nuts are discharged from the screw press.

Clarification and Purification

Crude palm oil extracted from the digested fruits by pressing contains varying amounts of water, solids, and dissolved impurities that must be removed. Fiber particles from the pressed crude oil are first removed by passing the oil over a vibrating screen, and sand and dirt are allowed to settle. Water is removed by settling or centrifuging and finally by vacuum drying. However the clarified crude oil still contains 0.1–0.25% of moisture, which helps to maintain oxidative stability and prevent the deposition of small amounts of soluble solids known as gums. The final oil is pumped to storage tanks pending despatch to the refinery.

The mill fibers (fibers from mesocarp after oil extraction) are partly burned to generate energy for mill-processing. The nuts are dried and cracked to obtain the kernels that can be further processed to yield palm kernel oil; the resultant cake left after oil extraction is used as animal feed.

REFINING AND FRACTIONATION

The minor components of crude palm oil are phospholipids, carbohydrates, trace metals, free fatty acids, mono- and diglycerides, sterols, carotenoid pigments, tocopherols, tocotrienols, and oxidized and odoriferous materials. Refining reduces those impurities of the crude oil that will adversely affect the quality of the end product, while retaining as much as possible of the tocopherols and tocotrienols because, as antioxidants, they confer oxidative stability to the end product. Crude palm oil is processed by two main methods, refining and fractionation (1,6).

Physical Refining

In Malaysia, physical refining, which subjects the oil to steam distillation under high temperature and vacuum, is the most widely adopted method for refining palm oil. The refining process consists of two major stages, pretreatment and distillation.

Pretreatment. Pretreatment involves conditioning with concentrated phosphoric acid, subsequent adsorption with activated bleaching earth under vacuum at a temperature of 90–130°C, followed by filtration. Pretreatment reduces the levels of phosphorus, trace metals, peroxides, carotenoid pigments, and their condensation products.

Deodorization and Distillation. The distillation step of physical refining involves steam distillation under vacuum at a temperature of 250–260°C to produce an oil of bland flavor and odor with oxidative stability. The process removes the volatile free fatty acids, aldehydes, and ketones (formed by oxidation of unsaturated fatty acids), degraded carotenoid pigments, hydrocarbons, sterols, tocopherols, and tocotrienols (vitamin E). However, not all the tocopherols and tocotrienols are lost in the distillate, as up to 62% of them is retained in the refined oil (7).

Fractionation

The triglyceride composition of palm oil is such that its major triglycerides include oleodipalmitin with a melting point of 37°C and palmitodiolein with a melting point of 19°C.

The aim of fractionation is to produce a liquid olein (slip melting point 21.6°C and cloud point 8.8°C) and a solid stearin. The olein is used mainly as a liquid vegetable oil for cooking and frying, while the stearin fraction is used in margarine, shortening, and frying fat.

In Malaysia, fractionation of palm oil into the low melting point olein and the high melting point stearin is done by two processes, referred to as dry fractionation and detergent fractionation.

During dry fractionation, the oil is first heated to 70–75°C to melt all crystal nuclei. The heated oil is then passed to a crystallizer equipped with a cooling jacket where it is cooled to 18–20°C for 4–8 h. The crystallization of stearin in liquid olein causes slurry formation. The liquid olein is separated from the solid stearin by membrane filtration of the slurry.

In detergent fractionation, the separation of olein and stearin is made possible by mixing the crystalline slurry with a detergent solution (sodium lauryl sulfate and magnesium sulfate). On centrifuging, the lighter olein is separated from the heavier aqueous phase containing the stearin. The liquid olein is washed, dried, and sent for storage while the stearin is recovered from the aqueous phase and the detergent is recycled. Special doubly fractionated olein (superolein) with a lower cloud point and higher iodine value and a softer stearin known as palm midfraction (PMF) can be obtained by a second fractionation. Crude palm oil can also undergo detergent fractionation, first to produce crude olein and crude stearin and thereafter refined to obtain the edible grades of palm olein and palm stearin.

THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF REFINED PALM OIL AND ITS FRACTIONS

The chemical and physical properties of an oil are important determinants of its applications. Palm oil consists of more than 99% glycerides with less than 1% nonglyceridic minor components. Table 1 shows the fatty acid composition and other characteristics, including the solid fat con-

Table 1. Characteristics of Refined Palm Oil and its Fractionated Products

Characteristic	Refined palm oil	RBD palm olein	RBD palm olein (doubly fractionated)	RBD palm stearin	Palm midfraction
Fatty acid composition, %					
Lauric 12:0	0.2	0.2	0.1	0.3	0.1
Myristic 14:0	1.1	1.0	1.0	1.5	1.2
Palmitic 16:0	44.0	39.8	36.6	52.0	51.0
Palmitoleic 16:1	0.1	0.2	0		
Stearic 18:0	4.5	4.4	4.0	4.9	5.6
Oleic 18:1	39.2	42.5	44.8	33.3	34.0
Linoleic 18:2	10.1	11.2	12.6	7.6	7.4
Linolenic 18:3	0.4	0.4	0.2	0.1	0.1
Arachidic 20:0	0.4	0.4	0.7	0.3	0.6
Total saturated	50.2	45.8	42.4	59.0	58.5
Total monounsaturated	39.2	42.5	44.8	33.3	34.0
Total polyunsaturated	10.5	11.6	12.8	7.7	7.5
Iodine value	53.3	58.0	61.4	44	42.5
Slip melting point °C	36.0	21.6	19.0	44–56	
Solid fat content, %					
5°C	62.2	51.1		67.2	
10°C	50.3	37.0	16.7	61.2	76.1
15°C	35.2	19.2	5.0	53.1	66.2
20°C	23.2	5.9	3.1	43.4	52.1
25°C	13.7			33.9	21.9
30°C	8.5			27.0	18.3
35°C	5.8			22.2	13.4
40°C	3.5			17.3	7.9

tent at varying temperatures, of refined, bleached, and deodorized (RBD) palm oil and its fractions (8–10).

Table 2 shows the content of the principal minor components, namely, the vitamin E and sterols (11–13) in refined palm oil.

It is of interest to point out that palm oil is unusually rich in the vitamin E tocotrienols (unsaturated analogues of tocopherol), with the tocotrienols constituting between 70–80% of the total vitamin E content as opposed to other vegetable oils in which tocopherols are the predominant vitamin E species (12).

The sterols identified in palm oil consist of cholesterol, campesterol, stigmasterol, and sitosterol, but their levels are considerably reduced after refining. Palm oil, like other vegetable oils, is generally regarded as cholesterol-free, although a level of 2 ppm has been reported in the refined oil from the initial levels of 7–13 ppm in the crude oil (9).

Table 2. Vitamin E and Sterols in Refined Palm Oil

	Mean/range in ppm
Vitamin E	716 (559–902)
α-Tocopherol	158
α-Tocotrienol	143
γ-Tocotrienol	329
δ-Tocotrienol	86
Sterols	
Cholesterol	2
Campesterol	26–30
Stigmasterol	12–23
Sitosterol	68–114

Crude palm oil is one of the richest natural sources of β-carotene (12), containing an average of 700 ppm of carotenoids (giving it the red color) mainly in the forms of β-carotene (56%) and α-carotene (35%). However the carotenoids are absent in the refined oil as they are heat-destroyed and removed during the deodorization stage of oil refining.

Palm kernel oil (PKO), as distinct from palm oil, is extracted from the kernel of the oil-palm fruit. About 9 tons of kernel oil are produced for every 100 tons of palm oil. Although derived from the same palm fruit, the physical and chemical characteristics of the two oils are quite different. PKO is grouped in the same class as coconut oil. It is particularly rich in the short-chain and medium-chain fatty acids, viz, 12:0 lauric acid (48%) and 14:0 myristic acid (16%).

Like palm oil, PKO can also be fractionated into palm kernel olein and palm kernel stearin, which greatly extends its food applications, particularly in specialty fats that require a high solid fat content between 20 and 30°C.

USES OF PALM OIL AND ITS FRACTIONS

Refined palm oil, RBD palm olein, and RBD palm stearin are the principal forms of processed palm oil that are now traded in the world vegetable oils market. Palm oil, palm olein, and palm stearin are, respectively, semisolid, liquid, and solid at 25°C. Because of their wide plastic range, palm oil and its fractions lend themselves to a wide range of food uses from liquid oils to the semisolid fats such as shortening, margarine, and vanaspati (vegetable ghee). In temperate countries, palm olein can also be used as a liquid oil by blending with domestic sources of polyunsaturated oils up to a level of 30%. Such a blend greatly increases the oxidative stability of the polyunsaturates.

The extreme versatility of palm oil and its products for edible and nonedible uses (10,14) are shown as follows:

Food Uses
Cooking/frying oil
Shortening and cooking fats
Vanaspati
Margarine
Cocoa butter substitute
Ice cream
Coffee whitener
Bakery and biscuit fats
Instant noodles
Filled milk
Soup mixes

Nonfood Uses
Oleochemicals (fatty acids, fatty alcohols, fatty amines, glycerol and methyl esters)
Detergents and surfactants
Soap and metal soap
Candles
Lubricating grease
Cosmetics
Tin plating
Diesel substitute
Plasticizer for plastics

NUTRITIONAL AND HEALTH ASPECTS OF PALM OIL

Recent years have seen a considerable amount of anti-palm-oil publicity, claiming that palm oil is unhealthy because of its saturated fat content. The allegation, however, has been challenged by recent animal and human feeding experiments (15). New findings now indicate that not only has palm oil little effect on raising blood cholesterol levels, but, in many instances, its consumption has led to a reduction in blood cholesterol levels (Table 3). The rationale for palm oil's lack of a cholesterol-raising effect despite the fact that it is classified as a saturated fat may be attributed to the following:

- Its conspicuous lack of the cholesterol-raising 12:0 lauric acid and 14:0 myristic acid (the two acids accounting for less than 1.5% of total).
- Its principal saturated fatty acid, the 16:0 palmitic acid, is now regarded as neutral and certainly much less cholesterolemic than previously thought (19).
- Moderate abundance of the cholesterol-lowering monounsaturated 18:1 oleic acid plus adequate amounts of the essential 18:2 linoleic acid.
- Presence of vitamin E tocotrienols that are known to suppress liver cholesterol synthesis, consequently reducing blood levels of cholesterol (23,24).

Table 3. Effects of Palm-Oil-Containing/Enriched Diets on Blood Cholesterol Levels

Effect	Reference
Chickens	
Palm oil lowered blood cholesterol compared to butter, lard, tallow, and coconut oil	16
Rats	
Palm oil lowered blood cholesterol compared to olive oil	17
Palm oil comparable to corn oil	18
Monkeys	
Palm oil lowered blood cholesterol compared to coconut oil and comparable to high-oleic safflower oil	19
Humans	
Palm oil lowered blood cholesterol compared to coconut oil	20
Palm oil lowered blood cholesterol compared to butterfat and vanaspati	21
Palm oil comparable to a Dutch Fat blend	22

In addition, palm-oil derived tocotrienols have also been demonstrated to inhibit platelet aggregation and thromboxane production (24), which may well explain the observation of a favorable antithrombotic effect found in palm-oil-fed animals (25,26).

BIBLIOGRAPHY

1. *PORAM Technical Brochure*, 4th ed., The Palm Oil Refiners Association of Malaysia, 1989.
2. S. Mielke, "Past and Prospective World Production and Exports of Palm Oil," in F. D. Gunstone, ed., *Palm Oil*, John Wiley & Sons, New York, 1987.
3. B. J. Wood, "Growth and Production of Oil Palm Fruits" in Ref. 2.
4. C. W. S. Hartley, *The Oil Palm*, 3rd ed., Longman Scientific & Technical, UK, 1988.
5. J. H. Maycock, "Extraction of Crude Palm Oil" in Ref. 2.
6. F. V. K. Young, "Refining and Fractionation of Palm Oil," in Ref. 2.
7. M. L. Wong, R. E. Timms, and E. M. Goh, "Colorimetric Determination of Total Tocopherols in Palm Oil, Olein and Stearin," *Journal of the American Oil Chemists' Society* **65**, 258–261 (1988).
8. B. K. Tan and C. H. Flingoh, "Malaysian Palm Oil, Chemical and Physical Characteristics," *PORIM Technology* **3**, **4**, (May 1981).
9. C. L. Chong, "Chemical and Physical Characteristics of Palm Oil," in *Lecture Series, Seventh Palm Oil Familiarisation Programme*, September 3–11, 1987, Palm Oil Research Institute of Malaysia, 1987.

10. S. A. Kheiri, "End Uses of Palm Oil—Human Food," in Ref. 2.
11. A. Gapor, *Palm Oil Development*.
12. S. H. Goh, Y. M. Choo, and S. H. Ong, "Minor Constituents of Palm Oil," *Journal of the American Oil Chemists' Society* **62**, 237–240 (1985).
13. B. Tan, "Palm Carotenoids, Tocopherol and Tocotrienols," *Journal of the American Oil Chemists' Society* **66**, 770–776 (1989).
14. R. J. deVries, "End Uses of Palm Oil—Industrial Uses," in Ref. 2.
15. "New Findings of Palm Oil," *Nutrition Reviews* **45**, 205–207 (1987).
16. A. S. H. Ong, N. Qureshi, A. A. Qureshi, et al., "Effects of Palm Oil and Other Dietary Fats on Cholesterol Regulation in Chickens," *The Federation of American Societies for Experimental Biology Journal* **2**, A1541 (1988).
17. M. Sugano, "One Counter Argument to the Theory that Tropical Oils are Harmful," *Yukagaku* (Journal of the Japanese Oil Chemists' Society, in Japanese) **40**, 48–51 (1987).
18. K. Sundram, H. T. Khor, and A. S. Ong, "Effect of Dietary Palm Oil and its Fractions on Rat Plasma and High Density Lipoproteins," *Lipids*.
19. K. C. Hayes, A. Pronczuk, and S. Lindsey, "Dietary Palmitic Acid Lowers Cholesterol by Comparison to Lauric and Myristic Acids in Monkeys," *Circulation* **80** (Suppl. 2) (1989).
20. T. K. W. Ng, Khalid Hassan, J. B. Lim, "Non-Hypercholesterolemic Effects of a Palm Oil Diet in Malaysian Volunteers," *Abstract in Nutrition and Health Aspects of Palm Oil*, 1989 PORIM International Palm Oil Development Conference, September 5–9, 1989, Kuala Lumpur, Malaysia.
21. S. A. Khan, A. B. Chugtai, L. Khalid, and S. A. Jaffery, "Comparative Physiological Evaluation of Palm Oil and Hydrogenated Vegetable Oils in Pakistan," *Abstract in Nutrition and Health Aspects of Palm Oil*, 1989 PORIM International Palm Oil Development Conference, September 5–9, 1989, Kuala Lumpur, Malaysia.
22. G. Hornstra, K. Sundram, and A. Kester, "The Effect of Dietary Palm Oil on Cardiovascular Risk in Man," *Abstract in Nutrition and Health Aspects of Palm Oil*, 1989 PORIM International Palm Oil Development Conference, September 5–9, 1989, Kuala Lumpur, Malaysia.
23. A. A. Qureshi et al. "The Structure of an Inhibitor of Cholesterol Biosynthesis Isolated from Barley," *Journal of Biological Chemistry* **261**, 10544–10550 (1986).
24. A. A. Qureshi, N. Qureshi, Z. Shen, et al., "Lowering of Serum Cholesterol in Hypercholesterolemic Humans by Palm Vitee," *Abstract in Nutrition and Health Aspects of Palm Oil*, 1989 PORIM International Palm Oil Development Conference, September 5–9, 1989, Kuala Lumpur, Malaysia.
25. G. Hornstra, "Dietary Lipids and Cardiovascular Disease: Effects of Palm Oil," *Oleagineux* **43**, 75–87 (1988).
26. M. L. Rand, A. A. M. Hennissen, and G. Hornstra, "Effects of Dietary Palm Oil on Arterial Thrombosis, Platelet Responses and Platelet Fluidity in Rats," *Lipids* **23**, 1019–1023 (1988).

YOON HIN CHONG
Palm Oil Research Institute of Malaysia
Selangor, Malaysia

See also FATS AND OILS: CHEMISTRY, PHYSICS, AND APPLICATIONS.

PARASITIC ORGANISMS

Although long-recognized as an important public-health problem, foodborne diseases are receiving an increasingly greater share of the public's attention. The Centers for Disease Control estimates that 6.5–33 million Americans (3–14% of the population) contract a foodborne infection annually, with more than 9,000 associated fatalities. Foodborne pathogens also constitute a major cause of morbidity and mortality worldwide. The World Health Organization estimates that more than 1 billion cases of acute diarrhea occur annually in children 1–5 years of age in the developing countries. The most important microorganisms and parasites transmitted by food, particularly meat, are listed in Table 1. In the United States, concern over protozoan and helminth parasites lies mainly with *Toxoplasma gondii* (toxoplasmosis), *Sarcocystis* spp. (sarcocystosis), *Trichinella spiralis* (trichnellosis), larval and adult

Table 1. Foodborne Disease Agents

Viruses
Hepatitis A virus
Norwalk agent
Other Norwalklike viruses
Rotaviruses
Adenoviruses[a]
Astroviruses[a]
Echoviruses
Snow-mountain agent
Cockle agent
Coxsackie B viruses
Caliciviruses[a]
Bacteria
Salmonella spp.
Shigella spp.
Campylobacter spp.
Escherichia coli
Vibrio parahaemolyticus
Listeria monocytogenes
Yersinia spp.
Helminths
Anaskis spp.
Eustrongylides spp.
Pseudoterranova spp.
Trichinella spiralis
Angiostrongylus spp.
Paragonimus spp.
Diphyllobothrium latum
Taenia spp. (*cysticercus*)
Protozoa
Amoeba
Giardia
Isospora
Cryptosporidia
Sarcocystis
Toxoplasma

[a] Viruses that cause gastroenteritis and that may be foodborne.

infections of *Taenia saginata* and *Taenia solium* (taeniasis and cysticercosis), and the fishborne nematodes (eg, anasakiasis). The importance of these parasites relates not only to their direct clinical effect, but also to the high economic costs associated with prevention of their entrance into the domestic food chain and with medical care. Estimated annual costs for inspection and condemnation at animal slaughter are shown in Table 2. Note that losses for fascioliasis and ascariasis represent condemnations for reasons that are more esthetic than public health ones.

All these losses have an important indirect consequence, namely lowering consumer confidence in meat safety and quality. Although reliable figures are difficult to obtain, some economists and public-health workers believe that the cost of foodborne parasites, in terms of public-health expenditures and lost wages, is very high. Estimated annual costs for human taeniasis (from bovine cysticerocosis) is $100,000; for trichinosis, $1.5–2.2 million; and for congenital toxoplasmosis, up to $215–323 million in terms of medical care and work loss (1).

Consumer attitudes, even in the absence of firm facts, can also have a severe economic impact on an industry. A good example of this is trichinellosis. It is thought that public awareness that pork might be infected with *Trichinella spiralis* costs pork producers several hundred million dollars annually in suppressed consumer demand and loss in exports (2). This despite the fact that less than one hog per 1,000 might be infected and only about 50 to 100 human infections are diagnosed each year in the United States. Obviously, the potential consequences of increased public concern over pork-transmitted *Toxoplasma gondii* will also be economically important. It should be noted that firm facts on the role of meat in human toxoplasmosis are still lacking, and research is underway to clarify the question of the importance of pork vis à vis cat feces in transmission to humans. All this underscores the fact that producers have much at stake in controlling and, in some cases, eradicating foodborne parasites, regardless of their direct role in animal and human health.

The rising public concern over food safety presents new and difficult challenges for the research community. Many current procedures and strategies for ensuring a healthful and safe meat supply will require marked improvement in order to meet expected demands; this is especially true for foodborne parasites (3). For the three most serious meat-transmitted parasitic diseases, toxoplasmosis, cysticercosis, and trichinellosis, truly effective or practical control strategies or technologies are not yet available in or applicable for the United States.

Table 2. Estimated Losses Due to Slaughterhouse Condemnations

Commodity	Annual ($ millions)
Cattle	
Livers (fascioliasis)	7.1
Carcasses (cysticercosis)	?[a]
Carcasses (sarcocystosis)	2.0
Swine	
Livers (ascariasis)	50

[a] No economic estimates available; however, in 1988 approximately 11,000 carcasses required refrigeration or cooking before passing inspection.

In many countries, strategies for control emphasize detection of parasites at the abattoir. Of the 74 waterborne and foodborne parasites identified in a current review, virtually all must be diagnosed by tedious gross or microscopic observation for individual organisms (4). The biotechnological revolution, however, is having a significant impact on the efforts to develop rapid and more sensitive inspection techniques. Hybridoma technology has facilitated identification and isolation of antigens with superior immunodiagnostic value. In many instances, however, the availability of these antigens for application is limited. Advances in recombinant DNA technology, however, may solve this problem by permitting large scale *in vitro* production.

It is also becoming apparent that for many, if not all, foodborne pathogens, the ultimate solution is prevention of infection of food at the production or farm level. This strategy is dependent, however, on a thorough understanding of the epidemiology of the parasites. The improvement in diagnostic tools through application of hybridoma and recombinant DNA technologies will have a great impact here, also. Of particular value has been the development of molecular probes (DNA, monoclonal antibodies) to identify cryptic infections, to characterize parasite strains, and to determine transmission dynamics. Prevention of infection strategies may, in many instances, also require the use of vaccines. Again, recombinant DNA technology is playing a vital role in the development of candidate vaccines for parasites such as toxoplasmosis and cysticercosis.

This article identifies the themes common to the control strategies for these diseases and highlights the role of biotechnology in developing those controls.

TOXOPLASMOSIS

General Concepts and Unsolved Problems

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is prevalent in humans and animals worldwide (5). *T. gondii* is transmitted by three routes: fecaloral, congenital, and carnivorism. Cats, the only definitive hosts, are the ultimate source of the infective oocyst stage, which is excreted in their feces. After a short period of exposure to air, oocysts sporulate (form sporozoites), becoming infectious for virtually all warm-blooded animals, including humans. Oocysts can survive in the environment for several months to a year. When oocysts from the environment are ingested the sporozoites, excyst from the oocyst in the intestine penetrate the wall then migrate to and grow inside st cells of the body. After several multiplication cycles, the parasite forms cysts in muscles, the liver, and the central nervous system (CNS). These tissue cysts can persist in humans and other animals virtually for life. The parasites within the tissue cysts are infectious to humans or animals if eaten with the meat. During pregnancy if a woman or animal acquires a primary infection with *Toxoplasma* there is a period when parasites circulate in the bloodstream, thereby rendering the fetus vulnerable to invasion. If cats ingest either infected meat or oocysts, the sexual stages develop in the small intestine. Oocysts eventually are produced and excreted.

Although 30–40% of adults in the United States have serum antibodies to *Toxoplasma*, most postnatally acquired *T. gondii* infections are asymptomatic or are manifested by mild flulike signs. However, toxoplasmosis can cause devastating illness in some adults and children. Loss of vision (chorioretinitis) and mental retardation are the two most important clinical symptoms in congenitally infected children. Although the child is infected before birth, such symptoms may not appear until adolescence. A fulminating, often fatal, illness may develop in patients with acquired immunodeficiency syndrome (AIDS) or in patients given immunosuppressive therapy while receiving organ transplants or treatment for malignancies. Toxoplasmosis is a widespread and important cause of abortion and neonatal death in sheep, goats, and pigs (5). Infection by live *T. gondii* parasites (live vaccine) induces a life-long immunity, thus there is optimism for developing a vaccine for animals, especially cats (6).

Control Strategies and Biotechnology

Strategies to control toxoplasmosis should include prevention of infection in livestock, identification of infected animals in the food chain and their removal or treatment to render the meat safe for human consumption, and prevention and treatment of toxoplasmosis in populations at risk (AIDS patients, transplant recipients, pregnant women and animals). Biotechnology can play an important role in all these strategies, as discussed below.

Identification and Characterization of Immunogens

Unlike the complex antigens of helminths (*Trichinella, Cysticercus*), *T. gondii* has only four major surface antigens (43, 30, 22, and 14 kDA); all strains of *T. gondii* so far investigated share these important antigens (6). Thus, there appears to be no potential strain-dependent vaccination or diagnostic problems. At least two proteins (P30 and P14) have been characterized. P30 is the major dominant antigen and constitutes 5% of the total surface protein (6,7). The gene encoding the P30 protein has been cloned, expressed in a vector, and the fusion protein is being tested for diagnosis. A preliminary study suggests that identification of high levels of specific IgA antibodies against P30 antigen is useful for diagnosing congenital toxoplasmosis in children (8).

DNA-Probes and Diagnosis

Cloned and amplified genes are proving to be a useful probe technology for diagnosis of infectious diseases. The B-1 gene probe of *T. gondii* has been cloned and amplified, and techniques have been developed to detect with it DNA from as little as one *Toxoplasma* organism. With refinements leading to mass production, the cost might be lowered so that this DNA probe can be used routinely for diagnosis (9).

Subunit Vaccines

Recent studies using mutant strains of *T. gondii* indicate that the persistence of live organisms in the host is not necessary for the maintenance of protective immunity (10). If the antigens that stimulate the protective response can be identified, cloned, and properly expressed in vectors, the expressed proteins can be tested as candidates for vaccination. The genes for two other major proteins, the P14 surface antigen and the P28 cytoplasmic antigen, have also been cloned and expressed in vectors. These fusion proteins are being tested as immunogens for possible inclusion in a *Toxoplasma* vaccine (10,11).

SARCOCYSTIS

Public-Health Significance

Two types of *Sarcocystis* infection (sarcocystis) are known in humans. One type is the intramuscular cyst stage probably acquired via feces of a carnivore, and the other type includes intestinal stages that develop after ingestion of raw infected meat. Because the actual sources of infection for the intramuscular cyst stage are unknown and because it is unlikely that human flesh is consumed often enough by carnivores to maintain a human–carnivore cycle in nature, it is hypothesized that such infections are accidental zoonoses, possibly existing in nature in a nonhuman primate–carnivore cycle (3). Because only about 40 such infections have been reported worldwide, most originating in tropical areas, this type of sarcocystosis does not represent a significant public-health problem. Two sources for the human intestinal infection are known: ingestion of raw beef and raw pork. However, little documentation exists regarding their public-health significance. There are several possible reasons for this lack of information. The life cycles and epidemiology of *Sarocystis* spp. have only recently been described. The sporocyst stage shed in the feces of infected humans is relatively small and often is overlooked in examination of feces. Finally, considering the excellent sanitation system used for disposal of human sewage in the United States, opportunities for infection are probably rare.

Biology and Life Cycle

Sarcocystis spp. are obligate two-host parasites, usually requiring a herbivorous intermediate host and a carnivorous final host. The specificity of these parasites is usually greater for the intermediate host than for the final host. Either host might be infected simultaneously with several species of *Sarcocystis*.

For *Sarcocystis hominis*, domestic cattle are the intermediate hosts; and for *Sarcocystis suihominis*, domestic swine are the intermediate hosts. Human and such nonhuman primates as cynomolgus and rhesus monkeys and chimpanzees serve as final hosts, which become infected by eating mature cysts containing zoites found in meat. The parasite undergoes development to the sexually reproducing stage in the lamnia propria of the small intestine. The offspring of the sexually distinct male and female gametes are called sporocysts (containing the infective sporozoite stage), which passes out in the feces. Cattle and pigs become infected when they ingest the sporocyst-containing sporozoites. After a complex migration and a sexual reproduction phase, a second-generation stage, termed the merozoite, invades the host's muscles where it develops to the cyst stage.

Diagnosis in Humans and Animals

In meat animals, the clinical signs of acute sarcocystosis provide a presumptive diagnosis. Animals may show excessive salivation or runny nose, loss of body hair, abortion, reduced milk production, or death. For a short time during peak acute illness the serum enzymes may be elevated. Serologic tests may be helpful in confirming the diagnosis; the indirect hemagglutination test and ELISA have been used in the laboratory but are not yet available commercially. Definitive diagnosis depends on finding parasites and lesions in sick animals. Hemorrhage and nonsuppurative inflammation are often found in tissues from accutely infected animals. The finding of large numbers of immature cysts in muscle biopsies or after death is indicative of a recent clinical infection.

Diagnosis of sarcocystosis in humans or other carnivores is based on finding sporocysts in the feces. This is best accomplished by standard laboratory fecal flotation procedures using saturated sugar solution or zinc sulfate followed by examination with bright-field microscopy.

Prevention and Control

The key to prevention and control of sarcocystosis in meat animals is the elimination from the environment of the sporocyst stage in carnivore feces. Any control measure designed to prevent carnivores from becoming infected and to prevent fecal contamination of feed and water will break the cycle. Such measures are also described for control of toxoplasmosis. In addition, every effort should be made to bury, incinerate, or otherwise remove dead livestock from farms, fields, or grazing areas to prevent domesticated and feral carnivores from acquiring infections.

To prevent human intestinal infection, meat should be cooked or frozen before it is eaten. In two studies of beef from retail food stores infectious *S. cruzi* (infectious for dogs but not humans) was present in various raw roasts, steaks, and hamburger but not in processed meats, such as frozen hamburger patties or minute steaks, beef bologna, frankfurters, or in hamburger cooked to 60°C (140°F) or higher.

TRICHINELLOSIS

Public Health Significance

Trichinellosis in the United States is not considered a major public-health problem in terms of number of human infections. But because the inspection of swine for *Trichinella spiralis* muscle larvae is not performed, as it is in Europe, the potential risk suppresses consumer demand for pork, resulting in considerable economic cost to the industry (2).

Life Cycle and Epidemiology

The most important features of this helminth's (nematode) life cycle is its obligatory transmission by ingestion of meat; there is no free-living stage, as exists in many other parasitic nematodes. *T. spiralis* is cosmopolitan in its distribution. Recent research has shown that the concept of only one species is wrong and that there are at least six to seven genetic types, some with distinct host specificities (12–14). The type that is normally associated with domestic swine is unique and appears to have evolved along with the domestication of *Sus scrofa* (pig) (12). Although there is some controversy as to the proper taxonomic status and host specificities of various isolates of *Trichinella* from domestic and wild animals, all appear to be infective for humans.

After ingestion, the larvae are digested out of the muscle capsule in gastric fluid and enter the small intestine, where within 4–6 days they develop into sexually mature males and females. Their offspring (newborn larvae) migrate via the circulatory system throughout the body, invade striated muscles, and eventually (17–21 days after initial infection) become infective, encapsulated larvae. Although larvae may invade smooth muscle and other tissue, they eventually die in these sites. The encapsulated larvae may persist for years in muscle, until they become calcified and die.

The degree of clinical disease in human trichinosis is somewhat dependent on the number of muscle larvae ingested. The ingestion of 500 or more larvae can produce moderate-to-severe and even life-threatening illness. During the first few weeks, if large numbers of worms are present, illness may be reflected by gastrointestinal signs such as nausea and abdominal pain. Subsequent to the production of the muscle-invading larval offspring, the acute muscle phase may be seen. This is usually characterized by muscular pain, facial edema, fever, and eosinophilia.

As pointed out above, the major source of *T. spiralis* for humans is the domestic pig, although game accounts for about a third of the annual cases. Periodic prevalence studies on swine have revealed a rather marked decline in infection rates among pigs in the United States during the past 85 years, although this varies considerably from one region to another. The prevalence in slaughter hogs has decreased from about 1.4% around the turn of the century to 0.63% in 1952 and 0.13% in 1970 (3). These rates are influenced by the preponderance, in the samples, of grain-fed Midwestern hogs, which account for the majority of production in the United States; prevalence surveys in the Midwest indicate only 0.001% of hogs are infected. Recent surveys (15) indicate that the rates in swine raised in the eastern United States, however, range from 0.5 to 1.0%; in this region swine husbandry includes more high-risk management practices, such a garbage feeding.

Control Strategies

There has been a resurgence of research on the epidemiology, biology, and control of *Trichinella spiralis* during the recent decade. A major ingredient in this renewed attention has been the biotechnological revolution and the new tools it has provided. Especially important has been the application of hybridoma technology to the development of better immunodiagnostic tests. The techniques of recombinant DNA have also been instrumental for providing a potential means to produce antigens and to develop probes for use in epidemiological research and systematics.

Because human infections derive mainly from the ingestion of infected pork, an immunodiagnostic test suitable for the abattoir has had high priority for both the Agricultural Research Service and the Food Safety and Inspection Service, USDA. The efforts of these agencies have been successful, and a commercial ELISA-type test is now available. The critical step in producing this test was the discovery of a simple parasite-cultivation procedure for obtaining the diagnostic antigen (16). The antigens have been purified using a monoclonal antibody in combination with immunoaffinity techniques. In field tests, the antigens proved to be highly specific and sensitive (17,18). Currently, research is underway to produce the antigens by recombinant methods, and preliminary results indicate these efforts will also be successful. This will enhance the commercial viability of the test.

This serological test has been valuable not only for national epidemiologic studies but also for the development of effective control strategies. An important aspect of the epidemiologic investigations has been the assessment of the role of the sylvatic trichinellosis in domestic pig infections. Considerable concern exists over the threat of wild-animal *Trichinella* infections as a source of infection for humans and domestic swine. Although many wild-animal isolates of *Trichinella* are poorly infective for swine, the highly infective pig-type strain has been isolated from wild animals (12). The high genetic variability among morphologically indistinguishable isolates of the *Trichinella* presents problems in interpreting epidemiologic data. However, these questions are being resolved through the application of such techniques as DNA restriction-enzyme fragment-length polymorphism analysis (RFLP) and the development of hybridization probes by cloning unique DNA sequences (12,19). It is clear that human encroachment on the habitat of wild animals has facilitated the introduction of the domestic pig type (*Trichinella spiralis sensu stricto*) into wild-animal populations and, hence, a sylvatic reservoir of parasites capable of infecting domestic swine has been established (20). Effective control strategies must take this potential threat into account.

Vaccines

A vaccine has been developed for swine trichinellosis (21). It will soon be subjected to field trials. However, the vaccine is based on whole, inactivated newborne larvae and can only be produced on a limited scale. If, however, the field trials are successful, efforts to produce the protective antigens by recombinant DNA methods can be anticipated.

CYSTICERCOSIS

Public-Health Significance

Two species of tapeworms may be transmitted to humans by ingestion of the larval or cysticercus stage encysted in the meat of livestock. These two species are *Taenia saginata*, the beef tapeworm, and *T. solium*, the pork tapeworm (3). The latter is of special significance because humans may also serve as an intermediate host for the cysticercus (larval) stage, where it often localizes in the brain, causing the disease termed neurocysticercosis. Neurocysticercosis is a major public-health problem in many areas of the world, especially Latin America (22). Neurocysticercosis, although an acute life-threatening disease in severe cases, is more often a long-lasting infection affecting the quality of the patient's life and social environment. The disease is of socioeconomic importance because 75% of patients with neurocysticercosis are at productive ages and are frequently unable to work soon after the onset of symptoms. Calculations of costs for medical care, such as hospitalization, chemotherapy, neurosurgery, and computed tomography, show that US$14.5 million were spent in Mexico during 1986 to treat only the 2,700 new hospitalized cases of neurocysticercosis. For these reasons, neurocysticercosis is recognized as being of major public-health significance. Furthermore, swine cysticerosis is considered a significant economic problem because of the condemnation of pig carcasses. In Mexico, more than US$43 million were lost in 1980, the equivalent of 68.5% of the total investment in pig stock production. In the southwest United States, human neurocysticerosis is increasingly encountered. The magnitude is suggested by the fact that in Los Angeles, fecal examinations revealed more than 60 positives out of 12,000 examined (23). In one neurologic service, 45 cases were reported over a 5-year period. High rates are also seen in other areas of the world. A 1984 survey in India revealed that 4% of humans were infected with adult *Taenia* and more than 17% of swine were infected with cysticerci of *T. solium* (24).

The bovine form, *T. saginata*, although less severe in humans because it is confined, as the adult worm stage, to the human intestine, does represent a considerable economic cost because inspection of beef carcasses for cysticerci is mandatory, although the incidence is very low. In the United States, the rate of bovine cysticercosis identified by carcass inspection has been less than 0.3/1,000 over the last 10 years. These rates frequently are higher in certain Western states because of sporadic outbreaks. For example, in 1984, the rate for Washington was 1.9/1000 (25).

Life Cycle and Epidemiology

The adult stage of *Taenia saginata* is a flat tapeworm that resides in the human small intestine (3). The tapeworm is composed of a chain (strobila) of proglottids or segments each of which contains male and female reproduction systems. As the proglottid matures, it produces a large number of eggs. Gavid proglottids detach from the strobila and pass out of the intestine through the host's anus. Generally, an infected person harbors one tapeworm, although in some regions of the world, such as, Asia Minor, multiple infections are frequent. The adult tapeworm normally localizes in the jejunum, about 50 cm below the duodenojejunal flexure, and rarely enters the gallbladder, appendix, or nasopharynx. The life span of *T. saginata* in humans may be as long as 30–40 years.

Taenia saginata is capable of producing a very large number of eggs, and an individual proglottid may contain about 80,000 eggs. Because each strobila can shed 6–9 gravid proglottids/day, a single infected individual may contaminate the pasture, lot, or barn with one-half million eggs or more each day. The mature egg contains the larval stage termed the onchosphere; immature eggs that are passed may complete their development outside the host. Ingestion of the egg by cattle is followed by disintegration of the outer embryophore and activation of oncosphere activity by gastric juice. Aided by its hooks and penetration glands, the onchosphere penetrates the bovine intestinal mucosa and within a couple of hours enters the blood or lymphatic system. The onchosphere utilizes the host's circulatory system to migrate throughout the host's body; however, successful development to the cyst or cysticercus stage usually occurs in skeletal muscle or the heart. The cysticercus, when fully developed, is composed of an invaginated scolex within a fluid-filled vesicle or bladder. When improperly cooked beef is eaten, the larvae are digested free in the gut and the scolex evaginates to attach by its suckers to the intestinal wall. The development of the strobila then commences, including proglottids with reproductive organs and eventually, eggs.

The life cycle of *T. solium* is similar but differs in several important respects (3). The gravid proglottids contain fewer eggs than do those of *T. saginata*. Another distinction is the lack of motility of *T. solium* proglottids, which results in their being shed in the feces as connected segments. The major difference from *T. saginata*, however, is the suitability of humans to serve as an intermediate host for *T. solium* cysticerci. As in pigs, the cysticerci are fairly evenly distributed throughout the liver, brain, central nervous system, skeletal muscle, and myocardium. The cysticercus of *T. solium* is bigger than that of *T. saginata* (5–20 mm in diameter). After 2–3 months of development, the cysticercus appears as a glistening pearly white cyst, with the scolex or head deeply invaginated into the fluid-filled bladder; the scolex bears four suckers and an apical crown of hooklets. When humans ingest infected pork, the larvae are digested out of the meat or tissue, the scolex evaginates from the bladder, attaches to the wall of the proximal portion of the ileum, and develops into a complete worm or strobila in about 3 months. The tapeworm begins to excrete proglottids after about 2 months and may live for many years.

Clinical Disease

Clinical disease associated with the intestinal adult form of *T. solium* is mild in humans and is similar to that of *T. saginata*. Far more serious may be infection with the larval cysticercus form, especially if the central nervous system is involved (3). Cerebral cysticercosis is usually classified according to its location: (1) ventricular-cisternal, (2) meningeal, (3) parenchymal, and (4) mixed. These localizations are often accompanied by a myriad of signs and symptoms, including seizures, hydrocephalus, headaches, dizziness, arterial thrombosis, loss of vision, and nausea. Cerebral cysticercosis is, on a worldwide basis, one of the most common parasitic diseases of the central nervous system (22).

Control Strategies

Current control strategies for these parasitic diseases are dependent on detecting infections in livestock in order to remove them from the food chain. However, procedures for this task are at present inadequate. For example, in the United States, the mandatory inspection of beef for cysticerci is known to be highly insensitive (26). Most current research on cysticercosis is aimed at developing rapid, more reliable immunodiagnostic tests for both livestock and humans.

Until recently, the prospects for an immunodiagnostic test for bovine cysticercosis were poor because of the low specificity of crude worm extracts as antigen reagents. Recently, biochemical fractionation approaches have greatly improved the specificity of these antigens. A genus-specific antigen from *T. hydatigena* (ThFAS) with a high degree of sensitivity and specificity for both bovine and porcine cysticercosis has been recently recovered (27,28). With this antigen, low infection levels in cattle could be detected as early as 3 weeks after exposure. Currently, efforts are underway to clone the gene for this antigen so that sufficient amounts of antigen for field tests can be made available.

The immunodiagnosis of human neurocysticercosis has also greatly improved. One of the most specific and sensitive test procedures is that described by Tsang and coworkers (29). This test is an enzyme-linked immunoelectrotransfer blot assay using glycoprotein antigens prepared by fractionation of *T. solium* cysticerci. In an evaluation using a large number of sera and cerebrospinal fluids, the test was 98% sensitive and 100% specific.

Recombinant DNA methods have had, to date, their greatest impact in human cysticercosis research in the area of taxonomy (30). As pointed out earlier, the systematics of the taeniid cestode group requires clarification because of its importance to understanding the epidemiology of these foodborne parasites. As various host and geographic isolates receive closer scrutiny, it is apparent that at least some of the species exist as complexes of intraspecific variants, frequently with variation in host specificity. Recently, a variety of geographic isolates of *T. solium*, have been analyzed with the aid of DNA probes (31). Considerable intraspecific genetic variation has been detected. Using a similar approach, distinct DNA differences between a *T. saginata*-like cestode from Taiwan and various other isolates of *T. saginata* have been observed (32). The human *Taenia* from Taiwan (and Korea) is unique in that it is more infective for swine than for cattle and has a predilection for the swine liver. The difference in DNA characteristics supports the interpretation that the Taiwan *Taenia* is genetically and biologically unique although closely related to *T. saginata*. It can be anticipated that these powerful methods will prove to be decisive in developing a greater understanding of the genetic variation and epidemiology of these important human parasites.

FISHBORNE PARASITES

The rising popularity of raw fish has increased the risk of a variety of new diseases, especially those caused by the larval stages of helminth parasites of fish. The majority of these seafood diseases occur in regions where seafood constitutes a major portion of the protein diet. The status of seafood zoonoses has recently been reviewed (33). The number of annual causes of anisakiasis (caused by *Anisakis* spp. and *Pseudoterranova* spp.) in Japan exceeded 3,100 in 1984. In the United States, although only about 50 cases have been reported since 1958, the annual number of new cases is increasing.

The most important freshwater fishborne parasites in the United States is the broad fish tapeworm *Diphyllobothrium latum*. Humans become infected by ingesting raw or inadequately cooked freshwater or andromous fish. Most commonly implicated are pike and walleys from the Great Lakes, where up to 50–70% may harbor the infective larval stage.

Currently, the control of these fishborne parasites relies on recommendations to the consumer for the preparation of safe fish meals and, in the case of marine fish, on some inspection by mechanical means. However, the pressure on government to develop mandatory, comprehensive inspection technologies for the inspection of marine and freshwater fish is increasing. Currently, accurate, rapid inspection methods do not exist. It can be expected, however, that if the need is sufficient, such inspection technology will be developed. Biotechnological tools may play an important role in this endeavor also.

BIBLIOGRAPHY

1. T. Roberts, "Microbial pathogens in raw pork, chicken and beef: benefit estimates for control using irradiation," *American Journal of Agric. Econ.* **67**, 957–965 (1985)
2. U.S. Department of Energy, "Trichina-safe pork by gamma irradiation processing: a feasibility study." Contract NO. DE-AC04-83AL-19411, Albuquerque, New Mexico, August 1983.
3. K. D. Murrell, R. Fayer, and J. P. Dubey, "Parasitic organisms," *Advances in Meat Research* **2**, 311–377 (1986).
4. R. Fayer, H. R. Gamble, J. R. Lichtenfels, and J. Bier, "Foodborne and waterborne parasites," in *APHA Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association. In press. 1990.
5. J P. Dubey and C. P. Beattie, *Toxoplasmosis of Animals and Man*, CRC Press, Boca Raton, Fla., 1988
6. A. M. Johnson, "Toxoplasma vaccines," in I. G. Wright, ed., *Veterinary Protozoan and Hemoparasite Vaccines*, CRC Press, Boca Raton, Fla., 1989, pp. 177–202.
7. L. H. Kasper, "Identification of stage-specific antigens of *Toxoplasma gondii*," *Infection and Immunity* **57**, 168 (1989).
8. A. DeCoster, F. Darcy, A. Capron, and A. Capron, "IgA antibodies against P30 as markers on congenital and acute toxoplasmosis," *Lancet* **2**, 1104 (1988).
9. J. L. Burg, C. M. Grover, P. Pouletty, and J. C. Boothroyd, "Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction," *Journal of Clinical Microbiology* **27**, 1787 (1989).
10. H Waldland and J. K. Frenkel, "Live and killed vaccines against toxoplasmosis in mice," *Journal of Parasitology* **G9**, 60 (1988).
11. J. B. Prince, F. G. Araujo, J. S. Remington, J. L. Burg, J. C. Boothroyd, and S. D. Sharma, "Cloning of cDNAs encoding a 28 kilodalton antigen of *Toxoplasma gondii*," *Molecular and Biochemical Parasitology* **34**, 3 (1989).
12. J. B. Dame, K. D. Murrell, D. E. Worley, and G A. Schad, "*Trichinella spiralis*: genetic evidence for synanthropic subspecies in sylvatic hosts," *Experimental Parasitology* **64**, 195 (1987).
13. E. Pozio, P. LaRosa, P. Rossi, and K. D. Murrell, "New taxonomic contributions to the genus *Trichinella* (Owen, 1835). I. Biochemical identification of seven clusters by gene-enzyme systems," in C. Tanner, ed., *Proceedings of the 7th International Conference on Trichinellosis*, CSIC Press, Madrid, Spain, 1989, pp. 76–82.
14. D. S. Zarlenga and K. D. Murrell, "Molecular cloning of *Trichinella spiralis* ribosomal RNA genes: application as genetic markers for isolate classification," in C. Tanner, ed., *Proceedings of the 7th International Conference on Trichinellosis*, CSIC Press, Madrid, Spain, 1989, pp 35–40
15. G. A. Schad, M. Kelly, D. A. Leiby, K. Blumrick, C. Duffy, and K. D. Murrell, "Swine trichinellosis in mid-Atlantic slaughterhouses: possible relationships to hog marketing systems," *Preventive Veterinary Medicine* **3**, 391–399 (1985).
16. H. R. Gamble, W. R. Anderson, C. E. Graham, and K. D. Murrell, "Diagnosis of swine trichinosis by enzyme-linked immunosorbent assay (ELISA) using an excretory-secretory antigen," *Veterinary Parasitology* **13**, 349 (1983).
17. H. R. Gamble and C. E. Graham, "Monoclonal antibody purified antigen for the immunodiagnosis of trichinosis." *American Journal of Veterinary Research* **45**, 67 (1984).
18. K. D. Murrell, W. R. Anderson, G. A. Schad, R. D. Hanbury, K. R. Kazacos, H. R. Gamble, and J. Brown, "Field evaluation of an ELISA test for swine trichinosis using an excretory-secretory antigen. *American Journal of Veterinary Research* **47**, 1046 (1986).
19. D. Boyd, T. deVos, G. Klassen, and T. Dick, "Characterization of the ribosomal DNA from *Trichinella spiralis*," *Molecular Biochemistry and Parasitology* **35**, 67 (1989).
20. K. D. Murrell, F Stringfellow, J. B. Dame, D. Luiby, and G. A. Schad, "*Trichinella spiralis*: evidence for natural transmission of *Trichinella spiralis* from domestic swine to wildlife," *Journal of Parasitology* **73**, 103 (1987).
21. H. P. Marti, K. D. Murrell, and H. R. Gamble, "*Trichinella spiralis*: immunization of pigs with newborn larval antigens," *Experimental Parasitology* **63**, 68 (1987).
22. A. Flisser, "Cysticercosis: a major threat to human health and livestock production," *Food Technology* **37**, 61 (1985).
23. W. J. Brown and M. Voge, "Cysticercosis, a modern day plague," *Pediatric Clinica of North America* **32**, 953–968 (1985).
24. K. M. L. Pathak, S. N. S. Gaur, and D. Kumar, "The epidemiology of strobillar and cystic phases of *Taenia solium* in certain parts of Uttar Pradesh (India)," *Indian Journal of Veterinary Medicine* **4**, 1748 (1984).
25. D. D. Hancock, S. E. Wikse, A. B. Lichtenwalner, R. B Wescott, and C. C. Gay, "Distribution of bovine cystercercosis in Washington," *American Journal of Veterinary Research* **50**, 564–570 (1989).
26. L. W. Dewhirst, "Antemortem diagnosis of bovine cysticercosis due to *Taenia saginata*," in *Proceedings of the 71st Annual Meeting, U.S. Livestock Sanitation Association*, 1968, pp. 540–545.
27. M. L. Rhoads, EIP Kamanga-Sollo, D. Rapic, K. D. Murrell, P. M. Schantz, and A. Beus, "Detection of antibody in humans and pigs to *T. solium* metacestodes using an antigenic fraction from *T. hydatigena* metacestodes," *Veterinary Archives* **57**, 143 (1987).

28. EIP Kamanga-Sollo, M. L. Rhoads, and K. D. Murrell, "Evaluation of an antigenic fraction of *Taenia hydatigena* metacestode cyst fluid for immunodiagnosis of bovine cysticercosis," *American Journal of Veterinary Research* **48**, 1206 (1987).
29. V. C. W. Tsang, J. A. Brand, and A. E. Boyer, "An enzyme-linked immunoelectrotransfer blot assay and glucoprotein antigens for diagnosing human cysticercosis (*Taenia solium*)," *Journal of Infectious Diseases* **154**, 50 (1989).
30. K. D. Murrell and M. L. Rhoads, "Application of biotechnology methods to the study of cestodes," *Southeast Asian Journal of Tropical Medicine and Health* **19**, 37 (1988).
31. A. K. Rishi and D. P. McManus, "Molecular cloning of *Taenia solium* DNA and characterization of taeniid cestodes by DNA analysis," *Parasitology* **97**, 161 (1988)
32. D. S. Zarlenga, D. P. McManus, P. C. Fan, and J. H. Cross, "DNA characterization of a prospective new species of *Taenia* from Taiwan using cloned ribosomal and repetitive DNA fragments," *Experimental Parasitology* **72**, 174–183 (1991).
33. T. L. Deardorff and R. M. Overstreet, "Sea-food transmitted zoonoses in the United States: the fishes, the dishes, and the worms," in C. R. Hackney and D. Ward, eds., *Microbiology of Marine Food Products*, Van Nostrand Reinhold, New York. In press.

GENERAL REFERENCES

Anonymous, "Issues in Pork Safety, Costs, Controls, and Incentives," *Agricultural Outlook* **201**, 28–32 (1993).

W. Buffolano et al., "Risk Factors for Recent Toxoplasma Infection in Pregnant Women in Naples," *Epidemiology and Infection* **116**, 347–351 (1996).

L. Bunetel et al., "Calibration of an in vitro Assay System Using a Nonadherent Cell Line to Evaluate the Effect of a Drug on *Toxoplasma gondii*," *Int. J. Parasitol.* **25**, 699–704 (1995).

D. Buxton and E. A. Innes, "A Commercial Vaccine for Ovine Toxoplasmosis," *Parasite Vaccines* S11–S16 (1995).

W. C. Campbell, "Meatborne Helminth Infections: Trichinellosis," in Y. H. Hui et al., eds., *Foodborne Disease Handbook, Diseases Caused by Viruses, Parasites and Fungi*, Vol. 2, Marcel Dekker Inc., New York, 1994, pp. 255–277.

A. Chapman et al., "Isolation and Characterization of Species-Specific DNA Probes from *Taenia solium* and *Taenia saginata* and Their Use in an Egg Detection Assay," *J. Clin. Microbiol.* **33**, 1283–1285 (1995)

J. L. De La Rosa et al., "Prevalence and Risk Factors Associated with Serum Antibodies Against *Trichinella spiralis*," *Int. J. Parasitol.* **28**, 317–327 (1998).

B. R. Dixon, "Prevalence and Control of Toxoplasmosis, A Canadian Perspective," *Food Control* **3**, 68–75 (1992).

J. P Dubey, "Toxoplasmosis," *J. Amer. Veterinary Med. Assoc.* **205**, 1593–1598 (1994).

J. P. Dubey et al., "Oocyst-Induced Murine Toxoplasmosis: Life Cycle, Pathogenicity, and Stage Conversion in Mice Fed *Toxoplasma gondii* Oocysts," *J. Parasitol.* **83**, 870–882 (1997).

J. F. Edwards, R. B. Simpson, and W. C. Brown, "Bacteriologic Culture and Histologic Examination of Samples Collected from Recumbent Cattle at Slaughter," *J. Amer. Veterinary Med. Assoc.* **207**, 1174–1176 (1995).

K. L. Ehnert et al., "Cysticercosis, First 12 Months of Reporting in California," *Bull. of the Pan Am Health Organ.* **26**, 156–172 (1992).

Flair-Flow Europe, "Fish Parasites of Public Health Significance," *Flair Flow Rep* **125**, 1 (1994).

A. A. Gajadhar, J. R. Bisaillon, and G. D. Appleyard, "Status of *Trichinella spiralis* in Domestic Swine and Wild Boar in Canada," *Can. J. Veterinary Res.* **61**, 256–259 (1997).

A. A. Gajadhar and W. C. Marquardt, "Ultrastructural and Transmission Evidence of *Sarcocystis cruzi* Associated with Eosinophilic Myositis in Cattle," *Can. J. Veterinary Res.* **56**, 41–46 (1992).

A. A. Gajadhar, W. C. Marquardt, and C. D. Blair, "Development of a Model Ribosomal RNA Hybridization Assay for the Detection of *Sarcocystis* and Other Coccidia," *Can. J. Veterinary Res.* **56**, 208–213 (1992).

H. R. Gamble, "Detection of Trichinellosis in Pigs by Artificial Digestion and Enzyme Immunoassay," *J. Food Protection* **59**, 295–298 (1996).

H. R. Gamble and I. V. Patrascu, "Whole Blood Serum and Tissue Fluids in an Enzyme Immunoassay for Swine Trichinellosis," *J. Food Protection* **59**, 1213–1217 (1996).

J. R. Isaac et al., "Detection of *Toxoplasma gondii* Oocysts in Drinking Water," *Appl. and Environmental Microbiol.* **64**, 2278–2280 (1998).

M. R. Lappin, "Diagnostic Testing for Toxoplasmosis," *Proc. North Amer. Veterinary Conf.* **9**, 221–223 (1995).

P. Mahannop et al., "Immunodiagnosis of Human Trichinellosis and Identification of Specific Antigen for *Trichinella spiralis*," *Int. J. Parasitol.* **25**, 87–94 (1995).

A. E. Marsh et al., "Sequence Analysis and Polymerase Chain Reaction Amplification of Small Subunit Ribosomal DNA from *Sarcocystis neurona*," *Amer. J. Veterinary Res.* **57**, 975–981 (1996).

A. Minniti et al., "Determination of Histamine in Common Sea Fish Parasites: *Anisakis simplex* and *Gymnorhynchus gigas*," *Ital. J. Food Sci.* **7**, 305–309 (1995).

K. D. Murrell, "Foodborne Parasites," *Int. J. Environmental Health Res.* **5**, 63–85 (1995).

Y. Ohnishi, T. Ono, and T. Shigehisa, "Histochemical and Morphological Studies on *Trichinella spiralis* Larvae Treated with High Hydrostatic Pressure," *Int. J. Parasitol.* **24**, 425–427 (1994).

K. S. Parmesh, D. W. Webert, and P. C. McCaskey, "Food Safety and Regulatory Aspects of Cattle and Swine Cysticercosis," *J. Food Protection* **60**, 447–453 (1997).

M. Pepin, P. Russo, and P. Pardon, "Public Health Hazards from Small Ruminant Meat Products in Europe," *Revue Scientifique et Technique Office International des Epizootes* **16**, 415–425 (1997).

J. H. Rim, "Field Investigation on Epidemiology and Control of Fish-Borne Parasites in Korea," *Int. J. Food Sci. and Technol.* **33**, 157–168 (1998).

F. Serrano et al., "Trichinella Strain, Pig Race and Other Parasitic Infections in the Reliability of ELISA for the Detection of Swine Trichinellosis," *Porositol.* **105**, 111–115 (1992).

L. Sloan, S. Schneider, and J. Rosenblatt, "Evaluation of Enzyme-Linked Immunoassay for Serological Diagnosis of Cysticercosis," *J. Clin. Microbiol.* **33**, 3124–3128 (1995).

H. J. Smith, K. E. Snowdon, and R. C. Finlay, "Serological Diagnosis of Cysticercosis by an Enzyme-Linked Immunosorbent Assay in Experimentally Infected Cattle," *Can. J. Veterinary Res.* **55**, 274–276 (1991).

J. L. Smith, "Documented Outbreaks of Toxoplasmosis Transmission of *Toxoplasma gondii* to Humans," *J. Food Protection* **56**, 630–639 (1993).

J. L. Smith, "*Taena solium* Neurocysticercosis," *J Food Protection* **57**, 831–844 (1994).

J. L. Smith, "Long-Term Consequences of Foodborne Toxoplasmosis: Effects on the Unborn, the Immunocompromised, the Elderly, and the Immunocompetent," *J. Food Protection* **60**, 1595–1611 (1997).

A. M. Tenter, "Current Research on *Sarcocystis* Species of Domestic Animals," *Int. J. Parasitol.* **25**, 1311–1330 (1995).

F. van Knapen, "European Proposal for Alternative *Trichinella* Control in Domestic Pigs," *Fleischwirtschaft* **78**, 338–339 (1998).

K. Darwin Murrell
USDA/ARS/NRRC
Peoria, Illinois

PASTA. See Cereals science and technology; Wheat science and technology.

PATENTS

It is obviously not possible to discuss the complex subject of food patents in a few thousand words. It is hoped that this article will provide an introduction to the basic procedure of obtaining a patent for an invention in the United States. However, it must be emphasized that the service of a patent attorney is the best assurance for obtaining proper protection for an invention. This discussion will use the term patent instead of food patent throughout. There are obviously scientific differences between a patent for a motor and one for a food packaging material. However, the principles underlying the applications for both are the same.

THE LAW

There are four general areas of legal protection available for the exploitation of intellectual property. These are (1) patents, (2) copyrights, (3) trade secrets, and (4) trademarks. The area or areas available for protecting any particular type of intellectual property will depend on the specific type under consideration.

The Constitution of the United States (Article 1, Section 8) gave Congress the right or power to enact laws concerning patents and copyrights. In particular Article 1, Section 8 states the following:

> The Congress shall have the power . . . to promote the progress of science and useful arts, by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries.

The basic philosophy behind both the patent laws and copyright laws is to encourage authors and inventors to publicly disclose their respective writings and discoveries. This disclosure, in turn, is to add to the total sum of knowledge publicly available. To encourage such disclosures, patent and copyright laws were enacted to provide certain rights to authors and inventors. The patent laws have been codified as Title 35 of the United States Code (USC) and are referred to as 35 USC. The laws directed to copyrights are codified in Title 17 of the United States Code and are referred to as 17 USC.

Because the entire field of patent law has been preempted by Congress, individual states are excluded from any legal control over patent law. In addition, since January 1, 1978, Congress has preempted the copyright law and, therefore, all rights created from January 1, 1978, and thereafter are subject to federal law.

DEFINITION AND RIGHTS

A U.S. patent is actually a contract between the inventor and the people of the United States represented by the government and specifically by the U.S. Patent and Trademark Office. The right conferred on the inventor is the right to exclude others from making, using, selling, offering for sale, or importing that which is covered in the claims of the patent. Such rights are actually negative rights in the sense that the patentee is not given the right to do anything except exclude others from making, using, or selling the invention for a limited period of time. Typically, but with certain exceptions, U.S. patents expire 20 years from the filing date of the application for patent. One such exception relates to patents involving drugs or any medical device, food additive, or color additive subject to regulation under the federal Food, Drug and Cosmetic Act or the Virus-Serum-Toxic Act for veterinary biological products, where it is possible to recapture that portion of the patent term, up to a maximum of 5 years, that was lost because of delays resulting from the Food and Drug Administration's or the Animal and Plant Health Inspection Service's review prior to approving commercial marketing or use of the drug, medical device, food additive, color additive, or biological product.

As in any contract each party is to receive something of value and each party is to give up something of value in exchange. In exchange for the rights granted by the government, the inventor is required to provide a disclosure of the invention. This is the inventor's "consideration" or "value" given up as part of the contractual relationship. The disclosure of the invention is made available to the public when the patent issues (that is, is granted by the government). This disclosure of the invention by the inventor must be a full description of the invention.

The general subject matter to which patents are directed is considered to be the useful arts. In particular patents are granted for inventions directed to processes, machines, manufactures, compositions of matter, or any new and useful improvements thereof. Printed copies of granted patents can be purchased from the U.S. Patent and Trademark Office for $3.00 per copy.

JURISDICTION

The U.S. Patent and Trademark Office is a governmental agency within the U.S. Department of Commerce. For ease of reference, this office will be referred to as "the office" in this article. The head is referred to as the commissioner. The office has the responsibility for reviewing patent applications and determining whether or not to grant or issue a patent thereon. It does not have jurisdiction to determine whether a patent is infringed. Moreover, except to the limited extent authorized by the patent reexamination procedure, it has no jurisdiction over issued patents and cannot make determinations of patent validity or invalidity.

F. van Knapen, "European Proposal for Alternative *Trichinella* Control in Domestic Pigs," *Fleischwirtschaft* **78**, 338–339 (1998).

K. Darwin Murrell
USDA/ARS/NRRC
Peoria, Illinois

PASTA. See Cereals science and technology; Wheat science and technology.

PATENTS

It is obviously not possible to discuss the complex subject of food patents in a few thousand words. It is hoped that this article will provide an introduction to the basic procedure of obtaining a patent for an invention in the United States. However, it must be emphasized that the service of a patent attorney is the best assurance for obtaining proper protection for an invention. This discussion will use the term patent instead of food patent throughout. There are obviously scientific differences between a patent for a motor and one for a food packaging material. However, the principles underlying the applications for both are the same.

THE LAW

There are four general areas of legal protection available for the exploitation of intellectual property. These are (1) patents, (2) copyrights, (3) trade secrets, and (4) trademarks. The area or areas available for protecting any particular type of intellectual property will depend on the specific type under consideration.

The Constitution of the United States (Article 1, Section 8) gave Congress the right or power to enact laws concerning patents and copyrights. In particular Article 1, Section 8 states the following:

> The Congress shall have the power . . . to promote the progress of science and useful arts, by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries.

The basic philosophy behind both the patent laws and copyright laws is to encourage authors and inventors to publicly disclose their respective writings and discoveries. This disclosure, in turn, is to add to the total sum of knowledge publicly available. To encourage such disclosures, patent and copyright laws were enacted to provide certain rights to authors and inventors. The patent laws have been codified as Title 35 of the United States Code (USC) and are referred to as 35 USC. The laws directed to copyrights are codified in Title 17 of the United States Code and are referred to as 17 USC.

Because the entire field of patent law has been preempted by Congress, individual states are excluded from any legal control over patent law. In addition, since January 1, 1978, Congress has preempted the copyright law and, therefore, all rights created from January 1, 1978, and thereafter are subject to federal law.

DEFINITION AND RIGHTS

A U.S. patent is actually a contract between the inventor and the people of the United States represented by the government and specifically by the U.S. Patent and Trademark Office. The right conferred on the inventor is the right to exclude others from making, using, selling, offering for sale, or importing that which is covered in the claims of the patent. Such rights are actually negative rights in the sense that the patentee is not given the right to do anything except exclude others from making, using, or selling the invention for a limited period of time. Typically, but with certain exceptions, U.S. patents expire 20 years from the filing date of the application for patent. One such exception relates to patents involving drugs or any medical device, food additive, or color additive subject to regulation under the federal Food, Drug and Cosmetic Act or the Virus-Serum-Toxic Act for veterinary biological products, where it is possible to recapture that portion of the patent term, up to a maximum of 5 years, that was lost because of delays resulting from the Food and Drug Administration's or the Animal and Plant Health Inspection Service's review prior to approving commercial marketing or use of the drug, medical device, food additive, color additive, or biological product.

As in any contract each party is to receive something of value and each party is to give up something of value in exchange. In exchange for the rights granted by the government, the inventor is required to provide a disclosure of the invention. This is the inventor's "consideration" or "value" given up as part of the contractual relationship. The disclosure of the invention is made available to the public when the patent issues (that is, is granted by the government). This disclosure of the invention by the inventor must be a full description of the invention.

The general subject matter to which patents are directed is considered to be the useful arts. In particular patents are granted for inventions directed to processes, machines, manufactures, compositions of matter, or any new and useful improvements thereof. Printed copies of granted patents can be purchased from the U.S. Patent and Trademark Office for $3.00 per copy.

JURISDICTION

The U.S. Patent and Trademark Office is a governmental agency within the U.S. Department of Commerce. For ease of reference, this office will be referred to as "the office" in this article. The head is referred to as the commissioner. The office has the responsibility for reviewing patent applications and determining whether or not to grant or issue a patent thereon. It does not have jurisdiction to determine whether a patent is infringed. Moreover, except to the limited extent authorized by the patent reexamination procedure, it has no jurisdiction over issued patents and cannot make determinations of patent validity or invalidity.

About 2,000 people with degrees in engineering or science are employed by the office to review and examine patent applications; they are referred to as patent examiners. Examiners conduct independent searches of the inventions described in the applications and then make decisions as to whether the invention described and claimed merits the granting of a patent.

The commissioner has the authority to promulgate rules and regulations for the purpose of administrating the patent and trademark laws so long as such rules are not inconsistent with these laws. In addition, the commissioner has the power to make rules that govern the recognition and conduct of those persons who represent applicants before the office. To be recognized as a patent agent or patent attorney and to advise and assist in the preparation or prosecution of applications and other business before the office, a person must comply with certain rules and regulations.

In the event an examiner refuses to allow or permit issuance of a patent for an application, the applicant has the opportunity at a particular stage of the proceedings to file an appeal from the examiner's decision to the U.S. Patent and Trademark Office Board of Patent Appeals and Interferences (hereafter referred to as "the board"). The board is composed of senior examiners, who are referred to as patent law administrative judges. Cases appealed to the board are considered by a three-member panel of the board.

A publication titled *Manual of Patent Examining Procedure (MPEP)* is available from the U.S. Government Printing Office. This publication is a guide to how the office interprets the laws, rules, and case law and how the patent examiners are to apply the same when examining and ruling on patent applications. Likewise, a similar publication available from the U.S. Government Printing Office titled *Trademark Manual of Examining Procedure (TMEP)* is a guide for trademark examiners in how to apply the laws, rules, and case law when ruling on trademark and service mark applications.

Another important publication available from the U.S. Government Printing Office is titled the *Official Gazette of the United States Patent and Trademark Office (OG)*. This is published each week in two separate sections. One is the patent section and the other is the trademark and service mark section. The patent section of the *OG* includes a short description of each patent granted during the week of publication, an index of the names of the patentees and the assignees for that week, notices of rule changes, and various other matters of interest with respect to patents.

Since 1929, published decisions in patent, trademark, and copyright cases have been reported in the *United States Patent Quarterly (USPQ and USPQ2d)*. This is published by the Bureau of National Affairs, Washington, D.C., which also publishes the weekly newsletter *Patent, Trademark, and Copyright Journal*. The latter contains news of matters of current development and interest in the patent, trademark, and copyright areas.

Decisions by the federal courts in trademark, copyright, and patent cases can also be found, depending on the particular court and the particular year in which the case was decided, in the *Federal Reporter* (F.), the *Federal Reporter second series* (F.2d), the *Federal Reporter third series* (F.3d), the *Federal Supplement* (F.Supp.), the *Supreme Court Reporter* (S.Ct.), and *The United States Reports* (U.S.).

PATENTABILITY

Specifications and Claims

To facilitate an understanding of the concept of patentability, some familiarity with the contents of patents and particularly with the specification and claims is desirable. The specification of a patent contains a detailed technical description of the invention involved. In addition, if an understanding of the invention would be facilitated by including drawings, the specification will do so and also provide an explanation of the drawings. The specification may also include a discussion of the background technology of the invention, the problems encountered in that technology, the state of the prior art, the objectives of the invention, and/or the advantages, if any, achieved by the invention.

The claim or claims of a patent appear at the end of the technical description of the patent. The function of the claim is particularly to point out and distinctly assert title to the subject matter that the applicant considers to be his or her contribution or invention for which he or she desires a patent grant. It is the claim or claims that are compared to the prior art to determine patentability. Likewise, it is the claim or claims that are compared to an accused activity to determine whether infringement actually exists.

What Can Be Patented

As required by 35 USC §101, an invention must be new and must be useful to be patentable. (Design patents need not be useful, however, to satisfy the requirements for patentability.) In addition, as required by 35 USC §101, the invention must be directed to a process, machine, manufacture, or composition of matter or to any new and useful improvement thereof. Patents are concerned with technology or the useful arts as contrasted to the liberal arts. A shorthand way to view the subject matter of a patent is that it represents the means by which a desired result is obtained.

The term process as used in the patent statute involves the treatment or manipulation of some material or materials or of information to cause some change to the material or information treated or manipulated by the process.

Novelty. Another requirement that an invention must satisfy to be patentable is that it must be new or novel (35 USC §102). Because patents are creatures of statutes, those conditions, referred to as prior art, that defeat the novelty of an invention are likewise defined in the patent laws. Only the more typically encountered ones are mentioned below.

The two most common categories of prior art are probably printed publications and patents. The publications and patents can be from any country and in any language. A patent or publication that describes an invention and is prior in time to it defeats the novelty of the invention. In the event a printed publication or patent describes an invention and is more than one year old when the patent application is filed in a patent office, such will render the invention of that patent application unpatentable no matter when the invention was actually made. In fact, the patent or publication could even be by the inventor and when such is more than one year before the actual filing of the patent application, granting of a patent is precluded. This situation is referred to as a statutory bar, because no matter whose patent or publication and no matter when the invention was made, a patent is barred. In essence the United States gives the inventor a one-year grace period after patenting or publication to file an application provided the invention was made prior to the patent or publication. However, most other countries do not give any grace period. Therefore, the patent application must already be on file before the invention is publically disclosed to be patentable in most other countries. Often an inventor desires to present or publish a paper describing some discovery. However, to preserve foreign rights, the paper should not be published until a patent application has been filed. After filing, it could be published without affecting rights.

Public use or public knowledge of an invention in the United States prior to the invention renders it unpatentable. Moreover a patent is precluded if the invention was in public use or on sale in the United States more than one year prior to filing the patent application. The public use or on sale activity could even be by the inventor and when it is more than one year before the filing of the patent application, such activity will prevent granting of a patent. This one-year period is an attempt to balance the desire for early disclosure of the invention to the public and the need to give the inventor some limited time to determine whether pursuing patent protection is justified.

Nonobviousness. The above-discussed novelty-defeating prior art items presuppose that the exact subject matter for which a patent is being sought is described by a single prior art item. However, even if the invention is not fully described by a single prior art item it still may not be patentable. In particular, the patent law also requires that the invention be nonobvious (35 USC §103). An invention is not patentable if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the technology of art that is the subject matter of the invention. Stated in other terms, the advance or difference between the subject matter sought to be patented and the prior art must not be so trivial to be readily apparent to persons working in that particular technology.

THE PATENT APPLICATION

The patent application, being a combination of a legal contract and a scientific paper, represents a unique written document. It is a scientific paper because it describes some technical achievement but differs from usual technical papers in that it must stand on its own to a greater extent than most technical papers. The patent represents a legal contract because it is actually a contract between the inventor and the people of the United States as represented by the government and, specifically, by the U.S. Patent and Trademark Office. In particular, the government gives the inventor the right to exclude others, for a limited period of time, from making, using, offering for sale, selling, or importing the invention as defined by the claims. The consideration given by the inventor in the contract is publicly disclosing information concerning the invention.

A patent application must include a specification, a drawing (in the event that the nature of the invention is such as to require a drawing for understanding), and a signed oath or declaration. The specification must contain both a written description of the invention and at least one claim. In addition, a government filing fee must be paid, which, of course, changes with time. The legal requirements for an application can be found in 35 USC §111. Moreover, by rule, an application must contain an abstract of the disclosure.

Abstract

The abstract is intended merely as a searching tool and should be a concise, informative statement that can give a quick and general idea of the nature and essence of an invention. The abstract has no legal effect on the interpretation of the claims of the patent or on the interpretation of the remainder of the disclosure of the patent.

Specification Description

The requirements for the specification of a patent are contained in 35 USC §112 and especially in ¶ 1 thereof. In particular, the requirements of the descriptive portion of the specification include the following:

1. A written description of the invention.
2. A written description of the manner and process of making and using the invention in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains or with which it is most nearly connected, to make and use the invention; a requirement usually referred to as the enablement requirement.
3. The best mode contemplated by the inventor of carrying out the invention; a requirement usually referred to as the best mode requirement.

This descriptive material is the consideration given by the inventor as the inventor's part of the bargain represented by the patent contract.

Written Description of the Invention

The requirements for a written description of the invention is that this specification must support the claims of the application. One way to insure this is to have the specification contain language that corresponds to the language employed in any of the claims or language that is its equivalent. Even if there is no explicit description of a generic invention in the specification, however, the mention of a representative component or a representative number of examples may provide an implicit description on which to base generic language. In particular, the specification, in addition to what it explicitly states, also implicitly discloses what would be apparent to those skilled in the art from a mere reading of it. It is not always safe to rely on what is implicit, however, because the outcome of having to convince another of the true import of an implicit disclosure is uncertain. In addition, it can be quite helpful, although not necessary, to include in the specification a discussion of the prior art or the efforts that have previously been made as well as a discussion of the advantages achieved by the invention or problems addressed by it. In fact, some courts have held that advantages that are not disclosed in the application to buttress the evidence of patentability cannot be relied on. In a sense the specification provides an opportunity to sell the patentability of the invention and, accordingly, can be used to that advantage.

Enablement

The specification must also include a written description that is sufficient to enable any person skilled in the art to make and use the invention. Although this enablement need only be addressed to those skilled in the art in explaining the technical aspects of the invention, it is desirable that it not be written in such a manner as to be understandable only by persons so skilled. This consideration may be ultimately important because the validity of a patent, if challenged, will initially be determined by a judge in a federal court who may have had little or no patent background and little or no technical background.

One way in the specification to teach persons skilled in the art how to make and use the invention is to include a number of representative examples. This represents only one way of teaching, however, and it is not the only means by which the requirements of the enablement portion of the statute can be satisfied. The specification need not convince those skilled in the art that its assertions are correct. There are instances, however, such as in certain chemical inventions where very broad claims are being advanced, that additional support may be requested to establish that the invention works as claimed.

A particular problem area involves inventions concerning biological materials such as microorganisms. Unless the biological material in question is known and available or can be readily produced by a known procedure without undue experimentation, it is usually not possible to explain how to obtain it merely by a written discussion. This problem of disclosure can be taken care of by depositing the material in an approved depository under conditions that will make it accessible once the patent issues. Making it accessible, however, does not give anyone the right to infringe the patent. In such a case, it is desirable for the biological material to be on deposit at the time the application is filed and for the application to refer to the access number given the microorganism by the approved depository. The description should also include any taxonomic information. Depositories in the United States include the following:

1. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852. Phone: 301-881-2600. Telex: 908-768.
2. National Research Culture Collection, U.S. Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604. Phone: 309-685-4011. Fax: 309-671-7814.

A deposit in either of the foregoing depositories can also be used for satisfying deposit requirements for certain other countries, such as those observing the Budapest Treaty.

For applications involving a computer program, the disclosure should include the program itself or at least a flow diagram that sets forth the sequence of operations the program is to perform. Even with a flow chart, the disclosure might be questioned by the examiner, particularly if the operations stated are very general or very complex.

A specification must also teach how to use the invention it describes. In drug cases, the office refuses to accept general statements of utility as satisfying this teaching requirements. General statements that have been considered unacceptable include those purporting pharmacological or therapeutical purposes or biological activities. To satisfy the teaching requirements in such cases, it is necessary to present more specific uses, such as the treatment of a particular ailment or disease. It is also desirable to disclose precise dosages and treatment methods.

Besides requiring a statement of utility, the office may also require proof depending on the type of utility asserted. For instance, the highest degree of proof of utility is usually required for those inventions stated to cure or treat diseases, such as cancers, that are generally considered, at the present time, as being difficult to treat. On the other hand, compositions whose properties are usually predictable from a knowledge of their constituents, such as laxatives, antacids, and certain topical preparations, require little or no clinical proof.

Best Mode

The specification is required to include the best mode contemplated by the inventor for carrying out the invention. This does not mean that the best way of carrying out the invention in any absolute sense must necessarily be disclosed, but only the best way contemplated by the inventor. This requirement also pertains to the point in time when the application was filed and need not be updated if a new best mode is subsequently contemplated by the inventor.

This requirement for disclosure of the best mode is to prevent inventors from obtaining the benefits of patent protection while maintaining for themselves, by concealing from the public, the preferred ways in which to carry out the invention. The best mode disclosure, as well as the enablement requirements previously discussed, represents the consideration given by the inventor in exchange for the benefits of the rights granted by the patent (ie, the right to exclude others from making, using, selling, offering for sale, or importing the invention).

In the event a specification does not satisfy the requirements of 35 USC §112, a patent can be held invalid. In addition, if the best mode was deliberately concealed, the issue of inequitable conduct against the office may be involved.

Drawings

If the nature of an invention is such as to lend itself to illustration, drawings become a required part of the application. Inventions that require drawings are those directed to machines, articles of manufacture, and certain processes. Such drawings are not required to be drawn to scale. Therefore, if dimensions or spatial relationships are important, these should be explicitly stated in the specification or on the drawings themselves. The drawings must also be described in the specification. There are some very specific rules with respect to the size and type of paper, the margins, and the cross-hatching to be used in all such drawings.

Claims

The claim or claims of a patent represent the metes, or bounds, of the property to be protected. In other words, as in real property, a patent claim stakes out that territory that the patentee considers his or her own, and any encroachment on that particular territory, as in real property, constitutes an infringement. The claims of a patent can be viewed as the word fence surrounding the invention. It is important not to confuse such claims with technical claims that state benefits or advantages.

The second paragraph of 35 USC §112 states the purposes of claims. Their basic functions are to point out, define, and distinctly lay title to the subject matter that the applicant regards as his or her invention, but not necessarily to describe that invention in any great detail. It is the descriptive portion of the specification that performs this function. In fact, the descriptive portion of the specification and of the claims differ chiefly in the degree of detail presented by each. In particular, claims usually omit mentioning nonessential features and, whenever possible, attempt to use generic language to describe the particular elements of the claimed invention instead of the far more specific language found in specifications.

It is important to recognize, however, that despite the desirability of obtaining the most generic claim coverage possible, varying claims of lesser scope are also important if the invention is to be adequately protected. A generic claim is more difficult for potential infringers to design around than a very specific claim is, but the latter is more difficult to invalidate in terms of prior art. On the other hand, a specific claim is easier to design around, and thus avoid literal infringement, than a generic claim.

It is helpful to make claims of both specific and intermediate scope to hedge against the possible invalidation of the patent's generic claim or claims. In such cases, the ability to establish infringement of valid claims of lesser scope may be important because such claims are often the only ones of any commercial value. This is why it is recommended that specific claims recite the best or preferred embodiments of the invention that are more fully disclosed in the description portion of the specification.

A claim must be written as a single sentence. In actuality, the claim is really the predicate noun that completes the sentence, "What I(we) claim is . . . " or "What is claimed is . . . "

Generally speaking, a claim can be divided into three major components. The first may be referred to as the introductory phrase or preamble, followed by a transitional phrase, and then the body of the claim.

Preamble of the Claim. The introductory phrase, or preamble, of a claim sets the stage for the remainder of the claim. It may merely make a general statement concerning the invention, the title of the invention, or the general class into which the invention falls. The introductory phrase might also include a statement of the intended use, object, purpose, or advantages or the invention. Some examples of the range of introductory phrases are as follows:

A process.
A process for extracting oil from fish.
A process for extracting omega-3 fatty acids from fish.

A composition.
A broth composition.
A chicken broth composition.

An apparatus.
An apparatus for separating grains of different sizes.
An apparatus for separating corns of different sizes.

As can be appreciated from these samples, the preamble may be as general or as specific as desired, depending on the particular invention involved, the prior art, and the intent of the preamble.

Transitional Phrase of the Claim. The degree to which a claim is considered open (inclusive) or closed (exclusive) is determined by its transitional phrase. This is an important distinction because the addition of a constituent or a step not explicitly recited in a closed claim can avoid its literal infringement. In an open claim, on the other hand, the presence of an additional constituent or step not explicitly recited in the claim will not necessarily avoid its literal infringement.

Claim terms that are considered to be closed are "consisting of" and "composed of." These terms mean that the presence or addition of something other than that which is explicitly recited in the claim will avoid its literal infringement. Claim terms considered open are "comprising," "including," and "containing." When such language is used, the inclusion of steps or constituents not explicitly recited will not necessarily avoid a literal infringement of the claim.

It is not necessary for a claim to be entirely closed or entirely open. In particular, the presence of the phrase, "consisting essentially of," is usually interpreted to mean that the claim covers not only the recited constituents of the process, composition, article, or apparatus but also any additional ones so long as the latter do not significantly interfere with the primary function or interrelationship of those constituents explicitly recited. If the material or component or step that is added to those recited is of a type that is usually employed with the kind of subject matter in question, then infringement is usually easy to establish.

Body of the Claim. The structural elements of the claim are presented in what is known as its body. These elements include, for instance, the steps of a process, the components of a composition, or the parts of a machine or apparatus that constitute the prime subject matter of the invention. The body of the claim specifies the configuration, spatial relationship between elements, and relative amounts of components that may be important to point out the subject matter.

It is not necessary that the elements of the claim be recited in the form of the actual structure, material, or procedural acts involved. Alternatively, the words *means* or *step* may be used followed by a description of the function to be performed by the means or step.

For instance, in a claim directed to a bioreactor, reciting "means for supporting the bottom of a bioreactor at a suitable distance from the floor" could be substituted for a description of bioreactor legs.

It is suggested that terms such as "about" and "approximately" be employed whenever reciting amounts, distances, or spatial relationships, because such terms may provide a somewhat greater scope in the interpretation of the breadth of the claim.

Types of Claims. The subject matter of claims can take the form of a process, an apparatus, an article of manufacture, or a composition. Moreover, the subject matter of a claim often referred to as a hybrid claim can take the form of any combination of these. For instance, a process claim might include a recitation of a certain apparatus or an apparatus claim might include recitations concerning materials treated by the apparatus. Usually, the patentability of such hybrid claims will depend only on those differences from the prior art of the principal subject matter of the claim rather than on the differences of the secondary recitations. For instance, the patentability of a process claim with apparatus recitations will depend primarily on the process steps and not on the apparatus recitations. Likewise, the patentability of an apparatus claim containing recitations of the material being worked on will depend on the structure of the apparatus rather than the material in question.

A particular type of hybrid claim sometimes found in chemical cases is referred to as a product-by-process claim. It is used to define a composition of matter or a material by referring to the particular process employed to prepare the product in question. The patentability of such a claim depends on whether the product differs from prior-art products, not whether the process steps differ from prior-art processes. The product-by-process claim can be important when no other way seems convenient to define a product that differs from prior art products. Because of the nature of the product, its differences from the prior art may be difficult to define other than by reference to the process itself. Also, the product-by-process claim may be important when the possibility exists that the manner in which the product has been described is not entirely correct.

The inventions resulting from the development of a new technology need not be restricted to any one of the preceding categories of inventions; they may, in fact, be claimed in any number of different ways. For instance, the development of a new composition of matter may not only result in a patentable invention with respect to the composition itself, but also to the process for preparing it, the apparatus used to carry out the process of preparing it, the process or processes for using it, finished articles containing it, and so on. Accordingly, any number of patents may be obtainable for any particular development. In fact, it is desirable to attempt to claim a development by making as many different types of claims as possible. This is important because a variety of claims can encompass different classes of direct infringers, and it may be more desirable to sue one kind of infringer rather than another in view of the potential recovery or convenience involved. For instance, it would certainly be more desirable to be able to sue the manufacturer of a composition rather than the users, particularly, if the users are individual consumers or the inventor's own customers. Also, certain types of claims may be more difficult to invalidate in view of the prior art than others, thereby hedging against loss of all of the potential protection available.

Each claim of a patent or patent application is considered as a separate invention and is examined independently from the other claims with respect to its patentability and validity. During the examination of an application, it is not uncommon for an examiner to reject some claims in an application while allowing others. Likewise, a court can find some claims valid, while finding others invalid.

Oath or Declaration

To complete the requirements of the application, it is necessary to have a declaration or oath signed by the inventor(s). This declaration need not be filed along with the initial application papers but must be filed within one month after notice by the office, requiring such filing or within two months of the initial filing, whichever is later.

SUMMARY

The patent system serves to promote the progress of technology by encouraging innovation and by making available to the public the information concerning patented inventions. This article is intended to provide inventors and their managers with some basic familiarity and comfort level with the patent system. In view of the relative complexity of this subject matter, this article is not intended to teach patent law or how to best protect an individual invention. In view of the significant cost of present-day scientific research and development and the global nature of implementing new technology, it is suggested that a patent attorney or patent agent be consulted as early as practical in order that the fruit of the innovative labors be adequately protected.

GENERAL REFERENCES

This article is adapted from B. A. Amernick, *Patent Law for the Nonlawyer: A Guide for the Engineer, Technologist, and Manager*, Van Nostrand Reinhold, copyright, 1986. Used with permission.

BURTON A. AMERNICK
Pollock, Vande Sande, and Amernick, RLLP
Washington, D.C.

PEACHES. See FRUITS, TEMPERATE.

PECTIC SUBSTANCES

Pectic substances are a group of complex polysaccharides localized to the middle lamella, intercellular crevices, and primary cell walls of most if not all higher plants (1). They are significant contributors to the texture of fruits and vegetables and their processed products (2). Pectin and/or pectin fragments have been reported to possess pharmacological activities that include immunostimulation, antimetastasis, hypoglycemic, and cholesterol-lowering effects (3). In addition to the obvious structural role of pectic polysaccharides in plants, pectic fragments act as chemical messengers in the development, growth, senescence, and biochemical protection of plants (4).

Because the gel-forming properties of pectin substances (*pectin* is derived from a Greek word meaning to congeal or solidify) were discovered before the development of modern organic and macromolecular chemistry, common rather than structural names were applied to pectic polysaccharides, and these persist (5).

DISTRIBUTION IN NATURE

The percentage of pectic substances in plants varies with species, variety, anatomy, and maturity. The highest concentrations of pectic substances are found in young tissue and in fruit tissue. Typically, whole mature fruit contains 3 to 7% pectic substances on a dry-weight basis and 0.13 to 1.1% on a fresh-weight basis. The relative high pectic and low caloric content of citrus fruits make them a good source of soluble dietary fiber in that pectic substances are not digested until they reach the lower gastrointestinal tract. Apple pomace (the residue of pressed apples) may contain up to 20% pectic substances, and the albedo of oranges (the white, spongy material on the inside of the peel) may contain 40% or more pectic substances on a dry-weight basis.

ISOLATION AND PURIFICATION

The relative ease of the isolation and purification of large quantities of food-grade pectic substances account for their early discovery. Commercially, apple pomace or citrus peels (eg, orange, grapefruit, and primarily lemon or lime) are extracted with the goal of producing food-grade pectin for gels (6). Typically, pectin is acid extracted at low pH (1.5–3) to inhibit degradation by endogenous enzymes, ester saponification, and alkaline degradation by beta-elimination reactions. Extracts are filtered or centrifuged to remove insolubles and precipitated with alcohol or polyvalent salts such as those from aluminum or copper.

To obtain pectins with maximum retention of native structure and properties for research purposes, milder techniques have been adopted at the expense of product yield and with considerable lengthening of the isolation-purification process. In these procedures, relatively intact cell walls are isolated by macerating plant tissues in aqueous alcohol, washing to remove extraneous debris and deactivate endogenous enzymes, then sequentially extracting with chelating agent and mild alkali. Attempts to reduce size heterogeneity by gel filtration chromatography and chemical heterogeneity by ion exchange chromatography have been met with limited success.

CHEMICAL COMPOSITION AND STRUCTURE

Mildly extracted pectic substance is primarily a helical block copolymer of *D*-galacturonic acid and its methyl ester (7). These comonomers are (1→4) *O*-linked as poly(*α*-*D*-galactopyranosyluronic acid) and its methyl ester. Blocks are interrupted by (1→2) *O*-linked *α*-*L*-rhamnopyranosyl inserts. Some of these features are incorporated in Figure 1. Neutral sugars associated with pectin other than rhamnose include arabinose, galactose, glucose, xylose, and mannose. At least three of these neutral sugars, arabinose, galactose, and xylose, have been found in pectin as short or oligomeric side chains that themselves may be branched (8). Some portions of the pectin backbone have a high density of side chains and have been designated as hairy regions, whereas other portions of the pectin backbone are completely devoid of side chains and have been labeled as smooth regions. The hairy regions contain most of the neutral sugars found in pectin, namely rhamnose, arabinose, galactose, and xylose. A few sources of pectin (eg, sugar beets) contain acetyl and feruloyl esters. Acetyl esters are linked to ring hydroxyls in the glacturonate backbone, whereas feruloyl esters appear to be linked to neutral sugar side chains. Approximately 80 to 90% (by weight) of total sugars in commercial citrus pectins are residues of galacturonic acid and its methyl ester with the remaining sugars being neutral. Degree of methyl esterification (DM) in pectic substances will vary with plant source and method of extraction. Commercial citrus and apple pectins with DM ranging from 80 to 18% are available. X-ray diffraction patterns of sodium polygalacturonate indicate that water molecules are bound to each galacturonate residue in the backbone (9). Thus, bound water may account for about 8% of pectin samples by weight.

METHODS OF ANALYSIS

Because of its complex composition, several methods are necessary to analyze for pectin (10). A few of the more important methods will be discussed here. Of the several colorimetric methods developed to analyze for galacturonic

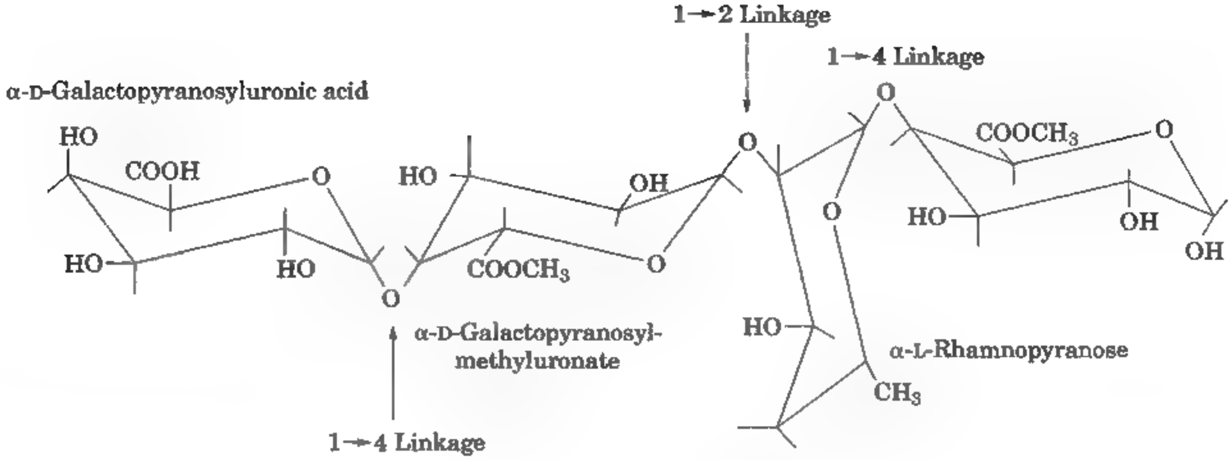


Figure 1. Portion of pectin molecule.

acid, the reaction of pectin with *m*-hydroxylbiphenyl in heated acids media to form a colored product is employed widely. Base titration before and after saponification is used for the simultaneous determination of DM and galacturonic content. Also, DM is determined by saponifying methyl ester groups and analyzing for free methanol by gas chromatography or by oxidizing the methanol to formaldehyde, which is determined colorimetrically. Carboxyl and methylester groups also may be determined by infrared spectroscopy in deuterated water provided there is a prior calibration against known standards. Often, neutral sugars are analyzed by gas chromatography after hydrolysis and conversion to volatile derivatives. Various derivatives and stationary phases have been employed. In one method, sugars are converted to their alditol acetates and chromatographed on a high-performance capillary column. Acetyl esters are analyzed by conversion to acetic acid, separation by steam distillation, and titration of the acid. Feruloyl esters are hydrolyzed to ferulic acid, which is analyzed by the Folin–Ciocalteu reagent.

CHEMICAL AND ENZYMATIC REACTIONS

The reactions of pectin are those characteristic of polysaccharides, esters, and organic acids (7). A select few of the more important reactions will be discussed briefly. Pectin undergoes acid-, base-, and enzyme-catalyzed depolymerizations. At acidic pH, glycosidic bonds other than those of (1→4) self-linked *α-D*-galacturonic acid are hydrolyzed preferentially. Thus commercial pectin is extracted from plant matrices by controlled acid hydrolysis. Prolonged acid hydrolysis can produce polygalacturonic acid with about 25 residues, whereas milder hydrolysis conditions have produced homogalacturonans with 70 to 100 galacturonan residues. Base-catalyzed depolymerizations occur at neutral and higher pH. These are beta-elimination reactions, which proceed with concurrent endodepolymerization, deesterification, and double bond formation (Fig. 2). The relatively high susceptibility to enzymatic and acid-catalyzed hydrolysis of the neutral sugar side chains and rhamnoglacturonan glycosidic bonds in the backbone of pectin may play an important role in plant metabolic processes. Evidence is accumulating that various pectic fragments act as biochemical messengers that initiate various biochemical reactions in plant development, senescence, and the defense of plants against pathogens (4). For example, evidence suggests that fragments from pectin-containing galactose may elicit ethylene production, which is important in the process of senescence. Endogenous endopolygalacturonases have been associated with fruits that ripen rapidly (eg, pears and freestone peaches), whereas those that contain only exopolygalacturonases (eg, apples and clingstone peaches) ripen more slowly. Polygalacturonases depolymerize deesterified pectins only. Most of the common pectin lyases depolymerize pectin in a fashion similar to beta-elimination reactions (Fig. 2). Commonly, pectin lyases are not found endogenously in plant cell walls but are produced by microorganisms. In addition to their biological importance, bacterial pectolytic enzymes have gained importance as probes to elucidate the neutral sugar side chain structure of pectin (11). For example, the action of *β*-(1,4)-galactanase has shown that apple pectin contains arabinogalactan side chains high in arabinose. More recently, fungal enzymes have been used to investigate the structure of the hairy regions in pectin (8). Pectin methyl esterase activity has been found in the plant cell wall, but the biological function of this enzyme is not clear. Reactions specific to the reducing sugar end group are important in that they permit determination of the number-average molecular weight and provide a method of following the course of depolymerization reactions. Chlorite oxidation of end groups has been used to determine pectin molecular weight, whereas several color reactions specific for reducing sugars have been used to assay for the galacturonic acid produced in depolymerization reactions (6). Ammonia partially amidates pectin through displacement of methyl ester groups. Amidated low methoxy pectic substances gel more readily than corresponding nonamidated low methoxy pectic substances (7). Pectin from sugar beet pulp has been gelled by intermolecular, oxidative coupling of feruloyl ester groups

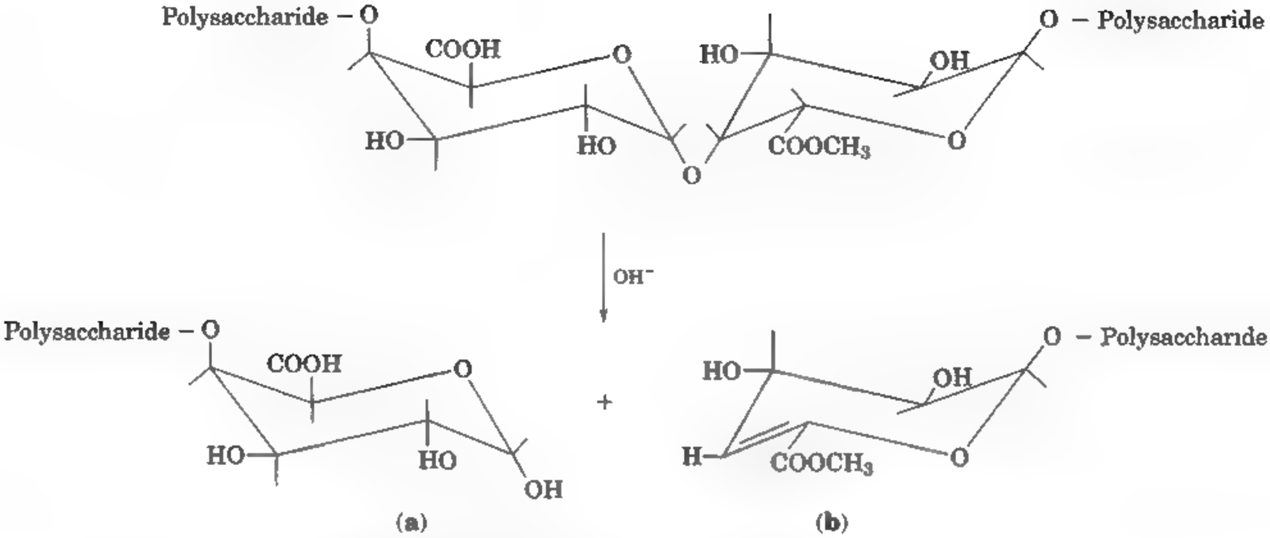


Figure 2. Schematic of β-elimination reaction: (**a**) is reducing sugar end group; (**b**) is unsaturated, nonreducing sugar end group.

through their aromatic rings. Coupling was achieved with a mixture of hydrogen peroxide and peroxidase (6). This reaction provides an opportunity for commercialization of beet pectin, because it is a poor gel former without oxidative coupling.

MOLECULAR WEIGHT, SIZE, AND SHAPE OF PECTINS

Over a period spanning more than 50 years of research, the molecular parameters of pectins have been measured extensively by numerous methods. Nevertheless, significant disagreements prevail concerning these parameters. The controversy may arise from the variability of pectin with plant source, whether dissolved pectin exists in solution as individual molecules or as aggregates, and the tendency of pectin to fragment. High methoxy (ca DM = 73) commercial citrus pectins give number-average molecular weights in the range 4–4.9 × 10^4 (12). A linear plot of reduced osmotic pressure against concentration appears to indicate a well-behaved unaggregated polymer. The second virial coefficient calculated from osmometry indicated a rodlike shape. Nevertheless, briefly heating pectin resulted in a slow concentration-dependent disaggregation that was also shown by osmometry. High-performance size-exclusion chromatography (HPSEC) with on-line viscometry detection on a series of commercial citrus pectins gave results consistent with the aggregated rod model (13). HPSEC studies of tomato cell wall pectins with computer-aided curve fitting of the chromatograms revealed that all pectins investigated were composed of a linear combination of five macromolecular subunits (14). The relative sizes of these subunits as obtained from their radii of gyration (R_g) were 1:2:4:8:16, with the smallest subunit having an R_g of about 25 Å. Dissociation of pectin into smaller-size fragments by dialysis against 0.05 *M* NaCl led to the conclusion that cell-wall pectin acted as if it were an aggregated mosaic, held together at least partially through noncovalent interactions. Superimposed and individual circular microgels in the micrometer size range were isolated from the cell walls of peaches and visualized by electron microscopy after rotary shadowing. Dilute NaCl and 50% aqueous glycerol disaggregated these microgels into rods, segmented rods, and kinked rods that collectively comprised the internal gel network of the microgels (15).

BINDING PROPERTIES

Many of pectin's unique properties result from its propensity to self-aggregate or to interact with cosolutes (6). A case in point is the pectin gels, which are three-dimensional networks capable of entrapping water. High-methoxyl pectins (HMP) with DM in the range 80 to 57% are induced to gel by adding sugars, which are thought to promote the self-aggregation of pectin by removing bound water, reducing ionic repulsions, and stiffening the polymer chain. These nonspecific interactions with sugars may be responsible for pectin's ability to reduce glucose intolerance in diabetics. For HMP at constant pH, gel strength and rate of gelling increase with DM, presumably through increased interchain hydrophobic interactions. HMPs of decreasing DM can be induced to gel by decreasing the pH, possibly through increased interchain hydrogen bonding. Low-methoxyl pectins (LMP), DM below 50%, gel with the addition of calcium or other divalent cations through interchain cross-links. Because decreasing DM increases the number of potential cross-links, LMP exhibits increased gel strength and higher gelling temperatures with decreasing DM. For HMP and LMP, gel strength increases with increased molecular size, possibly by cooperative self-interactions. It appears that all three major mechanisms exist for interchain interactions, namely divalent cation cross-links, hydrogen bonding, and hydrophobic interactions. Furthermore, for any mechanism to prevail, a critical number of functional groups must be present to promote cooperative interactions. Electron spin resonance studies support the concept that divalent cation binding to pectin in apple cell walls results in interchain bridges between adjacent smooth regions of pectin and that binding is a sequential cooperative process. Clinical studies suggest that pectin lowers blood cholesterol. This may be a case where the formation of divalent cation bridges is important in that calcium may form bridges between negatively charged pectin and bile acids. It has been suggested that binding and excretion of bile acids leads to a decrease of cholesterol in the bloodstream.

FOOD APPLICATIONS

A major application of pectin is in jams and jellies (16). A high-sugar jam contains 30 to 45% of fruit pulp and 0.20 to 0.4% pectin added as a gelling agent. Jams made with HMP must contain at least 60% soluble solids (sugars) to gel. Reduced-sugar or dietetic jams are manufactured with 55% or less soluble solids (even below 30%), by adding low methoxyl pectins (eg, in the range 0.75–1.0%). At very low soluble solids, a calcium salt often is added to aid gellation. Frequently, jellies are made from depectinized fruit concentrates with added pectin, water, and sugar. High-quality, tender confectionery jellies with excellent flavor-release characteristics contain pectin. Pectin is added to jams, fillings, and toppings as a gelling or thickening agent in the preparation of baked goods. HMP jams are useful in applications requiring resistance to the heat of baking such as occurs in producing tarts containing jam. Amidated low methoxyl pectins (ALMP) confer thermal reversibility to gels. These gels are useful as glazes for pastries or flans. Typically, these products are supplied as a paste containing ALMP, calcium diphosphate, and 65% sugar solids, which when diluted and melted can be reset to a clear, shiny glaze on cooling. In recent years, pectin has found increased application as an additive to dairy products. Yogurt containing fruit bases has been growing in popularity. Substituting pectin for modified starch as a thickening agent in yogurts will maintain a uniform distribution of fruit throughout the yogurt without masking delicate fruit flavors. Furthermore, unlike starches, pectin will not introduce a floury texture to yogurts. If the fruit bases contain 60% sugar, then HMP can be added. If the sugar content is lower than 60%, then ALMP is added. HMP stabilized casein against aggregation when heated at a pH less than 4.3. Thus it is added as a stabilizer in ultra-high-temperature-treated yogurt drinks and to milks blended with fruit juices. Pectin also stabilizes acidified soy milk drinks and whey products against protein precipitation. A low level of pectin is often added to low-calorie soft drinks to replace mouth-feel lost with the removal of sugar. Pectin is added to sorbet and ice pops to control ice crystal size; and to ice pops to prevent flavor and color from being sucked from the ice structure. Pectin is added to chutney and sauces to improve texture and batch-to-batch uniformity. LMP and ALMP gels can replace gelatin as a base in dessert jellies for the purpose of providing good flavor release. Pectin gels have higher melting points than gelatin gels and thus hold up better in warm weather.

Pectin has several applications in the pharmaceutical industry. It has been added to mixtures containing kaolin or bismuth compounds for the prevention of diarrhea. Pectin is added to maintain the viscosity of medicinal syrups. Recently, pectin has been employed as a filler in self-adhesive colostomy flanges and to promote healing by its addition to wound dusting powders and ulcer dressings.

GLOSSARY

Pectate. Pectate as in calcium or sodium pectate is pectic acid that is fully or partially neutralized with metal ions. Often referred to as polygalacturonate as in calcium or sodium polygalacturonate.

Pectic acid. Pectic acid is pure poly[(1-4)-*O*-linked (alpha-*D*-galactopyranosyl uronic acid)] and is often referred to as polygalacturonic acid.

Pectin. Pectin, as originally defined, is a water-soluble mixture of pectinic acids or partially neutralized pectinic acids capable of undergoing gel formation.

Pectinate. Pectinate as in calcium or sodium pectinate is pectinic acid that is partially or fully neutralized with metals ions.

Pectinic acid. Pectinic acid is a mixture of pectic substances, a significant portion of which is a copolymer of galacturonic acid and its methyl ester.

Protopectin. Protopectin refers to native, undissolved or insoluble plant tissue pectin.

DISCLOSURE STATEMENT

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

BIBLIOGRAPHY

1. M. C. Jarvis, "Structure and Properties of Pectin Gels in Plant Cell Walls," *Plant Cell Env.* **7**, 153–164 (1984).
2. G. B. Fincher and B. A. Stone, "Metabolism of Noncellulosic Polysaccharides," in W. Tanner and F. A. Loewus, eds., *Plant Carbohydrates II*, Vol. 13, Springer-Verlag, New York, 1981, pp. 103–105.
3. H. Yamada, "Contributions of Pectins on Health Care," in J. Visser and A. G. J. Voragen, eds., *Pectins and Pectinases*, New York, 1996, pp. 173–180.
4. E. A. Nothnagel et al., "XXII A Galacturonic Acid Oligosaccharide from Plant Cell Walls Phytoalexins," *Plant Physiol.* **71**, 916–1026 (1983).
5. Z. I. Kertesz, *The Pectic Substances*, Wiley-Interscience, New York, 1951, pp. 3–9.
6. M. L. Fishman, "Chemical and Physical Properties of Pectin," *ISI Atlas of Science: Biochemistry* **1**, 215–219 (1988).
7. J. N. BeMiller, "An Introduction to Pectins: Structure and Properties," in M. L. Fishman and J. J. Jen, eds., *Chemistry and Function of Pectins*, ACS Symposium Series **310**, American Chemical Society, Washington, D.C., 1986, pp. 2–12
8. H. A. Schols and A. G. J. Vorajen, "Complex Pectins: Structure Elucidation Using Enzymes," in J. Visser and A. G. J. Voragen, eds., *Pectins and Pectinases*, Elsevier, New York, 1996, pp. 3–19.

9. M. D. Walkinshaw and S. Arnott, "Conformations and Interactions of Pectins," *Molekulyarnaya Biologiya* **153**, 193–205 (1981).
10. L. W. Doner, "Analytical Methods for Determining Pectin Composition," in M. L. Fishman and J. J. Jen, eds., *Chemistry and Function of Pectins*, ACS Symposium, Series 310, American Chemical Society, Washington, D.C., 1986, pp. 13–21.
11. J. A. deVries et al., "Structural Features of the Neutral Sugar Side Chains of Apple Pectin Substances," *Carbohydr. Polym.* **3**, 193–205 (1983).
12. M. L. Fishman, L. Pepper, and P. E. Pfeffer, "Dilute Solution Properties of Pectin," in J. E. Glass, ed., *Water Soluble Polymers: Beauty with Performance*, Advances in Chemistry Series **213**, American Chemical Society, Washington, D.C., 1986, pp. 57–70.
13. M. L. Fishman et al., "Characterization of Pectins in Conjunction with Viscosity Detection," *J. Ag Food Chem.* **37**, 584–591 (1989).
14. M. L. Fishman, D. T. Gillespie, and S. M. Sondey, "Macromolecular Components of Tomato Fruit Pectin," *Arch. Biochem. Biophys.* **274**, 179–191 (1989).
15. M. L. Fishman et al., "Progressive Dissociation of Pectin," *Carbohydrate Res.* **248**, 303–316 (1993).
16. C. D. May, "Industrial Pectin Sources, Production and Applications," *Carbohydr Polym.* **12**, 79–99 (1990)

MARSHALL FISHMAN
USDA/ARS
Wyndmoor, Pennsylvania

PEPTIDES

Peptides have relevance to food science in various ways. Peptides contribute to the physical properties of food (physical aspect) and have taste (organoleptic aspect). Intestinal absorption of dipeptides or tripeptides is better than that of free amino acids (nutritional aspect). Biologically active peptides are found in food (physiological aspects). In this article the general aspects of peptides will be briefly summarized and various aspects of peptides as food constituents will be described.

GENERAL ASPECTS OF PEPTIDES

Definition

Peptide is a substance in which amino acids are bound together by peptide bonds, amide bonds between α-amino groups, and α-carboxyl groups of neighboring amino acids. Peptides containing fewer than 10 amino acids residues are called oligopeptides and those containing 10 amino acids or more are called polypeptides. Polypeptides containing more than 50 amino residues are called proteins.

Most peptides of natural origin are linear molecules containing L-amino acids. The structure of, for example, L-alanyl-L-threonyl-L-tyrosine is expressed as H-Ala-Thr-Tyr-OH or Ala-Thr-Tyr in three-letter abbreviations and as A-T-Y in simple one-letter abbreviations.

Usually, 20 different amino acids are found in proteins and peptides. Therefore, the number of possible peptides containing n residues of amino acid is 20^n.

Formation

Peptides are formed in four ways as described next.

1. Peptides are released by enzymatic digestion of proteins that have been synthesized on ribosomes according to the information coded in mRNA. This is the major route of peptide formation *in vivo*. Both the hydrolysis of food proteins in the digestive tract and also release of biologically active peptides in a variety of organs are classified in this category. The production of peptides by recombinant DNA technology essentially follows this route.
2. In living organisms there are peptide-synthesizing systems that are independent of mRNA and ribosomes. A few peptides (glutathione, kyotorphin, and cyclic peptide antibiotics) are synthesized in this way.
3. Under special *in vitro* conditions peptide bonds are also formed by protease-catalyzed reactions (1) (condensation and aminolyses of amides or esters).
4. Peptides are chemically synthesized by a condensation of amino acids in the presence of an activating reagent for carboxyl groups such as carbodiimides. If condensation between the carboxyl group of amino acid A and the amino group of amino acid B is to occur, the amino group of A and carboxyl group of B must be protected. Protection of other functional groups in the side chains of amino acids is also necessary. Stepwise syntheses of peptides are facilitated by a solid-phase synthesis (2). In the solid-phase synthesis developed by Merrifield, *t*-butyloxy-carbonyl (*t*-Boc) amino acid is coupled to chloromethyl polystyrene resin. After the acid-catalyzed removal of the *t*-Boc group from the resin, the next *t*-Boc amino acid is coupled to the resin in the presence of carbodiimide. Amino acids are thus coupled successively to the resin from the carboxyl terminus toward the amino terminus. Peptides are released from the resin in a strong acid such as anhydrous hydrogenfluoride. Protecting groups for amino acid side chains are also removed by this procedure. In a new procedure, the alkaline labile 9-fluorenyl-methoxycarbonyl (Fmoc) group is used as a blocking group for the α-amino group. In this case, recovery of the peptide from the resin is facilitated by trifluoroacetic acid.

CHEMISTRY AND ANALYSIS

The amino acid composition of a peptide is analyzed after acid- or base-catalyzed hydrolysis by an amino acid analyzer. The covalent structure of a linear peptide (primary structure) is analyzed by a stepwise chemical cleavage from the amino terminus of the peptide using phenyl isothiocyanate (Edman degradation) (3). This process is automatically done by a protein sequencer. The stepwise chemical cleavage method from the carboxyl terminus of peptide has also been reported (4). The primary structure of a peptide can be analyzed to some extent after the enzymatic cleavage by aminopeptidase or carboxypeptidase. The primary structure of a peptide can be also analyzed by X-ray crystallography. For the analysis of conformation of the peptides in solution, circular dichloism or nmr are available (5).

OCCURRENCE IN NATURE

A number of biologically active peptides occur in nature. Most of the biologically active peptides are synthesized as inactive precursor proteins and processed to an active form by a limited proteolysis. Acid- or base-catalyzed hydrolysis of proteins also produces peptides. Among heat-incoagulable peptides released by the digestion of proteins, those precipitable by the addition of ammonium sulfate are called proteoses and those soluble in saturated ammonium sulfate are called peptones.

Some peptides of microorganisms and plants have a cyclic structure and contain D-amino acid or amino alcohol or amino aldehyde. Those containing constituents other than amino acids are called depsipeptides; peptidic antibiotics belong to this category.

PEPTIDES IN PHARMACOLOGICAL USE

A number of biologically active peptides are used in pharmacology. These are classified into hormones, growth regulators, immunomodulators, neurotransmitters, substrates or inhibitors of enzymes, and so forth. Among them, some of the growth regulators and immunomodulators should be classified as proteins because of their molecular weights. The most widely used peptide drug is insulin. Not only agonists but also antagonists of these biologically active peptides show interesting pharmacological effects. Fragment peptides containing binding sites for receptors sometimes exhibit interesting biological effects as well. For example, Gly-Arg-Gly-Asp-Ser and Tyr-Ile-Gly-Ser-Arg are the fragment peptides of fibronectin and laminin, respectively. These peptides have been shown to prevent the metastasis of tumors. Asp-Ser-Asp-Pro-Arg (Hamburger peptide), a fragment peptide of human immunoglobulin E, shows an antiallergic effect. Peptide T (Ala-Ser-Thr-Thr-Asn-Tyr-Thr), a fragment peptide of the envelope glycoprotein of human immunodeficiency virus, inhibits the binding of the viral envelope to the CD-4 receptor of T-lymphocytes.

Inhibitor peptides for proteases show various pharmacological effects. Inhibitors for angiotensin I-converting enzyme or renin show antihypertensive effects, the aminopeptidase inhibitor bestatin shows an immunostimulating effect, and proryl endopeptidase inhibitor shows an antiamnesic affect.

Peptides are also promising as safe vaccines for the prevention of microbial or viral infections. Because of the difficulty of intestinal absorption and inactivation in the digestive tract, most peptide drugs are administered by injection. By suitable chemical modification, such as the incorporation of synthetic amino acids or acylation of the α-amino group or the amidation of the α-carboxyl group, some biologically active peptides are improved in specific activity, half-life, and proteolytic resistance. Some peptides become available for use through the oral or nasal route after such chemical modification or after physical modification such as emulsification and incorporation into liposomes. Another pharmacological use of peptide is as a solubilizer or emulsifier of insoluble drugs. Hydrolysates of gelation and casein have been shown to have such a potency (6).

PEPTIDES IN FOOD SCIENCE

Physical Aspect

Peptides contribute to the physical properties of food. For example, the smooth texture of cheese is dependent on the extent of proteolysis of casein during the maturation. Proteins and peptides have surface activities and show functional properties such as emulsifying and foaming. Amphiphatic properties, charge distribution, solubility, and the molecular weights of peptides are related to these functional properties. Usually the functional properties of food proteins are modified by proteolysis. In some proteins, like gluten, whose solubility is very low, the functional properties are improved by limited proteolysis. When a protein has good solubility, functional properties of the peptides derived from it tend to be lower than those of the parental proteins. Enzymatic digests of soybean proteins suppress thermal gelation and freezing-induced insolubilization of proteins. Soybean peptides protect starch from retrogradation.

Antioxidative Peptides

Amino acids such as tryptophan, tyrosine, histidine, methionine, proline, and branched-chain amino acids show antioxidative activity. Peptides containing these amino acids also show antioxidative activity (7,8). Antioxidative activities of peptides are sometimes higher than those of amino acids. This is partly because peptides have higher affinity for lipids than amino acids.

Taste of Peptides

Amino acids have taste. The taste of oligopeptides is sometimes more diverse and intense than that of amino acids. Oligopeptides elicit bitter, sweet, sour, umami, and salt (9).

Bitter Peptides. Hydrophobic acids (phenylalanine, tyrosine, tryptophan, leucine, valine, and isoleucine) of L-configulation taste bitter. Generally, oligopeptides consisting of these amino acids taste bitter. Bitter peptides are smaller than decapeptide in size and have two hydrophobic groups, or one hydrophobic group and one basic group, which are separated by 4.1 A (10). Hydrolysates of proteins, such as casein, soybean protein, and fish protein, sometimes taste bitter. Many bitter peptides have been isolated from hydrolysates of food proteins and their analogues have been synthesized. The bitterness of a protein hydrolysate is dependent on the types of protease used; formation of bitter peptide can be minimized by choosing protease (11). Carboxypeptidase W from wheat germ has been shown to be effective in debittering peptides. Oligopeptide rich in Glu is effective in masking a bitter taste.

Sweet Peptides. A typical sweet peptide (8) is aspartame (Asp-Phe-OMe), which is found during the synthesis of gastrin. Special arrangement of three functional groups (the basic α-amino group, the acidic β-carboxyl group of aspartyl residue, and the hydrophobic phenylalanyl residue) is required for sweetness. Phenyl-acetyl-glycyllysine and benzoyl-β-alanyllysine, which have a reverse arrangement of the three functional groups, have a sweetness that is one-fourth that of aspartame. Thaumatin, monellin, and curculin are sweet-tasting proteins isolated from tropical fruits. Curculin modifies sour taste into sweet taste. Miraculin, isolated from a plant native to western Africa, also modifies sour taste into sweet taste, although it is not sweet by itself. These proteins interact with the taste buds.

Sour Peptides. Glutamic acid and aspartic acid taste sour when their carboxyl groups are dissociated (8). Some oligopeptides containing Asp, Glu, or both also taste sour. Dipeptides formed by binding the γ-carboxyl group of Glu to the amino group of another amino acid have an astringent taste in addition to a sour taste.

Umami Peptides. Monosodium glutamate is a typical umami substance. Dipeptides containing Glu at the amino terminus elicit the umami taste in an aqueous solution at pH 6. The acidic oligopeptide fraction obtained from fish protein hydrolysates show an intense umami taste. An octapeptide, Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, which was isolated from papin hydrolysate of beef, has an umami taste and has been named a delicious peptide (12).

Salty Peptides. Hydrochlorides of basic dipeptides, such as Orn-β-Ala and Orn-Tau, potentiate the salty taste of sodium chloride and therefore may be useful in decreasing the amount of salt in foods used for hypertensive patients (13).

Nutritional Aspects of Peptides

A fairly large part of digested protein is absorbed from the intestinal mucosa not as free amino acids but as dipeptides and tripeptides. A carrier-mediated transport system for dipeptides and tripeptides in the intestinal mucosa is operated by a proton gradient (14). For example, absorption rate of Gly-Gly is higher than that of Gly. Furthermore, Gly-Gly-Gly is absorbed more quickly than Gly-Gly (15). In a patient with Hartnup disease, whose ability to absorb free aromatic amino acid is defective, Phe-Phe could be absorbed well (16). Solubility of hardly soluble amino acids is improved by incorporation into peptides. The taste of oligopeptide mixtures is milder than that of free amino acids. The osmotic pressure of peptides in solution is lower than that of an equal amount of free amino acids. For these reasons an oligopeptide mixture is better than an amino acid mixture for an enteral diet. A low phenylalanine peptide (LPP) mixture for phenylketonuremic patients is prepared by the digestion of the protein by protease followed by the removal of phenylalanine by the absorption to Sephadex or charcoal (17).

Biologically Active Peptides

There are many biologically active peptides in foods. These peptides are classified into two groups: peptides found in food in active form from the beginning, and those that are released from inactive proteins by the limited proteolysis during food processing, storage, and digestion (see Table 1).

Biologically Active Peptides Found in Foods. Foods of animal origin, especially milk, contain large numbers of peptides belonging to the first group. Today, many biologically active peptides such as epidermal growth factor, nerve growth factor, insulin, prolactin, somatoatatin, thyroid-releasing hormone, thyroid-stimulating hormone, growth hormone-releasing factor, luteinizing hormone-releasing hormone, adrenocorticotropic hormone, erythropoietin, bombesinlike peptides, calcitonin, and delta-sleep-inducing peptide are detected in milk by sensitive radioimmunoassay. In neonates, the proteolytic activity in the gastrointestinal tract is usually low and the intestine shows considerable permeability to large peptides. Therefore, these biologically active peptides found in milk might have a physiological function in the development of the gastrointestinal tract and other organs. In fact, epidermal growth factor given orally to newborn rats has been shown to increase the weight of the intestine, liver, heart, and kidney (18).

Some peptides of plant origin also show biological activity for animal cells. Soybean trypsin inhibitor has growth-promoting activity for endothelial cells.

Biologically Active Peptides Released by Limited Proteolysis of Food Proteins. Many types of biologically active peptides are released from food proteins. These are classified as (*1*) peptides controlling intestinal absorption, (*2*) ligands for receptors, (*3*) enzyme inhibitors, (*4*) antimicrobial peptides, and (*5*) others including antioxidative peptides. Biologically active peptides are released not only from animal proteins but also from plant proteins.

Peptides Controlling Intestinal Absorption. **Casein phosphopeptide**. The bioavailability of calcium in milk is better than that in other foods. This is partly attributable to lactose. However, in cheese, which is low in lactose, the availability of calcium is also good. This is explained by the phosphopeptides that are released from α_{s1}- and β-casein released by trypsin (19). Similar peptides are found in the intestine. Phosphopeptides promote intestinal absorption of calcium by solubilizing calcium phosphate. This peptide also facilitates absorption of ferric ion by the same mechanism. The peptide itself is not absorbed from the intestine.

Cholesterol-lowering peptide. Serum cholesterol–lowering peptide was isolated from an enzymatic digest of soybean protein (20). Hydrophobic core peptides remaining after the digestion of the protein adsorb bile salt and destabilizes lipid emulsion (21). Consequently, intestinal reabsorption of bile salt and absorption of cholesterol are

Table 1. Biologically Active Peptides Derived from Food Proteins

Peptides	Structure
Phosphopeptides that promote calcium absorption	
α-CPP	α_{s1}-casein (43–79)
β-CPP	β-casein (1–25)
Peptide that lowers serum cholesterol	
Fragment peptide of soybean protein	Unknown
Opioid peptides	
β-casomorphin 7	YPFPGPI
α-casein exorphin	RYLGYLE
Gluten exorphin A	GYYPT
Gluten exorphin B	YGGWL
Gluten exorphin C	YPISL
Opioid antagonists	
Casoxin A	YPSYGLNY
Casoxin B	YPYY
Ileum-contracting peptides	
Oryzatensin	GYPMYPLPR
Casoxin C	YIPIQYVLSR
Casoxin D	YVPFPPF
Albutensin A	ALKAWAVAR
β-lactotensin	HIRL
Vasorelaxing peptide	
Ovokinin	FRADHPFL
Peptides promoting phagocytosis	
β-casein (63–68)	PGPIPN
β-casein (191–193)	LLY
Glycinin A_{1a} (279–282)	QRPR
Soymetide: β-conglycinin α'(322–334)	MITLAIPVNKPGR
Immunosuppressive peptides	
Proline-rich polypeptide fragment	YVPLFP
Human lactoferin (231–245)	CPDNTRKPVDKFKDC
Caseinomacropeptide	
Peptides that inhibit platelet aggregation	
κ-casein (100–105)	PHLSF
κ-casein (106–116)	MAIPPKKNQDK
Human lactoferrin (39–42)	KRDS
Inhibitor peptides for Angiotensin I-converting enzyme	
α_{s1}-Casein (23–34)	FFVAPFPEVFGK
α_{s1}-casein (194–199)	TTMPLW
Casein-derived peptide from fermented milk	IPP, VPP
Tuna peptide	PTHIKWGD
Bonito peptide	LKPNM
Antimicrobial peptides	
Lactoferricin B	FKCRRWQWRMKKLGAPSITCVRRAF

inhibited, and the serum cholesterol levels of animals fed with plant proteins are lower than those fed with animal proteins (22)

Ligands for Receptors. Biologically active peptides derived from food proteins bind to receptors for various types of endogenous biologically active peptides. Usually, their receptor affinities are weaker than those of endogenous peptides. However, some of them show physiological effects after oral administration because of their resistance to peptidase and smallness in molecular size.

Opioid peptides. Opioid peptides have an affinity for opiate receptors. There are more than 20 endogenous opioid peptides in the human body. Besides analgesic activity, these peptides have various physiological effects such as control of gastrointestinal functions and hormone secretion. Many opioid peptides are released from food proteins by limited proteolysis. β-casomorphin 7 is released from β-casein (23) and α-casein exorphin, from bovine α_{s1}-casein (24). Gluten exorphins A, B, and C are released from wheat gluten (25,26). β-casomorphin exhibits analgesic activity when administered intracerebroventricularly. β-casomorphin increases postprandial insulin and somatostatin levels. It also elevates the heart rate.

Opioid antagonist peptides are also released from milk proteins. Casoxin A and B are derived from κ-casein (27). Casoxin C is a functional antiopioid peptide of which activity is mediated by complement C3a receptor (27,28).

Ileum-contracting peptides. Many endogenous peptides such as angiotensin II, substance P, bradykinin, and neurotensin induce contraction of the ileum. Ileum-contracting peptides were isolated from digests of food proteins. β-lactotensin is released from β-lactoglobulin by chymotryptic digestion (29). Oryzatensin and casoxin C, which are released by trypsin from rice albumin and κ-casein, respectively, induce ileum contraction through receptor for complement C3a (28,30). Albutensin A, which is released from serum albumin by trypsin, also contracts ileum through receptors for both complements C3a and C5a (31). Casoxin D also induces contraction of isolated guinea pig ileum (32).

Vasorelaxing peptide. Ovokinin released from ovalbumin by the action of pepsin is a typical vasorelaxing peptide that has antihypotensive activity after oral administration (33). Some peptides isolated as ileum-contracting peptides also show vasorelaxing activity.

Immunostimulating peptides. Phagocytosis-stimulating peptides are released from various food proteins (34–36). Albutensin A stimulates phagocytosis as a complement C5a agonist. The complement C3a agonists, oryzatensin and casoxin C, also stimulate phagocytosis, but to a lesser extent than C5a agonist.

Immunosuppressive peptides. Proline-rich polypeptide from ovine colostrum and its fragment peptide YVPLFP show immunosuppressive activity (37). The human lactoferrin[231–425] has a homology to thymopentin and suppresses immune response (38). Caseinomacropeptide also has immunosuppressive activities (39).

Inhibitors for platelet aggregation. Peptides derived from food proteins inhibiting platelet aggregation have been reported (40). Some of them are homologous in structure to RGD, which is a recognition sequence for receptors of the integrin family.

Others. Peptides having affinity to calmodulin and inhibiting cyclic nucleotide phosphodiesterase are released from α_{s2}-casein (41). A peptide-stimulating proliferation of fibroblast and potentiating glucagon is released from β-casein (42). Caseinomacropeptide released from κ-casein by the action of chymosin inhibits gastric secretion (43).

Enzyme Inhibitors. The most typical enzyme inhibitor found in enzymatic digests of food protein is that for angiotensin I-converting enzyme; most of the digests show apparent inhibitory activity. Inhibitors for prolylendopeptidase derived from food proteins have been also reported.

Inhibitors for angiotensin I-converting enzyme. Angiotensin I-converting enzyme (ACE) is a dipeptidyl carboxypeptidase that catalyzes the conversion of angiotensin I to angiotensin II, a strong pressor. Inhibitors for this enzyme lower blood pressure in hypertensive animals. Peptidic ACE inhibitor was first isolated from snake venom. ACE inhibitor peptides were isolated from enzymatic digest of gelatin (44), casein (45,46), fish protein (47,48), and many other proteins. Some of these peptides have an antihypertensive effect after oral administration. Potentiation of hypotensive activity was observed when the ACE inhibitor peptide fraction from fish meal was orally administered as a emulsion in egg yolk (49).

Antimicrobial Peptides. Because of its iron-binding ability, lactoferrin shows bacteriostatic effect. Lactoferricin, released from the amino terminus region of lactoforrin by the action of pepsin, has bactericidal activity for various types of microorganisms (50). Immunostimulating activity of lactoferricin may also contribute to its antimicrobial effect *in vivo*. The carbohydrate chain attached to caseinomacropeptide is reported to be effective in preventing viral and bacterial infections (51).

Food Allergy

Many food materials, for example, eggs, milk, soybeans, and rice, are allergenic. Some food allergens are attributable to certain primary structures of the proteins. Allergens can be destroyed by limited proteolysis. Therefore, an oligopeptide mixture prepared by hydrolysis of proteins can be used as a low-allergenic food. In some other cases, allergenic epitopes are conformational. Allergenic structures can be destroyed by physical denaturation in this case.

CURRENT AND FUTURE STATUS

Bioreactor systems for the continuous production of peptides by a hydrolysis of proteins have also been developed. Release of peptides can be monitored by a peptide sensor, which is composed of aminopeptidase and amino acid oxidase (52). Peptides can be synthesized in a bioreactor system using synthetic reaction of proteases. Aspartame can be synthesized by this method.

Many biologically active peptides of food origin have been found by using sensitive *in vitro* assay systems. Although it is quite probable that milk-derived peptides may have teleological functions for newborns, only a few of these peptides have been demonstrated to be effective after oral ingestion. For those peptides having truly desirable functions for human health, methods to increase their availability, such as the enhancement of absorbability or half-life, should be explored. Although their specific activities are usually lower than those of endogenous biologically active peptides, those derived from food proteins sometimes show new and unexpected structure-activity relationships.

Recombinant DNA technology has been used to produce and improve pharmacologically useful peptides in microbial, plant, and animal cells. Production of heterogenous proteins in plants or milk by the transgenic technique is an especially promising method for the production of food protein. The amino acid composition and physical properties of food proteins can be improved by the site-directed mutagenesis technique. Furthermore, it will be possible to introduce biologically active peptide sequences into food proteins and to remove allergenic sequences from them.

BIBLIOGRAPHY

1. K. Nakanishi and R. Matsuno, *Synthetic Peptides in Biotechnology*, Alan R. Liss, Inc., New York, 1988, pp. 173–202.
2. J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., 1984.
3. P. Edman, *Acta Chemical Scandinavica* **4**, 277–282 (1950).
4. D. H. Hawke et al., *Analytical Biochem.* **166**, 298–307 (1987).
5. G. D. Rose et al., *Advances in Protein Chem.* **37**, 1–109 (1985).
6. T. Imai et al., *Chem. Pharm. Bull.* **37**, 2251–2252 (1989).
7. S. J. Bishov and A. S. Henick, *J. Food Sci.* **37**, 873–875 (1972).
8. H.-M. Chen et al., *J. Agric. Food Chem.* **44**, 2619–2623 (1996).
9. T. Nishimura and H. Kato, *Food Rev. Int.* **4**, 175–194 (1988).
10. N. Ishibashi et al., *Agric. Biol. Chem.* **52**, 819–827 (1988).
11. J. Adler-Nissen, *Protein Tailoring for Food and Medical Uses*, Marcel Dekker, Inc., New York, 1986, pp. 97–122.
12. Y. Yamasaki and K. Maekawa, *Agric. Biol. Chem.* **42**, 1761–1765 (1978).
13. M. Tamura et al., *Agric. Biol. Chem.* **53**, 1625–1633 (1989).
14. T. Hoshi, *Ion Gradient-Coupled Transport*, Elsevier, Amsterdam, The Netherlands, 1986, pp. 183–191.
15. D. M. Matthews and J. W. Payne, *Current Topics in Membrane and Transport*, Vol. 14, Academic Press, Inc., Orlando, Fla., 1980, pp. 331–425.
16. A. M. Asatoor et al., *Gut* **11**, 380–387 (1970).
17. S. Arai et al., *Agric. Biol. Chem.* **50**, 2929–2931 (1986).
18. O. Koldovsky, *J. Nutrition* **119**, 1543–1551 (1989).
19. R. Sato et al., "Casein Phosphopeptide (CPP) Enhances Calcium Absorption from the Ligated Segment of Rat Small Intestine," *J. Nutr. Sci. Vitaminol.* **32**, 67–76 (1986).
20. M. Sugano et al., *Atherosclerosis* **72**, 115–122 (1988).
21. K. Iwami et al., *Agric. Biol. Chem.* **50**, 1217–1222 (1986).
22. K. K. Carroll and M. W. Huff, *Nutrition and Food Science: Present Knowledge and Utilization*, Vol 3, Plenum Publishing Corp., New York, 1980, pp. 379–385.
23. V. Brantl et al., *Hoppe-Seyler's Zeitschrift fur Physiologische Chemie* **360**, 1211–1216 (1979).
24. S. Loukas et al., *Biochem* **22**, 4567–4573 (1983).
25. S. Fukudome and M. Yoshikawa, *FEBS Lett.* **296**, 107–111 (1992).
26. S. Fukudome and M. Yoshikawa, *FEBS Lett.* **316**, 17–19 (1993).
27. H. Chiba et al., *J. Dairy Res.* **56**, 363–366 (1989).
28. M. Takahashi et al., *Peptides* **18**, 329–336 (1997).
29. M. Yoshikawa et al., *Nippon Nogeikagaku Kaishi* **64**, 557 (1990).
30. M. Takahashi et al., *Peptides* **17**, 5–12 (1996).
31. M. Takahashi et al., *Lett. Peptide Sci.* **5**, 29–35 (1998).
32. M. Yoshikawa et al., *Peptide Chem. 1992*, ESCOM Science Publishers B. V., Leiden, The Netherlands, 1993, pp. 572–575.
33. H. Fujita et al., *Peptides* **16**, 785–790 (1995).
34. D. Migliore-Samour and P. Jolles, *Experientia* **44**, 188–193 (1988).
35. M. Yoshikawa et al., *Ann. New York Acad. Sci.* **685**, 375–376 (1993).
36. M. Tanaka et al., *Nippon Nogeikagaku Kaishi* **68**, 341 (1994).
37. M. Janusz et al., *Mol. Immunol.* **24**, 1029–1031 (1987).
38. I. Z. Siemion, *J. Peptide Sci.* **1**, 295–302 (1995).
39. H. Otani et al., *J. Dairy Res.* **62**, 349–357 (1995).
40. A.-M. Fiat et al., *J. Dairy Res.* **53**, 351–355 (1989).
41. K. Kizawa et al., *J. Dairy Res.* **62**, 587–592 (1995).
42. N. Azuma et al., *Agric. Biol. Chem.* **53**, 2631–2634 (1989).
43. G. K. Shlygin et al., *Byull. Eksptl. Biol. Med.* **72**, 9–13 (1971).
44. G. Oshima et al., *Biochimica et Biophysica Acta* **566**, 128–137 (1979).
45. S. Maruyama and Suzuki, *Agric. Biol. Chem.* **49**, 1405–1409 (1985).
46. Y. Nakamura et al., *J. Dairy Sci.* **78**, 777–783 (1995).
47. Y. Kohama et al., *Biochemical and Biophysical Res. Comm.* **155**, 332–337 (1988).
48. K. Yokoyama et al., *Biosci. Biotechnol. Biochem.* **56**, 1541–1545 (1992).
49. K. Suetsuna and K. Osajima, *Nippon Eiyou Shokuryou Gakkaishi (in Japanese)* **42**, 47–51 (1989).
50. W. Bellamy et al., *J. Appl. Microbiol.* **73**, 472–479 (1992).
51. Y. Kawasaki et al., *Biosci. Biotechnol. Biochem.* **57**, 1214–1215 (1993).
52. T. Tsuchida et al., *Hakkokogaku (in Japanese)* **67**, 499–507 (1989).

M. YOSHIKAWA
Kyoto University
Kyoto, Japan
H. CHIBA
Kobe Women's University
Kobe, Japan

See also PROTEINS: AMINO ACIDS; PROTEINS: STRUCTURE AND FUNCTIONALITY.

PESTICIDE RESIDUES IN FOOD

The term pesticide is used to describe any chemical agent that controls pests. In the United States, more than 20,000 pesticide products are registered (1). Traditionally, pesticides are used in agriculture, but nonagricultural uses are also common and include garden and household pest control, sanitation, wood preservation, and mosquito abatement. Pesticides contribute large economic and health benefits to society through minimizing crop losses, protecting the nutritional integrity of food, ensuring year-round storage, and providing appealing foods (2). Since as early as 1000 B.C. the Chinese used sulfur as a fungicide to control mildew on fruit; today sulfur remains an important fungicide. In the sixteenth century, arsenical compounds were popular insecticides; and in the seventeenth century, nicotine, rotenone, and *Chrysanthemum* extracts were introduced as insecticides and are still in use (3). In the United States, widespread utilization of pesticides in agriculture occurred after the 1920s with the introduction of mechanized power in farms. It has been estimated that 40% of the world's food supply would be at risk without pesticides (4).

Of the total amount of pesticides used in the United States during 1995 (4.5 billion pounds), less than 21% was used in the production of food and fiber products (5). The agricultural use of pesticides fluctuates yearly depending on climatic conditions, pest outbreaks, and planted acreage of pesticide intensive crops.

Food safety has been one of the driving forces behind pesticide legislation and regulation. Pesticide residues remain a major public concern today; consumer attitude surveys indicate that 72 to 82% of Americans consider pesticide residues to be a major concern (6). Nevertheless, in terms of overall food safety, the U.S. Food and Drug Administration (FDA) ranks pesticides as its fifth food safety priority and of less concern than (*1*) microbiological contamination of foods, (*2*) nutritional imbalance, (*3*) environmental contaminants, and (*4*) naturally occurring toxins (7).

Besides residues in foods, there are other pesticide concerns, such as potential acute and/or chronic toxicity to humans from occupational exposure. Other indirect risks include the destruction of susceptible crops and natural vegetation, reduction of natural pest enemies, effects on fish and wildlife populations, livestock losses, honeybee losses, evolved pesticide resistance, and creation of secondary pest problems.

PESTICIDE USE AND CLASSIFICATION

A wide variety of the types of pesticides is used in food production. Modern pesticides include synthetically produced organic chemicals, naturally occurring organic and inorganic chemicals, and microbial agents (natural or obtained through genetic manipulation). Table 1 lists many types of pesticides and their targets. Weeds, insects, and fungi are the major pests responsible for damage to agriculture. In terms of pounds applied, herbicides accounted for 55% of U.S. pesticide use in 1995, followed by insecticides (32%), fungicides (7%), and other categories (6%) (8).

Table 1. Pesticide Types and Targets

Pesticide type	Pest controlled
Insecticide	Insect
Herbicide	Weeds
Fungicide	Fungi
Nematicide	Nematodes
Acaricide	Mites
Defoliant	Leaves
Bacteriocide	Bacteria
Rodenticide	Rodents
Molluscicide	Snails
Algacide	Algae

Insecticides comprise compounds of various chemical classes; some of the most common are listed in Table 2. Insecticides have a variety of mechanisms of action in insects, including affecting the insects' metabolism (nerve poisons, muscle poisons, dessicants, and sterilants) or through a physical effect such as clogging air passages. The most common classes of insecticides are chlorinated hydrocarbons, organophosphates, and carbamates. The chlorinated hydrocarbons DDT and aldrin, among others (Table 2), were developed in the 1930s and 1940s. Such pesticides were very potent (high insect toxicity), but presented simultaneously chronic health and environmental effects because of their resistance to environmental and metabolic breakdown, which led to widespread environmental contamination and buildup of residues in a variety

Table 2. Some Pesticide Classes and Examples of Each Class

Pesticide	Examples
Insecticides	
Chlorinated hydrocarbons	Dicofol, methoxychlor, DDT,[a] aldrin,[a] dieldrin,[a] chlordane[a]
Organophosphates	Parathion, malathion, phosdrin, diazinon, chlorpyrifos, azinphos-methyl
Carbamates	Aldicarb, carbaryl, carbofuran
Pyrethroids	Permethrin, cypermethrin
Herbicides	
Triazine	Atrazine, cyanazine
Phenoxy	2,4 D
Quaternary ammonium	Paraquat
Benzoic acids	Dicamba
Acetanilides	Alachlor, metolachlor
Ureas	Linuron
Fungicides	
Inorganic	Sulfur
Ethylenebisdithiocarbamates	Maneb, mancozeb
Chlorinated phenols	Pentachlorophenol

[a] Banned in the United States.

of animals, including humans. Most chlorinated hydrocarbons are no longer permitted for use in the United States, although a few of the least persistent members of the family are still allowed. Historically, the carbamate and organophosphate insecticides served as replacements for many of the chlorinated hydrocarbons. These insecticides exert their toxicity on both insects and mammals through inhibition of cholinesterase enzymes that normally function to regulate nervous system activity. Although more acutely toxic to nontarget organisms, including mammals, the organophosphate and carbamate insecticides are less persistent in the environment. Another class of insecticides, the pyrethroids, were introduced in the 1970s. They are considered excellent broad-spectrum insecticides; they are effective at low doses, exhibit low toxicity to mammals, and break down quite rapidly in the environment. Pyrethroids continue to be used extensively in agriculture but also have the disadvantages of being relatively costly and environmentally labile, and they commonly lose their effectiveness due to the development of insect resistance.

Herbicides include a variety of chemical compounds that act upon weeds through different toxicological mechanisms. In some cases the toxicity results from direct plant contact by destroying leaf and stem tissues. Other herbicides inhibit seed germination or seedling growth; damage leaf cells, causing weeds to dry up; or affect the weed's ability to perform photosynthesis (2).

Fungi can cause damage or stress to food crops, and massive infestations can occur during storage of many foods, including grains, when storage occurs under conditions of high temperature and/or moisture. A serious consequence of fungal attack can be the production of mycotoxins, such as aflatoxins and fumonisins, by *Aspergillus flavus* and *Fusarium moniliforme*, respectively. Some fungicides can destroy fungi that have already invaded the plant while others may prevent fungal infestations.

PESTICIDE REGULATION

In the United States, the first legislation concerning pesticides was passed with the creation of the FDA in 1938, through the Federal Food, Drug and Cosmetic Act (FFDCA). The FFDCA established the requirement for pesticide tolerances when pesticide use could result in residues on food or feed crops.

In 1947, Congress passed the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which grouped all pesticide products under one law and mandated labeling and registration requirements. The U.S. Department of Agriculture (USDA) was given the initial responsibility to administer FIFRA. Several amendments to FIFRA were passed (1975, 1978, 1980, 1984, 1988, and 1996), which included, among others, provisions for use restriction, pesticide reevaluation and re-registration, and toxicological and environmental impact studies. In 1958, the Delaney Clause was approved as an amendment to the FFDCA. It stipulated that any food additive shown to cause cancer in humans or laboratory animals could not be used. In 1972, the responsibility for FIFRA administration was transferred to the newly created Environmental Protection Agency (EPA). EPA is authorized to grant pesticide registrations, to establish pesticide tolerances, and to regulate pesticide residues in food and feed under FIFRA.

In 1996, after legal disputes over the Delaney Clause, a new act, the Food Quality Protection Act (FQPA) was signed into law. The FQPA mandates a single, health-based standard for all pesticides, eliminating the Delaney paradox that arose due to inconsistencies resulting in different methods for regulating pesticides in raw commodities and those found in processed foods (9). The FQPA establishes standards that apply to all types of risks, including cancer risks and endocrine disruption, and guides the establishment for setting allowable levels (tolerances) for all pesticide residues on raw agricultural commodities and processed food. Important new provisions of the FQPA include additional protection for infants and children, consideration of aggregate risks from food, water, and domestic exposure, and consideration of cumulative risks from pesticides with common mechanisms of toxicity. Additionally, FQPA expedites approval of safer pesticides, requires periodic re-evaluation of pesticide registrations and tolerances, provides a consumer right-to-know provision and creates incentives for the development and maintenance of effective crop protection tools for farmers (1).

Three major U.S. regulatory agencies have the primary responsibility for regulating pesticides:

1. EPA, which has developed a series of guidelines for the toxicological testing of pesticides, registers pesticides for use, prescribes labeling, and establishes allowable levels (tolerances) of pesticides on food and feed crops;
2. FDA, which monitors domestic and imported foods for pesticide residues and enforces tolerances; and
3. USDA, which enforces tolerances on meat, poultry, and some egg products and also conducts the Pesticide Data Program (PDP) that provides important information on pesticide residue levels of fruits and vegetables in ready-to-eat form that may be directly used by EPA as a risk assessment tool.

At the international level, the Food and Agriculture Organization of the World Health Organization (FAO/WHO) is the regulatory authority on pesticides and develops, through the Joint FAO/WHO Meeting on Pesticide Residues, regulatory standards for maximum residue levels (MRLs). These standards are used widely throughout the world, but their use is not universal; several countries, including the United States, adopt their own standards for pesticide residue levels and enforce their sovereign standards on food entering their countries from foreign lands.

TOXICOLOGICAL EVALUATION OF PESTICIDES

Pesticides used in food production require detailed study in a series of toxicological tests. Laboratory animals (often rodents, dogs, and nonhuman primates) are used to study the acute, subchronic, and chronic toxicity of pesticides. In these studies, the metabolic fate, mutagenicity, carcinogenicity, and teratogenicity of pesticides are determined in addition to a large number of other toxicological effects. Additionally, EPA requires studies on the environmental fate of pesticides and their breakdown products, and the effects on nontarget organisms. All the collected data is reviewed by EPA for evaluation of the risks associated with the pesticide. This is a long and costly process, which frequently involves more than 10 years before a pesticide registration may be granted and at a cost in the tens of millions of dollars.

As FIFRA is primarily a risk-balancing statute, the EPA registers pesticides under a statutory standard that requires balancing the benefits of the use of the pesticide in question (such as increased crop yield, lower food cost, or public health protection) with potential risks such as health effects of consumers or agricultural workers and environmental damage. Thus the EPA grants a registration and specifies the commodity or commodities for which the pesticide may be used, as well as appropriate conditions for use and disposal. Failure to obey such legal requirements (printed on the pesticide labels) is a federal offense. Once federal registration is granted, the pesticide may still be subject to restrictions or denied use within individual states. More stringent use restrictions are frequently applied in some states, such as California.

Tolerance Setting

When the use of a pesticide may have the potential to leave a residue on a food in the United States, a tolerance, representing the maximum allowable residue permitted, is usually established. Tolerances represent the maximum expected residues of a pesticide on a specified commodity resulting from legal applications of the pesticide under established conditions for its use. When such conditions are followed, it is highly unlikely that residues in excess of tolerances would be detected. Tolerances are established for specific pesticide/commodity combinations; as such, the same pesticide may have different tolerance levels established for different commodities, and the tolerances of a variety of pesticides on a single commodity frequently vary considerably.

It is important to emphasize that the tolerance level is not established based on safety, but rather represents the maximum residue anticipated from the legal use of the pesticide (10). Nevertheless, before granting a tolerance, the EPA makes assessments of potential human exposure resulting from all registered (and proposed) uses of the pesticide to calculate the theoretical maximum residue contribution (TMRC). The TMRC assumes that the specified pesticide is always applied to all acreage planted, that it is used on all commodities for which it is registered, that residues are always present at the tolerance level, and that there is no reduction on pesticide levels from the plant to the postharvest stage, up to the table. It is a typical worst-case scenario using highly conservative assumptions that may overestimate exposures by factors of 100 to 100,000 times (2).

The TMRC value is compared with the reference dose (RfD), which is a daily exposure level not considered to represent any appreciable level of risk. A pesticide is considered to pose a negligible risk and tolerances are usually approved when the TMRC is below the RfD, provided that the carcinogenic risk at the TMRC is below the level of one excess cancer per million. When the TMRC is found to be higher than the RfD, or when exposure at the TMRC leads to a cancer risk greater than one excess cancer per million, the EPA may adopt a more refined risk assessment to more accurately calculate exposure estimates. Such refinements commonly represent the anticipated residue contribution (ARC) and may include adjustments of actual pesticide use, more realistic residue data, and consideration of potential pesticide levels reduction through washing, peeling, cooking, processing, and so on. The tolerance is established if the ARC is below the reference dose, and the carcinogenic risk at such an exposure is below the negligible risk of one excess cancer per million.

The passage of the FQPA in 1996 has served to make the process for establishing tolerances for pesticides more complicated. Prior to FQPA, tolerances were established on a chemical-by-chemical basis and considered only dietary exposure to the chemical. FQPA stipulates that the EPA may establish tolerances only when the EPA assesses that the risks posed by pesticides represent a "reasonable certainty of no harm" with respect to both carcinogenic and noncarcinogenic risks. In determining whether the pesticides satisfy the reasonable certainty of no harm criteria, the EPA considers the *aggregate* exposure to the chemicals from dietary, drinking water, and residential sources as well as *cumulative* exposure from pesticides possessing a common mechanism of toxic action (such as the organophosphates), meaning that determinations may be made on entire families of chemicals rather than on a chemical-by-chemical basis. In addition, the EPA is required to consider applying an additional 10-fold uncertainty factor in cases where infants and children may be more susceptible than adults to specific pesticides; this effectively reduces the RfD by a factor of 10. It is clear that the new FQPA requirements will require EPA scientists and others to develop significantly more robust models for assessing human pesticide risks and that the more stringent reregistration requirements posed by FQPA may significantly reduce the amounts and types of pesticides that may be used on food crops in the near future.

PESTICIDE RESIDUE MONITORING

It is the responsibility of the FDA to enforce tolerances in domestic and imported foods. Domestic samples are usually collected near the source of production or at the wholesale level, whereas imported foods are sampled at the entry point in the United States. The FDA has a regulatory monitoring program and a second program, the Total Diet Study, which estimates human dietary intakes of pesticides.

Regulatory commodity monitoring programs used by the FDA comprise both surveillance and compliance monitoring.

Surveillance Monitoring

The objective of surveillance monitoring is to identify violative residues in foods (11). Most regulatory samples analyzed by the FDA are taken in the surveillance monitoring program, and sampling is designed to maximize the chances of encountering illegal residues rather than providing a statistically representative look at residues in the U.S. food supply.

Compliance Monitoring

In compliance monitoring the FDA usually obtains follow-up samples after illegal residues have already been determined.

Although the FDA analyzes some processed foods, tests are primarily performed on raw commodities (prior to washing or peeling). The analytical methodologies adopted can detect more than 200 possible pesticides, and results are known shortly after samples are received by the laboratories. Clearly, results from the FDA's surveillance monitoring program indicate that residue levels rarely approach the tolerances. Although infrequently found, the vast majority of illegal residues represent cases where pesticides that are legally allowed to be used in the United States are detected on commodities for which a tolerance is not established (8). It is critical to realize that illegal residues should not be construed as "unsafe" residues and more properly serve as an indicator of erroneous application practices or the incidental contamination of commodities on which the pesticides were not directly applied but migrated to through the action of drift or uptake from contaminated soil (10).

Additionally, since 1961 the FDA has been carrying out its Total Diet Study annually. In this study, foods are collected in a "market basket" approach, using four geographical regions and three cities in each region each year. A total of 261 different food samples are collected to comprise each market basket. Each collection of foods is prepared for table-ready consumption and then analyzed for pesticide residues (8). Results from the FDA's Total Diet Study commonly indicate that the average human dietary exposure to pesticides is well below the established RfDs.

Since 1991 the USDA's Pesticide Data Program (PDP) has relied on cooperation with states in all regions of the United States to develop residue data that could be more useful for risk assessments than that collected by the FDA. In its initial years, PDP tested fresh fruits and vegetables, which were prepared for analysis simulating practices used by consumers such as washing and/or peeling. Since 1994 PDP monitoring has given more attention to foods frequently consumed by infants and children and has included samples of canned and frozen fruits and vegetables, wheat, soybeans, whole milk, fruit juices, and corn syrup. PDP's data on pesticides in selected commodities are used by the EPA to support its dietary risk assessment and pesticide registration programs, and by the FDA to refine sampling for tolerance enforcement (12).

At the state level, individual monitoring programs have been established by many states. Currently, the largest state program is conducted in California. California results from 1995 showed illegal residues in only 1.6% of the samples analyzed; from those only about one-quarter represented overtolerance violations, while three-quarters referred to pesticides detected on commodities for which they were not registered. The vast majority of samples analyzed revealed no detectable residue, and on samples containing detected residues, most were present below 10% of the tolerance level (13).

BIBLIOGRAPHY

1. Environmental Protection Agency, "The Food Quality Protection Act of 1996," EPA Office of Pesticide Programs, 1999, ⟨*http://www.epa.gov/oppfead1/fqpa*⟩ (September 17, 1999).
2. S. O. Archibald and C. K. Winter, "Pesticides in Our Food: Assessing the Risks," in C. K. Winter, J. N. Seiber, and C. F. Nuckton, eds., *Chemicals in the Human Food Chain*, Van Nostrand Reinhold, New York, 1990.
3. D. J. Ecobichon, "Toxic Effects of Pesticides," in C. D. Klaassen, ed., *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York, 1996.
4. D. Pimentel et al., "Environmental and Economic Costs of Pesticide Use," *Bioscience* **42**, 750–760 (1992).
5. Environmental Protection Agency, "Pesticides and Toxic Substances," in *Pesticide Industry Sales and Usage, 1994 and 1995 Market Estimate*, EPA Office of Prevention, Washington, D.C., 1997.
6. C. M. Bruhn et al., "Consumer Response to Pesticide Food Safety Risk Statements: Implications for Consumer Education," *Dairy, Food, and Environmental Sanitation* **18**, 278–287 (1998).
7. C. K. Winter, "Lawmakers Should Recognize Uncertainties in Risk Assessment," *California Agriculture* **48**, 21–29 (1994).
8. Food and Drug Administration, "Pesticide Program Residue Monitoring 1997," 1998, ⟨*http//www.cfsan.fda.gov*⟩ (September 17, 1999).
9. National Research Council, *Regulating Pesticides in Food: The Delaney Paradox*, National Academy Press, Washington, D.C., 1987.
10. C. K. Winter, "Pesticide Tolerances and Their Relevance as Safety Standards," *Regul. Toxicol. Pharmacol.* **15**, 137–150 (1992).
11. D. V. Reed et al., "The FDA Pesticides Monitoring Program," *J. Assoc. Off. Anal. Chem.* **70**, 591–595 (1987).
12. U.S. Department of Agriculture, "USDA Pesticide Data Program: What Is PDP?" 1999, ⟨*http://www.ams.usda.gov/science/pdp/what.htm*⟩ (September 17, 1999).
13. California Department of Pesticide Regulation, "Residues in Fresh Produce," in *Executive Summary of 1995 Monitoring Program*, Sacramento, Calif., 1997.

Elisabeth L. Garcia
Carl K. Winter
University of California
Davis, California

See also Food safety and risk communication.

PHENOLIC COMPOUNDS

The term "phenolic compounds" embraces a wide range of compounds that possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives. Phenolic compounds are present in many plants. They are directly related to food characteristics such as taste, palatability, nutritional value, pharmacological and toxic effects, and microbial decomposition. Among the natural phenolic compounds, of which approximately 8000 are known to occur in plants, the flavonoids and their relatives form the largest group with more than 5000 known structures (1). This is considered only a fraction of the total number that are likely to be present in nature, since only a small percentage of plant species has been properly examined for their phenolic compounds. Phenolic compounds range from structures that are very lipophilic (eg, tangeretin) to those that are very water soluble (eg, quercetin 3-sulfate). The size of molecule varies greatly, ranging from monomer, catechol with molecular weight of 110, to the complex heavenly blue anthocyanin pigment of *Ipomaea coerulea*, which has molecular weight of 1759 (2,3). Considerable numbers of simple monocyclic phenols, phenolic quinones, lignans, and xanthones, as well as polymeric materials such as lignins, melanins, and tannins are considered to be important phenolic compounds.

Only a relatively few polyphenols are considered to be important to foods and feeds. They are *p*-coumaric, caffeic, ferulic, sinapic, gallic acids and their derivatives, and the common flavonoids and their glycosides. Anthocyanins and flavonols are important pigments in a variety of fruits and vegetables. While many common polyphenols are not pigments that enhance quality, some are important food constituents because they are responsible for off-colors, usually browning, that develop during the storage and processing of fruits and vegetables. Many phenolic compounds participate in both enzymatic and nonenzymatic browning reactions. The retention of fruit and vegetable color has become a matter of great importance because color is one of the primary factors in consumer acceptance of plant foods. In this respect the need to prevent the formation of undesirable color during processing has always posed a challenge to food technologists.

In addition to color, polyphenols also contribute to food flavor and other qualities. For example, astringency of polyphenols and its ratio with sugar and acid are important and useful criteria for determining the overall quality of fresh fruits, fruit beverages, and wines. Some polyphenols, such as chalcones and related compounds found in citrus fruits, are exceedingly sweet or bitter. Both bitterness and astringency of wine are due to phenolic compounds present in grapes. There have been many claims for the adverse effect of polyphenol compounds on dietary proteins. Tannins have the potential to affect many aspects of digestion due to their affinity for proteins, and the tannin-protein complex decreases its digestibility (4). Another important function of polyphenol compounds in terms of the human health benefit is the growing evidence that suggests that polyphenol compounds in the diet have a long-term health benefit and may prevent or reduce the risk of some chronic diseases. Certain naturally occurring phenolic compounds, often referred to as flavonoids, seem to function via one or more biochemical mechanisms to interfere with, or prevent, carcinogenesis. Due to limited space, only those common phenolic compounds found in fruits and vegetables and other plant foods reported in recent years will be discussed here.

STRUCTURE

Hydroxycinnamic acids are widely distributed in relatively high concentrations in most fruits and vegetables. The important acids are *p*-coumaric, caffeic, ferulic, and sinapic acids (Table 1). They rarely occur in free state, but rather as simple esters. The most common is quinic acid, especially 5-caffeoylquinic or 3-caffeoylquinic acid, which are commonly known as chlorogenic acid and neochlorogenic acid. Hydroxycinnamic acids often occur in fruits and vegetables as esters of malic acid, tartaric acid, hydroxycitric acid, tartronic acid, shikimic acid, galactaric acid, gluconic acid, and methoxyaldaric acid (5).

Flavonoids constitute one of the most distinctive groups of higher plant secondary metabolites. The term flavonoid embraces all those compounds whose structure is based on that of flavone (2-phenylchromone) (Fig. 1).

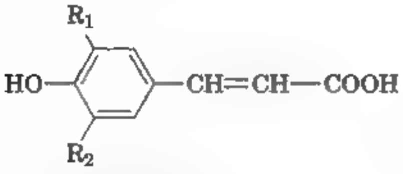
It can be seen that flavone consists of two benzene rings (A and B) joined together by a three-carbon link that is formed into a γ-pyrone ring. The various classes of flavonoid compounds differ from each other only by the state of oxidation of this 3-C link. Various states of oxidation at the link produce a number of different compounds found in plants, such as flavan-3-ols (catechins), and 3-hydroxyflavones (flavonols, such as quercetin). Also included in the flavonoids are flavanones and anthocyanidins, chalcones, and isoflavones, as shown in Table 1. In the flavanones (naringenin), the double bond between C-2 and C-3 is reduced.

The individual compounds within each class are also distinguished by the number of hydroxyl, methoxyl, and other groups substituted in the two benzene rings (A and B in Fig. 1). Hydroxyl groups of the A ring are substituted at either both C-5 and C-7, or only at C-7, while those of the B ring are usually substituted by either one, two, or three hydroxyl or methoxyl groups. The hydroxylation pattern of the B ring thus resembles that found in the commonly occurring cinnamic acids and coumarins. Most flavonoid compounds except catechins occur in the plant as glycosides in which some of the phenolic hydroxyl groups (usually C-3) are combined with sugar residues such as galactose, arabinose, xylose, glucose, and rhamnose. More than 720 flavone and flavonol glycosides are known to occur in the plant kingdom (6).

Anthocyanins are the most important group of water-soluble plant flavonoid pigments visible to the human eye. With a few exceptions, they are universal plant colorants and largely responsible for the cyanic colors of flower petals and fruits. Most of the approximately 300 anthocyanins that have been characterized consist mainly of 17 known aglycones (7,8). Of these aglycones, six are important food colorants, due to their common occurrence (9). The sugars substituted on the aglycone are glucose, rhamnose, xylose,

Table 1. Common Phenolic Compounds Found in Fruits and Vegetables

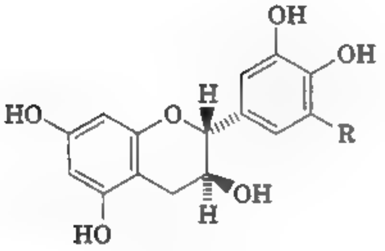
Hydroxycinnamic acid derivatives


R1
HO
CH=CH—COOH
R2

Cinnamic acids

R1 R2 = H, *p*-coumaric acid
R1 = OH, R2 = H, Caffeic acid
R1 = OMe, R2 = H, Ferulic acid
R1 R2 = OMe, Sinapic acid

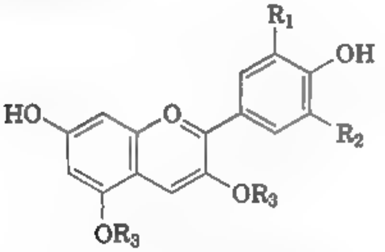
Catechins


OH
OH
HO
O
H
R
OH
H
HO

Catechins

R = H, (+) Catechin
R = OH (+) Gallocatechin
Opposite configuration at C-3
R = H (−) Epicatechin
R = OH (−) Epigallocatechin

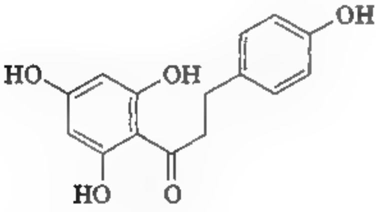
Anthocyanidins


R1
OH
HO
O
R2
OR3
OR3

Anthocyanidins (R3 = H)

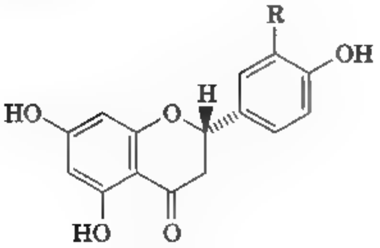
R1 R2 = H, Pelargonidin
R1 = OH, R2 = H, Cyanidin
R1 R2 = OH, Delphinidin
R3 = Sugar, Anthocyanins

Dihydrochalcones



Phloretin

Flavanones

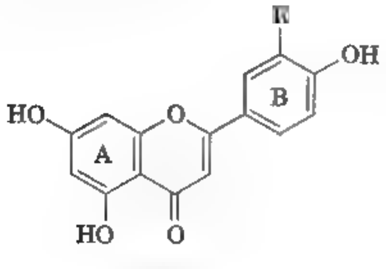

R
OH
H
HO
O
HO
O

Flavanones

R = H, Naringenin
R = OH, Eriodictyol

Table 1. Common Phenolic Compounds Found in Fruits and Vegetables *(continued)*

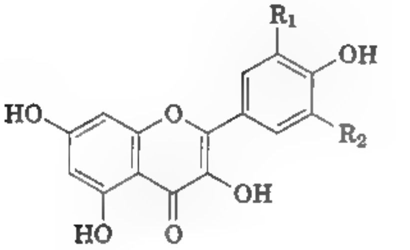
Flavones



Flavones

R = H, Apigenin
R = OH, Luteolin
Common glycosides have the sugar at C-7 hydroxyl group

Flavonols



Flavonols (3-hydroxyflavones)

R1 R2 = H, Kaempferol
R1 = OH, R2 = H, Quercetin
R1 R2 = OH, Myricetin

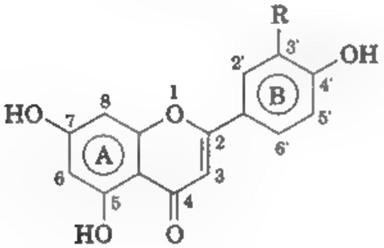


Figure 1. Flavones: the basic structure of flavonoids.

galactose, arabinose, and fructose. They occur as monoglycosides, diglycosides, and triglycosides. When the number of sugar residues is higher than three, they may attach to the basic molecule with alternating sugar and acyl acid linkages. The common acyl acids are coumaric, caffeic, ferulic, hydroxy benzoic, synapic, malonic, acetic, succinic, oxalic, and malic acids (9).

BIOSYNTHESIS

The biosynthesis of phenolic compounds occurs via various pathways. In recent years, considerable progress has been made in elucidating the biosynthesis of these compounds due to our rapidly developing knowledge of enzymology, and the improved quantification of substrates and products by high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy. All flavonoids derive their carbon skeleton from two basic compounds, malonyl-CoA and the CoA ester of a hydroxycinnamic acid (10). The origins of the direct flavonoid precursors, 4-coumaroyl-CoA and malonyl-CoA are derived from carbohydrates (Fig. 2). Malonyl-CoA is synthesized from the glycolysis intermediate acetyl-CoA and carbon dioxide, the reaction being catalyzed by acetyl-CoA caboxylase. The synthesis of 4-coumaroyl-CoA involves the shikimate/arogenate pathway, which is the main route to the aromatic amino acids, phenylalanine, tryptophan, and tyrosine in higher plants (10,11). The condensation of three molecules of malonyl-CoA with 4-coumaroyl-CoA to the C_{15} chalcone is catalyzed by chalcone synthase. Transformation by chalcone isomerase produces naringenin (flavonone) and other flavonoids via various steps. Dihydroflavonols formed by direct hydroxylation of flavanones in the 3 position are biosynthetic intermediates in the formation of flavonols, catechins, proanthocyanidins, and anthocyanidins.

Modification by hydroxylation of the A- and B-ring, methylation of hydroxyl groups as well as glycosylation and acylation reactions result in the immense diversity of flavonoids found in nature (10). The detailed biosynthesis steps of various flavonoids have been extensively described (12). Recent advances on flavonoid biosynthesis were restricted to specific steps and filled some of the remaining gaps in the metabolic pathway. Yet the key reaction to the anthocyanins as well as the formation of epicatechin, the proanthocyanidins, and some minor flavonoid-related compounds, such as aurones and dihydrochalcones, still remains a matter of debate (12).

ANALYSIS

Quantitative analysis of phenolic compounds in biological extracts can be carried out in many different ways. The

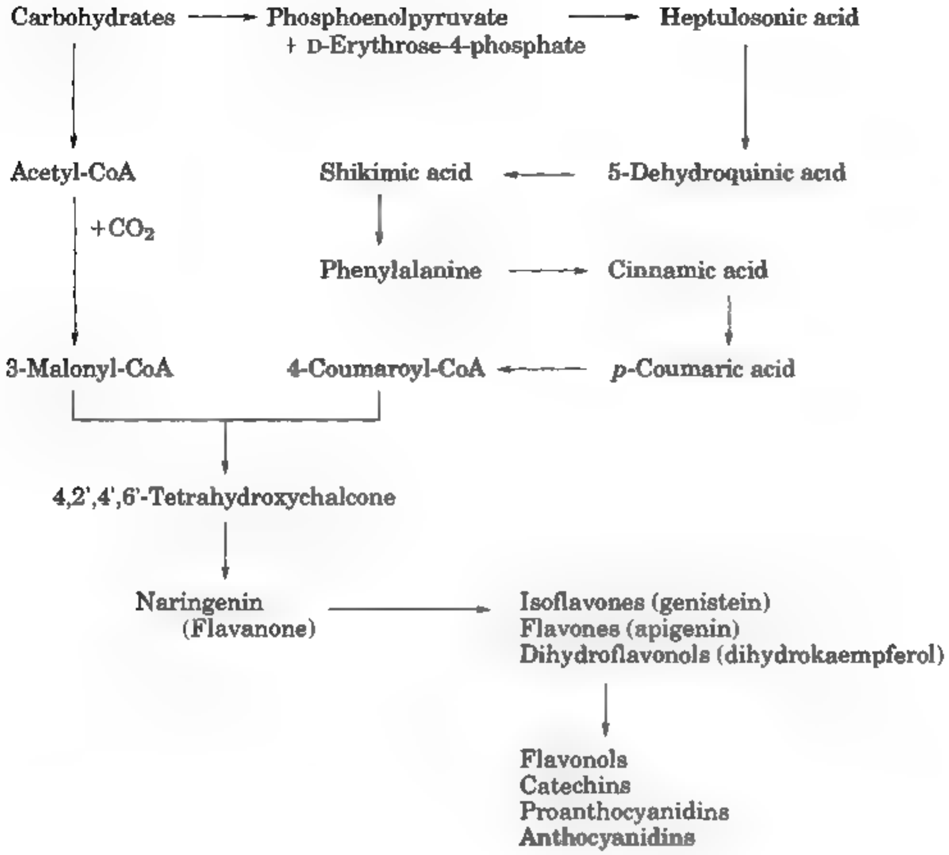

Carbohydrates
Phosphoenolpyruvate
+ D-Erythrose-4-phosphate
Heptulosonic acid
Acetyl-CoA
Shikimic acid
5-Dehydroquinic acid
$+CO_2$
Phenylalanine
Cinnamic acid
3-Malonyl-CoA
4-Coumaroyl-CoA
p-Coumaric acid
4,2',4',6'-Tetrahydroxychalcone
Naringenin
(Flavanone)
Isoflavones (genistein)
Flavones (apigenin)
Dihydroflavonols (dihydrokaempferol)
Flavonols
Catechins
Proanthocyanidins
Anthocyanidins

Figure 2. Biosynthetic pathways of phenolic compounds.

most reliable method for the determination of total phenols in food analysis laboratory is based on oxidation with the Folin-Ciocalteu reagent, which contains sodium phosphomolybdate and sodium tungstate. The intensity of the resulting blue complex can be estimated with a colorimeter or with a spectrophotometer (λmax at 725 nm). The colorless procoanthocyanidins can be estimated by heating them with acid and converting them to colored anthocyanidins. Individual phenolic compounds have been determined qualitatively by paper chromatography and semiquantitatively by densitometric analysis of the colored spots obtained by spraying two-dimensional chromatogram with a suitable reagent. Thin layer chromatography has been widely employed for polyphenols, because it is a highly effective, convenient, and inexpensive technique, especially for the separation of anthocyanin.

Today, it is possible to separate and quantify the individual polyphenols of fruits and vegetables by means of high-performance liquid chromatography (HPLC) with great success (13–15). HPLC has advantages of sensitivity, speed, and ease of use compared with other chromatographic procedures. When coupled with a diode array detector, HPLC provides an ideal procedure for accurately analyzing complex mixtures of polyphenols. Hydroxycinnamic acid esters in fruits and vegetables have been successfully separated by using a polyamide column (5). However, before using these procedures, the polyphenols must first be fractionated into several chemical groups to separate the individual polyphenols effectively (15,16). In addition, proton magnetic resonance, ^{13}C nuclear magnetic resonance (NMR), and mass spectrophotometry have been extensively used to determine the structures of polyphenols. Noteworthy achievements are the electron impact, chemical ionization, and fast atom bombardment (FAB) mass spectrometry that have been employed for the identification of various flavonoids, oligomeric hydrolyzable tannins, polyphenolic glycosides, and procyanidins (17). Previously, it had been difficult to obtain mass spectra of anthocyanins because they are not volatile and often present as salts. FAB mass spectrometry, however, gives molecular ions directly as $(M)^+$, and, therefore, its use has been extensive. The early use of CCl_4-soluble flavonoid trimethylsilyl ether derivatives for ^1H NMR spectroscopy and the advent of more sophisticated, higher-field spectrometers that have the capability to run ^{13}C as well as ^1H NMR spectra have been well reported (18,19). More recently, two-dimensional (2-D) homonuclear and heteronuclear spectra were produced for various flavonoids and its glycosides.

CONTENTS IN FRUITS AND VEGETABLES AND ITS PRODUCTS

The level of polyphenols in fruits and vegetables vary widely from species to species, cultivar to cultivar, season to season, and location to location. The accumulation of phenols in fruits may be higher or lower than in other parts of the plant, such as bark, leaves, or heartwood. The concentration of polyphenols often decreases as a fruit matures, but usually the amount per fruit increases. Fruits may contain considerable amounts of some types of polyphenols, such as anthocyanins, while other parts of the same plants, that is, leaves or bark, have very little or none. Concentrations of polyphenol compounds in various fruits and vegetables have been well documented (5,20) and some of them are presented in Table 2.

Among phenolic acids found in common fruits, chlorogenic acid is the major compound found in apples (62–385 ppm), pears (64–280 ppm), cherries (11–140 ppm), plums (15–142 ppm), peaches (43–282 ppm), and apricot (37–123 ppm). This is followed by neochlorogenic acid in cherries (73–628 ppm), plums (88–771 ppm), peaches (33–142 ppm), and apricots (26–132 ppm); and 3-*p*-coumaroylquinic acid in cherries (40–450 ppm), and plums (4–40 ppm). Among berries, blueberries are reported to contain the largest quantity of caffeoylquinic acids (1860–2080 ppm), followed by blackberries (45–53 ppm), and black currents (45–52 ppm) (5). The major phenolic acids in grapes are caffeoyltartaric acid, ranging from 46 to 397 ppm in white grapes (21) and from 50 to 435 ppm in the red cultivars (22).

Among vegetables, relatively large amounts of chlorogenic acid are found in eggplant (575–632 ppm), artichoke (433 ppm), endive (36–124 ppm), potatoes (22–71 ppm), carrots (23–121 ppm), lettuce (5–39 ppm), and tomatoes (12–71 ppm). The neochlorogenic acid content in Brussels sprouts is 70 to 120 ppm; red cabbage, 19 to 110 ppm; kale, 6 to 107 ppm; and broccoli, 58 ppm. Levels of sinapolyglucose have been reported in kale (16–273 ppm), red cabbage (28–84 ppm), Brussels sprouts (12–28 ppm), and broccoli (10 ppm). The levels of caffeoyltartaric acid in lettuce are 10 to 31 ppm while the dicaffeoyltartaric acid in endive is 38 to 334 ppm. Tomatoes contain a significant amount of caffeic acid-4-*o*-glucoside (15–48 ppm) and *p*-coumaric acid-*o*-glucoside (19–68 ppm) (5).

Among the various flavan-3-ols and their derivatives found in fruits and vegetables, apple skins mainly contain epicatechin (30–1010 ppm), procyanidins (30–910 ppm), phloretin glycosides (60–380 ppm), and quercetin glycosides (30–570 ppm) (23). A large quantity of epicatechin is also found in apricots (67–202 ppm), cherries (4–152 ppm), and pears (5–59 ppm) (5). Peaches are reported to contain mainly catechins (6–35 ppm) and its dimer, procyanidin B3 (4–85 ppm) (24). White grapes contain catechin gallates (3–105 ppm), epicatechin (7–63 ppm), and catechin (2–58 ppm) (21). As stated previously, the concentration of phenolic compounds in fruits and vegetables varies greatly depending on the tissue, cultivar, maturity, season, growing site, and various other factors such as cultivation practices.

Phenolic compounds constitute up to 35% of dry weight of tea, with major components such as epicatechin gallate, epigallocatechin, epigallocatechin gallate, catechin, quercetin, kaempferol, gallic acid, chlorogenic acid, and their derivatives. Green tea is known to contain more flavonols and their glycosides (25). Some fresh tea leaves (assamica variety) contain 9 to 13% epigallocatechin gallate, 3 to 6% epicatechin gallate, 3 to 6% epigallocatechin, and 1 to 2% epicatechin by dry weight as well as other flavonoids and their glycosides (26). Thearubigins, highly colored catechin oxidation products and their gallate are of major significance in determining the quality and flavor of tea. Black tea as consumed by humans contains about 36% thearubigins, 3% theaflavins, 5% epigallocatechin gallate, and 1% gallic acid by dry weight. Due to the large amounts of these phenolic compounds in tea, heavy drinkers of tea in Japan may consume 1 g of epigallocatechin gallate per day per person. Dry whole cocoa beans contain approximately 12 to 18% phenolic compounds and the major compound is epicatechin (27). Phenolic compounds in roasted coffee beans are produced during thermal processing from carbohydrates, chlorogenic acid, and lignins. Phenolic compounds in beer that contribute to bitterness, astringency, harshness, and the formation of haze are catechin and epicatechin (approximately 40 mg/L), gallocatechin (less than 15 mg/L), and hydroxycoumarins and anthocyanidins (less than 1 mg/L) (28). Phenolic compounds in wine derived from grape phenolics usually include derivatives of hydroxybenzoic and hydroxycinnamic acids, flavonoids such as flavan-3-ols, flavan-3, 4-diols, anthocyanins and anthocyanidins, flavonol, flavones, and condensed tannins (29). The composition of polyphenol compounds in wine depends on the type of grape used for vinification, extraction and wine making methods, and the chemical reactions that occur during the aging process. Individual phenolic compounds and their concentration in wine and grape are well documented (30).

EFFECTS OF FOOD PROCESSING

The phenolic compounds of fruit and vegetable products may be changed during storage and processing. From the view of the food processor, the tendency of polyphenols to undergo discoloration in fruits and vegetables is the main reason that they are significant constituents. They readily undergo color change because they serve as effective substrates for oxidation and react with other food components such as other polyphenols, thereby reducing sugars, metals and proteins. Phenolic compounds commonly found in plant foods are readily available substrates for polyphenol oxidase or peroxidase enzymes. Their reactivity lies not only in their molecular size and polyphenolic character, but also in their ability to complex strongly with protein, carbohydrates, nucleic acids, alkaloids, and minerals.

The oxidation of polyphenols during processing and storage of foods is accentuated by enzyme systems including catechol oxidase, laccase, peroxidases; also by alkali, especially in the presence of metal ions. The undesirable browning that occurs during processing of fruits and vegetables such as apples, apricots, banana, peaches, pears, and potatoes is due mainly to the oxidation of *o*-dihydroxyphenols by polyphenol oxidases and polymerization of the generated o-quinones (see the article BROWNING REACTION). The browning potential of many apple and peach cultivars correlates well with the quantity of certain phenolic compounds that is present. However, with certain cultivars, good correlation between browning potential and polyphenols is not always evident (31,32), presumably be-

Table 2. Concentration of Polyphenols in Fruits

Fruit	Hydroxybenzoic acid derivatives	Hydroxycinnamic acid derivatives	Anthocyanins	Flavonols	Falvon-3-ols	Tannins	Total phenolics
Apple		6.1–134	10–2160 (peel)	17.8–40 (peel) 154–285 (peel)	0.2–16.3 45.8 (peel)	40–350	50–1100 78–120 790–1160 (peel)
Avocado		2.8–46			3300 (peel)		2.8–28.7
				9 (pulp)		110–180	
Banana						0.6	150
Blackberry	0.9–3.0	6.2–8.8	82–180 234–326	0.85–2.15 20.7–31.4	7.3–17.7		
Blackcurrent	1.0–2.1	12.1–14.6	250	7.6–31.5	0.9–1.4	0.21–0.37%	
Cherry							
Sweet	0.05–4.1	28.5–140	350–450	1.1–3.1	2.0–7.0	70	360
Sour	0.1–0.8	27.0–67.1	28.8	4.2–6.0	15.2–20.2		200
Citrus							
Grapefruit	12.0–21.7						
Orange	13.6–16.3						
Gooseberry	1.1–2.1	2.5–14.8			1.7–3.3	0.06–0.1%	
Grape							
White	0.4 (skin, dw)	1.33–86.5 (skin)		0.81–8.19	1.4–52.7 (skin)	17.2 (skin)	350 (skin)
Red	24.7 (skin, dw)	10–109 (skin)	8–388	1.85–9.75	22–37 ppm	32.1–78.1 (skin)	900–950
Kiwi	60–100					35–60	
Peach		8.1–75		0.4–1.0	5.3–14.1		28–180
Pear		170	5–10 (peel)	4–160 (peel)	0.1–6.2	53	123–400
Persimmon					0.06% (dw)	0–2000	1–12% (dw)
Pineapple		5.3–14.9					
Plums	0.2–1.2	12.1–94	1.9–5.3	2–5.2	1.8–6.1	76–150	167–200
Raspberry, red	3.4–4.4	2–4	23–59	7.2–10.2	3.2–4.9	0.10–0.14	
Red currant	0.9–1.3	1–2.6	11.9–18.6	2–5.1	0.4–3.6		
Strawberry	0.7	1.4–3.1	28–70	2.7–17.4	2.4–9.6	110–150	
Tomato	0.15	7–23.2					45–57

Source: Ref. 20.
Note: All values are in mg/100 g fresh weight except where otherwise specified. dw dry weight.

cause of the presence of other substances that can modify the reactions. The contributions of different polyphenols to the color of grape and apple juices depend on the amounts of certain polyphenols in the fruit.

In general, the significance of phenolic compounds in food can be related to ascorbic acid destruction, color formation with heavy metals, and antioxidant activity. When both active polyphenol oxidases and phenolic substrates are present in fruits and vegetables, tissue discoloration takes place. But this reaction does not begin until all of the ascorbic acid has been destroyed. The reason for this is that the oxidized polyphenols, in the course of oxidation, can reversibly transfer oxygen to ascorbic acid, being itself reduced to its original state.

Flavonoids affect anthocyanin color by copigmentation and by copolymerization. Polyphenols contribute most to browning when enzymatically oxidized to quinones, and the quinones polymerize to form relatively stable polymers. Phenolic compounds also give characteristic color reactions with a number of heavy metals, such as ferric salts and aluminum ions (33). For example, black spotting of cashew kernels is caused by iron interaction with catechin, and red-brown precipitates in wine are caused by the copper-procoanthocyanidin reaction (34). Since copper, iron, and other complexing ions catalyze oxidation of polyphenols, they can promote oxidative browning. Quercetin, chlorogenic acid, and gallic acid form highly colored complexes with cyanidin-3-glucoside in the presence of aluminum ions (35). The combination of anthocyanins with flavonoids of another types occurs in red wine during aging (36).

One of the discolorations caused by reactions of colorless plant polyphenols is the reddening or pinking that occurs when colorless anthocyanogens are converted to anthocyanidins. Acidic media and heat are important factors in the conversion of proanthocyanidins to anthocyandins; these conditions often occur in foods, particularly canned fruits. Pink color in canned pears is associated with high procoanthocyanidin content and failure to cool the cans quickly after thermal processing (37). Pink color development in banana, cabbage, and broad beans is also related to polyphenols (38–40).

Browning and blackening in fruits and vegetables during processing and storage involves the production of many overlapping chromophores that have absorbances in the visible region. Reactions of polyphenols to given colored products are not necessarily oxidative, particularly those that involve reaction with the phloroglucinol portion of flavonoids. Phenol-aldehyde condensations are an example of this. Carbonyl-amine reactions can result in browning if the carbonyl is in a quinone molecule that can be observed in the Maillard sugar-amino acid type reaction.

Certain phenolic compounds are antioxidants that suppress activity of the enzymes, lipoxygenase (41) and β-galactosidase (42). Lipoxygenase causes oxidation of carotenoids in certain vegetables and thus lowers the vitamin A value of these foods. Among phenolic compounds, flavans exhibit a higher inhibitory effect on lipoxygenase than flavonols and phenolic acids (43). Catechin, chlorogenic acid, and quercetin glycosides suppress β-galactosidase in apple and retard softening during cold storage (42).

Another important reaction of polyphenols in foods is the complexing with protein. It has long been known that the o-dihydroxy phenolic compounds and the carbonyl groups of protein interact by hydrogen bonding (44) and that quinone-protein reactions also participate in this interaction (45). This polyphenols-protein interaction affects the protein quality of certain foods. The presence of tannins has been shown to reduce the nutritive value of various grains such as sorghum and fava beans. Vegetable proteins that have been exposed to *o*-dihydroxyphenols in oxidizing condition would be expected to be less readily digested and to have less biologically available lysine and cystine (46). When polymerized tannins combine with the proteins of food, the complex is less likely to be absorbed. Condensed tannins also may pass unchanged through the digestive tract.

Phenolic compounds and proteins reduce apple juice quality by the formation of haze and sediment during storage. Phenolic compounds participate in formation of haze during processing of beer and wine. Gelatin treatments have been used to remove some of the tannins and thus minimize the problem (47). Oxidized polyphenols may also react with proteins; the mechanisms of these quinone-protein reactions are not completely understood.

Some beneficial effects of polyphenols in relation to color and flavor of food products are associated with tea and coffee. In the cultivation of the tea plant, conditions are designed to produce tea leaves that are rich in epicatechin, epigallocatechin, and their gallate esters (46). A large number of phenolic compounds are produced during the roasting process of coffee, while some phenolics such as chlorogenic acid (which is known to contain as much as 4% by weight of coffee beans) is destroyed significantly (48).

Since bound phenolic residues are components of unlignified cell walls of many plants, some polyphenols may contribute to the fiber content of the human diet. Both primary and secondary cell walls of wheat endosperm, spinach, potato, and other vegetables contain polyphenols such as ferulic and coumaric residues that are linked to hemicelluloses or peptides (49).

BIOLOGICAL AND CHEMICAL ACTIVITIES

Phenolic compounds are known to possess several biological and chemical properties, including antioxidant activity, the ability to scavenge active oxygen species, the ability to scavenge electrophiles, the ability to inhibit nitrosation, the ability to chelate metals, the potential for autoxidation, producing hydrogen peroxide in the presence of certain metals, and the capability to modulate certain cellular enzyme activities. Various phenolic acids and its esters are effective in the prevention of microbial growth. Phenolics such as gallic acid and *p*-hydroxybenzoic acid esters showed inhibitory effects against the growth and toxin production of *Clostridium botulinum* types A and B (50). *p*-Coumaric acid (>250 ppm) and ferulic acid (250 ppm) inhibit the growth of *Saccharomyces cerevisiae* (51). Proanthocyanidins, flavonols, and benzoic acid extracted from cranberry showed antimicrobial effects on *Saccharo-*

myces bayanus and *Pseudomonas flurescens* (52). Some phenolic compounds in red grapes, such as catechin gallate, are known to have antimicrobial activity on aciduric, aerobic, and spore-forming bacteria isolated from apple juice (53).

Natural antioxidants occur in all higher plants and in all parts of the plant—wood, bark, stems, pods, leaves, fruit, root, flowers, pollen, and seeds. These are usually polyphenol compounds. Typical compounds that possess antioxidant activity include tocopherols, flavonoids, cinnamic acid derivatives, and other compounds. The major function of flavonoids and cinnamic acids is in their primary antioxidant activity as free radical acceptors and as chain-breaker. This includes antioxidative activity against lipoxygenase catalyzed reactions. Spices and herbs also have antioxidant activity that originates mainly from their polyphenolic constituents. The antioxidant activity of rosemary depends primarily on the concentration of carnosic acid and rosmaric acid, a derivative of caffeic acid (54).

Several flavonoids have been found to be mutagens *in vitro* test, while other investigators have failed to observe any *in vivo* genetic toxicity of flavonoids. Quercetin and its glycosides found in many fruits and vegetables showed antiviral activities against various viruses (55). A variety of dietary flavonoids have been found to inhibit tumor development in experimental animal models. The hydroxylated flavonoids have been found to (*1*) inhibit the metabolic activation of carcinogenes by modulation of cytochrom enzymes; (*2*) inactivate ultimate carcinogens; (*3*) inhibit generation of active oxygen species and act as scavengers of active oxygen species; (*4*) inhibit arachidonic acid metabolism; (*5*) inhibit protein kinase C and other kinase activity; and (*6*) reduce the bioavailability of carcinogenes (56). Phenolic compounds such as caffeic acid and ferulic acid can block the nitrosation of amines by reducing nitrite to nitric oxide or by forming C-nitroso compounds. Epigallocatechin-3-gallate, isolated from green tea, was found to reduce the incidence of chemically induced tumors in experimental animals in the liver, stomach, skin, lungs, and esophagus (56). Ellagic and chlorogenic acid also showed chemopreventive activity against liver, colon, and tongue carcinogens (57). Epidemiological studies in Finland and Netherlands indicate an inverse relationship between flavonoid intake and coronary heart disease (58–60). Quercetin was reported to be the main flavonoid consumed in these studies. Since numerous reports related to the positive effects of polyphenol compounds in human health are being published, and the topic of polyphenol compounds in relation to functional foods has been the major subject among many scientific communities in recent years, we expect to know more about the bioactivity of polyphenol compounds soon.

COMMERCIAL APPLICATIONS

The most important group of polyphenols for commercial application has been anthocyanins. Commercial food colorants have been used for many years, especially in the wine trade. To enhance the color of red wine and also as a general food colorant, anthocyanin pigments from grape skins have been extensively utilized. Grapes are the largest single fruit crop grown in the world, and, therefore, the major source of phenolic compounds among the different fruits and vegetables. Grape pigments have significant commercial value; approximately 10,000 tons of grape anthocyanins are utilized annually. Since anthocyanins are natural colorants, many processors prefer anthocyanins to synthetic red dyes. However, anthocyanins are less stable in many foods because light and pH affect them. They are also readily bleached by sulfur dioxide, which is often used in beverage and food processing as a preservative. This instability can be counteracted by reacting anthocyanins with carbonyl compounds, such as acetaldehyde, to stabilize them (9). Also, it is possible to use acylated anthocyanins, which are more stable to light than the simple glycosides.

Some phenolic compounds are also valuable as antioxidants, especially the highly hydroxylated types. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are synthetic phenolic compounds that are widely sued as antioxidant food additives, mainly in oils and food coating materials. Compounds such as alkyl gallates and nordihydroguaiaretic acid are well-known antioxidants. New phenolic compounds are currently being isolated and their chemical properties will be found. It is likely that some will have potent antioxidant properties that can be utilized in food processing.

Certain flavonoids have long been used as drugs in Eastern European and Southeastern Asia. The pharmacological activities of these polyphenols have been less specific and less pronounced than those of alkaloids or steroids. Therefore, doubt and controversy have always surrounded the therapeutic value of polyphenols. Many European countries and Japan have been leaders in the commercial production of herb phytomedicinals consisting mainly of polyphenol compounds. Germany accounts for the largest share of the European Union herbal drug market, with sales of $3 billion of an annual $6 billion total (61). Of the 10 best-selling herbs in the United States today, echinacea, garlic, ginseng, ginkgo, saw palmetto, and eleuthero have been popularized. This is primarily the result of European research, which takes place under a more favorable regulatory climate that permits reasonable evidence of efficacy. In the United States, demand for excess amounts of evidence regarding efficacy has discouraged research on those products and relegated them to the dietary supplement status (61). The Dietary Supplement Health and Education Act of 1994 permits manufacturers of supplements to make claims regarding the health benefits of the products, and numerous new products of dietary supplements that contain polyphenols are being produced. In the future, therefore, we expect to find more new health beneficial polyphenol compounds and will see the expanded use of natural phenolic compounds, both for the improvement of processed foods and also as new phytomedicines.

BIBLIOGRAPHY

1. J. B. Harborne, ed., *Flavonoids: Advances in Research since 1986*, Chapman & Hall, London, 1994.

2. J. B. Harborne, "Phenolics in the Environment: An Overview of Recent Progress," *Proc. 14th Int. Conf. Groupe Polyphenols*, Brock University, St. Catharines, Ontario, Canada, August 16–19, 1988.
3. J. B. Harborne, "Plant Polyphenols and Their Role in Plant Defense Mechanisms," in R. Brouillard, M. Jay, and A. Scalbert, eds., *Polyphenols 94*, INRA, Paris, 1995.
4. W. S. Pierpoint, "Reaction of Phenolic Compounds with Proteins and Their Relevance to the Production of Leaf Protein," in L. Telek and H. D. Graham, eds., *Leaf Protein Concentrates*, Avi, Westport, Conn., 1983.
5. K. Herrmann, "Occurrence and Content of Hydroxycinnamic and Hydroxybenzoic Acid Compounds in Foods," *Crit. Rev. Food Sci. Nutr.* **28**, 315–347 (1989).
6. J. B. Harborne and C. A. Williams, "Flavone and Flavonol Glycosides," in J. B. Harborne, ed., *The Flavonoids*, Chapman & Hall, London, 1988.
7. J. B. Harborne and R. J. Grayer, "The Anthocyanins," in J. B. Harborne, *The Flavonoids*, Chapman & Hall, London, 1988.
8. D. Strack and V. Wray, "The Anthocyanins," in J. B Harborne, ed., *Flavonoids: Advances in Research since 1986*, Chapman & Hall, London, 1994.
9. F. J. Francis, "Food Colorants: Anthocyanins," *Crit. Rev. Food Sci. Nutr* **28**, 273–314 (1989).
10. W. Heller and G. Forkmann, "Biosynthesis," in J. B. Harborne, ed., *The Flavonoids*, Chapman & Hall, London, 1988.
11. R. A. Jensen, "The Shikimate/Arogenate Pathway: Link between Carbohydrate Metabolism and Secondary Metabolism," *Physiol. Plant.* **66**, 164–186 (1985).
12. W. Heller and G. Forkmann, "Biosynthesis of Flavonoids," in J. B. Harborne, ed., *Flavonoids: Advances in Research since 1986*, Chapman & Hall, London, 1994.
13. B. Y. Ong and C. W. Nagel, "High-Pressure Liquid Chromatographic Analysis of Hydroxycinnamic Acid Tartaric Acid Esters and Their Glucose Esters in *Vitis vinifera*," *J. Chromatogr.* **157**, 345–341 (1978).
14. W. Brandl and K. Herrmann, "Analytical and Preparative High-Performance Liquid Chromatography of Hydroxycinnamic Acid Esters," *J. Chromatogr.* **260**, 447–451 (1983).
15. A. W. Jaworski and C. Y. Lee, "Fractionation and HPLC Determination of Grape Phenolics," *J. Agric. Food Chem.* **35**, 257–259 (1987).
16. J. Oszmianski and C. Y. Lee, "Isolation and HPLC Determination of Phenolic Compounds in Red Grapes," *Am. J. Enol. Vitic.* **41**, 204–206 (1990)
17. R. Self, "Fast Atom Bombardment Mass Spectrometry of Polyphenols," *Biomed. Environ. Mass Spectrom.* **13**, 449–469 (1986)
18. K. R. Markham, *Techniques of Flavonoid Identification*, Academic Press, London, 1982
19. K. R. Markhan and H. Geiger, "^{1}H Nuclear Magnetic Resonance Spectroscopy of Flavonoids and Their Glycosides in Hexadeuterodimethylsulfoxide," in J. B. Harborne, ed., *Flavonoids: Advances in Research since 1986*, Chapman & Hall, London, 1994.
20. J. I. Macheix, A. Fleuriet, and J. Billot, *Fruit Phenolics*, CRC Press, Boca Raton, Fla., 1990.
21. C. Y. Lee and A. W. Jaworski, "Phenolic Compounds in White Grapes Grown in New York," *Am. J Enol. Vitic.* **38**, 277–281 (1987).
22. V. L. Singleton, J. Zaya, and E. K. Trousdale, "Caftaric and Coutaric Acids in Fruit of Vitis," *Phytochemistry* **25**, 2127–2132 (1986).
23. S. Burda, W. Oleszek, and C. Y. Lee, "Phenolic Compounds and Their Changes in Apples during Maturation and Cold Storage," *J. Agri. Food Chem.* **38**, 945–948 (1990).
24. C. Y. Lee et al., "Enzymatic Browning in Relation to Phenolic Compounds and Polyphenoloxidase Activity among Various Peach Cultivars," *J. Agric. Food Chem.* **38**, 99–101 (1990).
25. D. A. Balentine, "Manufacturing and Chemistry of Tea," in C. T. Ho, C. Y. Lee, and M. T. Huang, eds., *Phenolic Compounds in Food and Their Effects on Health I*, American Chemical Society Symposium Series 506, ACS, Washington, D.C., 1992.
26. World Health Organization, International Agency for Research on Cancer, *Coffee, Tea, Mate, Methylxanthines and Methylglyoxal*, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, IARC Press, Lyon, France, Vol 51, 1991, pp. 207–271.
27. H. Kim and P. G. Keeney, "Epicatechin Content in Fermented and Unfermented Cocoa Beans," *J. Food Sci.* **49**, 1090–1092 (1984).
28. I. McMurrough, G. P. Roche, and K. G. Cleary, "Phenolic Acids in Beers and Worts," *J. Institute of Brewing* **90**, 181–184 (1984).
29. V. L. Singleton and P. Essau, *Phenolic Substances in Grapes and Wine, and Their Significance*, Academic Press, New York, 1969.
30. J. J. Macheix, J. C. Sapis, and A. Fleuriet, "Phenolic Compounds and Polyphenoloxidase in Relation to Browning in Grapes and Wines," *Crit. Rev. Food Sci. Nutr.* **30**, 441–486 (1991).
31. M. Y. CoSeteng and C. Y. Lee, "Changes in Apple Polyphenoloxidase and Polyphenol Concentration in Relation to Degree of Browning," *J. Food Sci.* **52**, 985–989 (1987).
32. C. Y. Lee and A. W. Jaworski, "Phenolics and Browning Potential of White Grapes Grown in New York," *Am. J. Enol. Vitic.* **39**, 337–340 (1988).
33. E. C. Bate-Smith, "Flavonoid Compounds in Foods," in E. N. Mrak and G. F. Stewart, eds., *Advances in Food Research*, Vol. 5, Academic Press, New York, 1954, pp. 161–257.
34. V. L. Singleton, "Common Plant Phenols other Than Anthocyanins, Contributions to Coloration and Discoloration," in C. O. Chichester, ed., *The Chemistry of Plant Pigments*, Academic Press, New York, 1972.
35. J. Jurd and S. Asen, "The Formation of Metal and Copigment Complexes of Cyanidin-3-Glucoside," *Phytochemistry* **5**, 1263–1271 (1966).
36. T. C. Sommers, "Pigmentation Profiles of Grapes and of Wines," *Vitis* **7**, 303–320 (1968).
37. B. S. Luh, S. J. Leonard, and D. S. Patel, "Pink Discoloration in Canned Bartlett Pears," *Food Technol.* **14**, 53–56 (1960).
38. R. E. Guyer and F. B. Erickson, "Canning of Acidified Banana Puree," *Food Technol.* **8**, 165–167 (1954).
39. S. Ranganna and V. S. Govindarajan, "Leucoanthocyanins in Cabbage and Pink Discoloration," *J. Food Sci. Technol.* **3**, 155–158 (1966).
40. D. Dickson, M. Knight, and D. I. Rees, "Varieties of Broad Beans Suitable for Canning," *Chemistry and Industry*, 1503 (1957).
41. K. S. Rhee and B. M. Watts, "Effect of Antioxidants on Lipoxygenase Activity in Model Systems and Pea Slurries," *J. Food Sci.* **31**, 669–674 1966.
42. A. J. Dick et al., "Quercetin Glycosides and Chlorogenic Acid: Inhibitors of Apple b-Galactosidase and of Apple Softening," *J. Agri. Food Chem.* **33**, 798–800 1985.
43. J. Oszmianski and C. Y. Lee, "Inhibitory Effect of Phenolics on Carotene Bleaching in Vegetables," *J. Agri. Food Chem.* **38**, 688–690 (1990).
44. K. H. Gustavson, "Interaction of Vegetable Tannins with Polyamides as Proof of the Dominant Function of the Peptide Bond of Collagen for Its Binding of Tannins," *J. Polym. Sci.* **12**, 317–324 (1954).

45. W D Loomis and J. Battaile, "Plant Phenolic Compounds and Isolation of Plant Enzymes," *Phytochemistry* **5**, 423–438 (1966).
46. W. S. Pierpoint, "Phenolics in Food and Feedstuffs," in C. F. Van Sumere and P. J. Lea, eds., *Annual Proceedings of the Phytochemical Society of Europe*, Clarendon Press, Oxford, 1985, pp 427–451.
47. A. Pollard and C. F. Timberlake, "Fruit Juice," in A. C. Hulme, ed., *The Biochemistry of Fruits and Their Products*, Vol. 2, Academic Press, New York, 1971.
48. I Flament, "Coffee, Cocoa, and Tea," *Food Rev. Int.* **5**, 317–414 (1989)
49. S. C. Fry, "Incorporation of Cinnamate into Hydrolase-Resistant Components of the Primary Cell Wall of Spinach," *Phytochemistry* **23**, 59–64 (1984).
50. M. D. Pierson and N. R. Reddy, "Inhibition of *Clostridium botulinum* by Antioxidants and Related Phenolic Compounds in Comminuted Pork," *J. Food Sci.* **47**, 1926–1929 (1982).
51. J. D. Baranowski et al., "Inhibition of *Saccharomyces cerevisiae* by Naturally Occurring Hydroxycinnamate," *J. Food Sci.* **45**, 592–594 (1980).
52. A. G. Marvan and C. W. Nagel, "Microbial Inhibitors of Cranberries," *J. Food Sci.* **51**, 1009–1013 (1986).
53. D. F. Splittstoesser, J. J. Churey, and C. Y. Lee, "Growth Characteristics of Aciduric Sporeforming *Bacilli* Isolated from Fruit Juices," *J. Food Prot.* **57**, 1080–1083 (1994).
54. D. E. Pratt, "Natural Antioxidants from Plant Material," in M. T. Huang, C. T. Ho, and C. Y. Lee eds., *Phenolic Compounds in Food and Their Effects on Health II*, American Chemical Society Symposium Series 507, ACS, Washington, D.C., 1992.
55. I. Musci, "Combined Antiviral Effect of Quercetin and Interferon on the Multiplication of Herpes Simplex Virus in Cell Culture," in L. Farkas, M. Gabor and F. Kallay, eds., *Flavonoids and Bioflavonoids*, Elsevier, Amsterdam, The Netherlands, 1986.
56. M. T. Huang and T. Ferraro, "Phenolic Compounds in Food and Cancer Prevention," in M. T. Huang, C. T. Ho, and C. Y. Lee, eds., *Phenolic Compounds in Food and Their Effects on Health II*, American Chemical Society Symposium Series 507, ACS, Washington, D.C., 1992.
57. T. Tanaka et al., "Protective Effects against Liver, Colon, and Tongue Carcinogenesis by Plant Phenols," in M. T. Huang, C T. Ho, and C. Y. Lee, eds., *Phenolic Compounds in Food and Their Effects on Health II*, American Chemical Society Symposium Series 507, ACS, Washington, D.C., 1992
58. M G. L. Hertog et al., "Dietary Antioxidant Flavonoids and Risk of Coronary Heart Disease: The Zutphen Elderly Study," *Lancet* **342**, 1007–1011 (1993)
59. P. Knekt et al., "Flavonoid Intake and Coronary Mortality in Finland: A Cohort Study," *Br. Med. J.* **312**, 478–481 (1996).
60. M. F. Muldoon and S. B. Kritchevsky, "Flavonoids and Heart Disease," *Br. Med. J.* **312**, 458–459 (1996).
61. V. E. Tyler, "Importance of European Phytomedicinals in the American Market: An Overview," in L. D. Lawson and R. Bauer, eds., *Phytomedicines of Europe*, American Chemical Society Symposium Series 691, ACS, Washington, D.C., 1998.

CHANG Y. LEE
Cornell University
Geneva, New York

See also COLORANTS: POLYPHENOLS; PHYTOCHEMICALS: BIOTECHNOLOGY OF PHENOLIC PHYTOCHEMICALS FOR FOOD PRESERVATIVES AND FUNCTIONAL FOOD APPLICATIONS.

PHOSPHATES AND FOOD PROCESSING

HISTORICAL DEVELOPMENT AND GENERAL USE

Phosphates exhibit functional properties in a wide variety of foods produced by all segments of the processed food industry. The availability of sodium, potassium, ammonium, calcium, and magnesium phosphates offers food technologists and food scientists formulation flexibility to control taste, nutritional, and other technical properties. The commercial use of food phosphates may be traced to 1864 when the first U.S. patent was granted for a phosphate-containing baking powder (1). The use of emulsifying salts in Europe for production of process cheese products began about 1895 and has been reviewed (2). Principal development in the United States began in 1916 (3). Since World War II new uses have developed in food products, including meats, poultry, seafood, beverages, dairy products, infant foods, cereals, desserts, produce, and nutritional supplements. Various detailed reviews have been published (4–6).

The largest market for food phosphates in the world is the United States where processing techniques and advanced food technology follow the consumer trends and demands for high-quality convenient foods. Phosphate use in food applications is an important segment of the overall industrial phosphate market. The U.S. consumption of food phosphates was estimated to be 87,000 t P_2O_5 in 1993, excluding phosphates sold into dentifrice applications and phosphoric acid sold directly into the food industry (7). The second largest market of food phosphates, Western Europe, is approximately 37,000 t P_2O_5 (1993), including the dentifrice market (7). The growth rate of food phosphates in the United States was predicted to be 1.5 to 2.3% annually (1993–1998, including phosphoric acid) while that predicted for Western Europe (1993–1997) was relatively flat (7). The bakery market segment represents approximately 40% of the food phosphates consumed in the United States while the meat, poultry, and seafood applications represent an estimated 30% of the phosphate consumed, followed by dairy (14%) and other (16%) (7).

NOMENCLATURE AND STRUCTURE

Of the phosphoric acids formed by the reaction between phosphorus pentoxide and water, orthophosphoric acid (H_3PO_4) is the simplest and most commonly encountered (8,9). One, two, or all three protons may be replaced by metal ions to form the orthophosphate salts. The acid orthophosphate salts can be dehydrated to form linear chains (polyphosphates) and rings (metaphosphates) wherein phosphate tetrahedrons share oxygen atoms. The polyphosphates have the general formula $M_{n+2}P_nO_{3n+1}$ (M equals one equivalent of hydrogen or metal ion), which approaches the formula of the cyclic metaphosphates as the chain length increases $(MPO_3)_n$.

Several systems of phosphate nomenclature exist, reflecting the historical changes in the understanding of the phosphate structures. Older names such as pyrophosphate and tripolyphosphate for the smallest of the polyphosphate anions are generally accepted in North American industry and commerce as opposed to the IUPAC names diphosphate and triphosphate, respectively, which are preferred in many other areas of the world. Some of the more common phosphates used in foods are listed in Table 1 along with their formulas.

MANUFACTURE

Phosphoric acid for food products can be produced either from elemental phosphorus or by the purification of wet-process phosphoric acid (9,11). Elemental phosphorus is produced by the reduction of phosphate rock with coke in the presence of silica in an electric furnace. Thermal (furnace) phosphoric acid is manufactured by the combustion and subsequent hydration of elemental phosphorus. For food use, thermal acid is treated with sulfide to remove traces of arsenic.

The North American production of technical- and food-grade phosphoric acid has shifted in the 1990s from almost exclusively thermal acid to a sizable proportion as purified wet-process acid. This shift was a continuation of a trend that had already taken place in many other parts of the world. In the wet process, phosphoric acid is made by reacting phosphate rock and sulfuric acid. The resultant acid contains percent levels of sulfate, fluoride, and metal impurities (Fe, Al, etc) and is therefore not suitable for food use. It is purified by a combination of solvent extraction and chemical treatment to yield a food-grade phosphoric acid. Phosphoric acid made by either the thermal or the wet acid purification route is typically available as 75 to 85% H_3PO_4.

The orthophosphate salts are made by neutralizing phosphoric acid with the appropriate base (Na_2CO_3, NaOH, KOH, NH_3, $CaCO_3$, $Ca(OH)_2$, $Mg(OH)_2$, etc) and separating the product by crystallization or drying. The condensed phosphate salts are made by thermal dehydration of the orthophosphate (9,11).

CHEMICAL PROPERTIES

Phosphates provide important functional properties for improving the processing and the final quality of foods. The use of a phosphate often improves more than a single property (4,8). The chemical effects of phosphates on foods are briefly outlined next. Physical properties of the phosphates (eg, particle size and rate of dissolution) are also important in many applications.

Acid-Base Properties

Various phosphates may provide acidity, alkalinity, or buffering, depending on the degree of neutralization and the structure of the phosphate. Orthophosphates and the short-chain polyphosphates have good buffering capacity, especially in the pH range of 6 to 8. Phosphoric acid is widely used as a beverage acidulant. Acidic phosphates are important leavening agents, and the basic phosphates such as sodium tripolyphosphate (STP) modify meat and dairy protein characteristics by altering their charge.

Table 1. Phosphates Commonly Used in Food Applications

Name	Formula
Orthophosphates	
Phosphoric acid	H_3PO_4
Monosodium phosphate (MSP)	NaH_2PO_4
Disodium phosphate (DSP)	Na_2HPO_4
Disodium phosphate dihydrate (DSPD)	$Na_2HPO_4 \cdot 2H_2O$
Trisodium phosphate (TSP)	Na_3PO_4
Trisodium phosphate dodecahydrate (TSPC)	$Na_3PO_4 \cdot 12H_2O \cdot 1/4NaOH$
Monopotassium phosphate (MKP)	KH_2PO_4
Dipotassium phosphate (DKP)	K_2HPO_4
Tripotassium phosphate (TKP)	K_3PO_4
Monoammonium phosphate (MAP)	$NH_4H_2PO_4$
Diammonium phosphate (DAP)	$(NH_4)_2HPO_4$
Monocalcium phosphate (MCPA)	$Ca(H_2PO_4)_2$
Monocalcium phosphate monohydrate (MCP)	$Ca(H_2PO_4)_2 \cdot H_2O$
Dicalcium phosphate (DCP)	$CaHPO_4$
Dicalcium phosphate dihydrate(DCPD)	$CaHPO_4 \cdot 2H_2O$
Tricalcium phosphate (TCP) (hydroxyapatite)	$Ca_{10}(PO_4)_6(OH)_2$
Dimagnesium phosphate (DMP)	$MgHPO_4 \cdot 3H_2O$
Sodium aluminum phosphates (SALP)	$NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$ $Na_3Al_2H_{15}(PO_4)_8$ $Na_{15}Al_3(PO_4)_8$
Polyphosphates	
Sodium acid pyrophosphate (SAPP)	$Na_2H_2P_2O_7$
Tetrasodium pyrophosphate (TSPP)	$Na_4P_2O_7$
Tetrapotassium pyrophosphate (TKPP)	$K_4P_2O_7$
Calcium acid pyrophosphate (CAPP)	$CaH_2P_2O_7$
Calcium pyrophosphate (TCPP)	$Ca_2P_2O_7$
Sodium tripolyphosphate (STP)	$Na_5P_3O_{10}$
Potassium tripolyphosphate (KTP)	$K_5P_3O_{10}$
Sodium potassium tripolyphosphate (SKTP)	$Na_3K_2P_3O_{10}$
Sodium hexametaphosphate (SHMP)[a] (Graham's salt)	$Na_{n+2}P_nO_{3n+1}$ (n ~ 6–20)
Insoluble sodium metaphosphate (IMP) (Maddrell's salt)	$(NaPO_3)_n$ ($n > 5,000$)
Potassium metaphosphate (KMP) (Kurrol's salt)	$(KPO_3)_n$ ($n \geq 1,000$)
Metaphosphate	
Sodium trimetaphosphate (STMP)	$(NaPO_3)_3$

Source: Refs. 9 and 10.

[a] Also known as sodium polyphosphates, glassy in *Food Chemicals Codex*.

Sequestration

Sequestration is the process of inactivating metal ions by forming soluble complexes. Polyphosphates chelate metal ions, thereby preventing the ions from entering into further (often undesirable) reactions. For example, polyphosphates inhibit the development of rancidity in oils and fats caused by iron (Fe^{3+}) catalyzed oxidation. The formation of precipitates and haze in beverages is eliminated by the addition of polyphosphates. Calcium ion sequestration aids in the removal of shells from shellfish and in the extraction of pectin from fruit.

Adsorption—Ionic Interaction

Phosphates (especially more basic condensed salts such as STP) may adsorb or complex with proteins and starches by virtue of their high charge. The charge and the extension of the protein is altered, thereby affecting its colloidal properties. Phosphates can, therefore, act to modify the processing characteristics or stability of proteins, for example, dispersion and peptization in dairy applications, improvement of water retention in meats, and emulsion stabilization in sausage and process cheese.

APPLICATIONS OF FOOD PHOSPHATES

Baking Industry Applications

The major function of phosphates in the baking industry is as leavening acids. Leavening is the process of making baked goods light by expanding or inflating the batter or dough during baking. Three general methods of leavening are employed: fermentation or yeast leavening, chemical decomposition, and chemical neutralization. In chemical neutralization, bicarbonate is neutralized by an acid salt such as monocalcium phosphate monohydrate (MCP). Although any acid component can be used to neutralize the bicarbonate, phosphate salts are preferred as they are manufactured to give controlled rates of reaction. The user can select the appropriate phosphate salt(s) so that carbon dioxide (CO_2) is released at the appropriate point in the baking process. Usually, CO_2 release will be desired during both mixing and while baking in the oven. Types of bakery product that are chemically leavened are listed in Table 2 along with typical use levels of sodium bicarbonate (10,12). The phosphate is used at a level to neutralize the bicarbonate. Other applications for phosphates in the baking industry include yeast food nutrients and dough conditioners.

Meat Industry Applications

The meat industry uses phosphate salts in processed meat, poultry, and seafood products to contribute a number of functionalities: pH change, buffering, sequestration of ions, and protein modification. The beneficial effects include an increase in water binding, which improves yield and reduces drip loss; emulsification of fats; solubilization of fibrous proteins, texture modification; flavor enhancement; color stabilization; and reduced rates of rancidity development. Increased pH and ionic strength modify protein properties to improve water-binding capacity. Applications in the meat, poultry, and seafood industries are summarized in Table 3.

Depending on the specific application, phosphates can be added to the muscle tissue in a number of ways. For most products, the phosphate is added in a pickling or curing solution by injection, vacuum tumbling, or through immersion of the tissue. It is important that the addition of the phosphate be followed by techniques to improve dispersion of the solution such as massaging or tumbling. This ensures uniformity and maximum yield and avoids localized pH effects, such as color change. U.S. Department of Agriculture (USDA) regulations do not allow phosphates to be added to fresh, uncooked meats or sausages. Phosphates are only permitted in frozen or processed meat products.

Dairy Industry Applications

In dairy processing, phosphates act as emulsifiers or melting salts accomplishing emulsification through protein modification. They help disperse ingredients in milk-based beverages and serve as acidifying and buffering agents in cheese production. Phosphates also prevent protein denaturation in dried and canned milks and coffee whiteners. In other applications, such as instant puddings or whey protein isolates, the ability of phosphates to promote the formation of protein complexes is used. By far the largest use of phosphates in the dairy industry is in the production of process cheese. In this application the phosphates modify proteins by sequestering calcium ions and adjusting pH. The modified proteins emulsify fats present in the cheese. As a result, the cheese is more uniform in flavor and exhibits controlled melt spread, and the fat does not separate when the cheese is melted. Some of the major applications for phosphates in the dairy industry are described in Table 4.

The Food and Drug Administration (FDA) standards of identity for process cheese, cheese food, and cheese spread allow phosphates to be added up to 3.0% by weight of the finished product. Phosphates permitted include disodium phosphate (anhydrous and dihydrate), trisodium phosphate (anhydrous and dodecahydrate), tetrasodium pyrophosphate, sodium hexametaphosphate, monosodium phosphate, and sodium acid pyrophosphate.

Miscellaneous Applications

Phosphoric acid and phosphates are useful as acidifying and buffering agents in beverages, preserves, and gelatin products. Powdered products may benefit from the flow-conditioning properties of tricalcium phosphate. Phosphates are used in a number of health-related applications where they contribute nutritional supplementation and fortification. Calcium and magnesium phosphates are used in infant foods for mineral supplementation. Calcium phosphates have also been used in several vitamin formulations. Both dicalcium phosphate (DCP) and tricalcium phosphate (TCP) have been used as excipients for tablets. In isotonic drinks, potassium orthophosphates are used to reestablish electrolyte balance. A summary of miscellaneous applications is listed in Table 5.

Table 2. Leavening Applications of Phosphates

Product	Typical $NaHCO_3$ level (%)	Leavening acid[a]
Baking powders	30–40	MCP, SAPP-28, SALP, DMP, MCPA
Biscuit doughs, frozen	1.5–2.0	SALP, SAPP-28, DMP
Biscuit and muffin mixes	1.5–2.0	SALP, DMP, MCPA, SAPP-28
Breading batter mixes	0.0–2 0	SAPP-40, SALP, MCP, DMP, SAPP-28, SAPP-RD
Cake mixes and bakery cakes	0.6–1.0	SALP, DMP, MCP, SAPP-28, DCPD, MCPA
Cookie mixes	0.0–2.0	SALP, MCPA, SAPP-28, SAPP RD
Doughnut mixes	0.5–1.0	SAPP-28, SAPP-40, SAPP-37, SALP, MCPA, SAPP-26, SAPP-43, MCP
Doughs, refrigerated	2.0–2.5[b]	SAPP RD, SAPP-26, SALP, DMP
Hush puppy mixes	1.5–2.0	SAPP-28, SAPP-40 MCPA, SALP
Pancake batters, frozen and refrigerated	1.7–2.2[b]	SALP, DMP, MCP, DCPD, SAPP-28
Pancake and waffle mix	1.5–2.0	SALP, DMP, MCP, MCPA, SAPP-28, SAPP-40
Phosphated flour		MCP, MCPA
Pizza mixes	0.3–1.2	SALP, DMP, MCP, SAPP-28, DCPD
Self-rising cornmeal	1.5–2.0	MCPA, SALP
Self-rising flour	1.2–1.5	SALP, MCPA, SAPP
Tortilla	0.2–1.0	SAPP, SALP, MCP

Source Ref 10.

[a] Refer to Table 1 for abbreviations, note that several grades of SAPP are produced as designated by the suffix -RD, -28, etc, which indicate different rates of CO_2 release.

[b] Percent of solids.

Table 3. Phosphates for Meat, Poultry, and Seafood

Food type	Phosphate[a]	Level (%)
Cooked sausages and other emulsion products	Sodium polyphosphate blends, TSPP	0.15–0.35
Ham	STP, sodium polyphosphate blends	0.4–0.5
Bacon	Sodium polyphosphate blends	0.4–0.5
Hamburger patties and ground beef patties (cooked or frozen)	Sodium polyphosphate blends, TSPP	0.25–0.4
Poultry, processed or frozen	STP, sodium polyphosphate blends	<0.5
Restructured poultry patties, rolls, nuggets	STP, sodium polyphosphate blends	0.15–0.35
Frozen shrimp	STP	<0.5
Frozen fish fillets	STP	<0.5
Surimi	STP, TSPP	0.1–0.5

Source Ref 10.

[a] refer to Table 1 for abbreviations

REGULATORY STATUS IN THE UNITED STATES AND OTHER COUNTRIES

The various food phosphates are classified as generally recognized as safe (GRAS) in the United States, with use limits set for foods not controlled by a Standard of Identity as that required for good manufacturing practice. Purity specifications have been defined (13). Regulations in all uses are defined by the FDA (14). Regulation of meats and poultry are administered by the USDA, which allows up to 0.5% of phosphate in all processed or frozen meats and poultry unless they are otherwise regulated (14). There are no regulated limits in seafood applications provided good manufacturing practices are followed. Process cheese products, phosphated flour, phosphated starch, and self-rising wheat and corn flours are foods with a Standard of Identity that defines and limits phosphate usage.

Many countries have regulations that are similar to those in the United States. There appears to be interest in standardized foods with regulatory limits for specific applications. Efforts are currently under way to harmonize the various national listings. This is being sponsored by the Food and Agricultural and World Health Organizations under UN sponsorship and is part of a broader program directed toward food phosphates in general. Similar efforts are under way in the European Economic Council.

TRENDS

The dynamics of the processed-food industry will continue to affect the need for food phosphates as consumers demand more convenient, nutritious, and healthy foods. Industry response to an aging population, more working parents, increased eating out, and other factors resulted in the introduction of 3400 new food products in the United States in 1988. Among these were many consumer products benefiting from the functionality of food phosphates, including frozen, microwaveable meat, poultry, and seafood entrees; dairy products and analogues; pet foods; instant desserts; and biscuits and other baked goods. Continued growth of fast foods and convenience foods should

Table 4. Phosphates for Dairy Applications

Application	Phosphate[a]	Level (%)
Evaporated or condensed milk or cream	DSP, DKP	0.02–0.10
Flavored milk powders	TSPP, TKPP	0.1–0.3
Nondairy coffee creamers	DKP, DSP	1.0–2.0 (dry) 0.1–1.0 (liquid)
Buttermilk	TSPP,TKPP, phosphoric acid	0.01–1.0
UHT concentrated milk	SHMP	0.1–1.0
Dried milk-drink products	DSP	2.0[b]
Sterile concentrated milk or cream	SHMP	0.1–1.0
Instant pudding or no-bake cheesecake	TSPP, DSP, DKP, MCP, SAPP, STP, TKPP	2–7
Milk foams	TSPP, TKPP, SHMP	1.5–2.0[b]
Imitation sour cream or chip dips	STP	0.05–0.2[c]
Spray-dried cheese	DSP, DKP, SHMP	1–3
Canned cream and cheese soups	DSP, DKP	0.2
Whipped toppings	TSPP, TKPP, DSP, DKP	0.025–1.0[d]
Process cheese	DSP or TSP blends with IMP, MSP, DSP, TSP, MKP, DKP, SAPP, TSPP, SHMP, TKPP	0.5–3.0
Cheese sauce	DSP, TSP, DKP, SHMP	0.5–3.0
Starter cultures	DSP, MAP, DAP, MKP, DKP	2–3
Frozen desserts and ice cream	DSP, TSPP, SHMP, DKP, TKPP	0.1–0.2
Direct-set cottage cheese	MCP, Phosphoric acid	0.03

Source: Ref. 10.

[a] Refer to Table 1 for abbreviations.

[b] Milk solids basis.

[c] Oil basis.

[d] Of liquid base.

Table 5. Miscellaneous Applications of Phosphates

Application	Phosphate[a]
Beverages	
Carbonated soft drinks	Phosphoric acid
Noncarbonated soft drinks	MCP
Fruit punch	TCP
Fruit juice	MKP
Produce	
Potatoes	SAPP, TSPP
Fruit	MCP, phosphoric acid, SHMP
Canned frozen vegetables	SHMP, TSPP, STP
Egg products (yolks, whole eggs, albumin, dried eggs)	STP, SHMP, MSP, MKP
Fats and oils	Phosphoric acid, TCP
Gelatin desserts	MSP, DSP
Salad dressings	Phosphoric acid
Jams and jellies	Phosphoric acid

[a] Refer to Table 1 for abbreviations.

result in growing demand for processed foods, many of which will benefit from the use of phosphates.

BIBLIOGRAPHY

1. U.S. Pat. 42,140 (March 29, 1864), E. N. Horford (to John H. Cheever).
2. F Kosikowski, *Cheese and Fermented Foods*, 2nd ed., Edwards Bros., Ann Arbor, Mich., 1977.
3. U.S. Pat. 1,186,524 (June 6, 1916), J. L. Kraft.
4. R. H. Ellinger, *Phosphates as Food Ingredients*, CRC Press, Boca Raton, Fla., 1972.
5. R. H. Ellinger, "Phosphates in Food Processing," in T. E. Furia, ed., *Handbook of Food Additives*, 2nd ed., R. C. Press, Cleveland, Ohio, 1972, pp. 617–780.
6. J. M. Deman and P. Melnychyn, eds., *Phosphates in Food Processing*, AVI, Westport, Conn., 1971.
7. M. Smart, "Industrial Phosphates," CEH Marketing Research Report, February, 1995, Chemical Economics Handbook-SRI International, Menlo Park, Calif.
8. J. R. Van Wazer, *Phosphorus and Its Compounds*, Vol. 1, Interscience, New York, 1958.
9. D. R. Gard, "Phosphoric Acid and Phosphates," in J. I. Kroschwitz and M. Howe-Grant, eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 18, 4th. ed., John Wiley & Sons, New York, 1996, pp. 669–718.
10. "Food Phosphates by Monsanto," Solutia Inc. Publication No. 7779226, St. Louis, Mo., 1994.
11. D. R. Gard, "Phosphoric Acid and Phosphates," in J. J. McKetta, ed., *Encyclopedia of Chemical Processing and Design*, Vol. 35, Marcel Dekker, New York, 1990, pp. 429–495.
12. J. F. Conn, "Chemical Leavening Systems in Flour Products," *Cereal Foods World* **26**, 119–123 (1981).
13. Committee on Food Chemicals Codex, *Food Chemicals Codex*, 4th ed., National Academy Press, Washington, D.C., 1996.
14. *Code of Federal Regulations*, Title 21, Part 182 and Title 9, Parts 318 and 381, Office of the Federal Register, National Archives, Washington, D.C., 1997.

BARBARA B. HEIDOLPH
DAVID R. GARD
Solutia, Inc.
St. Louis, Missouri

See also MEAT PRODUCTS.

PHOTOSYNTHESIS

Photosynthesis is a process by which radiant energy in the form of visible light is captured by photosynthetic organisms (including plants, algae, cyanobacteria, and bacteria) and converted to stable chemical energy. Photosynthetic organisms harvest and use light energy to make carbohydrate (sugars) by fixing carbon dioxide. Animals must obtain photosynthetically generated chemical energy by ingesting plants or by consuming other animals that feed on plants. Photosynthetic efficiency places a ceiling on the amount of energy that can be stored in our food reserves, and thus, the properties of photosynthesis are important to the scientist working in food technology and production. This review deals mostly with the fundamentals of the process of photosynthesis as they occur in higher plants. Detailed reviews on other aspects of photosynthesis have been published elsewhere (1–5).

The complete process of photosynthesis can be summarized by the reaction:

$$CO_2 + H_2O \rightarrow C(H_2O) + O_2$$

That is, light drives the conversion of carbon dioxide and water to carbohydrate and oxygen. Oxygenic photosynthetic organisms use H_2O as an electron donor to convert light energy into chemical energy. However, anoxygenic photosynthetic bacteria use inorganic (eg, H_2S) or organic (eg, succinate) hydrogen donors instead of H_2O. Without the energetic input of light, the conversion of CO_2 to $C(H_2O)$ is thermodynamically uphill by 114 kcal/mol. In plants and algae, photosynthesis is confined to a special organelle called the chloroplast (1). Photosynthesis can be divided into two distinct sets of reactions: the light reactions (light harvesting and electron transport) and the dark reactions (carbon reduction and biosynthesis).

STRUCTURE AND ORGANIZATION OF CHLOROPLASTS

Chloroplasts are cytoplasmic organelles that are enclosed by a double membrane or envelope, which gives them an ellipsoidal or oval shape of about 1 by 5 (1,6–8). They are structurally sound entities that can be isolated in functional form from many plants (9). The interior of the organelle consists of an extensive membrane network (thylakoids), and an aqueous phase (stroma). Thylakoid membranes are interconnected series of disk-shaped, membrane-limited vesicles that encase a lumenal region. The pigments and proteins required for light harvesting and photosynthetic electron transport are associated with the thylakoid membrane (10).

The dark reactions of photosynthesis occur in the chloroplast stroma (1,11). This soluble phase contains the metabolic pathways that utilize the chemical energy (ATP) and reducing power (NADPH) generated by light reactions. The most conspicuous stromal process is the reductive pentose phosphate (RPP) cycle (Calvin-Benson cycle, photosynthetic carbon reduction cycle), which converts carbon dioxide to carbohydrate (11,12). Other important stromal biosynthetic pathways include amino acid synthesis (13), reduction and metabolism of nitrate and ammonia (14), and reduction of sulfate (13).

Photosynthetic Membranes and Photosystems

In oxygenic plants, algae and cyanobacteria, photosynthesis is carried out with the help of pigment–protein complexes associated with specialized structures called thylakoid membranes. Thylakoid membranes in plants and algae are located in the chloroplast, whereas in cyanobacteria these membranes arise from the inner cell membrane into the cytoplasm. Thylakoids from plants and algae, but not cyanobacteria, have two morphologies: stacked membranes (granal lamellae) and nonstacked membranes (stromal lamellae) (1,6–8). The thylakoid membrane contains four distinct complexes (Fig. 1)—photosystem II (PSII), photosystem I (PSI), cytochrome b6/f, and the ATP synthase—that are each composed of several protein subunits, as well as chromophores and cofactors (eg, the chlorophylls, carotenoids, and hemes) (10,15,16). The complexes are spatially organized in the thylakoid membrane such that PSII is in the granal lamellae, and PSI and the ATP synthase are located only in the stromal lamellae. The cytochrome b6/f complex is distributed into both morphologies of thylakoids. Contrary to oxygenic photosynthetic organisms, the anoxygenic photosynthetic bacterial pigment–protein complexes are located either directly in the cell membrane or in the invaginations that arise from the membrane.

All oxygenic photosynthetic organisms, including plants, algae, and cyanobacteria, contain two photosystems, PS I and PS II (Fig. 2a). These photosystems are the sites for the photosynthetic electron transport and are embedded in the thylakoid membrane. Each photosystem is composed of pigments, proteins, and inorganic and organic cofactors that participate in the photosynthetic electron transport. Nonoxygenic photosynthetic bacteria contain only one photosystem (Figs. 2b and 2c), also composed of pigments, proteins, and other cofactors that participate in the electron transport.

Photosynthetic Pigments and Pigment-Protein Complexes

In oxygenic photosynthetic organisms, photosynthesis is initiated by visible radiation (400–700 nm) that is absorbed by pigments. The most abundant photosynthetic pigment is chlorophyll (Chl), a cyclic tetrapyrrole with a bound Mg^{+2} (Fig. 3a). Chl is utilized for both light collection and initiation of electron transport. In higher plants and green algae there are two forms of Chl, Chla and Chlb (17,18). They absorb in the blue (400–480 nm) and red (620–700 nm) spectral regions (Fig. 3b). Chla is involved in light capture and electron transport, while Chlb only has a light-harvesting role (15,19). Carotenoids, which absorb in the blue and green spectral regions, also serve as photosynthetic pigments in higher plants (19). Carotenoids, however, only play a modest role in light capture, because they are in relatively low concentration. Carotenoids also play a key role in protecting the chloroplast from excess light via the xanthophyll cycle (for more details see Refs. 20 and 21). All Chls and carotenoids used as pigments are bound to integral membrane proteins. The in-

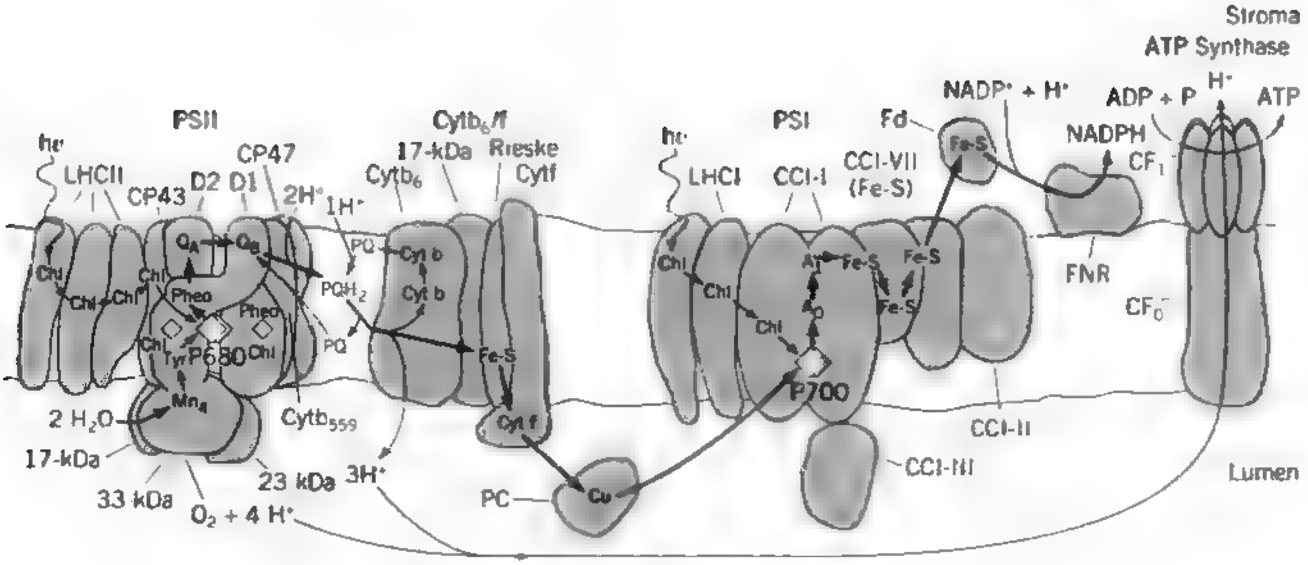


Figure 1. Organization of the four major thylakoid membrane complexes. PSII is constructed in two parts; the outer antenna (LHCII) and the core complex (the inner antenna [CP43 and CP47], cytochrome b559 and the D1/D2 reaction center). In PSII, electron flow is as indicated from water, through P680, to Q_B. The oxygen evolving Mn_4 cluster in the reaction center is stabilized by the 33-, 23- and 17-kDa polypeptides on the lumenal face of PSII. Although not depicted here, PSII is the stacked thylakoids. The cytochrome b_6/f complex is composed of four polypeptides. There are two electron pathways through this complex; one directly to PC and the other into the Q-cycle. PSI is also composed in two parts: the outer antenna (LHCI), and the core complex (CCI, which contains the inner antenna Chls, the reaction center Chls [P700], and electron acceptors). Electron flow is from PC to the Fd. CCI-III is a PC docking protein and CCI-II is an Fd docking protein. For both photosystems, light energy flows from the outer antenna, to the inner antenna, and finally to P680 or P700. The protons from water and PQH_2 oxidation drive ATP synthesis through the ATP synthase. Pigments and cofactors are as defined in Fig. 5 and the text.

teractions with protein widen absorbance bands of the pigments into the green spectral region (500–600 nm) (22).

Oxygenic photosynthetic cyanobacteria use Chla for light capture and initiation of electron transport. However, in place of Chlb-containing light-harvesting antennae, they possess special pigment–proteins, phycoerythrin, phycocyanin, and allophycocyanin, which serve a light-harvesting role. These pigment–proteins are organized into phycobilisomes and harvest red, blue, and green light (23). Contrary to the plant light-harvesting pigments that are embedded in the thylakoid membrane, the phycobilisomes are attached on the stromal surface of the thylakoid membrane.

All chlorophyll is bound exclusively to integral thylakoid membrane proteins (22,24). There are two classes of Chl-containing complexes: (*1*) antenna, which hold the vast majority of pigment (22,24,25) and (*2*) reaction centers, which have much less Chl but are the site of primary photochemistry initiated by special chlorophylls, P680 (PS II reaction center chlorophyll) and P700 (PS I reaction center chlorophyll) (16,26). Several antenna complexes combine with one reaction center to form a single photosystem.

The light-harvesting Chl-binding proteins of higher plants may be classified as either outer or inner antenna depending on their position relative to a reaction center (24–26). The outer antenna apoproteins, termed light-harvesting chlorophyll proteins (LHC) (Fig. 1), each bind a total of 7 to 10 Chla plus Chlb molecules. Those associated with PSII (LHCII) range in size from 24 to 29 kDa, while their PSI counterparts (LHCI) are generally smaller at 17 to 24 kDa (27,28). Each LHC polypeptide is proposed to contain three membrane spanning helices (29), and most LHCs share at least some primary amino acid sequence homology (30).

The inner antenna of PSII (part of the core complex [CC] of PSII [19,22,24,25]) is contained on two homologous polypeptides (CP43 and CP47, numbers refer to their molecular weight in kDa). These proteins are tightly bound to the PSII reaction center complex (Fig. 1). Each polypeptide binds approximately 25 Chla molecules (31). The inner antenna of PSI is composed of 40 to 50 Chla molecules, which are bound to the two reaction center apoproteins (CCI-subunit I) that contain P700 (25,32). The total antenna size (outer and inner antenna combined) of the respective photosystems is approximately 250 Chls per P680 and 150 Chls per P700 (15,25). The relative amount of excitation energy funneled to the two reaction centers from the antenna can be balanced by a process involving state I–state II transitions (1,33). Reversible binding of LHCII to PSII is associated with this phenomenon.

A photon of light is absorbed by an antenna Chl, and the energy is passed by resonance transfer from the outer antenna to the inner antenna and, finally, to P680 or P700 (Fig. 1). The movement of excitation energy is always

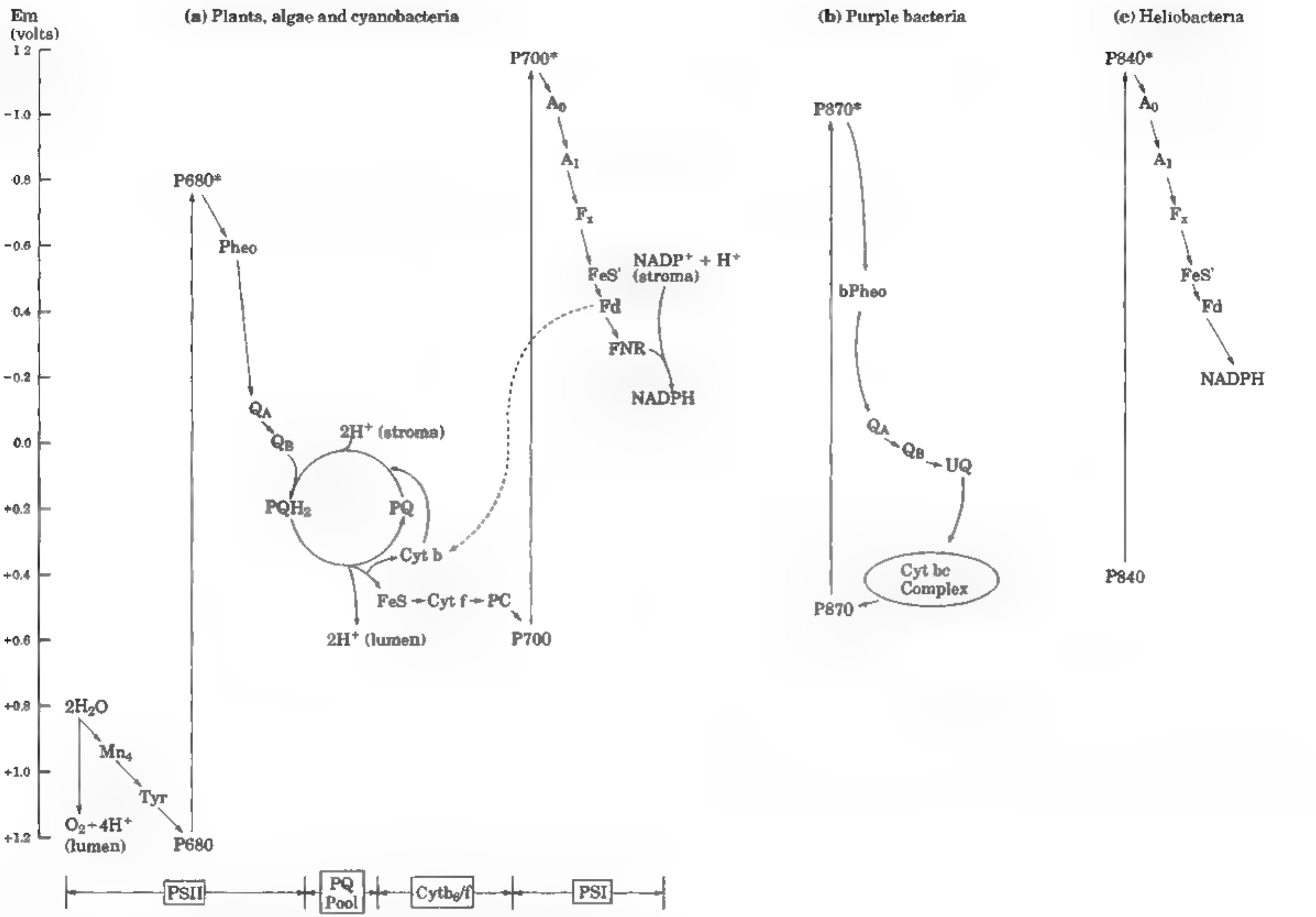

Em
(volts)
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
+0.2
+0.4
+0.6
+0.8
+1.0
+1.2
(a) Plants, algae and cyanobacteria
P680*
Pheo
Q_A
Q_B
$2H^+$ (stroma)
PQH_2
PQ
Cyt b
FeS → Cyt f → PC
$2H^+$ (lumen)
$2H_2O$
Mn_4
Tyr
$O_2 + 4H^+$
(lumen)
P680
P700*
A_0
A_1
F_x
FeS'
Fd
FNR
$NADP^+ + H^+$
(stroma)
NADPH
P700
PSII
PQ
Pool
$Cytb_6/f$
PSI
(b) Purple bacteria
P870*
bPheo
Q_A
Q_B
UQ
Cyt bc
Complex
P870
(c) Heliobacteria
P840*
A_0
A_1
F_x
FeS'
Fd
NADPH
P840

Figure 2. The schematic representation of photosynthetic electron transport. The ordinate represents the redox potential of the electron transport chain components. (**a**) Z-scheme of electron transport in plants, algae, and cyanobacteria. The electron flow from water to $NADP_+$ through two photosystems. The chlorophylls P680 and P700 are where light enters the system, raising the redox potential of the respective pigments. On the oxidizing side of PSII are water, the Mn_4 cluster and a tyr residue. On the reducing side of PSII are a pheophytin (Pheo) and two quinones (Q_A and Q_B). Mediating electron flow from PSII to PSI are the plastoquinone/plastoquinol (PQ/PQH_2) pool, the cytochrome b_6/f components and plastocyanin (PC). In the cytochrome complex, one electron goes directly to PC, while the other is cycled via cyt b back to the PQ pool (the Q-cycle). On the reducing side of PSI are a monomeric Chl (A_0), a phylloquinone (A_1), Fe-S centers and ferredoxin (Fd). Fd-$NADP^+$ reductase (FNR) catalyzes passage of electrons to $NADP^+$. Cyclic electron transport around PSI is connected by the dashed line. The protons consumed in the stroma and generated in the thylakoid lumen form the proton gradient used for ATP synthesis. (**b**) Photosynthetic electron transport in purple bacteria. There is only one photosystem involved in the electron transport which is analogous to PSII, because of the quinone type of electron carriers involved. (**c**) Photosynthetic electron transport in heliobacteria. Only one photosystem is involved in the electron transport that resembles the PSI, because of the iron-sulfur and ferrodoxins involved in the electron transport.

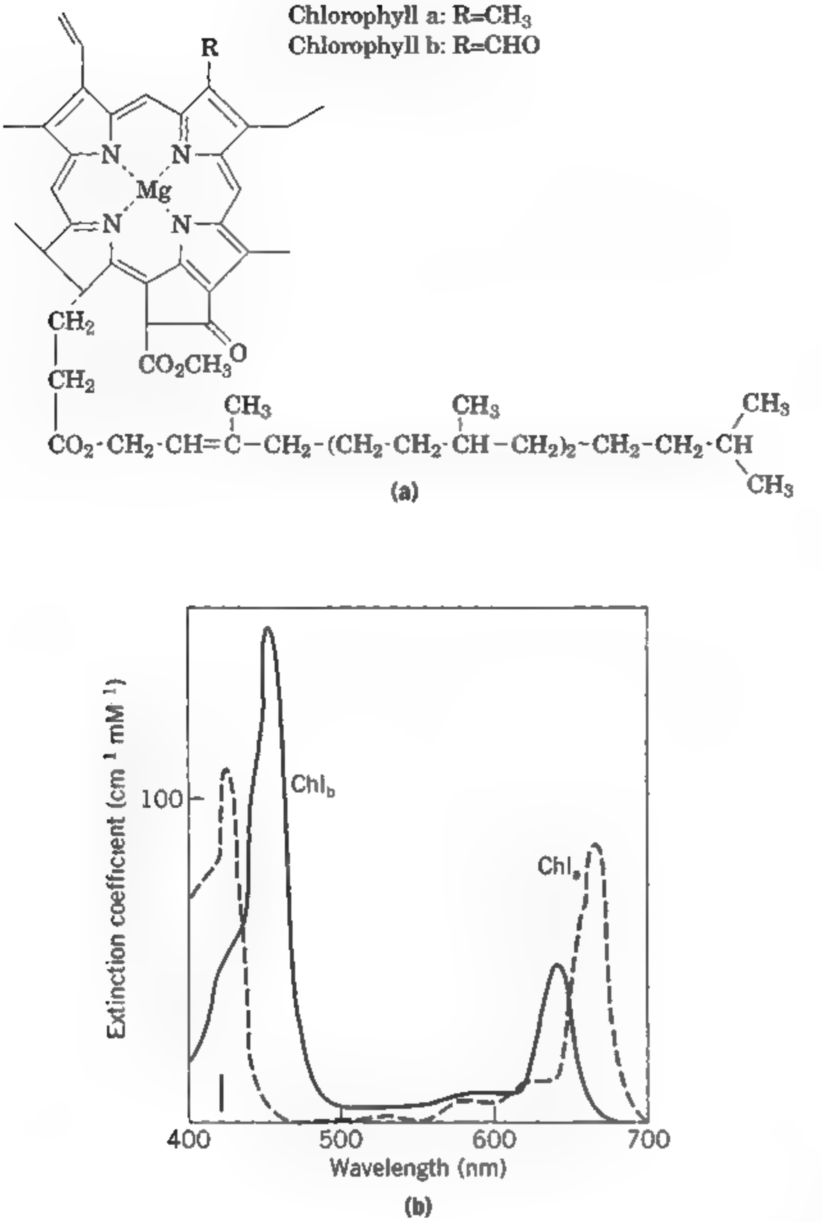


Figure 3. (**a**) Molecular model of chlorophyll a and b. (**b**) absorbance spectra of chlorophyll a and b.

downhill, progressing from pigments that absorb at shorter wavelengths (higher energy) to ones with longer wavelength absorbance bands (lower energy) (34). Photosynthetic electron transfer is initiated when the excitation energy reaches P680 or P700. The energy transfer from the light-harvesting complexes to the reaction centers is so efficient that more than 90% of the captured energy is passed on to the reaction center within a few hundred picoseconds.

The anoxygenic purple bacteria capture light with the help of bacteriochlorophyll (bChl) (22), which absorbs in the infrared (>700 nm), green (570 nm), and UVA (320–400 nm) spectral regions (17). These bacteria have different types of light-harvesting complexes and other mechanisms of regulating resonance transfer. However, their pigments still pass excitation energy down a gradient to the reaction center bChls. Like in plants, most anoxygenic bacteria contain two types of light-harvesting complexes: light-harvesting complex I (LH I) and light-harvesting complex II (LH II) (35).

Nonchlorophyll Thylakoid Complexes

The ATP synthase is the only major thylakoid membrane complex not directly involved in electron transport and does not contain chlorophyll (Fig. 1). It is a multisubunit protein complex consisting of two domains. CF0 is integral to the membrane and contains a proton channel. CF1 is extrinsic to the membrane and catalyzes ATP synthesis. ATP synthase utilizes the proton gradient across the thylakoid membrane during the light reaction to synthesize ATP (36). This chemiosmotic process of ATP synthesis in chloroplasts is very similar to the system mitochondria employ to generate ATP. This reaction in chloroplasts is called photophosphorylation and can be summarized as follows:

$$\mathrm{ADP} + \mathrm{Pi} \rightarrow \mathrm{ATP}$$

THE LIGHT-DRIVEN PHOTOSYNTHETIC REACTIONS

The individual components of the light reactions, which include light-harvesting pigments, electron carriers, and integral thylakoid membrane proteins, work efficiently together to convert light energy into ATP and NADPH. This whole process is basically carried over the two morphologies in the thylakoid membrane using PS II and PS I to drive the process.

Reaction Centers

Photosynthetic reaction centers are Chl-protein complexes that convert the energy of a photon into photochemical work. The key property of a reaction center is its ability to stabilize the photoinduced charge separation between the primary donor (a special Chl) and an electron acceptor. Our knowledge of photosynthetic reaction centers was revolutionized when Michel and Deisenhofer (37) solved the crystal structure of an anaerobic purple bacterial reaction center (research for which they won the 1988 Nobel Prize in chemistry). In this reaction center, the special Chls (P870) are a pair of bChla molecules (Fig. 4). They are bound to a heterodimer of two homologous proteins (L and M, 32 and 34 kDa, respectively), which also hold two bacteriopheophytins (bPheo), two accessory bChls, two quinones, and a nonheme iron. P870 is an energy trap because it absorbs at a longer wavelength (870 nm) than the antenna (800–850 nm). Upon excitation, P870 passes an electron to bPheo (the primary electron acceptor), forming bChl^+ and bPheo^-. The electron is quickly advanced to a secondary electron acceptor, Q_A, and finally to a third species, Q_B (Fig. 4). Thus, the reaction center bChls transfer one electron to Q_B with the input of one photon. The charge separation between bChl^+ and Q_B^- is kinetically stabilized by the reaction center. This allows time for reduction of $\mathrm{P870}^+$ by an electron donor (a cytochrome) back to P870. With the absorbance of a second photon, a second electron is transfered to Q_B^-, which then can diffuse out of the reaction center as a quinol (QH_2). Thus, Q_B is a constituent of the quinone pool that shuttles electrons from the reaction center to the cytochrome b-c complex (38).

The PSII of plants and purple bacterial reaction centers share several structural and functional properties (Fig. 4, see also Ref. 39). In PSII, the reaction center Chls (P680) are a special pair of Chla molecules (40,41), which absorb at a slightly longer wavelength than the antenna Chls. P680 is attached to a heterodimer of two homologous proteins (D1 and D2, 32 and 30 kDa, respectively), which also bind two pheophytins, two accessory Chls, Q_A, Q_B, and a nonheme iron (42). The D1 and D2 proteins and the L and M subunits are proposed to have similar structures in the photosynthetic membrane (Fig. 4, see also Refs. 39 and 43). D1 and D2 also share regions of important sequence homology with their bacterial counterparts (39,44). When P680 is excited, it reduces Pheo and the electron is rapidly transported across the membrane to Q_B via Q_A (Fig. 4). Concomitantly, $\mathrm{P680}^+$ is reduced by a tyrosine residue that is part of the D1 protein (45). The electron hole in tyr^+ is filled by the oxygen evolving complex (a Mn_4 cluster). Similar to the bacterial reaction center, Q_B is a two electron gate (38). After this quinone is reduced to QH_2 by a second electron, it dissociates from the reaction center, blends in with the PQ pool, and migrates to the cytochrome b_6/f complex (Fig. 1).

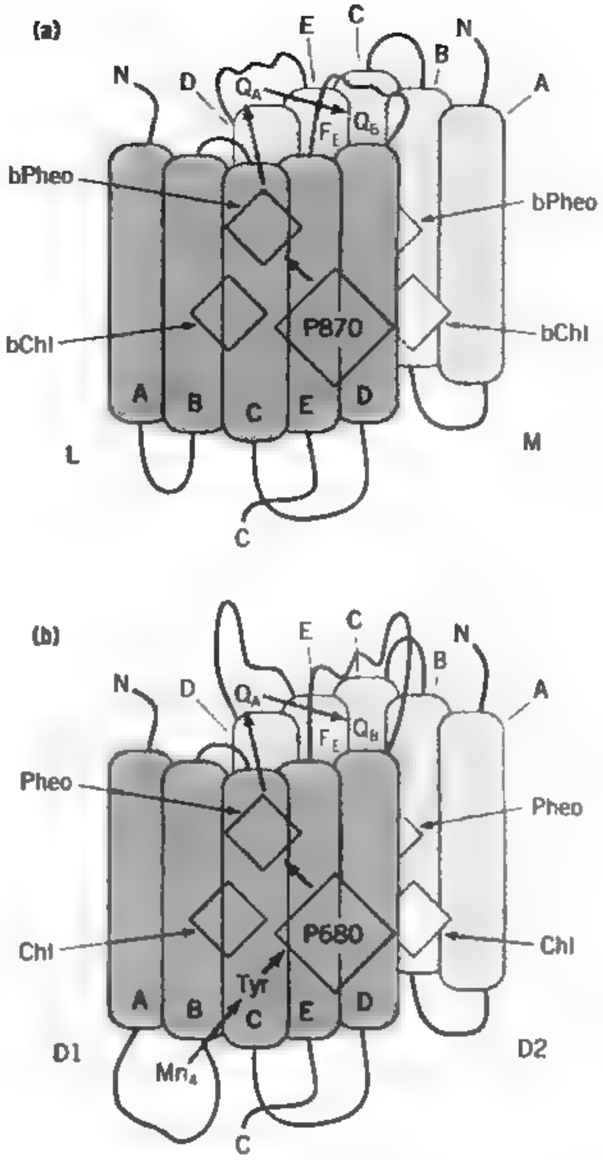


Figure 4. Structural comparison of the purple bacteria (a) and PSII (b) reaction centers. Each protein subunit (L, M, D1, and D2) has five membranes spanning helices labeled A through E. Both reaction centers contain the special pair Chls (P960 and P680), two Pheos, two accessory Chls, Q_A and Q_B, and a nonheme iron. The helices and cofactors are arranged with twofold rotational symmetry about an axis perpendicular to the membrane. The preferred pathway of electron transport through each complex is indicated.

A difference between the PSII and purple bacterial reaction centers is the rate of metabolism of the respective reaction center protein components (46). The D1 protein is subject to rapid, light dependent turnover, while all the proteins of the bacterial reaction center are kinetically stable. D1 degradation is one of the many effects associated with high light intensity damage to PSII (photoinhibition) (47). However, D1 turnover is also observed under low light intensities, including small amounts of UV radiation (48). Of environmental concern, PSII is a potential target of increased UV radiation from depletion of the ozone layer.

The PSI reaction center (Fig. 1) has a basic structure distinct from PSII (16,32,49). The reaction center Chls (P700) are a pair of Chla molecules that initiate primary photosynthesis (16,50). The primary electron acceptor (A_0) after P700 is a Chl (51), and the secondary acceptor (A_1) is a phylloquinone (52). These species and P700 are ligated to the heterodimer of 83 kDa apoproteins (CCI-subunit I) that also binds the inner antenna Chls (25,32). The electron passes from reduced phylloquinone through three Fe-S centers (one bound to CCI-subunit I and two bound to the 8 kDa CCI-subunit VII [53]) to ferredoxin (54). Ferredoxin in turn transfers the electron to $NADP^+$ (Figs. 1 and 2a).

Photosynthetic Electron Transport Chains

Electron transport begins in a photosystem. In oxygenic photosynthesis, two photosystems are used to oxidize water and reduce $NADP^+$ (26,55). Water oxidation is catalyzed by PSII, and reduction of $NADP^+$ is carried out by PSI (Fig. 2a). The formation of one oxygen molecule from two waters requires a four-photon input at PSII and pumps four electrons into the electron transport chain. Reduction of two $NADP^+$ molecules with these four electrons also depends on a four-photon input at PSI.

PSII, PSI, and the connecting electron carriers comprise the Z-scheme of photosynthesis (Fig. 2a). Electron transport is initiated when the energy of a photon excites a special Chl molecule, thereby increasing the reducing power of the pigment (34). In PSII, this species, P680, is probably a dimer of Chla molecules (40,41). P680 is excited to P680* by one photon. The latter transfers one electron to pheophytin (Pheo) which is a Chl without a bound Mg^{+2} (56). This results in $P680^+$ and $Pheo^-$. $P680^+$ is reduced by an electron from water. The electron on $Pheo^-$ is passed in series through two quinone (Q_A and Q_B) electron carriers (16,38). After Q_B is fully reduced, which requires two electrons (and thus two-photon events at P680), it leaves PSII as plastoquinol (PQH2). The electrons are transferred from PQH_2, in a semilinear fashion, through two cytochromes and a Fe-S center to plastocyanin (see the following and Fig. 2a). The two cytochrome proteins and the Fe-S protein collectively compose the cytochrome b_6/f complex (10). Plastocyanin in turn carries the electrons to PSI. PSI contains a special pair of Chla molecules (P700) (16,50) that harnesses the energy of a photon to elevate its redox potential. This drives an electron from the special Chls to A_0 (the primary acceptor) and subsequently to $NADP^+$.

Based on the Z-scheme, full-chain photosynthetic electron transport can be summarized by the reaction:

$$2H_2O + 2NADP^+ + 4ADP + 4Pi \xrightarrow{8\ \text{photons}} O_2 + 2NADPH + 4ATP$$

As the final component of the Z-scheme, NADPH is produced directly by electron transport. ATP, however, is generated by an ATP synthase that utilizes a proton gradient established across the thylakoid membrane by photosynthetic electron transport (Figs. 1 and 2a). This pH gradient is generated by oxidation of water on the lumenal side of the thylakoid and reduction of $NADP^+$ on the stromal face of the membrane. Additionally, protons are carried across the membrane by PQH_2.

Electrons can also be driven around a contained loop (the Q-cycle) (15,26) involving PSI, ferredoxin, plastoquinone (PQ), cytochrome b_6/f, and plastocyanin (cyclic electron transport, Fig. 1a). For this reason, electron transport through the cytochrome b_6/f complex is said to be semilinear (one electron goes linearly to plastocyanin and the other to the Q-cycle). ATP is generated by this process, as protons are carried across the thylakoid membrane by PQ. However, $NADP^+$ is not reduced, since there is not a net input of electrons.

Nonoxygenic photosynthetic bacteria have a single photosystem (26). For example, in the purple nonsulfur bacteria, electrons are passed around a cyclic pathway from a special pair of bChls (P870) through bacteriopheophytin (BPheo), two quinones (Q_A and Q_B), a membrane-bound cytochrome b-c complex, and back to P870 (Fig. 2b). The quinone carries protons across the photosynthetic membrane generating a pH gradient used for ATP synthesis. Although this electron transport chain appears to resemble PSI cyclic electron transport, the purple nonsulfur bacterial photosystem is more analogous to PSII. However, there are other nonoxygenic photosynthetic bacteria (green sulfur bacteria and heliobacteria) that can use sulfur compounds and organic hydrogen as electron donors. Their electron transport chain does not use BPheo and quinones, instead iron–sulfur centers and ferredoxin are used to reduce NAD^+ or $NADP^+$ (Fig. 2c). Thus, this electron transport chain resembles that of PSI.

Oxygen Evolution

Water is oxidized by a Mn_4 cluster bound to the D1–D2 reaction center proteins on the lumenal face of the thylakoid membrane (Figs. 1 and 2a, and Ref. 40). This oxygen-evolving complex is stably maintained on the PSII reaction center by three extrinsic polypeptides that bind to the lumenal face of PSII. After the tyr residue on D1 is oxidized by $P680^+$ the Mn cluster gives up one electron to reduce tyr^+ back to tyr. The oxygen-evolving complex has a four-electron capacity, effectively allowing it to reside in five different redox states (S-states), ranging from S_0 (fully reduced) to S_4 (fully oxidized) (Fig. 5). A photon is required to extract an electron during each S-state transition from S_0 to S_4. The S_4 state is spontaneously returned to the S_0 state in a radiationless transition by releasing one O_2 molecule and binding two water molecules. The four protons from the two oxidized water molecules remain in the lumen, contributing to the pH gradient across the thylakoid membrane.

THE DARK REACTIONS

The RPP Cycle

Fixation of CO_2 takes place with the ATP and reduced NADP generated during the photosynthetic electron trans-

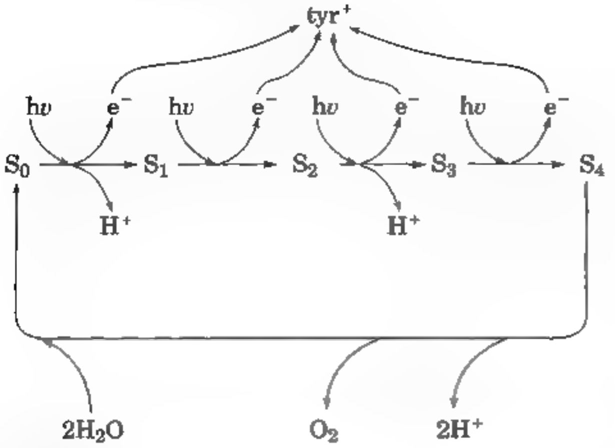


Figure 5. The S-state model for oxygen evolution in PSII. With the input of each photon, an electron is released and passed to tyr⁺ (see text). Protons are placed in the lumen of the thylakoid during three of the five steps. Oxygen is released and water taken up during the S_4 to S_0 transition.

port. The reactions occur in the stroma of the chloroplast. The overall reaction of CO_2 fixation can be summarized as follows:

$$
\begin{aligned}
&3CO_2 + 3RuBP + 9ATP + 6NADPH \\
&\quad + 9H_2O + 6H^+ \rightarrow C_3(H_2O) + 3RuBP \\
&\qquad + 9ADP + 9Pi + 6NADP^+
\end{aligned}
$$

The process is cyclic (the RPP cycle) (11,12), regenerating all the ribulose-bisphosphate (RuBP) consumed (Fig. 6). For each 3 CO_2s fixed, one extra C3 sugar is generated. ATP is utilized when 3-phosphoglycerate and ribulose-phosphate are phosphorylated. Diphosphoglycerate reduction to glyceraldehyde-3-phosphate is the only step in the RPP cycle involving NADPH.

Carboxylation of RuBP is carried out by RuBP carboxylase/oxygenase (Rubisco) (57), the most abundant enzyme in plants, if not the world. The enzyme is composed of eight large subunits (56 kDa) and eight small subunits (14 kDa). One catalytic domain is associated with each large subunit. Carboxylation of RuBP yields two C3 sugars (3-phosphoglycerate). Both 3-phosphoglycerates are reduced to glyceraldehyde-3-phosphate (GAP). GAP is necessary for the remaining metabolic reactions in the RPP cycle and those radiating from the cycle (11,12). Out of every six GAPs generated, five are converted back to RuBP (Fig. 6). The remaining GAP is either exported from the chloroplast, or it combines with another GAP and is stored as starch (58).

In addition to the carboxylation reaction, Rubisco can oxygenate RuBP (57). Although Rubisco has a lower affinity for O_2 than for CO_2, the O_2 concentration is high enough to make this oxygenation reaction significant. The products of RuBP oxygenation are 3-phosphoglycerate and phosphoglycolate. The process is called photorespiration because light drives the uptake of oxygen, and carbon dioxide is generated when phosphoglycolate is oxidized. A possible benefit of photorespiration is consumption of excess oxygen, which, if left unchecked, is potentially toxic (59). Also, it allows photosynthetic electron flow to continue at times when CO_2 is limiting.

Regulation of the RPP Cycle

Several key points in the RPP cycle are regulated in such a way that carbon fixation only proceeds in the light. For this reason, the "dark" reaction is a bit of a misnomer for the RPP cycle. Rubisco is activated in the light by Rubisco activase (60). For full activity, Rubisco must be stably carbamylated at a specific lys residue with a CO_2 that is distinct from the one used for carboxylation of RuBP. Rubisco activase probably plays a role in adding CO_2 to the lys. Rubisco is also inhibited by carboxyarabinitol 1-phosphate. This compound, which is produced in the dark, is a natural analogue of a carboxylation reaction intermediate. Activase probably catalyzes removal of carboxyarabinitol 1-phosphate from Rubisco in the light.

Four RPP cycle enzymes (glyceraldehyde-3-phosphate dehydrogenase, fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, and phosphoribulokinase, reactions 3, 6, 9, and 13 in Fig. 6) are activated when key disulfide bridges in the proteins are reduced by thioredoxin (58). Thioredoxin is a 12-kDa peptide with two redox active cysteines. In the light, ferredoxin usually reduces $NADP^+$ (Fig. 1). Occasionally, however, it passes redox equivalents to thioredoxin instead. Thus, this system detects light dependant electron transport in the thylakoid membrane.

C-4 Pathway of CO_2 Fixation

The C-4 plants, many of which are common to hot tropical climates, utilize a distinctive method of concentrating CO_2 in the chloroplast (11,61). These plants contain two cell types: bundle sheath cells surrounded by mesophyll cells. Carbon dioxide is added to phosphoenolpyruvate by phosphoenolpyruvate carboxylase in the cytoplasm of mesophyll cells. The product, oxaloacetate, is reduced to malate and subsequently is transported to the chloroplasts of bundle sheath cells. There it is decarboxylated and the liberated CO_2 is utilized by the RPP cycle. Pyruvate, which results from malate decarboxylation, moves back to the mesophyll cell, where it is phosphorylated, regenerating phosphoenolpyruvate. The C4-pathway allows efficient delivery of CO_2 to the RPP cycle, and, by keeping oxygen concentration in bundle sheath cells to a minimum, dramatically diminishes photorespiration.

CONCLUDING REMARKS

Photosynthesis is the process driven by the absorbance of light by chlorophyll and ending with the conversion of CO_2 into carbohydrate. Although there is a diversity among the photosynthetic organisms, there appears to be a great similarity in the process of photosynthetic electron transport and CO_2 fixation. Many reactions are employed to orchestrate the transfer of radiant energy to reduced carbon (chemical energy). The light reactions, which occur on thylakoid membranes (plants, algae, and cyanobacteria) and cell membranes (anoxygenic bacteria), begin with charge

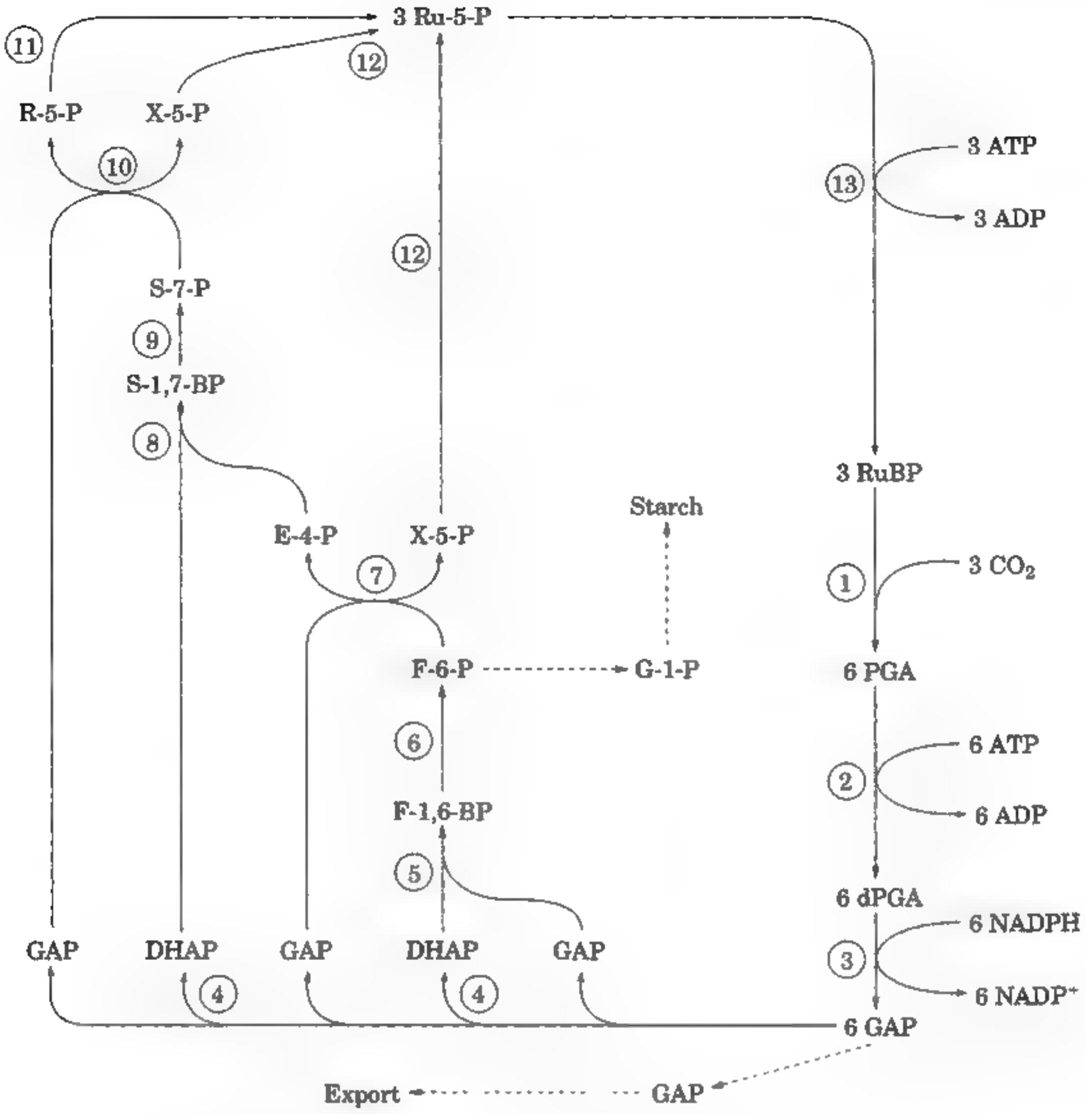


Figure 6. The reductive pentose phosphate cycle. The RPP cycle contains 13 reactions (see Robinson and Walker [Ref 12] for a description of the enzymes). The carboxylation and regeneration of three ribulose-1,5-bisphosphate (RuBP) molecules are shown. One extra C3 sugar is generated, which is either exported from the chloroplast or incorporated into starch. PGA: 3-phosphoglycerate; DPGA: 1,3-diphosphoglycerate; GAP: Glyceraldehyde-3-phosphate, DHAP: dihydroxyacetone phosphate; F-1,6-BP: fructose-1,6-bisphosphate, F-6-P: fructose-6-phosphate, X-5-P: xyulose-5-phosphate; E-4-P: erythrose-4-phosphate; S-1,7-BP: sedoheptulose-1,7-bisphosphate; S-7-P: sedoheptulose-7-phosphate; R-5-P: ribose-5-phosphate; G-1-P: glucose-1-phosphate.

separation in a reaction center and result in temporary storage of chemical energy as ATP and NADPH. This process alone requires the coordinated operation of pigment-protein complexes, as well as numerous photochemical and redox reactions. The ATP and NADPH are then used in a complex, highly regulated metabolic cycle that fixes CO_2. Thus, photosynthesis entails a long chain of reactions, with each step playing an equally important role in sustaining life on our planet.

BIBLIOGRAPHY

1. L. A. Staehelin, "Chloroplast Structure and Supramolecular Organization of Photosynthetic Membranes," in L. A. Staehelin and C. J. Arntzen, eds., *Encyclopedia of Plant Physiology*, New Series, Vol. 19, Springer-Verlag, Berlin, 1986, pp. 1–84.
2. M. D. Hatch and N. K. Boardman, eds., *The Biochemistry of Plants*, Vol. 10, Academic Press, San Diego, Calif., 1987
3. J. Amesz, ed., *Photosynthesis*, Elsevier Science, Amsterdam, The Netherlands, 1987.
4. R. P. F. Gregory, *Biochemistry of Photosynthesis*, Wiley, New York, 1989.
5. J. Amesz and A. J. Hoff, *Biophysical Techniques in Photosynthesis*, Kluwer Academic, Dordrecht, The Netherlands, 1995.
6. S. L. Wolfe, "Photosynthesis and the Chloroplast," in S. L. Wolfe, ed., *Introduction to Cell and Molecular Biology*, Brooks/Cole Wadsworth, Belmont, Calif., 1995, pp. 246–279

7. A. Dyson, "Andy Dyson's Home Page, A Level Biology, Module 1. Ultrastructure of a Eukaryotic Cell," URL: *http://www.biology.demon.co.uk/Biology/mod1/organelles* (last accessed August 30, 1999).
8. P. Scott, "Biols Home Page," URL: *http://www.biols.susx.ac.uk/Home/Peter_Scott/Index.html* (last accessed August 30, 1999).
9. M. Edelman, R. B. Hallick, and N. H. Chua, eds., *Methods in Chloroplast Molecular Biology*, Elsevier Biomedical Press, Amsterdam, The Netherlands, 1982.
10. J. M. Anderson, "Molecular Organization of Thylakoid Membranes," in J. Amesz, ed., *Photosynthesis*, Elsevier Science, Amsterdam, The Netherlands 1987, pp. 273–297.
11. F. D. MacDonald and B. B. Buchanan, "Carbon Dioxide Assimilation," in J. Amesz, ed., *Photosynthesis*, Elsevier Science, Amsterdam, The Netherlands, 1987, pp. 175–197.
12. S. P. Robinson and D. A. Walker, "Photosynthetic Carbon Reduction Cycle," in M. D. Hatch and N. K. Boardman, eds., *The Biochemistry of Plants*, Vol. 8., Academic Press, San Diego, Calif., 1981, pp. 193–236.
13. B. Halliwell, "Nitrogen and Sulfer Metabolism in Photosynthetic Tissues," in *Chloroplast Metabolism*, Clarendon Press, Oxford, England, 1981, pp. 227–244.
14. D. H. Turpin et al., "Interactions between Photosynthesis, Respiration, and Nitrogen Assimilation in Microalgae," *Can. J. Botany* **66**, 2083–2097 (1988).
15. D. R. Ort, "Energy Transduction in Oxygenic Photosynthesis," in L. A. Staehelin and C. J. Arntzen, eds., *Encyclopedia of Plant Physiology*, New Series, Vol. 19, Springer-Verlag, Berlin, 1986, pp. 143–196.
16. P. Mathis and A. W. Rutherford, "The Primary Reactions of Photosystems I and II of Algae and Higher Plants," in J. Amesz, ed., *Photosynthesis*, Elsevier Science, Amsterdam, The Netherlands, 1987, pp. 63–96.
17. J. C. Goedheer, "Visible Absorption and Fluorescence of Chlorophyll and Its Aggregates in Solution," in L. P. Vernon and G. R. Seely, eds., *The Chlorophylls*, Academic Press, New York, 1966, pp. 147–184.
18. W. Rudiger and S. Schoch, "Chlorophylls," in T. W. Goodwin, ed., *Plant Pigments*, Academic Press, San Diego, Calif, 1988, pp. 1–59.
19. R. Cogdell, "The Function of Pigments in Chloroplasts," in T. W. Goodwin, ed., *Plant Pigments*, Academic Press, San Diego, Calif., 1988, pp. 182–230.
20. B. Demmig-Adams, A. M. Gilmore, and W. W. Adams, "Carotenoids 3: *In vivo* Function of Carotenoids in Higher Plants," *FASEB J.* **10**, 403–412 (1996).
21. A. M. Gilmore, "Mechanistic Aspects of Xanthophyll-Cycle Dependent Protection in Higher Plant Chloroplasts and Leaves," *Physiol. Plant* **99**, 197–209 (1997).
22. H. Zuber, R. Brunisholz, and W. Sidler, "Structure and Function of Light-Harvesting Pigment-Protein Complexes," in J. Amesz, ed., *Photosynthesis*, Elsevier Science, Amsterdam The Netherlands 1987, pp. 233–271.
23. A. N. Glazer, "Phycobilisome: A Macromolecular Complex Optimized for Light Energy Transfer," *Biochim. Biophys. Acta* **768**, 29–51 (1984).
24. B. R. Green, "The Chlorophyll-Protein Complexes of Higher Plant Photosynthetic Membranes," *Photosynth. Res.* **15**, 3–32 (1988).
25. J. P. Thornber, "Biochemical Characterization and Structure of Pigment-Proteins of Photosynthetic Organism," in L. A. Staehelin and C. J. Arntzen, eds., *Encyclopedia of Plant Physiology*, New Series, Vol. 19, Springer-Verlag, Berlin, 1986, pp. 98–142.
26. N. K. Packham and J. Barber, "Structural and Functional Comparison of Anoxygenic and Oxygenic Organisms," in J. Barber, ed., *The Light Reactions*, Elsevier, Amsterdam, The Netherlands, 1987, pp. 1–30.
27. T. G. Dunahay and L. A. Staehelin, "Isolation and Characterization of a New Minor Chlorophyll a/b Protein Complex (CP24) from Spinach," *Plant Physiol.* **80**, 429–434 (1986).
28. A. Vainstein, C. C. Peterson, and J. P. Thornber, "Light-Harvesting Pigment-Proteins of Photosystem I in Maize," *J. Biol. Chem.* **264**, 4058–4063 (1989).
29. G. A. Karlin-Neumann et al., "A Chlorophyll a/b Protein Encoded by a Gene Containing an Intron with Characteristics of a Transposable Element," *J. Molecular and Applied Genetics* **3**, 45–61 (1985).
30. N. E. Hoffman et al., "A cDNA Clone Encoding a Photosystem I Protein with Homology to Photosystem II Chlorophyll a/b-Binding Polypeptides," *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8844–8848 (1987).
31. B. A. Diner and F.-A. Wollman, "Isolation of Highly Active Photosystem II Particles from a Mutant of Chlamydomonas reinhardtii," *Eur. J. Biochem.* **110**, 521–526 (1980).
32. R. Nechushtai et al., "Photosystem I Reaction Center from the Thermophilic Cyanobacterium Mastigocladus laminosus," *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1179–1183 (1983).
33. J. Bennett, K. E. Steinback, and C. J. Arntzen, "Chloroplast Phosphoproteins: Regulation of Excitation Energy Transfer by Phosphorylation of Thylakoid Membrane Polypeptides," *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5253–5257 (1980).
34. K. Sauer, "Photosynthetic Light Reactions: Physical Aspects," in L. A. Staehelin and C. J. Arntzen, eds., *Encyclopedia of Plant Physiology*, New Series, Vol. 19, Springer-Verlag, Berlin, 1986, pp. 85–97.
35. X. Hu, A. Damajanovic, and K. Schulten, "Architecture and Function of the Light Harvesting Apparatus of Purple Bacteria," *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5935–5941 (1998).
36. R. E. McCarty and C. M. Nalin, "Structure, Mechanism, and Regulation of the Chloroplast H+-ATPase (CF1-CF0)," in L. A. Staehelin and C. J. Arntzen, eds., *Encyclopedia of Plant Physiology*, New Series, Vol. 19, Springer-Verlag, Berlin, 1986, pp. 576–583.
37. J. Deisenhofer et al., "Structure of the Protein Subunits in the Photosynthetic Reaction Centre of *Rhodopseudomonas viridis* at 3 A Resolution" *Nature* **318**, 618–624 (1985).
38. P. R. Rich and D. A. Moss, "The Reactions of Quinones in Higher Plant Photosynthesis," in J. Barber, ed., *The Light Reactions*, Elsevier, Amsterdam, The Netherlands, 1987, pp. 421–445.
39. H. Michel and J. Deisenhofer, "Relevance of the Photosynthetic Reaction Center from Purple Bacteria to the Structure of Photosystem II," *Biochemistry* **27**, 1–7 (1988).
40. A. W. Rutherford, "Photosystem II, the Water-Splitting Enzyme," *Trends Biochem. Sci.* **44**, 227–232 (1989).
41. H. J. den Blanken et al., "High-Resolution Triplet-Minus-Singlet Absorbance Difference Spectrum of Photosystem II Particles," *FEBS Lett.* **157**, 21–27 (1983).
42. O. Nanba and K. Satoh, "Isolation of a Photosystem II Reaction Center Consisting of D-1 and D-2 Polypeptides and Cytochrome b-559," *Proc. Natl. Acad. Sci. U.S.A.* **84**, 109–112 (1987).
43. A. Trebst, "The Topology of the Plastoquinone and Herbicide Binding Peptides of Photosystem II in the Thylakoid Membrane," *Z. Naturforsch., C: Biosci.* **41**, 240–245 (1986).

44. D. C. Youvan et al., "Nucleotide and Deduced Polypeptide Sequences of the Photosynthetic Reaction-Center, B870 Antenna, and Flanking Polypeptides from *R. capsulata*," *Cell* **37**, 949–957 (1984)
45. R. J. Debus et al., "Site-Directed Mutagenesis Identifies a Tyrosine Radical Involved in the Photosynthetic Oxygen Evolving System," *Proc. Natl. Acad. Sci. U.S.A.* **85**, 427–430 (1987).
46. A. K. Mattoo, J. B. Marder, and M. Edelman, "Dynamics of the Photosystem II Reaction Center," *Cell* **56**, 241–246 (1989).
47. D. J. Kyle, I. Ohad, and C. J. Arntzen, "Membrane Protein Damage and Repair: Selective Loss of a Quinone-Protein Function in Chloroplast Membranes," *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4070–4074 (1984).
48. B. M. Greenberg et al., "Separate Photosensitizers Mediate Degradation of the 32-kDa Photosystem II Reaction Center Protein in the Visible and UV Spectral Regions," *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6617–6620 (1989).
49. B. D. Bruce and R. Malkin, "Subunit Stoichiometry of the Chloroplast Photosystem I Complex," *J. Biol. Chem.* **263**, 7302–7308 (1988).
50 K. D. Philipson, V. L. Sato, and K. Sauer, "Exciton Interaction in the Photosystem I Reaction Center from Spinach Chloroplasts: Absorption and Circular Dichroism Difference Spectra," *Biochemistry* **11**, 4591–4595 (1972).
51. M. R. Wasielewski, J. M. Fenton, and Govindjee, "The Rate of Formation of P700+-A0- in Photosystem I Particles from Spinach as Measured by Picosecond Transient Absorption Spectroscopy," *Photosynth. Res.* **12**, 181–190 (1987).
52. R. W. Mansfield, J. H. A. Nugent, and M. C. W. Evans, "ESR Characteristics of Photosystem I in Deuterium Oxide: Further Evidence That Electron Acceptor A1 Is a Quinone," *Biochim. Biophys. Acta* **894**, 515–523 (1987).
53. R. M. Wynn and R. Malkin, "Characterization of an Isolated Chloroplast Membrane Fe-S Protein and Its Identification as the Photosystem I Fe-SA/Fe-SB Binding Protein," *FEBS Lett.* **229**, 293–297 (1988).
54. W. Haehnel, "Plastocyanin," in L. A. Staehelin and C. J. Arntzen, eds., *Encyclopedia of Plant Physiology*, New Series, Vol. 19, Springer-Verlag, Berlin, 1986, pp. 547–559.
55. W. Haehnel, "Photosynthetic Electron Transport in Higher Plants," *Annual Rev. Plant Physiol.* **35**, 659–693 (1984).
56. V. V. Klimov et al., "Reduction of Pheophytin in the Primary Light Reaction of Photosystem II," *FEBS Lett.* **82**, 183–186 (1977).
57. T. J. Andrews and G. H. Lorimer, "Rubisco: Structure, Mechanisms, and Prospects for Improvement," in M. D. Hatch and N. K. Boardman, eds., *The Biochemistry of Plants*, Vol. 10, Academic Press, San Diego, Calif., 1987, pp. 131–218.
58. C. Cseke and B. B. Buchanan, "Regulation of the Formation and Utilization of Photosynthate in Leaves," *Biochim. Biophys. Acta* **853**, 43–63 (1986).
59. B. Halliwell, "Toxic Effects of Oxygen on Plant Tissues," in *Chloroplast Metabolism*, Clarendon Press, Oxford, England, 1981, pp. 179–205.
60. A. R. Portis, Jr., "Rubisco Activase," *Biochim. Biophys. Acta* **1015**, 15–28 (1990).
61. M. D. Hatch and C. R. Slack, "Photosynthetic CO2-Fixation Pathways," *Annual Rev. Plant Physiol.* **21**, 141–162 (1970).

T. SUDHAKAR BABU
BRUCE GREENBERG
University of Waterloo
Waterloo, Ontario
Canada

PHYTOCHEMICALS: ANTIOXIDANTS

The protective effects of fruit and vegetable consumption on diseases such as cancer and cardiovascular disease, among many other ailments, have been established for years. In recent history, the scientific community has been aggressively pursuing the basis of fruit and vegetable consumption and disease prevention to combat the prevalence of health disorders in today's society. Research studying the relationship between consumption of fruits and vegetables and cancer—and to a lesser extent cardiovascular disease—are the most abundant, because these conditions constitute the majority of health-related deaths in the industrialized world. Comprehensive reviews on the consumption of fruits and vegetables on the occurrence of cancer have resulted in 60 to 85% of the studies showing statistically significant associations in the decrease of cancer incidence (1). Individuals who consume the highest amount of fruits and vegetables have half the cancer rates as those who consume the least amount. A similar association has been seen with cardiovascular disease. Sixty percent of the studies reviewed by Ness and Powles (2) showed a statistically significant protective effect of fruit and vegetable consumption on coronary heart disease and stroke.

The consumption of an ample supply of fruits and vegetables (five to nine servings, as recommended by the U.S. Department of Agriculture Food Guide Pyramid) provides individuals with a wide variety of phytochemicals (both essential and nonessential nutrients from plants) that have been shown to have health benefits. A group of phytochemicals that have received significant attention for their health benefits are the antioxidants. These antioxidants include the nutritionally essential vitamins C (ascorbic acid), E (α-tocopherol), and β-carotene and also some nonessential compounds, such as the phenolics (most notably the flavonoids and isoflavones) and the carotenoids (Fig. 1). Because oxidative stress has been linked to many degenerative diseases, including cancer and cardiovascular disease, these antioxidants could potentially play a strong role in disease prevention. The fact that that only 9% of Americans consume at least five servings of fruits and vegetables per day is also substantial evidence that there is a large potential in preventing health problems through consumption of plant products (3).

Oxidative reactions that are harmful to health usually involve free radicals or reactive oxygen species that cause the oxidative modification of lipids, proteins, and nucleic acids (4). Free radicals can be produced as a by-product of normal metabolic reactions or from the decomposition of peroxides by transition metals or irradiation. In the case of lipid oxidation, the presence of free radicals that can oxidize unsaturated fatty acids will result in an autocatalytic reaction that involves the production of numerous free radical and lipid peroxide species. Reactive oxygen species are also produced as by-products of metabolic reactions and through the conversion of atmospheric oxygen to high-energy oxygen species by photosensitizers in the presence of light.

ANTIOXIDANT MECHANISMS OF PHYTOCHEMICALS

Phytochemical antioxidants can inhibit oxidative reactions by several different mechanisms. Both nutritionally non-

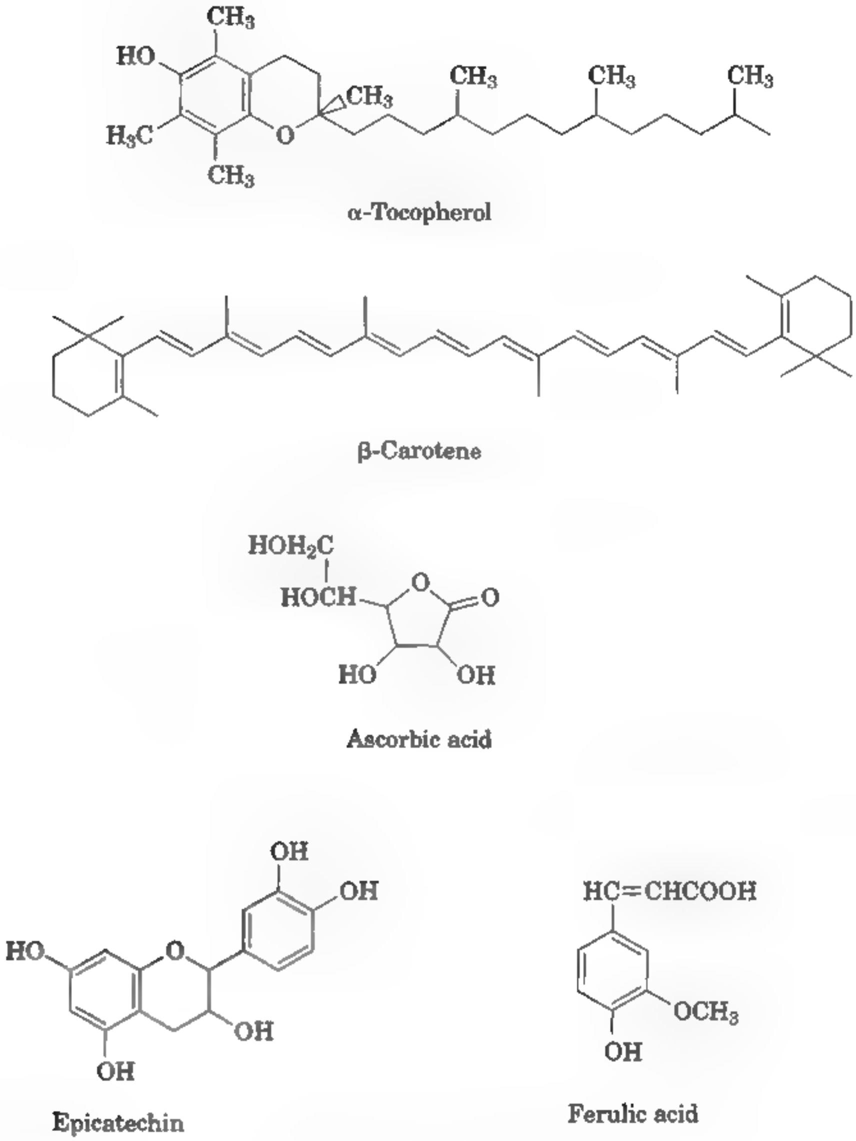


Figure 1. Structures of some common phytochemical antioxidants.

essential (eg, phenolic acids and isoflavones) and essential (α-tocopherol) phenolics inhibit oxidation by acting as chain-breaking antioxidants that are capable of inactivating free radicals and thus stopping the autocatalytic free-radical reaction (5). Phenolic compounds are efficient chain-breaking antioxidants because they are capable of scavenging free radicals, resulting in the formation of low-energy phenolic radicals that in turn do not have sufficient energy to further promote oxidative reactions. Ascorbic acid can also act as a chain-breaking antioxidant; however, it can also promote the reduction of transition metals (5). This metal-reducing activity will increase the pro-oxidant activity of metals because their reduced states rapidly promote the decomposition of peroxides into free-radical species.

Carotenoids can act as chain-breaking antioxidants when oxygen concentrations are low. However, in the presence of high oxygen concentrations, carotenoid radicals can actually increase oxidative reactions (6,7). Carotenoids are most effective at inhibiting oxidative reactions in the presence of systems that produce the reactive oxygen species, singlet oxygen. Singlet oxygen is produced from atmospheric oxygen when compounds known as photosensitizers (eg, chlorophyll, riboflavin, and myoglobin) are energized by light to an excited state. The excited state of the photosensitizer can then transfer its energy to oxygen to produce singlet oxygen that in turn oxidizes unsaturated fatty acids and amino acids (8). Carotenoids inhibit this reaction by physically absorbing energy from the excited photosensitizer or from singlet oxygen and then slowly returning the energy to the surrounding media through vibrational and rotational interactions. In order for a carotenoid to be able to inactivate singlet oxygen and activated photosensitizers through physical quenching, it must contain at least nine double bonds (9).

PHYTOCHEMICALS AND CANCER

Although there is abundant evidence implicating free radicals with cancer, the precise mechanism by which oxidative stress produces cancer is less clear. The free radicals (especially hydroxyl radicals), hydrogen peroxide, lipid peroxides, and singlet oxygen have been shown to cause DNA damage. The damage can occur through oxidative modification of the base and/or sugar moiety, single- and double-strand breaks, and DNA cross-linking (10). All this damage can be repaired through DNA repair enzymes, but problems may arise. Mutations following the incorrect repair or replication of DNA may lead to carcinogenesis. The body employs a series of enzymatic and structural antioxidant defenses along with exogenous dietary antioxidants to combat oxidative damage. However, these defenses are not perfect, and if the rate at which DNA becomes oxidized exceeds the rate at which it can be repaired, problems will arise. Over time, DNA damage becomes cumulative and is believed to account for the drastic increase in cancer rates seen with aging in humans and animals (3). Another possible mechanism may involve oxidative damage to critical proteins or enzymes associated with normal DNA repair. This damage may lead to erroneous repairs that may result in carcinogenic mutations (11). A third mechanism involves the enzymatic activation of compounds into carcinogens by free radicals, which is how many known procarcinogens are activated (10). These mechanisms are potential initiators of cancer and are believed to play a small role in the initiation stage of cancer. Free radicals are believed to play a larger role in the promotion of cancer through their ability to promote tumor development, stimulate oxygen radical production, and alter antioxidant defenses (10).

The ability of phytochemical antioxidants to act as free-radical scavengers *in vivo* demonstrates their potential as anticarcinogenic agents. By directly scavenging free radicals, oxidative stress in the body—and thus DNA damage—becomes diminished. Antioxidants have been shown to limit the chronic effects of smoking in animal models and reduce carcinogenic formation in chronic inflammation because smoking and inflammatory states both increase the exposure of tissues to free radicals (3). The protective effect of antioxidants is also related to their ability to decrease cellular division by limiting oxidants, which are known stimulants of cellular division (3). Rates of cellular division are strongly correlated to cancer rates because of increases in potential mutations with each replication of DNA. These mechanisms are the most important known effects of phytochemicals, but others that are either less understood or not as effective include bolstering enzymatic detoxification of carcinogens, directly hindering the formation of nitrosamines and other carcinogens, and maintaining normal DNA repair (1).

Despite the overwhelming protection seen in epidemiological studies relating fruit and vegetable consumption and cancer prevention, the results obtained by single or a combination of a few phytochemical antioxidants has been less encouraging. The next section presents an array of research, including epidemiological, clinical, animal, and *in vitro* studies in regard to phytochemical antioxidants and cancer. In-depth discussions of the research are impossible, and many limitations are inherent with each type of study. Animal studies often use extreme challenges on animals that do not relate to humans to obtain results. Epidemiological studies often ascertain nutrient values through questionnaires and dietary dairies that are difficult to interpret; therefore, it is often difficult to distinguish which phytochemicals are responsible for the observed effect. Intervention or clinical trials need to be very long and often run into problems of noncompliance, subjects are often at high risk (ie, smokers) for cancer development and may already be in the carcinogenic process before the study begins.

Carotenoids

Most of the epidemiological studies reviewed by Van Poppel and Goldbohm (12) that measure carotenoid intake from food and cancer risk have shown a strong protective effect, especially for lung, stomach, colorectal, and oral cancers (12). Intervention trials have not demonstrated such positive results. Recent studies observing lung and esophageal cancer have shown an increased risk or no effect with supplemental β-carotene. The α-Tocopherol, β-Carotene (ATBC) Cancer Prevention Study and the Carotene and Retinol Efficacy Trial (CARET) both demonstrated increases in lung cancer incidence and death in smokers among the β-carotene supplemented groups (13,14). A recent study that showed a protective effect was from Linxian, China, where esophageal and gastric cancers are prevalent (15). A combination of β-carotene, vitamin E, and selenium resulted in a 13% reduction in cancer deaths. A 6-year intervention trial in China, which was a follow up to the Linxian study, tested the ability of β-carotene to prevent esophageal and gastric cancers; it failed to show any reduction in cancer incidence (16). Studies on the recurrence of colorectal adenomas and skin cancer both failed to demonstrate any protection by β-carotene (17,18).

β-Carotene has been the predominant carotenoid studied, probably because of its availability in pill form, nontoxicity, and strong provitamin A activity, but other carotenoids have been shown to have stronger anticarcinogenic affects in animal models. In a study using chemical carcinogens as initiators and promoters of cancer, α-carotene was shown to be a more potent tumor suppressor in four locations (skin, lung, liver, and duodenum) compared to β-carotene (19). Palm carotene, which is a mixture of many carotenoids, was able to completely inhibit skin cancer development and was more effective in all sites, supporting evidence that a large mixture of phytochemicals are more potent in preventing cancer (19). Studies on high levels of carotenoids, such as lutein, lycopene, β-cryptoxanthin, and zeathin, in human blood have been linked to decreases in cancers of the prostate and breast (20,21), although other studies linking serum carotenoid levels with decreased risk of prostate and breast cancer have been less conclusive (22,23).

Ascorbic Acid

The ability of vitamin C to block carcinogenesis has been demonstrated in many animal models. Although its ability to promote or have no effect on cancer has also been demonstrated, overall vitamin C has shown anticarcinogenic activity. A study on female rats given chemical carcinogens resulted in the development of mammary tumors in the rats (24). When vitamin C only and vitamin C along with vitamin A, magnesium, and selenium were administered, tumorigenesis was reduced to 57.1% and 12%, respectively. A study by Pauling (25) that used hairless mutant mice irradiated with ultraviolet light resulted in cancer incidence being five times higher in rats not receiving vitamin C compared to rats receiving 10% vitamin C.

The epidemiological evidence supporting vitamin C has been promising for several types of cancer. From two comprehensive reviews of vitamin C and cancer incidence, the strongest association occurred with oral, esophageal, gastric, and pancreatic cancers (26,27). Of the 47 studies reviewed by Block (26) that reported vitamin C indexes or plasma ascorbate levels, 33 showed statistically significant protection, and none showed increased risk. Hormone-related cancers, such as breast, prostate, and ovarian cancers, have not been correlated to vitamin C intake. A study by Hunter et al. (28) of 89,494 woman concluded that large doses of vitamin C or E did not protect women from breast cancer. Intervention trials reviewed by Byers and Guerro (27) regarding supplemental vitamin C have been less encouraging, with most finding no or little protection. Clinical trials on the recurrence of cancer have been mixed. Survival times in cancer patients in Scotland receiving ascorbate supplements were twice as great as those of controls (29). In contrast, six intervention trials on the recurrence of colorectal polyps found no benefit from vitamin C supplementation (30).

Tocopherols

Animal studies evaluating the reduction of cancer with vitamin E (α-tocopherol) supplementation have been mildly supportive. A study using Wistar rats consuming diets with high vitamin E content found that rats developed fewer intestinal colorectal tumors induced by chemical carcinogens (31). Animal models testing skin and mammary glands have been inconclusive, but vitamin E has been shown to inhibit oral carcinomas (32). Observational epidemiological studies based on vitamin E consumed from the diet and intervention studies using supplemental vitamin E have been inconclusive (33). Colon cancer has received the most attention in regard to vitamin E and cancer prevention. There seems to be, at best, a weak beneficial effect with increased vitamin E intake. The Iowa Women's Health Study and the ATBC study, both large-scale trials with 35,000 and 29,000 participants, respectively, found vitamin E to be associated with a reduced risk of colon cancer (13,34). The Finnish Mobile Health Survey found a 1.5-fold increase in cancer incidence among individuals with the lowest serum α-tocopherol levels compared to the highest (33). Their findings supported a benefit only in the case of gastrointestinal cancers, with no effect seen with respiratory or hormone-related cancers. The Polyp Prevention Study Group, which assessed the benefit of vitamin E along with β-carotene in reducing colonic adenomas in 864 individuals, found no benefit from the supplementation (17).

Phenolics

Large-scale epidemiological or intervention studies regarding phenolics, as a group or individually, have yet to be performed. This may be due to the large diversity and abundance of phenolics found in our food supply or the limited availability of pharmaceutical sources of phenolics. Most of the research has been limited to *in vitro* and animal studies. The *in vitro* studies have proved the antioxidant effects of phenolics, and the animal studies have demonstrated their ability to block chemically induced carcinogenesis. Some examples of phenolics that have been shown to block carcinogenesis in animal models include quercetin, rutin, curcumin, caffeic acid, ellagic acid, genistein, and daidzein (35). Although phenolics have proved to be anticarcinogenic, studies have also demonstrated their ability to promote cancer. A study on rats that were fed high levels of caffeic acid, sesamol, and catechol resulted in the development of stomach cancer (36).

PHYTOCHEMICALS AND CARDIOVASCULAR DISEASE

Oxidative reactions have been linked to atherosclerosis through several different mechanisms. The most widely studied hypothesis of lipid oxidation and atherosclerosis involves the formation of oxidized, cytotoxic lipoproteins, particularly low-density lipoprotein (LDL) (37). During the oxidation of LDL, the lipoproteins become modified through either direct free-radical attack or formation of adducts between proteins and lipid oxidation products. The oxidized LDL can then be recognized and engulfed by macrophages, leading to the formation of foam cells that accumulate in arterial walls and form plaques (Fig. 2). Oxidized LDL has also been postulated to cause vascular inflammation and stimulate autoimmune reactions. Evidence supporting the relationship between LDL oxidation and cardiovascular disease is increasing; however, the importance of this mechanism to the development of cardiovascular disease has yet to be fully understood.

A second proposed mechanism for the link between oxidative reactions and atherosclerosis involves endothelium-derived relaxation factor (EDRF) (38). The endothelium, the cells that line the vascular lumenal surface, is a physiologically active organ that regulates vascular tone. The predominant regulator of vasodilation is EDRF, which

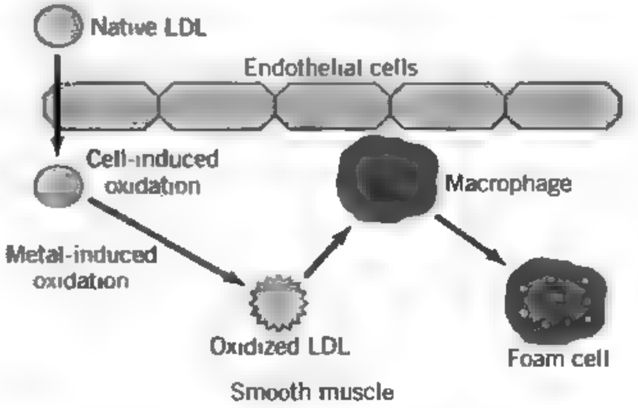


Figure 2. The proposed mechanism for promotion of coronary heart disease by oxidized LDL.

is thought to be identical to or similar to the free radical nitric oxide. Superoxide anion, another type of free radical, is believed to be vasoconstrictive owing to its ability to inactivate nitric oxide by converting it to peroxynitrite. Because peroxynitrite is a strong oxidizing agent, this can cause modification of proteins and DNA and thus damage endothelium function (Fig. 3). Therefore, the interaction of superoxide anion with nitric oxide leads to restriction in vascular dilation, which in turn can cause damage to the endothelium and lead to the development of atherosclerotic plaque formation. Theoretically, the introduction of antioxidants into the vasculature at suitable levels would negate the loss of dilation by inactivating superoxide anions and thus protect the vasodilatory activity of nitric oxide.

Carotenoids

Carotenoids, including β-carotene, are incorporated into LDL, but they only weakly inhibit LDL oxidation. Clinical and epidemiological studies have been largely inconclusive about the ability of carotenoids to inhibit atherosclerosis. These observations could be due to the weak antioxidant or even pro-oxidative activity of carotenoids (7). Recent research has suggested that foods high in carotenoids have been linked with decreased occurrence of atherosclerotic plaques, suggesting that carotenoids exert their protective effect later in the atherosclerotic process (39). However, this study was not able to specifically link dietary carotenoids with an antiatherogenic activity because the observed protective effects could be the result of other phytochemicals found in carotenoid-rich foods.

Ascorbic Acid

Vitamin C is an effective scavenger of superoxide anions and other free radicals, suggesting that it may protect endothelial function and inhibit the development of atherosclerosis. Ascorbate can inhibit the oxidation of LDL (40), and in studies on hypocholesterolemic individuals, ascorbic acid has been shown to improve vasodilation in subjects with chronic heart failure (41). However, epidemiological studies linking dietary vitamin C with prevention of atherosclerosis are inconclusive, and large-scale clinical trials with vitamin C alone have yet to be performed.

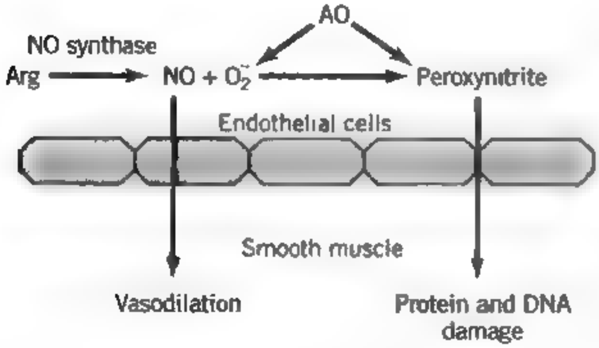


Figure 3. The proposed mechanism for the role of nitric oxide in the formation of coronary heart disease. Arg, arginine; AO, antioxidant; NO, nitric oxide; O_2^-, superoxide anion.

Tocopherols

Tocopherols, particularly vitamin E (α-tocopherol), have received much acclaim in the fight against atherosclerosis. Epidemiological evidence has shown a positive correlation between dietary α-tocopherol levels (>100 IU/day for 2 yr) and reduced incidence in cardiovascular disease (42,43). Clinical trials also support the idea that α-tocopherol is antiatherogenic, because 1000 IU of vitamin E for 2 yr decreased coronary lesions in men after bypass operations (44), and 400–800 IU vitamin E decreased heart attacks in 77% of men (45). The proposed mechanisms by which vitamin E protects against atherosclerosis includes its ability to inhibit LDL oxidation (46), maintain endothelial integrity (47), and inhibit monocyte adhesion to endothelial cells (48). In addition, vitamin E can protect the vasodilation activity of rat aortic strips (49).

Plant foods contain tocopherol isomers in addition to α-tocopherol, with γ-tocopherol being the most common. Tocopherol-binding protein is a liver protein responsible for tocopherol transport into very-low-density lipoprotein, which then carries tocopherol in the blood where it can be transported to peripheral tissues (50). The prevalence of α-tocopherol in most human tissues is due to the more than tenfold preference of tocopherol-binding protein for α-tocopherol compared to γ-tocopherol. Although γ-tocopherol inactivates most free radicals in a manner similar to α-tocopherol, it has been reported to have greater scavenging activity than α-tocopherol in the presence of peroxynitrite radicals and therefore has been suggested to be important in protecting endothelial function (51). This suggests that natural sources of tocopherols may be more beneficial to health because large doses of dietary α-tocopherol will result in very little γ-tocopherol being incorporated into tissue because of the large preference of tocopherol-binding protein for α-tocopherol.

Phenolics

Numerous plant phenolics, including those from tea, fruit, grains, and herbs, have been shown to inhibit the oxidation of LDL *in vitro* (52). The best studied of the plant phenolics are the catechins from tea, anthocyanins from grapes, and isoflavonoids from soybeans. Although many of these phenolics are absorbed from the diet into the blood, their retention time is normally very short (less than several hours). However, plasma metabolites of the plant phenolic quercetin have been shown to possess antioxidant activity, suggesting that the antioxidant effect of plant phenolics may be the result of a combination of the original compounds and their metabolites (53).

The ability of dietary catechins from tea to increase the antioxidant activity of blood plasma is unclear. Ingestion of green and black teas have been reported by Serafini et al. (54) and He and Kies (55) to increase the antioxidant activity of blood plasma, but McAnlis et al. (56) reported that black tea consumption did not affect plasma antioxidant capacity. Red grapes and red wine contain high amounts of anthocyanins that can inhibit both LDL oxidation (57) and platelet aggregation human subjects (58). The antiatherosclerotic benefits of grape anthocyanins have also been observed in clinical trials in which red wine was found to increase phenolics in blood and decrease lipid oxidation products in LDL (59) and in which grape juice consumption increased the antioxidant capacity of serum (60). Isoflavones can inhibit LDL oxidation and protect endothelial cells from the toxicity of oxidized LDL (61). Soy proteins containing isoflavones were found to decrease the occurrence of atherosclerotic lesions in monkeys (62).

CONCLUSION

Evidence continues to mount in support of the health benefits of phytochemicals. Unfortunately, the exact biochemical and physiological mechanisms for these health benefits are currently unclear. In fact, some of the phytochemicals, such as carotenoids and phenolics, have sometimes been shown to have pro-oxidative activity that could be harmful to health. In addition, very little is known about the absorption of the nonessential phytochemicals (eg, phenolics) through the gastrointestinal tract and their subsequent retention in tissues. Because of the lack of knowledge of the exact mechanisms and magnitude by which nutritionally nonessential antioxidants may affect health, consumption of large amounts of these compounds, in the form of either foods or supplements, may not be prudent until their bioactivity is better understood.

BIBLIOGRAPHY

1. K. A. Steinmetz and J. D. Potter, "Vegetables, fruit and cancer prevention: A Review," *J. Am. Dietetic Assoc.* **96**, 1027–1039 (1996).
2. A. R. Ness and J. W. Powles, "Fruit and Vegetables, and Cardiovascular Disease: A Review," *Int. J. Epidemiol.* **26**, 1–13 (1997).
3. B. N. Ames, M. K. Shigenaga, and T. M. Hagen, "Oxidants, antioxidants, and the degenerative diseases of aging," *Proc. Nat. Acad. Sci. U.S.A.* **90**, 7915–7922 (1993).
4. B. Halliwell and J. M. C. Gutteridge, "Role of Free Radicals and Catalytic Metal Ions in Human Disease: An Overview," *Methods Enzymol.* **186**, 1–88 (1990).
5. E. A. Decker, "Antioxidant Mechanisms," in C. C. Akoh and D. B. Min, eds., *Food Lipids, Chemistry, Nutrition, and Biotechnology*, Marcel Dekker, Inc., New York, 1998.
6. G. W. Burton and K. U. Ingold, "*β*-Carotene: An Unusual Type of Lipid Antioxidant," *Science* **235**, 1043–1046 (1987).
7. P. Palozza, "Prooxidant Actions of Carotenoids in Biologic Systems," *Nutr. Rev.* **56**, 257–265 (1998).
8. D. G. Bradley and D. B. Min "Singlet Oxygen Oxidation of Foods," *Crit. Rev. Food Sci. Nutr.* **31**, 211–236 (1992).
9. P. DiMascio, P. S. Kaiser, and H. Sies, "Lycopene as the Most Efficient Biological Carotenoid Singlet Oxygen Quencher," *Arch. Biochem. Biophys.* **274**, 532–538 (1989).
10. Y. Sun, "Free Radicals, Antioxidant Enzymes, and Carcinogenesis," *Free Radicals Biol. Med.* **8**, 583–599 (1990).
11. K. Frenler, "Oxidation of DNA Bases by Tumor Promoter Activated Processes," *Environ. Health Perspect.* **81**, 45–54 (1989).
12. G. Van Poppel and R. A. Goldbohm, "Epidemiological Evidence for Beta-Carotene and Cancer Prevention," *J. Am. Dietetic Assoc.* **62** (Suppl), 1393–1402 (1995).
13. The *α*-Tocopherol, *β*-Carotene Cancer Prevention Study Group, "The Effect of Vitamin E and Beta-Carotene on the Incidence of Lung Cancer and Other Cancers in Male Smokers," *N. Engl. J. Med.* **330**, 1029–1035 (1994).
14. G. S. Omenn et al., "The Beta-Carotene and Retinol Efficiency Trial (CARET) for Chemoprevention of Lung Cancer in High Risk Populations: Smokers and Asbestos Exposed Workers," *Cancer Res.* **54** (Suppl), 2038s–2043s (1994).
15. W. J. Blot et al., "Nutrition Intervention Trials in Linxian, China: Supplementation with Specific Vitamin/Mineral Combinations, Cancer Incidence, and Disease Specific Mortality in the General Population," *J. Nat. Cancer Inst.* **85**, 1483–1491 (1993).
16. J. Y. Li et al., "Nutrition Intervention Trials in Linxian, China: Multiple Vitamin/Mineral Supplementation, Cancer Incidence, and Disease Specific Mortality Among Adults with Esophageal Dysplasia," *J. Nat. Cancer Inst.* **85**, 1492–1498 (1993).
17. E. R. Greenberg et al. and the Polyp Prevention Study Group, "A Clinical Trial of Antioxidant Vitamins to Prevent Colorectal Adenoma," *N. Engl. J. Med.* **331**, 141–147 (1994).
18. E. R. Greenberg et al. and the Skin Cancer Prevention Group, "A Clinical Trial of *β*-Carotene to Prevent Basal Cell and Squamous Cell Cancer of the Skin," *N. Engl. J. Med.* **323**, 789–795 (1990).
19. H. Nishino, "Cancer Chemoprevention by Natural Carotenoids and Their Related Compounds," *J. Cell. Biochem.* **22** (Suppl), 231–235 (1995).
20. J. F. Dorgan et al., "Relationship of Serum Carotenoids, Retinol, *α*-Tocopherol, and Selenium with Breast Cancer Risk: Results from a Prospective Study in Columbia, Missouri (United States)," *Cancer Cases Control* **9**, 89–97 (1998).
21. E. Giovannucci et al., "Intake of Carotenoids and Retinol in Relation to Risk of Prostate Cancer," *J. Natl. Cancer Inst.* **87**, 1767–1776 (1995).
22. A. M. Nomura et al., "Serum Micronutrients and Prostate Cancer in Japanese Americans in Hawaii," *Cancer Epidemiol. Biomarkers Prev.* **6**, 487–491 (1997).
23. R. Jarvinen et al., "Diet and Breast Cancer Risk in a Cohort of Finnish Women," *Cancer Lett.* **114**, 251–253 (1997).
24. A. Ramesha et al., "Chemoprevention of 7,12-Dimethylbenz[a] Anthracene-Induced Mammary Carcinogenesis in Rat by the Combined Action of Selenium, Magnesium, Ascorbic Acid, and Retinyl Acetate," *Jpn. J. Cancer Res.* **81**, 1239–1246 (1990)
25. L. Pauling, "Effect of Ascorbic Acid on Incidence of Spontaneous Mammary Tumors and UV Light-Induced Skin Tumors in Mice," *Am. J. Clin. Nutr.* **54** (Suppl), 1252–1255 (1991).
26. G. Block, "Epidemiological Evidence Regarding Vitamin C and Cancer," *Am. J. Clin. Nutr.* **54** (Suppl), 1310s–1314s (1991).
27. T. Byers and N. Guerro "Epidemiological Evidence for Vitamin C and Vitamin E in Cancer Prevention," *Am. J. Clin. Nutr.* **62** (Suppl), 1385s–1392s (1995).
28. D. J. Hunter et al., "A Prospective Study of the Intake of Vitamins C, E, and A and the Risk of Breast Cancer," *N. Engl. J. Med.* **329**, 234–240 (1993).
29. E. Cameron and A. Campbell, "Innovation vs quality control: An 'unpublishable' clinical trial of supplemental ascorbate in incurable cancer," *Med. Hypotheses* **36**, 185–189 (1991)
30. S. N. Gershoft "Vitamin C (Ascorbic Acid): New Roles, New Requirements?" *Nutr. Rev.* **51**, 313–326 (1993).

31. M. G. Cook and P. McNamara "Effect of Dietary Vitamin E on Dimethylhydrazine-Induced Colonic Tumors in mice," *Cancer Res.* **40**, 1329–1331 (1980).
32. B. Toth and K. Patilk, "Enhancing Effect of Vitamin E on Murine Intestinal Tumorigenesis by 1,2-Dimethlylhydrazine Dihydrochloride," *J. Natl. Cancer Inst.* **70**, 531–536 (1983).
33. P. Knekt et al., "Vitamin E and Cancer Prevention," *Am. J. Clin. Nutr.* **53** (Suppl), 283s–286s (1991).
34. R. M. Bostizk et al., "Reduced Risk of Colon Cancer with High Intake of Vitamin E: The Iowa Women's Health Study," *Cancer Res.* **53**, 4230–4237 (1993).
35. H. L. Newmark "Plant Phenolics as Potential Cancer Prevention Agents," *Advances in Experimental Med. Biol.* **401**, 25–34 (1996).
36. M. Hirose et al., "Stomach Carcinogenicity of Caffeic Acid, Sesamol and Catechol in Rats and Mice," *Jpn. J. Cancer Res.* **81**, 207–212 (1990).
37. H. Esterbauer et al., "The Role of Lipid Peroxidation and Antioxidants in Oxidative Modification of LDL," *Free Radicals Biol. Med.* **13**, 341–390 (1992).
38. J. L. Mehta "Endothelium, Coronary Vasodilation, and Organic Nitrates, *Am. Heart J.* **129**, 382–391 (1995).
39. S. B. Kritchevsky et al., "Provitamin A, Carotenoid Intake and Carotid Artery Plaques: The Atherosclerosis Risk in Communities Study," *Am. J. Clin. Nutr.* **68**, 726–733 (1998).
40. A. Martin and B. Frei, "Both Intracellular and Extracellular Vitamin C Inhibits Atherogenic Modification of LDL by Human Vascular Endothelial Cells," *Arterioscler. Thromb. Vasc. Biol.* **17**, 1583–1590 (1997).
41. B. Hornig et al., "Vitamin C Improves Endothelial Function of Conduit Arteries in Patients with Chronic Heart Failure," *Circulation* **97**, 363–368 (1998).
42. M. J. Stampfer et al., "Vitamin E Consumption and the Risk to Coronary Disease in Women," *N. Engl. J. Med.* **328**, 1444–1449 (1993)
43. E. B. Rimm et al., "Vitamin E Consumption and the Risk of Coronary Heart Disease in Men," *N. Engl. J. Med.* **328**, 1450–1456 (1993).
44. H. N. Hodis et al., "Serial Coronary Angiographic Evidence That Antioxidant Vitamin Intake Reduces Progression of Coronary Artery Atherosclerosis," *JAMA* **273**, 1849–1854 (1995).
45. N. G. Stephens et al., "Randomized Controlled Trial of Vitamin E in Patients with Coronary Disease: Cambridge Heart Antioxidant Study," *Lancet* **23**, 781–786 (1996).
46. I. Jialal, "Evolving Lipoprotein Risk Factors: Lipoprotein (a) and Oxidized Low-Density Lipoprotein," *Clin. Chem.* **44**, 1827–1832 (1998).
47. B. Hennig, M. Toborek, and A. A. Cader, "Nutrition, Endothelial Cell Metabolism, and Atherosclerosis," *Crit. Rev. Food Sci. Nutr.* **34**, 253–282 (1994).
48. A. Martin et al., "Vitamin E Inhibits Low-Density Lipoprotein-Induced Adhesion of Monocytes to Human Aortic Endothelial Cells In Vitro," *Arterioscler. Thromb. Vasc. Biol.* **17**, 429–436 (1997).
49. C. Guarnieri et al., "α-Tocopherol Pretreatment Improves Endothelium-Dependent Vasodilation in Aortic Strips of Young and Aging Rats Exposed to Oxidative Stress," *Mol. Cell. Biochem.* **157**, 223–228 (1996).
50. M. G. Traber, "Determinants in Plasma Vitamin E Concentrations," *Free Radicals Biol. Med.* **16**, 229–239 (1994).
51. G. Wolf and D. Phil, "γ-Tocopherol: An Efficient Protector of Lipids Against Nitric Oxide-Initiated Peroxidative Damage," *Nutr. Rev.* **55**, 376–378 (1997).
52. J. A. Vinson et al., "Plant Flavonoids, Especially Tea Flavonals, Are Powerful Antioxidants Using an *In Vitro* Oxidation Model for Heart Disease," *J. Agric. Food Chem.* **43**, 2800–2802 (1995).
53. C. Morand et al., "Plasma Metabolites of Quercetin and Their Antioxidant Properties," *Am. J. Physiol.* **275**, R212–R219 (1998).
54. M. Serafini, A. Ghiselli, and A. Ferro-Luzzi, "Red Wine, Tea, and Antioxidants," *Eur. J. Clin. Nutr.* **50**, 28–32 (1996).
55. Y. H. He and C. Kies, "Green and Black Tea Consumption by Humans: Impact on Polyphenol Concentrations in Feces, Blood and Urine,"*Plant Foods Hum. Nutr.* **46**, 221–229 (1994).
56. G. T. McAnlis et al., "Black Tea Consumption Does Not Protect Low Density Lipoprotein from Oxidative Modification," *Eur. J. Clin. Nutr.* **52**, 202–206 (1998).
57. E. N. Frankel et al., "Inhibition of Oxidation of Human Low-Density Lipoprotein by Phenolic Substances in Red Wine," *Lancet* **341**, 454–457 (1993).
58. C. R. Pace-Asciak et al., "Wines and Grape Juices as Modulators of Platelet Aggregation in Healthy Human Subjects," *Clin. Chim. Acta* **246**, 163–182 (1996).
59. S. V. Nigdikar et al., "Consumption of Red Wine Polyphenols Reduces the Susceptibility of Low-Density Lipoproteins to Oxidation In Vivo," *Am. Soc. Clin. Nutr.* **68**, 258–265 (1998).
60. A. P. Day et al., "Effect of Concentrated Red Grape Juice Consumption on Serum Antioxidant Capacity and Low-Density Lipoprotein Oxidation," *Ann. Nutr. Metab.* **41**, 353–357 (1997).
61. S. Kapiotis et al., "Genistein, the Dietary-Derived Angiogenesis Inhibitor, Prevents LDL Oxidation and Protects Endothelial Cells from Damage by Atherogenic LDL," **17**, 2868–2874 (1997).
62. M. S. Anthony et al., "Soybean Isoflavones Improve Cardiovascular Risk Factors Without Affecting the Reproductive System of Peripubertal Rhesus Monkeys," *J. Nutr.* **126**, 43–50 (1996).

ROSS TOMAINO
ERIC A. DECKER
University of Massachusetts
Amherst, Massachusetts

See also ANTIOXIDANTS.

PHYTOCHEMICALS: BIOTECHNOLOGY OF PHENOLIC PHYTOCHEMICALS FOR FOOD PRESERVATIVES AND FUNCTIONAL FOOD APPLICATIONS

Phytochemicals from herbs and fermented legumes are excellent dietary sources of phenolic metabolites. These phenolics are important for food preservation and increasingly for therapeutic and pharmaceutical applications. The functional food-related objectives of the food biotechnology program at the University of Massachusetts are to elucidate the molecular and physiological mechanisms associated with the synthesis of important health-related, therapeutic phenolic metabolites in food-related plants and fermented plant foods. Current efforts focus on the elucidation of the role of the proline-linked pentose phosphate pathway in regulating the synthesis of the anti-

31. M G. Cook and P. McNamara "Effect of Dietary Vitamin E on Dimethylhydrazine-Induced Colonic Tumors in mice," *Cancer Res.* **40**, 1329–1331 (1980).
32. B Toth and K. Patilk, "Enhancing Effect of Vitamin E on Murine Intestinal Tumorigenesis by 1,2-Dimethlylhydrazine Dihydrochloride," *J. Natl. Cancer Inst.* **70**, 531–536 (1983).
33. P. Knekt et al., "Vitamin E and Cancer Prevention," *Am. J. Clin. Nutr.* **53** (Suppl), 283s–286s (1991).
34. R. M. Bostizk et al., "Reduced Risk of Colon Cancer with High Intake of Vitamin E: The Iowa Women's Health Study," *Cancer Res.* **53**, 4230–4237 (1993).
35. H. L. Newmark "Plant Phenolics as Potential Cancer Prevention Agents," *Advances in Experimental Med. Biol.* **401**, 25–34 (1996).
36. M Hirose et al., "Stomach Carcinogenicity of Caffeic Acid, Sesamol and Catechol in Rats and Mice," *Jpn. J. Cancer Res.* **81**, 207–212 (1990).
37. H. Esterbauer et al., "The Role of Lipid Peroxidation and Antioxidants in Oxidative Modification of LDL," *Free Radicals Biol. Med.* **13**, 341–390 (1992).
38. J. L. Mehta "Endothelium, Coronary Vasodilation, and Organic Nitrates, *Am. Heart J.* **129**, 382–391 (1995).
39. S. B. Kritchevsky et al., "Provitamin A, Carotenoid Intake and Carotid Artery Plaques: The Atherosclerosis Risk in Communities Study," *Am. J. Clin. Nutr.* **68**, 726–733 (1998).
40. A. Martin and B. Frei, "Both Intracellular and Extracellular Vitamin C Inhibits Atherogenic Modification of LDL by Human Vascular Endothelial Cells," *Arterioscler. Thromb. Vasc. Biol.* **17**, 1583–1590 (1997).
41. B. Hornig et al., "Vitamin C Improves Endothelial Function of Conduit Arteries in Patients with Chronic Heart Failure," *Circulation* **97**, 363–368 (1998).
42. M. J. Stampfer et al., "Vitamin E Consumption and the Risk to Coronary Disease in Women," *N. Engl. J. Med.* **328**, 1444–1449 (1993)
43. E. B. Rimm et al., "Vitamin E Consumption and the Risk of Coronary Heart Disease in Men," *N. Engl. J. Med.* **328**, 1450–1456 (1993).
44. H. N. Hodis et al., "Serial Coronary Angiographic Evidence That Antioxidant Vitamin Intake Reduces Progression of Coronary Artery Atherosclerosis," *JAMA* **273**, 1849–1854 (1995).
45. N. G. Stephens et al., "Randomized Controlled Trial of Vitamin E in Patients with Coronary Disease: Cambridge Heart Antioxidant Study," *Lancet* **23**, 781–786 (1996).
46. I. Jialal, "Evolving Lipoprotein Risk Factors: Lipoprotein (a) and Oxidized Low-Density Lipoprotein," *Clin. Chem.* **44**, 1827–1832 (1998).
47. B. Hennig, M. Toborek, and A. A. Cader, "Nutrition, Endothelial Cell Metabolism, and Atherosclerosis," *Crit. Rev. Food Sci. Nutr.* **34**, 253–282 (1994).
48. A. Martin et al., "Vitamin E Inhibits Low-Density Lipoprotein-Induced Adhesion of Monocytes to Human Aortic Endothelial Cells In Vitro," *Arterioscler. Thromb. Vasc. Biol.* **17**, 429–436 (1997).
49. C. Guarnieri et al., "α-Tocopherol Pretreatment Improves Endothelium-Dependent Vasodilation in Aortic Strips of Young and Aging Rats Exposed to Oxidative Stress," *Mol. Cell. Biochem.* **157**, 223–228 (1996).
50. M. G. Traber, "Determinants in Plasma Vitamin E Concentrations," *Free Radicals Biol. Med.* **16**, 229–239 (1994).
51. G. Wolf and D. Phil, "γ-Tocopherol: An Efficient Protector of Lipids Against Nitric Oxide-Initiated Peroxidative Damage," *Nutr. Rev.* **55**, 376–378 (1997).
52. J. A. Vinson et al., "Plant Flavonoids, Especially Tea Flavonals, Are Powerful Antioxidants Using an *In Vitro* Oxidation Model for Heart Disease," *J. Agric. Food Chem.* **43**, 2800–2802 (1995).
53. C. Morand et al., "Plasma Metabolites of Quercetin and Their Antioxidant Properties," *Am. J. Physiol.* **275**, R212–R219 (1998).
54. M. Serafini, A. Ghiselli, and A. Ferro-Luzzi, "Red Wine, Tea, and Antioxidants," *Eur. J. Clin. Nutr.* **50**, 28–32 (1996).
55. Y. H. He and C. Kies, "Green and Black Tea Consumption by Humans: Impact on Polyphenol Concentrations in Feces, Blood and Urine,"*Plant Foods Hum. Nutr.* **46**, 221–229 (1994).
56. G. T McAnlis et al., "Black Tea Consumption Does Not Protect Low Density Lipoprotein from Oxidative Modification," *Eur. J. Clin. Nutr.* **52**, 202–206 (1998).
57. E. N. Frankel et al., "Inhibition of Oxidation of Human Low-Density Lipoprotein by Phenolic Substances in Red Wine," *Lancet* **341**, 454–457 (1993).
58. C. R. Pace-Asciak et al., "Wines and Grape Juices as Modulators of Platelet Aggregation in Healthy Human Subjects," *Clin. Chim. Acta* **246**, 163–182 (1996).
59. S. V. Nigdikar et al., "Consumption of Red Wine Polyphenols Reduces the Susceptibility of Low-Density Lipoproteins to Oxidation In Vivo," *Am. Soc. Clin. Nutr.* **68**, 258–265 (1998).
60. A. P. Day et al., "Effect of Concentrated Red Grape Juice Consumption on Serum Antioxidant Capacity and Low-Density Lipoprotein Oxidation," *Ann. Nutr. Metab.* **41**, 353–357 (1997).
61. S Kapiotis et al., "Genistein, the Dietary-Derived Angiogenesis Inhibitor, Prevents LDL Oxidation and Protects Endothelial Cells from Damage by Atherogenic LDL," **17**, 2868–2874 (1997).
62. M. S. Anthony et al., "Soybean Isoflavones Improve Cardiovascular Risk Factors Without Affecting the Reproductive System of Peripubertal Rhesus Monkeys," *J. Nutr.* **126**, 43–50 (1996).

ROSS TOMAINO
ERIC A. DECKER
University of Massachusetts
Amherst, Massachusetts

See also ANTIOXIDANTS.

PHYTOCHEMICALS: BIOTECHNOLOGY OF PHENOLIC PHYTOCHEMICALS FOR FOOD PRESERVATIVES AND FUNCTIONAL FOOD APPLICATIONS

Phytochemicals from herbs and fermented legumes are excellent dietary sources of phenolic metabolites. These phenolics are important for food preservation and increasingly for therapeutic and pharmaceutical applications. The functional food-related objectives of the food biotechnology program at the University of Massachusetts are to elucidate the molecular and physiological mechanisms associated with the synthesis of important health-related, therapeutic phenolic metabolites in food-related plants and fermented plant foods. Current efforts focus on the elucidation of the role of the proline-linked pentose phosphate pathway in regulating the synthesis of the anti-inflammatory compound rosmarinic acid (RA). Specific aims of current research efforts are (*1*) to develop novel tissue-based screening and selection techniques to isolate high RA-producing, shoot-based clonal lines from genetically heterogenous, cross-pollinating species in the family Lamiaceae and (2) to target genetically uniform, regenerated shoot-based clonal lines for characterization of key enzymes and genes associated with the pentose phosphate pathway and linked to phenolic synthesis. These research objectives have substantial implications for harnessing the genetic and biochemical potential of genetically heterogenous, food-related medicinal plant species. The success of this research also provides novel methods and strategies to gain access to metabolic pathways of pharmaceutically important metabolites from ginger, curcuma, chili peppers, melon, and other food-related species with novel phenolics.

PLANT PHENOLICS AS PHYTOPHARMACEUTICALS

Plant phenolics are an important group of secondary metabolites that have diverse medicinal applications. Examples of the use of specific phenolics as antioxidant and anti-inflammatory compounds are curcumin from *Curcuma longa* (1), *Curcuma mannga* (2), and *Zingiber cassumunar* (3), as well as rosmarinic acid from *Rosmarinus officinalis* (4). The use of phenolics as cancer chemopreventive metabolites has been also established with curcumin from *Curcuma longa* (1), isoflavonoids from *Glycine max* (5), and galanigin from *Origanum vulgare* (6). Other medicinal uses of plant phenolics include lithospermic acid from *Lithospermum* sp. as antigonodotropic (7), salvianolic acid from *Salvia miltiorrhiza* as antiulcer (8), thymol from *Thymus vulgaris* as anticaries (9), anethole from *Pimpinella anisum* as antifungal (10), and hellicoside from *Plantago asiatica* as antiasthmatic (8).

Species belonging to the family Lamiaceae are important sources of phenolic-type pharmaceuticals. Therapeutic use of these species can be observed in several countries. Examples are provided in Table 1.

GENETIC HETEROGENEITY IN FAMILY LAMIACEAE

The major problem in the use of phytopharmaceuticals from the family Lamiaceae is the plant-to-plant variability of specific metabolites due to the genetic heterogeneity common to all species in this family. Much of this genetic heterogeneity is the result of gynodioecy, resulting in breeding character being influenced by natural cross-pollination (15). Floral diversity and bee pollination also contribute to high cross-pollination. This gives rise to substantial variability in active ingredient levels and quality (16,17). Therefore, the biochemical characterization of pathways and genetic access to specific metabolites in all species in Lamiaceae is difficult. Each plant within a given sample extract originates from a different heterozygous seed. Also, this makes breeding of elite varieties targeting enhancement of specific metabolites very challenging. Current genetic improvements have been limited to random selection and in some cases vegetative propagation (18).

ROSMARINIC ACID AND MEDICINAL APPLICATIONS

RA (Fig. 1) is an important caffeoyl ester (phenolic depside) with proven medicinal properties and well-characterized physiological functions. RA is found in substantial quantities in several species in the family Lamiaceae with medicinal uses. *Salvia lavandulifolia* is used as choleretic, antiseptic, astringent, and hypoglycemic drug in southern Europe and contains high quantities of rosmarinic acid (19). RA-containing *Ocimum sanctum* (holy basil) is widely used to reduce fevers and against gastrointestinal disease in India. In Mexico, high RA-containing *Hyptis verticillata* is widely used by Mixtec Indians against gastrointestinal disorders and skin infections (11). In Indonesia and several other parts of the southeast Asia, RA-containing *Orthosiphon aristatus* is known for its diuretic properties and is also used against bacterial infections and inflammations of the urinary system (14). *Salvia cavaleriei*, a high RA-containing species, is used in China for treatment of dysentery, boils, and injuries (20).

PHARMACOLOGICAL FUNCTIONS OF ROSMARINIC ACID

The pharmacological effect of RA through inhibition of several complement-dependent inflammatory processes has been clearly proven (4). Therefore, it has tremendous potential as a therapeutic agent for control of complement-activation diseases (4,21). RA has been reported to have effects on both the classical C3-convertase and on the cobra-venom-factor-induced alternative convertase pathway (4). Other *in vivo* studies show that RA inhibits sev-

Table 1. Examples of Species of Lamiaceae Used as Medicine

Species	Key metabolite	Use	Reference
Hyptis verticillata	Rosmarinic acid	Gastrointestinal disorders	11
Lavandula spp.	Rosmarinic acid	Anti-inflammatory	12
Lithospermum erythrorhizon	Rosmarinic acid	Anti-inflammatory	13
	Lithospermic acid	Antigonodotropic	8
Origanum vulgare	Galangin	Antimutagen	6
Orthosiphon aristatus	Rosmarinic acid	Diuretic and anti-inflammatory	14
Rosmarinus officinalis	Rosmarinic acid	Anti-inflammatory	4
Salvia miltiorrhiza	salvianolic acid	Antiulcer	8
Thymus vulgaris	Thymol	Anticaries	9

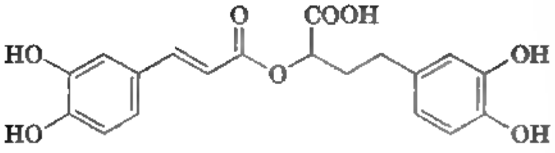


Figure 1. Structure of rosmarinic acid.

eral complement-dependent inflammatory processes, including paw edema induced by cobra venom factor and ovalbumin/antiovalbumin-mediated passive cutaneous anaphylaxis (21). It also inhibits prostacyclin synthesis induced by complement activation (22,23). It is also known to have complement-independent effects such as scavenging of oxygen free radicals (24) and inhibition of elastase. The relative safety of RA in relation to other methods of complement depletion is well documented (24). Among other actions of RA are antithyrotropic activity in tests with human thyroid membrane preparations, inhibition of complement-dependent components of endotoxin shock in rabbits, and the ability to react rapidly to viral coat proteins and so inactivate the virus (12,21). RA also inhibits Forskolin-induced activation of adenylate cyclase in cultured rat thyroid cells (25).

PRODUCTION OF ROSMARINIC ACID

RA has been targeted for production using undifferentiated cell suspension cultures of several species (12,14,26–30). The main purpose of cell suspension production of RA is the potential for large-scale production in bioreactors (31,32). Although large-scale production in bioreactors is feasible for RA, undifferentiated cell suspensions are not practical for metabolites produced in differentiated structures (eg, anethole in seeds, curcumin in rhizomes, and thymol in glandular cells of leaves). An additional disadvantage of undifferentiated callus-based suspension cultures is that the DNA is more error-prone; therefore, cell lines are genetically unstable (33). Even if problems of genetic stability and differentiation-linked metabolite production are solved, bioreactor-based production requires high initial operating costs and is not feasible in regions with poor industrial infrastructure. Gaining access to phenolic phytopharmaceuticals such as RA for all people at low cost can be best achieved by improving plant varieties for specific metabolites using modern biotechnology. Such improved, elite varieties could be incorporated into existing agricultural practices and systems. Keeping these perspectives in mind, the strategies outlined in this article are to isolate high RA-producing, genetically uniform, shoot-based clonal lines of several species belonging to the family Lamiaceae using tissue-culture techniques. Several species in this family are known to produce RA and are used in several parts of the world either as food additives, preservatives, or medicines. The high RA-producing, elite, shoot-based clonal lines will be screened based on resistance to a proline analogue and *Pseudomonas* spp. (34–36).

Selected elite, shoot-based clonal lines will be used to gain access to pathways important for biosynthesis of RA and for developing genetic transformation techniques. This will be used for subsequent engineering of RA biosynthesis using modern molecular biology techniques. Such a model for isolating genetically uniform, elite, shoot-based clonal lines from a heterogeneous and unknown genetic background can be extended for the metabolic engineering of other phytopharmaceuticals.

PATHWAYS ASSOCIATED WITH ROSMARINIC ACID BIOSYNTHESIS

It has been shown that two aromatic amino acids, phenylalanine and tyrosine, are precursors of RA biosynthesis (37). Using radioactive phenylalanine and tyrosine, it was established that they are incorporated into caffeic acid and 3,4-dihydroxyphenyllactic acid moieties, respectively (37). Steps in RA biosynthesis originating from phenylalanine and tyrosine have been characterized (Fig. 2) (13,14,30,38, 39). In several cell cultures, the activity of phenylalanine-ammonia-lyase was correlated to RA (13,14). Using *Anchusa officinalis* cell suspension cultures, it was reported that tyrosine aminotransferase catalyzes the first step of the transformation of tyrosine to 3,4-dihydroxyphenyllactic acid. Several isoforms of tyrosine aminotransferase were found to be active in cell suspension cultures of *Anchusa officinalis* (13,39). Prephenate aminotransferase in *Anchusa* cell suspension cultures was found to be important; its activity was affected by 3,4-dihydroxyphenyllactic acid (40). Other enzymes of late steps in the RA biosynthesis pathway, such as hydroxyphenylpyruvate reductase and RA synthase, were isolated and characterized in cell cultures of *Coleus blumei* (41–43).

Recently, microsomal hydroxylase activities that introduce hydroxyl groups at positions 3 and 3′ of the aromatic rings of ester 4-coumaroyl-4′-hydroxyphenyllactate to give rise to RA were isolated (30). This led to the proposed complete biosynthetic pathway for RA biosynthesis originating from phenylalanine and tyrosine.

These reports point to good success in understanding RA biosynthesis from phenylalanine and tyrosine using various cell suspension cultures. However, several major issues have to be addressed to gain access and to control the interacting metabolic fluxes critical to RA biosynthesis for subsequent metabolic engineering. There are major gaps in understanding and significant questions to be answered: (*1*) What is the role of primary metabolism, particularly the pentose phosphate pathway? (*2*) How is the pentose phosphate pathway regulated during RA synthesis? (*3*) What is the role of light in regulating RA biosynthesis? (*4*) How can the problems associated with genetic instability of undifferentiated callus cultures be resolved? (*5*) How can the understanding of metabolic pathways and subsequent engineering of efficient RA biosynthesis be used to develop elite varieties for traditional and contemporary agricultural production systems?

MODEL AND RATIONALE OF CURRENT RESEARCH

Selection of High RA-Producing, Shoot-Based Clonal Lines

The focus of current research is to develop techniques to isolate genetically uniform, high RA-containing, shoot-

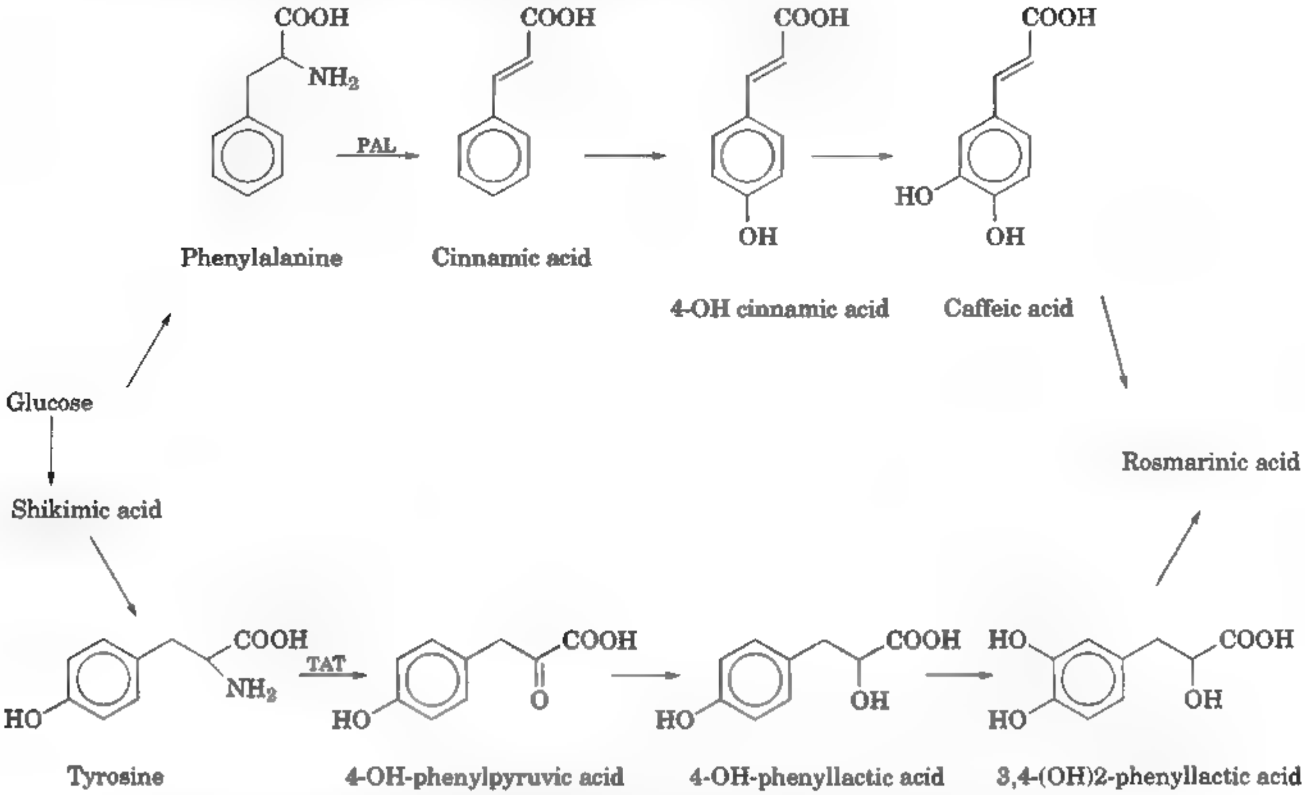


Figure 2. Rosmarinic acid biosynthesis from phenylalanine and tyrosine. PAL, phenylalanine ammonia lyase; TAT, tyrosine aminotransferase.

based clonal lines for metabolic pathway analysis and for developing gene transfer techniques for subsequent metabolic engineering. This will help provide excellent experimental systems and direction to fill gaps in knowledge of RA biosynthesis using several species used for food and medicinal applications. The basic strategy involves the isolation of genetically uniform, high RA-producing, shoot-based clonal lines from a heterogeneous genetic background. This heterogeneity is found in all species in the family Lamiaceae, and the breeding character is influenced by natural cross-pollination (15). The use of genetically uniform, shoot-based clonal lines envisioned in this article has the following advantages:

1. Shoot clones are genetically more stable than undifferentiated callus cultures.
2. Shoot clones allow the characterization of light-regulated pathways associated with RA synthesis.
3. Shoot clones can easily be targeted for large-scale greenhouse production of elite clonal lines or for incorporating into plant breeding programs to develop superior RA-producing seed varieties.
4. Shoot clones targeted for genetic engineering of metabolic pathways can be easily regenerated to whole plants for incorporation into plant-variety improvement programs.

Role of Proline-Linked Pentose Phosphate Pathway

High RA-producing, shoot-based clonal lines originating from a single heterozygous seed among a heterogeneous bulk-seed population are being selected based on tolerance to the proline analogue, azetidine-2-carboxylate (A-2-C) and a novel *Pseudomonas* sp. isolated from oregano. This strategy for selection of high RA clonal lines is based on the model that the proline-linked pentose phosphate pathway is critical for driving metabolic flux (erythrose-4-phosphate) toward shikimate and phenylpropanoid pathways (Fig. 3). Any clonal line with a deregulated proline synthesis pathway should have an overexpressed pentose phosphate pathway that allows excess metabolic flux to drive shikimate and phenylpropanoid pathways toward RA synthesis. Such proline-overexpressing clonal lines should be tolerant to A-2-C. If the metabolic flux to RA is overexpressed, it is likely to be stimulated in response to *Pseudomonas* sp. Therefore, such a clonal line is likely to be tolerant to *Pseudomonas* sp. Such a clonal line should also have high proline or proline oxidation (proline dehydrogenase) and RA content in response to A-2-C and *Pseudomonas* sp. In addition, in the presence of A-2-C or *Pseudomonas* sp., increased activity of key enzymes glucose-6-phosphate dehydrogenase (pentose phosphate pathway), pyrroline-5-carboxylate reductase (proline synthesis pathway), proline dehydrogenase (proline oxidation pathway), 3-deoxy-D-arabino heptulosonate-7-phosphate synthase (shikimate pathway), and phenylalanine ammonia-lyase (phenylpropanoid pathway) should be observed. The rationale for this model is based on the role of the pentose phosphate pathway in driving ribose-5-phosphate toward purine metabolism in cancer

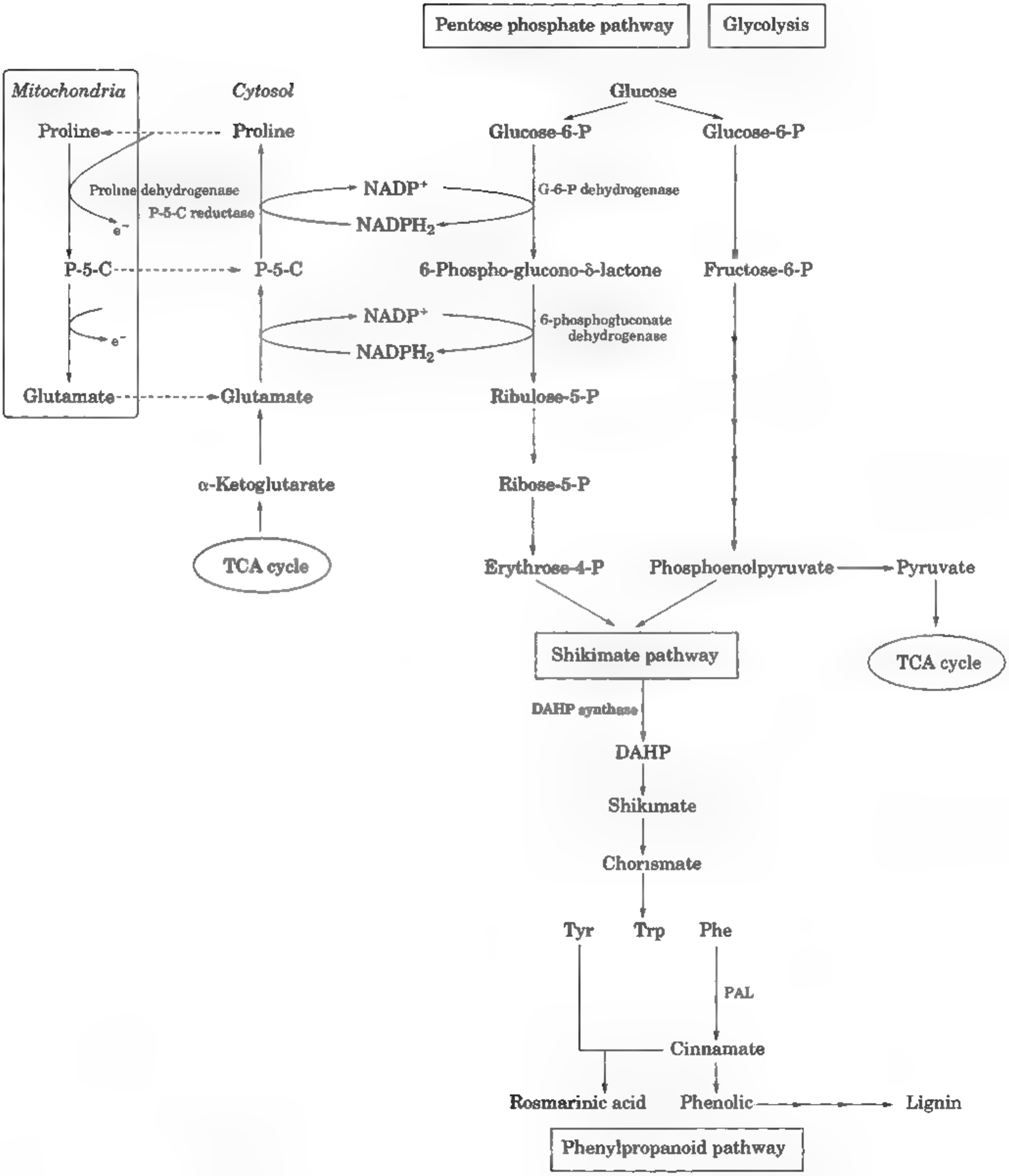

Pentose phosphate pathway
Glycolysis
Mitochondria
Cytosol
Glucose
Proline
Proline
Glucose-6-P
Glucose-6-P
Proline dehydrogenase
P-5-C reductase
NADP⁺
NADPH₂
G-6-P dehydrogenase
e⁻
P-5-C
P-5-C
6-Phospho-glucono-δ-lactone
Fructose-6-P
NADP⁺
NADPH₂
6-phosphogluconate dehydrogenase
e⁻
Glutamate
Glutamate
Ribulose-5-P
α-Ketoglutarate
Ribose-5-P
TCA cycle
Erythrose-4-P
Phosphoenolpyruvate
Pyruvate
Shikimate pathway
TCA cycle
DAHP synthase
DAHP
Shikimate
Chorismate
Tyr
Trp
Phe
PAL
Cinnamate
Rosmarinic acid
Phenolic
Lignin
Phenylpropanoid pathway

Figure 3. Proline-linked pentose phosphate pathway. TCA, tricarboxylic acid; PAL, phenylalanine ammonia lyase.

cells (44), differentiating animal tissues (45), and plant tissues (46). The hypothesis of this model is that the same metabolic flux from overexpression of the proline-linked pentose phosphate pathway regulates the interconversion of ribose-5-phosphate to the erythrose-4-phosphate-driving shikimate pathway. Shikimate pathway flux is critical for both auxin and phenylpropanoid biosynthesis, including RA. This hypothesis has been strengthened by preliminary results in which in several oregano clonal lines, RA biosynthesis was significantly stimulated by exogenous addition of A-2-C and ornithine (47). Such clonal lines are also tolerant to *Pseudomonas* spp. and respond to the bacterium by increasing RA and proline biosynthesis (34,48). High RA-producing clonal lines selected based on the model in this proposal will be targeted for preliminary characterization of key enzymes mentioned earlier. Such genetically uniform clonal lines will also be targeted for developing gene transformation techniques using *Agrobacterium* or particle gun bombardment. The success of this strategy will lead to access of critical interlinking metabolic pathways associated with RA biosynthesis. This will allow more detailed analysis that will lead to metabolic engineering for efficient RA biosynthesis. This strategy for RA biosynthesis can be the foundation for metabolic engineering of other phytopharmaceuticals from cross-pollinating, heterogeneous species.

BASIL AND FUNCTIONAL FOOD APPLICATIONS

The long-term goal of this project is to use the tools of biotechnology to develop improved clonal lines of dietary herbs and improved fermentation process for dietary legumes to generate consistent, nontoxic, and clinically relevant levels of phenolic metabolites for use as antimicrobials against chronic diseases caused by ulcer-associated *Helicobacter pylori* and urinary tract infection-associated *Escherichia coli*. Plant phenolic metabolites such as capsaicin from diet are known to be associated with low rate of ulcers through inhibition of *H. pylori* (49), and currently available synthetic drug treatments have significant side effects (50,51). Phenolics from cranberry have potential for use against urinary tract infections linked to *E. coli* (52). Use of dietary source of diverse antimicrobial-type plant phenolics could lead to reduced use of antibiotics and therefore reduce the potential increase in antibiotic-resistant, disease-causing bacteria. In addition, plant phenolic metabolites have antioxidant and anti-inflammatory properties (4) that contribute to cancer chemopreventive and immune-modulating potentials (eg, the French Paradox of reduced levels of high-fat diet-related diseases due to higher intake of phenolics from red wine and the reduced rate of breast cancer in Asian women due to high phenolics from a fermented soy-based diet). In order to overcome the problem of phytochemical inconsistency caused by genetic heterogeneity, plant-tissue-culture techniques have been developed to isolate a clonal pool of plants originating from a single seed (35). A single elite clonal line with superior phenolic profile (a combination of several bioactive phenolics) can then be selected based on tolerance to *Pseudomonas* spp. (34–36). In this project, this strategy will be used to select several elite clonal lines of basil (*Ocimum sanctum*), an herb species widely used in the diet and as medicine in several parts of the world. These elite lines (each clonal line originating from a different seed), following large-scale clonal propagation (micropropagation), will be targeted as dietary sources of phenolics (rosmarinic acid, eugenol, and methyl chavicol) to control chronic diseases associated with ulcer-linked *H. pylori* and urinary-tract-infection-linked *E. coli*. Additionally, the elite clonal lines will be screened for antioxidant activity.

THYME AND ANTIMICROBIAL APPLICATIONS IN FOOD

Therapeutic interventions for the treatment of *E. coli* 0157:H7 are limited, reinforcing the need to prevent its presence and reproduction in the food manufacturing process. The recent outbreaks of *E. coli* 0157·H7 through meat products has renewed interest in potential inhibitors of this organism (53,54). It is beyond the scope of this article to review all chemicals that are currently used for their antimicrobial activity. They include sorbates, benzoates, propionates, and sodium chloride. Others are added for color and flavor (nitrites) or for their antioxidant properties (BHA and BHT) but also possess antimicrobial activity. The food industry relies heavily on the use of synthetic antimicrobial agents to extend shelf life and preserve freshness. However, these synthetic preservatives in the food industry are coming under increasing scrutiny and reappraisal, resulting in the search for natural biochemicals from plants, including herbs and spices (55). Thus, there has been renewed interest in the antimicrobial properties of herbs and spices. In fact, about 20% of spices sold in the United States are used in the meat industry.

Most essential oils of spices and herbs are generally regarded as safe and are considered to contain the antimicrobial activity. The antimicrobial activity of plant extracts, including spices and essential oils, has been reviewed (56). Carvacrol and thymol are major volatile components of oregano, thyme, and savory. Generally, the thymol/carvacrol ratio in thyme is 10:1, whereas the carvacrol/thymol ratio in oregano is 20:1 (57). Carvacrol and thymol are known to have 1.5× and 20× the antimicrobial activity of phenol, respectively (8). In 1960, Katayama and Nagai (58) reported the antimicrobial activity of thymol and carvacrol against *Salmonella enteritidis*, *Staphylococcus aureus*, and *E. coli* (serotype not given). Similarly, Beuchat (59) in 1976 reported that the growth of *Vibrio parahaemolyticus* was delayed by the presence of 100 ppm of the essential oils of oregano and thyme. In 1989, Farag et al. (60) reported that of six essential oils examined (sage, rosemary, cumin, caraway, clove, and thyme), thyme oil was the most effective against three Gram-negative bacteria, with *E. coli* (serotype not given) being the most sensitive. The same workers (60) showed that thyme oil reduced the total bacterial count in butter stored at room temperature. This is the only report documenting the antibacterial effectiveness of essential oils in a food system.

The specific modes of action of plant extracts remain poorly defined, but there is some indication that they may cause a depletion of cellular energy (61). Because many of the components of essential oils, such as thymol, are similar in structure to phenolic antimicrobials, it seems reasonable that their modes of action would be similar.

Finally, environmental conditions may play a role in the effectiveness of natural antimicrobials. In particular, the antifungal properties of thymol and carvacrol were affected by pH (62). There is no similar information on its antibacterial properties, but its effectiveness in meat might be affected by such considerations. Our preliminary results demonstrating the effectiveness of thymol and carvacrol against *E. coli* in laboratory media were conducted at pH 6.5, near that of meat. This indicates that, at least with these two essential oils, pH alone would have a minimal adverse effect.

ELITE BASIL FOR POSTHARVEST PRESERVATION OF POTATOES

Preliminary studies conducted in several laboratories in the world indicate that naturally occurring plant extracts and natural compounds have demonstrated sprout inhibitory and antimicrobial properties (63–67). Several volatile monoterpenes that are primary components of essential oils inhibited sprouting when introduced as volatile into head space surrounding the potato tuber (66). Dimethylnapthalene (DMN) and diispropylnapthalene (DIPN) applied as thermal fog successfully suppressed sprouting under current storage conditions (68). DMN is reported to be a constituent of volatile released naturally by potatoes in storage (69,70).

Storage diseases of potatoes cause millions of dollars of loss each year. Fungicides are available to control some of the storage diseases, but none of the compounds currently registered for use function as sprout suppressants (63). *Helminthosporium solani*, the fungus that causes silver scurf on potatoes, caused a $8.6-million-dollar loss in 1992/1993 (71). *Fusarium* spp. (dry rot) and *Erwinia* spp. (soft rot) have become common ailments of the potato in storage, again causing loss of millions of dollars to the worldwide potato industry (72). Recent work from a few areas has demonstrated the dual advantage of natural products. The sprout suppressant properties and antimicrobial activity of ten essential oils were assessed on Spuntia (cv.) (73). Lavender (*Lavandula angustifolia*), sage (*Salvia fruticosa*), and rosemary (*Rosmarinus officinalis*) oils proved to be effective sprout inhibitors and also possesses antimicrobial activity (74). Observation during evaluation of naturally occurring aromatic compounds and monoterpenes for sprout suppression capabilities provided insight to this antimicrobial activity (66). Salicylaldehyde and 1,8-cineole-treated tubers were visibly free of storage diseases. Several *Fusarium* spp. strains tested in vivo were sensitive to DMN at ED_{50} values of 27 to 64 mL a.i L^{-1} and 78 to 124 mL a.i L^{-1} respectively (68). In the same study, salicylaldehyde suppressed growth and development of *H. solani*.

Natural products that potentially serve as sprout suppressants and disease-controlling agents will be an attractive proposition for postharvest use on potatoes. The potato industry would benefit from antimicrobial agent that could be fogged in or spray applied into a storage to disinfect exposed surfaces, including the surface layer of soil floor. Additionally, if seed potatoes could be sanitized of surface diseases such as silver scurf while in storage, the spread of inoculum in storage, to fields, and to subsequent crops could be decreased. Reduction or elimination of tuber surface defects caused by pathogens such as black scurf and silver scurf would be especially advantageous to the fresh market potato industry. The use of naturally occurring compounds for disease control would add to their acceptance by consumers. The priority in the industry today is to find a naturally occurring compound that (*1*) inhibits sprouting, (*2*) has no toxicity to humans at effective concentration, (*3*) has no adverse effect on potato quality, (*4*) requires little or no changes to existing postharvest methods, and (*5*) has additional benefits of disease suppression and additive health benefits that could be derived from the product itself.

Basil, which belongs to the family Lamiaceae, is an excellent culinary herb highly popular in gourmet food and in vegetable preparations such as pesto. The high phenolic content of basil, with chemicals such as eugenol methyl chavicol and rosmarinic acid, makes it an excellent natural preservative and a possible source of a natural disease-preventive phytochemical against chronic bacterial infections.

Development of new uses of crops such as basil through biotechnology for value-added preservation of potatoes will enhance the export potential of potatoes to the global markets. The current postharvest chemical used in potatoes, Chlorpropham®, is not permitted in Japan. Therefore, crops with preservative potentials, such as basil, are excellent targets for use of biotechnology to select elite lines with preservative qualities. The natural preservatives generated from basil will not only enhance the export potential of potatoes, but basil extracts can be potentially used for preservation of meats and seafood from oxidative deterioration (75). Basil-extract-treated food with consistent levels of specific phenolics like eugenol, methyl chavicol, and RA could also be a source of preventive medicinals against chronic infections such as ulcer-causing *H. pylori*.

BIBLIOGRAPHY

1. M. T. Huang, et al., "Inhibitory Effects of Curcumin on Tumor Promotion and Arachidonic Acid Metabolism in Mouse Epidermis," in L. Wattenberg et al., eds., *Cancer Chemoprevention*, CRC Press, Inc., Boca Raton, Fla., 1992, pp. 375–391.
2. A. Jitoe et al., "Antioxidant Activity of Tropical Ginger Extracts and Analysis of the Contained Curcuminoids," *J. Agric. Food Chem.* **40**, 1337–1340 (1992).
3. T. Masuda and A. Jitoe, "Antioxidative and Antiinflammatory Compounds from Tropical Gingers: Isolation, Structure Determination, and Activities of Cassumunins A, B and C, New Complex Curcuminoids from *Zingiber cassumunar*," *J. Agric. Food Chem.* **42**, 1850–1856 (1994).
4. P. W. Peake et al., "The Inhibitory Effect of Rosmarinic Acid on Complement Involves the C5 Convertase," *Int. J. Immunopharmacol.* **13**, 853–857 (1991)

5. A. M. Hutchins, J. L. Slavin, and J. W. Lampe, "Urinary Isoflavonoid Phytoestrogen and Lignan Excretion after Consumption of Fermented and Unfermented Soy Products," *J. Am. Dietetic Assoc.* **95**, 545–551 (1995).
6. K. Kanazawa et al., "Specific Desmutagens (Antimutagens) in Oregano against a Dietary Carcinogen, Trp-P-2, Are Galangin and quercetin," *J. Agric. Food Chem.* **43**, 404–409 (1995).
7. H Winterhoff, H. G. Gumbinger, and H. Sourgens, "On the Antiogonadotropic Activity of *Lithospermum* and *Lycopus* Species and Some of Their Phenolic Constituents," *Planta Medica* **54**, 101–106 (1988).
8. J. B. Harbone and H. Baxter, "Phenylpropanoids," in J. B. Harbone and H. Baxter, eds., *Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants*, Taylor and Francis, London, 1993, pp. 472–488.
9. S. Guggenheim and S. Shapiro, "The Action of Thymol on Oral Bacteria," *Oral Microbiol. Immunol.* **10**, 241–246 (1995).
10. M. Himejima and I. Kubo, "Fungicidal Activity of Polygodial in Combination with Anethole and Indole against *Candida albicans*," *J. Agric. Food Chem.* **41**, 1776–1779 (1993).
11. M. Kuhnt et al., "Biological and Pharmacological Activities and Further Constituents of *Hyptis verticillata*," *Planta Medica* **61**, 227–232 (1995).
12. T. Lopez-Arnaldos et al., "Spectrophotometric Determination of Rosmarinic Acid in Plant Cell Cultures by Complexation with Fe^{2+} Ions," *Fresenius' J. Anal. Chem.* **351**, 311–314 (1995).
13. H. Mizukami and B. E. Ellis, "Rosmarinic Acid Formation and Differential Expression of Tyrosine Aminotransferase Isoforms in *Anchusa officinalis* Cell Suspension Cultures," *Plant Cell Rep.* **10**, 321–324 (1991).
14. W. Sumaryono "Induction of Rosmarinic Acid Accumulation in Cell Suspension Cultures of *Orthosiphon aristatus* after Treatment with Yeast Extract," *Phytochemistry* **30**, 3267–3271 (1991).
15. A. J. Richards, "Gynodioecy," in A. J. Richards, ed., *Plant Breeding Systems*, G. Allen and Unwin Publishers, London, 1986, pp. 89–331.
16. A. Fleisher and N. Sneer, "Oregano Species and *Origanum* Chemotypes," *J. Sci. Food Agric.* **33**, 441–446 (1982).
17. K. P. Svoboda and S. G. Deans, "A Study of the Variability of Rosemary and Sage and Their Volatile Oil on the British Market: Their Antioxidant Properties," *Flavour Fragrance J.* **7**, 81–87 (1992).
18. Y. P. S. Bajaj, M. Furmanowa, and O. Olszowska, "Biotechnology of Micropropagation of Medicinal and Aromatic Plants," in Y. P. S. Bajaj, ed., *Biotechnology in Agriculture and Forestry*, vol. 4, Springer-Verlag, Berlin, 1988, pp. 60–97
19. S. Caniguera1 et al., "Phenolic Constituents of *Saliva lavandulifolia* ssp. *lavandulifolia*," *Planta Medica* **55**, 92 (1989)
20. H. J. Zhang and L. N. Li, "Salvianolic Acid I: A New Depside from *Salvia cavaleriei*," *Planta Medica* **60**, 70–72 (1994).
21. W. Engleberger et al., "Rosmarinic Acid. A New Inhibitor of Complement C3-Convertase with Anti-inflammatory Activity," *Int. J. Immunopharmacol.* **10**, 729–737 (1988).
22. H. Bult, A. G. Herrman, and M Rampart, "Modification of Endotoxin-Induced Haemodynamic and Haemolytical Changes in Rabbit by Melthylprednisolone, F(ab')$_2$ Fragments and Rosmarinic acid," *Br J. Pharmacol.* **84**, 317–327 (1985)
23. M. Rampart et al., "Complement-Dependent Stimulation of Prostacyclin Biosynthesis: Inhibition by Rosmarinic Acid," *Biochem. Pharmacol.* **35**, 1397–1400 (1986).
24. J. K. S. Nuytinck et al., "Inhibition of Experimentally Induced Microvascular Injury by Rosmarinic Acid," *Agents Actions* **17**, 373–374 (1985).
25. S. Kleemann and H. Winterhoff, "Rosmarinic Acid and Freeze-Dried Extract (FDE) of *Lycopus virginicus* Are Able to Inhibit Forskolin-Induced Activation of Adenylate Cyclase in Cultured Rat Thyroid Cells," *Planta Medica* **56**, 683 (1990).
26. M H. Zenk, H. El-Shagi, and B. Ulbrich, "Production of Rosmarinic Acid by Cell Suspension Cultures of *Coleus blumei*," *Naturwissenschaften* **64**, 585–586 (1977).
27 W. De-Eknamkul and B. E Ellis, "Rosmarinic Acid Production and Growth Characterization of *Anchusa officinalis* Cell Suspension Cultures," *Planta Medica* **50**, 346–350 (1984).
28. I. Hippolyte, et al., "Growth and Rosmarinic Acid Production in Cell Suspension Cultures of *Salvia officinalis*," *Plant Cell Rep.* **11**, 109–112 (1992).
29. H Mizukami, Y. Tabira, and B. E. Ellis, "Methyl Jasmonate-Induced Rosmarinic Acid Biosynthesis in *Lithospermum erythrorhizon* Cell Suspension Cultures," *Plant Cell Rep.* **12**, 706–709 (1993).
30. M. Petersen "Proposed Biosynthetic Pathway for Rosmarinic Acid in Cell Cultures of *Coleus blumei* Benth," *Planta* **189**, 10–14 (1993).
31. B. Ulbrich, W. Wiesner, and H. Arens, "Large-Scale Production of Rosmarinic Acid from Plant Cell Cultures of *Coleus blumei* Benth," in K. Neumann, ed., *Primary and Secondary Metabolism of Plant Cell Cultures*, Springer-Verlag, Berlin, 1985, pp. 293–303.
32. W Kreis and E. Reinhard, "The Production of Secondary Metabolites by Plant Cells Cultivated in Bioreactors," *Planta Medica* **55**, 409–416 (1989)
33. R. L. Phillips, S. M Kaeppler, and J. Olhoft, "Genetic Instability of Plant Tissue Cultures: Breakdown of Normal Controls," *Proc. Natl. Acad. Sci. U.S.A* **91**, 5222–5226 (1994).
34. K. Shetty et al., "Selection of High Phenolics-Containing Clones of Thyme (*Thymus vulgaris* L.) using *Pseudomonas* sp.," *J. Agric. Food Chem.* **40**, 3408–3411 (1996).
35. Y. Eguchi, O. F. Curtis, and K. Shetty, "Interaction of Hyperhydricity-Preventing *Pseudomonas* sp. with Oregano (*Origanum vulgare*) and Selection of High Phenolics and Rosmarinic Acid-Producing Clonal Lines," *Food Biotechnol.* **10**, 191–202 (1996).
36. R. Yang, O. F. Curtis, and K. Shetty, "Tissue-Culture-Based Selection of High Rosmarinic Acid-Producing Clonal Lines of Rosemary (*Rosmarinus officinalis*) using Hyperhydricity-Reducing *Pseudomonas*," *Food Biotechnol.* **11**, 73–88 (1997).
37. B. E. Ellis and G. H. N. Towers, "Biogenesis of Rosmarinic Acid in *Mentha*," *Biochem. J.* **118**, 287–291 (1970).
38. W. De-Eknamkul and B. E. Ellis, "Purification and Characterization of Tyrosine Aminotransferase Activities from *Anchusa officinalis* Cell Cultures," *Arch. Biochem. Biophys.* **257**, 430–438 (1987).
39. W. De-Eknamkul and B. E. Ellis, "Tyrosine Aminotransferase: The Entry Point Enzyme of the Tyrosine-Derived Pathway in Rosmarinic Acid Biosynthesis," *Phytochemistry* **26**, 1941–1946 (1987).
40. W. De-Eknamkul and B. E. Ellis, "Purification and Characterization of Prephenate Aminotransferase from *Anchusa officinalis* Cell Cultures," *Arch. Biochem. Biophys* **267**, 87–94 (1988).
41. M. Petersen and A. W. Alfermann, "Two New Enzymes of Rosmarinic Acid Biosynthesis from Cell Cultures of *Coleus blumei*: Hydroxyphenylpyruvate Reductase and Rosmarinic Acid Synthase," *Z. Naturforsch.* **43c**, 501–504 (1988).

42. E. Hausler, M. Petersen, and A. W. Alfermann, "Hydroxyphenylpyruvate Reductase from Cell Suspension Cultures of *Coleus blumei Benth*," *Z. Naturforsch.* **46C**, 371–376 (1991).

43. M. S. Petersen, "Characterization of Rosmarinic Acid Synthase from Cell Cultures of *Coleus blumei*," *Phytochemistry* **30**, 2877–2881 (1991).

44. J. M. Phang, "The Regulatory Functions of Proline and Pyrroline-5-Carboxylic Acid," *Curr. Top. Cell Regul.* **25**, 91–132 (1985).

45. A. Jost et al., "Experimental Control of the Differentiation of Leydig Cells in the Rat Fetal Testis," *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8094–8097 (1988).

46. D. H. Kohl et al., "Proline Metabolism in N_2-Fixing Nodules: Energy Transfer and Regulation of Purine Synthesis," *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2036–2040 (1988).

47. R. Yang and K. Shetty, "Stimulation of Rosmarinic Acid in Shoot Cultures of Oregano (*Origanum vulgare*) Clonal Line in Response to Proline, Proline Analogue, and Proline Precursors," *J. Agric. Food Chem.* **46**, 2888–2893 (1998).

48. P. Perry and K. Shetty, "A Model for Involvement of Proline During *Pseudomonas*-Mediated Stimulation of Rosmarinic Acid Levels in Oregano Shoot Clones," *Food Biotechnol.* **13**, 137–154 (1999).

49. N. L. Jones, S. Shabib, and P. M. Sherman, "Capsaicin as an Inhibitor of the Growth of the Gastric Pathogen *Helicobacter pylori*," *FEMS Microbiol. Lett.* **146**, 223–227 (1997).

50. D. Y. Graham et al., "Factors Influencing the Eradication of *Helicobacter pylori* with Triple Therapy," *Gastroenterology* **102**, 493–496 (1992).

51. J. Labenz, F. Leverkus, and G. Borsch, "Omeprazole plus Amoxycillin for the Cure of *Helicobacter pylori* Infection: Factors Influencing the Treatment Success," *Scand. J. Gastroenterol.* **29**, 1070–1075 (1994).

52. A. E. Sobota, "Inhibition of Bacterial Adherence by Cranberry Juice: Potential Use for the Treatment of Urinary Tract Infections," *J. Urol.* **131**, 1013–1016 (1984).

53. M. Cohen and R. Giannella, "Haemorrhagic colitis associated with *E. coli* 0157: H7," *Adv. Intern. Med.* **37**, 173–189 (1992).

54. M. Neill, "*E. coli* 0157:H7—Current Concepts and Future Prospects," *J. Food Safety* **10**, 99–110 (1989).

55. W. H. Stroh, "New Biotechnologies Set to Impact Industrial Food Preservative Market," *Genet. Eng. News* **13**, 8 (1993).

56. D. Conner, "Naturally Occurring Compounds," in P. M. Davidson and A. Branen, eds., *Antimicrobials in Foods*, Marcel Dekker, Inc., New York, 1993, p. 441.

57. U. Salzer, "The Analysis of Essential Oils and Extracts (Oleoresins) from Seasonings—A Critical Review," *Crit. Rev. Food Sci. Technol.* **17**, 345–371 (1977).

58. T. Katayama and I. Nagai, "Chemical Significance of the Volatile Components of Spices from the Food Preservation Standpoint. IV. Structure and Antimicrobial Activity of Some Terpenes," *Nippon Suisan Gakkaishi* **26**, 29–34 (1960).

59. L. Beuchat, "Sensitivity of *Vibrio parahaemolyticus* to Spices and Organic Acids," *J. Food Sci.* **41**, 899–902 (1976).

60. R. Farag et al., "Antimicrobial Activity of Some Egyptian Spice Essential Oils," *J. Food Prot.* **52**, 665 (1989).

61. D. Conner et al., "Effects of Essential Oils and Oleoresins of Plants on Ethanol Production, Respiration and Sporulation in yeasts," *Int. J. Food Microbiol.* **1**, 63–67 (1984).

62. D. Thompson, "Influence of pH on the Fungitoxic Activity of Naturally Occurring Compounds," *J. Food Prot.* **53**, 428–431 (1990).

63. M. D. Lewis, Ph.D Thesis, University of Idaho, 1996.

64. D. F. Meigh, A. A. E. Filmer, and R. Self, "Growth-Inhibitory Volatile Aromatic Compounds Produced by *Solanum tuberosum* Tubers," Phytochemistry **12**, 987–993 (1973).

65. K. K. Shetty et al., "Studies on Alternative Methods of Sprout Suppression for Russet Burbank Potatoes" (abstract), *Am. Potato J.* **68**, 634 (1991).

66. S. F. Vaughn and G. F. Spencer, "Volatile Monoterpenes Inhibit Potato Tuber Sprouting," *Am. Potato J.* **68**, 821–831 (1991).

67. A. A. E. Filmer and M. J. C. Rhodes, "Investigation of Sprout Growth-Inhibitory Compounds in the Volatile Fraction of Potato Tubers," *Potato Res.* **28**, 361–377 (1985).

68. M. D. Lewis, M. K. Thornton, and G. E. Kleinkopf, "Commercial Application of CIPC to Storage Potatoes," CIS Newsletter, University of Idaho College of Agriculture, Moscow, Idaho.

69. J. L. Beveridge, J. Dalziel, and H. J. Duncan, "The Assessment of Some Volatile Organic Compounds as Sprout Suppressants for Ware and Seed Potatoes," *Potato Res.* **24**, 61–67 (1981).

70. J. L. Beveridge, J. Dalziel, and H. J. Duncan, "Dimethylnaphthalene as a Sprout Suppressant for Seed and Ware Potatoes," *Potato Res.* **24**, 77–88 (1981).

71. K. K. Shetty and P. Patterson, A. E. Research Series No. 93-9, University of Idaho, Moscow, Idaho, p. 11.

72. A. E. W. Boyd, "Potato Storage Diseases," *Rev. Plant Pathol.* **51**, 297–321 (1972).

73. D. Vokon, S. Vareltzidou, and P. Katinakis, "Effects of Aromatic Plants on Potato Storage: Sprout Suppression and Antimicrobial Activity," *Agric. Ecosys. Environ.* **47**, 223–235 (1993).

74. A. M. Jenssen, J. J. C. Scheffer, and A. Baerheim Svendsen, "Antimicrobial Activity of Essential Oils: A 1976–1986 Literature Review, Aspects of the Test Methods," *Planta Medica* **53**, 395–398 (1987).

75. H. L. Madsen and G. Bertelson, "Spices as Antioxidants," *Trends Food Sci. Technol.* **61**, 271–276 (1995).

KALIDAS SHETTY
University of Massachusetts
Amherst, Massachusetts

PHYTOCHEMICALS: CAROTENOIDS

Carotenoids are isoprenoids biosynthesized by plants, bacteria, molds, and yeasts. As integral components of photosynthetic reaction centers, carotenoids exist in all photosynthetic organisms and in many animals by virtue of their passage up the food chain. Of the roughly 600 structurally distinct carotenoids identified to date, only a few dozen are commonly found in human foodstuffs, and many of these circulate in plasma lipoproteins at concentrations that generally reflect dietary intake. This article discusses properties and features of carotenoids that relate to their occurrence and use in foods, including structure and reactivity, analytical methodology, nutritional and other functional properties, and the impact of food processing. Carotenoid biosynthesis, chemical synthesis, and nomenclature are adequately presented and still relevant in the 1992 edition of the *Encyclopedia* (1) and in a recent series detailing carotenoid chemistry and analysis (2–4).

STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF CAROTENOIDS

Carotenoids are oligoterpenic lipids most commonly possessing 40 carbons and an extensive conjugated double-bond system. They are classified into two main groups: the hydrocarbon carotenes and the xanthophylls, the latter of which possess one or more oxygen-containing polar functional groups. Carotenoids may be bicyclic, monocyclic, or acyclic. Several common carotenoids naturally occurring in foods or used as color additives are illustrated in Figure 1. The conjugated double-bond system is the most influential structural feature of the carotenoids, affecting both hue and chemical stability. Carotenoids with six or more conjugated double bonds absorb visible light, and absorption maxima generally increase with increasing number of double bonds. Selected physical characteristics of common carotenoids are listed in Table 1.

The two chemical reactivities of carotenoids most relevant to food systems are oxidation and isomerization. Reaction with oxygen, either autocatalytically or enzymatically, is the major cause of carotenoid degradation in foods and affects both color capacity and stability. Oxidation can disrupt conjugation and result in bleaching of endogenous or added carotenoid pigments and in formation of off-flavors (5). Oxidative degradation of carotenoids can be initiated by those factors that stimulate oxidation of unsaturated fatty acids, including light, transition metals, and lipoxygenases. Environmental factors that affect the rate of oxidation include oxygen tension, temperature, water activity, and pH (6–9). Linear (zero-order) kinetics of color loss is generally observed in dehydrated foods, whereas first-order kinetics commonly apply to aqueous systems (8,10,11), indicating that water plays a protective role in oxidative decolorization of carotenoids. Carotenoids in plant foods tend to be stabilized by complexation with proteins or polysaccharides, and processes that disrupt the microstructure of foods can result in decreased chemical stability, as discussed later.

Carotenoids are synthesized, either naturally or chemically, predominantly in the all-*trans* configuration. However, in solution, carotenoids undergo isomerization to yield a variety of *cis* isomers, and thermodynamic constants have in some cases been determined (12,13). The rate of isomerization increases with temperature and oxygen exposure. *Trans*-to-*cis* isomerization generally results in small decreases in absorption maxima, and consequently slight changes in hue.

ANALYSIS OF CAROTENOIDS

Methods of carotenoid analysis vary according the nature of the sample, but they usually involve an extraction step followed by chromatographic separation and quantification, most commonly by high-pressure liquid chromatography (HPLC). General precautions, analytical strategies, applications, and artifacts have been extensively reviewed (2). The choice of solvent(s) for extraction is determined in part by the sample matrix and may involve single solvents or solvent mixtures. Petroleum ether is sufficient for the extraction of carotenes in oil-based products such as margarines (14). Complex matrixes such as plant and animal foods are often extracted with a mixture of solvents (14), although more recently, repeated tetrahydrofuran extraction has been successfully applied to many vegetables (15,16). In all cases, solvent purity, avoidance of oxygen and light exposure, and use of appropriate internal standards are essential. In many fruits, particularly citrus, carotenoids are present as fatty acid esters, and such samples must be saponified before chromatography since most HPLC methods assess only free carotenoids. For accurate determination of the provitamin A carotenoid content of foods, chromatographic systems that resolve provitamin A from nonprovitamin A carotenoids and the *cis* and *trans* isomers of β-carotene are required. Analytical approaches that fail to do so often overestimate the vitamin A potential of foods.

Given the large number of naturally occurring carotenoids and the lack of pure analytical standards for most, proper identification of carotenoids can be problematic. Photodiode array HPLC detectors are useful for routine structural confirmation of previously characterized carotenoids, but additional spectroscopic information is commonly required for structure elucidation of other carotenoids. Mass spectrometry, nuclear magnetic resonance, and circular dichroism are commonly employed for this purpose (3,17).

NUTRITIONAL PROPERTIES OF CAROTENOIDS

Carotenoids as Vitamin A Precursors

Although about 40 of the naturally occurring carotenoids can be metabolized to vitamin A, only 3 are quantitatively important with respect to total vitamin A intake in the United States. These are β-carotene, α-carotene, and cryptoxanthin, and of these, β-carotene is nutritionally most significant because of its prevalence in plant foods and subsequent higher frequency of consumption. Provitamin A carotenoids are estimated to account for 20–40% of total dietary vitamin A equivalents in the United States. Dietary carotenoids are freed from their food matrixes during digestion, diffuse into the intestinal mucosa with the aid of bile salt micelles, and undergo partial intracellular conversion to retinyl esters (18). Retinyl esters, along with nonmetabolized carotenoids and other lipids, are secreted in chylomicrons into the bloodstream, taken up by the liver, and either stored or complexed with retinol binding protein for transport to other tissues. The yield of vitamin A from any given food (actual vitamin A value) depends on the provitamin A carotenoid content of the food, the efficiency of digestion and uptake into the intestinal mucosa, and the extent of conversion to retinyl esters. At the present time, the true vitamin A value of specific plant foods is virtually unknown. Current values are based on the assumption that 6 mg of β-carotene consumed as food yields 1 mg retinol, or roughly 1 RDA. This equivalency, which assumes 33% absorption efficiency and 50% efficiency of conversion of β-carotene to vitamin A, is universally applied to all commodities, regardless of how they are prepared or consumed. It is generally held that oil solutions

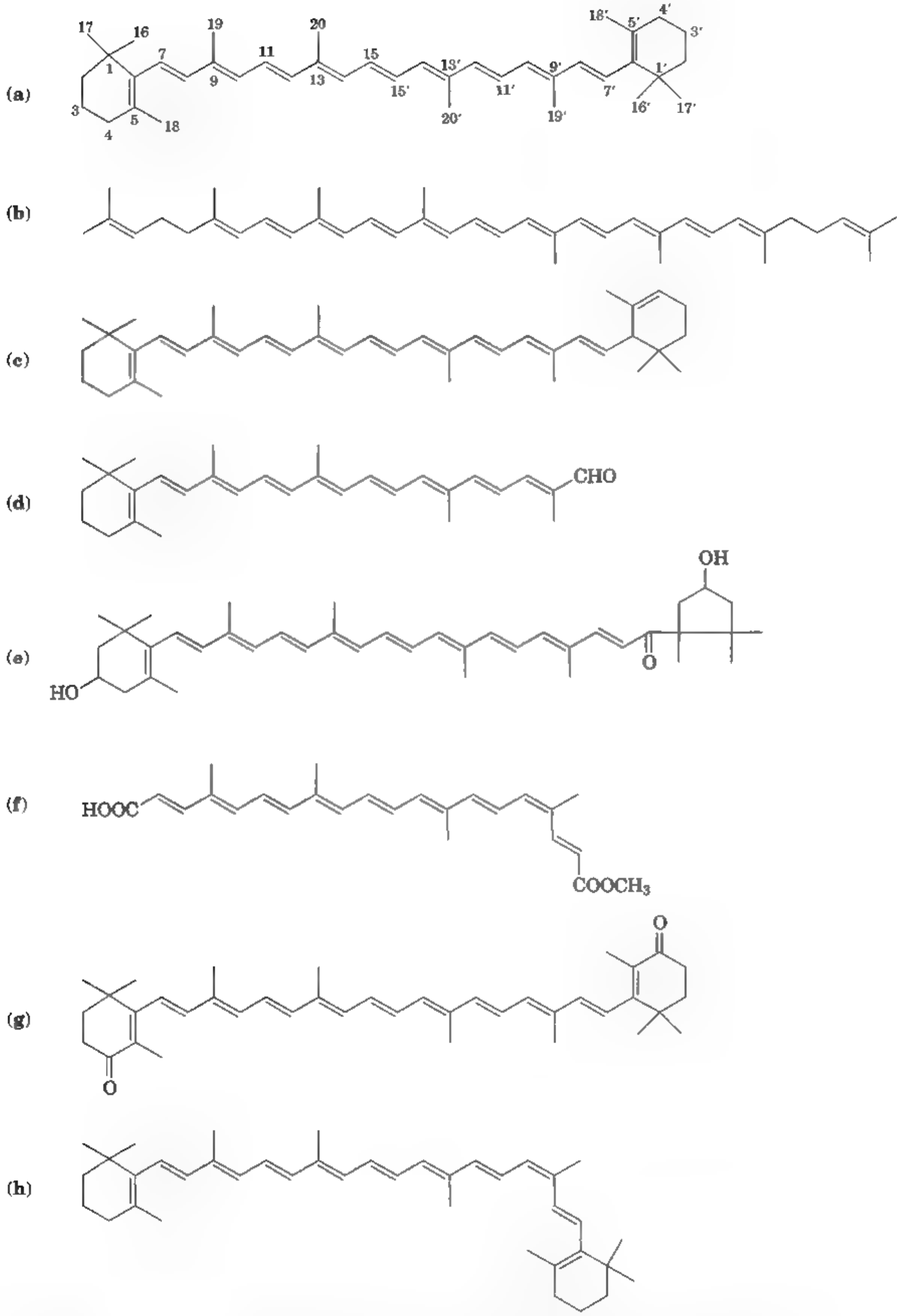

(a)
17
16
1
7
19
9
11
20
13
15
15′
13′
20′
11′
9′
19′
7′
18′
5′
4′
3′
1′
16′
17′
3
4
5
18
(b)
(c)
(d)
CHO
(e)
OH
HO
O
(f)
HOOC
COOCH$_3$
(g)
O
O
(h)

Figure 1. Chemical structure of common food carotenes and xanthophylls. (**a**) all-*trans*-*β*-Carotene; (**b**) lycopene; (**c**) *α*-carotene; (**d**) *β*-apo-8′-carotenal; (**e**) capsanthin; (**f**) bixin; (**g**) canthaxanthin, (**h**) 9-*cis*-*β*-carotene.

Table 1. Physical Characteristics of Several Common Food Carotenoids

Carotenoid	Color	λ_{max} (nm)[a]	$E^{1\%}\left(\frac{dL}{mg\ cm}\right)$[a]
β-Carotene	Light yellow to violet-red	452	2560 (hexanes)
α-Carotene	Orange-red	444	2800 (pet ether)
		477	2180 (CS2)
		445	2710 (hexanes)
Lycopene	Dark-red	472	3450 (hexanes)
		470	3450 (pet ether)
		487	3370 (benzene)
Canthaxanthin	Red-orange to brown-violet	472	2110 (hexanes)
		466	2200 (pet ether)
		480	2092 (benzene)
Bixin (annatto)	Yellow-orange to dark red	456	4200 (pet ether)
Capsanthin (paprika)	Orange red to dark red	483	2072 (benzene)
β-apo-8′-carotenal	Light orange to purplish black	460	2640 (pet ether)

[a] λ_{max}, maximum wavelength; $E^{1\%}$, absorptivity.

of carotenoids are more efficiently absorbed than those of fruits or vegetables. Even in healthy, well-nourished individuals, a substantial proportion of absorbed *β*-carotene undergoes conversion to vitamin A, indicating that conversion is not inhibited by adequate vitamin A nutriture (18).

Physiological Effects of Carotenoids in Humans

A select few carotenoids have been investigated as potential preventative or ameliorative agents with respect to a wide variety of diseases, as recently reviewed (19). Numerous epidemiological studies have found inverse associations between dietary intake of carotenoids (or blood levels of carotenoids) and risk for various cancers, although a causal role for carotenoids cannot be ascertained from such data. Recently, lycopene intake has been linked with reduced risk of prostate cancer (20). Lycopene is a major dietary carotenoid in the United States (21), and its plasma concentration often exceeds that of *β*-carotene (22). Only *β*-carotene, and in some cases canthaxanthin, have been studied using pure supplements, owing to the lack of availability of sufficient quantities of other pure carotenoids. Clinical intervention trials with *β*-carotene in high-risk populations expected to develop cancer within a few years have yielded mixed results, with no evidence for a protective effect in cancers of the skin or lung. Protective effects of *β*-carotene have been reported with premalignancies of the oral cavity.

Carotenoid supplementation has been used successfully in the amelioration of certain inherited photosensitivity disorders, the best example of which is erythropoietic protoporphyria. Elevated skin levels of *β*-carotene or canthaxanthin have been shown to be effective in increasing light tolerance in such patients (19). The effect of *β*-carotene supplementation on various aspects of immune functions has been studied rather intensively, again with mixed results. In most cases it has not been possible to ascertain if observed effects were attributable to *β*-carotene per se or to its potential retinoid metabolites. Lutein and zeaxanthin are found in the macular pigment of the eye and are under investigation with respect to risk of macular degeneration.

Bioavailability of Carotenoids

Disparate estimates of carotene absorption efficiency, ranging from 1 to 99%, have been reported over the past three decades (23). Although it is generally recognized that the true vitamin A value of all plant foods is probably not the same, methods to more precisely determine the bioavailability of provitamin A carotenoids from foods in humans have been lacking. Plant foods contribute up to 90% of total dietary vitamin A equivalents in some developing nations where consumption of animal products is low, although the bioavailability of some plant foods has been questioned (24). Heat processing of plant food products may increase the bioavailability of at least some carotenoids (25), perhaps by weakening protein–carotenoid interactions, dissolution or dispersal of crystalline carotenoids, or improving digestibility of the matrix. The absorption efficiency of lycopene from heat-processed tomatoes (paste) is substantially greater than from an equivalent dose of fresh tomato (26). Attempts to compare the vitamin A yield from any plant food as a function of processing have not been reported.

OCCURRENCE AND FUNCTIONAL PROPERTIES OF CAROTENOIDS IN FOODS

More than 600 structurally distinct carotenoids have been identified in nature, of which only a few dozen commonly occur in human foodstuffs. Although the provitamin A and some nonprovitamin A carotenoids are important as nutrients, most carotenoids significantly influence food quality through the hues that they impart to fruits, vegetables, and animal foods. In most plant or animal tissues, only a small number of distinct carotenoids usually account for more than 80% of the total carotenoid content; examples of commodities with large numbers of carotenoids in significant quantities are relatively rare. Although some carotenoids, such as *β*-carotene, occur in many different fruits and vegetables, others, such as lycopene (tomato) and *α*-carotene (carrot), occur in appreciable quantities in only a few commodities.

NATURALLY OCCURRING CAROTENOIDS IN PLANT AND ANIMAL FOODS

In plant foods, carotenoids exist in various physical and morphological states depending on the carotenoid and the commodity. In leafy vegetables, carotenoids are associated primarily with chloroplasts as integral components of the photosynthetic apparatus. In fruits, roots, and tubers, carotenoids associate with chromoplasts or other intracellular structures, although the precise nature of their molecular orientation is largely uncharacterized (27). Most plant-associated carotenoids appear to exist in complexation with proteins or polysaccharides, rather than as true solutions in oil droplets. Although most common vegetable oils contain low concentrations of soluble carotenoids that impart yellow hues, a notable exception is the highly pigmented red palm oil, which is used sparingly in foods in some tropical countries.

Sources of Variation in Carotenoid Content of Plant Foods

Published data on the carotenoid content of fruits and vegetables reflect considerable variation for most species. For example, the β-carotene content (mg per 100 g fresh weight) has been reported to vary from 4 to 10 for carrot, 0 3 to 8.0 for tomato, and 0.1 to 5.2 for sweet potato (28). Variety, maturation, source, and analytical accuracy all contribute, in usually unknown proportions, to the reported variation. Recently, a carotenoid composition database was compiled by the USDA Food Composition Laboratory that contains updated analytical data for many fruits, vegetables, and processed foods available in the United States (29). The data were obtained predominantly by HPLC, in some cases with structural verification by mass spectrometry. A compilation of existing data on carotenoid content of foods available in other countries is also available (30), although much of these data were obtained by methods that do not clearly distinguish between different carotenoids or carotenoid isomers. Older analytical techniques often overestimate the provitamin A carotenoid content of foods, particularly fruits (31). Actual varietal differences in β-carotene content can be as high as 100-fold in carrots and tomato, and breeding techniques have increased the β-carotene content of sweet potatoes by threefold in the past 50 years (28). Several tomato varieties are now marketed with widely varying concentrations of lycopene and β-carotene.

Data concerning seasonal variation in carotenoid content have been reported for many plant foods, including carrot, kale, lettuce, snap beans, and turnip greens. In general, carrots harvested in summer contain higher levels of carotenoids than those harvested in winter months, whereas leafy and green vegetables exhibit the opposite effect of season (28). The effect of maturation on the carotenoid content of plant foods also varies by commodity. In general, carotenoid concentration of fruits and nonleafy vegetables increases with maturity, whereas that of leafy vegetables increases to a lesser extent or decreases (28). Postharvest losses of carotenoids in fruits and vegetables tends to be low. For example, the loss of β-carotene in fresh broccoli and green beans is very slight up to 9 days after harvest under typical refrigeration conditions (32). However, physical damage to fresh plant foods that disrupts normal compartmentalization can release lipoxygenases and increase exposure to oxygen or light, resulting in carotenoid oxidation and changes in product hue (5,33).

Carotenoids in Animal Foods

Dietary intake of carotenoids from animal foods is low compared to that from plant foods because of the relatively low rates of deposition of feed-associated carotenoids in most animal tissues. Tissue concentrations are entirely dependent on the carotenoid content of the feed. In animal foods, the carotenoids are localized predominantly in adipocytes, and thus generally accumulate in fatty tissues to a greater extent than in lean tissues. Animal food sources of carotenoids include egg yolks, chicken, certain fish such as salmon, and crustaceans. Xanthophylls tend to be the predominant carotenoids in animal foods, because their efficiency of tissue deposition is usually higher than that of the carotenes. Examples of the deliberate pigmentation of animal tissues using carotenoids include the use of yellow corn or marigold extracts in chicken feed and crab (or shrimp) shell waste or synthetic astaxanthin in shrimp and salmon feeds (34).

CAROTENOIDS AS COLORANT ADDITIVES IN FOODS

Carotenoids are useful to impart yellow, orange, and red hues to foods with no or minimal effects on flavor. Because of their high extinction coefficients, carotenoids used as colorants are generally added to foods in concentrations ranging from 1 to 25 ppm. Because carotenoids are generally stable across a range of unit processing conditions, they are often used as alternatives to the certified color additives (35). Carotenoid preparations used as colorants include natural isolates manufactured from plant products of high carotenoid content or synthetic carotenoids compounded into oil- or water-dispersible forms.

Natural Isolates

A variety of plant commodities rich in carotenoids have been used in the manufacture of powders or oil-based preparations suitable for use as colorants in foods. Intensely colored carotenoid isolates derived from paprika are available either as powders or oleoresins and impart orange or red hues to many products, including salad dressings, sauces, soups, beverages, snacks, and confections. A major carotenoid in these preparations is capsanthin (Fig. 1). Extracts of the annatto seed containing high concentrations of bixin are also commonly used in such products as cheeses, instant soups, dairy products, and desserts (36). Other natural isolates include products derived from carrot and tomato. Tomato oleoresins have recently become available and contain high concentrations of lycopene. These products are used in Europe but are not yet approved in the United States.

Synthetic Carotenoid Preparations

Three synthetic carotenoids, identical in chemical structure to their naturally occurring counterparts, are permanently listed by FDA as exempt from the certification requirements applied to the synthetic FD&C dyes. These carotenoids are β-carotene, β-apo-8′-carotenal, and canthaxanthin and are available in both oil-soluble and water-dispersible forms. The latter are prepared by emulsification into low-moisture colloidal beadlets. The carotenoids in these preparations are preserved by physical barriers to oxygen exposure and by the inclusion of hydrophilic and/or hydrophobic antioxidants such as ascorbic acid, ascorbyl palmitate, and α-tocopherol.

Physical Formulations of Synthetic Carotenoid Additives Used in Foods

β-Carotene is commercially available to food manufactures as vegetable oil dispersions of micronized crystalline β-carotene at concentrations ranging from roughly 10 to 30% by weight. Such concentrates, in which a portion of the β-carotene is in true solution, is diluted into warm oil to achieve a stock solution of desired concentration. These products are suitable for coloration of other oils, margarines, cheeses, ice cream, soups, or bakery products. Tocopherols naturally present or added to the vegetable oil carrier enhance stability. Several water-dispersible forms of β-carotene are commercially available to food manufacturers. Such products are produced as low-moisture beadlets 60 to 500 μ in diameter, using gelatin proteins, polysaccharides (eg, dextrin), or some combination thereof as the hydrophilic matrix material. Most are 1 to 10% β-carotene by weight and may contain antioxidants such as ascorbic acid, ascorbyl palmitate, or α-tocopherol. These products are used to color fruit juices, other beverages, pasta, cheeses, and baked goods. Usage levels are typically low, in the range of 2.5 to 3.5 mg per lb.

β-Apo-8′-carotenal is a synthetic monocyclic provitamin A carotenoid (Fig. 1) approved by the FDA for use in foods in 1963. This carotenoid is available as micronized dispersions or concentrated solutions in vegetable oil, stabilized with added α-tocopherol. It is commonly used in processed cheeses and colored salad dressings to impart orange hues. Canthaxanthin is a nonprovitamin A xanthophyll approved for food use in 1969, and it is used at concentrations ranging from 5 to 60 ppm, depending on the application and desired color intensity. Its primary advantage over the certified colors FD&C Red No. 2, FD&C Red No. 3, and paprika oleoresin is its ability to impart truer tomato-red hues in tomato-based products (35). Unlike FD&C Red No. 3, canthaxanthin will not precipitate at low pH. This carotenoid is available in water-dispersible beadlet forms and is used for coloring a variety of foods, including tomato products, simulated meat products, and surimi.

Regulatory Issues Related to Carotenoids as Food Colorants

β-Carotene, β-apo-8′-carotenal and canthaxanthin are pigments exempt from FDA certification. Carotenoid preparations may not be used to pigment foods for which a standard of identity exists unless the carotenoid has been authorized by the standard (37). Maximum legal use rates are 15 mg/lb for β-apo-8′-carotenal, and 30 mg/lb for canthaxanthin (21 CFR 101.22), although these levels are rarely approached owing to their high tinctorial properties. β-Carotene usage is governed by good manufacturing practices in which the use is restricted to that needed to accomplish the desired effect. However, since β-carotene is a vitamin A source, its use in some circumstances may be determined by a combination of nutritional and pigmentation effects. According to 21 CFR 101.22, carotenoid color additives may be labeled using an appropriate descriptor such as "artificial color or "color added" or may be identified specifically by name.

Carotenoids as Nutritional Additives

Fortification of foods with carotenoids for nutritional purposes is an uncommon but growing practice in the United States. Such products include fruit or vegetable-based drinks in which the added carotenoids augment that contributed by the ingredient commodities. Other foods, such as breakfast cereals or sports bars, are not usual sources of carotenoids. Because of the high tinctorial properties of carotenoids, the usage levels tend to be low in products that cannot tolerate intense yellow, orange, or red hues.

EFFECTS OF PROCESSING ON NATURALLY OCCURRING CAROTENOIDS AND CAROTENOID COLORANTS IN FOODS

As discussed earlier, carotenoids tend to be sensitive to oxygen, light, and heat. However, cooking-associated losses generally tend to be less than 30% (38), depending on circumstances and product. Heat-intensive processing of fruits and vegetables, such as commercial sterilization (canning), have in some cases been reported to result in significant carotenoid losses (38). However, such data have often not accounted for changes in moisture content, which generally increase with canning and other cooking procedures involving addition of water. Accounting for alteration in moisture content, carotenoid retention during canning is generally high. The major reactions which occur during canning are those involving isomerization rather than oxidation. Carotene losses during storage of canned fruits or vegetables at ambient temperatures are minimal, probably because of low oxygen tension and high water content. In most foods, an acid pH tends to stabilize carotenoid pigments by inhibiting oxidation and stereomutations.

Carotenoid retention during common moist cooking procedures is generally high. Because of their low water solubility, leaching losses are low, and moist heat is insufficient to induce significant isomerization. Thus, carotenoid retention in vegetables during boiling is usually 85 to 100% after taking into account changes in moisture content (38). There are relatively little data on the effect of home or commercial frying operations on carotenoid stability, but losses could be expected to be higher than during other unit thermal processes because of the combination of high temperature and oxygen exposure. Losses of carotenoids with freeze drying are generally low to moderate, ranging from 4 to 13% for freeze-dried orange juice and carrots, respectively, whereas tray-drying followed by explosion puffing can result in losses of up to 25 percent (39). Carotenoids commonly exhibit differential stabilities. During both processing and dark storage of paprika, β-carotene is lost more rapidly than capsanthin, with losses variable across variety of pepper (40).

Carotenoid Isomerization During Thermal Processing

Thermal processing conditions can result in isomerization of one or more *trans* double bonds to the *cis* configuration, and carotenes appear to undergo isomerization more readily than xanthophylls (5). Newer analytical methods now permit more precise quantification of *cis* carotenoid isomers in foods. Typical canning procedures can result in isomerization of 10 to 40% of carotenes in some products, including carrot, sweet potato, broccoli, and green leafy vegetables (41). Isomerization results in slight decreases in extinction coefficient and absorption maxima, but these generally have less impact on hue than reactions involving oxidation. Nutritionally, the vitamin A value of *cis* isomers of the provitamin A carotenoids is substantially less than that of their all-*trans* counterparts.

BIBLIOGRAPHY

1. K. L. Simpson, "Carotenoids," in *Encyclopedia of Food Science and Technology*, Y. H. Hui, ed., John Wiley and Sons, Inc., vol. 1A-D, pp. 293–310 (1992).
2. G. Britton, S. Liaaen-Jensen, and H. Pfander, *Carotenoids, Volume 1A: Isolation and Analysis*, Birkhauser Verlag, Basel, 1995.
3. G. Britton, S. Liaaen-Jensen, and H. Pfander, *Carotenoids, Volume 1B:Spectroscopy*, Birkhauser Verlag, Basel, 1995.
4. G. Britton, S. Liaaen-Jensen, and H. Pfander, *Carotenoids, Volume 2: Synthesis*, Birkhauser Verlag, Basel, 1995.
5. J. Davidek, J. Velisek, and J. Pokorny, *Chemical Changes During Food Processing*, Elsevier, Amsterdam, 1990.
6. H. T. Gordon and J. C. Bauernfeind, "Carotenoids as Food Colorants," *Crit. Rev. Food Sci. Nutr.* **18**, 59–96 (1983).
7. J. M. Dietz and W. A. Gould, "Effects of Process Stage and Storage on Retention of Beta-Carotene in Tomato Juice," *J. Food Sci.* **51**, 847–848 (1986).
8. M. I. Minguez-Mosquera and M. Jaren-Galan, "Kinetics of the Decolouring of Carotenoid Pigments," *J Sci. Food Agric.* **67**, 153–161 (1995).
9. B. H. Chen, H. Y. Peng, and H. E. Chen, "Changes of Carotenoids, Color, and Vitamin A Contents during Processing Carrot Juice," *J. Agric. Food Chem.* **43**, 1912–1918 (1995).
10. L. A. Wagner and J. J. Warthesen, "Stability of Spray-Dried Encapsulated Carrot Carotenes," *J. Food Sci.* **60**, 1048–1053 (1995).
11. M. Goldman, B. Horev, and I. Saguy, "Decolorization of *β*-Carotene in Model Systems Simulating Dehydrated Foods. Mechanism and Kinetic Principles," *J. Food Sci.* **48**, 751–754 (1983).
12. W. E. Doering, D. Sotiriou-Leventis, and W. R. Roth, "Thermal Interconversions among 15-*cis*, 13-*cis* and all-*trans*-*β*-Carotene: Kinetics, Arrhenius Parameter, Thermochemistry, and Potential Relevance to Anticarcinogenicity of all-trans-*β*-Carotene," *J. Am. Chem. Soc.* **117**, 2747–2757 (1995).
13. M. Kuki, Y. Koyana, and H. Nagae, "Triplet-Sensitized and Thermal Isomerization of All-trans, 7-cis, 9-cis, 13-cis and 15-cis Isomers of *β*-Carotene: Configurational Dependence of the Quantum Yield of isomerization via the T1 State," *J. Phys. Chem.* **95**, 7171–7180 (1991).
14. E. De Ritter and A. E. Purcell, "Carotenoid Analytical Methods," in J. C. Bauernfeind, ed., *Carotenoids as Colorants and Vitamin A Precursers. Technological and Nutritional Applications*, Academic Press, Orlando, Fla., 1981, pp. 815–882.
15. F. Khackik, G R. Beecher, N. F. Whittaker. "Separation, Identification, and Quantification of the Major Carotenoid and Chlorophyll Constituents in Extracts of Several Green Vegetables by Liquid Chromatography," *J. Agric. Food Chem.* **34**, 603–6 (1986).
16. F. Khachik and G. R. Beecher. "Application of a C-45-*β*-Carotene as an Internal Standard for the Quantification of Carotenoids in Yellow/Orange Vegetables by Liquid Chromatography," *J. Agric. Food Chem.* **35**, 732–738 (1987).
17. K. Schiedt and S. Liaaen-Jensen, "Isolation and Analysis," in G. Brotton, S. Liaaen-Jensen, and H. Pfander, eds., *Carotenoids, Volume 1A: Isolation and Analysis*, Birkhauser Verlag, Basel, 1995, pp. 81–83.
18. R. S. Parker, "Absorption, Metabolism and Transport of Carotenoids," *FASEB J.* **10**, 542–551 (1996).
19. J. E. Swanson and R. S Parker, "Biological Effects of Carotenoids in Humans," in E. Cadenas and L. Packer, eds., *Handbook of Antioxidants*, Marcel Dekker, Inc., New York, NY, 1996.
20. E. Giovannucci, "Tomatoes, Tomato-Based Products, Lycopene, and Cancer: Review of the Epidemiologic Literature," *J. Natl. Cancer Inst.* **91**, 317–331 (1999).
21. L. C. Nebeling et al., "Changes in Carotenoid Intake in the United States: The 1987 and 1992 National Health Interview Surveys," *J. Am. Dietetic Assoc.* **9**, 991–996 (1997).
22. M. L. Nguyen and S. J. Schwartz, "Lycopene: Chemical and Biological Properties," *Food Technol.* **53**, 38–45 (1999).
23. S. de Pee and C. West, "Dietary Carotenoids and Their Role in Combating Vitamin A Deficiency: A Review of the Literature," *Eur. J. Clin. Nutr.* **50** (Suppl 3), S38–S53 (1996).
24. S. De Pee et al., "Lack of Improvement in Vitamin A Status with Increased Consumption of Dark-Green Leafy Vegetables," *Lancet* **346**, 75–81 (1995).
25. C. L. Rock et al., "Bioavailability of *β*-Carotene is Lower in Raw Than in Processed Carrots and Spinach in Women," *J. Nutr.* **128**, 913–916 (1998).
26. C. Gartner, W. Stahl, and H. Sies, "Lycopene is More Bioavailable from Tomato Paste than from Fresh Tomatoes," *Am. J. Clin. Nutr.* **66**, 116–122 (1997).
27. J. E. Bryant et al., "Isolation and Partial Characterization of *α*- and *β*-Carotene-Containing Carotenoprotein from Carrot (*Daucus carota* L.) Root Chromoplasts," *J. Agric. Food Chem.* **40**, 545–549 (1992).
28. A. Mozafar, *Plant Vitamins: Agronomic, Physiological and Nutritional Aspects*, CRC Press, Boca Raton, Fla., 1994.
29. A. R. Mangels et al., "Carotenoid Content of Fruits and Vegetables: An Evaluation of Analytic Data," *J. Am. Dietetic Assoc.* **93**, 284–296 (1993).
30. C. E. West and E. J. Poortvliet, *The Carotenoid Content of Foods with Special Reference to Developing Countries*, Office of Nutrition, Bureau for Research and Development, U.S. Agency for International Development, Washington, D.C., 1993.
31. S. L. Booth, T. Johns, and H. V. Kuhnlein, "Natural Food Sources of Vitamin A and Provitamin A," *Food Nutr. Bull.* **14**, 6–19 (1992).
32. Y. Wu, A. K. Perry, and B. P. Klein, "Vitamin C and *β*-Carotene in Fresh and Frozen Green Beans and Broccoli in a Simulated System," *J. Food Quality* **15**, 87 (1992).
33. C. A. Pesek and J. J. Warthesen, "Photodegradation of Carotenoids in a Vegetable Juice System," *J. Food Sci.* **52**, 744–746 (1987).
34. J. C. Bauernfeind, *Carotenoids as Colorants and Vitamin A Precursors. Technological and Nutritional Applications*, Academic Press, Orlando, Fla., 1981.
35. J. D. Dziezak, "Applications of Food Colorants," *Food Technol.* **41**, 78–88 (1987).

36. D. E. Pszczola, "Natural Colors: Pigments of Imagination," *Food Technol.* **52**, 70–76 (1998).
37. FDA, Title 21.73.95, April, Food and Drug Administration, Washington, D.C., 1986.
38. E. Karmas and R. S. Harris, *Nutritional Evaluation of Food Processing*, 3rd ed., AVI Books, Van Nostrand Reihold Co., New York, 1988.
39. P. M. Bluestein and T. P. Labuza, "Effects of Moisture Removal on Nutrients," in E. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., AVI Books, Van Nostrand Reihold Co., New York, 1988, pp. 393–422.
40. M. J. Minguez-Mosquera, M. Jaren-Galan, and J. Garrido-Fernandez, "Effect of Processing of Paprika on the Main Carotenes and Esterified Xanthophylls Present in the Fresh Fruit," *J. Agric. Food Chem.* **41**, 2120–2124 (1993).
41. W. J. Lessin, G. L. Catigani, and S. J. Schwartz, "Quantification of *cis-trans* Isomers of Provitamin A Carotenoids in Fresh and Processed Fruits and Vegetables," *J. Agric. Food Chem.* **45**, 3728–3732 (1997).

ROBERT PARKER
Cornell University
Ithaca, New York

See also CAROTENOID PIGMENTS; COLORANTS: CAROTENOIDS.

PHYTOCHEMICALS: LIPOIC ACID

α-Lipoic acid is a naturally occurring compound present as a cofactor in a number of mitochondrial enzymes that are involved in metabolism and energy production. In its free form, lipoic acid is a powerful antioxidant, functioning as a free-radical scavenger and an antioxidant "protector." Recently, attention has focused on lipoic acid as a cellular redox regulator, having the capacity to interact at various stages in signal transduction pathways. Such properties make this compound of potential therapeutic importance in conditions in which oxidative stress is involved. These properties and their implications are discussed in the article.

NATURAL SOURCES OF LIPOIC ACID

Lipoic acid is an eight-carbon compound containing two sulfur atoms in a (dithiolane) ring structure. The naturally occurring form is present bound to a lysine residue in the four α-keto-acid dehydrogenase complexes of the mitochondria, which catalyze the oxidative decarboxylation of pyruvate, α-ketoglutarate, and branched-chain α-keto acids, respectively. These complexes all share similarities in both structure and function (1). The role of lipoic acid is to transfer an acyl group from the decarboxylated acid to coenzyme A. During this process, the lipoyl moiety is reduced and then reoxidized by the enzyme lipoamide dehydrogenase in order to keep the catalytic cycle in motion. The redox properties of lipoic acid make it ideal for such a function. As such, lipoic acid plays a crucial role in metabolism.

Lipoic acid was first isolated in 1951 by Reed and colleagues (2) and was tentatively described as a vitamin until it was later discovered to be synthesized in both plant and animal tissues. Recently, the levels of naturally occurring lipoic acid (lipoyllysine) in both plant and animal tissues have been determined (3). In animal tissues, the content was found to be tissue-specific, the highest levels being found in the kidney, heart, and muscular tissues (2.6, ~1, and ~1 μg/g dry weight, respectively), while lower amounts were found in the brain and lung (0.2 μg/g dry weight). Since natural source lipoic acid is present in mitochondrial enzymes, it is highly likely that lipoic acid content will correlate with metabolic activity of the tissue. In plant tissues, the highest concentrations were found in green tissues, especially spinach (3.6 μg/g dry weight). Plants are unique in that they possess a chloroplastic form of lipoic acid; therefore, lipoic acid content, dependent on the type of tissue, will be determined by the density of mitochondria and chloroplasts. Such low levels of lipoic acid (~0.1% total weight of a mitochondrion) express the need for supplementation if the antioxidant properties of lipoic acid are to be gained. The antioxidant properties of lipoic acid can rarely be achieved through a normal diet.

BIOAVAILABILITY

When taken orally as a supplement or administered *in vitro*, α-lipoic acid is rapidly absorbed and taken up into cells where it is reduced to the more active form, dihydrolipoic acid. The uptake of exogenously supplied α-lipoic acid has been studied in the perfused rat liver and isolated hepatocytes (4). Two different transport mechanisms were reported: carrier-mediated uptake, which is prominent below 75 μM, and passive diffusion, which is prominent at higher concentrations. This study also showed that the carrier-mediated uptake can be blocked by medium-chain fatty acids, suggesting that the same translocator was in use (4). In vitro studies have investigated the various cellular reduction pathways of lipoic acid (5). Two mechanisms exist that are both tissue- and stereo-specific. The natural R enantiomer is preferentially reduced in the mitochondria by lipoamide dehydrogenase, a nicotinamide adenine nucleotide (NADH) dependent enzyme. The S form is predominantly reduced in the cytosol by glutathione and/or thioredoxin reductase, which are NADPH dependent. The various contributions of each pathway are dependent upon the tissue: heart muscle almost entirely reduces the R form, while in the liver there is an equal distribution between the two pathways.

Reduction contributes one mechanism of lipoate metabolism. Other routes have been demonstrated using radiolabeled lipoic acid administered either orally (6) or via injection (7) into the rat. Several components were identified, including the short-chain homologues bisnorlipoic and tetranorlipoic acids (7). Interestingly, no evidence for the oxidation of the dithiolane ring was observed. Administration of [1,6-^{14}C] lipoic acid has shown that lipoic acid is rapidly absorbed in the gut and passed to various tissues for catabolism (6). The localization of administered lipoate was greatest in the liver, other intestinal organs, and

36. D. E. Pszczola, "Natural Colors: Pigments of Imagination," *Food Technol.* **52**, 70–76 (1998).
37. FDA, Title 21.73.95, April, Food and Drug Administration, Washington, D.C., 1986.
38. E. Karmas and R. S. Harris, *Nutritional Evaluation of Food Processing*, 3rd ed., AVI Books, Van Nostrand Reihold Co., New York, 1988.
39. P. M. Bluestein and T. P. Labuza, "Effects of Moisture Removal on Nutrients," in E. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., AVI Books, Van Nostrand Reihold Co., New York, 1988, pp. 393–422.
40. M. J. Minguez-Mosquera, M. Jaren-Galan, and J. Garrido-Fernandez, "Effect of Processing of Paprika on the Main Carotenes and Esterified Xanthophylls Present in the Fresh Fruit," *J. Agric. Food Chem.* **41**, 2120–2124 (1993).
41. W J. Lessin, G. L. Catigani, and S. J. Schwartz, "Quantification of *cis-trans* Isomers of Provitamin A Carotenoids in Fresh and Processed Fruits and Vegetables," *J. Agric. Food Chem.* **45**, 3728–3732 (1997).

Robert Parker
Cornell University
Ithaca, New York

See also Carotenoid pigments; Colorants: carotenoids.

PHYTOCHEMICALS: LIPOIC ACID

α-Lipoic acid is a naturally occurring compound present as a cofactor in a number of mitochondrial enzymes that are involved in metabolism and energy production. In its free form, lipoic acid is a powerful antioxidant, functioning as a free-radical scavenger and an antioxidant "protector." Recently, attention has focused on lipoic acid as a cellular redox regulator, having the capacity to interact at various stages in signal transduction pathways. Such properties make this compound of potential therapeutic importance in conditions in which oxidative stress is involved. These properties and their implications are discussed in the article.

NATURAL SOURCES OF LIPOIC ACID

Lipoic acid is an eight-carbon compound containing two sulfur atoms in a (dithiolane) ring structure. The naturally occurring form is present bound to a lysine residue in the four α-keto-acid dehydrogenase complexes of the mitochondria, which catalyze the oxidative decarboxylation of pyruvate, α-ketoglutarate, and branched-chain α-keto acids, respectively. These complexes all share similarities in both structure and function (1). The role of lipoic acid is to transfer an acyl group from the decarboxylated acid to coenzyme A. During this process, the lipoyl moiety is reduced and then reoxidized by the enzyme lipoamide dehydrogenase in order to keep the catalytic cycle in motion. The redox properties of lipoic acid make it ideal for such a function. As such, lipoic acid plays a crucial role in metabolism.

Lipoic acid was first isolated in 1951 by Reed and colleagues (2) and was tentatively described as a vitamin until it was later discovered to be synthesized in both plant and animal tissues. Recently, the levels of naturally occurring lipoic acid (lipoyllysine) in both plant and animal tissues have been determined (3). In animal tissues, the content was found to be tissue-specific, the highest levels being found in the kidney, heart, and muscular tissues (2.6, ~1, and ~1 μg/g dry weight, respectively), while lower amounts were found in the brain and lung (0.2 μg/g dry weight). Since natural source lipoic acid is present in mitochondrial enzymes, it is highly likely that lipoic acid content will correlate with metabolic activity of the tissue. In plant tissues, the highest concentrations were found in green tissues, especially spinach (3.6 μg/g dry weight). Plants are unique in that they possess a chloroplastic form of lipoic acid; therefore, lipoic acid content, dependent on the type of tissue, will be determined by the density of mitochondria and chloroplasts. Such low levels of lipoic acid (~0.1% total weight of a mitochondrion) express the need for supplementation if the antioxidant properties of lipoic acid are to be gained. The antioxidant properties of lipoic acid can rarely be achieved through a normal diet.

BIOAVAILABILITY

When taken orally as a supplement or administered *in vitro*, α-lipoic acid is rapidly absorbed and taken up into cells where it is reduced to the more active form, dihydrolipoic acid. The uptake of exogenously supplied α-lipoic acid has been studied in the perfused rat liver and isolated hepatocytes (4). Two different transport mechanisms were reported: carrier-mediated uptake, which is prominent below 75 μM, and passive diffusion, which is prominent at higher concentrations. This study also showed that the carrier-mediated uptake can be blocked by medium-chain fatty acids, suggesting that the same translocator was in use (4). In vitro studies have investigated the various cellular reduction pathways of lipoic acid (5). Two mechanisms exist that are both tissue- and stereo-specific. The natural R enantiomer is preferentially reduced in the mitochondria by lipoamide dehydrogenase, a nicotinamide adenine nucleotide (NADH) dependent enzyme. The S form is predominantly reduced in the cytosol by glutathione and/or thioredoxin reductase, which are NADPH dependent. The various contributions of each pathway are dependent upon the tissue: heart muscle almost entirely reduces the R form, while in the liver there is an equal distribution between the two pathways.

Reduction contributes one mechanism of lipoate metabolism. Other routes have been demonstrated using radiolabeled lipoic acid administered either orally (6) or via injection (7) into the rat. Several components were identified, including the short-chain homologues bisnorlipoic and tetranorlipoic acids (7). Interestingly, no evidence for the oxidation of the dithiolane ring was observed. Administration of [1,6-^{14}C] lipoic acid has shown that lipoic acid is rapidly absorbed in the gut and passed to various tissues for catabolism (6). The localization of administered lipoate was greatest in the liver, other intestinal organs, and skeletal muscle (6). The presence of both oxidized and reduced forms has also been demonstrated in various tissues of vitamin-E-deficient mice after lipoic acid supplementation (8).

ANTIOXIDANT PROPERTIES OF LIPOIC ACID

The strained-ring conformation of lipoic acid enables the molecule to readily undergo oxidation and reduction, and indeed this is reflected in the high negative redox potential of the oxidized and reduced form (−0.32 V), which makes the reduced form (dihydrolipoate) a very powerful reductant. Such properties enable the lipoate couple to interact with a number of reactive oxygen species.

Various cellular pathways like those involved in the electron transport pathways of cell respiration and drug detoxification or activation of neutrophils and macrophages result in the formation of reactive oxygen and nitrogen species. If overproduced and not scavenged or converted to a nonreactive form, they can be potentially damaging to the cell. These include superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hyperchlorous acid (HOCl), hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals as well as nitric oxide ($NO^{\cdot}$) and peroxynitrite(ONOO). α-Lipoic acid and dihydrolipoic acid can scavenge both hydrogen peroxide and HOCl (9), while dihydrolipoic acid can also scavenge superoxide (9). Both α-lipoic and dihydrolipoic acid have been shown to scavenge hydroxyl radicals in a metal-catalysis system and in a metal-free reaction of ultraviolet irradiation induced by decomposition of the aromatic hydroperoxide model compound NP-III (9). Dihydrolipoic acid can also scavenge peroxyl radicals, formed from both lipophilic and hydrophilic peroxyl radical generators (9). Therefore, the lipoate couple represents a potent radical scavenging unit.

When antioxidants react with reactive oxygen species, the antioxidant is converted to a form that is no longer able to function and is said to be consumed. Therefore, this oxidized product needs to be recycled to this native form in order to function again. A number of antioxidants, including vitamin C, ubiquinols, and glutathione, can recycle vitamin E, the major chain-breaking antioxidant that protects biological membranes from lipid peroxidation. Dihydrolipoic acid has only a weak interaction with the tocopheroxyl radical, so the major recycling of vitamin E by dihydrolipoic acid occurs via the intermediary recycling of the ascorbyl radical by dihydrolipoic acid, which, in turn, recycles the vitamin E radical produced by oxidation. There is now evidence that lipoate supplementation increases tissue ubiquinol content, and ubiquinol can also recycle vitamin E. Therefore, there exists a network of antioxidants in which dihydrolipoic acid can interact and replenish in order to maintain both lipid and aqueous phase antioxidant status. This recycling by lipoate was shown as early as 1959 by Rosenberg and Culik (10), who demonstrated that lipoic acid supplementation could prevent the symptoms of both vitamin E and C deficiencies in guinea pigs. This work has since been repeated in vitamin-E-deficient mice (8).

Glutathione is a major intracellular antioxidant that acts as a sulfhydryl buffer, protecting cysteine residues in proteins from oxidation, the modulation of which has been discussed as a potential therapeutic strategy (11). α-Lipoate can interact with glutathione (GSH) both directly and indirectly. Dihydrolipoic acid (DHLA) can reduce oxidized glutathione (GSSG) to GSH, but GSH is incapable of reducing α-lipoate to DHLA (12). DHLA may also recycle vitamin E by reducing GSSG directly; the reduced GSH can then recycle vitamin E (13). Administration of α-lipoate to cells has been shown to cause an increase in intracellular GSH *in vitro* (14) and *in vivo* (15). This increase has been shown to be a consequence of increased GSH synthesis due to an improvement in cystine utilization (14).

SIGNAL TRANSDUCTION

Recently, attention has been focused on the role of oxidants and antioxidants in the regulation of intracellular signal transduction processes. Redox changes in cells trigger molecular responses, one such response being the activation of the transcription factor NF-κB. Most agents activating NF-κB tend to trigger the formation of reactive oxygen species or are oxidants themselves (eg, $O_2^{\cdot -}$, H_2O_2, or lipoxygenase products) (16). α-Lipoate can inhibit NF-κB activation induced by phorbol ester or tumor necrosis factor-α (TNF-α) in Jurkat T cells in a dose-dependent fashion (17), and both enantiomers of lipoate were found to be effective. It has recently been shown that the ability of α-lipoate to inhibit NF-κB was not dependent on increased intracellular glutathione levels (18). Following the activation of NF-κB, there is translocation into the nucleus and binding to specific areas of DNA, which elicits the response. α-Lipoate has been shown to inhibit the DNA binding of NF-κB (19); however, DHLA was found to enhance binding. Also, the inhibition of DNA binding by diamide can be overcome by the addition of DHLA. These observations suggest that two modes of redox regulation exist in cell signaling for NF-κB (20); the first, a requirement of oxidative processes in activating NF-κB; the second, a requirement for reductive processes in DNA binding.

THERAPEUTIC IMPLICATIONS

The chronic and degenerative diseases associated with aging that have been examined in detail have shown the involvement of free radicals or oxidants in their pathophysiology. Therefore, antioxidants may be important in slowing, and perhaps in the future will be of benefit in their treatment. All organ systems appear to be involved, from the eye, which is sensitive to photo-oxidative free-radical mediated reactions, to neurodegeneration, to initial stages of cancer, to cardiovascular diseases, and to viral activation, just to name a few. Redox and antioxidant regulation of physiological and pathophysiological processes are therefore assuming greater importance. These include redox-based antioxidants such as vitamin C and E; bioflavonoids and thiol antioxidants such as glutathione; and thioredoxin and α-lipoic acid, which are major substances that interact in the antioxidant network.

The properties of α-lipoate lead to the possibility that it may influence intracellular function via antioxidant actions and through affecting the redox status of thiol-containing proteins (9). Indeed, α-lipoic acid can be thought of as a metabolic antioxidant since it is a naturally occurring substance reduced by several cellular enzymatic systems. Beneficial effects of lipoic acid administration have been reported in diabetic complications (22,23), having been used in the treatment of diabetic neuropathy in Germany; ischemia-reperfusion injury (24); and liver disease (23). Furthermore, lipoic acid is be a good candidate for treatment in AIDS, neurodegenerative diseases (21), or heavy metal poisoning (9).

BIBLIOGRAPHY

1. M S. Patel and T. E. Roche, *FASEB J.* **4**, 3224–3233 (1990).
2. L. J. Reed et al., *Science* **114**, 93–94 (1951).
3. J. K. Lodge et al., *J. Appl. Nutr.* **49**, 3–11 (1997).
4. J. Peinado, H. Sies, and T. P. M. Akerboom, *Arch. Biochem. Biophys.* **273**, 389–395 (1989).
5. N. Haramaki et al., *Free Radic. Biol. Med.* **22**, 535–542 (1997).
6. E. H. Haramaki and D. B. McCormick, *Arch. Biochem. Biophys.* **160**, 514–522 (1974).
7. J. T. Spence and D. B. McCormick, *Arch. Biochem. Biophys.* **174**, 13–19 (1976).
8. M. Podda et al., *Biochem. Biophys. Res. Commun.* **204**, 98–104 (1994).
9. L. Packer, E. H. Witt, and H. J. Tritschler, *Free Radic. Biol. Med.* **19**, 227–250 (1995).
10. H. R. Rosenberg and R. Culik, *Arch. Biochem. Biophys.* **80**, 86–93 (1959).
11. A. Meister, in D. Dolphin, R. Poulson, and O. Avramovic, eds., *Glutathione: Chemical, Biochemical, and Medical Aspects*, New York, John Wiley & Sons, 1989, pp. 367–373.
12. P. C. Jocelyn, *Eur. J. Biochem.* **2**, 327–331 (1967).
13. A. Bast and G. R. M. M. Haenen, *Biochim. Biophys. Acta* **963**, 558–561 (1988).
14. D. Han et al., *Biofactors* **9**, 1–18 (1997).
15. E. Busse et al., *Arzneim. Forsch.* **43**, 829–831 (1992).
16. P. A. Baeuerle and T. Henkel, *Annu. Rev. Immunol.* **12**, 141–179 (1994).
17. Y. J. Suzuki, B. B. Aggarwal, and L. Packer, *Biochem. Biophys. Res. Commun.* **189**, 1709–1715 (1992).
18. C. K. Sen and L. Packer, *FASEB J.* **10**, 709–720 (1996).
19. Y. J. Suzuki et al., *Biochem. Mol. Biol. Int.* **36**, 241–246 (1995).
20. L. Packer, S Roy, and C. K. Sen, *Adv. Pharmacol.* **38**, 79–101 (1996).
21. L. Packer, H. J. Tritschler, and K. Wessel, *Free Radic. Biol. Med.* **22**, 359–378 (1997)
22. S. Jacob et al., *Exp. Clin. Endocrinol.* **104**, 284–288 (1996).
23. J. Bustamante et al., *Free Radic. Biol. Med.* **24**, 1023–1039 (1998).
24. M. Panigrahi *Brain Res.* **717**, 184–188 (1996).

JOHN K. LODGE
LESTER PACKER
University of California
Berkeley, California

PHYTOCHEMICALS: MELATONIN

N-acetyl-5-methoxytryptamine (melatonin) was first isolated from the pineal gland, a small organ in the brain of the mammal, 40 years ago. Since that time it has been characterized as the chief secretory product of the pineal gland of vertebrates (1) and linked to a variety of functions, including the regulation of seasonal reproduction, immune function stimulation, tumor inhibition, sleep induction, circadian rhythm regulation, and antioxidant function. In all vertebrates and most invertebrates where melatonin synthesis has been assessed, it is primarily produced in the pineal gland during the dark phase of the light/dark cycle. Because melatonin is normally released into the blood, circulating levels of melatonin are always higher at night than during the day.

Long before melatonin was found to exist in plants, the indoleamine was observed to have effects in plant cells similar to those in animals. Thus, in 1969 Jackson reported that melatonin increased the birefringence of the mitotic spindles in endosperm cells of *Haemanthus katherinae* just as it did in melanocytes obtained from a frog (2). Likewise, soon thereafter melatonin was reported to have similar cytoskeletal effects in onion root tips (3). Such cytoskeletal actions of melatonin may explain the effects of the indoleamine on the mitotic activity of cells from a variety of species.

IDENTIFICATION OF MELATONIN IN PLANTS

Melatonin was first detected and quantified in the dinoflagellate, *Gonyaulax polyedra* (4,5). In this species, melatonin was found to be at concentrations similar to those measured in the pineal gland of vertebrates. In addition to its obvious presence in this autotrophic organism, melatonin also exhibited a rhythm in these unicells like that observed in the pineal gland. Thus, melatonin levels measured either by high performance liquid chromatography or gas chromatography–mass spectrometry were higher at night than during the day. This finding stimulated research directed at identifying melatonin in higher plants, and in recent years, this indoleamine has been found in a remarkably large number of taxa (Table 1). Among angiosperms, melatonin has been identified in more than 30 species belonging to 19 different families, in both mono- and dicotyledons. Although melatonin has been reported in a rather small number of species, their diversity and the fact that almost all plants analyzed have been shown to contain melatonin suggests that this indoleamine may be ubiquitous in the plant kingdom. Likewise, within plant tissues, melatonin appears to be widespread, because it has been found in leaves, stems, roots, fruit, and seeds.

The concentration of melatonin in the plants studied varies widely, with the indoleamine being below detectable levels in potato tubers and very low in beet roots (2 pg/g weight) (12) to more than 5 ng/g weight in tall fescue (8). Even higher concentrations have been reported for some medicinal plants (Table 2). It is possible that some of the measurements actually underestimated the melatonin levels inasmuch as melatonin could easily have been de-

Table 1. Summary of the Species of Algae and Green Plants in Which Melatonin (*N*-acetyl-5-methoxytryptamine) Has Been Identified

Taxon	Species	Ref.
	Dinophyta	
	Gonyaulax polyedra	4,5
	Alexandrium lusitanicum	6
	Amphidinium carterae	6
	Phaeophyta	
	Pterygophora californica	7
	Chlorophyta angiospermae	
Rosaceae	*Malus domestica*	
	Fragaria magna	8
Brassicaceae	*Brassica oleracea*	
	Brassica compestris	
	Raphanus sativus	8
Apiaceae	*Angelica keiskej*	8
Actinidiaceae	*Actinidia chinensis*	8
Basellaceae	*Basella alba*	8
Chenopodiaceae	*Chenopoduim rubrum*	
	Beta vulgaris	9,10
Convolvulaceae	*Pharbitis nil*	11
Solanaceae	*Lycopersicon esculentum*	
	Lycospericon pimpinellifolium	
	Nicotiana tabacum	8,12
Cucurbitaceae	*Cucumis sativus*	8,12
Asteraceae	*Chrepanthemum coronarium*	
	Petasites japonicus	8
Liliaceae	*Allium cepa*	
	Allium fistulosum	
	Asparagus officinalis	8
Bromeliaceae	*Ananas comosus*	8
Poaceae	*Oryza sativa japonica*	8
	Hordeum vulgare	8
	Avena sativa	8
	Festuca arundinacea	8
	Zea mays	8
Zingiberaceae	*Zingiber officinale*	8
Musaceae	*Musa paradisiaca*	12
Araceae	*Colocasia esculenta*	8
Ateraceae	*Tanacetum parthenium*	13
Hypericaceae	*Hypericum perforatum*	13
Lamiaceae	*Scutellaria biacalensis*	13

Note: Melatonin was identified using a variety of techniques, including high-performance liquid chromatography, gas chromatography–mass spectroscopy, and radioimmunoassay.

graded during the extraction procedures used in these studies (14).

Table 2. Reported Melatonin Concentration in Commercially Obtained Medicinal Plants

Portion of plant	Melatonin (μg/g)
Tancetum parthenium	
Fresh green leaf	2.45
Fresh golden leaf	1.92
Freeze-dried green leaf	2.19
Freeze-dried golden leaf	1.61
Oven-dried green leaf	
Oven-dried golden leaf	1.69
Hypericum perforatum	
St. John's wort flower	4.39
St. John's wort leaf	1.75
Scutellaria biocalensis	
Huang-quin	7.11

Note: The three plants studied included feverfew (*Tanacetum parthenium*), St. John's wort (*Hypericum perforatum*), and Huang-qin (*Scutellaria biacalensis*).
Source: Reprinted with permission for Ref. 13.

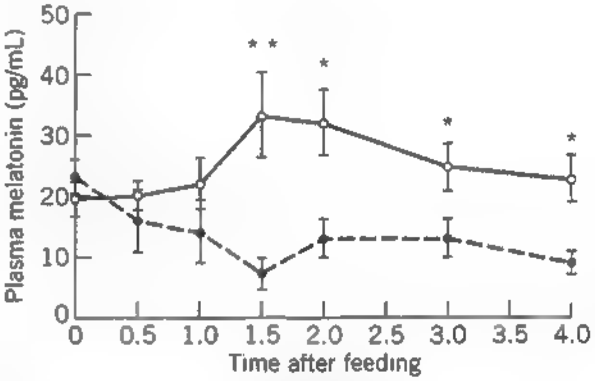


Figure 1. Blood levels of melatonin in chicks fed high-melatonin feed (3.5 mg/g) (open circle) compared to levels in chicks given low melatonin feed (<100 pg/g) (closed circle). This indicates that melatonin foods are absorbed and can increase circulating daytime levels of melatonin. The horizontal axis is in hours. $*p < 0.05$; $**p < 0.01$ is mean values in low-melatonin feed chicks. *Source:* Reprinted with permission from Ref. 8.

Among the normally consumed plant products that have been investigated to date, oats, rice, and barley products contain the largest amounts of measurable melatonin (8). When diets consisting of these plants were fed to chicks, levels of melatonin significantly increased in the circulation (Fig. 1) of the birds, and the authors of Reference 8 further showed that the ingested melatonin was bound to melatonin receptors in the animals. Clearly, the implication of these findings is that melatonin consumed in plant products is absorbed, enters the circulation, and could have physiological effects via specific receptors. Additionally, as will be discussed later, some of the melatonin's actions may be independent of receptors or binding sites so any ingested indoleamine may have a variety of actions, not only those that depend on its binding to receptors.

The lowest levels of melatonin are found in subterranean tubers, such as beet root and potato (12). According to R. Dubbels (personal communication), the concentrations of melatonin in tomatoes is inversely related to the degree of ripeness of the fruit. Thus, as fruit ripens, the concentration of melatonin seems to decrease accordingly.

TWENTY-FOUR-HOUR RHYTHMS IN PLANT MELATONIN

As already noted, a marked nocturnal rise in melatonin has been reported in the unicell, *Gonyaulax polyedra* (4).

The rise in melatonin in this organism begins with the onset of darkness, peaks near the middle of the dark phase of the light/dark cycle and then diminishes as morning approaches; this is highly reminiscent of the melatonin synthesis cycle in the pineal gland of mammals, including humans (15). A melatonin rhythm similar of that described in *G. polyedra* has also been measured in *Chenopoduim rubrum* (8) In this case, 15-day-old plants were maintained under a light/dark cycle of 12:12 (in hours). Melatonin concentrations were measured in the above-ground parts of the plant sampled at 2-hr intervals over a 24-hr period; during the dark period, the samples were collected with the aid of a dim green light. During the day, melatonin levels were uniformly low and in some cases undetectable. In contrast, during darkness, indoleamine concentrations began to increase to reach a peak at 4 to 6 hr after darkness onset; during the latter half of the dark period, melatonin levels diminished to day times values (Fig. 2). Whether the duration of elevated melatonin in either *G. polyedra* or *C. rubrum* would vary with the duration of darkness, as is the case in the mammalian pineal gland, has not been tested. Likewise, whether acute light exposure at night would alter the nocturnal increase in melatonin in plants is not known.

C. rubrum was specifically selected for the melatonin study because this obligate short-day plant exhibits pronounced and precise photoperiodic responses and rhythmic behavior; an endogenous rhythm in the flowering response, which is proportional to the duration of the dark period; and a sensitivity to night breaks with red light. Whether the melatonin rhythm relates to these cycles obviously remains unknown, as does whether other plants exhibit similar 24-hr rhythms in their melatonin content.

FUNCTION OF MELATONIN IN PLANTS

The functions of melatonin in animals are highly diverse and involve receptor and nonreceptor-related actions of this chemical mediator (1,15,16). It seems possible that melatonin in plants has some of these same functions. Melatonin's action at the level of the biological clock of mammals is well documented (17). Based on the observations of the pronounced melatonin rhythm in *C. rubrum*, it was assumed that this cycle may relate to other 24-hr metabolic and morphogenetic rhythms (10). However, because so little is known of the circadian oscillator in plants, a mechanism for how the cycle of melatonin relates to the oscillator(s) remains uninvestigated.

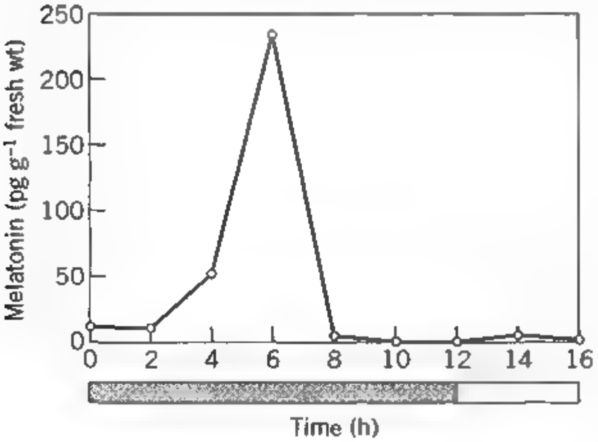


Figure 2. Nocturnal increase in the melatonin concentration in the above-ground parts of *Chenopodium rubrum* plants maintained under a light/dark cycle of 12:12. The dark bar at the bottom indicates the period of darkness. *Source:* Reprinted with permission from Ref. 10.

A likely function of melatonin in plants relates to its ability to scavenge free radicals and function as an antioxidant (18–20). Melatonin is a direct scavenger of the hydroxyl radical (·OH) (21–23), the peroxynitrite anion ($ONOO^-$) (24,25), and possibly the peroxyl radical (LOO·) (26) and singlet oxygen ($^1O_2$) (27). Likewise, it either increases mRNA levels and/or the activity of several antioxidative enzymes in mammalian tissues (28,29). In view of these findings, it is not surprising that melatonin would also be tested for its ability to resist oxidative damage in plants as well.

The herbicide paraquat generates reactive oxygen species and free radicals in a variety of plants and animals, including *G. polyedra* (30). When this bioluminescent dinoflagellate is incubated with paraquat, the herbicide reduces, in a concentration-dependent manner, the nocturnal glow peak of the organism (Fig. 3) (31), indicating that a regulator molecule required for the expression of the bioluminescence, such as 5-methoxytryptamine and/or its precursor molecule melatonin, is oxidized by the free radicals generated by paraquat. The addition of supplemental melatonin to the incubation medium prevents the inhibitory action of paraquat on bioluminescence. These findings are consistent with the restoration of the glow peak being a consequence of melatonin's ability to scavenge free radicals generated by paraquat.

In another model of lethal oxidative stress in *G. polyedra*, melatonin was found to protect the unicell from death (32). While basal levels of melatonin in *G. polyedra* vary between roughly 0.1 and 1 μM, depending on the circadian phase (4), in response to low temperature melatonin levels can increase by orders of magnitude reaching the μM range (33). When *G. polyedra* were incubated with hydrogen peroxide (H_2O_2), which is readily converted to the ·OH, the cells exhibited a several-fold enhanced bioluminescence for several hours, indicating severe oxidative damage to the cells. Adding melatonin to the incubation medium 1 hr before the addition of H_2O_2 prevented the intense light emission and cell death. In this case, the melatonin concentrations that were added, although in the mM range, were physiological for this species. These findings, then, provide strong evidence that melatonin functions as a physiological antioxidant in the dinoflagellate.

It has also been speculated that melatonin functions as an antioxidant in higher plants as well (12). Ozone, which generates free radicals and reduces plant growth, causes visible foliar damage and reduces the fresh weight of fruits when applied at the time of flowering (34,35). *Lycopersicon pimpinelliform* is highly sensitive to ozone (36) and contains very low levels of melatonin (12). Similarly, leaves of different varieties of *Nicotiana tabacum* are differentially

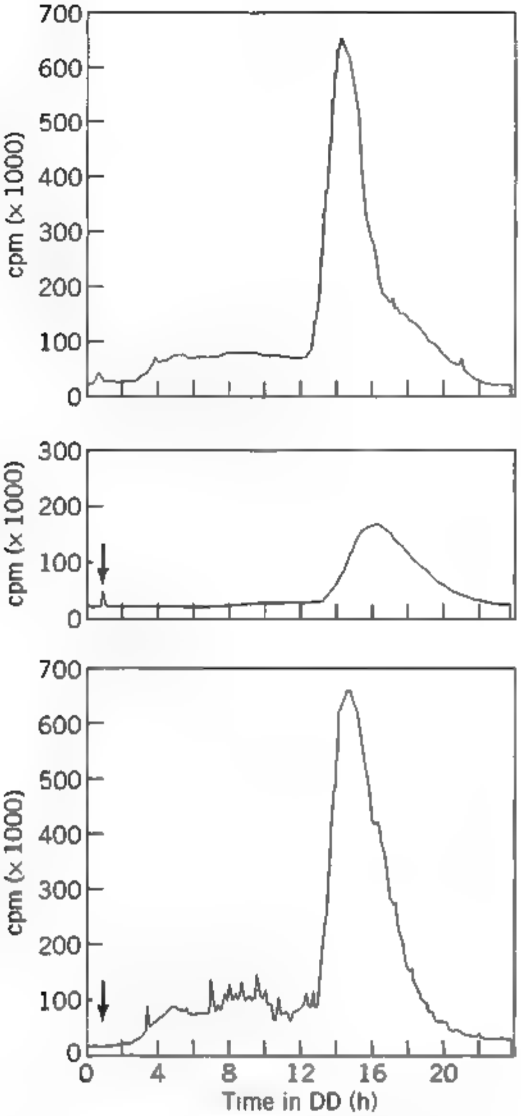


Figure 3. Spontaneous bioluminescence in *Gonyaulax polyedra* during darkness (DD). From top to bottom, control; middle, paraquat treatment that markedly suppresses to glow peak, bottom, combination of paraquat and melatonin showing that melatonin restored the glow peak. *Source:* Reprinted with permission from Ref. 31.

sensitive to ozone damage, and the sensitivity is lower in the leaves with the highest melatonin concentrations. This is consistent with the idea that melatonin in plants, as in animals, functions as an antioxidant.

Finally, considering melatonin's obvious function in animals as a modulator of circadian rhythms, the 24-hr fluctuations in this indoleamine in plants (9–11) and algae (4) indicates it may also be involved in the regulation of circadian rhythms and photoperiodicity in these species. Although there are no studies on these interactions in higher plants, in algae (18,37) the evidence is quite compelling for a role of melatonin in a variety of functions that vary over the light/dark cycle.

CONCLUSION

Relatively few plants have been examined as to their melatonin levels, but the preliminary data indicate the indoleamine is present and that its concentration may vary greatly over the light/dark cycle and between different species. Its presence in edible plants suggests that melatonin, like vitamin antioxidants, may be ingested in sufficient quantities to provide at least some protection against free radicals. In the plants themselves, melatonin is believed to function as a free-radical scavenger and in photoperiodism. This area of research is in its early stages of development, and the next several years will likely be associated with a substantial increase in the amount of information related to both the content and function of melatonin in plants.

BIBLIOGRAPHY

1. R. J. Reiter, "Pineal Melatonin: Cell Biology of Its Synthesis and of Its Molecular Interactions," *Endocrine Rev.* **12**, 151–180 (1991).
2. W. T. Jackson "Regulation of Mitosis. II. Interaction of Isopropyl *N*-Phenyl-Carbomate and Melatonin," *J. Cell Sci.* **5**, 745–755 (1969).
3. S. Banerjee and L. Margulis, "Mitotic Arrest by Melatonin," *Exp. Cell Res.* **78**, 314–318 (1973).
4. B. Poeggeler et al., "Pineal Hormone Melatonin Oscillates also in the Dinoflagellate *Gonyaulax polyedra*," *Naturwissenschaften* **78**, 268–269 (1991).
5. I. Balzer, B. Poeggeler, and R. Hardeland, "Circadian Rhythm of Indolemines in a Dinoflagellate, *Gonyaulax polyedra*: Persistence of Melatonin Rhythm in Constant Darkness and Relationships to 5-Methoxytryptamine," in Y. Touitou, J. Arendt, P. Pevet, eds., *Melatonin and the Pineal Gland*, Elsevier, Amsterdam, 1993
6. I. Balzer and R. Hardeland "Melatonin in Algae and Higher Plants—Possible New Roles as a Phytohormone and Antioxidant," *Botanica Acta* **109**, 180–183 (1996).
7. B. Fuhrberg et al., "The Vertebrate Pineal Hormone Melatonin Is Produced by the Brown Alga *Pterygophora californica* and Mimics Dark Effects on Growth Rate in the Light," *Planta* **200**, 125–131 (1996).
8. A. Hattori et al., "Identification of Melatonin in Plants and Its Effects on Plasma Melatonin Levels and Binding to Melatonin Receptors in Vertebrates," *Biochem. Mol. Biol. Int.* **35**, 627–634 (1995).
9. J. Kolar et al., "Melatonin in Higher Plants Determined by Radioimmunoassay and Liquid Chromatography-Mass Spectrometry," *Biol. Rhythm Res.* **26**, 406 (1996).
10. J. Kolar et al., "Melatonin. Occurrence, and Daily Rhythm in *Chenopodium rubrum*," *Phytochemistry* **44**, 1407-1414 (1997).
11. D. L. Van Tassel, J. Li, S. D. O'Neil "Melatonin: Identification of a Potential Dark Signal in Plants," *Plant Physiol.* **102** (Suppl. 1), 659 (1993).
12. R. Dubbels et al., "Melatonin in Edible Plants Identified by Radioimmunoassay and by High Performance Liquid Chromatography-Mass Spectroscopy," *J. Pineal Res.* **18**, 28–31 (1995).
13. S. J. Murch, C. B. Simmons, and P. K. Saxena, "Melatonin in Feverfew and Other Medicinal Plants," *Lancet* **350**, 1598–1599 (1997)

14. B. Poeggeler and R. Hardeland, "Detection and Quantification of Melatonin in a Dinoflagellate, *Gonyaulax polyedra*, Solutions to the Problem of Methoxyindole Destruction in Non-vertebrate Material," *J. Pineal Res.* **17**, 1–10 (1994).
15. R. J. Reiter "The Melatonin Rhythm: Both a Clock and a Calendar," *Experientia* **49**, 654–664 (1993).
16. J. Arendt, *Melatonin and the Mammalian Pineal Gland*, Chapman and Hall, London, 1995.
17. A. J. Lewy, R. L. Sack, and C. M. Singer, "Bright Light, Melatonin, and Biological Rhythms in Humans," in J. Montplaisir and R. Godbout, eds., *Sleep and Biological Rhythms*, Oxford Press, New York, 1990
18. R. Hardeland et al., "On the Primary Functions of Melatonin in Evolution, Mediation of Photoperiodic Signals in a Unicell, Photooxidation and Scavenging Free Radicals," *J. Pineal Res.* **18**, 104–111 (1995).
19. B. Poeggeler et al., "Melatonin and Structurally-Related, Endogenous Indoles Act as Potent Electron Donors and Radical Scavengers In Vitro," *Redox Rep.* **2**, 179–184 (1996).
20. R. J. Reiter et al., "Pharmacological Actions of Melatonin in Free Radical Pathophysiology," *Life Sci.* **60**, 2255–2271 (1997).
21. D. X. Tan et al., "Melatonin: A Potent, Endogenous Hydroxyl Radical Scavenger," *Endocrine J.* **1**, 57–60 (1993).
22. Z. Matuszak, K. J. Reszka, and C. F. Chignell, "Reaction of Melatonin and Related Indoles with Hydroxyl Radicals: EPR and Spin Trapping Investigations," *Free Radical Biol. Med.* **23**, 367–372 (1997).
23. P. Stasica, P. Ulanski, and J. M. Rosiak, "Melatonin as a Hydroxyl Radical Scavenger," *J. Pineal Res.* **25**, 65–66 (1998).
24. E. Gilad et al., "Melatonin Is a Scavenger of Peroxynitrite," *Life Sci.* **60**, PL169–174 (1997).
25. S. Cuzzocrea et al., "Protective Effect of Melatonin in Carrageenan-Induced Models of Local Inflammation," *J. Pineal Res.* **23**, 106–116 (1997)
26. C. Pieri et al., "Melatonin as an Efficient Antioxidant," *Arch. Gerontol. Geriatr.* **20**, 159–165 (1995).
27. C. M. Cagnoli et al., "Melatonin Protects Neurons from Singlet Oxygen-Induced Apoptosis," *J Pineal Res.* **18**, 222–226 (1996).
28. M. O. Kotler et al., "Melatonin Increases Gene Expression for Antioxidant Enzymes in Rat Brain Cortex," *J. Pineal Res.* **24**, 83–89 (1998).
29. R. J. Reiter, "Aging and Oxygen Toxicity: Relation to Changes in Melatonin," *Age* **20**, 201–213 (1997).
30. C. B. Ramalko, J. W. Hastings, and P. Colepicolo "Circadian Oscillation of Nitrate Reductase Activity in *Gonyaulax polyedra* Is Due to Changes in Cellular Protein Levels," *Plant Physiol.* **107**, 225–231 (1995).
31. I. Antolin and R. Hardeland, "Suppression of the *Gonyaulax* Glow Peak by Paraquat and Its Restoration by Melatonin," in R. Hardeland, ed., *Biological Rhythms and Antioxidant Protection*, Cuvillier Verlag, Göttingen, 1997.
32. I. Antolin et al., "Antioxidative Protection in a High-Melatonin Organism: The Dinoflagellate *Gonyaulax polyedra* Is Rescued from Lethal Oxidative Stress by Strongly Elevated, but Physiologically Possible Concentrations of Melatonin," *J. Pineal Res.* **23**, 182–190 (1997).
33. B. Fuhrberg et al., "Dramatic Rises of Melatonin and 5-Methoxytryptamine in *Gonyaulax* Exposed to Decreased Temperature," *Biol. Rhythm Res.* **28**, 144–150 (1997).
34. A. Z. Tenga, B. A. Marie, and D. P. Ormrod, "Recovery of Tomato Plants from Ozone Injury," *Hortiscience* **25**, 1230–1232 (1990).
35. L. M Mortensen, "Effects of Ozone Concentrations on Growth of Tomato at Various Light, Air Humidity and Carbon Dioxide Levels," *Sci. Hortic.* **49**, 17–24 (1992).
36. A. G. Gentile et al., "Susceptibility of *Lycopersion* ssp. to Ozone Injury," *J. Am. Soc. Hortic Sci.* **96**, 94–96 1971.
37. R. Hardeland et al., "Chronobiology of Indoleamines in the Dinoflagellate *Gonyaulax polyedra*: Metabolism and Effects Related to Circadian Rhythmicity and Photoperiodism," *Braz. J. Med. Biol. Res.* **29**, 119–123 (1996).

RUSSEL J. REITER
SEOK JOONG KIM
The University of Texas Health Science Center
San Antonio, Texas

PHYTOCHEMICALS: *VACCINIUM*

The genus *Vaccinium* belongs to the health family, and members include the blueberries (bilberries), cranberries, and whortleberries. Total world production is estimated to about 1 million tons per year. These widespread, small fruits are found nearly everywhere in the Northern Hemisphere.

Knowledge of the chemical composition of the plant part of *Vaccinium* spp. is relevant because some phytochemicals (from the Greek *phyton*, plant) have biological activity, and berries are used as food and as medicine.

HISTORY

In the antiquity, Vergil and Ovid wrote verses on blueberries. Pliny the Elder and Vitruvius described the use of bilberries as a dye. The word *Vaccinium* derives from the Latin word *vacca*, ie, cow, or it is a misprint of *bacca*, berry.

Several plant parts (berries, leaves, flowers) of *Vaccinium* spp. have also been used in natural medicine dating back to ancient times. Dioscorides (first century A.D.) described the use of bilberries in the treatment of the diarrhea. This traditional folk remedy is still implemented today. St. Hildegarde of Bingen (twelfth century A.D.) recommended the use of these wild fruits to promote menstruation. Blueberry was entered in the pharmacopoeia in the sixteenth century.

Blueberries have been eaten in America since prehistoric times. Native American tribes not only ate bilberries, but also used the berries as a dye and as a medicine. Cranberry fruits, too acidic to be eaten raw, were generally mixed with honey or maple syrup or smoked. The main use for blueberries, however, was as a preservative. The high content of phenolic compounds and benzoic acid provides the bacteriostatic, antifermentative, and antioxidant properties. By adding blueberries to bison meat or deer fat, natives could preserve the food for the winter. This preparation was known as *pemmican*. Another was *venison*, meat filled with blueberries that was then smoked and dried. Several tribes used blueberries in medicine, using both berries and leaves.

Consumption of blueberries was introduced to the Pilgrims by the natives, but blueberry cultivation did not begin until the end of the nineteenth century. On the U.S. East Coast, July now is known as Blueberry Month, a time devoted to the appreciation of this most popular fruit.

Of curious interest is that the economic fortune of blueberries blossomed during wartime. During the American Civil War, blueberry juice and cranberries were consumed in high quantities by the troops. In the United Kingdom during World War I, blueberries were used as a substitute for synthetic aniline dyes. During World War II, pilots of the Royal Air Force, when flying in the night bombing raids over Nazi Germany, observed that when their diets included blueberry jam, they had great visual capacity and no night blindness.

PLANT COMPOSITION

Hundreds of compounds have been identified in *Vaccinium*. They are divided into several main classes, which are discussed in this section.

Sugars

Two main carbohydrates occur in *Vaccinium* berries, glucose (1–3%) and fructose (0.5–2%) (1,2). In cranberry, glucose occurs in a much higher amount than fructose; this is unusual because usually fructose and glucose occur in equal amounts.

Acids

Major organic acids in *Vaccinium* spp. are citric, malic, quinic, and benzoic acids (3,4). Quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) and benzoic acid are the most important and characteristic acids of cranberry (*V. macrocarpom*). The mean benzoic acid content is 0.01%, occurring mainly as 6-benzoyl-D-glucose (vaccinin).

Phenolic Compounds

Phenolic compounds (4–6) are water-soluble compounds, sometimes present in glycosidic form, characterized by the occurrence of one or more hydroxyl residues in the molecule. They occur in monomeric or polymeric structures. They provide a high antioxidant potential in foods because they scavenge free radicals such as O_2^-, $HO^\cdot$, and $ROO^\cdot$. Phenolic compounds are also responsible for the color, taste, flavor, and astringency of foods.

Polymeric Phenols. The tannins comprise much of the phenolic content in *Vaccinium*. They have a molecular weight of up to 5000 and are characterized by their astringency, brown color, and ability to precipitate by bonding with proteins. Two types occur in vegetables, hydrolyzable and condensed. In *Vaccinium* they occur as condensed tannins.

Oligomeric Phenolic Compounds. Oligomeric (ie, of intermediate molecular weight that ranges from 500 to 1500) phenolic compounds (OPCs) (7,8) are dimeric, trimeric, or tetrameric forms of flavan-type phenols, such as catechin and epicatechin. They are called procyanidins or proanthocyanidins because they yield anthocyanidins after acid degradation. They occur as copolymer of all the possible combinations of the stereoisomer form of the monomeric flavans and are distinguished by an alphabetic letter (ie, B_1, B_2, B_3, and B_4 for the four-dimeric forms of [D,L]-catechin and [D,L]-epicatechin). With three monomers, the number of possible trimers would be 27, etc. A typical procyanidin, procyanidin B_1, that occurs in *Vaccinium* berries is represented in Figure 1.

Anthocyanins. The anthocyanins (5,6,9) are the pigments responsible for the red to dark blue color of the *Vaccinium* berries and their juice. Anthocyanins are flavonoids (a C_6-C_3-C_6 molecule) and occur in glycosidic form. The nature and the type of the attachment of the sugar may be different. The sugar can be also acylated, generally with an aromatic or an aliphatic acyl moiety. A typical anthocyanin is shown in Figure 2. The anthocyanin composition characterizes *Vaccinium* species with qualitative and quantitative differences. In highbush blueberry (*V. corymbosum* L.), the total anthocyanin content varies from 25 to 495 mg/100 g fresh weight (FW). In the lowbush blueberry (*V. angustifolium* L.), the anthocyanin content ranges from 120 mg/100 g in the variety Bloomingdon and 260 mg/100 g in the variety Chignecto. In *V. angustifolium*, it is important to observe the occurrence of up to 35% of anthocyanins acylated with acetic acid and the genetic relationship among varieties (10). In rabbiteye blueberry

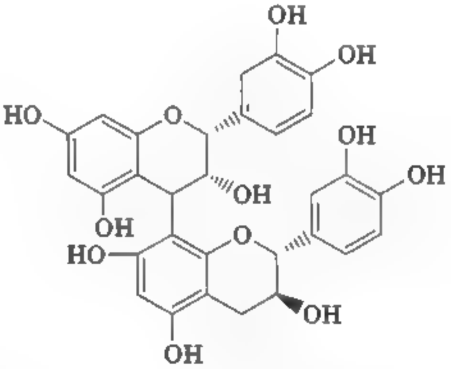


Figure 1. Procyanidin B_1, a catechin–epicatechin dimeric proanthocyanidin.

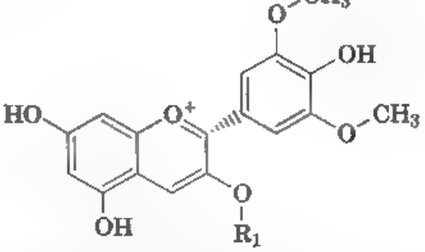


Figure 2. Malvidin, a typical *Vaccinium* anthocyanin. R_1, glucose, galactose, and arabinose.

(*V. ashei*), the total anthocyanin content is 210 mg/100 g in Tifblue berries and 272 mg/100 g in Bluegem berries.

Vaccinium elliotti, with an anthocyanin content of 760 mg/100 g, appears to be the species containing the highest amount of anthocyanins.

In *V. japonicum*, cyanidin 3-arabinoside (54%) and pelargonidin 3-arabinoside (39%) have been identified as two main pigments, they total 113 mg/100 g FW. This is the first report of pelargonidin glycosides in the genus *Vaccinium*.

In the fruit of *V. oxycoccus* L., or small cranberry, the main pigments present are peonidin 3-glucoside and cyanidin 3-glucoside, accounting for 41.9% and 38.3% of the total anthocyanin content, respectively. This anthocyanin pattern is rather different from that of the American cranberry (*V. macrocarpon*), which is rich in the 3-galactosides and the 3-arabinosides of peonidin and cyanidin; the total anthocyanin content of the fruit is about 78 mg/100 g FW. All possible combinations of cyanidin, delphinidin, petunidin, peonidin, and malvidin 3-galactosides, 3-glucosides, and 3-arabinosides have been found in the bilberry *V. myrtillus*. Quantitatively, the delphinidin glycosides are present in the largest quantities, and the peonidin glycosides are the least abundant. Fruit of bilberries from the Piedmont Alps in Italy contain 300–320 mg of anthocyanins/100 g FW. Bog whortleberry (*V. uliginosum*) contains 15 anthocyanins. The 3-glucoside of malvidin comprises the majority of these pigments (35.9%), and the total anthocyanin content is 256 mg/100 g FW. In Norwegian cowberries (*V. vitis-idaea* L.), the main anthocyanin is represented by cyanidin 3-galactoside (88.0%). The total anthocyanin content is 174 mg/100 g FW.

Phenolic Acids. *Vaccinium* berries contain a large number of phenolic acids as glycosides (6). The hydroxycinnamic acid derivatives (180–210 mg/100 g FW) are much higher in amount than the hydroxybenzoic derivatives (0.5–2.0 mg/100 g FW).

Simple Phenols. Arbutin (4-hydroxyphenyl-β-D-glucopyranoside) is a hydroquinone glycoside occurring in leaves and fruit (6). In the leaves of *V. vitis-idaea*, its content ranges from 40 to 90 mg g^{-1}. Its methyl ether, methylarbutin, occurs together with arbutin but in a lower amount.

Volatile Compounds

Several volatile compounds (11,12), including esters, hydrocarbons, aldehydes, ketones, cyclic ether, and sulfur-containing compounds, have been identified in different *Vaccinium* species, and there are qualitative and quantitative differences among them.

The impact compounds include *t*-2-hexenal, *t*-3-hexenol, and linalool in highbush blueberry. The impact character compounds in bilberry are *t*-2-hexenol, ethyl-3-methylbutanoate, and ethyl-2-methylbutanoate. Linalool contributes to the floral, rosy character unique to blueberry fruit, its concentration is less than 1 ppm. In cranberry, benzoic acid derivatives are responsible of the impact flavor character. As many as 50 volatile compounds have been identified in *Vaccinium* spp., but as many as 200 other compounds still remain unidentified.

Vitamins

Ascorbic acid is the vitamin occurring in highest amount in *Vaccinium*; its mean content in cranberry juice is 3 mg/100 mL (13,14). The folic acid content is 2.6 μg/100 g. The potential vitamin A content ranges from 50 to 100 IU/100 g. Trace amounts of thiamin, riboflavin, and niacin have also been detected.

Metals

The metal content includes potassium, sodium, calcium, rubidium, and magnesium, but *Vaccinium* berries are characterized by a high content of manganese, ranging from 28 to 250 ppm in *V. vitis-idaea* to 370 ppm in *V. myrtillus* (13,14).

NUTRITIONAL PROPERTIES

With their vivid color and their delicate flavor, *Vaccinium* berries stimulate the appetite. The energy supply of sugars, organic acids, minerals, and vitamins contained in them is important. However, an essential role is played by the phenolic compounds, particularly anthocyanins and OPCs. These phytochemicals are also responsible for the medicinal properties of bilberries (15–17).

An advantage of consuming large quantities of *Vaccinium* fruits may be an increase in antioxidative defenses in the body. The daily intake of phenolic compounds, including large quantities of anthocyanins, may prevent or lower the risk of a variety of ailments, particularly circulatory disease and stroke (17).

Flavonols and simple phenols retard the oxidation of ascorbic acid, tocopherols, and carotenoids, thus preserving their nutritional value and exerting a bacteriostatic action (18). Several scientific reports (19) have shown how phenolic substances, present in foods of plant origin, play an important role in nutrition. In *Vaccinium* berries, this fact is attributable to anthocyanins and procyanidins. They act as antioxidants in protecting the body from degenerative phenomena caused by the oxidation of lipids (cardiovascular diseases, atherosclerosis, etc.), and they have a chemopreventive action against the onset of cancer (20–31).

Manganese is an essential microelement for the synthesis of several enzymes, particularly superoxide dismutase (SOD), a very important enzyme that is the main front line defense against damaging free radicals (32).

PHARMACOLOGICAL PROPERTIES

The anthocyanins and OPCs are antioxidants; they are free radical scavengers and inhibitors of lipoxygenase, cyclooxygenases, hyaluronidases, and other oxidative enzymes, such as ascorbic oxidases. Anthocyanins also lower cholesterol levels and appear to have significant platelet antiaggregating activity as well as antithrombotic activity (33–39).

Ophthalmological studies done in the 1960s in France and Italy found that anthocyanins act directly on the speed of regeneration of the vision pigment. They have an indirect action by improving the blood flow to the strongly vascularized retina. Athletes and certain professionals (truck and bus drivers, video terminal operators), for whom good vision is critical, would benefit from high intake of *Vaccinium* anthocyanins (15).

A vast series of pharmacological and clinical studies conducted in Europe during the past 40 years has shown that the anthocyanin glucosides and OPCs have a marked action on the permeability and resistance of the capillary walls. In addition to the capillarotropic action, attributable to the so-called vitamin P factor, these compounds have a noticeable anti-inflammatory effect. They have been used successfully in capillary pathologies such as diabetes, atherosclerosis, arterial hypertension, and circulatory disturbances in superficial and deep veins and circulation problems during pregnancy (40–46).

Figure 3 shows some pharmaceutical formulations anthocyanin-containing from bilberries.

Other experimental and clinical studies have shown outstanding wound-healing, mucopoietic, capillaroprotective effects, not only on superficial wounds but also on the gastric mucosa and duodenum (40–46). Elderly persons, patients with diabetes, and persons with coronary disease, hypertension, or ulcers are among those who could greatly benefit from a diet rich in blueberries, cranberries, and whortleberries.

Recent clinical trials have also scientifically confirmed what has been known from popular experience. Cranberry juice and other *Vaccinium* berry juices prevent urinary tract infections in women and elderly individuals. The antiadhesion effect that impedes the proliferation of *Escherichia coli*, the bacteria responsible for the infection, has been proved to be due to the procyanidins contained in cranberry juice (47–50).

antocin 30
ANGIOREX

Figure 3. Pharmaceutical preparations based on bilberry anthocyanins.

CONCLUSION

Fresh and processed *Vaccinium* berries have been a part of our diet for a long time. In addition to their colorful appearance, they stimulate the appetite with flavorful and tasteful sensory properties and supply macronutrients and micronutrients to the body. The phytochemicals found in *Vaccinium* contribute significantly to cardiovascular disease prevention. They improve microcirculation, enhance eyesight, lower blood cholesterol levels, prevent lipid-oxidation-derived diseases (atherosclerosis, cancer, etc.), moderate the negative effects of aging, and have a synergistic action on all antioxidant vitamins (A, E, and C).

On the threshold of Third Millennium, Hippocrates' fifth-century B.C. statement is still topical:

> Let your food be your medicine, let your medicine be your food.

BIBLIOGRAPHY

1. W. Kalt and J. E. McDonald, "Chemical Composition of Lowbush Blueberry Cultivars," *J. Am. Soc. Hort. Sci.* **121**, 142–146 (1996).
2. A. B. Holmes and M. S. Starr, "Cranberry Juice," in S. Nagy, S. S. Chen, and P. E. Shaw, eds., *Fruit Juice Processing Technology*, Agriscience Inc., Auburndale, Fla., 1993, pp. 515–531.
3. E. D. Coppola, E. C. Conrad, and R. Cotter, "High Pressure Liquid Chromatographic Determination of Major Organic Acids in Cranberry Juice," *J. Assoc. Off. Anal. Chem.* **61**, 1490–1492 (1978).
4. V. Hong and R. E. Wrolstad, "Detection of Adulteration in Cranberry Juice Drinks and Concentrates," *J. Assoc. Off. Anal. Chem.* **69**, 208–213 (1986).
5. G. Mazza and E. Miniati, *Anthocyanins in Fruits, Vegetables and Grains*, CRC Press, Boca Raton, Fla., 1993.
6. J. J. Macheix, A. Fleuriet, and J. Billot, *Fruit Phenolics*, CRC Press, Boca Raton, Fla., 1990.
7. L. Y. Foo and L. J. Porter, "The Structure of Tannins of Some Edible Fruits," *J. Sci. Food Agric.* **32**, 711–716 (1981).
8. P. L. Wang, C. T. Du, and F. J. Francis, "Isolation and Characterization of Polyphenolic Compounds in Cranberries," *J. Food Sci.* **43**, 1402–1404 (1978).
9. J. Gross, *Pigments in Fruits*, Academic Press, London, 1987.
10. L. Gao and G. Mazza, "Quantitation and Distribution of Simple and Acylated Anthocyanins and Other Phenolics in Blueberries," *J. Food Sci.* **59**, 1057–1059 (1994).
11. W. D. Baloga, N. Vorsa, and L. Lawter, "Dynamic headspace gas chromatography-mass spectrometry analysis of volatile flavor compounds from wild diploid blueberry species," in R. L. Rouseff and M. M. Leahy, eds., *Fruit Flavors* American Chemical Society, Washington, D.C., 1994, pp. 235–247.
12. R. J. Horvath et al., "Comparison of Volatile Compounds from Rabbit Eye Blueberry (*Vaccinium ashei*) and Deerberry (*V. stamineum*) During Maturation," *J. Essent. Oil Res.* **8**, 645–648 (1996).
13. R. J. Bushway et al., "Mineral and Vitamin Content of Lowbush Blueberries (*Vaccinium angustifolium* Ait.)," *J. Food Sci.* **48**, 1878–1880 (1983).

14. R. R. Eitenmiller, R. F. Kuhl, and C. J. B. Smit, "Mineral and Water Soluble Vitamin Content of Rabbiteye Blueberries," *J. Food Sci.* **42**, 1311–1315 (1977).
15. E. Miniati and R. Coli, "Anthocyanins: Not Only Color for Foods," *Proc. 1st Int. Conf. on Natural Food Colorants*, University of Massachusetts, Amherst, Mass., 1994.
16. W. Kalt and D. Dufour, "Health Functionality of Blueberries," *Hort Technol.* **7**, 216–221 (1997).
17. P. Morazzoni and E. Bombardelli, "*Vaccinium myrtillus* L.," *Fitoterapia* **66**, 3–29 (1996).
18. C. A. Rice-Evans, N. J. Miller, and G. Paganga, "Structure–Antioxidant Activity Relationship of Flavonoids and Phenolic Acids," *Free Radiol Biol. Med.* **20**, 933–956 (1996).
19. M. T. Huang, C. T. Ho and C. Y Lee, "Phenolic Compounds in Food and their Effects on Health. II. Antioxidants and Cancer Prevention," ACS Symposium Series 507, American Chemical Society, Washington, D.C., 1992.
20. J. A. Bomser et al., "Induction of NAD(P)H:Quinone Acceptor Oxidoreductase in Murine Cells (Hepa 1c1c7) by Fruit (*Vaccinium*) Extracts," *FASEB J.* **9**, A993 (1995).
21. R. L. Prior et al., "Antioxidant Capacity as Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of *Vaccinium* Species," *J. Agric. Food Chem.* **46**, 2686–2693 (1998).
22. H. Wang, G. Cao, and R. L. Prior, "Oxygen Radical Absorbing Capacity of Anthocyanins," *J. Agric. Food Chem.* **45**, 304–309 (1997).
23. H. Wang, G. Cao, and R. L. Prior, "Total Antioxidant Capacity of Fruits," *J. Agric. Food Chem.* **44**, 701–705 (1996).
24. Y. S. Velioglu et al., "Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products," *J. Agric. Food Chem.* **46**, 4113–4117 (1998).
25. J. Bomser et al., "*In vitro* Anticancer Activity of Fruits Extracts from *Vaccinium* Species," *Planta Med.* **62**, 212–216 (1996).
26. M. T. Satue-Garcia, M. Heinonen, and E. N. Frankel, "Anthocyanins as Antioxidants on Human Low-Density Lipoprotein and Lecithin-liposome Systems," *J. Agric. Food Chem.* **45**, 3362–3367 (1997).
27. P. M. Laplaud, A. Leleubre, and M. J. Chapman, "Antioxidant Action of *Vaccinium myrtillus* Extract on Human Low Density Lipoproteins *In vitro*: Initial Observations," *Fundam. Clin. Pharmacol.* **11**, 35–40 (1997).
28. G. Cao et al., "Increases in Human Plasma Antioxidant Capacity After Consumption of Controlled Diets High in Fruit and Vegetables," *Am. J. Clin. Nutr.* **68**, 1081–1087 (1998)
29. M. T. Meunier, E. Duroux, and P. Bastide, "Activité Antiradicalaire D'Oligomères Procyanidoliques et D'Anthocyanosides Vis-à-vis de L'Anion Superoxyde et Vis-à-vis de La Lipoperoxydation," *Plant. Med. Phytothèr.* **23**, 267 -274 (1989).
30. L. Costantino et al., "Activity of Polyphenolic Crude Extracts as Scavengers of Superoxide Radicals and Inhibitors of Xanthine Oxidase," *Planta Med.* **58**, 342–344 (1992).
31. T. Wilson, J. P. Porcari, and D. Harbin, "Cranberry Extract Inhibits Low Density Lipoprotein Oxidation," *Life Sci.* **62**, 381–386 (1998).
32. B. Frei, *Natural Antioxidants in Human Health and Diseases*, Academic Press, San Diego, Calif., 1994.
33. C. Ghiringhelli, F. Gregoratti, and F. Marastoni, "Capillarothropic Activity of Anthocyanosides in High Doses in Phebopathic Stasis," *Minerva Cardioangiologica* **26**, 255–276 (1978).
34. G. Spinella, "Natural Anthocyanosides in Treatment of Peripheral Venous Insufficiency," *Arch. Med. Int.* **37**, 21–29 (1985).
35. V. Bettini et al., "Effects of *Vaccinium myrtillus* Anthocyanosides on Vascular Smooth Muscle," *Fitoterapia* **55**, 265–272 (1984).
36. D. Colombo and R. Vescovini, "Controlled Clinical Trial of Anthocyanosides from *Vaccinium myrtillus* in Primary Dysmenorrhea," *G. Ital. Ostetr. Ginecol.* **7**, 1033–1038 (1985).
37. S. Bertuglia, S. Malandrino, and A. Colantuoni, "Effect of *Vaccinium myrtillus* Anthocyanosides on Ischaemia Reperfusion Injury in Hamster Cheek Pouch Microcirculation," *Pharmacol. Res.* **31**, 183–187 (1995).
38. A. Colantuoni et al., "Effects of *Vaccinium myrtillus* Anthocyanosides on Arterial Vasomotion," *Arzneim. Forsch.* **41**, 905–909 (1991).
39. G. Pulliero et al., "*Ex vivo* Study of the Inhibitory Effect of *Vaccinium myrtillus* Anthocyanosides on Human Platelet Aggregation," *Fitoterapia* **60**, 69–75 (1989).
40. J. C. Monboisse et al., "Non-enzymatic Degradation of Acid-soluble Calf Skin Collagen by Superoxide Ion: Protective Effect of Flavonoids," *Biochem. Pharmacol.* **32**, 53–58 (1983).
41. R. Boniface and A. M. Robert, "Effect of Anthocyanins on Human Connective Tissue Metabolism in the Human," *Klin. Monatsbl. Augenheilkd.* **209**, 368–372 (1996).
42. Z. Detre et al., "Studies on Vascular Permeability in Hypertension: Action of Anthocyanosides," *Clin. Physiol. Biochem.* **4**, 143–149 (1995).
43. M. J. Magistretti, M. Conti, and A. Cristoni, "Antiulcer Activity of an Anthocyanidin from *Vaccinium myrtillus*," *Arzneim. Forsch.* **38**, 686–690 (1990).
44. A. Cristoni and M. J. Magistretti, "Antiulcer and Healing Activities of *Vaccinium myrtillus* Anthocyanosides," *Farmaco. Ed. Prat.* **42**, 29–43 (1987).
45. A. Lietti, A. Cristoni, and M. Picci, "Studies on *Vaccinium myrtillus* Anthocyanosides. I. Vasoprotective and Antiinflammatory Activity," *Arzneim. Forsch.* **26**, 829–832 (1976).
46. A. M. Robert et al., "Action of Anthocyanosides of *Vaccinium myrtillus* on the Permeability of the Blood Brain Barrier," *J. Med.* **8**, 312–332 (1977).
47. I. Ofek et al., "Anti-*Escherichia coli* Adhesion Activity of Cranberry and Blueberry Juices," *New Engl. J. Med.* **324**, 1599 (1991).
48. J. M. T. Hamilton-Miller, "Reduction of Bacteriuria and Pyuria after Ingestion of Cranberry Juice," *J. Am. Med. Assoc.* **272**, 588–590 (1994).
49. J. Avorn et al., "Reduction of Bacteriuria and Pyuria Using Cranberry Juice," *J. Am. Med. Assoc.* **271**, 751–754 (1994).
50. A. B. Howell et al., "Inhibition of the Adherence of P-Fimbriated *Escherichia coli* to Uroepithelial-cell Surfaces by Proanthocyanidin Extracts from Cranberries," *New Engl. J. Med.* **339**, 1085–1086 (1998).

ENRICO MINIATI
Perugia University
Perugia, Italy

See also COLORANTS: ANTHOCYANINS.

PHYTOCHEMICALS: WINE

The understanding of the term *phytochemicals* (chemicals of plants) is likely to be related to the discipline in which a person has an interest and/or training (1). For example, plant biochemists are to be more apt to think of phytochemicals as secondary metabolites, that is, other natural chemicals than the primary plant components of carbohydrates, proteins, or lipids; toxicologists may define phytochemicals as natural compounds that cause or inhibit mutagenicity, carcinogenicity, or teratogenicity; food technologists mainly consider phytochemicals in relation to food colors and taste; nutritionists are now using the term *phytonutrients*—phytochemicals that enhance human health over and above vitamins and minerals. Whatever the definition, phytochemicals are natural compounds in plant foods and plant food products, such as wine. It is known that many of them contribute to the desirable qualities of the food and wine and, more recently, to optimal health. Many phytochemicals of wine may differ from the phytochemicals of the grapes used for vinification. The most-studied are red grape pigments that slowly turn into new, modified, more stable pigments during "wine aging" (2). These substances, among others, are known as polyphenols and can take months or years to evolve during the aging process. Various types of polyphenols are found in a variety of colored fruits and vegetables as well as in tea and coffee.

A phenolic index ($E_{280} - 4$) has been used as an arbitrary division between two divisions of red wines, that is, light red wines and robust red wines (3). A low phenolic index of the former is usually from young wines marketed after a few months of vintage, whereas the latter indicates prolonged maturation and further development during bottle aging.

PHENOLICS IN WINE

The quality and characteristics of wine depend on its phenol content. The natural phenols and their derivatives are crucial to wine's color, astringency, bitterness, resistance to oxidation, and other characteristics that make wine interesting and diverse (4). Although wines differ greatly in phenol content, a gross comparison between white and red young *Vitis vinifera* wines is presented in Table 1. Phenolic compounds are usually within several chemical classes but are usually separated into two general classes, the flavonoids and the nonflavonoids, as seen in Table 1. High-performance liquid chromatography (HPLC) is presently very useful in the analysis of wine phenols. However, wine chromatographs show very large numbers of components, many that are not identified and many that may not be phenols. For reliable quantitation, a known standard sample of each phenol to be determined must be chromatographed for comparison under exactly the same conditions. A direct HPLC method for separation of all classes of wine phenolic compounds, without prepurification steps, has been developed (5).

Table 1. Gross Estimated Phenol Content for Typical Young, Light Table Wines (mg L^{-1})

	White	Red
Nonflavonoids		
Volatile phenols	1	5
Tyrosol	14	15
Gallic and other C6C1 acids	10	40
Caffeic acid/related compounds	140	140
Total	*165*	*200*
Flavonoids		
Catechins (flavan-3-ols)	25	75
Anthocyanin and derivative	0	400
Other monomeric flavonoids	Trace	25
Oligomeric flavonoids	5	500
Total	*35*	*1000*
Total phenols	*200*	*1200*

Source: Ref. 4.

Nonflavonoid Phenols

The two most prevalent types of nonflavonoids, as observed in Table 1, are gallic acid and caffeic acid, as well as their related compounds (Fig. 1a,b). Gallic acid, the major wine hydroxybenzoate compound; caffeic acid, the primary hydroxycinnamate compound; and the stilbene derivative, *trans*-resveratrol, are the major, important nonflavonoids in wine. Other hydroxybenzoates and hydroxycinnamates appear in wine with substituted molecules for hydrogen in the final acid moiety of these compounds. All wines have quite similar amounts of nonflavonoids, and these compounds can contribute important sensory properties and color stability to wine. Other nonflavonoids in wines are short-chain aldehydes, caftaric acids, vanillin, syringaldehyde, tyrosol, 4-vinylguaiacol, acetovanillone, eugenol, and 4-ethylphenol. Some of these appear to be degradation products of other phenols and increases during red wine aging (4).

trans-Resveratrol (Fig. 1c) levels in wines, which are somewhat higher in red wines than whites, was first shown to be generally under 1 mg/L (6). This compound is considered to be a phytoalexin. Phytoalexins in grapes and other plants are formed in response to stress, especially microorganisms causing disease, and are part of the plant's response mechanism for disease resistance. The importance of *trans*-resveratrol in wine is its antioxidant effect on human low-density lipoprotein (LDL) as well as a possible cancer chemopreventive agent (7). Probably with the combination of other antioxidant flavonoid compounds in wine resveratrol may be effective in inhibiting the pathogenesis of atherosclerosis (see "The French Paradox").

Some red Spanish wines, such as pinot noir and merlot, have been reported to have average levels of *trans*-resveratrol of 5 and 4 mg/L, respectively (8). The *cis* isomer of resveratrol has not been reported as a natural product in wine, but it is present and probably occurs from light exposure during winemaking and/or during storage (8).

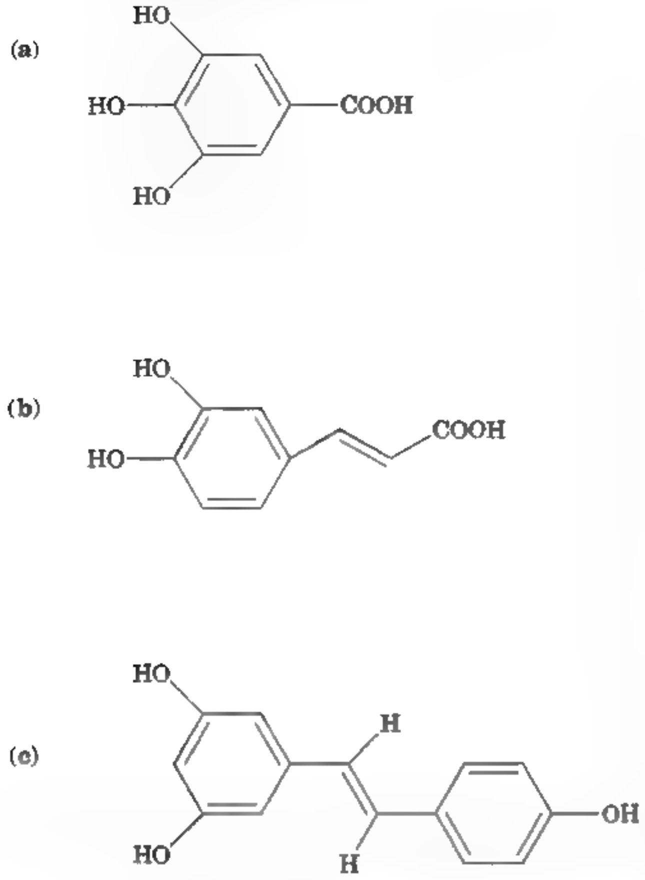


Figure 1. Examples of wine nonflavonoids. (a) Gallic acid; (b) Caffeic acid; (c) *trans*-Resveratrol.

There is also a 3 β-glycoside of transveratrol noted in the skin of grapes called piceid (9). It is not known whether these resveratrol derivatives all have the attributed health effects reported for the *trans* isomer or how much is actually absorbed and metabolized.

Flavonoids

The highest level (Table 1) and most important phenols in wine are the flavonoids (Fig. 2). Nearly 5000 individual flavonoids have been characterized. In most cases, the various kinds of flavonoids arise by glycosylation or other substitution, and a very large number of different sugars have been found to substitute flavonoids, especially the flavonol class (10).

A significant flavonoid class are the flavan-3-ols. The monomer is catechin (Fig. 2a), and the flavan-3-ol oligomer is called procyanidin (Fig. 2b). An isomer of catechin, epicatechin, comprises one-half of the procyanidins and a good portion of the polymers of flavan-3-ols, also known as condensed tannins. These tannins, sometimes called flavolans (4), are a major component of grape seeds, skins, and stems. Tannins are astringent polyphenols, with molecular weights ranging from about 500 to 5000. These flavan-3-ol polymers are different from hydrolyzable tannins that appear in wine from wood contact, usually from barrel aging. Gallic esters of the catechins are also present in wine but at lesser amounts and are rather unstable to oxidation.

Levels of catechin, the most abundant monomeric phenolic in wine, vary from year to year and within wine varieties. As seen in Figure 3, the average catechin levels in selected California red wines by vintage show these large yearly variations. Wines made from pinot noir grapes appear to have quite low catechin levels in the 1986 vintage, yet possess the highest catechin levels, 300 mg/L, in 1989 and 1990 as compared to the cabernet sauvignon, zinfandel, and merlot wines made in these same years (10).

Quercetin (Fig. 2c) and other flavonols all contain a 4-keto group and occur in grapes as glycosides and in wines partly as aglycones. Quercetin is relatively high in grape leaves and may be controlled to a large extent by sun exposure. Flavanonols, such as dihydroquercetin and dihydrokaempferol in the form of their 3-rhamnosides, have been observed in grapes and wines at very low concentrations (4). Quercetin also appears to vary in wine by grape variety and vintage year, with cabernet sauvignon containing the relatively highest level compared to California pinot noir, zinfandel, and merlot, as seen in Figure 4. The largest variation of quercetin appears to be present in pinot noir wines. This flavonol was not detected in white wine varieties (10).

Anthocyanins are important phenols that give color to red wines primarily by extraction of these pigments from grape skins during vinification. These phenols become quite complex and seem to be of decreased importance to wine color during aging. Anthocyanins do not occur free but are glucosides in grapes and wines. Following the original work of P. Ribereau-Gayon (11) that showed the European grape *Vitis vinifera* contains only anthocyanins that are 3-monoglucosides, whereas other grape species are 3,5-diglucosides, there are more than six reviews considering analyses of anthocyanins in wine (4). Recent analytical methodologies include HPLC and polarography. However, analysis of anthocyanins in wines are quite difficult and complex. There are a number of nondefined polymeric forms and rapid and complex equilibria regarding the red forms of the anthocyanins (flavylium ion) with the purple anhydro base together with a colorless carbinol base. The acidity, temperature, interaction with other compounds (eg, catechins), concentration of the total amount of anthocyanins, and a very quick polymerization of the red pigment during winemaking and storage complicates anthocyanin analysis.

Three major anthocyanin structures are shown in Figure 5a–c. Malvidin 3-glucoside and its acylated derivatives account for more than 60% of total anthocyanins in *V. vinifera* wines. This 3,5-diglucoside, found in native American grapes such as Concord and Niagara, is termed *malvin*. Delphinidin (Fig. 5b) and peonidin (Fig. 5c) are usually the next two major anthocyanins in most wines. There are five major and about 15 minor anthocyanins believed to be present in all red wines.

Figure 6 shows the average levels of malvidin-3-glucoside in four varietal red wines. The older red wines from the 1989 vintage have only a portion of the malvidin levels than the newer 1990 and 1991 wines (10). This indicates,

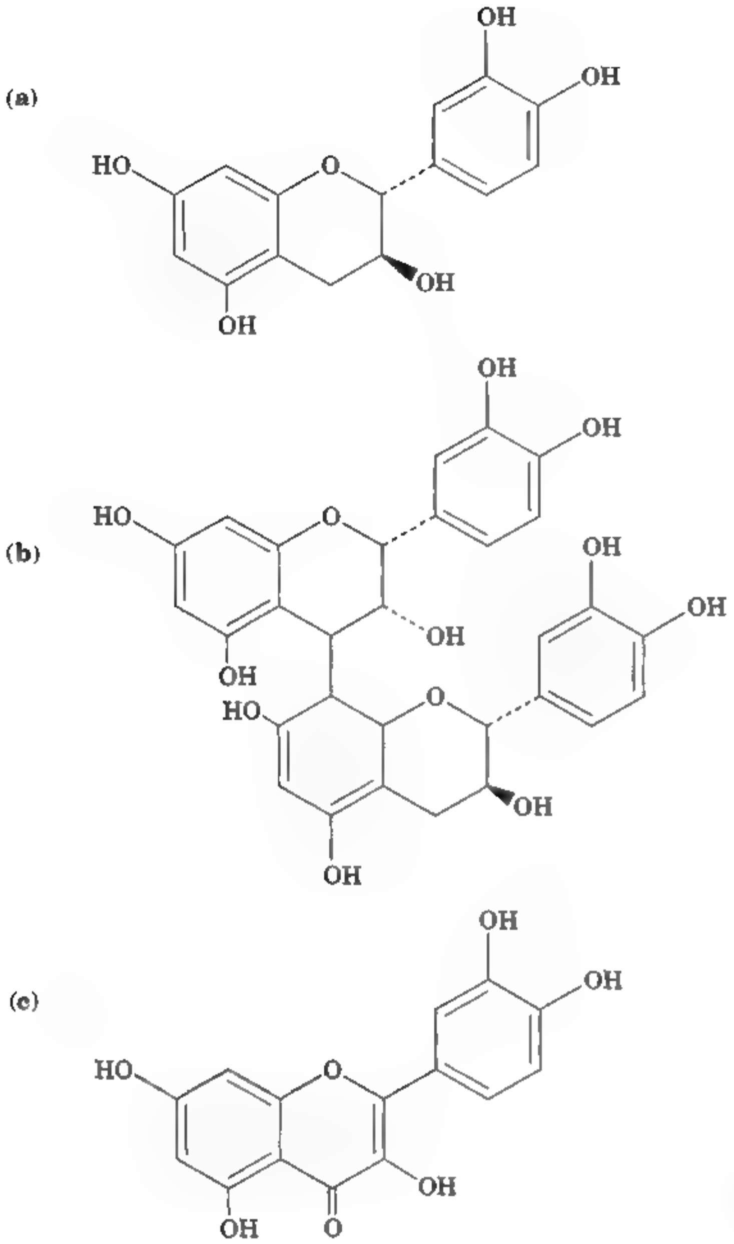


Figure 2. Examples of wine flavonoids. (**a**) Flavan-3-ol: catechin; (**b**) Flavan-3-ol oligomer: procyanidin; (**c**) Flavanol: quercetin.

as previously stated, that anthocyanins decrease with the age of wines.

VINIFICATION AND PHENOLICS

As seen in Figures 3, 4, and 6, the phenolic content of wines changes from vintage years and by grape varieties. Further phenolic changes in grapes have been noted from site of production, probably depending on a number of factors such as weather and soil conditions and grape degree of maturity. Catechins and procyanidins are observed at the highest level during the early stages of grape maturity and then decrease somewhat rapidly. Anthocyanins, developing in grape skins, steadily increase during maturity.

There are three main technological practices during vinification that change the flavonoid content of wines (12).

Destemming of Grape Clusters

After grape harvest, American winemakers usually first destem the grapes. Destemming in some European cellars is a current enological practice. Destemming grape clusters produces wines with less catechins and procyanidins. Some evidence indicates only a small reduction in epicatechins as compared to about a 25% reduction in catechin and procyanidins in wines from destemmed grapes.

Length of Maceration

Macerating red grapes with skins and seeds, and in some cases with stems, usually does not exceed 10 days because the maximum level of anthocyanin extraction then seems to occur. However, it has been noted that even with pro-

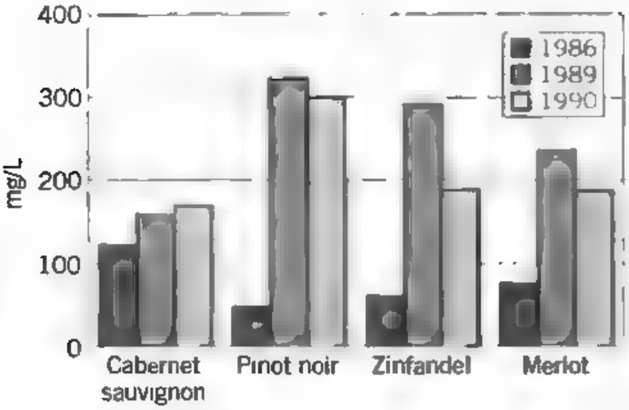


Figure 3. Average catechin levels in four red wines made in 1986, 1989, and 1990. *Source:* Reprinted with permission from Waterhouse and Teissedre, Ref. 10. Copyright 1997, American Chemical Society.

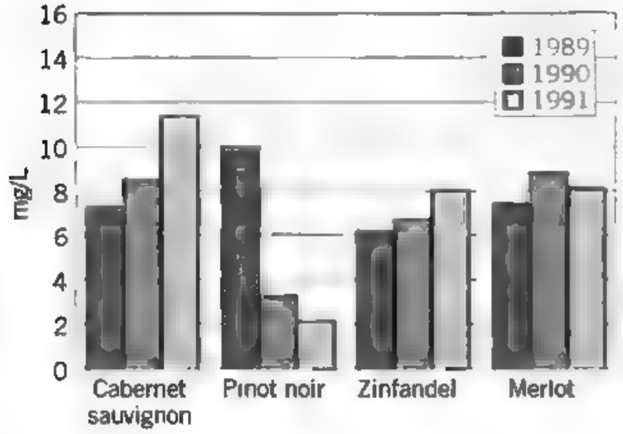


Figure 4. Average quercetin levels in four red wines made in 1989, 1990, and 1991. *Source:* Reprinted with permission from Waterhouse and Teissedre, Ref. 10. Copyright 1997, American Chemical Society.

longed skin contact and maceration, phenolic extraction rarely is more than 50% of the available grape amount. Maceration times, compared from 2 to 14 days, more than double the levels of catechin, epicatechin, and procyanidins in wine. These wines are more astringent because increased procyanidins and tannins causes high levels of astringency in young, red wines.

Fining of Wines

Fining or clarification of wines eliminates colloidal materials, usually involving phenolics and proteins, that cause turbidity and precipitates during aging. Clarification should be undertaken about 6 months after vintage, when both fermentation and malolactic fermentation have completed. The wine is then racked and filtered. Both red and white wines require fining, although some wines are now only filtered after centrifuging for faster marketing. Besides clarification of wine, certain fining agents stabilize wine against brown color development.

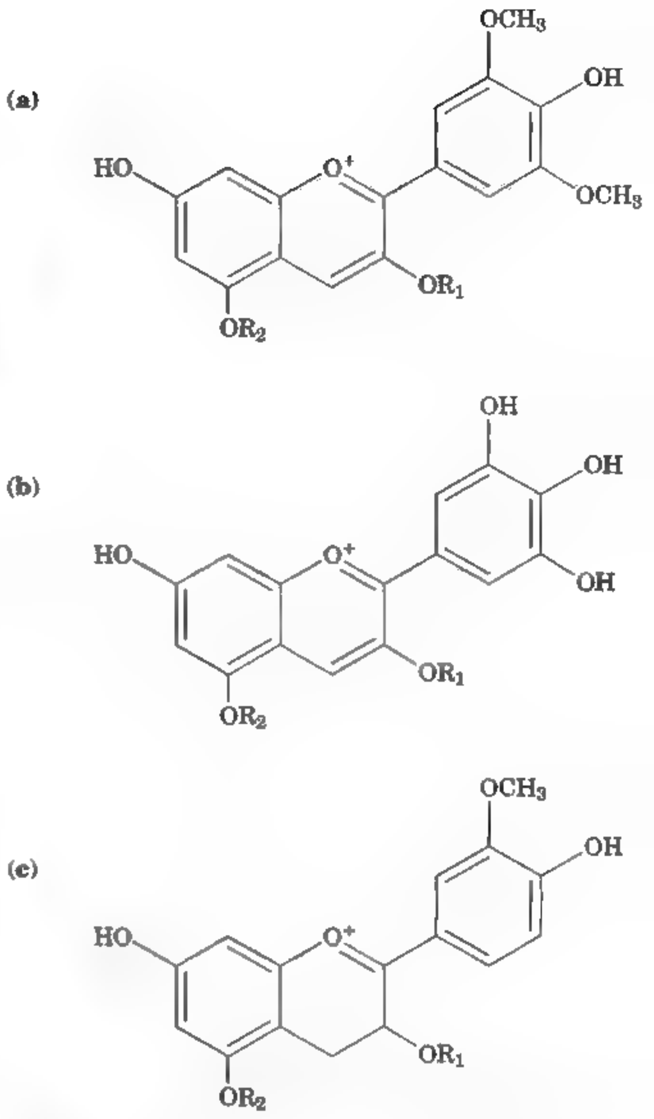


Figure 5. Examples of three major wine anthocyanin flavonoids. (a) Malvidin; (b) Delphinidin; (c) Peonidin. R_1 and R_2 are glucose (3,5-diglucosides), except in *V. vinifera* grapes and wine, where R_1 is glucose and R_2 is H (3-monoglucosides).

Bentonite, a common fining agent that is a clay consisting primarily of hydrated aluminum silicate, appears to be less effective in lowering catechins and procyanidins in wines than gelatin or poly(vinylpyrrolidone). However, the level of these and other phenols in wine as well as the amount of added fining agent used will affect the final amount of the finished wine's catechins and procyanidins.

WINE AND HEALTH

Wine is a rich source of polyphenolic antioxidants. Polyphenols are also widely present in fruits and vegetables.

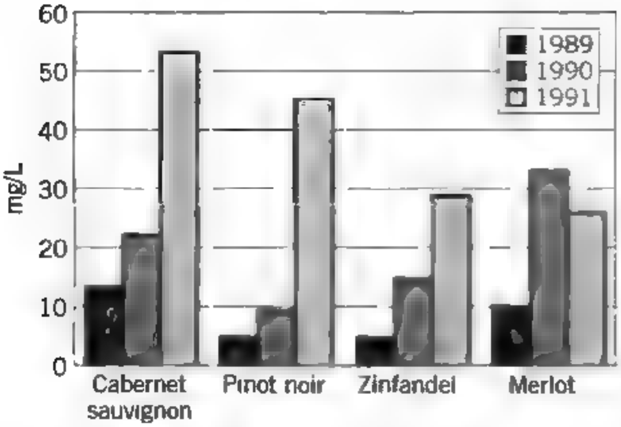


Figure 6. Average levels of malvidin-3-glucoside in four red wines made in 1989, 1990, and 1991. *Source:* Reprinted with permission from Waterhouse and Teissedre, Ref. 10. Copyright 1997, American Chemical Society.

Thus, healthy diets, as the Mediterranean diet, consists of large amounts of fruits and vegetables, with only limited levels of meat, and moderate amounts of red wine. Ethanol, the largest component of wine (<14%), and the polyphenols seem to be the healthful natural chemicals of wine as described in "The French Paradox."

Phytochemicals, such as the polyphenolics of food and wine that have disease-fighting and wellness-promoting qualities, have been recently termed *nutraceuticals*. The use of food as medicine was first presented in recorded history by the famous oath of Hippocrates, who stated he would use food first to treat disease and alleviate suffering in his patients. The use of wine in control of diseases of the heart and blood vessels has been advocated since the thirteenth century, but scientific studies published in 1904 first showed that consuming alcoholic beverages reduces the incidence of coronary artery diseases (13). Most physicians are hesitant to advise patients to drink moderate amounts of alcohol or wine, probably due to alcohol's potential abuse and known health problems in heavy drinkers. However, several population studies have shown that cardiovascular-related mortalities follow a U-shaped curve; light drinkers have lower cardiovascular-related mortalities than abstainers or heavy drinkers (14). Doll (15) has shown that drinking moderate amounts of alcohol daily, between one and four drinks for persons over the age of 45, reduces the risk of heart disease and premature death. There does not seem to be any positive effect from daily alcohol consumption for people under the age of 45.

The French Paradox

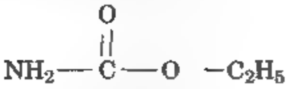
The French cardiologist S. Renaud first described a paradoxical situation in France, whereby there is a remarkably high intake of saturated fat but relatively low mortality from coronary artery disease. In most countries, a high intake of saturated fats is related to high mortality from heart disease. Renaud and de Lorgeril (16) indicated that the relatively high intake of wine in France explained this French paradox by the alcohol content of wine that inhibits blood platelet aggregation. Platelet aggregation is related to one of the conditions needed for the development of coronary disease. However, later studies by Frankel et al. (17) showed that the alcohol content of wine may not be the sole explanation for this protection. Red wine, containing antioxidant polyphenols, seemed to play an important role because *in vitro* studies showed that the red wine phenolics inhibited the oxidation of LDL. Continued studies by these investigators (18) showed that the two flavonoids, epicatechin and quercetin, had about twice the inhibiting potency as *trans*-resveratrol in LDL oxidation. The oxidation of LDL, commonly called the "bad cholesterol," is another condition usually required in the etiology of coronary disease.

Wine and Cancer

The study of food phytochemicals for cancer prevention and cure is a major research activity and will continue to expand as new knowledge develops (1). Most studies on chemical carcinogenesis in animals or with *in vitro* methodologies involve the pure phytochemical, including a number of polyphenols found in wine. Specifically, research on the flavonols, tannins (19), and resveratol (7) have shown anti-initiation, antipromotion, and/or antiprogression activities in chemical carcinogenesis studies with animal models.

The process of carcinogenesis is usually broadly divided into the three general stages of initiation, promotion, and progression. Initiation represents an alteration in the DNA of any cell by genetic mutations. Promotion is the process by which an initiated cell develops via changes of various enzyme systems and in intercellular communication patterns. Progression is the process by which a benign tumor progresses into a malignant one. The specific polyphenols, previously indicated, have shown antioxidant effects such as antimutagen mechanisms and the induction of phase II drug-metabolizing enzymes (anti-initiation effects), activation of membrane-stabilizing mechanisms (anti-inflammation responses), and inhibition of certain oxidase enzymes (antipromotion effects). Resveratrol inhibited tumorigenesis in a mouse skin cancer model and in carcinogen-treated mouse mammary glands in culture (antiprogression effects).

One recent study has shown a direct link to wine and cancer inhibition in a transgenic mouse model (20). These mice have a set of genetic disorders that cause tumors to grow along nerves similar to neurofibromatosis in humans. Dehydrated, dealcoholized red wine solids were fed as a supplement with a defined amino-acid-based diet to these mice successfully for three generations. Catechin was the major wine polyphenol, followed by gallic acid and epicatechin. These three phenols comprised about 93% of the total phenolics in the red wine used in this study. The mouse group fed the wine solids, compared to the unsupplemented controls, showed a significantly delayed tumor onset, and the major polyphenol, catechin, in the red wine was absorbed intact. This latter finding is important because if wine, or more specifically certain polyphenols in wine, has a role in the prevention of human cancer development, then future studies must show absorption, metab-



$NH_2-C(=O)-O-C_2H_5$

Figure 7. Ethyl carbamate.

olism, organ distribution, and excretion patterns of these polyphenolics and their metabolites. In addition, ethanol could play an interactive role in the enhancement of these processes

Ethyl Carbamate

Ethyl carbamate, also commonly known as urethane (Fig. 7), is produced at very low levels in many fermented foods and beverages, including bread, beer, soy sauce, yogurt, and red and white wine. It is developed in wine by the presence of urea with alcohol, by certain malolactic bacteria, or even by heating nonfermented grape juice with added ethanol (21).

Numerous wine surveys showed a range of ethyl carbamate levels from undetected to more than 100 μg/L. This compound is a water-soluble carcinogen as shown in numerous animal studies and is therefore considered a human dietary carcinogen. Thus, its content in foods and beverages should be as low as possible. In 1988, the Food and Drug Administration (FDA) established that American table wines should not have ethyl carbamate levels higher than 15 μg/L. The FDA also recommended that wine importers have some form of testing program so that foreign wines do not exceed this limit.

Carcinogenesis studies with mice have shown that the intake of ethanol in wine protects against the metabolite of ethyl carbamate, that is, the active or ultimate carcinogen, from forming through a competitive interaction mechanism. Also, lowered food and caloric intake with concomitant lowered body weights in mice seems to further reduce ethyl carbamate tumor development (22).

BIBLIOGRAPHY

1. M. Messina and V. Messina, "The Second Golden Age of Nutrition—Phytochemicals and Disease Prevention," in M.-T. Huang et al., eds., *Food Phytochemicals for Cancer Prevention I. Fruits and Vegetables,* ACS, Washington, D.C., 1994
2. R. Brouillard, F. George, and A. Fougerousse, "Polyphenols Produced During Red Wine Ageing," *Biofactors* **6**, 403–410 (1997).
3. T. C. Somers and E Verette, "Phenolic Composition of Natural Wine Types," in H. F. Linskens and J. F. Jackson, eds., *Wine Analysis*, Springer-Verlag, New York, 1988.
4. V. L. Singleton, "Wine Phenolics," in H. F. Linskens and J. F. Jackson, eds., *Wine Analysis*, Springer-Verlag, New York, 1988.
5. R. M. Lamuela-Raventos and A. L. Waterhouse, "A Direct HPLC Separation of Wine Phenolics," *Am. J. Enol. Vitic.* **45**, 1–5 (1994).
6. E. H. Siemann and L. L. Creasy, "Concentration of the Phytoalexin Resveratrol in Wine," *Am. J. Enol. Vitic.* **43**, 49–52 (1992).
7. M. Jang et al., "Cancer Chemopreventive Activity of Resveratrol, a Natural Product Derived from Grapes," *Science* **275**, 218–220 (1997).
8. R. M. Lamuela-Raventos et al., "Direct HPLC Analysis of *cis*- and *trans*-Resveratrol and Piceid Isomers in Spanish Red *Vitis vinifera* Wines," *J. Agric. Food Chem.* **43**, 281–283 (1995).
9. A. L. Waterhouse and R. M. Lamuela-Raventos, "The Occurrence of Piceid, a Stilbene Glucoside, in Grape Berries," *Phytochemistry* **37**, 571–573 (1994).
10. A. L. Waterhouse and P. -L. Teissedre, "Levels of Phenolics in California Varietal Wines," in T R. Watkins, ed., *Wine Nutritional and Therapeutic Benefits*, ACS, Washington, D.C., 1997.
11. P. Ribereau-Gayon, "*Recherches sur les Anthocyanes des Vegetaux. Application au Genre* Vitis," Librairie Gen. de l'Enseignement, Paris, 1959
12. E. Revilla, E. Alonso, and V. Kovac, "The Content of Catechins and Procyanidins in Grapes and Wines as Affected by Agroecological Factors and Technological Practices," in T. R. Watkins, ed., *Wine Nutritional and Therapeutic Benefits*, ACS, Washington, D.C., 1997.
13. R. T. Cabot, "The Relation of Alcohol to Arteriosclerosis," *JAMA* **43**, 774–775 (1904).
14. A. L. Klatsky, "The Epidemiology of Alcohol and Cardiovascular Diseases," in T. R. Watkins, ed., *Wine Nutritional and Therapeutic Benefits*, ACS, Washington, D.C., 1997.
15. R. Doll, "One for the Heart (Beneficial Effects of Alcohol Consumption on the Heart)," *Br. Med. J.* **315**, 1664–1668 (1997).
16. S. Renaud and M. de Lorgeril, "Wine, Alcohol, Platelets, and the French Paradox for Coronary Heart Disease," *Lancet* **339**, 1523–1526 (1992).
17. E. N. Frankel et al., "Inhibition of Oxidation of Human Low-Density Lipoprotein by Phenolic Substances in Red Wine," *Lancet* **341**, 454–457 (1993).
18. E. N. Frankel, A. L. Waterhouse, and J. E. Kinsella, "Inhibition of Human LDL Oxidation by Resveratrol," *Lancet* **341**, 1103–1104 (1993).
19. L. O. Dragsted, M. Strube, and J. C. Larsen. "Cancer-Protective Factors in Fruits and Vegetables: Biochemical and Biological Background," *Pharmacol. Toxicol.* **72** (Suppl. 1), 116–135 (1993).
20. A. J. Clifford et al., "Delayed Tumor Onset in Transgenic Mice Fed an Amino Acid-Based Diet Supplemented with Red Wine Solids," *Am. J. Clin. Nutr.* **64**, 748–756 (1996)
21. I.-M. Tegmo-Larsson and T. Henick-Kling, "Ethyl Carbamate Precursors in Grape Juice and the Efficiency of Acid Urease on Their Removal," *Am. J. Enol. Vitic* **41**, 189–192 (1990).
22. G. S. Stoewsand, J. L. Anderson and L. Munson, "Wine and the Effect of Body Weight on Ethyl Carbamate-Induced Tumorigenesis in Mice," *J. Wine Res.* **7**, 207–211 (1996)

G. S. STOEWSAND
Cornell University
Geneva, New York

PINEAPPLES. See FRUITS, TROPICAL.

POTATOES AND POTATO PROCESSING

Potatoes are members of the Solanaceae family. Of the many tuber-forming *Solanum* species, the one that is most widely cultivated is *Solanum tuberosum*. The edible portion of potato is a tuber or an underground stem. While the potato is referred to as the "Irish potato," the center of origin of cultivated potatoes is in the Andes Mountains of South America (1,2). Long before the Spaniards arrived in South America, potatoes were a major portion of diet and a component of the culture of the ancient civilizations of that part of the world. Potatoes were introduced in Europe by the mid- to late-1500s by the Spanish. It took some time for them to be accepted as a food. However, potatoes became a vital part of the food supply of Ireland in the 1600s. Unfortunately, potatoes were the primary component of the Irish diet by the late 1840s when late blight (*Phytophthora infestans*) destroyed a major portion of the crop. The famine forced many Irish to immigrate to North America. The importance of potatoes in Ireland is responsible for the term Irish potato.

Potato tubers are composed of approximately 75 to 80% water, 20 to 23% carbohydrates, and about 2% protein. Approximately 80% by weight of potato carbohydrate is starch and is composed of amylopectin (75–79%) and amylose (21–25%) (3,4). The nutritional value of the crop was demonstrated by the Irish diet prior to the famine. Currently most individuals underestimate the nutritional value of potatoes. Potatoes are a good source of vitamin C, B_6, potassium, and dietary fiber (Fig. 1). Although the protein content of potatoes is relatively low, it is a high-quality protein, and as demonstrated by Irish history, if enough potatoes are consumed, the requirement for protein can be satisfied. Because of the potato's ability to produce high yields under a wide range of climatic conditions, it is an important crop worldwide (5). It ranks after wheat, rice, and maize as the fourth most important crop and is the most widely grown vegetable in the world. The largest potato-producing countries in 1997 were China (46 million t), Russia (40 million t), Poland (25 million t), and the United States (20 million t) (6).

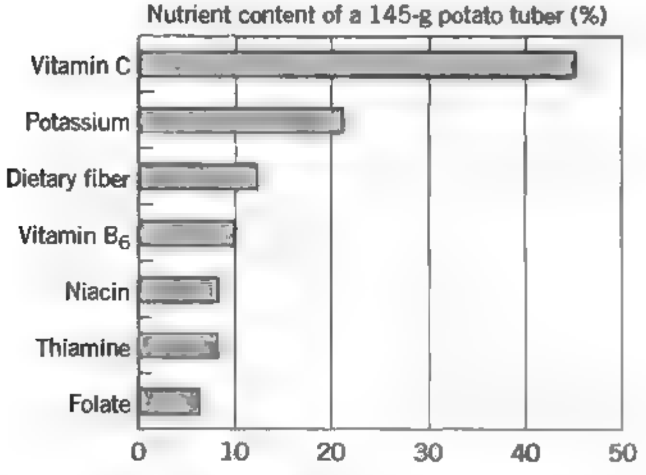


Figure 1. Nutritional contribution of a medium-sized potato to the daily requirements of 2000-calorie diet. *Source:* National Potato Board, Denver, Colo.

The per capita consumption of potatoes in the United States has increased from 46 kg in 1950 to 64 kg in 1997 (7). Approximately one-third of the United States crop is consumed in the fresh form while frozen products accounted for about 40% of the consumption (Fig. 2).

Nutritionally, potatoes are quite stable and are available year-round through modern storage and processing technology. Approximately 75% of the potatoes produced in the United States are stored for later consumption. Storage terms range from 2 to 11 months as required to match raw material supply with utilization. Because potatoes are highly perishable, potato storages must be designed and operated effectively and economically to limit losses in quality and quantity of the raw potatoes. Special storage environments are required to maintain potatoes for their intended end use (Table 1).

Potato varieties differ in their culinary characteristics. Potatoes with high dry matter (or specific gravity) are preferred for processing into frozen French fries, potato chips and dehydration, and as mealy, dry-textured baked potatoes. Potatoes used in potato salads or in canned products,

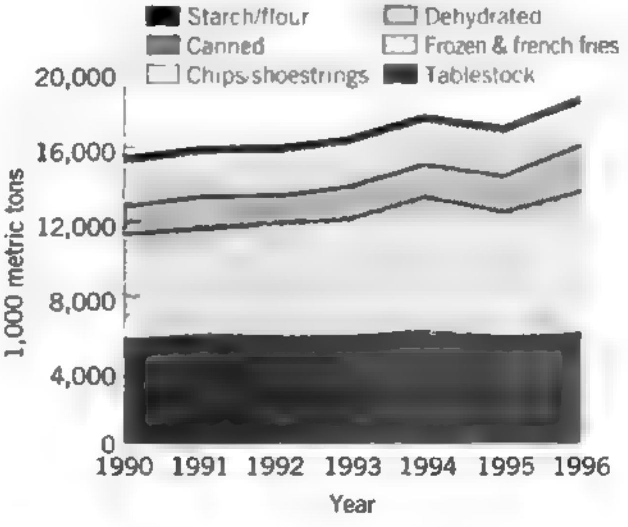


Figure 2. The fresh market equivalent of utilization of U.S. potatoes, 1990–1996.

Table 1. Environmental Requirements of Potatoes in Storage for Specific End Uses

End use	Temperatures, °C	Relative humidity, %	Airflow rate, m^3/kg-h
Seed	3–4	>90	0.02
Fresh	4	>90	0.02
French fries	7	>95	0.04
Chips	7–10	90–95	0.04

such as soup, should have low specific gravity. High-specific-gravity potatoes tend to fall apart when boiled.

The sugar content and composition of potatoes also determine how they are utilized in processing. Potatoes with a high reducing sugar level produce fried products that have a dark color.

PROCESSING OPERATIONS

Processing potatoes into finished food products involves a sequence of operations dependent on the product being prepared. Figure 3 illustrates major operations typically used for processing French fries, chips, and flakes. The initial stages of processing are common to the three major types of potato processing. Receiving, washing, peeling, inspection, and cutting/slicing potatoes are done in a similar manner, even though they may not be done in exactly the same. The steps outlined in Figure 3 are specific to the product being produced.

Receiving

Receiving operations are among the procedures that are common to all potato processing plants. Appropriate types and sizes of equipment and facilities are required for unloading (dump hoists, hoppers), handling (flumes, conveyors), and temporarily storing (bulk bins, pallet boxes) raw potatoes delivered to the plant by controlled-temperature highway trucks and railroad cars. Typical receiving operations consist of either wet fluming or dry conveying types of systems. Usually, some means of sampling the incoming potatoes is used to determine raw product quality.

Most potatoes are delivered to processing plants unwashed. Therefore, a system to remove soil and extraneous material is required at the plant. Forced water sprays combined with brushing are used to remove soil from the tubers. Destoners and trash elevators are normally incorporated into either the receiving operations or the initial washing operation at the plant.

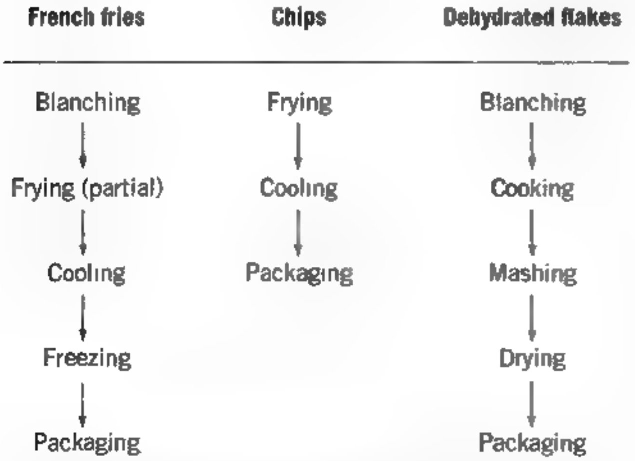


Figure 3. Flow diagram showing major operations in processing raw potatoes into French fries, chips, or dehydrated flakes.

Peeling

Peeling is a critical step in preparation for processing because the amount of material removed directly affects final product yield. Peel removal normally ranges from 1 to 10% by weight of raw product in chipping plants and from 10 to 25% in French fry plants. Steam peelers followed by abrasive peeling types are the most common. Abrasive peelers function by contact between tubers and an abrasive coating on moving rolls, drums, or disks to physically erode the peel from the tuber. The removed peel is then flushed away with flowing water. Steam peeling softens the peel at tuber surfaces via the action of heat and pressure in an enclosed vessel. Water sprays and brushes are used to remove the softened peel after the tubers exit the pressure vessel. Time and temperature settings are critical to controlling depth of peel softening and removal in the steam process.

Inspection

Removal of the peel often discloses blemishes within the potato tubers. Inspection for these defects is accomplished by visual means or electromechanical detection and removal. The number of inspectors or mechanical devices depends on product volume and/or defects. Defects such as bruised and discolored areas are commonly trimmed away or the entire tuber may be removed. Trimming of such defects from the tubers by hand is performed by the inspectors or by a photo/mechanical process. In some plants, a second inspection follows the cutting/slicing operation. Then optical detection and mechanical removal of only the defective portion of a French fry strip is performed.

Cutting/Slicing

Subsequent to the inspection operation, potatoes typically enter a cutting/slicing operation, which consists of equipment designed to produce cut potato pieces of appropriate size and shape from the raw tubers. A variety of sizes and shapes of slabs and strips may be obtained from a raw tubers by use of a basic industrial-type rotary vegetable slicer. In some plants, unique water knives, which cut peeled tubers into strips for French fries while being carried by flowing water in pipes, are used. Specially designed equipment provides for proper orientation of tubers relative to cutter blades and results in the longest possible cut strips. Multiple cutter units are used to meet plant capacity requirements.

Blanching/Cooking/Frying

Blanching is not used in chip processing but is a necessary step in French fry and flake production. Blanching (soaking in water at 70–80°C) accomplishes the gelatization of starch granules to improve product texture and limits subsequent cooking oil absorption. Additionally, blanching limits enzyme activity and leaches reducing sugars from the raw potato strips. The removal of reducing sugars is important in preventing dark-colored French fries. Cooling in water at approximately 10°C immediately following blanching is used in potato-flake processing to accomplish gelling of starch. This step is not needed in French fry production. To improve finished product quality (crispness and temperature retention), potato strips for French fries are dried after blanching and prior to frying. In some cases various coatings may be added prior to frying.

Although French fries and chips are both fried in cooking oil—times, temperatures, and equipment are different. Chips are fried as thin (0.125–0.175 cm) slices at times and temperatures that rapidly reduce their moisture content to less than 2%. Typical times and temperatures for chip frying might range from 1.5 to 3 min and from 155 to 190°C. French fries may be fried as strips ranging from 0.65 to 1.25 cm in thickness. Fry times are influenced by slice thickness and oil temperature, but range from 1 to 2.5 min in oil ranging from 160 to 190°C to accomplish an adequate par fry (partial frying), which removes moisture to less than 30 to 40%. Finish frying is done by the end-product user.

Cooling/Dehydrating

Products such as dehydrated potato flakes require cooking rather than frying. The cooking follows the blanching and cooling operations and use times and temperatures that best accomplish cell aggregation with minimal cell rupture. Typically, cooking involves the application of live steam (100°C) for times ranging from 20 to 30 min, dependent on slab thickness and potato solids content, to obtain a cooked product for mashing.

Potatoes are mashed immediately after cooking to minimize cell rupture. Additives such as emulsifiers that prevent gluiness/stickiness by complexing free starch (8), chelating agents that prevent after-cooking darkening by complexing metal ions (9), and antioxidants that prevent oxidation of potato components (10) are incorporated during the mashing process. Cooked potatoes are mashed in equipment that blends the potato tissue with additives until an appropriate consistency is obtained for application to the dehydrating drums for drying.

Dehydration to flakes consists of drying a thin layer of the mashed potato material to less than 6% moisture on a steam-heated rotating drying drum. This process results in a sheet of dried potato tissue 0.015 to 0.025 cm thick (11), which is then reduced to flakes via size-reduction/grinding equipment.

When processing potato chips or French fries, the frying operation is usually followed by a short cooling period before proceeding to the next operation. This cooling generally occurs on conveyor belts at ambient conditions in the plant (Fig. 4). French fries may undergo removal of excess surface oil in 70°C flowing air immediately following frying and before the cooling operation. Cooled French fries then enter a freezing operation. This operation rapidly reduces the product temperatures to −15 to −7°C with refrigerated air or by application of liquid nitrogen (−50°C) directly to the product surface.

Packaging

Packaging of all forms of processed potato products is accomplished by fully automated systems in most processing plants. Potato chips are packaged with form-fill equipment that currently uses special packing materials (foil laminates) that eliminate light and oxygen and provide for maintenance of injected nitrogen. This nitrogen serves both as an antioxidant for the contents and a cushion for the package. Frozen French fries are placed in retail or institutional packages via either form-fill or carton-fill equipment. Polyethylene materials and corrugated cardboard are commonly used for packaging frozen French fries. Potato flakes are filled into institutional or retail packages by automated fillers. Packages normally are made up of oxygen-barrier materials within corrugated boxes or polyethylene bags.

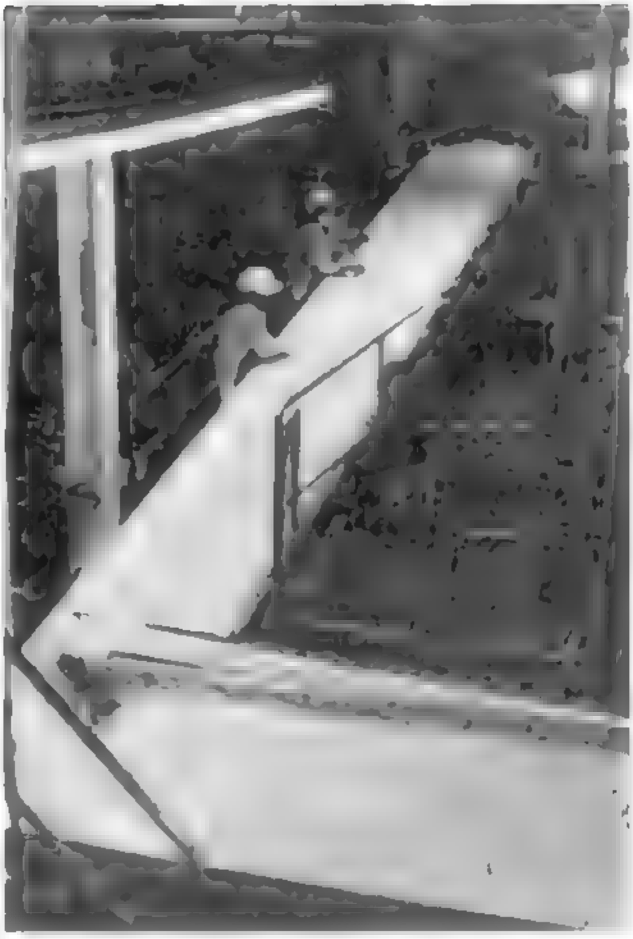


Figure 4. French fries cooling on conveyor belts. *Source:* Courtesy of J. R. Simplot Company.

Numerous types, sizes, and shapes of packages are used in the potato-processing industry to meet the myriad of marketing requirements. Individual packages are normally packed into cases that are further unitized into pallet loads for handling and transporting.

QUALITY

Recent additions of new processed products and heightened consumer awareness of quality aspects of existing processed products have drawn more attention to quality in all raw materials being processed. The primary raw material used in potato processing is, of course, the raw potato tuber. Finished product quality parameters such as color, flavor, texture, and even yield of finished product are directly related to specific quality characteristics of the raw tubers from which the products are processed (12).

Raw Product Quality

Physical characteristics of raw potatoes such as size, shape, smoothness, defects (greening, bruises), diseases (rots), and sprouts can influence the acceptance of potatoes for a particular processed potato product. For example, raw tuber length is extremely important for meeting the strip-length requirements of high-quality French fries. Conversely, smaller, round tubers are preferred for making potato chips, while neither tuber size nor shape is critical in flake processing.

Generally, chemical composition of the raw tuber is more critical across all forms of processed products. Dry-matter content (often measured as specific gravity) directly contributes to final yield of finished products in all processes and thus directly affects the economics of the processing plant (13). Typical ranges of acceptable specific gravity in raw potatoes for making various processed products are given in Table 2.

When the process involves frying of the potato tissue, a very high specific gravity is preferred. In addition to the concern for product yield, dry-matter content directly influences cooking oil absorption (15). In the production of French fries, however, specific gravities above 1.095 have been shown to contribute to poor texture and loss of desirable flavor in the final product.

The sugar content of raw potatoes for processing is another critically important chemical component, particularly when the process involves high-temperature frying. In such cases, the sugar content of potato tissue directly affects finished product color due to the Maillard reaction (16). In general, uniform light-colored finished products are desired (ie, chips, French fries); however, some products are required to exhibit some browning (hash browns, shoestrings). If the balance between sucrose and the reducing sugars, glucose and fructose, is unfavorable in the raw potatoes, the finished product will have poor color. Typically, a sucrose content less than 0.5% by weight and a glucose content less than 0.05% by weight are necessary for production of light-colored potato chips (17). Slightly higher concentrations of sugar are usable when producing French fries.

Finished Product Quality

Because raw-potato quality has a natural variability within any processing time interval, finished product quality will also exhibit variability. Therefore, monitoring of finished product quality versus some standard is practiced in most processing plants.

Table 2. Example Ranges of Specific Gravity for Selected Processed Products

Processed product	Specific gravity	Dry-matter content, %[a]
Chips	1.075–1.100	19.2–24.4
French fries	1.075–1.095	19.2–23.4
Flakes	1.075–1.100	19.2–24.4

Source Ref. 14.

[a] % dry matter = 24.182 + 211 04 (sp. gr. 1.0988)

Potato-chip color can be either subjectively or objectively evaluated. Subjective methods generally use color photographs of chips to which sample chips can be visually compared. Objective methods, such as spectrophotometric instruments, use measurement of light reflected from the finished chip material. Visual comparison to photographs is common in French-fry processing. French fries are also checked against a cut-length standard by sorting the sample. Sensory analyses of finished product samples are conducted to monitor flavor, texture, and appearance.

Oil content of fried products is determined via rapid methods such as refractometry or slower methods such as Soxhlet extraction. The oil content of chips and French fries will vary according to raw product dry-matter content, cut sizes, fry times, and temperatures, but it will normally range from 30 to 45% by weight in chips and from 4 to 7% in partial fried French fries.

BIBLIOGRAPHY

This article was initially written by Paul H. Orr, Red River Valley Potato Research Laboratory, East Grand Forks, Minnesota, and Jerry N. Cash, Michigan State University, East Lansing, Michigan

1. R. N. Salaman, *The History and Social Influence of the Potato*, Cambridge University Press, 1987
2. J. B. Sieczka and R. E. Thornton, "Commercial Potato Production in North America," in *Potato Association of America Handbook*, Potato Association of America, 1993.
3. B. B. Dean, *Managing the Potato Production System*, The Haworth Press, Inc., 1994.
4. A. van Es and K. J. Hartmans, "Starch and Sugars During Tuberization, Storage and Sprouting," in A. Rostovski et al., eds., *Storage of Potatoes*, Pudoc Wageningen, 1987.
5. D. Horton, "Potato Nutrition and Consumption," in *Potatoes Production Marketing and Program for Developing Countries*, Westview Press, 1987, pp. 93–110.
6. National Potato Council, *Potato Statistical Yearbook*, Englewood, Co., 1997.
7. National Potato Council, *Potato Statistical Yearbook*, Englewood, Co., 1998.
8. E. C. Lulai, "Interrelationships of Quality Testing Methods and Relative Effectiveness of Surfactants on Potato Flake Quality," *Amer. Potato J.* **60**, 441–448 (1983).
9. O. Smith and C. O. Davis, "Potato Quality, XIV. Prevention of Graying in Dehydrated Potato Products," *Amer. Potato J* **39**, 135–146 (1962).
10. G. M. Sapers et al., "Flavor Quality and Stability of Potato Flakes, Effects of Antioxidant Treatments," *J. Food Sci.* **40**, 797–799 (1975).
11. W. F. Talburt and O. Smith, *Potato Processing*, 4th ed., Van Nostrand Reinhold Co., New York, 1987
12. W. A. Gould and S. Plimpton, "Quality Evaluation of Potato Cultivars for Processing," *Ohio Agricultural Experimental Station Bulletin* **1172**, 1985.

13. P. H. Orr and C. K. Graham, "A Generalized Model for Determining Least-Cost Sources of Processing Potatoes," *Trans. Amer. Soc. Agricultural Engineers* **26**, 1875–1878 (1983).
14. C. von Scheele, G. Svensen, and J. Rasmussen, "Du Bestamming au Patatiseus, Starelse och Torrsubstanshalt wed Tilhjap av Dess Specifka VKT," *Nood. Jordr. Forsk*, 12–37 (1935).
15. E. C. Lulai and P. H. Orr, "Influence of Potato Specific Gravity on Yield and Oil Content of Chips," *Amer. Potato J.* **56**, 379–390 (1979).
16. R. S. Shallenberger, O. Smith, and R. H. Treadway, "Role of the Sugars in the Browning Reaction in Potato Chips," *J. Ag. Food Chem.* **7**, 274–277 (1959).
17. J. R. Sowokinos and D. A. Preston, "Maintenance of Potato Processing Quality by Chemical Maturity Monitoring (CMM)," *Minnesota Agricultural Experimental Station Bulletin* AD-SB-3441, 1988.

JOSEPH B. SIECZKA
Cornell University
Riverhead, New York

POULTRY FLAVORS

Poultry is among the most popular food products in the world. Its consumption has increased substantially in the last two decades. Consumer demand for poultry reflects the acceptability of the flavor of poultry products. Many factors influence the flavor quality of poultry foods, and numerous other factors affect the formation of flavors that evolve through complex chemical reactions during the heating process. This article summarizes pertinent production and processing factors associated with flavor variations and briefly reviews the chemical basis of poultry flavors.

For the purpose of this article, the flavor of poultry is defined as a blend of tastes and aromas of cooked poultry tissues, including lean dark and lean light meat, skin, and fat, primarily from chicken and turkey. In general, normal or conventionally produced and fresh-cooked poultry exhibit such flavor sensations as meaty, chickeny, chicken brothlike, and, to some extent, fatty-oily. On the other hand, any flavor notes that deviate from those characteristics normally expected from cooked poultry lead to the perception of off-flavors, such as fishy, stale, and rancid. However, there is no standard poultry flavor; sensory perception of a poultry food item with regular poultry flavor as judged by one panel in one region may be judged as weak or having no poultry flavor by another panel in a different region or as somewhat off in flavor by yet another panel. Hence, this article regarding flavor description and intensity is based mainly on individual reports in the literature.

EFFECT OF PRODUCTION AND PROCESSING METHODS

Production Factors

Production aspects that may influence the flavor of poultry include breed, sex, age, feed composition, and management. However, research findings regarding the effects of these factors are often inconsistent. An extensive investigation by the U.S. Department of Agriculture (USDA) in 1960 compared the flavor of 2,000 chickens grown under different production practices and concluded that modern birds (those grown in 1956) have as much flavor as old-style birds (1930s) regardless of the differences in breed and ration (1). On the contrary, some other reports indicated that broilers on rations containing dairy products produced meat with more flavor and that corn-fed poultry was more flavorful than poultry fed barley, oats, and wheat (2). Breast meat of older birds may have more flavor and odor intensity, whereas birds reared at low stocking density could produce thigh meat with stronger flavor (3).

Different species of poultry have different phospholipid profiles, and within one species the phospholipid profile is closely influenced by the fat composition of feed (4). One of the most significant findings concerning the effect of feed composition on poultry flavor was the determination of the responsible ingredients for the fishy off-flavor. It was found and confirmed that the fishy flavor was due to the presence of fish meal, fish oil, linseed oil, and other highly unsaturated fatty acids in the ration (2,5). The addition of alpha-tocopherol to feed reduces the fishy flavor to some extent. Dietary vitamin E also contributes to the oxidative stability of refrigerated and frozen turkey breast meat (6).

Processing, Preparation, and Cooking Effects

Many processing steps are required in the transformation of a live bird to a ready-to-cook, semiprepared (semicooked) or fully prepared (cooked) poultry product. Each processing step is also subjected to variations. Processing variables that have been investigated regarding the flavor of cooked poultry include chilling of eviscerated carcasses, application of phosphates, canning, refrigeration, and frozen storage of raw carcass or cooked meat.

The chilling method has little effect on cooked meat flavor provided that the meat is cooked within a short period after refrigeration or frozen storage. However, air-chilled chicken may not be as stable as immersion-chilled chicken over long storage periods (7). In general, frozen storage extends the storage time to 12 to 14 months without quality deterioration. Thawing and refreezing within a short period has no significant effect; the flavor of cooked meat was not affected when the raw carcass was thawed and frozen five times (8). Commercially processed fresh poultry items are usually distributed and marketed under refrigeration conditions. This short storage period before cooking facilitates the flavor development of cooked meat (9).

The flavor of cooked poultry also depends on the cooking and preparation methods. Flavor characteristics are different among boiled, roasted, and fried chicken, although all these items have similar chickeny flavor. Further-processed poultry products, such as refrigerated, precooked poultry meat, are susceptible to chemical deteriorations and the development of warmed-over flavor during storage. Frozen storage of cooked meats reduces the off-flavor problems to some extent. During chill storage, the flavor intensities of cardboard, warmed-over, and rancid/painty as well as overall off-flavor characteristics increased in broiler breast and thigh meat but not as much in skin (10). Mechanisms that have been suggested as being responsible for off-flavors include the breakdown of cell membranes during cooking and the autoxidation of polyunsaturated fatty acids of the membrane materials. Proper packaging and addition of antioxidants such as polyphosphate may retard off-flavor development (11). The stability of cooked, chill-stored poultry meat may also be affected by precooking temperature. Higher initial cooking endpoint temperatures accelerate the oxidative development during subsequent storage of cooked chicken (12,13).

CHEMICAL BASIS OF POULTRY FLAVORS

Development of Flavors

Raw meats, including poultry and red meats, generally have very little flavor except essentially a simple bloody aroma. During heating of the meat tissue, various chemical reactions take place, and the resulting numerous compounds are responsible for the cooked meat flavor. The precursors or the constituents in the raw tissue include proteins, peptides, free sugars, free amino acids, lipids, fatty acids, salts, minerals, nucleic acids, nucleotides, nucleosides, glycogen and amines, among others, with very few volatile compounds. The brothy taste of chicken is due to the presence of free amino acids and oligopeptides; these components are the flavor precursors (9). The removal of sulfur compounds from the volatile fraction of chicken meat and water slurries by passing the volatiles through mercuric chloride or mercuric cyanide solution resulted in an almost complete loss of "meaty odor." Removal of the carbonyl fraction via trapping in 2, 4-dinitrophenylhydrazine solution resulted in loss of "chickeny flavor," demonstrating a lipid role in species character (14).

The reactions and interactions of various constituents on heating, such as oxidation, decarboxylation, condensation, and cyclization, generate many volatiles. Major reactions involved in the development of cooked chicken flavor are the Maillard reaction and Strecker degradation, producing aldehydes, hydrogen sulfide, and ammonia. The Maillard reaction may result from the reaction of glycosidic hydroxyl groups of sugars and amino groups of amino acids, peptides, or proteins and can also occur between the hydroxyl groups of oxidized lipids and amino groups. Different tissues within the same species have different chemical compositions and undergo different chemical pathways (15). Hence, different volatiles and aqueous flavor compounds are generated on heating different raw tissues. Each compound may contribute a part of the blended flavor sensation. Numerous studies have attempted to qualitatively and quantitatively analyze all compounds related to poultry flavors and off-flavors.

Since the advent of analytical instrumentations, studies on the isolation and identifications of flavor compounds have increased rapidly. In 1972, a list of 178 compounds of raw and cooked poultry was compiled (16). Additional compounds were subsequently identified and 30 new compounds were found in chicken broth (17), including cyclic and acyclic hydrocarbons, alkylbenzenes, a terpene, and a nitrile. The number of total compounds of poultry flavors increased to more than 250 in 1982 (2). Later, 130 compounds, including many new ones, were isolated and identified in fried chicken that might originate from flour, oil, and/or the interaction products of chicken, flour, and oil (18). Eighty new compounds were reported in roasted chicken meat and skin (19) and 75 new compounds in fat drippings (20). Currently, more than 500 compounds, mostly volatiles, have been identified in heated poultry products. The major classifications and examples of compounds in each class are presented in Table 1.

Limited quantitative data have been reported. Although volatile components account for only a small fraction of chicken composition, these compounds are important to the flavor sensation. The aldehyde group is the predominant class of the total volatiles from roasted chicken meat and skin. Other important classes are the sulfur-containing compounds, alcohols, and ketones. The aldehydes are also abundant in the volatiles of fat drippings. The acid compounds are more important in fat drippings than in meat and skin. The identification of lactones in fat drippings deserve special attention in light of their absence in the meat and skin of roasted chicken. For a complete listing of all identified compounds, see Refs. 2 and 19 to 23. A brief discussion of major classes, their precursors, and the chemistry of flavor formation is presented in the following sections.

Hydrocarbons, Alcohols, and Carbonyl Compounds

Earlier studies of volatiles of cooked poultry identified a variety of hydrocarbons, alcohols, acids, aldehydes, and ketones. Of these classes, the carbonyl group (aldehydes and ketones) has often been suggested to be associated with meat flavor. Lipids are the principal precursors of these compounds, and many carbonyls probably originate from oleic, mevalonic, linoleic, and arachidonic acids (5,16). The flavor threshold values of some of the carbonyl compounds (up to C_9) are as low as a few parts per billion. The presence of highly unsaturated fatty acids, primarily in the phospholipids in chicken, is the basis for the development of the characteristic carbonyl profile that differentiates poultry flavor from the flavors of other species (14,24). At least nine compounds have been identified exclusively or predominantly in chicken, including 3-methyloctane, 2,2,6-trimethyloctane, 4-ethylbenzaldehyde, 1,2-dibutylcyclopentane, 2,6-bis(1,1-dimethylethyl)-4methylphenol, diethyl phthalate, 1,12-dodecanedial, 2(E), 4(E)-decadienal, and γ-dodecalactone (21,22). Aroma extract dilution analysis of volatiles obtained by the simultaneous distillation/extraction of chicken broth resulted in the identifications of nonanal, 2(E)-nonenal, 2(E), 4(E)-nonadienal, 2(E), 4(E)-decadienal, 2-undecenal, β-ionone, γ-decalactone, and γ-dodecalactone as primary chicken odorants. Alkanals, generated from turkey skin, contributed to meaty and turkeylike flavor, and the alk-2-enal group had a strong, oxidized, and brothlike flavor (25). Utilizing a supercritical carbon dioxide extraction technique followed by identification of compounds in isolated fractions, it was concluded that excess concentrations of enal aldehyde and ketones are related to the oxidized aroma and flavor of chicken broth (26).

In addition to the pathway of lipid oxidation, carbonyls can be derived from nonenzymatic browning (Maillard re-

Table 1. Representative Flavor Compounds in Poultry

Aliphatic Hydrocarbons (over 45 compounds)
n-decane
n-dodecane
n-heptane
1-octene
n-tridecane
Aromatic hydrocarbons (over 50 compounds)
benzene
n-propylbenzene
toluene
p-xylene
1,2,3-trimethylbenzene
Alcohols (over 45 compounds)
n-butanol
n-hexanol
n-heptanol
n-pentanol
2-methylpropanol-1-ol
Aldehydes (over 75 compounds)
acetaldehyde
n-butanal
2-methylbutanal
n-hexanal
trans-2-hexenal
Ketones (over 55 compounds)
acetone
2-butanone
2-heptanone
2,3-butanedione
3-octen-2-one
Amino acids, amines, and peptides (over 25 compounds)
cysteine
cystine
glutathione
methionine
dimethylamine
Fatty acids and esters (over 40 compounds)
pentanoic
ethyl acetate
hexanoic
2(3)-methylbutyric
ethyl lactate
Lactones (in fat drippings) (over 24 compounds)
4-hydroxybutanoic acid lactone
4-hydroxyhexanoic acid lactone
4-hydroxyoctanoic acid lactone
Pyrrols, pyridines, and pyrazines (over 50 compounds)
2-methylpyrazine
pyridine
pyrrole
3-ethylpyridine
2,6-dimethylpyrazine
Sulfur compounds (over 45 compounds)
methyl disulfide
thiophene
2-methyl thiophene
thiazole
ethane thiol
Furans (over 20 compounds)
2-butyl furan
2-hexyl furan
2-octyl furan
Miscellaneous (over 35 compounds)
hydrogen sulfide
ribose
inosine-monophosphate
chloroform
4,5-dimethyloxazole

action), Strecker degradation, and Amadori transformation reactions. The condensation of carbohydrates with amino acids during heating causes the formations of complex cyclic carbonyls. Carbonyl compounds could be important in secondary reactions; they resulted in furanones and mercapto compounds with roast meat aroma and meaty aroma (27).

Sulfur-Containing Compounds

Sulfur-containing compounds are also responsible for the species flavor (28), and they are important contributors for the meaty aroma in both dark and light chicken meat (14). The presence of hydrogen sulfide (H_2S) and its importance in chicken flavor have been confirmed in several studies (5). The low threshold level (10 ppb in water) makes H_2S an important factor in the cooked-meat aroma. H_2S may also contribute indirectly to the cooked poultry flavor by the formation of secondary products with other volatiles (29). During the heating process, sulfur compounds undergo decomposition and dissolve partially in the poultry lipid, giving the fat and the meat a characteristic sulfur flavor (30). It was found that 4,5-dimethylthiazole and 5-acetyl-2,4-dimethylthiazole have meaty, boiled poultry aroma (31). Also, using aroma extract dilution analysis, 2-methyl-3-furanthiol, 2-furfurythiol, methional, and 2,4,5-trimethylthiazole have been identified as the primary odorants of the chicken broth (22). The production of sulfur-containing volatiles and carbonyl compounds is also influenced by the cooking pH values; alkaline pH conditions increase the production of sulfur-containing volatile compounds but decrease the generation of carbonyls (32). The precursors of hydrogen sulfide in cooked poultry have been identified as mainly the cysteine and cystine of the proteins. Methional, dimethyl disulfide, and methanethiol are formed by Strecker degradation of methionine. But the identification of thiols and sulfides linked to alkyl groups indicate that sulfur-containing amino acids undergo breakdown by a more complex mechanism than the Strecker degradation (5).

Pyrazines and Furans

The pyrazine group has been regarded as one of the most important classes of flavor compounds (33). This group is usually produced by heating of appropriate precursors and exhibits a roasted or nutty aroma. The formation of pyrazines is probably due to the reaction of NH_3 or amino-containing compounds with sugars or other carbonyl compounds (25). It has also been shown that amino acid–N will react with sugar to produce different pyrazines and that degradation products of sugars can react with some amino acids to produce a number of pyrazines. At least one pyrazine, namely 5,7-dihydro-5,7-dimethylfuro (*3,4-b*)-pyrazine, has been incorporated in a chicken soup patent. Furans are derived in meat from the Amadori rearrangement, oxidation of unsaturated aldehydes, and the breakdown of 5′-ribonucleotides.

Other Compounds and Artificial Poultry Flavor

Many other classes of compounds, such as sugars, nucleotides, esters, fatty acids, and ammonia, also contribute to the total poultry flavor sensation. A number of them, for instance, inosinic acid, may contribute to mouth satisfaction and to the intensity of the flavor of other compounds. Other sulfur precursors that could possibly influence flavor include biotin, thiamine, and coenzyme A. A chicken flavor was developed by heating thiamine with S-containing polypeptides and alkanones or hydroxy-alkanones, then adding diacetyl and hexanal. Another formulation of artificial chicken flavor was generated from the combination of hexose, a bland fish or vegetable protein hydrolysate, cysteine, and arachidonic acid heated at 60°C for 10 min (2).

SUMMARY AND FUTURE DEVELOPMENT

Poultry flavors are composed of numerous chemical compounds. Some compounds, such as carbonyls and sulfur-containing compounds, have been shown to be indispensable in poultry flavors, but the literature has also suggested that other compounds play a role in the total flavor sensation of poultry. The flavors of various poultry products are affected by many factors: production, processing, and preparation conditions influence the tissue composition or the precursors of flavor compounds. The formation of flavor notes during heating of meat tissue involves complex chemical reactions; any variations in the precursors or heating conditions may affect the quality and quantity of the final reaction products—the flavor compounds. With the advances in analytical technologies and computer capabilities, it could be possible, in the near future, to identify those compounds critical to poultry flavor and to formulate poultry products with specific and desirable flavor characteristics.

BIBLIOGRAPHY

1. H. L. Hanson et al., "The Flavor of Modern and Old Type Chickens," *Poultry Sci.* **38**, 1071–1078 (1960).
2. H. S. Ramaswamy and J. F. Richards, "Flavor of Poultry Meat—A Review," *Can. Inst. Food Sci. and Technol. J.* **15**, 7–18 (1982).
3. L. J. Farmer et al., "Responses of Two Genotypes of Chicken to the Diets and Stocking Densities of Conventional U.K. and Label Rouge Production Systems. 2. Sensory Attributes," *Meat Sci.* **47**, 77–93 (1997).
4. G. Gandemer, "Muscle Lipids and Meal Quality—Phospholipids and Flavor," *Ocl-Oleagineux Corps Gras Lipides* **4**, 19–25 (1997).
5. E. L. Pippen, "Poultry Flavor," in H. W. Schultz, ed., *Symposium on Foods: The Chemistry and Physiology of Flavors*, AVI Publishing Co., Westport, Conn., 1967, pp. 251–266.
6. B. W. Sheldon et al., "Effect of Dietary Vitamin E on the Oxidative Stability, Flavor, Color, and Volatile Profiles of Refrigerated And Frozen Turkey Breast Meat," *Poultry Sci.* **76**, 634–641 (1997).
7. T. C. Grey et al., "The Effect of Chilling Procedure and Storage Temperature on the Quality of Chicken Carcasses," *Food Sci. Technol.* **15**, 362–365 (1982).
8. R. B. Baker et al., "Palatability and Other Characteristics of Repeatedly Refrozen Chicken Broilers," *J. Food Sci.* **41**, 443–445 (1976).
9. T. Nishimura et al., "Components Contributing to the Improvement of Meat Taste During Storage," *Agric. Biol. Chem.* **52**, 2323–2330 (1988).
10. C. Y. W. Ang and B. G. Lyon, "Evaluation of Warmed-Over Flavor During Chill Storage of Cooked Broiler Breast, Thigh and Skin by Chemical, Instrumental and Sensory Methods," *J. Food Sci.* **55**, 644–648, 673 (1990).
11. C. S. Rao et al., "Effects of Polyphosphates on the Flavor Volatiles of Poultry Meat," *J. Food Sci.* **40**, 847–849 (1975).
12. M. M. Mielche, "Development of Warmed-Over Flavour in Ground Turkey, Chicken and Pork Meat During Chill Storage, A Model of the Effects of Heating Temperature and Storage Time," *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* **200**, 186–189 (1995).
13. C. Y. W. Ang and Y. W. Huang, "Internal Temperature and Packaging System Affect Stability of Cooked Chicken Leg Patties during Refrigerated Storage," *J. Food Sci.* **58**, 265–269, 277 (1993).
14. L. J. Minor et al., "Chicken Flavor: The Identification of Some Chemical Components and the Importance of Sulfur Compounds in Cooked Volatile Fraction," *J. Food Sci.* **30**, 686–696 (1965).
15. C. Y. W. Ang, "Comparison of Broiler Tissues for Oxidative Changes After Cooking and Refrigerated Storage" *J. Food Sci.* **53**, 1072–1075 (1988).
16. R. A. Wilson and I. Katz, "Review of Literature on Chicken Flavor and Report of Isolation of Several New Chicken Flavor Components from Aqueous Cooked Chicken Broth," *J. Agric. Food Chem.* **20**, 742–747 (1972).
17. R. J. Horvat, "Identification of Some Volatile Compounds in Cooked Chicken," *J. Agric. Food Chem.* **24**, 953–958 (1976).
18. J. Tang et al., "Isolation and Identification of Volatile Compounds from Fried Chicken," *J. Agric. Food Chem.* **31**, 1287–1292 (1983).
19. I. Noleau and B. Toulemonde, "Quantitative Study of Roasted Chicken Flavour," *Lebensm.-Wiss. u.-Technol.* **19**, 122–125 (1986).
20. I. Noleau and B. Toulemonde, "Volatile Components of Roasted Chicken Fat," *Lebensm.-Wiss. u.-Technol.* **20**, 37–41 (1987).

21. N Ramarathnam, L. J. Rubin, and L. L. Diosady, "Studies on Meat Flavor. 4. Fractionation, Characterization, and Quantitation of Volatiles from Uncured and Cured Beef and Chicken," *J. Agric. Food Chem.* **41**, 939–945 (1993).
22. U. Gasser and W. Grosch, "Primary Odorants of Chicken Broth," *Z. Lebensm Unters Forsch.* **190**, 3–8 (1990).
23. D. L. Taylor and D. K. Larick, "Investigation Into the Effect of Supercritical Carbon Dioxide Extraction on the Fatty Acid and Volatile Profiles of Cooked Chicken," *J. Agric. Food Chem.* **43**, 2369–2374 (1995).
24. M. Rothe, E. Kirova, and G. Schischkoff, "Sensory Profile Studies on Broth Quality Problems," *Die Nahrung* **25**, 543–552 (1981).
25. J. H. MacNeil and P. S. Dimick, "Poultry Product Quality. 3. Organoleptic Evaluation of Cooked Chicken and Turkey Skin Fractions as Affected by Storage Time and Temperature," *J. Food Sci.* **35**, 191–195 (1970).
26. D. L. Taylor and D. K. Larick, "Volatile Content and Sensory Attributes of Supercritical Carbon Dioxide Extracts of Cooked Chicken Fat," *J. Food Sci.* **60**, 1197–1200, 1204 (1995).
27. A. E. Wasserman, "Chemical Basis for Meat Flavor, A Review," *J. Food Sci.* **44**, 6–11 (1979).
28. J. Schliemann et al., "Chicken Flavour—Formation, Composition and Production. I. Flavour Precursors," *Die Nahrung* **31**, 47–56 (1987).
29. E. L. Pippen and E. P. Mecchi, "Hydrogen Sulfide, A Direct and Potentially Indirect Constituents to Cooked Chicken Aroma," *J. Food Sci.* **34**, 443–446 (1969).
30. E. L. Pippen, E. P. Mecchi, and M. Nonaka, "Origin and Nature of Aroma in Fat of Cooked Poultry," *J. Food Sci.* **34**, 436–440 (1969).
31. S. Fors, "Sensory Properties of Volatile Maillard Reaction Products and Related Compounds, A Literature Review," in G. R. Waller and M. S. Feather, eds., *The Maillard Reactions in Foods and Nutrition*, American Chemical Society Symposium Series 215, Washington, D C., 1983, pp. 185–286.
32. C. S. Rao, E. J. Day, and T. C. Chen, "Effects of pH on the Flavor Volatiles of Poultry Meat During Cooking," *Poultry Sci.* **56**, 1034–1035 (1977).
33. S. S. Chang, and R. J. Paterson, "Recent Developments in the Flavor of Meat," *J. Food Sci.* **42**, 298–305 (1977).

CATHY Y. W. ANG
NCTR/FDA
Jefferson, Arkansas

DUANE K. LARICK
North Carolina State University
Raleigh, North Carolina

See also POULTRY: MEAT FROM AVIAN SPECIES.

POULTRY: MEAT FROM AVIAN SPECIES

The United States and China are the world leaders in meat animal production. The United States is the world's largest producer of beef, veal, broilers, and turkeys and is the second-largest producer of table eggs and pork. China is the world's largest producer of table eggs, swine, lamb and mutton, and horses, the second-largest in broilers, and third in beef and veal. None of that existed when Columbus first came to America (with the exception of possible domestication of turkeys in Mexico). Wild animals and birds were in abundance, and hunting easily supplied all the meat needs of the local inhabitants, and in future years a substantial part of the settlers' needs.

In his second voyage in 1493, Columbus brought to the West Indies livestock, which included chickens. In 1519 Cortez brought cattle and sheep to Mexico and brought turkeys back with him to Spain. De Soto brought horses and hogs to Florida in 1539, and later in the century missionaries brought these livestock to the Pacific Coast of North America. In the seventeenth century European settlers brought livestock and poultry to the United States. At that time pork was the main traditional meat source, partly because it could be well preserved without refrigeration. Chickens were used mostly for eggs and cockfights and less for meat. In 1641 the first meat-packing plant to produce salt pork was opened in Springfield, Massachusetts, by William Pynchon. Chickens were raised in the majority of households in small numbers. A hen laid about 60 eggs per year, mainly in the spring. Chickens provided the household with meat and eggs, and the surplus was bartered or sold in open markets.

In 1998 there were almost 9 billion meat-producing farm animals commercially raised on U.S. farms (Table 1). Many other animals were grown as specialty items for food, sport, and pleasure, mostly in low volume. Surprisingly, about 98% of all farm animals are birds. However, by amount of meat produced (in tons) and by revenue (in dollars), beef is still king, not only in the United States but also worldwide. Yet more people eat lamb, mutton, and sheep than any other animal flesh. The change of consumer preferences in meat consumption started slowly in the United States at the turn of the twentieth century and accelerated rapidly toward its end. The doubling of the American population since World War II from 132.1 million in 1940 to 269 million in 1998 strongly fueled the demand for poultry, resulting in the explosive growth in poultry production, meat consumption, and the emergence of a highly efficient vertically integrated poultry industry. Dramatic changes in lifestyle further increased the demand for poultry as a low-fat, convenient food. The majority of fat in poultry is deposited under the skin. Therefore substan-

Table 1. Major Meat-Producing Farm Animals On U.S. Farms (1998)

Broilers and roasters[a]	8,004,000,000
Layers	259,000,000
Turkeys	290,200,000
Ducks[b]	22,490,000
Beef and dairy cattle	89,485,000
Calves	10,016,000
Hogs	59,920,000
Sheep and lamb	7,616,000
Horses[c]	5,500,000
Total	*8,748,227,000*

Source: United States Department of Agriculture.
[a] Life span of a broiler is 6 weeks and of a roaster is 10 weeks.
[b] 1996 USDA figures.
[c] Most horsemeat is exported.

21. N Ramarathnam, L. J. Rubin, and L. L. Diosady, "Studies on Meat Flavor. 4. Fractionation, Characterization, and Quantitation of Volatiles from Uncured and Cured Beef and Chicken," *J. Agric. Food Chem.* **41**, 939–945 (1993).
22. U. Gasser and W. Grosch, "Primary Odorants of Chicken Broth," *Z. Lebensm Unters Forsch.* **190**, 3–8 (1990).
23. D. L. Taylor and D. K. Larick, "Investigation Into the Effect of Supercritical Carbon Dioxide Extraction on the Fatty Acid and Volatile Profiles of Cooked Chicken," *J. Agric. Food Chem.* **43**, 2369–2374 (1995).
24. M. Rothe, E. Kirova, and G. Schischkoff, "Sensory Profile Studies on Broth Quality Problems," *Die Nahrung* **25**, 543–552 (1981).
25. J. H. MacNeil and P. S. Dimick, "Poultry Product Quality. 3. Organoleptic Evaluation of Cooked Chicken and Turkey Skin Fractions as Affected by Storage Time and Temperature," *J. Food Sci.* **35**, 191–195 (1970).
26. D. L. Taylor and D. K. Larick, "Volatile Content and Sensory Attributes of Supercritical Carbon Dioxide Extracts of Cooked Chicken Fat," *J. Food Sci.* **60**, 1197–1200, 1204 (1995).
27. A. E. Wasserman, "Chemical Basis for Meat Flavor, A Review," *J. Food Sci.* **44**, 6–11 (1979).
28. J. Schliemann et al., "Chicken Flavour—Formation, Composition and Production. I. Flavour Precursors," *Die Nahrung* **31**, 47–56 (1987).
29. E. L. Pippen and E. P. Mecchi, "Hydrogen Sulfide, A Direct and Potentially Indirect Constituents to Cooked Chicken Aroma," *J. Food Sci.* **34**, 443–446 (1969).
30. E. L. Pippen, E. P. Mecchi, and M. Nonaka, "Origin and Nature of Aroma in Fat of Cooked Poultry," *J. Food Sci.* **34**, 436–440 (1969).
31. S. Fors, "Sensory Properties of Volatile Maillard Reaction Products and Related Compounds, A Literature Review," in G. R. Waller and M. S. Feather, eds., *The Maillard Reactions in Foods and Nutrition*, American Chemical Society Symposium Series 215, Washington, D C., 1983, pp. 185–286.
32. C. S. Rao, E. J. Day, and T. C. Chen, "Effects of pH on the Flavor Volatiles of Poultry Meat During Cooking," *Poultry Sci.* **56**, 1034–1035 (1977).
33. S. S. Chang, and R. J. Paterson, "Recent Developments in the Flavor of Meat," *J. Food Sci.* **42**, 298–305 (1977).

CATHY Y. W. ANG
NCTR/FDA
Jefferson, Arkansas

DUANE K. LARICK
North Carolina State University
Raleigh, North Carolina

See also POULTRY: MEAT FROM AVIAN SPECIES.

POULTRY: MEAT FROM AVIAN SPECIES

The United States and China are the world leaders in meat animal production. The United States is the world's largest producer of beef, veal, broilers, and turkeys and is the second-largest producer of table eggs and pork. China is the world's largest producer of table eggs, swine, lamb and mutton, and horses, the second-largest in broilers, and third in beef and veal. None of that existed when Columbus first came to America (with the exception of possible domestication of turkeys in Mexico). Wild animals and birds were in abundance, and hunting easily supplied all the meat needs of the local inhabitants, and in future years a substantial part of the settlers' needs.

In his second voyage in 1493, Columbus brought to the West Indies livestock, which included chickens. In 1519 Cortez brought cattle and sheep to Mexico and brought turkeys back with him to Spain. De Soto brought horses and hogs to Florida in 1539, and later in the century missionaries brought these livestock to the Pacific Coast of North America. In the seventeenth century European settlers brought livestock and poultry to the United States. At that time pork was the main traditional meat source, partly because it could be well preserved without refrigeration. Chickens were used mostly for eggs and cockfights and less for meat. In 1641 the first meat-packing plant to produce salt pork was opened in Springfield, Massachusetts, by William Pynchon. Chickens were raised in the majority of households in small numbers. A hen laid about 60 eggs per year, mainly in the spring. Chickens provided the household with meat and eggs, and the surplus was bartered or sold in open markets.

In 1998 there were almost 9 billion meat-producing farm animals commercially raised on U.S. farms (Table 1). Many other animals were grown as specialty items for food, sport, and pleasure, mostly in low volume. Surprisingly, about 98% of all farm animals are birds. However, by amount of meat produced (in tons) and by revenue (in dollars), beef is still king, not only in the United States but also worldwide. Yet more people eat lamb, mutton, and sheep than any other animal flesh. The change of consumer preferences in meat consumption started slowly in the United States at the turn of the twentieth century and accelerated rapidly toward its end. The doubling of the American population since World War II from 132.1 million in 1940 to 269 million in 1998 strongly fueled the demand for poultry, resulting in the explosive growth in poultry production, meat consumption, and the emergence of a highly efficient vertically integrated poultry industry. Dramatic changes in lifestyle further increased the demand for poultry as a low-fat, convenient food. The majority of fat in poultry is deposited under the skin. Therefore substan-

Table 1. Major Meat-Producing Farm Animals On U.S. Farms (1998)

Broilers and roasters[a]	8,004,000,000
Layers	259,000,000
Turkeys	290,200,000
Ducks[b]	22,490,000
Beef and dairy cattle	89,485,000
Calves	10,016,000
Hogs	59,920,000
Sheep and lamb	7,616,000
Horses[c]	5,500,000
Total	*8,748,227,000*

Source: United States Department of Agriculture.

[a] Life span of a broiler is 6 weeks and of a roaster is 10 weeks.

[b] 1996 USDA figures.

[c] Most horsemeat is exported.

tial fat reduction, sometimes to 2%, can easily be achieved through skin removal

Chicken and turkey consumption more than tripled during the twentieth century, and today chicken is the most-consumed meat when calculated on retail weight (Table 2). Beef and pork subsequently declined, mainly during the 1980s and early 1990s. Yet total red meat (beef, pork, veal, lamb, and mutton) consumed by Americans in 1997 was 52.7% of all meats, compared to poultry (broilers, roasters, turkeys, ducks, and geese) at 40.6% and fish at 6.7%. Another method to calculate meat consumption proposed by the beef industry and now in use is on a boneless basis. According to that method, poultry lost out, as the majority of its retail parts contain significant amounts of bone (Table 2). However, the gap is closing again as 80 lb of broilers and 64 lb of beef per American were consumed in 1998.

The worldwide picture is similar to that in the United States. Since the early 1960s, the number of chickens slaughtered worldwide rose about sixfold (from 6.5 billion in 1961 to 39 billion in 1997). Increased production of turkeys, ducks, and geese was also phenomenal but confined to certain world regions such as China, the Pacific Rim, and Europe. Strong world population growth (4.8 billion in 1985, 5.7 billion in 1995, 6.0 billion in 1999) and the doubling of per capita annual income even in developing countries (to $600 in 1996) fueled this growth, as a large portion of this income in developing countries and a smaller proportion in developed countries was spent to buy more meat. In the United States total meat consumption has increased by 14 lbs per capita since 1970.

Poultry has numerous advantages over ruminants in intensive production:

1. A very low feed conversion range, between 1.75 and 3.0 lb of feed/1 lb of live weight
2. Short production period
3. Vertically integrated industry
4. Highly automated and fast processing lines
5. Small requirement for land
6. Low product price

As a result of these advantages, self-sufficiency in poultry production became a goal in many countries. However, intensive poultry production requires a large volume of grain and oil seeds that are not available in many countries or must be used for human food. Soybeans and corn are the major feed components used in poultry husbandry. The majority of soybeans are produced by five countries (Table 3). World corn production is less concentrated, and 81% is produced by 20 countries. Yet the United States, China, and Brazil are the largest corn producers, with 69% of world production (Table 4). Not surprisingly, the United States, China, and Brazil are also the largest poultry meat and egg producers (Table 5), and India, the fifth largest soybean producer, is the fastest-growing egg producer. Moreover, these countries have some of the world's lowest

Table 2. Per Capita Consumption of Meat in the United States (1996)

	Retail Weight (lb)	Boneless equivalent (lb)
Chicken	73.0	50.9
Beef	68.0	63.8
Pork	49.0	45.6
Turkey	18.5	13.9
Veal	1.0	0.9
Lamb and mutton	1.0	0.8
Total meat and poultry	*210.5*	*175.9*

Table 3. World Soybean Production (1994)

Country	Quantity (million metric tons)	%
World soybean production	113.1	100.00
United States	49.2	43.50
Brazil	23.8	21.04
China	13.0	11.49
Argentina	12.2	10.79
India	4.5	3.98
Rest of the world	10.4	9.20
Total of top 5	*102.7*	*90.80*

Table 4. World Corn Production and Largest Producers (1998)

	Quantity (metric tons)[a]	Percent of world production (%)
World	594,709,169	100
United States	247,932,436	42
China	123,995,428	21
Europe	34,099,568	6
Brazil	33,497,587	6
Mexico	17,998,476	3
Argentina[b]	13,500,127	2
India	9,499,619	1
Rest of the world	114,218,948	19

Source. United States Department of Agriculture.

[a] To convert to bushels, multiply by 39.37.

[b] In 1999 Argentina will dramatically increase its corn production.

Table 5. The Largest Chicken Meat Producers (1997)

Country	Quantity (metric tons)	Percent of world production (%)
United States	12,574,000	24.3
China	8,582,000	16.6
Brazil	4,340,000	8.4
Mexico	1,442,000	2.8
Japan	1,235,000	2.4
France	1,215,000	2.4
United Kingdom	1,160,000	2.2
Indonesia[a]	1,053,000	2.0
Thailand	955,000	1.8
Spain	870,000	1.7
Ten largest		*64.6*

[a] In 1998 poultry production in Indonesia was drastically reduced after civil unrest and the departure of many Chinese who raised poultry.

meat and egg prices because feed is the costliest item in poultry production. Worldwide grain trading, as well as poultry meat imports, has dramatically increased during recent times. Moreover, more countries are banning or reducing local grain production for animals in favor of crops for human consumption. This has made the poultry industry in many developing countries vulnerable to the instability of financial markets. A major financial crisis started in 1994 in Thailand and spread throughout the Pacific Rim. Later it reached Russia, some of its former states, and several South American countries. The crisis had a devastating effect on poultry production in these countries as well as grain producers and poultry meat exporters around the world. Strong and rapid currency devaluation prohibited or strongly reduced these countries' capability to buy feed or meats.

In contrast, most meat-producing ruminants can be grown on pastures that do not compete with human food. The majority of the feeding is done by grazing on land that is not suitable for extensive grain or other crop production. The amount of land in the world that is fit for extensive crop production (arable land) is relatively small and represents 11% of the world land area (Table 6). Furthermore, the arable land available for crop production is reduced by urban development. The arable land is not equally distributed. Although the United States enjoys 20% arable land, a densely populated country like China has only 8% and must depend on feed importation and innovative feeding systems. A vast continent such as Africa has only 7% arable land. Land topography such as high mountains and very large lakes affects rainfall; the angle of the land surface area to the sun, geological history, and land conservation practices are among the parameters that strongly affect the amount of arable land in each region and crop yield. In contrast, 26% of the world's land can be used as pasture, which sustains ruminants without competing with other human food production. Pasture area continues to grow as people clear forests, such as the rain forest in Brazil, or convert wild animal–grazing land into pasture for domestic animals at the cost of endangering numerous plant and animal species and negatively impacting the ecology.

There is still room for increases in all segments of meat production, but the future balance between poultry and other species will be determined by land use, environmental considerations, human population growth rate, available capital, and the stability of financial markets.

CHICKEN

Chickens, especially broilers, are the backbone of the poultry industry. In 1997 more than 39 billion broilers were slaughtered worldwide, and a continuous increase is predicted for the foreseeable future (Table 7). To process this huge number of birds, fast and highly automated processing lines have been developed. A modern plant that can process up to 3.5 million birds per week is the new standard for the industry. This plant can also further process the broilers by cutting up the whole bird into four or eight pieces and reassembling the pieces back into a cut-up whole chicken. Because many of today's consumers prefer specific parts, the modern plant provides a large selection of fresh or cooked products. This significantly increases its profit margins. The plant also performs a retail packaging service to individual supermarket chains that includes printing the chain's specific prices for that week on each package. This enables retail markets to reduce or even eliminate from their meat departments butchers who used to perform the same job manually and at higher cost. This has made retail meat departments more profitable.

In comparison, the standard size of a processing plant during the 1960s that produced mostly ice-packaged whole birds was 50,000 birds per day. This type of plant and smaller ones still operate around the world in areas with lesser chicken production. To supply a processing plant with capacity of 3.5 million birds per week without interruption requires a complicated infrastructure. This includes parent breeder farms, hatcheries, broiler production barns, feed storage and feed mills, railroad access, and a transportation fleet. Railroad access is needed as major feed ingredients are purchased by trainload. The transportation fleet moves fertile eggs, chicks, and market-ready broilers from one facility to another as well as distributes the finished products to retail, fast-food, and institutional distribution centers and warehouses, from which they are distributed to stores.

As environmental-control parameters tighten, waste management becomes a high-priority consideration. This includes manure handling, wastewater treatment of effluents from production and processing, dead birds, hatcheries refuse, feathers and other unwanted biological materials, and odor control. Food-safety programs to control the growth and spread of pathogens are also addressed. In recent years, the detection of human pathogens in finished products has resulted in painfully expensive major product recalls. When food poisoning outbreaks have occurred, recall costs, penalties, and lawsuit judgments run in some cases to hundreds of millions of dollars. In recent years, plant closures, bankruptcies, and the sale of an entire company have occurred in the United States.

Table 6. World Land Distribution and Usage

Land type	Size (in thousand hectares[a])	Percentage (%)
World land	13,041,713	100
Arable land (cropland)	1,441,573	11
Pasture	3,357,520	26
Forests and woodland	3,861,081	30
Other	4,381,539	33

[a] Hectare is equal to 2 47 acres or 0.00386 square miles.

Table 7. World and Continent Production of Chicken Meat (1997)

	Quantity (metric tons)
World	51,645,000
Asia	17,300,000
North and Central America	15,546,000
Europe	8,750,000
South America	7,206,000
Africa	2,240,000
Oceania	603,000

The coordination of all poultry production and processing activities is of highest priority in that plant downtime (being idle) or operation at less than full capacity is extremely expensive. The only way to strictly control and coordinate these complicated operations is by vertically integrating most aspects of the business. This requires a large capital investment. In 1900 5 million chicken farmers were reported and registered in the United States (minimum of 300 chickens). In 1998 only 341 egg producers (minimum of 70,000 hens) and several thousand broiler farmers remained. Clearly the poultry industry is becoming highly concentrated in the United States. The 50 largest companies produce 99% of all broilers, the 10 largest produce 66% of all broilers, and the largest company (Tyson) produces and processes 25% of all broilers. Most of the small broiler farmers became contract growers associated with integrated meat companies.

The disappearance of the majority of the small processing plants caused the broiler farmers that remained in the business to lose their independence. Today, many broiler-production facilities are under contract to the poultry meat companies. The company usually provides the farmers with chicks, feed, and veterinary and nutritional services. The farmer provides the labor, buildings, equipment, litter, light, heat, and water. The farmer is paid by the pound of live weight produced and gets a premium for better-performing flocks. There are few meat companies in each region, so the farmer has limited contract options.

As profit margins have narrowed during the years, larger production units have become vital. However, economical expansion or renovation is strongly related to interest rates. In many countries chronic high interest rates limit potential growth. Financing the expansion from profits and from depreciation has proved to be too slow a response in the poultry business. The low interest rates in effect since the late 1980s have fueled the dramatic expansion and the further concentration of the American broiler industry.

Producers of specialty birds such as Poussin (young spring chicken), petit poulet, the oriental black chicken, and others, or those providing live poultry for oriental markets are the last stronghold of the independent producers in that they do not depend on the large processing plants to take their birds. They produce low volume at higher prices in a family-style operation. In most parts of the world the poultry industry is less concentrated and the market is more regulated than in the United States, and producers there enjoy more freedom of choice and better returns. However, regulated markets generally need protection from lower-cost imports. According to the General Agreement on Tariffs and Trade (GATT), trade barriers for poultry meat and eggs should be removed by 2005. This is creating a lot of political and social concern in countries like France, Poland, Canada, and others where small farms are still common.

History and Breeds of the Chicken

Wild-chicken habitat was the jungles of Southeast Asia—Burma, Laos, Malaysia, Cambodia, Thailand, and Indonesia—as well as India, Pakistan, Sri Lanka, and China. The oldest chicken remains date from 31 million years ago. Four species of the wild jungle fowl are known: the red, gray, Java, and Ceylon jungle fowls. All of these species are good flyers, have beautiful feather colors and patterns, and can still be seen in the wild. The red jungle fowl (*Gallus gallus*) was the main source for domestication, which was done around 2000 B.C. Old Chinese records indicated that in China domestication took place around 1500 B.C. In Malaysia red jungle fowl were kept in captivity long before that. There, roosters were captured or hatched for timekeeping in the jungle and for cockfights. They also became part of religious practices as sacrifices to the gods and often replaced human sacrifices. The chicken was not domesticated for eggs or for meat because the red jungle fowl laid only eight small eggs in a clutch and their 500- to 1100-gram carcasses were stringy and inedible.

The domesticated chicken (*G. domesticus*) moved westward from India to Persia and South Russia, from there to central and western Europe, and then south to the Mediterranean and North Africa. The oldest Greek picture of a chicken on amphorae is from 500 B.C. The first Roman records of cockfights are from 200 B.C.; earlier Roman history makes no mention of the chicken. The New World (the Bahamas) got its first chickens from Columbus while the U.S. mainland got the chickens 150 years later from the settlers.

The domestic chicken went through intensive breeding, and as a result hundreds of breeds exist. Because the chicken is a beautiful and in many cases exotic bird, most breeds were bred for showmanship and sport. Cockfights were highly popular around the world, in particular in Rome and in Byzantium. In the United States cockfighting used to be very popular, but it is now prohibited in many states. Abraham Lincoln got his nickname “Honest Abe” because he was a reputable cockfight referee.

Egg consumption became more common after breeding increased egg size and the number of eggs laid. Yet egg laying remained seasonal (spring). Chicken meat started to be consumed after the Greeks on the island of Kos developed a feed formula that fattened chickens. The technology remained unknown for many years until the Romans spread it throughout the Old World after conquering Greece. Around 100 B.C. eggs and chicken meat were staple foods throughout the Mediterranean and Europe.

The first commercial breeds for eggs and meat were pure breeds. The most common breeds used are the White Leghorn, an Italian breed used for white shell eggs, and Rhode Island Red, used for production of brown eggs. New Hampshire Red, White Plymouth Rock, Cornish, and British Light Sussex are often used for meat production. At the beginning of the century the dual-purpose breeds were popular because they provided high-quality meat and eggs. As intensive production took place, however, specific breeds for meat and eggs were developed separately for greater efficiency. In broilers, fast growth rates, improved feed-to-meat conversion ratio, white feathers, white or yellow skin, and year-round production were the main breeding goals. In 1935, about 16 weeks were needed to raise a 2.8-lb broiler with a feed conversion ratio of 4.4 lb feed per 1 lb live weight. In 1994, 6.5 weeks were needed to produce a 4.65-lb broiler with 1.9 feed conversion ratio. Laying hens went from 131 eggs per year in 1925 to 260 to 300 eggs in 1998. These are small birds with substantially less meat than broilers. About two dozen commercial breeders now supply most of the commercial meat stocks.

Production

Production and processing of chicken for meat and eggs became an extremely intensive high-volume operation in the United States. Low profit margins and the low product prices do not leave much room for errors or inefficiencies. To operate under these constraints, companies must continually increase their scale and efficiency. This trend started in the 1920s and 1930s when successful breeding turned broilers and laying chickens from seasonal into year-round egg and meat producers. It exploded during the 1980s and 1990s, taking advantage of the marvelous developments in computers and automation and the dramatic increase in consumer demand. The twenty-first century is expected to bring even greater efficiencies.

To supply a modern processing plant with 3.5 million crossbred broilers per week, a huge production pyramid system is required. At the top of the pyramid is the breeding company, which develops the male and female lines of the grandparent breeder lines. The grandparent males and females produce fertile eggs or live chicks of the male and female lines of parent breeder lines. These are sold directly to parent breeder farms, which belong to very large broiler companies or to their contracted farmers. Fertile eggs are produced and shipped to hatcheries, which generally belong to the broiler companies.

The standard broiler is raised for about 6 to 7 weeks. Therefore 22–25 million broilers at different ages must be raised at the same time to provide a steady supply of 3.5 million birds per week to the processing plant (projected broiler mortality is included). About 275,000 grandparent chickens and 750,000 parent breeder chickens are needed to maintain the steady stream of broiler chicks needed. The grandparents and parent birds are replaced at around 15 months of age when egg production decreases. A standard production house (growing barn) maintains 20,000 to 25,000 broilers (Fig. 1). Fewer birds are housed in summer to reduce heat stress. Therefore, 840 to 1,050 production houses are needed to support one plant. Around 36 plants of this magnitude will be needed to process the 6.3 billion broilers slaughtered in the United States annually (1997), although today many processing plants are smaller. Countries with smaller production facilities incur inefficiencies in their production systems, which results in higher production costs and higher finished product prices. Low standard of living, low local currency exchange rate against U.S. money, and low feed cost can improve prices in these countries when converted to U.S. dollars for comparison. However, when calculated as percentage of income spent on food, these prices are enormously high. Only highly vertically integrated large companies can have megaplants because large capital investment is needed. Smaller companies that are partially integrated need to buy part of their services from independent hatcheries or feed mills, which makes their operation more expensive. As a result the larger companies keep buying smaller ones and integrating them into their megaoperations. They also buy smaller food companies that further expand their processing and marketing capabilities. In many countries the rate of annual disappearance of poultry companies is 5 to 10% of all existing companies.

Figure 1. Mother breeder chicken production barn (laying nests are on the right and left sides).

Incubation is also a vital part of a modern megaproduction scale. The incubator was commercially developed around 500 B.C. by the Chinese, who improved the concept previously developed in what is now Malaysia. There, the jungle fowl eggs were gathered and incubated; because only cocks were wanted, all female pullets were sent free back to the jungle. The Chinese used a series of baskets that held about 1,200 eggs. Later, the Egyptians independently developed large hatcheries made from sun-dried mud bricks that held about 70,000 eggs at one time. The incubators were heated by burning coal or camel dung, and egg temperature was measured on the eyelid by an experienced operator. Controlling constant incubating egg temperature was an art passed down through families.

In the United States incubators started to be used in the late nineteenth century. During World War I a steep demand for eggs triggered the development of large incubators, as the hatching of eggs under sitting hens hit its limits. Among the most impressive developments was the million-egg room incubator developed in California. Incubation also substantially reduced the cost of chick production compared to mother hens. In 1928, 57% of all chicks were hatched in incubators; in 1959 96% of all eggs were incubated. Today incubators hold 80,000 to 90,000 eggs. They are fully automated and provide excellent control of temperature and other environmental parameters. Heating is done by electricity or hot water. Many modern incubators are of the tunnel type where the eggs are loaded into egg carts on one side and slowly move toward the hatcheries on the other. The chicks are hatched after 21 days. To supply the need of 7 million broilers per week, 10.5 million eggs must be incubated at any time. This requires 117 incubators. The efficiency of the hatchery is measured by hatchability percentage, which depends on parent breeding quality and also on the quality of the incubator operations. The average hatchability of broilers is 85 to 89%. The specific figures must be built into the production schedule of each operation by the addition of more incubators.

Harvesting

Harvesting is one of the most laborious jobs left in poultry production. Crews of bird catchers operate by night when the birds are immobilized due to their poor night vision. The catchers place the birds in shipping crates and load the crates on flatbed trucks. The accuracy and care of the catchers' operations strongly affect the processing plant yield in that bruises and broken limbs are trimmed off by the inspectors and used for pet food. A mechanical broiler loader was developed in England where a slow rotating drum with long rubber fingers carefully moves the birds into a tunnel equipped with conveyors that place the birds in the shipping crate. This equipment is operated by one person. Mechanical harvesters have not gained wide acceptance, but the diminishing labor force will require mechanical solutions in the future.

Other aspects of production are described in the turkey section that are similar in principle to those used for broilers. Processing is discussed in the article POULTRY MEAT PROCESSING AND PRODUCT TECHNOLOGY.

TURKEY

The wild turkey is native to North and Central America, and the discovery of America is associated with the discovery of the turkey. Turkeys roamed American forests long before humans crossed the Bering Strait. The oldest turkey fossils, which were found in California, are dated as over 10 million years old, late Pleistocene period (1). All turkeys belong to two species: the common turkey (*Meleagris gallopavo*), which is larger in size, and the ocellated turkey (*Agriocharis ocellata*), which is smaller and found mainly in Central America. All seven varieties still exist in the wild, however, the ocellated turkey is endangered.

The turkey used to be abundant in the United States during the Puritan era. In New England it was sometimes considered a pest to grain fields. At that time a whole wild turkey was sold for the very low price of 6 cents. Appreciation for the turkey drove Benjamin Franklin to propose it as the national bird, but it lost out to the eagle. Several factors could have led to the turkey's drastic population reduction: the disappearance of the chestnut, the turkey's main food source, due to the devastating chestnut blight; the excessive logging and drastic shrinking of the nation's forest areas, which provide the turkey's habitat; and excessive hunting. Fortunately, successful protection programs were implemented in time. The wild turkey was able to adjust to a different diet based on acorns, a variety of seeds, insects, and occasionally snakes. Today, population numbers are far below those at the time the Pilgrims landed in Plymouth, but there is no danger to the turkey's existence. About 84,000 wild turkeys are harvested annually in the United States.

The turkey can fly short distances up to a mile and uses this skill to reach higher tree branches for night resting after spending the day walking and running on the ground at speeds of up to 20 mph. The chicks, which are hatched on the ground, leave their nests after 1 day and are able to fly after 4 weeks. The mating season is in the spring, when a dominant male changes his external appearance by developing a thick red sac on his chest where fat is stored. He establishes a harem of several females and guards them and his territory from young challenging males. Fights for dominance may end with the death of the loser.

Domestication of the turkey was conducted mainly in Europe. The Native North American Indian did not domesticate the wild turkey, but it is documented that turkeys were kept in captivity in Mexico. The Aztec king Montezuma kept a large number of turkeys as well as other birds and animals for meat and for pleasure in zoological collections. However, the turkeys that were in abundance were also served as feed to other animals. Some believe that these turkeys were already domesticated. Montezuma's appreciation of the turkey was also expressed with turkey statues sculpted from pure gold. Columbus did not bring turkeys back with him to Spain, as turkeys did not exist in the West Indies. It is believed that Cortez brought them in the early 1500s from Mexico, followed by many other importations from all over the continent. Unlike tomatoes and potatoes, which weren't eaten until a century after their arrival due to suspicion that they were poisonous, turkeys were immediately well accepted. By 1570, domesticated turkeys were spread all over Europe and also returned to America with the settlers, where they were crossed with the eastern wild turkey (*Meleagris gallopavo silvestris*).

The meat of the early domesticated turkey was still tough, and most people did not know how to cook it to perfection. As a result, the literature of that time is split between negative opinions as well as praise-filled reports of turkey as a gourmet food. A major turning point was the wedding banquet of King Charles IX of France and Elizabeth of Austria in 1570. The cooked turkeys were so tasty that many of the noble guests started to raise turkeys on their estates. Even today, France remains the largest turkey producer in Europe. In the end, however, it was America that provided the turkey with eternal glory when the Pilgrims who landed in Plymouth, Massachusetts, celebrated Thanksgiving in November 1621 together with their Indian neighbors. In addition to giving thanks, the bountiful turkey was at the center of the celebration, which became an important holiday in the Puritans' life seeing they did not celebrate Christmas or other major holidays. Thanksgiving turned out to be a major civil holiday in the United States after proclamations by George Washington (1789), Abraham Lincoln (1863), and Franklin Roosevelt, who moved Thanksgiving to its present position on the calendar. Christmas, another holiday where turkey is a centerpiece food, had a tougher fight to be established as a holiday in the United States due to strong opposition. For 75 years, the U.S. Congress deliberately convened on Christmas Day. The glorification of the turkey as the holiday season bird placed extreme economic constraints on the turkey industry because most production was geared toward a short period of several meals per year. The marketing success of the chicken opened the door for the turkey. Many cut-up and value-added products with attractive nutritional profiles were developed, resulting in year-round consumption of 18 lb per capita annually in the U.S. (Table 8). In 1998 about 270 million turkeys were raised in the United States; 45 million were eaten at Thanksgiving, 22 million at Christmas, 19 million at Easter, and the remainder of 186 million were consumed year-round. However, 91% of the population ate turkey on Thanksgiving.

For a long time after the turkey was brought to Europe, it was thought that it was related to the guinea fowl, the West African bird that was brought to Europe by the Greeks from Egypt around 500 B.C. As a result both birds are called *meleagris* in Latin. The turkey was thought to be a cross between guinea fowl and male peacock, which is also reflected in its Latin name, *gallopavo* (Gallo = rooster, pavo = peacock). None other than Linnaeus, the father of scientific taxonomy, made this error. The English common name *turkey* also reflects the confusion between turkey and guinea fowl. Many exotic birds, including guinea fowls, as well as spices and other exotic goods came to Europe from India and frequently through Turkey. The turkey has many names in different languages. In Turkey the turkey is called "the American bird," whereas in Israel the name means "the rooster from India."

The 500 years of breeding has resulted in many varieties that exist all over the world. However, many of these varieties were developed for sport and showmanship and do not have much value as a commercial meat product. Commercial meat breeds were developed mainly in the twentieth century as the turkey industry was emerging from barnyard birds into vertically integrated, efficient commercial production and processing operations.

The following parameters are important to the turkey meat industry:

1. Rapid growth rate and efficient conversion ratio of feed into meat. Modern breeds can reach 40 lb in 20 weeks for the male (tom) and 20 lb for the female (hen). This is twice the weight in less time compared to wild or unimproved domesticated turkey growth (Table 9). The table demonstrates that hens eat less and gain less weight than toms but keep a similar feed conversion ratio. Hens are generally preferred when buying a whole bird. Europeans prefer smaller hens (12–15 lb) compared to Americans (15–18 lb).
2. Yield of breast meat. In the United States and in some European countries the white breast meat of turkey and chicken is overwhelmingly preferred over the dark thigh and leg meat and bears a much higher price. Therefore, effort was made to breed birds with a higher yield of white breast meat. The broad-breasted breed that was developed in England in the 1930s with no major impact on that market became a huge success in the United States and Canada. The wider skeletal structure allows larger breast muscles to be developed in a 40-lb tom. The huge breasts prevent the toms from mating, and artificial insemination is needed. Larger breast muscles can be further developed. However, to achieve this, selection for strains with good leg strength must occur simultaneously.

Table 8. Countries With Top Turkey Meat Consumption Per Capita (1998)

Country	Consumption (lb per capita)
Israel	27.8
United States	18.0
France	14.3
United Kingdom	11.5
Canada	9.7
Belgium-Luxemburg	7.5
Netherlands	3.9

Table 9. Weight Gain and Feed Conversion Standards for Tom and Hen Turkeys

Age	Tom average weight (lb)	Feed conversion[a]		Hen average weight (lb)
1	0.28	0.71	0.28	0.71
2	0.62	1.03	0.61	1.01
3	1.24	1.19	1.11	1.17
4	2.13	1.27	1.84	1.28
5	3.24	1.38	2.76	1.39
6	4.59	1.47	3.83	1.51
7	6.14	1.56	5.03	1.63
8	7.90	1.66	6.31	1.76
9	9.87	1.77	7.71	1.87
10	12.00	1.87	9.42	1.93
11	14.20	1.88	10.96	2.00
12	16.44	2.00	12.51	2.20
13	18.72	2.10	14.04	2.29
14	21.00	2.20	15.54	2.37
15	23.28	2.49	16.98	2.46
16	25.57	2.60	18.32	2.49
17	27.90	2.60	19.54	2.58
18	30.27	2.60	20.63	2.87
19	32.61	2.67	21.55	2.93
20	34.90	2.76	22.27	3.04
21	37.11	2.97	22.78	3.08
22	39.22	3.10	—	—

Source: Ref. 5.

[a] Feed conversion = pounds of feed/1 lb of live weight

3. Uniformity. The need for uniform carcass size is most important for the meat processor, because the processing line and especially the evisceration machine cannot be adjusted to large differences in bird size. Breeding and management techniques are able to control size variation, although not to perfection.
4. Appearance. The external appearance of a processed whole bird is a key parameter for consumer acceptance and for marketing promotion. During processing, scalding and mechanical feather plucking can provide the desired appearance. However, small black feathers (pinfeathers) and black pigment spots on the follicles can strongly downgrade appearance due to their strong contrast with the yellowish white skin. Breeding of white-feathered turkeys solved this problem.

Commercial Varieties

Commercial meat-producing turkey varieties are classified according to feather color, either bronze, black, or white, and by skeletal structure, the large broad breast and the smaller unimproved varieties.

The white varieties are now dominant in the United States and Canada. The White Holland and the Broad Breasted Bronze (BBB), were the most important source for breeding. Their descendant, the Broad Breast White, is today the main commercial breed in North America. The Beltsville Small White, with 15-lb toms and 10-lb hens, was popular in the 1950s, when many consumers preferred small birds. However, consumer preferences changed, and currently it holds a very small percentage.

The Bronze turkey is named after its unique color, a shimmering green-bronze that appears metallic in the sunlight. Two types exist, the Broad-breasted Bronze for commercial use and the unimproved variety, which is used in specialty small-scale production. The Broad-breasted Bronze was the world's main variety from the 1930s to 1950s but has been mostly replaced by the Broad-breasted White.

The Black turkey is a medium-size variety with a 27-lb tom and an 18-lb hen. In western Europe it is highly appreciated for its meat quality and therefore is intensely grown there, especially in France and England.

Production and Consumption

The turkey industry is composed of a small number of large vertically integrated companies. The entities own or contract out all major components of the businesses, such as hatcheries, production farms, feed mills, processing plants, and transportation fleets. In this way they can better control costs and quality and improve the efficiency of their operations by designing better production schedules.

In the commercial hatcheries eggs are placed in hatching trays, round edge up, and then secured in place. Environmental conditions and egg rotation are automatically controlled; chicks emerge after 28 days of incubation. The young poults go through sex determination, vaccinations against common diseases, and beak trimming to prevent cannibalism. Then they are placed in shipment packages, which are well ventilated and temperature controlled. Within 24 hours they are moved to brooder barns, where they live to about 6 weeks of age. During this time the poults need a special starter feed, supplemental heat from brooder stoves, adequate ventilation, and protection from exposure to diseases. Due to poult sensitivity to diseases, brooder barns are separated from hatcheries and growing barns. The growing barns are typically 500 feet long and 50 feet wide and hold about 10,000 hens or 7,000 toms (Fig. 2). Hens are grown for 14 to 16 weeks and toms 17 to 21 weeks before being marketed. In free-range operations, the birds can roam in a large fenced field adjacent to the barn, similar to free-range production of chickens (Fig. 3). In France, where the free-range system is well developed, other operational conditions besides outside space are required. These include no artificial lighting, large space allotment per bird inside and outside, no animal by-products in the feed, no antibiotics, no water chilling of carcasses during processing, and, most importantly, slow-growing breeds and a longer growth period for better flavor development. As a result, production costs are much higher, but devoted customers of free-range poultry are willing to pay this premium.

Figure 2. Broad-breast White turkey production barn.

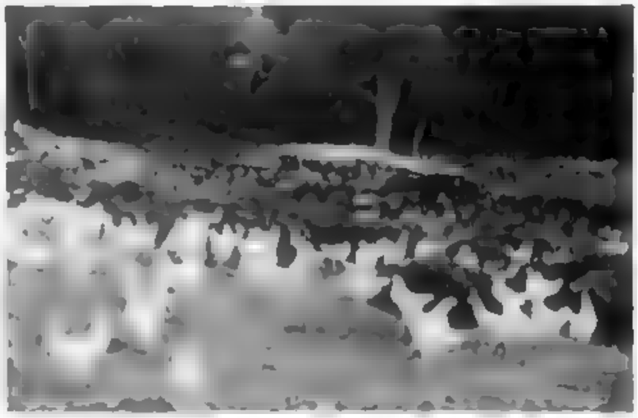


Figure 3. Free-range chicken farm in France.

Feed Mill Operation

Turkey production is very extensive, and profit margins are low. Feed is the most expensive component in raising turkeys and has a major effect on the performance of the flock and finished product quality. Therefore, a strict economically and scientifically based diet is needed. The feed mill formulates various grains and other ingredients on a least-cost basis for sound diets that are the most economical for that day. Four to eight different formulas are used regularly during the bird's lifetime. However, the ingredient composition of each formula may be changed frequently.

When processing time arrives, the birds are placed in transport cages and transported by truck to the processing plant (see the article POULTRY MEAT PROCESSING AND PRODUCT TECHNOLOGY). The entire growing barn is evacuated at the same time and then is cleaned, sanitized, and prepared for the next flock. Turkeys are now produced worldwide. However, large-scale production is still conducted according to historical development lines in North America and Europe (Table 10). The United States,

Table 10. Worldwide Turkey Meat Production (1997)

	Metric tons
World production	4,740,050
North and central america	2,653,310
Europe	1,777,270
South America	144,594
Asia	111,984
Africa	34,856
Oceania	18,036

Source. World Health Organization.

France, the United Kingdom, Italy, and Germany are the top producers (Table 11). Consumption follows production patterns with a single surprise, as Israel tops world consumption with 27.8 lb per capita in 1998 (Table 8).

DUCKS

Origin of Species

Virtually every domesticated duck kept in captivity today can trace its origins back to the wild mallard duck (*Anas platyrhynchos*). The exception to this is the Muscovy duck, which originated in Brazil and can still be found wild in Central and South America.

Habitat. The natural habitat of the wild duck is in areas near rivers, lakes, and streams. Both tree-dwelling and ground-dwelling ducks exist. Over the years, domesticated ducks have become quite capable of living in a wide range of habitats and climates. Although ducks adapt quite readily to living in a variety of environments, unlike their chicken counterparts, they are seldom housed in cages.

Domestication. The duck was most likely first domesticated in India, China, or in both countries. Though records are inconclusive, it would seem that the duck was domesticated approximately 3,000 to 4,500 years ago.

History of Association with Humans. Since being domesticated, the duck has served a variety of purposes. For centuries, flocks of ducks have been kept to supply both meat and eggs. In addition to supplying food, large flocks of ducks were often employed to rid fields and rice paddies of unwanted insects and foliage. One of the most famous culinary dishes made from duck, Pekin duck (Bejing duck), made history in the United States. In preparing the dish, the skin of the fresh duck is pre-dried so it will become very crispy after roasting. The roasted duck is then hung for 6 to 8 hours at room temperature. In accordance with its regulations, the United States Department of Agriculture (USDA) called for refrigeration of the roasted duck. The proponents of the traditional Bejing duck fought back in court and won. Today Pekin duck is the only perishable cooked meat exempt from refrigeration.

Table 11. The 10 Largest Turkey Meat Producers (1997)

Country	Metric Tons
United States	2,499,000
France	745,000
United Kingdom	297,000
Italy	258,000
Germany	206,000
Canada	142,000
Brazil	110,000
Israel	76,000
Romania	44,000
Hungary	39,000

Production Statistics in the United States and Worldwide

World production of duck meat stood in 1997 was 2.73 million t, and 83% of it was produced in Asia (Table 12). China is the largest producer with 72% of world production, followed by France (6.7%), Thailand (4%), and the United States (1.7%) (Table 13). The United States produced 40,000 t duck meat in 1997. However, there has been a slow increase in the number of ducks marketed for meat in the United States over the past few years.

Major Breeds

Thousands of years of selective breeding have resulted in ducks that come in a wide variety of shapes and colors. The differences in these ducks have come to be recognized as distinct breeds as well as different varieties within a particular breed. Several breeds have been developed particularly for meat purposes. Pekin (Fig. 4), Rouen, Aylesbury,

Table 12. World and Continent Duck Meat Production (1997)

	Quantity (metric tons)	Percentage of world production (%)
World	2,734,417	100
Asia	2,274,740	83.3
Europe	296,148	10.8
North and Central America	74,180	2.7
Africa	52,189	1.9
South America	30,978	1.1
Oceania	6,182	0.2

Table 13. World's 10 Largest Duck Meat Producers (1997)

Country	Quantity (metric tons)
China[a]	1,973,000
France	184,000
Thailand	111,000
United States	46,000
United Kingdom	38,000
Eritrea	37,180
Malaysia	36,800
Germany	27,500
Brazil	22,100
Mexico	20,000

[a] Hong Kong included.

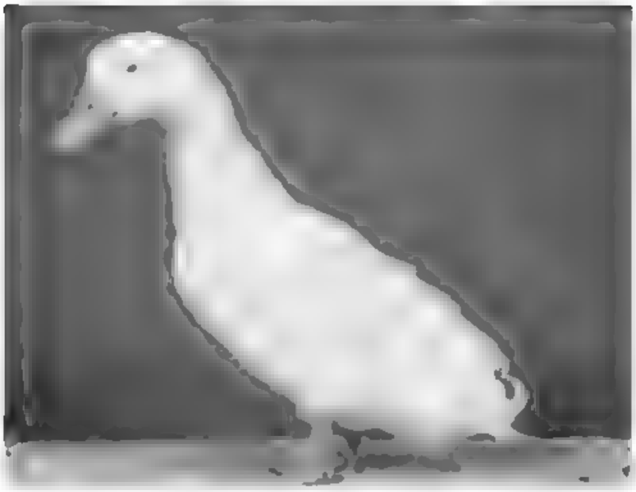


Figure 4. Heavy male Pekin duck (pure breed).

and Muscovy duck have all been selectively bred to provide a large carcass. Pekin duck is the most commonly marketed meat duck today. The Pekin is a large, pure white duck. It is typically marketed at 7 to 8 weeks of age. At this age, it generally weighs between 6.25 and 7.5 lb. The Muscovy duck has become more popular in recent years. Although it grows more slowly and therefore takes several weeks longer to reach optimum weight, it yields a much leaner carcass. The Muscovy also maintains a more efficient feed conversion ratio. The appearance of the Muscovy is quite unlike that of any other breed of duck. It is easily recognized by the large caruncles or fleshy red growths on its face. The Muscovy can be seen in a number of colors or varieties; however, the white Muscovy is most typically used for market purposes. Mule ducks, a sterile hybrid of the Muscovy and the Pekin (or occasionally another meat breed), are also becoming increasingly popular in the meat duck industry. The primary egg-producing breeds are Khaki Campbell and Indian Runner. The Campbell is primarily khaki colored, as the name implies. The Campbell has been bred for high production and exceeds even some of the best chicken breeds. It is not uncommon for some strains of Campbells to lay 300 or more eggs per year. The Indian Runner comes in a variety of colors, with white the most common. The average egg production of the Indian Runner is typically slightly less than that of the Khaki Campbell.

Production Practices

The production practices used in the duck meat industry follow the same general guidelines as those used in the chicken meat industry. The only significant difference is the picking process. The duck, with its thick feathers and down-covered body, is not as easily plucked as the chicken. To overcome this obstacle, many commercial processing plants have added a step to the processing line. After the birds are picked, they are run through hot wax. When the wax is cooled and hardened, it can be peeled away, thus removing any remaining feathers.

Products and By-products

In addition to supplying large quantities of meat and eggs, the duck industry provides numerous by-products. The feathers and down of ducks can be used to stuff clothing, pillows, sleeping bags, and comforters. It is quite common to use ducks, particularly the Muscovy, to produce liver pâté. In addition to this, a partially incubated duck egg is considered a delicacy in many countries.

Future Prospects

What does the future hold for the duck meat industry? Many would like to see an increase in the demand for duck meat and duck products. It is quite likely that artificial selection and well-managed breeding programs will provide us with a faster-growing duck that yields a larger carcass and requires less feed.

GOOSE

Origin of the Species

Habitat. The Romans referred to geese as *duplicis vitae*, or having two lives—being active both on land and in water. Indeed, geese are as comfortable on water as they are on land. Geese feed in both environments but are considered to be inefficient grazers because they cannot digest the cellulose of grasses.

Domestication. There is evidence that the Nile goose may have been domesticated by the end of the second millennium B.C. The Egyptians' utilization of the domesticated goose was short-lived; by the middle of the first millennium B.C. they had substituted domestic chicken meat for goose meat in their diet. The fact that wild waterfowl was so plentiful may have encouraged hunting of geese over farming of them. The domestic goose (*Anser domesticus*) is descended from the wild gray leg goose. This bird may have tended to linger, or lag, during its migrations, thus making it easier to catch and domesticate.

History of Association with Humans. As with other domesticated animals classified as household animals, the goose was probably domesticated by women and maintained in small numbers near the home. The ancient Greeks fattened geese, and goose meat was highly prized. Not only were geese kept by the Romans as a food source, they were also viewed as national heroes. The goose is said to have sounded the warning when the Gauls attacked Rome. The Romans consumed goose meat and eggs, learned how to produce fatty livers, used goose fat for medicinal purposes, and developed the goose quill pen. The Romans transported goose stock across Europe. Even after the fall of Rome, the goose was kept as a backyard/barnyard animal in western and central Europe. With the Europeans' colonization of North America came the goose as well. The domestic goose was kept in nearly every backyard through the early 1900s, for it offered more in return than any other animal or bird.

Production Statistics in the United States and Worldwide

A well-organized goose industry of any significant size does not currently exist in the United States. So relatively few geese are slaughtered under federal inspection that the USDA does not even have a separate listing for geese in its agricultural statistics report.

Europe and Asia continue to be the leaders in goose production. In Hungary, for example, geese represented 13.69% of all poultry slaughtered in 1991. The percentage for the rest of the world was 0.84%.

Major Breeds

Standardization of goose breeds has been ongoing since the late 1880s. The breed standards were set by poultry organizations whose members were concerned more with the "fancy" breeds (ie, breeding for show and competition) than with economic traits. Some standard breeds are Toulouse, the famous goose of France, and the blue-eyed German breed, Embden. Commercially raised meat geese in Europe are Rhenish and White Italians. The breeds used for the production of fatty livers, or foie gras, are Toulouse and Landaise. Both Rhenish and White Italians are white feathered, while Toulouse and Landaise are gray. A popular breed in China is Huoyan.

Production Practices

A variety of management systems are used for geese, depending on the country and the product of interest. It was once believed that the birds had to be at least partially maintained outdoors. A growing number of operations are raising their geese intensively, with no access to pasture. In Europe 10 to 20% of all the meat geese are being produced intensively. The birds may be kept on deep litter or slatted floors. A much smaller percentage of the geese kept for egg production are housed intensively with supplemental lighting. That figure is 5% or less in Europe (Metzer, personal communication, 1999). The birds kept for foie gras production are kept in a more restricted housing system during the period of intense feeding.

Products and By-products

Goose meat continues to have a place of prominence in many Asian and European menus. The ability of the goose, if properly managed, to produce an extremely large, fatty liver allows it to yield a product of extremely high value. The foie gras is sold whole or already cooked and made into pâté. Goose down is considered the premier down for the manufacture of clothing and bedding. Goose feathers are also utilized in similar products.

Future Prospects

Until recently, commercial goose production in the United States has been a relatively small-scale industry, with small flocks on many farms. The increasing diversity of the U.S. population, with many recent Asian and Eastern European immigrants, has already led to plans for and initiation of commercial goose operations in the United States. There appears to be room for growth and for opportunities to educate American palates about goose meat.

SQUAB (YOUNG PIGEON)

The squab is a young pigeon that is marketed at 26 to 30 days of age when it reaches 15 to 17 oz dressed weight. The meat has a distinctive delicate and unique flavor. Squab is preferred over adult pigeon because the meat is tender and has more flavorful fat. Squab meat is very light in color because the young bird has never flown. The squab is fed by both parents with pigeon milk, a thick, high-protein liquid produced in the parents' crop and fed directly to the squab. During the first several days of life, this is the only food the young bird receives. Later the pigeon milk is mixed in the crop with regular plant origin feed and, occasionally, insects. The feeding process continues until the squab leaves the nest in the wild or is sent to market.

Due to the unique feeding process, a pair of pigeons can have no more than two squabs at a time. In many cases one squab will become dominant, taking over the feeding or pushing its counterpart out of the nest, which could result in the death of the weaker squab. In captivity a pair of pigeons can produce up to 20 squabs per year, and modern breeding techniques have helped to increase the vitality of the weaker individuals and successfully bring most of them to market.

Pigeons and squabs have been eaten around the globe since ancient times. Wild pigeons could live almost everywhere and can be found from the Arctic Circle to the tip of South America, with the exception of Antarctica and the Hawaiian Islands. Pigeons and doves belong to the family Columbidae, which contains more than 300 species in the wild. The majority of the species nest in trees and bushes whereas other species reside in cavities in rocks or trees. Some of these species, such as the rock dove (*Columbia livia*), adapted to human surroundings and found shelter in barns, buildings, and the like. The rock dove, which originated in Europe, Africa, and Asia, is the bird most commonly seen in cities and towns around the world. This bird was domesticated and crossbred for many centuries, resulting in a variety of colors. The pure white dove became the symbol of purity and peace. Other breeds of this species were selected for racing, military and civil communication, and exhibitions. In searching for food, however, these birds became a pest in poultry farms and in food-processing plants. The birds can carry human pathogens such as *Salmonella* in their digestive systems and can recontaminate poultry feed and processed food with their feces. Another species, the mourning dove (*Zenidura macroura*), is found mainly in rural areas such as barns and is frequently seen sitting on telephone lines. Pigeons are good flyers, and some species migrate thousands of miles. The "homing" sense of certain species was used by humans for communication and racing. To support flying, the pigeon's breast muscles are large and dark and can reach up to one third of the bird's weight. A very large pigeon can weigh as much as 5 lb.

Pigeons are mentioned in early records of many nations from early biblical times (Noah and the ark), Greek mythology (the dove served ambrosia to Jupiter), and Roman records. Chinese records indicate that the Manchu emperors used to eat pigeons among five other game birds and their flesh had medicinal value. Until the twentieth century pigeons and squabs were hunted, not raised. As squabs became more popular, they were harvested from nests. As a result some species became extinct despite their initial huge numbers. One of the unlucky species was the passenger pigeon (*Ectopistes migratorius*), which at the beginning of the nineteenth century was the most abundant bird in North America. A report by the famous bird artist and naturalist John James Audubon describes a huge flock, estimated at 1.1 billion birds, in Kentucky (1813). When the flock became airborne, it eclipsed the sun. Flying at 60 mph the flock took 3 hours to pass him by, with a flock width estimated at 1 mile. When the birds nested in trees, more than 100 nests per tree were counted. The passenger pigeon was killed in huge numbers for food and hauled by trainloads to urban centers. The surplus was fed to hogs or rendered for fat to make soap. The expanding agriculture and the shrinking forests added to the disaster, turning the passenger pigeon into the pest of grain fields, and the last known free bird was killed in 1899. The bobwhite pigeon (*Colinus virginianus*) was also an important food source for settlers and Native Americans in the eastern United States and still exists in relatively small numbers.

Commercial breeding of pigeons was conducted first in France and Belgium, which developed the varieties of Bordeaux pigeon. In years to come these pioneer breeds were replaced by the French Mondain and the White Swiss Mondain. The United States followed in step by developing the White King, which produces a large full-breasted squab, and larger-scale production started in 1936. England used to be the traditional main market for squabs and pigeons and imported them from the United States and from the European continent. There the bird was served mainly to the well-to-do in restaurants and at parties and specialty events. In contrast, in China pigeons are the food of the common person.

Since the early 1980s California has become the largest center of squab production (Fig. 5). The climate in the central valley, a long, warm, and dry summer followed by a cool winter, is perfect for the locally grown King variants. The squab has a phenomenal growth rate and reaches almost 1 lb dressed weight in 1 month. Each parent pair produces up to 20 squabs per year.

Figure 5. Squab production pens. *Source:* Courtesy of the Squab Producers of California.

Squab production is a family operation conducted on a small to medium scale of 1,000 pairs on average. The pigeons are raised in a row of aviaries (pens), about 65 to 70 sq ft each, which hold 15 to 20 pairs in their individual nests. Management is relatively simple because the pigeons take care of each squab's needs. Feed and water are provided automatically; cleaning is conducted once annually. A pair's life span is about 5 years, and replacement of 20% of the flock annually is a management practice. In the United States about 1.6 to 2 million squabs are produced annually, mainly in California and South Carolina (about 1,000 short tons). Other major producers are France, Belgium, Switzerland, Canada, and China, but accurate production numbers are hard to come by. Most producers, with the exception of China, export a significant portion of their production.

In the United States squabs are sold mainly to white tablecloth restaurants and to oriental markets as well as exported frozen to many countries. The time that pigeons were a staple food in the United States are long gone, and the industry challenge is to introduce this meat again to a large customer base. Squabs bring the highest revenue and profit per bird than any other avian species, in part due to a year-round steady supply, control of surpluses, and devoted customers.

RATITES

Origin of the Species

The term *ratite* describes all the flightless birds and derives from the Latin word *ratis*, or raft. The birds in this grouping possess a sternum or breastbone that is flat, or shaped like a raft. This is in contrast to all other avian species, which are known as carinates. Carinates all have breastbones shaped like the keel (*carina* in Latin) of a boat. Though the ratite group is often described as large, flightless birds, this is partially incorrect. We commonly think of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*) as representative of the ratites, but the group also includes the much smaller rhea (*Rhea americana*, or greater rhea, and *Pterocnemi pennata*, or Darwin's rhea), cassowary (single wattled and double wattled), and the most diminutive, the kiwi (*Apteryx oweni, Apte haasti*, and *A. australis*). Only the ostrich, emu (Fig. 6), and rhea are raised commercially.

Habitat. Fossilized ostrich remains have been discovered in Europe, Asia, and Africa. All of the modern ostriches are native to sub-Saharan Africa. Emus are native to Australia, and both species of rhea are native to South America.

Domestication. Ostrich farming began in earnest ca 1863 in South Africa. Farmers in that area selected the wild-caught birds for certain traits associated with feather quality. After the turn of the century, these birds were crossed with imports from northern and eastern Africa, resulting in an ostrich with good feather quality and egg-laying capabilities. Although the emu had long been utilized by the aboriginal people of Australia and the rhea

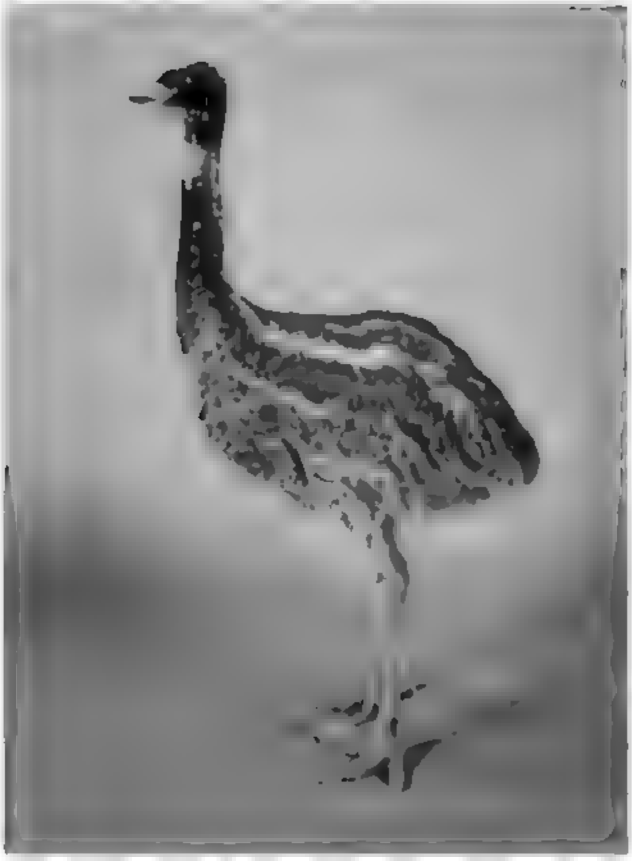


Figure 6. Emu, 2.5 months old.

had served as a meat source in South America, it is generally believed that neither the emu nor the rhea had been the subject of organized domestication efforts before 1988.

History of Association with Humans. The fact that humans have made use of ostrich feathers since ancient times is evidenced by biblical record, Egyptian hieroglyphs, and Greek writings. The efforts of South Africans as pioneers in ostrich farming are extensively documented. Though the South Africans were the first in the ostrich feather market, the Americans entered in the latter half of the nineteenth century. Ostrich farms in Pasadena and Los Angeles were southern California tourist attractions in the late 1800s and early 1900s.

With the collapse of the feather market in 1914, the South African industry experienced a great decline. No significant growth was observed until after 1960. The rise in environmental activism resulted in wildlife protection programs that prohibited the harvesting of rheas for meat in South America.

Production Statistics in the United States and Worldwide

Ratite production exploded onto the U.S. scene in the 1980s. Individuals with breeding stock touted what they considered the birds' strong selling points: valuable hides; a low-fat red meat; and by-products with unique characteristics, such as emu oil. Given the initially small number of ratite breeders but the extensive publicity, breeding stock commanded extremely high prices for several years. With the saturation of the breeder market, quick profits were no longer to be made by strictly selling birds. As the 1990s come to a close, ratite operators must make their money from selling product and not stock.

Major Breeds

Called the African Black by American producers, the farmed ostrich (*Struthio camelus* var. domedomesticus) are smaller than their wild counterparts. The farmed, blue-necked birds, however, have superior feather quality. The male and female have a height of 3.0 m and 2.5 m and a weight of 160 and 120 kg, respectively. The two-toed birds are sexually dimorphic. The male's coloration is black body, brown rump, and white wings and tail. The female is brownish gray. *Dromaius novaehollandiae* is the farmed emu. To date the commercial industry has not produced any specific emu strains or hybrids. The male and female have heights and weights of 1.5m, 36 to 38 kg and 1.8, 55 kg, respectively. The female is larger in these three-toed birds, but both sexes are grayish brown in color. Only the greater rhea is farmed in the United States and Europe. The male and female have heights of 1.7 m and 1.5 m, respectively. At 25 kg the male is slightly larger than the female. Both the male and female in these three-toed ratites are gray.

Production Practices

Traditional ostrich management in the Little Karoo in South Africa involved an extensive system, with the birds running on a natural field. The more intensive system in use today in South Africa involves taking the chicks from the incubator (42-day incubation period) and placing them in floor pens, either of concrete or on short lucerne pasture. From 6 weeks to 2 months the birds are placed in growing camps, where they do not have growing vegetation but rather are fed only a complete ration along with cut lucerne. From 6 months of age until slaughter, the birds are maintained in feedlots.

Management systems in the United States are much less uniform than in the well-established South African industry. As with South African operations, most producers in the United States use artificial incubation of the eggs and then place the newly hatched and dry chicks in chick pens. Concrete and sand are common substrates, and long narrow pens have been advocated to encourage exercise. Until the chicks can maintain their own body temperature, supplemental heat must be provided, and good ventilation is essential. In areas with a hospitable climate, the chicks can be turned out into enclosed pens during the warm part of the day. Chicks stay in the chick pens for 3 to 4 months. Juveniles are moved into larger pens that are well fenced. Breeding-stock birds are kept in pens in single pairs or in colonies of one male and several females. Breeder pens are 1/3 to 1/2 acre in size with a shelter. Again, good fencing is essential to prevent entry of predators, double fencing between adjacent pens can reduce male-to-male fighting. The feeding regimen involves diets containing 25.5% protein for chicks 0 to 2 months old. The protein content of the rations is decreased until the 10-month-old birds are eating a diet containing 8.596% protein. Ostriches are typically slaughtered at 10 to 14 months of age, at weights of 54 to 95 kg.

The majority of emu operators also use artificial incubation (50–52 day period) for their eggs. Housing systems for emus in the United States are similar to but smaller in scale than those for ostriches. A good percentage of the birds have been raised on small farms or large lots. The emu chicks are fed an 18 to 22% protein diet for the first 3 months. From that time on, they are placed on a 13% protein ration. Emus are typically slaughtered at 12 to 15 months of age, at a weight of 33.2 kg.

Rhea production is not an industry of any size or major significance. The incubation period for rhea eggs can vary from 20 to 43 days. Under natural conditions, the incubation and care of the young is performed by the male. Very little research has been conducted on the management of the rhea. For the most part, the birds have been raised more as a hobby than as a meat enterprise in the United States.

Products and By-products

Due to its reputed therapeutic value, emu oil is considered the emu's most valuable product. The oil must be rendered in a special manner before it can be used for medical and cosmetic purposes. Emu meat is another high-protein, low-fat red meat. However, the emu carcass does not yield large roasts and fillets such as can be cut from the ostrich carcass. Again, expert tanners are required to treat the emu hide. Bird size also imposes restrictions here. The hide is not large enough for the production of some leather goods but is well suited for the manufacture of small purses and accessories.

Those who have eaten rhea meat say it is a consistently flavorful product, and it has been used by South Americans for centuries. Some believe that rhea oil also has commercial possibilities.

All the ratites yield feathers at slaughter. Unless there is a rebirth of the millinery trade, the main outlet for the feathers will be costume and jewelry production. Producers often offer for sale blown-out eggs. The eggs are sought after by artists known as "eggers" who use eggs as their medium.

The growth of the ratite industries in the United States has been hampered by some of the same problems that face other young industries. The early years had too many unscrupulous promoters with no real dedication to seeing the industries survive as true agricultural enterprises. Those sincere producers who remain face developing markets, promotion, government-labeling restrictions, and an overall shortage of information. In addition to more publicity about the documented healthy nature of their meat products, the industries will also benefit from continuing research that seeks to verify the beneficial attributes of ratite oils.

GAME BIRDS

Game birds have long been consumed for food by hunters. In modern times certain species have become popular for domestic production (Fig. 7). The cost of raising these species in captivity varies with their reproductive biology, disease resistance, and adaptability to captive rearing. Artificial incubation and brooding (away from the mother) have dramatically reduced production costs. All of the popular species can be raised in confined houses, tiers of small wide floor brooders, or outdoor flight pens. Nutritional needs of the difference species vary, but game birds can generally be raised on feeds used for turkeys of a comparable age or breeding condition. Differences in behavior and disease susceptibility make it advisable for a novice to get professional advice before attempting to raise game birds commercially. Game birds, ratites, and squab have low fat levels, especially when they are skinless (Table 14). To overcome the effects of low fat content, they require special cooking techniques to produce a juicy and highly palatable entree.

Quail

Several species of quail are hunted for food throughout the world, but the Japanese quail (*Coturnix japonica*) and the bobwhite quail (*Colinus virginianus*) are two of the most commonly raised in North America for table use. The European quail (C. coturnix) has been widely consumed as food for centuries, but no accounts of its domestic rearing could be found.

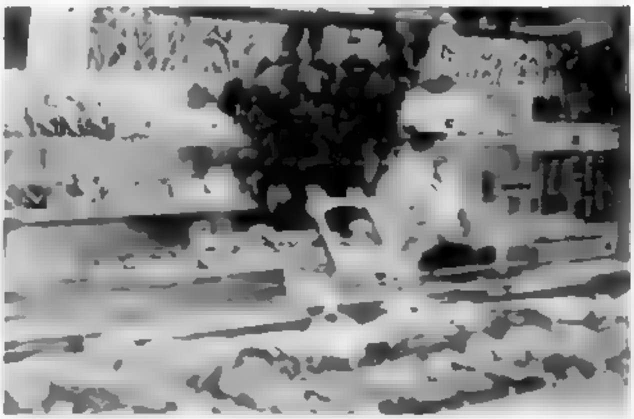


Figure 7. Partridge, grouse, and other game birds in continental supermarket in Tours, France.

Table 14. Nutritional Analysis of Deboned Meat of Game Birds (per 100 g)

Species	Calories (kcal)	Fat (g)	Protein (g)	Iron (mg)	Vitamins (g)
Pheasant	181	9.8	22.7	1.15	177
Pheasant (skinless)	133	3.6	23.6	1.15	165
Squab	294	23.8	18.5	3.5	165
Squab (skinless)	142	7.5	17.5	4.5	94
Guinea fowl	158	6.5	23.4	0.8	92
Guinea fowl (skinless)	110	2.5	20.6	0.8	41
Quail	192	12.1	19.6	4.0	248

Japanese Quail. Japanese quail is used for the production of eggs and meat throughout the world. Imports into the United States were made in the 1950s, but these birds are thought to have been raised in captivity in eastern Asia for as long as domestic chickens. The stock imported into North America soon led to the development of several color mutations and strains selected for meat type or egg production. The body weight of imported Japanese quail was about 100 g, but the meat strains have been increased by genetic selection to a range of 200 to 400 g or more. The rapid reproduction of Japanese quail (fertile eggs are often obtained at 6 weeks of age; incubation requires 17 days) makes this species popular for egg and meat production and for use as an experimental bird by laboratories. Except for the small chick size, which requires special feeding and watering equipment, they can be raised much like chickens. These quail are often raised to maturity in chicken starting batteries, which have ample headroom for adults.

The meat of Japanese quail is prized for table use, and many are marketed to upscale restaurants in North America. The breast muscle of this quail is darker than that of a chicken or pheasant, but the breast meat is less likely to be dry in texture when cooked. The number of Japanese quail raised for food is unknown, but certainly several million are grown annually in Asia and North America. Processed birds are sold whole bodied or partially boneless for $1.50 to $2.50 per bird.

The eggs of Japanese quail are widely sold for food. This quail produces an egg that is 8 to 10% of its body weight in size. In contrast, a chicken egg is 3 to 4% of the hen's body weight. When the quail is raised on typical poultry diets, its eggs taste like chicken eggs. The shell membranes of the eggs are quite tough, making them difficult to open for fresh consumption. Eggs are often hard cooked and eaten fresh or pickled. The small size makes the eggs readily, absorb the flavor of pickle solutions and they make attractive hors d'oeuvres.

Bobwhite Quail. Bobwhite quail are native to North and Central America. The quail are 9.5 to 10.5 in long and weigh about 6 to 7 oz, although larger birds are probably used commercially in that quail respond rapidly to weight selection. Male and female quail differ in plumage. Males have a white stripe that extends from the beak past the eye to the neck. The females have buff-colored chins, upper throats and eye stripes. This species reaches sexual maturity at about 16 weeks. The eggs hatch after 23 to 24 days incubation. They are often grown in batteries or wire floor pens to prevent disease. Birds to be sold for hunting are grown in flight pens, but meat birds can be grown entirely in confinement. Like Japanese quail, bobwhite are sold as a specialty poultry item, but they are much less popular due to their higher cost (unprocessed young mature birds typically sell for $4 each). Eggs of this species are sometimes used for food, although the eggs are smaller than Japanese quail eggs and are more expensive to produce because of the bird's larger body size and later sexual maturity.

Pheasant

Most pheasants grown in North America for meat are either Chinese ring-neck (*Phasianus colchicus lorquatus*) or Mongolian (*P. colchicus mongolicus*) or commercial stocks derived from these Pheasants are native to Asia and have been widely imported into North America as a game bird. The plumage of both species differs dramatically between males and females. Males have striking plumage color with a white ring around the neck. The Chinese male has a continuous white neck ring whereas the ring on the Mongolian male does not close under the beak. The Mongolian pheasant is about 10 to 20% larger in body weight than the Chinese. A white-feathered mutant pheasant is sometimes raised for meat in North America. This bird processes well, and no dark pinfeathers are visible. Under natural light pheasants produce about 50 eggs in the spring; reproduction then stops as the birds become refractory to the long days. The birds can be cycled to produce again in about 13 weeks by exposing them to a short day length for 9 to 10 weeks in a light-controlled facility. Pheasants are often sold in an eviscerated, whole-body package for $2.50 to $4 lb. They may be either young birds (called broilers) at 14 to 20 oz or adult birds at 2 to 4 lb. Some birds are sold smoked or fresh partially boned.

Partridge

Although several species of partridge can be reared in captivity, the chukar partridge (*Alectoris chukar*) is most commonly reared for hunting and table use. This bird, first imported into the United States in 1893, is native to Asia, where it inhabits mountains and valleys at elevations from 4,000 to 16,000 ft. Chukars have been established as a game bird in several western states. The plumage is predominately grey, with distinctive black-and-white markings on the head. Males and females are the same in appearance. Chukars are good fliers and are often released within private hunting clubs.

Chukars are widely raised throughout North America. Chukar eggs hatch in 23 days; hens reach sexual maturity at about 16 weeks and lay about 30 to 40 eggs in a season. Like the pheasant, the chukar hen becomes refractory to long days and can be cycled to lay again by exposure to a short day length for 8 to 10 weeks. Chicks can be brooded in wire floor or litter pens. Chukars are often raised on wire to prevent diseases. They are quite aggressive, and fighting and picking often injure pen mates. Beak trimming or use of antipicking devices may be necessary when the birds are raised in close confinement.

Chukars are excellent table birds and are often sold in an eviscerated, whole-body package for $3 to $4 per pound. They are often seen on the menus of upscale restaurants during the Christmas season.

Guinea Fowl

Guinea fowl (*Numida meleagris*) are native to Africa (Fig. 8). They are domestically raised and marketed as a specialty fowl in many parts of the world. Guinea females are reported to lay about 150 eggs in a 9-month season. Chicks are hatched artificially and raised much like meat chickens or turkeys. The meat of young guineas is reported to be excellent with a slightly gamy flavor. Eviscerated packaged birds typically weigh 3.5 to 4 lb and are often sold in an eviscerated, whole-body package for about $3 per pound.

Figure 8. Guinea fowl.

In France, guinea fowl are a staple food and are available in most supermarkets and many open markets around the country.

BIBLIOGRAPHY

1. G. Stocke, *Rancho La Brea: A Record of Pleistocene Life in California*, Los Angeles County Museum, Series 15, 5th ed., Los Angeles, Calif., 1953.
2. P. Ferket, "Turkey Growth Rate and Feed Consumption Standards: Turkey Performance Improves in 1998," *Turkey World*, 12–14 (January–February 1999).

GENERAL REFERENCES

U. Aldrovandi, *Aldrovandi on Chickens*, L. R. Lind, trans. and ed., University of Oklahoma Press, Norman, Okla., 1968.

A. W. Brant, "A Brief History of the Turkey World," *World's Poultry Sci. J.* **54**, 365–373 (1998).

The Broiler Industry, The Turkey Industry, and The Egg Industry: The Poultry Tribune Centennial Edition (1895–1995), Watt Mount Morris, Ill., 1995.

California Poultry Workgroup, *Turkey Care Practices*, Univ. of California, Cooperative Extension, Davis, Calif., 1998.

California Poultry Workgroup, *Broiler Care Practices*, 2nd ed., Univ. of California, Cooperative Extension, Davis, Calif., 1998.

Canadian Department of Agriculture, *Pictures Geese*, Publication No. 1345, Information Division of the Canadian Department of Agriculture, Ottawa, Canada, 1968.

E. Carbajo Garcia et al., "El sacrificio y la produccion de carne," E. Carbajo Garcia et al., eds., *Cria de Avestruces, Emues y Nandues*, Real Escuela de Avicultura, Barcelona, Spain, 1997, pp. 113–136.

E. Carbajo Garcia and F. Castello Fontova, "El Emu," E. Carbajo Garcia et al., eds., *Cria de Avestruces, Emues y Nandues*, Real Escuela de Avicultura, Barcelona, Spain, 1997, pp. 303–326.

E. Carbajo Garcia, E. F. Castello Fontova, and J. A. Castello Llobet, "Historia y Origen de la Produccion del Avestruz," in E. Carbajo Garcia et al., eds., *Cria de Avestruces, Emues y Nandues*, Real Escuela de Avicultura, Barcelona, Spain, 1987, pp. 19–40.

J. A. Crawford, "Foreword," in T. N. Tully, Jr. and S. M. Shane, eds., *Ratite Management, Medicine, and Surgery*, Krieger, Malabar, Fl., 1996.

R. D. Crawford, *Poultry Breeding and Genetics*, Elsevier, Amsterdam, The Netherlands, 1990.

A. Douglass, *Ostrich Farming in South Africa*, Cassell, Potter, Galpin, London, 1881.

Ducks and Geese, Reliable Poultry Journal, Quincy, Ill., 1910.

M. E. Ensminger, *Poultry Science*, Interstate, Danville, Ill., 1980.

I. Eric, *Geography of Domestication*, Prentice-Hall, Englewood Cliffs, N.J., 1970.

"Focus on the West," *Terra* **28**, 50–51 (1990).

F. D. Fowler, "Domesticated Geese," in *Ducks and Geese*, Reliable Poultry Journal, Quincy, Ill., 1910, p. 75.

M. E. Fowler, "Clinical Anatomy of Ratites," in T. N. Tully Jr. and S. M. Shane, eds., *Ratite Management, Medicine, and Surgery*, Krieger, Malabar, Fla., 1996, p. 1–10.

M. A. Hall, "Ratites," in M. A. Hall, ed., *National 4-H Avian Bowl Manual*, Clemson Univ., Clemson, S.C., 1998, pp. 171–173.

D. J. S. Hetzel, "Domestic Ducks: A Historical Perspective," in *Duck Production Science and World Practice*, University of New England Publishers, Armidale 1985.

F. W. Huchzermeyer, "Important Aspects of Ostrich Farming," in *Diseases of Ostriches and Other Ratites*, Agricultural Research Council, Ondersport, South Africa, 1998, pp. 77–119.

F. W. Huchzermeyer, "The Ostrich and Other Ratites," in *Diseases of Ostriches and Other Ratites*, Agricultural Research Council, Ondersport, South Africa, 1998, pp. 26–27.

L. M. Hurd, *Goose Raising*, Bulletin 823, State College of Agriculture, Ithaca, N.Y., 1951

J. Kozak, "Changes of the Goose Stock and the Goose Production in Hungary," *Proceedings of the XX World's Poultry Congress* **3**, 764–767 (1996).

W. M. Levi, *The Pigeon*, Levi, Sumter, S.C., 1945, reprint 1992.

J. P. Mackenzie, *Game Birds*, North Word Press, Minocqua, Wisc. 1989.

J. Mesia Garcia, "La alimentacion del avestrucez," in E. Carbajo Garcia et al., eds., *Cria de Avestruces, Emues y Nandues*, pp. 167–185.

Real Escuela de Avicultura, Barcelona, Spain, 1997, p. 167.

M. North and D. D. Bell, *Commercial Chicken Production Manual*, 4th ed., Chapman & Hall, New York, 1990.

C. G. Olentine, *Watt Poultry Statistics Yearbook*, Poultry International, Vol. 37, Watt Publishing Co., Mt. Morris, Ill., 1988.

M. E. Pennington, F. L. Platt, and C. G. Snyder, *Eggs*, Progress, Chicago, Ill., 1933.

W. Root, *Food: The Authoritative and Visual History and Dictionary of the Foods of the World*, Simon and Schuster, New York, 1980.

A. Rosinski et al., "Possibilities of Increasing Reproductive Performance and Meat Production in Geese," *Proceedings of the XX World's Poultry Congress* **111**, 724–735 (1996).

C. O. Sauer, *Seeds, Spades, Hearths, and Herds*, MIT Press, Cambridge, Mass., 1969.

A. W. Schorger, *The Wild Turkey: Its History and Domestication*, University of Oklahoma Press, Norman, Okla., 1966.

M. L. Scott, and W. F. Dean, *Nutrition and Management of Ducks*, M. L. Scott, Ithaca, N.Y., 1991.

D. J. v. Z. Smit, "Ostrich Farming Practice," in *Ostrich Farming in the Little Karoo*, Bulletin No. 358, Department of Agricultural Technical Services, Pretoria, South Africa, 1963, pp. 53–83.

A. Soyer, *The Pantropheon; or, A History of Food and Its Preparation in Ancient Times*, Paddington Press, London and New York, 1853; reprint 1977.

J. S. Stewart, "What Are the Kinds of Ostriches and Which One Is Best?" *Ostrich Report* **16**, 23 (May 1992).

L. Stromberg, *Poultry Oddities History and Folkore*, Stromberg, Pine River, Minn., 1982.

U.S. Department of Agriculture, *Agriculture Statistics*, Washington, D.C., 1998.

W. W. Van Wulffeten Palthe, *C. S. TH. Van Gink's Poultry Paintings, 1890–1968*, Dutch Branch of the World's Poultry Science Association, Beeklergen, The Netherlands, 1992.

D. W. Verwoerd, "Foreword," in F. W Huchzermeyer, ed., *Diseases of Ostriches and Other Ratites*, Agricultural Research Council, Ondersport, South Africa, 1998.

J. R. Wade, "Restraint and Handling of the Ostrich," in T. N. Tully, Jr. and S. M Shane, eds., *Ratite Management, Medicine, and Surgery*, Krieger, Malabar, Fla., 1996, pp. 37–45.

GIDEON ZEIDLER
University of California
Riverside, California
FRANCINE BRADLEY
RALPH ERNST
JENNIFER NEAR
University of California
Davis, California

POULTRY MEAT MICROBIOLOGY

CONTAMINATION OF POULTRY

The microflora of commercially processed raw poultry cannot be easily defined. Hundreds of different species of microorganisms have been isolated from live poultry and from fully processed, ready-to-eat carcasses and poultry parts. Nonpathogenic spoilage organisms and potential human pathogens are the two general categories of organisms that are of greatest concern to the poultry industry and the ultimate consumer. The following genera have been isolated from fully processed, raw poultry (1–4): *Pseudomonas, Achromobacter, Micrococcus, Flavobacterium, Alcaligenes, Proteus, Bacillus, Sarcina, Streptomyces, Penicillium, Oasora, Cryptococcus*, and *Rhodotorula*. These organisms originate from unlimited sources including the hatchery, the production environment, the feed or water, wild birds or animals, rodents, insects, domesticated farm animals, equipment, and even humans. In the United States and many other countries, almost all commercial broilers and turkeys are reared on the ground in large poultry houses. The outside surface of the live bird, including the feathers, feet, and skin, can support numerous different types of microorganisms. The contents of the intestinal tract of the bird is a constant source of contamination, which can easily be transferred to the live bird during production, transport, and processing. However, the skin of the carcass is more highly contaminated than the visceral cavity of freshly processed raw poultry (5,6). In the United States, the processing of almost all commercial poultry is extremely automated. This fact has caused considerable concern because birds move at considerable speed, up to 91 broilers/min. However, data suggest that commercially processed birds and birds processed by hand do not differ in the incidence or levels of microorganisms (7).

SHELF LIFE AND SPOILAGE

Freshly processed raw poultry carcasses or poultry parts typically harbor 1,000 to 10,000 microorganisms/cm^2 at the exit of the immersion chiller (5,8). At retail, total microbial counts can range from 10,000 to 10,000,000/cm^2 of skin surface area. Raw poultry is considered spoiled when total microbial counts exceed 10,000,000/cm^2 (9). At this level of bacterial growth, the product will be slimy and will have an uncharacteristic sweet smell (10). The two most important factors that influence shelf life are initial microbial levels and temperature of storage

Pseudomonas, Moraxella, and *Alcaligenes* are the principal microorganisms on spoiled poultry carcasses and parts (1,2,11–13). At the time when spoilage becomes evident by slime formation and off-odor, *Pseudomonas* may also have produced a green fluorescent pigment that is visible under ultraviolet light. Any off-odors are the result of biochemical changes in the protein and fat. The time between day of processing and spoilage at retail for nonfrozen poultry carcasses or parts is typically 10 to 14 days at 4°C. The retail consumer should expect only 1 to 2 days of shelf life once the product is brought home and stored in the refrigerator. Thus, if fresh poultry is not to be used on the purchase day or the day after purchase, the product should be stored in the freezer. Frozen whole turkeys or chickens can be stored in the home freezer for 1 year; poultry parts can be frozen for 6 to 9 months (14). Frozen storage recommendations are based on oxidative stability, not microbial spoilage.

Bacterial levels increase when poultry carcasses are segmented, filleted, deboned, or comminuted. In the home, ground turkey or chicken will have a refrigerated shelf life of 1 to 2 days. Ground poultry products can be stored frozen for 3 to 4 months. Again, frozen shelf life is based on oxidative stability. Frozen chicken, fried chicken parts, patties, or nuggets are very susceptible to oxidative rancidity and should be stored in the home refrigerator for 3 to 4 days or up to 4 months in the home freezer.

Numerous methods have been used for evaluating the numbers of microorganisms on poultry carcasses. Among them are pressing metal or plastic dishes filled with solidified agar, using cut tissue sections sliced to known dimensions, using a blender or stomacher, swabbing the skin surface, and rinsing the product with known amounts of diluent. Although counts will not be identical, results from most of these methods are comparable (15).

Studies have been made to determine the effect of temperature on the growth of bacteria on poultry carcasses. A shelf life of 2 days at 15°C and 30 days at 1°C for uneviscerated chickens has been reported (16). It has been noted that broiler carcasses remained acceptable for 9 to 19 days at 1°C, depending on a number of factors, notably the mode of processing and the type of wrapping (17). One study showed that when carcasses were stored at 0°C, it took approximately 18 days for spoilage to occur, and at 20°C only 2 days were required (18). It has been reported that chicken meat with an initial bacterial count of approximately 6.0×10^4 cells/in.^{2} spoiled after 16 days of storage at 0°C (9). Higher storage temperatures, such as 4.4 and 10°C, reduced the shelf life to 7 and 2 days, respectively.

A 10- to 12-day shelf life for immersion-chilled poultry meat and 18 days for hot-packaged poultry meat stored at 2 to 4°C been noted (3). Dry-chilling broiler carcasses by liquid nitrogen exposure resulted in a reduction of psy-

W. W. Van Wulffeten Palthe, *C. S. TH. Van Gink's Poultry Paintings, 1890–1968*, Dutch Branch of the World's Poultry Science Association, Beeklergen, The Netherlands, 1992.

D. W. Verwoerd, "Foreword," in F. W Huchzermeyer, ed., *Diseases of Ostriches and Other Ratites*, Agricultural Research Council, Ondersport, South Africa, 1998.

J. R. Wade, "Restraint and Handling of the Ostrich," in T. N. Tully, Jr. and S. M Shane, eds., *Ratite Management, Medicine, and Surgery*, Krieger, Malabar, Fla., 1996, pp. 37 45.

GIDEON ZEIDLER
University of California
Riverside, California
FRANCINE BRADLEY
RALPH ERNST
JENNIFER NEAR
University of California
Davis, California

POULTRY MEAT MICROBIOLOGY

CONTAMINATION OF POULTRY

The microflora of commercially processed raw poultry cannot be easily defined. Hundreds of different species of microorganisms have been isolated from live poultry and from fully processed, ready-to-eat carcasses and poultry parts. Nonpathogenic spoilage organisms and potential human pathogens are the two general categories of organisms that are of greatest concern to the poultry industry and the ultimate consumer. The following genera have been isolated from fully processed, raw poultry (1–4): *Pseudomonas, Achromobacter, Micrococcus, Flavobacterium, Alcaligenes, Proteus, Bacillus, Sarcina, Streptomyces, Penicillium, Oasora, Cryptococcus*, and *Rhodotorula*. These organisms originate from unlimited sources including the hatchery, the production environment, the feed or water, wild birds or animals, rodents, insects, domesticated farm animals, equipment, and even humans. In the United States and many other countries, almost all commercial broilers and turkeys are reared on the ground in large poultry houses. The outside surface of the live bird, including the feathers, feet, and skin, can support numerous different types of microorganisms. The contents of the intestinal tract of the bird is a constant source of contamination, which can easily be transferred to the live bird during production, transport, and processing. However, the skin of the carcass is more highly contaminated than the visceral cavity of freshly processed raw poultry (5,6). In the United States, the processing of almost all commercial poultry is extremely automated. This fact has caused considerable concern because birds move at considerable speed, up to 91 broilers/min. However, data suggest that commercially processed birds and birds processed by hand do not differ in the incidence or levels of microorganisms (7).

SHELF LIFE AND SPOILAGE

Freshly processed raw poultry carcasses or poultry parts typically harbor 1,000 to 10,000 microorganisms/cm^2 at the exit of the immersion chiller (5,8). At retail, total microbial counts can range from 10,000 to 10,000,000/cm^2 of skin surface area. Raw poultry is considered spoiled when total microbial counts exceed 10,000,000/cm^2 (9). At this level of bacterial growth, the product will be slimy and will have an uncharacteristic sweet smell (10). The two most important factors that influence shelf life are initial microbial levels and temperature of storage

Pseudomonas, Moraxella, and *Alcaligenes* are the principal microorganisms on spoiled poultry carcasses and parts (1,2,11–13). At the time when spoilage becomes evident by slime formation and off-odor, *Pseudomonas* may also have produced a green fluorescent pigment that is visible under ultraviolet light. Any off-odors are the result of biochemical changes in the protein and fat. The time between day of processing and spoilage at retail for nonfrozen poultry carcasses or parts is typically 10 to 14 days at 4°C. The retail consumer should expect only 1 to 2 days of shelf life once the product is brought home and stored in the refrigerator. Thus, if fresh poultry is not to be used on the purchase day or the day after purchase, the product should be stored in the freezer. Frozen whole turkeys or chickens can be stored in the home freezer for 1 year; poultry parts can be frozen for 6 to 9 months (14). Frozen storage recommendations are based on oxidative stability, not microbial spoilage.

Bacterial levels increase when poultry carcasses are segmented, filleted, deboned, or comminuted. In the home, ground turkey or chicken will have a refrigerated shelf life of 1 to 2 days. Ground poultry products can be stored frozen for 3 to 4 months. Again, frozen shelf life is based on oxidative stability. Frozen chicken, fried chicken parts, patties, or nuggets are very susceptible to oxidative rancidity and should be stored in the home refrigerator for 3 to 4 days or up to 4 months in the home freezer.

Numerous methods have been used for evaluating the numbers of microorganisms on poultry carcasses. Among them are pressing metal or plastic dishes filled with solidified agar, using cut tissue sections sliced to known dimensions, using a blender or stomacher, swabbing the skin surface, and rinsing the product with known amounts of diluent. Although counts will not be identical, results from most of these methods are comparable (15).

Studies have been made to determine the effect of temperature on the growth of bacteria on poultry carcasses. A shelf life of 2 days at 15°C and 30 days at 1°C for uneviscerated chickens has been reported (16). It has been noted that broiler carcasses remained acceptable for 9 to 19 days at 1°C, depending on a number of factors, notably the mode of processing and the type of wrapping (17). One study showed that when carcasses were stored at 0°C, it took approximately 18 days for spoilage to occur, and at 20°C only 2 days were required (18). It has been reported that chicken meat with an initial bacterial count of approximately 6.0×10^4 cells/in.^{2} spoiled after 16 days of storage at 0°C (9). Higher storage temperatures, such as 4.4 and 10°C, reduced the shelf life to 7 and 2 days, respectively.

A 10- to 12-day shelf life for immersion-chilled poultry meat and 18 days for hot-packaged poultry meat stored at 2 to 4°C been noted (3). Dry-chilling broiler carcasses by liquid nitrogen exposure resulted in a reduction of psychrophilic and memsophilic organisms initially and on storage at 2 to 4°C. An increase of approximately 2 days in shelf life of hot-packaged, liquid nitrogen—chilled cut-up parts was evident over the hot-packed parts (19). Lowering the storage temperature from 1.1 to 3.3°C to 2.2 to 1.1°C extended the shelf life for vacuum-CO_2—packed and the ice-packed broiler carcasses for approximately 5 and 4 days, respectively (4).

POTENTIAL HUMAN PATHOGENS

The microbiological population on poultry carcasses can, and often does, include potential human pathogens such as *Aeromonas* spp., *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus, Clostridium perfringens, Yersinia enterocolitica*, and *Listeria* spp. Fortunately, most of these organisms are present only at very low levels and tend to remain at low levels due to the fact that they reproduce less vigorously than the many nonpathogens, which outnumber them from the beginning.

Aeromonas

To date, no fully confirmed outbreaks of foodborne illness have been attributed to *Aeromonas*. However, substantial data suggest that this organism is capable of causing foodborne illness in humans. Data from the United States and other developed countries report the incidence of this potential pathogen on raw poultry to be greater than 50%. Levels of this organism on raw poultry tend to exceed 100 per gram or per milliliter. Unfortunately, this organism can grow during normal refrigeration. However, this organism is very sensitive to heat and radiation and would primarily be a problem only if poultry was eaten raw or undercooked.

Salmonella spp.

In terms of the scientific literature and the popular press, the most common poultry pathogen is *Salmonella*. In the United States, incidence of this organism on raw poultry ranges from 8 to 50% (20). In other countries, incidence rates approaching 100% have been recorded (21). Regardless of country, levels of this organism on raw poultry are almost always extremely low, less than 30 organisms per carcass. Thus, in most cases of human salmonellosis due to consumption of poultry, contributing factors include cross-contamination, improper cooking, and temperature abuse. Currently, *Salmonella* is the only organism with a performance standard (20% positive) for raw poultry in the United States.

Campylobacter spp.

The incidence of *Campylobacter* on raw poultry is exceptionally high, greater than 80% (8,22,23). In the last 10 years the reported incidence of *Campylobacter* on poultry products has increased. In reality, the methodology for recovery of this organism has greatly improved, thus increasing the chance of recovery. In the few studies where this organism has been enumerated, data suggest that levels are very high in comparison to levels of other common poultry-associated pathogens (20). And even though the organism is unlikely to grow on raw poultry due to its fastidious nature, initial levels (>500/mL) are in the range to cause human illness. Most cases of campylobacteriosis are sporadic, and contributing factors typically include cross-contamination and lack of proper handwashing. There is particular concern with campylobacteriosis because of increasing antibiotic resistance and the organisms' connection with Guillain–Barré syndrome.

Staphylococcus aureus

Data indicate that most raw fresh and frozen poultry, both chicken and turkey, are contaminated with *S. aureus* (20). And in most studies, the numbers reported are rather high (>1000 organisms/g or cm^2). This organism must multiply to reach even higher levels in a food and produce toxin in order for a case of foodborne illness to occur. Staphylococci are not good competitors, so even if initial levels are high, as bacterial competition increases during refrigerated storage (even during temperature-abuse situations), the number of staphylococci on raw poultry tends to decrease considerably. This organism becomes a concern on raw poultry only when the natural bacterial population is significantly decreased by some type of bactericidal treatment, especially when followed by temperature-abuse conditions. Because normal heating or reheating will not destroy the toxin produced by this organism, the easiest precaution for preventing staphylococcal foodborne illness is to refrigerate cooked poultry quickly.

Clostridium perfringens

The incidence of *C. perfringens* on raw poultry ranges from 10% to 80% (20). In studies where counts have been reported, the numbers have been very low (<10 organism/g or cm^2). In virtually all cases of foodborne illness attributed to *C. perfringens*, the principal problem is trying to cool large batches of a particular food. The cooking process heat-activates spores, which can germinate during cooling. Thus, fast cooling of cooked poultry products is very important.

Yersinia enterocolitica

Depending on the reporting country, there is a wide range in the incidence of *Y. enterocolitica* organism on raw poultry. In the United States, incidence of this organism on raw broilers at retail is 27% (24). Unfortunately, there is very little information regarding the level of this organism on raw poultry. It is important to remember that not all serovars of this organism are pathogenic, but pathogenic serovars have occasionally been isolated from frozen chickens at the retail level. Unfortunately, *Yersinia* is a psychrotroph and can survive better than other gram-negative organisms under extreme alkaline conditions such as might be present on poultry treated with trisodium phosphate.

Listeria spp.

Few data are available concerning the incidence of this potential pathogen on raw poultry before the late 1980s. Two early European studies, conducted before 1980, suggested that the intestinal tract of broilers, freshly processed broilers, fresh broilers at the time of sale, and frozen broilers were contaminated with this organism. Since 1989 numerous studies have been designed to evaluate the incidence of this pathogen on raw poultry products. Depending on the country where such studies were conducted as well as the isolation techniques utilized, incidence rates range from 2 to 94%. Of course, not all isolates recovered have been *L. monocytogenes* (2–50%), but the presence of any *Listeria* spp. would lead to the conclusion that pathogenic species could be present. However, it is important to note that the serotype most often incriminated in cases of human listeriosis (serotype 4b) has not been a problem in raw poultry. Data regarding the numbers of *Listeria* on raw poultry are limited but suggest that levels are quite low.

FURTHER-PROCESSED POULTRY PRODUCTS

Further-processed poultry products or items of poultry meat processed beyond the eviscerated carcass stage are subjected to many subsequent handlings that are major sources of microbial contamination. However, information concerning the microbiological quality of precooked chicken products is limited. The destruction of foodborne microorganisms is an essential part of the commercial cooking operation.

The microbiological quality of further-processed turkey products has been reported. In the 38 cooked products examined, neither *Salmonella* spp. nor *Escherichia coli* were found, whereas 16 out of 38 contained *C. perfringens*, and only 1 out of 38 was positive for *S. aureus* (25). It was reported that bacterial counts of ready-to-eat turkey rolls increased following storage at 5°C (26). *Salmonella* and coagulase-positive *Staphylococcus* were isolated from some of these samples.

Frozen fried chicken is usually subjected to subsequent handling, which provides a potential opportunity for microbial contamination of the product. The microbiological quality of frozen fried chicken products was studied; it was reported that the log total microbial count ranged from 2.74 to 4.66/gm (27). Neither mold nor yeast was detected, and all samples were *Salmonella* negative. *Micrococci* and *Staphylococci* have been isolated from both hot water and microwaved precooked chicken parts and commercial ready-to-eat sliced chicken products (28).

The total aerobic and coliform counts in chicken patties during refrigerated storage were studied (29). *Escherichia* was the predominant genus at day 0, but *Enterobacter* was predominant after 10 days of storage. The quality of prefried chicken patties and nuggets was studied (30). It was indicated that the predominant microflora in raw and fried chicken patties were gram-positive, rod-type organisms. In addition, vacuum packaging did not inhibit the ultimate psychrotrophic microflora growth on patties and nuggets after refrigerated storage.

Chicken ham cooked to internal temperatures of 68.3 and 71.1°C had a longer lag phase microbial growth at 2 to 4°C than products cooked to an internal temperature of 65.0°C. The shelf life of chicken ham at 2 to 4°C with 68.3 and 71.1°C end point internal temperatures was 33 and 35 days, respectively. Chilling the products in ice slush immediately after cooking extended the shelf life approximately 7 days. Vacuum packaging was found to be an effective method for extending the shelf life of cooked ham (31).

An interaction between frying temperature and internal temperature was significant for microbial counts of deep-fat fried chicken patties. When patties were fried to an internal temperature of 48.9°C, there was a significant decrease in microbial counts as frying temperature increased. This effect was not significant when patties were fried to an internal temperature of 60.0 or 71.1°C. An internal temperature of 71.1°C is required to be labeled "fully cooked," "ready-to-eat," "baked," or "roasted" poultry according to the USDA (32). Raw chicken patties had a mean total psychrotrophic count of log 5.1 colony forming unit (CFU)/gm. Chicken patties fried to an internal temperature of 71.1°C had microbial counts ranging from 1.52 to 1.75 CFU/gm.

RETARDING THE GROWTH OF MICROORGANISMS ON POULTRY

Temperature Control

Of all the methods to delay microbial spoilage, temperature control remains the most dependable and widely used. According to the USDA, broiler carcasses must be chilled to at least 4.4°C after 4 hours; turkeys, after 8 hours (32). In most commercial facilities this is accomplished through immersion chilling. Freezing poultry destroys 96 to 99% of the total surface microflora (33). Numbers continue to decrease during prolonged frozen storage. Freezing does not completely free poultry from salmonellae or other potential pathogens.

The bacteria responsible for the spoilage of poultry are not notably xerotolerant. Freezing will subject these organisms to water stress at temperatures close to their minima for growth. The synergistic effects of these two factors, temperature and water activity, probably insure that spoilage bacteria do not usually grow on frozen poultry, although some strains could possibly grow at temperatures below 2°C. The preservation of food by freezing is based on the retardation of microbial growth to the point at which decomposition due to microbial action does not occur (34). Pathogenic microorganisms do not grow below 2°C.

Preservatives

Numerous efforts have been made to use additives to reduce the microbial load on fresh poultry. Some of the techniques used have been chilling and washing of carcasses with chlorinated water (35–45), 70% alcohol dipping (46), immersion of carcasses in solutions of poly(hexamethylenebiguanide hydrochloride) (47), dipping in various organic acids (19,48–51), and chilling in polyphosphate solutions (52,53).

It was reported that when *Lactobacillus* cells were added to chilled water at a concentration of 100 million/mL, shelf life was increased as much as 3 days. Carcasses immersed in 0.12% lactic acid had a total bacterial count of 400 compared with a control group that had 130,000 organisms/g (50). Sorbic acid was an effective preservative when a 7.5% solution was sprayed onto the chilled poultry parts at 140°F in a ratio of 70:20:10 propylene glycol to water to glycerin (54). Nine acids were studied, and it was concluded that adipic and succinic acid increased the shelf life of broilers by 6 days more than the water-chilled samples when the pH of the chilling solution was 2.5 (49). Another study revealed that dipping cup-up broiler parts in 1% ascorbic acid solution for 3 min retarded microbial growth and increased the refrigerated life for 6 to 7 days compared to that of the control (19). It has been suggested that the use of polyphosphate solutions was effective in controlling the growth of gram-positive micrococci and staphylococci (28).

Of the materials tested for use in preserving chilled poultry carcasses, antibiotics have received the most attention. The addition of antibiotics to the chilling water had been widely accepted in the 1950s and the early 1960s. Chlorotetracycline or oxytetracycline was added to the chilling bath through which the birds passed following evisceration; the results indicated that these antibiotics lengthened the keeping time by at least several days, but that bacteria resistant to the antibiotics (eg, pigmented pseudomonads and certain *Alcalilgenes* species) grew on the birds and built up in the plant (55). The antibiotics had no effect on yeasts and molds that were also a part of the microflora of poultry carcasses (56). Because of the fact that small residues, about 0.5 ppm, may be left after cooking and the effect that such residues might have on the intestinal flora of humans, permission to use such compounds in foods was withdrawn by the FDA (57).

Potassium sorbate has been extensively investigated as an antibacterial agent for extending shelf life and inhibiting pathogen growth. The effectiveness of a 10% potassium sorbate dip in inhibiting bacterial growth of fresh chicken breast meat inoculated with *Salmonella* was demonstrated (58). It has also been reported that dipping broiler carcasses in a 5% potassium sorbate dip for 1 min was effective in inhibiting *Salmonella* and *S. aureus* (59).

Preparation of germicidal ice incorporated with food additives such as calcium hypochlorite, chloramine benzoic acid, formaldehyde, hydrogen peroxide, sodium propionate, and sodium nitrite for ice-packing purposes have been studied (60). It was proven that ice-containing glycol diformate and sorbic acid were more effective against microbial growth than was common ice or the acronizing process. It has been reported that the average shelf life of broiler parts ice-packed with 0.075% sorbic acid ice and 0.1% potassium sorbate ice was 11.5 and 3.6 days longer than the ice-packed controls (61). Both sorbic acid ice and potassium sorbate ice decreased the incidence of gram-negative rod-type organisms and increased the incidence of gram-positive cocci on broiler parts after 4 days of storage at 2 to 4°C.

Packaging

A number of studies have been made to determine the effect of packaging on the shelf life of poultry carcasses. One study reported that packaging materials had a significant effect on the shelf life of fresh poultry (62). Storage time for poultry carcasses wrapped in cellophane and cellophane—vinylidene chloride copolymer sheets were the same under nonvacuum conditions, but when the air was evacuated, the storage time increased 4 days beyond that obtained without vacuum (63). Fewer pseudomonads were found, but *Microbacterium thermosphactum* and atypical lactobacilli were a significant part of the spoilage flora on turkeys wrapped in impermeable film at 1°C (64). It has been reported that while the pseudomonads were the main spoilage organisms on the carcasses wrapped in the permeable film, the *Alternomonas putrefacians* predominated on the carcasses wrapped in the impermeable film.

The influence of packaging materials seems to affect the quality of fresh poultry. Bacterial growth was inhibited by using a shrinkable poly(vinyl chloride) film with a permeability to oxygen of approximately 500 mL/cm^2, 24 h, 1 atm (65). It was also concluded that shrinkable poly(vinyl chloride) film was better for retaining the quality and shelf life of chicken than any other material that was not air evacuated.

Another effective way of packaging is by employing carbon dioxide treatment. Chickens stored in 10 and 20% carbon dioxide at 1°C effectively extended the shelf life (66). It has been suggested that packaging broiler carcasses in nylon—surlyn film with a carbon dioxide addition rate of 3.6×10^{-4} and 7.22×10^{-4} m^3/kg carcass extended shelf life at 1.1°C of storage to 22 and 27 days, respectively (67). Vacuum-CO_2 packaging extended the shelf life of broiler carcasses for approximately 7 days when compared to those of the ice-packed controls (4). It was also reported that the major microorganism groups isolated from the spoiled ice-packed samples were members of the *Pseudomonas* species (93.3%); however, *Lactobacillus* spp. (74.0%) were found to be the dominant microflora on the vacuum-CO_2 packaged broilers after 28 days of storage at 1.1 to 3.3°C (4).

Other Treatments

Ozone treatment also has been used for preservation of foods, especially of fruits and vegetables. Ozone-treated broiler parts have consistently lower microbial counts than air-treated control parts during the entire refrigerated observation period (68). Using log total microbial counts of 7.0/cm^2 as spoilage criterion, broiler parts treated with ozone had a shelf life that was extended for 2.4 days. It was also indicated that ozone-treated carcasses contained about 52.7% gram-positive cocci, while air-treated controls had 39.6% gram-positive cocci. Studies using microflora from spoiled poultry meat have also demonstrated that ozone treatment preferentially destroyed gram-negative, rod-type organisms.

Radurization processing of fresh eviscerated poultry with a dose of 5 kGy has been reported to extend the shelf life at 5°C by approximately 14 days (69). It has been reported that at 4°C the shelf life of chicken carcass was 3 days for nonirradiated samples, 13 days for those irradiated at 3 kGy, and greater than 30 days for those irradiated at 7 kGy (70). Chicken carcasses irradiated with 5 to 10 kGy at 3 to 4°C could extend the shelf life by 2 to 4 weeks (71). Chicken breast meats were irradiated with 3.7 kGy at 0°C; a satisfactory quality for about 3 weeks was reported (72). Recently, it was noted that irradiation increased the shelf life of chicken fillets (73). The time required to reach a log number of 6.5/g was 15 days for the controls and 35 days for the fillets treated with 2 kGy, all stored at 3°C. The combination of vacuum skin packaging and 4 kGy irradiation dose resulted in fillets with a shelf life of more than 45 days at 3°C. Irradiation at 3 kGy destroyed all inoculated *S. typhimurium* and *E. coli* in ground chicken meat.

Antagonisms of microorganisms on broiler carcasses have been observed. The growth-interfering effect of spoilage microorganisms by gram-positive cocci on broiler carcasses has been reported (74). This growth-interfering effect was greater at 2 to 4°C and 5 to 7°C than at 19 to 21°C. The higher the ratio of the gram-positive cocci to the rod-type spoilage microorganisms, the more the growth-interfering effect was observed. The cell-free filtrates from gram-positive broth cultures interfered with the growth of the spoilage microorganisms at 2 to 4°C, and the causative agent was heat-sensitive metabolites.

BIBLIOGRAPHY

1. J. C. Ayres, W. S. Ogilvy, and G. F. Stewart, "Post-Mortem Changes in Stored Meats. Microorganisms Associated with Development of Slime on Eviscerated Cut-Up Poultry," *Food Technology* **4**, 199–205 (1950)
2. A. S. Arafa and T. C. Chen, "Effect of Vacuum Packaging on Microorganisms on Cut-Up Chicken and in Chicken Products," *Journal of Food Science* **40**, 50–52 (1975).
3. A. S. Arafa and T. C. Chen, *Evaluation of Final Washing and Immersion Chilling on Carcass Microflora from Fourteen Mississippi Poultry Processing Plants*, MAFES Research Report **3**, 1977.
4. K. Viseshsiri and T. C. Chen, *Microbiological Characteristics of Vacuum-CO_2 Packaged Broiler Carcasses*, MAFES Bulletin **910**, Mississippi State, Miss., 1982
5. H. W. Walker and J. C. Ayres, "Antibiotic Residuals and Microbial Resistance in Poultry Treated with Tetracyclines," *Food Research* **23**, 525–531 (1968).
6. M. Woodburn, "Incidence of Salmonellae in Dressed Broiler-Fryer Chickens," *Applied Microbiology* **12**, 492–495 (1964).
7. A. L. Izat, J M. Kopeck, and J. D. McGinnis, "Research Note: Incidence, Number, and Serotypes of Salmonella on Frozen Broiler Chickens in Retail," *Poultry Science* **70**, 1438–1440 (1991).
8. A. L. Waldroup, B. M. Rathgeber, and R. E. Hierholzer, "Effects of Reprocessing on Microbiological Quality of Commercial Prechill Broiler Caracasses," *Journal of Applied Poultry Research* **2**, 111–116 (1993).
9. R. P. Elliott and H. D. Michener, "Microbiological Standards and Handling Codes for Chilled and Frozen Foods. A Review," *Applied Microbiology* **9**, 452–468 (1961).
10. A. A. Kraft, "Microbiology of Poultry Products," *Journal of Milk and Food Technology* **34**, 23–29 (1971).
11. J. C. Ayres, "The Relationship of Organisms of the Genus *Pseudomonas* as the Spoilage of Meat, Poultry and Eggs. *Journal of Applied Bacteriology* **23**, 471–486 (1960).
12. E. M. Barnes and C. S. Impey, "Psychrophilic Spoilage Bacteria of Poultry," *Journal of Applied Bacteriology* **31**, 97–107 (1968).
13. T. A. McMeekin, "Spoilage Association of Chicken Leg Muscle," *Applied and Environmental Microbiology* **33**, 1244–1246 (1977).
14. USDA Safety Inspection Service, *The Safe Food Book: Your Kitchen Guide*, Home and Garden Bulletin No. 241, Washington, D.C., 1985.
15. A. L. Izat et al., "Effects of Sampling Method and Feed Withdrawal Period on Recovery of Microorganisms from Poultry Carcasses," *Journal of Food Protection* **52**, 480–483 (1989)
16. E. M. Barnes and D. H. Shrimpton, "Causes of Greening of Uneviscerated Poultry Carcasses during Storage," *Journal of Applied Bacteriology* **20**, 273 (1957).
17. E. O. Essary, W. E. C. Moore, and C. Y. Kramer, "Influence of Scald Temperatures, Chill Times, and Holding Temperatures on the Bacterial Flora and Shell Life of Fresh Chilled, Tray-Packed Poultry, *Food Technology* **12**, 684–687 (1958)
18. L. E. Dawson and W. J. Stadelman, *Microorganisms and Their Control of Fresh Poultry Meat*, NCM-7 Technical Bulletin **278**, Michigan State University, East Lansing, Mich., 1960.
19. A. S. Arafa and T. C. Chen, "Ascorbic Acid Dipping as a Means of Extending Shelf-Life and Improving Microbial Quality of Cut-Up Broiler Parts," *Poultry Science* **57**, 99–103 (1978).
20. A. L. Waldroup, "Contamination of Raw Poultry with Pathogens," *World Poultry Science Journal* **52**, 7–25 (1996).
21. A. S. Kamat et al., "Hygienization of Indian Chicken Meat by Ionizing Radiation," *Journal of Food Safety* **12**, 59–71 (1991).
22. N. J. Stern and J. E. Line, "Comparison of Three Methods for Recovery of *Campylobacter* spp. from Broiler Carcasses," *Journal of Food Protection* **55**, 663–666 (1992).
23. A. L. Waldroup, B. M. Rathberger, and R. H. Forsythe, "Effects of Six Modifications on the Incidence and Levels of Spoilage and Pathogenic Organisms on Commercially Produced Postchill Broilers," *Journal of Applied Poultry Research* **1**, 226–234 (1992).
24. N. A. Cox et al., "The Presence of *Yersinia enterocolitica* and Other *Yersinia* Species on the Carcasses of Market Broilers," *Poultry Science* **69**, 482–485 (1990).
25. E. A. Zottola and F. F. Busta, "Microbiological quality of further-processed turkey products," *Journal of Food Science* **36**, 1001–1004 (1971).
26. A. J. Mercuri, G. J. Banwart, J. A. Kinner, and A. R. Sessums, "Bacteriological Examination of Commercial Precooked Eastern Type Turkey Rolls," *Applied Microbiology* **19**, 768–771 (1970).
27. P. L. Wang, E. J. Day, and T C. Chen, "Microbiological Quality of Frozen Fried Chicken Products Obtained from a Retail Store," *Poultry Science* **55**, 1290 (1976).
28. T. C. Chen, J. T. Culotta, and W. S. Wang, "Effect of Water and Microwave Energy Precooking on Microbiology Quality of Chicken Parts," *Journal of Food Science* **38**, 155–157 (1973).
29. S. E. Craven and A. J. Mercuri, "Total Aerobic and Coliform Counts in Beef-Soy and Chicken-Soy Patties during Refrigerated Storage," *J. Food Prot.* **40**, 112–115 (1977).
30. Y. H. Yi, *Studies on the Quality of Prefried Chicken Patties During Refrigerated Storage*, Ph.D. dissertation, Mississippi State University, Mississippi State, Miss., 1986.
31. S. C. Yang and T. C. Chen, "Processing Yields and Shelf Life of Chicken Ham," *Taiwan Journal of Food Science* **15**, 133–141 (1988).

32. The U.S. Department of Agriculture, *Meat and Poultry Inspection Manual*, U.S. Department of Agriculture, Washington, D.C., 1973, Part 18, Subpart G, Article 37.
33. A. A. Kraft, J. C. Ayres, K. F. Weiss, W. W. Marion, S. L. Balloun, and R. H. Forsythe, "Effect of Method of Freezing on Survival of Microorganisms on Turkey," *Poultry Science* **48**, 128–136 (1963).
34. A. C. Peterson and R. E. Gunnerson, "Microbiological Critical Control Points in Frozen Foods," *Food Technology* **28**, 37–44 (1974).
35. E. E. Drewniak, M. A. Howe, Jr., H. E. Goresline, and E. R. Baucsh, *Studies on Sanitizing Methods for Use in Poultry Processing*, USDA Circular No. 930, 1954.
36. M. F. Gunderson, H. W. McFaddin, and T. S. Kyle, *The Bacteriology of Commercial Poultry Processing*. Burgess Publishing Co., Minneapolis, Minn., 1954.
37. L. E. Dawson, W. L. Mallmann, M. Frang, and S. Walters, "The Influence of Chlorine Treatments of Bacteria Population and Test Panel Evaluation of Chicken Fryers," *Poultry Science* **35**, 1140 (1956).
38. J. M. S. Dixon and F. E. Pooley, "The Effect of Chlorination on Chicken Carcass Infected with Salmonellae," *Journal of Hygiene* **59**, 343 (1961).
39. A. W. Kotula, J. E. Thompson, and J. A. Kinner, "Bacterial Counts Associated with the Chilling of Fryer Chickens," *Poultry Science* **41**, 818–821 (1962).
40. W. Mercer, "Product Protection by Chlorination and Other Safeguards," in *Proceedings of the Poultry and Egg Further-Processing Conference*, 1964, pp. 33–40.
41. M. D. Rankan, G. Clewlow, D. H. Shrimpton, and B. J. H. Stevens, "Chlorination in Poultry Processing," *British Poultry Science* **6**, 331–337 (1965).
42. J. E. Thomson, G. J. Banwart, D. H. Sanders, and A. J. Mercuri, "Effect of Chlorine, Antibiotics, Beta-Propolactone, Acids and Washing on *Salmonella typhimurium* on Eviscerated Fryer Chickens," *Poultry Science* **46**, 146–151 (1967).
43. C. J. Wabeck, D. V. Schwall, G. M. Evancho, J. C. Heck, and A. B. Rogers, "Salmonella Reduction, Chlorine Uptake, and Organoleptical Changes in Poultry Treated with Sodium Hypochlorite," *Poultry Science* **46**, 1333 (1967).
44. J. T. Patterson, "Bacterial Flora of Chicken Carcasses Treated with High Concentration of Chlorine," *Journal of Applied Bacteriology* **31**, 554–550 (1968).
45. R. M. Blood and B. Jarvis, "Chilling of Poultry: The Effects of Process Parameters on the Level of Bacteria in Spin-Chiller Waters," *Journal of Food Technology* **9**, 157–169 (1974).
46. K. N. Hall and J. V. Spencer, "The Effect of Ethanol on Shelf Life and Flavor of Chicken Meat," *Poultry Science* **43**, 573–576 (1964).
47. M. N. Islam and N. B. Islam, "Extension of Poultry Shelf Life by Poly(Hexamethylenebiguanide Hydrochloride), *J. Food Prot.* **42**, 416–419 (1979).
48. N. A. Cox, A. J. Mercuri, B. J. Juven, J. E. Thomson, and V. Chow, "Evaluation of Succinic Acid and Heat to Improve the Microbiological Quality of Poultry Meat," *Journal of Food Science* **39**, 985–987 (1974).
49. G. J. Mountney and J. E. O'Malley, "Acids as Poultry Meat Preservatives," *Poultry Science* **44**, 582–586 (1965).
50. U.S. Pat. (1962), J. F. Murphy and R. E. Murphy.
51. T. E. Minor and E. H. Marth, "*Staphylococcus aureus* and Staphylococcal Food Intoxications. A Review," *Journal of Milk and Food Technology* **35**, 228–241 (1972).
52. J. V. Spencer and L. E. Smith, "The Effect of Chilling Chicken Fryers in a Solution of Polyphosphates upon Moisture Uptake, Microbial Spoilage, Tenderness, Juiciness, and Flavor," *Poultry Science* **41**, 284–288 (1962).
53. R. P. Elliott, R. P. Straka, and J. A. Garibaldi, "Polyphosphate Inhibition of Growth of Pseudomonads from Poultry Meat," *Applied Microbiology* **12**, 517 (1964).
54. G. A. Perry, R. L. Lawrence, and D. Melnick, "Extension of Shelf Life of Poultry by Processing with Sorbic Acid," *Food Technology* **18**, 101–107 (1962).
55. H. W. Walker and J. C. Ayres, "Incidence and kids of microorganisms associated with commercially dressed poultry," *Applied Microbiology* **4**, 345–349 (1956).
56. R. H. Vaughn and G. F. Stewart, "Antibiotics as Food Preservatives," *Journal of the American Medical Association* **174**, 1308–1310 (1960).
57. J. T. Nickerson and A. J. Sinskey, *Microbiology of Foods and Food Processing*, Elsevier Publishing Co., Inc., New York, 1972.
58. M. C. Robach and F. J. Ivey, "Antimicrobial Efficacy of a Potassium Sorbate Dip on Freshly Processed Poultry," *J. Food Prot.* **41**, 284–288 (1978).
59. E. C. To and M. C. Robach, "Potassium Sorbate Dip as a Method of Extending Shelf Life and Inhibiting the Growth of *Salmonella* and *Staphylococcus aureus* on Fresh, Whole Broilers," *Food Technology* **37**, 107–110 (1980).
60. S. Kaloyereas, R. M. Crown, and C. S. McClesky, "Experiments on Preservation with a New Ice-Containing Glycol Diformate and Sorbic Acid," *Food Technology* **15**, 582–586
61. Y. L. Lin and T. C. Chen, *Effect of Sorbic Acid Ice on Microorganisms of Broiler Meat*, MAFES Research Report **14**, Mississippi State, Miss., 1989.
62. J. V. Spencer, M. W. Eklund, E. H. Suter, and M. M. Hard, "The Effect of Different Packaging Materials on the Shelf Life of Antibiotic Treated Chicken Fryers," *Poultry Science* **35**, 1173 (1956).
63. F. E. Wells, J. V. Spencer, and W. J. Stadelman, "Effect of Packaging Materials and Techniques on Shelf Life of Fresh Poultry Meat," *Food Technology* **12**, 425 (1958).
64. E. M. Barnes and D. H. Shrimpton, "The Effect of Processing and Marketing Procedures on the Bacteriological Condition and Shelf Life of Eviscerated Turkeys," *British Poultry Science* **9**, 243–251 (1968).
65. J. M. Debevere and J. P. Voets, "Influence of Packaging Materials on Quality of Fresh Poultry," *British Poultry Science* **14**, 17–22 (1973).
66. C. J. Wabeck, C. E. Parmella, and W. J. Stadelman, "Carbon Dioxide Preservation of Fresh Poultry," *Poultry Science* **47**, 468–474 (1968).
67. E. H. Sander and H. M. Soo, "Increasing Shelf Life by Carbon Dioxide Treatment and Low Temperature Storage of Bulk Pack Fresh Chickens Packaged in Nylon/Surlyn Film," *Journal of Food Science* **43**, 1519–1523, 1527 (1978).
68. P. P. W. Yang and T. C. Chen, "Effect of Ozone Treatment on Microflora of Poultry Meat," *J. Food Proc. and Preserv.* **3**, 177–185 (1979).
69. E. S. Idziak and K. Incze, "Radiation Treatment of Foods, Radurization of Fresh Eviscerated Poultry," *Applied Microbiology* **16**, 1061 (1968).
70. H. E. Bok and W. H. Holzapfel, "Extension of Shelf Life of Refrigerated Chicken Carcasses by Radurization," *Food Review* **11**, 69–71 (1984).

71. H. O. Cho, M. K. Lee, M. W. Bynn, J. H. Kwon, and J. G. Kim, "Radurization of the Microorganisms Contaminating Chicken," *Korean Journal of Food Science Technology* **17**, 170–174 (1985).
72. R. C. Baker, D. Scott-Kline, J. Hutchison, A. Goodman, and J. Charvat, "A Pilot Plant Study of the Effect of Four Cooking Methods on Acceptability and Yields of Prebrowned Battered and Breaded Broiler Parts," *Food Technology* **65**, 1322–1332 (1986).
73. Y. Zhou and T. C. Chen, *Effect of Vacuum Skin Packaging and Irradiation on Quality of Marinated Chicken Fillets*, M.S. thesis, Mississippi State University, Mississippi State, Miss., 1989.
74. T. C. Chen and K. Tanteeratarm, "The Growth Interfering Effects of Spoilage Microorganisms by Gram-Positive Cocci on Broiler Carcasses," in *Proceedings of the Seventeenth World Poultry Congress*, 1984, pp. 681–683.

T. C. CHEN
Mississippi State University
Mississippi State, Mississippi

A. L. WALDROUP
University of Arkansas
Fayetteville, Arkansas

See also MICROBIOLOGY OF FOODS.

POULTRY MEAT PROCESSING AND PRODUCT TECHNOLOGY

Poultry meat is consumed all around the world. In the last few decades poultry meat has increased in popularity in many countries. Among the reasons for this increase are the relatively low growing costs, the rapid growth rate of poultry, the high nutritional value of the meat, and the introduction of many new further processed products. Overall, the poultry industry has drastically changed since the beginning of the century. In the early 1920s most poultry was produced in small flocks mainly to support small farm units, and the poultry was sold live in the local area markets. Most birds were used both as a source of meat and eggs. This is still the situation in some countries; however, in most countries the poultry industry has grown and specializes in meat production breeds and egg production breeds. Over the years the number of small flocks has decreased dramatically as large operations specializing in raising poultry have emerged. Today it is not uncommon to find farmers specializing in only one phase of the growing stage (eg, breeding, hatching, raising pullets, growing operations). The growing operations are usually fairly large (ie, a few hundred thousand to a few million birds) and must be efficient in order to compete. Vertical integration of poultry operations (from hatching to further processing) is common in quite a few countries and helps in making the production more cost effective and competitive. Genetic improvements and selection for higher feed efficiency have also contributed to the ability of the industry to improve its product and maintain a competitive price in comparison with other red meat sources. At the beginning of the century, poultry was mainly selected and bred for exhibitions. Later some breeders started selecting birds for economical characteristics. At that point the chickens selected were used for the general purpose of supplying both meat and eggs. Selection for meat-type and egg-type birds started 10 to 15 years later. Today we are at the point where in many areas cockerels from a meat-type breed are not raised because they are no longer a profitable source of chicken meat (1). Over the years, birds were selected for fast growing characteristics, and as a result the average time to reach marketing weight for broilers was significantly reduced (12 weeks 40 years ago compared with about 7 weeks today).

Changes in lifestyle and consumption patterns have also helped to make poultry a more popular source of meat. Today consumers in the Western world are much more concerned with the caloric and nutritional value of their food. In this context, poultry has gained popularity because it is viewed as a good source of lean meat and its fat as more unsaturated than red meat. The poultry industry has also taken the initiative to develop new further processed products and to respond to the increasing consumer demand for convenient food items (ie, semi/fully cooked). The changes in poultry meat consumption in the United States over the last 85 years are presented in Table 1. As can be seen, the total poultry meat consumption doubled for the first time between 1940 and 1960 and the second time between 1960 to 1980. This increase has been seen only in poultry meat, whereas red meat consumption has either stayed constant or slightly declined over the same period of time (3). This tremendous increase in poultry meat also represents a change in marketing strategies. The proportion of cut-up poultry increased for example, from 25% in 1970 to 60% in 1980 and the proportion of further processed products increased from 5 to 25%. Exporting of poultry meat has also become an important international trade. Some of the leading countries are the United States (2090 metric tons in 1996), Brazil (530 tons), France (381 tons), Netherlands (195 tons), and Thailand (165 tons; Ref. 3).

In the following section the different processes involved in converting live poultry into meat, the nutritional value of poultry meat, the equipment used for further processing, and the production of poultry products are discussed.

TYPES OF POULTRY

The poultry industry markets different types of birds (Table 2). On a worldwide basis the most popular species in-

Table 1. Civilian per Capita Consumption of Poultry in United States Between 1910 and 1996

Year	Broilers	Turkeys	Total poultry
1910	—[a]	—	15
1940	2	3	17
1960	23.4	6	34
1980	47	10	61
1990	61.5	17	81
1996	71	18	90

Note. Poultry meat consumption based on ready to cook weights in pounds. Only broiler and turkey meat are presented.
Source: Refs. 2 and 3.
[a] No data available.

Table 2. Types of Commonly Available Poultry and Their Average RTC Weight

Type	Age (weeks)	Weight RTC (kg)
	Chickens	
Broiler or fryer	6–8	1.2–1.7
Roaster	8–10	3.0
Rock cornish game	3–4	0.6
Hen/stewing fowl	>52	1.4
Cock or mature rooster	>30	3.0
	Turkeys	
Broiler hen	12	4.2
Young hen	16	7.0
Young tom	17–18	12.5
Spend breeder	>52	11.0
	Ducks	
Broiler or fryer	7	2.5–3.8
	Geese	
Mature	12–16	5.0
	Guineas	
Mature	12	1.5
	Pigeons	
	4–5	0.4

Note: RTC = Ready-to-cook weight (ie, body weight excluding feathers, blood, digestive tract, head, and feet).

clude chickens, turkeys, and ducks. However, other species such as geese and pigeons can be found marketed on a large scale in certain areas. The average marketing age of some of the most common species and their ready-to-cook weights (weight without blood, feathers, viscera, feet, and head) are provided in Table 2.

PROCESSING FRESH POULTRY

Special large-scale plants for processing poultry are common around the world. These plants are specifically designed to process poultry and usually include a slaughtering facility, defeathering, evisceration, inspection, chilling, and packaging. In some cases they are built adjacent to meat processing plants.

The steps involved in processing poultry are illustrated in Figure 1. Usually the process starts with a bulk weighing of the birds received on the truck. The live weight subtracted from the eviscerated weight (minus the weight of the condemned birds) provides the yield value and is often used, together with the grades assigned, to determine the payment to the farmer. Unloading the birds from the crates and placing them on the shackle line is often done manually. Special care should be exercised at this stage to minimize bruising of the excited birds. This is commonly followed by stunning the birds; however, stunning is not always used, especially when religious considerations are involved (eg, according to the Jewish laws known as kosher processing, stunning is prohibited). When stunning is used, it is done by electrical current passing through the bird; the amount of current and duration depends on the size of the birds. In the case of broilers, a current in the range of 60 V for 5 to 10 s is common. It is important not to use too much current, since it can completely stop the heart activity. Gas stunning (by CO_2, argon) is also possible (4) and has the advantage of reduced bruising of the birds.

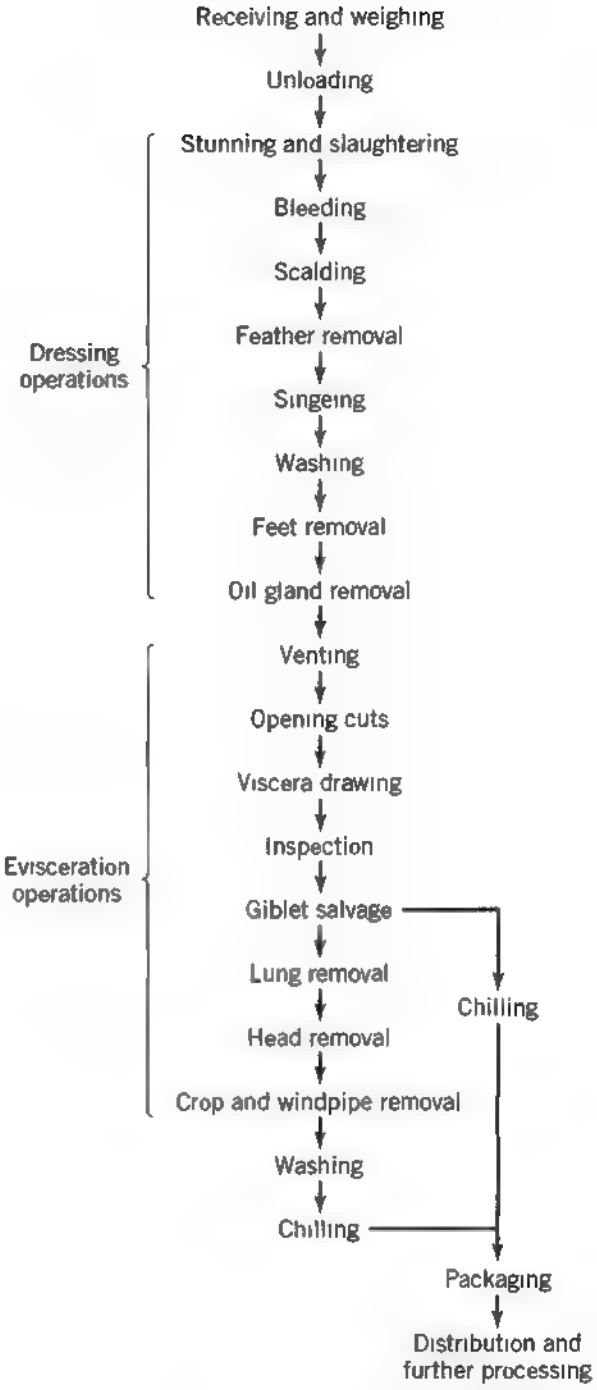

Receiving and weighing
Unloading
Stunning and slaughtering
Bleeding
Scalding
Feather removal
Singeing
Washing
Feet removal
Oil gland removal
Dressing operations
Venting
Opening cuts
Viscera drawing
Inspection
Giblet salvage
Lung removal
Head removal
Crop and windpipe removal
Evisceration operations
Chilling
Washing
Chilling
Packaging
Distribution and further processing

Figure 1. Unit operations in poultry processing.

Stunning, besides calming the birds, also helps in relaxing the muscles holding the feathers, which later facilitates feather removal.

The slaughtering itself can be done in different ways. The most commonly used method is the so-called modified kosher where the jugular vein is cut just below the jowls so that the esophagus and windpipe remain intact. According to the true kosher slaughtering procedure, the windpipe should pop out, and the whole procedure should be performed by a qualified person (*shochet*) and not by a machine. Decapitation and piercing through the roof of the mouth and brain are two other slaughtering methods that are seldomly used in Europe and North America today. After slaughtering, appropriate time should be allowed for bleeding since an excess amount of blood in the muscles will result in discoloration. About 35 to 50% of the total blood is removed during this stage, and it has been shown that the kosher or modified kosher slaughtering methods result in higher bleeding than decapitation.

Scalding is the process of immersing the birds in warm water to loosen the feathers. There are three commonly used temperatures for this purpose (Table 3), and they depend on the degree of difficulty in removing the feathers. The higher scalding temperature is the best for loosening feathers, but it is also the most harsh on the skin. In the case of the hard scalding, the outer layer of the skin (epidermis) is removed during plucking and the skin becomes discolored if dehydrated after processing. However, this method is the only satisfactory way to release the feathers of waterfowl. Relatively speaking, hard scalding does not cause as much discoloration in the thick skin waterfowl as it does in other poultry. Subscalding can remove part of the outer layer of the skin and leaves the skin sticky but will not result in excessive discoloration if the birds are kept in a moist environment. Semiscalding is used for young birds. It does not damage the outer layer of the skin while still allowing for relatively easy removal of the feathers in young birds. A true kosher processing, where scalding is prohibited, usually results in more skin tears because more force is required in the defeathering process. In large processing plants feather removal is done by large mechanical pickers equipped with rubber fingers that rub the feathers off the carcass. This is usually done while the carcass is hanging upside down and carried by a shackle line through the picker. The defeathering process can also be done in a batch-type operation by placing the carcasses in a drum equipped with rubber fingers. In small-scale operations, hand picking of the feathers is sometimes used. When pinfeathers are a problem (as with waterfowl), wax dipping after the picking process is common. Suspending the carcasses in hot wax, followed by cold water immersion, hardens the wax, which is later peeled in large pieces, pulling out the pinfeathers. The wax can be reused after reheating and filtering the feathers from the previous batch. When only minor problems of pinfeathers exist, singeing (the process of burning the small feathers) can be used. This is done by passing the carcass through a flame of a "clean" burning substance (eg, natural gas) that does not leave any off-odor or -flavor. The carcasses are then washed to remove all the soil left after the defeathering and singeing processes. Washing is usually followed by removing the oil gland. After this step, the carcasses are usually moved to another shackle line.

Table 3. Recommended Scalding Schedules for Defeathering

Scalding technique	Water temperature	Time (s)	Used for
Hard Scalding	>63°C	30–60	Waterfowl
Sub scalding	58–60°C	30–75	Mature birds
Semic scalding	52–54°C	30–75	Broilers, roasters, young turkeys

The evisceration process includes the opening of the body cavity and withdrawing the viscera. This process can be done manually by using a regular knife and a pair of scissors or automatically by using a circular saw and a scooplike arm to withdraw the viscera. In any case, special care should be taken not to pierce the viscera and contaminate the carcass. Such contamination can result, in some countries, in the condemnation of parts or the whole bird exposed to the spill. New automated equipment currently on the market allows viscera separation right after its withdrawal, which can improve the hygiene of the process. Once the viscera are exposed, the bird are inspected. The inspection is done at this point because certain diseases affect the intestine or liver. The inspection requirements differ among countries. In some countries it is required that each individual bird be inspected by a veterinarian; in other countries inspection is done on a whole flock basis and only a certain number of individual birds are inspected by a trained inspector. The viscera is removed after the inspection and giblets (liver, heart, and gizzard) are salvaged and washed in a separate line. This is followed by lung removal (manually or by a suction gun); head, crop, and windpipe removal; and a thorough washing of the carcass prior to chilling. The meat must then be chilled to minimize microbial growth. This step is mandatory in many countries, but where it is not, it is recommended that chilling take place as soon as possible. It is common to use long chillers with a counterflow of cold water or crushed ice to bring down the carcass temperature to about 4 to 5°C within 30 to 75 min. Air chillers are also used, but they might cause some surface dehydration if the relative humidity is not precisely controlled. In many countries, the amount of water pickup during the chilling process (when water is used) is regulated and is based on a certain percentage of the body weight. After chilling, the birds are usually packed or immediately deboned for further processing. Due to the relatively short time required for rigor mortis setup in poultry (as compared with red meat), no extra waiting time prior to chilling is required to prevent a phenomenon such as "cold shortening." Overall, the final meat quality is affected by different processing parameters (eg, stunning, chilling) as well as various growing and transporting parameters (1,5); all should be controlled for obtaining the best quality meat.

FRESH POULTRY MEAT

Composition and Nutritional Value

Poultry meat is widely accepted as a good source of high-quality protein, the B vitamins, and minerals such as iron.

The fact that poultry meat is considered to be a source of lean meat and has a higher level of unsaturated fat as compared with red meat has resulted in a significant increase in poultry meat consumption in North America (Table 1) and around the world. Overall, the composition of poultry meat is dependent on species, strain, sex, age, and diet. Table 4 illustrates that species vary in their fat content. Turkey meat is usually lower in fat than chicken, while goose and duck meat are higher in fat. In poultry fat is deposited either under the skin or in the abdominal cavity. Therefore it is easy to obtain very lean poultry meat by separating the skin from the flesh. This is different from red meat where marbling or intramuscular fat deposits are visible within a meat cut and are difficult to separate from the lean muscle. The diet of the monogastric birds can also significantly affect the composition of the meat. Carcass fat content and composition is particularly sensitive to the type of feed. In general, high-energy diets or low-protein diets have been shown to increase carcass fat. It is also possible to increase the proportion of unsaturated fat in poultry meat by manipulating the fat source in the diet.

The relatively high degree of fat unsaturation makes poultry meat susceptible to lipid oxidation during storage. Some processes, such as mechanical deboning, can further enhance the rate of lipid oxidation. In mechanically deboned poultry meat (MDPM), the release of heme from bone marrow, the aeration during the separation process, and extreme mechanical stress can further accelerate the oxidation process (7). The problem of oxidative rancidity can be partially controlled by the exclusion of oxygen by vacuum packaging, the addition of tocopherol to the diet of live birds (ie, vitamin E is deposited in the muscle tissue and later serves as an antioxidant), or the addition of synthetic or natural antioxidants to further processed products. It should be noted that synthetic antioxidants can be used only in countries where permitted.

The production of MDPM has increased substantially during the last three decades, mainly due to the development of equipment for harvesting the meat left on the skeletal frame after hand deboning and because of the increased demand for poultry meat. Today new aspects of mechanical deboning are investigated, such as the mechanical deboning of whole fowl and the subsequent washing of MDPM to obtain lighter meat. The latter is also less susceptible to lipid oxidation due to the removal of heme (eg, the process is similar to the production of fish-based surimi). MDPM is characterized by a pastelike texture and is readily available for the production of finely comminuted meat products (eg, frankfurters, bologna) where finely chopped muscle is traditionally used.

Cooking can also affect the final composition of the meat. This is basically due to the leaching of some of the meat components or the absorption of cooking media into the meat. Yields of cooked poultry average about 75%; the highest yields can be obtained by stewing (78%) followed by frying (77%) and roasting (69%) (6) However, since large variations exist in cooking methods, postslaughter practices (chilling and freezing), and preparation (with or without bone and skin), it is recommended that a reference to a specific cut and cooking procedure be sought.

Color of Fresh and Processed Poultry Meat

The basic color differences between muscles are a result of the relative amounts of white and red muscle fibers. These fibers have different characteristics, and the most noticeable difference to the consumer is their color. Meat color is largely dependent on the amount of meat pigment-myoglobin present in these fibers. Chicken breast muscle is predominantly composed of white fibers, which have a low level and therefore their color is light. On the other hand, thigh meat is mainly composed of red fibers and shows a darker color. Different poultry species also vary in the inherited amount of pigment in their muscles (chicken vs duck). The myoglobin is a complex molecule consisting of two major parts: the protein portion (called globin) and the nonprotein portion (called heme). The latter is capable of binding different compounds and, by that, changing the color of the meat. When the myoglobin is bound to oxygen, chicken leg meat will appear bright pink (Fig. 2). When the same fresh meat is packaged under vacuum it will develop a brownish color (the result of oxidizing the myoglobin into metmyoglobin). On cooking, the protein part of the meat pigment is denatured (similar to denaturation of egg proteins during cooking, which also results in a color and textural change) and gives the meat its typical greyish cooked color. When nitrite is added to the meat, prior to cooking, as is the case in many cured meat products (eg, turkey ham), a typical light pink color will develop. The difference between cured and noncured meat products can be clearly

Table 4. Composition and Nutritional Value of Four Raw Poultry Meats

Source of meat								
Species	Meat	Skin	Water (%)	Protein (%)	Fat (%)	Ash (%)	Iron (mg)	Calories (kcal)
Turkey	White	+	69.8	21.6	7.4	0.90	1.2	159
	White	−	73.8	23.5	1.6	1.00	1.2	115
	Dark	+	71.1	18.9	8.8	0.86	1.7	160
	Dark	−	74.5	20.1	4.4	0.93	1.7	125
Chicken	White	+	68.6	20.3	11.1	0.86	0.8	186
Broiler	Dark	+	65.4	16.7	18.3	0.76	0.98	237
Duck	All	+	48.5	11.5	39.3	0.68	2.4	400
Goose	All	+	50.0	15.9	33.5	0.87	2.5	371
	All	−	68.3	22.7	7.1	1.10	2.5	161

Source: Ref. 6. Expressed on a 100-g portion of meat and skin.

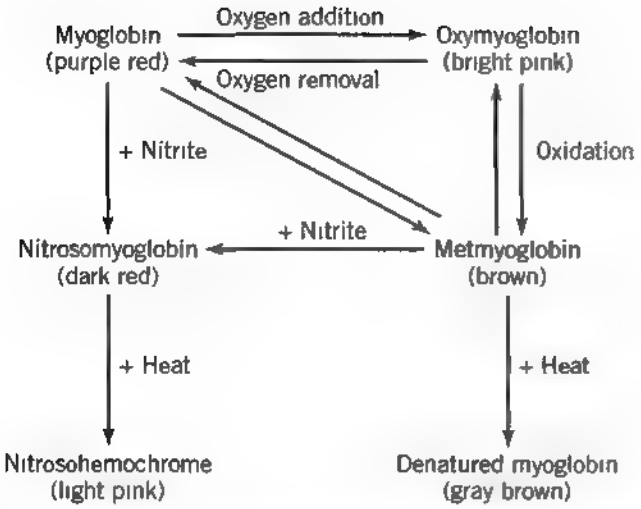


Figure 2. Colors of red poultry muscle (eg, chicken thigh meat).

illustrated when chicken leg meat is prepared at home (as a roast) resulting in a greyish color or when it is cured (as a turkey ham) in a processing plant resulting in the typical pink cured color.

Grading

Classifying poultry into ranges of quality is common in most countries around the world. The grades, based on certain standards, are usually developed by the government and can be voluntarily employed by the producers or imposed by a regulatory agency. Prior to the actual grading, poultry are divided into classes according to their species, sex, and age. This is done because each species has its own unique characteristics. The grading is usually done on the ready-to-cook bird; however, an inspection of the live birds can help in assessing the grade. Such an inspection usually includes: (*1*) general condition of the flock, (*2*) health of the flock, (*3*) feathering, (*4*) conformation, (*5*) flashing, and (*6*) lack of defects (1). The grading of the dressed ready-to-cook poultry is more accurate because the feathers have been removed. The five key areas considered in the grading process are (*1*) the conformation of the carcass, which is related to the presence of defects such as crooked bones, dented breast bones and swollen legs/wings; (*2*) fleshing, which refers to the amount of meat on the birds (well-fleshed birds are of the highest grade and the breast muscle is commonly used as the main indicator for the degree of fleshing); (*3*) fat covering, especially over the breast, back, and hips (although in young turkeys and chicken broilers only a moderate amount of fat is expected; (*4*) the presence of pinfeathers, which will lower the grade (ie, a grade A bird should be free of pinfeathers); and (*5*) the lack of defects such as bruises, broken bones, missing parts, tears, and discoloration of the skin. Additional information and precise details of the grading system used in the United States can be found in the U.S. Department of Agriculture (USDA) *Poultry Grading Manual* (8). In other countries consult the local inspection branch. All the grading systems include tolerances to compensate for interpretation variations and human error. It should be mentioned that in recent years a grading system based on meat quality attributes such as water-holding capacity and texture has been discussed. Such a system is of interest to further processors who are looking for meat that will hold added moisture and not fall apart during cooking regardless of skin tears or missing parts (5).

MEAT PROCESSING EQUIPMENT AND PROCEDURES

Different equipment is used in the fabrication of raw meat into further processed meat products. In the past most of the equipment was designed for a batch-type operation; however, today the trend is toward continuous operation. The latter speeds up the process, allows more volume to pass through a given plant, permits more automation, and, as a result, can save on operation costs. In this section the basic types of equipment used in meat processing plants and their operational principles will be reviewed.

Grinding or Size Reduction

Grinding or size reduction of the meat is the most common process in the manufacturing of meat products. Three major methods are used: grinding, flaking, and chopping.

Grinding. The meat is forced through a grinding plate that can have different opening sizes. An auger is used to convey the energy (manual or electric) to push the meat through the grinder plate (9).

Flaking. Partially frozen meat chunks or blocks are flaked by using a rotating cutting head. The machine has a circular cutting head and the meat is pushed close to the knives by centrifugal force. The size of the flakes is determined by the spacing in the cutting head. This eliminates the mechanical squeezing of the muscle fibers exerted in a conventional grinder, which results in higher moisture loss from the muscle (lean muscle contain about 70% moisture). The meat obtained from the flaker can be easily used in restructured meat products where a musclelike texture is reconstructed from small pieces of meat.

Chopping. The meat is passed through a set of cutting knives, and the degree of chopping is controlled by the length of cutting time. This process usually results in small particles mainly because of the small gap between the cutting knives and the bowl. Chopping is commonly used for fine comminution of meat products (frankfurters) and is not used to prepare products such as ground meat. The two popular designs include a bowl chopper and an emulsion mill. In a chopper, the meat is placed in a cutting bowl, which rotates at a relatively slow speed (15–30 rpm) while the meat is chopped by a set of semicircular knives (usually 3–15) at a speed of a few thousand revolutions per minute. In a mill, preground meat is passed through a fast rotating blade, positioned in a pretty similar way to a meat grinder, but operating at a much higher speed. Since emulsion mills operate at high speed and meat particles are subjected to considerable friction and relatively high temperature, special care should be given to the operating parameters.

Mixing

Mixing is another common step in further processing used to achieve four major goals: (*1*) provide uniformity when different meat sources are used, (*2*) mix nonmeat ingredients (eg, salt and spices) into the meat batter, (*3*) enhance absorption brine solution into the muscle structure, and (*4*) assist in extracting salt-soluble proteins from the muscles. Salt is usually added at the mixing stage because its uniform distribution is extremely important in manufacturing high-quality products (10,11). Briefly, the three functions of salt in meat processing are (*1*) extract salt-soluble proteins (mainly myosin and actin) required for binding and water holding, (*2*) provide flavor, and (*3*) serve as an antimicrobial agent.

Different mixer designs are available on the market. The paddle mixer uses a paddle to mix the meat in a stationary bowl, whereas the ribbon mixer has blades mounted on a horizontal shaft. With any mixer, blending should be precisely controlled to ensure uniform mixing and prevent overmixing, which can result in too much muscle fiber separation.

Tumbling and massaging are two newer procedures designed to achieve the same goals as mixing but in a gentler manner. In these processes the meat is subjected to a mild agitation in (*1*) a rotating drum (tumbling) and (*2*) a mixing vessel equipped with a slow moving paddle (massaging). This agitation, which is usually applied for a much longer period of time than in conventional mixing, helps disrupt some of the tissue structure, helps in distributing of the brine solution, and develops a protein exudate that binds meat chunks during cooking. These functions are further enhanced if the mechanical action is imposed in a vacuum (9).

Injection Machines

Injection machines are used to introduce the brine solution (water, salt, and flavorings) into large whole muscle chunks. In the past, the salt and spices were introduced by rubbing the dry ingredients onto the muscle surface or by immersing the muscle in a brine solution. These processes are time-consuming, especially when dealing with large muscles (eg, whole turkey breast). Today, mechanical injectors are commonly used in the industry. The injectors can consist of a single needle operated manually or have a few dozen needles automatically controlled to deliver a precise volume of brine. The needles must be narrow enough not to cause any damage to the appearance of the muscles. Special care should also be given to the uniformity of the injection process to prevent high salt and/or nitrite concentrations in localized areas. High concentrations of salt and nitrite can cause flavor and color defects (eg, nitrite burns) in the final product. It is common to tumble the meat after injecting the brine. This is done to ensure an even distribution of the brine, enhance brine absorption, and facilitate the extraction of salt soluble proteins (11).

Stuffing Machines

Machines are used to stuff the raw meat batter into the casings. Stuffers vary in size and degree of automation but generally can be divided into two basic types: piston and pump stuffers (12). In the piston stuffers, a piston driven by manual, hydraulic, or electrical energy forces the meat through a horn. The diameter of the horn, stuffing speed, and pressure are controlled by the operator and should match the size and type of the casings used. The piston-type stuffer is recommended for coarsely ground sausages and those having large particles such as fat and pickles, because they might be damaged by an impeller-type pump. In pump stuffers the meat is passed through an impeller-type pump that usually has feedback and pop-off connectors so a vacuum can be drawn to evacuate trapped air from the meat batter. Vacuum stuffers are more expensive but are advantageous in providing high-quality products since air pockets that might be filled with gelatin (melted connective tissue) or melted fat during the cooking process are eliminated. It is important to minimize these pockets because products with an excessive amount of pockets are rejected by the consumer.

Various types of casings are used for different types of meat products. They include:

1. Natural casings obtained from the gastrointestinal track of sheep, hogs, etc. They are digestible, permeable to moisture and smoke, and can shrink with the product when a drying step is employed. However, they are not uniform and are relatively difficult to handle especially when fast automated equipment is used.
2. Manufactured collagen casings that are made from natural collagen extracted from hides. They are uniform in size and usually stronger than natural casings.
3. Cellulose casings made from cotton fibers. They are also permeable to moisture and smoke, uniform, and very strong, but nondigestible. Overall, they are very popular where high-speed stuffing machines are used.
4. Plastic casings are moisture proof and used for either water or steam cooking. They are strong and can appear with printing on the outside surface; however, they are nondigestible and have to be removed prior to consumption.

Other casings are combinations of the preceding materials such as collagen reinforced with cotton, or cotton fibers coated with plastic. Rigid metal molds are used mainly for baking loaves.

Forming Machines

Forming machines are used to form products such as patties and nuggets. These products are made from a mixture of ground and emulsified meat and do not hold together very well in the raw state. The forming machine is basically a press with different templates that can be used to form any shape desired. To maintain the shape of the newly formed product it has to be either frozen immediately or cooked.

Battering and Breading Machines

Battering and breading machines are used to uniformly apply the batter and breading. These machines usually consist of a series of conveyor belts that carry the product (formed or whole cuts) through a series of ingredients used to coat the product. Some of the belts (usually a stainless steel mesh) can be shaken automatically to control the thickness of the breading layer.

Cooking

Most meat products are consumed after they have been cooked. This can be done in water, oil, by hot air, infrared, or microwave energy. Cooking results in distinct textural changes in the meat due to denaturation of different muscle proteins. Three major phases can be observed during cooking. The first increase in toughness (45–55°C) is due to myosin denaturation, the second (60–70°C) is due to sarcoplasmic protein and some connective tissue denaturation, while the third (above 75°C) actually results in reducing the toughness, which is the result of collagen transformation into gelatin (13). Differences exist in the denaturation profiles of different meats, and various muscles within the same animal. An example of this is the higher rigidity developed in white poultry meat (or products containing white meat) as compared with dark meat (14).

Hot air ovens, with/without a smokehouse attached, are very popular in the meat industry. The meat products are placed in a chamber and the heat generated from a gas burner or an electrical element is transferred into the products. The amount of moisture in the air, air flow, and temperature difference between the product and air will determine the rate of heating. Of particular importance is the relative humidity in the air (expressed as amount of moisture in the air at a given temperature). Water is a good conductor of heat and its presence helps in delivering the heat; however, a moist product surface results in a cooling effect due to evaporation. Therefore, a balance between the two factors should be always maintained.

Cooking in Water. Cooking in water is a faster way of transferring the heat into the product compared with hot air Steam kettles and water baths are used for this purpose. Commercial meat products are usually stuffed into a moisture-proof casing to eliminate cooking losses (consisting of protein, fat and moisture) from the product. However, some meat cuts are not packaged and are immersed directly in water/soup. This is done where moist heat is required to tenderize the tough connective tissue (eg, in boiling mature hen meat).

Frying. Frying is a very efficient way of transferring heat into the meat since the temperature of the fat can be raised well above 100°C. Frying in oil also provides a crisp texture on the outside of the product, which is desired in products such as fried chicken and breading on chicken nuggets.

Infrared Heating. Infrared heating is achieved by the use of an infrared lamp that heats up the surface of the product. The heat is then slowly transferred by conduction into the center of the product. This type of heating is mainly used for warming up cooked products, keeping products hot on a display counter, and in combination with microwave heating when surface browning is desired.

Microwave Heating. Microwave heating is a fast way of cooking whereby heating results from converting microwave energy to heat by friction of water molecules rotating due to rapid fluctuation in the electromagnetic field (915 and 2450 MHz are used commercially). Since cooking is very fast, there is usually not enough time to develop the typical brown color on the surface of the product. Therefore, other cooking methods, such as infrared, should be employed if a typical brown surface is required. Low microwave energy is also used to defrost meat. However, special care should be taken since there is a big difference between the heating profile of water and ice.

Smoking. Smoking is the application of wood-burning smoke compounds onto the product. Overall, more than 200 individual compounds have been identified in wood smoke (15). In the past, the only way of smoking was to expose the product to smoke derived from burning wood. Today, the processor can choose different preparations of liquid smoke extracts that can be directly added to the product, used as a dip, or be sprayed onto the product prior to cooking. In the past, smoking was mainly used as a means of preserving the meat that was also dried at the same time over wood fire. Some of the smoke components exert strong antimicrobial activity (eg, phenols) and therefore help in preserving the product. However, since these compounds can only penetrate a few millimeters into the product, they are basically protecting the surface. Today, smoking is mainly used to provide typical flavors to the product (eg, hickory smoke). The compounds that contribute to flavor include carbonyls, organic acids, and some of the phenols.

Smoking and cooking are considered to be two separate processes; however, they are usually discussed together because they often occur in immediate succession or simultaneously. It is important to realize that to achieve the best smoke penetration, the product should not be cooked because the denatured protein film, formed during cooking, will prevent smoke migration into the product. Therefore, smoking is done at low temperatures even though some heat is often applied to partially dry the surface. The latter is done to ensure that the smoke will not be washed off the product. When liquid smoke (smoke extracts) is used, the product is dipped or sprayed prior to cooking. In all cases, casings permeable to smoke (eg, collagen, cellulose) should be used. If liquid smoke is to be added directly into the raw batter, a special preparation (eg, pH adjusted) and low concentration should be used; and in this case there is no need to use permeable casings (9).

SANITATION IN POULTRY PROCESSING PLANTS

Meat is a perishable food item (Fig. 3) because it contains all the nutrients required for microorganism to grow, and

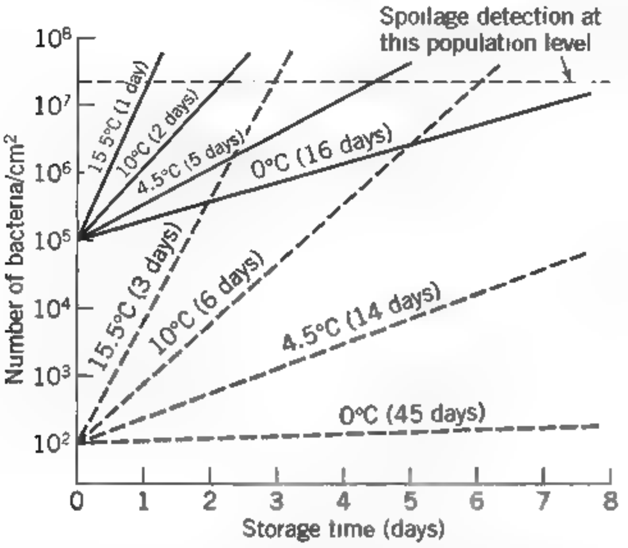


Figure 3. The time required for the spoilage of frankfurters that were contaminated with high and low levels of psychrophilic bacteria. Spoilage detection (by slime formation) was at a population level of 150 million bacteria/cm² of surface area. The high level of contamination was 1 million/cm², shown by the solid line; the low level was 100 bacteria/cm², shown by the broken line. *Source:* Ref. 16.

its pH (5.5–7.0) is not inhibitory to most microorganisms. The extensive fabrication and distribution of raw and processed meat further increases exposure to microbial contamination. It was reported that ground meat can be handled 10 to 15 times before it gets to the consumer.

One of the principal contamination sources during processing is slaughtering. The live healthy muscle is essentially free of microorganisms; however, after slaughtering the natural defense mechanisms no longer function. During slaughtering the sticking knife, which cuts through the skin, transfers microorganisms into the bloodstream. Because blood circulation is not immediately stopped, the microorganisms can still be distributed throughout the carcass. It is important to realize that 1 g of soil (dirt or manure) attached to the skin or feathers can contain 1 billion microorganisms. Another point of contamination is evisceration. The digestive tract harbors high numbers of microorganisms (about 200 million microorganisms per gram). If the gut contents are spilled on the carcass, high contamination levels can be expected. The process of defeathering can assist in the introduction of microorganisms into the skin. The scalding temperature used is insufficient to kill all the microorganisms, and the process of rubbing feathers off the skin actually helps to embed the microorganisms into the skin. Other potential contamination sources include people handling the meat, air, water used to rinse the carcasses, contact with equipment, and insects and rodents (17).

The method used for cleaning a food processing plant is based on the soil material present. Because meat contains mainly protein, fat, and moisture, alkaline solutions are the most common cleaning solutions used in the meat industry. An alkaline solution such as 1.5% sodium hydroxide can be used to saponify the fat and also to dipeptidize the protein deposits. Various alkaline phosphates and synthetic detergents are also used in meat processing plants. A new approach to cleaning involves the use of enzymes in a cleaning solution. A solution usually containing proteases (to break down protein deposits) is often used in a mild alkaline solution (to saponify the fat deposits). The main advantage of using enzymes is a significant reduction in corrosion in the plant. Because the alkalinity of the solution cannot be too high (otherwise will inactivate the enzymes), corrosion problems are minimized. However, it should be noted that enzyme solutions are more expensive to use, at least on a short-term basis.

The cleaning procedure in a meat plant includes:

1. *Removal of Heavy Soils from the Surface.* This step is usually done manually (scrapers) and can help in reducing waste loads and save on cleaning compounds.
2. *Rinse with Water.* High-pressure hoses can be used to facilitate this step. The water temperature should be below 55°C to prevent cooking the meat onto the surface.
3. *Wash with an Alkaline Solution or a Synthetic Detergent.* It is important to allow sufficient time for the chemical cleaning reaction(s) to take place. Usually a contact times of 5 to 10 min is recommended at a water temperature of 50 to 55°C. When vertical surfaces are cleaned, foaming agents are used to keep the cleaning solution in close contact with the surface. Enzyme solutions can also be applied at this stage; however, lower water temperature should be used to prevent enzyme inactivation.
4. *Rinse with Clean Water to Remove All the Alkaline and Detergent Solutions.*
5. *Acid Wash to Remove Scale Deposits.*
6. *Inspect to Ensure the Removal of All the Soil.*
7. *Sanitize the Plant.* It is crucial to start this step only after all the equipment is thoroughly cleaned, otherwise the sanitizer could not be in close contact with the surface and its activity is diminished. A chlorine solution (100–200 ppm), iodine (20–30 ppm), or quaternary ammonium solution (150–200 ppm) are commonly used.
8. *Rinsing and Drying.*
9. *Oiling, Only Areas Subject to Corrosion.*

Cleaning in place (CIP) is not very popular in meat plants because of plant outlay and design; however, where applicable (eg, a closed system such as a smokehouse) heavy duty detergents are used to effectively remove the soil deposits without exposing employees to harsh chemicals.

POULTRY PRODUCTS

Within the last 20 to 30 years many new processed poultry products have been introduced on the market. The poultry industry has taken the initiative to develop new products and also adopted some red meat recipes to increase consumption and to move away from seasonal demand. In the past whole turkeys were sold in the North American market mainly prior to Thanksgiving and Christmas. This kind of marketing significantly limited the increase in sales of poultry. Realizing these limitations (sale of whole birds and demand concentrated within 1–3 months) the poultry industry started to move methodically into further processing. At the beginning, red meat recipes were modified in order to manufacture poultry products (frankfurters), and later new technologies were developed exclusively for poultry (chicken nuggets). Chicken frankfurters were unheard of 25 years ago; however, after their introduction, they gained a significant market share, currently about 20% of the North American market. Overall, the poultry industry has moved into further processed poultry products, responding to consumer demand for more convenient food items such as semiprepared or fully prepared items. The increase in poultry meat consumption (Table 1) has been the result of aggressive marketing, the favorable nutrition profile of the meat, and the competitive price of the meat.

Today consumers can choose their meat products from a wide variety consisting of a few hundred different products. Becoming a knowledgable consumer can be a challenge. To assist the consumer, various systems have been suggested for classifying different meat products. One of the most common ways to classify the products is to group them into: (*1*) fresh: fresh poultry breakfast sausage, (*2*) uncooked and smoked: polish sausage, (*3*) cooked and smoked: frankfurter, bologna, (*4*) cooked: liver sausage, cooked salami, (*5*) dry/semidry or fermented: summer sausage, dry salami, and (*6*) cooked meat specialities: luncheon meats, jellied products, and loaves (9). However, a few recently developed technologies represent two additional categories: (*7*) restructured meat products: where small bits and pieces of meat are made into a steaklike product by using freezing and high pressure, and (*8*) surimi-like products: where the meat is minced, washed (to remove pigments and enzymes) and extruded to obtain a fiber muscle-like texture. In this section, examples of the major processed poultry products will be discussed (Table 5). Detailed information has been published elsewhere (1,2,18).

Smoked Turkey Breast

Smoked turkey breast is a premium product produced from the whole breast muscle. A brine solution (ie, water, salt, spices, and sometimes gums such as carrageenan) is added to the muscle, often by direct injection, prior to smoking and cooking. The meat is massaged or tumbled after brine injection to assist in absorbing the brine and achieving an even distribution within the muscle. The breast muscle is then stuffed into a netting (the meat can be covered with skin) and smoked and cooked in a smokehouse until an internal temperature of at least 70°C is reached. No nitrite is added to this product because it is desirable to maintain the white color of the cooked meats.

Poultry Rolls

Poultry rolls are made from dark meat, white meat, or their combination. The meat is obtained from whole chicken or turkey muscle, trimmings, and skin. In this product the pieces of meat are "glued" together to form a coherent product. Salt is added to extract the salt-soluble protein (mainly actin and myosin), which assists in binding the meat pieces and retaining the moisture and fat within the product. The skin and some of the trimmings are usually finely chopped (the term "emulsified" is used in the industry even though no true emulsion is formed) to facilitate the binding of the fat. Seasonings are added to provide flavor, and moisture is added to compensate for cooking losses and to improve the juiciness of the product. If added moisture exceeds a certain percentage (varies in different countries) of the raw meat moisture, the product should be labeled as a water-added product. The meat pieces are mixed together with the nonmeat ingredients until the meat becomes sticky (which is used as an indication of good protein extraction) and all the added moisture is absorbed. The mix is then stuffed into casings and the product is cooked either in a water bath or an oven.

Table 5. Examples of Various Further Processed Poultry Products

Product	Comments
Smoked turkey breast	Whole muscle, without nitrite
Poultry roll	White and dark meat
Turkey ham	Cured dark meat
Turkey bacon	Layers of light and dark meat
Summer sausage	Fermented product
Poultry frankfurter	Fine emulsion, in small diameter casings
Poultry bologna	Fine emulsion, in large diameter casings
Poultry patties	Hamburger type, not cured
Breakfast sausage	Ground product, sold fresh or frozen
Chicken nuggets	Whole muscle or restructured
Fried chicken	Battered and breaded, sold un- or precooked
Roasted, barbecued	Prepared with dry heat, crisp skin
Jellied chicken loaf	Cooked meat held in a gelatin matrix

Turkey Ham

Turkey ham is typically manufactured from turkey thigh meat. This product is much lower in fat content than the traditional pork ham. In the initial manufacturing step, a brine solution (ie, water, salt, phosphates, flavorings, and nitrite) is introduced either by injecting or marinating the meat. Tumbling is commonly used when a brine injection is employed and helps to get an even distribution of the curing ingredients and to extract the salt-soluble proteins to the surface. The meat is then stuffed into fibrous casings. The shape (round or oblong) and size are determined by the casings. The ham is smoked (smoke flavorings can also be added to the raw batter) and cooked to at least 68°C.

Summer Sausage and Salami

Summer sausage and salami are fermented poultry products made from dark poultry meat, skin, and fat. In this process microorganisms are used to reduce the pH of the product and add some typical flavor notes. In the past, microorganisms from a previous successful batch were introduced into a new batch and the product was allowed to ferment. Today the industry mainly uses starter cultures with a known composition of microorganisms (predominantly lactic acid bacteria). The producer can select from cultures that grow at different temperatures and produce specific flavors. Recently, a lot of progress has been made in the field of starter cultures, mainly through the use of genetic engineering where various characteristics can be included in one strain. The use of a starter culture is highly recommended because it ensures that lactic acid bacteria will dominate the fermentation, suppress pathogens, and produce the desired flavors. The degree of fermentation can be controlled by the quantity of the carbohydrate added (an energy source for the microorganisms) or by continuous pH monitoring. After the fermentation (1 to 2 days with a starter culture) the product is either smoked and cooked or only dried. The final product is usually shelf stable due to the low pH (4.6–5.1) and/or low water activity ($A_w < 0.90$).

Frankfurters and Bologna

Frankfurters and bologna are examples of finely comminuted meat products where the final product has a very homogeneous appearance. Dark muscle chunks and/or mechanically deboned meat are usually emulsified with the fat. A bowl chopper or an emulsion mill are used to achieve an efficient particle size reduction. Salt is added to extract the meat proteins, which is essential in binding the small meat particles and stabilizing the small fat globules within the protein matrix (10). Nitrite is added to inhibit *Clostridium botulinum* growth and to provide the typical cured meat color (Fig. 2). The meat batter is then stuffed into cellulose casings and smoked and cooked in a smokehouse. Since frankfurters are such a high volume item, some processors have dedicated an entire continuous line for this product. As with other meat products, low microbial contamination and refrigerated temperature can help prolong the shelf life (Fig. 3).

Chicken Nuggets

Chicken nuggets are one of the most successful poultry products introduced in the 1980s in North America. Originally, the product was prepared from a single piece of slightly marinated breast meat that was battered and breaded. Later, nuggets made from trimmings, dark meat, skin, mechanically deboned meat, and their combinations started to appear on the market. To prepare the products the meat pieces are marinated and mixed with a brine solution (containing salt and flavorings) until all the solution is absorbed by the meat. The meat is then put through a forming machine that creates the desired product shape, followed by battering and breading and deep fat frying. Frying is done after forming the product to preserve the product shape and to provide the typical crispy texture of the breading.

FUTURE DEVELOPMENT

In light of the material presented in the preceding sections and especially the continuous increase in poultry meat consumption (Table 1), it would seem that poultry meat is going to remain popular and might even gain a greater market share. Some predict that consumption level(s) will stabilize in this decade, and some forecast a further increase. One thing is certain, however; the poultry industry must be competitive to maintain its current market share. Poultry meat is also becoming more popular in many countries because of the ability of poultry to adapt to most areas of the world, the low economic value per unit and rapid growth rate (1,5). Therefore, it can be expected that on a worldwide basis, poultry consumption will increase.

Improvement in disease control will play an even more important role in the production of poultry as the trend toward larger growing units continues to increase. In addition, improvement in vaccination and feeding programs will further affect the competitiveness of the poultry industry. In the area of further processing, more automation and continuous operations will replace manual and batch operations. The meat industry is currently moving toward more automation and robotics to replace repetitive manual labor. Automated equipment for evisceration and cutting up poultry is already available on the market today. In the future, better cooperation between breeding and production personnel will help to produce a more uniform bird that will be easier to process in an automated plant.

Responding to emerging consumer demands, such as more convenient food items (eg, fully prepared in meat plants), microwave-ready food items and reduced-salt and -fat meat products will play a more important role in the future. Based on past responses of the poultry industry to consumer demands (eg, marketing fresh poultry, developing new products), it seems that the industry is well equipped to respond to future market trends.

BIBLIOGRAPHY

1. G. J. Mountney, *Poultry Products Technology*, Food Products Press, Binghampton, N.Y., 1989.
2. U.S. Department of Agriculture. *Labelling Policy Book*, USDA Food Safety Inspection Service, Meat and Poultry Inspection Technical Services, Washington, D.C., 1981.
3. American Meat Institute, *Meat and Poultry Facts*, Watt, Mt. Morris, Ill., 1997.
4. A. B. M. Raj, "Carcass and Meat Quality in Broilers either Killed with a Gas Mixture or Stunned with an Electric Current under Commercial Conditions," *British Poultry Sci.* **38**, 169–175 (1997).
5. S. Barbut, "Estimating the Magnitude of the Pale, Soft, and Exudative (PSE) Problem in Poultry. A Review," *J. Muscle Food* (1998).
6. U.S. Department of Agriculture. *Composition of Foods, Poultry Products: Raw, Processed, Prepared*, Agriculture Handbook 8-5, Washington, D.C., 1979.
7. G. W Froning, "Mechanical Deboning of Poultry and Fish," *Advances Food Res.* **27**, 109–145 (1981).
8. U.S. Department of Agriculture. *Poultry Grading Manual*, Agricultural Marketing Service, Handbook No. 31, Washington, D.C., 1989.

9. H. B. Hedrick, et al., *Principles of Meat Science*, Kendall/Hunt, Dubuque, Iowa, 1994.
10. S. Barbut, and C. J. Findlay, "Sodium Reduction in Poultry Products A Review," *CRC Crit. Rev. in Poultry Biology* **2**, 59–95 (1989).
11. S. Barbut, "Determining Water and Fat Holding," in G. M. Hall, ed., *Methods of Testing Protein Functionality*, Chapman & Hall, New York, 1996, pp. 186–225.
12. A. M. Pearson and F. W. Tauber, *Processed Meats*, AVI, Westport, Conn., 1984.
13. C. L. Davey and K. V. Gilbert. "Temperature Dependent Cooking Toughness in Beef," *J. Sci. Food Agric.* **25**, 931–937 (1974).
14. G. S. Mittal and S. Barbut, "Effects of Salt Reduction on the Rheological and Gelation Properties of White and Dark Poultry Meat," *J. Texture Stud.* **20**, 209–222 (1989).
15. J. A. Maga, *Smoke in Food Processing*, CRC Press, Boca Raton, Fla., 1989.
16. E. A. Zottola, *Introduction to Meat Microbiology*, American Meat Institute, Chicago, Ill., 1972.
17. N. G. Marriott, *Principles of Food Sanitation*, Chapman & Hall, New York, 1994.
18. W. J. Stadelman, *Egg and Poultry Meat Processing*, Ellis Horwood, Chichester, England, 1988.

SHAI BARBUT
University of Guelph
Guelph, Ontario
Canada

PROCESSED CHEESE

Processed cheese is manufactured by blending shredded natural cheeses of different types, sources, and degrees of maturity with emulsifying agents, adding water and heating the blend under partial vacuum with constant agitation until a homogeneous mixture is obtained. High shear mixing or homogenization could be used in addition. Besides natural cheeses, other dairy and nondairy ingredients may be included in the blend.

A number of natural cheese varieties were known and appreciated as a valuable food component in ancient Roman times, more than 2000 years ago. There are also quotations about cheeses in the Bible and in old Greek and Latin poetry. Compared with these data, processed cheese seems to be a brand new product, originating in this century, although based on natural cheeses.

Initially, processed cheese was manufactured without an emulsifying agent. The first attempt was as early as 1895, but only after the introduction of citrate (in Switzerland in 1912) as a calcium-sequestering agent did the industrial production of processed cheese become feasible. Actually, Swiss inventors first succeeded in changing the state of casein from coarsely dispersed calcium paracaseinate in the raw cheese, by using heat and sodium citrate as a peptizing agent, into a homogeneous, free-flowing condition: the sol state. Phosphates, which were introduced a few years later, enabled the real expansion of processed cheese manufacture. Quite independently, the production of processed Cheddar cheese started in the United States, developed by Kraft in Chicago in 1917, by using the mixture of phosphates and citrates as emulsifying agents.

The idea of processing was first meant to make use of natural cheeses that otherwise would be difficult or impossible to sell (cheeses with mechanical deformations and localized molds and trimmings produced during cheese formation, pressing, packaging, etc) and to obtain a product of better keeping quality. While soft cheese and semihard cheese could be canned, pasteurized, and consequently stored for a long time or exported overseas (even to the Tropics), such a procedure for hard cheeses has not been developed. All experiments with the aim to heat treat hard cheeses (eg, Emmentaler) failed, resulting in the breakdown of the cheese structure with exclusion of fat and water. Proteolysis and lipolysis continue beyond the stage where the optimum flavor has been developed in any given cheese. Advanced decomposition of proteins/lipids leads to a nutritionally inferior product, causing detrimental changes in sensory characteristics, ending up with an unsuitable cheese for consumption. The invention of processed cheese was the perfect solution for long storage of all native cheese varieties. Even longer storage could be achieved with processed cheese powder or sterilized processed cheese. The assortment of processed cheese was further expanded due to numerous possible combinations of various types of cheese and the inclusion of other dairy and nondairy components, which make it possible to produce processed cheese differing in consistency, flavour, size, and shape.

The main characteristics of processed cheese are composition, water contents, and consistency. According to these criteria, three main groups may be distinguished: processed cheese blocks, processed cheese foods, and processed cheese spreads (1–5) (Table 1). More recent subtypes are processed cheese slices and smoked processed cheese. Processed cheese analogues are imitation, simulated, or alternative groups of processed cheese that contain no cheese and are usually based on vegetable fat and casein blends.

Processed cheeses are advantageous compared with natural cheese primarily because of the following:

- Reduced refrigeration costs during storage and transport, especially important in hot climates
- Better keeping quality with less alterations during prolonged storage
- Great versatility of type and intensity of flavor, for example, from mild to sharp native cheese flavor or specific spices
- Milk replacement: excellent source of nutritively high valuable milk components for children or people who dislike milk
- Adjustable packaging for various usage, economical and imaginative.
- Suitability for home use as well as for fast-food restaurants, for example, in cheeseburgers, hot sandwiches, spreads, and dips

MANUFACTURING: PRINCIPLES AND TECHNIQUES

The manufacturing procedure for processed cheese consists of operations carried out in the following order: selec-

9. H. B. Hedrick, et al., *Principles of Meat Science*, Kendall/Hunt, Dubuque, Iowa, 1994.
10. S. Barbut, and C. J. Findlay, "Sodium Reduction in Poultry Products A Review," *CRC Crit. Rev. in Poultry Biology* **2**, 59–95 (1989).
11. S. Barbut, "Determining Water and Fat Holding," in G. M. Hall, ed., *Methods of Testing Protein Functionality*, Chapman & Hall, New York, 1996, pp. 186–225.
12. A. M. Pearson and F. W. Tauber, *Processed Meats*, AVI, Westport, Conn., 1984.
13. C. L. Davey and K. V. Gilbert. "Temperature Dependent Cooking Toughness in Beef," *J. Sci. Food Agric.* **25**, 931–937 (1974).
14. G. S. Mittal and S. Barbut, "Effects of Salt Reduction on the Rheological and Gelation Properties of White and Dark Poultry Meat," *J. Texture Stud.* **20**, 209–222 (1989).
15. J. A. Maga, *Smoke in Food Processing*, CRC Press, Boca Raton, Fla., 1989.
16. E. A. Zottola, *Introduction to Meat Microbiology*, American Meat Institute, Chicago, Ill., 1972.
17. N. G. Marriott, *Principles of Food Sanitation*, Chapman & Hall, New York, 1994.
18. W. J. Stadelman, *Egg and Poultry Meat Processing*, Ellis Horwood, Chichester, England, 1988.

SHAI BARBUT
University of Guelph
Guelph, Ontario
Canada

PROCESSED CHEESE

Processed cheese is manufactured by blending shredded natural cheeses of different types, sources, and degrees of maturity with emulsifying agents, adding water and heating the blend under partial vacuum with constant agitation until a homogeneous mixture is obtained. High shear mixing or homogenization could be used in addition. Besides natural cheeses, other dairy and nondairy ingredients may be included in the blend.

A number of natural cheese varieties were known and appreciated as a valuable food component in ancient Roman times, more than 2000 years ago. There are also quotations about cheeses in the Bible and in old Greek and Latin poetry. Compared with these data, processed cheese seems to be a brand new product, originating in this century, although based on natural cheeses.

Initially, processed cheese was manufactured without an emulsifying agent. The first attempt was as early as 1895, but only after the introduction of citrate (in Switzerland in 1912) as a calcium-sequestering agent did the industrial production of processed cheese become feasible. Actually, Swiss inventors first succeeded in changing the state of casein from coarsely dispersed calcium paracaseinate in the raw cheese, by using heat and sodium citrate as a peptizing agent, into a homogeneous, free-flowing condition: the sol state. Phosphates, which were introduced a few years later, enabled the real expansion of processed cheese manufacture. Quite independently, the production of processed Cheddar cheese started in the United States, developed by Kraft in Chicago in 1917, by using the mixture of phosphates and citrates as emulsifying agents.

The idea of processing was first meant to make use of natural cheeses that otherwise would be difficult or impossible to sell (cheeses with mechanical deformations and localized molds and trimmings produced during cheese formation, pressing, packaging, etc) and to obtain a product of better keeping quality. While soft cheese and semihard cheese could be canned, pasteurized, and consequently stored for a long time or exported overseas (even to the Tropics), such a procedure for hard cheeses has not been developed. All experiments with the aim to heat treat hard cheeses (eg, Emmentaler) failed, resulting in the breakdown of the cheese structure with exclusion of fat and water. Proteolysis and lipolysis continue beyond the stage where the optimum flavor has been developed in any given cheese. Advanced decomposition of proteins/lipids leads to a nutritionally inferior product, causing detrimental changes in sensory characteristics, ending up with an unsuitable cheese for consumption. The invention of processed cheese was the perfect solution for long storage of all native cheese varieties. Even longer storage could be achieved with processed cheese powder or sterilized processed cheese. The assortment of processed cheese was further expanded due to numerous possible combinations of various types of cheese and the inclusion of other dairy and nondairy components, which make it possible to produce processed cheese differing in consistency, flavour, size, and shape.

The main characteristics of processed cheese are composition, water contents, and consistency. According to these criteria, three main groups may be distinguished: processed cheese blocks, processed cheese foods, and processed cheese spreads (1–5) (Table 1). More recent subtypes are processed cheese slices and smoked processed cheese. Processed cheese analogues are imitation, simulated, or alternative groups of processed cheese that contain no cheese and are usually based on vegetable fat and casein blends.

Processed cheeses are advantageous compared with natural cheese primarily because of the following:

- Reduced refrigeration costs during storage and transport, especially important in hot climates
- Better keeping quality with less alterations during prolonged storage
- Great versatility of type and intensity of flavor, for example, from mild to sharp native cheese flavor or specific spices
- Milk replacement: excellent source of nutritively high valuable milk components for children or people who dislike milk
- Adjustable packaging for various usage, economical and imaginative.
- Suitability for home use as well as for fast-food restaurants, for example, in cheeseburgers, hot sandwiches, spreads, and dips

MANUFACTURING: PRINCIPLES AND TECHNIQUES

The manufacturing procedure for processed cheese consists of operations carried out in the following order: selec-

Table 1. Some Characteristics of Processed Cheese Types

Type of product	Ingredients	Cooking temperature, °C	Composition	pH	Ref.
Processed cheese block	Natural cheese, emulsifiers, NaCl, coloring	71–80	Moisture and fat contents correspond to the legal limits for natural cheese	5 6–5.8	1
		80–85		5.4–5.6	3
		74–85	≤45% moisture	5.4–5.7	2
Processed cheese food	Same as above plus optional ingredients such as milk, skim milk, whey, cream, albumin, skim milk cheese, organic acids	79–85	≤44% moisture, <23% fat	5.2–5.6	1
Processed cheese spread	Same as processed cheese food plus gums for water retention	88–91	≥44% and ≤60% moisture	5.2	1
		85–98		5.7–5.9	3
		90–95	≤55% moisture	5.8–6.0	2

Source: Ref. 4, courtesy of *Food Structure and Scanning Electron Microscopy.*

tion of natural cheese, computation of the ingredients, blending, shredding, addition of emulsifying agents, processing, homogenization (optional), packaging, cooling, and storage. A schematic representation of the processing procedure, including the possible facilities for particular operations, has been presented in Figure 1.

Selection of Natural Cheese

Proper selection of natural cheese is of special importance for the successful production of processed cheese. In some countries, processed cheeses manufactured from only one variety of cheese of different degrees of maturity are popular, for example, processed Cheddar cheese in the UK and Australia, Cheddar and Mozzarella in the United States and Canada, and Emmental in Western Europe. More frequently, processed cheeses are produced from a mix of various natural cheese types. Criteria for raw material selection are as follows (3):

- The type of flavour required
- The consistency and structure
- The fat content
- The variety and grade of fat in the raw material
- The maturity of the raw material
- The quantity of raw material in stock
- The nature and character of the additives
- The legal requirements
- The potential market
- Other, mostly economic, points

The combination of native cheeses, the processed cheese of proper maturity, is of special importance for the processed cheese quality, in terms of taste and flavor. Intact casein, present more in young cheese, has an emulsifying effect on milkfat and stabilizes the emulsion ("long structure"). This effect is not expressed by partially hydrolyzed protein, predominating in matured cheese ("short structure"). So, for example, high-fat spreadable processed cheese requires a larger proportion of young cheese in the blend with a correspondingly higher intact casein content.

Since it is possible to correct certain physical properties by skillful blending, some defective, but not harmful, cheeses can be used in processed cheese manufacture. Natural cheeses with microbial defects should not be selected for processing; spore-forming, gas-producing, and pathogenic bacteria are particularly hazardous. However, proper selection of good-quality natural cheeses is not, by itself, a guarantee that the processed cheese will have the desired high quality, if treated by improper process parameters.

Computation of Ingredients

The computation of the ingredients is based on fat and dry matter contents of natural cheese components as well as the composition of the final product. Formulation of the material balance of fat and dry matter, including all blend constituents, added water, and condensate from live steam used during processing, must be made in such a way as to yield a product with the desired composition. Additional adjustments of fat and dry matter during processing may be possible but this depends on the kind of equipment used.

Blending

Blending, which could be defined as designing the proper processed cheese blend composition and concerning, above all, selected cheese varieties, is highly influenced by the characteristics desired in the final product. However, there are no strict rules proscribed for designing the particular ratio among cheeses of different maturity in the blend. Only the general recommendations are suggested (1–3). For processed cheese blocks, mostly young to medium ripe cheese is used, whereas to produce processed cheese spread, a combination of young, semiripe and ripe cheese is preferred.

The main advantages of a high content of young cheese in the blend are as follows (2,5):

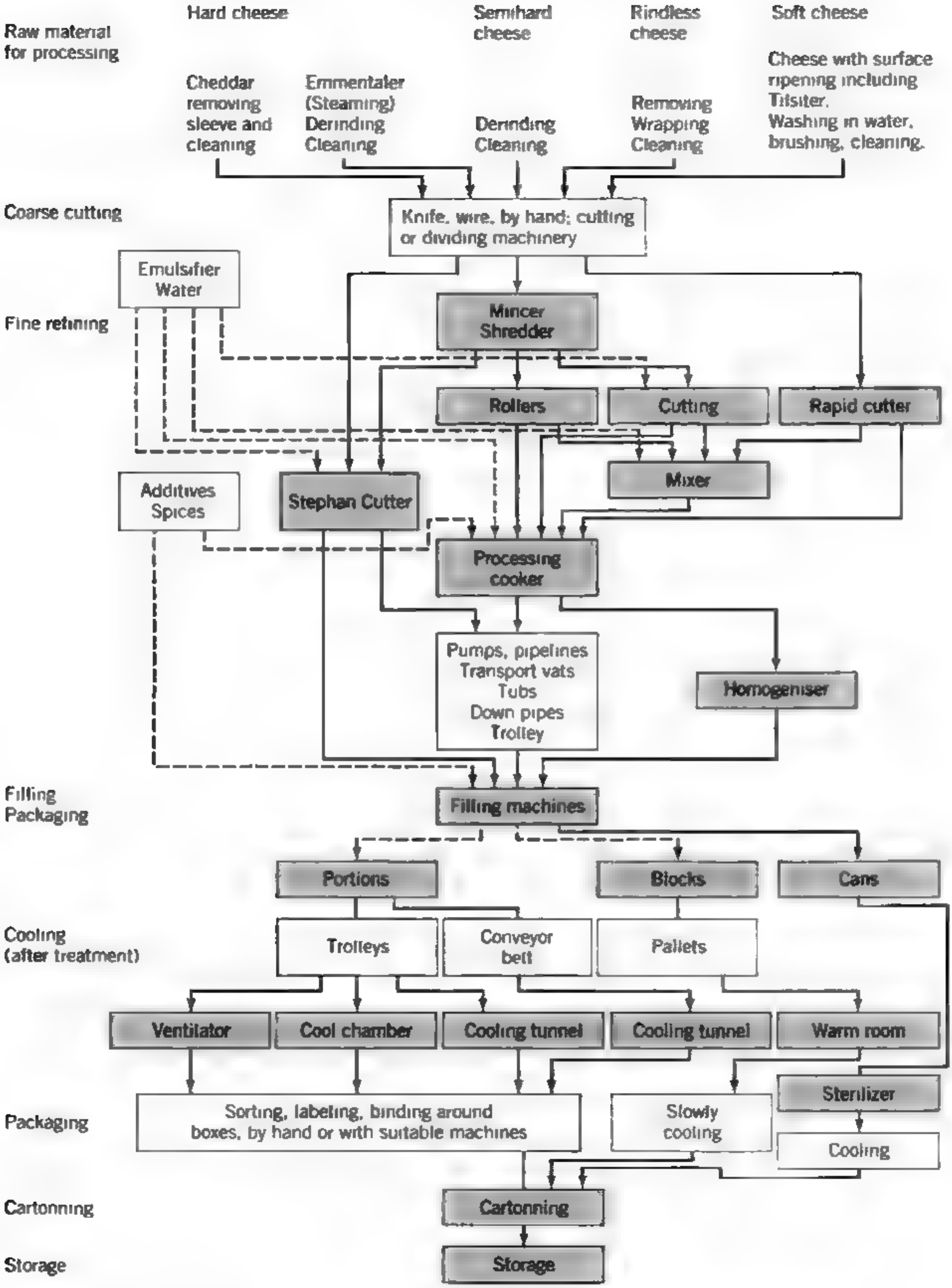

Raw material for processing
Hard cheese
Semihard cheese
Rindless cheese
Soft cheese
Cheddar removing sleeve and cleaning
Emmentaler (Steaming) Derinding Cleaning
Derinding Cleaning
Removing Wrapping Cleaning
Cheese with surface ripening including Tilsiter. Washing in water, brushing, cleaning.
Coarse cutting
Knife, wire, by hand; cutting or dividing machinery
Emulsifier Water
Fine refining
Mincer Shredder
Rollers
Cutting
Rapid cutter
Mixer
Additives Spices
Stephan Cutter
Processing cooker
Pumps, pipelines Transport vats Tubs Down pipes Trolley
Homogeniser
Filling Packaging
Filling machines
Portions
Blocks
Cans
Cooling (after treatment)
Trolleys
Conveyor belt
Pallets
Ventilator
Cool chamber
Cooling tunnel
Cooling tunnel
Warm room
Sterilizer
Packaging
Sorting, labeling, binding around boxes, by hand or with suitable machines
Slowly cooling
Cooling
Cartonning
Cartonning
Storage
Storage

Figure 1. Diagrammatic representation of the processing procedure. *Source:* Ref. 3, courtesy of Food Trade Press.

- The reduction of raw material costs
- The possibility of using cheeses with poor curing properties immediately after manufacture
- The formation of a stable emulsion with high water-binding capacity
- The production of a firm body, with good slicing properties of the finished product

The main disadvantages are

- the production of a tasteless cheese,
- an emulsifier-like off-flavor,
- excessive swelling,
- the tendency to harden during storage, and
- the presence of small air bubbles (developed due to the high viscosity of the blend).

The advantages of a high contents of extramature cheese in the blend include (2,5)

- the development of a full flavor,
- good flowing properties, and
- a high melting index (processed cheese melts easily).

The disadvantages are

- the possibility of sharp flavor development,
- A low emulsion stability, and
- a soft consistency.

To ensure permanent production, the processed cheese factory needs a good stock of native cheese varieties of a constant quality (physicochemical and microbial). If the native cheese is not rindless (packed and ripened in plastic wrapping), the rind must be removed.

In addition to natural cheeses, various other dairy and nondairy ingredients are used in the production of processed cheese products, as shown in Table 2. The list of ingredients varies from country to country and some restrictions may apply. Because the quality of the final product is influenced considerably by all the components present in the blend, the noncheese components must also fulfill certain qualitative requirements. The most frequently used dairy, but noncheese ingredients are concentrated skim milk or skim milk powder, casein, whey protein concentrates, coprecipitates, various whey products, milkfat products, and so on.

Skim milk powder promotes the creaming properties and improves the spreadability and stability of processed cheese, but, if used in quantities exceeding 12% of the total mass, it may adversely affect the consistency or may remain undissolved. However, skim milk powder may be reconstituted first, its casein precipitated by citric acid or proteolytic enzymes and the resulting curd added to the blend (2,5). Discoloration of processed cheese due to the Maillard reactions is excluded if total lactose content is not over 6% in the final product. Skim milk powder could also be used in processed cheese manufacture by recombination and native cheese production (destined for processing). Such a procedure was developed and published in Iraq in 1987 (6). Fat contents of the cheeses were standardized by using corn oil. Processed cheese produced from reconstituted skim milk cheese had a crumbly structure, which was overcome by blending reconstituted skim milk cheese with corn oil prior to processing. In addition to being low in price, this product is low in cholesterol and is recommended to heart disease patients.

Milk protein coprecipitates, characterized with high emulsifying capacity, if added to the blend, increase the stability of the cheese emulsion and improve the physical characteristics of the finished product. Acting as an emulsifying agent, they even enable the reduction of the amount of emulsifying salt added. This is important particularly for dietary and special food products, where limitation of the sodium content may be desirable. Milk protein coprecipitates should not exceed 5% in processed cheeses (5).

Whey products incorporated in processed cheese blends favorably influence both nutritive and economic characteristics of the finished product. Although ordinary whey powder is the most common whey product used in processed cheeses, in concentrations ranging up to 7% in the blend (7), whey protein products with lower mineral and lactose contents are preferable because they yield processed cheeses with better flavor characteristics. However, some other whey products could be successfully used in the processed cheese blend as well, such as whey concentrate (2–4%), precipitated whey proteins (up to 25% with flavor correction), and native whey protein concentrates obtained by ultrafiltration (5–20%) (7).

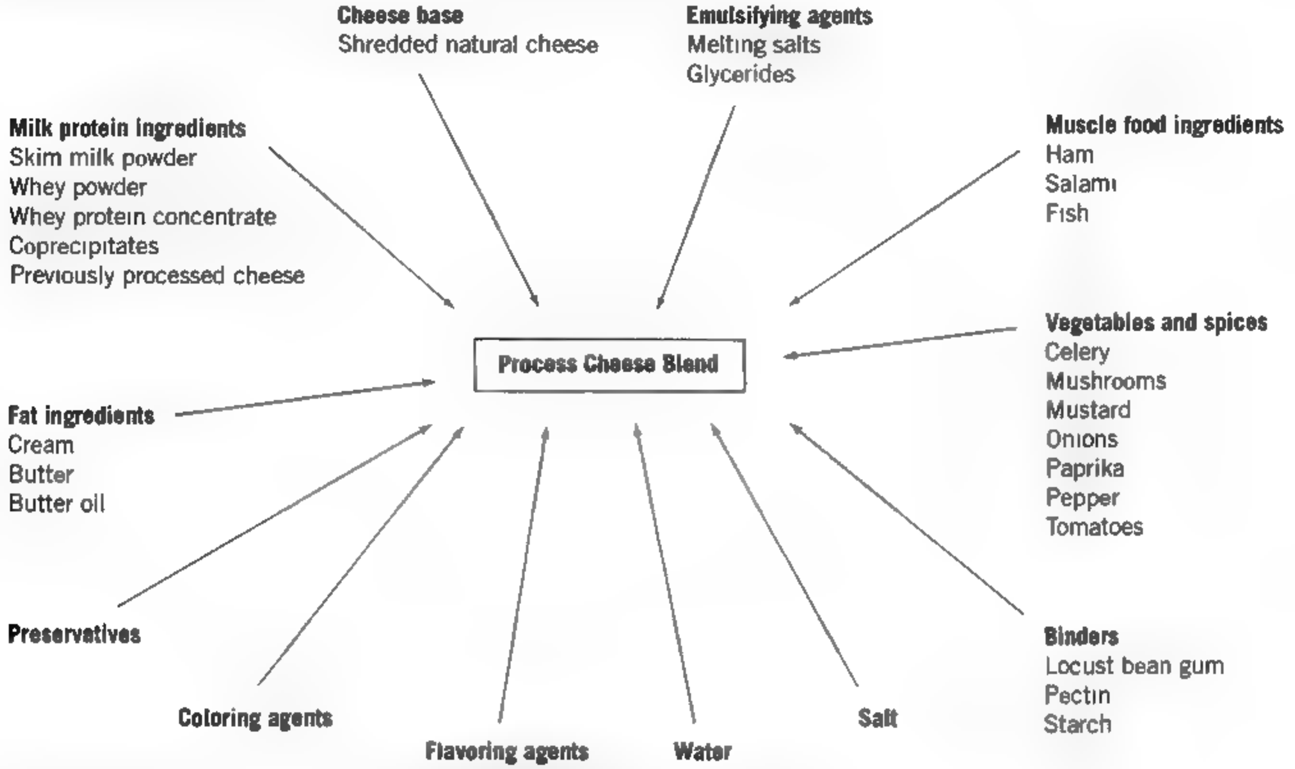
All milkfat ingredients (Table 2) used to adjust the fat content of the processed cheese to the desired level must be of high quality and free from off-flavors.

Attempts have recently been made to develop processed cheese blends with improved characteristics that can be produced at a lower cost. Egyptian authors (8) have produced processed cheese spreads with good spreadability by partially substituting calcium caseinate for natural cheese in the blend. Although full replacement, with cheese flavor added, failed to yield a spread with good characteristics, partial replacement improved spreadability. The best results were obtained using a blend composed of 6 to 8% skim milk powder, 5 to 7% calcium caseinate, 15% mature Cheddar cheese, 14% butter oil, and 3% emulsifying agent.

Numerous procedures have been developed for producing economically favorable cheese or cheese bases intended exclusively for processing. One of these processes for production of a cheese base by ultrafiltration (UF) and diafiltration of whole milk was developed and patented in Germany (9). The retentate (40% dry matter, 4.17% lactose) obtained after UF was pasteurized (high temperature–short time [HTST]), cooled to 30°C, inoculated with lactic starter, and, after 2 h, evaporated at 42°C to 62% dry matter. It was further incubated at 25°C until the pH reached 5.2 and was then packaged in plastic bags under vacuum. The vacuum-packaged cheese base can be stored and later used in processed cheese production in combination with ripe cheese, in a 80:20 ratio.

A group of authors from Utah State University produced a cheese for processing from ultrafiltered whole milk adjusted to pH 5.2 to 6.6. The melting properties of the

Table 2. Ingredients Used in the Manufacture of Processed Cheese


Cheese base
Shredded natural cheese
Emulsifying agents
Melting salts
Glycerides
Milk protein ingredients
Skim milk powder
Whey powder
Whey protein concentrate
Coprecipitates
Previously processed cheese
Muscle food ingredients
Ham
Salami
Fish
Vegetables and spices
Celery
Mushrooms
Mustard
Onions
Paprika
Pepper
Tomatoes
Process Cheese Blend
Fat ingredients
Cream
Butter
Butter oil
Preservatives
Binders
Locust bean gum
Pectin
Starch
Coloring agents
Salt
Flavoring agents
Water

Source: Ref. 4, courtesy of *Food Structure and Scanning Electron Microscopy.*

product improved with decreasing pH of the milk before UF, caused by reduced calcium concentration in the retentate. Chymosin treatment of the UF retentate adversely affected the meltability of cheese produced (5).

Investigators at Cornell University (10) used ultrafiltered skim milk retentate to constitute up to 60% of the formulation base. The casein is mostly in the insoluble state; thus, the retentates cannot be used alone for processing. However, cheese containing up to 60% of retentate solids (treated with fungal protease and lipase preparations at 45°C, 24 h) had better sensory attributes than control cheese; a combination of sodium citrate (2.7%) and citric acid (0.3%) was the best emulsifier for retentate-containing cheese. It has been shown by calculation that the UF application in preparing raw material for a processed cheese blend pays off the investment costs after only two years of operation (11).

A kind of curd product, called Schmelzpack, is manufactured in Germany especially for processed cheese production. Containing 90 to 100% unhydrolyzed casein (original casein from milk curd), it can be blended with natural cheeses of diverse sources, types, and maturity (3,5). Good results on the use of chicken pepsin for the peptonization of curd for incorporation into processed cheese have also been reported (5).

Some more recent investigations (12) showed the possibility of successful processed cheese manufacture from Cheddar cheese and skim milk powder cheese base. Cheese base was produced from reconstituted skim milk powder after UF. In one experiment cheese base was subjected to accelerated ripening by adding commercial proteolytic enzyme. All cheeses were of a good quality, except cheeses containing a large proportion of cheese base (more than 40%). The microstructure of all finished processed cheeses stored at 10°C were similar to one another.

Enzymic methods for accelerating the ripening of natural cheeses are developed for commercial use and have been reviewed in detail (13). In general, the need to accelerate cheese ripening orignates from the desire to decrease storage costs during ripening in cheese manufacture. Currently, there are three methods of proteinase addition in milk: direct blending with milk, encapsulated enzyme added to milk, and direct application to cheese curd. However, the investigations on accelerated cheese ripening are still being carried out and there is still the quality problem of maintaining the natural flavor balance and cheese texture. The importance of this problem diminishes if the cheese is manufactured exclusively for processing. For example, in Cheddar cheese manufacture, it was possible to reduce the duration of ripening from a few months to two to three weeks by proteinase addition (5). Pasteurized milk was coagulated at 36°C by chymosin and high quantities of starter culture (up to 4%). After cutting, cooking, and whey separation, the curd (at pH 5.1–5.3) was milled, mixed with an emulsifying agent to form a plastic mass, poured into containers, paraffined on top, and ripened for only a few weeks.

Authors from India (14) developed a processed cheese spread using accelerated-ripened Cheddar curd slurries.

The effect of pH; agitation; and the addition of reduced glutathione, cobalt, manganese, riboflavin, and diacetyl on flavor development in the slurries, as well as in processed cheese spread were investigated. Agitation and addition of glutathione had a positive effect on flavor development, whereas blending the slurries with 25% solids from fully ripened Cheddar cheese improved flavor, body, and texture of the finished product.

The relatively new possibility of achieving the wanted flavor in processed cheese is the addition of so-called modified cheese flavors (enzymatically modified cheese [EMC]). EMC are produced by inoculation and incubation of particular enzymes with specially designed media, followed by their heat inactivation. Flavor intensity of EMC is 10 to 30 times greater than the corresponding traditional cheese. EMC recently found application in various food products (confectionery, snack food, soups, sauces, etc). One study successfully substituted 15% ripe cheese in a processed cheese blend with a mixture of young cheese and whey powder by using Cheddar or Parmesan cheese flavor (7). Headspace analysis showed that experimental processed cheese produced with EMC, which had less ripened cheese in the blend, resulted in higher total aroma content than control samples. Thus, EMC application in processed cheese blends would be economically reasonable, resulting in a product of excellent quality. Egyptian authors (15) proved that the addition of modified Cheddar and Swiss cheese flavor affected the fatty acid composition of the produced processed cheeses. EMC caused the increase of short-chain fatty acid level, whereas Cheddar cheese flavor increased palmitic acid content as well.

A patent for chemical hydrolysis of cheese blends was granted in 1983 in the USSR. The trimmings were washed with hot water, centrifuged, comminuted, and hydrolyzed with 1.4 to 1.6 M HCl at 110 to 116°C for 3 to 5 h. A hydrolysate (about 20% TS with about 80% peptides in dry matter and 20% essential amino acids of total) was successfully incorporated in the blend, up to a maximum of 15% depending on the type and maturity of the natural cheese used (5).

As early as 1956, a rapid procedure for the production of raw material for processing based on curd acidification was patented in the United States (16). Milk was coagulated; the curd was mixed with lactic acid (0 2–1.5% acidity) and heated, with agitation, to 43°C for 6 to 40 min. The lactic acid solution was drained from the curd, which was washed with water, salted, and pressed. After two days' storage at 5°C, it was used in processed cheese manufacture. The same idea was explored in a recent French patent (17) in which the procedure for producing sliceable processed cheese of acidified milk or concentrated milk is described. The resulting curd is mixed with emulsifying agents, fat, and texture-modifying polysaccharides and further processed.

In addition to all the mentioned dairy-based products that could be included in the blend for processing, precooked cheese (also called *rework*) is an important blend component. Rework, or precooked, cheese is formerly processed cheese, or often it is the processed cheese from the previous charge. It is used to improve the texture and stability of the finished product, especially when very young or very mature raw cheese is used for processing. The percentage used in the blend varies between 1 and 30%, depending on the quality of the rework and the type of processed cheese wanted. Usually precooked cheese is used in the manufacture of processed cheese spreads to increase the creaming properties of the blend. Some dairies produce precooked cheese, which is stored in large containers. The presence of rework in processed cheese food has been detected by using electron microscopy (18).

The nondairy components of cheese blends can include spices, meat products, vegetables, and other ingredients. They all must be sterile and of the highest quality, with typical flavor. Their quantities must be properly prescribed for blending. The possible nondairy ingredients used in processed cheeses are shown in Table 2. Some attempts have been made to incorporate cottonseed flour (19) and dried vegetables as well (20).

Recent work from Alexandria University in Egypt (21) describes the texture and sensory characteristics of processed cheese made with palm oil (instead of butter) and Solva Complete B (instead of skim milk powder). The results were not encouraging.

Walnut is an attractive processed cheese additive; either shredded or cut in half, the quantity varies in the range of 8 to 10% (22,23). Additional sweet nondairy ingredients in processed cheese include fruit syrup, cocoa, honey, vanilla, hazelnut, and coffee extract (24).

Shredding (Grinding, Milling)

Shredding enables the emulsifying agent to come into close contact with the blend components during processing.

Addition of Emulsifying Agents

Addition of emulsifying agents is the last step in preparing the blend for processing. The quality of emulsifying agent added into the processed cheese blend depends on the type and age of cheese used in the blend (proportion of water and calcium) but is also determined by the final product group, which determines the sort of emulsifying agent as well. The amount of emulsifying salts that can be added to the cheese base are regulated by many countries and usually do not exceed 3 or 4%. Since the effects of emulsifying agents are responsible for the unique features of processed cheese production, their type and role will be treated in a separate section.

Processing

After all preparation treatments, the shredded, minced, and weighted raw material is transported to the cooker, where, by interaction with an emulsifying agent and water, processing is performed. Processing involves heat treatment of the blend with direct and/or indirect steam under partial vacuum. The product is constantly agitated through a continuous or batch method. If processing is carried out discontinuously, (ie, in a kettle), the temperature can reach 71 to 95°C for a period of 4 to 15 min (Table 1), depending on various parameters (3); this heating also provides pasteurization. In newly developed cookers it is also possible to reach the temperatures up to 140°C. A kettle, or cooker (Fig. 2), consists of two double-jacketed, round, stainless steel pans of various sizes (2 to 100 L), fitted with corresponding lids, three-stage switchable stirring equipment, and fittings for direct steam injection and vacuum draw. Double jackets enable indirect steam heating as well. There are specially designed units, similar to cutters used in meat processing, where cutting is completed prior to processing by the aid of rapidly rotating knives with simultaneous heating and homogenization of the product. In addition to this most common round design, the newer, horizontal, tube-shaped processing unit is also popular, particularly in the United States and Canada. This installation is fitted with one or two mixing arms (up to 4 m long); it is fed at one end and the final product is discharged at the opposite end, within 4 to 6 min. This type of unit is constructed for the batch operation, but it can be continuous and can process large capacities. A new programmed jacketed processor has been successfully developed (5), which is used to grind, mix, and process natural cheeses with other blend components, water, and emulsifiers using steam injection and vacuum, at 75°C for 5 min. The processed blend is discharged by either tilting the processor or by aseptic pumping to a packaging machine. This programmed batch processor acts via a punch card for blend formulation and cleaning in place.

By continuous processing, the blend is sterilized at temperatures of 130 to 145°C for 2 to 3 s in a battery of stainless steel tubes (1). Although continuous cheese cookers were developed as early as 1920, they are not commonly used in the processed cheese industry. The main reason is the great versatility in processed cheese products; therefore, changing over to a different product in continuous process, which requires intermediate cleaning, cannot be considered economical. A German patent describes a continuous process for simultaneous melting, homogenization, and sterilization in processed cheese production without application of pressure (25). A Japanese patent (26) describes a new method for the postprocessing heat treatment (to 100°C) of packed processed cheese, produced in the usual way.

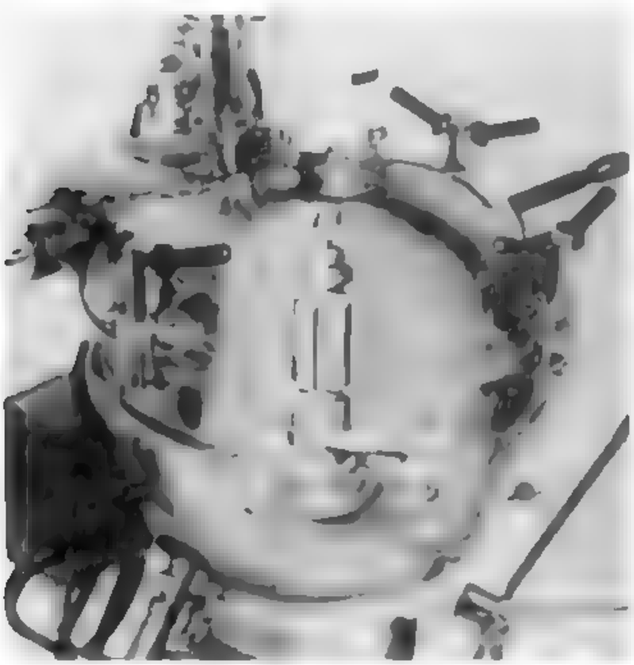


Figure 2. Universal machine for processed cheese production with cross section. *Source:* A. Stephan & Sons, Hameln, Germany.

A group of French authors (27) recently investigated the effects of blend variants and process conditions on protein–lipid interactions made by batch or extrusion cooker methods. Added emulsifying agents or premelted cheese mix increased lipid binding. Final cooling with slow mixing increased lipid binding in the extrusion cooker but not in the batch method. Proteolysis was greater in extruded than in the batch samples.

Regardless of the kind of facilities and technique of processing used, the emulsification of milkfat takes place. Transmission electron microscopy was used to observe the emulsification process (Fig. 3) (28).

The main chemical, mechanical, and thermal parameters in the cheese processing procedure are listed in Table 3. As evident from Table 3, the most important working conditions, which affect the processing and thus the quality of the final product, are as follows:

1. Temperature (heat induced by direct or indirect steam).
2. Duration of processing (depending on size and construction of the cooker, quality of raw material and blend composition, mechanical treatment, emulsifying agent used, desired keeping quality, etc).
3. Agitation (slow, at lowest speed of 60–90 rpm when producing processed cheese block, or fast, at 120–150 rpm for processed cheese spreads).
4. Acidity (pH) (a rather limited pH range; the increase of pH value, decrease of H+, causes better peptization of casein but can spoil keeping quality and flavor, whereas a decrease in pH value introduces

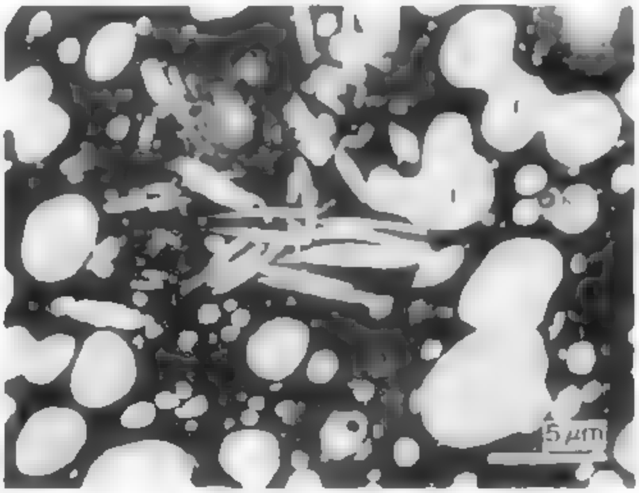


Figure 3. Transmission electron microscopy (TEM) of milkfat emulsification during cheese processing: f—fat; m—protein matrix; c—crystalline sodium citrate; p—calcium phosphate crystals; b—bacterium. *Source:* Ref. 28.

Table 3. Chemical, Mechanical, and Thermal Forces as Regulating Factors in the Cheese Processing Procedure

Process conditions	Firm slicing processed cheese, block cheese	Spreadable processed cheese, processed cheese spread
Raw material		
Average age	Young to medium ripe, predominantly young	Combination of young, medium ripe, overripe
Relative casein content	75–90%	60–75%
Structure	Predominantly long	Short to long
Emulsifying salt	Structure building not creaming, eg, high molecular polyphosphate C, SE, S7, PZ also citrate	Creaming, eg, lower and medium molecular polyphosphate, S9, S9 special, S10, S90
Water	10–25%	20–45%
Addition of water	All at once	In portions
Temperature	80–85°C	85–98°C (−150°C)
Duration of processing	4–8 min	8–15 min
pH	5.4–5.6	5.7–5.9
Agitation	Slow	Rapid
Precooked cheese	0–2.0%	5–20%
Milk powder or whey powder	0	5–10%
Homogenizing	None	Advantageous
Filling	5–15 min	10–30 min
Cooling	Slowly (10–20 h) at room temperature	Rapidly (15–30 min) in cool air
Treatment	Very light and carefully	Intensive treatment

Source: Ref. 3, courtesy of Food Trade Press.

thickening and solidifying of cheese structure). Higher pH values also favor more rapid product deterioration, in the event of postpasteurization contamination.

Homogenization (Optional)

Homogenization improves the stability of the fat emulsion by decreasing the average fat globule size. It also improves the consistency, structure, and appearance of the processed cheese. The flavor intensity is, however, reduced. If certain additives or spices are to retain their original form, they must be mixed into the product after homogenization. Because homogenization involves unnecessary additional capital, operational, and maintenance costs, and prolongs the production schedule, it is recommended only for blends with high fat contents. It is important to take care disinfecting the homogenizer, which could otherwise cause the reinfection of the final product.

Packaging

Processed cheese is usually packed and wrapped in laminated foil; in cardboard or plastic cartons; in tubes, cups, cans, and plastic containers; in sausage form; and occasionally in glass jars. Processed cheese, in sausage form, can be subjected to a smoking. A relatively new development is continuous slicing (Fig. 4) and packing of the cheese slices, suitable for sandwiches. Slices may also be obtained by mechanically slicing of rectangular processed cheese blocks. The common appearance of processed cheese in the European market is in a triangular portion (20–30 g), wrapped in heat-sealed laminated aluminum foil and assembled in round cardboard cartons. However, these packagings are quite rare in North America, where huge quantities of processed cheese block are packed in plastic foil, in the form of slices, for fast-food restaurants.

To avoid recontamination, finished processed cheese is transferred by means of stainless steel pipes to feed filling machines.

Cooling

The intensity and method of cooling is highly influenced by the type of processed cheese. Cooling of processed cheese spreads should be as fast as possible, while processed cheese blocks are cooled slowly, some time after production (rapid cooling softens the product). Cooling stops the creaming action by processed cheese spread, thus retaining creamy consistency and short structure. However, slow cooling can intensify Maillard reactions and promote the growth of spore-forming bacteria (29,30).

Storage

Processed cheese should be stored at temperatures in the range of 5 to 10°C, although such low temperatures may induce formation of calcium diphosphate–calcium pyrophosphate crystals. These crystals usually occur on the surface of product and can produce a gritty texture, but they are not harmful to the consumer.

EMULSIFYING AGENTS

Emulsifying agents (melting salts) are of major importance in processed cheese production. They provide a uniform structure during the melting process and affect the chemical, physical, and microbial quality of the product. Emulsifying agents are not emulsifiers in true chemical sense; that is, they are not surface-active compounds, although they help in emulsifying fat and stabilizing the emulsion. True emulsifiers like mono- and diglycerides may be included in commercially produced emulsifying agents, which are mixtures of selected compounds. The most com-

Figure 4. Continuous slicing machine, with two large cooling band units, to produce two different types of processed cheese. *Source:* Ref. 3, Sandvik Steel Inc, Fair Lawn, N.J. Courtesy of Food Trade Press.

mon components of the commercial salt mixtures are phosphates, polyphosphates, and citrates (Table 4), although compounds such as sodium potassium tartrate, complex sodium aluminum phosphate, sodium potassium tartrate, trihydroxyglutaric acid, and diglycolic acid could be used as well. The mixtures are of a constant and guaranteed quality; their composition is a secret and protected by the producer. The world's best-known commercial emulsifying agents producers both came from Germany: Benckiser-Knapsack, GmbH (Joha salts) and Giulini Chemie, and GmbH (Solva salts), which recently fused together in a single company entitled B. K. Giulini Chemie, in Ladenburg. Smaller producers come from a few other countries, such as Yugoslavia (KSS emulsifying salts, produced by Koteksprodukt) and the Czechoslovakia Republic (CI-FO emulsifying salts). However, some huge food companies and processed cheese plants, like Kraft in the United States and Canada Packers in Canada, design and use their own emulsifying agents.

The essential role of the emulsifying agents in the manufacture of processed cheese is to supplement the emulsifying capability of cheese proteins and can be summarized as follows (4,5):

1. Removing calcium from protein system by sequestering
2. Peptizing, solubilizing, and dispersing the proteins
3. Hydrating and swelling the proteins
4. Emulsifying the fat and stabilizing the emulsion
5. Controlling pH and stabilizing it
6. Forming an appropriate structure after cooling

The most important function of emulsifying agents is the ability to sequester calcium. Casein in cheese may be viewed as a molecule with a nonpolar, lipophilic end, whereas the other end, which contains calcium phosphate, is hydrophilic. Because of this structure, casein molecules function as emulsifiers (31). The solubility of casein in water, and hence its emulsifying capacity, are increased by reducing the calcium phosphate content. Calcium in the calcium paracaseinate complex of natural cheese is removed by the ion-exchange properties of melting salts, thus solubilizing the paracaseinate usually as sodium caseinate. Chemically, cheese processing could be observed as presented in Figure 5.

During processing, when higher temperatures are applied further polypeptide bonds are broken. Polyvalent anions of the emulsifying agents (eg, small ions of sodium diphosphate) attach themselves to the modified proteins, increasing their hydrophilic character. Protein molecules become larger, adsorb additional water, and thus increase the viscosity of the colloidal mass. This phenomenon is known as *creaming*. The affinity, that is, sequestering ability, of common emulsifiers for calcium increases in the following order: NaH_2PO_4, Na_2HPO_4, $Na_2H_2P_2O_7$, $Na_3HP_2O_7$, $Na_4P_2O_7$, $Na_5P_3O_{10}$ (5). The affinity of protein

Table 4. Emulsifying Salts Used in the Processing of Cheese

Group	Emulsifying salt	Formula	Molecular mass	P_2O_5 content, percent	Solubility at 20°C, percent	pH Value (1% solution)
Citrates	Trisodium citrate	$2Na_3C_6H_5O_7 \cdot 11H_2O$	714.31		High	6.23–6.26
Orthophosphates	Monosodium phosphate	$NaH_2PO_4 \cdot 2H_2O$	156.01	59.15	40	4.0–4.2
	Disodium phosphate	$Na_2HPO_4 \cdot 12H_2O$	358.14	19.80	18	8.9–9.1
Pyrophosphates	Disodium pyrophosphate	$Na_2H_2P_2O_7$	221.94	63.96	10.7	4.0–4.5
	Trisodium pyrophosphate	$Na_3HP_2O_7 \cdot 9H_2O$	406.06	34.95	32	6.7–7.5
	Tetrasodium pyrophosphate	$Na_4P_2O_7 \cdot 10H_2O$	446.05	31.82	10–12	10.2–10.4
Polyphosphates	Pentasodium tripolyphosphate	$Na_5P_3O_{10}$		57.88	14–15	9.3–9.3
	Sodium tetrapolyphosphate	$Na_6P_4O_{13}$		60.42	14–15	9.0–9.5
	Sodium hexametaphosphate (Graham's salt)	$(NaPO_3)_2$		69.60	Infinite	6.0–7.5

Source: Ref. 4, courtesy of Food Structure and Scanning Electron Microscopy.

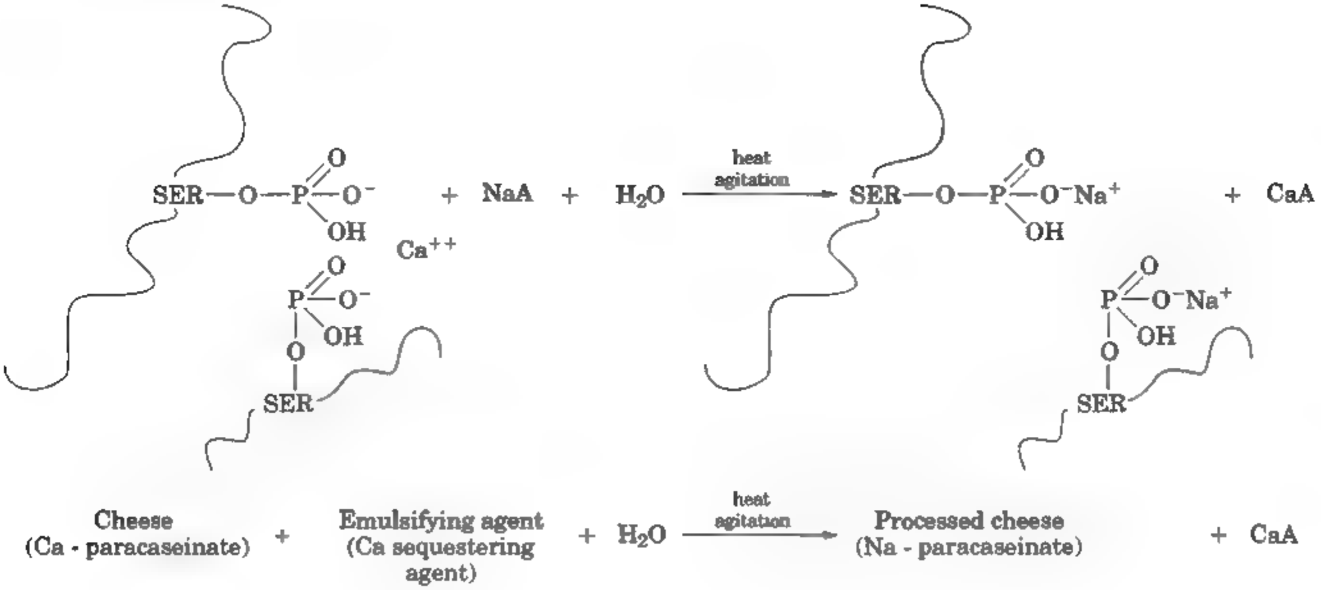

SER—O—P(=O)(OH)—O⁻
+ NaA + H₂O
heat
agitation
SER—O—P(=O)(OH)—O⁻Na⁺
+ CaA
Ca⁺⁺
SER
Cheese (Ca - paracaseinate)
+
Emulsifying agent (Ca sequestering agent)
+ H₂O
heat
agitation
Processed cheese (Na - paracaseinate)
+ CaA

Figure 5. Chemical reaction by cheese processing

for the cations and anions of melting salts is determined by the valency of these ions (4,5).

The main types and roles of emulsifying agents and their characteristics and possible combinations are discussed in detail elsewhere (3,5,32,33).

Generally, the application of polyphosphates yields processed cheese with superior structure and better keeping quality than other emulsifying agents. This may be due to their ability to solubilize calcium paracaseinate and sequester calcium. Pyrophosphates and, in particular, orthophosphates contribute undesirable sensory attributes to the processed cheese. Trisodiumcitrate appears to be as efficient an emulsifier as polyphosphates but lacks the bacteriostatic effect possessed by the polyphosphates.

QUALITY DEFECTS OF PROCESSED CHEESE

A high-quality processed cheese should have a smooth, homogeneous structure; shiny surfaces; uniform color; and no gas holes due to fermentation. However, there are numerous possible defects, of physicochemical or microbial origin, caused either by (*1*) unsuitable blend, or (*2*) inadequate processing.

An unsuitable blend comprises poor-quality or contaminated natural cheese; a bad relationship of blend components; poor-quality proteins; an improper protein-to-fat ratio; irregular quality or quantity of emulsifying agent; and incorrect values for pH, moisture content, and quantity of reworked cheese.

Inadequate processing means unsuitable time-temperature regimes, inadequate agitation, improper cooling, or unsuitable storage. Fortunately, most of the problems in processed cheese technology, when once properly detected, can be corrected. So the first task is to identify the cause responsible for the defect. As soon as the cause is determined and eliminated, the defect showed disappears in further processing.

The most common quality defects of physicochemical and microbial origins in processed cheese, their causes, and suggestions for their correction are presented in Table 5. A number of the cited defects originate in the natural cheese used in the blend, some of which can be avoided by proper processing. Processed cheese with certain minor defects can be recovered by reworking small quantities of it into subsequent batches. More serious defects cannot be corrected and render the product unsuitable for human consumption (eg, microbial changes, *Clostridium botulinum* toxin, presence of metal ions, and excessive Maillard browning).

Crystal formation, sometimes visible, is a serious defect in processed cheese. Most often, the reason for crystal formation is a low solubility of the emulsifying agent used. The situation is accentuated with excessive amounts of emulsifying agent, high calcium content in the natural cheese, high pH of the finished product, and storage of the processed cheese at low temperatures. Some emulsifying agents react with calcium in the cheese, producing insoluble calcium salts. Using sophisticated instrumental methods, such as electron microscopy, energy dispersive spectrometric analysis, and Debye-Scherrer X-ray analysis, crystals in processed cheese have been characterized. They have been chemically identified as calcium phosphate, calcium citrate, sodium calcium citrate, and disodium phosphate (3–5,34–36). Apparently, the most frequently formed crystals are calcium phosphate. During processing, calcium from the natural cheese reacts with phosphates of the emulsifying agents, thus resulting in insoluble calcium phosphate crystals (1). The growth of calcium phosphate crystals in processed cheese, when sodium diphosphate is used as an emulsifying agent, has been shown (28). In Figure 6, calcium diphosphate crystals are visualized (a) in processed cheese, (b) as isolated from cheese, and (c) as a chemically pure form (spray dried). Sheetlike citrate

Table 5. Most Common Quality Defects in Processed Cheese

Defect	Cause	Correction
	Flavor	
Moldy	Air contamination, moldy raw cheese	Use hermetically sealed foils, eliminate all moldy cheeses from the blend
Acid	Excess phosphates	Reduce emulsifier
Salty	Salty raw cheese or other components, too much emulsifier	Add young, unsalted cheese or fresh curd to blend, decrease the quantity of emulsifier
Soapy	High pH value (>6.2)	Add younger cheese (with lower pH value), use emulsifying agent with lower pH value
Burned with browning	Maillard reaction (lactose and amino acids); usually when very young cheese or whey products are present	Use processing temperatures <90°C, cool processed cheese immediately after packaging, avoid large containers, store <30°C, avoid high pH values in final product
	Texture (body, consistency)	
Too soft	High moisture, improper emulsifier, insufficient emulsifier, high pH, fast cooling, excess ripe cheese in blend, prolonged processing, slow agitation	Reduce water content, use suitable emulsifier, increase emulsifier content, decrease pH, slow down cooling, increase proportion of young cheese in blend, reduce processing time, increase agitation speed
Too hard	Low moisture, improper or excess emulsifier, low pH, slow cooling, improper blend, excess creamed or overcreamed, reworked cheese	Increase water content, use proper emulsifier, decrease emulsifier content, increase pH, speed up cooling, change blend composition, avoid addition of creamed or overcreamed reworked cheese
Hard, with water separation	Colloidal change in cheese structure (overcreaming), bacteriological action leading to reduced pH	Remove all factors that affect excess creaming, choose blend components carefully, keep processing temperatures >85°C
Inhomogeneous (grainy)	Unsuitable blend, improper emulsifier, insufficient or excess emulsifier, low pH, short processing time, low processing temperature, improper amount of added water, inadequate agitation, colloidal or bacteriological changes caused by improper storage	Add younger cheese, use suitable emulsifier, correct emulsifier quantity, correct pH, prolong processing time, increase processing temperature >85°C, increase the ammount of added water, continue agitation during processing and filling; proper cold storage
Sticky (adhering to lid foil)	Sticky foil, insufficiently impregnated, excessively high pH, processed mass left hot too long without agitation	Change aluminum foil, decrease water addition and add in two portions, increase proportion of ripe cheese or cause better creaming, keep pH <6.0, continue agitation until packaging
	Appearance	
Holes (blown)	Bacteriological changes (growth of *Clostridia*, coliform or propionic bacteria); physical changes (occluded air, CO_2 from emulsifier mixture (citrates), holes filled up with fluid from emulsifying agent having low solubility); chemical changes (hydrogen from reaction between processed cheese and aluminum foil)	Select cheese blend components carefully, keep processing temperatures >95°C, use proper vacuum, preheat citrate emulsifier before processing, extend processing time, test porosity of aluminum foil and if necessary change it
Crystals	Calcium diphosphate and calcium monophosphate crystals (when phosphates are used in emulsifying agent), calcium crystals (when citrates are used in emulsifying agent), crystals due to undissolved emulsifying salt, large crystals due to excess emulsifier, lactose crystal formation, caused by excess whey concentrates or low water content, light coloured, grainy precipitate of tyrosine (very mature cheese in blend)	Avoid monophosphates and diphosphates as emulsifying agents, or combinations with higher phosphates and polyphosphates; exclude citrates from emulsifying agent; exclude sandy reworked cheese from blend, distribute emulsifying agent better; increase processing time; add emulsifying agent in solution; use prescribed quantity of emulsifier, reduce level of whey products, and increase water content, exclude raw cheese that contains tyrosine crystals

Source: Refs. 1–3, and 5.

crystals are shown in commercial processed Gruyère cheese in Fig. 7. Quite recently crystalline monoclinic calcium pyrophosphate dihydrate was also found in processed cheese when emulsifying agents containing pyro- and polyphosphates were used (37). Authors assume that this crystalline product either comes from migration in the protein matrix of calcium ions and pyrophosphate anions, which are either directly introduced, or results from the hydrolysis of the polyphosphates. In addition to the emulsifying agents, lactose and free tyrosine may de-

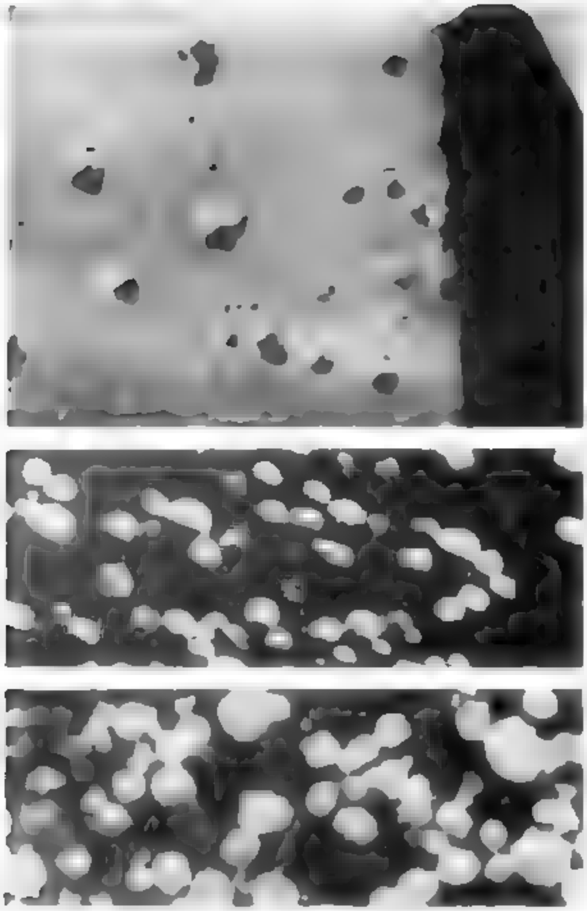


Figure 6. Calcium diphosphate crystals in (a) Gruyère processed cheese, (b) the isolated above diphosphate globular clumps, (c) chemically pure crystals (spray dried) (APV Anhydro A/S) *Source:* Ref. 3, courtesy of Food Trade Press.

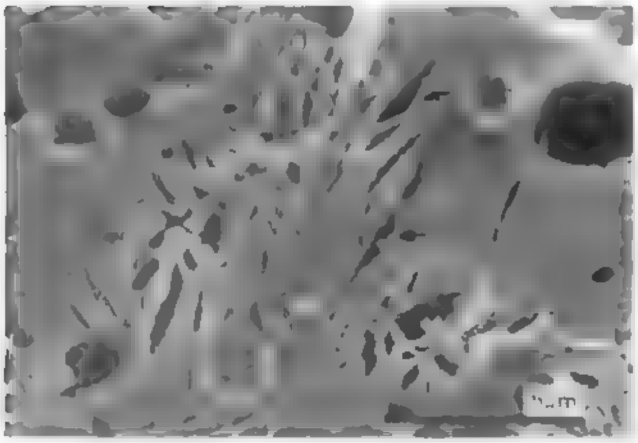


Figure 7. Imprints of sodium citrate crystals (arrows) in processed cheese. *Source:* Ref. 4, courtesy of Food Structure and Scanning Electron Microscopy.

velop crystals in processed cheese, if present at excessively high concentrations (1,2,5).

Discoloration or browning is a defect in processed cheese caused by the Maillard reactions (nonenzymic browning), when the product develops a dark brown or pink color. The exact mechanism and interrelations during all stages of Maillard reactions have been discussed in detail (30,38). Maillard reactions commence at elevated temperatures and continue autocatalytically. Because the main reactants in Maillard browning are amino acids and reducing sugars, the products most susceptible to these changes are blends containing high levels of young cheese; that is, high lactose concentration and other lactose-containing ingredients (particularly whey powder). Browning is more prevalent in processed cheese spreads because of higher processing temperatures, longer processing times, higher water and lactose contents, and higher pH. It has been shown that the intensity of the browning reaction can be reduced by using a galactose-fermenting strain of *Streptococcus salivarius* subsp. *thermophilus*, together with a mesophilic lactic starter culture, in curd production (39). A high NaCl content had the opposite effect, possibly by suppressing the activity of the lactic acid starter culture. Processed cheese that contains a high level of reworked cheese is exposed to more severe thermal treatment than usual and, consequently, the Maillard reactions are accentuated. Most noticeable are the levels of melanoidins, the main products of Maillard reactions, in sterilized processed cheese, even if cooled immediately after production, packed, and stored at low temperature (30).

Microbial defects in processed cheese are caused by spore-forming bacteria, which usually originate in the cheese milk and enter the process through the natural cheese used for blending. Other sources of microbial contamination include water supply, equipment, and additives (1–5). The normal thermal treatment during blend processing (Tables 1 and 3) does not eliminate viable spores from the product.

The spore-forming bacteria, which cause defects in processed cheese by producing gas, belong to the genera *Clostridium* (*C. butyricum*, *C. tyrobutyricum*, *C. histolyticum*, *C. sporogenes*, and *C. perfringens*) and *Bacillus* (*B. licheniformis* and *B. polymixa*). Especially hazardous is *C. botulinum*, producing a toxin that causes botulism. Whether produced continuously or in a batch cooker, processed cheese is not sterile. Germination of spores after processing is influenced by various factors; for example, blend composition, sodium chloride concentration, type and concentration of emulsifying agent, water level, pH, and the presence or absence of natural inhibitors. However, spore outgrowth can be prevented in a number of possible ways: preservatives in the cheese blend, sterilization of the processed cheese, or an increased redox potential of the blend. One of the most widely used methods of preventing spore outgrowth is the addition of preservative into the blend. There is, for example, a procedure patented in the United States where the outgrowth of *C. botulinum* spores and subsequent toxin formation is completely prevented in pasteurized processed cheese spread inoculated with 1000 spores per gram and incubated 48 weeks at 30°C, through the incorporation of nisin at the level of 250 ppm in the blend (40,41).

PROCESSED CHEESE ANALOGUES

There are two recognized groups of products not belonging to the classic range of dairy products: (1) modified dairy products, and (2) substitute (imitation or alternate) dairy products.

In modified dairy products, only one dairy component (eg, protein or fat) is substituted by a nondairy component for economic or nutritional reasons. Imitation products are based on novel components, frequently produced by newly developed technological procedures, using standard equipment but not containing the obligatory dairy component. In the United States, however, native cheeses are also labeled *imitation* if they do not meet the requirements proscribed by federal standards of identity for composition, which could result in confusion over terminology. Formulated or imitation products that closely resemble traditional dairy products are gaining in popularity. The main reason is that imitation products enable the utilization of milk components in combination with nonmilk ingredients, thus lowering production costs and resulting in the composition and nutritive characteristics as designed (42).

Substitution of one or more dairy macroconstituents in processed cheese manufacture usually does not cause any major technical problems. Processed cheese blends can easily be further enriched with desirable microcomponents (eg, vitamins and minerals). Blends can be tailored to yield less-expensive products. Essential components are casein and caseinates (Na, Ca, salts, etc), other proteins (soya, coconut, gluten, etc), suitable vegetable fats, flavorings, vitamins and minerals, food-grade acids (eg, lactic, citric) to correct the pH to 5.8 to 5.9, and emulsifying agents. A suitable blend, suggested for an imitation processed cheese product, is listed as follows (1):

Component	Quantity (%)
Water	100 00
Vegetable oil	3.50
Sodium caseinate	2 75
Dextrose or other sugar	3.80
Emulsifier	0.25
Stabilizer	0.40
Disodium phosphate	0.25
CaCl2	0.25
NaCl	0.15
Artificial color and flavor	Trace

Similar processing parameters and equipment are used as for conventional processed cheeses, processing temperature 79 to 85°C, giving a product of similar physicochemical and microbiological characteristics (1).

Numerous investigations have been carried out in the past decade to find new formulations (43–48). A method has been developed for a successful pilot plant production of a processed cheese based on dairy, noncheese components (43). Great similarity in components and in solubility, dispersibility, and colloidal stability of sodium and calcium caseinate dispersions and processed cheese have been found (44). Egyptian workers (45) included 20% Ras cheese, manufactured from recombined milk using a microbial enzyme, in a processed cheese blend containing butter oil as the lipid phase. Modification of processed cheese, by incorporating vegetable oil in the blend, improved cheese flavor and resulted in a 25 to 50% saving of butter (46). In a Russian patent, milkfat was substituted with a mixture of lard, beef fat, and sunflower oil up to 75% of the total fat content. The processed cheese obtained had clean flavor and mild aroma and was elastic with a uniform consistency (47). Various attempts have also been made to develop combined food products that contain processed cheese as a component. For example, a nutritious chocolate product, composed of 50% bitter chocolate and 50% processed cheese (emulsified, solidified, frozen, and coated with chocolate), was developed and patented in the UK (48). There are various processed cheese analogues in the U.S. market, even some with different colors and the look of a party cake.

NEW DEVELOPMENTS IN PROCESSED CHEESE PRODUCTS

In describing processed cheese analogues, constantly various new components and product modifications arise. However, new developments in classic processed cheese are targeted to obtain a product of better quality, to improve nutritive value of the product, or to widen the versatility of the production. The latest is achieved by introducing the nonconventional components in the blend or by developing a product of completely new characteristics by changing the technology. Some of these findings are still on a laboratory scale, while others are already patented and in industrial application.

In is well known that the addition of phosphates through emulsifying agents increases the phosphorus to calcium (P:Ca) ratio in the processed cheese, which is considered to be nutritionally detrimental. The P:Ca ratio has been reduced from 1.6 when sodium tripolyphosphate was used alone, to 1.1 when the same salt was used, but in combination with 1% surface active monoglyceride, which substituted half of the phosphate amount usually used (49). Another suggestion to correct the improper P:Ca ratio in processed cheese came from Japan (50). The idea of this patent is to fortify processed cheese with calcium by colloidal calcium carbonate addition.

On the other hand, there is a growing tendency to produce low-fat, low-sugar, and low-sodium food products. Consistent with this trend is the newly developed low-sodium processed cheese, manufactured with potassium-based emulsifying agent combined with δ-gluco-lactone (51) or low-fat sliced processed cheese with up to 50% less fat and no significant flavor loss, be it natural or herb-flavored processed cheese slices (41).

A recent review in processed cheese and related products (52) points to the development of a virtually fat-free processed cheese (53), virtually fat-free cheese analogues (54), as well as low-fat processed cheeses (55). The manufacture of virtually fat-free processed cheese (based on skim milk cheese, containing also skim milk powder, whey, and buttermilk powder) is covered by a U.S. patent (53).

The fat content of the finished cheese was approximately 1.67% (wt). Another U.S. patent (54) describes manufacture of a virtually fat-free cheese analogue that mimics the body, texture, and eating characteristics of ordinary processed cheese.

In the study from South Dakota State University (55), the composition and microstructure of some commercial full-fat and low-fat cheeses, including processed cheeses, have been investigated. The structure of low-fat cheeses was dominated by the protein matrix what explains the firmer texture of these cheeses. Commercial fat-reduced processed cheese spread in Germany contained 20% fat in total solids (TS), compared with classic processed cheese spread with 45% fat in TS (60% fat in TS in high-fat processed cheese) (56). The most recent U.S. patent (57) describes the processing of a fat-free high-moisture cheese sauce, based on a blend of skim milk cheese with milk protein and polyphosphate used as emulsifying agent.

However, some investigations at the University of Wisconsin have showed the advantages of particular milkfat components. It was reported that nine isomers of *cis*-9, *cis*-12-octadecadienoic acid (linoleic acid) were found in various processed cheese samples (58). The same synthetically prepared compound was effective in partial inhibition of mouse epidermal carcinogenesis.

There are numerous possibilities to enrich processed cheese with components of high nutritive value. One of these attempts conducted in Egypt (59) yielded a high-protein processed cheese in which 25, 20, 27.5, and 27.5% of nonfat solids were supplied from Cheddar cheese, whey protein concentrate, soy protein concentrate powder, and chickpea flour, respectively. It was reported that soy proteins, consumed at normal dietary levels, compare favorably with animal proteins for meeting human nutritional requirements. Chickpea seed lipids contain more polyunsaturated fatty acids, especially linoleic acid (62.44%). Blending of the selected vegetable proteins with whey proteins corrected their relative deficiency in sulfur-containing amino acids. The final product had fine consistency and high protein content and showed acceptable quality characteristics not only after production but also after two months of storage.

Excellent results were obtained in industrial-scale experiments, when isolated soy protein (Supro 710, Protein Technologies International) was included in the processed cheese blend (60). Compared with soy flour (about 56% proteins) or soy concentrate (about 72% proteins), soy protein isolate has remarkable nutritive, as well as functional, characteristics and does not adversely affect processed cheese products' flavor. The substitution rate of cheese dry matter was successful up to 15% in the blend, yielding products of high nutritive, sensory, and functional properties. Furthermore, if the flavoring agents are used in the processed cheese blend, the mentioned percentage could even be increased.

Additional new developments in processed cheese technology are predominantly targeted in the direction of obtaining processed cheese with natural cheese appearance. Some most recent findings of this type come from the well-known French cheese company Fromageries Bel and are covered by patents (61,62). Their process consists of melting a blend of cheeses, casein, skim milk, butter, and emulsifying agents; the mixture is agitated under an inert gas such as N_2, to give an expanded product, which is afterward partially defoamed, shaped, and rapidly cooled. The resulting processed cheese has an open texture similar to that of traditional cheeses with visible eyes approximately 0.5 mm in diameter.

Processed cheese, consisting mainly of a solution of dissolved proteins–peptides, has a high surface gloss, compared with natural cheese, consisting largely of undissolved suspended proteins–peptides with low surface gloss. By incorporation of milk concentrates obtained by membrane filtration, a new type of processed cheese was developed with the appearance (ie, low surface gloss) of natural cheese (63). The effect caused by undissolved suspended proteins–peptides was quantified by goniophotometric measurements. A German patent (64) prescribes the manufacture of foamed processed cheese, containing Cheddar, butter, yogurt, dried whey, acid casein, starch, emulsifying agents, and some optional flavorings, like fruits or meat products. The blend was processed in two stages, cooled, homogenized, and whipped, resulting in the completely new structure of the final product.

The U.S. processed cheese industry now produces colored, layered processed cheese tarts, nicely decorated and sweet in flavor. Several years ago the patent for continuous procedure for layered processed cheese tarts production was granted in Germany as well (65).

Processed cheese powder, convenient for application in the fast-food industry and snack foods such as popcorn, has been developed in the United States (66).

The demand for dried cheese product in the convenience foods industry is constantly increasing. For example, in Denmark the Cremo Cheese Company, MD FOODS, exports 97% of the 12,000 t annual production of cheese powder to be used as ingredients in biscuits and ready-made dishes and snacks (67).

The introduction of nonconventional components in the processed cheese blend, even the most recent attempts and findings such as modified cheese flavors, accelerated ripened cheese cheese produced of ultrafiltered or reconstituted milk, and various components other than cheese, are discussed in more detail in the section on manufacturing procedure ("Blending").

BIBLIOGRAPHY

Selected sections of this article have been adopted from Reference 5.

1. F. V. Kosikowski, *Cheese and Fermented Milk Foods*, Edward Brothers, Ann Arbor, Mich., 1982
2. M. A. Tomas, *The Processed Cheese Industry*, Department of Agriculture Bulletin D44, Sydney, New South Wales, Australia, 1977.
3. A. Meyer, *Processed Cheese Manufacture*, Food Trade Press, London, 1973.
4. M. Caric, M. Gantar, and M. Kalab, *Food Microstructure* **4**, 297–312 (1985).

5. M. Caric and M. Kalab, "Processed Cheese Products" in *Cheese: Chemistry, Physics, and Microbiology*, Vol. 2, 2nd ed., Chapman & Hall, London, 1993, pp. 467–505.
6. R. M. Saleem and K. A. Al-Banna, *Iraqi Journal of Agricultural Sciences "Zanco"* **5**, 161–168 (1987).
7. Lj. Kulic, "Modification of Composition of Cheese Blend Intended for Processing by Using Natural Cheese Flavours," M.Sc. Thesis, University of Novi Sad, Cara Lazara 1, Yugoslavia, 1989.
8. A. Gouda et al., *Egyptian Journal of Dairy Science* **13**, 115 (1985).
9. Ger. Pat. Appl. DE 32 24 364 AL (1983), J. Rubin and P. Bjerre.
10. V. K. Sood and F. V. Kosikowski, *J. Dairy Sci.* **62**, 1713–1718 (1979).
11. R. F. Madsen and P. Bjerre, *Nordeuropais Mejeri Tidsskrift* **47**, 135–139 (1981).
12. A. Y. Tamime et al., *Food Structure* **9**, 23–37 (1990).
13. B. A. Law, *Dairy Industries International* **45**, 5 (1980).
14. J. M. Saluja and S. Singh, *Journal of Food Science and Technology (India)* **26**, 29–31 (1989).
15. E. H. El-Bagoury and F. R. Helal, *Egyptian Journal of Food Science* **15**, 219–223 (1987).
16. U.S. Pat. 2,743,186 (1956), N. Kraft and P. J. Ward.
17. Fr. Pat. Appl. FR 87-15458 (87 1106) (1989), L. R. Rizzotti and B. Villaudy.
18. M. Kalab, J. Yun, and S. H. Yin, *Food Microstructure* **6**, 181–192 (1987).
19. S. A. Abou-Donia, A. F. Salam, and K. M. El-Sayed, *Indian Journal of Dairy Science* **36**, 119 (1983).
20. P. Brezani and K. Herian, *Zbornik Prac Vyskumneho Ustavu Mliekarskeho v Ziline* **8**, 173 (1984).
21. A. F. Salam, *Alexsandria Science Exchange* **9**, 167–175 (1988).
22. Brit. Pat. 1,452,253 (1976), R. Invernizzi and G. Prella.
23. Swiss Pat. 584,515 (1977), Societe des Produits Nestle SA.
24. V. A. Samodurov, *Molochnaya Promyshlennost* **51**, 17–19 (1985).
25. Ger. Pat. Appl. DE 31 24 725 A1 (1983), F. Zimmermann.
26. Jpn. Exam. Pat. JP 57 380 B2 (1982), T. Hayashi, N. Shibukawa, Y. Yoneda, K. Musashi.
27. G. Blond, E. Haury, and D. Lorient, *Science des Aliments* **8**, 325–340 (1988).
28. A. A. Rayan, M. Kalab, and C. A. Ernstrom, *Scanning Electron Microscopy* **3**, 635 (1980).
29. R. K. Robinson, *Dairy Microbiology*, Vol. 2, *Microbiology of Dairy Products*, Elsevier Applied Science, Barking, UK, 1980.
30. B. Milic, M. Caric, and B. Vujicic, *Non-Enzimatic Browning Reactions in Food Products*, Naucna knjiga, Belgrade, Yugoslavia, 1988.
31. L. A. Shimp, *Food Technol.* **39**, 63 (1985).
32. M. Caric and S. Milanovic, *Processed Cheese*, Naucna knjiga, Begrade, Yugoslavia, 1997.
33. W. Berger et al., *Processed Cheese Manufacture*, BK Ladenburg, Ladenburg, Germany, 1993.
34. M. A. Thomas et al., *J. Food Sci.* **45**, 458 (1980).
35. H. A. Morris, P. B. Manning, and R. Jenness, *J. Dairy Sci.* **52**, 900 (1969).
36. H. Klostermeyer, G. Uhlmann, and K. Merkenish, *Milchwissenschaft* **38**, 582 (1983).
37. J. F. Pommert et al., *J. Food Sci.* **53**, 1367–1369, 1447 (1988).
38. H. D. Belitz and W. Grosch, *Food Chemistry*, Springer-Verlag, Berlin, New York, 1987.
39. M. E. Bley, M. E. Johnson, and N. F. Olson, *J. Dairy Sci.* **68**, 555 (1985).
40. U.S. Pat. 4,584,199 (1986), S. L. Taylor.
41. E. J. Mann, *Dairy Industries International* **52**, 11–12 (1987).
42. M. Caric, *Concentrated and Dried Dairy Products*, VCH, New York, 1994.
43. J. R. Rosenau, *Energy Management and Membrane Technology in Food and Dairy Processing*, American Society of Agricultural Engineers, Chicago, Ill., 1983
44. O. Kirchmeier and F. X. Breit, *Milchwissenchaft* **38**, 80 (1982)
45. A. E. A. Hagrass et al., *Egyptian Journal of Food Science* **12**, 129 (1984).
46. USSR Pat. SU 971 216 A (1982), I. A. Snegireva et al.
47. USSR Pat. SU 1144 677 A (1985), V. A. Samodurov et al.
48. Brit. Pat. Appl. GR 2 113 969 A (1983), G. Vajda, L. Ravasz, B. Karacsonyi, and G. Tabajdi.
49. N. P. Zakharova, N. B. Gaurilova, and V. G. Dologoshchinova, *Trudy Uglich* **27**, 108–111, 121 (1979).
50. Jpn. Exam. Pat 5619967 (1981), Meiji Milk Products K.
51. R. C. Lindsay et al., *Proc. IDF Seminar, New Products via New Technology*, Atlanta, Ga., 1986.
52. E. J. Mann, *Dairy Industries International* **60**, 19–20 (1995).
53. U.S. Pat. 5,215,778 (1993), B. C. Davison.
54. U.S. Pat. 5,244,687 (1993), B. E. Rybinski.
55. V. V. Mistry et al., *Food Structure* **12**, 259 (1993).
56. B. K. Giulini Chemie Gmbh & Co, OHG, Ladenburg, *Technical Documentation* **6**, (1998).
57. U.S. Pat. 5,304,387 (1994), W. S. Hine.
58. Y. L. Ha, N. K. Grimm, and M. W. Pariza, *J. Agric. Food Chem.* **37**, 75–81 (1989).
59. A. A. El-Neshawy, S. M. Farahat, and H. A. Wahbah, *Food Chem.* **28**, 245–255 (1988).
60. M. Caric et al., *Mljekarstvo* **39**, 95–102 (1989).
61. Eur. Pat. Appl. EP 0 281 441 A1 (1988), J. Daurelles and J. Y. Bernard.
62. Fr. Pat. Appl. FR 2 610 794 A1 (1988), J. Daurelles and J. Y. Bernard.
63. K. H. Ney, *Alimenta* **26**, 123–124 (1987).
64. Ger. Pat. DE 33 14 551 C1 (1984), D. Bode
65. Ger. Pat. Appl. DE 37 27 660 A1 (1989), F. Zimmermann.
66. U.S. Pat. 5,227,187 (1993), J. A. Wiser, S. J. Terhune, B. E. Jr. Gilmartin, and J. A. Kintner
67. H. Mortensen, *Scandinavian Dairy Info* **8**, 24 (1994).

MARIJANA CARIC
Novi Sad University
Novi Sad, Yugoslavia

PROTEINS: AMINO ACIDS

Amino acids are fundamental to the pharmaceutical, food processing, and animal feed industries. Besides being integral to the health of humans, they are used in industry for their nutritive value, physiological metabolites, and taste.

In the food and animal feed industries, several essential amino acids, such as lysine, methionine, tryptophan, and threonine, are used in addition to L-glutamate, which accounts for more than 50% of total amino acid production. In the pharmaceutical industry, essential and nonessential amino acids are used, depending on the nutritional values desired. The demand for amino acids is expected to grow throughout the world as people seek improved lifestyles through diet.

L-Glutamate is used mainly as umami seasoning throughout the world. It was first produced in 1909 using an extraction method from the hydrolyzate of soybean with hydrochloric acid. A significant drawback to this method is that a large amount of by-products is produced, and it is necessary to deliver the solid matter as organic fertilizer and the amino acid moiety in solution as an alternative soybean source. In 1956, the fermentation method of L-glutamic acid from glucose using bacteria was reported, changing not only the production method but also the use of the amino acids themselves. The successful production of L-glutamic acid by fermentation was followed by production of other amino acids, using the same concept of regulation of the metabolic pathway of bacteria.

Methionine and glycine are produced by the chemical synthetic method. Methionine is mainly used as a feed additive in the DL-form because the D- and L-forms both have similar, nutritional values. Optical resolution is not necessary for glycine because it is achiral.

As the fermentation technology developed for L-glutamic acid production has been applied to other amino acids, the costs have become more reasonable. This has resulted in an increase in the distribution of L-glutamate, feed additives and L-lysine, and increased demand for amino acids by the pharmaceutical industry has been met.

USES OF AMINO ACIDS

Amino acids are used in animal feeds, food, parenteral and enteral nutrition, medicine, cosmetics, and raw materials for chemical industries.

Animal Feed

Although vegetable protein contains a high concentration of essential amino acids, it does not have a taste appreciated by many humans. When cooked with meat extract, the food's taste is improved, and its nutritive values for food animals are enhanced, thus providing an efficient concentration of essential amino acids that can be used to produce a tasty meat source throughout the world. The worldwide supply of free amino acids to increase the feed efficiency of feedstock has developed into a successful business in recent years.

Table 1 summarizes the limiting amino acids used as feed supplements (1). For grains such as maize, which is fed to both pigs and chickens, the first limiting amino acid is lysine, whereas in protein sources such as rapeseed meal, it is methionine, lysine, or tryptophan. There are three general methods of supplying lysine: adding soybean meal, which contains it in high quantity; adding a larger quantity of poor-quality protein such as corn gluten meal; or adding lysine directly. The first two approaches are more expensive and less economical in view of wasting nitrogen resources, so the direct addition of lysine has been adopted.

An optimal balance of amino acids is attained by adding deficient amino acids and reducing those in excess. When animals are fed corn–soybean meal (Fig. 1), about 2% of the feed protein contents can be saved without affecting the animal's growth response. One hundred kilograms of soybean meal contained in 2 tons of feed can be replaced by 97.5 kg of corn and 2.5 kg of lysine hydrochloride without lowering its nutritional value. When the price of the latter is lower than the former, the use of lysine increases.

Methionine. Fish meal and vegetable defatted meal are important protein sources of feed. The supply of fish meal has continuously decreased and cannot meet the demands for feed. Because of the decrease in fish meal for feed and the increased use of vegetable protein in vegetable oil meal, the demand for methionine has increased.

Lysine. As the demand for vegetable defatted meal has increased and the importance of amino acid balance in protein has been recognized, lysine has received increasing attention. Cereals are considered an energy source and comprise 60 to 80% of assorted feeds; they play an important role as protein sources but contain relatively small amounts of lysine. To compensate for this shortage, lysine has been used in place of fish meal.

When the ω-amino group of lysine reacts with a carbonyl group of a sugar derivative cmpound, which gives a positive Fehling reaction, a Schiff base is formed. These Schiff bases are not used as nutritional compounds. This reaction, which is accelerated by heat treatment, is usually performed to improve the protein efficiency.

Table 1. Limiting Amino Acids of Some Common Feeds for Pigs and Chickens

Ingredient	Crude protein (%)	Pig		Chicken	
		First	Second	First	Second
Maize	8.9	Lys	Trp	Lys	Trp
Sorghum	9.5	Lys	Thr	Lys	Arg
Barley	11.1	Lys		Lys	Met
Wheat	12.6	Lys	Thr	Lys	Thr
Soybean meal	46.2	Met	Thr	Met	Thr
Fish meal	64.3			Arg	
Rapeseed meal	35.3	Met		Lys	Arg
Peanut meal	47.4	Lys		Met	Lys
Sunflower seed meal	31.7	Lys		Met	Thr
Meat and bone meal	48.6	Lys		Trp	
Cottonseed meal	64.3	Lys	Thr	Lys	Met
Corn gluten meal	63.6	Lys	Trp	Lys	Trp

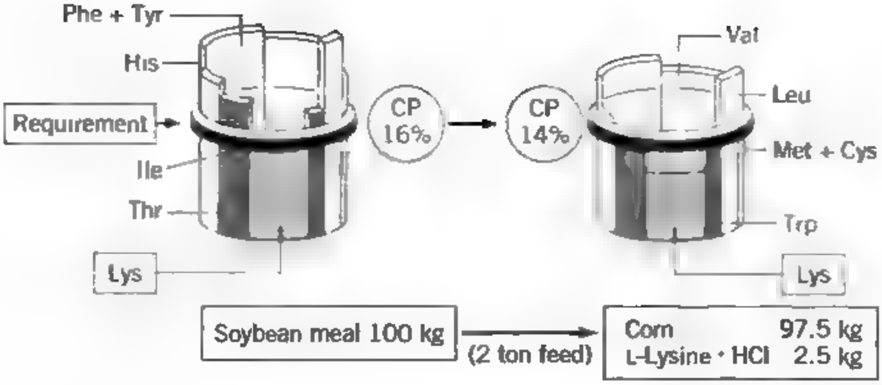


Figure 1. Balance of amino acids in feed for protein deficiency in a well-balanced diet.

When a group of animals received marginally deficient levels of lysine, the amount of weight gain was unchanged from the amount in the group receiving the required level of lysine. The lack of change in weight gain was due to the increase in fat deposition; nevertheless, protein retention decreased as the level of lysine decreased (2).

Fortification of Protein for Food

In southeast Asia, Africa, and South America, the population continues to increase, but no appropriate measures to improve the nutritional conditions in these societies have been implemented. The chronic shortage of protein, more than that of calorie intake, in these areas inhibits physical growth and mental development in children and deprives adults of the ability to perform physical labor. This makes impossible the development of a society with a sound economy. A 1957 report by the FAO to the United Nations stated that to make up the protein gap, it is necessary to provide a higher nutritional value of protein for the consumer (3). One concrete way to do this would be to fortify the amino acid content of cereal with seed protein, fish protein concentrate, and vegetable protein.

The principle of amino acid fortification is based on two factors. (*1*) Nutritionally efficient utilization of amino acids depends on the ratio of essential amino acids to all the amino acids contained in protein. (*2*) When the amino acid balance of a protein is corrected by the addition of certain amino acids, defined as limiting amino acids, significant improvements in the nutritional value of the protein are observed. The effects of fortification with limiting amino acids on the protein efficiency ratio (PER) for various cereals are summarized in Table 2 (4,5). The PER is defined as the ratio of the incremental weight gain in rats to the total amount of protein fed in certain intervals. The PER value for cereals such as rice, corn, barley, and wheat flour is approximately 1.0, which is lower than that of 2.5 for casein. The limiting amino acid for all cereals is lysine, in addition to threonine for rice, wheat, and barley flours and tryptophan for corn. When these limiting amino acids are added to cereals, the PERs for corresponding cereals are nearly the same as casein.

The limiting amino acid for soybean is methionine; for cotton seed, lysine and threonine; for sesame, lysine; and for peanuts, lysine, threonine, and methionine.

Table 2. The Effect of Adding Limiting Amino Acids on the Improvement of Protein Efficiency Ratio of Cereal

	Limiting amino acid added (%)			
Protein	L-Lysine HCl	D,L-Threonine	D,L-Tryptophan	Protein efficency ratio
Rice				1.50
	0.2	0.2	—	2.60
Wheat				0.65
	0.2	—	—	1.56
	0.4	0.3		2.67
Corn				0.85
	0.4	—	—	1.08
	0.4	—	0.07	2.55
Barley				1.66
	0.2	0.2	—	2.28
Kaoliang				0.69
	0.2	—	—	1.77

The idea of synthetic foods or chemical-defined foods composed of amino acids and other appropriate nutrients was investigated in the 1950s and applied to so-called space diets (6). The crystalline digestive diet, which contained crystalline amino acids as the nitrogen source as recommended by Greenstein, was the prototype of the space diet. It was investigated further in the U.S. space program, and from this the term *elemental diet* came into common use. When about 400 g of this synthetic food was administered to male adults for about half a year, no changes in pathophysiological or mental conditions were observed, except an extreme decrease in the amount of excretion, because no indigestible substances were contained in the synthetic food, and a decrease in several probiotic bacteria in the intestine (7,8).

Some infants exhibit lactose or cow's milk protein incompatibility. The formulas marketed for this condition often are based on isolated soybean protein and are supplemented with L-methionine to increase the nutritional value (9).

Umami Tastes

The specific tastes of foods are intimately related with their constituents, such as amino acids and peptides. The proteins contain about 20 kinds of amino acids, which are found in free form in dietary foods. As shown in Table 3, which summarizes the kinds of amino acids found in common foods, the differences in the amino acid patterns for each food are said to be key factors in their distinct tastes; the variety of tastes depends on the changes in the free amino acid components.

In urchin, amino acids such as glutamic acid, glycine, and alanine (and not so much methionine) contribute to specific sweet tastes. Green tea contains mainly glutamic acid and theanine; these two compounds are related to the umami tastes. These 20 or so amino acids found in food play an essential role in the harmonized mixed tastes of foods. The threshold value, which is defined as the limiting concentration distinguished from the intensity of specific taste, and the taste quality for various amino acids are summarized in Table 4 (10). L-Aspartic acid shows less intensity of umami taste than does L-glutamic acid; however, the sodium salt (monosodium aspartate), found in soybeans, enhances the umami tastes of soybeans usually associated with L-glutamate.

The specific taste of each amino acid generally remains unchanged when its concentration is changed. However, the specific tastes of L-alanine, L-arginine, L-glutamic acid, L-serine, and L-threonine, as shown in Table 5, change when their concentrations change (11,12).

Relationship between Umami Taste and Chemical Formula. The relationship between umami taste and the chemical structure of L-glutamate (Fig. 2) was investigated by Akabori and Kaneko (13–15). The threshold values for L-glutamate-related compounds for umami taste were as follows: 0.03% for L-glutamate, 0.16% for L-aspartate, 0.25% for sodium DL-α-amino adipic acid, 0.03% for sodium DL-threo-β-oxylglutamic acid, and 0.015% for sodium L-homocysteic acid.

Sodium L-aspartic acid has one less carbon than L-glutamate, and sodium L-amino pimeric acid has one more carbon than L-glutamate; these compounds as well as D-glutamate (Fig. 2b) show slight umami taste when compared with L-glutamate. When conversion of the α-amino bond occurs by acetylation of the carboxyl bond by esterification, or when the α-hydrogen bond is converted by methylation, these compounds lose the umami taste. When the hydrogen at the β-position is exchanged for an OH ion, the threo form keeps the umami taste, whereas the erythro form (Fig. 2f) loses the umami taste. When a γ-carboxyl bond was substituted for sulfonic acid (Fig. 2c), the umami taste was enhanced.

The intensity of umami taste depends on pH. When kept in the vicinity of pH 7, the intensity of umami taste is highest; when pH is acidic or basic, the intensity is lowered.

These variations are explained by Kaneko's five-membered-ring theory. For the umami taste to be present, the five-membered ring must have α-NH_3^+, and γ-carboxyl bonds that interact electrostatically. Moreover, it is important that three bonds α-NH_3^+, γ-COO^-, and α-H, are placed on the plane structure. D-Glutamate (Fig. 2b) and α-methyl L-glutamate (Fig. 2d) do not have this stereo structure and cannot interact with umami bud receptors because of this steric hindrance. For the stereo structure of β-hydroxyl L-glutamate, when the threo type (Fig. 2e) is formed, no hindrance of the OH bond for the formation of the five-membered-ring structure is observed, but in the erythro type (Fig. 2f), the umami taste is lost because the OH bond hinders the formation of the five-membered-ring structure.

In acidic regions, the γ-COO^- bond converts to COOH, and in basic regions, α-NH_3^+ bonds to NH_2, reducing the umami taste as a result of the reduction of the electrostatical interaction between COOH and NH_2 in both regions.

Flavor Enhancer

The free amino acids are used widely in food manufacturing for aromas and brown food colors (16). The flavors are

Table 3. Amino Acid Composition in Foods (mg%; dry base)

Amino acid	Pork	Beef	Urchin	Mackerel	Green tea	Seaweed
Alanine	4.19	11.28	261	37	25.2	1029
Arginine	—	—	316	6.1	142	90
Aspartic acid	1.37	0.28	4	9.8	136	230
Glutamic acid	1.95	4.63	103	20	668	640
Glycine	2.75	2.40	842	54	47	125
Histidine	2.55	4.10	54	563	6.7	0
Isoleucine	1.03	2.04	100	9.6	47	14
Leucine	2.68	3.81	176	14	34	20
Lysine	4.27	6.19	215	22	7.4	24
Methionine	0.69	2.01	47	7.3	0.6	0
Phenylalanine	0.51	1.36	79	9.2	10.1	15
Proline	0.64	—	26	5.4	18.3	62
Serine	2.95	7.53	130	6.9	81.1	53
Threonine	0.48	1.11	68	9.6	60.9	78
Tryptophan	—	—	39	2.2	11.6	0
Tyrosine	0.56	1.85	158	6.6	4.2	0
Valine	0.30	2.99	154	14	6.1	21
1-Methylhistidine	0.49	4.80	—	—	—	—
Theanine	—	—	—	—	1727	—

Table 4. Taste Profile of L-Amino Acids

		Taste quality				
L-Amino acid	Threshold value (mg/dL)	Sweet	Sour	Bitter	Salty	Umami
Gly	110	+ +				
Hyp	50	+ +		+ +		
β-Ala	60	+ +				+
Thr	260	+ +				
Pro	300	+ +		+ + +		
Ser	150	+ +				+
Lys HCl	50	+ +		+ +		+
Gln	250	+				+
Phe	150			+ + +		
Trp	90			+ + +		
Arg	10			+ + +		
Arg HCl	30	+		+ + +		
Ile	90			+ + +		
Val	150	+		+ + +		
Leu	380			+ + +		
Met	30			+ + +		+
His	20			+ +		
His HCl	5		+ + +	+	+	
Asp	3		+ + +			+
Glu	5		+ + +			+ +
Asn	100		+ +	+		
Monosodium glutamate	30	+			+	+ + +
Monosodium aspartate	100				+ +	+ +

Profiles of each basic taste intensity are expressed as follows: + + +, strongest; + +, stronger; and +, detectable. When a taste quality was not detectable, symbols and omitted.

Table 5. Change in Taste with Concentration of Amino Acids

	Concentration		Concentration	
Amino acid	Low (g/dL)	Taste	High (g/dL)	Taste
L-Alanine	0.5	Sweet	5.0	Sweet/umami
L-Arginine	0.2	Bitter/sweet	1.0	Bitter
L-Glutamic acid	0.025	Sour	0.2	Sour/umami
L-Serine	1.5	Sweet/sour	15.0	Sweet/umami/sweet
L-Threonine	2.0	Sweet/sour/bitter	7.0	Sweet/sour

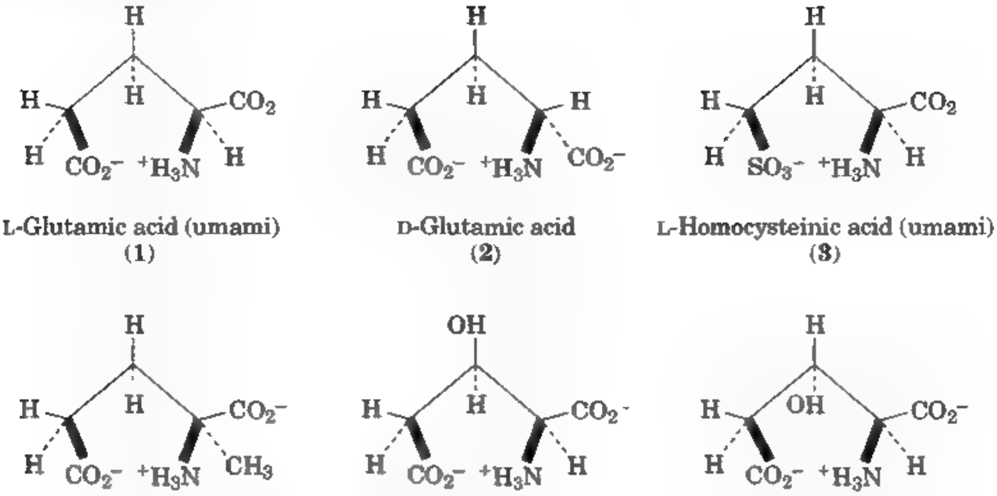
formed mainly during food processing (eg, the fermentation of alcoholic beverages) or cooking (frying, roasting, boiling) by the Maillard reaction between amino acids and reducing sugars. The Strecker degradation of amino acids plays a central role in this process. It is often possible to assign certain aromas to specific amino acids. The sulfur-containing amino acid cysteine is primarily responsible for meaty flavors, proline produces the aroma of bread crust, and phenylalanine and branched amino acids provide the characteristic flavor of chocolate. Valine and leucine are involved in the aroma of roast nuts. Methionine plays a key role in the aroma of french fries. The flavors of such products as precooked foods, snacks, and spices may be improved by the addition of the proper Maillard aromas (17).

Other applications of amino acids in foods are listed in Table 6. Amino acids are used in the food manufacturing industry for purposes other than supplementation and flavoring. L-Cysteine is used in bread baking as a flour additive to relax wheat gluten proteins, improve the texture of the baked product, and allow shorter kneading times.

Melanoidines, which are formed through the Maillard reaction, are stronger antioxidants than amino acids themselves. Glycine apparently exhibits a special preservative effect.

Pharmaceuticals

Elman and Weiner (18) attempted the clinical application of intravenous casein hydrolyzates in humans in 1939, and the nutritional requirement of amino acids was reported by Rose (19) in 1949. Stimulated by these investigations, various types of protein hydrolyzates containing amino acids were applied in clinical practice throughout the world. But because of the allergic reaction and asymptomatic hyperammonemia that resulted when protein hydrolyzates in the form of administered, crystalline amino acid solutions that did not contain unnecessary components such as peptides were needed. However, it was not until



L-Glutamic acid (umami)
(1)

D-Glutamic acid
(2)

L-Homocysteinic acid (umami)
(3)

α-Methyl-glutamic acid
(4)

β-Hydroxyl L-Glutamic acid
(threo type; umami)
(5)

β-hydroxyl L-Glutamic acid
(erythro type)
(6)

Figure 2. Relationship between the chemical structure of glutamic-acid-related substances and the appearance of umami taste.

1959 that such solutions were commercially available in Japan and became broadly used in daily clinical practice. This type of solution contains amino acids but no peptides, making it possible to have the exact amount of each amino acid in the solution.

The composition of amino acids in each solution was essentially based on recommendations by the Joint FAO/WHO Expert Group (20), which recommended a ratio of 1:1 between essential and nonessential amino acids in mixtures administered intravenously and those administered orally or through a feeding tube.

Meeting the increased branched-chain amino acid requirement in patients with severe surgical injuries has been demonstrated as being a means of nutritional support and treatment of disease itself (21).

CHEMICAL PROPERTIES

Formation of Salt

Because amino acid molecules have carboxyl groups and amino groups, they are able to react with both acids and bases to form a corresponding salt. When the salts react with weak acid, such as acetic acid, and weak alkali, such as ammonia, they are generally unable to separate as crystals. When not only mineral acids but also benzoic sulfonic acid and picric acid reacts with amino acids, chemically stable and characteristic crystalline salts easily form, which are used as tools for separation and identification of various amino acids. With metallic ions, such as silver, lead, zinc, copper, and tungsten, amino acids form sparingly soluble complex salts, which can be used as tools for separation and colorimetric analysis to identify the respective amino acids.

In the copper complex, the α-carbon atom is activated to react with aldehyde, for example, the glycine–copper complex reacts with acetaldehyde to yield threonine.

Esterification

When heated with alcohol in the presence of anhydrous hydrochloric acid, COOH in the α-position on the amino acid converts to the hydrochloride of the ester salt, corresponding to the alcohol used. This ester bond is easily released by treatment with alkali solution. The free ester of an amino acid eliminates alcohol on standing or warming to form cyclic 2,5-diketopiprazine, which converts to the corresponding peptide by hydrolysis with weak acidic. Moreover, when this amino acid ester reacts with hydroxylamine, it changes to hydroxamic acid, which on colorimetric analysis exhibits a specific color with $FeCl_3$.

Acylation

With acid anhydride or acyl chloride compounds under alkaline solution conditions (Schotten-Baumann conditions), amino acids react to form *N*-acyl α-amino acids, which are used mainly for protection of the amino group for peptide synthesis. Amino acids acylated with naturally occurring fatty acid residues are used industrially as easily degradable surfactants.

Schiff Base Formation

An example of a typical amino acid reaction is that when the amino acid combines with an aldehyde to form a Schiff base, a ketoimine-type compound. This Schiff base is important in view of the physiology of amino acids. Vitamin B_{12} enzymes, which control the decarbonation of amino acid and the amino group transformation between amino acid and keto acid, react after the formation of the Schiff

Table 6. Use of Amino Acids in Foods

Amino acid	Food	Function
L-Alanine	Synthetic sake	Seasoning
Monosodium L-aspartate	Seasoning	Seasoning
L-Arginine, L-glutamate	Green tea	Seasoning
L-Cysteine HCl	Bread	Enrichment of nutrition
Glycine	Natural fruit juice	Seasoning
	Fish paste product	Microbiostasis
	Pickles	
	Synthetic sake	
	Synthetic vinegar	
L-Glutamic acid	Meat product	Seasoning
	Canned food	Prevention of struvite
L-Histidine	Patient diet	Enrichment of nutrition
	Baby food	
L-Isoleucine	Cereal grain	Enrichment of nutrition
L-Lysine	Cereal grain, bread	Enrichment of nutrition
	Noodles, rice	
L-Lysine, L-aspartate	Cereal grain	Enrichment of nutrition
		Seasoning
L-Lysine, L-glutamate	Cereal grain	Enrichment of nutrition
		Seasoning
L-Methionine	Bread	Promotion of fermentation
L-Phenylalanine	Bread	Promotion of fermentation
L-Threonine	Bread	Promotion of fermentation
L-Tryptophan	Bread	Promotion of fermentation
L-Theanine	Green tea	Seasoning
L-Valine	Cereal grain	Enrichment of nutrition

base with pyridoxal phosphate to form the coenzyme of vitamin B_{12}.

Racemization

Racemization is one of the most important reactions for the seasoning industry: optically active amino acids are transformed into racemized amino acids. In general, this reaction easily occurs in alkaline conditions, and proceeds by the following scheme: the formation of a carbanion releases the α-H from the α-C by a base catalytic reaction, and this then equilibrates with the α,β-unsaturated form (22).

Degradation of Amino Acids

Amino acids do not have a defined melting point. They decompose over the broad range of 200-to 300°C but are comparatively stable at ambient temperature and occur as transparent or white crystals.

In the solid state, decarbonation mainly occurs when the amino acid is heated. The amino acid converts to the amine that has the same carbon number as the amino acid.

When an α-amino acid reacts with nitrous acid, it yields α-hydroxycarboxylic acid, and nitrogen gas. The amount of gas that is measured, is equivalent to the amino-nitrogen. This is the basis for the van Slyke method, used for the quantitative determination of amino acids.

When ninhydrin is added to an amino acid solution, the CO_2 released causes the color of the solution to turn of blue-violet called Ruhemann's purple, which has a maximum absorbency at 570 nm. This reaction is often used as a quantitative analysis of amino acids.

PHYSICAL PROPERTIES

Amphoteric Compounds

A common characteristic of amino acids is to have a dipolar ion (Zwitterion) in molecular form—not an uncharged form of H_2N-CHR-COOH but a dipolar ion, H_3N^+-CHR-COO^-—in both neutral solution and in solid state.

Generally, this is recognized by the following physical properties. Amino acids have (*1*) high melting points; (*2*) high dipole moments; (*3*) extremely low volatile characters; and (*4*) in solution, changes in the value of the dielectric constant of water and electrical strain are determined directly by Raman spectra and infrared spectra.

Dielectric Constant

Because of the dipolar ion structure of the amino acids, they are able to considerably increase the dielectric constant of the solvent in which they are dissolved (23–25). This increase is on such a high an order of magnitude that it is explicable only on the basis of the possession by these compounds of a large permanent electric moment.

The electric moments of the amino acids themselves cannot be determined by Debye's procedure because of their nonpolar solvents. This fact suggests the highly polar character of the amino acids.

The moment can be calculated from the spatial considerations on the basis of a dipolar ion structure. If the center of the positive charge is located on the α-nitrogen atom, and the center of the negative charge is midway between the two oxygens of the carboxyl group, the dipole distance is 2.96 Å, and the electric moment is the product of the elementary charge on the electrons

$$4.8 \times 10^{-10}\ \text{esu by}\ 2.96 \times 10^{-8}\ \text{cm} = 14.2 \times 10^{-18}\ \text{esu}$$

The Debye relation is $P = 4\pi N\mu^2/9$ kT for compounds with high permanent dipoles. P is the molar polarization and μ is the electric moment. The term P is a linear function of the dielectric constant of the medium, and the dielectric constant is related to the square of the electric moment per molecule of the compound.

The electric moment of a compound such as β-alanine would be expected from the Debye relation to be greater, and the effect on the dielectric constant of the medium would be expected to be greater than revealed by the isomeric compound α-alanine.

All α-amino acids produce an increase in the dielectric constant of water by about 23 per mol, which is independent of concentration. In the case of glycine, where the dielectric constant of the solution is 135, the increase is 2.5 mol/L. The dielectric increment per mole of solute, δ, is shown in the following equation:

$$\delta = D(\text{solution}) - D(\text{solvent})/\text{molarity}$$

For water at 25°C, the dielectric constant D_0 is 78.54; δ = 23 for α-alanine and $\delta = 35$ for β-alanine.

The value of δ at a pH in the range 4.5 to 7.5 for glycine was measured. δ falls steeply on either side of this (isoelectric) pH range as the initial dipolar ions convert into either cations or anions with simultaneous loss of dipolar ion character.

The values of δ for all α-amino acids range from 22.6 to 23.2; the values for the β-amino acids are about 35.

Raman Spectra Studies

In Raman spectra, the peaks at 1730 cm^{-1} for COOH and at 3320 cm^{-1} for NH_2 are not found in amino acids, but a peak at1400 cm^{-1} for COO^- is detected. In the amino acid hydrochloride salt, a peak of 1730 cm^{-1} for COOH is detected, but the peak for NH_3^+ is not detected because of the overlap with the absorbency of water.

The unionized COOH group frequency near 1730 cm^{-1} is a particular feature of the spectra. This COOH group would be expected to be present in all relatively unionized carboxylic acids, and if the dipolar ion character of the amino acids is true, this group would also be expected to occur in the hydrochlorides of the amino acids. When the carboxyl group is ionized, the 1730-cm^{-1} line disappears, thus further suggesting the dipolar ion character of the amino acids under these conditions (26–31). RCOOH or $NH_3^+RCOOH + Cl^-$ shows a 1730-cm^{-1} line, but $RCOO^-$ or $NH_3^+COO^-$ does not show a 1730-cm^{-1} line.

Dissociation Constant

In aqueous solution, amino acids are in a state of dissociation equilibrium that is dependent on the pH. In acidic conditions, they exist as cationic forms (A^+); in basic conditions, as anionic forms (A^-); in between, as mainly zwitterions ($A^\pm$), where the cationic and anionic forms are equal.

When an amino acid solution is in a neutral state and acidic or alkaline agents are added slowly, an abrupt change in pH at the inflection is observed that plateaus at constant value. The more added, the move abrupt the change in pH at the infliction. Amino acids show a double buffer action in both acidic and basic regions, which are named pK_1 and pK_2, corresponding to the inflection point in the titration curve.

The pK_1, pK_2, and pI values are calculated from the titration curves with the Henderson–Hasselbalch equation.

The equilibrium constant K_1 in acidic solution is expressed as $K_1 = [A^\pm][H^+]/[A^+]$. Conversely, in basic solution, the equilibrium constant is $K_2 = [A^-][H^+]/[A^\pm]$. When 50% of amino acid is dissociated, $[A^\pm] = [A^+]$; $[A^-] = [A^\pm]$, ie, $K_1 = [H^+]$ and $K_2 = [H^+]$, then pK_1 and pK_2 each show the pH in solution.

When a direct current is applied to an amino acid solution and the pH in solution is lower than pK_1', the amino acids transfer toward the cathode; when pH is higher than pK_2', they transfer toward the anode. This phenomenon is called electrophoresis and is used as a effective tool for the separation of amino acids. In between pK_1 and pK_2, no migration of amino acids is observed because the apparent charge of amino acid in solution is zero, which assumes that the concentration of cations in solution is equal to the concentration of anions:

$$[A^+] = [A^-]$$

$$K_1K_2 = [H^+]^2[A^-]/[A^+]$$

It follows that $[H^+]$ at the isoelectric point $= \sqrt{K_1K_2}$, and in negative logarithmic terms, where pI is pH at the isoelectric point, pI = pK_1 + pK_2/2. For neutral amino acids, such as glycine, an amphoteric ion theoretically exists only at the point of pI, but in the broad range of pH between 4.3 and 7.7, no amino acid transfer in electrophoresis observed. This range is said to be the isoelectric region, in which more than 99% of amino acids are dissociated as amphoteric compounds.

For acidic amino acids such as L-aspartic acid, the amino group and carboxyl group on the α-position and an additional carboxylic group on the R-site are dissociated. This compound has three pK values: pK_1 (α-carboxylic group), pK_2 (β-carboxylic group), and pK_3 (α-amino group), named in the order from the acidic region. The isoelectric point is defined as pI = pK_1 + pK_2/2.

On the other hand, a basic amino acid such as lysine has an additional amino group on the R-site, corresponding to pK' values that exhibit pK_1 for the α-carboxyl group, pK_2 for the α-amino group, and pK_3 for the ω-amino group, respectively. At neutral pH 7, lysine exists in cation form, when moved toward an alkaline condition, the ratio of cation form gradually decreases and reaches an isoelectric point of pI = pK_2 + p$K3$/2.

The pK_1 values show that the amino acids are considerably stronger acids than acetic acid. Because of intramolecular protonation of the amino moiety by the carboxyl group, the amino acids solutions are weakly acidic.

The differences in acidity and basicits are used in the separation of amino acid mixtures by ion exchange chromatography and electrodialysis. The dissociation constant pK and the isoelectric point pI for various amino acids are summarized in Table 7 (32).

Optical Rotation

Optical rotation is an important characteristic for determining the purity of an amino acid. The value measures changes that occur with changes in temperature, pH, the kind of solvent, and other conditions that affect related optically active substances. For polarimetric measurement of the specific rotation, the sodium D line (wavelength, 589 nm) is used as a light source and expressed as $[\alpha]$D.

The optical rotation for various amino acids is minimum at neutral conditions. For acidic and alkaline conditions, the value of the optical rotation of L-amino acids rotates toward a positive direction. For D-amino acids, conversely, the value rotates toward a negative direction. This rule, called Lutz's law or the Clough–Lutz–Jirgenson law, is used to determine the stereo allocation of an amino acid molecule. The stereo allocation also is attributed to the difference in the ionic form of the amino acid. Therefore, when acid is added, a change in the optical rotation value is observed up to 2 Eq, but above 2 Eq, no change in the optical rotation is observed.

When the temperature is elevated, the dextrorotary value of the optical rotation of L-aspartic acid decreases. At 75°C, the value is 0; conversely, the levorotary value increases with increases in temperature.

Optical rotatory dispersion (ORD), is the phenomenon that changes in optical rotation are observed when the wavelength (λ) is changed. When $[\alpha]$ is plotted as a function of λ^2, it is the so-called rotatory dispersion curve, if it obeys the Drude monominal formula, it is called monorotation dispersion. $[\alpha] = A/\lambda^2 - \lambda_0^2$, where A is the rotation constant, λ is the wavelength of the incident light, and λ_0 is the dispersion constant that corresponds to the wavelength of the nearest optically active absorption band (33).

In general, amino acids show a normal dispersion, but tyrosine shows an anomalous dispersion due to two rotatory contributions of opposite sign, ie, with decreasing wavelength, the rotation is minimal, becomes zero, changes sign, and approaches $+\infty$ (an effect known as the Cotton effect). In the case of tyrosine, good agreement is obtained from three constant Drude equations.

Further methods for investigating the structure of amino acid enantiomers include circular dichroism, which shows differing absorption for left- and right-handed circularly polarized light.

Isoleucine, threonine, and hydroxyproline contain two chiral carbon atoms each; therefore, they appear in four stereoisomeric forms. Cystine, which also contains two chiral carbons, has only three stereoisomers: L-, D-, and *meso*-cystine. The *meso* form has a plane symmetry.

Absorbency Spectra

Aliphatic amino acids exhibit no absorption in the UV region above 220 nm, with the exception of cystine (240 nm). The aromatic amino acids, phenylalanine, tyrosine, and tryptophan, have a UV absorbency above 220 nm. The exact position of the maximum and the molar extinction coefficient ϵ are affected by the pH of the solution.

On the other hand, for infrared spectra, amino acids have common absorbency and specific absorbency. The common absorbencies of infrared spectra for amino acids are in the vicinity of 3070 cm^{-1} for NH_3^+, 1620 cm^{-1} for COOH, and 1560 to 1600 cm^{-1} for COO^-. The common absorbency nearly always shows a similar value irrespective of the state of the amino acid—solid or solution.

Table 7. Dissociation Constant and Isoelectric Point of Amino Acids

Amino acid	pK_1	pK_2	pK_3	pK_4	pI
Alanine	2.34	9.69			6.00
Arginine	1.82	8.99	12.48		10.76
Asparagine	2.02	8.80			5.41
Aspartic acid	1.88	3.65	9.60		2.77
Cysteine	1.92	8.35	10.46		5.07
Cysine	1.04	2.05 (-COOH)	8.20 (-NH_2)	10.25	5.02
Glutamic acid	2.19	4.25	9.67	3.22	
Glutamine	2.17	9.13			5.65
Glycine	2.35	9.78			5.97
Histidine	1.78	5.97	8.97		7.59
Isoleucine	2.36	9.68			6.02
Leucine	2.36	9.60			5.98
Lysine	2.20	8.90	10.28		9.78
Methionine	2.13	9.28			5.74
Phenylalanine	2.16	9.18			5.48
Proline	1.95	10.64			6.30
Serine	2.19	9.21			5.68
Threonine	2.15	9.12			6.16
Tryptophan	2.38	9.39			5.89
Tyrosine	2.20	9.11	10.07		5.66
Valine	2.32	9.62			5.96

The common absorbency in acidic solutions is in the vicinity of 3030 cm^{-1} (NH_3^+), 2130 cm^{-1} (NH_3^+), and 1730 cm^{-1} (COOH); in alkaline solutions, it is 3300 cm^{-1} (NH_2), 1610 cm^{-1} (COO^-), and 1400 cm^{-1} (COO^-). These absorbency bands show the ionic form of the amino acid in each solution, ie, in solid or in solution, of the isoelectric point, which is the evidence that the amino acid exists as a dipolar ion. Common absorbencies at 1450 cm^{-1}, 1370 cm^{-1}, and 1330 cm^{-1} are attributed to C-N and C-H and do not vary with change in pH.

Solubility

The solubility in water for different amino acids varies greatly. Cystine is the most sparingly soluble, followed by tyrosine. Aspartic acid, glutamic acid, and leucine belong to the category of comparatively insoluble amino acids. The easily soluble amino acids are proline and hydroxyproline, and other amino acids belong to the relatively soluble category. The optical isomers for each amino acid—L,D, and D,L—demonstrate slight difference in solubility.

In general, the solubility increases when the temperature increases. Acidic amino acids especially show a remarkable dependence on temperature, for example, aspartic acid: 2.09 g/L at 0°C and 68.87 g/L at 100°C.

The solubility is expressed using a function of temperature as follows:

$$\log S = a + bt + ct^2$$

The empirical coefficients a, b, and c for various amino acids are summarized in Table 8 (32,34,35).

The solubility of amino acids varies with the change in pH and is minimal at the isoelectric point. The solubility increases when the isoelectric point is deviated toward alkaline or acid in solution. The change in solubility is governed by the ratio of the ionic form corresponding to the pH in solution. Therefore, the monochloride salts of both neutral amino acids and basic amino acids are more soluble than the free forms of the corresponding amino acids. The sodium salts of amino acids are also easily soluble compared with the free forms of amino acids.

Table 8. Parameter of Solubility for Various Amino Acids

Amino acid	a	$b \times 10^2$	$c \times 10^5$
Glycine	2.254	0.573	—
L-Alanine	2.1048	0.4669	—
D,L-Alanine	2.0830	0.5608	—
D,L-Valine	1.7749	0.2389	2.607
L-Leucine	1.3561	0.02233	3.727
D,L-Leucine	0.9013	0.2635	4.519
L-Isoleucine	1.5787	0.07682	2.594
D,L-Isoleucine	1.2616	0.2512	3.794
D,L-Serine	1.3432	1.520	−3.548
L-Aspartic acid	0.3194	1.519	—
D,L-Aspartic acid	0.4181	2.016	−5.000
L-Glutamic acid	0.5331	1.613	—
D,L-Glutamic acid	0.9317	1.523	—
L-Cystine	−1.299	1.357	—
L-Proline	3.1050	0.4206	—
L-Phenylalanine	1.2974	0.6982	—
L-Tryptophan	0.9456	0.4834	2.988

$\log S = a + bt + ct^2$ (S, g/L, t, °C).

The solubility of an amino acid tends to increase when there are lower levels of inorganic substances present, and it gradually decreases when there are higher levels of inorganic substances present. This tendency is similar to the salt-out effect on the solubility of protein, which is the change in the molecular form caused by the formation of molecular compounds between amino acids and inorganic substances. By varying the concentration of ammonium sulfate, the solubility of cystine increases at low levels of ammonium sulfate and decreases when the ionic strength is increased. When amino acids exist together, the solubility of each amino acid increases.

In organic solvents, almost all amino acids, except amino acid, proline, or hydroxyproline, are sparingly soluble. Proline is soluble up to 1.6 g/dL in ethanol at 20°C.

INDUSTRIAL PRODUCTION

Amino acids have been produced by four methods: (*1*) extraction from protein hydrolysate, (*2*) chemical synthesis, (*3*) fermentation, and (*4*) enzymatic synthesis. The amount produced and the methods used for each amino acid in one year (1997) are summarized in Table 9 (36).

Extraction

For several amino acids (cystine, tyrosine, leucine), isolation from natural raw materials is still an economical pro-

Table 9. Production of Amino Acids (ton/year, 1997)

Amino acid	Method	World
D,L-Alanine	C	1,500
L-Alanine	Enz	500
L-Arginine	Enz, F	1,200
L-Aspartic acid	Enz	7,000
L-Aspargine	Enz	1,200
L-Citrulline	Enz	
L-Cysteine	Ext, Enz	1,500
L-Cystine	Ext, Enz	
Glycine	C	22,000
L-Glutamic acid	F	1,000,000
L-Glutamine	F	1,300
L-Histidine	F	400
L-Isoleucine	F	400
L-Leucine	Ext, F	500
L-Lysine hydrochloride	F	250,000
D,L-Methionine	C	350,000
L-Methionine	Enz	300
L-Ornithine	F	
L-Phenylalanine	C, F, Enz	8,000
L-Proline	F	350
L-Serine	F	200
L-Threonine	C, F	4,000
L-Tryptophan	C, Enz, F	500
L-Tyrosine	Ext, F	120
L-Valine	C, F	500

Note: F, fermentation method; C, chemical method, Enz, enzymatic method, Ext, extraction method.

duction method. The procedure for isolating an amino acid from a protein hydrolyzate consists of passing the hydrolyzate over an ion exchange resin. After elution with aqueous ammonia, aminoacids are collected in fractions.

Chemical Synthetic Production

The D,L forms of alanine, methionine, and tryptophan available for living organisms are chemically produced. Glycine is also produced chemically because there is no asymmetric carbon in its structure. Because of the increasing demand for D,L-cysteine in hairdressing products, the chemical process for production of this amino acid has been developed.

Fermentation

In fermentation, an amino acid–producing bacterium is cultured in a medium that contains sugars and ammonium compounds as carbon and nitrogen sources under aerobic conditions with aeration and agitation. Other growth factors are also added if required. Three groups of bacteria are used in the industrial production of amino acids: (*1*) *Brevibacterium* sp., the glutamic acid bacteria; (*2*) enteric bacteria, *Escherichia coli*; and (*3*) *Bacillus* spp. (37).

The optimal culture condition depends on the nature of the bacteria used and on culture conditions medium composition, temperature, pH, agitation, and aeration.

Mechanism of Production. The metabolic pathway used to produce amino acids is regulated by key enzyme regulatory mechanisms, feedback inhibition by the end product, and repression. In wild-type bacteria, these mechanisms prevent the overproduction of amino acids. A permeability barrier prevents the excretion of amino acids outside the cell wall.

The overproduction of amino acids is achieved by changing the bacterial nature through mutation to release these metabolic regulators and by selecting optimal culture conditions.

Induction by Controlling the Culture Conditions

Glutamic acid-producing bacteria such as *B. lactofermentum* require biotin for growth. When the concentration of biotin is controlled in a limited range of 3 to 5 μg/L, the amount of L-glutamic acid accumulated reaches 80 g/L or more if more than 50% sugar is added). If the culture contains more or less than this biotin level, almost no accumulation of L-glutamic acid is observed.

When a small amount of penicillin is added to a culture with an excess amount of biotin, L-glutamic acid production is induced as a result of the inhibition of peptide glycan synthesis by penicillin in cell walls. When cane molasses is used as a carbon source in cultures in which a sufficient amount of biotin exists, L-glutamic acid is produced by this penicillin method.

Auxotrophic Mutant Strains

Many kinds of amino acids are accumulated by auxotrophic strains that mutated to require a growth factor. L-Lysine is produced by a homoserine auxotrophic mutant of glutamic acid-producing bacteria; *B. lactofermentum* and *Corynebacterium glutamicum* and typical examples. In glutamic acid bacteria, L-lysine is formed by way of the branched pathway shown in Figure 3. The aspartate kinase, which is the first key enzyme of lysine biosynthesis, is inhibited by the combination of L-threonine and L-lysine, and L-lysine is among the amino acids not overproduced. In the homoserine auxotroph, if the supply of L-threonine is limited, L-lysine formed by *de novo* synthesis does not inhibit the first enzyme without the aid of L-threonine In this mutant, the metabolic flux is genetically blocked to flow in the direction of threonine and methionine biosynthesis. This is one of the main reasons for the overproduction of L-lysine in mutant strains (38).

Regulatory Mutant Strains

When the feedback regulators in the biosynthetic pathway are genetically released, the overproduction of any amino acid is easily attained. These mutants are selected from among those resistant to the growth inhibition of amino acid analogues. *S*-(β-Aminoethyl)-cysteine (a lysine analogue)–resistant mutants of glutamic acid bacteria accumulate a large amount of L-lysine in the medium.

Cell Fusion

When protoplasts prepared from cells with two different genetic markers are mixed in the presence of poly(ethylene glycol) and $CaCl_2$, fused cells with different combinations of parental genetic markers are formed. By this method, a *B. lactofermentum* strain that rapidly produces L-lysine was obtained (39).

Gene Technology

The gene multiplication method with plasmid vectors has been applied to the breeding of amino acid producers. When a plasmid vector that can multiply within an amino acid producer is joined enzymatically to a DNA fragment carrying the genetic information from other strains of the same microorganism or other microorganisms, the resultant recombinant DNA is transferred to the amino acid producer to introduce new markers of the donor microorganisms. A plasmid such as pCG11 has sites sensitive to various restriction endonucleases and is easily joined to a DNA fragment prepared by digestion with corresponding restriction enzymes. Moreover, because it has the genes determining streptomycin resistance, it is easy to select strains with plasmids carrying these antibiotic resistance markers (40).

Enzymatic Process

Enzymatic catalysis is advantageous for cases where amino acids can be produced from relatively inexpensive chemicals with high specificity in a continuously operating process. The enzyme is recycled by binding it to a carrier and carrying out the reaction in a fixed-bed column.

Purification of Amino Acid from the Fermentation Broth

Ion exchange methods are usually used to recover amino acids from the fermentation broth. Because amino acids

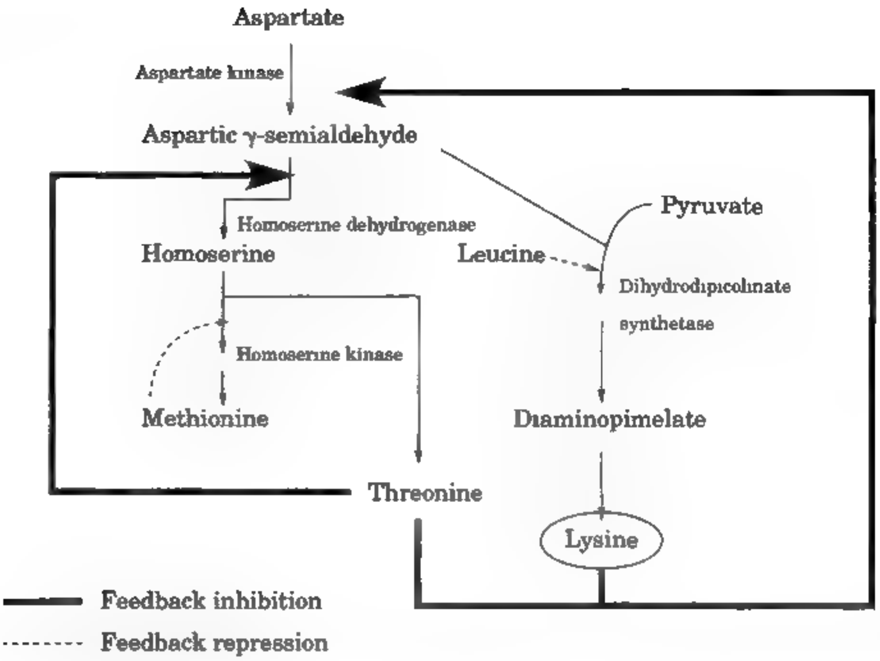


Figure 3. Regulation of lysine production in the metabolic pathway.

have an amphoteric character, when the pH is adjusted lower than pK_1, the amino acids change from zwitterion to cationic form. When a fermentation broth with a pH adjusted lower than pK_1 is passed through the strong cation exchange resin, the amino acid cation being exchanged with a counterion such as H^+ or ammonium ion is removed continuously until the concentration on the ion exchange resin reaches a value corresponding to equilibrium with that in the fermentation broth. The adsorbed amino acids are then eluted by ammonia water or other eluant. If ammonia water is used for the eluant, the counterion form of the ion exchange resin changes to the ammonium form simultaneously, and no generation process is necessary; moreover, almost no inorganic substances are contained in elution with the amino acid. The elution is condensed until the somewhat higher saturated concentration of amino acids is evaporated and crystallized to obtain amino acid crystals.

The crystallization method for an amino acid is selected on the basis of the characteristics of the amino acid and the degree of impurity contained in solution. The solubility minimum at the isoelectric point is useful for crystallizing amino acids. L-Aspartic acid and L-tyrosine, which show comparatively lower solubility, for example, are first dissolved in alkali or acid solution and then crystallized by neutralization to pI. On the other hand, L-lysine hydrochloride and L-glutamate, which show higher solubility, crystallize by the condensed crystallization method.

L-Leucine, L-isoleucine, and L-valine, branched-chain amino acids from which higher purity crystals are difficult to obtain, react with benzoic sulfonic acid derivatives to form corresponding complexes.

For pharmaceutical use, it is critical to prevent contamination by pyrogen substances and microorganisms. Pyrogen is a toxin consisting of polysaccharides and protein. It is excreted mainly by Gram-negative bacilli, and it is highly hydrophobic. The pyrogens are effectively adsorbed on activated carbon. Sterilization by heating and ultrafiltration membranes (molecular weight cutoff, 10,000 to 100,000) are used as well for rejection of pyrogens and microorganisms.

SAFETY

Physiological Effect of Imbalance

Excess amino acids are rapidly disposed of by increased metabolic degradation and are used to provide energy. The nitrogen is eliminated as urea.

When the component amino acids are imbalanced, alternation of the ribosome profile occurs in the liver, and ribonucleic acids are catabolized. Protein deficiency is usually coupled with calorie deficiency. The manifestation of chronic protein deficiency is known as marasmus and kwashiorkor. On the biochemical level, marasmus and kwaskiorkor result in a negative balance, indicating a reduction in protein inventory. The labile enzyme and plasma proteins are consumed, the greatest losses occurring first in the liver and then in the musculature. Brain, heart, and kidneys suffer minimal protein loss. The absence of a essential amino acid in the diet is more serious than protein deficiency because protein synthesis can then occur only by degradation of body protein.

Amino Acid Toxicity

When an excess amount of one or more amino acids is administered, some adaptive failure can lead to accumulation of the amino acids or certain metabolites in the organism, leading to anatomic or functional damage. The acute oral toxic levels have been studied by adding increasing quantities of individual amino acids to a protein diet (41)

Table 10. Acute Oral Toxicity of Amino Acids in Rats

Amino Acids	LD_{50} (g/kg weight of rat)
L-Alanine	>16
L-Arginine	~16
L-Asparagine	>16
L-Cysteine HCl	3.1 (2.7–3.6)
L-Cystine	11.2 (9.0–14.0)
L-glutamine	>16
Glycine	~16
L-Histidine	>16
L-Hydroxyproline	>16
L-Isoleucine	>16
L-Leucine	>16
L-Lysine HCl	10.6 (9.5–11.9)
L-Methionine	>16
L-Phenylalanine	~16
L-Proline	>16
L-Serine	14.0 (12.8–15.3)
L-Threonine	>16
L-Tryptophan	>16
L-Tyrosine	>16
L-Valine	>16
NaCl	3.75

(Table 10). The toxicity of individual amino acids depends on the total protein consumption.

The consumption of toxic amounts of amino acids increases their concentration in the plasma and brain. Because of the blood-brain barrier, however, the increase in the brain is not great (42).

BIBLIOGRAPHY

1. I. Chihata and K. Kawashima, in A. Yoshida et al., eds., *Nutrition: Proteins and Amino Acids*, Japan Scientific Societies Press, Springer-Verlag, New York, 1990, pp. 273–284
2. H. W. Brown, B. G. Harmon, and A. H. Jansen, *J. Anim. Sci.* **37**, 708 (1973).
3. FAO Committee on Protein Requirements, *FAO Nutritional Studies* No. 16, FAO, Washington, D.C., 1957.
4. E. E. Howe, G. R. Jansen, and C. W. Gelfillan, *Am. J. Clin Nutr.* **16**, 315 (1965).
5. E. E. Howe, G. R. Jansen, and M. L. Anson, *Am. J. Clin. Nutr.* **20**, 1134 (1967).
6. M. Winitz et al., *Nature* **205**, 741 (1965).
7. M. Winitz, D. A. Seedman, and J. Graff, *Am. J. Clin. Nutr.* **23**, 25 (1970)
8. M. Winitz et al., *Am. J Clin. Nutr.* **23**, 546 (1970).
9. S. J. Fomon, *Am. J. Clin. Nutr* **32**, 2460 (1979).
10. Y. Kobayashi, Y. Doi, and T. Takanami, in A. Yoshida et al., eds., *Nutrition: Proteins and Amino Acids*, Japan Scientific Societies Press, Springer-Verlag, New York, 1990, pp. 285–299.
11. Y. Komata, *Science of "Oishisa" and Taste* (in Japanese), Nippon Kougyou Shinbun Co., Ltd.
12. C. P. Berg, *Physiol. Rev.* **33**, 145 (1953).
13. S. Akabori, *Nippon Seikagaku Kaishi* **14**, 185 (1939).
14. T. Kaneko, R. Yoshida, and H. Katsura, *Nippon Kagaku Kaishi* **80**, 316 (1959).
15. U. K. Lee, T. Kaneko, *Bull. Chem. Soc. Jpn.* **46**, 3494 (1973)
16. W. J. Hertz and R. S. Shallenberger, *Food Res.* **25**, 491 (1960).
17. T. A. Rohan, *Food Technol.* **24**, 29 (1970)
18. R. Elman and D. O. Weiner, *J. Amer. Med. Assoc.* **112**, 795–802 (1939).
19. W. C. Rose, *Fed. Proc.* **8**, 546–552 (1949).
20. FAO/WHO Expert Group, *Protein Requirements*, WHO Technical Report Series No. 301, World Health Organization Geneva, Switzerland, 1965.
21. M. M. Meguid et al., in G. L. Blackburn, J. P. Grant, and V. R. Young, eds., *Amino acids, Metabolism and Medical Applications*, John Wright PSG Inc., 1983, pp. 147–154.
22. M. L. Anson, *Advances in Protein Chemistry* Vol. 4, 1948, p. 339.
23. J. Wyman, Jr., and T. L. McMeekin, *J. Am. Chem. Soc.* **55**, 908 (1933).
24. J. Wyman, Jr., *Chem. Revs.* **19**, 213 (1936).
25. K. J. Dunning and W. J. Shutt, *Trans. Faraday Soc.* **34**, 479 (1938).
26. T. Edsall, J. W. Otvos, and A. Rich, *J. Am. Chem. Soc.* **72**, 474 (1950).
27. F. Garfinkel and J. T. Edsall, *J. Am. Chem. Soc.* **80**, 3807 (1958).
28. E. Takeda et al., *J. Am. Chem. Soc* **80**, 3813 (1958).
29. F. Garfinkel and J. T. Edsall, *J. Am. Chem. Soc.* **80**, 3818 (1958).
30. F. Garfinkel and J. T. Edsall, *J. Am. Chem. Soc.* **80**, 3823 (1958).
31. F. Garfinkel, *J. Am. Chem. Soc.* **80**, 3827 (1958).
32. J. P. Greenstein and M. Winitz, eds., *Chemistry of the Amino Acids*, Vol. 1, John Wiley & Sons, Inc., New York, 1966.
33. E. Brand, *J. Am. Chem. Soc.* **76**, 5037 (1954)
34. W. M. Hoskins, M. Randall, and C. L. A. Schmidt, *J. Biol. Chem.* **88**, 215 (1930).
35. M. S. Dunn, F. J. Ross, and L. S. Read, *J. Biol. Chem.* **103**, 579 (1933).
36. *Amino Acids* Japan Essential Amino Acids Association, Inc., 1997.
37. S. Kinoshita, in A. L. Demain and N. A. Solomon, eds., *Biology of Industrial Microorganisms*, Benjamin/Commings Publishing Co., Inc., London, 1985, pp. 115–142.
38. K. Nakayama, in G. Reed, ed., *Prescott & Dunn's Industrial Microbiology*, 4th ed., AVI Publishing Co., Inc., Westport, Conn., 1982, pp. 748–801.
39. O. Tosaka and K. Takinami, K. Aida et al., eds., *Biotechnology of Amino Acid Production*, Kodansha, Ltd., Tokyo, 1986, pp. 152–172.
40. R. Katsumata et al., in O. M. Neijsel, R. R. van der Meer, and K. Ch. A. M. Layben, eds., *Proc. 4th European Congr. Biotechnol.* Vol. 4, *Proceedings of the 4th European Congress on Biotechnology*, Elsevier, Amsterdam, 1987, pp. 767–776.
41. *Acute Oral Toxicity of Rat to Twenty Five Amino Acids*, Huntingdon Research Center, 1971.
42. Y. Peng, J. K. Tews, and A. E. Herper, *Am. J. Pysiol.* **222**, 314–321 (1972).

GENERAL REFERENCES

G. L. Blackburn, J. P. Grant, and V. R. Young, eds., *Amino Acids, Metabolism and Medical Applications*, John Wright PSG, Inc., 1983.

J. P. Greenstein and M. Winitz, eds., *Chemistry of the Amino Acids*, Vol. 1, John Wiley & Sons, Inc., New York, 1966.

T. Kaneko et al., eds., *Amino Acid Industry*, Kodansha, Japan, 1973.

J. I. Kroschwitz and M. H. Grant, eds., *Encyclopedia of Chemical Technology*, 4th ed., Vol. 2, John Wiley & Sons, Inc., New York, 1992.

A. Yoshida et al., eds., *Nutrition: Proteins and Amino Acids*, Springer-Verlag, New York, 1990.

Tetsuya Kawakita
Ajinomoto Company, Inc.
Kawasaki, Japan

PROTEINS: DENATURATION AND FOOD PROCESSING

Food proteins constitute a wide range of protein types (eg, structural, storage, catalytic) and essentially include all those that are palatable, digestible, nontoxic, and accessible to humans. Although contributing to the nutritional value of foods by supplying a dietary source of essential and nonessential amino acids, proteins also serve as integral food components or ingredients by virtue of their diverse technological or functional properties (Table 1). These properties affect the behavior of the protein in a given food system during preparation, processing, or storage as judged by the quality attributes of the final product (1–3). They reflect a variety of interactions between physicochemical properties of the proteins per se (eg, molecular weight, amphiphilicity, structural flexibility), other food components (eg, water, carbohydrates, lipids, vitamins, minerals), and the environment in which these are associated. A variety of commodities and by-products may serve as the source of food proteins, yet their inclusion in the human diet usually necessitates some form of processing and/or preparation because exploitation of technological attributes normally requires the destruction and/or modification of the native protein structure (ie, denaturation). Processing affects not only each individual food component, but also the nature and intensity of interactions between components. Because many processing treatments can result in undesirable changes in the functional properties of food proteins and, therefore, contribute to poor final quality of protein-containing food products, an understanding of the relationship between processing factors and the changes in proteins that they induce is imperative. This article discusses protein stability and folding, protein denaturation, factors that influence it, and specific examples of food proteins denaturated during processing. Thorough discussions of various aspects of protein denaturation can also be found in References 4 to 10.

Table 1. Functional Properties of Proteins in Foods

General classification	Functional property
Organoleptic	Color, odor, flavor, texture, turbidity
Hydration	Solubility, dispersibility, wettability, water-holding capacity, water absorption
Surface	Emulsification, emulsion stability, foamability, whippability, film formation, fat absorption, binding properties
Thermal	Thermocoagulability, heat stability
Rheological	Elasticity, viscosity, gelation, cohesion, extrudability, adhesion, coagulation, aggregation, hardness, chewiness
Other	Compatability with additives, enzyme activity, susceptibility to modification

NATIVE PROTEIN STRUCTURE AND STABILITY

The functional properties of food proteins ultimately arise from their unique molecular conformations, which derive from all four levels of structural hierarchy (ie, primary, secondary, tertiary, quaternary) (11). During synthesis on the ribosome, the polypeptide chain, pending subsequent posttranslational processing (see section on protein folding, molten globules), adopts a unique molecular conformation traditionally referred to as the native conformation. This particular structure is dictated by the nature and sequence of amino acids in the polypeptide chain and is greatly influenced by solvent effects (12). The folding and resultant (native) conformation of a protein is largely governed by (equilibrium) thermodynamics. The third law of thermodynamics states that

$$\Delta G = \Delta H - T\Delta S \qquad (1)$$

where ΔG is the change in Gibbs free energy, ΔH is the change in enthalpy, T is absolute temperature (Kelvin), and ΔS is the change in entropy. To obey this law, the polypeptide chain must fold into a conformation such that the least amount of free energy is expended in maintaining it. In terms of free energy, hydrophobic effects contribute most to protein folding and stabilization. Hydrophobic interactions are nonspecific and result from the strong hydrogen-bonding properties of water. Nonpolar amino acid residues generally cannot participate in hydrogen-bonding; thus, water molecules surrounding nonpolar residues hydrogen bond with each other to form a highly ordered icelike structure that is associated with unfavorable (ie, low) entropy. The native polypeptide chain, therefore, tends to bury its hydrophobic residues within the interior of the molecule with exclusion of water from this core and to orient its hydrophilic amino acids toward the protein exterior. Exclusion of water from the protein interior

GENERAL REFERENCES

G. L. Blackburn, J. P. Grant, and V. R. Young, eds., *Amino Acids, Metabolism and Medical Applications*, John Wright PSG, Inc., 1983.

J. P. Greenstein and M. Winitz, eds., *Chemistry of the Amino Acids*, Vol. 1, John Wiley & Sons, Inc., New York, 1966.

T. Kaneko et al., eds., *Amino Acid Industry*, Kodansha, Japan, 1973.

J. I. Kroschwitz and M. H. Grant, eds., *Encyclopedia of Chemical Technology*, 4th ed., Vol. 2, John Wiley & Sons, Inc., New York, 1992.

A. Yoshida et al., eds., *Nutrition: Proteins and Amino Acids*, Springer-Verlag, New York, 1990.

TETSUYA KAWAKITA
Ajinomoto Company, Inc.
Kawasaki, Japan

PROTEINS: DENATURATION AND FOOD PROCESSING

Food proteins constitute a wide range of protein types (eg, structural, storage, catalytic) and essentially include all those that are palatable, digestible, nontoxic, and accessible to humans. Although contributing to the nutritional value of foods by supplying a dietary source of essential and nonessential amino acids, proteins also serve as integral food components or ingredients by virtue of their diverse technological or functional properties (Table 1). These properties affect the behavior of the protein in a given food system during preparation, processing, or storage as judged by the quality attributes of the final product (1–3). They reflect a variety of interactions between physicochemical properties of the proteins per se (eg, molecular weight, amphiphilicity, structural flexibility), other food components (eg, water, carbohydrates, lipids, vitamins, minerals), and the environment in which these are associated. A variety of commodities and by-products may serve as the source of food proteins, yet their inclusion in the human diet usually necessitates some form of processing and/or preparation because exploitation of technological attributes normally requires the destruction and/or modification of the native protein structure (ie, denaturation). Processing affects not only each individual food component, but also the nature and intensity of interactions between components. Because many processing treatments can result in undesirable changes in the functional properties of food proteins and, therefore, contribute to poor final quality of protein-containing food products, an understanding of the relationship between processing factors and the changes in proteins that they induce is imperative. This article discusses protein stability and folding, protein denaturation, factors that influence it, and specific examples of food proteins denaturated during processing. Thorough discussions of various aspects of protein denaturation can also be found in References 4 to 10.

Table 1. Functional Properties of Proteins in Foods

General classification	Functional property
Organoleptic	Color, odor, flavor, texture, turbidity
Hydration	Solubility, dispersibility, wettability, water-holding capacity, water absorption
Surface	Emulsification, emulsion stability, foamability, whippability, film formation, fat absorption, binding properties
Thermal	Thermocoagulability, heat stability
Rheological	Elasticity, viscosity, gelation, cohesion, extrudability, adhesion, coagulation, aggregation, hardness, chewiness
Other	Compatability with additives, enzyme activity, susceptibility to modification

NATIVE PROTEIN STRUCTURE AND STABILITY

The functional properties of food proteins ultimately arise from their unique molecular conformations, which derive from all four levels of structural hierarchy (ie, primary, secondary, tertiary, quaternary) (11). During synthesis on the ribosome, the polypeptide chain, pending subsequent posttranslational processing (see section on protein folding, molten globules), adopts a unique molecular conformation traditionally referred to as the native conformation. This particular structure is dictated by the nature and sequence of amino acids in the polypeptide chain and is greatly influenced by solvent effects (12). The folding and resultant (native) conformation of a protein is largely governed by (equilibrium) thermodynamics. The third law of thermodynamics states that

$$\Delta G = \Delta H - T\Delta S \qquad (1)$$

where ΔG is the change in Gibbs free energy, ΔH is the change in enthalpy, T is absolute temperature (Kelvin), and ΔS is the change in entropy. To obey this law, the polypeptide chain must fold into a conformation such that the least amount of free energy is expended in maintaining it. In terms of free energy, hydrophobic effects contribute most to protein folding and stabilization. Hydrophobic interactions are nonspecific and result from the strong hydrogen-bonding properties of water. Nonpolar amino acid residues generally cannot participate in hydrogen-bonding; thus, water molecules surrounding nonpolar residues hydrogen bond with each other to form a highly ordered icelike structure that is associated with unfavorable (ie, low) entropy. The native polypeptide chain, therefore, tends to bury its hydrophobic residues within the interior of the molecule with exclusion of water from this core and to orient its hydrophilic amino acids toward the protein exterior. Exclusion of water from the protein interior causes a large increase in the entropy of the previously structured surrounding water, while the close packing of residues in the protein interior generally leads to decreased enthalpy. The folding process may thus be regarded as an entropy-driven transition from a state of higher free energy (eg, random coil) to that of lower free energy (eg, native conformation). Although this transition is largely governed by hydrophobic effects, the particular folding patterns and final protein conformation(s) are governed by formation of (specific) hydrogen bonds, disulfide linkages, and electrostatic and van der Waals interactions within the protein. Maximization of these strongly interacting driving forces leads to the native conformation of proteins. The adopted conformation may not correspond to the global minimum of free energy because this structure may represent a metastable state (ie, local minimum). Therefore, in addition to thermodynamic constraints, protein folding and conformational stability are also governed by kinetic constraints.

The thermodynamic stability of the native protein is marginal, generally not exceeding 60 kJ/mol, or the equivalent strength of only 3 to 4 hydrogen bonds or a single electrostatic interaction (Table 2) (7,8). This lability suggests that such environmental and processing factors as pH, temperature, pressure, and solvent effects may readily alter protein conformation and, consequently, functional properties and product quality to varying degrees. The ability to predict and/or estimate protein stability and conformational potential ("the capacity of biopolymers to form intermolecular junction zones that generate the desired structural rheological and other physico-chemical properties of a given food system," [13]) is, therefore, of vital importance in protein isolation and in processing of protein-containing foodstuffs (9). For example, during isolation of food proteins (especially enzymes), it is generally desirable to avoid or to at least minimize disruption of native structure because native proteins possess higher conformational potential and, as a consequence, superior functional properties. During processing, knowledge of protein stability and of the extent of conformational (and functional) change resulting from manipulation of processing factors is advantageous and often necessary if high-quality protein-containing food products are to be produced and maintained throughout subsequent storage.

Protein Folding: An Overview

Prefolding State: The Molten Globule. In order for a protein to achieve a three-dimensional native state, it must undergo a large number of possible conformations rapidly, prior to achieving its final product. A problem for biochemists is understanding how a protein achieves its native state (N) or becomes folded. Anfinsen (14) discovered that a protein's amino acid sequence fully determines its three-dimensional structure and, thus, its biological function. Work from the late 1970s and early 1980s gave rise to the concept that the protein native (N) and unfolded states (U) were an all-or-none scenario (U ↔ N) (15,16). Yet, although this theory was generally accepted, strong experimental evidence of proteins treated with high guanidine hydrochloride (GdnCl) concentrations showed large differences in hydrodynamic and optical parameters when compared to little changes in the presence of high temperatures and/or low pH (plus addition of salts) (17). Evidence mounted for the existence of an intermediate equilibrium between the native and unfolded states. It was shown that temperature-denatured proteins are far from being completely unfolded and can undergo another cooperative transition when GdnCl or urea is added (18,19). Circular dichroism (CD) studies showed the existence of one or more equilibrium states that differed from the native state by the absence of the rigid aromatic side chains; at the same time, they differed from the unfolded states by the presence of secondary structure (18). The conclusion was made that these intermediates were unfolded, noncompact molecules with local secondary structure (20). These studies showed a physical state that existed between native and unfolded and was given the term *molten globule* (Fig. 1) (22).

Molten globule (MG), as a term, has been applied to all compact denatured states that have substantial secondary structure, but little or no tertiary structure. This can cover a wide range of ordered and disordered, partially folded proteins with and without disulfide bonds (23).

The molten globule conformation is frequently adopted rapidly where an unfolded protein is placed under refolding conditions before the appearance of the fully folded protein. Because of the prefolded state, occurrence of the molten globule (24) was thought to be an indicator of a kinetic intermediate and assigned the kinetic role of

$$\mathrm{U} \leftrightarrow \mathrm{MG} \leftrightarrow \mathrm{N}$$

This equation would imply that the molten globule is required for the formation of the native state. From this equation, the initial rate of forming N should be zero upon

Table 2. Linkages and Interactions Involved in Stabilizing Protein Conformation

	Energy (kJ/mol)	Enthalpy contribution	Entropy contribution	Disrupting agents
Covalent bonding (–S–S–)	330–380			Reducing agents
Electrostatic interactions	42–84	+ or −	+	Salts, high or low pH
Hydrogen bonding	8–30	−	−	Urea, detergents, heat
Hydrophobic interactions	4–12	+	+	Detergents, organic solvents, urea, guanidine-HCl, cooling
van der Waals forces	1–9	−	-	

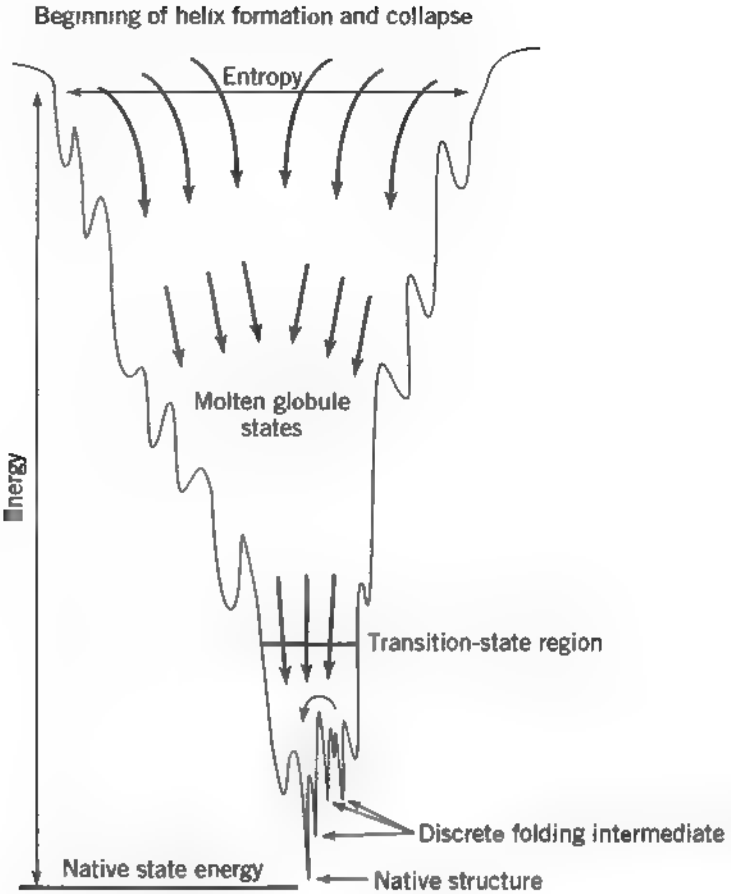


Figure 1. Molten globule state. *Source:* Ref. 21.

placement of the unfolded protein into refolding conditions. Only after the required molten globule intermediate has been generated should N appear. However, experimental data (25) have shown that under refolding conditions the equation should be rewritten to be

$$\begin{array}{rl} & \mathrm{U} \leftrightarrow \mathrm{N} \\ (\text{Fast}) & \updownarrow \\ & \mathrm{MG} \end{array}$$

The molten globule state in itself does not produce rapid folding, but has been speculated to be an off-pathway, nonproductive species that is the energetically preferred form of the unfolded protein under refolding conditions (25). Research has shown that the primary role for the molten globule state is to only slightly increase the overall rate of disulfide formation and to favor those interactions between cysteine residues distant in the polypeptide chain (26).

Physiological Role of the Molten Globule: Molecular Chaperones and Foldases. The physiological role of the molten globule has been speculated greatly during the past few years. Because of the properties that define the molten globule, proteins *in vivo* must adjust themselves to a large set of different conditions, eg, in the cytoplasm and/or near membranes where a number of these conditions are denaturing ones, ie, low pH or differing salt concentrations. The molten globule state permits proteins to exist in this state of flux, which allows for more pronounced small-scale fluctuations than the native state, which protects it from occasional loss of folding pattern by large-scale thermal fluctuations (27). The molten globule state and the newer, more expanded molten globule, the premolten globule (28), may be important for a class of proteins, chaperones, to trap proteins after their biosynthesis for self-assembly, transmembrane transport, and other processes that use protein molecules in a semiflexible (rather than in a rigid) state (27).

Molecular chaperones have been identified as a family of proteins that bind to and assist in the folding of proteins into their functional states. They do not form part of the final protein structure, nor do they possess steric information specifying a particular folding or assembly pathway (29). By recognizing unfolded or partially denatured proteins, the predominant role of chaperones seems to consist in the prevention of incorrect intra-and intermolecular associations of polypeptide chains that would result in their aggregation (30).

Consistent with their role in the folding of newly synthesized translocated proteins, many molecular chaperones are constitutively expressed. Under conditions that compromise protein folding and cell physiology, eg, heat shock, the synthesis of most molecular chaperones is induced to higher levels. It is then not surprising that many of the molecular chaperones were first identified in one or more organisms as heat shock proteins (HSPs) (31). However, it is now recognized that the same closely related proteins are frequently essential components of normal cells (30).

Chaperones themselves have aids or accessory proteins called co-chaperones that are responsible for mediating the activity of specific chaperones (32). They were first identified in *Escherichia coli*, and some have been shown to stimulate the rate of ATP hydrolysis (DnaJ) or act as a nucleotide exchange factor (as in the case for DnaK) (33).

The final group of protein folding helpers includes the array of proteins that act as foldases. Foldases include enzymes such as protein disulfide isomerases (PDLs) (34) and the immunophiles or peptidylprolyl isomerases (PPIs or rotamase) (35). These proteins have demonstrated catalytic activities that increase the rate of protein folding (36,37).

To perform its biological function, a protein cannot remain as a linear string of amino acids that has just come off of the protein-making ribosome. It must fold into its three-dimensional native state. The molten globule state is widely considered to be an important intermediate in protein folding and to have a polypeptide backbone with a nativelike topology. The importance of the molten globule is not in rapid protein folding, but for aiding in the trapping of the protein by molecular chaperones that assist in protein folding and targeting. The notion that chaperones are needed to assist protein folding is an interesting concept that extends rather than negates Anfinsen's findings (14) that proteins fold spontaneously based on their sequence. Although Anfinsen's findings were based on *in vitro* experiments, the chaperone (GroEL, in this example) appears to form a large central cavity inside its structure that allows the molten globule state protein the ability to self-assemble within the confines of the chaperone environment (38). The role of the chaperone (GroEL) is believed to be through assisting in the repeated binding and releasing of unfolded or partially folded polypeptide. During each binding interval, the chaperone sequesters the polypeptide and prevents formation of nonnative conformations. Eventually, when the protein has achieved its native state, it dissociates from the chaperone to perform its function(s).

When proteins are not in their native conformation, they suffer a loss in functionality. This loss of functionality is an important aspect not only in food processes but in nature.

PROTEIN DENATURATION

Protein denaturation has traditionally been defined as any modification of conformation that is not accompanied by cleavage of peptide bonds involved in primary structure. This definition is interpreted differently by various researchers and disciplines owing to a general inability to recognize the phenomenon when it occurs (5,6,10). Many problems in recognizing denaturation of food proteins are due to the fact that they are rarely pure entities, often precluding direct measurement of changes in their conformation; rather, they are usually a heterogeneous mixture in isolates and in food. In addition, a conformational change may not be sufficient to effect a detectable or significant change in functional properties of a food protein. However, a significant change in functional properties is usually the result of structural alteration. Thus, from a food science perspective, protein denaturation may best be defined operationally as any modification of conformation not accompanied by alteration of primary structure that results in a change in one or more of the functional properties of the protein. Certainly, direct measurement of changes in protein conformation is preferable; however, measurement of functional properties (Table 1) as a function of denaturing conditions may provide a meaningful assessment of food protein denaturation. Denaturation may also be assessed by measuring other properties of proteins (Table 3). Because the transition from the native (N) to denatured (D) state of a protein is accompanied by a change in energy, manifested by absorption or liberation of heat (ie, enthalpy), one of the most useful and direct methods of measuring parameters associated with food protein denaturation is differential scanning calorimetry (DSC) (6). A comprehensive review of thermal analysis of proteins is given by Harwalker and Ma (39). The primary advantage of DSC is that from thermograms generated, the following parameters can be obtained directly: heat capacity (C_p) of N and D states, change in heat capacity (ΔC_p) associated with the N → D transition, enthalpy change (ΔH) associated with the transition, and the temperature of denaturation (T_d) (Fig. 2).

Denaturation may ultimately lead to a completely unfolded polypeptide structure (ie, random coil), to a more compact globular structure, or to any of a number of intermediary and often short-lived conformations (eg, molten globule). Despite the fact that the native conformation of various (purified) proteins have been characterized by X-

Table 3. Properties of Proteins to Assess Denaturation

Functional
Thermodynamic
  Denaturation temperature
  Enthalphy
  Heat capacity
Hydrodynamic
  Sedimentation behavior
Electrophoretic
  Surface charge
Spectroscopic
  Ultraviolet, visible absorption
  Intrinsic fluorescence
  Extrinsic fluorescence
  Circular dichroism
  Optical rotary dispersion
  Light scattering
  Infrared
  Nuclear magnetic resonance
  Electron microscopy
Biological
  Catalytic/enzymatic
  Immunological
  Digestibility by proteases
Chemical reactivity of functional groups

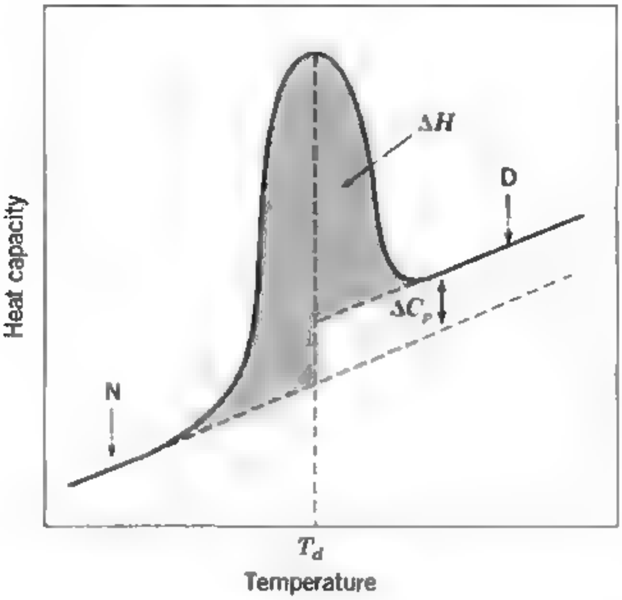


Figure 2. Parameters obtained from differential scanning calorimetry. N, Native state; ΔH, change in enthalpy; ΔC_p, change in specific heat capacity; T_d, denaturation temperature; D, denatured state.

Table 4. Denaturation Constants for the Transition State of Various Food and Food-Related Proteins

Protein	°r*H*° (kJ/mol)	$\Delta S°$ (J/mol/°C)
Pepsin	232.8	474.4
Lipase (pancreatic)	190.1	285.5
Amylase (malt)	174.2	219.0
Trypsin	168.3	187.1
Chymosin	373.9	870.9
Hemoglobin	316.5	639.3
Egg albumin	552.7	1,321.8
Peroxidase (milk)	775.8	1,951.0
Invertase (yeast)		
pH 3.0	311.5	638.1
pH 4.0	462.2	1,099.0
pH 5.2	361.7	774.6
pH 5.7	219.4	354.6

Source: Ref. 40.

ray crystallography and two-dimensional nuclear magnetic resonance, protein structures resulting from partial or complete denaturation are generally not well defined. The susceptibility of a protein to denaturation is dependent on the ability of the denaturant to break the bonds and/or interactions that stabilize the protein structure (Table 2). This requires energy input, ie, chemical or physical (Fig. 3). The relatively large values of denaturation constants in Table 4 (40) reflect the nature of denaturation (ie, N → D transition) in that many noncovalent interactions must be broken. Because every protein possesses a characteristic or unique structure, the effects of a denaturant are protein specific. For example, the denatured state of a protein may be transitional (eg, during thermal processing), after which it renatures with full recovery of its native (functional) properties (eg, many peroxidases). Conversely, the denatured state may be formed irreversibly and correspond to a new or technological state with predictable/desirable functional properties that differ from those of the native protein (eg, texturized plant proteins). Denaturation is generally reversible if the denatured protein (ie, unfolded polypeptide chain) is thermodynamically stabilized by the denaturant; removal of the denaturant allows the native conformation of the protein to be reestablished. In addition, small molecular weight proteins are more likely to renature than large molecular weight proteins. Denaturation is irreversible if the unfolded polypeptide chain is stabilized by interactions with other denatured proteins, as in acid or heat-induced protein aggregation. If disulfide bonds contribute to protein conformation and these are broken, denaturation is often irreversible.

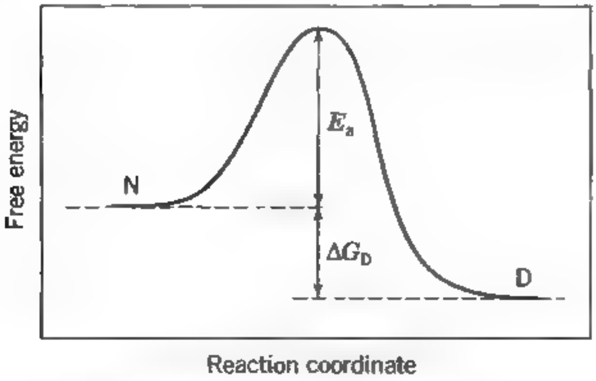


Figure 3. Energy output. N, Native state; ΔG_D, Gibbs free energy; E_a, activation energy; D, denatured state.

Protein denaturation is generally a cooperative multistate process (4) with many more-or-less unfolded intermediates (D_i) between the N and D states:

$$N \leftrightarrow D_1 \leftrightarrow D_2 \leftrightarrow D_3 \ \ldots \ \leftrightarrow D_n$$

These intermediate structures correspond to progressive stages in the alteration of the protein conformation. A protein does not have a rigid or static conformation but, rather, a dynamic conformation, undergoing rapid thermodynamic fluctuations about a mean conformation. Thus, each of the states in the $N \rightarrow D_i$ transition may be regarded as a population of structures of similar energy. Their existence or identification as discrete states requires that their populations not overlap.

Thermodynamic analysis of protein denaturation and/or conformational stability according to the foregoing scheme is complicated. Although often an oversimplification, it is useful to consider the denaturation process in terms of a two-state equilibrium (Fig. 4) (4,6–8). Use of a two-state model is valid if the DSC enthalpy change (ΔH, Fig. 2) approximates the van't Hoff enthalpy change. The equilibrium or denaturation rate constant for a two-state equilibrium can be written

$$k_D = \frac{(1 - f_N)}{f_N} = \frac{[D]}{[N]} \qquad (2)$$

where k_D is the equilibrium constant and f_N is the fraction of protein molecules in the native state. Reversible and

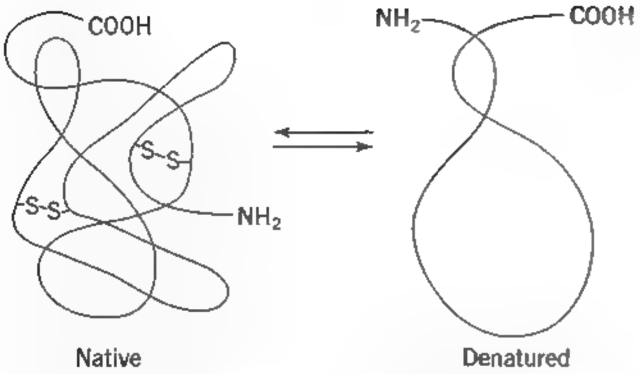


Figure 4. Two-state equilibrium model of the denaturation process.

irreversible denaturation are both initiated by reversible conformational change, eg, by unfolding. This initial conformational change is rate-limiting (41) and, in the case of irreversible denaturation, is followed by such secondary processes as aggregation or covalent modification (eg, proteolysis) resulting in a change in primary structure. If irreversible denaturation takes place, analysis of protein denaturation using the two-state model is precluded, because the two-state equilibrium is accompanied by an irreversible transition from the D state to an alternate structure(s) that cannot be readily characterized. Analysis of protein denaturation using the two-state model, therefore, requires that three criteria be met: (*1*) the N → D transition must be thermodynamically reversible; (*2*) an experimental technique must be used such that the transition be detected in the presence of the perturbing influence or denaturant; and (*3*) a reference conformational state, usually taken to be the N state, must be identified such that the stabilities of different proteins are compared. The limiting case for thermodynamic reversibility is an infinitely dilute solution. However, if thermodynamic reversibility can be assumed for the protein system in question, good approximation of the thermodynamic parameters $\Delta G°$, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ can normally be obtained from

$$\Delta G^{\circ}_{\mathrm{app}} = -RT \ln k_{\mathrm{D}} \qquad (3)$$

$$\Delta H^{\circ}_{\mathrm{app}} = -R\,\frac{d(\ln k_{\mathrm{D}})}{d(1/T)} \qquad (4)$$

$$\Delta C^{\circ}_{\mathrm{Papp}} = \frac{d(\Delta H^{\circ}_{\mathrm{app}})}{d(T)} = T\,\frac{d(\Delta S^{\circ}_{\mathrm{app}})}{d(T)} \qquad (5)$$

where $\Delta G^{\circ}_{\mathrm{app}}$ is the apparent change in standard free energy, $\Delta H^{\circ}_{\mathrm{app}}$ is the apparent change in enthalpy, $\Delta S^{\circ}_{\mathrm{app}}$ is the apparent change in entropy, $\Delta C^{\circ}_{\mathrm{Papp}}$ is the apparent change in heat capacity, k_{D} is the equilibrium or denaturation rate constant, R is the gas constant, and T is absolute temperature (Kelvin). Protein denaturation is normally accompanied by increases in (*1*) enthalpy, indicating that the N state possesses a lower free energy than the D state; (*2*) entropy, associated with the disorder that results from unfolding of the polypeptide chain; and (*3*) heat capacity, related to the transfer of apolar or hydrophobic groups to the aqueous environment during denaturation, with associated structuring of water. The stability of the N state relative to the D state, or the intrinsic conformational stability of the protein, is represented by ΔG_{app} (ΔG_{D}) (Fig. 3). A meaningful comparison of the stabilities of different proteins requires a knowledge of $\Delta G°$ and the derived thermodynamic quantities $\Delta H°$, $\Delta S°$ and $\Delta C_p°$ (4). These quantities are normally obtained from temperature, pressure, or concentration dependencies of ΔG_{app} (or k_{D}).

FACTORS AFFECTING PROTEIN DENATURATION

As previously stated, many food processing operations to which protein-containing foods are subjected, can result in protein denaturation. Such factors as temperature, pH, pressure, shear, irradiation, and the presence of salts and oxidizing/reducing agents can be used by the processor to produce desirable and high-quality products. However, these factors may also serve as processing hazards that must be avoided in order to maintain native protein structure and associated functional properties. In the broadest sense, processes that result in protein denaturation can be divided into two major categories: physical and chemical.

Physical Denaturants

Heat. Application of thermal energy is the most common means by which food is processed. Among the major components in food, proteins may be the most sensitive to temperature extremes. Thermal denaturation of proteins may be detrimental to product quality, as reflected in their reduced functional properties (Table 1), or desirable, as in the heat processing of whey for use in confections. The denaturing effects of increased temperature are dependent on many factors, including protein type and concentration, water activity, pH, ionic strength, and the nature of ions present. For most chemical reactions, rate increases approximately twofold for each 10°C rise in temperature. However, the rate of protein denaturation may increase 600-fold. This large difference in reaction rate is attributable to the low-energy bonds/interactions stabilizing protein conformation (Table 2). The dependence of the rate of protein denaturation on temperature can be determined from (first-order) thermal denaturation curves derived using any of a number of different methods to monitor changes in protein conformation (Table 3) These curves provide information needed to calculate such kinetic/processing parameters as activation energy and z values. These values are related by

$$E_a = \frac{2.303RTT_1}{z} \qquad (6)$$

where E_a is the activation energy (kJ/mol), R is the universal gas constant, T is temperature (Kelvin), T_1 is the temperature 10°C above T (Kelvin), $1/z$ is the slope of the denaturation curve, and z is the temperature change (°C) required to change the thermal denaturation rate by a factor of 10 (42,43). The energy required to denature the protein is represented by activation energy (Fig. 3, Table 4), which can be calculated from the Arrhenius law

$$E_a = RT^2 \frac{d(\ln k_D)}{d(T)} \qquad (7)$$

where k_D is the equilibrium or denaturation rate constant and T is absolute temperature (Kelvin). The values for transition-state enthalpy (ΔH) of various proteins listed in Table 4 are related to E_a by equation 8:

$$E_a = \Delta H + RT \qquad (8)$$

Values of E_a depend on the extent of denaturation, ie, the nature of the D state. For example, the E_a values associated with the thermal denaturation of proteins, from the N state to a completely unfolded state (eg, random coil), are large relative to other chemical reactions. Although covalent bonds other than disulfide cross-links are not broken during denaturation, a large number of low-energy noncovalent bonds and interactions are broken. The E_a values associated with thermal denaturation (ie, inactivation) of enzymes are relatively low, however, because the active sites of most enzymes are dependent on only a few low-energy bonds and/or interactions. Enzyme inactivation may be a direct consequence of thermally induced modification of protein conformation; however, the effects of temperature on substrate(s), activators, and inhibitors must also be considered.

Thermal denaturation of food and food-related proteins generally takes place between 55 and 80°C; enzymes tend to be more sensitive to the effects of heat and may begin to denature at a temperature as low as 45°C. Decreased solubility may accompany protein denaturation as heat-induced exposure of hydrophobic groups to solvent (ie, water) may result in the aggregation of unfolded protein molecules (7). A reduction or loss of biological activity and increased water absorption, susceptibility to protease digestion, and intrinsic viscosity may also accompany (thermal) denaturation. In addition, heat-mediated chemical alteration of proteins and their constituent amino acids can result (5,10). For example, dehydrogenation of serine, deamidation of glutamine and asparagine, and formation of new intra- and/or intermolecular covalent cross-links (eg, γ-glutamyl-ϵ-N-lysine) can significantly decrease the nutritional quality of proteins.

Cold. At some stage between postharvest/postslaughter and consumption, many foods are subjected to refrigeration and/or freezing so that product quality be maintained. However, just as the application of thermal energy can result in denaturation of proteins in foods, so too can the removal of thermal energy. Low-temperature denaturation of proteins is largely mediated by a reduction in hydrophobic interactions in conjunction with enhanced hydrogen bonding (Table 2). Thus, low temperatures can lead to aggregation and precipitation of proteins and/or to alteration of quaternary structure. For example, cold inactivation (ie, 10°C) of the glycolytic enzyme phosphofructokinase occurs by dissociation of the tetramer to two dimers as a result of weakened hydrophobic interactions between subunits.

The principal injurious effect of freezing is not low temperature per se, but the concomitant concentration of all soluble species as pure ice separates from the mixture. Concentration of acids and/or salts, resulting in large changes in pH and ionic strength, can have profound effects on proteins. At high subzero temperatures, the extent of protein denaturation is greatest, whereas at or below the eutectic temperature of the food system, minimal damage occurs (4). Fish proteins are particularly susceptible to destabilization by freezing temperatures. As a result, fish may become tough and exhibit excessive drip loss on thawing. Similarly, caseinate micelles of milk, which are relatively stable to heat, may be destabilized and coagulate during freezing. Not all proteins, however, are sensitive to freezing temperatures. In fact, several lipases and oxidases are resistant to freezing and remain active at subzero temperatures. In order to inactivate (ie, denature) these enzymes, foods are heat-treated (eg, blanching of vegetables) before frozen storage.

Pressure. Generally, proteins are not sensitive to pressure, and only when large pressures are applied do they exhibit changes. It appears that in most cases the denaturing effect is dependent on applied pressure, exposure time, pH, protein concentration, and temperature (44). Pressure-induced denaturation is thought to result from a decrease in protein volume accommodated by holes in the native protein structure and exposure of hydrophobic groups to solvent (45). The effects of shear on proteins are not unrelated to those for pressure. Shearing action during extrusion processing contributes to the texture of the final product; however, the denaturing effect of the shear plates is most likely secondary to the high temperatures and pressures used during texturization of the proteins. This subject has been reviewed (46). Shear denaturation can also occur during protein purification (eg, at pump heads and in chromatographic columns) and in immobilized enzyme reactors (4).

Interfaces. Gas–liquid, liquid–liquid, and liquid–solid interfaces commonly occur in food systems, eg, emulsions, foams, and aerosols. These interfaces are thermodynamically unstable; however, compounds may be present or added, with an affinity for each phase, that reduce energy at the interface and stabilize the system. Proteins, owing to their amphoteric nature and relatively high molecular weights, tend to migrate to interfaces and, in so doing, reduce interfacial tension between phases. The protein thereby adopts a higher energy state, ie, becomes denatured. This phenomenon is technologically exploited in the processing of such food products as milk, ice cream, butter, finely comminuted meats, cakes, and salad dressings. Yet denaturation of proteins at interfaces may also be detrimental, a phenomenon to be avoided or at least controlled during food processing.

The mechanism of denaturation at interfaces is a two-step process (Fig. 5), the first step involving rapid and diffusion-controlled sorption at the interface until a monolayer of concentration 2 to 3 mg/m^2 is attained. The pro-

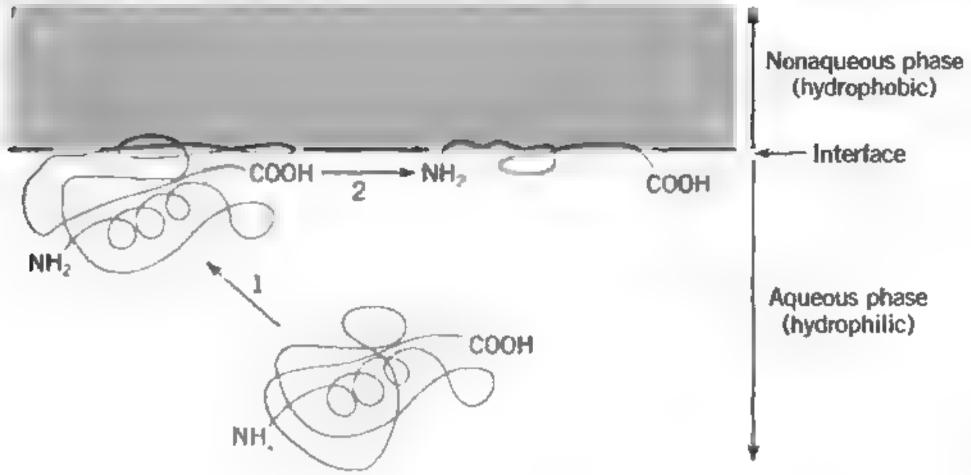


Figure 5. Two-step process of denaturation at interfaces.

pensity of different proteins to become sorbed at interfaces is dependent on their structures. Proteins that do not contain sizable hydrophobic or hydrophilic regions, or that possess disulfide cross-linked stabilized structures, tend not to adsorb at interfaces. When proteins are sorbed at interfaces, their sorbed structure depends on the stability of the native conformation. Once sorption has occurred, protein molecules reorganize (eg, unfold) and become denatured. This second step is rate-limiting, the rate being dependent on the conformational potential of the protein and its concentration. Detailed information on the denaturation behavior of β-casein, bovine serum albumin, and lysozyme at interfaces is reported in References 47 and 48.

Irradiation. Ionizing radiation (eg, γ, high-energy electrons) has been proposed for application in several areas of food processing, eg, inhibition of sprouting in onions, potatoes, and carrots; sterilization; and pasteurization. The effectiveness of irradiation as an antimicrobial agent has been well documented. However, at the levels of ionizing radiation required to destroy microorganisms (eg, 10 kGy), deterioration of sensory and nutritive quality may still result from autolysis (49). In general, the dose of ionizing radiation required for complete enzyme inactivation *in situ* is about 10-fold greater than that necessary to destroy microorganisms (50,51). Denaturation of proteins by irradiation is analogous to denaturation by other means; however, the specific effects are dependent on the wavelength and energy of the applied field. Other factors include the nature of the protein (enzyme), water activity, protein concentration and purity, oxygen tension, pH, and temperature (50). The structural alterations that irradiation can induce in proteins (eg, amino acid oxidation, ionization, free-radical formulation, polymerization) are often mediated by radiolysis of water (43). If the applied energy is sufficiently high, covalent bonds may be ruptured.

Chemical Denaturants

pH. Manipulation of pH is one of the oldest modes of food preservation, often used in combination with other preservation methods, eg, thermal processing, refrigeration, fermentation. pH is a critical determinant of protein conformation, stability, and function. Most proteins are stable over a characteristic pH range. Outside this range, denaturation can result from electrostatic repulsion of ionized groups within the protein molecule. The extent of denaturation is determined not only by the number of ionized groups but also by the location of these groups within the protein molecule (5,10). pH-induced denaturation of proteins is influenced by such factors as temperature, dielectric constant of the solvent, and ionic strength. In general, at high pH proteins remain soluble, largely because of the predominance of negative charges and their repulsion. In some instances, native conformation may be recovered when the pH is adjusted back to the characteristic range of the protein. Conversely, at low pH, proteins often aggregate, because positive charges rarely predominate within the pH ranges typically encountered during food processing.

Salts. Salts are used in many food processes and formulations for preservation and for development of flavor and texture. The denaturing effect of salts (ie, ions) on proteins in foods is difficult to assess: it is dependent on the nature of the protein, pH, and the size, charge, and concentration of the ion(s). Salts may react directly with proteins via electrostatic interaction, or they may alter the structure and orientation of water molecules around proteins. Ions may salt in (ie, solubilize) or salt out (ie, precipitate) proteins according to their location in the Hofmeister (lyotropic) series:

$$Ca^{2+} > Mg^{2+} > Li^{+} > Na^{+} > K^{+} > NH_4^{+}$$

$$PO_4^{3} > SO_4^{2-} > \text{citrate}^{2-} > \text{tartrate}^{2-} > \text{acetate}^{-} > Cl^{-} > Br^{-} > NO^{3-} > I^{-} > ClO_4^{-} > SCN^{-}$$

Salting-in ions (eg, Cl^{-}, ClO_4^{-}) denature proteins, whereas salting-out ions (Ca^{2+} SO_4^{2} PO_4^{3-}) stabilize native protein conformation. Salting out is believed to result from competition between proteins and ions for available water (4,5,10). Denaturation of proteins via salting-in arises from charge neutralization with concomitant reduction of hydrophobic effects.

Oxidation/Reduction. Various technological processes involve the use of oxidizing and reducing agents. Although not considered denaturants per se, these agents may lead to modification of amino acid residues (ie, cysteine, methionine, tryptophan, tyrosine, histidine), thereby resulting in protein denaturation. Food-grade oxidizing agents include peroxides (eg, H_2O_2, benzoyl peroxide), which are used for cold sterilization of milk and milk products and for bleaching of flour. Catalysts of lipid peroxidation (eg, light, heat, divalent metal ions, irradiation) also promote, directly and indirectly (ie, via reaction with lipid peroxides and their degradation products), oxidation of proteins. Bromates and oxidizing enzymes, which promote thiol-disulfide interchange through mild oxidation, are often used in the baking industry to improve the viscoelastic properties of gluten proteins. Reducing agents such as cysteine and ascorbic acid, which are commonly added to fruit juices, can similarly cause protein denaturation, but by disruption of disulfide cross-links. Alkaline conditions may also induce disulfide reduction in addition to lysinoalanine formation. Denaturation by reducing agents increases the susceptibility of the proteins to subsequent proteolysis and modification reactions.

DENATURATION OF FOOD PROTEINS

Controlled protein denaturation and associated reactions arising from processing of foods and isolated proteins are critical for some applications, eg, the formation of emulsions, foams, gels, and fibers. The following is a discussion of the effects of various environmental and processing factors on the major food protein classes (ie, meat and fish, milk, egg, plant).

Meat and Fish Proteins

The characteristics of proteins that affect their properties in comminuted meat products are not well defined (52). The ability of proteins to bind water and fat, as well as to retain these two components during heating and storage, is critical in the manufacture of processed meat products. Binding properties affect not only cook yield, but also final appearance and texture. Evaluation of individual muscle proteins suggests that the salt-soluble proteins (ie, myofibrillar proteins) are the major contributors in emulsification. Meat proteins generally display improved emulsification properties in the presence of increasing salt concentration, especially at pH values near or below their isoelectric points (pI). This apparent salt-induced shift in pI serves to increase or maintain protein solubility, an essential requirement for emulsification (53).

The emulsification properties of meat and fish protein are affected by changes in solubility, as induced by frozen storage, heating, and pH. However, solubility is not a good predictor of emulsification or other functional properties. One study reported that the amount of soluble protein from fresh meat sources was highly correlated with emulsification properties, irrespective of original meat source (54). For frozen or cooked meat, however, not only did the soluble protein content decrease, but the remaining noncoagulated soluble protein had lower emulsification properties compared to fresh meat sources. This observation was attributed to denaturation of soluble proteins that could have been caused by shearing during emulsification. Undenatured proteins are required for good emulsification properties since they possess greater conformational potential. Shear-induced protein denaturation may be followed by aggregation, this being intensified with increasing protein concentration. Dissociation of muscle protein complexes and subsequent protein denaturation without accompanying aggregation results in increased hydrophobicity of the salt-extractable proteins, with little change in their solubility or total sulfhydryl group content. Protein denaturation may be enhanced by hydrophobic association of the polypeptide chains at oil/water interfaces, resulting in a much larger available protein volume/surface area and increasing emulsifying capacity (1). Under moderate heating conditions (ie, <50°C), emulsification and functional properties of salt-extracted meat and fish proteins are often improved. At higher temperatures (ie, 50–70°C), functional properties are impaired, ie, proteins aggregate as reflected in decreased solubility and sulfhydryl group content. In addition, at temperatures above 70°C, proteins display decreased hydrophobicity (55).

The setting of a meat emulsion in comminuted products, in addition to binding of meat pieces in restructured or reformed products, is believed to be based on the establishment of a stable protein gel. Gelation arises from protein denaturation and subsequent association to form a three-dimensional matrix and is generally heat initiated, because raw meat pieces do not significantly bind to each other (56). An exception is the low-temperature (about 4°C) setting of sols from certain fish species that can occur on storage. During gel formation, exposure of sulfhydryl groups has been observed (55), in addition to shifts in pI caused by exposure of previously masked charged groups (57).

Perishability and compositional variations affect the use of fish muscle protein as a raw food material (eg, in the manufacture of kamoboko and surimi) (52). Aside from microorganisms and oxidative rancidity, the most conspicuous determinants of the quality of fish muscle proteins are alterations in functional properties, a direct reflection of protein denaturation (58). Formaldehyde is often produced during the storage of fish, especially gadoids, and has been implicated in the toughening of fish muscle during frozen storage. Through interaction with the side chain groups of fish muscle proteins, formaldehyde can increase the rate of protein denaturation, leading to aggregation of proteins and subsequent toughening (59). Denaturation of fish muscle proteins, especially on frozen storage, may also result from oxidation of free fatty acids and lipid peroxides or lipid–protein complex formation. When fish muscle proteins are thermally or surface denatured, the degree of denaturation is intensified in the presence of lipids. Yet, in other instances, intact lipids may function to stabilize and protect these proteins (53).

Milk Proteins

Milk proteins can be divided into two major groups: caseins and whey (or serum) proteins. Of these two, whey proteins, which are produced by heat precipitation and are used extensively as food ingredients, are extremely thermal labile (60). Thermal denaturation of whey proteins in fluid milk is manifested by the development of cooked flavor (61). Efficient use of whey as a functional food ingredient requires a knowledge of the denaturation behavior of individual whey proteins (62). Whey protein denaturation is considered a two-stage process: (*1*) disruption of tertiary and secondary structure, followed by (*2*) aggregation and coagulation, a phenomenon often associated with gel formation (5). Disulfide interchange reactions are largely responsible for aggregation and coagulation of β-lactoglobulin (a major component of whey) (63). The ability of whey proteins to form heat-induced gels is important in many food systems (eg, yogurt). Factors important in gelation include temperature, duration of thermal treatment, type and concentration of ions, state of the sulfur-containing amino acids, type of acidulant, and total solids concentration. In general, increases in total solids offer a protective effect by decreasing the rate and extent of whey protein denaturation (64). The presence of ions affects the thermal aggregation of β-lactoglobulin variably: phosphate and citrate inhibit, whereas calcium enhances, aggregation (65). Thermal denaturation of whey proteins modifies the course of milk coagulation and the rheological properties of the curd formed by acid or enzymes. In milk, thermally induced formation of intermolecular disulfide linkages occurs between unfolded β-lactoglobulin molecules and between unfolded β-lactoglobulin and casein micelles (ie, κ-casein and possibly α_{s1}-casein) (66). At temperatures in excess of 100°C, whey proteins may be extensively bound to casein micelles, thereby altering their surface properties.

Nonfat dry milk (NFDM) is used extensively in formulated foods and is produced from pasteurized skim milk, which is vacuum concentrated and spray-dried under conditions that result in either a low-heat or high-heat product. Low-heat NFDM is required for most applications that depend on a highly soluble protein (eg, emulsification) because it is processed under conditions that minimize whey protein denaturation and complexation with casein micelles (67). The casein proteins (ie, α-, β-, and κ-caseins) are generally not heat coagulable. In normal fluid milk, caseins resist coagulation for as long as 14 h at boiling temperatures or 1 h at 130°C. Thermocoagulation of casein in milk can occur as a result of compositional changes within the milk itself induced by sustained exposure to high temperatures (eg, increased acidity, a shift from soluble to colloidal forms of calcium and phosphates, denaturation, and hydrolysis of other milk proteins). Coagulation of milk is often attributed to the destabilization of casein micelles; however, this phenomenon may be the summation of many changes in the colloidal system (68).

Egg Albumin Proteins

The albumins (eg, ovalbumin, ovotransferrin, ovomucoid) are among the most widely used proteins in food formulation, owing to their exceptional gelling and whipping properties. Their functional diversity is directly attributable to their susceptibility to a variety of denaturants (69). Denaturation of albumins is a discrete phenomenon, the extent of which is dependent on temperature, pH, salt, and moisture content (69). When used in food formulations, egg protein gels that have been thermally set support and bind other ingredients within the matrix and contribute to the texture of the product (5,10). The extent of egg protein gelation (ie, denaturation) is influenced by the degree of oxidation of free sulfhydryls to form inter- or intramolecular disulfide linkages. For example, strong oxidizing agents, such as the metallic cations Fe^{3+} and Cu^{2+}, in addition to potassium iodate, enhance gel formation. However, hydrogen peroxide and potassium bromate, comparatively weak oxidizing agents, have little influence on gel strength. At alkaline pH values (eg, 9–10), reduction of disulfide linkages may occur that could also affect gel strength.

Egg albumin proteins are often pasteurized to facilitate their later use in formulated food products. Pasteurization generally does not affect their functional properties. However, ovotransferrin is more prone than ovalbumin to thermal denaturation. Ovalbumin is most stable at pH7.0, whereas ovotransferrin is least stable to thermal denaturation at this pH (5,10,69). At pH 6.8 and 50°C, up to 50% of the ovotransferrin is denatured in 4 to 5 min. In the presence of metals (eg, iron and aluminum), however, conformation of this protein is stabilized by a chelation-type mechanism. This protective effect is the impetus for the addition of aluminum and adjustment of pH to 7.0 with lactic acid before pasteurization of egg whites. Alternative pasteurization processes rely on lower temperatures and the addition of antibacterial agents (eg, hydrogen peroxide) (69). The native conformation of ovomucoid is resistant to extremes of pH and temperature. Prolonged exposure of ovomucoid to temperatures of 100°C does not alter its physicochemical properties. Its stability is attributed largely to intramolecular disulfide linkages (70). Denaturation of ovomucoid is suggested to be a three-stage process involving denaturation of each of three separate domains within the protein (71). If exposure to denaturing conditions is not prolonged, denaturation of ovomucoid is reversible (72).

Surface tension, important in such functional properties as foaming and emulsification, of egg and other proteins decreases markedly as denaturation proceeds. Thermal denaturation without coagulation improves surface properties (Table 1). This again implicates, as with meat proteins, the limited importance of solubility for functional (eg, surface) properties. Once sorbed at the interface, surface hydrophobicity plays a more dominant role in the foaming and emulsifying properties of egg proteins than does solubility.

Plant Proteins The impetus for utilization and development of (new) plant protein resources has been twofold: (*1*) to supplement, simulate, and/or replace muscle protein systems, and (*2*) to meet the increasing nutritional requirements of an increasing Third World population. Detailed reviews concerning denaturation of plant proteins in relation to their functional properties and food applications have been published (1,73).

Soybeans have been used in food systems, owing to their excellent functional and nutritional qualities. The major globulins of soy protein are conglycinin (7S) and glycinin

(11S). On heating, the subunits of both of these proteins can dissociate and reassociate in different ways. Formation of soluble aggregates of soy protein and the existence of sol, progel, gel, and metasol states and the roles of different forces in their formation have been discussed (74). The 11S globulin oligomer appears to undergo only minor changes on heating, retaining its quaternary structure and undergoing little obvious denaturation during formation of a gel when heated to 100°C (75). The importance of the overall hydrophobicity of unfolded proteins, rather than the surface hydrophobicity of undenatured proteins, for thermal functional properties has been noted for various proteins. High-heat treatment (121°C) of soy protein isolate at pH 5.5 led to a marked increase in overall hydrophobicity and a large reduction of solubility and emulsion stability (76). In contrast, the same heat treatment at pH 7.2 caused a moderate increase in overall hydrophobicity and a large increase in solubility and emulsifying properties. It is evident that environmental factors (eg, pH, heat) have a major impact on the functional properties of soy proteins.

Peanut and soy proteins that are heat denatured without any accompanying precipitation show enhanced foamability (77). Factors affecting foaming of these proteins include structural properties of the proteins per se (eg, flexibility, exposure of hydrophobic/hydrophilic groups, surface charge), ease of unfolding, and the Marangoni effect (ie, the ability to concentrate rapidly at a stress point). Also important are environmental factors such as temperature, pH, ionic strength, viscosity, and the presence of other components such as denaturants that could affect the intrinsic properties of the proteins (53).

The unique ability of wheat flour to form a cohesive and viscoelastic paste or dough when mixed and kneaded is due primarily to the properties of the two classes of principal storage proteins, gliadins and glutenins. These are collectively referred to as gluten proteins. Glutenins are responsible for the elasticity, cohesiveness, and mixing tolerance of the dough, whereas gliadins facilitate fluidity, extensibility, and expansion of the dough, thus contributing to loaf volume. A proper balance of both gluten proteins is essential for bread making. During the mixing and kneading of hydrated wheat, the gluten proteins orient, align, and partially unfold (ie, denature). Protein unfolding enhances both hydrophobic interactions and the formation of disulfide cross-links through disulfide interchange reactions, resulting in establishment of a three-dimensional matrix that serves to entrap starch granules and other dough components. The cleavage of disulfide cross-links by reducing agents such as cysteine destroys the cohesive structure of the hydrated dough. The addition of oxidizing agents such as bromate increases toughness and elasticity by promoting disulfide linkage formation. In addition to the gluten proteins, soluble proteins (ie, albumins and globulins) that are found in minor quantities denature and aggregate to aid in gel formation, thus contributing to setting of the bread crumb (78).

Drying, roasting, and cooking are thermal treatments routinely used to process plant proteins. These processes can cause substantial protein denaturation. Oat globulin, the major protein fraction in oats, has a quaternary structure similar to that of soy 11S globulin (glycinin), a heat-coagulable protein. Differential scanning calorimetry showed that oat globulin, heated under conditions inducing gelation, was not extensively denatured and exhibited highly cooperative transition characteristics (79). When 1% oat globulin was heated, aggregation and precipitation occurred. Ultraviolet and fluorescence spectra of soluble and insoluble fractions indicated no marked protein unfolding in the former fraction, but extensive denaturation in the insoluble aggregates. The insoluble fraction had significantly higher surface hydrophobicity than the soluble fraction and unheated protein (80).

Sonication has been used as a means to solubilize plant proteins and isolates/meals. Heat-treated, acid-precipitated soy proteins, intermediates in the commercial production of isolated proteins from defatted soybeans, have been dispersed by sonication (81). Changes in flow properties may have been derived from ultrasonic-induced dissociation of protein aggregates formed during thermal treatment. This was considered to be associated with partial cleavage of intermolecular hydrophobic interactions, but not cleavage of peptide or disulfide bonds. It was postulated that ultrasonically exposed hydrophobic regions are subsequently buried through rearrangement of the molecular structure, conferring greater hydrophilicity to the protein (81). However, it has been shown that sonication leads to agglomeration and aggregation, particularly of the 7S fraction (82). The ultrasonic action may have (*1*) promoted hydrophobic interactions between globular proteins; (*2*) induced formation of complex mixtures, as in the case of apolipoproteins; or (*3*) altered the equilibrium protein–protein and/or protein–lipid interactions, thereby favoring the formation of a cluster-type structure.

Thermoplastic extrusion technology has been used to texturize many plant proteins, especially soy, to produce fibrous structures that simulate meatlike products (83). The process begins with moistened, defatted soy flour, which is fed into an extruder where it is worked and heated, causing the protein molecules to denature and form new cross-linkages that result in a fibrous structure. The heated plasticized mass is forced through a die to form expanded texturized strands of vegetable proteins that have meatlike characteristics on rehydration (84). Extrusion of plant proteins has been reviewed (46).

STABILIZATION OF PROTEINS

The intrinsic instability of some proteins can be problematic for some food processing applications, eg, the use of a less-stable enzyme would require more time and larger amounts of enzyme to achieve the same processing goal as compared to a more stable enzyme. Following the introduction of site-directed mutagenesis in 1982 (85), research focused on the improvement of stability of proteins by site-specific mutations rather than random mutations. This approach was based on the hypothesis that the stability of proteins was governed by (*1*) bonds and interactions, (*2*) conformational factors, and (*3*) protection by modifications (86). To test this hypothesis, new disulfide bonds were introduced to stabilize various proteins, eg, T4 lysozyme

(87,88), subtilisin (89–91), dihydrofolate reductase (92), and λ repressor (93).

In a series of experiments, Matthews et al. stabilized T4 lysozyme using different strategies, eg, α-helix dipoles, entropy, conformation strain, and hydrophobic interactions (94,95). An 8°C increase in T_m (melting temperature) using a combination of strategic mutations was achieved (96–101). By changing the hydrophobic interaction between the subunits of L-lactate dehydrogenease, a 10°C increase in T_m was achieved (102). As an alternative type of hydrophobic interaction manipulation, site-specific glycosylation of hen egg white lysozyme resulted in a dramatic increase of thermostability (103). Suzuki et al. reported that the introduction of proline could stabilize protein by stabilizing the secondary structure (104). T4 lysozyme was stabilized by introducing a proline residue into an α-helix (105). Nakamura et al. also showed that *Bacillus* neutral protease was stabilized by introducting proline into a core α-helix (106).

Despite many successful reports on the stabilization of protein by site-directed mutagenesis, there is still no general theory to predict the site to be mutated. Gilis and Rooman used the solvent accessibility of the residues to determine the mutation site (107,108). The prediction of stabilization was based on a database containing the results of the mutation studies of various proteins.

Fersht's group, using barnase, conducted a series of mutation studies on their effects on stability and concluded the effects were varied even in a certain kind of the interactions, depending on the context (109,110). Furthermore, research based on the this hypothesis required that the three-dimensional structures be known in order to determine the mutation site.

Future research could provide a general theory on protein stabilization. Serrano et al. suggested one such possible theory. Systematic multiple mutation could stabilize proteins without the knowledge of the three-dimensional structures but would be dependent on two proteins with high sequence similarity (111). The reader is referred to the article by Jiminez-Flores and Bleck regarding recent advances in food protein biotechnology (112).

CONCLUSION

Various processing and environmental factors influence the functional properties of proteins; however, despite advances in molecular biology and analytical techniques the molecular basis for induced functional changes still remains ill defined. The reader is referred to articles by Jimenez-Flores and Bleck (112) and Yada et al. (113) for references regarding to recent advances in food biotechnology and analytical techniques, respectively. Although great gains have been made in the understanding of protein structure and its stabilization and, therefore, functionality, a need still exists to better define and characterize the denaturation of (specific) proteins if we are to control this phenomenon and take advantage of the technological attributes that proteins have to offer. Methodology by which to recognize denaturation when it occurs must be standardized. That food by its very nature is a multicomponent system in which a myriad of interactions can/have occurred makes this a foreboding task. Yet, in order to meet this mandate, the use of multicomponent or actual food systems is required. It must be stressed that although every protein has a unique structure and, therefore, unique conformational potential, the behavior of a protein in a given system is governed by thermodynamic and kinetic constraints.

ACKNOWLEDGMENTS

The financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

BIBLIOGRAPHY

1. J. E. Kinsella, "Functional Properties of Proteins in Foods," *CRC Crit. Rev. Food Sci. Nutr.* **7**, 219–280 (1976).
2. J. E. Kinsella, "Relationships between Structure and Functional Properties of Food Proteins," in P. F. Fox and J. J. Condon, eds., *Food Proteins*, Applied Science Publishers, New York, 1982, pp. 51–103.
3. S. Damodaran, "Functional Properties," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 167–234.
4. F. Franks, "Conformational Stability: Denaturation and Renaturation," in F. Franks, ed., *Characterization of Proteins*, Humana Press, Clifton, N.J., 1988, pp. 95–126.
5. A. Kilara and T. Sharkasi, "Effects of Temperature on Food Proteins and Its Implications on Functional Properties," *CRC Crit. Rev. Food Sci. Nutr.* **23**, 323–395 (1986).
6. C. N. Pace, "The Stability of Globular Proteins," *CRC Crit. Rev. Biochem.* **3**, 1–43 (1975).
7. C. N. Pace, "Protein Conformations and Their Stability," *J. Am. Oil Chem. Soc.* **60**, 970–975 (1983).
8. R. H. Pain, "The Conformation and Stability of Folded Globular Proteins," in F. Franks, ed., *Characterization of Protein Conformation and Function*, Symposium Press, London, 1979, pp. 19–36.
9. V. B. Tolstoguzov, "Some Physico-Chemical Aspects of Protein Processing into Foodstuffs," *Food Hydrocolloids* **2**, 339–370 (1988)
10. A. Kilara and V. R. Harwalkar, "Denaturation," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 71–165.
11. R. D. Ludescher, "Physical and Chemical Properties of Amino Acids and Proteins," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 23–70.
12. G. E. Schulz and R. H. Schirmer, *Principles of Protein Structure*, Springer-Verlag, New York, 1979
13. V. Tolstoguzov, "Structure–Property Relationships in Foods," in N. Parris et al., eds., *Macromolecular Interactions in Food Technology*, American Chemical Society, Washington, D.C., 1996, pp. 2–14.
14. C. B. Anfinsen, "Principles that Govern the Folding of Protein Chains," *Science* **181**, 223–230 (1973).
15. P. L. Privalov, "Stability of Proteins: Small Globular Proteins," *Adv. Protein Chem.* **33**, 167–241 (1979).
16. P. L. Privalov, "Stability of Proteins. Proteins Which Do Not Present a Single Cooperative System," *Adv. Protein Chem.* **35**, 1–104 (1982).

17. C. Tanford, "Protein Denaturation," *Adv. Protein Chem.* **23**, 121–282 (1968).
18. K. Kuwajima et al., "Three-State Denaturation of alpha-Lactalbumin by Guanidine Hydrochloride," *J. Mol. Biol.* **106**, 359–373 (1976).
19. M. Nozaka et al., "Detection and Characterization of the Intermediate on the Folding Pathway of Human alpha-Lactalbumin," *Biochemistry* **17**, 3753–3758 (1978).
20. K. Kuwajima, "A Folding Model of alpha-Lactalbumin Deduced from the Three State Denaturation Mechanism," *J. Mol. Biol.* **114**, 241–258 (1977).
21. J. N. Onuchic et al., "Towards an Outline of the Topology of a Realistic Protein-Folding Funnel," *Proc. Natl. Acad. Sci. USA* **92**, 3626–2630 (1995).
22. M. Ohgushi and A. Wada, "Molten Globule State: A Compact Form of Globular Proteins with Mobile Side-Chains," *FEBS Lett.* **164**, 21–24 (1983).
23. J. J. Ewbank et al., "What Is the Molten Globule," *Nature Struct. Biol.* **2**, 10–11 (1995).
24. L. C. Wu, Z. Peng, and P S. Kim, "Bipartite Structure of the alpha-Lactalbumin Molten Globule," *Nature Struct. Biol.* **2**, 281–286 (1995).
25. T. E. Creighton, "How Important Is the Molten Globule for Correct Protein Folding?" *Trends Biochem. Sci.* **22**, 6–10 (1997).
26. Z. Peng, L. C. Wu, and P. S. Kim, "Local Structure Preferences in the alpha-Lactalbumin Molten Globule," *Biochemistry* **34**, 3248–3252 (1995).
27. V. E. Bychkova, R. H. Pain, and O. B. Ptitsyn, "The Molten Globule State Is Involved in the Translocation of Proteins across the Membranes?" *FEBS Lett.* **238**, 231–234 (1988).
28. O. B. Ptitsyn, V. E. Bychkova, and V. N. Uversky, "Kinetic and Equilibrium Folding Intermediates," *Philos Trans. R. Soc. London B, Biol. Sci.* **348**, 35–41 (1995).
29. J. Ellis, "Proteins as Molecular Chaperones," *Nature (London)* **328**, 378–379 (1987).
30. J. Martin, "Protein Folding Assisted by the GroEL/GroES Chaperonin System," *Biochemistry (Moscow)* **63**, 374–381 (1998).
31. E. A. Craig, B. D. Gambill, and R. J. Nelson, "Heat Shock Proteins: Molecular Chaperones of Protein Biogenesis," *Microbiol. Rev.* **57**, 402–414 (1993).
32. C. Georgopoulos and W. J. Welch, "Role of the Major Heat Shock Proteins as Molecular Chaperones," *Ann. Rev. Cell Biol.* **9**, 601–634 (1993).
33. K. Liberek et al., "*Escherichia coli* DnaJ and GrpE Heat Shock Proteins Jointly Stimulate ATPase Activity of DnaK," *Proc. Natl. Acad. Sci. USA* **88**, 2874–2878 (1991).
34. R. B. Freedman, "Protein Disulfide Isomerase: Multiple Roles in the Modification of Nascent Secretory Proteins," *Cell* **57**, 1069–1072 (1989).
35. F. X. Schmid et al., "Prolyl Isomerases: Role in Protein Folding," *Adv. Protein Chem.* **44**, 25–66 (1993).
36. C. T Walsh, L. D. Zydowsky, and F. D. McKeon, "Cyclosporin A, the Cyclophilin Class of Peptidylprolyl Isomerases, and Blockade of T Cell Signal Transduction," *J. Biol. Chem.* **267**, 13115–13118 (1992).
37. H. F Gilbert, "Protein Chaperones and Protein Folding," *Current Opinions Biotechnol.* **5**, 534–539 (1994).
38. K. Braig et al., "The Crystal Structure of the Bacterial Chaperonin GroEL at 2.8 Å," *Nature* **371**, 578–586 (1994).
39. V. R. Harwalkar and C.-Y Ma, "Thermal Analysis: Principles and Applications," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 405–427.
40. A. E. Stearn, "Kinetics of Biological Reactions with Special Reference to Enzymatic Processes," *Adv. Enzymol.* **9**, 25–74 (1949).
41. D. P. Goldenberg and T E. Creighton, "Energetics of Protein Structure and Folding," *Biopolymers* **24**, 167–182 (1985).
42. D. B. Lund, "Heat Processing," in O. R. Fennema, ed., *Principles of Food Science Part II. Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975, pp. 31–92.
43. S. Schwimner, *Source Book of Food Enzymology*, AVI, Westport, Conn., 1981.
44. M. Joly, *A Physico-Chemical Approach to the Denaturation of Proteins (Phenomenological Aspects of Denaturation)*, Academic, New York, 1965
45. W Kauzmann, "Some Factors in the Interpretation of Protein Denaturation," *Adv. Prot. Chem.* **14**, 1–63 (1959).
46. D. W. Stanley, "Protein Reactions during Extrusion Processing," in C. Mercier, P. Linko, and J. M. Harper, eds., *Extrusion Cooking*, American Cereal Chemists, St. Paul, Minn., 1989, pp. 321–341.
47. D. E. Graham and M. C. Phillips, "Proteins at Liquid Interfaces. I. Kinetics of Adsorption and Surface Denaturation," *J. Colloid Interface Sci.* **70**, 403–413 (1979).
48. D. E. Graham and M. C. Phillips, "Proteins at Liquid Interfaces. II. Adsorption Isotherms," *J. Colloid Interface Sci.* **70**, 415–439 (1979).
49. R. Zender et al., "Aseptic Autolysis of Muscle: Biochemical and Microscopic Modifications Occurring in Rabbit and Lamb Muscle during Aseptic and Anaerobic Storage," *Food Res.* **23**, 305–326 (1958).
50. T. Richardson and D. B. Hyslop, "Enzymes," in O. R. Fennema, ed., *Food Chemistry*, 2nd ed., Marcel Dekker, New York, 1985, pp. 371–476.
51. J. R. Whitaker, "Enzymes," in O. R. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, pp. 431–530.
52. E. A. Foegeding, T. C. Lanier, and H. O. Hultin, "Characteristics of Edible Muscle Tissues," in O. R. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, 879–942.
53. S. Nakai and E. Li-Chan, *Hydrophobic Interactions in Food Systems*, CRC Press, Boca Raton, Fla., 1988.
54. T. A. Gillett et al., "Parameters Affecting Meat Protein Extraction and Interpretation of Model System Data for Meat Emulsion Formulation," *J. Sci.* **42**, 1606–1610 (1977).
55. R. Hamm, "Changes of Muscle Proteins during the Heating of Meat," in T Hoyem and O. Kvale, eds., *Physical, Chemical and Biological Changes in Food Caused by Thermal Processing*, Applied Science Publishers, London, 1977, pp. 101–134.
56. A. Ashgar, K. Samenjima, and T. Yasui, "Functionality of Muscle Protein in Gelation Mechanisms of Structured Meat Products," *CRC Crit. Rev. Food Sci. Nutr.* **22**, 27–106 (1986).
57. R. Hamm, "Heating of Muscle Systems," in E. J Briskey, R. G. Cassens, and J. C. Trautman, eds., *The Physiology and Biochemistry of Muscle as a Food*, University of Wisconsin Press, Madison, 1966, pp. 363–385.
58. J. Spinelli and J. A. Dassow, "Fish Proteins: Their Modification and Potential Uses in the Food Industry," in R. E. Martin et al., eds., *Chemistry and Biochemistry of Marine Food Products*, AVI, Westport, Conn., 1982.
59. J. F. Ang and H. O. Hultin, "Denaturation of Cod Myosin during Freezing after Modification with Formaldehyde," *J. Food Sci.* **54**, 814–818 (1989).

60. H. E. Swaisgood, "Characteristics of Milk," in O. R. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, pp. 841–878.

61. P. Walstra and R. Jennes, *Dairy Chemistry and Physics*, Wiley Interscience, New York, 1984.

62. F. Dannenberg and H.-G. Kessler, "Reaction Kinetics of the Denaturation of Whey Proteins in Milk," *J. Food Sci.* **53**, 258–263 (1988)

63. R. M. Hillier, R. L. J. Lyster, and C. C. Cheeseman, "Thermal Denaturation of α-Lactalbumin and β-Lactoglobulin in Cheese Whey: Effect of Total Solids Concentration and pH," *J. Dairy Res.* **46**, 103–111 (1979).

64. C. A. Zittle et al., "The Binding of Calcium Ions by β-Lactoglobulin Both before and after Aggregation by Heating in the Presence of Calcium Ions," *J. Am. Chem. Soc.* **79**, 4661–4666 (1957).

65. K. Watanabe and H. Klostermeyer, "Heat-Induced Changes in Sulfhydryl and Disulfide Levels of β-Lactoglobulin A and the Formation of Polymers," *J. Dairy Res.* **43**, 411–418 (1976).

66. S. I. Shalabi and J. V. Wheelock, "Effect of Sulfhydryl-Blocking Agents on the Primary Phase of Chymosin Action on Heated Casein Micelles and Heated Milk," *J. Dairy Res.* **44**, 351–355 (1977).

67. C. V. Morr, "Emulsifiers· Milk Proteins," in J. P. Cherry, ed., *Protein Functionality in Foods, ACS Symposium Series 147*, American Chemical Society, Washington, D.C., 1981, pp. 201–215.

68. R. M. Parry Jr., "Milk Coagulation and Protein Denaturation," in B. H. Webb, A. H. Johnson, and J. H. Alford, eds., *Fundamentals of Dairy Chemistry*, AVI, Westport, Conn., 1974, pp. 603–661.

69. D. V. Vadhera and K. R. Nath, "Eggs as a Source of Protein," *CRC Crit. Rev. Food Technol.* **4**, 193–309 (1973).

70. H. F. Déutsh and J. I. Moerton, "Physical-Chemical Studies of Soy Modified Ovomucoids," *Arch. Biochem. Biophys.* **931**, 654–660 (1961).

71. M. A. Baig and A. Salahuddin, "Occurrence and Characterization of Stable Intermediate State(s) in the Unfolding of Ovomucoid by Guanidine Hydrochloride," *Biochem. J.* **171**, 89–97 (1978).

72. T. Matsuda, K. Watanabe, and Y. Sate, "Temperature-Induced Structural Changes in Chicken Egg White Ovomucoid," *Agric. Biol. Chem.* **45**, 1609–1614 (1981).

73. Y. V. Wu and O. E. Inglett, "Denaturation of Plant Proteins Related to Functionality and Applications: A Review," *J. Food Sci.* **39**, 218–225 (1974).

74. N. Catsimpoolas and E. W. Meyer, "Gelation Phenomena of Soybean Globulins. I. Protein–Protein Interactions," *Cereal Chem.* **47**, 559–570 (1970).

75. T. Mori et al., "Differences in Subunit Composition of Glycinin among Soybean Cultivars," *J. Agric. Food Chem.* **29**, 20–23 (1981).

76. L. Voutsinas, S. Nakai, and V. P. Harwalker, "Relationships between Hydrophobicity and Thermal Functional Properties of Food Proteins," *Can. Inst. Food Sci. Technol. J.* **16**, 185–190 (1983)

77. J. P. Cherry and K. H. McWatters, "Whippability and Aeration," in J. P. Cherry, ed., *Protein Functionality in Foods, ACS Symposium Series 147*, American Chemical Society, Washington, D.C., 1981, pp. 149–176.

78. J. C. Cheftel, J.-L. Cuq, and D. Lorient, "Amino Acids, Peptides, and Proteins," in O. R. Fennema, ed., *Food Chemistry*, 2nd ed., Marcel Dekker, New York, 1985, pp. 245–369.

79. C.-Y. Ma and V. R. Harwalkar, "Chemical Characterization and Functionality Assessment of Oat Protein Fractions," *J. Agric. Food Chem.* **32**, 144–149 (1984).

80. C.-Y. Ma and V. R. Harwalkar, "Study of Thermal Denaturation of Oat Globulin by Ultraviolet and Fluorescence Spectroscopy," *J. Agric. Food Chem.* **36**, 155–160 (1988)

81. T. Furukawa and S. Ohta, "Ultrasonic-Induced Modification of Flow Properties of Soy Protein Dispersion," *Agric. Biol. Chem.* **47**, 745–750 (1983).

82. L. C. Wang, "Soybean Protein Agglomeration: Promotion by Ultrasonic Treatment," *J. Agric. Food Chem.* **29**, 177–180 (1981).

83. K. C. Rhee, C. K. Kuo, and E. W. Lusas, "Texturization," in J. P. Cherry, ed., *Protein Functionality in Foods, ACS Symposium Series 147*, American Chemical Society, Washington, D.C., 1981, pp. 51–88.

84. J. M. Harper, "Food Extrusion," *CRC Crit. Rev. Food Sci. Nutr.* **11**, 155–215 (1979).

85. M. J. Zoller and M. Smith, "Oligonucleotide-Directed Mutagenesis Using M13-Derived Vectors: An Efficient and General Procedure for the Production of Point Mutations in Any Fragment of DNA," *Nucl. Acids Res.* **10**, 6487–6500 (1982).

86. Y. Nosoh and T. Sekiguchi, "Protein Engineering for Thermostability," *Trends Biotechnol.* **8**, 16–20 (1990).

87. L. J. Perry and R. Wetzel, "Disulfide Bond Engineered into T4 Lysozyme: Stabilization of the Protein toward Thermal Inactivation," *Science* **226**, 555–557 (1984).

88. R. Wetzel et al., "Disulfide Bonds and Thermal Stability in T4 Lysozyme," *Proc. Natl. Acad. Sci. USA* **85**, 401–405 (1988).

89. J. A. Wells and D. B. Powers, "In vivo Formation and Stability of Engineered Disulfide Bonds in Subtilisin," *J. Biol. Chem.* **261**, 6564–6570 (1986).

90. M. W. Pantoliano et al., "Protein Engineering of Subtilisin BPN'· Enhanced Stabilization through the Introduction of Two Cysteines to Form a Disulfide Bond," *Biochemistry* **26**, 2077–2082 (1987).

91. C. Mitchinson and J. A. Wells, "Protein Engineering of Disulfide Bonds in Subtilisin BPN'," *Biochemistry* **28**, 4807–4815 (1989).

92. J. E. Villafranca et al., "An Engineered Disulfide Bond in Dihydrofolate Reductase," *Biochemistry* **26**, 2182–2189 (1987).

93. R. T. Sauer et al., "An Engineered Intersubunit Disulfide Enhances the Stability and DNA Binding of the N-Terminal Domain of λ Repressor," *Biochemistry* **25**, 5992–5998 (1986).

94. B. W. Matthews, "Genetic and Structural Analysis of the Protein Stability Problem," *Biochemistry* **26**, 6885–6888 (1987).

95. B. W. Matthews, "Structural and Genetic Analysis of Protein Stability," *Ann. Rev. Biochem.* **62**, 139–160 (1993).

96. B. W. Matthews, H. Nicholson, and W. Becktel, "Enhanced Protein Thermostability from Site-Directed Mutations That Decrease the Entropy of Unfolding," *Proc. Natl. Acad. Sci. USA* **84**, 6663–6667 (1987).

97. X.-J. Zhang et al., "Enhancement of Protein Stability by the Combination of Point Mutations in T4 Lysozyme Is Additive," *Protein Eng.* **8**, 1017–1022 (1995).

98. S. Dao-Pin, W. A. Baase, and B. W. Matthews, "A Mutant T4 Lysozyme (Val 131 → Ala) Designed to Increase Thermostability by the Reduction of Strain within an α-Helix," *Proteins* **7**, 198–204 (1990).

99. H. Nicholson et al., "Analysis of the Interaction between Charged Side Chains and the α-Helix Dipole Using Designed Thermostable Mutants of Phage T4 Lysozyme," *Biochemistry* **30**, 9816–9828 (1991).
100. H. Nicholson, W. J. Becktel, and B. W. Matthews, "Enhanced Protein Thermostability from Designed Mutations That Interact with α-Helix Dipoles," *Nature* **336**, 651–656 (1988).
101. M. Matsumura, W. J. Becktel, and B. W. Matthews, "Hydrophobic Stabilization in T4 Lysozyme Determined Directly by Multiple Substitutions of Ile3," *Nature* **334**, 406–410 (1988).
102. H. K. W. Kallwass et al., "Single Amino Acid Substitutions Can Further Increase the Stability of a Thermophilic L-Lactate Dehydrogenase," *Protein Eng.* **5**, 769–774 (1992).
103. S. Nakamura et al., "Hyperglycosylation of Hen Egg White Lysozyme in Yeast," *J. Biol. Chem.* **268**, 12706–12712 (1993).
104. Y. Suzuki et al., "A Strong Correlation between the Increase in Number of Proline Residues and the Rise in Thermostability of Five *Bacillus* Oligo-1,6,-glucosidase," *Appl. Microbiol. Biotechnol.* **26**, 546–551 (1987).
105. T. Herning et al., "Role of Proline Residues in Human Lysozyme Stability: A Scanning Calorimetric Study Combined with X-Ray Structure Analysis of Proline Mutants," *Biochemistry* **31**, 7077–7085 (1992).
106. S. Nakamura et al., "Improving the Thermostability of *Bacillus stearothermophilus* Neutral Protease by Introducing Proline into the Active Site Helix," *Protein Eng.* **10**, 1263–1269 (1997).
107. D. Gilis and M. Rooman, "Stability Changes upon Mutation of Solvent-Accessible Residues in Proteins Evaluated by Database-Derived Potentials," *J. Mol. Biol.* **257**, 1112–1126 (1996)
108. D. Gilis and M. Rooman, "Predicting Protein Stability Changes upon Mutation Using Database-Derived Potentials: Solvent Accessibility Determines the Importance of Local versus Non-Local Interactions along the Sequence," *J. Mol. Biol.* **272**, 276–290 (1997).
109. A. R. Fersht, A. Matouschek, and L. Serrano, "The Folding of an Enzyme. I. Theory of Protein Engineering Analysis of Stability and Pathway of Protein Folding," *J. Mol. Biol.* **224**, 771–782 (1992).
110. L. Serrano et al., "The Folding of an Enzyme. II. Substructure of Barnase and the Contribution of Different Interactions of Protein Stability," *J. Mol. Biol.* **224**, 783–804 (1992)
111. L. Serrano, A. G. Day, and A. R. Fersht, "Step-wise Mutation of Barnase to Binase. A Procedure for Engineering Increased Stability of Proteins and an Experimental Analysis of the Evolution of Protein Stability," *J. Mol. Biol.* **233**, 305–312 (1993).
112 R. Jiminez-Flores and G. T. Bleck, "Biotechnology," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 505–534.
113. R. Y. Yada et al., "Analysis: Quantitation and Physical Characterization," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 333–403.

RICKEY Y. YADA
ROBERT L. JACKMAN
JEFF L. SMITH
KENNETH G. PAYIE
TAKUJI TANAKA
University of Guelph
Guelph, Ontario
Canada

PROTEINS: STRUCTURE AND FUNCTIONALITY

This discussion of protein structure begins with amino acids as building blocks of proteins, followed by different levels of protein structure. Chemical forces that maintain the protein structure or change the structure level are then explained. Interactions of proteins, especially protein–protein, protein–lipid and protein–water interactions, are briefly reviewed. Finally, structure–function relationships are discussed. For more details, readers are referred to the work by R. D. Ludescher (1).

Four levels of structural organization in proteins can be distinguished: primary, secondary, tertiary, and quaternary structures. These terms refer to the amino acid sequence, the regular arrangements of the polypeptide backbone, the three-dimensional structure of the globular protein, and the structures of aggregates of globular proteins, respectively. In addition to this classification, the supersecondary structure is frequently described, which refers to association products of secondary structure, eg, coiled-coil α-helix in which two α-helixes are wound around each other, α–β mixed structure, and $\beta\alpha\beta$-unit structure (2). Furthermore, domain refers to a structurally independent unit that has the characteristics of a small globular protein (3). A domain is composed of supersecondary structures. Domains often have a specific function. A functional motif (sequences shorter than 25 amino acids) or domain (larger than 50 amino acids) is part of a protein that serves a particular function (4).

AMINO ACIDS

Twenty standard amino acid residues occur in proteins. These are listed in Table 1 in the order of their general abundance in proteins in nature (2). The three-letter and one-letter abbreviations that are commonly used are included.

Amino Acid Analysis

The amino acid composition of proteins is analyzed after hydrolysis of the proteins. Acids or proteases are used for the hydrolysis. Because of the lability of some amino acids (eg, tryptophan, especially in the presence of carbohydrates, and sulfur-containing amino acids), enzymatic hydrolysis is preferable. However, because of the difficulty in achieving complete hydrolysis and high costs of the required enzyme mixtures, acid hydrolysis is more popular. To avoid the destruction of those amino acids during hydrolysis, methanesulfonic acid (7), mercaptoethanesulfonic acid (8), or thioglycolic acid (9) may be used for hydrolysis instead of the otherwise commonly used 6 N hydrochloric acid.

High-performance liquid chromatography (HPLC), especially using a cation exchange column, has been the most popular method for amino acid analysis of the resultant protein hydrolysate. However, reverse-phase (RP) chromatography with a C_{18} or C_8 column before or after derivatization of amino acids with *o*-phthaldialdehyde is convenient for detection using a fluorometric detector (10). This is because of simpler solvent systems required for

99. H. Nicholson et al., "Analysis of the Interaction between Charged Side Chains and the α-Helix Dipole Using Designed Thermostable Mutants of Phage T4 Lysozyme," *Biochemistry* **30**, 9816–9828 (1991).
100. H. Nicholson, W. J. Becktel, and B. W. Matthews, "Enhanced Protein Thermostability from Designed Mutations That Interact with α-Helix Dipoles," *Nature* **336**, 651–656 (1988).
101. M. Matsumura, W. J. Becktel, and B. W. Matthews, "Hydrophobic Stabilization in T4 Lysozyme Determined Directly by Multiple Substitutions of Ile3," *Nature* **334**, 406–410 (1988).
102. H. K. W. Kallwass et al., "Single Amino Acid Substitutions Can Further Increase the Stability of a Thermophilic L-Lactate Dehydrogenase," *Protein Eng.* **5**, 769–774 (1992).
103. S. Nakamura et al., "Hyperglycosylation of Hen Egg White Lysozyme in Yeast," *J. Biol. Chem.* **268**, 12706–12712 (1993).
104. Y. Suzuki et al., "A Strong Correlation between the Increase in Number of Proline Residues and the Rise in Thermostability of Five *Bacillus* Oligo-1,6,-glucosidase," *Appl. Microbiol. Biotechnol.* **26**, 546–551 (1987).
105. T. Herning et al., "Role of Proline Residues in Human Lysozyme Stability: A Scanning Calorimetric Study Combined with X-Ray Structure Analysis of Proline Mutants," *Biochemistry* **31**, 7077–7085 (1992).
106. S. Nakamura et al., "Improving the Thermostability of *Bacillus stearothermophilus* Neutral Protease by Introducing Proline into the Active Site Helix," *Protein Eng.* **10**, 1263–1269 (1997).
107. D. Gilis and M. Rooman, "Stability Changes upon Mutation of Solvent-Accessible Residues in Proteins Evaluated by Database-Derived Potentials," *J. Mol. Biol.* **257**, 1112–1126 (1996)
108. D. Gilis and M. Rooman, "Predicting Protein Stability Changes upon Mutation Using Database-Derived Potentials: Solvent Accessibility Determines the Importance of Local versus Non-Local Interactions along the Sequence," *J. Mol. Biol.* **272**, 276–290 (1997).
109. A. R. Fersht, A. Matouschek, and L. Serrano, "The Folding of an Enzyme. I. Theory of Protein Engineering Analysis of Stability and Pathway of Protein Folding," *J. Mol. Biol.* **224**, 771–782 (1992).
110. L. Serrano et al., "The Folding of an Enzyme. II. Substructure of Barnase and the Contribution of Different Interactions of Protein Stability," *J. Mol. Biol.* **224**, 783–804 (1992)
111. L. Serrano, A. G. Day, and A. R. Fersht, "Step-wise Mutation of Barnase to Binase. A Procedure for Engineering Increased Stability of Proteins and an Experimental Analysis of the Evolution of Protein Stability," *J. Mol. Biol.* **233**, 305–312 (1993).
112 R. Jiminez-Flores and G. T. Bleck, "Biotechnology," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 505–534.
113. R. Y. Yada et al., "Analysis: Quantitation and Physical Characterization," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, Wiley-VCH, New York, 1996, pp. 333–403.

RICKEY Y. YADA
ROBERT L. JACKMAN
JEFF L. SMITH
KENNETH G. PAYIE
TAKUJI TANAKA
University of Guelph
Guelph, Ontario
Canada

PROTEINS: STRUCTURE AND FUNCTIONALITY

This discussion of protein structure begins with amino acids as building blocks of proteins, followed by different levels of protein structure. Chemical forces that maintain the protein structure or change the structure level are then explained. Interactions of proteins, especially protein–protein, protein–lipid and protein–water interactions, are briefly reviewed. Finally, structure–function relationships are discussed. For more details, readers are referred to the work by R. D. Ludescher (1).

Four levels of structural organization in proteins can be distinguished: primary, secondary, tertiary, and quaternary structures. These terms refer to the amino acid sequence, the regular arrangements of the polypeptide backbone, the three-dimensional structure of the globular protein, and the structures of aggregates of globular proteins, respectively. In addition to this classification, the supersecondary structure is frequently described, which refers to association products of secondary structure, eg, coiled-coil α-helix in which two α-helixes are wound around each other, α–β mixed structure, and βαβ-unit structure (2). Furthermore, domain refers to a structurally independent unit that has the characteristics of a small globular protein (3). A domain is composed of supersecondary structures. Domains often have a specific function. A functional motif (sequences shorter than 25 amino acids) or domain (larger than 50 amino acids) is part of a protein that serves a particular function (4).

AMINO ACIDS

Twenty standard amino acid residues occur in proteins. These are listed in Table 1 in the order of their general abundance in proteins in nature (2). The three-letter and one-letter abbreviations that are commonly used are included.

Amino Acid Analysis

The amino acid composition of proteins is analyzed after hydrolysis of the proteins. Acids or proteases are used for the hydrolysis. Because of the lability of some amino acids (eg, tryptophan, especially in the presence of carbohydrates, and sulfur-containing amino acids), enzymatic hydrolysis is preferable. However, because of the difficulty in achieving complete hydrolysis and high costs of the required enzyme mixtures, acid hydrolysis is more popular. To avoid the destruction of those amino acids during hydrolysis, methanesulfonic acid (7), mercaptoethanesulfonic acid (8), or thioglycolic acid (9) may be used for hydrolysis instead of the otherwise commonly used 6 N hydrochloric acid.

High-performance liquid chromatography (HPLC), especially using a cation exchange column, has been the most popular method for amino acid analysis of the resultant protein hydrolysate. However, reverse-phase (RP) chromatography with a C_{18} or C_8 column before or after derivatization of amino acids with *o*-phthaldialdehyde is convenient for detection using a fluorometric detector (10). This is because of simpler solvent systems required for

Table 1. Properties of the Amino Acid Residues of Proteins

Amino acid	Three-letter symbol	One-letter symbol		Molecular weight[a]	pK value of side chain	Hydrophobicity[b]	Bulkiness[c]
		Symbol	Mnemonics				
Alanine	Ala	A	Alanine	71		0.06	11.50
Glutamate	Glu	E	GluEtamate	128	4.3	−0.10	13.57
Glutamine	Gln	Q	Q-tamine	128		0.31	14.45
Either E or Q	Glx	Z					
Aspartate	Asp	D	AsparDate	114	3.9	−0.20	11.68
Asparagine	Asn	N	AsparagiNe	114		0.25	12.82
Either D or N	Asx	B					
Leucine	Leu	L	Leucine	113		3.50	21.40
Glycine	Gly	G	Glycine	57		0.21	3.40
Lysine	Lys	K	Before L	129	10.5	−1.62	15.71
Serine	Ser	S	Serine	87		−0.62	9.47
Valine	Val	V	Valine	99		1.59	21.57
Arginine	Arg	R	ARgining	157	12.5	−0.85	14.28
Threonine	Thr	T	Threonine	101		0.65	15.77
Proline	Pro	P	Proline	97		0.71	17.43
Isoleucine	Ile	I	Isoleucine	113		3.48	21.40
Methionine	Met	M	Methionine	131		0.21	16.25
Phenylalanine	Phe	F	Fenylalanine	147		4.80	19.80
Tyrosine	Tyr	Y	TYrosine	163	10.1	1.89	18.03
Cysteine	Cys	C	Cysteine	103		0.49	13.46
Tryptophan	Trp	W	tWO rings	186		2.29	21.61
Histidine	His	H	Histidine	137	6.0	2.24	13.67
Undetermined or nonstandard		X					

[a] Weighted mean of relative molecular weight of all residues. 108.7

[b] Hydrophobicity coefficients (X) derived from RP-HPLC of peptides, $\mathbf{X} = (\mathbf{A}^T\mathbf{b})(\mathbf{A}^T\mathbf{A})^{-1}$ where **A** is a sequence matrix and b is a mole fraction matrix (5).

[c] Bulkiness (Å) used for computation of protein compressibility (6).

eluting the amino acids from the column and quicker elution (less than 30 min) compared to ion exchange chromatography. The fluorometric RP chromatography can accurately quantify 5 pmol of 50-kDa protein, 2–3 times more sensitive than ion exchange/ninhydrin detector systems. Gas–liquid chromatography (GLC) is also being used for amino acid analysis after derivatization of amino acid. The purpose of this derivatization is to make the amino acids volatile by changing them to, for instance, *N(O,S)*-isobutoxycabonyl methyl esters among many other derivatization techniques. All serum amino acids were measured by gas chromatography (GC) with flame ionization detector using a DB017 capillary column in 9 min. Overall recoveries of amino acids added to serum samples were 88–108% (11). Recently, isotope dilution GC/electron capture ionization mass spectrometry was reported for less than 100 fmol amino acids in protein hydrolysates (12).

Progress has been made recently also in the RP/HPLC after precolumn derivatization. Rapid derivatization and direct injection without cleaning is now feasible by using 6-aminoquinolyl-*n*-hydroxysuccinimidyl carbamate for derivatization for fluorometric detectors (13). By using phenylisothiocyanate for derivatization, a UV detector that is more popular on HPLC instruments can be used with similar detectability as fluorometric detectors (14).

PRIMARY STRUCTURE

The backbone of polypeptide chains is described by two dihedral angles per residue: φ and ψ at the C^α atom. In a peptide linkage -NH-C^αH(R)-CO-NH-, the angles of -NH-C^α-, -C^α-CO-, and -CO-NH- are angles φ, ψ, and ω, respectively (Fig. 1). The two broken lines in Figure 1 show the location of peptide linkages with an amino acid residue -NH-C^αH(R)-CO- between these two linkages.

The genetic code involving the sequence of purine and pyrimidine bases in the DNA strand determines the se-

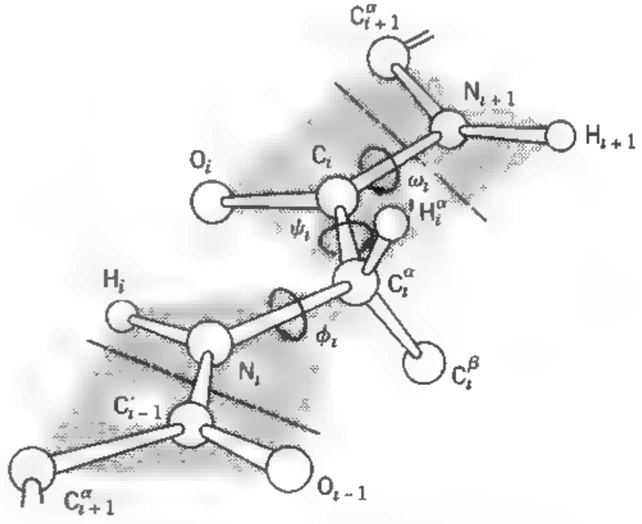


Figure 1. Polypeptide chain linking two peptide units. The chain is shown in a fully extended conformation with all φ_i, ψ_i, and ω_i angles greater than 180°. *Source:* Ref. 15

quence of amino acids along the protein chain. Each group of three bases along the messenger RNA strand specifies a particular amino acid, and the sequence of these triplet groups dictates the sequence of the amino acids in the proteins. The genetic code is, however, degenerate; that is, most amino acids are coded by more than one codon. Therefore, a nucleotide sequence cannot be derived from the colinear amino acid sequence. But an unknown amino acid sequence can be deduced from the colinear nucleotide sequences. Thus, protein sequence can be determined by analyzing the underlying nucleotide sequence.

Sequence Analysis

For peptide sequence analysis, solid-phase Edman degradation has been used. Lately, gas-phase microsequencing has become popular. First, a phenylthiocyanate or other similar isocyanate solution is added, and then lower alkylamines, eg, triethylamine, in vapor are delivered by a stream of argon gas to convert the peptide sample on a porous support into a phenyl thiocarbamylated peptide. Then trifluoroacetic acid or another similar fluoride-containing organic acid in vapor form is delivered to liberate the phenylthiohydantion (pth) amino acid derivatives. The resultant pth-amino acids are extracted and analyzed by RP/HPLC. This cycle is repeated until it reaches the COOH terminus of the peptide chain (16). In the case of larger protein molecules, fragmentation to small peptides is needed since there are limits on the chain length that can be analyzed.

The advent of capillary electrophoresis in conjunction with nanoelectrospray mass spectrometry enabled the Edman sequencing of peptides and proteins using 5–10 picomoles of materials (17).

Recent developments in nucleic acid cloning and sequencing techniques have made the determination of the amino acid sequence of a protein straightforward, inexpensive, accurate, and rapid (18). For analysis of DNA sequences, two methods are widely used: the enzymatic dideoxy method and the chemical method. The difference is primarily in the technique used to generate the ladder of oligonucleotides. In the enzymatic dideoxy sequencing method, a DNA polymerase is used to synthesize a labeled, complementary copy of a DNA template. In the chemical sequencing method, a labeled DNA strand is subjected to a set of base-specific chemical reagents. A set of radiolabeled single-stranded oligonucleotides is generated in four separate reactions, either enzymatically or chemically. In each of the four reactions, the oligonucleotides have one fixed and one end that terminates sequentially at each A, T, G, or C, respectively. The products of each reaction are fractionated by electrophoresis on adjacent lanes of a high-resolution polyacrylamide gel. After autoradiography, the DNA sequence can be read directly from the gel.

Characteristic Properties of Amino Acids in Protein Structure

Each amino acid residue has its own characteristic property that cannot be replaced by another residue. All amino acid residues, except glycine, have characteristic side chains on the main chain linked through peptide linkage (2). Glycine increases flexibility to the main chain because it has no side chain but with only two hydrogens. A polypeptide chain at a glycine residue has considerably more conformational freedom than at any other residue. Alanine is the smallest nonpolar residue that does not have much preference on whether it is located inside or on the surface of a protein molecule. The nonpolar side chains of valine, isoleucine, and leucine are branched, thus restricting internal flexibility. Phenylalanine has the largest nonpolar side chain. Because of the single methylene group on C^{β} as with the other two aromatic side chains (tyrosine and tryptophan), the side chain flexibility is restricted. Tyrosine has by far the most reactive side chain of the three aromatic residues because of its hydroxyl radical.

The side chain of proline is characteristic because the last atom of the side chain is bonded to the main chain N atom, forming a ring structure. This prevents the N atom from participating in hydrogen bonding and also provides a steric hindrance to the α-helical conformation (19). Consequently, proline has the smallest degree of conformational freedom of all the amino acids, and a polypeptide chain at a proline residue has appreciably less conformational freedom. This characteristic property of proline can be used for stabilizing protein structure; however, its location should be carefully chosen so that the new residue should neither create volume interferences nor destroy stabilizing noncovalent interactions (20). Proline introduction to the N-terminal end of active-site helix of *Bacillus stearothermophilus* neutral protease improved thermostability. The glycine residues on the *N*-terminal side of proline residue relaxed the possible strain that resulted from proline introduction, thereby increasing molecular rigidity (21). Methionine has a rather flexible side chain with a sulfur in a thioether bond. This sulfur introduces an electrical dipole moment.

All the larger nonpolar residues, namely valine, isoleucine, leucine, phenylalanine, proline, tryptophan, and, to a lesser extent, methionine, are predominantly in the inside of protein molecules. Polar (uncharged) side chains form hydrogen bonds, for instance, serine and threonine have hydroxyl groups that form hydrogen bonds (1). Cysteine plays a special role by forming disulfide bridges between different parts of the main chain. A group can be activated in protein through specific hydrogen bonds, such as serine acting as a donor to an unprotonated imidazole group of a histidine. Such charge relay systems form an essential part of the active site of serine proteases. In both serine and threonine, the hydroxyl group can react with acids to form esters via enzyme-catalyzed reactions. Both are common sites for phosphorylation, fatty acid esterification, and glycosylation in proteins. The acid amides of asparagine and glutamine can also form hydrogen bonds. The amido groups function as hydrogen donors and the carbonyl groups as acceptors. The side chain of glutamine is more flexible than that of asparagine because of its extra methylene group. The polar hydroxyl group of tyrosine forms relatively strong hydrogen bonds.

Histidine has a heterocyclic aromatic side chain with a p*K* value of 6.0. In the physiological pH range, its imidazole ring can be either uncharged or charged. This chemical equilibrium is suitable for catalyzing reactions. This is one of the reasons why histidine is found in several of the active sites of enzymes. Aspartate and glutamate, usually located at the molecular surface, are negatively charged at physiological pH. Because of the short side chain, the carboxyl groups of aspartate are relatively rigid. This may be a reason why the carboxyl groups of active sites of enzymes, eg, aspartyl proteinases, are mainly provided by aspartates and not glutamates.

Most of the positively charged lysine and arginine residues are also at the molecular surface. They are long and flexible and do not usually adopt a defined conformation. The surface net charges of these residues in counterbalance with surface hydrophobicity increase the solubility of globular proteins (22). Sometimes they participate in internal salt bridges or in catalysis. The ϵ-amino group of lysyl residues, and to a lesser extent the guanidinium groups of arginyl residues, are the target of enzyme action, which either modifies the side chain or cleaves the peptide chain at the carboxyl end of lysyl and arginyl residues of substrates. The ϵ-amino group of lysine is considered to be the second-most reactive group in proteins, second to the cysteine sulfhydryl group. The most famous reaction of the ϵ-amino group of lysine is the formation of a Schiff base by the so-called Maillard reaction with aldehydes.

The folding process of a polypeptide chain depends on the hydrophobicity of the side chains, because the formation of a hydrophobic core in the globule seems to be one of the essential driving forces in folding. The hydrophobicity of amino acid residues is dependent on water-accessible surface area and dipole content.

SECONDARY STRUCTURE

Secondary structures are regular arrangements of the backbone of the polypeptide chain. They are stabilized by hydrogen bonds between peptide amide (>N-H) and carbonyl (>C=O) groups. α-Helices and β-sheets make up about half the secondary structures of globular proteins, with the remainder occurring as tight turns, small loops, and random coils.

Helices

The backbone of a polypeptide chain forms a linear group, if its dihedral angles ($\varphi \cong -60°$ and $\psi \cong -50°$) are repeated. An α-helix with 3.6 amino acid residues per turn has a rise/residue ratio of 0.15 nm. The C=O group of each residue at position i is hydrogen bonded to the NH group at position $i + 4$. The polypeptide backbone forms a cylinder of 0.23 nm radius in which the backbone atoms are close-packed in van der Waals contact with each other inside the helix. The hydrogen-bonding pattern generates a loop of connectivity through covalent and hydrogen bonds that includes 13 atoms in four turns of the helix (a 4_{13} helix).

In addition to the α-helix, a shorter helix, a 3_{10} helix, is sometimes observed in protein molecules. Its energetically disadvantageous geometry restricts frequent occurrence of the 3_{10} helix in proteins; usually pieces of about one turn are observed. These pieces tend to be at the N and C termini of α-helixes.

A third helical structure is the polyproline II helix found in collagen (gelatin). This is an extended, left-handed helical structure with 3.3 residues per turn generated by dihedral angles of $\varphi \cong -80°$ and $\psi \cong +150°$. Stability of this structure relies on the specific conformational constraints of the proline residues by locking into $\varphi \cong -80°$ and not by hydrogen bonds.

β-Pleated Sheet

Extended polypeptide chains can associate by hydrogen bonding to form sheetlike structures in parallel β-sheets, with all β-strands pointed in the same direction, and in antiparallel β-pleated sheets, with alternate strands pointed in opposite direction. Individual β-strands or β-pleated sheets have a structure generated by a regular sequence of dihedral angles ($\varphi \cong -120°$ and $\psi \cong +140°$) that orient the NH and C=O bonds nearly perpendicular to the long axis of the polypeptide chain. Also, the idealized β-structure based on 2.0 residues per turn originally proposed was a flat pleated sheet structure; actual β-pleated sheets found in proteins usually have a left-handed twist. Globular proteins contain about 15% sheet structures. The average length of β-structure is about 6 residues, which is equal to 20 Å in length, corresponding approximately to the diameters of domains.

Reverse Turns (β-Turns)

An energetically economical and space-saving way of changing direction in a polypeptide chain involves four amino acid residues, which are often joined to chains in the β-sheet conformation with a 180° chain reversal. Reverse turns (or loops) consist mostly of hydrophilic residues. Reverse turns are common structural elements; approximately one-third of the amino acid residues of proteins is found in this conformation. The best-known examples are β-hairpins that link two adjacent strands of an antiparallel β-pleated sheet. They are stabilized by hydrogen bonds connecting the C=O on residue i to the NH on residue $i + 2$ or $i + 3$. At least, 15 different variants of reverse turns have been described and categorized (23). Because of the geometric constraints required for the turn, certain classes of turns have preferences for specific amino acids (glycine or proline) at specific positions. β-Turns linking contiguous β-strands are of considerable interest to biochemists because they are usually at the protein surface and often are involved in molecular recognition and antigenicity.

Examples of secondary structures are shown in Figure 2; the ribbon-like coils at both right and left sides are α-helices; parallel β-sheets are at the center; a β-turn links the antiparallel β-sheet pair at the upper-right-hand corner (this is the reason for naming of β-turn).

DETERMINATION OF SECONDARY STRUCTURE

A variety of methods have been used to determine secondary structures of proteins. This section outlines those methods.

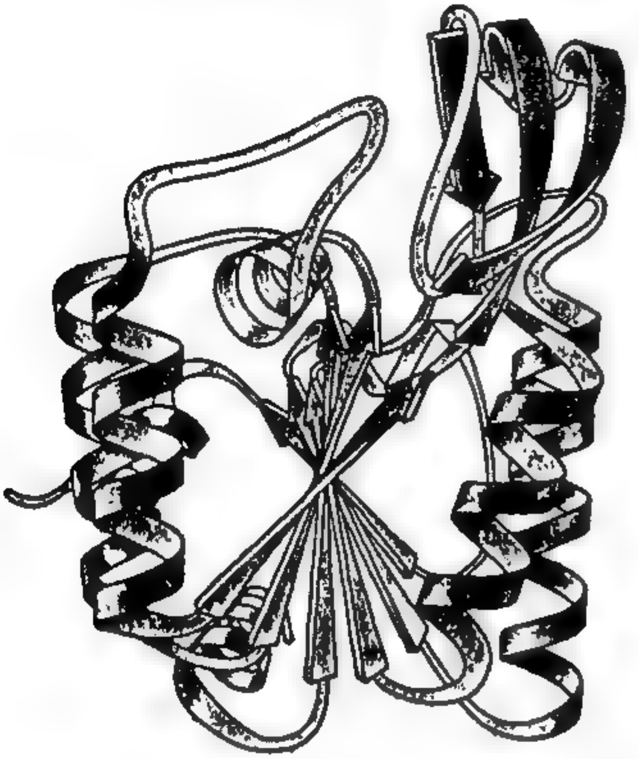


Figure 2. Secondary structure of phosphoglycerate kinase domain 2. *Source*. Ref. 24, with permission.

Infrared Spectroscopy

Assignments of the amide I band to various secondary structures in H_2O and D_2O are feasible by infrared spectroscopy. Amide I–III bands are caused by a vibration in the plane of the amide group of the polypeptide backbone; C=O stretch, N-H bend, and C-N stretch and N-H bend are the main vibrations for amide I, II, and III, respectively. The individual secondary structure fractions yield distinct maxima. However, the resolution of these structures in a globular protein that contains several of these structures is difficult because of the large half-widths of these structures, thereby interfering with their resolution. Least-squares optimization and Fourier deconvolution procedures have been used for the analysis of overlapping bands. Derivative spectroscopy is an alternative method for assessing the number and peak frequencies of the component peaks. The second derivative infrared spectra of various proteins have shown peaks associated with α-helical, β-sheet, and β-turn to be resolved and information pertaining to some amino acid side chains to be obtained.

Infrared spectra of 13 globular proteins in water were obtained in 1800–1480 cm^{-1}. High correlation coefficients of 0.80–0.99 were obtained between the infrared and X-ray estimates of ordered helix, disordered helix, ordered β-structure, disordered β-structure, turns, and remainder (25).

Laser Raman Spectroscopy

Raman and infrared techniques are complementary. Raman lines, derived-by a light-scattering effect measured by the emission of radiation from a vibrational exited state, are related to molecular polarizability originating from induced dipole moment, whereas infrared absorption is associated with the permanent dipole moment during molecular vibration. The amide I and II bands are strong in the infrared spectrum, whereas the amide I and III bands are prominent in the Raman spectrum. By analyzing the amide I Raman band directly as a linear combination of amide I bands of proteins whose secondary structures are known from X-ray data, excellent correlation between Raman and X-ray diffraction estimates was obtained for helix, β-strand, and β-turn composition (26).

Nuclear Magnetic Resonance Spectroscopy

The nuclear magnetic resonance (NMR) method for studying protein conformation is based on the fact that "nuclei of the same element in different chemical environments give rise to distinct chemically shifted spectral lines." The use of chemical shifts for the identification of specific secondary structures, however, has not been successful in determining secondary structures other than α-helix. The introduction of the nuclear Overhauser effect (NOE) into NMR has provided a network of short, intramolecular distance constraints between distinct locations along the polypeptide chain, which has allowed the characterization of other secondary structures. NOE is the enhancement in amplitude of the spectral line of nucleus observed during irradiation of one of a pair of coupled nuclear spins; irradiation of nuclei can cause changes in the population of the spin-state of the nuclei. Proton–proton NOE distance information was used for identification of polypeptide secondary structures in noncrystalline proteins. The combined information on all of the distances obtained from visual inspection of the two-dimensional NOE (NOESY) spectra was sufficient for determination of helical and β-sheet secondary structures in small globular proteins.

Although for larger proteins magnetic relaxation becomes a limiting factor, the benefits of using uniform high-level (>96%) deuteration was shown to inhibit relaxation processes (27). Complete deuteration provides significant signal-to-noise enhancement in heteronuclear NMR assignment and structure determination experiments, which uses the amide proton for detection. NMR pulse sequences lose sensitivity as the size of the protein under study increases above 25 kDa, mainly because of fast ^{13}C transverse relaxation via the strong dipolar coupling between a ^{13}C nucleus and its directly bonded protons. Since the gyromagnetic ratio of ^{2}H is 6.5 times smaller than that of ^{1}H, per deuteration dramatically reduces this relaxation.

A recent news release stated that "A technique called TROST (transverse relaxation–optimized spectroscopy) now greatly reduces NMR line broadening with increasing molecular size, permitting NMR analysis of molecules far beyond 100 kDa in size" (28).

Optical Rotatory Dispersion and Circular Dichroism

It is generally accepted that optical rotatory dispersion (ORD) and circular dichroism (CD) are a direct reflection of protein secondary structure. CD uses circularly polarized light (180–260 nm) to illuminate the specimen instead of plane polarized light in the case of ORD. The inherent problem of ORD curve analysis (ie, ORD absorption bands are infinitely broader compared to CD bands) has resulted in increasingly popularity of CD spectra analysis. Computer programs have been developed to compute the contents of α-helix, parallel and antiparallel β-sheets, β-turn, and random coil. Efforts have been made to expand the spectral range into the far ultraviolet, as low as 180 nm, for more detailed characterization of the CD spectra; thus, more accurate information can be obtained for the five forms of secondary structure. However, mechanical difficulty of maintaining high vacuum during measuring CD spectra restricts broad use of this approach. Instead, spectral analysis using advanced computer-aided classification (eg, artificial neural networks) is increasingly more popular (29).

PREDICTION OF SECONDARY STRUCTURE AND MOLECULAR MODELING

Many attempts have been made to predict protein structures from their primary sequences using a variety of parameters such as amino acid frequency, energy calculation, and φ and ψ values. As a result of these studies, it was possible to identify and quantify the secondary structure of proteins based on sequence data alone, with some degree of accuracy. In one of the most common of the secondary structure prediction methods, a statistical survey was conducted for 15 proteins in which the α-helix, β-sheet, and β-turn conformational potentials of all 20 amino acids were established (30). A set of empirical rules was then derived that allowed for the determination of the folding of the secondary structure regions in the proteins. Computer programs for this prediction, including programs for personal computers, have been published.

Although numerous prediction methods, in addition to the above Chou-Fasman approach (30), have been developed, the accuracy of these methods has not been very high, which is undoubtedly a result of the probabilistic nature of the methods (31). Moreover, protein structure hierarchy is not so rigorous as to assume that the secondary structure formation of a certain segment is entirely dependent on the sequence information alone; other segments at a distance along the chain also exert an influence.

To improve the correctness of prediction, attempts have been made by using sequence homology, multivariate analysis, and especially energy minimization. It is generally agreed that the maximum correctness is expected to be far less than 80%. In contrast, molecular modeling is based on known three-dimensional structures of homologous molecules with the expectation of maximum accuracy in prediction of about 80% (32).

TERTIARY AND QUATERNARY STRUCTURES

Tertiary structure refers to the three-dimensional structure of a polypeptide chain. The three-dimensional structure of multisubunit proteins is then described by the term quaternary structure, which refers to that structure resulting from the interactions between polypeptide chains, frequently through so-called self-association. However, in general, the three-dimensional structure includes all types of structure above the level of primary structure.

Three-dimensional structures of several hundred proteins have been determined by X-ray crystallography, yielding the location and bonding of all the atoms in the polypeptide chains. Detailed three-dimensional protein structure can also be determined by NMR methods (33), which are advantageous because the structure of proteins in solution can be determined in contrast to the requirement of protein crystals in X-ray diffraction measurement. It is now possible to determine the three-dimensional structure of proteins with molecular weights up to 20,000 routinely using NMR, provided that the protein can be obtained in large-enough quantities and is soluble and stable at room temperature over a period of days. This limit of molecular sizes is quickly increasing as described above (28).

Oligomeric proteins and multienzyme complexes such as pyruvate dehydrogenase are representative of the lowest level of macromolecular structural organization. "Supramolecular structures," such as ribosomes or the membranous components of the electron-transport chain, are examples of higher levels of macromolecular organization, which is the structural basis of life (3).

MOLECULAR FORCES

With the exception of disulfide bonds, molecular forces to maintain integrity of the structure are noncovalent, which are one to three orders of magnitude smaller than covalent energy.

Dispersion Forces

Dispersion forces occur between any pair of atoms. Each atom behaves like an oscillating dipole generated by electrons moving in relation to the nucleus of the atom. In a pair of atoms, the dipole in an atom polarizes the opposing atom. As a result, the oscillators are coupled, giving rise to an attractive force between the atoms. The theory of the attractive interaction between atoms is attributed to London (34), and such forces are often called London forces, or dispersion forces. They are long-range forces, arising from attractive forces between atoms over an extended distance.

It was shown by London, following the appearance of quantum theory, that this relationship is also applicable to the dispersion energy between two nonpolar molecules due to polarization of the electron density in one molecule by charge fluctuation in the other. Except in highly polar materials, it is the sum of London dispersion forces that contributes most to the total van der Waals attraction between macroscopic bodies.

Repulsive Forces

Short-range forces are repulsive, having their quantum mechanical origin in the overlap of electron density of adjacent molecules. Attractive and repulsive forces act simultaneously and cannot be isolated.

Electrostatic Interactions

Because covalent bonds between different types of atoms lead to an asymmetric bond electron distribution, most atoms of a molecule carry partial charges. Because a neutral molecule has no net charge, it contains only dipoles or higher multipoles. These multipoles interact with each other according to Coulomb's law.

$$U = kq_1q_2/Dr \qquad (1)$$

where U is the energy of association of two electric charges, q_1 and q_2, that are separated by the distance r. D is the dielectric constant of the medium.

Salt Bridges

The association of two ionic groups of opposite charge is known as an ion pair or salt bridge with a short r such as 4 Å in equation 1. There are only a few salt bridges in proteins, with the exception of phosphoproteins and some glycoproteins. These salt bridges amount to about 10–20 kcal/mol for adjacent carboxylate and ammonium groups. This value is higher than 3 kcal/mol of hydrogen bonds (35). Salt bridges are formed by about one-third of the charged residues; only 20% of these bridges are buried.

van der Waals Potentials

It is customary to combine all three noncovalent forces (ie, electron shell repulsion, dispersion forces, and electrostatic interactions) into a single simple potential function, or force field, that is called the van der Waals potential for historical reasons.

Hydrogen Bonds

The interatomic distance of monovalent atomic contacts are significantly shorter than the value calculated as the sum of the corresponding van der Waals radii when one of the atoms is hydrogen. Because the entire electron shell of hydrogen is appreciably shifted onto the atom to which hydrogen is covalently bound, the shell repulsion between contact partners is small, and the attracting charges can approach each other more closely. Such a short distance approach gives rise to a high attractive Coulomb energy and, therefore, a high dispersion energy.

Hydrophobic Interaction

The hydrophobic effect arises from the unfavorable interactions between water molecules and the nonpolar residues of a protein. When a hydrocarbon molecule is introduced into water, it induces changes in water structure, frequently decreasing its entropy. To minimize this unfavorable entropy change, the nonpolar molecules are forced to coalesce into dipoles or globules, reducing their surface of contact with water. As a result, the protein chain is forced to fold into a micellar structure with the hydrocarbon moiety on the inside of the globule and the polar groups on the outside. The removal of the nonpolar residues from contact with water makes a major contribution to the free energy of conformational stabilization. Thus, in this theory, the hydrophobic effect is purely the result of the phobia of water when contacting with hydrocarbons.

Disulfide Bonds

Disulfide bonds between pairs of cysteine residues can cross-link different chains of a protein, which gives rise to covalent chain assembles. Disulfide bonds occur between different segments within a single polypeptide chain as well. These bridges play an important role in rheological properties of food proteins. For instance, the cohesive elastic character of wheat flour doughs is based on the disulfide bonds of glutenin, and the three-dimensional network of disulfide bonds in glutenin is responsible for the difficulties encountered during wet milling of corn. Rearrangement of disulfide bonds in a protein by sulfhydryl–disulfide interchange reactions plays an important role in the regulation of activities of enzyme and immunoglobulins. By the catalytic action of sulfhydryl groups in low molecular weight compounds, eg, cysteine, glutathione and mercaptoethanol, proteins can be linked to each other to form large aggregates through a chain reaction. However, in the presence of higher concentrations of these reducing compounds, disulfide bridges in proteins are reduced to sulfhydryl groups, thereby decreasing the molecular weights as well as viscosity.

The measurement of sulfhydryl and disulfide contents of proteins is a very useful tool to assess protein structure. Raman spectroscopy is advantageous because sulfhydryl and disulfide groups appear as peaks in the spectrum, and even solid samples such as gels can be used for analysis with minimum pretreatment (36).

INTERACTIONS

Protein interactions are generally classified as the interactions of proteins with macromolecules and with small molecular weight compounds; a typical example of the former is a protein–protein association and that of the latter is a protein–lipid or protein–water interaction. They are an important property in elucidating the mechanisms of food protein functionality.

Protein–Protein Interaction

Gel filtration chromatography and gel electrophoresis are the most popular methods to determine changes in the molecular weight of proteins caused by interaction. However, to study chemical equilibrium, methods in which zonal separation occurs should be avoided. For this reason, frontal gel filtration (37), sedimentation equilibrium ultracentrifugation (38), light-scattering spectroscopy (39), and fluorescent polarization (40) are appropriate to use. Association constants of food proteins are in the order of $10^4\ M^{-1}$ as reported for κ–α_{s1}-casein and lysozyme–ovalbumin interactions (41). These values are lower than the order of 10^6–$10^7\ M^{-1}$ for inverse Michaelis-Menten constant ($1/K_m$) of enzymes vs substrates, 10^6–$10^9\ M^{-1}$ for antibodies vs antigens, and $10^{14}\ M^{-1}$ for avidin–biotin interaction, which is the strongest noncovalent interaction found in nature (38,42). The specific, strong avidin–biotin interaction has been used for chemical analysis, especially in immunoassay.

Protein–Lipid Interaction

Lipoproteins exist naturally in food systems such as egg yolk. However, lipids can also interact with proteins during food processing, thereby affecting the quality of foods such as bread and frozen fish. Protein–lipid interaction is the most important mechanism of emulsion formation. Analytical methods to measure this interaction include density gradient ultracentrifugation, fluorometry (43), gel filtration chromatography, and ultrafiltration. More sophisticated methods are electron spin-resonance spectroscopy using spin-labeling techniques with probes (44) and Raman spectroscopy (45).

Protein–Water Interaction

It has long been recognized that protein–water interactions play an important role in the determination and maintenance of the three-dimensional structure of proteins. Water in proteins involves stability, dynamics, and function of the proteins (46). Important states of water molecules surrounding protein molecules are structural water and monolayer water. The structural water refers to the water molecules that are part of the protein structure bound through hydrogen bonding. These 10–20 water molecules per protein molecule are unavailable for chemical reactions. The monolayer water refers to the water molecules tightly bound to the protein surface via dipole-induced dipole (hydrophobic hydration), ion–dipole (ionic hydration), and dipole–dipole (hydrogen bonding) interactions. At the saturated monolayer coverage, most proteins absorb about 0.3–0.5 g of water per gram of protein. This bound water is unfreezable and does not participate in any chemical reaction. Unlike the monolayer water, the multilayer water is available for chemical reactions, but some of these water molecules are unfreezable (47).

Most proteins exhibit the least water-binding capacity and solubility at isoelectric pH, presumably because of protonation of the carboxyl groups and enhanced hydrophobic interaction between protein molecules (47).

CHANGES IN PROTEIN STRUCTURE DURING FOOD PROCESSING

Physical treatments that modify protein structure include the use of thermal energy, mechanical energy, or pressure. Common examples of physical processes that alter food proteins are heating, freezing, radiating, extrusion, salting, fiber spinning, sonication, whipping, emulsification, and storage. These processes result in denaturation of the proteins. Denaturation is usually accompanied by unfolding of the protein molecules, without any apparent loss in solubility as long as they are monomeric. The unfolding step is frequently followed by aggregation, which may lead to loss of solubility. Thus, changes in solubility are frequently used as an indicator of protein denaturation. Determination of molecular weights by gel filtration chromatography and gel electrophoresis has been commonly used also for investigating protein denaturation (48).

The presence of a partially folded conformation called a molten globule state, that is, a kinetic intermediate of reversible denaturation equilibrium, was reported in many globular proteins (49). The molten globule has a native-like backbone secondary structure, and the side chain's environment undergoes a denaturation-like alteration. Hydrophobic clusters are exposed, as reflected in increasing binding of a hydrophobic fluorescent probe. It is postulated that molten globules are involved in the functional properties (eg, emulsification, foaming, and gelation) of food proteins (49).

In emulsions of oils in protein solutions, the proteins change from their native conformations to forms that depend largely on the hydrophobic interactions between the surface and the amino acid side chains in the protein. Ideally, hydrophobic side chains will be close to the oil surface and hydrophilic residues will favor the aqueous phase, but this will be constrained by the distribution of the amino acid residues in the protein. The conformation that is adopted also depends on the surface area that the protein is required to cover (47,50). Dickinson (51) classified milk proteins into two distinct classes: the disordered casein and the globular whey proteins. Substantial differences exist between these two classes in terms of adsorbed layer structure and surface rheological properties at the oil–water interface. Computer simulation showed promise for modeling the behavior of hypothetical proteins and peptides (51).

In the case of foam formation, the adsorption of proteins at the air–water interfaces has often been described in terms of three processes. They are transportation from bulk solution to the interface, penetration into the surface layer and reorganization of structure (surface denaturation) of the protein in the adsorbed layer (47,52). This process is similar to the case of emulsion formation, but the detail in structure changes at the air–water interface is unclear, mainly because of difficulty in analysis.

Conformational changes of proteins when the protein solutions are gelled upon heating are reviewed by Matsumura and Mori (53). It is interesting to note that β-sheet structure, which is mostly association units for gel formation, seems to be due to the formation of an intermolecular β-sheet structure induced by association between denatured molecules. That is, β-sheet formation itself probably has no direct relation to the denaturation step of globular proteins.

A similar phenomenon of aggregation of β-structure-forming (ie, cross β-fold) is observed in amyloid fibrinogenesis. Tissue deposition of soluble autologous proteins as insoluble amyloid fibrils is associated with serious diseases, including systemic amyloidosis, Alzheimer's diseases, and transmissible spongiform encephalopathy. Two naturally occurring human lysozyme variants were both amyloidogenic (54).

Modeling has been attempted to study the relationships between processing conditions and the resultant structural changes, using a variety of multivariate analysis techniques (55). The most advanced methods may be partial least squares regression (PLS), artificial neural networks, and genetic algorithm. Many commercial computer programs for these modeling methods are available.

For assessing the folding stability of protein molecules, in addition to the traditional spectroscopies (UV, fluorescence, and CD) calorimetry, solvent stabilization (56), a novel method of hydrogen exchange using NMR (57), and electrospray mass spectrometry (58) are being used. The exchange of the protons of buried backbone NH groups with those of the solvent is measured. Peptide NH groups that are exposed to solvent exchange rapidly, but those that form stable hydrogen bonds within the protein, or on occasion are simply buried, exchange slowly. The rate of $H \rightleftharpoons D$ exchange of individual groups can be measured conveniently using either normal protein dissolved in D_2O or D-labeled protein dissolved in H_2O.

STRUCTURE–FUNCTION RELATIONSHIPS

Structural changes of food proteins usually take place during processing. Because the structure closely relates to food quality, optimization of processing conditions to obtain the best quality is one of the most important objectives of food protein research. From the aspect of structure–function relationships of food proteins, there are three categories of structure changes. (*1*) The purpose of processing is not for changing structures, but the processing results in structure changes. An example is pasteurization or sterilization. (*2*) The structural changes are the main purpose of processing, such as in breadmaking. (*3*) A new type of structure change to be introduced, including chemical, enzymatic, and genetic changes of protein structure, optimizes these changes to achieve the best functions. The functions include all the chemical, physical, and biological properties of protein molecules. The use of bioactive peptides or proteins found in natural resources as ingredients in food is quickly becoming a new trend for contributing to human health. A new concept of chemopreventive agents is being introduced into diet to prevent diseases (59).

Meanwhile, a new paradigm is being discussed for protein research. A novel concept of protein substructure based on a knot-matrix construction principle revealed a much more powerful paradigm for protein research than that based on protein secondary structures (60). For macromolecular crystal structure analysis, the simple Debye-Waller model has been widely used. In this treatment of atomic motion, the probability of finding an atom in a given distance x from its equilibrium position x_0 is Gaussian. If it is assumed that the motion is isotropic, the model states that the motion in any direction can be characterized in terms of a mean-square vibration amplitude, $\langle x^2 \rangle$, also termed the mean-square displacement. The X-ray scattering from each atom is modified by a Gaussian function that is related to the mean-square displacement of that atom. The form of the Gaussian is

$$\exp(-B \sin^2\theta/\lambda^2)$$

where θ is the Bragg angle, λ is the wavelength of the incident radiation, and B is related to the mean-square displacement by

$$B = 8\pi^2\langle x^2 \rangle \qquad (2)$$

B is called Debye-Waller factor (59). The B factor provides useful information about conformational dynamics, which is calculated for each nonhydrogen atom.

Three groups of B factors are distinguished: group I, from 2 to 8 Å^2; group II, 8–14 Å^2; and group III, all higher values. Functional domains consist of one group I substructure (knot) to which is tethered most of the group II atoms (matrices) of the domain. Group III atoms are restricted to surface. In the knot-matrix construction principle, which explains properties of protein molecules, especially functions, knots determine palindromic B factor patterns and matrices put them to work. Palindromic patterns can be found on B factor vs atom-number plots as two functional domains against the residue lying halfway between them. The palindromic patterns control specificity of proteins such as enzymes and antibodies (60).

Optimization of Structural Changes

When structural changes are directly related to functions, such as loaf volume of bread, the model yielded from the correlation computation can be used to predict the structure parameters. Based on the response surface graphically illustrated, the optimum may be able to be located.

However, when the processing data are inadequate to conduct the modeling computation, such as creating new processing procedures or new products, computer-aided optimization could be the best solution. Again, there are many commercial computer programs available for optimization. If it is difficult to select the best method for achieving the objectives, contacting Network Enabled Optimization Center (NEOS) is recommended. NEOS is the optimization technology center that is a joint enterprise of Argonne National Laboratory (Argonne, Ill.) and Northwestern University (Evanston, Ill.).

Recently, a unique experimental optimization technique was proposed (62). Random-centroid optimization (RCO) uses simple algorithms since there is no need for complicated advanced algorithms. The method has been successfully applied to chemical synthesis as well as food processing projects.

Quantitative Structure–Activity Relationship Computation

Since the introduction of computer-aided study of quantitative structure–activity relationships (QSAR) to explain the functions of chemical compounds (63), many computer programs have been used for modeling. Instead of using processing conditions as independent variables, hydrophobic, steric, and electronic parameters were used in *QSAR* at the beginning.

In the case of food proteins, hydrophobicity and solubility were first used for *QSAR* computation (55) because of unavailability of data on structure and electronic parameters. This was one step ahead of the solubility–function relationship in the 1970s. Although the electronic parameter can be replaced by charge density, the steric parameter is still difficult to define for food proteins.

Chemical and Enzymatic Modification for Improving Functionality

To improve functions of proteins, modification of charge density, hydrophobicity, and molecular size has been conducted (64). Cross-linking and hydrolysis are two major techniques for modifying the size of protein molecules.

Genetic Engineering to Improve Protein Functions

Genetic engineering is quickly becoming an important technique in food processing for using engineered products as bioactive ingredients. This is because of successful developments of high-level expression techniques, thereby yielding near 1 g of recombinant proteins per liter of culture media (65) To avoid possible allergenicity or side effects, human peptides or proteins can be produced by genetic engineering to use in diet at reasonable costs. Examples are human lysozyme in infant formula and human cystatins as chemopreventive ingredients. The random-centroid optimization program modified for genetic study (RCG) was intended for use in site-directed mutagenesis (66). The RCG can be used to improve biological functions or to eliminate adverse effects. Structural changes could be a result of these mutations. Study on changes in molecular structure should be conducted to elucidate the mechanism of function of protein molecules under investigation. Since the difficulty in defining structure parameters is involved, the strategy to change structure to optimize function is usually hard to establish, despite the effort being made as described above (60). An advantage of RCG is that there is no need of *a priori* information on the structure–function relationships. Therefore, experimental optimization such as RCG can be a useful approach at the present stage of progress in protein chemistry.

ACKNOWLEDGMENTS

The author is greatly indebted to Dr. John R. Whitaker, University of California at Davis, for his invaluable advice. Without Dr. Whitaker's profound knowledge in this area, there would not be the present form of this article

BIBLIOGRAPHY

1. R. D. Ludescher, "2. Physical and Chemical Properties of Amino Acids and Proteins," in S. Nakai and H. W Modler, eds., *Food Proteins: Properties and Characterization*, VCH Publishers, Weinheim, Germany, 1996.
2. G. E. Schulz and R. H. Schimer, *Principles of Protein Structure*, Springer-Verlag, New York, 1979.
3. D. Voet and J. G. Voet, *Biochemistry*, John Wiley & Sons, New York, 1990.
4. H. R. Matthews, R. Freedland, and R. L. Miesfeld, *Biochemistry, A Short Course*, Wiley-Liss, New York, 1997.
5. M. C. J. Wilce, M.-I. Aguilar, and M. T. Hearn, "Physicochemical Basis of Amino Acid Hydrophobicity Scales: Evaluation of Four New Scales of Amino Acid Hydrophobicity Coefficients Derived from RP-HPLC of Peptides," *Analytical Chem.* **67**, 1210–1219 (1995).
6. M. M. Gromiha and P. K. Ponnuswamy, "Relationship between Amino Acid Properties and Protein Compressibility," *J. Theoretical Biol.* **165**, 87–100 (1993).
7. S. H. Chiou and K. T. Wang; "Simplified Protein Hydrolysis with Methanesulfonic Acid at Elevated Temperature for the Complete Amino Acid analysis of Proteins," *J. Chromatogr* **448**, 404–410 (1988).
8. J. Csapo et al., "Determination of the Cysteine Content of Foods and Feeds by Mercaptoethanesulphonic Acid Hydrolysis," *Acta Alimentaria Academiae Scientiarum Hungaricae* **15**, 227–235 (1986).
9. Y. Yokote et al., "Recovery of Tryptophan from 25-Minute Acid Hydrolysates of Protein," *Analytical Biochem.* **152**, 245–249 (1986).
10. J. Haginaka and J. Wakai, "Fluorimetric Determination of Amino Acids by High Performance Liquid Chromatography Using a Hollow-Fiber Membrane Reactor," *J. Chromatogr* **396**, 297–305 (1987).
11. S. Matsumura, H. Kataoka, and M. Makita, "Determination of Amino Acids in Human Serum by Capillary Gas Chromatography," *J. Chromatogr.* **681**, 375–380 (1996).
12. M. W. Duncan and A. Poljak, "Amino Acid Analysis of Peptides and Proteins on the Femtomole Scale by Gas Chromatography/Mass Spectrometry," *Analytical Chem.* **70**, 890–896 (1998).
13. M. Ward, "Amino Acid Analysis Using Precolumn Derivatization with 6-Aminoquinolyl-*N*-Hydroxysuccinimidyl Carbamate," in J M. Walker, ed., *The Protein Protocols Handbook*, Humana Press, Totowa, N.J., 1996, pp. 461–465
14. G B. Irvine, "Amino Acid Analysis Using Precolumn Derivatization with Phenylisothiocyanate," in J. M. Walker, ed., *The Protein Protocols Handbook*, Humana Press, Totowa, N.J., 1996, pp. 467–472.
15. A. G Walton, *Polypeptides and Protein Structure*, Elsevier Science Publishing, New York, 1981.
16. M. Haniu and J.E. Shively, "Microsequence Analysis of Peptides and Proteins. IX. Manual Gas-Phase Microsequencing of Multiple Samples," *Analytical Biochem.* **173**, 296–306 (1988).
17. M. D Bauer, S. Yiping, and F Wang, "Nano-electrospray Mass Spectrometry and Edman Sequencing of Peptides and Proteins Collected from Capillary Electrophoresis," in D. R. Marshak, ed., *Techniques in Protein Chemistry VIII*, Academic Press, New York, 1997, pp. 37–46.
18. F. M. Ausubel et al., eds., "DNA Sequencing," in *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 1998, pp. 7.01–7.7.31.
19. C. Branden and J. Tooze, *Introduction to Protein Structure*, Garland Publishing, New York, 1991.
20. C. Vieille and J. G. Zeikus, "Thermozymes: Identifying Molecular Determinants of Protein Structural and Functional Stability," *Trends in Biotechnol.* **14**, 183–190 (1996).
21. S. Nakamura et al., "Improving the Thermostability of *Bacillus stearothermophilus* Neutral Protease by Introducing Proline into the Active Site Helix," *Protein Eng.* **10**, 1263–1269 (1997).
22. S. Hayakawa and S. Nakai, "Relationship of Hydrophobicity and Net Charge to the Solubility of Milk and Soy Proteins," *J. Food Sci.* **50**, 486–491 (1985).
23. T E. Creighton, *Protein: Structures and Molecular Properties*, 2nd ed., Freeman, New York 1993.
24. J. King, "Deciphering the Rules of Protein Folding," *Chemical Eng. News* **67**(15), 32–54 (1989).
25. N. N. Kalnin, I. A. Baikalov, and S. Y. Venyaminov, "Quantitative IR Spectrophotometry of Peptide Compounds in Water Solutions. III. Estimation of the Protein Secondary Structure," *Biopolymers* **30**, 1273–1280 (1990).

26. R. W. Williams, "Protein Secondary Structure Analysis Using Raman Amide I and Amide III Spectra," *Methods in Enzymol.* **130**, 311–331 (1986).
27. R. A. Venters et al., "Strategies for NMR Assignment and Global Fold Determinations Using Perdeuterated Proteins," in D. R. Marshak, ed., *Techniques in Protein Chemistry VIII*, Academic Press, New York, 1997, pp. 605–615.
28. S. Borman, "Advance in NMR of Macromolecules, Technique Makes It Possible to Obtain Spectra of Much Larger Proteins," *Chemical and Eng. News* **76**, 55–56 (1998).
29. N. J. Greenfield, "Methods to Estimate the Conformation of Proteins and Polypeptides from Circular Dichroism Data," *Analytical Biochem.* **235**, 1–10 (1996).
30. P. Y. Chou and G. D. Fasman, "Empirical Prediction of Protein Conformation," *Annu. Rev. Biochem.* **47**, 251–276 (1978).
31. R. D. King, "Prediction of Secondary Structure," in M. J. E. Sternberg, ed., *Protein Structure Prediction*, Oxford University Press, Oxford, U.K., 1996, pp. 79–99.
32. N. Srinivasan, K. Gurprasad, and T. L. Blundell, "Comparative Modelling of Proteins," in M. J. E. Sterberg, ed., *Protein Structure Prediction*, Oxford University Press, Oxford, U.K., 1996, pp. 111–140.
33. D. G. Reid, ed., *NMR in Detail. Protein NMR Techniques*, Humana Press, Totowa, N.J., 1997
34. F. London, " Über einige Eigenshaften und Anwendungen der Molekularkräfte," *Zeitschrift für Phisikalische Chemie, Abteilung B* **11**, 222–251 (1930).
35. K. Hamaguchi, *The Protein Molecule, Conformation, Stability and Folding*, Japan Scientific Societies Press, Springer-Verlag, Tokyo, 1992.
36. M. Nonaka, E. Li-Chan, and S. Nakai, "Raman Spectroscopic Study of Thermally Induced Gelation of Whey Proteins," *J. Agric. Food Chem.* **41**, 1176–1181 (1993)
37. L. W. Nichol et al., "Evaluation of Gel Filtration Data on Systems Interacting Chemically and Physically," *Arch. Biochem. Biophysics* **121**, 517–521 (1967).
38. S. Nakai and M. Nonaka, "Computation of Molecular Weight and Weight Fraction of Five and Six Components in Mixtures from Model Equilibrium Ultracentrifugation Data," *J. Agric. Food Chem.* **40**, 824–829 (1992).
39. G.-M. Wu, D. Hummel, and A. Herman, "Analysis of the Solution Behavior of Protein Pharmaceuticals by Laser Light Scattering Photometry," in ACS Symposium 675, *Therapeutic Protein and Peptide Formulation and Delivery*, American Chemical Society, Washington, D.C., 1997, pp. 168–185.
40. K. L. Bentley et al., "Fluorescence Polarization: A General Method for Measuring Ligand Binding and Membrane Microviscosity," *Biotechniques* **3**, 356–366 (1985).
41. S. Nakai and C. M. Kason, "A Fluorescence Study of the Interactions between κ- and α_{s1}-Casein and between Lysozyme and Ovalbumin," *Biochimia et Biophysica Acta* **351**, 21–27 (1974).
42. F. M. Ausubel et al., eds., "Analysis of Protein Interactions," in *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 1998, pp. 20.01–20.5.9.
43. L. A. Sklar et al., "Conjugated Polyene Fatty Acids as Fluorescent Probes. Binding to Bovine Serum Albumin," *Biochem.* **16**, 5100–5108 (1977).
44. B. H. Robinson et al., "Application of EPR and Advanced EPR Techniques to Study of Protein Structure and Interactions," *EPR and Advanced EPR Studies of Biological Systems* **1985**, 183–255 (1985).
45. S. Nakai et al., "Quantification of Hydrophobicity for Elucidation of the Structure–Activity Relationships of Food Proteins," in *Interactions of Food Proteins*, ACS Symposium 454, American Chemical Society, Washington, D.C., 1991, pp. 42–58.
46. R. B. Gregory, "Protein Hydration and Glass Transfer Behavior," in R. B. Gregory, ed., *Protein-Solvent Interactions*, Marcel Dekker, New York, 1995.
47. S. Damodaran, "Functional Properties," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, VCH Publishers, Weinheim, Germany, 1996, pp. 167–234.
48. A. Kilara and V. R. Harwalkar, "Denaturation," in S. Nakai and H. W. Modler eds., *Food Proteins: Properties and Characterization*, VCH Publishers, Weinheim, Germany, 1996, pp. 71–165.
49. M. Hirose, "Molten Globule State of Food Proteins," *Trends in Food Sci. Technol.* **4**, 48–51 (1993).
50. D. D. Dalgleish, "Adsorption of Protein and the Stability of Emulsions," *Trends in Food Sci. Technol.* **8**, 1–6 (1997).
51. E. Dickinson, "Proteins at Interface and in Emulsions, Stability, Rheology and Interactions," *J. Chemical Soc. Faraday Trans.* **94**, 1657–1669 (1998).
52. P. J. Wilde and D. C. Clark, "Foam Formation and Stability," in G. M. Hall, ed., *Methods of Testing Protein Functionality*, Blackie Academic and Professional, London, 1996.
53. Y. Matsumura and T. Mori, "Gelation," in G. M. Hall, ed., *Methods of Testing Protein Functionality*, Blackie Academic and Professional, London, 1996.
54. D. R. Booth et al., "Instability, Unfolding and Aggregation of Human Lysozyme Variants Underlying Amyloid Fibrillogenesis," *Nature* **385**, 787–793 (1997).
55. S. Nakai, G. E. Arteaga, and E. C. Y. Li-Chan, "Computer-Aided Techniques for Quantitative Structure Activity Relationships Study of Food Proteins," in N. S. Hettiarachchy and G. R. Ziegler, eds., *Protein Functionality in Food Systems*, Marcel Dekker, New York, 1994, pp. 121–145.
56. B. A. Shirley, *Boiled Eggs and Molten Globules—Protein Stability and Folding: Theory and Practice*, Humana Press, Totowa, N.J., 1995.
57. J. Clarke, L. S. Itzhaki, and A. R. Fersht, "Hydrogen Exchange at Equilibrium: A Short Cut for Analysing Protein-Folding Pathways?," *Trends in Biological Sci.* **22**, 284–287 (1997).
58. Z. Zhang et al., "Higher-Order Structure and Dynamics of FK506-Binding Protein Probed by Backbone Amide-Hydrogen/Deuterium Exchange and Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy," in D. R. Marshak, ed., *Techniques in Protein Chemistry VIII*, Academic Press, New York, 1997
59. P. Greenwald, "Chemoprevention of Cancer," *Scientific American* **275**, 96–99 (1996).
60. R. Lumry, "The New Paradigm for Protein Research," in R. B. Gregory, ed., *Protein–Solvent Interactions*, Marcel Dekker, New York, 1995, pp. 1–141.
61. G. A. Petsko and D. Ringe, "Fluctuations in Protein Structure from X-Ray Diffraction," *Annu. Rev. Biophysics and Bioeng.* **13**, 331–371 (1984).
62. S. Nakai et al., "Optimization of Site-Directed Mutagenesis. 1. A New Random-Centroid Optimization Program for Windows Useful in Research and Development," *Agric. Food Chem.* **46**, 1642–1654 (1998).
63. A. J. Stuper, W. E. Bugger, and P. G. Jurs, eds., *Computer Assisted Studies of Chemical Structure and Biological Functions*, John Wiley and Sons, New York, 1979

64. S. Nakai, "An Overview," in S. Nakai and H. W. Modler, eds., *Food Proteins: Properties and Characterization*, VCH Publishers, Weinheim, Germany, 1996, pp. 1–21.
65. J. M. Cregg, T. S. Vedvick, and W. C. Raschke, "Recent Advances in Expression of Foreign Genes in *Pichia pastoris*," *Bio/Technol.* **11**, 905–910 (1993).
66. S. Nakai, S. Nakamura, C. H. Scaman, "Optimization of Site-Directed Mutagenesis. 2. Application of Random-Centroid Optimization to One-Site Mutation of *B. stearothermophilus* Neutral Protease to Improve Thermostability," *J. Agric. Food Chem.* **46**, 1655–1661 (1998).

S. Nakai
University of British Columbia
Vancouver, British Columbia
Canada

PULSED LIGHT PROCESSING

A new processing technology uses intense flashes of broad-spectrum white light to kill microorganisms on packaging and food surfaces and in air and water. The flashes have a duration of 100 to 300 millionths of a second and effectively kill all microorganisms (bacteria, fungi, spores, cysts, protozoa, and viruses) in less than a second. The process is effective for sterilization when the light can reach all surfaces (such as the surfaces of packaging materials) or the total volume of a product such as water and other transmissive fluids. Microbial levels on foods, including pathogens, are reduced to extend shelf life and reduce public health risk. The process is marketed under the trade name of PureBright® by PurePulse Technologies (a subsidiary of Maxwell Technologies Inc.), 4241 Ponderosa Avenue, San Diego, California 92123.

PULSED LIGHT

Electrical energy stored in a capacitor can be rapidly released to produce short, intense high-power pulses (Fig. 1). Such pulses are used to generate the intense flashes of PureBright light by electrically ionizing a xenon gas lamp. The broadband emitted light flash has wavelengths from far-UV (200–300 nm) through the near-UV (300–380 nm) and visible spectra (380–780 nm), to the infrared (780–1,200 nm). Approximately 25% of the light is in the UV wavelength range.

Flash duration is typically about 300 μs and the light intensity is greater than 20,000 times that of sunlight. A few flashes applied in a fraction of a second kills all exposed microorganisms, spores, and viruses.

Although the peak power of each flash is very high, the total energy consumed per flash is relatively low and the average operating power requirement modest.

All PureBright systems have a treatment-monitoring capability. Each pulse of light is monitored for total fluence and UV content. A feedback circuit is used to assure that proper treatment is provided and controlled. The process is essentially fail-safe.

MICROBICIDAL EFFECTS

PureBright antimicrobial mechanisms are based on the sensitivity of microorganisms to UV wavelengths that are readily absorbed by microbial nucleic acids and proteins, and the very high power and intensity of the flash.

The antimicrobial effects of one to several flashes are dramatic. PureBright treatment at 1.5 J/cm^2 kills 7 logs of *Staphylococcus aureus* ATCC 27661 suspended in 25-mL droplets dried on the surface of microbiological media.

Figure 2 shows the pulsed light kill kinetics obtained using the microdrop assay for a number of different bacteria and spores. The results show 7 to 9 logs/cm^2 reduction using a few flashes.

PureBright kill dynamics are superior to conventional ultraviolet light from a high-intensity mercury vapor lamp. Using relatively UV-resistant *Aspergillus niger* spores inoculated and dried on a packaging surface, conventional continuous wave UV kills 3.5 to 4.5 logs in 6 to 10 s with no appreciable increase in kill with additional treatment time. By comparison, PureBright kills more than 7 logs of *A. niger* spores with a few flashes applied in a fraction of

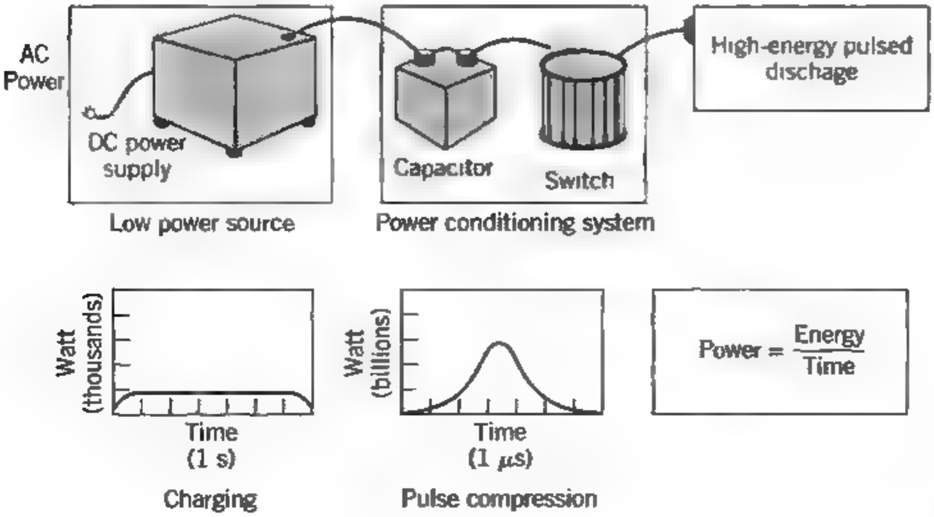


Figure 1. Pulsed energy processing.

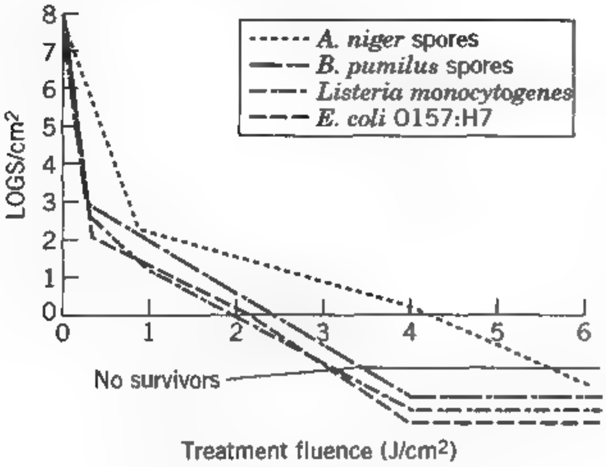


Figure 2. Pulsed light kill kinetics.

a second. This dynamic provides the effectiveness required for high sterility assurance (Fig. 3).

TREATMENT OF PACKAGING

PureBright can be used to sterilize packaging material surfaces, thus replacing hydrogen peroxide in many aseptic packaging applications. On most package surfaces, PureBright kills greater than 7 logs of *Bacillus pumilus* spores (a radiation-resistant bacterial spore) in challenge tests.

PureBright is also effective for package applications involving perishable, refrigerated, extended–shelf life food products, such as containers for yogurt, cottage cheese, or milk. Figure 4 shows a typical cup-and-lid-packaging line configuration that can be used for extending the shelf life of refrigerated desserts and other products.

Pulsed light is transmitted through many plastics, so certain packages can be sterilized on the inside by flashing the outside. Also, the light can be used to treat a filled and sealed product through the package if the product has good transmission properties such as water or other clear solutions. In general, colors will inhibit the UV transmission and microbial kill.

The olefins (polyethylene, polypropylene), acrylics, many polyamides and nylons, ethylene acrylic acid, EVA, EVOH, and similar plastics that transmit in the ultraviolet are good candidates for PureBright treatment through the package. Plastics with aromatic hydrocarbon backbones, side groups, or additives generally absorb UV too strongly to permit treatment through the package.

No adverse chemical or functionality effects on plastic packaging materials have been found using a variety of plastics treated with PureBright at levels in excess of those needed to provide appropriate microbial kill.

PureBright is also effective for treating metal or plastic bottle caps. Tests using four different types and configurations of caps have shown total kill of 4 to 5 logs of inoculated *A. niger* conidiospores using 2 to 6 flashes.

TREATMENT OF FOOD

PureBright reduces microbial loads on perishable foods such as baked goods, fresh and processed meats, poultry, fish, fresh fruit, and vegetables. Due to the generally opaque and irregular surfaces of foods, lower kill levels are realized than on packaging; however, significant shelf life extension is achieved and pathogenic microorganism levels reduced.

Microorganisms on fresh meat surfaces are reduced 1 to 3 logs by PureBright, resulting in extended refrigerator shelf life of 2 or more days. Similarly, the shelf life of baked goods is increased by the elimination of surface mold (Fig. 5).

The FDA has issued a food additive regulation that approves the use of PureBright on all foods for the control of bacteria, molds, yeast, and other microorganisms.

TREATMENT OF WATER

PureBright kills a wide range of microorganisms in clear water without the use of heat, chemicals, or fine filtration.

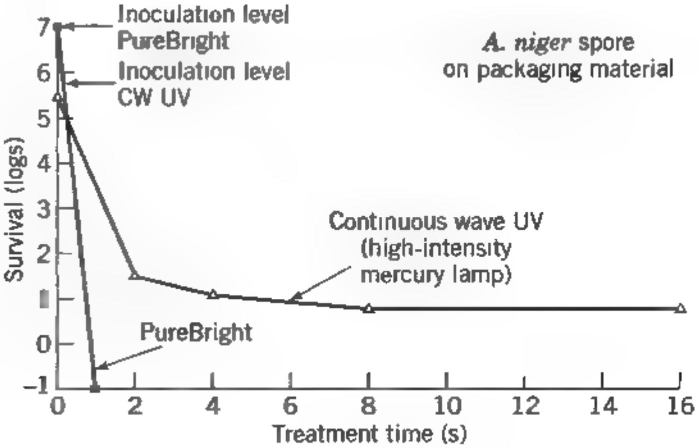


Figure 3. PureBright vs. high-power continuous-wave UV.

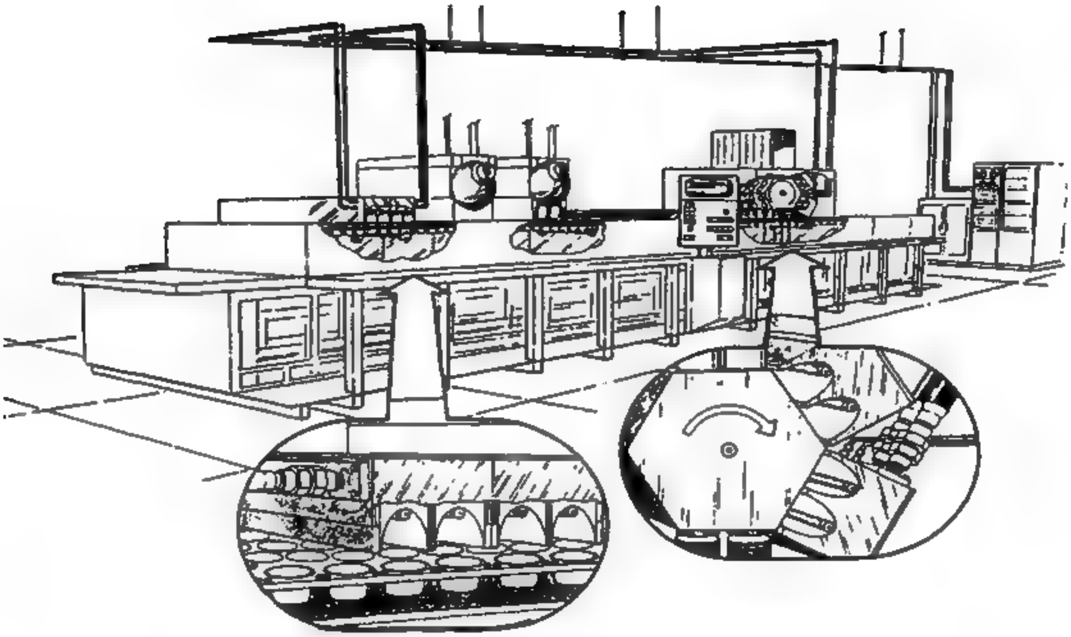


Figure 4. PureBright kills bacteria on packages in food-production line.

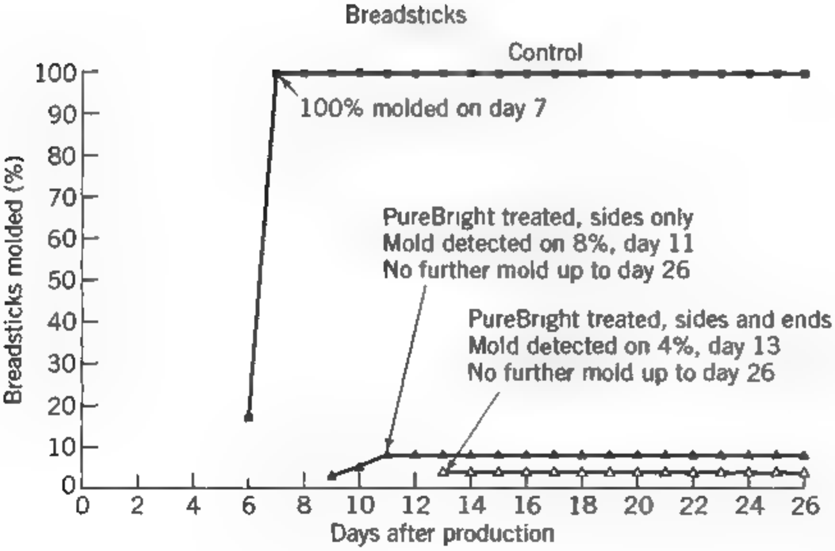


Figure 5. Extended shelf life of baked goods.

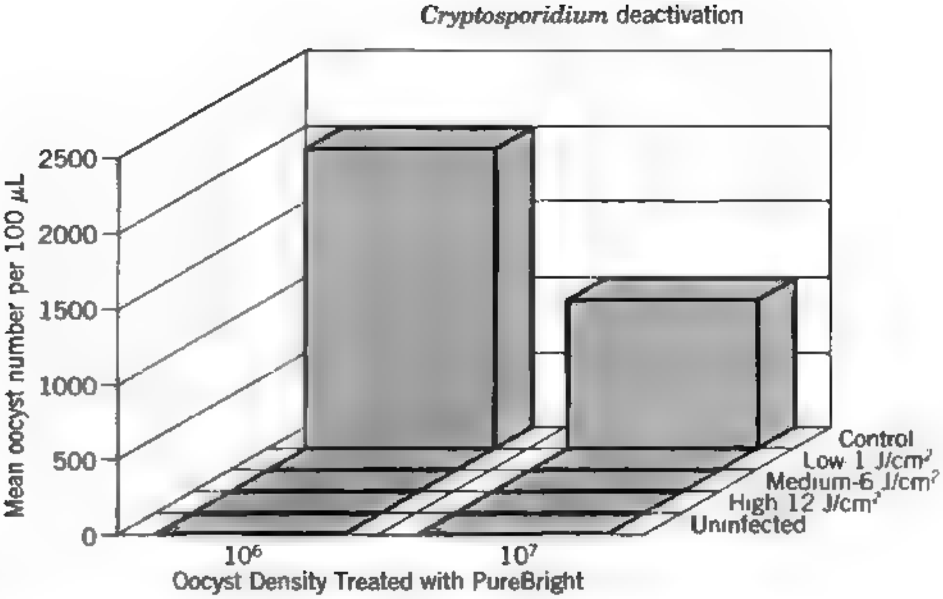


Figure 6. PureBright effect on *Cryptosporidium.*

Unlike other treatment methods, PureBright, at a relatively low level, has demonstrated efficient kill of *Cryptosporidium* oocyts and viruses (Fig. 6).

GENERAL REFERENCES

J. Dunn, T. Ott, and W. Clark, "Pulsed Light Treatment of Food and Packaging," *Food Technol.* **49**, 95–97 (1995).

J. Jagger, *Ultraviolet Photobiology*, Prentice Hall Inc., Englewood Cliffs, N.J., 1967.

L. Koller, *Ultraviolet Radiation*, John Wiley and Sons, Inc., New York, 1977.

K. Smith, *The Science of Photobiology*, Plenum Press, New York, 1977.

THOMAS M. OTT
Purepulse Technologies
Westwood, New Jersey

Q

QUALITY ASSURANCE

Americans spent about $337 billion on food purchased in retail food stores in 1994 and about $303 billion on food purchased in foodservice establishments (1). A variety of factors are responsible for the changes in U.S. consumption patterns, including changes in consumer preferences, relative prices, increases in real (adjusted for inflation) disposable income, and more food assistance to the poor. An expanded scientific base relating diet and health, new dietary guidelines for Americans designed to help people make food choices that promote health and prevent disease, and burgeoning interest in nutrition and food safety also influence marketing and consumption trends.

Consistent with dietary and health recommendations, Americans in 1996 consumed two-fifths more grain products and a fifth more fruits and vegetables per capita than they did in 1970, ate leaner meat, and drank lower-fat milk (2).

Though consumers do not want to spend a lot of time on food preparation, they also are not willing to scrimp on health and nutrition. The trend to lowering fat consumption has contributed to gains in poultry consumption and declines in red meat. Consumer concerns about food additives, chemicals, and preservatives have translated into a growing market for pesticide-free products, organic produce, and vegetarian choices. As consumer concerns about food safety have increased, so have the demands that government regulators increase efforts to assure food safety and guarantee consumers better and more accurate information about the food they eat. We have seen some major initiatives by the government such as the Food Code 1997, the Nutrition Labeling and Education Act (NLEA), United States Department of Agriculture (USDA) and Food and Drug Administration (FDA), Hogard Analysis Critical Control Point (HACCP) initiatives, and the President's National Food Safety Initiative. Environmental awareness has spawned a wave of recyclable, biodegradable, and nonpolluting products. Consumers are demanding "environmentally friendly" products. Manufacturers are using packaging and other environmental characteristics to appeal to consumers in advertising campaigns. From farm to table, technologies are being developed that contribute to sustainability of agriculture and environment (3).

Food service has shown tremendous growth. Industry growth is attributable in large part to the bundling of food products with convenience and quality.

A variety of factors are fueling the trends toward the use of more prepackaged and prepared items. In the commercial food service sector, the conflicting goals of expanding menu offerings and reducing operating costs are demanding that operators explore new types of products and packaging. It is expected that increased variety and greater speed will be the future direction of this industry. Increased variety will exist in many alternatives in the service and packaging methods. Speed will be reflected in the short time between when the food is bought and when it is consumed. Speed will also play a role in functions such as ordering and food preparation. Electronic systems will be increasingly critical to the operating process. Sophisticated heating and cooling mechanisms will be demanded. In the future, doing business will require science-based quality systems and the increased use of food technology. What will not change is the consumer's desire for good-tasting and healthy products that satisfy both nutritional and social needs. This is the service in food service that cannot be replaced by the increasingly sophisticated activities that are behind what is ultimately served.

THE FOOD QUALITY SYSTEM

The significance of any food quality system has to be discussed within the context of the food supply in its entirety. The quality of our food supply is of supreme importance to us all: consumers, farmers, processors, distributors, food service operators, and the regulatory agencies. Increasing affluence has given birth to a new breed of consumer, one accustomed to eating away from home frequently. This new consumer shows greater reliance on convenience and expects safe, high-quality foods for consumption (4).

The U.S. food supply is abundant. Our preservation processes have changed dramatically and include such developments as irradiation, genetic engineering, and controlled atmospheres. Distribution includes air transport, refrigerated railroad cars, trucks, freezers, and shipments by sea. We have a global system of distribution that makes, for example, a variety of out-of-season produce available year-round, with improved taste. This complexity has brought about concerns about food safety (5).

The consumer drives the food quality system. No quality program can be successful if it does not address consumer concerns, real or perceived. F. J. Francis discusses public perception of food safety in a detailed report and urges the scientific community to stand up and speak out when something is scientifically absurd. Epidemiologists have estimated the potential deaths due to pesticides as close as zero. The FDA and National Cancer Institute have repeatedly stated that risks from pesticides are insignificant. Yet USDA appropriated $40 million for pesticide monitoring, not to increase public safety but to reassure the public. It emphasizes the need for a science-based quality system that incorporates risk assessment and a mechanism to explain to the public the benefits of such a system through a core of knowledgeable food scientists (6).

A recent survey revealed that freshness is the most important quality consumers look at when purchasing refrigerated foods (7). In the writer's view, consumers associate freshness with quality, safety, and health. A USDA Economic Research Service (ERS) study (8) indicated that in 1995, 5.4 billion lb of food were lost at the retail level, and 91 billion lb were lost by consumers and food service. Fresh fruits and vegetables accounted for nearly 20% of consumer and food service losses. It was estimated that the total annual cost of unsalables to U.S. packaged goods manufactured in the 1996 supermarket channel was $1.96 billion (9). There is a need to improve the quality of our food system, maintain food safety, and return the benefits to the consumer. The various components of the food system are interdependent. The private sector, regulators, consumers, and universities need to work closely to insure that the goals of a science-based quality system are achieved.

SYSTEM DESIGN

Definitions

- Quality system: The organizational structure, responsibilities, procedures, and resources for implementing quality management
- Quality management: That aspect of overall management function that determines and implements the quality policy
- Quality policy: The overall quality intentions and direction of an organization, as regards quality, formally expressed by top management
- Quality control: The operational techniques and activities that are used to fulfill requirements for quality
- Quality assurance: All those planned and systematic actions necessary to provide adequate confidence that a product will satisfy given requirements

These terms are used to define quality systems and their implementation (10,11).

The elements of putting a system together are detailed in the publication *Food Processing Industry Quality System Guidelines* (12). These guidelines were written by a committee of experts and adapted from American National Standards Institute/American Society for Quality Control (ANSI/ASQC) Q9004-3-1993, *Quality Management and Quality System Elements—Guidelines for Processed Materials*. The monograph includes definitions of terms used in quality systems; management responsibility; quality systems principles; economics—quality-related cost considerations; quality in marketing, specification, design/development, procurement, and production; product verification; measuring and control of test equipment; nonconformity, corrective action, handling, and postproduction functions; quality documentation and records; personnel; product safety and liability; and use of quality methods.

The International Organization for Standardization (ISO) in Geneva, Switzerland, has developed a series that provides a framework in the development of quality plans. They are:

- ISO 9000 Quality Management and Quality Assurance Standards: Guidelines for Selection and Use
- ISO 9001 Quality Systems: Model for Quality Assurance in Design, Development, Production, Installation, and Servicing
- ISO 9002 Quality Systems: Model for Quality Assurance in Production, Installation, and Servicing
- ISO 9003 Quality Systems: Model for Quality Assurance in Final Inspection and Test
- ISO 9004 Quality Management and Quality System Element Guidelines
- ISO A 8402 Management and Quality Assurance Vocabulary

The Malcolm Baldridge National Quality Award is presented by the President of the United States to companies in the manufacturing and service industries. The criteria consist of seven categories: leadership, strategic planning, customer and market focus, information and analysis, human resources focus, process management, and business results. It is a good model and provides yardsticks by which companies can be recognized for both business excellence and quality achievements.

Although models provide a good framework in the development and implementation of quality plans, the models need to be adapted to individual business segments.

QUALITY COSTS

It is important to determine the impact of quality costs on company profitability. Cost assessment can be useful in evaluating not only the effectiveness of the quality system, but also criteria for internal improvement.

Costs can be divided into those for operating quality costs and those for external assurance. Operating quality costs are incurred by the company to attain and insure a specified quality level. They consist of prevention and appraisal costs (or investments) and failure costs (or losses). Prevention costs are incurred to prevent failures; some examples include ingredient sampling and analysis, equipment maintenance and design to maintain quality and food safety, and integrated pest management. Appraisal costs are incurred during testing and inspection to insure product quality; examples are laboratory testing and internal verification audits. External assurance costs are related to the demonstration and proof required as objective evidence by customers or regulatory agencies. Additional costs are opportunity costs, loss of business due to poor quality, and costs of exceeding requirements (11,12).

FOOD SAFETY

The Centers for Disease Control and Prevention (CDC) maintains statistics on foodborne outbreaks. From 1988 to 1992 (13), among outbreaks for which the etiology was determined, bacterial pathogens caused the largest percentage of outbreaks (79%) and the largest percentage of cases (90%); chemical agents caused 14% of outbreaks and 2% of cases; parasites, 2% of outbreaks and 1% of cases; and viruses, 4% of outbreaks and 6% of cases.

The Center for Science in the Public Interest compiled an inventory of 225 foodborne illness outbreaks (14) that occurred between 1990 and 1998. Ground beef caused 37 of 65 outbreaks; fruits and salads were the second most likely foods to be linked with an outbreak, with a total of 48 outbreaks. Seafood, both finfish and shellfish, was responsible for 32 outbreaks. Multi-ingredient foods, which included desserts, sauces, egg dishes, pasta dishes, and stuffing, were responsible for 63 outbreaks. In three-quarters of these outbreaks, the cause of illness was *Salmonella enteridis*. It is possible that emerging and re-emerging pathogens contributed to the large number of outbreaks of unknown etiology (14).

The Council for Agriculture and Science Technology (CAST) concludes that microbial pathogens in foods cause 6.5 to 44 million cases of human illnesses and up to 9000 deaths each year (15). It has been estimated that the costs of human illness for six bacterial pathogens—*Salmonella*, *Campylobacter*, *E. coli* 0157:H7, *Listeria*, *Staphylococcus aureus*, and *Clostridium pefigus*—are estimated to be $9.3 to $12.9 billion. Of these costs, $2.9 to $6.7 billion are attributed to foodborne bacteria (16). The estimated annual cost of *Campylobacter*-associated Guillain-Barré syndrome was $0.2 to $8.0 billion, with an estimation of $0.8 to $5.6 billion from food sources (17). Estimates are subject to errors because most of the comprehensive population-based studies have not attempted to determine which proportion of the reported illnesses are from food consumption and which are from other sources; also, foodborne illnesses can cause clinical conditions, not characteristically gastrointestinal symptoms (5,18).

Foodborne Diseases Active Surveillance Network (FoodNet) is a collaborative project among CDC; the health departments of California, Connecticut, Georgia, New York, Maryland, Minnesota, and Oregon; FDA; and USDA. The following bacteria have been targeted: *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* 0157·H7, *Listeria*, *Yersinia*, and *Vibrio* (19). FoodNet reports that *Campylobacter* was the most frequently isolated foodborne bacterium pathogen (49.4%), followed by *Salmonella* (27.4%), *Shigella* (15.7%), *E. coli* 0157:H7 (4.2%), *Yersinia* (1.7%), *Listeria* (1%), and *Vibrio* (0.6%) (20). FoodNet estimated that there were 8 million cases of these bacterial infections in 1997 in the United States (21).

EMERGING FOODBORNE DISEASES

Emerging and reemerging infections have been defined as new, recurring, or drug-resistant infections whose incidence in humans has increased in the past two decades, or whose incidence threatens to increase in the near future (22). This subject has been reviewed by Altekrusse (23). A committee of the Institute of Medicine recognized as early as 1992 the microbial threats to health in the United States of emerging infections and made recommendations (22). A number of factors contribute to the emergence of foodborne diseases (24).

A growing segment of the population is immunocompromised because of advancing age, chronic diseases, or infection with human immunodeficiency virus (HIV). Advances in medical sciences have extended life expectancies of persons with chronic diseases such as cancer, thus increasing their susceptibility to foodborne diseases.

Changes have occurred in industry, technology, agriculture, and lifestyles. There has been an increase in the consumption of fruits and vegetables. Produce is susceptible to microbial contamination during growth, harvest, and distribution. There has also been an increase in the demand for fresh-cut produce. The larger surface area makes this type of product more susceptible to contamination. Outbreaks have been associated with alfalfa sprouts, unpasteurized cider, fresh-squeezed orange juice, and frozen strawberries. Consumers have increased their spending on foods eaten away from home. Food service is a growing market. Improper cooking of items such as hamburgers, holding foods at improper temperatures, and cross-contamination are some of the causes of outbreaks. Extensive use of antibiotics has led to the emergence of multi-drug-resistant *Salmonella typhomurium* (DT) 104. Bovine spongiform encephalopathy (BSE) has emerged as an infectious agent, mainly in the form of adult dairy cattle in the United Kingdom. There is evidence that this prion-derived disease was a result of feeding cattle meat and bonemeal derived from dead ruminants such as sheep and slaughterhouse products infected with scrapie as a protein supplement. The problem was compounded by reduction of temperature used in heat treatment, and elimination of solvent extraction of animal tissue during meat and bonemeal processing led to a failure to inactivate BSE prion and thus allowed its contraction into the cattle population (24).

Allergenic mites are emerging as a food safety issue. Four species of food-contaminating mites have been reported to have caused allergenic reactions, including anaphylaxis in persons who consumed mite-contaminated foods. The mite species are the American house dust mite *Dermatophagoides farinae* Hughes (Acarina: Pyroglyphidae), the scaly grain mite *Suidasia* sp prob. *pontifica* Oudemans (Acarina: Suidasidae), an acarid mite *Thyreophagus entomophagus* Portus and Gomez (Ascarina: Acaridae), and the mold mite *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae) (25).

FDA guidance is delineated in Compliance Policy Guide 585.500, *Mushrooms, Canned or Dried (Freeze Dried or Dehydrated)—Adulteration Involving Maggots, Mites, Decomposition*. These levels are based on the assumption that the contaminants are harmless and unavoidable. The true level of safety to allergenic mites is unknown (25).

Miller mentions a number of pathogens that might potentially emerge, including prior induced variant of Kreutzfeld-Jakob disease, viruses, *Citrobacter freundii*, the newer genes of *Salmonella* such as *S. enteriditus* PT4 and *S. typhomurium* DT104, and parasites such as *Cyclospora*. Miller recommends strategies to anticipate the emerging pathogen challenge. They include intelligence, contingency, and strategic planning. Intelligence consists of gathering information through a global surveillance program. Contingency is rapid resource mobilization and assures that resources are available to quickly characterize the emerging pathogen. Strategic planning requires futuristic thinking to anticipate what may happen, taking into consideration the various factors such as society, economics, and technology.

NUTRITIONAL QUALITY

With increasing consumer awareness of nutrition, it becomes important to insure the proper handling of food products to maintain the claimed nutrition content. The Nutritional Labeling and Education Act (NLEA) defines the labeling requirements for food products, and quality systems need to be in place to insure that these requirements are met. Advances in food technology will result in minimal nutrient losses during harvest, processing, storage, and distribution. The challenge remains in educating the consumer about the importance of proper food handling (26).

HAZARD ANALYSIS: CRITICAL CONTROL POINT SYSTEMS

The scientific community accepts that HACCP systems, when used properly, will substantially enhance food safety. HACCP is defined as a management system in which food safety is addressed through the analysis and control of physical, chemical, and microbiological hazards from raw material production, procurement, and handling to manufacturing and consumption of finished product. The successful implementation of HACCP requires a firm commitment from top management (27). Bryan (28) provides a historical perspective on HACCP. The principles of HACCP were endorsed in the 1980s by the National Research Council (NRC) (29). NRC has issued a number of reports on meat, poultry, and seafood very supportive of the HACCP concept (30–32). A number of reports have been issued by the National Advisory Committee on Microbiological Criteria for Foods (33–38). The HACCP principles have gained recognition by industry and regulatory agencies. USDA has mandated the use of HACCP in meat and poultry (39), and FDA in seafood (40). Guidelines for fresh fruits and vegetables have been issued by FDA (41).

The FDA Food Code (42) incorporates the principles of HACCP. There have been a number of guidelines, manuals, and generic plans in the literature. The National Advisory Committee on Microbiological Criteria for Foods standardized the hazard principles as follows:

1. Conduct a hazard analysis
2. Determine the critical control points
3. Establish critical limits
4. Establish monitoring procedures
5. Establish corrective actions
6. Establish verification procedures
7. Establish record-keeping and documentation procedures

To facilitate the development of a HACCP plan, certain prerequisite requirements are suggested. The importance of education and training of management and employees cannot be overemphasized. An in-depth discussion on the preparation and implementation of HACCP plans is beyond the scope of this article. The HACCP steps, however, are briefly discussed here:

Conduct a Hazard Analysis. A hazard analysis requires identification of the hazards that might reasonably be expected to occur. The hazards must be critical to assure food safety. Examples include physical hazards (metal, glass), chemical hazards (pesticide residues, undeclared allergenic ingredients), and microbiological hazards (pathogens, parasites). Regulatory agencies have defect action levels such as pits and stems. They do not present a health hazard. Physical hazards have been reviewed.

Determine Critical Control Points (CCPs). Critical control points are defined as the steps that are applied to prevent, eliminate, or reduce a hazard to an acceptable level. Examples would be cooking, metal, or allergen controls.

Establish Critical Limits. Establishing critical limits involves determining the maximum and/or minimum values to which a physical, chemical, or microbiological hazard must be controlled at a determined CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard. Examples include cooking and cooling time and temperature limits, and metal detection limits.

Establish Monitoring Procedures. Monitoring procedures assess whether CCP is under control. They provide a record for future use in verification.

Establish Corrective Actions. Should deviations occur from the critical limits, then corrective action is needed to bring the critical limits into compliance. The steps include determining the cause and, of course, correcting it; disposing of the noncompliant product; and recording the corrective actions that need to be taken to prevent reoccurrence. These actions should be developed and be a part of the HACCP plan.

Establish Verification Procedures. Verification procedures are developed to determine whether the HACCP plan is valid. A well-designed, functional HACCP plan has enough safeguards built in so that end-product testing should be minimal.

Establish Record-Keeping and Documentation Procedures. Records are important in that they provide a history of the HACCP system performance and should include, among other information, results of the monitoring procedures, corrective actions, and validation records.

RISK ANALYSIS

Risk analysis is an important tool. It yields information on estimating and analyzing costs and benefits of various alternatives, provides direction on what should be allocated for long- and short-term benefits, and serves as a source of resource identification. The components of risk analysis are risk assessment, risk management, and risk communication. It is important to understand that risk analysis will not yield a process that will lead to the development of zero risk to the end user. There is an inherent risk in all food products. HACCP systems do use risk analysis. HACCP is a risk-management system. Microbiological risk assessments are complex because of the many complexities of the food system from farm to table (15).

PHYSICAL HAZARDS

Foods, as grown, can become naturally contaminated with rocks, stones, glass, metal, and so on. Additional contamination can occur throughout the food chain. Physical hazards are controlled by processes that use exclusion such as magnets, metal detectors, and screens. Plant policies such as preventive maintenance, glass control, and good manufacturing practices assist the process. Federal agencies have delineated defect action levels that include stems, pits in vegetables, and insect fragments. These levels are generally considered a health hazard.

CHEMICAL HAZARDS

Chemical hazards can be introduced into foods either directly or indirectly. These are also naturally occurring toxicants. Chemical hazards include pesticides used to control pests in crops, cleaning compounds and sanitizers used on food equipment surfaces, and environmental contaminants such as mercury in fish and polybrominated biphenyls in feed. Certain foods, such as peanuts, eggs, milk, fish, and wheat, contain allergens that can be harmful to an allergen-sensitive individual. There are questions as to the adequacy of current pesticide safety assessments for children (43). Consumer interest in dietary supplements has raised concerns about adverse reactions (44). Chemical hazards present a continuing challenge to the scientific community.

BIOLOGICAL HAZARDS

Bean and Griffin (45) summarized CDC statistics and reported that 66% of the outbreaks and 87% of cases were bacterial in nature. Some of the more common biological agents are *Salmonella*, *Sa. Aureus*, *Campylobacter*, *E. coli* 0157:H7, and viruses such as hepatitis A and Norwalk. There are concerns about some of the emerging pathogens such as *Listeria*. Hazard assessment will require an assessment of why they occur and survive in a particular food, and the determination of CCPs that would prevent these organisms from becoming a potential health hazard.

QUALITY MANAGEMENT

In a recent survey, 7 out of 10 (69%) consumers cited spoilage-related concerns, including bacterial contamination, as the most significant threat to food safety. Consumers are also taking action; almost half of survey respondents (45%) said they are doing something different as a result of the safe-handling labels on meat products. More respondents are washing their hands, cooking properly, not leaving meat out to thaw, and washing meat. More than 90% of the consumers surveyed indicated that the nutritional content of the foods they eat was important. Fat, salt, and cholesterol remain the top nutritional concerns (46,47). The consumer expects his or her food to be of high quality, safe, and nutritious. The consumer drives the food system, which in turns drives the industry to meet consumer expectations; hence the need for quality management.

The literature is replete with books and papers featuring quality buzzwords such as CP (critical path), PC (process control), SPC (statistical process control), and TQM (total quality management). Colleges and junior colleges offer differing curricula, as do associations, trainers, and consultants who offer courses and seminars.

The systems used for the implementation of quality will vary depending on the nature of the product, the process, and the end user. Agricultural commodities require implementation of good agricultural practices along with proper controls during distribution, such as temperature and humidity to maintain freshness and nutritional content. Similar precautions need to be taken with seafood products. Programs cannot be successful without management commitment and the appropriate allocations of funds to successfully implement programs. Food service offers unique challenges because of its complexity. Challenges will vary depending on the type and size of the company.

It is becoming increasingly evident that quality needs to be controlled in all its aspects, which includes all parameters that will directly or indirectly affect quality, namely, the raw materials, agricultural practices, manufacturing process, environment, finished product, product handling, and consumer guidance. Monitoring of these parameters needs to be directed toward minimizing product failures, prevention of substandard product, and production of uniform quality product that will meet defined criteria—organoleptic, physical, chemical, and microbiological.

Ingredients and packaging should be purchased based on specifications under a continuing food guarantee. The specifications should take into consideration the capabilities of the supplier to meet the specifications and the ultimate use of these ingredients in the finished product. The specification should define the physical, chemical, and microbiological requirements. The capabilities of the supplier should be taken into consideration, including the adequacy of HACCP and quality programs. The raw materials should be statistically sampled (48).

The increased production needs have increased demands for automated processes—complex equipment that requires the utilization of sophisticated techniques to sense and correct malfunctions—hence the need for statistical process controls. Statistical process control will increase the effectiveness of HACCP systems and assist in product improvement.

Finished products need to be tested on a periodic basis. An adequate process and HACCP controls will greatly minimize the need to test finished products.

It is important that environmental sanitation in the plant be taken seriously. A clean plant instills pride, increases productivity, and is conducive to the production of consistent-quality products. Continuous follow-up and inspections by trained personnel will determine the program's success or failure. The program should include equipment design and maintenance, cleaning, pest control, and personnel practices.

Equipment should be designed so that it is easily cleaned. Hard-to-clean spots can be focal points for microbial growth. Improperly maintained equipment is conducive to accidents and foreign material contamination. Through integrated pest management control, pesticides must be used judiciously. The extent of the programs depends on the nature of the food and the location of the manufacturing facility. Equipment must be properly cleaned and sanitized. The adequacy of cleaning and sanitizing needs to be monitored using a microbiological control program. Plants must maintain the highest standards of hygiene and follow good manufacturing practices.

The quality functions need to be working in an interdisciplinary manner with company functions such as research and development, operations, sales, marketing, financial, and human resources. All of the quality considerations driving manufacturing, packaging, and distribution will be rendered worthless if the product is mishandled by the consumer or the food service operator. Handling instructions need to be adequate and explicit.

The final link in the food chain is the consumer. Assuming that each American eats four times a day, there could be one billion opportunities each day for someone to contract or transmit a foodborne illness (49) or consume a substandard product. Therefore, a concerted effort to educate the consumer is very important. The successful quality program will require the implementation of a system approach from farm to fork (4).

CONCLUSION

The food industry has a long history, beginning when humans first served a meal from home. The industry has seen that event refined to its current state of a multisegmented domestic and international business. In addition to having to meet customer expectations in quality and price, the food industry must satisfy a number of requirements such as labeling, standards of identity, food safety, and so on. We have seen spiraling ingredient, labor, and regulatory costs; such increased costs lead to the pursuit of more efficient methods of manufacture and optimum formulations (50).

Thomas Jefferson prescribed the need for a wise and frugal government. We have initiatives undertaken by a number of presidents—Kennedy, Nixon, and most recently Clinton, with the Food Safety Initiative. The Center for the Study of American Business at Washington University estimates that the private sector will pay $688 billion in "hidden compliance costs" related to federal regulations in 1997, roughly 9% of the gross domestic product (51). The consumer will ultimately pay the price; yet government regulations are necessary. It is important, therefore, that the desired food quality be achieved through engineering quality into the process at minimum regulatory costs through such programs as HACCP and quality systems, and communicating and educating the consumer on how to handle the food product for optimum taste and nutrition.

BIBLIOGRAPHY

1. U.S. Department of Agriculture, *Food Marketing Review 1994–95*, Agricultural Economic Report No. 743, Washington, D.C., 1996.
2. J. Putnam, *Food Consumption, Prices, and Expenditures, 1970–75*, USDA Economic Research Service Report No. SB-939, Washington, D.C., 1997.
3. K. L. Lipton, W. Edmondson, and A. Manchester, *The Food and Fiber System: Contributing to the US and World Economics*, USDA Economic Research Service Agriculture Information Bulletin No. 742, Washington, D.C., 1998.
4. M. P de Figueiredo, "Quality Assurance of Food Safety," *Food Technol.* **44**, 58–59 (1981).
5. Institute of Medicine, *Ensuring Safe Food from Production to Consumption*, National Academy of Press, Washington, D.C., 1998.
6. F. J. Francis, "Public Perception of Food Safety Priorities," *American Council on Science and Health* **3**, 29–32 (1991)
7. S. Dowdell, "Looking for a Date: The Vital Statistics in a Recent Survey Reveal That Freshness Is the Most Important Quality Consumers Look For in a Perishables Department," *Supermarket News* **46**, 27–28 (1996).
8. L. S. Kantor et al., "Estimating and Addressing America's Food Losses," *Food Review* **20**, 2–12 (1997).
9. S. R. Matthews, "Is Damage Done," *Progressive Grocer* **76**, 63–68 (1997).
10. American Society for Quality, *ANSI/ISO/ASQC Q9000 Series Quality Standards*, ASQ Quality Press, Milwaukee, Wisc., 1994.
11. R. Early, *Guide to Quality Management Systems for the Food Industry*, Blackie Academic and Professional, Glasgow, Scotland, 1995.
12. American Society for Quality Food, Drug and Cosmetic Division, *Food Processing Industry Quality System Guidelines*, ASQ Quality Press, Milwaukee, Wisc., 1998.
13. N. H. Bean et al., "Surveillance for Foodborne Disease Outbreaks; United States 1988–1992," *Morbidity, Mortality Weekly Report* **45**, 16–20 (1996).
14. C. Smith de Waal, L. Aderton, and M. Jacobson, *Outbreak Alert: Closing the Gaps in Our Federal Food Safety Net*, Center for Science in the Public Interest, Washington, D.C., 1999.
15. Council for Agricultural Science and Technology, *Foodborne Pathogens*, CAST Task Force Report No. 122, Ames, Iowa, 1994.
16. J. Buzby et al., *Bacterial Foodborne Disease: Medical Costs and Productivity Losses*, USDA Agricultural Economic Report No. 741, Washington, D.C., 1996
17. J. Buzby, T. Roberts, and B. M. Allos, *Estimated Annual Costs of Campylobacter-Associated Guillain-Barre Syndrome*, USDA Agricultural Economic Report No. 756, Washington, D.C., 1997.
18. J. G. Morris and M. Potter, "Emergence of New Pathogens as a Function of Changes in Host Susceptibility," *Emerging Infectious Diseases* **3**, 435–441 (1997).
19. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, *FoodNet: CDC's Emerging Infections Program*, Washington, D.C., 1998.
20. U.S. Department of Agriculture, Food Safety and Inspection Service, Report to Congress, *FoodNet: An Active Surveillance System for Bacterial Foodborne Diseases in the United States*, Washington, D.C., 1998.
21. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Foodnet, CDC/USDA/FDA Foodborne Diseases Active Surveillance Network, *CDC's Emerging Infections Program: 1997 Surveillance Results*, Washington, D.C., 1997.

22. National Academy of Sciences, *Emerging Infections: Microbial Threats to Health in the US*, National Academy Press, Washington, D.C., 1993.
23. S. F. Altekrusse, M. L. Cohen, and D. L. Swerdlow, "Emerging Foodborne Diseases," *Emerging Infectious Diseases* **3**, 285–292 (1997).
24. A. J. Miller, J L. Smith, and R. L. Buchanan, "Factors Affecting the Emergence of New Pathogens and Research Strategies Leading to Their Control," *Journal of Food Safety* **18**, 243–263 (1998).
25. A. R. Olsen, "Regulatory Action Criteria," *Regul. Toxicol. Pharmacol.* **28**, 190–198 (1998).
26. E. Karmas and R. S. Harris, *Nutritional Evaluation of Food Processing*, Van Nostrand–Reinhold, New York, 1988.
27. National Advisory Committee on Microbiological Criteria for Foods, "Hazard Analysis and Critical Control Point Principles and Application Guidelines," *J. Food Prot.* **61**, 1246–1259 (1998).
28. F. Bryan, "HACCP Approach to Food Safety: Past, Present, and Future," *Food Testing Analysis* **5**, 13–19 (1999).
29. Subcommittee on Microbiological Criteria, Committee on Food Protection, Food and Nutrition Board, National Research Council, *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients*, National Academy Press, Washington, D.C., 1985.
30. Committee on Scientific Basis of Meat and Poultry Inspection, Food and Nutrition Board, National Research Council, *Scientific Basis of Meat and Poultry Inspection*, National Academy Press, Washington, D.C., 1985
31. Committee on Evaluation of USDA Streamland, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences, *Inspection System Guide for Cattle (515-C)*, National Academy Press, Washington, D.C., 1990.
32. Committee on Evaluation of the Safety of Fishery Products, Institute of Medicine, National Academy of Sciences, *Seafood Safety*, National Academy Press, Washington, D.C., 1991.
33 National Advisory Committee on Microbiological Criteria for Foods, "Outstanding Symposia in Food Science and Technology," *Food Technol.* **54**, 142–162 (1991).
34. "Microbiological Criteria for Raw Molluscan Shellfish," *J. Food Prot.* **55**, 463–480 (1992).
35. "Listeria Monocytogenes," *International Journal of Food Microbiology* **14**, 185–246 (1991).
36. "The Role of Regulatory Agencies and Industry in HACCP," *International Journal of Food Microbiology* **21**, 187–195 (1994)
37. "Generic HACCP for Raw Beef," *Food Microbiology* **10**, 449–488 (1993).
38 "Hazard Analysis and Critical Control Point System," *International Journal of Food Microbiology* **16**, 1–23 (1992).
39. U.S. Department of Agriculture, Food Safety and Inspection Service, "Pathogen Reduction: Hazard Analysis Critical Control Point Systems, Final Rule," *Fed. Regist.* **6**, 38805–38855 (1996).
40. U.S. Department of Health and Human Services, Food and Drug Administration, "Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products, Final Rule," *Fed. Regist.* **60**, 65095–65202 (1995).
41 U.S. Department of Health and Human Services, Food and Drug Administration, "Guidance for Industry to Minimize Food Safety Hazards," *Fed. Regist.* **63**, 58055–58056 (1998).
42. U.S. Department of Health and Human Services, Food and Drug Administration, *FDA Food Code 1997*, National Technical Information Service, Springfield, Va., 1997.
43. National Research Council, *Pesticides in the Diets of Infants and Children*, National Academy Press, Washington, D C., 1993.
44. Institute of Food Technologists, "IFT Says Flexibility Is Key to Food Safety," *IFT Voices* **1**, 1 (1999).
45. N. H. Bean and P. M. Griffin, "Foodborne Disease Outbreaks in the United States 1973–1987: Pathogens, Vehicles, and Trends," *J. Food Prot.* **53**, 804–817 (1990).
46. International Food Information Council, *Newsbites: 97 Consumer Trends*, Washington, D.C., 1998.
47. Food Marketing Institute, *Trends in the United States Consumer Attitudes and the Supermarket*, Washington, D.C., 1997.
48. C. Stannard, "Development and Use of Microbiological Criteria for Foods," *Food Science and Technology Today* **11**, 137–177 (1997).
49. A. M. Coulston, "President's Page: Personal Responsibility and Food Safety," *J. Am. Dietetic Assoc.* **99**, 236 (1999).
50. S. A. Goldblith, "Consumer, the Product the Premise," in S. R. Tannenbaum ed., *Nutritional Safety Aspects of Food Processing*, Marcel Dekker, New York, 1979, pp. 1–5.
51. Center for the Study of American Business, *1997 Annual Review*, Washington University, St. Louis, Mo., 1998.

MARIO P. DE FIGUEIREDO
Consultant
Chesterfield, Missouri

R

RAPID METHODS OF MICROBIOLOGICAL ANALYSIS

Rapid methods and automation in microbiology are dynamic fields of study that address the utilization of microbiological, chemical, biochemical, biophysical, immunological, and serological methods for the study of improving isolation, early detection, characterization, and enumeration of microorganisms and their products in clinical, food, industrial, and environmental samples. In the past 10 years, food microbiologists have started to adapt rapid and automated methods in their laboratories (Fig. 1). Conventional methods of detection, enumeration identification and characterization of microbes, are described in reference books such as *Compendium of Methods for the Microbiological Examination of Foods* (1), *Official Methods of Analysis of the AOAC* (2), *Bacteriological Analytical Manual* (3), *Standard Methods for the Examination of Dairy Products* (4), and *Modern Food Microbiology* (5). A comprehensive treatment of all areas of food microbiology was recently published by Doyle, et al. (6).

Important publications on the subject of rapid methods for medical specimens, water, food, industrial, and environmental samples are in a series of papers by Fung and colleagues (7–11) and books such as *Instrumental Methods for Quality Assurance in Foods* (12) and *Rapid Analysis Technique in Food Microbiology* (13). Hartman et al. (14) had an excellent chapter on rapid methods and automation in *Compendium* (1). Swaminathan and Feng (15) also provided updated materials in rapid methods.

The purpose of this article is to review the basic principles and practical applications of a variety of instruments and procedures directly and indirectly related to improved methods for microbiology in quality assurance and research in food microbiology.

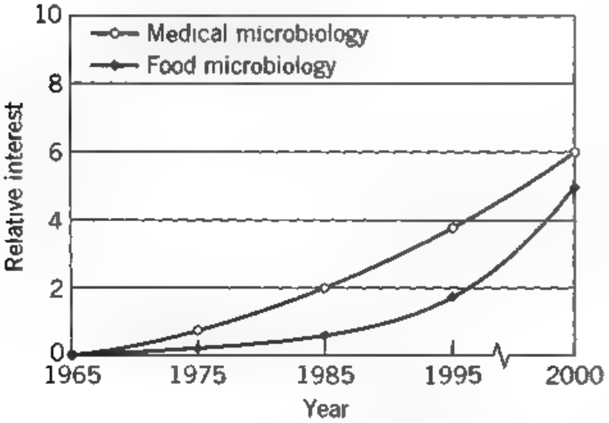


Figure 1. Relative interest in rapid methods among medical microbiologists and food microbiologists.

IMPROVEMENTS IN SAMPLING AND SAMPLE PREPARATION

One of the most useful instruments developed for sample preparation is the Stomacher (Tekmar, Cincinnati, Ohio). This instrument is designed to massage food samples in a sterile bag. The food sample is first placed in the sterile disposable plastic bag, and appropriate sterile diluents are added. The bag with the food is placed in the open chamber. After the chamber is closed, the bag is then massaged by two paddles for a suitable time, usually from 1 to 5 min. No contact occurs between the instrument and the sample. During massaging, microorganisms are dislodged into the diluent for further microbiological manipulation. Massaged slurries are then used for microbiological analysis. More than 30,000 units of the Stomacher have been sold worldwide since its introduction in 1975. A similar system named Masticator Homogenizer is marketed by IUL Instruments, Germany. Recently Sharpe invented a new instrument called Pulsifier that can dislodge bacteria from food by pulsification in a bag. Fung et al. (16) evaluated the Pulsifier versus the Stomacher and found that both instruments provided similar microbial counts from food samples. The Pulsifier resulted in less food debris in the sample, which is advantageous for pathogen detection using technology such as enzyme-linked immunosorbent assay (ELISA), Gene Probe, and polymerase chain reaction (PCR).

An instrument for sample preparation is the Gravimetric Diluter (Spiral Biotech, Bethesda, Maryland). One of the routine procedures in food microbiological work is to aseptically measure a sample of food (eg, 5 g of meat) and then aseptically add an exact amount of sterile diluent (eg, 45 mL) to make a desired dilution (1:10). With the Gravimetric Diluter, the analyst needs only to aseptically place an amount of food (eg, 5.3 g), into a Stomacher bag, set a desired dilution (1:10), and set the instrument to deliver the appropriate amount of sterile diluent (eg, 47.7 mL). Thus, the dilution operation can be automatically done. The dilution factor can be chosen by the analyst to satisfy the need (1:10, 1:50, 1:100, etc) simply by programming the instrument. Manninen and Fung (17) evaluated the Gravimetric Diluter and found that depending on the volume tested the accuracy of delivery for most samples was found to be in the range of 90 to 100%. A new version of this instrument is called Diluflo and has been in use satisfactorily in the author's laboratory since 1992. Recently Pbi of Italy marketed a similar instrument named Dilumacher for automatic microbiological dilution.

ALTERNATIVE METHODS FOR VIABLE CELL COUNT PROCEDURE

The conventional viable cell count or standard plate count method is time-consuming in terms of both operation and collection of data. Several methods have been explored to improve the efficiency of operation of the viable cell count procedure.

The spiral plating method is an automated system for obtaining viable cell counts (Spiral Biotech, Bethesda, Md.). The instrument can spread a liquid sample on the surface of agar contained in a petri dish in a spiral shape (the Archimedes spiral) with a concentration gradient starting from the center and decreasing as the spiral progresses outward on the rotating plate. The volume of liquid deposited at any segment of the agar plate is known. After the liquid containing microorganisms is spread, the agar plate is incubated overnight at an appropriate temperature for the colonies to develop. The colonies appearing along the spiral pathway can be counted either manually or electronically. Table 1 demonstrates that the spiral plating method provides the same counts as the conventional method. A skilled operator can perform 10 times more samples using the spiral system compared with conventional methods.

New versions of the spiral plater have recently been introduced as Autoplater (Spiral Biotech, Bethesda, Md.) and Whitley Automatic Spiral Plater (Bioscience International, Inc., Rockville, Md.). With these instruments an analyst need only present the liquid sample and the instrument completely and automatically processes the sample, including resterilizing the unit for the next sample.

Many instruments are now available for rapid enumeration of bacterial colonies in agar plate. Spiral Biotech (Bethesda, Md.) markets a laser system called CASBA II that has been in use in the author's laboratory for many years with satisfactory results. Recently the same company introduced CASBA III, which includes a high-resolution scanner with a high-performance personal computer and Windows-based colony image analysis software. Protos colony counter (Bioscience International, Inc., Rockville, Md.) is another automatic colony counter that can count 600 plates/hour at 0.5 s/plate. Many other companies also market automatic plate counters. Table 1 also shows that counting manually and counting by a laser counter provided the same number, but the laser counter can complete the task in a few seconds.

Another alternative method for viable cell count is the Isogrid system (QA Laboratories Ltd., San Diego, Calif.). This system consists of a square filter with hydrophobic grids printed on the filter to form 1,600 squares for each filter. Food samples are weighed, blended, and enzyme treated before passage through the membrane filter containing the hydrophobic grids by vacuum. The filter is then placed on agar containing a suitable nutrient for growth of the bacteria, yeasts, or molds. The hydrophobic grids prevent colonies from growing further than the square grids; thus all colonies have a square shape. This facilitates counting of the colonies both manually and electronically. This method has been successfully used to make viable cell counts for a variety of foods, including milk, meat, black pepper, flour, peanut butter, mushrooms, rice, fish, shrimp, and oyster (18–20). Other applications of the Isogrid system include determination of total coliforms, fecal coliforms, *Escherichia coli*, *Salmonella* spp., and other microorganisms (21).

Table 1. Comparison of Pour Plate and Spiral Plate Counted Manually and by Laser for Bacterial Cultures

	Log$_{10}$cfu/ml			
	Pour plate		Spiral plate	
Test cultures	Manual	Laser	Manual	Laser
Escherichia coli	8.86	8.85	8.73	8.85
Salmonella enteritidis	8.76	8.66	8.78	8.92
Pseudomonas aeruginosa	8.00	8.00	8.00	8.00
Staphylococcus aureus	8.04	7.78	8.18	8.18
Lactobacillus plantarum	9.48	9.40	9.60	9.69
Streptococcus sp.	7.73	7.66	8.00	8.08
Bacillus cereus	7.26	7.15	7.15	7.26
Micrococcus luteus	7.40	7.32	7.51	7.57

Rehydratable nutrients are embedded into a series of films in the Petrifilm (3M Co., St. Paul, Minn.) system. The top layer of the protective cover is lifted and 1 mL of liquid sample introduced to the center of the unit, and the cover is then replaced. A plastic template is placed on the cover to make a round mold. The rehydrated medium with nutrient will support the growth of microorganisms after suitable incubation time and temperature. The colonies can be counted directly in the unit. The unit is about the size and thickness of a plastic credit card, thus providing great savings of space in storage and incubation. Petrifilm units have been developed for total bacterial count, coliform count, fecal coliform count, yeast and mold counts, and hemorrhagic *E. coli* 0157:H7. Table 2 shows excellent correlation between Petrifilm and standard plate count method for the food.

Redigel system (RCR Scientific, Inc., Goshen, Ind.) is another convenient viable cell count system. This system consists of sterile nutrients with a pectin gel in a tube. The tube is ready to be used any time and no heat is needed to "melt" agar. A 1-mL food sample is first pipetted into the tube. After mixing, the entire content is poured into a special Petri dish previously coated with calcium. When liquid comes in contact with the calcium, a Ca-pectate gel is formed, and the complex swells to resemble conventional agar. After an appropriate incubation time and temperature, the colonies can be counted exactly like conventional standard plate count method. Besides total count, Redigel also has systems for coliform, fecal coliform, yeast and molds, lactic and bacteria, *Staphylococcus aureus*, and *Salmonella*.

The four aforementioned methods have potential as an alternative to the conventional agar pour plate method. Chain and Fung (23) made a comprehensive analysis of all four methods against the conventional method on seven different foods (skinless chicken breast, fresh ground beef, fresh ground pork, packaged whole shelled pecans, raw milk, thyme, and whole wheat flour, 20 samples each) and showed that the new systems and the conventional method were highly comparable and exhibited a high degree of accuracy and agreement (r – 0.95+). It should be noted that those performing these newer methods need some training and experience before satisfactory results can be obtained consistently. These are good methods if performed under careful operation.

In the direct epifluorescent filter technique (DEFT) method, the liquified sample is first passed through a filter that retains the microorganisms. The filter is then stained with acridine orange, and the slide is observed under ultraviolet microscopy. "Live" cells usually stain orange-red, orange-yellow, or orange-brown, whereas "dead" cells fluoresce green. The slides can be read by the eye, or by a semiautomated counting system marketed by Bio-Foss. A viable cell count can be made in less than an hour. With the use of an image analyzer, an operator can count 50 DEFT slides per hour (24). This method has been used satisfactorily for counting viable cells in milk and other food samples such as fish (25). Recently the Nordic countries used this method for quality assurance of ground beef. Tortorello and Gendel (26) further developed this procedure by using fluorescent antibodies in conjunction with DEFT to enumerate *E. coli* 0157:H7 in milk and juice.

Table 2. Comparison of the Standard Plate Count Method and the Petrifilm Method for Viable Cell Counts of Shrimp, Perch, Cod, and Whiting

	Colony-forming units/gm	
Sample	Standard plate count	Petrifilm SM
	Shrimp	
1	2.1×10^4	0.9×10^4
2	9.3×10^3	6.8×10^3
3	1.5×10^4	1.2×10^4
4	4.4×10^4	2.0×10^4
5	2.0×10^4	0.9×10^4
	Perch	
1	1.4×10^3	1.2×10^3
2	5.4×10^2	3.7×10^2
3	8.0×10^2	4.8×10^2
4	5.0×10^2	3.2×10^2
5	1.0×10^3	1.2×10^3
	Cod	
1	1.2×10^2	1.1×10^2
2	2.2×10^2	2.4×10^2
3	1.0×10^4	1.0×10^4
4	2.1×10^4	2.3×10^4
5	1.2×10^4	1.2×10^4
	Whiting	
1	3.0×10^2	3.1×10^2
2	5.8×10^2	5.6×10^2
3	1.2×10^3	1.1×10^3
4	5.4×10^2	5.2×10^2
5	8.8×10^2	8.4×10^2

Note: Samples were massaged in a Stomacher for 1 min in sterile diluent. Viable cells counts were made according to standard method. Incubation time was 48 h at 32°C. All samples were done in duplicate. Correlation coefficient between the two methods is $r = 0.99$.
Source: Ref. 22.

INSTRUMENTS FOR ESTIMATION OF MICROBIAL POPULATIONS AND BIOMASS

Many methods have been developed in recent years to estimate the total number of microorganisms by parameters other than the viable colony count as described in the previous section. For a new method to be acceptable, it should have some direct correlation with the total viable cell count. Thus, standard curves correlating parameters such as adenosine triphosphate (ATP) level, detection time of electrical impedance or conductance, generation of heat, radioactive CO_2, and so on, with viable cell counts of the same sample series must be made. In general, the larger the number of viable cells in the sample, the shorter the detection time of these systems. A scattergram is then plotted and used for further comparison of unknown samples. The assumption is that as the number of microorganisms increase in the sample, these physical, biophysical, and biochemical events will also increase accordingly. When a sample has 10^5 to 10^6 organisms/mL, detection can be achieved in about 4 to 6 h.

All living things utilize ATP. In the presence of a firefly enzyme system (luciferase and luciferin system), oxygen, and magnesium ions, ATP will facilitate the reaction to generate light. The amount of light generated by this reaction is proportional to the amount of ATP in the sample; thus, the light units can be used to estimate the biomass of cells in a sample. The light emitted by this process can be monitored by a variety of fluorimeters. These procedures can be automated for handling large numbers of samples. Some of the instruments can detect as little as 10^2 to 10^3 fg. The amount of ATP in one colony-forming unit has been reported as 0.47 fg with a range of 0.22 to 1.03 fg. Using this principle, many researchers have tested the efficacy of using ATP to estimate microbial cells in foods and beverages.

Lumac (Landgraaf, the Netherlands) markets several models of ATP instruments and provides customers with test kits with all necessary reagents, such as a fruit juice kit, hygiene monitoring kit, and so on. The reagents are injected into the instrument automatically, and readout is reported as relative light units (RLUs). By knowing the number of microorganisms responsible for generating known RLUs, one can estimate the number of microorganisms in the food sample. In some food systems, such as wine, the occurrence of any living matter is undesirable; thus, monitoring of ATP can be a useful tool for quality assurance in the winery. Recently, much interest has been expressed in using ATP estimation not for total viable numbers but as a sanitation check by companies such as Lumac, BioTrace (Plainsboro, N.J.), Lightning (IDEXX, Westbrook, Maine), Hy-Lite (Glengarry, Biotech, Cornwall, Canada), Charm 4000 Luminometer (Charm Sciences, Malden, Mass.), and others.

As microorganisms grow and metabolize nutrients, large molecules change to smaller molecules in a liquid system and cause a change in electrical conductivity and resistance in the liquid as well as at the interphase of electrodes. By measuring the changes in electrical impedance, capacitance, and conductance, scientists can estimate the number of microorganisms in the liquid because the larger the number of microorganisms in the fluid, the faster the change in these parameters that can be measured by sensitive instruments. A detailed analysis on the subject of impedance, capacitance, and conductance in relation to food microbiology has been made by Eden and Eden (27).

The Bactometer (bioMerieur Vitek, Inc., Hazelwood, Mo.) is an instrument designed to measure impedance changes in foods. Samples are placed in the wells of a 16-well module. After the module is completely or partially filled, it is plugged into the incubator unit to start the monitoring sequence. At first, there is a stabilization period for the instrument to adjust to the module, then a baseline is established. As the microorganisms metabolize the substrates and reach a critical number (10^5–10^6 cells/mL), change in impedance increases sharply, and the monitor screen shows a slope similar to the log phase of a growth curve. The point at which the change in impedance begins is the detection time, and this is measured in hours from the start of the experiment. The detection time is inversely proportional to the number of microorganisms in the sample. By knowing the number of microorganisms per milliliter in a series of liquid samples and the detection time of each sample, one can establish a standard curve. From the curve one can decide the cutoff points to monitor certain specifications of the food products.

Impedance methods have been used to estimate bacteria in milk, dairy products, meats, and other foods. This method has been used for determining the shelf life potential of pasteurized whole milk by Bishop et al. (28).

The Malthus system (Crawley, UK) works by measuring the conductance of the fluid as the organisms grow in the system. It also generates a conductance curve similar to the impedance curve of the Bactometer, and it also uses detection time in monitoring the density of the microorganisms in the food.

The Malthus system has been used for microbial monitoring of brewing liquids and hygiene monitoring. Gibson and colleagues (29–31) have done considerable work using the Malthus system to study seafood microbiology.

Besides estimating viable cells in foods, both the Bactometer and the Malthus systems can detect specific organisms by the use of selective and differential liquid media. An automatic instrument for measuring direct and indirect impedance has been developed in Europe and named RABIT (Rapid Automated Bacterial Impedance Technique).

An instrument called the Omnispec bioactivity monitor system (Wescor, Inc., Logan, Utah) is a tri-stimulus reflectance colorimeter that monitors dye pigmentation changes mediated by microbial activity. Dyes can be used that produce color changes as a result of pH changes, changes in the redox potential of the medium, or the presence of compounds with free amino groups. Samples are placed in microtiter wells or other types of containers and are scanned by an automated light source with computer interface during the growth stages (0–24 hours). The change of color or hue (a*, b*, L) can be monitored similar to impedance curve and conductance curve. Manninen and Fung (32) evaluated this system in a study of pure cultures of *Listeria monocytogenes* and food samples and found high correlation coefficients (r) of 0.90 to 0.99 for pure bacterial cultures and 0.82 for minced beef between the colony counts predicted by the colorimetric technique and the results of the traditional plate count method. They also showed that detection times for bacterial cultures such as *Enterobacter aerogenes, E. coli, Hafnia alvei*, and several strains of *L. monocytogenes* were substantially (2–24 h) shorter using the instrument than using the traditional method and concluded that the colorimetric detection technique employed by the Omnispec system simplifies the analyses, saves labor and materials, and provides a high sampling capacity. Tuitemwong (33) completed an extensive study using Omnispec 4000 to monitor growth responses of food pathogens in the presence or absence of membrane-bound enzymes. This instrument is highly efficient in large-scale studies of microbial interaction with different compounds in liquid and food.

The catalase test is another rapid method for estimation of microbial populations in certain foods. Catalase is a very reactive enzyme. Microorganisms can be divided into catalase positive and catalase negative. Both groups are important in food microbiology; however, under certain food-storage conditions, a certain group predominates. Most perishable foods (commercial as well as domestic) are cold stored under aerobic conditions. The organisms causing spoilage of these foods are psychrotrophs. The predominant psychrotrophic bacteria are *Pseudomonas* spp., which are strongly catalase positive. Other important psychrotrophs such as *Micrococcus, Staphylococcus*, and a variety of enterics are also catalase positive. Thus, one can make use of the presence of catalase to estimate the bacterial population. Catalase activity can be detected by a simple capillary tube method (34). Recently Binjasass and Fung (35) completed an extensive study using the capillary catalase tube method for monitoring microbial load and end-point cooking temperature of fish and found the method to be reliable and simple to use.

Ang et al. (36) also showed that heating poultry meat to 71°C (a legal requirement for these products) will destroy both bacterial and animal catalase. The test is 99% accurate and is simple and inexpensive to perform.

MINIATURIZED MICROBIOLOGICAL TECHNIQUES

Identification of microorganisms is an important part of quality assurance and control programs in the food industry. The author has developed many miniaturized methods to reduce the volume of reagents and media (from 5 to 10 mL to about 0.2 mL) for microbiological testing in microtiter plates. The basic components of the miniaturized system are the microtiter plates for test cultures, a multiple inoculation device, and containers to house solid media (large petri dishes) and liquid media (another series of microtiter plates). The procedure involves placing liquid cultures (pure cultures) to be studied into sterile wells of a microtiter plate to form a master plate. Each microtiter plate can hold up to 96 different cultures, 48 duplicate cultures, or various combinations as desired. The cultures are then transferred by a sterile multipoint inoculator (96 needles protruding from a template) to solid or liquid media.

Sterilization of the inoculator is by alcohol flaming. Each transfer represents 96 separate inoculations in the conventional method. After incubation at an appropriate temperature, the growth of cultures on solid media or liquid media can be observed and recorded, and the data can be analyzed. These miniaturized procedures save a considerable amount of time in operation, effort in manipulation, materials, labor, and space. These methods are ideal for studying large numbers of isolates or for research involving challenging large numbers of microbes against a host of test compounds.

The miniaturized methods have been used to study large numbers of isolates from foods (37–39) and to develop bacteriological media and procedures (40–43). Many useful microbiological media were discovered through this line of research. For example, an aniline blue *Candida albicans* medium was developed and marketed by Difco under the name Candida Isolation Agar. The sensitivity and specificity were 98.0 and 99.5%, respectively, with a predictive value of 99.1% (44).

On the commercial side, many diagnostic kits to identify microorganisms have been developed and marketed since the 1970s. Currently, API, Enterotube, R/B, Minitek, MicroID, and IDS are available. Most of these systems were first developed for the identification of enterics (*E. coli, Salmonella, Shigella, Proteus, Enterobacter* spp., etc). Later many of the companies expanded the capacity to identify nonfermentor, anaerobes, gram-positive organisms, and even yeasts and molds. Most of the early comparative analyses centered around evaluation of these kits for clinical specimens. Comparative analysis of diagnostic kits and selection criteria for miniaturized systems were made by Fung et al. (45) and Cox et al. (46). They concluded that these miniaturized systems are accurate, efficient, labor saving, space saving, and cheaper than the conventional procedure. Their usefulness in clinical and food microbiological laboratories will continue to be important.

TECHNIQUES INVOLVING INSTRUMENTS, IMMUNOLOGY, AND GENETIC TESTS

Many sophisticated instruments have been developed to identify isolates from clinical specimens such as Sensititre (Radiometer Amer, Westlake, Ohio) and Biolog (Hayward, Calif.). One of the most automated systems for the identification of isolates (clinical and foods) is the Vitek system. The system depends on the growth of target organisms in specially designed media housed in tiny chambers in a plastic card. The card is then inserted into the incubation chamber. The instrument periodically scans the wells of the cards and sends information to the computer, which then matches the database and identifies the unknown cultures in the cards. The system is entirely automated and computerized and provides hard copies for record keeping. The system is capable of identifying enterics, yeast, *Bacillus*, selected gram-positive pathogens, and other organisms.

The DNA probe (Genetrak, Farmingham, Mass.) is a sensitive method to detect pathogens such as *Campylobacter, Salmonella, Listeria*, and *E. coli*. At first, the system utilized radioactive compounds for assay. The second generation of probes uses enzymatic reactions to detect the presence of pathogens. Another major change in this area is the development of probes to detect target RNA. In a cell there is only one copy of DNA; however, there may be 1,000 to 10,000 copies of ribosomal RNA. Thus, the new generation of probes are designed to probe target RNA. After enrichment of cells (either in liquid or colonies on solid agar), DNA or RNA of target cells can be extracted and released into the liquid and then detected by the appropriate DNA and/or RNA probes. These methods are currently being automated. Currently, kits are available for *Salmonella, Listeria, Campylobacter, Yersinia*, and others. As the need arises more organisms will be added to the list.

Polymerase chain reaction (PCR) systems are the latest development in DNA amplification technology and recently have gained much attention in food microbiology. Originally the procedures are highly complicated, and a very clean environment is needed to perform the test. Recently, much research has been directed to simplifying the procedure for laboratory analysts. DuPont recently commercialized a system called BAX for PCR.

The BAX screening system is designed to work from overnight enrichment broths. In the case of the BAX system for *Salmonella*, the food is enriched overnight in any standard nonselective broth (lactose broth, buffered peptone water, etc). A 1:10 dilution into BHI is performed followed by a 3-h grow-back incubation. An aliquot is taken into a tube and treated with lytic enzymes and heat. An aliquot of the cell lysate is used to rehydrate the PCR reagents housed in a tube, and a control tablet and the sample is placed into the thermal cycler along with the corresponding control tube. Thermal cycling, UV visualization of the gel, and photography to document the results are then performed to detect PCR products.

PCR technology is now steadily moving into the food laboratories. Some obstacles of PCR technology include the occurrence of inhibitors in the food samples and the fact that DNA from dead cells can also be amplified, thus giving a false positive result for a food that may be safe to eat. For pure cultures this technology is very useful. Dilution of samples (1:10) will reduce the inhibitors, and enrichment of target culture will ensure amplication of live cells even in the presence of other organisms.

Another important development, also by Qualicon, is the RiboPrinter characterization system. The RiboPrinter is designed to accept isolated colonies of bacteria as the sample. A sample is prepared as follows:

1. A colony of bacteria is picked from an agar plate using a sterile plastic stick (provided in the sample kit).
2. Cells from the stick are suspended in a buffer solution by mechanical agitation.
3. An aliquot of the cell suspension is loaded into the sample carrier to be placed into the instrument. Each sample carrier has space for eight individual colony picks.

The patterns of organisms would allow an analyst to pinpoint the source of the pathogen. For example, even the pathogen *L. monocytogenes* has many different patterns. By matching the pattern of an isolate from food and patterns from isolates from a slicer or drain, the analyst can determine exactly the source of the *L. monocytogenes* in the finished product. This system received the Industry Award given by the Institute of Food Technologists in 1997.

Modification of the basic PCR procedure includes reverse transcriptase PCR, nested/multiplex PCR, randomly amplified polymorphic DNA (RAPD), fluorescent probes in PCR, among others. These methods are slowly finding their way to the food microbiology laboratory.

The enzyme-linked immunosorbent assay (ELISA) systems method commercialized by Organon Teknika (Durham, N.C.) utilizes two monoclonal antibodies specific for *Salmonella* detection. In a comparative study involving 1,289 samples, Eckner et al. (47) found no significant difference between the conventional method and the ELISA method for food samples except cake mix and raw shrimp. Another ELISA system, the Tecra system (International BioProducts, Redmond, Wash), was developed in Australia and uses polyclonal antibodies to detect *Salmonella* These methods have also been used to detect *Listeria* and *E. coli*. Many companies are providing a host of monoclonal and polyclonal antibodies for a variety of diagnostic tests, some including food pathogens (48).

One of the drawbacks of the ELISA test is the many steps necessary for adding reagents and washing test samples. In the Vitek ImmunoDiagnostic Assay System (VIDAS®) system, all intermediate steps are automated. VIDAS is a multiparametric immunoanalysis system that utilizes the enzyme-linked fluorescent immunoassay (ELFA) method. The end result of the test protocol is a fluorescent product, and the VIDAS reader utilizes a special optical scanner that measures the degree of fluorescence. From the moment the solid phase receptacles and the reagent strips are placed in the instrument, the VIDAS is fully automated. Many automated ELISA instruments are on the market now, including TECRA OPUS (International BioProducts, Redmond, Md.), Bio-tek Instruments (Highland Park, Vt.), Automated EIA Processor (BioControl, Bethell, Wash.), among others.

In a related development, several self-contained small units (REVEAL, VIP, etc) have been marketed recently. After enrichment (with or without an Oxyrase type of stimulation) an analyst need only apply a small aliquot (boiled or unboiled) to the kit. Reaction occurs in a few minutes. These kits have been used to rapidly screen *E. coli* 0157:H7 and *Salmonella* in ground beef.

UNIQUE system for *Salmonella* is another way of using immunocapture technology. A dipstick with antibody against *Salmonella* is applied to the pre-enriched liquid. The antibody captures the *Salmonella* if present. This charged dipstick is then placed in a fresh enrichment broth and the cells are allowed to multiply for a few hours. After the second enrichment step, the dipstick, with a much larger population of *Salmonella* attached to it, will be subject to further ELISA procedures. The entire test is housed in a convenient self-contained plastic unit. This type of method is very useful for a small laboratory with low-volume testing of pathogens.

Immunomagnetic capture methods have attracted much attention lately. VICAM and DYNAL developed magnetic beads coated with a variety of antibodies to capture target cells or cellular components in foods. After the magnetic beads have a chance to interact with potential target cells in a tumbling apparatus for an hour, the magnetic beads are physically separated from the food or liquid by a powerful magnet applied to the side of the test tube. Further microbiological procedures such as direct plating or ELISA tests can be made on these charged beads. Many test systems are now incorporating immunomagnetic capture technology to shorten the enrichment steps by at least 1 day. This is especially important for foods with very low number of target pathogen such as *Listeria* or *Salmonella*.

Motility enrichment is a very useful concept in rapid isolation and identification of food pathogens. Fung and Kraft (49) described a motility flask system for rapid detection and isolation of *Salmonella* spp. from mixed cultures and poultry products. The system involves a flask with a side arm that contains several agar layers. Lactose broth is placed in the flask, and then a sample (with or without salmonellae) is inoculated into the lactose broth. When salmonellae are present, they will swim through the first level of agar, which contains selenite cysteine and sodium lauryl sulfite that inhibits other organisms but allows salmonellae to pass through. Once salmonellae pass the first layer, they can grow and metabolize compounds in the second and third layers. By looking at the color changes in the second and third agar layers, one can make an assumption that salmonellae were in the sample that was put into the lactose broth. Further serological tests can then be performed to confirm the presence of *Salmonella*.

In more recent years, a commercial system called *Salmonella* 1-2 test (BioControl, Bothell, Wash.) was developed that utilizes motility as a form of selection. The food sample is first preenriched for 24 h in lactose broth, and then 0.1 mL is inoculated into one of the chambers in an L-shaped system. The chamber contains selective enrichment liquid medium. There is also a small hole connecting the liquid chamber with the rest of the system, which has a soft agar through which salmonellae can migrate. An opening on the top of the second chamber allows the analyst to deposit a drop of polyvalent anti-H antibody. If the sample contains salmonellae from the lower side of the L unit, salmonellae will migrate through the hole and up the agar column. Simultaneously, the antibody against flagella of salmonellae will move downward by gravity. When the antibody meets the salmonellae, they will form a visible immunoband. The presence of an immunoband in this system is a positive test for *Salmonella* spp. The system is easy to use and has gained popularity because of its simplicity.

Oxyrase (Mansfield, Ohio), a membrane fraction of *E. coli*, was found in the author's laboratory to stimulate the growth of a large number of important facultative anaerobic food pathogens. In the presence of a hydrogen donor such as lactate, Oxyrase can convert O_2 to H_2O, thereby reducing the oxygen tension of the medium and creating anaerobic conditions that favor the growth of facultative anaerobic organisms. In a medium containing 0.1 units/

mL of the enzyme, the growth of *L. monocytogenes*, *E. coli* 0157:H7, *S. typhimurium*, *S. faecalis*, and *Proteus vulgaris* were greatly enhanced; colony counts were greater by 1 to 2 log units, depending on the initial count and the strain studied, after incubation in the presence of the enzyme for 5 to 8 h at 35 to 42°C compared with control without Oxyrase.

By combining the oxyrase enzyme and a unique U-shaped tube, Yu and Fung (50–52) developed an effective method to detect *L. monocytogenes* and *Listeria* spp. from laboratory cultures and meat systems. Niroomand and Fung (53) studied the effects of oxyrase in stimulation of growth of *Campylobacter* from foods. Tuitemwong et al. (54) also found that these membrane fragments and those obtained from *Acetobacter* and *Gluconobacter* can stimulate growth of starter cultures in food fermentation.

There are many other systems that involve modern biochemistry, chemistry, and immunology. For example, one can use protein profiles for microbial fingerprinting (AMBIS system, San Diego, Calif.) or cell composition as a way to identify bacterial cultures (Hewlett-Packard, Palo Alto, Calif.).

CONCLUSIONS

This article describes a variety of methods that are designed to improve current methods, explore new ideas, and develop new concepts and technologies for the improvement of applied microbiology. Although many of these methods were first developed for clinical microbiology, they are being used for food microbiology. This field will certainly grow, and many food microbiologists will find these new methods very useful in their routine work in the immediate future. Many methods described here are already being used by applied microbiologists nationally and internationally.

ACKNOWLEDGMENTS

This material is based on work supported by the Cooperative State Research Service, U.S. Department of Agriculture, under Agreement No. 8890341874511, Agricultural Experiment Station, Kansas State University, Manhattan, Kansas 66506.

BIBLIOGRAPHY

1. C. Vanderzant and D. Splittstoesser, eds., *Compendium of Methods for the Examination of Foods*, American Public Health Association, Washington, D.C., 1992.
2. Association of Official Analytical Chemists, *Official Methods of Analysis of the AOAC (Association of Official Analytical Chemists)*, Vols. 1 and 2, Arlington, Va., 1990.
3. Food and Drug Administration, *Bacteriological Analytical Manual*, 6th ed., Association of Official Analytical Chemists, Food and Drug Administration, Arlington, Va., 1996.
4. American Public Health Association, *Standard Methods for the Examination of Dairy Products*, Washington, D.C., 1992.
5. J. M. Jay, *Modern Food Microbiology*, 4th ed. Van Nostrand-Reinhold, New York, 1992.
6. M. P. Doyle, L. R. Beuchat, and T. J. Montville, *Food Microbiology Fundamentals and Frontiers*, ASM Press, Washington, D.C., 1997.
7. D. Y. C. Fung, "Rapid Methods and Automation for Food Microbiology," in D. Y. C. Fung and R. F Matthews, eds , *Instrumental Methods for Quality Assurance in Foods*, Marcel Dekker, New York, 1991
8. D. Y. C. Fung, "Historical Development of Rapid Methods and Automation in Microbiology," *Journal of Rapid Methods and Automation in Microbiology* **1**, 1 (1992).
9. D Y. C. Fung, "Rapid Methods and Automation in Food Microbiology: A Review," *Food Rev. Int.* **10**, 357–375 (1994).
10. D. Y. C. Fung, "What's Needed in Rapid Detection of Food Borne Pathogens," *Food Technol.* **44**, 64–67 (1995).
11. D. Y. C. Fung et al., "Rapid Methods and Automation: A Survey of Professional Microbiologists," *J. Food Prot.* **52**, 65 (1989).
12. D. Y. C. Fung and R. F. Matthews, eds *Instrumental Methods for Quality Assurance in Foods*, Marcel Dekker, New York, 1991.
13. P. D. Patel, *Rapid Analysis Techniques in Food Microbiology*, Chapman & Hall, New York, 1994.
14. P. A. Hartman et al., "Rapid Methods and Automation," in C. Vanderzant and D. Splittstoesser, eds., *Compendium of Methods for the Examinations of Foods*, American Public Health Association, Washington, D.C., 1992.
15. B Swaminathan and P Feng, "Rapid Detection of Food-Borne Pathogenic Bacteria," *Rev. Microbiol.* **48**, 401 (1994).
16. D. Y. C. Fung et al., "The Pulsifier: A New Instrument for Preparing Food Suspensions for Microbiological Analyses," *Journal of Rapid Methods and Automation in Microbiology* **6**, 42–50 (1998).
17. M. T. Manninen and D. Y. C. Fung, "Use of the Gravimeter Diluter in Microbiological Work," *J. Food Prot.* **55**, 59 (1992).
18. P. Entis, "Enumeration of Coliforms in Nonfat Dry Milk and Canned Custard by Hydrophobic Grid Membrane Filter Method: Collaborative Study," *J. Assoc. Off. Anal. Chem.* **66**, 897 (1983).
19. P. Entis, "Enumeration of Total Coliforms and *Escherichia coli* in Foods by Hydrophobic Grid Membrane Filters Collaborative Study," *J. Assoc. Off. Anal. Chem.* **67**, 812 (1984).
20. A. W. Sharpe and P. I. Peterkin, *Membrane Filter Food Microbiology*, Research Studies Press, Letchworth, U K., 1988.
21. A. W. Sharpe, "Rapid Methods: Consideration to Adoption by Regulatory Agencies," *Proc. 105 AOAC Annu. Int Mtgs*, Phoenix, Ariz., August 12–15, 1991.
22. D. Y. C. Fung, R. A. Hart, and V Chain, "Rapid Methods and Automated Procedures for Microbiological Evaluation of Seafood," in D. E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier, Amsterdam, The Netherlands, 1987, pp. 247–253.

23. V. S. Chain and D. Y. C. Fung, "Comparison of Redigel, Petrifilm, Spiral Plate System, ISOGRID, and Standard Plate Count for the Aerobic Count on Selected Foods," *J. Food Prot.* **54**, 208 (1991).

24. G. L. Pettipher, "Review: The Direct Epifluorescent Filter Technique," *J. Food Technol.* **21**, 535 (1986).

25 G. L. Pettipher, "The Direct Epifluorescent Filter Technique," in M. R. Adams and C. F. A. Hope, eds., *Rapid Microbiological Methods*, Elsevier, New York, 1989.

26 M. Tortorello and S. M. Gendel, "Fluorescent Antibodies Applied to Direct Epifluorescent Filter Techniques for Microscopic Enumeration of *Escherichia coli* 0157:H7 in Milk and Juice," *J. Food Prot.* **56**, 672 (1993).

27. R. Eden and G. Eden, *Impedance Microbiology*, Research Studies Press, Letchworth, U.K., 1984.

28. J. R. Bishop, C. R. White, and R. Firstenberg-Eden, "A Rapid Impedimetric Method for Determining the Potential Shelf-Life of Pasteurized Whole Milk," *J. Food Prot.* **47**, 471 (1984).

29. D. M. Gibson and G. Hobbs, "Some Recent Developments in Microbiological Methods in Seafood Quality," in D. F. Kramer and J. Listen, eds., *Seafood Quality Determination*, Elsevier, Amsterdam, The Netherlands, 1987, pp. 283–298.

30. D. M. Gibson and I. D. Ogden, "Assessing Bacterial Quality of Fish by Conductance Measurement," *J. Appl. Bacteriol.* **49**, 12 (1980).

31. I. D. Ogden, "Use of Conductance Methods to Predict Bacterial Counts in Fish," *J. Appl. Bacteriol.* **61**, 36 (1986).

32. M. T. Manninen and D. Y. C. Fung, "Estimation of Microbial Numbers from Pure Bacterial Cultures and from Minced Beef Samples by Reflectance Colorimetry with Omnispec 4000," *Journal of Rapid Methods and Automation in Microbiology* **1**, 41 (1992).

33. K. Tuitemwong, "Characteristics of Food Grade Membrane Bound Enzymes and Applications in Food Microbiology and Food Safety," Ph.D. Dissertation, Kansas State University Library, Manhattan, Kans., 1993

34. D. Y. C. Fung, "Procedures and Methods for One-Day Analysis of Microbial Loads in Foods," in K.-O. Habermehl, ed., *Rapid Methods and Automation in Microbiology and Immunology*, Springer-Verlag, Berlin, 1985, pp. 656–664.

35. F. M. R. Binjasass and D. Y. C. Fung, "Catalase Activity as an Index of Microbial Load and End-Point Cooking of Temperature of Fish," *Journal of Rapid Methods and Automation in Microbiology* **6**.

36. C. Y. W. Ang et al., "Sensitive Catalase Test for End-Point Temperature of Heated Chicken Meat," *J. Food Sci.* (1994).

37 D. Y. C. Fung and P. A. Hartman, "Miniaturized Microbiology Techniques for Rapid Characterization of Bacteria," in C. G. Heden and T. Illeni, eds., *New Approaches to the Identification of Microorganisms*, Wiley, New York, 1975, pp. 347–370.

38. C. Y. Lee, D. Y. C. Fung, and C. L. Kastner, "Computer-Assisted Identification of Bacteria on Hot-Boned and Conventionally Processed Beef," *J. Food Sci.* **47**, 363 (1982).

39. C. Y. Lee, D. Y. C. Fung, and C. L. Kastner, "Computer-Assisted Identification of Microflora on Hot-Boned and Conventionally Processed Beef: Effect of Moderate and Slow Initial Chilling Rate," *J. Food Sci.* **50**, 553 (1985).

40. S. P. Chem and D. Y. C. Fung, "Acriflavin Violet Red Bile Agar for the Isolation and Enumeration of *Klebsiella pneumoniae*," *Food Microbiology* **7**, 73 (1991).

41. D. Y. C. Fung and C. Liang, "A New Fluorescent Agar for the Isolation of *Candida albicans*," *Bulletin d'information des Laboraores des Service Veterinaries (France)* **29/30**, 1–2 (1989).

42. C. C. S. Lin and D. Y. C. Fung, "Effect of Dyes on Growth of Food Yeast," *J. Food Sci.* **47**, 770 (1985).

43. C. C. S. Lin and D. Y. C. Fung, "Critical Review of Conventional and Rapid Methods for Yeast Identification," *CRC Crit. Rev. in Microbiol.* **14**, 273 (1987).

44. M. C. Goldschmidt, et al., "New Aniline Blue Dye Medium for Rapid Identification and Isolation of *Candida albicans*," *J. Clin. Microbiol.* **29**, 1095–1099 (1991).

45. D. Y. C. Fung, M. C. Goldschmidt, and N. A. Cox, "Evaluation of Bacterial Diagnostic Kits and Systems at an Instructional Workshop," *J. Food Prot.* **47**, 68 (1984).

46. N. A. Cox, "Selecting a Miniaturized System for Identification of *Enterobacteriaceae*," *J. Food Prot.* **47**, 74 (1984).

47. K. F. Eckner et al., "Comparison of *Salmonella* Bio-EnzaBead Immunoassay Method and Conventional Culture Procedure for Detection of *Salmonella* in Foods," *J. Food Prot.* **50**, 379 (1987).

48. D. Y. C. Fung, R. Bennett, and G. C. Lehleitner, "Rapid Diagnosis in Bacteriology: Contribution of Polyclonal and Monoclonal Antibodies," in M. M. Gaeteau, J. Henry, and G. Siest eds., *Biologie Prospective, le Colloque de Pont-e-Mousson*, John Libbey, London, 1988, pp. 21–26.

49. D. Y. C. Fung and A. A. Kraft, "A Rapid and Simple Method for the Detection and Isolation of *Salmonella* from Mixed Cultures and Poultry Products," *Poult. Sci.* **49**, 46 (1970).

50. L. S. L. Yu and D. Y. C. Fung, "Oxyrase Enzyme and Motility Enrichment Fung-Yu Tube for Rapid Detection of *Listeria monocytogenes* and *Listeria* spp.," *Journal of Food Safety* **11**, 149 (1991).

51. L. S. L. Yu and D. Y. C. Fung, "Effect of Oxyrase Enzyme in *Listeria monocytogenes* and Other Facultative Anaerobes," *Journal of Food Safety* **11**, 163 (1991).

52. L. S. L. Yu and D. Y. C. Fung, "Growth Kinetics of *Listeria* in the Presence of Oxyrase Enzyme in a Broth Model System," *Journal of Rapid Methods and Automation in Microbiology* **1**, 15 (1992).

53. F. Niroomand and D. Y. C. Fung, "Effect of Oxygen Reducing Membrane Fragments on Growth of *Capmylobacter*," *Journal of Rapid Methods and Automation in Microbiology* **2**, 247 (1994).

54. K. Tuitemwong, D. Y. C. Fung, and P. Tuitemwong, "Acceleration of Yoghurt Fermentation by Bacterial Membrane Fraction Biocatalysis," *Journal of Rapid Methods and Automation in Microbiology* **3**, 127 (1994).

DANIEL Y. C. FUNG
Kansas State University
Manhattan, Kansas

REFRIGERATED FOODS: FOOD FREEZING AND PROCESSING

In high latitudes it is possible to freeze foods simply by leaving the product outside in winter. This familiarity with food freezing resulted in a general feeling that such a simple process needs little planning or care in product selection, processing, packaging, or subsequent storage and can be accomplished with the minimum of skill. The enormous amount of scientific research put into these three P's (intrinsic Product quality, Processing, and Packaging) by food scientists has resulted in frozen raw materials and products, now increasingly available across the world, meeting the needs of the most sophisticated consumers. Equally important are the three T's (Time, Temperature, and Tolerance), which govern the practical shelf life (PSL) of all frozen products.

The advent of mechanical refrigeration (1) led by the Linde air compressor in the 1850s (capable of maintaining temperatures of about −9°C—the temperature at which mold activity can be ignored over short periods) made it possible to emulate winter conditions in high latitudes across the temperate and tropical regions irrespective of season. A frozen food industry developed, with its capability of transporting products such as beef, lamb, fish, shellfish, and butter around the world and particularly across the Tropics.

The pioneer of the retail frozen food industry, an American named Clarence Birdseye, invented a multiplate froster that enabled commercial quantities of food to be frozen rapidly and brought down to −18°C, which he specified as the warmest temperature at which this new "frosted" food should be stored (2). Today, some 70 years after the launch of the first commercially frozen retail frozen products in Springfield, Massachusetts (3), there has been only little change in the basic precepts of this now large industry. The results of a massive research program, carried out at the U.S. Department of Agriculture (USDA) Western Research Laboratory in Albany, California, in the 1950s, known as the Time–Temperature Tolerance (T–TT) program, validated Birdseye's original prescription of −18°C as the accepted temperature for the industry and has been immensely influential in defining custom and practice throughout the industry (4). Later researchers (5) favored colder temperatures for long-term storage of fish, especially fatty fish such as salmon. Many cold stores have been built capable of holding temperatures of −30°C and, in the case of surimi (to preserve the white color) for the Japanese market, temperatures as cold as −60°C.

The first textbook on food freezing (2) omitted two products that, when developed later, now play a large role in the industry—French fries and frozen concentrated orange juice. Sources of information on food freezing technology include the International Institute of Refrigeration (IIR), the proceedings of which cover the whole gamut of refrigerants; refrigerating equipment; cold store construction; road, rail, and sea transport of frozen foods; and the characteristics of various products during freezing, storage, and thawing (6). In the United States the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc. (ASHRAE) handbooks (7) summarize published research and provide essential background information on refrigerant characteristics. The IIR has also published a concise guide to the processing and handling of frozen foods (8).

Following the successful T–TT program in the 1950s, attention was drawn to the importance of other factors apart from time and temperature—the three P's (5). Subsequent publications (9,10) have elaborated on these developments and advanced our knowledge of the science and practice of food freezing, storage and transport.

T–TT

The concept that the rate of quality loss, as influenced by temperature, in most frozen foods during storage broadly follows the Arrhenius equation, as postulated in the T–TT studies (4), has stood the test of time. One refinement has been the investigation of the glass transition phenomenon in many frozen products (11). As temperature is reduced well below the freezing point, the ice crystals grow until the remaining unfrozen moisture, the freeze-concentrated unfrozen phase, becomes an increasingly highly concentrated and viscous solution. If temperature is lowered further, the point is reached (T_g) at which it becomes a "glass"—an amorphous solid, devoid of structure and totally unlike the highly ordered structure of ice crystals. Below the glass temperature (T_g) the rate of molecular mobility is markedly reduced and hence the rate of quality loss brought almost to a standstill. The kinetics of reactions associated with quality loss at temperatures close to the glass transition temperature (T_g) can be described by the Williams–Landel–Ferry (WLF) model, in which reaction rates (and hence quality loss) are a function of the temperature difference between the storage temperature and T_g (12). Shelf-life modeling of frozen foods is well described by Fu and Labuza (13). While the T_g of most frozen foods may be so cold as to render it too expensive (and unnecessary in view of the short storage periods—a few months—necessary to span the seasons or transport food across the world) to store at or near the T_g these findings open up new avenues of research to breed food plants and formulate prepared dishes with a "warm" T_g, thus increasing their PSL at conventional storage temperatures. Perhaps this research will even extend the range of products that can be successfully frozen.

PPP: PRODUCT QUALITY, PROCESSING, AND PACKAGING

Intrinsic Product Quality

Following Jul's work (5) that drew attention to the PPP factors, numerous researchers have confirmed and extended the understanding of the importance of initial product quality, principally in terms of freshness of the living tissue or formulated food. Perhaps the most dramatic claim showed that the PSL of squid held for only 24 h in ice between death and freezing was reduced by one-half compared with a similar squid frozen immediately on death (14). Cyroprotectants have an important part to play in modifying products to increase their PSL. Cryoprotectants commonly employed include sugars, amino acids, polyols, and so on. Indeed, the loss of PSL in the frozen state consequent upon loss of "freshness" in the raw material may be considered to be due to a loss of naturally occurring cryoprotectants. Antifreeze proteins have been found to depress the freezing point markedly (Antarctic fish can survive without freezing at −70°C), and the possibility that the use of antifreeze proteins may be able to improve the quality of frozen foods is being actively researched (15).

Processing

Any enzyme-mediated reactions will be greatly accelerated by freezing. The cryconcentration effect, caused by the removal of water in the form of ice from the medium, may be 5 or 10 times and is only feebly compensated for by the lowering of temperature. A cooked product, in which the enzymes have been destroyed, is more stable. This enhanced enzymic activity on freezing (which results in loss of color, flavor, and development of off-flavors in cold storage) calls for inactivating the enzymes in vegetables (such as sweet corn, peas, and green beans) by blanching; the choice of which indicator enzyme ensures adequate blanching is addressed by Whitaker (16).

Packaging

Oxygen is the great enemy of frozen food quality. Oxygen can be excluded by covering the product with sauce, by water glazing of fish or using packaging with a low oxygen permeability that can be reinforced by exhausting the package before sealing, by gas flushing, or by skin packaging (17), in which the preheated film is dropped onto the product, which is supported on a lower web of the same film. On withdrawing the air between the two films, a skin-tight package is formed, which is then heat sealed in a vacuum chamber. Figures 1 and 2 illustrate the effects of some common packaging materials on the stability of various products. Packaging may also serve as the cooking container, in a microwave oven or microwavable/dual ovenable packaging.

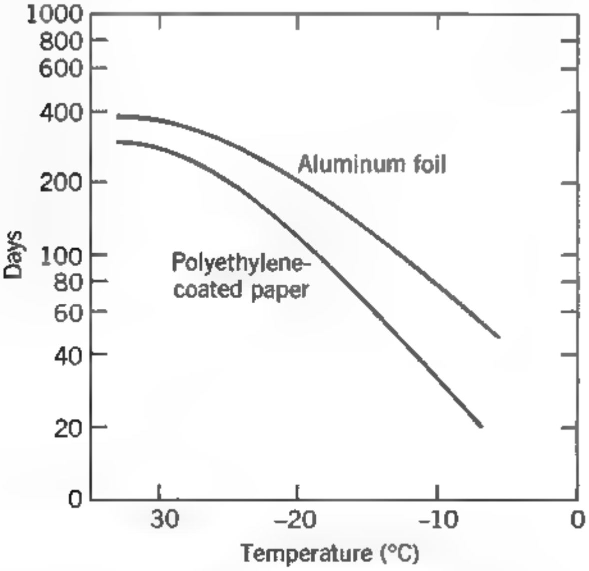


Figure 1. The effect of various packaging materials on shelf life of individually packaged hamburger meat.

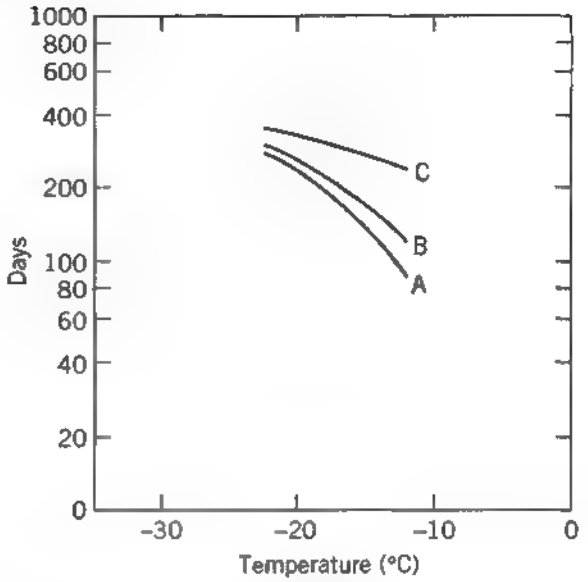


Figure 2. Acceptability time for pork chops. A wrapped in PE film and placed in master cartons; B same as A but a PA–PE laminate film; and C, vacuum packaged in PA–PE laminate (same material as in B).

BIBLIOGRAPHY

1. R. Thevenot, *A History of Refrigeration Throughout the World*, International Institute of Refrigeration, Paris, 1979.
2. D. K. Tressler and C. F. Evers, *The Freezing Preservation of Foods*, AVI Publishing, Westport, Conn., 1943.
3. E. W. Williams, *Frozen Foods: Biography of an Industry*, E. W. Williams Publications, New York, 1963.
4. W. B. Van Arsdel, M. J. Copley, and R. L. Olson, *Quality and Stability in Frozen Foods*, Wiley Interscience, New York, 1969.
5. M. Jul, *The Quality of Frozen Foods*, Academic Press, Orlando, Fla., 1984.
6. *Congress Proceedings of the International Institute of Refrigeration*, International Institute of Refrigeration, Paris, 1959–1995.
7. ASHRAE, *Refrigeration Systems and Applications*, Atlanta, Ga., 1986.
8. *Recommendations for the Processing and Handling of Frozen Foods*, 3rd. ed., International Institute of Refrigeration, Paris, 1986.
9. C. P. Mallet, ed., *Frozen Food Technology*, Blackie Academic and Professional, Glasgow, Scotland, 1993.
10. M. C. Erickson and Y.-C. Hung, eds., *Quality in Frozen Food*, Chapman and Hall, New York, 1997.
11. D. S. Reid, "Basic Physical Phenomena in the Freezing and Thawing of Plant and Animal Tissues," in C. P. Mallet, ed., *Frozen Food Technology*, Blackie Academic and Professional, Glasgow, Scotland, 1993.
12. H. D. Goff, "Measurement and Interpretation of the Glass Transition in Frozen Foods," in M. C. Erikson and Y.-C. Hung, eds., *Quality in Frozen Foods*, Chapman and Hall, New York, 1997.

13. B. Fu and T. P. Labuza, "Shelf-Life Testing: Procedures and Prediction Methods," in M. C. Erikson and Y.-C. Hung, eds., *Quality in Frozen Foods*, Chapman and Hall, New York, 1997.
14. J. Joseph, P. A. Perigreen, and M. R. Nair, "Effect of Raw Material Quality on the Shelf-Life of Frozen Squid (*Loligo duvaucelli*) Mantles," in *Storage Lives of Chilled and Frozen Fish and Fish Products*, International Institute of Refrigeration, Paris, 1984–1985.
15. G. A. MacDonald and T C. Lanier, "Cryoprotectants for Improving Frozen Food Quality," in M. C. Erikson and Y.-C. Hung, eds., *Quality in Frozen Foods*, Chapman and Hall, New York, 1997.
16. D. C Williams et al "Blanching of Vegetables for Freezing—Which Indicator Enzyme to Choose?," *Food Technol.* **40**, 130–140 (1986).
17. V. M. Balasubramanian and M S. Chinnan, "Role of Packaging in Quality Preservation of Frozen Foods," in M. C. Erikson and Y.-C. Hung, eds., *Quality in Frozen Foods*, Chapman and Hall, New York, 1997.

HUGH SYMONS
Kingston Upon Thames, England

REFRIGERATED FOODS: FOOD FREEZING AND WORLD FOOD SUPPLY

Freezing is widely regarded as the most elegant method of long-term preservation of a wide variety of highly perishable foods at economical cost. The list of products that cannot be frozen (salad vegetables, bananas, other whole fruits, etc) is much shorter than the list of products that are successfully frozen. Freezing enables perishable products (fish, shellfish, meats, vegetables, berries, and fruit juices) to be transported across the world and to span the seasons. It makes a multitude of high-quality perishable products available year-round anywhere in the world.

Freezing retains most nutrients, although a few thermolabile vitamins (Vitamin C, thiamine etc) are degraded by perhaps 10 to 30% over a year's storage at −18°C in most products (1).

MEAT

Most meats (beef, lamb, poultry, pork, and game) freeze excellently; that is, after freezing, frozen storage, and thawing, they are often indistinguishable from the original material. Quality retention during long-term storage is also very high, especially for meats containing highly saturated fat. Mammoth meat unearthed from the permafrost in Siberia after 20,000 years was found to be edible (2,3).

The frozen meat trade is vital to the major meat producing countries in the Southern Hemisphere (Argentina, Brazil, Australia, and New Zealand), as it enables them to export enormous tonnages of this vital export to the more densely populated countries in the Northern Hemisphere (North America and Europe). Before the advent of refrigerated transport, these countries could export only wool and tallow. Now butter, cheese, seafood, and vegetables have been added to the export of meat. At the dawn of the era of refrigerated transport the carrying temperature was −10°C, about the best that a Linde air compressor could achieve when relying on tropical seawater for condenser cooling. It is also the warmest temperature at which white and black molds fail to develop or only develop very slowly. This traditional "meat trade" carrying temperature has given way, largely due to containerization, to the customary frozen food temperature of −18°C. The impracticability of providing different temperatures in a container ship and the introduction of fish, frozen orange juice concentrate, vegetables, prepared meals, and so on, all of which demand at least −18°C, has led to standardization on this colder temperature.

SEAFOOD

Seafood remains the only substantial item of human diet a large part of which is still hunted, apart from small quantities of game. Aquaculture now provides substantial quantities of farmed salmon, tilapia, catfish, and trout to supplement the declining productivity of most fishing grounds. Wild seafood often occurs in greatest abundance at short seasons of the year and in areas remote from human populations desirous of consuming them. Several species (herring, cod, mackerel, and hake) are migratory, often present in concentrated shoals and in good eating condition for only a few months of the year. A preservation method is required to preserve them from the time of catching until ultimate consumption perhaps several thousands of miles away and several months later. Traditional methods such as salting, drying, and pickling (in either acid or alkali) have been largely supplanted by freezing.

The most heavily traded seafood commodity in world trade is the international fish block. Made from skinned and deboned fish, generally cod, pollack, or hake, which may be in fillets or sliced fish, often with tripolyphosphate added, this is the raw material from which portions, fish sticks, and fish fingers can be cut. These are generally battered and often breaded before sale to food service or retail outlets. Fish sticks, a relatively simple frozen fish product, developed in the United States in the early 1950s, found enthusiastic acceptance in many markets. Renamed fish fingers when launched in the rapidly growing frozen food market in the UK in 1955, this product became the second most popular item of diet in that country, second only to the traditional dish of fish and chips. Much whole, gutted fish is frozen at sea in upright plate freezers on board the trawler.

Aquaculture of salmon, catfish, trout, shrimp, mussels, and oysters relies almost exclusively on freezing to transport the catch after processing. Freezing has made seafood, often of hitherto exotic species, available to inland consumers to whom the product may have been unfamiliar. Seafood also makes a significant contribution to the nutritional status of the world. Species low in fat (cod, haddock, sole, pollack, hake, catfish, etc) constitute a rich source of animal protein coupled with a low caloric content. Species high in fat (salmon, herring, trout etc.) are very low in saturated fats, while the omega-3 fatty acids present are protective against cardiovascular disease. Surimi, a Japanese technology, depends on freezing for its production and distribution. This technology transforms otherwise disregarded species (eg, pollack in the Bering Sea) into washed, minced fish, which is then flavored and colored before binders are added; the material then simulates exotic species such as crab, lobster, King Crab, shrimp, and so on.

FRUIT AND FRUIT JUICES

Strawberries were the first popular frozen fruit in the United States. They were mixed with sugar in wooden barrels and placed in a cold store where the barrels were turned regularly to ensure proper mixing of the fruit/juice/sugar while being slowly frozen. Chilled whole fruit has eroded the market for this melange that is used in jams, jellies, ice cream, and so on. In the berry-producing countries of Eastern Europe the introduction of individually quick-frozen (IQF) berries, packed hygienically in tote bins each containing perhaps a thousand kilograms has superseded the sulfited pulps in wooden barrels used in the production of conserves and so on. Most whole fruit suffer a substantial loss of texture and mouth-feel during freezing and storage and are used in further manufacture: into pies, flans, jams, jellies, tortes, and so on.

The Florida citrus groves had to wait for the development of vacuum-concentrated juice technology before the enormously popular frozen citrus concentrates could be marketed. Frozen concentrated juice (FCOJ) now accounts for over 95% of the citrus crop in Florida, Israel, and Brazil. FCOJ is interesting from another point of view. In general the objective in freezing is to maintain the original quality of the raw material. Freezing does not seek to improve the raw material. In the case of FCOJ it is possible to blend, in the frozen state, the high acid–low sugar juice produced early in the season with low acid–high sugar juice produced later in the season to produce a consistent quality product. These juice concentrates are increasingly presented to the consumer as diluted to single strength, often as multiple blended chilled juices in single-serving containers.

FROZEN VEGETABLES

Development of a long-term preservation technique, such as freezing, coupled with economical temperature-controlled transport, has enabled the growing of each species of vegetable to be located in the area most conducive to obtaining high yields of high-quality crops often far removed from the urban communities that will consume the products. This is in contrast to the previous habit of clustering canneries around each conurbation.

The introduction of blast-freezing tunnels, and later the spiral tunnels (which occupy less factory floor space), enabled the products to be frozen in the IQF state. These bulk frozen vegetables can then be shipped across the world before being packed into consumer packages, blended, mixed with sauces, or forming one component of a multicomponent dish. In this way half a dozen different vegetables, grown in as many different continents, can be enjoyed year-round in any part of the world. Blanched and frozen within a few hours of harvesting, frozen vegetables often enjoy a superior nutritional profile—even after frozen storage for a year—than their fresh counterparts, which often spend several days before reaching the consumer and then may be stored in a refrigerator before being consumed. Not only is a year-round supply of nutritious vegetables made available but consumers can be introduced to exotic vegetables unfamiliar in their part of the world.

POTATOES

The pioneers of frozen foods in the 1930s and early 1940s, in the United States, knew that potatoes could not be successfully frozen. Maynard Joslyn is credited with developing the technology that enabled frozen French fries to be introduced into the United States market in 1947 (4). U.S. production alone now totals 8.4 billion pounds annually (Fig. 1). Much of this is consumed in fast-food outlets where four out of five customers order French fries, a greater proportion than those who order any other single product, even hamburgers! Instantly acceptable in every market to which they have been introduced, the quality standard for most of the world is the product processed from Russet Burbank potatoes in the northwestern states of Idaho, Washington, and Oregon in the United States.

BIBLIOGRAPHY

1. Harris and Karmes, *Nutritional Evaluation of Food Processing*, 2nd. ed., AVI Publishing Co. Inc., Westport, Conn., 1975.
2. B. Kurten, *How to Deep-Freeze a Mammoth*, Columbia University Press, New York, 1986.

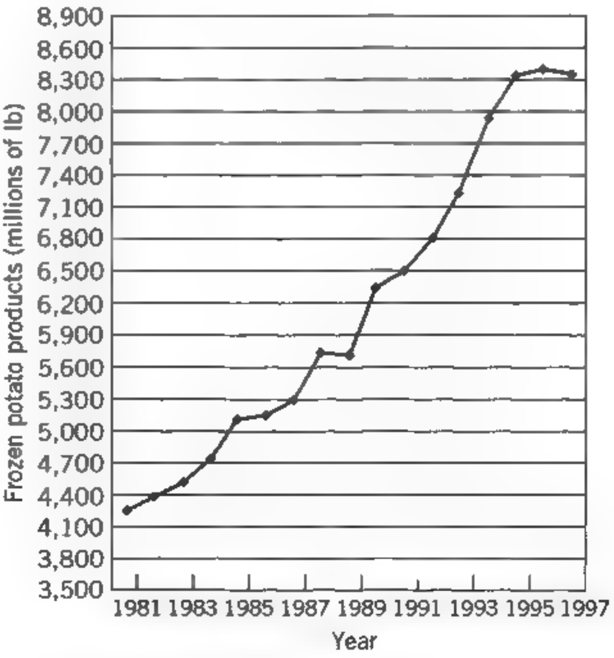


Figure 1. U.S. frozen potato production. *Source:* American Frozen Food Institute.

3. P. Mazur, "Frozen Neanderthals," *Nature* **342**, 23–52 (1989).
4. Talburt and Smith, *Potato Processing*, AVI Publishing Co., Inc. Westport, Conn., 1967.

HUGH SYMONS
Kingston Upon Thames, England

REFRIGERATED FOODS: HANDLING AND INVENTORY

POSTFREEZING OPERATIONS

The International Code of Practice for the Processing and Handling of Frozen Foods (1) elaborated by Codex Alimentarius forms the basis for any recommendations regarding these products. Product may be frozen before packaging, often in the individually quick-frozen (IQF) state, or go directly into the retail or food service package. IQF product may be stored in tote bins, containing around one ton of product, lined with high-density polyethylene liners of sufficient length to allow for sealing. As many postfreezing operations as possible should be carried out in cold store; any packaging operations carried out in chilled areas should be severely limited so that the frozen product spends as few seconds or minutes outside the cold store as possible. Two reports (2,3) produced under the EU FAIR program cover the cold chain and maximizing the quality of frozen foods.

CASING AND PALLETIZING

Casing is almost always carried out in a chilled area or ambient; mechanical palletizing is common, following which the pallet may be shrink-wrapped with polythene for stability during transport. Energy can be saved and product quality retained by introducing cases through a small aperture fitted with a guillotine door (which rises only high enough to admit the case) and palletizing in cold store. The use of forklift trucks, which necessitates two openings of a door sufficiently large to admit a truck for every pallet, incurs significant energy penalties. Vehicles are often sealed onto port doors opening into cold stores; making the interior of the vehicle contiguous with the cold store environment. Cold stores are often highly automated, using high-rise racking accessible with reach trucks fitted with closed cabins for driver comfort and radio communication informing the driver of his order-picking schedule (4).

INVENTORY CONTROL

Recent technological advances (bar coding, computerized control—Electronic Point of Sale, EPOS) have resulted in substantial changes in the management of the flow of product down the cold chain leading to Just-in-Time (JIT) inventory control. Instead of manufacturers producing stocks likely to be able to meet probable future sales to supermarkets or food service outlets and then awaiting sales orders, the sales outlets can now automatically call off the precise quantities needed, which in turn causes the manufacturers to schedule the required production. Reading the bar code of an individual package at a checkout automatically results in instructions passed back to the producing unit to replenish the stock.

OPEN SHELF-LIFE DATING

The EC Frozen Food Directive demands a minimum durability date on all frozen products. An evaluation of open-dating of foods (5) concluded:

> There is little evidence to support or to negate the contention that there is a direct relationship between open shelf-life dating and the actual freshness of food products when they are sold.

The International Institute of Refrigeration (IIR) (6) found a similar lack of evidence in support of open shelf-life dating when it stated:

> The antagonists of mandatory open shelf-life dating of frozen foods quote several disadvantages. The most important requirement for the retention of high quality in frozen foods is the maintenance of an adequately cold temperature. At sufficiently cold temperatures [at least −18°C (0°F) or colder] frozen foods possess a high quality life far in excess of the period such products spend in the cold chain between processing and consumption. Anything which detracts from good temperature management, such as unnecessary regard for product age, would be counterproductive to the objective of presenting the highest practicable quality to the ultimate consumer

A food stability survey (7) gives a comprehensive account of the custom and practice in various countries and made a number of recommendations including adoption of the AFDOUS Code (8). Open shelf-life dating of frozen foods, which often have a long practical shelf life (PSL), leads to the erroneous concept that the quality is in some way guaranteed up to the date given irrespective of storage temperature. An intending purchaser when faced with open dating can react in one of three ways. The date can be ignored, in which case the costs associated with open dating will have been in vain. Alternatively the purchaser may rummage to find the "youngest" product, which turns First In First Out (FIFO) into Last In First Out, (LIFO) and puts pressure on the retailer to display product with only one date. This will lead to more out-of-life stock to be disposed of. Finally the purchaser can select the oldest product knowing that this will assist the retailer in managing his inventory on a FIFO basis. Regrettably this last purchaser does not exist!

TIME-TEMPERATURE INTEGRATORS (T-TI)

Since time elapsed since freezing is an imperfect indicator of product quality, a device that will indicate the integrated effects of time and temperature (a T-TI) is obviously to be preferred and forms part of intelligent or *active* packaging

(9). The deteriorative changes (quality loss) in frozen foods are complex, but the reaction kinetics can be simplified into either pseudo-zero-order or pseudo-first-order kinetics (10). A large number of T-TIs have been patented; these have been reviewed by Taoukis (11). Only three seem to be currently available; the 3M Monitor Mark (which relies on the travel of a colored ink along a wick), the Life Lines T-TI (which relies on a progressive color change in a polymer) and the VITSAB T-TI (which relies on a color change from green to yellow mediated by an enzyme). Life Lines is used on frozen turkey rolls in the United States (10) and also, since 1991, on some 100 chilled products by the supermarket chain Monoprix in France (12). Several studies have shown a close correlation between the quality loss in various frozen and chilled foods and the response of T-TIs (13,14), and an industry specification for evaluating T-TIs for use in food has been elaborated (15). A British standard (16) sets out performance specification and reference testing for T-TIs. The biggest obstacle to the wider use of these inexpensive devices, which present quality assurance to the consumer, is probably the mandatory requirement for open dating in most countries and the resulting confusion that would arise between this almost totally meaningless and potentially misleading date and the far more useful reading of the T-TI.

IN-HOME STORAGE

Most domestic refrigerators possess a frozen food storage compartment while many households also have a home freezer, capable not only of storing frozen foods but also of freezing food. In Europe, star-marked refrigerators are common, a classification system that indicates the temperature in the compartment. One star indicates −12°C; two stars, −15°C; and three stars (the most common), −18°C under specified test conditions. These stars are repeated on the packaging of frozen foods, coupled with an indication of the maximum time the product should be stored according to the number of stars on the storage compartment. For the vast majority of products, the storage time is one week in a one-star, one month in a two-star, and three months in a three-star storage compartment. Home freezers carry three stars while some carry an additional star to denote that they are capable of freezing a substantial quantity of food without unduly elevating the temperature of the already frozen food. The commonest refrigerator-freezer in the United States permits adjustment of the thermostat and louvers that apportion the air from the evaporator between the two compartments so that the body of the refrigerator is maintained at 5°C and the freezer compartment at −18°C under specified test conditions.

THAWING

Most frozen foods requiring cooking are best cooked from the frozen state. Bulky products, such as poultry, whole fish, and so on, that require thawing are best thawed in the body of the refrigerator or in a microwave oven. These ovens often have a defrost program that allows periods of low or no energy to allow thermal equilibration to occur since ice and water absorb microwave energy very differently. It is important to allow extra cooking time if end cooking from the frozen state.

BIBLIOGRAPHY

1. Codex Alimentarius, *International Code of Practice for the Processing and Handling of Quick-Frozen Foods*, U.N. Food and Agriculture Organization, Rome, 1964.
2. R. Fuller, ed., *A Practical Guide to the Cold Chain from Factory to Consumer*, European Union FAIR Programme Concerted Action (CT96-1180), Leeds, United Kingdom, 1997.
3. C. J. Kennedy and G. P. Archer, eds., *Maximising Quality and Stability of Frozen Foods*, European Union FAIR Programme Concerted Action (CT96-1180), Leeds, United Kingdom, 1997.
4. A. F. Harvey, "Mechanical Handling," in C. V. J. Dellino, ed., *Cold and Chilled Storage Technology*, 2nd ed., Van Nostrand Reinhold, New York, 1997, p. 249.
5. Office of Technology Assessment, *Open Shelf-Life Dating of Food*, U.S. Government Printing Office, Washington, D.C., 1989.
6. *Recommendations for the Processing and Handling of Frozen Foods*, 3rd. ed., International Institute of Refrigeration, Paris, 1985.
7. *Food Stability Survey*, Vol. 2, U.S. Government Printing Office, Washington, D.C.
8. *Frozen Food Code*, Association of Food and Drug Officials of the United States, 1961.
9. J. D. Selman, "Time–Temperature Indicators," in M. L. Rooney, ed., *Active Food Packaging*, Blackie Academic and Professional, London, 1995, p. 215.
10. B. Fu and T. P. Labuza, "Shelf-Life Testing: Procedures and Prediction Methods," in M. C. Erikson and Y.-C. Hung, eds., *Quality in Frozen Food*, Chapman and Hall, New York, 1997, p. 377.
11. P. S. Taoukis, B. Fu, and T. P. Labuza, "Time–Temperature Indicators," *Food Technol.* **45**, 70–72 (1991).
12. P. Toursel, "La Puce, un Saut dans la Chaine du Froid," Process No. 1130, Paris, 1997.
13. R. P. Singh and J. H. Wells, "Use of Time–Temperature Indicators to Monitor Quality of Frozen Hamburger," *Food Technol.* **39**, 42–50 (1985).
14. R. P. Singh and J. P. Wells, "Monitoring Quality Changes in Frozen Strawberries with Time–Temperature Indicators," *Int. J. Refrig.* **10**, 296–300 (1987).
15. R. M. George and R. Shaw, "A Food Industry Specification for Defining the Technical Standard and Procedures for the Evaluation of Temperature and Time–Temperature Indicators," *Technical Manual No. 35*, Campden Food and Drink Research Association, United Kingdom, 1992.
16. British Standard 7908, "Packaging—Temperature and Time–Temperature Indicators—Performance Specification and Reference Testing," British Standards Institute, London, 1999.

HUGH SYMONS
Kingston Upon Thames, England

REFRIGERATED FOODS: TRANSPORTATION

The transportation of refrigerated raw materials, ingredients, and food products is an essential link between the food industry and consumers. Each food product possesses characteristics that decide its practical storage life (PSL). Temperature control is a vital factor in determining the PSL of all refrigerated products. Good transport meets these requirements in the chilled or frozen food chains. Some shorter-life products spend more than half their PSL in transport. For others the transit time is a small interval from production to consumption. Apart from temperature, other important factors in the maintenance of quality during transport are packaging, humidity, and protection from damaging product integrity.

Commercial pressures and the advance of computer technology (especially Electronic Point of Sale [EPOS] and Just-in-Time [JIT] inventory control) are helping to reduce the time many items are held in storage or transport. Transportation by sea, land, and air is becoming intermodal, with users demanding the mix that provides them with the optimum quality and cost in an acceptable time. There is keen competition among transportation providers that is quickening the development of improved technology and customer service.

SPECIAL REQUIREMENTS BY TYPE OF FOODSTUFF

Sensitive Chilled Foodstuffs

This growing sector includes a variety of meat, poultry, dairy, fish, and prepared food products. Some are carried within about 1°C of their freezing points (many foods start to freeze at about −1.5°C). The longest sensitive chill chain carries meat from New Zealand to Europe by way of Cape Horn. This involves at least 35 days. These cargoes do not respire, so they can be in tightly packed loads. Precooling to the carrying temperature is essential. These products need to be held within a narrow temperature band to minimize the loss of PSL (1).

Fresh Fruits and Vegetables

Products of this type are living and need transporting in equipment that can remove their heat of respiration, water vapor plus gases such as carbon dioxide and ethylene. The aim is to slow the rate of ripening by moving them at the coolest workable temperature without causing chill injury. This varies between products but can occur at temperatures as warm as 11°C. Products may need protecting from cold ambient temperatures in northern latitudes during the winter.

Some products benefit from being carried under controlled or modified atmosphere when the percentage of oxygen in the vehicle, or container, is reduced (2). Carbon dioxide levels can be changed by supplying measured amounts of the gas from a cylinder. Humidity control will become more important in the future, reducing cargo weight loss and helping to maintain quality longer. An evolving technology is the use of modified atmosphere packaging around the product. This material will prevent oxygen from passing through it but will allow carbon dioxide to flow out. As the product respires, the level of oxygen falls and the process slows down.

Other Chilled and Cooled Products

Modern transport systems allow product carriage under controlled conditions as warm as +30°C. The range continues to expand with increasing volumes of sugar confectionery, chocolate, biscuits, cheese, and shelf-stable products that need protection from extremes of temperature and humidity. Dehumidifiers are being fitted to refrigeration machinery that can help to dry the air surrounding products or packaging. This is useful when a low relative humidity will improve a product's shelf life (when it is not in fully sealed packaging) (3).

Frozen Foods and Ice Cream

The colder the temperatures, the longer these foods will maintain their practical storage lives. The old rule of keeping them colder than −18°C is being replaced by colder temperature requirements. The set point for some modern integral containers can be as cold as −27°C. A few specialized containers can achieve −60°C and are mainly used for carrying tuna to Japan (M. S. Walker, unpublished data). The aim is to reduce temperature fluctuations that cause losses in flavor, texture, and moisture from the surfaces of the produce. Moisture is usually deposited as ice crystals on the packaging or the evaporator coils of the refrigeration machinery (4). Recent research into the "glassy state" of frozen foods may result in demands for colder temperatures for specific foods in transit (5).

Good-quality packaging is essential to protect products from dehydration when exposed to moving refrigerated air within transport. Packaging must also withstand the coldest temperatures, retaining the product's properties while subject to vibration and impact shocks (6).

Compatibility of Mixed Loads

Precautions are needed to ensure that mixed loads can be carried together. The following define compatibility: transit temperature, relative humidity, emission of active gases such as ethylene, odor-absorbing qualities, and modified atmosphere requirements (6).

MODES OF REFRIGERATED TRANSPORT

Sea

Temperature-controlled cargo carriage is by intermodal containers and conventionally refrigerated ships. Some conventional refrigerated ships also carry integral containers (see Fig. 1). The world's container capacity is about the same as conventional ships and continues to expand. Some trades also use porthole-insulated containers in ships with central refrigeration systems.

Containers manufactured to international standards (ISO) provide a secure door-to-door delivery system with landside movements by road, rail, or barges (7). This in-

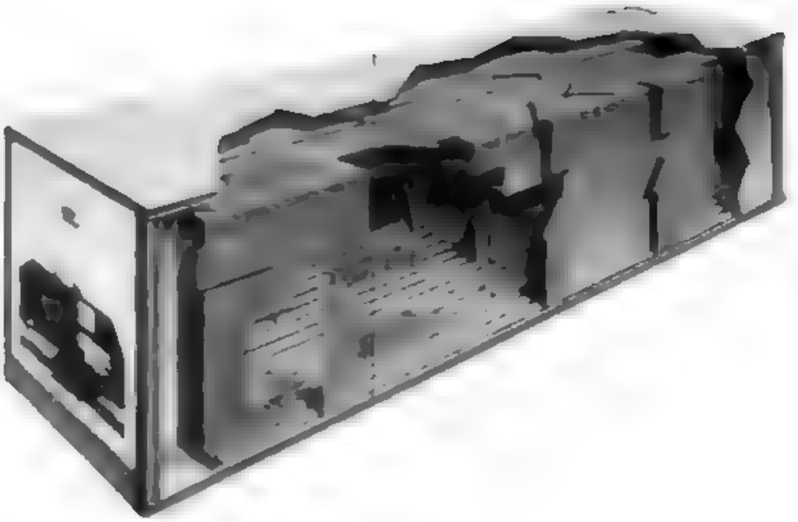


Figure 1. Cutaway view of a loaded integral container. *Source:* Carrier Transicold, reproduced with permission.

termodal chain depends on a supply of electricity from electrical mains or generators. Containers operate with air supplied from the refrigeration system along the T-section floor (see Fig. 2). It circulates around the cargo and in the space between the cargo and the doors. The air then returns along the space between the roof and the top of the cargo to the machinery section at the front (8).

In the chilled mode (warmer than −10°C), a sensing probe in the supply air controls the air temperature to the container. In the frozen mode (colder than −10°C), a sensing probe in the return air of the refrigeration unit controls the air temperature.

Porthole containers have air delivered through couplings to their bottom portholes. It passes around the container and returns through the top portholes. This older system continues to operate well, especially when high volumes of cargo are being carried. Clip-on units (see Fig. 3), towers, or total loss refrigerants such as liquid nitrogen or carbon dioxide provide refrigeration while ashore. This system is restricted to certain trade routes and ports.

Road Trailers

Most temperature-controlled trailers have mechanical refrigeration units powered by their own diesel engine. Major design advances have occurred in recent years due to noise restrictions, moves to more environmentally friendly refrigerants, microprocessor controls, and the need for smaller turning circles. They have resulted in better air temperature control, less defrosting, and lower power demands. Many countries have introduced less restricting maximum weights as road systems have improved, and vehicles have more axles.

Many trailers use refrigerated air blown over the top of the cargo (top-air delivery) and returned along the floor (see Fig. 4). In temperature climates, flat floors predominate, with return air passing through the pallet bases to the refrigeration unit (see Fig. 5). T-section floors are available for use in warmer climates. Air chutes, mounted under the roof, are sometimes used to provide better air distribution along the length of the unit (9). Some haulers use liquid nitrogen or carbon dioxide to provide refrigeration. These systems are almost noiseless in operation but need cargo close to the carriage temperature for efficient use.

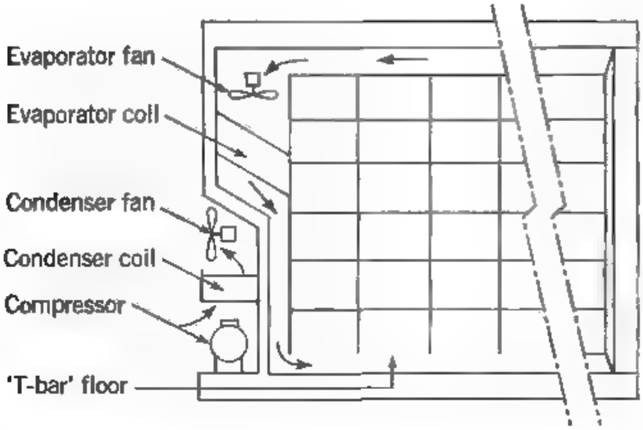


Figure 2. Schematic layout of a refrigerated container.

Figure 3. View of a porthole container with a clip-on unit.

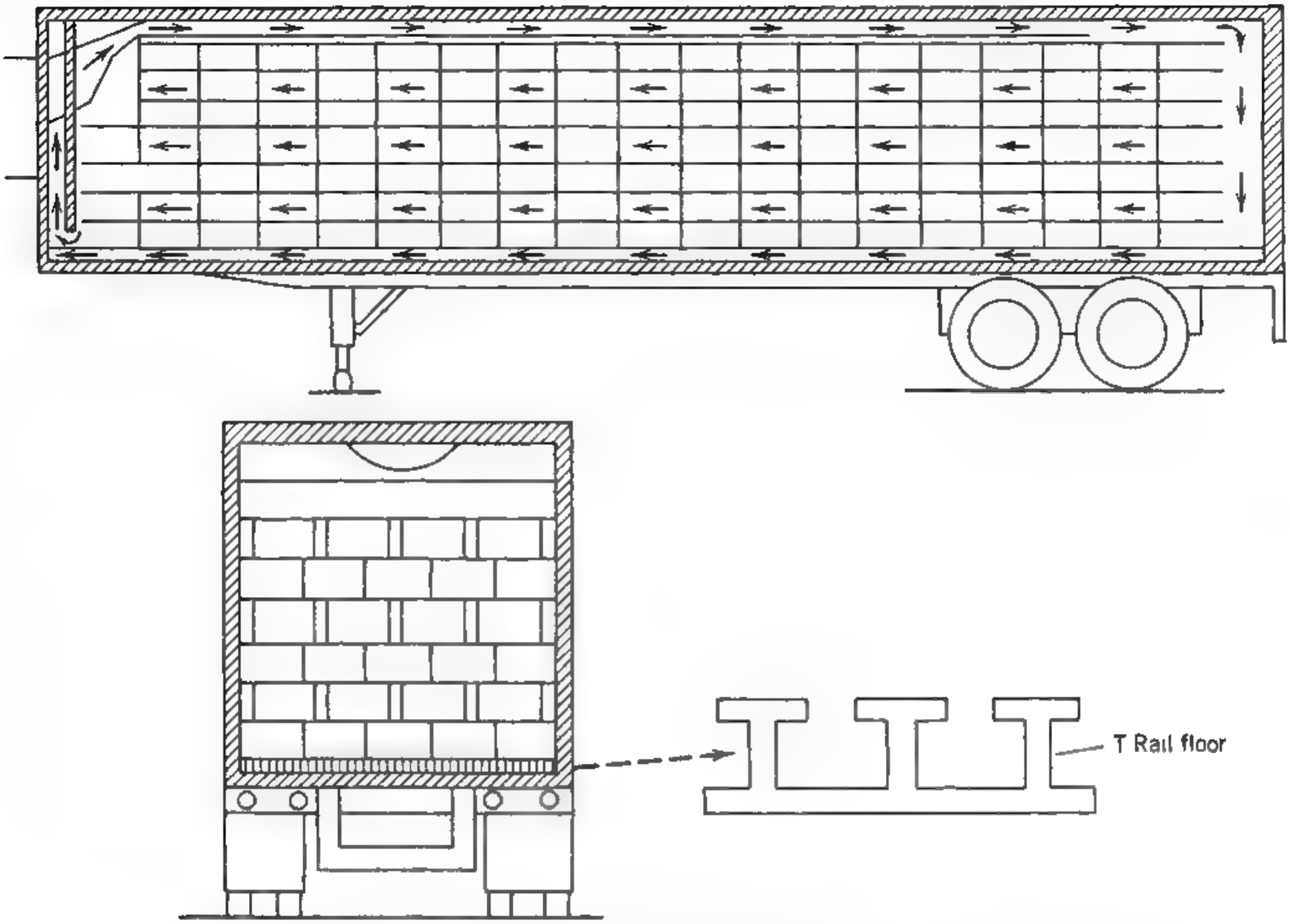


Figure 4. Pathways of air in a trailer equipped with a traditional top-air system and T-section floor.

Multicompartment vehicles are available for carrying products that need different temperatures. Movable bulkheads have also been designed to allow compartments to have variable volumes. If multiple deliveries occur, then it is important to ensure that the load on the tractor does not exceed legal limits. This can happen if the center of pressure of the trailer moves forward when products are taken away from the rear section.

Road Delivery Vehicles

There are many designs of rigid body insulated vehicles with refrigeration systems sized for the operational requirements. Multidelivery vehicles require adequate door protection such as plastic curtains to minimize heat entry when the driver is picking and removing products.

Some companies use a eutectic plate system built into the walls and ceiling. The eutectic is refrozen using an electrical main's electricity to power a small compressor when the vehicle returns to the depot. Ice can build up inside by freezing air that enters when the doors are open. It is removed when the vehicle body is allowed to warm up for routine cleaning.

Insulated pallet-sized containers with eutectic plates are used by some companies requiring small volumes of chilled, or frozen, products delivered to shops next to ambient commodities.

Air

Carriage by air provides a JIT service that can deliver perishable products to customers to meet market demands. It can also provide a quicker way of balancing stocks during times of peak demand. Air freight is very useful in supplying niche markets with products whose shelf life is too short for carriage by surface transport

Cargoes need to be, tightly packed, and consignments insulated, if a suitable container is not available. Refrigerants such as dry ice may be added, providing they do not freeze the cargo and the gases emitted do not damage sensitive products or provide a health hazard. Ice is also used to keep some cargoes cool. Drip loss during melting needs to be contained to prevent damage to packaging and the aircraft. Airlines normally need to give permission for the use of these refrigerants.

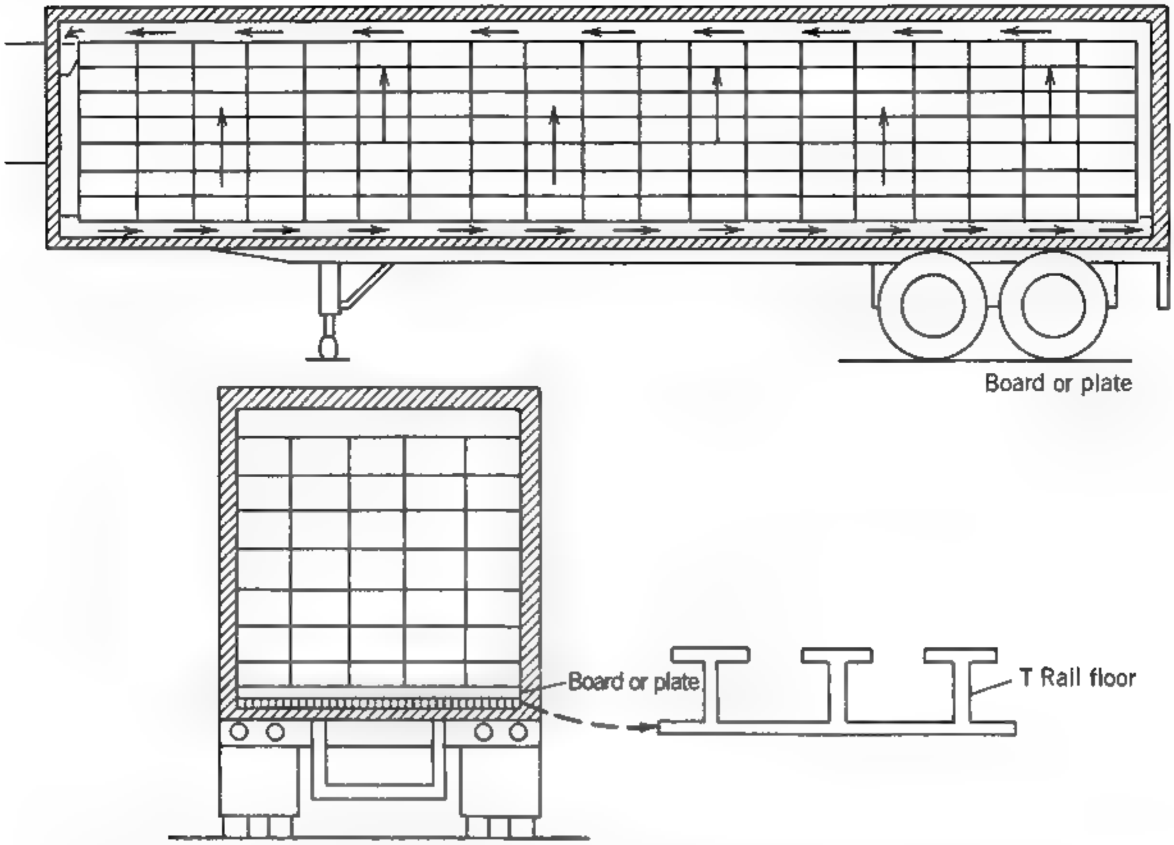


Figure 5. Pathways of air in a trailer equipped with a bottom-forced-air-supply system and a T-section floor.

Administration procedures must be efficient to avoid delays in cargo clearance. Completion of landside operations needs to be in time to prevent damage to the products.

Rail

The volumes of refrigerated cargo moved by rail varies greatly between various parts of the world. New Zealand and Australia have established routes for containers as part of an intermodal system. In the United States there are several routes where containers are stacked two high, giving improved utilization and cost economy.

Deregulation of rail freight services in many countries should lead in the future to more container movements by rail (10). The popularity of the hicube (9 ft 6 in) high container offers a challenge to the older rail systems with height limits. Special low loader flatbeds are being manufactured to contain them.

The former Soviet Union makes much use of refrigerated rail wagons. Trailers are carried on flat wagons in some areas. The Channel Tunnel between England and France requires fast trains that can carry complete road tractors and trailers. This quick drive on and off system has proved to be popular and efficient.

NEW TECHNOLOGY

Digital electronics is giving improved control of refrigeration systems and air temperatures. Data loggers are included in many systems, providing an accurate record of times on and off power plus supply and return air temperatures. Additional temperature-probe readings can also be supplied if required. Records of alarms caused by malfunctions in equipment can also be logged.

Integral containers fitted with modems can transmit data through the power cable to a host computer indirectly linked to the electrical supply (11). The technology exists for the transmission of data through a satellite to a central control room. Remote monitoring allows the checking of location and temperatures from many containers or vehicles continually. Alarms and events can also be transmitted. Routine adjustments of settings are also possible without reference to drivers or other staff. None of these systems obviate the need to check refrigeration systems periodically to ensure all mechanical parts are working.

ENVIRONMENT

Pressures to reduce environmental damage are leading to systems that consume less energy, have refrigerants and foam blowing agents that are more ozone friendly, and equipment with on/off options when reaching required carriage temperatures (9). These developments will continue.

QUALITY ASSURANCE

Demands for higher-quality foodstuffs, with a need for year-round supplies, are causing many retailers to source products worldwide. Food safety and quality requirements (12,13) plus a premium on "freshness" will continue to provide challenges to all sectors of the food transportation sector. Competition is leading to mergers and takeovers of companies with major transport companies operating globally.

BIBLIOGRAPHY

1. M. S. Walker, *Chilled Meat Technical Cold Chain Challenges*, Intermodal, Rotterdam, The Netherlands, 1998.
2. *Controlled Atmosphere in Marine Transport Refrigeration*, Cambridge Refrigeration Technology Seminar Cambridge, U.K., October 1, 1998.
3. Internal *Temperature Controlled Cargoes* P&O Nedlloyd Internal Procedures, London, 1997.
4. A. E. Hawkins, C. A. Pearson, and D. Raynor, "Advantages of Low Emissivity Materials to Products in Commercially Refrigerated Display Cabinets," *Proc. Inst. Refrig.* **69**, 54–67 (1973).
5. H. D. Goff, "Measurement and Interpretation of the Glass Transition in Frozen Foods," in M. C. Erickson and Y.-C. Hung, eds., *Quality in Frozen Foods*, Chapman and Hall, New York, 1997, pp. 29–46.
6. R. Heap, *Food Transportation*, Blackie Academic and Professional, London, 1998, pp. 75–96.
7. *International Standard for Thermal Containers*, ISO 1496 Part 2.2, October 1996.
8. J. P. Sheehan, "Meeting Diverse Reefer Cargo Demands with Marine Container Refrigeration Equipment," *Proc. Inst. Refrig.* **94**, 41–53 (1997–1998).
9. M. J. Heyward, *Food Transportation*, Blackie Academic and Professional, London, 1998, pp. 51–74.
10. T. C. Harris, *Inland Challenges Facing Deep Sea Carriers in Europe*, Intermodal, Rotterdam, The Netherlands, 1998.
11. *International Standard for Reefer Container Monitoring Using Power Cable Transmission*, ISO10368, 1992.
12. European Union General Food Hygiene Directive 94/43/EEC, *Off. J. European Community* **36**. 1–11 (1993).
13. Institute of Food Science and Technology, (U.K.) *Food and Drink Good Manufacturing Practice Guide*, London, 1998.

MICHAEL SANDERSON WALKER
P&O Nedlloyd
London, United Kingdom

RETORT POUCH

A retort pouch is a flat polymeric film laminate package designed to hermetically contain thermoprocessed food. Though some products are contained in pillow pack or bottom-gusseted designs, the predominant version has flat seals on all four sides. The laminate materials range from two-ply to four-ply depending on the desired shelf life, packaging and processing equipment, and product. The design as shown in Figure 1 resembles frozen boil-in-bag entrées but requires no refrigeration. In this context, the retort pouch is a shelf-stable item, the same as food products in hermetic metal cans or glass jars. Usually, a paperboard outer carton is used as an additional precautionary protective measure and to provide surfaces for graphics and print. Retort pouch is the predominant terminology for this concept, although synonyms are flex-pack, flex can, soft can, and pantry pack. Products packaged in the pouches are often referred to as ready meals; the term retort pouch usually encompasses products packaged in this manner.

The term retort correctly signifies that sterilization occurs after three traditional commercial canning steps: filling, headspace air removal, and sealing. This differentiates the retort pouch and its supporting technology from aseptic techniques and the term pouch from semirigid aluminum or thermoformable shallow polymeric tubs or trays for shelf-stable foods. The retort pouch postfilling thermoprocessing approach simplifies, to some extent, the sterilization operation but also imposes a heavier technological and performance burden on the basic package.

In the 1950s, the incentives for development were exploratory and the potential for a shelf-stable competitor for the then visible and strongly marketed frozen boil-in-bag concept. As feasibility approached reality, incentives beyond the convenience aspect of boil-in-bag included the potential for high-quality products, efficient retail shelf space utilization, and less energy usage for materials, processing, storage, and retail display.

The U.S. military, because of its unique combat ration requirements, saw potential advantages of the retort pouch (1): the flat shape fitted into field clothing and other gear conveniently; the pouch flexibility precluded injury if the soldier was falling, crawling, or crouching; opening the pouch required no separate tool or device; and cube and weight savings were possible. The thin cross-section, a nominal 0.75 in, would be suitable for high-quality products, especially those with conductive heating characteristics. Because the geometric center reached sterilization temperatures in a very short time, there would be less overprocessing in the pouch's peripheral areas. The ready-to-eat feature was appealing. The advent of the pouch

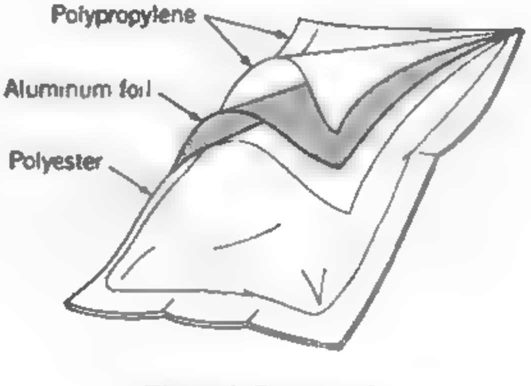


Figure 1. Retort pouch.

turned out to be fortuitous in that to meet the military's need for a long shelf life, procurement of suitable steel-plate cans was becoming problematical.

HISTORY

The history of the retort pouch covers initial curiosity-based exploratory experiments with polymeric films, recognition of the potential advantages as incentives to further delve into the concept, and performance of experiments to establish a database for the eventual design and production requirements of the pouch. The earliest formally recorded activity for applying flexible packaging to thermoprocessed foods is that of Hu and coworkers (2,3), which expanded on earlier graduate studies by Hu. Their results revealed that polymeric pouches of high- and low-acid foods could survive water processes using superimposed air to prevent bursting and also pointed out a need for stronger, heat-sealable materials with low oxygen- and water-permeability rates. Wallenberg and Jarnhall (4), showing European interest, confirmed the concept's feasibility. Limited production was reported in Italy as early as 1960 (5). In Japan, the growth of the retort pouch benefited from lack of competition from other preservation methods and from post–World War II development of pasteurized (up to 100°C) high-protein entrées (6).

During this period, up to approximately 1960, many fundamental questions were answered: materials were screened and their performance specifications established; durability of the basic package was confirmed, product formulations were optimized, processing techniques were further refined, and heat-sealing methods were studied and improved. Key contributions were made by film converters who developed and supplied the heat-resistant three-ply laminates. Equipment manufacturers proffered rotary and in-line equipment, ranging from use of preformed pouches to form, fill, and seal operations starting with roll stock film. Thermal processing centered on water cooks with superimposed air pressure.

Near the end of this early period of development, field tests performed by the military revealed that defects at the point of use occurred at a low frequency and were related to manufacturing deficiencies—mostly contaminated or leaking seals, but also body cuts from poor in-plant handling. In other words, if made and processed well, the pouch, as part of a ration meal, performed well. Similar manufacturing problems were reported in northern and southern Europe (7,8) and Japan (9).

The next sphere of activity, from 1961 through 1975, centered on transition of the retort pouch from a pilot plant operation and test production runs to commercial-level production systems. The approaches to success in this time frame universally reflected an acknowledgment that the production-oriented technology—as borrowed from the canning and frozen food industries—existed, and that standard equipment could be used, but that equipment and unit operation performance had to be at a high level. References 6 and 9–11 are reports on ensuing representative manufacturing system developments. If, for example, the pouch forming and filling operations started with roll stock and were performed on a standard intermittent motion machine, at least 14 additional functions need to be performed. A summary of production systems up to 1977 is presented in reference 12.

TECHNOLOGY BASE

Materials

During the earlier development phases, the film structure consisted of three plies, built to take advantage of and support the oxygen and water vapor barrier properties of aluminum foil. At that time—the mid-1950s to late 1980s—shelf life of over a year, and for the military, 3 to 5 years, was the given requirement. The three-ply materials consisted of:

1. The *outer ply*, offering some barrier and strength properties but mainly relied on for abrasion resistance, is usually polyethylene terephthalate (the polyester Mylar) in thicknesses of 9 to 12 μ. An adhesive system has been used to combine the outer ply with the aluminum foil.
2. The *center* material is dead soft aluminum foil in thicknesses of 9 to 12 μm. This ply is the barrier to oxygen and water vapor passage. Although some pinholes (foil breaks) are inevitable because of the foil's prelamination fragility, calculations of the percentage open area and storage tests both indicate that these breaks have no measurable adverse effect on performance. Neither do these breaks per se permit bacterial penetration; fractures must exist through all plies for bacteria to penetrate into the product.
3. The *inner ply* is the major performance contributor. It must be suitable for extrusion bonding to foil, where the bond must withstand thermoprocess temperatures as high as 270°F, transportation abuse, and exposure to a wide variety of troublesome food ingredients (lipids, salts, flavor oils) for long periods. It must be heat sealable on basically standard equipment; free of toxic or objectionable components, odor, or flavor; machinable; and capable of retaining its functional integrity at storage temperatures ranging from −25°F to over 110°F. The first inner food-contacting material used in any significant testing was polyvinyl chloride, adhered by an adhesive system to the foil layer.

Problems with delamination and off-odors with polyvinyl chloride led to two alternatives that prevailed until the late 1970s: a modified medium- to high-density polyolefin called C-79 by its producer (Continental Can Company) and a blend of ethylene–propylene copolymers. These two materials were also combined with the foil by an adhesive (a polyester–isocyanate system) (10). These materials, or minor modifications thereof, were used successfully worldwide.

In late 1974 and into 1975, there was a hiatus in retort pouch development while the U.S. Food and Drug Administration reassessed extractives attributable to the polyester–isocyanate adhesive system. Because of concern over the adhesive approach and the precautions necessary to produce an acceptable material, an extrusion lamination approach was perfected; the current universal inner film is a cast polypropylene that is then co-extruded with the foil

The three-ply foil laminate was satisfactory where preformed pouches were used or where pouches were made vertically on rotary or in-line form, fill, and seal machinery. Because the successful initial performance characteristics of the pouch were based on products made via such production procedures (both sides of the pouch equally manipulated and/or stressed), the only other film structures used were those without foil and, occasionally, also with a polyamide in lieu of the outer polyester. These other films were for products where a shorter shelf life (8–18 months) was acceptable.

In the late 1980s and into the 1990s, as experience and confidence accumulated with production procedures, distribution, and user performance of the retort pouch, some materials changes were made to accommodate horizontal form, fill and seal equipment, and the greater range of materials requirements engendered by consumer needs. Relative to use of horizontally oriented equipment, the alternate use of drawn semirigid polymer trays and the desirability of microwavability were also pertinent factors. Currently, for shelf life of a minimum of 3 years, such as required for the U.S. military's meals ready to eat combat ration, the cast polypropylene/foil/polyester structure is used for any pouches still vertically formed and filled and as the top or cover layer when horizontal machinery is used. The bottom or drawn laminate in the horizontal mode consists of cast polypropylene as the sealant ply, then foil, and oriented polypropylene as the outer ply. In Japan and impending in the United States is a four-layer material—a polyamide ply will be inserted between the foil and the polyester for preformed pouches or between the cast polypropylene and the foil for horizontal form, fill, and seal operations.

Where a shorter shelf life (8–18 months) is adequate, that is, for practically all applications except the military, the central aluminum foil ply is replaced by a high-barrier polymer, ethylene vinyl alcohol (EVOH). The resulting polypropylene/EVOH/polyester film—on horizontal systems—presents an adequate 18-month shelf life and a microwave reheat feature. EVOH meets U.S. regulatory requirements for direct food contact (13).

PACKAGE DESIGNS

The flat four-seal design has dominated retort pouch usage from the beginning. In addition to favorable marketing characteristics, such as flat surfaces for graphics and efficient shelf space usage, this design, whether implemented on vertical or horizontal equipment, facilitates visual inspection of the seals, minimizes seal junctions that are potential leak sites, provides the thin cross section needed for shorter thermoprocesses, and results in fast heating in hot water for serving. Tear notches provide an easy opening feature.

Except for some institutional packs where a sturdy bulk shipping case is used, an outer carton is used to provide additional handling protection. Experience has shown that gluing the pouch to the carton is not necessary.

Sacharow (14), reviewing Japanese experience, reported that a stand-up pouch featuring a bottom gusset is used for stews, soups, boiled vegetables, and oriental-style foods. In the United States, a juice drink product uses this bottom gusset design. The paperboard carton is absent in both of these applications.

PRODUCTS

The number and variety of products successfully packaged in retort pouches is very large. Lampi (12) listed more than 80 brand-name products marketed worldwide up to the year 1976. Since then, variations and forms of entrees, but probably not total numbers, have increased. Items include ready meals (eg, stews, meatballs in tomato sauce), straight sauces, meats with minimal fluids, vegetable packs, fruits, soups, and bakery items. The U.S. military's meals ready to eat combat ration currently has 24 menus—24 retort-pouched entrées supplemented with retort-pouched fruits, rice, and noodles. Shelf-stable bread and meat-containing sandwiches, though not basically thermoprocessed, exist in pouches of the same three-ply material.

Retort pouches continue to hold a share of the processed food market in Japan and are used for selected items in Europe. In the United States, following several test-market ventures, the market has narrowed to the military; items in support of weight control and health programs; microwavable meals for children; campers and hikers' items; specialty, high-quality-image items; and entrées and adjuncts for direct-networking marketing systems.

One of the cited advantages of the retort pouch has been a quality improvement over cylindrical cans because of reduced heat exposure to achieve sterilization. In actuality, conflicting experiences make this postulation difficult to confirm. The U.S. military, before accepting the retort pouch as a replacement for cans in its operational rations, gathered taste test data that showed a higher acceptance for the retort pouch (15). In addition to formal, controlled acceptance assessments, a perhaps truer indication of military acceptance, though anecdotal, was the posttest, ad libitum selection of leftover rations by the test participants; 4 out of 5 selected the pouched over the canned items. Undoubtedly, commercial entities, before entering into expensive test-marketing programs, satisfied themselves that quality, in addition to convenience and other user attributes, was high. And where there has been minimal competition from frozen foods, such as in Japan, the pouch has done well (16).

Beverly (17), summarizing opinions on various aspects of retort pouch processing, reported a range of conclusions. One large U.S. food processor reported that consumers found no quality difference between pouches and retail cans for the same product. This report also cited the internal evaluations by a second large processor as revealing a pouched food quality halfway between frozen and canned and a third stating they can achieve frozen food quality. Commercial processes of 10 oz of pouched product receive 70 to 83% of the total heat exposure of same-weight cans, which raised the question as to whether this decrease was significant enough to result in detectable product quality improvement.

Retention of thiamine in thermoprocessed foods has been used as a quantitative measure of quality. With pouches of sweet potato puree processed at 250°F, 77% of the thiamine was retained as compared to 60.4% for equal-volume cans (18). A computer model calculation for a conductive heating product indicated 84.6% thiamine retention at 250°F for a 12-oz pouch and 64% for a 12-oz can (19). Data comparing thickness of the product and thiamine retention confirmed these findings (20).

In summary, product quality of retort pouches can be better than that of cans, possibly equal to that expected from some frozen products. Commercial realization has been hindered by production inabilities to get pouch-to-pouch uniformity and repeatability of product heat treatment and quality. Current indications are that this gap is being overcome.

PRODUCTION SYSTEMS

The design of the retort pouch that was initially subjected to acceptance, storage, handling, and distribution durability trials was a flat four-seal version where both sides of the pouch structure were subjected to equal stress during filling and processing. Accordingly, when manufacturing systems were investigated, these systems were geared to manipulating opposing pouch walls equally and filling through the fourth seal position that had been left open. Then, after air removal, a closure seal was made. This principle, tied to vertical pouch handling, was applied to all production systems, whether predominantly manual or automated, up to the late 1980s, and whether preformed pouches or roll stock was the starting point.

Early user tests had shown that retort pouch defects were related to manufacturing deficiencies, primarily the need to fill pouches without seal-area contamination, remove residual air, and form a closure seal of adequate strength and high integrity. A judgment was also made that standard equipment principles could be used. To prove this point, a comprehensive development program was completed by a consortium of companies headed by Swift and Company. Program results are summarized in reference 21. Reference 12 describes production systems that existed up to 1977.

Since then, horizontally oriented equipment as shown in Figure 2 (courtesy of Multivac, Inc.) has been perfected and has gained acceptance to the point where it now prevails. The intermittent motion horizontal form, fill, and seal machine starts with roll stock film, as defined previously in the section on materials, where a lower web is formed into drawn pockets in dies assisted by compressed air or vacuum (and heat and plugs, if necessary). Each index movement forwards a set of pouches, for example, six. Next the pouches are filled in a single step or in multiple steps, automatically or manually, and, along with the upper web, are passed through a chamber where after closing, a vacuum is drawn and the top lidding film is heat sealed to the bottom web, seals being located where the die outline shows solid support. Pouches then exit the chamber and are cut into separate units, inspected, and transferred to the thermoprocessing operation.

The horizontal configuration is currently preferred because it is faster—a bigger pouch pocket area allows faster filling of several pockets or pouches at one time, and because the same equipment can be converted from pouches to drawn, semirigid polymer trays.

Whether prepared on vertically or horizontally oriented equipment, the finished pouched item must meet rigid specifications. References 22–25 provide requirements and criteria.

Filling

The filling of retort pouches can be divided into three steps or functions (12): (*1*) the use of pumps, augers, conveyors, and the like to move the product to the pouch opening; (*2*) the positioning and feeding of the product into the pouch through a nozzle designed or fitted with a positive cutoff or suck-back antidrip feature; and (*3*) the simultaneous presentation of the opened pouch or drawn pouch cavity to the filling station. Filling for vertical systems is discussed in detail in reference 12. The same steps and precautions apply to horizontal drawn pouch procedures. Controlling fill weight is critical because of its effect on the thermoprocess (26).

Air Removal

Before closure sealing, as much of the noncondensible gases as possible should be removed from the pouch for product stability, avoidance of pouch bursting during retorting (without resorting to high air counterpressures), assurance of product sterilization, easier detection of spoilage (swelling), and easier cartoning and casing. The air-removal step is carried out on equipment that also performs other steps such as filling or, more frequently, the closure seal operation. Specific residual gas levels are a function of product, occluded air, air-removal technique, and production rates. Recently, to accommodate solid, placeable items, the permissible residual air in an 8-oz pouch has been increased to 20 cc.

Air-removal techniques for vertical packaging systems used vacuum chambers, snorkel suction tubes, steam-flush air displacement, and counterpressure via opposing metal plates or waterhead pressure. On a horizontal drawn pouch machine, air is removed in an on-line hoodlike chamber where the four-sided sealing operation also takes place.

Sealing

Next to the basic pouch material itself, retort pouch seals have received the most attention relative to their assured performance through the processing steps and over the postprocess distribution period. There are two performance aspects to retort pouch sealing. The first is the creation of an inherently sound seal when a pouch is being vertically formed or formed over a lower web of horizontal pockets; that is, when seal surfaces are clean; the second,

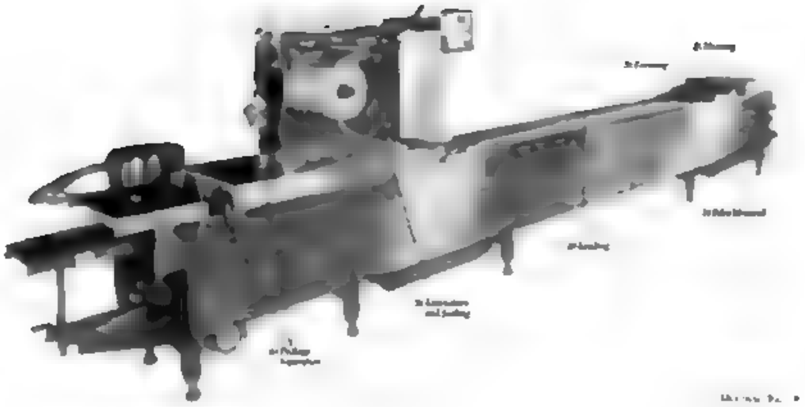


Figure 2. Horizontally oriented intermittent motion pouch form, fill, seal machine. *Source:* Courtesy of Multivac, Inc.

integral with the filling steps, is the creation of a sound seal free of contamination, severe distortion, or significant wrinkles, again true for vertical and horizontal approaches. These aspects of seals are covered by Lampi et al. (27). Young (28) presents a thorough discussion of the principles and options for sealing thermoplastics.

Both hot bar and impulse sealing techniques have been successfully used with retort pouches prepared on vertical systems. Improvements to bar design and control of sealing parameters have resulted in adequate pouch-forming seals, but most of the attention has been focused on the closure seal. This is where sealing occurs as an integral part of the air-removal operation and frequently has had to overcome minor product contamination or moisture (from steam flushing for air removal) in the seal area. Also, wrinkles have resulted from distortion during filling. Various tensioning grippers and forming bars have been used to maintain a taut, wrinkle-free seal area. Bars with a slightly transverse radiused cross-section used against a flat anvil bar can seal through water and grease contamination (29). In Japan, a superimposed triple hot bar technique where a second and a third bar flatten out blisters caused by vaporization of product from the initial hot bar sealing action has been used (9). With correct design and close surveillance of the filling, air-removal, and sealing operation, satisfactory closure seals can routinely be made.

On horizontal equipment, sealing dies are formed to match the outline and surface edges of the lower pockets for a set of pouches, for example, six. Once hot seals are made, there is a cooling pause or a movement to a second station where unheated bars provide cooling and stabilization of the seal area. Following that, the pouches are separated by a cutting die and moved on to the retorting operation.

RETORTING

Along with establishing consistently satisfactory materials and obtaining defect-free closure seals, the subject of retorting, the commercial sterilization step, has received considerable attention. During the early exploratory phases, enough studies, including inoculated packs, were performed so that apprehension over sterility was relieved and reliable cooking procedures were established. Then, as experience was accumulated and as productivity as well as quality became important for successful commercialization, retorting received more attention. For example, process times for retort pouches were established at 25 to 40% of those required for equivalent canned items; however, the total heat exposure in commercial processes was actually 70 to 83% of cans (26).

For determining thermoprocessing conditions (ie, cook time and temperature), the general and mathematical techniques for calculating cook times for traditional cylindrical cans based on heat-penetration data have been found to be generally suitable for retort pouches. The lethality levels, known as F_0, adequate for commercial cans were also found to be suitable for pouched products. Comparisons of the various process determination techniques and listings of precautions to note during their application to pouches (such as difficulty in locating the cold spot or occasional occurrence of nonlinear heating curves) have been published (12,19,30).

For successful retort pouch thermoprocesses, the following factors need to be closely controlled (19,26,31,32):

1. Pouch fill weight and residual gases
2. Pouch thickness during processing
3. Control and reproducibility of the entire cook cycle: come-up, process, and cool
4. The retort rack or other device that controls thickness during processing, and that must not adversely affect heat medium flow or temperature uniformity in the retort
5. Overriding or total pressure

A variety of processing techniques and equipment designs have been used with retort pouches, and each has its proponents. Still cooks under water with superimposed air pressure was the initial reliable procedure and is still used in some instances for some products. Pouches are positioned usually horizontally onto perforated pocketed trays and accumulated on carts that are wheeled into horizontal retort chambers. Most systems are designed so that rotation of the cart is possible. To conserve energy and reduce come-up time, the processing water is preheated to a high temperature in a separate tank and then fed into the retort chamber after loading. Processes are now computer controlled (33). Steam-air retorts are now proven, accepted, and more prevalent. They are basically the same design except that an air tank replaces the hot water preheating tank.

Other retort designs include a continuous cooker-cooler (trade-named Hydrolock) using steam-air as the heating medium, and a Universal Convenience Food Sterilizer (34), where pouches in retort carts are exposed to horizontally flowing hot water. Water is pumped from a hot water storage tank into each rectangular tanklike cart (as opposed to the entire chamber) fitted with a water flow distribution plate configured to assure uniform lateral water flow distribution.

RELIABILITY AND PERFORMANCE

Two initial concerns with the advent of interest in using flexible packaging for shelf-stable foods were:

1. Whether the pouch could be formed, filled, sealed, and processed at an acceptably low reject rate (i.e., be free of manufacturing defects), and
2. Whether the end product could withstand the rigors of postprocess handling and distribution (i.e., be free of leakers swells, and/or contamination). Although some technology and criteria were usable from metal and glass "canning" and from frozen and dry food flexible packaging, the retort pouch's characteristics were singular enough that new specifications and testing protocols were necessary.

The specific tests and the criteria required to assure that the material and the production line were in control and that items had been correctly packaged and to present a confidence that items would survive postprocess rigors changed over the years from the late 1950s to the present. These changes resulted from accumulated experience-based confidence with end-item performance; improvements in and institutionalization of manufacturing systems; improved materials converting; and the transition from vertical filling and sealing of pouches to horizontally oriented pouch drawing, filling, air removal, and sealing.

The initial performance standards from the mid-1960s to the late 1980s were built around a three-ply foil laminate, semiautomated packaging procedures, and the ability to resist military distribution abuse (considered the most rigorous). Lampi et al. (27) discuss these in detail. In summary, postretort pouch seals had to exhibit complete fusion, meet an internal pressure level of 20 psig for a 30-s hold time, meet a tensile strength of 12 lb/in for a 1/2-in sample width, and show no visual aberrations. Photographs of acceptable and nonacceptable seals were published as guides for visual inspection. Residual gases were initially kept at 5 cm^3 or less. An inspection plan suitable for monitoring production is shown and discussed by Lampi (12).

The key elements of the early test pack performance trials and supporting laboratory experiments and test have been incorporated into several documents:

1. National Food Processors Association Bulletin 41-L. *Flexible Package Integrity Bulletin*. (22).
2. *Classification of Visible Exterior Flexible Package Defects*. Poster. Association of Official Analytical Chemists (23). To be used in conjunction with NFPA Bulletin 41-L.
3. *Standard Guide for Use and Handling of Flexible Retort Pouches in the Manufacturing Environment*. ASTM F-1278-90 (24).
4. *Packaging and Thermoprocessing of Foods in Flexible Pouches*. Military Specification MIL-P-44073 (Latest version is E) (25).

The acceptance, and now prevalence, of horizontal form, fill, and seal machinery for pouches and the continuing compliance of films to specifications has resulted in emphasis on a burst test and two visual in-plant inspections as adequate in-plant control. The burst test is a simple puncture of the pouch with a needle, controlled pressure increase to a set level, and hold for 30 s. Visual inspections are pre- and postthermoprocessing.

The overall result of the maturation and stabilization of the processing technology, diligent use of key test(s), and trained visual inspection is that reports of retort pouch failures at the point of use are rare, so rare that the U.S. military reports "no problems" with their use of the pouch, and their usage rate is 24 million pouches per year. The absence of any failures is difficult to accept, but it most likely reflects that two visual examinations are more inclusive in picking out defects than anticipated and that field failures are so few that they are simply discarded without comment or complaint. Signs of postprocess failure parallel those of cans: obvious leakage, malodor, and swelling.

There has been interest in on-line defect-detection devices since the earliest establishment of a production procedure, primarily to replace visual examinations and their people-based frailties. Defects were defined as seal occlusions and leaks through seal areas or on the body of the pouch. Spencer and Bodman (35) surveyed leak detection methods, identified 23, and determined that helium gas as a tracer for "drier" foods and electrical conductivity changes in an external fluid for "wetter" foods were the best options to find a 10-μm hole on the body of the pouch. Lampi et al. (27) reported that the cost, size, and complexity of adaptation to a production scenario were prohibitive. Since then, interest has periodically resurfaced. The use of horizontal form, fill, and seal equipment provides the option, depending on the specific principle, of testing several pouches at once, favorably impacting on test equipment design. Floros and Gnanasekharan (36–38) discuss principles, technology, and applications to aseptic and flexible food packaging. In a symposium jointly sponsored by the Institute of Packaging Professionals and the Food Processors Institute (39), several aspects of package integrity and some candidate nondestructive defect-detection methods were discussed. To date (April 1998 at this writing) on-line defect detectors have not materialized, although some pressure differential profile procedures are promising, both technically and economically.

TRENDS

Retort-pouched foods, once they were proven viable, shelf-stable items, resulted in considerable commercial interest and several test-market trials by major food processors. Since the initial rush, commercial interest has subsided, and current applications are centered on specialty gourmet-image foods; liquid diets as used in hospitals; health and calorie-control entrées marketed in conjunction with health programs; and convenience entrées through direct-marketing network systems. Nearly all of these commercial pouches are foil free, with ethylene vinyl alcohol resins providing the oxygen and water vapor barrier. Shelf life is 18 months, and products are microwavable and hot-water heatable. The nature of these applications requires a high organoleptic quality, a major factor in their successes.

The U.S. military remains steadfast as one of the major users of retort-pouched foods; in the combat ration, the meals ready to eat, the retort pouch continues to meet the requirements of rigorous storage (including serving as war materiel reserves), distribution (which includes occasional free-fall air drops), climatic and geographical environmental extremes, and ultimate consumer preferences. Some variations among entrées have been introduced so that the ration can be used to alleviate indigenous food shortages in strife-torn areas. The meals ready to eat now feature 24 menus, and the ration includes a flameless ration heater (water-activated controlled electrochemical reaction). Through 1991, just under 400 million meals had been procured. Since then, consumption has hovered at 2 million cases (24 million meals) per year, and all indications are that these levels will remain constant.

Now that the retort pouch has been technically feasible for nearly 40 years and several attempts have been made at introducing it into major market segments, its future is, in all likelihood, to remain with the applications listed earlier. Some reasons for this are:

1. The frozen food distribution system in the United States from the manufacturer to the home freezer has been well established and has provided products that the retort pouch has had difficulty replacing. Conversely, the pouch has done well in Japan, where competitive processes do not exist to the same degree.
2. Consistency in quality (not quality per se) for some lines of flavor-sensitive products has not been satisfactory; that is, because of deficiencies (mostly related to inconsistent time variables with unit operations) in filling, sealing, and processing, some package-to-package product variations have been greater than the manufacturers felt was acceptable. In addition, equipment was not available for high-volume production.
3. The boil-in-bag approach, even with frozen foods, has not appealed to customers to the degree manufacturers expected.
4. The greatest deterrent has been an outgrowth of the pouch and its technology; namely, the shallow, semirigid, thermoprocessable polymer trays with barrier properties suitable for an acceptable 12- to 18-month shelf life. These packages have a thin cross section so that high-quality products are achieved with minimum thermoprocessing. They also provide structural protection for fragile, placeable items; easy, direct consumption from the trays (seals are peelable); and, most significantly, the capability to be heated in a microwave oven for serving.

The retort pouch has provided an essential package for the military and a viable one for selected commercial foods. Its applications have generally stabilized. Increased usage will depend on innovative products and marketing rather than on further technical improvements. Its development and technical acceptance has broadened shelf-stable food packaging by demonstrating that another materials class—polymers, in pouch, tray, or tub form—provides quality and performance at least equal to more traditional materials. The existence of these packaging options has resulted in the National Canners Association becoming the National Food Processors Association.

BIBLIOGRAPHY

1. F. J. Rubinate, "Flexible Containers for Heat Processed Foods," in *Proceedings of the Conference on Flexible Packages for Military Food Items*, National Academy of Science/Quartermaster Food and Container Institute for the Armed Forces, Washington, D.C., 1960, pp. 21–28.
2. K. H. Hu et al., "Feasibility of Using Plastic Film Packages for Heat-Processed Foods," *Food Technol.* **19**, 236–240 (1955).
3. A. I. Nelson, K. H. Hu, and M. P. Steinberg, "Heat Processable Food Films," *Modern Packaging* **20**, 173–179 (1956).
4. E. Wallenberg and B. Jarnhall, "Heat Sterilization in Plastics," *Modern Packaging* **31**, 165–167 (1957).
5. F. Nughes, E. Mantovani, and R. Merloni, "Nuova Serie di Controlli di Imballaggi per Cibi Pronti," *Imballaggio* **24**, 14–15 (1973).
6. Y. Tsutsumi, "Retort Pouch: Its Development and Application to Foods in Japan," *Journal of Plastics* **6**, 24–30 (1972).
7. S. F. T. Nieboer, "Flexible Vacuum Packs for Processed Vegetables," *Food Manufacturing* **45**, 60–64 (1970)
8. F. Nughes, "Perspective Report on Retortable Pouch Packaging in Europe," Report No. T-7313, Packaging Institute Seminar, St. Louis, Mo., 1973.
9. Y. Tsutsumi, "The Growth of Food Packed in Retortable Pouches in Japan," *Activities Report, Research and Development Associates for Military Food and Packaging Systems, Inc.* **27**, 149–153 (1975)
10. P. L. Goldfarb, "Pouch for Low Acid Foods," parts 1 and 2, *Modern Packaging* **43**, 70–76 (1970); **44**, 70–76 (1971).
11. D. D. Duxbury et al., "Reliability of Flexible Packaging for Thermoprocessed Foods under Production Conditions: Phase I, Feasibility," Technical Report No. 72-77-GP. U.S. Army Natick Laboratories, Natick, Mass., 1970
12. R. A. Lampi, "Flexible Packaging for Thermoprocessed Foods," *Advances in Food Research* **23**, 305–428 (1977).
13. R. H. Foster, "Polymers for Packaging, EVOH," in R. B. Holmgren and J. R. Russo, eds., *Packaging Encyclopedia*, Cahners, Newton, Mass., 1987, pp. 70–72, 75.
14. S. Sacharow, "Retortable Food Packaging: The Japanese Experience," *Prepared Foods* **154**, 29–30 (1985).

15. R. A. Lampi, "The Current Prognosis for Flexible Packaging of Military Rations," *Annu. Packaging Inst. Forum*, Chicago, Ill., October 7, 1974.
16. Y. Saito, "The Retort Pouch Is a Way of Life in Japan," *Activities Report, Research and Development Associates for Military Food and Packaging Systems, Inc.* **35**, 8–12 (1983).
17. R. G. Beverly, "Retort Pouch in the 80's," *Food Engineering* **52**, 100–103 (1980).
18. S. H. Rizvi, "Nutritional Retention of Retort Pouched Foods," *Activities Report, Research and Development Associates for Military Food and Packaging Systems, Inc.* **33**, 112–115 (1981).
19. R. Greene, "Designing Thermal Processes for Optimizing Product Quality in Retort Pouches," *Activities Report, Research and Development Associates for Military Food and Packaging Systems, Inc.* **35**, 19–24 (1983).
20. T. Ohlsson, "Optimal Sterilization Temperatures for Flat Containers," *J. Food Sci.* **45**, 848–852 (1980).
21. R. A. Lampi and F. J. Rubinate, "Thermoprocessed Foods in Flexible Packages: Transition to Production," *Package Development* **3**, 12–18 (1973).
22. National Food Processors Association, *Flexible Package Integrity Bulletin*, NFPA Bulletin 41-L, Washington, D.C., 1989.
23. Association of Official Analytical Chemists, *Classification of Visible Exterior Flexible Package Defects* (poster), Arlington, Va. 1989.
24. American Society of Testing and Materials, *Standard Guide for Use and Handling of Flexible Retort Food Pouches in the Manufacturing Environment*, ASTM F-1278-90, Philadelphia, Pa. 1990.
25. *Packaging and Thermoprocessing of Foods in Flexible Packages*, Military Specification MIL-P-44073E, U.S. Army Natick Research, Development and Engineering Center, Natick, Mass., 1995.
26. R. G. Beverly, J. Strasser, and B. Wright, "Critical Factors in Filling and Sterilizing in Institutional Pouches," *Food Technol.* **34**, 44–48 (1980).
27. R. A. Lampi et al., "Performance and Integrity of Retort Pouch Seals," *Food Technol.* **30**, 38–48 (1976).
28. W. T. Young, "Sealing," *Packaging* **23**, 200, 203–207 (1987).
29. G. L. Schulz and R. T. Mansur, "Sealing through Contaminated Pouch Surfaces," Technical Report No. 69-76-GP, U.S. Army Natick Development Center, Natick, Mass., 1969.
30. S. H. Spinak and R. C. Wiley, "Comparisons of the General and Ball Formula Methods for Retort Pouch Process Calculations," *J. Food Sci.* **47**, 880–884 (1982).
31. A. F. Badenhop, ed., *Proc. Conf. Using Retort Pouch Worldwide, Food Sciences Inst.*, Purdue University, Indianapolis, Ind., March 14–15, 1979.
32. A. A. Kopetz, C. A. Prange, and R. J. Flessner, "The Future Is in Our Hands: Critical Factors in Retort Pouch Thermal Process Assurance," *Activities Report, Research and Development Associates for Military Food and Packaging Systems, Inc.* **31**, 49–53 (1979).
33. R. Lingle, "Ameri Qual Foods: At Ease with MREs," *Prepared Foods* **158**, 144–146 (1989).
34. T. L. Heyliger, "Temperature Distribution Universal Sterilizer (Preliminary Studies)," Technical Memorandum No. 333, Food Research Department, Food Processing Machinery Division, FMC Corporation, Santa Clara, Calif., 1985.
35. W. T. Spencer and H. A. Bodman, "Nondestructive Testing of Packages of Thermoprocessed Foods: Final Report, Phase I (Draft)," Contract DAAG17-69-C-0013, U.S. Army Natick Laboratories, Natick, Mass., 1970.
36. J. D. Floros and V. Gnanasekharan, "Principles, Technology, and Application of Destructive and Nondestructive Package Integrity Testing," in R. K. Singh and P. E. Nelson, eds., *Advances in Aseptic Processing Technology*, Elsevier Science, London, 1991, pp. 157–188.
37. V. Gnanasekharan and J. D. Floros, "Package Integrity Evaluation: Part I, Criteria for Selecting a Method," *Packaging Technology and Engineering* **3**, 44–48 (1994).
38. V. Gnanasekharan and J. D. Floros, "Package Integrity Evaluation: Part II, Criteria for Selecting a Method," *Packaging Technology and Engineering* **3**, 67–72 (1994).
39. B. A. Blackistone and C. L. Harper, eds., *Plastic Packaging Integrity Testing*, Institute of Packaging Professionals and the Food Processors Institute, Herndon, Va., 1995.

RAUNO LAMPI
Westboro, Massachusetts

RHEOLOGY

Rheology is the study of deformation and flow of matter (1). In food applications, deformation is usually involved with assessing texture, while flow is generally associated with the property of viscosity (2). Generally texture is more loosely defined as "the response of the tactile senses to physical stimuli that result from contact between some part of the body and the food . . . a group of physical properties that derive from the structure of the food . . . properties expressed in terms of mass, distance and time only." In cheese grading, texture may refer to appearance while body may be the term used to denote rheological properties. Here, texture is used as a rheological term. Viscosity can be more rigorously defined as "the internal friction of a fluid or its tendency to resist flow." Often texture is used when describing the rheological properties of solid or semisolid foods such as cheese, fruit, bread, or cold ice cream, while viscosity is generally applied to foods that flow, such as pourable salad dressings, vegetable oils, syrups, or melted ice cream. Texture can also be used with a broader meaning, synonymously with rheology, including viscous properties of the food; this meaning of texture is especially common in describing semisolid foods such as ketchup, mayonnaise, or purées. Of the many sensory food attributes of concern to a food scientist, texture is the least well described (3). It has been observed that the consumer may also have difficulty describing texture but this difficulty does not lessen the importance of this attribute:

> When first asked about food texture, the consumer appears to exhibit very little spontaneous awareness. Flavor overshadows texture at the conscious level. People simply take the texture of a food for granted. . . . An average consumer may have difficulty in visualizing the concept of texture per se. . . . If the texture of a food is the way people have learned to expect it to be, and if it is psychologically and physiologically acceptable, then it will scarcely be noticed. If, however, the texture is not as it is expected to be . . . it becomes a focal point for criticism and rejection of the food. Care must be taken not to underestimate the importance of texture just because it is taken for granted when all is as it should be (4).

The science of rheology made significant advances since 1940 in the determination and explanation of the viscous and viscoelastic properties of polymers; this part of the field had its origins in the time of Newton and now has a solid theoretical and experimental basis (2,5,6). Analysis of the texture of foods is a more recent application of rheology. Modern food texture analysis could be said to have begun with the development of texture profile analysis (TPA, a scheme for measuring and classifying textural properties) (3,4,7–12). A texturometer (9), which evolved from a denture tenderometer built at MIT (13), has largely been replaced by tensile–compression testing machines made by Instron Corp. (14) and other similar instruments.

FLUID FOODS

The force required to make fluids flow depends on how fast they are made to flow. Conversely, the speed at which fluids flow depends on the force that is causing them to flow. The relationship between force and flow rate for a spreadable food such as mayonnaise can often be expressed as

$$\sigma = \sigma_y + m\dot{\gamma}^n \tag{1}$$

where σ is the shear stress (force per unit area) observed at a sheer rate $\dot{\gamma}$ (velocity gradient s^{-1}); σ_y (force per unit area) is a so-called yield stress, the minimum stress that must be applied to a material to initiate flow: and m and n are parameters characteristic of the material and are called the consistency coefficient and the flow behavior index, respectively. It has been argued that the concept of yield stress is not rigorously correct, that given enough time any material will flow under a small stress. In a real situation, as in consuming a food, time is not unlimited and many foods do exhibit a yield stress as will be seen in some of the following examples. The variables in equation 1 differ from simple force and flow rate in that the effects of geometry (of the instrument, pipeline, etc) have been taken into account.

Newtonian Flow

It is convenient to classify the rheological behavior of fluid foods according to their flow curves or rheograms, which are plots of shear stress against shear rate; that is, they are graphical portrayals of equation 1. Some liquid foods exhibit linear dependence of shear stress on shear rate, as shown in Figure 1 for 50 and 60% sucrose solutions. In the case of the sucrose solutions, there is no yield value and the flow curves are straight lines passing through the origin. Such fluids are called Newtonian after Newton who found that for such fluids equation 1 reduces to

$$\sigma = m\dot{\gamma}^1 \text{ or } \eta = \sigma/\dot{\gamma} \tag{2}$$

where m has been replaced by η, the Newtonian viscosity. As also shown in Figure 1, the viscosity of a Newtonian fluid is a constant and does not depend on shear rate. For most fluid foods, the ratio of $\sigma/\dot{\gamma}$ is not a constant, but changes (usually decreases) with increasing shear rate. Equation 2 may still be used to calculate an apparent viscosity of such materials at any shear rate.

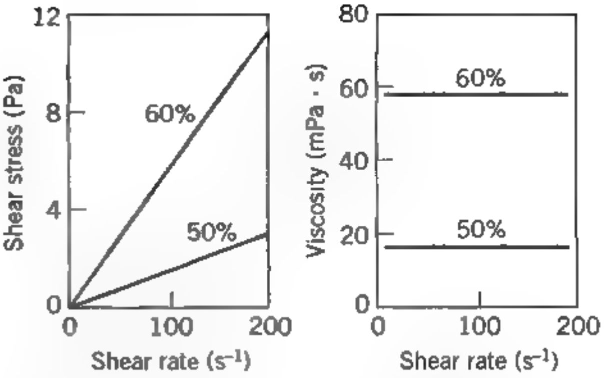


Figure 1. Rheograms for 50 and 60% sucrose solutions at 20°C showing ideal viscous flow.

Non-Newtonian Flow

Only a few fluid foods exhibit Newtonian flow behavior. Some syrups are approximately Newtonian over a limited shear rate range, but often their flow curves deviate from a straight line if the shear rate range is extended enough. Usually, syrups, oils, and most beverages do not have yield values even though they may not be Newtonian in their flow behavior. In the more general case, the flow behavior of a food is represented by equation 1 with a nonzero yield value. An example of such flow behavior is shown in Figure 2 for a tomato ketchup. This type of flow behavior is also shown by mayonnaise, viscous salad dressings, cheese sauces, and many other products. Products that exhibit decreasing apparent viscosity with increasing shear rate (ie, have a flow behavior index less than 1) are called pseudoplastic. If, as in Figure 2, the rheogram is curved and does not pass through the origin, the material is classified as pseudoplastic with a yield value. Non-Newtonian fluids that follow equation 1 (with or without yield values) are called power law fluids; however, many other equations have been developed to describe non-Newtonian flow. If the flow curve is a straight line intercepting the shear stress

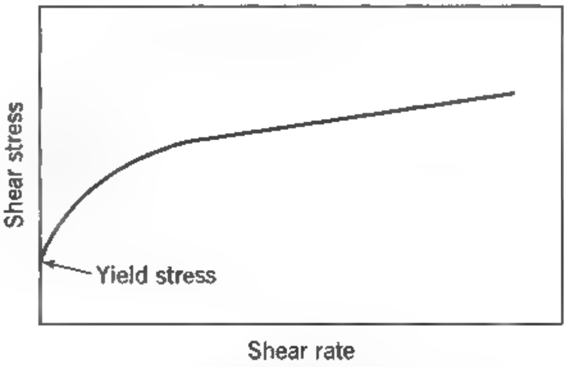


Figure 2. Rheogram for tomato ketchup plotted on arithmetic coordinates up to a shear rate of 500 s $^{-1}$.

axis at a nonzero value, then the material is said to be plastic, and if the rheogram is a linear function of shear rate, the material is said to show Bingham plastic behavior. Such behavior is unusual, although not unknown, in foods. Usually, the effect of shear is to break down the structure that may be present in a fluid at rest, so their apparent viscosity will decrease with increasing shear rate. This is especially evident in some gels that will flow like a normal fluid once the gel structure has been broken. Consumers commonly shake containers of products such as ketchup or Thousand Island salad dressing because the shearing makes such products more fluid.

A few products (some starch slurries, for example) show dilatant flow behavior, wherein the apparent viscosity increases with increasing shear rate. One explanation of non-Newtonian flow behavior is that long molecules or asymmetric particles may tend to align themselves so their long axes are parallel to the shear field and this orientation leads to a change in resistance to flow.

Some products are slow to recover from the effects of shearing, and under some circumstances some products may not recover at all. The time lag in recovering an initial structure after it has been broken by shear is the reason it is possible to pour ketchup following shaking the bottle. The shearing breaks the initial structure and it stays broken for a short time after the shearing has stopped. (If a bottle is completely filled, it may be difficult to apply much shear to the product and shaking may not initiate flow.) Such time dependence in recovering the initial structure is called thixotropy. If there is no recovery, the process is called rheodestruction. Dilatant fluids may also relax slowly to their unsheared (in this case, lower viscosity) initial state and that time dependence is called rheopexy. Shearing may be applied to a product at a fixed level for a variable amount of time, at levels that vary linearly as a function of time, or at any shear rate and time combination. While the process of applying shear to a product is somewhat arbitrary, for rigorous rheological analysis of the data, it is essential that the shearing history be exactly known; furthermore, certain controlled applications of shear to the product will give data that are easier to analyze, so in practice a specific, not arbitrary, shearing process is followed. Idealized rheograms for thixotropic and rheodestructive dispersions are shown in Figure 3 for processes in which a constant shear rate is applied for a fixed time and then stopped. Idealized rheograms for thixotropic and rheopectic fluids are shown in Figure 4 for shearing processes in which the shear rate is increased and then decreased linearly with time while recording the resultant shear stress. Instruments are commercially available for recording flow curves in this manner, using shear rate as the independent variable, or for recording shear rate as a function of shear stress. The choice of instrument type depends on the objective of the measurement.

Principles of Flow Measurement

The schematic diagram of a parallel plate viscometer shown in Figure 5 will be used to illustrate how rheological data are obtained. If a fluid is placed in the gap y between the parallel plates (each of area A) and a force F is applied to the (movable) upper plate, it will move with a velocity v relative to the (stationary) lower plate. The shear strain (γ) is a measure of the relative distortion of the sample (x/y, or in differential form, dx/dy); the shear rate, or more precisely the shear strain rate, is the change in strain as a function of time ($d\gamma/dt$ or dv/dy); and the shear stress is the force per unit area ($F/A = \sigma$); the apparent viscosity (defined earlier) is the ratio of shear stress to shear rate. The fluid next to the upper plate moves with it at a velocity v and the fluid next to the lower plate is stationary. Be-

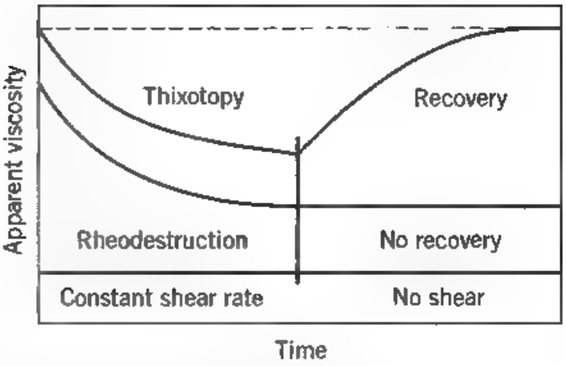


Figure 3. Idealized rheograms for thixotropic and completely rheodestructive dispersions.

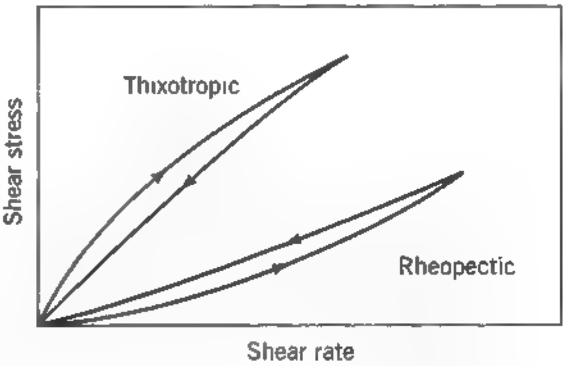
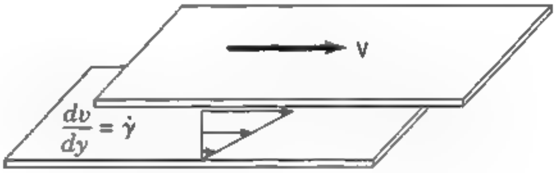


Figure 4. Idealized rheograms of time-dependent flow in continuous up curve and down curve experiments.



$$\text{Shear strain} = \frac{dx}{dy} = \gamma$$

$$\text{Shear rate} = \frac{d\gamma}{dt} = \dot{\gamma}$$

Figure 5. Velocity profile for an ideal viscous fluid in steady laminar shearing flow between two parallel plates.

tween the plates there exists a velocity gradient; for a Newtonian material the gradient is linear as shown in Figure 5 but the gradient will have a more complex shape for other types of flow behavior.

While parallel plate viscometers are available commercially, in practice this is sometimes not a convenient form of the instrument. Coaxial cylinder viscometers are more commonly used than are parallel plate viscometers. It is possible to imagine constructing a coaxial cylinder viscometer from a parallel plate viscometer by forming one of the plates into a cylinder and then shaping the second plate into a cylinder around the first one. Then one cylinder is rotated while the other is stationary and the fluid fills the gap between the cylinders. Parameters are obtained in basically the same way as discussed in the preceding paragraph for the parallel plate viscometer, except the factors that account for geometric effects are somewhat more complicated. A rotating bob immersion viscometer could be regarded as a simplified version of the coaxial cylinder viscometer in which the inner cylinder rotates and the outer cylinder has been replaced with the wall of the container. If the container is large enough, it can be assumed that the gap between the rotating bob and the container wall is infinitely large. In fact, with a 2-cm diameter bob, this assumption is fair even if the container is a medium size (say a 250-mL) beaker. If the assumption can be made, the data analysis is somewhat simplified. Such immersion viscometers are simple to use and very popular in industry. While it is more difficult to obtain rheologically rigorous parameters from immersion viscometers, their readings can be very useful in quality assurance and process control.

Temperature Effects on Flow

Temperature effects can be critical in rheological measurements. Some viscometers have a built-in means for controlling the sample temperature, such as a thermostatic jacket. Sometimes the measurements can be made in a room where the temperature is well controlled. At any rate, temperature control is necessary; this is illustrated in Figure 6, which shows the effects of temperature on the consistency coefficient of plain and salted liquid egg yolk. Note that the ordinate scale in Figure 6 is logarithmic, showing that the consistency coefficients for these samples decrease exponentially with increasing temperature, so a relatively small error in temperature control could lead to a much larger (proportionally) error in the rheological parameters. In general, the viscosities of Newtonian liquids decrease logarithmically as the temperature is raised, and this dependence can be expressed as an Arrhenius-type relationship:

$$\eta = Ae^{E/RT} \tag{3}$$

where E is the activation energy of flow (E is an energy barrier that must be overcome before the elementary flow processes can occur and is related to the coherence of the molecules in the liquid), A is a constant, R is the gas constant (8.314 J/K mole or 1.987 cal/K mole), and T is the absolute temperature (K) (6,15).

An Arrhenius-type plot is shown in Figure 7 for a 40% sucrose solution over a temperature range of 0–80°C. In this example, the activation energy of flow (E) is 23.8 kJ/mole. If data can be fitted in this manner, it is possible to use equation 3 to predict the viscosity of a Newtonian fluid at any temperature in the range. Moreover, because E represents molecular associations within a fluid, this may be a useful tool in the study of interactions in fluid systems, and their changes with the application of heat. (It should be remembered that equation 3 is an empirical equation and the activation energy is not derived from fundamental molecular theory, even though it is thought to represent molecular-level phenomena.)

For non-Newtonian fluids, temperature effects on flow behavior may be more complex. An approach to analyzing such effects was indicated by the egg yolk data (Fig. 6) where the temperature dependence of the consistency coefficient (m) was shown. Similarly, the temperature dependence of the flow index parameter (n) could be determined and, from equation 1, the apparent viscosity could be calculated at any temperature–shear rate combination; if the yield value were nonzero, it would also need to be determined as a function of temperature.

SOLID FOODS

Viscoelasticity

The rheology of solid foods is usually considered in the area of food texture (2,7,8,16,17). Most solid foods are viscoelas-

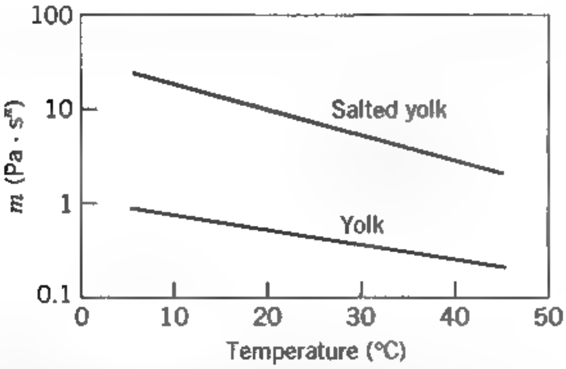


Figure 6. Effect of temperature on consistency coefficient for liquid egg yolk and salted yolk.

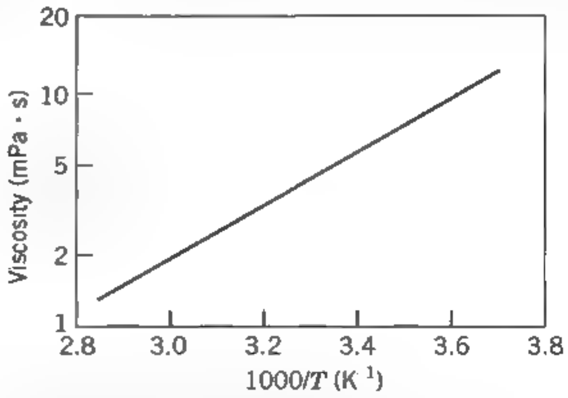


Figure 7. Arrhenius-type plot of viscosity for 40% w/w sucrose solutions between 0 and 80°C.

tic, that is, they exhibit viscous flow behavior (as discussed), but also behave somewhat as elastic solids. The viscoelastic aspect of rheological behavior is particularly relevant to material response in situations of unsteady motion, that is, when stresses or strains are changing with time. Examples of this are cutting, mashing, chewing, and swallowing of solid materials, and mixing, pouring, spreading, or pumping of fluid systems.

Elasticity can be defined as that property of a food by virtue of which, after deformation and on removal of stress, it tends to recover part or all of its original size, shape, or both (18). Clearly many products, such as cheese, are elastic if the stresses are small, but if the stress is high so that the product is crushed to only a fraction of its original height, for example, then there will be little recovery. The limit of stress, beyond which a food loses its elasticity, is called the elastic limit. On mastication the elastic limit of any food will be exceeded, but the elasticity of many foods (such as Mozzarella cheese, marshmallows, gelatin desserts) is an important sensory aspect of the food. Products, such as margarines, which tend to flow when the elastic limit is exceeded are viscoelastic in their rheological behavior. A food's viscoelasticity can be measured and expressed in terms of its storage and loss moduli. The storage modulus G' is a measure of elasticity, or energy stored, and the loss modulus G'' is a measure of viscosity, or energy lost, by a food when it is subjected to stress and strain (19). The ratio of the loss and storage moduli is called the loss tangent.

$$\tan\delta = G''/G' \tag{4}$$

Viscoelastic parameters depend on food composition and temperature, so, as with other rheological measurements, the sample and test conditions must be described in detail (8).

Principles of Measuring Viscoelastic Properties

There are different techniques for measuring viscoelastic properties of foods, but probably the most commonly used is dynamic testing wherein linear viscoelastic response to very small oscillatory shear is observed. Idealized dynamic responses of elastic, viscous, and viscoelastic systems to sinusoidal oscillatory shear are shown in Figure 8. In such tests, the strain γ is a sinusoidal function of time t:

$$\gamma = \gamma_0 \sin(\omega t) \tag{5}$$

where ω is the oscillatory frequency and γ_0 is the maximum strain amplitude. The strain rate, or shear rate, will be the first derivative of strain with respect to time:

$$d\gamma/dt = \dot{\gamma} = \omega\gamma_0 \cos(\omega t) \tag{6}$$

If the food behaves as an ideal elastic (Hookean) solid, stress is directly proportional to strain:

$$\sigma = k\gamma_0 \sin(\omega t) \tag{7}$$

but in an ideal viscous (Newtonian) fluid, the stress is directly proportional to shear rate:

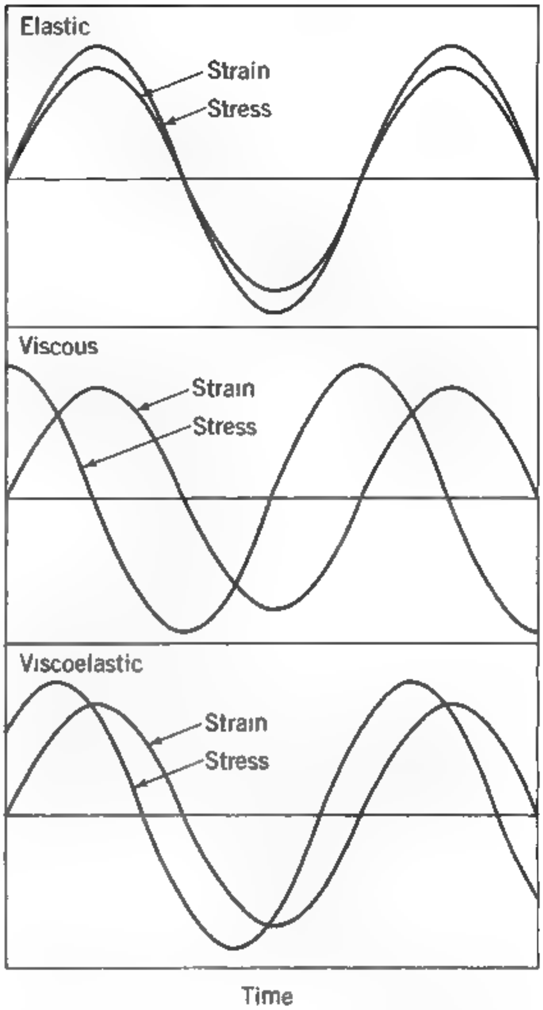


Figure 8. Idealized dynamic response of elastic, viscous, and viscoelastic systems to sinusoidal oscillatory shear.

$$\sigma = \eta\omega\gamma_0 \cos(\omega t) \tag{8}$$

Most foods exhibit both viscous and elastic properties at the same time and will have a viscoelastic stress response that combines the features of equations 7 and 8:

$$\sigma = \gamma_0[G' \sin(\omega t) + G'' \cos(\omega t)] \tag{9}$$

where the moduli G' and G'' are as defined previously. On softening a food (increasing the temperature of a cheese or increasing the water content of a dough, for example) the food will become more liquidlike and less solidlike and its loss tangent (G''/G', eq. 4) will increase. Such objective characterization, putting numbers on what is observed sensorily, can be very valuable in product development and in quality control.

Texture Profile Analysis

Dynamic testing, as described in the preceding paragraph, can give a rigorously correct rheological characterization of a food, and it may be possible to correlate viscoelastic parameters with fundamental, molecular-level properties. The analysis of dynamic testing data becomes highly complex if the elastic limit of the product is exceeded. As indicated earlier, mastication almost always exceeds the elastic limit of solid or semisolid foods, and so do many food processing operations. A rheological method was needed to imitate the mastication (chewing) process and still provide objective data; therefore, texture profile analysis (TPA) was developed in response to this need.

In a TPA measurement, a food sample of specific dimensions is compressed, the compressive force is removed and the sample is compressed again. This two-bite sequence is imitative of the chewing process. During the test, the amount of compression (distance) and compressive force are recorded. The resultant force vs distance plot (sometimes compressive stress, force per unit area, is used instead of force) is called a TPA curve. An Instron TPA curve for Cheddar cheese is shown in Figure 9. In that measurement, a cylinder of cheese 25 mm high and 21 mm in diameter was compressed 20 mm (80%) until it was only 5 mm high. Then the platen was lifted off the cheese and lowered again to the same point. Several TPA parameters may be derived from the TPA curve: the maximum force H, which occurs at the end of the first compression, is called the hardness; the force of the first maximum F is called the fracturability (not every food shows a fracturability peak); the work done to compress the sample on the "first bite" is given as the area A_1, and on the second bite, A_2, and the ratio A_2/A_1 is called the cohesiveness C; the distance S is called the springiness; the negative or tensile area A_D is the adhesion or stickiness; gumminess G is the product of hardness times cohesiveness; and chewiness is the product of hardness times cohesiveness times springiness. One advantage of the TPA method is that the parameters derived from it have been shown to correlate with sensory perception; it really is a way to replace or supplement subjective evaluation of foods with numbers and so has proven invaluable in product characterization and development.

FUTURE DEVELOPMENTS

TPA parameters depend on experimental conditions; compression rate, temperature, sample size, and nature of the platen surfaces. These conditions have not been standardized between laboratories and, until they are, it will remain difficult to compare TPA data obtained by different investigators. Such standardization is an urgent need.

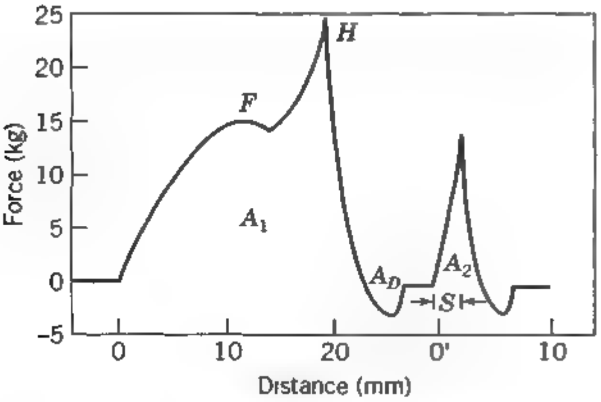


Figure 9. Instron TPA curve for cheddar cheese.

In recent years, significant progress has been made in correlating sensory and objective evaluation of food rheology; the texts cited in the bibliography all include information on sensory–instrument correlation. Much work is still needed in this area. The effects of temperature, composition, and other variables on food rheology can be extremely complicated, and consequently have not been extensively investigated. Work is needed in this area, especially with the objective of drawing general conclusions that can be applied to a variety of foods.

Rheological instruments for in-line measurement and control of food processes are generally not completely satisfactory. Instruments are needed in this area, particularly to handle non-Newtonian, viscoelastic systems, and the influence on results caused by temperature variations. Such instrumentation must be designed to permit measurement of complex rheological properties that will be consistent with the processing or sensory conditions of interest, and to comply with good manufacturing practice regulations.

Correlations between food rheology and microstructure and molecular interactions have rarely been entirely successful. Rheological behavior and microstructure are expressions of molecular and structural organization within a food that should be further explored by these methodologies to address both fundamental and applied challenges in food science. This is an area that should receive more attention.

BIBLIOGRAPHY

1. S. L. Passman, *J. Rheology* **28**, 663 (1984).
2. M. C. Bourne, *Food Texture and Viscosity: Concept and Measurement*, Academic Press, Inc., Orlando, Fla., 1982.
3. A. S. Szczesniak, *J. Food Sci.* **28**, 385 (1963).
4. A. S. Szczesniak and E. L. Kahn, *J. Texture Studies* **2**, 280 (1971).
5. J. D. Ferry, *Viscoelastic Properties of Polymers*, 3rd ed., John Wiley & Sons, Inc., New York, 1980.
6. J. R. Van Wazer, J. W. Lyons, K. Y. Kim, and R. E. Colwell, *Viscosity and Flow Measurement: A Laboratory Handbook of Rheology*, Wiley-Interscience, New York, 1963.
7. A. Kramer and A. S. Szczesniak, eds., *Texture Measurements of Foods*, Reidel Publishers, Dordrecht, The Netherlands, 1973.
8. P. Sherman, ed., *Food Texture and Rheology*, Academic Press, Orlando, Fla., 1979.
9. H. H. Friedman, J. E. Whitney, and A. S. Szczesniak, *J. Food Sci.* **28**, 390 (1963).
10. A. S. Szczesniak, M. A. Brandt, and H. H. Friedman, *J. Food Sci.* **28**, 397 (1963).
11. A. S. Szczesniak, *J. Food Sci.* **28**, 410 (1963).
12. A. S. Szczesniak, *J. Texture Studies* **6**, 5 (1975).
13. B. E. Proctor, S. Davison, G. J. Malecki, and M. Welch, *Food Technol.* **9**, 471 (1955).
14. M. C. Bourne, *J. Food Sci.* **32** (1967)

15. I. Tinoco, Jr., K. Sauer, and J. C. Wang, *Physical Chemistry: Principles and Applications in Biological Sciences*, Prentice Hall Press, Englewood Cliffs, N.J., 1978.
16. H. R. Moskowitz, ed., *Food Texture: Instrumental and Sensory Measurement*, Marcel Dekker, Inc., New York, 1987.
17. M. Peleg and E. B. Bagley, eds., *Physical Properties of Foods*, AVI Publishing Co., Inc., Westport, Conn., 1983.
18. J. M. deMan, P. W. Voisey, V. F. Rasper, and D. W. Stanley, eds., *Rheology and Texture in Food Quality*, AVI Publishing Co., Westport, Conn., 1976.
19. H. Faridi and J. M. Faubion, eds., *Fundamentals of Dough Rheology*, American Association of Cereal Chemists, St. Paul, Minn., 1986.

DAVID N. HOLCOMB
Kraft General Foods Technology
Glenview, Illinois

MARVIN A. TUNG
Technical University of Nova Scotia
Halifax, Nova Scotia
Canada

S

SALAD OILS. See FATS AND OILS: PROPERTIES, PROCESSING TECHNOLOGY, AND COMMERICAL SHORTENINGS.

SALMONELLA. See FOODBORNE DISEASES.

SANITIZERS. See DISINFECTANTS.

SAUSAGES

Sausages are a diverse group of foods made from ground or comminuted meats, salt and spices. They originated during prehistoric times when our ancestors discovered that addition of salt and drying would delay spoilage. Adding various spices improved palatability and stuffing them into intestines enhanced convenience. Homer mentions sausages in the Odyssey and the word, sausage, come from Latin meaning salted or meat preserved by salting. Over the centuries virtually every culture developed distinctive sausages depending on their live stock, spices, climate, and culture. The European origin of most of our sausages is indicated by the city names associated with them: Bologna, Genoa, Braunschweig, and Thuringen. Dried sausages originated in southern Europe where warm climates required a more spoilage-resistant sausage. Northern Europeans, having a cooler climate, created cooked and summer sausages. Americans contributed Lebanon bologna and the popularization of the hot dog.

Sausages are an excellent source of high quality proteins. In addition, they contribute iron, zinc, and B vitamins, particularly folic acid, B_6 and B_{12}. Controversy has existed over the addition of nitrite and possible subsequent formation of nitrosamines, sodium content and fat levels, especially saturated fats.

Americans manufactured 6.0 billion pounds of sausages in 1987, 10% of the total amount of all red meats. Frankfurters amounted to 1.5 billion pounds, bologna 640 million, uncooked cured sausages 24 million, dried and semidried 445 million, liver sausages 96 million, loaf products 1.3 billion, and other sausage and loaf products 1.0 billion pounds. Poultry based luncheon meats totaled 151 million pounds and poultry frankfurters were 280 million pounds.

Classification of sausages is not clear cut because of the many variations in materials, spices and processes used. Meat is ground or finely chopped (comminuted) and mixed with salt and spices. It may have curing salts added and may then be stuffed, smoked, cooked, fermented, or dried. The United States Department of Agriculture classifies sausages as fresh; uncooked-smoked; cooked-smoked; cooked; dry/semi-dry; and luncheon meats, loaves, and jellied products.

Fresh sausages are made from uncured (no nitrate or nitrite added) meats, usually pork. They are ground and mixed with salt and spices, then stuffed into chubs, natural casings or manufactured casings. They have a limited shelf-life even when refrigerated and must be cooked before serving.

Uncooked-smoked sausages (country-style sausage and kielbasa) are usually cured, require refrigeration for preservation and are cooked before serving.

Cooked-smoked sausages (frankfurters and bologna) are cured and cooked in casings inside a smokehouse. When properly refrigerated they have a long storage life and do not require final cooking by the consumer.

Cooked sausages (liver sausage and Braunschweiger) may be cured and are usually comminuted, seasoned, stuffed, and cooked. They are generally consumed cold.

Dry/semi-dry sausages utilize bacteria to ferment sugars into lactic acid. This increases the sausages' acidity and contributes to their characteristic flavor and to their bacterial preservation. They are then dried to varying extents. These sausages do not need cooking before serving. Semidried sausages (summer sausages, cervelat, thuringer) need refrigeration for optimal storage. Dry sausages (hard salami, Genoa salami and pepperoni) need little or no refrigeration. Lebanon bologna is fermented and smoked but not appreciably dried.

The category of luncheon meats encompasses a wide variety of popular products. Typically a mixture of chopped meats with extenders and other ingredients is processed into a loaf form. Pimento loaf, olive loaf, and honey loaf are examples. Also included in this group are sandwich spreads, which are cooked, soft mixtures packaged in chubs. Jellied products, such as jellied tongue, souse and head cheese, consist of chunks of cooked and cured meats held in a loaf form by gelatin.

INGREDIENTS

Meats

Beef and pork are the primary meats, but goat and sheep are used by several cultures and fish sausages are consumed in the Orient. Poultry luncheon meats and frankfurters are increasing in popularity. Skeletal muscle has the highest binding and water holding properties compared to other meats and non-meat extenders. Other meats (lips, tripe, cardiac muscle), variety meats (tongue, livers, pork stomachs, etc) and mechanically deboned meats must be labelled if used. Pork trimmings and backfat are important ingredients, because too little fat results in a dry, unpalatable product. Maximum levels of fat for different sausages are regulated by the U.S. Department of Agriculture; for example, frankfurters must have less than 30% fat.

Salt

Salt is necessary for extracting the meat proteins to bind fat and water and to form the sausages' texture. Salt also contributes to the sausages' flavor and retards microbiological growth. Most sausages contain 2.5 to 3.0% salt, although fresh sausages contain only about 0.75%. To reduce the sodium content, potassium chloride may be substituted for salt (sodium chloride). The practical limit to the amount of potassium chloride that can be added is half of the total salt because of the bitter taste of the potassium. Addition of phosphates is another way to improve the binding and water holding of the proteins. Pyrophosphates, tripolyphosphate, and metaphosphates may be added to a combined maximum of 0.5%.

Curing Salts

Originally part of natural salts, sodium and potassium nitrate and nitrite causes formation of the cured meat color and flavors. The nitrate is converted by naturally occurring bacteria to nitrite, which in turn forms nitric oxide. This binds to the heme iron of myoglobin to form the pink color of cured meats. Nitrite also contributes to the flavor of cured meats and retards rancidity. Nitrite inhibits the outgrowth of *Clostridium botulinum* spores; growth of these bacteria can produce a potentially fatal toxin. Nitrite addition is strictly limited because of the potential for formation of mutagenic nitrosamines. Use of sodium ascorbate or erythorbate prevents formation of nitrosamines by competing with secondary amines for nitrite, speeds the curing reactions, and stabilizes the cured meat color.

Spices

The flavor of a particular sausage is strongly dependent on the spices added; every sausage maker has a special mixture. Sage gives the flavor to fresh breakfast sausages; white pepper, mustard, coriander and nutmeg flavor frankfurters; garlic is added to Italian sausages; and cayenne pepper, paprika and garlic flavor pepperoni.

Sugars

Sucrose (common sugar), dextrose, corn syrups, or sugar derivatives may be added to many sausages. About 1% is necessary in fermented sausages for growth of the lactic acid bacteria. Sugars contribute to the flavors by their sweetness, participation in browning flavors, and masking (mellowing) the saltiness.

Water

Often added as ice to chill the meats, addition of water permits more efficient chopping and mixing, aids in dissolving the salts, and improves the fluidity of the mixture for proper filling of the casings. The texture of sausages is greatly enhanced by having the proper amount of moisture.

Additives

Acidulents, such as glucono-delta-lactone, are occasionally added to increase the acidity rapidly. This accelerates the curing reaction in quickly processed products such as frankfurters, or controls the growth of undesirable microorganisms in fermented sausages until fermentation occurs. Soy proteins, milk proteins (casein and whey), yeast protein, flours, and starch are permitted in limited amounts in some sausages where they improve emulsion stability, fat and water binding, flavor, cooking yield, and textural characteristics. Antioxidants to retard rancidity and mold inhibitors are added to some products. All of these additives must be declared on the label if they are used.

PROCESS

Grinding and Comminuting

The meat for fresh and fermented sausages is ground through a grinder and then mixed with salt and any other ingredients. Some loaf and luncheon meat products use chunks of meat bound together by the extracted meat proteins that coat the surface after mixing with salt and water.

Frankfurters and bolognas are comminuted or finely chopped with the salt and ice to extract/solubilize the proteins. Then the fat and remaining ingredients are added and chopped further to form a smooth batter.

Casings

The sausage mixture or batter is stuffed into casings of various types, sizes and shapes for further processing, storage, and consumer convenience. Originally natural or animal casings made from stomachs, intestines and bladders of cattle, hogs and sheep were used, and are still associated with many traditional, high quality sausages. They are in limited supply and highly perishable. They are less uniform in size, more fragile and require more care during stuffing than manufactured casings. The latter are either cellulose from cotton linters (cotton by-products) or collagen from the corium layer of beef hides. Cellulose casings are available in many sizes, strengths, and properties. Small types are used to cook frankfurters in a smokehouse and then removed before packaging. Large fibrous cellulose casings, which remain on luncheon meats and fermented and dried sausages, are removed by the consumer. Small collagen casings can be consumed and are used for breakfast and other uncooked sausages.

Stuffing and Linking

The sausage mixtures and frankfurter batters can be pumped through stuffers by either impeller or piston pumps. The casings are placed over a stuffing horn and the sausage extruded into the casing. Links are formed by twisting the casing or by using string ties or metal clasps.

Fermenting and Drying

Originally the bacteria for fermented meats were natural contaminants of the meats, equipment and facilities. Small amounts of successfully fermented products were often added back to new batches to start the fermentation. Most manufacturers now add cultures of selected bacteria and a fermentable sugar and then hold the mixture at 70–82°F and 80% relative humidity for 2–3 days to ensure a desirable fermentation. The bacteria produce lactic acid which gives the sausages their tangy flavor and reduces the pH to 4.6–5.4, aiding preservation. The cured color also forms at this time. The sausages then go into the drying room where they are held at 45–55°F and 69–72% relative humidity. Semi-dry sausages are dried for 10 to 21 days and contain 50% moisture. Dry sausages are dried for 60 to 120 days and contain only 35% moisture.

Smoking and Cooking

Smoke is no longer as important as a preservative, but it contributes to flavor, color, and microbial preservation. Controlled burning of hardwood chips produces a mixture of organic acids, carbonyls, and phenols. The acids cause surface coagulation of the proteins forming a skin on the product. Carbonyls contribute a brownish color and phenols plus some carbonyls add the smoke flavors. Many continuous and large volume processes use an aerosol of liquid smoke which is more consistent and easier to use. It may also be added directly as a flavoring or applied to the surface. In addition, liquid smoke does not contain any of the carcinogenic polycyclic compounds that may be formed if the burning temperatures are too high.

Frankfurters are smoked during the beginning of a cooking sequence. The temperature is raised in steps with humidity control and good circulation until the internal temperature reaches 155–160°F. The frankfurters are then showered with cold water to cool.

Packaging

Most sausages are sold in the casings they were stuffed into. Frankfurters, however, are stripped of the cellulose casing after cooking in the smokehouse. Many sausages are vacuum packaged to extend shelf life by preventing water loss, retarding oxidation, and slowing microbial growth.

Developments

Sausages continue to have great popularity because of their appealing flavor and convenience. The increasing variety available in the retail stores reflect consumer interest in convenience and specialty foods. Salt levels have been reduced although many sausages still contain high amounts of sodium. Research is underway to reduce or replace the animal fat while retaining the expected flavor and textural feel of the products. Meat inspection and quality assurance programs are under revision as the inspection agencies include more microbial and residue testing with the traditional visual inspection. Hazard analysis-critical control point (HACCP) procedures are particularly important in controlling pathogenic microorganisms such as *Salmonella, Staphylococci* and *Listeria*.

GENERAL REFERENCES

H. W. Ockerman, *Sausage and Processed Meat Formulations*, AVI Publishing Co., Inc., Westport, Conn., 1989.

A. M. Pearson and F. W. Tauber, *Processed Meats*, 2nd ed., AVI Publishing Co., Inc., Westport, Conn., 1984.

R. E. Rust, "Sausage Products," in J. F. Price and B. S. Schweigert, eds., *The Science of Meat and Meat Products*, 3rd ed., Food & Nutrition Press, Inc., Westport, Conn., 1987.

U.S.D.A., *Agriculture Statistics*, U.S. Government Printing Office, Washington, D.C., 1988.

R. C. WHITING
A. J. MILLER
United States Department of Agriculture
Philadelphia, Pennsylvania

SEAFOOD: FLAVORS AND QUALITY

BACKGROUND

With few but important exceptions, the majority of seafoods are relatively mild in flavor intensity when compared to foods prepared from beef, poultry, pork, and lamb. Indeed, American and European consumer preferences for seafoods seem to favor varieties that have mild flavor profiles, for example, various white-fleshed species such as soles, cods, snappers, and rock fishes (1). Because these flavors are so subtle, they can be overwhelmed by very small quantities of exogenous or endogenous flavors due to contamination or spoilage, such as fuel oils, rancidity, ammoniacal, acetic acid, and earthy flavors. In addition, because of this subtlety and low intensity, the presence of flavor enhancers and modifiers, such as monosodium glutamate or 5′-ribonucleotides (disodium 5′-inosinates[5′-IMP] and 5′-guanylates[5′-GMP]), can exert enormous and profound influences on the perception of quality of a seafood. Added to this complex interplay is the creation of new flavors that can occur during processing that are identified with the final product, for example, canned salmon flavor, that may or may not have been present in the original seafood.

For the purposes of this review, some definitions and discussion of flavor and quality are in order. We will use the description of flavor put forward by Hall (2) and Chang (3). Hall (2) defined flavor as the "sum of those characteristics of any material taken in the mouth, perceived principally by the senses of taste and smell, and also by the general pain and tactile receptors in mouth, as reviewed and interpreted by the brain." The reader is particularly directed to a recent review by Kinsella (4) for a biochemical/biophysical discussion of flavor and the role of binding of the flavor compounds to physiological sites. Although Chang (3) and Hall (2) identify three components to flavor (taste, odor, and "pain-tactile"), we will limit our discussion of seafood flavors to only taste and odor. The pain-tactile responses of pungency, heat, cooling, or other nonspecific or trigeminal neural responses are not *directly* associated with commonly consumed seafoods themselves but are usually associated with external materials added to a finished seafood, such as condiments, peppers, herbs, and spices.

When a seafood, or any food, for that matter, is consumed, a series of conscious and subconscious responses to flavor and texture stimuli are integrated into a single unified perception. Because of this facility of the brain to integrate many stimuli into a single unifying concept, the definition of seafood quality becomes elusive and difficult. Typically, discussions of quality usually end with the statement, "but I'll know it when I see it." Therefore, any attempt to discuss seafood quality objectively must include a compilation of various measurable physical, chemical, and sensory attributes that have been correlated with predetermined quality assessments.

The underlying assumption of all quality assessment or assurance is that if a series of standards are met upon analysis, the product under evaluation is then considered to be of a certain level of quality. Unfortunately, these standards defining levels of quality (eg, excellent, good, bad, poor, decomposed, or passable) for measured attributes have been difficult to develop. Part of the reason is that the human perception of quality in seafoods is multifactorial, comprising a large number of attributes that are integrated in a *judgmental* response. This judgmental response, particularly in the evaluation of sensory attributes such as flavor and texture, can be strongly influenced or biased by personal, cultural, and societal factors. For example, some popular fermented fish/vegetable sauces would be assessed as spoiled or rotten by some palates yet be considered highly desirable by others. In short, what is "spoilage" to one is "fermentation" to another. Because of these cultural differences, it is sometimes difficult to arrive at a precise and consistent international set of standards, particularly for attributes that are the result of sensory judgment.

Today a great reliance is still placed on subjective assessments of quality in seafoods, such as presence of decomposition or quality grades. Inspectors and graders, in both government agencies and the seafood industry, are trained to various degrees in sensory techniques to conduct these evaluations. In many cases, potentially large sums of money and the health and safety of many consumers may ride on the nose, eyes, and hands of a few seafood inspectors (in some cases, only one inspector). Clearly, it would be desirable to minimize reliance on these subjective methods with all their inherent drawbacks, namely, fatigue, boredom, and sensory overload, and use chemically and physically measurable indicators of seafood quality.

Therefore, due to the elusive and complex nature of quality as it applies to seafood, the emphasis of this review will be on specific identifiable flavor/odors and their relationship to quality insofar as information or standards may exist. Our approach in this chapter will be to first identify those components that can be identified as specific flavors/odors that characterize specific seafoods. We will then discuss various modifiers and enhancers that influence the integration of the basic flavor components. Finally we will discuss various efforts to use seafood flavors to classify fish species. We feel that this last section is necessary because of the large number of marine species that complicate the seafood market. Recently, various efforts have been made to simplify the marketing and retailing of seafoods due to the overwhelming array of species that can confront the consumer. These efforts range from species groupings derived from statistical analysis of trained sensory panels to categories developed by consumer groups or knowledgeable seafood-industry personnel. Therefore we have divided the review into three sections:

1. Chemical compounds responsible for specific flavors found in seafoods.
2. Compounds responsible for modifying and/or influencing seafood flavors.
3. Attempts to use seafood flavors and texture attributes to classify fish.

Though the major portion of this chapter will involve a discussion of seafoods, that is, fishery products taken from the marine environment, we will include, of necessity, many products that derive from freshwater sources due to the enormous growth of aquaculture (eg, catfish, trout, and salmon) and the introduction of anadromous marine species (Coho salmon, *Oncorhynchus kisutch*; pink salmon, *Oncorhynchus gorbuscha*; chinook salmon, *Oncorhynchus tshawytscha*) into freshwater systems such as the Great Lakes. In fact, some of the more serious flavor problems arise in aquaculture systems due to feed components and environmental sources such as algae. In addition, the tremendous growth of marine aquaculture in the past 10 years has introduced similar problems to seawater-raised species.

Clearly, significant use of sensory-evaluation techniques are employed in seafood flavor and odor research. It is not our intent to review basic sensory methodologies and associated techniques and utilities; to that end the reader is encouraged to examine several excellent texts by O'Mahony (5), Jellinek (6), Warren and Walradt (7), and Boudreau (8). The book by O'Mahony (5) is particularly recommended for its statistical methods and procedures used in sensory evaluation. For developing sensory panels and programs, the reader is especially directed to the series of monographs published by the American Society for Testing and Materials (ASTM) for particularly useful training techniques and requirements of sensory panels (9–15). Recently, an excellent monograph on "Flavor and Lipid Chemistry of Seafoods", the result of an American Chemical Society symposium, has just appeared (16).

CHEMICAL COMPOUNDS RESPONSIBLE FOR FLAVORS FOUND IN SEAFOODS

Quality Flavor Attributes

Chemical compounds responsible for seafood flavors can be of either an endogenous or exogenous origin. Endogenous compounds are those that are present in fish and shellfish as the result of their inherent metabolism, whereas exogenous compounds arise from the diet, from immediate contact with the aquatic environment, for example, skin absorption and adsorption, or from both. The common endogenous compounds usually found in fish give rise to the characteristics that distinguish one fish species from another, such as salmon from sole, and are responsible for our perception of seafood quality attributes such as fresh, stale, and rancid. On the other hand, exogenous compounds are present accidentally in the fish, commonly arising from the environment, and are usually considered taints or contaminants.

Up until the 1970s high-resolution gas chromatography (HRGC) and mass spectrometry (MS) were available as expensive research tools only to well-financed institutions or corporations. With the advent of new technologies in engineering, manufacturing, and computers, HRGC and MS came within the reach of many research laboratories, and as a result came a rapid increase in flavor and odor research. Before the use of these new techniques, research into odors and flavors associated with fishery products were relegated largely to "scratch-and-sniff" techniques. Although these early techniques may appear crude and unsophisticated by today's standards, they were in their limited way remarkably productive and accurate. Many of these early studies were based on the abilities of very perceptive sensory analysts who could detect various odors and flavors present in a food and then search for simple chemical compounds that duplicated the flavor or odor. Usually for these studies the chemical compounds were selected on their probability of being present in the food, perhaps due to oxidative breakdown of fats and oils, spoilage, or other possible biochemical changes. These techniques (so-called "P and P," or postulate and prove, and "I and I," or isolate and identify; see Forss [17], p. 191) were similar to those used in the fragrance industry in the formulation of duplicate perfumes.

To overcome some of these past empirical approaches, Stansby and Jellinek (18) attempted to place fish odors and flavors research on a more rational but still descriptive basis. They classified nine types of flavors or odors: A, amine; B, burnt; F, freshwater-fish; G, green; I, iodine; N, natural species; O, pure oxidation types; P, putrid; and S, sweet. These descriptive terms are still in use today in the search for the responsible chemical compounds in seafood flavor and odor research. During this study, Stansby and Jellinek also noted that the green flavor of fish oils was similar to that of *cis*-3-hexen-1-ol and *cis*-3-hexenal, the latter found in the reversion of soybean oil (19). Other odors identified in their studies was that of green cucumber, similar to that of hex-2-enal and *trans*-2-*cis*-6-nonadienal (2,6-nonadien-1-al) found by Forss et al. (20).

As will be seen from the preceding brief historical perspective in research of flavors and odors in fishery products, these early studies were remarkably accurate in identifying specific flavor compounds.

In terms of quality, what is a fresh fish? In a redundant way, a fresh fish has a fresh-fish flavor or odor. What compound or compounds are responsible for the fresh-fish aroma? At the same time, poor-quality fish are said to have "fishy" odors and flavors. This section will cover the compounds responsible for fresh-fish flavor/odor, "fishy" flavors, rancidity/scorch flavors, and other miscellaneous flavors.

Fresh-Fish Flavor/Odor. After catch, a series of metabolic changes begin in the tissues of fish and shellfish (21,22). Part of the catabolic changes is the formation of odors indicative of decomposition. In general, the odor of a fillet removed immediately from a landed fish has a "fresh" odor; with time this odor disappears and "sweet" (perfumelike) odors begin to appear (23). Eventually, depending on holding conditions and length of time, odors such as fruity, sour, and putrid develop (24–26). However, the disappearance of the fresh-fish aroma is usually the first sign of a quality change in the fish and has been a subject of considerable interest to flavor/odor investigators (27).

Josephson et al. (28–30) found a variety of odorous compounds (Table 1), but three families of compounds were major contributors to the characterization of Lake Michigan whitefish (*Coregonus clupeaformis*): C_6 aldehydes and alcohols contributed green and plantlike aromas (eg, 2-hexenal and 1-hexanol), C_9 aldehydes and alcohols contributed cucumber and melon aromas (eg, 2-nonenal, 2,6-nonadienal,6-nonen-1-ol, and 3,6-nonadien-1-ol), and the C_8 ketones and alcohols provided a characteristic mushroom aroma (eg, 1-octen-3-one, 1,5-octadien-3-one, 1-octen-3-ol, 1,5-octadien-3-ol, 2-octen-1-ol, and 2,5-octadien-1-ol). In this work, they found that increasing amounts of corresponding alcohols (1,5-octadien-3-ol and 3,6-nonadien-1-ol) and a concomitant decrease in carbonyls (1,5-octadien-3-one and 3,6-nonadienal) was associated with the change in aroma from that of very fresh whitefish to aromas that were characterized as "suppressed, flat, and sweet melonlike." Josephson et al. (27) found that greater losses of fresh-fish carbonyl compounds were observed in fish held under air (2°C, 7 days) than under a carbon dioxide atmosphere.

In a study comparing freshwater species (whitefish [*C. clupeaformis*], smelt [*Osmerus mordax*], black crappie [*Pomoxis nigromaculatus*], bluegill [*Lepomis macrochirus*], muskellunge [*Esox masquinongy*], perch [*Perca flavescens*], northern pike [*E. lucius*], ciscoe [*Coregonus artedii*], walleye pike [*Stizostedion vitreum*], sauger [*Stizostedion canadense*], rainbow trout [*Oncorhynchus mykiss*], and emerald shiners [*Notropis atherinoides*]) and saltwater species (ocean perch [*Sebastes marinus*], cod [*Gadus morhua*], petrale sole [*Eopsetta jordani*], and haddock [*Melanogramus aeglefinus*]), Josephson et al. (29) found a common occurrence of hexanal, 1-octen-3-ol, 1,5-octadien-3-ol, and 2,5-octadien-1-ol, whereas freshwater species contained in addition 1-octen-3-one and 1,5-octadien-3-one. Interestingly, Whitfield et al. (36) found that (Z)-1,5-octadien-3-ol and 1-octen-3-ol were involved in the metallic off-flavor of deep sea prawn (*Hymenopenaeus sibogae*) and sand lobster (*Ibacus peronii*). Also Berra et al. (37) found that (E,Z)-2,6-nonadienal was responsible for the cucumber aroma of Australian grayling (*Prototroctes maraena*).

It is postulated that the origin of these fresh-fish flavor/odor compounds is the enzymes and nonenzymic oxidation of polyunsaturated lipid fractions in the flesh (28,29,38). Josephson et al. (39) found that the formation of carbonyls and alcohols that characterize the fresh-fish aroma of emerald shiners (*N. atherinoides*) were almost completely inhibited when the fish were sacrificed and then immediately exposed to acetylsalicylic acid (aspirin) or stannous chloride, both specific inhibitors of fatty acid cyclooxygenase and lipoxygenase, respectively. They concluded that these observations implicated the involvement of enzymic conversions of ω-6 and ω-3 fatty acids to the development of volatile aroma compounds in fresh fish. Josephson et al. (39) found that the highest concentrations of volatiles from

Table 1. Volatile Compounds Identified in Fresh Whitefish (*C. clupeaformis*)

Peak no.	Compounds	I_E	Estimated concentration[a], ppb	GC effluent odor quality (packed column)	Odor threshold[b], ppb	Identification means
1	2,3-Butandione	3.16	1.7	Butterlike	8.6	MS,Rt,odor
2XO[c]	Hexanal	4.49	20.5 ± 9.2	Green	4.5	MS,Rt,odor
3	1-Penten-3-ol	5.08	9.5 ± 2.1		400	MS,Rt
4	3-Heptanone	5.20	4.0	Earthy		MS,Rt
5	2-Methyl-2-pentenal	5.24	1.95 ± 0.19			MS,Rt
6	2-Heptanone	5.40	3.2 ± 3.1		300	MS,Rt
7	Dodecane	5.50	0.6 ± 0.1			MS,Rt
8	Heptanal	5.51	1.8 ± 0.6		3.0	MS,Rt
9	3-Methyl-1-butanol	5.61	Internal standard (1.25 ppm)			MS,Rt
10O	(*E*)-2-Hexenal	5.83	1.4 ± 0.6		17	MS,RT
11	1-Pentanol	6.03	1.6 ± 0.2		120	MS,RT
12X	3-Octanone	6.18	2.0 ± 1.1		50	MS,Rt
13	Ethenylbenzene	6.26	1.4			MS,Rt
14O	Trimethylbenzene	6.40	3.4 ± 0.2			MS
15	Acetoin	6.46	Not measured			MS,Rt
16	2-Octanone	6.52	1.0 ± 0.1	Peaks 16 and 17-green, earthy, aldehyde	150	MS,Rt
17	Octanal	6.57	0.6 ± 0.1		0.7	MS,Rt
18X	1-Octen-3-one	6.57	2.6 ± 1.5	Boiled mushrooms	0.09	MS,Rt,odor
19	Tridecane	6.71	0.8 ± 0.2			MS,Rt
20	2,3-Octanedione	6.89	2.0 ± 1.6			MS,Rt
21	1-Hexanol	7.14	1.6 ± 0.8		4870	MS,Rt
22X	1,5-Octadien-3-one	7.42	1.3 ± 0.3	Geranium leaves	0.001	MS,Rt,odor
23X	3-Octanol	7.54	1.0 ± 0.6		18	MS,Rt
24O	Nonanal	7.62	3.2 ± 1.1	Planty, aldehyde	1.0	MS,Rt,odor
25	Tetradecane	7.73	0.8 ± 0.2			MS,Rt
26X	(*Z*)-2-Octenal	7.91	1.4 ± 0.3	Peaks 26 and 27-fatty, heavy, green	3.0	MS,Rt
27	Acetic acid	7.95	4.5 ± 0.6		7.0	MS,Rt
28X	1-Octen-3-ol	8.12	18.6 ± 4.1	Raw mushrooms	10	MS,Rt,odor
29X	1,5-Octadien-3-ol	8.45	24.8 ± 2.0	Earthy, mushroom	10	MS,Rt,odor
30XO	Benzaldehyde	8.69	1.0	Peaks 30, 31, and 32-slight cucumber, green, vinelike	0.44	MS,Rt
31O	Decanal	8.69	2.6 ± 0.4		0.1	MS,Rt
32	Pentadecane	8.75	2.4 ± 0.2			MS,Rt
33O	(*E*)-2-Nonenal	9.00	5.8 ± 1.5	Cardboardlike, cucumber	0.08	MS,Rt,odor
34X	1-Octanol	9.11	0.5		480	MS,Rt
35	2,3-Butandiol	9.15	6.4 ± 2.6			MS,Rt
36O	(*E*,*Z*)-2,6-Nonadienal	9.43	17.2 ± 0.1	Cucumber rind, peeling	0.01	MS,Rt,odor
37X	2-Octen-1-ol	9.72	6.3 ± 0.4	Peaks 37 and 38-green, musty	40	MS,Rt,odor
38	Hexadecane	9.74	1.4 ± 0.2			MS,Rt
39O	1-Nonanol	10.19	0.8			
40X	2,5-Octadien-1-ol	10.34	3.8 ± 1.5	Peaks 40 and 41-fresh fish undertone	MS,Rt	
41O	6-Nonen-1-ol	10.41	4.8 ± 0.8			MS,Rt
42	Heptadecane	10.67	2.0 ± 1.0			MS,Rt
43O	3,6-Nonadien-1-ol	11.05	5.7 ± 0.7	Clean cucumber	10	MS,Rt
44	Naphthalene	11.14	1.3 ± 0.2			MS,Rt
45	Octadecane	11.62	1.6 ± 0.7			MS,Rt
46	Nexanoic acid	11.82	1.1			
47	Unknown	12.38	12.4 ± 4.1	Green, cucumberlike		43(100), 71(60), 41(35), 56(32), 55(30), 83(21), 39(14), 89(12), 98(10)

Source Reprinted from Ref. 28 with permission from *J. Agric. Food Chem.* Copyright 1983 American Chemical Society. Data also from Refs. 31–35.

[a] Based on duplicate determinations; μg/L slime-water extract.

[b] Threshold in water.

[c] X = previously identified in mushrooms. O = previously identified in cucumber/melon.

enzymic conversions appear to be associated with the slime and the surfaces of the fish. In their model, polyunsaturated fatty acids (eg, eicosapentaenoic acid; $C_{20:5}$, ω-3) undergo enzymic oxidation to fresh-fish aromas by 12-lipoxygenase or 15-lipoxygenase at the skin-water interface because these enzymically derived volatiles appear to occur at higher concentrations in the skin than in fish muscle. They speculated that the enzymic oxidation and cleavage of the C_{20} polyunsaturated fatty acids may be involved with the induction of cellular repair because of the close involvement of the mucus or slime layer. In addition, these volatile compounds present in the slime may also provide signals for feeding, schooling, or fright responses.

Research on enzyme-mediated oxidations of fatty acids has been pursued in recent years (40–43) German and Kinsella (44) demonstrated the ability of lipoxygenase from trout tissue to catalyze the oxidation of polyunsaturated fatty acids (PUFA) typically associated with fish (arachidonic, eicosapentaenoic, and docosahexaenoic acid) to acyl peroxides. Hsieh and Kinsella (45), working with gill preparations from rainbow trout (*O. mykiss*), focused on the properties of enzyme-initiated lipid oxidation in tissues, specifically lipoxygenase, to produce specific oxidative flavor compounds in fish tissues. The major volatile compounds generated from the PUFA were 1-octen-3-ol, 2-octenal, 2-nonenal, 2-nonadienal, 1,5-octadien-3-ol, and 2,5-octadien-1-ol. They concluded that these compounds may be important in imparting fresh aroma to fish and also in causing off-flavors. Following the proposal of Josephson et al^{34}, Hsieh et al^{35} demonstrated the presence of 12-lipoxygenase activity in fish skin, which may play a role in generating off-flavors. Some fish have little of the lipoxygenase activity in the skin, according to Hsieh et al. (43). For example, white bass (*Morone chrysops*), rock bass (*Ambloplites rupestris*), sheepshead (presumably Archosargus probatocephalus), and bluegill (*L. macrochirus*) did not have detectable activities in the skin but did have the enzyme present in the gill tissue. With the exception of rainbow trout, most species examined by Hsieh et al. (43) had higher lipoxygenase activity in the gills than in the skin. These studies point out the need for proper postharvest processing, such as low-temperature storage, and perhaps most importantly, the avoidance of damage to skin and muscle tissue, such as bruising, cutting, and punctures. Hsieh and Kinsella (45) also found that antioxidants such as butylated *tert*-hydroxyanisole (BHA), *tert*-butylated hydroxytoluene (BHT), esculetin (6,7-dihydroxycoumarin), and esculin (esculetin 6-glucoside) could inhibit lipoxygenase activity. Esculetin was found to be a potent inhibitor of lipoxygenase activity, whereas BHT had negligible inhibitory effect. The use of these compounds could effectively control lipid oxidation and slow the deterioration of fish after landing.

Earlier workers noted that the aroma of freshly harvested fish had some recognizable components, described in such terms as green, cucumber, and leafy. In turn, various compounds were suggested and identified that might logically be present to play a role in this aroma. Perhaps the most significant finding in the series of papers by Josephson et al. (27–30,39) is that the fresh-fish aroma is composed of a variety of chemical compounds that are produced by a dynamic system involving a complex of lipids, enzymic and nonenzymic reactions, and physiological compartments in the fish. What role these compounds play in the life of the fish is not yet clear, but Josephson et al. (39) and Josephson and Lindsay (30) suggest, not unreasonably, that these compounds may play some biologically active role, such as trauma recovery or defense.

It is well known that during cooking and storage of cooked foods, significant flavor changes occur. In some cases, new and favorable flavors are developed, such as browning flavors during baking or boiling. However, after a food is cooked, degradative changes can also occur that yield undesirable flavors. Although the baking industry has expended a large effort on staling and its prevention, little work has been done toward similar research on seafood products. Recently, Josephson and Lindsay (46) reported on some lipid changes in chicken and fish. Briefly, they found that 2,4-alkadecadienals were formed from the autoxidation of polyunsaturated fatty acids. Though these compounds are thought to contribute desirable aromas to various foods, they are also found in foods with undesirable flavors such as warmed-over, stale, and oxidized. In model systems, Josephson and Lindsay (46) demonstrated that 2,4-decadienal was degraded to 2-octenal and ethanal by a water-mediated alpha/beta double-bond hydration, retro-aldo condensation reaction series. The resulting 2-octenal was further degraded to hexanal and ethanal. Working with battered, deep-fried Alaskan pollock fillets, they found that immediately after frying and after 3 days of refrigerated storage (6°C), the 2,4-decadienals were higher than in fried chicken. In both these foods, the concentration of the 2,4-decadienals declined after refrigerated storage with concomitant increases in 2-octenal. A major difference was observed between chicken and fish in hexenal levels. In the chicken, an initially large hexenal concentration tripled during storage, but hexenal levels declined in fish during storage. Josephson and Lindsay (46) suggest that the staling flavors that are formed during refrigerated storage may arise from retro-aldol-related reactions. Kubota and Kobayashi (47) reported two ketones in the aroma concentrate of cooked shrimp (*Euphausia pacifica, E. superba*, and *Sergia lucens*) and reported the structures as tentatively (5Z, 8Z, 11Z)- and (5E, 8Z, 11Z)-5,8,11-tetradecatrien-2-ol. Recently, in more detail, Kobayashi et al. (48) synthesized a series of tetradecatriens, isomers of 5,8,11-tetradecatriene-2-one, determined their structures, and related their sensory aroma characteristics to the chemical structures. The descriptions of the isomers of 5,8,11-tetradecatrien-2-one are listed in Table 2. Compounds I and II were the naturally occurring isomers reported earlier by Kubota and Kobayachi (47).

Though alcohols and ketones are indeed responsible for the variety of odors and flavors noted in seafoods, other compounds containing nitrogen and sulfur are also involved in fresh, cooked, and fermented products. In a similar vein, Tanchotikul and Hsieh (49) examined the volatile flavor components in crayfish (*Procambarus clarkii*) waste. Using dynamic headspace sampling with gas chromatography (GC)/MS techniques, they identified 117 compounds; of these compounds, 106 were positively identified and the other 11 were tentatively identified in the head-

Table 2. Aroma Characteristics of Isomers of 5,8,11-Tetradecatrien-2-One

Isomer	Aroma characteristic
I (5Z, 8Z, 11Z)	Shrimp, crab, shellfish
II (5E, 8Z, 11Z)	Shrimp, crab, sea cucumber
III (5Z, 8E, 11Z)	Fruity, oily, seafood
IV (5Z, 8Z, 11E)	Fruity, oily, milk
V (5E, 8E, 11Z)	Short-legged clam, seafood
VI (5E, 8Z, 11E)	Short-legged, clam, oily
VII (5Z, 8E, 11E)	Fruity, cucumber, white-meat fish
VIII (5E, 8E, 11E)	Oily, fishy, dry bonito

space of crayfish waste. Interestingly, they found a large amount of phenol, which appeared to give the sample a medicinal aroma. In addition to a variety of compounds resulting from oxidation of polyunsaturated fatty acids, Tanchotikul and Hsieh (49) also identified nitrogen-containing compounds, pyrazines and pyrroles. 2,5-dimethylpyrazine was the most abundant nitrogen-containing compound they detected.

Recent work by Vejaphan et al. (50) with cooked crayfish (*P. clarkii*) tail meat revealed 70 volatile compounds in many different chemical classes. These authors identified green, grassy, woody, buttery, metallic, or sulfurlike aromas. Although ketones were the major components found in crayfish waste and in tail meat, significant sulfur-containing compounds were also found. Buttery et al. (51) identified two straight-chain sulfur compounds, dimethyl disulfide and dimethyl trisulfide, that gave the odor of cooked cabbage and spoilage. Indeed, these compounds are found as flavor/odor components in cabbage, broccoli, and cauliflower (51). In addition, the organic sulfides (dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide) are also involved with anaerobic putrefactive decomposition of many foods (26). Clearly, these compounds would not be expected to contribute desirable aromas to cooked crayfish.

Defect Flavor Attributes

Fishy Flavors and Odors Whereas the fresh-fish flavor is a desirable component of high-quality fish, "fishy" flavors and odors represent the other end of the quality spectrum, namely, poor or low quality. The fishy odors and flavors discussed here are in contrast to decomposition or spoilage aromas that involve bacterial and catabolic changes in fish that would be declared unfit for human consumption. In general, a fishy odor or flavor in either the raw or the cooked product marks a seafood as low quality. However, there are marked, and perhaps unusual, exceptions to this rule. For example, the authors of this article and Jellinek (6, p. 76) have observed sensory panelists (American and German) who were raised in environments where fresh fish was not available reject freshly caught fish as being too "bland." Clearly, the perception of fishy flavors and odors is somewhat culturally biased. Nevertheless, in the broad mainstream of seafood quality, the presence of fishy flavors and odors marks a seafood of low quality.

In 1962, a pioneer in fish flavors and odors, M. E. Stansby (52,53), commented, "With few exceptions . . . the relation of fishy odors and flavors to specific chemical substances in fish has been largely neglected in recent years." At this time, Stansby (53) pursued earlier work by Davies and Gill (54) and Davies (55) relating fishy odors in fish to nitrogenous compounds (ie, trimethylamine) and fat in the flesh. Besides basic organoleptic work on the odor of trimethylamine (TMA), Stansby (52,53) related the interaction of TMA with fish oils in attempting to define the odor that most people identify as fishy. Stansby concluded that probably no one compound was responsible for the fishy odor and that TMA, fish oil, and air were necessary for the development of fishy odors.

TMA is undoubtedly responsible for the major portion of the fishy odors noted in most cooked samples of marine fish. TMA usually arises from the reduction of TMA oxide (TMAO) in fish tissues (56). This reduction proceeds quite easily, due to either enzymic reactions or simple redox reactions utilizing ferrous ion (Fe^{2+}) complexed in some way in the tissue (57). With heating at 80°C, EDTA-complexed FE^{2+}, can quantitatively reduce TMAO to TMA within 5 min and serves as the basis of a method for TMAO/TMA analysis (58). TMAO occurs primarily in the marine fishes, particularly the gadoids (cods, haddock, hakes, and pollock), whereas in freshwater fishes the TMAO content is low to nondetectable. Even in species that contain large amounts of TMAO (ie, the gadoids), the variation in muscle concentration of TMAO is highly variable (57). TMAO can also break down to formaldehyde and dimethylamine. This latter reaction is thought responsible for toughening found in some fish fillets during frozen storage due to cross-linking reactions of the protein and formaldehyde (59). The odor of dimethylamine (bp 7°C) is similar to that of TMA. The origin and role of TMAO in fish is not well understood at present, and the interested reader is directed to the review by Hebard et al. (57) for a more complete discussion concerning origin and occurrences of TMAO.

TMAO itself has a moderately intense odor but it is not fishy; its odor is similar to that of stale air released from an automobile tire. On the other hand, TMA (bp 4°C) is ammoniacal in high concentration, but as the concentration is lowered a fishy note appears. Nevertheless, although diluted TMA has an odor that is somewhat fishy, it is not quite identical to that observed in deteriorating fish (52,53). At least in fish with high levels of TMAO, TMA is probably responsible for most of the undesirable fishy aromas (60,61) but other compounds may also be involved, such as acetic, butyric, and valeric acids (61). TMA or TMAO may not be completely necessary for the development of a fishy aroma. For example, one of the authors (J. C. W.) noted that when freeze-dried bovine serum albumin was coated with a molecularly distilled fish oil fraction and stored at −20°C for several weeks under nitrogen in a ground glass stoppered flask, a distinctly fishy aroma was observed in the headspace of the container. It was assumed that oxidative free radical reactions interacted with the proteinaceous material to yield unknown odorous compounds.

Clearly the production of the fishy aroma is complex, involving a multiplicity of compounds and reactions, perhaps similar to those described earlier in the production of fresh-fish aromas and flavors, that can produce a myriad of compounds (eg, acids, alcohols, esters, aldehydes, and ketones). These compounds in turn mix with TMA to yield the distinct fishy odors that may vary from one fish species to another.

Rancidity In plant oils and fats, two kinds of rancidity are noted (62): oxidative and hydrolytic. Oxidative rancidity is caused by the presence of oxygen and its reaction with the unsaturated lipid fraction, producing typical off-flavors and odors. Hydrolytic rancidity is the result of the hydrolysis of triglycerides, producing free fatty acids. These free fatty acids, particularly capric, lauric, and myristic acids, have very strong off-flavors. While hydrolysis of fish lipid triglycerides occurs, the resulting fatty acids have much higher molecular weights (ca 300 daltons) with concomitant lower flavor and odor intensities (63).

By and large the development of rancidity in a seafood is noted by flavor more than odor. However, in some frozen fatty species of fish, definite rancid odors can be detected quite easily. Nevertheless, for most low- to moderate-fat species, rancidity is usually noticed first by taste when the product is eaten, particularly in the fattier tissues. Many of the odors and flavors *and* reaction mechanisms that we described earlier that play a role in the fresh-fish flavor also play a role in rancidity. This is not surprising in that many of the fresh-fish flavors are developed through hydroperoxidation reactions of the polyunsaturated lipids present in seafoods. It is not our intent here to go into details of the reactions involved in the oxidation of fats and oils. The interested reader is directed to several excellent texts by Hamilton (64), Hardy (65), and Sargent et al. (66).

The flavors we associate with rancidity arise from the oxidation of lipids and are very complex in nature, similar to those responsible for fresh-fish flavor. These reactions of oxygen and the double bonds in seafood lipids can be catalyzed by trace metals, such as Fe^{2+} complexed in the heme molecule (67). A large portion of the work on rancid flavors and odors has been conducted on plant lipids, using linolenic or linoleic acids as model compounds that contain 3 and 2 *cis*-double bonds, respectively. Marine fish, on the other hand, contain not only both linolenic ($C_{18:3}$) and linoleic ($C_{18:2}$) acids but also other highly unsaturated fatty acids containing 4, 5, and 6 *cis*-double bonds (arachidonic—$C_{20:4}$, eicosapentaenoic—$C_{20:5}$, and docosahexaenoic acids—$C_{22:6}$, respectively). Furthermore, these C_{20} unsaturated acids are highly labile to both enzymic and nonenzymic oxidation (68–70). If one considers the production of compounds from the oxidation of C_{18} fatty acids complex, the complexity of products from the C_{20} and C_{22} unsaturates must be increased by at least an order of magnitude. Therefore, much of the work on the production of odorous and flavor compounds in model systems has understandably been done using linolenate ($C_{18:3}$) and linoleate systems ($C_{18:2}$).

In a model system Ullrich and Grosch (71) identified the most intense odor compounds in autoxidized methyl linoleate at room temperature using flavor units (FU) and flavor dilution factors (FD). After a reaction time of 48 h, 20 mol percent of the methyl linolenate was converted into hydroperoxides. Ullrich and Grosch (71) found that *trans,cis*-2,6-nonadienal followed by 1,*cis*-5-octadien-3-one, *trans,cis*-3,5-octadien-2-one and *cis*-3-hexanal had the highest flavor units. However, after 102 h, 1,*cis*-5-octadien-3-one was by far the most important odor compound, which was followed by *cis*-3-hexenal and *trans,cis*-2,6-nonadienal. 1,*cis*-5-octadien-3-one has a potent geranium-like note that gives a metallic off-flavor (72). Ullrich and Grosch (71) found that the odor was so potent that no peak could be detected for this compound in the gas chromatographic chart until enrichment procedures were employed. They postulate that 1,*cis*-5-octadien-3-one is the oxidation product of the 1,*cis*-5-octadien-3-ol

Hsieh et al. (73) identified volatile compounds in undeoderized menhaden fish (*Brevoortia tyrants*) oil. They used a dynamic headspace sampler and stripped the volatiles at 65°C onto a Tenax TA sorbent cartridge held at ambient temperature. Desorbed compounds were separated and identified using either gas chromatography, mass spectrometry, or both. In their method, the low-molecular-weight alkanes (C_1 to C_7) were not detected due to insufficient cold trapping. Though the major portion of the volatiles found were alkenals and alkadienals, the largest component was heptan-3-one at 8530 ppb (sickly sweet odor) followed by butanoic acid (8110 ppb, odor of dirty socks). The volatile short-chain fatty acids (C_2–C_6) gave very intense and objectionable sweaty odors. The origin of these low-molecular-weight acids (ie, C_2–C_6) was not discussed, but they could arise from hydrolytic cleavage of short-chain triglycerides or from the oxidation of short-chain aldehydes arising from the hydroperoxidation of unsaturated fatty acids. In addition, they found significant amounts of decadienals, decatrienals, and nonatrienals that have oxidized oil odors and are characteristic of crude menhaden oil.

Tainting Flavors Unlike their terrestrial counterparts, fish are intimately associated with their environment. Given this situation, whatever is present in that aquatic environment has more than ample opportunity to enter the living aquatic organism. In this way, fish and shellfish are particularly prone to tainting, or the acquisition of environmental odors and flavors. For example, oil spills into the marine environment near aquaculture pens or fish raised in closed-pond aquaculture will sometimes produce earthy, muddy, or musty odors or flavors in the fish (74) Some of these flavors can be acquired through the food, but most originate from microorganisms (75) living in the water, soil, or pond detritus. These muddy or tainting flavors can present economic problems to fish growers. For example, sometimes commercially reared catfish (*Ictalurus punctatus*) will develop such an intense musty odor or flavor that the fish cannot be marketed. A lexicon of flavor descriptors has been developed for pond-raised catfish by Johnsen et al. (76). Fish reared in closed-pond cultures are susceptible to acquiring odorous compounds when these ponds become eutrophic, usually caused by high feeding rates, temperatures, and stocking densities and low aeration. Though tainting occurs in small ponds, stagnant water, or both, tainted fish have also been reported from lakes and salt water. Yurkowski and Tabachek (77,78) reported that the muddy flavor in rainbow trout (*O. mykiss*) and other species, taken from some western Canadian lakes, was due to geosmin and methylisoborneol. In saltwater studies, Persson (79) correlated the muddy flavor of bream (*Abramis brama*) with an increase in the biomass of *Oscillatoria agardhii* in the Gulf of Finland. Clearly, the tainting of seafood products can also occur due to the presence of human-made pollution, such as petroleum hydrocarbons from oil spills or the discharge of other odorous chemicals as by-products of industrial production. Although we believe that the human-made taints can and do seriously impact seafood quality, a detailed discussion of this particular complex problem is beyond the intent of this chapter. Therefore, we have limited our discussion of seafood flavors and quality to those compounds arising from natural environmental origins and leave a discussion of the contribution of human-made contaminants to others.

Several heterocyclic compounds have been reported to be involved in muddy or earthy odors: 2-methylisoborneol (80,81), 2-methylenebornane, and 2-methyl-2-bornene (82). Examination of the commercial catfish ponds revealed 2-methylisoborneol in both water and mud. Martin et al. (81) report that they found the highest levels of 2-methylborneol in catfish flesh (284 ng/g), whereas mud and water were much lower, 14.9 and 10.06 ng/g, respectively. The range for the 2-methylisoborneol was 5.0 to 284 ng/g in the fish flesh, whereas water ranged from a low of 0.1 ng/g to a high of 13.32 and mud ranged from a low of 1.0 ng/g to 14.9 ng/g. They determined a bioconcentration factor (concentration in water/concentration in fish) for 2-methylisoborneol of 28.1 ± 14.0 (± std dev) for the channel catfish. An unsaturated aliphatic compound, 1,10-*trans*-dimethyl-*trans*-(9)-decalol or geosmin (83), has been isolated from muddy-tasting catfish. These compounds, the 2-methylisoborneol and geosmin, are the products of the metabolism of certain species of cyanobacteria and actinomyces (75,84). Gerber (75) proposed the name geosmin for 1,10-*trans*-dimethyl-*trans*-(9)-decalol based on the Greek words *ge*, meaning earth, and *osme*, odor. Geosmin and methylisoborneol have been reported to be produced by *Microbispora rosea* 3758, *Nocardia* spp., and *Streptomyces* spp. (75,85). These organisms are readily found in water and soils (86), so it is not surprising that geosmin and methylisoborneol have been found in other foods besides fish, for example, soil-grown vegetables such as beets (87,88). Blue-green algae are also responsible for the production of various compounds that are the cause of off-flavors in water (89,90).

Lovell and Sackey (91) were able to induce earthy-musty flavors in channel catfish by culturing the fish in tanks containing a geosmin-producing blue-green alga, *Symploca muscorum*; however, they did not identify the chemical compounds responsible for the off-flavor. Lovell et al. (83) attempted with mixed success to correlate sensory findings of earthy-musty flavors in catfish with geosmin content. In their study, 10 of 13 fish were judged to have an earthy-musty taint and were found by instrumental analysis to contain geosmin. Using an inverse scoring system (highest intensity of earthy-musty flavor receives a 0; no off-flavor receives a score of 10), they found that one fish that contained over 200 μg/kg geosmin had a score of 2.7, whereas a fish containing 15.6 μg/kg was given a score of 1.7. Clearly, other compounds, such as the bornanes, may also be present in these fish that may contribute to the scoring of flavor quality.

Sulfur-containing compounds, such as dimethyl disulfide and dimethyl trisulfide, are also responsible for tainting. However, of these two compounds, dimethyl disulfide is somewhat of a two-edged sword in that depending on concentration, it can play both beneficial and detrimental roles. For example, at low concentrations, it is responsible for the typical odor of fresh oysters, but at higher concentrations it has been reported to be responsible for a petroleum refinery odor in Nova Scotia mackerel (*Scomber scombrus*) (92), a petroleum odor in canned salmon (93), and a blackberry odor in Labrador cod (94). In Nova Scotia mackerel, levels of 0.2 to 0.3 μg/g of dimethyl sulfide were found in tail muscle whereas stomach contents had levels as high as 17 μg/g. The high levels of dimethyl sulfide were thought to arise from the mackerel's consumption of large quantities of the pteropod *Spiratella retroversa*. The metabolic precursor was probably dimethyl-β-propiothetin because large quantities were also found in the stomach contents.

Another sulfur compound, dimethyl trisulfide ($CH_3SCH_2SSCH_3$), was implicated in the off-flavor of some Australian red prawns (*Hymenopenaeusi sibogae*) (95). The prawns exhibited a strong disagreeable cooked-onion flavor and aroma. Close inspection of the lot revealed that some of the prawns were damaged, the flesh was discolored, and the shellfish were slimy. Initial speculation was that the onionlike odor was due to microbial spoilage, possibly as a result of poor handling. Whitfield et al. (95) analyzed the product and found that dimethyl trisulfide was responsible for the off-flavor. In their studies, they collected approximately 10 μg of dimethyl trisulfide from 100 g of shrimp, giving an estimated level of 100 μg/kg in the original product. The odor threshold for dimethyl trisulfide is very low; Buttery et al. (51) reported it to be 0.01 μg kg^{-1}, or 0.01 ppb in water. Interestingly, adding dimethyl trisulfide to minced samples of fresh normal-flavored shrimp at levels of 1 to 10 μg kg^{-1} followed by cooking for 3 min at 100°C gave a product with a distinctive and unpleasant onion flavor; however, when it was added at 100 μg kg^{-1}, the material became inedible. Because the shrimp were heavily damaged and had high indole levels (50 μg kg^{-1}), they speculated that two pseudomonads, *Pseudomonas putrifaciens* and *P. perolens*, might be possible causative agents. Both *P. putrifaciens* and *P. perolens* have been reported to produce dimethyl trisulfide when inoculated onto sterile fish muscle and incubated at 0°C (96,97).

During the early stages of spoilage, chilled fish muscle will develop a musty, potatolike odor (98,99). Castell et al. (100) determined the causative organism to be *P. perolens*. Miller et al. (96) found a variety of odorous compounds in spoiling rockfish (*Sebastes melanops*) muscle that had been inoculated with *P. perolens* ATCC 10757 and allowed to incubate at 5, 15, and 25°C. The following compounds were detected and identified: methyl, dimethyl disulfide, dimethyl trisulfide, 3-methyl-1-butanol, butanone, and 2-methoxy-3-isopropylpyrazine. Also tentatively identified were 1-penten-3-ol and 2-methoxy-3-isopropylpyrazine. The musty, potatolike odor was primarily caused by 2-methoxy-3-isopropylpyrazine (96). In a slightly later work, again using sterile *S. melanops* muscle tissue, Miller et al. (97) found that *P. putrifaciens, P. fluorescens*, and an *Achromobacter* species (strain H15) produced methyl mercaptan, dimethyl disulfide, dimethyl trisulfide, 3-methyl-1-butanol, and trimethylamine. The bacterial strains used in these studies were isolated from the environment. The *P. fluorescens* was isolated from ocean perch whereas the *P. putrifaciens* strain H6 and the *Achromobacter* strain H15 were isolated from Dungeness crab. Only the *Achromobacter* species did not produce dimethyl trisulfide. The major sulfur-containing compounds produced by *P. fluorescens* were methyl mercaptan and dimethyl disulfide.

Other sulfur-containing compounds, such as heterocyclic sulfur compounds, have been associated with meaty aromas in meat products and may also be important in crustaceans (101–103). Kubota et al. (101) found that the cooked-odor concentrate from boiled antarctic krill (*Euphausia superba* Dana) contained 11 sulfur-containing compounds. Significant odorous sulfur compounds were 3,5-dimethyl-1,2,4-trihiolane (onionlike to garlicky) depending on replacement of the methyl groups with ethyl groups. Dimethylthiolanes have been identified in the volatiles of cooked beef, beef broth, cooked mutton, and cooked chicken. As in the case with the dimethyl disulfide, in concentrated form they have a sulfide odor, but upon dilution they yield a meaty aroma. 3-ethyl-5-methyl-1,2,4-trithiolane and 3,5-diethyl-1,2,4-thiolane have been identified in the flavor compounds of onion. In a later work, Kubota et al. (102) studied the volatile components of roasted shrimp (*Pleoticus muelleri*). In this work, they found 77 compounds, including isovaleric acid, alkyl pyrazines, isovaleramide, ketones, and some sulfur-containing compounds. In roasted shrimp, 3-methylthiopropanol was present at the highest level, but in boiled shrimp, dimethyl disulfide was present whereas the 3-methylthiopropanol was absent.

In another study that examined both cooked and fermented shrimp (*Acetes japonicus*) Choi et al. (103) found that sulfur compounds and pyrazines seemed to be important contributors to the cooked odor of the fermented product. In their work, shrimp were allowed to ferment in 20% salt at 20°C for 3 months, while the shrimp were kept frozen (−25°C) until used. Volatiles from both the raw and fermented shrimp were collected using a combined steam distillation-extraction (water–diethyl ether = 1000:100 mL) for 4 h. The main components of the volatile concentrate made from the nonfermented shrimp were thialdine (dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine, 41.4%), isovaleraldehyde + ethanol (22.5%) and pyrazines (6%), whereas in the fermented shrimp the main odor constituents were the pyrazines (32%), isovaleraldehyde + ethanol (26.5%), and furfuryl alcohol (25.1%). Indole was low (0.3%) in the nonfermented shrimp and present only in trace amounts in the fermented product. The presence of furfuryl alcohol in the fermented product would probably account for its nutty flavor notes. Choi et al. (103) speculate that the origin of sulfur and nitrogen compounds in the fermented shrimp arise from free amino acids released during the fermentation process, yielding smaller molecular compounds that in turn condense to form products such as the pyrazines, thialdines, trithiolanes, and thiazoles.

Rancidity. Perhaps the most common defect in all foods, and specifically in seafoods, that consumers recognize most readily is rancidity. In both formal studies and informal interviews, rancidity is commonly reported by both trained and untrained panelists. Unfortunately, although it can be easily identified organoleptically, its chemical measurement and correlation with sensory observation has been somewhat more difficult. Attempts at measuring the components of oxidative rancidity and correlating them with sensory findings has proved difficult because of the compounds' transient nature, particularly in a complex food matrix. Many of the compounds responsible for rancidity, such as malonaldehyde, are very reactive and are presented with a variety of substrates in foods Numerous methods have been proposed and used to measure the degree of rancidity of foods, and the reader is directed to Rossell (62), particularly pages 25 to 34. The oxidation of oils, particularly in a food matrix, is a very dynamic system, producing many highly labile compounds that in turn can react with each other or with other components in the food. All of these yield odorous or flavor compounds that are characteristic of rancidity.

The presence of highly unsaturated fatty acids (eg, $C_{22:6}$ and $C_{20:5}$) in seafoods makes for one of the most complex systems for study. In the early stages of oxidative rancidity, peroxides are formed at the double bonds in the unsaturated lipids, yielding compounds that are odorless and flavorless. Through further oxidation, these compounds are eventually converted to aldehydes and ketones, many of which have undesirable flavors, such as fishy or rancid (104). For the measurement of the early stages of oxidation, the peroxide value (PV) is useful, whereas for intermediate stages, the thiobarbituric acid value (TBA) method, which is claimed to be specific for malonaldehyde, is most useful. Many of these oxidation products, namely, the aldehydes and ketones, are volatile and highly reactive. In addition, they are present in a very complex food matrix with many available and similarly reactive functional groups (eg, amino groups, $—NH_2$). Therefore, as rancidity proceeds, the TBA values will increase and then reach a maximum and begin to decline as the malonaldehyde is consumed in the food matrix. For a discussion of these methods, the reader is directed to Rossell (62, pp. 25 and 29). Official methods for PV (105,106) exist, but methods for estimating malonaldehyde using TBA are still largely empirical. In our laboratory the method of Lemon (107) based on the method of Vyncke (108), developed at the Canadian Fisheries laboratory at Halifax, Nova Scotia, has been found useful. Researchers must be aware that peroxides, and particularly malonaldehyde, can increase and then decline in food samples. Consequently, some degree of knowledge about the history of the food sample is necessary to ensure meaningful interpretation of PV and/or TBA values.

COMPOUNDS RESPONSIBLE FOR MODIFYING SEAFOOD FLAVORS AND TASTES

Typically when seafoods are described by either trained or untrained sensory panels, a set of common descriptors are usually found, such as "sweetness," "saltiness," "briny," and "salty-briny" (109,110). The cause of these tastes has been variously ascribed to the presence of sodium chloride or carbohydrates in the fish flesh. However, recent work indicates that it may not be necessary to rely on sodium chloride and simple sugars to explain some of these taste phenomena. For example, the peptide N-L-α-aspartyl-L-phenylalanine 1-methyl ester, or aspartame as it is commonly known, is now widely used as an artificial sweetener because it is about 160 times sweeter than sucrose. It has also been recognized that peptides and nucleotides can be responsible for bitter tastes. Furthermore, Tada et al. (111) reported L-ornithyltaurine as a salty-tasting peptide. More recently, Kawasaki et al. (112) and Seki et al. (113) reported further studies on salty peptides, such as L-ornithyl-β-alanine and a series of synthesized peptides and amino acid derivatives. Some of these compounds appear to have the ability to potentiate the salty taste. Though some controversy appears to surround the early work on salty peptides, recent papers by Kawasaki et al. (112) and Seki et al. (113) confirm that peptides and amino acid derivatives can have a salty taste or potentiate the salty perception. Clearly, seeking to profile foods by merely measuring the quantities of sodium chloride, simple carbohydrates, and other low-molecular-weight compounds ought to be reassessed. The saltiness and sweetness that are commonly attributed to fresh fish may possibly have their origins in some of these peptides.

Umami

An important taste sensation associated with monosodium glutamate (MSG) is described as "meaty" or "mouth-filling." Many who are aficionados of Asian cuisines are perhaps very familiar with this flavor. Besides MSG, other compounds, such as the 5′-nucleotides, also have the same flavor and in addition have some capability of potentiating the taste of MSG (114). A recent computer search of the literature by the authors indicates that up to two-thirds of the reports on the taste of MSG and 5′-nucleotides originated in either Japan or Asia. Because of the Japanese keen interest and large body of work on this flavor, we will use here the Japanese term *umami* to describe the taste sensation of MSG and the 5′-nucleotides (5′-ribonucleotides and their derivatives such as disodium 5′-inosinate [IMP] and 5′-guanylate [GMP]). For more information about the derivation and description of umami, the reader is directed to the review by Yamaguchi (114,115).

Although MSG contributes the basic taste sensation of umami to a food, the 5′-nucleotides may play just as important a role. Some of the nucleotides have the umami flavor, but their ability to potentiate this flavor may be more important. The 5′-nucleotides are naturally present in many foodstuffs and have probably been used for their flavor-enhancing abilities long before their role was understood. For example, Table 3 lists some values for the nucleotides in common foods. It can be seen from this data that the use of meat and poultry broths or soups prepared with shiitake mushrooms would have a potentiating effect, that is, one similar to that of the commercially produced Ribotide® (Takeda Chemical Industries, Ltd.), if used with a food high in glutamic acid. The prevalence of these broths in the cooking traditions of various cultures, particularly Chinese and French, may be due to the presence of these naturally occurring enhancers. In fact, fermented fish sauces, which are common throughout Asia, are prepared from fish in the sardine family, a group of fish high in 5′-IMP. In Thailand, these fish sauces are prepared by layering salt and fish, then permitting autolysis to occur slowly, sometimes over a 2-year period at tropical temperatures (27°C–35°C). After the fermentation process is finished, the liquefied fish portion is drawn off and filtered if necessary. The finished sauce will contain a variety of amino acids, nucleotides, salt, and other components. A high-quality fish sauce will be clear, almost odorless, and moderate to dark brown in color and be used as a condiment with various foods; typically pieces of food are dipped in the sauce before being eaten. In some cases, the fish sauce may be added as an ingredient to soups or cooking broths. In Japan, shaved or small pieces of dried bonito are a common ingredient added to miso soups; the bonito's very high 5′-IMP content obviously contributes to the miso profile.

In addition to IMP and other 5′-nucleotides, peptides may also be responsible for flavor enhancement. Fujimaki et al. (117) isolated pleasant brothy tastes from the hydrolysis of fish protein concentrate using commercial peptidase enzymes. Molsin® (obtained from Seishin Seiyaku Co.), Rapidase® (obtained from Takeda Chemical Co.), and Pronase® (obtained from Kaken Kagaku Co.) enzymes gave the strongest brothy flavor. The release of brothy taste enhancers appeared enzyme dependent in that pepsin yielded little of the enhancing flavor. Noguchi et al. (118) found that peptides from fish protein hydrolysates having flavor activity qualitatively similar to MSG had high molar ratios of glutamic acid residues. Arai et al. (119) suggest that highly acidic (hydrophilic) L-glutamyl oligopeptides possibly possess a brothy taste and contribute to the favorable taste of food protein hydrolysates. Yamasaki and Maekawa (120) isolated what they term a "delicious" peptide from the gravy of beef meat having a proposed structure of H-Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala-OH. Later Yamasaki and Maekawa (121) synthesized this peptide and compared the synthetic compound with that isolated from beef and found that the sensory properties were the same. During this work they examined several precursor peptides and found that removal of the Lys-Gly group, yielding Asp-Glu-Glu-Ser-Leu-Ala, created a sour-tasting peptide, whereas the tripeptide Ser-Leu-Ala produced a bitter-tasting peptide. The peptide Glu-Ser-Leu-Ala was both sour and astringent. More recently, Tamura et al. (122) studied the interactions of the "delicious" peptide's amino acid groups. They confirmed that the umami and salty taste were produced by a combination of the basic and acidic groups of the peptide. They proposed that the cationic and anionic groups must be located next to each other to produce the salty and umami taste.

In careful determinations, Yamaguchi et al. (123) reported a threshold value for the umami at 0.012 g/100 mL or 6.25×10^{-4} M. Under the conditions of their testing, the MSG threshold was higher than that of quinine sulfate or tartaric acid and lower than sucrose, but similar to that

Table 3. Nucleotide Content of Some Foodstuffs

	Nucleotides, mg %					
Food	5′-CMP	5′-UMP	5′-IMP	5′-GMP	5′-AMP	ADP + ATP
Sardine			192.6		6.6	15.4
Bonito			285.2		7.6	15.8
Salmon			154.5	0.0	6.9	
Perch			124.9	0.0	8.4	
Sea bream			214.8			30.0
Codfish			43.8		23.9	295.6
Swordfish			19.9	0.0	3.1	
Rainbow trout			117.0		14.6	223.4
Trout			187.0		4.2	27.7
Oyster	+	31	0	0	21	53
Crab			0		10.1	372.5
Prawn (Shrimp)					11.5	701.5
Beef	1.0	1.6	106.9	2.2	6.6	17.4
Pork	1.9	1.6	122.2	2.5	7.6	12.2
Chicken	2.3	3.6	212	3.6	5.2	30.1
Asparagus		72			27	77
Tomato	0.5	2.2			10.4	
Green beans	0.8	2.6			1.8	
Cucumber	±	0.6			0.5	
Onion	±	0.5	±		0.8	
Mushroom (dry)[a]	114.2	135.2		156.5	131.6	

Note. Abbreviations used: 5′-CMP, 5′-cytidylic acid; 5′-UMP, 5′-uridylic acid; 5′-IMP, 5′-inosinic acid; 5′-GMP, 5′-guanylic acid; 5′-AMP, 5′-Adenylic acid; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate.

[a] Hot water extract of dried *shiitake*.

Source: Ref. 116.

of sodium chloride. MSG appears to interact with the four tastes. In this experiment, the thresholds for the four tastes were measured again in a 5-mM solution of MSG or IMP. The thresholds for sucrose and sodium chloride were unaffected by either of the umami substances, but IMP raised the threshold of tartaric acid from 0.94 mg % to 30 mg % (nearly a 30-fold increase), whereas MSG raised the tartaric acid threshold to 1.9 mg % (approximately 2-fold increase). MSG had no apparent effect on the quinine sulfate threshold (0.049 mg %), but IMP raised the threshold to 0.4 mg % (about an 8-fold increase). Perhaps the most important aspect of the umami flavor is the synergistic relationship between MSG and IMP. Yamaguchi (114) found that when mixtures of MSG and IMP containing 25 mg % MSG and 25 mg % IMP were rated by a sensory panel, the flavor intensity was equivalent to 780 mg % MSG alone, a 16-fold increase in apparent flavor. This synergistic effect was not affected by the four taste substances (sucrose, sodium chloride, tartaric acid, and quinine sulfate). He did find that the basic amino acids, histadine and arginine, could suppress the synergistic effect of IMP; however, when the pH was adjusted to between 5 and 6.5, the synergism was recovered.

At a more practical level, Yamaguchi (114), in extensive taste tests with untrained sensory personnel, examined the effect of umami substances on food flavors. These tests employed from 180 to 300 people in panels that varied from 25 to 50 persons. The results of these tests indicated that MSG has no effect on the aroma of food but does increase the total taste intensity. MSG enhances certain flavor characteristics of foods, such as continuity, mouth fullness, impact, mildness, and thickness. Specific food flavors are also increased, such as meatiness. Probably most importantly, MSG increases the palatability of foods.

The presence of 5′-nucleotides, particularly IMP, may significantly influence our perception of the flavor, and hence the quality, of seafoods. Some of these nucleotides arise from the metabolism of adenosine-5′-triphosphate (ATP):

$$\begin{aligned}\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP (HxR)} \rightarrow \text{hypoxanthine (Hx)}\\ \rightarrow \text{xanthine (Xa)} \rightarrow \text{uric acid (UA)}\end{aligned}$$

In resting muscle tissue, ATP is the predominant nucleotide. ATP undergoes a series of enzymatic dephosphorylation steps, forming adenosine-5′-diphosphate (ADP) and finally adenosine-5′-monophosphate (AMP). At this stage, deamination of AMP by AMP deaminase yields inosine-5′-monophosphate (124). These reactions proceed very rapidly, even during slow freezing of fish muscle (125). In trawl-caught fish, most ATP is depleted during capture and is converted to IMP within a day or two if the fish is stored on ice (126). IMP is then dephosphorylated by 5′-nucleotidase to form inosine (HxR); however, this reaction is slow compared to the formation of IMP. Inosine is cleaved by muscle ribosidase to form hypoxanthine and ribose; however, this reaction appears to be quite variable and depends on the fish species. For example, in lemon sole, petrale sole, and redfish very little HxR is seen (125,127,128). In other species, the further metabolism of inosine is much slower than the dephosphorylation of IMP, causing inosine to accumulate. In haddock, plaice, and pacific salmon inosine accumulates and then very slowly breaks down to hypoxanthine (125,129,130). The further oxidation of hypoxanthine to xanthine and uric acid is also slow, probably due to microbial metabolism (123). A measure of the various ATP metabolites has been adopted as an indicator of freshness or quality of seafoods—the K value. The K value is defined as:

$$K = \frac{[\mathrm{Hx}] + [\mathrm{HxR}]}{[\mathrm{HxR}] + [\mathrm{Hx}] + [\mathrm{ATP}] + [\mathrm{ADP}] + [\mathrm{AMP}] + [\mathrm{IMP}]} \times 100$$

where the concentrations of the various nucleotides in the fish muscle are presented in brackets. In some cases, because the tissue concentrations of ATP, ADP, and AMP are so low, the equation is shortened to:

$$K_1 = \frac{[\mathrm{HxR}] + [\mathrm{Hx}]}{[\mathrm{Hxr}] + [\mathrm{Hx}] + [\mathrm{IMP}]} \times 100$$

The K value has been supported by the Japanese government in an experimental project since 1982 aimed at improving the distribution of fish by controlling its freshness (131). Apparently, in this project, the K values of fish were measured and then shown on packaging labels for the benefit of consumers. Though the K value appears to be accepted in Japan, it has not been widely adopted in the United States, where freshness or quality of seafoods is still determined by empirical observation based on experience. Unfortunately, determining K values requires relatively expensive laboratory equipment and procedures that are beyond the financial resources of many small processors and retailers, which may account for its current lack of widespread use. Nevertheless, the K value does appear to be a potentially useful tool that could find application in determining seafood quality in Western countries if the chemical analyses could be simplified, automated, or both. It is beyond the scope of this chapter to detail the use of K values in determining fish freshness; therefore, the reader is directed to the chapter by Ehira and Uchiyama (131).

Though it is clear that freshness of fish depends on the mixture of nucleotides, the levels of IMP in fish tissue appear to be directly related to acceptability. Bremmer et al. (132) found that flavor acceptability scores were positively correlated with IMP levels in four species of North West Shelf Australian tropical fish. The study involved long-spined sea bream (*Argyrops spinifer*), painted sweetlip (*Diagramma pictum*), snapper (*Lutjanus vittus*), and threadfin bream (*Nemipterus furcosus*). The fish were maintained on ice for up to 24 days. As expected, IMP levels declined in almost a linear fashion while sensory panelists' scores also decreased. In two species, long-spined sea bream and threadfin bream, hypoxanthine, a catabolite of IMP, was low and did not exhibit much change. The snapper and painted sweetlip, on the other hand, showed dramatic increases in hypoxanthine and would correlate negatively with flavor acceptability. Initial bacterial loads on the fish samples were low, typically of the order of 10^2 to 10^3 cfu/cm^2 and were identified to be *Moraxella, Vibrios, Aeromonas*, and *Flavobacterium*; at the end of 23 days of storage the predominant species were *Pseudomonas, Moraxella*, and *Aeromonas* and were present at levels of 10^6 cfu/cm^2, with a high of 10^7. Nevertheless, Bremner et al. (129) concluded that acceptability or shelf life was more related to IMP loss rather than bacterial spoilage. In temperate waters off Alaska, Greene and Bernatt-Byrne (133) found that both inosine and 5′-inosine monophosphate were positively correlated with overall desirability of both Pacific cod (*Gadus macrocephalus*) and pollock (*Theragra chalcogramma*). On the other hand, hypoxanthine was negatively correlated with flavor and desirability similar to the findings of Bremner et al. (132).

Over the past decade, there has been considerable interest in the taste of shellfish in Japan, probably due to the development and manufacture of crustacean and molluskan surimi analogs. Konosu (134) reviewed the role of amino acids, low-molecular-weight organic acids, and nucleotides on shellfish tastes. In work on synthetically reconstituted extracts that paralleled extracts from abalone (*Haliotis gigantea discus*), Konosu and Maeda (135) found that glycine and glycine betaine contributed to the sweetness and umami of abalone. As expected, the major contributor to umami in these extracts was glutamic acid. The presence of 5′-AMP also contributed to the umami taste. Glycogen, which was present in high concentration in the extract (7.4%), appeared to contribute a body effect on the taste, although glycogen itself was tasteless. Konosu (134) concluded that the taste characteristic of abalone is due to umami produced by glutamic acid and AMP; the sweetness is derived from glycine and glycine betaine. Glycogen produces a harmonizing and smoothing of the taste. Konosu claimed that AMP is accumulated in marine invertebrates, in contrast to fish where IMP accumulates, because the muscle lacks AMP aminohydrolase, or if it is present, its activity is low.

Free amino acids apparently also play a role in the desirability of squid. Like Konosu and Maeda's (135) earlier work with abalone, Endo et al. (136) found in squid (Hirakensaki-Ika, *Logigo chinensis*; Kensaki-Ika, *L. Kensaki*; Aori-Ika, *Sepioteusthis lessoniana*; and Ko-Ika *S. esculenta*) that high levels of free amino acids (glycine, alanine, and proline) were correlated with palatability. The role of glutamic acid and umami in the palatability of these squid was unclear in that it was high in one species (*Ommastrephes sloani pacificus*, Surume-Ika) considered inferior in taste, yet in another preferred species (*S. lessoniana*, Aori-Ika), glutamic acid was low. In two species that were considered inferior in taste (Sode-Ika, *T. rhombus* and Surume-Ika, *O. sloani pacificus*), TMA oxide content was about twice that found in the desirable species.

Reddy et al. (137) found that freeze-dried wash water from clam-processing plants could be added to clam dips in place of clam meat and juice without any significant changes in overall acceptability, taste, odor, texture, and appearance. Major amino acid components in these wash water samples were glycine, glutamic acid, alanine, arginine, serine, and in some cases aspartic acid. In their work, Reddy et al. (137) determined gas chromatographic volatile profiles but did not identify individual components that may be responsible for the clam flavor profile in these freeze-dried extracts.

In processing the 5′-nucleotide can have a significant role in potentiating flavor. Hashida et al. (138) studied the application of 5′-nucleotides to canned seafoods. In canned short-necked clams (*Venerupis semidecusata*), oyster (*Ostrea giges*), and red crab (*Acanthodes armatus*) the flavor of the seafood was enhanced at the following levels: 0.04 or 0.08% Ribotide® (a 1:1 mixture of 5′-IMP · Na_2 and 5′-GMP · Na_2, Takeda Chemical Industries Ltd.) for short-necked clam and red crab meat. The 5′-nucleotides appeared to be somewhat stable to heating and storage. Canned red crab meat retained 77 to 88% of added nucleotides during storage for 28 days, and canned short-necked clams retained 52 to 61% after 189 days.

ATTEMPTS TO USE SEAFOOD FLAVOR AND TEXTURE ATTRIBUTES TO CLASSIFY FISH

Due to a greatly expanded U.S. fishery, the American seafood marketplace contains a variety of new commercially harvested fish with little name familiarity, which has created a minor identity crisis in the industry and for the consumer. Although the National Marine Fisheries Service of the U.S. Department of Commerce (NMFS), the American Fisheries Society, and the International Congress of Zoology maintain scientific names of glossaries of common fish names, these are generally unsuited for use in market identification. To make matters worse, common names for fish species used in the United States contain little or no useful information for the consumer (139). As an example of the problem, some fish have many common names (as many as 5) from as many different locations, and many popular commercial fish have several common names, such king salmon which is also known as chinook, blackmouth, and spring salmon. Also, commonly used names in one region of the United States may be misleading in another, for example, the fish known as "snapper" along the Pacific coast is a species of rockfish (*Sebastes* spp.), not the acceptable *Lutjanidae* spp. from the Gulf of Mexico and Caribbean that the Food and Drug Administration has officially designated as snapper. In other cases, names may be unattractive or pejorative or may reflect ethnic slurs or epithets (eg, ratfish, hagfish, wolffish, jewfish, etc). As a result, consumers can learn little about the edibility characteristics of a product from its name, thus a large variety of seafoods remain effectively unavailable to them. Faced with a bewildering array of common names, it is not surprising that consumers confine their purchases to a few familiar items rather than deal with the unknown.

Because the American consumer was perceived as resisting the purchase of new seafoods that appeared in the marketplace because of a lack of information about what they taste like, NMFS launched a research project to determine the feasibility of developing a national nomenclature system that would group fish species according to their edibility characteristics (flavor and texture). It was felt that both processors and retailers could employ such a system to group together fish that share the same edibility characteristics so that consumers, both new and experienced, would be able to consistently and knowledgeably select their seafood. The system could work much like our classification of wines in the United States (ie, Chablis, Burgundy, claret, etc). Each of these wine classifications can contain one or more specific grape varietals, for example, claret can be made from Cabernet sauvignon, Merlot, or both.

It is a common human trait to classify and group that which interests us. For example, we readily group foods based on ethnic origin (eg, Italian, Mexican, Chinese, Japanese); we even break down the cuisines of a single country (eg, French/Provençal, Chinese/Szechuan, American/Southern, Mexican/"Tex-Mex"). The classification of seafoods is no exception. It is quite common to hear a buyer in the fish market ask, "What is this fish like for eating?" Questions of this type are not at all unreasonable considering the large number of fish species that crowd both Western and Asian marketplaces (more than 1,000 worldwide, 500 in the U.S. alone). Inevitably, the answers to such questions will involve one or more flavor attributes (eg, sweet, mild, strong, etc). Clearly, the flavor of a fish species is one of the most important criteria that the consumer exercises when he or she purchases seafood.

It should be clear that the various chemical compounds that impart characteristic flavors and odors to a particular species of fish are many and complex. Despite the efforts of scientists, certainly as detailed in this chapter, the relationships of specific chemical compounds and observed flavors and odors are yet to be completely understood. To date, the marketplace must ultimately rely on the consumer to determine what a fish "should be." However, to be "knowledgeable" about fish has, in recent years, become increasingly more difficult because of a large increase in the number of fish species and products finding their way into the marketplace (136). Demands on the consumer's discriminatory abilities will be increased as even more fish species are introduced into the marketplace, both in the United States and elsewhere. This increase is due to a variety of political, economic, social, and health reasons. Economic factors are perhaps the easiest to identify. In the past, fishermen, particularly those of developed countries, would keep only the most desirable fish, such as halibut, salmon, and cod, in the catch and return the less-desirable species, the so-called "by-catch," to the sea. However, with increased global demand for fishery products as well as improved (and sometimes costlier) fishing technology and processing methods, many species of fish in the "by-catch" heretofore not always utilized are now harvested and processed into edible products.

To help resolve these problems of nomenclature, NMFS, in its role of providing technical and marketing assistance to the fishery industry, organized and coordinated a program to attempt to clarify existing marketing names and provide improved procedures for establishing a uniform seafood nomenclature. As part of this project, NMFS funded a study that eventually involved elements of the U.S. Army Research Laboratories at Natick, Massachusetts (NLABS); the University of Massachusetts; Arthur D. Little Co.; and NMFS laboratories in Gloucester, Massachusetts; Charleston, South Carolina; and Seattle, Washington. As envisioned by NMFS and its collaborators, may be responsible for the clam flavor profile in these freeze-dried extracts.

In processing the 5′-nucleotide can have a significant role in potentiating flavor. Hashida et al. (138) studied the application of 5′-nucleotides to canned seafoods. In canned short-necked clams (*Venerupis semidecusata*), oyster (*Ostrea giges*), and red crab (*Acanthodes armatus*) the flavor of the seafood was enhanced at the following levels: 0.04 or 0.08% Ribotide® (a 1:1 mixture of 5′-IMP · Na_2 and 5′-GMP · Na_2, Takeda Chemical Industries Ltd.) for short-necked clam and red crab meat. The 5′-nucleotides appeared to be somewhat stable to heating and storage. Canned red crab meat retained 77 to 88% of added nucleotides during storage for 28 days, and canned short-necked clams retained 52 to 61% after 189 days.

ATTEMPTS TO USE SEAFOOD FLAVOR AND TEXTURE ATTRIBUTES TO CLASSIFY FISH

Due to a greatly expanded U.S. fishery, the American seafood marketplace contains a variety of new commercially harvested fish with little name familiarity, which has created a minor identity crisis in the industry and for the consumer. Although the National Marine Fisheries Service of the U.S. Department of Commerce (NMFS), the American Fisheries Society, and the International Congress of Zoology maintain scientific names of glossaries of common fish names, these are generally unsuited for use in market identification. To make matters worse, common names for fish species used in the United States contain little or no useful information for the consumer (139). As an example of the problem, some fish have many common names (as many as 5) from as many different locations, and many popular commercial fish have several common names, such king salmon which is also known as chinook, blackmouth, and spring salmon. Also, commonly used names in one region of the United States may be misleading in another, for example, the fish known as "snapper" along the Pacific coast is a species of rockfish (*Sebastes* spp.), not the acceptable *Lutjanidae* spp. from the Gulf of Mexico and Caribbean that the Food and Drug Administration has officially designated as snapper. In other cases, names may be unattractive or pejorative or may reflect ethnic slurs or epithets (eg, ratfish, hagfish, wolffish, jewfish, etc). As a result, consumers can learn little about the edibility characteristics of a product from its name, thus a large variety of seafoods remain effectively unavailable to them. Faced with a bewildering array of common names, it is not surprising that consumers confine their purchases to a few familiar items rather than deal with the unknown.

Because the American consumer was perceived as resisting the purchase of new seafoods that appeared in the marketplace because of a lack of information about what they taste like, NMFS launched a research project to determine the feasibility of developing a national nomenclature system that would group fish species according to their edibility characteristics (flavor and texture). It was felt that both processors and retailers could employ such a system to group together fish that share the same edibility characteristics so that consumers, both new and experienced, would be able to consistently and knowledgeably select their seafood. The system could work much like our classification of wines in the United States (ie, Chablis, Burgundy, claret, etc). Each of these wine classifications can contain one or more specific grape varietals, for example, claret can be made from Cabernet sauvignon, Merlot, or both.

It is a common human trait to classify and group that which interests us. For example, we readily group foods based on ethnic origin (eg, Italian, Mexican, Chinese, Japanese); we even break down the cuisines of a single country (eg, French/Provençal, Chinese/Szechuan, American/Southern, Mexican/"Tex-Mex"). The classification of seafoods is no exception. It is quite common to hear a buyer in the fish market ask, "What is this fish like for eating?" Questions of this type are not at all unreasonable considering the large number of fish species that crowd both Western and Asian marketplaces (more than 1,000 worldwide, 500 in the U.S. alone). Inevitably, the answers to such questions will involve one or more flavor attributes (eg, sweet, mild, strong, etc). Clearly, the flavor of a fish species is one of the most important criteria that the consumer exercises when he or she purchases seafood.

It should be clear that the various chemical compounds that impart characteristic flavors and odors to a particular species of fish are many and complex. Despite the efforts of scientists, certainly as detailed in this chapter, the relationships of specific chemical compounds and observed flavors and odors are yet to be completely understood. To date, the marketplace must ultimately rely on the consumer to determine what a fish "should be." However, to be "knowledgeable" about fish has, in recent years, become increasingly more difficult because of a large increase in the number of fish species and products finding their way into the marketplace (136). Demands on the consumer's discriminatory abilities will be increased as even more fish species are introduced into the marketplace, both in the United States and elsewhere. This increase is due to a variety of political, economic, social, and health reasons. Economic factors are perhaps the easiest to identify. In the past, fishermen, particularly those of developed countries, would keep only the most desirable fish, such as halibut, salmon, and cod, in the catch and return the less-desirable species, the so-called "by-catch," to the sea. However, with increased global demand for fishery products as well as improved (and sometimes costlier) fishing technology and processing methods, many species of fish in the "by-catch" heretofore not always utilized are now harvested and processed into edible products.

To help resolve these problems of nomenclature, NMFS, in its role of providing technical and marketing assistance to the fishery industry, organized and coordinated a program to attempt to clarify existing marketing names and provide improved procedures for establishing a uniform seafood nomenclature. As part of this project, NMFS funded a study that eventually involved elements of the U.S. Army Research Laboratories at Natick, Massachusetts (NLABS); the University of Massachusetts; Arthur D. Little Co.; and NMFS laboratories in Gloucester, Massachusetts; Charleston, South Carolina; and Seattle, Washington. As envisioned by NMFS and its collaborators, the system would be based on similarities in edibility characteristics among the various fish species produced by U.S. commercial fisheries. The system would enable consumers and the fishing industry to make informed choices among species (familiar and unfamiliar) by providing comparative sensory data consumers need to select a desired texture, flavor, and so on, of fish (140,141).

Although sensory analyses are commonly used in fisheries research, quality control, and inspection (142), it was recognized at the beginning that there was no comprehensive set of sensory terms available that was meaningful and useful *to consumers* for making comparisons between different species (141). In addition, because sensory comparisons were to be a part of this project, there was a need for a reliable scaling method that permitted the development of quantifiable numeric sensory profiles that could be statistically manipulated into valid groups. This work included research in methods of cooking; sensory panel techniques; and the development of sensory attributes to describe fish samples, including the development of descriptive terms and selection of a psychophysical method for scaling the magnitude of sensory attributes. Ultimately, a complex protocol was developed for classifying fish based on edibility characteristics, including texture, flavor, and chemical compositional attributes. Although this edibility profile involved both flavor and texture attributes, for purposes of this chapter only the work with flavor will be discussed. More detail will be given to this work because we feel that the sensory methods combined with statistical methods, such as cluster analysis, and the descriptors developed for this project have useful application to other areas of seafood technology.

Sensory Panels

Because of the consumer-oriented nature of this project, a comprehensive list of sensory attributes that could be applied to a wide range of edible fish species (141) was developed. This was achieved through the use of both extensive consumer and trained-taste panel assessments. The descriptive sensory analysis of aroma, flavor-by-mouth, and aftertaste attributes of cooked fish muscle was performed by the Natick flavor profile panel using the Arthur D. Little Flavor Profile Method (143,144). From these panels and related activities, a list of descriptive terms that was applicable to the description of the edibility characteristics of fish was generated through the use of statistical methods that were able to identify attributes that best discriminated among the various species of fish. Concomitantly, the researchers had to select a reliable scaling method for the tasters to use to describe the magnitude of the sensory attributes. The method selected was based on a 7-point category scale of intensity where 1 = slight, 4 = moderate, and 7 = extreme. Complete details of the procedures used to develop the scalar method can be found in Kapsalis (140), Cardello et al. (145), and Sawyer et al. (146). As the result of extensive testing and winnowing, a list of flavor descriptors emerged (Table 4).

Table 4. Sixteen Flavor Attributes Identified by Consumers and Trained Panelists as Important to the Characterization of Fish

Flavor attributes
Overall flavor intensity[a]
Delicate or fresh fish
Heavy or gamey fish[a]
Fish (old fish)
Sweet
Briny, salty[a]
Sour[a]
Seaweed
Bitter
Fish oil[a]
Buttery
Nutty
Musty
Ammonia
Metallic
Shellfish[a]

[a] These flavor attributes were found to be significant discriminators on the basis of stepwise discriminant analysis.

The resulting system was tested by applying the method to a model system of 17 species of North Atlantic fish. Among the species of fish tested were Atlantic whiting (*Merluccius bilinearis*), Atlantic mackerel (*Scomber scombrus*), monkfish (goosefish) (*Lophius americanus*), Atlantic pollock (*Pollachius virens*), and others. Once completed, the results of the texture and flavor panel analyses were compiled as mean texture attributes and consensus ratings for flavor attributes of the 17 species of fish (139). From the compiled data, it was possible to compare and contrast the various species using a composite profile such as that shown in Figure 1 (137), where weakfish and cusk are contrasted. The model was further analyzed to identify groups of fish with similar characteristics using cluster analysis (147). The tree diagram in Figure 2 (140) shows the results of cluster analysis of the combined sensory data. Although limited in scope by the low number of fish evaluated, the purpose of the tree is to show the order of combination of the species. Pollock and tilefish, located opposite number 1 on the left side of the diagram, are paired

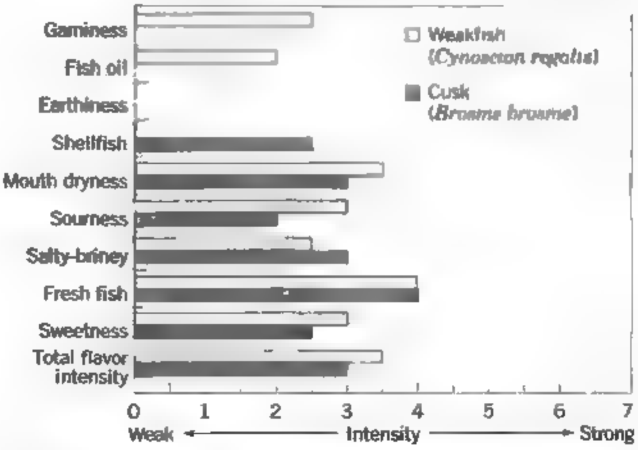


Figure 1. Composite (flavor/texture) sensory profile for weakfish (*Cynoscion regalis*) and cusk (*Brosme brosme*).

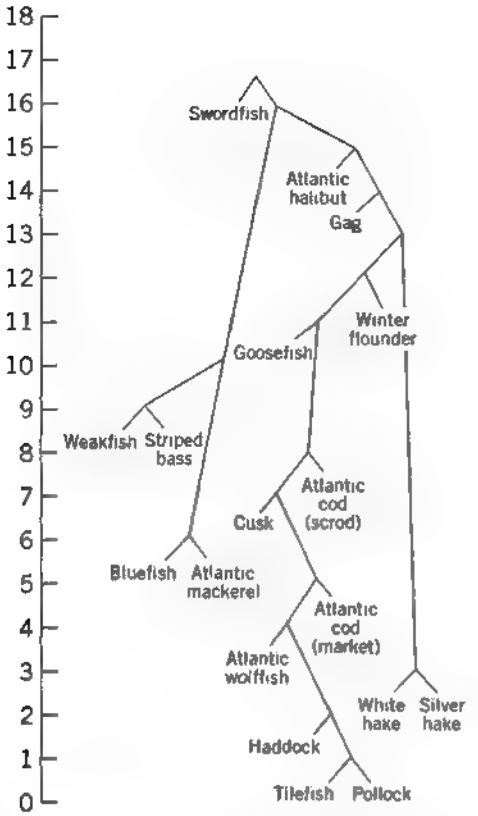


Figure 2. Tree diagram depicting the results of the cluster analysis of combined (flavor/texture) sensory data for 17 species of North Atlantic fish.

together because of their strong sensory similarities. Haddock, appearing opposite step 2, was next most similar to those paired in step 1 whereas white hake and whiting, grouped together, were next in line of sensory similarity (step 3). Hake and whiting were shown together because their profiles were more alike than either tilefish, pollock, or haddock. The amalgamation of all the species was continued until the cluster diagram was completed. The last few species (halibut, grouper, and swordfish) represent fish with the least similar sensory attributes and are weak linkages in the branching system. Figure 2 was divided into two major groups of fish. The first group consists of fish with low fat content, flavor intensity, and white flesh (tilefish, pollock, haddock, wolffish, cod, etc). The second major group includes species such as bluefish, mackerel, weakfish, and striped bass. This group is characterized by high fat content, high flavor intensity, and dark flesh. The third group was represented by swordfish. Within the two major groups of white flesh fish are two subgroups. These are represented by tilefish, pollock, haddock, wolffish, cod, cusk, monkfish, and flounder on one branch and hake and whiting on the adjacent branch.

After the 17 fish species were arranged by cluster analysis according to their characteristic sensory attributes, the original sensory data matrix was used to determine the "reasons" that the various species of fish were grouped together through the use of multidimensional unfolding (148). With this technique, it was possible to visualize the relationships among stimulus objects (individual fish species) and sensory variables (the various flavor or texture attributes) by constructing a statistical map. The resulting map (Figure 3) has embedded within it both the sensory profile attributes as well as the 17 species of fish that were evaluated. Fish species in close proximity to one another are perceptually similar in texture, flavor, and appearance. The proximity of any fish species to any attribute point is an index of the perceived amount of that attribute in that fish species. For a more complete description of these methods and interpretation of the results, the reader is directed to the final report by NLABS summarizing the nomenclature project (140). For a more detailed explanation of the application and uses of cluster analysis, the text by Romesburg (147) is recommended.

After the initial phase of the work was completed, personnel at NLABS trained teams from three NMFS laboratories (Gloucester, Charleston, and Seattle) in their profiling methods. The NMFS laboratories began profiling commercially harvested fish species from their respective regions. Although the NMFS nomenclature program called for a comprehensive study of edibility characteristics, including chemical composition, instrumental color and texture measurements, and evaluation of sensory texture characteristics, we will deal with only the flavor attributes.

For the purposes of edibility profiling, the best-quality fish were used, usually 1 to 3 days postharvest; however, in some cases frozen fish fillets were used because they represented typical commercial product. Nevertheless, all samples used were carefully identified as to species, location, and time (date) of catch. Open consensus sensory panels (with 8–12 members) were run as described by Kapsalis (140) and Prell et al. (110). After scoring each sample, scores were tabulated in common and the results discussed to clarify individual profiles. Where there was disagreement, panelists were encouraged by the panel leader to reach a consensus. Sometimes this was difficult to achieve because of real organoleptic differences between samples of the same species. Each fish sample was replicated a minimum of 3 times during the course of this work. Therefore, the sensory profile of each species described here is a composite of data determined by the taste panel after discussion and agreement. All the combined flavor data describing each species of fish evaluated by NMFS laboratories were subjected to cluster analysis using the hierarchical method employing the BMDP-P2M "Cluster Analysis of Cases" computer program (149).

In all, 74 combined species of fish were analyzed. Results of the initial sensory evaluations are presented in the cluster analysis diagram in Figure 4 and were based on raw, unweighted data. Numbers appearing at the bottom of the diagram indicate the strength of relationships (amalgamations) based on similarities. Low numbers, or early amalgamation, indicate strong similarities between fish species and their flavor characteristics, whereas larger numbers represent fish that were quite dissimilar. From this cluster diagram, it is clear that complex relationships between fish species and their respective flavor profiles were found. Because of the large number of fish species represented in Figure 4 and the complex nature of their

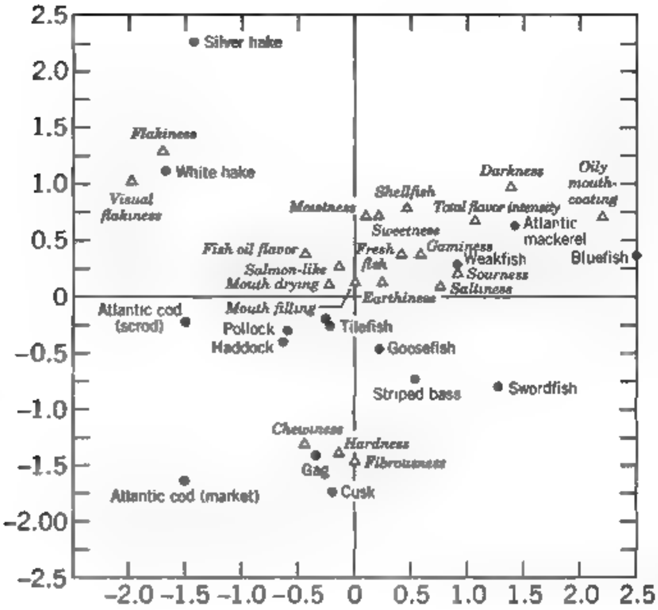


Figure 3. Multidimensional unfolding of analysis (two-dimensional) of relationships among 17 species of North Atlantic fish and estimates of combined sensory (flavor/texture) attributes.

flavor profile relationships, it became obvious that it was necessary to further simplify the cluster diagram in order to provide a far less complicated basis for describing general flavor characteristics, particularly for marketing purposes.

A less-complex cluster diagram or tree could be achieved by analyzing the same group of fish but using only four of the most significant sensory terms from Table 5 (total flavor intensity, sweetness, salty, and sourness). Results of that analysis are shown in Figure 5. Using only these four flavor attributes, the cluster analysis yielded three distinct major groups of fish. Each group, in turn, was composed of a series of subgroups of fish with increasing similarities in sensory attributes. Associations of species are different from those in Figure 4 in that only four attributes were used. We will discuss the three groups that appear in Figure 5 to give a feel for what cluster analysis can yield in its application to flavor profiling. In the discussion of each of these three groups, we will make use of the other flavor attributes, not used in the cluster analysis, as an aid in describing each group.

In Figure 5, the overall flavor profile of fish represented in group 1 was generally characterized by a moderate to moderately low total flavor intensity (TFI); that is, early impact of combined flavors, with a predominant sweet and salty-briny flavor. The intensity of a persistent, but very low, sour flavor was also observed in group 1 fish. Other flavor notes encountered in group 1 fish were gamey (as in fresh-cooked venison and opposed to beef); earthy, fish oil, and mouth drying (astringency), particularly in widow rockfish (*Sebastes entomales*). The first three flavor attributes were considered low to very low, but the sensation of mouth drying was more pronounced. Chinook salmon (*O. tshawytscha*), on the other hand, has a very low earthy flavor note but was characterized by the ever-present fish oil flavor associated with the more oily species. Mouth drying in chinook salmon had a moderately low impact. Pen-raised (in salt water) Atlantic salmon (*Salmo salar*) tended to have slightly more exaggerated minor flavor notes as compared to the king salmon and a more pronounced earthy flavor. The flavor notes fish oil and earthy were also more pronounced (low intensity) in the Atlantic salmon than in the chinook salmon. The effect of mouth drying at the point of swallowing was moderately low. A low-intensity shellfish (lobster, clam), earthy, and fish oil flavor note characterized longspine porgy (*Stenotomus caprinus*), a species with a moderate TFI. However, the mouth drying flavor attribute was found to be very low in this species. The most predominant flavor characteristic of king mackerel (*Scomberomus cavalla*) was a moderately low gamey note followed by a low-intensity earthy flavor and mouth-drying sensation. Although mackerel was considered to be a somewhat flavorful species, in this grouping it falls in the moderate group, due in part to the presence of the tunas and sharks with their high sour notes.

Although defined by a moderate TFI, the flavor profile of spot (*Leiostomus xanthurus*) was most influenced by a slightly low salty flavor, a low gamey note, and a sweet afterflavor. Mouth drying was very minimal in spot. Residual aftertaste of earthy and fish oil were very low intensity. Monkfish (*L. americanus*), or goosefish, had a moderately low overall TFI with a slight salty, sweet flavor and near threshold sour note. The most notable sensory characteristic of this fish was its moderate shellfish flavor note, which is responsible for the fish being referred to as "poor man's lobster." The fish has a persistent shellfish aftertaste while mouth drying is low.

Unlike group 1 fish, which have a higher overall TFI, fish in group 2 are generally characterized by a low TFI and are typically described as mild in flavor. These fish have moderately low to low salty-briny and/or sweet flavor notes, and sourness was generally noticeable as a very low to just barely detectable flavor note. A typical species in

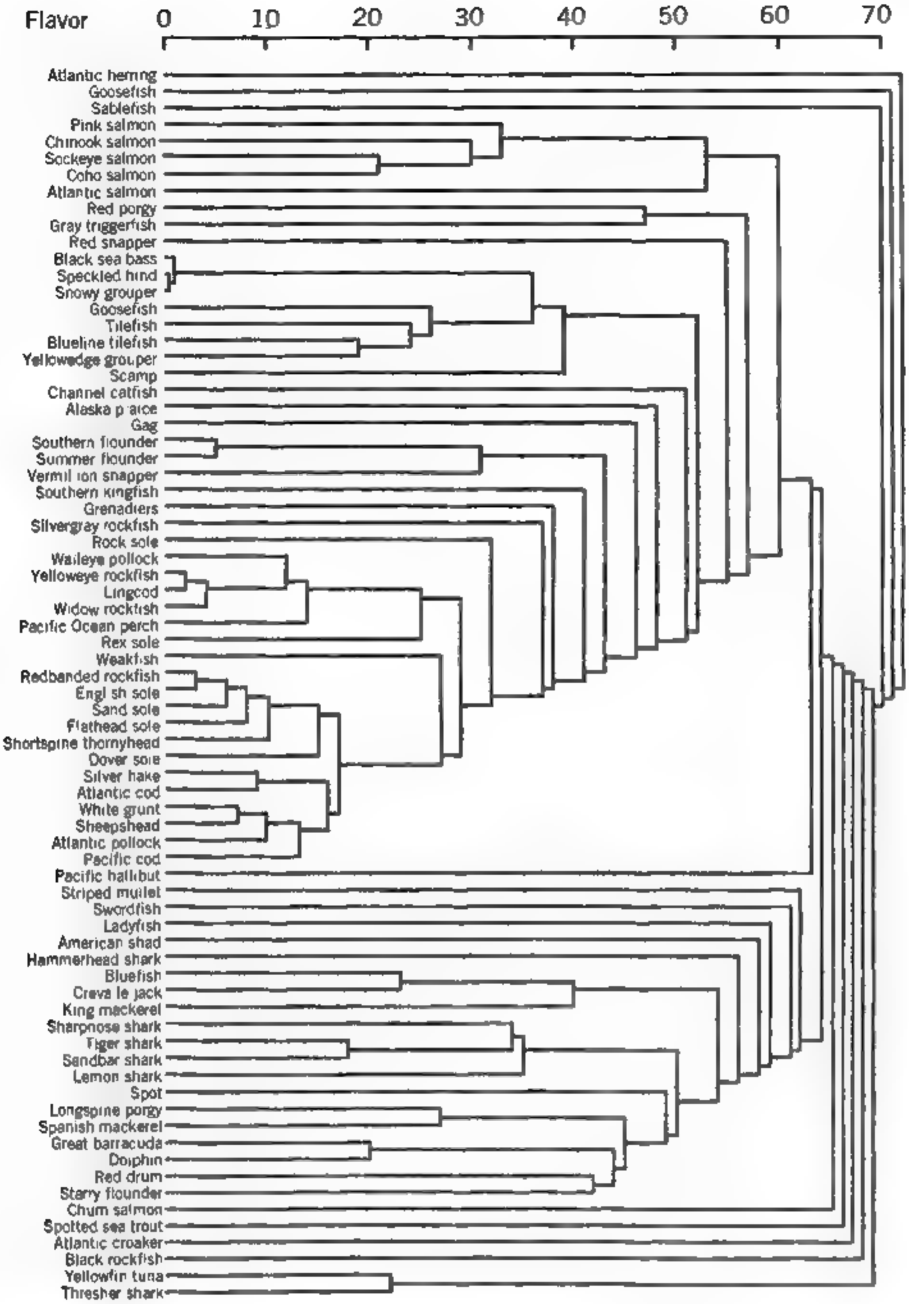

Flavor
0
10
20
30
40
50
60
70
Atlantic herring
Goosefish
Sablefish
Pink salmon
Chinook salmon
Sockeye salmon
Coho salmon
Atlantic salmon
Red porgy
Gray triggerfish
Red snapper
Black sea bass
Speckled hind
Snowy grouper
Goosefish
Tilefish
Blueline tilefish
Yellowedge grouper
Scamp
Channel catfish
Alaska plaice
Gag
Southern flounder
Summer flounder
Vermilion snapper
Southern kingfish
Grenadiers
Silvergray rockfish
Rock sole
Walleye pollock
Yelloweye rockfish
Lingcod
Widow rockfish
Pacific Ocean perch
Rex sole
Weakfish
Redbanded rockfish
English sole
Sand sole
Flathead sole
Shortspine thornyhead
Dover sole
Silver hake
Atlantic cod
White grunt
Sheepshead
Atlantic pollock
Pacific cod
Pacific halibut
Striped mullet
Swordfish
Ladyfish
American shad
Hammerhead shark
Bluefish
Crevalle jack
King mackerel
Sharpnose shark
Tiger shark
Sandbar shark
Lemon shark
Spot
Longspine porgy
Spanish mackerel
Great barracuda
Dolphin
Red drum
Starry flounder
Chum salmon
Spotted sea trout
Atlantic croaker
Black rockfish
Yellowfin tuna
Thresher shark

Figure 4. Expanded cluster analysis diagram (tree) showing relationships (amalgamations) between 74 species and their individual flavor profiles.

this group is English sole (*Parophrys vetulus girard*), which has a low TFI characterized by a somewhat sweet-salty flavor. It also has a very light or threshold gamey-shellfish flavor and is sometimes known to possess a very low earthy note. A bitter or iodine aftertaste was the mouth-drying characteristic in these samples. Similarly, Dover sole (*Microstomus pacificus*) was also a very mild flavored fish with a low TFI with a characteristic slight salty-sweet flavor and a hint of sour and threshold levels of earthy-gamey flavors. The effect of mouth drying was also low and sometimes accompanied by a low iodine and/or bitter aftertaste. Atlantic cod (*Gadus morhua*), also characterized by a low TFI, often had a slight earthy flavor and mouth-drying note and was sometimes characterized by a low shellfish flavor note or a slight sour aftertaste. All of these attributes were found at low intensity levels when encountered. With a slightly stronger TFI (low) than the aforementioned fish, summer flounder (*Paralichthys dentatus*) and southern flounder (*Paralichthys lethostigma*) were relatively mild-flavored fish characterized by a low salty-briny and sour flavor with a very low level of shellfish, sweet, or sometimes earthy flavor notes observed. A fish oil flavor note was sometimes detected in the dark flesh but at or near threshold levels. Mouth drying was observed but at a low intensity. Scamp grouper (*Myteroperca phenox*) was also found to be a species with a low TFI, with a mild salty-sweet flavor and a very low sour note. Scamp was characterized by a slight shellfish flavor

Table 5. Sensory Terms for the Description of Fish Flavor

Total flavor intensity	
The initial or early total impact of flavors	
Salty-brine	A combination of the taste sensations of sodium chloride and the other salt compounds found in ocean water.
Sour	The taste sensation produced by acids. The taste of vinegar or lemon are typical examples.
Shellfish	The flavor associated with any cooked shellfish, such as lobster, clam, or scallop.
Gamey fish	The flavor associated with the heavy, gamey characteristics of some cooked fish such as Atlantic mackerel, as opposed to a delicate flavor such as sole. Analogous to the relationship of the heavy, gamey characteristics of fresh cooked venison compared to fresh cooked beef, or duck to chicken.
Fish oil	The flavor associated with fish oil, such as found in mackerel and canned sardines or cod liver oil.
Sweet	The basic taste sensation of which the taste of sucrose is typical.
Earthy	The flavor associated with slightly undercooked boiled potato, soil, or muddy fish.
Mouth drying	The sensation of dry skin surfaces of the oral cavity; dry feeling in the mouth after swallowing; astringency.
Other flavor notes that may be encountered	
Bitter	The taste sensation of which the taste of a solution of caffeine, quinine, or iodine are typical examples.
Metallic	The taste sensation suggesting the use of slightly oxidized metal, such as tin or iron.
Nutty-buttery	The flavor associated with the rich, full flavor of chopped nuts, such as pecans and warm melted butter.
Canned salmon	The flavor associated with the salmon character in canned salmon.
Old fish	The flavor associated with cooked fish, with an off-note related to trimethylamine.
Stale fish	The flavor associated with cooked fish that is getting "off," but is not yet old.

note and a very low earthy and fish oil aftertaste. Mouth drying was very low in intensity.

Two species of fish in group 2, black rockfish (*Sebastes melanops*) from the Pacific Ocean and spot (*L. xanthurus*) from the Atlantic Ocean, were noted for the mildly heightened TFI as compared to other fish species represented in group 2. Both species were considered to be low salty-sweet flavored with very low sour notes. These fish were also characterized for their slight gamey and earthy flavor notes. Black rockfish was also found to have a very slight fish oil flavor associated with its dark flesh. This species had a moderately low mouth-drying aftertaste whereas spot had a very low level of mouth drying.

As a family, fish in group 3 tended to have a much higher TFI profile than fish from the preceding groups. In this analysis, group 3 represents all of the shark species tested interspersed with several nonshark species, including tuna and herring. The least flavorful in this group were the starry flounder (*Platichthys stellatus*) and sandbar shark (*Carcharhinus plumbeus*). These two different species were characterized by a moderately low TFI and very low sweetness; however, this was accentuated by low salty and/or sour flavors that were more likely to be associated with sharks. Mouth drying or astringency was low but definitely present. Afterflavors of gamey, earthy, and fish oil were often present but were found at very low concentrations. Somewhat stronger-flavored scalloped hammerhead shark (*Spherna lewini*), ladyfish (*Elops sarus*), and lemon shark (*Negaprion brevirostris*) were characterized by a moderately low TFI that was somewhat influenced by a low salty-sour flavor. The effect of mouth drying was slight, and the gamey and earthy notes were predominate afterflavors but at low intensities. Perhaps the most flavorful species in group 3 were yellowfin tuna (*Thunnus albacores*) and thresher shark (*Alopias verlpinus*). These moderately flavored fish had a relatively strong TFI that was characterized by a moderate to moderately strong initial sour flavor. Usually equated with early impact of sour was a salty-briny note, which was found at low levels of flavor intensity. At the same level of intensity was a gamey and/or meaty flavor that was itself somewhat modified by a very slight sweet flavor. An earthy note was also part of the overall flavor profile but at very low levels. The impact of mouth drying was moderately low, and the aftertaste was sour-salty with some gamey note.

It is essentially from the results of research by other workers studying the chemistry of flavors that we are beginning to understand that those characteristics that distinguish one fish from another, such as salmon from sole, are attributed to chemical compounds that are endogenous in origin. We also know that exogenous flavor compounds from the environment can accidentally manifest themselves in fish, but these are generally considered as taints. Chemical compounds responsible for flavor attributes such as sweet, salty, or salty-briny, flavor characteristics common to the soles, are usually identified with the presence of sodium chloride (salt) or carbohydrates. However, the same flavors can also be caused by other chemical components such as peptides (aspartame) or nucleotides (IMP), chemicals known for their ability to impart or potentiate salty or sweet flavors in fish. Peptides are also known to influence bitter, sour, and acidic flavors common to tunas and sharks. Low pH, caused by the accumulation of lactic acid in fish muscle resulting from the breakdown of glycogen, can also produce sour flavors in fish.

Other flavor notes such as clamlike, shellfishlike, buttery, grassy, metallic, or sulfurlike, are the result of chemicals known as organic sulfides. One especially, dimethylsulfide (DMS), has been identified as producing the clam or shellfish flavor in certain gadoids and salmon when present in very small concentrations. Organic sulfides, which by their very nature can be complex, have also been

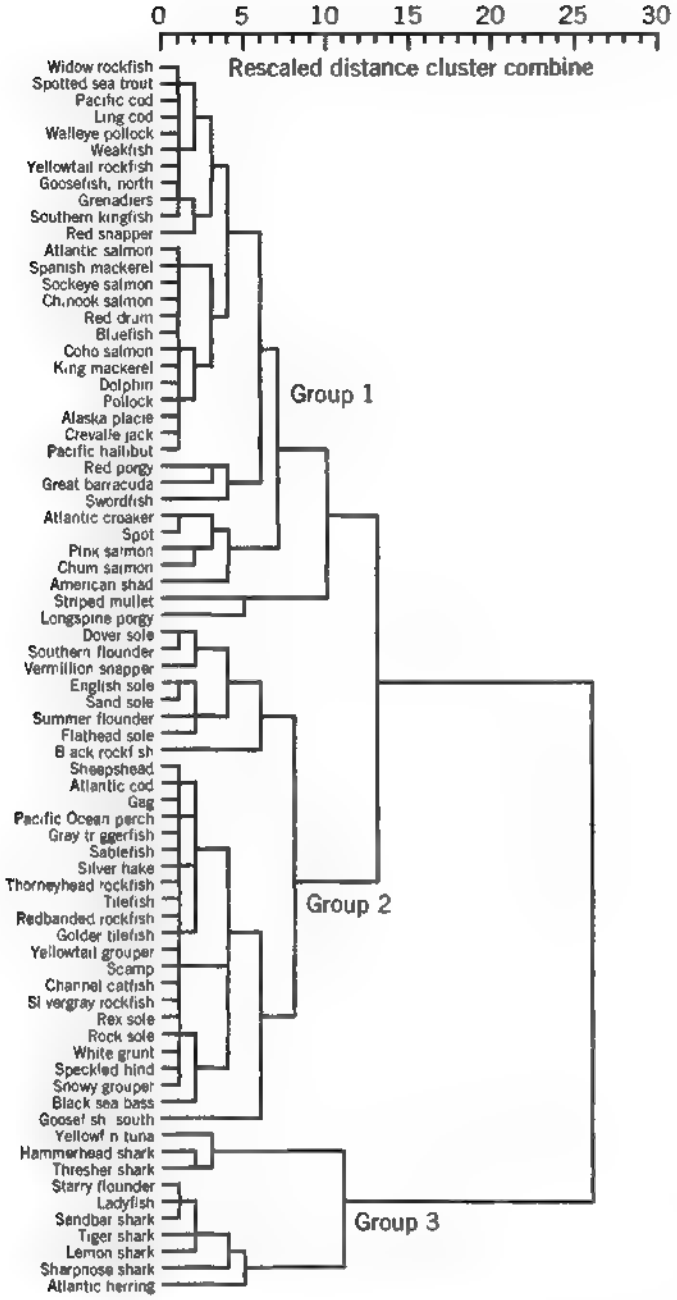


Figure 5. Grouping diagram (modified tree) of 74 species by cluster analysis showing their relationships (amalgamations) based on four significant flavor attributes.

identified with the meaty flavors prominent in some species of tuna and shark. Although organic sulfides occur naturally in fish, natural feeds such as *Spiratella heliciana*, a plankton that contains dimethyl-β-propiothetin that can be converted to DMS, can also contribute to different flavors in fish.

In addition to the various flavors attributed to organic sulfides, alcohols, ketones, and phenols have also been identified as producing their own set of characteristic flavor notes in fish. Phenols are known to cause medicinal flavors and aromas in shrimp and crayfish and possibly are responsible for the metallic-bitter or iodoformic odors and flavors sometimes seen in flatfish, such as Dover or English sole. These flavors and odors are also found in natural feeds, such as algaes and seaweeds.

Fishy odors and flavors, in contradistinction to the desirable fresh-fish flavor, predominantly found in cooked dark-muscled fish like herring or mackerel and sometimes in gadoids (cods, haddocks, hakes, and pollock), are generally caused by the presence of TMA. TMA is easily formed by the breakdown of trimethylamine oxide, which can be accumulated through the diet. The combination of lipid oxidation with TMA appears to contribute to the overall fishy odor and flavor.

The fish oil flavor, identified in the NMFS nomenclature work, was associated with the initial oxidation of fish lipids (triglycerides and fatty acids) and is best described as resembling a light cod liver oil similar to the oils used in packing sardines, and also containing grassy notes (odor of fresh-mown grass) as in fresh king or coho salmon, or a nutty-buttery or light creamy flavor characteristic of the mild-flavored black cod (sablefish).

Because of their close relationship to the environment, fish frequently reflect this connection with off-flavors or taints. For example, descriptors such as earthy, muddy, or musty are often used to describe the flavor of catfish or trout that are raised in artificial environments. Although these flavor notes are often attributed to artificial feeds, the flavors can also be caused by microorganisms living in the water, soil, or pond detritus where the fish are raised. In the marine environment, muddy flavor found in bream were identified as coming from the blue-green algae *Osculatoria agardhii*. These off-flavors are also thought to be produced by heterocyclic organic compounds such as 1,10-*trans*-dimethyl-*trans*-(9)-decolol or geosmin and 2-methyllisoborneal and are the metabolic products of certain species of cyanobacteria and actinomyces.

It is easy to see that edibility profiling combined with the clustering technique provides useful tools for establishing a rational basis for the development of fish edibility groups that can be conveniently used in marketing fishery products. Results from the nomenclature studies conducted by NMFS laboratories show that, although they are complex, fish can indeed be classified into groups according to their inherent flavor characteristics. These studies also suggest that flavor profiling can be used to classify new or commercially underutilized species of fish into edibility groups as they enter the market. Because we limited ourselves to the subject of flavor profiling in this section, only a small portion of the NMFS nomenclature work was described here. However, more species were evaluated but were not included in this discussion because the minimum replicate requirements were not met. Considerably more work remains to be done to complete the edibility profiling.

FUTURE WORK

As we updated this chapter, it became clear that efforts devoted to identifying the chemical components of seafood flavors appeared to have reached a plateau in the late 1980s and very early 1990s, based at least in part on the number of recent publications. At least part of the reason for this may lie in the fact that in the decade since the first edition chapter was written, some very significant changes have occurred within the seafood industry related to the outstanding growth and importance of value-added convenience food products. Part of the cause of these changes have been cultural, such as the increased numbers of families with both spouses working; political, for example, passage of the 1976 Magnuson Fishery Conservation and Management Act (MFCMA) (150); and environmental/economic, for example, overfished, high-valued species such as salmon.

Demand for value-added convenience foods in both North America and Europe is evidenced by the increased popularity of fast-food restaurants worldwide and sales of ready-to-eat and prepared entrées, particularly those that minimize and simplify both home and institutional food preparation—that is, "heat and eat" foods. To lower costs and reduce time, institutional users (restaurants, hospitals, school lunch programs) rely less on in-house food preparation, preferring to utilize centralized production of whole fresh or frozen entrées. Many of these entrées involve new combinations of food forms. Convenience foods have been a staple in supermarkets, but now warehouse/club stores, which may have exclusively serviced the restaurant trade (both fast-food and white-tablecloth types), are now marketing these same items for home consumption. To service all these market segments, the food industry has created new and improved, value-added, complex-formulated food forms. To maintain market niches, the seafood industry has followed suit. New seafood product forms include the use of analogue simulated traditional forms (eg, crabmeat and shrimp), flavored breadings, sauces, and stuffings applied to traditional seafood forms, such as steaks and fillets.

Clearly, interest in seafood flavors and flavor components is now complicated by the presence of other food components, such as extracts, spices, herbs, induced cooking flavors (eg, browning of fried and baked breadings). In such complex cases, the inherent mild flavors of most fish and shellfish are completely lost or submerged in the final presentation.

Passage of MFCMA effectively extended U.S. jurisdiction over the waters of all coastal states and U.S. marine territories. The effect of this legislation was to establish a fisheries conservation zone that enabled the United States to greatly expand its commercial fisheries, most notably in Alaska, where an estimated 10 billion lb of fish and shellfish were available to U.S. fishers. Although much of this fishery pre-existed before the act, namely, cod, halibut, salmon, and crab, a biomass in excess of 5 billion lb of groundfish consisting of walleye pollock, assorted rockfish (*Sebastes* spp.), soles, and flounders was opened up to the U.S. seafood industry when foreign entities were excluded from the newly formed economic zones. At the same time, extension of the Pacific coast's economic zones expanded a fishery for Pacific whiting (200,000 MT) (151), a white fish, facilitating the growth of a new industry in the eastern Pacific. Passage of this act led to one of the most significant developments in the seafood processing industry: the appearance of surimi-based analogue products, primarily artificial crab and shrimp forms. Produced from very mild flavored pollock or whiting, surimi (160 MT in 1997) (155) is finely minced, deboned, washed fish flesh containing stabilizers to which crab or shrimp meats or flavorings are added to make simulated products. These can be used in seafood salads, casseroles, hors d'oeuvres, and other dishes. Here the role of extractable flavorings and the fates of flavor components is of major importance and will need future study.

Stresses on our marine fishery resources, such as overfishing, environmental factors, and economics, have led to the creation of new fisheries enterprises and modified traditional ones. For example, aquaculture and fish culturing are now major contributors to the seafood marketplace. In the past 10 years, there has been outstanding growth in the production of farmed salmon. The largest producer of farmed salmon (500,000 MT) (152) is Norway. Norway competes with Chile, which is the fastest-growing producer of farmed salmon, as well as with Scotland and Canada for sustainable markets. A considerable portion of the salmon consumed in the United States (855 million lb) (152), however, still comes from the capture fisheries. But within the last 10 years, farmed salmon produced in the United States and imported has successfully taken a significant market share. Although salmon, catfish, and trout have been successfully farmed in the United States, other species are now being investigated for possible cultivation. Convenience, value added, and institutional processors are major forces behind this growth. They in turn are being driven by the need for quality, portion and cost control, and year-round availability.

Fish culture raises some interesting questions concerning flavor research. It has been known for over 30 years that cultured fish, such as catfish and salmon, can take on both physical and flavor characteristics of their diets, for example, astaxanthin and canthaxanthin are used to achieve the pink or reddish color expected with salmon. Cultured trout or salmon fed diets high in soybean meal sometimes accrued a "beany" flavor. We have also recognized that a similar phenomenon occurs in wild fish, such as salmon, where unusual flavor notes, or taints, are observed. We have discussed some of these in this chapter. Fish can, at least from a flavor standpoint, become what they eat. This raises the potential of including desired and selective flavor components in the diet of cultured fish, which might include herbs, spices, and other extractables, to achieve new, flavorful seafood products. If not the inclusion of specific flavor components, perhaps alteration of the lipid content could also achieve similar, subtle flavor changes.

CONCLUSIONS

The consumer, acting as the ultimate sensory evaluator, is the final judge of the quality of *any* food. In this capacity, seafood flavor plays a central role in the evaluation of seafood quality. Because of this intimate relationship between quality and flavor, a clear understanding of the components that constitute flavor are significant and important. In this chapter, we have attempted to point out that consumers use seafood flavor for two broad decision-making functions: to classify fish species to determine their use and to determine acceptability. In a broad sense, the consumer asks first, "*Will* I like it?" followed by "*Do* I like it?" The answer to these questions determines repeat purchases of the item, on which rests the future of the seafood industry. Clearly, flavor science and technology has matured during the past decade, primarily due to the advent of modern instrumentation capable of observing and measuring compounds in the nanomole to femtomole range. The combined use of sensitive mass spectroscopy and high-resolution gas and liquid chromatography have helped our understanding of the flavor components associated with varying quality levels of seafoods. In addition, the combined use of sophisticated statistical tools along with sensory scoring techniques has enabled food technologists to put classification of fish species on a rational basis.

It would appear from a brief perusal of this chapter that the data generated over these last two decades on flavor compounds has diverged from the work of the sensory specialist. What then for the future? It is our belief that the dichotomy between flavor and sensory data is more apparent than real. New capabilities (both instrumental and chemical) will continue to be developed and applied to flavor research. In the last decade, instrumentation such as GC–mass spectroscopy, once the domain only of sophisticated academic research laboratories, has become increasingly available because it has decreased remarkably in price while becoming far easier to use and maintain. As with most current technology, this trend is expected to continue, and these methods will find their way into everyday routine Q&A procedures. In addition, the phenomenal and dramatic decrease in the costs of computation has placed extremely powerful analytical and statistical programs on virtually every desk in most laboratories. All of this taken together should yield even more data and information on chemical compounds responsible for flavor. However, even with all of this technical firepower, the role of the human judge or sensory specialist will not be replaced but will become more important. It will be the sensory judges and consumers who will in the final analysis have to integrate the myriad of data observations into a cohesive role for flavor in quality.

BIBLIOGRAPHY

1. M. Hamilton and R. K. Bennett, "Consumer Preferences for Fresh White Fish Species," *J. Consumer Studies and Home Economics* 8, 243–249 (1984).
2. R. J. Hall, "Food Flavors: Benefits and Problems," *Food Technol.* **22**, 1388–1392 (1968).
3. S. S. Chang, "Food Flavors: A Scientific Status Summary by the Institute of Food Technologists' Expert Panel on Food Safety & Nutrition," *Food Technol.* **43**, 99–106 (1989).
4. J. E. Kinsella, "Flavor Perception and Binding," *Inform* **1**, 215–226 (1990).
5. M. O'Mahony, *Sensory Evaluation of Food—Statistical Methods and Procedures*, Marcel Dekker, New York, 1986.
6. G. Jellinek, *Sensory Evaluation of Food—Theory and Practice*, Ellis Horwood, Chichester, United Kingdom, 1985.
7. C. B. Warren and J. P. Walradt, eds., *Computers in Flavor and Fragrance Research*, ACS Symposium Series 261, American Chemical Society, Washington, D.C., 1984.
8. J. C. Boudreau, ed., *Food Taste Chemistry*, ACS Symposium Series 115, American Chemical Society, Washington, D.C., 1979.
9. *Guidelines for the Selection and Training of Sensory Panel Members*, ASTM Special Technical Publication 758, American Society for Testing and Materials, Philadelphia, Pa., 1981.
10. *ASTM Manual on Consumer Sensory Evaluation*, ASTM Special Technical Publication 682, American Society for Testing and Materials, Philadelphia, Pa., 1979.
11. *Compilation of Odor and Taste Threshold Values Data*, ASTM Publication DS 48A, American Society for Testing and Materials, Philadelphia, Pa., 1978
12. *Correlating Sensory Objective Measurements*, ASTM Special Technical Publication 594, American Society for Testing and Materials, Philadelphia, Pa., 1976.

13. *Sensory Evaluation of Appearance of Materials*, ASTM Special Technical Publication 545, American Society for Testing and Materials, Philadelphia, Pa., 1973.
14. *Manual on Sensory Testing Methods*, ASTM Special Technical Publication 434, American Society for Testing and Materials, Philadelphia, Pa., 1968.
15. *Basic Principles of Sensory Evaluation*, ASTM Special Technical Publication 433, American Society for Testing and Materials, Philadelphia, Pa., 1968.
16. F. Shahidi and K. R. Cadwallader, eds., *Flavor and Lipid Chemistry of Seafoods*, ACS Symposium Series 674, American Chemical Society, Washington, D.C., 1997.
17. D. A. Forss, "Odor and Flavor Compounds from Lipids," in R. T. Holman, ed., *Progress in the Chemistry of Fats and Oils and Other Lipids*, Vol. 13, Pergamon Press, Oxford, United Kingdom, 1972, pp. 177–258.
18. M. E. Stansby and G. Jellinek, "Flavor and Odor Characteristics of Fishery Products with Particular Reference to Early Oxidative Changes in Menhaden Oil," in R. Kreuzer, ed., *The Technology of Fish Utilization*, The Fishing News (Books) Ltd., London, 1965, pp. 171–176.
19. G. Hoffmann, "3-*cis*-Hexenal, The 'Green' Reversion Flavor of Soybean Oil," *J. Amer. Oil Chem. Soc.* **38**, 1–3 (1961).
20. D. A. Forss et al., "The Flavor of Cucumbers," *J. Food Sci.* **27**, 90–93 (1962).
21. R. M. Love, *The Food Fishes—Their Intrinsic Variation and Practical Implications*, Van Nostrand Reinhold, New York, 1988.
22. L. F. Jacober and A. G. Rand, "Biochemical Evaluation of Seafood," in R. E. Martin et al., eds., *Chemistry and Biochemistry of Marine Food Products*, AVI Publishing Co., Westport, Conn., 1982, pp. 347–365.
23. C. H. Castell and R. E. Triggs, "Spoilage of Haddock in the Trawlers at Sea: The Measurement of Spoilage and Standards of Quality," *J. Fish. Res. Board Canada* **12**, 329–341 (1955).
24. J. M. Shewan et al., "The Development of a Numerical Scoring System for the Sensory Assessment of the Spoilage of Wet White Fish Stored in Ice," *J. Sci. Food Agric.* **4**, 283–298 (1953).
25. B. G. Shaw and J. M. Shewan, "Psychrophilic Spoilage Bacteria of Fish," *J. Appl. Bacteriol.* **31**, 89–96 (1968).
26. A. Kamiya and Y. Ose, "Study of Odorous Compounds Produced by Putrefaction of Foods. V. Fatty acids, Sulphur Compounds and Amines," *J. Chromatogr.* **292**, 383–391 (1984).
27. D. B. Josephson, R. C. Lindsay, and G. Olafsdottir, "Measurement of Volatile Aroma Constituents as a Means for Following Sensory Deterioration of Fresh Fish and Fishery Products," in D. E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier Science Publishers, Amsterdam, The Netherlands, 1986, pp. 27–47.
28. D. B. Josephson, R. C. Lindsay, and D. A. Stuiber, "Identification of Compounds Characterizing the Aroma of Fresh Whitefish (*Coregonus clupeaformis*)," *J. Agric. Food Chem.* **31**, 326–330 (1983).
29. D. B. Johsephson, R. C. Lindsay, and D. A. Stuiber, "Variations in Occurrences of Enzymatically Derived Volatile Aroma Compounds in Salt- and Freshwater Fish," *J. Agric. Food Chem.* **32**, 1344–1347 (1984).
30. D. B. Josephson and R. C. Lindsay, "Enzymic Generation of Volatile Aroma Compounds from Fresh Fish," in T. H. Parliment and R. Croteau, eds., *Biogeneration of Aromas*, ACS Symposium Series 317, American Chemical Society, Washington, D.C., 1986, pp. 201–219.
31. F. A. Frazzalari, ed., *Compilation of Odor and Taste Threshold Value Data*, American Society for Testing and Materials, Philadelphia, Pa., 1978, pp. 79–80, 84, 116–117, 120.
32. H. Pyysalo and M. Suihko, *Lebensm.-Wiss. Technol.* **9**, 371 (1976).
33. P. A. T. Swoboda and K. E. Peers, *J. Sci. Food Agr.* **28**, 1019 (1977).
34. F. B. Whitfield et al., *Aust. J. Chem.* **35**, 373 (1982).
35. R. G. Buttery, in R. Teranishi, R. A. Flath, and H. Sugisawa, eds., *Flavor Research—Recent Advances*, Marcel Dekker, New York, 1981, pp. 180–184.
36. F. B. Whitfield et al., "Oct-1-en-3-ol and (5Z)-Octa-1,5-dien-3-ol, Compounds Important in the Flavour of Prawns and Sand-Lobsters," *Austr. J. Chem.* **35**, 373–383 (1982).
37. T. M. Berra, J. F. Smith, and J. D. Morrison, "Probable Identification of the Cucumber Odor of the Australian Grayling *Prototroctes maraena*," *Trans. Amer. Fisheries Soc.* **111**, 78–82 (1982)
38. C. Karahadian and R. C. Lindsay, "Role of Oxidative Processes in the Formation and Stability of Fish Flavors," in R. Teranishi, R. G. Buttery, and F. Shahidi, eds., *Flavor Chemistry Trends and Developments* ACS Symposium Series 388, American Chemical Society, Washington D.C., 1989, pp. 60–75.
39. D. B. Josephson, R. C. Lindsay, and D. A. Stuiber, "Biogenesis of Lipid Derived Volatile Compounds in the Emerald Shiner (*Notropis atherinodes*)," *J. Agric. Food Chem.* **32**, 1347–1352 (1984).
40. J. Kanner and J. E. Kinsella, "Lipid Deterioration Initiated by Phagocytic Cells in Muscle Foods: β-Carotene Destruction by a Myeloperoxidase-Hydrogen Peroxide-Halide System," *J. Agric. Food Chem.* **31**, 370–376 (1983).
41. J. B. German and J. E. Kinsella, "Lipid Oxidation in Fish Tissue. Enzymatic Initiation via Lipoxygenase," *J. Agric. Food Chem.* **24**, 680–683 (1985).
42. J. Kanner, J. B. German, and J. E. Kinsella, "Initiation of Lipid Peroxidation in Biological Systems," *CRC Crit. Rev. Food Sci. Nutrition* **25**, 317–364 (1987).
43. R. J. Hsieh, J. B. German, and J. E. Kinsella, "Lipoxygenase in Fish Tissue: Some Properties and the 12-Lipoxygenase from Trout Gill," *J. Agric. Food Chem.* **36**, 680–685 (1988).
44. J. B. German and J. E. Kinsella, "Hydroperoxide Metabolism in Trout Gill Tissue: Effect of Glutathione on Lipoxygenase Products Generated from Arachidonic Acid and Docosahexaenoic Acid," *Biochim. Biophys Acta* **879**, 378–387 (1986).
45. R. J. Hsieh and J. E. Kinsella, "Lipoxygenase Generation of Specific Volatile Flavor Carbonyl Compounds in Fish Tissues," *J. Agric. Food Chem.* **37**, 279–286 (1989).
46. D. B. Josephson and R. C. Lindsay, "Retro-Aldol Related Degradations of 2,4-Decadienal in the Development of Staling Flavors in Fried Foods," *J. Food Sci.* **52**, 1186–1190, 1218 (1987).
47. K. Kubota and A. Kobayashi, "Identification of Unknown Methyl Ketones in Volatile Flavor Components from Cooked Small Shrimp," *J. Agric. Food Chem.* **36**, 121–123 (1988).
48. A. Kobayachi et al., "Syntheses and Sensory Characterization of 5,8,11-Tetradecatrien-2-One Isomers," *J. Agric. Food Chem.* **37**, 151–154 (1989).
49. U. Tanchotikul and T. C. Y. Hsieh, "Volatile Flavor Components in Crayfish Waste," *J. Food Sci.* **54**, 1515–1520 (1989)
50. W. Vejaphan, T. C. Y. Hsieh, and S. S. Williams, "Volatile Flavor Components from Boiled Crayfish (*Procambarus clarkii*) Tail Meat," *J. Food Sci.* **53**, 1666–1670 (1988).

51. R. G. Buttery et al., "Additional Volatile Components of Cabbage, Broccoli, and Cauliflower," *J. Agric. Food Chem.* **24**, 829–832 (1976).
52. M. E. Stansby, "Odors and Flavors," in M. E. Stansby, ed., *Fish Oils, Their Chemistry, Technology, Stability, Nutritional Properties, and Uses*, AVI Publishing Co., Westport, Conn., 1967, pp. 171–180
53. M. E. Stansby, "Speculations on Fishy Odors and Flavors," *Food Technol.* **16**, 28–32 (1962).
54. W. L. Davies and E. Gill, "Investigations on Fishy Flavors," *Chem. and Ind.* (London) **55**, 141T (1936).
55. W. L. Davies, "Fishiness as a Flavor and Taint," *Flavours* **2**, 18 (1939).
56. J. M. Regenstein and M. A. Schlosser, in R. E. Martin et al., eds., *Chemistry and Biochemistry of Marine Food Products*, AVI Publishing Co., Westport, Conn., 1982, pp. 137–148.
57. C. E. Hebard, G. J. Flick, and R. E. Martin, in R. E. Martin et al., eds., *Chemistry and Biochemistry of Marine Food Products*, AVI Publishing Co., Westport, Conn., 1982, pp. 149–304
58. J. C. Wekell and H. J. Barnett, "A New Method for the Analysis of Trimethylamine Oxide using Ferrous Sulfate and EDTA," *J. Food Sci.* **56**, 132–135, 138 (1991).
59. J. Spinelli and B. Koury, "Nonenzymic Formation of Dimethylamine in Dried Fishery Products," *J. Agric. Food Chem.* **27**, 1104–1108 (1979).
60. N. R. Jones, "Fish Flavors," in H. W. Schultz, E. A. Day, and L. M. Libbey, eds., *Symposium on Food: The Chemistry and Physiology of Flavors*, AVI Publishing Co., Westport, Conn., 1967, pp. 267–295.
61. T. Kikuchi, S. Wada, and H. Suzuki, "Significance of Volatile Bases and Volatile Acids in the Development of Off-flavor of Fish Meat," *J. Jpn. Soc. Food. Nutrition* **29**, 147–152 (1976).
62. J. B. Rossell, "Measurement of Rancidity," in J. C. Allen and R. J. Hamilton, eds., *Rancidity in Foods*, Applied Science Publishers, London, 1983, pp. 21–45.
63. C. Karahadian and R. C. Lindsay, "Evaluation of Compounds Contributing Characterizing Fishy Flavors in Fish Oils," *J. Am. Oil Chemists Soc.* **66**, 953–960 (1989).
64. R. J. Hamilton, "The Chemistry of Rancidity in Foods," in J. C. Allen and R. J. Hamilton, eds., *Rancidity in Foods*, Applied Science Publishers, London, 1983, pp. 1–20.
65. R. Hardy, "Fish Lipids Part 2," in J. J. Connell, ed., *Advances in Fish Science and Technology*, Fishing News Books, Surrey, United Kingdom, 1980, pp. 103–111.
66. J. Sargent, R. J. Henderson, and D. R. Tocher, "The Lipids," in J. E. Halver, ed., *Fish Nutrition*, 2nd ed., Academic Press, San Diego, Calif., 1989, pp. 154–218
67. T. Roubal, *Lipid Peroxidation Damage to Biological Materials*, Ph.D. Dissertation, University of California, Davis, Calif., 1965.
68. A. Khayat and D. Schwall, "Lipid Oxidation in Seafood," *Food Technol.* **37**, 130–140 (1983).
69. H. O. Hultin, R. E. McDonald, and S. D. Kelleher, "Lipid Oxidation in Fish Muscle Microsomes," in R. E. Martin et al., eds., *Chemistry and Biochemistry of Marine Food Products*, AVI Publishing Co., Westport, Conn., 1982, pp. 1–11.
70. C. H. Castell, "Metal Catalyzed Lipid Oxidation and Changes of Proteins in Fish," *J. Am. Oil Chemists Soc.* **48**, 645–649 (1971).
71. F. Ullrich and W. Grosch, "Identification of the Most Intense Odor Compounds Formed During Autoxidation of Methyl Linolenate at Room Temperature," *J. Am. Oil Chemists Soc.* **65**, 1313–1317 (1988).
72. P. A. T. Swoboda and K. E. Peers, "Metallic Odour caused by Vinyl Ketones Formed in the Oxidation of Butterfat. The Identification of Octa-1,*cis*-5-dien-3-One," *J. Sci. Food. Agric.* **28**, 1019–1024 (1977).
73. T. C. Y. Hsieh et al., "Characterization of Volatile Components of Menhaden Fish (*Brevoortia tyrannus*) Oil," *J. Am. Oil Chemists Soc.* **66**, 114–117 (1989).
74. N. N. Gerber, "Geosmin, An Earthy-smelling Substance Isolated from *Actinomycetes*," *Biotechnol. Bioeng.* **9**, 321–327 (1967).
75. N. N. Gerber, "Volatile Substances from *Actinomycetes*: Their Role in the Odor of Pollution of Water," *Water Sci. Tech.* **15**, 115–125 (1983).
76. P. B. Johnsen, G. V. Civille, and J. R. Vercellotti, "A Lexicon of Pond-raised Catfish Flavor Descriptors," *J. Sensory Studies* **2**, 85–91 (1987).
77. M. Yurkowski and J.-A. L. Tabachek, "Identification, Analysis, and Removal Ofgeosmin from Muddy Flavoured Trout," *J. Fish. Res. Board Can.* **31**, 1851–1858 (1974).
78. M. Yurkowski and J.-A. L. Tabachek, "Geosmin and 2-Methylisoborneol Implicated as a Cause of Muddy Odor and Flavor in Commercial Fish from Cedar Lake, Manitoba," *Can. J. Fish. Aquat. Sci.* **37**, 1449–1450 (1980).
79. P. E. Persson, "The Source of Muddy Odor in Bream (*Abramis brama*) from the Porvoo Sea Area (Gulf of Finland)," *J. Fish. Res. Board Can.* **36**, 883–890 (1979).
80. N. Sugiura, O. Yagi, and R. Suda, "Musty Odor from Blue-green Alga, *Phormidium tenue* in Lake Kasumigaura," *Environ. Tech. Lett.* **7**, 77–86 (1986).
81. J. F. Martin et al., "Analysis of 2-Methylisoborneol in Water, Mud, and Channel Catfish (*Ictalurus punctatus*) from Commercial Culture Ponds in Mississippi," *Can. J. Aquat. Sci.* **44**, 909–912 (1987).
82. J. F. Martin, T. H. Fisher, and L. W. Bennett, "Musty Odor in Chronically Off-Flavored Channel Catfish: Isolation of 2-Methyleneboronane and 2-Methyl-2-Bornene," *J. Agric. Food Chem.* **36**, 1257–1260 (1988).
83. R. T. Lovell et al., "Geosmin and Musty-Muddy Flavors in Pond-Raised Channel Catfish," *Trans. Amer. Fisheries Soc.* **115**, 485–489 (1986).
84. N. N. Gerber and H. A. Lechevalier, "Production of Geosmin in Fermentors and Extraction with Ion-Exchange Resin," *Appl. Microbiol.* **34**, 857–858 (1977).
85. K. Sivonen, "Factors Influencing Odour Production by *Actinomycetes*," *Hydrobiologia* **86**, 165–170 (1982).
86. R. G. Buttery and J. A. Garibaldi, "Geosmin and Methylsioborneol in Garden Soil," *J. Agric. Food Chem.* **24**, 1246–1247 (1976).
87. T. E. Acree et al., "Geosmin, the Earthy Component of Table Beet Odor," *J. Agric. Food Chem.* **24**, 419–430 (1976).
88. K. E. Murray, P. A. Bannister, and R. G. Buttery, "Geosmin: An Important Volatile Constituent of Beetroot (*Beta vulgaris*)," *Chem. Ind.* (London), 973 (1975).
89. G. P. Slater and V. C. Blok, "Volatile Compounds of the Cyanophyceae—A Review," *Water Sci. Techn.* **15**, 181–190 (1983).
90. G. Izaguirre et al., "Production of 2-Methylisoborneol by Two Benthic Cyanophyta," *Water Sci. Tech.* **15**, 211–220 (1983).
91. R. T. Lovell and L. A. Sackey, "Absorption of Musty Flavor by Channel Catfish Held in Monospecies Cultures of Blue-

Green Algae," *Trans. Amer. Fisheries Soc.* **102**, 774–777 (1973).

92. R. G. Ackman, J. Hingley, and K. T. McKay, "Dimethyul Sulfide as an Odor Component in Nova Scotia Fall Mackerel," *J. Fish. Res. Board Can.* **29**, 1085–1088 (1972).
93. T. Motohiro, "Studies on the Petroleum Odour in Canned Chum Salmon," referenced in S. Ikeda in J. J. Connell, ed., *Advances in Fish Science and Technology*, Fishery News Books Ltd., Surrey, United Kingdom, 1980.
94. J. C Spios and R. G. Ackman, "Association of Dimethyl Sulphide with the 'Blackberry Odour' in Cod from the Labrador Area," *J. Fish. Res Board Can.* **21**, 423–425 (1964).
95. F B. Whitfield, D. J. Freeman, and P. A. Bannister, "Dimethyl Trisulphide: An Important Off-flavour Component in the Royal Red Prawn (*Hymenopenaeus sibogae*)," *Chem. Ind.* (London), 692–693 (1981).
96. A. Miller, III, et al., "Volatile Compounds Produced in Sterile Fish Muscle (*Sebastes melanops*) by *Pseudomonas perolens*," *Appl. Microbiol.* **25**, 257–261 (1973).
97. A. Miller, III, et al., "Volatile Compounds Produced in Sterile Fish Muscle (*Sebastes melanops*) by *Pseudomonas purtrefaciens, Pseudomonas fluorescens*, and an *Achromobacter* Species," *Appl. Microbiol* **26**, 18–21 (1973).
98. C. H. Castell and M. F. Greenough, "The Action of *Pseudomonas* on Fish Muscle I. Organisms Responsible for Odours Produced During Incipient Spoilage of Chilled Fish Muscle," *J. Fish. Res. Board Can.* **14**, 617–625 (1957).
99. C. H. Castell and M. F. Greenough, "The Action of *Pseudomonas* on Fish Muscle. IV. Relation Between Substrate Composition and the Development of Odours by *Pseudomonas fragi*," *J. Fish. Res. Board Can.* **16**, 21–31 (1959).
100. C. H. Castell, M. F. Greenough, and N. L. Jenkin, "The Action of *Pseudomonas* on Fish Muscle. II. Musty and Potatoe-like Odours," *J. Fish. Res Board Can.* **14**, 775–782 (1957).
101. K. Kubota, A. Kobayashi, and T. Ymanishi, "Some Sulfur Containing Compounds in Cooked Odor Concentrate from Boiled Antarctic Krills (*Euphausia superba* Dana)," *Agric. Biol. Chem.* **44**, 2677–2682 (1980).
102. K. Kubota, H. Shyimaya, and A. Kobayashi, "Volatile Components of Roasted Shrimp," *Agric. Biol. Chem.* **50**, 2867–2873 (1986).
103. S. H. Choi, A. Kobayashi, and T. Yamanishi, "Odor of Cooked Small Shrimp, *Acetes japonica* Kishinouye: Difference Between Raw Material and Fermented Product," *Agric. Biol Chem.* **47**, 337–342 (1983).
104. H. H. Huss, "Fresh Fish—Quality and Quality Changes," FAO Fisheries Series No. 29, Food and Agriculture Organization of the United Nations, Danish International Development Agency, Rome, Italy, 1988.
105. "Peroxide Value of Oils and Fats Titration Method Final Action," American Oil Chemists Society Method 965.33, in K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Vol. 2, Association of Analytical Chemists, Inc., Arlington, Va., 1990.
106. *British Standard 684*, British Standards Institution, London, 1976.
107. D. W Lemon, "An Improved TBA Test for Rancidity," New Series Circular No. 51, Canadian Fisheries and Marine Service Halifax, Nova Scotia, 1975.
108. W. Vyncke, "Evaluation of the Direct Thobarbituric Acid Extraction Method for Determining Oxidative Rancidity in Mackerel (*Scomber scrombus* L.)," *Fette, Seifen, Anstrichm.* **77**, 239–240 (1975).
109. F. M. Sawyer, "Sensory Methodology for Estimating Quality Attributes of Seafoods," in D E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier Science Publishers, Amsterdam, The Netherlands, 1987, pp. 89–97.
110. P. A. Prell and F. M. Sawyer, "Flavor Profiles of 17 species of North Atlantic Fish," *J. Food Sci.* **53**, 1036–1042 (1988).
111. M. Tada, I. Shinoda, and H. Okai, "L-Ornithyltaurine, A New Salty Peptide," *J. Agric. Food Chem.* **32**, 992–996 (1984).
112. Y. Kawasaki et al., "Glycine Methyl Ester Hydrochloride as the Simplest Examples of Salty Peptides and Their Derivatives," *Agric. Biol. Chem.* **52**, 2679–2681 (1988).
113. T. Seki et al., "Further Study on the Salty Peptide Ornithyl-β-Alanine. Some Effects of pH and Additive Ions on the Saltiness," *J. Agric. Food Chem.* **38**, 25–29 (1990).
114. S. Yamaguchi, "The Umami Taste." in J. C. Boudreau, ed., *Food Taste Chemistry*, ACS Symposium Series 115, American Chemical Society, Washington D.C., 1979, pp. 33–51.
115. S. Yamaguchi and K. Ninomiya, "Umami and Palatability," abstract for 216th American Chemical Society Meeting, Boston, Mass., August 23–27, 1998
116. *Ribotide, A Flavor Enhancer*, Takeda Chemical Industries, Ltd.
117. M. Fujimaki et al., "Taste Peptide Fractionation from a Fish Protein Hydrolysate," *Agric. Biol. Chem.* **37**, 2891–2898 (1973).
118. M. Noguchi et al., "Isolation and Identification of Acidic Oligopeptides Occurring in a Flavor Potentiating Fraction from a Fish Protein Hydrolysate," *J. Agric. Food Chem.* **23**, 49–53 (1975).
119. S. Arai et al., "Tastes of L-Glutamyl Oligopeptides in Relation to Their Chromatographic Properties," *Agric. Biol. Chem.* **37**, 151–156 (1973).
120. Y. Yamsaki and K. Maekawa, "A Peptide with Delicious Taste," *Agric. Biol. Chem.* **42**, 1761–1765 (1978).
121. Y. Yamasaki and K. Maekawa, "Synthesis of a Peptide with Delicious Taste," *Agric. Biol. Chem.* **44**, 93–97 (1980).
122. M. Tamura et al., "The Relationship Between Taste and Primary Structure of 'Delicious Peptide' (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) from Beef Soup," *Agric. Biol. Chem.* **53**, 319–325 (1989).
123. S. Yamaguchi et al., "Measurement of the Relative Taste Intensity of some L-α-Amino Acids and 5′-Nucleotides," *J. Food Sci.* **36**, 846–849 (1971).
124. J. M. Kennish and D. E. Kramer, "A Review of High-Pressure Liquid Chromatographic Methods for Measuring Nucleotide Degradation in Fish Muscle," in D. E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier Science Publishing, New York, 1987.
125. T. Saito and K. Arai, "Studies on the Organic Phosphates in Muscle of Aquatic Animals. V: Changes in Muscular Nucleotides of Carp During Freezing and Storage," *Bull. Jpn. Soc Sci. Fish.* **23**, 265–268 (1957).
126. B.-O. Kassemsarn et al., "Nucleotide Degradation in the Muscle of Iced Haddock (*Gadus aeglefinus*), Lemon Sole (*Pleuronectes microcephalus*), and Plaice (*Pleuronectes platessa*)," *J. Food Sci.* **28**, 28–37 (1963).
127. J. Spinelli, M. Eklund, and D. Miyauchi, "Measurement of Hypoxanthine in Fish as a Method of Assessing Freshness," *J. Fish. Res. Board Can.* **29**, 710–714 (1964)
128. D. I. Fraser, S. G. Simpson, and W. J. Dyer, "Very Rapid Accumulation of Hypoxanthine in the Muscle of Redfish Stored in Ice," *J. Food Sci.* **25**, 817–821 (1968)

129. D. E. Kramer et al., data presented at the 4th International Congress on Engineering and Food, Edmonton, Alberta, Canada, July, 1985.
130. V. M. Creelman and N. Tomlinson, "Inosine in the Muscle of Pacific Salmon Stored in Ice," *J. Fish. Res. Board Can.* **17**, 449–451 (1960).
131. S. Ehira and H. Uchiyama, "Determination of Fish Freshness Using the *K* Value and Comments on Some Other Biochemical Changes in Relation to Freshness," in D. E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier Science Publishing, New York, 1987, pp. 185–207.
132. H. A. Bremner et al., "Nucleotide Catabolism: Influence on the Storage Life of Tropical Species of Fish from the North West Shelf of Australia," *J. Food Sci.* **53**, 6–11 (1988).
133. D. H. Greene and E. I. Bernatt-Byrne, "Adenosine Triphosphate Catabolites as Flavor Compounds and Freshness Indicators in Pacific Cod (*Gadus macrocephalus*) and Pollock (*Theragra chalcogramma*)," *J. Food Sci.* **55**, 257–258 (1990).
134. S. Konosu, "The Taste of Fish and Shellfish," in J. C. Boudreau, ed., *Food Taste Chemistry* ACS Symposium Series 115, American Chemical Society, Washington D.C., 1979, pp. 185–203.
135. S. Konosu and Y. Maeda, "Muscle Extracts of Aquatic Animals. IV. Distribution of Nitrogenous Constituents in the Muscle Extracts of an Abalone, *Haliotis gigantea discus* Reeve," *Bull. Jpn. Soc. Sci. Fish.* **27**, 251–254 (1961).
136. K. Endo, M. Hujita, and W. Simidu, "Studies on Muscle of Aquatic Animals. Free Amino Acids, Trimethylamine oxide, and Betaine in Squids," *Bull. Jpn. Soc. Sci. Fish.* **28**, 833–836 (1962).
137. N. R. Reddy et al., "Characterization and Utilization of Dehydrated Wash Waters from Clam Processing Plants as Flavoring Agents," *J. Food. Sci.* **54**, 55–59, 182 (1989).
138. W. Hashida, T. Mouri, and I. Shiga, "Application of 5′-Ribonucleotides to Canned Seafoods," *Food Technol.* **22**, 1436–1441 (1968).
139. R. E. Martin, W. H. Doyle, and J. R. Brooker, "Toward and Improved Seafood Nomenclature System," *Mar. Fish. Rev.* **45**, 1–20 (1983).
140. J. G. Kapsalis, "Consumer and Instrumental Edibility Measures for Grouping of Fish Species," Final Report, National Technical Information Service, U.S. Dept. of Commerce, Springfield, Va., 1980.
141. A. V. Cardello et al., "Sensory Methodology for the Classification of Fish According to Edibility Characteristics," *Lebensm. Wiss. und Technol.* **16**, 190–194 (1983).
142. F. J. King et al., "Consumer and Instrumental Edibility Measures for Grouping Fish Species," in J. J. Connell, ed., *Advances in Fish Science and Technology*, Fishing News (Books), Ltd., London, 1980.
143. S. E. Cairncross and L. Sjostrom, "Flavor Profiles—New Approach to Flavor Problems," *Food Technol.* **4**, 308–311 (1950).
144. J. F. Caul, "The Profile Method of Flavor Analysis," E. M. Mrak and G. F. Stewart, eds., in *Advances in Food Research*, Academic Press, New York, 1957.
145. A. V. Cardello et al., "Sensory Evaluation of the Texture and Appearance of 17 Species of North Atlantic Fish," *J. Food Sci.* **47**, 1818–1823 (1982).
146. F. M. Sawyer, A. V. Cardello, and P. A. Prell, "Consumer Evaluation of the Sensory Properties of Fish," *J. Food Sci.* **53**, 12–18, 24 (1988).
147. H. C. Romesburg, *Cluster Analysis for Researchers*, Van Nostrand Reinhold, New York, 1984.
148. C. J. Coombs, *A Theory of Data*, Wiley, New York, 1964.
149. L. Engelman, "Cluster Analysis of Cases," in W. J. Dixon and M. B. Brown, eds., *Biomedical Computer Programs, P-Services*, University of California Press, Berkeley, Calif., 1977.
150. National Marine Fisheries Service, *Our Living Oceans. The Economic Status of U.S. Fisheries*, Technical Memorandum NMFS F/SPO-22, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, 1996.
151. National Marine Fisheries Service, *Fisheries of the United States, 1996*, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, D.C., 1997.
152. H. M. Johnson et al., *1998 Annual Report on the United States Seafood Industry*, 6th ed., H. M. Johnson and Associates, Bellevue, Wash., 1998.

JOHN C. WEKELL
HAROLD J. BARNETT
National Oceanic and Atmospheric Administration
Seattle, Washington

See also SENSORY SCIENCE: PRINCIPLES AND APPLICATIONS.

SEAFOOD: SENSORY EVALUATION AND FRESHNESS

The term fresh seafood refers to a concept, not a distinct object or a specified actuality. Therefore it has many different definitions, such as (*1*) seafood that has never been frozen, cooked, cured, or otherwise preserved (1,2); (*2*) seafood that has the characteristics of being newly harvested and is not the opposite of stale (3); (*3*) a seafood that is the opposite of stale (4); (*4*) seafood that arrived at the store at a particular time (5); and (*5*) raw product that has not progressed beyond a certain degree of microbiological or chemical degradation (6). Depending on which definition is used, it may be very difficult to objectively determine if a specific seafood is actually "fresh." However, when freshness is defined in terms of the sensory characteristics (appearance, flavor, odor, and/or texture) of the specific seafood being evaluated, it is very possible to objectively determine the freshness of that seafood. A seafood of optimum freshness would be one that possesses the characteristics concerning appearance, flavor, odor, and/or texture that are normally associated with that particular seafood product or species, that is, it is caught at the best time of year; caught in the best location; caught by the best method; and handled, processed, prepared, and served in the best manner.

Seafood freshness is considered an extremely important factor in determining overall quality of a particular seafood item (7–9). Depending on the particular seafood item being purchased (eg, chilled, frozen, or canned), the buyer may or may not be able to readily determine the freshness of that seafood item. However, each user determines, either consciously or unconsciously, the freshness (characteristics regarding appearance, flavor, odor, and/or texture) of each

129. D. E. Kramer et al., data presented at the 4th International Congress on Engineering and Food, Edmonton, Alberta, Canada, July, 1985.
130. V. M. Creelman and N. Tomlinson, "Inosine in the Muscle of Pacific Salmon Stored in Ice," *J. Fish. Res. Board Can.* **17**, 449–451 (1960).
131. S. Ehira and H. Uchiyama, "Determination of Fish Freshness Using the *K* Value and Comments on Some Other Biochemical Changes in Relation to Freshness," in D. E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier Science Publishing, New York, 1987, pp. 185–207.
132. H. A. Bremner et al., "Nucleotide Catabolism: Influence on the Storage Life of Tropical Species of Fish from the North West Shelf of Australia," *J. Food Sci.* **53**, 6–11 (1988).
133. D. H. Greene and E. I. Bernatt-Byrne, "Adenosine Triphosphate Catabolites as Flavor Compounds and Freshness Indicators in Pacific Cod (*Gadus macrocephalus*) and Pollock (*Theragra chalcogramma*)," *J. Food Sci.* **55**, 257–258 (1990).
134. S. Konosu, "The Taste of Fish and Shellfish," in J. C. Boudreau, ed., *Food Taste Chemistry* ACS Symposium Series 115, American Chemical Society, Washington D.C., 1979, pp. 185–203.
135. S. Konosu and Y. Maeda, "Muscle Extracts of Aquatic Animals. IV. Distribution of Nitrogenous Constituents in the Muscle Extracts of an Abalone, *Haliotis gigantea discus* Reeve," *Bull. Jpn. Soc. Sci. Fish.* **27**, 251–254 (1961).
136. K. Endo, M. Hujita, and W. Simidu, "Studies on Muscle of Aquatic Animals. Free Amino Acids, Trimethylamine oxide, and Betaine in Squids," *Bull. Jpn. Soc. Sci. Fish.* **28**, 833–836 (1962).
137. N. R. Reddy et al., "Characterization and Utilization of Dehydrated Wash Waters from Clam Processing Plants as Flavoring Agents," *J. Food. Sci.* **54**, 55–59, 182 (1989).
138. W. Hashida, T. Mouri, and I. Shiga, "Application of 5′-Ribonucleotides to Canned Seafoods," *Food Technol.* **22**, 1436–1441 (1968).
139. R. E. Martin, W. H. Doyle, and J. R. Brooker, "Toward and Improved Seafood Nomenclature System," *Mar. Fish. Rev.* **45**, 1–20 (1983).
140. J. G. Kapsalis, "Consumer and Instrumental Edibility Measures for Grouping of Fish Species," Final Report, National Technical Information Service, U.S. Dept. of Commerce, Springfield, Va., 1980.
141. A. V. Cardello et al., "Sensory Methodology for the Classification of Fish According to Edibility Characteristics," *Lebensm. Wiss. und Technol.* **16**, 190–194 (1983).
142. F. J. King et al., "Consumer and Instrumental Edibility Measures for Grouping Fish Species," in J. J. Connell, ed., *Advances in Fish Science and Technology*, Fishing News (Books), Ltd., London, 1980.
143. S. E. Cairncross and L. Sjostrom, "Flavor Profiles—New Approach to Flavor Problems," *Food Technol.* **4**, 308–311 (1950).
144. J. F. Caul, "The Profile Method of Flavor Analysis," E. M. Mrak and G. F. Stewart, eds., in *Advances in Food Research*, Academic Press, New York, 1957.
145. A. V. Cardello et al., "Sensory Evaluation of the Texture and Appearance of 17 Species of North Atlantic Fish," *J. Food Sci.* **47**, 1818–1823 (1982).
146. F. M. Sawyer, A. V. Cardello, and P. A. Prell, "Consumer Evaluation of the Sensory Properties of Fish," *J. Food Sci.* **53**, 12–18, 24 (1988).
147. H. C. Romesburg, *Cluster Analysis for Researchers*, Van Nostrand Reinhold, New York, 1984.
148. C. J. Coombs, *A Theory of Data*, Wiley, New York, 1964.
149. L. Engelman, "Cluster Analysis of Cases," in W. J. Dixon and M. B. Brown, eds., *Biomedical Computer Programs, P-Services*, University of California Press, Berkeley, Calif., 1977.
150. National Marine Fisheries Service, *Our Living Oceans. The Economic Status of U.S. Fisheries*, Technical Memorandum NMFS F/SPO-22, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, 1996.
151. National Marine Fisheries Service, *Fisheries of the United States, 1996*, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, D.C., 1997.
152. H. M. Johnson et al., *1998 Annual Report on the United States Seafood Industry*, 6th ed., H. M. Johnson and Associates, Bellevue, Wash., 1998.

JOHN C. WEKELL
HAROLD J. BARNETT
National Oceanic and Atmospheric Administration
Seattle, Washington

See also SENSORY SCIENCE: PRINCIPLES AND APPLICATIONS.

SEAFOOD: SENSORY EVALUATION AND FRESHNESS

The term fresh seafood refers to a concept, not a distinct object or a specified actuality. Therefore it has many different definitions, such as (*1*) seafood that has never been frozen, cooked, cured, or otherwise preserved (1,2); (*2*) seafood that has the characteristics of being newly harvested and is not the opposite of stale (3); (*3*) a seafood that is the opposite of stale (4); (*4*) seafood that arrived at the store at a particular time (5); and (*5*) raw product that has not progressed beyond a certain degree of microbiological or chemical degradation (6). Depending on which definition is used, it may be very difficult to objectively determine if a specific seafood is actually "fresh." However, when freshness is defined in terms of the sensory characteristics (appearance, flavor, odor, and/or texture) of the specific seafood being evaluated, it is very possible to objectively determine the freshness of that seafood. A seafood of optimum freshness would be one that possesses the characteristics concerning appearance, flavor, odor, and/or texture that are normally associated with that particular seafood product or species, that is, it is caught at the best time of year; caught in the best location; caught by the best method; and handled, processed, prepared, and served in the best manner.

Seafood freshness is considered an extremely important factor in determining overall quality of a particular seafood item (7–9). Depending on the particular seafood item being purchased (eg, chilled, frozen, or canned), the buyer may or may not be able to readily determine the freshness of that seafood item. However, each user determines, either consciously or unconsciously, the freshness (characteristics regarding appearance, flavor, odor, and/or texture) of each seafood item as it is consumed (9). Thus, the degree to which the freshness of a seafood item meets the user's expectations will greatly affect whether the user will purchase that seafood item again (9–12).

INDIRECT DETERMINATION OF SEAFOOD FRESHNESS

Seafood freshness may be indirectly determined by chemically analyzing a sample to determine the concentration of a specific chemical(s) within the sample or by measuring the magnitude of one or more physical parameters (eg, color, distance, force) of a sample. This observed concentration of a chemical or magnitude of a physical parameter is used to indirectly measure (predict) the level of a specific sensory attribute, which allows for the immediate determination of the freshness once the predicted level of the sensory attribute is compared to that specified in the appropriate product standards. Because the measurement of chemical concentrations and physical magnitudes are often very precise (exact), these types of methods have often been considered to be objective methods and therefore superior (because they are less variable) to methods involving sensory evaluation (13–15), which have often been referred to as subjective methods. It is very important to realize that just because the results of these objective methods of determining seafood freshness may be less variable does not necessarily mean the results are thereby more accurate (16). The tremendous practical importance of understanding the difference between results that are precise and results that are accurate is clearly demonstrated by this example: "The archer or marksman may group shots close together, thus being precise, but the shots may be at the rim of the target instead of at the bull's-eye, thus being inaccurate."

Depending on the circumstances, objective chemical and physical methods may be very useful, but only if scientifically sound studies have definitely revealed that a close relationship between the results of the specific chemical or physical method and objective sensory evaluation methods does indeed exist. The results proving such a close relationship apply only to the conditions (method of catching and handling, time of season during which the seafood was harvested, species being examined, specified chemical/physical method, specified sensory evaluation method, etc) under which the investigation was conducted. Altering only one of these conditions may alter the relationship to such a degree that the close relationship may no longer apply, causing the estimated level of the sensory attribute to be of questionable value. Also during these investigations, the sensory evaluations must have been conducted using scientifically sound procedures. Whenever a chemical or physical method has met these requirements, it can be extremely useful. For additional information concerning chemical and/or physical methods of determining seafood freshness, see references 17–23.

DIRECT DETERMINATION OF SEAFOOD FRESHNESS

In addition to its use in confirming the validity of a specific chemical or physical method of indirectly determining seafood freshness, sensory evaluation procedures are frequently used to directly measure seafood freshness.

The results of a sensory evaluation depend on the type of sensory test used. In general, the many different sensory tests may be grouped into a few categories, each with a common purpose. These categories are discriminative or difference tests (which are used to determine whether an overall sensory difference exists between samples or how a particular sensory attribute differs between samples), descriptive tests (which attempt to identify sensory characteristics concerning appearance, odor, flavor, texture, or sound of a food product and to quantify them), and affective tests (which are used to determine which sample is preferred and how acceptable a particular sample is) (24–26). Although affective tests are subjective, both discriminative and descriptive (including grading) methods are objective (24–29). Thus, arbitrarily classifying all sensory methods as subjective is now widely recognized as wrong. Grading (a descriptive test) is the major sensory evaluation method used to determine seafood freshness.

Freshness grading is a technique that directly determines the level of one or more specific sensory attributes. This is achieved by having a small number of graders (who have been highly trained) who individually use their appropriate senses (sight, smell, taste, and/or touch) and a written grade standard (consisting of words that clearly define specific sensory properties) to sort the seafood being evaluated into clearly defined categories (grades) (3,27,30–32). Although this type of sensory evaluation is used in a wide variety of food industries, these assessors are usually trained to evaluate only one class of product, such as coffee, dairy products, seafood, tea, or wine (31).

Regardless of the seafood being evaluated, technologically sound freshness grading directly measures freshness by quantifying the characteristics of appropriate sensory attributes. This is achieved by using a single type of sensory test (ie, a structured category scale, which is commonly called a grade standard) and a small number of highly specialized graders who have been trained to function as analytical instruments (31,32).

Just because freshness grading is an objective procedure does not necessarily mean the result will be accurate (ie, hit the bull's-eye). Whenever freshness grading is being conducted, extreme care must be taken to ensure that all graders always function as analytical instruments (21,33). It is extremely difficult to ensure the accuracy of the graders' assessments without first clearly stating, in writing, all of the reasons why the seafood freshness of a particular product is being graded (29). The importance of each factor that affects the objectivity of the assessments can then be readily established by comparing it with these reasons.

Ensuring Graders Function as Analytical Instruments

Ensuring that graders function as analytical instruments can best be achieved by properly screening all graders, properly training all graders, reducing the effects of the immediate surroundings, minimizing the effects of psychological factors, and monitoring the accuracy of the assigned grades.

Screening primarily involves testing the potential grader's basic ability to assess each type of attribute (appearance, odor, flavor, and/or texture) that the grader will likely be required to evaluate by subjecting the person to a broad range of triangle tests, which vary only in appearance, aroma, flavor, or texture (34). This will determine if the potential grader is actually capable of accurately individually assessing appearance, odor, flavor, and texture. However, screening also involves assessing an individual's health, general attitudes, availability, and ability to communicate (32,34).

Because it depends on the specific situation, the extent of training that is actually necessary is best established by critically examining the written reasons for determining freshness. This is very important because if the extent of training is not closely related to the duties expected, a person may receive costly training for a specific grading duty that he or she may never perform, or conversely, the grader may be asked to conduct a specific grading duty for which he or she has not been adequately trained. For example, extensive training is necessary whenever graders are required to assess specific off-odors and off-flavors, commonly referred to as taints (35).

Training involves teaching the potential grader (*1*) the general principles and practices of sensory evaluation; (*2*) to readily identify and accurately assess each sensory characteristic defined in each of the different product standards that he or she will be expected to use; (3) to have a long-term memory so that standards that have not been recently used can be readily and accurately implemented; (*4*) to grade a large number of samples at a single session, and (*5*) to evaluate his or her performance (35,36).

The grader's learning of general principles and practices usually involves formal lectures and demonstrations concerning topics such as the operation of each of the different senses (sight, smell, taste, touch); how the perception of color (and other types of appearances), flavor, odor, and texture is affected by a wide range of variables; the different types (affective, discriminative, descriptive) of sensory assessment; and technologically valid testing procedures (31,35). The importance of graders possessing a sound knowledge of the principles and practices of sensory evaluation must not be underestimated.

The assessment area may greatly affect the grader's ability to function as an analytical instrument. Any detrimental effects of the immediate surroundings may be minimized by (*1*) ensuring that all of the surfaces (booths, cabinets, ceilings, countertops, doors, floors, tables, walls, etc) are of a neutral color; (*2*) ensuring that there is no distraction by noise (including talking); (*3*) ensuring that the assessment area is both well ventilated and cleaned using only non-odor-generating cleaners, thereby preventing/eliminating foreign odors; (*4*) ensuring that the samples are not prepared but are only evaluated within the assessment area; and (*5*) ensuring that the assessment area is illuminated to give an intensity of approximately 1,000 lx/m^2 and a color temperature of 5,000 to 5,500 K (29,33,35). Whenever the entire assessment area is not properly illuminated, a properly illuminated grading cabinet must be used.

The negative effect of psychological factors (Table 1) must not be ignored but must always be seriously considered. For example, the grader(s) who assess the seafood must not have been involved with actually obtaining the seafood (30). This is because knowledge concerning where the seafood was caught, how the seafood was handled, and the length of time the seafood was stored can cause the grader to have specific expectations concerning the results, and the grader would no longer be acting as an analytical device (25). In addition, (*1*) graders should not be involved with the preparation of the sample; (*2*) each sample should be labeled with a three-digit random number; and (*3*) the samples should be presented in a random order, ensuring that the order in which the samples are graded by the grader is balanced so that each different product appears in a given position an equal number of times.

Table 1. Psychological Factors Influencing Sensory Measurements

Factor	Explanation
Expectation error	Any information panelists receive about the test can influence the lts
Stimulus error	The desire to be right may cause the panelists' judgment to be influenced by irrelevant characteristics of the samples.
Logical error	Can cause a panelist to assign ratings to particular characteristics because they appear to be logically associated with other characteristics.
Leniency error	Occurs when panelists rate products based on their feelings about the researcher, thereby ignoring product differences.
Halo effect	Tendency of the rating of one factor to influence the rating of another factor when panelists are asked to evaluate odor, texture, color, and taste simultaneously.
Suggestion effect	Occurs when the response of one panelist is influenced by reactions of other panelists.
Order effect	Tendency of panelists to score the second sample (of a set of samples) higher or lower than expected.
Contrast effect	Two products that are markedly different cause panelists to exaggerate the difference in their scores.
Convergence error	Occurs when a large difference between two products masks small differences between other samples in the test.
Proximity error	When a set of samples is being rated on several characteristics, panelists usually rate as more similar the characteristics that follow one another (in close proximity) on the ballot sheet than those that are either far apart or read alone.
Central tendency error	Occurs when panelists score samples in the mid-range of a scale to avoid extremes.
Motivation	An interested panelist is always more efficient and reliable.

Source. Ref. 37.

Although grading different products in a balanced manner sounds very simple, it can be extremely difficult to achieve, particularly when a large number of different products are to be assessed at one time. For example, when the number of different products being evaluated at any one time is increased from 1 to 2 to 3 to 4 to 5, the required number of different combinations necessary to achieve a balanced presentation increases from 1 to 2 to 6 to 24 to 120, respectively. Thus, when more than three different products are evaluated at the same time, the large number of combinations necessary to achieve a balanced presentation becomes very cumbersome.

Regardless of the extent to which the graders have been screened and trained and the effects of the surroundings, and the psychological factors have been reduced, the grader must accurately implement the grade standards. The necessity of monitoring the assessments of graders who have been screened and trained to operate as analytical instruments is similar to the necessity of periodically checking the accuracy of a pH meter (or other instruments used for chemical analysis) even though it had previously been calibrated (38–40). Monitoring the accuracy of assigned grades may be achieved by having the grader check his or her consistency, comparing the grader's assessments with those of other graders, and conducting formal examinations (30). A grader's performance may also be monitored by having the grader, without his or her knowledge, assess samples with sensory characteristics of a known level.

Defined Grade Standards

In addition to having graders use their senses (sight, smell, taste, and/or touch) as analytical instruments to sort the seafood into specified categories, the second extremely essential part of seafood freshness grading is the defined grade standard that describes each category. Even if the effects of both immediate surroundings and psychological factors are minimized and the graders are both well screened and well trained, the assigned grades will probably not be valid unless the grade standards clearly allow the grader to objectively assess the samples.

The traditional grade standard used to grade intact (ie, not filleted, split, or steaked) gutted fish consists of specified sensory characteristics of three to six different criteria (eg, visual characteristics of the eyes, gills, outer slime, peritoneum, and skin and odorous characteristics of the gills) associated with the fish (41). Depending on the specific grade standard, each criterion of each sample being assessed is individually categorized as being one of two grades (accept or reject), three grades (A, B, or C), or four grades (A, B, C, or D), and so on, whichever is specified in the grade standard being used. Each grade description, for each of the three to six criteria, consists of three to six terms. Only when the grade for each criterion has been determined can the final grade (based on the grades assigned each of the different criteria) be assigned. However, the sensory characteristics of a criterion being assessed may not agree with all of the three to six terms used to describe the specific grade of a specific criterion. Whenever this lack of agreement occurs, it often causes the grader to be confused and therefore increases the time it takes to decide which grade to assign, decreasing the objectivity of this type of grading. Consequently, methods that avoid this serious disadvantage were eventually developed.

One method of increasing both the speed and the objectivity of a seafood freshness system was to increase both the number of grades within a specific criterion and the number of different criteria to be assessed (42). Rather than having four different grades for each of six different criteria (41), six different grades for each of 11 criteria were used (42). These changes reduce the number of terms required to describe each grade of each criterion and thereby (*1*) increase the chances that a sample's sensory characteristics agree with all of the terms used to describe a specific grade of a specific criterion; (*2*); decrease confusion, (*3*) increase objectivity, and (*4*) increase speed of assessment. However, depending on both the system and the sample being assessed, the increased number of grades for each criterion may increase the probability that an individual sample's sensory characteristics (of one or more specific criteria) agree with the terms of more than one specific grade of that specific criterion. This would also cause the grader to be confused, resulting in decreased speed and reduced objectivity.

An alternate method of increasing both the speed and the objectivity of the traditional freshness grading system is to greatly increase the number of criteria but to also decrease the number of grades within each criterion. This method was first used by Australian scientists (43). Eighteen different criteria (appearance of surface, skin, scales, slime, stiffness, clarity of eyes, shape of eyes, iris, blood of eyes, color of gills, mucus of gills, smell, belly discoloration, firmness, condition of vent, odor of vent, nature of stains in belly cavity, and color of blood) were used, but only two to four grades (demerit points) were used within each criterion (43). Once the sensory characteristic of a criterion was determined, it was immediately assigned a demerit point ranging from 0 to 3. Every description of each demerit point was very brief, usually involving only one or two words, and, if possible, was very precise. Thus, while assessing each criterion, a grader was exposed to minimum confusion. Another unique feature of this system was that the final freshness grade of a sample was based not on the average of the different grades assigned the different criteria, but on the total number of demerit points assigned the sample assessed (43). Individuals using this system have been reported to assess the freshness of intact fish both rapidly and objectively (43–46). Graders may readily use a pencil and paper to record their assessments of each sample, but programming this grading system into a handheld computer helps ensure that all questions have been answered and decrease the time required to assess a fish (47). Other important aspects of this system initially reported by Branch and Vail (43) are that (*1*) no undue emphasis is placed on a single feature, and the sample cannot be rejected on the basis of a single criterion; (*2*) minor differences in any one criterion being assessed do not unduly influence the total score; and (*3*) the combination of the different criteria and different demerit scores gives a total possible score of a reasonable magnitude (47).

Although the grading system initially reported by Branch and Vail (43) is both objective and nondestructive, it is extremely rapid, requiring only 5 minutes to grade 10 fish (45). Since its development this system has been reported to be a successful method of objectively determining the freshness grade of intact anchovy, Atlantic cod, herring, hoki, plaice, redfish, saithe, sardine, and whiting (44–

46,48,49). In addition to being a useful method of grading intact fish, this procedure has also been reported to be useful for grading fillets (44). Thirteen different criteria (fillet color, bloodstains, clotting, skin color, texture, gaping, bruising, autolysis discoloration, parasite infestation, other discoloration or contaminations, ease of filleting, ease of skinning, and wetness) with two to four demerit points for each criterion was used (44).

It is extremely important to stress that whenever one intends to use this system for a new species, preliminary studies must be conducted to ensure that all the criteria and their corresponding defined characteristics incorporated in the grade standard are appropriate and will actually be used (45,46,48,49).

CONCLUSION

Sensory evaluation (eg, grading) of seafood is an extremely useful rapid and objective method of both directly determining seafood freshness and verifying the accuracy of chemical and/or physical methods used to indirectly measure seafood freshness. However, if sensory grading is being used to objectively determine seafood freshness, it is extremely important that all graders be appropriately screened, trained, and monitored; all effects of the immediate surroundings be minimized; the effects of psychological factors be minimized; and an appropriate grade standard (one that actually allows the graders to function as analytical instruments) be used. Otherwise the procedure may not be objective, and the results may not hit the bull's-eye (ie, be accurate).

BIBLIOGRAPHY

1. C. Perkins, ed., *The Advanced Seafood Handbook*, Seafood Business Magazine, Rockland, Maine, 1992.
2. Canada Department of Fisheries and Oceans, *Fish Inspection Regulations Amended February 25, 1992*, Government of Canada, Ottawa, Canada, 1992.
3. J. J. Waterman, *Composition and Quality of Fish: A Dictionary*, Torry Advisory Note No. 87, U.K. Ministry of Agriculture Fisheries and Food, Torry Research Station, Her Majesty's Stationery Office, Edinburgh, Scotland, 1982.
4. I. Dore, *Fish and Shellfish Quality Assessment*, Van Nostrand–Reinhold, New York, 1991.
5. J. M. Regenstein and C. E. Regenstein, *Introduction to Fish Technology*, Van Nostrand–Reinhold, New York, 1991.
6. F. W. Wheaton and T. B. Lawson, *Processing Aquatic Food Products*, Wiley, New York, 1985.
7. N. K. Sorsensen, "Physical and Instrumental Methods for Assessing Seafood Quality," in H. H. Huss, M. Jakobsen, and J. Liston, eds., *Quality Assurance in the Fish Industry*, Elsevier Science, Amsterdam, The Netherlands, 1992, pp. 321–332.
8. M. Sakaguchi and A. Koike, "Freshness Assessment of Fish Fillets Using the Torrymeter and K-Values," in H. H. Huss, M. Jakobsen, and J. Liston, eds., *Quality Assurance in the Fish Industry*, Elsevier Science, Amsterdam, The Netherlands, 1992, pp. 333–338.
9. P. F. Howgate, "Quality Assessment and Quality Control," in A. Aitken et al., eds., *Fish Handling and Processing*, Her Majesty's Stationery Office, Edinburgh, Scotland, 1982, pp. 177–186.
10. L. K. Freeman, "The Quest for Quality," *Food Engineering* **62**, 62–71 (1990).
11. R. Martin, "Contaminants in Relation to Quality of Seafood," *Food Technol.* **42**, 104–108 (1988)
12. K. McNutt, "Consumer Attitudes and the Quality Control Function," *Food Technol.* **42**, 97–98 (1988).
13. T. A. Gill, "Objective Analysis of Seafood Quality," *Food Rev. Int.* **6**, 681–714 (1990).
14. T. A. Gill, "Biochemical and Chemical Indices of Seafood Quality," in H. H. Huss, M. Jakobsen, and J. Liston, eds., *Quality Assurance in the Fish Industry*, Elsevier Science, Amsterdam, The Netherlands, 1992, pp. 377–388.
15. L. F. Pivarnik et al., "Freshness Assessment of Six New England Fish Species Using the Torrymeter," *J. Food Sci.* **55**, 79–82 (1990).
16. F. Garfield, "Sampling and Sample Analysis," *Quality Assurance Principles for Analytical Laboratories*, Association of Official Analytical Chemists, Arlington, Va. 85 1991, pp. 64–85.
17. J. R. Botta, "Chemical Methods of Evaluating Freshness Quality," in J. R. Botta, *Evaluation of Seafood Freshness Quality*, VCH, New York, 1995, pp. 9–33.
18. J. R. Botta, "Physical Methods of Evaluating Freshness Quality," in *Evaluation of Seafood Freshness Quality*, VCH, New York, 1995, pp. 35–63.
19. F. R. Jack, A. Paterson, and J. R. Piggott, "Perceived Texture: Direct and Indirect Methods for Use in Product Development," *International Journal of Food Science and Technology* **30**, 1–12 (1995).
20. J. Oehlenschlager, "Evaluation of Some Well Established and Some Underrated Indices for the Determination of Freshness and/or Spoilage in Ice Stored Wet Fish," in H. H. Huss, M. Jakobsen, and J. Liston, eds., *Quality Assurance in the Fish Industry*, Elsevier Science, Amsterdam, The Netherlands, 1992, pp. 339–350.
21. H. H. Huss, ed., *Quality and Quality Changes in Fresh Fish*, Fisheries Technical Paper No. 348, Food and Agriculture Organization (FAO) of the United Nations, Rome, 1995.
22. G. Olafsdottir et al., "Methods to Evaluate Fish Freshness in Research and Industry," *Trends Food Sci. Technol.* **8**, 258–265 (1997).
23. C. N. G. Scotter, "Non-Destructive Spectroscopic Techniques for the Measurement of Food Quality," *Trends Food Sci. Technol.* **8**, 285–292 (1997).
24. Institute of Food Technologists, "Sensory Evaluation Guide for Testing Food and Beverage Products," *Food Technol.* **35**, 50–59 (1981).
25. M. Meilgaard, G. V. Civille, and B. T. Carr, *Sensory Evaluation Techniques*, 2nd ed., CRC Press, Boca Raton, Fla., 1991.
26. H. Stone and J. Sidel, *Sensory Evaluation Practices*, Academic Press, New York, 1985
27. J. J. Connell and P. F. Howgate. "Fish and Fish Products," in S. M. Herschdoerfer, ed., *Quality Control in the Food Industry*, Vol. 2, Academic Press, London, 1986, pp. 347–405.
28. G. Jellinek, *Sensory Evaluation of Food: Theory and Practice*, Ellis Horwood, Chichester, U.K., 1993.
29. E. Larmond, "Sensory Evaluation Can Be Objective," in J. K. Kapsalis ed., *Objective Methods in Food Quality Assessment*, CRC Press, Boca Raton, Fla., 1986, pp. 3–14

30. K. Straus, "Seafood Standards," in K. Straus, ed., *The Seafood Handbook: Seafood Standards*, Seafood Business Magazine, Rockland, Maine, 1991, pp. 7–17.
31. R. K. York, "Canadian Fish Products: Fish Inspection and Sensory Evaluation," *Canadian Institute of Food Science and Technology Journal* **22**, AT441–AT444 (1989).
32. International Standards Organization, *Sensory Analysis: General Guidance for the Selection, Training, and Monitoring of Assessors, Part II: Experts, Draft International Standards*, International Standards Organization, Geneva, Switzerland, 1991.
33. J. J. Connell, *Control of Fish Quality*, 3rd ed. Fishing News Books, London, 1990.
34. American Society of Testing and Materials, *Guidelines for the Selection and Training of Sensory Panel Members*, American Society of Testing and Materials Special Technical Publication No. 758, Philadelphia, Pa., 1981.
35. P. Howgate, *Review of Inspection Procedures (Sensoric Evaluation) for Fish and Shellfish*, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Committee on Fish and Fishery Products, Bergen, Norway, 1992.
36. International Standards Organization, *International Standard: Sensory Analysis—Vocabulary*, ISO Reference No. ISO 5492, International Standards Organization, Geneva, Switzerland, 1992.
37. L. M. Poste et al., *Laboratory Methods for Sensory Analysis of Food* Publication No. 1864E, Government of Canada, Research Branch, Department of Agriculture, Ottawa, Ontario, 1991.
38. J. K. Taylor, "Validation of Analytical Methods," *Anal. Chem.* **55**, 600A–605A (1983).
39. J. K. Taylor, "Role of Collaborative Studies in Evaluation of Analytical Methods," *J. Assoc. Off. Anal. Chem.* **69**, 398–400 (1986).
40. P. J. Wagstaffe, "Errors in Analytical Methods: Use of Intercomparisons to Locate Sources of Error and to Improve Accuracy in Food Analysis," *Analysis* **17**, 455–459 (1989).
41. P. Howgate, A. Johnston, and A. Whittle, eds., *Multilingual Guide to EC Freshness Grades for Fishery Products*, Torry Research Station, Food Safety Directorate, Ministry of Agriculture Fisheries and Food Aberdeen, Scotland, 1992.
42. H. M. Lupin et al., "Storage of Chilled Patagonian Hake (*Merluccius hubbsi*)," *J. Food Technol.* **15**, 285–300 (1980).
43. A. C. Branch and A. M. A. Vail, "Bring Fish Inspection into the Computer Age," *Food Technology in Australia* **37**, 352–355 (1985).
44. H. A. Brenner, "A Convenient Easy-to-Use System for Estimating the Quality of Chilled Seafoods," *Fish Processing Bulletin* **7**, 59–70 (1985).
45. E. Larsen et al., "Development of a Method for Quality Assessment of Fish for Human Consumption Based on Sensory Evaluation," in H. H. Huss, M. Jakobsen, and J. Liston, eds., *Quality Assurance in the Fish Industry*, Elsevier Science, Amsterdam, The Netherlands, 1992, pp. 351–358.
46. J. Nielsen, et al., "Development of Methods for Quality Index of Fresh Fish," *FAR Meet.*, Noordwijkkerhort, The Netherlands, 1992.
47. H. A. Brenner, J. Olley, and A. M. A. Vail, "Estimating Time-Temperature Effects by a Rapid Systemic Sensory Method," in D. E. Kramer and J. Liston, eds., *Seafood Quality Determination*, Elsevier Science, Amsterdam, The Netherlands, 1987, pp. 413–435.
48. E. Martinsdottir and A. Atnason, "Redfish, Kapittel 4, Slutraport," in *Nordic Industrial Fund, Quality Standards for Fish, Final Report Phase II*, 1992, pp. 21–35.
49. S. Jonsdottir, "Kvalitetsnomer pa sild, Slutrapport," in *Nordic Industrial Fund, Quality Standards for Fish: Final Report Phase II*, 1992, pp. 36–69.

J. R. Botta
Moncton Food Quality Investigations, Inc.
Moncton, New Brunswick
Canada

SEAWEED AQUACULTURE: TAIWAN

INTRODUCTION

Aquaculture of seaweed in Taiwan started in 1961 with the cultivation of *Gracilaria*. Presently one species of *Gracilaria, G. tenuistipitata* Chang et Xia var. *liui* Chang et Xia (Fig. 1); two species of *Porphyra, P. dentata* Kjellman (Fig. 2) and *P. haitanensis* T. J. Chang et Zhang Baofu; and two species of brown algae, *Undaria undarioides* (Yendo) Okamura and *Laminaria japonica* Areschong are cultivated. But only *Gracilaria* and *Porphyra* are cultivated on a commercial scale and *Undaria* and *Laminaria* are still in the

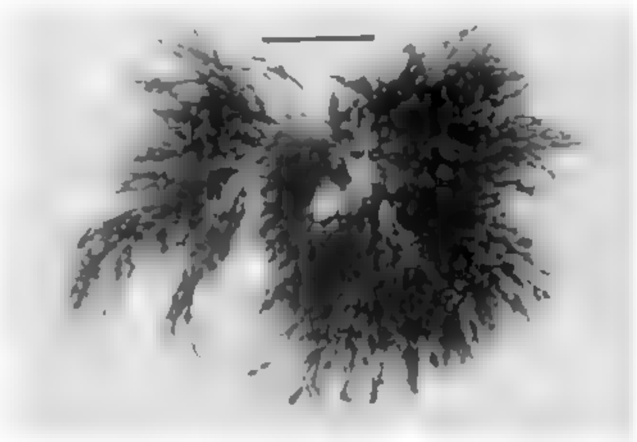


Figure 1. Habit of *Gracilaria tenuistipitata* var. *liui* from Anping (scale = 1 cm).

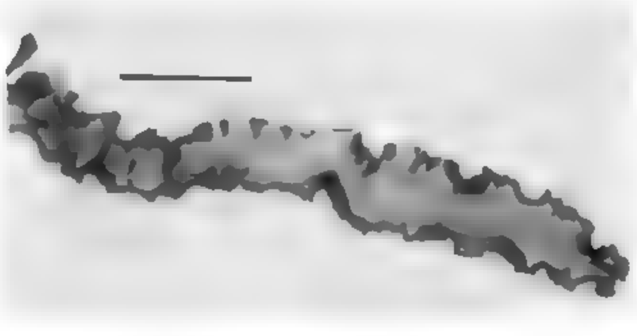


Figure 2. Habit of *Porphyra dentata* from Penghu (scale = 1 cm).

experimental stages. The actual methods of cultivating *Gracilaria* and *Prophyra* practiced in Taiwan are described as follows.

CULTIVATION OF *GRACILARIA*

Gracilaria is a red algal genus, widely distributed in many temperate to tropical regions and many species of this genus are cultivated for manufacturing an industrial colloid, agar, in many parts of the world (1–3). Species of *Gracilaria* are popularly known in Taiwan as "Long-Shi-Tsai" (meaning vegetable of dragon's beard). Originally it was commercially cultivated in ponds for extracting agar but now it has been cultivated chiefly for feeding small abalone, *Haliotis diversiolar* Lischke. There are thirteen species of *Gracilaria* reported from the coast of Taiwan and its offshore islands (4). Among them, *G. tenuistipitata* var. *liui* (Fig. 1) (5) is the main species cultivated, but sometimes plants of *G. gigas* Harvey and *G. lichenoides* (L.) Harvey can also be found among the cultivated species in the same pond (1).

In Taiwan, cultivation of *Gracilaria* started in early 1960 (6). Then, the raw material for agar extraction had decreased significantly due to overexploitation of natural agarophyte, *Gelidium* resources. Thus, in order to meet the increasing demand for agarophyte from the agar industry, the Taiwan Fisheries Bureau supported the Chilou Fishermen's Association in carrying out experimental cultivation of *Gracilaria* in Hsinta-Kang Bay (Kauhsiung Hsien) in 1961. The initial method of cultivation was by inserting branches of the seaweed into ropes and growing them in shallow coastal water. Later, in the same year (6), another experiment was done at Si-Kun-Shen (Tainan Hsien) growing the seaweeds by scattering them on the bottom of shallow, brackish water ponds in which milkfish, *Chanos chanos* Forskal, were cultivated. Subsequent experiments proved that the latter method of cultivation was better than the former one.

In 1970s, with the assistance from the Joint Commission on Rural Reconstruction, the methods of cultivation were improved greatly. Therefore, during this period both the cultural area and the production of the seaweed had increased. According to the Taiwan Fisheries Year Book, the total culture area in 1972 was about 111 ha and had increased to more than 240 ha in 1980. The Anping area (Tainan City) (Fig. 3) alone reached about 200 ha and the annual production increased from about 3,200 tons of fresh seaweed in 1972 to about 9,600 tons in 1980. In the mid 1980s, the area of cultivation decreased significantly to less than 200 ha due to the fact that many *Gracilaria* ponds were utilized to cultivate grass shrimps, *Penaeus monodon* Fabricius. But from 1988, prevalent shrimp diseases caused many former *Gracilaria* ponds to return to growing seaweed again. At the same time, the number of small abalone farms has increased greatly, and the increase in the numbers of abalone farms has brought about a great demand for fresh *Gracilaria* material. Thus, having been stimulated by the increasing demands for fresh seaweed, the number of *Gracilaria* ponds was also increased. Now the total area of cultivation is about 350 ha and it produced about 9,310 tons of fresh seaweed in 1989.

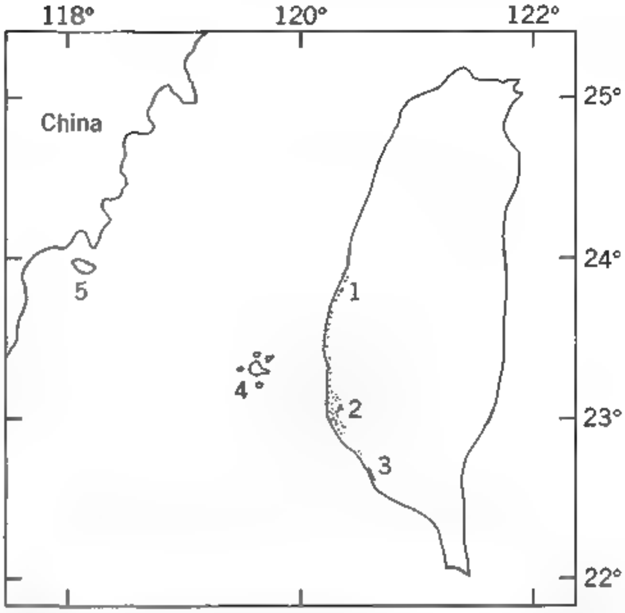


Figure 3. Map of Taiwan showing principal areas of seaweed cultivation. 1, Chanhua Hsien; 2, Anping, Tainan City; 3, Pington Hsien; 4, Penghu; 5, Kinmen. 1–3, *Gracilaria* cultivation. 4, *Porphyra dentata*; 5, *P. haitanensis*.

All *Gracilaria* ponds are located on the west coast of Taiwan, covering the coastal area from Chan-hua to Pington Hsien (counties) (Fig. 3), mainly in the Anping area (Tainan City) which occupies about 250 ha. The farming method employed is a polyculture system. That is, plants of *Gracilaria* are scattered randomly on the bottom of ponds in which milkfish, *Chanos chanos* are cultivated. Sometimes, grass shrimps, *Penaeus monodon* and crabs, *Scylla serrata* Forskal are also cultivated together to increase the income of the pond. Presently almost all seaweed produced is used for feeding small abalone culture. Only a small portion of the seaweed which is harvested from late autumn to winter goes to the agar industry. Sketches of the commercial cultivation of *Gracilaria* are presented as follows:

The Pond

These were formerly used in cultivation of milkfish or grass shrimp. The best sites for *Gracilaria* cultivation are those where no strong wind prevails and where both sea and fresh water can be obtained easily. A place with sufficient tidal difference to facilitate exchange of water is preferable to ones with little tidal difference. Most of the ponds are rectangular and are quite different in size. Smaller ponds are preferable to larger ones, because the former are easier to manage when shrimps and/or crabs are additionally cultivated. Each pond has an inlet and an outlet to facilitate the exchange of water.

Ponds are generally about 50 to 80 cm deep with a flat bottom of clay loam, sand silt loam, sandy loam or loamy sand (7). Seaweed growing in the pond with a sandy bottom can be buried in the sand during windy days. Therefore, the size of the pond should be smaller and the water should be deeper to minimize the effects of wind. Since seaweed in larger ponds (larger than one hectare) generally pile up at one corner of the pond by the wind, installation of one or two rows of wind breaks made of bamboo pieces perpendicular to the direction of the wind in the center of the pond is needed to minimize the damage which might be caused by the wind.

Seeding

At present *Gracilaria tenuistipitata* var. *liui* (Fig. 1) is the species most extensively cultivated.

The procedures of cultivation are as follows: (*1*) Drying the pond in the sun for several days. At the same time the bottom of the pond is flattened. These tasks are usually finished during early spring. Once the cultivation is started and goes well, the seaweed can be grown continuously in the same pond for many years without drying the pond again. (*2*) Introduction of water. Depending on the season, about 30 to 60 cm deep of water is introduced. (*3*) Seeding. It is usually done from March to May. Cultured strains are used as planting stock. Depending on the fertility of the ponds, about 5,000 to 12,000 kg/ha of fresh material are added by tearing them randomly into pieces and scattered evenly into the pond. (*4*) Introduction of milkfish to control epiphytes. Generally about 1,000 fish (about 15 cm long) per hectare are introduced before the appearance of epiphytes.

Pond Management

The depth of the pond water (the distance between the top of the seaweed and the water surface) is usually kept at 30 to 40 cm. But during the summer time, the water level is elevated to 50 to 60 cm high to prevent damage from rise in water temperature. While in the winter, the water level is lowered about 20 to 30 cm to receive more sunlight and to raise water temperature for the seaweed.

Frequent change of water is needed to keep a proper range of salinity and to provide nutrients for the growth of the seaweed. One change of water every two or three days is the usual practice. Only one half to two-thirds of the water is changed. The optimum salinity of the pond water is about 15 to 25 ppt (7). In some extreme cases the salinity can be as low as 4 ppt during the rainy season or as high as 50 ppt in the winter. Under such extreme conditions, even though they may not cause death of the seaweed in one or two days, the pond water should be changed more often.

To accelerate the growth of the seaweed, about 3 kg/ha of urea or 120 to 180 kg/ha of fermented manure of pigs or chickens are applied every two or three days at the time of the introduction of new water. The amount of fertilizer application are based on the color of the thalli of *Gracilaria*. If they are yellowish brown, it means they need fertilizer. When they are dark reddish-brown, it means the seaweed are healthy (8).

The main pests of *Gracilaria* are species of *Enteromorpha, Chaetomorpha*, and *Ectocarpus*, with the former two being the most harmful and most difficult to control. At present, control of epiphytes with milkfish seems quite satisfactory. However, milkfish may also eat *Gracilaria* after the epiphytes are gone. When this is the case, the larger fish are removed and smaller ones are introduced again. During winter because of lower grazing power of the fish, epiphytes, especially plants of the species *Ectocarpus*, may prevail. Fortunately plants of *Ectocarpus* are small and their influence on the seaweed is relatively small. Besides epiphytes, *Tilapia mossambica* Peters may become a pest of *Gracilaria. Tilapia* are widely distributed in southern Taiwan and get into *Gracilaria* ponds very easily during the changing of water. Because of their rapid propagation, the population of *Tilapia* usually becomes very large in a short time and causes great damage to seaweed because of their strong grazing ability and their digging holes into the bottom of the pond. Therefore, efforts should be made to catch as many *Tilapia* every two or three days to keep their population down.

Depending on the season, the quantity of seaweeds seeded, and the fertility of the pond, seaweeds are harvested once every 16 to 20 days during summer, or 35–45 days during the winter. In general, (in fertile ponds) about one third to one half of the seaweed is harvested each time. Harvesting is done with a scoop net which collects the seaweed in rows and washes them with pond water. The cleaned seaweed is brought to the bank of the pond with a raft and is put into nylon bags. Each bag contains about 60 kg of fresh seaweed. They are shipped to abalone farms in trucks. If the seaweed is going to agar factories, it is sun-dried on bamboo screens or plastic sheets. About 6 kg of fresh plants yield 1 kg of dry seaweed. After harvesting, the plants remaining in the pond are torn into pieces and are seeded again. Generally about 16,000 to 50,000 kg/yr/ha of fresh *Gracilaria* are produced.

In conclusion, the cultivation of *Gracilaria* in Taiwan is quite successful with vegetative propagation in brackish-water ponds with milkfish. It seems that the present technique is adequate and the production is profitable to the fisherman. Previously the seaweed produced was used as raw material for the domestic agar industry and production was quite stable. But since 1988, the increased demand for fresh seaweed for the small abalone, *Haliotis diversiolor* mariculture has changed the situation greatly. Now almost all of the seaweed produced is used for abalone cultivation, and the *Gracilaria* needed for agar production has to be imported.

AQUACULTURE OF *PORPHYRA*

Plants of the genus *Porphyra* are known as "Tsu-tsai" in China, "nori" in Japan and "purple larve" in the west. They are highly appreciated as food by many oriental (Asian) people, and are cultivated on a large scale in China mainland, Japan, and Korea (9).

Every year Taiwan has to import a large amount of "Tsu-tsai" for human consumption. In 1980 about USD 2.0 million dollars worth of "Tsu-tsai" was imported, but this increased to about USD 3.0 million in 1987.

Cultivation of *Porphyra* started in 1970, in the Penghu Islands (Fig. 3). Due to a lack of basic studies on the biology of the cultivated species, during the first several years the whole process and method of the cultivation took place through much trial and error. Later on, (the biology of) some local species of *Porphyra*, such as *P. angusta* Ueda and *P. dentata* Kjellman, were studied both in the laboratory and in the field. Based on the results of these studies, the methods of cultivation were improved and gave very good result. Encouraged by the successful cultivation in Penghu (Islands), the cultivation of this red alga was tried in Kinmen (Fig. 3) in 1978 with a local species, *P. haitanensis*. The prospect of developing the *Porphyra* industry in this country appears to be very promising. Now *P. dentata* and *P. haitanensis* are the main species cultivated in Penghu and Kinmen, respectively.

CULTIVATION OF *CONCHOCELIS*

Cultivation of *Porphyra* begins with the culture of filamentous sporophytic phase, or *Conchocelis*. The culture of *Conchocelis* begins at the end of February to the middle of March depending on the water temperature. During this time the water temperature (17–22°C) becomes suitable for the germination of carpospores and the growth of the conchocelis plants. The flat upper parts of oyster shells are used as substrate for the carpospores.

The attachment of carpospores to the shells is initiated by sprinkling carpospore suspension over the shells placed side by side on the bottom of small shallow tanks which are 40 × 61 × 15 cm in size, containing seawater about 10 cm deep. The suspension of carpospores is prepared by filtering the suspension of pulverized mature plants of *Porphyra* crushed in a grinder. After the completion of seeding carpospores on shells, the tank is placed on a woody shelf under 1000 to 1500 lux light intensity for one week to allow the carpospores to germinate on the shells. The water then is changed. The carpospores germinate into conchocelis plants and burrow into the pearl layer. The conchocelis are cultured in the tank until the end of October for seeding conchospoes on the net. During the cultivation of conchocelis, the water in the shell-tank should be changed once every two to three weeks, and the shells should be cleaned with a brush every one to two months depending on the growth of epiphytes on the shells. Once the conchocelis grow into the shell, the growing conditions of the conchocelis and epiphytes can be controlled by changing light intensity. During summer, special attention should also be paid to the temperature of the nursery room to avoid undue rise in temperature of the tank water to affect on the growth of the conchocelis. If the room temperature rises to higher than 30°C, seawater is used to lower the temperature by spraying it on the floor, at the same time, the light intensity of the room is lowered to about 400 lux or less.

Another method of growing conchocelis is seeding the conchocelis fragments on shells. Seeding conchocelis fragments on the shells starts during the middle of May through the middle of June, by grinding several conchocelis colonies which are growing in a flask and kept in the laboratory. The colonies are cut in a sterile grinder with 500-mL filtered seawater for about 30 seconds. Then conchocelis-fragments solution is diluted with about 30 times of filtered seawater and spread thinly on the shells to allow the conchocelis to grow into it.

Seeding Conchospores on Nets and Cultivation of Leafy *Porphyra*

Conditions for conchospore formation and liberation of these *Porphyra* species are still not well understood. Therefore, seeding conchospores on cultivation nets is still (at the mercy of nature) and the annual production fluctuates greatly. It usually starts in the middle of October at Penghu, or from the end of October through early November at Kinmen.

The attachment of conchospores on the net is accomplished by putting the mature conchocelis-bearing shells into troughs of bamboo which form the frames of the net rafts, or into plastic bags opened at top which are attached beneath the net. About 75 to 100 shells are needed for each net. At Penghu, the floating raft type (9) is employed in the cultivation of leafy plants. The net rafts are floated with anchor ropes fixed in the middle part of the intertidal zone. The net rafts are made of bamboo. They are rectangular in shape, about 2.0 × 9.0 m in size and with six bamboo legs of about one meter long. The cultivation nets are made of synthetic strings. They are rectangular in shape and measures 1.5 × 8.0 m (Penghu) or 2 × 20 m (Kinmen) in size. In general, used old nets are preferable to new ones, because the surface of new strings are too slippery for the conchospores to attach. Two nets which are stacked one upon another are fastened on a net raft.

About 40 days after seeding, the seedings of *Porphyra* plants may reach 15 to 30 cm long and can be harvested. They are cultivated until March of the next year and can then be harvested 3 or 4 times, producing about 18 to 30 kg of fresh seaweed per raft (2 nets).

In Taiwan, fishermen usually cultivate *Porphyra* as profitable side job during winter when they cannot go out fishing. Since Mainland China produces large quantity of very cheap dried "Tsu-tsai" the fishermen in Taiwan are gradually losing their interest to cultivate *Porphyra* and the area of cultivation in Taiwan is decreasing.

BIBLIOGRAPHY

1. Y. M. Chiang, "Cultivation of *Gracilaria* (Rhodophyta, Gigartinales) in Taiwain," in T. Levering, ed., *Proceedings 10th International Seaweed Symposium*, 1981, pp. 569–574.
2. M. D. Hanisak, B. E. Lapointe, and J. H. Ryther, "Cultivation of Unattached Macroalgae in Florida," *J Phycol.* **21**, 15 (1985).
3. A. Pizarro and H. Barrales, "Field Assessment of Two Methods for Planting the Agar-Containing Seaweed, *Gracilaria*, in Northern Chile," *Aquaculture* **59**, 31–43 (1986).
4. H. N. Yang and Y. M. Chiang, "Taxonomical Study on the *Gracilaria* of Taiwan," *J. Fish. Soc. Taiwan* **9**, 55–71 (1982).
5. Y. M. Chiang and J. L. Lin, "Nitrate Uptake by Nitrogen-Starved Plants of the Red Alga *Gracilaria tenuistipitata* var. *liui*, *Jp. J. Phycol (Sorui)* **37**, 187–193 (1989).
6. W. H. Kuan, "*Gracilaria* Cultivation," *China Fish. Monthly* **3**, 3–9, 1962. (in Chinese).
7. S. P. Huang, "Effects of Salinity on the Growth and Uptake of Nitrogen of *Gracilaria verrucosa* and *Gelidium japonicum*," M. S. Thesis, National Taiwan University, 1980 (in Chinese), 6 pp.

8. B. E. Lapointe and J. H. Ryther, "Some Aspects of the Growth and Yield of *Gracilaria tikvahiae* in Culture," *Aquaculture* **15**, 185–193 (1979).
9. C. K. Tseng, "Commercial Cultivation," in C. S. Lobban and M. J. Wynne, eds., *The Biology of Seaweeds*, University of California Press, Berkeley and Los Angeles, 1981.

YOUNG-MENG CHIANG
National Taiwan University
Taipei, Taiwan

SENSORS AND FOOD PROCESSING OPERATIONS

Sensors link the real world with the electronic and computer world. The effectiveness of a process monitoring and control system is greatly dependent on the accuracy, repeatability and the rate of response of the various sensors employed to provide the input parameters (eg, values of temperature, moisture, composition, flow, pH, etc.). Unavailability of the right sensors (1) is one reason why the U.S. food industry has not benefitted as much from computer-based monitoring and control systems that have dramatically improved efficiency, energy savings and labor savings in most other manufacturing sectors (2–4). Automated process monitoring and control can also help to meet some of the unique process quality control requirements of the food industry such as the rigid standards of identity and regulatory requirements of the United States Food and Drug Administration. Need for automation is also felt as more batch operations in food industry are replaced with continuous processing. Development of better sensing systems are an integral part of such automation.

The unique and unsatisfied sensing needs of the food industry are often related to measuring composition of materials, usually in the solid state. One study (5) in the food industry revealed that almost 40% of the needs were for moisture sensors, 23% were for fat sensors, 14% were for protein sensors, 6% were for solids content sensors, and less than 3% were for acidity sensors. The remaining 14% were for a number of different sensors. The urgent need for the composition sensors had blocked out requests for temperature, pressure, flow and level sensors although it is known that there are unsatisfied needs in these areas. Composition sensing involves high specificity and sensitivity in the assay of food ingredients, trace compounds, additives, contaminants, toxins, microbial state, and extent of oxidative reactions such as fat rancidity. It is the need for sensitivity to very specific chemical, biochemical, or biological components that makes each composition sensing process in the food industry truly unique and difficult.

Some of the sensing techniques having limited success in rapid composition sensing include spectroscopic, microwave, ultrasonic, and fiber-optic methods (6). Of these, the near infrared spectroscopic technique has been the most successful in analyzing food components, but the process frequently requires sample preparation and is generally off-line and more expensive. Microwave, ultrasonic, and fiber-optic sensing techniques are primarily unable to provide the high specificity and sensitivity need for numerous food components. Off-line laboratory (wet chemistry) procedures are available for most measurement needs in the food industry. Typically, samples are removed from the process, taken to a laboratory, and answers received minutes or hours later, as opposed to providing immediate results to allow the utilization of computer controlled processes. For example, several techniques exist for moisture measurement. These include oven-dry, Karl-Fischer, electrical conductance, electrical capacitance, radio frequency absorption, microwave absorption and infrared absorption. Of these, only the microwave absorption method is reasonably rapid and can be used on-line. Off-line techniques similarly exist for measurements of fat, protein, etc, but their continuous on-line sensing is limited by the lack of suitable sensors.

Chemical and biological sensors can provide some of the needed specificity and sensitivity for rapid sensing of various components in the food industry. They minimize chances of 'mistaken identity' and produce faster results with accuracy comparable to conventional laboratory techniques. They are generally small, easy to use and can be made highly sensitive. For example, some biosensors can be made sensitive down to parts-per-billion and parts-per-trillion detection level (7). Information on composition sensors, which is a more immediate necessity in food processing, is harder to locate (8) compared to sensing techniques for physical parameters (eg, temperature, flow). This is due to the very specific nature of chemical and biological reactions exploited in these sensors and the resulting limited range of use. Also, due to the interdisciplinary nature of such sensing processes, the little information that is available and has possible relevance to food applications (9,10) is scattered over literature sources spanning several scientific disciplines. This study attempts to identify such novel and existing composition sensing techniques from a great variety of literature sources, categorize the techniques, and discuss their potential for rapid monitoring of food industry processes.

CHEMICAL SENSORS

The three main types of chemical sensors—ion selective electrodes, chemically sensitive field effect transistors, and metal oxide gas sensors—will be discussed.

Ion-Selective Electrodes

Ion-selective electrodes (ISEs) are electrochemical devices (11) whose voltage output, at virtually zero current, is directly related to the concentration of some ionic species around the electrode. They have been in use since the early 1930s and are discussed here since they form the basis of many advanced chemical and biological sensors. The ISEs have a membrane that selectively allows only certain ions to pass through. This selective migration of ions from a higher to a lower concentration results in an uneven charge distribution across the membrane, with a consequential building up of a potential across the membrane in a direction which opposes further movement of the ions, and an equilibrium is reached. At equilibrium, the resulting potential would exactly balance the net ionic flow in each direction, and no further net flow of charge would occur. In a system in which no current can flow, the total number of ions required to develop this opposing potential is extremely small compared to usual solution concentrations. This potential difference developed across the membrane is of the same form as that described for redox electrodes and may be written as:

$$E - E^0 + \frac{RT}{n\mathrm{F}} \ln A$$

where E is the observed potential; E^0 is a constant potential which varies with the choice of reference electrodes; R is the gas constant; T is the temperature; n is the integral value of the charge on the measured species, A; F is the Faraday constant; and A is the thermodynamic activity of the measured species.

The properties and performance of the electrodes depend on the composition and choice of membrane material. The most well-known ISE is the glass electrode used for pH measurements (Fig. 1). A typical glass electrode consists of a glass tube sealed in one end with a very thin glass membrane and filled with a reference electrolyte. Electrical contact with the inner solution is normally made with a metal wire coated with Ag/AgCl. The glass electrode is immersed in the analyte together with a reference electrode. Hydrogen ions build up a pH dependent voltage across the glass membrane and this voltage is measured by connecting the pH electrode and the reference electrode to a very high input resistance voltmeter.

An example of a food application is the measurement of the sodium content of foods (12). This study described a glass electrode with special composition and having selectivity and sensitivity for sodium ions. The operation was performed in a range where hydrogen ion activity was negligible compared to sodium ion activity. Sodium glass-membrane electrodes contained 11% Na_2O, 18% Al_2O_3 and 71% SiO_2 and could detect sodium ion concentrations as low as 0.23 ppm. Varying percentages of these three oxides caused a desired selectivity for other monovalent ions such as potassium. The electrode was successfully used to find sodium in meat samples. Use of this electrode required less sample preparation than other analytical methods but was still an off-line method for solids since they needed sample preparation. Other reported ion-selective electrodes include measuring sodium and potassium in practically all types of foods (13,14) and measuring nitrite content of animal feed (15).

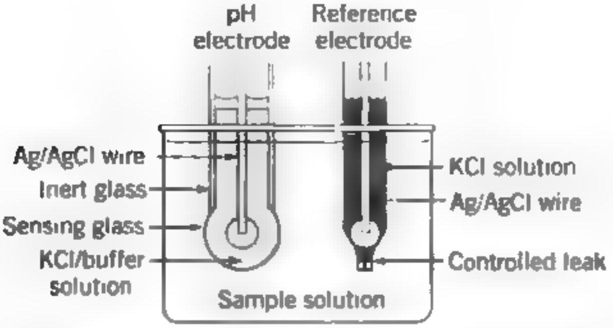


Figure 1. Schematic representation of a pH sensing electrode with a pH electrode and a reference electrode. *Source:* Ref. 11.

Practical use of ion-selective electrodes in foods have not been very successful due to an incomplete understanding of the underlying principles and due to the poor performance of some of the older instruments. They are normally not suitable for aggressive environments and sample preparation is often required with these electrodes. However, they are still faster and less expensive than more traditional wet chemistry techniques and have potential for future on-line use in food and agricultural processing, particularly in composition sensing of fluids.

Chemically Sensitive Field Effect Transistor (CHEMFET)

Chemical parameters can be measured with semiconductor devices. The ion-selective electrodes can be combined with a transistor to develop an ion-selective field effect transistor (ISFET). The relationship between ISEs and ISFETs is shown in Figure 2 (16), in which the conventional ISE and a reference electrode 'R' are first shown connected to the insulated gate field effect transistor 2(IGFET) input of a voltmeter (Fig. 2a). An IGFET is commonly called MOSFET (Metal-oxide-semiconductor field effect transistor). It has very high input impedance and is used in ultra-high-input impedance amplifiers as needed in the potentiometric electrode measurement to minimize the flow of current. The integration process is achieved by attaching the ion-selective membrane 'M' directly to the gate of the input transistor (Fig. 2b,c). The resulting device is called an ion-selective field effect transistor (ISFET). The ISFET (or CHEMFET) chip is completely insulated except for the gate whose surface complexation with the specific electrolyte in solution determines the ionic selectivity of the chip.

Food application of CHEMFETs include on-line measurement of pH. Due to the chemical selectivity of the ion-selective membrane, these sensors can potentially be made to respond to other food components (liquid and gaseous) and therefore measure their concentration on-line. Compared to ion-selective electrodes, CHEMFETs offer several advantages including flexibility due to small size, a fast response, and the requirement of less complex labor-intensive calibration and preconditioning procedures (18). At present, they are used as research tools in physiology

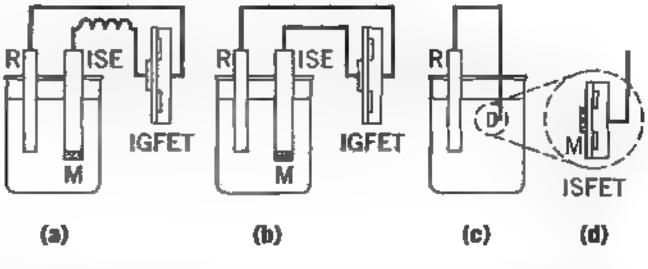


Figure 2. Evolution of ISFET (ion-selective field effect transistor, (c) and (d)) from ISE (ion-selective electrode) and IGFET (insulated gate field effect transistor) in (a) and (b). *Source:* Ref. 17.

and medicine, surface adsorption studies, gas detection, and the study of stochastic processes at various interfaces. The compatibility of the ion-selective membranes and semiconductor materials used in the construction of CHEMFETs is the major practical problem standing in the way of wide usage of these devices. By deliberate choice both the electrochemical element (the membrane) and the electronic preamplifier (FET chip) are exposed to a very hostile environment, the electrolyte solution. In such a situation, the requirements that are placed on the encapsulation materials are much more stringent than those required for ISEs. Once the encapsulation and membrane attachment problems are solved, design of sensor packages that will include data acquisition as well as data-processing elements will be feasible (16).

Using an operational amplifier, Perez-Olmos (19) improved the sensitivity of ISE for measuring potassium and calcium in wines. Knee and Srivastava (20) adapted an ISE to measure calcium in apple fruit tissue; calcium is a critical mineral affecting postharvest handling and quality. A fully automated battery-operated computerized field-based ISE method for monitoring fluoride in water was reported by Bond et al. (21).

Metal Oxide Gas Sensors

The most widely used sensors for combustible gases today are based on semiconducting metal oxides. Semiconducting gas sensors are usually based on the surface properties of the oxides of tin or zinc (SnO_2 or ZnO). It is generally agreed that the surface conductivity of semiconductors can be markedly changed by the adsorption and subsequent reaction of gases with already-adsorbed atmospheric oxygen. This implies that for an *n*-type material (where electrical conduction is associated with electrons, as opposed to holes) such as SnO_2 or ZnO, the concentration of electrons available for conduction can be changed by either oxidation or reduction processes. At elevated temperatures, atmospheric oxygen is adsorbed and it accepts electrons to become O_2^-, O^-, or O^{2-}. If a reducing gas is then also adsorbed, it may either simply donate electrons and become a positively charged species; or it may react with oxygen, thus releasing bound electrons. In either case, electrons become available for conduction and the resistance of the surface layers decreases drastically. For an oxidizing gas, the converse mechanisms will operate and the resistance will rise. This conductivity versus gas concentration response is exploited in the metal oxide gas sensors.

An example of a possible food industry use of a gas sensor can be seen in the work of Mandenius and Mattiasson (22) for the on-line monitoring of ethanol during fermentation processes. A sensor (Fig. 3) similar to the one used by them is described by Watson (23). The sensor consisted of a small tubular ceramic former having an interdigitated metallization pattern on the outer surface, upon which was deposited the active materials, which was largely tin oxide plus catalytic dopants, notably palladium. A heater filament was put inside the tube, while the gold alloy sensor leads were bonded to annuli on the outer surface at the ends. These annuli were in fact part of the interdigitated metallization deposited on the outer surface coated with the sintered, but porous, tin oxide mix. In the work of Mandenius and Mattiasson (22), ethanol, which is a reducing gas, was allowed to flow over the tin oxide coated surface of the sensor. The gas was absorbed onto the sensor surface and produced a marked decrease in the surface electrical resistance. By using a continuous dilution of the gas flow streams, the lower limit of detection of ethanol was extended by their work to operate within the concentration ranges of importance in biotechnological processes. A catalytic gas sensor device combined with a carrier gas flow control was recently developed into an integrated ethanol-sensing system (25). This permitted online monitoring of ethanol during the fermentation operation in a brewery. Gas detectors can be potentially made sensitive to other gases or volatiles present in biotechnological processes (for example, butanol, formate, acetate, and formaldehyde). For example, using metal oxide semiconductor gas sensors, it was possible to analyze wine vapors (26) or coffee aroma (27).

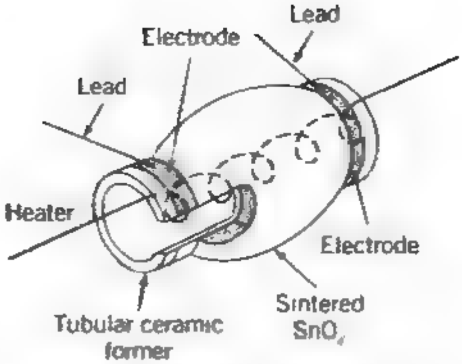


Figure 3. Configuration of a semiconductor gas sensor. *Source:* Refs. 23 and 24.

The advantages of this form of sensor include small size, convenient operation from low voltage power supplies, very high sensitivity in most applications and the need for only simple associated electronics. The disadvantages include a continuous power drain for sensor heating, sensitivity to ambient temperature and humidity and the presence of long-term drift. The first generation of gas sensors has suffered from poor selectivity, but many improvements are taking place, and the above example illustrates their potential on-line uses for food and agricultural processing.

BIOSENSORS

A biosensor comprises a biologically sensitive material immobilized in intimate contact with a suitable transducing system which converts the biochemical signal into a quantifiable and processible electrical signal (28,29). The biologically sensitive material is typically an enzyme, multienzyme system, antibody, membrane component, organelle, bacterial or other cell, or whole slices of mammalian or plant tissues. To produce an electrical signal, the biological interacting system is placed in close proximity to a suitable transducer (Fig. 4). When biological molecules and medicine, surface adsorption studies, gas detection, and the study of stochastic processes at various interfaces. The compatibility of the ion-selective membranes and semiconductor materials used in the construction of CHEMFETs is the major practical problem standing in the way of wide usage of these devices. By deliberate choice both the electrochemical element (the membrane) and the electronic preamplifier (FET chip) are exposed to a very hostile environment, the electrolyte solution. In such a situation, the requirements that are placed on the encapsulation materials are much more stringent than those required for ISEs. Once the encapsulation and membrane attachment problems are solved, design of sensor packages that will include data acquisition as well as data-processing elements will be feasible (16).

Using an operational amplifier, Perez-Olmos (19) improved the sensitivity of ISE for measuring potassium and calcium in wines. Knee and Srivastava (20) adapted an ISE to measure calcium in apple fruit tissue; calcium is a critical mineral affecting postharvest handling and quality. A fully automated battery-operated computerized field-based ISE method for monitoring fluoride in water was reported by Bond et al. (21).

Metal Oxide Gas Sensors

The most widely used sensors for combustible gases today are based on semiconducting metal oxides. Semiconducting gas sensors are usually based on the surface properties of the oxides of tin or zinc (SnO_2 or ZnO). It is generally agreed that the surface conductivity of semiconductors can be markedly changed by the adsorption and subsequent reaction of gases with already-adsorbed atmospheric oxygen. This implies that for an *n*-type material (where electrical conduction is associated with electrons, as opposed to holes) such as SnO_2 or ZnO, the concentration of electrons available for conduction can be changed by either oxidation or reduction processes. At elevated temperatures, atmospheric oxygen is adsorbed and it accepts electrons to become O_2^-, O^-, or O^{2-}. If a reducing gas is then also adsorbed, it may either simply donate electrons and become a positively charged species; or it may react with oxygen, thus releasing bound electrons. In either case, electrons become available for conduction and the resistance of the surface layers decreases drastically. For an oxidizing gas, the converse mechanisms will operate and the resistance will rise. This conductivity versus gas concentration response is exploited in the metal oxide gas sensors.

An example of a possible food industry use of a gas sensor can be seen in the work of Mandenius and Mattiasson (22) for the on-line monitoring of ethanol during fermentation processes. A sensor (Fig. 3) similar to the one used by them is described by Watson (23). The sensor consisted of a small tubular ceramic former having an interdigitated metallization pattern on the outer surface, upon which was deposited the active materials, which was largely tin oxide plus catalytic dopants, notably palladium. A heater filament was put inside the tube, while the gold alloy sensor leads were bonded to annuli on the outer surface at the ends. These annuli were in fact part of the interdigitated metallization deposited on the outer surface coated with the sintered, but porous, tin oxide mix. In the work of Mandenius and Mattiasson (22), ethanol, which is a reducing gas, was allowed to flow over the tin oxide coated surface of the sensor. The gas was absorbed onto the sensor surface and produced a marked decrease in the surface electrical resistance. By using a continuous dilution of the gas flow streams, the lower limit of detection of ethanol was extended by their work to operate within the concentration ranges of importance in biotechnological processes. A catalytic gas sensor device combined with a carrier gas flow control was recently developed into an integrated ethanol-sensing system (25). This permitted online monitoring of ethanol during the fermentation operation in a brewery. Gas detectors can be potentially made sensitive to other gases or volatiles present in biotechnological processes (for example, butanol, formate, acetate, and formaldehyde). For example, using metal oxide semiconductor gas sensors, it was possible to analyze wine vapors (26) or coffee aroma (27).

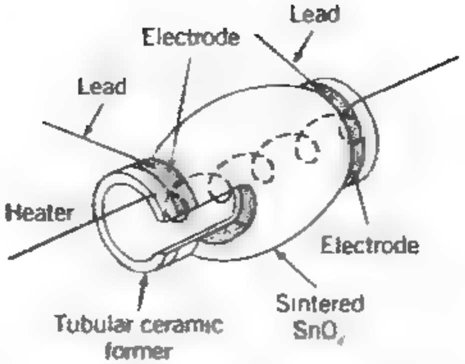


Figure 3. Configuration of a semiconductor gas sensor. *Source:* Refs. 23 and 24.

The advantages of this form of sensor include small size, convenient operation from low voltage power supplies, very high sensitivity in most applications and the need for only simple associated electronics. The disadvantages include a continuous power drain for sensor heating, sensitivity to ambient temperature and humidity and the presence of long-term drift. The first generation of gas sensors has suffered from poor selectivity, but many improvements are taking place, and the above example illustrates their potential on-line uses for food and agricultural processing.

BIOSENSORS

A biosensor comprises a biologically sensitive material immobilized in intimate contact with a suitable transducing system which converts the biochemical signal into a quantifiable and processible electrical signal (28,29). The biologically sensitive material is typically an enzyme, multienzyme system, antibody, membrane component, organelle, bacterial or other cell, or whole slices of mammalian or plant tissues. To produce an electrical signal, the biological interacting system is placed in close proximity to a suitable transducer (Fig. 4). When biological molecules

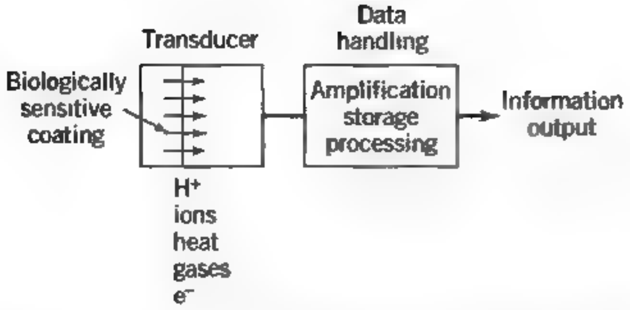

Transducer
Data handling
Biologically sensitive coating
Amplification storage processing
Information output
H+
ions
heat
gases
e⁻

Figure 4. A generalized biosensor configuration. The biologically sensitive coating comprising an antibody, receptor protein or biocatalyst, such as an enzyme, organelle, whole cell or tissue slice, generates a physico-chemical change in response to the analyte which is converted into an electrical signal by the transducer, amplified and subsequently processed and outputted. *Source:* Ref. 3.

interact specifically and reversibly, a change takes place in one or more physico-chemical parameters associated with the interaction, such as a change in proton concentration, release or uptake of gases (O_2, CO_2, NH_3), specific ions (NH_4^+, monovalent cations, anions), heat, optical density, non-specific ions or electron transfer which if generated in close proximity to a suitable transducer, may be converted into an electrical signal. Specific interactions with the biological material can be exploited in the biosensors for sensing specific food ingredients as well as trace compounds, additives or contaminants, toxins, and marker chemicals indicating the microbial state of the food (28,31,32).

Intimate contact between the biosystem and the transducer is quite important. This is achieved by immobilization of the biological material (biocatalyst) at the device surface, generally by one of several methods. First, by chemically cross-linking the biocatalyst with an inert, generally proteinaceous, material with a bifunctional reagent to form intermolecular bonds between the catalyst and the inert protein. An alternative and commonly employed procedure, physically restrains the biocatalyst at the transducer surface by entrapment in polymer matrices such as polyacrylamide or agarose, or by retention with a polymer membrane comprising cellophane, cellulose acetate/nitrate, poly(vinyl alcohol) or polyurethane. Finally, a preferred procedure in some cases is to covalently attach the biologically sensitive system directly to the surface of the transducer and thereby achieve intimate contact without incurring the diffusional limitations, sometimes observed with membrane or matrix entrapped systems (30).

The transducer is an electrical device which responds to the products of the biocatalytic process and outputs the response in a form which can subsequently be electrically amplified and displayed. The design of the transducer should accommodate the following features: it must be highly specific for the analyte of interest and respond in an appropriate concentration range; it should have a moderately fast response time, typically 1–60 s; it must be amenable to miniaturization and should ideally compensate internally for adverse environmental effects such as temperature dependency, drift, etc.

Three major types of biosensors currently available are biosensors based on either potentiometric or amperometric transducers (33,34) and those combined with optical fibers (35).

Potentiometric Biosensors

Potentiometric devices operate under equilibrium conditions and measure the accumulation of charge at the electrode surface brought about by some selective process. The best known potentiometric biosensor is based on the ion-selective electrode (ISE) discussed earlier, where an immobilized enzyme is coated over the electrode, making it a potentiometric enzyme electrode. The specificity and sensitivity of various enzymes in catalyzing different reactions are exploited here and the substrates or products are measured as they are produced or consumed at the electrode surface. The glass pH electrode was the first ISE to be used with enzymatic reactions that proceed with the consumption or production of hydrogen ions, although other electrodes have subsequently been utilized. For example, the enzyme urease catalyzes the hydrolysis of urea and may be exploited in the determination of urea by immobilization around a pH, NH_4^+, HCO_3^- or NH_3-gas electrode (30).

The integration of an ion-selective membrane with a solid state FET results in the ion-selective field effect transistor (ISFET) as described under Chemical Sensors. It is possible to immobilize enzymes directly over the gate of an ISFET. The resulting device would be called an enzymatically sensitive field effect transistor (ENFET). A major advantage of the ENFET over an equivalent enzyme electrode is its small size. Other advantages of ENFETs are similar to those of ISFETs described earlier. Caras et al. (36–38) reported ENFETs sensitive to glucose and penicillin. In their glucose ENFET (Fig. 5), they had immobilized glucose oxidase enzyme by covalently linking it to an open, polyacrylamide gel matrix and placing it on the gate of a pH-sensitive transistor. The figure shows two pH-sensitive ISFETs on a chip which are identical except one of them has the glucose oxidase enzyme added to its gel while the other does not have any enzyme added. The two ISFETs

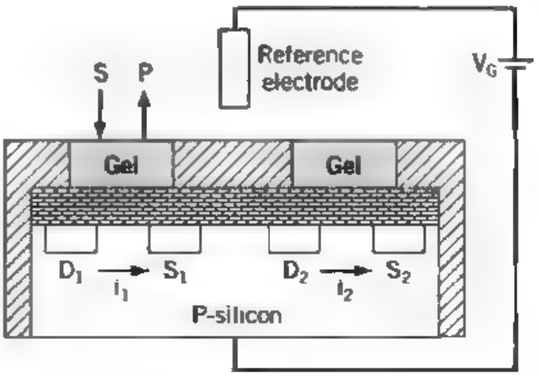


Figure 5. Schematic diagram of the ENFET chip sensitive to glucose. One of the gels has glucose oxidase enzyme for glucose sensing. D and S are transistor drain and source, respectively. V_G is the applied gate voltage and I is the drain-to-source current. *Source:* Ref. 38.

were put side-by-side so that the non-specific variations in the transistor outputs which were caused by variations in the ambient temperature, pH, and common noise could be eliminated by differential measurement. Such devices are under development and have future potential for on-line monitoring of glucose and other components in various food processes (39).

Amperometric Biosensors

Amperometric electrodes measure the flux of electroactive species. An example of an amperometric biosensor for food applications is a microbial sensor for detection of fish freshness (40). During storage of fish, larger molecular weight compounds such as proteins and glycogen are gradually degraded into smaller molecular weight compounds, which can be utilized more readily by microorganisms. The sensor (Fig. 6) was prepared by immobilizing spoilage causing bacteria, *A. putrefaciens*, on a membrane filter. The filter was fixed at the tip of an oxygen electrode and covered with a cellulose acetate membrane. The extent of the assimilation of organic substances (resulting from deterioration of fish during storage) by these immobilized microorganisms can be determined from the respiratory activity of the microorganisms by directly measuring the consumption of oxygen in the oxygen electrode. When extract from a stored fish was allowed to flow over the membranes, oxygen consumption due to the increased respiratory activity of the microorganisms caused a decrease in dissolved oxygen around the microbial membrane and consequently brought a marked decrease in the output current of the oxygen electrode. The current decrease between the initial and the minimum current was used as the measure of fish freshness. The output of the sensor, like many other sensors, is influenced by the number of immobilized living cells, pH, temperature, etc. The fish freshness sensor is one of the few biosensors developed for specific applications to the food industry. A xanthine oxidase-immobilized and carbon-based screen-printed electrode was recently developed for determining fish freshness (41). Amperometric measurements of uric acid and hypoxanthine in fish muscle exudates were effective in calculating *K* value, the index of fish freshness based on the levels of uric acid and hypoxanthine.

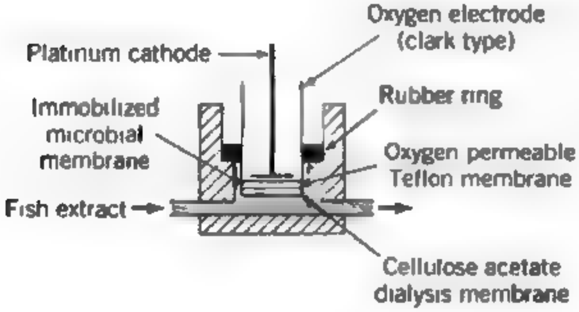

Oxygen electrode (clark type)
Platinum cathode
Rubber ring
Immobilized microbial membrane
Oxygen permeable Teflon membrane
Fish extract
Cellulose acetate dialysis membrane

Figure 6. Schematic diagram of a microbial sensor (an amperometric enzyme electrode) system for fish freshness determination. *Source:* Ref. 40.

Another example of an amperometric enzyme electrode is a sensor for rapid in-line determination of lactose and other milk components (42). The sensor was prepared by immobilizing glucose oxidase and β-galactosidase on a nylon membrane and placing it on the platinum electrode surface. A cellulose acetate membrane was placed between the electrode surface and immobilized enzyme to eliminate electroactive compounds such as ascorbic acid. The layer of immobilized enzymes were covered with a cellulose acetate dialysis membrane in order to prevent microbial attack and leaching of the enzymes. The platinum electrode surface can measure hydrogen peroxide concentration amperometrically. Lactose from milk entered through the cellulose acetate dialysis membrane and first changed to glucose by the action of β-galactosidase. The glucose formed reacted with glucose oxidase and formed hydrogen peroxide at the platinum electrode which was reflected by a change in current in the electrode. This method of lactose determination in milk samples does not require any preliminary sample treatment, is inexpensive and also very simple and quick compared to existing titrimetric methods. It is likely to be useful for obtaining rapid analytical data on milk in dairy farms and can be used in-line. More recent applications of this technology include the measurement of aspartame in diet beverages (43), nitrate in drinking water (44), glutamate in seasonings (45), and malate in grape musts and wines (46).

Fiber-Optic Biosensors

Optical fibers are being used for numerous sensing applications (35). The fibers are rugged, more resistant to corrosion than other sensors and immune to electromagnetic interference. The transparent fibers of glass are generally used to guide a light signal to the point of measurement where the light signal changes its parameters in response to physical, chemical, and biological changes at the point of measurement. This modified light signal transmitted back along the fibers is analyzed to derive information about the physical, chemical, and biological changes. Common fiber-optic-based biochemical sensing techniques involve the use of enzymes or substrates immobilized on the tip of an optical fiber for substrates or enzyme activity measurement respectively. Trettank et al. (47) described an optical fiber sensor capable of continuously monitoring glucose. In this feasibility study, glucose oxidase enzyme was physically immobilized in a sensing layer at the end of a fiber optic light guide (Fig. 7). The enzyme catalyzed the oxidation of glucose to give gluconic acid, which in turn, lowered the pH in the microenvironment of the sensor. By following the changes in the fluorescence of a pH sensitive dye incorporated in the sensing layer, the enzymatic reaction could be monitored. The optical isolation layer prevented ambient light or intrinsic sample fluorescence from interfering. The pH sensitive dye used was 1-hydroxypyrene-3,6,8-trisulfonate (HPTS) which had an almost linear decrease in bright green fluorescence over the pH 7-6 range. The food industry, with a need for glucose sensing for fermentation monitoring and control, can benefit from such developments. A highly specific and sensitive fluorometric fiber-optic biosensor immobilized with glucose-

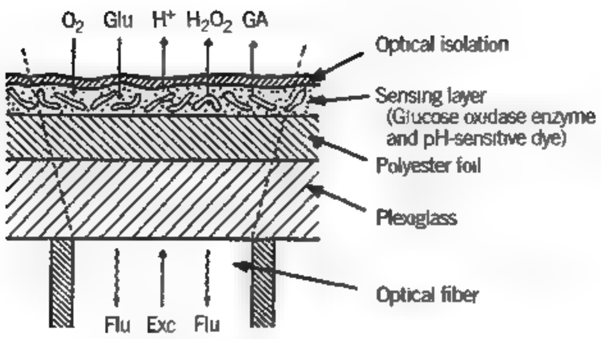


Figure 7. Cross-section through the sensing layer of the fiber-optic glucose sensor. The directions of the exciting light (Exc) and fluorescence (Flu) are also shown. *Source*. Ref 47.

fructose-oxidoreductase from *Zymomonas mobilis* was later developed for the dual analysis of glucose and fructose (48).

Another type of fiber-optic-based biological sensor involves bioluminescence, where light is emitted during highly sensitive and specific enzymatic reactions. For example, a bio-luminiscent system (47) for the determination of NADH was produced using the bacterial luciferase and NAD(P)H:FMN oxidoreductase commobilized on a polyamide membrane. The bioluminiscent system was maintained in close contact with one end of a optical-fiber bundle. The light intensity obtained during reactions was analyzed using a photomultiplier tube which is a simpler instrumentation as compared to the need for a light source and monochromators in many other types of fiber-optic-based biosensors. The maximum light intensity correlated linearly with the concentration of NADH in this study. Such systems are quite new but hold future promises for composition sensing. An extremely sensitive dual-enzyme fiber-optic biosensor immobilized with glutamate dehydrogenase and glutamate–pyruvate transaminase was reported for measuring glutamate based on reduced NAD luminescence (49). A fiber-optic biosensor immobilized with xanthine oxidase and peroxidase was subsequently reported for assaying the quality of seafood products (50). Krug et al. (51) developed a fiber-optic system to measure total and free cholesterol. Immobilized cholesterol esterase cleaved cholesterol esters, while cholesterol oxidase converted cholesterol to cholest-4-ene-3-one and hydrogen peroxide. Hydrogen peroxide formed a colored complex with a dye that was measured by fiber-optic instruments.

A variety of fiber-optic biosensors have been reported capable of detecting bacterial and mold toxins including *Clostridium botulinum* toxin A (52), Staphylococcal enterotoxin B (53), *Eschericha coli* lipopolysaccharide (54), and the mycotoxin fumonisin B1 (55).

Advantages of biosensors are that they do not require highly technically qualified users in order to generate precise results, they are inexpensive, they generate results in a short period of time thus enabling better control of processes or better information for users, and that they can be used in remote locations. Estimates of possible markets of biosensors in the 1990s for food and other industrial process monitoring have been put at $59 million. Novel biosensors are currently under development at several institutions around the world. Although much of this development is geared towards biomedical needs, food processing can significantly benefit from them due to the similar types of measurements involved. Future research related to biosensors will focus on immobilization techniques, the matching of biosensitive detection substances to analytes, proper transducing mechanisms, proper packaging of the sensor for use in hostile environments, and the optimization of the sensor response.

SUMMARY

The food industry has unique and unsatisfied needs for rapid, on-line sensing of process parameters, particularly the composition of various food components. Chemical and biological sensing techniques have been identified that can provide the specificity for a particular reaction and can be fast, accurate, inexpensive, and easier-to-use when compared to standard laboratory tests. The chemical sensors explored include semiconductor gas sensor, ion selective field effect transistors, and ion selective electrodes. Also included are enzyme electrodes, enzyme field effect transistors, and fiber-optic biosensors. These future sensing techniques have potential for on-line monitoring and control of food and agricultural processing operations.

ACKNOWLEDGMENTS

This research was supported through Hatch project 408, USDA. Source of this article is A. K. Datta, "Novel Chemical and Biological Sensors for Monitoring and Control of Food Processing Operations," *J. Food Eng.* **12** (1990). Copyright 1990. Reproduced with permission, Elsevier Science Publishers, England.

BIBLIOGRAPHY

1. H. C. J. Frost, The Food Industry's Unsatisfied Needs for Sensing Systems, *Prepared Foods*, Gorman Publishing Co., Chicago, July 1984.
2. J. P. Clark, K. J. Valentas, and D. B. Lund, Report of the Workshop Session on Food Process Engineering/Automation, *Food Technology* **39**, 21R–22R (1985).
3. G. W. Isaacs, Research Needs in Sensor Technology for Agriculture, Experiment Station Committee on Organization and Policy, 1986.
4. L. D. Pederson, W. W. Rose, H. Redsun, and S. H. Bogosian, Assessment of Sensors Used in the Food Industry Interim Report, National Food Processors Association, Dublin, Calif., 1989.
5. H. C. J. Frost, "Unsatisfied Needs for Continuous Sensing Systems. Instrumentation in the Food and Pharmaceutical Industries," *Proceedings of the Process Automation Symposium sponsored by the Instrument Society of America*, Instrument Society of America, N C., 3–4 April 1985.
6. A. K. Datta, Sensor Needs and Their Development for On-Line Control of Food Processing Operations, *Paper no. 87-6530*, American Society of Agricultural Engineers, 1987
7. D. Best, "Biosensors Revolutionize Quality Control," *Prepared Foods* **10**, 182–186 (1987).

8. L. Gould, Sensors in Food Processing: Part Two, *Sensors* **6**, 75–84 (1989).
9. E. Kress-Rogers, "Instrumentation in the Food Industry I: Chemical, Biochemical and Immunological Determinants," *Journal of Physics E: Scientific Instrumentation* **19**, 13–21 (1986)
10. E. Kress-Rogers, Instrumentation in the Food Industry. II: Physical Determinants in Quality and Process Control *Journal of Physics E: Scientific Instrumentation* **19**, 105–109 (1986).
11. M. S. Frant, "Ion-Selective Electrodes, in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 13, Wiley-Interscience, New York, 1978.
12. W F. Averill, Ion-Sensitive Electrode System Measures Sodium Content of Foods, *Food Technology* **37**, 44–2 (1983).
13. E. Rabe, Zur natrium-und kaliumbestimmung mit ionensensitiven elektroden. (Determination of Sodium and Potassium by Ion-Sensitive Electrodes.) *Zeitscheuft fur Lebensmittel-Untersuchung und-Forschung* **76**, 270–274 (1983).
14. Y. Yamamoto et al., "A Volatile Amine Sensor Based on the Amperometric Ion Selective Electrode," *Buneski-Kagaku* **38**, 589–595 (1989).
15. H. J. Issaq, G. M. Muschik, and N. H. Risser, "Determination of Nitrite in Animal Feed by Spectrophotometry or Potentiometry with a Nitrite-Selective Electrode," *Analytica Chimica Acta* **154**, 335–339 (1983).
16. J. Janata, Chemically Sensitive Field Effect Transistors, in J. Janata and R. J. Huber, eds., *Solid State Chemical Sensors*, Academic Press, Inc., New York, 1985.
17. J. Janata and R. J. Huber, Ion-Selective Field Effect Transistors, *Ion-Selective Electrode Review* 1, 31–79 (1979).
18. S. Moire, "Chemfet Sensors in Industrial Process Control," *Sensors* **5**, 39–44 (1988).
19. R. Perez-Olmos et al., "Construction and Evaluation of Ion-Selective Electrodes for Potassium and Calcium with a Summing Operational Amplifier, Application to Wine Analysis," *Journal of Analytical Chemistry* **360**, 659–663 (1998).
20. M. Knee and P. Srivastava, "Binding of Calcium by Cell Walls and Estimation of Calcium in Apple Fruit Tissue with an Ion Selective Electrode," *Postharvest Biology and Technology* **5**, 19–27 (1994).
21. A. M. Bond et al., "Development and Application of a Fully Automated Battery-Operated Computerized Field-Based Fluoride Monitor," *Analytical Chimica Acta* **237**, 345–352 (1990).
22. C. F. Mandenius and Mattiasson, Improved Membrane Gas Sensor Systems for On-Line Analysis of Ethanol and Other Volatile Organic Compounds in Fermentation Media," *European Journal of Applied Microbiology and Biotechnology* **18**, 197–200 (1983).
23. J. Watson, "The Tin Oxide Gas Sensor and its Applications," *Sensors and Actuators* **5**, 29–42 (1984).
24. U.S. Pat 3,631,436 (to Figaro Gas Sensor).
25. G. D. Austin et al., "A Gas-Sensor-Based On-Line Ethanol Meter for Breweries," *Journal of the American Society of Brewing Chemists* **54**, 212–215 (1996).
26. C. di Natale et al., "Metal Oxide Semiconductor Gas Sensors Array as a Tool for the Analysis of Wine Vapors, Current and Future Trends," *Proceedings of Euro Food Chem VIII*, Vol. 1, Vienna, Austria, 1995, pp. 131–134.
27. T. Aishima, "Aroma Discrimination by Pattern Recognition Analysis of Responses from Semiconductor Gas Sensor Array," *Journal of Agricultural and Food Chemistry* **39**, 752–756 (1991).
28. S. S. Deshpande and R. M. Rocco, "Biosensors and Their Potential Use in Food Quality Control," *Food Technology* **48**, 146–150 (1994).
29. D. L. Wise, *Applied Biosensors*, Butterworth Scientific Ltd., U.K., 1989.
30. C. R. Lowe, An Introduction to the Concept and Technology of Biosensors, *Biosensors* **1**, 3–16 (1985)
31. E. Kress-Rogers, and E. J. D'Costa, Biosensors for the Food Industry. *Analytical Proceedings* **23**, 149–151 (1986).
32. G. Wagner and G. G. Guilbault, *Food Biosensor Analysis*, Marcel Dekker, New York, 1993
33. M. K. Y. Ho and G. A. Rechnitz, "An Introduction to Biosensors," in R. M. Nakamura, Y. Kasahara, and G. A. Rechnitz, eds., *Immunochemical Assays and Biosensor Technology for the 1990s*, American Society of Microbiologists, Washington, D.C., 1992, pp. 275–290.
34. A. N. Reshetilov, "Models of Biosensors Based on Principles of Potentiometric and Amperometric Transducers: Use in Medicine, Biotechnology, and Environmental Monitoring (Review)," *Applied Biochemistry and Microbiology* **32**, 72–85 (1996).
35. G. Yazbak, "Fiberoptic Sensors Solve Measurement Problems," *Food Technology* **45**, 76–78 (1991).
36. S. D. Caras and J. Janata, "pH-Based Enzyme Potentiometric Sensors, Part 3, Penicillin-Sensitive Field Effect Transistor," *Analytical Chemistry* **57**, 1924–1925 (1985).
37. S. D. Caras, J. Janata, D. Saupe, and K. Schmidt, "pH-Based Enzyme Potentiometric Sensors, Part I, Theory," *Analytical Chemistry* **57**, 1917–1920 (1985).
38. S. D. Caras, D. Petelenz, and J. Janata, "pH-Based Enzyme Potentiometric Sensors, Part 2, Glucose-Sensitive Field Effect Transistor," *Analytical Chemistry* **57**, 1920–1923 (1985).
39. R. Ulber et al., "ISFET Biosensors, Applications in Analysis and Biological Process Control," *Lebensmittel & Biotechnologie* **13**, 187–189 (1996).
40. E. Watanabe, A. Nagumo, M. Hoshi, S. Konagaya, and M. Tanana, "Microbial Sensors for Detection of Fish Freshness," *Journal of Food Science* **52**, 592–595 (1987).
41. M. A. Carole, G. Volpe, and M. Mascini, "Amperometric Detection of Uric Acid and Hypoxanthine with Xanthine Oxidase and Carbon Based Screen-Printed Electrode, Application for Fish Freshness Determination," *Talanta* **44**, 2151–2159 (1997).
42. M. Mascini, D. Moscone, G. Palleschi, and R. Pilloton, "In-Line Determination of Metabolites and Milk Components with Electrochemical Biosensors," *Analytica Chimica Acta* **213**, 101–111 (1988).
43. R. L. Villarta, A. A. Suleiman, and G. G. Guilbault, "Amperometric Enzyme Electrode for the Determination of Aspartame in Diet Food," *Microchemical Journal* **48**, 60–64 (1993).
44. S. A. Glazier, E. R. Campbell, and W. H. Campbell, "Construction and Characterization of Nitrate Reductase-Based Amperometric Electrode and Nitrate Assay of Fertilizers and Drinking Water," *Analytical Chemistry* **70**, 1511–1515 (1998).
45. R. L. Villarta, D. D. Cunningham, and G. G. Guilbault, "Amperometric Enzyme Electrodes for the Determination of L-Glutamate," *Talanta* **38**, 49–55 (1991).
46. M. C. Messia et al., "A Bienzyme Electrode for Malate," *Analytical Chemistry* **68**, 360–365 (1996).
47. W. Trettank, M. J. P. Leiner, and O. S. Wolfbeis, Fiber-Optic Glucose Sensor with a pH Optrode as the Transducer," *Biosensors* **4**, 15–26 (1988).

48. L. Sei-Jin et al., "A Fluoremetric Fiber-Optic Biosensor for Dual Analysis of Glucose and Fructose Using Glucose-Fructose-Oxidoreductase from *Zymomonas mobilis*," *Journal of Biotechnology* **36**, 39–44 (1994).
49. A. J. Wang and M. A. Arnold, "Dual-Enzyme Fiber-Optic Biosensor for Glutamate Based on Reduced Nicotinamide Adenine Dinucleotide Luminescence," *Analytical Chemistry* **64**, 1051–1055 (1992).
50. C. Coppersmith, P. Pivarnik, and A. G. Rand, "Fiber-Optic Chemiluminescent Biosensor for the Analysis of Quality Status in Seafood Products," IFT Annual Meeting, 1996, Abstract p. 132.
51. A. Krug et al., "Colorimetric Determination of Free and Total Cholesterol by Flow Injection Analysis with a Fiber Optic Detector," *Enzyme and Microbial Technology* **14**, 313–317 (1992).
52. R. A. Ogert et al., "Detection of *Clostridium botulinum* Toxin A Using a Fiber Optic-Based Biosensor," *Analytical Biochemistry* **205**, 306–312 (1992).
53. L. A. Tempelman et al., "Quantitating Staphylococcal Enterotoxin B in Diverse Media Using a Portable Fiber-Optic Biosensor," *Analytical Biochemistry* **233**, 50–57 (1996).
54. E. A. James, K. Scmeltzer, and F. S. Ligler, "Detection of Endotoxin Using an Evanescent Wave Fiber-Optic Biosensor," *Applied Biochemistry and Biotechnology* **60**, 189–202 (1996).
55. V. S. Thompson and C. M. Maragos, "Fiber-Optic Immunosensor for the Detection of Fumonisin B1," *Journal of Agricultural and Food Chemistry* **44**, 1041–1046 (1996).

GENERAL REFERENCES

W. J. Albery, P. N. Bartlett, A. E. G. Cass, and K. W. Sim, "Amperometric Enzyme Electrodes. Part IV. An Enzyme Electrode for Ethanol," *Journal of Electroanalytical Chemistry* **218**, 127–134 (1987).

R. C. Hochberg, "Fiber-Optic Sensors," *IEEE Transactions on Instrumentation and Measurement* **IM-35**, 447–450 (1986).

C. Nylander, Chemical and Biological Sensors, *Journal of Physics E: Scientific Instrumentation* **18**, 736–749 (1985).

F. W. Scheller and R. Renneberg, Glucose-Eliminating Enzyme Electrode for Direct Sucrose Determination of Glucose-Containing Samples, *Anal. Chim. Acta* **152**, 265–269 (1983).

SENSORY SCIENCE: PRINCIPLES AND APPLICATIONS

INTRODUCTION

Over the past fifty years sensory analysis of foods has grown from an informal "taste test" performed by bench top chemists and product developers to a science comprising basic tenets, accepted methods, and defined statistical analyses. The scientific literature of sensory analysis comprises investigations of sensory processes (the way we respond to physical stimuli), methodological studies of "taste testing" (how to assess reactions to food products in a test situation), and statistical experimental design (how to set up systematically varied formulations of products). The actual physiology and psychophysics of the sensory systems involved in sensory analysis (viz, taste, smell, touch, appearance) are dealt with elsewhere in this volume. Data about the physical characteristics of food belong properly to the domain of food science. Sensory analysis is the nexus where these different disciplines join.

THE THREE AREAS OF SENSORY ANALYSIS

The easiest approach to sensory analysis for both theory and practice divides it into three major subject areas:

1. *Descriptive analysis.* The language of food and drink, and how we describe our perceptions.
2. *Intensity measurement.* Perception of the sensory characteristics of foods on a scale, which shows how we process physical stimulus magnitudes to generate sensory responses.
3. *Hedonic measurement.* Our likes/dislikes.

Each of these three topics has been addressed by numerous investigations in a large scientific literature stretching back centuries, but with much of that literature appearing in the ever burgeoning science of the 1950s and later (1,2). In the main this article deals with both conventional and new techniques available to the sensory analyst.

DESCRIPTIVE ANALYSIS

At the heart of sensory analysis lies the complex field of language or descriptive analysis. While at first blush it may seem easy to describe a food, individuals familiar with a specific food use many terms to describe the fine nuances. Some of these terms are idiosyncratic, but many are standard terms easily understood. There is no single descriptive language of general and universal application, but rather attempts to capture on paper and numerically the elusive character of different foods (3).

Since ancient times researchers have tried to create standardized lists of terms by which to summarize (and classify) sensory perceptions. Researchers using any of these lists soon find that the set of terms in any list is unbalanced and necessarily incomplete, comprising too many unusable terms for the specific product being studied, but too few relevant terms to capture the key "note" or attributes. Human beings use a stretchable "rubber bag" of terms to describe each product. The number of terms grows or shrinks to fit the specific product, but the list for any product may be sure to contain terms that would never be found in a general list.

People process a constant amount of "information" when they describe products. When forced to describe a wide range of qualitatively different products during a single test panelists overlook or jettison the nuances and focus on the more general terms that differentiate this broad array of qualitatively different stimuli. For products which vary only slightly from each other (eg, different samples of strawberry aroma) panelists employ the more rarely used terms, focusing on the minor differences and highlighting them with these terms.

Fixed Descriptor Systems Versus User Developed Systems For Flavor

Tables 1–3 shows lists of descriptors for smell (4). With a rating scale the panelist rates each odor or flavor on a limited set of characteristics. The investigator may compare two or more samples across the profile of the descriptors to determine their similarity (or difference). Academic researchers, mainly, use the ready-made lists, often to profile the sensory characteristics of pure odorous stimuli (so-called "model systems.) Table 4 shows some of the profiles obtained by this method.

Applied researchers interested in specific foods and their nuances prefer to develop their own lists customized to each product in order to capture the appropriate characteristics. A general list of descriptors selected to apply (potentially) to all flavors would not contain many of these unique terms. In order to develop a list comprising these "relevant" terms researchers follow a protocol designed to elicit many candidate terms, and then cull the list to select specific terms which truly apply to the specific product. One protocol comprises the three steps laid out below:

Initial List Development. Panelists assess the products, and either individually record all the terms that they feel would apply, or in a group discuss the product (in which case the terms would emerge from the discussion). By requiring panelists to list the terms in isolation (without interacting with other panelists) the researcher need not worry about the panelist being influenced by opinions of others. In contrast, by eliciting the terms in a "focus group" (eg, 4–12 panelists participating in a structured discussion) the researcher may discover that the panelists are "sparked" to search for other terms. The interaction among the panelists inspires an extra measure of creativity from which emerge new terms that would ordinarily not come to the fore.

Culling the List to the Final Set. Once the full set of possible terms is collected, panelists or the researcher cull the list to select those descriptors that are meaningful (rather than being idiosyncratic to one panelist). The final list is often developed by polling the panelists separately to see which terms in the reduced list apply, and retaining only those terms which receive at least a certain minimum number of votes. Panelists may discuss each term in a follow-up group, and decide by group consensus whether or not each term belongs in the list.

Scale Development. The final step consists of erecting scales for each term as well as selecting reference samples to define that term (or at least explicating the term in a paragraph). This third step ensures that the panelists comprehend the meaning of the term, and have available a reference sample to represent the characteristic. Without references or explication, panelists may have different ideas about what a descriptor term means. This final step ensures that all panelists share the same concept of the attribute (6).

Three systems have been developed commercially using the methods described above. Historically, the first one was the A.D. Little Inc. "Flavor Profile" (7,8). The Flavor Profile method demands intense discussion by panelists of the flavor aspects of a product, followed by the development of standards. Panelists use an easily understood intensity scale with defined levels. In addition to assessing the flavor notes, panelists also evaluate the blend of the attributes and the order in which the attributes appear. The Flavor Profile method gained wide acceptance and support from industry because it partially solved the pressing problem of measuring "flavor." Requiring extensive training and being fairly expensive, the Flavor Profile technique has generally been limited to industry, rather than academia.

More recently, sensory analysts have had the benefit of other methods, such as the QDA technique (Quantitative Descriptive Analysis; (9,10)) and Spectrum (11). These two systems use better scales, making their data more amenable to statistical analyses. QDA, Spectrum and the Flavor Profile all operate according to the same principles, however. Furthermore, the consultants at Arthur D. Little Inc. have presented a variant of the Flavor Profile technique more appropriate for statistical analysis. They call the approach PAA (Profile Attribute Analysis), which is an offshoot of the Flavor Profile. PAA is developed in the same way (viz, by group discussion), but has scale properties appropriate for a wide range of statistical procedures (8).

Flavor, in contrast to appearance and texture, has enjoyed the primary attention of researchers involved in developing descriptive language because there is no immediate language of flavor. Most flavor words are either too general without easy definition (eg, "aromatic," "harsh"), or too specific and refer to an object (eg, "winy" or "goaty"). There are no easily understood terms that are general, as there are for taste (eg, sweet, salty, sour, bitter) and texture (eg, hard, soft, liquid). Descriptive analysis thus provides the necessary language to capture the sensory nuances of flavor.

Descriptive Analysis For The Other Senses

Texture has received attention by researchers, who patterned their efforts after the efforts expended for flavor. Following the development of the Flavor Profile, Szczesniak and her colleagues at the General Foods Corporation created the Texture Profile (12). Unlike the Flavor Profile which was developed specifically for each food product, the Texture Profile comprised a standard set of descriptor terms, along with different reference samples to represent each scale term. Table 5 shows the relevant scales and standard reference stimuli to accompany each scale point.

The texture profile has been expanded to include cosmetics as well (13). Since, however, the textural characteristics of cosmetics differ (because we invoke different mechanical forces when we apply cosmetics to the skin) the descriptive terms differ. Table 6 shows how the Texture Profile has been modified to accommodate a cosmetic product that is applied by rubbing, rather than a food product that is chewed and ingested.

The Contribution Of Statistics—Multivariate Analysis

Recognizing that there are many descriptors that we can use for product evaluation, psychologists and statisticians

Table 1. Classification Systems for Odorants

Zwaardemaker (1895): 30 subclasses	Linnaeus (1758): 7 classes	Henning (1915): 6 classes	Croker and Henderson (1927): 4 classes	Amoore (1952): 7 classes	Schuts (1964): 9 classes	Wright and Michaela (1964): 8 classes	Harper and co-workers (1968): 44 classes	Misc. add.
1 Fruity						Hexyl-acetate	Fruity	
2 Waxy							Soapy	
3 Ethereal				Ethereal	Etherish		Etherish, solvent	
4 Camphor				Camphor			Camphor, mothballs	
5 Clove	Aromatic						Aromatic	
6 Cinnamon		Spicy				Spice	Spicy	
7 Aniseed						Benzo-thiazole		
8 Minty				Minty			Minty	
9 Thyme								
10 Rosy								
11 Citrus		Fruity				Citral	Citrus	
12 Almond					Spicy		Almond	
13 Jasmine		Flowery		Floral			Floral	
14 Orange blossom	Fragrant		Fragrant		Fragrant		Fragrant	
15 Lily								
16 Violet								
17 Vanilla					Sweet		Vanilla, sweet	
18 Amber							Animal	
19 Musky	Ambrosial			Musky			Musk	
20 Leek	Alliaceous						Garlic	
21 Fishy							Ammonia	Fishy
22 Bromine								
23 Burnt		Burnt	Burnt		Burnt	Affective	Burnt	
24 Phenolic							Carbolic	
25 Caproic	Hircine		Caprylic				Sweety	
26 Cat-urine								
27 Narcotic	Repulsive							
28 Bed-bug								
29 Carrion	Nauseous						Sickly	
30 Fecal							Fecal	
31		Resinous				Resinous	Resinous; paint	
32		Foul		Putrid	Sulfurous	Unpleasant	Putrid sulfurous	
33			Acid				Acid	
34					Oily		Oily	
35					Rancid		Rancid	
36					Metallic		Metallic	
37							Meaty	
38							Moldy	
39							Grassy	
40							Bloody	
41							Cooked-vegetable	
42								Sandal
43								Watery
44								Urinous
(Nonolfactory)				(Pungent)		(Trigeminal)	(Pungent and 5 others)	

Source: Ref. 4.

have questioned the "independence" of these descriptors. Are these descriptive terms truly independent of each other? Or, do many of the terms overlap with each other, so that the full array of many terms simply reflects different combinations of the same and limited number of "primaries."

Color science provides us with a model showing how the many colors of the rainbow can be expressed as combinations of three distinct primaries, whether these primaries be the familiar red, blue, and yellow, or more esoteric combinations of wavelengths whose combination in various ways regenerates all colors.

In the late 1930s statisticians, especially those involved with psychological issues, began to recognize that they could reduce data from many psychological tests to just a few primary dimensions using the statistical procedure of factor analysis. According to factor analytical theory, the many tests of mental abilities, as an example, all measure just a few simple "primary" aspects of intelligence. Each test comprises a unique and specifiable combination of

Table 2. Examples of Attributes for a Variety

Eucalyptus
Buttery
Like burnt paper
Cologne
Caraway
Orange (fruit)
Household gas
Peanut butter
Violets
Tea-leaflike
Wet wool, wet dog
Chalky
Leatherlike
Pear (fruit)
Stale tobacco smoke
Raw cucumber-like
Raw potatolike
Mouselike
Pepperlike
Bananalike
Burnt rubberlike
Geranium leaves
Urinelike
Beery (beerlike)
Cedarwoodlike
Coconutlike
Ropelike
Seminal, spermlike
Like cleaning fluid (Carbone)
Cardboardlike
Lemon (fruit)
Dirty linenlike
Kippery (smoked fish)
Caramel
Sauerkrautlike
Crushed grass
Chocolate
Molasses
Strawberrylike
Stale
Corklike
Lavender
Cat-urinelike
Barklike, birchlike
Roselike
Celery
Burnt candle
Mushroomlike
Pineapple (fruit)
Fresh cigarette smoke
Nutty (walnut, etc)
Fried fat
Wet paperlike
Coffeelike
Peach (fruit)
Laurel leaves
Scorched milk
Sewer odor
Sooty
Crushed weeds
Rubbery (new rubber)
Bakery (fresh bread)
Oak wood, cognaclike
Grapefruit
Grapejuicelike
Eggy (fresh eggs)
Bitter
Cadaverous, like dead animal
Maple (as in syrup)
Seasoning (for meat)
Apple (fruit)
Soup
Grainy (as grain)
Raisins
Hay
Kerosene

Table 3. Fragrance Classifications

Class	Subclass
1. Citrus	Classic
	Modern
2. Green	Fresh
	Balsam
	Floral
3. Floral	Fresh
	Floral
	Heady
	Sweet
4. Aldehydic	Fresh
	Floral
	Sweet
	Dry
5. Oriental	Spicy
	Sweet
6. Chypre	Fresh
	Floral-animal
	Sweet
7. Fougere	Classic
	Modern
8. Woody	Dry
	Warm
9. Tobacco/leather	
10. Musk	

these primaries. Factor analysis uses the correlations (or covariances) of the tests with each other to estimate the number of these "primaries" and their respective contributions to each test. The factor analysis approach *does not name the primaries*. Rather it demonstrated the existence of the primaries, and shows both the number of these primaries, and the correlation of each test with each primary. It is left up to the ingenuity of the investigator to name the statistically uncovered primaries.

Factor analysis lends itself to analyzing the perception of odors, tastes and textures. The panelist profiles the stimuli (eg, pure odorants) on a list of characteristics using one or another scale. The investigator then processes the data, to uncover the underlying "factors" or "attributes." Table 7 shows results for perfumes (14). Table 8 and 9 show factor analysis of texture. Table 8 shows results for 79 food names each rated on 40 texture attributes (15). Table 9 shows results for 50 nonfood stimuli, each profiled on 20 texture attributes (16).

Beginning in the late 1950s and early 1960s with the work of two psychologists, Clyde Coombs at the University of Michigan (17) and Roger Shepard at Bell Laboratories (18) alternate ways to derive these "fundamental dimensions" were developed. They developed statistical procedures known as "proximities analysis." Rather than instructing panelists to rate the samples on predefined scales, panelists rated (or ranked) the perceived "distance" or dissimilarity of pairs of stimuli. Either the actual ratings of "distance" or the rank order of dissimilarity between pairs were processed to yield the basic set of dimensions. The analysis produced a geometrical map in which the stimuli could be placed. Distances between pairs of stimuli in this geometrical map correspond to subjective dissimilarity between the pairs of stimuli.

Overall multivariate techniques nicely display dissimilarities between different stimuli. The data plots show which stimuli are similar and which are different, as well as an idea of the nature of dimensions. As a method for uncovering basic sensory dimensions, however the statistical procedures have not lived up to their promise. They have found use primarily as devices for data reduction of many attributes to a simple set.

Descriptor Systems for Sensory Analysis—Their Outlook

Historically, descriptor systems began as a method to learn how the senses work. The Linnean approach (classification as a way to understand the physiology of the senses), gave way to description as a way to understand products. Today we have available many systems, appropriate either when we desire a "ready made" descriptor system, or when we need to target a descriptor system to a specific product.

We can best understand the future of descriptive analysis from the problems which it helps to solve.

Table 4. Examples of Attribute Profiles for a Variety of Odorants

Experienced panelist		Inexperienced panelist	
	(A) Musk xylol (solid)[a]		
Fragrant	2.6 (13/15)	Light	2.3 (12/20)
Sweet	1.8 (12/15)	Fragrant	2.0 (13/20)
Floral	1.3 (12/15)	Sweet	1.9 (11/20)
Musklike	1.0 (6/15)	Floral	1.8 (11/20)
Dry, powdery	0.7 (7/15)	Dry, powdery	1.2 (8/20)
		Aromatic	1.0 (7/20)
	(B) Naphthalene (1% in DNP)[a,b]		
Like mothballs	4.1 (15/15)	Like mothballs	4.3 (20/20)
Camphorlike	1.7 (9/15)	Camphorlike	2.4 (13/20)
Cool, cooling	0.7 (8/15)	Cool, cooling	1.6 (11/20)
Etherish, anaesthetic	0.6 (8/15)	Heavy	1.5 (10/20)
		Aromatic	1.1 (11/20)
		Sickly	0.8 (9/20)
	(C) Octanol (0.5% in DNP)[a,b]		
Soapy	2.3 (9/15)	Soapy	2.4 (12/20)
		Light	1.8 (13/20)
		Cool, cooling	1.6 (11/20)
		Sweet	1.4 (10/20)
		Oily, fatty	1.3 (9/20)
		Sickly	1.9 (11/20)
		Fragrant	1.0 (7/20)
	(D) Phenylacetic Acid (1.5% in DNP)[a,b]		
Sickly	1.3 (9/15)	Sickly	1.7 (14/20)
Sharp, pungent, acid	1.1 (10/15)	Sweaty	1.7 (13/20)
Floral	1.1 (7/15)	Putrid, foul decayed	1.4 (13/20)
Sweaty	0.9 (8/15)	Heavy	1.4 (10/20)
Sweet	0.9 (8/15)	Sharp, pungent acid	1.4 (9/20)
Fragrant	0.9 (7/15)	Warm	1.3 (10/20)
Sour, acid, vinegar	0.8 (7/15)	Sour, acid, vinegar	1.3 (9/20)
		Animal	1.2 (8/20)
		Sweet	1.1 (9/20)
		Rancid	1.1 (9/20)
		Fecal (like dung)	1.0 (8/20)
	(E) Phenylethanol (0.5% in DNP)[a,b]		
Floral	2.7 (11/15)	Floral	3.1 (17/20)
Fragrant	2.2 (10/15)	Fragrant	3.0 (19/20)
Sweet	2.0 (13/15)	Sweet	2.4 (17/20)
		Light	2.2 (12/20)
		Herbal, green, cut, grass, etc	1.4 (11/20)
		Aromatic	1.3 (12/20)
		Cool, cooling	1.2 (12/20)
	(F) Nitrobenzene (10% in DNP)[a,b]		
Almondlike	3.1 (13/15)	Almondlike	3.7 (17/20)
Aromatic	1.2 (9/15)	Sweet	1.9 (13/20)
Petro, chemical solvent	0.9 (8/15)	Heavy	1.8 (10/20)
Vanillalike	0.9 (7/15)	Oily, fatty	1.6 (11/20)
Etherish, anaesthetic	0.7 (7/15)	Sickly	1.5 (14/20)
		Fragrant	1.5 (12/20)
		Aromatic	1.1 (8/20)
		Like mothballs	1.0 (8/20)

Source: Ref. 5.

[a] First number = mean rating; ratio = number in panel using that term.

[b] DNP = dinitrophthalene.

Table 5. The General Foods Texture Profile System for Foods

Standardized rating scales for mechanical properties with generalized standards (intensity of parameter increases downward)

Hardness	*Fracturability*	*Chewiness*
Cream cheese	Corn muffin	Rye bread
Velveeta cheese	Egg Jumbos	Frankfurter
Frankfurters	Graham crackers	Large gumdrops
Cheddar cheese	Melba toast	Well-done round steak
Giant stuffed olives	Bordeaux cookies	Nut chews
Cocktail peanuts	Ginger snaps	Tootsie Rolls
Shelled almonds	Treacle brittle	

Adhesiveness	*Viscosity*
Hydrogenated shortening	Water
Cheese Whiz	Light cream
Cream cheese	Evaporated milk
Marshmallow topping	Maple syrup
Peanut butter	Chocolate syrup
	Cool'n Creamy pudding
	Condensed milk

Geometrical properties related to particle size and shape		Geometrical properties related to particle size and orientation	
Property	Example	Property	Example
Powdery	Confectioner's sugar	Flaky	Flaky pastry
Chalky	Tooth powder	Fibrous	Breast of chicken
Grainy	Cooked Cream of Wheat	Pulpy	Orange sections
Gritty	Pears	Cellular	Apples, cake
Course	Cooked oatmeal	Aerated	Whipped cream
Lumpy	Cottage cheese	Puffy	Rice pudding
Beady	Cooked tapioca	Crystalline	Granulated sugar

Source: Ref. 12.

Quality Control (eg, plant to plant variation, modifications of an ingredient or a process by new suppliers or new machinery). Descriptive analysis develops a sensory "fingerprint" of the product, which can then be compared to standards on file. Often it is vital that descriptive analysis record the sensory characteristics of those products which defy description by chemical or physical analysis. It is difficult, if not impossible, to assess some products (eg, coffee), simply from the tracings of the gas chromatograph, (which records several hundred ingredient components in the product, each at its own concentration). Sensory analysis, using an experienced evaluator (in contrast to instrumental analysis) can more efficiently classify the sample as "same" or "different" from a "gold" standard reference. To the degree that quality assurance becomes increasingly important in the food industry we may expect to see increasing use of descriptive analysis to maintain sensory identity. The descriptive analysis report will be of the sort generated by the Flavor Profile technique, or the QDA technique, ie, designed to capture the sensory nuances of the particular product.

New Product Development. Product developers searching for new creations use descriptive analysis to help them achieve their goal. For instance, in the creation of a new cola beverage, descriptive analysis may highlight specific flavor notes such as winy, pruny, etc, which differentiate one cola from another. Descriptive analysis can point out the need for the specific characteristic to be present. Descriptive analysis proves even more valuable when it shows the product developer that he has, indeed, incorporated the desired note into the product. In a parallel fashion, descriptive analysis proves useful when it shows that the new product possesses an undefined "off-note." Identifying and labeling that note helps the product developer to find the cause and remove the offending compound, flavor or process.

Relating Sensory Characteristics to Physical Measures. Much scientific interest has focused on the relation between what we subjectively perceive and the objective physical (or chemical) properties of products. Chemists analyze the aroma of food products into hundreds of constituents by using gas chromatography and mass spectrometry. A trained panelist sniffing the "effluent" or separated chemicals at the exit port of the chromatograph (after the components have been separated) can describe what each component smells like. This dual analysis, combining objective and subjective measures, is often done by analytical chemists to characterize aromas.

Texture researchers correlate well defined physical characteristics that they measure instrumentally (eg, force applied, deformation obtained) with the sensory attributes. The "Instrumental Texture Profile" proposed by Bourne (19) as an extension of Szczesniak's subjective texture profile (12), exemplifies this subjective-objective type of correlation. Corresponding to each of Szczesniak's terms, Bourne recommends a well-defined instrumental measure. Szczesniak also has provided "Instrumental Analogs" of texture perceptions, using the General Foods Texturometer.

MEASUREMENT OF PERCEIVED INTENSITY

Intensity measurement (quantification) comprises the second major thrust of sensory analysis. Quantification takes many forms, including determination of threshold (the lowest level of a stimulus that one can either detect or recognize), assessment of discrimination ability (the smallest physical change between two samples that is just noticeable), and scaling of suprathreshold intensity (how strong does the stimulus seem).

Is It Possible to Measure Perceived Intensity Validly?

Scientists agree that they can measure "objective" characteristics such as temperature and mass using well-accepted scientific procedures. It is more problematic for sensations. We cannot point to a sensory perception in the way that we can point to a physical stimulus. Measurement of "private sensory experience" does not, intuitively, appear to be "public" or open to validation and replication in the same way that we understand scientific measurement to be.

Table 6. The Texture Profile System Modified for Use in Skin Care Product Evaluation

Stage of evaluation parameters	Skin care product attributes and definition	Texture profile
Pick-up: product removed from container, product poured or squeezed from bottle onto fingertips, or products lifted from jar with forefinger	Thickness: perceived denseness of product; evaluated as force required to squeeze between thumb and forefinger, rated as thin-medium-thick Consistency perceived structure of product; evaluated as resistance to deformation and difficulty of lifting from container; rated as light-medium-heavy	Viscosity (for lotions) or Hardness, cohesiveness
Rubout (application): spread of product over and into skin with fingertips using gentle circular motion at a rate of two rubs per second for a specified period of time, depending on the product	Spreadability: ease of moving product from point of application over rest of face; evaluated as resistance to pressure; rated or described as: "slips"—very easy to spread "glides"—moderately easy "drags"—difficult to spread Absorbency: rate at which product perceived as absorbed into skin; evaluated by noting changes in character or product and in amount of product remaining (tactile and visual) and by changes in skin surfaces; rated as slow-moderate-fast	Viscosity, cohesiveness, springiness, gumminess, adheaveness Other characteristics—oil and water content of product
After-feel (and appearance): evaluation of skin surface with fingertips, visually and kinesthetically immediately after product application and possibly at varying intervals thereafter	After feel: type and intensity of product residue left on skin; changes in skin feel; product residue described by type ie, film (oily or greasy), coating (waxy or dry), flaky or powdery particles, the amount of such residue, identified as slight-moderate-large Skin feel described as dry (taut, pulled tight), moist (supple, pliant), oily (dirty, clogged) Other sensations also noted and identified where applicable (ie, clean, stimulated, irritated, etc)	Other characteristics—oil and water content Geometrical characteristic—gritty, powdery, etc

Source: Ref. 18.

Despite these roadblocks, however, researchers have developed an array of reliable and thus far valid techniques to measure perception (20). Each measurement technique is grounded in specific assumptions. Each technique yields numerical scale values for perception which permit decisions to be made—whether pertaining to the perceiver (eg, the panelist can or cannot perceive the stimulus, two stimuli are or are not "different," the stimulus is strong or weak), or to the product (eg, the product developer should increase or decrease the sensory characteristic of an ingredient, by a specific amount).

Measurement of Thresholds

The most fundamental and intuitively obvious measurement is the threshold, defined as the lowest physical intensity of a stimulus that a panelist can correctly detect. Occasionally other definitions may apply (eg, recognition of stimulus quality rather than detection). Threshold measurement requires the least intellectual effort on the part of the panelist. The panelist simply responds that either he detects or he does not detect the stimulus. There are no other intellectual demands. By varying the physical intensity of the stimulus and determining how many panelists detect the stimulus (or recognize its quality—a different task, but one requiring the same response) the investigator determines the level of the stimulus that is just detectable. The physical intensity at which the percentage detecting correctly equals 50% is defined as the threshold.

The early psychology literature is replete with reports on the measurement of threshold. In practice and prior to the widespread use of subjective intensity scales, food scientists assumed (albeit wrongly) that the threshold corresponded to relative intensity. For instance, artificial sweeteners such as saccharin possess a threshold concentration hundreds of times lower than the threshold concentration of sugar. Researchers misinterpreted this concentration ratio to mean that saccharin was several hundred times sweeter than sugar. Thresholds only indicate the physical intensity of a stimulus which generates a fixed sensory intensity—namely "just perceptible." Thresholds do not, and cannot provide a direct measure of subjective intensity, however.

Discrimination Testing and Sensory Measurement

A second milestone in sensory measurement was reached when investigators quantified the ability of panelists to discriminate. Discrimination testing traces its beginning to research conducted more than one hundred fifty years ago. E. H. Weber (21), a German physiologist, reported that stimuli which are just noticeably different from each other often lie in a constant ratio. That is, if the ratio is 1.05, then it requires an approximately 5% increase in a stim-

Table 7. Results of a Factor Analysis of Perfumes

Odorants tested

McCall's scheme of perfumes, which classifies perfumes according to user groups

Example

Group 1 = single florals (eg, White Rose, Jasmin, Heliotropin)
Group 2 = floral bouquet (eg, Arpège, Paris, Amour Amour)
Group 3 = modern blends (eg, Chanel No. 5, l'Aimant)
Group 4 = oriental blends (eg, Emeraude, Shalimar, Tabu, l'Origan)
Group 5 = woodsy-mossy-leafy (eg, Soir de Paris, Mitsuoko, Cypre)
Group 6 = spicy bouquet (eg, Capri)
Group 7 = fruity bouquet (eg, Fleurs de Rocailles)

Subjects

25 women

Scaling

Each subject rated each perfume on a series of 25 bipolar scales (ie, semantic differential scales).

Results

Four dimension extracted but not named:

1. Chanel No. 5 vs White Rose (on opposite ends)
2. l'Origan vs Aspège (on opposite ends)
3. Aspège vs Cypre (on opposite ends)
4. Not specified

Source: Ref. 14.

Table 8. Results of a Factor Analysis of Texture Food Names

Food names used in questionnaire

79 names profiled and 40 attributes

1. Hard
2. Soft
3. Juicy
4. Chewy
5. Not chewy
6. Warm
7. Cold
8. Oily
9. Greasy
10. Viscous
11. Sticky
12. Moist
13. Elastic
14. Gummy
15. Creamy
16. Flaky
17. Fibrous
18. Thick
19. Light
20. Heavy
21. Slippery
22. Crunchy
23. Melting
24. Brittle
25. Slippery
26. Crunchy (other term)
27. Sprinkling
28. Rustling
29. Saku (Japanese)
30. Coarse
31. Sticky
32. Slushy
33. Mushy
34. Sticky (other term)
35. Fluffy
36. Hoku (Japanese)
37. Smooth
38. Lumpy
39. Crisp
40. Sticky (other term)

Results

8 factors:
Hard-soft
Cold-warm
Oily-juicy
Elastic
Flaky
Heavy
Viscous
Smooth

Source: Ref. 15.

Table 9. Results of a Factor Analysis of Texture-nonfoods

Samples tested

Felt, line, handkerchief, tricot, nylon, broadcloth, silk, acetate, satin, etc (50 different samples)

Scales

20 bipolar scales; panelists rated each sample on every one of the bipolar scales, each of which contained 7 points

Subjects

25 untrained female students

Results

Four factors extracted:

1. Coldness (attributes loading high included painful, stiff, nonplastic, cold, coarse, heavy, massive, substantial)
2. Wet and smooth (attributes loading high included were, heavy, substantial, smooth, massive)
3. Hardness (attributes loading high included sharp, stiff, hard, plastic)
4. Opposition of tactual impression of viscosity, elasticity, and plasticity from visual aesthetic impression of luster or gloss, smoothness

Source: Ref. 16.

ulus intensity before panelists will recognize the change and report that the second stimulus seems stronger.

It took the genius of another German physiologist and physicist, G. T. Fechner (22), to recognize that discrimination ability could become the foundation for a scale of subjective sensory magnitude. Fechner conjectured that to create a sensory scale one should add successive physical intensities that are just discriminable from each other. Each stimulus increment, (corresponding to one just noticeable difference), would equal one psychological unit of perceived intensity. One would then cumulate just noticeable differences to develop a true sensory scale. Mathematically sensory intensity relates to physical intensity by a simple function, a logarithmic function, written as:

$$\text{Sensory Intensity} = A[\log(\text{Physical Intensity})] + B$$

Researchers erected psychological scales of sensory intensity using the cumbersome discrimination testing (23). As a method for applied sensory analysis, however, discrimination tests found more use in the practical realm, with problems such as deciding whether or not two stimuli were perceptibly different from each other. Applied researchers were not particularly interested in erecting a sensory scale based on discrimination testing. They, however, did need discrimination tests to compare products which differed in terms of ingredients, processing conditions, storage time, etc. The analysis of differences were far more statistical however, invoking procedures such as the T test for difference of means or percentages, or the analysis of variance (ANOVA) to assess differences among many samples.

Direct Scales of Sensory Magnitude

More productive uses of scaling have been made with "direct scaling," in which the investigator instructs the panelist to rate the stimulus intensity using a numbering system. The intensity scale may comprise graded categories (eg, from weak to strong, on a set of categories ranging from as few as 3, up to as many as 100 categories or more), or require the panelist to place a mark on a line of fixed length to denote intensity (with one end of the line denoting weak, and the opposite end denoting strong), or even require the panelist to assign numbers so that the ratios of the numbers assigned reflect the ratios of perceived intensity (the method of magnitude estimation (24). The investigator need not use numbers nor line length as the scale measure. The panelists can adjust the intensity of a stimulus such as white noise so that the intensity of the adjusted stimulus matches the perceived intensity of the stimulus being measured (25,26).

Direct scaling methods (in contrast to threshold and discrimination tests) enable the panelist to behave as a true measuring instrument. Data can be collected from many panelists relatively quickly and painlessly. The investigator straightforwardly treats the data by conventional statistical procedures, including computing the average and measures of variability (standard deviation, variance), determining differences among samples ("t" tests, analysis of variance), and modeling the relation between perceived intensity and physical measures by regression analysis (curve fitting).

In practical terms, scaling procedures provide a great deal of useful information. Consider the data shown in Table 10, which shows average consumer ratings of five cereals, on a variety of attributes. (Some of the attributes in Table 10 are sensory attributes, some are liking attributes, whereas some are "image" attributes). Panelists used a 0–100 scale. When panelists rate each product on all of the attributes the investigator generates a "report card" for both marketed products and test prototypes.

Profiles of products on many attributes enable the researcher to compare the full profile of one product to the full profile of another and assess the degree of difference. Graphical display of data using the so-called spider plots, (Fig. 1) shows the difference between products. The "spider plot" is not analytical—it does not show the user anything beyond the fact that the two samples being plotted overlap, or do not. (One gets this same information from tables such as Table 10). The Spider plots, however, make the data come alive and increase its impact.

HEDONICS—MEASUREMENT OF LIKING

Acceptance measurement, the third branch of sensory analysis, may well be, the most vital aspect. We select and eat foods on the basis of their visual appeal and palatability. A large and growing body of scientific literature has been published on the measurement of acceptance. Appropriate measures of liking or purchase intent, and the assessment of possible consumer boredom with a product are critical issues when applying hedonic tests in the world of commercial research.

The Search for the Liking Scale

Buried in the archives of scientific literature are numerous attempts to create scales which measure acceptance, and techniques beyond scaling which probe other aspects of hedonics. Table 11 shows a variety of scales, ranging from classification (like/dislike) up to refinements which go beyond liking to probe other activities such as the expected effort one would make to eat the food (eg, FACT scale) (27,28). Still other efforts have focused on the assessment of the boredom factor in food—how time itself and repeated consumptions of the food item modify acceptance (29).

Directional Liking Scales

Although researchers use many different liking scales, other scales also incorporate aspects of liking. The "directional scale," often used to guide product development is a prime example. Directional scales combine intensity with liking, as shown by the following statement:

> "Considering the amount of visible spice in this product, is the amount of spice: Too little, Just right, Too much? (Choose the appropriate answer.)"

Directional questions assume that the panelist knows the "optimal level" or "ideal level" of the attribute. When evaluating the product the panelist also estimates deviations from that optimum. Directional scales appeal to product developers because they appear, superficially, to provide accurate direction. Ostensibly the developer need only know the degree to which the product under-delivers or over-delivers an attribute. Table 12 shows data obtained from directional scales for a complex product—spaghetti and meatballs rated on a variety of directional attributes.

In many cases the panelists cannot accurately gauge the direction of change, however. For attributes which have a strong emotional positive or negative connotation (eg, chocolate intensity, coffee bitterness, etc) the product never has enough or always has more than one desires. As a result chocolate products are often rated as not having enough "chocolate" on the directional scales, whereas coffee is too often rated as having too much bitterness, even when it has too little bitter taste to make it palatable. Panelists do not know their ideal points for these emotion laden attributes. They find it hard to provide direction to product developers, who consequently continue to interpret literally the panelist's desire for more of the attribute, (eg, add more chocolate), never quite reaching the goal of "just right." Furthermore, all too often consumers may request several incompatible changes in the product, making the data less usable. Despite these inadequacies and failings, however, directional scales are widely used in product development to guide consumer driven reformulation. They are easy to use, they incorporate hedonics, and they do provide some guidance.

EXPERIMENTAL DESIGN AND SENSORY PRODUCT OPTIMIZATION

Sensory analysis has achieved its major commercial impact by providing reliable tests results from panelists.

Direct Scales of Sensory Magnitude

More productive uses of scaling have been made with "direct scaling," in which the investigator instructs the panelist to rate the stimulus intensity using a numbering system. The intensity scale may comprise graded categories (eg, from weak to strong, on a set of categories ranging from as few as 3, up to as many as 100 categories or more), or require the panelist to place a mark on a line of fixed length to denote intensity (with one end of the line denoting weak, and the opposite end denoting strong), or even require the panelist to assign numbers so that the ratios of the numbers assigned reflect the ratios of perceived intensity (the method of magnitude estimation (24). The investigator need not use numbers nor line length as the scale measure. The panelists can adjust the intensity of a stimulus such as white noise so that the intensity of the adjusted stimulus matches the perceived intensity of the stimulus being measured (25,26).

Direct scaling methods (in contrast to threshold and discrimination tests) enable the panelist to behave as a true measuring instrument. Data can be collected from many panelists relatively quickly and painlessly. The investigator straightforwardly treats the data by conventional statistical procedures, including computing the average and measures of variability (standard deviation, variance), determining differences among samples ("t" tests, analysis of variance), and modeling the relation between perceived intensity and physical measures by regression analysis (curve fitting).

In practical terms, scaling procedures provide a great deal of useful information. Consider the data shown in Table 10, which shows average consumer ratings of five cereals, on a variety of attributes. (Some of the attributes in Table 10 are sensory attributes, some are liking attributes, whereas some are "image" attributes). Panelists used a 0–100 scale. When panelists rate each product on all of the attributes the investigator generates a "report card" for both marketed products and test prototypes.

Profiles of products on many attributes enable the researcher to compare the full profile of one product to the full profile of another and assess the degree of difference. Graphical display of data using the so-called spider plots, (Fig. 1) shows the difference between products. The "spider plot" is not analytical—it does not show the user anything beyond the fact that the two samples being plotted overlap, or do not. (One gets this same information from tables such as Table 10). The Spider plots, however, make the data come alive and increase its impact.

HEDONICS—MEASUREMENT OF LIKING

Acceptance measurement, the third branch of sensory analysis, may well be, the most vital aspect. We select and eat foods on the basis of their visual appeal and palatability. A large and growing body of scientific literature has been published on the measurement of acceptance. Appropriate measures of liking or purchase intent, and the assessment of possible consumer boredom with a product are critical issues when applying hedonic tests in the world of commercial research.

The Search for the Liking Scale

Buried in the archives of scientific literature are numerous attempts to create scales which measure acceptance, and techniques beyond scaling which probe other aspects of hedonics. Table 11 shows a variety of scales, ranging from classification (like/dislike) up to refinements which go beyond liking to probe other activities such as the expected effort one would make to eat the food (eg, FACT scale) (27,28). Still other efforts have focused on the assessment of the boredom factor in food—how time itself and repeated consumptions of the food item modify acceptance (29).

Directional Liking Scales

Although researchers use many different liking scales, other scales also incorporate aspects of liking. The "directional scale," often used to guide product development is a prime example. Directional scales combine intensity with liking, as shown by the following statement:

> "Considering the amount of visible spice in this product, is the amount of spice: Too little, Just right, Too much? (Choose the appropriate answer.)"

Directional questions assume that the panelist knows the "optimal level" or "ideal level" of the attribute. When evaluating the product the panelist also estimates deviations from that optimum. Directional scales appeal to product developers because they appear, superficially, to provide accurate direction. Ostensibly the developer need only know the degree to which the product under-delivers or over-delivers an attribute. Table 12 shows data obtained from directional scales for a complex product—spaghetti and meatballs rated on a variety of directional attributes.

In many cases the panelists cannot accurately gauge the direction of change, however. For attributes which have a strong emotional positive or negative connotation (eg, chocolate intensity, coffee bitterness, etc) the product never has enough or always has more than one desires. As a result chocolate products are often rated as not having enough "chocolate" on the directional scales, whereas coffee is too often rated as having too much bitterness, even when it has too little bitter taste to make it palatable. Panelists do not know their ideal points for these emotion laden attributes. They find it hard to provide direction to product developers, who consequently continue to interpret literally the panelist's desire for more of the attribute, (eg, add more chocolate), never quite reaching the goal of "just right." Furthermore, all too often consumers may request several incompatible changes in the product, making the data less usable. Despite these inadequacies and failings, however, directional scales are widely used in product development to guide consumer driven reformulation. They are easy to use, they incorporate hedonics, and they do provide some guidance.

EXPERIMENTAL DESIGN AND SENSORY PRODUCT OPTIMIZATION

Sensory analysis has achieved its major commercial impact by providing reliable tests results from panelists.

Table 10. Ratings of Five Cereals on Attributes Comparison of Attribute Profiles—High versus Low Scorers[a]

	High-scoring cereals			Low scoring cereals	
	100% Natural	Cheerios	Honey Nut Cheerios	100% Bran	Grape Nuts
			Overall		
Purchase interest	72	66	63	15	28
Liking—dry	78	62	68	31	24
Liking—w/milk	77	68	66	31	27
			Appearance		
Like appearance—dry	65	65	65	14	26
Like appearance—wet	68	63	63	22	23
Size of pieces	28	37	37	12	4
Thickness	36	47	47	18	20
Darkness	43	28	28	75	45
Familiar looking	54	95	95	28	46
			Flavor		
Like flavor—dry	74	57	65	36	25
Like flavor—w/milk	76	61	67	36	31
Like wheat flavor	43	24	34	25	31
Intensity—dry	75	46	60	50	56
Nutty—dry	82	33	36	34	45
Intensity—w/milk	74	52	64	47	57
Nutty—w/milk	84	25	37	34	47
Sweet	66	13	80	33	17
Spicy	46	15	32	18	15
			Texture		
Like texture—dry	72	61	72	29	23
Like texture—milk	74	60	65	22	28
Crunchy—dry	83	60	75	49	91
Amt. milk absorbed	28	39	28	79	57
Quickly absorbs milk	23	36	32	75	59
Holds together	83	61	69	38	76
Crunchy—milk	86	46	66	28	97
Crispy—milk	87	40	59	15	81
			Image		
Approp. as dry snack	80	59	70	19	12
Nutritious	76	60	50	51	65
Quality	83	71	77	52	66
Satisfy as snack	81	55	70	11	14
Calorie	69	37	73	39	44
Unique	59	23	38	55	51
Tire of it (boredom)	33	46	44	73	60
Kids would like	65	67	81	9	7
Teens would like	70	59	70	13	20

[a] Percent of panelists who rate the product as definitely or probably would buy on a 5-point buy scale (1 = definitely not buy to 5 = definitely would buy.) Remaining scales = 0–100.

When coupled with experimental design and modeling (by regression analysis), sensory analysis becomes a powerful tool to guide product development.

Experimental Design

An alternative approach beyond directional scales to develop products using consumers, consists of systematically varying the formula ingredients in a way which allows one to assess the effects of each ingredient, and the interactions among ingredients on acceptance and attribute perceptions.

Experimental designs are properly the province of statisticians (30). Sensory analysts and product developers have used experimental designs with great success to understand consumer reactions to test prototypes comprising known ingredients and processes. Product categories amenable to these designs range from simple food systems such as a fruit-flavored beverage, to complex systems such as pizza, apple pie, and sausage. In all cases the assessment

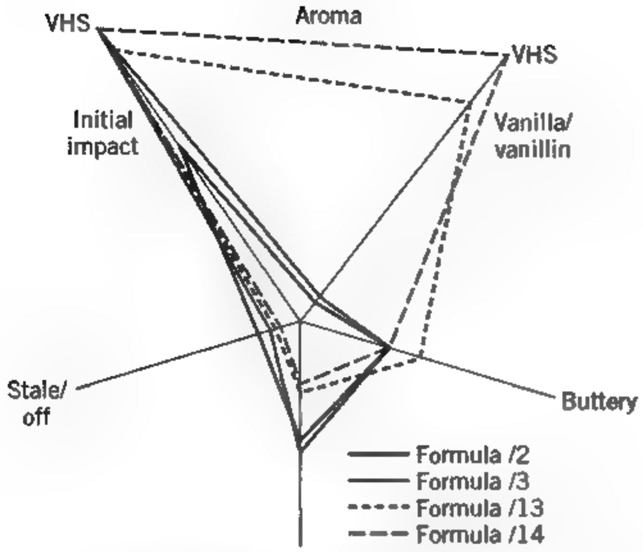


Figure 1. Example of quantitative descriptor analysis "spider plot" of the aroma of a sample. Scale: 0 at center to 30–40 at edge. *Source:* Ref. 10.

Table 11. Verbal Descriptors for Hedonic Scales

Number of scale points descriptors[a]	
2	Dislike, unfamiliar
3	Acceptable, dislike, not tried
3	Like a lot, dislike, do not know
4	Well liked, indifferent, disliked, seldom if ever used
5	Like very much, like moderately, neutral, dislike moderately, dislike very much
5	Very good, good, moderate, dislike, tolerate
5	Very good, good, moderately well, tolerate, dislike
6	Very good, good, moderate, tolerate, dislike, never tried
9	Like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

Food action (fact) rating scale[b]

Eat every opportunity
Eat very often
Frequently eat
Eat now and then
Eat if available
Do not like—eat on occasion
Hardly every eat
Eat if no other choice
Eat if forced

Source. [a]Ref. 27 and [b]Ref. 28.

Table 12. Example of Attribute Profile Ratings Using Different Attributes Liking, Image, and Directional, Product: Spaghetti and Meatballs[a]

Attribute		Product[b]	
		#1	#2
	Overall		
Like	Overall liking of product	55	63
	Appearance		
Like	Appearance	63	75
Like	Appearance of meatballs	63	66
Dir	Darkness of sauce	15	9
Dir	Size of portion	−8	0
	Overall taste		
Like	Overall taste of product	55	12
Dir	Strength of afterstate	15	4
	Meatballs		
Like	Taste of meatballs	57	53
Like	Texture of meatballs	63	50
Dir	Amount of meatballs	−3	−1
Dir	Size of meatballs	−5	−2
Dir	Moistness of meatballs	0	−11
Dir	Softness vs firmness of meatballs	1	13
	Sauce (taste)		
Like	Overall flavor of sauce	53	62
Dir	Strength of tomato flavor	11	−1
Dir	Spiciness of sauce	7	−3
Dir	Sweetness of sauce	−4	−1
Dir	Amount of seasoning	7	−5
Dir	Amount of salt	6	2
	Sauce (texture, amount)		
Like	Overall texture of sauce	65	65
Dir	Amount of sauce	18	−1
Dir	Thickness of sauce	7	2
Dir	Smoothness vs chunkiness of sauce	0	2
	Pasta		
Dir	Amount of pasta	−11	3
Dir	Softness vs firmness of pasta	−14	−5

[a]Explanation of ratings: Panelists rated each product on a variety of directional questions (0 = much too little, 50 = on target, 100 = much too much).
Data for directional (DIR) questions shown, after 50 was subtracted from each rating. Positive numbers = over-delivery on the attribute. Negative numbers = under-delivery on the attribute. Like = liking rating (0 = hate, 100 = love).
[b]Base size: 112.

of systematically varied alternatives has educated the researcher and provided concrete direction for product modification.

A Case History Using Sensory Analysis—Tomato Sauce

The easiest way to understand experimental design and its link with sensory analysis is with a case history. This case history concerns tomato sauce. The issue was to develop a new formulation to take advantage of a new processing technology.

Phase 1. Formula Selection and Design

Phase 1 comprises the selection of appropriate variables, and the recommended array of formula combinations. R&D selected 6 variables to investigate. Different experimental designs can be used to investigate the 6 variables, depending upon the expected relation between formula variables and consumer ratings. If one expects the attributes rated by consumers to change only linearly with ingredients, with no interaction among the variables, as few as 12 prototypes need to be developed using the Plackett Burman screening design. Table 13 (part A) shows this design (31). If one expects curvature in the data, ie, the attributes (whether sensory or liking) do not follow a simple straight line versus physical changes, then one may wish to fit a quadratic curve, necessitating a quadratic design. If one expects curvature and interactions among the variables than the central composite screening design shown in Table 13 (part B) is appropriate (32). When there are simply too many prototypes to create, but one wishes to use the central composite design (because it captures curvature in the response surface and allows for interaction between pairs of ingredients) one would select the reduced design such as that shown in Table 13 (part C). R&D selected the design, shown in Table 10 (part C), encompassing 29 prototypes.

Along with the experimentally designed products, researchers test in-market competitors which provide "anchors" for the category. Panelists do not know which samples belong to the experimental design and which are actual in-markets products. All products are tested "blind," and in a randomized order. The only clues come from the panelists' sensory perceptions.

Attribute Selection for the Questionnaire

Attributes define the characteristics to which consumers will attend when evaluating the sample. It is important to select appropriate attributes, but the choice of attributes in an experimental design is not as critical as would be the case were the products to simply represent unconnected "rifle shots."

The key attribute is usually the overall criterion of acceptance, whether this be overall liking, purchase intent, or some integrative measure such as "high quality." The key attribute measures the single response that is of real interest to the investigator, and usually corresponds to the characteristic of the product that is being optimized.

The questionnaire should also deal with relevant sensory characteristics. Optimization methods can uncover formulations which are highly acceptable and simultaneously possess specific, required sensory characteristics (eg, to support an advertising position). The questionnaire can encompass image attributes as well (eg, "refreshing" for a beverage, "caloric" for a snack, etc). Image attributes are more complex cognitive characteristics.

Often there are other data available for the experimentally varied products. Additional data may include objective physical measurements, cost of goods, storage stability, ratings by experts which describe qualitative nuances, and evaluations by a quality control panel. If available, these other data sets can be added to the data file. The augmented attribute set appears in Table 14.

In this study data was available from all of these sources. Generally, however, studies involving experimental design and optimization are of a more modest scale. The

Table 13. Plackett Burman Screen Design

Product	A	B	C	D	E	F
			A. Six Variables in 12 Prototypes[a]			
101	1	1	0	1	1	1
102	0	1	1	0	1	1
103	1	0	1	1	0	1
104	0	1	0	1	1	0
105	0	0	1	0	1	1
106	0	0	0	1	0	1
107	1	0	0	0	1	0
108	1	1	0	0	0	1
109	1	1	1	0	0	0
110	0	1	1	1	0	0
111	1	0	1	1	1	0
112	0	0	0	0	0	0
			B. Six Variables—Full Replicate[b]			
301	1	1	1	1	1	1
302	1	1	1	1	1	−1
303	1	1	1	1	−1	1
304	1	1	1	1	−1	−1
305	1	1	1	−1	1	1
306	1	1	1	−1	1	−1
307	1	1	1	−1	−1	1
308	1	1	1	−1	−1	−1
309	1	1	−1	1	1	1
310	1	1	−1	1	1	−1
311	1	1	−1	1	−1	1
312	1	1	−1	1	−1	−1
313	1	1	−1	−1	1	1
314	1	1	−1	−1	1	−1
315	1	1	−1	−1	−1	1
316	1	1	−1	−1	−1	−1
317	1	−1	1	1	1	1
318	1	−1	1	1	1	−1
319	1	−1	1	1	−1	1
320	1	−1	1	1	−1	−1
321	1	−1	1	−1	1	1
322	1	−1	1	−1	1	−1
323	1	−1	1	−1	−1	1
324	1	−1	1	−1	−1	−1
325	1	−1	−1	1	1	1
326	1	−1	−1	1	1	−1
327	1	−1	−1	1	−1	1
328	1	−1	−1	1	−1	−1
329	1	−1	−1	−1	1	1
330	1	−1	−1	−1	1	−1
331	1	−1	−1	−1	−1	1
332	1	−1	−1	−1	−1	−1
333	−1	1	1	1	1	1
334	−1	1	1	1	1	−1
335	−1	1	1	1	−1	1
336	−1	1	1	1	−1	−1
337	−1	1	1	−1	1	1
338	−1	1	1	−1	1	−1
339	−1	1	1	−1	−1	1
340	−1	1	1	−1	−1	−1
341	−1	1	−1	1	1	1
342	−1	1	−1	1	1	−1
343	−1	1	−1	1	−1	1
344	−1	1	−1	1	−1	−1
345	−1	1	−1	−1	1	1
346	−1	1	−1	−1	1	−1
347	−1	1	−1	−1	−1	1
348	−1	1	−1	−1	−1	−1

Table 13. Plackett Burman Screen Design *(continued)*

Product	A	B	C	D	E	F
349	−1	−1	1	1	1	1
350	−1	−1	1	1	−1	1
351	−1	−1	1	1	−1	1
352	−1	−1	1	1	−1	−1
353	−1	−1	1	−1	1	1
354	−1	−1	1	−1	1	−1
355	−1	−1	1	−1	−1	−1
356	−1	−1	1	−1	−1	−1
357	−1	−1	−1	1	1	1
358	−1	−1	−1	1	1	−1
359	−1	−1	−1	1	−1	1
360	−1	−1	−1	1	−1	−1
361	−1	−1	−1	−1	1	1
362	−1	−1	−1	−1	1	−1
363	−1	−1	−1	−1	−1	1
364	−1	−1	−1	−1	−1	−1
365	1	0	0	0	0	0
366	−1	0	0	0	0	0
367	0	1	0	0	0	0
368	0	−1	0	0	0	0
369	0	0	1	0	0	0
370	0	0	−1	0	0	0
371	0	0	0	1	0	0
372	0	0	0	−1	0	0
373	0	0	0	0	1	0
374	0	0	0	0	−1	0
375	0	0	0	0	0	1
376	0	0	0	0	0	−1
377	0	0	0	0	0	0

C. Six Variables—Central Composite, Quarter Replicate[c]

Product	A	B	C	D	E	F
101	1	1	1	1	1	1
102	1	1	1	−1	1	−1
103	1	1	−1	1	−1	−1
104	1	1	−1	−1	−1	1
105	1	−1	1	1	−1	−1
106	1	−1	1	−1	−1	1
107	1	−1	−1	1	1	1
108	1	−1	−1	−1	1	−1
109	−1	1	1	1	−1	1
110	−1	1	1	−1	−1	−1
111	−1	1	−1	1	1	−1
112	−1	1	−1	−1	1	1
113	−1	−1	1	1	1	−1
114	−1	−1	1	−1	1	1
115	−1	−1	−1	1	−1	1
116	−1	−1	−1	−1	−1	−1
117	1	0	0	0	0	0
118	−1	0	0	0	0	0
119	0	1	0	0	0	0
120	0	−1	0	0	0	0
121	0	0	1	0	0	0
122	0	0	−1	0	0	0
123	0	0	0	1	0	0
124	0	0	0	−1	0	0
125	0	0	0	0	1	0
126	0	0	0	0	−1	0
127	0	0	0	0	0	1
128	0	0	0	0	0	−1
129	0	0	0	0	0	0

[a] 1 = high; 0 = low.

[b] 1 = high; 0 = medium; −1 = low.

[c] 1 = high; 0 = medium; −1 = low, E in this section = ABC, F = BCD.

Table 14. Attributes Used in the Sauce Study

Independent Variable

- *Ingredient A*
- *Ingredient B*
- *Ingredient C*
- *Ingredient D*
- *Ingredient E*
- *Cook-process*
- *Cost-of-Goods*

Consumer-Data[a]

- C/Like/Overall
- C/Like/Segment-A
- C/Like/Segment-B
- C/Like/Segment-C
- C/Like/Appearance
- C/Like/Aroma
- C/Like/Flavor
- C/Like/Texture
- C/Image/Fresh
- C/Image/Authentic
- C/Image/Sophisticated
- C/Sen/Darkness
- C/Sen/Aroma
- C/Sen/Taste
- C/Sen/Sweet
- C/Sen/Spicy
- C/Sen/Aftertaste
- C/Sen/Chunky
- C/Sen/Grainy

Quality Control[b]

- QC/Coarse
- QC/Visible-spice
- QC/Pieces
- QC/Thick
- QC/Flavor
- QC/Bitter
- QC/Burnt
- QC/Metallic

Instrumental-Readings[c]

- I/Pieces-surface
- I/Color
- I/NaCl
- I/Acid
- I/Consistency

Expert-R&D-Panel[d]

- E/Particulates
- E/Burnt-sweet
- E/Onion-flavor
- E/Pepper-flavor
- E/Sweet-taste
- E/Sour-taste
- E/Salt-taste
- E/Bitter-taste
- E/Throat-burn

[a] C = Consumer rating.
Like = liking.
Sen = sensory.
Segment = sensory preference segments (showing different patterns of liking).

[b] QC = Quality Control Panel.

[c] I = Instrumental measure.

[d] E = Expert panel (QDA Method).

studies involve fewer variables (often no more than 2–3), and may use only one panel (eg, consumers from a research guidance panel). However the data becomes more variable once the data set is augmented to comprise consumer and instrumental data.

Panel Selection

Depending upon when the research is undertaken (early versus late in the development cycle) the researcher will work with different consumer panels. In the earliest development stages researchers work with an in-house group of consumers or experts. Often, however, it is necessary to obtain ratings from users of different and competing products in the category. It is also instructive to run the study in different geographical areas (or even in different countries). The researcher hires field services in the different markets and "tailors" a consumer panel with specific demographic and usage characteristics. Fewer consumers participate in these guidance tests than in subsequent large scale market tests. This study tested products among 120 female consumers, 40 consumers in 3 markets, (in order to ensure representativeness). Optimization studies are usually of this magnitude, with panel sizes ranging from 50 to 200 participants.

Panels for optimization studies usually comprise users of the category, occasionally (and more specifically) users of the product being optimized and/or users of competitive brands. Here half the panel comprised consumers who used the manufacturer's product most often, and the other half comprised consumers who used competitor products. By incorporating different groups of consumers in the same panel, (all of whom test the same array of products), the investigator can compare ratings assigned to the same products by the two groups. The comparison can be used to develop optimal products which satisfy one target group, or both groups simultaneously.

Activities During the Evaluation

Depending upon the product being tested, panelists may either participate in a supervised session in a central location, or test the products at home, without supervision.

Panelists followed the protocol presented in Table 15. A central location setup makes it easy to monitor the quality of the data as the panelists assign their ratings. Close supervision of the evaluation ensures that panelists remain alert and motivated. Neither the interviewers nor the panelists "know the correct answer." Panelists must, perforce, answer honestly because there are no other cues to aid them other than their sensory perceptions. The ongoing question and answer dialogue between the panelist and the interviewer (after each product is rated) maintains motivation. For home use tests (appropriate when panelists prepare the product themselves, or use the product as an ingredient), other procedures are necessary. These procedures include giving the panelist one or two products to use, instructing the panelist to rate each product just after eating it, and then requiring the panelist to return to the test site where the panelist is questioned about the ratings that he or she assigned. Upon returning to the test site the panelist receives the next set of products, and follows the same protocol. Control in a home use test is maintained by the careful allocation of products to panelists, along with an orientation session at the start of the study (before actual home use). Motivation is maintained by payment for the successful completion of the test and by the ongoing question and answer interchanges between panelists and the interviewers.

Table 15. Protocol for the Sauce Study

- Panelists prerecruited to participate, by a telephone call.
- Panelists are screened to be appropriate (viz, category users, interested in the concept which an interviewer reads to them).
- Panelists show up, prepared for a 4-hour session.
  - Panelists show up, in groups of 15–20 (randomly recruited, so that the panelists do not know each other).
- Panelists are oriented in scaling by a short practice exercise.
  - Panelists rate water and cracker on sensory and liking attributes.
- Panelists try the first product, rating it on all attributes.
  - Products are randomized to reduce order bias. Products are rated on attributes as they appear (viz, appearance first, then aroma, then texture). Liking ratings and sensory ratings are interdigitated.
- Ratings checked by an attending interviewer on a panelist-by-panelist basis, to ensure panelist comprehension.
- Panelist waits 15 minutes and goes to the next product.
- After panelist finishes, the panelist is paid and dismissed.
- Each panelist rates 11 products (randomized from the full set).

Initial Data Analysis—Product × Attribute Matrix

The initial data comprises a matrix of mean ratings of each product by each attribute. Table 16 shows part of this matrix. Statisticians may prefer to adjust the means in Table 16 to account for the fact that each panelist evaluated only a randomized subset of products. These "adjusted means" rather than the actual means, would appear in Table 16.

Table 16 alone teaches a lot about the products because the researcher can compare products, on the different attributes. Even without an experimental design several instructive analyses (correlation, regression, and histogram plotting) highlight key dimensions of the category.

Questions investigators can answer with Table 16 (in its entirety) include:

1. For a specific attribute do the products differ a great deal or relatively little? What is the range of scores? By looking at the highest and lowest means across products on the attribute, and graphing the distribution of the means within that range the investigator sees the "spread" of products. If the raw data is also available, then a 1 way analysis of variance (ANOVA) provides a measure of discrimination, (viz, the F ratio). The F value measures the "signal to noise" ratio, or variability due to product differences versus variability due to random error (eg, panelist differences).
2. What is the distribution of the mean liking ratings across products? If competitor products score in the 50s and 60s (on a 0–100 point scale), whereas most of the prototypes (from the experimental design) score in the 30s and 40s, then the optimized product will probably not score higher than the competitors. The only way that one could develop a superb product would be if the optimal formulation lies in a region of ingredient values not tested in the original experimental design. (In that case the optimal solution would be suspect).
3. Which liking attributes covary with overall liking? This analysis reveals important attributes. Figure 2 shows a schematic plot of overall liking (ordinate) versus attribute liking (abscissa). The correlation between the two attributes describes the strength of a *linear relation* between attribute liking and overall liking, but does not show the slope. A straight line shows the quantitative nature of the relation. The steeper the slope of the straight line the more small changes in attribute liking covary with large changes in overall liking (and therefore the more important the attribute).
4. Which sensory attributes covary with overall liking? As a formula variable or sensory attribute changes, liking also changes. Often this relation exhibits an

Table 16. Partial Data Base for Sauces

Product	110	111	112	113	114	115	116	117
Independent variables[a]								
I/Ingred-A	3	2	2	1	1	1	2	3
I/Ingred-B	1	2	3	1	1	1	2	3
I/Ingred-C	1	2	2	1	3	3	2	1
I/Ingred-D	3	3	2	3	3	1	2	1
I/Ingred-E	1	2	2	1	1	1	1	3
I/Cook-process	1	2	2	3	0	3	2	3
O / Cost-of-Goods	116	138	132	116	141	132	119	123
Consumer-data[b]								
C/Like/Overall	44	63	67	57	56	49	42	61
C/Like/Segment-A	52	61	78	63	48	57	53	56
C/Like/Segment-B	39	65	73	53	58	50	42	63
C/Like/Segment-C	47	60	51	58	56	43	37	59
C/Like/Appearance	48	61	66	55	66	60	56	59
C/Image/Sophisticated	46	47	47	49	52	43	43	56
C/Sen/Darkness	47	57	71	51	59	55	53	69
C/Sen/Aroma	39	44	49	42	46	45	43	51
C/Sen/Taste	51	65	64	57	58	44	54	72
C/Sen/Sweet	33	48	73	39	39	43	38	48
C/Sen/Spicy	42	55	52	52	46	36	36	43
C/Sen/Afterstate	40	44	32	39	38	32	40	41
C/Sen/Thick	53	72	70	55	76	55	51	59
Quality-Control[c]								
QC/Coarse	56	77	74	68	68	63	68	81
QC/Visible-spice	39	52	48	44	42	47	30	68
QC/Pieces	25	32	39	24	48	50	33	37
QC/Thick	62	82	73	67	85	64	71	70
QC/Flavor	59	63	59	61	68	52	72	65
QC/Bitter	13	12	14	13	20	17	13	23
QC/Burnt	55	56	53	54	54	50	58	53
QC/Metallic	41	27	28	36	32	40	45	34
Instrument-reading								
I/Pieces-surface	26	30	33	24	38	51	30	31
I/Color	46	52	63	44	53	51	47	64
I/NaCl	42	42	35	41	38	23	29	38
I/Acid	53	41	29	42	46	32	43	46
I/Consistency	57	26	39	53	27	59	46	56
Expert-R&D-QDA[d]								
E/Particulates	35	47	49	41	62	49	50	34
E/Burnt-sweet	17	32	28	16	23	12	14	23
E/Salt-taste	60	54	49	57	56	28	53	55
E/Bitter-taste	1	8	5	5	6	1	1	7
E/Throat-burn	19	20	21	23	17	12	16	21

[a] I = instrumental, O = objective, 3 = high 2 = medium 1 = low.
[b] C = consumer.
[d] QC = quality control.
[d] E = expert panel.

inverted U shape (32,33). One can plot the relation between sensory attribute level (abscissa) and liking (ordinate). The data will scatter around the curve but the underlying data should follow a pattern that can be extracted. Fitting a parabola [liking = A + B(Sensory Level) + C(Sensory Level^2)] to the data, uncovers this relation. The fitted curve shows the *probable* nature of the relation, as Figure 3 illustrates (schematically).

Developing Relations Between Variables (Modeling)

Modeling creates a mathematical relation between the systematically varied independent variables and dependent

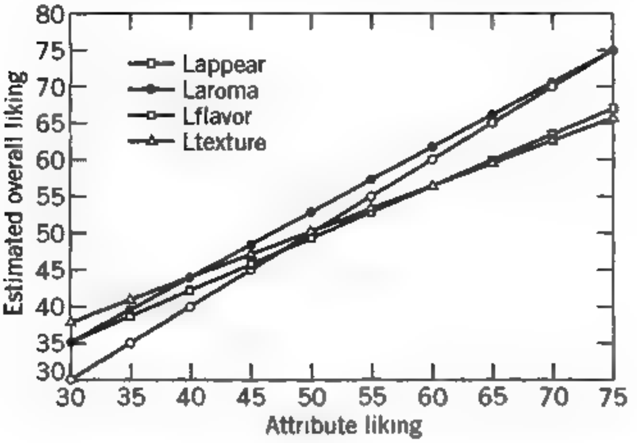


Figure 2. Relation between attribute liking and overall liking. The steeper the slope of the straight line the more important is the attribute.

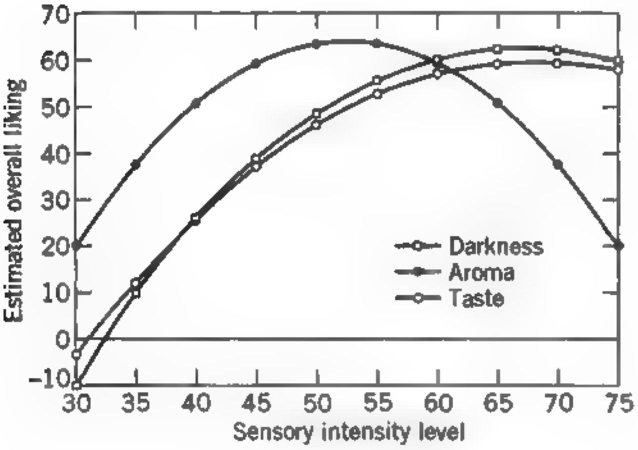


Figure 3. Schematic relation between sensory attribute level and overall liking. The relation usually follows an inverted U shaped curve. Large areas under the curve (subtended by the curve) correspond to important attributes.

variables. Dependent variables can be sensory attributes, acceptance ratings (all from consumers), or physical measures (including cost of goods), etc.

Relations between variables are expressed by equations. Researchers can use one of many available equations, depending upon the expected relation between the independent variable and the dependent variable. Some of these equations are

The Linear Equation. Changes in the dependent variable correspond to *linear* changes in the independent variable. The linear equation often emerges when we vary one ingredient (eg, sucrose) in a simple food system (eg, sweetened carbonated beverage). Over the limited concentration range consumer ratings of perceived sweetness versus sweetener concentration follow a straight line. Although a straight line might not describe the "true, underlying" relation between the two variables (sucrose level versus perceived sweetness) it is an adequate and useful representation.

We express the relation by the simple linear equation:

$$\text{Dependent Variable} = A + B(\text{Independent Variable})$$

Depending upon how closely the data points fall to the line, the equation may fit the data well or poorly. Of critical importance is the value of slope, B, which shows how unit changes in the independent variable correspond to changes in the dependent variable. When B is high there is great sensitivity to the independent variable. When B is low there is little sensitivity. Large changes in the independent variable correspond to small changes in the dependent variable.

Quadratic Relation. As the independent variable increases the rating first increases, peaks and then drops down. The parabolic or quadratic equation is a good summary of how liking varies with physical level (33). The parameters of the quadratic equation are unique to each product. Parabolic equations allow for increases, peaks, and then decreases (the classical inverted U curve), or simply increase and flatten (or the corresponding decrease and flattening). The equation is expressed as:

$$\text{Dependent Variable} = A + B(\text{Independent}) + C(\text{Independent})^2$$

The Plane (for Two or More Independent Variables, Jointly Predicting the Dependent Variable). The plane generalizes the line. We can envision it easily as a flat sheet in 3 dimensions. The slope of the plane in any dimension (corresponding to a single independent variable) is given by the coefficient for that independent variable. The plane equation does not account for interactions among the variables. The plane assumes that each variable acts entirely independently to determine the dependent variable. We write the equation of the plane as a multiple linear equation:

$$\text{Dependent} = A + B(\text{Independent 1}) + C(\text{Independent 2}). \;\ldots$$

In the absence of theory about the relation between independent variables and dependent variable it is prudent to use the multiple linear equation. The equation makes no assumptions other than there exists a simple linear relation between the dependent variable and each of the independent variables.

Quadratic Surface. Like the plane the quadratic surface also relates several independent variables simultaneously to the dependent variable. We assume that there exists a parabolic or curvilinear relation between the independent variables and the dependent variable (eg, for overall liking). However, for a quadratic or parabolic surface not only do we have linear terms, but also square terms.

If the equation comprises only linear terms and square terms, then we assume that the variables act independently of each other to determine the value of the dependent variable. The square terms allow each independent variable to act in a nonlinear fashion. There may be a flattening of the surface, so that as an independent variable increases the dependent variable can first increase, but then flatten out, and then perhaps even decrease. If there are no interactions between the independent variables, then we write the equation as:

$$\begin{aligned}\text{Dependent} = {} & \text{A} + \text{B}(\text{Independent 1}) + \text{C}(\text{Independent 1})^2 \\ & + \text{D}(\text{Independent 2}) + \text{E}(\text{Independent 2})^2 \\ & + \ldots .\end{aligned}$$

Like the plane equation, the parabolic or quadratic equation can be extended to accommodate many independent variables simultaneously. If there exists interaction between pairs of independent variables, we can write the equation as above, but also include a multiplicative term [(Independent 1) × (Independent 2)] to account for interactions (here between the first two independent variables). Many such pairwise interactions may exist. The equation can accommodate them.

There are many other equations that an investigator can use in order to model the relations between independent variables and dependent variables. For product testing, however, we generally do not really know the true form of the relation (viz, how variables interact with each other). It is prudent to use the most parsimonious equation. Most researchers opt for the linear equation, and add square terms and pairwise interactive (multiplicative) terms only when specifically required, usually for one of two reasons:

1. *Theory*. We know from experimentation that liking does not continue to increase linearly with physical level *ad infinitum*, but eventually peaks, plateaus, and then drops. Curvature requires the quadratic term in the equation.
2. *Parsimony*. The quadratic equation is relatively parsimonious, allowing both curvature and interactions between variables. Other equations might fit the data better, but would demand more terms.

Whether the investigator fits a linear or quadratic model to the data, the utility of the model is the same—it predicts responses to combinations of independent variables, even when the combinations were not actually tested but lie within the range tested.

Tables 17 and 18 show the equations developed for the products (darkness, liking). The equations are simply mathematical statements. They lack substantive meaning for the product developer, until the developer substitutes meaningful values for the independent variables and estimates the attribute ratings.

Optimizing a Response by Finding the Highest Value

A major benefit of modeling is the ability to discover the particular combination of physical variables which correspond to a desired condition, such as the highest (optimal) rating on an attribute (eg, liking). Table 19 shows several optimizations, including overall optimization of liking (column A), three optimizations for liking subject to imposed cost constraints (columns B,C,D), and optimization of lik-

Table 17. Model (Equation) Relating Formula and Process Variables to Liking[a]

Variable[b]	Coefficient	Std error	T value[c]	P value[d]
Constant	16.5	11.9	1.3	0.1
A	−2.3	4.5	−0.5	0.6
B	6.1	4.5	1.3	0.1
C	−0.5	4.5	−0.1	0.9
D	15.1	4.6	3.2	0.0
E	15.5	4.5	3.4	0.0
F	−2.6	2.0	−1.2	0.2
A*A	0.2	1.1	−0.2	0.8
B*B	−0.5	1.1	−0.4	0.6
C*C	0.9	1.1	0.8	0.3
D*D	−2.9	1.1	−2.6	0.0
E*E	−3.3	1.1	−3.0	0.0
F*F	0.6	0.5	1.3	0.1

Analysis of variance

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Regression	2376.813	12	198.068	6.836	0.000
Residual	898.187	31	28.974		

[a] Multiple R: .85 Squared multiple R: .73
Adjusted squared multiple R: .62 Standard error of estimate: 5.3

[b] A–E = Ingredients A to E, respectively; F = Cook process.

[c] T Value = T test for significance of the coefficient. (T values in excess of +1 or −1 denote significant coefficients—vis, those significantly different from 0).

[d] P value = probability that the coefficient is really "0". (Low P value means that the coefficient is significantly different from 0).

Table 18. Model Relating Formula and Process Variables to Perceived Darkness[a]

Variable[b]	Coefficient	STD error	T[b]	P[b]
Constant	36.7	7.1	5.1	0.0
A	−0.6	2.7	−0.2	0.0
B	3.0	2.7	1.1	0.2
C	−0.5	2.7	−0.1	0.8
D	9.0	2.8	3.2	0.0
E	−0.2	2.7	−0.1	0.9
F	0.1	1.2	0.1	0.8
A*A	−0.1	0.6	−0.2	0.8
B*B	−0.0	0.6	−0.0	0.9
C*C	0.7	0.6	1.0	0.2
D*D	−1.9	0.6	−2.7	0.0
E*E	1.7	0.6	2.5	0.0
F*F	0.0	0.3	0.1	0.8

Analysis of variance

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Regression	2717.953	12	226.496	21.499	0.000
Residual	326.593	31	10.535		

[a] Multiple R: .94 Squared multiple R: .89
Adjusted squared multiple R: .85 Standard error of estimate; 3.2

[b] See footnotes in Table 17 for explanation.

Table 19. Sequence of Optimal Formulations (Maximal Liking) Under Reduced Cost of Goods and High Spiciness

	No constraints	Cost constraints			Spiciness constraint
	A[a]	*B*[b]	*C*[c]	*D*[d]	*E*[e]
I/Ingred-A	1.0	1.0	1.0	1.0	1.0
I/Ingred-B	3.0	3.0	3.0	3.0	3.0
I/Ingred-C	3.0	2.3	1.4	1.0	3.0
I/Ingred-D	2.5	2.4	2.5	2.1	2.5
I/Ingred-E	2.3	2.1	2.3	2.0	2.7
I/Cook Process	3.0	3.0	3.0	3.0	3.0
O/Cost-of goods	151	140	130	120	154
		Consumer data			
C/Like/Overall	71	68	65	64	70
C/Like/Appearance	74	69	64	60	74
C/Like/Aroma	63	59	57	55	64
C/Like/Flavor	71	68	65	64	70
C/Like/Texture	76	72	65	60	77
C/Image/Fresh	68	64	62	61	67
C/Image/Authentic	63	59	59	54	64
C/Image/Sophisticated	55	52	51	49	58
C/Sen/Darkness	70	66	66	63	74
C/Sen/Aroma	54	50	50	48	57
C/Sen/Taste	67	65	65	62	70
C/Sen/Sweet	56	56	55	54	56
C/Sen/Spicy	61	58	68	55	65
C/Sen/Aftertaste	35	36	37	35	38
C/Sen/Thick	79	72	65	58	82
C/Sen/Chunky	60	53	44	38	61
C/Sen/Grainy	30	29	28	24	33
		Quality control			
QC/Coarse	82	77	76	72	86
QC/Visible-Spices	59	53	54	50	66
QC/Pieces	50	43	32	28	50
QC/Thick	83	77	73	67	86
QC/Flavor	64	62	59	58	64
QC/Bitter	16	14	15	13	20
QC/Burnt	53	52	52	52	54
QC/Metallic	21	23	25	25	25
		Instrument reading			
I/Pieces-Surface	47	40	30	25	48
I/Color	62	60	60	57	66
I/NaCl	37	37	38	37	38
I/Acid	34	36	37	36	35
I/Consistency	28	36	41	48	24
		Expert R&D			
E/Particulates	61	54	43	35	63
E/Burnt-sweet	29	27	26	23	31
E/Onion-flavor	30	29	31	29	32
E/Pepper-flavor	12	11	11	10	12
E/Sweet-taste	33	32	31	30	33
E/Sour-taste	52	54	55	56	51
E/Salt-taste	48	48	50	61	48
E/Bitter-taste	9	8	9	6	12
E/Throat-burn	21	21	22	21	21

[a] A = No constraints.
[b] B = Cost of goods less than 140.
[c] C = Cost of goods less than 130.
[d] D = Cost of goods less than 120.
[e] E = Perceived spiciness greater than 65.

ing for a product that must be perceived as highly spiced (column E). Optimization yields the likely formulations, which are then used to estimate the likely response profile on all of the attributes. If the model includes attributes derived from physical measures, then one can calculate the profile of physical measures corresponding to the optimal formulation. This link between physical measures and ratings can be used to develop a quality control system (see below).

Fitting a Predesignated "Goal" Profile

The models allow one to prescribe a desired goal profile of subjective attributes, and then discover the particular combination of physical variables which generates that desired goal profile. Rather than optimizing a single response (eg, overall liking), the optimizer system varies the independent variables until the expected response profile matches as closely as possible the predesignated goal profile. (The closeness of fit can be expressed either in absolute terms, or in percentage terms. Percentage for "closeness of fit" is preferable when the units of measurement for the response attributes differ from attribute to attribute). Table 20 shows the solution, for two goal profiles. The first profile comes from consumers who evaluated a competitor product that the researcher wishes to "match" using the set of ingredients. The second goal profile was generated at the plant by instrumental probes which recorded the characteristics of each batch as it was produced. The objective was to estimate consumer reactions to this batch, using the instruments as quality probes.

Profile fitting enables the investigator to estimate the likely profile for one data set, given the profile for another data set. Each target profile constitutes a goal. It is possible to:

1. *Relate Difference Sources of Data*. Given a target profile from one source (eg, instrumental measures) the investigator can estimate the corresponding attribute profile which would emerge from another source of data for the same product (eg, consumer sensory ratings).
2. *Program a set of "probes" (in-line) in a processing plant to respond to the formulation as consumers would*. Given the profile of responses to a batch obtained from the probe, the quality assurance engineer can estimate the likely formulation that would have yielded the profile, and then from that formulation estimate the likely consumer ratings.

Table 20. Example of Goal Fitting

	Consumer goals		Instrumental goals	
	Ingredient			
I/Ingred-A	1.0		2.5	
I/Ingred-B	1.9		1.3	
I/Ingred-C	2.1		2.8	
I/Ingred-D	1.0		1.0	
I/Ingred-E	1.6		1.0	
I/Cook-process	3.0		3.0	
O/Cost-of-goods	125		139	
	Consumer data			
C/Like/Overall	56		54	
C/Image/Fresh	54		56	
C/Image/Authentic	46		49	
C/Image/Sophisticated	46	Goal	52	
C/Sen/Darkness	56	50	55	
C/Sen/Aroma	45	60	45	
C/Sen/Taste	57	50	60	
C/Sen/Sweet	45	60	39	
C/Sen/Spicy	50	50	49	
C/Sen/Aftertaste	36	60	40	
C/Sen/Thick	50	50	66	
C/Sen/Chunky	42	60	49	
C/Sen/Grainy	24	50	26	
	Quality control			
QC/Coarse	62		73	
QC/Visible-Spices	45		45	
QC/Pieces	38		43	
QC/Thick	58		78	
QC/Flavor	56		64	
QC/Metallic	29		82	
	Instrument reading			
				Goal
I/Pieces-surface	37		40	40
I/Color	52		49	45
I/NaCl	37		39	30
I/Acid	40		45	55
I/Consistency	58		40	40
	Expert R&D panel			
E/Particulates	40		50	
E/Burnt-sweet	19		22	
E/Sweet-taste	27		26	
E/Sour-taste	57		58	
E/Salt-taste	48		55	
E/Throat-Burn	18		18	

Sensitivity Analysis Around a Point

Estimates of responses to a single combination of variables do not tell the whole story. Often the formulation projected by the optimizer corresponds to the absolutely highest point of what may be a very wide plane of virtually equally acceptable optima. Furthermore, changes in one physical variable may not change ratings, whereas changes in another variable may dramatically change ratings.

Sensitivity analysis estimates effects due to one variable, with all other variables held constant. For changes in one independent variable the investigator estimates the attribute profile using the equations.

Table 21 shows the sensitivity analysis. The table shows how the dependent variable covaries with the independent variable.

Using the Product Model to Assure Quality

The product model relates independent attributes (under research or production control) to dependent variables, be

Table 21. Sensitivity Analysis (From midpoint)

	1.0	1.3	1.7	2.0	2.3	2.7	3.0
			Ingredient A				
O/Cost-of-goods	132	132	132	132	132	132	133
C/Like/Overall	61	61	60	60	59	59	58
C/Sen/Darkness	61	61	60	60	59	59	58
C/Sen/Aroma	48	47	47	47	47	47	47
C/Sen/Taste	63	63	64	64	65	66	66
C/Sen/Sweet	47	47	47	46	46	45	44
C/Sen/Spicy	55	55	56	56	56	57	57
C/Sen/Aftertaste	38	38	39	40	41	42	42
C/Sen/Thick	64	64	64	64	64	63	63
C/Sen/Chunky	46	45	45	45	45	45	45
C/Sen/Grainy	27	27	27	27	28	28	28
			Ingredient B				
O/Cost-of-Goods	131	132	132	132	132	133	133
C/Like/Overall	55	57	58	60	61	62	63
C/Sen/Darkness	57	58	59	60	61	62	63
C/Sen/Aroma	46	46	47	47	47	47	48
C/Sen/Taste	63	63	64	64	65	65	65
C/Sen/Sweet	40	42	44	46	49	52	55
C/Sen/Spicy	54	55	55	56	56	56	56
C/Sen/Aftertaste	41	41	41	40	39	39	38
C/Sen/Thick	60	61	63	64	65	65	66
C/Sen/Chunky	44	44	45	45	45	46	47
C/Sen/Grainy	27	27	27	27	27	27	27
			Ingredient C				
O/Cost-of-Goods	118	123	128	132	137	142	146
C/Like/Overall	57	58	58	60	61	62	64
C/Sen/Darkness	58	59	59	60	61	62	63
C/Sen/Aroma	45	46	46	47	48	48	49
C/Sen/Taste	62	63	64	64	65	65	65
C/Sen/Sweet	45	45	46	46	47	47	47
C/Sen/Spicy	54	55	55	56	56	57	58
C/Sen/Aftertaste	39	40	40	40	40	39	39
C/Sen/Thick	54	57	61	64	67	70	72
C/Sen/Chunky	34	38	41	45	48	52	55
C/Sen/Grainy	24	25	27	27	28	28	28
			Ingredient D				
O/Cost-of-Goods	127	128	130	132	134	136	138
C/Like/Overall	53	56	58	60	60	60	60
C/Sen/Darkness	57	58	59	60	60	60	59
C/Sen/Aroma	45	46	46	47	47	47	47
C/Sen/Taste	61	62	64	64	65	64	63
C/Sen/Sweet	45	46	46	46	47	46	46
C/Sen/Spicy	53	54	55	56	56	56	55
C/Sen/Afterstate	39	39	40	40	40	40	40
C/Sen/Thick	51	56	61	64	66	66	66
C/Sen/Chunky	40	42	44	45	45	45	44
C/Sen/Grainy	25	26	27	27	28	28	28
			Ingredient E				
O/Cost-of-Goods	126	128	130	132	134	136	138
C/Like/Overall	54	57	58	60	60	59	58
C/Sen/Darkness	55	56	58	60	62	65	68
C/Sen/Aroma	43	44	45	47	49	51	54
C/Sen/Taste	59	61	62	64	67	69	71
C/Sen/Sweet	44	45	46	46	46	46	45
C/Sen/Spicy	48	50	53	56	59	62	65
C/Sen/Aftertaste	38	38	39	40	42	44	46
C/Sen/Thick	58	60	62	64	66	67	69
C/Sen/Chunky	41	42	44	45	46	47	48
C/Sen/Grainy	25	25	26	27	29	31	34
			Cool process				
O/Cost-of-Goods	133	32	132	132	132	132	132
C/Like/Overall	60	60	60	60	60	60	60
C/Sen/Darkness	60	60	60	60	60	60	60
C/Sen/Aroma	46	47	47	47	47	47	47
C/Sen/Taste	64	64	64	64	64	64	64
C/Sen/Sweet	46	46	46	46	47	47	47
C/Sen/Spicy	56	56	56	56	56	56	57
C/Sen/Aftertaste	40	40	40	40	40	40	40
C/Sen/Thick	65	64	64	64	64	64	64
C/Sen/Chunky	46	46	45	45	45	45	46
C/Sen/Grainy	27	27	27	27	28	28	28

Note: selected attriutes shown.
All ingredients held constant at the midpoint of the design (each variable held at "2"). Then each independent variable (ingredients A–E, cooking process was separately increased in small steps from a low of 1 to a high of 3. The numbers in the table show changes in attributes, including cost of goods and consumer ratings.

these consumer ratings, expert panel ratings, etc. The product model plays a role in assuring quality, because it can be used in two distinct ways to control production:

1. *Process Control*. At the most basic level sensitivity analysis reveals how process or ingredient variations affect liking. Once the desired formulation has been located within the grid of alternative formulations, and the range of independent variables set (high to low for each independent variable), the investigator computes the sensitivity curves for each independent variable, holding the other independent variables fixed (at the prescribed level for production). The changes in the sensory ratings show how the independent variable affects the sensory character of the product (viz, sensory attributes). The change in liking indicates how changes in the independent variable affect acceptance.

   Quality control tables based upon sensitivity analyses highlight those independent variables which produce noticeable sensory changes, and the degree to which those changes generate acceptance changes in their wake. The manufacturer should maintain tighter control over the key critical variables (perhaps at greater cost) and maintain looser control (at lower cost) over the less important variables (where departure from the optimal or production specifications do not reduce acceptance nor affect sensory integrity).

   The approach is *consumer driven*, because it is based upon the reactions of consumers to actual variations of the product, rather than hypothesis of what production variables might be important. It might well turn out that some variables play little or no role in determining acceptance, and can either be reduced to save money, or ignored by quality assurance over a wide range of levels. Conversely, more attention would then be paid to those important variables which influence acceptance.

2. *Batch Analysis*. The inter-relation among formula variables, consumer responses, instrumental variables, expert panel responses, and quality control panel responses assures quality at the production level on a batch-to-batch basis. Each batch is measured, either by experts, by the quality control panel, or by instruments. The measurements generate a goal profile. The goal profile determines a corresponding set of estimated ingredients or process variables which would generate that profile. Once the levels of the independent variables are estimated the model estimates the corresponding consumer sensory profile and/or the likely acceptance rating. As each batch emerges from the production line quality assurance can calculate the expected difference from the reference or "gold standard" on a sensory or acceptance basis. At the time of the measurement, plant personnel decide whether the product is sufficiently similar to a target standard (or reasonably acceptable) to warrant shipment, or whether the batch must be reworked (or even discarded).

AN OVERVIEW

Sensory analysis in the last decade of the Twentieth Century has matured considerably since its informal beginnings almost ninety years before. At its inception the field comprised individuals who had little experience in evaluation, who were practitioners of other disciplines, and who made sensory analysis a professional avocation. Techniques developed haltingly as the field developed, with various home grown approaches proposed, implemented, the best culled for further use, and the remainder discarded. The archival early literature of sensory analysis reveals these discarded byways.

Today, thanks to the continued importance of sensory satisfaction placed on products by the food processor and the need to remain competitive in a marketplace of intensely competing brands, manufacturers have refined their test methods. By combining contributions from academia, statistical procedures, psychological and psychophysical test methods, and applying the tests procedures in a business environment which needs accurate and actionable feedback, practitioners have advanced the field considerably. What was once an informal bioassay of a product to determine palatability has become, over the past years, a computer-based technology which quantitatively guides product development to improve consumer acceptance.

Sensory analysis comprises many different methods. Its strength coming from a body of diverse approaches is also its inherent weakness. There is no single set of approaches deemed "appropriate, correct and true" in sensory analysis, but rather different approaches to the same problem, proffered by practitioners. Sensory analysis is a science in development, and a technology in practice. The slow development towards a science is witnessed by the recent emergence of journals in the U.S., and the U.K devoted to sensory analysis as a science. These journals are belated witnesses to that recognition, and to the evolving nature of the field. Whether it takes a year or another lifetime, however, the field will become a true science. The wealth of methods presented to the users in product development, and the literature now available certainly today qualify sensory analysis as a dynamic, evolving, intellectually stimulating and business-relevant technology which has a vital place in product development and marketing. Sensory analysis has earned a key role in food science, and continues to be worthy of that role.

BIBLIOGRAPHY

1. M. A. Amerine, R. M. Pangborn, and E. B. Roessler, *Principles of Sensory Evaluation of Food*, New York, Academic Press, New York, 1965.
2. R. M. Pangborn and I. Trabue, "Bibliography of the Sense of Taste," in M. R. Kare and O. Maller, eds., *The Chemical Senses and Nutrition*, Johns Hopkins Press, Baltimore, 1967.
3. R. Harper, E. C. Bate-Smith, and D. G. Land, *Odour Description and Odour Classification*, American Elsevier, New York, 1968.
4. J. E. Amoore, "A Plan to Identify Most of the Primary Odors," in C. Pfaffmann, ed., *Olfaction and Taste IV*, Rockefeller University Press, New York, 1969, pp. 158–171.
5. R. Harper, E. C. Bate-Smith, and N. M. Griffiths, "Odour qualities: A glossary of usage," *British Journal Of Psychology* **59**, 231–252 (1968).
6. H. Sokolow, "Qualitative Methods for Language Development, in H. Moskowitz, ed., *Applied Sensory Analysis of Foods*, Vol I, CRC Press, Boca Raton, Fla., 1988, pp. 3–20.
7. J. F. Caul, "The Profile Method of Flavor Analysis," *Advances in Food Research*, 1957 pp. 1–40.
8. A. J. Neilson, V. B. Ferguson, and D. A. Kendall, "Profile methods: Flavor profile and profile attribute analysis," in H. R. Moskowitz, ed., *Applied Sensory Analysis of Foods*, Vol I, CRC Press, Boca Raton, Fla., 1988, pp. 22–41.
9. H. Stone, J. L. Sidel, S. Oliver, A. Woolsey, and R. Singleton, "Sensory Evaluation by Quantitative Descriptive Analysis," *Food Technology* **28**, 24–34 (1974).
10. K. L. Zook and J. H. Pearce, "Quantitative Descriptive Analysis," in H. R. Moskowitz, ed., *Applied Sensory Analysis of Foods*, Vol I, CRC Press, Boca Raton, Fla., 1988, pp. 44–71.
11. M. Meilgaard, G. V. Civille, and B. T. Carr, *Sensory Evaluation Techniques*, CRC Press, Boca Raton, Fla., 1987.
12. A. S. Szczesniak, M A. Brandt, and H. H. Friedman, "Development of standard rating scales for mechanical parameters of texture and correlation between the objective and sensory methods of texture evaluation," *Journal of Food Science* **28**, 397–403 (1963).
13. N. Schwartz, "Adaptation of the Sensory Texture Profile to Skin Care Products," *Journal of Texture Studies* **6**, 33 (1975).
14. M. Yoshida, "Studies in the Psychometric Classification of Odor," *Japanese Psychological Research* **6**, 111–115 (1964).
15. M. Yoshida, "Dimensions of Tactile Perceptions," *Japanese Psychological Research* **10**, 157–173 (1968).

16. S. Yoshikawa, S. Nishimaru, T. Tashiro, and M. Yoshida, "Collection and Classification of Words for Description of Food Texture, I, II, III," *Journal of Texture Studies* 1, 437–463 (1970).
17. C. Coombs, *A Theory of Data*, John Wiley & Sons, Inc., New York, 1964.
18. R. N. Shepard, "The Analysis of Proximities: Multidimensional Scaling with an Unknown Distance Function," *Psychometrika* **27**, 219–246 (1962).
19. M. Bourne, personal communication, 1975.
20. G. A. Gescheider, *Psychophysics: Method and Theory*, Lawrence Erlbaum Associates, Hillsdale, N.J. 1976.
21. E. H. Weber, *De Pulsu Resorptime, Auditor, et Tache. Annotations Anaomatical et Physiological*, Leipzig, Koehler, 1834, (cited by E. G. Boring, *Sensation and Perception In; The History of Experimental Psychology*, New York, Appleton-Century Crofts, New York, 1942.
22. G. T. Fechner, *Elemente der Psychophysik*, Breitkopf und Hartel, Leipzig, 1860.
23. F. Lemberger, "Psychophysiche Untersuchungen uber den Geschmack von Zucker und Saccharin," *Pfleuger's Archiv fur die Gesamte Physiologie* **123**, 293–311 (1908).
24. S. S. Stevens, "On the Brightness of Lights and the Loudness of Sounds," *Science* **118**, 576 (1953).
25. B. Bond and S. S. Stevens, "Cross Modality Matches of Brightness and Loudness by 5 Year Olds," *Perception & Psychophysics* **6**, 337–339 (1969).
26. H. R. Moskowitz, "Intensity Scales for Pure Tastes and Taste Mixtures," *Perception & Psychophysics* **9**, 51–56 (1971).
27. H. L. Meiselman, "Scales for Measuring Food Preference," in M. S. Petersen and A. H. Johnson, eds., *Encyclopedia of Food Science*, Avi, Westport, Conn., 1978, pp. 675–678.
28. H. G. Schutz, "Food Action Rating Scale for Measuring Food Acceptance," *Journal of Food Science* **30**, 365–374 (1965).
29. J. L. Balintfy, P. Sinha, H. R. Moskowitz, and J. G. Rogozenski, *The Time Dependence of Food Preferences*, Food Product Development, Nov. 1975.
30. G. E. Box, W. G. Hunter, and J. S. Hunter, *Statistics For Experimenters*, John Wiley & Sons, Inc., New York, 1978.
31. R. L. Plackett and J. D. Burman, "The Design of Optimum Multifactorial Experiments," *Biometrika* **33**, 305–325 (1946).
32. J. G. Beebe-Center, *The Psychology of Pleasantness And Unpleasantness*, Van Nostrand Reinhold, New York, 1932.
33. H. R. Moskowitz, "Relative Importance of Perceptual Factors to Consumer Acceptance: Linear Versus Quadratic Analysis," *Journal of Food Science* **46**, 244–248 (1981).

HOWARD R. MOSKOWITZ
Moskowitz Jacobs Inc.
White Plains, New York

SENSORY SCIENCE: STANDARDIZATION AND INSTRUMENTATION

Whenever the sensory properties of a substance are at issue, the sine qua non is that sensory evaluation is supreme. No chemical, physical, or instrumental test can substitute for sensory judgment under all circumstances. Humans and other animals are the only ones who can make decisions about acceptability or other sensory judgments. Notwithstanding that, there have been efforts as long as chemical and sensory measurements have coexisted for technologists and others to attempt to substitute chemical or physical measurements for sensory ones. Some reasons are obvious. Others are more illusory than factual. Among the obvious ones is the fact that chemical and mechanical tests usually can be conducted at any time whereas the "expert" tea taster, fish smeller, or the sensory panel—if the firm is more in the modern mode—are often available only during the daytime shift. Among the reasons more illusory than factual is the conception that chemical tests are more objective and precise; they often are, but that is not necessarily better. As in target shooting, there are differences between precision and accuracy. The archer or marksman may group shots close together, thus being precise, but the shots may be at the rim of the target instead of at the bull's-eye. Although a chemical test may be very precise in itself, it may miss the target simply because it is measuring an attribute not particularly well correlated with sensory quality. The purpose of this article is to describe how correlations may be established, if they exist, and how to use those that do exist most effectively as substitutes for, or complements to, sensory evaluation. The statement made above that no instrumental test can substitute for sensory evaluation under all circumstances is true, but fortunately there are many instances where instrumental measurements, once shown to be correlated with sensory judgment, can be used beneficially as replacements for sensory evaluation provided from time to time the instrumental test is restandardized or validated against sensory evaluation. In spite of some of the limitations just mentioned, the use of instrumental tests to supplement sensory evaluation for quality-control purposes is already a well-established industrial practice. In basic research, such tests are even more valuable. If we ever hope to understand sensory perception, most certainly knowledge of the role played by specific compounds, functional groups, and various forms of interaction among compounds must be ascertained. The interaction referred to here is not the chemical reaction between compounds but the perceptual interaction in the brain where there are often additive, masking, or synergistic effects. While correlations themselves are not adequate, they are a step toward establishing cause-and-effect relations.

EMERGING AND COALESCING TECHNOLOGIES

A happy set of circumstances has made sensory–instrumental correlations both easier to detect and more useful than was true 45 yr ago. Hereafter the term instrumental will be used in a generic sense to encompass all forms of chemical, mechanical, physical, spectral, or auditory tests where there is some type of physical measurement being obtained to match against sensory evaluation. In other instances, the particular form of physical measurement will be named where that is necessary to provide accuracy. That is so for the first development to be described. The detection and isolation of chemical compounds became easier once gas chromatography was established (1). Prior to that time, analytical chemists often had to spend weeks isolating and identifying even a single substance. Inas-

16. S. Yoshikawa, S. Nishimaru, T. Tashiro, and M. Yoshida, "Collection and Classification of Words for Description of Food Texture, I, II, III," *Journal of Texture Studies* 1, 437–463 (1970).
17. C. Coombs, *A Theory of Data*, John Wiley & Sons, Inc., New York, 1964.
18. R. N. Shepard, "The Analysis of Proximities: Multidimensional Scaling with an Unknown Distance Function," *Psychometrika* **27**, 219–246 (1962).
19. M. Bourne, personal communication, 1975.
20. G. A. Gescheider, *Psychophysics: Method and Theory*, Lawrence Erlbaum Associates, Hillsdale, N.J. 1976.
21. E. H. Weber, *De Pulsu Resorptime, Auditor, et Tache. Annotations Anaomatical et Physiological*, Leipzig, Koehler, 1834, (cited by E. G. Boring, *Sensation and Perception In; The History of Experimental Psychology*, New York, Appleton-Century Crofts, New York, 1942.
22. G. T. Fechner, *Elemente der Psychophysik*, Breitkopf und Hartel, Leipzig, 1860.
23. F. Lemberger, "Psychophysiche Untersuchungen uber den Geschmack von Zucker und Saccharin," *Pfleuger's Archiv fur die Gesamte Physiologie* **123**, 293–311 (1908).
24. S. S. Stevens, "On the Brightness of Lights and the Loudness of Sounds," *Science* **118**, 576 (1953).
25. B. Bond and S. S. Stevens, "Cross Modality Matches of Brightness and Loudness by 5 Year Olds," *Perception & Psychophysics* **6**, 337–339 (1969).
26. H. R. Moskowitz, "Intensity Scales for Pure Tastes and Taste Mixtures," *Perception & Psychophysics* **9**, 51–56 (1971).
27. H. L. Meiselman, "Scales for Measuring Food Preference," in M. S. Petersen and A. H. Johnson, eds., *Encyclopedis of Food Science*, Avi, Westport, Conn., 1978, pp. 675–678.
28. H. G. Schutz, "Food Action Rating Scale for Measuring Food Acceptance," *Journal of Food Science* **30**, 365–374 (1965).
29. J. L. Balintfy, P. Sinha, H. R. Moskowitz, and J. G. Rogozenski, *The Time Dependence of Food Preferences*, Food Product Development, Nov. 1975.
30. G. E. Box, W. G. Hunter, and J. S. Hunter, *Statistics For Experimenters*, John Wiley & Sons, Inc., New York, 1978.
31. R. L. Plackett and J. D. Burman, "The Design of Optimum Multifactorial Experiments," *Biometrika* **33**, 305–325 (1946).
32. J. G. Beebe-Center, *The Psychology of Pleasantness And Unpleasantness*, Van Nostrand Reinhold, New York, 1932.
33. H. R. Moskowitz, "Relative Importance of Perceptual Factors to Consumer Acceptance: Linear Versus Quadratic Analysis," *Journal of Food Science* **46**, 244–248 (1981).

HOWARD R. MOSKOWITZ
Moskowitz Jacobs Inc.
White Plains, New York

SENSORY SCIENCE: STANDARDIZATION AND INSTRUMENTATION

Whenever the sensory properties of a substance are at issue, the sine qua non is that sensory evaluation is supreme. No chemical, physical, or instrumental test can substitute for sensory judgment under all circumstances. Humans and other animals are the only ones who can make decisions about acceptability or other sensory judgments. Notwithstanding that, there have been efforts as long as chemical and sensory measurements have coexisted for technologists and others to attempt to substitute chemical or physical measurements for sensory ones. Some reasons are obvious. Others are more illusory than factual. Among the obvious ones is the fact that chemical and mechanical tests usually can be conducted at any time whereas the "expert" tea taster, fish smeller, or the sensory panel—if the firm is more in the modern mode—are often available only during the daytime shift. Among the reasons more illusory than factual is the conception that chemical tests are more objective and precise; they often are, but that is not necessarily better. As in target shooting, there are differences between precision and accuracy. The archer or marksman may group shots close together, thus being precise, but the shots may be at the rim of the target instead of at the bull's-eye. Although a chemical test may be very precise in itself, it may miss the target simply because it is measuring an attribute not particularly well correlated with sensory quality. The purpose of this article is to describe how correlations may be established, if they exist, and how to use those that do exist most effectively as substitutes for, or complements to, sensory evaluation. The statement made above that no instrumental test can substitute for sensory evaluation under all circumstances is true, but fortunately there are many instances where instrumental measurements, once shown to be correlated with sensory judgment, can be used beneficially as replacements for sensory evaluation provided from time to time the instrumental test is restandardized or validated against sensory evaluation. In spite of some of the limitations just mentioned, the use of instrumental tests to supplement sensory evaluation for quality-control purposes is already a well-established industrial practice. In basic research, such tests are even more valuable. If we ever hope to understand sensory perception, most certainly knowledge of the role played by specific compounds, functional groups, and various forms of interaction among compounds must be ascertained. The interaction referred to here is not the chemical reaction between compounds but the perceptual interaction in the brain where there are often additive, masking, or synergistic effects. While correlations themselves are not adequate, they are a step toward establishing cause-and-effect relations.

EMERGING AND COALESCING TECHNOLOGIES

A happy set of circumstances has made sensory–instrumental correlations both easier to detect and more useful than was true 45 yr ago. Hereafter the term instrumental will be used in a generic sense to encompass all forms of chemical, mechanical, physical, spectral, or auditory tests where there is some type of physical measurement being obtained to match against sensory evaluation. In other instances, the particular form of physical measurement will be named where that is necessary to provide accuracy. That is so for the first development to be described. The detection and isolation of chemical compounds became easier once gas chromatography was established (1). Prior to that time, analytical chemists often had to spend weeks isolating and identifying even a single substance. Inasmuch as almost every food contains hundreds of compounds, if not thousands, and most of these have sensory import, there was little prospect of being able to establish good correlations when only a few compounds in a given kind of food could be identified and quantified. Fortunately, sometime before gas chromatography enabled chemistry to go "multicomponent," food and other sensory substances began to be looked at in the same way.

Prior to 1949, sensory judgments were generally quite simple. Expert tea tasters or wine judges rendered a composite judgment that this tea or that wine met their company's buying specifications, perhaps with a few comments as to particularly pertinent qualities. Generally, they kept the basis for their judgments to themselves. In fact, their status as an expert depended on the imperiousness of their judgments. In some industries, sensory panels came into use and worked in parallel with experts. In other industries, the first sensory analyses were those provided by sensory panels. In either case, the panels also rendered simple judgments. At the most they examined the material for odor, taste, texture, or color or for such specific attributes as sweetness, bitterness, saltiness, and sourness. Even the latter attributes are often not as specific as is desirable, because different substances can have different nuances of bitterness. The same is true of the other character notes.

The development that changed things in 1949 was the development of the flavor profile method (FPM) (2). The method required panelists to attempt to pick out from the melange of sensory notes comprising odor or flavor the particular odor or taste character notes that were there. FPM set the feet of sensory scientists on the right path (3), but it was inadequate in some respects. The responses sought were only semiquantitative. Furthermore, one of the objectives of the method was to get the panelists to arrive at a consensus as to which sensory notes were present, the order of their perception, and their intensities. Semiquantitative data and the merging into a consensus differences expressed by the individual panelists made difficult the establishment of sound correlations between the sensory–instrumental measurements. The deficiency of strict quantitative data to match against the quantitative data of instrumental methods changed in 1974 when quantitative descriptive analysis (QDA) was devised (4). QDA requires that the panelists make their judgments independently, and the rather crude scoring system of the FPM was replaced with more carefully defined scoring systems. Other differences between the FPM and QDA procedures as well as the quantitative sensory profiling (QSP) methods that have been developed more recently have been described (3). The FPM and QDA procedures were singular developments for the influence they had on sensory analysis.

EXAMINATION OF MULTICOMPONENT DATA SETS

In 1966 there was a seminal development (5,6), which was the use of statistical multivariate analysis (MVA) to seek out from all the data from gas chromatography those peaks most effective for classifying samples into the sensory category to which they belonged. Earlier efforts had been made to find the peaks most closely correlated with sensory quality, but they were often futile or only partially successful because simple, univariate statistical methods, such as ξ^2, were employed rather than MVA procedures. The new method used discriminant analysis (6). Almost immediately other researchers began to follow this lead; sometimes they used discriminant analysis, but quite logically they began to examine other forms of MVA for their applicability to sensory–instrumental analysis. The result is that today several multivariate methods play a role in examining and correlating sensory and instrumental data.

The flux that enabled multicomponent sensory data to be effectively melded with multicomponent instrumental data was the development of computers. Some of MVA procedures had existed since early in the twentieth century, but they were not widely used because calculation was too onerous and tedious a task when done by hand or by the hand-cranked calculating machines then extant. In the 1960s and the 1970s the critical components to effective correlation of sensory and instrumental data fused. The development of gas chromatography in 1952 (1), the application of discriminant analysis to multicomponent data in 1966 (5,6), and the refining of multicomponent sensory evaluation in 1974 (4) were all encompassed by advances in computer technology during the same period, and subsequent to that time, and set the stage for these individual achievements to coalesce into a major thoroughfare of application. Their coalescing had an influence much like the confluence of the Ohio, the Missouri, and other rivers with the Mississippi, enabling it to bear a heavier traffic load than it could if other rivers did not add to its majesty.

KINDS OF MULTIVARIATE METHODS

There are at least 14 kinds of MVA procedures used to examine sensory–instrumental data. Some are of chief benefit for exploratory examination of data to gleam from large data sets certain components or relations for more detailed examination or later use. Others are employed to learn whether sample differences exist. Among the procedures are

Principal component analysis
Cluster analysis
Multidimensional scaling
Discriminant analysis
Multiple regression analysis
Partial least squares analysis
Response surface analysis
Procrustes analysis
Canonical analysis
Correspondence analysis
Multivariate analysis of variance
Fuzzy logic (mathematics)
Neural network
Factor analysis

Of the above procedures, principal component analysis (PCA), cluster analysis (CA), and factor analysis (FA) are known as methods of internal analysis. They are used to study the relations of variables within the same data set, ie, a set of sensory measurements or a set of instrumental measurements, but not sensory compared with instrumental measurements. Discriminant analysis (DA) and multiple regression (MRA) perform just the opposite functions. DA is a procedure for assigning products, or some other entity, to a class based on measurements made on those products, with prior knowledge that classes do exist. For example, yellow and red globe onions and Vidalia (Grano–Granex) onions were analyzed gas chromatographically to learn if they could be so classified (7). That species differences existed was, of course, known in advance. MRA works somewhat analogously to DA except it applies to things that progress in some manner rather than things in discrete classes, as species of onions are. An illustration would be predicting the intensity of vanilla flavor in ice cream when different levels of vanilla are added. DA and MRA are the most useful of the MVA methods for predicting the identity or state of a sample. They are useful because once a discriminant function or a multiple regression equation has been calculated, the identity or state of a new sample can be predicted by merely inserting its measurement values in the equation. The other methods listed above are more commonly used to study relations between sensory and instrumental measurements. Some, such as canonical analysis or partial least squares regression can be used for prediction purposes, but generally they are used to examine relations among variables rather than to pinpoint the identity of a single sample.

PRELIMINARY OR EXPLORATORY USES

While not always confined to being exploratory methods, PCA, CA, and FA are often used for that purpose. Of the three, PCA is probably the one most used to examine data to learn how the data should be subsequently examined to secure the greatest amount of value from them. Basically, PCA is a procedure for examining the variance of data to learn which variables go together and which others belong to a different group. The procedure thus performs two functions. It establishes whether there is correlation among some of the variables (sensory scores and instrumental measurements); then it attempts to reduce the dimensionality of the data, ie, groups together in a component those things that are correlated with each other.

Variance, or dispersion, should perhaps be defined. It is a measure of the degree to which measurement values should in theory be the same rather than differ slightly, as they usually do. The variation represents error, not in the sense that the measurement is wrong but in the sense that measurements made on the same sample are usually not identical. Among causes of error, generally minor, are periodic inconsistencies in the performance of the instrument. Slightly more common are variations in results caused by the operator of the instrument not being entirely consistent in his or her handling of the instrument or samples. The most prevalent cause of variance is the inherent differences that characterize all biological materials—and a very high proportion of specimens examined for sensory qualities are biological in nature: nearly all our foods and beverages are. Other materials such a perfumes may be compounded from differences substances, but many of the most desirable ingredients are extracted from natural substances. Such things as air or water pollutants, although not necessarily biologically derived, likewise vary according to the way the wind blows and how an industrial plant is being operated. Error should not, therefore, be looked on as "wrongness" but rather "rightness" in the sense that all things exhibit some variation in their properties and performance, and the error term is merely reflecting that.

CALCULATION AND FUNCTIONING OF PCA

PCA operates by searching for the variables accounting for the greatest amount of variance. The items under investigation (samples, people, and descriptive terms), correlated and contributing the greatest amount of variance in ratio to the total, are grouped into the first principal component. The second component is derived in the same way. It is a measure of the variance remaining after the first component has been extracted and accounts for the next greatest amount of variance. The process continues in that fashion until all the variance has been accounted for. In practice, the investigator is rarely interested in all the components. Usually, the first few account for so much of the existing total variance that the other components can be ignored because they are not supplying enough additional information to justify being retained for further use or evaluation. The consequence of PCA is that the volume and complexity of the data set is reduced. The investigator may give up 5–15% of the information contained in the whole mass of data. In turn, there will be fewer entities to work with in the future, maybe three components, instead of the original number of samples (people, descriptive terms, or whatever was being subjected to PCA). Not only may data reduction be involved but PCA is useful to learn whether products thought to be different are in fact different. A common problem is to judge whether products sold under different brand names are indeed different sensorially. The same producer may, for example, market the same product under two or more different labels.

Not quite in line with the illustration above is another type of problem (8). Nine brands of Scotch whisky and one of Irish whiskey were analyzed. Upon applying PCA it was learned that some of the brands were so close together in their sensory qualities they should be treated as one set of samples, not two or three different samples. A cooperative research project was conducted between the food chemistry and technology department of the University of Helsinki and the University of Georgia (9). The Finns had selected five kinds of Finnish sour rye bread, which they considered to be representative of different types, for sensorial and chemical examination. The chemical data permitted the five breads to be separated except for breads no. 1 and 4. The sensory data did the same except breads no. 1 and 2 could not be separated. Putting together the two kinds of data permitted sample resolution of the five kinds of bread, but a pictorial representation of just how close together the five kinds were was desired. Figure 1 illus-

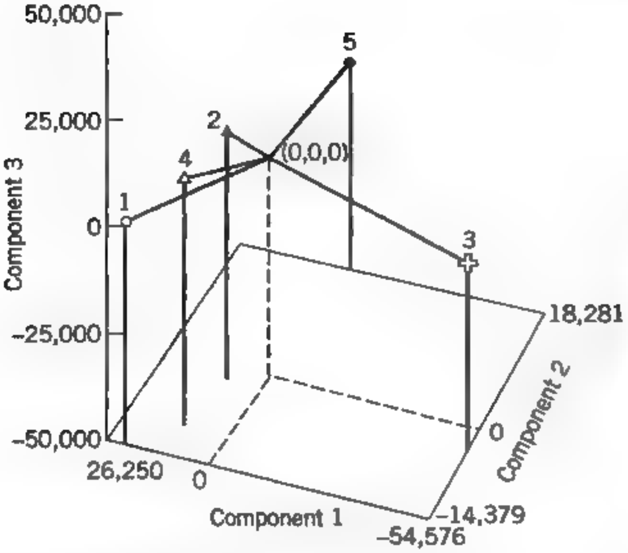


Figure 1. Spatial relations of five types of Finnish wholemeal sour rye bread in three-dimensional space based on principal component analysis. Data set reduced from responses by 20 panelists for 12 attributes, replicated four times. Numerals designate the five kinds of bread.

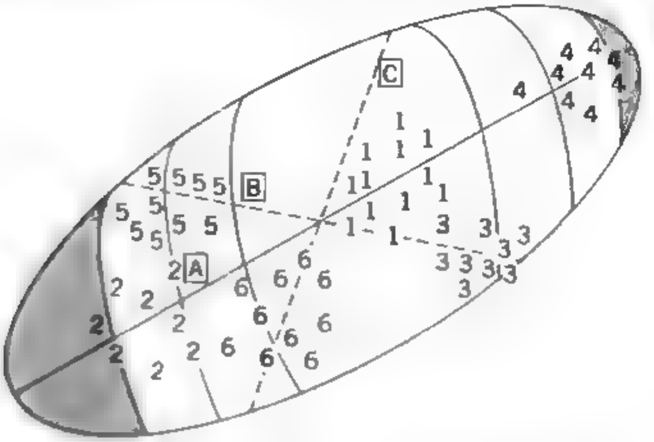


Figure 2. Depiction of basis for calculation of principal components. Regression curve through measurement types 2, 1, and 4 establishes first component, regression curve through measurement types 5 and 3 establishes second component, curve through measurement type 6 illustrates formation of a third component, the regression curve for each being orthogonal to the others.

trates the positions of the five types of bread, one to the other, in three-dimensional space. Other ways of providing evidence that the samples differed will be described later. PCA was used here as a preliminary check on the validity of the assumption that the five kinds of bread were different.

Perhaps the theory underlying PCA should have been given first, but it is hoped that the reader will find the explanation of what PCA is about more understandable having first seen some of the results of its application. In the case of the Finnish sour rye bread, the data mass was reduced from judgments by 20 panelists for 12 sensory characteristics and several chemical measurements to five components, ie, the five coordinates of the five kinds of bread. Figure 2 depicts what is going on. The kinds of measurements represented by the numbers 1, 2, and 4 are correlated with each other because they lie along the same, and the longest, axis. They account for the greatest amount of variance; thus they represent the first component. Measurement values 3 and 5 are along the next longest axis, and they account for the next greatest amount of variance, once the variance of the first component has been removed. They thus constitute the second component. Measurement value 6 is off by itself. It, therefore, constitutes the third component. Although the three axes are not at right angles to each other, one of the goals of PCA is to derive components that are orthogonal, ie, at right angles, to each other. The drawing was purposely made not to be ideal in terms of orthogonality, because in practice the axis of each component is not always exactly at a right angle to the other axes. The drawing depicts the way PCA was originally calculated. The problem of calculating principal components was solved by fitting a line to the longest axis of the ellipsoid of objects such that the sum of the squared residual distances was at a minimum for highly correlated objects (10). The same was then done for the next longest axis and those next most highly correlated. The reason the components are orthogonal to each other is that once the first component has been extracted, subsequent ones extracted are not correlated, ie, are orthogonal, because the variance of the first is no longer in play to allow correlation to exist. To carry on PCA calculations today, Hotelling's (11) procedure is generally used. It depends on transposing and inverting the variance–covariance matrix, or the correlation matrix, to accomplish the same purpose. The procedure selects the variables accounting for the greatest proportion of the variance, it arrays them and then rotates them in such a way that they are in the order of decreasing variance and are orthogonal.

Figure 3 provides an illustration of the use of PCA to reduce data dimensionality. This study was mentioned above (7). Forty-five gc peaks were useful to resolve product differences, but only 7 peaks were needed to be 100% correct in classifying 47 samples of the three different kinds of onions. For many, a pictorial display is more convincing than numerical data. To take advantage of the information contained in the 7 peaks, yet still be able to show that the onions did indeed differ, PCA was applied to the 7 peaks to reduce them to three components. The graph shows quite clearly that the three kinds of onions occupy three distinct locations in space.

A more useful purpose of PCA than merely to reduce a number of variables to a lesser number of components to permit visual display is the use of it to classify panelists undergoing training for possible membership on a sensory panel. Reference was made above to biological differences often being the cause of errors in measurement. Humans are no exception. Every one is an individual, responding in different ways when called on to be a sensory panelist. That is true even after training. Some of the variation can be overcome by training panelists to respond nearly alike

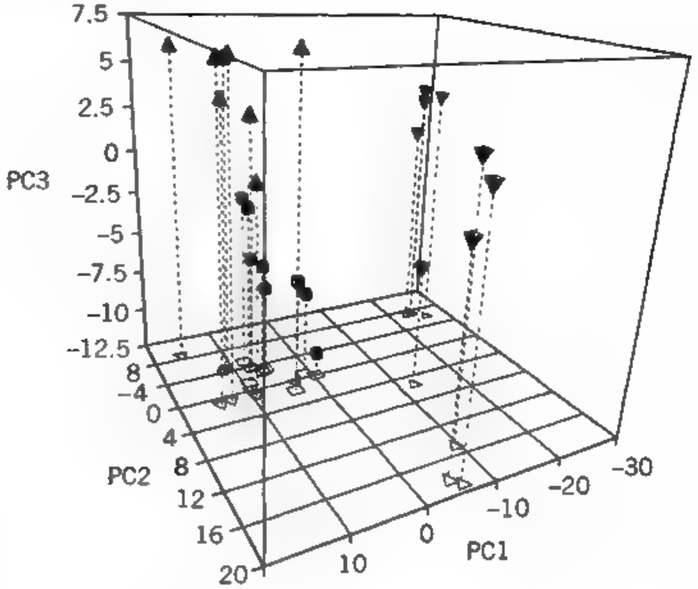


Figure 3. Principal component analysis applied to yellow and red globe onions and to Vidalia onions based on reducing seven gas chromatographic peaks to three components, four replications each.

to the same stimulus, but it can never be fully overcome. In deciding whether to admit an individual to membership on a sensory panel, it must be considered whether the individual will be in reasonable concert with other members of the panel, or if the individual will respond so individually as to be a statistical outlier. Figure 4 shows PCA applied to the results of 11 panelists when they examined a baked bean product for the intensity of 14 attributes (12). Panelist 9 is clearly an outlier. Panelists 1, 2, and 3 do not agree with the others as closely as is desirable. If panelist 9 is to be retained as a panelist, he will have to receive additional training in an effort to bring his responses into line with the rest of the panel. The sensory leader might decide that panelists 1, 2 and 3 might likewise improve in performance with additional guidance and training. One of the advantages sensory technologists have today is that PCA can be readily applied to the responses of panelists, and if there is a program to graph the results, a visual display can be created so that panelists can see how far they are from the rest. Knowing that, often panelists can readjust their mental scaling processes to bring their responses into line with the rest of the panel. For any of the illustrations above, the basic information is supplied as numerical output. The investigator generally just examines it to make decisions. Naturally the numerical output is the only way of examining the output beyond the third component. Graphic illustrations were given here merely to make clear the kinds of information obtainable from PCA.

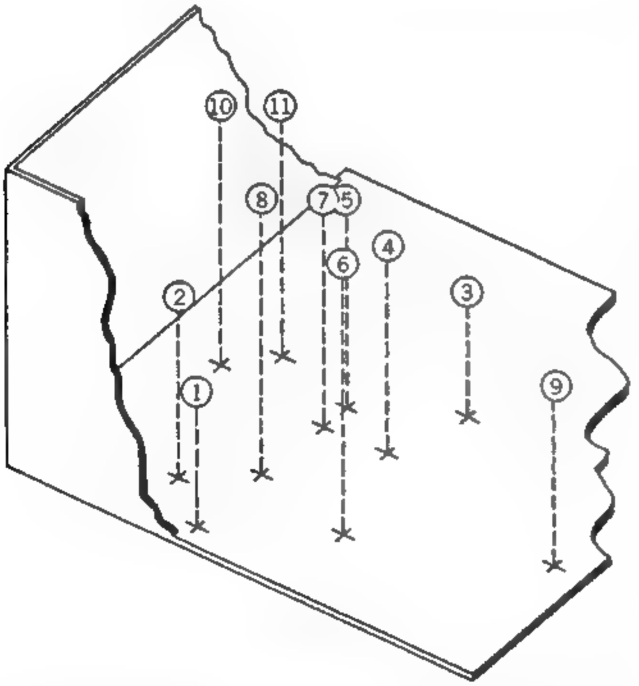


Figure 4. Interspatial relations of 11 panelists based on principal component analysis, eight baked bean products evaluated for 14 attributes, four replications.

In reducing the dimensionality of the data, some information is given up, but usually not much. In return for the loss of information, relations among the entities being evaluated, whatever they are, are made clearer. The entities can be products as in the first illustration, gas chromatographic peaks in the third, the responses of people in the fourth, or any pertinent category such as descriptive terms, geographical location, or demographic information.

CLUSTER ANALYSIS

Cluster analysis (CA) is another data-reduction method. It, too, can be used to examine the responses of panelists to learn whether panel members are responding alike. CA can take several different forms. Whereas PCA depends on examination of variance, CA is basically a measure of proximity. It can be calculated on the basis of distance, correlation, or the effect they have on variance by lumping together or separating other individual elements or tentative clusters. There are several forms of depicting proximity. There are single linkage, centroid linkage, average linkage, density linkage, and minimum variance. One of the most useful is the agglomerative hierarchical clustering technique, of which Ward's minimum variance method is one. Ward's method uses the square of the Euclidean distance as the proximity measure. Some publications refer to the representation as a tree; others call it an icicle. Whether distance, correlation, or variance, the basic purpose of CA is the same. Things having characteristics in common form a cluster; things dissimilar to the first have to go into a second cluster, etc. Having characteristics in common does not necessarily mean that the objects in a cluster are related. Sensory leaders hope that their panelists will form a single cluster; but just being grouped together does not mean the panelists are related. They merely happen to be together because they respond alike; only rarely are panel members kin. Table 1 shows cluster analysis applied to the 20 panelists who examined the five kinds of Finnish sour rye bread for 12 attributes (13). Tables 2 and 3 show the kinds of output obtained by different clustering processes. There are some differences because hierarchial clustering was used for the icicle formation (Table 2) and the VARCLUS routine of the SAS package (14) was used for Table 3. Either one of the clustering processes serves the purpose of the sensory analyst, who is generally interested in only the major groups formed. Here there are a few differences in the assignment of panelists, but they reveal essentially the same information. Both processes said that panelists 7 and 12 responded least like any of the others. From other information (15) every one of the 20 panelists was an accomplished sensory judge who could reproduce his or her assignment of scores. The only flaw was that the panel did not operate as one unit. The panelists of each of the clusters arrived at the same decision, three of the kinds of bread were sensorially different, but they didn't reach the same conclusion by the same pathway.

Sometimes CA is used to learn if resolution of product differences can be stepped up. By applying CA to a group

Table 1. Ward's Minimum Variance Cluster Analysis Applied to the Scale Values of 20 Panelists Evaluating 12 Attributes of Finnish Wholemeal Sour Rye Bread

```
                                              Panelists
i—Partial R^2 1   13  3   12  7   5   19  8   15  9   2   6   17  20  4   18  10  11  14  16
0.35
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
0.3           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
0.25          XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
0.2           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
0.15          XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXXXXXXX
0.1           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXXXXXX   XXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXX       XXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXX       XXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXX       XXXXXXXXX   XXXXXXXXXXXX   XXXXXXXXXXXXXXXXXX
0.05          XXXXXXXXXXXXXXXXXXXX       XXXXXXXXX   XXXXXXXX       XXXXXXXXXXXXXXXXXX
              XXXXXXXXXXXXXXXXXXXX       XXXXXXXXX   XXXXXXXX       XXXX    XXXXXXXXXX
              XXXXX   XXXXXXXX           XXXXXXXXX   XXX            XXXX         XXXXX
              XXXXX   XXXXXXXX           XXXXXX                     XXXX         XXXXX
                      XXX
```

Table 2. Tabular Output of Ward's Minimum Variance Cluster Analysis[a]

	Eigenvalues of the covariance matrix			
Variables	Eigenvalue	Difference	Proportion	Cumulative
1	796.987	313.406	0.429565	0.42956
2	485.580	254.313	0.260643	0.69021
3	229.267	132.981	0.123572	0.81378
4	96.286	22.873	0.051897	0.86568
5	73.413	25.975	0.039569	0.90525
6	47.438	9.109	0.025568	0.93081
7	38.329	7.307	0.020659	0.95147
8	31.023	6.098	0.016721	0.98163
9	24.925	8.704	0.013434	0.98163
10	16.221	5.445	0.008743	0.99037
11	10.776	3.687	0.005808	0.99618
12	7.089		0.003821	1.00000

Number of clusters	Clusters joined				Frequency of new cluster	Semipartial R^2	R^2
19		3		12	2	0.010001	0.989999
18	CL19	11		14	2	0.011672	0.978327
17				7	3	0.014082	0.964245
16		4		18	2	0.014111	0.950134
15		8		15	2	0.014587	0.935547
14		1		13	2	0.016947	0.918600
13		2		6	2	0.019158	0.899442
12	CL15			9	3	0.024987	0.874454
11		10	CL18		3	0.025403	0.849052
10	CL17			5	4	0.027946	0.821106
9	CL14		CL10		6	0.029373	0.792732
8	CL13			17	3	0.030646	0.761086
7	CL16		CL11		5	0.034671	0.726215
6	CL8			20	4	0.041325	0.685890
5	CL9			19	7	0.054941	0.684890
4	CL7			16	6	0.060708	0.569241
3	CL5		CL12		10	0.062688	0.506553
2	CL6		CL4		10	0.189603	0.316950
1	CL3		CL2		20	0.316950	0.000000

[a] Root-mean-square total-sample standard deviation = 12.4343; Root-mean-square distance between observations = 60.9153.

of panelists, it was possible to demonstrate more conclusively that three cheese products were different (16). Before the panel was split into three clusters, classification of 180 samples by discriminant analysis into the product category to which they belonged was 76% correct. When DA was applied to each of the clusters separately, the success level was raised to 90%. Either PCA or CA can be used for the purpose of determining whether samples differ. Table 4 shows Ward's minimum variance cluster analysis applied to five grades of tea to learn how much alike or different they are. Tea no. 5 was a "fine" tea selected by an expert tea buyer. Tea no. 1 was the poorest as judged by the tea buyer. Note that the CA tells the investigator that the panelists can most readily tell apart teas no. 1 and 5. The fact that teas nos. 2, 3 and 4 did not break off from each other as readily indicates that the panelists had more difficulty in distinguishing among them.

MULTIDIMENSIONAL SCALING

Multidimensional scaling (MDS) will be explained at this point chiefly because it, too, is a form of proximity measurement. It is a method for finding a configuration of points for individuals from information about the distance between the points. The usual metric is similarity or dissimilarity judgments, but correlation matrices, attribute ratings, or Euclidean distances can be used. The $r \times c$ matrix is generally not one of $n \times p$ but rather $n \times n$. It has been stated that the strategy in MDS is to look for the solution with the smallest dimensionality in which the differences between the reconstructed distances and the original proximities are acceptably small (17). Smaller stress values indicate more satisfactory fit. Various programs for the analysis of proximity data have been described (18). It has been pointed out that MDS and correspondence analysis have some features in common with PCA (19). For MDS, if $n > p$, then a PCA should generally be preferred because it is easier to find the eigenvectors of a $p \times p$ matrix than the larger $n \times n$ matrix. The theory and methods of MDS have been published (20).

DISCRIMINANT ANALYSIS

FA and correspondence analysis will be discussed later. Although PCA and CA can be used to depict product differ-

Table 3. Varclus (Oblique Principal Component) Clustering of 20 Panelists Based on Their Correlation Matrices

		R^2 with		
	Variable (panelists)	Own cluster	Next closest	1-R^2 ratio
Cluster 1	2	0.6955	0.4487	0.5524
	4	0.6727	0.5217	0.6842
	10	0.4667	0.2598	0.7205
	11	0.5618	0.3385	0 6625
	14	0.5137	0.2311	0.6325
	16	0.6652	0.3906	0.5495
	17	0.6051	0.3610	0.6180
	18	0.6990	0.3981	0.5000
	20	0.7621	0.3548	0.3687
Cluster 2	1	0.4873	0.2060	0.6458
	3	0.4340	0.2197	0.7253
	5	0.5326	0.3257	0.6931
	6	0 6405	0.5085	0.7314
	8	0.4476	0.2818	0.7691
	9	0.5211	0.2681	0.6544
	13	0.5267	0.3455	0.7231
	15	0.5462	0.3316	0.6788
	19	0.5076	0.2401	0.6480
Cluster 3	7	0.6645	0.1541	0.3967
	12	0.6645	0.1898	0.4142

		Cluster summary for 3 clusters			
Cluster	Members	Cluster variation	Variation explained	Proportion explained	Second eigenvalue
1	9	9.00000	5.64174	0.6269	0.71773
2	9	9.00000	4.64371	0.5160	0.91497
3	2	2.00000	1.32892	0.6645	0.67108
Total variation explain = 11.6144					Proportion = 0.580718

Source Refs. 13 and 14.

ences, DA and MRA serve that purpose more admirably. DA is a procedure that depends for its effectiveness on transposing the measurement values in such a way that the linear distance between samples is maximized. There are four kinds of DA: stepwise (SDA), linear or multiple (LDA), canonical, and nearest-neighbor. Normally SDA is used to winnow from the measurements those that are not effective as discriminators, then ferret from among those retained the ones that are most effective. In actuality, computer programs search for the most effective discriminator among the measurements, eg, gas chromatographic peaks, liquid chromatographic peaks, or mechanical measurements. Having selected the best single discriminator, the program then looks for a second measurement to go with the first to form a pair of measurements for discrimination. The process goes on in that fashion until enough measurements have been selected to permit all the samples to be assigned to the proper class, or else the process fails because it runs out of discriminating variables before full resolution of product differences is obtained. Table 5 shows how the process works schematically and Table 6 lists the kind of output obtained. The data are for the classification of a certain industrial produced in four branch plants. The objective was to learn whether the products could be assigned by chemical measurement to the plant where each was actually produced. Sometimes sample differences cannot be resolved by instrumental measurements. Generally where there are many measurements available such as gas chromatographic or liquid chromatographic analysis yields, there is no difficulty in finding sufficient discriminating variables to permit the samples to be correctly classified. When sensory scores for various attributes (such as crumbliness, graininess, sweetness, a sulfidic odor, and a grassy note) are the kinds of quantitative values available, there is generally less success in attaining 100% classification. Sometimes 100% success can be achieved, but usually the maximum is approximately 85%.

LINEAR DISCRIMINANT ANALYSIS

SDA is really a screening operation. The investigator wants to find out which measurements are useful to resolve product differences. Then either the discriminant or the classification coefficients are utilized to form a discriminant function. The form of the equation is

$$Z = \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 + \lambda_4 X_4 - C$$

where the λ's are weighting values, the X's are the measurement values, C is a correction factor, and Z is the weighted mean. The magnitude of the weighted mean determines the class to which the sample is assigned. The correction term is provided (it is a part of the computer output) to enable original (raw) measurement values to be used. As a part of the DA calculation, the measurement

Table 4. Ward's Minimum Variance Cluster Analysis Applied to Five Grades of Tea, Grade Five Being a High-Quality Tea, Grade One Being the Tea Lowest in Quality

```
                                Product
                    1       2       4       3      5
Semipartial R² 0.7 +
                   |
                   |
                   |
                   |
                   |
                   |XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
               0.6 +XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
               0.5 +XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
               0.4 +XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |XXXXXXXXXXXXXXXXXXXXXXXXX      X
               0.3 +XXXXXXXXXXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
               0.2 +X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
               0.1 +X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXXXXXXXX      X
                   |X       XXXXXXXXXXX            X
                   |X       XXXXXXXXXXX            X
                   |X       XXXXXXXXXXX            X
                   |X       XXXXXXXXXXX            X
                   |X                              X
               0   +X                              X
```

Table 5. Illustration of Selecting and Combining Variables to Improve Percent Success in Classifying Products[a]

Step	Gc peak(s) selected	Percent correct jackknifed classification
1	4	63.1
2	4 + 36	68.4
3	4 + 36 + 26	75.9
4	4 + 36 + 26 + 25	88.6
5	4 + 36 + 26 + 25 + 2	95.8
6	4 + 36 + 26 + 25 + 2 + 33	95.8
7	4 + 36 + 26 + 25 + 2 + 33 + 17	100.0

[a] Three onion varieties: yellow and red globe onions and Vidalia (Grano-Granex) onions.

Table 6. Order of Selection and Deletion of Variables to Classify Product Produced in Four Branch Plants[a]

Step	Gc peak		*F*-value	*U*-statistic	Multivariate *F*-value
	Enter	Delete			
1	20		361.90	0.0153	361.90
2	11		134.93	0.0006	219.15
3	10		16.70	0.0001	126.72
4	6		32.02	0.0000	101.73
5	42		24.95	0.0000	87.97
6	3		32.30	0.0000	76 93
7	41		7.35	0.0000	69.08
8	12		8.59	0.0000	62.55
9	18		12.97	0.0000	57.66
10		3			52 54
11	2		3.51	0.0000	49.79

[a] *F*-value = univariate *F*-value for that peak; *U*-statistic is a measure of the error, ie, the degree to which the classification is approaching 100% correctness, values rounded off to fourth decimal, multivariate *F*-value = *F*-value for values as combined.

values are standardized. The correction constant adjusts for that fact so that the raw, ie, nonstandardized, values can be inserted in the equation.

SDA was used to learn whether instrumental measurements could be used as a purchasing specification for an herb bought from five different countries (21). It was demonstrated that the chemical means was quite effective at distinguishing the same kind of an herb grown in five different geographical countries of the world. At intervals, the chemical means need to be revalidated by matching the instrumental output with sensory judgments in case growers have made some change in their growing practices or in the way the product is stored or shipped following harvesting, or for other reasons. However, if the equation is established with sufficient replication of measurements and samples to start with, it is usually quite robust.

The opposite approach has also been used (22). Chemical analysis has been used in an attempt to standardize more closely batches of distillers' spirits. It was decided that sensory evaluation coupled with LDA was a more suitable way to maintain a high level of quality control. The procedure is a more direct means of ascertaining sensory quality than going through chemical analysis, which in turn must be correlated with sensory quality. It is less suitable for high-speed lines or for plants that run 16 or 24 h/day. A disadvantage to using sensory panelists is that if a panelist leaves the panel, the SDA process and development of a LDA equation should be recalculated to be sure the replacement panelist has not caused a change in the weighting values. Recalculation, however, is not a time-consuming operation. In fact, if the original panelists were selected carefully to be sure they were in good agreement in their responses and if records of performance have been kept (as they should be), replacing a panelist is no more a touchy subject than it is for replacement of any panelist. A comparison of the new panelist's performance with the prior history of the panel should tell the sensory analyst whether the panel mean is likely to be influenced.

MULTIPLE REGRESSION ANALYSIS

MRA is used similarly to LDA. The chief difference is that MRA is appropriate for applications where the dependent variable is along a continuum and the measurement values are continua, whereas LDA is suitable for assigning a sample to a disjoint set of categories, ie, classes. In the typical regression application, flavor intensity or something that progressively changes is predicted from several measurement values that themselves can be any value along continua. The item being predicted is called the dependent value; the predictors are the independent values. A multiple regression equation takes the form:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + e$$

where Y is the value of the dependent variable; a is the intercept of the regression line; the b's are the slopes, ie, the weighting values attached to each predicting variable; e is residual error; and the X's are the measurement values. Stepwise regression analysis (SRA) can be applied just as can SDA. The difference is that an estimate is given for the intercept and for the b values (the weighting value) for regression of each of the independent values as they are added to the total number of independent regressors included in the equation.

Premises to Use of SDA or MRA

The MVA procedures above are risky ventures unless relations among the variables are based on a firm foundation of replication of measurement and the initial set of samples is truly representative of those likely to be encountered in the future. If there is not adequate sampling and replication of measurements, there is the risk that relations that appear to exist may be spurious. The usual statistical risk is set at a probability level of 0.05. When there are 7 or 10 or 20 measurement values, each one of which will fluctuate some from determination to determination, there is no practical way to calculate the total risk involved in making a wrong decision. The only ways to overcome the uncertainty are to carry on determinations long enough in time, to evaluate a large number of samples, and to choose the sources of the samples carefully to be fairly sure of having selected a fair representation of nature's variability. Although on first use an equation may appear to be a good predictor of the class to which a product belongs or the degree of some quality it has, it should be looked at with suspicion. Some industrial firms that have replaced routine sensory evaluation with instrumental analysis conducted sensory and instrumental analyses side by side for five years to be sure that the first indications of effectiveness were not just a case of good luck at the first try. That kind of effort is not wasted. As data accumulates, the predicting equation can be refined to make it even more applicable and robust than it probably was originally. It has been pointed out that the process of validating an equation by accumulating more and more data is much like the building up of a delta (23). Eventually through accretion, the shifting sands of random happenings become firm earth on which one can build with confidence. The caveat is to be cautious at first, no matter how good an equation seems to be, until it has proved itself on repeated occasions.

There are some features of MVA that the user should be aware of. Novices to the use of SDA and SRA are sometimes disconcerted because the process of selecting good discriminators appears to be overlooking some that are good. The step process bypasses a measurement that by itself is moderately effective as a discriminator in favor of one less of a discriminator by itself, because the computer program is looking for a discriminator not correlated with one already selected. If discriminating measurements are correlated, use of both of them adds little to the discrimination. All that is being provided is redundant information. Table 7 provides an illustration of bypassing measurements that have a high F-value, ie, are effective discriminators, in favor of measurements that are not correlated to one already selected. A noncorrelated variable is adding more to the discrimination needed than would one merely providing redundant information. Note from Table 7 that often a term not appearing to be particularly discriminating by itself is selected ahead of terms appearing to be better discriminators because the one selected in conjunction with the terms already selected adds more to discrimination than its betters would have. Sometimes a

Table 7. Illustration of Order Variables Selected to Avoid Covariation[a]

Gc peak	*F*-value	Order selected	Gc peak	*F*-value	Order selected
12	175.8	1	16	25.4	
39	139.2		6	23.2	4
5	96.8		32	22.3	
38	94.9	2	21	19.5	
46	70.7		4	18.6	
9	69.2		26	17.4	
48	67.4		34	14.4	
22	60.9	5	43	12.4	10
36	60.3	9	37	11.8	
28	56.2		17	11.4	
30	49.7	8	45	9.7	
2	37 2		8	9.0	
33	36.7		11	7.7	
25	34.3		7	6.8	
29	30.9	6	44	6.7	
14	30.3		7	5.2	3
10	30.0		3	4.2	7
31	26.9				

[a] *F*-value specified to enter or delete variable, <4.0. *F*-value is univariable *F*-value.

good term, once selected, may be later eliminated (Table 6) because in combination with the set of terms then at play, the term doing the replacing steps up the discriminating power more than the term selected earlier did.

Table 8 shows for SRA the same sort of thing as described above. At the fourth step, the computer had to go over possible combinations again and again to find the combination that yielded the highest coefficient of determination. In this instance the gain in effectiveness of prediction is so slight that most investigators would decide to go with a three- or four-term equation rather than one having a larger number of terms.

In some cases, investigators may not select the most efficient LDA or MRA equation because while there is one best equation there usually is a whole tier of second-best equations. A user of LDA or MRA may decide that one of the tests among the combination yielding the most efficient equation is too lengthy or too expensive to run, that a slight sacrifice in efficiency is merited in exchange for a more speedy or less costly test. In one example, an attempt was made to determine which compounds in oil of onion were the essential ones to an oil of onion flavor so that a synthetic product could be formulated (24). The most effective equation generated did not include compounds that the literature and the experience of the investigators indicated were compounds likely to be essential to onion flavor. The ones not selected were colinearly related (see the discussion above concerning correlated versus orthogonal terms) to the ones selected; thus both would not be included in the same equation. The researchers, therefore, forced into the equation the gas chromatographic peaks they thought were critical to authenticity of onion flavor. This goes back to the statement made in the opening paragraph that a chemical test may be precise but miss the target because it is not measuring a key sensory quality. Computer programs are blind. They will select the most efficient equation. Sensory technologists and chemists sometimes must interpose their judgments into the process to obtain a formula that fits the task. In fact, in every case the analyst should examine the variables selected to be sure to have an equation that preferably is related to sensory quality.

Table 8. R^2 Improvement and F-Value Decline as Variables are Added, Stepwise Regression

Step	Variable added	Variable deleted	R^2	Regression F-value
1	GC5		0.954	458.60
2	GC16		0.969	326.36
3	GC9		0.975	256.28
4	GC38		0.978	209.93
4	GC33	GC9	0.979	244.66
4	GC12	GC16	0.979	244.88
4	GC7	GC5	0.981	247.92
4	GC26	GC33	0.983	276.43
4	GC30	GC38	0.984	296.00
5	GC44		0.986	245.02
6	GC9		0.987	213.13
20[a]			0.994	25.11

Note: Product H with various amounts of ingredients added; dependent variable is intensity of flavor

[a] Beyond 20 variables, the SAS stepwise regression program became quite costly (52 variables available here). Normally, a limitation on the number of steps would be specified. Actually, Step 3 was the last step where all the variables entered had probabilities < 0.05.

There is a parallel to the matter of accepting a secondary equation. Sometimes an equation extending over the whole range of samples fails to discriminate well somewhere along the range. By calculating an equation for the part of the range giving trouble, the second equation permits the samples to then be readily separated. An illustration of that procedure to make product resolution tractable has been given (25). Being able to do that is one of the benefits of having analyzed sufficient samples to know where the problems are and having the information at hand to overcome the problem.

PARTIAL LEAST SQUARES REGRESSION

A procedure that has come to the fore in recent years is partial least squares regression analysis (PLS). The calculation and functioning have been described (26). Assume one has two blocks of variables: sensory and instrumental. PSC searches for a few latent orthogonal variables (factors) to describe the interrelationships between the two blocks of variables (27). It extracts from the regressor matrix (ie, the instrumental block), those factors relevant to prediction of the sensory parameters of the food. The procedure may be looked upon as a combination of PCA and multiple linear regression performed simultaneously on the latent variables. Several research publications are available that detail applications in sensory-instrumental correlation (26,27).

RESPONSE SURFACE METHODOLOGY

The response surface method (RSM) will be mentioned at this point, too, because it is another of the regression methods. The basic idea underlying RSM is to find the combination of several ingredients that lead to optimal sensory quality. An elementary description of the procedures for setting up a response surface trial and of analyzing the results have been given (28). Other treatises have been published (29,30). Sometimes the resulting configuration is not in the form of a mountain peak. The area of optimal performance may be a ridge. At other times a saddle formation is evident. RSM is highly effective in permitting reduction of the number of trials that must be carried out; unfortunately, for sensory purposes its application is generally limited to a few variables only, because otherwise the amount of sensory testing would be beyond the capability of most laboratories to handle the enormous number of samples that would have to be evaluated at any one session. Figures 5, 6, and 7 illustrate some of the kinds of response surfaces that can result (31).

PROCRUSTES ANALYSIS

Like PLS, generalized Procrustes analysis (GPA) has come to play a major role in the relating of one set of variables

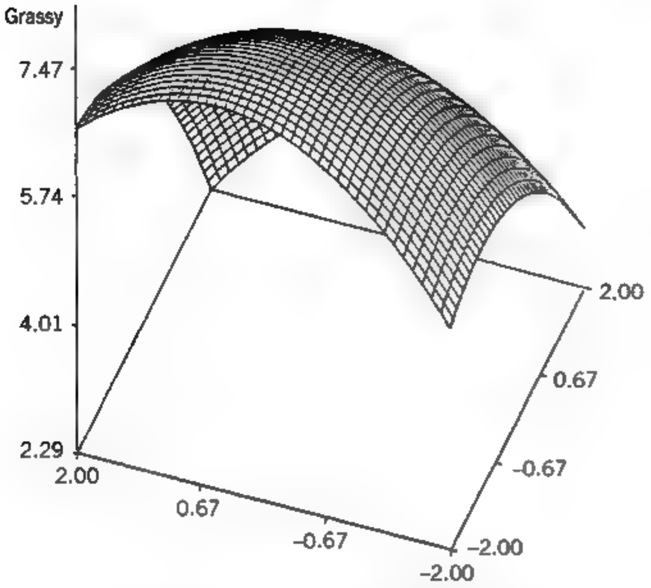


Figure 5. Illustration of response surface showing a peak. The vertical axis shows sensory evaluation for a grassy note in tea as influenced by varying amounts of linalool (abscissa) and epicatechin (ordinate).

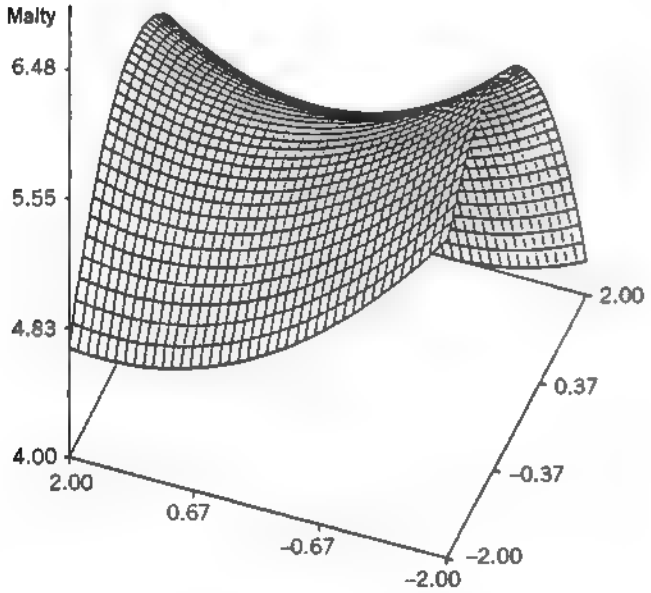


Figure 6. Illustration of a saddle formation, response surface analysis The vertical axis shows degree of maltiness imparted to tea on addition of varying amounts of epicatechin (abscissa) and 1-penten-3-ol (ordinate).

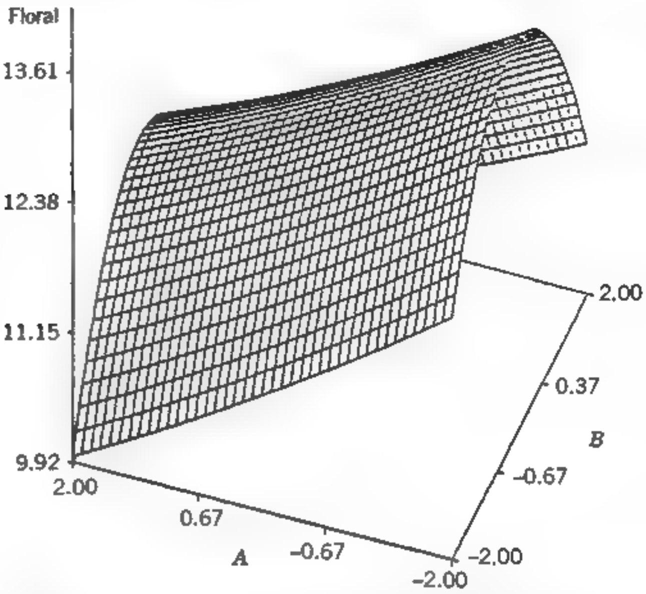


Figure 7. Illustration of ridge formation, response surface analysis. The vertical axis shows the degree of a floral note imparted to tea after being treated with varying amounts of 1-penten-3-ol (abscissa) and *cis*-3-hexen-1-ol (ordinate).

to another. Dijksterhuis (32) has explained the principles and techniques underlying GPA. GPA has especial application when consumers are allowed to use "free-choice profiling" to describe their perceptions of the qualities of a set of foods (33,34). Trained panelists are normally asked to rate the intensities of an established set of terms. There are good reasons for not training consumers to use a restricted set of terms. In free-choice profiling, the panelists are free to use whatever terms seem appropriate to them to describe the sensations they are perceiving. A consequence is that the configuration of terms and intensities applied to the sensation almost always differ for each panelist. GPA involves rescaling of the intensity values and rotation of the configurations to bring them as closely as possible into alignment with each other. The investigator thereby secures a consensus configuration of the terms and of the relationships of the products to each other. The process derives its name from the mythical innkeeper Procrustes, who made all his guests exactly fit the beds. If the guest were longer than the bed, Procrustes cut off the guest's legs at the appropriate place, if the guest were smaller than the bed, Procrustes used a rack to stretch the unwary guest. Kaye (35) commented that "in a procrustean approach to a theory, the scientist stretches and pulverizes any data until it [they] fits the preconceived ideas." That is not a valid criticism applied to free-choice profiling. The investigator might have a preconceived notion as to the pattern of the consensus configuration, but free-choice profiling hardly favors much in the way of a firm idea of the pattern to be evolved. Others, in only two publications that will be cited (36,37), have commented upon or reported comparisons between GPA and other multivariate forms of analysis.

MULTIVARIATE ANALYSIS OF VARIANCE

Multivariate analysis of variance (MANOVA) is another of the MVA procedures of use in analyzing multicomponent data. It is akin to analysis of variance (ANOVA). In ANOVA, the total sum of squared deviations about the grand mean, SS (total), is partitioned into a sum of squares due to one or more sources and a residual sum of squares. In MANOVA, the p-dimensional multivariate analysis is based on the same design, there are pSS (total)'s to partition, one for each component measured. In addition, there are the measures of covariance between pairs of components presented as sums of products. MANOVA is concerned with partitioning of both of these measures, the variance and covariance, collected in a matrix of sums of squares and of products. The result is that MANOVA yields a global estimate as to whether there are any significant differences among the different variables or their correlations. MANOVA can be separated into ANOVAs yielding the same information. The number of ANOVA calculations required is $p(p + 1)/2$. To illustrate, if there are seven dimensions, 28 ANOVAs would yield the same information as the one MANOVA.

The effect of the above is that ANOVA for one type of measurement acts as if the others did not exist whereas MANOVA takes all of the different kinds of measurements made into account in one operation. The global aspect means that if the multivariate F-value is not significant, there is not a significant difference anywhere among the variables. If the multivariate F-value is significant, there is statistical significance somewhere. For most purposes, the analyst then must apply other tests to ascertain where significance lies.

A use of MANOVA that has value in sensory analysis is to ascertain just how effective a judge has been at performing several tasks. When a prospective sensory judge has been trained to assign scores to several different attributes of the sensory material, there is a problem at the end of the training period to decide just how effective the judge should be for the various tests. Must each judge be significant for each one of the attributes examined, or must the sensory leader be willing to accept the fact that not every judge can be statistically significant for each of the tasks. The usual way is to look at all the probability values that go with the F-test (ANOVA) for each of the tasks, then make a judgment based on a composite opinion of overall performance. MANOVA puts a little objectivity into the process. The thing to do is to calculate the multivariate F-value; from it, some of the tediousness of examining a whole matrix of F-values or probability values for each judge can be eliminated. If a judge exhibits a multivariate F that has a high degree of probability, it is almost certainly possible to eliminate that judge as a suitable candidate for appointment to the sensory panel. If the probability is very low, the judge should be retained. Only for judges intermediate in performance need the sensory leader go back to the ANOVA matrix to make the final decision. Perhaps the judge is a poor performer for only one attribute in which case the leader might decide to accept the judge in spite of a questionable multivariate F-value. If the ANOVA documents that performance has been spotty across the board, the sensory leader more likely will reject the judge. Illustrations of the joint use of the frequency of significant probability according to ANOVA calculations and by MANOVA calculation have been given (15,38,39). MANOVA's merit is chiefly one of telling the analyst to stop at a particular point, because significance doesn't exist, or to go on, because significance does exist, but other methods of analysis must be applied to determine where or how often.

FUZZY LOGIC

Fuzzy logic will barely be mentioned, for its use in the sensory field is not as common as the other methods described here. The theory behind fuzzy logic is that uncertain phenomena can be treated mathematically to offset ambiguous or conflicting information to yield instead a yes-or-no answer to allow the best product, choice of ingredients, or process method to be selected (40).

NEURAL NETWORKS

Neural networks (NN) involves making decisions by learning the results of past performance. In that regard, NN is not much different from most of the methods discussed previously where experimentation is used to yield a data set from which, in turn, a predicting equation may be derived for future application. Although, as was pointed out earlier, instrumental approaches to relating sensory-to-instrumental data are often blind in the sense that the chemical/physical predictor(s) chosen may not be at all related to sensory quality, that risk is even greater for NN. NN consists of three parts: input (measurement) variables, hidden interrelations, and output variables (41,42). The output values are the result of examination of the thousands of permutations arising from even a moderate number of different measurement values. The process is spoken of as "training." By means of 10,000 or 100,000 iterations—or even more—interrelations among the hidden values are repeatedly evaluated until a set is found that correlates with the known differences in the test samples, Based on evaluation of numerous test samples, output values are eventually found that allow categorization of an unknown to possible classes, ie, the best product, a product matching the traditional product, one suffering from flaws such as off-flavor, or some one of the sensory notes being so dominant as to be objectionable. Once the necessary output value(s) have been obtained, NN may then be used for routine evaluation such as in quality control.

CANONICAL ANALYSIS

Canonical analysis is the counterpart of simple correlation analysis. In simple correlation two things increase jointly, decrease jointly, or one increases as the other decreases. Whatever they do, they stay in step with each other. Canonical correlation works the same way. The difference is that in canonical correlation sets of variables are correlated with each other instead of individual items. Many pairs of correlates may exist among the different variables. The intent is to find the set with maximum linear correlation. The canonical variates are derived in essentially the same manner as principal components. In PCA the intent is to account for as much of the variance as possible within one set. In canonical correlation, the intent is to account for the maximum amount of correlation between sets. The analysis process searches out the first canonical variate from the first set and the first from the second set that are maximally correlated with each other. It then does the same for the second set and all that follow. Like simple correlation, the two first correlates may be plotted against each other; so too may the second set, and any others that exist. Like PCA, only the first few correlates may be of interest. Correlation may be between sets of sensory measurements, sets of instrumental measurements, or more commonly between sensory and instrumental measurements. Canonical analysis has some similarity to MRA. In MRA the dependent variable is singular whereas the independent variables are multiple; in canonical correlation, both sets are multiple.

CANONICAL DISCRIMINANT ANALYSIS

A form of analysis that is particularly valuable is canonical DA. Table 9 shows canonical DA applied to the five kinds of rye bread referred to earlier. The values above the diagonal give the interspatial distance. The values below the line give the multivariate *F*-value. All are significant. If some are significant and some not significant, it is more informative in that case to give the probability values that go with the *F*-values. When all are significant, differences between products are easier to discern from the *F*-values themselves. In determining interspatial relations, canonical DA is preferable to PCA because canonical analysis allows the setting of statistical limits.

CORRESPONDENCE ANALYSIS

Correspondence analysis is a procedure for the analysis of contingency tables. The cells of the table contain frequencies or concentrations. They are given unit weight by dividing by the grand sum. The contingency table is essentially a two-way classification where neither the rows nor columns need be regarded as the variables and measurements. Both are commonly called elements. To permit plotting of both sets of elements on one plot, a fully symmetrical treatment of the data is employed. That can be done by calculating a χ^2 distance as the similarity measure for both rows and columns. Once that is done, PCA and principal coordinate analysis, another name for MDS, are applied to yield a factor analysis in terms of the χ^2 profile measurements. A short description of correspondence analysis with two simple illustrations has been presented (43). There have been extensive publications on correspondence analysis (44); it is a method of analysis particularly favored in France. A book has also been published on the subject (45).

Table 9. Canonical Discriminant Analysis Applied to Five Types of Finnish Wholemeal Sour Rye Bread

	Products				
Products	1	2	3	4	5
1		1.11	4.50	1.11	1.80
2	3.91		4.23	1.16	1.60
3	59.67	52.21		4.25	3.31
4	3.73	4.00	56.17		1.50
5	9.17	7.56	33.50	6.81	

Note: Values above the diagonal line show the Mahalanobis distances between products; values below the diagonal line list the multivariate *F*-values (probability < 0.0001). Evaluation was by 20 panelists for 12 attributes in four replications.

FACTOR ANALYSIS

Factor analysis (FA) has been left to the last because it is a method about which an unusual amount of controversy swirls. Some statisticians look on all the internal analysis methods with some suspicion on the grounds that the results are high provincial simply because only one data set is involved. Others frown on FA particularly because of indeterminacy in factor solutions (19). There is no doubt that different solutions can be obtained according to the particular method of factor analysis employed. Notwithstanding such criticisms, FA is a procedure that is used more and more in sensory–instrumental analysis. FA is different from PCA in that PCA is concerned with variance only. FA requires a statistical model and is concerned with explaining the covariance structure as well as variance. Unlike PCA where there is no particular assumption about possible underlying structure among the variables, FA is based on the faith that the observed correlations are mainly the result of some underlying regularity in the data. The various factoring techniques initially extract common factors, but generally they are uninterpretable. To enhance interpretability, there are several models for rotation and determination of the number of factors. The ideal situation is to have a variable highly loaded on one factor and scarcely loaded on the others.

There are three kinds of matrices involved in FA. One is the factor pattern. It provides the weights to estimate variables from the factors. A second one is the factor score. That matrix provides weights to estimate factors from the variables. The third kind of a matrix is the factor structure. It gives the correlations between the factors and the variables. The pattern matrix delineates more clearly the groupings or clustering of variables than the structure matrix.

If two rotations give rise to different relationships, the two interpretations should not be looked on as conflicting. They are two different ways of looking at the same thing, ie, they are two different points of view in the common-factor space. The basic impetus to employing any rotational method is the same: somehow to achieve simpler and theoretically more meaningful factor patterns. Orthogonal factors are mathematically simpler to handle; oblique factors are empirically more realistic.

Although FA is frowned on by some statisticians, some of their reasons are highly suspect because provinciality does not apply to many sensory–instrumental data sets. Most of the applications of FA in the past dealt with such things as determining people's opinion or some similar data where there was no opportunity to replicate responses. Sensory panel results are different. Often there is replication; thus the analyst has the opportunity to determine whether the panelist is responding randomly or consistently for the same sample given at different times. The objection to FA that it is likely to be provincial may still apply to the factoring of consumer responses, because in most instances there is no replication. If, however, the survey is large enough, that objection loses some of its validity.

Whether or not replication is involved, there is one phase of FA that has been overlooked by most investigators. As is true for all methods of analysis where a mean is calculated, the following question should be asked. Is the mean merely the average of a group of disparate responses or do the responses tend to agree with each other? To illustrate, if a particular perfume receives scores of 7, 7, 6, 8, 8, 6, and 6 out of a possible score of 10, or an average score of 6.86, and another perfume receives scores of 10, 10, 4, 5, 10, 6, and 3, or an average score of 6.86, the conclusion should be entirely different although the means are the same. The first perfume is likely to have general acceptance whereas the second will appeal to some and be scorned by others. In the same fashion, rather than just generate a factor pattern for the whole sensory panel and assume that it represents the pattern for all members of the panel, the patterns yielded by the different panelists should be examined to learn if they agree in general or the pattern for the panel is merely the mean for a group of disparate patterns. Rarely, have factor patterns been examined in that light. Early studies began to look at the homogeneity of the factor patterns of a panel in that manner. One study visually examined the eigenvalues to learn if they were similar (46). Another study applied statistical tests to learn objectively how well they agree (38). Later other studies (13,47,48) refined the process even more with the result that today factor patterns should be examined as most other sensory–instrumental data are, to learn if the individual patterns are quite homogeneous or if they are heterogeneous. If they are heterogeneous, then, of course, considerably less credence should be put into their value and representativeness of how things really are. Figure 8 shows the extent of agreement between a panel of 18 and a subset of 8 from the same panel. Often it is difficult to compare the pattern for an individual member versus the panel because of lack of degrees of freedom to carry on the analysis for the individual's responses. To apply some of the FA methods there must be at least limited pooling of responses to provide sufficient degrees of freedom to enable a comparison to be made. Figure 9, which is a little easier to interpret than Figure 8, shows one comparison (45). While a little harder to visualize the agreement, Figure 8 is more realistic of the pattern that exists. Note that the cube standing for total flavor fits in factor 1 or 2 as does sour flavor. That is as it should be. Pointed out in the beginning is the fact that an attribute may be partly in one factor and partly in another, because FA involves both variance and covariance. The same sort of standing in one factor with one foot and in another with the other foot applies to chemical data as well. A chemical may have more than one type of functional group; consequently its covariance may be shared by two different factors reflecting the two kinds of properties caused by different functional groups. A major use both of PCA and FA is to discern redundancies in the choice of descriptive terms. Table 10 shows a factor that was derived in one study. Panelists evaluated canned and frozen green beans for 27 attributes. The seven terms comprising Factor 4 reveal that the panelists had been put to a lot of unnecessary work. In theory, any one of the 7 terms could substitute for all the others. In practice, a sensory technologists might not want to go that far and might still want to use three or four different terms in case some panelists are more adept at distinguishing among products based on one descriptor whereas a different one is more meaningful to other panelists. It can readily be understood that crispness and tenderness are the complements of each other, but it is not as clear that slimy, juicy, and soggy represent nearly the same characteristic.

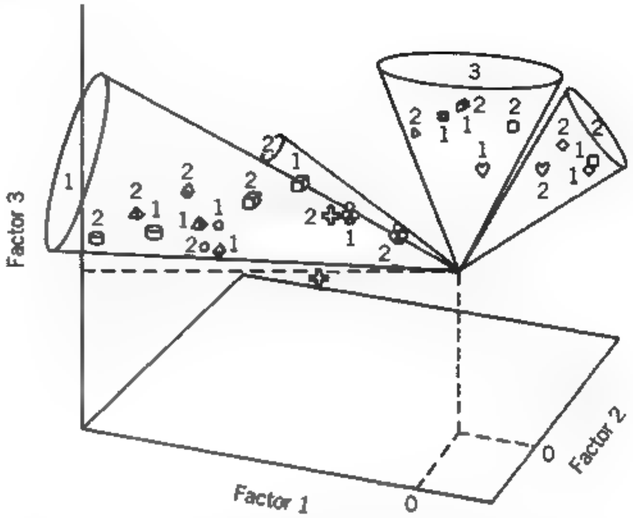


Figure 8. Comparison of factor patterns exhibited by a panel of 18 and a subset consisting of 8 of the panel members. The numbers in the opening of each cone stand for the factor pattern, the symbols within a cone represent the different attributes evaluated. pyramid = rye flavor; spade = rye aroma; circle = total aroma; cube = total flavor; cross = sour aroma; club symbol = sour flavor; triangle = sweet aroma; flower = sweet flavor; heart = saltiness; diamond = bitter flavor; square = flour flavor; cylinder = desirability.

WHITHER SENSORY–INSTRUMENTAL CORRELATION?

The tools to carry on sensory–instrumental analysis are pretty much in place. Some industrial firms have made good use of the tools available by working out for themselves beneficial applications involving the complementary uses of sensory and instrumental analysis. There has been little full substitution of instrumental methods for sensory ones because chemical and other analytical methods themselves are not complete. There are instances of as many as 400 or more peaks being discernible on gas chromatograms, but that number often falls short of the number of compounds comprising the sensory character of a food product; thus no chemical analysis is probably ever really

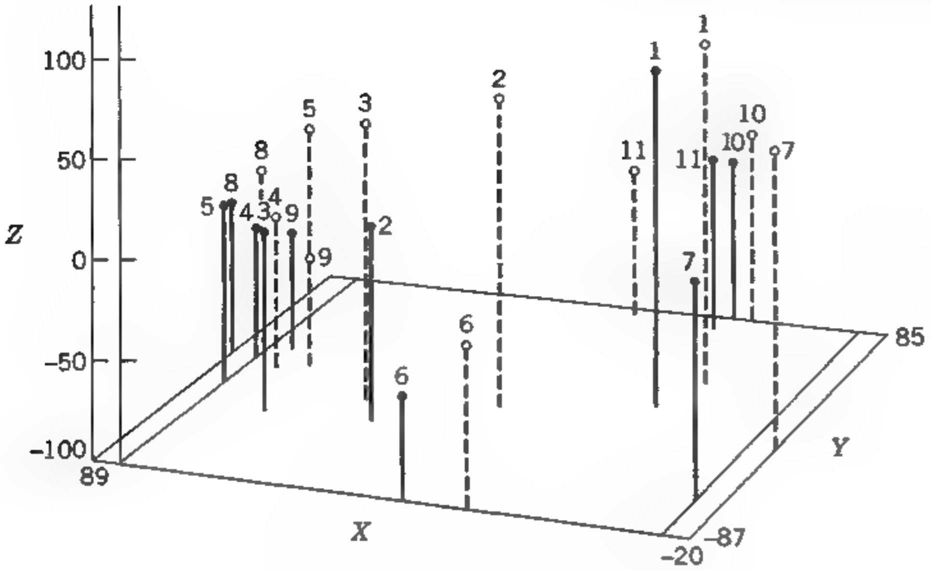


Figure 9. Comparison of factor patterns: ○, panel results pooled; ●, two of the panelists.

Table 10. Texture Factor Pattern for Canned, Frozen, and Fresh Green Beans as Judged by 21 Panelists, Four Replications (Factor Pattern Derived from among 21 Attributes Evaluated)

Term	Correlation with factor
Coarse	0.57
Fibrous	0.57
Crisp	0.53
Slimy	−0.53
Juicy	−0.53
Soggy	−0.63
Tender	−0.66

exhaustive of all the compounds in a food. More important, no chemical analysis reveals the exact pattern of the chemicals contained within a food or most other sensory materials. Not every compound is detected to the same extent by gas chromatography or liquid chromatography. Thus the exact ratios between compounds is often not known. In concentrating materials for analysis so as to bring the concentrations within the range of the instrument, the ratios among compounds are initially changed because of effects of vapor pressure or solubility in the extracting medium. The interrelations of factors affecting texture present even greater difficulties. Characteristics such as gumminess and the hardness of embedded particles need not be related to each other, thus it is not possible to speak of total texture in the same way as it is possible to speak of total flavor. There are some difficulties in measuring color, but on the whole color can be more reliably expressed physically than is true for the other sense modalities. Relations between acoustical properties and sensory characteristics have been extensively studied (49). The point of mentioning each of the senses is that a food or a perfume or the odor of a barnyard is usually judged by more than one sense. That is true even for the seemingly simple response to the odor of a barnyard. It is thought that the sensation perceived is odor, but actually generally more than odor is involved. Some of the volatile substances reaching the nose are compounds possessing trigeminal characteristics causing a stinging sensation or even pain. Considering all that is not known, it is remarkable that sensory–instrumental applications have been as useful as they are.

To make correlation methods even better, it is necessary to know more about the sensory receptors and how the brain processes information. Humans and other animals probably utilize a process somewhat like PCA. Hundreds of sensory signals are detected and these sensory signals must be combined in various ways to form patterns of varying complexity; in other words, data reduction is carried out. Like PCA components, some of the patterns may encompass signals from only a dozen or so sensory notes, other may encompass several dozen. The point is that humans and other animals are responding to several patterns representing different kinds of sensations and patterns of varying size, then somehow this semiprocessed information is ultimately taken and put together as a composite sensation that tells the mind that this food is delectable, that food is almost without taste, and that food has a trace of cinnamon in it. In the brain a whole series of complex interaction effects have taken place. They are not the chemical interactions a chemist thinks of, a reaction between this physical substance and that. They are chimera, not amenable to understanding even if all the kinds of chemical reactions that could occur among the hundreds of chemicals comprising flavor were known, because they are perceptive reactions not physical ones.

Before sensory–instrumental correlation can reach the ultimate in applicability, knowledge of sensing, brain processing of information, and all the other sciences (physiology, psychology, biochemistry, and anatomy) that impinge on the reception, the processing, and the analyzing of sensory information must be greatly expanded. At present, statistical methods are adequate. There must be enlistment of a greater number of statisticians who have an interest in sensory research and are willing to work around some of the limitations that sensory analysis imposes. That which is ideal is often not practical in sensory analysis, such as the evaluation of a large number of samples all at one session. For the time being, present-day computers can handle most of the data sets presented to them, but the day will come when computing demands will be far greater than they are now. If perceptual interactions are begun to be understood, far more will be demanded of computing capacity and complexity than presently exists. Chemical measurement of food components must be made to be "true" to avoid giving a distorted picture of the relations among the hundreds of compounds comprising foods and other natural substances of sensory importance. The characteristics of compounds that make them be sensory substances must be learned. There is fair knowledge of the functional grouping that must exist for substances to be sweet, the properties of chemicals that make them be sour or salty are known with even more certainty, but by and large it is not really known why this compound has the property of being a sensory substance and that one does not. It is necessary know as much about the laws that govern sensory properties as does a synthetic chemist who is seeking to synthesize a new compound. From knowledge of the properties of chemicals, their functional groupings, and possible pathways to foster reactions otherwise particularly difficult to make occur, the chemist can predict in advance the likelihood of success in synthesizing a new compound. Chemists, once they know the identity of a compound, have at their fingertips, or at least readily ascertainable, a whole series of other information about properties of that substance. Sensory properties are not hard facts like vapor pressure and molecular weight. They must be described in terms of perception. Their description is mutable according to who is doing the describing. The description should be an immutable property, not merely a perception. Notwithstanding as important as are all these other considerations, there is nothing to stop the far wider use of multivariate analysis of sensory–instrumental relations. There must be a will on the part of practitioners to stay at the task for some time to avoid having their results be parochial because of acquiring insufficient data. Once sufficient data has been secured, to be sure the results represent real relations, sensory–instrumental correlations can be of great practical importance for present industrial operations such as ensuring ingredients purchased meet the company's buying specifications or the finished product meets its quality specifications for marketing. Of greater importance, correlations so determined can serve as a base for the advancement of fundamental knowledge. From such correlations will ultimately arise an understanding of cause-and-effect relations. Still more important, they in turn will provide a good share of the base of knowledge required to advance comprehension of the detection of sensory signals, their analysis and data processing within the brain.

BIBLIOGRAPHY

1. A. T. James, A. J. F. Martin and G. H. Smith, "Gas Liquid Partition Chromatography. The Separation and Microestimation of Ammonia and Methylamines," *Biochemistry Journal* **52**, 238–247 (1952).
2. S. E. Cairncross and L. B. Sjostrom, "Flavor Profiles—A New Approach to Flavour Problems. Annual Meeting, Institute of Food Technologists (1949)." *Food Technology* 4, 308–311 (1950).
3. J. Powers, "Descriptive Methods of Analysis," in J. R. Piggott, ed., *Sensory Analysis of Foods*, 2nd ed , Elsevier Applied Science Publishers, Barking, UK, 1988.
4. H. Stone, J. L. Sidel, S. Oliver, A. Woolsey, and R. C. Singleton, "Sensory Evaluation by Quantitative Descriptive Analysis," *Food Technology* **28**, 24–34 (1974).
5. J. J. Powers and E. S. Keith, "Evaluation of the Flavour of Foods from Gas Chromatographic Data," in *Abstracts of Papers*, Second International Congress of Food Science and Technology, Warsaw, 1966.
6. J. J. Powers and E. S. Keith, "Stepwise Discriminant Analysis of Gas Chromatographic Data," *Journal of Food Science* **33**, 207–213 (1968).
7. H. W. Dodo, "Sensory and Chemical Attributes of Three Varieties of Onions (*Allium cepa*)," Master of Science thesis, University of Georgia, Athens, 1985.
8. J. R. Piggott and S. P. Jardine, "Descriptive Sensory Analysis of Whisky Flavour," *Journal of the Inst. Brew.* **85**, 82–85 (1979).
9. U. Hellemann, J. J. Powers, H. Salovaara, K. Shinholser, and M. Ellila, "Relation of Sensory Sourness to Chemically Analyzed Acidity in Wholemeal Sour Rye Bread," *Journal of Sensory Studies* **3**, 95–111 (1988).
10. K. Pearson, "On Lines and Planes of Closest Fit to Systems of Points in Space," *Phil. Mag.* **6**, 559–572 (1901).
11. H. Hotelling, "Analysis of a Complex of Statistical Variables into Principal Components," *Journal of Educational Psychology* **24**, 417–441, 498–520 (1933).
12. J. J. Powers, S. Cenciarelli, and K. Shinholser, "El uso de programas estadisticos generales en la evaluacion de los resultados sensoriales," *Rev. Agroquim. Technol. Aliment.* **24**, 469–484 (1984).
13. K. Shinholser, U. Hellerman, H. Salvaara, M. Ellila, and J. J. Powers, "Factor Patterns Yielded by Subsets of Panelists Examining Finnish Sour Rye Bread," *Journal of Sensory Studies* **2**, 119–213 (1987).
14. *SAS User's Guide: Statistics*, Version 5 ed., SAS Institute Inc., Cary, N.C., 1985.
15 J. J. Powers, "Uses of Multivariate Methods in Screening and Training Sensory Panelists," *Food Technology* **42**, 123–127 (1988).
16. I. K. Hwang Choi, "A Case Study of the Use of Standard Methods to Analyze Multivariate Data," Ph.D. dissertation, University of Georgia, Athens, 1982.
17. S. L. Bieber and D. V. Smith, "Multivariate Analysis of Sensory Data: A Comparison of Methods," *Chemical Senses* **11**, 19–47 (1986).
18. *SPSS (Statistical Package for the Social Sciences)*, 2nd ed., SPSS, Inc., Chicago, Ill., 1986.
19. C. Chatfield and A. J. Collins, *Introduction to Multivariate Analysis*, Chapman and Hall, London, 1980.
20. S. S. Schiffman, M. L. Reynolds, and F W. Young, *Introduction to Multidimensional Scaling, Theory, Methods and Applications*, Academic Press, Inc., Orlando, Fla., 1981.
21. M. Gillette, "Applications of Descriptive Analysis," *Journal of Food Protection* **47**, 403–409 (1984).

22. G. D Wilkin, M. A. Weber, and E. A. Lafferty, "Appraisal of Industrial Continuous Still Products," in J. R. Piggott, ed., *Flavour of Distilled Beverages*, Ellis Horwood Ltd., Chichester, UK, 1983.

23. J J Powers and G. O. Ware, "Discriminant Analysis," in J. R. Piggott, ed., *Statistical Procedures in Food Research*, Elsevier Applied Science Publishers Ltd., Barking, UK, 1986.

24. W. G. Galetto and A. Bednarczyk, "Relative Flavour Contribution of Individual Volatile Components of the Oil of Onion (*Allium cepa*)," *Journal of Food Science* **40**, 1165–1167 (1976).

25. J. J. Powers, "Techniques of Analysis of Flavours: Integration of Sensory and Instrumental Methods," in I. D. Morton and A. J. MacLeod, eds., *Food Flavors, Part A. Introduction*, Elsevier, Amsterdam, The Netherlands, 1982.

26 M. Martens and H. Martens, "Partial Least Squares Regression," in J. R. Piggott, ed., *Statistical Procedures in Food Research*, Elsevier Applied Science Publishers Ltd., Barking, UK, 1986.

27. M. Martens and Eeke van der Burg, "Relating Sensory and Instrumental Data from Vegetables Using Different Multivariate Techniques," in J. Adda, ed., *Progress in Flavour Research*, Elsevier, Amsterdam, The Netherlands, 1985, pp. 131–148.

28. M. E. Wells, "Response Surface Methodology and Subjective Data," in J. J. Powers and H. R. Moskowitz, eds., *Correlating Sensory Objective Measurements–New Methods for Answering Old Problems*, Publication STP 594, American Society for Testing and Materials, Philadelphia, 1976.

[illegible] G. E. P. Box, W. G. Hunter, and J. S. Hunter, *Statistics for Experimenters—An Introduction to Design, Data Analysis and Model Building*, John Wiley & Sons, Inc., New York, 1978.

30. L. Vuataz, "Response Surface Methods," in Ref. 23.

31. D. R. Godwin, "Relationships between Sensory Response and Chemical Composition of Tea," Ph.D. dissertation, University of Georgia, Athens, 1984.

32. G. B. Dijksterhuis, *Multivariate Data Analysis in Sensory and Consumer Science*, Food & Nutrition Press, Inc., Trumbull, Conn., 1997.

33. G. M. Arnold and A. A. Williams, "The Use of Generalised Procrustes Techniques in Sensory Analysis," in J. Adda, ed., *Progress in Flavour Research*, Elsevier, Amsterdam, The Netherlands, 1985, pp. 35–50

34. H. T. Lawless and H. Heymann, *Sensory Evaluation of Food*, Chapman & Hall, New York, 1998.

35. B. Kaye, *Chaos & Complexity*, VCH Publishers, New York, 1993.

36. E. A. Hunter and D. D. Muir, "A Comparison of Two Multivariate Methods for the Analysis of Sensory Profile Data," *Journal of Sensory Studies* **10**, 89–104 (1995).

37. R. Popper, H. Heymann and Frank Rossi, "Three Multivariate Approaches to Relating Consumer to Descriptive Data," in A. M. Munoz, ed., *Relating Consumer, Descriptive, and Laboratory Data*, Manual 30, ASTM, West Conshohocken, Pa., 1997, pp. 39–61.

38. J. J. Powers, K. Shinholser, and D R. Godwin, "Evaluating Assessors' Performance and Panel Homogeneity Using Univariate and Multivariate Statistical Analysis," in J. Adds, ed., *Progress in Flavour Research 1984*, Elsevier, Amsterdam, The Netherlands, 1985.

39. J. J. Powers, "Utilizing General Statistical Programs to Evaluate Sensory Data," *Food Technology* **38**, 74–84 (1984).

40. Q. Zhang and J. B. Litchfield, "Applying Fuzzy Mathematics to Product Development and Comparison," *Food Technology* **45**, 108–118 (1991).

41. C. N. Thai and R. L. Shewfelt, "Modeling Sensory Color Quality of Tomato and Peach. Neural Networks and Statistical Regressionk," *Transactions of the ASAE* **34**, 950–955 (1991).

42. G. Peters et al., "Linear Regression, Neural Network and Industion Analysis to Determine Harvesting and Processing Effects on Surimi Quality," *Journal of Food Science* **61**, 876–880 (1996).

43. D. L. Hoffman and G. R. Franke, "Correspondence Analysis: Graphical Representation of Categorical Data in Marketing Research," *Journal of Marketing Research* **23**, 213–227 (1986).

44. J. P. Benzecri et al., *L'Analyse des donnees, Vol 11. L'Analyse des correspondances*, Dunod, Paris, 1973.

45. M. J. Greenacre, *Theory and Application of Correspondence Analysis*, Academic Press, Inc., Orlando, Fla., 1984.

46. J. M. Harries, D. N. Rhodes, and B. B. Chrystall, "Meat Texture. 1. Subjective Assessment of the Texture of Cooked Beef," *Journal of Text. Studies* **3**, 101–114 (1972)

47. E. I. Fischman, K. J. Shinholser, and J. J. Powers, "Examining Methods to Test Factor Patterns for Concordance," *Journal of Food Science* **52**, 448–454 (1987).

48. J. J. Powers, K. Shinholser, and D. Brett, "Testing Factor Patterns for Agreement," *Proceedings Tecno Alimentaria, Barcelona* (1987).

49. Z. M. Vickers, "Sensory, Acoustical, and Force-Deformation Measurements of Potato Chip Crispness," *Journal of Food Science* **52**, 138–140 (1987).

GENERAL REFERENCES

P. L. Bernstein, *Against the Gods, The Remarkable Story of Risk*, John Wiley and Sons, Inc., New York, 1996.

BMDP Biomedical Computer Programs, P-series, *University of California Press*, 1979.

J. Caul, "The Profile Method of Flavor Analysis," *Advances in Food Research* **7**, 1–40 (1957).

A. Dravnieks, L. Keith, B. K. Krotoszynski, and J. Shah, "Vaginal Odors: GLC Assay Method for Evaluating Odor Changes." *Journal of Pharmaceutical Science* **63**, 36–40 (1974).

M. C. Gacula, Jr., *Descriptive Sensory Analysis in Practice*, Food and Nutrition Press, Inc., Trumbull, Conn., 1997.

P. E. Green, *Analyzing Multivariate Data*, Dryden Press, Hinsdale, Ill., 1978.

T. Persson and E. von Sydow, "A Quality Comparison of Frozen and Refrigerated Cooked Sliced Beef. 2 Relationships between Gas Chromatographic Data and Flavor Scores," *Journal of Food Science* **37**, 234–239 (1972).

J. J. Powers, "Applying Multivariate Statistical Methods to Enhance Information Obtainable from Flavor Analysis Results," in David B. Min and Thomas H. Smouse, eds., *Flavor Chemistry of Lipid Foods*, American Oil Chemists' Society, Champaign, Ill., 1989.

B. G. Tabachnick and L. S. Fidell, *Using Multivariate Statistics*, Harper & Row, New York, 1983.

L. Vuataz, "Some Points of Methodology in Multidimensional Data Analysis as Applied to Sensory Evaluation," *Nestlé Research News 1976/77*, Nestec Ltd., Vevey, Switzerland, 1977.

JOHN J. POWERS
University of Georgia
Athens, Georgia

SHELLFISH

This article concerns three major groups of shellfish: oysters, scallops, and shrimps. When discussing these popular groups of seafoods, the government, academics, and industry are interested in harvesting, processing, sanitation, and economic considerations. Limited by space, this article will discuss the quality control of processing oysters, scallops, and shrimps with some emphasis on definitions and standards, general processing, sanitary practices, and sizing. It is obvious that the thrust of this article is most applicable to the United States.

OYSTERS

For many years packers of canned oysters in the Gulf of Mexico area of the United States have labeled their output with a declaration of the drained weight of oysters in the containers. Packers in other areas have marketed canned oysters with a declaration of the total weight of the contents of the container. Under present-day practice consumers generally do not discard the liquid packing medium, but use it as a part of the food. Compliance with the label declaration of quantity of contents requirement will be met by an accurate declaration of the total weight of the contents of the can.

General Definitions

Oysters, raw oysters, and shucked oysters, are the class of foods each of which is obtained by shucking shell oysters and preparing them in a prescribed manner. If water or salt water containing <0.75% salt is used in any vessel into which the oysters are shucked, the combined volume of oysters and liquid should not be less than four times the volume of the water or salt water. Any liquid accumulated with the oysters is removed. The oysters are washed, by blowing or otherwise, in water, salt water, or both. The total time that the oysters are in contact with the water or salt water after leaving the shucker, including the time of washing, rinsing, and any other contact with water or salt water is not more than 30 min. In computing the time of contact with water or salt water, the length of time that oysters are in contact with water or salt water that is agitated by blowing or otherwise, should be calculated at twice its actual length. Any period of time that oysters are in contact with salt water containing not less than 0.75% salt before contact with oysters should not be included in computing the time. Before packing into containers for shipment, or other delivery for consumption, the oysters are thoroughly drained and are packed without any added substance.

Shell oysters means live oysters of any of the species *Ostrea virginica*, *Ostrea gigas*, or *Ostrea lurida*, in the shell, which, after removal from their beds, have not been floated or otherwise held under conditions that result in the addition of water. The oysters are drained on a strainer or skimmer that has an area of not less than 300 in.^{2}/gal of oysters, drained, and has perforations of at least 0.25 in. in diameter and not more than 1.25 in. apart, or perforations of equivalent areas and distribution. The oysters are distributed evenly over the draining surface of the skimmer and drained for not less than 5 min.

Extra Large Oysters

Extra large oysters, oysters counts (or plants), extra large raw oysters, raw oysters counts (or plants), extra large shucked oysters, and shucked oysters counts (or plants) are of the species *O. virginica*. One gallon of this size oyster contains not more than 160 oysters and a quart of the smallest of these oysters contains not more than 44 oysters.

Large Oysters

Large oysters, oysters extra selects, large raw oysters, raw oysters extra selects, large shucked oysters, and shucked oysters extra selects are of the species *O. virginica* and 1 gal contains more than 160 oysters but not more than 210 oysters. A quart of the smallest of these oysters contains not more than 58 oysters, and a quart of the largest oysters contains more than 36 oysters.

Medium Oysters

Medium oysters, oysters selected, medium raw oysters, raw oysters selects, medium shucked oysters, and shucked oysters selects are of the species *O. virginica*. One gallon contains more than 210 oysters but not more than 300 oysters; a quart of the smallest oysters contains not more than 83 oysters, and a quart of the largest oysters contains more than 46 oysters.

Small Oysters

Small oysters, oysters standards, small raw oysters, raw oysters standards, small shucked oysters, and shucked oysters standards are of the species *O. virginica*; 1 gal contains more than 300 oysters but not more than 500 oysters. A quart of the smallest oysters contains not more than 138 oysters, and a quart of the largest oysters contains more than 68 oysters.

Very Small Oysters

Very small oysters, very small raw oysters, and very small shucked oysters are of the species *O. virginica*; 1 gal contains more than 500 oysters and a quart of the largest oysters contains more than 112 oysters.

Olympia Oysters

Olympia oysters, raw Olympia oysters, and shucked Olympia oysters are of the species *O. lurida*.

Large Pacific Oysters

Large Pacific oysters, large raw Pacific oysters, and large shucked Pacific oysters are of the species *O. gigas*, and 1 gal contains not more than 64 oysters. The largest oyster in the container is not more than twice the weight of the smallest oyster therein.

Medium Pacific Oysters

Medium Pacific oysters, medium raw Pacific oysters, and medium shucked Pacific oysters are of the species *O. gigas*, and 1 gal contains more than 64 oysters and not more than 96 oysters. The largest oyster in the container is not more than twice the weight of the smallest oyster therein.

Small Pacific Oysters

Small Pacific oysters, small raw Pacific oysters, and small shucked Pacific oysters are of the species *O. gigas*. One gallon contains more than 96 oysters and not more than 144 oysters; the largest oyster in the container is not more than twice the weight of the smallest oyster therein.

Extra Small Pacific Oysters

Extra small Pacific oysters, extra small raw Pacific oysters, and extra small shucked Pacific oysters are of the species *O. gigas*, and 1 gal contains more than 144 oysters. The largest oyster in the container is not more than twice the weight of the smallest oyster therein.

Canned Oysters

Canned oysters contain one or any mixture of two or all of the forms of oysters described above in a packing medium of water, the watery liquid draining from oysters before or during processing, or a mixture of such liquid and water. The food may be seasoned with salt. It is sealed in containers and so processed by heat as to prevent spoilage. The forms of oysters referred to are prepared from oysters that have been removed from their shells and washed and that may be steamed while in the shell or steamed or blanched or both after removal therefrom, and are as follows:

1. Whole oysters with such broken pieces of oysters as normally occur in removing oysters from their shells, washing, and packing.
2. Pieces of oysters obtained by segregating pieces of oysters broken in shucking, washing, or packing whole oysters.
3. Cut oysters obtained by cutting whole oysters.

The name of the food is oysters or cove oysters if the species is *O. virginica*; oysters or Pacific oysters if the species is *O. gigas*; and oysters or Olympia oysters if the species is *O. lurida*.

The standard of fill of container for canned oysters is a fill such that the drained weight of oysters taken from each container is not less than 59% of the water capacity of the container. If canned oysters fall below the standard of fill of container, the label should bear the general statement of substandard fill: "A can of this size should contain _ oz. of oysters. This can contains only _ oz.," the blanks being filled in with the applicable figures.

Abbreviated Inspections

Microbiological considerations are of prime importance in any shellfish gathering and processing plant. Time-temperature abuses enter into most problems with the products. However, the high value of these products has made economic violations even more profitable to the unethical operator. The FDA conducts both abbreviated and comprehensive inspections of oyster processing plants.

When conducting an abbreviated inspection, the FDA uses the following critical factors:

1. Check for evidence of contamination from the presence of cats, dogs, birds, or vermin in the plant.
2. Check results of any testing conducted on incoming oysters including filth, decomposition, pesticides, or bacteria.
3. Check for possible incorporation of excessive fresh water through prolonged contact with water or by insufficient drainage.
4. Be alert to misbranding of oysters by size.
5. Determine if employee sanitation practices preclude adding contamination (clean dress and proper use of 100 ppm chlorine equivalent hand sanitizers).
6. Determine if equipment is washed and sanitized about every two hours.
7. Check for time-temperature abuses that may cause rapid bacterial growth.
8. Weigh 20 retail packages for net weight declaration, and check accuracy of labeling statements at this time.

Comprehensive Inspection

During a comprehensive inspection of an oyster processing plant, an FDA inspector pays particular attention to the points listed below.

1. Check insanitation in chucking plants and decomposition of oysters, by conventional means. Pay particular attention to frozen oysters rolled in batter ready to cook. When dealing with economic violations, emphasis should be placed on consumer-size packages, pints, and quarts, with less attention to larger-size institutional packs.
2. The abuses most often encountered in the marketing of shucked oysters are
   a. The incorporation of excessive fresh water into the bodies of the oysters by keeping them in prolonged contact with fresh water.
   b. Increasing the output of oysters, usually by insufficient drainage before packing.
   c. Short volume.
   d. Misbranding as to size. Actions on misbranding as to size should be based only on significant evidence of financial gain.
3. Cover in detail the amount of water in shucking pails, the total length of time of contact with water, length of blowing time, length of time in contact with fresh water when not being blown, length of draining time, description of skimmer and draining process, and a count of the oysters in one or more sizes. De-

termine the source of oysters and whether they are all from the same locality.

SCALLOPS

The scallop industry encompasses three primary species:

1. Sea scallops.
2. Bay scallops.
3. Calico scallops.

The processing of sea scallops is accomplished on board the vessel actually harvesting the product. Boats that process sea scallops remain at sea from 3 to 12 days depending on area and catch. In most cases, the calico scallops are harvested daily and processed at shore processing plants rather than on board the vessel. The trend, however, is toward on board processing for this species also. Bay scallops pose a unique problem in that they may be processed in a commercial plant or at home.

Comprehensive Inspection

During a comprehensive inspection of scallop processors, the following will be covered.

1. Raw materials, determine
   a. Geographical area where the scallops are harvested.
   b. Type of scallops harvested and processed by common or species name.
   c. How scallops are handled between harvesting and processing.
2. Processing
   a. Observe in detail the scallop processing operation. Make a flow plan.
   b. Check shucking and evisceration process and see if this process is physically separated from the packaging and other operations.
   c. Determine source of water used in the scallop washing and rinsing operations. If treated by the processor, determine nature and extent of treatment.
   d. See if equipment used in processing operation is of proper construction and design.
   e. Check firm's equipment cleaning and sanitizing operation.
   f. Determine time and temperature of processing operation. Check
      i. How long between harvest and chuckling and the temperature of the scallops.
      ii. How long scallops are held at ambient air temperature and determine the ambient temperature.
      iii. How long between shucking and rinsing and the temperature of the scallops.
      iv. After being iced, how long before scallops reach an internal temperature below 40°F.
   g. Check finished product packaging.
   h. Determine source of ice used in icing operation and if bagged ice is used, source and type of bag, condition of bags, and conditions storage.
   i. Check finished product storage facilities and condition.
   j. Obtain any coding system used and key.
   k. Check on the use of any food additives to determine if used at allowable levels.
3. Sanitation
   a. See if building or vessel is free from rodent or insect activity.
   b. Check that toilets and handwashing facilities provided are properly located and maintained.
   c. Determine strength and type of hand sanitizing solutions used and the sanitizer's location.
   d. Note any employee practices that could lead to the contamination of the scallops with filth or bacteria.
   e. See if water and ice used in the process is from an approved source and list source.
   f. Determine method of shell and waste material disposal.
   g. Evaluate the firm's operation for compliance with FDA's Human Foods (Sanitation) Good Manufacturing Practice Regulations.
   h. Document any insanitary conditions noted that could lead to the contamination of this firm's products with filth and/or bacteria.
   i. Obtain distribution for the collection of official samples.

Frozen Raw Scallops

Frozen raw scallops are clean, wholesome, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species. The portion of the scallop used should be only the adductor muscle eye, which controls the shell movement. Scallops should be washed, drained, packed, and frozen in accordance with good manufacturing practices and are maintained at temperatures necessary for the preservation of the product. Only scallops of a single species should be used within a lot.

Solid pack scallops are frozen together into a solid mass that may be glazed or not glazed. Individually quick-frozen pack (IQF) scallops are individually quick frozen. Individual scallops can be separated without thawing. Again, they may or may not be glazed.

Scallops are of two types: adductor muscle and adductor muscle with catch (gristle or sweet meat) portion removed. Good flavor and odor (essential requirements for a U.S. grade A product) means that the product has the typical flavor and odor of the species and is free from bitterness, staleness, and off-flavor and off-odors of any kind. Reasonably good flavor and odor (minimum requirements for a U.S. Grade B product) means the product is lacking in good flavor and odor but is free from objectionable off-flavors and off-odors of any kind. Dehydration refers to the loss of moisture from the scallops surface during frozen storage.

Small degree of dehydration is color-masking but can be easily scraped off. Large degree of dehydration is deep, color-masking, and requires a knife or other instrument to scrape it off.

Undesirable small pieces are pieces that will pass through the openings in a 0.75-in. sieve for larger-size scallops. For the smaller by scallops, undesirable pieces are pieces of scallops that do not have the general conformation of the other scallops. The total weight of these pieces within a sample unit will be obtained. These pieces should not be used for determining the weight ratio.

Color refers to reasonably uniform color characteristics of the species used within an individual container. Only noticeable variation in color from the predominating color of the scallops in the container is considered. Medium gray to black colored scallops are not to be graded.

Extraneous materials are pieces or fragments of undesirable material that are naturally present in or on the scallops and that should be removed during processing. An instance of minor extraneous material includes each occurrence of intestines, seaweed, etc, and each aggregate of sand and grit up to 0.5-in.^{2} and located on the scallop surface. An instance of major extraneous material includes each instance of shell or aggregate of embedded sand or other extraneous embedded material that affects the appearance or eating quality of the product. Texture refers to the firmness, tenderness, and moistness of the cooked scallop meat that is characteristic of the species.

Frozen Raw Breaded Scallops and Frozen Fried Scallops

Frozen raw breaded or fried scallops are

1. Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading.
2. Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation.
3. Composed of a minimum of 50% by weight of scallop meat.

The styles of frozen raw breaded scallops and frozen fried scallops include

1. *Random Pack*. Scallops in a package are reasonably uniform in weight or shape. The weight or shape of individual scallops are not specified.
2. *Uniform Pack*. Scallops in a package consist of uniformly shaped pieces that are of specified weight or range of weights.

The scallops are of two types: adductor muscle and adductor muscle with catch (gristle or sweet meat) portion removed.

Condition of the package refers to freedom from packaging defects and the presence in the package of oil, loose breading, or frost. Ease of separation refers to the difficulty of separating scallops that are frozen together after the frying operation and during freezing.

Continuity refers to the completeness of the coating of the product in the cooked state. Lack of continuity is exemplified by breaks, ridges, or lumps of breading. Each 1/16-in.^{2} area of any break, ridge, or lump of breading is considered an instance of lack of continuity. Individual breaks, ridges, or lumps of breading measuring less than 1/16 in.^{2} are not considered objectionable. Deduction points are based on the percentage of the scallops within the package that contain small or large instances of lack of continuity.

Workmanship defects refer to the degree of freedom from doubled and misshaped scallops and extraneous material. The defects of doubled and misshaped scallops are determined by examining the frozen product, while the defects of extraneous materials are determined by examining the product in the cooked state. Doubled scallops are two or more scallops that are joined together during the breading or frying operations. Misshaped scallops are elongated, flattened, mashed, or damaged scallop meats.

Extraneous materials are pieces or fragments of undesirable material that are naturally present in or on the scallops and that should be removed during processing. Examples of minor extraneous material include intestines, seaweed, and each aggregate of sand and grit within an area of 0.5 in.^{2} Examples of major extraneous material include shell and aggregate of embedded sand or other extraneous embedded material that affects the appearance or eating quality of the product.

Character refers to the texture of the scallop meat and of the coating and the presence of gristle in the cooked state. Gristle is the tough elastic tissue usually attached to the scallop meat. Each instance of gristle is an occurrence. Texture refers to the firmness, tenderness, and moistness of the cooked scallop meat and to the crispness and tenderness of the coating of the cooked product. The texture of the scallop meat may be classified as a degree of mushiness, toughness, and fibrousness. The texture of the coating may be classified as a degree of pastiness, toughness, dryness, mushiness, or oiliness.

SHRIMP

Canned Wet Pack Shrimp in Transparent or Nontransparent Containers

Canned wet pack shrimp is the food consisting of the processed meat of peeled shrimp; free of heads; and, to the extent practicable under good manufacturing practice, free of shells, legs, and antennae. One or any combination of species may be canned, prepared in one of the styles specified, in sufficient water or other suitable aqueous packing medium to fill the interstices and permit proper processing in accordance with good manufacturing practice.

Canned shrimp may contain one or more of the optional ingredients specified. It is packed in hermetically sealed transparent or nontransparent containers and so processed by heat as to prevent spoilage.

The species of shrimp that may be used in the food are of the families Penaeidae, Pandalidae, Crangonidae, and

Palaemonidae. Canned shrimp is prepared in one of the following styles:

1. Shrimp with readily visible dark vein (dorsal tract, back vein, or sand vein).
2. Deveined shrimp containing not less than 95% by weight of shrimp prepared, by removing the dark vein from the first five segments by deliberate cutting action.
3. Shrimp, other than "deveined" as described, containing not less than 95% by weight of shrimp with no readily visible dark vein within the first five segments.
4. Broken shrimp, consisting of less than four segments and otherwise conforming to one of the styles described.

The following safe and suitable optional ingredients may be used:

1. Salt.
2. Lemon juice.
3. Organic acids.
4. Nutritive carbohydrate sweeteners.
5. Spices or spice oils or spice extracts.
6. Flavorings.
7. Sodium bisulfite.
8. Calcium disodium ethylenediaminetetraacetate (EDTA), complying with prescribed regulations.

The name of the food is shrimp, or shrimps. The word *prawns* may appear on the label in parentheses immediately after the word *shrimp*, or shrimps, if the shrimp are of large or extra large size. When the food is of the style described above, the words *cleaned, cleaned (deveined), deveined*, or *contains no dark veins* may appear. The name of the food should include a declaration of any flavoring that characterizes the food and the term spiced if spice characterizes the food. Each of the ingredients used should be declared on the label as required.

Frozen Raw Breaded Shrimp: Definitions

Frozen raw breaded shrimp is the food prepared by coating one of the optional forms of shrimp with safe and suitable batter and breading ingredients. The food is frozen. The food tests not less than 50% of shrimp material. The term shrimp means the tail portion of properly prepared shrimp of commercial species. Except for composite units, each shrimp unit is individually coated. The optional forms of shrimp are

1. *Fantail or Butterfly*. Prepared by splitting the shrimp; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.
2. *Butterfly, Tail Off*. Prepared by splitting the shrimp; tail fins and all shell segments are removed.
3. *Round*. Round shrimp, not split; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.
4. *Round, Tail Off*. Round shrimp, not split; tail fins and all shell segments are removed.
5. *Pieces*. Each unit consists of a piece or a part of a shrimp; tail fins and all shell segments are removed.
6. *Composite Units*. Each unit consists of two or more whole shrimp or pieces of shrimp, or both, formed and pressed into composite units prior to coating; tail fins and all shell segments are removed; large composite units, prior to coating, may be cut into smaller units.

The batter and breading ingredients referred to are the fluid constituents and the solid constituents of the coating around the shrimp. These ingredients consist of suitable substances that may or may not be food additives. Chemical preservatives that are suitable are

1. Ascorbic acid, which may be used in a quantity sufficient to retard development of dark spots on the shrimp.
2. Antioxidant preservatives may be used to retard development of rancidity of the fat content of the food, in amounts within the limits prescribed.

The label should name the food as follows:

1. Breaded fantail shrimp (the word *butterfly* may be used in lieu of fantail in the name).
2. Breaded butterfly shrimp, tail off.
3. Breaded round shrimp.
4. Breaded round shrimp, tail.
5. Breaded shrimp pieces.
6. Composite units.

If the composite units are in a shape similar to that of breaded fish sticks the name is breaded shrimp sticks; if they are in the shape of meat cutlets, the name is breaded shrimp cutlets. If prepared in a shape other than that of sticks or cutlets, the name is breaded shrimp _, the blank to be filled in with the word or phrase that accurately describes the shape, but that is not misleading.

The word *prawns* may be added in parentheses immediately after the word *shrimp* in the name of the food if the shrimp are of large size; for example, "fantail breaded shrimp (prawns)." If the shrimp are from a single geographical area, the adjectival designation of that area may appear as part of the name; for example, "breaded Alaska shrimp sticks."

Frozen Raw Lightly Breaded Shrimp: Definitions

Frozen raw lightly breaded shrimp differs from the regularly breaded ones in that it contains not less than 65% of shrimp material and that the word *lightly* immediately precedes the words *breaded shrimp* on the label.

Shrimp: General Processing Quality and Standards

These standards should apply to clean, wholesome shrimp of the regular processed commercial species that are fresh or frozen, raw or cooked. Such shrimp are processed and maintained in accordance with good commercial practice at temperatures necessary for the preservation of the product. Types of fresh shrimp include

1. Frozen individually (IQF) glazed or unglazed.
2. Frozen solid pack, glazed or unglazed.

The styles of the shrimp can be as follows:

1. *Raw*. Uncoagulated protein.
2. *Parboiled*. Heated for a period of time such that the surface of the product reaches a temperature adequate to coagulate the protein.
3. *Cooked*. Heated for a period of time such that the surface of the product reaches a temperature adequate to coagulate the protein.
4. *Cooked*. Heated for a period of time such that the thermal center of the shrimp reaches a temperature adequate to coagulate the protein.

The market forms of shrimp include

1. Heads on (head, shell, tail fins on).
2. Headless (only head removed; shell, tail fins on).
3. Peeled, round, tail off (all shell and tail fins removed with segments unslit and vein not removed).
4. Peeled, round, tail off (all shell and tail fins removed with segments unslit and vein not removed).
5. Peeled and deveined, round, tail off (all shell and tail fins removed with segments shallowly slit to last segment and vein removed to last segment).
6. Peeled and deveined, round, tail on (all shell removed except last shell segment, and tail fins, with segments shallowly slit to last segment and vein removed to last segment).
7. Peeled and deveined, fantail or butterfly tail off (all shell and tail fin removed with segments deeply slit to last segment and vein removed to last segment).
8. Peeled and deveined, fantail or butterfly, tail on (all shell removed except last shell segment and tail fins, with segments deeply slit to last segment and vein removed to last segment).
9. Peeled and deveined, western (all shell removed except last shell segment and tail fins, with segments split completely to last segment and vein removed to last segment).
10. Shell on pieces (head removed, shell and tail fins when existing not removed).
11. Peeled and deveined, round pieces (all shell removed with segments shallowly slit except last segment when existing; vein removed except last segment removed with segments deeply slit except last segment when existing; vein removed except last segment when existing).
12. Peeled undeveined pieces (all shell removed).

Evaluation of Flavor and Odor. Sensory evaluation of flavor and odor on each of the sample units should be carried out only by those trained to do so. For raw odor evaluation, frozen shrimp should be thawed. Fresh or thawed shrimp are broken and the broken flesh is held close to the nose immediately to detect off-odor. Cooked style shrimp should be evaluated for flavor and odor as is, thawed if frozen. Sensory evaluation of cooked samples should be completed as soon as practical after cooking while the samples are still warm.

Good flavor and odor means that the product has the normal, pleasant flavor and odor characteristic of freshly caught shrimp that is free from off-flavors and odors of any kind. A natural odor or flavor reminiscent of iodoform is acceptable unless excessive. Reasonably good flavor and odor means that the product may be somewhat lacking in good flavor and odor characteristic of freshly caught shrimp but is free from objectionable off-flavors and off-odors of any kind. Minimum acceptable flavor and odor means that the raw product and the cooked product have a moderate storage-induced odor (for the raw product) and flavor and odor (for the raw product) and flavor and odor (for the cooked product), but the product is reasonably free from any objectionable off-flavors and off-odors that may be indicative of spoilage or decomposition.

Evaluation of Physical Characteristics and Defects. Each sample unit should be evaluated as to physical characteristics and defects in accordance with the following definitions and methods of analysis. Dehydration refers to a general desiccation of the shrimp flesh that is noticeable after the shell and glaze are removed. It is evaluated by noting any detectable change from the normal, characteristic bright appearance of freshly caught, properly iced or properly processed shrimp. The degree of dehydration is as follows:

1. Slight dehydration means scarcely noticeable dessication of the shrimp flesh that will not affect the desirability or eating quality of the shrimp.
2. Moderate dehydration means conspicuous desiccation of the shrimp flesh that will not seriously affect the desirability or eatng quality of the shrimp.
3. Severe dehydration means conspicuous dessication that will seriously affect the desirability or eating quality of the shrimp.

Deterioration is evaluated by noting any detectable change in the normal good odor of freshly caught, properly iced or properly processed shrimp.

1. Slight deterioration means that overall the sample unit lacks the normal characteristic pleasant odor of freshly caught, properly iced or properly processed shrimp; the desirability or eating quality of the shrimp is not affected.

2. Moderate deterioration means that overall the sample unit has scarcely noticeable odors of prolonged storage off-odors that materially affect the desirability or eating quality of the shrimp.
3. Severe deterioration means that overall the sample unit has definite odors of prolonged storage or spoilage odors that seriously affect the desirability or eating quality of the shrimp.

Fresh or thawed shrimp (glaze removed) should be visually examined for the presence of broken or damaged shrimp. Broken or damaged shrimp, identified as follows, are grouped together and evaluated by noting the percent by weight of such broken or damaged shrimp in the total net weight of the sample unit.

1. Broken means a shrimp having a break in the flesh greater than one-third of the thickness of the shrimp measured where the break occurs.
2. Damaged means a shrimp that is crushed or mutilated so as to materially affect its appearance or usability.

Frozen Raw Breaded Shrimp: Processing Quality and Standards

Frozen raw breaded shrimp are whole, clean, wholesome, headless, and peeled shrimp that have been deveined, where applicable, of the regular commercial species, coated with a wholesome, suitable batter or breading. Whole shrimp consist of five or more segments of unmutilated shrimp flesh. They are prepared and frozen in accordance with good manufacturing practice and are maintained at temperatures necessary for the preservation of the product.

Frozen raw breaded shrimp should contain not less than 50% by weight of shrimp material. The styles of shrimp are as follows:

1. Regular breaded shrimp are frozen raw breaded shrimp containing a minimum of 50% of shrimp material.
2. Lightly breaded shrimp are frozen raw breaded shrimp containing a minimum of 65% of shrimp material.

Types of breaded shrimp are breaded fantail shrimp and breaded round shrimp. Breaded fantail shrimp includes the following:

1. Split (butterfly) shrimp with the tail fin and the shell segment immediately adjacent to the tail fin.
2. Split (butterfly) shrimp with the tail fin but free of all shell segments.
3. Split (butterfly) shrimp without attached tail fin or shell segments.

Breaded round shrimp includes the following:

1. Round shrimp with the tail fin and the shell segment immediately adjacent to the tail fin.
2. Round shrimp with the tail fin but free of all shell segments.
3. Round shrimp without attached tail fin or shell segments.

Hygienic processing of frozen raw breaded shrimp includes processing and maintenance in accordance with the applicable requirements of the good manufacturing practice regulations. Good flavor and odor means that the cooked product has flavor and odor characteristics of freshly caught or well-refrigerated shrimp and the breading is free from staleness and off-flavors and off-odors of any kind. Iodoform is not to be considered in evaluating the product for flavor and odor. Reasonably good flavor and odor means that the cooked product may be somewhat lacking in the good flavor and odor characteristics of freshly caught or well-refrigerated shrimp but is free from objectionable off-flavors and objectionable off-odors of any kind.

Dehydration refers to the occurrence of whitish areas on the exposed ends of the shrimp (due to the drying of the affected area) and to a generally desiccated appearance of the meat after the breading is removed. Deterioration refers to any detectable change from the normal good quality of freshly caught shrimp. It is evaluated by noting in the thawed product deviations from the normal odor and appearance of freshly caught shrimp.

Extraneous material consists of nonedible material such as sticks, seaweed, shrimp thorax, or other objects that may be accidentally present in the package. Halo means an easily recognized fringe of excess batter and breading extending beyond the shrimp flesh and adhering around the perimeter or flat edges of a split (butterfly) breaded shrimp. Balling up means the adherence of lumps of the breading material to the surface of the breaded coating, causing the coating to appear rough, uneven, and lumpy. Holidays means voids in the breaded coating as evidenced by bare or naked spots. Damaged frozen raw breaded shrimp means frozen raw breaded shrimp that have been separated into two or more parts or that have been crushed or otherwise mutilated to the extent that their appearance is materially affected. Black spot means any blackened area that is markedly apparent on the flesh of the shrimp. Sand vein means any black or dark sand vein that has not been removed, except for that portion under the shell segment adjacent to the tail fin when present.

Breading of shrimp has long posed a problem from an economic standpoint. In addition, the time–temperature abuses present a great potential for food poisoning organisms. The growing scarcity and consequential high value of the raw material make the breading standards even more important.

Abbreviated Inspection

During an abbreviation inspection of a shrimp processing plant, an FDA inspector is advised of the following:

1. Check for the presence of cats, dogs, birds, or vermin in the plant.

2. Review testing of incoming shrimp. Check results of tests for decomposition, bacterial load, pesticides, and other possible adulterants.
3. Evaluate operation for compliance with standards for raw breaded shrimp.
4. Watch for any time—temperature abuses in the handling of seafood.
5. Determine that employee hygienic practices are satisfactory, eg, clean dress, washing of hands, and use of 100 ppm chlorine equivalent hand sanitizers.
6. Note any equipment defects that cause seafood to lodge, decompose, then dislodge into the pack.
7. Observe breading operations for suspected excesses, lack of coolant to keep batter mix below 50°F in an open system and below 40°F in a closed system.
8. Check labeling declarations while weighing 20 retail units against the net weight statement on four different products or sizes.

Comprehensive Inspection

During a comprehensive inspection, the FDA inspector will note the following.

Raw Materials: Receipt and Storage. The inspector will determine if:

1. Shrimp and other raw materials are inspected on receipt for decomposition, microbial load, pesticides, and filth.
2. Raw materials susceptible to microbial contamination are received under a suppliers guarantee.
3. Raw material specifications exist and only wholesome raw materials are accepted into active inventory; determine disposition of rejected raw materials.
4. Shrimp receiving and storage facilities are physically adequate.
5. Frozen shrimp are stored at −18°C (0°F) or below.
6. Fresh or partially processed shrimp are iced or otherwise refrigerated to maintain a temperature of 4°C (40°F) or below until they are ready to be processed.
7. Decomposed shrimp are being processed.
   a. Examine shrimp as received and again after sorting, for decomposition; classify as passable (class 1), decomposed (class 2), or advanced decomposition (class 3); less-experienced inspectional personnel should submit some of class 2 and class 3 shrimp for confirmation by the laboratory.
   b. Prompt handling and adequate sorting is necessary to prevent decomposition; check times and temperatures.
   c. Where decomposed shrimp are going into canned or cooked-peeled shrimp, collect investigational samples of the finished pack; give attention to disposition of loads showing a high percentage of decomposition that cannot be adequately sorted, and to disposition of reject shrimp; make certain that bait shrimp is denatured.
8. Fresh raw shrimp are washed and chilled to 4°C (40°F) or below within 2 h of receipt; frozen shrimp should be held at −18°C (0°F) or below; determine if they are examined organoleptically when received.
9. Peeled and deveined shrimp are promptly chilled to 4°C (40°F) or below.

Plant Sanitation. The inspector will determine if:

1. The water (ice) is
   a. From an approved source.
   b. Disinfected and contains residual chlorine.
   c. Sampled and analyzed for contamination.
   d. Handled in a sanitary manner.
2. Drainage facilities are adequate to accommodate all seepage and wash water.
3. The plant has readily cleanable floors that are sloped and equipped with trap drains.
4. The plant is free of the presence of vermin, dogs, cats, or birds.
5. The screening and fly control are adequate.
6. Offal, debris, refuse is placed in covered containers and removed at least daily or continuously.
7. Adequate handwashing and sanitizing facilities are located in processing area and are easily accessible to the preparation, peeling, and subsequent processing operations.
8. Signs are posted directing employees handling shrimp and other raw materials to wash and sanitize their hands after each absence from the workstation.
9. Employees actually wash and sanitize their hands as necessary (before starting work, after absences from the workstation, when hands become soiled, etc).
10. Hand-sanitizing solutions are maintained at 100 ppm available chlorine or the equivalent and are used.
11. Persons handling food or food contact surfaces wear clean outer garments, maintain a high degree of personal cleanliness, and conform to good hygienic practices.
12. Management prevents any person known to be affected with boils, sores, infected wounds, or other sources of microbiological contamination from working in any capacity in which there is a reasonable probability of contaminating the food.
13. The product is processed to prevent contamination by exposure to areas involved in earlier processing steps, refuse, or other objectionable conditions or areas.
14. Food contact surfaces are constructed of metal or other readily cleanable materials.
15. Seams are smoothly bonded to prevent accumulation of shrimp, shrimp material or other debris.

16. Each freezer and cold storage compartment used for raw materials, in process or finished product is fitted with required temperature indicating devices.
17. Unenclosed batter application equipment is flushed and sanitized at least every 4 h during plant operations and all batter application equipment is cleaned and sanitized at the end of and the beginning of the days operation.
18 Breading application equipment and utensils are thoroughly cleaned and sanitized at the end of the days operations.
19. Utensils used in processing and product contact surfaces of equipment are thoroughly cleaned and sanitized at least every 4 h during operation.
20. All utensils and product contact surfaces excluding breading application equipment and utensils are rinsed and sanitized before beginning the days operation.
21. Containers used to convey or store food are handled in a manner to preclude direct or indirect contamination of the contents.
22. The nesting of containers is prohibited.

Processing. The inspector will determine if:

1. Raw frozen shrimp are defrosted at recommended temperatures: air defrosting at 7°C (45°F) or below or in running water at 21°C (70°F) or below in less than 2 h.
2. Fresh raw shrimp are washed in clean potable water and chilled to 4°C (40°F) or below.
3. Fresh shrimp are adequately washed, culled, and inspected.
4. Every lot of shrimp that has been partially processed in another plant, including frozen shrimp, is inspected for wholesomeness and cleanliness.
5. Shrimp entering thaw tank are free from exterior packaging material and substantially free of liner material.
6. On removal from thaw tank, shrimp are washed with a vigorous potable water spray.
7. Shrimp are removed from thaw tank within 30 min after they are thawed.
8. During the grading, sizing, or peeling operation the:
    a. Equipment is cleaned and sanitized before use.
    b. Water is maintained at proper chemical strength and temperature.
    c. Raw materials are protected from contamination.
9. Sanitary drainage is provided to remove liquid waste from peeling tables.
10. Firm prohibits the practice of salvaging shrimp (ie, repacking the accumulated hulls and shells for missed shrimp or shrimp pieces).
11. Peeled and deveined shrimp are promptly chilled to 4°C (40°F) or below.
12. Peeled shrimp are transported from peeling machines or tables immediately, or, if containerized, within 20 min.
13. Peeled shrimp containers, if applicable, are cleaned and sanitized as often as necessary, but in no case less frequently than every 3 h.
14. When a peeler is absent from his duty post, his container is cleaned and sanitized prior to resuming peeling.
15. Peeled shrimp that are transported from one building to another are properly iced or refrigerated, covered, and protected.
16. Shrimp are handled minimally and protected from contamination.
17. Shrimp that drop off processing line are discarded or reclaimed.
18. Shrimp are washed with a low-velocity spray or in unrecirculated flowing water at 10°C (50°F) or below just prior to the initial batter or breading application, whichever comes first, except in cases where a predust application is included in the process.
19. Removal of batter or breading mixes or other dry ingredients from multiwalled bags is accomplished in an acceptable manner.
20. Batter in enclosed equipment is insured a temperature of not more than 4°C (40°F) and disposed of at the end of each work day, but in no circumstances less often than every 12 h.
21. Batter in an unenclosed system is maintained at or below 10°C (50°F) and disposed of at least every 4 h and at the end of the day's operation.
22. Breading reused during a day's operation is sifted thru a 0.5-in. or smaller mesh screen.
23. Breading remaining in the breading application equipment at the end of a day's operation is reused within 20 h and is sifted as above and stored in a freezer in a covered sanitary manner.
24. Hand batter pans are cleaned, sanitized, and rinsed between each filling with batter or breading.

Finished Product Process and Quality Assurance. The inspector will determine if:

1. Processing and handling of finished product is
    a. Performed in a sanitary manner.
    b. Protected from contamination.
    c. Arranged to facilitate rapid freezing.
2. Manual manipulation of breaded shrimp is kept to a minimum.
3. Aggregate processing time, excluding the time required for thawing frozen material, is less than 2 h, exclusive of iced or refrigerated storage time.
4. Breaded shrimp are placed into freezer within 30 min of packaging.
5. Breaded shrimp are frozen in a plate or blast freezer at −29°C (−20°F) or below.

6. Storage freezer is maintained at or below −18°C (0°F).
7. In-line, environmental, and finished product samples are analyzed and evaluated at least weekly for microbial conditions; review these analytical records if available.
8. Firm has established microbiological specifications for the final product; if so, review and report these specifications.
9. Firm withholds from distribution lots that do not meet their established microbiological standards.
10. Finished product is handled and stored in a manner that precludes contamination.
11. Labels bear a cautionary statement to keep product frozen.
12. Packaged finished product bears a label code; report interpretation or breakdown of firm's coding.

GENERAL REFERENCES

Code of Federal Regulations, Titles 21 and 50.
Y. H. Hui, *United States Food Laws and Regulations*, 2 vols., Wiley-Interscience, New York, 1986.

Y. H. Hui
American Food and Nutrition Center
Cutten, California

SNACK FOOD TECHNOLOGY

Snacks can be considered as tasty, savory, or sweet foods eaten at nonmeal occasions. Indeed, *Snack World's* 1987 Consumer Attitude Survey indicated that salted snacks are most often eaten while watching television, at parties, or between meals (1). With the trend today toward grazing, the boundaries of snack foods are being broadened. In such a view it is possible to consider the influx of finger foods and microwaveable items such as dough enrobed meat and cheese, pizza, and hot dogs as snacks. *Snack Food* magazine (2) includes the following categories: candy, cookies–crackers, frozen pizza, and snack cakes–pies, along with such traditional salted items as potato chips, tortilla chips, nuts, pretzels, popcorn, granola items, dried fruit, extruded snacks, and meat snacks in its annual review of the snack food market. While a strong case can be made for including all of these items, only the technology for potato chips, tortilla chips, popcorn, pretzels, extruded items, and meat snacks will be considered in this overview due to space constraints.

BUSINESS AND TRENDS

Of the salted snacks, potato chips have the greatest share of the market, followed by corn and tortilla chips, nuts, dried fruit, popcorn, extruded snacks, meat snacks, pretzels, and granola. Estimated sales (2) for representative items are found in Table 1. The strong growth of the popcorn sector is directly attributable to microwave popcorn sales. Tortilla and corn chips have shown sales growth in each year from 1978 to 1989. Total sales have more than doubled in that period. During the mid-1980s granola product sales climbed to $439 million but have been declining since. Currently, dried fruit snack sales are increasing, presumably due to their wholesome image. There also has been a sharp increase in imported items and healthful products. Rice cakes have had a strong market impact because they are low in salt, fat, and calories. In addition, there has been a proliferation of miscellaneous items including flat breads, plantain and apple chips, bagel crisps, and sourdough bread crisps.

POTATO CHIPS

Potato chip manufacture is much more involved than the mere frying of thinly sliced potatoes. Potatoes are living organisms and as such have respiration and energy requirements. Because it is impossible to provide a year-round supply of potatoes to the chipper, potatoes must be stored. During storage, the plant's energy requirements are met by conversion of starch to sugars through enzymatic action. After a short curing period that allows cuts and bruises to heal, chipping potatoes are placed in well-ventilated cold storage (95% RH and 50–55°F) (3). However, during storage there is an accumulation of the reducing sugars, glucose, and fructose, which lead to unacceptably dark chips (4). Stored potatoes must be reconditioned to reconvert the reducing sugars back to starch through enzymatic action. Reconditioning is carried out by holding potatoes at 65–70°F until frying trials produce chips of the desired color, usually in one to four weeks (3). Potatoes with higher specific gravities are desired because they have increased chip yield and reduced oil consumption (4).

The block flow diagram of Figure 1 outlines a large commercial chipping process. After dumping, the potatoes are conveyed to a washer–destoner. Often, the conveying system is a water flume where a large centrifugal pump moves potatoes and water around the plant. The turbulence of the water helps remove dirt and stones, which are separated by gravity and are removed by a conveyor. Other types of destoner include an elevating screw and a bubble unit in which jets of air or water tumble the potatoes and dislodge

Table 1. Estimated Sales by Manufacturers (Millions of Dollars)

Snack food	1988	1987	1986
Potato chips	2,890	2,850	2,651
Corn and tortilla chips	1,401	1,324	1,283
Snack nut meats	1,098	1,160	1,160
Dried fruit snacks	587	508	418
Popcorn	554	458	365
Extruded snacks	472	467	415
Meat snacks	424	400	325
Pretzels	303	293	271
Granola snacks	298	359	439

Source: Ref. 2.

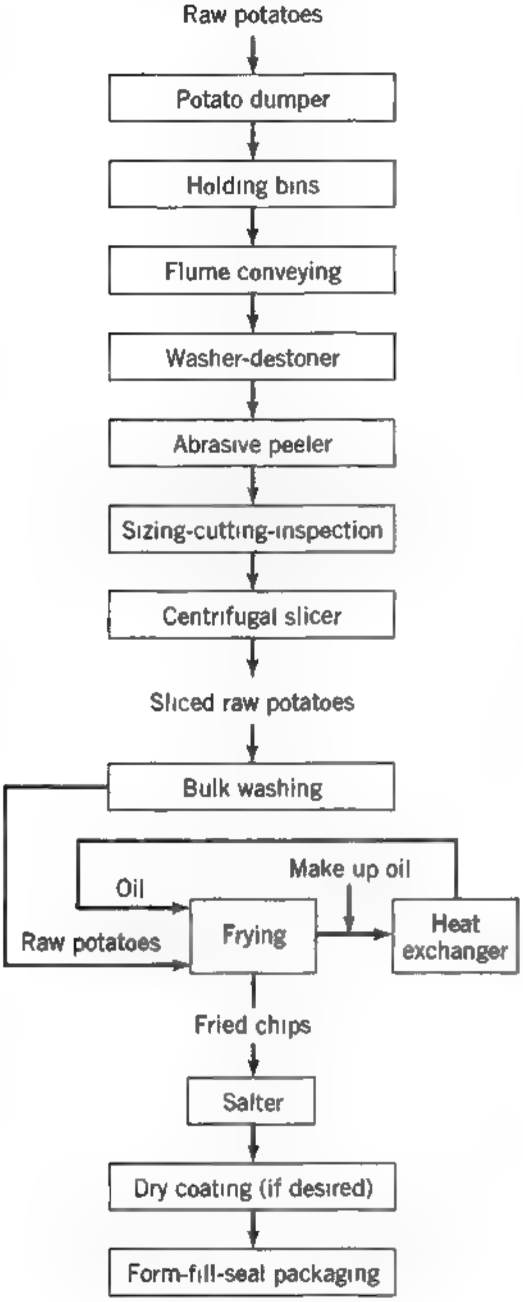


Figure 1. Commercial potato chip process flow diagram.

debris. Once washed, the potatoes are abrasively peeled. The peeler contains long cylindrical rollers covered with a rough material. The units are designed to provide uniform tumbling of potatoes against the rollers. Steam and chemical (lye) peelers are generally not used with chips because such methods lead to unsightly rings, while chemicals can carry over to the oil. Peeled potatoes can be sized, if necessary. Most North American manufacturers use rotary centrifugal slicers. Potatoes are fed into the center of a rotating bowl where centrifugal action and carefully designed impellers force the potatoes against knives mounted in the stationary outer housing. A large variety of knives are available including straight, corrugated, wave, and julienne types. To maintain efficient cutting, blades must be replaced frequently. Raw slice thickness is about 1/16 in. Slice thickness must be adjusted to compensate for such variables as potato variety, physical condition, specific gravity, consumer preference, and ease of processing. Sliced potatoes are washed and sprayed with either cold or hot water to remove surface starch and sugars. After draining, they are fried.

Modern potato fryers incorporate a variety of devices to insure long oil life and uniformity in frying conditions. Hot oil, 360–380°F, is pumped into the front end of the fryer where the washed slices enter. The turbulent action of water escaping help separate the slices. The velocity of the oil and the action of conveying paddles helps move the chips downstream. To achieve final frying to less than 1.5% moisture, the last section of the fryer contains a hold-down conveyor to uniformly remove the last traces of water from the buoyant chips. During frying the oil temperature drops by about 40°F. External heat exchangers are used to bring the oil back to the desired inlet temperature (5). After frying, chips are salted and optionally flavored before they are packaged. Packaging for chips should include moisture, oxygen, and light barriers.

KETTLE CHIPS

A recent market trend has been the emergence of crunchy and crispy, kettle type, homestyle, or hand cooked chips. Kettle chips achieve their desired crispy texture and stronger taste through batch frying. Raw, unwashed sliced potatoes are added to a batch or kettle of hot oil, which causes the oil temperature to drop. The chips fry as the oil temperature recovers. Frying times in the kettle are almost three times longer than in the continuous fryer (nine minutes vs three). It is believed that some large producers have simulated kettle fry conditions in the large continuous units.

TORTILLA CHIPS AND CORN CHIPS

Modern methods for the production of corn snacks such as tortilla chips and corn chips have origins in the ancient Aztec process of nixtamalization or alkaline cooking and steeping of corn (6). In nixtamalization whole corn is cooked in excess water containing lime. A mixture of white and yellow corn is used because corn chips made from 100% yellow corn have an unacceptable flavor profile probably resulting from carotenoid degradation (7). After cooking, the corn is allowed to steep overnight. During the process, some starch is gelatinized, the pericarp or hull disintegrates, and the endosperm hydrates and softens. After washing, the cooked corn or the nixtamal is stone ground into masa (6,7).

A modern tortilla chip process is outlined in Figure 2 and a more detailed account follows. Corn is cooked in excess water and lime (1% of corn weight) at temperatures above 200°F. Water-to-corn weight ratios range from 1.5 to 2.0:1. Cooking times vary depending on end use of the masa but are typically in the 10–30 min range. After cook-

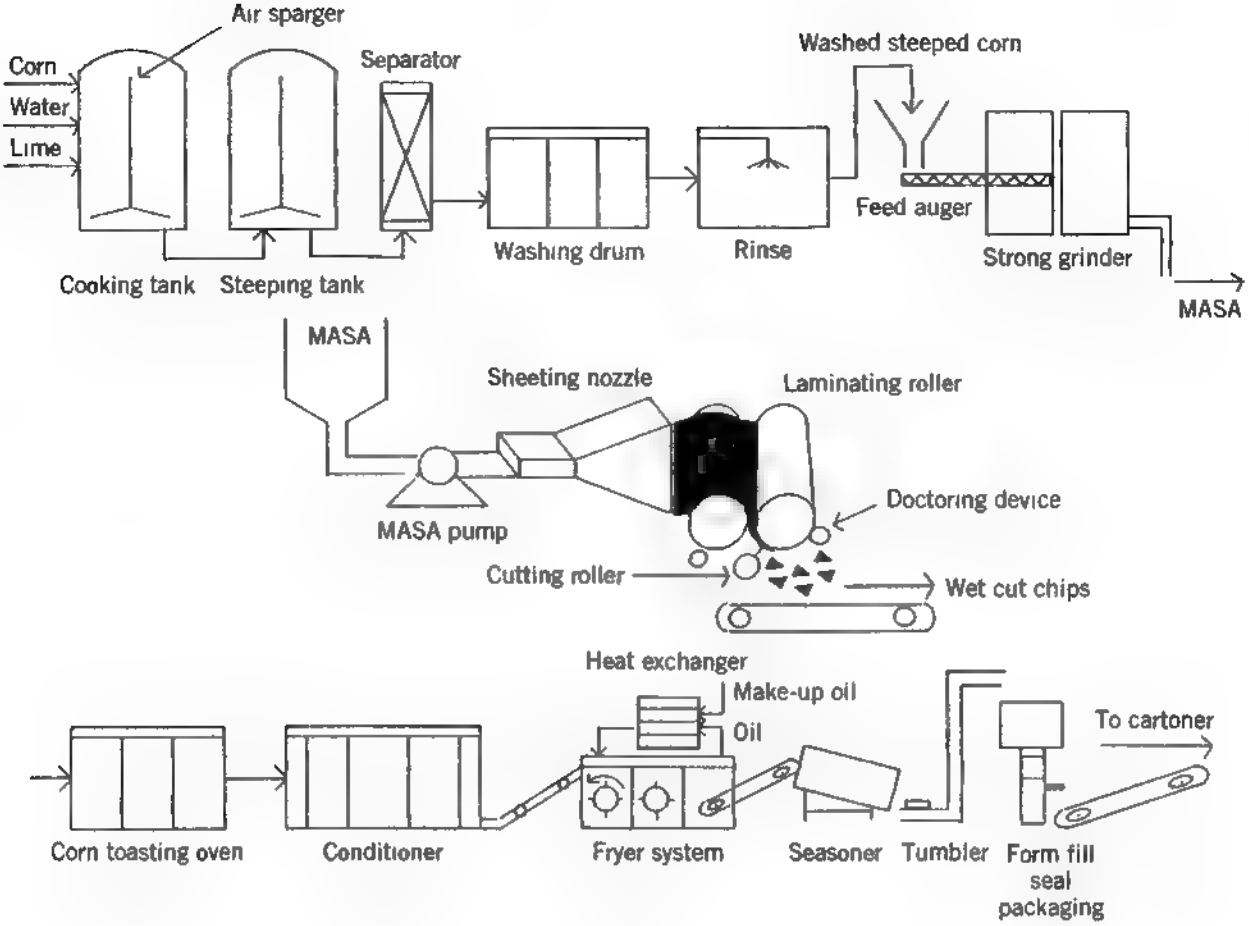

Air sparger
Corn
Water
Lime
Separator
Washed steeped corn
Feed auger
Washing drum
Rinse
Strong grinder
Cooking tank
Steeping tank
MASA
MASA
Sheeting nozzle
Laminating roller
Doctoring device
MASA pump
Cutting roller
Wet cut chips
Heat exchanger
Make-up oil
Oil
To cartoner
Corn toasting oven
Conditioner
Fryer system
Seasoner
Tumbler
Form fill seal packaging

Figure 2. Tortilla chip process outline.

ing, the corn is quenched to below 150°F to stop starch gelatinization. The cooked corn is then held in water or steeped from 8 to 24 h. At this point the moisture content has reached 45–50%. After steeping, the corn is pumped to a washing drum where lime and loose hulls are removed. The nixtamal is ground to masa in a stone grinding mill. Stone grinding is preferred because it provides the range of particle sizes needed for optimum frying performance and texture. Water added to the mill to facilitate grinding brings moisture of the ground corn or masa to 50–54%. The ground masa is immediately pumped to a spreading device that deposits a 0.5–1-in.-thick sheet onto sizing rollers. The rollers extrude the masa into an extremely thin sheet (less than 1/16 in), which is cut into the desired shape with roller cutters. The wet, newly cut chips, which lie on the belt in a monolayer are baked at extremely high temperatures (600–850°F) for 10–20 s. During the baking some moisture is driven off and a dry skin is formed around a moist interior. To prepare the baked chips for frying, the chips pass through a conditioning chamber where the moisture of the chip equilibrates and the chip cools. If moisture equilibration did not take place, the chips would expand and blister in the fryer. Conditioning equipment varies widely and consequently equilibration times range from 15 s to 15 min. In the conditioner the baked chips loose an additional 2% water and enter the fryer at 35–38% (7).

Tortilla chip fryers resemble potato chip fryers. They usually contain paddles to keep the chips moving downstream. The last section of the fryer contains a hold-down belt, which submerges the buoyant chips and ensures that all the chips will contain less than 1.5% water. Due to the lower water content of the tortilla chips, frying times are shorter than for potato chips, about 1 min at 350–370°F. The fat content of fried tortilla chips is about 25%. Chips are seasoned and coated while hot, then packaged. The process for fried corn chips is very similar to the tortilla chip process. Longer cook times and an increased water-to-corn ratio produces a somewhat softer corn. After stone grinding, the resulting masa contains 50–52% water and has a particle size range somewhat more coarse than the tortilla masa. The wet masa is extruded and cut into pieces that fall into very hot frying oil, about 400–410°F. The coarse structure of the masa ensures that pathways exist for the water to escape. Otherwise an expanded blistered chip would result. The fried chips are more friable than the tortilla chips and contain about 35% fat.

POPCORN

Why does popcorn pop when heated at atmospheric pressure and why do other cereal grains such as wheat, barley, rice, and dent corn fail to do so? The answer lies in the structure of the popcorn kernel itself. Popcorn kernels contain a strong pericarp or hull and a high percentage of translucent endosperm cells where densely packed starch granules are located. Most other grains contain opaque cells that have many intergranular spaces. During popping, the hull serves as a pressure vessel that allows the moisture held in the kernel to turn into superheated steam. Eventually the hull can no longer withstand the internal pressure and it fails. Rupture occurs at temperatures in the range 350–375°F (8). These temperatures correspond to saturated steam pressures inside the kernel of 135–185 psi. Microscopic studies have shown that after popping, cell walls are generally intact (8,9). It is believed that the tight packing of the translucent cells helps the escaping steam to create the foamed network. Opaque starch contains air spaces that provide channels for the escaping steam; consequently it does not foam or expand.

Popcorn quality, as perceived by the consumer, is strongly related to its expanded volume. Highly expanded popcorn is desired for its tenderness and "melt-in-the-mouth" characteristics. Kernel moisture and kernel test weight (bulk density or number of kernels per 10 g) both strongly influence the amount of expansion. Maximum expansion of popcorn typically occurs in the 12.5–15% total moisture range (8). However, each hybrid has its own optimum moisture content. The level of expansion at moistures below 10% is poor (8). Among hybrids, those with the highest test weights have the greatest expanded volumes (10). The percentage of totally popped kernels is strongly related to both popping temperature and moisture content. In one study, when the temperature of cooking oil was raised from 345 to 350°F the percentage of totally popped kernels rose from 70 to 94%. Similarly reduction in moisture content from 11.1 to 9.9% caused a reduction from 100% popping to 82% (8).

Commercial Popping

Today, most large commercial poppers use continuous dry or hot air poppers. Continuous poppers generally consist of a rotating drum with helical flights for conveying the popcorn. Hot air (410–430°F) is blown in from the feed end and heats the tumbling kernels, which are metered into the drum from above. Unpopped kernels, small pieces and hulls are separated with a screen, then the popped kernels are cooled and coated if desired. Because fresh popcorn is extremely hygroscopic, it must be immediately packaged in a moisture barrier, eg, foil, to preserve freshness. Coatings range from mixtures of oil, salt, seasonings, and coloring to candy caramel and nuts. Caramel corn is made by combining fresh popcorn with caramel, a molten mixture of sugar, glucose, butter, sodium bicarbonate, and flavor in a specialized agitated vessel. After coating, the caramel corn is cooled and separated into the appropriate cluster size before packaging.

Microwave Popcorn

Through the late 1980s microwave popcorn has been one of the fastest growing grocery product categories in the United States. By the end of 1987 annual microwave popcorn sales were estimated at $351 million, up from $124 million in 1985 and $246 million in 1986 (2). Most microwave popcorn is packed in an expandable bag that incorporates design features that focus or concentrate the microwave energy on a mixture of solid fat, popcorn and salt. Popcorn-to-fat ratios are approximately 3:1. The bags are generally designed to expand to allow room for the popped corn (11). Early commercial microwave popcorn products were refrigerated or frozen to provide fat and moisture stability. Newer packaging materials such as polyester-lined kraft paper provide about six months shelf stability for the fat-covered kernels and eliminate the need for freezing or refrigeration. The introduction of susceptors (metallized films that are good absorbers of microwave energy) to the microwave popcorn package has improved poppability by providing a localized source of intense heat (400–450°F) beneath the kernel–oil mixture.

EXTRUDED SNACKS

Extruded snack products fall into two broad categories: directly expanded or puffed, and pellets (dense half-products). Extrusion literally means forcing out. Many food formers such as hydraulic presses and double rollers are called extruders. However, the extruders used in snack food processing resemble those of the plastic industry. Rotating screws enclosed in a barrel convey a cereal dough and force it through a die. Products are cut to size with rotating knives. Both single and twin-screw extruders are used. Twin-screw extruders are more complex, more flexible, more easily controlled and operate over a wider range of moisture conditions. However, they are more costly. Single-screw extruders still are used extensively, particularly in the manufacture of expanded corn snacks and pellets.

Direct Expansion

Directly expanded snacks are made by extruding cereal grains and flours at rather low moisture levels (12–16%) and forcing them through a die. Corn grits are the most common ingredient. Rice flour, wheat, potato starch flour, and tapioca all expand well. Due to its high native fat content, oat flour does not provide the expanded puffy texture desired in a snack base. In puffing extrusion friction and shear combine to heat and plasticize the grains or flours into a pressurized mass of molten gelatinized dough. The pressure at the die end of the extruder is much greater than the saturated pressure of steam at the temperature where the dough is extruded, about 350°F. Thus the water becomes superheated. Consequently when the dough is forced through the die into the atmosphere, water and steam are released explosively and cause the gelatinized starch to expand into a foamed structure that sets up on cooling. The extruders used for puffed snacks range from extremely short length single-screw collet extruders that have a length-to-diameter ratio (*L/D*) of only 2–3 to 1 to long single- and twin-screw models of 20:30 *L* /*D* (12). Expanded puffs contain 5–9% water and must be dried or baked to below 2% moisture to achieve the desired crispness. Once dried, puffs are coated with oil and flavors. Cheese puffs are an example of a baked, coated expanded snack.

Fried Expanded

In a variation of direct expansion, corn grits of 15–18% water are extruded in a collet type extruder that contains a die or auxiliary rolling device modified to restrict the expansion of corn grit extrudate. The extrudate, in the form of irregular rods, is deep fried to remove residual water, impart a fried flavor, and slightly expand the extrudate. After frying the rods are coated, usually with cheese flavoring.

Pellets

The use of extruders to manufacture pellets is actually a new version of an old process. A number of Asian cultures make a starch paste of about 50% water containing flavoring such as shrimp. The paste is molded, gelatinized with steam, sliced, dried, and fried to a puff. Extruded pellets are made in much the same way. A blend of flours and starches that have good puffing characteristics, such as corn and potato, is combined with water and flavor (if desired) and extrusion cooked to gelatinize the starch. Moisture content ranges from 25 to 35%. The cooked mass must be cooled before it is formed to avoid expansion and produce a dense, glassy pellet. If a twin-screw extruder is used the dough can be cooled by venting steam and by external heat transfer before it is forced through a pasta-type die and knife assembly. In another version, two single-screw extruders are used (13). The first cooks and gelatinizes. As the dough exits the first extruder, water evaporates, and cools the dough mass that has foamed. The second extruder recompresses the mass and forces it through a shaping die. Pellet shapes are virtually unlimited. Shapes include grills, wagon wheels, spirals, shells, tubes, and playing card suits among others. Freshly extruded pellets are tacky, therefore they must be predried with air to prevent sticking in the final dryer. Final drying takes place in a rotating drum dryer equipped with temperature and humidity control. Pellets of about 10% moisture emerge after 6–8 h of drying (14). The shelf-stable pellets are extremely versatile and can be fried in only 10–15 s, puffed in hot air without any fat and can even be puffed in a microwave. Fried pellets contain 20–25% fat.

PRETZELS

Pretzels are a baked item made from a very stiff dough containing flour, shortening, malt, yeast, salt and sodium bicarbonate. Intricate machinery is used to make the classic twist pretzels from cut lengths of dough. Today many twist pretzels are extruded. Multiple sets of individually controlled augers force the dough through a die where the exiting dough is cut by wires. The pretzel receives its characteristic glaze and dark brown color as the result of a caustic dip. Before baking the raw, shaped pretzels are conveyed through a hot, 200°F, sodium hydroxide bath (0.5–1.25%) for about 10–15 s (15). The dipped pretzels are immediately sprinkled with enough salt so that about 2% remains on the finished product. Baking ovens vary considerably. In the United States many companies use a two-pass oven. In the first pass pretzels are baked at 425–475°F for about 5 min. At the end of the first pass the pretzels, now at 15% water, are removed from the baking belt with a doctor blade. A second belt returns the pretzels under the baking zone to dry them down to approximately 2% water.

NUTS

In the United States, peanuts are the major snack nut, commanding over a 50% share. Other major snack nuts include cashews, almonds, pistachios, macadamias, and sunflower seeds. Walnuts, pecans, and filberts are viewed primarily as in shell or baking commodities—not snacks. Most snack nuts are blanched (skin removed) and roasted to develop a nutty flavor and crunchy texture. Nuts are either roasted dry in air or in oil, in units that resemble fryers. Oil-roasted nuts are salted when the nuts are still warm to promote adhesion. A recent market trend has been honey roasting, where nuts are covered with a honey–water solution and coated with a sugar–starch mixture prior to roasting (16). Due to their high fat level nuts are extremely susceptible to oxidative rancidity. Consequently many snack nuts are vacuum packed.

MEAT-BASED SNACKS

Meat snack sales have grown steadily through the 1980s and now account for roughly 5% of the salted snack market. Major constituents of the segment are beef sticks, beef jerky, and popped pork skins.

Popped Pork Skins

Popped pork skins are produced from diced, rendered green pork skins. After a hot brine dip, skins are drained, cooled and diced into 0.5–1-in. pieces. Diced skins must be rendered, to remove fat and moisture. Skins are rendered by heating at 230–240°F in oil or lard that contains antioxidants and antifoaming agents (17). After 4 h or so of rendering, the diced pellets rise and are removed. Following cooling and draining the pellets are fried at 400–425°F to create a puffed product.

Jerky

Jerky probably has its origins with native Americans who simply stripped or jerked the muscle from game animals and dried it slowly in the wind or sun or over smoky fires. This has evolved to a process where beef is marinated, optionally smoked, and carefully dried. Restructured jerkys, made from ground meat, are gaining in popularity owing to ease of manufacture. Ground meat is mixed with curing aids, seasonings such as pepper and garlic, sugar, salt, dextrose, sodium nitrite and sodium erythorbate and extruded or formed into thin strips. Formed strips, which may be frozen are cured and dried for approximately 12 h at 150°F (18).

Beef Sticks

Beef sticks and other related meat stick products such as snack-size pepperoni are classified as fermented sausages. However, some meat snack sticks are acidulated, often with glucose-δ-lactone to achieve the desired pH and sharp taste. Chilled meat (32–34°F) is ground or chopped with a silent cutter into coarse pieces. Salt, spices, flavorings, curing salts and a fermentable carbohydrate such as dextrose is blended in along with a lactic acid starter culture, or A_w acidulant. In preparing fermented sausages it is crucial to control the amount of carbohydrate and the distribution of the starter culture (19). Good distribution ensures uniformity, whereas the amount of dextrose dictates the final pH. After stuffing, the sausages ferment at temperatures that range from 65 to 120°F but typically 90 to 110°F depending on the culture. Sausages can be stuffed into strippable cellulose casings or coextruded into reconstituted collagen. The end of fermentation is signaled by achievement of the final desired pH, eg, 4.8. Following fermentation, which may include smoking, the sticks are dried. Drying temperatures vary considerably. However, sausages that contain pork must reach an internal temperature of 137°F to destroy trichinae parasites (19).

PACKAGING

Snack packaging must answer to many challenges. Snacks are generally high in fat, very low in moisture, and often fragile. Both oxygen and light barriers are needed to combat rancidity. Potato chips in particular are subject to light damage. Moisture barriers are needed to prevent sogginess and to maintain crispness. Metallized films that protect against these elements have become the packaging material of choice. The opacity of the metallized film also offers the ability to hide crumbs from the consumer. Polyester and polypropylene laminates are common. Polypropylene is preferred when it is necessary to protect the bag or pouch from punctures from sharp snack pieces such as tortilla chips. Pouches or bags made on form–fill–seal machines have become the primary package for fragile, salted snacks due to their low relative cost, their ability to display attractive graphics and their ability to cushion. In the most modern plants, computer controlled, multiple head scaling units dose products into pouches with a high level of accuracy.

TRENDS

Although snacks are an indulgent item, they have not been immune from consumer demands for less fat, more fiber, low salt, and no cholesterol. Nutritional snacks such as rice cakes, dried fruit, and sunflower seeds have established strong market niches. No salt potato chips and pretzels are on the market, while the resurgence of popcorn may be due to its high fiber content. It is anticipated that more snacks will incorporate fiber. There is ample opportunity for introducing fiber into fabricated chips, extruded pellets, and tortilla chips. Technology is available for producing lower fat potato chips. It is expected that consumer demand will soon establish a segment for lower-calorie fried snacks despite the superior taste and texture of the full-fat products. Over the last few years flavors have become more sophisticated and spicier. Cajun, Mexican, cream cheese and dill, seafood, Italian (herbs and cheese), Indian (curry), and even hot pepper flavored snacks have all been introduced. Shape and texture are also becoming increasingly important. The introduction of extruded pellet technology has dramatically opened up the possibilities of snack shapes. Kettle potato chips have established a crunchier, tastier potato chip category. Rippled, ridged, corrugated, and undulated cuts offer diversity in texture. At the same time other snack segments are moving toward simplicity. Such products include white popcorn and uncoated tortilla chips. Sweet tastes have intruded into the salted snack market. Honey coating, introduced with nuts, has helped launch sweet coated popcorn and potato chips. Despite all the innovations, it is expected that traditional fried, salted potato chips will continue to lead the salted snack market.

BIBLIOGRAPHY

1. "1987 Snack Food Association Consumer Attitude Report," *Snack World* **44**, 1–35 (Sept. 1987).
2. "20th Annual State of the Snack Food Industries Report," *Snack Food* **77**, M1–M40 (June 1988).
3. R. E. Hardenburg, A. E. Watanda, and C. Y. Weng, *The Commercial Storage of Fruits and Vegetables, and Florist and Nursery Stocks*, USDA Agriculture Handbook no. **66**, U.S. Government Printing Office, Washington, D.C., 1986.
4. O. Smith, "Potato Chips," in W. F. Talburt and O. Smith, eds., *Potato Processing*, 3rd ed., AVI Publishing Co., Inc., Westport, Conn., 1975.
5. "*Potato Chip Processing*," Heat and Control, Inc., So. San Francisco, 1986.
6. M. S. Bedolla and L. W. Rooney, "Cooking Maize for Masa Production," *Cereal Foods World* **27**, 219–221 (May 1982).
7. M. H. Gomez, L. W. Rooney, R. D. Waniska, and R. L. Pflugfelder, "Dry Corn Masa Flours for Tortilla and Snack Food Production," *Cereal Foods World* **32**, 372–377 (May 1987).
8. R. C. Hoseney, K. Zeleenak, and A. Abdelrahman, "Mechanism of Popcorn Popping," *Journal of Cereal Science* **1**, 43–52 (Jan. 1983).
9. R. M. Reeve and H. G. Walker, "The Microscopic Structure of Popped Cereals," *Cereal Chemistry* **46**, 227–241 (May 1969)
10. C. G. Haugh, R. M. Lien, R. E. Hanes, and R. B. Ashman, "Physical Properties of Popcorn," *Trans. ASAE* **19**, 168–171, 176 (Jan.–Feb. 1976).
11. U.S. Pat. 4,450,180 (May 22, 1984), J. D. Watkins (to Golden Valley Foods, Inc.).
12. G. Toft, "Snack Foods: Continuous Processing Techniques," *Cereal Foods World* **24**, 142–143 (Apr. 1979).
13. O. B. Smith, "Extrusion—Cooked Snacks in a Fast Growing Market," *Cereal Science Today* **19**, 312–316 (Aug. 1974).

14. M. Byrne, "The Shape of Things to Come," *Food Manufacture*, 59–61 (Oct. 1982).
15. S. A. Matz, *Snack Food Technology*, 2nd ed., AVI Publishing Company, Inc., Westport, Conn., 1984, pp. 180–182.
16. U.S. Pat. 4,161,545 (July 17, 1979), W. M. Green and M. W. Hoover
17. S. A. Matz, *Snack Food Technology*, 2nd ed., AVI Publishing Company, Inc., Westport, Conn., 1984, pp. 130–133.
18. U.S. Pat. 4,239,785 (Dec. 16, 1980), E. N. Roth.
19. L. Long, S. L. Komarik, and D. K. Tressler, *Food Products Formulary, Vol. I, Meats, Poultry, Fish, Shellfish*, 2nd ed., AVI Publishing Company, Inc., Westport, Conn., 1982.

GENERAL REFERENCES

M. Byrne, "The Boom in Snack Foods," *Food Eng. Int.* **18**, 49–50, 52, 54, 57–58 (1993).

J. I. Duffy, ed., *Snack Food Technology Recent Developments*, Noyes Data Corporation, Park Ridge, N.J., 1981.

B. Gebhardt, "Oils and Fats in Snack Foods," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., John Wiley & Sons, New York, 1996, pp. 409–427.

R. C. E. Guy, "Snack Foods Product Design II, Snack Food Formulation," *Food Ingred. Analysis Int.*, 25–26 (1997).

J. M. Harper, *Extrusion of Foods*, Vols. 1 and 2, CRC Press, Inc., Boca Raton, Fla., 1981.

M. Hilliam, "Trends in Snack Foods," *Food Processing* **66**, 14–15 (1997)

R. Holding, "Coextruded Three Dimensional Snack Foods," *Proc. Grain Processing Asia* **95**, 56–57 (1995).

P. Hollingsworth, "Snack Foods," *Food Technol.* **49**, 58, 60, 62 (1995)

D. Howling, "Convenience and Snack Foods," *Eur. Food and Drink Rev.*, 69, 71 (1991).

K. J. Lee, "Utilization of Korean Maizes in Production of Alkaline Processed Snack Foods," *J. Food Sci. Nutrition* **2**, 11–16 (1997).

S. A. Matz, *Snack Food Technology*, 2nd. ed., AVI Publishing Co., Westport, Conn., 1984.

T. M. Midden, "Impingement Air Baking for Snack Foods," *Cereal Foods World* **40**, 532–535 (1995).

M. N. Riaz, "Technology of Producing Snack Foods by Extrusion," *Tech. Bull. Amer. Inst. Baking Res. Dept.* **19**, 1–8 (1997).

T. P. Shukla, "Future Snacks and Snack Food Technology," *Cereal Foods World* **39**, 704–705 (1994).

W. F. Talburt and O. Smith, *Potato Processing*, 3rd. ed., AVI Publishing Co., Westport, Conn., 1975.

P. Tettweiler, "Snack Foods Worldwide," *Food Technol.* **45**, 58, 60, 62 (1991).

Robert E. Altomare
D. Mark Kettunen
Charles J. Cante
General Foods USA
Tarrytown, New York

SOLID-STATE FERMENTATION AND VALUE-ADDED UTILIZATION OF FRUIT AND VEGETABLE PROCESSING BY-PRODUCTS

SOLID-STATE FERMENTATION

Definition

Solid-state fermentation, or solid-substrate fermentation (SSF), while difficult to define precisely, is generally referred to as the process in which microbial growth and product formation occur on the surface of solid materials in the absence or near-absence of free water, and the substrate contains certain moisture that exists in absorbed form within the solid matrix (1,2).

Solid-state fermentation deals with the utilization of water-insoluble materials for microbial growth and metabolic activities. It is different from surface culture, which uses either a solid or liquid substrate, and refers primarily to the mode of growth (3). It is also distinguished from submerged liquid fermentation/culture by the fact that microbial growth and product formation occur at or near the surfaces of solid materials with low moisture contents. Microbial activities cease at a certain low level of moisture content, and this establishes the lower limit at which solid-state fermentation can take place (3). The upper limit for solid-state fermentation is a function of absorbency of the medium which varies with the substrate material type (3).

Solid-state fermentations are not as well as characterized on a fundamental scientific or engineering basis as are the submerged liquid cultures that have been used almost exclusively in the West for the industrial production of microbial metabolites (2). They are, however, widely used in the orient for thousands of years, and traditional methods used in food processing have been modernized and extended to nontraditional products (1,2). Today solid-state fermentation is increasingly gaining interest in the world for development of value-added products from a variety of cheap materials, and for bioremediation of agricultural and industrial wastes.

History

Solid-state fermentation has been used long before the underlying microbiological or biochemical processes involved were understood. The use of naturally occurring microorganisms in the preparation of foods such as bread and cheese, or directly as food such as mushrooms, dates back many centuries, and these are some examples of traditional solid-state fermentation systems (4). As early as 2600 B.C., Egyptians were making bread by methods essentially similar to those of today (4). In Asia, cheese had been prepared as food for several hundred years before the birth of Christ (4). The preparation of koji for soy sauce and miso production in Japan and Southeast Asia goes back as far as 1000 years ago and probably 3000 years ago in China (4,5). Preservation of fish, meat, and other animal products by solid-state fermentation goes back about 2500 years (4). Vinegar was produced by solid-state fermentation from fruit pomace in the eighteenth century (5). The production of gallic acid is another early example of solid-state fermentation, and its discovery was made in the eighteenth century (4). Solid-state composting was used for sewage treatment in the late nineteenth century (4,5). The production of fungal and other microbial enzymes by solid-state fermentation started during the early twentieth century. The new fermentation introduced from 1920 to 1940 was the production of gluconic acid, citric acid, and enzymes, as well as the development of rotary drum fermenter (4,5). Between 1940 and 1950, the fermentation industry developed very rapidly, and the first clinically useful

14. M. Byrne, "The Shape of Things to Come," *Food Manufacture*, 59–61 (Oct. 1982).
15. S. A. Matz, *Snack Food Technology*, 2nd ed., AVI Publishing Company, Inc., Westport, Conn., 1984, pp. 180–182.
16. U.S. Pat. 4,161,545 (July 17, 1979), W. M. Green and M. W. Hoover
17. S. A. Matz, *Snack Food Technology*, 2nd ed., AVI Publishing Company, Inc., Westport, Conn., 1984, pp. 130–133.
18. U.S. Pat. 4,239,785 (Dec. 16, 1980), E. N. Roth.
19. L. Long, S. L. Komarik, and D. K. Tressler, *Food Products Formulary, Vol. I, Meats, Poultry, Fish, Shellfish*, 2nd ed., AVI Publishing Company, Inc., Westport, Conn., 1982.

GENERAL REFERENCES

M. Byrne, "The Boom in Snack Foods," *Food Eng. Int.* **18**, 49–50, 52, 54, 57–58 (1993).

J. I. Duffy, ed., *Snack Food Technology Recent Developments*, Noyes Data Corporation, Park Ridge, N.J., 1981.

B. Gebhardt, "Oils and Fats in Snack Foods," in Y. H. Hui, ed., *Bailey's Industrial Oil and Fat Products*, Vol. 3, 5th ed., John Wiley & Sons, New York, 1996, pp. 409–427.

R. C. E. Guy, "Snack Foods Product Design II, Snack Food Formulation," *Food Ingred. Analysis Int.*, 25–26 (1997).

J. M. Harper, *Extrusion of Foods*, Vols. 1 and 2, CRC Press, Inc., Boca Raton, Fla., 1981.

M. Hilliam, "Trends in Snack Foods," *Food Processing* **66**, 14–15 (1997)

R. Holding, "Coextruded Three Dimensional Snack Foods," *Proc. Grain Processing Asia* **95**, 56–57 (1995).

P. Hollingsworth, "Snack Foods," *Food Technol.* **49**, 58, 60, 62 (1995)

D. Howling, "Convenience and Snack Foods," *Eur. Food and Drink Rev.*, 69, 71 (1991).

K. J. Lee, "Utilization of Korean Maizes in Production of Alkaline Processed Snack Foods," *J. Food Sci. Nutrition* **2**, 11–16 (1997).

S. A. Matz, *Snack Food Technology*, 2nd. ed., AVI Publishing Co., Westport, Conn., 1984.

T. M. Midden, "Impingement Air Baking for Snack Foods," *Cereal Foods World* **40**, 532–535 (1995).

M. N. Riaz, "Technology of Producing Snack Foods by Extrusion," *Tech. Bull. Amer. Inst. Baking Res. Dept.* **19**, 1–8 (1997).

T. P. Shukla, "Future Snacks and Snack Food Technology," *Cereal Foods World* **39**, 704–705 (1994).

W. F. Talburt and O. Smith, *Potato Processing*, 3rd. ed., AVI Publishing Co., Westport, Conn., 1975.

P. Tettweiler, "Snack Foods Worldwide," *Food Technol.* **45**, 58, 60, 62 (1991).

ROBERT E. ALTOMARE
D. MARK KETTUNEN
CHARLES J. CANTE
General Foods USA
Tarrytown, New York

SOLID-STATE FERMENTATION AND VALUE-ADDED UTILIZATION OF FRUIT AND VEGETABLE PROCESSING BY-PRODUCTS

SOLID-STATE FERMENTATION

Definition

Solid-state fermentation, or solid-substrate fermentation (SSF), while difficult to define precisely, is generally referred to as the process in which microbial growth and product formation occur on the surface of solid materials in the absence or near-absence of free water, and the substrate contains certain moisture that exists in absorbed form within the solid matrix (1,2).

Solid-state fermentation deals with the utilization of water-insoluble materials for microbial growth and metabolic activities. It is different from surface culture, which uses either a solid or liquid substrate, and refers primarily to the mode of growth (3). It is also distinguished from submerged liquid fermentation/culture by the fact that microbial growth and product formation occur at or near the surfaces of solid materials with low moisture contents. Microbial activities cease at a certain low level of moisture content, and this establishes the lower limit at which solid-state fermentation can take place (3). The upper limit for solid-state fermentation is a function of absorbency of the medium which varies with the substrate material type (3).

Solid-state fermentations are not as well as characterized on a fundamental scientific or engineering basis as are the submerged liquid cultures that have been used almost exclusively in the West for the industrial production of microbial metabolites (2). They are, however, widely used in the orient for thousands of years, and traditional methods used in food processing have been modernized and extended to nontraditional products (1,2). Today solid-state fermentation is increasingly gaining interest in the world for development of value-added products from a variety of cheap materials, and for bioremediation of agricultural and industrial wastes.

History

Solid-state fermentation has been used long before the underlying microbiological or biochemical processes involved were understood. The use of naturally occurring microorganisms in the preparation of foods such as bread and cheese, or directly as food such as mushrooms, dates back many centuries, and these are some examples of traditional solid-state fermentation systems (4). As early as 2600 B.C., Egyptians were making bread by methods essentially similar to those of today (4). In Asia, cheese had been prepared as food for several hundred years before the birth of Christ (4). The preparation of koji for soy sauce and miso production in Japan and Southeast Asia goes back as far as 1000 years ago and probably 3000 years ago in China (4,5). Preservation of fish, meat, and other animal products by solid-state fermentation goes back about 2500 years (4). Vinegar was produced by solid-state fermentation from fruit pomace in the eighteenth century (5). The production of gallic acid is another early example of solid-state fermentation, and its discovery was made in the eighteenth century (4). Solid-state composting was used for sewage treatment in the late nineteenth century (4,5). The production of fungal and other microbial enzymes by solid-state fermentation started during the early twentieth century. The new fermentation introduced from 1920 to 1940 was the production of gluconic acid, citric acid, and enzymes, as well as the development of rotary drum fermenter (4,5). Between 1940 and 1950, the fermentation industry developed very rapidly, and the first clinically useful antibiotic, penicillin, was produced by both solid-state fermentation and submerged culture method (4). During the decade from 1950 to 1960, steroid transformation by fungal spores, which was produced by solid-state fermentation, was developed (4). From 1960 to 1980, many important new solid-state fermentation processes were developed, which include the production of mycotoxins and the treatment and reuse of animal, plant, and domestic wastes (4,5). Since 1980 the developments of solid-state fermentation have been made in every aspect: expansion of microbial types, utilization of wastes as cheaper substrates, improvement of fermenter design, and discovery of new products. The history and recent developments in solid-state fermentation have been reviewed in detail by several authors (3–5).

Microbial Types

Although a wide range of microorganisms are able to grow on solid substrates, a relatively few genera and species are employed in the main commercial systems. Filamentous microorganisms are most widely used in solid-state fermentations. The ability of such microorganisms to colonize substrates by apical growth and penetration gives them a considerable ecological advantage over nonmotile bacteria and yeasts, which are less able to multiply and colonize on low-moisture substrates (6). Among the filamentous fungi, three classes have gained the most practical importance in SSF: the phycomycetes such as the genera *Mucor*, and *Rhizopus*, the ascomycetes with the genera *Aspergillus* and *Penicillium*, and the basidiomycetes, especially the white rot fungi such as edible mushrooms (3).

Bacteria and yeasts usually grow on solid substrates at the 40 to 70% moisture levels and can play important roles in some solid-state fermentations (3,6). In composting, thermophilic bacteria grow predominantly when the temperature exceeds 60°C, while ensiling processes are predominated by lactic acid bacteria (7). In food fermentations, the best-known yeast genera such as *Saccharomyces*, *Candida*, and bacterial genera such as *Lactobacillus* and *Bacillus*, have established their commercial roles (6).

Very few actinomycetes were used in solid-state fermentation. Jermini and Demain (8) first developed a solid-state fermentation system for the production of cephalosporin antibiotics by *Streptomyces clavuligerus* grown on barley, and approximately 300 μg of cephalosporins per gram of substrate was produced under the optimal conditions for seven days. Sircar et al. (9) recently optimized a solid-state fermentation medium for the production of clavulanic acid antibiotics by *Streptomyces clavuligerus* grown on a medium consisted of wheat rawa, soya flour, dipotassium hydrogen phosphate, and sunflower oil cake.

Substrates

Both natural and synthetic substances can be used in solid-state fermentation. The main substrates for solid-state fermentation are insoluble in water, and water is absorbed into substrate particles that can be used by microorganisms for growth and metabolic activity. Bacteria and yeasts grow on the surface of the substrate while fungal mycelia penetrate into the particles of the substrate (5). The substrates used are, with the exception of synthetic media, usually cheap agricultural raw materials and its by-products (10).

The most widely used substrates for solid-state fermentation in practice are mainly materials of plant origin, which include starchy materials such as grains, rice, corn, roots, tubers and legumes, and cellulosic, lignin, proteins, and lipid materials (6). These organic materials in nature are mixtures of polymeric compounds in structure, which act as a source of carbon, nitrogen, and other nutrients as well as providing anchorage for the microorganisms (5). Recent interest in solid-state fermentation has put more weight on cheaper substrates, including various agricultural or agroindustrial by-products. Agricultural and food processing wastes such as wheat bran, cassava, sugar beet pulp, bagasse, citrus peel, corn cob, banana waste, sawdust, wheat and rice straw, and fruit pomace are the most commonly used substrates for solid-state fermentation. Such by-products are usually lignocellulosic and pectin-rich wastes in nature and have been used in SSF to produce many value-added products.

Products

Several products are generated from solid-state fermentation. Some products are directly used as foods. For example, many kinds of higher fungi, edible mushrooms, have been cultivated and used as human food for centuries in China and Japan. They usually grow on agricultural residues, such as wheat straw, wood sawdust, grasses, horse manure, cotton seed crust, and fruit pomaces (11,12). In ancient time, the bread was made through natural solid-state fermentation by indigenous microorganisms. Today people use the pure culture of baker's yeast to produce bread (13). Roquefort and Camembert represent the two important types of mold-fermented cheese. *Penicillium roqueforti* is the blue mold of Roquefort cheese while *Penicillium camemberti* is the white mold of Camembert (14). *Penicillium* species and *Mucor* species are often used in fermented sausage production (15,16).

Among many oriental fermented products, soy sauce is probably the most well known in the diet of Western countries (17). Soy sauce is made from soybeans and wheat flour fermented by molds. The main microorganisms include *Aspergillus oryzae* (koji) and yeasts and lactic acid bacteria (18). Tempeh, a vegetarian meat analogue and source of vitamin B-12 (generally lacking in vegetarian diets), is yet another product made in the orient by solid-state fermentation (19,20).

Solid-state fermentation can be used not only as a tool for nutritional enrichment but also as a means of reducing toxins in raw substrates (21). It was reported that the tempeh mold *Rhizopus oligosporus* could decrease the aflatoxin content of peanut presscake by 70% during fermentation (22). A significant reduction in antinutritional and toxic components in breads and other plant-derived foods by solid-state fermentation was observed (23). Biomass production is another important aspect of bioconversion from wastes to value-added feed or food through solid-state processes (24). A recent study shows that carotenoid could be produced from cornmeal by solid-state fermentation using *Penicillium* species, with a maximum yield of 5.26 mg of carotenoid per 100 g of substrate (25).

Solid-state fermentation has been used for producing several industrially important products today. Production of many kinds of important organic acids has been successfully achieved via solid-state fermentation. Such organic acids include citric acid (26), lactic acid, acetic acid, gluconic acid, glutamic acid (27), linolenic acid (28), and other fatty acids (29), and phenolics such as ferulic acid (30). In addition, Hesseltine (31) used rice as the substrate to produce aflatoxin by *Aspergillus flavus* and *Aspergillus parasiticus* via solid-state fermentation.

The best potential application of solid-state fermentation, however, is the production of various industrial enzymes such as amylases, proteases, cellulases, pectinases, rennet, invertase, lactase, peroxidase, and so on. The production of cellulase, α-amylase and β-glucosidase from agricultural by-products by solid-state fermentation was investigated by Zheng et al. (32,33). Glucoamylases were produced by solid-state fermentation from cassava starch using *Rhizopus* species (34) or corn flour and wheat bran (35). A thermostable α-amylase was produced by thermophilic *Bacillus coagulans* in solid-state fermentation (36). A thermostable α-L-arabinofuranosidase was produced by solid-state fermentation with *Thermoascus aurantiacus* on sugar beet pulp (37). α-Galactosidase was formed by *Aspergillus niger* on wheat and rice bran–based solid-state medium (38). A novel carbohydrase complex was produced from solid-state fermentation by the aerobic fungus *Penicillium capsulatum* (39). A protease was produced from wheat bran by *Rhizopus oryzae*, with a maximum enzyme activity of 341 units per gram of wheat bran (40). A lipase was produced by solid-state fermentation using gingelly oil cake as the substrate from *A. niger*, and the enzyme activity of 363.6 units per gram of dry substrate was obtained under optimal conditions (41).

Numerous products were produced by solid-state fermentation from fruit processing wastes, and they will be discussed later in this article.

Factors Affecting SSF

Solid-state fermentations are in general carried out in fermenters of simple construction and operation and without the range of control units found in liquid fermentation systems. Laboratory studies are usually carried out in conical flasks, beakers, Rowx bottles, jars, or glass incubators. For a pilot-plant or large-scale SSF, however, the design of bioreactors in batch or continuous mode has been empirical in nature (42). For a solid-state fermentation with a selected microorganism, the success of the process will depend on culture condition with the controlled parameters including mainly nutrient supplements, moisture content, temperature, pH, aeration, and agitation.

Nutritional factors usually limit the growth of fungi. In solid-state fermentation, this limitation is much more severe due to the limited diffusion rate of the substrate and the limited access of the fungus to the substrate. An important indicator of nutritional regulation of growth in solid-state fermentation is the C/N ratio. Optimal C/N ratio varies in a wide range from 10 to 100 or higher in various SSF processes, but the availability of C and N can be more important than the ratio (3). In most SSF systems, the C source comes from the natural starchy or cellulosic materials while the N source is added. The most commonly used synthetic N sources are NH_4Cl, $(NH_4)SO_4$, NH_4NO_3 and urea, while some organic N sources such as soybean cake and yeast extract are often used in solid-state fermentation.

The moisture level of the substrate will have a determining influence on the success of the overall process. A better way of expressing moisture content is water activity (a_w), which gives the availability of water for growth of microorganisms. In general, the types of the microorganisms that can grow in SSF systems are determined by the water activity. Most bacteria grow at higher a_w values while filamentous fungi and some yeasts can grow at lower a_w level. The microorganisms, which can grow and are capable of carrying out their metabolic activities at lower a_w level, are suitable for SSF processes. Water activity level in SSF is governed by the nature of substrate, the type of end product, and the requirement of microorganisms. A high a_w level results in decreased porosity or intracellular spaces, lower oxygen diffusion, decreased gas exchange, enhanced formation of aerial mycelium, decreased substrate degradation and an increased risk of bacterial contamination (43). In contrast, low a_w will lead to decreased substrate swelling and decreased microbial growth (43).

Temperature is another critical parameter that can affect SSF processes, since every microorganism has its own optimal temperatures for growth and for metabolism, which are often different. During solid-state fermentation, a large quantity of metabolic heat is generated and this is directly related to the metabolic activities of the microorganism (43,44). It is not difficult to control the temperature for solid-state fermentation in the laboratory; in large-scale SSF, however, due to high substrate concentration, microbial heat generation is much greater per unit volume than in liquid fermentation, and therefore, constant heat removal for temperature control is a major problem (43,44).

Although pH is one of the critical factors, the monitoring and control of pH during solid-state fermentation is not usually attempted. It is difficult to monitor and control pH in the solid-state fermentation, since pH electrodes able to measure the pH of the moist solids, in the absence of free water, are not available (43). On the other hand, good buffering capacity of some of the substrates used in SSF helps in eliminating the need for pH control during fermentation. This advantage is, therefore, exploited in the initial adjustment of the pH of the solids in the range of 4.4 to 5.0 during moistening by using water at the desired pH level (43). Another approach in counteracting the acidification of the fermenting mass is the use of urea as the nitrogen source rather than ammonium salts (44).

For most solid-state fermentations, oxygen is essential and is usually achieved by free air exchange or forcing sterile air under pressure through the fermenting mass (43). The mechanism of O_2 transfer from the circulating air into the aerobic microorganisms is unclear, but most probably it occurs via the O_2 dissolved in the water film around the particle surface (43,44). Aeration not only provides oxygen but also simultaneously removes carbon dioxide, other volatile metabolites and heat from the fermenter (44).

Agitation is not employed in many solid-state fermentation processes; however, it is usually an essential part of periodically or continuously agitated aerobic solid-state fermentations (44). Agitation of the fermenting mass can ensure homogeneity with respect to temperature and gaseous environment and promote heat and gas transfers. It also helps for the uniform distribution of nutrients during the course of fermentation (43,44).

Comparison to Liquid Fermentation

Solid-state fermentation has numerous advantages over the conventional stirred or aerated liquid fermentation both on a laboratory and large scale. First of all, the medium is relatively simple (4). For instance, cereal grain, legumes, or other plant and animal products, various agricultural and food processing wastes, are all potential substrates of solid-state fermentation. Usually the only other medium component required in SSF is water, although occasionally other nutrients such as nitrogen sources or minerals may be added (4). Other advantages of solid-state fermentation over liquid fermentation include higher substrate concentration, less probability of contamination, superior productivity, improved product recovery, reduced energy and water requirements and wastewater output, and lower capital investment (4,45).

Despite solid-state fermentation being both economically and environmentally attractive, their biotechnological exploitation has been rather limited (5). Compared with liquid fermentation, the major problems associated with solid-state fermentation include the limitations on microorganisms, medium heterogeneity, heat and mass transfer control, growth measurement and monitoring, and scale-up problems (4,44,45).

On the whole, SSF may be the exclusive tool, especially when demand for the product is limited, as is the case for some industrial enzymes. Furthermore, SSF has demonstrated a great potential in bioconversion of agricultural and industrial wastes into useful value-added products. In addition to obtaining a variety of value-added products, SSF system can also be used in environmental remediation of agricultural by-products.

FRUIT AND VEGETABLE PROCESSING WASTES

Apple Pomace

Apple pomace is the residual left after juice extraction and represents about 25% of the original fruit. It is rich in carbohydrate but low in protein and fat contents. The freshly pressed apple pomace has a low pH ranging from 3.1 to 3.8, with a bulk density of 935 kg/m^3 (46,47). Since it is produced wet with high moisture content, it is susceptible to rapid growth of microorganisms. The major composition of apple pomace is shown in Table 1.

Table 1. Chemical Composition of Apple Pomace

Constituent	Fresh pomace (%)	Dried pomace (%)
Moisture	66.4–78.2	11.0–12.5
Carbohydrates	9.5–22.0	—
Nitrogen-free extract	—	54.8–59.3
Pectin	1.5–2.5	15.0–18.0
Crude fiber	4.3–10.5	15.3–20.6
Proteins	1.03–1.82	4.45–5.67
Fat (ether extract)	0.82–1.43	3.75–4.65
Ash	0.56–2.27	2.11–3.50
Potassium (as K_2O)	0.2–0.6	—
Phosphorus (as P_2O_5)	0.4–0.7	—

Source: Ref. 46.

It is estimated that nearly 36 million tons of apples are produced annually in the United States, and approximately 45% of which is used for processing purposes, with a primary by-product of apple pomace, which results from processing for juice, cider, applesauce, or slices (46). More than 500 apple-processing plants in the United States generate a total of about 1.3 million metric tons of apple pomace each year, and the annual disposal fees for apple pomace alone exceed $10 million in the United States (47,48). The high level of organic contents in apple pomace will result in environmental pollution if they are disposed directly to the environment.

Other Fruit and Vegetable Processing Wastes

The food processing industry in the United States generates large amount of various by-products as wastes each year, a large portion of which comes from fruit and vegetable processing industries. It is estimated that approximately 9 million tons of solid residuals are generated annually from the fruit and vegetable processing industry in the United States (49). In addition to apple pomace, other major fruit and vegetable processing wastes come from citrus, grape, cherries, berries, banana, olive, peach, pear, pineapple, kiwifruit, apricot, sweet corn, tomato, potato, asparagus, beans, peas, carrots, beet, pumpkin, squash, spinach, and sauerkraut processing industries (49–56). The primary sources of solid residues resulting from fruit and vegetable processing operations include sorting, cutting, slicing, peeling, pulping, and pressing (49).

Traditionally about 79% of the fruit and vegetable processing wastes is used for animal feed, and the remaining 21% is handled as waste (49). The solid wastes contain mainly soluble sugar and other hydrolyzable materials with small amounts of crude fiber. Disposal of such wastes may present an added cost to processors, and direct disposal to soil or in a landfill may not continue to be acceptable. Thus, exploration of potential value-added uses for pomace is highly attractive.

UTILIZATION OF FRUIT AND VEGETABLE PROCESSING WASTES VIA SOLID-STATE FERMENTATION

Fertilizer

Although direct disposal of fruit processing wastes in landfills has become environmentally unacceptable, some fruit processing wastes can be composted under anaerobic condition, and then used as fertilizer in landfills, because such wastes were readily degraded under anaerobic digestion conditions (57,58). Solid-state fermentation can also be used for composting of fruit and vegetable processing wastes such as apple waste (59) and tomato pomace (60). Composted apple pomace and other fruit and vegetable processing wastes can be used in nursery potting mixes and as field soil amendments (61). Composted grape pomace was used as an organic fertilizer in vineyards for growing grapes (62).

Animal Feed and Single-Cell Protein

Apple pomace was directly used as a feed for cows and sheep, but its value as an animal feed is less than $7 per ton (47). Apple pomace is a poor animal feed supplement because of its low protein content. The nutritional value of apple pomace was improved by solid-state fermentation with a food yeast, *Candida utilis* (62). Yeast fermentation resulted in a 2.5-fold increase in protein, 3.4-fold increase in niacin, 10-fold increase in pantothenic acid, 1.5-fold increase in riboflavin, and 1.2-fold increase in thiamine (62). An improved stock feed was produced from apple pomace by solid-state fermentation with *Kloeckera apiculate* and *C. utilis* (63,64).

A solid-state fermentation of orange processing wastes with *Aspergillus niger* and *Rhizopus* species enriched the protein content by 300%, and the fermented product could be readily sold as animal feed (65). Banana wastes were also used for protein enrichment and protein and biomass production by solid-state fermentation using *A. niger* (66), yeast *Pichia spartinae* (67), and *Saccharomyces uvarum* (68).

As the world population increases, there is a greater demand for new sources of protein for formulating new types of food. For this reason, there is a great interest in the use of single-cell protein as a source of protein for animals or humans. Apple pomace is a potential substrate for producing single-cell protein. The microorganisms that can be used for single-cell protein production include yeast, filamentous fungi, bacteria, and algae. It is well recognized that these microorganisms have the ability to produce protein and other essential nutrients (49). Yeasts have been studied extensively and are already widely used as a source of protein for animals and humans. The production of "food yeast," *C. utilis*, has long been accepted for use in foods by the regulatory authorities. *C. utilis* has high nutritive value and is able to grow rapidly in high yields utilizing a variety of carbon and nitrogen sources, with high tolerance to low pH. A general review on the production of feed and food yeasts from various plant materials has been published (69). A single-cell protein of *C. utilis* was successfully produced from apple pomace (70).

Mushrooms

Mushroom production is one of the few large-scale commercial applications of microbial technology profitable for bioconversion of cellulosic waste materials to valuable foods (11). Apple pomace was found to be a good substrate for the cultivation of edible mushrooms. Various oyster mushrooms (*Pleurotus* species) were growing very well on apple pomace with a biological efficiency ranging between 30 and 40% (71). The biological efficiency was a measurement of the conversion rate, a ratio of the fruit body yield to the weight of the cultivation medium. In another experiment, a mixture of apple pomace and sawdust was used as a substrate for production of shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus ostreatus*) on synthetic logs (72). Apple pomace is complex and has readily usable carbohydrates that complement the nutritional properties of sawdust. As a result, the mushroom mycelia grew faster and more densely in logs containing apple pomace than in sawdust alone (73).

Ethanol

In general, ethanol is produced from a liquid substrate by a culture of *Saccharomyces cerevisiae*. Because of its physical nature, apple pomace is not readily amenable to submerged yeast fermentation (74). A solid-state fermentation system for the production of ethanol from apple pomace with a strain of *S. cerevisiae* was devised (74–76). The yield of ethanol varied from about 29 g to more than 40 g per kilogram of apple pomace fermented at 30°C in 24 h, depending on the samples fermented; the fermentation efficiency of this process was approximately 89% (76). The spent pomace resulting from the separation of alcohol should be a better animal feed supplement, because its protein content was enriched by the yeast biomass. A novel process for concomitant production of ethanol and animal feed from apple pomace by solid-state fermentation was developed (77). Ethanol production from other fruit processing wastes such as corn fiber (78,79), carob (80,81), and banana waste (68) has also been reported.

Gupta et al. (80) further studied the effect of nutrition variables on solid-state alcoholic fermentation of apple pomace by several strains of yeasts and improved the fermentation efficiency by adding various phosphates, nitrogen sources, or trace elements. Ngadi and Correia (82) studied the effect of factors such as moisture and bioreactor mixing speed on the ethanol production from apple pomace. They even proposed a mathematical model to describe the kinetics of solid-state ethanol fermentation from apple pomace and suggested a logistic function of cell growth and ethanol production during solid-state fermentation (83).

Biogas

Fruit and vegetable processing wastes were readily degraded under anaerobic digestion conditions (57,58,84). Jewell and Cummings (47) investigated the biodegradability of apple pomace using long-term digestion experiments and by graphic analysis. A total of 12 steady-state loading conditions were examined, and kinetic analysis indicated that a hydraulic retention time of 45 days would result in nearly 90% conversion of the biodegradable organics to biogas, and the methane content of the biogas was 60% (47). Under these conditions, the net energy yield from pomace would range from about 0.7 to 1.7 million kcal per wet metric ton, with dry matter contents varying between 20 and 40% of the wet weight, and the energy has a value of between $12 and $30 per wet metric ton of apple pomace (47).

Orange processing waste was also used to produce biogas by solid-state fermentation. Srilatha et al. (85) reported that pretreatment of orange processing waste by solid-state fermentation using selected strains of *Sporotrichum, Aspergillus, Fusarium*, and *Penicillium* improved overall productivity of biogas and methane.

Citric Acid and Other Organic Acids

Citric acid, a tricarboxylic acid, has a wide range of applications in the food, pharmaceutical, and beverage industries as an acidifying and flavor-enhancing agent. The use of apple pomace as a substrate for microbial production of citric acid under solid-state fermentation conditions has been reported (86,87). In the experiment, 40 g of apple pomace was introduced into 500-mL Erlenmeyer flasks and inoculated with *A. niger* spore suspension, and then incubated at 30°C for five days under stationary conditions. Methanol was added to the substrate before fermentation to enhance the yield of citric acid. Under the optimal fermentation conditions, this process yielded up to 90 g of citric acid per kilogram of wet pomace fermented, with a conversion rate of more than 88% based on the amount of sugar consumed (46). A process for leaching citric acid from apple pomace fermented with *A. niger* was also devised (88).

Pineapple waste could be a better substrate for citric acid production in solid-state fermentation than apple pomace (89), and *A. niger* was used in the process (90,91). The highest citric acid yield achieved on pineapple waste in four days was 161 g per kilogram of dried pineapple waste of 70% moisture content, and in the presence of 3% methanol, and the conversion rate was 62.4% based on the sugar consumed (89).

Kiwifruit peel is a by-product resulting from the manufacture of kiwifruit into nectars or slices and represents nearly 10 to 16% of the weight of the original fruit, depending on the peeling method used (52). Like apple or pineapple processing wastes, kiwifruit peel waste can be a good substrate for citric acid production by solid-state fermentation, and about 100 g citric acid per kilogram of kiwifruit peel was produced by *A. niger* in the presence of 2% methanol at 30°C in four days. The yield was more than 60% based on the amount of fermented sugar consumed (52).

Corn wastes were used as a substrate for citric acid production by *A. niger* with a maximum yield of 250 g/kg dry matter of corn cobs after 72 h of growth at 30°C (92). Carob kibble, a waste material from carob pod fruit was also an attractive substrate for citric acid production by solid-state fermentation (93,94). Orange processing wastes were also used as a substrate to produce citric acid by *A. niger* (95). During citric acid fermentation, the presence of methanol was a critical factor in increasing citric acid yield, and this appears to be a general phenomenon with strains of *A. niger* (52,95).

Other organic acids were also produced by solid-state fermentation from fruit processing wastes. Grape pomace could also be used for citric acid production by solid-state fermentation (96). The production of tartaric acid from grape pomace was also attempted (97), while banana wastes were targeted as a substrate for lactic acid production (98).

Butanol

The acetone-butanol fermentation is a well-known process that is being looked at with renewed interest recently, owing to the increasing value of oil products. The classical substrates used for butanol production by fermentation are a variety of starchy materials and molasses, which are of relatively high cost. Apple pomace was used as the substrate for butanol production by solid-state fermentation with the strains of *Clostridium acetobutylicum* and *C. butylicum*, and the yields of butanol from apple pomace were between 1.9 and 2.2% of fresh apple pomace (99).

Volatile Compounds

During solid-state fermentation with edible fungus *Rhizopus oryzae* on apple pomace and cassave bagasse, substantial volatile compounds were produced in association with the fungal growth (100). The major volatile metabolites produced by *R. oryzae* from apple pomace were identified as acetaldehyde, 1-propanol, ethyl acetate, ethyl propinioate and 3-methyl butanol (100). Ethyl acetate and ethyl propionate probably originated from the esterification between ethanol and acetic acid and propionic acid, respectively, which are the products of carbohydrate metabolism (100).

Enzymes

Pectic enzymes derived from *Aspergillus* species have been widely used to increase juice yields and clarify juices. The commercial enzyme preparations are produced by cultivating the mold in a synthetic medium under submerged fermentation conditions. Recently apple pomace has been reported to be an attractive raw material for the production of pectinases by *Aspergillus foetidus* in solid-state cultures (101). To obtain maximum enzyme yields, it is essential to supplement apple pomace with a sufficient amount of an organic nitrogen constituent such as corn steep, yeast extract, or peptone (102). Berovic and Ostroversnik (103) developed a solid-state bioprocess for production of pectolytic enzymes from apple pomace and the process parameters such as inoculation, influence of mixing, aeration, temperature, and moisture content on pectolytic enzymes production were studied. Under the optimal conditions, maximal amounts of 15 g of polygalacturonase and 200 mg of pectinesterase per kilogram of solid medium were obtained (103). Polygalacturonase is one of the most important pectinases and is widely used in juice processing industries (104). Apple pomace was used as the substrate of solid-state fermentation to produce polygalacturonase by *A. niger*, and the highest yield of 25,000 units per kilogram of fermented apple pomace was achieved (105). In addition to apple pomace, citrus processing wastes were also alternative substrates for pectinase production in solid-state fermentation (106,107).

Amylases are enzymes catalyzing the hydrolysis of α-1,4-glucosidic linkages of polysaccharides and have wide applications in the food and beverage processing indus-

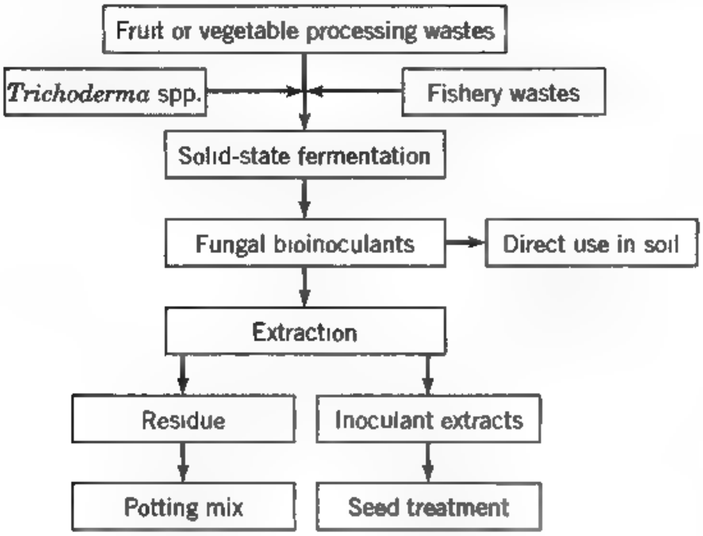


Figure 1. A value-added approach to utilization of fruit and vegetable processing wastes.

tries. Banana waste was a good substrate for α-amylase production by *Bacillus subtilis* under solid-state fermentation (108).

β-Glucosidase (β-D-glucoside glucohydrolase) can catalyze the hydrolysis of glycosidic linkages in aryl and alkyl β-D-glucosides as well as glycosides containing only carbohydrate residues (109,110), and it has been used to increase the concentration of aroma volatile compounds from wine and fruit juice through enzymatic hydrolysis of nonvolatile precursors (111,112). A recent study by Hang and Woodams (113) shows that apple pomace is a potential substrate for production of β-glucosidase by solid-state fermentation, and the highest yield of 900 units of the enzyme per kilogram of fermented apple pomace was obtained by using *A. foetidus*.

β-Fructofuranosidase catalyzes the enzymatic hydrolysis of a fructofuranoside to an alcohol and D-fructose. This enzyme is used commercially in the conversion of sucrose to glucose and fructose and in the manufacture of chocolate-coated soft cream candies (114). Apple pomace was used as the substrate for β-fructofuranosidase production by solid-state fermentation using *Aspergillus* species with the highest yield of 2700 units per kilogram of fermented apple pomace (115).

Lipases (triacylglycerol acylhydrolases) catalyze the hydrolysis of triglycerides to glycerol and free fatty acid at an oil–water interface. This enzyme was produced by solid-state fermentation of olive cake and sugar cane bagasse (116).

A multienzyme complex containing pectinase, cellulase, and xylanase enzymes was produced by a coculture of six fungal isolates grown on orange peels as the sole carbon source (117). This multienzyme preparation also contains insignificant levels of amylase and lipase activities, suggesting potential uses in the extraction of the major components such as starches and lipids form plant materials (117). A cellulase-free xylanase was produced by solid-state fermentation from *Thermomyces lanuginosus* grown on corncobs (118).

A New Value-Added Approach to Utilization of Fruit and Vegetable Processing Wastes

Recently, Zheng and Shetty (119,120) proposed a novel strategy for better utilization of apple and cranberry processing wastes. In this process, a solid-state bioconversion of such wastes into various fungal bioinoculants, which have multiple applications in agricultural and environmental industries, was suggested. For example, *Trichoderma* inoculants produced from apple pomace significantly enhanced the seedling vigor of peas germinated in potting soil (Z. Zheng and K. Shetty, unpublished data, 1999). Commercial seed treatments are being viewed as a means to substantially increase the value of the seed and to improve plant growth and productivity. As a result of enhanced seedling vigor, the plant growth and productivity may be significantly improved. The seed vigor-enhancing compounds could also be extracted with water from fermented fruit pomace, and the residue remaining after extraction is being targeted for use as potting soil mix for several plant species. Therefore, the results of this research have showed value-added application potential for agricultural and environmental industries. A schematic diagram of the new value-added approach for utilization of food processing wastes is shown in Fig. 1.

BIBLIOGRAPHY

1. E. Cannel and M. Moo-Young, "Solid State Fermentation Systems," *Process Biochem.* 4, 2–7 (1980).
2. R. E. Mudgett, "Solid-State Fermentations," in A. L. Demain and N. A. Solomon, eds., *Manual of Industrial Microbiology and Biotechnology*, American Society for Microbiology, Washington, D.C., 1986, pp. 66–83.
3. M. Moo-Young, A. R. Moreira, and R. P. Tengerdy, "Principle of Solid-Substrate Fermentation," in J. E. Smith, D. R. Berry, and B. Kristiansen, eds., *The Filamentous Fungi*, vol. 4, *Fungal Technology*, E. Arnold, London, 1983, pp. 117–144.

4. K. E. Aidoo, R. Hendry, and B. J. B. Wood, "Solid Substrate Fermentation," *Adv. Appl. Microbiol.* **28**, 201–237 (1982).
5. A. Pandey, "Recent Progress Developments in Solid-State Fermentation," *Process Biochem.* **27**, 109–117 (1992).
6. J. E. Smith and K. E. Aidoo, "Growth of Fungi on Solid Substrates," in D. R. Berry, ed., *Physiology of Industrial Fungi*, Blackwell, Oxford, England, 1988, pp. 249–269.
7. D. A. Mitchell, "Microbial Basis of Processes," in H. W. Doelle, D. A. Mitchell, and C. E. Rolz, eds., *Solid Substrate Cultivation*, Elsevier, Essex, England, 1992.
8. M. F. G. Jermini and A. L. Demain, "Solid State Fermentation for Cephalosporin Production by *Streptomyces clavuligerus* and *Cephalosporium acremonium*," *Experientia* **45**, 1061–1065 (1989).
9. A. Sircar, P. Sridhar, and P. K. Das, "Optimization of Solid State Medium for the Production of Clavulanic Acid by *Streptomyces clavuligerus, Process Biochem.* **33**, 283–289 (1998).
10. J. P. Smits et al., "Solid-State Fermentation—A Mini Review," *Agro Food Industry Hi-Technol.* **9**, 29–36 (1998)
11. E. Moyson and H. Verachtert, "Growth of Higher Fungi on Wheat Straw and Their Impact on the Digestibility of the Substrate," *Appl. Microbiol. Biotechnol.* **36**, 421–424 (1991).
12. S. Ohga and Y. Kitamoto, "Future of Mushroom Production and Biotechnology," *Food Rev. Int.* **13**, 461–469 (1997).
13. G. Reed, "Production of Bakers' Yeast," in G. Reed, ed., *Prescott and Dunn's Industrial Microbiology*, 4th ed., AVI, Westport, Conn., 1982, pp. 593–633.
14. C. S. Pederson, *Microbiology of Food Fermentations*, 2nd ed., AVI, Westport, Conn., 1979.
15. M. D. Selgas et al., "Potential Technological Interest of a *Mucor* Strain to Be Used in Dry Fermented Sausage Production," *Food Res. Int.* **28**, 77–82 (1995).
16. F. K. Lucke, "Fermented Meat Products," *Food Res. Int.* **27**, 299–307 (1994).
17. H. L. Wang and C. W Hesseltine, "Oriental Fermented Foods," in G. Reed, ed., *Prescott & Dunn's Industrial Microbiology*, 4th ed., AVI, Westport, Conn., 1982, pp. 492–538.
18. B. J. Wood, "Technology Transfer and Indigenous Fermented Foods," *Food Res. Int.* **27**, 269–280 (1994)
19. G. Campbell-Platt, "Fermented Foods—A World Perspective," *Food Res. Int.* **27**, 253–257 (1994)
20. S. Keuth and B. Bisping, "Vitamin B12 Production by *Citrobacter freundii* or *Klebsiella pneumoniae* during Tempeh Fermentation and Proof of Enterotoxin Absence by PCR," *Appl. Environ. Microbiol.* **60**, 1495–1499 (1994).
21. K. H. Steinkraus, "Nutritional Significance of Fermented Foods," *Food Res. Int.* **27**, 259–267 (1994).
22. A. G. Van-Veen, D. C. W. Graham, and K. H. Steinkraus, "Fermented Peanut Presscake," *Cereal Sci. Today* **13**, 96–99 (1968).
23. N. R. Reddy, "Reduction in Antinutritional and Toxic Compounds in Plant Foods by Fermentation," *Food Res. Int.* **27**, 281–290 (1994).
24. A. Noomhorm, S. Ilangantileke, and M. B. Bautista, "Factors in the Protein Enrichment of Cassava by Solid State Fermentation," *J. Sci. Food Agri.* **58**, 117–123 (1992).
25. J. R. Han, "Sclerotia Growth and Carotenoid Production of *Penicillium* sp. PT95 during Solid State Fermentation of Corn Meal," *Biotechnol. Lett.* **20**, 1063–1065 (1998).
26. M. Gutierrez-Rojas et al. "Heat Transfer in Citric Acid Production by Solid State Fermentation," *Process Biochem.* **4**, 363–369 (1996).
27. K. M. Nampoothiri and A. Pandey, "Solid State Fermentation for L-Glutamic Acid Production Using *Brevibacterium* sp.," *Biotechnol. Lett.* **18**, 199–204 (1996).
28. E. V. Emelyanova, "γ-Linolenic Acid Production by *Cunninghamella japonica* in Solid State Fermentation," *Process Biochem.* **31**, 431–434 (1996).
29. S. Argelier, J. P. Delgenes, and R. Moletta, "Design of Acidogenic Reactors for the Anaerobic Treatment of the Organic Fraction of Solid Food Waste," *Bioprocess Eng.* **18**, 309–315 (1998).
30. C. B. Faulds and G. Williamson, "Release of Ferulic Acid from Wheat Bran by a Ferulic Acid Esterase (FAE-III) from *Aspergillus niger*," *Appl. Microbiol. Biotechnol.* **43**, 1082–1087 (1995).
31. C. W. Hesseltine, "Biotechnology Report: Solid State Fermentation," *Biotechnol. Bioeng.* **14**, 517–532 (1972).
32. Z. Zheng, F. B. Elegado, and Y. Fujio, "Production and Some Properties of Cellulase from *Rhizopus japonicus* IFO5318," *Annu. Rep. ICBiotech* **16**, 223–232 (1993).
33. Z. Zheng et al., "Screening of Cellulase-Rich Microbial Strains and the Conditions for Enzyme Production," *J. Microbiol.* **16**, 35–38 (1996).
34. C. R. Soccol et al. "Breeding and Growth of *Rhizopus* in Raw Cassava by Solid State Fermentation," *Appl. Microbiol. Biotechnol.* **41**, 330–336 (1994).
35. A. Pandey, P. Selvakumar, and L. Ashakumary, "Performance of a Column Bioreactor for Glucoamylase Synthesis by *Aspergillus niger* in SSF," *Process Biochem.* **31**, 43–46 (1996).
36. K. R. Babu and T. Satyanarayana, "α-Amylase Production by Thermophilic *Bacillus coagulans* in Solid State Fermentation," *Process Biochem.* **30**, 305–309 (1995)
37. N. Roche, C. Desgranges, and A. Durand, "Study on the Solid-State Production of a Thermostable α-L-Arabinofuranosidase of *Thermoascus aurantiacus* on Sugar Beet Pulp," *J Biotechnol.* **38**, 43–50 (1994).
38. R. I. Somiari and E. Balogh, "Production of an Extracellular Glycosidase of *Aspergillus niger* Suitable for Removal of Oligosaccharides from Cowpea Meal," *Enzyme Microbiol. Technol.* **17**, 311–316 (1995).
39. I. C. Connelly et al., "Novel Carbohydrase 'Complex' from Solid-State Cultures of the Aerobic Fungus *Penicillium capsulatum*," *Enzyme Microbiol. Technol.* **13**, 470–477 (1991).
40. R. Tunga, R. Banerjee, and B. C. Bhattacharyya, "Optimizing Some Factors Affecting Protease Production under Solid State Fermentation," *Bioprocess Bioeng* **19**, 187–190 (1998).
41. N. R. Kamini, J. G. S. Mala, and R. Puvanakrishnan, "Lipase Production from *Aspergillus niger* by Solid-State Fermentation Using Gingelly Oil Cake," *Process Biochem.* **33**, 505–511 (1998).
42. A. Pandey, "Aspects of Fermenter Design for Solid-State Fermentations," *Process Biochem.* **26**, 355–361 (1991).

43. B. K. Lonsane et al., "Engineering Aspects of Solid State Fermentation," *Enzyme Microbiol. Technol.* **7**, 258–265 (1985).

44. B. K. Lonsane et al., "Scale-up Strategies for Solid-State Fermentation Systems," *Process Biochem.* **27**, 259–273 (1992).

45. P. E. Cook, "Fermented Foods as Biotechnological Resources," *Food Res. Int.* **27**, 309–316 (1994).

46. Y. D. Hang, "Production of Fuels and Chemicals from Apple Pomace," *Food Technol.* 41, 115–117 (1987).

47. W. J. Jewell and R. J. Cummings, "Apple Pomace Energy and Solids Recovery," *J. Food Sci.* **48**, 407–410 (1984).

48. K. J. Carson, J. L. Collins, and M. P. Penfield, "Unrefined, Dried Apple Pomace as a Potential Food Ingredient," *J. Food Sci.* **59**, 1213–1215 (1994).

49. Y. D. Hang, "Production of Single-Cell Protein from Food Processing Wastes," in J. H. Green and A. Kramer, ed., *Food Processing Management*, AVI, Westport, Conn., 1979, pp. 442–455.

50. R. H. Walter and R. M. Sherman, "Fuel Value of Grape and Apple Processing Wastes," *J. Agric. Food Chem.* **24**, 1244–1245 (1976).

51. H. Al-Wandawi, M. Abdul-Rahman, and K. Al-Shaikhly, "Tomato Processing Wastes as Essential Raw Materials Source," *J. Agric. Food Chem.* **33**, 804–807 (1985).

52. Y. D. Hang, B. S. Luh, and E. E. Woodams, "Microbial Production of Citric Acid by Solid State Fermentation of Kiwifruit Peel," *J. Food Sci.* **52**, 226–227 (1987).

53. S. N. Onyeneho and N. S. Hettiarachchy, "Antioxidant Activity, Fatty Acids and Phenolic Acids Compositions of Potato Peels," *J. Sci. Food Agric.* **62**, 345–350 (1993).

54. F. Carvalheiro, J. C. Roseiro, and M. T. A. Collaco, "Biological Conversion of Tomato Pomace by Pure and Mixed Fungal Cultures, *Process Biochem.* **29**, 601–605 (1994).

55. W. P. Weiss, D. L. Frobose, and M. E. Koch, "Wet Tomato Pomace Ensiled with Corn Plants for Dairy Cows," *J. Dairy Sci.* **80**, 2896–2900 (1997).

56. G. A. Fenton and M. J. Kennedy, "Rapid Dry Weight Determination of Kiwifruit Pomace and Apple Pomace Using an Infrared Drying Technique," *N. Z. J. Crop Hortic Sci.* **26**, 35–38 (1998).

57. W. Knol, M. M. Van der Most, and J. de Waart, "Biogas Production by Anaerobic Digestion of Fruit and Vegetable Wastes," *J. Food Sci. Agric.* **29**, 822–827 (1978).

58. A. G. Lane, "Methanol, Anaerobic Digestion of Fruit and Vegetable Pressing Wastes," *Food Technol. Australia* **31**, 201–206 (1979)

59. S. F. Barrington, K. Elmoueddeb, and B. Porter, "Improving Small-Scale Composting of Apple Waste," *Can. Agr. Eng.* **39**, 9–16 (1997).

60. C. Chong, "Experiences with the Utilization of Wastes in Nursery Potting Mixes and as Field Soil Amendments," *Can. J. Plant Sci.* **79**, 139–148 (1999).

61. G. Logsdon, "Pomace Is a Grape Resource," *Biocycle* **33**, 40–41 (1992).

62. Y. D. Hang, "Improvement of the Nutritional Value of Apple Pomace by Fermentation," *Nutr. Rep. Int.* **38**, 207–211 (1988).

63. H. Rahmat, R. A. Hodge, and G. J. Manderson, "Solid-Substrate Fermentation of *Kloeckera apiculate* and *Candida utilis* on Apple Pomace to Produce an Improved Stock-Feed," *World J. Microbiol. Biotechnol.* 11, 168–170 (1995).

64. V. K. Joshi and D. K. Sandhu, "Preparation and Evaluation of an Animal Feed Byproduct Produced by Solid-State Fermentation of Apple Pomace," *Bioresource Technol.* **56**, 251–255 (1996).

65. T. J. B. Menezes et al., "Protein Enrichment of Citrus Wastes by Solid Substrate Fermentation," *Process Biochem.* **24**, 167–171 (1989).

66. J. Baldensperger et al., "Solid State Fermentation of Banana Wastes," *Biotechnol. Lett.* **7**, 743–748 (1985).

67. S. L. Chung and S. P. Meyer, "Bioprotein from Banana Wastes," *Dev. Ind. Microbiol.* **20**, 723–731 (1979)

68. O. Enwefa, "Biomass Production from Banana Skins," *Appl. Microbiol. Biotechnol.* **36**, 283–284 (1991)

69 G. Reed and H. J. Peppler, *Yeast Technology*, AVI, Westport, Conn., 1973.

70. P. J. Fellows and J. T. Worgan, "Growth of *Saccharomycopsis fibuliger* and *Candida utilis* in Mixed Culture on Apple Processing Wastes," *Enzyme Microbiol. Technol.* **9**, 434–437 (1987).

71. R. C. Upadhyay and H. S. Sohi, "Apple Pomace—A Good Substrate for the Cultivation of Edible Mushrooms," *Curr. Sci.* **57**, 1189–1190 (1988).

72. J. J. Worrall and C. S. Yang, "Shiitake and Oyster Mushroom Production on Apple Pomace and Sawdust," *HortScience* **27**, 1131–1133 (1992).

73. E. Gabler et al., "Feasibility of Ethanol Production from Cheese Whey and Fruit Pomace in New York," in *Proceedings of Feed and Fuel from Ethanol Production Symposium*, NRAES Report no. 17, Cornell University, Ithaca, N.Y., 1982, p. 13.

74. Y. D. Hang, "Production of Alcohol from Apple Pomace," *Appl. Environ. Microbiol.* **42**, 1128–1129 (1981).

75. Y. D. Hang et al., "Production of Alcohol from Apple Pomace," *Appl. Environ. Microbiol.* **42**, 1128–1129 (1981).

76. Y. D. Hang, "A Solid State Fermentation System for Production of Ethanol from Apple Pomace," *J. Food Sci.* **47**, 1851–1852 (1982)

77. D. K. Sandhu and V. K. Joshi, "Solid State Fermentation of Apple Pomace for Concomitant Production of Ethanol and Animal Feed," *J. Sci. Ind. Res India* **56**, 86–90 (1997).

78. R. J. Bothast and B. C. Saha, "Ethanol Production from Agricultural Biomass Substrates," *Adv. Appl. Microbiol.* **44**, 261–286 (1997).

79. B. C. Saha, B. S. Dien, and R. J. Bothast, "Fuel Ethanol Production from Corn Fiber-Current Status and Technical Prospects," *Appl. Biochem. Biotechnol.* **70**, 115–125 (1998).

80. L. K. Gupta, G. Pathak, and R. P. Tiwari, "Effect of Nutrition Variations on Solid State Alcoholic Fermentation of Apple Pomace by Yeasts," *J. Sci. Food Agric.* **50**, 55–62 (1990).

81. T. Roukas, "Solid-State Fermentation of Carob Pods for Ethanol Production," *Appl. Microbiol. Biotechnol.* 41, 296–301 (1994).

82. M. O. Ngadi and L. R. Correia, "Solid State Ethanol Fermentation of Apple Pomace as Affected by Moisture and Bioreactor Mixing Speed," *J. Food Sci.* **57**, 667–670 (1992).

83. M. O. Ngadi and L. R. Correia, "Kinetics of Solid-State Ethanol Fermentation from Apple Pomace," *J. Food Eng.* **17**, 97–116 (1992).

84. R. Borja and C. J. Banks, "Kinetic Study of Anaerobic Digestion of Fruit Processing Wastewater in Immobilized-Cell Bioreactors," *Biotechnol. Appl. Biochem.* **20**, 79–92 (1994).

85. H. R. Srilatha et al., "Fungal Pretreatment of Orange Processing Waste by Solid-State Fermentation for Improved Production of Methane," *Process Biochem.* **30**, 327–331 (1995).
86. Y. D. Hang and E. E. Woodams, "Apple Pomace. A Potential Substrate for Citric Acid Production by *Aspergillus niger*," *Biotechnol. Lett.* **6**, 763–764 (1984).
87. Y. D. Hang and E. E. Woodams, "Solid State Fermentation of Apple Pomace for Citric Acid Production," *J. Appl. Microbiol. Biotechnol.* **2**, 281–285 (1986).
88. Y. D. Hang and E. E. Woodams, "A Process for Leaching Citric Acid from Apple Pomace Fermented with *Aspergillus niger* in Solid-State Culture," *Mircen J. Appl. Microbiol.* **5**, 379–382 (1989).
89. C. T. Tran and D. A. Mitchell, "Pineapple Waste—A Novel Substrate for Citric Acid Production by Solid State Fermentation," *Biotechnol. Lett.* **17**, 1107–1110 (1995).
90. V. DeLima, T. Stamford, and A. Salgueiro, "Citric Acid Production from Pineapple Waste by Solid State Fermentation Using *Aspergillus niger*," *Arq. Biol. Technol.* **38**, 773–783 (1995).
91. C. T. Tran, L. I. Sly, and D. A. Mitchell, "Selection of a Strain of *Aspergillus* for the Production of Citric Acid from Pineapple Waste in Solid-State Fermentation," *World J. Microbiol. Biotechnol.* **14**, 399–404 (1998).
92. Y. D. Hang and E. E. Woodams, "Production of Citric Acid from Corncobs by *Aspergillus niger*," *Bioresource Technol.* **65**, 251–253 (1998).
93. T. Roukas, "Carob Pod: A New Substrate for Citric Acid Production by *Aspergillus niger*," *Appl. Biochem. Biotechnol.* **74**, 43–53 (1998).
94. T. Roukas, "Citric Acid Production from Carob Pod by Solid-State Fermentation," *Enzyme Microbiol. Technol.* **24**, 54–59 (1999).
95. G. Aravantinos-Zafiris et al., "Fermentation of Orange Processing Wastes for Citric Acid Production," *J. Sci. Food Agric.* **65**, 117–120 (1994).
96. Y. D. Hang, "Recovery of Food Ingredients from Grape Pomace," *Process Biochem.* **23**, 2–4 (1988).
97. C. Nurgel and A. Canbas, "Production of Tartaric Acid from Pomace of Some *Anatolian* Grape Cultivars," *Am. J. Enol. Vitic.* **49**, 95–99 (1998).
98. A. Lopez-Baca and J. Gomez, "Fermentation Pattern of Whole Banana Waste Liquor with Four Inocula," *J. Sci. Food Agric.* **60**, 85–89 (1992).
99. C. E. Voget, C. F. Mignone, and R. J. Ertola, "Butanol Production from Apple Pomace," *Biotechnol. Lett.* **7**, 43–46 (1985).
100. A. Bramorski et al., "Production of Volatile Compounds by the Edible *Rhizopus oryzae* during Solid State Cultivation on Tropical Agro-Industrial Substrates," *Biotechnol. Lett.* **20**, 359–362 (1998).
101. R. A. Hours, C. E. Voget, and R. J. Ertola, "Apple Pomace as Raw-Material for Pectinases Production in Solid-State Fermentation," *Biol. Wastes* **23**, 221–228 (1988).
102. R. A. Hours, C. E. Voget, and R. J. Ertola, "Some Factors Affecting Pectinase Production from Apple Pomace in Solid-State Cultures," *Biol. Wastes* **24**, 147–157 (1988).
103. M. Berovic and H. Ostroversnik, "Production of *Aspergillus niger* Pectolytic Enzymes by Solid State Bioprocessing of Apple Pomace," *J. Biotechnol.* **53**, 47–53 (1997).
104. F. M. Rombouts and W. Pilnik, "Enzymes in Fruit and Vegetable Juice Technology," *Process Biochem.* **13**, 9–13 (1978).
105. Y. D. Hang and E. E. Woodams, "Production of Fungal Polygalacturonase from Apple Pomace," *Food Sci. Technol.* **27**, 194–196 (1994).
106. C. G. Garzon and R. A. Hours, "Citrus Waste—An Alternative Substrate for Pectinase Production in Solid-State Culture," *Bioresource Technol.* **39**, 93–95 (1992).
107. H. E. Hart et al., "Orange Finisher Pulp as a Substrate for Polygalacturonase Production by *Rhizopus oryzae*," *J. Food Sci.* **56**, 480–483 (1991).
108. C. Krishna and M. Chandrasekaran, "Banana Waste as Substrate for α-Amylase Production by *Bacillus subtilis* (CBTK 106) under Solid-State Fermentation," *Appl. Microbiol. Biotechnol.* **46**, 106–111 (1996).
109. J. Woodward, "Fungal and Other β-D-Glucosidase—Their Properties and Applications," *Enzyme Microb. Technol.* **4**, 73–79 (1982).
110. T. R. Yan, Y. H. Lin, and C. L. Lin, "Purification and Characterization of an Extracellular β-Glucosidase II with High Hydrolysis and Transglucosylation Activities from *Aspergillus niger*," *J. Agric. Food Chem.* **46**, 431–437 (1998).
111. P. J. Williams, "Hydrolytic Flavor Release in Fruit and Wines through Hydrolysis of Nonvolatile Precursors," in T. Acree and R. Teranishi, eds., *Flavor Science: Sensible Principles and Techniques*, American Chemical Society, Washington, D.C., 1993.
112. Y. Z. Gunata et al., "Hydrolysis of Grape Monoterpenyl β-Glucosides by Various β-Glucosidases," *J. Agric. Food Chem.* **38**, 1232–1236 (1990)
113. Y. D. Hang and E. E. Woodams, "Apple Pomace: A Potential Substrate for Production of β-Glucosidase by *Aspergillus foetidus*," *Food Sci. Technol.* **27**, 587–589 (1994).
114. G. Reed, *Enzymes in Food Processing*, Academic, New York, 1966.
115. Y. D. Hang and E. E. Woodams, "β-Fructofuranosidase Production by *Aspergillus* Species from Apple Pomace," *Food Sci. Technol.* **28**, 340–342 (1995).
116. J. Cordova, M. Nemmaoui, and M. Ismaili-Alaoui, "Lipase Production by Solid State Fermentation of Olive Cake and Sugar Cane Bagasse," *J. Mol. Catal. B-Enzyme* **5**, 75–78 (1998).
117. A. M. S. Ismail, "Utilization of Orange Peels for the Preparation of Multienzyme Complexes by Some Fungal Strains," *Process Biochem.* **31**, 645–650 (1996).
118. H. Purkarthofer, M. Sinner, and W. Steiner, "Cellulase-Free Xylanase from *Thermomyces lanuginosus*: Optimization of Production in Submerged and Solid-State Culture," *Enzyme Microbiol. Technol.* **15**, 677–682 (1993).
119. Z. Zheng and K. Shetty, "Solid-State Production of Beneficial Fungi on Apple Processing Wastes Using Glucosamine as the Indicator of Growth," *J. Agric. Food Chem.* **46**, 783–787 (1998).
120. Z. Zheng and K. Shetty, "Cranberry Processing Waste for Production of Solid State Fungal Inoculants Production," *Process Biochem.* **33**, 323–329 (1998).

ZUOXING ZHENG
KALIDAS SHETTY
University of Massachusetts
Amherst, Massachusetts

SOY FOODS, FERMENTED

Fermented soy foods are an important part of the human diet in Asia. The fermented soy foods that originated many thousands of years ago in the Asian countries are often referred to as indigenous fermented foods (1,2). Some of the fermented soy foods are now popular in the West (3). The increased palatability of fermented soy foods is due to desirable changes in soybean properties, including texture and organoleptic characteristics (flavor, aroma, and appearance or consistency). Elimination of beany flavors, improvement in digestibility, enhanced keeping quality of the product, improved safety (absence of toxins and partial and/or complete elimination of antinutritional factors), improved nutrition, and reduced cooking time (3) are results of fermentation of soybeans. Fermentation makes the organoleptic characteristics of soybean more attractive to the consumer than the raw soybean.

Of the great number of fermented soybeans consumed in Asia, only a few have been studied in the West; these are described in Table 1 (3,4). Some of these products are used primarily as flavoring agents that add some protein or amino acid to generally bland and low-protein diets. Others, such as tempe and natto, are served as staples. The origin of some fermented and nonfermented soy foods is presented in Table 2. The majority of soy foods, such as soy milk, soybean sprout, tofu, fermented tofu, soy sauce, and miso, originated in China. Natto is a soy food of Japanese origin, whereas tempeh originated in Indonesia (5).

SOY SAUCE

History

The recorded use of soy sauce dates back 2,700 years in China. The prototypes of soy sauce are believed to have been introduced from China to Japan more than 1,300

Table 1. Asian Fermented Soyfoods

Foods	Local names	Microorganisms involved	Substrate(s)	Use and nature of product
Soy sauce	Chiang-Yu (Chinese) Inyu (Chinese) Shoyu (Japanese) Ketjap (Indonesian) Kecap (Indonesian) Kanjang (Korean) Tayo (Philippine) See-ieu (Thai)	*Aspergillus* *Pediococcus* *Torulopsis* *Saccharomyces*	Whole soybean Defatted soyflake Wheat	Seasoning, a flavoring agent Dark reddish brown liquid, salty taste suggesting the meat extract
Miso	Chiang (Chinese) Tou-chiang (Chinese) Wei-cheng (Chinese) Miso (Japanese) Doenjang (Korean) Tauco (Indonesian) Tao-chieo (Thai) Soybean paste	*Aspergillus* *Pediococcus* *Streptococcus* *Saccharomyces* *Torulopsis*	Whole soybean Rice Barley	Flavoring agent, soup base Light yellow to dark reddish brown paste, smooth or chunky, salty and strongly flavored, resembling soy sauce
Sufu	Sufu, Furu, Fuju, Toufuru, Tao-yu (Chinese) Fu-nyu (Japanese) Tohuri (Philippine) Chinese cheese Soybean cheese Fermented tofu	*Actinomucor* *Mucor* *Rhizopus* *Aspergillus* *Monascus*	Soybean curd (firm tofu)	Condiment Creamy cheese-type cubes, salty, served with or without further cooking
Natto	Natto (Japanese) Tau-nou or Thua-nao (Thai) Kenima (Nepalese)	*Bacillus natto* *Bacillus subtilis*	Whole soybean	Served with cooked rice as a main dish or snack Cooked beans bound together and covered with viscous, sticky substance produced by the bacteria, ammonium odor, musty flavor
Tempe	Tempe kedelee (Indonesian) Tenpei (Japanese)	*Rhizopus oligosporus*	Whole soybean	Cooked and served as main dish or snack Cooked soft soybeans bound together by mycelia as cake, a clean fresh and yeasty odor
Taosi	Tou-shih (Chinese) Tao-si (Philippine) Tao-cheo (Malaysian) Taotjo (Indonesian) Hamanatto (Japanese)	*Aspergillus* *Streptococcus* *Pediococcus*	Whole soybean Wheat flour	Condiment, seasoning Nearly black soft bean, salty flavor resembling soy sauce
Meitauza	Meitauza (Chinese)	*Mucor* *Actinomucor*	Soybean presscake	Snack food
Meju	Meju (Korean)	*Aspergillus* *Rhizopus*	Black soybean	Seasoning agent

Source: Refs. 3 and 4.

Table 2. The Origin of Soyfoods

Soyfoods	Country of origin	Date of origin
Soy milk	China	164 B.C.
Soybean sprout	China	
Tofu	China	164 B.C.
Fermented tofu	China	Before 1400 A.D.
Soy sauce	China	700 B.C.
Miso	China	722 B.C.
Natto	Japan	Before 1286 A.D.
Tempe	Indonesia	Before 1750 A.D.

Source Ref. 5.

years ago. The history of soy sauce in China and Japan is summarized in Table 3 (5,8).

Table 3. History of Soy Sauce

China	Japan
Shu-Ching (700 B.C.)[a] Chu[a] Chiang (meat from fish, bird, or meat)	
Chi-Min-Yao-Shu (532–549 A.D.)[a] Chu (made from crushed wheat, or wheat flour made into balls or cakes, or cooked rice) Chiang (made from soybeans or wheat) Shi and Shi-tche	Manyo-shu (350–759)[a] Koji (same as chu) Hishio (same as chiang, made from fish, meat, or soybean) Koma-hishio and miso
Tang dynasty (618–906) Ben-chao-gong mu (1590)[a] Chiang-yu Tao-yu	Taiho-law (701)[a] Soybean-hishio, miso, kuki same as shi), taremiso, usudare, misodamari Ekirinbon-setsuyoshu (1598)[a] Shoyu (same Chinese charactors as chiang-yu) Honcho-shokukan Shoyu, miso, tamari Industrial production of Koikuchi-shoyu in Noda (1561) and Chochi (1616), that of usukuchi shoyu in Sendai (1645); export of shoyu from Nagasaki, Japan (1668)

Source Ref 6.

[a] Names of old references

Note Chu. mold-cultured cereals; chiang: a mixture of chu, proteinous foodstuffs, and salt; shi· mold-cultured soybeans with or without salt; shi-tche: the saltwater extract of shi; chiang-yu. the liquid separated from chiang; tao-yu: the liquid separated from soybean chiang.

Country of Consumption

Soy sauce is produced and consumed mostly in Asian countries. Owing to progress in world trade, soy sauce is now consumed almost worldwide. In 1978, soy sauce was exported from Hong Kong, the Korean Republic, Singapore, and Japan to a total of 97 countries (7,8).

Preparation

Different countries have different methods of preparing soy sauce. Even in Japan, there are five kinds of soy sauce: koikuchi, usukuchi, tamari, saishikomi, and shiro. The preparation of koikuchi soy sauce is outlined in Figure 1. The process includes (*1*) the treatment of raw materials, (*2*) koji making, (*3*) mash production and aging, (*4*) pressing, and (*5*) refining and pasteurization (8).

Treatment of Raw Materials. Cleaned soybeans or soybean meal is moistened (add 120–135% water) and cooked with steam under pressure. Wheat kernels are roasted at 170 to 180°C for several minutes and coarsely crushed into four or five pieces.

Koji Making. Equal parts of the resultant cooked soybeans or defatted soybean meal and roasted cracked wheat are mixed together and inoculated with 1 to 2% wt/wt of the seed koji or a pure culture of *Aspergillus oryzae* or *A. sojae*. This mixture, containing 45% water, is spread on larger perforated stainless steel or wooden trays to a depth of 30 to 40 cm and incubated in a room at 25 to 30°C with humidity control for 2 to 3 days. During this period, the temperature, moisture, and aeration are controlled to al-

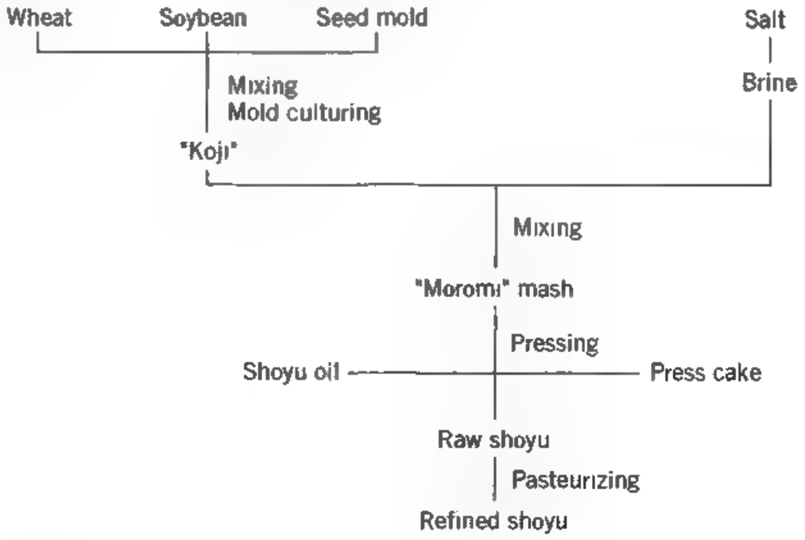


Figure 1. Preparation of Japanese fermented koikuchi shoyu. *Source* Ref. 8.

low the seed koji to grow on the mixture, to prevent development of *Mucor* spp. or bacteria, and to enhance production of proteolytic and amylolytic enzymes. A rather high moisture level (30–32%) is necessary for good mycelial growth and enzyme activity. The resulting end product (clear yellow to yellowish green in color), is called koji, which is a mixture of hydrolytic enzymes and the substrate.

Mash (Moromi) Production and Aging. The koji is mixed with salt water, which has a 22 to 23% salt content and a volume 120 to 130% that of the raw materials. The mash or moromi is transferred to deep fermentation tanks. Approximately 5- to 10-kL wooden kegs or 10- to 20-kL concrete tanks for soy sauce fermentataon are now being replaced by resin-coated iron tanks of 50 to 300 kL. The moromi is held for 4 to 8 months, depending on its temperature, with occasional agitation with compressed air to mix the dissolving contents uniformly and to promote the microbial growth. During the fermentation period, the enzymes from koji mold hydrolyze most of the protein to amino acids and low molecular weight peptides. Approximately 20% of the starch is consumed by the mold during koji cultivation, but almost all the remaining starch is converted into simple sugars. More than half this is fermented to lactic acid and alcohol by lactobacilli and yeasts, respectively. The initial pH value drops from 6.5–7.0 to 4.7–4.9. The lactic acid fermentation carried out in the beginning stage is gradually replaced by yeast fermentation. Pure culture of *Pediococcus halophylus* and *Saccharomyces rouxii* are sometimes added to the mash. The salt concentration of mash remains at 17 to 18% (weight per volume) after 1 or 2 months. The high concentration of mash effectively limits the growth to only a few desirable types of microorganisms.

Pressing. Once the aging of mash is completed, the liquid (raw shoyu) is separated from the mash by a hydraulic filter press in commercial operations or by a simple mechanical press on a domestic level. Sometimes, fresh salt water is added to the press cake, and a second fermentation is allowed to proceed for 1 to 2 months before a second pressed shoyu is produced, which is of lower quality than the first one. The final moisture content of the press cake is less than 30%. The press cake is called shoya cake, which is used as animal feed.

Refining and Pasteurization. Raw shoyu is separated into three layers (sediment at the bottom, clear shoyu in the middle, and an oil layer at the top) on standing in tanks. The oil layer is called shoyu oil and is removed by decantation. The middle layer, clear shoyu, is pasteurized at 80 to 85°C in a kettle or in a heat exchanger to kill vegetative microbial cells, denature enzymes, and coagulate proteins. Sometimes a filter aid is added to the pasteurized shoyu to enhance clarification. The clear shoyu is filtered, bottled, and marketed. Preservatives such as benzoic acid or propyl- or butyl p-hydroxy benzoate are sometimes added to the filtered shoyu during pasteurization. Steps for the industrial preparation of shoyu in Japan are mechanized, which prevents contamination by undesirable microorganisms.

The preparation of usukuchi, tamari, and shiro shoyu are almost the same as those of koikuchi shoyu, except procedures for usukuchi are directed at getting lighter color and aroma in the final product. Tamari shoyu uses soybean with a small amount of wheat (20:3) and dryer koji. Tamari mash cannot be agitated with compressed air. The liquid is obtained by dripping, not by pressing. Shiro shoyu is made mostly from wheat with less soybean, the volume of which is 10 to 20% that of wheat. Saishikomi is made from enzymatically degraded soybeans and wheat instead of the usual salt water.

Soy Sauce Produced in Other Oriental Countries

The fermented soy sauce industrially produced in Korea is similar to the Japanese koikuchi type. Almost the same amount of soy sauce is prepared at home as is industrially produced. The homemade soy sauce is prepared by a traditional method in which cooked soybeans are mashed and made into small balls, then subjected to natural inoculation of *Aspergillus* and *Rhizopus* molds, a process taking several months in winter. When spring comes these mold-cultured materials are extracted with salt water. The liquid part is boiled and fermented under the sun to make soy sauce. The residue of extraction is mixed with salt and stored to make miso, sometimes along with red pepper (6). Of Taiwan soy sauce, 5 to 10% is estimated to be inyu, which is made only from soybeans. The remarkable characteristics of inyu are that it is prepared from black soybeans instead of yellow soybeans and that the black bean koji is washed with water before it is mixed with salt water to make mash. Fermented soy sauce similar to inyu and tamari is still being produced in the southern part of China, and it seems to be the prototype of the soy sauce prepared only from soybeans. In Japan, tamari mash is usually fermented in wooden kegs, but the inyu in Taiwan, Singapore, and the southern part of China is fermented in big china pots placed under sunlight.

However, most of the soy sauce made in Peking and Shanghai today is prepared differently. The koji is prepared by using a large-scale method to culture *A. oryzae* with a mixture of steamed soybeans and wheat or wheat bran (6:4), and the koji is mixed with salt water to make hard mash, the moisture content of which is about 80% and the salt concentration about 6 to 7%. This hard and low-salt mash is kept at 45 to 50°C for about 3 weeks for enzymatic digestion. The digested mash is extracted with hot salt water and then with plain hot water. The residue without salt is good for animal feed. There is no alcoholic fermentation of mash or processing of mash as in Japanese shoyu manufacture.

The soy sauce produced in Indonesia is called kecap. The soybean koji is put in a wooden tank containing a salt solution of 20% and left to soak for 3 to 4 weeks at room temperature. The mash is boiled in a certain volume of water and then filtered. The filtrate liquor is cooked in water, two to three times the amount of liquor, and mixed with caramel and other spices (9).

New Development

Recent research and technology advances can be summarized as follows (8,10):

1. Greater use of defatted soybean instead of whole beans
2. Increase in protein digestibility of raw materials from 65 to 90% as the result of improved cooking methods of soybeans and wheat; the selection and mutation of starter molds; improved conditions for culturing molds or making koji; and the control of mash in terms of the temperature, pH, types and behavior of lactobacilli and yeasts; and the chemical components
3. Reduction of the time for koji cultivation from 72 to 48 hours
4. Decrease of fermentation period of mash from 1 to 3 years to about 6 months
5. The use of pure cultured starters of lactobacilli and yeasts
6. Mechanization of the equipment and expansion of the production scale
7. Improvement of quality and reduction of cost

Composition

The typical composition of the five types of shoyu are presented in Table 4. Both koikuchi and usukuchi shoyu contain appreciable amounts of total nitrogen in the range of 1.2 to 1.6%, of which 80 to 90% are lower peptides, peptones, and amino acids. Ammonia makes up the remaining nitrogen. Lactic, pyroglutamic, acetic, succinic, and formic acids are present in the koikuchi and usukachi shoyu. Shoyu contains neutral sugars such as glucose, galactose, arabinose, and xylose. A total of 271 flavor components have been found in Japanese shoyu in which 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF) possesses a shoyulike sweet flavor (11).

Organisms Used

The microorganisms used in koji are *A. oryzae* and *A. sojae*. The molds produce a great variety of enzymes including α-amylase, proteases (acid, neutral, and alkaline), nucleases, sulfatases, phosphatases, transglycosidases, peptidases, acylase, ribonucleo-depolymerases, mononucleotide phosphatase, adenyl-deaminases, and purine nucleosidases (12).

The predominant active microbes in shoyu mash are salt-tolerant lactobacilli and yeasts such as *Zygosaccharomyces rouxii* and *Candida (Torulopsis) versatillis* or *C. etchellsii*. The major lactobacilli are *P. soyae* or *P. halophylus*. Good results have also been obtained by adding pure cultured lactobacilli to the new mash. The initial pH value of mash, 6.5 to 7.0, gradually decreases as the raw materials are degraded and lactic acid fermentation proceeds, and at around pH 5.5, yeast fermentation takes the place of lactic acid fermentation. To accelerate the alcoholic fermentation and to shorten its development time, pure cultured yeasts, *Z. rouxii* or *Torulopsis*, are sometimes added to the shoyu mash when its pH value reaches about 5.3, usually 3 to 4 weeks after the mash making.

Safety

Soy sauce mold mostly belongs to the *A. oryzae, A. sojae*, and *A. tamarii*, whereas all the aflatoxin-producing molds belong to *A. parasiticus* and *A. flavus*. Although fluorescent compounds are produced by *Aspergillus* molds with Rf values resembling those of aflatoxins, there is no hazard in human consumption of soy sauce (1,7,9,11).

Future Development

1. Soy sauce–like seasonings can be prepared from a mixture of plant proteins and starches other than soybeans and wheat.
2. An effort to find better strains that can degrade plant tissue is needed.
3. A much greater reduction in fermentation time is necessary for economic reasons.
4. Supplementing the enzyme systems would help reduce both the fermentation period and the viscosity of mash.
5. The heat-coagulant substances produced by pasteurization still pose a problem of refining.
6. It would be interesting to produce flavor compounds biochemically.
7. To increase the stability of fermented soy sauce is a difficult but important problem (7).

MISO

History

There is evidence to suggest that miso fermentation predates the production of soy sauce (10,12,13). Soy sauce was first called tamari miso. Tamari literally means a liquid drip and is the name given to the liquid that drips to the bottom of a miso keg while miso is aging. Thus, tamari, as the original form of soy sauce in Japan, was made from miso (14).

Countries of Consumption

Miso is consumed in China, Taiwan, Japan, the Philippines, Indonesia, and Korea. In these countries, miso is called by different names (Table 1).

Table 4. Typical Composition of Different Types of Shoyu

Types	Salt % (w/v)	Total nitrogen % (w/v)	Formyl nitrogen % (w/v)	Reducing sugars % (w/v)	Alcohol % (w/v)	pH
Koikuchi	16.95	1.58	0.92	2.99	2.45	4.79
Usukuchi	19.95	1.21	0.82	3.88	2.87	4.79
Tamari	16.85	2.35	1.14	6.98	0.68	4.85
Shiro	17.86	0.51	0.28	17.21	0.11	4.74
Saishikomi	14.30	2.35	1.17	9.43	1.33	4.84

Source: Ref. 8.

Preparation

There are many varieties of miso that differ from one another depending on the manufacturing process. Rice miso is the most popular type. The manufacturing method of salty rice miso is presented in Figure 2 (12,14). Miso fermentation is about the same as that of soy sauce. Unlike soy sauce, however, miso is a paste product and is mainly consumed as a miso soup. Most recently, there has been new development in the process of the brine fermentation of miso by an automatic system (10).

In Indonesia a similar product is called tauco. It uses soybean as raw material. The fermented product is cooked with palm sugar with two times the amount of salt used in the brine fermentation.

Composition

The approximate composition of various types of miso is presented in Table 5. The moisture content ranges from 42.6 to 45.7%. Sweet and barley miso have higher amounts of carbohydrates compared to others. This is due to a short fermentation period (12,14).

Organisms Used

The principal and indispensable microorganisms in miso fermentation are koji molds, salt-tolerant yeasts, and lactic acid bacteria. The koji molds are *A. oryzae* and *A. sojae*. The bacteria *Streptococcus faecalis* and *S. faecium* are the dominant weak salt-tolerant lactic acid bacteria. *P. halophilus* and *P. acidi lactici* are the most dominant lactic acid types. They play an important role in the fermentation of miso. These organisms can survive in 23% brine. Because the acid tolerance of these bacteria is comparatively weak, they cannot survive below pH 5.0. The role of these bacteria in miso fermentation is to produce organic acids from carbohydrates, which lowers the pH of the fermenting miso and accelerates the growth of salt-tolerant yeasts. *Z. rouxii*, *T. versatilis*, and *T. etchellsii* are the salt-tolerant yeasts. *Z. rouxii* grows in a high-salt medium (18% sodium chloride) of pH 4.0 to 5.0 and is the main yeast involved in the fermentation of miso. The yeasts produce ethanol from sugars and phenolic compounds such as 4-ethylguaiacol, 4-ethylphenol, and 2-phenylethanol. These compounds give a characteristic flavor to the fermented miso. *Z. rouxii* var halomembranis nov var, *Pichia* spp., and *Hansenula* spp. are the aerobic film yeasts, which often produce films on substrate surfaces, resulting in off-flavor of the products.

Safety

No aflatoxin-producing mold strains were found in mold strains collected from miso factories. Similarly, no aflatoxin was detected in kojis from miso factories (12,14).

Future Development

Miso production in Japan decreased from 590,000 tons in 1973 to 572,000 tons in 1983. The decrease may be due to

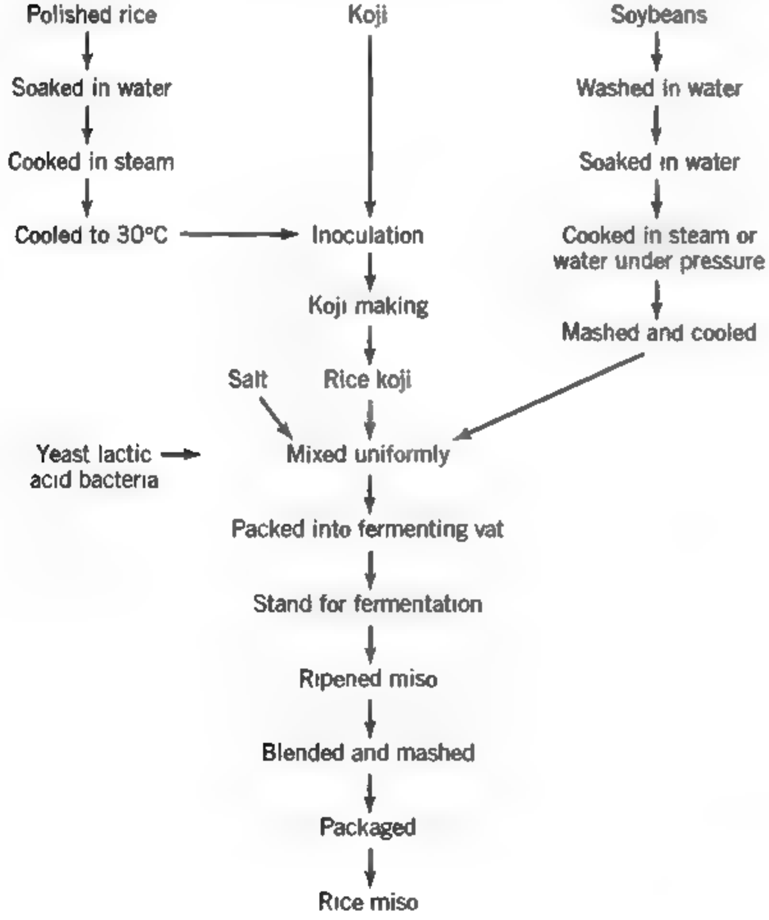

Polished rice
Soaked in water
Cooked in steam
Cooled to 30°C
Koji
Inoculation
Koji making
Rice koji
Salt
Soybeans
Washed in water
Soaked in water
Cooked in steam or water under pressure
Mashed and cooled
Yeast lactic acid bacteria
Mixed uniformly
Packed into fermenting vat
Stand for fermentation
Ripened miso
Blended and mashed
Packaged
Rice miso

Figure 2. Flow chart of the manufacture of salty rice miso. *Source:* Ref. 15.

Table 5. Chemical Composition of Various Types of Miso

Component	Sweet miso	Salty light-yellow miso	Salty red miso	Barley miso	Soybean miso
Moisture (%)	42.6	45.4	45.7	44.0	44.9
Protein (%)	9.7	12.5	13.1	9.7	17.2
Fat (%)	3.0	6.0	5.5	4.3	10.5
Carbohydrate (%)	36.7	19.4	19.1	28.3	11.3
Crude fiber (%)	1.2	2.5	2.0	1.7	3.2
Ash (%)	6.8	14.2	14.6	12.0	12.9
Salt (%)	6.1	12.4	13.0	10.7	10.9
Calcium (mg/100 g)	80.0	100.0	130.0	80.0	150.0
Phosphorus (mg/100 g)	130.0	170.0	200.0	120.0	250.0
Iron (mg/100 g)	3.4	4.0	4.3	3.0	6.8
Thiamin (mg/100 g)	0.05	0.03	0.03	0.04	0.04
Riboflavin (mg/100 g)	0.1	0.1	0.1	0.1	0.12
Niacin (mg/100 g)	1.5	1.5	1.5	1.5	1.2
Vitamin B_{12} (μg/100 g)	—	0.17	—	—	—

Source: Ref. 15.

the high sodium chloride content. If the salt level could be lowered, some of the problems related to sodium would be lessened and consumption probably would increase. Therefore, further studies should be conducted to find new flavoring materials to replace salt.

SUFU

History

Manufacture of tofu (unfermented soybean curd) began during the era of the Han dynasty. The Pen Ts'ao, or Chinese Materia, of 1596, compiled by Li Shi-Chin, indicated that tofu was invented by Liu An (179 B.C. to 122 B.C.), king of Wainan. It is not known when sufu production began (16). Sufu is produced and consumed in China and Taiwan. It is used directly as a condiment or is cooked with vegetables or meats. Because of its creamy texture, it is suitable for use as a cracker spread or as an ingredient for dips and dressings in the Western world (16,17).

Preparation

The preparation of sufu consists of five steps: (*1*) preparation of tofu, (*2*) preparation of pehtze (freshly prepared tofu grown with the mold) (*3*) salting, (*4*) processing, and (*5*) aging. A flow sheet for preparation of sufu is shown in Figure 3 (16,18,19).

Preparation of Tofu. Soybeans are washed, soaked overnight in water, and ground with water to a milky slurry. A water-to-bean ratio of 10:1 is common. The ground slurry is boiled or steamed and filtered to separate soy milk. A coagulant such as calcium sulfate, magnesium sulfate, or sea salt (about 2.5–3.5% of dry weight of soybean) is added to the hot soy milk. Generally, 20% higher weight of coagulant is used to produce tofu for sufu preparation than that used for regular tofu. After the addition of coagulant to the soy milk, the mixture is agitated vigorously to break the curd into small pieces. The agitated mixture is set aside for 10 min to complete the coagulation process. The coagulated material is referred to as tofu. Tofu is separated from the whey by filtering and pressing the coagulated mixture. Pressing is done in a cloth-lined box to form firm

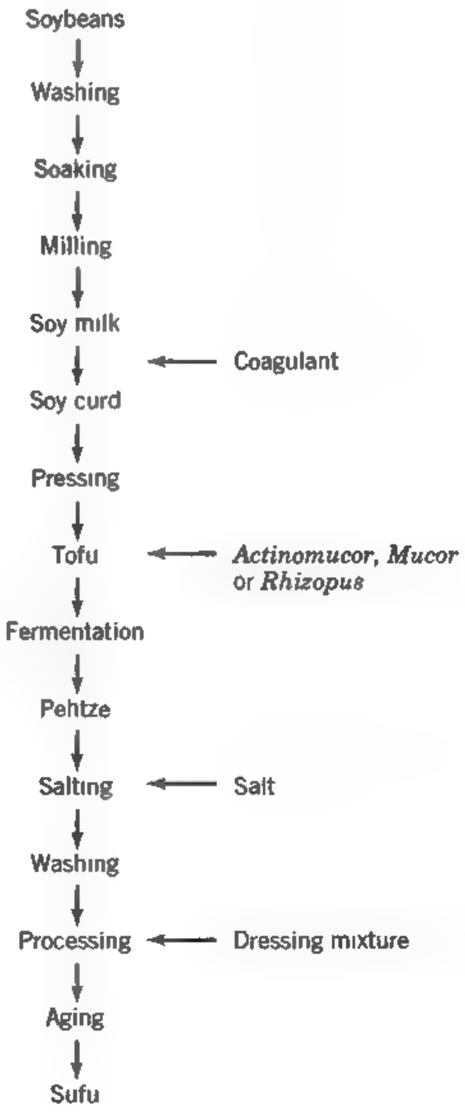


Figure 3. Flow sheet for preparation of sufu. *Source:* Ref. 18.

blocks of desired size. The firm blocks of tofu are cut into small cubes (2 × 4 × 4 cm) weighing approximately 18 g. Because adept tofu workers are becoming scarce, the automatic tofu machine was developed in recent years. This machine saves a lot of labor and unifies the quality of tofu.

Preparation of Pehtze. Pehtze is tofu overgrown with grayish hairlike mycelium of molds of the genus *Actinomucor* spp., *Mucor* spp., or *Rhizopus* spp. To avoid contamination and bacterial spoilage, tofu cubes are exposed to bright sunlight for several hours before inoculation with mold. Instead of being sun dried, tofu cubes can be dried in a hot-air oven at 100°C for about 15 min (17). Before hot-air drying, tofu cubes may be immersed in an acidic saline solution (prepared by dissolving 6 g NaCl and 2.5 g citric acid in 100 mL water) (20). The hot tofu cubes are cooled to 20°C. A pure culture of molds is inoculated onto the surface of the tofu cubes. The inoculated tofu cubes are put in a tray for incubation. The room temperature is maintained at 10 to 20°C. After 3 to 7 days, white fungal mycelium can be seen on the surface of tofu cubes, and at that time they are taken out and immediately salted.

Salting. Pehtze is transferred to large earthenware jars (having a volume of 7 hL) for further curing. It is put in layers in the jars. Each layer of pehtze is sprinkled with a layer of salt and kept for 3 to 4 days. During this period, the pehtze absorbs most of the salt. The salted pehtze is removed from the jars, washed with water, and transferred to other jars for further processing.

Processing and Aging. Pehtze is processed and aged in earthenware jars containing a dressing mixture. The most commonly used dressing mixture consists of 2 kg salt, 1 kg soy sauce mash, 0.6 kg red koji, 0.6 kg jaggery, and 6 kg water. Additional essence can be added to the dressing mixture to give a special flavor. For aging, alternate layers of pehtze and dressing mixture are packed into the jar to 80% of its volume, then the jar is filled with brine containing 12 to 20% salt. The mouth of the jar is wrapped with sheath leaves of bamboo shoots and sealed with clay. The sealed jars are aged for 1 to 3 months or longer for further fermentation before packing into small containers for marketing (16,19). The salt in the dressing mixture imparts a salty taste to the sufu and retards the growth of contaminating microorganisms and mold. Shelf life is a major problem with sufu. Alcohol in the dressing mixture helps to preserve sufu; however, it gives a harsh flavor to the sufu. Therefore it is important to find a method for preservation of fermented pehtze and sufu without the use of alcohol in the dressing mixture.

Composition

The chemical composition of fresh and dried tofu, pehtze, and sufu is presented in Table 6. Enzymes such as invertase, emulsin, trypsin-like protease, pepsin-like protease, oxidase, and catalase are found in pehtze. These enzymes are produced by the mold during fermentation and act on their respective substrates. During the first 10 days of aging, the mold enzymes act on substrates such as proteins and lipids to produce various hydrolyzed products. These hydrolyzed products provide the principal constituents for development of mild characteristic flavors of sufu.

Table 6. Composition of Tofu, Pehtze, and Sufu

	Tofu		Pehtze		Sufu	
Component (%)	Fresh	Dried	Fresh	Dried	Fresh	Dried
Moisture	75.8	—	70.0	—	59.7	—
Protein	16.0	66.0	17.9	59.7	15.9	39.4
Fat	7.2	29.7	9.8	32.8	20.3	50.4
Carbohydrate	0.1	0.4	0.5	1.7	0.0	0.0
Fiber	0.0	0.0	0.4	1.3	1.1	3.7
Ash	0.9	3.9	1.4	4.5	3.0	7.4

Source: Ref. 18.

Organisms Used

In the preparation of pehtze, a variety of fungi are involved. They are *Rhizopus chinensis* var. chungyuen, *Actinomucor elegans*, *Mucor hiemalis*, *M. silvaticus*, and *M. praini*. The fungi, namely *R. chinensis* var chungyuen, are most commonly present in commercial sufu. In the dressing mixture, the fungi *Monascus anka* or *M. purpureus* is present in the red koji, and the microorganisms used for the processing of soy sauce are present in the soy mash.

Safety

When Sprague-Dawley mice were fed 30 g sufu per kg body weight, the mice did not die, and there were no toxic or growth effects. If properly fermented, sufu and its related products are not hazardous to health. The microorganisms involved in sufu fermentation are not known to produce harmful toxins (16).

Future Development

Sufu is a creamy, cheeselike product that has a mild flavor. It can be used in the same way as cheese. If further improvements in taste, flavor, and texture are made, it may become more widely accepted. Although a pure culture method for preparing sufu has been developed, future studies are needed to develop uniform high-quality products and well-defined economical processes to manufacture sufu. Nutritional quality evaluation of sufu needs further investigation (16).

NATTO

History

The earliest record for the term natto appeared in 1068, but there was no description of the preparation method. Natto, during early periods of use, was prepared simply by wrapping cooked soybeans with rice-straw bundles and fermenting the wrapped soybeans at ambient temperature (21,22). In the natural fermentation of soybeans, mold usually dominates, but natto is one of the few products in which bacteria predominate during fermentation. *Bacillus natto*, identified as *B. subtilis*, is the organism responsible for natto fermentation. Consequently, natto possesses the characteristic odor and persistent musty flavor of this organism and is also covered with viscous, sticky polymers produced by *B. subtilis*. Because of its characteristic odor, flavor, and slimy appearance, natto, even though it is well known in Japan, is not as popular nor as widely consumed as miso (23). A similar product, kenima, is known in Nepal, Sikkim, and the Darjeeling districts of India. No yeasts or filamentous fungi were recovered from the samples analyzed from Darjeeling, but two rod-shaped, acid-producing bacteria, present at levels of 10^6 to 10^7/g of wet weight were recovered. Uncooked kenima was unappealing to the Western taste, but when deep-fried and salted, it had a pleasant, nutlike flavor. Thai thua-mao and Nigerian dawadawa are also alkaline-fermented foods closely related to Japanese natto and Indian kenima (22).

Preparation

The manufacture of natto is very simple, as shown in Figure 4. The spores of *B. natto* are sprayed on the cooked soybeans. The resultant soybeans are put into a porous plastic container with a cover and packed. The packed container is put into a fermentation room and kept for 14 to 18 h. The cooked whole soybean grains are kept in the original shape, and the surface of each cooked grain is covered with a very viscous, stringy substance, which is produced by *B. natto* (10).

Composition

There is no change in fat and fiber contents of soybeans during a 24-h period of fermentation, but carbohydrate almost totally disappears. A great increase in water-soluble and ammonia nitrogen is noted during fermentation as well as during storage. The amino acid composition remains the same. Boiling markedly decreases the thiamine level of soybeans, but fermentation by *B. natto* enhances the thiamine content of natto approximately to the same level of soybeans. Riboflavin in natto greatly exceeds that in soybeans. Vitamin B_{12} in natto was found to be higher than in soybeans (23).

TEMPEH

History

Even though tempeh has been produced and consumed in Indonesia for centuries, there are no written records of its origin. The central and east Java areas of Indonesia are still the major tempeh producers. For that reason, several scientists thought that tempeh probably originated in these areas. Besides these areas, it is produced and consumed in other parts of Indonesia (1,9,24).

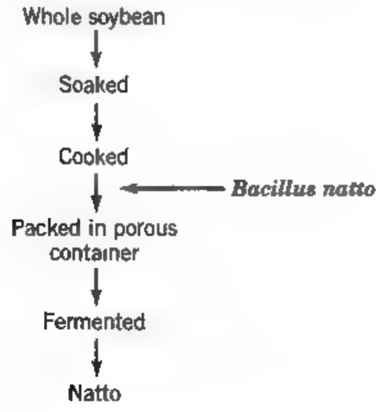


Figure 4. Manufacturing process of natto. *Source:* Ref. 10.

Countries of Consumption

Tempeh is relatively unknown in surrounding countries such as Thailand, China, and Japan, where soybeans form an important part of the diet. Tempeh is produced in small quantities and consumed by immigrants from Indonesia in Malaysia, Surinam, Canada, and the Netherlands. It is becoming a popular food for many vegetarians in the United States. Tempeh experienced a sudden rise in popularity in the United States in 1975, although it was studied extensively and known in the United States as early as 1950 (25). In 1971, soybeans and the fermented food tempeh were introduced into the Republic of Zambia to improve the nutritional status of that country. Tempeh was introduced recently into Sri Lanka through trainees who received special training in production and processing technologies of tempeh in Indonesia.

Preparation

The essential steps for traditional preparation of tempeh are presented in Figure 5. At first, the soybeans are soaked and cooked for 30 min in boiling water to loosen the soybean seed coats. The seed coats of cooked soybeans are hand removed or rubbed with feet to loosen the seed coats and washed with water to separate beans from seed coats. The dehulled beans are again soaked overnight to be hydrated and to allow bacterial acid fermentation. The soaked, dehulled beans are cooked again, drained, cooled, and inoculated with a starter culture or an inoculum from

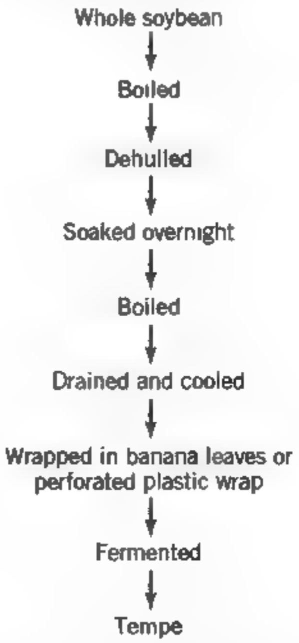


Figure 5. Flow sheet: Indonesian household tempe process. *Source:* Ref. 24.

Table 7. Composition of Nutrients Per 100 g of Tempe

Tempe type	Food energy (cal)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)	Ash (%)
Soy tempe fresh	157	60.4	19.5	7.5	9.9	1.4	1.3
Soy tempe, sun-dried		8.9	43.1	18.0	26.2	3.8	
Soy tempe, freeze-dried		1.9	46.2	23.4	25.8	2.7	2.7
Soy tempe, dry basis		0.0	54.6	14.1	27.9	3.1	3.5
Soy tempe, deep fried		50.0	23.0	18.0	8.0	2.0	1.0

Source: Ref. 24.

a previous batch, wrapped in banana leaves or perforated plastic bags, and incubated for up to 48 h at room temperature (1).

The tempeh should be harvested as soon as the bean cotyledons have been completely overgrown with mycelium and knitted into a compact cake. Freshly made tempe can be stored for a day or two at room temperature without changing many of its qualities or flavor characteristics. Traditionally in Indonesia, tempeh is consumed on the day it is made. If the fresh tempeh is stored for longer periods (more than 2 days) at room temperature, it becomes unsuitable for consumption because of off-flavors and odors produced by excessive proteolysis or by contaminating bacteria and other microorganisms.

Composition

Tempeh is an excellent source of proteins, vitamins, and minerals. It is often used in the diet of diabetics because of the low utilizable carbohydrate content (1,25). During a 72-h fermentation of tempeh, total soluble solids and soluble nitrogen increase, respectively, from 13 to 28% and 0.5 to 2.5%, while the total nitrogen remains fairly constant. The nutrient composition of tempeh is presented in Table 7 (24).

Organisms Used

At least four species of *Rhizopus* (*R. Oligosporus saito*, *R. oryzae*, *R. stolonifer (Ehrenbex Fries) Lind*, and *R. arrhizus* (Fisher) could be used in the preparation of tempeh. In addition to the mold *R. oligosporus*, numerous bacteria (both spore-forming and non-spore-forming types) and yeasts are reported to exist in tempeh. The presence of bacteria in the tempeh fermentation causes the off-odors and flavors of tempeh.

Safety

It is reported that the mold species responsible for tempeh fermentation do not produce aflatoxin. There have been no reports of toxin found in foods prepared from soybean. The mold required for tempeh fermentation is reported to protect tempeh and tempeh-like foods against aflatoxin production and aflatoxin-producing molds. The tempeh mold *R. oligosporus* prevents accumulation of aflatoxin in tempeh by hydrolyzing it (9).

Future Development

Tempeh has great potential as a key protein source in developing countries owing to its low cost and easy preparation. In the future, tempeh may play a major role in satisfying the protein deficiency problems in underdeveloped countries. During tempeh preparation, two major problems are usually encountered: large energy requirement and losses of solids during preparation. There is a need for research on how to reduce energy input and losses of solids.

TAOSI

Taosi is a product made by fermenting whole soybeans with strains of *A. oryzae*. The product is known as toushih by the Chinese, taotjo by the East Indians, and hamanatto by the Japanese (23). In the United States, such fermented beans are often referred to as black beans because of their

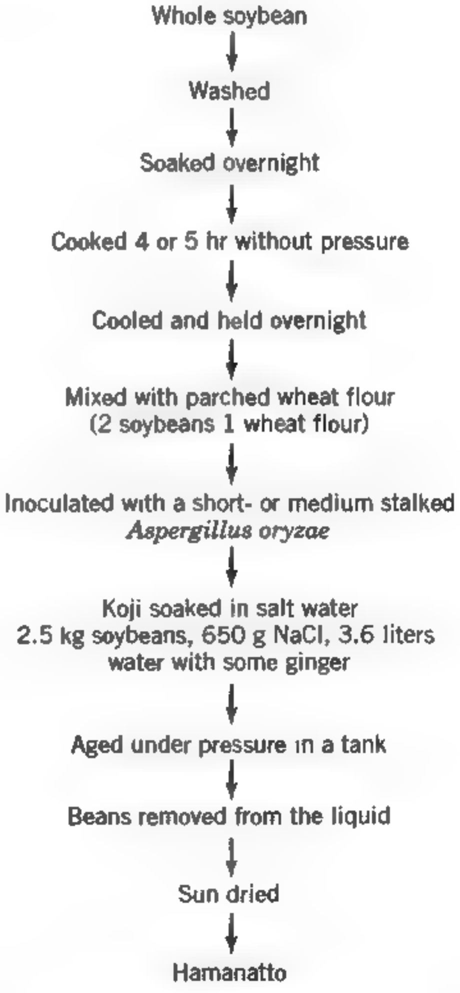

Whole soybean
Washed
Soaked overnight
Cooked 4 or 5 hr without pressure
Cooled and held overnight
Mixed with parched wheat flour (2 soybeans 1 wheat flour)
Inoculated with a short- or medium stalked *Aspergillus oryzae*
Koji soaked in salt water 2.5 kg soybeans, 650 g NaCl, 3.6 liters water with some ginger
Aged under pressure in a tank
Beans removed from the liquid
Sun dried
Hamanatto

Figure 6. Process for making Hamanatto. *Source:* Ref. 23.

color. In China, the product is described as "shi" in the old literature and Chi-Min-Yao-Shu (Table 3) (15). It is mainly consumed as a side dish. The methods of preparing soybeans for fermentation and the composition of the brine may vary from country to country, but the essential features are similar. Soybeans are soaked and steamed until soft, drained, cooled, mixed with parched wheat flour, and then inoculated with a strain of *A. oryzae*. After incubation, the beans are packed with the desired amount of salt, spices, wine, and water and aged for several weeks or months. The finished products are blackish, they have a salty taste, and their flavor resembles that of shoyu. However, they may differ in salt and moisture contents. Hamanatto is rather soft, having a high moisture content. Toushih has a much lower moisture content than that of hamanatto and therefore is not as soft as hamanatto. Taotjo tends to have a sweet taste because sugar is often added to the brine (23).

A typical process for making hamanatto in Japan is outlined in Figure 6. The finished product has a salt content of 13% and a moisture content of 38%. The fermented beans can be used as an appetizer to be consumed with bland foods, such as rice gruel, or they can be cooked with vegetables, meats, and seafoods as a flavoring agent (23).

BIBLIOGRAPHY

1. K. H. Steinkraus, ed., *Handbook of Indigenous Fermented Foods*, 2nd ed., Marcel Dekker, New York, 1996.
2. C. W. Hesseltine and H. L. Wang, "Fermented Foods," *Chem. and Industry* **12**, 393 (1979).
3. N. R. Reddy et al., "Legume-based Fermented Foods: Their Preparation and Nutritional Quality," *CRC Crit. Rev. Food Sci. Nutrition* **17**, 335 (1982).
4. N. R. Reddy, M. D. Pierson, and D. K. Salunkhe, eds., *Legume-Based Fermented Foods*, CRC Press, Boca Raton, Fla., 1986.
5. S. Chen, "Soy Foods in the Far East and USA," *The 1st European Soy Foods Workshop*, Amsterdam, Netherlands, September, 1984, pp. 227–228.
6. H. L. Wang, "Tofu and Tempe as Potential Protein Sources in the Western Diet," *J. Am. Oil Chemists Soc.* **61**, 528–534 (1984).
7. T. Yokotsuka, "Soy Sauce Biochemistry," in C. O. Chichester, E. M. Mrak, and B. S. Schweigert, eds., *Advances in Food Research*, Vol. 30, Academic Press, Orlando, Fla., 1986, pp. 196–329.
8. D. Fukushima, "Industrialization of Fermented Soy Sauce Production Centering Around Japanese Shoyu," in K. H. Steinkraus, ed., Industrialization of Indigenous Fermented Foods, Marcel Dekker, New York, 1989, pp. 1–88.
9. F. G. Winarno, "Traditional Fermented Soy Foods," in F. G. Winarno, ed., *International Soy Foods Symposium*, Yogyakarta, Indonesia, September, 1986.
10. D. Fukushima, "New Development in the Process of Traditional Soy Foods in Japan," in F. G. Winarno, ed., *International Soy Foods Symposium*, Yogyakarta, Indonesia, September, 1986.
11. N. Nunomura and M. Sasaki, "Soy Sauce," in N. R. Reddy, M. D. Pierson and D. K. Salunkhe, eds., *Legume-Based Fermented Foods*, CRC Press, Boca Raton, Fla., 1986, pp. 5–46.
12. H. Ebino, "Industrialization of Japanese Miso Fermentation," in *Industrialization of Indigenous Fermented Foods*, in K. H. Steinkraus, ed., Marcel Dekker, New York, 1989, pp. 89–126.
13. W. Shurtleff and A. Aoyagi, *The Book of Miso*, Autumn Press, Kanagawa-ken, Japan, 1976.
14. S. H. Abiose, M. C. Allan, and B. J. B. Wood, "Microbiology and Biochemistry of Miso Fermentation," in A. I. Laskin, ed., *Advances in Applied Microbiology*, Vol. 28, Academic Press, New York, 1982, pp. 239–265
15. H. Ebine, "Miso," in N. R. Reddy, M. D. Pierson, and D. K. Salunkhe, eds., *Legume-Based Fermented Foods*, CRC Press, Boca Raton, Fla., 1986, pp. 47–68.
16. Y.-C. Su, "Sufu," in N. R. Reddy, M. D. Pierson and D. K. Salunkhe, eds., *Legume-Based Fermented Foods*, CRC Press, Boca Raton, Fla., 1986, pp. 69–83.
17. H. L. Wang and C. W. Hesseltine, "Mold-Modified Foods," in H. J. Peppler and D. Perlman, eds., *Microbial Technology*, Vol. 2, 2nd ed., Academic Press, New York, 1979.
18. Y. C. Su, "Traditional Fermented Foods in Taiwan," *Proc. Oriental Fermented Foods*, Food Industry Research and Development Institute, Hsinchu, Taiwan, **15** (1980).
19. N. S. Wai, "Investigation of the Various Processes Used in Preparing Chinese Cheese by the Fermentation of Soybean Curd with *Mucor* and Other Fungi," Final Technical Report, UR-A6-(40)-1, U.S. Department of Agriculture, Washington, D.C., 1968.
20. W. Shurtleff and A. Aoyagi, *The Book of Tofu*, Ten Speed Press, Berkeley, Calif., 1983.
21. T. Ohta, "Natto," in N. R. Reddy, M. D. Pierson, and D. K. Salunkhe, eds., *Legume-Based Fermented Foods*, CRC Press, Boca Raton, Fla., 1986, pp. 85–93.
22. K. H. Steinkraus, "African Alkaline Fermented Foods and Their Relation to Similar Fermented Foods in Other Parts of the World," in A. Westby and P. J. A. Reilly, eds., *Traditional African Foods Quality & Nutrition*, International Foundation for Science, Stockholm, Sweden, 1991, pp. 87–92.
23. C. W. Hesseltine and H. L. Wang, "Fermented Soybean Food Products," in A. K. Smith and S. J. Circle, eds., *Soybeans: Chemistry and Technology*, AVI Publishing Co., Westport, Conn., 1972, pp. 389–419.
24. F. G. Winarno and N. R. Reddy, "Tempe," in N. R. Reddy, M. D. Pierson, and D. K. Salunkhe, eds., *Legume-Based Fermented Foods*, CRC Press, Boca Raton, Fla., 1986, pp. 95–117.
25. W. Shurtleff and A. Aoyagi, *The Book of Tempeh: A Super Soy Food from Indonesia*, Harper & Row Publishers, New York, 1979.

KEITH H. STEINKRAUS
Cornell University
Ithaca, New York

WEN-LIAN CHEN
Food Industry Research and Development Institute
Hsinchu, Taiwan

SOYBEANS AND SOYBEAN PROCESSING

The four principal oilseed crops grown in the United States are soybeans, cottonseed, peanuts, and sunflowers. Some are consumed directly as foods, and all serve as sources of edible oils. After removal of these oils, the resulting meals are rich in proteins and are used mainly for animal feeds. Soybeans belong to the family Leguminosae (legume) and the genus and species of *Glycine max Merrill*. Soybeans are principally produced in the United States, Brazil, People's Republic of China, and Argentina. They are used for edible oil, animal feed, food, edible proteins and industrial oil.

Soybeans are now the principal oilseed crop in the United States. They are believed to have been domesticated in the eastern half of northern China around the eleventh century B.C. or earlier. They were later introduced and established in Japan and other parts of Asia, brought to Europe, and were introduced in the United States in the late eighteenth century or early nineteenth century. Soybeans became established as an oilseed crop in the United States in the late 1920s and attained major commercial and importance during World War II.

PROPERTIES AND COMPOSITIONS

A common feature of the structure of soybean seed is storage of the bulk of the protein and oil in distinct membrane-bound, subcellular organelles called protein bodies and lipid bodies, (spherosomes) as illustrated in Figure 1. Although not shown in Figure 1, the protein bodies contain inclusions referred to as globoids (>0.1 μm) that are storage sites for phytate and cations such as potassium, magnesium, and calcium. During germination, the contents of the storage organelles are mobilized and utilized by the growing seedling. Soybeans grow on erect, bushy annual plants, 6.3–2.0 m high with hairy stems and trifoliolate leaves. The flowers are white or purple or combinations of white and purple. Growing season varies with latitude (120–130 days in central Illinois). Seeds are produced in pods, usually containing three almost spherical to oval seeds weighing 0.1–0.2 g. Commercial varieties have a yellow seed coat plus two cotyledons, plumule, and hypocotyl-radicle. Cotyledons contain protein and lipid bodies.

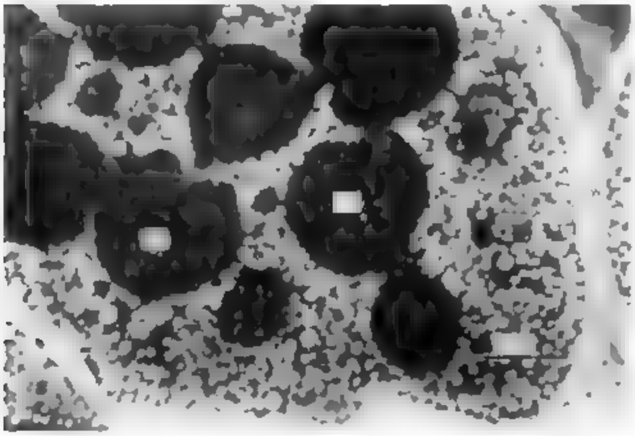


Figure 1. Transmission electron micrograph of a section of a mature, hydrated soybean cotyledon. Protein bodies (PB), lipid bodies (LB), and cell wall (CW) are identified. *Source:* Ref. 1.

On a moisture-free basis, soybeans contain approximately 8% hulls, 20% oil, 43% protein ($N \times 6.25$), 5% ash, and 52% protein in dehulled, and defatted meal (variable depending on efficiency of dehulling and oil extraction, variety of seed, and climatic conditions during growth). The proteins found in soybeans are complex mixtures consisting of four characteristic fractions with molecular weights of ca 8,000–700,000, as illustrated by the ultracentrifuge pattern (Fig. 2). The 7 S and 11 S fractions usually predominate. The 7 S and 11 S fractions are considered to be storage proteins and are located in the protein bodies. The principal portion of soybean 7 S fraction, β-conglycinin, consists of at least seven isomers resulting from various combinations of three subunits (α, α', and β) $\alpha\beta$, $\alpha'\beta2$, $\alpha\alpha'\beta$, $\alpha2\beta$, $\alpha'\alpha2$, $\alpha3$, and $\beta3$. Based on the sizes of the subunits, the β-conglycinin isomers have molecular weights between 126,000 and 171,000.

The 11 S molecule, also called glycinin, is more complex than β-conglycinin. It consists of ca 12 polypeptides, half of which have acidic isoelectric points (molecular weight ca 37,000–44,000) and half of which have basic isoelectric points (molecular weight ca 17,000–22,000); six different acidic and five different basic polypeptides have been separated and identified. Some acidic and basic subunits are linked in nonrandom pairs through disulfide bonds. They are apparently synthesized as single-chain proteins that are subsequently modified by proteolysis to form the acidic and basic polypeptide chains.

In addition to the storage proteins, soybeans contain a variety of minor proteins, including trypsin inhibitors, hemagglutinins, and enzymes (eg, urease and lipoxygenase). Amino acid compositions of soybeans are given in Table 1 along with the essential amino acid pattern for a high-quality protein that meets human requirements as established by the Food and Nutrition Board of the National

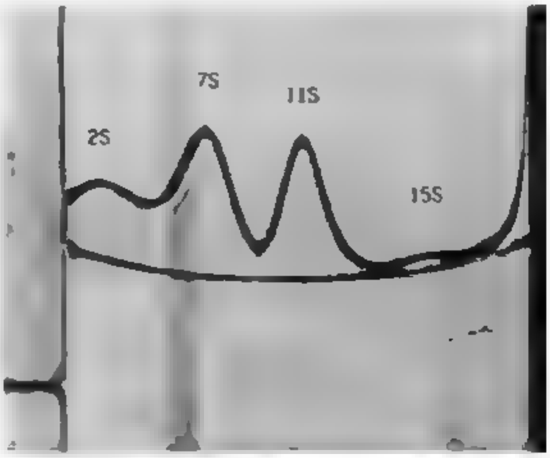


Figure 2. Ultracentrifugal pattern for the water-extractable proteins of defatted soybean meal in pH 7.6, 0.5 ionic strength buffer. Numbers across the top of the pattern are sedimentation coefficients in Svedberg units (S). Molecular weight ranges for the fractions are 2 S: 8,000–50,000; 7 S: 100,000–180,000; 11 S: 300,000–350,000; and 15 S: 700,000 *Source:* Ref. 2.

Table 1. Ammonia and Amino Acid Composition of Defatted Oilseed Meals, g/16 g Nitrogen

Amino acid	Soybean[a]	FNB Pattern[b]
Lysine	6.4	5.1
Histidine	2.6	1.7
Ammonia	1.9	
Arginine	7.3	
Aspartic acid	11.8	
Threonine	3.9	3.5
Serine	5.5	
Glutamic acid	18.6	
Proline	5.5	
Glycine	4.3	
Alanine	4.3	
Valine	4.6	4.8
Cystine	1.4	
Methionine	1.1	2.6
Isoleucine	4.6	4.2
Leucine	7.8	7.0
Tyrosine	3.8	
Phenylalanine	5.0	7.3
Tryptophan	1.4	1.1

[a] Means for seven varieties (3)

[b] Essential amino acid pattern (Food and Nutrition Board pattern) for high-quality protein for humans. An essential amino acid cannot be synthesized by an organism at a sufficiently rapid rate to meet metabolic needs (4).

Research Council. Soybeans meet or exceed the reference pattern except for valine and the sulfur amino acids, which are only slightly low.

Representative fatty acid compositions of the unprocessed triglyceride oils found in soybeans are given in Table 2. Although the oleic and linoleic acid content of soybean oil are high, soybean oil is distinguished from the others by a content of 4–10% linolenic acid, and hence is called a linolenic acid oil.

In addition to the triglycerides, soybeans also contain phosphatides that consist mainly of phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and phosphatidylserines. Generally, ca one-half the phosphatides are extracted from soybeans with hexane.

Table 2. Fatty Acid Composition of Unprocessed Oilseed Oils, Percentage

Carboxylic acid	Soybean
Saturated fatty acids	
10:0	
12:0	0.10
14:0	0.16
16:0	10.7
18:0	3.87
20:0	0.22
22:0	
24:0	
Unsaturated fatty acids	
16:1	0.29
18:1	22.8
18:2	50.8
18:3	6.76
20:1	

Source: Ref. 5

Sterols are present in concentrations of 0.2–0.4% in soybean oil in the following compositions: campesterol, 20%; stigmasterol, 20%; β-sitosterol, 53%; and δ^5 avenasterol, 3%. The sterols exist in the seeds in four forms: free, esterified, nonacylated glucosides, and acylated glucosides. Soybeans contain a total of 0.16% of these sterol forms in the ratio of ca 3:1:2:2.

Soybeans contain two types of carbohydrates: soluble monosaccharides and oligosaccharides and insoluble polysaccharides. The contents of soluble sugars and total carbohydrates in the defatted oilseed meals are given in Table 3; values for intact seeds are different because of their oil and seed-coat contents. Sucrose, raffinose, and stachyose are the principal soluble sugars present. The polysaccharide (not including crude fiber) content is roughly equal to the total carbohydrates minus total soluble sugars, and it ranges from 11 to 19% of the flours.

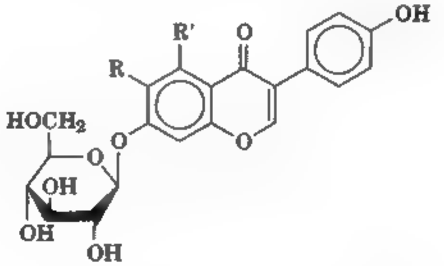
Soybeans contain minor components that affect the defatted seeds, especially when used for feed and food. Soybean seeds contain 1.0–1.5% phytic acid. The isoflavone glucosides genistin, daidzin, and glycetein-7-β-glucoside, plus small amounts of the corresponding aglycones, are constituents of soybeans. Isoflavone contents range from 0.047 to 6.36% (Fig. 3). Soybeans also contain saponins. Recent results indicate that saponin content of soybeans is 5.6% and that of defatted soy flour 2.2–2.5%; these values are ca tenfold higher than those obtained in earlier investigations.

Table 3. Carbohydrate Contents of Defatted Oilseed Flours, Percentage

Constituent	Soybean
Soluble Sugars	
Glucose	trace
Sucrose	7.80
Trehalose	
Raffinose	1.25
Stachyose	6.30
Total	*15.35*
Total carbohydrate[a]	*31.0*

Source: Ref. 6.

[a] Obtained by different: 100 − (protein + oil + ash + crude fiber) = nitrogen-free extract.



Daidzin, R = R′ = H
Genistin, R = H, R′ = OH
Glycetein-7β-glucoside, R = OCH_3, R′ = H

Figure 3. Daidzen, Genisten and glycetein-7β-glucoside.

HARVESTING AND STORAGE

The U.S. soybean crop normally is harvested in September or October. Ideal moisture content for harvesting is 13%, and the crop can be successfully stored at this moisture content until the following summer. Soybeans at <12% moisture can be stored for 2 yr or more with no significant deterioration. Beans with moisture above safe storage limits are dried or placed in aerated bins for gradual moisture reduction.

Soybeans are trucked from the farm to country elevators. From there they are moved by truck or rail to processing plants, subterminal elevators, or terminal elevators. From the terminal elevators, the beans are shipped by rail or barge to export elevators or processors. Soybeans are stored in concrete silos 6–12 m in diameter with heights of > 46 m. The silos are often arranged in multiple rows, and the resulting interstitial silo areas are likewise used for storage. In bulk storage, seasonal temperature changes cause variations in temperature between the different portions of the grain mass (eg, in the winter, soybeans next to the outer walls are colder than those in the center of the silo). Such temperature differences initiate air currents that transfer moisture from warm to cold portions of the seed mass. Thus bulk soybeans originally at safe moisture concentrations may, after storage, have localized regions of higher moisture that cause growth of microorganisms, which in turn can lead to heating. Under these conditions, the beans turn black and may even ignite. Such seasonal moisture transfer also occurs with other oilseeds. In commercial practice the temperature is carefully monitored and, when it rises, the beans are either remixed or processed. Aflatoxin contamination is not a problem as it is with cottonseed and peanuts. Although fungi invade soybeans stored at high moisture and temperatures. *Aspergillus flavus* does not grow well on soybeans and aflatoxins are negligible.

PROCESSING

Virtually all soybeans processed in the United States are solvent extracted (Fig. 4). Beans arriving at the plant are cleaned and dried if necessary before storage. When the beans move from storage to processing, they are cleaned further, cracked to loosen the seed coat or hulls, dehulled, and then conditioned to 10–11% moisture. The conditioned meats are flaked and extracted with hexane to remove the oil. Hexane and oil in the miscella are separated by evaporation and the hexane is recovered. Residual hexane in the flakes is removed by steam treatment in a desolventizer-toaster. The heat treatment inactivates antinutritional factors (trypsin inhibitors) in the raw flakes and increases protein digestibility. A metric ton of soybeans yields ca 180 kg oil and 790 kg meal.

NUTRITION AND TOXICITY

Oil

Because of their high linoleic acid contents, unhydrogenated and partially hydrogenated soybean oils are good sources of this essential fatty acid. Soybean oil is the principal vegetable oil consumed 4.66×10^6 t in 1985), and ca 75% is partially hydrogenated to impart high temperature stability to cooking oil, extend shelf life, and improve flavor stability and physical and plastic properties. Linoleic and linolenic acid contents of soybean oil are reduced by hydrogenation, but more important from a nutritional viewpoint are the migration of double bonds up and down the carbon chain and the conversion of cis to trans isomers, ie, positional and geometrical isomerization. Although long-term studies with rats and short-term tests with humans have failed to reveal toxic effects on ingestion of partially hydrogenated soybean oil, this complex problem is under active investigation.

Heating and oxidation of fats, especially under severe conditions, results in the formation of a variety of compounds including hydrocarbons, cyclic hydrocarbons, alcohols, cyclic dimeric acids, and polymeric fatty acids. Some of these compounds are toxic, but the present consensus is that an oil such as soybean oil is safe and nontoxic when used under normal cooking conditions.

Proteins and Meals

Nutritional properties of the oilseed protein meals and their derived products are determined by the amino acid compositions, content of biologically active proteins, and various nonprotein constituents found in the defatted meals. Phytic acid, which is common to soybean meals, is believed to interfere with dietary absorption of minerals such as zinc, calcium, and iron. Numerous studies have demonstrated that methionine is the first limiting amino acid in soybean protein, ie, it is in greatest deficit for meeting the nutritional requirements of a given species. Although it is common practice to add synthetic methionine to broiler feed to compensate for this deficiency, there is growing evidence that this is not necessary when soy proteins are fed to humans, with the possible exception of infants. The presence of trypsin inhibitors in soybeans is well documented, and when ingested, their primary physiological effect is to enlarge the pancreas. They are largely inactivated by moist heat, and there are no documented cases where ingestion of soybean proteins by humans has affected the pancreas. Long-term effects of ingestion of soy products by rats are under study.

PRODUCTS AND USES

Oil

Most crude oil obtained from oilseeds is processed further and converted into edible products. Only a small fraction of the total oils is utilized for industrial (nonedible) purposes. For edible purposes, oilseed oils are processed into salad and cooking oils, shortenings, and margarines. These products are prepared by a series of steps as shown for soybean oil in Figure 5.

Degumming removes the phosphatides and gums, which are refined into commercial lecithin or returned to the defatted flakes just before the solvent removal and toasting step. Next, free fatty acids, color bodies, and metallic prooxidants are removed with aqueous alkali. Some processors omit the water-degumming step and remove the phosphatides and free fatty acids with alkali in a single

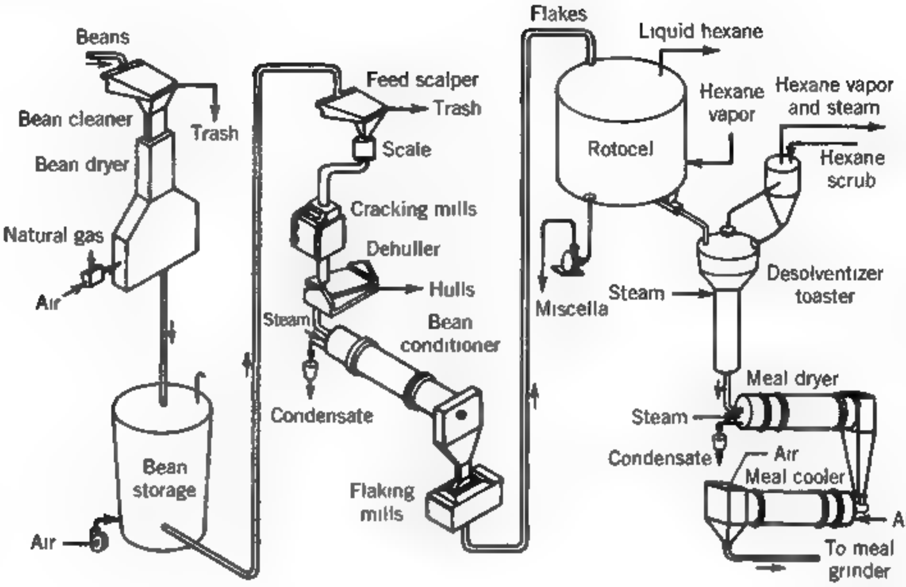

Beans
Bean cleaner
Trash
Bean dryer
Natural gas
Air
Bean storage
Air
Feed scalper
Trash
Scale
Cracking mills
Dehuller
Hulls
Steam
Bean conditioner
Condensate
Flaking mills
Flakes
Liquid hexane
Rotocel
Hexane vapor
Hexane vapor and steam
Hexane scrub
Miscella
Steam
Desolventizer toaster
Meal dryer
Steam
Condensate
Air
Meal cooler
Air
To meal grinder

Figure 4. Schematic outline for processing soybean into oil and meal by solvent extraction. *Source:* Courtesy of Dravo Corp.

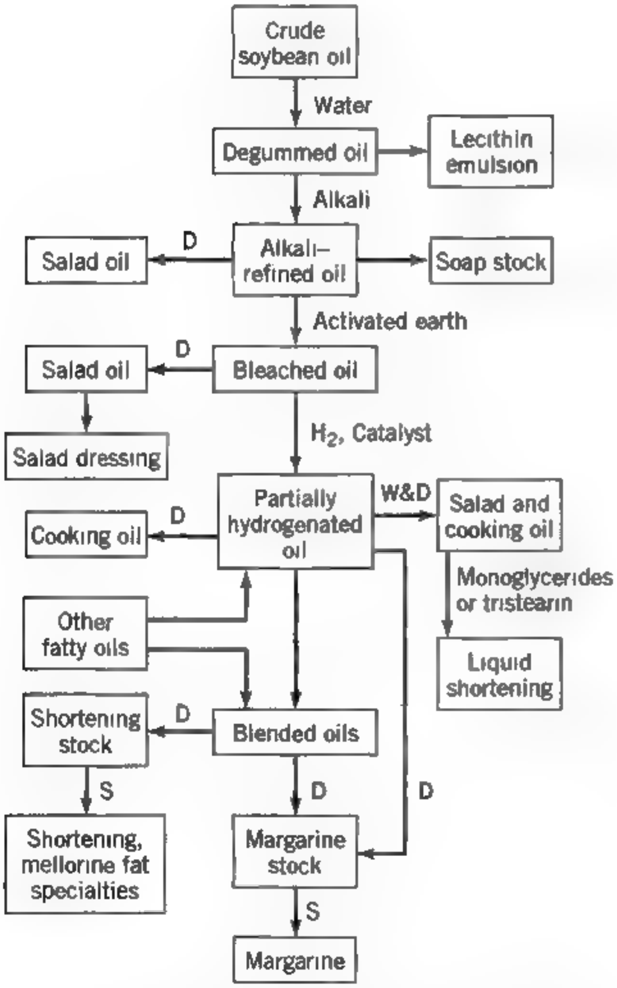

Crude soybean oil
Water
Degummed oil
Lecithin emulsion
Alkali
Salad oil
D
Alkali-refined oil
Soap stock
Activated earth
Salad oil
D
Bleached oil
Salad dressing
H₂, Catalyst
Partially hydrogenated oil
W&D
Salad and cooking oil
Cooking oil
D
Monoglycerides or tristearin
Other fatty oils
Liquid shortening
Shortening stock
D
Blended oils
S
D
D
Shortening, mellorine fat specialties
Margarine stock
S
Margarine

Figure 5. Schematic outline for manufacture of edible soybean oil products. D, deodorization; W, winterization; S, solidification. *Source:* Courtesy of the American Soybean Association and the American Oil Chemists' Society.

operation. High, vacuum steam distillation in the deodorization step removes undesirable flavors to yield a product suited for salad oil. Partial hydrogenation, under conditions where linolenate is selectively hydrogenated, results in an oil with greater stability to oxidation and flavor deterioration. After winterization (cooling and removal of solids that crystallize in the cold), the product is suited for use as salad and cooking oils. Alternatively, soybean oil can be hydrogenated under selective or nonselective conditions to increase its melting point and produce hardened fats. Such a partially hydrogenated soybean oil, by itself or in a blend with other vegetable oils or animal fats, is used for shortening and margarine. Blends of soybean or other oils of varying melting point ranges are utilized to obtain desired physical characteristics, eg, mouthfeel and plastic melting ranges, and the least expensive formulation.

Polyunsaturated fatty acids in vegetable oils are subject to oxidative deterioration. Linolenic esters in soybean oil are particularly sensitive to oxidation; even a slight degree of oxidation, commonly referred to as flavor reversion, results in undesirable flavors, eg, beany, grassy, painty, or fishy. Oxidation is controlled by the exclusion of metal contaminants (iron and copper), addition of metal inactivators (citric acid), minimum-exposure to air, protection from light, and selective hydrogenation to decrease the linolenate content to ca 3%.

Nonfood Uses

Vegetable oils are utilized in a variety of nonedible applications, but only 2–3% of U.S. soybean oil production is used for such products. Soybean oil can be converted into alkyd resins for protective coatings, plasticizers, dimer acids, surfactants, and a number of other products.

Protein Products

Most of the meal obtained in processing of soybeans is used as protein supplements in animal feeds. Only in the last two decades have appreciable amounts been converted into products for human consumption, and these have been almost exclusively derived from defatted soybean flakes.

Feeds

Because of their high content of protein, protein meals are essential ingredients of poultry and livestock feeds. Proximate compositions are shown in Table 4. In contrast, dehulled soybean meal is low in crude fiber and high in protein. Although limiting in methionine, soybean meal is high in lysine is a key ingredient for blending with corn in formulating feeds for nonruminats, eg, poultry and swine. The two proteins complement each other; soy supplies the lysine and corn the methionine necessary to provide a balanced ration at relatively low cost.

Edible Products

At present, only defatted soybean flakes are converted into edible-grade products. Defatted soybean flakes for edible purposes are prepared essentially as outlined in Figure 4, except that more attention is paid to sanitation than in processing for feed use, and the solvent is removed in a vapor desolventizer—deodorizer or flash desolventizer to permit preparation of flakes ranging from raw to fully cooked. Desolventizer–toasters are used to prepare fully cooked (toasted) flakes to give maximum nutritive value. Degree of cooking is determined by estimating the amount of water-soluble protein remaining after a moist heat treatment with the protein dispersibility index (PDI) or the nitrogen solubility index (NSI). A raw, uncooked flake has a PDI or NSI of ca 90, whereas a fully cooked flake has values of 5–15.

Defatted soybean flakes give three classes of products differing in minimum protein content (expressed on a dry basis) flours and grits (50% protein); protein concentrates (70% protein); and protein isolates (90% protein). Typical analyses are shown in Table 5. Flours and grits are made by grinding and sieving flakes. Concentrates are prepared by extracting and removing the soluble sugars from defatted flakes by leaching with dilute acid at pH 4.5 or leaching with aqueous ethanol. Isolated soy proteins are obtained by extracting the soluble proteins with water at pH 8–9, precipitating at pH 4.5, centrifuging the resulting protein curd, washing, redispersing in water (with or without adjusting the pH to ca 7), and finally spray drying (Fig. 6). Flours and concentrates are also specially processed into textured products that are used as meat extenders and substitutes. Typical composition of soybean protein products and their uses are summarized in Table 5.

Oilseed proteins are used in foods at concentrations of 1–2 to nearly 100%. Because of their high protein contents, textured soy flours and concentrates serve as meat substitutes. At low concentrations, the proteins are added primarily for their functional properties, eg, emulsification, fat absorption, water absorption, texture, dough formation, adhesion, cohesion, elasticity, film formation, and aeration. The use of some oilseed proteins in foods is limited by flavor, color, and flatus problems. Raw soybeans, for example, taste grassy, beany, and bitter. Even after processing, residues of these flavors may limit the amounts of soybean proteins that can be added to a given food. Flatus production by defatted soy flours has been attributed to raffinose and stachyose. These sugars are removed by processing the defatted flours into concentrates and isolates.

Table 4. Proximate Compositions of Soybean Meals, wt %

Meal	Process	Dry matter	Crude protein	Crude fat	Crude fiber	Ash
		Soybean				
With hulls	Solvent	89.6	44.0	0.5	7.0	6.0
Dehulled	Solvent	89.3	47.5	0.5	3.0	6.0

Source: Ref. 7.

Table 5. Typical Compositions of Soybean Protein Products and Their Uses,[a] wt %

Constituents	Defatted flours and grits[b]	Protein	
		Concentrates[c]	Isolated[d]
Protein[e]	56.0	72.0	96.0
Fat	1.0	1.0	0.1
Fiber	3.5	4.5	0.1
Ash	6.0	5.0	3.5
Soluble carbohydrates	14.0	2.5	0
Insoluble carbohydrates	19.5	15.0	0.3

[a] Analytical values on a moisture-free basis (8).

[b] For baked goods, ground and processed meats, breakfast cereals, diet foods, infant foods, meat substitutes, confections, and milk replacers.

[c] For ground and processed meats and infant foods.

[d] For processed meats, meat substitutes, infant foods, and dairy substitutes.

[e] $N \times 6.25$.

Industrial Products

In the United States, only soybean protein isolates are used for industrial applications. They are available commercially in unhydrolyzed types are made by the process outlined in Figure 6; the acid-precipitated curd is washed, dried, and then ground. For the hydrolyzed types, the acid-precipitate curd is suspended in alkali and heated to dissociate the proteins into subunits and partially hydrolyze the polypeptide chains. The reaction is terminated by acidifying to a pH of ca 4.5 to precipitate the modified protein, which is dried. The chemically modified isolates are made by proprietary processes. Isolates are employed primarily as adhesives for clays used in coating of paper and paperboard to render surfaces suitable for printing.

Food Products

Soybeans seeds are consumed as such or are processed into edible products. Small amounts of soybeans are roasted and salted for snacks. Nut substitutes for baked products and confections are also manufactured from soybeans. Larger amounts are used in Oriental foods, some of which are becoming increasingly popular in the United States.

Soy Milk. In the traditional Chinese process, soybeans are soaked in water, ground into a slurry, cooked, and filtered to remove the insoluble cell wall and hull fractions. A number of process modifications have been made since the early 1960s, including a heat treatment before or during grinding to inactivate the enzyme lipoxygenase and thus prevent formation of grassy and beany flavors. Markets for the blander products made by the new processes

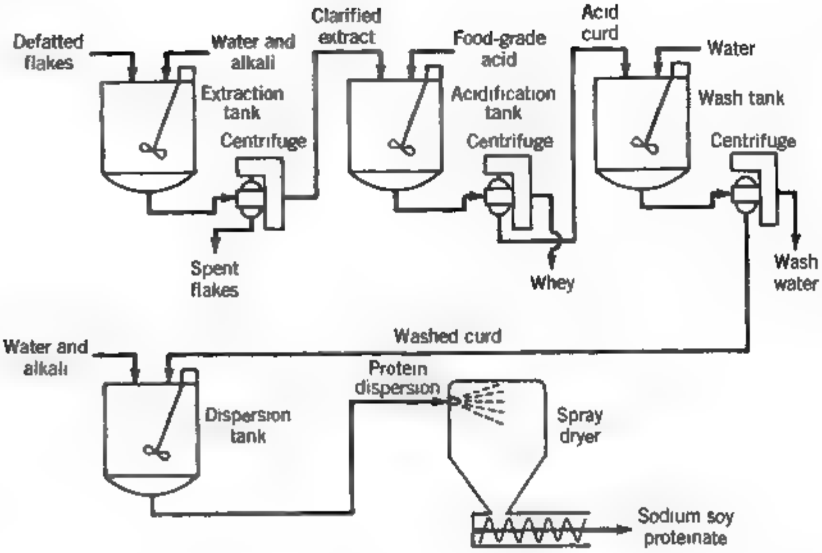


Figure 6. Schematic outline for manufacture of soybean protein isolates.

have developed rapidly in Japan since the late 1970s. Some of the products are sweetened and flavored; fermented (yogurtlike) soy milks are also available in Japan and other parts of Asia.

Tofu. Tofu is prepared by adding a coagulant such as calcium sulfate to soy milk to precipitate the protein and oil into a gelatinous curd. The curd is then separated from the soluble portion (whey), pressed, and washed to yield a market-ready product that is a traditional food in Japan and throughout Asia. It has become popular in the United States since the late 1970s and was produced in 1981 by more than 150 shops.

Miso. Miso is a pastelike food that resembles peanut butter in consistency and is made by fermenting cooked soybeans and salt with or without a cereal such as rice or barley. It is used as a base for soups and is consumed in Japan, China, Indochina, Indonesia, and the East Indies. In 1990, it was produced by ca 20 small establishments in the United States.

Tempeh. Cooked dehulled beans are inoculated with the mold *Rhizopus oligosporus* and allowed to ferment for 24 h. The mold mycellium binds the soybean cotyledons together. When sliced and deep fried in fat, the product is crisp and golden brown. Tempeh, a traditional food of Indonesia, was made by more than 30 concerns in the United States in 1981.

Soy Sauce. This condiment, well known to U.S. consumers, is made by fermentation or acid hydrolysis. In the fermentation process, cooked soybeans or defatted soybean meal are mixed with roasted wheat and the mixture is blended with a pure culture of *Aspergillus oryzae*. Brine is added and the mixture is allowed to ferment 6–8 months. The product is then filtered and pasteurized. In the acid hydrolysis process, defatted soybean flour is refluxed with hydrochloric acid until the proteins are hydrolyzed. The hydrolysate is then filtered, neutralized, and bottled.

BIBLIOGRAPHY

Adapted from W. J. Wolfe, "Soybeans and Other Oilseeds." *ECT*, 3rd ed., Vol. 21, p. 407. Refer to the original document for specific reference citation.

1. *Nippon Shokuhin Kogyo Gakkai-shi* **15**, 290 (1968).
2. *Journal of Agricultural and Food Chemistry* **18**, 969 (1970).
3. *Journal of the American Chemical Society* **45**, 876 (1968).
4. *RDA*, 10th ed., FNB/NAS, 1990.
5. *Journal of the American Dietetic Association* **68**, 224 (1976).
6. *Journal of the American Chemical Society* **54**, 150 (1977).
7. *Feedstuffs* **53**, 25 (1981).
8. *Journal of the American Oil Chemists' Society* **51**, 67A (1974).

GENERAL REFERENCES

R. L. Anderson and W. J. Wolf, "Compositional Changes in Trypsin Inhibitors, Phytic acid, Saponins and Isoflavones Related to Soybean Processing," *J. Nutrition* **125**, 581S–588S (1995).

D. R. Erickson, *Practical Handbook of Soybean Processing and Utilization*, American Oil Chemists' Press, Champaign, Ill., 1995.

W. J. Huei and P. A. Murphy, "Mass Balance Study of Isoflavones During Soybean Processing," *J. Ag. and Food Chem.* **44**, 2377–2383 (1996)

S. H. Lence, D. J. Hayes, and W. H. Meyers, "Futures Markets and Marketing Firms: The U.S. Soybean-Processing Industry," *Amer. J. Ag. Econ.* **74**, 716–725 (1992).

H. Miyamura, Y. Takenaka, and T. Takenaka, "Fibrinolytic Activity of Akara Fermented by *Bacillus subtilis*. II. The Utility of Akara, A By-Product of the Soybean Processing Industry," *J. Jap. Soc. for Food Sci. and Technol.* **45**, 100–107 (1998).

Z. L. Nikolov et al., "Potential Applications for Supercritical Carbon Dioxide Separations in Soybean Processing," *Developments in Food Sci.* **29**, 595–616 (1992).

G. A. Sulebele, "Soybean Processing Industry, An Update," *Indian Food Industry* **10**, 23–26 (1991).

L. Thomas and A. Hohn, "Beneficial Use of Enzymes in Soybean Processing," *Food Marketing and Technol.* **11**, 14, 16, 18 (1997).

Y. H. Hui
American Food and Nutrition Center
Cutten, California

SPICES AND HERBS: NATURAL EXTRACTIVES

The importance of herbs and spices in flavor and color applications cannot be overstated, but there are shortcomings as well. The natural extractives of spices and herbs are designed to overcome many of the difficulties inherent in the use of whole or ground plant material in food and flavor applications.

The aroma and flavor produced by the use of plant material varies, depending not only on the variety and source of the raw material, but also on the growing conditions and weather patterns of a given year. This variability makes the consistent application in food products very difficult. In addition, flavor components in plant tissue are unstable, leading to a decrease in quality due to volatility losses, oxidation, isomerization, hydrolysis, and other chemical changes. Plant tissue can also support molds and other types of undesirable microbial contamination.

Spices and herbs require storage under regulated conditions, such as refrigeration, that will minimize deterioration with time. Given the large bulk volume, this can be an expensive proposition. Extractives of spices and herbs are highly concentrated, which simplifies storage conditions and greatly decreases the amount of additive needed in product formulations. They are more stable in terms of flavor strength and have lower microbiological activity.

The biggest advantage of extractives versus dried spices, however, is the increased control of the flavoring agents. A more consistent flavoring agent is available due to the quality control of the extraction process. A broader range of flavor effects is made possible by blending or further processing of the extractives. Natural extractives are divided into essential oils, oleoresins, and products made from these.

ESSENTIAL OILS

Essential oils can be defined as the volatile materials present in plants. These are complex mixtures of flavor and fragrance substances usually obtained by distillation. U.S. import statistics are published periodically (1).

Manufacturing Process

Essential oils, due to their relatively high boiling points, are usually obtained by steam distillation, by codistillation with water, or by the combined use of steam and water (2,3). In steam distillation the plant material (whole or ground) is packed into a still, and the essential oil is removed by passing steam through the plant material. The steam pressure is most commonly atmospheric, although both higher and lower pressures are used.

Hydrodistillation, or direct-heating distillation, is the process in which plant material is soaked in the boiler while heat is applied to codistill essential oil with water (4). The plant material is supported above boiling water. This method is used for collecting the volatile oil of green plant material.

On cooling, the raw distillate separates into aqueous layer and an organic layer. The organic layer may require further water removal by centrifugation or by the use of a drying agent. The essential oil can be purified and refined to obtain the desired flavor and aroma characteristics and to remove undesired flavor components. Vacuum distillation, liquid/liquid extraction, and adsorption are the most commonly used refinement techniques.

Vacuum distillation provides for the separation of the components with less thermal degradation than redistillation at atmospheric pressure. At reduced pressures the essential oil is redistilled or fractionated to remove undesired compounds and to concentrate desired compounds. Several types of products are obtained depending on the distillation conditions used:

1. Rectified oils are redistilled with rejection of the last distillation fraction (single rectified) or the first and last fractions (double rectified). Usually only a small percentage of the raw essential oil containing water, color, off-aromas, and other undesirable components are rejected.
2. Terpeneless oils can be produced if efficient separation conditions can be achieved so that the terpene fraction can be removed from the higher boiling fractions that contain the more valuable flavor compounds.

Liquid/liquid extraction is a process using differences in polarity and solubility to separate oxygenated compounds from terpenes. A mixture of the essential oil and solvent is agitated and allowed to settle into two liquid layers that are then separated. The more polar compounds will be concentrated in the polar solvent and recovered after solvent removal. In cold-solvent extraction of citrus oils, for example, the oxygenated compounds are concentrated into aqueous alcohol. The use of a second, nonpolar solvent to dilute the terpenes may improve the separation and selectivity of the extraction.

Adsorption collects and concentrates selected compounds according to their affinities to a solid, for example, silica gel. The essential oil, with or without the use of a solvent, is passed through a solid material that selectively adsorbs some components. These compounds are then stripped from the solid phase by a suitable solvent and concentrated by removal of the solvent.

When the essential oil has been enriched in the ingredients that are responsible for the characteristic flavor, it is commonly referred to as a folded oil. Although this term originally referred to concentration by distillation, it is currently used without specification of the method used to increase the flavor strength.

Physical Characteristics

Physical characteristics, such as refractive index, optical rotation, specific gravity, among others, of essential oils are tabulated in various listed references (5–7). It is important to remember that the physical characteristics do not necessarily determine quality, flavor, or natural origin, although they can be a useful part of quality control.

Chemical Composition

Essential oils are complex mixtures of compounds; some essential oils have been separated into hundreds of different components by modern techniques (6,8,9). Although some essential oils have only one main constituent, it is not necessary that the main component determine the characteristic aroma. In some cases a minor component can be the most important for defining an aroma, for example, dill ether in dill seed oil. In general, the minor components can be expected to determine the quality of the aroma, or at least the completeness of its sensory impact (10,11). The constituents of essential oils can be grouped into three major categories:

1. Hydrocarbons, usually olefinic, are compounds whose structures are made up of isoprene units. Terpenes, sesquiterpenes, and diterpenes are in this group. They have a low contribution to aroma and flavor strength even though they may constitute the major percentage of the oil. The unsaturated nature of these compounds can lead to deterioration due to oxidation and polymerization.
2. Oxygenated hydrocarbons, which can be subcategorized as alcohols, esters, aldehydes, ethers, ketones, phenols, and so on, have much lower sensory thresholds than hydrocarbons. In some cases an individual oxygenated compound gives the characteristic aroma of a spice or herb, and its level in the essential oil can be correlated to flavor strength.
3. Other compounds containing sulfur or nitrogen can be important for the flavor of essential oils or can impart off-flavors at extremely low levels.

Gas chromatography (GC) is by far the most widely used technique in the study of the components of essential oils (12–14). These compounds are separated by boiling point and by interactions with the stationary-phase coating of the GC columns. The improved resolution possible with capillary columns compared to packed columns has greatly improved separations. One of the advantages of GC is the flexibility and selectivity possible in the detection of the compounds as they are eluted from the column. A flame ionization detector can provide universal and sensitive detection of all compounds in an essential oil. However, a detector specific for sulfur- or oxygen-containing compounds can also be used. Using a gas chromatograph coupled to a mass spectrometer or a Fourier transform infrared spectrometer can provide compound identification as well as quantification. The use of a computerized library search greatly simplifies the identification of previously characterized compounds. The gas chromatograph is not a suitable instrument for all essential oils; thermally labile compounds can decompose during the assay.

OLEORESINS

Oleoresins are the solvent extractables of the botanical raw material after solvent removal. They contain the volatile and nonvolatile active ingredients of the raw materials and have been an item of commerce for more than 50 years (15). As with spices and herbs, numerous oleoresins are available to the flavor-food chemist to achieve the desired impact. The usage of oleoresins parallels the volume of raw commodities as listed in U.S. government import statistics (16). Major oleoresins are black pepper, capsicum, celery, ginger, nutmeg, paprika, and turmeric. Minor oleoresins are basil, bay, cinnamon, clove, coriander, mace, marjoram, oregano, rosemary, sage, and thyme. A more complete listing of the products can be found in Title 21 *Code of Federal Regulations* (CFR), part 182, section 182.20, and part 184 (17).

MANUFACTURING PROCESS

Individual manufactures differ in the process details used for production, but all have in common the following stages:

grinding > extraction > desolventization > standardization

The grinding operation is employed to make the extractives available for dissolution into the solvent. Grinding also increases the surface area available for extraction. Many types of grinding equipment are used, depending on the raw material and the extraction process. This information is not readily available from manufacturers because it is one of the most critical steps in the process and is considered proprietary by most manufacturers. What is important to remember is that the grinding must have minimum deleterious effect on the aroma and flavor attributes of the raw material but must be effective in insuring exhaustive extraction of the components contributing to the essence. Because heat is generated in most grinding processes, cryogenic techniques are often employed to retain volatile fractions.

The extraction process can be subdivided into two types: single stage and double stage. The choice for the individual manufacturer depends on the solvent used to perform the extraction and solvent removal capability. The permitted solvents for extraction are listed in 21 *CFR*, part 173, and include acetone, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene (17). Use of chlorinated solvents is being reevaluated within the industry and will be further discussed under the section on regulatory issues.

In single-stage extraction, the ground product is transferred to the extraction vessel and washed with solvent to remove the essence, and the exhausted material (marc) is desolventized to recover entrained solvent. The extraction vessel may be batch type, where all of the extraction functions occur in a steam-jacketed pot containing a basket. The ground material is loaded into a basket, sealed, and washed with solvent. The oleoresin is then desolventized; the marc is removed and desolventized separately. Alternatively, this process may be done on a continuous basis, with the ground material introduced via an air lock to the extractor and conveyed through solvent wash stages; the marc passes through another air lock for desolventization.

Double-stage extraction utilizes the batch-type extractor. The difference is that the volatile fraction is removed by steam distillation prior to solvent extraction of the resinous fraction. These two fractions are then recombined in the standardization stage. This procedure is most often used with the manufacture of oleoresin black pepper; the advantage of this process is that the resinous fraction can be desolventized under more rigorous conditions, because the preservation of the volatile fraction is not an issue. The disadvantage is that the process limits the extraction solvent to the chlorinated hydrocarbons to produce commercially acceptable product, because residual water from the first stage inhibits extraction of the resinous fraction (18).

The desolventization process reduces the extraction solvent in the oleoresin to permissible levels, as defined in the applicable regulations (17). Various methods are employed, dependent on the physical characteristics of the oleoresin. Two basic strategies are used. The first, where preservation of the volatile fraction is not required and viscosity of the product is low, is called wiped-film evaporation. The oleoresin-laden solvent, or miscella, is pumped through a column and spread by an impeller along the inside surface. The column is heated via a steam jacket and partial vacuum is applied to aid in solvent removal. The miscella is introduced at the top of the column, with the thickness of the film controlled by addition rate, speed of the impeller, viscosity, and temperature. The evaporated solvent flows countercurrent to the oleoresin and is recovered for reuse. Oleoresins that are processed in this manner include the capsicums and turmeric.

The second method, where retention of the volatile fraction is desired or the viscosity is high, is called batch vacuum distillation. The miscella is loaded into a steam-jacketed vacuum vessel, which is fitted with a fractionation column. This equipment is normally called a still. Steam is applied to heat the vessel to the atmospheric boiling point of the solvent. The solvent vapors are refluxed to return the volatile fraction back to the product. After the majority of the solvent is removed from the product (which is indicated by a temperature rise in the still), vacuum is applied to further reduce the solvent content to acceptable levels. Although care is taken to exclude the volatile fraction from the distillate, it is necessary with some products to fractionate the distillate to recover some of the volatiles. These light fractions are reserved for blending in the standardization step.

Standardization is necessary to re-create the flavor and aroma profile of the raw material while providing the customer with a consistent product. Edible oils, such as soybean or cottonseed, are used to standardize flavor intensity. Emulsifiers are also incorporated with some products at this stage to make for easier application. Standardization occurs on a batch basis, combining the extractives into a mixing vessel. If the manufacturer uses standardization agents, then they must be clearly stated on the label. The ingredients are sequentially combined based on decreasing viscosity, with the recovered volatile fractions being added last. The blending may be achieved by use of a high shear stirrer, colloidal mill, or homogenizer. Mixing times are product dependent. Samples are drawn for testing to assure conformance to requirements of the final mixture before packaging.

Physical Characteristics

Oleoresins are liquids; viscosities vary from that of vegetable oil, as with capsicum and celery, to that of a semisolid paste, as with nutmeg. The oleoresins are concentrated essences of the raw material with an increase of flavor strength in the range of 6-fold for celery to greater than 40-fold for cinnamon. This is due to the varying amount of extractables within each raw material and to the fact that the aroma and flavor components on extraction from the plant matrix are more readily incorporated into the end product. Along with the information provided by the manufacturer, many of the products have criteria established in the *Food Chemical Codex* (19) to aid the user in developing meaningful specifications.

Chemical Composition

Unlike the essential oils, where only the volatile notes are available for aroma and flavor effect, oleoresins contain the volatile fraction, active compounds and fixed compounds. The volatile fraction is very similar in composition to the previously discussed essential oils. The active compounds are defined, for the purpose of this article, as the nonvolatile constituents perceived predominantly in the mouth and not by the nasal membranes. To give a few examples, these compounds are the pungent alkaloid group related to piperine in black pepper and white pepper, the amide group related to capsaicin in capsicums, and the keto-alcohols related to gingerol in ginger. The fixed compounds are defined as the nonvolatile, nonpungent compounds. Examples of these are coloring compounds, such as chlorophyll found in herbs, cartenoids found in capsicums, and curcuminoids found in turmeric. The fixed compounds also include various saturated and unsaturated fats, which lend balance to the flavor and aroma profiles of the oleoresins, making them more representative of the starting raw material. The extraction process does not yield the carbohydrate or protein fraction of the raw material, for the most part, and therefore these fractions are not an important component of the oleoresin.

FORMS AND APPLICATIONS

The common form of both essential oils and oleoresins is an oil-dispersible liquid. Because most foods contain a high amount of water, homogenous distribution is difficult. Concentrated products are required in small dosages to achieve the desired effect, making it doubly difficult to ensure consistency. To overcome these difficulties, various means have been developed to incorporate the extractives into commercial products:

1. Dispersed products (dry-soluble seasonings) are prepared by dispersing the essential oils and oleoresins on an edible carrier: salt, dextrose, or flour. Limited in concentration by the ability of the carrier to remain flowable, these blends can incorporate up to 10% liquids.
2. Solubilized products are blended with polysorbate esters, such as polysorbate 80; these products are clearly dispersible in deionized water. Such formulations may contain up to 50% extractives.

3. Emulsions are blended into water with gum arabic or other permitted emulsifying agents; these products have a limited shelf life.
4. Encapsulation (spray drying) forms products in which essential oils or oleoresins are homogenized into water with vegetable gums or food starches, then atomized into a drying chamber. The resultant dry powder may contain up to 20% liquids, with the encapsulation providing some increased shelf life stability in comparison to dispersed products.
5. Water-dispersible liquids are also available. Since 1980, advances in emulsifier technology have led to products that are dispersible in water without the use of polysorbate esters. These products contain about 50% extractives and are much more shelf stable than many of the other products listed in this section. They are easily incorporated into the water phase of the finished product. Some manufacturer formulations are also soluble in the oil-fat phase of a product, evenly distributing the extractives in both phases.

Decisions must be made by the product formulator as to which form of the extractives is most suitable for the application. The standard essential oils and oleoresins have been successfully used in oil-fat-based foods, such as salad dressings, without special incorporation techniques.

Traditionally the dispersed products have had the widest application in food systems, being mixed in as dry ingredients in early processing. Most formulated meats are flavored in this manner, along with bakery and breading goods and seasonings for snack foods. Pickling processors use the oleoresins solubilized with polysorbate esters to flavor the brine, which migrates to the product during retort and storage. Emulsions are not widely used, although they do have limited applications in emulsified meats such as bologna and hot dogs. Encapsulated products are used in dry mixes (soups, salad dressings, drinks, etc) where extended shelf life is required.

Water-dispersible liquids can replace most of the aforementioned application needs, though only to a limited degree in pickling and dry-mix applications. Most extractive producers provide technical support based on their applications research and experience with a variety of processes. This can be a valuable asset to the flavor formulator.

NUTRITIONAL INFORMATION

It is not possible to generalize the nutritional value of essential oils or oleoresins. Nutritional information is available from the manufacturer for individual products. In foods, the weight percentage of these added flavor ingredients does not usually make a significant contribution to overall nutrition. Essential oils, because they are both ether soluble and combustible, assay as fat. Oleoresins contain the natural vegetable oils extracted with aroma and flavor components, predominantly unsaturated fats normally found in vegetable sources. Carriers, emulsifiers, and diluents also need to be considered in nutritional data in that they often make up more than 50% of commercial forms. Specific components may assay as protein, such as piperine in black pepper, although they are not types of protein. Mineral and vitamin content is not usually significant, with the exception of vitamins A and C in some products.

Herbs and spices contain many compounds with proven antioxidant, anti-inflammatory, and antimutagenic activity. Nutraceuticals is a term used to describe the beneficial components in spices and herbs as part of a diet or as food supplements (19).

METHODS OF ANALYSIS

The most important criteria for evaluation of extractives are the flavor and aroma profiles. Organoleptic evaluation is the best means by which to judge these flavor additives. Even though it is difficult and expensive to do on a routine basis in production operation, it is necessary. As a prescreening tool, physical measurements can reduce the number of organoleptic evaluations somewhat. It is critical that the analytical methods to be employed are agreed on by both customer and supplier, because different methods give different results, although in theory they are measuring the same attributes. Control criteria (20) and methods of analysis (21,22) have been published.

REGULATORY STATUS

In the United States, Title 21 of the *CFR* governs usage of spice extractives in foods and drugs. Under part 182.20 and part 184 are listed the essential oils and oleoresins that are considered to be generally recognized as safe (GRAS) and their requirements. Oleoresins used as vegetable colors are listed under separate headings of part 73. Under part 173, the maximum residue for the solvent used in extraction is as follows:

Solvent	Level (ppm)
Acetone	30
Ethylene dichloride	30
Isopropyl alcohol	50
Methyl alcohol	50
Methylene chloride	30
Hexane	25
Trichloroethylene	30

Some countries do not permit the use of extracts made with chlorinated solvents. This must be taken into account when finished goods are to be exported. Because the list of areas in the world that enforce this ban is growing, any listing here would be obsolete before publication. The standardizing agents used in the extracts are considered incidental additives. The labels of finished foods in which these products are used need only state "natural flavors" or "natural flavoring." It is best to check with legal counsel to confirm labeling status.

BIBLIOGRAPHY

1. United States Department of Agriculture, *U.S. Essential Oil Trade*, USDA Foreign Agricultural Service, Washington, D.C., 1980–1989.
2. K. Bauer and D. Garbe, *Common Fragrance and Flavor Materials*, VCH Verlagsgesellschaft, Germany, 1985.
3. H. B. Heath, *Flavor Technology: Profiles, Products, Applications*, AVI Publishing Co., Inc., Westport, Conn., 1978.
4. E. F. K. Denny, "Hydro-distillation of Oils from Aromatic Herbs," *Perfumer and Flavorist* **14**, 57–63 (1989).
5. G. Fenaroli, *Fenaroli's Handbook of Flavor Ingredients*, T. E. Furia and N. Bellanca, eds. and trans., The Chemical Rubber Co., Cleveland, Ohio, 1971.
6. B. M. Lawrence, ed., *Essential Oils (1976–1978), (1979–1980), (1981–1987), (1989–1991), (1992–1994)*, Allured Publishing Co., Carol Stream, Ill.
7. *EOA Book of Standards and Specifications*, Essential Oil Association of USA, Inc., New York, 1979.
8. S. R. Srinivas, *Atlas of Essential Oils*, published by the author, New York, 1986.
9. E. Guenther, *The Essential Oils*, Vols. 1–6, Van Nostrand Reinhold Co., Inc., New York, 1949.
10. F. A. Fazzalari, ed., *Compilation of Odor and Taste Threshold Values Data*, American Society for Testing and Materials, Philadelphia, Pa., 1978.
11. A. Dravnieks, *Atlas for Odor Character Profiles*, American Society for Testing Materials, Philadelphia, Pa., 1985.
12. W. Jennings and T. Shibamoto, *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*, Academic Press, Inc., Orlando, Fla., 1980.
13. Y. Masada, *Analysis of Essential Oils by Gas Chromatography and Mass Spectroscopy*, John Wiley & Sons, Inc., New York, 1976.
14. P. Sandra and C. Bicchi, eds., *Capillary Gas Chromatography in Essential Oil Analysis*, Heutig, New York, 1987.
15. H. B. Heath, *Source Book of Flavors*, AVI Publishing Co., Inc., Westport, Conn., 1981, p. 212.
16. United States Department of Agriculture, *U.S. Spice Trade*, USDA Foreign Agricultural Service, Washington, D.C
17. Office of the Federal Register National Archives and Records Administration, *Code of Federal Regulations, Title 21 Food and Drugs*, U.S. Government Printing Office, Washington, D.C., 1997.
18. V. S. Govindarajan, "Pepper—Chemistry, Technology, and Quality Evaluation," *CRC Critical Review of Food Science and Nutrition*, 115–225, 1977.
19. I. Goldberg, ed., *Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals*, Chapman and Hall, New York, 1994.
20. Committee on Codex Specifications, *Food Chemical Codex*, 4th. ed., National Academy Press, Washington, D.C., 1996.
21. Association of Official Analytical Chemists, *Method of Analysis of the AOAC*, 16th ed., AOAC, Arlington, Va., 1995.
22. American Spice Trade Association, *Official Analytical Methods of the American Spice Trade Association*, 4th ed., ASTA, Englewood Cliffs, N.J., 1997.

JAMES A. GUZINSKI
TED M LUPINA
Kalsec, Inc.
Kalamazoo, Michigan

SPICES AND SEASONINGS

Spices are the whole or ground seed, fruit, bark, or root of a plant. Examples are cumin, allspice, cinnamon, and ginger. Herbs are the leafy parts of annual or perennial shrubs or plants. Oregano, marjoram, and basil would fit this classification. Seasonings, on the other hand, are blends of spices with other functional and nonfunctional ingredients. Seasonings are usually blended with other food items to produce a finished food product. Spices and seasonings will be discussed separately.

SPICES

The use of all spices and herbs has increased dramatically over the years. According to the United States Department of Agriculture (USDA), imports of spices in 1997 was more than 638 million lb (1).

Definition

The Food & Drug Administration (FDA) has defined *spice* as any "aromatic vegetable substance in the whole, broken or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic, and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed" (2). The Code of Federal Regulations (CFR) goes on to define the specific spices and herbs as "spices." In addition, paprika, saffron, and turmeric are identified by the FDA also as colors and must be declared as such on ingredient labels (2). See Table 1 for a listing of the common spices.

Table 1. Spice Classification

Spices	
Allspice	Dill seed
Anise	Fennel seed
Anise, star	Fenugreek
Caraway seed	Ginger
Cardamom	Mustard seed
Celery seed	Nutmeg
Cinnamon	Mace
Cloves	Pepper, black & white
Coriander	Pepper, red
Cumin seed	
Herbs	
Basil	Rosemary
Bay leaves	Sage
Dill weed	Savory
Marjoram	Tarragon
Oregano	Thyme
Colors	
Paprika	Turmeric
Saffron	

Economic Factors

Because spices are agricultural commodities, various political and climatic conditions can cause prices to rise and fall due to supply and demand. If a war or other political situation occurs, often the spice cannot be exported. For example, during the 1979 Iran hostage crisis, cumin was difficult to obtain and prices rose substantially due to U.S. trade restrictions. Cumin was often shipped to Pakistan and France from Iran, before shipping to the United States.

If growing conditions are poor in countries where a spice is usually imported from, prices may increase dramatically, or the spice may be unavailable completely. In 1988 and 1989, Dundicut chillies, commonly used to make red pepper, were unavailable from Pakistan due to a drought. Beginning in 1992, the quality of sage imported from the former Yugoslavia decreased dramatically due to internal ethnic conflicts and the resulting division of the country. In addition, in recent years, black pepper prices have risen about 50%. This is mostly due to poor growing conditions over a multiyear period. In addition, low commodity prices prior to the increase did not give farmers an incentive to grow pepper.

When purchasing spices from overseas, either direct from source or through brokers in New York, it is important to keep abreast of supply problems and not restrict specifications to a particular country of origin unless absolutely necessary.

Cleaning and Production

Spices are commonly brought into the United States through New York brokers and sold to spice processors who clean and sometimes grind the product. Large spice companies import direct from processors overseas. The spices are then sold to other food processors, packaged and sold to consumers or food service establishments, or blended with other products to produce seasonings.

The United States has cleanliness specifications that all imported spices must meet. These are the FDA Defect Action Levels (DALs) (3). An excellent discussion of how spices are legally imported and passed by FDA can be found in *Spices and Seasonings, A Food Technology Handbook* (4). The most commonly used cleanliness guidelines are the American Spice Trade Association (ASTA) Cleanliness Specifications (5). Both the DALs and the ASTA specifications are designed to be the minimum measure of cleanliness and to realize that spices, being agricultural commodities, cannot be free of foreign material and do require additional cleaning by processors. Table 2 shows the current ASTA specifications. Processors receive spices in bales or often burlap bags and are responsible for cleaning and grinding (if required) to meet their internal specifications or those set by their customer. Reputable spice processors first check the product for cleanliness in the whole form by sampling a representative number of containers and physically checking for extraneous material such as live and dead insects, stones, excreta, hair, metal, and glass. If the product is found to have these items present, it must be cleaned. Infestations with insects are treated with methyl bromide or heat to kill them, then removed as described next. Metal is generally removed by magnets, which should be present on all cleaning and grinding equipment. See Figure 1 for a flowchart of spice processing.

Although a variety of equipment is used to clean spices, depending on the foreign matter present and the spice being cleaned, three basic types of separation techniques are used: air separators, gravity separators, and centrifugal separators. Air separators use the movement of air to separate material according to the velocity of air required to remove the foreign material. Centrifugal separators use centrifugal force to separate product, and gravity separators separate particles by differences in weight or specific gravity of the product. Most equipment uses a combination of these three methods to clean spices. The most commonly used cleaning equipment is the vacuum gravity separator, or air table. This piece of equipment uses two separation techniques: air separation and gravity separation (Fig. 2).

In a vacuum gravity separator, product is metered onto a porous deck where air is forced through, suspending the product. The air and deck motion causes the particles to separate into layers of different densities. The heavy material moves uphill and the light material moves downhill. This piece of equipment can be adjusted to separate product into many different density fractions. More information on cleaning spices is available from ASTA (6).

After cleaning, the product is typically checked again for cleanliness. If the product passes, it is checked for quality attributes and either sold whole or ground. Grinding can be done to any specific size by using screens to separate the product into specific fractions.

Spice Quality

Chemical and Physical. Quality attributes for spices are usually present on a specification from the processor. These include volatile oil, moisture, heavy and light filth, ash, and acid-insoluble ash. Product specific tests include Scoville heat units for red peppers, ASTA color units for paprika, and curcumin content for turmeric. ASTA has published the *Official Analytical Methods of the American Spice Trade Association* (7). The most common methods are outlined in the following:

Volatile oil is the major aroma and flavor component of all spices. It will volatilize at low temperatures, and most spices contain up to 20 or more different components in the volatile oil. A spice generally contains 0.5 to 3.0% volatile oil, and typical levels are higher in seed spices than in herbs. Whole spices have a higher volatile oil level than ground spices. When a spice is ground, it loses its volatile oil content quickly due to the crushing of the protective cell structure present in the whole spice. In addition, the heat produced by grinding can vaporize the volatile oil during processing. Volatile oil content is an important quality parameter in spices. The higher the volatile oil content, the more flavor the spice has. Volatile oil is lost with age, and it can vary between different crop years, origins, and even within lots. In the industry, a minimum level is usually set that the spice must adhere to. Using liquid nitrogen to cool the mill head during grinding can help retain spice volatiles.

Moisture levels also indicate spice quality. A high moisture content indicates improper storage or inadequate dry-

Table 2. ASTA Cleanliness Specifications

Name of spice seed, or herb	Whole insects, dead by count[k]	Excreta, mammalian by mg/lb)	Excreta, other (by mg/lb)	Mold (% by wgt)	Insect defiled/infested (% by wgt)	Extraneous/foreign matter (% by wgt)
Allspice	2	5	5.0	2.00	1.00	0.50
Anise	4	3	5.0	1.00	1.00	1.00
Sweet Basil	2	1	2.0	1.00	1.00	0.50[l]
Caraway	4	3	10.0	1.00	1.00	0.50
Cardamom	4	3	1.0	1.00	1.00	0.50
Cassia	2	1	1.0	5.00	2.50	0.50
Cinnamon	2	1	2.0	1.00	1.00	0.50
Celery Seed	4	3	3.0	1.00	1.00	0.50
Chillies	4	1	8.0	3.00	2.50	0.50
Cloves[a]	4	5	8.0	1.00	1.00	1.00[a]
Coriander	4	3	10.0	1.00	1.00	0.50
Cumin Seed	4	3	5.0	1.00	1.00	0.50
Dill Seed	4	3	2.0	1.00	1.00	0.50
Fennel Seed	SF[e]	SF[e]	SF[e]	1.00	1.00	0.50
Ginger	4	3	3.0	SF[f]	SF[f]	1.00
Laurel Leaves[b]	2	1	10.0	2.00	2.50	0.50
Mace	4	3	1.0	2.00	1.00	0.50
Marjoram	3	1	10.0	1.00	1.00	1.00[l]
Nutmeg (Broken)	4	5	1.0	SF[g]	SF[g]	0.50
Nutmeg (Whole)	4	0	0.0	SF[h]	SF[h]	0.00
Oregano[c]	3	1	10.0	1.00	1.00	1.00[l]
Black Pepper	2	1	5.0	SF[i]	SF[i]	1.00
White Pepper[d]	2	1	1.0	SF[j]	SF[j]	0.50
Poppy Seed	2	3	3.0	1.00	1.00	0.50
Rosemary Leaves	2	1	4.0	1.00	1.00	0.50[l]
Sage[b]	2	1	4.0	1.00	1.00	0.50
Savory	2	1	10.0	1.00	1.00	0.50[l]
Sesame Seed	4	5	10.0	1.00	1.00	0.50
Sesame Seed, Hulled	4	5	1.0	1.00	1.00	0.50
Tarragon	2	1	1.0	1.00	1.00	0.50[l]
Thyme	4	1	5.0	1.00	1.00	0.50[l]
Turmeric	3	5	5.0	3.00	2.50	0.50

[a] Clove stems: A 5% allowance by weight for unattached clove stems over and above the tolerance for Other Extraneous Matter is permitted.

[b] Laurel Leaves and Sage: "Stems" will be reported separately for economic purposes and will not represent a pass/fail criteria.

[c] Oregano: Analysis for presence of Sumac shall not be mandatory if samples are marked "Product of Mexico."

[d] White Pepper: "Percent Black Pepper" will be reported separately for economic purposes and will not represent a pass/fail criteria.

[e] Fennel Seed. In the case of Fennel Seed, if more than 20% of the subsamples contain any rodent, other excreta or whole insects, or an average of 3 mg/lb of mammalian excreta, the lot must be reconditioned.

[f] Ginger: More than 3% moldy pieces and/or insect infested pieces by weight.

[g] Broken Nutmeg: More than 5% mold/insect defiled combined by weight.

[h] Whole Nutmeg: More than 10% insect infested and/or moldy pieces, with a maximum of 5% insect defiled pieces by count.

[i] Black Pepper: 1% moldy and/or infested pieces by weight.

[j] White Pepper: 1% moldy and/or infested pieces by weight.

[k] Whole Insects, Dead: Cannot exceed the limits shown.

[l] Extraneous Matter: Includes other plant material, eg foreign leaves

ing. High moisture contents can cause mold or microbiological growth. Testing for moisture can be done by two methods: vacuum oven drying or toluene distillation. Vacuum oven drying generally gives a higher result because some of the volatile oils may be driven off as well as the moisture. Therefore, this method is generally only recommended for capsicums and dehydrated vegetables where volatile oil is not an important parameter.

Heavy and light filth indicates the amount of foreign material present in the spice. Metal shavings, rocks, insect fragments, and glass will all be found by these methods. Filth testing is especially important in ground spices because these items may be difficult to identify by other methods. The spice to be analyzed is placed in a dense organic solvent such as carbon tetrachloride. The heavy foreign matter will sink and the spice will float. By physically separating these two fractions, heavy and light filth can be determined.

Total ash is the residue left after ignition of the spice. Acid-insoluble ash is the residue left after treating the total ash with hydrochloric acid. These tests also determine the amount of foreign material present. Rocks, metal, and other foreign items will remain behind as residue. Ash is a measure of metal, sand, and minerals naturally occurring in the spice. Acid-insoluble ash is a measure of only metal and sand present.

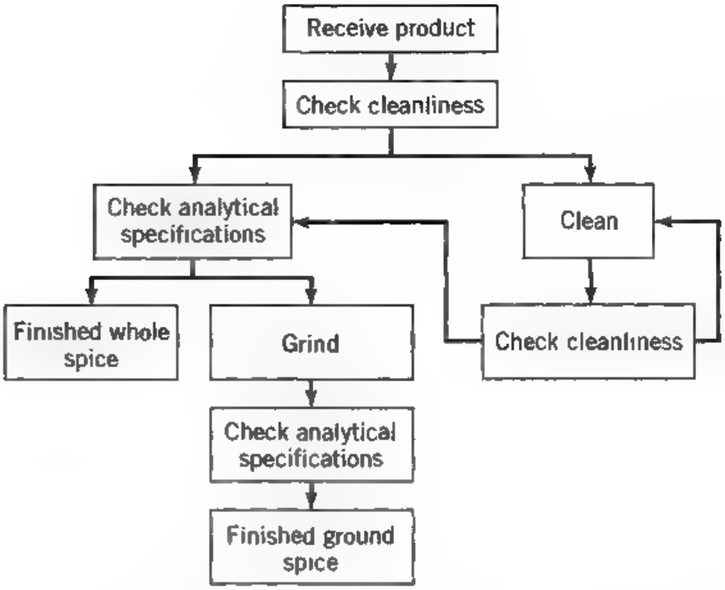


Figure 1. Flowchart of spice processing.

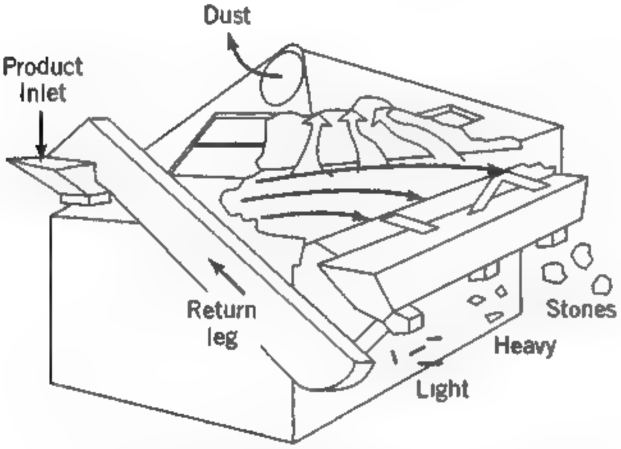


Figure 2. Vacuum gravity separator (air table).

Heat units indicate the relative heat of red peppers. The higher the number, the hotter the red pepper. The Scoville test is a threshold taste test of a red pepper ethanol extraction using five trained panelists. Scoville test has been the most common method for determining the heat level in red peppers, although variance between labs can be very high. ASTA has recently dropped the approval for the Scoville method. A more accurate and ASTA-approved method is high-pressure liquid chromatography (HPLC), which is an analytical test instead of an organoleptic method. The correlation between Scoville and HPLC methods is questionable.

Extractable color of paprika is determined by an extraction with acetone followed by a spectrophotometric test. This method results in the ASTA color of paprika, such as 100 ASTA paprika. These units do not necessarily correlate with surface color. Curcumin content of turmeric is determined by a similar method.

Microbiological. Spices can contain very high levels of bacteria. Most come from third world countries where sanitary conditions are poor. Often spices are laid out in fields, sides of roads, or banks of rivers to dry and are subject to a high degree of contamination. It is not uncommon to have total plate counts on black pepper up to 40 million per gram. Even onion powder grown in the United States can have counts as high as 1 million per gram. The highest counts are usually found on allspice, black pepper, caraway, celery seed, cumin, paprika, and onion powder. Typical microbiological tests performed on natural spices are total plate count, yeast, mold, total coliforms, *Escherichia coli*, and *Salmonella*.

Often spices are treated with sterilizing agents to reduce bacterial loads. The two most common treatments are ethylene oxide and irradiation. Some spice suppliers also use steam to sterilize spices. Both ethylene oxide and irradiation have disadvantages; however, microbial counts can be significantly reduced by using one of these methods.

Ethylene oxide is a gas that is used to reduce microbial loads in spices. It can cause about a 90% reduction in microbial counts. This chemical has been found to be a carcinogen and may be physically retained on the spice after the process has been completed. Chlorohydrins that are toxic can also form. The FDA has a 50 ppm residual tolerance. Currently the FDA and Environmental Protection Agency (EPA) are debating its continued approved use.

Irradiation is an ionizing radiation that is used to kill microorganisms in spices. It is a very effective treatment, providing almost 100% kill of bacteria (8). However, certain consumer groups are very vocal about their opposition to this treatment due to its misguided association with radiation. Spices can be irradiated with levels up to 3 Mrad. FDA regulations state that an irradiated spice must be labeled as "treated with/by irradiation" and the irradiation logo prominently displayed. If a food contains irradiated ingredients, the finished product is not required to be labeled as irradiated (9). With the increasing *E. coli* incidence in meats, this may be an ideal time to influence consumer perception about this very effective treatment. FDA is currently considering alternate labeling regulations.

Description of Whole Spices

Allspice, Pimenta dioica L. (formerly *Pimenta officinalis*) is the dried, unripe berry of a shiny leafed evergreen tree indigenous to the Western Hemisphere. The berries are reddish brown, round and smooth, and about 1 cm in diameter. The name allspice comes from the flavor description: a combination of cloves, cinnamon, and nutmeg. Another, less common term for allspice is pimento. Allspice is imported from Jamaica, Guatemala, Mexico, and Honduras. In 1997, the United States imported about 2.3 million lb of allspice (1).

Anise seed, Pimpinella anisum L., is the seed of a small annual herb of the parsley family. The grayish-brown oval seeds are about 0.5 cm long. Anise seed has a strong licorice-like flavor and is grown primarily in Spain, Egypt, and Turkey. The United States imported about 2.6 million lb in 1997 (1).

Basil, Ocimum basilicum L., is the leaf of an annual herb of the mint family native to India and the Persian countries. The leaves are greenish gray and about 5 cm long and 2 cm wide before drying. When purchased whole, basil is actually dried pieces of leaves. Primary sources are France, Egypt, and the United States. Domestic basil is much more expensive than the imported varieties because it is mechanically harvested and dried. Basil has seen phenomenal growth in the last 30 years. Only 40,000 lb were imported into the United States in 1964 compared with 6.1 million lb in 1997! (1).

Bay leaves or laurel leaves, *Laurus nobilis* L., are large light-green elliptical leaves, up to 8 cm in length and 3 to 4 cm wide. The flavor is fragrant and sweet with a bitter note and is used most commonly in soups and stews as well as pickling spices. Principal countries exporting to the United States are Turkey and Greece. There is also a domestic bay leaf that is packed for retail sale, *Laurus californica*, which does has a slightly different flavor and is longer and narrower than its imported counterpart.

Capsicum peppers, Capsicum frutescens L., include red peppers, chili peppers, and sweet bell peppers. Red pepper, also known as cayenne, is ground chili pods, generally the small elongated and hottest varieties, which are blended to produce a product with standard heat units. Heat levels, expressed as either Scoville heat units or HPLC heat units, in whole pods vary from crop to crop. The hottest varieties are grown in Africa, China, and India. Chili pepper is the ground product of larger, milder peppers, primarily grown in California, New Mexico, and Mexico. Variances in color are primarily due to a caramelization process that processors employ and pepper variety.

Caraway seed, Carum carvi L., is the fruit of a biennial plant of the parsley family. Each seed is a half of the fruit and is about 0.75 cm long, curved, with ridges lengthwise. Caraway is native to Europe, Asia, and North Africa, although it is primarily exported now from The Netherlands. The flavor is warm and acrid, similar to dill seed. The United States imported about 6.9 million lb in 1997 (1).

Cardamon, Elettaria cardamomum Maton, is a large perennial belonging to the ginger family. The spice is imported whole in oval, greenish pods, 1 to 2 cm long, which contain 15 to 20 brownish-black seeds. These seeds produce the sweet, aromatic flavor of cardamon. Most cardamon is grown in India, Guatemala, and Ceylon. It is a minor spice, with the United States only importing about 0.5 million lb a year (1).

Celery seed, Apium graveolens L., is the dried fruit of a biennial herb native to southern Europe called smallage. It is different from Pascal celery, which is eaten as a vegetable in the United States. The seed is oval, brown, and very small, about 0.25 cm in length. It has a warm, bitter flavor. It is now primarily imported from India.

Cinnamon. There are many species of cinnamon or cassia. The type most commonly imported into the United States is not cinnamon at all, but rather cassia (*Cinnamomum cassia* Presl—Chinese cassia or *Cinnamomum burmannii* Blume, Indonesian or Korintji cassia). Cinnamon (*Cinnamomum verum* Presl—Ceylon or Seychelles cinnamon) is lighter in color and milder than the two varieties of cassia previously mentioned. It is most commonly exported to Great Britain and other countries. Both cinnamon and cassia are the bark of evergreen trees, which is peeled off and allowed to curl into cinnamon sticks. Another, less common type is Saigon cinnamon, *Cinnamomum loureirii*, which recently is being imported from Vietnam again. Before the Vietnam War, this was a much more common supply for cinnamon.

Cloves (*Eugenia caryophyllata*, Thunb.) are the dried, unopened flower buds of an evergreen tree of the myrtle family. Cloves are about 1 cm long and resemble a rounded top nail. The flavor is strong and aromatic. Cloves are grown in many parts of the world; however, the chief exporters are Madagascar and Brazil.

Coriander seed (*Coriandrum sativum*) is the dried ripe fruit of an annual herb native to southern Europe and the Mediterranean region. Coriander is a round light brown seed about 0.5 cm in diameter with vertical ridges. The leaves of the coriander plant are known as cilantro, which is used extensively in Latin American, Thai, and Vietnamese cuisine. The flavor of coriander seed is mild and fragrant. Most coriander is currently imported from Canada and Morocco.

Cumin, Cuminium cyminum L., is a small annual herb of the parsley family. The dried fruit, commonly referred to as the seed, is oval, about 0.5 cm long and similar in appearance to caraway. Cumin is used extensively in Mexican and Indian foods and has a strong earthy and bitter flavor. Cumin is grown and exported from the Middle East, India, and Turkey.

Dill, Anethum graveolens L., is an annual herb related to the other spices of the parsley family. Dill seed and dill weed are from the same plant. The seeds are oval, tan, and about 0.5 cm long. The flavor is reminiscent of caraway. Dill is grown in Egypt, India, and the United States.

Fennel seed, Foeniculum vulgare Mill., is the dried fruit of a perennial plant native to southern Europe. The seed is oval and curved, about 0.75 cm long, and greenish in color. The flavor is similar to anise, but less sweet. The consumption of fennel in the United States has increased significantly in the last 20 years, primarily due to its use in pizza topping and Italian sausage. Fennel is imported primarily from India and Egypt, about 7.5 million lb in 1997 (1).

Fenugreek (*Trigonella foenum-graecum* L.) is a little-used spice in the United States. Its flavor is maplelike and bitter. The spice itself is a hard, tan pod containing 10 to 20 seeds. It is primarily used to make imitation maple flavor and curry powders in the United States. Fenugreek is imported from India and Morocco.

Ginger, Zingiber officinale, is a perennial herb with thick, tuberous roots or rhizomes. These tubers are often described as shaped like deer antlers and are tan. To cultivate, the rhizomes are dug up, washed, dried, and often peeled. The flavor is pungent and spicy. Ginger is available fresh, crystallized, and dried. It is imported from China, India, and Jamaica. More than 29.5 million lb (including fresh) were imported in 1997 (1).

Marjoram (*Marjorana hortensis*, Moench) is the leaves of a perennial of the mint family and is a close relative of oregano. Its leaves are green-gray and about 1.25 cm long. The flavor is aromatic, camphoraceous, and bitter. Marjoram is native to western Asia and the Mediterranean region. Principal sources today are France and Egypt.

Mustard seed (*Brassica hirta*—white or yellow mustard and *Brassica juncea*—Oriental or brown mustard) is small in size, about 3 mm in diameter. Color ranges from a light tan (*B. hirta*) to a reddish brown (*B. juncea*). Both types of mustard contain the enzyme myrosinase, which reacts with glycosidic compounds (sinalbin in *B. hirta* and sinigrin in *B. juncea*) in the presence of moisture to release its characteristic pungency (10). Acidic liquids do not trigger this reaction, due to the lowered pH inactivating the myrosinase, but will preserve the pungency if added after it first develops in water. *B. juncea*, upon the enzymatic reaction, produces the compound allylisothiocyanate, which gives a powerful aroma, much like horseradish (10).

Mustard seeds are available in three primary forms: whole mustard, often used in pickling spices; ground mustard, which is used mainly in the sausage industry; and mustard flour. Ground mustard can be treated with heat to deactivate the myrosinase enzyme. This type of ground mustard is used in fresh sausages because the active enzyme can react with the protein in the meat. Mustard flour is the ground seed after the husk or bran is removed. Mustard flour is generally milled to a very fine flour and is a blend of yellow and Oriental mustard seeds. Mustard flour is used primarily in the salad dressing and sauce industries. The principal producing countries of mustard are Canada and the northern plains of the United States.

Nutmeg and *mace* (*Myristica fragrans*, Houtt) are both from an evergreen tree native to the islands of the East Indian archipelago. The tree produces an apricot-like fruit containing a large brown seed about 4 cm long and 2 cm wide. This is the nutmeg seed. Mace is a lacy, netlike orange covering over the seed. The flavor of both is very similar: sweet and warm. Nutmeg is generally milder and sweeter while mace has a sharper flavor. Nutmeg and mace are primarily grown in Indonesia, Grenada, and Trinidad. Nutmeg is the product used most often in the United States. Import volumes from 1997 show it 14 times the volume of mace (1).

Oregano. Two types of oregano are commonly used in the United States. *Origanum vulgare* L. is a perennial of the mint family and is indigenous to the Mediterranean region. Its leaves are dark green, hairy, and about 1.5 cm long. The flavor is bitter with a green note. It is grown primarily in Greece and Turkey. Mexican oregano, *Lippia graveolens* has a distinctly different flavor. Its leaves are larger than the Mediterranean variety. Mexican oregano is commonly available on the West Coast and used most often in Mexican, Southwest, and Tex Mex recipes.

Parsley. Petroselinum sativum L. and *Petroselinum crispum* are two varieties of parsley. The former is the curly leaf variety grown primarily in California and the most common type dried today. The flakes are available in a variety of sizes and bright green in color. The flavor is very mild.

Paprika, Capsicum annum L., is the ground dried fruit pods of a small bushy plant. The pods are orange to bright red and used primarily for their coloring properties. Most paprika is sweet and mild and is grown primarily in California, Spain, Morocco and Hungary.

Pepper, black and white. Piper nigrum L. is the berry of an evergreen-climbing vine native to the coast of southwestern India. The berries are shriveled, dark brown to black, and about 0.75 to 1.0 cm in diameter. Black and white pepper both come from the same plant. White pepper is a ripe peppercorn with the dark hull removed. Black pepper is the unripe berry, which is simply dried. Green peppercorns, growing in popularity recently, are the immature berry of the same vine and are often freeze-dried. Primary sources of black pepper are the Malabar Coast of India (often called Tellicherry if the berry is large enough), the Lampung district of Sumatra in Indonesia, and Brazil. Vietnam has recently emerged as a viable source for black pepper. White pepper is usually imported from the Muntok area of Indonesia. Black pepper is the largest volume spice imported into the United States. In 1997, 112 million lb were imported (1)!

Rosemary, Rosmarinus officinalis L., is an evergreen shrub of the mint family. The leaves are green, narrow, and about 2 cm long, resembling pine needles. It is currently exported from Albania, France, and Spain.

Saffron, Crocus sativus L., is the most expensive spice, costing an average wholesale price of about $200/lb in 1997. It is the dried stigmas of the flower of a purple crocus. The cost is due to the hand harvesting of the three delicate stigmas on each flower. It takes about 250,000 stigmas to produce one pound of saffron (11). Saffron has a bitter flavor but is most prized for its bright yellow color. Spain is the primary producer.

Sage, Salvia officinalis L., is an evergreen shrub of the mint family. The leaves are silver gray to green and velvety to the touch, ranging from 5 to 7 cm long. Sage is primarily used in poultry dishes and sausage, especially fresh pork sausage. In 1997, 4.4 million lb were imported into the United States (1), primarily from Albania and Turkey.

Savory, Satureja hortensis L., is an annual herb of the mint family. It has narrow, dark green leaves about 0.5 to 1.0 cm long. Savory is used very little in the United States. The flavor is sharp, aromatic, and resinous. The major producing countries are Albania and France.

Tarragon, Artemisia dracunculus L., has grown in popularity in recent years. It is a small perennial plant of the sunflower family. Its leaves are large (about 5 cm long), narrow, and dark green. The flavor is fragrant and bittersweet with a licorice-like undertone. It is grown in California and also imported from France.

Thyme, Thymus vulgaris L., is a perennial shrub native to the Mediterranean region. The leaves are narrow, grayish green, and only about 0.5 cm long. The flavor is aromatic and pungent, often used in poultry seasonings and Creole dishes. Thyme is imported primarily from Spain and France.

Turmeric, Curcuma longa L., is a perennial herb of the ginger family. Its thick underground rhizome is boiled, cleaned, dried, and ground into a powder. It has a musty, earthy flavor and is mainly utilized for its bright yellow color. Turmeric is used in curry powders and prepared mustard. There are two types grown in India, Alleppy and Madras. Alleppy is most commonly imported into the United States and has a higher curcumin content than the Madras type.

Other Spice Products

Extractives of spices are the oil-soluble flavor extract of the natural spice. Two types are available: essential oils and oleoresins. Essential oils are the steam-distilled fraction of the oil from the spice. Spice oleoresins are the solvent extract of the spice, containing the essential oil as well as other nonvolatile extractives.

Oleoresins provide the user with a more rounded, closer duplication of the natural spice flavor than essential oils. For example, oleoresin black pepper contains not only its volatile oil, but also piperine, which is the compound that gives it the black pepper bite, or pungency. Black pepper essential oil contains the volatile flavor components only, with no piperine present. Other nonvolatile components present in spice oleoresins include heat components (capsaicin-red pepper), fixatives (from the fatty oils from the seeds of celery, anise, fennel, etc), antioxidants (rosemary and sage), and pigments (paprika and turmeric) (12).

Both essential oils and oleoresins are very concentrated forms of the spice flavor, with usage levels in finished products usually less than 0.01%. Using extractives will standardize the flavor of the finished product compared with natural spices, which can vary from years, origins, or lots. Spice extractives are free from microbial contamination and enzyme activity found in cinnamon and black pepper (12). Extractives also provide flavor with no particulates present in a finished food product. Shipping and storage are easier with extractives, and shelf life is longer than natural spices. The flavor of extractives, however, is not as complex as in their natural spice counterparts.

Many extractives are also available in water-soluble versions from suppliers for use in products where water is the aqueous phase. Most commonly available products contain emulsifiers to make them water soluble.

Spice extractives can be difficult to use in food processing because they are very concentrated. Oleoresin black pepper, for example, is very viscous and hard to pour. Soluble spices were developed by the spice industry to alleviate these problems. Soluble spices are spice extractives plated on a salt or dextrose carrier. They are free-flowing powders, often formulated to approximate a one-to-one replacement to the ground spice. Other products that have been developed for manufacturers include spray-dried extractives and spray-dried products mixed with a carrier to again approximate a one-to-one replacement ratio.

SEASONINGS

Seasoning blends can be defined as dry mix products containing spices; other flavoring agents; and functional ingredients such as salt, sugars, and starches that enhance or provide flavor to a food item. A seasoning blend can be spices only, spices with starches, salt, hydrolyzed vegetable proteins, monosodium glutamate, and flavors such as a gravy mix, or even a blend that has no spices present at all, such as a cheese snack seasoning.

The spice industry has defined analytical testing procedures and standards due to the efforts of ASTA. The seasoning industry does not. It does require experienced, creative food technologists with a well-rounded knowledge of food chemistry, food processing, and laws governing labeling and legal limits of ingredients. The technologist must know how ingredients react in the finished food system, how the finished product is cooked or processed to select the proper ingredients, the intended target market, and finally have a well-developed palate to produce the wide variety of seasonings desired by food processors and consumers.

Seasoning blends are used by food processors for a variety of reasons, primarily for convenience. Seasoning blends can be purchased in batch-sized units, thus reducing ingredient inventories. In addition, fewer quality control personnel are needed for raw material inspection. The responsibility for batching each ingredient separately is eliminated, thus reducing the possibility of error. Consumers purchase seasoning blends for many of the same reasons, most commonly convenience and flavor.

Food Industry

Seasonings are used in the food industry in almost every product category, most commonly in those industries where dry ingredients must be blended with wet ingredients or topically applied. Processed meats, snacks, sauces, gravies, dips, soups, and salad dressing manufacturers are the major users of seasonings. When you think of all the items that are composed of these basic products, the possibilities are endless. Frozen entrees, pizza, boxed side dish items, and retorted sauces and entrees are just a few examples.

Table 3 lists some of the more common meat, snack, and gravy products and the basic spices and other ingredients associated with them. The meat items highlight the spices, although other functional and flavor imparting ingredients (salt, dextrose, sugar, sodium erythorbate) are usually included in the formula. In the snack and gravy area, spices as well as other ingredients are listed because the spices themselves do not produce all the flavors associated with the seasonings. To formulate these products, the flavors

Table 3. Common Seasoning Ingredients

Meat seasonings	
Wiener and bologna	*Italian sausage*
Coriander	Fennel
Nutmeg	Anise
Allspice	Black pepper
Ginger	Red pepper
Clove	Garlic
Black pepper	
Paprika	
Corned beef	*Pork sausage*
Garlic	Sage
Allspice	Black pepper
Clove	Red pepper
Bay	
Cassia	
Snack seasonings	
Barbecue	*Cheese*
Sugar	Cheese solids
Salt	Whey and other dairy ingredients
Citric acid	Dehydrated shortening
Chili pepper	Buttermilk
Clove	Salt
Allspice	Onion
Smoke	Flavorings
Yeast (Torula)	Yeast (Torula)
Red and black pepper	Salt
Tomato	
Garlic	
Onion	
Sour cream and onion	
Sour cream solids	
Buttermilk	
Whey and other dairy ingredients	
Artificial flavors	
Citric acid	
Onion	
Parsley	
Salt	
Gravies	
Thickeners (starches and flours)	Celery seed
Hydrolyzed vegetable proteins	Black pepper
Meat flavors	Onion
Dehydrated meat or meat stock	Turmeric (chicken gravy)
Shortening or nondairy creamers	Caramel color (brown gravy)
Milk solids	Salt

must be balanced to achieve the exact flavor profile desired. This is where the knowledge of an experienced seasoning food technologist is important. There are literally hundreds of variations of each type of flavor.

Ethnic food products are always popular. Everyone is looking for the next "hot" trend. Table 4 lists five basic ethnic flavors and the spices needed to create them. By first formulating a base product, such as a marinade, with the proper amount of salt, hydrolyzed vegetable protein, sugar, gum or starch, phosphate, and so on, the spices listed in Table 4 can then be added and balanced to produce the desired flavor of the finished product. It should be noted that seasonings not only use the natural spice for flavor but also oleoresins and essential oils to produce a product with the exact profile desired.

Specifications

When purchasing seasonings, the specification should adequately reflect the important attributes of the blend. Generally, flavor profile, appearance, granulation, salt, and color are included, as well as other tests such as pH if acid is present; cold, hot, or retort viscosity where applicable; or heat units if red pepper is present in appreciable amounts. If the food item is very sensitive to microbial loads, such as a sour cream dip, then microbiological spec

Table 4. Common Ethnic Seasonings

Italian	*Asian*
Oregano	Ginger
Basil	Garlic
Marjoram	Sesame
Garlic	Red pepper
Fennel	5 spice (cinnamon, anise, ginger, fennel, cloves)
Anise	
Red pepper	
Mexican	*Indian*
Chili pepper	Cumin
Cumin	Coriander
Oregano	Fenugreek
Garlic	Cardamom
Onion	Turmeric
Red pepper	Red pepper
Paprika	Black pepper
Cinnamon	Garlic
Red pepper	Onion
Cajun	*Caribbean*
Red pepper	Red pepper
White pepper	Allspice
Black pepper	Ginger
Thyme	Nutmeg
Oregano	Thyme
Garlic	Bay leaves
Onion	Onion
Paprika	Garlic
Sassafras	Chili pepper
	Cumin
	Coriander
	Turmeric

ifications should be considered. When writing or reviewing specifications, it is essential to know what the important parameters are that the seasoning blend is contributing to the finished food.

FUTURE TRENDS

With the increasing demand for high-quality and unique food items, the need for the seasoning company is more evident than ever before. Although many seasoning companies began by servicing the needs of processed meat manufacturers, the trend for reduced red meat consumption, as well as competition within the industry, has limited growth in this area. Conversely, snacks, specialty sauces, marinades, glazes, and seasonings are on the rise. These types of products hold the future for seasoning manufacturers.

In addition, leveraged buyouts and consequent cutbacks in research and development staffs should cause a higher demand for outside product development work. Finally, small start-up companies often require help with new products. Seasoning companies can offer their services in new product development. The new millennium promises a bright future for the seasoning industry.

BIBLIOGRAPHY

1. United States Department of Agriculture, *United States: Imports of Specified Condiments, Seasonings and Flavoring Materials*, U.S. Government Printing Office, Washington, D.C., 1998.
2. *CFR* 21:101.22 & 182.10, U.S. Government Printing Office, Washington, D.C., 1996.
3. Food and Drug Administration, Center for Food Safety and Applied Nutrition, *The Food Defect Action Levels* (HFS-565), U.S. Government Printing Office, Washington, D.C., 1997.
4. D. R. Tainter and A. T. Grenis, *Spices and Seasonings; A Food Technology Handbook*, VCH-Wiley, New York, 1993
5. *ASTA Cleanliness Specifications for Spices, Seeds, and Herbs*, American Spice Trade Association, Englewood Cliffs, N.J., 1997.
6. *Cleaning and Reconditioning of Spices, Seeds, and Herbs*, American Spice Trade Association, Englewood Cliffs, N.J.
7. *Official Analytical Methods of the American Spice Trade Association*, American Spice Trade Association, Englewood Cliffs, N.J., 1997.
8. M. Vajdi and R. R. Pereira, "Comparative Effects of Ethylene Oxide, Gamma Irradiation and Microwave Treatments on Selected Spices," *J. Food Sci.* **38**, 893–895 (1973).
9. *CFR* 21:179.26, U.S. Government Printing Office, Washington, D.C., 1996.
10. *Mustard Flour*, R. T. French Co., Rochester, N.Y., 1988.
11. J. D Dziezak, "Spices," *Food Technol.* **43**, 102–116 (1989).
12. G. D. Deline, "Modern Spice Alternatives," *Cereal Foods World* **30**, 697–700 (1985).

GENERAL REFERENCES

K. T. Farrell, *Spices, Condiments and Seasonings*, 2nd ed., Van Nostrand Reinhold, New York, 1990.

J. Giese, "Spices and Seasoning Blends: A Taste for All Seasons," *Food Technol.* **48**, 88–98 (1994).

S. Hegenbart, "Spices and Seasoning Blends," *Food Product Design* **3**, 43–64 (1993).

E. L. Ortiz, *The Encyclopedia of Herbs, Spices, and Flavorings*, Dorling Kindersley, New York, 1992.

J. W. Purseglove et al., *Spices*, Vols. 1 and 2, Longman, Essex, England, 1987.

E. W. Underriner and I. R. Hume, *Handbook of Industrial Seasonings*, Chapman and Hall, London, 1993.

DONNA R. TAINTER
Tone Brothers, Inc.
Ankeny, Iowa

STARCH

Next to water, starch is the most abundant constituent in the human diet. Starch is abundantly available, is inexpensive, is a desirable source of energy, and occurs in the form of granules (see the section "Granular Nature, Cooking Characteristics, and Solution and Gel Properties"). Starch occurs naturally in most plant tissues, including roots and tubers, cereal grains, vegetables, and fruits. Large amounts of starches are consumed as components of wheat, corn (maize), and rye flours and as components of potatoes and whole kernels of corn, rice, and barley. Starch composition and properties vary with the plant sources from which they are obtained. Each starch from each source is unique in terms of its behavior and characteristics. Starches may be isolated and added to food products as ingredients. Often, they are modified after isolation and added during the preparation process in modified form.

Native and modified starches serve a variety of roles in food products. They are added primarily to modify texture and consistency. Principally they serve to bind water, to thicken, and to form soft, spoonable gels, all controllable properties. Starches generally must be cooked in order to realize their physicochemical properties and to impart their functionalities. Heating starch granules in the presence of water causes the granules to swell, lose their crystallinity, and in some cases, break apart (see the section "Granular Nature, Cooking Characteristics, and Solution and Gel Properties"). When a starch is cooked in excess water, the resulting dispersion is called a paste.

Corn (maize) is the principal source of commercial starch in the United States (1–3). Lesser amounts of potato, tapioca (cassava, manioc), sorghum (milo), wheat, rice, and arrowroot starches are used in the United States (1), but a starch such as one from potato, wheat, or cassava may be the major starch in other countries. Minor amounts of sago, barley, sweet potato, mung bean, and rye starches find their way into commerce elsewhere.

MOLECULAR COMPONENTS

Although commercial starch granules, especially those from cereal grains, contain small amounts of protein, lipids (especially phosphoglycerides and free fatty acids), and other components, the principal components are amylose and/or amylopectin (Tables 1 and 2) (1,4).

ifications should be considered. When writing or reviewing specifications, it is essential to know what the important parameters are that the seasoning blend is contributing to the finished food.

FUTURE TRENDS

With the increasing demand for high-quality and unique food items, the need for the seasoning company is more evident than ever before. Although many seasoning companies began by servicing the needs of processed meat manufacturers, the trend for reduced red meat consumption, as well as competition within the industry, has limited growth in this area. Conversely, snacks, specialty sauces, marinades, glazes, and seasonings are on the rise. These types of products hold the future for seasoning manufacturers.

In addition, leveraged buyouts and consequent cutbacks in research and development staffs should cause a higher demand for outside product development work. Finally, small start-up companies often require help with new products. Seasoning companies can offer their services in new product development. The new millennium promises a bright future for the seasoning industry.

BIBLIOGRAPHY

1. United States Department of Agriculture, *United States: Imports of Specified Condiments, Seasonings and Flavoring Materials*, U.S. Government Printing Office, Washington, D.C., 1998.
2. *CFR* 21:101.22 & 182.10, U.S. Government Printing Office, Washington, D.C., 1996.
3. Food and Drug Administration, Center for Food Safety and Applied Nutrition, *The Food Defect Action Levels* (HFS-565), U.S. Government Printing Office, Washington, D.C., 1997.
4. D. R. Tainter and A. T. Grenis, *Spices and Seasonings; A Food Technology Handbook*, VCH-Wiley, New York, 1993
5. *ASTA Cleanliness Specifications for Spices, Seeds, and Herbs*, American Spice Trade Association, Englewood Cliffs, N.J., 1997.
6. *Cleaning and Reconditioning of Spices, Seeds, and Herbs*, American Spice Trade Association, Englewood Cliffs, N.J.
7. *Official Analytical Methods of the American Spice Trade Association*, American Spice Trade Association, Englewood Cliffs, N.J., 1997.
8. M. Vajdi and R. R. Pereira, "Comparative Effects of Ethylene Oxide, Gamma Irradiation and Microwave Treatments on Selected Spices," *J. Food Sci.* **38**, 893–895 (1973).
9. *CFR* 21:179.26, U.S. Government Printing Office, Washington, D.C., 1996.
10. *Mustard Flour*, R. T. French Co., Rochester, N.Y., 1988.
11. J. D Dziezak, "Spices," *Food Technol.* **43**, 102–116 (1989).
12. G. D. Deline, "Modern Spice Alternatives," *Cereal Foods World* **30**, 697–700 (1985).

GENERAL REFERENCES

K. T. Farrell, *Spices, Condiments and Seasonings*, 2nd ed., Van Nostrand Reinhold, New York, 1990.

J. Giese, "Spices and Seasoning Blends: A Taste for All Seasons," *Food Technol.* **48**, 88–98 (1994).

S. Hegenbart, "Spices and Seasoning Blends," *Food Product Design* **3**, 43–64 (1993).

E. L. Ortiz, *The Encyclopedia of Herbs, Spices, and Flavorings*, Dorling Kindersley, New York, 1992.

J. W. Purseglove et al., *Spices*, Vols. 1 and 2, Longman, Essex, England, 1987.

E. W. Underriner and I. R. Hume, *Handbook of Industrial Seasonings*, Chapman and Hall, London, 1993.

DONNA R. TAINTER
Tone Brothers, Inc.
Ankeny, Iowa

STARCH

Next to water, starch is the most abundant constituent in the human diet. Starch is abundantly available, is inexpensive, is a desirable source of energy, and occurs in the form of granules (see the section "Granular Nature, Cooking Characteristics, and Solution and Gel Properties"). Starch occurs naturally in most plant tissues, including roots and tubers, cereal grains, vegetables, and fruits. Large amounts of starches are consumed as components of wheat, corn (maize), and rye flours and as components of potatoes and whole kernels of corn, rice, and barley. Starch composition and properties vary with the plant sources from which they are obtained. Each starch from each source is unique in terms of its behavior and characteristics. Starches may be isolated and added to food products as ingredients. Often, they are modified after isolation and added during the preparation process in modified form.

Native and modified starches serve a variety of roles in food products. They are added primarily to modify texture and consistency. Principally they serve to bind water, to thicken, and to form soft, spoonable gels, all controllable properties. Starches generally must be cooked in order to realize their physicochemical properties and to impart their functionalities. Heating starch granules in the presence of water causes the granules to swell, lose their crystallinity, and in some cases, break apart (see the section "Granular Nature, Cooking Characteristics, and Solution and Gel Properties"). When a starch is cooked in excess water, the resulting dispersion is called a paste.

Corn (maize) is the principal source of commercial starch in the United States (1–3). Lesser amounts of potato, tapioca (cassava, manioc), sorghum (milo), wheat, rice, and arrowroot starches are used in the United States (1), but a starch such as one from potato, wheat, or cassava may be the major starch in other countries. Minor amounts of sago, barley, sweet potato, mung bean, and rye starches find their way into commerce elsewhere.

MOLECULAR COMPONENTS

Although commercial starch granules, especially those from cereal grains, contain small amounts of protein, lipids (especially phosphoglycerides and free fatty acids), and other components, the principal components are amylose and/or amylopectin (Tables 1 and 2) (1,4).

Amylose is an essentially linear polysaccharide composed of (1→4)-linked α-D-glucopyranosyl units (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). Its degree of polymerization (DP) is 1,000 to 16,000 (MW 160,000–2,650,000), depending on the source and method of preparation. Amylose can have several conformations. In the solid state, it probably exists most often as a left-handed, sixfold helix. In solution, it seems to be a loosely wound and extended helix that behaves as a random coil. Because of its helical structure, amylose is able to complex with hydrophobic molecules. In foods, amylose often complexes with mono- and diglycerides and/or free fatty acids or their salts. Complexed amylose molecules retrograde less effectively (see the section "Retrogradation"). Hence, molecules complexed with hydrocarbon chains provide greater stability to foods, so emulsifiers and other surfactants are added to baked goods to retard staling, which at least in part results from retrogradation.

Amylopectin has a branch-on-branch structure. Amylopectin molecules are composed of chains of (1→4)-linked α-D-glucopyranosyl units; branches are formed by joining these chains with α-D-(1→6) linkages. The average chain length is 20 to 30 units, although branch points are not equally spaced. The currently accepted architecture of an amylopectin molecule is that of a long main chain to which are attached clusters of branches, some of which are themselves branched (Fig. 1). This model has been termed the cluster model. The molecular weight of amylopectin has been measured as $5 \times 10^7 - 4 \times 10^8$ (DP $3 \times 10^5 - 2.5 \times 10^6$), depending on the source and method of preparation. Potato starch amylopectin occurs as a natural phosphate ester.

Corn and rice cultivars with altered polysaccharide composition have been developed. Normal corn starch is composed of approximately 28% of the linear polysaccharide amylose and approximately 72% of the branched polysaccharide amylopectin. Those starches that result from a mutation that makes them composed of essentially 100% amylopectin are designated waxy starches. Waxy maize (waxy corn) starch is an important food starch. Other all-amylopectin starches have been produced but are not commercially available. High-amylose corn starches containing approximately 55% and 70% amylose are in commercial production and find some food use. Starches from other special corn cultivars are both available and under development.

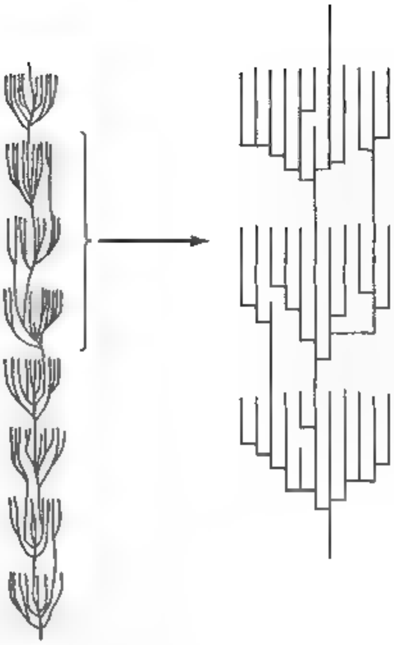


Figure 1. Structures of segments of an amylopectin molecule.

The physical properties of amylose and amylopectin (Table 2) are reflected in starches. For example, high-amylose starches are difficult to gelatinize (because of the extra energy needed to dissociate and hydrate the aggregates of amylose); form firm, opaque gels; and can be used

Table 1. General Properties of Some Native Starch Granules and Their Pastes

	Common corn	Waxy maize	High-amylose corn	Potato	Tapioca
Amylose content, %	28	<2	50–70	21	17
Cooking temperature, °C	62–78	63–72	160–170	58–65	52–65
Relative viscosity	Medium	Medium high	Very low[a]	Very high	High
Paste rheology (body)	Short	Long	Short	Very long	Long
Paste clarity	Opaque	Slightly turbid	Opaque	Translucent	Translucent
Relative shear stability	Stable	Unstable	Stable	Unstable	Unstable
Tendency to gel	Strong	Weak	Very strong	Weak	Medium

[a] Under ordinary cooking conditions (100°C).

Table 2. General Properties of the Two Starch Polysaccharides (Unmodified)

Property	Amylose	Amylopectin
Solution stability	Unstable (precipitates)	Stable
Gels	Soft, reversible, flowable gels become irreversibly stiffer with time; undergo syneresis	Solutions do not gel
Solubility	Difficult soluble; high-temperature solubility only	Soluble
Complex formation	Complexes with iodine, lipids, and various polar organic molecules	Does not complex

to make strong, tough films. Their solutions and gels undergo retrogradation rapidly. Waxy maize starches, even when unmodified, gelatinize easily and yield viscous, almost transparent solutions that do not gel.

In general, the properties of a starch paste or gel are a function of the amounts of amylose and amylopectin dispersed or solubilized, the amounts of insoluble swollen granules and granule fragments, and interactions between components. Amylose increases gel strength; amylopectin decreases gel strength and viscosity.

GRANULAR NATURE, COOKING CHARACTERISTICS, AND SOLUTION AND GEL PROPERTIES

All green plants package and store carbohydrate (D-glucose) in the form of discrete particles of starch called granules (1,5). In granule form, starch is insoluble in cold water and only slightly hydrated. The sizes and shapes of granules are specific for the plant of origin and can be identified microscopically (1,6). Diameters (in micrometers) of some commercial starches are in the following ranges: rice, 3 to 8; corn, 5 to 25; tapioca, 5 to 35; potato, 15 to 100.

Occurrence in granule form makes starch unique. Starch granules can be dispersed in water, producing low-viscosity slurries that can be easily mixed and pumped. Starch granules can be reacted while slurried. They can be isolated/recovered, either with or without reaction, by filtration or centrifugation and resuspended for cooking. The thickening power of granular starch is realized only when the slurry is cooked.

Starch granules reversibly absorb water and swell slightly but remain as granules until an aqueous suspension (slurry) is heated. When dry corn starch (normal moisture content 10–12%) is placed in cold water, the moisture content of its granules increases to approximately 30%, and the average granule size of normal yellow dent corn starch increases by ca 9% and of waxy maize starch approximately 23%.

When heated in water, starch granules gelatinize. Gelatinization is the collapse (disruption) of molecular orders within granules resulting in irreversible changes in properties such as granule swelling, native crystallite melting, loss of birefringence, and leaching of soluble components (primarily amylose) (7). (Some amylose leaching can occur at temperatures below the gelatinization temperature.) The temperature of initial gelatinization and the range over which gelatinization occurs depends on the method used to determine it and is governed by the starch type and concentration and heterogeneities within the granule population under observation (Table 1).

Pasting is the phenomenon following gelatinization when a starch slurry containing excess water is heated (7). It involves further granule swelling, additional leaching of soluble components, and eventually, especially with the application of shear, total disruption of granules, resulting in molecules and aggregates of molecules in dispersion or solution. In most if not all cases, some granule remnants remain.

The cooking behavior of starches and the viscosity of the resulting pastes is most often followed with an instrument with which a starch suspension is heated at a designated rate to a designated temperature while continuously measuring and recording the viscosity. The resulting paste is held at the designated temperature (usually 95°C) for a designated time and then cooled. The resulting curve reveals the pasting temperature, rate of viscosity development, peak viscosity, rate and extent of viscosity breakdown, and rate and extent of viscosity development during paste cooling (Fig. 2).

The property of forming thick pastes or gels is the basis of most starch uses. The extent of starch gelatinization and pasting is the principal factor controlling texture, other product properties such as storageability, and digestibility. In some baked goods, many starch granules remain ungelatinized (as much as 90% in pie crust and some cookies that are high in fat and low in water content). In the processing and preparation of most other food products containing starch, which usually occur in the presence of excess water, starch granules swell beyond the reversible point. On heating a starch slurry (usually 2–5% starch), granules absorb water until almost all water is within highly swollen granules, forcing the granules to press against one another, filling the container with a highly viscous paste or gel. These highly swollen granules are fragile. Stirring then effects granule rupture and disintegration and a decrease in viscosity.

Starches from different sources have different cooking characteristics (Table 1). Tuber and root (potato and tapioca) starches gelatinize more easily than do cereal starches and produce more viscous pastes that easily and rapidly lose viscosity on application of shear. These pastes are rather clear and slow to gel and have a bland flavor. Ordinary corn starch produces an opaque, cohesive gel that undergoes syneresis and has a slight cereal flavor. The properties of waxy maize starch pastes are in between those of potato starch and corn starch. Rice and wheat starches also produce opaque pastes. All starches can be overcooked to give stringy pastes; they can also be undercooked.

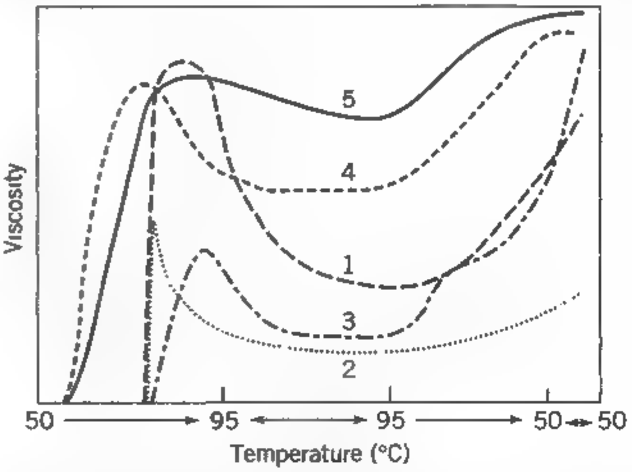


Figure 2. Generalized curves showing the cooking behaviors of selected starches; 1, potato starch; 2, waxy maize starch, 3, common corn starch; 4, stabilized common corn starch; 5, moderately crosslinked and stabilized common corn starch.

The viscosity obtained by cooking a suspension of starch is determined by starch type, type and amount of modification, solids concentration, pH, amount of agitation during heating, rate of heating, maximum temperature reached, time held at that temperature, agitation during holding, and the presence of other ingredients. High sugar concentrations decrease the rate of gelatinization and lower the peak viscosity and gel strength. Emulsifiers such as monoacylglycerols and lipids that complex with amylose inhibit granule swelling, increase both the gelatinization temperature and the temperature needed to produce maximum viscosity, and decrease both the temperature needed for gel formation and gel strength.

Pregelatinized Starches

Starches can be cooked and the paste dried in a way that destroys granular structure and allows the product to be redispersed (dissolved) in water at temperatures below the gelatinization temperature. Various types of these pregelatinized (instant) starches that do not require cooking to achieve thickening are produced. Some will produce smooth dispersions; others produce pulpy or grainy dispersions and find use in fruit drinks and tomato products. Pregelatinized starches are often used in dry mixes because they disperse readily, even when mixed with other ingredients such as sugar.

Cold Water-Swellable Starches

By heating common corn starch in media containing a limited amount of water, granular products that swell extensively in cold water can be made. Cold water-swellable starches can be used in instant products of various kinds. When they are dispersed in sugar syrups with rapid stirring and the mixture is poured into molds, a rigid gel that can be sliced is formed.

Retrogradation

Starch molecules in an unordered state (in solution, in a dispersion, or in gelatinized granules) will undergo a process called retrogradation. Retrogradation (setback) is the reassociation of starch polymer molecules. It occurs when molecules that have become disordered during cooking begin to reassociate in an ordered structure (8). In the initial phases of retrogradation, linear segments of two or more starch chains may form a simple juncture point that may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears. The result is gelation or precipitation. Generally, extensive retrogradation is undesirable. Various approaches are used to retard retrogradation; one is the complexation described earlier (see the section "Molecular Components").

Derivatization to produce stabilized starch is also used to reduce the tendency for retrogradation (see the section "Stabilized Starch").

MODIFIED FOOD STARCH

In the native form, starches do not have the properties desired by food processors. For example, when aqueous slurries of native starches are heated, the starch granules swell, then rupture—causing the viscosity to increase rapidly, then fall. Cooling of the cook produces weak-bodied, cohesive pastes or gels. Modification can correct such a defect. According to the U.S. *Code of Federal Regulations* (21 *CFR* 172.892), modified food starch (1,9) may be prepared using the following treatments: acid modification with hydrochloric and/or sulfuric acids; bleaching with hydrogen peroxide, peracetic acid, potassium permanganate, or sodium hypochlorite; oxidation with sodium hypochlorite; esterification with acetic anhydride, adipic–acetic mixed anhydride, monosodium orthophosphate, 1-octenylsuccinic anhydride, phosphoryl chloride, sodium tripolyphosphate and/or sodium trimetaphosphate, or succinic anhydride; etherification with propylene oxide; or various combinations of the preceding. Also allowed under the regulations are bleaching with ammonium persulfate or sodium chlorite, acetylation using vinyl acetate, and etherification with acrolein or epichlorohydrin (crosslinking); but these reactions are not currently used to produce food starches. Limitations of modifications are given in the regulations. Most U.S. modified food starch is prepared from waxy maize, common corn, or potato starch. In the United States, the ingredient label of the product containing one of these food starches must designate "modified food starch" or "food starch modified" as one of the ingredients but does not need to indicate the source of the starch or the modification(s).

In general, derivatization increases solution and gel clarity, reduces the tendency to gel and/or crystallize, improves water binding, increases freeze-thaw stability, reduces the gelatinization temperature, and increases the peak viscosity. Combinations of derivatizations and other modifications are used to obtain desired properties for specific applications. The useful properties of starches that can be modified and controlled by various treatments are their property of forming a suspension of insoluble granules, without thickening, until the slurry is heated; their ability to thicken aqueous systems and to form a paste on heating; their ability to form semisolid gels on cooling of pastes; their ability to form strong, adhesive films and coatings and to act as a binder; the ability of their pastes to disperse and suspend fats, oils, and particulate matter; and the ability of their pastes and gels to provide important textural qualities to prepared foods.

Crosslinked Starch

Crosslinking is the most important modificaton applied to food starches. It is used to control texture and provide heat, acid, and shear tolerance and, thus, to provide better control over end-product quality and greater flexibility in dealing with formulations and processing and storage conditions. Diphosphate esters, which can be introduced by reaction of starch with either phosphoryl chloride or sodium trimetaphosphate (Fig. 3), are the most common crosslinks. Crosslinking of the polymer chains of starch granules is also done by making an adipic acid diester.

Crosslinking strengthens the granule. A small amount of crosslinking (eg, treatment with only 0.0025% sodium trimetaphosphate) greatly reduces both the rate and the

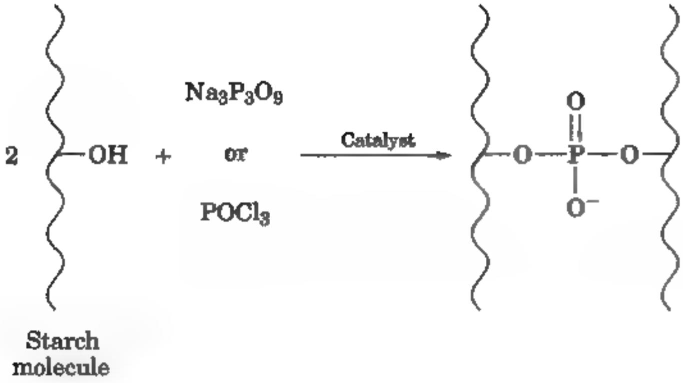


Figure 3. Crosslinking of molecular chains of starch granules with phosphate diester linkages.

degree of granule swelling and the sensitivity of starch pastes to processing conditions (high temperature; extended cooking times; high shear during mixing, milling, homogenization, or pumping; low pH). Pastes of crosslinked starches are more viscous, more stable, shorter-textured (more pseudoplastic), and generally heavier-bodied than those of native starch from which they were prepared. Crosslinked waxy maize starch products are especially popular in the food industry. Starches with a range of properties are prepared by varying the kind and degree of crosslinking (Table 3). In general, when choosing a starch for a particular application, one should select a starch that is crosslinked sufficiently to enable it to withstand the processing conditions to which it will be subjected and still give optimum viscosity in the final product. Crosslinked starches can be pregelatinized.

Stabilized Starch

Pastes of ordinary starch will gel, and the gels will generally be cohesive, long-textured, and prone to syneresis—all undesirable characteristics. Pastes of underivatized waxy maize starch, which has no amylose, are less inclined to retrograde or gel; however, in the refrigerator or freezer, they will eventually increase in opacity and become chunky. This is especially true if processing or cooking conditions effect cleavage of chains, producing linear fragments.

To improve textural aesthetics and/or other properties, other starch derivatives are prepared (Table 4). Few of the hydroxyl groups of modified food starches are derivatized, in other words, ether or ester groups are present in small amounts. Most modified food starches contain, on average, one substituent group per every 5 to 10 α-D-glucopyranosyl units, a DS (degree of substitution, the average number of hydroxyl groups derivatized per monomeric unit of the polymer, of 0.1–0.2). These small degrees of derivatization change dramatically the properties of the starch and increase its range of application.

Table 3. General Reasons for Crosslinking Starches

Changes in granule properties
Modification of cooking characteristics
Inhibition of swelling
Changes in paste properties
Reduction of cohesiveness
Inhibition of gel formation
Improvement of acid stability
Improvement of heat stability
Improvement of shear tolerance

The derivatives most commonly used to prepare a stabilized starch are the hydroxypropyl ether, the acetate ester, and the monophosphate ester. Addition of these groups reduces the ability of starch molecules to form junction zones (intermolecular associations).

Crosslinked Stabilized Starches

Often, it is desired to improve both processing and cooking tolerances and shelf and storage stability, so many food starches are derivatized with two reagents to introduce both crosslinking and non-crosslinking substituent groups.

Addition of Hydrophobic Groups

Other derivatives are made to add specific functional properties. For example, nonwetting hydrophobic starches are used as release agents for dusting on dough sheets or as processing aids. Other starch products with hydrophobic groups are used as emulsifiers and emulsion stabilizers.

Acid-Modified Starches

Acid-modified (acid-thinned, thin-boiling) starches are prepared by treating a suspension of native or derivatized

Table 4. General Reasons for Making Stabilized Starches

Changes in granule properties
Reduction in energy required to cook
Changes in paste properties
Increased stability
Increased freeze-thaw stability
Enhancement of clarity
Increased sheen
Inhibition of gel formation
Reduction of gel syneresis
Increased viscosity
Improved interactions with other substances
Improvement in stabilizing properties

starch with dilute mineral acid at temperatures below the gelatinization temperature for varying periods. When it is determined that a product that gives the desired viscosity after cooking has been produced, the acid is neutralized and the starch is recovered by centrifugation or filtration, washed, and dried. In this process, a small amount of glycosidic bond hydrolysis occurs, resulting in products that produce much less viscosity. A concurrent weakening of the granule structure occurs. The result is that there is more granule disintegration when acid-modified starches are heated in water; and although they have reduced viscosity-imparting power, they form gels with increased strength and improved clarity.

These so-called thin-boiling starches are used when strong gel strength is desired, such as in gum candies (eg, jelly beans, orange slices, and spearmint leaves) and in processed cheese loaves. Where especially strong or fast-setting gels are desired, high-amylose starches are used. Other of these low-viscosity products, which allow higher-concentration pastes to be formed, are used as film formers and adhesives in products such as pan-coated nuts and candies.

Bleaching

Absolute whiteness, particularly of corn starch, requires bleaching. The bleach most commonly used is sodium hypochlorite. During the bleaching operation, the starch is oxidized. Small amounts of carboxyl and carbonyl groups are introduced and some glycosidic bonds are cleaved. The result is decreased pasting temperature, thickening power, and tendency to retrograde.

USES

Uses of starches and starch products in foods are extensive. In the granular form (dry powder), starches are used as anticaking agents and diluents and for dusting and molding. When nearly fully cooked to a state approaching a molecular dispersion, starches are used to provide viscosity (thickening), impart texture, suspend solids, and form films. Pastes are also employed as binders, processing aids, protective colloids, and encapsulating agents. As swollen granules (produced by heating slurries of cross-linked granules), starches are used to provide viscosity, impart texture, and suspend solids and as binders and processing aids. Use of a high-amylose starch will make breaded products crispier. Table 5 lists important properties exhibited by starch in both the dry and cooked states and a few examples of products containing a starch or modified food starch.

Table 5. Important Properties of Specific Food Starch Products

Property	Product Examples
Adhesion	Breaded products, pan-coated nuts and candies
Anticaking (flowing aid)	Baking powder, powdered sugar, salt
Antistaling	Baked goods
Binding	Extruded foods, meat products
Clouding	Beverages, cream fillings
Crispiness	Breaded products
Dusting	Bread, chewing gum, rolls
Foam strengthening	Confectioneries
Gelling	Gum candies, processed cheeses, puddings, spoonable dressings
Glazing	Nuts
Hydration	Dry mixes
Moisture retention	Breadings
Molding	Gumdrops
Oil absorption	Peanut butter
Pulpiness	Fruit drinks, tomato products
Shaping	Meat products, pet foods
Stabilizing emulsions and suspensions	Beverages, salad dressings
Thickening	Baby foods, cream-style corn, gravies, pie fillings, sauces, soups, yogurt

DIGESTION

Essentially, only cooked starch can be digested effectively by humans. Amylases are the enzymes that catalyze the hydrolysis of the glycosidic bonds of the polysaccharides of starch (10). α-Amylases are endo-enzymes, that is, enzymes that catalyze the hydrolysis of internal bonds of starch polysaccharides. Although saliva contains an α-amylase, very little starch hydrolysis occurs in the mouth because of the short dwell time.

Almost all starch digestion and absorption takes place in the small intestine. The pancreatic juice secreted into the small intestine contains another α-amylase. This enzyme effects a rapid reduction in molecular weight of the starch polysaccharides, producing starch oligosaccharides (maltooligosaccharides), primarily of six and seven α-D-glucopyranosyl units (Fig. 4). The α-amylase then acts more slowly on these oligosaccharides to reduce them to smaller fragments (maltose and maltotriose). The enzyme acts even more slowly on maltotriose; it does not catalyze the hydrolysis of the glycosidic bond of the disaccharide

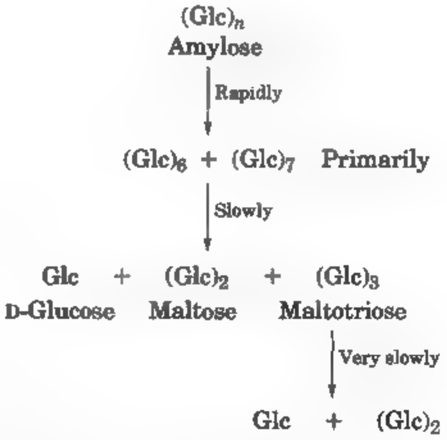

(Glc)$_n$
Amylose
Rapidly
(Glc)$_6$ + (Glc)$_7$ Primarily
Slowly
Glc + (Glc)$_2$ + (Glc)$_3$
D-Glucose Maltose Maltotriose
Very slowly
Glc + (Glc)$_2$

Figure 4. Action of α-amylase on amylose. Glc = D-glucose or α-D-glucosyl unit.

maltose. α-Amylases catalyze the hydrolysis of α-D-(1→4) linkages only, never α-D-(1→6) linkages. Therefore, the products of the action of pancreatic α-amylase on amylose and amylopectin are both linear and branched maltooligosaccharides.

Other enzymes are then needed to catalyze the hydrolysis of the maltooligosaccharides. Complete hydrolysis to D-glucose is required because only monosaccharides can be absorbed. Disaccharidases are located on the surface of cells lining the inner surface of the small intestine. Maltase catalyzes the hydrolysis of maltose, 4-*O*-(α-D-glucopyranosyl)-D-glucose (see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING), to D-glucose. Isomaltase catalyzes the hydrolysis of isomaltose, 6-*O*-(α-D-glucopyranosyl)-D-glucose, to D-glucose. Both enzymes act on higher oligosaccharides but more slowly than they do on the disaccharides.

Starch that resists and escapes digestion by endogenous enzymes of the upper gastrointestinal tract and is thus available to the large intestine as a fermentable substrate is called resistant starch. Resistant starch has physiological benefits, primary among which may be a decreased incidence of colorectal cancer and ulcerative colitis. The resistant starch content of foods can be manipulated by choice of the botanical source of starch, processing, cooking, and storage conditions.

STARCH-RELATED PRODUCTS

Starches can be converted into various products of depolymerization, namely, D-glucose (dextrose), dextrins, maltodextrins, syrup solids, and syrups, including high-fructose syrups (see the articles CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING and SWEETENERS: NUTRITIVE) (1,11).

BIBLIOGRAPHY

1. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds., *Starch: Chemistry and Technology*, 2nd ed., Academic Press, Orlando, Fla., 1984.
2. J. B. May, "Wet Milling: Process and Products," in S. A. Watson and P. E. Ramsad, eds., *Corn. Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987, pp. 377–397.
3. F. T. Orthoefer, "Corn Starch Modification and Uses," in S. A. Watson and P. E Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987, pp. 479–499.
4. A. Guilbot and C. Mercier, "Starch," in G. O. Aspinall, ed., *The Polysaccharides*, Vol. 3, Academic Press, Orlando, Fla., 1985, pp. 209–282
5. L. F. Hood, "Current Concepts of Starch Structure," in D. R. Lineback and G. E. Inglett, eds., *Food Carbohydrates*, AVI Publishing Co., Westport, Conn., 1982, pp. 217–236.
6. J. Seidemann, *Starke-Atlas*, Paul Parey, Berlin, 1966.
7. W. A. Atwell et al., "The Terminology and Methodology Associated with Basic Starch Phenomena," *Cereal Foods World* **33**, 306–311 (1988)
8. V. J. Morris, "Starch Gelation and Retrogradation," *Trends in Food Sci. Technol.* 1, 2–6 (1990).
9. P. S. Smith, "Starch Derivatives and Their Use in Foods," in D. R. Lineback and G. E. Inglett, eds., *Food Carbohydrates*, AVI Publishing Co., Westport, Conn., 1982, pp. 237–269.
10. The Amylase Research Society of Japan, eds., *Handbook of Amylases and Related Enzymes*, Pergamon Press, Oxford, 1988.
11. G. M. A. van Beynum and J. A. Roels, eds., *Starch Conversion Technology*, Marcel Dekker, New York, 1985.

J. N. BEMILLER
Purdue University
West Lafayette, Indiana

See also CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING; CARBOHYDRATES: FUNCTIONALITY AND PHYSIOLOGICAL SIGNIFICANCE.

SUGAR: SUBSTITUTES, BULK, REDUCED CALORIE

Absorption of polyols is slower than absorption of their carbohydrate analogs, allowing a portion to reach the large intestine where metabolism by the colonic bacteria yields short-chain fatty acids and reduced calories to the host. Measuring the caloric value of a polyol in humans is a difficult task, and regulatory agencies may reach different conclusions despite having similar databases from which to render an opinion. The Life Sciences Research Office Expert Panel of the Federation of American Societies for Experimental Biology prepared a report titled "The Evaluation of the Energy of Certain Sugar Alcohols Used as Food ingredients" in June 1994. The Expert Panel estimated the net energy of sugar alcohols from 1.6 to 3.3 kcal/g. The European Union promulgated a Nutritional Labeling Directive stating that all polyols have a caloric value of 2.4 cal per gram.

Polyols are resistant to metabolism by oral bacteria that break down sugars and starches to release organic acids, leading to erosion of tooth enamel and eventually to dental cavities. Polyols are noncariogenic. Polyols are useful alternatives to sugars and facilitate formulation of sweet, but not cariogenic snacks.

Polyols are slowly and poorly absorbed, compared with simple sugars, and do not raise blood glucose levels following consumption. Compared with sucrose or starch, both of which raise blood glucose levels, polyols do not. The major polyols are described next.

Hydrogenated Starch Hydrolysates

Hydrogenated starch hydrolysates (HSHs) including hydrogenated glucose syrups, maltitol syrups, and sorbitol syrups, are a family of products found in a wide variety of foods. They serve a number of functional roles, including use as bulk sweeteners, viscosity or bodying agents, humectants, crystallization modifiers, cryoprotectants, and rehydration aids.

HSHs are produced by the partial hydrolysis of corn, wheat, or potato starch and subsequent hydrogenation of the hydrolysate at high temperature under pressure. The end product is composed of sorbitol, maltitol, and higher hydrogenated saccharides. By varying the manufacturing conditions and by controlling the extent of hydrolysis, one can tailor the chemical species produced. The term HSH is applied to describe the broad groups of polyols that contain substantial quantities of hydrogenated oligo- and polysaccharides in addition to any monomeric or dimeric polyols.

Because hydrolysis and subsequent hydrogenation does not yield a single compound but, rather, a variety of compounds, manufacturers have adopted a practice of using the prevalent polyol to name the product. For example, polyols containing sorbitol as the majority component are called sorbitol syrups; those with maltitol as the majority are maltitol syrups or maltitol solutions. Polyols without a single polyol at 50% or more of the total continue to be referred to by the general term HSH.

HSHs are outstanding humectants that do not crystallize, enabling the production of sugar-free confections with the same cooking and handling systems used to produce sugar candies. These products are used extensively in confections, baked goods, a broad range of other foods, dentifrices, and mouthwashes.

HSHs provide 40 to 90% of the sweetness of sugar, texture, and bulk to a variety of sugarless products.

Isomalt

Isomalt is derived from sucrose in a two-step process. In step one, the normal linkage of 1 (glucose) to 5 (fructose) is changed to a 1 to 6 linkage. In step two, hydrogenation converts the aldehyde to one of two alcohols, either gluco-mannitol or gluco-sorbitol in approximately equal proportions as shown in Figure 1.

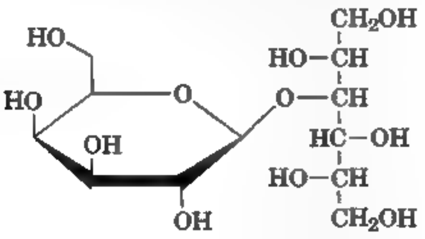
The use of isomalt in place of sucrose retains the volume and textural qualities of sucrose. Because isomalt can be heated without losing its sweetness or being broken down, it is used in products that are boiled, baked, or subjected to high temperatures.

Isomalt absorbs very little water. Therefore, products made with isomalt tend not to be as sticky as products made with sucrose. Extended shelf life is observed with isomalt-containing foods.

Isomalt's sweetening power depends on its concentration, temperature, and the form of the product in which it is used. When used alone, it contributes 45 to 65% of the sweetness that would result from the same amount of sucrose. For nutrition labeling purposes, isomalt has a caloric density of 2.0 cal per gram, one-half that of sucrose. Isomalt's lower caloric value is partly due to the fact that intestinal enzymes are not able to easily hydrolyze its more stable disaccharide bond. Therefore, less is absorbed from the small intestine into the blood, and absorption takes place at a slower rate than with sucrose.

Lactitol

Lactitol is manufactured by reducing the glucose half of the disaccharide lactose (2).



The resulting compound has two more hydrogen atoms than does lactose. Unlike lactose, lactitol is not hydrolyzed by lactase enzyme. It is neither hydrolyzed nor absorbed in the small intestine. Lactitol is metabolized by bacteria in the large intestine, where it is converted into carbon dioxide, short-chain fatty acids, and a small amount of hydrogen.

The organic acids are further metabolized resulting in a calorie contribution of 2 cal per gram. The Scientific Committee for Food of the European Union has provided a Nutrition Labeling Directive stating that all sugar alcohols, including lactitol, have a caloric value of 2.4 kcal/g. Canada has assigned a 2.6 kcal/g value to lactitol.

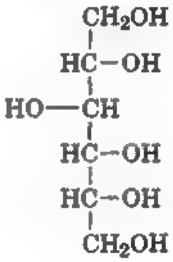
As a replacement for sucrose in formulated foods, lactitol does not induce an increase in blood glucose or insulin levels. Control of blood glucose, lipids, and weight are the three major goals in diabetes management. Foods using lactitol to replace sugar can be used by people with diabetes, giving them a wider variety of low-calorie and sugar-free choices.

Lactitol is not metabolized by oral bacteria that break down sugars and starches to release acids that may lead to cavities.

Due to its stability, solubility, and similar taste to sucrose, lactitol can be used in a variety of low-calorie, low-fat and/or sugar-free foods such as ice cream, chocolate, hard and soft candies, baked goods, sugar-reduced preserves, chewing gum, and sugar substitutes.

Sorbitol

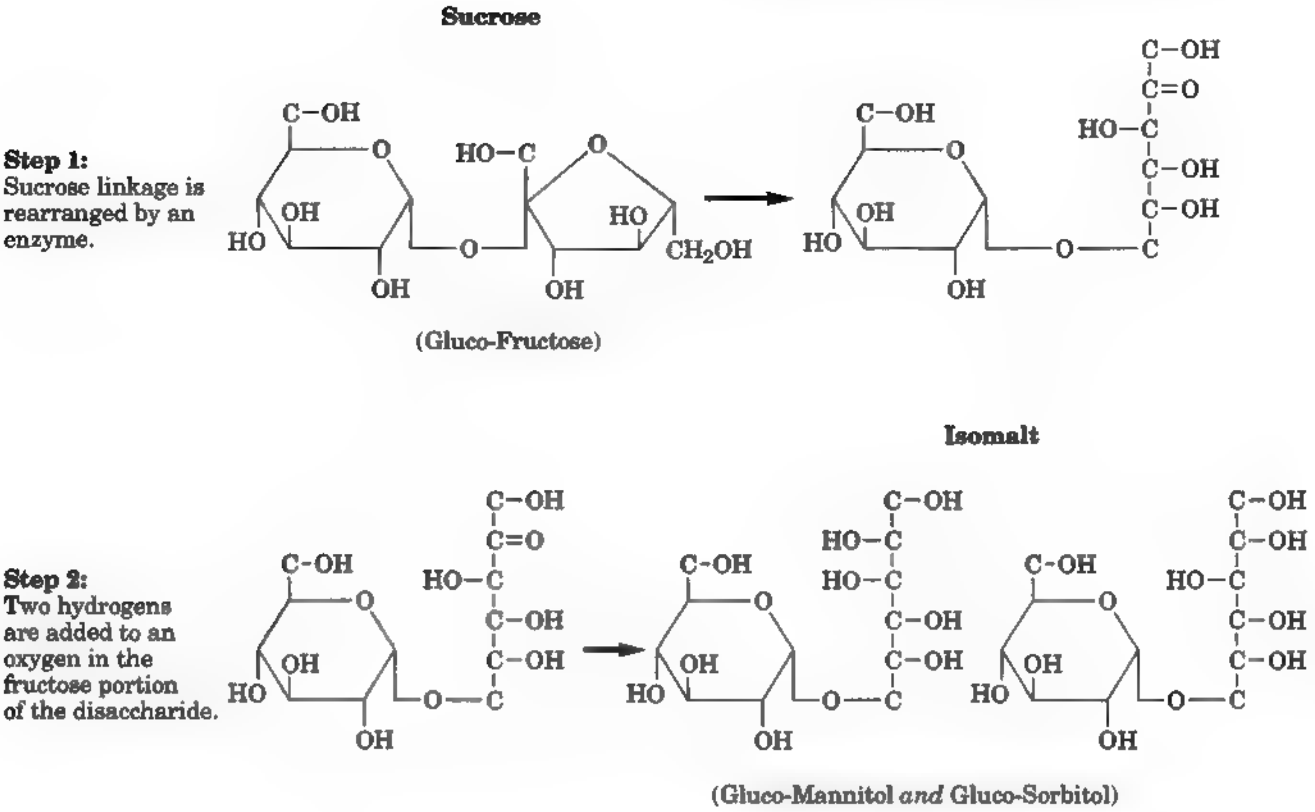
Sorbitol is manufactured from glucose by high-pressure hydrogenation or by electrolytic reduction, which results in the addition of two hydrogens per molecule. The chemical structure is shown as follows (2):



Sorbitol is about 60% as sweet as sucrose with fewer calories, 2.6 cal per gram in the United States, and 2.4 cal per gram in the European Union. Sorbitol is a natural constituent of a wide variety of fruits and berries.

Sorbitol is used as a humectant in many types of products for protection against loss of moisture content. The moisture-stabilizing and textural properties of sorbitol are used in the production of confectionery, baked goods, and chocolate where products tend to become dry or harden. Its moisture-stabilizing action protects these products from drying and maintains their initial freshness during storage.

Sorbitol is stable and chemically unreactive. Sorbitol is resistant to metabolism by oral bacteria that break down sugars and starches to release acids. Sorbitol can with-



Step 1: Sucrose linkage is rearranged by an enzyme.

Step 2: Two hydrogens are added to an oxygen in the fructose portion of the disaccharide.

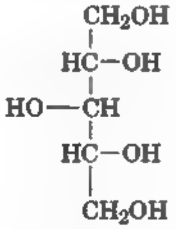
Figure 1. How isomalt is produced. *Source:* Ref. 1.

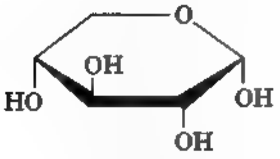
stand high temperatures and does not participate in browning reactions.

Sorbitol functions well in many food products such as chewing gums, candies, frozen desserts, cookies, cakes, icings, and fillings as well as oral care products including toothpaste and mouthwash.

Xylitol

Xylitol is a five-carbon sugar alcohol prepared by the reduction of xylose. The chemical structures of xylitol and xylose are shown as follows (2):





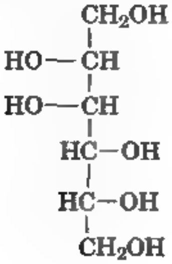
α-D-Xylose

Xylitol occurs naturally in many fruits and vegetables and is produced in the liver during metabolism of D-glucose. Produced commercially from plants such as birch and other hardwood trees and fibrous vegetation, xylitol has the same sweetness and bulk as sucrose with reduced calories (about 2.4 cal/g in the United States and European Union). Xylitol quickly dissolves and produces a cooling sensation in the mouth.

Xylitol is a caries fighter. In clinical tests, the consumption of xylitol-containing foods between meals was associated with significantly reduced new caries formation, even when participants were already practicing good oral hygiene. Xylitol inhibits the growth of *S. mutans*, the primary bacterium associated with dental caries. Use of xylitol-containing foods was correlated with a significant decrease in plaque accumulation. In addition, the sweetness and cooling effect of xylitol-sweetened products such as mints or chewing gum creates an increase in salivary flow. The combination of demonstrated increased salivary flow, retardation of plaque growth, and caries prevention qualifies xylitol as a good-for-your-teeth food ingredient.

Mannitol

Mannitol is synthesized from hydrogenation of invert sugar, monosaccharides, and sucrose. It is used in the food industry as an anticaking and free-flow agent, flavoring agent, lubricant and release agent, stabilizer, and thickener. Its chemical structure is shown as follows (2):

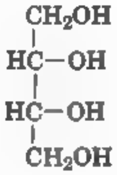


$$\begin{array}{r@{}c@{}l} & CH_2OH & \\ & | & \\ HO- & CH & \\ & | & \\ HO- & CH & \\ & | & \\ & HC & -OH \\ & | & \\ & HC & -OH \\ & | & \\ & CH_2OH & \end{array}$$

The caloric density of mannitol is 1.6 cal per gram in the United States.

Erythritol

Erythritol is a four-carbon sugar alcohol with the following chemical structure (2):



$$\begin{array}{c@{}l} CH_2OH & \\ | & \\ HC & -OH \\ | & \\ HC & -OH \\ | & \\ CH_2OH & \end{array}$$

Erythritol has the lowest caloric density of the polyols—0.2 cal per gram in the United States.

Polyols as a group provide the bulk of sugar, without as many calories as sugar. Since the compounds as a class are poorly absorbed, substantial percentages my enter the large intestine and undergo metabolism by the colonic bacteria. This can cause adverse gastrointestinal events. Gastrointestinal events are usually mild and temporary. These events are controlled by either reducing the polyol intake, and/or by gradually increasing the intake to achieve the desired level over time. This provides an opportunity for the colonic bacteria to adapt to the presence of the polyol in the large intestine.

Compared with sucrose, polyols as a class are lower in calories, have little effect on blood glucose levels, require moderate to no insulin for metabolism, are not cariogenic, replace the bulk of sugar on a one-to-one basis, and have valuable food processing properties. For sugar-reduced and -free food products, the polyols are central to food formulation.

Polydextrose

Polydextrose is a randomly bonded polymer of dextrose containing minor amounts of bound sorbitol and citric acid. It was designed to replace the body and texture lost when sugar or carbohydrates were removed from a traditional food or beverage. Important in its commercialization and utilization is its reduced calorie content, deemed as 1 kcal/g by the U.S. Food and Drug Administration.

Polydextrose is made from food-grade starting materials by vacuum polycondensation. Dextrose is thermally polymerized using sorbitol as a plasticizer and citric acid as a catalyst. The resulting product is a slightly acidic, water-soluble polymer. Through further processing, various grades are produced with bland, desirable features. Polydextrose is not sweet.

Polydextrose is used in confectionery to achieve calorie and sugar reduction in hard and chewy candy; to replace sucrose in chocolate; to replace the bulk, creaminess, smoothness, and mouth-feel of sugar and fat in frozen desserts; to provide viscosity without gumminess in cultured dairy products; to replace sugars and some fat in baked applications; and to replace sugars and build solids in fruit spreads and filling applications.

Food ingredient manufacturers have responded to the desire for reduced calorie/fat nutrition claims with a diverse array of products that help food processors reduce the level of fat in formulated foods. These include lipid or lipidlike materials including salatrim and olestra, microparticulated proteins (Simplesse), and carbohydrate-based ingredients. The carbohydrate-based food ingredients are reviewed here.

Fiber

Fiber sources from cereal products—oat, wheat, corn and combinations of the three—are available. Through a series of purification steps, manufacturers can provide products that are light in color, essentially bland in flavor, and neutral in odor. Typically these products have the ability to absorb water, from 2 to more than 6 g of water per gram of fiber. The hydrated fiber products are frequently used in place of fats and oils on a gram for gram basis.

Similar products are available based on starch technology. Combinations of starch and water are able to replace fats and oils in some low-heat applications, ice cream, and processed meats. Limitations in this technology are the inability to carry flavors, and the inability to cook and fry with these reduced-fat products.

Hydrocolloids

Hydrocolloids have also found favor in replacing fat and oils in formulated food products. M. Glicksman classified hydrocolloids into a number of major classes including (*1*) exudates (gum Arabic), (*2*) extracts from seed weed (carrageenans) and land plants (pectin) and animals (gelatin), (*3*) flours from seeds (guar) and cereals (starches), (*4*) biosynthesis or fermentation (xanthan gum), and (*5*) cellulose and chemically modified cellulose derivatives.

Food hydrocolloids provide a wide array of technological benefits in formulated food products. In calorie-reduced foods, reduced in fats and/or sugars, these products restore the eating qualities associated with full-fat and full-sugar formulations. In an era of full nutritional disclosure, the bulking agents can facilitate the reduction or removal of fats and oils while achieving products that are acceptable to calorie- and fat-conscious consumers.

Hydrocolloids can perform many and varied functions, including as adhesives, binding agents, bodying agents, crystallization inhibitors, clarifying agents, clouding agents, fibers, emulsifiers, encapsulating agents, film formers, flocculating agents, foam stabilizers, gelling agents, moldings, flavor emulsifiers, suspending agents, swelling agents, syneresis inhibitors, thickening agents, whipping agents, and more.

Exudates. Exudates from various plants are among the oldest gums available to humans. The gums are exuded in teardrop or flake shapes that are normally harvested by hand, brought to a central location for sorting, and ground into powder for final sale. For food applications, it is common to spray-dry the gums to reduce the bacterial content and yield a clean, white powder. The four commercial gums include arabic, ghatti, karaya, and traagacanth.

Gum arabic is exuded from the Acacia trees where it forms in scars or wounds on the tree. Gum arabic is a complex, highly branched, globular molecule with low viscosity, and extremely high solubility in water, up to 55%. The stabilizing and emulsifying properties of gum arabic are highly prized characteristics. The other three gums are much more viscous in aqueous solutions. Gum arabic is used for many important functions, including for flavor fixation, for prevention of sugar crystallization and to keep fatty components distributed in confectionery products, as an emulsifier in flavor emulsion concentrates, for preparation of glazes and toppings in baked applications, and to prepare coatings for vitamin and mineral supplements.

Extracts. Extract examples include carrageenan, pectin, and gelatin. Carrageenan is isolated from certain members of the class Rhodophyceae (red seaweeds). It is a hydrocolloid consisting mainly of the potassium, sodium and magnesium, calcium, and ammonium sulfate esters of galactose, and 3,6-anhydrogalactose copolymers. Carrageenans are soluble in hot water and hot milk. Carrageenan reacts with the casein fraction of milk and is very effective in stabilizing milk-based products. Carrageenans are used in the formulation of milk and milk-based beverages, ice cream products, milk puddings, dessert gels, meat analogs, salad dressings, and more.

Pectin is the designation for a group of valuable polysaccharides extracted from edible plant material and used extensively as gelling agents and stabilizers. Pectic substances are abundant in fruits and vegetables and to a large extent are responsible for firmness and form retention of their tissue. Pectin and pectic substances are heteropolysaccharides mainly consisting of galacturonic acid and galacturonic acid methyl ester residues. Pectin is obtained by aqueous extraction of citrus peels and apple pomace.

Pectin solutions show relatively low viscosity compared with other plant hydrocolloids; hence, pectin has limited use as a thickener. Pectin is primarily used as a gelling agent to impart texture to jams, jellies, and preserves. Other applications include bakery fillings and glazings, yogurt fruit preparations, fruit beverages and sauces, fruit jellies and jelly centers, and dairy products.

Gelatin is extracted from cowhides, pigskins, and animal bones. Gelatin is prepared by either alkaline or acid treatment of collagenous tissue and is a form of hydrolyzed, denatured collagen; it is well known for its ability to form food gels. Collagen has an unusual amino acid distribution, containing repeating triplets of -(Gly-X-Y)-where a large percentage of X and Y are proline or hydroxyproline residues. *In vivo* collagen self-assembles to give a triple helical structure; the structure is stabilized by the formation of chemical cross-links. Above about 40°C, cross-linked collagen unwinds and forms a random, denatured configuration. On cooling, such denatured collagen gels. Gelation is thought to occur through development of junction zones with partial reformation of the collagen triple helix structure.

Gelatin can be used as a thickening agent at low concentrations, and a gelling agent at high concentrations. Gelatin is unique in the spectrum of gelling agents in that it can form aqueous gels with water at any pH and without the need for any other additives. Gelatin gels are thermally reversible and can be melted and reset by heating and cooling.

Flours from Seeds and Cereals. Flours containing gums such as guar and starch are separated by mechanical means from the plant seed or cereal. The guar plant, *Cyamposis tetragonolobus*, is a member of the legume family. The endosperm of guar seed is an important hydrocolloid. The guar molecule is a straight-chain galactomannan with galactose on every other mannose unit. Guar gums form a colloidal dispersion to yield a highly viscous system. Guar gum solutions of 1% concentrations or higher are thixotropic with thixotropy decreasing below 1%. In comparison with other hydrocolloids, guar gum at equivalent solids in water has the highest viscosity.

Guar gum modifies the behavior of water in food systems in a highly efficient manner. It reduces and minimizes friction in food products, thereby aiding processing and palatability of foods. Guar viscosity aids in the control of crystal size in saturated sugar solutions. Guar imparts smoothness to ice cream by promoting small ice crystals. Guar yields a homogeneous finished texture to cottage cheese. The addition of guar to cold-packed cheese eliminates syneresis and results in more uniform texture and flavors.

Biosynthesis or Fermentation. Fermentation or biosynthesis is the route of production of xanthan gum. Microorganisms that produce extracellular polysaccharides are widely distributed in marine and land environments. *Xanthomonas campestris*, a naturally occurring bacterium originally isolated from the rutabaga plant, is grown in fermentation vats to produce xanthan gum, a high-molecular-weight polysaccharide gum. The gum is extracted with isopropyl alcohol, dried, and milled. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid, and is prepared as the sodium, potassium, or calcium salt. The polymer backbone consists of 1,4-linked β-D-glucose and is, therefore, identical to that of cellulose. At the 3-position of alternate glucose monomer units is a trisaccharide side chain containing a glucuronic acid residue between two mannose units.

Xanthan gum is completely soluble in hot or cold water. Low concentrations of xanthan gum exhibit high viscosity. Solutions of xanthan gum at 1% or higher concentration appear almost gel-like at rest, yet these solutions pour readily and have low resistance to mixing and pumping.

Xanthan gum has found application in bakery fillings and icings; in beverages to build body, clouding agent, and to suspend insoluble ingredients; in confectionery in processing starch jelly candies and in xylitol-coated chewing gum; in dairy products where it performs as a stabilizer;

in dairy substitutes as a stabilizer; and in pourable dressings as an emulsion stabilizer.

Powdered Cellulose and Cellulose Derivatives. Powdered cellulose is a polymer composed of carbon, hydrogen, and oxygen. Chemically, it is a chain of glucose units linked in a 4-(*β*-D-glucosido)-D-glucose, not to be confused with *α*-1,4-glucan, which is common in starch. The manufacture of powdered cellulose begins with wood pulp that undergoes a series of bleaching steps and drying. Following drying, the pure white, virgin cellulose is cut to the desired fiber length by cutters or by a ball mill. The lengths are varied to achieve specific applications in the food industry. Due to its nature, powdered cellulose is more than 99% fiber and considered to have zero calories.

Powdered cellulose has a number of interesting properties, including *(1)* a greater affinity for water than fat, *(2)* hydrogen bonding that restricts displacement of water by fat, *(3)* no nonenzyme browning, and *(4)* increased pliability of powdered cellulose–containing foods.

Powdered cellulose has a number of food applications, including *(1)* increasing the fiber content of formulated foods, *(2)* reducing the caloric content of the food by displacement of fats, and *(3)* increasing water retention capacity and viscosity.

Being a white, flavorless, and odorless powder enhances the use of powdered cellulose as a noncaloric bulking agent in food products. Low-level inclusion of powdered cellulose in fried food formulations can result in reduced fat pickup during frying. This is attributed to the hydrophilic nature of powdered cellulose and its molecular structure, which permits the formation of significant amounts of additional hydrogen bonds that require more energy to break during the frying process.

Other uses for powdered cellulose include binding and thickening (with gums and stabilizers), anticaking, antisticking extrusion aid, enhancing the volume of baked goods, as a texturizing agent, and more.

There are a variety of chemically modified cellulose products, including carboxymethylcellulose, methylcellulose, hydroxypropylcellulose, and hydroxypropylmethylcellulose with food applications.

There are a variety of reasons a food scientist may wish to reduce the content of simple sugars and or fat in food products. Among the reasons are to reduce calories, reduce simple sugars, reduce fat, attain nutrition labeling advantages, attract consumers who seek diet or dietetic foods, and more. In the case of sugar-reduced foods, these are normally formulated with a high-intensity sweetener and certain bulking agents that fill the space occupied by sugar(s).

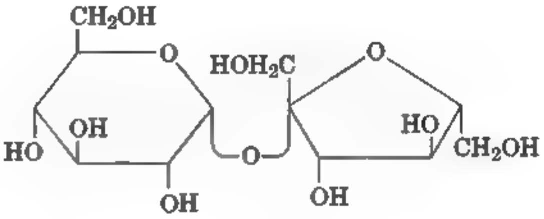
BIBLIOGRAPHY

1. K. McNutt and Sentko, "Sugar Replacers, A Growing Group of Sweeteners in the United States," *Nutrition Today* **31**, 255–261 (1996).
2. S. Budavari, ed., *The Merck Index*, 11th ed., Merck & Co., Inc., Rahway, N.J., 1989, pp. 1375, 1591, 3614, 5633, 9996.

Robert Harkins
North Brunswick, New Jersey

SUGAR: SUCROSE

Sugar (sucrose) is a common food ingredient, often used to satisfy the innate human desire for sweetness (see the article Sweeteners: nutritive) (1–3). In most of its forms, sugar is a very pure food ingredient. It is also a versatile ingredient and, as a digestible carbohydrate, a macronutrient.



STRUCTURE

The chemical name of sucrose is *α*-D-glucopyranosyl *β*-D-fructofuranoside or *β*-D-fructofuranosyl *α*-D-glucopyranoside (4). In a sucrose molecule, the two monosaccharide units are linked reducing end to reducing end, that is, anomeric carbon atom to anomeric carbon atom, by a glycosidic linkage. Because both the potential aldehydo group of the D-glucosyl unit and the potential keto group of the D-fructosyl unit are covalently bound in a mutual glycosidic bond, sucrose has no reducing end, as do essentially all other oligo- and polysaccharides (with few exceptions), so it is classified as a nonreducing carbohydrate.

SOURCES

Sucrose is produced by all green plants, but only sugar cane and sugar beets are used to provide commercial quantities. (Maple syrup, fruits, honey, etc provide a relatively insignificant amount of additional sucrose to the human diet.)

Sugar cane (12–18 months old) is brought to the mill and crushed as soon as possible to reduce exposure to microorganisms, which hydrolyze sugar by means of the enzyme invertase as a first step in using it as a carbon and energy source. Some organisms also convert a portion of the sugar to dextran, a soluble polysaccharide that thickens the sugar solution and causes clogging of filters and other mechanical problems.

To obtain sugar from the cane, it is crushed between rolls (5). A spray of water helps remove the juice. The juice, which is approximately 16% sucrose, is made slightly alkaline by addition of lime (calcium hydroxide) to prevent hydrolysis of the acid-labile glycosidic linkage, and the mixture is heated to coagulate proteins. A heavy scum or cake containing a variety of impurities results. The mixture is filtered and concentrated under reduced pressure at a carefully controlled temperature to approximately 50% solids. When crystals of about 300-μm diameter develop, they are removed by centrifugation and washed. The mother liquor is further concentrated to obtain another crop of crystals (6). Concentration and crystallization is continued until impurities build up to the point where the remaining sucrose will not crystallize. Usually this occurs after two or three crops of crystals are obtained. The final mother liquor, termed black strap molasses, is dark, black, heavy, bitter-flavored, and high in ash.

The light brown product, raw sugar, is shipped, usually across an ocean, to a refining mill for purification. First, the crystals are washed to remove the film of molasses from the surfaces of the crystals. The washed raw sugar is dissolved, and the solution is treated with lime and carbon dioxide or lime and phosphates and filtered to produce a clear, golden solution, which is decolorized by passing it through a column of activated carbon. The colorless solution is then evaporated to supersaturation. By controlling the rate of evaporation, crystal size can be controlled. Crystals are collected, washed, and dried.

Sugar beets contain approximately 76% water, 16% sucrose, 5.5% insoluble pulp, and 2.5% other solubles (7). Sugar is obtained by countercurrent extraction of V-shaped slices of beets called cosettes. Heating weakens cell walls and enhances diffusion of sugar from cells. Approximately 98% of the sugar is extracted in this operation. The extract contains approximately 12% sucrose by weight and 2% soluble impurities. Among these impurities are a trisaccharide, raffinose, which has a single D-galactopyranosyl unit attached to sucrose, and a tetrasaccharide, stachyose, which contains an addition D-galactosyl unit attached to the first galactosyl unit. Lime and carbon dioxide are added for clarification of the extract and removal of some soluble impurities, just as they are in the cane sugar purification process. Following centrifugation and filtration to remove precipitated impurities, the clarified extract (approximately 13% dissolved solids) is treated again with carbon dioxide to remove residual calcium ions. Sulfur dioxide is added to minimize color formation during subsequent processing steps. Then, the solution is evaporated to 60 to 65% concentration for crystallization as in the cane sugar process, and as in the cane sugar process, several crops of crystals are obtained by successive repetitions of the concentration and crystallization steps. About 83% of the sugar present in fresh sugar beets is obtained as pure, white sugar.

FORMS

Refined sugar from either source is pure crystalline sucrose. Although refined sugar is available in a variety of forms, each is at least 99.8% sucrose, with some forms being 99.96% sucrose. Crystalline refined sugar is produced in a variety of crystal size distributions (granulations) (8). There is no standard definition of granulation grades, so grades with the same designation from different manufacturers may not have the same mesh-size distribution. From the largest to the smallest crystal sizes, the most common grades are as follows. Coarse granulated sugar, generally the highest purity crystalline product with the lowest color, is used in fondants and other formulations where a colorless product is desired. Sanding granulated sugar, with a crystal size that normally ranges between U.S. 20 and U.S. 40 mesh, is used mainly as sprinkle on baked goods and as sanding on starch gum confectioneries. Extra Fine (X-Fine) or Fine sugar normally has a crystal size that ranges between U.S. 20 and U.S. 100 mesh. It is the largest volume industrial granulated sugar because it is best for bulk-handling without being susceptible to caking. Fruit granulated sugar normally has a crystal size that ranges between U.S. 40 and U.S. 100 mesh, making it slightly finer than Extra Fine. It is used mainly in dry mixes such as gelatin dessert, pudding, and drink mixes. Bakers Special sugar, with a size distribution normally between U.S. 50 and U.S. 140 mesh, is used for sugaring of cookies and in doughs. Because it dissolves more rapidly than the other granulated forms, it is often used to sweeten bar drinks. Powdered confectioners' sugar is produced by grinding or milling granulated sugar with starch, which is added to prevent caking. It is used for dusting and to form wet fondants. Other grades and granulated forms with other names are also marketed.

Some food or beverage processors, rather than dissolve crystalline sucrose, prefer to purchase sugar in solution. Liquid sucrose is at least 99.5% sucrose at a concentration of at least 67%. It can be used whenever a dissolved granulated sugar product might be used. Amber liquid sugar, which is darker in color and higher in ash than is liquid sucrose, is used where color and minor inorganic impurities can be tolerated. In liquid invert sugar, about half of the sugar has been inverted, that is, converted into equimolar amounts of D-glucose and D-fructose (see the article SWEETENERS: NUTRITIVE), making liquid invert sugar an approximately equimolar mixture of sucrose, D-glucose, and D-fructose. It is sweeter than liquid sucrose and will not crystallize.

Brown sugars are used to impart molasses flavor to cookies, candies, and other products. Brown sugar is composed of very fine sugar crystals in a thin film of syrup. Brown sugar is produced in two different ways. Soft brown sugars (light/golden and dark) are crystallized directly from dark syrups of cane sugar selected for color and flavor during the refining process. In an alternative method, cane or beet sugar crystals are coated with a proper cane syrup or molasses.

Less-refined sugars, available under a variety of names, are essentially first, second, or third crops of crystals. Dried cane juice is prepared by vacuum evaporation of filtered cane juice to a dry solid that is ground. Sugarcane syrups are prepared by evaporation of unfiltered sugarcane juice.

Molasses is both the syrup (concentrated juice) that remains after cane sugar has been recovered by crystallization and the product of raw cane sugar manufacture. It is 40 to 60% sucrose, D-glucose, and D-fructose. It is available in several grades based on color and flavors and can be used to flavor food products. It may be unsulfured or lightly sulfured. Most molasses is used in animal feed supplements.

SWEETNESS

The relative sweetness of nutritive sweeteners is a function of concentration, pH, and temperature (see the article

SWEETENERS: NUTRITIVE). Nevertheless, relative sweetnesses of the common nutritive sweeteners can be roughly determined (Table 1). The clean, pure sweet taste of sucrose, without aftertaste or unpleasant secondary reactions, is the standard by which all other sweeteners are judged (9,10). Sucrose also balances other flavor components (11).

OTHER PROPERTIES

Crystalline sucrose, in addition to being sweet tasting, is colorless and odorless (12,13). Dry crystalline sucrose is stable up to its melting point. At temperatures of approximately 160 to 186°C (320–367°F), sucrose crystals melt and the molecules decompose with formation of a family of dark-brown carmelization products.

The glycosidic bond joining the two sugar units is essentially a high-energy bond and unstable; its cleavage is the first step in the thermal decompositions mentioned in the preceding paragraph. The bond is also very susceptible to acid-catalyzed hydrolysis into an equimolar mixture of D-glucose and D-fructose. This process is called inversion and the product invert or invert sugar. The products of hydrolysis, both of which are reducing sugars, will react with amino acids, peptides, proteins, and ammonium ions in typical Maillard reactions, producing other caramels and resulting in the brown colors, aromas, and flavors associated with cooking and baking.

In addition to acid-catalyzed hydrolysis, the extent of which is a function of time, temperature, and pH, sucrose undergoes enzyme-catalyzed hydrolysis to the same products. Yeast and bacterial hydrolytic enzymes are called invertases; the enzyme of the human digestive tract, which operates by a slightly different mechanism, is called a sucrase.

Its high degree of water solubility is a key property of sucrose (14). As a result, several methods have been developed for determining its concentration in solution, primary among which are measurement of specific gravity and refractive index. Equations and tables have been developed to determine its saturation concentration at various temperatures; and scales have been developed to report concentrations, the primary one being the Brix scale, in which the percentage by weight of sucrose in a water solution is expressed as degrees Brix.

Table 1. Approximate Relative Sweetnesses of Common Nutritive Sweeteners

Sweetener	Approximate relative sweetness
D-Fructose (crystalline, initial)	140
55% High-fructose corn syrup	120
Invert sugar syrups	105
Xylitol	105
Sucrose	100
D-Glucose	75
Sorbitol (D-glucitol)	50
Maltose	40
D-Mannitol	40
Lactose	25

Also important to sugar producers and food processors are the specific heat and heat of crystallization of sucrose, and again equations and tables have been developed to determine each as functions of temperature and concentration. Other tables relate sucrose concentration to the freezing point (depressed) and boiling point (elevated) of its solutions. Surface tension increases and the vapor pressure of water and water activity decreases with increasing sugar concentration (12,13).

USES

Sucrose is a macronutrient food ingredient that provides a variety of functionalities in processed food products. It always provides sweetness and may be used to provide bulk, body, and texture. It may also function as a moisturizing agent, dispersing agent, stabilizer, fermentation substrate, flavor carrier, browning agent, decorative agent, and preservative. Important characteristics of sucrose related to its use in processed foods are (*1*) its solubility in water; (*2*) its ease of crystallization upon evaporation of water; (*3*) its function as a precursor to caramel and Maillard reaction colors, flavors, and aromas; (*4*) its stability and nonhygroscopic nature, which allows it to be used as a diluent, dispersant, and bulking agent in dry mixes; (*5*) its being a readily fermentable substrate for yeasts and other specific microorganisms; and (*6*) its ability to decrease water activity appreciably and thereby to provide protection from chemical and/or microbiological deterioration and extend shelf life (15).

The largest amount of sucrose is used in bakery and breakfast cereal products. In bakery products, in addition to providing sweetness and being a source of fermentable carbohydrate, sucrose may provide one or more of the following functionalities: crumb tenderization, moisture retention, texture improvement, formation of crust color, shelf-life extension, and an aid to whipping and creaming (16). It is used in both crystalline and liquid forms.

In yeast-leavened products, the amount of sugar used can range from approximately 1% to approximately 11% (based on flour) in breads and can be as much as 20% in Danish sweet products. In these products, sucrose is used as a source of fermentable carbohydrate and to enhance flavor, improve crust color and toasting properties, improve crumb texture and moistness, and extend shelf life. The principal contributors to bread flavor are fermentation and crust browning, both of which involve sugar. Browning is the result of both carmelization (thermal decomposition) and the Maillard reaction.

Two processes are involved in producing crumb texture: gluten hydration and starch gelatinization. With increasing amounts of sugar in bread doughs, less and less water is available for gluten hydration, the rate of gluten development is reduced, and the tenderness of the bread crumb increases. Sucrose also inhibits starch gelatinization in a concentration-dependent manner. A delay of starch gelatinization is important in cake making, but its role in bread making is unclear. In some cake formulations, sugar acts as a creaming and whipping aid. It also modifies crumb texture, aids in crust color formation, and retains moisture. In cookie manufacture, sucrose increases the temperature at which starch gelatinizes, prevents hydration of wheat flour proteins, and retards gluten formation. Sucrose also serves as a creaming aid in mixing cookie dough, provides flavor and browning, increases cookie volume and spread of the dough, imparts crispness through crystallization, and produces the characteristic surface cracking pattern of some cookies. Substitution with, or addition of a sufficient amount of, a corn syrup to prevent sucrose recrystallization will produce a cookie with a soft (rather than a crisp) texture and prevent surface cracking. In icings, frostings, and glazes, sucrose provides sweetness, browning, stability, and a pleasing appearance and balances flavor.

Sucrose is blended with other ingredients and added to grain during cooking, prior to flaking, or in the dough stage of ready-to-eat (RTE) breakfast cereals to contribute binding, flavor, browning, and texture (18). It is also a potentiator of other flavors. However, by far the major use of sucrose in RTE cereals is for frosting or sugar coating.

Confectionery production consumes the second greatest amount of sucrose (17). The production of hard candies depends on the solubility of sucrose in water and the fact that, as water is boiled off a sugar solution, the boiling point increases. Therefore, the boiling temperature can be used as a determinant of sugar concentration; for example, when the boiling point of a sugar solution is 93°C (200°F), the concentration of sucrose is approximately 82% by weight. In hard candies, sucrose is combined with glucose, a corn syrup, and/or a maltodextrin to achieve the proper consistency. Soft candies consist of very small crystals of sucrose embedded in a matrix of the other ingredients. Very small crystals of sucrose are also a major ingredient in chocolate, which requires a dry sweetener with low hygroscopicity. Cocrystallization of citric acid with sucrose provides a nonhygroscopic sanding material.

Sucrose is also used in the preparation of other food products. Gelation of pectin in nondietetic jams, jellies, preserves, and marmalades requires a pH in the range 3.0 to 3.4 and soluble solids of approximately 65%, which is most often met with sucrose, making it the principal ingredient (19). Because of its high concentration, it also functions as preservative. Sucrose is used in very few carbonated beverages in the United States, but it is the sweetener used in most "non-diet" carbonated beverages in the rest of the world, dry beverage mixes, and liqueurs (20). In ice cream and other frozen dairy products, sucrose provides a clean, sweet taste and contributes to the creamy texture and body (21). Sucrose is also used in canned fruit, condiments (salad dressings, tomato catsup, mayonnaise, barbecue and steak sauces, etc), cured meats and sausage products, and many other products such as puddings, pie fillings, and granola bars (22).

PHYSIOLOGICAL EFFECTS

While several specific cause-and-effect relationships between consumption of sucrose and pathological conditions have been suggested, they have not been substantiated by research (23). Only the link between sucrose consumption and the incidence of dental caries has been documented.

BIBLIOGRAPHY

1. M. A. Godshall, "Use of Sucrose as a Sweetener in Foods," *Cereal Foods World* **35**, 384–389 (1990).
2. N. L. Pennington and C. W. Baker, eds., *Sugar, a User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990.
3. M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995.
4. A. B. Rizzuto, "Cane Sugar Refining," in N. L. Pennington and C. W. Baker, eds., *Sugar, a User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 22–25.
5. S. Perez, "The Structure of Sucrose in the Crystal and in Solution," in M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995, pp. 11–32.
6. G. Vaccari and G. Mantovani, "Sucrose Crystallisation," in M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995, pp. 33–74.
7. S. E. Bichsel, "Beet Sugar Production," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 26–35.
8. J. F. Dowling, "Sugar Products," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 36–45.
9. R. L. Knecht, "The Flavor of Sucrose in Foods," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 66–70.
10. I. Ramirez, "The Sweetness of Sugar," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 71–81.
11. M. A. Godshall, "Role of Sucrose in Retention of Aroma and Enhancing Flavor of Foods," in M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995, pp. 248–263.
12. R. L. Knecht, "Properties of Sugar," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 46–65.
13. P. Reiser, G. G. Birch, and M. Mathlouthi, "Physical Properties," in M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995, pp. 186–222.
14. Z. Bubnik and P. Kadlec, "Sucrose Solubility," in M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995, pp. 101–125.
15. A. L. Raoult-Wack, G. Rios, and S. Guilbert, "Sucrose and Osmotic Dehydration," in M. Mathlouthi and P. Reiser, eds., *Sucrose: Properties and Applications*, Blackie Academic & Professional, London, 1995, pp. 279–290.
16. J. G. Ponte, Jr., "Sugar in Bakery Foods," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 130–151.
17. C. M. Barnett, "Sugar in Confectionery," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 103–129.
18. C. E. Walker, "Sugar in Ready-to-Eat Breakfast Cereals," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 182–197.
19. E. E. Meschter, "Sugar in Preserves and Jellies," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 212–227

20. G J. Marov and J. F. Dowling, "Sugar in Beverages," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 198–211.
21. D. E. Smith, "Sugar in Dairy Products," in N. L. Pennington and C W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 152–164.
22. N. D. Pintauro, "Sugar in Processed Foods," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 165–181.
23. T. Cayle, "Sugar in the Body," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 82–102.

J. N. BeMiller
Purdue University
West Lafayette, Indiana

See also Carbohydrates: classification, chemistry, labeling; Carbohydrates: functionality and physiological significance.

SULFITES AND FOOD

Sulfites, or alternatively, sulfiting agents, refers to sulfur dioxide (SO_2) and some inorganic sulfites that will release SO_2 under conditions of use. Sulfites have been widely used in foods for various purposes, including control of enzymatic and/or nonenzymatic browning; inhibition of microbial growth; and as antioxidants and reducing and bleaching agents. Typical applications of sulfites in foods may be seen in such cases as preservation of fresh fruits and vegetables, prevention of discoloration of dehydrated fruits and vegetables, inhibition of undesirable microorganisms in wine brewing, and bleaching of food starches.

Since 1959 sulfites have been classified by the Food and Drug Administration of the United States (USFDA) as GRAS (generally recognized as safe) substances when they are used in foods according to the Code of Federal Regulations (CFR). However, the numerous reports of sulfite-induced asthma in some cases of sulfite sensitivity had prompted USFDA to commission an expert panel to reexamine the GRAS status of sulfites in 1984. Consequently, on August 8, 1986 USFDA banned the use of sulfites in fruits and vegetables intended to be served or sold raw to consumers and on January 9, 1987 announced that any foods containing a detectable level of residual sulfites (ie $\geq$10 ppm SO_2 based on the currently official method), either directly from addition during processing or indirectly from other ingredients, must be labeled with sulfite content disclosed.

According to CFR, six sulfites are currently listed as GRAS that may be used in foods, except for meats and other foods recognized as a main source of thiamine and for fruits and vegetables intended to be served and sold raw to consumers: sulfur dioxide (SO_2), sodium bisulfite ($NaHSO_3$), sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium sulfite (Na_2SO_3), and potassium bisulfite ($KHSO_3$). Potassium sulfite (K_2SO_3) and sulfurous acid (H_2SO_3), which are not listed as GRAS, are specifically allowed for use only in caramel processing.

The species of sulfites approved for food use may vary with country. For instance, the officially approved sulfites in Taiwan ROC (Republic of China) for use in foods are potassium sulfite (K_2SO_3), sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), sodium hydrosulfite ($Na_2S_2O_4$), potassium metabisulfite ($K_2S_2O_5$), sodium metabisulfite ($Na_2S_2O_5$), and anhydrous sodium sulfite (1).

CHEMICAL REACTIONS OF SULFITES IN FOOD SYSTEM

Basic Chemistry of Sulfites

In aqueous solution, sulfites may exist in various forms as shown in the following formulas depending on the pH value of the solution (2,3):

$$H_2O + SO_2 \rightleftharpoons H_2SO_3 \quad (SO_2 \text{ dissolves readily in } H_2O)$$

$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^-, \quad pK_1 = 2$$

$$HSO_3^- \rightleftharpoons H^+ + SO_3^{2-}, \quad pK_2 = 7$$

From the p*K* values of sulfurous acid (H_2SO_3) and the pH value of the solution, one can calculate the approximate percentage of various species of sulfites (see Table 1).

Since most food systems have a pH of 3–7, the predominant form of sulfites is HSO_3^-. Sulfurous acid and SO_2 gas may exist only in high-acid foods with pH values less than 4.

Reactions of Sulfites with Food Components

Sulfites react readily with many food components such as aldehydes, ketones, reducing sugars, proteins, and amino acids and form various combined organic sulfites (4). Some of the reactions are desirable and some are undesirable.

Reactions between sulfites and carbonyls such as aldehydes and ketones will primarily form hydroxysulfonates which are very stable at pH 1–8 (5). This reaction enables sulfites to prevent the formation of enzymatic and nonenzymatic browning by binding the carbonyl intermediates of browning reactions (6–8). In general, all carbonyl hydroxysulfonates have good stability, with α,β-unsaturated carbonyl hydroxysulfonates as the most stable (7).

Reducing sugars do not readily react with sulfites as carbonyls. To form sugar hydroxysulfonates, a considerable excess in molar equivalent of reducing sugar is required (4). Sugar hydroxysulfonates are much less stable than carbonyl hydroxysulfonates (2,3). At lower pH, sugar hydroxysulfonates have better stability (2).

Table 1. Percentage of Four Sulfite Species

	Species of sulfites			
pH	SO_2	H_2SO_3	HSO_3^-	SO_3^{2-}
2	37	31.5	31.5	
3	6	8.5	85	
4	0.5	1	98	
5			99	1
6			91	9
7			50	50

20. G J. Marov and J. F. Dowling, "Sugar in Beverages," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 198–211.
21. D. E. Smith, "Sugar in Dairy Products," in N. L. Pennington and C W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 152–164.
22. N. D. Pintauro, "Sugar in Processed Foods," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 165–181.
23. T. Cayle, "Sugar in the Body," in N. L. Pennington and C. W. Baker, eds., *Sugar, A User's Guide to Sucrose*, Van Nostrand Reinhold, New York, 1990, pp. 82–102.

J. N. BEMILLER
Purdue University
West Lafayette, Indiana

See also CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING; CARBOHYDRATES: FUNCTIONALITY AND PHYSIOLOGICAL SIGNIFICANCE.

SULFITES AND FOOD

Sulfites, or alternatively, sulfiting agents, refers to sulfur dioxide (SO_2) and some inorganic sulfites that will release SO_2 under conditions of use. Sulfites have been widely used in foods for various purposes, including control of enzymatic and/or nonenzymatic browning; inhibition of microbial growth; and as antioxidants and reducing and bleaching agents. Typical applications of sulfites in foods may be seen in such cases as preservation of fresh fruits and vegetables, prevention of discoloration of dehydrated fruits and vegetables, inhibition of undesirable microorganisms in wine brewing, and bleaching of food starches.

Since 1959 sulfites have been classified by the Food and Drug Administration of the United States (USFDA) as GRAS (generally recognized as safe) substances when they are used in foods according to the Code of Federal Regulations (CFR). However, the numerous reports of sulfite-induced asthma in some cases of sulfite sensitivity had prompted USFDA to commission an expert panel to reexamine the GRAS status of sulfites in 1984. Consequently, on August 8, 1986 USFDA banned the use of sulfites in fruits and vegetables intended to be served or sold raw to consumers and on January 9, 1987 announced that any foods containing a detectable level of residual sulfites (ie $\geq$10 ppm SO_2 based on the currently official method), either directly from addition during processing or indirectly from other ingredients, must be labeled with sulfite content disclosed.

According to CFR, six sulfites are currently listed as GRAS that may be used in foods, except for meats and other foods recognized as a main source of thiamine and for fruits and vegetables intended to be served and sold raw to consumers: sulfur dioxide (SO_2), sodium bisulfite ($NaHSO_3$), sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium sulfite (Na_2SO_3), and potassium bisulfite ($KHSO_3$). Potassium sulfite (K_2SO_3) and sulfurous acid (H_2SO_3), which are not listed as GRAS, are specifically allowed for use only in caramel processing.

The species of sulfites approved for food use may vary with country. For instance, the officially approved sulfites in Taiwan ROC (Republic of China) for use in foods are potassium sulfite (K_2SO_3), sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), sodium hydrosulfite ($Na_2S_2O_4$), potassium metabisulfite ($K_2S_2O_5$), sodium metabisulfite ($Na_2S_2O_5$), and anhydrous sodium sulfite (1).

CHEMICAL REACTIONS OF SULFITES IN FOOD SYSTEM

Basic Chemistry of Sulfites

In aqueous solution, sulfites may exist in various forms as shown in the following formulas depending on the pH value of the solution (2,3):

$$H_2O + SO_2 \rightleftharpoons H_2SO_3 \quad (SO_2 \text{ dissolves readily in } H_2O)$$
$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^-, \quad pK_1 = 2$$
$$HSO_3^- \rightleftharpoons H^+ + SO_3^{2-}, \quad pK_2 = 7$$

From the pK values of sulfurous acid (H_2SO_3) and the pH value of the solution, one can calculate the approximate percentage of various species of sulfites (see Table 1).

Since most food systems have a pH of 3–7, the predominant form of sulfites is HSO_3^-. Sulfurous acid and SO_2 gas may exist only in high-acid foods with pH values less than 4.

Reactions of Sulfites with Food Components

Sulfites react readily with many food components such as aldehydes, ketones, reducing sugars, proteins, and amino acids and form various combined organic sulfites (4). Some of the reactions are desirable and some are undesirable.

Reactions between sulfites and carbonyls such as aldehydes and ketones will primarily form hydroxysulfonates which are very stable at pH 1–8 (5). This reaction enables sulfites to prevent the formation of enzymatic and nonenzymatic browning by binding the carbonyl intermediates of browning reactions (6–8). In general, all carbonyl hydroxysulfonates have good stability, with α,β-unsaturated carbonyl hydroxysulfonates as the most stable (7).

Reducing sugars do not readily react with sulfites as carbonyls. To form sugar hydroxysulfonates, a considerable excess in molar equivalent of reducing sugar is required (4). Sugar hydroxysulfonates are much less stable than carbonyl hydroxysulfonates (2,3). At lower pH, sugar hydroxysulfonates have better stability (2).

Table 1. Percentage of Four Sulfite Species

	Species of sulfites			
pH	SO_2	H_2SO_3	HSO_3^-	SO_3^{2-}
2	37	31.5	31.5	
3	6	8.5	85	
4	0.5	1	98	
5			99	1
6			91	9
7			50	50

Sulfites can break down the disulfide bonds of proteins and amino acids and form thiol (R-SH) and *S*-sulfonate ($R\text{-}SSO_3^-$) (9). Tertiary amines and Schiff's bases produced from browning reactions also react with sulfites and form amine bisulfites (3). Methionine will be oxidized and converted to methionine sulfoxide by sulfites through free-radical reaction (10).

In addition to these reactions, sulfites react with many vitamins, including B_1, B_{12}, C, and K, resulting in loss of vitamin activity (4).

APPLICATION OF SULFITES IN THE FOOD INDUSTRY

By making use of the reactions with various food components, sulfites can be applied to the food industry for one or more of the following purposes.

Inhibition and Control of Microorganisms

Sulfites are often used in wine brewing to prevent undesirable bacterial fermentation. Table grapes and fruits intended for jam and juice are also often preserved by sulfites to prevent decay (11,12).

The inhibitory action of sulfites is more effective to acetic acid bacteria, lactic acid bacteria, and various molds and is less effective in yeasts (3). The antimicrobial activity of sulfites is generally in the following order. Gram-negative bacteria > Gram-positive bacteria > molds > yeasts (13). The mechanisms of the antimicrobial action of the sulfites is not well understood; however, it is believed that undissociated sulfurous acid is the active form possessing antimicrobial activity (14). With this regard, the pH of the food system should be low enough (≤ 4) to have a significantly inhibitory effect. The combined sulfites do not have antimicrobial action.

Inhibition of Enzymatic and/or Nonenzymatic Browning

Sulfites can effectively prevent or minimize nonenzymatic browning by forming stable hydroxysulfonates with carbonyls and reducing sugars as mentioned in the previous section. In this respect, sulfites are widely used in wine making and in dehydrated fruits and vegetables to prevent the discoloration of the finished products (3,7,8). Sulfites can also inhibit some oxidative enzymes such as polyphenoloxidase, ascorbate oxidase, lipogenase, and peroxidase (6) and therefore can retard the enzymatic browning resulting from polyphenoloxidase. In addition, sulfites can form stable hydroxysulfonates with browning intermediates such as quinones, and prevent further reaction to form browning pigment (6). Thus, sulfites are commonly used in fresh vegetables for salad bars (this application has been banned by USFDA), peeled and sliced potatoes, apple dice and other fruits used in bakery products, fresh mushroom for processing, and table grapes and fresh shrimp, where enzymatic browning presents a serious problem.

Use as Antioxidants and Reducing Agents

As an antioxidant, sulfites can be used in citrus juices (12), beer (13), and peas (6) to prevent the oxidative off-flavor. As an reducing agent, sulfites are widely employed as dough conditioners in the baking industry to break down the disulfide bonds of the gluten fraction and facilitate the processing of biscuits, crackers, and cookies and obtain the desired texture properties of the finished products (15).

TREATMENT AND RESIDUAL LEVELS OF SULFITES IN FOODS

It is difficult to establish criteria for the treatment and residual levels of sulfites used in foods because of the complexity of foods, the wide variations in individual daily intake of sulfite-treated foods, and the dependence of the residual level of sulfites on food composition, processing methods, and storage conditions (4). In spite of this, it is believed that the residual amount of sulfites at the point of consumption is very limited provided foods are treated under normal conditions (2,3).

Currently the treatment and residual level of sulfites in most foods is not strictly limited in the United States. The residual levels or treatment levels are regulated only for those food products that are commonly consumed. These include glucose syrup, dextrose monohydrate, and grape wine, and the maximum residual levels are 40, 20, and 350 ppm (SO_2), respectively. For food starch bleaching the treatment of SO_2 has been established at $\leq 0.05\%$ (4). Regulations in this regard may vary with countries. For instance, the official regulation on maximum residual levels of sulfites in Taiwan, ROC for various foods is as follows (all calculated as SO_2); dehydrated fruits and vegetables and gelatin, 500 ppm; candies, 300 ppm; shrimps and shellfish, 100 ppm; fruit wines, 25 ppm and other processed foods, 30 ppm; and in beverages other than fruit juices, wheat flour, and flour products, the use of sulfites is prohibited (1).

After treatment, sulfites may react with various food components forming combine sulfites, may be oxidized into sulfates (SO_4^{2-}), liberated as SO_2 gas, or may still exist in free inorganic sulfites depending on food composition, pH, processing conditions, and storage conditions. Combined sulfites predominate in most food systems.

BIBLIOGRAPHY

1. Department of Health of Taiwan ROC, *Regulations on Scope and Application Level of Food Additives* (in Chinese), Department of Health of ROC, 1993, pp. 18-4-1–18-4-6.
2. L. F. Green, "Sulfur Dioxide and Food Preservation (a Review)," *Food Chemistry* **1**, 103–104 (1976).
3. M. A. Joslyn and J. B. S. Braverman, "The Chemistry and Technology of the Pretreatment and Preservation of Fruits and Vegetable Products with Sulfur Dioxide and Sulfites," *Advances in Food Research* **5**, 97–160 (1954).
4. S. L. Taylor, N. A. Higley, and R. K. Bush, "Sulfites in Foods," *Advances in Food Research* **30**, 2–64 (1986).
5. L. F. Burroughs and A. H. Sparks, "Sulfite-Binding Power of Wines and Ciders," *Journal of the Science of Food and Agriculture* **24**, 187–198 (1973).
6. D. R. Haisman, "The Effect of Sulfur Dioxide on Oxidizing Enzyme Systems in Plant Tissues," *Journal of the Science of Food and Agriculture* **25**, 803–810 (1974).

7. D. J. McWeeny, M. E. Knowles, and J. F. Hearne, "The Chemistry of Non-enzymatic Browning in Foods and Its Control by Sulfites," *Journal of the Science of Food and Agriculture* **25**, 735–746 (1974).
8. B. L. Wedzicha, O. Lamikanva, J. C. Herrera, and S. Panahi, "Recent Developments in the Understanding of the Chemistry of Sulphur(IV) Oxospecies in Dehydrated Vegetables," *Food Chemistry* **15**, 141–155 (1984).
9. G. W. Means and R. E. Feeney, *Chemical Modification of Proteins*, Holden-Day, San Francisco, 1971, p. 152.
10. S. F. Yang, "Sulfoxide Formation from Methionine or Its Sulfide Analogs during Aerobic Oxidation of Sulfite," *Biochemistry* **9**, 5008–5014 (1970).
11. K. E. Nelson and M. Ahmedullah, "Packaging and Decay Control Systems for Storage and Transit of Table Grapes for Export," *American Journal of Enology and Viticulture* **27**, 74–79 (1976).
12. A. C. Roberts and D. McWeeny, "The Uses of Sulfur Dioxide in the Food Industry (a Review)," *Journal of Food Technology* **7**, 221–238 (1972).
13. R. S. Applebaum, "Sulfites and Food (a Review)," *The Manufacturing Confectioner*, 45–55 (November 1985).
14. J. G. Carr et al. *Journal of Applied Bacteriology* **40**, 201–212 (1976).
15. P. Wade, "Action of Sodium Metabisulfite on the Properties of Hard Sweet Biscuit Dough," *Journal of the Science of Food and Agriculture* **23**, 333–336 (1972).

PING YANG CHANG
Food Industry Research and Development Institute
Hsinchu, Taiwan

See also FOOD ADDITIVES.

SUPERCRITICAL FLUID TECHNOLOGY

Supercritical fluid (SCF) technology has many potential applications in extraction and fractionation processes as an alternative to conventional separation processes. The chemical, pharmaceutical, polymer, and food industries are actively engaged in developing and evaluating this technology.

The ability of a SCF to dissolve low-vapor-pressure materials was first reported in 1879 (1). Solubility experiments carried out in high-pressure glass cells showed that several inorganic salts such as cobalt chloride, potassium iodide, potassium bromide, and ferric chloride could be dissolved or precipitated solely by changes in pressure on ethanol above its critical point. Increasing the pressure of the system caused the solutes to dissolve; decreasing the pressure caused the dissolved materials to nucleate and precipitate. An overview of the background, early findings, and historical development of the technology up to the mid-1980s has been presented (2).

Intensive industrial interest in the application of supercritical fluid extraction (SCFE) used in food and biological products resulted in numerous patents, and a number of journal articles have been written to introduce or to review the subject (3–7).

Some of the motivations to employ SCF technology as a viable separation technique are (*1*) tightening governmental regulations on solvent residues, and pollution control; (*2*) consumer concerns over the use of chemical solvents in food manufacture; (*3*) increased demand for higher quality products that traditional processing techniques cannot meet; and (*4*) the increased cost of energy. SCFE can enhance product recovery, improve product quality and safety, and minimize energy requirements. There are many potential applications, especially in the food and pharmaceutical fields. Today, SCFE is applied on a large scale in the decaffeination of coffee (8,9), and in the recovery of hop extracts used in beer brewing (10). Both of these applications employ SC CO_2 to replace conventional liquid solvents such as methylene chloride, which is under toxicological investigation by the U.S. Food and Drug Administration (FDA) and faces increasing restrictions on its use. In addition, SCF chromatography is being developed as an analysis tool (11).

FUNDAMENTALS OF SUPERCRITICAL FLUID EXTRACTION

Definitions and Properties

The critical temperature T_c of a substance is the temperature above which it cannot be liquefied, no matter how high the pressure. There is a corresponding pressure on the *P-T* diagram called the critical pressure, P_c (Fig. 1). The region on the P-T diagram above both T_c and P_c is called the supercritical region. In this region, the compressed gas is called a SCF, having characteristics of both gases and liquids. Fluids under SC conditions exhibit enhanced dissolving power and have transport properties that favor high extraction capabilities (Table 1). The SCF has a density similar to that of a liquid and functions like a liquid solvent, but it diffuses easily like a gas because its viscosity is low (9).

Thermodynamic Principles

The high density of a SCF allows it to dissolve relatively large quantities of organic compounds that normally have

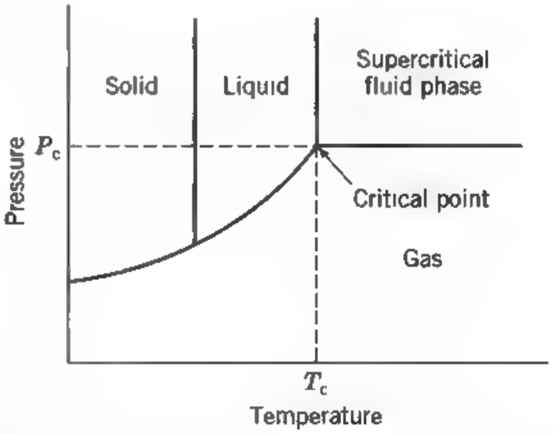


Figure 1. Pressure-temperature phase diagram of a pure substance.

Table 1. Ranges of Some Physical Properties Associated with Different Fluid States

	Density, kg/m^3	Diffusivity, m^2/s	Viscosity, MPa.s
		Gas	
$P = 101.3$ kPa $T = 288$–303 K	0.6–2	$(0.1–0.4) \times 10^{-4}$	$(1–3) \times 10^{-4}$
		Supercritical	
$T = T_c P = P_c$	200–500	0.7×10^{-7}	$(1–3) \times 10^{-4}$
$T = T_c P = 4P_c$	400–900	0.2×10^{-7}	$(3–9) \times 10^{-4}$
		Liquid	
$T = 288$–303 K	600–1600	$(0\ 22) \times 10^{-9}$	$(0.2–3) \times 10^{-3}$

Source. Ref. 11.

low solubility in the ordinary gaseous state of the same fluid. However, the solubility of compounds in a SCF also depends on solute and solvent properties. The dissolved compounds can be recovered from the fluid by decreasing the pressure or increasing the temperature, both of which reduce fluid density and allow it to be separated from the dissolved compound.

The reason SCF technology has been slow to find commercial applications has been due in part to the lack of available thermodynamic data to develop large-scale operations. Thermodynamic principles that can be used for quantitative and mathematical descriptions of solvent power of the SCF are models of phase equilibria and equations of state. For a process that involves the dissolution of a solute or a mixture of solutes from a solid matrix or a liquid mixture at constant temperature and pressure, the equilibrium conditions for the system would be given by

$$\hat{f}_i^{food}(T, P, x_i) = \hat{f}_i^{SCF}(T, P, y_i) \qquad (1)$$

where

$$\hat{f}_i^{food} = \text{food system component fugacity}$$
$$\hat{f}_i^{SCF} = \text{SCF component fugacity}$$

T is the temperature, P is the pressure, x_i is the mole fraction of solute in food, and y_i is the mole fraction of solute in SCF phase.

Earlier studies have considered the food as an inert solid containing the soluble material. Then, the problem was treated similarly to the calculation of water activity (12). Later, it was confirmed that the solid matrix interacts with the soluble material; therefore, it is not appropriate to consider it as inert. For example, the solubility of pure caffeine in CO_2 can be 200 times larger than its solubility in CO_2 when dissolved in coffee beans (13). Therefore, for complex materials such as black pepper, clove buds, ginger rhizomes, and so on, an empirical treatment is advisable, since currently there is no equation available to describe their thermodynamic behavior. If, however, the system involves extraction or concentration of solutes present in a liquid phase, equation 1 can be used together with any appropriate cubic equation of state (EOS), such as Peng-Robinson (PR) or Soave–Redlich–Kwong (SRK). A simplified thermodynamic description of the calculation of solubility of a food system in CO_2 follows (14,15). The solute can be a mixture of substances like orange essential oil, a mixture of fatty acids or any similar system. For constant temperature and pressure, equation 1 is written for each component of the mixture, plus one for CO_2. Therefore, for a mixture with the following composition:

$$Z^* = (Z_1^*, Z_2^*, \ \ldots \ , Z_{n_c-1}^*) \qquad (2)$$

n_c equations similar to equation 1 should be written, where Z^* is the composition vector, Z_i^* is the molar fraction of component in the mixture, and $(n_c - 1)$ is the number of substances from the original mixture that will be treated with CO_2.

The data required for SCF plant scale-up is the solubility of the mixture (food system) with the composition represented by equation 2 in the SC solvent for a given temperature and pressure. This is accomplished by combining equation 1 with an appropriate EOS capable of calculating the fugacity for a given temperature, pressure, and composition. From thermodynamics we have (14,16):

$$\ln(\hat{\varphi}_i) = \ln\left(\frac{\hat{f}_i}{x_i P}\right) = \frac{1}{RT}\int_{\underline{V}=\infty}^{\underline{V}} \left[\frac{RT}{\underline{V}} - P\right] d\underline{V} - \ln(Z) + (Z - 1) \qquad (3)$$

where $\hat{\varphi}_i$ is the fugacity coefficient of component i, $\hat{f}_i$ is the fugacity of component, i, T is the temperature, P is the pressure, x_i is the mole fraction of component i, $\underline{V}$ is the specific volume of the mixture, and $Z = PV/RT$ is the compressibility factor.

To evaluate the fugacity coefficients for all substances that form the mixture, the PR EOS for a mixture can be used:

$$P = \frac{RT}{\underline{V} - b} - \frac{a(T)}{\underline{V}(\underline{V} + b) + b(\underline{V} - b)} \qquad (4)$$

Using the compressibility factor Z, equation 4 can be written as (17):

$$Z^3 + \alpha' Z^2 + \beta' Z + \gamma' = 0 \qquad (5)$$

where $Z = P\underline{V}/RT$, $\alpha' = (-1 + B)$, $\beta' = (A - 3B^2 - 2B)$, $\gamma' = (-AB + B^2 + B^3)$, $A = a(T)P/(RT)^2$, and $B = bP/RT$. In general, the parameter $a(T)$ is referred to as the attractive parameter and b as the volumetric parameter, and they are given by:

$$a(T) = \sum_{i,j} x_i x_j a_{i,j} \quad a_{i,j} = a_{j,i} = \sqrt{a_{i,i} a_{j,j}}\,(1 - k_{a_{i,j}}) \qquad (6)$$

$$b = \sum_{i,j} x_i x_j b_{i,j} \quad b_{i,j} = b_{j,i} = \left[\frac{b_{i,i} + b_{j,j}}{2}\right](1 - k_{b_{i,j}}) \qquad (7)$$

where a_{ij} is the attractive parameter for binary i-j, b_{ij} is the volumetric parameter for the binary i-j, a_{ii} is the attractive parameter for component i, b_{ii} is the volumetric parameter for component i, $k_{a_{i,j}}$ is the attractive binary interaction parameter, and $k_{b_{i,j}}$ is the volumetric binary interaction parameter. The pure components' attractive and volumetric parameters are calculated using the critical properties of the solute. For instance (17):

$$a_{ii}(T) = 0.45724R^2T_c^2/P_c\alpha(T) \quad (8)$$

$$b_{ii} = 0.07780RT_c/P_c \quad (9)$$

$$\sqrt{\alpha(T)} = 1 + \kappa(1 - \sqrt{T/T_c}) \quad (10)$$

and

$$\kappa = 0.37464 + 1.54226\omega - 0.226992\omega^2 \quad (11)$$

where w is the acentric factor.

To solve equation 1, which is a nonlinear problem, a nonlinear trial-and-error solution is required. The objective function to be minimized is defined accordingly with the available experimental data. The following information is needed: (*1*) the system temperature and pressure, (*2*) the composition vector Z^*, and (*3*) the matrices of binary attractive and volumetric interaction parameters given by equations 6 and 7. This should include binary systems such as component-i/CO_2 and component-i/component-j. Then, there will be n_c binary parameters of the kind component i-CO_2, plus $(n_c)^2/2$ parameters of the kind component-i/component-j. Binary liquid-SC and liquid-vapor equilibrium data are required to evaluate these parameters. Currently, scarce information is available in the literature for systems involving foods; therefore, some difficulties can be anticipated to solve the preceding equations. Finally, one should be aware that to solve equation 1, equation 4 is solved twice, once for each phase in equilibrium.

The procedure just described was used to evaluate the solubility of orange essential oil in CO_2 (15,16). The problem was treated as a dewpoint problem; therefore, the oil solubility in CO_2 was considered to be the amount of carbon dioxide required to dissolve a given oil sample. Defining α as the essential oil fraction in the final SC mixture (essential oil and CO_2), the composition vector in the CO_2 rich phase (SCF) when the oil phase is completely dissolved is given by:

$$y_{i+1} = \alpha Z_i^* \quad i = 1, (n_c - 1) \quad (12)$$

The solubility of the essential oil in the SC phase will be:

$$S_b = \frac{\sum_{i=2}^{n_c} m_i}{m_{CO_2}} = \frac{\sum_{i=1}^{n_c-1} y_{i+1}M_{i+1}}{y_{CO_2}M_{CO_2}} = \frac{\alpha \sum_{i=1}^{n_c-1} Z_i^* M_i}{(1-\alpha)M_{CO_2}} \quad (13)$$

where S_b is the solubility of the oil (mg of oil/g of CO_2), m_i are the mass fractions of essential oil components, M_i are the molecular mass of essential oil components, and y_i are molar fractions of essential oil components, respectively. Figure 2 shows the experimental and calculated solubilities of the orange oil (oil-phase) in CO_2 made using both PR and SRK equations of state. The calculations were done using the oil with the composition given in Table 2 (18) and the composition given in Table 3 (19).

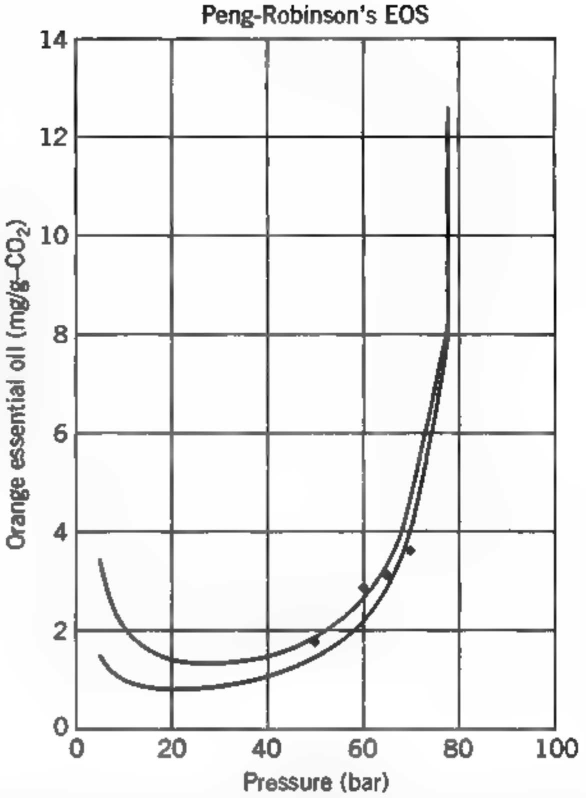


Figure 2. Predicted solubility of orange essential oil in CO_2 according to PR and SRK equations of state. *Source:* Experimental solubility determined by Santana (Ref. 19) at 308.15 K.—Calculated using orange essential oil molar composition determined by Marques (Ref. 18);—Calculated using orange essential oil molar composition determined by Santana (Ref. 19).

Experimental data on the physical properties of essential oil components is scarce in the literature. In addition, many of these substances are thermally degraded before T_c can be measured. Therefore, T_c, P_c, and acentric factor (w) used in equations 8 and 9 were predicted. The choice of methods to be used in these calculations depends on the available experimental data on pure components. The binary parameters used are given in Table 4 (16). For binary systems (orange essential oil component/CO_2 or essential oil component/essential oil component) where experimental data is missing, interaction parameters were set equal to zero. This choice does not imply that there is no interaction or that the interaction are negligible between these binary systems, but this simplifying assumption was required to make the problem solvable.

Although the orange essential oil used by Marques (18) and Santana (19) have different compositions, their calculated solubilities did not differ much from the experimental value measured by Santana (19), as can be observed in Figure 2. The PR and SRK equations gave similar solubility values. In this case, the calculated solubilities were not sensitive to the initial oil composition. The

Table 2. Molar Composition of Orange Essential Oil Determined by Marques (18) and Pure Component Properties

Component	Z_i^*	T_{eb} (*K*)	T_c (*K*)	P_c (bar)	*w*
Ethanol	0.0886	351.65[a]	513.92[d]	61.48[d]	0.6452[d]
Linalool	0.00586	472.15[a]	635.99[c]	25.82[b]	0.7617[f]
α-Terpineol	0.00055	493.15[a]	675.59[c]	29.50[b]	0.7133[f]
trans-2, Hexenal	0.00012	419.65[a]	615.15[c]	35.94[b]	0.4199[f]
Octanal	0.00361	444.15[a]	620.10[e]	27.35[b]	0.5558[f]
Nonanal	0.00053	464.15[a]	637.67[e]	24.80[b]	0.6053[f]
Decanal	0.00287	481.65[a]	651.94[e]	22.59[b]	0.6536[f]
Dodecanal	0.00027	484.94[b]	675.98[b]	18.97[b]	0.7585[f]
Citronelal	0.00050	480.65[a]	663.86[e]	24.05[b]	0.5570[f]
Neral	0.00051	502.15[c]	699.97	25.25[b]	0.7174[f]
Geranial	0.00125	502.15[a]	699.97[e]	25.25[b]	0.7174[f]
β-Simensel	0.00012	592.58[b]	782.72[b]	17.64[b]	0.6853[f]
α-Simensel	0.00015	600.06[b]	794.25[b]	17.79[b]	0.6749[f]
Ethyl butyrate	0.00100	369.15[a]	571.00[d]	30.60[b]	0.4190[d]
α-Pinene	0.00421	429.35[a]	630.87[e]	28.90[b]	0.3242[f]
Δ-3-Carene	0.00102	440.15[b]	646.74[b]	28.90[b]	0.3242[f]
β-Mircene	0.01620	440.15[a]	642.32[e]	28.08[b]	0.3425[f]
Valencene	0.00342	564.52[b]	780.55[b]	18.97[b]	0.4324[f]
d-Limonene	0.94895	451.15[a]	661.11[e]	27.56[b]	0.3170[f]

[a] T_{eb} from Ref. 20

[b] P_c calculated using Ref. 18.

[c] Ref. 21.

[d] Ref. 22.

[e] T_c calculated using Ref 18 and T_{eb} from Ref 20

[f] Lee-Kesler method to calculate acentric factor, Ref. 23.

Table 3. Molar Composition of Orange Essential Oil Determined by Santana (19) and Pure Components Properties

Component	Z_i^*	T_{eb} (*K*)	T_c (*K*)	P_c (bar)	*w*
Linalool	0.00727	472.15[a]	635.99[a]	25.82[b]	0.7617[f]
α-Pinene	0.00610	429.35[e]	630.87[e]	28.90[b]	0.3242[f]
β-Mircene[2]	0.00726	440.15[e]	642.32[e]	28.08[b]	0.3425[f]
Sabinene	0.00177	437.15	640.12	29.35	0.3647
Limonene	0.97760	451.15[a]	661.11[e]	27.56[b]	0.3170[f]

[a] T_{eb} from Ref. 20.

[b] P_c calculated using Ref. 19

[c] Ref. 21.

[d] Ref. 22.

[e] T_c calculated using Ref. 19 and T_{eb} from Ref. 20.

[f] Lee-Kesler method used to calculate acentric factor (23).

Table 4. Binary Interaction Parameters Available for the System: Orange Oil/CO_2.

	PR		SRK	
	$k_{aij}10^2$	$k_{bij}10^2$	$k_{aij}10^2$	$k_{bij}10^2$
CO_2/Ethanol	9.048	−1.414	8.543	−1.412
CO_2/Linalool	4.281	−3.156	4.363	−3.249
CO_2/α-Pinene	9.482	−2.820	10.28	−2.805
CO_2/*d*-Linomene	10.15	1.960	9.921	−1.415

Source. Ref. 16.

PR and SRK equations gave similar solubility values. This is expected since, in general, these mixtures are formed from the same family of substances.

Different substances have different critical temperatures and pressures, important in selecting the appropriate fluid and operating conditions for an application (Table 5). If the critical pressure is too high, processing will be more difficult and costly because equipment must be designed for high pressures. If the critical temperature is too high, heat-sensitive materials such as many food constituents will be altered or destroyed.

Carbon dioxide is the solvent of choice for food applications for many reasons (25). It is *(1)* inert and noncorrosive in dry environments, *(2)* nonflammable and nonexplosive, *(3)* abundant and inexpensive, *(4)* nontoxic and accepted as a harmless ingredient of foods and beverages, *(5)* easy to separate the solute from the solvent, and *(6)* has desirable physical properties such as low T_c (31°C) and low P_c (7.39 MPa), low viscosity, low surface tension, and high diffusivity. When pressure is decreased, CO_2 will separate out from the solute as gas. CO_2 can also protect foods from oxygen and limit oxidation reactions. The solubilities of some substances in liquid CO_2 are given in Table 6. This can be extrapolated to the SC CO_2 extraction. Since the CO_2 molecule is nonpolar, at high densities it is a good solvent of lipophilic organic substances and esters, ethers, and lactones. In general, hydroxyl and carboxyl groups reduce the solubility of a substance in SC CO_2. Polar or charged materials such as sugars and amino acids are not soluble. Large molecules such as cellulose and proteins are also insoluble. The solubilities of substances such as waxes, resins, and pigments increase with an increase in the density of SC CO_2. Solubility is also affected by the intermolecular forces of the solute molecules, which enable the CO_2 molecules to surround and hold them in solution (5). In general, as the number of carbon–carbon double

Table 5. Critical Property Data for Some Supercritical Solvents

Substance	Critical temperature[a] (K)	Critical pressure[b] (MPa)	Critical density[b] (kg/m^3)
Methane	190.6	4.60	162
Ethylene	282.4	5.03	218
Chlorotrifluoromethane	302.0	3.92	579
Carbon dioxide	304.2	7.38	468
Ethane	305.4	4.88	203
Propylene	365.0	4.62	233
Propane	369.8	4.42	217
Ammonia	405.6	11.30	235
Diethyl ether	467.7	3.64	265
n-Pentane	469.6	3.37	237
Acetone	508.1	4.70	278
Methanol	512.6	8.09	272
Benzene	562.6	4.89	302
Toluene	591.7	4.11	292
Pyridine	620.0	5.63	312
Water	647.3	22.00	322

[a] Ref. 24.

[b] Ref. 4.

Table 6. Solubilities of Some Compounds in liquid CO_2

Fully miscible	Partially miscible (% by wt)		Low solubility
H_2S, SO_2	Water	0.1	β-carotene
Limonene	Iodine	0.2	Urea
Benzene	Oleic acid	2.0	Glycine (not soluble)
Acetic acid	Lactic acid	0.5	Citric acid
Ethyl alcohol	Glycerol	0.05	Ascorbic acid
Benzaldehyde	Aldol	11.0	Sucrose (not soluble)
	Orange oil (308, 15K)[a]		
	5.0 MPa	0.17	
	6.0 MPa	0.28	
	6.5 MPa	0.31	
	7.0 MPa	0.36	

Source: Ref. 26.

[a] Ref. 19.

bonds increases, solubility decreases. Substances belonging to the same chemical class are dissolved in the SC phase in order of their increasing boiling points. The use of cosolvents or entrainers such as water or methanol enhances the solubility of substances normally not miscible with SC CO_2.

PROCESS SYSTEMS AND OPERATION CHARACTERISTICS

A simplified diagram of a process that uses a SCF as a pressure-dependent, variable-power solvent for extraction is shown in Figure 3. A typical system consists of an extraction vessel at high pressure, expansion valves, separator vessels, and a compressor or pump. The feed enters the extraction vessel and contacts the SC CO_2. The pressure and temperature are controlled. Since its density is high, the SCF dissolves some components and separates them from the feed. The rest is obtained as residue. The SC phase containing dissolved materials flows through an expansion valve where the pressure is reduced. Since its density is reduced, the SCF deposits some dissolved components into the separation vessel as product 1. The remaining solutes in the SC phase are separated from CO_2 in the last separator and collected as product 2. The CO_2 is now at a low pressure, and carries no dissolved material. It is recycled by going through the pump, and reentering the extraction vessel. Therefore, multiple stages can be used to fractionate different compounds from the original feed. However, fractionation of extracts is possible only if the constituents exhibit large differences in vapor pressure.

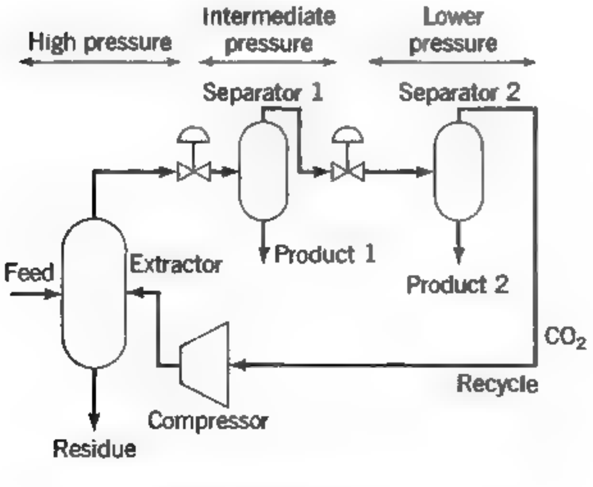


Figure 3. Typical process flow diagram of SCFE.

As an alternative to fractionation by pressure reduction, the process can also be operated at constant pressure with temperature changes in the SCF to cause separation. Such a system is shown in Figure 4.

Operation Characteristics

The conditions of pressure and temperature for extraction and fractionation applications using CO_2 vary greatly with the materials to be extracted. Typical ranges of pressure and temperature for food applications are given in Figure 5. To evaluate the technical and economic feasibility for SCF extraction of a specific material, a pilot-plant study is carried out to determine factors such as solubility and selectivity properties, yields, quality, and operation condi-

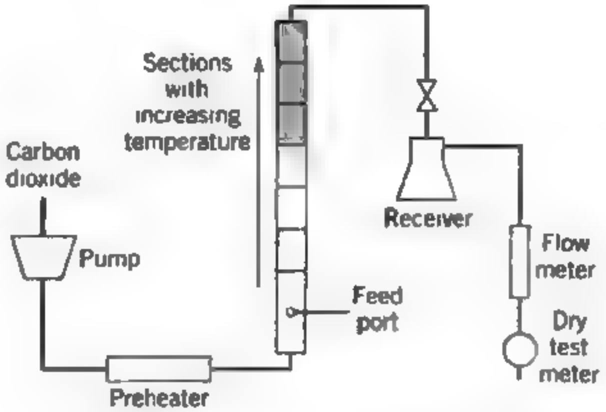


Figure 4. Column-type separation by supercritical fluids.

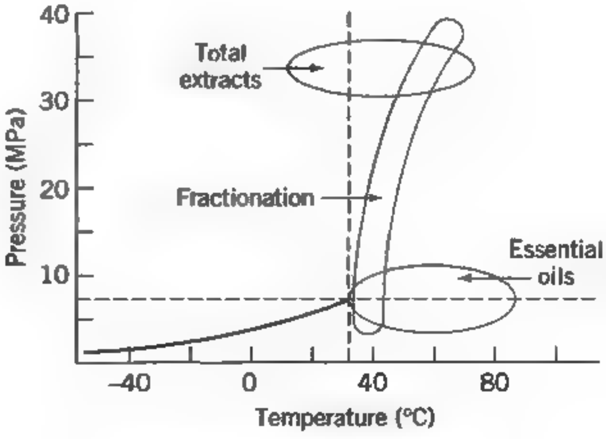


Figure 5. Typical applications of SCFE.

tions. Analysis of SCF operations applicable to the food industry, design and economic considerations, and optimization of SCFE operations have been discussed in the literature. The products of SCFE processes show different characteristics as compared with those obtained by traditional vacuum distillation processes. In general, the product quality of SCFE processes may or may not be superior to those produced by conventional methods.

COMMERCIAL APPLICATIONS

Coffee

In the decaffeination process of coffee, green coffee beans are treated with CO_2. The coffee beans are not roasted prior to the extraction. Thus, the inherent moisture in the beans acts as a chemical agent to free the caffeine from its bound form (2). Presoaked green coffee beans are treated with CO_2 at 16 to 22 MPa. The CO_2 is continuously recycled until the caffeine diffuses out of the beans into the SC CO_2 phase. The caffeine is washed out with water at 70 to 90°C. Caffeine content in the bean can be decreased from the initial value in the range of 0.7 to 3% to a value as low as 0.02%.

Hop Extraction

The use of hop extracts by the brewing industry is a recent, commercial application of SC extractions. The soft resin constituents of hops that contain the flavor components, humulones and lupulones, are conventionally extracted with dichloromethane. This results in a pasty, dark-green to black-green mass. In addition, the dichloromethane must be removed by evaporation after the resins have been extracted. In contrast, SC CO_2 extraction of hops between 35 and 80°C and 80 to 300 atm results in an "olive-green, pasty extract with an intense aroma of hops" (10)

POTENTIAL APPLICATIONS

Extraction of Flavors

As compared with conventional solvent extraction, SCFE could be a potential alternative to extract and fractionate flavors and fragrances from fermentation-induced and natural products (8,27). SC CO_2 has been used to extract the following materials: essential oils and piperine from black pepper, alkaloids from chili pepper, eugenol from cloves, cinnamic aldehyde from cinnamon, limonene and carvone from caraway, sesamin from sesame seeds, vanillin from vanilla pods, menthol and menthone from peppermint leaves, and geraniol and citronella from roses. Supercritical CO_2 was used for concentration of aroma and flavor compounds in citrus oils (28). Citrus oils, like many other essential oils, consist of mixtures of hydrocarbons of the terpene and sesquiterpene groups, oxygenated compounds, and nonvolatile residues. The compounds that provide much of the characteristic flavor are mostly oxygenated compounds that account for less than 4% of the orange oil. Thus, the concentration requirement is high and the amount of yield is relatively low.

EDIBLE OILS AND FATTY ACIDS

There is intense interest in the extraction and fractionation by SC CO_2 of vegetable, animal, and seed oils used for human consumption. In conventional extraction, hexane residue in the material leftover from extraction is routinely detected in ppm levels. Concerns about possible carcinogenicity, hazards, and cost associated with hexane have prompted researchers to develop alternative methods. To design a SCFE system, two types of information are necessary: equilibrium distribution of the oil between the SC phase and the plant matrix, and mass-transfer rate of the oil from the oil source to the solvent. Soybean oil, canola seed oil, palm oil, wheat germ oil, mustard oil, corn oil, and cottonseed oil have been extracted with SC CO_2. The effects of moisture content and particle size of the seeds, CO_2 flow rate, chamber dimensions, and other factors on the oil yield have been investigated (29). For soybeans, oxidative stability of SC CO_2 extracted oil was lower than conventional hexane extracts, since phosphatides that are natural antioxidants were not extracted with SC CO_2. Extraction of freeze-dried phytoplankton by SC CO_2 at 40°C and between 17 and 32 MPa resulted in an orange-red oil rich in omega-3 fatty acids, without extracting chlorophyll (30). Conventional separation or concentration of fatty acids, which have low vapor pressures and normally high boiling points, requires vacuum distillation at high operating temperatures. Some thermal degradation is unavoidable in this process. In contrast, it has been shown that the separation of fatty acid ethyl esters differing by two carbon atoms is possible by using SC CO_2 under mild conditions (31). Cholesterol is readily soluble in SC CO_2. There are intense efforts to remove it from foods from animal origin, without damaging their quality (32). For all of its potential benefits, there are challenges for the food scientist and engineer to implement the SCF technology. The lack of accumulated process and scale-up experience, the existence of many patents in the field, and high capital and maintenance costs are some of these challenges.

BIBLIOGRAPHY

1. J. B. Hannay and J. Hogarth, "On the Solubility of Solids in Gases," *Proc R. Soc.* London **29**, 324–326 (1879)
2. M. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction—Principles and Practice*, Butterworth, Boston, Mass., 1986.
3. G. M. Schneider, E. Stahl, and G. Wilke, eds., *Extraction with Supercritical Gasses*, Verlag Chemie, Deerfield Beach, Fla., 1980.
4. D. F. Williams, "Extraction with Supercritical Gases," *Chem. Eng. Sci.* **36**, 1769–1788 (1981).
5. M. E. Paulaitis et al., "Supercritical Fluid Extraction," *Rev. Chem. Eng.* **1**, 179–250 (1983).
6. S. S. H. Rizvi et al., "Supercritical Fluid Extraction. Fundamental Principles and Modeling Methods," *Food Technol.* **40**, 55–65 (1986).
7. S. S. H. Rizvi et al., "Supercritical Fluid Extraction: Operating Principles and Food Applications," *Food Technol.* **40**, 57–64 (1986).

8. R. A. Novak and R. J. Robey, "Supercritical Fluid Extraction of Flavoring Materials: Design and Economics," paper presented at the AIChE 1988 Annual Meeting in Washington, D.C., November 27–December 2, 1988.
9. K. Zosel, "Separation with Supercritical Gases: Practical Applications," in G. M. Schneider, E. Stahl, and G. Wilke, eds., *Extraction with Supercritical Gases*, Verlag Chemie, Deerfield Beach, Fla., 1980.
10. P. Hubert and O. G. Vitzthum, "Fluid Extraction of Hops, Spices, and Tobacco with Supercritical Gases," in G. M. Schneider, E. Stahl, and G Wilke, eds., *Extraction with Supercritical Gases*, Verlag Chemie, Deerfield Beach Fla., 1980.
11. L. G. Randall, "The Present Status of Dense (Supercritical) Gas Extraction and Dense Gas Chromatography: Impetus for DGC/MS Development," *Separation Sci. Technol.* **17**, 1–118 (1982).
12. M. A., A. Meireles and Z. Nikolov, "Extraction and Fractionation of Essential Oils with Liquid Carbon Dioxide," in G. Charalambous, ed., *Herbs, Spices, and Edible Fungi*, Elsevier, Amsterdam, The Netherlands, 1994, pp. 171–199.
13. G. Brunner, *Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and the Applications to Separation Process*, Steinkopff, Darmstadt, Germany, 1994.
14. F. A. Cabral and M. A. A. Meireles, "The Solubility and the Phase Equilibria of Essential Oil with CO_2 Calculated Using a Cubic Equation of State," in G. Charalambous, ed., *Food Flavors: Generation, Analysis and Processing Influence*, Elsevier, Amsterdam, The Netherlands, 1995, pp. 331–354.
15. L. Cardozo-Filho, F. Wolff, and M. A. A. Meireles, "High Pressure Phase Equilibrium: Prediction of Essential Oil Solubility," *Ciência e Tecnologia de Alimentos* 17, 485–488 (1997).
16. L. Cardozo-Filho et al., "The Generalized Maximum Likelihood Method Applied to High Pressure Phase Equilibrium," *Ciência e Tecnologia de Alimentos* **17**, 481–484 (1997).
17. S. I. Sandler, *Chemical Engineering Thermodynamics*, 2nd ed., Wiley, New York, 1989.
18. D. S. Marques, "Terpene Reduction in Orange Essential Oil by Preparative Chromatography and Supercritical Carbon Dioxide Extraction," Master's Thesis, Faculty of Food Engineering, State University of Campinas, São Paulo, Brazil, 1997, 331 pp.
19. H. B. Santana, "Determination of the Solubility of Essential Oil in CO_2," Master's Thesis, Faculty of Food Engineering, State University of Campinas, São Paulo, Brazil, 1996, 119 pp.
20. R. C. Weast and M. J. Astle, *CRC—Handbook of Data on Organic Compounds*, Vols. 1 and 2, CRC Press, Boca Raton, Fla., 1992.
21. R. H. Perry and C. H. Chilton, *Chemical Engineers' Handbook*, 5th ed., McGraw-Hill, New York, 1973.
22. R. P. Danner and T. E. Daubert, *Data Compilation Tables of Properties of Pure Compounds*, DIPPR, The Pennsylvania State University, University Park, 1984.
23. R. C. Reid, J. M. Prausnitz, and B. E. Pouling, *The Properties of Gases and Liquids*, 4th ed., McGraw-Hill, New York, 1987.
24. R. C. Reid, J. M. Prausnitz, and T P. Sherwood, *The Properties of Gases and Liquids*, McGraw-Hill, New York, 1977.
25. H Broyle, "CO_2 as a Solvent Its Properties and Application," *Chem. Industry (London)* 19, 385–390 (1982).
26. W. G. Schultz and J. M. Randall, "Liquid Carbon Dioxide for Selective Aroma Extraction," *Food Technol.* **24**, 1282–1287 (1970).
27. V. J. Krukonis, "Supercritical Fluid Extraction in Flavors Applications," in D. D. Bills and C. J. Mussinan, eds., *Characterization and Measurement of Flavor Compounds*, ACS Symposium Series, 289, American Chemical Society, Washington, D.C., 1985, pp. 154–175.
28. F. Temelli, C. S. Chen, and R. J. Braddock, "Supercritical Fluid Extraction in Citrus Oil Processing," *Food Technol.* **42**, 145–150 (1988).
29. J. P. Friedrich, G. R. List, and A. J. Heaking, "Petroleum-Free Extraction of Oil from Soybeans with Supercritical CO_2," *J. Am. Oil Chem. Soc.* 59, 288 (1982)
30. J. T. Polak et al., "SC CO_2 Extraction of Lipids from Algae," in K. P. Johnston and J. M. L. Penninger, eds., *Supercritical Fluid Science and Technology*, ACS Symposium Series, 406, American Chemical Society, Washington, D C., 1989, pp. 449–467.
31. K. Arai and S. Saito, "Fractionation of Fatty Acids and Their Derivatives by Extractive Crystallization Using Supercritical Gas as Solvent," paper presented during the 1986 Annual AIChE meeting in Miami Beach, Fla., November 2–7, 1986.
32. J. M. Wong and K. P. Johnston, "Solubilization of Biomolecules in CO_2 Based Supercritical Fluids," *Biotechnol. Prog.* **2**, 29 (1986).

M. O. BALABAN
University of Florida
Gainesville, Florida

M. A. A. MEIRELES
State University of Campinas—UNICAMP
São Paulo, Brazil

SURFACE (INTERFACIAL) TENSION

Surface tension can be referred to as free energy per unit area or force per unit length. Customary units are either ergs per square centimeter or dynes per centimeter. In SI (International System of Units) units, they are joules per square meter or newtons per meter (J/m^2, N/m).

The mathematical notation used for surface tension is usually γ, and in performing dimensional analysis, γ is represented by M/t^2, where M is mass and t is time. The methods available for the measurement of surface tension include the capillary rise method, the capillary wave method, the ring method, and the tensiometer method (1).

Surface energy (tension) at the boundary of two phases, such as solid–liquid in skim milk, liquid–liquid in salad dressing, gas–liquid in meringue, gas–solid in foam candy, and solid–gas in smoke, is an important parameter for studying the manufacturing and the shelf-life stability of food systems (2). Furthermore, many chemical and physical processes can occur at the boundary; for example, dissolution and crystallization, heterogeneous catalysis, and phenomena related to the colloidal state. Surface energy at the boundary often determines whether the process will occur.

London, van der Waals, or cohesive forces act among the molecules of all substances irrespective of their state of aggregation. Such forces are significant only when the molecules are rather close to each other, say, only several nanometers apart. For polar compounds, such as water, hydrogen bonding is also a significant cohesive force. In a single bulk phase, intermolecular cohesive forces are balanced. This is because molecules in the interior of a phase are attracted equally in all directions to the other molecules in their vicinity. However, in either solids, liquids, or gases, if the atoms, ions, or molecules exist in the interface, they are exposed to the action of unbalanced forces due to both phases. In other words, those at the interface are not surrounded completely by other entities of the same type or same physical state. Thus, the surface molecules or atoms are in an energy state different from that of those in the bulk phase. This additional energy, generally called surface energy, imparts to the surface region distinct features that are unique to the region. Surface tension acts parallel to the substance's surface, opposing any attempt to expand the surface area. A component of surface energy causes forces to act normally at the phase boundary, resulting in an inward attraction, which, in turn, tends to reduce the number of molecules at the interface and to reduce the interfacial area to a minimum. A liquid, for example, has a tendency to obtain a spherical shape, the shape with the smallest ratio of surface to volume, if it is not contained. On the other hand, if the liquid is contained, it does not alter to the spherical shape because gravity imposes an added requirement. The surface must be uppermost and parallel to the plane of the earth, as it takes the shape of its container. Work or energy is required to increase the area of a surface or of an interface. This requires molecules to be moved from the body of the substance to the surface (interface). The total surface energy required to expand a surface by 1 cm^2 is the sum of the work required to overcome the surface tension and the amount of heat that must be supplied to maintain the expanding surface at a constant temperature. If the surface of water is at 20°C, the thermal energy required is 47.7 $ergs/cm^2$, and since surface tension, $\gamma = 72.8$ $ergs/cm^2$, the total energy is 120.5 $ergs/cm^2$ (2). With an increase in temperature, the kinetic energy of the molecules increases and the cohesive forces among them decreases, which leads to a decreased surface tension. The influence of temperature on the surface tension of water and vegetable oils is shown in Table 1 (3). The surface tensions shown in Table 1 are those between the liquid (water or oil) phase and the gaseous phase (air). For making stable food emulsions, the interfacial tensions between edible oils and water, as shown in Table 2 (4,5), are important factors. An important consequence of surface tension (and its existence) is that there is a pressure difference on the two sides of a surface. This can be visualized by considering a gas-filled balloon stretched by the internal pressure of the gas. It experiences an elastic force parallel to the surface that counteracts the stretching; otherwise the balloon would pop. The internal pressure of a balloon must be larger than the external pressure since the gas is not only counteracting the external pressure but also stretching the balloon. This force is analogous to the surface tension of a liquid, which arises due to the rubber molecules' attraction or the liquid's molecular attraction with each other.

Table 1. Influence of Temperature on the Surface Tension (dyne/cm) of Water and Vegetable Oil

Temperature, °C	Water	Cottonseed oil	Coconut oil	Olive oil
0	72.6			
20	72.8	35.4	33.4	33.0
30	71.2			
50	67.9			
80	62.6	31.3	28.4	
100	58.9			
130		27.5	24.0	

Source: Ref. 3

Table 2. Interfacial Tension (dyne/cm) Between Water and Various Oils

Purified Oil	25°C	75°C
Triolein	14.6	13.5
1,3-Dioleo-2-palmitin	14.5	12.3
Peanut (screw press)	18.1	
Peanut (solvent, extracted)	18.5	
Cottonseed (screw press)	14.9	
Olive	17.6	
Coconut	12.8	

Source: Refs. 4 and 5.

In general, the smaller the interfacial tension, the less energy is required to create an emulsion between the phases. As a rule of thumb, the interfacial tension between water and edible oil should be below 10 dyne/cm to facilitate emulsification of the two phases (2). To meet this requirement, surface-active agents, or surfactants, are often added to reduce the interfacial tensions between the two phases. Surfactants in small quantities are known to reduce interfacial tensions. However, there are other solutes, such as inorganic salts and compounds with a large number of hydroxyl groups, such as sugars, when added to water, the surface tension of the solution increases slightly as the concentration of the solute increases. The molecular-level explanations for such distinctive phenomena are the following. Surface-active chemicals exhibit positive adsorption phenomena that lead to a higher concentration of the added chemicals at the surface (or interface) than in the bulk. For sugars and salts that show a negative adsorption phenomenon in water, the concentration in the bulk is higher than that in the surface (or interface). Not only are their effects in opposite directions, but the effects of surface-active agents on the surface (or interfacial) tension are much more drastic than those caused by sugars and salts. Consequently, small amounts of surfactants are normally used. At the molecular level, surfactant molecules are oriented at the surface (or interface) by pointing their polar (hydrophilic) groups toward the aqueous phase and their apolar (hydrophobic) groups toward the gaseous (nonaqueous) phase. In this process, the surface free energy is minimized. The surfactant molecules will form a monolayer or a film on the surface until the surface is completely covered and will form micelles with organized structures in the bulk solution as the concentration of surfactant exceeds the critical micelle concentration, which is in the range 0.004 to 0.15 mol/L for many surfactants (2).

Surfactants are either natural or synthetic, nonionic, or ionic. Food-grade surfactants are generally nonionic and are preferably natural. Soaps and detergents act as surfactants in order to lower the surface tension, which helps to remove oily dirt particles from solid surfaces (including skin). This enables these products to do what they do best—clean! Many proteins are good surfactants. Protein molecules can be adsorbed on the interface to form either a monolayer or multiple layers, depending on the individual protein. This is related to the fact that different proteins have different abilities in reducing the surface tension of water. Table 3 shows the equilibrium surface tension of four different protein solutions at different concentrations.

Similarly, Table 4 shows the influence of several milk proteins on the interfacial tension between water and butter oil. The values of surface and interfacial tension shown in Table 3 and 4 are both equilibrium values that were obtained after a transient period of surface (or interfacial) tension reduction after the addition of the protein. An equilibrium is reached when the surface tension measured stays at a constant value over an arbitrarily selected time period, such as 30 min.

Table 3. The Equilibrium Surface Tension of Protein Solutions at Various Concentrations

Protein	Protein concentration, %w/v	Equilibrium surface tension[a]
Ovalbumin	8.0	41.9
	4.0	40.3
	2.0	41.7
	1.0	44.0
Soybean protein	3.0	41.2
	2.0	41.7
	1.0	42.2
	0.3	42.5
	0.1	42.9
Bovine serum albumin	2.0	49.4
	1.5	49.1
	1.0	48.6
	0.5	49.7
Casein	3.0	48.6
	2.0	42.2
	1.0	46.5
	0.6	48.4
	0.3	47.0
	0.1	47.6

Source. Ref 6.

[a] Surface tension values (dyne/cm) attained and maintained constant for more than 30 min.

Table 4. Influence of Milk Proteins on the Interfacial Tension Between Water and Butter Oil at 40°C

Protein	Concentration, %	Interfacial tension, dyne/cm
None	—	19.2
Euglobulin	0.2	18
	0.6	18
β-Lactoglobulin	0.2	14
	0.6	14
α-Lactalbumin	0.2	11
	0.6	11
Interface protein	0.2	11
	0.6	9

Source: Ref. 7.

When protein molecules are adsorbed onto the interface, they may change tertiary structure to suit the new environment, such as specific orientation and film formation to obey the law of thermodynamics. Specifically this results in the reduction of surface energy, which makes the achieved thermodynamic state more stable. Compounded by the fact that proteins are slow-diffusing molecules, the kinetics of reducing interfacial tension by the addition of protein is not a fast one. According to Ghosh and Bull (8), the surface tension of 0.06% egg albumin solution (pH 4.9, 30°C) dropped from 62 to 48 dyn/cm within a period of 55 min. Graham and Phillips (9) also reported that the process of reduction of surface tension due to the addition of relatively low protein concentrations can take between 10 and 60 min. Kitabatake and Doi (6) reported that the reduction of surface tension due to added protein follows first-order kinetics. They also found that the formability of the protein solution correlated better with the first-order rate constant of the surface tension reduction process than with the equilibrium surface tension of the solution. In other words, formability involves the capacity to entrap air on whipping, which is promoted by proteins that will cause a fast reduction in the surface tension of water.

There are structural similarities between foams and emulsions; surface tension and interfacial tension are important physical parameters to be dealt with in food processing for the former and the latter, respectively.

BIBLIOGRAPHY

1. A. W. Adamson, *Physical Chemistry of Surfaces*, 4th ed., John Wiley and Sons, New York, 1982.
2. W. D. Powrie and M. A. Tung, "Food Dispersions," in O. R. Fennema, ed., *Food Dispersions in Principles of Food Science Part I: Food Chemistry*, Marcel Dekker, New York, 1976, pp. 185–194.
3. A. Halpern, "The Surface Tension of Oils," *J. Physical Colloid Chem.* **53**, 895–897 (1949).
4. R. R. Benerito, W. S. Singleton, and R. O. Feuge, "Surface and Interfacial Tensions of Synthetic Glycerides of Known Composition and Configuration," *J. Physical Chem.* **58**, 831–834 (1954).
5. W. S. Singleton and R. R. Benerito, "Surface Phenomena of Fats for Parenteral Nutrition," *J. Amer. Oil Chemists' Soc.* **32**, 23–36 (1955).
6. N. Kitabatake and E. Doi, "Surface Tension and Foamability of Protein and Surfactant Solutions," *J. Food Sci.* **53**, 1542–1545 (1988).
7. R. H. Jackson and M. Pallansch, "Influence of Milk Proteins on Interfacial Tension Between Butter Oil and Various Aqueous Phases," *J. Agricultural and Food Chem.* **9**, 424–427 (1961).
8. S. Ghosh and H. B. Bull, "Adsorbed Films of Bovine Serum Albumin: Tensions at Air-Water Surfaces and Paraffin Water Interfaces," *Biochemica et Biophysica Acta* **66**, 150–157 (1963).
9. D. E. Graham and M. C. Phillips, "Proteins at Liquid Interfaces I, Kinetics of Adsorption and Surface Denaturation," *J. Colloid Interface Sci.* **70**, 403–414 (1979).

Shaw Wang
Rutgers University
Piscataway, New Jersey

SURIMI: SCIENCE AND TECHNOLOGY

Surimi is a Japanese term for mechanically deboned fish mince that has been washed with water and mixed with cryoprotectants for a long frozen shelf life. It is used as an intermediate product for a variety of shellfish analog products, such as crab legs, shrimp, and lobster. Minced fish, on the other hand, is a mechanically separated flesh that has not been washed and does not have good freeze storability. Washing not only removes fat and undesirable matter, such as blood, pigments, and odorous substances but, more important, increases the concentration of myofibrillar proteins (primarily actomyosin) through removal of water-soluble sarcoplasmic proteins. As a result, washing improves gel strength and elasticity, essential properties for surimi-based products.

Unlike soy protein, surimi, because of its high concentration of myofibrillar proteins, produces an elastic and chewy texture that can be made to resemble that of shellfish. Because it has this unique property, surimi has been used extensively in Japan for many centuries in a variety of traditional as well as new fabricated products. Surimi technology has led to the development of commercially acceptable shellfish analogs that were not successful in the U.S. market when soy protein was used. It appears that surimi has great potential as a functional protein ingredient that can be substituted for a variety of traditional animal and vegetable proteins. The potential of surimi in developing new products is not limited to shellfish analogs; it has already been realized in developing products based on surimi–meat blends. The virtually unlimited resources of underutilized fish species will ensure a sufficient supply of surimi at a reasonable cost to meet the need for base material for surimi-based products.

The development of surimi technology in the United States began in the early 1980s, utilizing Alaska pollock (*Theragra chalcogramma*). Within a short period, the U.S. surimi industry established a strong commercial base with technology development assisted by the government and university research laboratories. As a result, U.S. surimi production from Alaska pollock has grown from about 4,000 t in 1984 to an estimated 160,000 t in 1997, employing 19 factory ships and four shore plants. In nine years, the U.S. consumption of surimi analog products rose from 2,700 t (6 million lb) in 1980 to an estimated 80,000 t (176 million lb) in 1996 (1). The world surimi production topped 540,000 t (2) in 1992. As the Alaska pollock resource weakens, Southern blue whiting (*Micromesistius australis*) caught off Argentine and Pacific whiting (*Merluccius productus*) off the U.S. West Coast became the next largest sources for surimi production.

The interest of the U.S. industry in these products stems from the following: the surimi market continues to grow worldwide; new products with high profit margins can be developed; nontraditional fish species can be processed at a profit; and the nonseafood food companies can enter the manufacturing of surimi seafood products without having to become involved in fish processing. This article focuses on the historical background of surimi technology, and the important physical and chemical principles behind the process for manufacturing surimi and surimi-based seafood products.

Traditionally, Japanese surimi was freshly prepared from fresh fish and immediately processed into kamaboko products. Kamaboko is a generic term that includes a variety of products prepared from surimi and is distinguished from texturized shellfish meat analogs, which are a new breed of surimi-based products. The technique for making kamaboko products from minced and washed fish evolved around A.D. 1100, when Japanese fishermen discovered that they could keep the product longer if washed minced fish was mixed with salt, ground, and steam cooked or broiled (Fig. 1). Although all products made from surimi are generally called kamaboko, strictly speaking, kamaboko is one that is mounted on a wood plate and steamed or broiled. There are other narrowly defined products such as chikuwa, which is broiled, and tempura, which is fried.

The traditional surimi production was run on a day-to-day basis, depending on the supply of fresh fish. Consequently, the surimi industry could not expand to any great extent and remained a small-scale operation. However, in 1959, the surimi industry took a new turn when a group of scientists at the Hokkaido Fisheries Laboratories discovered a technique to stabilize frozen surimi (3,4). This discovery was made from an incidental finding of a cryoprotectant that kept the surimi from freeze denaturation during frozen storage. This technique enabled Japanese manufacturers to stockpile surimi. Previously, most of the surimi was produced on shore, but subsequently about half of the surimi has been produced on processing ships as a result of an intensive joint effort by government and industry to mechanize on-board surimi production. Subsequently, frozen surimi production increased from 32,000 t in 1965 to 380,000 t in 1975, together with a record production of 1.1 million t of kamaboko (5,6).

THE SURIMI PROCESS

A typical surimi process on a pilot-plant scale is shown in Figure 2. The fish is headed, gutted, and cleaned in a washing tank. The washed fish is then put through a belt-drum-type meat separator that separates the flesh from the bone and skin. The fillets processed by a high-speed filleter are often favored over headed and gutted fish for surimi of superior quality. The diameter of the drum perforations ranges from 4 to 7 mm and is selected in accordance with the size and freshness of the fish (7). For the production of high-quality surimi with limited washing, particularly in the case of on-board processing, the backbone is mechanically removed, or the fillet is used. Filleting is done mechanically using a filleting machine at a fast speed.

Basically, surimi is produced by repeatedly washing mechanically separated fish flesh with chilled water (10°C) until it becomes odorless and colorless or, technically, until most of the water-soluble proteins are removed. The water temperature does not need to be kept unnecessarily low. It should be determined by the type of fish (species), specifically the thermostability of fish protein; for example, 10°C for Alaska pollock (a cold-water fish) and 15°C for red hake (*Urophycis chuss*, a temperate-water fish) (8). On the basis

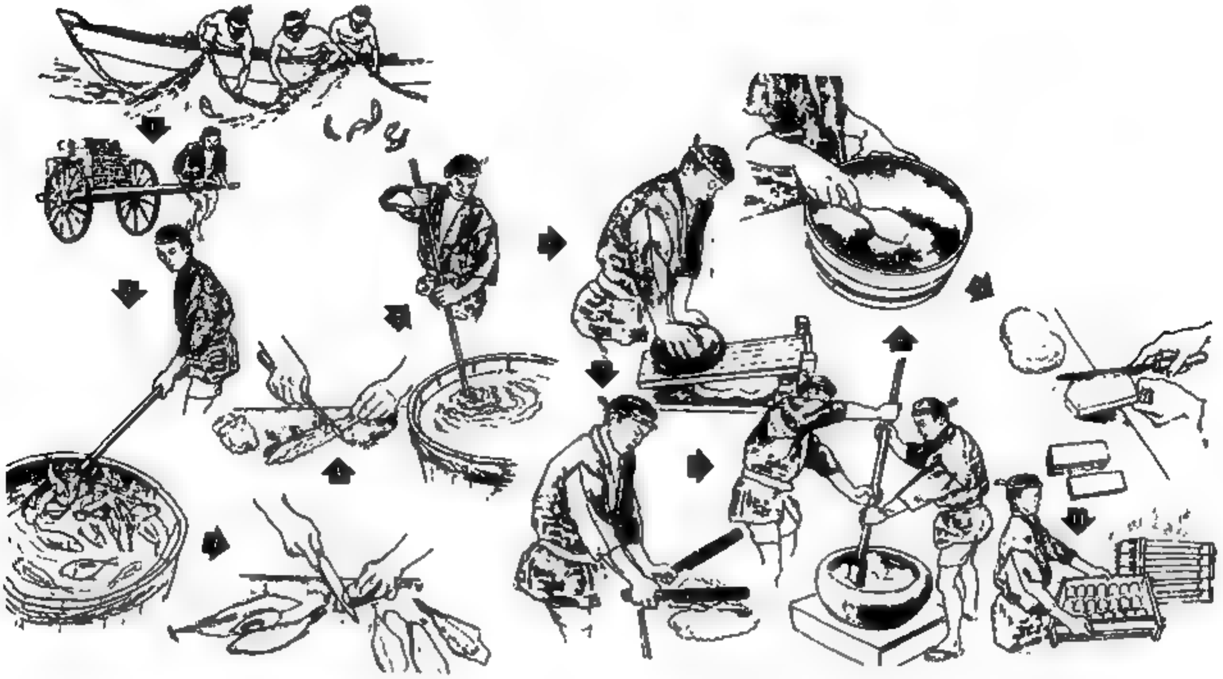


Figure 1. An old surimi and kamaboko making process; (1) fish harvest, (2) gutting and cleaning, (3) filleting, (4) mincing, (5) washing, (6) dewatering, (7) chopping, (8) stone grinding with salt and spices, (9) straining, (10) shaping of kamaboko, and (11) steaming of kamaboko. *Source:* Courtesy of Suzuhiro Kamaboko Kogyo Co., Ltd., Odawara, Japan.

of the relationship between species and thermostability of actomyosin ATPase (9), it can be assumed that warm-water fish can tolerate a higher water temperature than cold-water fish without a reduction in protein functionality.

In a washing process, the number of washings, the volume of water, and the washing time will vary with the fish species, the initial condition of the fish (freshness), the type of washing unit, the ratio of water to meat weight, and the desired quality of surimi (10). Generally, in the shore plant process three washings are recommended at a 4:1 ratio of water to meat, while in the factory ship process two washings are done at two to three parts water to one part meat because of fresh water shortage. Based on 5 min for each washing and two to three washing cycles, 15 to 20 min for the entire leaching process is appropriate for a commercial surimi manufacturing operation.

In a commercial process, the washing is done continuously, with mechanical agitation in a series of washing tanks and a rotary screen rinser. During repeated washings with continuous agitation, much of the water-soluble proteins are removed, along with undesirable substances and enzymes, causing the level of actomyosin to increase. The level of functional actomyosin is a measure of the gel-forming ability of the surimi. This explains why surimi gives a more elastic texture than unwashed minced fish meat. For the last washing, a 0.1 to 0.3% NaCl solution is used to ease the removal of the water. The washing is followed by refining with the aid of a refiner that removes connective tissue, black skin, bone, and scale. The white flesh passed through the refiner's perforations is collected and referred to as first grade, while the portion rejected is put through the refiner again to recover the additional refined flesh, which is darker and less functional than the first grade. The second-time refined mince is, therefore, referred to as second grade.

The refined flesh is transferred to a screw press, which removes excess water. At this point, the flesh should be white, odorless, and residue free and have 82 to 84% moisture. Using a silent cutter, cryoprotectants sucrose, sorbitol, and sodium tripolyphosphate are mixed into the dewatered flesh at levels of 4, 4 to 5, and 0.2 to 0.3%, respectively. The levels and ratio of sucrose and sorbitol can be adjusted depending on the type of product to be made and the sweetness desired. Some surimi are prepared only with sorbitol, while some are produced only with sucrose but at a level no more than 5% because of sweetness. The principal steps of the rotary screen surimi manufacturing process are summarized in Figure 2.

The material balance of surimi manufacturing is shown in Figure 3. Most of losses occur during washing in the form of water-soluble proteins and secondarily from refining in which connective tissue is removed. Fine particles of water-insoluble myofibrillar proteins are also lost through the screen during draining. A variety of surimi are made from more than 60 different fish species. Each species requires slightly different processing techniques. Primary species include Alaska pollock, Southern blue whiting, Pacific whiting, and treadfin bream (*Nemipterus tambuloides*) in Thailand, which are processed in commercial volume for the production of frozen surimi.

Pacific whiting is caught off the U.S. West Coast, and its estimated production has exceeded 30,000 t in 1997.

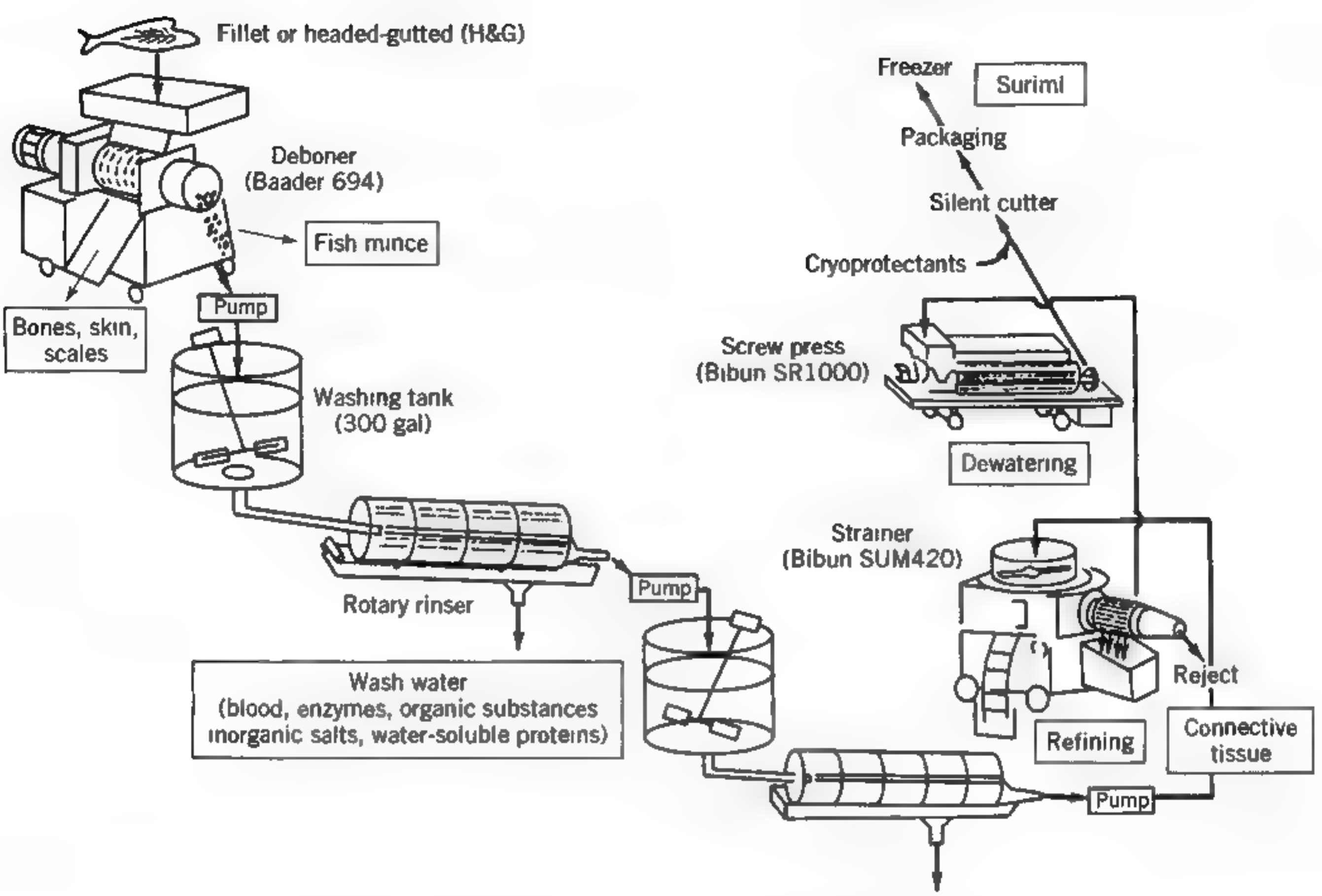

Fillet or headed-gutted (H&G)
Deboner
(Baader 694)
Fish mince
Bones, skin, scales
Pump
Washing tank
(300 gal)
Rotary rinser
Wash water
(blood, enzymes, organic substances
inorganic salts, water-soluble proteins)
Pump
Pump
Strainer
(Bibun SUM420)
Reject
Refining
Connective tissue
Dewatering
Screw press
(Bibun SR1000)
Cryoprotectants
Silent cutter
Packaging
Freezer
Surimi

Figure 2. Flowchart of a pilot-plant surimi manufacturing process. *Source:* Courtesy of the Department of Food Science and Nutrition, University of Rhode Island.

Due to a strong parasitic proteolytic activity, Pacific whiting requires a special process that includes holding fish at below 5°C and the use of enzyme inhibitor such as beef plasma protein (1%) or egg white (2%) in mince prior to freezing. Currently, the surimi produced from Alaska pollock and Southern blue whiting make up the majority of the commercial production and command superior quality in terms of color, odor, and gel-forming ability.

The factory ship–processed surimi always commands high quality because of the freshness of the fish used and the prior removal of the backbone and belly flap. This leaves a minimum amount of blood and cavity residues with no autolysis (proteolytic degradation) in deboned fish mince, requiring less water in washing. Due to limited water availability, it is an absolute necessity for factory ships to have minimum washing using the freshest fish mince with minimum organ residues, whereas shore plants process boat-delivered fish, which could be one to five days old, depending on the length of the fishing trip. Thus, sometimes these shore-processed fish could lack freshness and produce a low-grade surimi. In the shore process, fish is often deboned without prior removal of the backbone and belly flap. Fish that is less fresh and has inadequate deboning requires more extensive washing to remove proteolyzed products, blood, and other undesirable organ residues and fluids. The preceding explains why only two washing cycles are employed in the ship process, compared with three to four cycles in the shore process. The modern factory ship (Fig. 4) has the capability of processing surimi, as well as by-products such as fish meal and fish oil. The only limitation of the ship process is the water supply, which must be generated from seawater by desalinization.

Cryoprotectants were originally incorporated into the dewatered meat by a kneader or stone grinder; however, this incorporation is now accomplished by a high-speed cutter, which is more effective and faster than the kneader. Caution must be taken not to allow the temperature of the mix to exceed 10°C, above which protein functionality could be damaged.

PROCESS WASTEWATER

Surimi making requires a large amount of fresh water during the washing process; this ranges from 10 to 20 times the weight of the deboned meat, depending on the species, condition of fish, and extent of cleaning required. Currently, all the water used during the surimi process is discharged as wastewater. This is of concern because the wastewater contains an average of 3.4 g of protein per liter

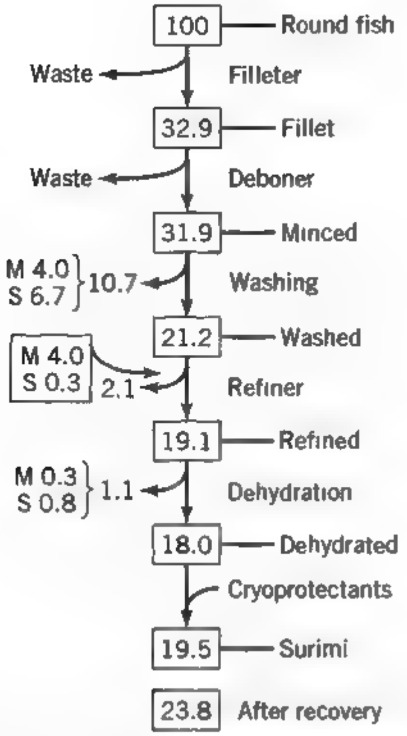

100
Round fish
Waste
Filleter
32.9
Fillet
Waste
Deboner
31.9
Minced
M 4.0
S 6.7
10.7
Washing
21.2
Washed
M 4.0
S 0.3
2.1
Refiner
19.1
Refined
M 0.3
S 0.8
1.1
Dehydration
18.0
Dehydrated
Cryoprotectants
19.5
Surimi
23.8
After recovery

Figure 3. Material balance of surimi manufacturing process. M, myofibrillar proteins; S, sarcoplasmic proteins. *Source:* Ref. 11.

(0.34%). About 80% of the protein present in the wastewater is water soluble. The total protein lost accounts for approximately 30% of the deboned meat weight (12) and varies from plant to plant, depending on the amount of water used and the number of washing cycles employed. The wastewater generated from the shore plants must be treated to reduce its biological oxygen demand (BOD) level before being discharged.

PROTEIN RECOVERY

During washing of minced meat, a considerable amount of water-soluble and some insoluble proteins are lost, as much as 33% (on a minced meat weight basis, Fig. 3), of which water-insoluble myofibrillar proteins in fine particles unretained by a rotary screen can be recovered by a decanter centrifuge or a settling tank with a conical bottom (10). Both have been successfully used to improve the yield in recent years. Some efforts were made without commercial success to recover water-soluble proteins employing an ultrafiltration (UF) membrane system.

GEL QUALITY EVALUATION

Surimi is highly concentrated with myofibrillar proteins, primarily actomyosin. The myofibrillar proteins are solubilized by salt during chopping, which is an initial step of the manufacturing shellfish analog products. The solubilized protein sol, or paste, gels on heating. The gel-forming

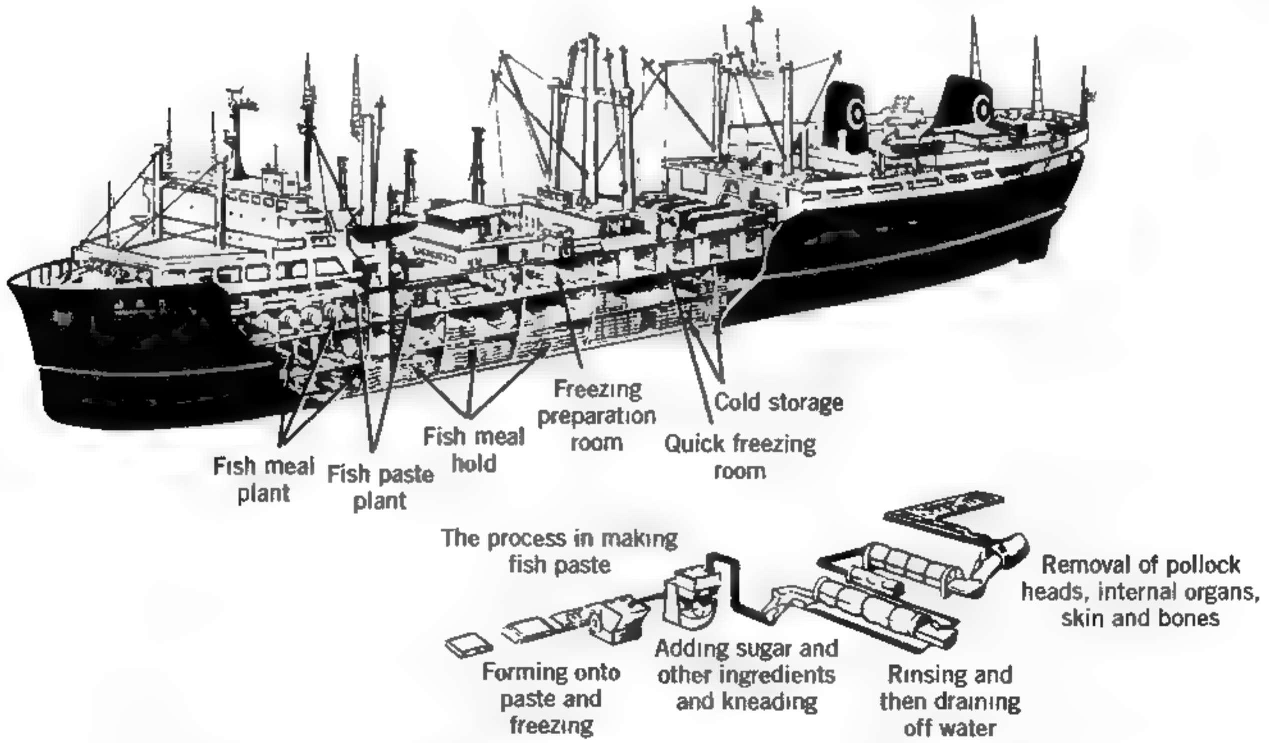

Freezing preparation room
Cold storage
Fish meal hold
Quick freezing room
Fish meal plant
Fish paste plant
The process in making fish paste
Removal of pollock heads, internal organs, skin and bones
Forming onto paste and freezing
Adding sugar and other ingredients and kneading
Rinsing and then draining off water

Figure 4. Surimi factory ship, the 21,000-ton Mineshima Maru. *Source:* Courtesy of Hippon Suisan Kaisha Co., Ltd., Tokyo, Japan.

ability, measured by the water-binding capacity of the comminuted tissue (protein solubilization) and gel strength, is determined by the level of functional actomyosin. The level of functional actomyosin, measured as extractable actomyosin or ATPase activity, increases with an increase in the number of washing cycles and decreases as the freshness of the fish decreases; such a decrease in extractable actomyosin is caused by tissue autolysis, which is proteolytic breakdown of myofibrillar protein by catheptic protease. The proteolytic breakdown increases with extended storage or during the spawning season. The quality of surimi during frozen storage is affected by storage temperature, storage period, the level of remaining moisture, the type and level of cryoprotectants used, and remaining inorganic salts (10,13).

The quality of surimi is graded on the basis of its gel-forming ability and the chemical and visual conditions of raw surimi. A new version of the Japanese grading system for frozen surimi was developed initially by Tokai Regional Fisheries Research Laboratory (1980) and adopted by various individual companies with some modifications for its internal standards. The modified standard procedures of Japan Fisheries Ltd. (14) and some of the procedures from the Tokai Regional Fisheries Laboratory are summarized in Table 1.

In the United States, on the other hand, methods for specifying properties of raw surimi instead of a grading system were proposed by the U.S. surimi technical committee under the direction of the National Fisheries Institute (15). Additional testing procedures for surimi (16) and fiberized analog products (17) were also proposed. They were intended not only to supplement the existing Japanese grading system but also to make procedures more practical for the United States, where the system is intended for specification rather than grading. In this way, a suitable type of surimi, which meets the gel-forming requirements of specific formulations, can be selected.

USE OF CRYOPROTECTANTS

Freezing of surimi become commercially possible after the discovery of the cryoprotective role of sucrose, which prevents muscle protein, particularly actomyosin, from denaturation during frozen storage. Initially, 8% sucrose was used, but it made the surimi too sweet and caused a color change during frozen storage. Subsequently, the level of sucrose was reduced to 4%, and 4% sorbitol was added to compensate for this reduction (3). The increased ratio of sorbitol to sucrose resulted in an increase in the gel-forming ability (compressive force), suggesting that sorbitol is more effective cryoprotectant than sucrose. The most desirable sweetness was attained at a 6:2 ratio of sorbitol to sucrose (18). The cryoprotective effect of these sugars was greatly enhanced by adding polyphosphate at a level of 0.2 to 0.3%.

Prevention of protein denaturation by sugars can be explained by their ability to increase the surface tension of water (19) and the amount of bound water, resulting in increased hydration of protein molecules. This prevents withdrawal of water molecules from the protein, thus stabilizing the protein (20).

CHANGES DURING FROZEN STORAGE

The gel-forming ability of surimi made from fresh (one- to two-day-old) fish in good condition does not change significantly for up to one year when held at a constant temperature below −20°C (21,22). However, when the surimi is stored at higher temperatures, the gel-forming ability gradually decreases; this is attributed to a decrease in extractable actomyosin subsequent to freeze denaturation of proteins (22). Temperature fluctuation during short-term transportation does not significantly reduce surimi quality. However, extended periods of temperature fluctuation (more than three weeks) do cause significant loss in quality (23).

GEL FORMATION

Myofibrillar proteins, primarily actomyosin, in surimi need to be solubilized by salt during chopping to form an extrudable paste (sol) essential for fabrication. The surimi paste must be extruded right after chopping so that it can be shaped or formed before it sets to a gel. The extruded paste is subjected to partial heat setting for fiberization, followed by cooking. With or without heat, the surimi sol undergoes gelation where randomly arranged peptide chains irreversibly transform to an ordered state with the aid of both hydrogen and hydrophobic bonds (5,24). This irreversible transition from sol to gel is called *setting*. Hydrogen bonding is primarily involved in setting at low temperature, while hydrophobic bonding dominates in subsequent gel setting at high temperature (25). In a commercial practice, a partial heat-setting is done at temperatures of 40 to 50°C before a final cooking at 80 to 90°C. The textural quality of the finished product is highly dependent upon the way the partial heat setting is carried out. A proper partial heat setting with a right temperature–time relationship is required for development of a desirable texture. Gel-setting behavior and gel strength vary from species to species (26).

Gel setting (swari) takes place at temperatures up to 50°C without cooking and occurs even below room temperature. However, when a comminuted fish (paste) passes through a heating zone of 60 to 70°C, part of the gel structure is altered such that the gel becomes soft. This phenomenon is called softening (modori). Such softening is believed to be associated with proteolytic activity of alkaline protease, because this enzyme is present in many fish species and has an optimum activity at 60 to 70°C (27–29). The degree of gel softening is significantly reduced after washing the fish mince. This further confirms the involvement of protease in gel softening and suggests that gel softening is specific to the type and amount of water-soluble proteins (sarcoplasmic) in the fish tissue. This theory has been challenged by a series of reports in which softening of the texture still occurred after protease was inhibited (30,31). This led to a theory of temperature-dependent gel-setting behavior. At a fast heating rate, a tight, cohesive network with a large number of small aggregates is formed, whereas at a slow heating rate, as in the case of 60 to 70°C heating, a loose network with a small number of large aggregates is formed (32).

Table 1. Japanese Grading System for Quality of Frozen Surimi

Item	Procedure
	Chemical and visual conditions
Moisture	5 g of half-thawed surimi is dried either in the oven at 105°C or in their moisture balance, and the moisture loss determined
pH	10 g of thawed surimi is homogenized with 90 mL of distilled water, and pH is measured
Impurities	The presence of impurities is scored by counting the number of black skin and bone residues in a 10 cm × 10 cm field, which is prepared by spreading 10 g of surimi in 1 mm thickness
Whiteness	The whiteness of surimi gel is measured by a Hunter color meter (model C-1) with red filter (620 mm)
	Physical properties
Expressible drip	50 g of thawed surimi is placed in a cylinder (35 mm in diameter × 150 mm long) having a base with a 3 mm mesh and pressed at a 500-g load for 5–10 min and at an additional 500-g load for 20 min, expressible drip (%) = (drip weight/sample weight) × 100
Viscosity	143 g of thawed surimi is homogenized for 8 min at 8–10°C with 857 mL of 3.5% NaCl solution in an antifoaming mixer having cooling capability; after 40 min at room temperature, the viscosity is measured using a Brookfield viscometer (Tokyo Instruments, Model C) at 10°C at 4 rps
	Gel-forming ability
Gel preparation	A gel is prepared using 2–5 kg of thawed surimi and 3% salt by grinding in a kneader for 30 min or chopping in a silent cutter for 15 min; during chopping, the temperature of the surimi paste must be kept below 10°C to preserve the functionality of actomyosin; potato starch is added according to the grade: none to AA grade, 3% to A grade, and 5% to B grade; the resulting paste is stuffed into a 30-mm-diameter polyvinylidene chloride casing, linked in 25-cm lengths, and immediately heated in a water bath at 90°C for 40 min; it is then cooled in cold water and left at room temperature for 18–48 h until tested
Gel strength	The gel strength is measured by either a rheometer or an Okada gelometer using a cylindrical specimen (30 mm in diameter × 25 mm long) and a 5-mm-diameter plunger with a round end, gel strength (g-cm) = breaking force × deformation
Folding test	Grade is determined according to the extent of crack when a 33-mm-thick slice of gel is folded between thumb and index finger AA = No crack occurs after folding twice A = Crack occurs after folding twice, but no crack occurs after folding once B = Crack occurs gradually after folding once C = Crack occurs immediately after folding once D = Breakable by finger press without folding
Sensory score test	Firmness is scored by the bite test: Extremely firm = 10 Very firm = 8 Moderately firm = 6 Slightly soft = 4 Very soft = 2 Extremely soft = 1

The texture of gels prepared from a given surimi system is affected by the moisture content of the surimi, the levels of added salt and polyphosphates, the extent of actomyosin solubilization (chopping time), pH, and heating schedule. Generally, the gel weakens as the moisture content increases. The salt concentration required for gel formation ranges from a minimum of 2% to a maximum of 3% of the weight of surimi, depending on the process and saltiness requirements; the average level used in the commercial process is 2.5%, where the formula contains other ingredients in addition to surimi. When only surimi and water are used in the formula, the gel strength of Alaska pollock surimi reaches a maximum at 0.26 M NaCl (1.5%) on a gel weight basis and gradually decreased as a salting-out effect occurred on further increases in salt concentration (8). Solubilization of actomyosin increases with chopping time and reaches a maximum. The chopping time for maximum solubilization is determined by the amount of surimi used and the size and speed of chopper. Beyond this maximum chopping time, the mechanical shear damage and temperature rise occur along with protein–protein interaction, resulting in a decrease in gel-forming ability (33,34). Therefore, chopping should be stopped when a sufficient solubilization is achieved, while keeping the temperature of the batter below the temperature that protein can tolerate. The present Japanese practice calls for chopping at 10°C as a maximum tolerable temperature for Alaska pollock. The maximum allowable chopping temperature thus depends on the type of fish species. Temperate species such as red hake and croaker can tolerate a higher temperature than cold-water species such as Alaska pollock (8). Thermostability of actomyosin is closely related to the body temperature of the fish and the temperature of the water where the fish lives. The lower the water temperature, the more unstable the fish muscle actomyosin is (9,35).

Elasticity and resilience of surimi gel increase with an increase in the concentration of actomyosin, but decrease with an increase in the concentration of water-soluble (sarcoplasmic) proteins. The presence of water-soluble proteins retards gel setting by interfering with the actomyosin cross-linking process. A mechanism was proposed whereby water-soluble proteins bind actomyosin, making it less available for the cross-linking process (36,37).

The strength of surimi gel is affected by the pH of the comminuted surimi paste. The optimum pH varies with the fish species from which surimi is prepared, as well as with formulation, because ingredients interact with one another. A pH around 7 with a moderate salt concentration gives maximum gel strength. A pH below 7 results in a sharper decrease in the gel strength than above 7 (5). The pH dependency of gel formation is related to the dependence of water-binding ability of myofibrillar protein on pH (38).

PRODUCTION OF SURIMI-BASED PRODUCTS

Surimi-based products are prepared by extruding the surimi paste into various shapes that resemble shellfish meat such as crab, lobster, scallop, or shrimp. The closer the simulation desired, the greater the sophistication of the extrusion technique that is required. The products may be divided into four major categories according to their fabrication and structural features: molded, fiberized, composite-molded, and emulsified (Fig. 5).

Molded Products

These products are produced by molding the chopped surimi paste into the desired shape and allowing it to set and form an elastic gel. Molding may be done by either a single extrusion or a coextrusion. For the former, the paste is extruded through a single opening of the nozzle without concurrent texturization. For the latter, the paste is extruded through a nozzle having many separate openings such that strings of extrudate are laid over one another during forming. Coextrusion, therefore, gives a meatlike texture, whereas the single extrusion results in a uniform and rather rubbery mouthfeel.

Fiberized Products

As shown in Figure 6, these products are produced by extruding the paste into a thin sheet through a rectangular nozzle having a narrow opening (1–2 mm). The extruded sheet is then partially heat set and cut into strips of desired width by a cutter, similar to a noodle cutter, having a clearance that allows only partial cutting (<4/5 of the thickness), so that a sheet of strips results. The surimi used in this process should be of high grade so that the paste remains sufficiently cohesive and elastic while it is stretched, cut, and pulled during a fabrication process. The greatest pulling tension occurs between the cutter and wrapper. Strip width is determined by the type of finished product desired. Fine strips are preferred for the fibrous

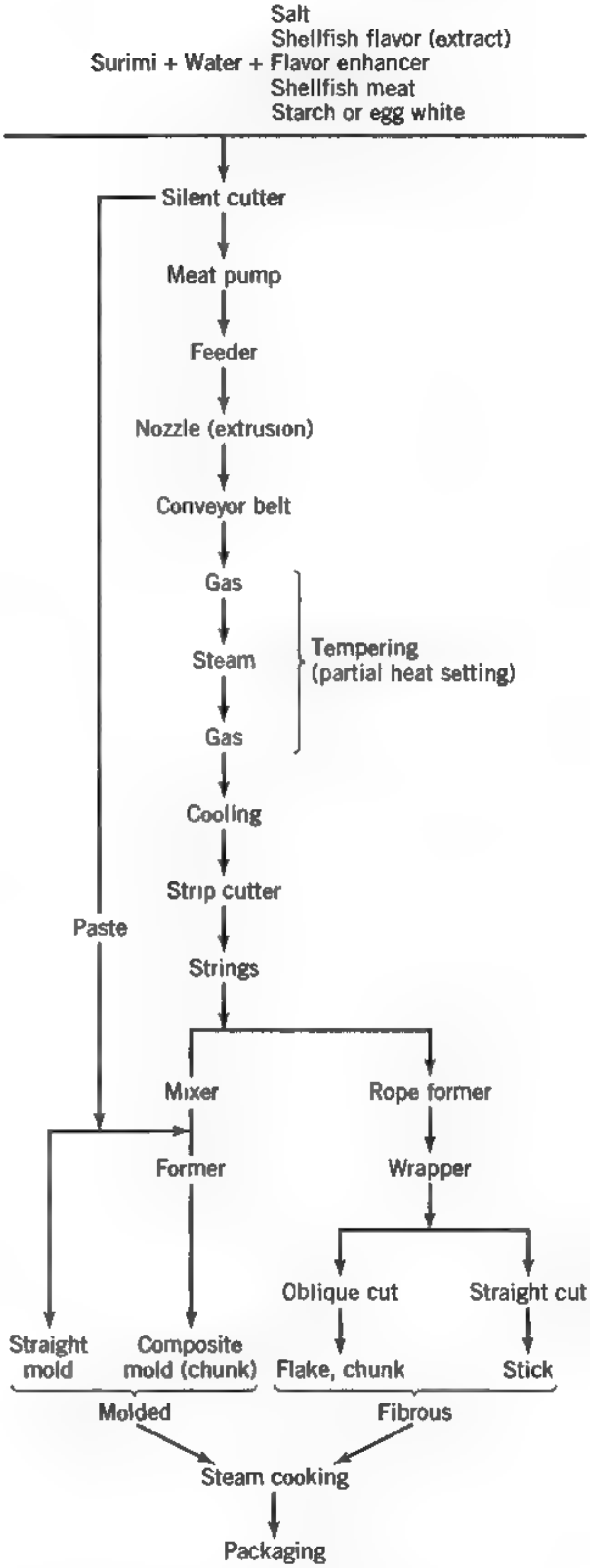

Surimi + Water + Salt
Shellfish flavor (extract)
Flavor enhancer
Shellfish meat
Starch or egg white
Silent cutter
Meat pump
Feeder
Nozzle (extrusion)
Conveyor belt
Gas
Steam
Gas
Tempering (partial heat setting)
Cooling
Strip cutter
Paste
Strings
Mixer
Rope former
Former
Wrapper
Oblique cut
Straight cut
Straight mold
Composite mold (chunk)
Flake, chunk
Stick
Molded
Fibrous
Steam cooking
Packaging

Figure 5. Flowchart of process for fibrous, flake, chunk, and composite-mold-type products. *Source:* Courtesy of the Institute of Food Technologists.

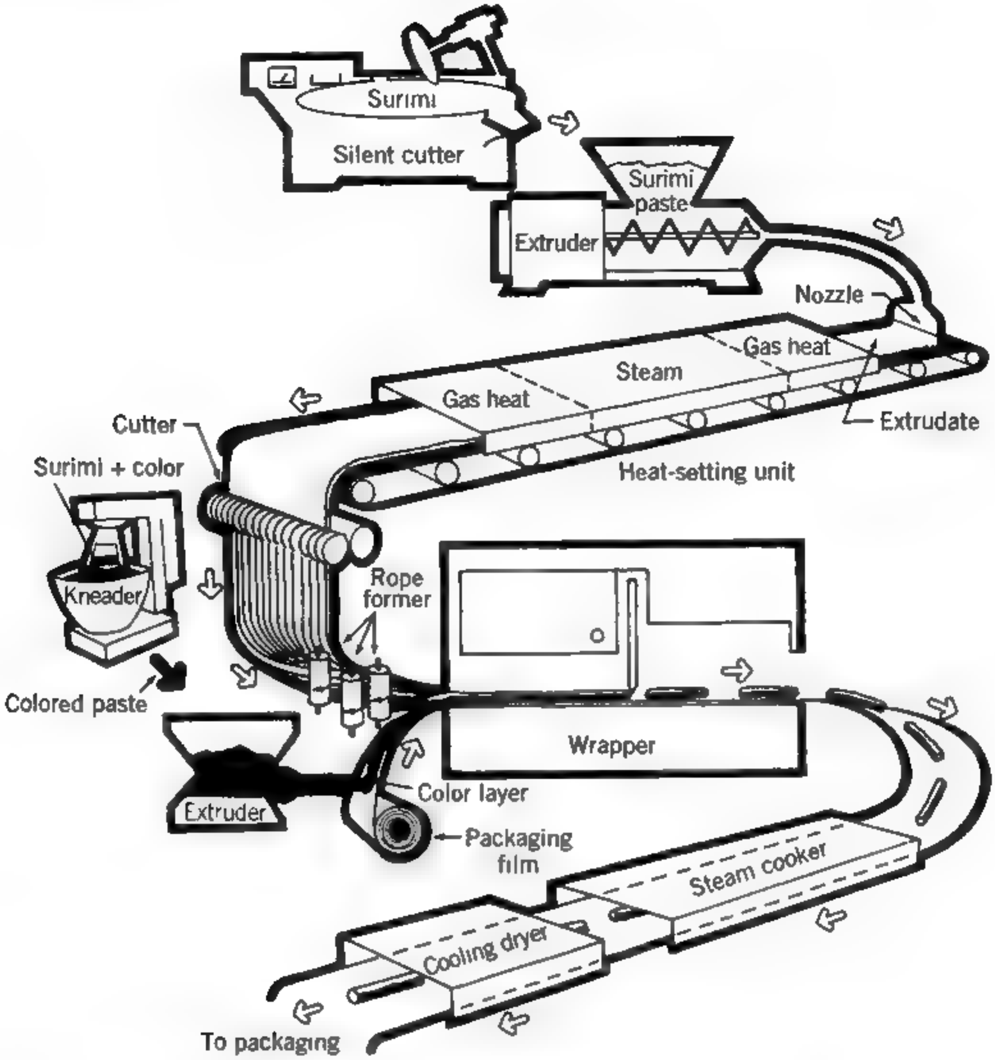

Surimi
Silent cutter
Surimi paste
Extruder
Nozzle
Gas heat
Steam
Gas heat
Extrudate
Heat-setting unit
Cutter
Surimi + color
Kneader
Rope former
Colored paste
Extruder
Color layer
Packaging film
Wrapper
Steam cooker
Cooling dryer
To packaging

Figure 6. Schematic of small-scale crab leg processing line (capacity 1 t/day). The color layer is transferred from the film to the meat surface during steam heating. *Source:* Courtesy of the Institute of Food Technologists.

crab leg product, whereas wider strips are more suitable for the crab flakes and chunks as well as for scallop analogs.

The resulting sheet of strips is folded into a rope (a bundle of fibers) by a simple narrowing device called a rope former. The rope is then colored, wrapped, and cut into a desired length by a wrapping machine. Coloring is done by applying a thin layer of colored paste onto a wrapping film. The film of colored paste sticks onto the surface of the rope as wrapping and cooking progress. Coloring is also done by directly applying a thin colored extrudate onto the rope without wrapping film. The crab leg product is produced by a straight cut, whereas the flake and chunk types are formed by an oblique cut. During the folding process, the texture of the finished products can be further altered by manipulating the adhesion between the folded layers. Recently, a fiberization by the twin-screw extrusion was developed in Japan. In this process, protein salt-solubilization is followed by texturization of surimi protein, both done in an extruder. Although the texturization by extrusion is found to be efficient and less labor intensive, the process has not been universally accepted due to inferior texture and flavor retention compared to the conventional fiberization method.

Composite-Molded Products

For these products, the strings or shreds of desired length are mixed with surimi paste and extruded into a desired shape. Strings or shreds are produced either by the method just described or by shredding a block of surimi gel into thin rectangular pieces (<1 mm thick). Texture can be manipulated by adjusting the mixing ratio of strings and shreds and surimi paste (binder). This type of product gives a better bite than the strictly molded variety, which often tends to be rubbery and uniform in texture. Composite-molded products are found in stick form and sold mixed with fiberized products. Most typical products under this category that can be found in the market are shrimp and lobster analogs. Another type of composite-molded product called *fish ham* is prepared by mixing the dice of cured tuna and pork into the fish paste before extrusion.

Emulsified Products

To make this type of product, surimi is treated in a manner similar to that used when meat is processed for emulsion products. The level of fat added is usually less than 10%, and the type of fat used is not limited to animal fat. In fact, vegetable oil is often added, because unlike mammal and bird meat, fish meat readily produces a stable emulsion with oil (39). The wiener-type products have been developed and successfully marketed in Japan. Sausage-type products can be produced by a method similar to that used for composite-molded products, as illustrated in Figure 7.

EFFECT OF INGREDIENTS ON TEXTURE

The sequence of incorporation of ingredients affects the textural quality of the final product and varies with the type of formulation. In general, salts and a portion of ice-chilled water (one-third) are added first, then the mixture is chopped for the first third of the chopping period to allow the solubilization of myofibrillar protein. Next, for the improvement of texture and water binding, starch and egg white (raw, pasteurized, and frozen) are added with the remaining water, and the mixture is chopped for the remainder of the chopping period. Flavor is added during the last third of the chopping period for maximum delivery.

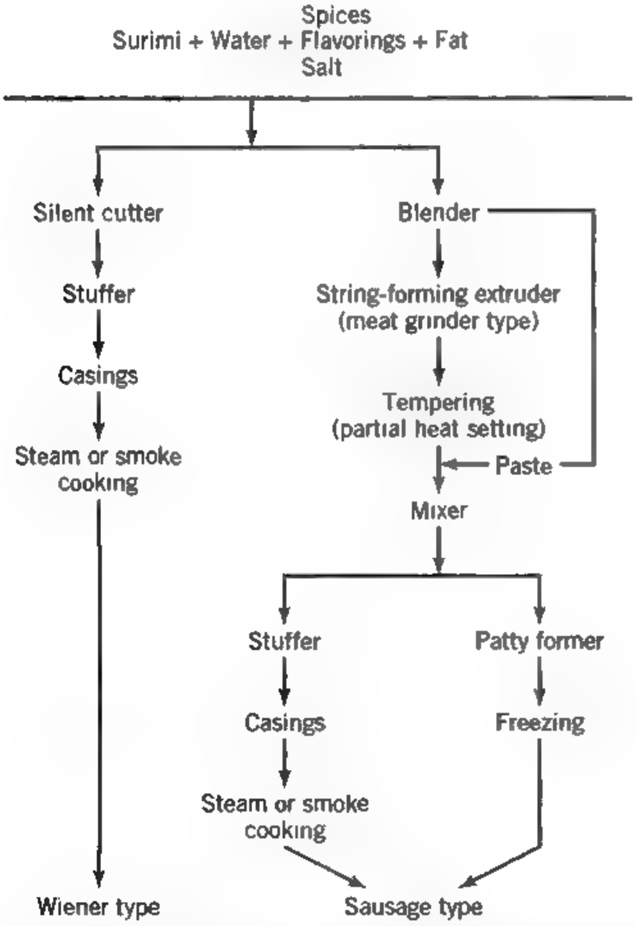


Figure 7. Flowchart of process for surimi-based emulsion-type products. *Source:* Courtesy of the Institute of Food Technologists.

Starch

Currently, corn or wheat starch is used at around 6% to improve the textural properties of surimi gel, or at higher levels for cost reduction. Starch must be uniformly dispersed and gelatinized to strengthen the gel. Starch participates in gel formation as a dispersed phase, whereas protein does so as a continuous phase. Starch increases gel strength and elasticity through composite reinforcement and water binding. Gel-strengthening ability varies greatly from starch to starch. The gel-strengthening ability of starch is affected by its water-binding capacity during gelatinization and the viscosity of the gelatinized starch (40). The greater the water-binding capacity and viscosity of the starch, the greater is its gel-strengthening ability.

In general, strong, elastic gels are produced from unmodified high amylopectin starches, such as potato and waxy corn starches, which are less prone to retrogradation at room temperature. In addition to the water-binding capacity and rheological properties of starch, its retrogradiation behavior and interaction with protein during gel network formation are also important factors that should be considered in studying the role of starch in surimi gel formation.

Despite the ability of potato starch and waxy maize to produce strong, elastic gels, the gels prepared with these starches have poor freeze–thaw stability and become rubbery and rigid with extended frozen storage. Addition of unretrogradable modified starch (hydroxypropylated type) greatly improves freeze–thaw stability, but it weakens gel strength in terms of elasticity and firmness. It is, therefore, advisable to use a proper mix of unmodified and modified starch to produce a desirable balance between gel strength and freeze–thaw stability. Starch modification through cross-linking in addition to hydroxypropylation has been a commercial practice to overcome gel-weakening problem associated with use of starch that is only hydroxypropylated.

Proteins

Addition of nonmuscle proteins tends to reduce gel strength and rubberiness. Nonmuscle proteins include egg white, milk protein, soy protein, and wheat gluten. The appropriate reduction in such textural properties leads to texture modifications and often results in an improvement of sensory quality. The gel-texture-modifying effect of nonfish proteins is closely related to their water-binding properties (41,42). Egg white is most commonly used in surimi-based products. Egg white is added in a raw form (<10%) on a surimi weight basis to enhance gel strength. When dry egg white is used, an appropriate amount of water should be added to adjust the moisture content in the finished product. Another important function of egg white is its ability to make the product whiter and glossier.

Hydrocolloids (Gums)

Gums being considered for use in surimi-based seafoods include carrageenan and alginate (extracted from seaweeds) and curdlan and xanthan gum (microbial extract). Among these ι-carrageenan gum has shown a gel strength improvement effect when used in either a dry or a cold hydrated form as low as at 0.2% (dry powder) (43,44). The gel-strengthening ability of gums highly depends on their firmness and elastic properties (45).

Cross-linking Agents

Microbially produced transglutaminase is being applied in surimi to increase the gel strength. The gel-strengthening mechanism involves cross-linking of myosins through the formation of nonsulfide covalent bonds between the carboxyl group of glutamic acid and ϵ-amino group of lysine during a cold setting, which requires several hours (Seki and Kimura). Therefore, it may not be useful for a high-speed production of crab analog, but suitable for some surimi-based products that require a cold setting as part of the preparation step.

Sodium ascorbate, not ascorbic acid, increases surimi gel strength with maximum at a 0.2% level (46). Its gel-strengthening effect is believed to be from cross-linking through oxidation of sulfhydryl (-SH) groups in proteins. Despite its gel-strengthening effect, ascorbate causes the surimi gel to undergo freeze syneresis with increased expressible moisture and rubbery texture development.

IMPORTANT PROCESSING CONSIDERATIONS

There are a number of important steps and considerations in making surimi-based seafood products:

1. Use of high-quality surimi is essential in making fiberized products that require a highly elastic and resilient texture. Surimi of good quality can be readily recognizable, as its paste becomes tacky, glossy, and translucent on chopping with salt and is extruded smoothly. In contrast, surimi of poor quality produces a dull, opaque, and less tacky paste that breaks easily when extruded.
2. Selection of ingredients is determined by the formulation needs, primarily gel strength and freeze–thaw stability requirements. Type and level to be used should be carefully determined so that a desirable texture can be obtained in the finished products.
3. Water may be added so that the moisture level remains at 78 to 80%, as a maximum, in the paste. The amount of water to be added depends on the quality of the surimi, particularly its water-holding capacity. In a given surimi system, the amount of water to be added must be determined on the basis of the moisture content of surimi, the amount of starch and other water-absorbing ingredients added, and the desired texture, particularly the firmness and elasticity of the finished products.
4. Chopping must be sufficient for maximum solubilization of actomyosin, and carried out below the temperature that fish actomyosin tolerates; for example, 10°C for Alaska pollock.
5. Setting of the paste extrudate should occur slowly and to a moderate degree without surface drying. The setting time is generally controlled by the length and width of the conveyor belt. The longer the setting takes, the better the product becomes. If setting is achieved in a short time at a high temperature, the extrudate becomes less manageable for fiberization, and the texture of finished products may be less elastic and resilient. The flavor may also be thermally altered by the elevated temperature. An optimum temperature–time relationship must be determined to obtain a desirable setting condition.
6. In selecting formulation, several considerations must be made as to what the product requirements are, what form of product will be manufactured (fiberized, molded, or composite-molded), what kind of texture is required (soft-moist, rubbery, fibrous, etc), and what form of product will be marketed (refrigerated or frozen). If it is to be distributed frozen, the expected time span on the market shelf and the kind of freeze–thaw stability needed for that time span must be considered. Other considerations are the type of formulation (starch, protein, starch–protein combination, low sugar, and salt), the nutritional equivalency, and the level of ingredients to be used (low or high). These are all interrelated and the optimization of formulation would vary with each ingredient group, namely, starch, protein, or starch–protein.

BIBLIOGRAPHY

1. National Marine Fisheries Service, *Fishery Market News Report*, National Marine Fisheries Service, Washington, D.C., 1990.
2. L. de Franssu, "The World Surimi Industry Prospects for Europe," *FAO/Globefish Research Programme*, Vol. 18, FAO, Rome, 1991.
3. Jpn. Pat. 306,857 (1961), K. Nishiya.
4. K. Tamoto et al., "Studies on Freezing of Surimi and Its Application. IV. On the Effect of Sugar upon the Keeping Quality of Frozen Alaska Pollock Meat," *Bull. Hokkaido Regional Fish Res. Lab.* **23**, 50–60 (1961).
5. M. Okada, "History of Surimi Technology in Japan," in T. C. Lanier and C. M. Lee, eds., *Surimi Technology*, Marcel Dekker, New York, 1992, pp. 3–22
6. J. J. Matsumoto, "Minced Fish Technology and Its Potential for Developing Countries," in *Proceedings on Fish Utilization Technology and Marketing*, Vol. 18, Indo-Pacific Fishery Commission, Bangkok, 1978, sec. HI, p. 267.
7. K. Toyoda et al., "The Surimi Manufacturing Process," in T. C. Lanier and C. M. Lee, eds., *Surimi Technology*, Marcel Dekker, New York, 1992, pp. 79–112.
8. M. Douglas-Schwarz and C. M. Lee, "Comparison of the Thermostability of Red Hake and Alaska Pollock Surimi during Processing," *J. Food Sci.* **53**, 1347–1351 (1988).
9. K. Arai, K. Kawamura, and C. Hayashi, "The Relative Thermostabilities of the Actomyosin-ATPase from the Dorsal Muscles of Various Fish Species," *Bull. Jpn. Soc. Sci. Fish* **39**, 1077–1085 (1973).

10. C. M. Lee, "Surimi Processing from Lean Fish," in F. Shahidi and J. R. Botta, eds., *Seafoods: Chemistry, Processing Technology and Quality*, Blackie Academic & Professional, London, 1994, pp. 263–287.
11. C. M. Lee, "Surimi Manufacturing and Fabrication of Surimi-Based Products," *Food Technol.* **40**(3), 115–124 (1986).
12. H. Watanabe et al., "An Estimation of the Amount of Protein Lost in the Effluent from Frozen Surimi Manufacture," *Bull. Jpn. Soc. Sci. Fish* **48**, 869–871 (1982).
13 T. Tamoto, "Effect of Leaching on the Freezing Denaturation of Surimi Proteins," *New Food Industry* **13**, 61–69 (1971).
14. Nippon Suisan Kaisha, Ltd., *Standard Procedure for Quality Evaluation of Frozen Surimi*, Nippon Suisan Kaisha, Ltd., Tokyo, 1980.
15. National Fisheries Institute, *A Manual of Standard Methods for Measuring and Specifying the Properties of Surimi*, National Fisheries Institute, Washington, D.C., 1991.
16. C. M. Lee, "Surimi Process Technology," *Food Technol.* **38**(11), 69–80 (1984).
17. K. S. Yoon and C. M. Lee, "Effect of Powdered Cellulose on the Texture and Freeze–Thaw Stability of Surimi-Based Shellfish Analog Products," *J. Food Sci.* **55**, 87–91 (1990).
18. P. Akengo and C. M. Lee, "Assessment of Cryoprotective Effectiveness of Various Combinations of Sucrose and Sorbitol and the Manner of Incorporation," paper presented at the 30th Atlantic Fisheries Technological Conference, Boston, Mass., August 25–29, 1985.
19. T. Arakawa and S. N. Timasheff, "Stabilization of Protein Structure by Sugars," *Biochemistry* **21**, 6536–6544 (1982).
20. J. J. Matsumoto and S. F. Noguchi, "Cryostabilization of Protein in Surimi," in T. C. Lanier and C. M. Lee, eds., *Surimi Technology*, Marcel Dekker, New York, 1992, pp. 357–388.
21. K. Iwata et al., "Influences of Storage Temperatures on Quality of Frozen Alaska Pollock Surimi," *Reigto (Refrigeration)* **43**, 1145–1150 (1968).
22. K. Iwata et al., "Study of the Quality of Frozen Stored Alaska Pollock Surimi. I. The Influence of Freshness of the Material and Changes in Storage Temperature," *Bull. Jpn. Soc. Sci. Fish* **37**, 626–633 (1971)
23. M. Okada et al., "Influences of Transportation on the Quality of Frozen Alaska Pollock Surimi," *Reito (Refrigeration)* **43**, 1270–1277 (1968).
24. E. Niwa and M Miyake, "Physico-Chemical Behavior of Fish Meat Proteins. I. Behavior of Polypeptide Chains of Proteins during Setting of Fish Meat Paste," *Bull. Jpn. Soc. Sci. Fish* **37**, 877–883 (1971).
25 E Niwa, Y. Matsubara, and I. Hamada, "Hydrogen and Other Polar Bondings in Fish Flesh Gel and Setting Gel," *Bull. Jpn. Soc. Sci. Fish* **48**, 667–670 (1982)
26. Y. Shimizu, R. Machida, and S. Takenami, "Species Variations in the Gel-Forming Characteristics of Fish Meat Paste," *Bull. Jpn Soc. Sci. Fish* **47**, 95–104 (1981).
27 Y Makinodan and S Ikeda, "Studies on Fish Muscle Protease. IV Relationship between Himodori of Kamaboko and Muscle Protease," *Bull. Jpn. Soc. Sci. Fish* **37**, 518–523 (1971).
28. C. S. Cheng, D. D Hammann, and N. B. Webb, "Effect of Thermal Processing on Minced Fish Gel Texture," *J. Food Sci.* **44**, 1080–1086 (1979).
29. T. C. Lanier et al., "Effects of Alkaline Protease in Minced Fish on Texture of Heat-Processed Gels," *J. Food Sci* **46**, 1643–1645 (1981).
30. K. Iwata, K. Kobashi, and J. Hase, "Studies on Muscle Alkaline Protease. H. Sime Enzymatic Properties of Carp Muscular Alkaline Protease," *Bull. Jpn. Soc. Sci. Fish* **40**, 189–200 (1974).
31. C. M. Lee, "Differentiation between Enzymatic and Thermal Effects on Textural Modification in Fish Gels," paper presented at the 43rd Annual Meeting of the Institute of Food Technologists, New Orleans, La., June 19–22, 1983.
32. E. Niwa, "Functional Aspect of Surimi," in R. E Martin and R. L. Collete, eds., *Proceedings of the International Symposium on Engineered Seafood-Including Surimi*, National Fisheries Institute, Washington, D C., 1985, pp. 136–142.
33. J. C. Deng, R. T. Toledo, and D. A. Lillard, "The Effect of Temperature and pH on Protein–Protein Interactions in Actomyosin Solutions," *J. Food Sci.* **41**, 273–277 (1976).
34. C. M. Lee and R. T. Toledo, "Factors Affecting Textural Characteristics of Cooked Comminuted Fish Muscle," *J. Food Sci.* **41**, 391–397 (1976).
35. I. Kimura, T. Murozuka, and K. Arai, "Comparative Studies on Biochemical Properties of Myosins from Frozen Muscle of Marine Fishes," *Bull. Jpn. Soc. Sci. Fish* **43**, 315–321 (1977).
36. M. Okada, "Effect of Washing on the Jelly Forming Ability of Fish Meat," *Bull. Jpn. Soc. Sci. Fish* **30**, 255–261 (1964).
37. Y. Shimizu and F. Nishioka, "Interactions between Horse Mackerel Actomyosin and Sarcoplasmic Proteins during Heat Coagulation," *Bull. Jpn. Soc. Sci. Fish* **40**, 231–234 (1974).
38. R. Hamm, "Functional Properties of the Myofibrillar System and Their Measurements," in P. J. Bechtel, ed., *Muscle as Food*, Academic, Orlando, Fla., 1986, pp. 135–199.
39. C. M. Lee and A. Abdollahi, "Effect of Physical Properties of Plastic Fat on Structure and Material Properties of Fish Protein Gels," *J. Food Sci.* **46**, 1755–1759 (1981).
40. J. M. Kim and C. M. Lee, "Effect of Starch on Textural Properties of Surimi Gel," *J. Food Sci.* **52**, 722–725 (1987).
41. K. H. Chung and C. M. Lee, "Relationship between Physio-Chemical Properties of Nonfish Protein and Textural Properties of Protein-Incorporated Surimi Gel," *J. Food Sci.* **55**, 972–975 (1990).
42. C. M. Lee and K. H. Chung, "The Role of Hydrodynamic Properties of Biopolymers in Texture Strengthening/Modification and Freeze–Thaw Stabilizing of Surimi Gel," in M. N. Voigt and R. Botta, eds., *Advances in Fisheries, Technology and Biotechnology for Increased Profitability*, Technomic Publishing, Lancaster, Pa., 1990, pp 397–412.
43. C. W. Bullens et al., "The Function of Carrageenan-Based Stabilizers to Improve Quality of Fabricated Seafood Products," in M. N. Voigt and R. Botta, eds., *Advances in Fisheries, Technology and Biotechnology for Increased Profitability*, Technomic Publishing, Lancaster, Pa., 1990, pp. 313–323
44. I. Filipi and C. M. Lee, "Preactivated Iota-carrageenan and Its Rheological Effects in Composite Surimi Gel," *Lebensm.-Wiss. u.-Technol.* **31**, 129–137 (1998).
45. C. M. Lee, "Relationships of Rheological and Hydrodynamic Properties of Biopolymeric Ingredients to the Composite Surimi Gel Texture," in T. Yano, R. Matsuno and K. Nakamura, eds., *Developments in Food Engineering—Part 1*, Blackie Academic & Professional, London, 1994, pp. 99–101.
46. H. G. Lee et al., "Sodium Ascorbate Affects Surimi Gel-Forming Properties," *J. Food Sci.* **57**, 1343–1347 (1992).

CHONG M. LEE
University of Rhode Island
Kingston, Rhode Island

SWEETENERS: NUTRITIVE

Sweeteners are used to satisfy the inborn human desire for sweet taste. They also make sour and bitter foods more palatable. And the functionalities non-high-intensity sweeteners impart to prepared foods is often as important as, or more important than, their taste characteristics. Each sweetener, either natural or synthetic, has specific properties and therefore specific applications and limitations. For this reason and because the demand and hence the market for sweeteners is so great, many different sweeteners have been developed, are available, and are used.

Those sweeteners that provide calories to humans are categorized as nutritive sweeteners (1); those that do not are nonnutritive sweeteners (see the article SWEETENERS: NONNUTRITIVE). Nutritive sweeteners are covered in this article with one exception. Peptide sweeteners such as aspartame (APM) are classified as nutritive by the U.S. Food and Drug Administration because they, like other peptides and proteins, contribute approximately the same number of calories per gram as do carbohydrates. However, because they are high-intensity sweeteners, they need only be used in small amounts and therefore contribute an insignificant number of calories to the products in which they are used; so in this work, they are considered as nonnutritive sweeteners.

Table 1 lists the nutritive sweeteners available for food products. Relative amounts used depend on the country and/or region and are a function of the food type, such as bakery product, beverage, or canned fruit. In the remainder of this article, the term sweetener refers to the sweeteners in Table 1, that is, nutritive sweeteners as defined previously.

Table 1. Non-High-Intensity, Nutritive Sweeteners

Naturally occurring	
Sucrose	Maple syrup
Honey	
Derived from starch	
Glucose (corn) syrups	Glucose (dextrose)
High-fructosee syrups	Fructose (levulose)
Corn syrup solids	Reduced corn syrups
Maltodextrins	Trehalose
Polyhydric alcohols	
Sorbitol	Reduced corn syrups
Mannitol	Erythritol
Xylitol	
Derived from sucrose	
Invent sugar	
Byproduct of sucrose refining	
Molasses	
Derived from inulin	
Fructose	Inulin syrups

In the United States, sucrose (ca 45%), glucose (corn) syrups (ca 13%), high-fructose syrups (HFCS) (ca 40%), and glucose (dextrose) (ca 2.5%) account for 99% of total sweetener use (2). About 40 billion lb (20×10^9 kg) of these products are produced and used in beverages (ca 36%), pharmaceuticals and miscellaneous products (ca 17%), confections (ca 12.5%), bakery products (ca 10%), breakfast cereals (ca 8.5%), dairy products (ca 8%), and preserved fruits and vegetables (ca 8%) (2). (About two-thirds of the sugar used to make alcoholic beverages and bakery products is not actually consumed by humans but used up in fermentation.)

Liquid sweeteners are usually analyzed for and graded in terms of color, clarity, concentration/solids content, pH, sulfur dioxide content, presence of other dissolved solids, and viscosity. If they are produced by hydrolysis of starch, that is, if they are glucose (corn) syrups, the dextrose equivalency (DE) and saccharide distribution is also determined and reported. Crystalline sweeteners are graded by particle size. They are dissolved and analyzed as the liquids are.

In baked goods, sweeteners provide sweetness, texture, and humectancy (1). Providing sweetness is most important in sweet breads, sweet rolls, doughnuts, cakes, and cookies. Sweeteners provide the fermentable substrate for yeast-leavened products. Sweeteners also reduce/retard starch gelatinization (completely in some cases) (see the article STARCH) and gluten development. Moisture content/retention is key to texture (soft and moist vs crisp, for example). The primary determinant of texture is the glass transition temperatures of the ingredients (especially starch, sugar, or both), and the primary determinant of the glass transition temperature is the moisture content. By raising the sugar concentration, the glass transition temperature of wheat starch granules is raised and the degree of starch gelatinization at baking temperatures is reduced. The degree to which this occurs is also a function of the type of sweetener used.

About 55% of the sweetener used to make bakery products is sucrose. Sucrose provides sweetness, bulk, flavor enhancement, texture/crumb structure (by influencing starch gelatinization and gluten development), shelf life, and the fermentable substrate for yeast-leavened products. HFCS, which accounts for ca 25% of the total sweetener used in bakery products, can often replace sucrose, except in certain cookies where crispness results from low moisture content and the glassy state of sucrose. Glucose (corn) syrups (ca 11%) provide moisture retention, freeze-thaw stability, and dough viscosity and control sugar crystallization. Glucose (dextrose) is the most efficient sweetener in terms of promoting browning, that is, crust color development.

Sucrose is also the most important ingredient in confections, in which it is usually present in either a crystalline or glassy state or a mixture of the two states. Crystals are often an important contributor to texture, and thus crystal size is important. Glucose (corn) syrup is often used to prevent crystallization so that the product is a glassy solid. Carbohydrate sweeteners also function as humectants.

Other applications of sweeteners are in the preparation of jams, jellies, preserves, and marmalades; in beverages; in frozen desserts; in the preservation of canned fruits; and in the preparation of breakfast cereals.

SUCROSE

Sucrose may be obtained from different sources, primarily from sugarcane and sugar beet, and is available in a variety of forms. For example, maple syrup is a solution of sucrose (58–66%) and less than 10% of other constituents. Because of its overall dominance worldwide as a sweetener, sucrose is presented separately (see the article SUGAR: SUCROSE).

FRUCTOSE

D-Fructose (levulose) is available in both crystalline and solution forms and is the sweetest (on a weight basis) and most soluble of the natural sugars (1–3). A 5 to 15% aqueous solution of D-fructose at room temperature is about 1.15 to 1.25 times as sweet as an equal concentration of sucrose. (Actual relative sweetness is a function of temperature, pH, and concentration.) In its pure crystalline form, β-D-fructopyranose (see Fig. 1), fructose has 1.8 times the sweetness of sucrose on an equal-weight basis; but few, if any, foods are prepared with crystalline fructose as the sole sweetener. Fructose dissolves rapidly and has a rapidly forming and intense sweet taste. Its sweetness perception dissipates before that of sucrose peaks. This makes fructose an ideal sweetener for products such as sorbets in which lingering sweetness is not desired. Fructose intensifies perceived flavor and acid tastes, that is, both flavor and acid levels can be reduced when products such as puddings, dessert gels, pie fillings, and beverages are formulated with fructose. A blend of sucrose and fructose is sweeter than the sweetness of either sugar used alone (3).

As both fructose itself and sucrose–fructose blends are sweeter than sucrose alone, less of them is needed for the same degree of sweetness. Fructose and high-fructose syrups are used, therefore, in reduced-calorie foods and beverages, and because fructose is sweeter than sucrose, use of crystalline fructose, usually in combination with granulated sugar, in sweetened, dry-mix fruit drinks reduces package size and weight and shipping costs.

Because of its water-binding capacity, which is greater than that of other sugars, fructose can be used as a humectant in baked goods (where it reduces staling) and in jams, jellies, pet foods, and certain confections. Crystalline fructose can also reduce the water activity of intermediate-moisture foods such as granola bars and fruit products and thus can act as a preservative, but because of its hygroscopic nature, it requires special handling and storage conditions (3). In addition, higher-solids solutions can be prepared with fructose than with sucrose (83% vs 71% at 30°C [86°F], 90% vs 76% at 60°C [140°F]). When used with food starches, fructose provides more rapid development of vis-

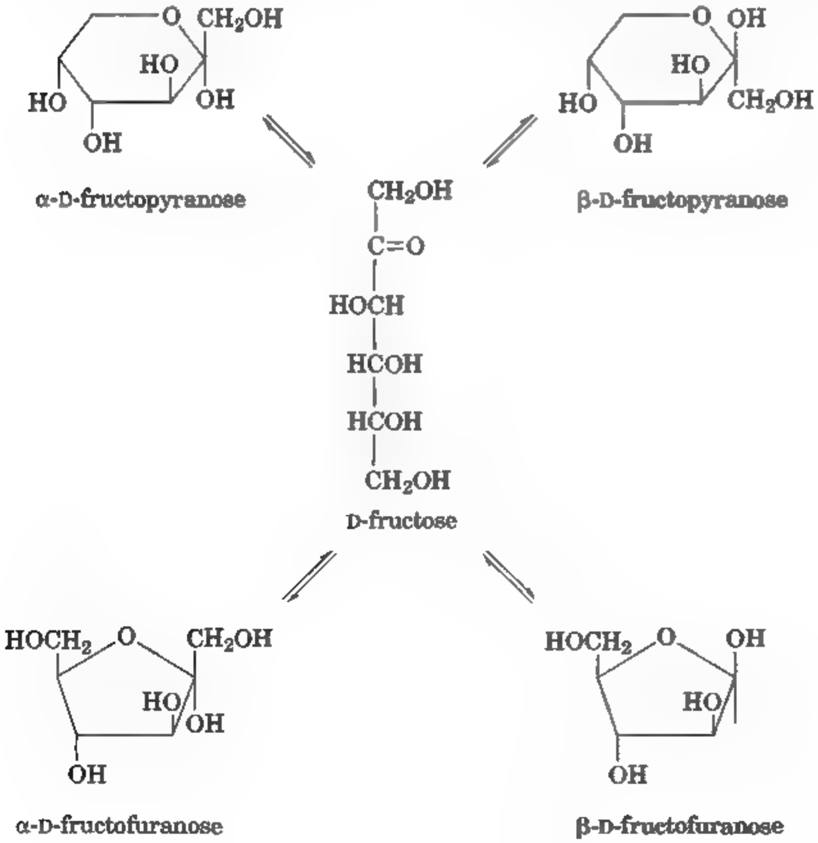


Figure 1.

cosity and increased final gel strength, as compared to sucrose on an equal-weight basis.

Fructose is less glucogenic than is glucose or sucrose, so it has been recommended as a replacement for those sweeteners in the diets of diabetic and obese persons. However, fructose is more lipogenic than is glucose or starches and can cause increases in blood pressure, uric acid, and lactic acid in susceptible segments of the population (4).

In Europe small amounts of inulin syrup, which is primarily fructose with some fructooligosaccharides, is made and used. The primary source of the inulin is chicory. Jerusalem artichoke is an alternative source.

GLUCOSE

D-glucose (dextrose) occurs naturally in low concentrations in many fruits and other plant tissues (Table 2). Glucose used in processed food formulations is produced by enzyme-catalyzed hydrolysis of starch, usually to produce dextrose syrups of DE 95 or DE 99; see the section "Glucose (Corn) Syrups." Crystalline products of DE 93 to DE 99 are available in various granulations. Solutions of glucose (2–50%) are 0.50 to 0.90 times as sweet as those of equal concentrations of sucrose. Its negative heat of solution effects a cool sensation that enhances fruit flavors. It promotes browning. Glucose is available commercially as crystalline anhydrous dextrose and dextrose monohydrate (ca 8.5% water) and in solution, microcrystalline, and agglomerated forms.

Glucose is used in a wide variety of products, particularly in the confectionery industry in jellies, gums, marshmallows, pressed products, panned products, chocolate, and compound coatings; as a bulking agent for high-intensity sweeteners; as a coating for dried fruits such as raisins, dates, apricots, and pears; as a binder in tabletted confections; and as a carrier for liquid flavors and oils. It is used to improve product density in some cakes and cookies.

GLUCOSE (CORN) SYRUPS

Syrups are produced from starch by enzyme-catalyzed hydrolysis or a combination of acid-catalyzed and enzyme-catalyzed hydrolyses (see the section on "Fructose-Glucose Syrups," also see the article CARBOHYDRATES: CLASSIFICATION, CHEMISTRY, LABELING). In the United States, where these syrups are prepared from corn starch, they are known as corn syrups. In Europe, where they may be made from potato or wheat starch, in addition to corn starch, they are known as glucose syrups.

Glucose syrups are described primarily by the term dextrose equivalence (DE), which is defined as the percent reducing sugar calculated as glucose on a dry-weight basis and is an indicator of the degree of hydrolysis. Glucose has a DE value of 100 and starch a DE value of 0. Corn syrups (also labeled corn sweetener) with DE values of from ca 24 to at least 80 are available, those with DE values of 42 and 62 being the most common types. All are colorless, viscous liquids of 74 to 85% solids. Those with the lower DE values have the highest-average-molecular-weight saccharides and those with the higher DE values contain the lowest-average-molecular-weight saccharides (glucose, maltose, etc). Low-DE syrups are the least hygroscopic and impart less stickiness but bind water, preventing its loss. High-DE syrups are the most hygroscopic and the sweetest. A DE 24 syrup contains ca 5% glucose, 6% maltose, 11% maltotriose, and 78% oligosaccharides with degrees of polymerization greater than 3. A DE 80 syrup contains ca 56% glucose.

Corn syrups provide sweetening and viscosity, control humectancy and water activity, give surface glaze, and impart desirable texture to a wide variety of foods, beverages, confectionery, and bakery products, including canned and frozen desserts, meat products, peanut butter, pickles, salad dressings, table syrups, and pet foods.

Syrups can also be described by their saccharide composition (6,7). High-maltose syrups can contain 28 to 65% maltose and little glucose. They provide reduced browning and hygroscopicity when compared with a glucose syrup of equivalent DE. Maltose, or malt sugar, is a disaccharide. It is created in yeast doughs by the action of enzymes on the starch or flour. Barley malt, in which barley starch has been broken down to a high percentage of maltose during germination of the grain, is used as an adjunct in brewing to enhance the color and flavor of beer. Products containing maltose produced by malting or by the action of yeast have a distinctive flavor.

FRUCTOSE–GLUCOSE SYRUPS

Fructose and glucose occur together in HFCS (known outside the United States as high fructose [HF] and isoglucose syrups), invert sugar, honey, and certain fruits and vegetables. HFCS is made from starch by the action of combinations of enzymes (8,9). First, a mixture of α-amylase and glucoamylase, and often a debranching enzyme (pullulan-

Table 2. Sweetener Composition of Some Fruits

	Apple	Grape	Peach	Strawberry
Glucose	2.03 (1.40–2.35)	7.18 (3.98–9.05)	1.03 (0.72–1.50)	2.17 (1.90–2.33)
Fructose	5.74 (4.80–6.40)	7.44 (3.86–9.30)	1.23 (0.86–1.80)	2.30 (2.13–2.40)
Sucrose	2.55 (0.54–2.78)	0.43 (0.18–1.61)	5.73 (4.50–6.80)	1.00 (0.08–1.45)
Sorbitol	0.51 (0.51–0.58)	0.20	0.89 (0.50–0.90)	0.32
Xylitol				0.28

Note. Percent of fresh weight. mean (range).
Source: Ref. 5.

ase or isoamylase [amylo-1,6-glucosidase]) is used to convert native or acid-thinned starch to a solution that is as close to 100% D-glucose as can be obtained (6,7,10). A heat-stable α-amylase is generally used for the initial breakdown (liquefaction) of a native starch paste, which is performed at 80 to 95°C (185–205°F). The resulting glucose solution can be concentrated to a syrup and is the source of crystalline glucose (6,7).

The glucose solution is then treated with an immobilized enzyme commonly known as glucose isomerase (6,7) to convert a portion of the glucose into fructose, that is, to achieve thermodynamic equlibrium. An HFCS containing 42% fructose, 53% glucose, and 5% maltooligosaccharides is prepared in this way. Using an industrial-scale chromatographic separation, fructose pure enough for crystallization (ca 90%) is obtained. By blending a 42% fructose solution with a 90% fructose solution, a 55% fructose (41% glucose and 5% maltooligosaccharides) solution is obtained. In the U.S. soft drink industry, 55% fructose syrup, designated HFCS-55 (HF-55), is the main nutritive sweetener used; some 42% fructose syrups are also used. HFCS-55 has a sweetness ca 1.2 times that of sucrose; therefore, less HFCS, as compared to sucrose, is required to give equivalent sweetness, resulting in products with fewer calories. HFCS is used in many other food, confectionery, and beverage products, including soft cookies, where its humectancy and noncrystallizability are important; other baked goods; salad dressings; pickle products; catsup; table syrups; canned and frozen fruits; desserts; soft chewy candies; and granola bars. HFCS is easy to blend, store, and ship.

INVERT SUGAR

True invert sugar is a 50:50 mixture of glucose and fructose produced by cleavage of the glycosidic linkage of sucrose (see the article SUGAR: SUCROSE) with dilute acid or the enzyme invertase. It too is sweeter than sucrose. Invert sugar and HFCS-50 (HF-50) are identical in composition and properties. Invert sugar acts as a humectant and a water binder and promotes nonenzymic browning. However, although an invert sugar syrup composed predominately of invert sugar can be obtained, more commonly used is a sugar solution with only a portion of the sucrose hydrolyzed. Invert sugar is used in the confectionery industry to control or prevent crystallization of sucrose. In the presence of the proper amount of invert sugar, only small crystals of sucrose that are below the perceptual threshold of the mouth form.

Inversion, a term used to denote the hydrolysis of sucrose to glucose plus fructose, often occurs during processing. Deliberate postprocessing conversion is used, for example, in producing chocolate-covered cherries, where invertase in the fondant acts on sucrose after the product is enrobed with chocolate, turning the fondant into a liquid syrup that has a reduced tendency to undergo fermentation. (Invert sugar is also available in a creamy fondant form.) Inadvertent inversion caused by heating too long or at too high a temperature or in the presence of acid can negatively effect stickiness, graininess, color formation, and shelf life.

Invert sugar can be used in creme centers, fudge, marshmallows, caramels, chewy candies, nougats, and jellies. It can be added to the ingredient blend but is often formed during candy manufacture.

HONEY AND MOLASSES

Honey and molasses are used primarily to impart their flavors. Honey contains about 38% fructose and 31% glucose. In addition to providing its unique flavor and humectancy, honey can enhance spicy or fruity flavors in such products as gum and fruit jelly candies and mint creams. It is a more costly ingredient than HFCS, sucrose, or invert sugar.

Molasses, a by-product of cane sugar refining, also contains a high percentage of invert sugar. Molasses is available in several grades. Lighter-colored molasses are generally used in foods and confectioneries requiring long cooking times. Darker-colored molasses are generally added at the end of processing to products requiring its robust flavor. Both honey and molasses are available in liquid and dry powder forms.

CORN SYRUP SOLIDS

Corn syrup solids, those products in the approximate range of DE 20 to DE 36, are produced by less breakdown of starch than occurs during the manufacture of corn syrups. They have less reducing power and hence a lesser tendency to brown, only slight sweetness, and low to moderate hygroscopicity. They are used as binders and crystallization inhibitors; to impart a chewy texture and improved mouthfeel and flavor; and to provide easy digestibility and control of sweetness in such products as infant formulas, chocolate drinks, coffee whiteners, whipped toppings, imitation sour creams, frozen foods, prepared meats, and granola bars. In frozen novelties, corn syrup solids provide viscosity, an increased freezing point, controlled water crystallization, more body, slower meltdown, better stability, and a smoother texture.

MALTODEXTRINS

Maltodextrins have DE values from ca 5 to ca 15 and are even less sweet than corn syrup solids. They find application because of their quick dispersion, rapid solubility, film-forming ability, low hygroscopicity, crystallization-inhibitor property, ability to impart body, low browning potential, low osmolality, resistance to caking, flavor-carrying ability, good binding power, fat dispersibility, textural effects, and ability to provide clean flavor in food products such as baked goods (cream-type fillings, glazes, frostings), powdered mixes (soups, salad dressings, sauces, flavors, spice blends), frozen foods and novelties, meat spreads, sauces (white, cheese, pizza), salad dressings, imitation cheeses and sour creams, confectioneries (chewy candies, hard candies, pan coatings, nut and snack coatings), sports beverages, and infant formulas. A key to their use in powdered mixes is their ability to be spray-dried and to produce agglomerated products. Because of their high solubility and low hygroscopicity and their ability to form complexes with some hydrophobic substances, maltodextrins are often used as carriers, bulking agents, or both. Special crystalline maltodextrins find use as fat replacers.

LACTOSE

Lactose (see the article LACTOSE) is only slightly sweet and is used where only low sweetness is desired, for its aroma- and flavor-binding properties, or for a combination of these qualities.

TREHALOSE

Trehalose, a naturally occurring disaccharide of D-glucosyl units that can be produced from starch enzymically, has ca 45% of the sweetness of sucrose and low hygroscopicity. It is noncariogenic and does not contribute to browning. It is reported to be the most effective sugar in protecting proteins against changes effected by drying or freezing and in reducing the rate of starch retrogradation. It is approved for use as a food ingredient in Japan.

SUGAR ALCOHOLS

Alditols (also known as sugar alcohols, polyhydric alcohols, and polyols) with a sweet taste include glucitol (sorbitol), mannitol, xylitol, erythritol, and hydrogenated corn syrups, especially high-DE and high-maltose syrups.

Sorbitol, the most widely used alditol, is one-half to two-thirds as sweet as sucrose, depending on concentration and temperature. It is available in crystalline form and as a 70% solution. Sorbitol can be used as a humectant. It is used in special dietary foods, breath mints, cough drops, hard and soft candies, and chewing gum. Its negative heat of solution provides a cooling effect. Excessive consumption (>50–80 g/day) may have a laxative effect.

Mannitol is also about two-thirds as sweet as sucrose. It is used in sugarless chewing gum as a dusting agent because of its low solubility and low hygroscopicity. It is also used to a limited extent as a bulking agent in powdered foods.

Xylitol has about the same sweetness as sucrose. Commercial xylitol is produced by reduction of D-xylose obtained from corncobs, sugarcane bagasse, birch wood, or a combination of these. Of all nutritive sweeteners, xylitol has the largest negative heat of solution and provides the greatest cooling effect. Xylitol is used in sugarless chewing gum and mints, other confectioneries, dietetic foods, and foods for diabetics.

Erythritol is the newest alditol to be introduced. Like other alditols, it is classified as a nutritive sweetener because it does have some caloric value, although it is less than 5% that of sucrose. The sweetness of a 10% solution is 50 to 60% that of sucrose at the same concentration. Crystalline erythritol has a strong cooling effect. When used with high-intensity sweeteners, the sweet taste is improved. It is proposed for use in chewing gum as a softener that extends shelf life.

Sorbitol, mannitol, xylitol, and erythritol all have good heat stability, resistance to thermal and nonenzymic browning, and a low tendency to undergo fermentation with the production of acids and are considered to be noncariogenic. There is evidence that xylitol may actually be effective in reducing the number of new dental caries (11). All sugar alcohols have about the same caloric content as sucrose but are absorbed more slowly from the digestive tract and do not raise postprandial blood sugar and insulin levels; thus they are suitable for the diets of diabetics.

Chemical reduction (hydrogenation) of syrups, especially high-maltose syrups, produces products available as viscous 75% solutions with a sweetness about three-fourths that of sucrose. These syrups, like regular glucose syrups, do not crystallize, prevent crystallization of other ingredients, and impart hygroscopicity. They can be used to prepare sugarless chewing gum and hard, chewy, fondant, and jellied candies. Crystalline maltitol is produced and used in Japan.

Lactitol, the reduced form of lactose, is about 40% as sweet as sucrose; has a clean, sweet taste; is produced in the Netherlands; and is approved for food use in the United Kingdom as a sweetener for dietetic foods and foods for diabetics.

BIBLIOGRAPHY

1. R. J. Alexander, *Sweeteners: Nutritive*, Eagen Press, St. Paul, Minn., 1998.
2. R. J. Alexander, "Carbohydrate Sweeteners," *Cereal Foods World* **42**, 420 (1997).
3. "Crystalline Fructose: A Breakthrough in Corn Sweetener Process Technology," *Food Technol.* **41**, 66, 67, 72 (1987).
4. J. Hallfrisch, "Metabolic Effects of Dietary Fructose," *FASEB J.* **4**, 2652–2660 (1990).
5. H. Scherz and F. Senser, eds., *Food Composition and Nutrition Tables*, 5th ed., CRC Press, Boca Raton, Fla., 1994.
6. N. E. Loyd and W. J. Nelson, "Glucose- and Fructose-containing Sweeteners from Starch," in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds., *Starch: Chemistry and Technology*, 2nd ed., Academic Press, Orlando, Fla., 1984, pp. 611–660.
7. G. M. A. van Beynum and J. A. Roels, eds., *Starch Conversion Technology*, Marcel Dekker, New York, 1985.
8. R. Bernetti, "From Corn Syrup to Fructose," *Cereal Foods World* **35**, 390 (1990).
9. J. P. Casey, "High Fructose Corn Syrup: A Case History of Innovation," *Starch* **29**, 196 (1977).
10. Amylose Research Society of Japan, *Handbook of Amylases and Related Enzymes*, Pergamon Press, Oxford, 1988.
11. A. Bar, "Caries Prevention with Xylitol," *World Rev. Nutrition and Diet* **55**, 183–209 (1988).

J. N. BEMILLER
Purdue University
West Lafayette, Indiana

SWEETENERS: NONNUTRITIVE

The sense of sweet taste has directed people, as well as many types of animals, insects and probably even microorganisms, to nutritive substances since early in time. Although it is almost certain that the correlation of sweet taste with nutrition was noted earlier, it appears the first written record of this recognition was made by Aristotle in 330 B.C. (1). He speculated that the taste system could be subdivided into the basic taste qualities of sweet, bitter, sour, salty, astringent, pungent, and harsh and that it was the function of this sensory system to direct organisms to sources of nutrition. In modern times, at least for most of the western hemisphere, adequate nourishment has not been an issue. On the contrary, 20th-century westerners generally tend to consume excess calories. Obesity is very common and people are increasingly developing illnesses that are at least partly attributed to excess calorie consumption (eg, cardiovascular disease, diabetes, and cancer), although decreased physical activity is also a factor.

Consumers are very interested in low calorie food and beverage products. Such products require low or zero calorie replacements for full-calorie ingredients. Significant contributors to the caloric content of many foods are sucrose and other nutritive carbohydrate sweeteners. The discovery of the potent sweet taste of saccharin by Fahlberg in 1878 made possible, for the first time, the formulation of zero- or low-calorie equivalents to full calorie food products (2). Since 1878, chemists have synthesized, or identified from nature, thousands of novel, potently sweet organic compounds. Many of these sweeteners are substantially better than saccharin in taste quality. Saccharin's sweetness is limited by the presence of bitter and metallic off tastes.

The sweetener discovery of greatest commercial significance is that of aspartame, a peptide-type sweetener that was serendipitously discovered in 1965 by Schlatter, a chemist at G.D. Searle & Company (3). Aspartame's sweetness is not limited by off-tastes and as such allowed, for the first time, formulation of many low-calorie food products with minimal compromise in taste. Because aspartame so closely mimics sucrose taste, it is not surprising that aspartame has revolutionized the low-calorie food and beverage industry. It must be pointed out that aspartame, because of its natural amino acid content, is a nutritive rather than nonnutritive sweetener. Aspartame is metabolized to provide energy proportional to the amount ingested. However, since about 200 g of sucrose may be replaced by 1 g of aspartame, it functions, in effect, as a nonnutritive sweetener.

Aspartame, saccharin, and other sweeteners that are substantially more potent than sucrose are generally referred to as high-potency sweeteners. These sweeteners are often erroneously called intense or high-intensity sweeteners. This latter terminology is confusing and therefore not recommended because no sweet substances are known to exhibit a higher intensity of sweetness than is possible with sucrose. Occasionally, high-potency sweeteners are referred to as artificial sweeteners. This term is also inaccurate because sweet taste from any source is real and not artificial. It is recommended that sweeteners be referred to as nutritive, nonnutritive, or high-potency, as accurately describes the particular substance. This review will focus on nonnutritive and low-calorie sweeteners, among which sweetness potencies vary from less than 1 to more than 10,000 times that of sucrose.

In some food systems, such as confections, frozen desserts, and baked goods, acceptable products cannot be prepared by simply replacing sugar with high-potency sweeteners. In these systems, sucrose and other nutritive carbohydrate sweeteners provide functionality beyond sweet taste. In confections, sucrose may provide nearly 100% of the product bulk, and in baked goods, the contribution to product bulk is important but somewhat less so. In some baked goods, sucrose is a substrate for yeast because the action of yeast is essential for development of the airy texture. Sucrose also provides, following hydrolysis, the necessary reducing sugar needed for Maillard browning of baked goods. In frozen desserts, sucrose performs the key function of freezing-point depression. Without sucrose or other sugars, frozen desserts are not malleable. Thus, for many foods, it is not possible to prepare low-calorie alternatives with high-potency sweeteners. Necessary for the formulation of such food products are nonnutritive sweeteners that are one-for-one substitutions for sucrose. These sweeteners are often referred to as sugar macronutrient substitutes or sugar MNSs.

This article begins with a discussion of the properties required for commercial viability of nonnutritive sweeteners. The nonnutritive sweeteners of broadest use in the food industry today are saccharin salts, cyclamate salts, aspartame, and acesulfame-K. Two sweeteners recently approved in many countries and that may experience significant use in the future are sucralose and alitame. These six sweeteners are discussed in detail. Five nonnutritive sweeteners that have experienced limited application in foods are neohesperidin dihydrochalcone, thaumatin, glycyrrhizin, the stevia sweeteners (stevioside, rebaudioside A, etc) and Lo Han Kuo sweetener (mogroside). Short summaries of these sweeteners are given. These 11 sweeteners are all high-potency sweeteners. Five sugar MNSs that are being used in foods are the polyols maltitol, lactitol, isomalt, fructooligosaccharide sweetener, and erythritol. These sweeteners are also discussed. Finally, the article concludes with a summary of the most significant recent developments in the field of nonnutritive sweeteners.

REQUIREMENTS FOR COMMERCIAL VIABILITY OF NONNUTRITIVE SWEETENERS

Taste Quality

The number of food products sweetened with nonnutritive sweeteners has increased dramatically since the 1960s. The most successful of these products are beverages, especially carbonated soft drinks (CSDs). Good tasting zero- or near-zero-calorie CSDs are now available as alternatives to the sucrose and high-fructose corn syrup sweetened products that have approximately 150 calories per 12-oz serving. This commercial success only occurred, however, once good-tasting noncaloric sweeteners approaching the quality of sucrose were identified. Consumers have never shown any significant willingness to sacrifice taste quality in their choices of food products. Helgren of Abbott Laboratories made a key discovery in 1957, that greatly facilitated the 1960s burst of growth in low-calorie foods (4). He found that the combination of sodium saccharin with sodium or calcium cyclamate, in a ratio such that each sweetener contributed equal sweetness to the mixture, resulted in a product with improved taste quality relative to either sweetener taken separately. This blend was one of approximately 10 parts cyclamate salt to 1 part of the saccharin salt, since the latter is roughly 10 times more potent than the former. Cyclamate and especially saccharin salts exhibit off-tastes that are often described as bitter and metallic. As a result of these off-tastes, neither saccharin- nor cyclamate-sweetened products approach the taste quality of sugar-sweetened products. Helgren found, however, that these off-tastes are substantially ameliorated in the 10:1 cyclamate/saccharin blend. In fact, the improvement was so significant that, for the first time, zero- and low-calorie alternatives to many food and beverage products were possible without major compromise in taste.

The taste quality of a sweetener is really only meaningful in the context of a food product, and the taste quality of a food product is best assessed by consumer studies. However, less resource-intensive techniques are often used to predict taste quality. Flavor profile analysis (FPA) is such a technique (5). In FPA, pioneered at the Arthur D. Little Company in the 1940s, expert sensory panels are trained to break down complex and multiple-flavor-attribute systems and to rate component intensities. The FPA technique has also been used to assess the taste attributes of sweeteners. As examples, the flavor profiles of saccharin and cyclamate are illustrated in Figure 1. These data, as well as most other quantitative sensory data provided in this review, were generated by an expert sensory panel of The NutraSweet Company. The sensory studies were conducted according to the methodology described in the work by Carr et al. (6).

Some nonnutritive sweeteners are quite similar to sucrose in flavor profile. One might expect that such sweeteners would make possible diet products equivalent in taste to sucrose-sweetened products. However, this is not the case. One factor contributing to the difference in taste between the nonnutritive-sweetener- and sucrose-sweetened products is sweetener temporal profile (7). DuBois et al. have comprehensively studied this effect in the flavonoid glycoside class of sweeteners, the best-known member of which is neohesperidin dihydrochalcone (8). In the case of this sweetener, 9 s are required to reach the sweetness intensity maximum for a solution *iso*-sweet with 10% sucrose. This time has been defined as the sweet-taste appearance time (AT). For comparison, 10% sucrose exhibits an AT of 4 s. In addition to a delayed AT, neohesperidin dihydrochalcone shows a prolonged sweet aftertaste. This effect has been defined as the time required for the perceived sweetness intensity to decline from a 10% sucrose equivalent to the low, but greater than threshold, 2% sucrose equivalent. This time has been defined as the extinction time (ET). Neohesperidin dihydrochalcone exhibits an ET of 40 s in comparison to 14 s for *iso*-sweet 10% sucrose. These temporal effects combine to cause neohesperidin dihydrochalcone to be a substantially less acceptable sweetener than sucrose in most food systems. Thus, it is not sufficient to know only a sweetener's flavor profile to predict viability. The temporal profile must also be known. Ultimately, of course, the taste quality of a sweetener may be appreciated only after consumer acceptability studies in the food category of interest.

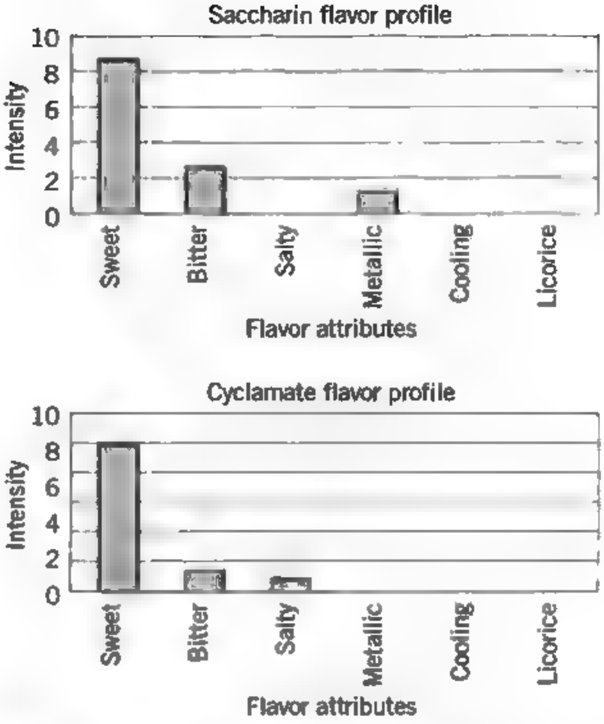


Figure 1. FPA of sodium saccharin (384 ppm) and sodium cyclamate (5930 ppm) in water.

Safety

In the United States, the use of sweeteners is regulated by the 1958 Food Additives Amendment to the Food, Drug and Cosmetic Act of 1938. This legislation and its effects on the regulation of sweeteners and other food additives has been reviewed (9,10). By this act, saccharin and cyclamates were exempted as generally recognized as safe (GRAS) food ingredients. Not all sweeteners are included on the GRAS list, however, which in its original form, listed 675 food ingredients. Surprisingly, even sucrose is not included. However, the omission of sucrose and many other obviously safe food ingredients prompted the following official FDA comment:

> It is impractical to list all substances that are generally recognized as safe for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder and monosodium glutamates as safe for their intended use. (11)

In order to achieve GRAS status, a sweetener or any food ingredient may be generally recognized as safe among

> . . . experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (*1*) scientific procedures or (*2*) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. (12)

For the cases of substances not included on the GRAS list, two tracks toward approval for use in foods are defined. First, and somewhat simpler where applicable, is the GRAS Affirmation Process. To qualify for this track, it must be either (*1*) demonstrated that the substance was in common use in food in the United States prior to 1958 or (*2*) be based on expert judgment of safety demonstrated by published safety studies. If the available safety data are not sufficient to support the requested uses or increase in projected exposure levels, the FDA may affirm GRAS status but limit levels of use until further safety data are established. Examples of sweeteners for which GRAS affirmation has been requested are discussed in the latter part of this article.

The second track toward approval of a sweetener for use in foods is the food additive petition (FAP) process. The FAP process requires extensive safety studies in test animals as well as studies in humans. One objective of these studies is the determination of the highest dose that may be given without adverse effects. This dose is termed the no observed adverse effect level (NOAEL). The NOAEL, in the most sensitive animal species evaluated, is then used by the FDA to regulate the level at which the food additive may be used in foods. This level, termed the acceptable daily intake (ADI), is defined as 0.01 of the NOAEL in the most sensitive animal species. These NOAEL and ADI exposure levels are given in milligrams per kilogram of body weight. Thus, as an example, if the NOAEL of a proposed new sweetener is determined to be 500 mg/kg in the most sensitive animal species evaluated, an ADI of 5 mg/kg would be allowed. The question then naturally arises as to the meaning of a 5 mg/kg ADI allowance. This level is then employed by the FDA to determine the food categories and levels in those categories in which the new sweetener may be used. The objective of this exercise is to ensure that the 5 mg/kg exposure level is not exceeded on a chronic basis. In order to do this, 90th percentile, 14-day average food category consumption data are employed. Approval of the new sweetener may then be granted for use in food categories where, in the aggregate, 90th percentile, 14-day average consumption data on these categories does not exceed the ADI. Examples of sweeteners that have successfully undergone, or are currently undergoing the FAP Process, are discussed in this article. Marshall and Pollard have comprehensively reviewed the category and level approvals that have been granted in the United States and abroad for many of the sweeteners discussed herein (13). Broulik reviewed the regulatory issues related to new sweetener development in the United States in 1996 (14).

Although individual countries assume responsibility for the regulation of food additives within their boundaries, there has been an attempt at international standardization of food additive regulation. Vettorazzi reviewed this process in 1989 (15). In 1956, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The fundamental objective of JECFA is the establishment of ADIs for food additives following the assembly and interpretation of all relevant biological and toxicological data. It is important to recognize that ADIs provide a wide margin of safety. According to JECFA, "an ADI provides a sufficiently large safety margin to ensure that there need be no undue concern about occasionally exceeding it provided the average intake over longer periods of time does not exceed it" (16). Over its lifetime, more than 700 intentional food additives have been evaluated by JECFA.

Solubility

Many sweeteners are insufficiently water soluble to be of general utility. Commonly, sweetness intensity levels at least equivalent to 10% sucrose are required. In some systems (eg, frozen desserts), soluble sweetener levels are required that match the sweetness of 15 to 20% sucrose. In addition, for many food systems, manufacturers may require that sweeteners dissolve sufficiently rapidly so as to not interfere with current manufacturing processes. A particularly relevant illustration of requirements imposed by manufacturing processes is that for CSD products. In this case, it is necessary that a concentrate solution of the sweetener–flavor system complex be rapidly attainable. Thus, high solubility and rapid dissolution rates are very desirable properties for nonnutritive sweeteners.

Stability

To be commercially viable, a sweetener must be stable to the intended conditions of use. Degradation may be from hydrolytic, pyrolytic, or photochemical processes depending on the food application. Stability is required for two reasons. First, the sweetener must not degrade such that the level of sweetness of the food product would be substantially reduced during the product lifetime. Also, the sweetener must not break down to cause any off taste. The second reason for the stability requirement relates to sweeteners that are defined as food additives. By the FAP process, degradation products must be shown to be safe. Currently, for most organic compounds, if exposure to the degradation product may reach or exceed 0.0063 mg/kg, then the requisite safety assessment studies would be equal to those mandated for the sweetener itself (17). As such, a sweetener's stability is a very important factor when assessing its viability for development as a new food additive.

Cost

Nonnutritive sweeteners are always compared to sucrose, the consumer's standard. With sucrose in plentiful supply, presently at 36 cents/lb, potential replacements must either be cost-competitive or present sufficient advantages to justify a premium (18). The effective cost to the food product manufacturer of an alternative sweetener is equivalent to the wholesale price divided by the potency of the alternate product in delivering the desired property. In most cases, the desired property is sweet taste intensity. Thus, the effective cost or cost per sucrose equivalent (CSE) is the quotient of wholesale price and sweetness potency (P). P is generally expressed as a multiple of the P of sucrose, which we define to be 1. Although sometimes P is expressed on a molar basis, most commonly P is expressed on a weight basis (P_w). It is important to recognize that although P_w is not constant for high-potency sweeteners, it often is for sugars and other polyol sweeteners of low potency (19). P_w for a high-potency sweetener varies according to the sucrose reference concentration. Concentration/response (C/R) functions demonstrating saccharin's and cyclamate's nonlinear dependencies of P_w on sucrose reference concentration are illustrated in Figure 2. DuBois et al. demonstrated that the law of mass action hyperbolic function $R = R_mC/(k_d + C)$ provides a good fit for high-potency sweetener C/R function data and that the simple linear function $R = P_wC + b$ provides a good fit for most sugar and polyol sweetener C/R function data (20). When sweetener C/R data are fit to these equations, the constant terms provide very useful information. If a sweetener's C/R data are well modeled by $R = P_wC + b$, then P_w for the sweetener is a constant and is determined to be the slope of the line. On the other hand, if a sweetener's C/R data are well modeled by $R = R_mC/(k_d + C)$, then P_w is not a constant and is dependent on sucrose reference concentration. It is important to note, however, that the parameters R_m (maximal response) and k_d (apparent sweetener/receptor dissociation constant) in this equation provide useful information on the properties of the sweetener. R_m is the maximal response, in sucrose equivalents, possible for that sweetener. Thus, for sodium saccharin and sodium cyclamate, the equations given in Figure 2 show that the maximum sweetness responses are equivalent to 10.1 and 15.2% sucrose, respectively. The R_m value for saccharin is rather low and indicates that it would be difficult to formulate a food product based on saccharin as the sole sweetener. k_d also provides useful information. It is the sweetener concentration in milligrams per liter that gives 50% of the maximal response. Thus, for sodium saccharin and sodium cyclamate, the equations given in Figure 2 show that 115 and 3140 mg/L are equivalent to 5.0 and 7.6% sucrose, respectively. These values are useful in assessment of the potential of a sweetener as a sole sweetener or as a component of a blend system.

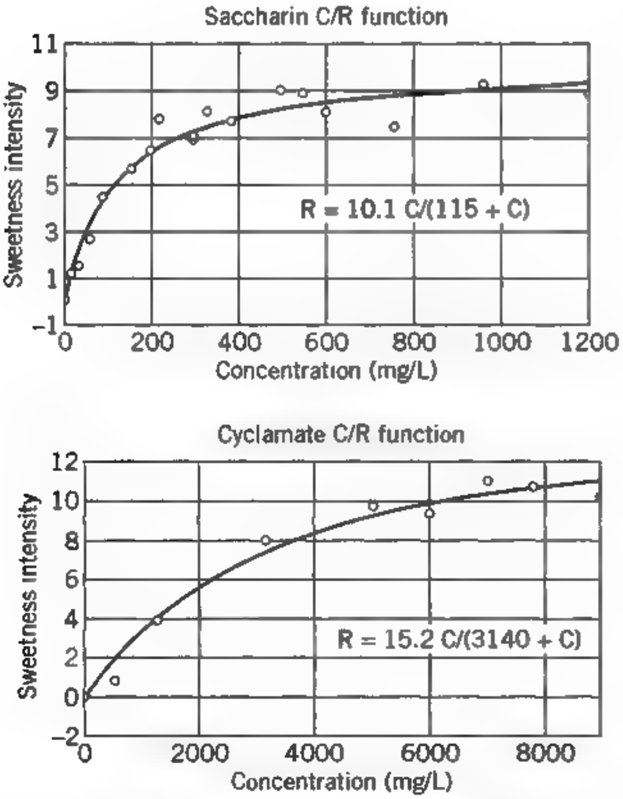


Figure 2. C/R functions for sodium saccharin and sodium cyclamate in water.

In addition to the reference concentration effect on P, substantial effects can also be found that are due to food systems and temperature. As an example of the food system effect, it has been reported that in water, aspartame is 133 times more potent than sucrose (10% reference), whereas in CSDs, it is 180 times sucrose at the same reference concentration level (21). As an example of the temperature effect, it has been reported, and is generally accepted, that fructose is more potent than sucrose (22). At elevated temperatures, however, sucrose is more potent than fructose. Thus, calculation of sweetener CSE requires specification of the application to ensure that the relevant value of P_w is used.

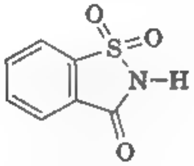
Carbohydrate sweeteners (glucose, maltose, etc) generally are less potent than sucrose. As such, CSE values for carbohydrate sweeteners usually exceed that of sucrose. During the past century, many high-potency noncarbohydrate sweeteners have been discovered. As a consequence, very low CSE values are often realized for these sweeteners. For illustration, consider sodium saccharin, which currently is available in the United States for a wholesale price of \$3.90/lb (18) and exhibits P_w (8) in water of 180 times that of sucrose. The CSE of sodium saccharin, used in water at an 8% sucrose sweetness equivalent level, would therefore be \$3.90/180 = 2.2 cents/lb. This contrasts quite sharply with sucrose, which is presently available for a wholesale price of 36 cents/lb (18). Clearly, such cost factors attainable with high-potency sweeteners offer food manufacturers substantial increased profitability in the low-calorie sector of product categories. Although sweetener cost is certainly an important consideration for the food manufacturer, it is discussed here only for the cases of saccharin and cyclamates, which, as commodity chemicals, have relatively stable prices.

HIGH-POTENCY NONNUTRITIVE SWEETENERS OF MAJOR COMMERCIAL SIGNIFICANCE

The four nonnutritive sweeteners saccharin, cyclamate, aspartame, and acesulfame-K are used extensively today. In addition, sucralose and alitame are newcomers that may see significant use in the future. These six sweeteners are discussed in detail in this section. These sweeteners are approved for general use in the United States except for alitame. The chemical composition, a history of discovery and development, and discussions of taste quality, safety, solubility, and stability issues are presented for each.

Saccharin

Saccharin (1) is the first sweet-tasting organic compound identified that is significantly more potent than sucrose. It was discovered in 1878 by Fahlberg while he was working in the Remsen's laboratory at Johns Hopkins University (2). Saccharin is exemplary of the *N*-sulfonyl amide structural class of sweeteners that is distinguished by the common $-CONHSO_2-$ substructural unit.



(1)

After its discovery, saccharin soon found its way into many food applications. It was particularly popular among persons with diabetes as well as obese persons. Saccharin use was limited, however, by a taste quality substantially inferior to that of sucrose. Its sweet taste is accompanied by significant bitter and metallic taste attributes. Interestingly, it has been found that the population is heterogeneous in this respect, approximately one-third is hypersensitive to these off tastes, and the remaining two-thirds is moderately sensitive to insensitive (23–26). Saccharin's FPA data and C/R function are illustrated in Figures 1 and 2, respectively, and are also given in Table 1. As has been generally discussed earlier, sweetness potency is strongly dependent on the concentration of sucrose used as reference. For saccharin, the falloff in sweetness potency as a function of increasing sucrose reference concentration is particularly dramatic. Thus, from the equation in Figure 2, saccharin P_w values of 710, 180, and 9 are calculated relative to sucrose references of 2, 8, and 10%, respectively. The abrupt drop in sweetness potency shown here for saccharin is due to its low R_m of 10.1. Persons hypersensitive to saccharin bitterness do not experience an R_m that even approaches 10, whereas individuals insensitive to this off-taste experience a much higher R_m. However, the average result for the expert sensory panel used for the development of the data in this study is 10.1. Although the reason for a low R_m for some panelists is not known, it seems likely that it may be due to mixture suppression effects. This phenomenon of suppression of one taste attribute (eg, sweetness) by another (eg, bitter or sour) is well known (19). The temporal profile of saccharin is essentially identical to that of sucrose. ATs of 4 and ETs of 14 s, respectively, have been determined for both saccharin and sucrose (7). Thus, with the exception of a marginal flavor profile, which is particularly objectionable for some subjects, the taste quality of saccharin mimics that of sucrose sufficiently to be useful in food applications.

Questions concerning the safety of saccharin date back to the early 1900s. A staunch defender of saccharin safety was President Theodore Roosevelt. In response to those who questioned its safety, Roosevelt stated, "Anybody who says saccharin is injurious to health is an idiot" (27). Despite such high-level support, however, saccharin has continued to come under fire. The chronologies of safety studies and regulatory agency actions concerning saccharin have been comprehensively reviewed (28,29). The principal events in this chronology have had many ups and downs. In 1958, saccharin was listed as one of the 675 substances on the original GRAS list. In 1972, however, the FDA retracted the GRAS status of saccharin based on concern over results in a long-term rat feeding study conducted by the Wisconsin Alumni Research Foundation. Then, in 1977, as a result of a Canadian multigeneration rat study in which bladder tumors were found in the second-generation animals, the FDA announced its intention to ban saccharin. The FDA held that it had no choice other than to ban saccharin because of the Delaney Clause of the Food Additive Amendment to the Food, Drug and Cosmetic Act. This law requires that if any food additive, at any dose, in any animal species, is found to cause cancer, its further use is to be outlawed. Acting FDA Commissioner Gardner stated,

> We have no evidence that saccharin has ever caused cancer in human beings. But we do now have clear evidence that the

Table 1. FPA and C/R Function Data on Major High-Potency Nonnutritive Sweeteners

Sweetener	C/R function data		FPA data					
	R_m	k_d	Sweet	Bitter	Salty	Metallic	Cooling	Licorice
Saccharin[a]	10.1	115	8.6	2.6	0.0	1.2	0.0	0.0
Cyclamate[b]	15.2	3240	10.0	1.2	0.6	0.0	0.0	0.0
Aspartame[c]	16.0	562	8.4	0.0	0.0	0.0	0.0	0.0
Acesulfame-K[d]	11.6	472	7.7	2.8	0.0	1.3	0.0	0.0
Sucralose[e]	14.7	142	8.7	0.0	0.0	0.0	0.0	0.0
Alitame[f]	14.6	28.1	8.4	0.0	0.0	0.0	0.0	0.0

[a] Sodium salt; 384 mg/L for FPA.
[b] Sodium salt; 5930 mg/L for FPA.
[c] 560 mg/L for FPA.
[d] 974 mg/L for FPA.
[e] 172 mg/L for FPA.
[f] 29 mg/L for FPA.

safety of saccharin does not meet the standards for food additives established by Congress.

Acting in response to strong consumer and industry pressure to ensure the continued availability of saccharin, Congress placed a moratorium on the FDA ban pending additional research. This moratorium on the saccharin ban has since been extended several times, and the one presently in place is set to expire on May 1, 2002. During this period of moratorium, the regulatory climate has evolved considerably such that, at the present time, a policy that considers both risks and benefits is now considered as more appropriate than the absolute statements of the Delaney Clause. In addition, risk is now recognized to be more of a function of dose than an all-or-none phenomenon as was previously considered. Mechanism of action is also recognized as an important factor to consider. Since the 1970s, research has been conducted in effort to elucidate the mechanism whereby saccharin initiates bladder cancer in rats. Interestingly, in one important study, evidence was obtained that the effect, which could only be demonstrated in rats, is due to the sodium component of sodium saccharin and not due to saccharin, the sweetener (30). In this and related studies, sodium ascorbate (vitamin C), sodium chloride (salt), and other sodium salts were shown to promote similar effects on the rat bladder. Most scientists think that the FDA should withdraw its requirement of a cancer warning label for food products containing saccharin in the very near future.

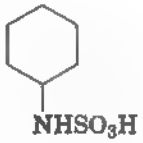
Temporary U.S. regulatory restrictions regarding the use of saccharin have been defined (31). For example, in beverages, saccharin may be used up to a level of 12 mg/oz (406 ppm). JECFA has established 5 mg/kg as an ADI for saccharin and stated that the rodent bladder tumors caused by sodium saccharin are not relevant to humans.

The physical properties of saccharin are ideal for use as a nonnutritive sweetener. First, it is extremely stable toward all the conditions to which it may be exposed in food applications. In studies conducted at the Sherwin Williams Company, it was determined that at pH 3.3 and 120°C, 18 and 69% loss occurred at 27 and 219 h, respectively (M. L. Mitchell, unpublished results, 1989). The exclusive product of this hydrolysis is 2-sulfobenzoic acid, which is also present as a low-level contaminant in commercial samples of saccharin. At pH 7.0 and 120°C, even greater stability was found with no significant degradation after 27 h and only 6% loss after 219 h. At pH 9.0 and 120°C, stability was also high with 2 and 12% losses, respectively, found after 27 and 219 h. The sole degradation product formed under neutral and alkaline pH conditions is 2-sulfonamidobenzoic acid, which is also a low-level contaminant in commercial samples of saccharin. As a consequence of this high stability, neither loss of sweetness during food product lifetime nor degradation product safety is a significant concern for saccharin. Second, saccharin is highly water soluble. As the sodium salt, 1200 g is soluble in 1 L of water, whereas in the acid form, a solution of 3.4 g/L may be obtained (32). If we consider saccharin P_w (8) to be 180, as an example for applications where an 8% sucrose level of sweetness intensity is required, it can be calculated that saccharin is either 2700 or 8 times as soluble as necessary, depending on whether the sodium salt or free-acid form is employed.

At the present time, no sweetener matches saccharin in terms of cost. As discussed earlier in the general discussion on sweetener cost, the current price of $3.90/lb (18) coupled with $P_w(8) = 180$ combine to result in a saccharin CSE of 2.2 cents/lb. Thus, saccharin is a very inexpensive sweetener.

Cyclamates

Although many sweet-tasting organic compounds were found during the 59 years between the discovery of saccharin and 1937, none achieved significant usage in foods. Then, in 1937, Sveda, a graduate student in Audrieth's laboratory at the University of Illinois, discovered that metal salts of cyclohexylsulfamic acid are sweet (33). These salts have become known as cyclamate salts or, more commonly, cyclamate. Cyclamate, in the acid form, has the chemical structure **2** and was the first of the sulfamate structural class of sweeteners to be discovered. Sweeteners of this class exhibit the —NH-SO_3—moiety as the common structural feature. Cyclamate is generally used in foods as either the sodium or calcium salt. Since 1937, many sweet analogues of **2** have been synthesized, although none have been developed for use in foods.



(**2**)

Sensory panel studies on cyclamate salts demonstrate them to better reproduce the taste quality of sucrose than does saccharin. Nonetheless, a significant bitter taste attribute is noted for cyclamate. The flavor profile of sodium cyclamate is illustrated in Figure 1. Interestingly, a salty attribute is also present in the sodium cyclamate profile. This factor is almost certainly due to the high sodium ion concentration present in intensely sweet solutions. The C/R function for sodium cyclamate is illustrated in Figure 2. As is true for all high-potency sweeteners, the potency of sodium cyclamate is dependent on sucrose reference concentration. Thus, $P_w(2) = 42$, $P_w(8) = 23$, and $P_w(10) = 17$ are calculated from the equation given in Figure 2. The temporal profile of sodium cyclamate is very similar to that of sucrose. ATs of 4 and ETs of 14s have been determined for both substances (7). Thus, although sodium cyclamate exhibits a flavor profile with significant bitter and salty notes, it exhibits a sucrose-like temporal profile and, in the aggregate, mimics the taste of sucrose sufficiently to be generally useful in food applications.

The history of the evaluation of cyclamate safety has been reviewed comprehensively (34,35). Cyclamates were first approved in 1951 for use as drugs for use by people with diabetes and others who had to restrict their sugar intake. In 1958, they were listed by the FDA on the original GRAS list. As was discussed earlier, the use of cyclamate sweeteners experienced explosive growth during the 1960s after it was found that a 10:1 mixture of cyclamate and saccharin exhibits taste quality superior to either sweetener individually and approaches that of sucrose. In 1969, the FDA became concerned about cyclamate safety. At that time, the FDA was advised of a rat study in which the commonly used 10:1 cyclamate/saccharin mixture was shown to induce bladder tumors. These results were interpreted by the FDA to indicate that cyclamate salts (ie, not saccharin salts) are bladder carcinogens in rats. As a consequence, cyclamates were immediately removed from the GRAS list and, in 1970, were banned from use. At the same time, however, cyclamates have remained on the market in more than 50 countries.

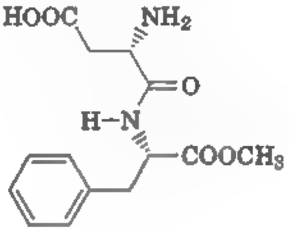
Since the 1970 FDA action, Abbott Laboratories and the Calorie Control Council have maintained continuous efforts to have cyclamates reapproved. As a result of this effort, it is now generally accepted that cyclamates are not carcinogens (34). The principal remaining areas of concern are related to the biological activity of cyclohexylamine, the major cyclamate metabolite. In early studies, hypertensive activity and testicular atrophy effects were noted for this metabolite. However, in recent studies, no adverse hypertensive or reproductive effects have been observed in humans. Nonetheless, these weak biological effects for cyclohexylamine may limit the ADI to be granted by the FDA in the event that it is reapproved. The ADI established by JECFA for cyclamate is 11.0 mg/kg. In the European community, cyclamate levels in CSDs are restricted to a maximum of 400 ppm (36). From the equation given in Figure 2, it can be calculated that 400 ppm cyclamate is equivalent to 1.7% sucrose in sweetness intensity. Thus, although cyclamates may be reapproved in the United States, restrictions may limit their application to sweetener blend systems.

Cyclamates exhibit excellent solubility and stability characteristics for use in essentially all imaginable applications. The sodium and calcium salt forms are both commercially available. Although the acid form is sufficiently water soluble (13.3 g/100 mL), its high acidity results in preference for the very soluble sodium (20 g/100 mL) or calcium (25 g/100 mL) salts (37). Since $P_w(8) = 23$, as an example for applications where an 8% sucrose equivalent level of sweetness is desired, it can be calculated that sodium cyclamate is 570 times as soluble as necessary. Hydrolytic degradation of cyclamate salts proceeds to yield cyclohexylamine and inorganic sulfate. As a consequence of the adverse biological activity of cyclohexylamine, FDA scientists conducted a comprehensive evaluation of cyclohexylamine levels in a spectrum of food products (38). Cyclohexylamine was found in the majority of these products albeit at low levels. Interestingly, even in the most acidic samples (cola CSDs), cyclohexylamine levels increased insignificantly during 4 months of ambient-temperature storage. Cyclohexylamine in food products appears to be substantially derived as a cyclamate sweetener contaminant. Data have been reported on the hydrolysis of cyclamate under extreme conditions (39). After 1 h at 100°C, 13.7, 8.1, 0.98, 0.10, 0.52, and 0.58% of cyclamate sweetener is lost at pH values of 0.9, 1.6, 2.5, 4.5, 5.3, and 6.5, respectively. In summary, cyclamate sweeteners are quite stable. No significant loss of sweetness or generation of unsafe degradation products is expected in any common applications.

Cyclamates are economical sweeteners. On the world market, sodium cyclamate is presently available for $1.04/lb (40). On a CSE basis, this is equivalent to 4.5 cents/lb if one employs $P_w(8) = 23$ times that of sucrose as the relevant sweetness potency. Thus, although sodium cyclamate is approximately two times more expensive than saccharin, it still represents a very substantial economy over sucrose.

Aspartame

L-Aspartyl-L-phenylalanine methyl ester (3) is an example of one of several novel structural types of sweet-tasting organic compounds identified in the 1960s. It is known under the generic name aspartame, is often abbreviated as APM, and is very well known to consumers as NutraSweet®, a brand name of Monsanto. As was true for all the sweeteners described herein that are not of botanical origin, the sweetness of APM was discovered by accident. The discovery was made by Schlatter while working under the direction of Mazur of G.D. Searle & Company (3). APM was prepared by Schlatter as an intermediate in a drug discovery program aimed at finding new ulcer treatments. Interestingly, this compound had been prepared some years earlier by chemists at ICI in Great Britain. Its sweet taste had not been noted, however.



(3)

Aspartame was the first sweet-tasting member of a structural class of sweeteners generally known as the dipeptides. Peptides are oligomers of α-amino acids. Aspartame is simply derived from the two natural amino acids L-aspartic acid and L-phenylalanine. It is ironic that even though substantially more than 1000 sweet-tasting analogues of aspartame have been prepared since 1965, none are more advantageous than aspartame after consideration of all the properties requisite for commercial viability.

The phenomenal commercial success of aspartame is easily explained by its exceptional sucrose mimicry. As suggested by its flavor profile summarized in Table 1, it is essentially indistinguishable from sucrose; no nonsweet taste attributes are observed. Interestingly, however, the temporal profile of aspartame is different from sucrose. Aspartame exhibits both a slightly delayed AT [5 s (APM) vs. 4 s (sucrose)] and a protracted ET [19 s (APM) vs. 14 s (sucrose)] (7). These differences are not sufficient, however, to adversely affect acceptability in food products. An interesting and useful manner in which aspartame has an advantage over other high-potency sweeteners is that it enhances other flavor attributes. This effect was noted early on and has been systematically studied by Baldwin and Korschgen (41) and also by Wiseman and McDaniel (42). It has been hypothesized that this unique advantageous property of aspartame is due to its atypical temporal profile (43). More specifically, aspartame's flavor enhancement properties are suggested to be consequences of its slightly delayed AT relative to that of sucrose. Thus, greater temporal resolution of sweetness (taste) and flavor (olfaction) signals to the brain occurs for APM than is the case for sucrose. This increased resolution of neural olfactory and taste signals for APM over sucrose may result in the flavor intensity to be judged higher in the better-resolved aspartame-sweetened system. This effect is then interpreted as a flavor enhancement effect of aspartame.

The sweetness potency of aspartame is again dependent on the sucrose reference concentration, although somewhat less so than for the other sweeteners discussed in this section. Thus, from the APM C/R function given in Table 1, it is calculated that $P_w(2) = 250$, $P_w(8) = 140$, and $P_w(10) = 107$. Disparities between these potencies and literature potencies quoted earlier are a consequence of differences in methodology.

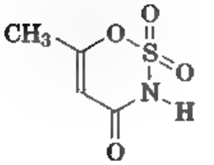
The safety of aspartame has been as extensively studied as any food additive. Most of the principal metabolism, preclinical, and clinical studies have been reviewed (44–46). On the basis of evaluation of all the safety assessment studies, aspartame was approved by the FDA in 1981 with an ADI of 20 mg/kg. This ADI is based on a NOAEL in preclinical studies of greater than 2,000–4,000 mg/kg. In 1983, based on clinical studies, the ADI for aspartame was raised by the FDA to 50 mg/kg. The ADI established by JECFA for aspartame is 40 mg/kg. No effects were noted in the clinical studies at doses many times greater than approved for human consumption. Consideration of aspartame metabolism explains why this is the case. On ingestion, aspartame is completely broken down to the two natural amino acid building blocks and methanol. The safety of the amino acids L-aspartic acid and L-phenylalanine is, of course, no surprise since normal dietary protein (from meat, milk, eggs, etc) provides substantially greater quantities of these nutrients. Methanol also is not a safety concern when exposure occurs as a metabolite of aspartame. Beverages formulated with aspartame to a sweetness level matching 10% sucrose contain the equivalent of 50 to 60 mg/L of methanol; this is substantially less than the average of 140 mg/L content for fruit juices. Thus, the safety of aspartame should be no surprise to anyone. Consumption of aspartame does not result in exposure of the internal body tissues to any novel substances. Natural subunit assembly and metabolic subunit disassembly is a unique high-potency sweetener concept presently exemplified only by aspartame. Despite the safety of aspartame, however, products containing it are required to carry an informational statement for people with a rare genetic disease known as phenylketonuria (PKU). Approximately 1 of 15,000 people has this disorder, which involves an inability to metabolize phenylalanine. Unchecked, PKU results in mental retardation. In the United States, this disorder is detected at birth, if present. The normal treatment is a phenylalanine-restricted diet from infancy through childhood and sometimes into adulthood. It should be emphasized, however, that phenylalanine consumption is only restricted, not eliminated, since it is an essential amino acid and, as such, is necessary for life. Persons with PKU must carefully monitor their consumption of phenylalanine from all dietary sources, including aspartame.

Aspartame exhibits sufficient solubility for all food applications. At ambient temperature in water, a solubility of approximately 1.0% can be attained at pH 4 and also at neutral pH; solubility reaches a minimum at pH 5.5, the isoelectric point (47). Thus, using $P_w(8) = 140$, it can be calculated that aspartame is greater than 70 times more soluble than necessary to provide an 8% sucrose level of sweetness intensity.

If aspartame has a drawback, it is hydrolytic stability. At 25°C (77°F), its hydrolytic half-lives in aqueous buffer are 116, 260, 242, 82, and 2 days for pHs of 3, 4, 5, 6 and 7, respectively (21). Clearly, maximum utility would be expected in the pH range 3 to 5. Fortuitously, this is a representative range for most food systems. It is particularly fortuitous that none of the three principal degradation products, L-aspartyl-L-phenylalanine (AP), 3-benzyl-6-carboxymethyl-2,5-diketopiperazine (DKP), and β-L-aspartyl-L phenylalanine methyl ester (β-APM) exhibit either off-tastes in aged food products or safety problems. All the degradation products have been tested and demonstrated to be safe. Interestingly, β-L-aspartyl-L-phenylalanine (β-AP), the product of ester hydrolysis of β-APM, has been demonstrated to be naturally produced on metabolism of dietary protein (48). In summary, aspartame is sufficiently stable for use in acidic food products. On the other hand, the neutral pH range encountered in baked goods is problematic. An encapsulated form of aspartame has been developed, however, to address this limitation (49).

Acesulfame

The discovery of acesulfame was accidental, just as was the case for saccharin, cyclamate, and aspartame. In 1967, Clauss and Jensen, while working in the laboratories of Hoechst AG on the reactions of fluorosulfonylisocyanate with acetylenes, obtained the product 5,6-dimethyl-1,2,3-oxathiazin-4(3*H*)-one-2,2-dioxide and noted it to be sweet (50). As a consequence of the 1969 ban on cyclamates in the United States, Hoechst initiated a systematic program to optimize the properties found in this compound. In the end, it was determined that the preferred product candidate was 6-methyl-1,2,3-oxathiazin-4(3*H*)-one-2,2-dioxide, which has since been given the generic name acesulfame and has chemical structure 4. The potassium salt of acesulfame is known as acesulfame-K, is often referred to as ACE-K, and has been given the brand name Sunette®. Acesulfame is structurally related to saccharin in that both exhibit the essential-for-activity N-sulfonyl amide structural motif (ie, $-CONHSO_2-$) and thus are both members of the N-sulfonyl amide sweetener class. The chemistry, safety assessment, sensory properties, and applications of acesulfame-K have been reviewed (51).



(4)

The structural analogy of acesulfame with saccharin is not the only area of commonality. ACE-K exhibits a flavor profile with bitter and metallic attributes that are equivalent to or stronger than those of saccharin. The FPA data for ACE-K are summarized in Table 1. Interestingly, it has been found that the population is heterogeneous with respect to its sensitivity to the bitter and metallic off-tastes of acesulfame-K, as has also been noted for saccharin. Particularly striking in this study is the finding that the same individuals who are sensitive to saccharin off-taste characteristics are also sensitive to those of ACE-K. As a consequence, ACE-K, like saccharin, has minimal utility as a sole sweetener in food applications. Although quantitative temporal profile data on acesulfame-K are not available, it appears, qualitatively, to be quite similar to sucrose in this dimension. In summary, it is expected that ACE-K will find a niche in food applications as an alternative to saccharin. Thus, its principal application should be as a component of blends with aspartame and other sweeteners with sucroselike flavor profiles.

C/R function data on acesulfame-K are provided in Table 1. The sweetness potency of ACE-K drops off with increasing sucrose reference concentration just as is the case for saccharin, cyclamate, and aspartame. The rate of this decrease is rapid, very much as is the case for saccharin. Thus, from the C/R function for ACE-K given in Table 1, $P_w(2) = 204$, $P_w(8) = 76$, and $P_w(10) = 34$ are calculated.

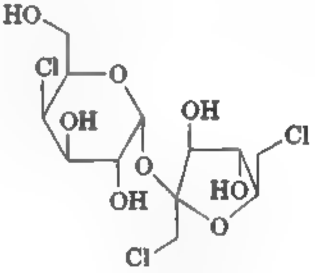
ACE-K received its first approval in the United States for tabletop use in 1988 and finally received approval for all food categories in 1998. An ADI of 15 mg/kg was established by the FDA after review of the safety assessment studies submitted by Hoechst. An ADI of 15 mg/kg has also been established by JECFA. The safety studies conducted on ACE-K have been reviewed (52).

The solubility of acesulfame-K is quite high, with 27 g dissolving in 100 mL of water (53). If, for illustration, the $P_w(8) = 76$ value of acesulfame-K is employed, it can be calculated that acesulfame-K is more than 250 times more soluble than necessary to match the sweetness intensity of 8% sucrose.

The hydrolytic stability of acesulfame-K is substantially less than that of saccharin. For example, under conditions designed to model a cola CSD application, 15% is lost in 1 year at room temperature and 25% is lost in 3 months at 40°C (104°F) (54). Products that have been identified from ACE-K degradation include acetoacetamide-N-sulfonic acid, acetoacetamide, acetoacetic acid, and acetone. In summary, it can be said that although ACE-K is not completely stable toward hydrolytic breakdown, its slight instability does not create any problems (eg, loss of sweetness) from an application perspective.

Sucralose

Until the discovery of saccharin, nearly all known sweet substances were carbohydrate based. In 1976, in a collaborative program between Hough's laboratory at Queen Elizabeth College in the University of London and scientists at Tate & Lyle, it was discovered that certain halodeoxysucrose derivatives are potently sweet (55). This collaboration led to the selection of a trichlorinated derivative of sucrose that they named sucralose (**5**) as a product candidate (56). The halodeoxysucroses, of which sucralose is the best known example, are the first carbohydrate-based sweeteners that are substantially more potent than the simple sugars. The commercialization of **5** was pursued by a joint venture of Tate & Lyle and McNeil Specialty Products Company, a Johnson & Johnson subsidiary. Sucralose is known under the brand name of Splenda®. The properties of sucralose were reviewed in 1996 (57).



(**5**)

FPA data on sucralose are provided in Table 1. As can be seen by inspection of these data, sucralose exhibits a clean sweet taste equivalent to that of sucrose. No off-tastes are observed. Unfortunately, temporal profile data on sucralose are not available, and so it is not possible to quantitatively-compare it to sucrose in this dimension. In general, however, sucralose's sweetness appears to linger somewhat similar to the behavior of aspartame.

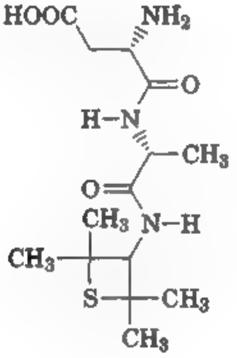
C/R function data on sucralose are provided in Table 1. Its sweetness potency drops off with increasing sucrose reference concentration just as is the case for saccharin, cyclamate, aspartame, and acesulfame-K. Thus, from the C/R function given in Table 1, $P_w(2) = 910$, $P_w(8) = 470$, and $P_w(10) = 330$ are calculated for sucralose.

In 1998, the FDA granted approval for the general use of sucralose in foods and beverages. An ADI of 5 mg/kg was established by the FDA after review of the safety assessment studies submitted. In contrast, an ADI of 15 mg/kg has been recommended by JECFA. Sucralose slowly hydrolyzes in acidic food and beverage products to the monosaccharides 4-chloro-4-deoxy-galactose and 1,6-dichloro-dideoxy-fructose, and for this reason, it was required that safety studies be carried out on sucralose and the two degradation products. The solubility of sucralose in water is very high and is not significantly affected by pH. Sucralose is commercially available as a 25% (w/v) solution. If, for illustration, the $P_w(8) = 470$ value of sucralose is employed, it can be calculated that sucralose is >1400 times more soluble than necessary to match the sweetness intensity of 8% sucrose.

The hydrolytic stability of sucralose is high, though not as high as that of saccharin. As already mentioned, it does slowly hydrolyze to the two chlorinated monosaccharides 4-chloro-4-deoxy-galactose and 1,6-dichloro-dideoxy-fructose in acidic food systems. However, the loss of sucralose over 1 year is <5%, and so there is no significant loss of sweetness. In the solid form, sucralose does break down with loss of hydrochloric acid, causing the solid product to caramelize. This problem has substantially been addressed by reducing the particle size of the solid material to less than 12 μM. The microparticulated material has adequate shelf life for common food and beverage manufacturing operations. In summary, it can be said that although sucralose is not completely stable, its slight instability does not create any problems (eg, loss of sweetness) from an application perspective.

Alitame

Alitame is the product of an intensive research program carried out by Hendrick and his coworkers at Pfizer Central Research during the 1970s (58). The program was aimed at the identification of a stable, cost-effective analogue of aspartame that, of course, still retains the quality taste of aspartame. Alitame was selected from a group of hundreds of aspartame analogues, which were synthesized by the Pfizer chemists. Alitame has recently been reviewed (59).



(6)

FPA data on alitame are provided in Table 1. As can be seen by inspection of these data, alitame exhibits a clean, sweet taste comparable to sucrose taste. No off-tastes are observed. Unfortunately, temporal profile data on alitame are not available, and so it is not possible to quantitatively compare it to sucrose in this dimension. In general, however, alitame's sweetness appears to linger somewhat, much as is the case for aspartame.

C/R function data on alitame are provided in Table 1. Its sweetness potency drops off with increasing sucrose reference concentration just as is the case for saccharin, cyclamate, aspartame, acesulfame-K, and sucralose. Thus, from the C/R function given in Table 1, $P_w(2) = 4440$, $P_w(8) = 2350$, and $P_w(10) = 1640$ are calculated for alitame.

In 1986, Pfizer submitted an FAP to the U.S. FDA requesting broad approval for use in foods and beverages. At about the same time, approval was also requested in many other countries. At this point, approval for use in the United States has not yet been granted. However, broad clearance for use has been obtained in several countries, including Australia, Chili, China, Columbia, Indonesia, Mexico, and New Zealand. An ADI for alitame of 1 mg/kg was recommended by JECFA after review of all the safety assessment studies conducted. Alitame slowly breaks down in acidic food and beverage products to L-β-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanine amide and N-(2,2,4,4-tetramethyl-3-thietanyl-D-alanine amide, and for this reason, it was required that safety studies be carried out on alitame and these two degradation products.

Alitame's solubility in water is high (131g/L w/v) and is only slightly sensitive to pH. If, for illustration, the $P_w(8) = 2350$ value of alitame is employed, it can be calculated that alitame is >3800 times as soluble as necessary to match the sweetness intensity of 8% sucrose.

The stability of alitame is substantially better than that of aspartame, though not as high as that of saccharin. Its hydrolytic stability is very sensitive to pH. In the pH range of 2 to 4, alitame is 2 to 3 times more stable than aspartame, while in the pH range of 5 to 8, it is >1000 times more stable. As already mentioned, it does slowly break down to L-β-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanine amide and N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanine amide in acidic food systems. However, the loss of alitame is insufficient to significantly affect the shelf life of most products. Alitame is limited, however, in that it does break down in acidic systems, by an unknown pathway, to produce trace levels of a sulfurous breakdown product or products. This limitation is particularly evident in cola beverages. In summary, it can be said that alitame is sufficiently stable for most food applications but is not an alternative for acidic products that require a long shelf life.

Summary

At the present time, there are four high-potency nonnutritive sweeteners that have achieved widespread use in the food industry, and there are two newcomers that are expected to see significant use in the future. Comparative data on these sweeteners are given in Table 2.

OTHER HIGH-POTENCY NONNUTRITIVE SWEETENERS OF COMMERCIAL SIGNIFICANCE

In this section, the five nonnutritive high-potency sweeteners neohesperidin dihydrochalcone, stevia sweeteners, glycyrrhizin, thaumatin, and Lo Han Kuo sweetener are discussed. In view of the limited approvals for use of these sweeteners in the United States, discussions of them will be abbreviated. Comparative data on these sweeteners are given in Table 3.

Neohesperidin Dihydrochalcone

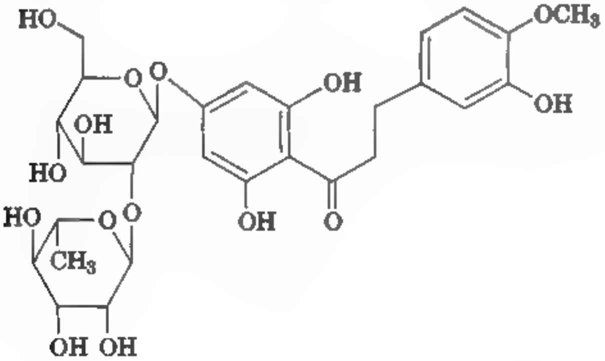
As was the case for the high-potency sweeteners mentioned above, neohesperidin dihydrochalcone (NDC) also was discovered in a serendipitous manner. Horowitz and Gentili, of the USDA laboratory of Pasadena, California,

Table 2. Summary of High-Potency Nonnutritive Sweeteners of Major Commercial Significance

Sweetener	Year of discovery	Taste quality	Safety concern	Solubility	Hydrolytic stability	Cost[a]	U.S. regulatory status	Other properties
Saccharin	1878	Fair; bitter and metallic off-taste	None	Excellent	Excellent	2.2	Food additive permitted on an interim basis	
Cyclamates	1937	Good; weak, bitter and salty off-taste	Metabolite associated with testicular atrophy and hypertension	Excellent	Excellent	4.5	Not approved	
Aspartame	1965	Excellent	None	Good	Fair		ADI = 50 mg/kg	Flavor enhancement
Acesulfame-K	1967	Fair, bitter and metallic off-taste	None	Excellent	Good		ADI = 15 mg/kg	
Sucralose	1976	Excellent	None	Excellent	Good		ADI = 5 mg/kg	
Alitame	1979	Excellent	None	Excellent	Good		Not approved	

[a] Cost in cents/lb on a cost per sucrose equivalent basis.

as part of an investigation on the bitter tastes of citrus flavonoid glycosides, made a surprising observation (60). Based on a model of bitter taste structure–activity relationships they were developing in the early 1960s, they predicted that naringin dihydrochalcone would be bitter like naringin, the bitter constituent of grapefruit rinds. Naringin dihydrochalcone was then synthesized, and the USDA investigators were astonished to find it to be sweet. Subsequent studies, initiated in attempt to optimize the properties of sweeteners of this type, resulted in the identification of NDC (7) as the preferred product candidate. Neohesperidin, the flavanone glycoside precursor of 7, occurs naturally in the Seville orange. NDC and other related sweeteners have been reviewed (61).



(7)

NDC, until later discoveries in the 1970s, was one of the most potent sweeteners known. At near threshold levels of sweetness intensity, Horowitz and Gentili reported NDC to be 7 times more potent than saccharin on a weight basis. Problematical, however, are the presence of bitter, cooling, and licorice taste attributes (8). In addition to a flavor profile quite different from that of sucrose, NDC exhibits a substantially delayed and protracted temporal profile relative to that of sucrose. An AT = 9 s (sucrose, 4 s) and an ET = 40 s (sucrose, 14 s) have been measured (7). In food systems, the complex flavor and atypical temporal profiles of NDC combine to provide a very unnatural taste. Thus, the utility of NDC in food systems is marginal unless employed as a minor component in combination with other more sucroselike sweeteners.

NDC is not approved in the United States for use as a sweetener, and no FAP for such use has been filed. The Flavor and Extract Manufacturers' Association of the United States (FEMA) has, however, approved it as GRAS. By this approval, it may be used as a flavor modifier within the range of 1 to 5 ppm in a variety of foods and beverages (62). At these low concentrations, NDC has been reported to enhance flavors, to enhance mouth-feel, and to make many nonnutritive sweeteners taste more sugarlike. These properties of NDC have recently been reviewed (63).

Stevia Sweeteners

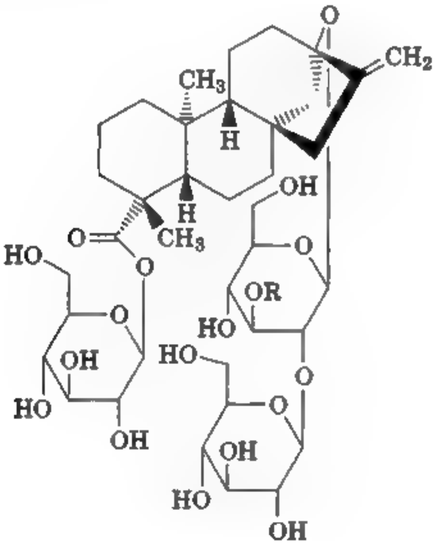
In Paraguay is found a small shrub known as *Stevia rebaudiana* (Bertoni), which is distinguished by its intensely sweet leaves. Investigations began to identify the sweet component of these leaves in the early part of this century. These efforts have been reviewed (64). The first significant work was by the French researchers Bridel and Lavieille, who isolated a crystalline sweet product. They determined this substance to be 300 times as potent as sucrose and to be a glycoside composed of three glucose moieties and a diterpenoid aglycone. This diterpenoid glycoside is now known as stevioside. The structure determination of stevioside as **8a** was completed in the 1950s and early 1960s by a combination of the efforts of Mosettig and Wood. Since that time, Tanaka et al. at Hiroshima University in Japan have isolated and characterized at least eight additional sweet glycosidic analogues of **8a** that are present in the

Table 3. FPA and C/R Function Data on Other Nonnutritive Sweeteners

Sweetener	C/R function data		FPA Data					
	R_m	k_d	Sweet	Bitter	Salty	Metallic	Cooling	Licorice
Neohesperidin dihydrochalcone[a]	9.8	53.2	7.2	0.5	0.0	0.0	3.3	2.3
Stevioside[b]	9.9	406	7.0	3.6	0.0	0.0	0.0	0.0
Rebaudioside A[c]	10.0	200	8.5	2.6	0.0	0.0	0.0	1.3
Glycyrrhizin[d]	7.8	240	7.2	1.6	1.0	0.0	1.2	1.3
Thaumatin[e]	10.1	3.6	8.7	1.2	0.0	0.0	1.8	0.0

[a] 160 mg/L for FPA.
[b] 1000 mg/L for FPA.
[c] 400 mg/L for FPA.
[d] 300 mg/L for FPA.
[e] 35 mg/L for FPA.

plant source. The most important of these is rebaudioside A (**8b**) because of its somewhat improved flavor profile. Also of particular significance are the oligomeric glucose conjugates of **8a**, which have been described by Tanaka and associates. These compounds are obtained on reaction of stevioside with starch and glucosyl transferase enzymes. This work was reviewed in 1991 (65). All these natural and enzyme-modified diterpenoid glycosides are approved for use in Japan. The plant-derived glycosides are also approved in South Korea, the Peoples Republic of China, Brazil, and Paraguay. Stevia sweeteners have been comprehensively reviewed (66,67).



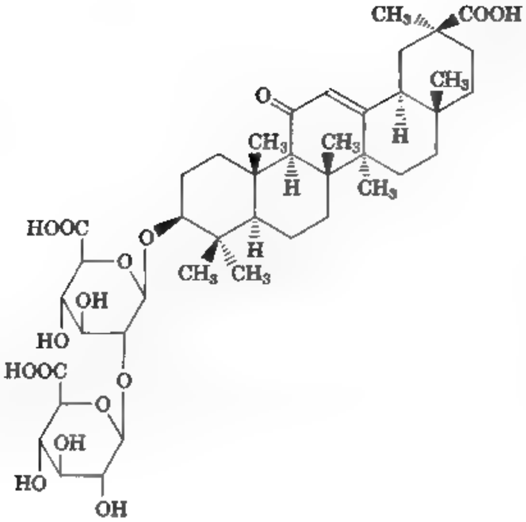
(8a) R=H
(8b) R=β-D-Glu

Of the natural stevia sweeteners, rebaudioside A is preferred because of its improved flavor profile relative to stevioside. Even rebaudioside A, however, exhibits substantial bitter and cooling taste attributes. In general, the stevia sweeteners exhibit temporal profiles that are sucroselike in AT (stevioside AT = 4 s; sucrose AT = 4 s) but not in ET (stevioside ET = 22 s; sucrose ET = 14 s) (7). Thus, as a consequence of flavor and temporal profiles that are rather unlike those of sucrose, rebaudioside A is not likely to find broad utility in food applications unless as a component of a blend.

At the present time, no stevia sweeteners are approved for use in the United States as either food additives or flavors. Furthermore, it is not expected that any will be approved since it is apparent, for a combination of performance, economic, and competitive reasons, that the comprehensive studies requisite for a FAP are not being undertaken. At the same time, however, stevia sweeteners are being used in the United States as dietary supplements. As such, they may be sold in the form of the plant leaves, but they may not be added to foods where their principal use is to provide sweetness.

Glycyrrhizin

Glycyrrhizin, a mixture of calcium, magnesium, and potassium salts of glycyrrhizic acid, occurs at a level of 6 to 14% in the roots of the European and Central Asian shrub *Glycyrrhiza glabra* Linn. (Fabaceae). The crude extract of the plant is well known as licorice. Glycyrrhizin is obtained from licorice extract by sulfuric acid precipitation. Ammoniated glycyrrhizin (AG) is obtained by dissolution of glycyrrhizin in aqueous ammonia, concentration, and recrystallization from alcohol. AG, which contains more than one molar equivalent of ammonia, may be converted to the monoammonium salt known as monoammonium glycyrrhizinate (MAG) by careful recrystallization. Glycyrrhizic acid is a triterpenoid glycoside of structure **9**. The original structural work leading to this structural assignment was completed by Lythgoe and Trippett in 1950 (68). MAG has been reported to have $P_w(10) = 33$ times that of sucrose and to exhibit significant bitter, licorice, and cooling flavor attributes (69). The temporal profile of MAG is not at all sucroselike. A very slow AT of 16 s (sucrose AT = 4 s) and a very prolonged ET of 69 s (sucrose ET = 14 s) have been reported (7). As a consequence of these strong deviations from sucroselike sweetness, MAG and the related licorice-derived products have only very limited utility in food applications. These limited applications have been reviewed (70).



(9)

In 1985, the glycyrrhizic-acid-derived sweeteners were confirmed in the United States as GRAS (71). However, the approval is specific with regard to their use as flavoring agents, flavor enhancers, and surfactants. Specifically excluded from this approval is as a component of sugar substitutes. As a consequence of this limited approval, the applications of glycyrrhizin-type sweeteners are extremely limited. Limiting utility also is the lack of sucroselike taste quality.

Thaumatin

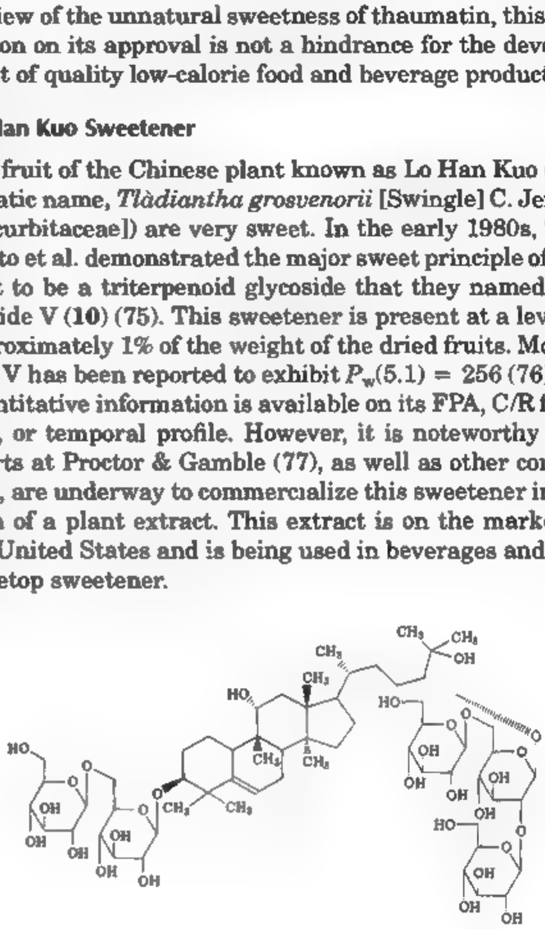
Thaumatin, currently marketed under the trademark Talin®, is a protein that occurs naturally in the fruit of the West African plant *Thaumatococcus daniellii*. This protein is a single chain of 207 amino acids with eight disulfide bridges and a molecular weight of 22,209. van der Wel and colleagues at Unilever carried out most of the classical structure determination work on thaumatin. Their work has been reviewed (72). More recently, X-ray crystallographic studies by Sung-Hou Kim at the University of California, Berkeley, have completed the structure determination (73).

On a molar basis, thaumatin is one of the most potent sweet substances ever found. A molar basis potency of 100,000 times that of sucrose has been reported relative to a threshold concentration of sucrose. On a weight basis, however, and relative to a 10% sucrose reference, thaumatin's potency is only 1,600 times that of sucrose. In flavor profile studies, thaumatin is, in many respects, similar to the glycyrrhizin sweeteners. Significant licorice and cooling taste attributes are observed. Particularly problematical is a very atypical, temporal profile relative to sucrose. The sweetness AT is significantly delayed and the sweetness ET, very protracted. As a consequence of these strong deviations from sucrose taste, thaumatin has only limited utility.

Thaumatin was approved in the United States as GRAS by FEMA in 1984 and has approvals in many other countries as well (74). FEMA's approval was extended in 1996 to include recombinant thaumatin (62). These approvals are limited to the use of thaumatin as a flavor modifier, and it may now be used in a broad range of food and beverage products where it provides this functionality. It may not be used, however, where its function is as a sweetener. In view of the unnatural sweetness of thaumatin, this limitation on its approval is not a hindrance for the development of quality low-calorie food and beverage products.

Lo Han Kuo Sweetener

The fruit of the Chinese plant known as Lo Han Kuo (systematic name, *Tlàdiantha grosvenorii* [Swingle] C. Jeffrey [Cucurbitaceae]) are very sweet. In the early 1980s, Takemoto et al. demonstrated the major sweet principle of this fruit to be a triterpenoid glycoside that they named mogroside V (**10**) (75). This sweetener is present at a level of approximately 1% of the weight of the dried fruits. Mogroside V has been reported to exhibit $P_w(5.1) = 256$ (76). No quantitative information is available on its FPA, C/R function, or temporal profile. However, it is noteworthy that efforts at Proctor & Gamble (77), as well as other companies, are underway to commercialize this sweetener in the form of a plant extract. This extract is on the market in the United States and is being used in beverages and as a tabletop sweetener.



(10)

SUGAR MACRONUTRIENT SUBSTITUTES OF COMMERCIAL SIGNIFICANCE

In this section, the five sweeteners maltitol, lactitol, isomalt, fructooligosaccharide sweetener, and erythritol are discussed. Each substance attempts to deliver all the functionality of sucrose; thus, they are sugar macronutrient substitutes (MNSs). All these sweeteners are approved for use following the GRAS affirmation process rather than the FAP process. The bioavailable calorie content of the sugar MNSs is a subject of substantial debate. In the United States, specific bioavailable calorie contents have been assigned to each sugar MNS, whereas in the European Union, a value of 2.4 cal/g has been assigned for all sugar MNSs. Comparative data on these sweeteners are presented in Table 4.

Maltitol

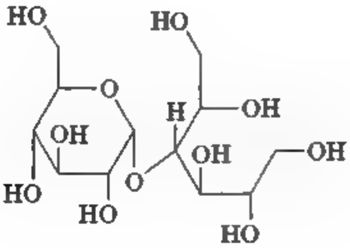
Maltitol is a disaccharide alcohol having structure 11. Maltitol is prepared commercially by hydrogenation of maltose, which in turn is obtained from starch by a combination of enzymatic hydrolysis and chromatography. It is generally agreed to have a sweetness potency of approximately 0.9 times that of sucrose and is relatively invariant

Table 4. FPA and C/R Function Data on Sucrose, Glucose, and Sugar MNS Sweeteners

	C/R function data		FPA data					
Sweetener	P_w	b	Sweet	Bitter	Salty	Metallic	Cooling	Licorice
Sucrose[a]	0.98	0.63	10.0	0.0	0.0	0.0	0.0	0.0
Glucose[b]	0.52	0.52						
Maltitol[c]	0.46	1.33	8.0	0.0	0.0	0.0	0.0	0.0
Lactitol[d]	0.40	−0.58	9.6	0.0	0.0	0.0	0.0	0.0
Isomalt[e]	0.42	−0.02						
FO sweetener[f]	0.27	−0.50	2.3	0.0	0.0	0.0	0.0	0.0

[a] 10.0% (w/v) for FPA.
[b] 10.0% (w/v) for FPA.
[c] 10.0% (w/v) for FPA.
[d] 25.5% (w/v) for FPA.
[e] 10.0% (w/v) for FPA.
[f] 8.0% (w/v) for FPA.

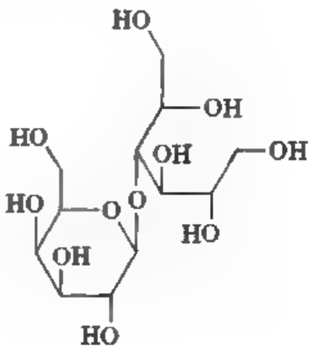
with respect to sucrose reference concentration and application. Pure maltitol was first described as a sweetener for reduced-calorie foods by Mitsuhashi and coworkers of Hyashibara Company in 1973 (78). Crystalline maltitol is known principally under the Hyashibara brand name Malbit®. Maltitol is currently approved for food applications in Denmark, Norway, Finland, the United Kingdom, Switzerland, Sweden, Belgium, France, Austria, and Italy. A GRAS affirmation petition was filed with the FDA by Towa Chemical Industry Company in 1986. Commercial development of maltitol as a food ingredient in the United States awaits action on this petition. The historical development, physical properties, safety assessment studies, metabolism, and food applications studies on maltitol have been comprehensively reviewed and will not be discussed further here (79,80). Particularly relevant to the present discussion, however, are bioavailable calorie content and laxative effects. Substantial controversy has developed over the bioavailable calorie issue, and it is unlikely that an absolute number of calories per gram will ever be agreed on. Arguments on this subject have been summarized by Secard and LeBot (81). As a result of a consideration of the rates of cleavage and absorption in all regions of the gastrointestinal tract, Ziesenitz and Siebert suggest that a value of 2.4 cal/g may be approximately correct (82). The generally accepted bioavailable calorie content in the United States, however, is 3.0 cal/g. Thus, for maltitol, only an approximate 25% reduction in calories may be possible relative to fully nutritive carbohydrate sweeteners. Ziesenitz and Siebert also indicate the intestinal discomfort (laxation, flatulence, etc) experienced from maltitol to be relatively mild.



(11)

Lactitol

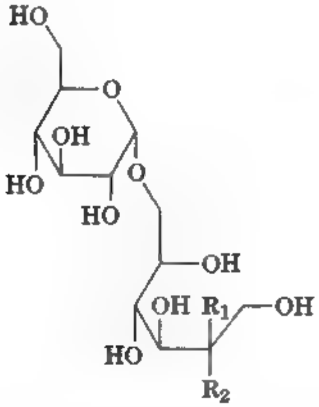
Lactitol is a disaccharide alcohol of structure **12**. It is obtained commercially by the hydrogenation of lactose (milk sugar), which is available in quantity as a by-product of the dairy industry. Lactitol exhibits a sweetness potency that varies only slightly with sucrose reference concentration; $P_w(2) = 0.30$ and $P_w(8) = 0.39$ have been reported in a general review on this sweetener (83). Lactitol has been commercially developed by CCA Biochem of The Netherlands and is known under the brand name Lacty®. Lactitol was affirmed as GRAS in the United States in 1993. In an attempt to understand the bioavailable calorie content of lactitol, studies have been conducted on the rate of lactitol cleavage in the small intestine. In comparison to lactose and isomaltose, the cleavage rate was sharply reduced. Thus, lactitol passes largely unchanged into the large intestine. Anaerobic microbial fermentation at this point converts it into organic acids and gases. The organic acids are, in part, absorbed by the host as an energy source. In general, after review of the results of many animal and human studies, it is accepted that lactitol has a bioavailable calorie content of approximately 2.0 cal/g. According to Ziesenitz and Siebert, the intestinal discomfort to be expected from lactitol is high (82).



(12)

Isomalt

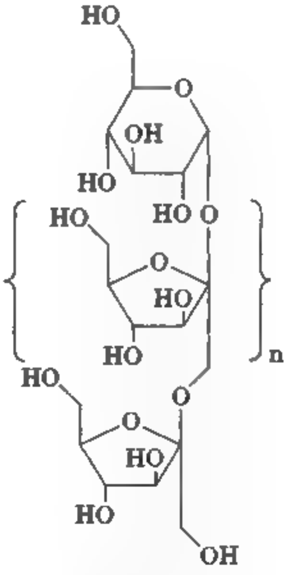
Isomalt is approximately a 1:1 mixture of the two disaccharide alcohols 1-*O*-α-D-glucopyranosyl-mannitol (**13a**) and 1-*O*-α-D-glucopyranosyl-sorbitol (**13b**). The development and properties of isomalt have been reviewed (84–87). Isomalt is best known under the trade name Palatinit® of Palatinit Sussungsmittel GmbH, a wholly owned subsidiary of Sudzucker AG. Isomalt was discovered at Suddeutsche Zucker in the early 1950s. In the late 1970s, Bayer AG joined with Suddeutsche Zucker in the development of isomalt in the joint venture Palatinit Sussungsmittel GmbH. Isomalt is obtained from sucrose in a two-step process in which it is first enzymatically rearranged to produce the reducing disaccharide isomaltulose, which is then hydrogenated to give the **13a**/**13b** mixture known generically as isomalt. The sweetness potency of isomalt is moderately dependent on sucrose reference concentration; $P_w(1) = 0.28$ and $P_w(10) = 0.45$ have been reported. Isomalt is generally approved for food usage in a number of countries, including the United Kingdom, Switzerland, France, Israel, The Netherlands, South Africa, Austria, Australia, Denmark, Finland, Sweden, West Germany, Italy, and Norway. In addition, specific category approvals have been given in a number of other countries. In the United States, isomalt was affirmed as GRAS in 1990 following the publication of the last of four scientific papers detailing the results of safety assessment studies on isomalt (W. Irwin, personal communication, 1990). As is the case for all sweeteners of this type, the bioavailable energy content for isomalt is controversial. Although some studies suggest isomalt to be fully caloric, it is generally accepted that 2.0 cal/g is approximately correct. Thus, isomalt use may permit only a 50% reduction of caloric intake when used as a substitute for fully nutritive sweeteners. Ziesenitz and Siebert have indicated the intestinal discomfort experienced as a consequence of isomalt consumption to be relatively mild (82).



(**13a**) $R_1 = OH, R_2 = H$
(**13b**) $R_1 = H, R_2 = OH$

Fructooligosaccharide Sweetener

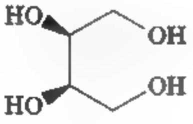
Fructooligosaccharide sweetener (FO sweetener) is a mixture of fructooligosaccharides of the type found naturally in onions, asparagus, Jerusalem artichoke tubers, and wheat. Specifically, it is largely a mixture of kestose (**14a**), nystose (**14b**), and 1-β-fructofuranosyl-nytose (**14c**). Typically, the ratio of these sugars in FO sweetener is 28:60:12. Meiji Seika Company of Japan has conducted most of the development work of FO sweetener, to which they have given the brand name Neosugar®. The sweetness potency of FO sweetener has been reported to be 0.4 to 0.6 times that of sucrose, depending on reference concentration. FO sweetener is prepared from sucrose by treatment with the microbial enzyme fructosyl transferase. To date, FO sweetener is approved for food use only in Japan. It has been reported that the glycolytic breakdown of FO sweetener in the small intestine is slight and that FO sweetener is therefore a nonnutritive sweetener (88). In other studies, however, it has been demonstrated that FO sweetener is extensively fermented in the large intestine (82). Studies specifically conducted on nystose suggest a very substantial calorie provision to the host following anaerobic microbial fermentation in the large intestine (81). The allocation of a precise value for the bioavailable calories of FO sweetener is difficult; a value of at least 2.0 cal/g is probably realistic, however. The intestinal discomfort and laxation effects promoted by FO sweetener were indicated to be high by Ziesenitz and Siebert (82). This report is consistent with the results of a clinical study by Stone-Dorshow and Levitt (89).



(**14a**) n = 1
(**14b**) n = 2
(**14c**) n = 3

Erythritol

Erythritol is a four-carbon polyol of structure **15**. It is present naturally in a wide range of microorganisms, plants, and animals and, as such, is present in many foods. This natural form of erythritol is the symmetrical isomer and is more properly referred to as *meso*-erythritol. Erythritol has been commercially developed by at least two companies, Mitsubishi in Japan and Eridania Beghin-Say in Europe. In the United States, Mitsubishi Chemical Company and Cargill have formed a partnership known as M&C Sweeteners for the purpose of commercializing erythritol. Erythritol's properties and food applications have recently been reviewed (90). The sweetness potency of erythritol has been reported to be 0.6 to 0.7 times that of sucrose. Unfortunately, flavor and temporal profile data, as have been reported for most of the other sweeteners discussed in this review, are not available for erythritol. However, it is the general consensus that erythritol exhibits a clean sweet taste with a sugarlike temporal profile. Erythritol has fair solubility in water (37% at 25°C) and is very stable. It is prepared on yeast fermentation of glucose. It is easily isolated in a purity of >99% on recrystallization from the filtered fermentation broth. At this time, erythritol has already experienced significant use in foods and beverages in Japan and is expected to see use in many other countries. It was affirmed as GRAS in the United States in 1997. Of all the sugar MNSs that have been commercially developed, erythritol is the most promising because it is lowest in bioavailable calories and it is also the lowest in intestinal discomfort. It has been conservatively estimated that the bioavailable calorie content of erythritol is 0.2 cal/g. This estimate assumes that the portion of a normal dose (ie, 20–50 g in a single intake) that is not absorbed is completely fermented to short-chain fatty acids by the anaerobic bacteria of the colon. In fact, however, erythritol is very poorly fermented by these bacteria (G. DuBois and G. Stark, unpublished results, 1988), and therefore the actual bioavailable calorie content of erythritol is zero or near zero. The minimal intestinal discomfort of erythritol follows from the fact that it is readily absorbed from the small intestine, taken up into the bloodstream, and excreted in the urine. Thus, most of the fermentative and osmotic effects of a large quantity of sugar MNS in the colon are avoided.



(15)

Summary

Only the high-potency sweeteners (saccharin, cyclamates, acesulfame-K, aspartame, sucralose, alitame, etc) allow the food technologist the opportunity to formulate noncaloric products. Even this opportunity, however, is limited to a few product categories (eg, CSDs) where the product bulking and other nonsweet taste properties of sucrose are not required. Other food product categories require properties of sucrose in addition to sweet taste (eg, bulking and freezing-point depression), and these can be realized only when employing sugar MNSs (eg, maltitol or isomalt), perhaps in combination with a high-potency sweetener. Most of these one-for-one sugar substitutes are substantially caloric, however, and therefore only reduced calorie food products may be formulated in product categories requiring their use. In addition, most sugar MNSs may have laxative effects. Thus, at this point in time, fully satisfactory zero- or low-calorie food products may be formulated only in product categories where sweet taste is the sole property of sucrose required. Other food product categories will be significantly impacted by the drive for zero- or low-calorie products only on the identification of a new ingredient. To meet this need, this new substance must deliver all or most of the properties of sucrose and, at the same time, be both truly noncaloric and without laxative effects. A strong need exists for such a food ingredient.

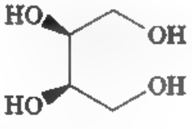
NEW DEVELOPMENTS IN NONNUTRITIVE SWEETENERS

Significant progress has been made in the identification of novel high-potency nonnutritive sweeteners. Most of the effort aimed at this objective has been focused on the dipeptide structural class of sweeteners in follow-up of the 1965 discovery of aspartame, the first dipeptide sweetener. The search for analogues of aspartame was intensified by the 1975 report by Fujino and coworkers of Takeda Chemical Industries that dipeptide ester **16** exhibits a clean sweet taste, with $P_w(8.3) = 33{,}200$ times that of sucrose (91). This represents more than a 200-fold potency increase over aspartame. Problematical, however, was that **16** is much less stable than aspartame. Nonetheless, **16**, along with aspartame, are exciting lead compounds that stimulated research in at least eight laboratories, including the following:

1. *Pfizer Pharmaceutical Company*: Hendrick and coworkers discovered alitame (**6**), as has already been described (58).
2. *Proctor & Gamble Company*: Rizzi and coworkers discovered dipeptide ester **17** (92). They reported **17** to exhibit $P_w(8–10) = 16{,}450$ and to be substantially more stable than aspartame. Problematical for **17**, however, is a delayed AT and a prolonged ET.
3. *Shanghai Institute of Organic Chemistry*: Zheng and coworkers discovered dipeptide ester **18** (93). This compound was said to be stable and to exhibit $P_w(10) = 2000$.
4. *General Foods Corporation*: In 1988, Roy et al. also discovered dipeptide ester **18** as well as analogue **19**. They reported these compounds to be stable and to exhibit $P_w(8.6) = 2300$ and $P_w(8.5) = 1133$ times that of sucrose, respectively (94).
5. *Takasago Corporation*: Takasago chemists disclosed their discovery of the same compounds (ie, **18** and **19**) as were discovered by the General Foods chemists (95).

Erythritol

Erythritol is a four-carbon polyol of structure **15**. It is present naturally in a wide range of microorganisms, plants, and animals and, as such, is present in many foods. This natural form of erythritol is the symmetrical isomer and is more properly referred to as *meso*-erythritol. Erythritol has been commercially developed by at least two companies, Mitsubishi in Japan and Eridania Beghin-Say in Europe. In the United States, Mitsubishi Chemical Company and Cargill have formed a partnership known as M&C Sweeteners for the purpose of commercializing erythritol. Erythritol's properties and food applications have recently been reviewed (90). The sweetness potency of erythritol has been reported to be 0.6 to 0.7 times that of sucrose. Unfortunately, flavor and temporal profile data, as have been reported for most of the other sweeteners discussed in this review, are not available for erythritol. However, it is the general consensus that erythritol exhibits a clean sweet taste with a sugarlike temporal profile. Erythritol has fair solubility in water (37% at 25°C) and is very stable. It is prepared on yeast fermentation of glucose. It is easily isolated in a purity of >99% on recrystallization from the filtered fermentation broth. At this time, erythritol has already experienced significant use in foods and beverages in Japan and is expected to see use in many other countries. It was affirmed as GRAS in the United States in 1997. Of all the sugar MNSs that have been commercially developed, erythritol is the most promising because it is lowest in bioavailable calories and it is also the lowest in intestinal discomfort. It has been conservatively estimated that the bioavailable calorie content of erythritol is 0.2 cal/g. This estimate assumes that the portion of a normal dose (ie, 20–50 g in a single intake) that is not absorbed is completely fermented to short-chain fatty acids by the anaerobic bacteria of the colon. In fact, however, erythritol is very poorly fermented by these bacteria (G. DuBois and G. Stark, unpublished results, 1988), and therefore the actual bioavailable calorie content of erythritol is zero or near zero. The minimal intestinal discomfort of erythritol follows from the fact that it is readily absorbed from the small intestine, taken up into the bloodstream, and excreted in the urine. Thus, most of the fermentative and osmotic effects of a large quantity of sugar MNS in the colon are avoided.



(**15**)

Summary

Only the high-potency sweeteners (saccharin, cyclamates, acesulfame-K, aspartame, sucralose, alitame, etc) allow the food technologist the opportunity to formulate noncaloric products. Even this opportunity, however, is limited to a few product categories (eg, CSDs) where the product bulking and other nonsweet taste properties of sucrose are not required. Other food product categories require properties of sucrose in addition to sweet taste (eg, bulking and freezing-point depression), and these can be realized only when employing sugar MNSs (eg, maltitol or isomalt), perhaps in combination with a high-potency sweetener. Most of these one-for-one sugar substitutes are substantially caloric, however, and therefore only reduced calorie food products may be formulated in product categories requiring their use. In addition, most sugar MNSs may have laxative effects. Thus, at this point in time, fully satisfactory zero- or low-calorie food products may be formulated only in product categories where sweet taste is the sole property of sucrose required. Other food product categories will be significantly impacted by the drive for zero- or low-calorie products only on the identification of a new ingredient. To meet this need, this new substance must deliver all or most of the properties of sucrose and, at the same time, be both truly noncaloric and without laxative effects. A strong need exists for such a food ingredient.

NEW DEVELOPMENTS IN NONNUTRITIVE SWEETENERS

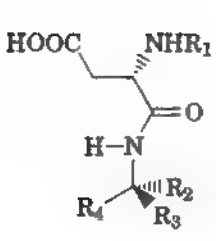
Significant progress has been made in the identification of novel high-potency nonnutritive sweeteners. Most of the effort aimed at this objective has been focused on the dipeptide structural class of sweeteners in follow-up of the 1965 discovery of aspartame, the first dipeptide sweetener. The search for analogues of aspartame was intensified by the 1975 report by Fujino and coworkers of Takeda Chemical Industries that dipeptide ester **16** exhibits a clean sweet taste, with $P_w(8.3) = 33{,}200$ times that of sucrose (91). This represents more than a 200-fold potency increase over aspartame. Problematical, however, was that **16** is much less stable than aspartame. Nonetheless, **16**, along with aspartame, are exciting lead compounds that stimulated research in at least eight laboratories, including the following:

1. *Pfizer Pharmaceutical Company*: Hendrick and coworkers discovered alitame (**6**), as has already been described (58).
2. *Proctor & Gamble Company*: Rizzi and coworkers discovered dipeptide ester **17** (92). They reported **17** to exhibit $P_w(8\text{–}10) = 16{,}450$ and to be substantially more stable than aspartame. Problematical for **17**, however, is a delayed AT and a prolonged ET.
3. *Shanghai Institute of Organic Chemistry*: Zheng and coworkers discovered dipeptide ester **18** (93). This compound was said to be stable and to exhibit $P_w(10) = 2000$.
4. *General Foods Corporation*: In 1988, Roy et al. also discovered dipeptide ester **18** as well as analogue **19**. They reported these compounds to be stable and to exhibit $P_w(8.6) = 2300$ and $P_w(8.5) = 1133$ times that of sucrose, respectively (94).
5. *Takasago Corporation*: Takasago chemists disclosed their discovery of the same compounds (ie, **18** and **19**) as were discovered by the General Foods chemists (95).

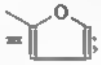
6. *Coca-Cola Company*: Iacobucci and coworkers reported dipeptide ester **20** (P_w = 1930) (96) in 1988 and dipeptide amide **21** (P_w = 2500) (97) in 1994.
7. *University of California at San Diego*: Goodman and coworkers discovered **22** as the preferred product candidate (98,99). Sweetener **22** was reported to be 800 to 1000 times more potent than sucrose and also to be much more stable than aspartame.
8. *Université Claude Bernard*: Nofre and Tinti discovered aspartame derivative **23**, which they named neotame and which they report exhibits $P_w(10)$ = 10,000 (100). This sweetener is reported to be equivalent to aspartame in sweetness quality, although it is not more stable than aspartame in acidic products. This new sweetener has been commercially developed by Monsanto. A FAP for tabletop use was filed on neotame in December 1997, and a second petition for general use was filed in January 1999.

A rational approach to engineering safety into a high-potency nonnutritive sweetener is to construct it from building blocks that are common components of the diet. Aspartame is exemplary of such a rationale. Clearly, a protein sweetener would also be consistent with this idea because of the natural amino acid subunit composition. This logic led to a program on the synthesis of thaumatin variants at International Genetic Engineering, Inc. (Ingene) (101). The objective was to identify analogues having a more sucroselike taste. Although the preparation of improved thaumatin analogues has been reported, it appears that the level of improvement is insufficient to justify commercialization. A similar program was undertaken by Kim at the University of California at Berkeley based on another sweet protein known as monellin (102,103). Monellin is a two-chain protein having a molecular weight of 10,700; it is roughly equivalent in sweetness potency to thaumatin and is similarly disadvantaged by an atypical temporal profile. Guided by X-ray crystallographic data, the Kim group designed and genetically engineered an equally potent, and much more stable, single-chain analogue of monellin. This sweetener has also been expressed in plants, including lettuce and tomatoes. Several other sweet proteins have been identified in nature, including the mabinlins (104), curculin (105), and brazzein (106). All these proteins exhibit a sweetness slow to develop that lingers relative to that of sucrose. Nonetheless, studies aimed at the identification of analogues with a more sugarlike taste may be a fruitful area for future research.

Progress has also been made in the identification of novel sugar MNSs. One type of carbohydrate-based nonnutritive sweetener that has been pursued is based on the L- or so-called left-handed sugars (107). It is argued that these substances, being enantiomerically related to the natural D- or right-handed sugars, will be nonmetabolizable and therefore nonnutritive. However, evidence has been presented that one of these sweeteners, L-glucose, is fermented by human fecal bacteria (82). Thus, L-glucose, and perhaps all the L-sugars, are partially caloric and may cause significant intestinal discomfort as well. Of greater

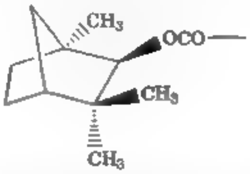


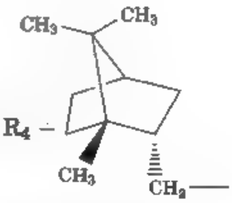
(16) $R_1 = H$; $R_2(R_3) = COOCH_3$; $R_3(R_2) = H$; R_4 = FnOCO

(17) $R_1 = H$; $R_2(R_3) =$  ; $R_3(R_2) = H$; R_4 = FnOCO

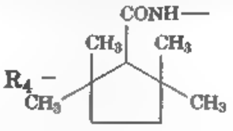
(18) $R_1 = H$; $R_2 = CH_3$; $R_3 = H$; R_4 = FnOCO

(19) $R_1 = H$; $R_2 = CH_3$; $R_3 = CH_3$; R_4 = FnOCO

FnOCO = 

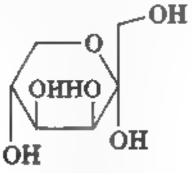
(20) $R_1 = H$; $R_2(R_3) = COOCH_3$; $R_3(R_2) = H$; R_4 = 

(21) $R_1 = H$; $R_2 = CH_2CH_3$; R_3 = CONH-(S)-CH(CH_2CH_3)Ph

(22) $R_1 = H$; $R_2 = CH_3$; $R_3 = H$; R_4 = 

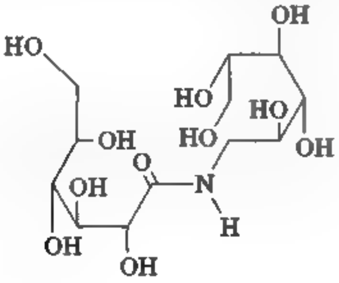
(23) $R_1 = (CH_2)_2C(CH_3)_3$; $R_2 = COOCH_3$; $R_3 = H$; $R_4 = CH_2Ph$

potential interest is D-tagatose (**24**), a hexose sugar formed from lactose in heated milk and is claimed by Zehner and associates to be noncaloric (108,109). MD Food Ingredients has developed a commercial process to prepare **24** from lactose and is initiating a GRAS affirmation petition on it for general use in foods and beverages. D-Tagatose is claimed to exhibit a clean sweet taste, with $P_w = 0.5$. In rigorous studies, D-tagatose was estimated by MD Food Ingredient scientists to exhibit a bioavailable calorie content of 2.0 Cal/g.



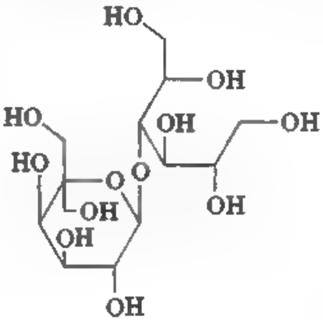
(**24**)

A breakthrough in the design of novel sugar MNSs was described by DuBois et al. in 1992 (110). They identified a wide variety of cleanly sweet sugar MNSs. This work focused on the design of replacements for sucrose and, therefore, to reproduce the colligative properties of sucrose (freezing point depression, etc), novel molecules with molecular weight comparable to that of sucrose were designed. In addition, this work was based on recognition of the fact that neutral, polyhydroxylic organic compounds of low molecular weight, in general, are sweet. Thus, in the attempt to reproduce the taste of sucrose, novel molecules with the same ratio of C atoms to OH groups as exhibited by sucrose (ie, C/OH = 1.5) were identified as targets for synthesis and evaluation. One of the preferred sugar MNS sweeteners identified in this work is polyol **25**. This sweetener exhibits a clean sweet taste and a P_w of 0.46 which is independent of sucrose reference concentration. This polyol is very stable and quite water soluble. Sugar MNS **25** is easily prepared in quantitative yield in one step on reaction of gluconolactone with 1-amino-1-deoxy-sorbitol. The latter starting material is commercially available and is easily prepared by reductive amination of glucose with ammonia. Of particular interest is the fact that **25** is not fermented at all by human fecal bacteria and therefore has an apparent bioavailable calorie content of 0 Cal/g.



(**25**)

At about the same time as the work on **25** and related compounds was described by DuBois et al., Mazur and associates at Proctor & Gamble described another series of sugar MNSs that also appear to have 0 Cal/g bioavailable calorie content (111). They referred to these new sugar MNSs as the 5-C-(hydroxymethyl)hexoses. The preferred compound in the Mazur series of compounds is **26**, which is prepared in three steps from lactose. Sugar MNS **26** is claimed to exhibit a clean sweetness, a P_w of 0.5, and it is not fermented at all by human fecal bacteria. Further, it is claimed to function well as a sugar replacement in a broad range of food products.



(**26**)

CONCLUSION AND FUTURE DEVELOPMENT

In summary, the nonnutritive sweetener market in the United States is dominated by aspartame and saccharin. In the near future, it is reasonable to expect that the recently approved acesulfame-K and sucralose, as well as cyclamates and alitame, which are not yet approved, may be competitors in this market. In the longer term, it is likely that blends of these sweeteners will be the most favored nonnutritive sweetener systems. Sweetener cost, as a consequence of the competition among these sweeteners as well as the substantial sweetness synergies realized in sweetener blends, is thus likely to be only a minor component of food product manufacturing cost. This statement is applicable, however, only to food categories in which the sucrose function may be fulfilled by a high-potency sweetener alone. Categories that require the type of functionality delivered by a sugar MNS such as maltitol, isomalt, lactitol, fructooligosaccharide sweetener, and erythritol are very unlikely ever to be favored by low-sweetener cost. The colligative properties of sucrose that are requisite for such applications necessitate one-for-one replacement. These materials have high manufacturing cost, relative to sucrose. In addition, the laxative effects when consumed at high levels are a disadvantage. A strong need exists for a sugar MNS that is truly noncaloric and nonlaxative.

BIBLIOGRAPHY

1. G. R. T. Ross, ed., and trans., "Aristotle," in *Aristotle: De-Sensu and de Memoria*, Cambridge University Press, Cambridge, U.K., 1906, pp. 41–99 (originally published ca. 330 B.C.).
2. C. Fahlberg and I. Remsen, *Berichte* **12**, 469–473 (1879).

3. R. H. Mazur, J. M. Schlatter, and A. H. Goldkamp, *J. Am. Chem. Soc.* **91**, 2684 (1969).
4. U.S. Pat. 2,803,551 (August 20, 1957), F. J. Helgren (to Abbott Laboratories).
5. M. Meilgaard, G. Vance Civille, and B. T. Carr, *Sensory Evaluation Techniques*, Vol. II, CRC Press, Boca Raton, Fla., 1987, pp. 5–6.
6. B. T. Carr et al., in C.-T. Ho and C. H. Manley, eds., *Flavor Measurement*, Marcel Dekker, New York, 1993, pp. 219–237.
7. G. E. DuBois and J. F. Lee, *Chem. Senses* **7**, 237–246 (1983).
8. G. E. DuBois, G. A. Crosby, and R. A. Stephenson, *J. Med. Chem.* **24**, 408–428 (1981).
9. R. J. Ronk, in J. H. Shaw and G. G. Roussos, eds., *Sweeteners and Dental Caries*, Information Retrieval, Inc., Washington, D.C., 1978, pp. 131–144.
10. H. W. Schultz, *Food Law Handbook*, AVI Publishing Company, Westport, Conn., 1981.
11. *Code of Federal Regulations*, Title 21 (Food and Drugs), Section 182.1, U.S. Government Printing Office, Washington, D.C., 1988, pp. 385–386.
12. *Code of Federal Regulations*, Title 21 (Food and Drugs), Section 570.30, p. 611 U.S. Government Printing Office, Washington, D.C., 1989, p. 611.
13. J. P. Marshall and J. A. Pollard, *Food Legislation Surveys No. 1: Sweeteners—International Legislation*, 4th ed., Leatherhead Food, R. A., United Kingdom (1987).
14. F. J. Broulik, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic & Professional, London, 1996, pp. 35–55.
15. G. Vettorazzi, in R. D. Middlekauf and P. Shubik, eds., *International Food Regulation Handbook*, Marcel Dekker, New York, 1989.
16. "Toxicological Evaluation of Certain Food Additives with a Review of General Principles and of Specifications," World Health Organization Technical Report Series No. 539, Geneva, Switzerland, 1974, p. 11.
17. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, U.S. Food and Drug Administration, Bureau of Foods, 1982, pp. 1–19.
18. *Chemical Marketing Reporter*, Vol. 256, Schnell Publishing Company, New York, 1999.
19. H. R. Moskowitz, ed., *Product Testing and Sensory Evaluation of Foods*, Food and Nutrition Press, Westport, Conn., 1983.
20. G. E. DuBois et al., in D. E. Walters, F. T. Orthoefer, and G. E. DuBois, eds., *Sweeteners: Discovery, Molecular Design, and Chemoreception*, American Chemical Society, Washington, D. C., 1991, pp. 261–276.
21. B. E. Homler, in L. D. Stegink and L. J. Filer Jr., eds., *Aspartame: Physiology and Biochemistry*, Marcel Dekker, New York, 1984.
22. L. Hyvonen and P. Koivistoinen, in G. G. Birch and K. J. Parker, eds., *Nutritive Sweeteners*, Applied Science Publishers, Englewood, N.J., 1982, pp. 135–137.
23. F. J. Helgren, M. F. Lynch, and F. J. Kirchmeyer, *J. Am. Pharm. Assoc. (Sci. Ed.)* **44**, 353–355 (1955).
24. L. M. Bartoshuk, *Science* **205**, 934–935 (1979).
25. J. F. Gent and L. M. Bartoshuk, *Chem. Senses* **7**, 265–272 (1983).
26. S. Pecore and B. T. Carr, "Sweeteners: Carbohydrate and Low Calorie, International Conference," paper presented at The Agricultural and Food Chemistry Division of the American Chemical Society Symposium, Los Angeles, Calif., September 22–25, 1988.
27. E. M. Whelan, *The Conference Board Magazine* **16**, 54 (1977).
28. G. J. Walter and M. L. Mitchell, in L. O'Brien Nabors and R. C. Gelardi, eds., *Alternative Sweeteners*, Marcel Dekker, New York, 1986, pp. 15–41.
29. A. G. Renwick, *Comments Toxicol.* **3**, 289–305 (1989).
30. S. M. Cohen et al., *Cancer Res.* **58**, 2557–2561 (1998).
31. *Code of Federal Regulations*, Title 21 (Food and Drugs), Section 180.37, U.S. Government Printing Office, Washington, D.C., 1988, pp. 378–379.
32. *The Merck Index*, 10th ed., Merck & Co., Rahway, N.J., 1983.
33. L. F. Audrieth and M. Sveda, *J. Org. Chem.* **9**, 89 (1944).
34. B. A. Bopp, R. C. Sonders, and J. W. Kesterson, *CRC Crit. Rev. Toxicol.* **16**, 213–306 (1986).
35. L. O'Brien-Nabors and W. T. Miller, *Comments Toxicol.* **314**, 307–315 (1989).
36. *Food Chemical News*, November 13, 1989, p. 19.
37. K. M. Beck, in T. E. Furia, eds., *CRC Handbook of Food Additives*, Vol. 11, 2nd ed., CRC Press, Boca Raton, Fla., 1980, p. 125.
38. T. Fazio, J. W. Howard, and E. O. Haenni, *J. Assoc. Off. Anal. Chem.* **53**, 1120–1128 (1970).
39. "Cyclamates," Hearings before Subcommittee No. 2 of the Committee on the Judiciary House of Representatives, Serial No. 2, Ninety-Second Congress, Washington, D.C., September 29, 30 and October 6, 1971.
40. Zhong Hua Fang BA (Hong Kong, China), quotation for sodium cyclamate: $2.30/kg (April 28, 1999).
41. R. E. Baldwin and B. M. Korschgen, *J. Food Sci.* **44**, 938–939 (1979).
42. J. J. Wiseman and M. R. McDaniel, paper presented at the Institute of Food Technology Meeting, Chicago, Ill. (1989)
43. G. DuBois, "Sweeteners, Nonnutritive," in Y. H. Hui, ed., *Encyclopedia of Food Science and Technology*, John Wiley & Sons, New York, 1991.
44. L. D. Stegink and L. J. Filer, Jr., eds., *Aspartame, Physiology and Biochemistry*, Marcel Dekker, New York, 1984, pp. 29–199, 289–653.
45. H. H. Butchko and F. N. Kotsonis, *Comments Toxicol.* **3**, 253–278 (1989).
46. C. Tschanz et al., eds., *The Clinical Evaluation of a Food Additive: Assessment of Aspartame*, CRC Press, Boca Raton, Fla., 1996.
47. *NutraSweet Technical Applications Manual*, The NutraSweet Company, Deerfield, Ill., 1987, p. 5.
48. E. G. Burton et al., *J. Nutr.* **119**, 713–721 (1989).
49. U.S. Pat. 4,704,288 (November 3, 1987), J. H. Tsau and J. G. Young (to The NutraSweet Company).
50. K. Clauss and Harald Jensen, *Angew. Chem.* (International Edition in English) **12**, 869–876 (1973).
51. D. G. Mayer and F. H. Kemper, eds., *Acesulfame-K*, Marcel Dekker, New York, 1991.
52. G.-W. von Rymon Lipinski and D. Maver, *Comments Toxicol.* **3**, 279–287 (1989).
53. G.-W. von Rymon Lipinski and B. E. Huddart, *Chem. Ind.* **6**, 427–432 (1983).
54. *United States Food Additive Petition*, Hoechst Celanese Corporation, Somerville, N.J.
55. L. Hough and S. P. Phadnis, *Nature* (London) **263**, 800 (1976).
56. U.S. Pat. 4,435,440 (March 6, 1984), L. Hough (to Tate & Lyle Public Limited Company, Reading, England).

57. M. R Jenner, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic and Professional, London, 1996, pp. 253–262.
58. U.S. Pat. 4,411,925 (October 25, 1983), T. M. Brennan and M. E. Hendrick (to Pfizer, Inc.).
59. M E. Hendrick, H. L. Mitchell, and P. R. Murray, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic and Professional, London, 1996, pp 226–239.
60. R. M. Horowitz and B. Gentili, *J Agric. Food Chem.* **17**, 696–700 (1969).
61. R. M. Horowitz and B. Gentili, in *Product Testing and Sensory Evaluation of Foods*, Food and Nutrition Press, Westport, Conn., 1983, pp. 135–153.
62. R. L. Smith et al., *Food Technol.* **10**, 72–81 (1996).
63. M. G. Lindley, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic & Professional, London, 1996, pp. 240–252.
64. G. A. Crosby and R. E. Wingard, Jr., in C. A. M. Hough, K. J. Parker, and A. J. Vlitos, eds., *Developments in Sweeteners-1*, Applied Science Publishers, New York, 1979, pp. 137–140.
65. S.-H. Kim and G. E. DuBois, in S. Marie and J. R. Piggott, eds., *Handbook of Sweeteners*, Blackie, Glasgow, 1991, pp. 116–185.
66. K. C. Phillips, in T. H. Grenby, ed., *Developments in Sweeteners-3*, Elsevier Applied Science, New York, 1987, pp. 1–43.
67. B. Crammer and R. Ikan, in T. H. Grenby, ed., *Developments in Sweeteners-3*, Elsevier Applied Science, New York, 1987, pp. 45–64.
68. B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983–1990 (1950).
69. G. E. DuBois, in H. J. Hess, ed., *Annual Reports in Medicinal Chemistry*, Vol. 17, Academic Press, New York, 1982, pp. 323–332.
70. L. O'Brien Nabors and G. E. Inglett, in *Product Testing and Sensory Evaluation of Foods*, Food and Nutrition Press, Westport, Conn., 1983, pp. 311–313.
71. *Fed. Regist.* **50**, 21043–21045 (1985).
72. J. D. Higginbotham, in *Product Testing and Sensory Evaluation of Foods*, Food and Nutrition Press, Westport, Conn., 1983, pp. 103–134.
73. S.-H. Kim, A. Devos, and C. Ogata, *Trends Biol. Sci.* **13**, 13–15 (1988).
74. B. L. Oser, R. A. Ford, and B K. Bernard, *Food Technol.* **38**, 66–89 (1984).
75. T. Takemoto et al., *Yakugaku Zasshi* **103**, 1167–1173 (1983).
76. A. D. Kinghorn and D. D. Soejarto, *CRC Crit. Rev. Plant Sci.* **4**, 79–120 (1986).
77. M. J. Mohlenkamp, oral presentation, *International Symposium on Sweeteners*, Jerusalem, Israel, July 14–20, 1996.
78. U.S. Pat. 3,741,776 (June 26, 1973), M. Mitsuhashi, M. Hirao, and K. Sugimoto (to Hyashibara Company, Okayama, Japan).
79. I. Fabry, in T H. Grenby, ed., *Developments in Sweeteners-3*, Elsevier Applied Science, New York, 1987, pp. 83–108.
80. M. Heume and A. Rapaille, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic & Professional, London, 1996, pp 85–108.
81. P. J. Secard and Y. LeBot, paper presented at the Agricultural and Food Chemistry Division of the American Chemical Society Symposium, *Sweeteners: Carbohydrate and Low Calorie*, International Conference, Los Angeles, Calif., September 22–25, 1988.
82. S. C. Ziesenitz and G Siebert, in T. H. Grenby, ed., *Developments in Sweeteners-3*, Elsevier Applied Science, New York, 1987, pp. 83–108.
83. C. H. Den Uyl, in T. H. Grenby, ed., *Developments in Sweeteners-3*, Elsevier Applied Science, New York, 1987, pp. 83–108.
84. W. E. Irwin, paper presented at The Agricultural and Food Chemistry Division of theamerican Chemical Society Symposium, *Sweeteners: Carbohydrate and Low Calorie*, International Conference, Los Angeles, Calif., September 22–25, 1988.
85. P. J. Strater, in *Product Testing and Sensory Evaluation of Foods*, Food and Nutrition Press, Westport, Conn., 1983, pp. 217–244.
86. S. C. Ziesenitz, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic and Professional, London, 1996, pp. 109–133.
87. I. Willibald-Ettle and H. Schiweck, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic and Professional, London, 1996, pp. 134–149.
88. T. Oku, T. Tokunaga, and N. Hosoya, *J. Nutr.* **114**, 1574–1581 (1984).
89. T. Stone-Dorshow and M. D. Levitt, *Am. J. Clin. Nutr.* **46**, 61–65 (1987).
90. J. Goossens and M. Gonze, in T. H. Grenby, ed., *Advances in Sweeteners*, Blackie Academic and Professional, London, 1996, pp. 150–186.
91. M. Fujino et al., *Chem. Pharm. Bull.* **24**, 2112–2117 (1976).
92. U.S Pat. 4,692,513 (September 8, 1987), R. B. Blum et al. (to The Proctor & Gamble Company).
93. G. Z. Zeng and S. T. Wei, *Molecular Recognition of Taste*, Science Publisher, Beijing, China, 1984
94. U.S. Pat. 4,766,246 (August 23, 1988), P. R. Zanno, R. E. Barnett, and G. M. Roy (to General Foods Corporation).
95. Jpn. Pat. 61291596 (December 22, 1986), A. Nagakura et al. (to Takasago Perfumery Co., Ltd., Japan).
96. U.S. Pat. 4,788,069 (November 29, 1988), G. A. Iacobucci, J. G. Sweeny, and J. G. King III (to The Coca-Cola Company).
97. U.S. Pat. 5,286,509 (February 15, 1994), L. L. D'Angelo and J. G. Sweeny (to The Coca-Cola Company).
98. U.S. Pat. 4,571,345 (February 18, 1986), M. S. Verlander, W. D. Fuller, and M. Goodman (to Cumberland Packing Corp.).
99. W. D. Fuller, M. Goodman, and M. S. Verlander, *J. Am. Chem. Soc.* **107**, 5821–5822 (1985).
100. U.S. Pat. 5,480,668 (January 1, 1996), C. Nofre and J.-M. Tinti (to Universite Claude Bernard).
101. International Pat. Application W085/01746 (April 25, 1985), M. Grunstein, S. Holienberg, R. Koduri, and T. Date (to Beatrice Companies, Inc.).
102. International Pat. Application W088/10265 (December 29, 1988), S.-H. Kim and J. M. Cho (to Lucky Biotech Corp., Emeryville, Calif.).
103. S.-H. Kim et al., *Prot. Eng.* **2**, 571–575 (1989).
104. X. Liu et al., *Eur. J. Biochem.* **211**, 281–287 (1993).
105. H. Yamashita, S. Theerasilp, and Y. Kurihara, *Tenth International Symposium on Olfaction and Taste*, Oslo, Norway, 1989, p. 77.

106. U.S. Pat. 5,346,998 (September 13, 1994), B. G. Hellekant and D. Ming (to Wisconsin Alumni Research Foundation).
107. U.S. Pat. 4,262,032 (April 14, 1981), G. V. Levin (to Biospherics, Inc., Rockville, Md.).
108. G. V. Levin et al., *Am. J. Clin. Nutrition* **62**, 11615–11685 (1995).
109. U.S. Pat. 4,786,722 (November 22, 1988), L. R. Zehner (to Biospherics, Inc.).
110. G. E. DuBois et al., *J. Chem. Soc. Chem. Commun.*, 1604–1605 (1992).
111. A. W. Mazur and M. J. Mohlenkamp, in M. Yalpani, ed., *New Technologies for Healthy Foods & Nutraceuticals*, ATL Press, Mt. Prospect, Ill., 1997, pp. 124–142.

GRANT E. DUBOIS
The Coca-Cola Company
Atlanta, Georgia

SYRUPS (STARCH SWEETENERS AND OTHER SYRUPS)

Pure crystalline glucose (dextrose) and glucose-containing syrups are hydrolysis products of starch and can be referred to as starch-based sweeteners or starch sugars. Crystalline maltose and high maltose content syrups are also produced by starch hydrolysis. Starch-based sweeteners are produced almost exclusively from corn starch in the United States, and the syrups are usually called corn syrups. Elsewhere, other starch sources are also used, such as wheat, rice, potato, and tapioca, to name a few. Although the commercial products are analytically distinguishable, they have in common the general methods of preparation and many properties and applications.

Starch-based sweeteners display increasing sweetness with increasing degree of conversion until complete conversion to D-glucose is achieved. Additional sweetness can be gained by isomerizing the glucose molecule to fructose. Sweetness is impacted by concentration as well as the content and stereochemical configuration of the individual saccharides. When tasted by different taste panels at approximately 10 wt % solids concentration, the various starch sweeteners exhibit a range of sweetness relative to sucrose. This range is illustrated in Figure 1. The degree of conversion can be quantified by the dextrose equivalent (DE) of the products. From the lesser converted maltodextrins through the corn syrups to pure glucose, sweetness increases from about zero to 60 to 85% that of sucrose. Then, as glucose is isomerized to fructose, sweetness continues to rise, up to a value of 105 to 135% that of sucrose when tasting pure fructose solutions.

Dextrose is the common or commercial name for D-glucose, the monosaccharide liberated by complete hydrolysis of starch. Maltose is a disaccharide that is produced by the action of β-amylase enzyme on liquefied starch. Corn syrups (starch hydrolysates having a DE value of 20 or greater) are clear, colorless, viscous liquids prepared by hydrolysis of starch to solutions of dextrose, maltose, and higher molecular weight saccharides. Maltodextrins have a lower degree of hydrolysis than corn syrups, with DE values less than 20. High-fructose corn syrups (HFCS) are products containing fructose produced by enzymatic isomerization of dextrose. Starch sugar production and pricing data for a number of countries around the world are shown in Table 1. It should be noted that accurate production data are sometimes difficult to obtain, prices are usually published list prices, and quantity discounts are normal. Production and price data shown in this article should be considered as approximations only.

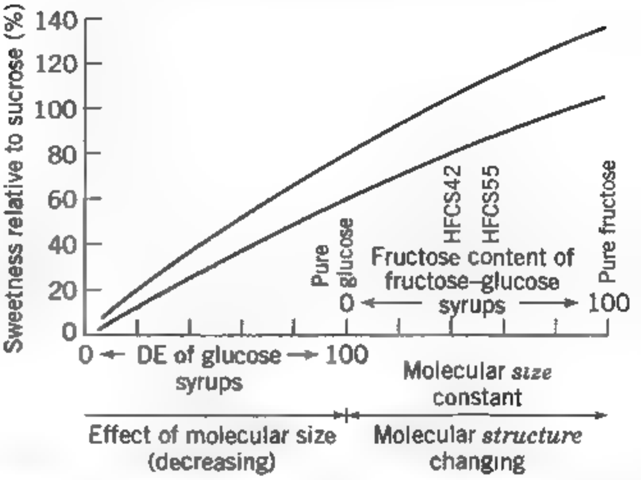


Figure 1. Range of sweetness of starch sugars relative to sucrose. All data at about 10 wt % solids water solutions. *Source:* Ref. 1, reproduced with permission.

Maple syrup is a nutritive sweetener produced by evaporating maple tree sap to a concentrated solution of carbohydrate (nearly all sucrose). Molasses is a syrup produced as a by-product during sugar purification. Because of differing sucrose recoveries and the presence of gums, minerals, and nitrogenous materials, molasses composition can vary. Sorgo syrup is made from the juice extracted from the canes of the sorghum plant. Palatable syrups can also be made from artichokes by hydrolyzing the inulin contained in aqueous extracts to form fructose-rich syrups. Today, inulin-based fructose is produced commercially, but the primary source is reported to be chicory.

The per capita consumption of the major nutritive sweeteners is shown in Figure 2. Before the development of HFCS in the late 1960s, starch sugars comprised less than one-fifth of the total; sucrose was the sweetener of choice. As HFCS rapidly captured market share, primarily in the beverage industry, and consumption of dextrose, corn syrups, and maltodextrins continued to grow, starch sugars increased their market share. Around 1985, they captured more than half of the total nutritive sweetener market. Today, they hold about 60% of the nutritive sweetener market in the United States. The growth of starch sugars in various market segments is shown in Figure 3. The growth of the beverage segment and its dominant position today can be clearly seen.

DEXTROSE

Dextrose (D-glucose, corn sugar, starch sugar, blood sugar, grape sugar) is by far the most abundant sugar in nature It occurs either in the free state (monosaccharide form) or chemically linked with other sugar moieties. In the free

Table 1. Production and Pricing of Starch-Based Sweeteners by Country

Country	Glucose syrups	Maltose syrups	Crystalline glucose		Total sugar	Fructose-glucose syrup	
			Monohydrate	Anhydrous		42%	55%
			United States				
Production	3200		645	25	68	2300	5000
Price, US$/kg ds	0.30		0.62	1.25	0.88	0.41	0.45
			Europe (EU15)				
Production	2200[a,b]		320[a,b]		45	330[a,b]	
Price, US$/kg ds	0.73[c]		0.96[c]		0.88	0.69[c]	
			Canada				
Production	118[d]					300[e]	
			People's Republic of China				
Production	250	100	250[f]		50	100[e]	
Price, RMB[g]/kg ds	4	4	6		3	6[h]	
			Japan				
Production		440[e,i]	35[d]	39[d]	44[d]	845[a,e]	
Price, yen/kg/ds	34[a]	46[a]	95[a]	126[a]	113[d]	48[a]	54[a]
			Latin America[d]				
Production	285		9			36	155
Price, US$/kg ds	0.30		2.86			0.17	0.40
			Korea				
Production	200[j]		33[d,k]	3[d,k]	55[d,k]	250[e,j]	
Price, won/kg ds	384[l]		420[l]	420[l]	390[m]	390[l]	
			South Africa				
Production		175[j]	3				
Price, US$/kg ds	0.43[n]		0.68				
			Australia[o]				
Production	57					4.3	
			Malaysia[o]				
Production	3.8		2.4				
Price, US$/kg ds	0.57		0.59				

Note: all production values in 10^3 t of dry substance-per year; 1996 values used except where noted.
Source. Ref. 2, reproduced with permission.

[a] 1995 data.
[b] 90 + % of the volume shown is sold within the EU15; less than 10% is sold in other European countries.
[c] 1983 data.
[d] 1986 data.
[e] Combined production of 42% db and 55% db fructose-bearing syrups shown.
[f] Most crystalline (pure) dextrose is monohydrate; some is anhydrous. Main uses are medical in nature.
[g] At the time of the preparation of this chart, US$1.00 = 8.5 RMB.
[h] Price for 42% db fructose syrup shown; only one known producer of 55% fructose syrup in the China.
[i] Combined production data for glucose and maltose syrups.
[j] 1992 data.
[k] Totals for the production of these three products in 1992 virtually unchanged from 1986, individual product data not available in 1992 so 1986 values are shown.
[l] 1994 data.
[m] 1991 data.
[n] 1996 list price for 43 DE glucose syrup.
[o] 1987 data.

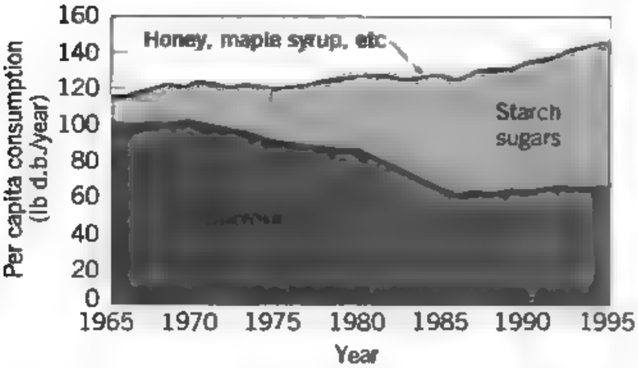


Figure 2. Per capita U.S. sweetener consumption by type. *Source:* Ref. 1, reproduced with permission.

state, it occurs in substantial quantities in honey, fruits, and berries. As a polymer of anhydrodextrose units, it occurs in starch, cellulose, and glycogen. Sucrose is a disaccharide of dextrose and fructose. Commercial production of dextrose by hydrolysis of starch yields white crystalline sugars that are either anhydrous ($C_6H_{12}O_6$) or hydrated by a single water molecule ($C_6H_{12}O_6 \cdot H_2O$). Dextrose monohydrate, with its one molecule of water of crystallization per molecule of sugar, separates from concentrated solutions at <50°C (122°F). Anhydrous D-glucose does not contain water of crystallization and separates at 50 to 115°C (122 to 239°F). Another anhydrous form, β-D-glucose, separates if crystallization is carried out at temperatures >110 to 115°C (230 to 239°F).

In one of the first attempts to prepare commercial dextrose, grapes were used as the starting material (3). It is generally conceded that Kirchoff's work in 1811 was the forerunner of the starch hydrolysate industry (4). It was first reported in 1815 that acid conversion of starch to sugar was the result of hydrolysis of the starch rather than dehydration, and that the starch sugar was identical with grape sugar (5). It was not until 1842, however, that starch hydrolysis was first practiced commercially in the United States. Crystalline dextrose became a main industrial product when a commercially feasible crystallization process was patented in 1923 (6). This patent was one of the rare instances of a crystal structure being the subject of a patent claim. Fifty metric tons of monohydrate dextrose were sold in the United States in 1923. Today, the annual production is more than 500,000 metric tonnes, a 10,000-fold increase.

Properties

Physical properties of the three crystalline forms of dextrose are listed in Table 2. In solution, dextrose exists in both the α and β forms. When α-dextrose dissolves in water, its optical rotation diminishes gradually as a result of mutarotation until, after a prolonged time, an equilibrium value is reached (see arrows, Table 2). At this point, about 62% of the dextrose is present in the β form. This equilibrium value is not significantly changed over a wide range of temperatures and concentrations. The same equilibrium value apparently exists in anhydrous melts as well as in glassy materials. Pure crystalline β-dextrose is quite sensitive to moisture, and it changes to the more stable α-form if exposed to high humidity. At 25°C (77°F), α-dextrose monohydrate dissolves fairly rapidly, yielding a solution containing about 30 wt % dextrose. Very slowly thereafter, further quantities of dextrose dissolve until a saturated solution containing ca 50 wt % dextrose is obtained. The first phase of the dissolving process results from the limited solubility of α-dextrose. The slow subsequent dissolution is caused by the transformation of part of the dissolved α-dextrose to the more soluble β form. When saturation is finally reached, a mixture of α- and β-dextrose in solution is in equilibrium with solid α-dextrose monohydrate. At 25°C (77°F), anhydrous α-dextrose dissolves rapidly and beyond the limit of solubility of α-dextrose monohydrate. Since the monohydrate is the stable form at

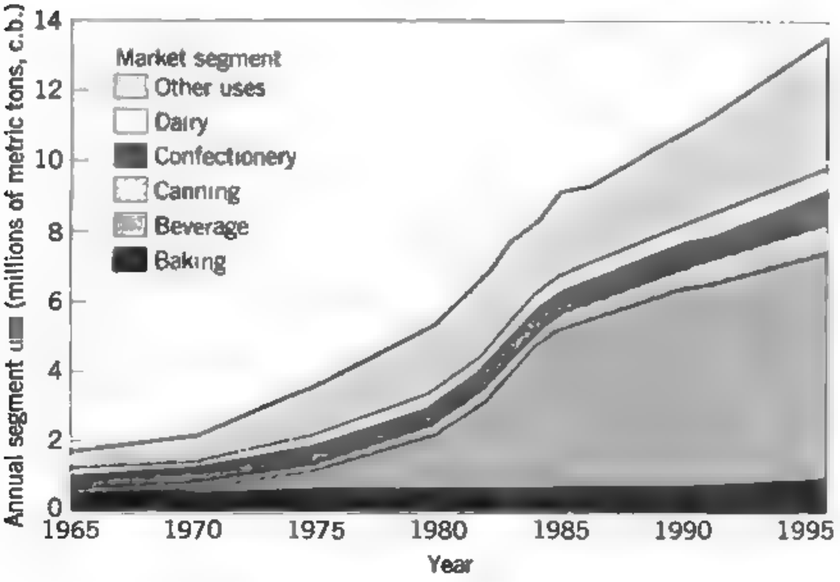


Figure 3. Applications of starch sugars in the United States, 1965–1996. *Source:* Ref. 1, reproduced with permission.

Table 2. Physical Properties of D-Glucose

Property	α-D-Glucose	α-D-Glucose hydrate	β-D-Glucose
Molecular formula	$C_6H_{12}O_6$	$C_6H_{12}O_6 \cdot H_2O$	$C_6H_{12}O_6$
Melting point (°C)	146	83	150
Solubility (at 25°C), g/100-g solution	$62 \rightarrow 30.2 \rightarrow 51.2^{a}$	$30.2 \rightarrow 51.2^{a,b}$	$72 \rightarrow 51.2^{a}$
Optical rotation, $[\alpha]_D$	$112.2 \rightarrow 52.7^{a}$	$112.2 \rightarrow 52.7^{a,b}$	$18.7 \rightarrow 52.7^{a}$
Heat of solution (at 25°C), J/g[c]	+59.4	+105.1	+26.0

[a] Equilibrium value.
[b] Anhydrous basis.
[c] To convert joules to calories, divide by 4.184.

this temperature, crystallization of the hydrate occurs to its limit of solubility and the pattern then follows that of the monohydrate as described earlier. The rate of attainment of equilibrium is increased by heating or in the presence of acids or bases. Data for the solubility of the equilibrium mixtures, as interpolated from previous data (7), are listed in Table 3.

Dextrose in solution or in solid form exists in the pyranose structural conformation. In solution, a small amount of the open-chain aldehyde form exists in equilibrium with the two cyclic forms (Fig. 4) and is responsible for the reducing properties of dextrose (8).

Dextrose exhibits the reactions of an aldehyde, a primary alcohol, a secondary alcohol, and a polyhydric alcohol. In acid solution, either after standing for a prolonged time or after heating, dextrose undergoes polycondensation, i.e., dehydration. This reaction yields a mixture of di- and oligosaccharides, most of which are the disaccharides gentiobiose and isomaltose. In acid solution and at high temperature, dehydration leads to formation of 5-hydroxymethylfurfural, which is a water-soluble, high-boiling, and relatively unstable compound. Polymerization of 5-hydroxymethylfurfural yields dark-colored compounds, and is an intermediate in the discoloration of sugar solutions (9). Dextrose decomposition under these conditions also yields levulinic and formic acids.

In mildly alkaline solution, the principal reaction of dextrose is partial transformation, i.e., isomerization, to fructose and other ketoses. D-Mannose, saccharinic acids, and other decomposition products are formed to a lesser extent. Highly alkaline solutions, particularly in the presence of atmospheric oxygen, can form a complex mixture of products of decomposition and rearrangement. Mild oxidation in slightly alkaline solution gives D-gluconic acid in quantitative yield. More vigorous oxidation with nitric acid yields glucaric acid, tartaric acid, oxalic acid, and other compounds resulting from fragmentation of the dextrose molecule. Alkaline Fehling's solution is reduced by dextrose with roughly 5 atoms of copper reduced per molecule of dextrose. Electrolytic reduction or catalytic hydrogenation of dextrose is practiced commercially to manufacture sorbitol.

When dextrose is heated with methanol containing a small amount of anhydrous hydrogen chloride, α-methyl-D-glucoside is obtained in good yield and can be isolated by crystallization. Similar reactions occur with higher alcohols, but the reaction products are more difficult to isolate by crystallization. Dextrose reacts with acid anhydrides in the presence of basic catalysts, yielding esters. The complete reaction gives the pentaacetylated derivative.

The reaction of dextrose with a nitrogen-containing compound, for example, amino acids or proteins, yields a series of intermediates that form pigments of varied molecular weight (Maillard reaction). The type of pigments produced is dependent on reaction conditions such as pH, temperature, and concentration of reactants (10).

Dextrose is the common intermediary metabolite in carbohydrate metabolism because other utilizable monosaccharides are converted to dextrose before they are further metabolized. Starch, glycogen, and the common monosaccharides are hydrolyzed enzymatically in the alimentary canal. Dextrose is normally absorbed into the portal-vein blood, by which it is transported first to the liver and then circulated to all parts of the body. Before dextrose or any other monosaccharide can be utilized metabolically, it must be phosphorylated and enter the glycolytic cycle. Other monosaccharides, such as galactose and fructose, eventually are converted to glucose-6-phosphate and metabolized like dextrose.

Manufacture

Until 1960, a commercial high dextrose content syrup was produced by acid-catalyzed hydrolysis of starch at elevated

Table 3. Solubility of Dextrose in Water

Temperature (°C)	Dextrose in solution (wt %)	Temperature (°C)	Dextrose in solution (wt %)
0	34.9	30	54.6
5	38.0	40	61.8
10	41.2	50	70.9
15	44.5	60	74.8
20	47.8	70	78.2
25	51.3	80	81.3

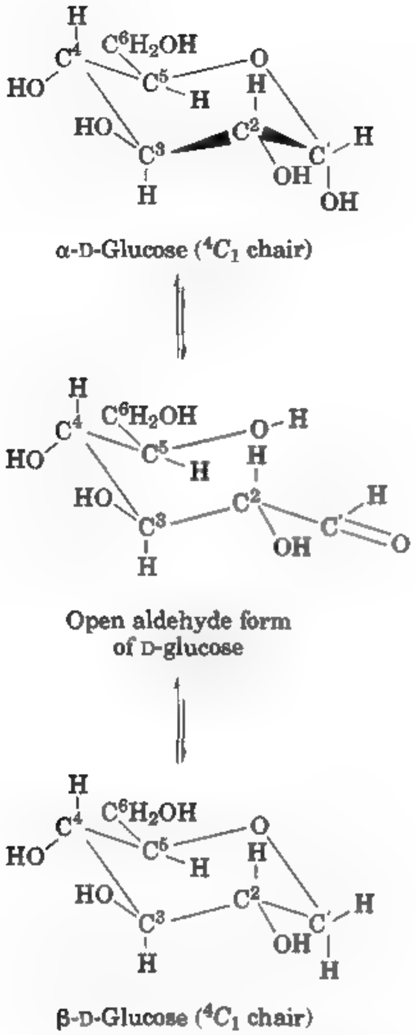

α-D-Glucose ($^4C_1$ chair)
Open aldehyde form of D-glucose
β-D-Glucose ($^4C_1$ chair)

Figure 4. Structures of D-glucose.

temperatures and pressures. Dextrose content of the starch hydrolysate was limited to about 86% dry basis (db) as a result of the formation of degradation products (11). Dextrose content was increased by partial or complete replacement of acid with one or more enzymes. These processes are referred to as acid–enzyme (A–E) or enzyme–enzyme (E–E), depending on whether initial starch hydrolysis (the starch thinning or liquefaction step) is conducted with acid or a bacterial α-amylase. In either case, subsequent conversion to dextrose (saccharification) is achieved with glucoamylase, a fungal enzyme that releases dextrose from the nonreducing end of starch polymers by successive hydrolysis of α-1,4 and 1,6 glucosidic linkages.

By 1960, the A–E process was in general use, and hydrolysate dextrose content was increased to 92 to 94% db (12). A typical process involved thinning a 30 to 35 wt % starch slurry with acid (HCl or H_2SO_4) at a temperature and pressure necessary to achieve a DE level of 10 to 20. DE is a measure of the reducing-sugar content of a starch hydrolysate calculated as dextrose and expressed as a percentage of the total dry substance. Formation of acid-reversion products during thinning limited the dextrose yield attained during saccharification. Higher dextrose content was achieved by the E–E process that was developed in the 1960s. This process used a heat-resistant bacterial α-amylase for starch thinning. Lower temperature and nearly neutral liquefaction conditions limited side reactions and resulted in hydrolysate dextrose contents of 95 to 97% db (11,13). Initial processes were based on α-amylase derived from the bacterium *Bacillus subtilis*. In a typical process, 30 to 40 wt % starch is thinned at 85°C (185°F) for 1 h followed by a short heat treatment at 120 to 140°C (248 to 284°F). Then, a second enzyme addition is made, and reaction at 85°C (185°F) is continued to complete the liquefaction step (Fig. 5). The resulting 10 to 15 DE hydrolysate is then saccharified with glucoamylase. The high temperature heat treatment is required to solubilize insoluble starch particles that are believed to be amylose–fatty acid complexes formed during initial liquefaction (14). Since the high temperature inactivates the enzyme, a second enzyme addition is necessary to continue hydrolysis before the complex reforms.

Elimination of the heat treatment step was made possible by the commercialization of α-amylases that are extremely thermostable and capable of efficient starch hydrolysis at a temperature above 100°C (212°F). Enzymes derived from *Bacillus stearothermophilus* (15) and *Bacillus licheniformis* (16) are used for this purpose (Fig. 6). Starch slurry is thinned continuously with a steam-injection heater at 30 to 40 wt % solids, pH 6 to 6.5, and 103 to 107°C (217 to 225°F) for 6 to 10 min. A 1 to 2 h hold at about 95°C (203°F) completes the thinning and yields a 10 to 15 DE

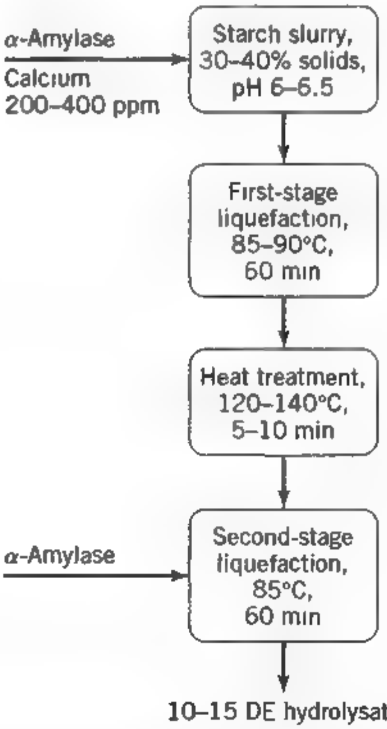

α-Amylase
Calcium 200–400 ppm
Starch slurry, 30–40% solids, pH 6–6.5
First-stage liquefaction, 85–90°C, 60 min
Heat treatment, 120–140°C, 5–10 min
α-Amylase
Second-stage liquefaction, 85°C, 60 min
10–15 DE hydrolysate

Figure 5. Simplified process flowsheet. Starch liquefaction using *Bacillus subtilis* α-amylase.

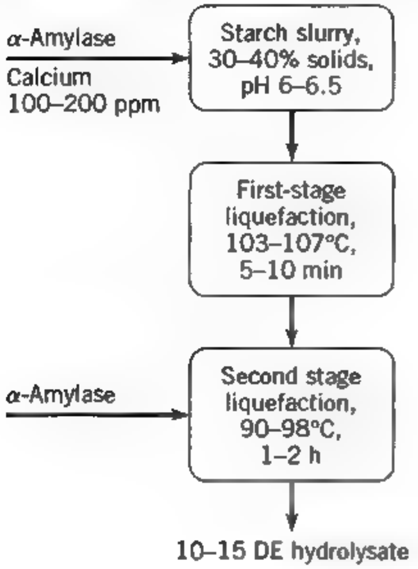

α-Amylase
Calcium
100–200 ppm
Starch slurry, 30–40% solids, pH 6–6.5
First-stage liquefaction, 103–107°C, 5–10 min
α-Amylase
Second stage liquefaction, 90–98°C, 1–2 h
10–15 DE hydrolysate

Figure 6. Simplified process flowsheet. Starch liquefaction using *Bacillus stearothermophilus* or *Bacillus licheniformis* α-amylase.

hydrolysate for saccharification. The high initial temperature is sufficient to disrupt the amylose–lipid complex. Concurrently, the thermostable enzyme hydrolyzes the starch to a point where reassociation of the complex cannot occur. The thermostable α-amylases are able to survive the initial liquefaction with little loss in activity since the half-life of α-amylases derived from *B. stearothermophilus* and *B. licheniformis* is about 2 h (15) and 1 h (16), respectively, at 105°C (221°F).

Regardless of the type of thinning utilized, dextrose is produced by the action of glucoamylase during saccharification (17). Glucoamylase is produced from strains of *Aspergillus niger* in submerged fermentation. A broth is obtained containing two or more glucoamylase isozymes, α-amylase, transglucosidase, and other enzymes, eg, protease and cellulase. The α-amylase assists in saccharification (11); however, transglucosidase catalyzes the formation of isomaltose and, therefore, must be removed before use. Removal of the enzyme is accomplished by adsorption on a clay mineral or by other techniques. Alternatively, selection for an *Aspergillus* mutant that does not produce transglucosidase eliminates the need for a removal step (18). Glucoamylase is also produced from a *Rhizopus* organism in Japan by surface fermentation; however, enzyme properties are somewhat inferior to those of *Aspergillus*.

Saccharification of liquefied starch hydrolysate is conducted by batch or continuous processes in large agitated reactors that are often several million liters in size. The reaction is conducted at 58 to 61°C (136 to 142°F), pH 4 to 4.5 with a glucoamylase dosage that is sufficient to produce the maximum dextrose yield in 1 to 4 days. At the normal solids level of 30 to 35 wt %, maximum hydrolysate dextrose content is about 95 to 96% db. During saccharification, isomaltose and maltose are produced by the reverse reaction (reversion) of glucoamylase, in which two dextrose molecules are combined. Consequently, if the reaction is extended beyond the time needed to achieve maximum dextrose content, the dextrose level decreases as a result of excessive formation of these reversion products.

Dextrose content can be increased by conducting saccharification at lower solids. As solids level is reduced, the forward reaction is favored and a higher dextrose content is achieved. At a solids level of 10 to 12 wt %, a dextrose content of 98 to 99% db can be attained (19). However, operation at low solids is not commercially viable because of increased evaporation cost, the need for larger saccharification tanks, and the risk of microbial infection.

Dextrose level can also be increased by using enzymes that enhance the action of glucoamylase during saccharification. Enzymes available for this purpose are pullulanase and *Bacillus megaterium* amylase. Pullulanase is specific for the hydrolysis of α-1,6 glucosidic linkages and, when used in combination with glucoamylase, increases the rate and extent of dextrose production (20). A *B. megaterium* enzyme has been commercialized that combines hydrolysis and transferase activity to assist glucoamylase in increasing dextrose content (21). Either enzyme is effective in increasing hydrolysate dextrose content by 0.5 to 1.0% db.

After saccharification, the high dextrose content hydrolysate is clarified by centrifugation or filtration (usually either precoat or membrane) to remove insolubles. Concentration (usually by evaporation) to lower the water content and refining to reduce color and ionic contaminants are the next steps. Refining can be done with activated carbon (either powdered or granular), or with ion exchange resins (decolorizing and/or demineralizing resins), either singly or in combination. A simplified flowsheet for the manufacture of high dextrose content products is shown in Figure 7.

Commercial dextrose products include crystalline monohydrate or anhydrous dextrose and liquid dextrose. In addition, the hydrolysate can be refined and evaporated to a high DE corn syrup or dried to a solid product. Compositions are listed in Table 4.

Crystalline dextrose products can be manufactured using either batch or continuous crystallization technology. Sites for crystal growth can be provided either by the addition of seed crystals or by spontaneous nucleation. Subsequent crystal growth is encouraged by maintaining a controlled degree of supersaturation. Control of the entire process is critical to ensure that the resulting crystals are of a size and shape that will readily separate from the remaining mother liquor in perforate bowl centrifuges to provide a dry product of high purity.

The predominant technique for the production of monohydrate dextrose uses batch cooling-type crystallizers, although continuous crystallization using either evaporation (23) or cooling (24) to maintain supersaturation have been described. A process employing a continuous precrystallizer supplying batch crystallizers has been used to increase productivity (25).

In the dominant process, clarified, refined, and concentrated liquor having a dextrose content above about 80% db is mixed with a substantial bed of seed crystals (typically 20 to 30 wt %) left from the previous batch in the

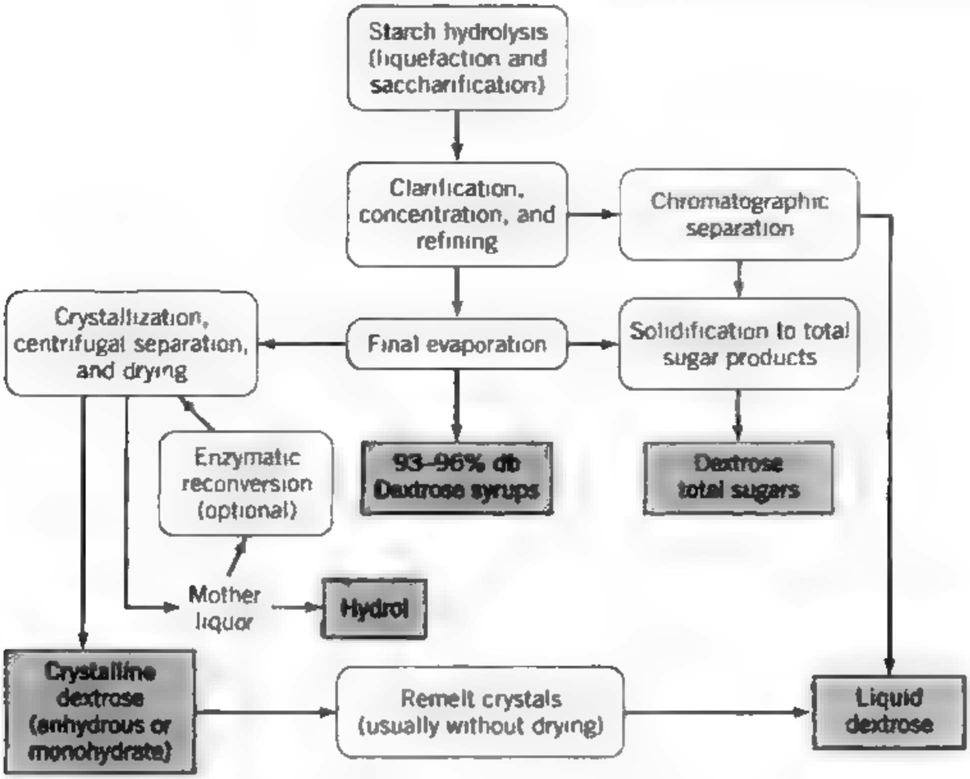

Starch hydrolysis (liquefaction and saccharification)
Clarification, concentration, and refining
Chromatographic separation
Crystallization, centrifugal separation, and drying
Final evaporation
Solidification to total sugar products
Enzymatic reconversion (optional)
93–96% db Dextrose syrups
Dextrose total sugars
Mother liquor
Hydrol
Crystalline dextrose (anhydrous or monohydrate)
Remelt crystals (usually without drying)
Liquid dextrose

Figure 7. Simplified process flowsheet for the manufacture of high dextrose content products.

Table 4. Saccharide composition of dextrose, high-fructose corn syrups, corn syrups, and maltodextrins (% db)

		Constituent							
Sweetener type	DE	Fructose	Dextrose	DP-2	DP-3	DP-4	DP-5	DP-6	DP-7+
Dextrose	>99.5	0	>99.5	<0.25	DP-3+ totals <0.1				
High DE corn syrup	97	0	95	<4	<1	DP-4+ totals <1			
HFCS42		43	52	DP-2+ totals 5					
HFCS55		55	40	DP-2+ totals 5					
HFCS90		90	7	DP-2+ totals 3					
Acid-converted corn syrup	25	0	2.5	7	8.5	6	6	13	57
	33	0	9	10	12	9	7	6	47
	43	0	19	14	12	10	8	7	30
Acid–enzyme corn syrup	43	0	6	45	12	3	2	2	30
Enzyme–enzyme corn syrup	28	0	3	11	14	DP-4+ totals 72			
	43	0	2	52	15	1	1	1	28
High-maltose corn syrup	50	0	4	65	15	DP-4+ totals 16			
	53	0	3	75	13	DP-4+ totals 9			
High-conversion corn syrup	70	0	46	29	7	5	3	2	8
Maltodextrin	1	0	0.3	0.1	0.2	DP-4+ totals 99.4			
	5	0	0.9	0.9	1	1.1	1.3	1.4	93.4
	10	0	0.5	2.5	4.2	3.4	3.6	5.4	81.4
	15	0	1.4	4.2	6.2	4.4	4.4	9.2	70.2
	18	0	1.6	4.7	6.5	4.6	4.6	10	68

Source: Refs. 2, 22, and Manufacturer's Product Data Sheets.
Notes: DP is the degree of polymerization (DP-2 represents disaccharides, DP-3, trisaccharides, etc). Values are typical for the syrup types shown but will vary depending on the process conditions used during manufacture. Values are expressed as weight percent dry basis.

crystallizer. The common form of crystallizer is a horizontal cylindrical tank fitted with a slowly turning agitator and a means of cooling the contents by indirect heat transfer. The initial temperature of the mixture is kept below 50°C (122°F) to ensure that crystals grow in the monohydrate form. The mass is slowly cooled, usually over a period of days, until about 50 to 60 wt % of the dry substance has crystallized out as monohydrate crystals of a form suitable for separation and washing.

The values for concentration, initial and final temperature and rate of cooling vary depending primarily on the purity of the supply material. Monohydrate dextrose crystals can be successfully grown in liquors of purity ranging from pure dextrose to syrups containing less than 60% db dextrose. Dextrose crystallizes to form monohydrate α-dextrose although the existence of a hydrated β-dextrose has been reported (26).

The resulting magma in the crystallizers is passed into perforated screen centrifuge baskets where it is spun to remove as much of the mother liquor as practical. Then a spray of water is applied to the rapidly rotating cake to wash off residual mother liquor. After spinning an additional period to remove as much liquid as practical, the cake is plowed out of the basket and fed to a dryer to reduce the moisture content to about 8.5 to 9.0%, slightly below the theoretical monohydrate moisture content.

Monohydrate crystallization can be done in several sequential steps starting with high purity hydrolysate to maximize the production of higher value crystalline product and to minimize the amount of mother liquor (usually sold as a lower value process co-product called Hydrol) that contains the polysaccharides that result from incomplete starch hydrolysis. Or, a portion of the mother liquor can be recycled and mixed with the incoming hydrolysate to provide an equivalent yield of crystals, usually about 75 to 80% db of the incoming hydrolysate. Such processes still result in 20 to 25 wt % of the hydrolysate supplied to the crystallization process being sold as Hydrol. Enzymatic reconversion of the mother liquor has been reported to allow nearly complete elimination of the production of lower value Hydrol (27).

The anhydrous form of dextrose is more fastidious than is the monohydrate and does not form easily separated crystals when crystallized from liquors below about 88 to 90% db dextrose content. Although it can be produced by batch crystallization, the predominant method uses vacuum evaporative crystallizers operated either continuously or in a batch mode. And because the starting material requires a minimum purity of about 95% db to produce purgable crystals, the dominant production process begins with a supply produced by remelting monohydrate crystals, resulting in the production of a very high purity product that meets the requirements of the *United States Pharmacopeia*. When producing USP-grade dextrose, the supply liquor can be passed through an ultrafilter membrane filter to reject pyrogens and make the final product acceptable for injection into the human body. Anhydrous α-dextrose is produced when the crystallization temperature is between about 55 and 100°C (about 131 and 212°F), preferably about 65°C (149°F) to minimize color development during the 6 to 8 h crystallization cycle required to produce a magma containing about 50% of the dry substance on the crystalline form. Crystals are separated, washed, and dried much like the monohydrate variety, although centrifuge cycles are usually shorter. Anhydrous β-dextrose crystallizes at temperatures above about 100°C (212°F), although the β-form will continue to crystallize at lower temperatures if all α-form crystals have been excluded from the crystallizer. The β-form is the most soluble and rapidly dissolves in water.

Clarified, refined high dextrose content hydrolysate can be evaporated to about 71% ds and sold as a liquid product. Or, it can be solidified to either a monohydrate or anhydrous total sugar product (containing all the polysaccharides that result from incomplete starch hydrolysis) using a variety of processes (28). If a higher purity product is desired, chromatographic separation technology can be used to separate the high dextrose content syrup into a very pure (>99.5% db dextrose) product and a raffinate stream that contains nearly all the polysaccharides that result from incomplete starch hydrolysis. The raffinate stream can either be concentrated and sold as a lower value (Hydrol-type) product or be enzymatically reconverted. The very pure dextrose syrup can be sold as a liquid product or converted to a very pure total sugar. Liquid dextrose can also be produced by remelting crystalline dextrose.

Dry dextrose products can be shipped in vapor-resistant bags usually containing 50 or 100 pounds, or the approximately equivalent metric sizes of 20, 25, and 40 kg. Reusable containers holding about 1 metric ton of material are used for customers who do not require bulk delivery but who do not want the bother and expense of bag opening and disposal. Bulk transport via truck or railcar (jumbo railcars can hold as much as 82 metric tons or 180,000 lb) is also used. Solid products should be stored below 40°C (104°F), and rapid temperature changes and extremes should be avoided to minimize product caking.

Although most liquid products are shipped in bulk trucks and railcars (the largest railcars holding as much as 90 metric tons), some products are sold in 200-L (55 gallon) drums and 1000-L (250-gallon) disposable containers. Most small customers are supplied the dry equivalent (where available) of the desired syrup until their needs grow large enough to justify the installation of facilities for bulk truck delivery.

Liquid product storage facilities must be carefully designed to minimize product oxidation and microbial problems, especially in the headspace of storage tanks. Typical storage conditions of common liquid starch sweeteners are shown in Table 5. Storage temperatures selected are a compromise between a low temperature that would minimize product color development with time and the higher temperatures needed to either reduce the viscosity of the lower DE products to allow pumping or to prevent formation of crystals in the higher DE syrups and HFCS.

Economic Aspects

The price of starch is a major factor in the cost of production of starch sugars such as crystalline dextrose. Equipment investment, notably for crystallizers, is another im-

Table 5. Typical Storage Conditions of Liquid Starch Sweeteners

Syrup type	Solids content (wt %)	Temp. (°C)	Factor-controlling storage conditions
42 DE acid syrup	80	38	Product viscosity
42 DE acid syrup	83	49	Product viscosity
60 DE acid–enzyme syrup	82	32	Product viscosity
60 DE acid–enzyme syrup	84	41	Product viscosity
95 DE high-dextrose syrup	71	49	Dextrose crystallization
HFCS42	71	32	Dextrose crystallization
HFCS55	77	27	Dextrose crystallization
HFCS90	80	16	Fructose crystallization
Liquid dextrose	71	52	Dextrose crystallization

portant factor. Since Hydrol usually sells for a small fraction of the price of dextrose, optimizing process yield also has a meaningful impact on profits. Nearly eliminating the concomitant production of Hydrol by enzymatic mother liquor reconversion has been shown to provide a double-digit profit increase (27).

The price of dextrose monohydrate since 1975 is given in Table 6. Although demand, processing capacity, and production costs no doubt exert some effect on pricing, dextrose price has averaged about 95% of sucrose price on a dry basis for the period 1975 to 1996. Compared to dextrose monohydrate, anhydrous dextrose sells at a premium; prices in early 1989 were $1.12/kg (50 cents/lb) for anhydrous dextrose and $1.21/kg (55 cents/lb) for USP anhydrous dextrose.

Analysis and Specifications

The United States and international specifications for crystalline dextrose are given in References 29 to 32, respectively. High-quality anhydrous dextrose produced for the pharmaceutical industry is prepared in accordance with specifications given in Reference 33. Typical product analyses include reducing sugar value, solution color, solution clarity, ash, particle size distribution, and moisture. High-performance liquid chromatography (HPLC) is used to measure the concentration of dextrose and other saccharides in solution.

Table 6. Wholesale List Prices for Some Starch Sugars in the United States ($/kg db)

Year	Dextrose	HFCS42	HFCS55	Corn syrup
1975	0.46	0.50	NA	0.34
1976	0.34	0.31	NA	0.25
1977	0.31	0.27	NA	0.18
1978	0.36	0.27	NA	0.19
1979	0.38	0.29	NA	0.22
1980	0.64	0.52	NA	0.32
1981	0.65	0.47	0.52	0.35
1982	0.60	0.31	0.41	0.30
1983	0.58	0.41	0.46	0.28
1984	0.58	0.44	0.50	0.28
1985	0.53	0.39	0.44	0.24
1986	0.52	0.40	0.44	0.23
1987	0.50	0.36	0.38	0.22
1988	0.56	0.36	0.41	0.26
1989	0.56	0.42	0.47	0.30
1990	0.54	0.43	0.48	0.31
1991	0.54	0.46	0.51	0.34
1992	0.54	0.46	0.51	0.33
1993	0.54	0.41	0.46	0.29
1994	0.57	0.44	0.49	0.34
1995	0.56	0.37	0.42	0.32
1996	0.56	0.41	0.45	0.36

Source: Data from USDA Economic Research Service publications.

Uses

The main use of dextrose is in the food-processing industry, where it is of value for its physical, chemical, and nutritive properties. Distribution of dextrose to various industries in the United States is shown in Table 7. In the baking industry, it serves primarily as a fermentable sugar that contributes to crust color. In the beverage industry, it is used as a source of fermentables in low-calorie beer and as a sweetener in beverage powders. In the canning industry, it supplies sweetness, body, and osmotic pressure and also contributes to better natural color retention in certain products. In the confectionery industry, it is used to supply sweetness and softness control and to regulate crystallization. Dextrose is also used in tableted products; flavor is often enhanced by the cooling effect obtained when dextrose hydrate dissolves in the mouth. In frozen desserts, it prevents oversweetness and improves flavor.

In many cases, dextrose is used in conjunction with sucrose. Although dextrose by itself is somewhat less sweet than sucrose, the combination with sucrose may be as sweet as pure sucrose at the same concentration. Dextrose is also used in the pharmaceutical industry for intravenous feeding as well as tableting and other formulations. In fermentation, dextrose is a raw material for biochemical synthesis, and, chemically, it is a raw material for sorbitol, mannitol, and methyl glucoside production. Dextrose in the form of unrefined hydrolysate is used as a raw material in yeast fermentations for production of alcohol that is used as an octane enhancer in gasoline.

HIGH-FRUCTOSE CORN SYRUPS

High-fructose corn syrups (HFCS, isosyrup, isoglucose) are concentrated solutions containing primarily fructose and dextrose with lesser quantities of higher molecular weight saccharides. HFCS is produced by partial enzymatic isomerization of dextrose hydrolysates followed by refining and

Table 5. Typical Storage Conditions of Liquid Starch Sweeteners

Syrup type	Solids content (wt %)	Temp. (°C)	Factor-controlling storage conditions
42 DE acid syrup	80	38	Product viscosity
42 DE acid syrup	83	49	Product viscosity
60 DE acid–enzyme syrup	82	32	Product viscosity
60 DE acid–enzyme syrup	84	41	Product viscosity
95 DE high-dextrose syrup	71	49	Dextrose crystallization
HFCS42	71	32	Dextrose crystallization
HFCS55	77	27	Dextrose crystallization
HFCS90	80	16	Fructose crystallization
Liquid dextrose	71	52	Dextrose crystallization

portant factor. Since Hydrol usually sells for a small fraction of the price of dextrose, optimizing process yield also has a meaningful impact on profits. Nearly eliminating the concomitant production of Hydrol by enzymatic mother liquor reconversion has been shown to provide a double-digit profit increase (27).

The price of dextrose monohydrate since 1975 is given in Table 6. Although demand, processing capacity, and production costs no doubt exert some effect on pricing, dextrose price has averaged about 95% of sucrose price on a dry basis for the period 1975 to 1996. Compared to dextrose monohydrate, anhydrous dextrose sells at a premium; prices in early 1989 were $1.12/kg (50 cents/lb) for anhydrous dextrose and $1.21/kg (55 cents/lb) for USP anhydrous dextrose.

Analysis and Specifications

The United States and international specifications for crystalline dextrose are given in References 29 to 32, respectively. High-quality anhydrous dextrose produced for the pharmaceutical industry is prepared in accordance with specifications given in Reference 33. Typical product analyses include reducing sugar value, solution color, solution clarity, ash, particle size distribution, and moisture. High-performance liquid chromatography (HPLC) is used to measure the concentration of dextrose and other saccharides in solution.

Table 6. Wholesale List Prices for Some Starch Sugars in the United States ($/kg db)

Year	Dextrose	HFCS42	HFCS55	Corn syrup
1975	0.46	0.50	NA	0.34
1976	0.34	0.31	NA	0.25
1977	0.31	0.27	NA	0.18
1978	0.36	0.27	NA	0.19
1979	0.38	0.29	NA	0.22
1980	0.64	0.52	NA	0.32
1981	0.65	0.47	0.52	0.35
1982	0.60	0.31	0.41	0.30
1983	0.58	0.41	0.46	0.28
1984	0.58	0.44	0.50	0.28
1985	0.53	0.39	0.44	0.24
1986	0.52	0.40	0.44	0.23
1987	0.50	0.36	0.38	0.22
1988	0.56	0.36	0.41	0.26
1989	0.56	0.42	0.47	0.30
1990	0.54	0.43	0.48	0.31
1991	0.54	0.46	0.51	0.34
1992	0.54	0.46	0.51	0.33
1993	0.54	0.41	0.46	0.29
1994	0.57	0.44	0.49	0.34
1995	0.56	0.37	0.42	0.32
1996	0.56	0.41	0.45	0.36

Source: Data from USDA Economic Research Service publications.

Uses

The main use of dextrose is in the food-processing industry, where it is of value for its physical, chemical, and nutritive properties. Distribution of dextrose to various industries in the United States is shown in Table 7. In the baking industry, it serves primarily as a fermentable sugar that contributes to crust color. In the beverage industry, it is used as a source of fermentables in low-calorie beer and as a sweetener in beverage powders. In the canning industry, it supplies sweetness, body, and osmotic pressure and also contributes to better natural color retention in certain products. In the confectionery industry, it is used to supply sweetness and softness control and to regulate crystallization. Dextrose is also used in tableted products; flavor is often enhanced by the cooling effect obtained when dextrose hydrate dissolves in the mouth. In frozen desserts, it prevents oversweetness and improves flavor.

In many cases, dextrose is used in conjunction with sucrose. Although dextrose by itself is somewhat less sweet than sucrose, the combination with sucrose may be as sweet as pure sucrose at the same concentration. Dextrose is also used in the pharmaceutical industry for intravenous feeding as well as tableting and other formulations. In fermentation, dextrose is a raw material for biochemical synthesis, and, chemically, it is a raw material for sorbitol, mannitol, and methyl glucoside production. Dextrose in the form of unrefined hydrolysate is used as a raw material in yeast fermentations for production of alcohol that is used as an octane enhancer in gasoline.

HIGH-FRUCTOSE CORN SYRUPS

High-fructose corn syrups (HFCS, isosyrup, isoglucose) are concentrated solutions containing primarily fructose and dextrose with lesser quantities of higher molecular weight saccharides. HFCS is produced by partial enzymatic isomerization of dextrose hydrolysates followed by refining and

Table 7. Utilization of Starch Sugars by Market Segment in the United States

Year	Baking	Beverage	Canning	Confectionery	Dairy	Total[a]
			Dextrose, thousands of metric ton/year[b]			
1965	178	8	21	37	6	468
1970	174	8	23	52	7	547
1975	157	18	15	62	7	561
1980	51	66	4	55	2	513
1985	56	81	2	58	1	479
1988	71	88	3	66	1	565
1996	—	—	—	—	—	700
			HFCS (all types), thousands of metric ton/year[c]			
1970	18	39	9	0.5	5	99
1975	129	279	64	4	36	716
1980	365	1,039	235	15	140	2,659
1985	428	4,246	288	39	213	6372
1988	410	5,097	394	60	253	7288
1996	—	—	—	—	—	9560
			Corn syrup (all types), thousands of metric ton/year[c]			
1965	209	36	104	410	159	1211
1970	222	93	98	439	213	1449
1975	315	205	174	416	278	2278
1980	196	384	126	446	241	2201
1985	151	377	150	496	290	2469
1988	166	400	150	456	238	2677
1996	—	—	—	—	—	3295

Note. Dash indicates data on individual market segments not available. 1996 data are author's personal estimates of U.S. production.

[a] Includes other market segments/applications not listed in table.

[b] Monohydrate basis (8.5–9.0% moisture).

[c] Commercial basis.

concentration. Commercial syrup products contain 42, 55, or 90% fructose on a dry-weight basis (db). Crystalline fructose is also commercially available in large quantities.

In nature, fructose (levulose, fruit sugar) is the main sugar in many fruits and vegetables. Honey contains about 50 wt % fructose on a dry-weight basis. Sucrose is composed of one unit each of fructose and dextrose combined to form the disaccharide. Fructose exists in polymeric form as inulin in plants such as Jerusalem artichokes, chicory, dahlias, and dandelions and is liberated by treatment with acid or enzyme (34).

Fructose was first isolated in 1847 (35). In 1874, it was recognized that fructose had advantages over sucrose as a sweetener for diabetics (36). Following the discovery of the alkaline conversion of dextrose to fructose in 1895 (37), a considerable number of investigations were conducted in an attempt to develop a commercial process (38). However, because of problems associated with color, off-flavor, degradation products, and low fructose yield, a process was never commercialized. Enzymatic conversion of dextrose to fructose with glucose isomerase was first reported in 1957 and patented in 1960 (39). Research in this area continued for several years in Japan, resulting in commercial production in 1966 (40) and a U.S. patent in 1971 (41). Japanese technology was licensed by Standard Brands and production was initiated in the United States in 1967 by a batch process. A 15 wt % fructose syrup was manufactured initially, followed in 1968 by a 42% db fructose product. In 1972, a continuous system was initiated using an immobilized enzyme process (42,43).

Properties

Fructose, a ketohexose monosaccharide, crystallizes as β-D-fructopyranose and has a molecular weight of 180 and a melting point of 102 to 104°C (216 to 219°F). In solution, fructose undergoes rapid mutarotation to a mixture of α-D- and β-D-fructopyranose and α-D- and β-D-fructofuranose (Fig. 8), during which time specific rotation changes from $-132°$ to $-96°$ (45). At equilibrium, about 60 to 70% of the fructose is in the β-pyranose form, and 20 to 30% is in the β-furanose form. The remainder exists as α-D-fructose. Relative amounts of each depend on temperature and pH. At equilibrium, the solubility of fructose in water is 80 wt % at 25°C (77°F). The crystalline form of fructose is the sweetest of the tautomeric forms, 1.8 times sweeter than sucrose. Because the crystalline form is the sweetest form, relative sweetness may change when the fructose dissolves in a high moisture or liquid food product. Regardless of the tautomeric form, fructose is sweeter than sucrose and thus can be used in reduced quantities to attain equivalent sweetness. A discussion of the metabolism of fructose is given in Reference 46.

HFCS is sweeter than conventional corn syrups because of the presence of fructose, although intensity of sweetness results from many factors, eg, temperature, pH, and concentration. In general, 42% db HFCS (HFCS42) has the same sweetness as sucrose, 55% db HFCS (HFCS55) is as sweet as medium invert and 2 to 5% sweeter than sucrose, and 90% db HFCS (HFCS90) is 15 to 30% sweeter than sucrose (47). Other properties of HFCS include high solubility, which reduces the possibility of crystallization during shipment; humectant properties allowing for increased shelf life of bakery products; easier decomposition of fructose during baking, resulting in improved color and flavor; and high osmotic pressure for containment of microbial growth.

Manufacture

HFCS42 is produced commercially by continuous isomerization of clarified and refined dextrose hydrolysate with immobilized glucose isomerase. Enriched syrup containing 90% db fructose is prepared by chromatographic separation and blended with HFCS42 to obtain HFCS55 (Fig. 9).

During the initial development of glucose isomerase, it became obvious that batch reactions would not be commercially feasible because of several factors. The long residence time required with soluble enzyme caused the production of color, off-flavors, and by-products, eg, psicose, which is a nonmetabolizable sugar formed under alkaline conditions. In addition, enzyme cost was high, and it became necessary to develop a process that allowed reuse of enzyme. Consequently, many different methods of immobilization were examined in an attempt to develop a continuous reaction system. As a result of these studies, two types of immobilization processes were developed for commercial use: whole-cell and soluble-enzyme processes.

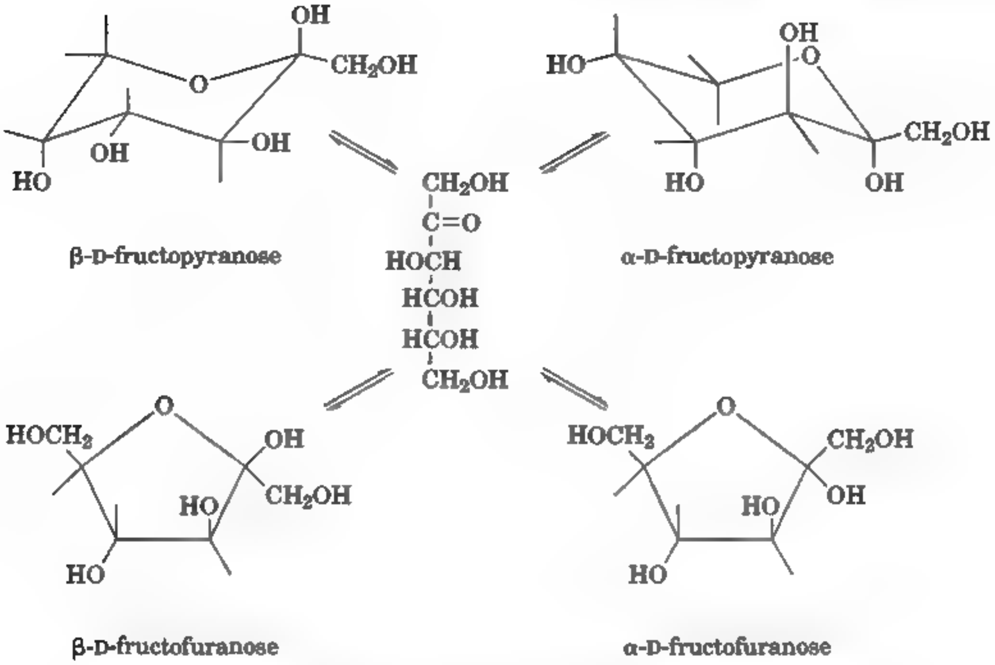

β-D-fructopyranose
α-D-fructopyranose
β-D-fructofuranose
α-D-fructofuranose
CH₂OH
C=O
HOCH
HCOH
HCOH
CH₂OH

Figure 8. Structures of fructose. *Source:* Ref. 44, reproduced with permission.

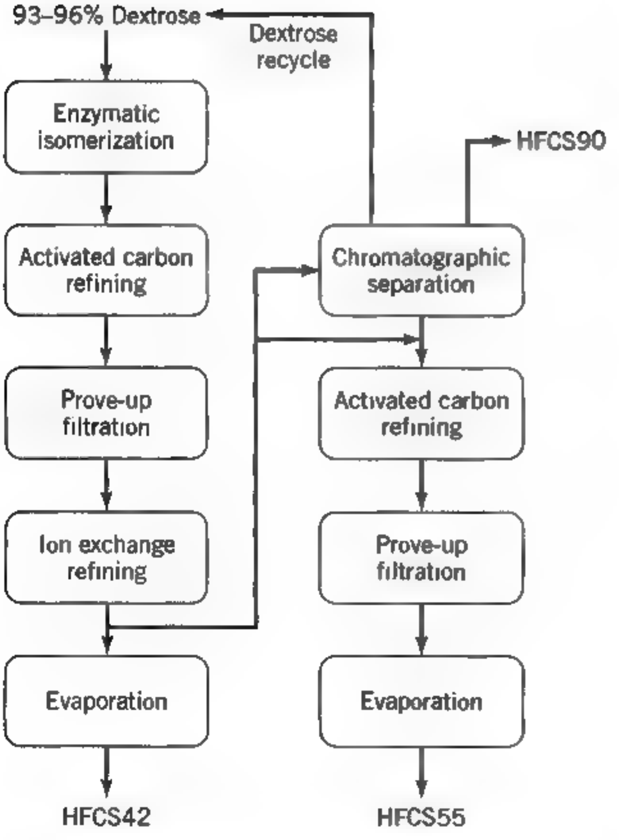

93–96% Dextrose
Dextrose recycle
Enzymatic isomerization
Activated carbon refining
Prove-up filtration
Ion exchange refining
Evaporation
HFCS42
HFCS90
Chromatographic separation
Activated carbon refining
Prove-up filtration
Evaporation
HFCS55

Figure 9. Simplified process flowsheet for fructose production.

In the whole-cell process, the microbial cell containing intracellular enzyme is recovered from the fermentation broth and treated to preserve enzymatic activity and maintain particle integrity. In one process (48), culture broth is centrifuged, and the concentrated cells are disrupted by homogenization and cross-linked with glutaraldehyde. The material is then diluted and flocculated with a cationic flocculant, and a final product is prepared by extrusion, drying, and sieving. Another method (49) involves fixing the cells in gelatin and cross-linking with glutaraldehyde, followed by washing, particle size classification, and drying.

In the soluble-enzyme process, enzyme is separated from the cells and purified before immobilization. Since glucose isomerase is an intracellular enzyme, the cell wall is first disrupted by physical (sonification, homogenization) or enzymatic methods. The solubilized glucose isomerase is then separated by centrifugation and/or filtration and concentrated by ultrafiltration. In one method of immobilization (50), binding takes place by simply contacting the soluble enzyme with a particle containing a combination of DEAE cellulose, titanium dioxide, and polystyrene. Other supports for glucose isomerase include anion-exchange resin, anion-exchange cellulose, porous ceramics, and porous alumina.

Glucose isomerase enzymes have been isolated from a variety of microbial sources (51,52). However, only a few bacterial organisms have been used to produce glucose isomerase for commercial use. These include *Streptomyces olivochromogenes, S. murinus, S. rubiginosus, Bacillus coagulans, Actinoplanes missouriensis,* and *Microbacterium arborescens.* Enzyme properties vary depending on the source, but all are similar in terms of operational pH and temperature.

HFCS is produced from dextrose hydrolysate that has been clarified, refined (including demineralization), and evaporated to 40 to 50 wt % dry substance (ds), as shown in Figure 9. Magnesium is added as a cofactor to maintain isomerase stability and to prevent inhibition by trace amounts of residual calcium. If the calcium level is below 1 ppm, a magnesium level (added as $MgSO_4$) of 0.4 mM is sufficient, although a higher calcium level requires additional magnesium. Hydrolysate is passed through a fixed bed of immobilized isomerase at a controlled flow rate. Process conditions depend on the particular enzyme system used but are generally 55 to 65°C (131 to 149°F), pH 7.5 to 8.5, and an initial reaction time of 1 h or less.

Maximum fructose content at equilibrium is 50 to 55% db; however, residence time is adjusted to attain 42 to 45% db fructose since a greatly increased reaction time is required to attain higher levels. The enzyme can be used for as long as several months, and compensation for loss in activity during operation is made by regulating the residence time, ie, flow rate through the column. Enzyme reactors are operated in parallel or in series until activity is reduced to about 10% of the initial level. Isomerized hydrolysate containing about 42% db fructose is adjusted to pH 4 to 5, refined to remove color and salts using resins either alone or in combination with activated carbon, and concentrated by evaporation to about 71 wt % solids. Product shipment is by tank trucks or by rail. Since the dextrose content in the HFCS product is more than 50% db, storage at 32 °C (90°F) is required to prevent crystallization. If crystallization occurs, the syrup is heated to 38°C (100°F) to dissolve crystals before unloading.

Products containing higher levels of fructose are produced by chromatographic separation of HFCS42 and were first commercially available in limited quantities in 1976. Enrichment is accomplished by passing HFCS42 through a column of absorbent (usually a strong-acid cation-exchange resin) containing calcium or other cation groups acting as a counter ion. Fructose is retained to a greater degree than dextrose or oligosaccharides, and therefore a stream containing most of the nonfructose is collected first in a raffinate stream, followed by elution of a fructose-enriched extract with water. Separation can be achieved using batchwise, semicontinuous, or continuous operation; commercial operations favor continuous processes. The continuous procedure involves a simulated moving bed in which feed and desorbent enter the column at different points while fructose and raffinate streams are withdrawn at others. Points of entry and withdrawal are changed periodically to correspond to flow through the column, and hence separation efficiency is maximized. Typical operation involves the addition of HFCS42 at a dry substance content of 50 to 60% to a column at 50 to 70°C (122 to 158°F). Dextrose-rich raffinate is recycled to isomerization columns for additional production of HFCS42. Enriched HFCS is blended with HFCS42 to produce a product containing 55% db fructose. A solids level of 77% ds is suitable for shipment at 27°C (81°F) with minimal dextrose crystallization problems. In addition, enriched HFCS at about 90% db fructose is evaporated to 80% ds for shipment as an essentially noncrystallizable syrup at normal room temperature.

Fructose of >99% purity is produced by crystallization to the β-D-fructopyranose form. In one process, chromatographic separation of 42% HFCS is used to produce a fraction containing 97% fructose. The material is evaporated to 70% solids, and 50% of the fructose is recovered by crystallization in 80 to 100 h (53).

Economic Aspects

Published U.S. wholesale prices of 42 and 55% HFCS since 1980 are listed in Table 6. The 1989 price for 90% fructose was about $1/kg (45 cents/lb) on a commercial basis. The price of HFCS has not been intimately tied to the price of sucrose for a number of years. Supply and demand seem to be the most important factors influencing price.

During the early 1970s, the price of refined sugar increased dramatically, resulting in a concomitant increase in the price of HFCS and the construction of new production facilities. When sugar prices fell during 1975 to 1978, HFCS prices also dropped, falling to a level of about 30 to 35% below sugar rather than the 15 to 20% discount that had existed previously. Excess production capacity also lowered HFCS prices, delaying the start-up of some new plants. However, during the late 1970s through the early 1980s, demand for HFCS increased significantly in the soft-drink industry, and the discount to sucrose decreased, especially for the sweeter HFCS55 product.

As can be seen from Table 7, the new sweetener also took market share from existing starch sugars in some segments, especially the more expensive dextrose. Dextrose consumption in the United States actually fell for a time before rebounding in the late 1980s.

Because of the increased demand and higher sucrose prices, processing capacity was again increased throughout the industry. From 1980 to 1985, per capita consumption of HFCS increased from 8.7 to 20.5 kg (19 to 45 lb) due primarily to 100% substitution for sugar in many soft drinks. Because of this near maximum penetration of the soft drink market, HFCS growth slowed to 2 to 4% per year through the end of the 1990s, and prices relative to sucrose fell again.

Then, in 1991, consumption accelerated to a level of 5 to 6% annual growth, and prices firmed until the industry once more made substantial capacity increases that caused prices relative to sucrose to fall. This was particularly painful in 1996 when corn prices increased dramatically, averaging nearly $4.00/bushel ($160/metric ton) for the year. Prices in 1997 reached lows not seen in 20 years but have begun to firm as demand continues to grow by about 1 billion pounds per year. HFCS prices in the United States are expected to continue to be influenced primarily by factory utilization and to a lesser degree by sugar prices and the cost of corn.

Analysis and Specifications

International (including U.S.) specifications for HFCS are the same as those for corn syrup. Fructose content is determined by high-performance liquid chromatography; dry substance, by refractive index; and color, by spectroscopy.

High-fructose corn syrup is used as a partial or complete replacement for sucrose or invert sugar in food applications to provide sweetness, flavor enhancement, fermentables, or humectant properties. It is used in soft drinks, baking, canned fruit, dairy products, and confections (Table 7). HFCS is used in combination with sucrose as well as other corn sweeteners. The main application of HFCS55 is in soft drinks. HFCS containing 90 wt % fructose is used in low calorie or specialty foods because of its high sweetness and, therefore, reduced usage level and lower caloric value. Other uses are as a liquid tabletop sweetener or as a honey-flavored product for use in baked goods and confections. Crystalline fructose is essentially pure and is used at a level that provides sweetness at a lower caloric level than that in other sweeteners. Uses include health foods and medicines.

CORN SYRUPS AND MALTODEXTRINS

Corn syrups (glucose syrup, starch syrup) are concentrated solutions of partially hydrolysed starch (having a DE value greater than 20) that contain dextrose, maltose, and higher molecular weight saccharides. Corn syrups are generally sold in the form of viscous liquid products, although solid forms are also available, especially in the lower DE ranges. They vary in physical properties (eg, viscosity, humectancy, hygroscopicity, sweetness, and fermentability), primarily as a result of their individual saccharide distributions. Maltodextrins are starch hydrolysates having a DE value less than 20.

Properties

Corn syrups are defined on the basis of reducing-sugar content as having a DE of 20 or greater. They are classified into four types: Type I syrups have DE values from 20 up to 38; type II, from 38 up to 58; type III, from 58 up to 73, and type IV, 73 and above. Syrups may also be classified as acid-conversion, acid-enzyme-conversion, high-maltose, etc. However, as shown in Table 4, the most adequate characterization is with respect to their content of individual saccharides. In many cases, it is the content of individual saccharides or groups of saccharides that determines the characteristics of syrups. Functional properties of corn syrups include fermentability, viscosity, humectancy and hygroscopicity, sweetness, colligative properties, and browning reactions.

Fermentability of corn syrups by yeast is important in certain food applications, eg, baking and brewing. The fermentable sugars present in corn syrup are dextrose, maltose, and maltotriose. Fermentability of maltose or maltotriose depends on the specific fermentation process and organism. In general, greater fermentability is obtained at the higher DE levels.

Viscosity of corn syrup is a function of DE value, temperature, and solids concentration. Viscosity decreases with increasing DE and temperature and increases with increasing concentration. For a 43-DE corn syrup, viscosity at 1.42 sp gr (43°Bé) is 56,000 mPa · s (= cP) at 27°C (81°F), 14,500 mPa · s at 38°C (100°F), and 4900 mPa · s at 49°C (120°F). Corresponding values for a 55-DE syrup at the same density and temperatures are 31,500 mPa · s, 8500 mPa · s, and 2900 mPa · s.

The hygroscopic and humectant properties of corn syrups are of great importance in many applications. Depending on the type of syrup and on the specific conditions of temperature and humidity, the products may either resist or facilitate moisture loss or moisture absorption. The ability to attract moisture or retard its loss increases with increasing DE value. Prevention of moisture pickup is more characteristic of syrups with low DE values.

Sweetness is primarily a function of the levels of dextrose and maltose present and therefore is related to DE (see Fig. 1). Other properties that increase with increasing DE value are flavor enhancement, flavor transfer, freezing-point depression, and osmotic pressure. Properties that increase with decreasing DE value are bodying contribution, cohesiveness, foam stabilization, and prevention of sugar crystallization.

Manufacture

Acid hydrolysis of starch is conducted by batch or continuous processes. Batchwise conversion is carried out in large cookers or converters that are usually built of manganese bronze and have capacities of about 10 m^3 (2650 gallons). A suspension of starch at 35 to 40 wt % solids is placed in the converter. Hydrochloric acid is added to provide a concentration of 0.015 to 0.02 N, and the converter is steam heated until a temperature of 140 to 160°C (284 to 320°F) is reached. The mixture is held at this temperature for a period of time, usually 15 to 20 min, to produce the desired degree of hydrolysis.

Improved process control and, therefore, better product uniformity, is achieved by continuous processes using indirect heating. Acidified starch slurry is pumped at a constant rate through a series of heat exchangers at reaction conditions similar to those used in batch operations. In either process, hydrolysate is neutralized to pH 4 to 5.5 by addition of soda ash, clarified by centrifugation or filtration, refined (using activated carbon and/or ion exchange resins) to remove color and acid degradation products, and concentrated to 77 to 85 wt % solids. Sulfur dioxide used to be added during evaporation to some grades of syrup to reduce color development, but improved refining techniques have all but eliminated this practice.

Syrups produced by straight acid hydrolysis do not exceed 55 DE because of the formation of difficult-to-remove color and flavor by-products created at higher DE levels. Some syrup compositions are given in Table 4. Syrups are also produced by A–E or E–E processes. Starch is first hydrolyzed by acid, as described above, or by enzyme, as described for dextrose, followed by saccharification to the desired composition with one or more enzymes.

Maltose syrup, for example, is prepared from about 20 DE, partially hydrolyzed starch by saccharification with a maltose-producing enzyme at ca 55°C (131°F) and pH 5. Maltogenic enzymes, eg, β-amylase obtained from barley, wheat, or soybeans or fungal α-amylase derived from *Aspergillus oryzae*, are used to produce a hydrolysate containing about 45 to 60 wt % maltose (see Table 4). Higher levels of maltose (up to 80 to 90 wt %) are produced by saccharification with a combination of enzymes, including a maltogenic enzyme and a debranching enzyme, eg, pullulanase.

High-maltose syrups can be separated chromatographically to produce a 95 to 97% db maltose stream from which pure maltose can be crystallized. The sweetness of maltose is 30 to 40% that of sucrose. Maltose exists as both α- and β-forms, and aqueous solutions equilibrate at 42% α- and 58% β-form. At 20°C (68°F), crystalline α-anhydrous maltose will dissolve to about 64% ds, whereas β-monohydrate crystals dissolve to initially form a 27% ds solution. At equilibrium, a 42% α- and 58% β-form solution has a solubility of about 62% ds at 20°C (68°F) (50).

High-conversion syrups of 60 to 70 DE containing high levels of dextrose and maltose are also produced (Table 4). These types of syrups are produced in the same manner as maltose syrup, except a combination of glucoamylase and maltogenic enzyme is used. Maltose and high-conversion syrups are refined by the same procedures as described earlier and shipped in rail cars and tank trucks. Syrups are generally heated to about 30 to 50°C (86 to 122°F) to facilitate unloading.

Some syrups, particularly those with a limited extent of hydrolysis, are reduced to dry form by spray-drying or roll-drying. These products are commonly called dry maltodextrins (DE less than 20), corn syrup solids, and total sugars (for the highly converted products containing predominantly one saccharide). They are usually shipped in moisture-resistant bags.

Products of <20 DE are referred to as maltodextrins or hydrolyzed cereal solids (Table 4). Maltodextrins are usually prepared from regular or waxy corn starch, although some recently introduced products use high-amylose starch to enhance the retrogradation of the linear polymers to form maltodextrins resistant to digestion. Maltodextrins are produced by enzyme or acid hydrolysis processes to products of 5 to 20 DE. They are clarified, refined, and either spray-dried to a moisture content of 3 to 5 wt % and shipped in 100-lb (45.4-kg) bags or sold as a syrup of about 75 wt % solids. These products are bland-tasting, and the solid forms are free-flowing and nonhygroscopic.

Economic Aspects

Prices of corn syrup in the United States since 1975 are listed in Table 6. In the early 1970s, competition depressed prices to a very low level (12.4$/cwt, equal to $273/metric ton, in 1972). Pricing recovery followed when sucrose prices increased, and production capacity was reduced because of the manufacture of other competitive products. Recent prices have been about 50 to 60% that of sucrose.

Analysis and Specifications

International (including U.S.) specifications for corn syrups and dried corn syrups are given in References 55 to 57. A list of maltodextrins and their physical properties for U.S. and non-U.S. producers is available in Reference 58.

Corn syrups are usually sold with a specification of the Baumé measurement, and these can be related to the solids content. The most common value is sp gr 1.42 (43°Bé), corresponding to 80.3 wt % solids for a 42-DE acid-converted syrup. Solids contents for such a syrup at sp gr 1.39, 1.41, 1.44, and 1 45 (41, 42, 44, 45°Bé) are 76.3, 78.3, 80.3, 82.3, and 84.3 wt %, respectively. Higher DE syrups have slightly higher solids contents at the same densities. Saccharide composition is determined by high-performance liquid chromatography. Other important analyses include color, iron, and pH (although with the development of totally demineralized syrups, pH measurement has much less meaning).

Uses

Principal uses of corn syrups are shown in Table 7. Corn syrups are often used in combination with sucrose, dextrose, or HFCS. The specific type of syrups used depends on the properties desired in the final product. Properties listed above are important to varying degrees in the various products, and changes in formulation can affect the choice of corn syrup required to supply the most desirable properties. In many cases, corn syrups are used as supplementary sweeteners, with sucrose remaining the primary one.

In the confectionery industry, corn syrups are used extensively in nearly every type of confection, ranging from hard candy to marshmallows. In hard candies, which are essentially solid solutions of nearly pure carbohydrates, corn syrup contributes resistance to heat discoloration, prevents sucrose crystallization, and controls hygroscopicity, viscosity, texture, and sweetness. Maltose syrups, high-conversion syrups, and acid-converted syrups (36 and 42 DE) are all used for this application.

In the canning and preserving industries, corn syrups are used to prevent crystallization of sucrose, provide body, accentuate true fruit flavors, and improve color and texture. In the beverage industry, the predominant use is in the beer and malt-liquor areas. High-conversion syrups are used to replace dry-cereal adjuncts, provide fermentable sugars, enhance flavor, and provide body. These syrups contain controlled amounts of dextrose and maltose for proper fermentation.

Corn syrups used in baking are generally of the highly converted type. They are incorporated into cakes, cookies, icings, and fillings to increase the amount of moisture retained, retard crystal growth of other sugars, enhance tenderness, and increase shelf life. In yeast-raised goods, fermentability is of importance, and therefore only high DE syrups are used.

Corn syrups used in ice cream and frozen desserts are generally 36- or 42-DE acid-converted syrups. The syrup serves primarily to provide maximum flexibility in adjusting flavor, texture, body, and smoothness; it also aids in grain control and in the modification of melt-down and shrinkage characteristics of the frozen product.

Syrups of 25 to 30 DE are used as spray-drying aids in products such as coffee whiteners. High-conversion syrup, maltose syrup, and 42-DE syrup are used in jams and jellies. Additional uses of corn syrup include applications in table syrups, baby food, meat packing, breakfast foods, salad dressing, pickles, dehydrated powdered foods, medicinal syrups, textile furnishings, adhesives, and numerous other products and processes.

MAPLE SYRUP

Maple syrup is prepared by evaporating sap from the maple tree to a concentrated solution containing predominantly sucrose. Its characteristic flavor and color are formed during evaporation. Maple syrup is produced from the sap of several varieties of mature maple trees, eg, the sugar maple (*Acer saccharum*) and black maple (*Acer nigrum*). Main producing areas include the northeastern and upper midwestern United States and eastern Canada.

Manufacture and Uses

Collection of sap is made sometime between late fall and midspring, depending on weather; the best time is when the temperature is about 7°C (45°F) during the day and below freezing at night. Sap generally contains 2 to 3 wt % solids, of which about 96% is sucrose and the remainder other carbohydrates, organic acids, ash, protein, and ligninlike materials. A taphole is drilled into the tree, the hole is sanitized with a germicidal pellet, and a spout is driven in. Sap is collected in a bucket or bag or alternatively in plastic tubes directed to a centralized collection tank by gravity or vacuum.

Evaporation is conducted at atmospheric pressure until a boiling point of 104°C (219°F) is reached to produce a syrup meeting federal specifications of at least 66 wt % solids (59). Control of syrup concentration is critical. A syrup concentrated to only 65 wt % of solids has a thin taste, and one concentrated to more than 67 wt % crystallizes when cooled. A refractometer or hydrometer is used to measure concentration. Final specific gravity should be 1.35 (37.75°Bé) at 15.6°C (60°F). Flavor and color develop during evaporation as a result of reactions occurring between sugar and other components.

If change in the sucrose content is minimized during evaporation, a light-colored syrup of delicate maple flavor is produced. Darker syrups usually suffer from greater sucrose inversion and the production of caramelized off-flavors. Color is the principal grading factor, and the USDA has developed glass color filter standards referred to as light, medium, and dark amber. Syrup is clarified, graded as to color, flavor, and density, and finally packaged in small containers for retail sale as table syrup. Typically, the product contains 88 to 99% db sucrose and 0 to 12% db invert sugar. Maple syrup is also used in candy manufacture by blending with sucrose.

Other applications include addition to cookies, cakes, ice cream, baked beans, baked ham, and baked apples. Maple sugar is prepared by evaporating sap to a high solids content, ie, a boiling temperature of 116 to 121°C (241 to 250°F), and then allowing the supersaturated solution to crystallize or solidify during cooling. Maple cream or maple butter is made by stirring a supersaturated solution while cooling rapidly to produce a product of creamy texture.

Production and Economics

Maple syrup production in the United States averaged 4.9 million L (1.3 million gal) per year between 1989 and 1998. Canada produced about three times this amount. Production in the United States was as high as 6.4 million L in 1992 and 6.1 million L in 1996. Prices have increased from $6.00 per liter in 1992 to $7.00 per liter in 1998 (60).

MOLASSES

Molasses, another type of syrup, is a by-product of the sugar industry; it is the mother liquor remaining after crystallization and removal of sucrose from the juices of sugarcane or sugar beet and is used in a variety of food and nonfood applications. Molasses was first produced from sugarcane in China and India centuries ago, later spreading into Europe and Africa. It was introduced as the by-product of cane sugar production into Hispaniola (island Española in modern West Indies) by Columbus in 1493. During colonial times, molasses was very important to the American colonies for the production of rum. In 1733, the British Parliament passed the Molasses Act to tax importation of molasses from foreign countries. This attempt to restrict trade was ignored by the colonies and was, in part, responsible for the American Revolution.

Manufacture

Raw sugar is produced from sugarcane by a process that involves extraction of the sugar in water, treatment to remove impurities, concentration, and several crystallizations. After the first crystallization and removal of first sugar, the mother liquor is called first molasses. First molasses is recrystallized to obtain a second lower-quality sucrose (second sugar) and a second molasses. After a third crystallization, the third molasses contains considerable nonsucrose material, and additional recovery of sucrose is not economically feasible. The third molasses is sold as blackstrap, final, or cane molasses. Raw sugar obtained from this process is mixed with water to dissolve residual molasses and is separated by centrifugation. This process is called affination, and the syrup is referred to as affination liquor. The sugar is dissolved in water, treated to remove color and impurities, and subjected to several crystallizations to obtain refined sugar. The mother liquor from the final crystallization is combined with affination liquor and crystallized to produce a dark sugar (remelts) that is recycled to raw sugar. The remaining mother liquor, called refiners molasses, is similar to final molasses but usually of better quality.

In beet sugar manufacture, the beet juice does not contain reducing sugars (fructose and glucose) that are present in cane juice but may contain raffinose. Because of the absence of reducing sugars, the sucrose level in beet molasses is not reduced to the same extent as with cane.

Final molasses from beet contains about 60 wt % sucrose (dry basis) compared to 30 wt % sucrose (dry basis) in cane molasses. Treatment of diluted beet molasses with calcium oxide precipitates sucrose as tricalcium sucrate (Steffen process), which is recycled to the incoming hot beet juice. During recycling, raffinose accumulates in the final molasses and retards crystallization if not removed. Therefore, a portion of the final molasses is periodically removed and called discard molasses. High-test molasses (invert molasses) is produced from cane sugar when sucrose manufacture is restricted because of overproduction. The cane sugar at about 55 wt % solids is enzymatically converted to invert syrup to prevent crystallization and is evaporated to a syrup. The product is used in the same applications as blackstrap molasses.

Nearly 8% of the beet sugar production forecast for fiscal 1996/1997 is sucrose recovered from molasses. First employed in 1989, molasses desugarization using ion exchange technology is performed at eight facilities across the United States (61). After recovery of about 85% of the sucrose contained in the molasses feedstock, the process can also produce two co-products, CMSB (consolidated molasses solids from beets) that contains 18 to 21% sucrose and betaine, used as a growth stimulant for poultry and fish.

Molasses from other sources include citrus and corn sugar (Hydrol) molasses. Citrus molasses is produced from citrus waste and contains 60 to 75% sugars. Corn sugar molasses, commonly called Hydrol, is the mother liquor remaining after dextrose crystallization and contains a minimum of 43% reducing sugars expressed as dextrose. Molasses is shipped in drums, barrels, tank trucks, tank cars, barges, and ocean-going vessels. Because of high viscosity, molasses must be heated in some situations to facilitate pumping. However, prolonged heating must be avoided to prevent carmelization.

Composition

Molasses composition depends on several factors, eg, locality, variety, soil, climate, and processing. Cane molasses is generally at pH 5.5 to 6.5 and contains 30 to 40 wt % sucrose and 15 to 20 wt % reducing sugars. Beet molasses is about 7.5 to 8.6 pH, and contains about 50 to 60 wt % sucrose, a trace of reducing sugars, and 0.5 to 2.0 wt % raffinose. Cane molasses contains less ash, less nitrogenous material, but considerably more vitamins than beet molasses. Composition of selected molasses products is listed in Table 8.

Uses

The primary use of molasses is in animal feed. Molasses provides a carbohydrate source, salts, protein, vitamins, and palatability and may be used directly or mixed with other feeds.

The carbohydrate content of 24.6 L (6.5 gallons) of blackstrap molasses is considered to be equal to 0.035 m^3 (one bushel) of corn as measured by the energy produced from 0.035 m^3 of corn and the amount of molasses required to produce the same amount of energy. When molasses is less expensive than corn, sales increase; when the reverse is true, sales decrease. Relative corn and molasses costs are given in Table 9 for 1981 to 1986.

Table 8. Molasses Composition

Molasses Type	Solids (%)	Total sugars as invert (% db)	Crude protein (% db)	Total ash (% db)
Cane (Louisiana)	80.8	59.5	3.0	7.2
Cane (refiner's)	75.4	55.9	2.1	8.6
High test (Cuba)	80.4		0.7	1.4
Beet (Wisconsin)	78.6	52.7	11.4	9.3
Corn	74.9	50.3	0.4	8.9
Citrus	71.4	42.4	4.7	4.8

The second main use for molasses is in fermentation processes as an inexpensive source of carbohydrate. Molasses is the basic raw material for rum production and is also used for production of yeast and citric acid.

Food applications utilize first and second molasses in baking (bread, cakes, cookies) for flavor. Molasses is also used in curing of tobacco and meats, in confections such as toffees and caramels, and in baked beans and glazes. Distribution of molasses to various industries is shown in Table 10.

Production and Economics

Molasses production along with imports and exports in the United States are shown in Table 11. Principal molasses producers are listed in Table 12. Molasses prices since 1980 are given in Table 13. Because of declining price, desugaring processes have been developed using ion exchange technology to recover about 85% of the sucrose content of molasses as refined sugar. When desugaring techniques are used, more than 90% of the sucrose contained in the beet feedstock is recovered as beet sugar. About 8% of the beet sugar production forecast for fiscal 1996/1997 is sucrose recovered from molasses.

SORGHUM SYRUPS

Sorghum syrup, also known as sorgo, is made from the juice of the sorghum plant that is related to the sugar cane. The finished syrup flavor is similar to that of cane syrup but has a sharper tang. Some sorghum syrups exhibit a strong, distinctive flavor. For this reason, blends of sorghum and corn syrup are found to be preferred by some (62).

Although sorghum has been grown for grain and animal feed for many centuries, syrup production is a relatively recent use that began in the United States shortly before the Civil War. In 1859, nearly 7 million gallons (26 million L) were produced, increasing to 28 million gallons by 1879 (63). By 1938, production had decreased to about 15 million gallons annually. Today, only 1 to 2 million gallons are produced each year. Much of this sorghum syrup is produced by small-scale producers for local consumption. Because of the fragmented nature of the market, it is difficult to obtain an accurate measure of the amount of sorghum syrup produced.

Although many variations exist on the process for sorghum syrup production, typically the sorghum plants are topped and the leaves are removed from the canes. The juice is then pressed from the canes, usually in a roller mill essentially like the mills used for crushing sugar cane. A ton of sorghum yields enough juice to produce between 10 and 20 gallons (38 and 76 L) of finished syrup, depending on the growing and crushing conditions as well as the sorghum variety grown. Sweet sorghum varieties yield more syrup than forage varieties.

Table 9. Price Comparisons between Molasses and No. 2 Yellow Corn

	Minneapolis, Minnesota			Chicago, Illinois			Stockton, California		
Year	Corn, 0.035 m^3 1 bushel	Molasses 24.6 L	Difference	Corn, 0.035 m^3 1 bushel	Molasses 24.6 L	Difference	Corn, 0.035 m^3 1 bushel	Molasses 24.6 L	Difference
1981	293.2	422.7	−129.5	315.8	420.0	−104.2	393.1	339.1	+54.0
1982	240.0	269.2	−29.2	250.8	269.1	−18.3	341.0	230.0	+111.0
1983	308.1	292.3	+15.8	317.3	294.6	+22.7	397.0	267.6	+129.4
1984	309.7	307.9	+1.8	324.0	307.5	+16.5	390.3	273.5	+116.8
1985	254.1	276.8	−22.7	267.3	271.2	−3.9	340.5	237.5	+103.0
1986	192.8	349.8	−157.0	208.2	348.0	−139.8	289 5	279.2	+10.3

Note. Prices are shown in cents.

Table 10. Estimated Utilization of Molasses in the United States, 1000 Metric Ton

Year	Distilled spirits	Yeast and citric acid	Pharmaceuticals and edible molasses	Sub total	Mixed feeds direct feeding, and silage	Total utilization
1981	31.4	400.4	101.8	533.6	2,496.4	3,030.0
1982	75.1	435.1	82.5	592.7	2,291.4	2,884.1
1983	266.3	489.9	102.2	858.4	2,334.0	3,192.4
1984	372.8	505.9	37.3	916.0	2,305.2	3,221.2
1985	372.8	532.5	34.6	939.0	2,455.9	3,395.8
1986	410.0	484.6	59.1	953.7	2,014.0	2,967.7

Table 11. U.S. Molasses Production, Imports, and Exports

	Production					
Year	Mainland cane	Hawaii cane	Domestic beet	Refiners blackstrap	Imports	Exports
1985	502	168	625	97	1186	97
1986	520	195	729	91	945	275
1987	517	197	758	79	710	237
1988	527	127	NA	NA	729	210

Note: Data are expressed as 10^6 L.

Table 12. World Production of Industrial Molasses, 1000 Metric Ton

Country	1984/1985	1985/1986	1986/1987	1987/1988
Brazil	4,800	3,986	2,587	4,000
USSR	3,200	2,900	3,100	3,300
India	2,500	2,850	3,100	3,280
China	1,130	1,909	1,814	1,850
Cuba	2,000	2,000	1,750	1,750
United States[a]	1,725	1,716	1,710	1,700
Mexico	1,400	1,608	1,590	1,630
Thailand	1,356	1,194	1,205	1,300
France	1,165	1,126	1,013	1,000
Argentina	702	854	950	950
Indonesia	720	840	950	930
South Africa	852	796	735	770
Poland	708	636	675	700
Australia	736	658	693	680
FRG	640	751	715	600
Philippines	744	551	518	580
Others	10,173	9,970	10,661	10,675
Totals	*34,551*	*34,345*	*33,766*	*35,695*

[a] Does not include Puerto Rico or Hawaii.

Table 13. Molasses Price, US$/Ton

Year	Blackstrap[a]	Beet[b]
1980	106.37	105.38
1985	55.39	NA
1986	76.68	88.49
1987	55.64	66.44
1988	71.26	80 36
1997 (Nov.)	57.50	NA

[a] New Orleans.

[b] Colorado.

The juice contains starch and is sometimes treated with a hydrolyzing enzyme to convert the starch to soluble sugars. Treatment with invertase can be used to invert the sucrose into glucose and fructose to reduce the tendency of the finished syrup to crystallize. The juice is clarified and boiled in an atmospheric evaporating pan similar to a maple syrup concentrator. The traditional sorghum pan is a shallow rectangular pan 2-1/2 to 4 feet (3/4 to 1-1/4 m) wide and 12 to 18 feet (3-3/4 to 5-1/2 m) in length divided internally by staggered strips of metal to impart a zigzag flow to the syrup. Heat can be supplied by direct fire beneath the pan or from a steam jacket. The syrup flow is regulated so that product discharges at the desired solids level. The syrup is concentrated to about 76 to 80% solids and is then cooled and transferred to cans, bottles, or jars for sale.

INULIN-BASED PRODUCTS

Inulin is a naturally occurring polymer usually consisting of 17 to 30 anhydrofructose units and a terminal anhydroglucose unit that replaces starch as the food reserve in many plants of the family Compositae, such as Jerusalem artichoke, chicory, salsify, cardoon elecampane, burdock, dahlia, and ti plants. The polymer chain length varies during the growing season and consequently, the glucose–fructose ratio of products of inulin hydrolysis is not constant (64). Although the Jerusalem artichoke is reported to produce tuber yields as high as 100 ton/hectare (65), present commercial sources of inulin are primarily obtained from chicory (66).

A number of processes have been described for the production of crystalline fructose and fructose-rich syrups from inulin-containing plant material (34,67–69). Most commercial crystalline fructose and fructose-rich syrups are produced by isomerization of glucose obtained from starch hydrolysis and enriched by chromatographic separation. Although some fructose is being produced from inulin, much of the inulin being recovered today is partially enzymatically hydrolyzed to make a range of nondigestible oligofructose ingredients that are finding food use as fat mimetics.

ACKNOWLEDGMENTS

The author acknowledges that a portion of the information contained in this article was the work of Ronald E. Hebeda, previously published as the chapter entitled "Syrups" that appeared in *Encyclopedia of Food Science and Technology*, 3rd ed., John Wiley & Sons, New York, 1992, pp. 2490–2504.

BIBLIOGRAPHY

1. F. W. Schenck, "Worldwide Starch Sugars—Past and Future," *Corn Annual*, Corn Refiners Assoc., Inc., Washington, D.C., 1997, pp. 9–12.
2. F. W. Schenck, "Glucose and Glucose-containing Syrups," in *Ullman's Encyclopedia of Industrial Chemistry*, 6th ed., Wiley-VCH, New York.
3. H. Wichelhaus, *Der Starkezucker*, Akademische Verlagsgesellschaft, Leipzig, Germany, 1913.
4. G. S. C. Kirchoff, *Memoirs of the Imperial Academy of Science at St. Petersbourg* 4, 27 (1811).
5. T. de Saussere, *Ann. Phys.* **49**, 129 (1815).
6. U.S. Pat. 1,471,347 (1923), W. B. Newkirk (to Corn Products Refining Company).
7. R. F. Jackson and C. G. Silsbee, *Natl. Bureau Standards (U.S.) Sci. Pap.* **437**, 715 (1922).
8. S. M. Cantor and Q. P. Peniston, *J. Am. Chem. Soc.* **62**, 2113 (1940).
9. B. Singh, G. R. Dean, and S. M. Cantor, *J. Am. Chem. Soc.* **70**, 517 (1948).
10. D. J. McWeeny, "The Role of Carbohydrate in Non-Enzyme Browning," in G. G. Birch and L. F. Green, eds., *Molecular Structure and Function of Food Carbohydrate*, Wiley, New York, 1973, p. 21.
11. E. R. Kooi and F. C. Armbruster, "Production and Use of Dextrose," in R. L. Whistler and E. F. Paschall, eds., *Starch Chemistry and Technology*, Vol. II, Academic Press, New York, 1967, p. 562.
12. A. D. Woolhouse, *Starch as a Source of Carbohydrate Sweeteners*, Report No. CD 2237, Department of Scientific and Industrial Research, New Zealand, 1976, p. 20.
13. R. V. MacAllister, "Nutritive Sweeteners Made from Starch," in R. S. Tipson and D. Horton, eds., *Advances in Carbohydrate Chemistry and Biochemistry*, Vol. 36, Academic Press, New York, 1979, p. 40.
14. R. E. Hebeda and H. W. Leach, "The Nature of Insoluble Starch Particles in Liquefied Corn-Starch Hydrolysates," *Cereal Chem.* **51**, 272–278 (1974).
15. W. E. Henderson and W. M. Teague, "A Kinetic Model of *Bacillus stearothermophilus* α-Amylase under Process Conditions," *Starch / Stärke* **40**, 412–418 (1988).
16. P. Rosendal, B. H. Nielsen, and N. K. Lange, "Stability of Bacterial α-Amylase in the Starch Liquefaction Process," *Starch / Stärke* **31**, 368–372 (1979).
17. B. C. Saha and J. G. Zeikus, "Microbial Glucoamylases: Biochemical and Biotechnological Features," *Starch / Stärke* **41**, 57–64 (1989).
18. B. E. Norman, "The Application of Polysaccharide Degrading Enzymes in the Starch Industry," in R. C. W. Berkeley, G. W. Gooday, and D. C. Ellwood, eds., *Microbial Polysaccharides and Polysaccharases*, Academic Press, New York, 1979; p. 359.
19. U.S. Pat. 4,017,363 (April 12, 1977), W. H. McMullen and R. Andino (to Novo Industri A/S).
20. U.S. Pat. 4,560,651 (December 24, 1985), G. C. Nielsen et al., (to Novo Industri A/S).
21. R. E. Hebeda, C. R. Styrlund, and W. M. Tezgue, "Benefits of *Bacillus megaterium* Amylase in Dextrose Production," *Starch / Stärke* **40**, 33–36 (1988).
22. R. E. Hebeda, "Syrups," in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed., Vol. 22, John Wiley and Sons, New York, 1983.
23. U.S. Pat. 3,257,665 (1966), L. R. Idaszak (to Corn Products Company).
24. U.S. Pat. 4,620,880 (1986), G. Bodele et al., (to Roquette Freres).
25. U.S. Pat. 4,357,172 (1982), L. W. Edwards (to CPC International).
26. G. R. Dean, "An Unstable Crystalline Phase in the D-Glucose-Water System," *Carbohydr. Res.* **34**, 315–322 (1974).
27. A. F. M. Mahbubar Rahman and F. W. Schenck, "Reconversion Improves Monohydrate Dextrose Economics," paper 13 presented at the 1997 AACC Annual Meeting, San Diego, Calif., October 13, 1997.
28. F. W. Schenck, "Solid Starch Hydrolysates," *Cereal Foods World* **41**, 388–390 (1996).
29. *Code of Federal Regulations*, 21 CFR 168.110 and 21 CFR 168.111, U.S. Government Printing Office, Washington, D.C.
30. Codex Alimentarius Commission, *Dextrose Monohydrate*, CAC Vol. III, 1st ed., Codex Standard 8-1981, FAO/WHO, Rome, 1981.

31. Codex Alimentarius Commission, *Dextrose Anhydrous*, CAC Vol. III, 1st ed., Codex Standard 7-1981, FAO/WHO, Rome, 1981.
32. Codex Alimentarius Commission, *Powdered Dextrose (Icing Dextrose)*, CAC Vol. III, 1st ed., Codex Standard 54-1981, FAO/WHO, Rome, 1931.
33. *The United States Pharmacopoeia*, 21st revision (USP XXI NF XVI), United States Pharmacopoeial Convention, Inc., Rockville, Md., 1985, p. 300.
34. M. Kierstan, "Production of Fructose Syrups from Inulin," *Process Biochem.* **2** (1980)
35. T. Doty, "Fructose: The Rationale for Traditional and Modern Uses," in P. Koivistoinen and L. Hyvonen, eds., *Carbohydrate Sweeteners in Foods and Nutrition*, Academic Press, London, 1980, p. 259
36. C. Morris, "America's Gold," *Food Eng.* **95** (1980).
37. C. A. L. DeBruyn and W. A. van Eckenstein, *Recueil des Travaux Chimiques des Pays-Bas* **14**, 203 (1895).
38. M. Seidman, "New Technological Developments in D-Fructose Production," in G. G. Birch and R. S. Shallenberger, eds., *Developments in Food Carbohydrate*, Vol. 1, Applied Science Publishers, London, 1977, p. 19.
39. U.S. Pat. 2,950,228 (August 23, 1960), R. O. Marshall (to Corn Products Co.).
40. Y. Takasaki, "Studies on Sugar-Isomerizing Enzyme Production and Utilization of Glucose Isomerase from *Streptomyces sp.*", *Agric. Biol. Chem.* **30**, 1247 (1966).
41. U.S. Pat. 3,616,221 (October 26, 1971), Y. Takasaki and O. Tanabe (to Agency of Industrial Science and Technology, Tokyo).
42. U.S. Pat. 3,694,314 (September 26, 1972), N. E. Lloyd, L. T. Lewis, R. M. Logan, and D. N. Patel (to Standard Brands, Inc.)
43. U.S. Pat. 3,788,945 (January 29, 1974), K. N. Thompson, R. A. Johnson, and N. E. Lloyd (to Standard Brands, Inc.).
44. L. M. Hanover, "Crystalline Fructose: Production, Properties, and Applications," in F. W. Schenck and R. E. Hebeda, eds., *Starch Hydrolysis Products*, Wiley-VCH, New York, 1992, pp. 201–231.
45. R. V. MacAllister and E. K. Wardrip, "Fructose and High Fructose Corn Syrups," in M. S. Peterson and A. H. Johnson, eds., *Encyclopedia of Food Science*, AVI Publishing Co., Westport, Conn., 1978, p. 330.
46. G. L. S. Pawan, "Fructose," in G. G. Birch and L. F. Green, eds., *Molecular Structure and Function of Food Carbohydrate*, Wiley, New York, 1973, p. 65.
47. *Food Product Development* **39** (April 1978).
48. O. B Jorgensen et al., "A New Immobilized Glucose Isomerase with High Productivity Produced by a Strain of *Streptomyces murinus*," *Starch / Stärke* **40**, 307–313 (1988).
49. J. V. Hupkes, "Practical Process Conditions for the Use of Immobilized Glucose Isomerase," *Starch / Stärke* **30**, 24–28 (1978).
50. R. L. Antrim and A. L. Auterinen, "A New Regenerable Immobilized Glucose Isomerase," *Starch / Stärke* **38**, 132–137 (1986)
51. R. van Tilburg, "Enzymatic Isomerization of Corn Starch Based Glucose Syrups," in G. M. A. Van Beynum and J. A. Roels, eds., *Starch Conversion Technology*, Marcel Dekker, New York, 1985.
52 Y. Takasaki, "Data on Individual Related Enzymes" in *The Amylase Research Society of Japan*, Pergamon Press, New York, 1988.
53. C. E. Morris, "First Crystalline-Fructose Plant in U.S.," *Food Eng.* **53**, 70–71 (1981)
54. M. Okada and T. Nakakuki, "Oligosaccharides: Production, Properties, and Applications," in F. W. Schenck and R. E. Hebeda, eds., *Starch Hydrolysis Products*, VCH, New York, 1992, pp. 351–352.
55. *Code of Federal Regulations*, 21 CFR 168.120 and 21 CFR 168.121, U.S. Government Printing Office, Washington, D.C.
56. Codex Alimentarius Commission, *Glucose Syrup*, CAC Vol. III, 1st ed., Codex Standard 9-1981, FAO/WHO, Rome, 1981.
57. Codex Alimentarius Commission, *Dried Glucose Syrup*, CAC Vol. III, 1st ed., Codex Standard 10-1981, FAO/WHO, Rome.
58. R. J. Alexander, "Maltodextrins: Production, Properties, and Applications," in F. W. Schenck and R. E. Hebeda, eds., *Starch Hydrolysis Products*, VCH, New York, 1992, Tables 8.1–8.6, pp. 241–248.
59. *Code of Federal Regulations*, 21 CFR 168.140, U.S. Government Printing Office, Washington, D.C.
60. *Sugar and Sweetener Situation and Outlook Report*, ERS, U.S. Department of Agriculture, Washington, D.C., 1999
61. *Sugar and Sweetener Situation and Outlook Report*, ERS, U.S. Department of Agriculture, Washington, D.C., 1996.
62. J. L. Collins, I. C. Yachouh, and I. E. McCarty, "Quality of Sorghum-Corn Syrup Blends," *Tennessee Farm Home Sci.*, 10–13 (1980).
63. L. R. Wilhelm and I. E. McCarty, *An Evaluation of Sorghum Syrup Processing Operations in Tennessee*, University of Tennessee Ag. Exp. Station Research Report 85-01 (1995).
64. J. S. Bacon, *Biochem. J.* **51**, 208 (1952).
65. M. Parameswaran, "Jerusalem Artichoke," *Food Australia* **46**, 473–475 (1994).
66. P. Coussement, *Food Ingredients and Analysis International* **18**, 25–27 (1996).
67. J. W. Eichinger et al., *Ind. Eng. Chem.* **24**, 41 (1932).
68. P. V. Golovin, N. A. Bryukhanova, and A. T. Fridman, *J. Sugar Ind. USSR* **3**, 140 (1929).
69. F. A. Dykins et al., *Ind. Eng Chem.* **25**, 937 (1933).

GENERAL REFERENCES

G. G. Birch, L. F. Green, and C. B. Coulson, eds., *Glucose Syrups and Related Carbohydrates*, Elsevier, Amsterdam, The Netherlands, 1970.

R. T. Foulds, Jr., "Maple Sugar," in A. H. Johnson end M. S. Peterson, eds., *Encyclopedia of Food Technology*, AVI Publishing Co., Westport, Conn., 1974.

R. E. Hebeda, "Corn Sweeteners," in S. A. Watson and P. E. Ramstad, eds., *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1987.

S. H. Hemmingsen and B. E. Norman, "Enzyme Technology in the Manufacture of Sugars from Cereals," in G. E. Inglett and L. Munck, eds., *Cereals for Food and Beverages*, Academic Press, New York, 1980.

D. Howling, "The General Science and Technology of Glucose Syrups," in G. G. Birch and K. J. Parker, eds., *Sugar: Science and Technology*, Applied Science Publishers, London, 1979.

A. Lachmann, "Molasses," in A. H. Johnson and M. S. Peterson, eds., *Encyclopedia of Food Technology*, AVI Publishing Co., Westport, Conn., 1974.

N. E. Lloyd and W. J. Nelson, "Glucose and Fructose Containing Sweeteners from Starch," in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds., *Starch: Chemistry and Technology*, 2nd ed., Academic Press, New York, 1984.

R. V. MacAllister, "Manufacture of High Fructose Corn Syrup Using Immobilized Glucose Isomerase," in W. H. Pitcher, Jr., ed., *Immobilized Enzymes for Food Processing*, CRC Press, Boca Raton, Fla., 1980.

R. V. MacAllister, E. K. Wardrip, and B. J. Schnyder, "Modified Starches, Corn Syrups Containing Glucose and Maltose, Corn Syrups Containing Glucose and Fructose, and Crystalline Dextrose," in G. Reed, ed., *Enzymes in Food Processing*, 2nd ed., Academic Press, New York, 1975.

R. A. McGinnis, ed., *Beet-Sugar Technology*, 3rd ed., Beet gar Development Foundation, Fort Collins, Colo., 1982.

G. P. Meade and J. C. P. Chen, *Cane Sugar Handbook*, 10th ed., John Wiley & Sons, New York, 1977, pp. 359–377.

Molasses Market News, Market Summary 1966, Agricultural Marketing Service, USDA, Denver, Colo., 1966.

H. M. Pancoast and W R Junk, eds., *Handbook of Sugars*, 2nd ed., AVI Publishing Co., Westport, Conn., 1980.

F. W. Schenck and R. E. Hebeda, eds., *Starch Hydrolysis Products*, VCH, New York, 1992.

J. W. White, Jr. and J. C. Underwood, "Maple Syrup and Honey," in G. E. Inglett, ed., *Symposium: Sweeteners*, AVI Publishing Co., Westport, Conn., 1974.

C. O. Willits and C H. Hills, eds., *Maple Syrup Producers Manual*, Agricultural Handbook No. 134, Agricultural Research Service, USDA, Washington, D.C., 1976.

"*Enzyme Technology*," in L. B. Wingard, Jr., E. Katchalski-Katzir, and L. Goldstein, eds., *Applied Biochemistry and Bioengineering*, Vol. 2, Academic Press, New York, 1979.

G. M A. Van Beynum and J. A. Roels, eds., *Starch Conversion Technology*, Marcel Dekker, New York, 1985.

Fred W. Schenck
Sun City, Arizona

TANNINS

BACKGROUND

Tannins have been defined as water-soluble polyphenolic compounds with molecular weights between 500 and 3000 that have the ability to precipitate alkaloids, gelatin, and other proteins (1). The usefulness of such a definition stems from the original interest in these substances in the tanning process, that is, the conversion of hides to leather, and is enhanced by the recent recognition of other important chemical and physical properties. The tanning of hides has been practiced since around 1500 B.C. The process involves the cross-linking of adjacent collagen chains with polyphenol attachment at several points. The molecular weight limitations apply because a minimum molecular size is required to engage protein chains; too large a molecule cannot easily diffuse into the collagen fibers (2). The plant polyphenols that exhibit the property of tannage also interact with other proteins, peroxides, and many other biological molecules. This has broad implications for fields as diverse as plant evolution, animal nutrition, other aspects of animal physiology, food technology, and, possibly, human therapeutic applications. The significance of the plant polyphenols referred to as tannins goes far beyond their role in the production of leather.

Tannin molecules are complex. Not all have been completely characterized or even recognized. Basically, there are two types: proanthocyanidins, often referred to as condensed; and glucose polyesters of gallic or hexahydroxydiphenic acids, also known as hydrolyzable. The polyphenols are secondary metabolites and not integral parts of the primary growth, energy producing, or reproductive processes of the plant.

Examples of the two major types of tannins are shown in Figure 1.

PROANTHOCYANIDINS

The proanthocyanidins are condensation products of flavanols, chiefly the catechins. Acid hydrolysis under stringent conditions ruptures carbon–carbon bonds connecting flavanol moities. Anthocyanidins and catechins are the final products. Alkaline hydrolysis results in the formation of quinone intermediates that may then be converted to anthocyanidins (3). The acid hydrolysis reaction is demonstrated in Figure 2.

The proanthocyanidins are almost invariably associated with their corresponding flavanols (catechins) from which they are derived. During flavanol biosynthesis a portion of the cation intermediate becomes attached to an already synthesized flavanol molecule to form a dimer rather than undergoing enzymic reduction to the monomer. Trimers and higher oligomers (condensation products of flavanols or other tannin units) are formed in an analogous fashion (4). The proportion of the various species produced is largely a function of the availability of the necessary reducing enzyme.

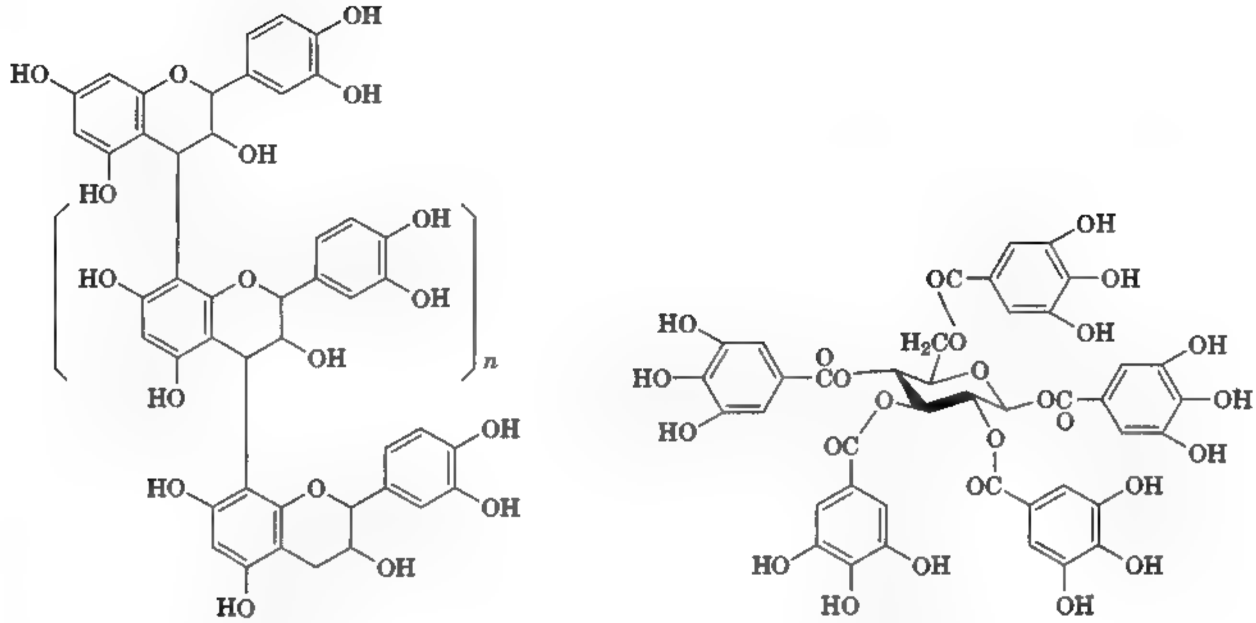
It has also been suggested that quinone, rather than cation, intermediates participate in proanthocyanidin formation (3). The quinones involved also react with proteins in a process that has been called quinone tanning. The reactions are irreversible with the formation of new covalent bonds. Insects exploit quinone tanning to the ultimate extent. Their bodies are covered with a hard exoskeleton consisting of quinone–protein adducts that provide protection from desiccation and predators and that account for their biological success (5).

Formation of a dimeric condensation product by the cation mechanism is illustrated in Figure 3. Procyanidins are the most frequently encountered group of the proanthocyanidins. They are derived from the simple catechins (R = H). Prodelphinidins are not as widespread. They are derived from the gallocatechins (R = OH).

Biosynthesis of the parent flavanol proceeds through a complex pathway starting with the conversion of phenylalanine to cinnamic acid. Incorporation of acetate units leads to the formation of chalcones. Ring closure results in flavonoid structures from which catechins are derived (4).

The procyanidins in foods occur in soluble free forms and in insoluble combinations with polysaccharides. Such adducts are the result of covalent bonds formed during the biosynthesis of the procyanidins. Dimeric procyanidins, barely within the molecular size range of tannins, and trimeric forms are widely distributed in berries and in the leaves of many fruit plants at levels of 50 to 500 mg/100 g fresh weight (6). They are often accompanied by dimeric prodelphinidins, which predominate in strawberries. Fruits and grains are also rich in higher oligomers of catechins. As molecular size increases, the solubility of the proanthocyanidins decreases. Varieties of sorghum, one of the most widely consumed of all grains by humans, differ in tannin levels by a factor of 100. Some fodder varieties contain as much as 6% tannin (7).

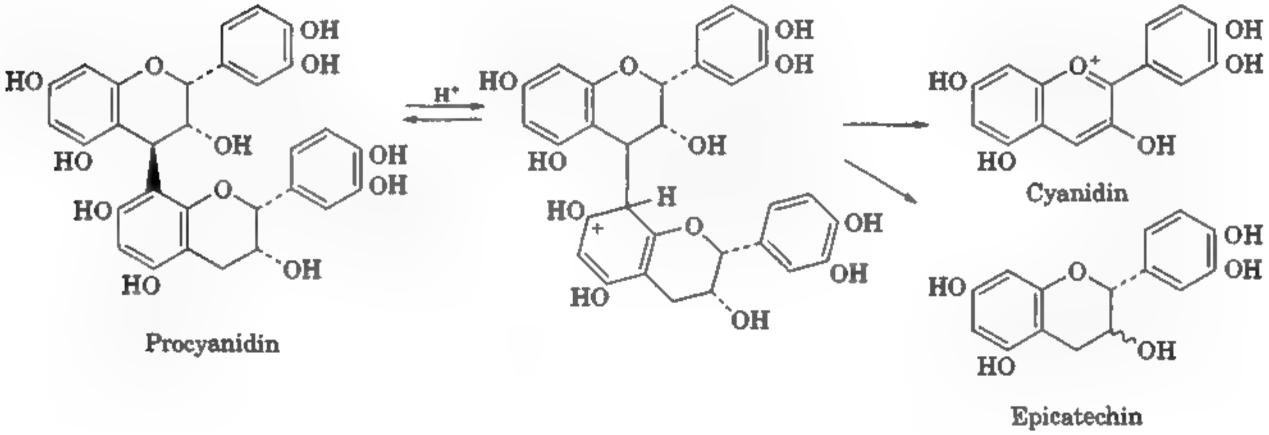
Proanthocyanidins and their parent flavanols that occur in human food undergo changes during preparation and storage that often lead to the formation of highly colored polymeric material. Enzymic browning occurs when susceptible polyphenols come in contact with plant oxidases in the presence of atmospheric oxygen as a result of cell disruption caused by spoilage, insect infestation, or other mechanical damage. This effect is exploited in the manufacture of black tea. The cell structure of fresh green tea leaf is disrupted mechanically to promote contact between the abundant cellular flavanol supply and oxidizing enzymes. Proanthocyanidins constitute one fraction of the oxidized catechin group and are responsible for some of the desirable organoleptic properties of black tea. Procyanidins are also important constituents of cocoa. They undergo many changes during the processing of the bean that impact on flavor and color (6). Gallotannins are also present and constitute over 4% of the dried fermented cotyledons.



Proanthocyanidin

Pentagalloyl glucose

Figure 1. Tannins.

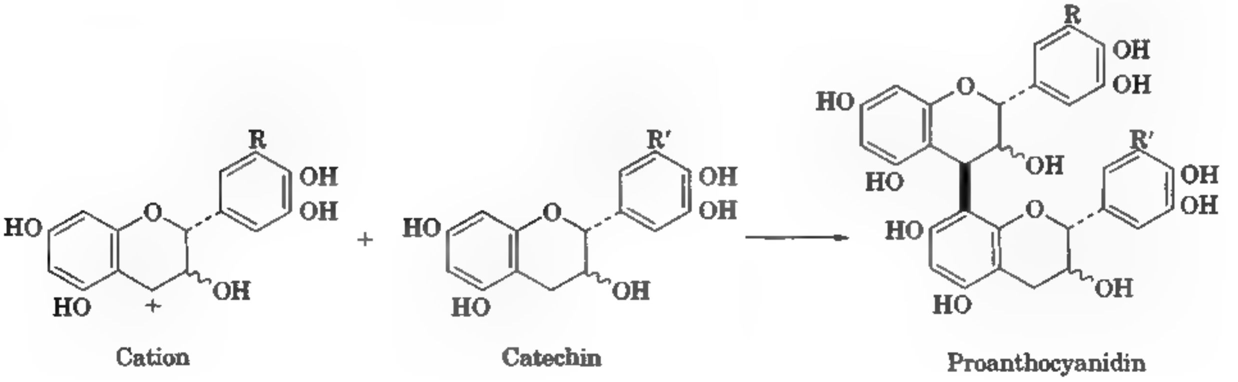


Procyanidin

Cyanidin

Epicatechin

Figure 2. Acid hydrolysis of proanthocyanidins.



Cation

Catechin

Proanthocyanidin

Figure 3. Formation of proanthocyanidins.

In other food products enzymic browning may be disadvantageous. It can be prevented by enzyme inactivation (often blanching), by the use of antioxidants, or by the rigorous exclusion of oxygen. Wine technology is largely an exercise in tannin control (6). White wine production is managed so as to exclude the polyphenol-rich grape skins and seeds from the juice in order to minimize enzymic browning. Procyanidins are present at about 10 mg/L and may be desirable at this low level. Red wine is produced so as to include phenolic components in the fermenting juice. Final procyanidin content is in the range of 40 to 65 mg/L. Color changes that occur during the aging of red wine include the diminution of the original color (primarily from anthocyanins) and the formation of new, more stable, oligomeric pigments (8). Procyanidin dimers in beer originate from the malt and are troublesome haze formers. The tannins involved are primarily dimers of catechin and epicatechin (9).

Fruit, vegetables, grain, and many beverages are sources of condensed tannins in the diet as shown by a few examples in Table 1.

Table 1. Proanthocyanidin Content of Foods

Food	Proanthocyanidins (mg/100 g)
Strawberries	110–150
Raspberries	100–140
Black currants	350
Persimmons	800–1700
White wine	1–2
Red wine	4–7
Sorghum	100–1000
Barley	20
Tea (black, leaf)	15,000
Cocoa (powder)	4500

POLYESTERS

Most of the hydrolyzable tannins are essentially polygallates of glucose. Hydroxyl groups of the gallate segments can be further esterified with gallic acid to build up large complex molecules. Gallotannins occur in chestnut, oak, sumac, and in several plant galls. The Peruvian plant, tara, is an important commercial source. The gallotannins of commerce are known as tannic acid. They contain 5 to 10 esterified gallic acid residues. Tannic acid is used as a mordant in the dyeing of cotton, as a clarifying agent for wine and beer, and as a food flavorant at low concentrations. It is approved for use in the United States as a GRAS additive as shown in Table 2.

Table 2. Approved Use Levels for Tannic Acid

Product	Percentage of product as used
Baked goods	0.01
Alcoholic beverages	0.015
Nonalcoholic beverages, beverage bases, gelatins, puddings, fillings	0.005
Frozen desserts and soft candy	0.04
Hard candy and cough drops	0.013
Meat products	0.001

When galloyl groups of glucose esters are appropriately oriented, oxidative coupling may take place during plant metabolism to form ellagitannins that contain hexahydroxydiphenic acid groups. Hydrolysis yields ellagic acid as well as gallic acid (10). The ellagitannins are rigid molecules in contrast to the gallotannins, which exhibit a great degree of flexibility. The structural relationships among the ellagitannis, casuaricitin, and pentagalloyl glucose that are depicted in Figure 4. provide an indication of the biosynthetic pathway for the formation of ellagitannins.

Many ellagitannins yield additional phenolic compounds on hydrolysis such as chebulic, dehydrodigallic, and flavogallonic acids. These substances are also formed by modification of galloyl groups attached to glucose. In some ellagitannins, glucose is in its open-chain state. The ellagitannins occur in the leaf, bark, and heartwood of many plant species.

The complexity and large molecular size of some of the ellagitannins is demonstrated by the isolation of a series of newly found substances in the flower buds of plants of the *Camellia* sp (11). One of these, *Camelliin B*, while only a dimer, exhibits a complex macroring structure as shown in Figure 5. Several other macroring dimeric ellagitannins have been isolated from other plants and are part of this newly recognized class of tannins.

As of 1993 more than 400 discrete ellagitannins had been reported (12,13). Taxonomic studies have shown that each of these tannins is associated with specific plant species that follow particular paths of plant evolution (14).

Gallotannins and ellagitannins are not present in food at the high levels observed for the proanthocyanidins. Although *tannic acid* has often been referred to as a component of tea and coffee, none is present. Gallic acid is formed in tea, however, by hydrolysis of gallated flavonoids.

ASTRINGENCY

The reaction of tannins with protein is responsible for their astringency—a property of considerable importance in human and animal feeding. It can be described as the complex of sensations due to shrinking, drawing, or puckering of the epithelium in the buccal cavity. Astringency is not one of the basic taste sensations, as its effect is noticed away from the site of the primary taste receptors. It is a tactile sensation arising from reduced lubrication of the tongue and soft palate (15). The primary reaction that leads to astringency is the precipitation of proteins and mucopolysaccharides in saliva. The astringency of tannins is their most noticeable organoleptic property and is correlated with their protein complexation ability. The simple glucose–gallic acid esters bind more strongly to protein than do the proanthocyanidins because of conformational restraints imposed by the interflavan bonds of the latter group. The gallotannins are, accordingly, more astringent than the proanthocyanidins. It has been shown that proteins that are rich in the amino acid proline, and that possess open, flexible structures have the highest affinity for polyphenols. The presence of these proteins in human saliva is protective against the entrance of excessive quantities of polyphenols into the digestive tract. There is evidence that the prevalence of tannins in the human diet tends to increase the formation of the proline-rich proteins (16).

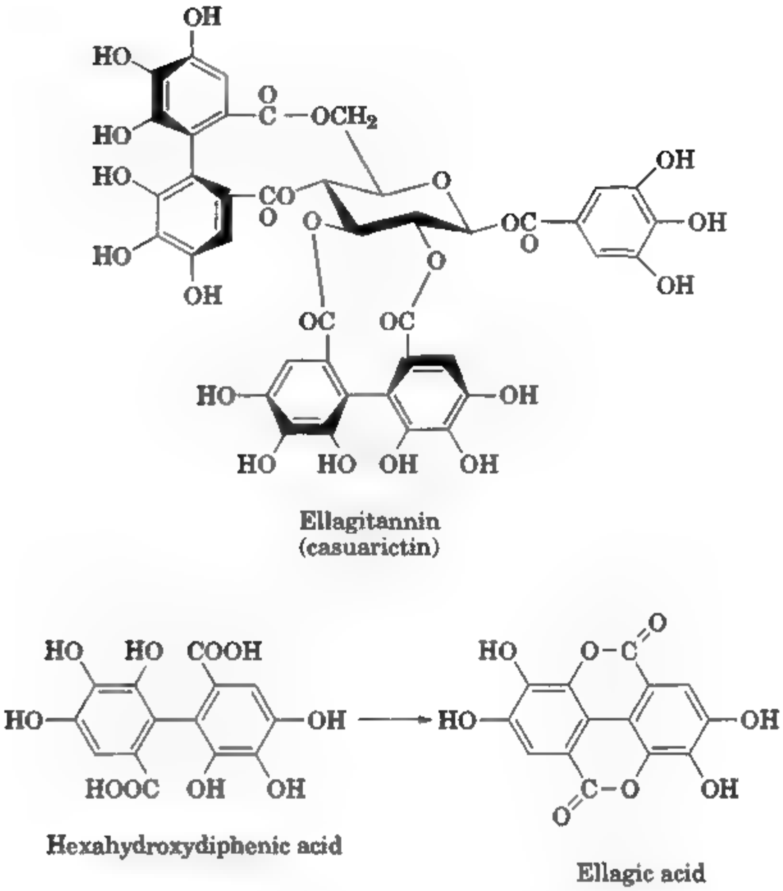


Figure 4. Ellagitannins and component relationships.

The structural feature of a tannin responsible for the phenomenon of astringency has been stated as "the accumulation within a molecule of moderate size, of a substantial number of phenolic groups, many of which are associated with 1,2-dihydroxy or 1,2,3-trihydroxy orientation within a phenyl ring" (16).

The human diet basically includes two groups of tannins: the ellagic acid esters of glucose (ellagitannins) found in the persimmon, pomegranate, and chestnut; and flavanol-derived substances such as condensed catechin oligomers occurring in many fruits, and compounds produced by the enzymic oxidation of the flavanols accompanied by condensation reactions. These are found in wine, beer, and black tea.

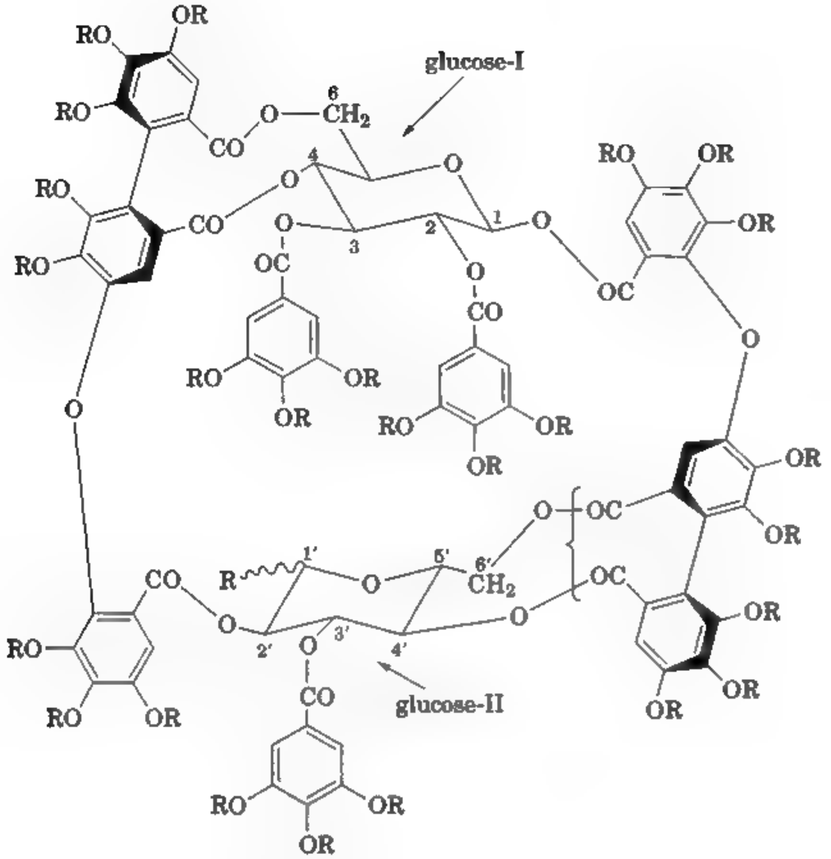
The more water-soluble tannins, such as the ellagitannins, are less efficient in precipitating protein than the polygalloyl glucose esters. Molecular size is also important because of its effect on the orientation of the di/trihydroxylphenyl groups with respect to the proline-rich proteins. Astringency decreases because of steric hindrance as the number of epicatechin units in an oligomer exceeds six or seven. The loss of astringency that occurs during fruit ripening may be caused by further polymerization or by complexation with increased amounts of sugar.

ANALYSIS

The isolation, determination of structure, and quantitation of tannins have been exhaustively investigated because of concerns with their possible physiological effects and because of their interest as pharmaceutical agents. The separation of closely related oligomers has been accomplished with droplet countercurrent and centrifugal partition chromatography (17). By use of these techniques, large molecules, such as nobotanin K, consisting of 4 glucose and 20 gallic acid groups have been isolated in the pure state. Accurate molecular mass determination of these nonvolatile substances has been perfected with the use of fast atomic bombardment (FAB) and mass spectrometry (MS). Nuclear magnetic resonance (NMR) spectroscopy has also provided details of the intricate molecular structures of large complex species. The use of fast atom bombardment mass spectrometry (FABMS) has allowed for the accurate structure determination of large tannin molecules—up to molecular weight of 3200 (18). The most significant methods for the quantitation of tannins are based on measurements of protein precipitation, since their most important properties are attributed to this effect. Procedures based on interaction with bovine serum albumen measure both tannins and protein in the precipitate (19). As little of 5 μg of tannic acid can be detected.

TANNIN–PROTEIN INTERACTIONS

The primary mode for tannin–protein interaction is hydrogen bond formation (20). It is largely a surface phenomenon. Bonding occurs between the amide carbonyl groups of



R=H, R′=OH

Figure 5. Camelliin B.

the protein and the hydroxyl groups of the polyphenol. The polyfunctional tannin molecule is able to bind at many sites on the surface of the protein molecule, thus increasing its hydrophobic nature and promoting precipitation. Inhibition of enzymic activity is often a result of tannin–protein complexation. At high protein concentrations the interaction results in cross-linking. There is also evidence for some hydrophobic bonding.

The formation of the tannin–protein complex is pH dependent and reaches a maximum at the isoelectric point of the protein. Most complex formation is reversed at pH >9. Solvents, such as acetone, that tend to form hydrogen bonds competitively inhibit the interaction. Polyphenol molecules with a high degree of flexibility are most reactive. Proanthocyanidins are more rigid than the gallotannins and are less active complexing agents on a molar basis.

Tannin–protein complexation is favored as the polyphenol solubility is decreased. Proteins with a high proline or hydroxyproline content, such as gelatine, have a greater affinity for polyphenols as these amino acids impart flexibility to the protein molecule and form strong hydrogen bonds. The tendency of a protein to bind to a tannin may be determined by the degree to which the protein competitively prevents inhibition of a specific enzymic activity by the tannin.

Complexation and precipitation of caffeine by tannins has been recognized as the most important cause of "cream" formation when infusions of black tea are allowed to cool. The enzyme tannase catalyzes the cleavage of the gallate linkage in tannin molecules and has been used to solubilize caffeine–polyphenol adducts during preparation of instant tea (see the article TEA). It may be induced in large quantities in some strains of *Aspergillus* molds. Caffeine–polyphenol systems have been used as models for the investigation of tannin–protein interactions because there is a resemblance between the molecular structures of caffeine and peptides.

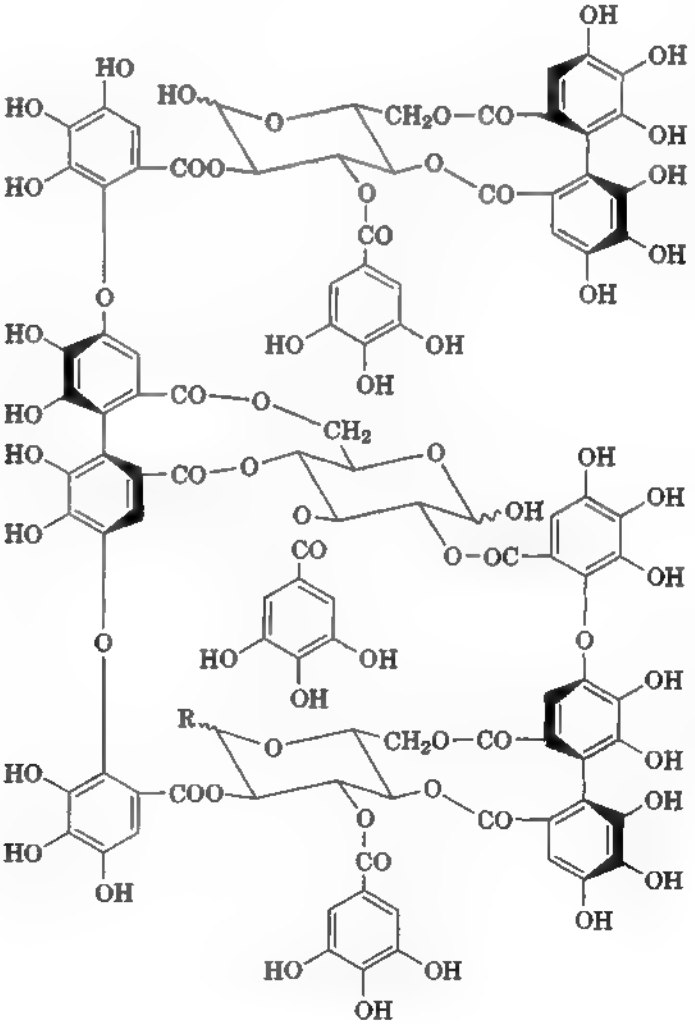
The tanning of animal hides with vegetable extracts accounts for only a small proportion of the leather now produced in the United States. The use of chromium salts has supplanted the traditional process for most applications. Vegetable tannage depends on packing the amorphous regions of collagen fibers with large quantities of tannins so as to bring about extensive cross-linking. The collagen may adsorb up to 50% of its weight of tannins. Tanning renders the mass impermeable to bacterial attack, decreases water penetration, and increases tensile strength. Vegetable-tanned leather has superior qualities of resilience. The extracts used are derived from oak galls, chestnuts, and the bark and heartwood of trees such as quebracho, gambier, and mimosa. Wastewater from tanneries is highly contaminated. Its purification before discharge is made difficult by the inhibitory action of residual tannins on the microbiological organisms normally utilized for anaerobic fermentation (21).

PHYSIOLOGICAL EFFECTS

Dietary tannin decreases the growth rate of many mammals and the net energy available to domestic grazers (22). For ruminants there is a compensating effect since complexation of dietary protein diminishes its microbial degradation in the rumen. Egg production by hens is decreased. The antinutritional effects probably result from the formation of less digestible complexes.

The presence of tannins in plants imparts some degree of protection against insects and grazing animals because of their negative nutritional impact. It is suggested that this may be the evolutionary basis for their presence (20). Conversely, some insects and higher animals have developed compensatory mechanisms that enable them to consume tannins with no antinutritional effects.

The widespread consumption of *Sorghum bicolor* in tropical semiarid zones provides large human populations with their major source of calories and protein. The nutritional consequences of high sorghum diets have been studied extensively and much specie variation has been observed (22). The rat goes through an adaptation period during which salivary glands are stimulated to produce proline-rich proteins. These compete effectively for tannins and inhibit dietary protein complexation. In other animal species, different salivary proteins are produced that have similar inhibitory effects. The hamster has no compensatory mechanism and soon succumbs. Several techniques have been utilized to minimize the antinutritional effects of the tannins in high-sorghum human diets. Cooking is not effective. Dehulling is carried out in many cultures with considerable benefit since tannin is concentrated in the layer just below the seed coat. Alkaline treatment of



Oenothein A: R = OH
Woodfordin D: R = (α)-OG

Figure 6. Oenthein A and Woodfordin D.

the grain and soaking in dilute formaldehyde are also effective procedures for improving its nutritional value.

The polyphenolic content of the tannins imparts antioxidant activity to biological systems. The ability of condensed tannins from several plant sources to scavenge oxygen-containing free radicals has been demonstrated (23). Exploitation of this effect to produce beneficial consequences for humans has been suggested since peroxidation has been implicated in tumerogenesis. Some dimeric ellagitannins exhibit antitumor activity in mice. Structure and activity have been correlated (23). It has been suggested that possible mechanisms for pharmacological activity of tannins may depend on several tannin propensities: complexation with transition-metal ions; free-radical quenching; complexation with proteins and peptides (25).

Inhibition of tumor growth in rodents by the administration of some oligomeric hydrolyzable tannins after intraperitoneal inoculation of tumor cells has been observed. Examples of these active ellagitannins are oenothein and woodfordin, shown in Figure 6. This effect is an example of host-mediated antitumor activity as interleukin-1 is induced in the treated animals (26,27).

Life expectancy of stroke-prone hypertensive rats has been extended by feeding persimmon tannin. Grape seed procyanidin exhibits anti-inflammatory activity in experimental animals. (28).

Some selected purified tannins have been tested and shown to be nonmutagenic by the Ames Test (29).

BIBLIOGRAPHY

1. E. Haslam, "Twenty-Second Procter Memorial Lecture: Vegetable Tannins—Renaissance and Reappraisal," *J. Soc. Leather Technol. Chem.* **72**, 45–64 (1988).
2. T. White, *The Chemistry of Vegetable Tannins, Society of Leather Trades Chemists*, Croydon, London, 1956, p. 7.
3. R. W. Hemingway and L. Y. Foo, "Condensed Tannins: Quinone Methide Intermediates in Procyanidin Synthesis," *J. Chem. Soc. Commun.* **18**, 1035–1036 (1983).
4. E. Haslam, "Symmetry and Promiscuity in Procyanidin Biochemistry," *Phytochemistry* **16**, 1625–1640 (1977).
5. M. Sugumaran, "Quinone Methide Sclerotization: A Revised Mechanism for β-Sclerotization of Insect Cuticle," *Bio-Org. Chem.* **15**, 194–211 (1987).
6. W. S. Pierpoint, "Flavonoids in the Human Diet," in V. Cody, E. Middleton Jr. and J. B. Harborne, eds., *Progress in Clinical and Biological Research*, Vol. 213, Alan R. Liss, New York, 1986, pp. 125–140.
7. R. Gujer, D. P. Magnolato, and R. Self, "Proanthocyanidins and Polymeric Flavonoids from Sorghum," in V. Cody, E. Middleton Jr., and J. B. Harborne, eds., *Progress in Clinical and Biological Research*, Vol. 213, Alan R. Liss, New York, 1986, pp. 159–166.
8. T. C. Somers and E. Verette, "Phenolic Composition of Natural Wine Types," in H. F. Linkskens and J. F. Jackson, eds., *Modern Methods of Plant Analysis, New Series Wine Analysis*, Vol. 6, Springer-Verlag, Berlin, 1988, pp. 219–257.
9. K. J. Siebert, A. Carrasco, and P. Y. Lynn, "Formation of Protein-Polyphenol Haze in Beverages," *J. Agric. Food Chem.* **44**, 1997–2005 (1996).
10. E. Haslam, "Vegetable Tannins," in T. Swain, J. B. Harborne, and C. V. Van Sumere, eds., *Recent Advances in Phytochemistry*, vol. 12, *Biochemistry of Plant Phenolics*, Plenum Press, New York, 1979, pp. 475–523.
11. T. Yoshida et al., "Tannins of Theaceous Plants II. Camelliiians A and B. Two New Dimeric Hydrolyzable Tannins from Flower Buds of Camellia-japonica and Camellia-sasanqua THUNB," *Chem. Pharm. Bull.* **38**, 2681–2686 (1990).
12. E. Haslam, "Polyphenols—Phytochemical Chameleons," *Proc. Phytochem. Soc. Eur.* **34**, 219 (1993).
13. T. Okuda, T. Yoshida, and T. Hatano, "Hydrolyzable Tanins and Related Polyphenols," *Prog. Chem. Org. Nat. Prod.* **66**, 1–117 (1995).
14. T. Okuda, T. Yoshida, and T. Hatano, "Classification of Oligomeric Hydrolyzable Tannins and Specificity of Their Occurrence in Plants," *Phytochemistry* **32**, 507–521 (1993).
15. P. A. S. Breslin et al., *Chem. Senses* **18**, 405–407 (1993).
16. M. N. Clifford, "Astringency," *Proc. Phytochem. Soc. Eur.* **41**, 87–107 (1997).
17. T. Okuda, T. Yoshida, and T. Hatano, "New Methods of Analyzing Tannins," *J. Natural Products* **52**, 1–31 (1989).
18. T. Okuda, T. Yoshida, and T. Hatano, "Hydrolyzable Tannins and Related Polyphenols," *Prog. Chem. Org. Nat. Prod.* **66**, 62 (1995), pp. 2–117.
19. H. P. S. Makkar, "Protein Precipitation Methods for Quantitation of Tannins: A Review," *J. Agr. Food Chem.* **37**, 1197–1202 (1989).
20. C. M. Spencer et al., "Polyphenol Complexation—Some Thoughts and Observations," *Phytochemistry* **27**, 2397–2409 (1988).
21. H. S. Bajwa and C. F. Forster, "The Inhibition of Anaerobic Processes by Vegetable Tanning Agents," *Environ. Technol. Lett.* **9**, 1245–1256 (1988).
22. L. G. Butler et al., "Dietary Effects of Caffeine," in V. Cody, E. Middleton Jr., and J. B. Harborne, eds., *Progress in Clinical and Biological Research*, Vol. 213, Alan R. Liss, New York, 1986, pp. 141–157.
23. S. Uchida et al., "Radical Scavenging Action of Condensed Tannins," *Neurosciences (Kobe, Japan)* **14**, 243–245 (1988).
24. K. Miyamoto et al., "Relationship between the Structures and the Antitumor Activities of Tannins," *Chem. Pharm. Bull.* **35**, 814–822 (1987).
25. E. Haslam, "Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action," *J. Nat. Prod.* **59**, 205–215 (1996).
26. K. T. Miyamoto et al., "Antitumor Activity and Interleukin-1 Induction by Tannins," *Anticancer Res.* **31**, 37–42 (1993).
27. T. Yoshida et al., "Woodfordin D and Oenothien A, Trimeric Hydrolyzable Tannins of Macro-Ring Structure with Antitumor Activity," *Chem. Pharm. Bull.* **39**, 1157–1162 (1991).
28. M. Gábor, "Anti-allergic and Anti-inflammatory Properties of Flavonoids," in V. Cody, E. Middleton Jr., and J. B. Harborne, eds., *Progress in Clinical and Biological Research*, Vol. 213, Alan R. Liss, New York, 1986, pp. 471–480.
29. C. L. Yu and B. Swaminathan, "Mutagenicity of Proanthocyanidins," *Food Chem. Toxicity* **25**, 135–139 (1987).

HAROLD N. GRAHAM
New York City, New York

See also TEA.

TASTE AND ODOR. See SENSORY SCIENCE: PRINCIPLES AND APPLICATIONS; SENSORY SCIENCE: STANDARDIZATION AND INSTRUMENTATION.

TEA

The term tea refers to the plant *Camellia sinensis*, the dried, processed leaf manufactured from it, extracts derived from the leaf, and beverages prepared from leaf or extract of this species. Tea is the most widely consumed of all beverages aside from water. Annual worldwide per capita consumption, although geographically far from uniform, is 40 to 50 L (based on total known tea production of 2.667 million tons (1) and a beverage yield of ca 100 L/kg). Apart from its extensive usage, it is of interest to the food technologist as well as to the biochemist, organic chemist, and physiologist because of the dependence of the final product characteristics on the unusual chemical composition of the fresh leaf and because of the complex series of biochemical and organochemical reactions that occur during processing. There has also been increasing interest in its health benefits.

Two major varieties of *C. sinensis* are recognized: *assamica*, a large-leaved (15–20 cm) plant, and *sinensis*, a smaller-leaved (5–12 cm) plant. Intervarietal hybrids are common. There are also hybrids with a related species, *Camellia irrawadiensis*. Tea originated in southeast Asia in an area that includes parts of China and India, and probably Myanmar, Laos, and Vietnam.

The first authenticated consumption of tea dates from the fourth century A.D. in China, although legend dates it much earlier. Its use spread to Japan, where cultivation started in the eighth century. Tea from China reached Europe in the sixteenth century, and by the end of the seventeenth century it had become a popular beverage served in London teahouses. Tea was discovered growing wild in Assam Province of India in 1823 and cultivation followed rapidly. The plant was introduced into the Dutch East Indies (Indonesia) and then into Ceylon (Sri Lanka) after the destruction through disease of that country's coffee plantings in the 1870s. Cultivation of tea on a large scale subsequently spread to other parts of Asia, to Georgia in the former USSR, and to Africa, South America, and several tropical islands. Tea production was carried out on a very small scale in South Carolina around the beginning of the twentieth century; at its peak, ca 10,000 lb/yr was produced (2). Commercial operation was discontinued in 1915 but resumed in 1987 (3).

AGRICULTURE

Tea grows best in tropical and subtropical areas where adequate rainfall (200 cm), good drainage, and slightly acid soils prevail. *Sinensis* variety is the more cold resistant. In hot tropical areas, tea quality is improved by planting at high altitudes, as practiced in India and Sri Lanka, where tea is cultivated at elevations up to 2,000 m. The tea plant is maintained by pruning as a low shrub (1–1.5 m) at a density of 5,000 to 10,000 plants/ha. Tea is now primarily propagated from leaf cuttings rather than from seed. The establishment of desirable clones is accomplished by selection on the basis of beverage quality, yield, pest and frost resistance, and other criteria dictated by local conditions and marketing considerations. Yields have been greatly increased and exceed 6,000 kg/ha in some clonal fields, although national averages are very much lower (1). Tissue culture of tea is under investigation as a method for producing and rapidly replicating new clonal material. Plants have been regenerated and set out in soil (4). Tea can remain productive for many decades; some 100-year-old plantings are still being harvested. The use of irrigation, fertilizers, mulches, and pesticides has been extensively researched in many tea-growing areas and the results reported in the journals of the various tea research institutes (5–8). Pesticides banned in the United States are not used (9).

New tea growth (flush) is harvested at intervals of 6 to 12 days depending on climatic conditions. In cooler areas such as North India, Japan, and Georgia there is a dormancy period. Plucking is a hand operation on most estates. Varying degrees of mechanization are being applied in some countries where terrain and labor costs are appropriate. These range from handheld cutters powered by small back-packed gasoline engines to large self-propelled harvesters that straddle the tea row. Removing the apical bud and two leaves below it ("two and a bud") constitutes the ideal plucking practice, but it is not always realized, and never with mechanical harvesting. Tea estates range in size from small peasant holdings of 1 ha or less to large establishments of up to 1,000 ha. The larger estates usually include housing, schools, hospitals, and other facilities for the workers employed in this labor-intensive activity. Labor utilization is often at the rate of 3 persons/ha. Plucking may account for half of the total requirement.

FRESH LEAF COMPOSITION

Tea leaf contains all of the enzymes, metabolic intermediates, and structural elements normally associated with photosynthesis and plant growth. In addition, the tea plant contains a large quantity of substances that are responsible for its unique properties as a beverage. Chief among these are polyphenols (mostly flavonoids) and caffeine. A representative analysis of young shoots from fresh *assamica* leaf is depicted in Table 1.

Flavanols

The most striking chemical characteristic of tea flush is its very high content of flavanols. Although amounts vary, depending on all of the factors that influence plant metabolism, such as light, rainfall, temperature, nutrient availability, leaf age, and genetic makeup, a small number of flavanols alone may constitute more than 20% of the dry matter. The most important group of the tea flavanols are the catechins. These include the free catechins as well as monogallate and digallate esters and a single caffeic acid ester (12). Free catechins and the monogallate esters predominate, and their reaction products are the best known (Fig. 1).

Table 1. Composition of Tea Flush (% Dry Weight)

Flavanols	25
Flavonols and flavonol glycosides	3
Flavone C-glycosides	0.2
Polyphenolic acids and depsides	5
Other polyphenols	3
Caffeine	3
Theobromine	0.2
Amino acids	4
Organic acids	0.5
Monosaccharides	4
Polysaccharides	14
Protein	15
Cellulose	7
Lignin	6
Lipids, waxes, sterols	3.5
Chlorophyll and other pigments	0.5
Ash	5
Volatiles	<0.1

Source: Refs. 10 and 11.

The tea flavanols are water-soluble, colorless, astringent substances that oxidize rapidly, especially in an alkaline environment. They occur in the cytoplasmic vacuoles of the leaf cells. They form complexes with many other substances (13). Epigallocatechin gallate constitutes a high proportion of fresh-leaf weight. Tea beverage quality is correlated with fresh-leaf flavanol content, especially with the gallated compounds that decrease in quantity as the leaf ages on the growing plant. They are the most important components in the black-tea manufacturing process.

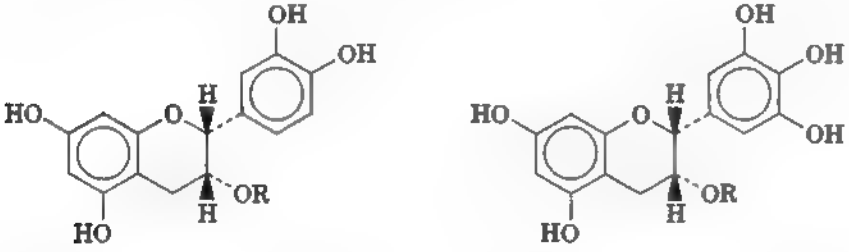
Catechins are notable antioxidants, a property also exhibited by their oxidation products present in black tea and by other flavonoid compounds (14). This is demonstrated by their ability to scavenge free radicals generated in many *in vitro* systems. The physiological significance of this property will be discussed later in this article.

Other Polyphenols

Other polyphenols include gallic acid (**7**) and quinic acid; flavonols, such as kaemferol (**11**), quercitin (**12**), myricetin (**13**), and their glycosides; a group of chalcan–flavan dimers; proanthocyanidins; a group of flavone-*C*-glycosides (10); and a group of bisflavanols also known as theasinensins (12). Depsides present include chlorogenic acid (**8**), *p*-coumarylquinic acid (**9**), and theogallin (3-galloylquinic acid) (**10**), which is unique to tea (Fig. 2). It occurs at relatively high concentrations (1% of dry weight) and is said to correlate with quality. Most of the polyphenols participate in the important reactions that occur during manufacture.

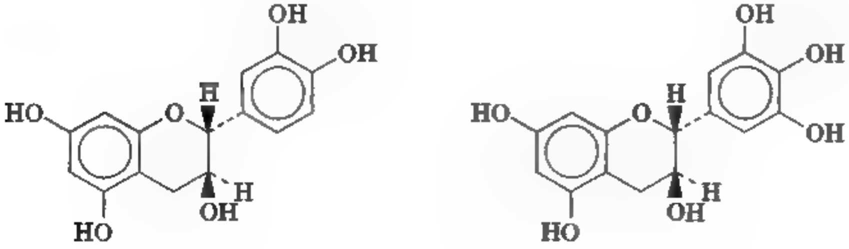
Caffeine and Other Xanthines

Tea flush contains 2 to 4.5% caffeine and much smaller amounts of theobromine. Theophylline may be present but in small quantities. Caffeine is responsible for the mild stimulatory properties of tea beverage and is generally



(1) (−)-epicatechin;
R = H; 1–3% of dry wt
(2) (−)-epicatechin gallate;
R = 3,4,5-trihydroxybenzoyl;
3–6% of dry wt

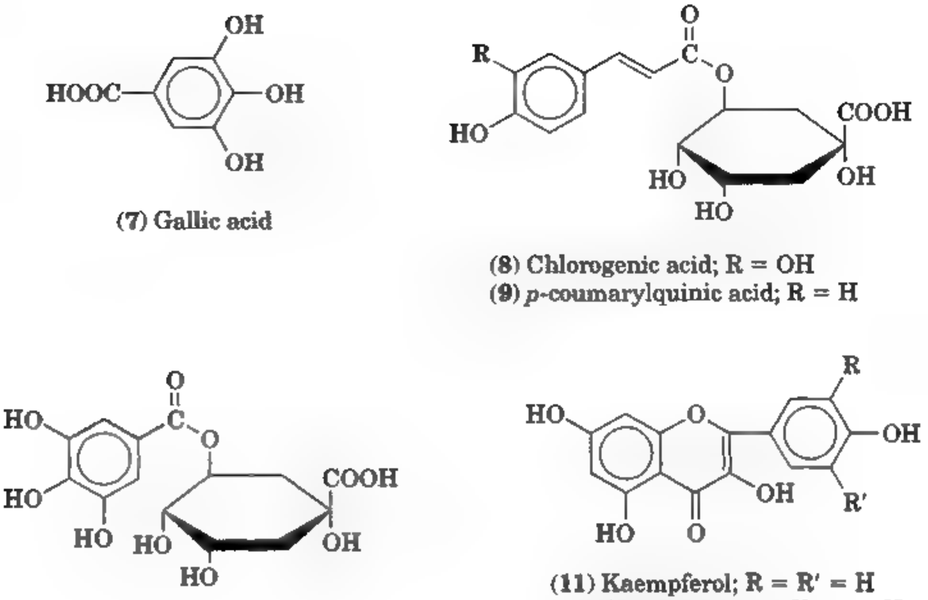
(3) (−)-epigallocatechin;
R = H; 3–6% of dry wt
(4) (−)-epigallocatechin gallate;
R = 3,4,5-trihydroxybenzoyl;
9–13% of dry wt



(5) (+)-catechin; 1–2% of dry wt

(6) (+)-gallocatechin; 3–4% of dry wt

Figure 1. The flavanols occurring in tea.



(7) Gallic acid

(8) Chlorogenic acid; R = OH
(9) *p*-coumarylquinic acid; R = H

(10) Theogallin

(11) Kaempferol; R = R′ = H
(12) Quercitin; R = OH, R′ = H
(13) Myricetin; R = R′ = OH

Figure 2. Other polyphenols occurring in tea.

$HO_2CCH(NH_2)CH_2CH_2CONHC_2H$

Figure 3. Theanine (**14**).

present at a level of 30 to 45 mg/cup. Its tendency to complex with polyphenols affects the organoleptic properties of tea beverage and the technology of instant-tea manufacture. The properties of caffeine and tea decaffeination processes are not discussed in this article.

Amino Acids

In addition to the normal plant leaf amino acids, theanine (5-*N*-ethylglutamine) (**14**) (Fig. 3) is unique to tea and correlates with green-tea quality (15). Several of the other amino acids in the leaf take part in aroma formation during manufacture.

Carotenoids

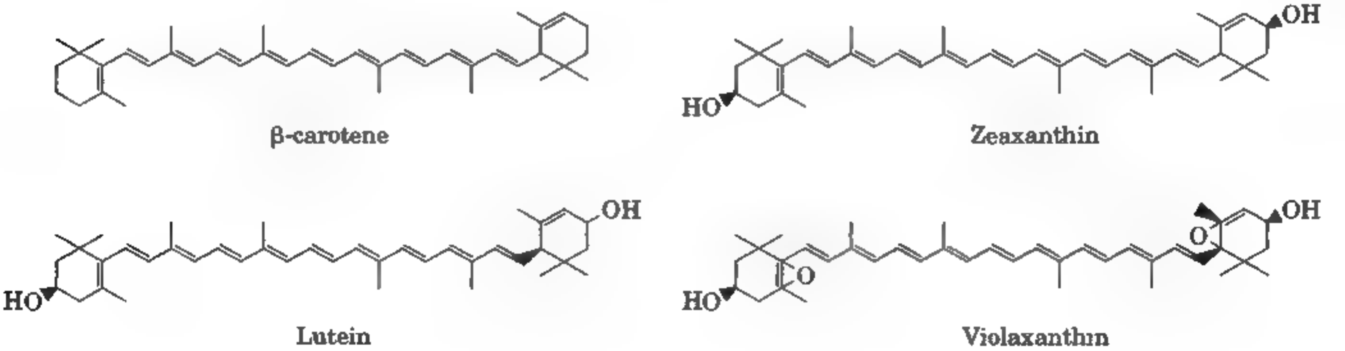
Carotenoid compounds constitute only 0.03 to 0.06% of tea flush but are important in aroma generation during manufacture. They include *β*-carotene, lutein, zeaxanthine, and violaxanthine (Fig. 4) (16).

Volatiles

More than 50 volatile compounds have been reported in fresh leaf, although some of these may be artifacts of the isolation procedures (17). Aroma components, their origin, and their significance will be discussed in the manufacturing section as it is during leaf processing that most aroma character is developed.

Lipids, Waxes, and Pigments

The presence of unsaturated fatty acids is also of significance to aroma generation, as will be shown. Linoleic and linolenic acids are present at a level of ca 1.2% in fresh leaf



β-carotene

Zeaxanthin

Lutein

Violaxanthin

Figure 4. Tea carotenoids.

(18). Other hydrophobic compounds such as plant waxes and sterols may be sufficiently extracted to affect beverage appearance (19). Chlorophyll is of significance as a possible precursor of some dark-colored pigments in manufactured tea.

Enzymes

Tea leaf polyphenol oxidase that catalyzes the aerobic oxidation of the catechins during manufacture of black tea is of prime importance. It contains copper (0.32%) and exists as a mixture of several isoenzymes. Its main component has a molecular weight of 142,000 (20). Peroxidase also takes part in the polyphenol oxidation processes (21).

The complex enzyme systems involved in the synthesis of the flavanols and related tea leaf components have been described in two reviews (22,23). Alcohol dehydrogenase (24) and leucine α-ketoglutarate transaminase (25) contribute to the development of aroma components during manufacture. Glycosidases catalyze the breakdown of several glucosides to yield other important aroma components (26).

Minerals

In addition to the common plant minerals, the tea plant is rich in potassium and fluoride and also accumulates aluminum if soil conditions so favor (27).

MANUFACTURE

The manufacturing process converts freshly harvested leaf to products of commerce. Black tea is the most widely consumed form of tea. It is produced by promoting the aerobic oxidation of fresh leaf catechins in reactions catalyzed by tea polyphenol oxidase. Green tea, consumed mostly in China, Japan, the Middle East, and North Africa, is processed so as to prevent the oxidation of catechins. Oolong tea is partially oxidized. It is manufactured and consumed primarily in China and Taiwan.

Black Tea

The most significant change that occurs during the manufacture of black tea is the conversion of the colorless catechins to a complex mixture of yellow-orange to red-brown substances accompanied by the development of a large number of volatile compounds. The process was historically referred to as fermentation, but no microbiological processes take place. The changes result in a dark-colored leaf that produces an amber-colored beverage, less astringent than that derived from fresh leaf. The product possesses an exceedingly complex flavor profile.

Catechin Oxidation

The first step in catechin transformation is oxidation to highly reactive quinones (Fig. 5) (28). All catechins undergo this reaction, but the gallocatechins (3) and (4) are preferentially oxidized. Quinone formation is the primary driving force for the transformation of catechins to black-tea components.

Theaflavins

During fermentation, quinones derived from a simple catechin (1), (2), or (5) react with quinones derived from gallocatechins (3), (4), or (6) to form compounds with a seven-membered benztropolone ring known as theaflavins (Fig. 6). The predicted theaflavins derivable from each of the possible quinone combinations have been isolated (29,30). In solution, theaflavins are bright orange-red in color and provide "brightness," a desirable quality, to the beverage. (Many of the named attributes of tea arise from the tea taster's lexicon and are difficult to define objectively (31).

The distinctively colored theaflavins are determined spectrophotometrically in a tea brew (32). Total theaflavin concentration in black tea does not exceed 2.5% and is sometimes as low as 0.3% (33). Prolongation of the oxidation period and high fermentation temperatures decrease theaflavin content (34). Theaflavins are oxidized by epicatechin quinone, formed late in the oxidation process because of its high oxidation potential (35). Low oxygen tension also results in decreased theaflavin formation (36). At most, only 10% of the original catechin content of tea flush is accounted for as theaflavins in black tea.

Theaflavic Acids

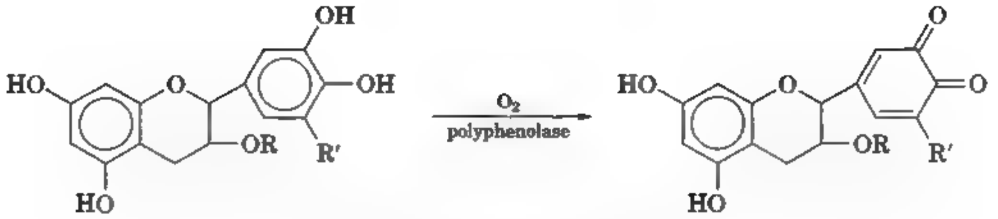
Theaflavic acids are formed by the reaction between quinones derived from (1), (2), or (5) and gallic acid quinone (Fig. 7). It is postulated that gallic acid is formed by degallation during processing. Although it is not directly oxidized by tea polyphenol oxidase, it is converted to a quinone by the catechin quinones that possess a higher oxidation potential, such as that of epicatechin (37,38). The theaflavic acids are bright red, acidic substances present in black tea only in very small quantities because of their reactivity (39). Theaflagallins originate through a similar route but involve the gallocatechins (3), (4), or (6). They are also present in very small quantities (40).

Bisflavanols

Coupling of quinones derived from (3) and (4) produces a series of colorless substances known as bisflavanols (35). They are highly reactive and occur only at low levels in black tea (Fig. 8). The bisflavanols are also known as theasinensins. There have been some reports of small amounts of theasinensins in fresh leaf (12).

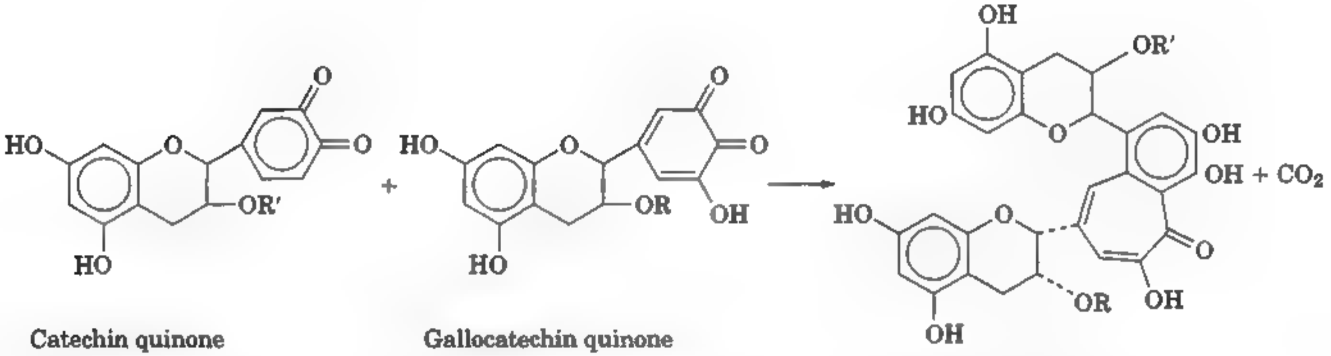
Thearubigins

In the course of black-tea formation, ca 10 to 15% of the leaf catechin fraction remains unchanged and ca 10% is accounted for by theaflavin, theaflavic acid, and bisflavanol formation. Seventy five to 80% of the original catechin fraction is converted to a complex mixture, incompletely defined and largely intractable to resolution. This red-brown fraction was named thearubigen when first described in 1957 (42). Investigations of its chemistry over a period of four decades have resulted in conflicting data and conclusions, attesting to the complexity of the mixture. Molecular weight determinations have indicated a range of 700 to 40,000 (43), but this must be considered in context with the known increase in apparent molecular weight



R = H or 3,4,5-trihydroxybenzoyl
R′ = H or OH

Figure 5. Enzymic oxidation of catechins.

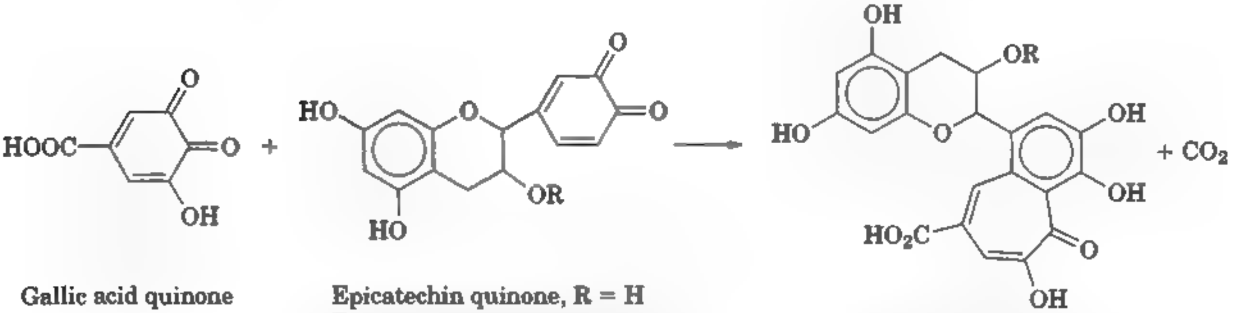


Catechin quinone
R′ = H
Catechin gallate quinone
R′ = 3,4,5-trihydroxybenzoyl

Gallocatechin quinone
R = H
Gallocatechin gallate quinone
R = 3,4,5-trihydroxybenzoyl

(**15**) Theaflavin; R= R′ = H
(**16**) Theaflavin gallate A; R= 3,4,5-trihydroxybenzoyl, R′ = H
(**17**) Theaflavin gallate B; R= H R′ = 3,4,5-trihydroxybenzoyl
(**18**) Theaflavin digallate; R= R′ = 3,4,5-trihydroxybenzoyl

Figure 6. Formation of theaflavins.



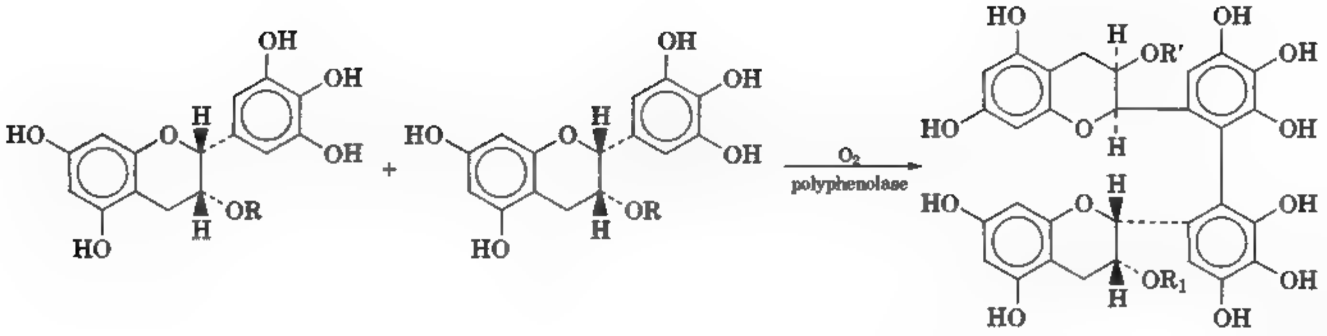
Gallic acid quinone

Epicatechin quinone, R = H
Epicatechin gallate quinone
R = 3,4,5-trihydroxybenzoyl

(**19**) Epitheaflavic acid; R = H
(**20**) Epitheaflavic acid-3′-gallate; R = 3,4,5-trihydroxybenzoyl

Figure 7. Formation of theaflavic acids.

that occurs when tea infusions are allowed to stand. Polyphenolic compounds in this molecular weight range are active complexing agents and are therefore likely to be present in combination with other substances. Flavonol glycosides have been shown to constitute one group of compounds that appear as part of the thearubigin fraction as originally designated, but, as colorless compounds, they are not usefully regarded as such. New high-performance liquid chromatography (HPLC) techniques have allowed for the isolation of several other components of this mass.



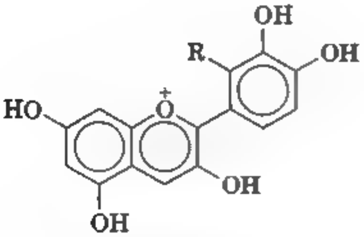
(**21**) Bisflavanol A; R = R′ =3,4,5-trihydroxybenzoyl
(**22**) Bisflavanol B; R = 3,4,5-trihydroxybenzoyl; R′ = H
(**23**) Bisflavanol C; R = R′ = H

Figure 8. Formation of bisflavanols.

A fraction designated as theafulvin has been separated but not resolved. It accounts for only a small proportion of the thearubigin fraction. It is a buff-colored material containing some proanthocyanidin groups, but most of its mass is not completely characterized (44). Theacitrins have also been isolated. They are red substances, similar to the theaflavins but possibly containing additional carboxylic acid groups. They are also present in very small quantities (45,46). Separation techniques have not yet provided methodology for the resolution of the major portion of the thearubigin mixture.

Degradative treatments of the unresolved material have been helpful in piecing together more information. Acid hydrolysis results in the formation of cyanidin and delphinidin (Fig. 9), suggesting proanthocyanidin structures (47). Other degradative procedures have provided evidence for the presence of heterogeneous polymers of flavanols and flavanol gallates (48).

Decreases in theaflavins and bisflavanols during fermentation suggest that these compounds are further oxidized and subsequently incorporated into the thearubigin complex. It is also likely that catechin quinones oxidize (**8**), (**10**), and glycosides of (**11**), (**12**), and (**13**), leading to incorporation of their oxidation products as well (29). In addition, minor amounts of hydrogen peroxide generated during fermentation may be activated by the peroxidase enzyme and bring about further oxidative condensations that augment the thearubigin fraction (49). Model system studies in which single catechins and known catechin mixtures are enzymatically oxidized *in vitro* have shown some promise for further structure elucidation (50).



(**24**) Cyanidin; R = H
(**25**) Delphinidin; R = OH

Figure 9. Anthocyanidins from tea thearubigins.

This fraction constitutes the largest group of compounds in black tea (up to 20% of dry weight) and contributes significantly to color, strength, and mouth-feel of the beverage, but is its least well defined. It has been suggested that thearubigin may not be a useful term for the chemical characterization of this tea fraction because of its heterogeneity and the more recently observed facts that some unchanged fresh-leaf components are included (13). It does refer, however, to a group of substances that have significance to the tea taster with regard to beverage organoleptic properties.

BLACK TEA

Black-tea manufacture is carried out in several stages known as withering, rolling, fermenting, firing, and sorting. An understanding of the changes that take place during these processes has been gradually accumulated since the 1930s.

Withering

Plucked leaf arrives at the estate factory within a few hours of its harvest. It is handled carefully to prevent premature bruising and to promote dissipation of heat generated during continued respiration. Moisture level is reduced from 75–80% to 55–65% without heat buildup, usually in troughs designed for effective airflow through leaf beds up to 30 cm thick. Moisture reduction converts the turgid leaf to a flaccid material that is easily handled without excessive fracture. The process takes place over a period of 6 to 18 h, depending on factory equipment and weather conditions. Important chemical changes occur during withering: cell membrane permeability is increased, which allows for more efficient disruption of cell structure during the next stage of manufacture; amino acid and organic acid levels increase; and polyphenol oxidase activity is enhanced. These changes have been referred to as chemical wither. Degree of physical wither (moisture loss) is estimated by leaf texture or by checking the weight loss of an isolated leaf batch. The withering process affects final product value (51).

Rolling

Rolling serves to establish proper conditions for the enzymic oxidation of the flavanols by atmospheric oxygen. This is accomplished by disrupting cell structure to effect enzyme–substrate contact. The leaf mass must also be maintained in a physical state conducive to oxygen diffusion. Rolling processes are designed to meet these requirements. The conventional roller consists of a circular platform, 1 to 1.3 m in diameter, equipped with battens. Above this, a smaller circular sleeve moves eccentrically over the surface of the platform. Withered leaf is charged into the sleeve and a pressure cap is lowered into it. The rates of movement and the pressure applied are chosen in accordance with leaf properties and atmospheric conditions. Intermittently, sufficiently rolled leaf (dhool) is removed by sieving and the remainder is rerolled. Leaf juices become well distributed on the surface of the cut, twisted leaf. Teas manufactured by this process are known as orthodox. They include almost all Ceylon teas.

More modern equipment has come into use in most of the other tea-growing areas. The McTear Rotorvane consists of a cylinder 20 or 37.5 cm in diameter, lined with vanes, through which a vaned rotorshaft propels the withered leaf. Constriction at the end applies back pressure and increases the work done on the leaf. Cutter blades are sometimes added to the shaft. From the Rotorvane, tea is usually passed through a CTC (crush, tear, curl) machine, which consists of a pair of ridged cylindrical rollers revolving at different speeds. There is a narrow, adjustable clearance between the rollers. The Rotorvane-CTC combination provides efficient and uniform maceration and is well suited to the establishment of a continuous manufacturing process. The macerated leaf is fluffed up by passage through a high-speed Rotorvane, with no back pressure, to eliminate matting and facilitate air diffusion. It is desirable to maintain leaf temperature below 35°C during maceration to prevent flavor deterioration. The Legg Cutter, originally designed for processing tobacco, is used in India to process unwithered or lightly withered leaf. The Lawrie Tea Processor (LTP) is essentially a hammer mill. It is used in Africa for lightly withered leaf.

The final physical state of the leaf is dependent on the maceration conditions employed and affects the course of the oxidation reactions. This, in turn, affects the chemical composition and some aspects of the quality of the final product, especially the theaflavin-to-thearubigin ratio (52).

Fermentation

The term fermentation arose from the erroneous concept of black-tea production as a microbial process (53). Around 1901 the conversion of green tea to black tea became recognized as an oxidative process initiated by tea enzymes (54). The process actually starts at the onset of maceration and is allowed to continue under ambient conditions in most instances, but leaf temperatures are preferably controlled so as not to exceed 25 to 30°C. Lower temperatures (15–25°C) improve flavor (34). Temperature control and air diffusion are facilitated by spreading the macerated leaf in layers 5 to 8 cm thick on racked trays in a fermentation room maintained at high humidity and at the lowest feasible temperature. Depending on the nature of the leaf, maceration technique, ambient temperature, and the style of tea desired, the fermentation time ranges from 45 min to 3 h, although the shorter process times are more common. Completion of oxidation is usually judged by the development of the characteristic coppery color and the fermented tea aroma. More highly controlled fermentation systems have been described. They depend on the time-controlled conveyance of rolled leaf on mesh belts through which there is forced air circulation. In a few factories the fermentation belt is part of a closed system that allows for control of temperature, aeration, and humidity (55).

Firing

Fermentation is terminated by firing in large ovens with hot forced-air circulation. In most driers, loaded trays traverse the drying zone countercurrent to the airflow. Incoming air temperature is ca 90°C and emergent air is ca 50°C. Time-temperature profiles are controlled to effect drying in 18 to 20 min with little energy wastage. Fluid-bed driers are sometimes utilized (56). During the firing process leaf moisture is reduced to 2 to 3%. Proper drying control is crucial to product quality. Many organochemical processes are accelerated during this period, as are the enzymic reactions before total thermal inactivation occurs. Incomplete enzyme inactivation can cause accelerated deterioration during storage. One noticeable effect of firing is the color change brought about by the conversion of chlorophyll to pheophytins and pheophorbide, which are dark brown and black, respectively. Small amounts of caffeine are lost through sublimation. A high proportion of the aroma of black tea is generated during the firing process.

Grading and Packaging

Fired tea is graded by the use of a series of oscillating screens. The grades most frequently produced, in descending particle size order, are orange pekoe (OP), pekoe (P), broken orange pekoe (BOP), broken orange pekoe fannings (BOPF), fannings, and dust. Fine dust, debris, and some fiber are removed by winnowing. Stalky materials tend to retain moisture and may be removed from the dryer tea fractions by use of an electrostatic sorter. It is essential to protect tea from heat, moisture, and light to insure reasonable shelf life. Color, taste, and aroma deteriorate if poor storage conditions prevail. Theaflavin decrease is marked (57). Aroma quality also deteriorates. Multilayered natural Kraft bags lined with aluminum foil have come into general use in shipment.

Blending

Most branded black tea is blended by the packer. This is essential to insure constancy of taste, appearance, and, to the greatest extent possible, cost. Seasonal, climatic, and market changes would make a constant product impossible to achieve unless the tea blender had access to many different teas for use in a single blend. This allows for necessary substitutions without departure from product image. The special talents and experience of the tea taster are still required. Instrumental analysis is not yet adequate to define and control all of the attributes important to the consumer.

Aroma Generation

Black-tea aroma is a complex system in which well over 500 components have been identified (58). The continuing improvement of analytical techniques results in the proliferation of known tea volatiles, for example, one research paper reported the identification of 133 new components, all of which are present in quantities <10 μg/kg of beverage (59). Aroma ingredients may originate either as components of the fresh leaf, as products of enzymic or organochemical activity during the manufacturing processes, or as products of the high-temperature firing step. Almost all aroma components develop during postharvest processes. It is probable that only a few dozen are significant to tea aroma quality.

Aldehydes are generated in fresh leaf by the oxidative breakdown of unsaturated fatty acids catalyzed by lipoxygenases. The short-chain unsaturated aldehydes are generally deleterious to tea flavor. Leaf alcohol dehydrogenase systems catalyze their partial reduction to alcohols that exhibit a characteristic green leaf flavor not desirable at high levels (60). The more volatile of these compounds are partially lost during the firing stage. Aldehydes are also formed during black-tea manufacture by a Strecker-type synthesis resulting from the reaction of catechin quinones with amino acids. Phenylacetaldehyde is formed from phenylalanine in this manner (61).

Terpene alcohols, phenylethyl alcohol, methyl salicylate, and other important aroma components are generated by enzymic hydrolysis of their glycosides (62). Their reported presence in fresh leaf may be partially the result of initiating hydrolysis during analytical procedures. These compounds contribute significantly to the character of tea aroma. Several aromatic compounds present in fine teas are formed by the oxidation of the tea carotenoids by catechin quinones. These include *cis*-jasmone, theaspirone, β-ionone, and dihydroactinodiolide (61). Carotenoids are also thermally decomposed to produce these and other aroma components during firing.

Other thermal reactions are responsible for the development of a large group of cyclic aroma components. These include pyrazines, pyrroles, pyridines, and quinolines (63). Their significance is not known. Some compounds that impart the most desirable flavor characteristics to black tea are shown in Table 2. Tea tasters use the term quality to refer to tea with desirable aroma character. Black-tea aroma also contains the group of short-chain (5–7 carbon atoms) aldehydes and alcohols that arise from lipid oxidation. An abundance of these substances is undesirable. Aroma quality is determined by the relative quantities of the two groups of components (58,64).

Table 2. Desirable Black-Tea Aroma Components

Benzyl alcohol
Dihydroactinodiolide
Geraniol
cis-3-hexenyl hexanoate
β-ionone
cis-jasmone
Linalool
Linalool oxides
Methyl salicylate
Nerolidol
Phenylacetaldehyde
2-phenylethanol
1-terpineol
Theaspirone

BLACK TEA BEVERAGE

Preparation and Properties

Black-tea beverage is characterized by its amber color and distinctive aroma. In the United States it is generally prepared from tea bags containing 2.27 g of tea (200 servings/lb). Tea bag paper must be sufficiently permeable to provide rapid infusion but also retain small leaf particles. It should not impart taste to the beverage, it must be consistent in weight and thickness to insure machinability, and it must have adequate dry and wet strength. Abaca fiber obtained from a plant grown in Ecuador and the Philippines is commonly included. Tea bagging machines are able to produce up to 1,000 bags/min.

Infusing tea bags with boiling water in a 6- to 8-oz cup for 3 min provides a beverage containing 0.25 to 0.35% solids. (The use of packaged tea in the United States has declined greatly and now accounts for only approximately 3% of retail sales). Beverage concentration is highly dependent on extraction time, temperature, and water-to-tea ratio. Caffeine is extracted proportionally with the other soluble components. Excessively hard water produces a murky beverage. Black-tea beverage color is one of the useful indicators of value in that it reflects the quality of the cultivar, the success of the manufacturing procedure, and the control of storage conditions. Desirable tea beverage color requires the use of fresh leaf that contains a sufficient level of polyphenols and oxidizing enzyme held under conditions favorable to achieving adequate oxidation, and proper theaflavin-to-thearubigin ratios. Inferior aroma may result from suboptimal manufacturing or storage conditions. Brewed tea beverage is relatively unstable. Storage for more than 30 to 60 min results in noticeable flavor deterioration. Theaflavin levels decrease during long-term holding at elevated temperatures (65).

When black-tea beverages are allowed to cool, cloudiness slowly develops, and eventually a precipitate becomes visible. The tea taster refers to this as cream and considers it a normal characteristic of most acceptable teas. The composition of cream and its significance to instant tea manufacture will be discussed under that topic.

Composition

The composition of a characteristic black-tea beverage is shown in Table 3.

GREEN TEA

The primary objective in green-tea manufacture is to prevent catechin oxidation to the greatest extent possible while imparting an attractive, twisted appearance to the leaf and developing the characteristic green tea aroma. There are many styles of green tea. In Japan sencha is the most extensively drunk. Gyokuro, grown under shade, is the most highly esteemed. Gunpowder tea is a pelleted green tea consumed in China.

Enzyme Inactivation

The leaf enzymes may be inactivated either by steaming, most often carried out in Japan, or by panning, the procedure of choice in China. The pan, however, has been superseded by 5-m-long, open, rotating cylinders that are heated during the passage of the tea over a 7- to 10-min period. Catechin oxidation is prevented and the leaf retains its green color and almost all of its original polyphenol content.

Rolling

The primary function of the rolling process in green-tea manufacture is to impart the desirable twisted appearance to the leaf. Lighter equipment is used than that required for black-tea production as small-leaved varieties are generally utilized, and there is no need for cell disruption and oxygen permeability.

Table 3. Composition of Black-Tea Beverage (Weight % of Extract Solids)

Thearubigins	36.0
Theaflavins	2.6
Theaflavic acids	Trace
Bisflavanols	Trace
Catechins	11.2
Flavonol glycosides	Trace
Gallic acid	1.1
Chlorogenic acid	0.2
Caffeine	7.6
Theobromine	0.7
Oxalic acid	1.5
Other aliphatic acids	1.2
Lipids and waxes	4.8
Sugars	6.9
Polysaccharides	4.2
Pectin	0.2
Peptides	6.0
Theanine	3.5
Other amino acids	3.0
Potassium	4.8
Other minerals	4.7
Aroma	<0.1

Source: Ref. 66.

Firing and Aroma Development

Green teas are often fired in horizontal rotating drums. The chemical changes that take place are primarily responsible for the development of aroma (67).

Beverage Composition

The composition of a representative green tea beverage is shown in Table 4.

Approximately 300 components of green-tea aroma have been identified. The most important and a representative aroma composition is shown in Table 5.

OOLONG TEA

Oolong teas are only partially oxidized and retain a considerable amount of the original polyphenolic material so

Table 4. Composition of Green-Tea Beverage (Weight % Solids)

Epigallocatechin gallate	20.3
Epicatechin gallate	5.2
Epigallocatechin	8.4
Epicatechin	2.0
Flavonols	2.2
Caffeine	7.4
Theanine	4.7
Glutamic acid	0.5
Aspartic acid	0.5
Argenine	0.7
Other amino acids	0.8
Sugars	6.7
Alcohol precipitatable material	12.2
Potassium	4.0
Volatiles	<0.1

Source. Ref. 68.

Table 5. Significant Green-Tea Aroma Components (% Total in a Representative Sample)

Geraniol	17.9
Linalool oxides	16.0
Linalool	9.5
Nerolidol	8.8
cis-jasmone	7.5
2,6,6,trimethyl-2-hydroxycyclohexane-1-one	7.0
β-ionone	5.5
Benzyl alcohol	4.7
cis-3-hexenyl hexanoate	3.5
5,6-epoxy-*β*-ionone	2.7
1-pentene-3-al	2.7
1-terpineol	2.2
cis-3-hexene-1-ol	2.0
Acetylpyrrole	1.8
2-phenylethanol	1.3
cis-pentene-1-ol	1.1
Pentane-1-ol	0.7

Source: Ref. 69.

there is little or no cream formation from beverage-strength extracts.

Manufacture is most often a cottage industry involving a series of withering, gentle rolling, and drying steps what vary greatly from facility to facility. Sun drying is often utilized as the first step. Appearance of leaf is considered an important aspect of quality. Hand labor is often utilized.

Composition of Oolong Tea

The color of oolong tea is intermediate between that of green and black teas, and to some extent its composition reflects a similar relationship. Residual catechin levels vary with the intensity and duration of the oxidation processes. There is little or no theaflavin or thearubigin. It is interesting to note, however, that there are also substances present that have not been found in either green or black teas. These may represent intermediates on the oxidative pathways that would lead to theaflavins or thearubigins on further oxidation or end products only formed under the gentle oxidation conditions prevailing because of less-severe cell disruption. These conditions result in lower quinone concentrations that may favor different oxidative pathways.

Components of oolong teas include several theasinensins and their oxidation products, known as oolongtheanins and oolonghomobisflavans (methylene-bridged bisflavanols) (70,71).

WORLD PRODUCTION AND TEA TRADE

Total world tea production has increased over the last few decades largely in proportion to population growth, but not uniformly. Increases have been greatest where investment and technology have been encouraged, such as in Kenya and north India, and declining where political and economic stability are minimal, such as in Sri Lanka and Georgia. Table 6 is a compilation of tea-production data for the major producing countries in 1997.

Tea consumption throughout the world is far from uniform. In Europe it ranges from ca 3 kg per capita annually in the United Kingdom and Ireland to less than 0.1 kg in Italy. Similar variations occur in the other continents. U.S. consumption is 0.36 kg; in Canada it is 0.68 kg. Tea consumption has been slowly declining in the traditional Anglo-Saxon markets where the popularity of coffee and soft drinks has been increasing. It has increased markedly in India as the economic situation has improved.

Importation of tea into the United States has remained fairly stable on a per capita basis over the period from 1938 to 1998. Product origin has changed considerably. China, Indonesia, Argentina, Sri Lanka, Kenya, and India are now the major suppliers to the U.S. tea market (1). Tea is imported into the United States without duty. Standards established by the International Standards Organization (ISO) and Codex are intended to function as guidelines for teas in international trade. These include extractable solids (32% minimum), ash (4–8%), and crude fiber (16.5% maximum). The real price of tea has tended to decline with only infrequent increases occasioned by temporary shortages. This has caused problems for producing countries, many of which depend on tea as a major source of foreign currency. Efforts to control tea prices through international agreements among the producing countries have never been successful.

Table 6. World Tea Production in 1997 (1000 t dry leaf)

India	811
China	613
Sri Lanka	277
Indonesia	131
Turkey	110
Japan	91
Iran	60
Bangladesh	53
Other Asia	73
Total Asia	*2,219*
Kenya	221
Malawi	44
Tanzania	22
Uganda	21
Zimbabwe	17
Other Africa	36
Total Africa	*361*
Argentina	55
Other S. America	14
Total S. America	*69*
Other	18
Total world	*2,667 (ca 600 is green tea)*

Source. Ref. 1.

The consumption pattern has also undergone large changes. Iced tea use in the United States has grown from a very small proportion of the total to approximately 80%. This factor has contributed greatly to the importance of convenience products based on cold-water-soluble instant teas and ready-to-drink (RTD) beverages.

INSTANT TEA

Instant-tea powder is generally prepared by the aqueous extraction of black tea followed by concentration and drying. It is also possible to prepare instant tea from green tea or from oxidized leaf prior to the drying step. As most tea consumed in the United States is drunk as a cold beverage, the cold-water insolubility of the important cream fraction introduces technological problems in manufacture. A product prepared simply by extraction with hot water, concentration, and drying will not rehydrate completely without applied heat or the use of additives. Cold-water extraction does not recover sufficient solids to meet organoleptic or economic criteria. Most instant-tea technology is concerned with ways to resolve this problem. Tea cream, the fraction that precipitates when a black-tea solution is cooled, constitutes 15 to 30% of the original extract solids. The quantity varies with the type of tea, the extract concentration, and the temperature to which the extract is cooled. Beverage color, caffeine content, and taste are disproportionately concentrated in the cream fraction. Tea cream is a complex of the oxidized polyphenolic material with caffeine along with other occluded substances. The aggregates that develop on standing include other components of tea extract, such as proteinaceous material, lipids, and waxes. Freshly precipitated cream can be resolubilized by heating. The first instant teas that appeared on the U.S. market were prepared by discarding the cream after cooling the hot extract. The cream problem was solved in many ways as indicated by a copious patent literature. Most approaches are based on the disruption of the caffeine–tannin complexes.

Manufacture

Tea is extracted with hot water in a countercurrent mode, either batchwise in kettles or in columns. Continuous extraction systems have been described (72). Aroma fractions are collected by vacuum stripping and condensation or by separation and preservation of the first column effluents. They are retained for later addition to the product stream. Vacuum concentration to the desired solids level for adequate cream separation may then be carried out. Prolonged storage of extracts at elevated temperatures is avoided to prevent oxidative and microbiological deterioration. Tea cream is solubilized or avoided by several different procedures. Treatment of cream with the enzyme tannase results in the selective hydrolysis of gallate linkages, thereby increasing solubility (73). Similarly, it is possible to treat black tea with tannase to produce a cold-water-extractable product (74). The addition of unoxidized (green tea) catechins to black-tea extract prevents the formation of insoluble substances by competitive complexation (75).

Several oxidative procedures for the conversion of green-tea extracts to black tea have been described. These include the use of oxygen, ozone, or hydrogen peroxide (76–78). The reactions are carried out in an alkaline medium so that oxidation and hydrolysis occur simultaneously. These oxidized green-tea extracts are useful in instant-tea manufacture because color and solubility control are easily effected by their addition to the black-tea extract stream.

If cream has been solubilized apart from the remainder of the extract, the fractions are recombined and the total extract is brought to the concentration desired for drying. Vacuum or freeze concentration may be utilized. Concentration by reverse osmosis has also been described as useful for flavor conservation (79). Retained aroma fractions, often concentrated, are added to the drier feed.

Most instant tea is spray-dried. Pectins extracted from tea or other sources have been used to control final product density (80). Inflation of particles with carbon dioxide during spray-drying controls density and wall thickness. Both parameters affect product solubility. Instant tea may also be freeze-dried. The treatment of spent tea leaf with a β-gluconase or cellulase to hydrolyze leaf components adds to the yield of extract solids and decreases the disposal load. The additional use of proteases enhances this effect. The extraction of fermented green leaf, omitting the firing stage, is an attractive process for use in tea-growing countries to conserve energy. Minor amounts of instant tea are prepared for hot beverage consumption. Processing is simpler and taste is more authentic.

Product Characteristics

Instant-tea powders are usually manufactured with a density of ca 0.1 g/mL. Products are soluble in cold tap water and produce a clear solution with an authentic tea color and taste. Instant-tea mixes that contain sugar or a noncaloric sweetener and various fruit flavors are widely used. Canned iced tea beverages containing sweeteners and flavors are also based on instant tea. Decaffeinated instant tea has also come into use. Most instant tea is manufactured and consumed in the United States, where it accounts for ca 15% of total tea usage. Production in 1998 was ca 2,500 t. Kenya, India, and Sri Lanka together manufacture ca 5,000 t, more than half of which is exported to the United States (1).

READY-TO-DRINK (RTD) TEA

RTD teas represent the fastest-growing mode of tea consumption in the United States. In 1997 ca 1 billion L was produced. They are usually presented as a sweetened, flavored, slightly acidified beverage. Taste stability and maintenance of clarity under refrigeration pose major technical problems in their manufacture. The addition of high methoxy pectin to an acidified, flavored tea extract at normal beverage strength (ca 0.3%) results in a clear, cold-water-stable product (81). Gum arabic provides the same benefits (82).

PHYSIOLOGICAL EFFECTS

Soon after the introduction of tea into England, extreme positive and negative effects were attributed to it. A London teahouse advertisement stated that "Tea removeth lassitude, vanquisheth heavy dreams, easeth the frame, and strengtheneth the memory. It overcometh superfluous sleep, and prevents sleepiness in general, so that without trouble whole nights may be passed in study." Though some doctors extolled its curative powers, others attacked tea for causing "palsies, impotence, leanness, sterility, and nervousness" (83).

Tea is now considered to be a healthful beverage. It is a useful source of fluoride and potassium. Its vitamin content is too low to be significant except for heavy users (5 cups per day), who may obtain 5 to 10% of the RDA for riboflavin, niacin, folic acid, and pantothenic acid.

Catechins and their oxidation products have been shown to be effective antioxidants. They exhibit significant quenching of active oxygen species such as hydroxyl and peroxyl radicals as well as more stable free radicals. In lipophilic systems they inhibit the free radical–mediated oxidation of low-density lipoproteins (LDL). Active oxygen radicals are known to play a role in mutagenesis and chemical carcinogenesis. Oxidized LDL is believed to promote atherosclerotic lesions. Tea has therefore been studied for its possible usefulness in preventing these developments. It has exhibited protective effects on laboratory animals treated with mutagenic, carcinogenic, or atherogenic agents.

Prior administration of green-tea extracts inhibits the chromosome aberrations normally induced by aflatoxin B_1

in rat bone marrow cells (84). Green-tea polyphenol consumption exhibits protective effects for rodents treated with carcinogens (85). Topical application of green-tea polyphenols is protective against the initiation of skin tumors in mice treated with 3-methylcholanthrene (86). Though most of these studies have been conducted with green tea or its components, black tea also exhibits protective effects in biological systems (87). Extension of these studies to humans (primarily with epidemiological approaches) has not produced definitive results, partly due to the confounding factors inherent in studies of this type. Epidemiological studies carried out in different parts of the world are difficult to compare and evaluate because of dietary differences. There is often a lack of quantification concerning the tea solids consumed. In a few such investigations, however, some evidence for its anticarcinogenic effect for humans has been obtained (88).

Green tea and green-tea polyphenols lower serum cholesterol levels of rats maintained on a cholesterogenic diet (89). A strong negative correlation has been observed between green-tea consumption and the onset of stroke in a large Japanese population (90).

Tea may be inhibitory to the absorption of iron. Borderline iron deficiencies may be accentuated by high levels of intake (91). A similar effect occurs with thiamine (92). Reports linking tea consumption with esophageal cancer have been based on faulty epidemiological studies: In some areas where the condition is rampant and where tea consumption is high, such as in parts of China, dietary contamination with carcinogenic molds and nitrosamines is widespread (93); in an area of Iran, beverages are commonly consumed scaldingly hot—a practice in itself conducive to esophageal cancer.

Confusion of herbal teas with *Camellia sinensis* and tannic acid with tea components has led to further misunderstanding concerning the effects of tea consumption.

ACKNOWLEDGMENTS

The author is grateful for the considerate and expert assistance provided by Terris Binder and Diane Cassatly of the Lipton Library and Information Services.

BIBLIOGRAPHY

1. *Annual Bulletin of Tea Statistics*, International Tea Committee, London, 1998.
2. G. F. Mitchell, *The Cultivation and Manufacture of Tea in the United States*, Bulletin No. 234, USDA Bureau of Plant Industry, Washington, D.C., 1912.
3. S. F. McLester, *Tea and Coffee Trade Journal* **169**, 78–81 (1997).
4. P. V Arulpragasam, R. Latiff, and P. Seneviratne, "Studies on the Tissue Culture of Tea," *Sri Lanka Journal of Tea Science* **1**, 20–23 (1988).
5. *Annual Reports*, Tea Research Institute of East Africa, Kericho, Kenya.
6. *Tea Quarterly*, Journal of the Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka.
7. *Two and a Bud*, Tocklai Experimental Station, Jorhat, Assam, India.
8. *Annual Reports*, United Planters Association of South India, Cinchona P.O., Coimbatore District, South India.
9. B. Banerjee, "Revised List of Approved Pesticides," *Two and a Bud* **27**, 80–84 (1980).
10. D. A. Balentine, M. E. Harbowy, and H. N. Graham, "Tea: The Plant and Its Manufacture, Chemistry, and Consumption of the Beverage," in G. Spiller, ed., *Caffeine*, CRC Press, New York, 1998, pp. 35–72.
11. U. H. Engelhardt, A. Finger, and S. Kuhr, "Determination of Flavone C-glycosides in Tea," *Z. Lebensm.-Unters.-Forsch.* **197**, 239–244 (1993).
12. F. Hashimoto, G. Nonaka, and I Nishioka, "Tannins and Related Compounds: LXXVII," *Chem. Pharm. Bull.* **37**, 77–85 (1989).
13. M. E. Harbowy and D. A. Balentine, "Tea Chemistry," *Critical Reviews in Plant Science* **16**, 415–478 (1997).
14. S. A. Wiseman, D. A. Balentine, and B Frei, "Antioxidants in Tea," *Critical Rev. Food Sci. Nutr* **37**, 705–718 (1997).
15. R. A. Cartwright, E. A. H. Roberts, and D. J. Wood, "Theanine, an Amino Acid N-Ethylamide, Present in Tea," *J. Sci. Food Agric.* **5**, 597–599 (1954).
16. S. Venkatakrishna, B. R. Premachandra, and H. R. Cama, "Distribution of Carotenoid Pigments in Tea Leaves," *Tea Quarterly* **47**, 28–31 (1977).
17. H. Yingfang et al., "Study on the Chemical Constituents of the Volatile Oils from the Fresh Leaves of *Camellia sinensis*," *Acta Botanica Sinica* **24**, 440–450 (1982).
18. A. C. Liyanage, M. J. de Silva,and A. Ekanayake, "Analysis of Major Fatty Acids in Tea," *Sri Lanka Journal of Tea Science* **57**, 46–49 (1988).
19. R. Seshadri and N. Dhanaraj, "New Hydrophobic Lipid Interactions in Tea Cream," *J. Sci. Food Agric.* **45**, 79–86 (1988).
20. S. R. Whitaker, "Polyphenol Oxidase," *Food Sci. Technol.* **61**, 543–556 (1994)
21. A. Finger, "*In vitro* Studies on the Effect of Polyphenol Oxidase and Peroxidase on the Formation of Polyphenolic Black Tea Constituents," *J. Sci. Food Agric.* **66**, 293–305 (1994).
22. D. A. Balentine, "Tea," in *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th edition, John Wiley & Sons, New York, 1997, pp. 747–768.
23. W Heller and G. Forkmann, "Biosynthesis of Flavonoids," in J. B. Harborne, ed., *The Flavonoids: Advances in Research since 1986*, Chapman & Hall, London, 1994, pp. 495–535.
24. A. Hatanaka and T. Harada, "Formation of *cis*-3-Hexenal, *trans*-2-Hexenal, and *cis*-3-Hexenol in Macerated *Thea sinensis* Leaves," *Phytochemistry* **12**, 2341–2346 (1973).
25. R. L. Wickremasinghe, "The Mechanism of Action of Climatic Factors in the Biogenesis of Tea Flavor," *Phytochemistry* **13**, 2057–2063 (1974).
26. K. Morita et al., "Aglycone Constituents in Fresh Tea Leaves Cultivated for Green and Black Tea," *Biosci. Biotechnol. Biochem.* **58**, 687–690 (1994).
27. T. Nagata, M. Hayatsu, and N. Kosuge, "Aluminum Kinetics in the Tea Pland Using ^{27}Al and ^{19}F NMR," *Phytochemistry* **32**, 771–775 (1993).
28. G. W. Sanderson, "The Chemistry of Tea and Tea Manufacturing," in V. C. Runeckles, ed., *Structural and Functional Aspects of Phytochemistry*, Academic Press, Orlando, Fla., 1972, pp. 247–316.
29. Y. Takino, "Reddish Orange Pigments of Black Tea: Structure and Oxidative Formation from Catechins," *Japanese Agric. Res. Quarterly* **12**, 94 (1978).
30. D. J. Cattell and H. E. Nursten, "Separation of Thearubigens on Sephadex LH-20," *Phytochemistry* **16**, 1269–1272 (1977).

31 International Organizations for Standards, *Black Tea Vocabulary*, International Standards Organization, London, 1982.

32. D. L. Whitehead and C. M. J. Temple, "Rapid Method for Measuring Thearubigins and Theaflavins in Black Tea Using C_{18} Sorbent Cartridges," *J. Sci. Food Agric.* **58**, 149–152 (1992)

33. E. A. H. Roberts and R. F. Smith, "The Phenolic Substances of Manufactured Tea: IX, The Spectrophotometric Evaluation of Tea Liquors," *J. Sci. Food Agric.* **14**, 689–700 (1963).

34. J. B. Cloughley, "The Effect of Fermentation Temperature on the Quality Parameters and Price Evaluation of Central African Black Teas," *J. Sci. Food Agric.* **31**, 911–919 (1980).

35. K. L. Bajaj et al., "Effects of (−)-Epicatechin on Oxidation of Theaflavins by Polyphenol Oxidase from Tea Leaves," *Agric. Biol. Chem.* **51**, 1767–1772 (1987).

36. A. Robertson, "Effects of Physical and Chemical Conditions on the *in vitro* Oxidation of Tea Leaf Catechins," *Phytochemistry* **22**, 889–896 (1983).

37. J. E. Berkowitz, P. Coggon, and G. W. Sanderson, "Formation of Epitheaflavic Acid and Its Transformation to Thearubigens during Tea Fermentation," *Phytochemistry* **10**, 2271–2278 (1971).

38. D. T. Coxon, A. Holmes, and W. D. Ollis, "Theaflavic and Epitheaflavic Acids," *Tetrahedron Lett.* **60**, 5247–5250 (1970).

39. T. Bryce et al., "Three New Theaflavins from Black Tea," *Tetrahedron Lett.* **72**, 463–466 (1972).

40. G. Nonaka, F. Hashimuto, and I. Nishioka, "Tannins and Related Compounds: XXXVI, Isolation and Structures of Theaflagallins, New Red Pigments from Black Tea," *Chem. Pharm. Bull.* **34**, 61–65 (1986)

41. G. Nonaka, O. Kawahara, and I. Nishioka, "Tannins and Related Compounds: XV, A New Class of Dimeric Flavan-3-ol Gallates, Theasinensins A and B, and Proanthocyanidin Gallates from Green Tea Leaf," *Chem. Pharm. Bull.* **31**, 3906–3914 (1983).

42. E. A. H. Roberts, R. A. Cartwright, and M. Oldschool, "Fractionation and Paper Chromatography of Water Soluble Substances from Manufactured Tea," *J. Sci. Food Agric.* **8**, 72–80 (1957).

43. D. J. Millin and D. W. Rustidge, "Tea Manufacture," *Process Biochemistry* **2**, 9–13 (1967).

44. R. G. Bailey, H. E. Nursten, and I. McDowell, "Isolation and Analysis of a Polymeric Thearubigin Fraction from Tea," *J. Sci. Food Agric.* **59**, 365–375 (1992).

45. A. L. Davis et al., "A Polyphenolic Pigment from Black Tea," *Phytochemistry* **46**, 1397–1402 (1997).

46. C. Powell et al., "Fractionation of Thearubigins: Isolation of Theacitrins," *Symp. Recent Advances Chem. Biochem. Tea*, School of Biological Sciences, University of Surrey, Guilford, Surrey, U.K., March 24, 1994.

47. A. G. Brown et al., "The Identification of the Thearubigens as Polymeric Proanthocyanidins," *Phytochemistry* **8**, 2333–2340 (1969).

48. T. Ozawa et al., "Elucidation of the Partial Structure of Thearubigins from Black Tea by Chemical Degradation," *Biosci. Biotechnol. Biochem.* **60**, 2023–2027 (1996).

49. S. C. Opie, M. N. Clifford, and A. Robertston, "Formation of Black Tea Thearubigins by *in vitro* Enzymic Oxidation of Flavan-3-ols," *Symp. Recent Advances Chem. Biochem. Tea*, School of Biological Sciences, University of Surrey, Surrey, England, Month day(s), 1994.

50. M. A. Dix et al., "Fermentation of Tea in Aqueous Suspension: Influence of Tea Peroxidase," *J. Sci. Food Agric.* **32**, 920–932 (1981).

51. K. L. Tomlins and A. Mashingaidze, "Influence of Withering, including Leaf Handling, on the Manufacturing and Quality of Black Teas: A Review," *Food Chem.* **60**, 573–580 (1997)

52. P. O. Owuor and C. O. Othieno, "Effects of Maceration on the Chemical Composition and Quality of Clonal Black Teas," *J. Sci. Food Agric.* **49**, 87–94 (1989).

53. *The Tea Cyclopedia*, Whittingham, London, 1882.

54. W. H. Ukers, *All about Tea*, Tea and Coffee Trade Journal, New York, 1935.

55. Brit. Pat. 1,274,002 (May 10, 1972), A. C. K. Krishnaswami and C. Hariprasad.

56. Brit. Pat. 1,484,540 (September 1, 1977), D. Kirtisinghe and D. P. Ranasinghe (to Tea Research Institute).

57. J. B. Cloughley, "Storage Deterioration in Central African Tea: Changes in Chemical Composition, Sensory Characteristics, and Price Evaluation," *J. Sci. Food Agric.* **32**, 1213–1223 (1981).

58. J. M. Robinson and P. O. Owuor, "Tea Aroma," in K. C. Wilson and M. N. Clifford, eds., *Tea: Cultivation to Consumption*, Chapman & Hall, London, 1992, pp. 603–647.

59. W. Mick and P. Schreier, "Additional Volatiles of Black Tea Aroma," *J. Agric. Food Chem.* **32**, 924–929 (1984).

60. P. O. Owuor, "Flavor of Black Tea: A Review," *Tea* **7**, 29–42 (1986).

61. G. W. Sanderson and H. N. Graham, "On the Formation of Black Tea Aroma" *J. Agric. Food Chem.* **21**, 576–584 (1973).

62. N. Fischer, S. Nitz, and F. Drawert, "Freie und Gebundene Aromastoffe in Grünem und Schwarzem Tee," *Z. Lebensm.-Unters.-Forschung* **185**, 195–201 (1987).

63. O. G. Vitzhum, P. Werkhoff, and P. Hurbert, "New Volatile Constituents of Black Tea Aroma," *J. Agric. Food Chem.* **23**, 999–1003 (1975).

64. M. A. Bokuchava and N. I. Skobeleva, "The Flavor of Beverages," in I. D. Morton and A. J. Mcleod, eds., *Food Flavours: Part B*, Elsevier Science, Amsterdam, The Netherlands, 1986, pp. 49–84.

65. G. R. Roberts and C. C. Rajasingham, "Changes in the Polyphenolic Constituents of Tea Liquors during Storage," *Tea Quarterly* **50**, 71–73 (1981).

66. G. W. Sanderson et al., "Contribution of Polyphenolic Compounds to the Taste of Tea," in G. Charalambous and I. Katz, eds., *Sulfur and Nitrogen Compounds in Food Flavors*, ACS Symposium, Vol. **26**, American Chemical Society, Washington, D.C., 1976, pp. 14–46.

67. T. Anan, "Changes of Chemical Compounds during Green Tea Manufacturing," *Japanese Agric. Res. Quart.* **22**, 195–199 (1988).

68. M. Nakagawa, "Relationship between Chemical Constituents and the Taste of Green Tea," *Tea Res. J. (Japan)* **40**, 1 (1973).

69. M. Kosuge, H. Aisaka, and T. Yamanishi, "Flavor Constituents of Chinese and Japanese Pan-Fried Green Teas," *Eiyot Shokuryo* **34**, 545–549 (1981).

70. F. Hashimoto, G. Nonaka, and I. Nishioka, "Tannins and Related Compounds: LXIX, Isolation and Structure Elucidation of B,B′-Linked Bisflavanols, Theasinensins D-G and Oolongtheanin from Oolong Tea (2)," *Chem. Pharm. Bull.* **36**, 1676–1684 (1988).

71. F. Hashimoto, G. Nonaka, and I. Mishioka, "Tannins and Related Compounds: XC, 8-C-Ascorbyl(−)-Epigallocatechin 3-O-Gallate and Novel Dimeric Flavan-3-ols, Oolonghomobisflavans A and B from Oolong Tea (3)," *Chem. Pharm. Bull.* **37**, 3255–3263 (1989).

72. U.S. Pat. 3,992,983 (November 23, 1976), R. J. Gasser and S. N. Watercutter (to Socit d'Assistance Technique pour Produits Nestle SA).
73. Brit. Pat. 1,380,135 (January 8, 1975), P. Coggon, H. N. Graham, and G. W. Sanderson (to Unilever, Ltd.).
74. U.S. Pat. 4,051,254 (September 27, 1977), G. W. Sanderson, A. C. Hoefler, and H. N. Graham (to Thomas J. Lipton, Inc.).
75. U.S. Pat. 4,680,193 (July 14, 1987), T. L. Lunder and C. M. Nielsen (to Nestec SA).
76. U.S. Pat. 3,484,246 (December 16, 1969), T. R. Moore, Jr., H. N. Graham, and M. Gurkin (to Thomas J. Lipton, Inc.).
77. U.S. Pat. 3,484,247 (December 16, 1969), H. N. Graham, V. V. Studer, and M. Gurkin (to Thomas J. Lipton, Inc.)
78. U.S. Pat. 3,484,248 (December 16, 1969), H. N. Graham, V. V. Studer, and M. Gurkin (to Thomas J. Lipton, Inc.).
79. Eur. Pat. 267660 (May 18, 1988), B. Hoogstad (to Unilever NV).
80. U.S. Pat. 3,666,484 (May 30, 1972), M Gurkin, G. W. Sanderson, and H. N. Graham (to Thomas J. Lipton, Inc.).
81. U.S. Pat. 5,529,796 (June 25, 1996), D. A. Balentine, S. A. Gobbo, and J. W. Tobin (to Thomas J. Lipton, Inc.).
82. Eur. Pat. 675,683 (October 11 1995), D. A. Balentine and J. W. Tobin (to Unilever PLC).
83. J. F. Greden, "The Tea Controversy in America," *JAMA* **236**, 63–66 (1976).
84. Y. Ito, S. Ohnishi, and K. Fujie, "Chromosome Aberrations Induced by Aflotoxin B-1 in Rat Bone Marrow Cells *in vivo* and Their Suppression by Green Tea," *Mutat. Res.* **222**, 253–261 (1989).
85. S. K. Katiyar and H. Mukhtar, "Tea Consumption and Cancer," in A. P. Simopoulos, ed., *Metabolic Consequences of Changing Dietary Patterns. World Rev. Nutr. Diet*, Vol. 79, Karger, Basel, Switzerland, 1996, pp. 154–184.
86. Z. Y. Wang et al., "Protection against Polycyclic Aromatic Hydrocarbon-Induced Skin Tumor Initiation in Mice by Green Tea Polyphenols," *Carcinogenesis* **10**, 411–415 (1989).
87. Z. Y. Wang et al., "Inhibition of N-Nitrosobenzylamine-induced Esophageal Tumorigenesis in Rats by Green and Black Tea," *Carcinogenesis* **16**, 2143–2148 (1995).
88. Y. T. Gao et al., "Reduced Risk of Esophageal Cancer Associated with Green Tea Consumption," *J. Natl. Cancer Inst.* **86**, 855 (1994).
89. H. Matsuda et al., "Effects of Crude Drugs on Experimental Hypercholesterolemia: I, Tea and Its Active Principles," *Journal of Ethnopharmacology* **17**, 213–224 (1986).
90. Y. Sato et al., "Possible Contribution of Green Tea Drinking Habits to the Prevention of Stroke," *Tohoku J. Experimental Med.* **157**, 337–343 (1989).
91. C. S. Farkas, "Survey of Tea Intake by Northern Canadian Indians with Emphasis on the Implications for Iron Status," *J. Can. Diet Assoc.* 4, 318 (1980).
92. R. B H. Wills and K. J. McBrien, "Antithiamin Activity of Tea Fractions," *Food Chem.* **6**, 111–114 (1980).
93. "Mold Linked to Esophageal Cancer," *Science News* **130** (1986).

HAROLD N. GRAHAM
New York, New York

THERMAL PROCESSING OF FOOD

Thermal processing of canned foods has been one of the most widely used methods of food preservation during this century. In its broadest sense, it refers to the technology of using heat sterilization to preserve ready-to-eat foods and other biological products so that they can remain safe and wholesome under long-term extended storage at room temperature without chemical additives or preservatives. Foods preserved in this manner have become so commonplace in the human diet that the health of the world population now depends in great measure on the safety and wholesomeness of these foods. Thermal processing consists of heating food containers in pressurized retorts at specified temperatures for prescribed lengths of time. These process times are calculated on the basis of achieving sufficient bacterial inactivation (lethality) in each container to comply with public health standards and to ensure that the probability of spoilage will be less than some minimum. Associated with each thermal process are some degradation of heat-sensitive vitamins and other undesirable quality factors. Because of these quality and safety factors, great care is taken in the calculation of these process times and in the control of time and temperature during processing to avoid either underprocessing or overprocessing. The heat transfer considerations that govern the temperature profiles achieved within the container of food are critical factors in the determination of time and temperature requirements for sterilization. The sections that follow explain the distinction between sterilization and pasteurization, provide a brief history describing the evolution of canning technology leading to the current state of the art, a description of the various types of commercial equipment systems operating today in modern food processing factories, a review of the scientific principles that must be understood in order to establish these processes and specify necessary process conditions, and a look at future trends and perspectives for this important food preservation technology.

STERILIZATION VERSUS PASTEURIZATION

Thermal processing covers the broad area of food preservation technology in which heat treatments are used to inactivate microorganisms to accomplish either commercial sterilization or pasteurization. Sterilization processes are used with canning to preserve the safety and wholesomeness of ready-to-eat foods over long terms of extended storage at normal room temperature (nonrefrigerated) without additives or preservatives, and pasteurization processes are used to extend the refrigerated storage life of fresh foods. Although both processes make use of heat treatments for the purpose of inactivating microorganisms, they differ widely with respect to the classification or type of microorganisms targeted, and thus the range of temperatures that must be used and the type of equipment systems capable of achieving such temperatures.

Pasteurization

Pasteurization is a relatively mild heat treatment given to foods with the purpose of destroying selected vegetative

72. U.S. Pat. 3,992,983 (November 23, 1976), R. J. Gasser and S. N. Watercutter (to Socit d'Assistance Technique pour Produits Nestle SA).
73. Brit. Pat. 1,380,135 (January 8, 1975), P. Coggon, H. N. Graham, and G. W. Sanderson (to Unilever, Ltd.).
74. U.S. Pat. 4,051,254 (September 27, 1977), G. W. Sanderson, A. C. Hoefler, and H. N. Graham (to Thomas J. Lipton, Inc.).
75. U.S. Pat. 4,680,193 (July 14, 1987), T. L. Lunder and C. M. Nielsen (to Nestec SA).
76. U.S. Pat. 3,484,246 (December 16, 1969), T. R. Moore, Jr., H. N. Graham, and M. Gurkin (to Thomas J. Lipton, Inc.).
77. U.S. Pat. 3,484,247 (December 16, 1969), H. N. Graham, V. V. Studer, and M. Gurkin (to Thomas J. Lipton, Inc.)
78. U.S. Pat. 3,484,248 (December 16, 1969), H. N. Graham, V. V. Studer, and M. Gurkin (to Thomas J. Lipton, Inc.).
79. Eur. Pat. 267660 (May 18, 1988), B. Hoogstad (to Unilever NV).
80. U.S. Pat. 3,666,484 (May 30, 1972), M Gurkin, G. W. Sanderson, and H. N. Graham (to Thomas J. Lipton, Inc.).
81. U.S. Pat. 5,529,796 (June 25, 1996), D. A. Balentine, S. A. Gobbo, and J. W. Tobin (to Thomas J. Lipton, Inc.).
82. Eur. Pat. 675,683 (October 11 1995), D. A. Balentine and J. W. Tobin (to Unilever PLC).
83. J. F. Greden, "The Tea Controversy in America," *JAMA* **236**, 63–66 (1976).
84. Y. Ito, S. Ohnishi, and K. Fujie, "Chromosome Aberrations Induced by Aflotoxin B-1 in Rat Bone Marrow Cells *in vivo* and Their Suppression by Green Tea," *Mutat. Res.* **222**, 253–261 (1989).
85. S. K. Katiyar and H. Mukhtar, "Tea Consumption and Cancer," in A. P. Simopoulos, ed., *Metabolic Consequences of Changing Dietary Patterns. World Rev. Nutr. Diet*, Vol. 79, Karger, Basel, Switzerland, 1996, pp. 154–184.
86. Z. Y. Wang et al., "Protection against Polycyclic Aromatic Hydrocarbon-Induced Skin Tumor Initiation in Mice by Green Tea Polyphenols," *Carcinogenesis* **10**, 411–415 (1989).
87. Z. Y. Wang et al., "Inhibition of N-Nitrosobenzylamine-induced Esophageal Tumorigenesis in Rats by Green and Black Tea," *Carcinogenesis* **16**, 2143–2148 (1995).
88. Y. T. Gao et al., "Reduced Risk of Esophageal Cancer Associated with Green Tea Consumption," *J. Natl. Cancer Inst.* **86**, 855 (1994).
89. H. Matsuda et al., "Effects of Crude Drugs on Experimental Hypercholesterolemia: I, Tea and Its Active Principles," *Journal of Ethnopharmacology* **17**, 213–224 (1986).
90. Y. Sato et al., "Possible Contribution of Green Tea Drinking Habits to the Prevention of Stroke," *Tohoku J. Experimental Med.* **157**, 337–343 (1989).
91. C. S. Farkas, "Survey of Tea Intake by Northern Canadian Indians with Emphasis on the Implications for Iron Status," *J. Can. Diet Assoc.* 4, 318 (1980).
92. R. B H. Wills and K. J. McBrien, "Antithiamin Activity of Tea Fractions," *Food Chem.* **6**, 111–114 (1980).
93. "Mold Linked to Esophageal Cancer," *Science News* **130** (1986).

Harold N. Graham
New York, New York

THERMAL PROCESSING OF FOOD

Thermal processing of canned foods has been one of the most widely used methods of food preservation during this century. In its broadest sense, it refers to the technology of using heat sterilization to preserve ready-to-eat foods and other biological products so that they can remain safe and wholesome under long-term extended storage at room temperature without chemical additives or preservatives. Foods preserved in this manner have become so commonplace in the human diet that the health of the world population now depends in great measure on the safety and wholesomeness of these foods. Thermal processing consists of heating food containers in pressurized retorts at specified temperatures for prescribed lengths of time. These process times are calculated on the basis of achieving sufficient bacterial inactivation (lethality) in each container to comply with public health standards and to ensure that the probability of spoilage will be less than some minimum. Associated with each thermal process are some degradation of heat-sensitive vitamins and other undesirable quality factors. Because of these quality and safety factors, great care is taken in the calculation of these process times and in the control of time and temperature during processing to avoid either underprocessing or overprocessing. The heat transfer considerations that govern the temperature profiles achieved within the container of food are critical factors in the determination of time and temperature requirements for sterilization. The sections that follow explain the distinction between sterilization and pasteurization, provide a brief history describing the evolution of canning technology leading to the current state of the art, a description of the various types of commercial equipment systems operating today in modern food processing factories, a review of the scientific principles that must be understood in order to establish these processes and specify necessary process conditions, and a look at future trends and perspectives for this important food preservation technology.

STERILIZATION VERSUS PASTEURIZATION

Thermal processing covers the broad area of food preservation technology in which heat treatments are used to inactivate microorganisms to accomplish either commercial sterilization or pasteurization. Sterilization processes are used with canning to preserve the safety and wholesomeness of ready-to-eat foods over long terms of extended storage at normal room temperature (nonrefrigerated) without additives or preservatives, and pasteurization processes are used to extend the refrigerated storage life of fresh foods. Although both processes make use of heat treatments for the purpose of inactivating microorganisms, they differ widely with respect to the classification or type of microorganisms targeted, and thus the range of temperatures that must be used and the type of equipment systems capable of achieving such temperatures.

Pasteurization

Pasteurization is a relatively mild heat treatment given to foods with the purpose of destroying selected vegetative microbial species (especially the pathogens) and inactivating the enzymes. Because the process does not eliminate all the vegetative microbial population and almost none of the spore formers, pasteurized foods must be contained and stored under conditions of refrigeration with chemical additives or modified atmosphere packaging, which minimize microbial growth. Depending on the type of product, the shelf life of pasteurized foods could range from several days (milk) to several months (fruit juices). Because only mild heat treatment is involved, the sensory characteristics and nutritive value of the food are minimally affected. The severity of the heat treatment and the length of storage depends on the nature of the product, pH conditions, the resistance of the target microorganism or enzyme, the sensitivity of the product, and the method of heating. Some of these are summarized in Table 1 (1).

Most pasteurization operations involving liquids (milk, milk products, beer, fruit juices, liquid egg, etc) are carried out in continuous heat exchangers. The product temperature is quickly raised to the pasteurization levels in the first heat exchanger, held for the required length of time in the holding tube, and quickly cooled in a second heat exchanger. For viscous fluids, a swept surface heat exchanger is often used to promote faster heat transfer and to prevent surface fouling problems. In-package pasteurization is similar to conventional thermal processing of foods except that it is carried out at lower temperatures. The thermal processing of high acid foods (natural or acidified) is also sometimes termed pasteurization to indicate that relatively milder heat treatment is involved (generally carried out at boiling water temperatures).

Sterilization

Sterilization implies the destruction of all viable microorganisms and is not the appropriate word to be used for thermal processing of foods, because these foods are far from being sterile in the medical sense of the word. The success of thermal processing does not lie in destroying all viable microorganisms but in the fact that together with the nature of the food (pH), environment (vacuum), hermetic packaging, and storage temperature, the given heat process prevents the growth of microorganisms of spoilage and public health concern. In essence, it represents a thermal process in which foods are exposed to a high-enough temperature for a sufficiently long time to render them commercially sterile. The process takes into account the heat resistance of the spore formers in addition to their growth sensitivity to oxygen, pH, and temperature. The presence of vacuum in cans prevents the growth of most aerobic microorganisms, and if the storage temperature is kept below 25°C, the heat-resistant thermophiles pose little or no problem. From the public health perspective, the most important microorganism in low-acid ($pH > 4.5$) foods is *Clostridium botulinum*, a heat-resistant, spore-forming, anaerobic pathogen that, if it survives processing, can potentially grow and produce the deadly botulism toxin in foods. Because *C. botulinum* and most spore formers do not grow at $pH < 4.5$ (acid and medium-acid foods), the thermal processing criterion for these foods is the destruction of heat-resistant yeasts and molds, vegetative microorganisms, or enzymes. The temperature and pH requirements of some common spoilage microorganisms are summarized in Table 2 (2). Because spore formers generally have high heat resistance, the low-acid foods that support their growth are processed at elevated temperatures (115–125°C), whereas acid foods need only to be brought to 80–90°C for adequate inactivation of enzymes or destruction of vegetative cells, yeasts, and molds.

STERILIZATION EQUIPMENT SYSTEMS

Historical Perspectives

The practice of canning as a method of food preservation originated in the early 1800s in France when Emperor Napoleon Bonaparte offered a prize of 12,000 francs to anyone who could develop better and more diversified foods to feed his troops on their military campaigns. A man named Nicholas Appert won the prize for successfully preserving, for the first time, a variety of perishable food products by heat-processing them in glass jars and bottles. At the time of Appert's discovery, the reasons for food spoilage were not known; process times and temperatures were selected by trial and error. Pasteur discovered the existence of microscopic organisms many years later in 1860.

From Appert's work, plus the invention of the metal container and the pressure cooker or retort, evolved the present-day thermal processing technology. The development of metal and glass containers capable of withstanding more than 1 atm of added internal pressure was a ma-

Table 1. Pasteurization Objectives and Conditions for Selected Foods

Food	Purpose	Typical processing conditions
Fruit juice	Inactivation of enzymes (pectinesterase and polygalacturonase)	65°C for 30 min; 77°C for 1 min, 88°C for 15 s
Beer	Destruction of spoilage microorganisms (wild yeasts, *Lactobacillus* sp.), and residual yeasts (*Saccharomyces* sp.)	65–68°C for 20 min (in bottle); 72–75°C for 1–4 min at 900–1,000 kPa
Milk	Destruction of pathogens: *Brucella abortis, Mycobacterium tuberculosis,* *Coxiella burnettii*	63°C for 30 min; 71.5°C for 15 s
Liquid egg	Destruction of pathogens *Salmonella seftenburg*	64.4°C for 2.5 min; 60°C for 3.5 min
Ice cream	Destruction of pathogens	65°C for 30 min; 71°C for 10 min; 80°C for 15 s

Source: Ref. 1.

Table 2. Spore-Forming Bacteria Important in Spoilage of Food

	Acidity of food	
Approximate temperature (°C) range for vigorous growth	Acid 3.7 < pH < 4.5	Low acid pH > 4.5
Thermophilic (55–35°)	*B. coagulans*	*C. thermosaccharolyticum* *C. nigrificans* *Bacillus stearothermophilus*
Mesophilic (40–10°)	*C. butyricum* *C. pasteurianum* *B. mascerans* *B. polymyxa*	*C. botulinum*, A and B *C. sporogenes* *B. licheniformis* *B. subtilis*
Psychrophilic (35–<5°)		*C. botulinum*, E

Source: Ref. 2.

jor breakthrough. This allowed a concurrent development of devices permitting the exposure of the filled and sealed containers to steam pressures above atmospheric, namely, processing temperatures of 120°C instead of only 100°C (boiling water). Because the thermal inactivation of food spoilage organisms is a function of both time and temperature, the higher temperature under pressure allowed a very considerable reduction in the time necessary to ensure product sterility. Equally important was the marked improvement of the canned food quality. Commercial equipment systems in use today for heat sterilization of canned foods are described in the following sections.

Retort Processing

Batch Systems. There are two fundamentally different process methods by which canning is accomplished in the food industry. These two methods are known as retort processing and aseptic processing. In retort processing, foods to be sterilized are first filled and hermetically sealed in cans, jars, or other retortable containers. Then, they are heated in their containers using hot steam or water under pressure so that heat penetrates the product from the can wall inward, and both product and can wall become sterilized together. In aseptic processing, a liquid food is first sterilized outside the container by pumping it through heat exchangers that deliver very rapid heating and cooling rates. Then, the cool sterile product is filled and sealed in a separately sterilized package under a sterile environment at room temperature. Thus, retort processing can be thought of as in-container or in-can sterilization, and aseptic processing can be thought of as out-of-container sterilization.

In retort processing (in-can sterilization), the food to be sterilized is first filled and hermetically sealed in rigid, flexible, or semirigid containers such as metal cans, glass jars, retort pouches, or plastic bowls or trays that are placed within large steam retorts, sometimes called autoclaves. These are pressure vessels that work like giant pressure cookers, as shown in Figure 1. Once the retorts are full of containers to be sterilized, the retort doors are closed tightly and the air is replaced by hot steam under pressure to achieve temperatures above the atmospheric boiling point of water. A common retort temperature for sterilizing canned foods is 121°C (250°F), at approximately 1 atm of added internal pressure. After the containers have been exposed to the sterilizing temperature for sufficient time to achieve the desired level of sterilization, the steam is shut off, and cooling water is introduced to cool the containers and reduce the pressure, thus ending the process. Once the retort pressure has returned to atmosphere, the doors can be opened, and the processed containers are removed for labeling, case-packing, and warehousing to await distribution to the marketplace.

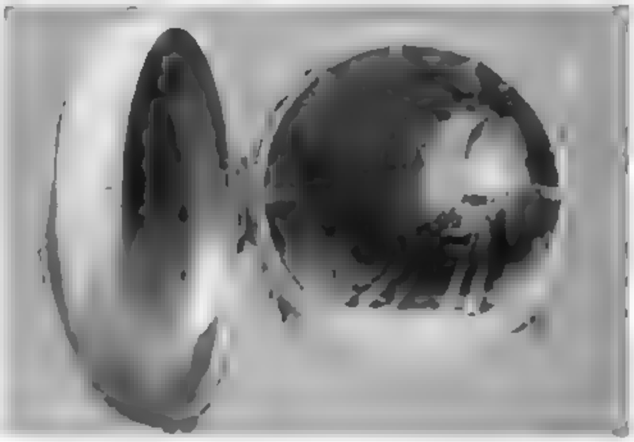


Figure 1. Empty batch retort with doors ajar showing interior rails for entry and exit of wheeled crates used in loading and unloading operations. *Source:* Courtesy FMC Food Process Systems Division, Madera, Calif.

Although the unloading and reloading operations are labor intensive, a well-managed cook room can operate with surprising efficiency. The cook room is the room or area within a food canning factory in which the retorts are located (Fig. 2). Some cook rooms are known to have more than 100 retorts operating at full production. Although each retort is a batch cook operation, the cook room as a whole operates as a continuous production system in that filled and sealed unsterilized cans enter the cook room continuously from the filling line operations, and fully processed sterilized cans leave the cook room continuously enroute to subsequent case packing and warehousing. Within the cook room, teams of factory workers move from retort to retort to carry out loading and unloading operations, and retort operators are responsible for a given number or bank of retorts. These operators carefully monitor the

Figure 2. Exterior view of operating batch retort (above), and commercial system of batch retorts showing automated loading/unloading operations in a typical food processing plant cook room (below). *Source:* Courtesy FMC Food Process Systems Division, Madera, Calif.

operation of each retort to make sure the scheduled process is delivered for each batch. For convection-heating products that benefit from mechanical agitation during processing, agitating batch retorts are available that accomplish axial rotation of the cans or end-over-end agitation by rotating the entire crate during processing operations (3,4).

Continuous Retort Systems. Continuous retort operations require some means by which cans are automatically and continuously moved from atmospheric conditions into a pressurized steam environment, held or conveyed through that environment for the specified process time, and then returned to atmospheric conditions for further handling operations. The most well-known commercially available systems that accomplish these requirements are the crateless retort, the continuous rotary cooker, and the hydrostatic sterilizer (3,4).

Crateless Retorts. A crateless retort system is, in a sense, an automatic cook room in that the system is made up of a series of individual retorts, each operating in a batch mode, with loading, unloading, and process scheduling operations all carried out automatically without the use of crates. An individual crateless retort is shown schematically in Figure 3. When ready to load, the top hatch opens automatically, and cans fed from an incoming conveyor literally fall into the retort, which is filled with hot water to cushion the fall. Once fully charged, the hatch is closed while the incoming conveyor diverts the flow of cans to another retort that is ready for loading. Steam entering from the top displaces the cushion water out the bottom. When the cushion water has been fully displaced, all valves are closed and processing begins. At the end of the process time, the retort is refilled with warm water and the bottom hatch, which lies beneath the water level in the discharge cooling canal, is opened to let the cans gently fall onto the moving discharge conveyor in the cooling canal. After all cans are discharged, the bottom hatch is reclosed, and the retort is ready to begin a new cycle. A commercial system of crateless retorts would consist of several such retorts in a row sharing a common infeed and discharge conveyor system to achieve continuous operation of any design capacity.

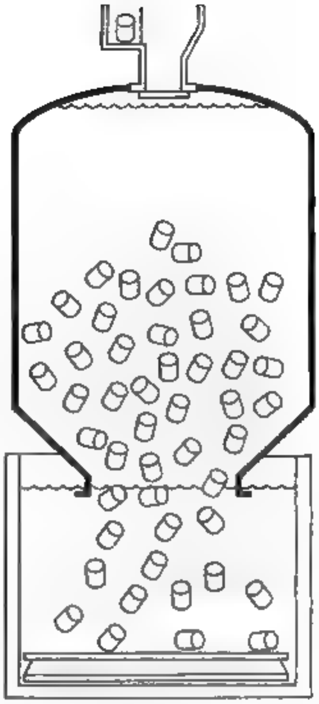


Figure 3. Operating schematic of a crateless retort showing "free fall" discharge of sterilized food containers onto submerged cooling canal exit conveyor.

Continuous Rotary Cookers. The continuous rotary pressure sterilizer or "cooker" is a horizontal rotating retort through which the cans are conveyed while they rotate about their own axis through a spiral path on a revolving reel mechanism. Residence time through the sterilizer is controlled by the rotating speed of the reel, which can be adjusted to accomplish the specified process time. This, in turn, sets the line speed for the entire system. Cans are transferred from an incoming can conveyor through a synchronized feeding device to a rotary pressure sealed transfer valve, which indexes the cans into the sterilizer while preventing the escape of steam and loss of pressure (much like a revolving door). Once cans have entered the sterilizer, they travel in the annular space between the reel and the shell. They are held between spines on the reel and a helical or spiral track welded to the interior shell wall. In this way, the cans are carried by the reel around the inner circumference of the shell imparting a rotation about their own axes, while the spiral track in the shell directs the cans forward along the length of the sterilizer by one can length for each revolution of the reel. At the end of the sterilizer, cans are ejected from the reel into another rotary valve and into the next shell for either additional cooking or cooling (Figs. 4 and 5).

Most common systems require at least three shells in series to accomplish controlled cooling through both a pres-

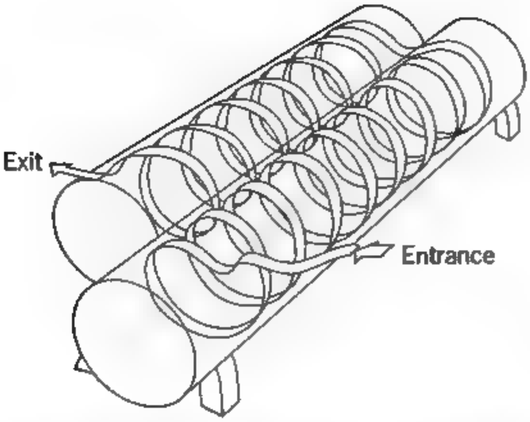


Figure 4. Schematic of helical path traveled by food containers moving through a series of rotary cooker/cooler shells in a continuous rotary sterilizer system.

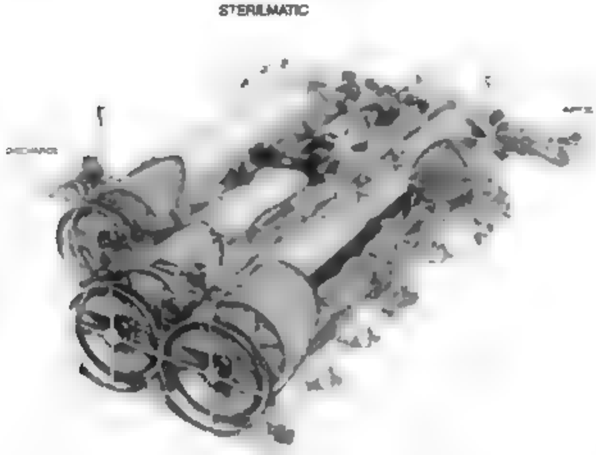


Figure 5. Continuous rotary sterilizer system with cutaway views showing rotary pressure-seal valves and internal helical spline and real conveying mechanism. *Source:* Courtesy FMC Food Process Systems Division, Madera, Calif.

sure cool shell and an atmospheric cool shell following the cooker or sterilizer. For cold-fill products that require controlled preheating, as many as five shells may be required in order to deliver an atmospheric preheat, pressure preheat, pressure cook, pressure cool, and atmospheric cool. By nature of its design and principal of operation, a continuous rotary sterilizer system is manufactured to accommodate a specified can size and cannot be easily adapted to other sizes. For this reason, it is not uncommon to see several systems in operation in one food canning plant, each system dedicated to a different can size filling line.

Hydrostatic Sterilizers. These systems are so named because steam pressure is controlled hydrostatically by the height of a leg of water. Because of the height of water leg required, these sterilizers are usually installed outdoors adjacent to a canning plant. They are self-contained structures with the external appearance of a rectangular tower as shown in Figure 6. They are basically made up of four chambers: a hydrostatic bring-up leg, a sterilizing steam dome, a hydrostatic bring-down leg, and a cooling section.

The principal of operation for a hydrostatic sterilizer can be explained with reference to the schematic in Figure 7. Containers are conveyed through the sterilizer on carriers connected to a continuous chain link mechanism that provides positive line speed control. This provides residence time control to achieve specified process time in the steam dome. Carriers are loaded automatically from incoming can conveyors and travel to the top of the sterilizer

Figure 6. Exterior view of continuous hydrostatic sterilizer. *Source:* Courtesy FMC Food Process Systems Division, Madera, Calif.

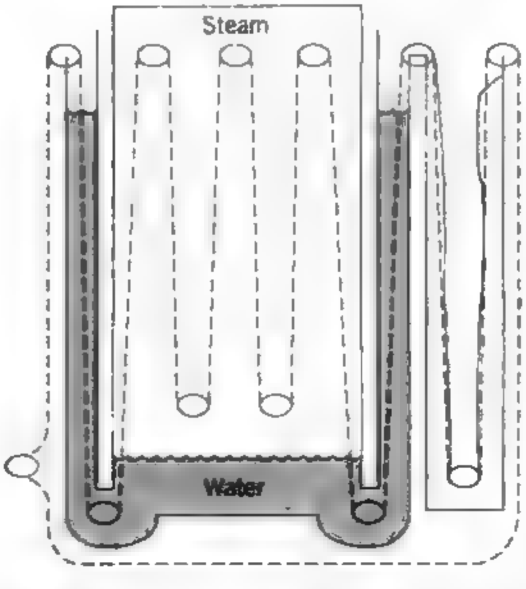


Figure 7. Schematic of continuous conveyor path traveled by food containers through inlet water leg, sterilizing steam dome, outlet water leg, and cooling section of a continuous hydrostatic retort.

where they enter the bring-up water leg. They travel downward through this leg as they encounter progressively hotter water. As they enter the bottom of the steam dome, the water temperature will be in equilibrium with steam temperature at the water steam interface. In the steam dome, the cans are exposed to the specified process or "retort" temperature controlled by the hydrostatic pressure for the prescribed process time controlled by the carrier line speed. When cans exit the steam dome, they again pass through the water steam interface at the bottom and travel upward through the bring-down leg as they encounter progressively cooler water until they exit at the top. Cans are then sprayed with cooling water as the carriers travel down the outside of the sterilizer on their return to the discharge conveyor station.

Aseptic Processing

Aseptic canning systems have rapidly developed in recent years primarily to allow for the marketing of shelf-stable foods in novel or more economical packaging systems that cannot withstand normal retort processing conditions. The primary goal in earlier development work on aseptic canning systems was to use the high temperature–short time (HTST) benefit of aseptic processing to minimize quality losses that occur in the slow heating of foods processed in conventional retorting systems. In either case, aseptic canning circumvents the need for retort operations by sterilizing the product outside of the container through heat exchanger systems before it is filled aseptically into separately sterilized containers or packaging systems.

Heat Exchangers. The benefits of HTST processing have been known for a number of years and have led to the rapid development of aseptic canning systems wherever possible. These methods generally apply only to fluid products that can be pumped through heat exchangers capable of applying ultra-high temperature–short time (UHT) heating conditions to the product before it is filled and sealed aseptically. The general types of heat exchangers commonly used with aseptic canning systems fall into the two basic categories of direct and indirect heating. In direct heating, the product is brought into direct contact with live steam through either steam injection or steam infusion heaters followed by holding and flash cooling under controlled pressure (Fig. 8). In indirect heating, the product contacts the heated metal surfaces of a heat exchanger, which separate the product from direct contact with the heat exchange medium. Either plate, tubular, or swept-surface heat exchangers are most often used for this purpose (Fig. 9). The residence time experienced by the product as it flows through an insulated holding tube or holding section between the heating and cooling heat exchangers accomplishes the necessary process time for delivering the specified sterilizing value or lethality, and is controlled by flow rate.

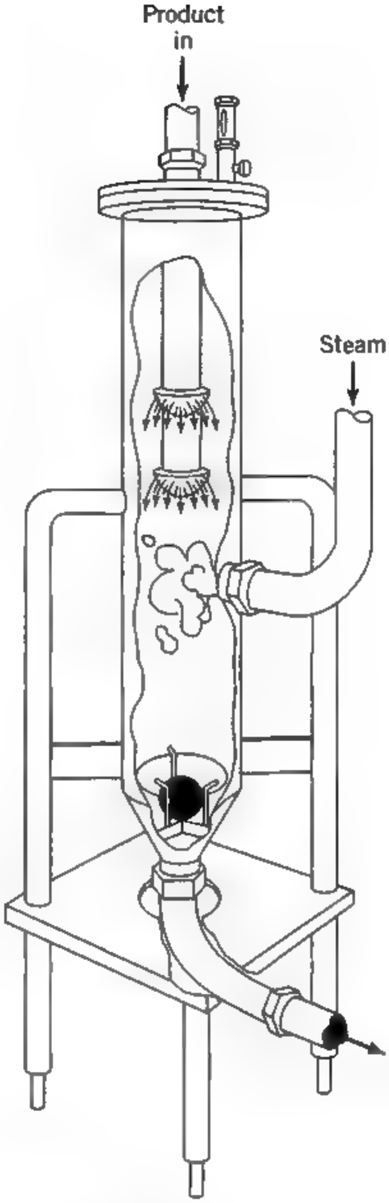


Figure 8. Steam-infusion heat exchangers. *Source:* Ref. 5, courtesy of Crepaco, Inc.

Aseptic Processing Systems. Among the first commercially successful aseptic canning systems is the Dole system, illustrated schematically in Figure 10. The system was designed to aseptically fill conventional steel cans, and made use of superheated steam chambers to sterilize empty can bodies and covers as they were slowly conveyed to the filling chamber. The filling chamber was also maintained sterile by superheated steam under positive pressure and received cool sterile product from the heat exchangers in the product sterilizing subsystem. The entire system was presterilized before operation by passing superheated steam through the can tunnel, cover and closing chamber, and filling chamber for a prescribed start-up program of specified times and temperatures. The product sterilizing line was presterilized by passing pressurized hot water through the cooling heat exchanger (with coolant turned off), product filling line, and filler heads. This start-up procedure had to be repeated every time a compromise in sterility occurred at any system component. Careful

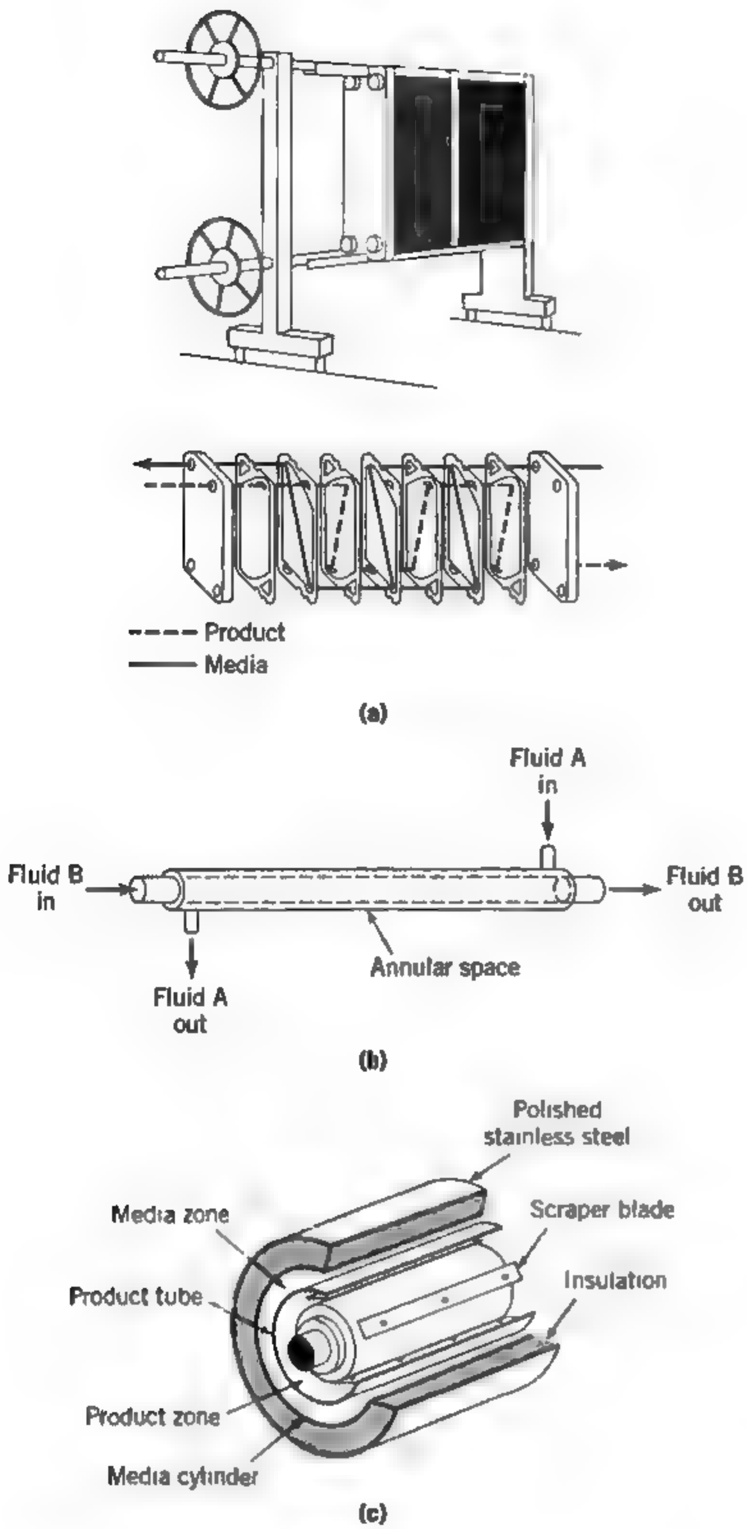

Product
Media
(a)
Fluid A in
Fluid B in
Fluid B out
Annular space
Fluid A out
(b)
Polished stainless steel
Media zone
Scraper blade
Insulation
Product tube
Product zone
Media cylinder
(c)

Figure 9. (a) Plate heat exchangers with product flow schematic, (b) schematic of tubular heat exchangers, and (c) cutaway section of swept surface heat exchangers. *Source:* Ref. 5, courtesy of Cherry-Burrell.

monitoring and control by skillful and highly trained operators is a must for such an intricately orchestrated system.

Although the Dole system had been the mainstay for aseptic filling into metal cans, subsequent regulatory approval for the use of chemical sterilants such as hydrogen peroxide to sterilize the surfaces of various paper, plastics, and laminated packaging materials opened the door to a wide array of commercially available aseptic filling systems that produce shelf-stable liquid foods in a variety of gable-topped, brick-packed, and other novel package configurations. Filling machines designed for these packaging

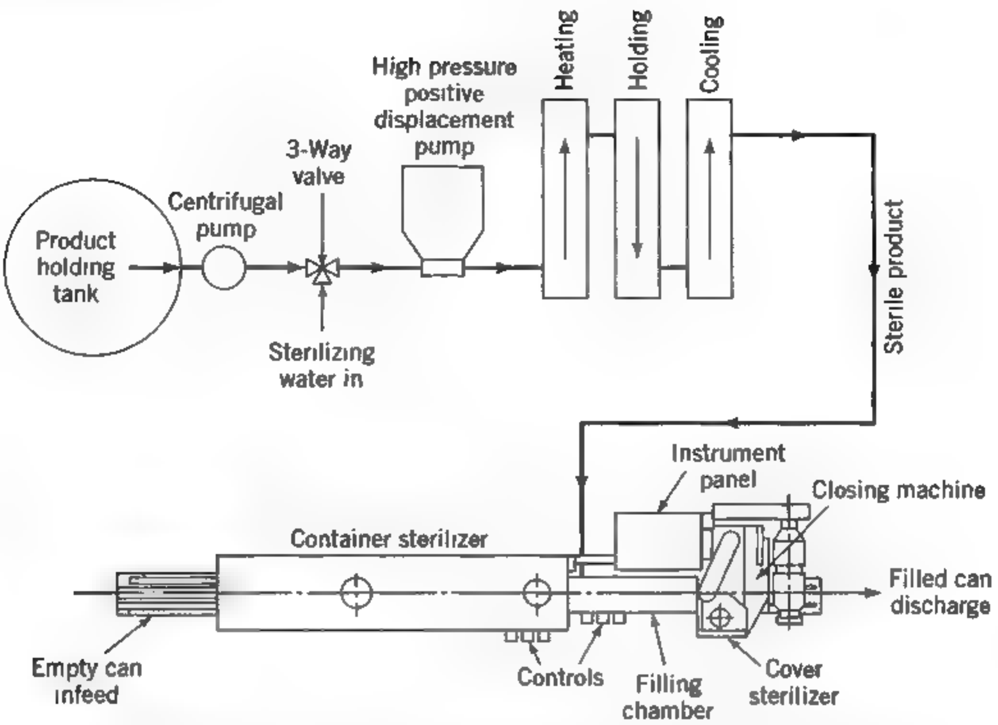


Figure 10. Dole Aseptic Canning Line. *Source:* Ref. 4, courtesy of CTI Publications, Inc.

systems are usually based on the use of form-fill-seal operations. In these machines, the packaging material is fed from either precut blanks or directly from roll stock, passed through a chemical sterilant bath or spray treatment, formed into the final package shape while being filled with cool sterile product from the product sterilizing system, and then sealed and discharged, all within a controlled aseptic environment.

Another important commercial application of aseptic processing technology is in the storage and handling of large bulk quantities of sterilized food ingredients, such as tomato paste, fruit purees, and other liquid food concentrates that need to be purchased by food processors or institutional end users for use as ingredients in further processed prepared foods. The containers for such applications can range in size from the classic 55-gallon steel drum to railroad tank cars or stationary silo storage tanks. Specially designed aseptic transfer valves and related handling systems make it possible to transfer sterile product from one such container to another without compromising sterility (Fig. 11).

Pasteurization Processes Systems

Pasteurization can be carried out by either in-container or out-of-container processes. The main difference from sterilization is that the lower temperatures used for pasteurization do not require the need for operating under pressure. Thus, the equipment systems needed for pasteurization are much simpler in design and easier to operate and maintain.

Normally, liquid foods with delicate heat-sensitive quality attributes, such as milk and fruit juices, are pasteurized out-of-container using HTST heat exchangers to pasteurize with minimum quality degradation before filling in clean packages. These HTST pasteurization systems are similar to the aseptic process systems used in sterilization except that they operate at lower temperatures and at atmospheric pressure, and they do not require rigid aseptic filling conditions. Some liquid dairy products, such as dairy cream and coffee whitener, are given a sterilization heat treatment by operating the heat exchanger under pressure to achieve sterilizing temperatures, but are filled into conventional sanitary cartons without aseptic filling systems. Such products are marketed as ultrapasteurized with markedly longer storage life under refrigeration.

Less heat-sensitive foods as well as most nonliquid foods are pasteurized in-container much like the retort process for sterilization, except that an open tank of hot or near-boiling water is sufficient, and there is no requirement to use pressure vessels like retorts or autoclaves. A third method of pasteurization, known as hot fill, makes use of the high pasteurizing temperature reached by the product in a batch tank or mixing kettle as part of the product preparation. The clean empty containers are filled with the hot product and sealed. They are held upright for a few minutes to transfer sufficient heat to the container walls and bottom, and then they are inverted for an additional few minutes to complete pasteurization of the container lid and seal area using heat transferred from the still hot product. Most canned fruits, fruit preserves, and acidified (pickled) products are pasteurized in this way.

Note that the food examples given for the hot fill method of pasteurization are nonrefrigerated foods that enjoy long-term storage at room temperature without the use of sterilization heat treatments. That is because they are high-

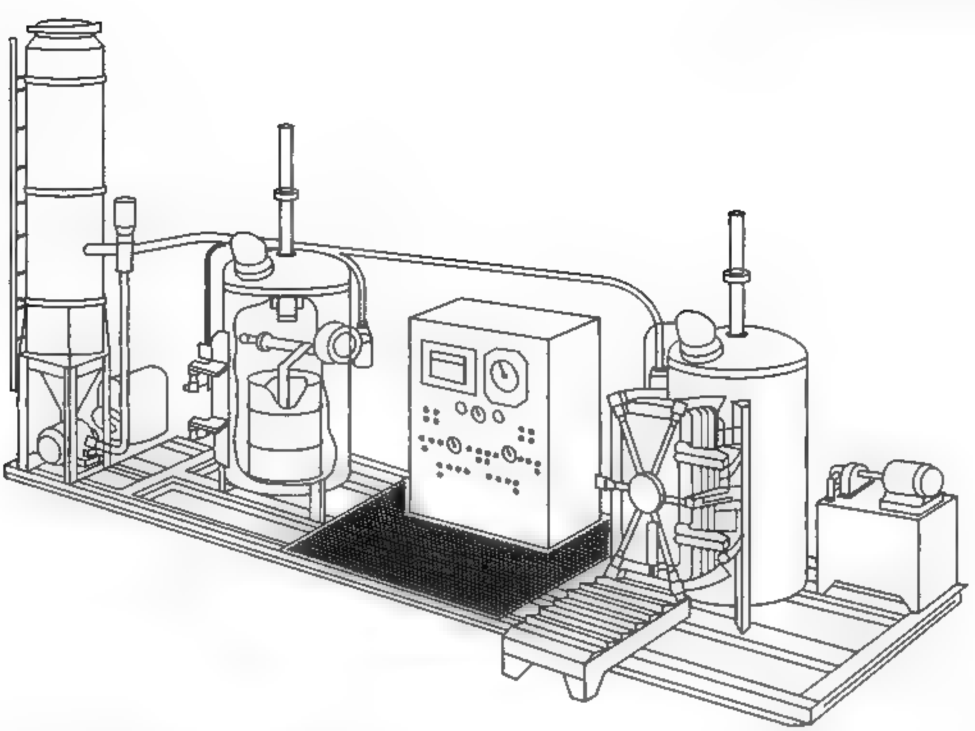


Figure 11. Aseptic filling system for 55-gallon drums. *Source:* Ref. 4, courtesy of Cherry-Burrell Corporation and CTI Publications.

acid foods (pH < 4.5) that cannot support the growth of heat-resistant spore forming pathogens. High-acid foods are subject to spoilage principally by yeasts and molds, which have low heat resistance and can be inactivated by pasteurization heat treatments alone. These are technically canned foods, but are essentially processed by the use of pasteurization technology. That is why it is important to distinguish between high-acid and low-acid canned foods in the context of thermal processing.

SCIENTIFIC PRINCIPLES OF THERMAL PROCESSING

Important Interrelationships

An understanding of two distinct bodies of knowledge is required to appreciate the basic principles involved in thermal process calculation. The first of these is an understanding of the thermal inactivation kinetics (heat resistance) of food-spoilage-causing organisms. The second body of knowledge is an understanding of the heat transfer considerations that govern the temperature profiles achieved within the food container during the process, commonly referred to in the canning industry as heat penetration.

Figure 12 conceptually illustrates the interdependence between the thermal inactivation kinetics of bacterial spores and the heat transfer considerations in the food product. Thermal inactivation of bacteria generally follows first-order kinetics and can be described by a logarithmic reduction in the population or concentration of bacterial spores with time for any given lethal temperature, as shown in the upper family of curves in Figure 12. These

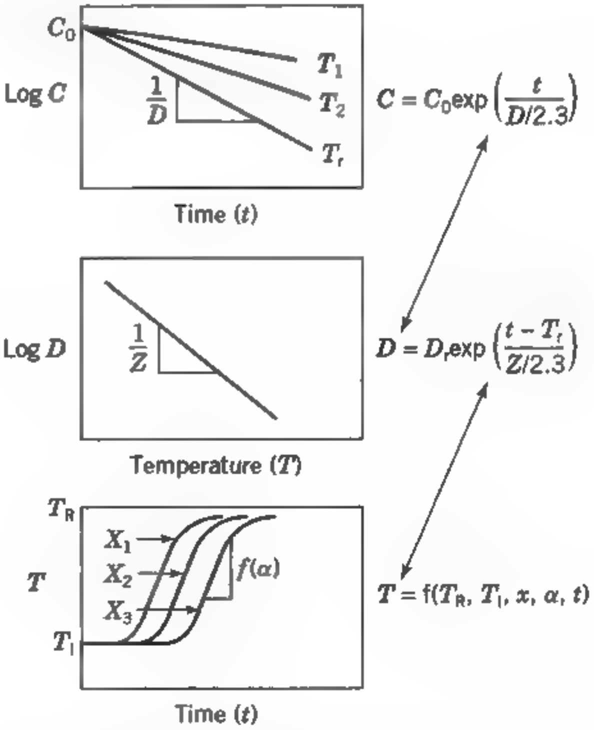


Figure 12. Time and temperature dependence of the thermal inactivation kinetics of bacterial spores in the thermal processing of canned foods. *Source:* Ref. 3, reprinted with permission, copyright 1992 by Marcel Dekker, Inc., New York.

are known as survivor curves. The decimal reduction time, *D*, is expressed as the time in minutes to achieve 1 log cycle of reduction in concentration, *C*. As suggested by the family of curves shown, *D* is temperature dependent and varies logarithmically with temperature, as shown in the second graph. This is known as a thermal death time (TDT) curve and is essentially a straight line over the range of temperatures used in food sterilization. The slope of the line that describes this relationship is expressed as the temperature difference, *Z*, required for the line to traverse 1 log cycle (10-fold change in *D*). The temperature in the food product, in turn, is a function of the retort temperature (T_R), initial product temperature (T_I), location within the container (x), thermal diffusivity of the product (α), and time (t), as shown by the heat penetration curves at the bottom of Figure 12. Thus, the concentration of viable bacterial spores during thermal processing decreases as a function of the inactivation kinetics, which are a function of temperature. The temperature, in turn, is a function of the heat transfer considerations, involving time, space, thermal properties of the product, and initial and boundary conditions of the process.

Microbiological Considerations

Heat Resistance. The heat resistance of microorganisms varies considerably. At any given temperature, this is generally expressed as a decimal reduction time (*D*-value), which is the heating time required to reduce the number of microorganisms by 90% (or to one-tenth of the initial). The temperature sensitivity of these *D*-values is expressed in terms of a *Z*-value, which represents the temperature range that results in a 10-fold change in the *D*-values. These two values can be realized as negative reciprocal slopes of logarithm of surviving microbial numbers vs time (*D*-value or survivor curve) and logarithm of *D*-values vs temperature (*Z*-value curve) as described by the functional expressions in Figure 12. Some typical *D*- and *Z*-values for selected microorganisms are given in Table 3.

As mentioned earlier, the *Z*-value curve, which describes the temperature dependency of the *D*-value, is often referred to as the TDT curve. It forms the basis upon which thermal process times and temperatures are determined and is shown in more detail in Figure 13. Once the TDT curve has been established for a given microorganism in a given food product, it can be used to calculate the time and temperature requirements for any idealized thermal process. For example, assume a process is required that will achieve a 6-log-cycle reduction in the population of bacterial spores whose kinetics are described by the TDT curve in Figure 13, and that a temperature of 235°F has been chosen for the process. The TDT curve shows that the *D*-value at 235°F is 10 min. This means that 10 min will be required for each log cycle reduction in population at that temperature. If a 6-log-cycle reduction is required, a total of 60 min is required for the process. If a temperature of 270°F had been chosen for the process, the *D*-value at that temperature is approximately 0.1 min, and only 0.6 min (or 36 s) would be required at that temperature to accomplish the same 6-log-cycle reduction.

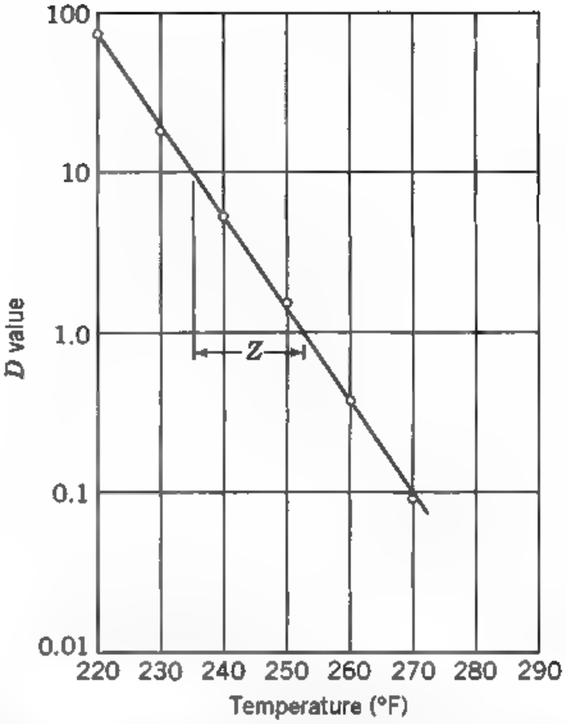


Figure 13. Thermal death time (TDT) curve showing temperature dependency of *D* value (decimal reduction time of microorganisms) given by temperature change (*Z*) required for tenfold change in *D* value. *Source:* Ref. 3, reprinted with permission, copyright 1992 by Marcel Dekker, Inc., New York.

Table 3. Thermal Resistance of Spore Formers Used as a Basis for Thermal Processing

Microorganism	D_{250}-value, min	z-value, °C
B. stearothermophilus	4.0	7.0
B. subtilis	0.48–0.76	7.4–13.0
B. cereus	0.0065	9.7
B. megaterium	0.04	8.8
C. perfringens		10.0
C. sporogenes	0.15	13.0
C. sorogenes (PA 3679)	0.48–1.4	10.6
C. botulinum	0.21	9.9
C. thermosaccharolyticum	3.0–4.0	8.9–12.2

Sterilization F-Value (Lethality). The example process calculations carried out using the TDT curve in Figure 13 showed clearly how two widely different processes (60 min at 235°F and 0.6 min at 270°F) were equivalent with respect to their ability to achieve a 6-log-cycle reduction for that organism. Therefore, for a given Z-value, the specification of any one point on the line is sufficient to specify the sterilizing value of any process combination of time and temperature on that line. The reference point that has been adopted for this purpose is the time in minutes at the reference temperature of 250°F (121°C), or the sterilizing *F*-value (lethality) for the process. Since the *F*-value is expressed in minutes at 250°F (121°C), the unit of lethality is 1 min at 250°F (121°C). Thus, if a process is assigned an F-value of 6, it means that the integrated lethality achieved by whatever time–temperature history employed by the process must be equivalent to the lethality achieved by 6 min of exposure to 250°F.

To illustrate, the example process calculation using the TDT curve in Figure 13 will be repeated by specifying the F-value for the required process. Recall from that example that the process was required to accomplish a 6-log-cycle reduction in spore population. All that is required to specify the F-value is to determine how many minutes at 250°F will be required to achieve that level of log-cycle reduction. The D_{250}-value is used for this purpose, since it represents the number of minutes at 250°F to accomplish 1 log-cycle reduction. Thus, the F-value is equal to D_{250} multiplied by the number of log cycles required in population reduction, or

$$F = D_{250}(\log a - \log b) \quad (1)$$

where a is the initial number of viable spores and b is the final number of viable spores (or survivors).

In this example, $D_{250} = 1.16$ min as taken from the TDT curve in Figure 13, and $(\log a - \log b) = 6$. Thus, $F = 1.16(6) = 7$ min, and the sterilizing value for this process has been specified as $F = 7$ min. This is normally the way in which a thermal process is specified for subsequent calculation of a process time at some other temperature. In this way, information regarding specific microorganisms or numbers of log cycles reduction can be replaced by the F value as a process specification. Note also that this F-value serves as the reference point to specify the equivalent process curve discussed earlier. By plotting a point at 7 min on the vertical line passing through 250°F in Figure 13 and drawing a curve with a slope of $1/z$ parallel to the TDT curve through this point, the line will pass through the two equivalent process points that were calculated earlier (60 min at 235°F and 0.6 min at 270°F). Alternatively, the equation of this straight line can be used to calculate the process time (t) at some other constant temperature (T) when F is specified:

$$F = 10^{[(T-250)/Z]}t \quad (2)$$

Equation 3 becomes important in the general case when the product temperature varies with time during a process, and the F-value delivered by the process must be integrated mathematically, such as at the center of a container of solid food.

$$dF = \int_0^t 10^{[(T-250)/Z]}dt \quad (3)$$

Specification of Process Lethality. Establishing the lethality (F-value to be specified for a low-acid canned food) is undoubtedly one of the most critical responsibilities taken on by a food scientist or engineer acting on behalf of a food company in the role of a competent thermal processing authority. The steps normally taken for this purpose are outlined here.

There are two types of bacterial populations of concern in canned food sterilization. First is the population of organisms of public health significance. In low-acid foods with pH above 4.5, the chief organism of concern is *Clostridium botulinum*. A safe level of survival probability that has been accepted for this organism is 10^{-12}, or one survivor in 10^{12} cans processed. This is known as the 12-D concept for a botulinum cook. Since the highest D_{250} value known for this organism in foods is 0.21 min, the minimum process sterilizing value for a botulinum cook assuming an initial spore load of one organism per can is

$$F = 0.21 \times 12 = 2.52 \text{ min}$$

Essentially, all low-acid foods are processed far beyond the minimum botulinum cook in order to deal with spoilage-causing bacteria of much greater heat resistance. For these organisms, acceptable levels of spoilage probability are usually dictated by economic considerations. Most food companies accept a spoilage probability of 10^{-5} from mesophilic spore-formers (organisms that grow and spoil food at room temperature). The organism most frequently used to characterize this classification of food spoilage is a strain of *Clostridium sporogenese* known as PA 3679 with a maximum D_{250} value of 1.00. Thus, a minimum process sterilizing value for a mesophilic spoilage cook assuming an initial spore load of one spore per can is

$$F = 1.00 \times 5 = 5.00 \text{ min}$$

Where thermophilic spoilage is a problem, more severe processes may be necessary because of the high heat resistance of thermophilic spores. Fortunately, most thermophiles do not grow readily at room temperature; they require incubation at unusually high storage temperatures (110–130°F) in order to cause food spoilage. Generally, foods that show no more than 1% spoilage (spoilage probability of 10^{-2}) upon incubation after processing will show less than the minimum 10^{-5} spoilage probability in normal commerce. Therefore, when thermophilic spoilage is a concern, the target value for the final number of survivors is usually taken as 10^{-2}, and the initial spore load needs to be determined through microbiological analysis since contamination from these organisms varies greatly. For a situation with an initial thermophilic spore load of 100 spores per can and an average D_{250} value of 4.00, the process sterilizing value required would be

$$F = 4.00(\log 100 - \log 0.01)$$
$$F = 4.00(4) = 16 \text{ min}$$

These procedural steps are guidelines for average conditions; they often need to be adjusted up or down in view of the types of contaminating bacteria that may be present, the initial level of contamination or bioburden of the most resistant types, the spoilage risk accepted, and the nature of the food product.

Heat Transfer Considerations

In traditional thermal processing of canned foods, containers are placed in steam retorts that apply heat to the outside wall. The product temperature cannot respond instantaneously, but will gradually rise in an effort to approach the temperature at the wall followed by a gradual fall in response to cooling at the wall. In this situation, the sterilizing value delivered by the process will be the integrated result of the time–temperature profile experienced at the slowest heating point of the container. This profile shape will depend in large part upon the mode of heat transfer experienced by the product.

Modes of Heat Transfer

Conduction-Heating. Solid-packed foods in which there is essentially no product movement within the container, even when agitated, heat largely by conduction heat transfer. Because of the lack of product movement and the low thermal diffusivity of most foods, these products heat very slowly and exhibit a nonuniform temperature distribution during heating and cooling, which is caused by the temperature gradient set up between the can wall and geometric center. For conduction-heating products, the geometric center is the slowest heating point in the container. Therefore, process calculations are based on the temperature history experienced by the product at the can center. Solid-packed foods such as canned fish and meats, baby foods, pet foods, pumpkin, and squash fall into this category. These foods are usually processed in still cook or continuous hydrostatic retorts that provide no mechanical agitation.

Convection-Heating. Thin-bodied liquid products packed in cans, such as soups, sauces, and gravies, will heat by either natural or forced convection heat transfer, depending upon use of mechanical agitation during processing. In a still cook retort that provides no agitation, product movement will still occur within the container because of natural convective currents induced by density differences between the warmer liquid near the hot can wall and the cooler liquid near the can center. The rate of heat transfer in nearly all convection heating products can be increased substantially by inducing forced convection through mechanical agitation. For this reason, most convection-heating foods are processed in agitating retorts designed to provide either axial or end-over-end can rotation. Normally, end-over-end rotation is preferred and can be provided in batch retorts; continuous rotary retorts can provide only limited axial rotation. Unlike conduction heating products, because of product movement in forced convection-heating products, the temperature distribution throughout the product is reasonably uniform under mechanical agitation. In natural convection, the slowest heating point is somewhat below the geometric center and should be located experimentally in each new case.

Broken-Heating. Broken-heating canned food products exhibit a break between the two modes of heat transfer; they will heat part of the time by convection and part of the time by conduction. The more common of these foods are those that initially heat by convection, then, because of starch gelatinization or other thickening agent activity, they set-up or thicken and proceed to heat by conduction. Less common are products that begin heating first by conduction, then for the remainder of the period heat by convection. Generally, these are products with solid pieces in a liquid brine that settle and pack into the lower two-thirds or so of the container when placed in the retort. After some time of heating, when convective currents become sufficiently strong, the solid pieces are lifted and disperse to begin moving with the liquid phase.

Heat Penetration Measurement. The primary objective of heat penetration measurements is to obtain an accurate recording of the product temperature at the can cold spot over time while the container is being heated under a controlled set of retort processing conditions. This is normally accomplished through the use of copper constantan thermocouples inserted through the can wall so as to have the junction located at the can geometric center. Thermocouple lead wires pass through a packing gland in the wall of the retort for connection to an appropriate data acquisition system in the case of a still cook retort. For agitating retorts, the thermocouple lead wires are connected to a rotating shaft for electrical signal pick up from the rotating armature outside the retort.

The precise temperature–time profile experienced by the product at the can center will depend on the physical and thermal properties of the product, size and shape of the container, and retort operating conditions. Therefore, it is imperative that test cans of product used in heat penetration tests be truly representative of the commercial product with respect to ingredient formulation, fill weight, head space, and can size and that the laboratory or pilot plant retort being used is capable of accurately simulating the operating conditions that will be experienced during commercial processing on the production-scale retort systems intended for the product. If this is not possible, then heat penetration tests should be carried out using the actual production retort during scheduled breaks in production operations.

Heat Penetration Curves. During a heat penetration test, both the retort temperature history and product temperature history at the can center are measured and recorded over time. A typical test process will include venting of the retort with live steam to remove all atmospheric air and then closing of vents to bring the retort up to operating pressure and temperature. This is the point at which process time begins, and the retort temperature is held constant over this period of time. At the end of the prescribed process time, steam is shut off, and cooling water is introduced under overriding air pressure to prevent sudden pressure drop in the retort. This begins the cooling phase of the process, which ends when the retort pressure returns to atmosphere and the product temperature in the can has reached a safe low level for removal from the retort. A typical temperature–time plot of these data is shown in Figure 14, which illustrates the degree to which the product center temperature can lag behind the retort temperature during both heating and cooling. The product center temperature history can be taken directly from this plot to perform a process calculation by numerical integration of equation 3; this will be discussed in further detail later.

A heat balance between the heat absorbed by the product and the heat transferred across the can wall from the

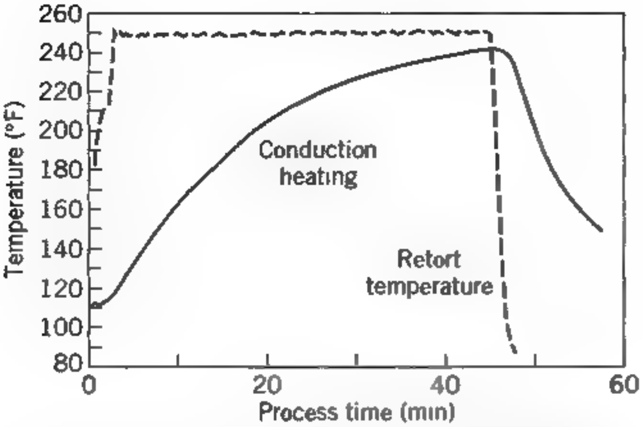


Figure 14. Center temperature profile in cylindrical container of conduction-heating food product in response to constant retort temperature process during heating and cooling.

steam retort could be expressed as follows for an element of food volume facing the can wall of surface area A and thickness L:

$$\rho LAC_p \frac{dT}{dt} = \frac{k}{L} A(T_r - T) \qquad (4)$$

where T is product temperature, T_r is retort temperature, and ρ, C_p, and k are density, specific heat, and thermal conductivity of the product, respectively. Because of the high surface heat transfer coefficient of condensing steam at the can wall and high thermal conductivity of the metal can, overall surface resistance to heat transfer can be assumed negligible in contrast to the product's resistance to heat transfer. After rearranging terms, expression 4 can be written in the form of an ordinary differential equation:

$$\frac{dT}{dt} = \left(\frac{k}{\rho C_p}\right) L(T_r - T) \qquad (5)$$

By letting the thermal diffusivity (α) represent the combination of thermal and physical properties ($k/\rho C_p$) and letting T_o represent the initial product temperature, the solution to equation 5 becomes

$$\frac{T_r - T}{T_r - T_o} = \exp\left(\frac{\alpha}{L^2} t\right) \qquad (6)$$

Thus, the product center temperature can be seen to be an exponential function of time; a semilog plot of the temperature difference $[T_r - T]$ against time would produce a straight line sloping downward having a slope related to the product's thermal diffusivity and can dimensions (Fig. 15). Because the heat penetration rate factor, f_h, is the reciprocal slope of the heat penetration curve, it is related to the product's thermal diffusivity and container dimensions. For a finite cylinder, the following relationship can be used to obtain the thermal diffusivity from a heat penetration curve:

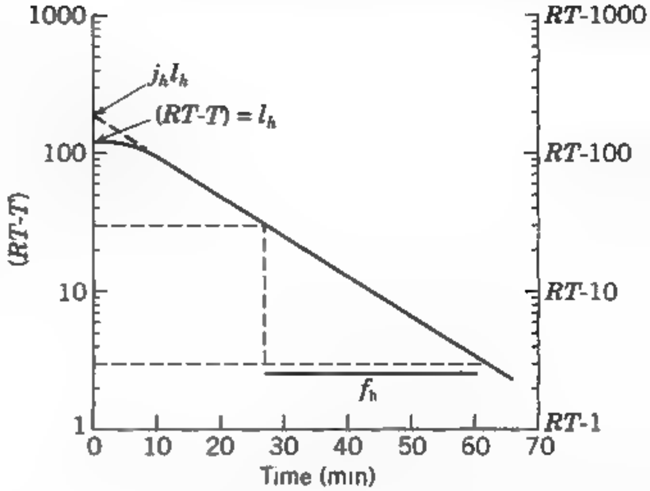


Figure 15. Semilogarithmic heat penetration curve showing predominent log-linear decay of difference between retort and product temperature over process heating time.

$$\alpha = \frac{2.303}{\left[f_h\left(\frac{2.4048^2}{R^2} + \frac{\pi^2}{L^2}\right)\right]} \qquad (7)$$

where R = can radius, L = one-half can height, and f_h is the heating rate factor in minutes. This relationship is also useful to determine the heating rate factor, f_h, for the same product in a different sized container, since the thermal diffusivity is a combination of physical properties that characterize the product and its ingredient formulation and remains unaffected by different container sizes.

COMPUTER SIMULATION

Numerical Models

Another purpose for obtaining the thermal diffusivity of products from a heat penetration curve is to make use of numerical computer models capable of simulating the heat transfer in canned foods. One of the primary advantages of these models is that once the thermal diffusivity has been determined, the model can be used to predict the product temperature history at any specified location within the can for any set of processing conditions and container size. With the use of such models, it is unnecessary to carry out repeated heat penetration tests in the laboratory or pilot plant in order to determine the heat penetration curve for a different retort temperature or can size. A second advantage of even greater importance is that the retort temperature need not be held constant, but can vary in any prescribed manner throughout the process, and the model will predict the correct product temperature history at the can center. Use of these models has been invaluable for simulating the process conditions experienced in continuous sterilizer systems, in which cans pass from one chamber to another experiencing a changing retort temperature at the can wall as they pass through the system. Another important application of these models is in the rapid evaluation of an unscheduled process deviation, such as when an unexpected drop in retort temperature occurs during the course of the process. The model can quickly predict the product center temperature history in response to such a deviation and calculate the delivered F-value for comparison with the sterilizing value specified for the product (6–8).

The first published numerical computer model for simulating the thermal processing of canned foods made use of a numerical solution by finite differences of the two-dimensional partial differential equation (equation 8) that describes conduction heat transfer in a finite cylinder (9). Temperature in the food product is a function of the retort temperature (T_R), initial product temperature (T_I), location within the container (x), thermal diffusivity of the product (α), and time (t) in the case of a conduction-heating food. In practice, α is obtained from the slope of the heat penetration curve (f_h) and is readily known. Because heat is applied only at the can surface, temperatures will rise first only in regions near the can walls, and temperatures near the can center will begin to respond only after a considerable time. Mathematically, the temperature (T) is a distributed parameter in that at any point in time (t) during heating, the temperature takes on a different value with location in the can (r,y), in any one location, the temperature changes with time as heat gradually penetrates the product in accordance with the thermal diffusivity (α).

Equation 8 can be written in the form of finite differences for numerical solution by digital computer, as shown in equation 9. The finite differences are discrete increments of time and space defined as small fractions of process time and container height and radius (Δt, Δh, and Δr, respectively). As a framework for computer iterations, the cylindrical container is imagined to be subdivided into volume elements that appear as layers of concentric rings having rectangular cross-sections, as illustrated in Figure 16 for the upper half of the container. Temperatures nodes are assigned at the corners of each volume element on a vertical plane as shown in Figure 17, where i and j are used to denote the sequence of radial and vertical volume elements, respectively. By assigning appropriate boundary and initial conditions to all the temperature nodes (interior nodes set at initial product temperature and surface nodes set at retort temperature), the new temperature reached at each node can be calculated after a short time interval (Δt) that would be consistent with the thermal diffusivity of the product obtained from heat penetration data (f_h). This new temperature distribution is then taken to replace the initial one, and the procedure is repeated to calculate the temperature distribution after another time interval. In this way, the temperature at any point in the container at any instant in time is obtained. At the end of process time, when steam is shut off and cooling water is admitted to the retort, the cooling process is simulated by simply changing the boundary conditions from retort temperature to cooling temperature at the surface nodes and continuing with the computer iterations described earlier.

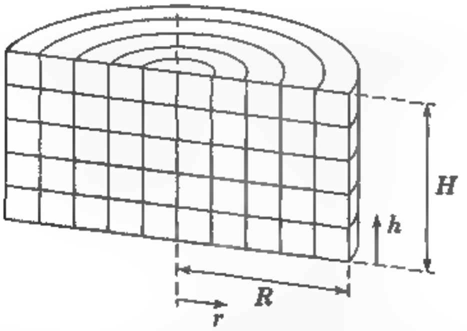


Figure 16. Subdivision of a cylindrical container for application of finite differences for numerical solution of heat conduction equation in a finite cylinder. *Source:* Ref. 3, reprinted with permission, copyright 1992 by Marcel Dekker, Inc., New York.

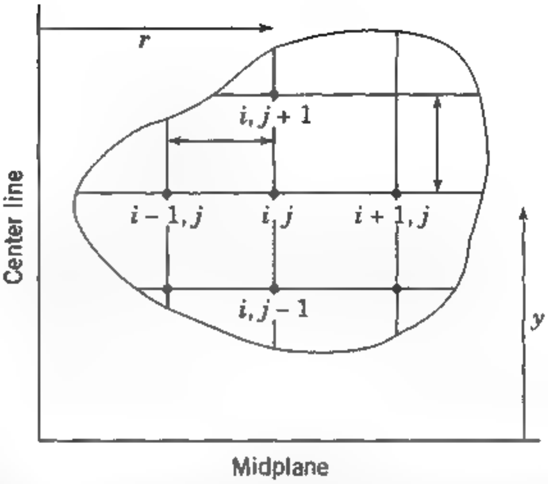


Figure 17. Labeling of grid nodes in matrix of volume elements on a vertical plane for application of finite differences. *Source:* Ref. 3, reprinted with permission, copyright 1992 by Marcel Dekker, Inc., New York.

$$\frac{dT}{dt} = \alpha\left[\frac{d^2T}{dr^2} + \frac{1}{r}\frac{dT}{dr} + \frac{d^2T}{dy^2}\right] \tag{8}$$

$$\begin{aligned} T_{ij}^{(t+\Delta t)} = T_{ij}^{(t)} &+ \frac{\alpha\Delta t}{\Delta r^2}\,[T_{i-1j} - 2T_{ij} + T_{i+1j}]^{(t)} \\ &+ \frac{\alpha\Delta t}{2r\Delta r}\,[T_{i-1j} - T_{i+1j}]^{(t)} \\ &+ \frac{\alpha\Delta t}{\Delta h^2}\,[T_{ij-1} - 2T_{ij} + T_{ij+1}]^{(t)} \end{aligned} \tag{9}$$

In this way, the temperature at the can center can be calculated after each time interval to produce a heat penetration curve upon which the process lethality or F-value can be calculated. When the numerical computer model is used to calculated the process time required at a given retort temperature to achieve a specified lethality, the computer follows a programmed search routine of assumed process times that quickly converges on the precise time at which cooling should begin in order to achieve the specified F-value. Thus, the model can be used to determine the process time required for any given set of constant or variable retort temperature conditions. The next section describes the method for calculating the process time required at any given retort temperature to deliver a specified process sterilizing value or lethality.

Process Calculation

The numerical integration of equation 3 is the most versatile method of process calculation because it is universally applicable to essentially any type of thermal processing situation. It makes direct use of the product temperature history at the slowest heating point of the container obtained from a heat penetration test or predicted by a computer model for calculating the process sterilizing value delivered by a given temperature–time history. This method is particularly useful in taking maximum advantage of computer-based data acquisition systems used in connection with heat penetration tests. Such systems are capable of reading temperature signals received directly from thermocouples monitoring both retort and product center temperature and processing these signals through the computer. Both retort temperature and product center temperature are plotted against time without any data transformation. This allows the operator to see what has actually happened throughout the duration of the process test. As the data are being read by the computer, additional programming instructions call for calculation of the incremental process sterilizing value at each time interval between temperature readings and summing these over time as the process is under way (numerical integration of equation 3). As a result, the accumulated sterilizing F-value is known at any time during the process and can be plotted on the graph along with the temperature histories to show the final value reached at the end of the process. An example of the computer printout from such a heat penetration test is shown in Figure 18.

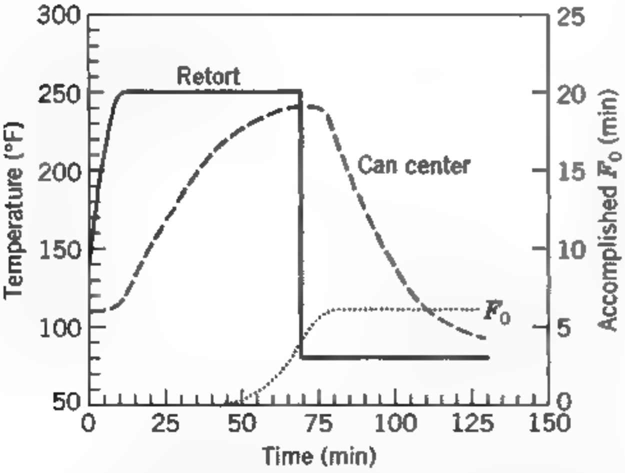


Figure 18. Computer-generated plot of measured retort temperature and calculated center temperature and accomplished F_0 for a given thermal process. *Source:* Ref. 3, reprinted with permission, copyright 1992 by Marcel Dekker, Inc., New York.

Process Optimization

The principle objective of thermal process optimization is to maximize product quality, minimize undesirable changes, minimize cost, and maximize profits. At all times, a minimal process must be maintained to exclude the danger from microorganisms of public health and spoilage concern. Five elements common to all optimization problems are performance or objective function (nutrients, texture, and sensory characteristics), decision variables (retort temperature and process time), constraints (practical limits for temperatures and required minimal lethality), mathematical model (analytical, finite differences, and finite element), and optimization technique (search, response surface, and linear or nonlinear programming).

Optimization theory makes use of the different temperature sensitivity of microbial and quality factor destruction rates. Microorganisms have lower decimal reduction time (less resistant to heat) and a lower Z-value (more sensitive to temperature) than most quality factors. Hence, a higher temperature will result in preferential destruction of microorganisms over the quality factor. Especially applied to liquid product either in a batch in-container mode or in continuous aseptic systems, the higher temperature with shorter time offers a great potential for quality optimization. However, for conduction heating foods, one of the major limitations is the slower heating. All higher temperatures do not necessarily favor the best quality retention because they also expose the product nearer the surface to more severe temperature than the product at the center, which might result in diminished overall quality. Using a finite differences computer simulation program, it has been demonstrated that the optimal process temperature is around 250°F (121°C) for maximized thiamin retention. In fact, processing at temperatures beyond 265°F was shown to result in poorer thiamine retention than processing at 240°F (9).

Product quality optimization can also be accomplished by promoting faster and more uniform heating in the product by other means, such as optimized container geometry in the form of appropriate height-to-diameter ratios, alternate packaging materials and shave such as the thin-profile retort pouch or semirigid container, or an agitated process for foods that are normally conduction heated but can flow under the influence of agitation (6).

On-Line Computer Control

Traditional control of thermal process operations has consisted of maintaining specified operating conditions that have been predetermined from product and process development research, such as the process calculations for the time and temperature of a batch cook. Sometimes unexpected changes can occur during the course of the process operation or at some point upstream in a processing sequence such that prespecified processing conditions are no longer valid or appropriate, and off-specification product that is produced must be either reprocessed or destroyed at appreciable economic loss. These types of situations can be of critical importance in food processing operations because the physical process variables that can be measured and controlled are often only indicators of complex biochemical reactions that are required to take place under the specified process conditions.

Because of the important emphasis placed on the public safety of canned foods, processors operate in strict compliance with the Food and Drug Administration's Low-Acid Canned Food Regulations. Among other things, these regulations require strict documentation and record-keeping of all critical control points in the processing of each retort load or batch of canned product. Particular emphasis is placed on product batches that experience an unscheduled process deviation, such as when a drop in retort temperature occurs during the course of the process that may result from loss of steam pressure. In such a case, the product will not have received the established scheduled process and must be either destroyed, fully reprocessed, or set aside for evaluation by a competent processing authority. If the product is judged to be safe, then batch records must contain documentation showing how that judgement was reached. If judged unsafe, then the product must be fully reprocessed or destroyed. Such practices are costly.

In recent years, food engineers knowledgeable in the use of engineering mathematics and scientific principles of heat transfer have developed computer models (described earlier in this section) capable of simulating thermal processing of conduction-heated canned foods. These models make use of numerical solutions to mathematical heat transfer equations capable of predicting accurately the internal product cold spot temperature in response to any dynamic temperature experienced by the retort during the process. The accomplished lethality (F_o) for any thermal process is easily calculated by numerical integration of the predicted cold spot temperature over time, as explained previously. Thus, if the cold spot temperature can be accurately predicted over time, so can accumulated process lethality.

Computer-based intelligent on-line control systems make use of these models as part of the decision-making software in a computer-based on-line control system. Instead of specifying the retort temperature as a constant boundary condition, the actual retort temperature is read directly from sensors located in the retort and is continually updated with each iteration of the numerical solution. Using only the measured retort temperature as input to the control system, the model operates as a subroutine calculating the internal product cold spot temperature at small time intervals for computer iteration in carrying out the numerical solution to the heat conduction equation by finite differences. At the same time, the model also calculates the accomplishing process lethality associated with cold spot temperature in real time as the process is under way. At each time step, the subroutine simulates the additional lethality that will be contributed by the cooling phase if cooling were to begin at that time. In this way, the control system decision of when to end heating and begin cooling is withheld until the model has determined that final target process lethality will be reached at the end of cooling (10).

By programming the control logic to continue heating until the accumulated lethality has reached some designated target value, the process will always end with the desired level of sterilization (F_o) regardless of an unscheduled process temperature deviation. At the end of the process, complete documentation of measured retort temperature history, calculated center temperature history, and accomplished sterilization (F_o) can be generated in compliance with regulatory record-keeping requirements. Such documents are shown in Figure 19 for a normal process and for the same intended process with an unexpected deviation.

FUTURE TRENDS

Thermal processing has been in use as a predominant method of food preservation since the middle of the nineteenth century. People throughout the world have become

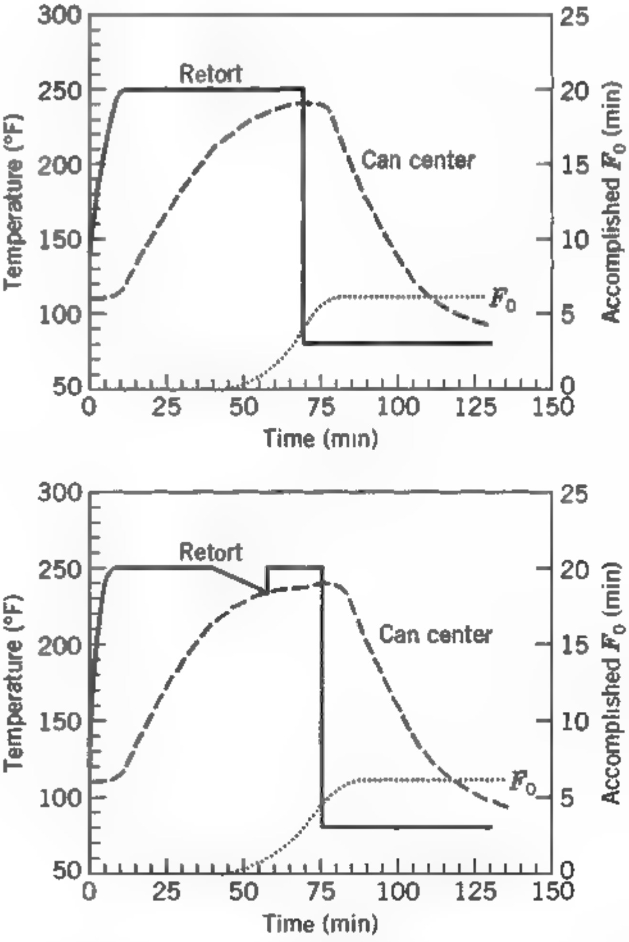


Figure 19. Output documentation of computer-based on-line control system showing scheduled heating time of 68 minutes for normal process (above), and heating time extended automatically to 76 minutes in compensation for unscheduled temporary loss of retort temperature (process deviation) (below).

quite familiar with canned foods packed in traditional metal cans and glass jars. Perhaps less apparent is the important role that this technology has had and continues to have in promoting and sustaining the health and well-being of populations throughout the world. Although the major portion of canned foods produced throughout history has been used to help feed the consuming public, this technology has also played a very strategic role in major world events. The famous C-rations that supported military troops through the World Wars I and II were canned food rations. These rations remained safe and wholesome for consumption after long periods of storage and handling under highly stressful and abusive conditions.

Developments in new packaging materials and retort systems have brought about a host of innovative new canned food products that are often not recognized as being canned foods. Most canned food rations for today's military troops are packaged in flexible retort pouches; they are convenient, comfortable (soft), and lightweight when being carried on maneuvers. For feeding large numbers of military troops in field kitchens, canned foods come as fully prepared meals in large institutional-sized rectangular steam-table trays ready to heat and serve.

The increasing popularity of the microwave oven has created a demand for canned foods in microwavable containers that are ready to "pop and zap." This has led to the increasing success of complete prepared meals in attractive lunch bowls or dinner trays that can be placed in the microwave oven and be taken directly to the dinner table. Such products are hardly recognizable as traditional canned foods, but they are.

Perhaps least recognizable, however, is the important role that thermal processing technology has played in the pharmaceutical and health care industry. Large quantities and varieties of sterile solutions are required daily for surgical and patient care procedures. Sterile saline solutions, irrigation solutions, intravenous solutions with dextrose or glucose, and dialysate solutions, along with a host of other large-volume parenteral solutions in glass, plastic, flexible and semirigid containers, are sterilized in retort systems using the technology of canning for food preservation. Such products, of course, are neither thought of nor considered to be canned foods, but they are in fact a very important use of thermal processing technology throughout the world.

BIBLIOGRAPHY

1. P. Fellows, *Food Processing Technology: Principles and Practices*, Ellis Horwood Ltd., Chichester, United Kingdom, 1988.
2. D. B. Lund, "Heat Processing," in M. Karel, O. R. Fennema, and D. B. Lund, eds., *Principles of Food Science, Part II: Physical Principles of Food Preservation*, Marcel Dekker, Inc., New York, 1975.
3. A. A. Teixeira, "Thermal Process Calculations," in D. R. Heldman and D. B. Lund, eds., *Food Engineering Handbook*, Marcel Dekker, New York, 1992, pp. 563–620.
4. A. Lopez, *A Complete Course in Canning and Related Processes*, CTI Publications, Baltimore, Md., 1987.
5. R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, San Diego, Calif., 1984.
6. A. A. Teixeira, G. E. Zinsmeister, and J. W. Zahradnik, "Computer Simulation of a Variable Retort Control and Container Geometry as a Possible Means of Improving Thiamine Retention in Thermally Processed Foods," *J. Food Sci.* **40**, 656–659 (1975).
7. A. A. Teixeira and J. E. Manson, "Computer Control of Batch Retort Operations with On-line Correction of Process Deviations," *Food Technol.* **36**, 85–90 (1982).
8. A. K. Datta, A. A. Teixeira and J. E. Manson, "Computer-based Retort Control Logic for On-line Correction of Process Deviations," *J. Food Sci.* **51**, 480–483, 507 (1986).
9. A. A. Teixeira et al., "Computer Optimization of Nutrient Retention in Thermal Processing of Conduction-heated Foods," *Food Technol.* **23**, 137–142 (1969).
10. A. A. Teixeira and C. F. Shoemaker, *Computerized Food Processing Operations*, Van Nostrand Reinhold, New York, 1989.

A. A. Teixeira
University of Florida
Gainesville, Florida

See also Canning: regulatory and safety consderations.

THERMAL STERILIZATION OF CANNED LIQUID FOODS

Sterilization of food involves the destruction of microorganisms carried in it so that the food can be stored longer and be safe to consume. Thermal sterilization is the most often used and most time-tested method of sterilization. It involves use of heat to destroy the harmful microorganisms. But heat also affects nutrients and other desirable food properties adversely. Design of sterilization processes involves selection and implementation of heating temperature and duration to minimize the destruction of desirable food components while maintaining the required level of destruction of the harmful microorganisms (1,2).

Due to the finite size of any food material, time-temperature histories will always vary spatially, the exact variation being a function of the food properties, the design of the machinery, and the properties of container (batch) or tube (continuous). Only by analyzing all of these factors, which requires detailed heat transfer studies, would the required time-temperature history be known. Sterilization of liquids is further complicated because it is not the time-temperature history of locations but that of the individual moving liquid particles that determine the extent of sterilization, the latter being difficult to observe experimentally or to calculate theoretically.

The sterilization literature spans more than 100 years. Most studies are product, process, or equipment specific. The studies generally do not explain the underlying physics in sufficient detail to be able to make generalized conclusions about a large category of problems. This article synthesizes the engineering studies of liquid food sterilization to provide insight into the underlying physics of the process. After introducing the general concepts involving quite familiar with canned foods packed in traditional metal cans and glass jars. Perhaps less apparent is the important role that this technology has had and continues to have in promoting and sustaining the health and well-being of populations throughout the world. Although the major portion of canned foods produced throughout history has been used to help feed the consuming public, this technology has also played a very strategic role in major world events. The famous C-rations that supported military troops through the World Wars I and II were canned food rations. These rations remained safe and wholesome for consumption after long periods of storage and handling under highly stressful and abusive conditions.

Developments in new packaging materials and retort systems have brought about a host of innovative new canned food products that are often not recognized as being canned foods. Most canned food rations for today's military troops are packaged in flexible retort pouches; they are convenient, comfortable (soft), and lightweight when being carried on maneuvers. For feeding large numbers of military troops in field kitchens, canned foods come as fully prepared meals in large institutional-sized rectangular steam-table trays ready to heat and serve.

The increasing popularity of the microwave oven has created a demand for canned foods in microwavable containers that are ready to "pop and zap." This has led to the increasing success of complete prepared meals in attractive lunch bowls or dinner trays that can be placed in the microwave oven and be taken directly to the dinner table. Such products are hardly recognizable as traditional canned foods, but they are.

Perhaps least recognizable, however, is the important role that thermal processing technology has played in the pharmaceutical and health care industry. Large quantities and varieties of sterile solutions are required daily for surgical and patient care procedures. Sterile saline solutions, irrigation solutions, intravenous solutions with dextrose or glucose, and dialysate solutions, along with a host of other large-volume parenteral solutions in glass, plastic, flexible and semirigid containers, are sterilized in retort systems using the technology of canning for food preservation. Such products, of course, are neither thought of nor considered to be canned foods, but they are in fact a very important use of thermal processing technology throughout the world.

BIBLIOGRAPHY

1. P. Fellows, *Food Processing Technology: Principles and Practices*, Ellis Horwood Ltd., Chichester, United Kingdom, 1988
2. D. B. Lund, "Heat Processing," in M. Karel, O. R. Fennema, and D. B. Lund, eds., *Principles of Food Science, Part II: Physical Principles of Food Preservation*, Marcel Dekker, Inc., New York, 1975.
3. A. A. Teixeira, "Thermal Process Calculations," in D. R. Heldman and D. B. Lund, eds., *Food Engineering Handbook*, Marcel Dekker, New York, 1992, pp. 563–620.
4. A. Lopez, *A Complete Course in Canning and Related Processes*, CTI Publications, Baltimore, Md., 1987.
5. R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, San Diego, Calif., 1984.
6. A. A. Teixeira, G. E. Zinsmeister, and J. W. Zahradnik, "Computer Simulation of a Variable Retort Control and Container Geometry as a Possible Means of Improving Thiamine Retention in Thermally Processed Foods," *J. Food Sci.* **40**, 656–659 (1975).
7. A. A. Teixeira and J. E. Manson, "Computer Control of Batch Retort Operations with On-line Correction of Process Deviations," *Food Technol.* **36**, 85–90 (1982).
8. A. K. Datta, A. A. Teixeira and J. E. Manson, "Computer-based Retort Control Logic for On-line Correction of Process Deviations," *J. Food Sci.* **51**, 480–483, 507 (1986).
9. A. A. Teixeira et al., "Computer Optimization of Nutrient Retention in Thermal Processing of Conduction-heated Foods," *Food Technol.* **23**, 137–142 (1969).
10. A. A. Teixeira and C. F. Shoemaker, *Computerized Food Processing Operations*, Van Nostrand Reinhold, New York, 1989.

A. A. TEIXEIRA
University of Florida
Gainesville, Florida

See also CANNING: REGULATORY AND SAFETY CONSDERATIONS.

THERMAL STERILIZATION OF CANNED LIQUID FOODS

Sterilization of food involves the destruction of microorganisms carried in it so that the food can be stored longer and be safe to consume. Thermal sterilization is the most often used and most time-tested method of sterilization. It involves use of heat to destroy the harmful microorganisms. But heat also affects nutrients and other desirable food properties adversely. Design of sterilization processes involves selection and implementation of heating temperature and duration to minimize the destruction of desirable food components while maintaining the required level of destruction of the harmful microorganisms (1,2).

Due to the finite size of any food material, time-temperature histories will always vary spatially, the exact variation being a function of the food properties, the design of the machinery, and the properties of container (batch) or tube (continuous). Only by analyzing all of these factors, which requires detailed heat transfer studies, would the required time-temperature history be known. Sterilization of liquids is further complicated because it is not the time-temperature history of locations but that of the individual moving liquid particles that determine the extent of sterilization, the latter being difficult to observe experimentally or to calculate theoretically.

The sterilization literature spans more than 100 years. Most studies are product, process, or equipment specific. The studies generally do not explain the underlying physics in sufficient detail to be able to make generalized conclusions about a large category of problems. This article synthesizes the engineering studies of liquid food sterilization to provide insight into the underlying physics of the process. After introducing the general concepts involving heating of fluids, three broad categories of liquid food sterilization systems are discussed in detail. These are continuous heating, agitated containerized heating, and unagitated containerized heating. Included under each category are a brief description of the processing equipment, methods of heating, flow and temperature profiles, sterilization values, effect of non-Newtonian liquid behavior, and effect of presence of particulates.

QUANTITATIVE MEASURE OF STERILIZATION

The rates of reactions in foods, such as the destruction of microorganisms or nutrients, are generally first-order reactions described by

$$-\frac{dc}{dt} = k_T c \tag{1}$$

where c is the concentration of any component at time t and k_T is the rate constant at temperature T. The temperature dependency of the rate constant is given by the well-known Arrhenius law

$$k_T = k_0 e^{-E_a/RT} \tag{2}$$

where k_0 is a constant called frequency factor, E_a is the activation energy, and R is the gas constant. The temperature T normally varies in a heating process. To obtain the final concentration in changing temperature, equation 1 is integrated between initial concentration c_i and final concentration c at time t to get

$$\ln \frac{c_i}{c} = k_0 \int_0^t e^{-E_a/RT} dt \tag{3}$$

In the food literature, equation 3 is used in a different form. Instead of referring to a final concentration, an equivalent heating time of F_0 is used that gives the same final concentration when temperature T is constant at a reference value. For a reference temperature of T_R, if the rate constant is k_{T_R},

$$F_0 = \frac{\ln(c_i/c)}{k_{T_R}} = \frac{k_0 \int_0^t e^{-E_a/RT} dt}{k_0 e^{-E_a/RT_R}} = \int_0^t e^{[(E_a/R)(1/T_R - 1/T)]dt} \tag{4}$$

When the process temperature T stays close to T_R, equation 4 can be simplified by noting $TT_R \sim T_R^2$. A new parameter Z is defined in terms of the activation energy E_a to describe the temperature dependence of reaction as

$$Z = \frac{2.303RT_R^2}{E_a} \tag{5}$$

Using this simplification and the definition of parameter Z, equation 4 can be rewritten as

$$F_0 = \int_0^t 10^{(T-T_R)/Z} dt \tag{6}$$

This is the familiar equation in food sterilization that provides the equivalent heating time F_0 at a reference temperature T_R for a process whose actual temperature T varies with time t.

In any finite mass of liquid (or any material), the temperature history $T(t)$ varies spatially throughout the mass. Thus F_0 as given by equation 6 will vary spatially at any given time. Thus in reality, there is always a distribution of F_0 values due to spatial variation of temperature history. For bacterial destruction the lowest value of F_0 is taken as the measure of the extent of sterilization. The average F_0 for nutrients, which is sometimes referred to as the cook value, provides a measure of the nutritional quality of the processed food material.

The complete distribution of quality parameters such as sterilization and nutrient retention is generally quite difficult to obtain due to the complex variations of temperature T with position and time in most processing situations of practical interest. Under these circumstances, design of the heating process is aimed at minimizing the spatial variation of temperature, for example, by introducing turbulence. For many practical calculations, where spatial variation is ignored, mean temperature is used in equation 6.

SPECIAL CONSIDERATIONS FOR LIQUIDS

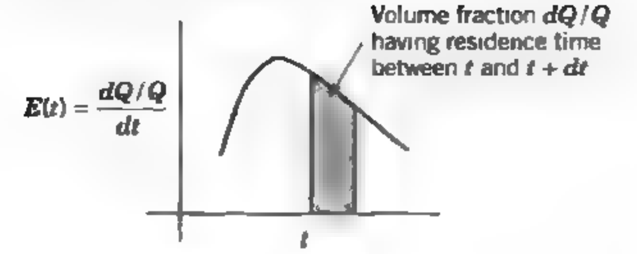
Several factors uniquely complicate the study of liquid sterilization as compared to solids, which are now discussed.

Distribution of Residence Time and Thermal Time

Chemical and biological reactions such as destructions of microorganisms and nutrients associated with sterilization refer to elements in the fluid and solid food. For pure conduction heating in solids, the elements stay in a fixed location. Thus sterilization of material particles becomes synonymous with sterilization at various locations. To obtain the extent of these reactions in liquids that are often flowing, material elements would need to be followed conceptually. Generally speaking, for every liquid particle the temperature history as well as the duration of heating would be different.

The durations of heating are studied using the concepts of residence-time distributions (RTDs). The time it takes a molecule to pass through the sterilization vessel is called its residence time. In continuous operations, all liquid particles do not remain in the equipment for the same time. Particles near the walls (boundary layer) or in dead space, for example, move more slowly than particles farther away. Such behavior of liquids result in a distribution of residence times for various liquid elements. From equation 6 the extent of sterilization would vary for the various liquid elements. It is therefore important to have an idea about the RTD to insure that the liquid residing for the shortest time receives adequate sterilization. Figure 1 shows an RTD where the area between any two residence times is

Residence-time distribution



Thermal-time distribution

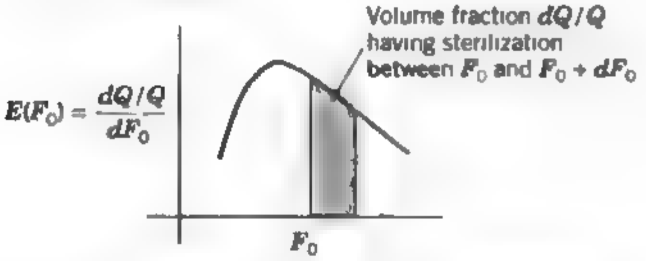


Figure 1. Representations of residence-time distributions and thermal-time distributions of fluids during a sterilization process. The symbol Q refers to either the volumetric flow rate or the total volume of liquid being sterilized.

the volume (or mass) fraction of liquid that resides between those two times. RTDs are described in detail in various sources (3–5).

But RTDs have only a duration of heating and not a temperature history and therefore cannot provide information on sterilization distribution in general. In analogy to RTDs, thermal-time distributions (TTDs) have been defined (6) that include the effect of temperature history. Figure 1 shows a typical TTD. The area under the curve between two values of sterilization is the fraction of liquid volume between those sterilization values. Unlike RTDs, TTDs have complete information on the temperature history in addition to duration of heating. Three parameters that can characterize the distribution are the lowest value, range, and average values. Average values are of interest in estimates of nutrient retention. For sterilization, the lowest value is of interest. The lowest value in the distribution corresponds to the particle whose time-temperature history combines (as given by equation 6) to be the lowest F_0 value. This least value of sterilization of a particle is difficult to obtain experimentally. In practice, least value of sterilizations obtained at various locations in the flow domain is used as the least sterilization for the process.

It is important to note the conceptual distinction between the time-temperature histories of location as opposed to a liquid particle (7). Time-temperature history at a location in a moving liquid would not correspond to a single liquid particle in general. Instead it would correspond to all particles passing through that point over time. Depending on the nature of the flow, point sterilization can be different from particle sterilization. From the food safety point of view, point sterilization is inherently safe because it assumes a particle is stagnant at a location where temperature always stays the least. All physical particles stay only some time at the slowest heating point, and no particle is expected to stay all the time in the slowest heating point. Thus all particles in the system obtain a sterilization more than or equal to the sterilization calculated based on the temperature at the slowest heating location. It becomes difficult to refer to slowest heating location when no one location stays the coldest over the entire period, as in some complex recirculating flows described later. In practice, the region over which the coldest point moves over time is called the slowest heating zone. The time-temperature history at any location in the slowest-heating zone is considered to provide the least value of sterilization.

Rates of Convective Heat Transfer

In heating of liquids, whether as continuous flow in a heat exchanger or as batch processing in a can, it is useful to think of the heat transfer process as comprising several thermal resistances. These thermal resistances are related to the commonly used heat transfer coefficients that describe a heating process involving liquids, that is, in flowing media. Thus, the total thermal resistance between a fluid food and the heating medium is made up of internal thermal resistance between the fluid food and the wall of the heat exchanger or the container holding the fluid, resistance of the wall of the heat exchanger or the container, and the thermal resistance between the outside wall of the heat exchanger or the container and the heating medium such as steam. Symbolically, we can write (8)

$$\underbrace{1/U}_{\substack{\text{total}\\ \text{resistance}}} = \underbrace{1/h_i}_{\substack{\text{resistance of}\\ \text{liquid food}\\ \text{inside the}\\ \text{container}}} + \underbrace{\Delta x/k}_{\substack{\text{resistance of}\\ \text{container wall}}} + \underbrace{1/h_o}_{\substack{\text{resistance of}\\ \text{heating fluid}\\ \text{outside the}\\ \text{container}}} \qquad (7)$$

Here U is the overall heat transfer coefficient between the fluid food and the heating medium, h_i is the heat transfer coefficient between the fluid food and the container or exchanger wall, k and Δx are the thermal conductivities and thicknesses, respectively, of the container or exchanger wall, and h_o is the heat transfer coefficient between the container or exchanger outside wall and the heating medium. The internal convective heat transfer coefficient, h_i, is generally small and the most significant one. The purpose of agitation is to significantly enhance it. The contribution due to wall conductivity and thickness can be ignored for metal cans with large thermal conductivity but can be significant with nonmetallic materials such as pouches. The external convective heat transfer coefficient is very large for steam condensation, and therefore its contribution can be ignored for steam heating. For heating with other liquids, the external heat transfer resistance can be significant.

The overall heat transfer coefficient U can be used to make an energy balance to calculate the change in food temperature. For a continuously flowing liquid, this energy balance can be written as

$$\dot{m}c_p dT = U dA(T_m - T) \qquad (8)$$

where dA is a small area of the heat exchanger surface over which heat transfer takes place and during which time the temperature of the liquid changes by an amount dT. The mass flow rate of the liquid being heated is $\dot{m}$, its specific heat is c_p, and T_m is the temperature of the heating medium. For batch heating of a liquid of mass m in a can, the energy balance can be written as

$$mc_p dT = UA(T_m - T)dt \qquad (9)$$

where A is typically the surface area of the can and m is the total mass of liquid that changes its temperature by an amount dT during the time dt. Integrating equation 8 over the complete area of the heat exchanger or integrating equation 9 over the total duration of heating gives the time-temperature history $T(t)$ of the liquid to be used with equation 6 to obtain the net sterilization of the liquid. This is particularly useful in complex heat transfer situations where spatial variation in temperature is either small or quite difficult to obtain.

Newtonian and Non-Newtonian Liquids

The relationship between shear stress and shear rate of a large number of liquids can be represented as

$$\sigma = \sigma_0 + K\left(-\frac{dv}{dz}\right)^n \qquad (10)$$

where σ is the shear stress, σ_0 is the shear stress needed to initiate flow, dv/dz is the velocity gradient of shear rate, K is called the consistency coefficient, and n is called the flow behavior index. When σ_0 is zero and n is equal to one, the liquid is called Newtonian and K becomes the viscosity of the liquid. For all other values of σ_0 and n, the liquid is described as non-Newtonian. Both Newtonian and non-Newtonian behavior are common to liquid foods (9). Among non-Newtonian liquid foods, a more common type is a pseudoplastic liquid for which σ_0 is zero and n is less than one. Many of the studies on non-Newtonian liquids referred to in this article are pseudoplastic type.

Laminar and Turbulent Flow

Turbulent flow is generally desired. Mechanical agitation is one way of getting turbulent flow as in a scraped-surface heat exchanger or in an agitating retort. In continuous flow, tube dimensions, flow rates, and liquid properties are adjusted to get a Reynolds number in the turbulent range. There are times, however, when turbulent flow is uneconomical to achieve, as in the case of many non-Newtonian liquids. These liquids exhibit high apparent viscosity, and pumping pressures required to obtain fully turbulent conditions are not economic for production rates of interest (10). Likewise, extent of agitation required for containerized non-Newtonian liquid to attain turbulent flow may not always be feasible for practical industrial applications.

Presence of Solid Particles in the Liquid

When solid particles are present in the liquid, a very complex heat-transfer system results. The particles in the liquid would need to be sterilized besides the liquid itself. Additional conductive resistance for the inside of the solid food and convective resistance between solid food surface and the liquid carrying it are added to the three resistances on the right side of equation 7. The thermal resistance represented by the surface heat transfer coefficient between the solid particles and the fluid carrying it is difficult to obtain experimentally or estimate from theoretical considerations. Additionally, the interior of the solid particles heat by a slow conduction process. The combined effect is that the sterilization of the food is limited by the sterilization of the particles.

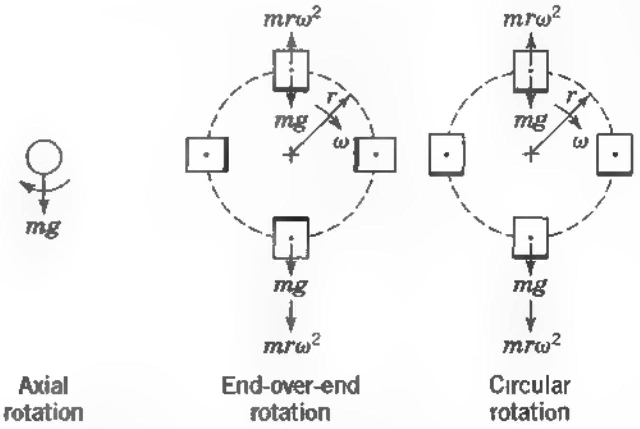
AGITATED BATCH (IN-CONTAINER) HEATING OF LIQUIDS

Processing Equipment and Methods of Heating

Mechanical agitation is frequently used to enhance heat transfer by increasing the surface heat transfer coefficient between the container surface and the liquid in it. Processing time can be markedly shortened this way, generally resulting in improvement of quality. Quality also may improve because there is less chance for food to cook onto the can walls because the can contents are in motion. The critical factors that decide the effective agitation are the headspace or the amount of airspace in the can, consistency of the liquid, and speed and mode of agitation (11).

Several modes of agitation are used in the industry, some of which are shown in Figure 2. In retorts known as sterilmatic type (13), the can rotates part of the time about its own axis and part of the time about an axis parallel to its own, as it travels through the retort. As the can rolls, the headspace travels along the cylindrical contours of the can, resulting in the agitation of the contents. In a spin cooker the can is axially rotated (Fig. 2) at speeds as high as 500 rpm as it travels through the length of the retort.

In the other major type of agitation called end-over-end (EOE) agitation, the can is rotated around an axis perpen-



Axial rotation

End-over-end rotation

Circular rotation

Figure 2. Some common types of can rotation. *Source:* Ref. 12.

dicular to its own (Fig. 2). The axis of rotation is also located externally to the container. A positive movement of the headspace bubble is created throughout the rotating cycle. Because the headspace is moving along the length of the can and reverses direction every half a rotation, excellent mixing of the contents is achieved. In another rotating method of agitation (12) the cans are rotated in a circular path (Fig. 2) in such a way that the can orientation remains fixed. Agitation forces similar to those in EOE agitation are produced in this system.

The heating medium generally used is steam. Heating in direct flame with agitation of the cans is also used (14,15). The flame temperatures can be close to 2,000°C, and this high temperature is the primary contributor to rapid heat transfer.

Flow and Temperature Profiles

The complex nature of the agitation provided by the equipment discussed previously has generally defied any theoretical analysis to obtain detailed temperature and flow profiles. The temperature and flow patterns are affected by the headspace, fill of the container, solid-to-liquid ratio when solid particles are present, consistency of the liquid, and speed and type of agitation (11). There is only one theoretical study of liquid flow and heat transfer in a container that incorporates some agitations of the container similar to the sterilmatic-type retort discussed previously (16). The effects of container rotation about its own axis were considered to have the dominant effect on heat transfer and liquid flow over simultaneous rotation about an axis parallel to its own. Thus only container axial rotation was considered. Faster heating rates were observed in presence of rotation (centrifugally driven flows).

Experimental studies have measured temperature at several locations and correlated the heat transfer coefficient with other parameters. Nusselt-Prandtl correlations for obtaining heat transfer coefficient in axially rotated cans in a steam retort using water and silicone oil as model liquids have been provided (17). The temperature distribution in direct-flame heating of axially rotating cans was very uniform (15). Transient temperatures in an axially rotating can heated in a steam retort was shown to be quite uniform (Fig. 3). Temperature measurements in an end-over-end rotating can were also similar (Fig. 4) and showed uniformity, which further improved when the direction of rotation was reversed at intervals. It has been noted that the heat transfer coefficient was much higher in EOE as compared to axial rotation (18). It was also noted that the mere presence of a minimal-size headspace in EOE rotation markedly increases the heat transfer coefficient, although its contribution becomes progressively smaller (Fig. 5).

Due to the complexities of a theoretical or an experimental study, and the presence of fairly uniform temperatures inside the agitated container, detailed spatial variations of temperature and velocities are often bypassed. Instead, the energy balance in equation 9 is based on T being the mean fluid temperature. Using this formulation, overall heat transfer coefficient, U, is made available from experiment often in the form of standard Nusselt-Prandtl number correlations. The coefficient U depends on a number of processing parameters. For example, in EOE agitation, increasing the retort temperature increased the overall heat transfer coefficient, U, as shown in Figure 6, probably by lowering the viscosity of the fluid. Rotational speed also increased the heat transfer coefficient (Fig. 7), due to enhanced mixing. Figure 7 also shows the expected reduction in overall heat transfer coefficient for a more viscous fluid (oil).

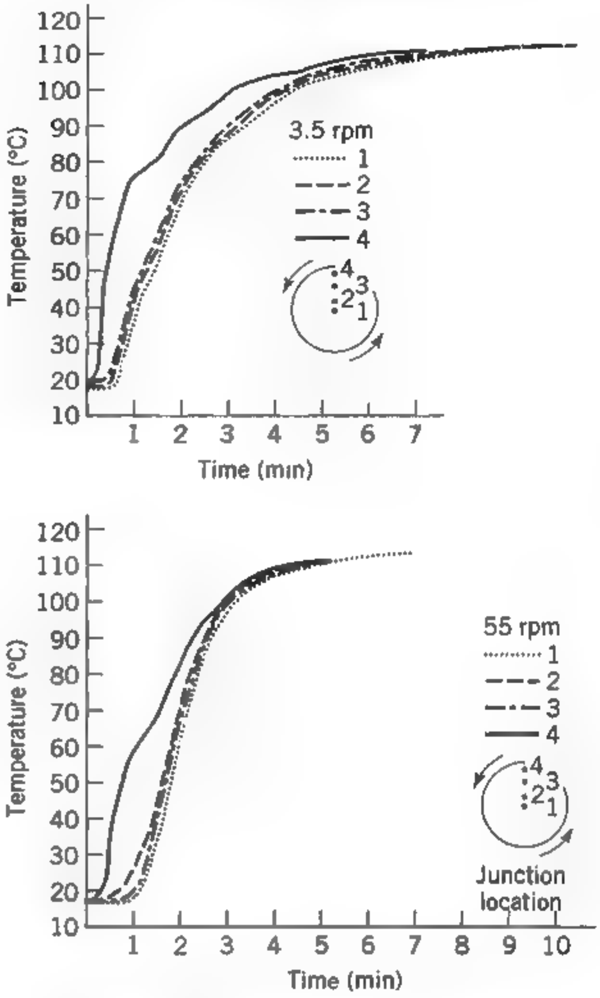


Figure 3. Temperature distribution at radial locations in an axially rotating horizontal can. *Source:* Ref. 18.

The solution to equation 9 when starting from a uniform initial temperature of T_0 is

$$\frac{T - T_m}{T_0 - T_m} = e^{-(UA/mc_p)t} \tag{11}$$

which can also be written in the form

$$\log\left(\frac{T - T_m}{j(T_0 - T_m)}\right) = -t/f \tag{12}$$

where $j = 1$ and $f = mc_p/2.303UA$. Thus a plot of $\log((T - T_m)/(T_o - T_m))$ versus t would be a straight line charac-

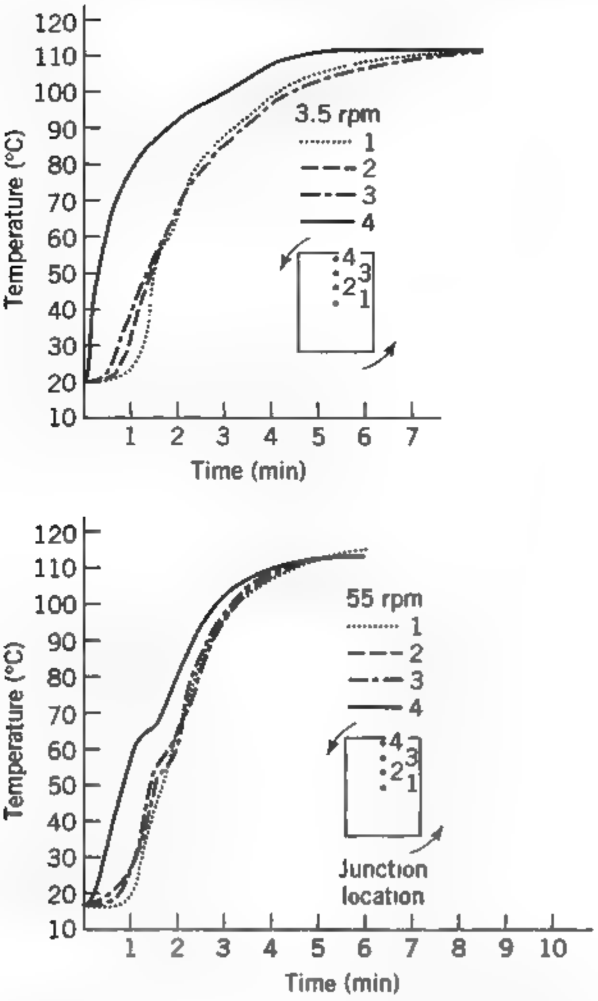


Figure 4. Temperature distribution at axial locations in an end-over-end agitated can. *Source:* Ref. 18.

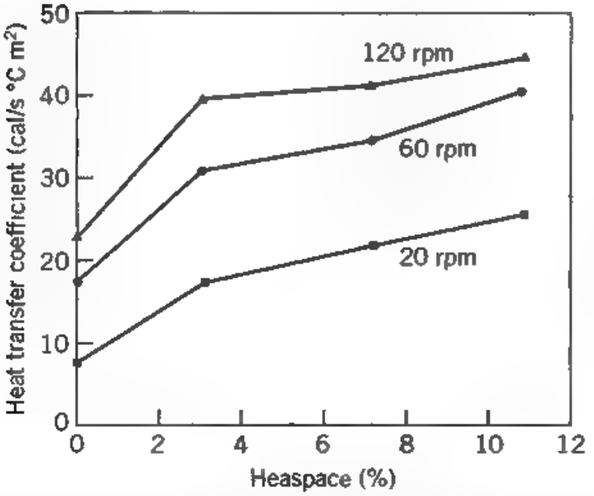


Figure 5. Inside surface heat transfer coefficient of an end-over-end agitated can, as influenced by headspace. *Source:* Ref. 19.

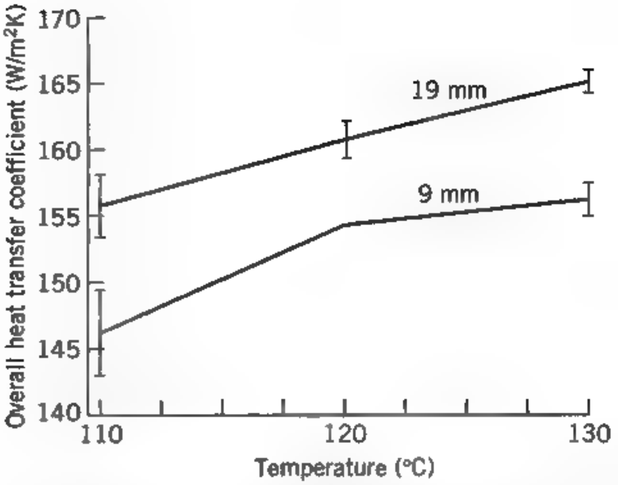


Figure 6. Overall heat transfer coefficient during end-over-end rotation at two (9 and 19 cm) radii of rotation at a rotational speed of 15 rpm as influenced by the retort (heating medium) temperature. *Source:* Ref. 20.

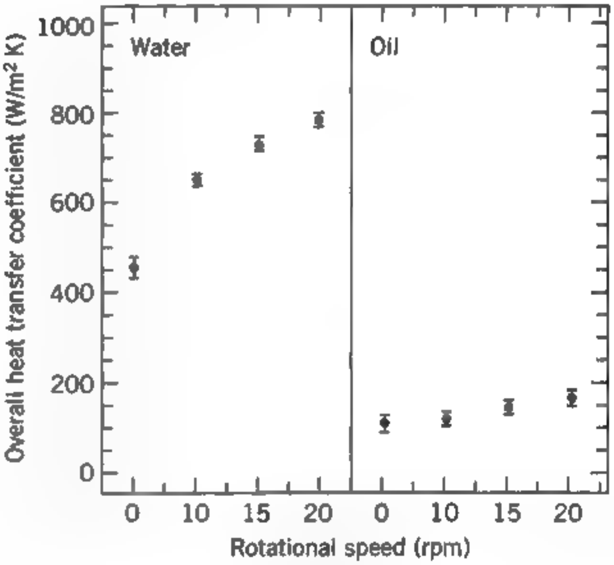


Figure 7. Overall heat transfer coefficient between a canned liquid and the heating medium during end-over-end rotation as influenced by the rotational speed of the can and the liquid viscosity. *Source:* Ref. 21.

terized by the slope $-1/f$ and intercept j. This enables the f and j values to be obtained experimentally from measured $T(t)$ data. These f and j values are used to calculate sterilization time for a process as discussed in books such as Ball and Olson (1).

Sterilization Values and Distribution

Because the detailed temperature and flow patterns have not been solved or experimentally obtained for the various complex heating situations, knowledge of distribution of sterilization (TTD) inside the agitated containers is not available. Due to the presence of agitation, liquid would generally have a smaller spatial variation of temperature within the container and therefore a smaller spread in sterilization values. The precise cold point in the can is generally unavailable for specific heating situations due to unavailability of detailed spatial variation in temperature. In axially rotating cans under flame sterilization (15), the cold points were on the axis near the ends of the can, although temperatures at other locations varied very little except near the wall. The center of the can is generally assumed to be the cold point for agitated heating, although for small variations in temperature within the container this is somewhat irrelevant.

Effect of Non-Newtonian Liquid Behavior

Several non-Newtonian liquids in the steritort have been studied, and correlations similar to Nusselt-Prandtl number correlations for Newtonian liquids have been provided. Several other studies have also included non-Newtonian liquids, and these studies have been summarized (11). Studies in nonfood applications indicate that the convective heat transfer characteristics of non-Newtonian liquids are generally similar to those for Newtonian liquids (22,23)

Effect of Presence of Particulates

Several authors have studied agitated canned liquids in the presence of particulates, although rigorous engineering studies providing detailed temperature and velocity profiles in the liquid in presence of particulates are generally lacking (24,25). The additional critical parameter due to the presence of particles is, of course, the convective surface heat transfer coefficient between the fluid and the particles. It was noted that for agitations in a steritort, this surface heat transfer coefficient depended on the ratio of particle to container diameter, rotational speed of steritort, and liquid properties (26). In axial rotation of horizontal cylindrical containers, liquid to particle heat transfer coefficient for a physically restrained particle was found to be nearly invariant at 160 ± 30W/m^{2}K (27).

In EOE agitation, the overall heat transfer coefficient is shown (Fig. 8) to increase initially as particle concentration is increased from a single particle. This is attributed to the additional particles aiding the fluid mixing as compared to a single particle. After some concentration value, further increase in concentration reduces the overall heat transfer coefficient. Such decrease was also observed by others (26,27) and is attributed to the decreased ability of the fluid to move inside the can (28). The overall heat transfer coefficient decreased with increase in particle size (28), as shown in Figure 9. This trend is supported by reference 29 but is opposite that reported by reference 26. Spherical-shaped particles lead to a higher overall heat transfer coefficient, followed by cylindrical shape, with the cubic shape producing the smallest heat transfer coefficient (30).

NONAGITATED BATCH (IN-CONTAINER) HEATING OF LIQUIDS

Processing Equipment and Methods of Heating

Nonagitating or still retorts are used when agitators are to be avoided to keep the product or package integrity or for economical reasons in small production volume. Due to slow movement of the liquid from natural convection, heating times required for sterilization are somewhat long. Steam is generally the heating medium, however, hot water is also used.

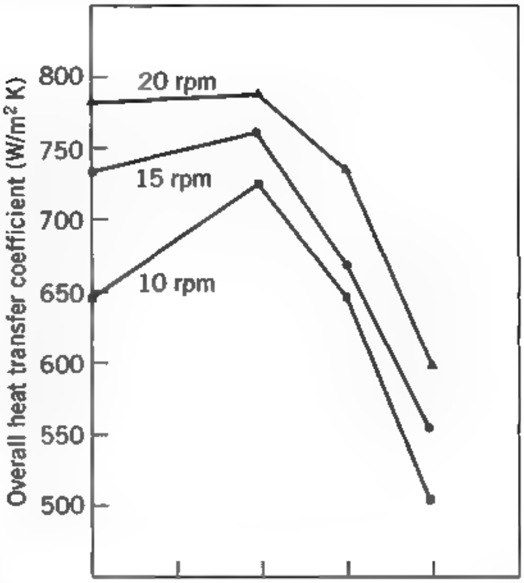


Figure 8. Overall heat transfer coefficient between solid food particles in a canned liquid and the heating medium as influenced by particle concentration during end-over-end rotation. *Source:* Ref. 28.

Flow and Temperature Profiles

In natural convection heat transfer, the liquid moves only due to the buoyancy induced by the change in temperature. This couples the flow and temperature fields and makes their computation a challenging task, as can be seen for pasteurization of beer in bottles (31) and sterilization of canned Newtonian (32) and non-Newtonian (33,34) fluids. Figures 10 and 11 show the typical flow patterns and temperature profiles in such nonagitated heating starting from a fluid at rest at a uniform temperature and raising its walls to the retort temperature. The liquid next to the sidewall, the top wall, and the bottom wall receives heat from the hot walls. As the liquid is heated, it expands and thus gets lighter. Liquid farther from the sidewall is still at a much lower temperature. The buoyancy force created by the liquids at different temperatures forces the hot liquid, next to the wall, upward. A sharp drop in temperature occurs from the wall to the core, creating a boundary layer. The buoyancy force resulting from this large temperature difference contributes to the largest velocities near the wall. The hot liquid going up is interrupted by the top wall and then travels radially toward the core. The core liquid, being heavier, moves downward and toward the wall. Thus a recirculating flow is created with a boundary layer at the sidewall, core flow at the centerline, and a mixing region at the top. Because the liquid is going up only through a small cross-sectional area (the thickness of the boundary

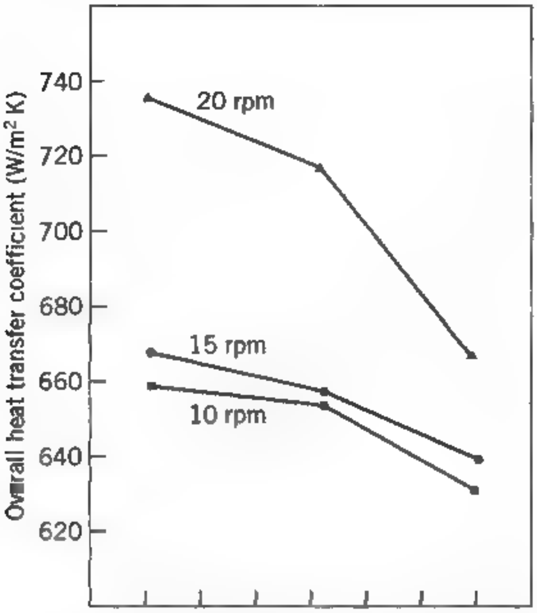


Figure 9. Overall heat transfer coefficient between solid food particles in a canned liquid and the heating medium as influenced by particle size during end-over-end rotation. *Source:* Ref. 28.

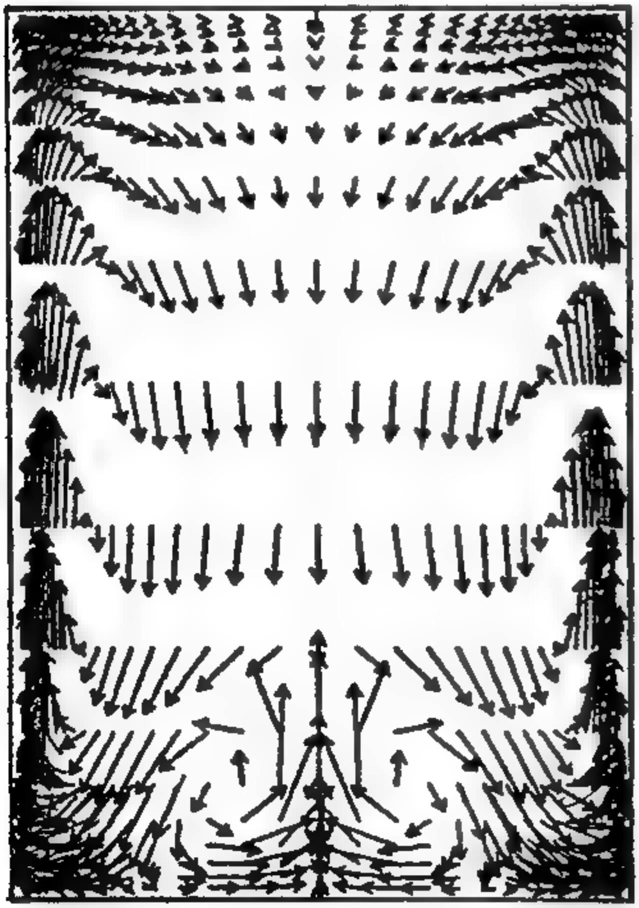


Figure 10. Computed velocity vectors in natural convection heating of liquid in a cylindrical can heated from all sides. *Source:* Ref. 32.

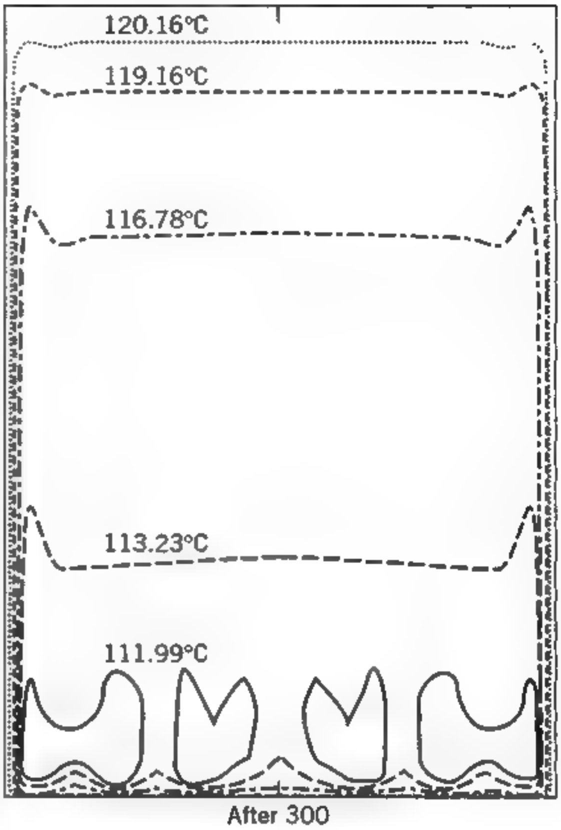


Figure 11. Computed transient temperature contours in natural convection heating of liquid in a cylindrical can heated from all sides with the wall temperature set at 121°C. *Source:* Ref. 32.

layer) while its downward movement is over a much larger area at the core, the downward velocity is much smaller.

Temperature values along the axis show that due to deposition of hot liquid, the top stays consistently at a higher temperature. However, at the very bottom, because the bottom wall was heated, the temperature is higher again. The cold point, therefore, exists somewhere in between and was found not to stay at the same location over time. These cold points were seen to migrate within the bottom 15% of can height with no particular pattern of migration (35). Similar location of cold points were experimentally observed (36–38).

Experimental studies of flow patterns during natural convection heating of a fluid food in a container have been performed. These are mostly qualitative visual observations, except in (37), where velocities were measured using a laser–Doppler velocimeter. Temperature profiles were measured in these studies and were often represented as the slope $-1/f$ and intercept j of semi–log time-temperature line as shown in equation 12. It has been shown (39) that use of such f and j values to represent time-temperature data for natural convection heating has been merely an extension of the formulas for conduction (1) and forced convection (equation 12) heating and is empirical

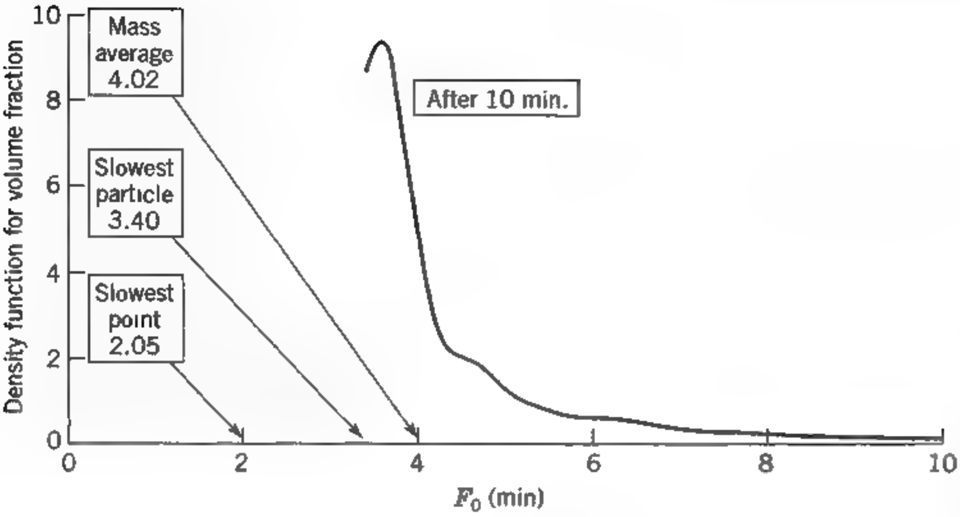


Figure 12. Computed distribution of sterilization (thermal-time-distribution) in liquid for the conditions in Figures 10 and 11.

without any physical or mathematical justification. Nevertheless, the *f* and *j* values measured from experiment are used to calculate sterilization time, as described in Ball and Olson (1).

Sterilization Values and Distribution

A typical transient distribution of sterilization (TTD described previously) in a cylindrical container (35) is shown in Figure 12. This was calculated by implicitly following the liquid elements (7) throughout the duration of heating. The volume (or mass) average sterilization lies within the range of the distribution, as expected. However, the sterilization calculated based on the temperature at the slowest heating point lies completely outside the distribution. This is because all physical fluid particles stay only some time in the slowest heating zone, and no particle stays all the time in the slowest heating zone. Thus all particles in the system obtain a sterilization more than what is calculated based on the slowest heating zone. Because sterilization at the slowest heating zone is easily measured by placing a thermocouple, this is the temperature used in practice to calculate sterilization. The slowest point value in Figure 12 demonstrates that use of such slowest heating thermocouple data provides additional overprocessing (and safety) beyond the true least sterilization of a fluid.

Effect of Non-Newtonian Liquid Behavior

For non-Newtonian (pseudoplastic) liquid with temperature-dependent viscosity, the recirculation pattern, radial and axial profile, and location of the slowest heating points were found (36) to be qualitatively similar to that of Newtonian liquid (35) discussed previously. As could be expected for the higher effective viscosity of pseudoplastic liquids, the maximum axial velocity near the wall was lower, being of the order of 10^{-4} m/s as opposed to 10^{-2} m/s for Newtonian liquid. The non-Newtonian nature itself was not expected to change the physics considerably because at the low shear rates involved in a naturally convective flow, the liquid behaves close to a Newtonian liquid.

Starch gelatinization has been included (34,40) in unagitated heating of a canned 3.5% cornstarch dispersion. The thermorheological behavior of the starch dispersion was described in terms of three phases: (*1*) a pregelatinization phase where the magnitudes of apparent viscosity were assumed to be those of the continuous phase, (*2*) a gelatinization phase where the magnitudes of apparent viscosity increased, and (*3*) a postgelatinization phase where the magnitudes of apparent viscosity decreased. Although the spatial velocity and temperature profiles were qualitatively similar to the previous studies of unagitated heating (32), they (34,40) showed for the first time how the well-known broken heating curves (temperature-time curves with drastic changes in slope) develop during heating of starch-containing products, due to changes in the product apparent viscosity with temperature. The complex thermorheological behavior of the starch dispersion made it difficult to develop prediction equations for temperature-time histories using the commonly used *f* and *j* parameters.

Effect of Presence of Particulates

The complexities of natural convection heating are considerably amplified when solid particles are present in the liquid food, for which little information is available. If the overall heat transfer coefficient between the solid particles inside the container and the heating medium is available, equations for conduction heating can be used to estimate the particle temperatures and insure sterility. Dimensionless Nusselt-Prandtl type correlations for the overall heat transfer coefficients between mushroom-shaped particles and water in a still retort have been provided (41), although there was considerable scatter in the data. Presence of spherical glass particles reduced natural convection in viscous liquid such as silicone, whereas the particles had very little effect on convection in thin liquid such as water (37). Smaller particles caused greater reduction in the convective flow.

BIBLIOGRAPHY

1. C. O Ball and F. C. W. Olson, *Sterilization in Food Technology*, McGraw-Hill, New York, 1957.
2. C. R. Stumbo, *Thermobacteriology in Food Processing*, Academic Press, Orlando, Fla., 1965.
3. J. M. Smith, *Engineering Kinetics*, McGraw-Hill, New York, 1981.
4. A. M. Scalzo et al., "Residence Time of Egg Products in Holding Tubes of Egg Pasteurizers," *Food Technol.* **23**, 678–681 (1969).
5. M. A. Rao and M. A. Loncin, "Residence Time Distribution and Its Role in Continuous Pasteurization, Part 1," *Lebensm.-Wiss. Technol.* **7**, 5–13 (1974).
6. E B. Nauman, "Nonisothermal Reactors: Theory and Applications of Thermal Time Distributions," *Chem. Eng. Sci.* **32**, 359–367 (1977).
7. A. K. Datta, "Integrated Thermokinetic Modeling of Processed Liquid Food Quality," in W. E. L. Spiess and H Schubert, eds., *Engineering and Food*, Vol. 1, *Physical Properties and Process Control*, Elsevier Applied Science, New York, 1989, pp. 838–847.
8. F. P. Incropera and D. P. Dewitt, *Introduction to Heat Transfer*, Wiley, New York, 1984.
9. M. A. Rao and S. S. H. Rizvi, *Engineering Properties of Foods*, Marcel Dekker, New York, 1986.
10. A. H. P. Skelland, *Non-Newtonian Fluid Flow and Heat Transfer*, Wiley, New York, 1967.
11. M. A. Rao and R. C. Anantheswaran, "Convective Heat Transfer to Fluid Foods in Cans," *Advances in Food Research* **32**, 39–84 (1988).
12. P. Parchomchuk, "A Simplified Method for Agitation Processing of Canned Foods," *J. Food Sci.* **42**, 265–268 (1977).
13. A. Lopez, *A Complete Course in Canning: Book 1, Basic Information on Canning*, Canning Trade, Baltimore, Md., 1981.
14. D. J. Casimir, "Flame Sterilization," *CSIRO Food Research Quarterly* **35**, 34–39 (1975).
15. R. L. Merson et al., "Temperature Distributions and Liquid Side Heat Transfer Coefficients in Model Liquid Foods in Cans Undergoing Flame Sterilization Heating," *Journal of Food Process Engineering* **4**, 85–98 (1981).
16. F. Ladiende, "Studies on Thermal Convection in Self-Gravitating and Rotating Horizontal Cylinders in a Vertical External Gravity Field," Ph.D. Dissertation, Cornell University, Ithaca, N.Y., 1988.
17. C. L. Soule and R. L. Merson, "Heat Transfer Coefficients to Liquids in Axially Rotated Cans," *Journal of Food Process Engineering* **8**, 33–46 (1985).
18. S. Hotani and T. Mihori, "Some Thermal Engineering Aspects of the Rotation Method in Sterilization," in T. Motohiro and K. Hayakawa, eds., *Heat Sterilization of Food*, Koseisha-Koseikaku, Tokyo, 1983
19. D. Naveh and I. J. Kopelman, "Effects of Some Processing Parameters on the Heat Transfer Coefficients in a Rotating Autoclave," *Journal of Food Processing and Preservation* **4**, 67–77 (1980).
20. S. S. Sablani and H. S. Ramaswamy, "Particle Heat Transfer Coefficients under Various Retort Operating Conditions with End-over-End Rotation," *Journal of Food Process Engineering* **19**, 403–424 (1996).
21. H. S. Ramaswamy and S. S. Sablani, "Particle Motion and Heat Transfer in Cans during End-over-End Rotation: Influence of Physical Parameters and Rotational Speed," *Journal of Food Process Engineering* **21**, 105–127 (1997).
22. E. M. Parmentier, "A Study of Thermal Convection in Non-Newtonian Fluids," *J. Fluid Mech.* **84**, 1–11 (1978).
23. S. F. Liang and A. Acrivos, "Experiments on Buoyancy Driven Convection in Non-Newtonian Fluid," *Rheol. Acta* **9**, 447–454 (1970).
24. N. G. Stoforos and R. L. Merson, "Estimating Heat Transfer Coefficients in Liquid/Particulate Canned Foods Using Only Liquid Temperature Data," *J. Food Sci.* **55**, 478–483 (1990).
25. A. N. Lekwauwa and K. Hayakawa, "Computerized Model for the Prediction of Thermal Responses of Packaged Solid Liquid Food Mixture Undergoing Thermal Processes," *J. Food Sci.* **51**, 1042–1049 (1986).
26. M. K. Lenz and D. B. Lund, "The Lethality-Fourier Number Method: Heating Rate Variations and Lethality Confidence Intervals for Forced-Convection Heated Foods in Containers," *Journal of Food Process Engineering* **2**, 227–271 (1978).
27. M. F. Deniston, B. H. Hassan, and R. L. Merson, "Heat Transfer Coefficients to Liquids with Food Particulates in Axially Rotating Cans," *J. Food Sci.* **52**, 962–966 (1987).
28. S. S. Sablani and H. S. Ramaswamy, "Heat Transfer to Particles in Cans with End-over-End Rotation: Influence of Particle Size and Concentration (% v/v), *Journal of Food Process Engineering* **20**, 265–283 (1997).
29. B. Hassan, "Heat Transfer Coefficients for Particles in Liquid in Axially Rotating Cans," Ph.D. Dissertation, University of California, Davis, 1984.
30. H. S. Ramaswamy and S. S. Sablani, "Particle Shape Influence on Heat Transfer in Cans Containing Liquid Particle Mixtures Subjected to End-over-End Rotation," *Lebensm.-Wiss. Technol.* **30**, 525–535 (1997).
31. M. S. Engelman and R. L. Sani, "Finite-Element Simulation of an In-Package Pasteurization Process," *Numerical Heat Transfer* **6**, 41–54 (1983).
32. A. K. Datta and A. Teixeira, "Numerical Modeling of Natural Convection Heating in Canned Liquid Foods," *Transactions of the ASAE* **30**, 1542–1551 (1987).
33. A. Kumar, M. Bhattacharya, and J. Blaylock, "Numerical Simulation of Natural Convection Heating of Canned Thick Viscous Liquid Products," *J. Food Sci.* **55**, 1403–1410 (1990).
34. W. H. Yang and M. A. Rao, "Numerical Study of Parameters Affecting Broken Heating Curve," *Journal of Food Engineering* **37**, 43–61 (1998).
35. A. K. Datta and A. Teixeira, "Numerically Predicted Transient Temperature and Velocity Profiles during Natural Convection Heating of Canned Liquid Foods," *J. Food Sci.* **53**, 191–195 (1988).
36. J. L. Blaisdell and W. J. Harper, "Location of Slowest Cooling Zone and Rate Cooling of Homogenized Milk in Various Containers," *Transactions of ASAE* **13**, 433–435 (1970).
37. J. Hiddink, *Natural Convection Heating of Liquids, with Reference to Sterilization of Canned Foods*, Agricultural Research Reports No. 839, Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands, 1975.
38. L. G. Zechman and I. J. Pflug, "Location of the Slowest Heating Zone for Natural-Convection-Heating Fluids in Metal Containers," *J. Food Sci.* **54**, 205–209 (1989).
39. A. K. Datta, "On the Theoretical Basis of the Asymptomatic Semilogarithmic Heat Penetration Curves Used in Food Processing," *Journal of Food Engineering* **12**, 177–190 (1990).

40. W. H. Yang and M. A. Rao, "Transient Natural Convection Heat Transfer to Starch Dispersion in a Cylindrical Container: Numerical Solution and Experiment," *Journal of Food Engineering* 36, 395–415 (1998).

41. S. K. Sastry, "Convective Heat Transfer Coefficients for Canned Mushrooms Processed in Still Retorts," *1984 Winter Meet. ASAE*, New Orleans, La., December 11–14, 1984.

ASHIM K. DATTA
Cornell University
Ithaca, New York

THERMODYNAMICS

Thermodynamics is the science of the dynamics of heat and the quantitative relationship between heat and other forms of energy. It is the basis for:

1. Analyzing and studying the transformation of energy from one form to another.
2. The use and availability of energy to do useful work.
3. The stability and equilibrium associated with chemical substances.

Thermodynamics relationships pertain to processes in which matter changes state or in equilibria in which matter remains fixed. The application of thermodynamic relationships are most frequently used in food processing operations that involve transferring heat and changing the state of materials.

Processing changes of state take place during heating and cooling, expanding and contracting, vaporizing and condensing, and when chemical reactions result from reactions in a food system. Since all forms of energy tend to change into heat, the quantitative measurement of temperature is a most important controlling factor in studying and controlling food systems.

There must always be a driving force or energy potential difference between two systems before a useful relationship can be realized. The end point of any process is reached when mechanical, physical, thermal, or chemical equilibrium is reached or when the two systems are disengaged. These concepts are embodied in the Laws of Thermodynamics, the first law being the basis of the science.

LAWS OF THERMODYNAMICS

There are four laws of thermodynamics which are developed into basic equations describing property relationships, energy balances, entropy balances, and mass balances. An excellent way of stating the four laws of thermodynamics can be demonstrated by visualizing three bodies, A, B, and C (1). A and C are identical rigid hot bodies at the same temperature and body B is a cold body below the temperature of A and C. In discussing the laws and the applications, especially in food processing, it is useful to visualize the transfer of heat between these bodies.

Zeroth Law

When bodies A and C are placed in contact with each other, no change will occur. This is a relatively recent law, added to the original three laws, to emphasize the fact that when two bodies or systems are at the same state of energy, no useful work can be obtained by putting the two systems in contact with each other. From this, it follows that the zeroth law also states that two systems in thermal equilibrium with a third are in thermal equilibrium with each other.

First Law

If A is placed in contact with B, there will be an increase in the internal energy of B equaling a decrease in the internal energy of A. The first law as originally stated is that the sum of all the energies in an isolated system is constant or that energy may be transformed from one form into another, but it cannot be created or destroyed.

This law is particularly important as applied to food processing in which the basis for operations is the transfer of mass and energy. Food processing involves heating, cooling, changing state (freezing and evaporating), adding chemicals that may cause internal energy changes, and irradiation.

Second Law

After A has been in contact with B, the initial conditions will never be restored without outside influence. The cooling of A and heating of B constitute a spontaneous, irreversible process resulting in loss of ability to do work. Simply stated the second law is that all systems tend to approach a state of equilibrium.

Third Law

It is impossible by any procedure, no matter how idealized, to reduce any system to the absolute zero of temperature in a finite number of operations. As in the case of the zeroth law, the third law has little practical application in determining energy relationships involving food processing. It is primarily applied to the physics of very low temperatures.

USING THERMODYNAMIC RELATIONSHIPS IN FOOD PROCESSING

Prior to discussing specific food processing operations, it is necessary to define the world or system in which the process takes place. That is, boundaries must be defined as a means of isolating the process from the surrounding environment. There is usually some interchange between a system and the surroundings but they do not affect the principal relationships within the system. For example, if a food is being heated in a retort the heat balance must account for the heat interchange between the source and the food plus allow for a heat loss through the insulated or lagged walls of the retort.

The most important thermodynamic applications reflected in food processing involve: (a) transferring mass within the processing environment or between phases in a process, (b) transferring heat to heat or cool a food and, (c) transferring heat to change the phase of a component in a food processing system.

Basic Relationships

In considering units of measurement, it is necessary to define base values from which one can compare changes. For example, when one is standing on a ladder, the height of 3 meters would be compared to the base value of zero, the floor. Otherwise 3 meters would have no meaning. Likewise, the base for measuring temperature, and thus being able to determine heat energy changes in a body, is absolute zero.

The heat content or enthalpy of a food and the change of this parameter is basic to processing. It is defined as:

$$H = E + pv \tag{1}$$

where E is the internal energy, p is the absolute pressure, and v is the volume. The changes in enthalpy and not the absolute values are important in food processing. Hence, at constant pressure, which is the case in most food processing:

$$\Delta H = \Delta E + p\Delta v \tag{2}$$

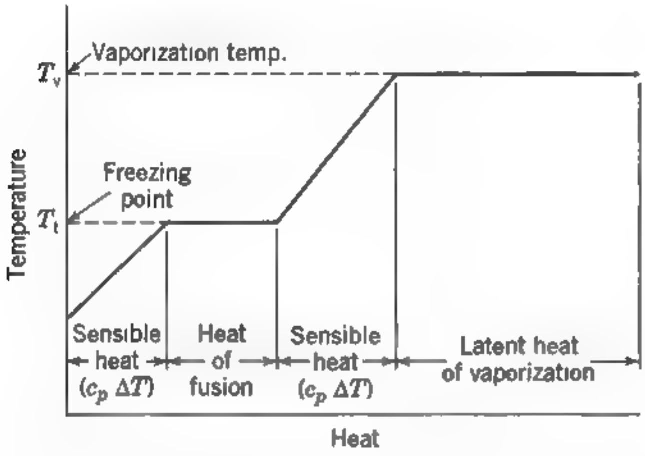


Figure 1. Heat stages when heating a food from a frozen solid to a vapor at constant pressure.

Table 1. Physical Constants of Water and Ice

Molecular weight	18.01534
	Phase transition properties
Melting point at 101.3 kPa (1 atm)	0.000°C
Boiling point at 101.3 kPa (1 atm)	100.000°C
Critical temperature	374.15°C
Triple point	0.0099°C and 610.4 kPa (4.579 mm Hg)
Heat of fusion at 0°C	6.012 kJ (1.436 kcal)/mol
Heat of vaporization at 100°C	40.63 kJ (9.705 kcal)/mol
Heat of sublimation at 0°C	50.91 kJ (12.16 kcal)/mol

Source: Refs. 5 and 6.

Often in a food process, there is no pv work so that the change in internal energy is equal to the change in enthalpy.

The specific heat of a food is the change in heat content per unit mass per unit temperature change. At constant volume

$$c_{v\ \text{Avg}} = \frac{Q_v}{T_2 - T_1} = \frac{E_2 - E_1}{T_2 - T_1} \tag{3}$$

and at constant pressure

$$c_{p\ \text{Avg.}} = \frac{Q_p}{T_2 - T_1} = \frac{H_2 - H_1}{H_2 - H_1} \tag{4}$$

In actuality, specific heat is not constant over significant temperature and pressure ranges and each of the above equations must be considered in integral form for precise definitions. However, over most ranges of temperature and pressure at which food process and auxiliary food operations take place, the specific heats can be considered constant and the average value is satisfactory.

Entropy (S) expresses the second law in precise mathematical terms. The first law is concerned with the accounting of the many kinds of energy that are involved in a given process whereas the second law is concerned with how available the energy of this system is for doing useful work. The concept of entropy is hard to visualize since it is not defined in terms which one can equate to normal physical entities such as a mass of material or a given amount of heat. It is expressed as follows:

$$dS = \frac{dq}{T} \tag{5}$$

The value of entropy is always positive and is actually a measure of the decline of the universe.

The total energy balance of a fluid flowing at steady state in a closed system (eg, food flowing through a pipe or chamber) accounts for the various changes that effect the thermodynamic state of the system between two given locations. An energy balance between the two sections of the fluid which is flowing is simplified for most food processing conditions in which the food is incompressible and the specific volume is essentially constant:

$$\begin{aligned} x_1 + E_1 + p_1v_1 + V_1^2/2g + W \\ = x_2 + E_2 + p_2v_2 + V_2^2/2g + F \end{aligned} \tag{6}$$

x is the potential energy due to height above reference plane, E is the internal energy, pv is the pv work done by the system, $V^2/2g$ is the kinetic energy of the flowing fluid, W is the work done by the system, and F is the friction loss due to contact with walls of the vessel. The total change in

Table 2. Useful Properties of Water

			Properties of saturated water			
T (°C)	ρ (kg/m^3)	C_p (J/kg · K)	k (W/m · K)	μ (Pa · s)	α (m^{2}s)	Pr
0	1002	4218	0.552	17.9×10^{-4}	1.31×10^{7}	13.06
20	1001	4182	0.597	10.1	1.43	7.02
40	995	4178	0.628	6.55	1.51	4.34
60	985	4184	0.651	4.71	1.55	3.02
80	974	4196	0.668	3.55	1.64	2.22
100	960	4216	0.680	2.82	1.68	1.74
120	945	4250	0.685	2.33	1.71	1.45
140	928	4283	0.684	1.99	1.72	1.24
160	910	4342	0.680	1.73	1.73	1.10
180	889	4417	0.675	1.54	1.72	1.00
200	867	4505	0.665	1.39	1.71	0.94
220	842	4610	0.652	1.26	1.68	0.89
240	816	4756	0.635	1.17	1.64	0.88
260	786	4949	0.611	1.08	1.58	0.87
280	753	5208	0.580	1.02	1.48	0.91
300	714	5728	0.540	0.96	1.32	1.02

Source: From Ref. 7. Reprinted with permission.

heat content of the system or the amount of heat transferred is, therefore:

$$Q = \Delta PE + \Delta E + \Delta pv + \Delta KE - W - F \qquad (7)$$

ΔPE is the change in potential energy, $x_2 - x_1$, ΔE is the change in internal energy, $E_2 - E_1$, Δpv is the change in pv work, $p_2v_2 - p_1v_1$, and ΔKE is the change in kinetic energy, $V_2^2/2g - V_1^2/2g$.

In considering food processes, there are many simplifications to the rigorous thermodynamic relationships. This is possible since most of the fluids are being heated or cooled and there are negligible changes in specific volume, and kinetic energy and no shaft work is being done by the flowing product. Therefore, since $\Delta E + \Delta pv = \Delta H$ = enthalpy, the work required for compressing a gas or the heat change in cooling or heating is equal to the enthalpy and equation 7 becomes:

$$W = Q = \Delta H \qquad (8)$$

Heating or Cooling Foods

The amount of heat required to raise or lower the temperature of a food is dependent on the specific heat. As previously discussed, over the temperature ranges encountered in food processing, the specific heat (c_p) can be considered constant and the heat added or lost (called sensible heat) is

$$Q = c_p(T - T) \qquad (9)$$

When a change in phase takes place, there is an additional heat input required called the "latent" heat. Hence, the latent heat of vaporization or the latent heat of fusion must be considered when a product is being evaporated or frozen. Although certain foods (eg, pure fats) have independent latent heat values for phase change, the latent heat of most foods is associated with water. When foods are dehydrated or frozen, water is normally the major component that determines the latent heat required for the phase change. In fact, water is the sole consideration in dehydration processes since that is the only component that is removed by a change of state.

Figure 1 is a generalized diagram showing the stages of heat change taking place as a product is heated and goes through phases changes as follows:

1. Heating in frozen stage to freezing point, $Q_1 = c_p\Delta T$ (c_p is that for the frozen product).
2. Changing from frozen to solid or liquid food, Q_2 = Latent heat of fusion.
3. Heating liquid or solid food, $Q_3 = c_p\Delta T$. (c_p is that for the nonfrozen product).
4. Changing to vapor phase, Q = Latent heat or vaporization

$$Q_T = Q_1 + Q_2 + Q_3 + Q_4$$

Values for thermodynamic properties of foods and other substances are available in many scientific handbooks. These give the thermal properties as related to temperature, pressure, and volume. Most frequently used are Tables giving the basic properties of water (Tables 1, Table 2) and saturated steam (Table 3). The base or reference point for published thermodynamic properties have been established by international agreements as $E = 0$ and entropy at 0.01°C.

NOMENCLATURE

c	specific heat of substance, J/(kg)(K) or Btu/(lb)(F).
c_p	specific heat at constant pressure, J/(kg)(K) or Btu/(lb)(F).
c_v	specific heat at constant volume, J/(kg)(K) or Btu/(lb)(F).

Table 3. Properties of Saturated Steam: Temperature Table (SI Units)

		Specific volume m^3/kg		Internal energy kJ/kg			Enthalpy kJ/kg			Entropy kJ/kg · K		
Temp. (°C) T	Press. (kPa) P	Sat. liquid v_f	Sat. vapor v_g	Sat. liquid u_f	Evap. u_{fg}	Sat. vapor u_g	Sat. liquid h_f	Evap. h_{fg}	Sat. vapor h_g	Sat. liquid s_f	Evap. s_{fg}	Sat. vapor s_g
0.01	0.6113	0.001 000	206.14	0.00	2375.3	2375.3	0.01	2501.3	2501.4	0.0000	9.1562	9.1562
5	0.8721	0 001 000	147.12	20.97	2361.3	2382.3	20.98	2489.6	2510.6	0.0761	8.9496	9.0257
10	1 2276	0.001 000	106.38	42.00	2347.2	2389.2	42.01	2477.7	2519.8	0.1510	8.7498	8.9008
15	1.7051	0.001 001	77 93	62.99	2333.1	2396.1	62.99	2465.9	2528.9	0.2245	8.5569	8.7814
20	2.339	0.001 002	57.79	83.95	2319.0	2402.9	83.96	2454.1	2538.1	0.2966	8.3706	8.6672
25	3.169	0.001 003	43.36	104.88	2304.9	2409.8	104.89	2442.3	2547.2	0.3674	8.1905	8.5580
30	4.246	0.001 004	32.89	125.78	2290.8	2416.6	125.79	2430.5	2556.3	0.4369	8.0164	8.4533
35	5 628	0.001 006	25.22	146.67	2276.7	2423.4	146.68	2418.6	2565.3	0.5053	7.8478	8.3531
40	7.384	0.001 008	19.52	167.56	2262.6	2430.1	167.57	2406.7	2574.3	0.5725	7.6845	8.2570
45	9.593	0.001 010	15.26	188 44	2248.4	2436.8	188.45	2394.8	2583.2	0.6387	7.5261	8.1648
50	12.349	0.001 012	12.03	209.32	2234.2	2443.5	209.33	2382.7	2592.1	0.7038	7.3725	8.0763
55	15.758	0.001 015	9.568	230.21	2219.9	2450.1	230.23	2370.7	2600.9	0.7679	7.2234	7.9913
60	19 940	0.001 017	7.671	251.11	2205.5	2456.6	251.13	2358.5	2609.6	0 8312	7.0784	7.9096
65	25 03	0.001 020	6.197	272.02	2191.1	2463.1	272 06	2346.2	2618.3	0.8935	6.9375	7.8310
70	31.19	0.001 023	5.042	292.95	2176.6	2469.6	292.98	2333.8	2626.8	0.9549	6.8004	7.7553
75	38.58	0.001 026	4.131	313.90	2162.0	2475.9	313.93	2321.4	2635.3	1.0155	6.6669	7.6824
80	47.39	0.001 029	3.407	334.86	2147.4	2482.2	334.91	2308.8	2643.7	1.0753	6.5369	7.6122
85	57.83	0.001 033	2.828	355.84	2132.6	2488.4	355.90	2296.0	2651.9	1.1343	6.4102	7.5445
90	70.14	0.001 036	2.361	376.85	2117.7	2494.5	376.92	2283.2	2660.1	1.1925	6.2866	7.4791
95	84.55	0.001 040	1.982	397.88	2102.7	2500.6	397.96	2270.2	2668.1	1.2500	6.1659	7.4159
	MPa											
100	0.101 35	0.001 044	1.6729	418.94	2087.6	2506 5	419.04	2257.0	2676.1	1.3069	6.0480	7.3549
105	0.120 82	0.001 048	1.4194	440.02	2072.3	2512.4	440.15	2243.7	2683.8	1.3630	5.9328	7.2958
110	0.143 27	0.001 052	1.2102	461.14	2057.0	2518.1	461.30	2230.2	2691.5	1.4185	5.8202	7.2387
115	0.169 06	0.001 056	1.0366	482.30	2041.4	2523.7	482.48	2216.5	2699.0	1.4734	5.7100	7.1833
120	0.198 53	0.001 060	0.8919	503.50	2025.8	2529.3	503.71	2202.6	2706.3	1.5276	5.6020	7.1296
125	0.2321	0.001 065	0.7706	524.74	2009.9	2534.6	524.99	2188.5	2713.5	1.5813	5.4962	7.0775
130	0.2701	0.001 070	0.6685	546.02	1993.9	2539.9	546.31	2174.2	2720.5	1.6344	5.3925	7.0269
135	0.3130	0.001 075	0.5822	567.35	1977.7	2545.0	567.69	2159.6	2727.3	1.6870	5.2907	6.9777
140	0.3613	0.001 080	0.5089	588.74	1961.3	2550 0	589.13	2144.7	2733 9	1.7391	5.1908	6.9299
145	0.4154	0.001 085	0.4463	610.18	1944.7	2554.9	610.68	2129.6	2740.3	1.7907	5.0926	6.8833
150	0.4758	0.001 091	0.3928	631.68	1927.9	2559.5	632.20	2114.3	2746.5	1.8418	4.9960	6.8379
155	0.5431	0.001 096	0.3468	653.24	1910.8	2564.1	653.84	2098.6	2752.4	1.8925	4.9010	6.7935
160	0.6178	0.001 102	0.3071	674.87	1893.5	2568.4	675.55	2082.6	2758.1	1.9427	4.8075	6.7502
165	0.7005	0.001 108	0.2727	696.56	1876.0	2572.5	697.34	2066.2	2763.5	1.9925	4.7153	6 7078
170	0.7917	0.001 114	0.2428	718.33	1858.1	2576.5	719.21	2049.5	2768.73	2.0419	4.6244	6.6663
175	0.8920	0.001 121	0.2168	740.17	1840.0	2580.2	741.17	2032.4	2773.6	2.0909	4.5347	6.6256
180	1.0021	0.001 127	0.194 05	762.09	1821.6	2583.7	763.22	2015.0	2778.2	2.1396	4.4461	6.5857
185	1.1227	0.001 134	0.174 09	784.10	1802.9	2587.0	785.37	1997.1	2782.4	2.1879	4.3586	6.5465
190	1.2544	0 001 141	0.156 54	806.19	1783.8	2590.0	807.62	1978.8	2786.4	2.2359	4.2720	6.5079
195	1.3978	0.001 149	0.141 05	828.37	1764.4	2592.8	829.98	1960.0	2790.0	2.2835	4.1863	6.4698
200	1.5538	0.001 157	0.127 36	850.65	1744.7	2595.3	852.45	1940.7	2793.2	2.3309	4.1014	6.4323
205	1.7230	0.001 164	0.115 21	873.04	1724.5	2597.5	875.04	1921.0	2796.0	2.3780	4.0172	6.3952
210	1.9062	0 001 173	0.104 41	895.53	1703.9	2599.5	897.76	1900.7	2798.5	2.4248	3.9337	6.3585
215	2.104	0.001 181	0.094 79	918.14	1682.9	2601.1	920.62	1879.9	2800.5	2.4714	3.8507	6.3221
220	2.318	0.001 190	0.086 19	940.87	1661.5	2602.4	943.62	1858.5	2802.1	2.5178	3.7683	6.2861
225	2.548	0.001 199	0.078 49	963.73	1639.6	2603.3	966.78	1836.5	2803.3	2.5639	3.6863	6.2503
230	2.795	0.001 209	0.071 58	986.74	1617.2	2603.9	990.12	1813.8	2804.0	2.6099	3.6047	6.2146
235	3.060	0.001 219	0.065 37	1009.89	1594.2	2604.1	1013.62	1790.5	2804.2	2.6558	3.5233	6.1791
240	3.344	0.001 229	0.059 76	1033.21	1570.8	2604.0	1037.32	1766.5	2803.8	2.7015	3.4422	6.1437
245	3.648	0.001 240	0.054 71	1056.71	1546.7	2603.4	1061.23	1741.7	2803.0	2.7472	3.3612	6.1083
250	3.973	0.001 251	0.050 13	1080.39	1522.0	2602.4	1085.36	1716 2	2801.5	2.7927	3.2802	6.0730
255	4.319	0.001 263	0.045 98	1104.28	1496.7	2600.9	1109.73	1689.8	2799.5	2.8383	3.1992	6.0375
260	4.688	0.001 276	0 042 21	1128.39	1470.6	2599.0	1134.37	1662.5	2796.9	2.8838	3.1181	6.0019
265	5.081	0.001 289	0.038 77	1152.74	1443.9	2596.6	1159.28	1634.4	2793.6	2.9294	3.0368	5.9662
270	5.499	0.001 302	0.035 64	1177.36	1416.3	2593.7	1184.51	1605.2	2789.7	2.9751	2 9551	5.9301
275	5.942	0.001 317	0.032 79	1202.25	1387.9	2590.2	1210.07	1574.9	2785.0	3.0208	2.8730	5.8938
280	6.412	0.001 332	0.030 17	1227.46	1358.7	2586.1	1235.99	1543.6	2779.6	3.0668	2.7903	5.8571
285	6.909	0.001 348	0 027 77	1253.00	1328.4	2581.4	1262.31	1511.0	2773.3	3.1130	2.7070	5.8199

Table 3. Properties of Saturated Steam: Temperature Table (SI Units) *(continued)*

		Specific volume m³/kg		Internal energy kJ/kg			Enthalpy kJ/kg			Entropy kJ/kg · K		
Temp. (°C) T	Press. (kPa) P	Sat. liquid v_f	Sat. vapor v_g	Sat. liquid u_f	Evap. u_{fg}	Sat. vapor u_g	Sat. liquid h_f	Evap. h_{fg}	Sat. vapor h_g	Sat. liquid s_f	Evap. s_{fg}	Sat. vapor s_g
	MPa											
290	7.436	0.001 366	0.025 57	1278.92	1297.1	2576.0	1289.07	1477.1	2766.2	3.1594	2.6227	5.7821
295	7.993	0.001 384	0.023 54	1305.2	1264.7	2569.9	1316.3	1441.8	2758.1	3.2062	2.5375	5.7437
300	8.581	0.001 404	0.021 67	1332.0	1231.0	2563.0	1344.0	1404.9	2749.0	3.2534	2.4511	5.7045
305	9.202	0.001 425	0.019 948	1359.3	1195.9	2555.2	1372.4	1366.4	2738.7	3.3010	2.3633	5.6643
310	9.856	0.001 447	0.018 350	1387.1	1159.4	2546.4	1401.3	1326.0	2727.3	3.3493	2.2737	5.6230
315	10.547	0.001 472	0.016 867	1415.5	1121.1	2536.6	1431.0	1283.5	2714.5	3.3982	2.1821	5.5804
320	11.274	0.001 499	0 015 488	1444.6	1080.9	2525.5	1461.5	1238.6	2700.1	3.4480	2.0882	5.5362
330	12.845	0.001 561	0.012 996	1505.3	993.7	2498.9	1525.3	1140.6	2665.9	3.5507	1.8909	5.4417
340	14.586	0.001 638	0.010 797	1570.3	894.3	2464.6	1594.2	1027.9	2622.0	3.6594	1.6763	5.3357
350	16.513	0.001 740	0.008 813	1641.9	776.6	2418.4	1670.6	893.4	2563.9	3.7777	1.4335	5.2112
360	18.651	0.001 893	0.006 945	1725.2	626.3	2351.5	1760.5	720.5	2481.0	3.9147	1.1379	5.0526
370	21.03	0.002 213	0.004 925	1844.0	384.5	2228.5	1896.5	441.6	2332.1	4.1106	0.6865	4.7971
374.14	22.09	0.003 155	0.003 155	2029.6	0	2029.6	2099.3	0	2099.3	4.4298	0	4.4298

Source: Adapted from Ref 8.

F	friction work, J/kg or Btu/lb.
H	enthalpy of product, kJ/kg or Btu/lb.
KE	kinetic energy, J/kg or Btu/lb.
m	mass, kg or lb.
p	pressure, Pa (N/m) or lb/in. (psi).
PE	potential energy, J/kg or Btu/lb.
Q	quantity of heat transferred, J/kg or Btu/lb.
T	temperature, °C or °F.
E	internal energy, J/kg or Btu/lb.
v	volume, m or ft or specific volume/unit mass, m/kg or ft/lb.
W	work, J/kg or Btu/lb.
Δ	change in a property (eg, temperature, enthalpy).

BIBLIOGRAPHY

1. J. J. Martin, *Thermodynamics Program, A.I.Ch.E. Teaching Aids*, American Institute of Chemical Engineers, New York, 1965.
2. E. L. Watson and J. C. Harper, *Elements of Food Engineering*, Van Nostrand Reinhold Company, New York, 1986.
3. D. R. Heldman, *Food Process Engineering*, AVI Publishing Company, Inc., Westport, Conn., 1977.
4. G. M. Pigott, compilation of classroom lecture notes and unit operations laboratory experiments over 28 years of teaching Food Engineering, University of Washington, Seattle, 1989.
5. F. Franks, ed., *Water—A Comprehensive Treatise*, 6 vols., Plenum Press, New York, 1972–1979.
6. R. C. Weast and M. J. Astle, eds., *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, Fla., 1981.
7. J. A. Duffie and W. A. Beckman, *Solar Engineering of Thermal Processes*, Wiley-Interscience, New York, 1980.
8. J. H. Keenan, F. G. Keyes, P. G. Hill, and J. G. Moore, *Steam Tables*, John Wiley & Sons, Inc., New York, 1969; Reprinted in G. J. Van Wylen and R. E. Sonntag, *Fundamentals of Classical Thermodynamics*, John Wiley & Sons, Inc., New York, 1978.

GENERAL REFERENCES

H. Cabezas, Jr., M. B. Kabiri, and D. C. Szlag, "Statistical Thermodynamics of Phase Separation and Ion Partitioning in Aqueous Two-Phase Systems," *Bioseparation* **1**, 227–233 (1990).

M. Certel and M. E. Ertugay, "The Thermodynamics of Water Activity in Foods," *Gida* **21**, 193–199 (1996).

A. Cesaro, "The Role of Conformation on the Thermodynamics and Rheology of Aqueous Solutions of Carbohydrate Polymers," *J. Food Eng.* **22**, 27–42 (1994).

G. Grasso, "Modellistic Description of Food Materials and Their Technologies, II: The Macrostructural Approach," *Industrie Alimentari* **35**, 923–932 (1996).

C. Kapseu et al., "Thermodynamics of Solid Formation in Cottonseed Oil," *J. American Oil Chemists' Society* **68**, 237–240 (1991).

H. Kumagai et al., "Application of Solution Thermodynamics to the Water Sorption Isotherms of Food Materials," *Bioscience Biotechnol. and Biochem.* **58**, 475–481 (1994).

H. Kumugai, A. Mizuno, and T. Yano, "Analysis of Water Sorption Isotherms of Superabsorbent Polymers by Solution Thermodynamics," *Bioscience Biotechnol. and Biochem.* **61**, 936–941 (1997).

M. Rutgers, K. D. Van, and H. V. Westerhoff, "Control and Thermodynamics of Microbial Growth Rational Tools for Bioengineering," *CRC Critical Rev. in Biotechnol.* **11**, 367–395 (1991).

V. A. Taran, O. G. Fedorov, and A. I. Pokatilov, "Mathematical Formulae for Determining the Dessication Thermodynamics of Chilled Foods by Means of Energy Entropy," *Kholodil'naya Tekhnika* **12**, 21–24 (1988).

George M. Pigott
University of Washington
Seattle, Washington

TOXICANTS, NATURAL

In addition to the many well-known major components (protein, fat, carbohydrate, and fiber) and trace nutrients (vitamins, minerals, and nonessential compounds), our food contains thousands of naturally present toxic compounds. Although these chemicals are in every meal we eat and are present in much greater quantities than residues of synthetic chemicals such as PCBs and pesticides, they have traditionally received relatively little attention compared to these well-known human-made chemicals. Furthermore, the mechanisms of action of natural toxins—metabolic activation, interaction with critical cellular macromolecules—are no different from those of synthetic toxins. In short, our bodies handle toxins similarly regardless of their origin. Many of the toxins presented here are known to cause or strongly suspected of causing cancer in laboratory animals and are therefore potentially carcinogenic in people. The human health risk posed by individual natural toxins varies considerably due to a variety of factors such as dose, inherent potency, variety of the diet, and presence of detoxifying factors. In any event, natural toxins pose a far greater health risk than do synthetic chemicals in our foods, despite the popular notion that "natural is good."

An issue of major concern is the relationship between diet and various human diseases, including cancer. As the single most important cancer variable to be identified from epidemiology studies, diet contributes to approximately 35% of the variation in cancer rates among individuals and populations in the United States. Other factors such as food additives, genetic predisposition, industrial pollution, and pesticide contamination play comparatively minor roles in human cancer rates. Excluding smoking, diet-related factors account for over 50% of all remaining cancer deaths in this country, or 150,000 food-related cancer deaths per year in the United States alone.

Natural toxicants are defined here as naturally occurring substances in plants or other food products that exert undesirable or unhealthy effects when they are consumed. Although not intended to be an exhaustive, all-inclusive discussion of natural toxins, this section will center on those that are particularly well studied and characterized or are of contemporary interest in food toxicology. The focus of this chapter will be not on bacterial toxins, but on three major categories of food toxicants: (*1*) toxins from plant or plant-derived foods, (2) mold-produced toxins (or mycotoxins), and (*3*) toxic substances created during cooking or other processing of the food (or induced toxins). An additional yet important category of induced toxicants is endogenous toxic substances that may appear inadvertently in genetically manipulated plant materials that result from efforts to alter plant quality. This latter category will also not be considered here.

Our food also contains natural chemicals that can counteract and thereby prevent the adverse effects of many natural and synthetic toxins. Though much more work on these chemopreventives is needed, the data suggesting that some plant foods can actually reduce the incidence of certain types of cancer thus far are very encouraging. Hundreds of animal and epidemiological studies have identified several foods or specific compounds that offer protection against the carcinogenic effects of a wide variety of natural and synthetic chemicals. A few compounds have been shown to actually reverse the carcinogenic process in animals. As might be imagined, the field of chemoprevention is one of the most exciting areas in nutritional toxicology and cancer research. The reader is encouraged to consult a review on food chemopreventives.

NATURAL PLANT TOXINS IN FOODS

The following is a survey of some of the most well studied and characterized plant toxins.

Allyl Isothiocyanates

Allyl isothiocyanates are a group of major naturally occurring compounds that confer the pungent flavor to foods such as mustard and horseradish, where it is present at about 50 to 100 ppm. These compounds are in *Brassica* vegetables such as broccoli and cabbage, and in cassava and other tropical staple foods, but at much lower concentrations. Normal dietary exposure to isothiocyanate-containing foods releases milligram amounts of isothiocyanates. Nominal processing steps (chopping, rinsing, milling) renders the food safe when wash water is discarded. In high doses, isothiocyanates are carcinogenic in rats but nonmutagenic in bacteria. Isothiocyanates do not occur in foods per se but occur as glucosinolate conjugates that are hydrolyzed when the plant releases enzymes as it is disturbed, such as during chopping, processing, or ingestion (Fig. 1). The major concern with isothiocyanates is their goitrogenic properties in that they inhibit binding of iodine in the thyroid gland. Because iodine is required for the formation of the critical thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), isothiocyanate-induced hyperthyroidism (goiter) mimics iodine deficiency. Hyperthyroidism is a physiological response as the thyroid attempts to compensate for reductions in both T_4 and T_3 production.

Endemic goiter is seen in geographical areas like India and Africa where consumption of poorly processed foods is coincident with iodine deficiency. Like many food toxins, allyl isothiocyanates are "double-edged swords" in that they have been shown to be chemopreventive in certain animal-testing protocols.

Canavanine

Despite their reputation as being the ultimate health food, alfalfa sprouts contain up to 15,000 ppm canavanine, an arginine analogue that can substitute for this amino acid in cellular proteins, thereby altering their function. Canavanine is also produced in other legumes such as the jack bean. Canavanine inhibits nitric oxide synthetase and induces heat-shock proteins in human cells *in vitro* (1). By virtue of its antimetabolic action, canavanine is under current consideration as an antitumor drug in combination with other antimetabolites such as 5-fluorouracil, but it has not yet been tested for carcinogenicity. Canavanine may cause autoimmune disorders such as lupus erythematosus in people (2). Primates fed alfalfa sprouts develop a severe toxic syndrome resembling human lupus.

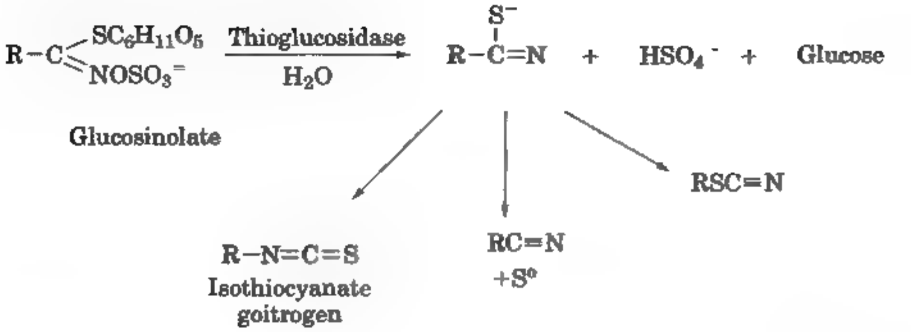


Figure 1. Mechanism of formation of isothiocyanate goitrogen from glucosinolates.

Cyanogenic Glycosides

Seeds from apples, apricots, cherries, peaches, pears, plums, and quinces as well as almonds, sorghum, lima beans, cassava, corn, yams, chickpeas, cashew nuts, and kirsch contain compounds that are toxic due to their release of free hydrogen cyanide, which occurs when the plant tissue is disturbed as during chopping, processing, or ingestion. These conditions initiate the hydrolysis of the glycoside by the action of β-glucuronidases and other enzymes naturally present in the plant tissue and in the intestinal lumen. Although acid also initiates this process, it doesn't appear to occur in the digestive tract to any great extent, despite the acid environment in the stomach. Hydrolysis by β-glucuronidases produce the sugar and a cyanohydrin, the latter spontaneously or enzymatically degrading to form free hydrogen cyanide (Fig. 2). There are several such cyanogenic glycosides, of which linamarin, amygdalin, and dhurrin are examples (Fig. 2). In the 1970s, amygdalin, as laetrile, was a fad remedy touted as a cure and/or preventive for cancer and other ailments. Underground "clinics" briefly flourished where patients were given large quantities of amygdalin or amygdalin-rich seeds and nuts.

Cyanide is one of the most acutely toxic chemicals. It binds to and inactivates heme enzymes, specifically mitochondrial cytochrome aa_3 oxidase, resulting in an acute, life-threatening anoxia. The two-step therapy is initiated with sodium nitrite, which induces methemoglobinemia, permitting the release of cyanide from heme proteins, followed by sodium thiosulfate, which acts as a substrate for rhodanese, an endogenous hepatic enzyme that catalyzes the conversion of free cyanide to the less toxic thiocyanate.

Cases of acute human poisoning from the cyanide released from certain varieties of lima beans, cassava, and bitter almonds are a regular occurrence (3). Due to the importance of cassava as a subsistence crop in Africa and South America, cyanogenic glycosides in that food probably represent the greatest health risk. High-cyanide varieties of cassava, distinguished by their bitter taste, may contain over 600 ppm cyanide on a dry weight basis, whereas "sweet" varieties contain significantly less. Processing steps such as sun drying, soaking, boiling, and fer-

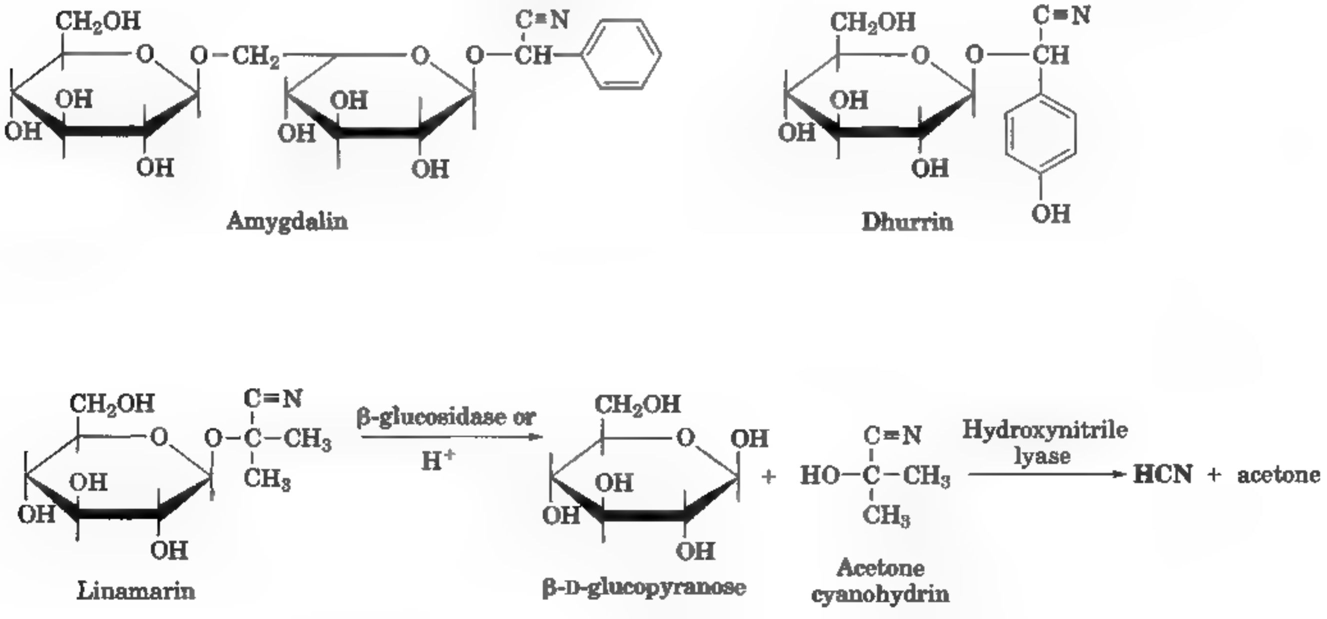


Figure 2. Natural cyanogenic glycosides and mechanism of formation of hydrogen cyanide.

menting render the food safe by eliminating most of the cyanide (4). In addition to regular cases of human deaths, cyanogenic glycosides in cassava may be responsible for birth defects, endemic goiter, and konzo, an upper myelopathic motor neuron disease endemic to East Africa (5). Cyanogenic glycosides have also been implicated as a causative agent of diabetes. The risk associated with cyanide poisoning due to cassava is negligible in the United States because cassava-based foods (such as tapioca pudding) are highly processed and rarely eaten here.

Hydrazines and Other Toxins in Edible Mushrooms

Commercial mushrooms, such as the cultivated mushroom (*Agaricus bisporus*), the shiitake mushroom (*Cortinellus shiitake*), and the false morel (*Gyromitra esculenta*), all contain substantial amounts of compounds in the hydrazine family (Fig. 3). Many hydrazines are potent liver toxins and animal carcinogens. Commonly found in concentrations of 500 ppm in mushrooms, *N*-methyl-*N* formylhydrazine is a lung carcinogen in mice and is also carcinogenic in hamsters. People eating a 100-gram serving of mushrooms (therefore ingesting 50 mg *N*-methyl-*N* formylhydrazine) get nearly the same dose (on a per-kilogram-body-weight basis) that will cause cancer in mice on sustained daily exposure.

Shiitake and *Agaricus* mushrooms contain up to 3000 ppm agaritine, a metabolic product of which (a diazonium derivative) is a potent carcinogen and a mutagen. The major carcinogenic hydrazine in the false morel, gyromitrin (acetaldehyde-*N*-methyl-*N*-formylhydrazone), is also present in similar concentrations. Other carcinogenic hydrazines include *p*-hydrazinobenzoate (present in *A. bisporus* at 10 ppm) and 4-(hydroxymethyl) benzenediazoate (HMBD), the latter shown to induce DNA strand breaks, presumably through a carbon-centered free-radical intermediate, a possible mechanism of the carcinogenic action of hydrazines in general (6). Another carcinogenic hydrazine, methylhydrazine, is present in smaller concentrations (14 ppm).

Whole mushrooms have been shown in numerous studies to cause cancer in laboratory animals, but whether they are a significant cause of cancer in people is uncertain. Rats fed a diet of whole *A. bisporus* mushrooms (30% of total diet) did not have a significant increase in tumors compared to control animals.

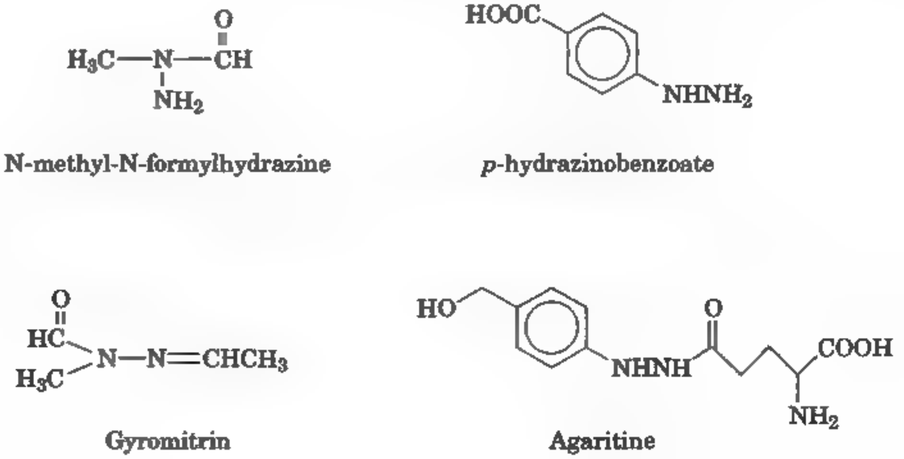
Toxic Substances in Spices and Flavoring Agents

Safrole, estragole, myristicin, β-asarone, piperine, and isosafrole (Fig. 4) are closely related alkenylbenzenes found in many spices, essential oils, and herbs. They are also present, in much lower levels, in parsnips, parsley, and sesame seeds. All are weak to moderate rodent hepatocarcinogens.

Oil of sassafras (*Sassafras albidum*), which was once used to flavor root beer, contains approximately 85% safrole. The oil has been banned as a flavor additive since 1960 but Safrole is also a minor, natural component of nutmeg, mace, star anise, cinnamon, and black pepper. Safrole is also found in sassafras tea. Sassafras bark is an ingredient in filé powder used to make gumbo, a spicy Cajun stew. A procarcinogen, safrole is activated by endogenous enzymes to a DNA-reactive intermediate that forms covalent adducts with guanine *in vitro* (7). Estragole, a related aromatic flavor agent, is found in tarragon, basil, and fennel and is likewise a weak carcinogen.

Isosafrole, a component of ylang-ylang (*Cananga odorata*) oil, a flavorant and scent, is carcinogenic in mice. Many of these alkenylbenzenes interact with cytochrome P-450 (CYP)–mediated metabolism. For example, both isosafrole and safrole are powerful inducers of CYP 1A enzymes. Safrole and isosafrole also inhibit CYP 2E1 enzymes and in so doing protect against carbon tetrachloride liver toxicity in mice. β-asarone is a major component of oil of calamus (derived from the *Acorus calamus* root, which is a folk remedy for indigestion) and was once used to flavor vermouth and bitters. It is an intestinal carcinogen in rats.

Myristicin is a major flavor component of nutmeg, which is derived from the dried seed of the *Myristica fragrans* tree. The world's principal commercial supply of nutmeg is grown in the Malay peninsula. Approximately 2% of nutmeg is myristicin, which is present in the steam-distilled volatile oil produced from the dried seeds. Mace, a closely related spice, is derived from the outer coating of the seed. Myristicin is also found in black pepper, parsley,



N-methyl-N-formylhydrazine

p-hydrazinobenzoate

Gyromitrin

Agaritine

Figure 3. Representative hydrazines in mushrooms.

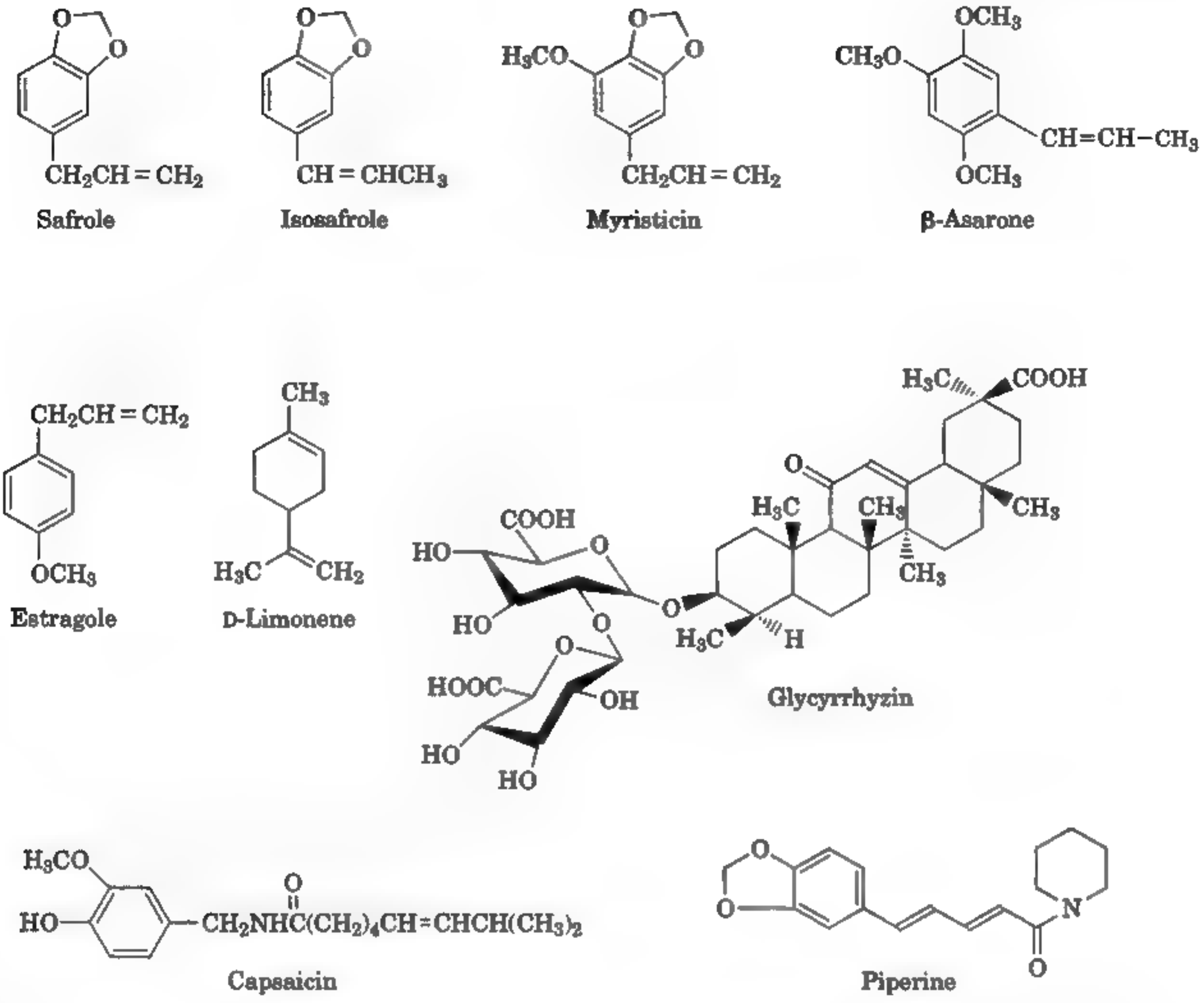


Figure 4. Toxins in spices and flavoring agents.

celery, dill, and carrots. Though not thought to be carcinogenic, large amounts of nutmeg, equivalent to two whole nutmeg seeds (ca 15 g), are intoxicating and allegedly hallucinogenic, and large doses cause undesirable side effects such as tachycardia, flushed skin, and dry mouth. Because pure myristicin is not as hallucinogenic as nutmeg, other components in nutmeg are believed to contribute to its reported psychoactive properties.

Piperine, an alkaloid present in high concentrations (10%) in black pepper (*Piper nigrum* and other spp.), is largely responsible for the pungent "bite" of this condiment. Powdered *P. cubeba* berries are added to cigarettes and smoked as a remedy for throat irritation, and oil derived from these berries is added to some throat lozenges. Reports of the cancer-causing ability of this compound are conflicting. Extracts of black pepper caused cancer in mice at several sites in skin painting tests, whereas orally injected piperine did not (8). Furthermore, piperine is not mutagenic in a number of *in vitro* screening assays. Under appropriate conditions, however, piperine is chemically converted to potentially carcinogenic intermediates. In the presence of nitrite, piperine is nitrosated to form highly mutagenic nitrosamine intermediates *in vitro* that may have potential carcinogenic activity. Like the related alkenylbenzenes, piperine also affects CYP expression and activity. Piperine specifically inhibits CYP 2E1 while specifically inducing the expression and activities of CYP 1A and 2B (9).

The ingredient responsible for the pungency of red and yellow chili peppers (*Capsicum frutescens*, *C. conoides*, and *C. annum*) is capsaicin, which represents up to 0.5% of the weight of the fruit. Aerosol capsaicin sprays are popular dog repellents for mail carriers. Topical creams containing capsaicin (0.025%) are commercially available as analgesics. Although its pain-relieving qualities are debatable, capsaicin has been shown to cause a local depletion of substance P, an endogenous neuropeptide known to transmit pain impulses. Thus, even though the physiological conditions causing pain may persist, capsaicin prevents pain impulses from reaching the brain.

Capsaicin may be a weak carcinogen. It is a bacterial mutagen in the Ames test and causes benign digestive tract adenomas in mice given lifelong dietary exposure at 0.03125%. Intraperitoneal injections of capsaicin causes the formation of sister-chromatid exchanges and micronuclei in mice. Sister-chromatid exchanges and micronuclei are genotoxic endpoints presumably associated with cancer risk. One possible toxic mechanism is that CYP 2E1 converts capsaicin to an active phenoxy radical intermediate that has the potential for alkylating tissue macromolecules such as DNA and protein (10).

Glycyrrhyzin is a saponin-like glycoside found in licorice (*Glycyrrhiza glabra*). Licorice is one of the oldest traditional medicines; it has been used as an expectorant, a flavoring agent (also used to mask the bitter taste of medicines), and a demulcent. Reportedly, licorice was used by the Sumerians as far back as 4000 B.C., and pieces of licorice root were found in King Tut's tomb. The one caveat to the many benefits of licorice is that ingesting large amounts of it promotes hypertension.

Glycyrrhizin is thought to be responsible for the hypertensive properties of licorice. The mechanism is through inhibition of the enzyme 11β-hydroxysteroid dehydrogenase, which acts as a protective modulator by metabolizing receptor-active glucocorticoids such as cortisol to 11-keto derivatives (eg, cortisone) that are not receptor agonists. Inhibition of this enzyme mimics aldosterone excess, which results in severe sodium retention, loss of potassium (hypokalemia), and hypertension (11). Licorice binges reportedly have been responsible for fatal episodes of acute hypertension in people. Consequently, people with heart problems or hypertension should avoid licorice; as little as 100 to 200 g/day can cause persistent, heightened mineralocorticoid activity. Recently, a healthy Salt Lake City man was hospitalized with congestive heart failure and pulmonary edema for eating 21/2 lb of licorice in 3 days.

A major component of citrus oils (orange, grapefruit, lemon, and others) is d-limonene, which is also found in much lower amounts in other fruits and vegetables. Citrus peel oil can contain as much as 95% d-limonene. Citrus oils or pure d-limonene is the major constituent in flavoring agents and/or fragrances in perfumes and soaps and in a variety of foods such as ice cream, soft drinks, baked goods, gelatin, chewing gum, and puddings. It is also the active ingredient in "natural" citrus-based degreasing solvents and in insect repellents. d-limonene is specifically toxic to the kidneys of male rats. d-limonene binds specifically but reversibly to α_{2u}-globulin, which is the major low-molecular-weight protein produced by the renal proximal tubules and hence excreted in the urine of the male rat. Female rats excrete much less α_{2u}-globulin. Accordingly, male rats that do not excrete α_{2u}-globulin (NBR strain) do not exhibit nephrotoxicity following d-limonene treatment.

Some studies indicate that d-limonene causes renal tumors in rodents. When administered orally, d-limonene induced renal adenomas and carcinomas in male rats but not in mice. Oral d-limonene was also shown to significantly promote the development of N-nitrosoethylhydroxyethylamine-induced renal tumors in male rats. However, the toxicity and carcinogenicity of d-limonene appears to be absolutely species- and gender-specific due to the specific binding of this natural compound with α_{2u}-globulin. Because humans do not excrete α_{2u}-globulin, d-limonene is not thought to be harmful to people.

Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids (PAs) are common plant toxins produced by more than 200 species of flowering plants, from genera such as *Senecio*, *Crotalaria*, and *Cynoglossum*. They are often present at very high concentrations—as much as 5% of the plant's dry weight. PA-containing plants pose significant health hazards to people who consume some kinds of "natural" herbal teas and traditional folk remedies and those who eat grain-based foods contaminated with PA-containing plant parts. Some PAs have been investigated in clinical trials for their anticancer potential.

These chemicals are acutely and chronically hepatotoxic, and many are carcinogenic, mutagenic, and teratogenic. PAs are derivatives of a necine base like retronecine, otonecine, or heliotriine esterified to various necic acid substituents (Fig. 5). PAs are activated by CYPs primarily of the 3A4 subfamily to reactive bifunctional pyrrolic electrophiles that form covalent cross-links to a variety of cellular nucleophiles, such as DNA and proteins. Pyrrolic intermediates then reportedly from electrophilic carbonium ions at atoms 7 and 9 and cross-link cellular nucleophiles at these positions. Cytochrome P450s also convert PAs to the less toxic and more easily excreted N-oxides that do not interact with cellular constituents (Fig. 6). Accordingly, animals that metabolize PAs to produce proportionally more N-oxides (such as sheep) appear to be relatively resistant to the toxic effects of PAs compared to animals that produce more of the pyrrole (rats and horses). Other hydrolysis reactions may also occur that decrease the toxicity of PAs.

DNA cross-links are probably a critical event in PA bioactivity in that the cytotoxic, antimitotic, and megalocytic activity of PAs closely corresponds with the formation of cross-links *in vitro*. PAs form both DNA interstrand and DNA–protein cross-links in equal amounts *in vitro* (12). Structure-activity studies have revealed that the presence of a continuous macrocyclic diester and α,β-unsaturation are important structural determinants for DNA cross-link formation (13). Thus PAs like senecionine are more potent cross-linkers than monocrotaline, which is more potent than open diesters such as latifoline and heliosupine. Of those examined, the simple necine retronecine is the least active cross-linker. The pattern of proteins cross-linked by PAs is similar to those cross-linked by other bifunctional compounds, such as *cis*platinum and mitomycin C. Actin was recently found to be the major protein involved in the PA-induced cross-links in mammalian cells (14).

Petasitenine is found in *Petasites japonicus*, a medicinal herb used as an expectorant and cough suppressant. The flower stalks of this herb are used as a food or herbal remedy. When incorporated into the diet, dried stalks are hepatocarcinogenic to rats. Purified petasitenine is also hepatocarcinogenic in rats as well as mutagenic in bacteria.

Coltsfoot (*Tussilago farfara*) is a common herb that has been used for centuries in Europe and Asia as a medicine for coughs and bronchitis. (*Tussilago* is the ancient Roman term for "cough suppress.") The plant contains the PA senkirkine at concentrations as high as 150 ppm, as well as high concentrations of senecionine, another very hepatotoxic (and carcinogenic) PA. Again, both the dried buds of coltsfoot (when ground and mixed in the diet) and purified senkirkine or senecionine cause liver tumors in rats, and both are bacterial mutagens.

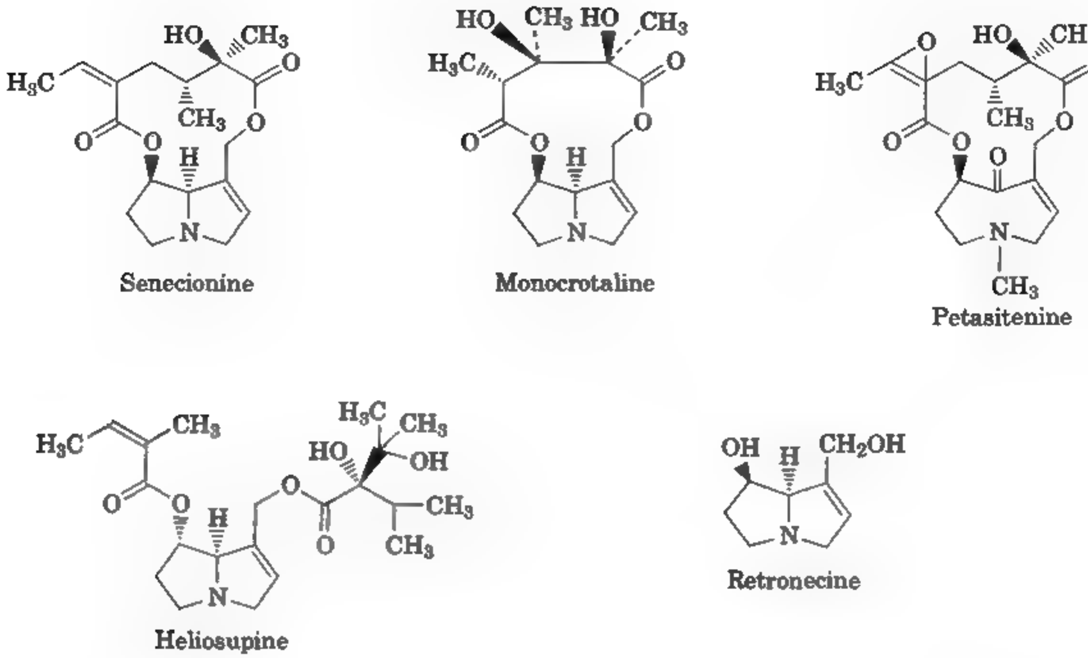


Figure 5. Five representative pyrrolizidine alkaloids.

Human intoxication by PA-containing plants is well recognized and reported in the medical literature and is endemic in Jamaica, India, and parts of Africa. Liver cirrhosis, veno-occlusive disease, and liver cancer are linked to consumption of PA-containing plants. Hispanic and Native American populations in the western and southwestern United States are at high risk for PA intoxications due to their traditional widespread use of herbs, occasional lack of confidence in traditional medicine, and, more commonly, lack of access to medical care.

Comfrey (*Symphytum officinale*) is a nearly universal herb commonly sold not only in health food stores and by herbalists but also in supermarkets. Since ancient Greek and Roman times, both leaves and roots have been used to make teas and compress pastes to treat a variety of external and internal diseases, such as wounds, skin disorders, and respiratory diseases. Numerous vegetarian recipes call for comfrey leaves in the preparation of soufflés, salads, and breads. Comfrey leaves and roots contain up to 0.29% of PAs such as intermedine, lycopsamine, symphytine, and others. Diets containing powder from dried leaves and roots cause liver tumors in rats. Additionally, these pure PAs are animal carcinogens and bacterial mutagens. Several cases are cited in the medical literature of comfrey-related intoxications in people. The well-known toxicity and carcinogenicity of comfrey is such a significant cause for concern that the governments of Australia, Canada, Great Britain, and Germany either restrict comfrey's availability or have banned its sale entirely. The U.S. Food and Drug Administration (FDA) has not yet acted to restrict the sale of PA-containing foods.

Substances in Bracken Fern

Bracken fern (*Pteridium aquilinum*, *P. esculentum*, and others) is widely used as greens or in salads in places like New Zealand, Australia, Canada, the United States, and especially Japan. It is also a forage plant for sheep and cattle. Veterinarians first noticed severe toxicity—bladder cancer, bone marrow depression, severe leukemia, thrombocytopenia, and a hemorrhagic syndrome—in livestock grazing on this plant. Bracken is a potent bladder, lung, and intestinal carcinogen in rodents. Lactating cows fed bracken fern produced milk that was carcinogenic to rats, showing that human exposure may also occur through cow's milk. Human consumption of bracken fern has been linked to an increased incidence of esophageal cancer in Japan. Ptaquiloside, potent norsesqiterpenoid glucoside (Fig. 7), often present at up to 1.3% dry weight, is thought to be the major carcinogen in bracken. Like other natural carcinogens, ptaquiloside alkylates DNA at codon 61 in the *Ha*-ras oncogene (15).

Solanum Alkaloids

Members of the family Solanaceae contain a variety of toxic steroidal glycoalkaloids. Potatoes (*Solanum tuberosum*) are an important food staple in many parts of the world, and under certain conditions they produce a variety of glycoalkaloids. Concentrations of solanum alkaloids vary considerably between species and strain of potatoes, but more important factors include wound damage, fungal or bacterial infection, exposure to light, and whether the potatoes have sprouted. The major glycoalkaloids are α-solanine and α-chaconine (Fig. 8), which may be present at concentrations over 100 ppm. Like physostigmine, solanine and chaconine are potent inhibitors of the enzyme acetylcholine esterase. Higher amounts of α-solanine and α-chaconine are present in the potato greens (tops). Healthy potatoes contain negligible amounts of these toxins.

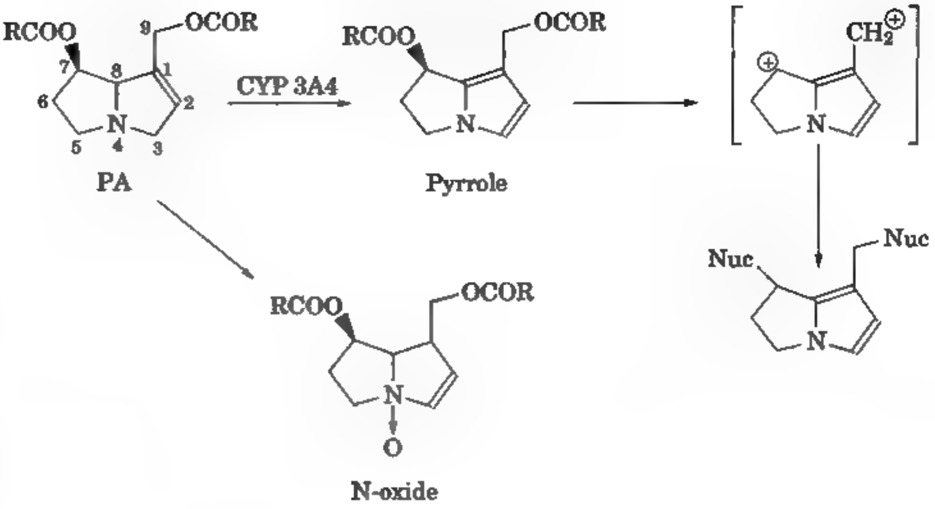
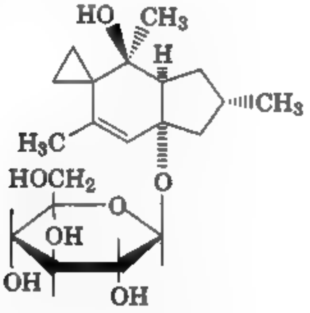


Figure 6. Metabolic formation of pyrroles and N-oxides from pyrrolizidine alkaloids. Nuc = cellular nucleophile such as glutathione, DNA, or protein.



Ptaquiloside

Figure 7. Chemical structure of the bracken carcinogen ptaquiloside.

Episodes of human poisoning by green potatoes have been documented. Fatalities from solanum alkaloid are rare, but gastrointestinal symptoms—gastric pain, weakness, nausea, vomiting, and labored breathing—that are consistent with acetylcholinesterase inhibition are more common. These symptoms have been duplicated in clinical trials with human volunteers. Studies have indicated that the acetylcholinesterase inhibitory activity of solanine is probably insufficient to cause these toxic effects, which are probably due to the combined toxicity of solanine with other cholinesterae inhibitors in the potato, such as chaconine.

Most cases of human poisoning and deaths have occurred in Europe, but they are occasionally seen in the Western Hemisphere. Livestock fed damaged potatoes or peel, greens, or trim are occasionally poisoned. A small number of studies in which animals are fed toxic doses of blighted potatoes or pure glycoalkaloid have indicated that these compounds may have weak teratogenic activity. For example, solanine and chaconine, and their aglycone derivative solanidine, induced craniofacial malformations (exencephaly, encephalocele, and anophthalmia) in Syrian hamsters (16). In that study, solanidine was a much stronger teratogen than solanine and chaconine, which were classified as weakly teratogenic. The teratogenic and embryotoxic effects of solanine and chaconine appear to be synergistic (17).

Caffeic Acid and Chlorogenic Acid

High concentrations (often over 1,500 ppm) of caffeic acid and its quinic acid conjugate, chlorogenic acid (Fig. 9), occur in an extremely wide range of vegetables (eg, lettuce, potatoes, radishes, and celery), fruits (eg, grapes, berries, eggplant, and tomatoes), and seasonings (eg, thyme, basil, anise, caraway, rosemary, tarragon, marjoram, sage, and dill). Coffee is especially rich in caffeic and chlorogenic acid, as well as other compounds, such as caffeine. A cup of coffee contains about 190 mg of chlorogenic acid. Other minor conjugates of caffeic acid also exist.

Chlorogenic acid is hydrolyzed in the gastrointestinal tract to caffeic and quinic acids. In people, caffeic acid is metabolized to the o-methylated derivatives ferulic, dihydroferulic, and vanillic acids and *meta*-hydroxyphenyl derivatives, which are excreted in the urine. Caffeic acid inhibits 5-lipoxygenase, which is a key enzyme in the biosynthesis of various eicosanoids such as leukotrienes and thromboxanes. These eicosanoids are mediators of a wide variety of physiological and disease states and are involved in immunoregulation, asthma, inflammation, and platelet aggregation. At high doses (2% in the diet), caffeic acid causes forestomach squamous cell papillomas and carcinomas in both sexes of rats and mice, renal tubular cell hyperplasia in male rats and female mice, and alveolar type II cell tumors in male mice (18). Chlorogenic acid is a bacteria mutagen but has not been tested for carcinogenicity.

Coumarin and Furocoumarin

Coumarin (Fig. 9) is a natural anticoagulant found in a variety of plant foods such as cabbage, radish, and spinach and in flavoring agents such as lavender and sweet woodruff (*Asperula odorata*), the latter an essential herb in making German May wine. Coumarin is found in herb teas based on tonka beans (*Dipteryx odorata*) and sweet clover

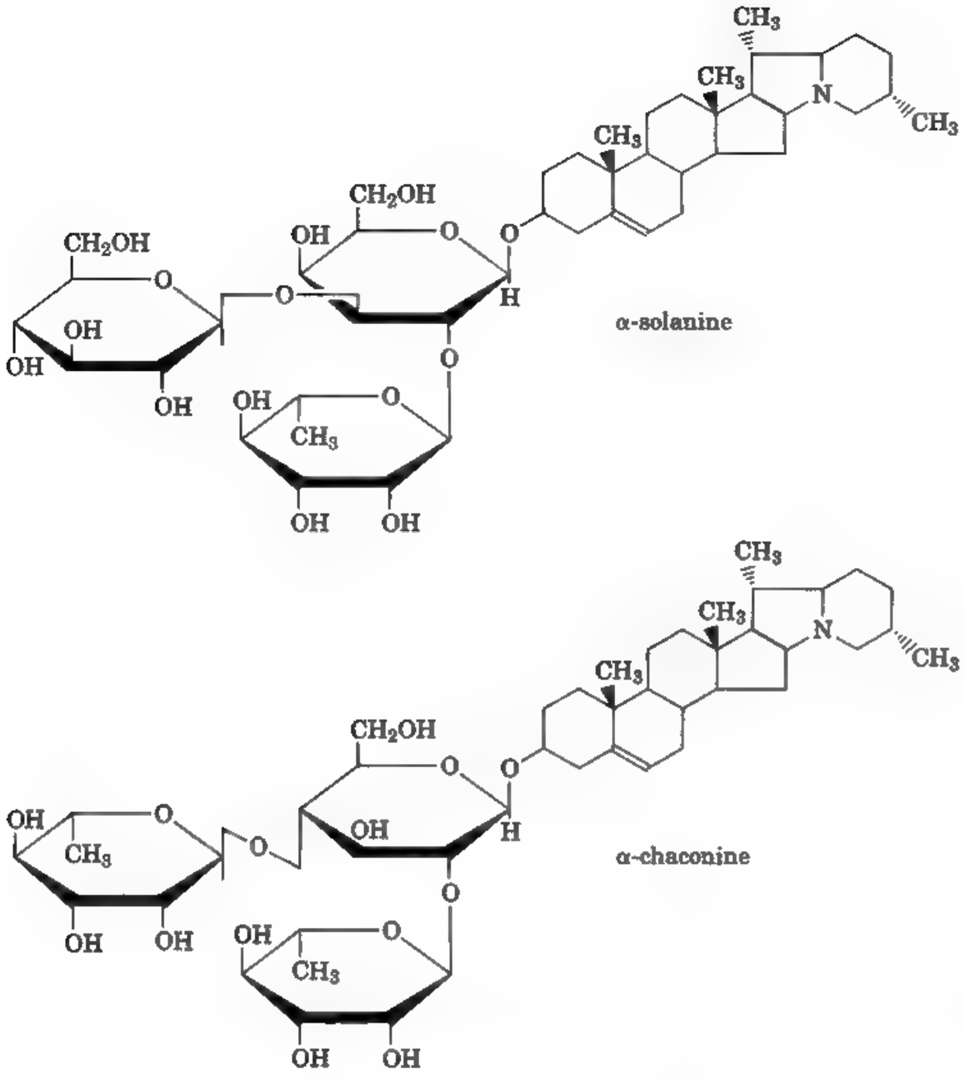


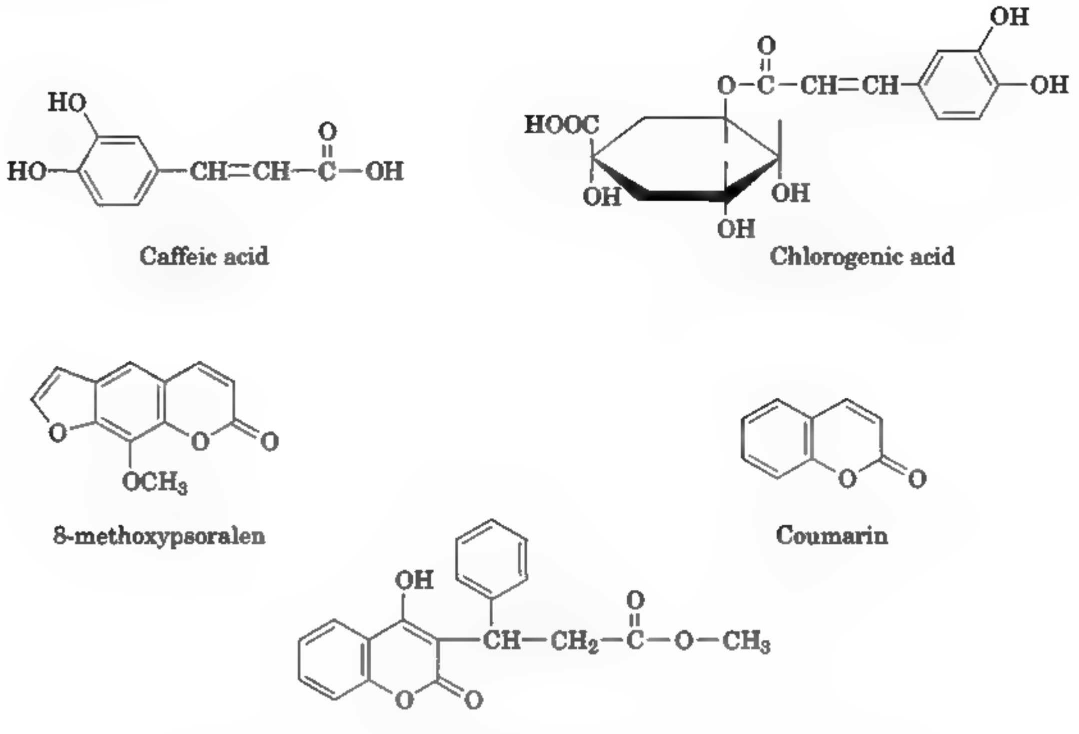
Figure 8. Acetylcholinesterase inhibitors in potatoes—α-solanine and α-chaconine.

(*Melilotus albus* and *M. officinalis*) called melilot. (In fact, the name coumarin originates from *coumarou*, the Carribean name for tonka beans.) Purified coumarin was once used as a food additive but was banned by the FDA after it was discovered that high doses caused liver damage in test animals. Coumarin has also been reported to cause bile duct carcinomas in rats. The anticoagulant action of coumarin is based on its interference with the action of vitamin K in the synthesis of clotting factors II, VII, IX, and X. The anticoagulant nature of coumarin was discovered when cows grazing on sweet clover developed bruising and internal bleeding. Shortly after it was found that rodents (which were used to bioassay for the then-unknown toxic principle in clover) were extremely susceptible to clover, the newly isolated 3-(α-acetonylbenzyl)-4-hydroxycoumarin, known as warfarin (a named derived from the acronym for Wisconsin Alumni Research Fund), was developed as a rodent bait. Known as coumadin, warfarin is also used in human medicines as a blood-thinning agent and to prevent the formation of blood clots.

Psoralens are a group of phototoxic furocoumarins widespread in a number of plant families such as Apiaceae (formerly Umbelliferae—celery and parsnips), Rutaceae (eg, bergamot, limes, cloves), and Moraceae (eg, figs). Celery contains 100 ppb psoralens, whereas parsnips contain approximately 40 ppm. When activated by sunlight, psoralens are mutagenic, presumably due to their ability to form interstrand and protein cross-links with DNA. Many members of this chemical family are carcinogenic as well, including 5-methoxypsoralen and 8-methoxypsoralen (also called methoxsalen and xanthotoxin, respectively, Fig. 9). Methoxsalen, in addition to forming DNA cross-links, causes a specific mutation in the tumor suppressor gene p53. Dietary exposure to psoralens is probably not a significant health risk; however, the margin of safety is thought to be narrow. Human volunteers who ingested 300 g of celery roots (with a total phototoxic furocoumarin content of 28 ppm) experienced no skin reactions after UVA exposure, and the blood levels of psoralen, methoxsalen, and 5-methoxypsoralen were below the analytical detection limit.

MYCOTOXINS

Mycotoxins are a diverse group of mold-produced chemicals that elicit a wide range of toxic responses in animals



Caffeic acid

Chlorogenic acid

8-methoxypsoralen

Coumarin

Warfarin

Figure 9. Compounds in coffee (chlorogenic and caffeic acid) and coumarins.

and humans. Pre- or postharvest contamination of various food crops by mycotoxigenic fungi is a common problem; approximately 25% of the world's food supply is contaminated by mycotoxins annually. The severity of mycotoxin contamination of agricultural commodities varies from year to year, depending on factors such as excessive moisture in the field and in storage, temperature extremes, humidity, drought, variations in harvesting practices, and insect infestation. Although the actual resultant economic loss to agriculture is difficult to determine with accuracy, it is considerable.

Many mycotoxins have been implicated in outbreaks of human diseases. Some are potent animal and presumed human carcinogens. In domestic animals, such as dairy cattle, swine, and poultry, mycotoxin contamination is known to reduce growth efficiency, lower feed conversion, lower reproductive rates, impair resistance to infectious diseases, reduce vaccination efficacy, and induce pathologic damage to the liver and other organs. For these reasons, mycotoxins pose a major threat to public and animal health. A few classes of mycotoxins that are problems in foods will be considered here.

Aflatoxin B_1

Aflatoxin B_1 (AFB_1) represents a group of potent mycotoxins produced by strains of the filamentous fungus *Aspergillus flavus* and *A. parasiticus*. Of the *Aspergillus* mycotoxins, AFB_1 has generated the greatest concern and has stimulated the most research because of its extreme toxicity and its widespread occurrence in staple foods and feeds (such as corn, peanuts, and cottonseed). For these reasons, the U.S. FDA regulates AFB_1 in foods. The current action level, the concentration above which the commodity is condemned and discarded, is 20 ppb of total aflatoxins. This regulatory value was established in the 1960s, in large part from the analytical detection limits at that time. The action level for the major AFB_1 metabolite present in milk and milk products, aflatoxin M_1 (AFM_1), is 0.5 ppb in fluid milk. Other regulatory guidelines for AFB_1 include 20 ppb in corn for dairy cows, 300 ppb in corn for finishing beef cattle and swine, and 100 ppb for breeding stock. Permissible AFB_1 concentrations in cottonseed for beef cattle, swine, and poultry is 300 ppb. These regulatory concentrations generally preclude detectable AFB_1 in the various products from these animals. Worldwide, established tolerances for AFB_1 in animal feeds range from 10 to 600 ppb.

Prevention of *Aspergillus* infection in foods and feeds is the most desirable method of reducing contamination. Despite the best agricultural practices, however, AFB_1 contamination is mostly unavoidable. Thus, several methods of reducing postharvest product contamination have been developed. Some of these methods involve early identification and segregation of grossly contaminated kernels of corn or peanuts, or electronic devices to identify and reject grains that exhibit fluorescence due to AFB_1. Ammoniation results in nearly complete elimination of aflatoxins and associated toxicity in commodities, and it is suitable for treating large batches of product (19). This method has not yet been approved by the FDA for interstate shipments, but it is in use in some states. Another experimental strategy is the use of inorganic adsorbent feed additives that prevent absorption of mycotoxins in animals. Hydrated sodium calcium aluminosilicate (HSCAS), an FDA-approved anticaking agent, significantly reduces AFB_1 bioavailability as well as many of its specific toxic effects in pigs (20).

The many toxic effects of AFB_1 are initiated by its conversion, principally by hepatic and extrahepatic microsomal CYPs, to a variety of metabolites (Fig. 10). The reputed carcinogenic intermediate is the AFB_1-8,9-epoxide. Presumably because of its extreme reactivity (it has a half-life of approximately 0.5 s), the AFB_1-8,9-epoxide has been isolated only indirectly from biological systems as adducts of glutathione (GSH), DNA bases, or other macromolecules. Most other metabolic products are less toxic than parent AFB_1, the most prevalent of which is AFM_1, so named for its appearance in the milk of dairy cows that consume AFB_1-contaminated feeds. Other detoxified metabolites produced from the CYP oxidation of AFB_1 include aflatoxin Q_1 (AFQ_1) and aflatoxin P_1 (Fig. 10). Soluble NADPH-dependent cytosolic enzymes reduce AFB_1 to produce aflatoxicol (AFL), which is nearly as toxic, mutagenic, and carcinogenic as the parent compound. Thus AFL is not considered a detoxified metabolite. Because the reduction is reversible, AFL is postulated to represent a storage form of AFB_1.

The most critical detoxification AFB_1 route is via glutathione S-transferase (GST) using GSH as cofactor. Species whose GST has a high affinity for the AFB_1-8,9-epoxide are generally protected from the toxic and carcinogenic effects of AFB_1, regardless of how well the epoxide is formed by CYPs. The AFB_1-8,9-epoxide is a substrate for GST, producing some form of nontoxic AFB_1-GSH adduct that is often the simple sulfhydryl derivative of AFB_1-GSH. Aflatoxin B_1 may also be detoxified via conjugation with sulfates and glucuronic acid. The AFB_1-8,9-epoxide may also be catalytically (by epoxide hydrolase) or spontaneously hydrolyzed to the AFB_1-8,9-dihydrodiol. A soluble AFB_1 aldo-keto reductase (AFAR) has also been isolated from liver and extrahepatic tissues from human, rat, and other species that has strong affinity for reducing the AFB_1-diol, thereby contributing to epoxide detoxification (21).

The electrophilic and highly reactive AFB_1-8,9-epoxide is reportedly responsible for the carcinogenic and mutagenic action of AFB_1. This intermediate binds to cellular nucleophiles, such as DNA. Activated AFB_1 binds exclusively to guanyl residues, and the AFB_1-N^7-guanine (AFB_1-N^7 Gua) adduct is the most predominant (Fig. 10). Additional adducts have been isolated, of which the "ring-opened" derivative of AFB_1-N^7-Gua, the formamidopyrimidine, or AFB_1-FAPyr, is the most common. In hepatic DNA from livers of rats injected with AFB_1, approximately 80% of the adducts present are AFB_1-N^7-Gua, whereas the AFB_1-FAPyr comprises approximately 7% (22). The formation of these adducts is the presumed first step in the development of heritable mutations from which tumors may arise. Repair of these genetic lesions occurs in living cells enzymatically or spontaneously, and the removed adduct is excreted in the urine. The ring-opened adduct appears to be more resistant to DNA repair enzymes. In rats treated with a single dose of AFB_1, the AFB_1-N^7-Gua was rapidly removed with an apparent half-life of 7.5 h, whereas other adducts, such as FAPyr, were removed much more slowly (23).

Aflatoxin B_1 is a potent acute toxin that primarily targets the liver. The primary lesions include hemorrhagic necrosis, fatty infiltration, and bile duct proliferation. In pigs, guinea pigs, and dogs, these effects are found most commonly in the centrilobular region, whereas in ducklings and rats, the periportal region is the site of action. Vertebrate species show considerable variation in susceptibility to the acute effects of AFB_1, and no species appears to be totally resistant. Poultry, rainbow trout, rats, and monkeys are particularly sensitive to the acute effects of AFB_1. Mice and hamsters are much less sensitive (24).

Aflatoxin B_1 is carcinogenic in a wide variety of animals. As is the case following acute exposures, the major target organ is the liver, although tumors in other organs result from long-term dietary exposure to AFB_1. Aflatoxin B_1 at 0.4 ppb fed over a 14-month period resulted in a 14% incidence of hepatocellular carcinomas in rainbow trout, the most sensitive animal species known to the carcinogenic effects of this mycotoxin. By contrast, only a 5% tumor incidence was observed during a similar time frame in Fischer rats exposed to 5 ppb.

Epidemiological data indicate that at least in sub-Saharan Africa and Southeast Asia, where AFB_1 contamination in foods is considerable, dietary AFB_1 is an important risk factor for human hepatocellular carcinoma (HCC). In these geographical areas there is a linear relationship between levels of AFB_1 contamination of food and the incidence of HCC. A factor that complicates epidemiology is that the incidence of hepatitis B virus infection, which is another reputed factor for HCC in humans, is also high in these regions. Specific biomarkers of human exposure to AFB_1, such as adducts of DNA and serum albumin, have proven to be valuable tools in the study of the role of dietary AFB_1 in human cancer. Measurement of these biomarkers in samples of blood or urine has allowed a direct determination of actual AFB_1 exposure in populations, which is an improvement over performing random dietary analysis for AFB_1 and imprecise dietary recall surveys.

Although the majority of interest in possible health effects has correctly focused on dietary exposure to AFB_1, workers in food and grain production, harvest, transport, and processing industries are also exposed to considerable amounts of airborne, respirable AFB_1-contaminated grain dusts. For example, airborne dust sampled in a corn-processing plant contained 107 ng/m^3 AFB_1, and the daily occupational exposure to this toxin was estimated to be between 40 and 856 ng (25). In another survey, concentrations of AFB_1 in smaller, more easily retained airborne grain particles were found to contain more AFB_1 than did larger grain particles; AFB_1 in particles under 7 μm were as high as 1,814 ppb, whereas particles in the size range of 7 to 11 μm had an average content of 695 ppb (26).

Some studies indicate that inhalation exposure to AFB_1 may result in adverse health effects to those exposed. Aflatoxin-contaminated peanut dusts have been associated with liver and lung cancer in Dutch peanut-processing workers who were continually exposed to be-

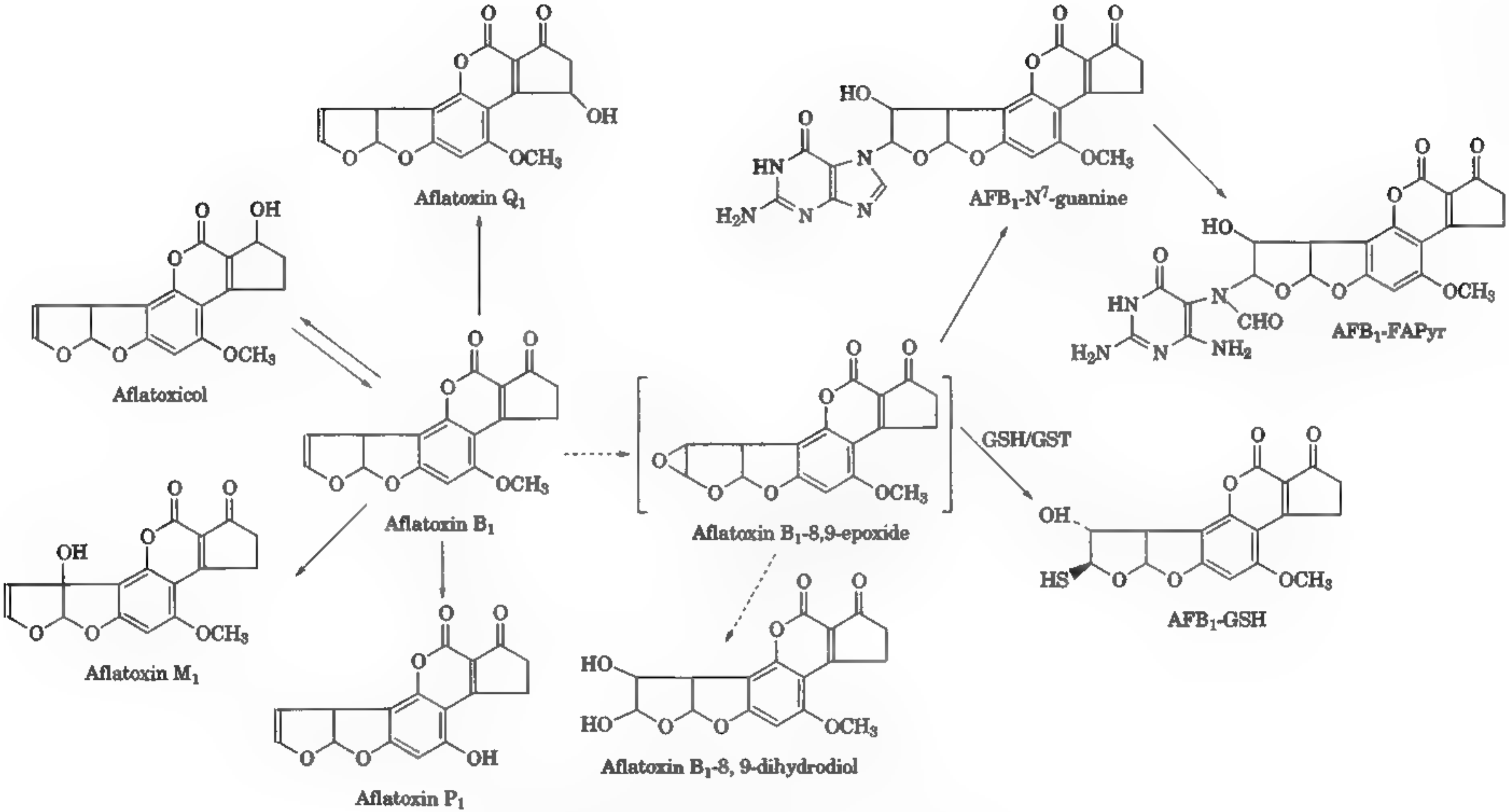

Aflatoxin Q$_1$
AFB$_1$-N^7-guanine
Aflatoxicol
AFB$_1$-FAPyr
Aflatoxin B$_1$
Aflatoxin B$_1$-8,9-epoxide
GSH/GST
Aflatoxin M$_1$
AFB$_1$-GSH
Aflatoxin P$_1$
Aflatoxin B$_1$-8, 9-dihydrodiol
OCH$_3$
OH
HO
HS
CHO
NH$_2$
H$_2$N
HN
N
O

Figure 10. Major metabolites and fate of aflatoxin B$_1$ in animals.

tween 0.04 and 2.5 μg AFB_1 per week, compared to unexposed cohorts. In laboratory studies, AFB_1 has been shown to be converted to stable metabolites and activated to mutagenic and DNA-binding intermediates by cells of the mammalian respiratory epithelium. In cultured airway epithelium from a variety of mammalian species, AFB_1 forms AFB_1-N^7-Gua and FAPyr in patterns similar to those found in hepatic systems. Compared to liver, human airway tissue has comparatively little AFB_1-metabolizing ability, however (27).

Tricothecenes

Another economically and toxicologically significant class of mycotoxins are the tricothecenes, which are a group of more than 150 structurally related compounds produced by several genera of fungi, the most common of which are *Fusarium sporotrichioides* and *F. graminearum*. Tricothecenes are common contaminants of grains such as corn, wheat, and barley. In the United States, corn from the upper Midwest is especially affected. Low temperatures, high moisture, and humidity appear to increase toxin production. Tricothecenes possess a sesquiterpenoids tetracyclic 12,13-epoxy-tricothecane skeleton. Examples of important tricothecenes include T-2 toxin, nivalenol, and deoxynivalenol (Fig. 11). T-2 toxin was the first tricothecene discovered in grains, but of the tricothecenes, the incidence of deoxynivalenol (DEN; vomitoxin) is more widespread worldwide.

The most acutely toxic tricothecene is verrucarin J, which has a LD_{50} of 0.5 mg/kg (iv) in the mouse. In mice, T-2 and nivalenol are nearly equitoxic; LD_{50} values are 5.2 and 4.1 mg/kg (ip), respectively. The *in vivo* toxicity of T-2 and nivalenol are approximately 10 times that of deoxynivalenol. In a variety of *in vitro* systems, T-2 toxin consistently has been the most toxic, followed by nivalenol and then deoxynivalenol (28).

Acute tricothecene toxicity is characterized by vomiting, diarrhea, and inflammation. Dermal irritation, feed refusal, abortion, and hematological sequelae such as anemia and leukopenia also are common. In cattle, dietary T-2 toxin at 0.64 ppm for 20 days results in death with bloody feces, enteritis, and abomasal and ruminal ulcers. In poultry, tricothecenes at levels as low as 5 ppm cause oral necrosis and reduced body weight gains. Decreased egg production and shell quality result when poultry are fed a diet of 20 ppm T-2. Tricothecenes also are toxic when administered dermally and cause symptoms similar to those seen when these mycotoxins are administered orally.

Several widespread cases of human tricothecene poisoning have occurred. Known as *alimentary toxic aleukia*, the disease follows a multistage pathogenesis. Shortly after ingestion of contaminated cereal grains, initial symptoms include a burning sensation in the mouth, tongue, throat, esophagus, and stomach and gastrointestinal disturbances. Severe hematologic effects, such as leukopenia and granulopenia, may then follow, which may progress to white cell counts as low as 100/μL of blood. Continuous exposure to trichothecenes results in rashes on the skin and elsewhere that may progress to severe necrotic lesions.

Tricothecenes have widespread adverse effects on synthesis of proteins and other macormolecules and on membrane and immune functions. Tricothecenes inhibit all steps in protein synthesis (initiation, elongation, and termination). Protein synthesis inhibition likely is a result of binding of the toxin to ribosomes. The binding of T-2 to ribosomes is specific and saturable (0.3 nM), and the stoichiometry is 1 toxin molecule bound per ribosome (29).

The amphipathic nature of T-2 has led some to believe that it could be incorporated into the lipid or protein moieties of cellular plasma membranes, thereby interfering with membrane function. Myoblasts treated with T-2 toxin had reduced uptake of calcium, glucose, leucine, and tyrosine within 10 min of exposure, effects apparently independent of protein synthesis inhibition.

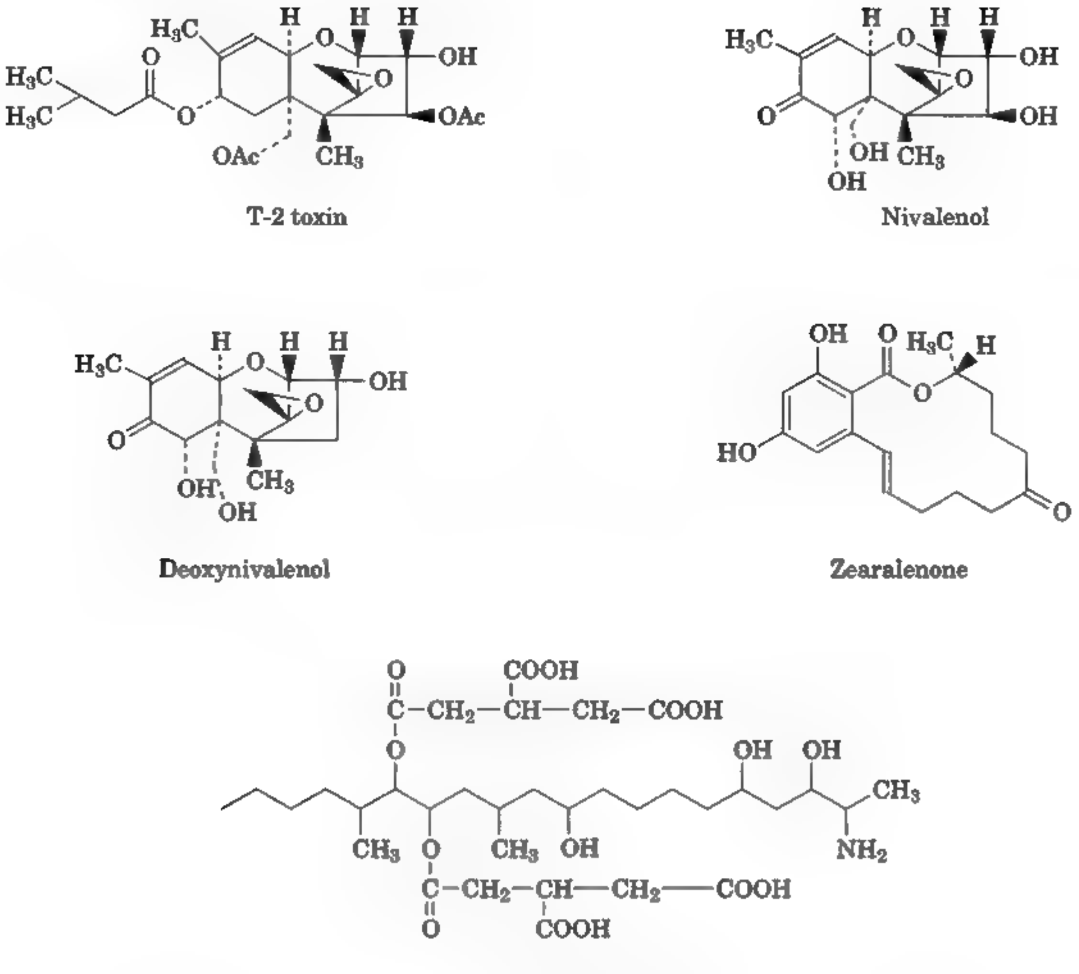
The toxin significantly alters several immune parameters, and the major effects appear to be associated with the cellular immune response. Specifically, effects such as an inhibition of the mitogen response, reductions of protein, DNA and interleukin-2 synthesis in concanavalin A–treated normal spleen cells, thymic atrophy, and reductions in plaque-forming spleen cells have been demonstrated. In animals, decreases in resistance to many challenges that are cellular immune dependent have been observed, such as to bacterial, fungal, and mycobacterial infections and skin grafts.

Zearalenone

Zearalenone (ZEN) is a phenolic resorcyclic acid lactone (Fig. 11) produced by strains of *Fusarium*, primarily by *F. graminearum* and *F. sporotrichiodes*. It is a natural contaminant of corn, wheat, barley, oats, sorghum, and hay. As with most mycotoxins, significant ZEN production is promoted by high humidity and low temperatures, common conditions in the upper Midwest during autumn harvest. Zearalenone is a nearly universal corn contaminant and is often found in the same samples with tricothecenes. Despite its dissimilarity to steroid compounds, ZEN produces potent hyperestrogenic responses in susceptible animals. Thus the toxicity of this compound is unique among known mycotoxins.

The animal most affected by ZEN is swine, but other animals, such as cattle, poultry, and laboratory animals, are affected to a lesser degree. Symptoms of ZEN poisoning include uterine enlargement and swollen vulva and mammae. In pigs, symptoms of hyperestrogenism generally appear when contamination of ZEN in corn exceeds 1 ppm, but it can occur as low as 0.1 ppm. In ewes, ZEN exposure results in decreased ovulation rate and cycle length and an increase in duration of estrus. Young male pigs exposed to ZEN undergo symptoms of feminization, such as enlarged nipples and testicular atrophy.

Zearalenone causes significant adverse reproductive effects in animals. For example, dairy heifers exposed to ZEN have reduced conception rates; in rats, ZEN (10 mg/kg in the diet) causes decreased growth rate, food intake, fertility, fetal resorptions, stillbirths, abortion and fetal bone malformations. ZEN reduces egg production in hens. In newborn female mice, ZEN (1 μg daily for 5 days) results in significant ovary-dependent reproductive tract



T-2 toxin

Nivalenol

Deoxynivalenol

Zearalenone

Fumonisin B_1

Figure 11. Representative *Fusarium* mycotoxins.

alterations at 8 month posttreatment; the majority of treated animals lacked corpus lutea and uterine glands and exhibited squamous metaplasia of the uterine luminal epithelium. Ovarectomized, ZEN-treated animals showed none of these ovary-dependent alterations.

The mechanism of the estrogenic effect of ZEN appears to be mediated via binding of ZEN or metabolite(s) to the cytoplasmic estrogen receptor. In rat uterine tissue, *trans*- and *cis*-ZEN and two ZEN derivatives compete with 17β-estradiol for binding with the cytosolic receptor, but with a lesser affinity that that of estradiol. Accordingly, ZEN appears to have a greater affinity for estrogen receptors from animals that are more susceptible to the estrogenic effects of the mycotoxin. The affinity of ZEN to uterine and oviduct estrogen receptors follows the pattern pig, rat, and then chicken.

Fumonisins

Fumonisins are a group of recently discovered mycotoxins that have been associated with toxicity and mortality in horses and pigs following ingestion of *Fusaria*-contaminated corn-based feeds. Fumonisin B_1 (FB_1) is the most toxic representative of the fumonisins (Fig. 11). One such animal disease is the neurotoxic syndrome Equine Leukoencephalomalacia (ELEM), which is characterized by facial paralysis, nervousness, lameness, ataxia, and an inability to eat or drink. The principal pathologic lesions include severe cerebral edema, focal malacia, and liquefaction of cerebral white matter. The onset of such severe symptoms can be as short as a few hours. ELEM syndrome often is epizootic and nearly always is associated with ingestion of moldy corn.

Several studies have positively linked fumonisins to ELEM. A majority of horses fed a corn-based feed contaminated with 37 to 122 ppm FB_1 developed fatal ELEM (30). Hepatic involvement is often coincident with central nervous involvement in horses and swine. Intravenous or dietary administration of FB_1 causes both pulmonary edema and hydrothorax in swine (31). In swine, lower doses of FB_1 result in a slowly progressive hepatic necrosis, whereas higher doses result in acute pulmonary edema coincident with hepatic toxicity. That fumonisins are potent inhibitors of sphingosine biosynthesis in cultured hepatocytes has been postulated to account for both the hepatotoxic and central nervous system effects of this toxin.

Besides the neurotoxic and hepatotoxic effects, FB_1 is a potent rodent carcinogen. Initial studies showed that a diet containing culture material inoculated with *F. moniliforme* was carcinogenic in rats. Dietary FB_1 not only initiates γ-glutamyltranspeptidase-positive (γ-GT +) hepatic foci, but also promotes those induced by compounds such as diethylnitrosamine (32). Long-term dietary FB_1 (50 ppm) induces a high incidence (66%) of HCC along with metastases to the heart, lungs, or kidneys (33). Symptoms of hepatic involvement such as macronodular cirrhosis and cholangiofibrosis are also observed. Later studies have led to the conclusion that FB_1 is probably only a modest initiator of liver tumors, because γ-GT^+ foci and hepatocellular nodules were observed only after prolonged feeding of very high (0.1%) FB_1 in rats. It was postulated that the carcinogenic effect of FB_1 likely involves promotion and the selection of initiated hepatocytes, events that occur during the postinitiation phase of hepatocarcinogenesis. Additionally, FB_1 carcinogenicity does not appear to involve interaction with DNA. Neither FB_1 nor FB_2 elicits unscheduled DNA repair in primary rat hepatocyte cultures treated either *in vitro* or *in vivo* (34). It is therefore possible that the carcinogenic activity of fumonisins is mediated via epigenetic mechanisms, such as is the case of peroxisome proliferators.

Research on the health effects of fumonisins is relatively modest, and information on the mechanism of fumonisin toxicity or on the mechanisms underlying species sensitivity is only beginning to be compiled. Obviously, more information is needed to provide a fuller understanding of the extent of the adverse effects of fumonisins on human and animal health. Because corn is a staple in many parts of the world, and because *Fusarium* contamination of corn is nearly universal, it is likely that fumonisins may be involved in human toxicoses and other health effects. *Fusarium*-contaminated corn has been epidemiologically associated with human esophageal cancer in some regions of South Africa.

Ergot Alkaloids

Documented since the Middle Ages, the fungus *Claviceps purpurea* has caused periodic outbreaks of mycotoxicoses in many parts of the world. This mold, which grows in grasses and cereal products, is especially prevalent in overwintered rye and wheat. Growth of the fungus takes the form of hard, black-purple sclerotia (or ergots) that germinate in the spring. *Claviceps* produces several ergot alkaloids, which are derivatives of lysergic acid (ergotamine; Fig. 12). The major effect of these compounds is vasoconstriction, resulting in a reduction of blood flow and subsequent gangrene in the feet, legs, and hands. Symptoms of ergot poisoning include a burning sensation in the arms and legs, gangrene, abortion, vomiting, diarrhea, weakness, and sometimes hallucinations.

Ochratoxins

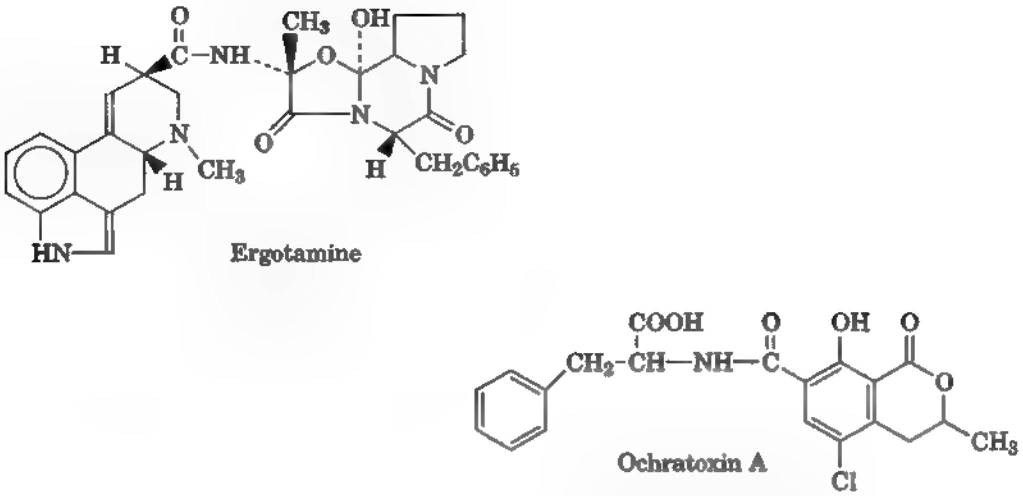
Ochratoxins are produced by *Aspergillus ochraceus* and selected *Penicillium* species. Ochratoxin A (Fig. 12), which is the most toxicologically significant of the ochratoxins, is produced in a variety of cereal grains (wheat, barley, oats, corn), dry beans, peanuts, and cheese. Ochratoxin A is a potent nephrotoxin in nearly all animals studied and is a probable etiologic agent for Balkan endemic nephropathy (BEN), a fatal chronic renal disease. The syndrome, which is associated with nephritis and associated urinary tumors, is especially prevalent in Bulgaria, Romania, and Yugoslavia. Pigs exposed to ochratoxin A experimentally present a nephropathy strikingly similar to BEN.

Surveys have repeatedly shown that a significant percentage of food supplies from several Balkan countries are contaminated with ochratoxin. In addition to the aforementioned cereal and other products, ochratoxin is often found in animal products such as sausage, bacon, and ham.

HETEROCYCLIC AMINES IN COOKED FOODS

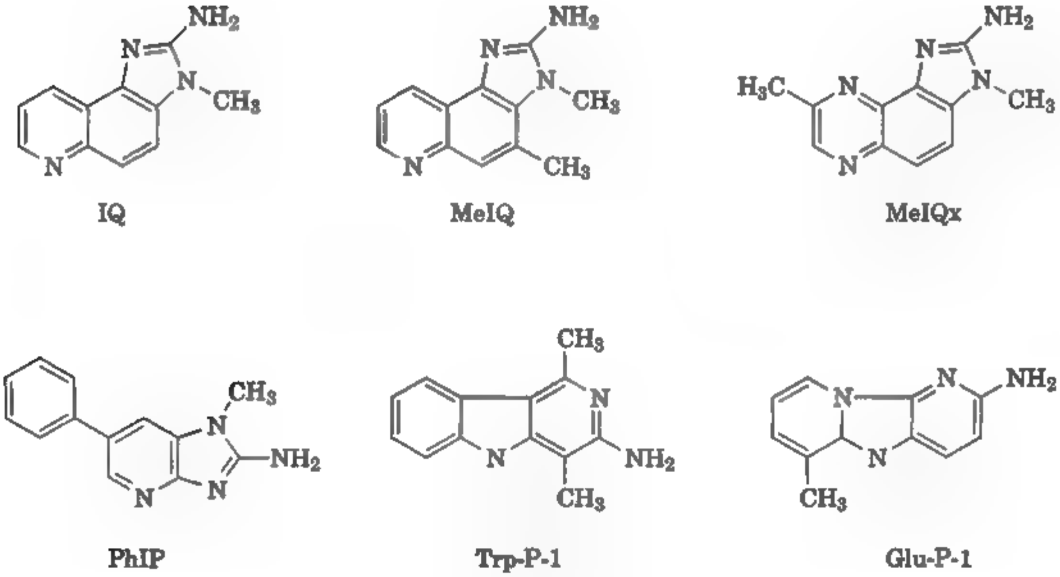
It has been known for years that heating food produces several classes of toxic compounds. For example, polycyclic aromatic hydrocarbons, the same compounds present in chimney soot, are also produced by charbroiling meat, chicken, and fish. More recently, however, a new class of heat-produced compounds, the heterocyclic amines (HCA), have been discovered and characterized. Like polycyclic aromatic hydrocarbons, HCAs are produced by pyrrolysis reactions that occur under ordinary cooking conditions. HCAs have been found in meat, fish, and poultry. Components in raw food that react to form HCAs include creatinine, sugars, and amino acids. Both creatinine and amino acids appear to be rate limiting, but the role of sugars in formation of HCAs is uncertain (35). Cooking practices involving higher temperatures, such as flame broiling or panfrying, produce higher amounts of these toxicants, which are stable in food under most storage situations. In addition to temperature, cooking time and type of food are factors important in HCA formation.

More than 20 HCAs have been isolated from cooked foods, nearly all of which are powerful mutagens, and in most cases animal carcinogens. In fact, HCAs are among the most potent bacterial mutagens known. Some of the most common and intensely studied include IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline), methyl IQ (MeIQ; 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline), methyl IQx (MeIQx; 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole), and Glu-P-1 (2-amino-6-methyldipyridol[1,2-*a*:3′,2′-*d*]imidazole) (Fig. 13). Heterocyclic amines are well absorbed from the intestine and, like many other natural carcinogens, require hepatic metabolic activation for toxic action. Cytochrome P450 1A2 has been identified as an important isozyme that catalyses the N-hydroxylation of the exocyclic amino groups of HCAs. Reactive electrophilic intermediates are produced by subsequent ester formation with either acetic acid, sulfuric acid, or proline (Fig. 14). The mutagenic and carcinogenic action of HCAs are thought to occur via binding of reactive nitreuniom ion intermediates with the C-8 atom of guanine in DNA. This resultant bulky adduct causes frameshift mutations by small base deletions.


Ergotamine

Ochratoxin A

Figure 12. Chemical structures of mycotoxins ergotamine and ochratoxin A.


IQ MeIQ MeIQx

PhIP Trp-P-1 Glu-P-1

Figure 13. Major carcinogenic heterocyclic amines in cooked foods.

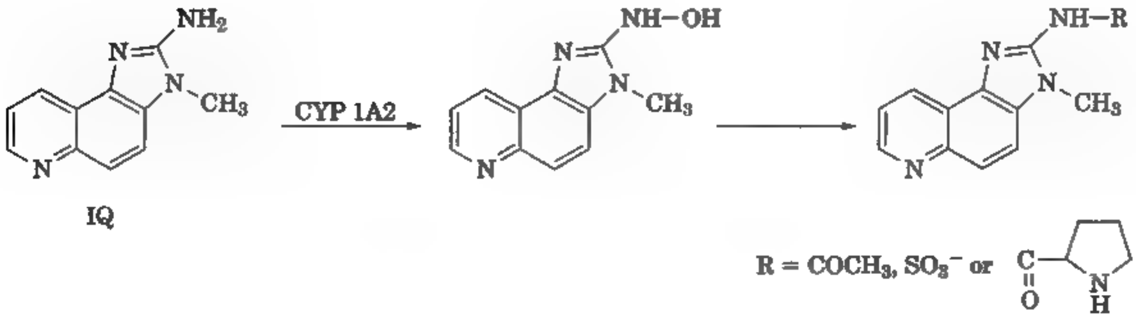


Figure 14. Mechanism of metabolic activation of IQ.

As animal carcinogens, heterocyclic amines are not as potent as would be expected from their impressive mutagenic activity. However, HCAs are still potent rodent carcinogens, causing tumors in a wide variety of tissues, mainly the liver, blood vessels, intestines, and forestomach. The most abundant HCA in cooked food, PhIP, causes tumors primarily of the large intestines, mammary gland, and lymphoid tissue (36).

Although the intake of HCAs is unavoidable, the average amount consumed in people varies dramatically. In the general population, the average intake of HCAs has been calculated to be between 0.4 and 16 μg/day (37). Urinary HCAs have been isolated in all healthy volunteers who eat normal diets. Whether HCAs are in fact carcinogenic in people at the nominal exposure levels is uncertain and is difficult to ascertain. Amounts of individual HCAs in the human diet are probably insufficient to cause cancer per se. Differences in diet, meat intake, and cooking methods can result in 50,000 to 100,000-fold differences in HCA exposure among individuals (38). Animal studies seem to indicate that HCAs act additively, or perhaps synergistically. Therefore, mixtures of HCAs, which would be present in most cooked foods, may be associated with human cancer risk.

ACKNOWLEDGMENTS

The author wishes to acknowledge support from grant ES04813 from the National Institute of Environmental Health Sciences, NIH; from National Research Initiative—National Competitive Program grants 970-3081 and 980-3754 from the USDA; and from the Utah Agricultural Experiment Station, Utah State University, Logan, Utah, 84322-4810, where this is designated as document number 7089.

BIBLIOGRAPHY

1. E. Mattei et al., "Stress Response, Survival and Enhancement of Heat Sensitivity in a Human Melanoma Cell Line Treated with L-Canavanine," *Anticancer Res.* **12**, 757–762 (1992).
2. A. Montanaro and E. J. Bardana, Jr., "Dietary Amino Acid-Induced Systemic Lupus Erythematosus," *Rheum. Dis. Clin. North Amer.* **17**, 323–332 (1991).
3. A. Akintonwa and O. L. Tunwashe, "Fatal Cyanide Poisoning from Cassava-Based Meal," *Hum. Exp. Toxicol.* **11**, 47–49 (1992).
4. O. C. Kemdirim, O. A. Chukwu, and S. C. Achinewhu, "Effect of Traditional Processing of Cassava on the Cyanide Content of Gari and Cassava Flour," *Plant Foods Hum. Nutr.* **48**, 335–339 (1995).
5. T. Tylleskar et al., "Cassava Cyanogens and Konzo, an Upper Motoneuron Disease Found in Africa," *Lancet* **339**, 208–211 (1992); published erratum appears in *Lancet* **339**, 440 (1992)
6. K. Hiramoto et al., "DNA Strand Breaking by the Carbon-Centered Radical Generated from 4-(Hydroxymethyl) Benzenediazonium Salt, a Carcinogen in Mushroom *Agaricus bisporus*," *Chem. Biol. Interact.* **94**, 21–36 (1995).
7. G. Luo and T. M. Guenthner, "Covalent Binding to DNA *In vitro* of 2′,3′-Oxides Derived from Allylbenzene Analogs," *Drug Metab. Dispos.* **24**, 1020–1027 (1996).
8. H. Wrba et al., "Carcinogenicity Testing of Some Constituents of Black Pepper (*Piper nigrum*)," *Exp. Toxicol. Pathol.* **44**, 61–65 (1992).
9. M. H. Kang et al., "Piperine Effects on the Expression of P4502E1, P4502B and P4501A in Rat," *Xenobiotica* **24**, 1195–1204 (1994).
10. Y. J. Surh and S. S. Lee, "Capsaicin, A Double-Edged Sword: Toxicity, Metabolism, and Chemopreventive Potential," *Life Sci.* **56**, 1845–1855 (1995).
11. B. R. Walker and C. R. Edwards, "Licorice-Induced Hypertension and Syndromes of Apparent Mineralocorticoid Excess," *Endocrinol. Metab. Clin. North Am.* **23**, 359–377 (1994).
12. J. R. Hincks et al., "DNA Cross-linking in Mammalian Cells by Pyrrolizidine Alkaloids: Structure–Activity Relationships," *Toxicol. Appl. Pharmacol.* **111**, 90–98 (1991).
13. H. Y. Kim et al., "Structural Influences on Pyrrolizidine Alkaloid-Induced Cytopathology," *Toxicol. Appl. Pharmacol.* **122**, 61–69 (1993).
14. R. A. Coulombe, Jr., G. L. Drew, and F. R. Stermitz, "Pyrrolizidine Alkaloids Cross-link DNA with Actin," *Toxicol. Appl. Pharmacol.* **54**, 198–202 (1999).
15. A. S. Prakash et al., "Mechanism of Bracken Fern Carcinogenesis: Evidence for H-*ras* Activation via Initial Adenine Alkylation by Ptaquiloside," *Nat. Toxins* **4**, 221–227 (1996).
16. W. Gaffield and R. F. Keeler, "Induction of Terata in Hamsters by Solanidane Alkaloids Derived from *Solanum tuberosum*," *Chem. Res. Toxicol.* **9**, 426–433 (1996).
17. J. R. Rayburn, M. Friedman, and J. A. Bantle, "Synergistic Interaction of Glycoalkaloids α-Chaconine and α-solanine on Developmental Toxicity in *Xenopus* Embryos," *Food. Chem. Toxicol.* **33**, 1013–1019 (1995).
18. A. Hagiwara et al., "Forestomach and Kidney Carcinogenicity of Caffeic Acid in F344 Rats and C57BL/6N × C3H/HeN F1 Mice," *Cancer Res.* **51**, 5655–5660 (1991).
19. D. L. Park et al., "Review of the Decontamination of Aflatoxins by Ammoniation: Current Status and Regulation," *J. Assoc. Off. Anal. Chem.* **71**, 685–703 (1988).
20. R. W. Beaver et al., "Distribution of Aflatoxins in Tissues of Growing Pigs Fed an Aflatoxin-Contaminated Diet Amended With a High Affinity Aluminosilicate Sorbent," *Vet. Human Toxicol.* **32**, 16–18 (1990).
21. E. M. Ellis and J. D. Hayes, "Substrate Specificity of an Aflatoxin-Metabolizing Aldehyde Reductase," *Biochem. J.* **312**, 535–541 (1995).
22. J. M. Essigmann et al., "Metabolic Activation of Aflatoxin B_1: Patterns of DNA Adduct Formation, Removal, and Excretion in Relation to Carcinogenesis," *Drug Metab. Rev.* **13**, 581–602 (1982).
23. R. G. Croy and G. N. Wogan, "Temporal Patterns of Covalent DNA Adducts in Rat Liver after Single and Multiple Doses of Aflatoxin B_1," *Cancer Res.* **41**, 197–203 (1981).
24. D. S. P. Patterson, "Metabolism as a Factor in Determining the Toxic Action of the Aflatoxins in Different Animal Species," *Food Cosmet. Toxicol.* **11**, 287–294 (1973).
25. W. R. Burg, O. L. Shotwell, and B. E. Saltzman, "Measurements of Airborne Aflatoxins during the Handling of Contaminated Corn," *Amer. Indust. Hyg. Assoc.* **42**, 1–11 (1981).
26. W. G. Sorenson, J. P. Simpson and M. J. Peach, III, "Aflatoxin in Respirable Corn Dust Particles," *J. Toxicol. Environ. Health* **7**, 669–672 (1981).
27. J. D. Kelly et al., "Aflatoxin B_1 Activation in Human Lung," *Toxicol. Appl. Pharmacol.* **144**, 88–95 (1997).
28. Y. Ueno, "Toxicological Features of T-2 Toxin and Related Trichothecenes," *Fund. Appl. Toxicol.* **4**, S124–S132 (1984).
29. J. L. Middlebrook and D. L. Leatherman, "Specific Association of T-2 Toxins with Mammalian Cells," *Biochem. Pharmacol.* **38**, 3093–3102 (1989).

30. T. M. Wilson and P. F. Ross, "Fumonisin Mycotoxins and Equine Leukoencephalmalacia," *J. Amer. Vet. Med. Assoc.* **198**, 1104 (1991).
31. P. F. Ross et al., "Production of Fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* Isolated Associated with Equine Leukoencephalomalacia and a Pulmonary Edema Syndrome in Swine," *Appl. Environ. Microbiol.* **56**, 3225–3226 (1990).
32. W. C. A. Gelderblom et al., "Fumonisins: Isolation, Chemical Characterization and Biological Effects," *Mycopathologia* **117**, 11–16 (1992).
33. W. C. A. Gelderblom et al., "Toxicity and Carcinogenicity of the *Fusarium moniliforme* Metabolite, Fumonisin B_1, in Rats," *Carcinogenesis* **12**, 1247–1251 (1991).
34. W. C. A. Gelderblom et al., "The Cancer-Initiating Potential of the Fumonisin B Mycotoxins," *Carcinogenesis* **13**, 433–437 (1992).
35. F. T. Hatch, M. G. Knize, and J. S. Felton, "Quantitative Structure–Activity Relationships of Heterocyclic Amine Mutagens Formed During the Cooking of Food," *Environ. Molecular Mutagenesis* **17**, 4–19 (1991).
36. T. Sugimura, "Overview of Carcinogenic Heterocyclic Amines," *Mutation Res.* **376**, 211–219 (1997).
37. K. Wakabayashi et al., "Food-Derived Mutagens and Carcinogens," *Cancer Res.* **52**, 2092S–2098S (1992).
38. J. S. Felton et al., "Health Risks of Heterocyclic Amines," *Mutation Res.* **376**, 37–41 (1997).

ROGER A. COULOMBE, JR.
Utah State University
Logan, Utah

See also MYCOTOXINS.

TOXICOLOGY AND RISK ASSESSMENT

Risk assessment is the process used to determine the probability, type, and magnitude of human toxic effects anticipated from exposure to specific levels of chemicals. All chemicals have the potential to produce adverse effects on health under some conditions of exposure. According to a sixteenth-century principle expressed by Paracelsus (ca 1493–1541), it is the right dose that differentiates a poison and a remedy. There is, however, a great variation of potency among chemicals in regard to their ability to cause adverse health effects; some chemicals may cause toxicological effects at very low doses, whereas others require much higher doses before effects are produced. All our food is composed of chemicals, and there are numerous types of chemicals that have been subject to risk assessment and/or regulation (Table 1).

The U.S. Food and Drug Administration (FDA) manages risks from food additives and veterinary drugs that may persist as residues in foods of animal origin (milk, meat, and eggs). The FDA has published comprehensive guidelines for safety assessment of direct food and color additives (1). The U.S. Environmental Protection Agency (EPA) is responsible for the control of several chemicals that may contaminate food, including pesticides and water pollutants. The EPA has developed a variety of guidelines for the toxicological testing of pesticides. At the international level, the regulatory authorities include different committees of the Food and Agriculture Organization of the World Health Organization (FAO/WHO), such as the Joint FAO/WHO Expert Committee on Food Additives, the Joint FAO/WHO Meeting on Pesticide Residues, and the Codex Alimentarius.

Table 1. Food Chemicals Considered in Risk Analysis

Food additives
Preservatives
Colors
Flavors
Stabilizers
Supplements
Biogenic compounds
Plant toxins or phytotoxins
Bacterial toxins
Enterotoxins
Mycotoxins
Marine toxins (eg, algal toxins responsible for paralytic shellfish poisoning and diarrhetic shellfish poisoning)
Residues
Processing aids (catalysts, filtration aids, antifoaming agents, extraction solvents, etc)
Pesticides
Animal feed additives
Veterinary or therapeutic drugs (medicines, antibiotics, hormones)
Environmental contaminants (heavy metals, industrial chemicals such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons)
Contaminants
Processing contaminants (eg, mutagenic heterocyclic amines)
Food packaging migrants (plastics, waxes, inks, etc); also called indirect food additive
Others
Adulterants, chemicals used for tampering

TOXICITY: TYPES AND TARGETS

Based on the duration of exposure to chemicals, toxicity is often classified as acute (involving a single dose), chronic (generally involving exposure over a lifetime), and subchronic (repeated exposures).

Different organs can be damaged by chemicals, including the liver, kidney, skin, brain, lungs, stomach, spleen, intestines, bladder, eyes, blood, and blood vessels. Different systems can also be affected, including the cardiovascular, nervous, immune, and reproductive systems, as well as the developing embryo or fetus. Although some toxicological effects can be reversed, others are irreversible. Carcinogens are chemicals capable of producing tumors, teratogens cause birth defects (2).

Toxicity Testing of Food Chemicals

In early times, trial-and-error methods were required to allow the distinction between safe and unsafe foods. In fact, early risk assessors worked for ancient pharaohs as food tasters. Currently, the preferred method for predicting potential health risks is human epidemiology, which involves statistical treatment of data collected from human populations. Epidemiological studies have been used to correlate human cancers with various factors. It is estimated that one-third of cancers can be attributed to diet (3) and that dietary changes can bring a substantial decrease in the rate of certain cancers (4).

Despite its usefulness in predicting risks from human exposure, epidemiology is limited by several factors, such as ethical considerations that prohibit human studies, the difficulty in measuring health effects with low probabilities of occurrence, inaccuracy due to recall bias, problems in identifying control groups that have not been exposed to the chemical under study, and the impossibility of obtaining human data on newly developed chemicals prior to their release.

As the preferred substitute for epidemiological data, animal toxicology studies are commonly performed in a variety of animal species, and the results of such studies are extrapolated to allow risk assessors to predict potential human health risks.

The major types of toxicology studies commonly conducted are whole animal studies and *in vitro* studies.

Whole Animal Studies. For practical reasons, most whole animal toxicology studies are conducted in rodents (mice and rats), although tests in other nonrodent species such as dogs and nonhuman primates are also commonly performed.

The most comprehensive toxicology test performed is the lifetime animal bioassay using rodent species administered daily doses of the test material over their normal life span. Typical dosing schemes involve a high dose (the maximum tolerated dose, or MTD), a lower dose (often one-fourth to one-half of the MTD), and a control (zero) dose. (5). In testing many food chemicals, high doses are often used to maximize the potential to identify toxicological effects; many of these doses are several orders of magnitude greater than the typical human intake.

In vitro Studies. Commonly, a number of *in vitro* studies are also performed that may utilize specific components of mammalian systems (for example, liver enzymes obtained from whole animals) or using other living systems such as bacterial colonies. *In vitro* studies are frequently employed to identify the potential metabolic pathways that a specific chemical is subject to and to screen for various forms of toxicity such as mutations and endocrine disruption. Results from initial *in vitro* studies may provide preliminary information for chemical and pharmaceutical manufacturers that allow them to identify suitable chemical candidates they may wish to study in expanded and costlier whole animal studies. Some *in vitro* studies also allow risk assessors to determine the similarities and differences between test animals and humans in their abilities to metabolize the chemicals under investigation. Specific tests have been developed to determine the DNA-damaging properties of certain chemicals and DNA-repair tests (6). It is anticipated that increasingly sophisticated methods, with major inputs from molecular biology, will play a more extensive and important role in risk assessment (7).

RISK ASSESSMENT

In 1983, the National Research Council (NRC) of the National Academy of Sciences published the first U.S. federal guidelines for performing chemical risk assessments (8). The NRC identified four major components of the risk assessment process: hazard identification, dose-response assessment, exposure assessment, and risk characterization.

Hazard Identification

Hazard identification is the determination of whether a particular chemical is or is not causally linked to specific health effects. This first step involves a critical evaluation of all the available information on the chemical under study, including epidemiological (human) data and experimental (animal) toxicity data, conditions of exposure under which the effects may be produced, and studies identifying possible biochemical mechanisms of toxic action. The information may have been developed from acute, subchronic, or chronic toxicology studies and might include a variety of harmful effects, such as cancer, allergic reactions, birth defects, developmental defects, reproductive abnormalities, neurotoxicity, immunotoxicity, or toxicity to the liver, kidney, or lung.

A critical component of hazard identification is the determination of whether a chemical does or does not cause cancer. This distinction is important because risk assessment practices use different criteria for carcinogens (cancer-causing chemicals) and noncarcinogens. Cancer studies usually are performed using the chronic lifetime rodent bioassays and frequently make use of the MTD. The MTD is usually determined following the results of 90-day toxicity studies and is roughly described as the highest dose that does not alter the test animal's longevity or well-being because of noncancer effects (9). However, it has been argued that many chemicals may induce cancer at the MTD through biological mechanisms that do not occur at lower dose. Such mechanisms include increased cell proliferation rates in response to high-dose toxicity (10). Exposure at lower doses, where these mechanisms are not active, would not result in cancer.

Dose-Response Assessment

Once a specific toxicological hazard has been identified, it is possible to determine the relation between the level of exposure and the probability of occurrence of the adverse effects in question. The procedures used to establish this dose-response relationship are governed by the type of hazard; noncarcinogenic and carcinogenic hazards are typically treated differently.

There are two general models used to determine the dose-response relationships: (1) the threshold model and (2) the nonthreshold model.

Threshold Model. This model is adopted for noncarcinogenic hazards and assumes that toxic effects will not be observed until a minimum, or threshold, dose is reached.

To estimate the threshold, toxicology studies generally try to identify two dose levels: one above the threshold at which effects are seen (known as the lowest observed effect level, or LOEL) and one presumably below the threshold at which no effects are seen (known as the no observed effect level, or NOEL). The degree to which the NOEL and LOEL estimates approximate the threshold cannot be determined because of statistical and biological limitations as well as limitations in the number of dose levels used in toxicology studies. As a prudent measure, the NOEL is generally used as a conservative estimate of the threshold (11).

To determine acceptable levels of human exposure following identification of the NOEL value from animal studies, uncertainty factors (often called safety factors) are also incorporated into the process. Such factors presumably account for differences in animal to human extrapolation and consider biological variability among human individuals.

The NOEL values are derived from toxicology studies involving small homogeneous groups of animals and, therefore, may not represent appropriate thresholds for large and nonhomogeneous human populations. The choice of uncertainty factors is governed by the availability of human data; the nature, severity, and chronicity of the effect; the quality of animal toxicology data; and the need to accommodate human response variability for sensitive subgroups. Overall uncertainty factors may range from 1 to 10,000 (12).

The most common uncertainty factors is 100, which is rationalized as a 10-fold uncertainty factor for species variation (assuming humans are 10 times more sensitive than the animals studied) multiplied by another 10-fold uncertainty factor for human variation (assuming some humans are 10 times more sensitive than the average humans). It has been recommended that up to an additional 10-fold uncertainty factor be incorporated to provide greater protection for infants and children from possible effects of pesticide residue exposure if data relating the relative sensitivities of infants and children to the sensitivities of adults are not available (12).

An acceptable daily intake (ADI) is calculated by dividing the NOEL by the selected uncertainty factor. The ADI is defined as an estimate of the amount of a food chemical (such as an additive or pesticide) that can be ingested by humans on a daily basis over a lifetime without anticipated harm. The EPA has recently replaced the term ADI with an analogous term, the toxicity reference dose, or RfD, thereby removing the inference of acceptability, which may carry the connotation of a nonscientific, value judgment (2). For metal contaminants with cumulative properties (such as mercury or lead), the concept of provisional tolerable weekly intake (PTWI) was established by the WHO/FAO (13). For chemicals that are essential nutrients but cause toxicological effects when consumed at high levels, the WHO (14) established the concept of provisional maximum tolerable daily intakes (PMTDI).

As an alternative to the use of the NOEL approach in the dose-response assessment, the concept of the benchmark dose has been proposed (15). This approach is intended to reduce the uncertainty related to the NOEL determination. The benchmark dose is defined as the lower confidence limit for the effective dose that produces a specific increase in the incidence over control (background) levels. The benchmark dose provides a consistent basis for calculating the RfD, considers the dose-response model, and uses all available experimental data in contrast to the NOEL approach, which ignores the shape of the dose-response curve. The benchmark dose approach can also be applied to the risk assessment of carcinogens.

Nonthreshold Model. This model is used for assessment of the dose-response relationship for carcinogenic hazards. It is assumed that any level of exposure to a carcinogen may provide some probability for the development of cancer (although the probability is still related to the dose), which implies that a NOEL does not exist for carcinogens. Mechanistically, it is assumed that many carcinogens act as mutagens that cause direct damage to the genes; it has been proposed, in what is often called the one-hit model of carcinogenesis, that exposure to a single molecule of a carcinogen could ultimately lead to a mutation that could develop into cancer.

As mentioned previously, the study of cancer-causing chemicals usually involves long-term rodent (eg, rat and mouse) assays. In an effort to maximize the chance of detecting cancer in the animal studies, special strains of animals that may be more susceptible to developing cancer are often used; this practice raises questions about the validity of extrapolating such results to humans (16). Moreover, although cancer itself requires tumors to invade other tissues, benign (noninvasive) tumors are also usually considered evidence of potential carcinogenicity.

The notion that carcinogens lack toxicity thresholds is controversial. Chemicals may, in fact, use several biological mechanisms to cause cancer (17). Genotoxic chemicals, which cause mutations, may indeed lack threshold doses. In other cases, such as the induction of thyroid tumors (18) or the induction of tumors resulting from increased cell proliferation (10), it is argued that such carcinogenic effects are exerted through mechanisms consistent with a threshold hypothesis. Currently, however, most carcinogenicity risk assessment models still rely on the nonthreshold assumption.

Typical human exposure to animal carcinogens is often several thousand times lower than doses that produce tumors in experimental studies. Toxicology testing protocols for carcinogens usually involve the administration of massive levels of chemicals (at the MTD) to animals throughout their lifetimes. Calculation of carcinogenic risks therefore requires the results of high-dose animal studies to be extrapolated to predict human risks at low exposures. A number of mathematical models have been developed for the dose-response assessment of carcinogens; each yield a value known as the cancer potency factor (19). Cancer potency factors may vary widely depending on the choice of the model and its assumptions. The most commonly used model is the linearized multistage model that assumes a cell, which may be a target for a carcinogenic chemical, goes through a specific number of different stages and that the probability of a carcinogenic "hit" on the cell, which leads to the development of cancer, is stage-specific. At the lowest level of exposure, the relationship between exposure level and excess cancer is linear. Also commonly performed are statistical corrections that express cancer risks on the basis of the upper 95% confidence interval of the slope of the dose-response curve, adding an additional element of conservatism to the risks (20). Upper-confidence-level cancer risks may be orders of magnitude greater than the best estimates obtained using the mathematical models.

Exposure Assessment

Before risks from exposure to chemicals in food can be properly assessed, the information derived from the hazard identification and dose-response assessment processes must be combined with an estimate of the likely amount of human exposure, or dose, before the levels of risk may be assessed. To obtain reliable information on dietary exposure to chemicals in food, it is necessary to obtain information on the frequency of occurrence and levels of the chemicals in food and the amounts and types of various food items that are consumed. Human exposure to chemicals in the diet is typically expressed as the product of the concentration of the chemical in various foods and the amounts of the foods consumed. Estimation of both factors requires several assumptions and involves considerable uncertainty.

The exposure assessment depends on the type of food chemical under study. With food additives, the concentration of a chemical in food may be well known and relatively constant. For incidental chemicals in foods (eg, pesticide residues, hormones, antibiotics, and environmental contaminants), however, the concentration may vary dramatically from sample to sample, making an accurate estimate of the actual level of concentration difficult to obtain. The choice of assumptions used to predict the concentration levels may also be related to the availability of reliable monitoring data.

Several techniques are available to estimate pesticide residue levels in foods. The highly theoretical and conservative assumption (worst case) is that all pesticides are present at a predetermined level, typically at the maximum allowable level throughout the whole production system, including the raw food commodity, processed forms of the food, and the food in table-ready form. More complex data-intensive approaches are based upon actual measurements of residue levels at the time the food is ready to be consumed. Also of use are a variety of intermediate techniques that consider factors such as residue results from field monitoring studies, effects of postharvest factors on residue levels, and incorporation of actual pesticide-use data (20). Results from the use of the various techniques may differ dramatically. Archibald and Winter (21), using realistic pesticide residue data, reported exposure levels ranging from hundreds to tens of thousands of times lower than those estimated using much more conservative assumptions.

Obtaining useful and accurate food consumption data represents another scientific challenge. Most food consumption data are collected from national food consumption surveys. Typically, such surveys rely on eight general methods that are used to assess food consumption: food disappearance data (correcting food production and import data for food exports, waste, storage, and nonhuman food use), household disappearance data, dietary histories, dietary frequencies, 24-hour recalls, food records, weighted intakes, and duplicate portions (22). The method used depends upon the purposes of the study and availability of resources. For dietary risk assessment purposes, the most common food consumption estimates are derived from the results of U.S. Department of Agriculture nationwide food consumption surveys based on three-day dietary records describing the types and amounts of foods consumed, both at home and away from home. Such surveys involve tens of thousands of individuals in the 48 continental states and Hawaii, Alaska, and Puerto Rico. The amount of each food item consumed and the individual's weight are specified. Additional information concerning demographic and socioeconomic background, age, gender, ethnicity, and geographic location are tabulated. The accuracy of such food consumption estimates is directly related to the accuracy of those surveyed in identifying the amounts and types of foods they actually consumed during the survey period.

A critical step in the exposure assessment phase is to identify the target audience exposed to the food chemical under study. It is widely accepted that dietary chemical exposures of different population subgroups may vary dramatically due to differences in food consumption patterns. One of the limitations of data from nationwide food consumption surveys is the ability to accurately identify the food consumption patterns of population subgroups for which the sample size is limited in the overall data set.

During the past decade, the assessment of food chemical risks to infants (up to 1 y) and children (1–12 y) has been a key issue in risk assessment. Infants and children represent a unique subpopulation group, and concerns are common not only because of their small body size, but also because of their physiological and developmental differences. Infants and children eat fewer foods than adults, but consume more food on a per-body-weight basis. Hence, their exposure to pesticide residues is often greater than that for adults (12). Other vulnerable population subgroups are represented by the elderly and people with existing health conditions.

The main focus of chemical food safety evaluation has been on pesticides. In the case of acute (short-term) risk assessments, the diets of the most highly exposed individuals—those representing the upper 90th, 95th, or 99th percentiles (high-level consumers)—are often considered rather than median consumers, and chemical concentrations are often considered at highest detected levels rather than at median levels.

Traditional approaches to estimate food chemical exposure have relied upon "point estimates" derived by assuming a particular concentration level of the chemical and a specific food consumption estimate. In reality, both chemical levels and food consumption patterns may vary dramatically among different food samples and individual consumers and are more appropriately presented as distributions. Through the process of statistical convolution, it is possible to combine the distributions of chemical levels and food consumption patterns to develop an exposure distribution rather than simply a point estimate. Through such a process, for example, it is possible to identify what percentage of a population (or subpopulation) is exposed to a specific amount of a chemical (such as the RfD) on a given day or in a short time period. Such a distributional analysis approach is also more amenable to the assessment of exposure to multiple chemicals that may have similar mechanisms of toxic action (12).

Risk Characterization

The final stage of the risk assessment process involves describing the nature, and often the magnitude, of risk and optimally includes statements reflecting the uncertainty in the final risk estimates. The core of the risk characterization stage is a comparison between estimated human exposures with levels of toxicological significance. Risk may be expressed as a probability, but optimally, risk characterization should include qualitative evaluation in addition to single numerical depictions of risk or ranges of numerical depictions. Such qualitative factors include the strength of the evidence that a chemical produces the particular effect from which the risk was estimated. The numerous uncertainties and assumptions inherent in the risk assessment process should also be discussed (23). In fact, the analysis of uncertainty in the risk estimate is an essential component of risk characterization and may provide valuable information for risk managers.

For noncarcinogens, risk characterization typically relates the estimated exposure to the RfD or ADI. It is critical to understand that the RfD or ADI is not a toxicity threshold level in itself that provides a cutoff between "safe" and "unsafe" human exposures. Exposure at the RfD or ADI presents a very low risk, although "very low" is undefined (2). Qualitatively, risk increases at levels above the RfD or ADI, with greater exposure resulting in greater potential risk.

For carcinogens, estimated cancer risks are obtained by multiplying exposure estimates by cancer potency factors. This practice often results in the reporting of cancer risks indicating, for example, a specific number of cancers (above normal cancer rates) per 1 million persons exposed. The actual numbers presented usually represent the upper bounds of cancer risks calculated using conservative treatment of various uncertainties (such as nonthreshold behavior) and, as such, probably do not accurately reflect true cancer rates (24). Although fairly arbitrary, a cancer risk of 1 excess cancer per million persons exposed typically represents a level of risk considered to be "negligible" (19).

BIBLIOGRAPHY

1. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, Redbook II, U.S. Food and Drug Administration, Washington, D.C., 1993.
2. J. V. Rodricks, *Calculated Risks: The Toxicity and Human Health Risks of Chemicals in Our Environment*, Cambridge Univ. Press, New York, 1992.
3. B N. Ames and L. S. Gold, "The Causes and Prevention of Cancer: Gaining Perspective," *Environ. Health Persp.* **105** (Suppl. 4), 865–873 (1997).
4. R. Doll, "The Lessons of Life: Keynote Address to the Nutrition and Cancer Conference," *Cancer Res.* **52**, 2024s–2029s (1992).
5. M. W. Pariza, "Risk Assessment," *Crit. Rev. Food Sci. Nutr.* **31**, 205–209 (1992).
6. L. A. P. Hoogenboom and H. A. Kuiper, "The Use of *In Vitro* Models for Assessing the Presence and Safety of Residues of Xenobiotics in Food," *Trends Food Sci. Technol.* **8**, 157–166 (1997).
7. D. R. Tennant, "Food Chemicals and Risk Analysis," in D. R. Tennant, ed., *Food Chemical Risk Analysis*, Blackie Academic & Professional, London, 1997, pp. 3–18.
8. *Risk Assessment in the Federal Government: Managing the Process*, National Research Council, National Academy Press, Washington, D.C., 1983.
9. *Issues in Risk Assessment*, National Research Council, National Academy Press, Washington, D C., 1993
10. B. N. Ames and L. S. Gold, "Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis," *Science* **249**, 970–971 (1990).
11. Environ, *Elements of Toxicology and Chemical Risk Assessment*, Environ Corp., Washington, D.C., 1986.
12. *Pesticides in the Diets of Infants and Children*, National Research Council, National Academy Press, Washington, D.C., 1993.
13. WHO/FAO, *Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium*, 16th Report, WHO Technical Report, Series No. 505, Geneva, Switzerland, 1972.
14. WHO, *Evaluation of Certain Food Additives and Contaminants*, 26th Report, WHO Technical Report, Series No. 683, 1982.
15. K. S. Crump, "A New Method for Determining Allowable Daily Intakes," *Fundam. Appl. Toxicol.* **4**, 854–871 (1984).
16. P. H Abelson, "Health Risk Assessment," *Reg Toxicol. Pharmacol.* **17**, 219–223 (1993).
17. G. B. Gori, "Cancer Risk Assessment: The Science That Is Not," *Reg. Toxicol. Pharmacol.* **16**, 10–20 (1992).
18. O. E. Paynter et al., "Goitrogens and Thyroid Follicular Cell Neoplasia: Evidence for a Threshold Process," *Reg. Toxicol. Pharmacol.* **8**, 102–119 (1988).
19. *Regulating Pesticides in Food: The Delaney Paradox*, National Research Council, National Academy Press, Washington, D.C., 1987.
20. C. K. Winter, "Dietary Pesticide Risk Assessment," *Rev. Environ. Contam. Toxicol.* **127**, 23–67 (1992).
21. S. O. Archibald and C. K. Winter, "Pesticides in Our Foods: Assessing the Risks," in C. K. Winter, J. N Seiber, and C. F. Nuckton, eds., *Chemicals in the Human Food Chain*, Van Nostrand Reinhold, New York, 1990, pp. 1–50.
22. J. A. T. Pennington, "Methods for Obtaining Food Consumption Information," in I. Macdonald, ed., *Monitoring Dietary Intakes*, Springer-Verlag, New York, 1991, pp. 3–8.
23. F. D Hoerger, "Presentation of Risk Assessments," *Risk Anal.* **10**, 359–361 (1990).
24. C. K. Winter, "Lawmakers Should Recognize Uncertainties in Risk Assessment," *Calif. Agric.* **48**, 21–29 (1994).

ELISABETH L. GARCIA
CARL K. WINTER
University of California
Davis, California

See also FOOD SAFETY AND RISK COMMUNICATION; FOOD SAFETY AND RISK MANAGEMENT; FOOD TOXICOLOGY.

ULTRAFILTRATION AND REVERSE OSMOSIS

BACKGROUND INFORMATION

Ultrafiltration (UF) and reverse osmosis (RO) are unit operations in which water or some solutes in a solution are selectively removed through a semipermeable membrane. Both processes are similar in that the driving force for the transport of water and solutes across the membrane is the pressure applied to the feed solution. However, the membrane and pressure used and the separation achieved are quite different. Membranes used in both UF and RO have a very thin skin layer or an asymmetric structure formed by phase-inversion processes. In RO, the thin skin layer consists of a very dense film; thus, virtually all suspended matter and most dissolved species are retained by the membrane, and only water passes through the membrane. The operating pressure is in the order of 700 kPa or higher to overcome the high osmotic pressure of the low molecular weight solutes. UF membranes have a thin skin layer that is microporous and, hence, they separate species based on molecular size. In addition to water, some dissolved low molecular weight species can pass through the membrane. The driving force for the separation is the pressure difference across the membrane, typically in the range of 70 to 700 kPa. Another important difference between UF and RO is the effect of the chemical nature of the membrane polymer. In UF, the chemical nature of the membrane polymer has only a small effect on the separation process since separation is based on a sieving mechanism. By contrast, RO separations are based on preferential sorption or solution/diffusion phenomena and are greatly influenced by the chemical nature of the polymer membrane.

The advantages of UF and RO, comparing to other separation or dewatering processes, have been summarized by Porter and Nelson (1). First, they do not require a phase change in solvent to effect the separation or dewatering processes. Second, UF and RO can be conducted at ambient or other selected temperatures. These can result in considerable savings in processing energy, elimination of thermal or oxidation degradation problems associated with evaporation and collapse of gels, breaking of emulsions, and mechanical damages associated with freezing. For UF processes, in addition, changes in ionic strength and pH that occur during other methods of separation or dewatering are also avoided because low molecular weight acids, bases, or salts are not retained and are freely permeable to the UF membranes.

The main limitations of UF and RO are (*1*) fouling of the membranes, which reduces the operating time between periods of cleaning; (*2*) the maximum concentration is limited to 30% total solids; (*3*) higher capital costs than evaporation; and (*4*) variation in the product flow rate when changes occur in the concentration of feed solution (2).

The history of UF and RO has been reviewed by Sourirajan (3), Matsuura and Sourirajan (4), and Cheryan (5), and the costs of their operations has been reviewed by Cheryan (5).

MEMBRANES AND MEMBRANE MODULES

UF and RO membranes are usually asymmetric, which means the pores change in size from one surface of the membrane to the other. They also differ from microporous membranes, which have pores of uniform size throughout the membrane. The two techniques have been developed to manufacture asymmetric membranes: one utilizes the phase-inversion process (6) and the other forms a composite structure by depositing a very thin homogeneous solid polymer film on a microporous membrane (7). The phase-inversion process consists of three steps: (*1*) a polymer is dissolved in an appropriate solvent to form a solution containing 10 to 30 wt % polymer; (*2*) the solution is cast into a film about 100 to 500 μm thickness; and (*3*) the film is quenched in a nonsolvent, which for most polymers is typically water or an aqueous solution (3,8,9). Variations of the phase-inversion process such as wet, dry, or melt spinning processes have been reviewed (5). More than 90 different homopolymers, copolymers, and blends have been investigated for the manufacture of the phase-inversion membranes (10). Cellulose acetate, polysulfone, and polyamide membranes are the most common types used commercially.

The methods of forming composite membranes have been reviewed (5,11). These methods can be briefly summarized as follows: (*1*) casting of the barrier layer separately, which is followed by lamination to the support film; (*2*) dip-coating of the support film in a polymer solution and drying, or in a reactive monomer or prepolymer solution followed by curing with heat or radiation; (*3*) gas-phase deposition of the barrier layer from a glow-discharge plasma; and (*4*) interfacial polymerization of reactive monomers on the surface of the support film.

Mineral or ceramic membranes are another class of membranes that are lately receiving increased attention. They are formed by the deposition of inorganic solutes, such as zirconium oxide, onto reusable porous carbon tubes or porous metal tubes. Because they are made of inorganic materials, they have little or none of the disadvantages associated with polymeric membranes. They possess a high degree of resistance to chemical and abrasion degradation and tolerate a wide range of pH and temperature (5).

For the successful application of UF and RO, the design of the membrane module and the layout of the system in which the module is installed are as important as the selection of the proper membranes (9). There are four important membrane module designs used in both UF and RO: tubular, plate-and-frame, spiral-wound, and capillary or hollow-fiber.

The tubular membrane module is shown in Figure 1 and consists of a membrane cast on the inside of a porous fi-

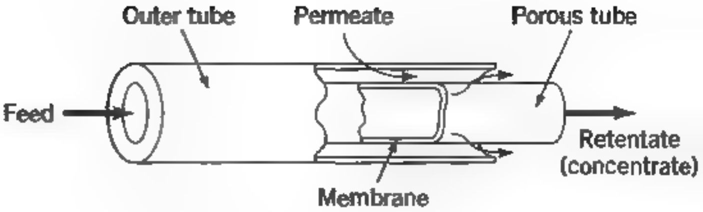


Figure 1. A tubular membrane module.

berglass reinforced plastic tube or a synthetic fiber tube and then inserted into a supporting, perforated stainless steel tube. These tubes are usually 12.5 mm (0.5 in.) to 25 mm (1 in.) in internal diameter and 0.6 to 6.4 m (2 to 20 ft) in length. A number of these tubes are then placed inside of a PVC or stainless steel sleeve or shroud to form a module. This type of membrane module provides a good control of the concentration polarization and membrane fouling. The system can usually tolerate large quantities of suspended solid matter in the feed and can be cleaned by foam swabs without dismantling the equipment. However, it has a lower membrane surface per module volume than other types of membrane module design.

A typical plate-and-frame membrane module is shown in Figure 2. It is similar to the conventional filter press. The membranes, porous support plates, and spacers with curved ribs forming the feed flow channels are clamped together and stacked between two end plates in a vertical or horizontal frame. Permeate is led out through fine, stainless steel tubes from the edge of the plates. All plate-and-frame membrane modules provide a larger membrane surface per unit volume than tubular membrane modules, but the control of concentration polarization is more difficult. Handling of solutions with suspended solids may cause plugging of the flow channel.

Figure 3 shows a spiral-wound membrane module. Two flat membrane sheets, separated by a porous membrane support, are placed together with their active sides facing away from each other. The three sides are then sealed together and the fourth side is attached to a perforated center stainless tube. A cover leaf and a spacer screen are then placed on each side of the membrane and the whole assembly is rolled around the center tube and placed inside a PVC or stainless steel housing fitted with manifolds to form a spiral-wound module. The feed is pumped into one end of the module and flows in parallel to the axis of the module. The permeate flows radially through both the membrane and porous membrane support and finally into the perforations of the center tube. The retentate or concentrate then flows out from the other end of the membrane module. The membrane surface per unit volume is higher than the plate-and-frame type, but the control of concentration polarization effects is more difficult. Severe membrane fouling may occur even with moderate concentrations of suspended solid materials. Thus, the spiral-wound membrane module is usually used in RO applications, and its use in UF is limited (9).

The hollow-fiber geometry is a newer approach to the membrane module design (Fig. 4). The membrane is a self-supporting tube with the active surface on the inside of the tube. Each hollow fiber has a fairly uniform bore and is bonded on each end in an epoxy tube sheet and is housed in a clear polysulfone or translucent PVC shell to form a membrane cartridge. The feed enters the inside of a group of hollow-fiber tubes, and the permeate passes through the tube and enters the shell side of the chamber for collection. Hollow-fiber membrane modules provide the largest membrane surface per unit volume of all membrane module design. However, the small fiber tubes are highly susceptible to fouling and plugging. Pretreatment or prefiltering of the

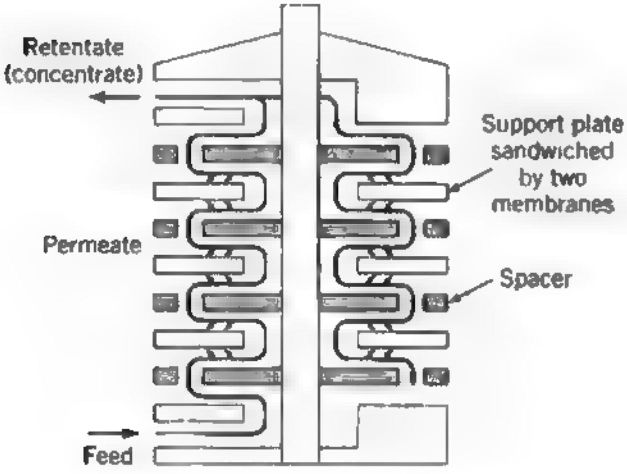


Figure 2. A plate-and-frame membrane module.

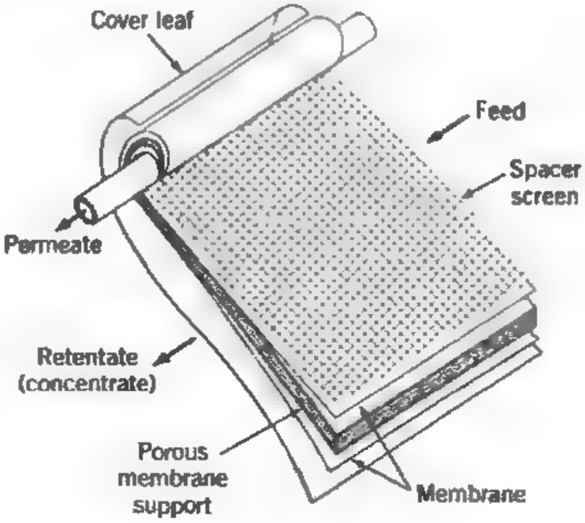


Figure 3. A spiral-wound membrane module.

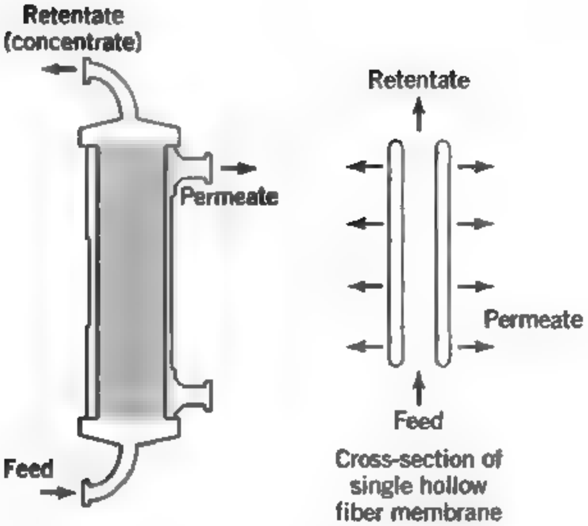

Retentate (concentrate)
Permeate
Feed
Retentate
Permeate
Feed
Cross-section of single hollow fiber membrane

Figure 4. A hollow-fiber membrane cartridge.

feed is often required and, furthermore, the flow rate of the feed is limited because it is proportional to the inlet pressure, which is limited to 1.8 atm (25 psig) maximum operating pressure.

PROCESS DESCRIPTION

UF and RO can be conducted either as a single-pass, batch, feed-and-bleed, or multistage recycle system (5). In single-pass systems, the feed solution is brought to contact with the membrane module only once (Fig. 5). This limits the attainable concentration of the retentate. Batch systems return the retentate continuously to the feed tank for recycling through the membrane module (Fig. 6). Because the permeate is collected in another tank, the retentate is concentrated continuously until the desirable retentate concentration is reached. Feed-and-bleed systems are essentially a combination of the batch and single-pass operations (Fig. 7). Initially, the retentate is totally recycled similar to a batch system until the final retentate concentration is reached. At this point, a portion of the retentate is bled from the system, and fresh feed is added to the recycle loop. The quantity of feed into the loop is controlled at a rate equal to the permeate flowing out of the loop plus the amount of the retentate being taken out of the system. Multistage operations are achieved by linking two or more feed-and-bleed stages together (Fig. 8). The stages are operated in series with respect to the retentate/feed flow but in parallel for permeate flow. Each stage operates at a constant feed concentration, which increases from the first to

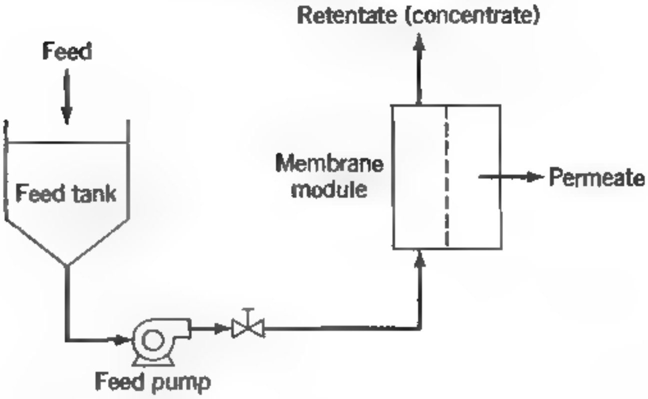

Retentate (concentrate)
Feed
Feed tank
Membrane module
Permeate
Feed pump

Figure 5. A schematic diagram of single-pass UF or RO system.

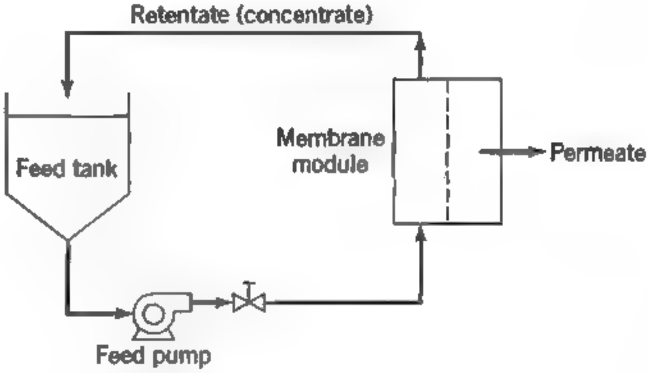

Retentate (concentrate)
Feed tank
Membrane module
Permeate
Feed pump

Figure 6. A schematic diagram of batch UF or RO system.

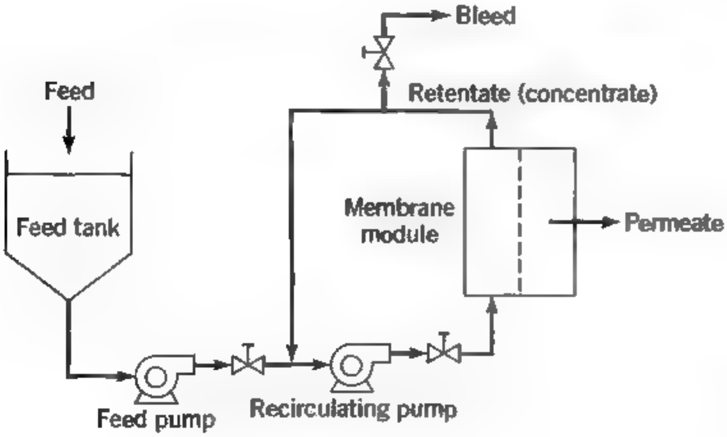

Bleed
Feed
Retentate (concentrate)
Feed tank
Membrane module
Permeate
Feed pump
Recirculating pump

Figure 7. A schematic diagram of feed-and-bleed UF or RO system.

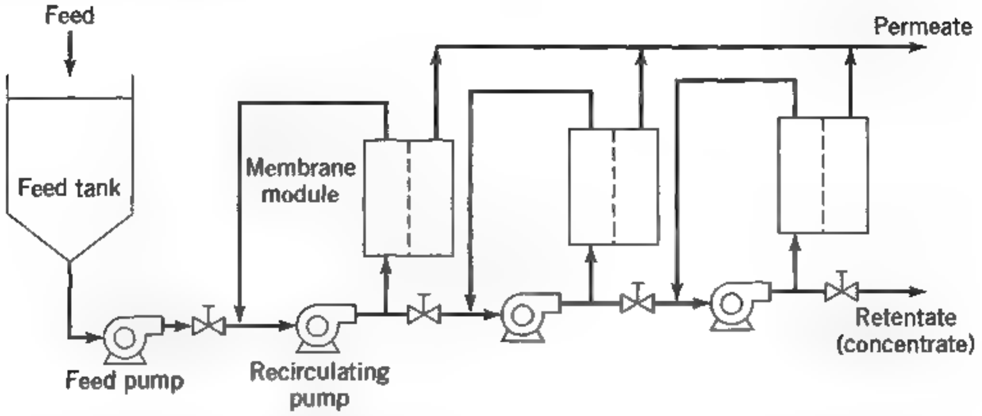


Figure 8. A schematic diagram of multistage recycle UF or RO system (only three stages are shown).

the last stage. The final stage operates at the desired retentate concentration. The advantage of the multistage system is that the retentate can be concentrated to high levels without the long residence time that occurs in a batch system, which can lead to potential microbial problems (12).

EFFECTS OF OPERATING PARAMETERS

The performance of a UF system is quantified by permeate flux. There are four important operating parameters that affect the flux: pressure, temperature, feed concentration, and flow rate. The pressure-flux behavior in typical UF processes is pressure dependent at low pressures and pressure independent at high pressures (5); increasing pressure increases permeate flux when the pressure is low, but permeate flux becomes independent of pressure when the pressure is high. Both the resistance model and the osmotic pressure model describe correctly the observed pressure-flux behavior of the UF processes (13). In the resistance model, the increase in pressure increases the flux and compacts the boundary layer. The compaction of the boundary layer increases its hydraulic resistance and thus opposes further increases in flux. On the other hand, the osmotic model does not consider the hydraulic resistance of the boundary layer. The increase in pressure increases the concentration of solute at the membrane surface and, therefore, the osmotic pressure. The increase in the osmotic pressure opposes the increase in flux. The mathematical expressions for the resistance model and the osmotic pressure model are shown in the following equations (5,13). The resistance model:

$$J = \frac{\Delta P}{r_m + r_b} = \frac{\Delta P}{r_m + \varphi \Delta P}$$

The osmotic pressure model:

$$J = \frac{\Delta P - \Delta\pi}{r_m}$$

where J is the permeate flux, ΔP is the pressure difference, r_m is the membrane resistance, r_b is the resistance due to the boundary layer and gel-polarized layer, φ is the proportional coefficient between r_b and ΔP, and $\Delta\pi$ is the osmotic pressure.

Higher temperatures generally favors the increase in flux of the UF processes. This is because increasing temperature reduces the solution viscosity. Cheryan (5) suggested that it is best to operate UF at the highest possible temperature that is within the limits of the feed solution and the membrane. Increasing feed concentration increases the viscosity and density of the solution, but reduces the solute diffusivity; the net results are increased concentration polarization and increased thickness of the boundary layer. Observed experimental results, in general, show that the flux decreases exponentially with increasing feed concentration (5). On the other hand, increasing flow rate or turbulence tends to remove the accumulated solids near the membrane surface and reduce concentration polarization near the membrane surface and the thickness of the boundary layer. Therefore, the permeate flux increases.

In addition to permeate flux, the performance of a reverse osmosis (RO) system is also quantified by solute rejection. Similar to a UF system, pressure, temperature, feed concentration, and flow rate are four major influencing processing parameters. The effect of pressure on permeate flux and solute rejection is complex. Although permeate flux increases with pressure, the osmotic pressure at the membrane surface also increases because of increased solute concentration. The latter tends to reduce the increase of permeate flux. Because the solute flux is not driven by pressure, it is not affected by increasing pressure. Therefore, increasing pressure increases solute rejection. However, increased concentration at the membrane surface indirectly increases the solute flux, which tends to moderate the increase in solute rejection.

Temperature increases both permeate flux and solute flux. This is because increasing temperature reduces viscosity of the permeate and increases diffusivity and solubility of the solute in the membrane (13). Because the increase in solute flux with temperature is usually faster than the increase in permeate flux, solute rejection decreases with increasing temperature.

Increasing feed concentration reduces permeate flux by increasing osmotic pressure at the membrane surface because of increased solute concentration. Because solute flux is concentration driven, increased solute concentration or concentration polarization at the membrane surface increases solute flux. Both increased solute flux and reduced permeate flux cause rapid reduction in solute rejection.

The effect of flow rate on permeate flux and solute rejection is similar to that of pressure (13). Increasing flow rate reduces concentration polarization near the membrane surface and the thickness of the boundary layer. Thus, permeate flux increases, causing the osmotic pressure to increase; this tends to reduce or moderate the permeate flux. The initial reduction in the concentration polarization caused by increase in flow rate decreases solute flux and hence increases solute rejection. However, this is also reduced or moderated by the osmotic pressure increases since solute flux is concentration driven.

APPLICATIONS IN FOOD INDUSTRIES

Dairy Products

UF and RO are finding increased applications in the dairy products. Milk and skim milk are concentrated for the manufacture of soft and hard cheeses (14–16), dried whole milk and skim milk (17), ice cream (18), milk beverages (19), condensed milk (20), and yogurt (14,21,22). Proteins recovered from sweet and acid wheys are by-products from the manufacture of the various kinds of cheeses. Lactose can be separated from wheys for uses in alcohol or beer production (23).

Cereal and Oilseeds

For cereal and oilseeds, UF and RO are used for (*1*) recovery of proteins from ground coconut meat (24), cottonseed and soy flour (25–27), and rapeseed meal (28); (*2*) removal of undesirable components such as raffinose, stachyose, and phytic acid from soy milk (29); (*3*) treatment and processing of cottonseed and soy whey (30,31); (*4*) protein modification (32); and (*5*) degumming of edible oils (33).

Agricultural Raw Materials and By-products

For agricultural raw materials and by-products, notable applications for UF and RO are (*1*) recovery of meat proteins from animal blood and meat waste (1); (*2*) recovery of useful by-products from potato, sweet potato, and wheat starch processes (34–36); and (*3*) removal of glucose while concentrating egg white (37–39).

Others

UF and RO have found many other applications. Examples include concentration fractionation, or purification of alfalfa juice (40), apple juice (41–44), beet juice (45,46), cane juice (47), citrus juices (48), maple sap (49), onion juice (50), orange juice (51), passion fruit juice (52), perilla anthocyanins (53), strawberry juice (54), tomato juices (55), and vegetable juices (56). UF and RO are also finding many applications in biotechnology (5,57) and removal of toxic components from rapeseed meal extracts (58). The discussion is beyond the scope of this article, however.

BIBLIOGRAPHY

1. M. C. Porter and L. Nelson, "Ultrafiltration in the Chemical, Food Processing, Pharmaceutical, and Medical Industries," in N. N. Li, ed., *Recent Developments in Separation Science*, Vol. 2, CRC Press, Boca Raton, Fla., 1972, pp 227–267.
2. P. Fellows, "Membrane Concentration," in P. Fellows, ed., *Food Processing Technology*, Ellis Horwood, Chichester, London, 1988, pp. 148–158.
3. S. Sourirajan, *Reverse Osmosis*, Academic Press, New York, 1970.
4. T. Matsuura and S. Sourirajan, *Advances in Reverse Osmosis and Ultrafiltration*, National Research Council Canada Publ., Ottawa, Canada, 1989.
5. M. Cheryan, *Ultrafiltration and Microfiltration Handbook*, Technomic Publ. Co., Lancaster, Pa., 1997.
6. R. E. Kesting, *Synthetic Polymeric Membranes*, McGraw-Hill, New York, 1971.
7. R. L. Riley, G. R. Hightower, and C. R. Lyons, "Preparation, Morphology, and Transport Properties of Composite Reverse Osmosis Membranes for Seawater Desalination," in H. K. Longsdale and H. E. Podall, eds., *Reverse Osmosis Membrane Research*, Plenum Press, New York, 1972, pp. 437–456.
8. T. Matsuura, *Synthetic Membranes and Membrane Separation Processes*, CRC Press, Boca Raton, Fla., 1994.
9. H Strathmann, "Membrane Separation Processes," *J. Membrane Sci.* **9**, 121–189 (1981).
10. D. R. Lloyd and T. B. Meluch, "Selection and Evolution of Membrane Materials for Liquid Separations," in D. R. Lloyd, ed., *Materials Science of Synthetic Membranes*, American Chemical Society, Washington, D.C., 1985, pp. 47–79.
11. J. E. Cadotte, "Evolution of Composite Reverse Osmosis Membranes," in D. R. Lloyd, ed., *Materials Science of Synthetic Membranes*, American Chemical Society, Washington, D.C., 1985, pp. 273–294.
12. F. V. Kosikowski, "Membrane Separations in Food Processing," in W. C. McGregor, ed., *Membrane Separations in Biotechnology*, Marcel Dekker, New York, 1986, pp. 201–254.
13. J. D. Mannapperuma, "Design and Performance Evaluation of Membrane Systems," in K. J. Valentas, E. Rotstein, and R. P. Singh, eds., *Handbook of Food Engineering Practice*, CRC Press, Boca Raton, Fla., 1997, pp 167–209.
14. H R. Chapman et al., "Use of Milk Concentrated by Ultrafiltration for Making Hard Cheese, Soft Cheese and Yogurt," *J. Soc. Dairy Technol.* **27**, 151–155 (1974)
15. F. L. Davies, P. A. Shankar, and H M. Underwood, "The Use of Milk Concentrated by Reverse Osmosis for the Manufacture of Yogurt," *J. Soc. Dairy Technol.* **30**, 23–28 (1977).
16. D. J. Phillips, "Application of Membrane Processing to Cheese Manufacture," in R. W. Field and J. A. Howell, eds., *Process Engineering in the Food Industry*, Elsevier Applied Science, London, 1989, pp 249–258.
17. J. Abbot et al., "Application of Reverse Osmosis to the Manufacture of Dried Whole Milk and Skim Milk," *J. Dairy Res.* **46**, 663–672 (1979).
18. A. Bundgaard, "Hyperfiltration of Skim Milk for Ice Cream Manufacture," *Dairy Ind.* **39**, 119–122 (1974).
19. F. V. Kosikowski, "Low Lactose Yogurts and Milk Beverages by Ultrafiltration," *J. Dairy Sci.* **62**, 41–46 (1979).

20. D. Serpa Alverez, M. Bennasar, and B. De la Fuente, "Application of Ultrafiltration to the Manufacture of Sweetened Condensed Milk," *Le Lait* **59**, 376–386 (1979).
21. G. Morgensen, "Production and Properties of Yogurt and Ymer Made from Ultrafiltered Milk," *Desalination* **35**, 213–222 (1980).
22. P. H. Ferguson, "Membrane Processing in the Food and Dairy Industries," in R. W. Field and J. A. Howell, eds., *Process Engineering in the Food Industry*, Elsevier Applied Science, London, 1989, pp. 237–247.
23. T. I. Hedrick, "Reverse Osmosis and Ultrafiltration in the Food Industry: Review," *Drying Technol.* **2**, 329–352 (1984).
24. P. Chakraborty, "Functional Properties of Coconut Protein Isolate by Ultrafiltration," in M. Le Maguer and P. Jelen, eds., *Food Engineering and Process Applications*, Vol. 2, Elsevier Applied Science, London, 1986, pp. 305–315.
25. J. T. Lawhon et al., "Production of Protein Isolates and Concentrates from Oilseed Flour Extracts Using Industrial Ultrafiltration and Reverse Osmosis Systems," *J. Food Sci.* **42**, 389–394 (1977).
26. O. Omosaiye and M. Cheryan, "Ultrafiltration of Soybean Water Extracts: Processing Characteristics and Yields," *J. Food Sci.* **44**, 1027–1031 (1979).
27. N. Devereux and M. Hoare, "Membrane Recovery of Soluble and of Precipitated Soya Proteins," in M. Le Maguer and P. Jelen, eds., *Food Engineering and Process Applications*, Vol 2, Elsevier Applied Science, London, 1986.
28. Y. Tzeng, L. L. Diosady, and L. J. Rubin, "Preparation of Rapeseed Protein Isolate by Sodium Hexametaphosphate Extraction, Ultrafiltration, Diafiltration, and Ion-Exchange," *J. Food Sci.* **53**, 1537–1541 (1988).
29. H. G. Ang, W. L. Kwik, and C. K. Lee, "Ultrafiltration Studies of Foods: Part 1—The Removal of Undesirable Components in Soymilk and the Effects on the Quality of the Spray-Dried Powder," *Food Chem.* **20**, 183–199 (1986).
30. E. S. K. Chian and M. A. Aschauer, "Effect of Freezing Soybean Whey upon the Performance of Ultrafiltration Process," in G. F. Bennett, ed., *Industrial Waste Water Treatment*, AIChE Symp. Ser., No. 144, 1974, pp. 163–169.
31. J. T. Lawhon, et al., "Fractionation and Recovery of Cottonseed Whey Constitutes by Ultrafiltration and Reverse Osmosis," *Cereal Chem.* **52**, 34–41 (1975).
32. S. D. Cunningham, C. M. Cater, and K. F. Mattil, "Cottonseed Protein Modification in an Ultrafiltration Cell," *J. Food Sci.* **43**, 1477–1480 (1978).
33. C. D. O'Donnell, "Lipid Science: Refining Knowledge of Fats & Oils," *Prepared Foods* **167**, 87–90 (1998).
34. E. O. Strolle et al., "Recovering Useful Byproducts from Potato Starch Factory Waste Effluents," *Food Technol.* **34**, 90–95 (1980).
35. F. Meuser and F. Köbler,"Membrane Filtration of Process Water from Potato and Wheat Starch Plants," in C. Cantarelli and C. Peri, eds., *Progress in Food Engineering*, Forster-Verlag AG, Küsnacht, Switzerland, 1983, pp. 335–340.
36. B. H. Chiang and W. D. Pan, "Ultrafiltration and Reverse Osmosis of the Water from Sweet Potato Starch Process," *J. Food Sci.* **51**, 971–974 (1986).
37. E. Lowe et al., "Egg White Concentrated by Reverse Osmosis," *Food Technol.* **23**, 45–54 (1969).
38. R. E. Payne, C. G. Hill Jr., and C. H. Amundson, "Concentration of Egg White by Ultrafiltration," *J. Milk Food Technol.* **36**, 359–363 (1973).
39. L. S. Tsai, K. Ijichi, and M. W. Harris, "Concentration of Egg White by Ultrafiltration," *J. Food Protect.* **40**, 449–455 (1977).
40. B. E. Knuckles et al., "Pilot Scale Ultrafiltration of Clarified Alfalfa Juice," *J. Food Sci.* **45**, 730–734, 739 (1980).
41. F. Chou, R. C. Wiley, and D. V. Schlimme, "Reverse Osmosis and Flavor Retention in Apple Juice Concentration," *J. Food Sci.* **56**, 484–487 (1991).
42. M. J. Sheu and R. C. Wiley, "Preconcentration of Apple Juice by Reverse Osmosis," *J. Food Sci.* **48**, 422–429 (1983).
43. O. I. Padilla and M. R. McLellan, "Molecular Weight Cut-off of Ultrafiltration Membranes and the Quality and Stability of Apple Juice," *J. Food Sci.* **54**, 1250–1254 (1989).
44. V. Gokmen, Z. Bornemman, and H. H. Nijhuis, "Improved Ultrafiltration for Color Reduction and Stabilization of Apple Juice," *J. Food Sci.* **63**, 504–507 (1998).
45. S. Landi et al., "Purification of Beet Raw Juice by Means of Ultrafiltration Membranes," *Z. Zuckerind* **24**, 585–591 (1974).
46. A. Bayındirli, F. Yildız, and M. Özilgen, "Modeling of Sequential Batch Ultrafiltration of Red Beet Extract," *J. Food Sci.* **53**, 1418–1422 (1988).
47. R. F. Madsen, "Application of Ultrafiltration and Reverse Osmosis to Cane Juice," *Int. Sugar J.* **75**, 163–167 (1973).
48. R. J. Braddock, S. Nikdel, and S. Nagy, "Composition of Some Organic and Inorganic Compounds in Reverse Osmosis-Concentrated Citrus Juices," *J. Food Sci.* **53**, 508–512 (1988).
49. C. O. Willits, J. C. Underwood, and U. Merten, "Concentration by Reverse Osmosis of Maple Sap," *Food Technol.* **21**, 24–26 (1967).
50. J. S. Nuss and D. E. Guyer, "Concentration of Onion Juice Volatiles by Reverse Osmosis and Its Effects on Supercritical CO_2 Extraction," *J. Food Process Eng.* **20**, 125–139 (1997).
51. B. G. Medina and A. Garcia, "Concentration of Orange Juice by Reverse Osmosis," *J. Food Process Eng.* **10**, 217–230 (1988).
52. Z. R. Yu and B. H. Chiang, "Passion Fruit Juice Concentration by Ultrafiltration and Evaporation," *J. Food Sci.* **51**, 1501–1505 (1986).
53. M. Y. Chung, L. S. Hwang, and B. H. Chiang, "Concentration of *Perilla Anthocyanins* by Ultrafiltration," *J. Food Sci.* **51**, 1494–1497, 1510 (1986).
54. S. Rwabahizi and R. E. Wrolstad, "Effects of Mold Contamination and Ultrafiltration on the Color Stability of Strawberry Juice and Concentrate," *J. Food Sci.* **53**, 857–861, 872 (1988).
55. D. Pepper, A. C. J. Orchard, and A. J. Merry, "Concentration of Tomato Juices and Other Fruit Juices by Reverse Osmosis," in R. W. Field and J. A. Howell, eds., *Process Engineering in the Food Industry*, Elsevier Applied Science, London, 1989, pp. 281–290.
56. S. S. Köseoglu, J. T. Lawhon, and E. W. Lusas "Vegetable Juices Produced with Membrane Technology," *Food Technol.* **45**, 124–130 (1991).
57. W. C. McGregor, *Membrane Separations in Biotechnology*, Marcel Dekker, New York, 1986.
58. M. J. Lewis and T. J. A. Finnigan, "Removal of Toxic Components Using Ultrafiltration," in R. W. Field and J. A. Howell, eds., *Process Engineering in the Food Industry*, Elsevier Applied Science, London, 1989, pp. 291–306.

FU-HUNG HSIEH
University of Missouri
Columbia, Missouri

UNITED STATES: ARMED FORCES FOOD RESEARCH AND DEVELOPMENT

Food and foodservice equipment research and development for the Army, Navy Air Force, Marine Corps and the Defense Logistics Agency is conducted at the U.S. Army Natick Research, Development and Engineering Center in Natick, Massachusetts. Since 1970, Natick has served as the executive agent for the Department of Defense Food Program, conducting research, development and engineering to meet the unique feeding needs of the Armed Forces through a combination of in-house and contractual efforts.

Research and Development conducted under the DOD Food Program can generally be divided into two categories: Technology Base and Military Service Requirements. Technology Base efforts encompass research in areas of known or anticipated technology gaps that impede solution of current problems or development of future systems to meet military feeding needs. Military Service Requirements are recognized, validated needs identified by one or more of the Services and generally address known current deficiencies or needs contemplated in the next three to seven years.

The DOD Food and Nutrition Research and Engineering Board (FNREB), consisting of representatives of each of the four military Services and other involved organizations, functions as a board of directors, providing direction to Natick in formulation of the DOD Food Program. The FNREB meets annually to prioritize the projects, including initiation of effort on new requirements identified by the Services during the preceding year and revalidation of the need for continuation of ongoing work.

Each Service maintains a full-time representative at Natick as a member of the Joint Technical Staff (JTS). These representatives provide military guidance to the Natick scientists relative to their Services' projects and provide liaison between Natick personnel and the military community. They assist in matters such as organizing field tests and provide a communication link between the item developer (Natick) and the customers, the military planners and men and women in the field, aboard ships and on aircraft. The JTS members represent the FNREB on day-to-day actions and participate fully in all aspects of the DOD Food Program formulation and execution.

The annual program, formulated and prioritized as described above, is executed in three Natick elements, the Food Engineering Directorate, the Soldier Science Directorate and the Advanced Systems Concepts Directorate, whose specific functions are summarized below.

FOOD ENGINEERING DIRECTORATE

The Food Engineering Directorate (FED) is responsible for design, development and evaluation of military rations and foodservice equipment, to fulfill the requirements of the Army, Navy, Air Force, and Marine Corps, as well as special requirements to support the Defense Logistics Agency. Response to identified needs of the above Services involves long-range research, item development and engineering support to procurement agencies for food and foodservice equipment during the entire life of the item. The end products of FED's effort are Technical Data Packages (specification, drawings, etc), which are used to procure items from commercial suppliers.

The Food Engineering Directorate is divided into three product divisions and a support division. The product-oriented divisions are the Technology Acquisition Division, the Food Technology Division, and the Food Equipment and Systems Division.

The Technology Acquisition Division (TAD) is structured and staffed to conduct basic research and exploratory development involving new technologies for food preservation and processing, and for foodservice equipment and operations. The TAD's function is to explore, assess, adapt and refine new materials, equipment and operational concepts as basis for establishing new technological opportunities for exploitation to meet near- and long-term military feeding requirements. As with all FED Divisions, the emphasis on R&D conducted by TAD is on military-unique requirements, for which there are no civilian counterparts.

Exploratory and feasibility studies are conducted on new foods and new processing techniques needed to provide optimally configured and formulated field rations, which can be assembled from a limited number of basic components, to meet specific needs, such as high altitude or low temperature operating environments, for future military scenarios. Objectives of studies on foods and processes include, in addition to optimizing nutrient content, extending shelf life, improving product functionality and reducing overall food costs through means such as automating processing technologies and developing methods to objectively monitor quality and serviceability of food products in storage.

Research and engineering studies on food service equipment encompass diverse areas, including combustion technology, energy conversion technologies, robotics, electronics, material science and sanitation technologies. The goal of these studies is to assure that new and emerging technologies are applied to military food service equipment needs, and to develop new technology as necessary to meet anticipated unique military needs for feeding military personnel on the "high-tech" battlefields of the future.

The Food Technology Division (FTD) conducts exploratory development, advanced development and engineering programs to meet current and future military feeding needs. Division responsibilities include development of new or improved foods, food processes and operational rations in response to specific Military Service Requirements. Disciplines include food technology, packaging technology, and physical sciences. Product development and evaluation are focused primarily on operational rations for field feeding and include design of rations to meet designated nutritional content, acceptance by troops, serviceability, storage life, and convenience of use under field conditions. Commercial items are evaluated for suitability and are utilized wherever possible, or modified, if feasible, to meet any special requirements resulting from the unique military environment. This Division is responsible for preparation of technical requirements for approximately 700 procurement documents and for providing engineering and technical support to the Defense Personnel

Support Center, the agency responsible for purchasing foods and operational rations used by all military services.

The Food Equipment and Systems Division (FESD) is responsible for development, testing and fielding of new or improved foodservice equipment and systems in support of Military Services Requirements. Its mission includes evaluation of commercial foodservice equipment and methods, including foreign military equipment, for applicability to military requirements. Advanced and engineering development tasks are accomplished through in-house design and contracted design and fabrication of prototype equipment. Development of foodservice equipment encompasses all aspects of evaluation, including laboratory and field testing, to assure that all requirements are met.

In addition to specific equipment development and testing, this Division is responsible for managing the development of selected systems throughout the acquisition cycle. Systems engineering activities involve integration of components, ie, food, packaging, preparation and serving equipment, into a total feeding system. Engineering support is provided to procuring activities throughout the life cycle of the hardware system.

ADVANCED SYSTEMS CONCEPTS DIRECTORATE

The Advanced Systems Concepts Directorate (ASCD) combines the functions of advanced systems development, long-range planning, and interfacing with military customers. With respect to foodservice systems, ASCD works closely with each of the uniformed Services through the DOD Food and Nutrition Research, Development and Engineering Program. From its creation in 1970, as the Operations Research and Systems Analysis Office, ASCD has evolved into an organization employing roughly 20 individuals working in the foodservice concept development and long-range planning arena. Typically, there are 10 to 15 projects being pursued in ASCD's portion of the food program. These projects place ASCD in the forefront of developing the most advanced feeding system concepts for land, sea, and air military missions.

ASCD applies a systems approach, which uniquely combines appropriate statistical and mathematical modeling techniques with field verification when possible. This approach provides analyses of the benefits and characteristics of the existing system and alternatives, and validates the performance of the selected candidate system with field experiments. The success of this approach has been demonstrated in the development and rapid adoption of a new foodservice system concept for aircraft carriers, the development and fielding of a new concept for combat field feeding in the Army, and the development and deployment of a new feeding concept for Air Force Ground-Launched Cruise Missiles.

Current systems analysis development projects include a foodservice concept for the proposed Peacekeeper Rail Garrison system. Peacekeeper units must operate without assigned foodservice personnel, without resupply, and, despite extreme space constraints, must still provide high quality food for the crews. The problems of providing quality foodservice to the Navy's antisubmarine warfare squadrons is the subject of another project wherein the current system cannot provide the flexibility required by air crews engaged in long surveillance and combat missions. Also for the Navy, ASCD is developing a new concept for disposal of plastic waste products. Under international treaty, by 1993 the Navy was to cease disposing of plastics at sea. With the large volume of plastic packaging generated by aircraft carriers, which are essentially floating cities containing over 5,000 individuals, and with industry's trend toward increasing use of plastics packaging material, this is a crucial project addressing a highly visible problem.

In addition to projects involving long-term improvements to current systems, ASCD is involved in the definition and evaluation of "Next Generation" (1990s) and "Future Systems" (2000 and beyond), recognizing future threats and warfighting concepts. One example is a project to explore concepts for undersea subsistence storage and resupply. Another project is looking at the value of concepts involving mobile, automated food production systems in terms of futuristic battlefield threats and warfighting concepts.

Working closely with representatives from the Food Engineering and Soldier Science Directorates, ASCD also conducts a proactive program to obtain feedback from users in the field. For this purpose ASCD representatives makes a large number of visits to Army installations worldwide. These visits familiarize General Officers and their staffs with Natick's products as well as providing opportunities for collecting survey data from soldiers using those products in field exercises.

SOLDIER SCIENCE DIRECTORATE

The Soldier Science Directorate has the mission of science and technology leadership for all of Natick's products and systems in support of the individual soldier. The Directorate, a multidisciplinary research organization, draws from the behavioral, biological, physical, chemical, and engineering sciences to maintain a decided edge in technology in order to avoid surprise and to ensure the timely transfer of information to Natick's product Directorates, other Army research and development activities, industry, and academia. The SSD thrusts in food science and technology are aimed at providing soldiers with acceptable, nutritionally complete and environmentally stable meals for the whole spectrum of operational scenarios for the battlefield of the future.

The Directorate is organized into three divisions known as the Behavioral Sciences Division, the Biological Sciences Division, and the Physical Sciences Division. Approximately half of the Directorate's 129 permanent civilian and 17 military personnel have doctorate or master's degrees.

The Behavioral Sciences Division provides human factors support to all projects and programs at Natick. Its Food Systems Human Factors Branch has responsibility for supporting ration design and development. Modern marketing research techniques are being introduced into the behavioral research efforts in order to develop sensory and behavioral rules, criteria, and limits for optimizing the acceptance of novel rations and to engineer into these rations the desirable sensory characteristics that will ensure their acceptance and consumption in the targeted field environment. Methods are being developed to attenuate or eliminate the effects of jet lag on individuals by modifying diet or feeding patterns prior to, during, or after time zone changes. Artificial intelligence technology is being implemented to aid in configuring ration components that optimally meet the nutrition and load-carrying requirements for a given mission. An expert system is being developed to aid soldiers who have to forage for food in unfamiliar and hostile environments.

The Biotechnology Branch of the Biological Sciences Division focuses on the development of new food packaging based on biopolymers. These materials, which provide long-storage stability due to low oxygen permeability, can be microwaved and are readily biodegradable. Efforts are also underway to develop a survival kit with the capability to convert environmental substrates into edible materials through the action of enzymes and/or microorganisms.

The Biohazard Assessment and Control Branch of the Biological Sciences Division studies the nutritional adequacy and stability of rations and the microbiological quality of military rations stored for varying lengths of time under a wide range of temperature and humidity levels. The research focus is on developing preservative systems that minimize oxidative events causing membrane damage during processing or storage. Other projects are directed toward developing a nonanimal assay to aid in the selection of fats for use in energy-dense military foods, improved thermal processing techniques using combinations of pH, water activity, and preservatives to prevent microbial growth in current and future combat ration components, and water extraction, recovery, and generation concepts for enhanced ability to supply potable drinking water under combat feeding conditions.

The primary contribution of the Physical Sciences Division to rations research is in the development of new or improved techniques for materials testing. Among other important contributions, the Physical Sciences Division has developed more sensitive as well as more precise methodology for measuring the amount of oxygen permeation of packaging materials. This division also possesses the capability to provide analytical support plus textural engineering via electron microscopy characterization of membranous materials used for encapsulating and packaging rations.

The Divisions within the Soldier Science Directorate do not pursue their scientific and technological objectives in a vacuum. Rather, they work closely with each other, other product directorates at Natick, and outside agencies. The Directorate maintains agreements with other federal agencies, academia, and industry for the pursuit of solutions to common perplexing scientific and engineering problems. The focus of all these efforts is to ensure that new rations, materials, and packaging processes are consistent with improving the total performance, health, and safety of today's and tomorrow's soldier, whether operating under peacetime training or extremely hostile combat conditions.

GERALD L. SCHULZ
D. PAUL LEITCH
STANLEY HOLGATE
U.S. Army Natick Research, Development and Engineering Center
Natick, Massachusetts

UNITED STATES DEPARTMENT OF AGRICULTURE

In the United States, the principal in-house research agency of the U.S. Department of Agriculture (USDA) is the Agriculture Research Service (ARS). It is one of the four component agencies of the Research, Education, and Economic (REE) mission area.

This article provides an overview of research activities conducted by the USDA in cooperation with universities in the country. The information has been adapted from its website, *http://www.ars.usda.gov*.

MISSION OF AGRICULTURE RESEARCH SERVICE

ARS conducts research to develop and transfer solutions to agricultural problems of high national priority and provides information access and dissemination to:

- Ensure high-quality, safe food and other agricultural products
- Assess the nutritional needs of Americans
- Sustain a competitive agricultural economy
- Enhance the natural resource base and the environment
- Provide economic opportunities for rural citizens, communities, and society as a whole

GOALS OF AGRICULTURE RESEARCH

ARS goals are:

1. To enhance the production, value, and safety of foods and other products derived from animals that have a major impact on the American economy, world markets, and the U.S. balance of trade
2. To enhance the quality of the environment through a better understanding of and building on agriculture's complex links with soil, air, and biotic resources
3. To enhance the production, value, and safety of foods and other products derived from plants that have a major impact on the American economy, world markets, and the U.S. balance of trade.

The remaining part of this article will discuss the details of these goals as expressed by the USDA national programs under the following three research areas:

1. Animal production, product value, and safety (APPVS)
2. Natural resources and sustainable agricultural systems (NRSAS)
3. Crop production, product value, and safety (CPPVS)

ANIMAL PRODUCTION, PRODUCT VALUE, AND SAFETY

Multidisciplinary research conducted by ARS in these areas is built on the following national programs discussed in the sections that follow.

Animal Genome, Germ Plasm, Reproduction, and Development

Program Rationale. Production of foods derived from animals has a major impact on the U.S. agricultural economy. Annual cash receipts of $93 billion from livestock and poultry products account for about 50% of all agricultural products. The main components of this program are designed to identify and use livestock and poultry with appropriate genotypes that will have a major impact on the quality of animal products used for food, international competitiveness, and efficiency of production.

Program Components

Animal Germ Plasm. A major goal is to identify, facilitate, acquire, and characterize potentially useful germ plasm and selection to improve efficiency of production and to retain genetic diversity of animals for future generations. Another major goal is to store gametes or embryos on a long-term basis (eg, 25 years or more). The research effort emphasizes germ plasm cryopreservation and vitrification to enhance the feasibility and efficiency under which desired germ plasm would be stored. The program includes research on the sexing of male and female sperm so as to provide sexed gametes and embryos for storage. It also includes research on stem cells, primordial cells, cell lines, and DNA as they relate to the overall objective of the national program.

Animal Genome. A major goal is to develop genomic maps and associated DNA markers to improve accuracy of selection, increase frequency of desirable genes in populations, and characterize valuable germ plasm populations. For each livestock species genetic maps of polymorphic loci must be developed with sufficient resolution to permit location, definition, and use of genes affecting economically important traits. Related program components include analyses of the fine structure of candidate genes and gene families, definition of the genetic basis of quantitative trait loci to be used to implement genetic marker–assisted selection, and development of new experimental technologies for utilization of genome information.

Reproductive Efficiency. The overall goal is to improve reproductive efficiency of livestock, poultry, and aquaculture species. Research will focus on improving reproductive performance of animals through genetics, nutrition, and health management and on management of environmental factors such as temperature and humidity. Research advances and new biotechnologies will be developed to reduce losses due to reproduction problems in all species, and to maximize reproductive output of animals that produce high-quality products.

National Animal Germ Plasm Database. The overall goal is to develop a fully operational national animal germ plasm database system that will be linked with a central and satellite database that is now part of the USDA-ARS Genetic Resources Information Network (GRIN) System at Beltsville, Maryland. Access is readily available to the public through the Internet at *http://www.ars-grin.gov/nag*. An important component of the program is to expand the database to contain descriptors and characteristics of the world's animal genetic resources and DNA sequences or mapping information on relevant animal genomes. An additional program component is development of cooperative arrangements with outside groups such as the National Association of Animal Breeders (NAAB) and the American Livestock Breeds Conservancy to fully develop the database and to establish, for each species, national species committees representing industry, scientists, and commodities.

Animal Gene Bank/Repository. The purpose of the animal gene bank/repository is to develop long-term storage space for sperm, embryos, oocytes, stem cells, cell lines, and DNA from designated genotypes covering a wide range of domestic animal species and aquaculture. An important component of this program is to establish a national committee on cryopreservation to work with species committees. This national committee will establish priorities for genetic material to be maintained in repositories and will define long-term storage requirements for this material.

Animal-Production Systems

Program Rationale Producers are challenged with integrating knowledge from diverse disciplines into production practices suitable for their individual operation. Research on food animal–production systems assesses the interactions between nutrition, genetics, reproduction, physiology, microbiology, immunology, and molecular biology and related effects on animal health, productivity, and the environment. Improvements in the production efficiency of individual components are often realized in the total system and ensure a continuing supply of economical nutrient-dense products for the consumer. Research leading to discoveries and applications in production efficiency, sustainability, animal and environmental well-being, and high-quality products are imperative if animal agriculture is to be economically viable.

Program Components

Animal Nutrition. Nutrition is the single most costly component in modern animal production. Suboptimal nutrition is a significant factor in the failure to realize genetic potential for production and increased susceptibility to disease. Economically optimizing nutrient supply and use is imperative for improving growth and reproduction and maximizing overall production efficiency. Research is needed in the following areas:

1. Chemical composition and availability of nutrients in feedstuffs

2. Nutritional requirements of grazing and nongrazing animals
3. More efficient use of nutrients
4. Specific attention to improving the use of environmentally sensitive nutrients such as phosphorus, nitrogen, copper, and potassium
5. Increasing understanding of nutrient partitioning between biological functions (eg, reproduction, growth, and lactation)

Integrated Animal Systems Research. Production output and efficiency of the whole animal represent the biological integration of nutritional, physiological, and genetic components. Factors that affect an individual component influence animal production, either partially or totally, through their effects on other biological components. It is imperative that research be conducted that develops our knowledge of how animals integrate nutrition, endocrinology, immunology, and genetic factors to optimize efficiency of nutrient use, reproduction efficiency, and product quantity and quality. With this knowledge, one can understand the animal as a production unit in developing new approaches and decision aids applicable to improving the sustainability of animal-production systems.

Integrated Information for Animal-Production Systems. The overwhelming amount of information about animal-production efficiency is difficult to use without the aid of computer-based technology. This technology application is needed to improve management decisions and strategies that will yield the greatest economic returns. New-generation computer models are needed for evaluating production options. Decision aids are needed to integrate the components of animal production in modular forms compatible with farming systems programs. These decision aids must be useful to farmers and producers.

Animal Diseases

Program Rationale. Animal disease is the single greatest hindrance to efficient livestock and poultry production, with economic losses estimated to be 17% of production costs in the developed world and more than twice this figure in the developing world. Rapid diagnostic tests, novel genetic vaccines, immune modulatory strategies, disease-resistant genes, and increased biosecurity measures are needed to prevent or control outbreaks and the spread of animal diseases and to protect people from zoonotic diseases. Protecting the livestock industry from animal diseases ensures competitiveness of the U.S. animal agriculture in the global marketplace and promotes a sustainable and profitable production system in the United States.

Program Components

Pathogen Detection. New generations of rapid, sensitive, and specific serological and molecular diagnostic tests are needed to:

- Detect newly emerged disease pathogens
- Identify new variants of known disease pathogens
- Control or eradicate zoonotic diseases
- Control diseases that impact production trade

Epidemiology of Disease. Epidemiological surveillance can better define the economic impact of livestock diseases, enabling animal producers to better understand the ecology of emerging disease and natural transmission cycles. This is needed to develop strategies to prevent disease. The emergence or introduction of an exotic disease into the United States could rapidly escalate into an epidemic due to lack of resistance in host animals, absence of vaccines or effective drugs, and limited resources to effectively manage the spread of these pathogens.

Mechanism of Disease. A better understanding of the molecular and cellular mechanisms responsible for disease requires studies of disease agents, the host animal's response, and the environment. The genetic basis for pathogenicity differences among selected viral, bacterial, mycotic, and parasitic agents affecting livestock is needed to define the molecular factors that promote the emergence of highly virulent pathogens and to provide an understanding of how virulence correlates with pathologic manifestation of disease. The interaction of host-specific and environmental factors such as the role of air quality and incidence of respiratory disease is needed to develop immune modulatory strategies for improved vaccine efficiency. Mechanisms controlling vector–pathogen–host interactions will be investigated. Molecular tools will aid in determining the method and frequency of pathogen persistence in recovered and subclinically infected animals.

Genetic Resistance to Disease. The genetic basis, when present, for disease resistance to parasitic diseases; arthropod-vectored viral diseases; oncogenic viral diseases, such as Marek's disease; bacterial diseases, such as Johne's disease and scrapie will be investigated. Host immunological and physiological responses will be related to genes and genetic markers. Immunogenetic studies will lead to the identification of genes of pathologic significance that can be used to develop novel non-immunity-based disease-control strategies.

Disease Prevention/Control through Vaccines and Novel Strategies. To prevent animal disease, the biology and ecology of pathogens must be understood and their weaknesses exploited. The knowledge of the variation in genetic sequences of genes from pathogens could explain the basis for some vaccine failures as well as provide a rational approach to developing improved vaccines. Modification of existing vaccines to enhance effectiveness and safety will be adequate in some cases. Development and testing of new subunit, whole virus, viral-vectored, deletion mutants, naked DNA, and peptide vaccines for the prevention of important livestock diseases will lead to a new generation of more-effective vaccines. The evaluation of new vaccination schemes and immune modulators to improve herd protection against selected diseases will provide better disease protection. Identification of environmental factors that contribute to disease and the design of improved management practices to control diseases are an integral part of disease-control programs.

Arthropod Pests of Animals and Humans

Program Rationale Animal pests and pathogens are a serious threat to the U.S. agricultural economy, to food safety, and to human health. Losses caused by pests and pathogens amount to hundreds of millions of dollars annually and comprise productivity losses and damage to the quality of animal products. These problems restrain the exportation of animals and animal products from the United States and lead to higher prices in the domestic market. Arthropods that transmit human disease pose risks to the health of U.S. military troops deployed overseas and to the global human population. Pesticides and drugs used for the control of pests and pathogens pose a distinct and identifiable threat to animal and human health. Research is needed to develop safe, efficacious, environmentally benign, and user-friendly methods for the detection, surveillance, and control of pests and for the development of new and effective vaccines and immunomodulators, biologically based pest- and pathogen-control strategies, and the development and use of pest- and pathogen-resistant livestock.

Program Components

Ticks and Mites (Cattle Fever Ticks, Lyme Ticks, and Mange Ticks). Research focus is on the development and application of technologies for detection, surveillance, monitoring, pesticide-resistance management, and biologically and ecologically based area-wide control.

Blood-Sucking and Filth Flies. Research thrust is on the development, integration, application of technologies for detection, surveillance, monitoring, pesticide-resistance management, and biologically and ecologically based control.

Athropod-Borne Animal Pathogens. Research thrust is on the development and application of technologies for detection, surveillance, and control of pathogens on methods to enhance production, quality, and safety of animals and animal products; and on the development of a database to support research on pests and pathogens of veterinary and medical importance.

Arthropod-Borne Diseases. Research thrust is on the development of information technology leading to intervention strategies to prevent or reduce arthropod-borne pathogen infections in animals and humans and to reduce the risk of U.S. animal livestock from domestic, exotic, and emerging arthropod-borne animal pathogens.

Repellent for Personal Protection from Biting Insects and Arthropod-Borne Diseases. Research thrust is on the development of new personal-protection technology for use by U.S. military troops and the American public and on the development of alternatives to tropical repellents, such as deet, and permethrin, which is a toxicant used on clothing.

Household Pests. Research focus is on development, integration, and application of tools for detection, surveillance, monitoring, pesticide-resistance management, and biologically and ecologically based control.

Structural Pests. Research focus is on the development, integration, and application of tools for the detection, surveillance, monitoring, pesticide-resistance management, and biologically and ecologically based technologies for termite control.

Imported Fire Ants. Research thrust is on development and application of areawide fire ant–management technologies.

Screwworm Eradication. Research focus is on development and application of improved technologies for sterile fly production and development and application of improved technologies for detection, surveillance, monitoring, and eradication of flies.

Animal Well-Being and Stress-Control Systems

Program Rationale. Measures of well-being are needed to give producers and consumers the information they need to evaluate management practices and determine which techniques best assess the well-being of animals used for food production. Development of scientific measures of well-being and an enhanced ability to interpret such measures are crucial to the evaluation of current agricultural practices and development of improved alternatives. Stress caused by social and environmental stressors needs to be understood to limit negative impacts on production efficiency and well-being. Animal well-being research will benefit animals, producers, and ultimately consumers by reducing animal health-care costs and by improving food-production efficiencies. Lack of sensitivity to animal-welfare issues may be used in domestic marketing and as an artificial trade of animal products in world markets.

Program Components

Scientific Measures of Well-Being. Measures of well-being of food-producing animals are needed to make scientific assessments. These measures must be scientifically sound and relevant. The measurements will integrate behavioral, physiological, and productivity parameters of economic importance.

Adaptation and Adaptedness. Most food animals have been domesticated for thousands of years. Selection under intensive management conditions has occurred only recently and is oriented primarily toward the improvement of production traits. Research in this area will determine the roles that genetics and environment play in well-being. Research information on adaptedness will serve as a basis for modifying management practices. Genetic research will be evaluated to improve animal fitness and determine the basis of adaptation to environmental stressors such as heat and cold. Marker-assisted selection techniques will be explored.

Social Behavior and Spacing. With the intensification of animal agriculture and the greater number of animals at each location or in production units, a major question is whether the intensive management adversely affects an animal's well-being. Research will be conducted to provide a scientific basis for understanding the social behavior of food animals and how the quality and quantity of space influences behavior. Research to show consequences of the change in patterns of social interactions and space utilization will require an integrated research approach.

Cognition and Motivation. The mental state, fear, frustration, suffering, pleasure, and boredom of animals are major concerns of the public; however, there is currently little scientific information that can be used as a basis for addressing these concerns. Research is needed to learn how animals perceive and process sensory information from the environment and what animals learn.

Evaluate Practices and Systems to Improve Well-Being. Management practices such as transportation and slaughter, and special agricultural practices such as beak trimming, deboning, branding, tail docking, and castration, are important and necessary elements of animal management in current production systems. These practices affect the well-being of animals. Research will address evaluation of the current practices and alternative practices concerning potential pain, stress or discomfort, and production efficiency. Alternative environment systems and current management practices will be evaluated for their effect on farm animal well-being and overall goals to improve animal comfort, well-being, and production efficiency. Research to improve both production efficiency and animal well-being will be conducted.

Bioenergetic Criteria for Environmental Management. Adverse environmental conditions cause livestock and poultry losses, decreased production efficiency, and decreased animal well-being. Available technology needs to be adopted for proactively managing environmental stressors. Research to develop decision-support tools is needed to help producers deal with environmental stressors, provide protective measures, recognize livestock and poultry in distress, and take appropriate management action.

Aquaculture

Program Rationale. With increasing seafood demand, declining capture fisheries, and a fisheries trade deficit exceeding $4 billion annually, aquaculture is poised to become a major U.S. growth industry in the twenty-first century. The confirmed growth and competitiveness of U.S. aquaculture will be related to the resources invested in research and technology development. A strong research and development program for U.S. agriculture, led by ARS would offer significant benefits to both producers and consumers by enhancing production efficiency, profitability, and quality of aquaculture products and systems.

Program Components

Genetic Improvement. There has been limited genetic improvement of aquaculture, so there are major opportunities for improvement through traditional animal breeding, broodstock development, germ plasm preservation, molecular genetics, and allied technologies. Research will address improvement in growth rates, feed efficiency, survival, disease resistance, fecundity, yield, and product quality; genetic characterization and gene mapping; and conservation utilization of important aquatic germ plasm.

Integrated Aquatic Animal Health Management. Despite progress in aquatic animals, significant losses to disease still occur. Research will address improvement of survival vigor and well-being of cultivated aquatic animal stocks through integrated aquatic and health research; improved technologies and practices, such as population health management; and development of health-management products, including vaccines and therapeutics disease detection/diagnostic techniques.

Reproduction and Early Development. There are major opportunities for improved reproduction and early development of cultivated aquatic organisms. Research will advance year-round maturation and production of gametes and fry of economically valuable species; development of specialized brood stock; and improvement of systems to enhance reproduction efficiency.

Growth, Development, and Nutrition. There are substantial opportunities to improve growth, development, and nutrition of cultivated aquatic organisms. Research will advance improving survival, growth rates, feed conversion, environmental tolerances, and feed formulations and feeding strategies to reduce dependence on marine fish–based protein aquaculture diets.

Aquaculture Production Systems. There are opportunities to improve the performance of aquaculture production systems through development and application of innovative early approaches and technologies. Research will address development and successful application to aquaculture of new technologies as well as relevant existing technologies and engineering presently employed in other sectors of the economy.

Sustainability and Environmental Compatibility of Aquaculture. The goal is to protect and conserve the nation's water resources and natural environments by conducting research and technology transfer to improve the sustainability and environmental compatibility of production systems. Of primary concern is the protection and conservation of the nation's resources.

Quality, Safety, and Variety of Aquaculture Products to Consumers. The goal is to improve the quality, safety, and variety of aquaculture products through research and technology transfer. Research will address improvement of safety, freshness, flavor, texture and nutritional characteristics, and shelf life of cultivated fish and shellfish; and development of value-added products and processes.

Information and Technology Transfer. Aquaculture in the United States can benefit greatly from improvements in technology-transfer programs, information dissemination, and access information and technology generated and adopted in foreign countries. Programs that address international information-retrieval networks and partnerships improve access to important technology and development of appropriate databases linked to electronic systems to enhance information exchange.

HUMAN NUTRITION REQUIREMENTS, FOOD COMPOSITION, AND INTAKE

Program Rationale

A viable agricultural enterprise demands that producers target consumers' needs for desirable, safe, and nutritious foods.

Human nutrition science has moved from a focus on the prevention of nutrient deficiencies to an emphasis on health maintenance and reduced risks of chronic disease. Diet affects growth, development, aging, and the ability to enjoy life to its fullest. Dietary intake has been linked to risks for development of a variety of common chronic diseases. The annual economic impact of cardiovascular disease in the United States exceeds $8 billion; obesity, $86 billion; osteoporosis, $10 billion; cancer, $104 billion; and cataracts, $4 billion. The American Cancer Society esti-

Evaluate Practices and Systems to Improve Well-Being. Management practices such as transportation and slaughter, and special agricultural practices such as beak trimming, deboning, branding, tail docking, and castration, are important and necessary elements of animal management in current production systems. These practices affect the well-being of animals. Research will address evaluation of the current practices and alternative practices concerning potential pain, stress or discomfort, and production efficiency. Alternative environment systems and current management practices will be evaluated for their effect on farm animal well-being and overall goals to improve animal comfort, well-being, and production efficiency. Research to improve both production efficiency and animal well-being will be conducted.

Bioenergetic Criteria for Environmental Management. Adverse environmental conditions cause livestock and poultry losses, decreased production efficiency, and decreased animal well-being. Available technology needs to be adopted for proactively managing environmental stressors. Research to develop decision-support tools is needed to help producers deal with environmental stressors, provide protective measures, recognize livestock and poultry in distress, and take appropriate management action.

Aquaculture

Program Rationale. With increasing seafood demand, declining capture fisheries, and a fisheries trade deficit exceeding $4 billion annually, aquaculture is poised to become a major U.S. growth industry in the twenty-first century. The confirmed growth and competitiveness of U.S. aquaculture will be related to the resources invested in research and technology development. A strong research and development program for U.S. agriculture, led by ARS would offer significant benefits to both producers and consumers by enhancing production efficiency, profitability, and quality of aquaculture products and systems.

Program Components

Genetic Improvement. There has been limited genetic improvement of aquaculture, so there are major opportunities for improvement through traditional animal breeding, broodstock development, germ plasm preservation, molecular genetics, and allied technologies. Research will address improvement in growth rates, feed efficiency, survival, disease resistance, fecundity, yield, and product quality; genetic characterization and gene mapping; and conservation utilization of important aquatic germ plasm.

Integrated Aquatic Animal Health Management. Despite progress in aquatic animals, significant losses to disease still occur. Research will address improvement of survival vigor and well-being of cultivated aquatic animal stocks through integrated aquatic and health research; improved technologies and practices, such as population health management; and development of health-management products, including vaccines and therapeutics disease detection/diagnostic techniques.

Reproduction and Early Development. There are major opportunities for improved reproduction and early development of cultivated aquatic organisms. Research will advance year-round maturation and production of gametes and fry of economically valuable species; development of specialized brood stock; and improvement of systems to enhance reproduction efficiency.

Growth, Development, and Nutrition. There are substantial opportunities to improve growth, development, and nutrition of cultivated aquatic organisms. Research will advance improving survival, growth rates, feed conversion, environmental tolerances, and feed formulations and feeding strategies to reduce dependence on marine fish–based protein aquaculture diets.

Aquaculture Production Systems. There are opportunities to improve the performance of aquaculture production systems through development and application of innovative early approaches and technologies. Research will address development and successful application to aquaculture of new technologies as well as relevant existing technologies and engineering presently employed in other sectors of the economy.

Sustainability and Environmental Compatibility of Aquaculture. The goal is to protect and conserve the nation's water resources and natural environments by conducting research and technology transfer to improve the sustainability and environmental compatibility of production systems. Of primary concern is the protection and conservation of the nation's resources.

Quality, Safety, and Variety of Aquaculture Products to Consumers. The goal is to improve the quality, safety, and variety of aquaculture products through research and technology transfer. Research will address improvement of safety, freshness, flavor, texture and nutritional characteristics, and shelf life of cultivated fish and shellfish; and development of value-added products and processes.

Information and Technology Transfer. Aquaculture in the United States can benefit greatly from improvements in technology-transfer programs, information dissemination, and access information and technology generated and adopted in foreign countries. Programs that address international information-retrieval networks and partnerships improve access to important technology and development of appropriate databases linked to electronic systems to enhance information exchange.

HUMAN NUTRITION REQUIREMENTS, FOOD COMPOSITION, AND INTAKE

Program Rationale

A viable agricultural enterprise demands that producers target consumers' needs for desirable, safe, and nutritious foods.

Human nutrition science has moved from a focus on the prevention of nutrient deficiencies to an emphasis on health maintenance and reduced risks of chronic disease. Diet affects growth, development, aging, and the ability to enjoy life to its fullest. Dietary intake has been linked to risks for development of a variety of common chronic diseases. The annual economic impact of cardiovascular disease in the United States exceeds $8 billion; obesity, $86 billion; osteoporosis, $10 billion; cancer, $104 billion; and cataracts, $4 billion. The American Cancer Society estimated in 1996 that one-third of the 500,000 cancer deaths annually in the United States are due to dietary factors.

Changing population dynamics, lifestyle habits, food technologies, animal- and crop-production capabilities, and the globalization of the market for foods all demand revisions in targeting food production, distribution, and marketing strategies to assure optimization of health through nutrition. Nutrition research design and capabilities are increasingly needed to address the changing dynamics and needs of the populations to be fed and the dynamics of the food supply.

ARS is an ideal location for human nutrition research because of the proximity to research on basic agriculture. A nutrition research program within the USDA focused on dietary intake and its health significance will offer significant benefits to producers, consumers, and industry by strengthening the rational basis for agricultural and public health programs and policy.

Program Components

Changes in Needs for Nutrients Throughout the Life Cycle. One needs to understand the nutrient requirements of infants, children, adolescents, and adults to understand the effects of nutrition at different points in the life cycle on the need for nutrients later in life and the prevention of chronic disease.

What Do Americans Eat and What Are the Trends for Changes in Diet? Research is needed on the nature of the diet of individuals and populations to understand their particular risk for disease at various stages of life, as a basis for modifying food and agriculture policy and practice, and as a strategy in marketing.

Composition of the Diet. There is a need for more extensive information about key foods, which are major contributors of the U.S. population's intake of nutrients of public health significance. A national nutrient databank is needed to accurately reflect the dynamic diet of the United States, with representative data on a variety of foods, including effects of biotechnologies and ethnic, restaurant-prepared, and carry-home foods. Nutritionally significant components of food must be identified, followed by the development of analytical methods to quantify those nutrients.

Function and Metabolism of Nutrients for Cognitive and Physical Behavior. Information is required to provide an understanding of how various dietary components act to improve learning and physical performance potential.

Definition of Marginal Deficiencies or Borderline Deficiencies. Frank nutritional deficiencies are rare in the United States, but there is abundant evidence that subclinical deficiencies have profound negative effects on health, intellectual development, growth, and disease resistance. Research is needed to define borderline deficiencies and to update indicators of deficiency.

Bioavailability of Nutrients in Foods. A need exists to understand how nutrients interact and how the effects of agricultural practices including postharvest handling and cooking affect nutrient content and bioavailability. It is important in this context that nutrition research methods be adequate to meet the growing demands and opportunities for genetic engineering in plant and animal production strategies.

What Are Effective Intervention Strategies? Simply knowing the nutritional requirements for humans will not ensure adequate nutrition in the population for a variety of reasons, including poor education, low income, and inadequate access to food, among others. Research on biological outcomes is needed to identify effective ways of communicating food and nutrition knowledge to individuals and populations to elicit changes in food intake and to design effective food-assistance programs.

Nutritional Needs of a Diverse Population. Research is needed to provide an understanding of the nutritional needs of diverse individuals that differ based on gender, race, genetic backgrounds, environment, behavior, and lifestyle.

Food Safety

Program Rationale. The U.S. food supply is among the world's safest and most wholesome. However, significant food-safety problems can cause either human illness or economic losses and threaten the international competitiveness of American agriculture. The recent food safety initiative (FSI) has highlighted food safety, and in particular, the control of food-borne pathogens, as an important concern of the entire federal government. Reduction in the potential health risks to consumers from human pathogens in food is the most important food-safety goal.

Program Components

Microbial Pathogens. Determining how to reduce microbial pathogens in food products throughout food operations from farm to fork is the most urgent food-safety problem today. A major goal of this program is to develop tests that are precise, reliable, and rapid enough to detect contamination in all foods before they enter into commerce. Equally important is the development of effective, reliable, and cost-effective methods to control or eliminate pathogens in and on food-producing animals throughout production and processing. Additionally, recently recognized pathogen problems to be addressed include:

- The presence of pathogens in fruits and vegetables
- The presence and persistence of pathogens in animal waste
- Pathogen resistance to traditional processing techniques and to drugs
- The need for development of pathogen growth and survival models to support the risk-assessment process

Chemical Residues. The objective of the chemical residue program is to reduce the risks of chemical residues from animal drugs, food additives, herbicides, and pesticides and environmental contaminants that are potentially present in foods. A major goal is to develop reliable, effective, accurate, user-friendly, cost-effective residue-detection methodology that requires minimal amounts of organic solvents to detect these residues. The program also increases knowledge of the adsorption, distribution, metabolism, and excretion and toxicity of certain chemicals and environmental contaminants in food-producing animals so that animal producers gain the knowledge and the means to control their residues in foods.

Mycotoxins. The presence and potential for the presence of mycotoxins in crops not only is a direct food-safety problem but also threatens the competitiveness of U.S. agriculture in the world market. Major goals are to control aflatoxin in peanuts, corn, cottonseed, tree nuts, and figs; fumonisins in corn; and deoxynivalenol in wheat and barley through an understanding of the biology of plant–fungus interactions and toxin production in the field. Specific approaches include combined altered agronomic practices, chemical and biological control, improved plant resistance to mycotoxins through conventional plant breeding and transgenic approaches, and reduction of insect damage leading to fungal infections. This program also develops methods to measure mycotoxins in important crops.

Toxic Plants. This program component seeks to minimize exposure of animals and humans to natural toxins from poisonous plants. The research focuses on:

- Identifying toxic principles in plants to which animals might be exposed (from range or pasture)
- Determining toxic manifestations of these plants in animals
- Developing appropriate technology to reduce losses in livestock
- Developing appropriate technology to control elevated cadmium in sunflower seeds and wheat that concern some foreign markets
- Using biotechnology to reduce the presence of solanaceous alkaloids in new varieties of potatoes

NATURAL RESOURCES AND SUSTAINABLE AGRICULTURAL SYSTEMS

ARS seeks to enhance the quality of the environment through seeking a better understanding of and building on agriculture's complex links with soil, water, air, and biotic resources. Multidisciplinary research is conducted to solve problems arising from the interaction between agriculture and the environment. New practices and technologies will be developed to conserve the nation's natural resource base and balance production efficiency and environmental quality. ARS collaborates with foreign research entities to address global environmental quality problems. This research area is built on the national programs discussed in the sections that follow.

Water Quality and Management

Program Rationale. Life depends on water. Safe drinking water is mandated by the 1996 amendments to the Safe Drinking Water Act; clean water in lakes, rivers, streams, and aquifers is mandated by the 1972 Clean Water Act; and safe, dependable, and abundant food and fiber depends on safeguarding our nation's soil and water resources. Agricultural production systems that sustain and protect our national water resources while satisfying our needs for clean water and safe, dependable food and fiber supply are paramount national issues. Agriculture, however, is identified as a major nonpoint source of water contamination. Water quality can be degraded by agricultural chemicals and crop nutrients as well as by salts, toxic trace elements, and waterborne pathogens. Water and soils can be degraded by application of waste products and waters from municipal and industrial sources as well as from agricultural processing plants and agricultural animal-production practices. Innovations and improvements in American agriculture are needed to sustain our vital interests in a safe, abundant, and reliable food and fiber supply while enhancing our nation's water quality and sustaining our precious water resources.

In February 1998, the President of the United States released a clean water action plan that contained 111 action items addressing land and environmental issues. ARS targets the following national programs:

Program Components

Land Area Issues: Watershed Management on Agricultural Lands. By fostering research on weather and climate characterization, hydrological processes and watershed characteristics, and watershed management, this component focuses on the interaction between the environment and agricultural production on irrigated and rain-fed croplands and grazing lands at the field, farm, and watershed scales. For instance, research exploring the natural variability of weather is a critical component of determining the reliability of water supplies, the economic risks associated with farming and ranching operations, as well as the frequency and severity of natural hazards. Research on the interaction of water with soil and vegetation will help assess natural and human-made impacts on the environment and the nation's natural resource base. Research on watershed management will promote more effective use of precipitation in food and fiber production, better allocation and assessment of irrigation water resources, and other advances in integrated ecosystem management. Land use policy, management, and stewardship reflect responsible consideration of the ability of rain-fed crop production and grazing land practices to provide high-quality water in sufficient amounts to fulfill on- and off-site requirements. Research in advancing strategies for water management is needed to promote and safeguard the amount and quality of these water supplies. Research will quantify the impacts of rain-fed and grazing lands tactics and determine how they affect the hydrologic cycle.

Land Resource Issues: Irrigation and Drainage Management. Existing and future water resources for irrigation are projected to decline even further, which further emphasizes the need to improve irrigation and drainage practices to enhance water quality and sustain American food production for strategic national economic and social benefits. Innovative irrigation and drainage techniques and management are required that can improve water quality, reduce soil erosion, conserve water, and reduce energy requirements while enhancing and sustaining crop production and water use efficiency. Advanced technologies, like precision agriculture, site-specific management, remote sensing, and decision-support systems, are needed to address the water quantity and quality needs in irrigated agriculture.

Water Resource Issues: Water Quality Management on Agricultural Lands. The decline in the expansion of agriculture can be attributed in part to the high costs of developing new water resources. Research is needed to develop technologies for use by growers and by water-management agencies and regulatory agencies at all levels of government. Research must focus on improving the efficiency and safety of structures used to store and regulate water flows. With these needed improvements, rural and urban communities will be better protected from the worst ravages of floods, loss of productive irrigated and rain-fed croplands from stream bank failures, and landscape degradation from concentrated flow erosion. In addition, the development and implementation of more effective water-use strategies for irrigated and dryland farming, including water measurement and conservation, wetland and riparian area management, and water reuse are necessary priorities in order to enhance crop production and improve its efficiency, increase biodiversity in the environment, and conserve water on agricultural lands in an economic and socially responsible manner.

Water Resource Issues: Water-Quality Management on Agricultural Lands. New and improved strategies are needed to reduce water contamination from agricultural lands. Improved technologies are required for the management of wastewaters and for transferring specific farming-management systems from one geographic area to another. Field practices will be developed to reduce impacts of pesticides, nutrients, sediments, salts, toxic trace elements, and bacterial contaminants in surface waters and groundwaters. New strategies will be developed that use management systems evaluation areas to determine the water quality and other environmental benefits of alternative farming systems at field, farm, watershed, and river basin scales on irrigated and rain-fed croplands and grazing lands. Water-resource management to improve water quality will be evaluated on regional ecosystems in the context of its diversity and risk management in sustainable agricultural systems.

Water Resource Issues: Water Resource Databases, Decision-Support Systems, and Cross-Cutting Technologies. The development and validation of improved water resource–management technologies requires long-term databases. Models and decision-support systems are needed to develop optimal strategies for managing water resources in irrigated and dryland agriculture to:

- Establish the benefits of precision agriculture and integrated farming technologies
- Resolve conflict among competing water demands when the supplies are limited
- Determine the socioeconomic and environmental impact of proposed water, nutrient, and pesticide management programs and policies

Science-based results and technologies will be transferred to various user groups and policy makers and integrated into other ARS national programs.

Soil Assessment and Management

Program Rationale. The thin layer of soil at the surface of the earth functions as the central natural resource to sustain life. Soil management determines plant productivity, which in turn sustains animal productivity. Soils also remove impurities to benefit water and air quality. It is imperative that a balance be reached between the short-term use of the soil and the long-term sustainability of this critical resource. Protective, preservation, and management practices need to be developed to overcome limitations to productivity while maintaining or enhancing environmental quality. Tools need to be developed to assess the overall quality of a soil to determine the effectiveness and sustainability of soil-management practices. The overall goal of this national program is to develop and transfer science-based knowledge regarding the physical, chemical, and biological properties and processes within soil resources to ensure sustainable and economically viable food, feed, and fiber production throughout the nation and world.

Program Components

Resource Conservation. Protection of our soil resource is a key component of this program. Control of water, wind, and tillage-induced erosion needs to be developed to stop and reverse soil degradation (eg, loss of organic matter, soil compaction, infertility, and poor water retention). An improved understanding is needed of how soil influences greenhouse gases through carbon sequestration. Techniques are needed to measure soil salinity and develop remediation strategies at field and watershed scales. A greater understanding of the impact of nonagricultural activities on soil productivity is needed. Although individual soil properties can be measured, the capability to assess soil quality or health for a range of uses and functions is not actual yet. Soil quality assessment will encompass studies of soil properties, processes, and indicators; measurement tests and protocols; and interpretive tools and decision-support aids.

Water Use Efficiency. Improving soil water infiltration, storage, and use by crops has economic and environmental benefits. Research to increase soil infiltration and reduce runoff and sediment transport of agricultural chemicals to surface waters is one focus of this component. Improving soil physical properties to improve soil water storage and use by crops will also be a focus of this program component (eg, tillage and residue management systems and biological systems to improve crop rooting). Coordinating research to improve our fundamental understanding of soil crusting and sealing processes will result in the development of methods to reduce the impact of these processes on runoff and infiltration. Other research will focus on the development of techniques to evaluate the impact of irrigation practices on soil and other ecosystem resources. Especially critical in this area will be research to effectively use wastewater for irrigation without degrading soil physical, chemical, or biological properties and processes.

Nutrient-Use Efficiency. Efficient crop nutrient use results in increased profitability and reduced potential for negatively impacting the environment. Research to improve our understanding of how tillage (primary and secondary), crop rotation, cover crops, and residue management affect nutrient cycling and fertilization requirements will result in improved management systems and decision aids. Coordinating research to improve nutrient (both inorganic and organic) input, application, and utilization efficiencies will also be conducted, including site-specific and precision nutrient management. Synchronizing nutrient supply in soil with crop need and developing quick, reliable tests for nutrient availability in soil and organic matter, manures, wastes and amendments, and cover crops will also be addressed. Another focus of this program component is research to improve our understanding of how soil organic matter fractions influence soil structure, water relations, and nutrient cycling. Quantification of how nutrient-use practices coordinate to soil-management practices to affect environment quality will also be addressed in this program. Research to evaluate effects of grazing and livestock systems on nutrient cycling and other indicators of soil quality is also critical to this program component.

Resource-Based Profitability. Soil-management assessments and practices that sustain the soil resource must be economically viable or they will not be adopted. This research component will identify and develop management systems that improve profitability and competitiveness by increasing productivity (output per unit input). In addition, research will develop quantitative economic assessments at various scales for impacts of soil-management practices both on-farm and off-farm. These results can be used by action agencies to increase acceptance of sustainable practices. Finally, research to identify and develop production systems that are less dependent on nonrenewable resources will be conducted.

Air Quality

Program Rationale. The health of humans, plants, and animals, as well as other aspects of the environment, are affected by dusts, odors, and other volatilized substances released to the atmosphere by agricultural activities. Excess levels of ozone damage plants. The air-quality national program conducts research on both these issues:

- To understand contaminant releases and their impacts, and how to minimize them
- How ozone affects plants, and how to reduce the impact

In many areas, dusts are intimately related to wind erosion, the effects of which are deleterious to soils and to downwind environments.

Program Components

Dust Emission-PM-10. These are dusts that are regulated by the Environmental Protection Agency (EPA) because they pass through the human nasal air-filtering system and lodge in the lungs. This research program seeks to elucidate the biological, physical, and chemical mechanisms by which 10-μm and smaller particles are generated, how they are transported and suspended in the air, and their patterns of movement. As understanding of these mechanisms improves, advances are made in technology that can be used on farms to reduce or prevent the emission of dusts to the atmosphere. Research is also conducted on identifying geographical areas that significantly emit dusts, so land managers and policy makers can facilitate application of controls where they are most needed. As resources become available, the air-quality national program will be expanded to consider dust emissions of such agricultural operations as tillage and harvest.

Effect of Ozone on Crop Systems. High levels of ozone in the atmosphere result in reduction of crop yields by as much as 20%. ARS research assesses the effects of ozone on crop production and plant health and develops techniques for mitigating the problems. Carbon dioxide is intimately associated with ozone problems, and the effects of this gap on crop physiology and productivity are also under study.

Emission of Odors from Agricultural Operations. Most animal-production enterprises and some crop operations result in unpleasant odors that are carried downwind and impact neighboring households and communities. Basic research is needed to understand the biological and chemical processes that produce odors and emit them to the atmosphere. This will permit the development of mitigating measures to be applied at the emitting source.

Emission of Volatilized Pesticides by Agriculture. Volatilized pesticides are a complex of very fine liquid particles and gaseous phase compounds, or they may be attached to particulates from other agricultural or nonagricultural sources. The research objectives are:

- To understand the chemical and biological mechanisms that influence pesticide volatilization and transport
- To understand transport processes
- To understand the impacts of deposited volatiles
- To develop means to reduce volatile emissions

Wind Erosion. High winds on exposed soils give rise to dust storms that are locally devastating in terms of denuding productive fields of their soils and depositing large amounts of soil sediment in the immediate vicinity of the storm. A large storm may also result in emitting into the atmosphere large amounts of PM-10 that can travel for thousands of miles. Research objectives include:

- Understanding the biological, physical, and chemical mechanisms of wind erosion
- Developing effective control measures for application on farm fields

Develop Wind-Erosion and Air-Quality Simulation Models and Aids for Agricultural Decision Making. Simulation models are based on scientific knowledge developed as a result of research discussed in the air quality problem areas. The aim of simulation models is to integrate and express science knowledge to facilitate the selection of the best management practices. One may test various combinations of control measures (such as roughness of a land surface in relation to wind breaks, or surface area versus depth of waste lagoon) to find the optimum emission-controlling combinations for any given farm or farm component. This capability allows the land manager to select practices with a high degree of confidence so that they will have the desired impact on the farm's emissions or on lessening excess ozone impacts.

Global Change

Program Rationale. The term global change is a shortened form of the phrase global environmental change. It refers to large-scale changes in the earth's biological, geological, and atmospheric systems. Agriculture is vulnerable to these environmental changes. Successfully adapting to change depends on a quantitative and predictive understanding of the ways that agriculture and food supplies may be affected by climate change, rising atmospheric carbon dioxide and ozone levels, and increased UV-B radiation. Title XXIV of the 1990 farm bill directed the Secretary of Agriculture to "study the effects of global climate change on agriculture," including "the effects of simultaneous increases in temperature and carbon dioxide on crops of economic importance; the effects of more frequent or more severe weather events on such crops; the effects of potential changes in hydrological regimes on current crop yields," and other possible impacts.

Program Components

Climate Change Effects on Agriculture. Research conducted under this component focuses on the potential impacts of a changing climate on crop and livestock production. Both direct and indirect effects are considered.

Direct effects include positive and negative influences of rising temperatures, altered precipitation amounts and patterns, increased cloudiness, and similar climatic changes on:

- Geographical areas of crop and forage production
- Geographical distribution of weeds, insects, and diseases
- Plant physiology and crop yields
- Animal performance

Implications of an increase in the frequency of extreme weather conditions and climatic events, as predicted by some climate models, are also considered here. Important is the possibility of increases in the occurrence level of various stresses and the interaction of additional stresses induced by climate change with existing ones. Many such interactions can be envisioned in that determining the significance of those with potential to intensify adverse or positive effects of climate change is an important goal.

Agriculture's Role in Greenhouse Gas Emissions and Sequestration. Changes in agricultural land uses and increased use of production practices that conserve natural resources may be reducing greenhouse gas emissions (or removing them from the atmosphere and sequestering them in vegetation and soils). Increased productivity of vegetation due to the "fertilization effect" of rising atmospheric carbon dioxide levels also may be removing atmospheric carbon and storing it in soils as organic matter. Limited studies show soil carbon increased after conversion of cropland from conventional to limited or no-tillage systems, reduction or elimination of fallow periods, the use of cover crops, and plantings of perennial grasses on cropland under the conservation reserve program. However, effects of these new and larger sinks on net greenhouse gas emissions are not well quantified and have not been related to the spatial distributions of such practices in ways that allow the magnitude of carbon sequestering to be compared with estimates of agricultural and other sources of atmospheric carbon.

Effects of Rising Atmospheric Carbon Dioxide Levels on Plants and Ecosystems. Many questions remain concerning the reality or magnitude of the carbon dioxide fertilization effect on vegetation. Until these are resolved by additional experimentation, a wide range of assumptions must be accommodated in predictive models, and model output will remain very general. Will increases in growth and productivity due to rising carbon dioxide eventually decline, because limiting availability of water and nutrients precludes continued responsiveness of crop stands and plant communities? To what extent do the favorable responses of plants to elevated carbon dioxide, observed under controlled conditions, also occur in the real world? Do the carbon dioxide–induced increases in growth and production observed at a doubling of current carbon dioxide levels extend to a tripling, or beyond? ARS conducts research on the effects of tropospheric ozone on plant physiology and crop yield as a part of the air-quality national program because ozone is a pollutant and ozone damage to plants is most appropriately considered an air-quality problem.

UV-B Radiation. Fundamental questions about UV-B effects on plants and animal health also remain unresolved. No one understands how plants cope with current background levels of UV-B, so it is not possible to predict the extent to which crops and native vegetation can adapt to higher levels or repair more extensive damage to DNA and chlorophyll. Seedlings of annual species were used in most of the research conducted to date, so little is known about long-term effects or responses of perennial or woody species. Of special interest are the interactions of current and increased levels of UV-B with other plant stresses as global change effects. For instance, deleterious effects of UV-B are eliminated when plants are grown in carbon dioxide concentrations expected in the next century. The question of how to experimentally control UV-B radiation is an issue. Source lamps and filters may not provide the proper spectral quality, and radiation levels (dosages) calculated from the available action spectrum may not be appropriate or accurate.

Grazing-Land Management

Program Rationale. More than half of the earth's land surface is grazed. In the United States, rangelands, pastures, and hay lands together make up about 55% of the total land surface. More than 85% of the 307 million acres of publicly owned lands in the western United States are grazed. These lands, the plants that grow on them, and the domestic and wild animals that graze them all contribute to the environmental, economic, and social well-being of our nation. The estimated annual value of U.S. hay production alone is $11 billion, third in value behind corn and soybeans and exceeding the value of wheat, vegetables, cotton, and noncitrus fruits. Livestock numbers vary with time, but grazing lands are usually home to more than 100 million cattle and 8 million sheep, supporting a livestock industry that annually contributes $70 billion in farm sales to the U.S. economy. Grazing lands also function as watersheds. Maintaining adequate supply of clean water for irrigated agriculture, industrial uses, and urban areas is critical to society, and much of our water supply originates as rainfall or snowmelt on rangeland watersheds. A strong ARS research effort will be required to maintain the economic stability and environmental acceptability of the use of grazing lands for animal agriculture.

Program Components

Forage Germ Plasm. Limitations in the availability of adapted, nutritious, highly productive forages continue to hamper the production potential of the nation's grazing lands. New forage, pasture, and rangeland varieties with higher nutritive quality and resistance to diseases, insects, and weeds are needed to maximize the utilization efficiency of the nation's grazing lands.

Forage Management. Management of timely forage availability in hay fields, in pastures, and on rangelands to maximize seasonal distribution, yield, and quality is one of the greatest limitations in enhancing the productivity of the nation's grazing lands. New forage-management technologies and more efficient approaches to forage management are needed to help managers maximize economic efficiencies and facilitate the integration of grazing lands into livestock-production systems.

Grazing Management. Environmentally sound management of grazing animals on the nation's grazing lands has become a public concern. Grazing-management systems are needed that help grazing-lands managers maximize economic efficiencies in grazing-animal production while minimizing or avoiding negative impacts on the environment.

Ecosystem Processes. Increased knowledge of the natural processes (competition, fire, herbivory, carbon and nutrient cycling, water use, energy capture and flow, and vegetation change) that control productivity and promote stability of these grazing lands is required to develop better approaches to their management. A thorough understanding of the basic biology of grazing lands is needed to provide land managers with the best information for managing pastures and, particularly, rangelands.

Grazing-Lands Databases, Simulation Models, and Decision-Support Systems. Management decision making by the grazing lands (particularly rangelands) landowners and managers would be greatly enhanced by the availability of decision-support systems. Databases that provide necessary inputs to decision-support tools are sorely needed.

Conservation of Grazing Lands as a Natural Resource. Conservation practices on grazing lands are critical to the long-term preservation of the earth's soil, wildlife, biodiversity, and air and water quality. Efforts are needed to provide the best information on the management of grazing lands to enhance the environment as well as provide a feed source for livestock.

Manure and By-Product Utilization

Program Rationale. Our nation's farms, cities, and industries generate over 1 billion tons of by-products each year. Animal production in large confinement units, often concentrated on relatively small land areas, has exacerbated the already difficult problem of how best to manage manure. Odors and other emissions to the atmosphere from confinement units as well as other by-product sources are of increasing concern. Accumulation of large amounts of animal, municipal, and industrial by-products at the production site or inappropriate subsequent disposal of the by-products can harm soil, water, and air quality. Many by-products, however, also contain essential nutrients (nitrogen, phosphorus, etc) that can help meet crop requirements if properly applied to soil. Organic by-products can serve as soil conditioners and increase soil organic matter levels. Research on the development of systems to optimally integrate animal manure and other products into sustainable agricultural practices is indispensable to agriculture today. Manures and by-products can be agricultural resources rather than wastes if they are appropriately managed. The goal of the manure and by-product utilization national program is to develop agronomic management practices that effectively utilize manure and other by-products and are protective of the total environment (soil, water, air, etc) and public and animal health.

Program Components

Nutrient Management. Movement of nutrients in excess amounts from manure and other by-products to water and air can cause significant environmental problems. These nutrient losses to the environment can occur during manure handling and storage and during and after field application. Nitrogen and phosphorus from manure and other sources have been associated with algal bloom and accelerated eutrophication of lakes and streams. Proposed regulations and congressional legislation will limit application of manure nitrogen and phosphorus to levels that will protect water quality. Historically, manure applications have been based on meeting crop nitrogen needs, but recently phosphorus has become a greater concern in many areas. A variety of research areas will need to be addressed to protect soil, water, and air from excess nutrients in manure and other by-products, including:

- A more efficient use of nutrients in animal feed
- Improved manure handling, storage, and treatment technologies
- Improved tests for nutrients in manure and soil treated with manure
- Development of soil threshold nutrient levels that protect water quality
- Development of methods to identify areas in a watershed that are most susceptible to nutrient losses
- Improved methods for precise application of manure

- Development of integrated animal and cropping systems to effectively use and recycle nutrients

Atmospheric Emissions. Odor-causing volatile organic compounds and greenhouse gases are generated at most animal-production facilities. These were of less concern when the volume of manure generated at a farm or production site was less. Because volatile organic compounds are often immediately irritating, they are the primary concern of the general populace at many locations. Furthermore, they are now more frequently perceived as real threats to air quality and therefore are subject to air-quality regulations. Although they can be generated at any place in the process, it is in the handling and storage system (eg, lagoons) that the bulk of the odors and trace gases are emitted. Improved management practices, new treatment technologies, or alternative production systems are needed to solve these atmospheric emission problems. This will require an increased understanding of the processes involved in the generation and transport of volatile organic compounds and greenhouse gases.

Pathogens. Contamination of water, food, and air by pathogens potentially present in manure and other by-products is an increasing concern. Most of these outbreaks were from more virulent strains of pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Campylobacter jejuni*, *Listeria monocytogens*, *Cryptosporidium parvum*, *Cyclospora*, and *Yersinia enterocolitica*. In addition, viruses remain a concern, and *Pfiesteria* outbreaks on the east coast of the United States may have been related to animal waste mismanagement. The survival, transport, and ultimate fate of pathogens from manure and other by-products need to be determined. The effects of manure management on other harmful organisms like *Pfiesteria* also need to be determined. The entire production process needs investigation to ascertain where the greatest risks occur and to develop the most effective controls so as to protect public and animal health.

Integrated Farming Systems

Program Rationale. Because of narrow profit margins and due to their fixed geographical locations, most farms and ranches have only limited flexibility to respond to changing factors in their environments. Every farm or ranch is a complex system of interacting components residing in a natural and socioeconomic environment. A high degree of management skill is required of modern producers. The primary products of the integrated farming systems national program are information and tools for use by farmers and by consultants in farm planning and decision making within the complex socioeconomic-physical-technical environment of farm or farms for which they are responsible. The overall problem to be addressed is provision of science-based information and its effective retrieval, management, and analysis for agricultural management decision making. This includes historic, recent, and real-time information and data. The goal is to help farmers retrieve this information, understand it, and apply it to the development and effective management of whole farm systems within the context of their ecosystems and communities. Effective response to issues concerning farms and ranches requires that partnerships be established in which research is conducted jointly with farmers and with participation from community stakeholders.

Program Components

Interactions among Components of Whole Farm Systems. A collection of components selected on the basis of their individual descriptions may form a farming system, but the operation of the whole system greatly depends on how the components interact—whether they are mutually compatible and supportive or whether one or more of them conflicts with the effectiveness of others. Considerable knowledge is available regarding many individual agricultural components. An essential aspect of the systems program is to understand interactions among and between the components and then to effectively apply this understanding. The integrated farming systems national program focuses on projects that foster understanding and managing of interactions among system components and how these components in turn act together within the larger process or system. On-farm research will often be used to ascertain system functions.

System Impacts. The integrated farm system is viewed as a component of a larger system, such as an ecological or watershed system. Phenomena such as eroded sediments, pathways of pesticides, and speed and sites of breakdown of pesticides become potentially important to ecological and watershed systems beyond the farm. The integrated farming systems national program will address this issue with the aim of assisting farmers and consultants to manage their environmental impacts. In a similar manner, with the assistance of farmers, consultants, economists, and social scientists, issues related to farm systems and economic viability and rural communities will be assessed. There is a critical need for knowledge of how management practices influence system processes and interactions.

Development of Integrated Farming Systems. At meetings with farmers and ranchers, a common refrain is that data and information are the primary need, and that farmers and ranchers will develop their own farm or ranch systems, given full information regarding components and their interactions. However, there may be situations in which it is appropriate for the agricultural research community to design and propose management strategies for whole or partial systems for use in agriculture. For example, there appears to be a special need for information on the transition from farming with high inputs to farming with reduced nonrenewable inputs, such as organic farming. The entire farming system needs to be evaluated to provide optimal entry points for farmers to make such a transition. The adoption of scientific management practices, whether in a precision or a sustainable agriculture context or both, is fostered by an understanding of how the site-specific practices affect the entire operation.

Decision-Support Systems. Integrated farming systems is a national program whose primary organizational function is to integrate knowledge and information developed in ARS and elsewhere in world agricultural research for direct application in decision making at farm (and field) level. The target audiences are farmers, ranchers, and their primary enterprise consultants, but benefits will also accrue to policymakers, financial and other service providers, and regulators. Possible outcomes include Internet systems, agricultural-management gaming programs, individual computer-based decision aids, printed guidelines, sustainable agriculture decision support, precision agriculture, and other products. These products will take multiple forms so that they will be accessible by farmers and others with a wide variety of educational backgrounds, skills, economic positions, and types of agricultural enterprises. New delivery technologies will be sought, but methods that have worked in the past, such as field days, county farmer meetings, and on-farm consultations and demonstrations, will continue to be used.

CROP PRODUCTION, PRODUCT VALUE, AND SAFETY

ARS seeks to enhance the production, value, and safety of foods and other products derived from plants that have a major impact on the American economy, world markets, and the U.S. balance of trade. The ARS scientific program in CPPVS consists of multidisciplinary research to solve problems of high national priority that threaten the security, safety, and productivity of U.S. agriculture. In the area of human nutrition, foods from plants are a major contributor of vital nutrients and fiber in the diets of Americans. ARS collaborates with public, private, academic, and foreign research entities to increase crop production and improve product quality and safety. Through these efforts, the United States can preserve its preeminent role as food provider to the world and overcome artificial trade barriers in world markets. Research in this area is based on the national programs discussed in the sections that follow.

Plant Microbial and Insect Germ Plasm, Conservation, and Development

Program Rationale. Genetic diversity is the basis of a sustainable agriculture. Habitat destruction, emerging pests and disease, changes in climate, and human-made pollution affecting soil and water are threatening the occurrence and distribution of traditional agricultural systems and natural plant communities on a global scale. The disappearance of natural plant habitats has a major impact on agriculture and industrial production. Genetic resources of plants play a major role in crop improvement for the introduction of new genes to increase resistance to pests and diseases and improve both productivity and crop quality. Microbial resources are essential for cycling the elements essential for long-term sustainability and productivity. New technologies are needed to develop more rapid and efficient methods of identifying useful properties of genes for manipulating genetic material. New approaches in the characterization and classification of genetic material and in the application of new biotechnologies will support the development of new crops and more efficient use of microbes and beneficial insects. The national program in genetic resources encompasses conservation, enhancement, and the development of new approaches for breeding and genetic improvement utilizing molecular technologies.

Program Components

Germ Plasm Acquisition, Maintenance, and Management. Higher plant, microbial, and insect germ plasm should be acquired and safeguarded through the expansion of gene banks or *in situ* preserves for long-term accessibility. The genetic makeup of acquired germ plasm should be characterized to insure broad-spectrum genetic variability while minimizing redundancy.

Germ Plasm Enhancement and Manipulation. Sources of useful and important genes should be identified and incorporated into crop germ plasm. The genetic bases of crop, microbial, and beneficial insect gene pools should be broadened through genetic improvement to combat potential losses due to pests, diseases, and stresses imposed by the natural environment. The long-term economic value of genetic material for use by plant breeders and other scientists will be increased.

Genomics and Database Management. Genomic databases are an essential component in the management of new information that is generated at an increasingly rapid rate. Genomic and DNA sequence analysis should be emphasized, and the development of software to manage DNA sequence information should be continuously updated. Software to store, process, and organize the data generated by these activities should also be further developed. New DNA probes and primers will be needed to facilitate germ plasm characterization. Infrastructure will be developed that provides collaborative links through computer networks among ARS laboratories, universities, the private sector, and other government research facilities.

Germ Plasm Resources Information Network. GRIN is the official database of the National Plant Germplasm System (NPGS) and is currently maintained on a minicomputer at Beltsville, Maryland. Data in GRIN are available to any plant scientist or researcher worldwide. The functions of GRIN are:

- To act as a repository for all information of interest about plant germ plasm maintained by the NPGS
- To unify the NPGS with regard to data standards, crop location, and movement of germplasm
- To allow fast access to the most current data available for all users of the germ plasm and its accompanying information
- To facilitate and track the distribution of germplasm
- To provide germ plasm maintenance sites with a system of inventory management.

Improving Plant Biological and Molecular Processes

Program Rationale. Products of American agriculture are increasingly marketed to consumers around the globe. Increasing population and improving economic status of other countries, as well as changing environmental and other safeguards domestically, have set American farmers on a course of rapid change. Better food safety and security, crop protection, crop yield, and crop quality require advances at the frontiers of agricultural sciences. Yesterday's scientific advances support the rapid pace of change today; today's research breakthroughs will support tomorrow's innovations. This program is organized around fundamental long-term research needed for breakthrough advances in crop production, protection, product value, and safety to meet the changing needs of society and the global marketplace.

Program Components

Increased Crop Productivity and Stress Tolerance. Research will provide fundamental scientific knowledge required for continued increases in crop productivity and production efficiency to keep abreast with the growing demand for food. Basic information will be developed to increase the efficiency by which inputs are used by crops and to reduce unnecessary depletion of resources, extra expense in crop production, and contamination of the agroecosystem with excess nutrients or water not used by the crop. Innovative approaches will be developed to increase crop productive capacity in the future while safeguarding the environment. Mechanisms of crop responses to environmental stress will be identified and the processes improved to minimize yield losses and yield variability. New knowledge of plant gene organization and function will fuel more effective genetic improvement of crops.

Improved Crop Protection. Research focuses on providing fundamental plant knowledge to control pests (primarily insects, weeds, and nematodes) and pathogens in a more environmentally friendly manner than presently available. The relationships among host plants, pests and pathogens, and beneficial organisms will be characterized and expanded into new pest-management technology that will be sustainable over the long term. The processes to be investigated include sensing of pests and pathogens by the host plant, triggering of plant defense reactions, signaling processes within or between individual plants or between species that contribute to plant health, transfer and expression of resistance genes, and other fundamental plant processes that determine plant interactions with pests and pathogens. The research is expected to provide new approaches to crop protection that will be more effective, less costly, more targeted to the pest or pathogen species, and less risky to the environment.

Enhanced Crop Product Value, Quality, and Safety. Research will provide basic plant information to increase the diversity of products from crop plants, to improve crop product quality, and to prevent occurrence of contaminants (from either microorganisms or from plants themselves) that make plants and plant products unsuitable for consumption. Research within this program will lead to development of new crops and crop products. In addition, it will delineate the properties of crop products and their roles in product utilization as well as innovative ways of measuring quality and safety determinants. This advance in knowledge will be the basis for identifying limitations to achieving best product quality and developing novel ways to overcome those limitations.

Plant Disease

Program Rationale. Since the beginning of agriculture, diseases have reduced the yield, quality, and value of plant products in agricultural, landscape, and forest settings. Despite considerable success over the years in managing them, plant diseases still cause more than $9 billion in annual losses in the United States alone. This is largely because:

1. Current control strategies are not 100% effective.
2. Pathogens evolve and overcome once-effective management tactics.
3. Exotic pathogens are introduced.

Thus an ongoing research program is needed to devise effective management strategies to keep up with the changing disease situation.

Identification and Classification of Pathogens. Effective disease control usually depends on rapid and accurate identification of the pathogens involved so that appropriate control measures may be taken. Accurate identification of pathogens is also critical for making sound decisions regarding quarantines of imported and exported plant material commodities. Knowing how pathogens are related to each other can be helpful in suggesting possible control strategies.

Biological Control. Biological control is a strategy that uses beneficial microorganisms to block pathogens from causing disease. In addition to finding such organisms and, in some cases, genetically improving them, it is necessary to learn how they are influenced by factors such as weather, soil type, conventional pesticide application, and crop variety to make biological control as effective and reliable as possible. Also needed are commercially feasible methods to mass produce, store, and apply biological control agents.

Cultural Control. Changing the way a crop is managed (soil preparation, time of planting, irrigation regime, storage conditions, etc) often has a significant impact on disease severity. Knowledge of how such practices affect pathogen survival and disease progression can be used to devise cultural systems that allow acceptable crop production yet are less favorable for disease development.

Pathogen Biology, Genetics, Population Dynamics, Spread, and Relationship with Hosts and Vectors. Research on the processes that take place during disease development can uncover vulnerable steps in the life cycles of pathogens where control measures can be successfully used. Similarly, understanding how pathogens move from plant to plant in the field or within harvested commodities in storage, how they survive in the absence of host material, or how they are affected by their environment can suggest possible control strategies. Knowledge of how and where pathogens survive on plant parts and seeds is essential for developing methods to reduce the domestic and international spread of disease.

Host Plant Resistance to Disease. Enhancing genetic resistance to disease in plants is sometimes the only disease-management option and is often the most cost-effective, environmentally benign one. This approach depends on research to identify and characterize genes for resistance in crop species themselves; in plants closely related to them; and in unrelated, alien species. Incorporating such genes into commercially acceptable varieties can be accomplished through conventional plant breeding and genetic engineering.

Crop and Commodity Pest Biology, Control, and Quarantine

Program Rationale. Economic loss of food and fiber due to damage caused by insect, mite, and weed pests costs U.S. consumers and producers billions of dollars every year. Besides direct losses, pests may further reduce the quality and value of products, increase the costs of production, restrict U.S. products from foreign markets, damage environmental areas, and place native species at risk. Technology developed through the crop and commodity pest biology, control, and quarantine national program provides the pest-control methods used to devise effective and sustainable integrated pest management (IPM) programs that support agricultural-production systems for a diversity of situations throughout the country. The overall philosophy of this national program is to develop and help implement sustainable approaches to managing pests through a combination of biological, cultural, mechanical, and chemical tactics that reduce pest populations to acceptable levels while minimizing economic loss, impact on human health, and environmental risk.

Program Components

Identification and Classification of Insects, Mites, Microbes, and Plants. Accurate taxonomic identification and classification of organisms is the first step in providing information on the biology and control of known and new pests; in determining the geographic origin of introduced pest species; and in the location, collection, and importation of effective biological control agents for IPM programs. ARS scientists will maintain specimen and germ plasm collections of agriculturally important insects, mites, microbes, and plants, conduct taxonomic assessments of important groups of organisms; develop identification keys; and support private and governmental action agencies in characterizing critical pest problems.

Investigation of Pest Biology. Inadequate knowledge of pest biology, movement, genetics, physiology, biochemistry, organismal biology, reproduction, and population ecology currently limits our ability to develop and implement biologically sound and environmentally compatible pest-control technologies. Continued ARS research on insect, mite, microbe, and plant biology and on important individual pest species is necessary to provide the scientific knowledge base to create new and effective pest-control alternatives that are safer for humans, nontarget organisms, and the overall environment.

Understanding Pest/Host Plant Interactions and Economic Impact. ARS scientists are discovering how insect, mite, and weed pests interact with valuable crops and commodities to cause losses and are determining the biological mechanisms that allow pests to cause this damage. The economic assessment of pest attack and the associated costs of pest control provide further vital information on pest-control alternatives and action thresholds for IPM programs. This research will lead to new cost-effective methods of pest control such as pest-resistant crops, biological control agents, or other environmentally friendly technologies.

Investigation of Pest Exclusion and Quarantine Treatment Procedures. Development of pest detection, exclusion, and quarantine treatment technologies is important both in keeping new pests from invading the United States and in treating our crops and commodities so they can be shipped to and sold in foreign markets. In this era of expanded free trade and global shipping, the movement, introduction, and establishment of exotic pests is an ever-increasing threat that will be addressed through research that supports action and regulatory agencies such USDA Animal and Plant Health Inspection Service.

Development of New and Improved Pest-Control Technologies. Research and development of new pest-control technologies, particularly biologically based methods such as host plant resistance, biological control, semiochemical treatment methods (eg, pheromones, mating disruption), and cultural strategies will be expanded. Additionally, pest and natural enemy sampling will be improved, pesticide formulation technology will be made more efficient, new precision agriculture methods will be evaluated, and the impact of these technologies on both target and nontarget organisms will be evaluated and optimized. The new technology resulting from this research will provide the tools to protect ourselves and future generations from the continuous pressure of pest damage that threatens our food and fiber supply each and every year.

Integration of Component Technologies for IPM Systems. IPM is the best method known to control pest problems; however, research on combining different pest-control technologies into effective, economical, and sustainable IPM systems needs to be explicitly conducted or integration often fails. Although integration of pest-control tactics is the primary responsibility of other ARS national programs, consideration is given in this national program to the planning of holistic IPM systems early in the development of each component pest-control technology.

Integrated Crop Production and Protection Systems

Program Rationale. Production capacity, production efficiency, and crop protection are major pillars supporting national crop productivity. A high priority is the development and subsequent transfer to customers of efficient crop production and sustainable cropping systems. Overall challenges are:

- To substantially increase the knowledge base of and sustainable technology for crop production and cropping systems
- To improve the delivery of technologies generated
- To promote the use of these systems.

Meeting these goals will require bringing emerging technological capabilities together to support immediate and long-range strategies aimed at future crop production, protection, and food-safety challenges for small, medium, and large farms. It will also require substantive collaborations with the customers in program planning, research, and evaluation. The program closely ties in the whole farm-management strategies of the integrated farming systems national program and with the components of the crop protection and quarantine national program.

Program Components

Crop Management and Production Efficiency. The goal is to develop new cutting-edge crop-production principles and practices and provide the necessary agronomic principles, technologies, and approaches associated with the efficient production of crops. Research undertaken in this component will (*1*) help fill the knowledge gap needed for applying appropriate principles and practices under differing ecological and climatic conditions, and (*2*) furnish the agronomic principles needed for crop production.

Bees and Pollination. The goal is to develop new technologies leading to sustainable strategies for using bees as pollinators to increase crop production. At the same time these technologies will help maintain the profitability for providers of bees that are used in the pollination and honey-production industry and will help U.S. farms to remain competitive in the global marketplace. The discovery of two parasitic mites of honeybees in the 1980s, the invasion of the Africanized honeybee in 1990, and diseases of both Apis and non-Apis bees is seriously impacting the availability of pollinators. Technology for the rapid detection and control of parasitic mites and other diseases of pollinators will enhance pollination efficiency and honey production.

Agroengineering, Agrochemical, and Related Technology. The goal is to develop more effective production, harvesting, and tillage equipment; sensor technology; and pesticide-application methodologies and other tools for crop production. The component also includes developing appropriate practices for the use in and incorporation into cropping systems of products generated through biotechnology.

Models and Other Decision Aids. The goal is to provide user-friendly crop-production models and decision aids for (*1*) assessing alternative biological, economic, and related production and management practices; (*2*) maximizing processing; and (*3*) increasing energy-use efficiency.

New Uses, Quality, and Marketability of Plant and Animal Products

Program Rationale. American agriculture faces increasing, intense competition in the global marketplace. Worldwide, agricultural production has increased faster than demand in many areas, resulting in current commodity surpluses, low prices, and unreliable profitability. Recent shifts in U.S. farm policy to remove price supports emphasize the need for American agriculture to move beyond production of ever-larger quantities of ever-cheaper commodities. In response, farmers and ranchers must be able to produce higher-quality products that can be differentiated from lower-value commodities, commodities and co-products must be converted into useful value-added food and nonfood products, and products must be protected from contamination or loss of quality after harvest to ensure marketability. Research must also be responsive to consumer demands for high-quality, safe products; government and consumer pressure to provide products that are "environmentally friendly" or that are produced using processes that are "friendly" to people, animals, and the environment; and the need for a sustainable and profitable agricultural-production system. In this program, research will develop knowledge and technology for crop and animal product quality measurement and maintenance or enhancement during processing and marketing, commodity and co-product processing into value-added materials, and new specialty products from crops and animals.

Program Components

Intrinsic Product Quality. Fundamental research will clarify the roles of product composition, molecular structure, and physical state in determining end-use quality and functionality. Genetic improvement of crops and animals for quality attributes will lead to the best possible product quality at the farm gate. Applied research will develop new processes to maintain or enhance product quality during harvest, storage, transport, and marketing.

Pest and Disease Control. The goal is to provide information that will alleviate commodity trade barriers and quality and economic losses attributed to microbial or insect infestation. The major thrust of this research is to prevent spoilage or contamination by managing or eliminating postharvest pests and pathogens, especially those resulting in formation of mycotoxins. This effort includes both basic and applied research to improve pest and disease resistance, new treatments to eliminate pests and pathogens (especially treatments that do not rely on synthetic chemical pesticides), and biological control of pests and pathogens. The focus of this work is on preservation of harvested farm products. Research is specifically aimed at overcoming quarantine barriers to export or ensuring that food safety is found in different programs.

Product Handling and Grading. Efficient technologies and improved or new equipment will be developed to preserve the quality of agricultural commodities during harvest, transport, and storage. Research will establish basic needs for material handling that preserves quality characteristics, and processes and equipment will be developed that meet the requirements. Research will also develop new technology for product grading to provide rapid, accurate, and reproducible information on quality. The research in this component of the program will lead to both expanded and better-documented value of agricultural products. The improved quality and the improved grading will make agricultural products from the United States more competitive and marketable domestically and overseas.

New Processes, New Uses, and Value-Added Products. The goal is to develop new uses of agricultural products and co-products. Innovative new processes will be created and existing ones adapted for the extraction and purification or the manufacture of superior products from agricultural commodities. Application of these innovative technologies will expand the range and value of agricultural products and reduce the cost of their production, making processed goods from the United States more competitive. Sources of natural products will be identified for use as nutriceuticals, pharmaceuticals, biopesticides, or other high-value uses. This program will identify alternative sources and create technology leading to an expanded, diverse range of value-added food and nonfood products from commodities and undervalued by-products of agriculture. New crops will be "mined" for valuable materials.

Bioenergy and Energy Alternatives

Program Rationale. The last decade has been characterized by huge U.S. trade deficits. In fact, petroleum import for transportation purposes alone was $50 billion in 1996. America's dependency on foreign oil (now at 50% and rising) is not only an economic issue but is one of national security as well, particularly in times of global unrest. These factors, coupled with environmental concerns regarding the use of fossil fuels and production of CO_2, fostered the expansion of the fuel ethanol industry. The capacity of the U.S. industry now exceeds 1.5 billion gallons per year. The industry has become an important partner with American agriculture and the USDA estimates that 17,000 jobs are created for every billion gallons of ethanol produced. Similarly, a nascent biodiesel industry has been developing in recent years.

Current use of ethanol and biodiesel as fuel additives or alternative fuels (eg, E-85, a good, neat biodiesel) depends on many factors, including political actions, tax policies, agricultural practices, regulatory issues, and international economic trends. The relatively high cost of ethanol production and the very high cost of biodiesel production, however, remain as important constraints on their use. Removing technical constraints is the key to a viable biofuel industry of the future.

A likely market for alternative energies is within agriculture itself. Low-cost alternative fuels can be used to power farm tractors and small agricultural production and processing facilities within rural communities. Wind and solar energy, as well as biofuels, may also be utilized to supply or supplement electrical energy for water pumping, small-scale irrigation systems, and other farmstead needs.

Program Components

Ethanol. Advances in enzymology; microbiology; and chemical, biochemical, and process engineering are required to underpin this effort. Technologies are needed to reduce the cost of producing ethanol from cornstarch (95% of production). In addition, the conversion of biomass, a vastly more abundant feedstock, is an economic necessity. This program's first biomass target is corn fiber, due to its ready availability at ethanol plants and extremely low value. Immediate integration of corn fiber to ethanol at the plant is feasible and would result in at least a 10% production increase. These research findings will be applicable to other forms of biomass as well, ranging from agricultural and forestry wastes to fast-growing crops that could be grown solely for energy production. Research will focus on, but not be limited to, developing new enzymic processes for saccharification and improved microorganisms to ferment the multiple sugars found in biomass. To improve process economics, value-added co-products will be developed from current by-products, and separation technologies will be improved for ethanol as well as co-product recovery.

Biodiesel. Vegetable oils and animal fats and their derivatives (biodiesel) are attractive as alternative fuels, extenders, and additives for compression ignition (diesel) engines. Opportunities for biodiesel include off-road markets such as underground mines, marine applications, mass transit (subways, trains), and stationary power generation. However, research is needed to improve cold start-up and operability, to identify and reduce harmful exhaust emissions (eg, nitrogen oxides), to develop a rapid and low-cost fuel-quality test, and to reduce feedstock and formulation costs.

Energy Alternatives for Rural Practices. Autonomous water-pumping systems are needed to supply water to livestock in semiarid areas where precipitation is sufficient for forage production but is not adequate for watering livestock. Renewable energy systems are well suited to this application because they are independent of environmentally hazardous, expensive distribution systems. Remote renewable energy systems are needed to supply additional electric power to rural areas that are undersupplied by overload rural electric distribution systems. On-farm electric-generating systems powered by a combination of wind, solar, and biofuels will be developed.

Methyl Bromide Alternatives

Program Rationale. Methyl bromide (MB) is a highly efficacious soil fumigant for more than 100 crops and foreign ornamental nurseries. It is also used to kill arthropod pests in postharvest commodities fumigate structures. The U.S. EPA published a listing of MB as an ozone depletor, banning the production and importation of MB in January 2001. The development of alternatives to MB will have a major impact on the U.S. agricultural industry and alternatives to its use must be found.

Program Components

Preplant Soil Fumigation Alternatives. The loss of methyl bromide for soil fumigation will result in serious disease, pest, and weed problems. These problems will likely be highly variable and will depend on crop, soil type, environment, and cropping conditions. Therefore, separate research efforts will be required to develop multiple management strategies. Approaches will include host plant resistance, biological control, cultural practice modifications, alternative chemicals, and combinations of all of these.

Postharvest Commodity Treatment (Including Structural). The loss of MB in 2001 will have significant negative impacts on U.S. domestic and international trade. The time frame is very short given that treatments will have to be developed commodity by commodity, and many years may be required to gain appropriate new commodity treatment by a trading partner country. Approaches will include heat; controlled atmospheres; irradiation; chemicals, including other fumigants; exclusion; as well as exotic pest-eradication technology.

UNITED STATES FOOD MARKETING SYSTEM.

See UNITED STATES FOOD MARKETING SYSTEM in the Supplement section.

Contents

Preface v

Preface to the First Edition vii

Contributors ix

Conversion Factors, Abbreviations, and Unit Symbols xvii

A **1**

- Acidulants 1
  - Citric Acid 1
  - Malic Acid 1
  - Tartaric Acid 1
  - Fumaric Acid 2
  - Functions of Food Acids 2
  - Conclusion 5
  - Bibliography 5
- Affluence, Food Excess, and Nutritional Disorders 6
  - Obesity 6
  - Hypertension 7
  - Diabetes Mellitus 8
  - Cardiovascular Disease 8
  - Role of Diet in Cardiovascular Disease 8
  - Cancer 9
  - Diet and Cancer Risk 9
  - Anorexia Nervosa and Bulimia Nervosa 9
  - Conclusion 10
  - Bibliography 11
- Aflatoxins 13
- Agglomeration and Agglomerator Systems 13
  - General Aspects 13
  - Undesired Agglomeration – Caking 13
  - Agglomeration Methods 14

- Characterization of Agglomerated Powders 16
- Product Examples 17
- Bibliography 18

Alcohol, Polyhydric 18

Alcoholic Beverages and Human Responses 19

- General Metabolism of Alcohol 19
- How Alcohol Is Consumed 19
- Absorption of Alcohol 21
- Physiological Actions of Alcohol 22
- Bibliography 26
- General References 26

Alkaloids 26

- Classification and Properties 26
- Bibliography 34
- General References 34

American Dietetic Association 35

Amino Acids 35

Animal By-Product Processing 35

- Blood 36
- Rendering 36
- Fine Chemicals and Pharmaceuticals 42
- Sausage Casings 42
- Pet Food 42
- Bibliography 42
- General References 43

Animal Science and Livestock Production 43

- Business Aspects of Livestock Production 43
- The Discipline of Animal Science 44
- Principles of Animal Reproduction and Breeding 45
- Principles of Animal Nutrition and Feeding 46
- Red Meat Production 47
- Milk Production 48
- Future Developments 52
- Bibliography 53
- General References 54

Antibiotics in Foods of Animal Origin 54

- Sample Preparation and Residue Isolation 55
- Analytical Methodology 56

This page has been reformatted by Knovel to provide easier navigation.

- Future Trends 60
- Bibliography 60

Antimicrobial Compounds 63

- Sodium Chloride 64
- Organic Acids and Esters 64
- Nitrites 66
- Parahydroxybenzoic Acid Esters (Parabens) 66
- Sulfites 67
- Dimethyl Dicarbonate 67
- Phenolic Antioxidants 67
- Phosphates 68
- Naturally Occurring Compounds and Systems 68
- Onions and Garlic 71
- Bibliography 73

Antioxidants 75

- Chain-Breaking Antioxidants 76
- Inhibiting Lipid Oxidation Catalysts 76
- Control of Reactive Oxygen Species 78
- Effective Utilization of Antioxidants in Foods 78
- Bibliography 78

AOAC International 79

- History 79
- Membership 79
- Technical Services 80
- Technical Divisions 80
- Meetings and Education 80
- Publications 80

Aphrodisiacs and Stimulants 81

- Components of Food 81
- Smell and Sexual Arousal 81
- Bibliography 85

Appetite 87

- Physiological Influences 88
- Metabolic and Hormonal Influences 88
- Conditioned Hunger 90
- Specific Appetites and Nutrient Depletions 90
- Sensory and Perceptual Factors 90
- Emotional Factors 92

This page has been reformatted by Knovel to provide easier navigation.

- Social Factors 93
- Cognitive Factors 93
- Interactions Among Relevant Factors 93
- Conclusions 93
- Bibliography 94

Apples 95

Apricots 95

Aquaculture 95
- Global Aquaculture Producers and Production 96
- Species under Cultivation 96
- Economics 98
- Regulation 99
- Culture Systems 100
- Water Sources and Quality 104
- Nutrition and Feeding 106
- Reproduction and Genetics 108
- Diseases and Their Control 109
- Harvesting, Processing, and Marketing 110
- Bibliography 110

Aquaculture: Engineering and Construction 110
- Background 110
- Definition 111
- System Design 111
- Holding Systems 112
- Quantifying Processes 114
- Water Conditioning 115
- Future Developments 117
- Bibliography 117

Aromatherapy 118
- General Reference 119

Artificial Intelligence 119
- Background 119
- Examples 121
- Advantages/Future Development 121
- Bibliography 122

Aseptic Processing and Packaging Systems 122
- Definitions 122
- Basic Aseptic System 122

This page has been reformatted by Knovel to provide easier navigation.

Description of the Aseptic Processing System 122
Nonthermal Processing 126
Aseptic Packaging Systems 126
Summary 127
General References 127
Aseptic Processing: Ohmic Heating 128
Principle of Ohmic Heating 128
Design of the Ohmic Heater 128
Measurement of Electrical Conductivity of Particulate Food Products 129
Temperature Control of the Ohmic Heater 129
Aseptic Processing Using the Ohmic Heater 129
Product Quality 130
Bibliography 132

B 133

Bakery Leavening Agents 133
Chemical Leavening 133
Biological Leavening 137
Bibliography 138
Bakery Specialty Products 139
Native Flat Breads 139
Pita Bread 139
Pizza Dough 140
Bagels 140
Pretzels 141
English Muffins 141
Doughnuts 142
Eclairs 143
Layered Dough Products 143
Pies 144
General References 145
Bamboo Shoots 145
Family and Related Species 145
Practical Description 145
Origin, Distribution, and Production 145
Culture 145
Harvest 146
Storage 146

- Production Season 146
- Food Uses 147
- Nutritional Value 147
- Bibliography 147

Bananas 148

Barley 148

Beekeeping 148
- History 148
- Biology of Honey Bees 148
- Workers 149
- Queens 149
- Drones 149
- Mating 149
- Swarming 149
- Diseases and Pests 149
- Africanized Honey Bees 150
- Pollination 151
- Products of the Hive 151
- Future Development 152
- Bibliography 153

Beer 153
- History 153
- Raw Materials of Brewing 155
- Unmalted Cereals (Adjuncts) 155
- Special Malts 156
- Hops 156
- Yeast 159
- The Brewing Process 160
- Plant Operations 168
- Quality Control 168
- World Beer Production 169
- Glossary 169
- General References 171

Beverages: Carbonated 171
- Description 171
- Ingredients 172
- Nutritional Value 173
- Availability 173

- Terminology 173
- History of Carbonated Beverages 174
- Soft Drink Industry 174
- Soft Drink Bottler Associations 175
- Soft Drink Production 175
- Marketing 176
- General References 176

Beverages: Noncarbonated 177
- Beverage Types 177
- History 177
- U.S. Beverage Market 178
- Recent Developments 178
- Other Trends Affecting Beverages 179
- General References 179

Biologically Stable Intermediates 179
- Dried Codfish 180
- Sulfited Fruits 180
- Tomato Products 181
- Grape Juice 182
- Frozen Foods 182
- Bibliography 182

Biscuit and Cracker Technology 183
- Ingredients 183
- Formulas and Procedures 186
- Equipment 187
- Packaging, Storage, and Distribution 189
- Current and Future Trends 189
- Bibliography 189
- General References 190

Blanching 190
- Blanching Methods 190
- Objectives for Blancher Control 192
- Effects of Blanching 192
- Bibliography 196
- General References 198

Blood 198

Blueberries 198

Botulism 198

This page has been reformatted by Knovel to provide easier navigation.

Bovine Spongiform Encephalopathy (BSE) 198
  Overview 198
  Transmissible Spongiform Encephalopathies 199
  BSE Link to Human Disease 199
  The Causative Agent 200
  The BSE Epizootic 201
  Activities to Protect the Food Supply 202
  Glossary 203
  Bibliography 203
Breakfast Cereals 204
  Ready-to-Eat Breakfast Cereals 204
  Hot Breakfast Cereals 205
  Nutritional Considerations 206
  Stability of Breakfast Cereals 207
  Breakfast Cereal Packaging 207
  Bibliography 208
  General References 208
Browning Reaction, Enzymatic 208
  Polyphenol Oxidase 209
  PPO Analysis 210
  PPO Location in Plant 210
  Extraction and Purification 210
  Physiochemical Properties 211
  Control of Browning in Foods 214
  Bibliography 216
Bulking Agents 218
Butter and Butter Products 218
  Butter Consumption 218
  Definitions and Grading of Butter 219
  Composition 220
  Modification of Milk Fat 221
  Processing 223
  Butter Products 225
  Bibliography 229

This page has been reformatted by Knovel to provide easier navigation.

C **231**

Cacao Bean 231

Caffeine 231

- Properties 231
- Analysis 232
- Occurrence 232
- Biogenesis 232
- Caffeine in the Human Diet 232
- Caffeine Production 233
- Metabolism of Caffeine 234
- Pharmacology 234
- Mutagenicity 237
- Teratogenicity 237
- Carcinogenicity 237
- Decaffeination 237
- Bibliography 238

Cancer Risk and Diet 239

- Macronutrients 240
- Carbohydrate 240
- Fat and Energy 240
- Fiber 241
- Fruits and Vegetables 241
- Minerals 242
- Summary 242
- Bibliography 242
- General References 243

Canning: Regulatory and Safety Considerations 243

- Regulatory Controls 243
- Safety Concerns 244
- Safeguards 247
- General References 248

Canola Oil 248

- Background 248
- Horticulture-Postharvest Handling 249
- Breeding Canola for Improved Yield and Quality – Role of Biotechnology 251
- Comparison of Canola with Other Oilseeds 253
- Processing 254
- Edible By-Products 257

This page has been reformatted by Knovel to provide easier navigation.

- Nonedible By-Products 258
- Standards and Safety 258
- Future of Canola 259
- Bibliography 260
- General References 262

Caramel 262

Carbohydrates: Classification, Chemistry, and Labeling 262
- Monosaccharides 262
- Oligosaccharides 265
- Polysaccharides 267
- Bibliography 268

Carbohydrates: Functionality and Physiological Significance 268
- Description 268
- Food Functionality 269
- Basic Physiological Effects 269
- Health Maintenance and Disease Prevention 270
- Bibliography 271

Carotenoid Pigments 272
- Carotenoid Nomenclature 272
- Biosynthesis 274
- Occurrence in Nature 275
- Effect of Agricultural Practices 277
- Analysis 277
- Effect of Food Processing 278
- Carotenoids as Food Colors 279
- Conversion of Carotenoids to Vitamin A 279
- Carotenoids by Chemical Synthesis 280
- Function of Carotenoids 280
- Bibliography 281

Centrifuges: Principles and Applications 282
- Stokes' Law 286
- Screening the Various Centrifuge Types 286
- Centrifuge Types and Conclusion 286

Cereals, Nutrients, and Agricultural Practices 286
- Cereal Grain Production and Composition 286
- Wheat 287
- Corn 287
- Rice 288

- Oats 288
- Barley 288
- Sorghum 288
- Rye 288
- Disclosure Statement 288
- Bibliography 289
- General References 290

Cereals Science and Technology 290
- Grains 290
- Physical Properties and Structure 292
- Standards and Classification 297
- Bibliography 298

Cheese 298
- Origin 298
- Classification 299
- Cheesemaking – General Steps 299
- Recent Developments 301
- Microbiological and Biochemical Changes During Cheese Making and Cheese Ripening 303
- Accelerated Cheese Ripening 307
- Cheese Defects 307
- Process Cheese 308
- Cheese Analogues: History 311
- Definitions 311
- Commercial Use 311
- Ingredients in Cheese Analogues 312
- Protein Sources 312
- Characteristics 313
- Bibliography 313
- General References 314

Cheese Rheology 315
- First Principles 315
- Definitions 316
- Models of Rheological Behavior 317
- Empirical Instrumental Tests 318
- Physical Measurements 321
- Relaxation 325
- Rheology and Structure 326

This page has been reformatted by Knovel to provide easier navigation.

- Some Experimental Results 332
- Variation with Age 333
- Subjective Assessments 334
- Conclusion 334
- Disclosure Statement 335
- Bibliography 335
- General References 336

Cherries 337

Chilled Foods 337
- Market for Chilled Foods 337
- United States 337
- European and Japanese Markets 338
- Processing of Chilled Foods 339
- Intrinsic Product Factors 340
- Chilling Techniques 341
- Packaging of Chilled Foods 341
- Distribution of Chilled Foods 342
- Temperature Control and Monitoring 343
- Microbiology of Chilled Foods 344
- Bibliography 347

Chlorophyll 348

Chocolate and Cocoa 348
- White Chocolate 348
- Cocoa Beans 348
- Chocolate Liquor 348
- Cocoa Powder 349
- Cocoa Butter 349
- Sweet and Milk Chocolate 350
- Theobromine and Caffeine 350
- Nutritional Properties of Chocolate Products 350
- Economic Aspects 350
- General References 351

Citrus 351

Cleaning-in-Place (CIP) 351
- Requirements for CIP 351
- Types of CIP Sets/Systems 352
- Control System 353
- Energy and Cost Considerations 353

Bibliography 353
Coconuts 353
Coffee 354
Modern Coffee Production 354
Economic Importance of Coffee 354
Processing and Packaging 354
Roasting Technology 357
Brewing 358
Instant Coffee 359
Decaffeinated Coffee 360
Bibliography 360
General References 361
Colloid Mills 361
History 361
General Description of Design 361
Theory of Operation 362
Products 362
Bibliography 363
Color and Food 363
Importance of Food Color 363
Color Theory 364
Color Measurement 365
Sample Presentation 368
Data Interpretation 368
Color Measurement of Specific Foods 369
Role of Colorants 369
Effect of Color on Sensory Perceptions 370
Marketing Implications 371
Bibliography 371
Colorants 372
Colorants: Introduction 372
Colorants: Anthocyanins 375
Colorants: Anthraquinones 378
Colorants: Betalains 380
Colorants: Caramel 383
Colorants: Carotenoids 385
Colorants: Carthamin 390
Colorants: Chlorophylls 391

This page has been reformatted by Knovel to provide easier navigation.

- Colorants: Food, Drug, and Cosmetic Colorants 393
- Colorants: Haems 396
- Colorants: Iridoids 397
- Colorants: Monascus 398
- Colorants: Phycobilins 399
- Colorants: Polyphenols 400
- Colorants: Turmeric 401
- Colorants: Miscellaneous Colorants 402

Computer Applications in the Food Industry 404
- Background and Definitions 404
- Food Industry Applications 404
- Future Developments 407
- Bibliography 407

Controlled Atmospheres for Fresh Fruits and Vegetables 408
- Types of CA Storage 408
- Beneficial Effects of CA Storage 409
- Effects on Physiological Disorders 409
- Conclusion 409
- Bibliography 412
- General References 413

Corn 414

Corrosion and Food Processing 414
- Materials of Construction 414
- Selecting Materials of Construction 417
- Types of Corrosion 418
- Corrosion of Specific Environments 428
- Corrosion by Insulating Materials 433
- Corrosion of Rubbers 435
- Bibliography 437

Crabs and Crab Processing 437
- King Crab 438
- Snow Crab 439
- Dungeness Crab 440
- Blue Crab 440
- Bibliography 441

Cranberries 442

Cultural Nutrition 442
- A Literal Melting Pot 442

Regional Differences 442
Ethnic, Cultural, and Religious Influences 442
People and Their Land 449
Bibliography 449
General References 449
Cultured Milk Products 449
Microorganisms 450
Nutrition 451
Bibliography 451
Cyclospora 451

D **453**

Dairy Flavors 453
Overview of Dairy Products 453
Conclusion 458
Bibliography 458
Dairy Ingredients: Applications in Meat, Poultry, and Seafoods 460
Processed Meat Products 460
Dairy Ingredients in Meat Products 460
Finely Comminuted Meat Products 461
Sectioned and Formed Meat Products 464
Interaction of Milk Proteins with Meat Proteins 464
Poultry 466
Seafood 467
Bibliography 467
General References 468
Dairy Ingredients for Foods 469
Forms of Dairy Ingredients 470
The Ingredient Range 470
Products in Which Milkfat Is the Dominant Component 472
Milk Proteins 472
Cheese and Cultured Products 474
Lactose 475
The Nutritionally Valuable Minor Components of Milk 475
Prepared Ingredients for Foods 476
Conclusion 477
Acknowledgments 477
Bibliography 477

- General References 479
Dehydration 480
- Theory 480
- Equipment 488
- Effects on Foods 493
- Rehydration 497
- Nomenclature 497
- Acknowledgments 497
- Bibliography 497
- General References 498
Detergents 498
- Types of Detergent 498
- Detergent Properties 499
- Detergent Components 499
- Cleaning 499
- Disposal 500
- Food Safety 500
- Worker Safety 500
- Bibliography 501
- General References 501
Disinfectants 501
- Definitions 501
- Chemical Disinfecting Agents 502
- Physical Disinfecting Agents 506
- Evaluation of Disinfectants 506
- Modular Systems of Disinfection 507
- Legislation 507
- Bibliography 508
Distillation: Technology and Engineering 509
- Distillation Terminology 509
- System Components: Columns, Internals, Instrumentation, and Auxiliary Equipment 511
- Packaged Distillation Systems 515
- Batch Distillation 516
- Column Design 516
- Bibliography 518
Distilled Beverage Spirits 519
- History 519
- Taxation, Government Regulations 521

This page has been reformatted by Knovel to provide easier navigation.

Production and Consumption 522
Standards of Identity 522
The Manufacturing Process 529
Analysis 535
Packaging 538
Exports of Distilled Spirits 539
Glossary 539
Bibliography 539
General References 540
Dry Milk 540
History 540
Processing 540
The Product 541
Utilization 542
General References 542
Dryers: Technology and Engineering 542
Batch Dryers 542
Continuous Dryers 544
Spray Dryers 545
Rotary Dryers 548
Pneumatic Dryers 550
Band Dryers 550
Selection 553
Efficient Energy Utilization in Drying 553
Spray Drying: New Developments, New Economics 558
Spray Dryers: Fluid-Bed Agglomeration 567
Spin Flash Dryers 571
Partial List of Products Processed on Various Types of APV Dryers 577
Bibliography 578

E **579**

E. Coli 0157 579
Edible Films and Coatings 579
Protein Films 579
Polysaccharide Films 580
Lipids and Resins 581
Composite Films 582
Bibliography 582

Eel 584
  Biology 584
  Life History 585
  Ecology of Elvers 587
  Culture Techniques 587
  Culture Systems 588
  Consumption and Processing 590
  Skin Processing 591
  Future Development 591
  Bibliography 592
Eggs and Egg Products 593
  Development of the Egg Industry 593
  Egg Formation and Structure 593
  Composition of the Egg 594
  Production Practices 594
  Shell Egg Processing 595
  Conversion of Shell Eggs to Liquid Product 596
  Nutritional and Functional Properties of Eggs 596
  Production of Egg-Rich Convenience Foods 597
  Modifying the Composition of Eggs by Processing 598
  Eggs as a Source of Pharmaceutical Products 599
  Bibliography 599
Elastins and Meat Ligaments 599
  Morphology 600
  Chemistry and Biochemistry 600
  Industrial Applications 601
  Bibliography 601
Emulsifier Technology in Foods 602
  Bibliography 611
Emulsifiers, Stabilizers, and Thickeners 611
  Functions of Emulsifiers, Stabilizers, and Thickeners 612
  Types of Emulsifier 614
  Uses of Emulsifiers 619
  Conclusion 620
  Bibliography 620
Encapsulation Techniques 620
  Definition and Technology Development 620
  Spray Drying 621

This page has been reformatted by Knovel to provide easier navigation.

  - Solvent Dehydration 622
  - Encapsulation by Extrusion 622
  - Air Suspension Coating 622
  - Centrifugal Extrusion 622
  - Coacervation 623
  - Inclusion Complexation 623
  - Spray Chilling 623
  - Basic Criteria for Encapsulating Food Ingredients 623
  - Encapsulated Ingredients – Commercial Types and Applications 624
  - Future Development 626
  - Bibliography 626
- Energy Usage in Food Processing Plants 627
  - Milk Processing 627
  - Liquid Food Concentration 628
  - Food Blanching 629
  - Poultry Processing 629
  - Fish Processing 630
  - Canning 630
  - Baking Industry 631
  - Bibliography 632
- Enteral Formulas and Feeding Systems 633
  - Enteral Formulas 633
  - Condition-Specific Nutritionals 634
  - Delivery Systems 636
  - Processing Technology 638
  - Regulatory Activities 639
  - Acknowledgments 640
  - Bibliography 640
- Enzyme Assays for Food Scientists 643
  - Reasons for Assaying Enzymes 643
  - Theoretical Aspects of Enzyme Assays 644
  - Specific Enzyme Assays 646
  - Bibliography 650
- Enzymes in Food Production 652
  - General 652
  - Baked Goods 653
  - Fresh Fruits and Vegetables 654
  - Beverages 654

Dairy Foods 655
Endogenous Enzymes as Processing Indicators 656
Browning Reactions – Color and Flavor 657
Bibliography 657
General References 657
Enzymology 658
Kinetics 658
Applications in Food Processing 658
Bibliography 661
General References 661
Evaporated Milk 661
History 661
The Product 661
Product Processing 662
Industry Production 662
General References 662
Evaporation 663
Theory 663
Equipment 665
Effect on Foods 674
Nomenclature 675
Bibliography 675
Evaporators: Technology and Engineering 676
Types of Evaporators 676
Selection of Evaporators 680
Evaporation Configuration and Energy Conservation 683
Residence Time in Film Evaporation 686
Engineering Conversion 686
Plate-Type Evaporators 688
Mechanical Vapor Recompression Evaporators 691
Bibliography 696
Extraction 696
Principles of Extraction 697
Liquid-Solid Extraction (Leaching) 697
Liquid-Liquid Extraction 698
Special Extraction Operations 699
Bibliography 699

Extrusion 699
- Definition and History of Usage 699
- Food Applications 699
- General Description of Equipment 700
- Bibliography 701

Extrusion Cooking 702
- Background 702
- Effects of Processing Parameters 702
- Changes in Food after Extrusion 702
- HTST Extrusion Cookers 703
- Methodology for HTST Extrusion Cooking 703
- Applications and Description for Extrusion Cooking of Different Categories of Products 704
- Bibliography 705

Extrusion Processing: Texture and Rheology 706
- Texture 707
- Rheology 710
- Texture and Rheology 715
- Conclusion 716
- Nomenclature 716
- Bibliography 716
- General References 720

F **721**

Fats and Oils: Chemistry, Physics, and Applications 721
- Component Fatty Acids 721
- Component Glycerides 723
- Phospholipids 724
- Unsaponifiable Components 724
- Processing 724
- Physical Properties 727
- Autoxidation and Antioxidants 728
- Emulsions and Emulsifiers 729
- Novel Fats and Oils 729
- Bibliography 730
- General References 731

Fats and Oils: Flavors 731
- Mechanisms of Autoxidation 731
- Mechanisms of Photosensitized Oxidation 732

This page has been reformatted by Knovel to provide easier navigation.

- Thermal Oxidation of Fats and Oils 732
- Factors Affecting Oxidation 733
- Flavor Formation by Enzymatic Reactions 733
- Flavor Properties of Fats and Oils 733
- Contribution of Specific Oils to Flavor 733
- Determination of Flavor Compounds 734
- Sensory Evaluation 734
- Flavor Evaluation by Gas Chromatography 734
- Bibliography 735

Fats and Oils: Properties, Processing Technology, and Commercial Shortenings 736
- Chemistry and Structure 736
- Processing Aids and Additives 740
- Fats and Oils Functionality 740
- Salad and Cooking Oils 740
- Frying Shortenings 741
- Griddle and Pan Frying Shortenings 741
- Bakery Shortenings 741
- Other Applications 742
- Butter and Margarines 743
- U.S. Fat Consumption 743
- Nutrition 744
- Bibliography 744
- General References 746

Fats and Oils: Substitutes 746
- Role of Fat Replacers in Food 746
- Composition of Fat Replacers 747
- Nutritional Impact of Fat Replacers 748
- Regulating Fat Replacers 751
- Conclusion 753
- Bibliography 753

Fiber, Dietary 754
- Components 754
- Analysis 754
- Physicochemical Properties 754
- Physiologic and Metabolic Effects 755
- Recommendations 755
- Nutritional Value 755
- Uses in the Food Industry 756

This page has been reformatted by Knovel to provide easier navigation.

Dangers of High-Fiber Diets 756
Bibliography 756
Filth and Extraneous Matter in Food 757
The Federal Food Laws 757
Enforcement of the Act 757
Modes of Contamination 758
Keeping Filth Out of Food 759
How Does Filth in Food Affect Human Health? 760
Filth in Food in Perspective 760
The Paradox of Filth in Food 760
Bibliography 761
Fish and Shellfish Microbiology 763
Microorganisms in Finfish 763
Fish Spoilage and Changes in Bacterial Flora of Fish During Cold Storage 764
Microorganisms in Shellfish 764
Microbiology and Quality 766
Seafood Irradiation 768
Seafood Microorganisms of Public Health Significance 769
Microbiological Criteria and Inspection 771
Bibliography 772
Fish and Shellfish Products 776
The Seafood Chain 776
U.S. Fishery 777
Maintaining Quality of the Catch 783
Processing Seafood 786
Seafood Safety 797
Future Development 798
Bibliography 798
Fish Cakes 799
Fishes: Anatomy and Physiology 799
Gross Anatomy and Orientation 800
Digestive System 800
Renal System 802
Cardiovascular System 804
Respiratory System 805
Nervous System 806
Endocrine System 808
Reproductive System 809

This page has been reformatted by Knovel to provide easier navigation.

- Immune System 812
- Bibliography 813
- General References 813

Fishes: Species of Economic Importance 813
- Anchovy 814
- Striped Bass 814
- Cod Fishes 815
- Flatfishes 818
- Herrings and Sardines 821
- Menhaden 823
- Mullet 823
- Salmon 825
- Smelt 829
- Tuna and Swordfish 831
- Bibliography 833
- General References 833

Fish, Minced 834

Flavor Chemistry 834
- Overview of Flavor Compounds 834
- Sensory Basis of Flavor 835
- Classification and Origin of Flavor Compounds 836
- Occurrence and Organoleptic Properties of Heterocyclic Compounds in Food 838
- Maillard Reaction and Formation of Heterocyclic Flavor Compounds in Food 839
- Flavor Formation by Enzymes and Microorganisms 840
- Analysis of Flavor Compounds 842
- Bibliography 844

Foams and Silicones in Food Processing 844
- Foams 844
- Silicone Antifoams 845
- Basic Guidelines 845
- Future Development 845
- Bibliography 846
- General References 846

Food Additives 846
- Functionality of Food Additives 847
- Government Regulations 849
- Description of Major Food Additives 852
- General References 862

This page has been reformatted by Knovel to provide easier navigation.

Food Allergy 863
  Ice-Mediated Food Allergies 863
  Cell-Mediated Food Allergies 865
  Conclusion 866
  General References 866
Food Analysis 866
  Information Sources 866
  Sampling 867
  Selection of an Analytical Method 867
  Analytical Methods 868
  Major Food Components 870
  Bibliography 871
Food and Nutrition Science Alliance (FANSA) 872
Food and Nutrition Science Alliance Organizations 872
  The American Dietetic Association 872
  American Society for Clinical Nutrition 873
  American Society for Nutritional Sciences 873
Food Chemistry and Biochemistry 874
  Education 874
  Publications 875
  Bibliography 876
Food Chemistry: Mechanism and Theory 877
  Carbohydrates 877
  Lipids 880
  Proteins 881
  Colorants 883
  Flavors 884
  Natural Toxicants 885
  Conclusions 887
  Disclosure Statement 887
  Bibliography 887
Food Consumption Surveys in the U.S. Department of Agriculture 889
  Uses of the Data 889
  Recent USDA Food Consumption Surveys 890
  History of USDA Food Consumption Survey Methodology 891
  Trends in Food Consumption 893
  Pyramid Servings Data 893
  The National Nutrition Monitoring and Related Research Program 895

This page has been reformatted by Knovel to provide easier navigation.

Bibliography 896

Food Crops: Nondestructive Quality Evaluation 897

Principles Used for Nondestructive Instrumental Quality Evaluation 897

Description and Application of Methods 897

Future Developments 900

Bibliography 900

Food Crops: Postharvest Deterioration 902

Causes of Losses 902

Prevention of Losses 903

Bibliography 904

General References 905

Food Crops: Storage 905

Factors Affecting Storage Life 905

Injuries 906

Supplemental Methods to Cold Storage 907

Future Developments 907

Bibliography 907

General References 908

Food Crops: Varietal Differences, Maturation, Ripening, and Senescence 908

Varietal Differences 908

Maturation 909

Ripening 909

Senescence 910

Bibliography 911

General References 911

Food Engineering 911

Professional Responsibilities of a Food Engineer 911

Educational Requirements of a Food Engineer 912

Research and Development Areas in Food Engineering 912

Food Engineering as Related to Other Disciplines and Professional Societies 913

Food, Drug, and Cosmetic Colorants 913

Food Fermentation 913

Definition 913

History 914

Microorganisms Used in Fermented Foods 914

Types of Fermentation Processes 914

Therapeutic Value of Cultured Milk 916

Fermented Solid Dairy Products – Cheese 916

This page has been reformatted by Knovel to provide easier navigation.

- Alcoholic Beverages ... 918
- The Role of Genetic Engineering in Food Fermentation ... 922
- Bibliography ... 922
- General References ... 923

Food Freezing ... 923
- Refrigeration History ... 923
- The Removal of Heat (Refrigeration) ... 924
- The Food Freezing Process ... 929
- Bibliography ... 933
- General References ... 933

Food Marketing ... 934
- Marketing Philosophy ... 934
- The Marketing Management Process ... 934
- Marketing Strategy ... 935
- Marketing Environment ... 935
- Marketing Mix (the Four *P*'s) ... 935
- Marketing Research ... 938
- Conclusion ... 938
- Bibliography ... 938

Food Microstructure ... 939
- Light Microscopy ... 939
- Electron Microscopy ... 941
- Atomic Force Microscopy ... 942
- Dynamic Light Scattering ... 943
- Image Analysis ... 944
- Bibliography ... 944

Food Plant Design and Construction ... 946
- Critical Issues ... 946
- Project Approach ... 946
- Phase I – Feasibility Study ... 946
- Phase II – Engineering and Architectural Design ... 949
- Phase III – Project and Construction Management ... 951
- Bibliography ... 953
- General References ... 953

Food Preservation ... 953
- Causes and Manifestations of Food Deterioration ... 953
- Preservation Principles and Applications ... 955
- Bibliography ... 958

Food Processing 959
  Food Composition 959
  Food Laws and Regulations 961
  Preservation of Living Tissue 961
  Preservation of Nonliving Tissue 962
  Heat Treatment 963
  Nonthermal Processes Yielding Microbial Inactivation 964
  Freezing 964
  Reduction of Water Activity (Dehydration) 966
  Mechanical Food Processes Enhancing Preservation 966
  Chemical Preservation Methods 967
  Toxicological Implication of Processed Food 967
  Food-Processing Facilities 968
  Food Packaging 968
  Bibliography 969
  General References 969
Food Processing: Effect on Nutritional Quality 969
  Thermal Processing 970
  Food Preservation Through Control of Water 976
  Emerging Food Processing Technologies and Their Implications on Quality 978
  Reaction Kinetics in Food Systems 979
  Basic Principles of Kinetics 980
  Final Remarks 983
  Bibliography 984
Food Processing: Standard Industrial Classification 986
  Standard Industrial Classification Manual 986
  Meat Products (IGN 201) 987
  Dairy Products (IGN 202) 988
  Canned, Frozen, and Preserved Fruits, Vegetables, and Food Specialties (IGN 203) 990
  Grain Mill Products (IGN 204) 991
  Bakery Products (IGN 205) 993
  Sugar and Confectionery Products (IGN 206) 994
  Fats and Oils (IGN 207) 995
  Beverages (IGN 208) 996
  Miscellaneous Food Preparations and Kindred Products (IGN 209) 997
  Bibliography 999
Food Processing: Technology, Engineering, and Management 999
  Controlling Food Qualities 999

- The Optimizing Decision Maker 1001
- Examples 1001
- Conclusion 1006
- Bibliography 1006

Food Regulations: International, Codex Alimentarius 1006

- History 1006
- Conclusion 1009
- Bibliography 1009
- General References 1009

Food Safety and Risk Communication 1009

- Risk Messages 1010
- Risk Comparisons 1010
- Risk and Consumer Perceptions 1010
- Bibliography 1011

Food Safety and Risk Management 1012

- Consideration of Risk Characterization 1012
- Risk, Costs, and Benefits 1012
- Prevention, Intervention, and Control Actions 1012
- United States Regulations 1013
- International Regulations 1014
- Bibliography 1014

Food Safety Education: Consumer 1014

- Sources of Consumer Information 1014
- Consumer Concerns 1015
- Consumer Safe-Handling Knowledge 1016
- Consumer Perception of the Source of Foodborne Illness 1016
- U.S. National Food Safety Program 1016
- Bibliography 1017

Food Science and Technology: Definition and Development 1017

- Food Research Before the Term "Food Science" Arose 1018
- The First of the Teaching Departments 1018
- Food Science and Technology as a Title 1019
- Positions Filled by Food Scientists/Technologists 1019
- Bibliography 1020

Food Science and Technology: the Profession 1020

- The Profession 1021
- Strengthening Value-Added Components 1022
- Newer Processes 1022

This page has been reformatted by Knovel to provide easier navigation.

- Nutraceuticals 1023
- Governmental Activities 1023
- Governmental Regulation 1023
- Summary 1024
- Bibliography 1024

Food Spoilage 1025

- Organoleptic Description of Spoilage 1025
- Food Spoilage Mechanisms 1027
- Tools for Detection of Spoilage 1032
- Tools for Prediction of Spoilage 1033
- Measures to Prevent Spoilage 1034
- Bibliography 1035

Food Surface Sanitation 1035

- Food Soils 1036
- Fundamentals 1036
- Sanitation Procedure 1038
- Chemicals 1038
- Methods 1041
- Evaluation of Effectiveness of Sanitation Systems 1043
- Evaluation of the Effectiveness of Disinfection 1044
- Future Development 1045
- Bibliography 1047
- General References 1048

Food Toxicology 1048

- Toxicology: Principles, Definitions, and Scope 1048
- Food Toxicology 1050
- Safety Evaluation and Risk Assessment 1053
- Conclusions 1056
- Bibliography 1057

Food Utilization 1057

- The Alimentary System 1058
- Carbohydrates 1059
- Proteins 1066
- Fats 1073
- Nucleic Acids 1077
- Water, Vitamins, and Minerals 1077
- Bibliography 1077
- General References 1077

Foodborne Diseases 1078

- Definitions 1078
- Food Intoxications 1079
- Bacterial and Microbial Intoxication 1079
- Bacterial Infection 1081
- Summary 1088
- Bibliography 1088
- General References 1088

Foodborne Microorganisms: Detection and Identification 1089

- Detection Methods That Require Viable Cells 1089
- Detection of Organisms and/or Their Products by Methods That Do Not Require Viable Cells 1092
- Bibliography 1094

Foodservice Systems 1098

- Overview 1098
- Menu System 1100
- Purchasing, Receiving, and Storage Systems 1100
- Production System 1101
- The Service System 1101
- Human Resource System 1102
- Equipment and Energy System 1102
- Quality Assurance Control System 1102
- General References 1102

Freeze Concentration 1103

- Products 1103
- Freezing Point Depression 1103
- Cleanness of Separation 1103
- Ice Crystal Formation and Growth 1104
- Ice Production 1105
- Freeze-Concentration Systems 1105
- Bibliography 1106

Freeze Drying 1106

- Products 1106
- Advantages and Disadvantages 1107
- System Components 1107
- Preparation 1107
- Basic Process 1108
- Heat Transfer 1108

Pressure Drop 1108
Sublimation Temperature 1108
Product Surface Temperature 1109
Drying Time 1109
Collapse 1109
Particulate Products 1109
Foamed Products 1110
Condensation 1110
Batch Freeze Driers 1110
Continuous Freeze Driers 1110
Alternative Freeze Drying Methods 1110
Bibliography 1111
Freezing Systems for the Food Industry 1112
Fundamentals 1112
History of Refrigeration 1112
Fundamental Changes During Freezing 1113
Definitions 1116
Freezing Equipment 1118
Thermodynamic Properties 1125
Determination of Holding Time 1130
Major Considerations in Freezer Design 1130
Refrigeration Systems 1134
Bibliography 1138
Fresh-Cut Fruits and Vegetables: Modified Atmosphere Packaging 1139
Partial Processing of Fresh Produce 1139
The Rationale for Modified Atmosphere Packaging of Fresh-Cut Produce 1139
Problems and Concerns Associated with MAP of Fresh-Cut Produce 1140
Modes of MAP 1140
Shelf-Life Requirements of MAP Fresh-Cut Products 1141
Selection of MAP Materials 1141
Safety Considerations 1141
Bibliography 1141
Fruit Dehydration 1142
Principles 1142
Drying Methods 1143
Predrying Treatments 1145
Postdrying Treatments 1146
Packaging 1146

- Procedures for Selected Commodities 1146
- Bibliography 1148

Fruit Preserves and Jellies 1149
- Definitions and Standards 1149
- Gelation-Pectin Mechanism 1149
- Carbohydrate Sweeteners 1150
- Processing Techniques 1151
- Quality Parameters 1152
- Current Trends Worldwide 1153
- Summary 1153
- Bibliography 1153
- General References 1153

Fruits, Semi-Tropical 1154
- Acerola 1154
- Bael 1154
- Calamondin 1154
- Carob 1154
- Chironja 1154
- Fig 1154
- Grapefruit 1154
- Kumquat 1154
- Lemon 1155
- Litchi 1155
- Longan 1155
- Loquat 1155
- Mandarin 1155
- Medlar 1155
- Orange 1155
- Pomegranate 1156
- Pummelo 1156
- Sour Orange 1156
- Sugar Apple 1156
- Tangerine 1156
- Ugli 1156
- Bibliography 1156

Fruits, Temperate 1156
- Apple 1156
- Apricot 1157

- Bilberry 1158
- Blackberry 1158
- Blueberry 1158
- Cherry 1158
- Chokeberry 1158
- Cranberry 1159
- Currant 1159
- Gooseberry 1159
- Grape 1159
- Huckleberry 1160
- Kiwi 1160
- Loganberry 1160
- Mulberry 1160
- Peach 1160
- Pear 1161
- Persimmon 1161
- Plum 1161
- Prune 1161
- Quince 1161
- Raisin 1162
- Rose Hip 1162
- Strawberries 1162
- Bibliography 1162

Fruits, Tropical 1163
- Avocado 1163
- Banana 1163
- Breadfruit 1163
- Carambola 1164
- Cashew Apple 1164
- Ceriman 1164
- Cherimoya 1164
- Custard Apple 1164
- Date 1164
- Durian 1164
- Feijoa 1165
- Guava 1165
- Ilama 1165
- Jabotica 1165

- Jackfruit 1165
- Java Plum 1165
- Jujube 1165
- Limes 1165
- Mamey 1165
- Mango 1165
- Mangosteen 1166
- Miracle Fruit 1166
- Papaya 1166
- Passion Fruit 1166
- Pineapple 1166
- Plantain 1166
- Prjckly Pear 1167
- Pulasan 1167
- Rambutan 1167
- Rose Apple 1167
- Santol 1167
- Sapodilla 1167
- Soursop 1167
- Star Apple 1167
- Tamarind 1167
- Tree Tomato 1167
- Wax Apple 1167
- Bibliography 1167

Frying Technology 1168
- Process Analysis 1168
- Engineering Principles 1168
- Fryers 1169
- Food and Oil Quality Interactions 1169
- Frying Oil Degradation 1169
- Food Quality 1171
- Nutrition and Toxicology 1171
- Regulatory 1171
- Frying Research 1171
- Process Optimization 1172
- Frying Oil Substitutes 1172
- Frying Oil Controversies 1172
- Bibliography 1172

This page has been reformatted by Knovel to provide easier navigation.

Fumigants 1172
  Available Fumigants 1173
  Properties of Metal Phosphides 1173
  Methyl Bromide 1174
  Controlled Atmospheres 1175
  Mixtures of Gases 1176
  Bibliography 1176
  General References 1176
Functional Foods 1176
  Functional Foods from Plant Sources 1177
  Functional Foods from Animal Sources 1179
  Current Trends 1181
  Bibliography 1181

G 1183

Gelatin 1183
  Chemistry and Biochemistry 1183
  Gelatin Manufacture 1184
  Gelatin Safety 1184
  Gelatin Properties and Uses 1185
  Gelatin Testing Methods 1186
  Bibliography 1188
  General References 1188
Genetic Engineering: Animals 1188
Genetic Engineering: Food Flavors 1188
  Enzyme Mixtures and Purified Enzymes 1189
  Microbial Cells 1191
  Plant Catalysts 1194
  Commercial Bioflavors 1196
  Bibliography 1196
Genetic Engineering: Principles and Applications 1198
  History 1199
  Principles of Genetics 1199
  Genetic Engineering Technologies 1205
  Applications of Genetic Engineering in Food Science 1210
  Bibliography 1211
Grains and Protectants 1212
  Bibliography 1215

Grapefruit 1216
Gums 1216
  Algins 1217
  Carboxymethylcelluloses 1217
  Carrageenans, Agar, and Furcellaran 1219
  Gelatin 1220
  Gellan Gum 1220
  Guar and Locust Bean (Carob) Gums 1221
  Gum Arabics 1222
  Gum Tragacanth 1222
  Hydroxypropylcelluloses 1222
  Konjac Mannan 1222
  Methylcelluloses and Hydroxypropylmethylcelluloses 1223
  Pectins 1223
  Xanthan Gums 1224
  Bibliography 1224

H 1225

Hazard Analysis and Critical Control Points (HACCP) 1225
Heat 1225
  Processing Foods by Adding or Removing Heat 1226
  Changing the State of a Food Product 1226
  Bibliography 1227
Heat Exchangers 1227
  Heat Exchangers 1227
  Heat Exchangers: Fouling 1231
  Heat Exchangers: Paraflow 1237
  Heat Exchangers: Plate Versus Tubular 1246
  Heat Exchangers: Scraped Surface 1254
Heat Transfer 1270
  Background 1270
  Processing Foods by Conduction Heat Transfer 1272
  Processing Foods by Convection Heat Transfer 1274
  Combined Convection and Conduction Heat Transfer 1275
  Radiation Heat Transfer 1276
  Transferring Heat in Foods 1277
  Bibliography 1278
  General Reference 1278

This page has been reformatted by Knovel to provide easier navigation.

High-Pressure Processing 1278
- Technology of Food Preservation by High Pressure 1279
- Packaging Foods for High-Pressure Treatment 1280
- Product Safety and Regulatory Issues 1280
- Economic Considerations 1280
- General References 1281

History of Foods 1281
- Background 1282
- Paleolithic 1283
- Conclusion 1287
- Glossary 1288
- Bibliography 1289

Homogenizers 1289
- Early History 1289
- Description 1290
- Homogenizing Valve 1291
- Theories of Homogenization 1292
- Homogenizing Valve Design 1292
- Applications 1292
- Operation 1294
- Testing 1294
- Summary 1294
- Bibliography 1294

Hunger, Food Deprivation, and Nutritional Disorders 1295
- Assessment of Nutritional Status 1295
- Global Nutrition Situation 1295
- Malnutrition and Nutritional Disorders 1296
- The Future 1301
- Bibliography 1301

Hurdle Technology 1302
- Principles of Hurdle Technology 1302
- Basic Aspects 1303
- Applications of Hurdle Technology 1305
- Food Design 1306
- Bibliography 1306

Hydrogen-Ion Activity (pH) 1307
- pH Determination 1307
- pH Measurement System Electrodes 1309

pH Instrumentation 1311
Temperature Effects 1311
Nonaqueous Solvents 1312
Indicator pH Measurements 1312
Industrial Process Control 1312
Bibliography 1313
General References 1313
Hydrogenation 1313
Reaction Mechanism 1313
Selectivity 1313
Effects of Process Conditions 1314
Catalysis 1315
Catalyst Poisons 1317
Catalysts 1318
Hydrogenation in Practice 1319
General References 1321
Hydrophobicity in Food Protein Systems 1321
Background 1321
Theory and Measurement of Protein Hydrophobicity 1322
Spectroscopic Methods: Intrinsic Fluorescence 1324
Hydrophobicity of Food Proteins 1326
Importance of Hydrophobicity in Functionality of Food Proteins 1326
Future Trends 1329
Bibliography 1329
General References 1331

I **1333**

Ice Cream and Frozen Dessert 1333
Background and Definitions 1333
Ice Cream Composition and Physical Properties 1333
Ice Cream Processing – Mix Making, Freezing, and Hardening 1337
Ice Cream Defects 1339
Novelties 1340
Sherbets and Sorbets (Ices) 1340
Other Frozen Desserts 1340
Future Trends 1341
Bibliography 1341

Immobilized Enzymes 1342
  Methods for Immobilization 1342
  Current Applications in Food Processing 1343
  Food Analysis 1344
  Potential Applications and Future Developments 1345
  Bibliography 1345
  General References 1345
Immunological Methodology 1345
  Background 1346
  Food Constituents 1348
  Natural Contaminants 1352
  Microbial Contaminants 1356
  Chemical Agents 1357
  Conclusion 1359
  Bibliography 1360
  General References 1367
Infant Foods 1368
  Infant Nutrition 1368
  History of Foods and Feeding Practice 1369
  Infant Formula 1369
  Baby Foods 1369
  Controversies 1370
  Consumption Patterns 1370
  Alternatives 1371
  General References 1371
Institute of Food Technologists (IFT) 1371
Institute of Food Technologists: Awards 1372
  Nicholas Appert Award 1372
  Babcock-Hart Award 1372
  Samuel Cate Prescott Award 1373
  International Award 1373
  William V. Cruess Award 1373
  Carl R. Fellers Award 1373
  Food Technology Industrial Achievement Award 1374
  Calvert L. Willey Distinguished Service Award 1374
  Stephen S. Chang Award for Lipid or Flavor Science 1374
  Industrial Scientist Award 1374
  Research and Development Award 1375

- Elizabeth Fleming Stier Award 1375
- Bernard L. Oser Food Ingredient Safety Award 1375

Institute of Food Technologists: History and Perspectives 1375
- Formation of the Institute 1376
- Beyond Broaching an Idea 1376
- Annual Meeting Thereafter 1376
- Early-Current Officers 1376
- IFT Organizational Structure 1376
- Membership Classes 1377
- Programs of the Institute 1377
- Science Communication Program 1377
- The IFT Foundation 1377
- IFT Publications 1378
- Perspectives 1378
- IFT and IUFoST 1378
- Bibliography 1378

Integrated Pest Management 1379

International Development 1379
- International Development: Australia 1379
- International Development: Canada 1383
- International Development: Finland 1388
- International Development: Germany 1393
- International Development: Ireland 1399
- International Development: New Zealand 1402
- International Development: South Africa 1406
- International Development: Spain 1410
- International Development: Switzerland 1413
- International Development: Taiwan 1416
- International Development: United Kingdom 1421

International Food Information Service (IFIS) 1425
- Structure and Aims 1425
- Databases and Coverage 1425
- Additional Products and Services 1426

International Union of Food Science and Technology 1426
- Objectives 1426
- Adhering Bodies to IUFoST 1427
- History 1427
- Organization 1427

This page has been reformatted by Knovel to provide easier navigation.

Irradiation of Foods 1428
  The Evolving Nature of Food Processing Technology 1428
  The Concept of Food Irradiation Has a Long History 1428
  Current Applications of Radiation Technology in Agriculture 1428
  Growing Interest in Food Irradiation 1429
  Technical Aspects 1429
  Safety and Wholesomeness 1431
  Overview of Implementation Progress 1432
  Current Status 1435
  Future Outlook 1435
  Bibliography 1436
  General References 1436

K **1437**

Kinetics 1437
  Mathematization of Kinetic Behavior 1437
  Basic Chemical Kinetics 1437
  First-Order Rate Processes 1438
  Enzyme- and Cell-Catalyzed Reactions 1439
  Starch Conversion 1442
  Effects of Energy Inputs 1443
  Bioreactor 1445
  Biological Reactor 1445
  Food Storage and Prediction of Shelf Life 1447
  Bibliography 1448
Kosher Foods and Food Processing 1449
  The Kosher Market 1449
  The Kosher Dietary Laws 1449
  Allowed Animals and Prohibition of Blood 1449
  Prohibition of Mixing of Milk and Meat 1450
  Passover 1450
  Equipment Koshering 1450
  Industrial Jewish Cooking 1451
  Dealing with Kosher Supervision Agencies 1451
  Gelatin 1452
  Biotechnology 1452
  Federal and State Regulations 1452
  Allergies 1453

This page has been reformatted by Knovel to provide easier navigation.

Bibliography 1453
General References 1453
Krill Protein Processing 1453
Chemical Composition 1453
Processing 1454
Bibliography 1456

L 1457

Laboratory Robotics and Automation 1457
Robot Types 1457
Robot Programming 1457
Applications 1458
Implementation 1459
Bibliography 1460
General References 1460
Lactose 1460
Source 1460
Properties 1460
Lactose Digestion and Lactose Intolerance 1460
Uses 1461
Products from Lactose 1461
Properties 1461
Analysis 1461
Bibliography 1462
Lecithins 1462
Commercial Lecithin 1462
Bibliography 1465
Licorice Confectionery 1466
By-Products 1466
Candy 1466
Types of Licorice 1467
Ingredients 1467
Batch Process Manufacture 1467
Continuous Process Manufacture 1468
Domestic and Foreign Standards and Regulations 1468
Limonin 1470
History of Limonin in Citrus Juices and Its Determination 1470
Taste Threshold Levels 1471

This page has been reformatted by Knovel to provide easier navigation.

Regulatory Aspects for Limonin in Grapefruit Juice 1472
Limonin Content of Grapefruit Juice 1472
Effect of Several Factors on Limonin Content of Grapefruit Juice 1472
Effect of Limonin in Grapefruit Juice on Sensory Flavor Quality 1473
Interaction Between Brix:Acid Ratio and Limonin Content in Affecting Sensory Flavor of Grapefruit Juice 1475
Methods for Reducing the Limonin Content of Grapefruit Juice 1476
Summary, Conclusions, and Recommendations 1477
Acknowledgments 1478
Bibliography 1478
Lipids: Nutrition 1479
Fat Effects on Health 1480
Fat and Fatty Acid Intake 1484
Summary 1484
Bibliography 1484
Livestock Feeds 1485
Farming Unconventional Animals 1487
Companion Animals 1487
Nutrient Requirements 1487
Computers 1488
The Feed Manufacturing Industry 1488
Feeding Systems Used in Livestock Production 1488
Feed Ingredients 1491
Feed Processing 1493
Diet Formulation and Example Diets 1494
Future Developments 1494
Bibliography 1495
Lobster: Biology and Technology 1496
Homarus Americanus 1496
Nephrops Norvegicus 1496
Palinuridae 1496
Catching Methods 1496
Live Holding 1497
Shipping 1497
Processing 1498
Aquaculture 1498
Crayfish 1498
Bibliography 1499

This page has been reformatted by Knovel to provide easier navigation.

Low-Acid and Acidified Foods 1501
  pH of Various Foods 1501
  Microbiology of Low-Acid and Acidified Foods 1501
  Thermobacteriology 1503
  Regulations Governing Low-Acid and Acidified Foods 1504
  Establishing a Process 1505
  Types of Containers 1507
  Deviation Management 1507
  Processing Systems for Low-Acid Canned Foods 1507
  Processing Acidified Foods 1508
  Aseptic Processing and Packaging 1508
  Spoilage of Canned Foods – Determination of Cause 1508
  Conclusion 1509
  Bibliography 1509

M 1511

Machine Vision Systems: Process and Quality Control 1511
  The System and Its Components 1511
  Applications for MVS 1513
  Functions of Machine Vision Systems 1515
  Configuration and Types of MVS 1515
  Bibliography 1516
Malts and Malting 1517
  Changes Occurring in Malting Grains 1517
  Malting Technologies 1518
  Malt Types 1519
  Bibliography 1520
Margarine 1521
  Historical Milestones 1521
  Types of Products 1521
  The Regulatory Identity of Margarine 1522
  Margarine Fats 1522
  Other Fatty Type Ingredients 1523
  Aqueous Components 1524
  Manufacturing Process 1524
  Quality Testing 1524
  General References 1525

This page has been reformatted by Knovel to provide easier navigation.

Marine Enzymes 1525

  Enzyme Types, Definitions, Sources, and Some Properties 1525

  Applications, Current Use, and Impact of Biotechnology 1529

  Bibliography 1530

  General References 1533

Marine Toxins 1534

  Biotoxins 1534

  Occurrence 1535

  Health Effects 1535

  Detection 1536

  Controls 1536

  Other Disorders 1537

  Bibliography 1537

Mass Transfer and Diffusion in Foods 1538

  Molecular Diffusion 1539

  Diffusion with Bulk Flow and Chemical Reactions 1542

  Unsteady-State Mass Transfer 1543

  Mass Transfer in Two-Phase Systems 1543

  Determination of Diffusivity and Overall Mass Transfer Coefficient 1544

  Applications of Diffusion and Mass Transfer in Food Processing 1547

  Nomenclature 1548

  Bibliography 1548

  General References 1549

Meat and Electrical Stimulation 1549

  History 1549

  Description 1549

  Meat Tenderness-Toughness 1551

  Scientific Basis 1552

  Factors Affecting ΔpH 1554

  Factors Affecting dpH/dt 1555

  Practical Results of Stimulation 1555

  Role of Electrical Stimulation in Hot Boning 1557

  Bibliography 1558

Meat, Modified Atmosphere Packaging 1561

  Types of MAP Packages 1561

  Muscle Scavenging of Oxygen 1562

  Dynamic MAP Systems 1563

  Active Packaging 1563

- The Reactions of Spoilage Floras and Pathogenic Bacteria to Modified Atmospheres 1563
- Muscle Pigment Reactions in Modified Atmosphere 1564
- Summary 1566
- Bibliography 1566

Meat Processing: Technology and Engineering 1567
- Injecting 1567
- Massaging and Tumbling 1567
- Grinding 1568
- Chopping and Emulsifying 1569
- Mixing 1569
- Stuffing 1570
- Heat Processing 1570
- Packaging 1573
- Bibliography 1574
- General References 1575

Meat Products 1576
- Formulations 1577
- Restructured Meat Products 1577
- Curing 1578
- Acidification 1579
- Smoking 1579
- Preservation Methods 1580
- Home Meal Replacement 1581
- By-Products 1581
- Regulations and Standards 1582
- Bibliography 1583
- General References 1583

Meat Science 1584
- What Is Meat Science? 1584
- Principles of Meat Science 1584
- Muscle Structure and Function 1584
- Muscle to Meat 1586
- Myofibrillar Aspects of Meat Quality 1588
- Role of Connective Tissue in Meat Quality 1591
- Cooking and Meat Tenderness 1592
- Animal Factors Affecting Meat Quality 1594
- Meat Color 1596
- Meat Storage and Flavor Changes 1598

- Nutritional Aspects of Meat 1600
- Bibliography 1600

Meat Slaughtering and Processing Equipment 1604
- Ovines 1604
- Pigs 1609
- Poultry 1613
- Beef 1614
- Meat Starter Cultures and Meat Product Manufacturing 1614
- Fermented Sausage Manufacture 1615
- The Influence of Starter Cultures in Fermented Sausages 1615
- Public Health Aspects of Dry-Cured Sausages 1618
- Raw Ham Manufacture 1619
- Manufacture of Meat Starter Cultures 1620
- Future Prospects 1620
- Bibliography 1620

Membrane Filtration Systems 1622
- Membrane Spread Technique 1622
- Direct Epifluorescent Technique 1622
- Hydrophobic Grid Membrane Filtration 1622
- Bibliography 1625
- General References 1627

Membrane Processing 1627
- Important Factors 1627
- Food Applications 1628
- Bibliography 1628

Microbiology of Foods 1629
- Numbers and Kinds of Microbes in Foods 1629
- Bacteria 1630
- Yeasts and Molds 1632
- Intrinsic and Extrinsic Parameters of Foods 1632
- Food-Preservation Techniques 1633
- Bibliography 1634
- General References 1634

Microwave Science and Technology 1635
- Fundamentals of Microwave Heating 1635
- Effect of Various Physical Factors 1636
- Food-Processing Applications 1638
- Advantages of Microwave Processing 1638

Examples of Microwave Processing Applications 1638
Future Development 1640
Bibliography 1641
General References 1641
Military Food 1641
Military Subsistence Program 1641
Logistics 1642
Current Rations 1643
Technology and Science 1644
Sensory Analysis of Rations 1646
Ration Testing and Evaluation 1646
Nutrient Stability 1647
Microbiological Stability 1649
Quality Monitoring of Stored Foods 1649
Future Concepts 1650
Bibliography 1652
Milk and Milk Products 1653
Composition and Properties 1653
Processing 1654
Cleaning Systems 1656
Packaging 1656
Analysis and Testing 1656
Manufactured Products 1657
Bibliography 1659
General References 1660
Minerals: Micronutrients 1660
Micromineral Toxicity 1661
Micromineral Deficiency 1662
Antagonisms and Interactions Among Trace Minerals 1662
Mineral Absorption 1663
Bioavailability 1663
Apparent Absorption 1663
Mineral Uptake and Transport 1663
Functions of Microminerals 1664
Minerals and Gene Expression 1665
Bibliography 1666
Molecular Modeling: Computer Modeling of Functional Properties 1666
Computer Modeling of Biopolymers 1667

Application of Molecular Mechanics Calculations to Food Systems 1669
Conclusions 1670
Bibliography 1670
Monosodium Glutamate (MSG) 1671
Muscle as Food 1671
Composition of Muscle Tissue 1671
Conversion of Muscle to Meat 1672
Processing and Fabrication 1672
General References 1672
Mushrooms: Cultivation 1673
Description 1673
Conservation of Culture 1673
Cultivation 1674
Bibliography 1677
Mushrooms: Processing 1678
Processing 1678
Bibliography 1681
Muslim Dietary Laws: Food Processing and Marketing 1682
Muslim Dietary Laws 1682
Halal Food Processing 1682
Bibliography 1683
Mycotoxin Analysis 1684
General Analytical Procedures 1684
Sampling and Subsampling 1684
Extraction 1685
Cleanup Methods 1685
Analysis: Separation, Detection, Quantitation 1686
Chemical Methods 1687
Capillary Electrophoresis 1689
Immunochemical Methods 1689
Biological Methods 1691
Rapid Screening Methods 1692
Future Development 1693
Bibliography 1693
Mycotoxins 1698
Deoxynivalenol and Zearalenone 1699
Aflatoxin 1700
Fumonisins 1701

Ochratoxin 1702
Other Mycotoxins That Can Occur in Food 1702
Bibliography 1703

N 1707

Nitrosamines 1707
Chemistry of Nitrosation 1707
Formation of Nitrosamines in Foods 1708
N-Nitrosamines in Food 1709
Trends 1712
Dietary Exposure to *N*-Nitroso Compounds 1714
Nitrosamine Exposure and Human Cancer 1715
Bibliography 1715
General References 1715
Nucleotides 1717
Synergism of 5′-Nucleotides for Umami Taste 1717
Structure of Nucleic Acids 1720
The Structure of Nucleic Acid Polymers 1720
Optical Properties of Nucleic Acid 1722
Contents of Nucleotides in the Ingredients of Foods 1724
Properties of Nucleic Acids 1726
Bibliography 1731
General References 1732
Nutritional Labeling 1732
Some Key Milestones in Food Labeling 1732
The Nutrition Labeling and Education Act of 1990 1733
Current U.S. Food-Product Labeling Requirements 1734
Nutrition Information Based on NLEA 1736
Future Issues and Concerns 1741
Additional Information 1742
Bibliography 1742
General References 1742
Nuts 1743
Nutritional Quality and Food Processing 1743

O 1745

Oilseeds and Vegetable Oils 1745
Processing Oilseeds 1745

This page has been reformatted by Knovel to provide easier navigation.

- Specific Oils 1747
- Bibliography 1754
- General References 1754

Olestra 1755

Olives and Olive Oil 1755
- The Olive Tree 1756
- Olive Oil 1759
- Table Olives 1765
- Reduction and Reuse of Wastes 1775
- The International Olive Oil Council 1775
- Summary 1776
- Bibliography 1776

Omega-3 Fatty Acids 1779

Optimization Methods in Food Processing and Engineering 1779
- The Best 1779
- The Elements of Optimization Problems 1779
- Optimization Methods 1781
- Application and Implementation 1787
- Bibliography 1788

Oranges 1789

Osteoporosis 1789
- Definition 1789
- Symptoms 1789
- Risk Factors 1789
- Bone Development over a Lifetime 1789
- Bone Composition 1791
- Genetic and Environmental Factors 1791
- Diagnosis 1791
- Prevention of Osteoporosis 1792
- Resources for More Information 1794
- Bibliography 1794

Oxidation 1796
- Oxidation in Proteins 1797
- Enzyme-Catalyzed Oxidation 1798
- Oxidation of Vitamins 1799
- Bibliography 1800

Ozone and Food Processing 1801
- Ozone Background 1801

This page has been reformatted by Knovel to provide easier navigation.

Ozone Disinfection Properties 1802
Gras Approval of Ozone 1802
Food Processing 1803
Ozone Applications for Food Storage 1803
Ozone Applications for Food Processing 1803
Recommendations 1804
Bibliography 1804

P **1807**

Packaging: Part I – General Considerations 1807
Marketplace Demands and Driving Forces 1807
Functions of a Food Packaging System 1807
Gas Barrier Protection and Shelf Life 1807
Physical Protection 1809
Food-Package Interactions 1809
Packaging and Waste Disposal 1810
Regulations 1810
Other Considerations 1810
Information Technology and Packaging 1810
Bibliography 1811
Packaging: Part II – Labeling 1811
Key Differences Between FDA and USDA 1811
Principal Display Panel 1813
Product Identity Statement 1814
Declaration of Net Quantity of Contents – FDA 1815
Declaration of Net Quantity of Contents – USDA 1817
Claims-Related Statements 1817
Additional USDA Labeling Requirements 1819
Information Panel 1820
Nutrition Facts 1820
Ingredient List 1820
Manufacturer Identity 1821
Percent-Juice Declaration 1821
USDA Safe-Handling Instructions 1821
Other Labeling Requirements 1822
Acknowledgments 1824
General References 1824

This page has been reformatted by Knovel to provide easier navigation.

Packaging: Part III – Materials 1824
  Glass 1824
  Metal 1825
  Paper and Paperboard 1825
  Plastic 1826
  Bibliography 1829
Packaging: Part IV – Controlled/Modified Atmosphere/Vacuum Food Packaging 1830
  Growth of MAP Technology 1830
  Methods of Atmosphere Modification 1831
  Gas-Packaging Equipment 1831
  Roles of Gases Used in Modified Atmosphere Packaging 1832
  Antimicrobial Effects of CO_2 1833
  Factors Influencing the Antimicrobial Effect of CO_2 1833
  Application of Gas Packaging for Shelf-Life Extension of Food 1835
  Advantages and Disadvantages of MAP 1838
  Microbiological Safety of MAP Foods 1838
  Conclusion 1838
  Bibliography 1839
  General References 1839
Palm Oil 1839
  The Oil Palm and Its Fruits 1840
  Extraction of Crude Palm Oil 1840
  Refining and Fractionation 1841
  The Chemical and Physical Characteristics of Refined Palm Oil and Its Fractions 1841
  Uses of Palm Oil and Its Fractions 1842
  Nutritional and Health Aspects of Palm Oil 1843
  Bibliography 1843
Parasitic Organisms 1844
  Toxoplasmosis 1845
  Sarcocystis 1846
  Trichinellosis 1847
  Cysticercosis 1848
  Fishborne Parasites 1849
  Bibliography 1850
  General References 1851
Pasta 1852
Patents 1852
  The Law 1852

This page has been reformatted by Knovel to provide easier navigation.

- Definition and Rights ... 1852
- Jurisdiction ... 1852
- Patentability ... 1853
- The Patent Application ... 1854
- Summary ... 1857
- General References ... 1858

Peaches ... 1858

Pectic Substances ... 1858
- Distribution in Nature ... 1858
- Isolation and Purification ... 1858
- Chemical Composition and Structure ... 1858
- Methods of Analysis ... 1858
- Chemical and Enzymatic Reactions ... 1859
- Molecular Weight, Size, and Shape of Pectins ... 1860
- Binding Properties ... 1860
- Food Applications ... 1861
- Glossary ... 1861
- Disclosure Statement ... 1861
- Bibliography ... 1861

Peptides ... 1862
- General Aspects of Peptides ... 1862
- Chemistry and Analysis ... 1862
- Occurrence in Nature ... 1863
- Peptides in Pharmacological Use ... 1863
- Peptides in Food Science ... 1863
- Current and Future Status ... 1866
- Bibliography ... 1867

Pesticide Residues in Food ... 1868
- Pesticide Use and Classification ... 1868
- Pesticide Regulation ... 1869
- Toxicological Evaluation of Pesticides ... 1869
- Pesticide Residue Monitoring ... 1870
- Bibliography ... 1871

Phenolic Compounds ... 1872
- Structure ... 1872
- Biosynthesis ... 1874
- Analysis ... 1874
- Contents in Fruits and Vegetables and Its Products ... 1875

This page has been reformatted by Knovel to provide easier navigation.

- Effects of Food Processing 1876
- Biological and Chemical Activities 1878
- Commercial Applications 1879
- Bibliography 1879

Phosphates and Food Processing 1881

- Historical Development and General Use 1881
- Nomenclature and Structure 1881
- Manufacture 1882
- Chemical Properties 1882
- Applications of Food Phosphates 1883
- Regulatory Status in the United States and Other Countries 1884
- Trends 1884
- Bibliography 1885

Photosynthesis 1886

- Structure and Organization of Chloroplasts 1886
- The Light-Driven Photosynthetic Reactions 1890
- The Dark Reactions 1891
- Concluding Remarks 1892
- Bibliography 1893

Phytochemicals: Antioxidants 1895

- Antioxidant Mechanisms of Phytochemicals 1895
- Phytochemicals and Cancer 1896
- Phytochemicals and Cardiovascular Disease 1898
- Conclusion 1900
- Bibliography 1900

Phytochemicals: Biotechnology of Phenolic Phytochemicals for Food Preservatives and Functional Food Applications 1901

- Plant Phenolics as Phytopharmaceuticals 1902
- Genetic Heterogeneity in Family Lamiaceae 1902
- Rosmarinic Acid and Medicinal Applications 1902
- Pharmacological Functions of Rosmarinic Acid 1902
- Production of Rosmarinic Acid 1903
- Pathways Associated with Rosmarinic Acid Biosynthesis 1903
- Model and Rationale of Current Research 1903
- Basil and Functional Food Applications 1906
- Thyme and Antimicrobial Applications in Food 1906
- Elite Basil for Postharvest Preservation of Potatoes 1907
- Bibliography 1907

This page has been reformatted by Knovel to provide easier navigation.

Phytochemicals: Carotenoids 1909

- Structure and Physicochemical Properties of Carotenoids 1910
- Analysis of Carotenoids 1910
- Nutritional Properties of Carotenoids 1910
- Occurrence and Functional Properties of Carotenoids in Foods 1912
- Naturally Occurring Carotenoids in Plant and Animal Foods 1912
- Carotenoids as Colorant Additives in Foods 1913
- Effects of Processing on Naturally Occurring Carotenoids and Carotenoid Colorants in Foods 1914
- Bibliography 1915

Phytochemicals: Lipoic Acid 1916

- Natural Sources of Lipoic Acid 1916
- Bioavailability 1916
- Antioxidant Properties of Lipoic Acid 1917
- Signal Transduction 1917
- Therapeutic Implications 1917
- Bibliography 1918

Phytochemicals: Melatonin 1918

- Identification of Melatonin in Plants 1918
- Twenty-Four-Hour Rhythms in Plant Melatonin 1919
- Function of Melatonin in Plants 1920
- Conclusion 1921
- Bibliography 1921

Phytochemicals: *Vaccinium* 1922

- History 1922
- Plant Composition 1923
- Nutritional Properties 1924
- Pharmacological Properties 1924
- Conclusion 1925
- Bibliography 1925

Phytochemicals: Wine 1927

- Phenolics in Wine 1927
- Vinification and Phenolics 1929
- Wine and Health 1930
- Bibliography 1932

Pineapples 1933

Potatoes and Potato Processing 1933

- Processing Operations 1934

- Quality 1935
- Bibliography 1936

Poultry Flavors 1937
- Effect of Production and Processing Methods 1937
- Chemical Basis of Poultry Flavors 1938
- Summary and Future Development 1940
- Bibliography 1940

Poultry: Meat From Avian Species 1941
- Chicken 1943
- Turkey 1946
- Ducks 1949
- Goose 1950
- Squab (Young Pigeon) 1951
- Ratites 1952
- Game Birds 1954
- Bibliography 1956
- General References 1956

Poultry Meat Microbiology 1957
- Contamination of Poultry 1957
- Shelf Life and Spoilage 1957
- Potential Human Pathogens 1958
- Further-Processed Poultry Products 1959
- Retarding the Growth of Microorganisms on Poultry 1959
- Bibliography 1961

Poultry Meat Processing and Product Technology 1963
- Types of Poultry 1963
- Processing Fresh Poultry 1964
- Fresh Poultry Meat 1965
- Meat Processing Equipment and Procedures 1967
- Sanitation in Poultry Processing Plants 1969
- Poultry Products 1970
- Future Development 1972
- Bibliography 1972

Processed Cheese 1973
- Manufacturing: Principles and Techniques 1973
- Emulsifying Agents 1980
- Quality Defects of Processed Cheese 1982
- Processed Cheese Analogues 1985

- New Developments in Processed Cheese Products 1985
- Bibliography 1986

Proteins: Amino Acids 1988
- Uses of Amino Acids 1988
- Chemical Properties 1992
- Physical Properties 1993
- Industrial Production 1996
- Safety 1998
- Bibliography 1999
- General References 2000

Proteins: Denaturation and Food Processing 2000
- Native Protein Structure and Stability 2000
- Protein Denaturation 2003
- Factors Affecting Protein Denaturation 2005
- Denaturation of Food Proteins 2008
- Stabilization of Proteins 2010
- Conclusion 2011
- Acknowledgments 2011
- Bibliography 2011

Proteins: Structure and Functionality 2014
- Amino Acids 2014
- Primary Structure 2015
- Secondary Structure 2017
- Determination of Secondary Structure 2017
- Prediction of Secondary Structure and Molecular Modeling 2019
- Tertiary and Quaternary Structures 2019
- Molecular Forces 2019
- Interactions 2020
- Changes in Protein Structure During Food Processing 2021
- Structure-Function Relationships 2022
- Acknowledgments 2023
- Bibliography 2023

Pulsed Light Processing 2025
- Pulsed Light 2025
- Microbicidal Effects 2025
- Treatment of Packaging 2026
- Treatment of Food 2026
- Treatment of Water 2026

General References 2028

Q 2029

Quality Assurance 2029
- The Food Quality System 2029
- System Design 2030
- Quality Costs 2030
- Food Safety 2030
- Emerging Foodborne Diseases 2031
- Nutritional Quality 2031
- Hazard Analysis: Critical Control Point Systems 2032
- Risk Analysis 2032
- Physical Hazards 2033
- Chemical Hazards 2033
- Biological Hazards 2033
- Quality Management 2033
- Conclusion 2034
- Bibliography 2034

R 2037

Rapid Methods of Microbiological Analysis 2037
- Improvements in Sampling and Sample Preparation 2037
- Alternative Methods for Viable Cell Count Procedure 2037
- Instruments for Estimation of Microbial Populations and Biomass 2039
- Miniaturized Microbiological Techniques 2040
- Techniques Involving Instruments, Immunology, and Genetic Tests 2041
- Conclusions 2043
- Acknowledgments 2043
- Bibliography 2043

Refrigerated Foods: Food Freezing and Processing 2045
- T-TT 2045
- PPP: Product Quality, Processing, and Packaging 2045
- Bibliography 2046

Refrigerated Foods: Food Freezing and World Food Supply 2047
- Meat 2047
- Seafood 2047
- Fruit and Fruit Juices 2048
- Frozen Vegetables 2048

This page has been reformatted by Knovel to provide easier navigation.

- Potatoes 2048
- Bibliography 2048

Refrigerated Foods: Handling and Inventory 2049
- Postfreezing Operations 2049
- Casing and Palletizing 2049
- Inventory Control 2049
- Open Shelf-Life Dating 2049
- Time-Temperature Integrators (T-TI) 2049
- In-Home Storage 2050
- Thawing 2050
- Bibliography 2050

Refrigerated Foods: Transportation 2051
- Special Requirements by Type of Foodstuff 2051
- Modes of Refrigerated Transport 2051
- New Technology 2054
- Environment 2054
- Quality Assurance 2055
- Bibliography 2055

Retort Pouch 2055
- History 2056
- Technology Base 2056
- Package Designs 2057
- Products 2057
- Production Systems 2058
- Retorting 2059
- Reliability and Performance 2060
- Trends 2061
- Bibliography 2061

Rheology 2062
- Fluid Foods 2063
- Solid Foods 2065
- Future Developments 2067
- Bibliography 2067

This page has been reformatted by Knovel to provide easier navigation.

S **2069**
Salad Oils 2069
Salmonella 2069
Sanitizers 2069
Sausages 2069
    Ingredients 2069
    Process 2070
    General References 2071
Seafood: Flavors and Quality 2071
    Background 2071
    Chemical Compounds Responsible for Flavors Found in Seafoods 2072
    Compounds Responsible for Modifying Seafood Flavors and Tastes 2079
    Attempts to Use Seafood Flavor and Texture Attributes to Classify Fish 2083
    Future Work 2090
    Conclusions 2091
    Bibliography 2091
Seafood: Sensory Evaluation and Freshness 2095
    Indirect Determination of Seafood Freshness 2096
    Direct Determination of Seafood Freshness 2096
    Conclusion 2099
    Bibliography 2099
Seaweed Aquaculture: Taiwan 2100
    Introduction 2100
    Cultivation of *Gracilaria* 2101
    Aquaculture of *Porphyra* 2102
    Cultivation of *Conchocelis* 2103
    Bibliography 2103
Sensors and Food Processing Operations 2104
    Chemical Sensors 2104
    Biosensors 2106
    Summary 2109
    Acknowledgments 2109
    Bibliography 2109
    General References 2111
Sensory Science: Principles and Applications 2111
    Introduction 2111
    The Three Areas of Sensory Analysis 2111
    Descriptive Analysis 2111

- Measurement of Perceived Intensity 2116
- Hedonics – Measurement of Liking 2119
- Experimental Design and Sensory Product Optimization 2119
- An Overview 2131
- Bibliography 2131

Sensory Science: Standardization and Instrumentation 2132

- Emerging and Coalescing Technologies 2132
- Examination of Multicomponent Data Sets 2133
- Kinds of Multivariate Methods 2133
- Preliminary or Exploratory Uses 2134
- Calculation and Functioning of PCA 2134
- Cluster Analysis 2136
- Multidimensional Scaling 2138
- Discriminant Analysis 2138
- Linear Discriminant Analysis 2139
- Multiple Regression Analysis 2141
- Partial Least Squares Regression 2142
- Response Surface Methodology 2142
- Procrustes Analysis 2142
- Multivariate Analysis of Variance 2143
- Fuzzy Logic 2144
- Neural Networks 2144
- Canonical Analysis 2144
- Canonical Discriminant Analysis 2145
- Correspondence Analysis 2145
- Factor Analysis 2145
- Whither Sensory-Instrumental Correlation? 2146
- Bibliography 2148
- General References 2149

Shellfish 2150

- Oysters 2150
- Scallops 2152
- Shrimp 2153
- General References 2159

Snack Food Technology 2159

- Business and Trends 2159
- Potato Chips 2159
- Kettle Chips 2160

This page has been reformatted by Knovel to provide easier navigation.

Tortilla Chips and Corn Chips 2160
Popcorn 2162
Extruded Snacks 2162
Pretzels 2163
Nuts 2163
Meat-Based Snacks 2163
Packaging 2164
Trends 2164
Bibliography 2164
General References 2165
Solid-State Fermentation and Value-Added Utilization of Fruit and Vegetable Processing By-Products 2165
Solid-State Fermentation 2165
Fruit and Vegetable Processing Wastes 2168
Utilization of Fruit and Vegetable Processing Wastes Via Solid-State Fermentation 2168
Bibliography 2171
Soy Foods, Fermented 2175
Soy Sauce 2175
Miso 2178
Sufu 2180
Natto 2181
Tempeh 2182
Taosi 2183
Bibliography 2184
Soybeans and Soybean Processing 2185
Properties and Compositions 2185
Harvesting and Storage 2187
Processing 2187
Nutrition and Toxicity 2187
Products and Uses 2187
Bibliography 2190
General References 2190
Spices and Herbs: Natural Extractives 2191
Essential Oils 2191
Oleoresins 2192
Manufacturing Process 2192
Forms and Applications 2193
Nutritional Information 2194

- Methods of Analysis 2194
- Regulatory Status 2194
- Bibliography 2195

Spices and Seasonings 2195
- Spices 2195
- Seasonings 2201
- Future Trends 2203
- Bibliography 2203
- General References 2203

Starch 2203
- Molecular Components 2203
- Granular Nature, Cooking Characteristics, and Solution and Gel Properties 2205
- Modified Food Starch 2206
- Uses 2208
- Digestion 2208
- Starch-Related Products 2209
- Bibliography 2209

Sugar: Substitutes, Bulk, Reduced Calorie 2209
- Bibliography 2214

Sugar: Sucrose 2214
- Structure 2214
- Sources 2214
- Forms 2215
- Sweetness 2215
- Other Properties 2216
- Uses 2216
- Physiological Effects 2217
- Bibliography 2217

Sulfites and Food 2218
- Chemical Reactions of Sulfites in Food System 2218
- Application of Sulfites in the Food Industry 2219
- Treatment and Residual Levels of Sulfites in Foods 2219
- Bibliography 2219

Supercritical Fluid Technology 2220
- Fundamentals of Supercritical Fluid Extraction 2220
- Process Systems and Operation Characteristics 2224
- Commercial Applications 2225
- Potential Applications 2225

- Edible Oils and Fatty Acids 2225
- Bibliography 2225

Surface (Interfacial) Tension 2226
- Bibliography 2228

Surimi: Science and Technology 2229
- The Surimi Process 2229
- Process Wastewater 2231
- Protein Recovery 2232
- Gel Quality Evaluation 2232
- Use of Cryoprotectants 2233
- Changes During Frozen Storage 2233
- Gel Formation 2233
- Production of Surimi-Based Products 2235
- Effect of Ingredients on Texture 2237
- Important Processing Considerations 2238
- Bibliography 2238

Sweeteners: Nutritive 2240
- Sucrose 2241
- Fructose 2241
- Glucose 2242
- Glucose (Corn) Syrups 2242
- Fructose-Glucose Syrups 2242
- Invert Sugar 2243
- Honey and Molasses 2243
- Corn Syrup Solids 2243
- Maltodextrins 2243
- Lactose 2244
- Trehalose 2244
- Sugar Alcohols 2244
- Bibliography 2244

Sweeteners: Nonnutritive 2245
- Requirements for Commercial Viability of Nonnutritive Sweeteners 2245
- High-Potency Nonnutritive Sweeteners of Major Commercial Significance 2248
- Other High-Potency Nonnutritive Sweeteners of Commercial Significance 2254
- Sugar Macronutrient Substitutes of Commercial Significance` 2257
- New Developments in Nonnutritive Sweeteners 2260
- Conclusion and Future Development 2262
- Bibliography 2262

This page has been reformatted by Knovel to provide easier navigation.

Syrups (Starch Sweeteners and Other Syrups) 2265
  Dextrose 2265
  High-Fructose Corn Syrups 2273
  Corn Syrups and Maltodextrins 2277
  Maple Syrup 2278
  Molasses 2279
  Sorghum Syrups 2280
  Inulin-Based Products 2282
  Acknowledgments 2282
  Bibliography 2282
  General References 2283

T 2285

Tannins 2285
  Background 2285
  Proanthocyanidins 2285
  Polyesters 2287
  Astringency 2287
  Analysis 2288
  Tannin-Protein Interactions 2288
  Physiological Effects 2290
  Bibliography 2291
Taste and Odor 2292
Tea 2292
  Agriculture 2292
  Fresh Leaf Composition 2292
  Manufacture 2295
  Black Tea 2297
  Black Tea Beverage 2299
  Green Tea 2300
  Oolong Tea 2300
  World Production and Tea Trade 2301
  Instant Tea 2301
  Ready-to-Drink (RTD) Tea 2302
  Physiological Effects 2302
  Acknowledgments 2303
  Bibliography 2303

This page has been reformatted by Knovel to provide easier navigation.

Thermal Processing of Food 2305
- Sterilization Versus Pasteurization 2305
- Sterilization Equipment Systems 2306
- Scientific Principles of Thermal Processing 2313
- Computer Simulation 2317
- Future Trends 2320
- Bibliography 2321

Thermal Sterilization of Canned Liquid Foods 2321
- Quantitative Measure of Sterilization 2322
- Special Considerations for Liquids 2322
- Agitated Batch (In-Container) Heating of Liquids 2324
- Nonagitated Batch (In-Container) Heating of Liquids 2327
- Bibliography 2330

Thermodynamics 2331
- Laws of Thermodynamics 2331
- Using Thermodynamic Relationships in Food Processing 2331
- Nomenclature 2333
- Bibliography 2335
- General References 2335

Toxicants, Natural 2336
- Natural Plant Toxins in Foods 2336
- Mycotoxins 2343
- Heterocyclic Amines in Cooked Foods 2349
- Acknowledgments 2351
- Bibliography 2351

Toxicology and Risk Assessment 2352
- Toxicity: Types and Targets 2352
- Risk Assessment 2353
- Bibliography 2356

U **2357**

Ultrafiltration and Reverse Osmosis 2357
- Background Information 2357
- Membranes and Membrane Modules 2357
- Process Description 2359
- Effects of Operating Parameters 2360
- Applications in Food Industries 2361
- Bibliography 2361

United States: Armed Forces Food Research and Development 2363
  Food Engineering Directorate 2363
  Advanced Systems Concepts Directorate 2364
  Soldier Science Directorate 2364
United States Department of Agriculture 2365
  Mission of Agriculture Research Service 2365
  Goals of Agriculture Research 2365
  Animal Production, Product Value, and Safety 2366
  Human Nutrition Requirements, Food Composition, and Intake 2369
  Natural Resources and Sustainable Agricultural Systems 2371
  Crop Production, Product Value, and Safety 2377
United States Food Marketing System 2381

V **2383**

Vanilla Extract 2383
  History of Vanilla 2383
  Horticulture 2383
  Curing 2385
  Extraction 2388
  Flavor 2390
  The Vanilla Market 2396
  Bibliography 2398
  General References 2399
Vegetable Dehydration 2399
  Predrying Treatments 2400
  Drying Methods 2400
  Packaging and Storage of Dehydrated Vegetables 2402
  Quality Control 2403
  Dehydration Procedures for Selected Commodities 2403
  General References 2405
Vegetable Processing 2406
  General Principles 2406
  Microbial Spoilage of Foods 2406
  Chemical and Biological Changes 2406
  Freeze Drying 2408
  Harvesting, Handling, and Storing 2408
  Preparing Vegetables for Processing 2408
  Processing 2409

This page has been reformatted by Knovel to provide easier navigation.

- Some Illustrative Samples of Vegetable Processing 2411
- Trends in Vegetable-Processing Development 2415
- Bibliography 2415

Vegetable Production 2416
- Definition 2416
- Classification of Vegetables 2416
- Production Statistics 2417
- Vegetable Consumption 2420
- Vegetable Improvement 2421
- Vegetable Growth Requirements 2422
- Propagation of Vegetables 2423
- Vegetable-Production Systems 2423
- Field Production of Vegetables 2424
- Vegetable Harvest 2425
- Postharvest Handling 2426
- Projections for the Future 2427
- Bibliography 2427
- General References 2427

Vegetables, Pickling 2427
- History 2427
- Processing 2428
- Fermentation and Microbiology 2430
- Nutritive Value 2431
- Shelf Life and Bulk Storage 2432
- Safety 2432
- Starter Cultures 2433
- Bibliography 2433
- General References 2433

Vegetarian and Vegetarian-Aware Eating Trends 2434
- What Vegetarian Means 2434
- A Brief History 2434
- Nutritionally Misunderstood 2434
- Vegetarian-Aware Shoppers 2434
- Meat on the Menu 2434
- Market Size 2435
- Demographics of Vegetarian and Vegetarian Aware Shoppers 2435
- Dietary Priorities 2435
- Heart-Healthy, Lowfat, and Low-Calorie Diets 2436

Health Issues 2436
Health Concerns 2436
Health Problems 2437
Shopping Habits 2437
Package Information 2438
Label Claims 2438
A Growing Market 2439
Bibliography 2439
Vitamins 2440
Vitamins: Survey 2440
Vitamins: Ascorbic Acid 2449
Vitamins: Biotin 2467
Vitamins: Folic Acid 2478
Vitamins: Niacin, Nicotinamide, and Nicotinic Acid 2487
Vitamins: Pantothenic Acid 2496
Vitamins: Pyridoxine (B_6) 2505
Vitamins: Riboflavin (B_2) 2515
Vitamins: Thiamine (B_1) 2527
Vitamins: Vitamin A 2538
Vitamins: Vitamin B_{12} 2551
Vitamins: Vitamin D 2562
Vitamins: Vitamin E 2585
Vitamins: Vitamin K 2592

W 2601
Waste Management: Animal Processing 2601
Dairy-Processing-Related Waste Management 2601
Egg-Processing-Related Waste Management 2603
Meat- and Poultry-Processing-Related Waste Management 2606
Bibliography 2608
Waste Management: Fruits and Vegetables 2609
Sources and Characteristics of Fruit and Vegetable Wastes 2609
Conclusion 2613
Bibliography 2613
Water Activity: Food Texture 2614
Food Texture 2614
Water Activity 2614
General Considerations 2615

This page has been reformatted by Knovel to provide easier navigation.

- Sorption Isotherms 2615
- Apples 2616
- Beef 2618
- Cereal Grains 2620
- Cereal-Based Foods 2622
- Powders 2624
- Sugars 2625
- Miscellaneous Foods 2626
- Summary 2628
- Bibliography 2628
- General References 2630

Water Activity: Good Manufacturing Practice 2630
- Water Activity and Control of Microbial Growth 2631
- Establishing Scheduled Processes 2631
- FDA GMP Regulations Governing the Processing Requirement 2632
- Humectants and Their Regulatory Use 2633
- Water Activity Determinations 2633
- Disclosure Statement 2634
- Acknowledgments 2634
- Bibliography 2634
- General References 2634

Water Activity: Microbiology 2635
- Definition of Water Activity 2635
- Water Activity as an Indicator of Microbial Safety and Shelf Life 2635
- Osmotic Stress and Osmoregulation in Microorganisms 2637
- Water Activity in Detection and Enumeration of Microorganisms 2637
- Influence of Water Activity on Microbial Death and Survival 2638
- Water Activity Measurement 2638
- Prediction of Water Activity 2640
- Final Remarks 2640
- Bibliography 2640

Wheat Science and Technology 2641
- Wheat 2641
- Production, Trade, and Uses 2641
- Gross Composition 2642
- Milling 2645
- Flour Types 2648
- Bibliography 2651

Whey: Composition, Properties, Processing, and Uses 2652
- Types and Forms of Whey 2652
- Other By-Products of Milk and Whey Processing 2652
- Composition and Properties 2652
- Industrial Processing of Whey 2655
- Nutritive Value and Uses of Whey 2659
- Bibliography 2660

Wine 2662
- Introduction 2662
- Regulations and Appellations 2662
- Viticulture (the Science of Grape Growing) 2662
- Microorganisms and Fermentation 2663
- Wine Making 2663
- Sensory Properties 2664
- Reading Wine Labels 2667
- Production and Consumption Statistics 2669
- Bibliography 2669
- General References 2670

Supplement 2671

Genetic Engineering: Animals 2671
- Agricultural Applications 2673
- Domestic Animals as Bioreactors 2675
- The Future 2675
- Bibliography 2675
- General References 2675

Nuts 2676
- Where the Science Began 2676
- Peanuts in a Weight-Loss Diet 2677
- Nuts Help People Stick to Weight-Loss Diets 2677
- Peanuts and Nuts Satisfy Hunger 2677
- Nut and Peanut Eaters Have Better Body Profiles 2677
- Peanuts and Nuts Decrease the Risk of a Second Heart Attack 2677
- Nutrients Recently Found in Peanuts and Nuts 2678
- Peanuts 2678
- Cashews 2681
- Almonds 2681
- Research 2682

This page has been reformatted by Knovel to provide easier navigation.

- Brazil Nuts 2682
- Hazelnuts 2682
- Macadamia Nuts 2683
- Pecans 2685
- Pine Nuts 2687
- Pistachio 2687
- Walnuts 2688
- Nuts in Diet Patterns 2688
- Mediterranean Diet Pyramid 2688
- Peanuts and Nuts in World Cuisines 2688
- Allergies 2689
- Bibliography 2689
- General References 2689

Postharvest Integrated Pest Management 2690
- What Is IPM? 2690
- Development of IPM Programs 2691
- Setting the Stage for IPM: the Pesticide ERA 2691
- Setting the Stage for IPM: Entering the IPM ERA 2692
- Food Safety and IPM 2692
- IPM for Fresh Commodities with Zero Pest Tolerance 2694
- Postharvest IPM for Raw, Durable Commodities 2694
- IPM for Food Processing 2697
- IPM for Value-Added Food Products 2698
- Adopting IPM: Challenge and Necessity 2699
- Bibliography 2699

United States Food Marketing System 2701
- An Overview of Trends 2702
- Production 2706
- Processing and Manufacturing 2707
- Food Wholesaling, Retailing, and Service 2711
- Food Retailing and Food Service 2714
- Future Forces for Continual Change 2718
- Bibliography 2722

Index **2725**

This page has been reformatted by Knovel to provide easier navigation.

V

VANILLA EXTRACT

HISTORY OF VANILLA

Vanilla, the world's most prized flavor, is one of the most valuable treasures the Europeans brought back from the New World. When Cortez landed his army in Eastern Mexico in 1519, he formed an alliance with the local Indians, who then helped lead the Spanish troops against Montezuma and his Aztec empire (Fig. 1). Montezuma royally welcomed Cortez with a vanilla-cocoa brew. This drink, chocolatl, was concocted from cocoa beans, ground corn, honey and Tlilxochitl (vanilla pods). Cortez took Montezuma's life and his treasures, including the secret of vanilla, which was brought back to the Old World. For several hundred years thereafter, cured vanilla beans were imported from Mexico to Europe for the production of vanilla flavor and perfume. Although the Spaniards had this supply of cured beans, they did not have the complete secret of how to grow and cure vanilla. It was over a hundred years before the Europeans could successfully cultivate the vanilla plant in greenhouses. Propagation through cuttings was somewhat successful in the early 1700s, although the plants seldom flowered and never produced fruits.

Plants were started in tropical regions, including Indonesia, also with no fruit. Eventually, in 1836, a botanist noted that the flowers needed to be individually hand pollinated in order to fruit. In Mexico, this may have been done naturally by bees, hummingbirds and/or a species of leaf-cutting ant. There is no experimental proof that any insect is actually effective in pollinating the vanilla flowers (1,2).

By the mid 1800s improvements in the human hand-pollination techniques had been developed which led to successful vanilla plantations in Madagascar, Reunion, Mauritius, the Seychelles Islands, Tahiti, the Comoros Islands, Ceylon, Java, the Philippines, and parts of Africa. By the 19th century more vanilla was being grown in Madagascar and tropical Asia than in Mexico, breaking the monopoly enjoyed by Mexico for over 200 years. Mexico continued to be a major producer of vanilla beans until the mid-1900s.

HORTICULTURE

Vanilla is the common term for the alcoholic extract of the vanilla bean. The vanilla bean is actually the fruit of a thick, tropical orchid vine. Of the 35,000 or more species in the orchid family, *Orchidacae*, the vanilla orchids produce the only edible fruit. There are over 50 vanilla orchid species, of which only two are of commercial use. *Vanilla planifolia* Andrews (also known as *Vanilla fragrans* (Salisbury) Ames) is the species responsible for 99% of the vanilla imported into the United States (Fig. 2). The other species, *Vanilla tahitensis* (Tahitian Vanilla) grows on the French Pacific Islands and is visually quite different. The tahitensis pods are shorter, have a thicker skin, less seeds, and are much broader than the planifolia beans. Tahitian vanilla beans are primarily exported to France and Europe, although roughly nine tons are imported into the United States.

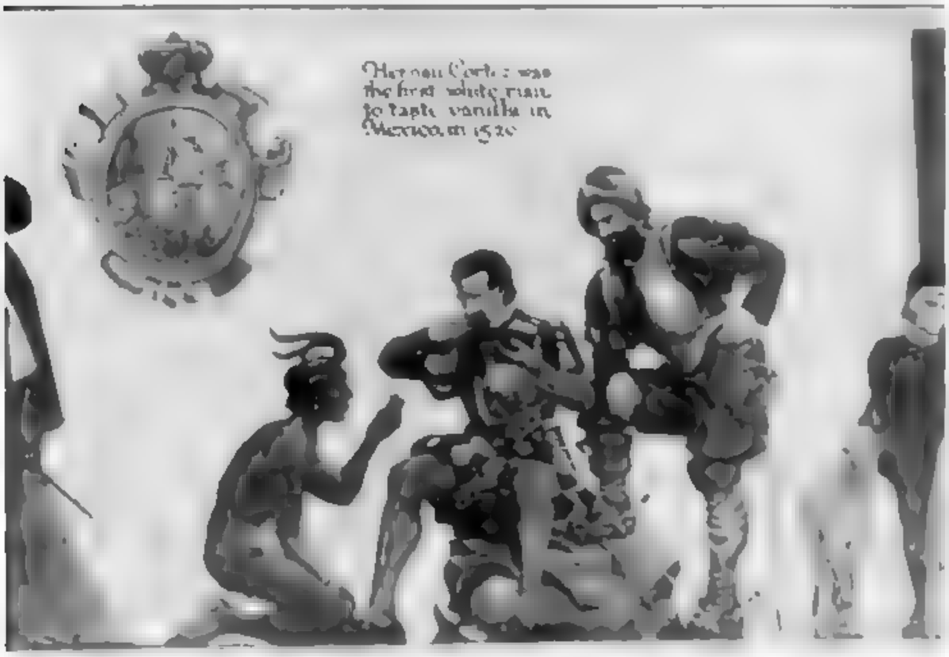


Figure 1. The Spanish troops led by Cortez conquered Montezuma and stole the secret of vanilla. *Source:* Photograph courtesy of McCormick/Schilling Spices.

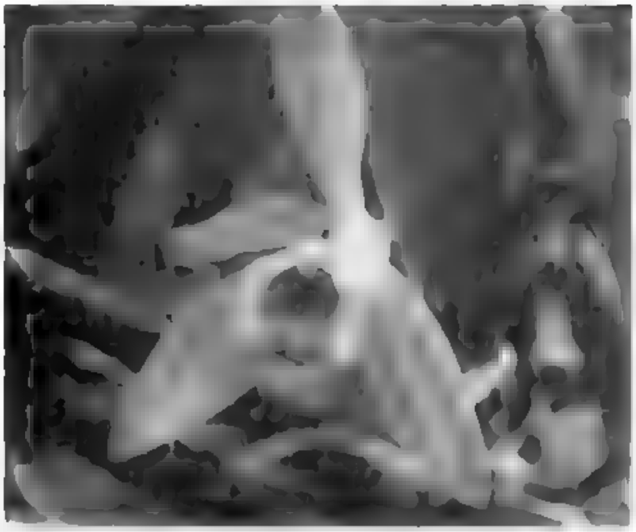


Figure 2. The vanilla orchid. *Source:* Photograph courtesy of McCormick/Schilling Spices.

Vanilla pompona is frequently cited as a third commercial species of vanilla orchid used in perfumes. Today, this species is rarely seen and is mostly a curiosity. Visually, the pods resemble small bananas (2).

Vanilla planifolia is indigenous to southeastern Mexico, the West Indies, Central America and the northern part of South America. Vanilla vines will grow between 25° north and south of the equator; in hot, moist tropical climates; in a 50/50 mixture of sun and shade; from sea level to 2,000 ft. altitude; in areas with frequent moderate rainfall and no extended droughts or high winds; and with gentle slopes for drainage. For commercial production of vanilla beans, it is optimum to have the rainfall evenly distributed throughout 10 months of the year followed by a two-month dry spell to check vegetative growth and spur flower formation. These conditions describe a typical tropical island climate. Today, virtually all vanilla beans are grown on islands such as Madagascar and the Indonesian Islands, where temperatures range between 70–90°F, with 80–100 in. of rain per year.

Vanilla planifolia has smooth, succulent bright green leaves and aerial roots which cling to some type of support. If left untended, vanilla vines will grow 75 ft to the tree tops. On vanilla plantations they are pruned or bent downward to keep the flowers and beans in reach of the workers for pollination and harvest. The pruning and bending also seems to increase flowering.

Commercially, vanilla is propagated entirely by means of 3 ft cuttings, 8 to 12 nodes in length. Longer cuttings usually bring the vines into early production. Vanilla is very difficult to grow from seeds (1–3).

The vines are cultivated from cuttings on trellises and supports, but most frequently on living trees. The species of tree depends upon the geographical area. Small trees with low branches make the best supports, and those with smaller leaves will allow an appropriate amount of sunlight through. Quick growing banana trees are commonly used to provide shade as well (4). Even when man-made supports are used, trees are still planted for partial shading of the vines. The character and growth of the vines depends greatly on the amount of shade and exposure (1).

Healthy vines produce up to 1000 satiny-yellow orchid blossoms, of which only 5–30% will be hand-pollinated depending upon the age and health of the plant. The orchids flower in the morning and die by sundown if not fertilized. Although the flowering period extends over 2 months, each blossom has only this small time-window in which to be fertilized. An expert can pollinate 1000 blossoms a day. If too many blossoms bear fruit, the vines will be weakened for subsequent production. Roughly 100 beans are the maximum number that should be expected from a cultivated plant.

After pollination, the pods ripen in seven to nine months. The vanilla beans are green, long, smooth and slender, somewhat resembling a large green bean filled with thousands of tiny seeds (Fig. 3). As the beans mature they lengthen to up to 12 in. (by 6 months) and gradually turn yellow in the 9th month. The beans are usually harvested before they fully ripen, when only the blossom-end tips are pale yellow (Fig. 4). The best quality vanilla extract comes from blossom-end-yellow beans rather than less ripened green beans (5,6).

At this time, the beans are anywhere from 5–10 in. long and about one-inch in circumference. Longer beans are more desirable as they bring in more poundage per bean. If the beans are picked too early, their flavor precursor content and their quality will be low. They will also become too woody in flavor character and will be more prone to molding. If they are allowed to fully ripen prior to harvest (blossom-end-brown) they split. Splits are very susceptible to molding. Although splits are often considered inferior quality and bring a lower price, if not moldy their vanillin content and resulting extracts can be superior to whole beans (7,8). This may be due to their fully ripe nature.

A very important consideration in the ripening of the vanilla fruit is the formation of the vanillin precursor, Glucovanillin, and most importantly the enzyme B-glucosidase. During the curing process glucovanillin is en-

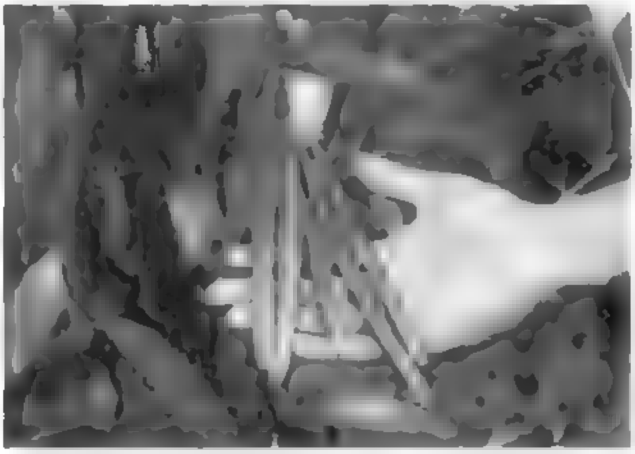


Figure 3. Unripe, green, three month old Madagascar vanilla beans. *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

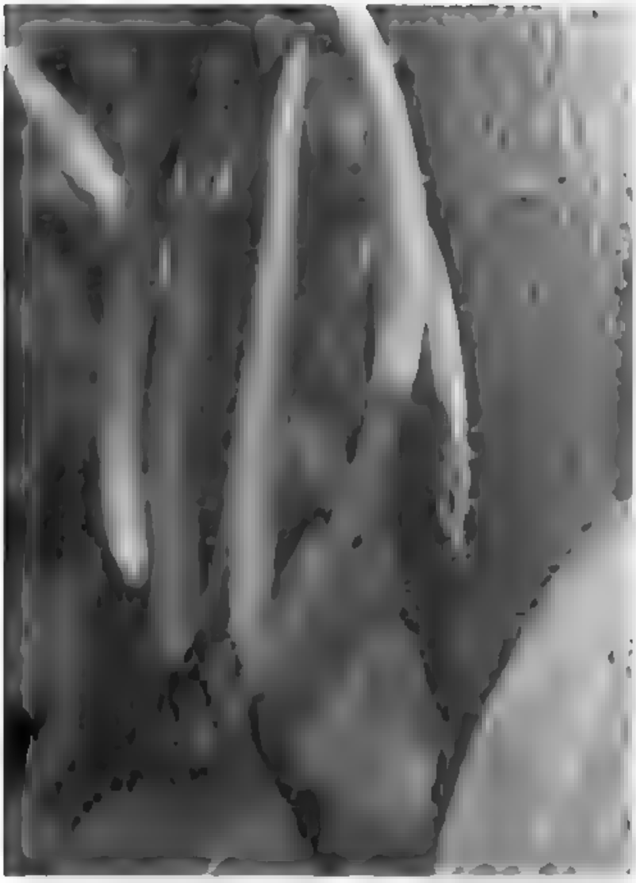


Figure 4. Vanilla beans on the vine showing mature (blossom-end-yellow) split (blossom-end-brown and split) and unripe green pods. *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

zymatically converted by the glucosidase to glucose and the aldehyde vanillin, the single most characteristic component of vanilla extract flavor. The longer a bean ripens before harvest the more concentrated the glucosidase is, and thus the more concentrated the vanillin and other important flavor precursors are after curing (9). Higher vanillin beans are considered higher quality and command a higher market value.

Privately funded bioengineering research on vanilla has progressed slowly over the past 5 years. Research has focused on improving the resilience of the plant and on manipulating its cells to mass produce natural vanilla flavors in the lab. No patents have been issued yet, nor has the technology been commercialized (10).

CURING

The vanilla orchid and the mature vanilla bean have no aroma; it is the curing process that develops the characteristic flavor of vanilla beans. Vanilla beans left on the vine will cure naturally, but the pod splits, loses contents, and ultimately decreases in flavor and value.

The Mexican Indians developed the original, very labor-intensive process for curing green vanilla beans. The French made slight modifications to this original process, and this Bourbon process is generally practiced in the Madagascar and Comoros Islands today. Other modifications are practiced in Indonesia. No geographical source of vanilla beans strictly employs one method of curing and many parts of the process are interchanged (mixed and matched). The curing operation is not a regulated, large-scale, sophisticated procedure. It is crude, nonhygienic, subject to personal modifications, and is practiced by individual small farmers up to larger scale curer-exporters. Any given shipment of cured beans may represent the composite curing operations of dozens of individual producers. During the curing process, each bean is individually handled and inspected at least a dozen times (2,11).

In 1952, 1952, and in 1972, McCormick & Company, Inc. patented processes for rapid, controlled curing of vanilla beans (12–14). However, none of these modern methods are commercially practiced in full today. Portions of the process, modified to use whole beans, are being commercially applied in some parts of Java and Bali (2).

All curing methods involve four basic phases:

1. *Wilting* or *killing* of the beans, which stops the natural respiratory metabolism and vegetative life of the pod (Figs. 5 and 6). The specific technique used to kill the beans will affect subsequent vanillin contents (6,15,16). After wilting, the beans may have begun to turn chocolate brown in color.
2. *Sweating* the wilted beans until they are flexible in the hand and can be easily wrapped around the finger. This step involves a fairly rapid dehydration and slow fermentation. The characteristic flavor compounds develop here. Key enzymatic and nonenzymatic reactions occur during this phase forming sugars, phenols, quinones, pigments, vanillin and other aromatic compounds. After sweating, the beans are deep chocolate brown.
3. *Drying* of the sweated beans very slowly at low temperature to 20–25% finished moisture (Fig. 7). Beans should still be flexible; over-drying, or too rapid drying, reduces flavor quality and value.
4. *Conditioning* of the dried beans in closed boxes for a few months, where they finish the development of their characteristic fragrance. Unless moldy, beans can be kept indefinitely in this state.

Generally about five to seven pounds of green vanilla beans yields one pound of finished product, or about one hundred twenty pounds of finished beans per acre of vanilla vines.

The basic steps in the curing process are summarized, by method, Table 1 with illustration in Figure 8. Many bean-curers employ a combination of methods and/or their own customized approach. Thus, there is a certain amount of variation in quality even within one growing region in any given year (2).

When properly cured, vanilla beans should resemble long, very thin cigarillos; supple, very dark brown, a raisin-like texture and a somewhat oily sheen (Figs. 8–10). High quality beans may have white crystals of vanillin clinging

Figure 5. Killing of the beans in Madagascar. *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

to the outside, but this is rarely seen today. The beans should be free of mold and insects (2,7,8).

Beans should be less than 25–30% moisture or a greenish-white mold will develop. If mold does develop, it is removed by washing with alcohol in mild cases, or by cutting off the affected portion if severe. The odor of mold from a few beans can permeate an entire box. This reduces the quality and the value of the beans.

After curing, the vanilla beans can be divided into the following four quality grades by appearance (the numbers of, and names given to, these grades differ according to source).

1. *Whole beans, no defects*; oily sheen, smooth exterior, moist, aromatic, very dark brown.
2. *Whole beans, some defects*; rough exterior, somewhat reddish color, spotted, dry.
3. *Splits*; whole split beans.
4. *Cuts*; beans chopped into 1–2 in. lengths, may include very small whole beans.

The sorted beans are grouped by quality grade, packed loosely or in bundles of 50–100 in tin, wooden or cardboard boxes, sealed and shipped to their destination (Fig. 11).

Figure 6. Killed beans are poured into sweating chambers in Madagascar. *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

Figure 7. Vanilla beans sundrying in Madagascar. *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

The bulk of global vanilla bean trading is handled by only a handful of importer-dealers. Internationally, all beans are traded in the cured form. Locally, many vanilla farmers sell their crops of green beans to regional curing operations, but green beans are not traded internationally (2).

Table 1. Vanilla Curing: Four Typical Methods

Step	Mexican[a]	Bourbon[b]	McCormick[c]	Java[d]	Miscellaneous[e]
Wilting or killing	Stored in shed for a few days until the beans shrivel; then *Sun / traditional* Green beans spread on wool blankets or straw mats in the direct sun until too hot to hold (ca 1 h). During the dry season; Nov.–May, or *Oven* (generally used today) Beans rolled in wet blankets, placed in humid oven or room at 60°C for 36 – 48 h. Indirect heat from firewood.	Beans placed in straw or metal baskets *or* in blankets, immersed in hot (150–190°F) water for 2–3 minutes, drained.	Beans are chopped into 1/2 in. lengths	Process extremely variable between regions Immerse in boiling water, or Nothing—no killing	Freezing for 3–4 h Ethylene gas 100 ppm for 8–16 h Scratch bean with pin (surface wounding) Place beans in ashes of fire (Guiana method)
Sweating	*Sun / traditional* Spread on blankets or straw mats and exposed daily to sunshine for 1–2 h, then placed the remainder of each day inside blanket and/or inside a shelter. Repeated daily for several weeks until proper aroma and texture are obtained. *Oven / current procedure* Sweating continues in the closed warm room/oven until brown. Beans in blankets may be transferred into chests.	Hot, drained beans packed into chests, or boxes covered with tarp, retained temperature about 122°F. Removed after several days when brown. Then placed in sun as in Mexico, but on elevated platforms, for 6–8 days.	Chopped beans placed into perforated trays, trays are stacked in curing tank, cured at 140°F for about 72 h, until beans are no longer green.	McCormick process/ whole beans, *or* Nothing—no sweating, or Sun-sweating as in traditional Mexico	
Drying	Sweated beans spread on grass mats or blankets in the sun every day for a few hours for 2 months or longer; then dried to finish inside a shelter.	Same as in Mexico, but often on elevated platforms.	Dried in rotary or fluidized dryer with forced air at 140°F until moisture content is 35–40%.	McCormick process, or In tobacco curing sheds	
Conditioning	Beans are sorted; placed in closed boxes for several months.	Placed in closed tin boxes or chests for several months.	Remove from rotary drier, spread 4 in. deep in perforated conditioner. Blow room temp/ humidity air through conditioner until moisture is 20–25%. Place in loosely covered container for 3 months (during shipping).	No conditioning in some cases, *or* McCormick process, or Placed in closed boxes for several months.	
Packaging	Bundles of 50–130 beans in paper or metal boxes.	Bundled 50–100 beans/ bundle in tin or cardboard boxes.	Metal or cardboard box, not hermetically sealed.	Highly variable, whatever the farmer has on hand.	
Total time	5–6 months	5–8 months	3 months	2 weeks to 2 months	

Source: [a]Refs. 2, 4, 16, and 17.
[b]Refs. 2, 4, and 17.
[c]Refs. 12–14.
[d]Ref. 2.
[e]Ref. 5

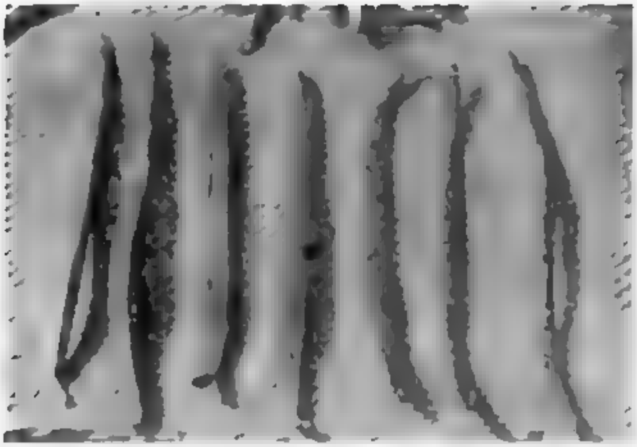


Figure 8. Vanilla bean at various stages of curing; green, killed, sweated, dried, and conditioned (left to right). *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

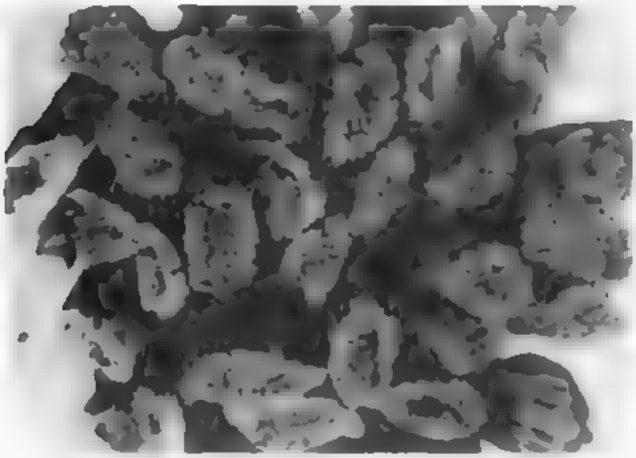


Figure 9. Cross section of cured vanilla beans showing vanillin, resins, and seeds. *Source:* Photograph courtesy of McCormick/Schilling Spices.

Moisture and vanillin contents vary from crop year to year, but are fairly stable within a given period. Table 2 illustrates the typical ranges in 1988–1990.

EXTRACTION

Regulatory

Vanilla extract is the only flavoring material with a U.S. FDA standard of identity. It is included in the *Code of Federal Regulations* (21-CFR-169). The standard was developed and promulgated concurrent, and in close relationship, with the ice cream standard (21-CFR-135.110). This coordination was necessary since vanilla extract and related flavorings are ice cream's most widely used flavorants; and the labeling of ice cream is dependent on the type of flavoring used. Category I (21-CFR-135) vanilla ice cream contains only pure vanilla components and no arti-

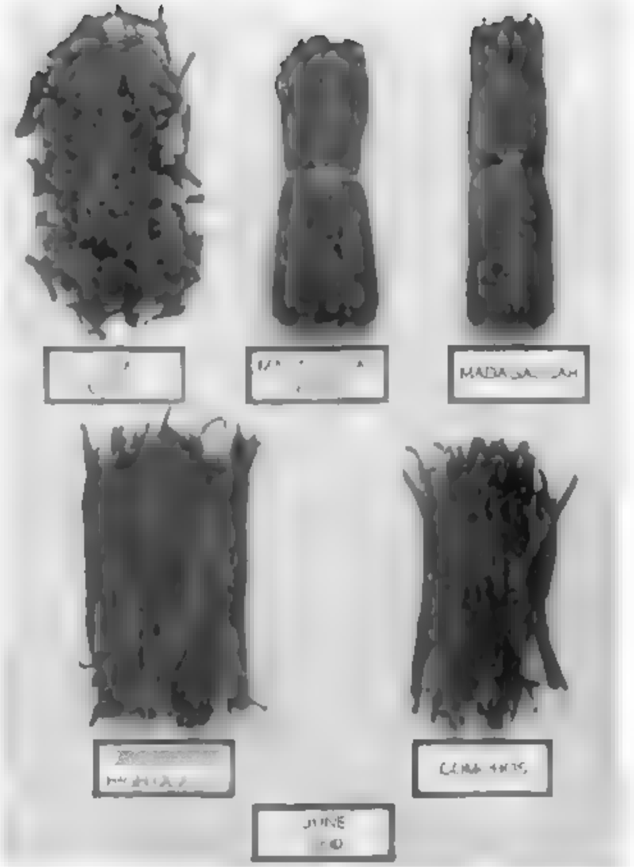


Figure 10. Cured vanilla beans from Madagascar, Comoros, and Java; whole splits and cuts. *Source:* Photograph courtesy of McCormick/Schilling Spices.

Figure 11. Sorting of vanilla beans in Madagascar. *Source:* Photograph courtesy of Benjamin H. Kaestner III, McCormick/Schilling Spices.

Table 2. Moisture and Vanillin Contents Ranges in 1988–1990

Vanilla bean origin	Moisture, bean (%)	Vanillin, one-fold extract (% weight/volume)
Madagascar, whole	13–30	0.09–0.26
Comoros, whole	16–26	0.14–0.25
Indonesian, whole superior quality	12–30	0.09–0.25
Indonesian, whole average quality	10–19	0.04–0.13
Indonesian, whole poor quality	9–11	0.04–0.06
Mexican	17–23	0.14–0.17
Java cuts	8–12	0.02–0.09
Bourbon cuts	11–16	0.06–0.13

ficial flavors. This product can be labeled "Vanilla Ice Cream." Category II (21-CFR-135) vanilla ice cream can be flavored with up to one ounce of synthetic vanillin per unit (defined below) of vanilla extract. This natural and artificial product, where the natural is the characterizing and predominant contributor to the flavor, must be labelled "Vanilla Flavored Ice Cream." Finally, Category III (21-CFR-135) ice cream contains predominantly or exclusively an artificial vanilla flavoring which includes primarily synthetic vanillin. This product must be labelled "Artificially Flavored" or "Artificial Vanilla."

Both the ice cream standard and the vanilla standard nomenclatures rely heavily on the definition of a unit of vanilla constituent. This term is defined by the Vanilla Standard 21-CFR-169.3. The types of vanilla beans are identified as "the properly cured and dried fruit pods of *Vanilla planifolia* Andrews and of *Vanilla Tahitensis* Moore." But the term properly cured and dried is not defined. The quantity of beans necessary to make a unit weight of vanilla beans is also identified. This last part has led to confusion in that the definition of quantity was set at 13.35 oz of 25% moisture vanilla beans. This is 10 oz of dry weight solids. If the beans contain greater than 25% moisture, logic states (as does the standard) that the quantity of beans in a unit be based on 10 oz of dry weight solids, although the wording refers again to the 25% moisture vanilla beans. However, when the moisture content of the beans used is below 25% moisture, the standard requires 13.35 oz regardless of the dry weight solids. No guidance is given concerning when the moisture content should be measured, when packed, when purchased, when received, or when used. There has been much discussion within the industry recently concerning the relationship between moisture content of the beans and the flavor of the extract. A positive correlation (eg, increase in moisture accompanies an increase or improvement in flavor) would be necessary to require an adjustment in the quantity and quality of bean usage in the extraction. The intent of the regulation was to provide a consistent product to the consumer, and therefore, an analytically valid dry weight standard would be more appropriate and defendable. This debate will probably go on for several more years. Until resolution, the government is evaluating and approving appropriate industrial permits as requested by vanilla extract producers.

Because the moisture content is so important to policing the standards (and will be regardless of which refinement prevails), the standard goes on to define how moisture should be analyzed—azeotropic distillation with toluene/benzene. This, also, leads to a concern in that most, if not all, extraction companies and analytical labs no longer permit the use of benzene in their facilities. Therefore, the moisture analysis needs to be revised as the entire standard is being overhauled. Currently, the Technical Committee of the Flavor Extract Manufacturer's Association of the United States (FEMA) has undertaken this task. In addition, alternative methods to the wet technique should be investigated and incorporated as appropriate. These include near infrared (NIR), electroconductivity, nuclear magnetic resonance (MMR), etc.

The remainder of the standard is involved with describing in general terms how the extract is made and what other ingredients can be used. The standard also defines other products related to pure vanilla extract—what constitutes them and how they can be labeled:

- Concentrated vanilla extract
- Vanilla flavoring
- Concentrated vanilla flavoring
- Vanilla powder
- Vanilla–vanillin extract
- Vanilla–vanillin flavoring
- Vanilla–vanillin powder

The more significant statements in the standard, which should be noted, require that the finished extract have not less than 35% ethyl alcohol and contain no less than one unit of vanilla beans per gallon.

As a federal regulation, the FDA standard is enforceable by agencies of the Executive Branch. Because this product uses potable alcohol subject to tax controls, the Bureau of Alcohol, Tobacco and Firearms (BATF) has been in the forefront of assuring compliance to the standard. In addition, the Flavor Extract Manufacturing Association of the United States (FEMA) has a self-policing policy aimed at maintaining quality and integrity of vanilla extracts and natural vanilla flavors

As a result of this unique standard, the manufacture of vanilla extract is tightly controlled and requires a means for assuring compliance via analytically supported criteria.

Manufacture

As a result of the restrictions imposed by the Vanilla Standard, there is a limited number of methods to produce this regulated product. All methods are required (1) to extract the total rapid and odorous principles extractable by 35% alcohol and (2) to use no less than a unit quantity of vanilla beans in a gallon of extract. Therefore, the manufacturing variations involve how the alcohol and vanilla beans are brought into contact, the temperature during the extraction, the percent alcohol in the miscella (extracting solution) and the length of time the vanilla beans and solvent are in contact.

The temperature is usually between ambient and 60°C and the alcohol concentration between 40% and 60% (vol/vol). The extraction time can be days, weeks, or months. Economics inhibit the longer extraction times and encourage higher temperatures and other innovations to accelerate extraction of the desired vanilla constituents. The alcohol concentration is controlled in order to encourage efficient extraction of the desirable constituents, while inhibiting the non-polar lipids and waxes which are soluble in the higher proof alcohol solvents. Some extractors prefer those processes which encourage rapid, indiscriminating conditions followed by settling tanks, filters, and/or centrifuges to remove constituents not soluble at ambient temperature and in the final 35% alcohol solution.

Maceration (soaking) and percolation are the two methods for contacting vanilla beans and solvents. Soaking involves mixing the chopped beans with the solvent and allowing these to set and extract for a given time period. The first extract is removed and the beans reextracted. This is repeated until the desired level of extraction is achieved. The combined solutions produce a complete and full-bodied extract.

However, soaking has been replaced almost entirely by percolation (Fig. 12). As the name implies, percolation involves a continuous circulation of the aqueous ethanol through the chopped vanilla beans. Unlike coffee percolation, the heat applied, if any, is not enough to cause the solvent circulation. The solvent is pumped mechanically through the beds of vanilla beans. The temperatures can vary between ambient and 60°C and the drip percolation can be hours to days.

The entire extraction process can be divided into four subroutines (*1*) sample preparation, (*2*) extraction, (*3*) adjustment and/or concentration (where a concentrate is desired), and (*4*) solvent recovery. Sample preparation involves chopping or shredding the vanilla beans. The extraction process has also been detailed previously. Concentration involves removing all or part of the extraction solvent. This is called folding and is usually accomplished by solvent distillation under vacuum. A one-fold (1X) extract contains one unit of vanilla beans per gallon. The extract removed is usually over one-fold and over 35% alcohol. This permits the adjustment to the optimum flavor and cost for a minimum standard product as well as compliance with the standard and customer specifications. This also allows for the preparation of a concentrate with lower energy use. Some higher fold vanilla extracts can be made by adjusting the ratio of beans to alcohol, and by using counter-current extraction methods. Extracts of four (4X) fold or less are attainable in this manner. Higher folds are best made by producing an oleoresin through total solvent removal, followed by dilution to the desired 5X, 10X or 20X. One-fold extracts are sold retail; the concentrated or multifolded extracts are favored by industrial users.

Figure 12. Vanilla extract percolators. *Source:* Photograph courtesy of McCormick/Schilling Spices.

The solvent recovery step involves the recovery of residual solvent from the exhausted or spent vanilla bean material. Economics and regulation demand this for two reasons—the most obvious being the ability to reuse the costly solvent for further extraction. In addition, the U.S. Bureau of Alcohol, Tobacco & Firearms (BATF) stipulates that the spent beans be free of recoverable alcohol. The alcohol is often recovered from the spent material through distillation.

More modern methods of extraction and concentration have been used. These include supercritical carbon dioxide extraction and reverse osmosis for concentration. These methods produce useful products for industrial flavoring, but they either fail to fit the regulatory requirements for the standardized product, and/or are very expensive. These specialized products differ in solubility, flavor profile and appearance, but add to the list of natural vanilla flavorings available to food and beverage manufacturers.

Finally, vanilla extract, like a fine wine, improves on aging! Storage in oak barrels (new or previously used for whiskey) is a traditional method for vanilla. Modern technology can accelerate the process (19,20).

FLAVOR

Chemistry

The chemistry and analysis of vanilla has been studied extensively for two main reasons; first, in order to understand what causes this popular delicate unique flavor which also enhances many other flavors, and secondly, to protect the integrity and therefore the quality of this product from unscrupulous manufacturers and suppliers who are willing to adulterate vanilla or even present a completely synthetic imitation product as natural. A complete review of past endeavors is beyond the limits of this review, so a brief historic background and summary of the more current results of applications that have been developed from these many efforts and discoveries over the past 100 years are presented.

Flavor Chemistry

Vanillin is the most abundant of the vanilla flavor constituents. However, since its discovery by Golby in 1858 (21), there has been much controversy as to its contribution to the overall flavor impact and quality of vanilla extract. Klimes and Lamparsky (22), who at the time utilized very innovative nanogram techniques, identified 169 components, most below 1 ppm, in vanilla beans. Other than to state the general importance of the trace constituents to the overall sensation of vanilla flavour, no specifics on individual constituent contributions to flavor were identified. This has been the general trend in vanilla research: identify the unique constituents but ignore their ultimate sensory contributions. This intentional withholding of information is a result of proprietary and costly research undertaken by various companies whose livelihood and future is tied in part to understanding and exploiting vanilla flavor.

However, Galetto and Hoffman (23) synthesized the methyl and ethyl ethers of *p*-hydroxybenzyl alcohol and vanillyl alcohol found in vanilla (Compounds I, II, III & IV in Fig. 13). They described them as imparting "sweet, vanilla-like flavor notes with creamy, coconut secondary flavor which could contribute to the 'character' of vanilla." Schulte-Elte and co-workers (24) stereoselectively synthesized two diastereoisomers of vitisporanes (Compound V, Fig. 13) which had been found to be "important aroma components of vanilla." They report the odors of each to be "unmistakably different." The cis-compound is fresher and more intense, is reminiscent of green chrysanthemum and has a flowery-fruity wine note. The trans has the heavy scent of exotic flowers with an earthy-woody undertone and a note of dry wines as in marc. Feyertag and Hutchins (25) isolated and identified a trimethypiperidonene with two possible isomers (Compound VI A or B, Fig. 13) which they report as having a camphoraceous minty odor and a burning taste.

Walbaum (26) identified the oxidative series of anisyl compounds—alcohol, aldehyde and acid in Tahitian vanilla (Compounds VII, VIII, IX in Fig. 13). Gnadinger (20) confirmed that the alcohol was the major component. These could account for the unique floral aroma of the extract from Tahitian beans. Purseglove and co-workers (27) have done a commendable job of comparing the constituents of three species of vanilla as reported in the literature. They observed that "differences in volatile-component composition between species are probably quantitative rather than qualitative. . . . "

As of 1989, we have reviewed 98 articles and patents on vanilla which identify about 190 constituents in vanilla. Riley and Kleyn (28), in 1989, report that over 250 volatile flavor components had been identified, but did not specify which.

Despite all this research, the key constituents responsible for vanilla's characteristic flavor remain undefined. The general belief in the industry is that 1 oz of synthetic vanillin is equivalent in flavor strength, not character, to one gallon of vanilla extract. However, 1 gal of pure extract contains approximately 7 g of vanillin, or only one-quarter of an ounce. Therefore, these other undefined constituents

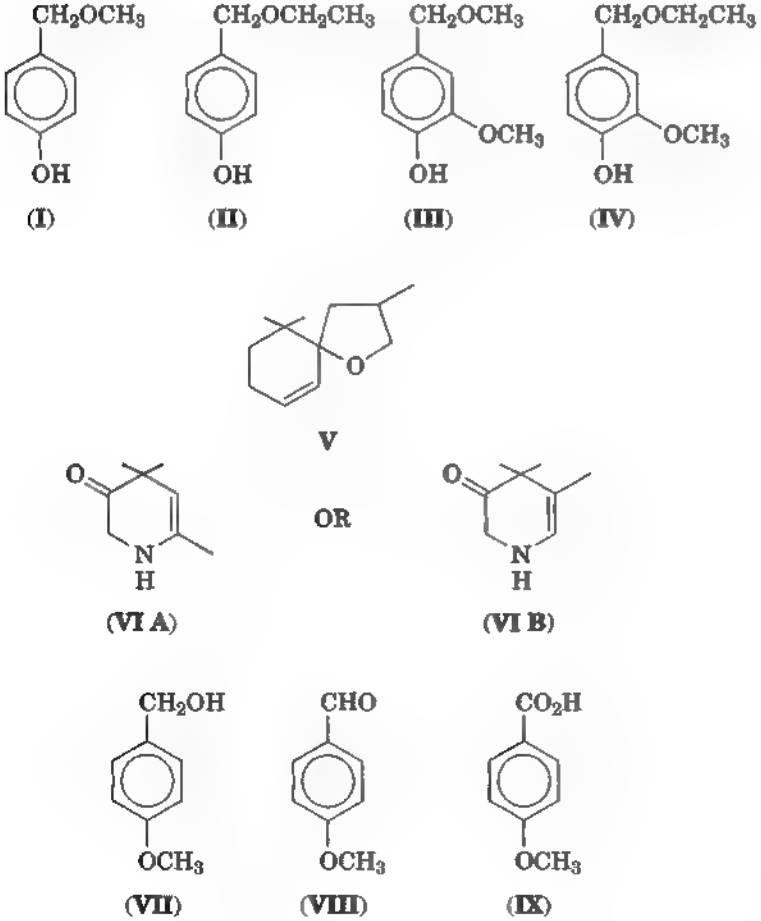


Figure 13. Vanilla flavor compounds.

provide the remaining 75% of the flavor strength and character (18). The vanilla standard implies agreement with this ratio in the definition of vanila–vanillin extract (21-CFR-169.180).

Analytical Methodology

Methods to analyze vanilla have evolved over the years, guided by two forces—regulation of the vanilla standard and specification of the industrial user. Before adoption of the FDA Standard of Identity, manufacture and "enforcement" were guided by the *U.S. Pharmacopoeia* and expectations of the customer. The late nineteenth century and early twentieth saw reliance on lead numbers (Wichmann), vanillin content, and the determination of coloring content (Marsh Test) and related resins content. These analyses could detect the lack of vanilla bean extractives, the use of prune juice, caramel, wine or artificial colors and the addition of coumarin (29–31). The lead number which by the 1930s was used as a measure of quality was investigated in detail by Purcell (32). He separated the individual fractions contributing to the lead number and demonstrated a very small percentage of the constituents contributed to vanilla flavor. Broderick (31) confirmed and expanded on Purcell's efforts in an attempt to establish analytically supported quality measurements. He supported the use of the related color and resin analyses. However, lead number is still used as an analytical index, primarily as an indicator of geographic origin.

With the promulgation of the vanilla standard in 1962, new authentication methods became available, as did more sophisticated adulteration techniques. The use of St. John's Wort, cherry bark extracts and the addition of organic acids to by-pass existing detection methods required the application of paper chromatographic techniques (33,34) and gas chromatography (35,36) to detect the adulteration.

Each of the new methods reduced the number of adulterated products, but also challenged the "creative" manufacturers.

Many of these techniques and others have been upgraded and refined and are contained in the Official Methods of Analysis (1990), published by the Association of Official Analytical Chemists (AOAC), Section 36. Flavors (37):

973.23 Alcohol in Flavors-Gas Chromatographic Method

920.127 Specific Gravity of Vanilla Extract-Pycnometer Method

920.128 Alcohol in Vanilla Extract

920.129 Glycerol in Vanilla Extract

947.09 Propylene Glycol in Vanilla Extract

946.10 Vanillin in Vanilla Extract—Ultraviolet Screening Method

966.12 Vanillin in Vanilla Extract—Ultraviolet Spectrophotometric Method

966.13 Vanillin in Ethyl Vanillin in Vanilla Extract—Paper Chromatographic Method

920.130 Coumarin in Vanilla Extract—Photometric Method

955.31 Vanillin, Ethyl Vanillin, and Coumarin in Vanilla Extract—Chromatographic Separation Method

968.15 Lead Number (Wichmann) of Vanilla Extract—Titrimetric Method

920.01 Solids (Total) in Vanilla Extract

920.131 Ash of Vanilla Extract

926.09 Vanilla Resins in Vanilla Extract—Quantitative Method

960.36 Vanilla Resins in Vanilla Extract—Paper Chromatographic Qualitative Test

920.132 Methanol in Vanilla Extract—Titrimetric Method

920.133 Color (Insoluble in Amyl Alcohol) in Vanilla Extract—Colorimetric Method

960.37 Plant Material (Foreign) in Vanilla Extract—Paper Chromatographic Method

Table 3. Vanillin Carbon SIRA

Vanillin source	δ^{13}C 0/00 PDB
Madagascar	−20.4
Java	−18.7
Mexico	−20.3
Tahiti	−16.8
Lignin	−27.0
Eugenol	−30.8
Guaiacol	−32.7

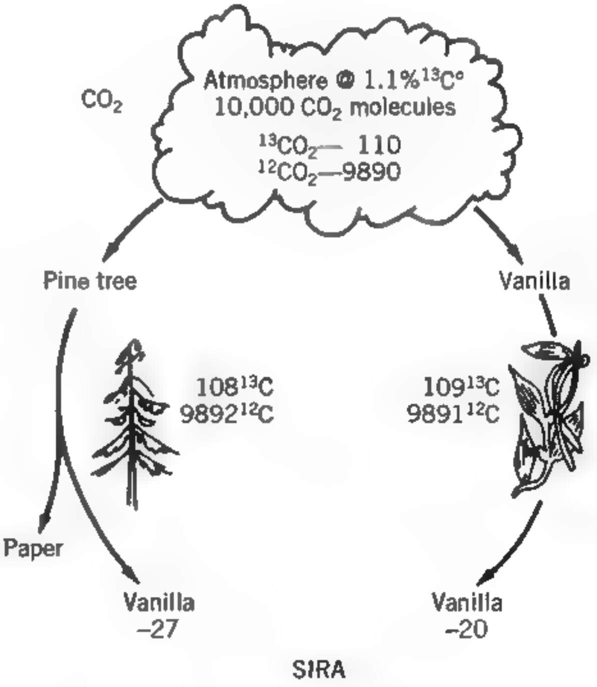


Figure 14. Biosynthesis of vanillin.

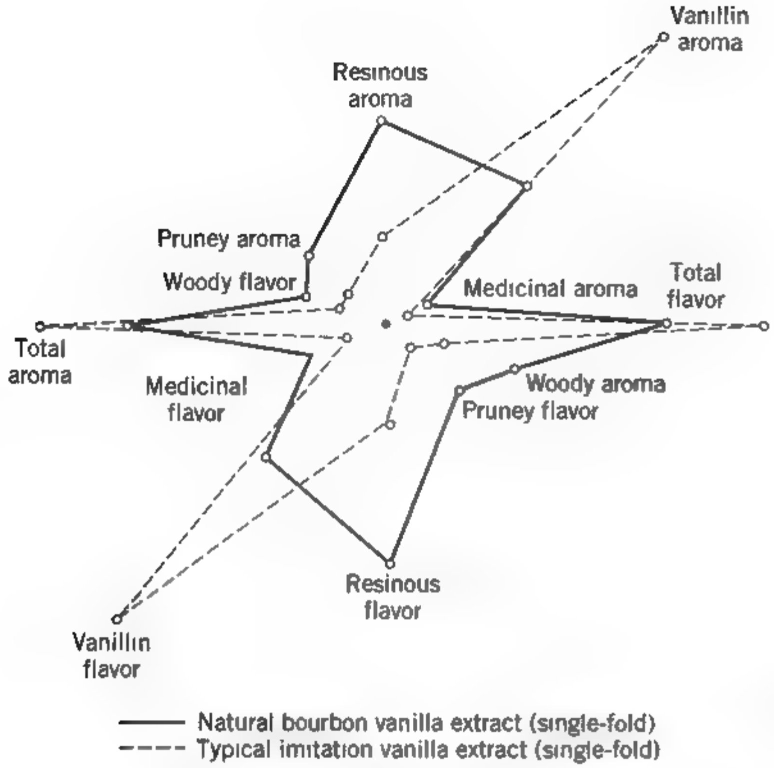


Figure 15. Vanilla aroma and flavor sensory profiles. Products evaluated at 0.5% in water. Intensity of flavor and aroma characteristics (eg, resinous, pruney) increases with distance from center point.

964.11 Flavoring Additives in Vanilla Extract—Thin Layer Chromatographic Method

963.16 Organic Acids in Vanilla Extract—Paper Chromatographic Sorting Method

969.22 Organic Acids in Vanilla Extract—Derivative Gas Chromatographic Method

971.12 Nonvanillin Vanilla Volatiles in Vanilla Extract—Direct Gas Chromatographic Method

These methods, and the technique for moisture analyses elaborated in the standard of identity can be used for quality control of manufacturing and customer specification. They are also useful for identifying gross, but not the newer more sophisticated adulterations, of vanilla products.

High pressure liquid chromatography was used to authenticate vanilla product by several workers. Herrmann and Stocki (38) utilized a reverse-phase gradient system and reported the quantitation of 4-hydroxybenzyl alcohol, vanillyl alcohol, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, and vanillin.

Guarino and Brown (39) developed an HPLC method which separated *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, vanillin and ethyl vanillin. The method was evaluated by six industrial laboratories and the authors recommended a collaboration of this method as an alternative method for the quantitation of vanillin in extracts.

Archer (40) developed an HPLC procedure with phenoxyacetic acid as an internal standard. He quantitated *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxybenzaldehyde and vanillin in a series of oleoresins, vanilla extracts and vanilla essences. From this data, he suggested a range of ratios for authentic essences.

In 1975, Martin and co-workers (41) proposed the use of identification ratios for determining authentic products. He demonstrated that from the analyses of vanillin, potassium, inorganic phosphates, and nitrogen and the interrelationship of their ratios, the BATF could not only determine adulteration, but also the strength of an extract (units of vanilla beans per gallon). Martin and co-workers (42) elaborated further on this by including the analyses of the amino acids: alanine, valine, glycine, isoleucine, leucine, proline, threonine, serine, methionine, hydroxyproline, phenylalanine, aspartic acid, glutamic acid, tyrosine, lysine, histidine, arginine and tryptophan.

Martin's analyses for the 1975 vanilla crop found the lead number for Madagascar, Mexican and Comoros extracts to be approximately 1.0, whereas Java extracts were around 1.5 and Tahitian about 0.8.

Bricout (43), and later, Hoffman and Salb (44) investigated the high resolution mass spectrophotometric method based on the analyses of the stable isotopes of carbon (C-12 and C-13). Vanillin from vanilla bean origin as a result of the biosynthesis, has a greater C-13 concentration, and therefore, less negative C-13/C-12 ratio than vanillin from synthetic sources—eugenol/clove oil, guaiacol or lignin (Table 3, Fig. 14). However, this was circumvented through the use of synthetic vanillin doped with C-13 labeled material. In an attempt to stay at least even with the fraudulent vendors, additional techniques were developed to detect each carbon position which could be labelled (45–47). In addition, several workers, including a group at the University of Georgia (CAIS) funded by FEMA, are looking at

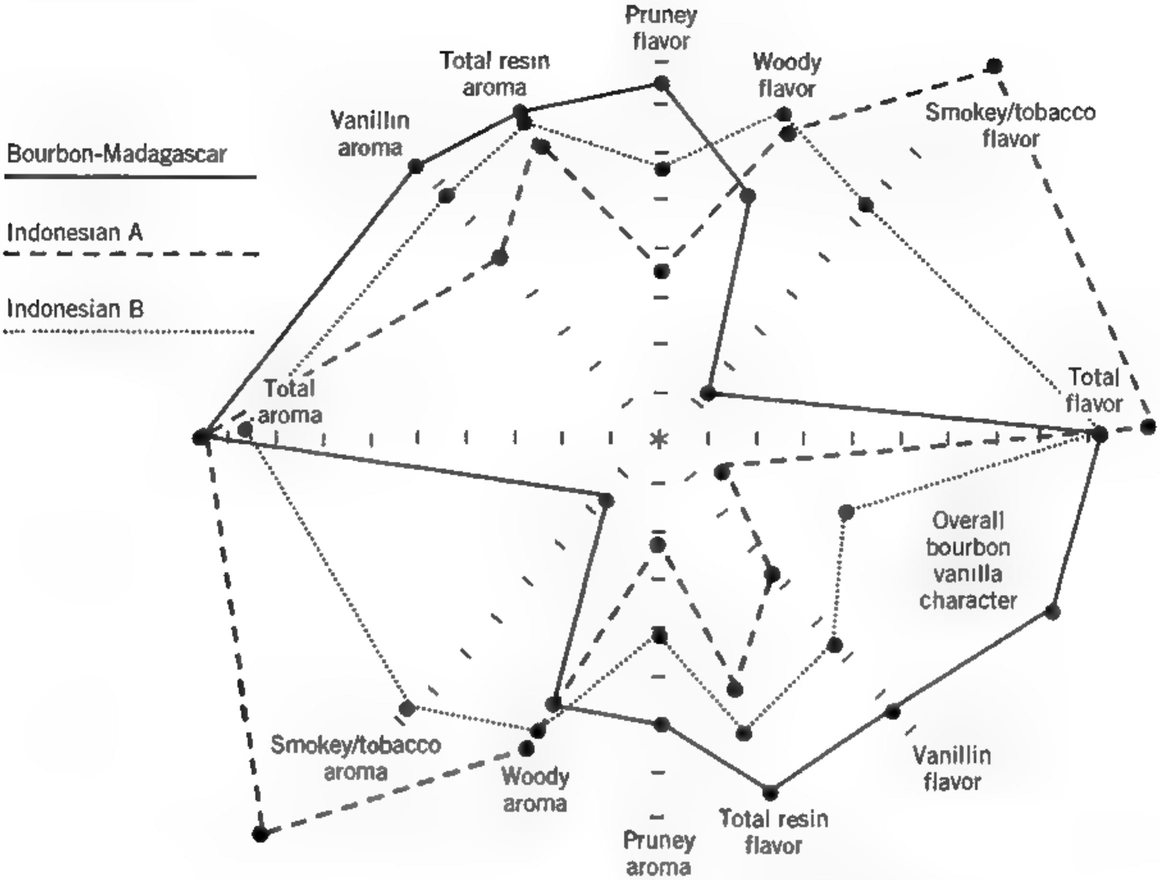


Figure 16. Vanilla aroma and flavor profiles for Bourbon and Indonesian pure vanilla extracts; tested at 1.0% in room temp. spring water, Apr. 25, 1990.

Table 4. Flavor Characteristics of Pure Vanilla Extract

Flavor character	Bourbon beans	Java beans	Tahitian beans
Vanillin	Slight	Very slight	Slight
Resinous/leathery	Slight-moderate	Moderate	Very slight
Woody	Slight	Moderate	Very slight
Pruney	Slight	Very slight	Very slight
Fruity	Very slight	None	Moderate
Chocolate	Very slight	Very slight	None
Smokey–tobacco	None	Moderate	None
Bourbon–rummy	Moderate	Very slight	Slight
Sweet–floral	Very slight	None	Moderate

Note These descriptions reflect the norm. Flavor quality does very between lots, years, and manufacturers, depending upon a variety of factors (see text).

other isotopic techniques—C-14 (radiocarbon), D/H and 0-16/0-18, each merely a stop gap; none absolute (48).

A battery of tests is possibly the only solution to eliminating most of the fraud. Martin and co-workers (49) demonstrated this technique when they coupled their vanillin/potassium ratios with the stable carbon isotopic method. Fayet and co-workers (50), coupled stable isotope ratio analyses of both vanillin and *p*-hydroxybenzaldehyde and HPLC analyses of vanillin, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic and vanillic acids.

Toulemonde (51) investigated the application of high resolution deuterium NMR for the detection of adulterated vanilla products and postulated that the aldehydic deuterium should be investigated further. Maubert and co-workers (52) in France developed site-specific isotopic fractionation-nuclear magnetic resonance or SNIF-NMR. This technique uses multivariant analysis of the deuterium concentration of each site on the vanillin molecule in order to differentiate synthetic and natural materials.

Sensory

If beauty is in the eye of the beholder, then flavor quality is in the mouth of the consumer! The flavor of vanilla extracts does vary considerably, depending primarily upon country of origin, but also upon crop-year, curing techniques used, storage conditions, extraction method and age of the vanilla extract itself. It is difficult to state what is the best quality vanilla, as taste is largely a matter of personal opinion. Most experts generally consider the flavor of a high vanillin (≥0.20%), high moisture (≥20%) bourbon bean to deliver the best quality extract. The lower the vanillin content, the lower the flavor quality of the bean will be, not just because of the vanillin itself, but also due to

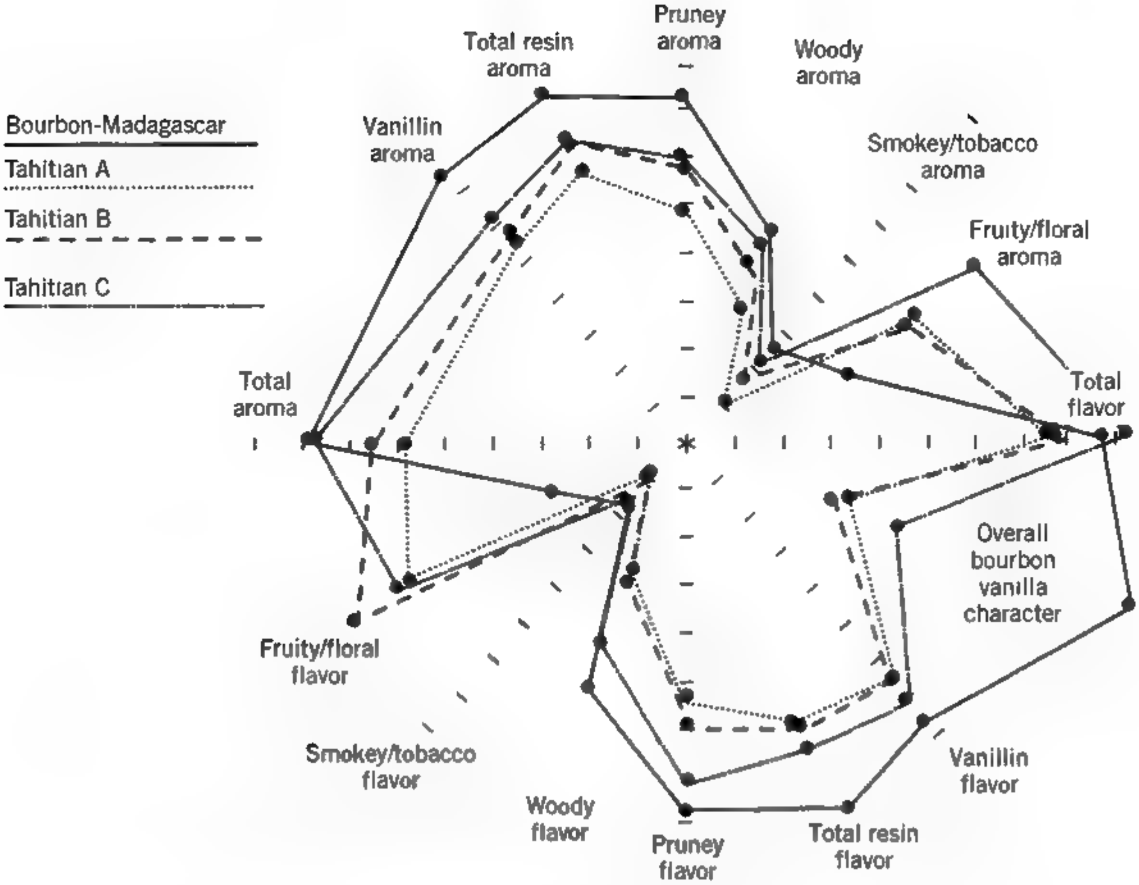


Figure 17. Vanilla aroma and flavor profiles for Bourbon and Tahitian pure vanilla extracts; tested at 1.0% in room temp. spring water, May 23, 1990.

the other flavor notes which develop along with vanillin during curing. These flavor notes include characteristic woody, pruney, resinous, leathery, floral and fruity aromatics. These flavor characteristics do not develop and/or are lost, in over-dried (low moisture) beans.

With undue emphasis, vanillin content is generally used as the prime indicator of flavor quality of beans and extracts. As a result, this focus on vanillin content has distracted many in the vanilla business from due consideration of the remaining aromatics. Collectively, these other flavor notes such as pruney, woody, floral, fruity and rummy are even more important than vanillin character alone. This point is illustrated quite clearly in Mexican vanilla bean extract which is traditionally quite low in vanillin yet superior in flavor quality. On the other hand, most imitation vanilla extracts are very high in vanillin content, but lacking in the remaining full-bodied character notes (Fig. 15). Vanillin alone is probably responsible for no more than 25% of the character of quality vanilla extract (18).

Over the past 30 years, the best quality vanilla beans have come most consistently from the islands of Madagascar, Comoros and Reunion ("Bourbon" beans). The quality of beans from the Indonesian islands has been generally low, but that of some lots has improved significantly over the past 2–3 years. The quality of Java beans has typically been low for several reasons: (1) the beans are picked too green for optimal formation of glucovanillin, beta-glucosidase and other flavor precursors, and/or (2) the beans pick up a typical smokey-tobacco flavor during curing, and/or (3) the beans are shipped without the conditioning phase. The quality of beans from certain growers/processors in Indonesia is improving due to the implementation of parts of the quick cure (McCormick) process (Table 1), allowing beans to ripen on the vine and to improved handling in general. However, the majority of beans coming from Indonesia today are still lower in vanillin content and exhibit an undesirable smokey flavor (Fig. 16).

Tahitian vanilla has a very different flavor profile in comparison to *vanilla planifolia* (Table 4). It is very floral, fruity/cherry and perfumey, and less resinous/leathery than *vanilla planifolia*. Tahitian vanilla extract is not easily found in the United States because production volumes of the beans are relatively small and the flavor is not substitutable for *vanilla planifolia* (Fig. 17). Mexican vanilla beans are also rarely used in the United States due to high price and low production volumes. They produce a relatively good quality woody/resinous flavored extract (Fig. 18) although lower than Bourbon in vanillin content (0.14–

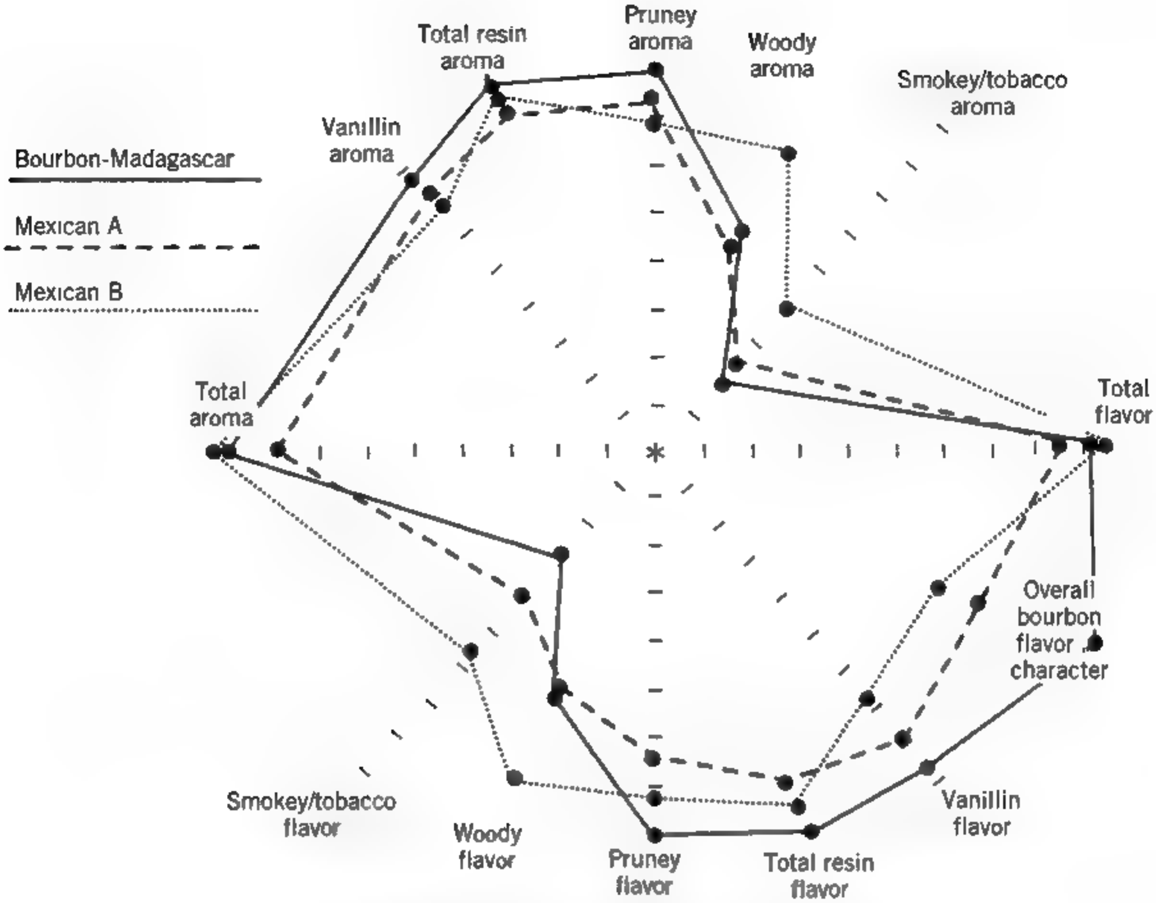


Figure 18. Vanilla aroma and flavor for Bourbon and Mexican pure vanilla extracts; tested at 1.0% in room temp. spring water, June 20, 1990.

0.17). These fine quality Mexican extracts should not be confused with the inexpensive, often coumarin-containing imitation "extracts" from Mexico which are popular with U.S. tourists.

Descriptive flavor profiles (Figs. 16–18) illustrate the differences in flavor notes and flavor balance between vanilla extract samples made from beans of different origin.

The flavor of vanilla extracts improves with aging. A minimum of 60 days aging of a finished extract is advisable to develop the resinous, bourbon character fully. The extract continues to improve over a several year period until it more resembles a delightful liquor than vanilla extract. Although aging improves a good vanilla extract, it does not compensate for poor starting material (poor beans). Likewise, aging of good beans improves their quality, but aging does not compensate for or improve poor quality beans.

THE VANILLA MARKET

The bulk of global vanilla trade is in the form of whole or cut cured beans. Further processing into vanilla extract is generally done in the consuming countries. The bean itself is used in Europe to flavor home recipes while in North America the pure extract of vanilla is widely used by retail customers. In Japan, vanilla remains virtually undiscovered. Imitation and artificial vanilla flavors (vanillin-based) are heavily used in the North American and European industrial markets.

Madagascar normally produces at least 70% of the world supply of cured vanilla beans (53). The United States is the largest consumer of vanilla bean exports, importing more than 50% of total worldwide production, followed by France at 15–20% and West Germany at 10–15% (53). Over the past decade, the proportion of vanilla beans used by the United States that are from Madagascar has dropped consistently from 60–70% of vanilla bean imports to 38% in 1989, while imports of Indonesian beans have increased from less than 20% to almost 50% in 1989. Less than 10% of United States beans comes from the Comoros and Reunion islands and negligible amounts from other sources (Table 5).

Historically, wide fluctuations in supply and thus, price, have characterized the vanilla bean market. Over the past 30 years, U.S. bean prices have ranged from $2/lb up to $90/lb (2,54). Traditionally, the Tahitian beans are the most expensive; the Java beans the least expensive in price, 1990–1991 U.S. bulk prices for cured beans were (2):

Table 5. Vanilla Import Figures (metric tons)

Country of origin	1980[a]	1981[b]	1982[b]	1983[b]	1984[b]	1985[b]	1986[c]	1987[c]	1988[c]	1989[c]	1990[c]
Madagascar	205	438	580	651	603	462	672	820	578	421	398
Indonesia	90	123	103	240	192	184	306	443	433	526	482
Comoros and Reunion	31	64	99	59	34	87	49	97	185	107	59
Malaysia			56	12							
France	7	4	23	2							
Mexico						2.78	1.58	11	10	16	1.6
Other Pacific Islands (Tonga)						6.72	14	10	7.68	28	2.6
French Pacific Islands						1.04	2.28	4.48	2.59	8.67	3.8
French Indian Ocean Areas							0.75	1.22			5.0
Costa Rica								0.16			
Argentina							2.02				
Pacific Islands including Caroline							0.05				
Belgium									2.0		
New Caledonia									0.51	0.34	
Total	*343*	*640*	*884*	*977*	*841*	*743*	*1048*	*1387*	*1219*	*1107*	*975*

[a] Data from the General Agreement on Tariffs & Trade (GATT), *Spices, A Survey of the World Market*, 1982.
[b] Data from the General Agreement on Tariffs & Trade (GATT), *Spices, A Survey of the World Market*, 1986.
[c] Data from the U.S. Department of Commerce, Bureau of Trade Statistics, 1987–1989.

Java $8/lb–24/lb (depending upon flavor quality)
Tonga $28.00–30.00/lb
Comoros 35.50/lb
Madagascar $37.50/lb
Tahitian $55.00/lb

In order to stabilize vanilla prices, the Vanilla Alliance was formed with Madagascar, the Comoros and Reunion (Bourbon Islands) as members. This organization regulates prices and inventories of Bourbon beans. The pricing of Bourbon vanilla is set by the producing countries with little or no negotiation possible. In Indonesia and other pro-

Table 6. Vanilla Extract Usage

Market/application	Usage level[a]	% of supply used in this application
Food service		5
Retail		20
Industrial		75
Ice cream	1 oz/10 gal mix (10×) 2.5–3.0 oz/10 gal mix (5×) 8–12 oz/10 gal mix (1×) 1 oz/10 gal mix (1×)	30
Soft beverages		17
Cola	0.05% (1×)	
Cream soda	0.1% (1×)	
Alcoholic beverages	0.25% (1×)	11
Yogurt	1 oz/10 gal mix (10×) 2.5–3.0 oz/10 gal mix (5×) 14 oz/10 gal mix (1×) 1 oz/10 gal mix (1×)	10
Others		7
Cakes	0.15–0.2% (5×) 1.0% (1×)	
Candy, cream	0.5% (1×)	
Cookies	0.5% (1×)	
Cream sauces	0.1% (1×)	
Frosting	0.1% (10×) 0.5% (1×)	
Pudding	1.0% (1×) 0.5% (1×)	
Cereal	0.25% (10×) 2.0% (1×)	
Tobacco products	0.04% (1×)	

Source: McCormick & Co., Inc. Industrial Marketing Estimates, 1988.
[a] 10× = 10 fold concentration, 5× = 5 fold concentration, etc.

ducing countries, vanilla trade is still conducted through traditional spice trading channels (shippers, brokers, dealers, merchants).

Roughly 75% of the 3.2 million gallons of U.S. vanilla extract is distributed through industrial channels, 20% through retail and 5% through Food Service (Table 6). Industrially, pure vanilla extract's primary use is in dairy desserts, particularly ice cream (40%). Soft beverages account for 23% of the industrial market and alcoholic beverages for 15%. The retail consumer of vanilla extract uses it primarily for baking of cookies and cakes.

Ice cream and ice cream novelties are clearly the largest category of vanilla extract usage in the United States. Category I and II ice creams rely in total or in part on pure vanilla extract from a legal as well as a marketing standpoint (see Regulatory section). About one-third of all ice cream is vanilla flavored. Additionally, vanilla is found as an ingredient in other flavors such as chocolate, strawberry, fudge-swirl, butter pecan, etc.

The soft beverage industry uses vanilla extract in cola, cream, pepper-type, root beer and other sodas.

Natural flavors such as vanilla extract are required by BATF regulations for spirits and specialty liquors. Vanilla can be used to round-out harsh flavor profiles in whiskey products. The alcoholic beverage industry generally uses the more concentrated forms of vanilla extract; 3-fold, 5-fold and 10-fold.

Cereals, bakery products, confectionery and tobacco products also use vanilla extract, although they use more artificials or imitations than natural. The yogurt industry, which is rapidly growing, is another major user of vanilla.

Table 6 summarizes major markets and usages for pure vanilla extract in 1988–1989. Vanilla has many other nontraditional uses, such as its use as a paint additive to improve the aroma of a freshly painted room and for grafting an orphaned lamb to an ewe by dabbing the ewe's nose and the lamb's rump with vanilla!

BIBLIOGRAPHY

This article is used with permission. Copyright 1990, McCormick & Company, Inc.

1. N. Childers, H. Cibes, and E. Hernandez-Medina, "Vanilla–The Orchid of Commerce," in C. Withner, ed., *The Orchids–A Scientific Survey*, Ronald Press Company, New York, 1959, pp. 477–508.
2. B Kaestner, Director of Spice Procurement, McCormick & Co., Inc., Personal interview, April 1990.
3. L. Knudson, "Germination of Seeds of Vanilla," *American Orchid Society Bulletin* **19**, 241–247 (1950).
4. D. Correll, "Vanilla—It's Botany, History, Cultivation and Economic Import," *Economic Botany* **7**, 291–358 (1953).
5. F. Arana, *Vanilla Curing*, Circular #25, Federal Experiment Station, Washington, D.C., May 1945.
6 M. Jones and G. Vicente, "Criteria for Testing Vanilla in Relation to Killing and Curing Methods," *J. Agr. Res.* **78**, 425–450 (1949).
7. M. Anzalone, Specifications Supervisor, McCormick & Co., Inc., personal interview, 1990.
8. K. Lentz, Laboratory Technician, McCormick & Co., Inc., personal interview, 1990.
9. F. Arana and A. Kevorkian, "Relation of Moisture Content to Quality of Vanilla Beans," *The Journal of Agriculture at the University of Puerto Rico* **27**, 105–114 (July 1943).
10. "Biotech Vanilla's Promise Still Remains Down the Road," *Chemical Marketing Reports*, 36 (March 1990).
11. J. Sullivan, retired Director of Technical Services, McCormick & Co., Inc., personal interview, 1990.
12. U.S. Pat. 2,621,127, (Dec. 9, 1952), L. Towt (to McCormick & Co., Inc.).
13. U.S. Pat. 2,835,591 (May 20, 1958), R. Graves, R. Hall, and A. Karas (to McCormick & Co., Inc.).
14. U.S. Pat. 3,663,238 (May 16, 1972), A. Karas et al., (to McCormick & Co., Inc.).
15. F. Arana, *Vanilla Curing and Its Chemistry*, Bulletin #4, Federal Experiment Station of the USDA, Mayaguez, Puerto Rico, 1944.
16. F. Arana, "Action of a Beta-Glucosidase in the Curing of Vanilla" *Food Research* **8**, 343–351. (July–Aug. 1943).
17. A. Karas, retired Principal Scientist, McCormick & Co., Inc., personal interview, 1990
18. E. Merwin, retired Principal Scientist, McCormick & Co., Inc., personal interview, 1990.
19. J. Broderick, "The Chemistry of Vanilla," *Coffee Tea Ind. Flavor Field* **78**, 74 (1955).
20. C. Gnadinger, "Identification of Sources of Vanilla Extracts," *Ind. Eng. Chem.* **17**, 303 (1927).
21. P. M. Golby, "Research on the Principal Odor Constituents in Vanilla" *J. Pharm. Chem.* **34**, 401 (1858).
22. I. Klimes and D. Lamparskey, "Vanilla Volatiles—A Comprehensive Analysis," *Int. Flavours Food Additives* **7**, 272–291 (1976).
23. W. Galetto and P. Hoffman, "Some Benzyl Ethers Present in the Extract of Vanilla (*Vanilla plainfolia*)," *J. Agr. Food Chem.* **26**, 195–197 (1978).
24. K. Shulte-Elte, "Vitispiranes, Important Constituents of Vanilla Aroma," *Helvetica Chim. Acta* **61**, 1125–1133 (1978).
25. E. Feyertag and R. Hutchins, "Isolation and Tentative Identification of a New Constituent of Bourbon Vanilla Bean Extract," *J. Food Chem.* **7**, 311–315 (1981).
26. H. Walbaum, "Das Vorkommen von Anis-alkohol, und Anisaldehyd in den Fruchten der Tahitivanille," *Wallachs Fastschrift*, 649–653 (*Ber. Schimmel*, 140–141 Oct. 1909)
27. J W. Purseglove, E. G. Brown, C. L. Green, and S. R. J. Robbins, in *Spices*, Vol. 2, *Vanilla*, Longman, Inc., New York, 1981.
28. K. Riley and D. Kleyn, "Fundamental Principles of Vanilla/Vanilla Extract Processing and Methods of Detecting Adulteration in Vanilla Extracts," *Food Technol.* **43**, 64 (1989).
29. A. Wenton and E. Berry, *The Chemical Composition of Authentic Vanilla Extracts, Together With Analytical Methods and Limits of Constants*, U.S. Department of Agriculture, Bureau of Chemistry, Bulletin No. 147, 146–159 (1912).
30. W. Denis, "The Detection of Prune Juice and Caramel in Vanilla Flavoring Extracts," *The Journal of Industrial and Engineering Chemistry* **III**, 254–255 (1911).
31. J. Broderick, Jr., "Vanilla Color," *The Flavor Field* (Feb. 1954); J. Broderick, Jr., "Lead Number and Vanilla Quality", *The Flavor Field* (March 1954); J. Broderick, Jr., "The Relationship of Vanilla Resin to Vanilla Color," *The Flavor Field* (May 1954).
32. C. Purcell, "More On The Lead Number," *The Spice Mill Flavors*, 694–695 (1934).

33 W. Stahl, W. Voelker, "Analysis of Vanilla Extracts II. Preparation and Reproduction of Two Dimensional Fluorescence Chromatograms," *J. AOAC* **43**, 606–610 (1960).

34. W Stahl et al., "Analysis of Van. Extracts III. Use of Paper Chromatography in the Analysis of Pure and Adulterated Van. Ext." *J. AOAC* **44**, 549 (1961).

35. J. Fitelson and G. Bowden, "Determination of Organic Acids in Vanilla Extracts by GLC Separation of Trimethylsilylated Derivatives," *J AOAC* **51**, 1224 (1968).

36. K. Schoen, "Organic Acids in Vanilla by Gas-Liquid Chromatography: Interpretation of Analytical Data," *J. AOAC* **55**, 1150 (1972).

37. K. Helrich, *Official Methods of Analysis 1990*, 36, 15th ed. *Flavors*, AOAC, Arlington, Va., 1990.

38. A. Hermann and M. Stockli, "Rapid Control of Vanilla-Containing Products Using High Performance Liquid Chromatography," *J. Chromatog* **246**, 313–316 (1982).

39. P. Guarino and S. Brown, "Liquid Chromatographic Determination of Vanillin and Related Flavor Compounds in Vanilla Extract: Cooperative Study," *J. AOAC* **68**, 1198 (1985).

40. A. Archer, "Analysis of Vanilla Essences by High Performance Liquid Chromatography, *J. Chromatog.* **462**, 461–466 (1989).

41. G. Martin et al., "Determining the Authenticity of Vanilla Extracts," *Food Technol.* **29**, 54 (1975).

42. G. Martin et al., "Determining the Authenticity of Vanilla Extracts," *J. Food Science* **42**, 1580–1586 (1977).

43. J. Bricout, J. C. Fontes, and L. Merlivat, "Detection of Synthetic Vanillin in Vanilla Extracts by Isotopic Analysis," *J. AOAC* **57**, 713 (1974).

44. P. Hoffman and M. Salb, "Isolation and Stable Isotope Ratio Analysis of Vanillin," *J. Agr. Food Chem.* **27**, 352 (1979).

45. D. Krueger and H. Krueger, "Carbon Isotopes in Vanillin and the Detection of Falsified "Natural" Vanillin," *J. Agr. Food Chem.* **31**, 1265–1268 (1983).

46. D. Krueger and H. Krueger, "Detection of Fraudulent Vanillin Labeled with ^{13}C in the Carbonyl Carbon," *J. Agr. Food Chem.* **33**, 323:25 (1985).

47. J. Bricout et al., "New Possibilities for the Analysis of Stable Carbon Isotopes in Monitoring Vanilla Quality," *Ann. Falsif. Expert. Chem. Toxicol.* **74**, 691 (1981).

48. R. Culp and J. Noakes, "Identification of Isotopically Manipulated Cinnamic Aldehyde and Benzaldehyde," *J. Agr. Food Chem.* **38**, 1249–1255 (May 1990).

49. G. Martin, F. Alfonso, D. Figert and J. Burggraff, "Stable Isotope Ratio Determination of the Origin of Vanillin in Vanilla Extracts and Its Relationship to Vanillin/Potassium Ratios," *J. AOAC* **64**, 1149–1153 (1981).

50. B. Feyet et al., "New Analytical Specifications In The Study of Vanilla Beans," *Analysis* **15**, 217–226 (1987).

51. B. Toulemonde, "Differentiation Between Natural and Synthetic Vanillin Samples Using ^{2}H-NMR," *Helvetica Chim. Acta* **66**, 2342 (1983).

52. C. Maubert et al., "Determination of the Origin of Vanillin By Multivariate Analysis of the Site-specific Natural Isotope Fractionation Factors of Hydrogen," *Analysis* **16**, 434–439 (1988).

53. N. Anand and A. Smith, *The Market for Vanilla*, Report of the Tropical Development and Research Institute, London, England, G198, 1986, V + 33p.

54. R. Braceklein, Team Leader Extract Manufacture, McCormick & Co., Inc., personal interview, 1990.

GENERAL REFERENCES

Ref. 27 is a good general reference.

A. K. Balls and F. E. Arana, "The Curing of Vanilla," *Industrial and Engineering Chemistry* **33**, 1073–1075 (1941).

M Mariani, A. Scotti, and E. Colombo, "Vanilla: From the Origin to Its Analysis," in Italian and English, *Quintessenza* **17**, 3–25 (1982).

P. Rain, *Vanilla Cookbook*, Celestial Arts Publisher, Berkeley, Calif. 1986.

F. Rosengarten, Jr., *The Book of Spices*. Pyramid Communications, Inc., New York, 1973.

R. Sax, "All About Vanilla," *Bon Appetit*, 132–138 (March 1986).

Standard #5565, International Standards Organization. *Vanilla (Vanilla Fragrans, Salisbury Ames) Specification*, 1982-12-01, Ref. #ISO 5565-1982(E), 1982.

R. Theodose, "Traditional Methods of Vanilla Preparation and Their Improvement," *Trop. Sci.* **15**, 47–57 (1973).

Marianne H. Gellette
Patrick G. Hoffman
McCormick & Co., Inc.
Hunt Valley, Maryland

See also Flavor chemistry.

VEGETABLE DEHYDRATION

Dehydration involves the removal of the water normally present in the tissues by evaporation. Vegetable dehydration has one or both of the following purposes: (*1*) to preserve the perishable raw commodity against deterioration, and (*2*) to reduce the cost of packaging, handling, storing, and transporting the material by converting it to a dry solid, thus reducing its weight and also, usually but not necessarily, its volume.

Essential to the dehydration process is the application of heat to vaporize water, and some means of removing water vapor after its separation from the plant tissue. The removal of water involves mass transfer, and the application of heat in some manner also involves heat transfer. Three basic methods of heat transfer are used in dryers in varying degrees of prominence and combinations. These are convection, conduction, and radiation. Dehydration terms are described in the article Fruit dehydration.

Dehydrated vegetables are an important and steadily growing portion of the food industry. Vegetables commonly dehydrated include potatoes, beets, cabbage, carrots, parsley, chili peppers, horseradish, onion, garlic, bell peppers, green beans, turnips, parsnips, sweet corn, mushrooms and celery.

The U.S. vegetable-dehydration industry may be divided into four groups:

1. Onions and garlic, almost exclusively in California
2. Potatoes, primarily in Idaho and also in Washington, Oregon, and a few other states
3. All other vegetable products, almost exclusively in California

33 W. Stahl, W. Voelker, "Analysis of Vanilla Extracts II. Preparation and Reproduction of Two Dimensional Fluorescence Chromatograms," *J. AOAC* **43**, 606–610 (1960).

34. W Stahl et al., "Analysis of Van. Extracts III. Use of Paper Chromatography in the Analysis of Pure and Adulterated Van. Ext." *J. AOAC* **44**, 549 (1961).

35. J. Fitelson and G. Bowden, "Determination of Organic Acids in Vanilla Extracts by GLC Separation of Trimethylsilylated Derivatives," *J AOAC* **51**, 1224 (1968).

36. K. Schoen, "Organic Acids in Vanilla by Gas-Liquid Chromatography: Interpretation of Analytical Data," *J. AOAC* **55**, 1150 (1972).

37. K. Helrich, *Official Methods of Analysis 1990*, 36, 15th ed. *Flavors*, AOAC, Arlington, Va., 1990.

38. A. Hermann and M. Stockli, "Rapid Control of Vanilla-Containing Products Using High Performance Liquid Chromatography," *J. Chromatog.* **246**, 313–316 (1982).

39. P. Guarino and S. Brown, "Liquid Chromatographic Determination of Vanillin and Related Flavor Compounds in Vanilla Extract: Cooperative Study," *J. AOAC* **68**, 1198 (1985).

40. A. Archer, "Analysis of Vanilla Essences by High Performance Liquid Chromatography, *J. Chromatog.* **462**, 461–466 (1989).

41. G. Martin et al., "Determining the Authenticity of Vanilla Extracts," *Food Technol.* **29**, 54 (1975).

42. G. Martin et al., "Determining the Authenticity of Vanilla Extracts," *J. Food Science* **42**, 1580–1586 (1977).

43. J. Bricout, J. C. Fontes, and L. Merlivat, "Detection of Synthetic Vanillin in Vanilla Extracts by Isotopic Analysis," *J. AOAC* **57**, 713 (1974).

44. P. Hoffman and M. Salb, "Isolation and Stable Isotope Ratio Analysis of Vanillin," *J. Agr. Food Chem.* **27**, 352 (1979).

45. D. Krueger and H. Krueger, "Carbon Isotopes in Vanillin and the Detection of Falsified "Natural" Vanillin," *J. Agr. Food Chem.* **31**, 1265–1268 (1983).

46. D. Krueger and H. Krueger, "Detection of Fraudulent Vanillin Labeled with ^{13}C in the Carbonyl Carbon," *J. Agr. Food Chem.* **33**, 323:25 (1985).

47. J. Bricout et al., "New Possibilities for the Analysis of Stable Carbon Isotopes in Monitoring Vanilla Quality," *Ann. Falsif. Expert. Chem. Toxicol.* **74**, 691 (1981).

48. R. Culp and J. Noakes, "Identification of Isotopically Manipulated Cinnamic Aldehyde and Benzaldehyde," *J. Agr. Food Chem.* **38**, 1249–1255 (May 1990).

49. G. Martin, F. Alfonso, D. Figert and J. Burggraff, "Stable Isotope Ratio Determination of the Origin of Vanillin in Vanilla Extracts and Its Relationship to Vanillin/Potassium Ratios," *J. AOAC* **64**, 1149–1153 (1981).

50. B. Feyet et al., "New Analytical Specifications In The Study of Vanilla Beans," *Analysis* **15**, 217–226 (1987).

51. B. Toulemonde, "Differentiation Between Natural and Synthetic Vanillin Samples Using ^{2}H-NMR," *Helvetica Chim. Acta* **66**, 2342 (1983).

52. C. Maubert et al., "Determination of the Origin of Vanillin By Multivariate Analysis of the Site-specific Natural Isotope Fractionation Factors of Hydrogen," *Analysis* **16**, 434–439 (1988).

53. N. Anand and A. Smith, *The Market for Vanilla*, Report of the Tropical Development and Research Institute, London, England, G198, 1986, V + 33p.

54. R. Braceklein, Team Leader Extract Manufacture, McCormick & Co., Inc., personal interview, 1990.

GENERAL REFERENCES

Ref. 27 is a good general reference.

A. K. Balls and F. E. Arana, "The Curing of Vanilla," *Industrial and Engineering Chemistry* **33**, 1073–1075 (1941).

M Mariani, A. Scotti, and E. Colombo, "Vanilla: From the Origin to Its Analysis," in Italian and English, *Quintessenza* **17**, 3–25 (1982).

P. Rain, *Vanilla Cookbook*, Celestial Arts Publisher, Berkeley, Calif. 1986.

F. Rosengarten, Jr., *The Book of Spices*. Pyramid Communications, Inc., New York, 1973.

R. Sax, "All About Vanilla," *Bon Appetit*, 132–138 (March 1986).

Standard #5565, International Standards Organization. *Vanilla (Vanilla Fragrans, Salisbury Ames) Specification*, 1982-12-01, Ref. #ISO 5565-1982(E), 1982.

R. Theodose, "Traditional Methods of Vanilla Preparation and Their Improvement," *Trop. Sci.* **15**, 47–57 (1973).

MARIANNE H. GELLETTE
PATRICK G. HOFFMAN
McCormick & Co., Inc.
Hunt Valley, Maryland

See also FLAVOR CHEMISTRY.

VEGETABLE DEHYDRATION

Dehydration involves the removal of the water normally present in the tissues by evaporation. Vegetable dehydration has one or both of the following purposes: (*1*) to preserve the perishable raw commodity against deterioration, and (2) to reduce the cost of packaging, handling, storing, and transporting the material by converting it to a dry solid, thus reducing its weight and also, usually but not necessarily, its volume.

Essential to the dehydration process is the application of heat to vaporize water, and some means of removing water vapor after its separation from the plant tissue. The removal of water involves mass transfer, and the application of heat in some manner also involves heat transfer. Three basic methods of heat transfer are used in dryers in varying degrees of prominence and combinations. These are convection, conduction, and radiation. Dehydration terms are described in the article FRUIT DEHYDRATION.

Dehydrated vegetables are an important and steadily growing portion of the food industry. Vegetables commonly dehydrated include potatoes, beets, cabbage, carrots, parsley, chili peppers, horseradish, onion, garlic, bell peppers, green beans, turnips, parsnips, sweet corn, mushrooms and celery.

The U.S. vegetable-dehydration industry may be divided into four groups:

1. Onions and garlic, almost exclusively in California
2. Potatoes, primarily in Idaho and also in Washington, Oregon, and a few other states
3. All other vegetable products, almost exclusively in California

4. Freeze-drying of certain high-value crops, mainly in California and Oregon

Dehydrated vegetables are used in dry soup mixes, canned soups and sauces, frozen entrées, processed meats, baby foods, dairy products, snack foods, and seasoning blends.

PREDRYING TREATMENTS

Almost all vegetable crops are dehydrated fresh from the field, although some items such as green beans may be dehydrated from the frozen state. Harvesting, washing, sorting, grading, peeling, and cutting operations are essentially identical to those described for canned or frozen foods, and the raw material is handled by a similar method.

Some exceptions to standard operating conditions are that most vegetables are blanched before drying; beets are not peeled—all dehydrated beets are sold as dried ground powder; and parsley is dried in a specially designed hot-air tower for a short time to discharge to a more conventional dryer for final moisture equalization.

Enzyme Inactivation

Vegetables contain catalase, peroxidase, polyphenoloxidase (PPO) and many other enzymes. At the time of cutting and peeling, enzymatic reactions are accelerated and discoloration will often occur. Enzymes can be inactivated by heating or acidification to low pH values. Inactivation by heat treatment (blanching) is usually done by immersing the vegetables in hot water at 95 to 100°C for a few minutes, or exposing it to steam. Vegetables have also been successfully blanched by hot air, infrared, or microwave radiation. In addition to enzyme inactivation, blanching serves to remove the gases between the tissues, soften tissues, decrease the amount of microorganisms, and cleanse the vegetables.

Commercially continuous blanchers are favored over the batch type, which involve a 2- to 10-min exposure to live steam. A conveyor or an exhausting box is suitable for the continuous blanching process. The effectiveness of blanching is controlled by measuring the activity of catalase in cabbage and PPO in other vegetables, and a guaiacol test is used as an index for the activity of peroxidase. If fresh vegetables are not properly blanched, enzymatic deterioration of the dehydrated products may take place during processing or storage or after reconstitution. Flavor changes resulting from lipid oxidation may take place during storage. Browning reactions may occur if the dried product is stored at temperatures higher than 200°C and when the moisture content is higher than 5%.

Sulfuring

Vegetables undergo browning due to enzymatic or nonenzymatic reactions that occur during processing and storage. Until recently the addition of sodium sulfite or metabisulfite to cut vegetables before dehydration has been a standard practice to control both enzymatic and nonenzymatic browning. Sulfites act as a PPO enzyme inhibitor and also react with intermediates to prevent pigment formation. Also, sulfiting agents control the growth of microorganisms, act as bleaching agents, and provide other technical functions. Sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium bisulfite, and potassium metabisulfite salts are generally recognized as safe (GRAS) compounds for use in foods by the FDA. For small-scale operations, blanched vegetables may be dipped for a specified time in a sulfite salt solution. Spraying, however, is the most practical method. Cabbage, potatoes, and carrots are routinely sulfite treated before dehydration. The highest sulfite levels are required for cabbage, between 750 and 1500 ppm. Limits of 200 to 500 ppm are imposed on potatoes and carrots. Soaking in 500 to 1000 ppm sodium bisulfate solution for 5 to 10 min before slicing retained good color of mushrooms. The residual sulfur dioxide after such treatment was less than 10 ppm in the dry mushrooms.

Sulfites have been associated with allergylike reactions in some asthmatics, prompting limit of their use. Because of these reactions, and the legal requirement to declare on the label of packaged food that the product has been treated with sulfites, food processors are seeking alternative methods for producing high-quality dehydrated vegetables. However, sulfites are multifunctional agents. The search for alternatives has yielded compounds that are effective substitutes for only one or two of the functionalities obtained with sulfites. Perhaps the best alternative to sulfiting agents is the use of ascorbic acid or erythorbic acid as a browning inhibitor. Blends of citric/ascorbic or citric/erythorbic acids are also effective and somewhat less expensive. These compounds can be applied to prepared vegetables by immersion in a water solution containing 1% citric and 0.5 to 1% ascorbic or erythorbic acid for 30 to 60 s. The penetration of these browning inhibitors may be enhanced by vacuum infiltration of treatment solutions, which also removes air from within the product's void spaces.

DRYING METHODS

Selection of a drying method for a given food product is determined by quality requirements, raw material characteristics, and economic factors. In general, dryers are suitable for either batch or continuous operation. Batch-operated equipment usually is related to small production runs or to operations requiring great flexibility.

As most vegetables are dehydrated to under 5% (low moisture) level, sun drying with vegetables has only limited application, except for a small quantity of home-dried mushrooms and limited use of solar dryers for carrots, celery, and the like. Most dehydrated vegetables are processed by the forced air–drying method. A disadvantage of air-dried vegetables is their low rate of rehydration. The process of rehydration after drying is not a simple reversal of the drying mechanism. Some of the changes produced by drying are irreversible; also, the swelling of outside layers as water is reabsorbed puts severe stress on the softened outside layers. Irreversible changes of the colloidal components of vegetable tissue occur if the material is held for a period of time at high temperature, even if the exposure is insufficient to produce scorching. However, depending on the particular use of dehydrated vegetables, slow rehydration may not be of critical importance. Large quantities of carrots, onions, garlic, potatoes, and other vegetables are forced air–dried. For rapid rehydration and for highly heat-sensitive vegetables (chives, mushrooms, etc), the freeze-drying process may be justified.

For liquid, purees, or the flowable suspension form of vegetable material, spray drying is the most important dehydration method. Among vegetable substances, concentrated tomato puree is commercially spray-dried. Drum drying, a high-temperature, short-time method, is an inexpensive way to dry pureed foods. Drum drying is successfully used for drying mashed potatoes and tomato concentrates.

The common kinds of vegetable-drying equipment are cabinet, tunnel, continuous conveyor belt, pneumatic, belt trough, fluidized bed, bin, drum, spray, and freeze dryers.

Cabinet Drying

A cabinet dryer is essentially a small batch tray dryer. The trays are made of stainless angle iron and stainless net or apertured plate with a height of about 5 cm. The dryer usually loads more than 10 trays vertically. The heat source usually is a steam coil or electrical coil through which the air from a fan passes across the trays loaded with the raw vegetables. Cabinet dryers are usually held at a constant temperature, though humidity may decrease during the process of drying. Airflow may be across or through the trays with or without recirculation. Cabinet dryers are flexible as to type and size of operation, although production may be lower and labor requirements greater than for a tunnel dehydrator.

Tunnel Drying

A tunnel dryer is basically a group of truck-and-tray dryers operated in a programmed series so as to be semicontinuous. Truckloads of freshly prepared material are moved at intervals into one end of the long, closely fit enclosure; the string of trucks is periodically advanced a step; and the dried truckloads are removed at the other end of the tunnel. The hot drying air is supplied to the tunnel in any of several ways: counterflow, concurrent or parallel flow, center exhaust, multistage, and compartment arrangements.

Continuous Conveyor Drying

The continuous conveyor dryer consists of an endless belt that carries the material to be dried through a tunnel of warm circulating air. The speed of the conveyor is variable to suit both the product and the heat conditions. Most dryers have several sections, allowing different flow rates, humidities, and temperatures to be set to each section and the rotation of the product when it moves from one section belt to the next. This drying method has the advantage of essentially automatic operation, which minimizes labor requirements.

These dryers are being used extensively in vegetable dehydration plants for such crops as beets, carrots, onions, garlic, and potato pieces. The conveyor dryer is best adapted to the large-scale drying of a single commodity for the whole operating season; it is not well suited to operations in which the raw material or drying conditions are changed frequently.

Pneumatic Drying

The method of drying finely divided solids while they are being conveyed in a hot airstream has been used for the first stage or rough drying of granulated cooked potatoes in the manufacture of potato granules. The partial drying desired takes place in a few seconds for finely granulated wet potato, whose initial moisture content generally has been reduced to around 35 to 40% by an admixture of fresh mash with recycled dry product. Most of the material is finer than 60 mesh, but a small portion may be 20 mesh or coarser. The moist granulated material is usually sucked into the conveying pipeline at a low-pressure throat. Air velocity in the pipeline is just enough to overcome the free-fall velocity of the coarser particles to keep them suspended while avoiding unnecessary abrasion damage to the delicate potato cells. The pipe is sufficiently long to give the necessary retention time at that air velocity.

The operating section is generally terminated by a diverting section that helps to separate the light, completely dry particles from the heavier, still somewhat moist ones and gives the latter more time in the hot air. The conveying riser ends with a cyclone collector where the granular solid is separated from the outgoing airstream.

Belt-Trough Drying

Belt-through drying is a type of continuous trough-flow drying that can be used for a wide range of vegetables that have a firm texture in piece form. It consists of a wire-mesh belt running on sprockets mounted on three driveshafts. During part of its path of travel, the belt forms a trough whose bottom rests on an inclined, flat-surfaced air gate. The material to be dried is continuously cascaded in this trough while a current of hot air blows up through the grade and bed of material. High operating temperatures result in high drying rates without causing appreciable heat damage to vegetable pieces because the constant agitation keeps any individual piece from being exposed to the very hot dry air for more than a moment. Then each piece is surrounded by air at much lower temperatures for a longer time before again moving into the zone of intense drying.

Fluidized-Bed Drying

In fluidized-bed drying, heated air is blown up through the food particles with just enough force to suspend the particles in a gentle motion. Semidry particles such as potato granules gradually migrate through the apparatus until they are discharged dry. Heated air is introduced through a porous plate that supports the bed of granules. The moist air is exhausted at the top. This process is continuous, and the depth of the bed and other means can regulate the amount of time that the particles remain in the dryer. This type of drying can be used to dehydrate peas and other vegetable particles also.

Bin Drying

The bin drying method is widely used as a finishing step in the dehydration of piece-form vegetables when they constitute a deep bed of granular, nonsticky material. Bin dryers are used to complete the drying operation after most of the moisture has been removed by other drying techniques. Typically, they reduce the moisture content of a partially dried, 10 to 15% moisture cut vegetable to 3 to 6% or even lower in the case of onion slices and possibly cabbage shreds.

A characteristic unit consists of a metal or wooden box equipped with an air inlet at the bottom and a wire mesh deck or false bottom with an air supply duct below it, arranged so that warm dry air can be passed through the nearly dry product piled on the deck.

Drum Drying

Drum dryers are designed to handle solutions, vegetable purees, and pastes. Drum drying processes are used successfully in the production of powdered tomato, pea and bean soups, potato flour, tomato juice flakes, mashed potato flakes, and sweet potato flakes.

A drum dryer consists of one or two hollow drums, which are fitted so that a heating medium, usually steam, or a heat-transfer liquid can be circulated through them. The drums are mounted to rotate about the symmetrical axis and are customarily driven with a variable-speed mechanism. A feeding device applies a thin, uniform layer of the material to be dried on the hot drum surface. A knife is fitted to the drum at an appropriate location. The material is dried as the heated drum rotates toward the blade, which scrapes the thin layer of dry material from the drum surface.

Spray Drying

In spray drying, the fluid to be dried is dispersed into a stream of heated air. The dry particles are separated from the air and collected, and the moist, cold air is exhausted. The process is used to dehydrate vegetable juices. Materials that are easily damaged by heat or oxidation can be handled in a properly designed and operated spray dryer.

Freeze Drying

Freeze dehydration consists of freezing the product and then evaporating the water in the form of ice (by sublimation) directly into the vapor phase without liquid interim. Water vapor is removed from the frozen food, which is achieved by reducing the pressure in a sealed drying chamber by a vacuum pump to 50 to 1000 mm mercury. This pressure holds the vegetables below their eutectic temperature, facilitates rapid evaporation of water molecules from the frozen state, and condenses the vapor either within the chamber or in a separate chamber. Heat energy applied to the frozen product causes the water molecules to break loose and migrate to the block of ice on the condenser, where their energy is removed by cooling. Heat may be applied to the frozen product by conduction or radiation, or both.

Freeze dehydration produces one of the highest-quality food products obtainable by any drying method. The porous, nonshrunken structure of the freeze-dried vegetable facilitates rapid and nearly complete rehydration when water is added. The low processing temperatures and the rapid transition minimizes the extent of various degradative reactions associated with other drying methods and also help reduce the loss of volatile flavor compounds. However, freeze drying is an expensive way to evaporate water, and in addition freeze-dried vegetables must be protected from deterioration by adequate packaging.

PACKAGING AND STORAGE OF DEHYDRATED VEGETABLES

Oxygen concentration, moisture content, temperature, exposure to light, and the length of storage all affect deterioration of dehydrated vegetables. The relative importance of these parameters vary from commodity to commodity. For instance, carrots and other high-carotene-containing tissues have been found to be very sensitive to the presence of oxygen.

Because freeze-dried foods differ significantly in physical and chemical properties from food dried by conventional methods, they must be stored differently than other dehydrated products. Moisture absorption and changes in quality due to oxidation are the most important problems encountered in the storage of freeze-dried vegetables.

Packaging Materials

Cartons, cans, glass containers, and pouches can be used to package dehydrated vegetables. At the time of packaging, dried vegetables should not be above 4% moisture, and in some cases a maximum of 3% is preferable. Most dried vegetables are packed in paper cartons lined with some moisture-proof material, preferably in high-density polyethylene bags that are heat sealed and placed in the cartons.

To destroy insects, dehydrated vegetables may be fumigated with methyl bromide as they come off the drying trays, while they are still hot and before insects have had the opportunity to lay eggs on them. Two serious objections to the use of cartons for packing dehydrated vegetables are their susceptibility to later insect infestation and to penetration of moisture.

The ideal container for dehydrated vegetables is the key-top can such as is used for canned meats and for coffee. If the cans are reinforced to prevent collapse under vacuum, or if the cans are tightly filled so that the dried product will support the walls, the filled cans may be sealed under high vacuum. The vacuum protects against oxidative changes and will prevent insect infestation. Friction-top cans of the type used for paint are usually insectproof and reasonably moistureproof. They have been used very successfully for dried vegetables. Powdered vegetables should be packed only in airtight tin or glass containers to prevent not only insect infestation but also the absorption of moisture and subsequent caking.

Dehydrated vegetables can also be packed in 5-gal cans of rectangular shape. Glass jars are moisture- and insect-proof. Some can be sealed under vacuum, and they are preferable to other types for dehydrated foods. One great advantage of glass is the visibility of the products; another is the resealability of most glass jars. However, the greater weight and fragility of these containers are disadvantages. Plastic pouches, laminated pouches, and aluminum–film combination pouches may be used for packaging dehydrated vegetables.

Freeze- or vacuum-dried foods must be kept in airtight containers made from a material that is impermeable to oxygen and light. Transparent packaging materials are usually unsuitable for this purpose. Because freeze-dried products are fragile, the packaging materials should protect them from mechanical damage.

In-Package Desiccants, Anticaking Agents, and Compression

Use of in-package desiccants to bring moisture content to 2% or lower should permit storage of dehydrated vegetables for an extended time. Addition of fumed silica at a concentration of 1 to 2% or calcium stearate at 0.1 to 0.2% is recommended in powdered vegetables to prevent agglomerating or caking of spray-dried tomato powder.

Dried vegetables may be compressed into dense bricks or cylinders. These can be wrapped in any suitable manner, for example, in paper with an outer aluminum foil wrap, in cellophane or plastic, or in cartons or cans. If compressed to high density, the products are resistant to insect attack and represent a substantial saving of storage space. Compression technology utilizes hydraulic press to reduce product volumes to as little as 1/16th of the original volume. Carrots, green beans, spinach, cabbage, and celery are suitable for utilizing this process.

QUALITY CONTROL

Important laboratory tests specific for dehydrated food are moisture, sulfite contents, particle size distribution, enzyme activity determinations, and behavior on rehydration.

Moisture

Moisture may be determined in about 10 min with an analytical balance, which uses an infrared lamp and a calibrated dial to indicate weight loss in powdered foods. The Karl Fischer titration method is recommended for moisture determinations on low-moisture products.

Sulfur Dioxide

Sulfur dioxide residue can be determined by the Monier-Williams method, which has a detection limit of 50 ppm. The ion-chromatographic technique is accurate to 1 ppm, and it has a short analysis time of 25 min.

Particle Size Distribution

Particle size distribution, an important characteristic of powdered products in particular, is determined by a screen analysis. In this procedure, the product is shaken for a specified time (usually 3–5 min) through a series of standard sieves and the residue on each sieve is determined.

Enzymes

The enzyme peroxidase and catalase have been used as indicators of blanching adequacy before dehydration. Some vegetables such as green peas may be overblanched when peroxidase is used as an indicator, and lipoxygenase has been proposed as a new indicator to determine the effectiveness of blanching. It takes less than half the heat to destroy lipoxygenase as it does to destroy peroxidase.

Rehydration Test For the rehydration test, a 50-g sample from tray, bin, or final package is placed in a beaker or jar. For such products as peas, potatoes, corn, and sweet potatoes about 200 mL of cold water is added; for other vegetables, 300 to 500 mL depending on the drying ratio. If the pieces float, a wire screen cut to fit the beaker will keep them submerged. After standing 12 h at room temperature the samples are drained for 3 to 4 min on a screen and again weighed. After experience with different lots, a minimum drained weight for each vegetable can be established and used as an indication of rehydration value.

DEHYDRATION PROCEDURES FOR SELECTED COMMODITIES

Potatoes

In the United States about 20% of the processing potatoes (about 2 million t in 1997) are dehydrated. To achieve maximum use and efficiency of manufacturing facilities, the processor attempts to operate the dehydration plant year-round. Thus it is necessary for large quantities of potatoes to be placed in storage at the end of each harvest. A high relative humidity must be maintained to prevent dehydration of stored potatoes, which is associated with as much as 10% loss of carbohydrates.

From storage or the field, the potatoes are directed to a water flume or other transport system by high-pressure water hoses. A metering wheel feeds the potatoes into the process system. The potatoes are washed by passing them through a rotary drum or cylindrical washer where the potatoes are scrubbed either with brushes or merely by tumbling them together. Water sprays remove additional foreign material and soil particles. Following the washer the potatoes pass over a short draining belt, which permits internal recirculation of the wash water. An inspection of the potatoes is made on the drainage belt, and the undesirable whole potatoes are removed.

Four systems are used to remove potato peel: abrasive peeling, steam peeling, lye (caustic) or wet lye peeling, and dry caustic peeling. The peel loss, including trimming, can result in a 15 to 30% loss of the potatoes processed. The trimming process should be considered part of the peel removal. In this process the presence of eyes, blemishes, and remaining peel are often detected electronically, which directs the imperfect potato to the trim table, where the imperfections are manually cut out of the potato. After being peeled, the potato is sliced or diced, depending on the desired end product. In any cutting process a number of potato cells are ruptured, releasing a considerable amount of starch. Many processors are installing hydrocyclones to remove the starch from the wash water in the form of slurry.

After the potato is peeled and sliced, the pieces are blanched to deactivate the enzymes, to remove surface air, to partially cook the potato, and to remove excess sugars from the potato pieces. The potato slices or dices are dehydrated as individual pieces in an atmospheric recirculating air tunnel or conveyor dryer. If granules or flakes are to be processed, then blanched potato pieces are mashed and conditioned before drying as flakes on a drum dryer or as granules in a fluid bed dryer.

Onions and Garlic

The harvest period is from mid-May to November for onions and from mid-June until the beginning of October for garlic. Topped and screened bulbs are transported to the plant, where they are screened to remove dirt and loose stems and then gently conveyed to sorting bins. These bins are fitted with fans that circulate warm air through the onions (from bottom to top) in order to dry remaining tops and stems, the outer layer of skin, and the bulbs themselves to prevent microbial spoilage.

The dehydration of onions involves cleaning them with both wet and dry methods. Dry cleaning is used to remove dried tops, some loose skins, and dirt. Thus machinery usually consists of a series of vibrating screens, parallel rollers, or air aspirators or a combination of all three. Dried tops are usually pinched off by a series of rollers. Wet cleaning is usually done by a series of dip-and-soak tanks and high-pressure water sprays. Hand trimming is sometimes employed to further remove tops, defective parts of bulbs, or other undesirable blemishes.

Garlic normally contains more dirt than onions do, and dry cleaning is essential. This involves a series of rollers or screens, which both screen the dirt and aspirate the skins from the bulk. A cracking device is included to break the bulbs into individual cloves. It is the cloves that are eventually sliced and dried into a finished product. The cloves are inspected visually (culls, trash, and foreign debris removed) and conveyed through a riffle washer, the purpose of which is to wash the individual cloves and separate them from small rocks and any adhering dirt. They then enter a flotation tank, where they are immersed in water. The good cloves sink, whereas defective (dry) clove and loose skin float and are simmered off as solid waste.

Onions may be size graded as a part of the dry-cleaning operation, or they may be sorted by size and quality after wet washing. In some cases the larger onions are separated from the main stream and used primarily in the production of larger onion pieces (ie, large sliced, sliced, and large chopped portion). These bulbs are normally cored by rotating circular saws, which remove the root and root crown from the bulb, and the dry outer scales are removed by revolving high-pressure washers or by a flame peeler.

Following grading, sorting, and washing, the onion bulbs or garlic cloves are conveyed to specially designed machines that slice the whole bulbs and cloves into thin layers. A belt or vibratory conveyor then transfers these layers to a continuous-belt dryer. All onions and garlic dehydrated in the United States are now dried on continuous-belt conveyors. They are usually long and multistaged with baffled chambers that blow heated and sometimes desiccated air from over and under the bed depth of the raw slices. Residence time in this type of dryer is usually 19 to 20 h, resulting in a product that has a finished moisture content of no greater than 4.25% for onions and 6.0% for garlic. Alternatively, the onion and garlic slices may be taken from the final stage of drying at a slightly higher moisture and reduced to the desired moisture content by bin drying. After dehydration, the dried slices are usually screened, milled, aspirated, separated, and ground in various mechanical combinations to achieve the final desired piece size.

Dehydrated onions are sold in a variety of sizes. Classification includes sliced, chopped, minced, granulated, and powdered. Care is taken during dehydration to insure minimal powder production because of its low value. Pieces are separated by screening or air classification. Screening, grinding, and packaging operations are carried out in special dehumidified rooms because of the hygroscopic nature of the dried onions. Air in these rooms is kept below 30% RH.

Mushrooms

Mushrooms are one of the important vegetables (in terms of value) to be preserved by dehydration. *Agaricus bisporus* is the most frequently used for drying. The process of commercial drying begins with trimming the stalks that are contaminated with soil and hard to wash, followed by soaking whole mushrooms in 0.05 to 0.1% sodium sulfite for 5 to 10 min before they are cut into slices to retard discoloration. The residual sulfur dioxide of dried mushrooms is less than 10 ppm. The mushroom is sliced longitudinally with a slicer machine to a thickness of 4 mm. Two drying methods are applied to mushrooms air drying and freeze drying. Air drying involves two stages: the first is drying at 40 to 45°C for 13 h and then at 70°C for 2 h, both with air velocity of outlet of about 70 m/min. Drying at a higher initial temperature could cause browning of the product. In freeze drying two stages are involved also. The first is to freeze the mushrooms at −34°C and then dehydrate with a freeze dryer until the moisture content of the products is reduced to 2 to 3%. The temperature may be raised gradually to about 60 to 70°C in 10 to 12 h. The drying ratio of mushrooms is 11 to 11.75 and the yield is about 8.5 to 9.0%. The dried product should be stored in airtight containers at 20°C.

Peppers

The principal dried peppers are hot red peppers for cayenne and sometimes pimientos for paprika. Ancho is the pepper that is partially or completely dried and used as the principal flavor source in chili powders. Peppers are harvested by hand. At the plant they are washed, inspected, and sorted for size and color. They are then cored and sliced and the seeds removed by screening and water sprays. Stems and core material are also removed. Sulfiting to a final sulfite level of 1000 to 2500 ppm is applied by spraying. Pimientos are not sulfited, but due to their very thick and tough skin, pimientos must be peeled before dehydration. This is accomplished in the same manner as for canning, which may involve flame, hot oil, or lye peeling.

The initial temperature in dehydrators can be as high as 76.6°C with a finishing temperature of 63°C or below. Moisture is removed so that a final level of 5% is accomplished in sweat or finishing bins. The dried product is used to flavor and improve the appearance of various processed foods. Some sliced peppers are partially dried and mixed with salt for preservation for ultimate use in various processed products. Grinding of sweet peppers for paprika and of hot peppers for cayenne pepper is carried out immediately at a very low moisture content because dried peppers rapidly absorb moisture and become tough.

Bell peppers are handpicked and delivered to the plant in large wooden bins. Bell peppers change color and solids content as they mature on the plant. The first portion of the harvest results in the prime green bell pepper. As the season grows longer, peppers remaining on the vine begin to develop yellow and yellow-orange stripes and blotches. Reaching full maturity, they develop an intense red color. Typically, then, bell peppers are run as three distinct products: green, mixed, and red cut into dice, slice, and so on. Dehydrated bell peppers are used in soup and gravy mixes, salad dressings, dip mixes, and spreads.

The peppers are initially graded according to size to facilitate cracking of the pods and later core separation. Grading is usually done by parallel rollers, the smaller vegetables dropping to another processing line. The peppers are then usually washed in immersion-type or spray-type washers and conveyed to a popper or cracker. This can be a device consisting of two closely spaced belts moving in the same direction (one over the other to pull the peppers in and crack them), or it may be two revolving wheels between which the peppers fall and are popped. Separation of pods and cores is usually accomplished by means of flotation. The very light and buoyant core floats whereas the flesh of the pepper is denser and sinks.

The cores are skimmed and go to solid waste. Inspection for defects and hand sorting of remaining attached cores is then accomplished before a final wash to remove the remaining bits of core and seeds. The peppers are diced, sliced, or cut into the desired piece size and sprayed with a sodium bisulfite solution to give a final sulfur dioxide content of 1000 to 2500 ppm. Most bell pepper products are dried on a continuous-belt dryer. The dice are removed from the belt at about 10% moisture content, and the final moisture content of 5% is achieved in bin drying. Packing is usually done in bulk (drums), although the peppers may be repacked into smaller packages at a later date.

Other Vegetable Crops

Other vegetables commonly dehydrated include beets, cabbage, carrots, parsley, horseradish, turnips, parsnips, and celery. Additionally, other vegetables such as asparagus, tomatoes, green beans, spinach, and green onion tops may be dehydrated on commercial demand. These items are commonly used as ingredients for various food specialties, baby food, and dry soup mix.

Almost all crops are dehydrated fresh from the field, although some items such as green beans may be dehydrated from the frozen state. As examples, the dehydration process for celery and carrots are described in detail.

Dehydrated celery is commercially available as 1/8-in. stalk dice, stalk and leaf flakes, stalk and leaf granules, and powder. Celery is delivered to the plant in bulk fresh from the field. The butt and leaf ends are trimmed by mechanical circular saws. Following trimming the inner yellow stalks are separated by hand and typically discarded. The celery stalks are then washed in immersion-type or spray-type washers and conveyed directly to slicers or dicers. The cut fractions are sprayed with sodium bisulfite solution (to preserve color) and fed in a steady stream to continuous-belt dryers. The dried product may be further dried in forced-air bin dryers or may be packed directly into bulk containers.

Carrots are size graded, inspected, washed, trimmed, and peeled almost exactly as for canning or freezing. After final inspection and wash, they are conveyed to a dicer or slicer. Diced carrots are sold in various commercial sizes, cross-cut slices made at right angles to the axis of the carrot, and so on. Immediately after cutting the carrot pieces are blanched, usually in a steam blancher, by being spread on a continuous stainless-steel mesh belt at a loading of about 4 lb/sq ft and heating in flowing steam for 6 to 8 min and spraying with sodium bisulfite solution of about 1% concentration. Blanching is critical, and both catalase and peroxidase enzymes must be inactivated. The carrot pieces are usually dried in conveyor or tunnel-type dehydrators; the initial temperature can be 46 to 93°C and the finished temperature 96°C. Four percent moisture is the desirable endpoint for adequate storage and retention of carotene. The dehydrated pieces are packed in airtight containers and filled with inert gas such as carbon dioxide or nitrogen. One hundred kilos of raw carrots will yield approximately 9 kg of low-moisture carrots (11:1 drying ratio).

GENERAL REFERENCES

Anonymous, "New Insight into Blanching Vegetables," *Food Eng.* **55**, 161 (1984).

C. Andres, "Compressed Dehydrated Cabbage has 'Fresh' Color and Texture," *Food Process.* **37**, 93 (1977).

J. G. Brennan, *Food Dehydration, A Dictionary and Guide*, Technomic Publishing Co., Lancaster, Pa., 1994.

T. P. Labuza, S. R. Tannenbaum, and M. Karel, "Water Content and Stability of Low Moisture and Intermediate Moisture Foods," *Food Technol.* **24**, 543–550 (1970).

H. S. Lambrecht, "Sulfite Substitutes for the Prevention of Enzymatic Browning in Foods," C. Y. Lee and J. R. Whitaker, eds., *American Chemical Society Symposium Series 600*, Washington, D.C., 1995, pp. 313–323.

S. B. Lin, "Drying and Freeze-Drying of Vegetables," D. S. Smith et al., eds., *Processing Vegetables, Science and Technology*, Technomic Publishing Co, Lancaster, Pa., 1997.

G. M. Sapers, "Browning of Foods: Control by Sulfites, Antioxidants, and Other Means," *Food Technol.* **47**, 75–84 (1993).

L. P. Somogyi and B. S. Luh, "Vegetable Dehydration," in B. S. Luh and G. J. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988.

W. F. Talburt and O. Smith, *Potato Processing*, Van Nostrand Reinhold, New York, 1987.

S. L. Taylor and R. K. Bush, "Sulfites as Food Ingredients," *Food Technol.* **40**, 47–52 (1986).

W. B. Van Arsdel, M. J. Copley, and A. L. Morgan, *Food Dehydration*, 2nd ed., Vols. 1 and 2, AVI Publishing Co., Westport, Conn., 1973.

LASZLO P. SOMOGYI
Consulting Food Scientist
Kensington, California

VEGETABLE PROCESSING

The vegetable processing industry in the United States has made technological progress during the past decades. More computers are being used in the handling, processing, and marketing of processed vegetables (1). Increasing recognition of the importance of vegetables to human nutrition has stimulated greater consumer interest and the food industry in improving quality of processed vegetables.

Vegetable processing has greatly expanded the farm produce markets through conversion of perishable produce into various stable forms that can be stored and shipped to distant markets. Canning, freezing, dehydration, pickling, and freeze drying are the basic methods of preserving vegetables. The ability to lengthen the period of vegetables' availability in a preserved form that retains their nutritive value and palatability improves human health, adds variety to the human diet, and reduces the time for preparation. Equally important is that these gains have been achieved at low cost due to mechanization and automation of the processing methods. This article updates the recent developments in vegetable processing.

GENERAL PRINCIPLES

The preservation of vegetables in a stable form that can be stored and shipped to distant markets is a primary objective. The large quantity of canned vegetables preserved every year helps growers as well as consumers (2,3). Frozen vegetables can maintain better color, texture, and flavor after processing. Dehydrated vegetables, such as beans, corn, and soy beans, are also very important items.

Pickling is a good process for preserving cucumbers, olives, and cabbages. The principles of preserving vegetables by canning depends on killing the microorganisms in sealed containers (4). Frozen vegetables are preserved by freezing at −18°C or lower and storage of the product in containers to prevent moisture loss. Dehydrated vegetables are preserved by decreasing the moisture content so that no microorganisms can grow. Storage temperature (usually at 20°C or lower) and packaging materials are very important to the shelf life of canned vegetables. In the pickling of vegetables, lactic acid fermentation lowers the pH of the vegetable to 3.0 to 4.0 so that the products can be easily canned or dehydrated.

MICROBIAL SPOILAGE OF FOODS

There are several methods of preventing spoilage of foods. The most common method is to destroy the microorganisms in the food and prevent recontamination by microorganisms from the outside of the container. Canned vegetables are sterilized by heating the prepared product in vacuum-sealed containers in a pressure cooker. Due to the high pH value of most vegetables, usually above 4.5, it is necessary to heat the container under pressure at 116 to 121°C so that the heat-resistant bacterial spores are killed. The pH of fruits is usually below 4.5, and the vegetative non-spore-forming microbes present in fruits are readily killed in boiling water at 100°C (212°F). The details for the operation of the pressure cooker in vegetable canning were described by Luh and Kean (4). In general, the safety of canned vegetables on the market is under the strict control of the Food and Drug Administration, the Environmental Protection Agency, and the health authority in each state.

The second method of preventing food spoilage is to alter the environment so as to prevent or retard the growth of undesirable microorganisms. Frozen vegetables are preserved by preheating the prepared vegetable with steam or in boiling water sufficient to inactivate the undesirable enzymes, followed by cooling, packaging, and freezing. Rapid freezing of the packaged foods by low-temperature air blast is very important to prevent undesirable textural changes.

Dehydration is probably the oldest method used in food preservation. Removal of water from foods is usually accomplished by dehydration in a tunnel with hot air of low relative humidity, or by sun drying. It is very important to control the dehydration process so that undesirable oxidation catalyzed by polyphenolase, peroxidase, and lipoxygenase enzymes are prevented.

Preparation and handling of foods before canning, freezing, and dehydration may greatly affect the quality of the preserved foods. Packaging and postprocessing storage conditions are very important to the keeping quality of canned, frozen, and dehydrated foods. Vegetables are dehydrated for protection against spoilage by microorganisms by controlling the moisture content and water activity in the preserved foods. Large quantities of soybeans, corn, rice, and wheat are preserved by dehydration each year for export. Ray (5) published a book entitled *Fundamental Food Microbiology* that contains detailed information on food microbiology.

CHEMICAL AND BIOLOGICAL CHANGES

The most common chemical and biological change in foods that causes degradation in sensory quality is oxidation of unsaturated oil and fats and flavoring compounds. These oxidation reactions occur much more rapidly when catalyzed by lipase, lipoxygenase, and polyphenoloxidase when the tissues are damaged by mechanical impact or by physiological changes during postharvest storage. It is extremely important to destroy the enzymes by blanching with steam or in boiling water to inactivate the undesirable enzymatic deterioration during the canning and freezing processes (Table 1). Removal of molecular oxygen from a container of foods also retards oxidation.

A common form of texture breakdown in processed foods is related to the breakdown of the pectic materials in the

Table 1. Blanching Time for Vegetable Freezing

Vegetables	Min. at 100°C	Vegetables	Min. at 100°C
Artichokes	3–10	Kale	2
Asparagus	2–4	Mustard greens	2
Beans, green or wax	3	Okra	3–4
Beans, lima	2–4	Peas, black-eyed	2
Broccoli	3	Peas, green	1-1/2
Brussels sprouts	3–5	Peppers, green & red	2–3
Carrots (cut)	2	Rhubarb	1
Cauliflower	3	Spinach	1-1/2–2
Celery	3	Squash, summer	3
Collards	3	Turnip greens	2
Corn (cut)	4	Turnips	2
Corn on the cob	7–11		

Source. Ref. 6.

cell wall caused by pectic enzymes. Softening of pickled cucumbers and olives can be attributed to degradation of cell walls by pectin esterase and polygalacturase from microorganisms or by excessive heating in the presence of acid. In addition to quality attributes, such as flavor and color, the nutritive values of the processed foods must be maintained as much as possible. The use of sulfur dioxide in dried-fruit processing has been a common practice in preventing oxidation of ascorbic acid. Another advantage in the application of sulfur dioxide at 1,000- to 2,000-ppm levels in dehydrated vegetables is to prevent the Maillard type of browning reaction. Because of the allergic effect (irritation with respiration) that sulfur dioxide produces in some people, recent trends have been toward using other antioxidants to accomplish the maintenance of dehydrated vegetables (7–9). The ideal in developing a new processing technique or a new food product would involve preventing growth of microorganisms through control of moisture and water activity.

Freezing

Microorganisms will not grow in the frozen state. The key to holding the fresh flavor of frozen foods was not freezing per se, but rather proper inactivation of flavor-deteriorating enzymes by blanching before freezing (Table 1). The most common method is to test for any residual enzyme activity of peroxidase, catalase, or lipoxygenase in the blanched foods. Recent research interests of the frozen food industry includes searching for better blanching conditions to stop undesirable biochemical changes during processing and improving storage stability of frozen foods (6,10,11).

Dehydration

Removal of water from vegetables is accomplished primarily by dehydration in a tunnel with hot air of low relative humidity, or by sun drying (12). Microorganisms will not grow if the moisture content of the foods is reduced to a safety zone that varies with the chemical components of the food. The principle is to control the water activity of the final product, and also to retain the sensory quality and storage stability of the dehydrated product. The processor must control the moisture content and water activity (a_w) of the dehydrated product. Most foods will not spoil if the a_w is at 0.60 or lower. The limitation is dependent on the chemical components of the foods. For better keeping quality, dehydrated onions must be at 3 to 4% moisture content. Water activity is a measure of the free moisture in a product. It is determined by dividing water vapor pressure of the substance by the vapor pressure of pure water at the same temperature. The dehydrated vegetables should be packaged in containers that will prevent moisture, air, and light from entering the package. Use of sulfur dioxide at proper levels in dehydrated vegetables will inhibit the browning reaction, thus lengthening the shelf life of the product. Several methods of dehydration are used commercially, namely, forced hot air, drum drying, spray drying, vacuum drying, and freeze-drying (3,12). Use of continuous-belt convey and hot-air dryers has increased in recent years. Addition of sodium sulfite or metabisulfite to cut vegetables before dehydration has been a standard practice. In addition to improving storage stability of the dehydrated product, the presence of a small amount of sulfite in a blanched cut vegetable makes it possible to increase the drying temperature and thus shorten the drying time, increasing the production capacity of the dehydrator (12,13). More recently, use of sulfite in food dehydration has received considerable scrutiny throughout the food industry (8,9,12). Processors are looking for alternative methods for producing high-quality dehydrated vegetables. Lambrecht (7) reported that the family of erythorbates, erythorbic acid and sodium erythorbate, are steriosomers of the ascorbates and function in a similar manner to that of antioxidants. These compounds are reducing agents and are preferentially oxidized in foods, thus preventing or minimizing oxidative flavor and color deterioration. Erythorbates can prevent enzymatic browning in many products such as fruits, vegetables, and beverages. It is a prime requirement in any vegetable-dehydration process that no opportunity exist for the development of bacterial toxins by *Clostridium* spp. and other causative organisms that may be present. Such toxin formation is most likely to occur when a moist product, usually with some soil contamination, is given a heat treatment followed by prolonged holding under moderately warm conditions without access to air. Thorough washing and proper blanching are required to obtain a satisfactorily low level of microorganisms. The second requirement is the absence of pathogenic bacteria such as *Salmonella* and *Staphylococcus aureus*. The third requirement is maintenance of reasonably low general bacteriological content so that no undesirable odor or flavor develops in processing. The quality of the finished dehydrated product is reflected not only in its texture, flavor, and color but also in its ability to rehydrate readily. Storage temperature is very important to the keeping quality of dehydrated vegetables. Even though dehydrated vegetables will not spoil at room temperature, better-quality retention can be achieved by proper packaging and storage at temperatures lower than 20°C.

Pickling

Pickled food may be defined as a product to which an edible acid, for example, lactic or acetic acid in the form of vinegar, has been added. A fermented food is a food in which the acid is produced from the sugars in foods by the fermentation of lactic acid bacteria (14).

FREEZE DRYING

The freeze-drying process consists of removing moisture from foods in the frozen state by sublimation under high vacuum. The low temperature used in the process inhibits undesirable chemical and biochemical reactions and minimizes loss of aromatic compounds. The dried product is light in weight and can be stored in airtight containers for long periods without refrigeration.

The important steps in making freeze-dried vegetables include raw material selection, grading, size separation, washing, removal of undesirable parts, sorting, slicing, blanching, freezing, freeze drying, inspection, screening, packaging, and marketing.

Freeze drying is applicable to vegetables of high intrinsic value such as asparagus, mushroom, and parsley. For bell peppers and pimientos, a common commercial practice is to remove part of the water in the prepared raw produce in a hot-air dehydrator, followed by freeze drying. This two-stage method greatly reduces the cost of production without significant loss of quality in the finished product.

HARVESTING, HANDLING, AND STORING

Vegetables reach the peak of quality at a definite time, depending on the varietal characteristics, time of planting, location, temperature, soil type, available water, adequate fertilizer application, and cultural practices (15).

By the application of a formula that must be worked out for each variety, a succession of plantings of the same variety may be made at intervals to assure a continuous supply of raw produce during the season. The processing may be further extended by the use of early, midseason, and late varieties.

The growers as well as the processors have to know how to harvest their crops within a narrow time limit. This can be coordinated by the fieldpersons of the processors with the growers. In the United States, slightly more than half of the vegetable crops are processed. Beets, corn, cucumbers, green peas, potatoes, lima beans, potatoes, tomatoes, and several other crops are mechanically harvested.

Bulk bins are used for holding field-run vegetables until they can be transported to the processing plant and for holding precooled vegetables in refrigeration until processing is completed.

Each bulk bin can hold 25 to 27 field boxes of vegetables. When filled, the bins weigh about 1,000 lb. The bins are emptied by mechanical dumpers either onto inspection/grader belts or into washers.

PREPARING VEGETABLES FOR PROCESSING

The operations involved in processing vary somewhat with the type of vegetable and the method to be used, for example, canning, freezing, dehydration, freeze drying, or pickling. The general preparation procedures for vegetable freezing may be summarized as follows:

1. *Harvest at the optimum maturity stage before any portion becomes fibrous and tough.* Objective testing methods should be applied before harvest. The decision must be made by experienced field workers as to when and how to harvest. Vegetables at the peak of quality are highly perishable and should be harvested, transported, and processed promptly after harvest.
2. *Sorting and grading.* This operation removes diseased, insect-infested, and trash materials. This may involve a roller grader, air blower, rod shaker, or any mechanical device followed by sorting on conveyor belts.
3. *Washing.* Rinsing with water will remove dirt, insects, and small trash. Food-grade detergents may be used when washing vegetables taken from the soil such as potatoes, red beets, mechanically harvested tomatoes, and some leafy vegetables.
4. *Preparation such as peeling, shelling, trimming, cutting, and dicing.* Lye peeling of potatoes and mechanically harvested tomatoes has been a common practice. Due to greater restrictions on waste disposal, peeling waste that contains sodium hydroxide will pose a difficult problem with regard to disposal. There is a trend to return to steam peeling for mechanically harvested tomatoes.
5. *Blanching.* This is very important step to inactivate the enzymes present in the vegetables. The blanching process inactivates the enzymes that will cause discoloration and the formation of off-flavor and off-aroma during freezing storage and reduces the number of microorganisms. This process makes the product easier to pack into the container. The blanching process can also remove harsh flavors in collards, snap beans, and spinach for freezing preservation. The blanching time required for freezing some vegetables is presented in Table 1.

Test for Adequacy of Blanching

For the canning and freezing of vegetables, it is very important to have an adequate blanching procedure to avoid development of off-flavor and off-aroma in the product during postprocessing storage. Several enzymes, such as catalase, peroxidase, polyphenoloxidase, and lipoxygenase, are present in vegetable tissues. These enzymes should be properly controlled by blanching in order to produce a stable frozen vegetable. A simple method, but not quantitative in nature, is to add a drop of 0.5 to 3.0% hydrogen peroxide to the freshly cut surface of the blanched vegetable. The appearance of pinkish-orange color indicates the presence of peroxidase, whereas a perfusion of bubbles indicates the presence of oxidases.

Robinson (16) reviewed the literature on peroxidases in fruit and vegetables. Thermal inactivation of peroxidases in horseradish and other vegetables has been studied in detail. Although thermostable enzymes may represent only a small proportion of the total enzymatic activity in fresh foods, for blanched vegetables their effects on the quality may be quite substantial.

Peroxidase Assay

Peroxidase activity in green asparagus extract can be measured spectrophotometrically at 470 nm. The assay mixture contains 0.225 mL 0.3% H_2O_2, 0.225 mL 1% guaiacol in water, and 2.4 mL 0.2 M sodium acetate (pH 4.5). The reaction is initiated at 30°C after addition of 0.01 to 0.02 mL enzyme solution. A Perkin-Elmer spectrophotometer equipped with thermostatical cell compartment and recorder is used to monitor absorbance change with time at 470 nm. Enzyme activity was calculated from the linear portion of the curve. One unit of peroxidase activity is defined as 1 optical density/min at 470 nm.

The peroxidase paper test developed by the USDA Western Regional Research Laboratory, Albany, California, can be used by the frozen vegetable–processing industry for any residual peroxidase activity in the blanched vegetables.

Lipoxygenase Assay

Most assays for lipoxygenase employ the substrate, linoleic acid, with measurement of the disappearance of dissolved oxygen or by the increase in absorbance at 234 nm. In one assay, the bleaching of indigo carmine by the hydroperoxide reaction product was monitored. Lipoxygenase assay has been used by the industry with an intention of reducing the blanching time for preparing frozen vegetables.

PROCESSING

Canning

Important vegetables being preserved by canning are asparagus, beans, beets, carrots, corn, hominy, mushrooms, okra, olives, peas, pimientos, potatoes, spinach, whole tomatoes, tomato paste, and various other types of tomato products (4). Tin cans and glass bottles are used as containers for processed vegetables. The quantities of some selected canned vegetables in the United States (1977–1990) are presented in Table 2. Corn, beans, and olives were separated on a moving screen with different mesh sizes, or over differently spaced rollers. Separation into groups according to degree of ripeness or perfection of shape may also be done on conveyor belts. Green peas and lima beans are frequently separated into more or less mature portions by flotation in a salt solution, the operation being performed continuously in automatic equipment. Trimming, if necessary, is done by hand operators well trained in locating and removing blemishes. In many cases, vegetables are sliced, diced, halved, or peeled, usually by machines specially designed for each type of produce. In each of these steps, the raw vegetable is continuously inspected, and a final inspection is made to pick out mashed or broken pieces as well as any foreign matter that may have passed the cleaning, washing, and trimming operations.

Blanching

The prepared vegetables are immersed in hot water or exposed to live steam for a proper amount of time in an operation known as blanching. The process serves to inactivate enzymes and thus arrest changes in flavor, and to soften the product so that more may be filled into the container. Proper blanching removes the occluded gases in the tissue and reduces strain on the seam of the can during processing. Hot-water blanching causes loss of some nutrients and formation of large volumes of liquid waste. Microwave and hot-air blanching may also be used to minimize wastewater problems. The processor has to select the best blanching method for the particular type of vegetable. Lin and Schynes (18) studied the influence of blanching on the texture and color of 11 kinds of sterilized vegetables. They observed that low-temperature-long-time (LTLT) blanching significantly increased the firmness of seven kinds of canned vegetables, including carrot, endive, beetroot, leek, green bean, onion, and white cabbage. Among these, the texture of the canned carrot, endive, leek, and green bean was improved to a great extent. Analysis of variance revealed that LTLT treatment in solution containing calcium and zinc ions significantly affected the texture and color retention of sterilized green beans and endive. The reason for the improvement may be related to the activation of pectin–esterase enzyme during the blanching process, which enhances the firming action of demethylated pectin by calcium ions.

Filling

Metal or glass containers are conveyed to the point where they are filled with the prepared vegetables. Before being filled, the containers are cleaned by air blast.

Most filling is done by machine, although some vegetables such as asparagus may be put into containers by hand. The container is filled with the solid product and then usually topped with a liquid, which can be juice, as for whole peeled tomatoes; brine; or water that contains ingredients to improve the sensory quality of the processed foods. The choice of ingredients includes sucrose, high-fructose corn syrup, salt, organic acids, spices, and thickening agents. Sucrose is added to enhance the flavor of the product, to blend with other flavor notes, and to suppress the effects of undesirable compounds. A slightly sweet taste is desirable in some vegetables such as corn, green peas, red beets, and peeled tomatoes. Sucrose acts as a seasoning agent rather than as a sweetener in vegetable canning.

Brines are added to the cans during canning. If sucrose is incorporated into the brine, an interaction between the two ingredients may take place that has a beneficial effect on the flavor acceptance of the canned product. Canned and frozen vegetables are regulated by USDA and FDA standards (Table 3) that relate to the produce itself and all dry and liquid ingredients incorporated into the brine.

Vacuum in the Container

The purpose of keeping a vacuum in canned foods is to remove air so that the pressure inside the container follow-

Table 2. Quantity (United States pack—24/303 basis) of Selected Vegetables Canned from 1977 to 1990

Year	Asparagus	Beans, lima	Beans, snap	Beets (× 1,000 cases)	Carrots	Corn, sweet	Peas, green	Tomatoes[a]
1977	3,705	2,657	54,494	11,348	5,973	56,300	30,238	54,124
1978	3,382	3,356	57,121	12,834	6,609	57,907	25,269	49,241
1979	2,819	3,061	66,281	14,990	6,298	60,022	36,492	52,896
1980	2,535	2,833	59,689	11,322	5,084	50,574	30,056	53,096
1981	2,844	2,602	52,808	9,555	4,639	57,949	27,296	51,937
1982	2,727	2,275	48,832	11,034	6,222	60,522	24,790	42,968
1983	2,549	2,338	46,506	9,035	4,958	51,595	20,827	37,597
1984	2,928	2,316	52,089	9,292	4,832	56,789	23,312	43,170
1985	3,097	1,966	55,503	9,386	4,787	55,729	29,306	37,485
1986	3,154	1,738	48,939	9,042	4,321	55,090	20,919	38,438
1987	3,361	1,326	56,081	12,127	5,249	59,117	23,057	44,634
1988	3,846	1,267	46,610	8,519	4,505	50,831	12,494	50,626
1989	3,060	1,586	60,500	9,175	N.A.	65,437	24,336	N.A.
1990	N.A.	N A.	58,750	N.A.	N.A.	62,216	24,298	N.A.
Average	3,077	2,255	63,684	1,089	5,342	57,148	25,192	46,351
%	1.5	1.1	31.2	0.5	2.6	28 0	12.3	22.7

Source: Ref. 17.

[a] Excludes soups and baby foods.

ing heat treatment and cooling will be less than atmospheric. The vacuum helps to reduce strain on containers during heat treatment and cooling and minimizes the level of oxygen remaining in the headspace.

Vacuum in the container can be obtained by heating the filled container through a steam blancher for 5 to 7 min to 80 to 90°C, followed by sealing the container, while it is still hot. The alternative is to seal the can in a mechanical vacuum seamer at room temperature. Another method is to replace the air in the headspace with steam before double seaming. The steam in the headspace condenses after heat processing and thereby creates a vacuum.

The vacuum in the canned, cooled container will vary with the type of container, the depth of the headspace, and the type of product. Quality-assurance personnel should always check the vacuum, headspace, and drained weight according to USDA regulations.

Sealing

To seal the lids onto the flange of the metal can, a double seam is created by interlocking the curl of the lid and the flange of the can. Various types of closing machines (double seamers) can create the vacuum in the headspace either mechanically in a vacuum-sealing chamber or by steam flow before the sealing.

Glass containers are sealed under vacuum created mechanically or by steam flow. The bottles are sealed with a close-fitting cover of tin-plate or a threaded or lug cap. Sealing speeds of 800 to 1,000 bottles/min have been achieved for baby foods.

Heat Processing

Heat sterilization of the canned vegetables either in metal cans or glass jars applied after hermetic (airtight) sealing is called the *process*. During the process, microorganisms that can cause food spoilage are destroyed by heat in either stationary or rotary pressure cookers. The temperature and time of heat processing vary with the nature of the product and the size of the container (4,19). In Table 4, the time–temperature requirement for canning selected vegetables is presented. The calculated sterilizing values for heat processing some vegetable in commercial practices are presented in Table 5.

Acid products with pH values below 4.5 are readily preserved at the temperature of boiling water. The containers holding these products are processed in atmospheric steam or hot-water cookers. Vegetables and other low-acid foods require higher temperatures for sterilization in steam-tight pressure cookers (retorts or continuous pressure cookers) usually controlled by automatic devices. The size of the container is an important factor in determining the correct combination of time and temperature in processing. Obviously, heat will penetrate to the center of a small can more quickly than to the center of a large one. Because it is vital for canning processes to be exact for every size of container as well as for every type of product, precise information about the rate of heat penetration is required (Table 5).

Steam is usually used to sterilize canned foods. Improved methods of heat processing are being used extensively. These include continuous-agitating pressure cookers and hydrostatic cookers in which the necessary temperature is maintained by the pressure of the water column. Another new process is flame sterilization, in which the cans are exposed to direct flames while rotating rapidly. After being held for the time necessary to ensure sterilization, the cans are cooled by means of water sprays.

Cooling

Containers are cooled quickly to prevent overcooking. This may be done by adding water to the cooker under air pressure or by conveying containers from the cooker to a rotary cooler equipped with a cold-water spray. Cooling water should be treated with 1 to 2 ppm free chlorine to avoid recontamination of the canned foods.

Table 3. Processed Vegetables for which U.S. Standards of Grades Have Been Established

Asparagus, canned (2541)
Asparagus, frozen (381)
Beans, canned baked (6461)
Beans, canned dried (411)
Beans, green and wax, frozen (2321)
Beans, canned lima (471)
Beans, frozen lima (501)
Beans, frozen speckled butter (lima) (5241)
Beets, canned (521)
Broccoli, frozen (631)
Brussels sprouts, frozen (651)
Carrots, canned (671)
Carrots, frozen (701)
Cauliflower, frozen (721)
Chili sauce, canned (2191)
Corn, canned cream-style (851)
Corn on the cob, frozen (931)
Corn, canned whole-kernel (881)
Corn, frozen whole-kernel (911)
Hominy, canned (3281)
Leafy greens (other than spinach), frozen (1371)
Mushrooms, canned (1481)
Okra, canned (3331)
Okra, frozen (1511)
Okra and tomatoes (tomato and okra), canned (3421)
Olives, canned green (5441)
Olives, canned ripe (3751)
Onion rings, frozen breaded (4061)
Onions, canned (3041)
Peas, canned (2281)
Peas, frozen (3551)
Peas and carrots, canned (6201)
Peas and carrots, frozen (2501)
Peas (field and black-eyed), canned, (1641)
Peas (field and black-eyed), frozen (1661)
Peppers, frozen sweet (3001)
Pickles (1681)
Pimientos, canned (2681)
Pork and beans, canned (6441)
Potatoes, frozen French-fried (2391)
Potatoes, hash brown (6401)
Potatoes, canned white (1811)
Pumpkin (squash), canned (2741)
Sauerkraut, canned (2951)
Sauerkraut, bulk (3451)
Spinach, canned (1901)
Spinach, frozen (1921)
Squash, frozen cooked (1941)
Squash, canned summer-type (3581)
Squash, frozen summer-type (1961)
Succotash, canned (6001)
Succotash, frozen (2011)
Sweet potatoes, canned (2041)
Sweet potatoes, frozen (5001)
Tomatoes, canned (5161)
Tomato catsup (2101)
Tomato juice, canned (3621)
Tomato juice, concentrated (5201)
Tomato paste, canned (5041)
Tomato puree (tomato pulp), canned (5081)
Tomato sauce, canned (2371)
Turnip greens with turnips, frozen (3731)
Vegetables, frozen mixed (2131)

Note: Numbers in parentheses refer to the section in the Code of Federal Regulations (7 CFR 52) in which the standard is published.

Labeling and Casing

After the cooking, cooling, and drying operations, the containers are ready for labeling. Labeling machines apply glue and labels in one high-speed operation. The labeled cans or jars are conveyed to devices that pack them into shipping cartons.

Aseptic Processing and Packaging

In aseptic processing, the problems of chemical and quality changes resulting from the slow heat penetration inherent in an in-container process are avoided by sterilizing and cooling the food separately from the container. Presterilized containers are filled with the sterilized and cooled product and the containers are sealed in a sterile atmosphere with a sterile cover. Because aseptic processing is a continuous operation, the behavior of one part of the system can affect the overall performance of the entire system. As a result, numerous critical factors are associated with aseptic processing and packaging, often requiring automated control systems. The processor must consider the sterilization of the product, the processing equipment, sterilization of the packaging materials, and maintenance of sterile conditions throughout the aseptic system (21). Aseptically canned products are superior to retorted products in color, aroma, flavor, and nutrient retention.

Reuter (22) published a book entitled *Aseptic Packaging of Foods*. Aseptic processing of liquid with small particles (a few millimeters in size) and liquids with larger particles (15–25 mm in size) were treated differently. In liquids with small particles, plate and tubular heat exchangers are used for low-viscosity liquids in heating and cooling, whereas scraped-surface heat exchangers are used for high-viscosity liquids. There is a well-known process in which the carrier liquid of high viscosity is separately ultraheat treated by continuous method in a scraped-surface heat exchanger and the solid product is first sterilized in a discontinuous aseptic heater by direct steam injection. After sterilization, the two components are reunited aseptically, mixed, and then aseptically packaged. Because of the complicated process, this method still experiences only limited usage by the industry. Aseptic processing of tomato paste in bulk (50-gal or 300-gal containers) is practiced in commercial processing because of the improvement of the sensory quality of the product compared with those made by conventional heating and cooling methods.

SOME ILLUSTRATIVE SAMPLES OF VEGETABLE PROCESSING

Beet Canning

The annual pack of canned beets (*Beta vulgaris* L.) is 10 to 12 million cases in the United States (17). Beets for canning should have a uniform deep red color. Detroit Dark Red is the most important cultivar for canning. The beet-canning season starts in August and ends in the early part of November. The mechanical harvester travels along the row digging, cleaning, topping, and discharging beets into a truck ready for transport to the cannery. If storage is necessary, beets for canning should be stored in slatted bins in a cool, well-ventilated warehouse. Under proper conditions beets may be stored for several months.

Canning Process. After delivery to the cannery, beets are washed thoroughly in a rotary-drum spray washer. Beets are usually graded into three size classes by a Magnuson Shufflo beet sizer or other rod-and-reel sizer. They are peeled by a two-stage procedure in which they are first heated sufficiently to loosen the skin without overcooking the flesh and are then peeled in an abrasion peeler. The peeled beets are inspected and trimmed. Smaller beets, 46 mm or less in diameter, are canned whole; the medium sizes, between 46 and 70 mm, are sliced; and the large sizes, over 70 mm, are diced or cut into shoestring strips. The dices and julienne cuts are washed under sprays to

Table 3. Processed Vegetables for which U.S. Standards of Grades Have Been Established

Asparagus, canned (2541)
Asparagus, frozen (381)
Beans, canned baked (6461)
Beans, canned dried (411)
Beans, green and wax, frozen (2321)
Beans, canned lima (471)
Beans, frozen lima (501)
Beans, frozen speckled butter (lima) (5241)
Beets, canned (521)
Broccoli, frozen (631)
Brussels sprouts, frozen (651)
Carrots, canned (671)
Carrots, frozen (701)
Cauliflower, frozen (721)
Chili sauce, canned (2191)
Corn, canned cream-style (851)
Corn on the cob, frozen (931)
Corn, canned whole-kernel (881)
Corn, frozen whole-kernel (911)
Hominy, canned (3281)
Leafy greens (other than spinach), frozen (1371)
Mushrooms, canned (1481)
Okra, canned (3331)
Okra, frozen (1511)
Okra and tomatoes (tomato and okra), canned (3421)
Olives, canned green (5441)
Olives, canned ripe (3751)
Onion rings, frozen breaded (4061)
Onions, canned (3041)
Peas, canned (2281)
Peas, frozen (3551)
Peas and carrots, canned (6201)
Peas and carrots, frozen (2501)
Peas (field and black-eyed), canned, (1641)
Peas (field and black-eyed), frozen (1661)
Peppers, frozen sweet (3001)
Pickles (1681)
Pimientos, canned (2681)
Pork and beans, canned (6441)
Potatoes, frozen French-fried (2391)
Potatoes, hash brown (6401)
Potatoes, canned white (1811)
Pumpkin (squash), canned (2741)
Sauerkraut, canned (2951)
Sauerkraut, bulk (3451)
Spinach, canned (1901)
Spinach, frozen (1921)
Squash, frozen cooked (1941)
Squash, canned summer-type (3581)
Squash, frozen summer-type (1961)
Succotash, canned (6001)
Succotash, frozen (2011)
Sweet potatoes, canned (2041)
Sweet potatoes, frozen (5001)
Tomatoes, canned (5161)
Tomato catsup (2101)
Tomato juice, canned (3621)
Tomato juice, concentrated (5201)
Tomato paste, canned (5041)
Tomato puree (tomato pulp), canned (5081)
Tomato sauce, canned (2371)
Turnip greens with turnips, frozen (3731)
Vegetables, frozen mixed (2131)

Note: Numbers in parentheses refer to the section in the Code of Federal Regulations (7 CFR 52) in which the standard is published.

Labeling and Casing

After the cooking, cooling, and drying operations, the containers are ready for labeling. Labeling machines apply glue and labels in one high-speed operation. The labeled cans or jars are conveyed to devices that pack them into shipping cartons.

Aseptic Processing and Packaging

In aseptic processing, the problems of chemical and quality changes resulting from the slow heat penetration inherent in an in-container process are avoided by sterilizing and cooling the food separately from the container. Presterilized containers are filled with the sterilized and cooled product and the containers are sealed in a sterile atmosphere with a sterile cover. Because aseptic processing is a continuous operation, the behavior of one part of the system can affect the overall performance of the entire system. As a result, numerous critical factors are associated with aseptic processing and packaging, often requiring automated control systems. The processor must consider the sterilization of the product, the processing equipment, sterilization of the packaging materials, and maintenance of sterile conditions throughout the aseptic system (21). Aseptically canned products are superior to retorted products in color, aroma, flavor, and nutrient retention.

Reuter (22) published a book entitled *Aseptic Packaging of Foods*. Aseptic processing of liquid with small particles (a few millimeters in size) and liquids with larger particles (15–25 mm in size) were treated differently. In liquids with small particles, plate and tubular heat exchangers are used for low-viscosity liquids in heating and cooling, whereas scraped-surface heat exchangers are used for high-viscosity liquids. There is a well-known process in which the carrier liquid of high viscosity is separately ultraheat treated by continuous method in a scraped-surface heat exchanger and the solid product is first sterilized in a discontinuous aseptic heater by direct steam injection. After sterilization, the two components are reunited aseptically, mixed, and then aseptically packaged. Because of the complicated process, this method still experiences only limited usage by the industry. Aseptic processing of tomato paste in bulk (50-gal or 300-gal containers) is practiced in commercial processing because of the improvement of the sensory quality of the product compared with those made by conventional heating and cooling methods.

SOME ILLUSTRATIVE SAMPLES OF VEGETABLE PROCESSING

Beet Canning

The annual pack of canned beets (*Beta vulgaris* L.) is 10 to 12 million cases in the United States (17). Beets for canning should have a uniform deep red color. Detroit Dark Red is the most important cultivar for canning. The beet-canning season starts in August and ends in the early part of November. The mechanical harvester travels along the row digging, cleaning, topping, and discharging beets into a truck ready for transport to the cannery. If storage is necessary, beets for canning should be stored in slatted bins in a cool, well-ventilated warehouse. Under proper conditions beets may be stored for several months.

Canning Process. After delivery to the cannery, beets are washed thoroughly in a rotary-drum spray washer. Beets are usually graded into three size classes by a Magnuson Shufflo beet sizer or other rod-and-reel sizer. They are peeled by a two-stage procedure in which they are first heated sufficiently to loosen the skin without overcooking the flesh and are then peeled in an abrasion peeler. The peeled beets are inspected and trimmed. Smaller beets, 46 mm or less in diameter, are canned whole; the medium sizes, between 46 and 70 mm, are sliced; and the large sizes, over 70 mm, are diced or cut into shoestring strips. The dices and julienne cuts are washed under sprays to

Table 4. Time–Temperature Requirement for Canning Selected Vegetables

Vegetables	Can sizes	Fill weight (oz)	Initial tempreature (°F)	Retort time (min) 240°F	Retort time (min) 250°F
Asparagus cuts, in brine	300 × 409	11.0	120	27	17
Beans, green/waxy, whole/cuts, in brine	303 × 406	12.5	120	21	12
Beans, lima, succulent, in brine	303 × 406	11.0	120	46	23
Beets, whole/cuts, in brine	303 × 406	10.9	140	31	19
Carrots, whole/cuts, in brine	303 × 406	11.6	140	30	20
Carrots and peas, in brine	303 × 406	11.5	140	37	18
Corn, whole kernel, in brine	303 × 406	12.0	140	46	22
Mushrooms, sliced, in brine	300 × 400	9.0	140	—	31
Peas, green, succulent, in brine	303 × 406	11.5	140	38	19
Potatoes, white, whole, in brine	303 × 406	11.6	140	34	22
Spinach, in brine	303 × 406	12.6	140	71	55

Source: Ref. 20.

Table 5. Calculated Sterilizing Values (F_0) for Some Commercial Processes

Product	Can sizes	Approximate: calculated sterilizing value (F_0)
Asparagus	All	2–4
Green beans, brine packed	No. 2	3.5
Green beans, brine packed	No. 10	6
Chicken, boned	All	6–8
Corn, whole kernel, brine packed	No. 2	9
Corn, whole kernel, brine packed	No. 10	15
Cream-style corn	No. 2	5–6
Cream-style corn	No. 10	2.3
Dog food	No. 2	12
Dog food	No. 10	6
Mackerel in brine	301 × 411	2.9–3.6
Meat loaf	No. 2	6
Peas, brine packed	No. 2	7
Peas, brine packed	No. 10	11
Sausage, Vienna, in brine	Various	5
Chili corn carne	Various	6

Source: Courtesy of American Can Co.

remove fines. Beets are canned in enamel-lined cans to prevent bleaching of the color. Peeled beets should be handled in stainless-steel equipment. In some plants, beets are washed, heated in a high-pressure steam peeler for a short time, and then released to atmospheric pressure. The skins are loosened in this process and are removed in a rotary-drum washer under heavy sprays of water. They are then inspected, sorted, and trimmed.

Filling and Heat Processing. Sliced, diced, or julienne-cut beets are packed into cans by machine; whole beets are packed by hand or a hand-pack filler. A light brine is added with a small amount of added sucrose. The amount of sweetener added, together with the natural sugar contents of the beets, should result in a level of about 7% sugar in the beet pack. After brining, the cans are exhausted in steam, sealed, and heat processed in a retort. The cans are sealed by the steam-flow method. Beets in no. 303 cans are usually processed in a continuous-agitating Food Machinery and Chemical Corporation (FMC) retort at 115°C for 22 min and cooled in a two-stage continuous water cooler. No. 10 cans (1-gal can) are processed for 33 min at 115°C in the same type of retort and then cooled in water. Sliced beets packed in light syrup are processed at 115°C for 35 min in no. 303 jars and for 40 min in no. 2-1/2 jars.

Green Beans

Two types of green beans (*Phaseolus vulgaris* L.) are cultivated: bush and climbing, or pole, types. Most commercial cultivars are of the bush type because they can be machine harvested. The Blue Lake cultivar is preferred for canning or freezing.

Canning Process. After arrival at the factory, green beans are conveyed to size graders. These consist of revolving cylinders with slots of various diameters through which the beans fall onto conveyors that carry them to the shippers. The beans are separated into the following sizes: no. 0, 4.8 mm or less; no. 1, 4.8 to 5.8 mm; no. 2, 5.8 to 7.3 mm; no. 3, 7.3 to 8.3 mm; no. 4, 8.3 to 9.5 mm; no. 5, 9.5 to 10.7 mm; and no. 6, over 10.7 mm.

Snipping. After size grading, the beans are carried through snippers consisting of metal cylinders having narrow slots. As the cylinder revolves, the ends of the beans are caught in the slots, and fixed knives lying snugly against the lower surface of the cylinder cut off the tips and stems that have been caught in the slots.

Inspection. The snipped beans then pass over inspection belts, where defective beans are removed. Those that escape snipping are returned to the snipping machine again.

Cutting. Smaller beans (no. 1, 2, and 3) are canned whole. The larger ones are cut crosswise by machine into lengths of 25 to 28 mm for regular-cut beans. Some beans of smaller size are cut lengthwise after blanching; these are called French-cut beans. Cross-cut beans are cut before blanching. The cut beans are screened to remove fine fragments and short pieces, which are canned as a special small-cut pack.

Blanching. The cut beans and whole no. 1, 2, and 3 sizes are blanched for 1.5 to 2 min, usually in hot water at 82°C to give better-filled cans. Blanched whole beans may be returned to French-style cutters and cut into shoestring-shaped pieces.

Filling and Heat Processing. Cut blanched beans are packed to volume mechanically. A plunger beyond the filler automatically levels the fill of each can. The cans are then filled with hot water, and dry salt is added by a dispenser. The cans are steam exhausted for about 5 min and are sealed hot or are steam flow sealed. The 303, no. 1 tall and no. 10 cans are the most popular. The processing time for no. 2 (307 × 409) and smaller cans with initial temperature at 49°C are 22 min at 115°C and 12 min at 121°C; for no. 10 cans, 27 min at 115°C, and 15 min at 121°C. For French-cut green beans, the processing time for no. 2 cans (307 × 409) is 26 min at 115°C; for no. 10 cans (603 × 700) 36 min with the initial temperature at 49°C (4).

Frozen Green Beans. In 1988, the total pack of frozen green beans was 235,986,000 lb. The preparation procedures and varietal characteristics of green beans for freezing are similar to those for canned beans, except for the last step in the terminal end of the preservation (6,20).

Corn

Sweet corn (*Zea mays* L.) is one of the common vegetables being preserved by canning, freezing, and dehydration. In 1988, the pack of frozen cut corn was 423,399,000 lb and that of frozen corn on the cob was 345,869,000 lb. The important corn-canning states are Illinois, Minnesota, Wisconsin, Indiana, Iowa, Maine, Maryland, New York, Washington, and Michigan. The corn cultivars suitable for canning are Country Gentleman, Crosob, Golden Bantam, Golden Cream, Potters Excelsior, Stowell's Evergreen (white cultivars), and Yellow Evergreen. Golden Bantam was more popular before the hybrid cultivars came in general use. Yellow cultivars have large kernels that make an attractive pack. For canning, the corn should be young and tender and the ears well formed. Nearly all commercially grown corn is now from hybrid cultivars. These give increased yield; better uniformity; and excellent flavor, color, and canning quality. Examples of single crosses are Golden Cross Bantam, Ioana, and Golden Hybrid 2409.

Styles of Canned Corn. Canned corn is packed mostly as *Maine style* or *Maryland style*. Maine style is obtained by cutting through the kernels, scraping the remaining portions of the kernels from the cobs, and mixing the scrapings with the cut kernels. Water flavored with salt and sugar is added to give the desired consistency. Starch is also usually added. The canned product is creamier in texture and is the more popular of the two styles. It is also called *cream-style* corn. Maryland-style, or whole kernel, corn consists of the whole kernels cut from the cob and canned in brine; the cobs are not scraped. A third style, *double-cut* corn, is made from overmature corn or from large kernels. The corn is cut from the cob, and then the kernels are cut a second time with special knives to produce a cream-corn effect. *Brineless whole kernel corn*, vacuum sealed in cans, is now being favorably received.

Harvesting. Corn may be at best maturity for canning about 15 to 19 days after the appearance of the silk. The fieldperson uses the thumbnail test by noting the pressure necessary to break the hull of the kernel with the thumbnail. Experienced persons can judge maturity quite well based on the thumbnail test plus assessment of the appearance and feel of the ears. A pressure tester can also be used to test the toughness of the corn and hence its fitness for canning. The Succulometer measures the volume of liquid expressible from a weighed sample of kernels; other tests used are sugar content, starch content, tenderometer readings, and percentage of alcohol-insoluble matter. The FMC harvester cuts the cornstalks near ground level and elevates them to a mechanism that cuts the ears off the stalks. An additional machine separates the ears from the leaves and stalk. A corn harvester can harvest 10 to 12 acres/day.

Husking and Silking. Conveying systems are arranged so that the corn is handled mechanically throughout the entire canning process. The husking machine has a pair of rapidly revolving rubber or milled steel rolls that catch the husks and remove them. Modern huskers are equipped with two sets of rolls and are known as double huskers. The rolls in a husking machine are spaced so that an ear cannot pass between them; friction of the rolls against the ear loosens the husk, which is caught between the rolls and torn off. The rolls are slightly wider apart at the upper end, where the larger portion of the husks is removed. The rolls are mounted so that they can spread apart sufficiently to allow the husks to pass between them but are then returned instantly to their former position by powerful springs. They are kept wet with a spray of water during operation to prevent fouling. Fixed knives cut off both the butt and the silk ends. A hooklike device loosens and opens the husk on the ear before it reaches the rolls. The rolls then easily catch the husk and are apt to do a cleaner job of husking and removing the silks. The capacity of most single-husking machines is rated at 60 to 80 ears/min and of double machines at 120 to 160 ears/min (approximately 2 t/h). In some canneries, the husked ears are passed through a silking machine equipped with revolving brushes and rolls. The ears are carried forward through the brushes, which remove nearly all the silk and deliver the silked ears to the washing machine. Silking of the machine can be omitted if the work of the mechanical huskers and the trimmers is well done.

Sorting and Washing. In the first sorting, improperly husked ears are returned to the husking department, where the culls and trash are removed. In the second sorting, the ears may be sorted into A and B grades, the A grade being used for "fancy" and "choice" canned corn and the B grade for "standard" quality. The more mature ears are sorted out for cream-style corn. There are two satisfactory methods of washing husked ears: (1) by means of a silker-brusher washer, and (2) by means of a revolving cylinder or conical reel. The corn is passed under a heavy spray of water.

Canning Whole-Kernel Corn. In making whole-kernel corn, some canners blanch the husked ears in water near the boiling point to coagulate the juice before cutting. The ears are fed, small end first, through curved knives that accommodate themselves by means of springs to the size of the ear. The cut corn is inspected on a slowly moving belt and the unfit material discarded.

Cut whole-kernel corn is next passed through a shallow tank filled with water in which a paddle stirrer moves slowly. The kernels sink, and the floating trash is removed automatically. The kernels are then conveyed to the silking and washing reels (cob reels). As the reels revolve, the kernels fall through the screen to the product-collecting pan. From this point, they are pumped to a dewatering screen, and the dewatered corn is delivered to the filling machine.

Whole-kernel corn is heated by steam in a tubular heater on its way to the can filler and is filled hot. The cans are lined on the inside with C-enamel (corn enamel), which contains zinc oxide to prevent discoloration caused by formation of black FeS. The FMC 15-station filler, a gravity-type filling and brining machine, is commonly used. It is equipped with 15 telescopic product-measuring pockets mounted on a revolving turret. The pockets measure the exact required amount of product and discharge it through filling tunnels into cans. Cutoff plates automatically open and close the measuring pockets. The pockets rotate in succession under a supply hopper to receive their load. The hopper rotates and is equipped with an offset stud that agitates the corn and thus prevents bridging, ensuring uniform fill pockets and cans. Below the hopper is a special drain that drains away any water entering the hopper with the corn. The cans travel on supporting tracks that can be vibrated to settle the product in the cans. Filled cans travel from the filler to the briner. Hollow cylindrical measuring tubes within the brine tank separate a column of brine and fill it into the cans. Up to 350 cans/min can be filled and brined. The brines contains 2.0 to 2.7% salt and 6 to 8% sucrose.

The brine may contain as little as 1.5% sugar depending on the grade and the processor's standards. Recently, the amount of salt in the canned product may be reduced by 50 to 70% to suit the needs of the public. Cans are filled with brine at 46 to 49°C. Corn has also been canned in butter and cheese sauce to improve the flavor acceptance. To each no. 2 can, 383 to 400 g of whole-kernel corn is added. The filled cans pass beneath a topper, which is a leveling plunger that automatically gives the proper headspace in the can. In many plants, the cans are sealed in a steam-flow (or steam-vac) sealer. In some plants they are well exhausted in a steam-exhaust box and sealed hot. The process time for canned whole-kernel corn in no. 2 cans (303 × 409) is 46 min at 115°C or 22 min at 121°C, provided that the initial temperature is at 60°C. For no. 10 cans (603 × 700) the process time is 81 min at 115°C and 43 min at 121°C.

Frozen Corn. The harvest and preparation procedures for frozen corn are similar to those for canned corn, except for the last terminal step in freezing. Yellow corn cultivars such as Golden Cross Bantam are usually preferred for freezing. Sweet corn loses its quality rapidly after harvest and should be frozen within a few hours of harvest. Blanched whole-kernel corn is produced in a number of ways: (*1*) the corn may be completely blanched (7–11 min) on the cob before cutting; (*2*) it may be partially blanched on the cob to set the milk, then cut and blanched again; or (*3*) it may be cut and subsequently blanched. This last practice results in loss of flavor, lower yield, and microbiological problems. The split blanch is recommended to keep the bacterial counts at a minimum. After the corn is cut, the kernels must be cleaned to remove bits of kernels; husk; silk; light fiber; and dry, immature kernels. Both froth-washing and brine-flotation graders are excellent for this purpose. The corn kernels are removed at the sink or discharge end while the trash floats off the top discharge or is picked up by a rotating reel (6).

Whole-kernel corn is usually individually quick-frozen and either packaged directly into cartons and polyethylene bags or bulk stored. A fluidized freezing process can be used for both cut corn or cobbed corn.

Green Peas

The U.S. pack of canned peas was 24,298,000 cases (24 × no. 303 cans) in 1990 (Table 2). The pack of frozen green peas was 358,779,000 lb in 1988. There appears to be a gradual shifting of canned peas to frozen peas in the past few years. Wisconsin, New York, Minnesota, Illinois, Washington, and Oregon are important pea-producing states. Peas (*Pisum sativum* L.) are grown as a field crop by tractor cultivation. The most important cultivar is Alaska; the most widely used wrinkle-seeded cultivars are Perfection, Horsford Market Garden, Advancer, and Admiral. Alaska is a favorite in the eastern United States because it is a more reliable producer than most wrinkled cultivars. Popular cultivars in the Midwest are Early Perfection, Prince of Wales, and Green Giant s-537. In Oregon and Washington, Perfection is the principal cultivar used for canning. The words *smooth* and *wrinkled* apply to the ripe, dry peas; before drying, all are smooth.

Green Pea Canning. Green peas are harvested and vined at vining stations in the field, thereby avoiding long haulage of the vines. The correct time for harvesting is determined by the appearance of the pods, which should be swollen and well filled with young tender peas. Most canners use the tenderometer to determine maturity and grade of peas.

The shelled peas are collected at the viners in bins of 45.5-kg capacity. Normally no more than 4 h elapse between vining in the field and canning of the peas. The shelled peas are emptied onto a conveyor that moves them to a broad vibrating screen. The peas drop through the screen, but leaves, clods, pods, and other trash pass over the end of the screen. When peas are vined at the cannery, the shelled peas go directly from the viner over the scalper screen. After screening, the peas are cleaned in a clipper cleaner, which operates on the same principle as a grain-cleaning mill. A strong blast of air from a fan blows light trash away, and screens remove coarser waste material. The peas fall through the screen and are conveyed into stainless-steel tanks of water to which has been added a special soap in the form of a paste and a highly refined mineral oil. The separation tank is in the form of two cones, one inverted and the other upright, with a cylindrical portion between the cones. A special pump violently aerates and circulates the liquid, converting it into a frothy mixture. As the peas drop to the bottom of the lower cone, they are picked up by a pump and delivered to a squirrel-cage spray washer in which any adhering trash is thoroughly removed. The trash flows to the surface and overflows from the upper cone. Cleaned, shelled peas are size graded in a slowly revolving screen cylinder or through a series of nested or parallel screens; in either case the screens have holes of varying diameters.

Blanching. Hot-water blanching is done in a pea blancher of special design for 3 min at 88 to 93°C. If the peas are fairly mature, the blanching time may be increased to 4 to 5 min; young tender peas are blanched for 1.5 min. Water for blanching should be soft because the calcium in hard water will harden the peas. A continuous fluidized-bed steam-blanching process for peas can be used. Steam blanching for over 30 s prepares peas adequately for canning. However, steam blanching does not have as great a cleansing and plumping action as water blanching.

Quality Grading and Sorting. Only the largest peas of any cultivar need to be quality graded. After the blanched peas are cooled, the larger ones are fed into a brine of 38 to 40° Salometer (9.5–10.0% salt). The younger tender peas are lower in density than the riper peas and will float in brine of this concentration. The more mature grade B peas will sink; they are removed from the bottom of the brine separator continuously and are pumped or flumed to the next operation. The lighter grade A peas are carried over into the overflow and conveyed to the next stage. Both classes are thoroughly washed immediately after brine separation to remove the adhering brine.

Filling and Heat Processing. The sorted peas are fed by gravity to cone-shaped hoppers on the can-filling machine. The peas fall into the cups of the automatic rotary can filler. Each cup delivers exactly the desired volume of peas into a can. The cans are carried to the briner. The cans for peas are usually enameled with C-enamel to prevent blackening of the tinplate due to FeS formation. The brine for canned peas usually contains sugar as well as salt. The composition of the brine differs considerably according to the preference of the distributors and canners and the maturity of the peas. As less-mature peas are sweeter, they need less sugar in the brine. The brine is added at 71°C by adjustable cups. Filled cans are sealed under steam flow by the steam-vac procedure. The recommended heat-process time for peas in no. 2 cans (303 × 409) is 31 min at 115°C, or 15 min at 121°C; for no. 10 cans, 48 min at 115°C or 21 min at 121°C, provided that the initial temperature is at 60°C. Canned peas should be cooled rapidly following sterilization to avoid excessive softening and the formation of a cloudy brine through gelatinization of starch.

Frozen Green Peas. The procedures for freezing green peas are similar to those for canned peas except for the step of freezing preservation (6).

TRENDS IN VEGETABLE-PROCESSING DEVELOPMENT

1. Studies on plant genetics of vegetables is becoming a very important topic. The objectives are to improve the varietal characteristics of the vegetable seeds regarding yield, flavor, quality, texture, resistance to pathogens, and adaptability to stress. Tailoring genes for crop improvement and genetic engineering of plants are attracting more horticultural scientists to pursue further studies on vegetable improvement.
2. Value-added vegetable products have been of great research interest to various research institutes, university and agricultural experiment stations, and the biotechnology industries. Products such as vegetable juices, sauces, soups, and pickled vegetables also attract more attention within industrial research programs. The success of the vegetable industry depends on quality control and sanitation departments to keep up the high levels of quality (23) and nutrition demanded by the consumer as well as the government. The ultimate goal is to have processed vegetables with good storage life, quality retention, nutritive value, and safety for the consumer. Use of computers for process control and record is now a must for modern vegetable processors.

BIBLIOGRAPHY

1. A. Przybyla, "Computers in Food Processing," in B. S. Luh and J. G. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988, pp. 741–764.
2. J. M. Connor, *Food Processing—An Industrial Powerhouse in Transition*, D. C. Heath and Company, Lexington, Mass., 1988.
3. D. S. Smith et al., "Processing Vegetables," in D. S. Smith, eds., *Science and Technology*, Technomic Publishing Co., Lancaster, Pa., 1997.
4. B. S. Luh and C. E. Kean, "Canning of Vegetables," in B. S. Luh and J G. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988, pp. 195–285.
5. B. Ray, *Fundamental Food Microbiology*, CRC Press, Boca Raton, Fla., 1996.
6. B. S. Luh and M. C. Lorenzo, "Freezing of Vegetables," in B. S. Luh and J. G. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988, pp 343–386.
7. H. S. Lambrecht, "Sulfite Substitutes for the Prevention of Enzymatic Browning in Foods," in C. Y. Lee and J. R. Whitaker, eds., *Enzymatic Browning and Its Prevention*, pp. 313–323, ACS Symposium Series 600, American Chemical Society, Washington, D.C., 1995, pp. 313–323

8. S. J. Pintauro, S. L. Taylor and C. O. Chichester, A review of the safety of sulfites as food additives, The Nutrition Foundation Status Report, The Nutrition Foundation, Inc., Washington, DC, 1983.
9. S. L. Taylor and R. K. Bush, "Sulfites as Food Ingredients," *Food Technol.* **40**, 47–52 (1986).
10. D. P. Judge, *The Almanac of the Canning, Freezing, Preserving Industries*, 78th ed., Edward E. Judge and Sons, Inc., Westminster, Md., 1995.
11. C. P. Mallett, *Frozen Food Technology*, Blackie Academic and Professional, London, 1993.
12. L. P. Somogyi and B. S. Luh, "Vegetable Dehydration," in B. S. Luh and J. G. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988, pp. 387–473.
13. W. F. Talburt and O. Smith, *Potato Processing*, 4th ed., Van Nostrand Reinhold, New York, 1987.
14. B. S. Luh and J. G. Woodroof, *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988.
15. J. G. Woodroof, "Harvesting, Handling and Storing Vegetables," in B. S. Luh and J. G. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988, pp. 135–174.
16. D. S. Robinson, "Peroxidases and Their Significance in Fruits and Vegetables," in P. F. Fox, ed., *Food Enzymology*, Vol. 1, Elsevier Applied Science, London, 1991, pp. 399–426.
17. *Agricultural Statistics*, U.S. Department of Agriculture, Washington, D.C., 1992.
18. Z. M. Lin and E. Schyens, "Influence of Blanching Treatments on the Texture and Color of Sterilized Vegetables," in *Symp. Second International Conference Food Science and Technology*, Wuxi Institute of Light Industry, Wuxi, China, 1994.
19. D. Downing, *A Complete Course in Canning and Related Processes*, 13th ed., Book III, *Processing Procedures for Canned Food Products*, The Canning Trade Inc., Timonium, Md., 1996.
20. A. Lopez, "Canning of Vegetables," in *A Complete Course in Canning*, Book III, 12th ed., The Canning Trade Inc., Baltimore, Md., 1987.
21. *Canned Foods—Principles of Thermal Process Control, Acidification and Container Closure Evaluation*, 5th ed., The Food Processors Institute, Washington, D.C., 1988.
22. H. Reuter, *Aseptic Packaging of Food*, Technomic Publishing Co., Inc., Lancaster, Pa., 1988.
23. H. Patino and B. S. Luh, "Quality Control," in B. S. Luh and J. G. Woodroof, eds., *Commercial Vegetable Processing*, 2nd ed., Van Nostrand Reinhold, New York, 1988, pp. 561–609.

B. S. Luh
University of California
Davis, California

VEGETABLE PRODUCTION

The focus of this article is on the commercial production of vegetables for sale. Commercial producers range from those growers that operate on a very limited scale to those with thousands of hectares in several locations. Current data on production and consumption are provided and the contributions of vegetables to human health discussed. The utilization of genetic resources in concert with various environmental situations to optimize production is considered along with environmentally sensitive pest-management strategies. Harvest and postharvest handling practices that provide for timely delivery of high-quality, nutritious products to consumers are included.

DEFINITION

Vegetables are so diverse (Fig. 1) and their use so ubiquitous that it is difficult to contrive a definition that will not have exceptions. One authority (1) proclaims in desperation, "The terms are impossible of close definition because the plants that fall within their scopes are so various. The best definition is an enumeration of the plants." One attempt at defining vegetable has been proposed by Maynard (2): " . . . a vegetable is a herbaceous plant, some parts of which may be eaten. Plant parts usually eaten, and representative vegetables, include roots (beet, carrot, turnip); stems (asparagus, kohlrabi); tubers (potato); bulbs (leek, onion); leaf petioles (celery, rhubarb); entire leaves (cabbage, lettuce, spinach); flower parts (broccoli, cauliflower); immature fruit (cucumber, snowpea, summer squash); and mature fruit (cantaloupe, tomato, winter squash)." A more complete listing of vegetables with designated parts used for food is shown in Table 1. The foregoing definition, although quite encompassing, excludes one important vegetable—mushroom—which is not herbaceous. The pileus (cap) and stipe (stalk) are the edible portions of the common mushroom.

CLASSIFICATION OF VEGETABLES

Vegetables, because of their diversity, can be classified in many ways for the convenience of the grower, crop adviser, shipper, or marketer. One consideration for classification, based on plant part consumed, has already been illustrated in Table 1. Another classification scheme (Table 2) is based on familial relationships that focus on characteristics of the flower. The appearance of the plant may be

Figure 1. Vegetable display at Hunt's Point Wholesale Market in New York City.

Table 1. Vegetable Plant Parts Used as Food

Below-ground structures		Above-ground structures		
Bulb	*Root*	*Leaf*	*Leaf petiole*	*Immature fruit*
Garlic	Beet	Cabbage	Celery	Cucumber
Leek	Carrot	Chard	Rhubarb	Eggplant
Onion	Celeriac	Chicory		Okra
Shallot	Horseradish	Chinese cabbage	*Stem*	Snap bean
	Jicama	Chive		Snowpea
Corm	Parsnip	Collards	Asparagus	Summer squash
Taro	Rutabaga	Endive	Kohlrabi	
	Sweet potato	Kale		*Mature fruit*
Rhizome	Turnip	Lettuce	*Flower bud*	
		Mustard	Broccoli	Cantaloupe
Ginger		Parsley	Cauliflower	Chayote
		Spinach	Globe artichoke	Pepper
Tuber				Pumpkin
			Immature seed	Tomato
Jerusalem artichoke				Watermelon
Potato			Lima bean	Winter squash
Yam			Pea	
			Sweet corn	

drastically altered by environmental extremes, but the structure of the flower remains unchanged, making it a constant factor for classification purposes. The principal vegetables of commerce in developed temperate regions are found in a relative handful of plant families. The most important of these are Solanaceae (potato, tomato, pepper), Brassicaceae (cabbage, Chinese cabbage, broccoli), Cucurbitaceae (cucumber, squash, watermelon), Alliaceae (onion, garlic), and Fabaceae (peas, beans).

Even though commercial vegetable production is realized in a relatively small number of plant families, the potential is enormous. Kays and Silva Dias (3) list an astounding 389 species in 69 families as vegetables (not necessarily on a commercial basis) cultivated in the world. In China alone, Chen, Zhu, Fu, and Wang (4) report that 177 species in 65 families may be grown as vegetables, but only about 20% of these are now grown commercially. Clearly, the potential for diversity in the diet is available if demand can be generated and production practices developed. Aside from their familial relationships, related vegetables may have similar growth habits that make for common cultural practices, for example, the vining crops cantaloupe, squash, and watermelon of the gourd family have similar spatial requirements. Similarly, tomato, pepper, and eggplant in the nightshade family grow best in a long, warm, frost-free season. Hazards to crop production such as plant diseases and insects may find common hosts within a family, for example, similar pests attack a number of vegetables within the mustard family.

PRODUCTION STATISTICS

United States

The current status of the commercially important vegetables in the United States (5) is shown in Table 3. The area (1000 ha) harvested, production per ha (t) and in total (1000 t), and value per ha (U.S. dollars) and in total (million U.S. dollars) is shown for each vegetable. Eggplant, escarole/endive, and brussels sprouts are grown on the smallest area whereas dry bean, potato, and sweet corn are grown on the largest area. Production per hectare is lowest for dry pea, asparagus, and lima bean and highest for celery, tomato, and onion. Total production varies on the low side with escarole/endive, brussels sprouts, and eggplant to potato, tomato, and sweet corn on the high side. Crop value per hectare is low for dry pea, dry bean, and lima bean and high for celery, bell pepper, and artichoke. On a total value basis beet, escarole/endive, and eggplant are lowest while potato, tomato, and lettuce are highest. Overall, vegetables were harvested from almost 3 million ha that produced over 56 million t and had a farm value exceeding $13 billion U.S. in 1997. Accordingly, the U.S. vegetable industry has a very great economic impact on the country.

The localization of the U.S. industry (5) is illustrated in Figure 2. By any standard, California is the most important state for vegetable production for fresh and processing use. California is favored by a variety of climatic niches, from cool coastal areas to warm interior valleys, and changing seasons related to the extreme south–north situation of the state. For example, the Mediterranean climate necessary for successful artichoke production is found in coastal areas south of San Francisco whereas the long, warm season that favors tomato production is found in the vast Central Valley.

Florida, Georgia, Arizona, and Texas are major contributors to the fresh-produce industry. Production in these states occurs primarily in the "off-season" fall, winter, and spring months when local production is not possible in the northeastern and Midwest population centers.

For processing, Wisconsin, Minnesota, Washington, and Oregon are important producing states. High yields, essential for profitability of processing crops, are favored by the long days of summer in these northern states.

Table 2. Common Vegetable Family Groupings

Family	Common name	Vegetables
Aizoaceae	Carpetweed family	New Zealand spinach
Alliaceae	Onion family	Leek, shallot, onion, garlic, chive
Apiaceae	Carrot family	Celery, carrot, parsnip, parsley
Araceae	Arum family	Taro
Asteraceae	Sunflower family	Endive, chicory, cardoon, globe artichoke, lettuce, Jerusalem artichoke, salsify
Brassicaceae	Mustard family	Horseradish, mustard, kale, rutabaga, collards, cauliflower, cabbage, brussels sprouts, kohlrabi, broccoli, Chinese cabbage, turnip, radish
Cactaceae	Cactus family	Prickly pear
Chenopodiaceae	Goosefoot family	Chard, garden beet, spinach
Convolvulaceae	Bindweed family	Sweet potato
Cucurbitaceae	Gourd family	Watermelon, cantaloupe, cucumber, pumpkin, gourd, squash, chayote
Dioscoreaceae	Yam family	Yam
Fabaceae	Pea family	Jicama, lima bean, French bean, pea, gram, southern pea
Liliaceae	Lily family	Asparagus
Malvaceae	Mallow family	Okra
Polygonaceae	Buckwheat family	Rhubarb
Poaceae	Grass family	Sweet corn
Solanaceae	Nightshade family	Pepper, tomato, eggplant, sweet pepino, potato
Zingiberaceae	Ginger family	Ginger

Table 3. U.S. Vegetable Industry—Area, Production, and Value (1997)

		Production		Value	
Vegetable	Area harvested (1000 ha)	Ha (t)	Total (1,000 t)	Ha (USD)	Total (million USD)
Artichoke[a]	3.68	10.66	39.24	18,375	67.62
Asparagus[b]	29.76	3.02	89.77	6,089	181.22
Bean, dry[b]	787.32	5.95	132.22	745	586.45
Bean, lima[b]	22.96	3.31	76.00	1,740	39.96
Bean, snap[b]	111.51	7.51	836.88	2,566	286.13
Beet[c]	3.02	36.70	110.84	2,695	8.14
Broccoli[b]	54.31	14.46	785.40	9,124	495.52
Brussels sprouts[a]	1.70	16.81	28.58	15,765	26.80
Cabbage[b]	34.53	40.81	1,409.24	8,329	287.59
Carrot[b]	48.43	41.80	2,024.29	9,883	478.64
Cauliflower[b]	19.18	15.33	294.06	10,321	197.96
Celery[a]	10.90	75.16	819.28	25,087	273.45
Corn, sweet[b]	278.03	14.53	4,039.60	2324	646.12
Cucumber[b]	64.60	16.39	1,058.63	5128	331.24
Eggplant[a]	1.05	31.89	33.48	16,724	17.56
Escarole/Endive[a]	1.26	20.02	25.22	10,413	13.12
Garlic[a]	14.97	16.82	251.74	17,470	261.52
Lettuce[a]	112.37	35.14	3,949.07	14,271	1,603.63
Melons					
Cantaloupe[a]	46.04	23.21	1,068.48	9,076	417.86
Honeydew[a]	11.90	22.09	262.86	9,192	109.39
Watermelon[a]	74.71	24.73	1,847.66	4,139	309.23
Mushroom[b]	–	–	352.61	–	730.28
Onion[a]	62.70	46.22	2,897.69	10,342	648.44
Pea, dry[b]	148.68	2.06	306.59	289	43.02
Pea, green[b]	108.82	3.97	431.77	1,259	137.00
Pepper, bell[a]	26.57	28.63	760.81	18,916	502.60
Potato[b]	536.83	38.86	20,857.01	4,855	2,604.19
Spinach[b]	13.98	15.71	219.62	5,323	74.41
Sweet potato[b]	33.74	18.17	612.77	6,327	213.49
Tomato[b]	165.41	65.06	10,762.03	11,198	1,852.19
Total	*2,828.96*		*56,383.44*		*13,444.77*

[a] Includes product for the fresh market only.

[b] Includes product for the fresh and processing markets.

[c] Includes product for the processing market only.

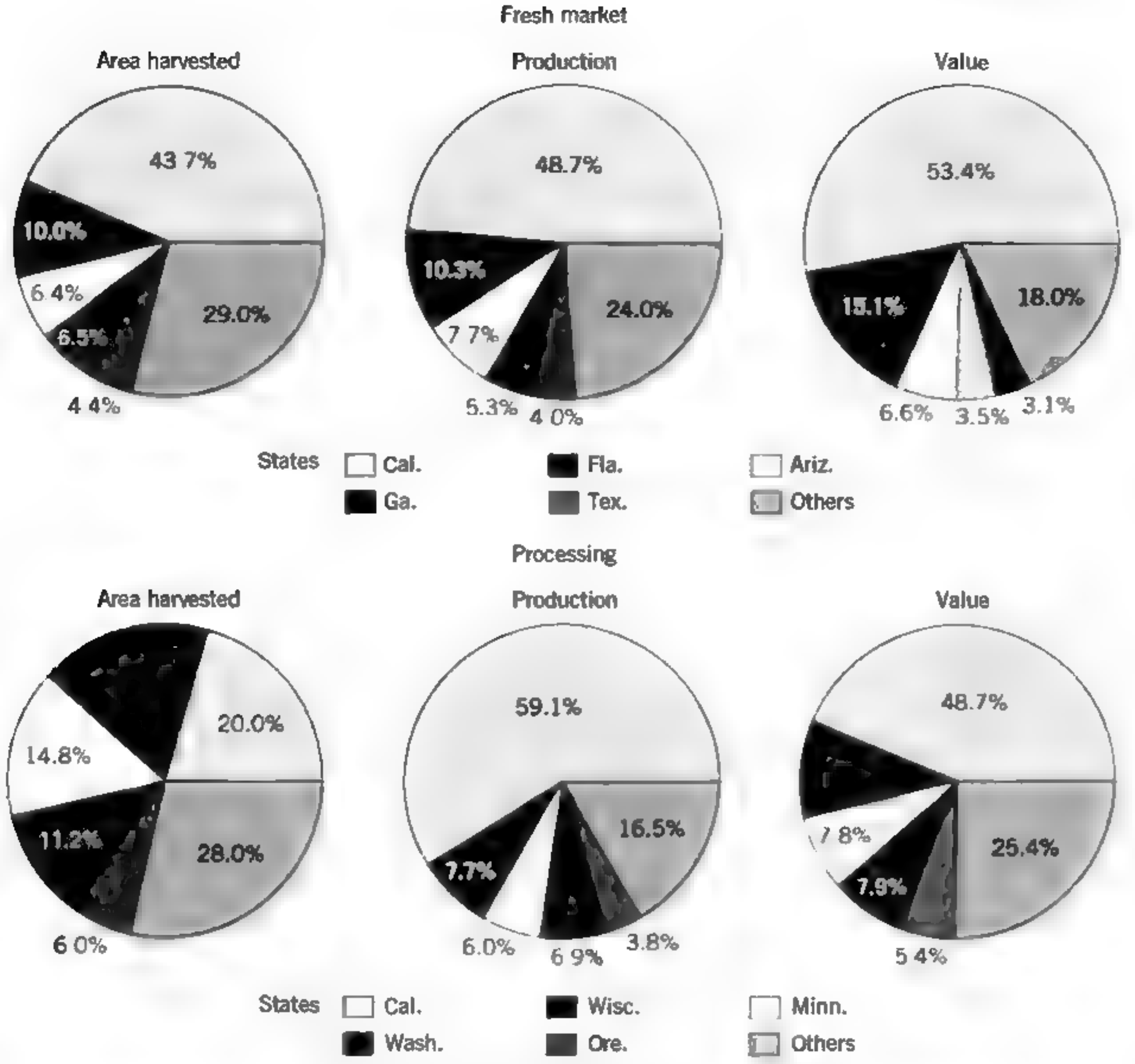


Figure 2. Important U.S. vegetable-producing states, 1997.

World

The area harvested (1,000 ha), yield (kg/ha), and total production (1,000 t) of the world's most important vegetables (6) are shown in Table 4. In terms of area harvested, dry bean, potato, and cassava are the most important crops, whereas artichoke, green bean, and cauliflower are relatively unimportant. Highest yields are obtained from tomato, cabbage, and carrot. The dry legumes—bean, chickpea, and lentil—produce the lowest yields per hectare because of their low moisture content. Total world production is highest for potato, sweet potato, and cassava—the root crops that provide massive amounts of energy and can be stored for extended periods to provide foodstuff when other foods are unavailable. Vegetables entering into commerce on a global basis are harvested from over 118 million ha and produce more than 1 billion t. Because much of the population in developing countries is engaged in subsistence farming where farm produce, including vegetables, does not enter into traditional commerce, it is clear that total worldwide production is much greater than that reported.

The ranking of the primary countries in production (t) of vegetables (6) is shown in Table 5. This listing is related to the size of the country, its population, presence of a favorable climate, and ethnic culinary habits of the population. Even a casual examination of Table 5 will reveal the overwhelming importance of China (Fig. 3) as a vegetable-producing country. Of 25 vegetables or vegetable groups listed, China ranks first in 15. India, the next most frequent listing, appears four times as the leading producer. According to data developed by the Chinese Ministry of Agricultural Statistics and quoted by Chen and Liu (7), the 21 major vegetables in China were grown on 4,365,000 ha, which produced more than 142 million t of vegetables in 1991.

Table 4. World Vegetable Production—Principal Vegetables (1996)

Vegetable	Area harvested (1,000 ha)	Yield (kg/ha)	Production (1000 t)
Artichoke	106	10,836	1,150
Cabbage	1,974	23,632	46,656
Cantaloupe and other melons	994	16,283	16,190
Carrot	770	21,396	16,477
Cassava	16,322	9,983	162,942
Cauliflower	681	18,681	12,725
Chickpea	12,009	742	8,908
Chilies and pepper, green	1271	11,064	14,068
Cucumber and gherkin	1424	16,182	23,051
Dry bean	27,470	679	18,639
Dry broad bean	2,355	1,499	3,531
Dry pea	6,515	1,680	10,945
Eggplant	722	16,589	11,981
Garlic	986	10,549	10,401
Green bean	491	7,376	3,620
Green pea	806	6,467	5,214
Lentil	3,389	832	2,819
Onion, dry	2204	16,174	35,644
Potato	18,353	16,065	294,834
Pumpkin, squash, and gourd	768	12,789	9,822
Sweet potato	9,156	14,662	134,244
Taro	1,016	5,650	5,739
Tomato	3,094	27,435	84,873
Watermelon	2,393	16,601	39,725
Yam	3,173	10,435	33,110
Total	*118,442*	–	*1,007,308*

Figure 3. A retail vegetable display in Shanghai, China.

VEGETABLE CONSUMPTION

Estimated per capita consumption of commercially produced vegetables in the United States in 1997 (8) is shown in Table 6. Total consumption varies from 0.2 kg each for artichoke, eggplant, and dry pea to 64.7 kg for potato. Tomato at 41.7 kg is the second most popular vegetable in the United States. Substantial quantities of sweet corn and lettuce are also consumed. The large number of distinct vegetables for which data are available is indicative of the diversity available to American consumers. In addition, the "other vegetable" category accounts for several additional vegetables. Total U.S. per capita consumption

Table 5. World Vegetable Production—Leading Countries (1996)

Vegetable	Rank 1	2	3	4	5
Artichoke	Italy	Spain	Argentina	France	United States
Cabbage	China	Russian Fed.	India	Korea Rep.	Japan
Cantaloupe and other melons	China	Turkey	Iran	United States	Spain
Carrot	China	United States	Russian Fed.	Poland	United Kingdom
Cassava	Nigeria	Brazil	Zaire	Thailand	Indonesia
Cauliflower	India	China	France	Italy	United Kingdom
Chickpea	India	Turkey	Pakistan	Iran	Australia
Chilies and peppers, green	China	Turkey	Nigeria	Mexico	Spain
Cucumber and gherkin	China	Iran	Turkey	United States	Japan
Dry bean	India	Brazil	China	Mexico	United States
Dry broad bean	China	Egypt	Ethiopia	Morocco	Italy
Dry pea	France	Ukraine	Canada	China	Russian Fed.
Eggplant	China	Turkey	Japan	Egypt	Italy
Garlic	China	Korea Rep.	India	United States	Spain
Green bean	China	Turkey	Indonesia	Spain	Italy
Green pea	United States	China	France	United Kingdom	India
Lentil	India	Turkey	Canada	Bangladesh	Syria
Onion, dry	China	India	United States	Turkey	Japan
Potato	China	Russian Fed.	United States	Poland	Ukraine
Pumpkin, squash, and gourd	China	Ukraine	Turkey	Mexico	Egypt
Sweet potato	China	Indonesia	Uganda	Vietnam	Rwanda
Taro	Ghana	China	Nigeria	Ivory Coast	Japan
Tomato	China	United States	Turkey	Italy	Egypt
Watermelon	China	Turkey	Iran	United States	Korea Rep.
Yam	Nigeria	Ivory Coast	Ghana	Benin	Togo

Table 6. Per Capita Annual Consumption of Commercially Produced Vegetables—United States (1997)

	Amount (kg)			
Vegetable	Fresh	Canned	Frozen	Total
Artichoke, all	–	–	–	0.2
Asparagus	0.3	0.1	0.1	0.5
Bean, dry, all	–	–	–	3.5
Bean, snap	0.7	1.8	0.9	3.3
Broccoli	2.3	–	1.1	3.4
Cabbage	4.8	0.6	–	5.4
Cantaloupe	5.5	–	–	5.5
Carrot	5.8	0.6	1.2	7.6
Cauliflower	0.7	–	0.2	1.0
Celery	3.0	–	–	2.7
Corn, sweet	3.7	4.8	4.3	12.8
Cucumber	2.8	2.3	–	5.1
Eggplant, all	–	–	–	0.2
Garlic, all	–	–	–	0.9
Honeydew melon	1.2	–	–	1.2
Lettuce, head	10.9	–	–	10.9
Lettuce, leaf and romaine	2.9	–	–	2.9
Mushroom	1.0	0.8	–	1.8
Onion[a]	8.6	–	–	8.6
Pea, green	–	0.7	1.0	1.6
Pea & lentil, dry, all	–	–	–	0.2
Pepper, bell	3.2	–	–	3.2
Pepper, chile	–	2.8	–	2.8
Potato[b]	21.8	–	42.9	64.7
Spinach	0.3	0.3	0.3	0.8
Sweet potato, all	–	–	–	2.1
Tomato	8.6	33.2	–	41.7
Watermelon	7.7	–	–	7.7
Other vegetables, all	–	–	–	4.8
Total	*95.8*	*48.0*	*9.1*	*207.1*

[a] Includes fresh and processed onion.

[b] Includes all processed potato.

Table 7. U.S. Per Capita Annual Use of Commercially Produced Vegetables, 1970–1998

	Amount (kg)			
Year	Fresh[a]	Canned	Frozen[b]	Total
1970	83.1	44.4	34.1	161.6
1975	78.6	42.9	38.5	160.0
1980	79.2	45.1	36.2	160.5
1985	86.1	43.4	43.2	172.7
1990	91.1	48.7	44.3	184.1
1995	96.3	48.3	58.8	203.4
1998	103.6	47.1	53.5	204.2

[a] Includes fresh and processed sweet potato and mushroom.

[b] Includes canned and frozen potato.

of over 200 kg is impressive. For comparison, per capita consumption of Chinese cabbage, cucumber, tomato, cabbage, eggplant, kidney bean, celery, sweet pepper, and Chinese chive was 195 kg in Beijing, China, in 1995, according to Chen and Liu (7).

The data in Table 6 present a snapshot of U.S. per capita vegetable consumption in 1997. But what have been the trends in vegetable consumption in recent times? Per capita consumption of fresh, canned, frozen, and total vegetables (8) from 1970 to the present at 5-year intervals is shown in Table 7. Over the period fresh vegetable consumption increased from about 83 to 104 kg, canned vegetable consumption from about 44 to 47 kg, frozen vegetable consumption from 34 to 54 kg, and total consumption from 162 to 204 kg. Accordingly, there have been major increases in fresh and frozen vegetable consumption, but not much change in the consumption of canned vegetables over the period 1970 through 1998. Much of the increase in frozen vegetable consumption, however, can be attributed to the increased popularity of potato products, notably french fries.

There were major increases in consumption of certain vegetables during the period. For example, broccoli consumption increased 500%, carrot consumption 75%, melons (watermelon, cantaloupe, honeydew) 47%, lettuce 37%, onion 68%, bell pepper 322%, tomato 24%, potato 17%, and mushrooms 307%. Only a few vegetables recorded less consumption during the period, notably celery, green peas, and sweet potato (8).

What factors have fueled the increases in vegetable consumption? The industry-financed "Five a Day" program has focused attention on the health benefits of vegetable and fruit consumption. This and other educational programs have promoted vegetables in general and even specific constituents such as antioxidants, lycopene, and beta-carotene for their general and specific health benefits. The trend toward increased vegetable consumption is expected to continue as consumers gain a greater understanding of the role of vegetables in human health.

VEGETABLE IMPROVEMENT

Vegetables cultivated by the very first farmers were similar to those that had been collected in the wild by their immediate predecessors. Improvement came slowly as the result of careful observation by the best farmers or by acquisition from neighbors via trade transactions. Surely the need for food during famine necessitated consumption of valuable seeds or other propagating material, so the improvement process was temporarily set back. Early horticulturists would prize and select for such qualities as ability to thrive under stressful growing conditions, good keeping quality, and good taste. The development of the earliest varieties occurred over time by careful selection and isolation of germplasm. The seed trade probably had its beginning in barter but gradually became a distinct enterprise toward the end of the eighteenth century. According to Nonnecke (9), David Landreth of Philadelphia established the first commercial seed business in the United States in 1784. The area around Philadelphia became the center of the vegetable seed business in the early nineteenth century with the establishment of several other firms including Bernard McMahon. Large-scale commercial bean and pea seed production was initiated in New York state by the Keeney Seed Company shortly thereafter.

As the country developed westward, it was found that the low humidity and low rainfall there during the growing season resulted in greatly improved quality, including disease-free seed. Much of the open-pollinated and some of the hybrid vegetable seed production is still centered in the western United States.

Continued improvement in vegetable varieties took place during the nineteenth and early twentieth centuries by careful selection. As knowledge of the new science of genetics became more commonplace, its power was utilized to make controlled and planned improvements in the performance of vegetable varieties. Because these newly developed varieties were all true breeding, the successes obtained by one company could quickly be utilized by competitors.

All of this changed beginning in 1934 when Glen Smith at Purdue University developed the first widely accepted vegetable hybrid, Golden Cross Bantam sweet corn. Hybrids, because they are not true breeding, offer the opportunity for exclusivity by the originator and, if successful, a very substantial financial incentive. The grower also benefits from hybrids by use of seed that produces a uniform crop with useful characters, such as disease resistance from both parents combined into the single hybrid, albeit at higher seeds costs. From this early beginning, hybrids have become the norm in many of the important vegetables—beet, broccoli, cabbage, carrot, sweet corn, cucumber, eggplant, melons, onion, pepper, spinach, and tomato. The promise of exclusivity with hybrids has contributed to the development of major national and international firms devoted to vegetable seed production and sales.

Currently, the use of molecular genetics is being exploited in vegetable improvement. Using these techniques, foreign genes can be introduced into vegetable germplasm. Despite some public concern, products such as virus-resistant summer squash, beetle- and virus-resistant potato, and worm-resistant sweet corn are now entering the marketplace. The possibilities for vegetable improvement using these techniques in concert with the knowledge of the trained horticulturist appear to be very great.

VEGETABLE GROWTH REQUIREMENTS

Vegetable plants, like all plants, thrive and produce successful crops when factors required for growth are present in suitable proportions. These factors are enumerated and discussed briefly.

Light

The ambient light conditions are employed for field production of vegetables. Field production is timed for favorable temperatures that coincide with summertimelike conditions and ample light for maximum growth and yield. Protected culture, on the other hand, occurs during the off-season, and crops produced in greenhouses, plastichouses, or tunnels may respond favorably to artificial light.

Water

Plants require water to maintain turgidity and replace that lost through transpiration to the surrounding environment. The water molecule is cleaved in photosynthesis to free H^+, which combines with CO_2 to produce simple sugars that can then be transformed into the myriad of compounds produced by plants. So crop yields are dependent on an adequate water supply provided through either rainfall or irrigation. Because of their high value, virtually all vegetable production in developed countries is irrigated. The recent introduction of microirrigation (Fig. 4), as a system for delivery of water directly to the crop in thin, polyethylene tubes, has enhanced the use of irrigation in developing countries where water for irrigation is often in critically short supply. The frequency and intensity of irrigation is dependent on the soil's water-holding capacity; prevailing weather, especially temperature, wind, and relative humidity; and characteristics of the plant (11). Those crops with shallow root systems (Table 8) require more frequent, lower volume irrigations than those with deep root systems.

Essential Elements

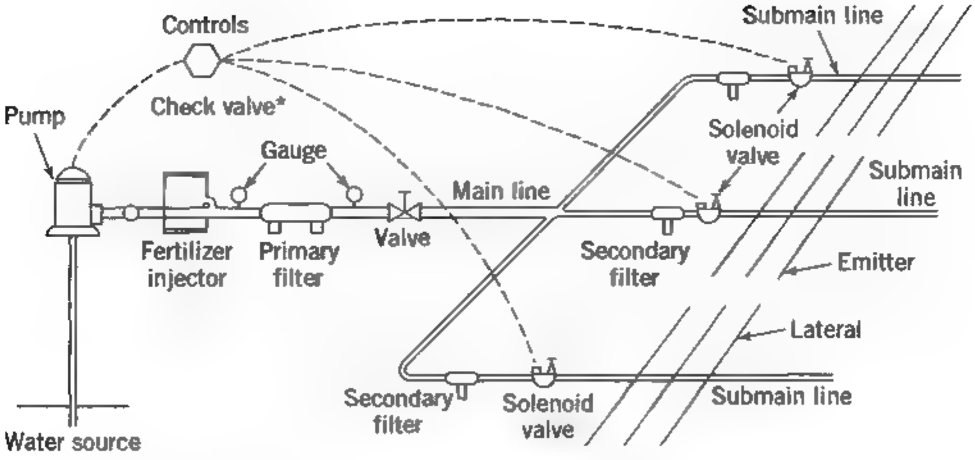
In addition to the organic elements carbon, hydrogen, and oxygen provided from carbon dioxide from the atmosphere and from water, certain inorganic elements are necessary for growth of all plants. The macronutrients nitrogen, phosphorous, and potassium are required in highest concentrations. The secondary elements calcium, magnesium, and sulfur are also necessary in substantial quantity, but in addition they affect soil pH, which has a profound effect on availability of other elements. Iron, boron, manganese, zinc, copper, and molybdenum are required in minute concentrations by plants and collectively are termed micronutrients.

Medium

The traditional medium for field and protected culture of vegetables has been native soil, often amended with organic matter and inorganic fertilizer and sometimes treated with limestone or sulfur compounds for pH adjustment. This is still the situation for field culture but is rarely so for protected culture. Various materials, including peat, perlite, rockwool, straw bales, and liquid cultures, have replaced soil as a growing medium in protected culture. These materials provide superior physical characteristics and are generally free of pathogenic organisms. Essential elements are provided on a scheduled basis when soil substitutes are used for vegetable culture.

Carbon Dioxide

Carbon dioxide (CO_2) is present in the atmosphere at an average concentration of 0.03%. There is no practical way yet of altering the ambient CO_2 concentrations under field conditions. Experimental results have been inconclusive when CO_2-enriched water has been introduced into low tunnels in the field. There has been considerable success in growth and yield enhancement, however, when CO_2 concentrations are elevated to about 0.10% in protected culture. This practice is universal in areas like western Europe, where ventilation of structures is not required on a continuous basis and the additional CO_2 is trapped in the structure.



*A backflow preventer or vacuum breaker is required in some areas.

Figure 4. Microirrigation system components. *Source:* Ref. 10, reprinted with permission.

Table 8. Characteristic Maximum Rooting Depths of Various Vegetables

Shallow (45–60 cm)	Moderately deep (90–120 cm)	Deep (>120 cm)
Broccoli	Bean, bush	Artichoke
Brussels sprouts	Bean, pole	Asparagus
Cabbage	Beet	Bean, lima
Cauliflower	Cantaloupe	Parsnip
Celery	Carrot	Pumpkin
Chinese cabbage	Chard	Squash, winter
Corn	Cucumber	Sweet potato
Endive	Eggplant	Tomato
Garlic	Mustard	Watermelon
Leek	Pea	
Lettuce	Pepper	
Onion	Rutabaga	
Parsley	Squash, summer	
Potato	Turnip	
Radish		
Spinach		

Source. Ref. 10, reprinted with permission.

Oxygen

Postharvest life of certain vegetables is enhanced by use of controlled-atmosphere storage whereby O_2 concentration is lowered with concomitant increases in CO_2 and H_2, which reduces respiration rates.

Temperature

Each vegetable has a specific minimum, maximum, and optimum temperature for growth at a particular life cycle stage. These cardinal temperatures may be quite different as the plant passes through seed germination, establishment, vegetative growth, reproductive growth, and maturation. Consequently, a general scheme (Table 9) giving a range of temperatures usually suffices (11). The frost sensitivity of vegetables is of special interest in that it determines time of planting in areas subject to frosts. For example, one would feel quite comfortable planting peas any place in New England in April, but it would be extremely unwise to plant tomatoes in the field at that time. Peas are extremely frost tolerant; in my own experience peas 8 cm high were totally unaffected by a late-season snowfall of about the same depth. On the other hand, tomato will be irreparably damaged at temperatures slightly below 0°C.

PROPAGATION OF VEGETABLES

Most vegetables are propagated by seed, either by planting directly in the field or transplanting seedlings that were started from seeds in protected culture (Fig. 5) some weeks previously. The latter procedure is more conservative of costly seeds, provides a more hospitable environment for fragile seedlings, and lengthens the growing season in areas having a restricted period of favorable weather. Vegetative propagation (Table 10) is necessary for some vegetables that do not breed true from seeds or do not readily produce seeds. Various organs or organ sections may be used for propagation, depending on the crop. Although seeds may be used to propagate globe artichoke and potato, vegetative organs are by far the most common in commercial uses. Vegetative propagation is used only when sexual (seed) propagation is not possible because, at least in temperate areas, the expense of storage from one season to the next is extremely great. Vegetative planting stock may be stored *in situ* in tropical areas.

VEGETABLE-PRODUCTION SYSTEMS

Methods employed for commercial vegetable production vary with geographical location, the crop being grown, season of production, and sometimes by the grower and resources available for production. Consequently, it is possible to discuss production systems not in specific terms but only in generalities.

Table 9. Temperature Demands and Frost Sensitivities of Vegetables

	Frost sensitivity		Frost sensitivity
Hot: Optimum range 25–27°C; growth range 18–35°C			
Okra	+	Cantaloupe	+
Roselle	+	Hot pepper	+
Watermelon	+	Sweet potato	+
Warm: Optimum range 20–25°C; growth range 12–35°C			
Cucumber	+	Sweet corn	+
Eggplant	+	Tomato	+
Sweet pepper	+	Bean	+
Pumpkin, squash	+	Purslane	u
New Zealand spinach	u		
Cool-hot: Optimum range 20–25°C; growth range 7–30°C			
Taro	u	Chicory	−
Globe artichoke	u	Bok choy	−
Onion, shallot	−	Scorzonera	−
Leek	−	Chives	−
Garlic	−		
Cool-warm: Optimum range 18–25°C; growth range 7–25°C			
Pea	−	Red beet	(−)
Broad bean	−	Swiss chard	(−)
Cauliflower	−	Spinach	−
Broccoli	−	Lettuce	(+)
Cabbage	−	Endive	−
Kohlrabi	−	Carrot	−
Kale	−	Celery, celeriac	(+)
Brussels sprout	−	Parsnip	−
Turnip	−	Potato	+
Rutabaga	−	Lamb's lettuce	−
Chinese cabbage	−	Rhubarb	−
Parsley	−	Asparagus	(+)
Fennel	(+)	Horseradish	−
Dill	(+)	Garden cress	(+)
Radish	(+)		

Note: + indicates sensitive to weak frost; − indicates relatively insensitive; ( ) indicates uncertain; u indicates undetermined.

Figure 5. Production of vegetable transplants for field setting has become a specialized segment of the vegetable industry.

Table 10. Vegetative Propagation of Vegetables

Vegetable	Propagule
Cassava	Stem cuttings
Ginger	Rhizome divisions
Globe artichoke	Divisions, suckers, seeds
Horseradish	Rhizome divisions
Jerusalem artichoke	Tubers
Potato	Tubers, seeds
Rhubarb	Divisions
Sweet potato	Slips from roots, vine cuttings
Taro	Corms, cormels
Yam	Tubers, crowns, sets

FIELD PRODUCTION OF VEGETABLES

Traditional Practices

Vegetables are high-value crops with little or no tolerance for blemished products in the marketplace. Accordingly, vegetable growers have used the best agricultural practices of the day, including such things as large additions of composted manure to the soil, use of appropriate crop rotations, use of cover crops in the off-season to prevent soil erosion and to trap residual fertilizers, and early disposal of crop residue to assist in pest control. In addition, successful vegetable growers routinely have provided inputs such as quality seeds, abundant fertilizer, pesticides, irrigation, and weed control together with extensive use of labor and a high level of management for profitable yields of quality produce. Advanced growers use south-facing slopes to produce earlier crops, planted (Fig. 6) or constructed windbreaks to restrict damaging winds early in the season, and various techniques for frost prevention.

Enhanced Inputs

Research is continually developing new technologies for crop production. To remain competitive and enhance effi-

Figure 6. A sugarcane windbreak creates a favorable microclimate for pepper production in south Florida.

ciencies, the most advanced growers adopt these technologies as soon as available. For some growers use of polyethylene mulch with microirrigation/fertigation is new technology even though it has been commonplace for some time in other production areas. Integrated crop management (ICM) is a concept whereby all production and pest-management decisions are considered before invoking a single variable. Best management practices for judicious use of fertilizers is an integral part of ICM, as is precision farming that provides for chemical applications where needed rather than farmwide. Enhanced germplasm fits particularly well into the ICM concept. Together these practices provide for sustainability of the vegetable enterprise.

Organic Production

Some consumers desire products that are produced without chemicals, even though growers use extreme care in chemical use and governmental agencies monitor pesticide residues. This has led to an alternative cultural system known as organic farming. Organic farming is a production system that avoids or excludes the use of synthetically compounded fertilizers, pesticides, or growth regulators. It relies on crop rotations, mechanical cultivation, and biological pest control to maintain soil productivity; to supply nutrients; and to control insects, diseases, and weeds. Specific aspects of organic farming are defined in several state laws. National organic-farming guidelines are being developed. Organic vegetable production represents a very small but rapidly growing part of the industry.

Protected Culture

Vegetable production in glasshouses, plastichouses (Fig. 7), and high tunnels (Fig. 8) is the most rapidly growing segment of the entire vegetable industry. Nearly complete control of the growing environment is achieved in modern growing structures. When combined with superior genetic material, yields are manyfold those of field-grown vegetables. For example, tomato yields in the Netherlands from protected culture are over 7 times greater than in the United States, mostly from field culture. Products derived from protected culture such as tomato, pepper, eggplant, cucumber, and lettuce are sold to discriminating, affluent buyers around the world.

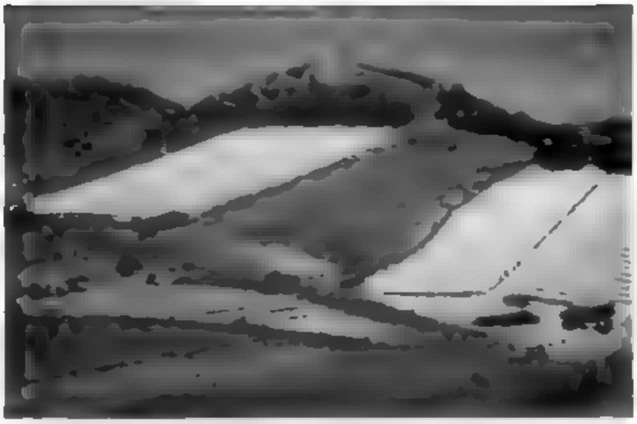


Figure 8. High tunnels for vegetable production in southern Spain.

VEGETABLE HARVEST

Vegetables grown for fresh market have a high value and a low tolerance for blemishes, so they are mostly hand harvested (12). Selection, sorting, grading, trimming if necessary, and packing usually are done in the field for some vegetables such as lettuce, cabbage, broccoli, and endive. Other vegetables like asparagus, tomato, and snap beans usually are brought to a central packing area where these tasks are performed. Some vegetables destined for the fresh market are usually harvested mechanically, including carrot, potato, brussels sprouts, and sweet potato. Snap beans for fresh market are hand harvested when prices are high and machine harvested (Fig. 9) when prices are low.

Whenever possible, vegetables destined for processing are harvested mechanically to reduce costs and provide the volume of product necessary for efficient plant operation. Virtually all of the beet, carrot, pea, bean, sweet corn, spin-

Figure 7. A vast expanse of plastichouses for vegetable production cover the landscape in southern Spain.

Figure 9. Machine harvest of snap beans in Miami, Dade County, Florida.

Table 11. Status of Hand versus Mechanical Harvest of Vegetables

Area hand harvested (%)	Vegetable			
76–100	Artichoke	Asparagus	Broccoli[a]	Cabbage
	Cauliflower	Celery	Cucumber[a]	Lettuce
	Green onion	Collards	Cress	Dandelion
	Eggplant	Endive	Escarole	Fennel
	Kale	Kohlrabi	Mushroom[a]	Okra
	Pepper	Rapini	Rhubarb[a]	Romaine
	Sorrel	Squash	Watercress	Cassava
	Celeriac	Ginger	Parsley root	Parsnip
	Rutabaga	Salsify	Turnip	Taro[a]
	Jerusalem artichoke			
51–75	Sweet potato	Mustard greens	Parsley	Swiss chard
	Turnip greeens			
26–50	Dry onion[a]	Pumpkin[a]	Tomato[a]	
0–25	Beet[a]	Carrot	Potato[a]	Lima bean[a]
	Snap bean[a]	Sweet corn[a]	Spinach[a]	Horseradish[a]
	Pea[a]	Garlic	Brussels sprouts[a]	Malanga
	Boniato	Radish		

[a] More than 50% of the crop is processed.

Source: Ref. 13, reprinted with permission.

ach, and potato crops for processing are harvested by mechanical means. A summary of the status of hand versus mechanical vegetable harvest is shown in Table 11.

POSTHARVEST HANDLING

Vegetables are perishable commodities. Maximum postharvest life varies from a few days to several months depending on the vegetable. Harvesting at the correct maturity, careful handling at harvest and during packing and shipping, good sanitation, timely and proper cooling (Fig. 10), shipping and storing at optimal temperature and relative humidity, and freedom from exposure to ethylene gas for some commodities will help to assure maximum postharvest life. The importance of rapid cooling and maintenance of recommended temperature throughout the postharvest period to the consumer cannot be overemphasized as it is the most important factor in determining life of the product.

Shipping containers provide a measure of volume or weight and must be designed to protect the product. Their shape should conform to standard pallets (Fig. 11) for convenient vehicle loading and unloading. Vegetable-shipping

Figure 10. Forced-air cooling provides rapid cooling of beans and other products.

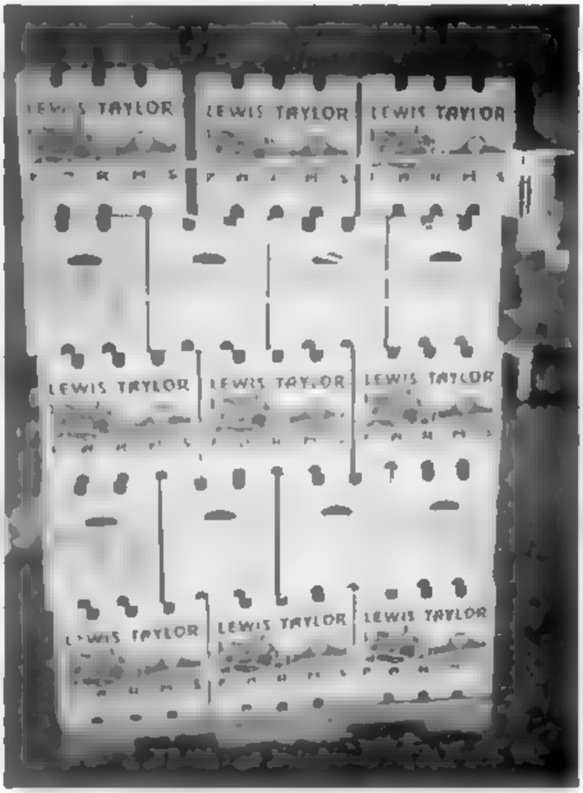


Figure 11. Pepper cartons designed to utilize the full pallet area are used in south Georgia.

containers may be constructed of burlap, paper, fiberboard, wood, or plastic. Because of the mass of materials at receiving points, there is increasing emphasis on materials that are recyclable or reusable.

PROJECTIONS FOR THE FUTURE

What are the expectations for the commercial vegetable industry as we enter the twenty-first century? Some thoughts on this question follow: Vegetables will continue to be consumed in greater quantities; there will be an ever-increasing variety of vegetables from which consumers can choose; organically grown vegetables will command a greater proportion of the market; the vegetable industry will be global rather than national as trade barriers are loosened; there will be fewer new pesticides available to growers and increased monitoring of pesticide usage; the use of genetically enhanced vegetable varieties will become commonplace; and the number of vegetable enterprises will decrease, but those remaining will be larger.

BIBLIOGRAPHY

Reprinted from the Florida Agricultural Experiment Station Journal Series No. R-06636.

1. The Staff of The Liberty Hyde Bailey Hortorum, *Hortus Third*, MacMillan Publishing Co., New York, 1976.
2. D. N. Maynard, "Vegetable," in S. J. Foderaro, ed., *Academic American Encyclopedia*, Vol. 19, Aretê Publishing Co., Princeton, N.J., 1980, pp. 531–534.
3. S. J. Kays and J. C. Silva Dias, *Cultivated Vegetables of the World*, Exon Press, Athens, Ga., 1996.
4. H. Chen et al., "Diversity of Edible Vegetables in China," in V. E. Rubatzky, H. Chen, and J. Y. Peron, eds., *Proc. Third Int. Symp. on Diversification of Vegetable Crops*, Acta Horticulturae No. 467, International Society for Horticultural Science, Leuven, Belgium, 1998.
5. Anonymous, *Vegetables, 1997 Summary*, Vg 1-2(98), U.S. Department of Agriculture Washington, D.C., 1998.
6. Anonymous, *FAO Production Yearbook 1996*, FAO Statistics Series No. 135, Food and Agriculture Organization of the United Nations, Rome, 1997.
7. D Chen and M. Liu. "An Overlook at Vegetable Production, Consumption, Distribution and its Progress in China," in V. E. Rubatzky, H. Chen, and J. Y. Peron, eds. *Proc. Third Int. Symp. Diversification of Vegetable Crops*, Acta Horticulturae No. 467, International Society for Horticultural Science, Leuven, Belgium, 1998.
8. G. Lucier, *Vegetables and Specialties. Situation and Outlook*, VGS-275, U.S. Department of Agriculture, Washington, D.C., 1998.
9. I. L. Nonnecke, *Vegetable Production*, Van Nostrand Reinhold, New York, 1989
10. D N. Maynard and G. J. Hochmuth, *Knott's Handbook for Vegetable Growers*, 4th ed., John Wiley & Sons, 1997.
11. H. Krug, "Environmental Influences on Development, Growth and Yield," in H. C. Wein, ed., *The Physiology of Vegetable Crops*, CAB International, New York, 1997.
12. J. F. Thompson, "Harvesting Systems," in A. A. Kader, ed., *Postharvest Technology of Horticultural Crops*, 2nd ed. University of California, Oakland, Calif., 1992.

GENERAL REFERENCES

J. L. Brewster, *Onions and Other Vegetable Alliums*, CAB International, New York, 1994.

A. A. Kader, ed., *Postharvest Technology of Horticultural Crops*, University of California, Oakland, Calif., 1992.

D. N Maynard and G. J. Hochmuth, *Knott's Handbook for Vegetable Growers*, 4th ed., John Wiley & Sons, New York, 1997.

R. W. Robinson and D. S. Decker-Walters, *Cucurbits*, CAB International, New York, 1997.

V. E. Rubatzky and M. Yamaguchi, *World Vegetables, Principles, Production, and Nutritive Value*, 2nd ed., Chapman and Hall, New York, 1997.

E. J. Ryder, *Lettuce, Endive, and Chicory*, CAB International, New York, 1999.

H. C. Wein, ed., *The Physiology of Vegetable Crops*, CAB International, New York, 1997.

R. Wills et al., *Postharvest, An Introduction to the Physiology & Handling of Fruit, Vegetables, & Ornamentals*, 4th ed., CAB International, New York, 1998.

DONALD N. MAYNARD
University of Florida
Bradenton, Florida

VEGETABLES, PICKLING

Vegetables may be preserved by fermentation, direct acidification, or a combination of these along with other processing conditions and additives to yield products that are referred to as pickles. Pasteurization and refrigeration are used to ensure stability of certain products. Organic acids and salt (NaCl) are primary preservatives for most types of pickles. Lactic acid is produced naturally in fermented products. Acetic acid (or vinegar) is the usual acid added to pasteurized, nonfermented (fresh-pack) pickles. Acetic acid also is added to many products made from fermented (salt-stock) cucumbers. Other preservatives such as sodium benzoate, potassium sorbate, and sulfur dioxide may be added to finished products. Although the term *pickles* in the United States generally refers to pickled cucumbers, the term is used herein in a broader sense to refer to all vegetables that are preserved by fermentation or direct acidification. Cucumbers, cabbage, olives, and peppers account for the largest volume of vegetables and fruits commercially pickled. Lesser quantities of onions, tomatoes, cauliflower, carrots, melon rinds, okra, artichokes, beans, and other produce also are pickled.

Although this article emphasizes pickling of cucumbers and cabbage, which are major vegetable commodities commercially preserved and consumed in the United States and Europe, and kimchi, which is a mixture of fermented vegetables consumed in Korea, most vegetables have been preserved by pickling, either commercially or in the home.

HISTORY

The preservation of vegetables by fermentation is thought to have originated before recorded history, and the tech-containers may be constructed of burlap, paper, fiberboard, wood, or plastic. Because of the mass of materials at receiving points, there is increasing emphasis on materials that are recyclable or reusable.

PROJECTIONS FOR THE FUTURE

What are the expectations for the commercial vegetable industry as we enter the twenty-first century? Some thoughts on this question follow: Vegetables will continue to be consumed in greater quantities; there will be an ever-increasing variety of vegetables from which consumers can choose; organically grown vegetables will command a greater proportion of the market; the vegetable industry will be global rather than national as trade barriers are loosened; there will be fewer new pesticides available to growers and increased monitoring of pesticide usage; the use of genetically enhanced vegetable varieties will become commonplace; and the number of vegetable enterprises will decrease, but those remaining will be larger.

BIBLIOGRAPHY

Reprinted from the Florida Agricultural Experiment Station Journal Series No. R-06636.

1. The Staff of The Liberty Hyde Bailey Hortorum, *Hortus Third*, MacMillan Publishing Co., New York, 1976.
2. D. N. Maynard, "Vegetable," in S. J. Foderaro, ed., *Academic American Encyclopedia*, Vol. 19, Aretê Publishing Co., Princeton, N.J., 1980, pp. 531–534.
3. S. J. Kays and J. C. Silva Dias, *Cultivated Vegetables of the World*, Exon Press, Athens, Ga., 1996.
4. H. Chen et al., "Diversity of Edible Vegetables in China," in V. E. Rubatzky, H. Chen, and J. Y. Peron, eds., *Proc. Third Int. Symp. on Diversification of Vegetable Crops*, Acta Horticulturae No. 467, International Society for Horticultural Science, Leuven, Belgium, 1998.
5. Anonymous, *Vegetables, 1997 Summary*, Vg 1-2(98), U.S. Department of Agriculture Washington, D.C., 1998.
6. Anonymous, *FAO Production Yearbook 1996*, FAO Statistics Series No. 135, Food and Agriculture Organization of the United Nations, Rome, 1997.
7. D. Chen and M. Liu. "An Overlook at Vegetable Production, Consumption, Distribution and its Progress in China," in V. E. Rubatzky, H. Chen, and J. Y. Peron, eds. *Proc. Third Int. Symp. Diversification of Vegetable Crops*, Acta Horticulturae No. 467, International Society for Horticultural Science, Leuven, Belgium, 1998.
8. G. Lucier, *Vegetables and Specialties. Situation and Outlook*, VGS-275, U.S. Department of Agriculture, Washington, D.C., 1998.
9. I. L. Nonnecke, *Vegetable Production*, Van Nostrand Reinhold, New York, 1989.
10. D. N. Maynard and G. J. Hochmuth, *Knott's Handbook for Vegetable Growers*, 4th ed., John Wiley & Sons, 1997.
11. H. Krug, "Environmental Influences on Development, Growth and Yield," in H. C. Wein, ed., *The Physiology of Vegetable Crops*, CAB International, New York, 1997.
12. J. F. Thompson, "Harvesting Systems," in A. A. Kader, ed., *Postharvest Technology of Horticultural Crops*, 2nd ed. University of California, Oakland, Calif., 1992.

GENERAL REFERENCES

J. L. Brewster, *Onions and Other Vegetable Alliums*, CAB International, New York, 1994.

A. A. Kader, ed., *Postharvest Technology of Horticultural Crops*, University of California, Oakland, Calif., 1992.

D. N. Maynard and G. J. Hochmuth, *Knott's Handbook for Vegetable Growers*, 4th ed., John Wiley & Sons, New York, 1997.

R. W. Robinson and D. S. Decker-Walters, *Cucurbits*, CAB International, New York, 1997.

V. E. Rubatzky and M. Yamaguchi, *World Vegetables, Principles, Production, and Nutritive Value*, 2nd ed., Chapman and Hall, New York, 1997.

E. J. Ryder, *Lettuce, Endive, and Chicory*, CAB International, New York, 1999.

H. C. Wein, ed., *The Physiology of Vegetable Crops*, CAB International, New York, 1997.

R. Wills et al., *Postharvest, An Introduction to the Physiology & Handling of Fruit, Vegetables, & Ornamentals*, 4th ed., CAB International, New York, 1998.

DONALD N. MAYNARD
University of Florida
Bradenton, Florida

VEGETABLES, PICKLING

Vegetables may be preserved by fermentation, direct acidification, or a combination of these along with other processing conditions and additives to yield products that are referred to as pickles. Pasteurization and refrigeration are used to ensure stability of certain products. Organic acids and salt (NaCl) are primary preservatives for most types of pickles. Lactic acid is produced naturally in fermented products. Acetic acid (or vinegar) is the usual acid added to pasteurized, nonfermented (fresh-pack) pickles. Acetic acid also is added to many products made from fermented (salt-stock) cucumbers. Other preservatives such as sodium benzoate, potassium sorbate, and sulfur dioxide may be added to finished products. Although the term *pickles* in the United States generally refers to pickled cucumbers, the term is used herein in a broader sense to refer to all vegetables that are preserved by fermentation or direct acidification. Cucumbers, cabbage, olives, and peppers account for the largest volume of vegetables and fruits commercially pickled. Lesser quantities of onions, tomatoes, cauliflower, carrots, melon rinds, okra, artichokes, beans, and other produce also are pickled.

Although this article emphasizes pickling of cucumbers and cabbage, which are major vegetable commodities commercially preserved and consumed in the United States and Europe, and kimchi, which is a mixture of fermented vegetables consumed in Korea, most vegetables have been preserved by pickling, either commercially or in the home.

HISTORY

The preservation of vegetables by fermentation is thought to have originated before recorded history, and the technology was developed by trial and error. Pederson (1) presumed that early humans observed that when vegetables were flavored with salt or brine and packed tightly in a vessel, they changed in character but remained appetizing and nutritious. Pederson concluded that the Chinese were the first to preserve vegetables in this manner and assumed that fermentation in salt brines occurred first and that dry salting came later. The Chinese have been credited with introducing fermented vegetables into Europe.

Pasteurization, as a means of preserving acidified vegetables, was introduced in the United States in the 1940s. Before then, all pickled vegetables were preserved commercially by fermentation. Pasteurization resulted in a doubling of the per capita consumption of pickled vegetables by the 1950s. Refrigerated pickles were introduced into U.S. commerce in the 1960s and have been an increasing segment of the industry since.

PROCESSING

Processing methods for the preservation of cucumbers, cabbage, and kimchi will serve to illustrate the principles involved in vegetable pickling.

Cucumbers

Various authors have attributed the origin of the cucumber (*Cucumis sativus* L.) to Africa, China, India, or the Near East (2). Later domestication occurred throughout Europe, and cucumbers are now grown throughout the world using field or greenhouse culture, but with various characteristics, depending upon region.

Cucumbers are bred either for fresh market or processing (pickling). The fresh market varieties possess a relatively tough skin, which serves to extend their storage life as fresh produce. Pickling varieties, however, possess a thin, relatively tender skin. Pickling cucumbers are harvested in a relatively immature stage, before the seeds mature and before the seed area becomes soft and starts to liquify.

Cucumbers are harvested by hand or mechanical means, depending upon availability of labor, land size and conformation, and other factors. Cucumbers are a seasonal crop grown in various geographical regions and shipped to the processor. Great changes have occurred in the United States during the past 20 years as to the origin of the fruit that a processor receives. Although once a mainly regional and seasonal enterprise, some large processors now receive fresh cucumbers nearly the entire year. The fruit is grown from Mexico to Canada and shipped fresh to processors according to their demands. Cucumbers grown near the processor may be processed within 24 hours. Cucumbers are shipped under refrigeration if grown at distant locations from the processor. The demand for a year-round supply of fresh cucumbers varies according to the types of products that the processors manufacture. Brined cucumbers, being more stable, are transported intercontinentally.

Pickling cucumbers are preserved by three basic methods: fermentation (40% of overall production), pasteurization (40%), and refrigeration (20%), as outlined in Figure 1 (3). After pasteurization of fresh cucumber pickles was introduced into the U.S. industry in the 1940s, the consumption of pickles increased significantly because of the milder acid flavor and more uniform quality of the pasteurized product. The process involves heating properly acidified cucumbers to an internal temperature of 74°C and holding for 15 min (4). Some processors deviate from this standard process, depending upon their products and experiences. Fermented cucumbers also may be pasteurized to increase shelf stability, but at lower temperatures and times. Refrigerated pickles are preserved by addition of low concentrations of vinegar and a chemical preservative (eg, sodium benzoate), in addition to refrigeration at 1 to 5°C. Microbial growth in these products is not desired. Nonacidified, refrigerated pickles, originally popular among certain ethnic groups, also are marketed in some metropolitan areas. These products may or may not be allowed to undergo fermentation before refrigeration. After packaging, these nonacidified pickles undergo a slow lactic acid fermentation while under refrigeration.

Cabbage

The modern, hard-head cultivars of cabbage (*Brassica oleracea*) are reported to have descended from wild, nonheading *Brassias* originating in the eastern Mediterranean and in Asia Minor (5). Cabbage is grown for the fresh market and for the production of sauerkraut. For use in the production of sauerkraut, it is desired that the cabbage heads be large (typically 8–12 lb) and compact (ie, dense), have a minimum of green outer leaves, and possess desirable flavor, color, and textural properties when converted into sauerkraut.

Fresh cabbage for sauerkraut is harvested mechanically or by hand mostly from August to November. The cabbage is transported to the processor, where it is graded, cored, trimmed, shredded, and salted. The waste from the coring and trimming operations typically is returned to the field, where it is plowed into the soil. This waste constitutes about 30% of the weight of the fresh cabbage.

After shredding (about 1-mm thick), the cabbage is conveyed by belt, where salt is added, to the fermentation tanks. The tanks typically hold 20 to 180 tons of shredded cabbage. Most tanks today are constructed of reinforced concrete, but some wooden tanks remain. The tank is uniformly filled, heaped to extend slightly above the top of the tank, and loosely covered with plastic sheeting. After about 24 h, brine generated and located at the bottom of the tank is allowed to drain from the tank to allow the top of the cabbage to settle below the top of the tank. Then, the cabbage is manually distributed to create a slightly concave surface. Plastic sheeting then is placed on the surface, and water is added on top to weight it down and to provide an anaerobic seal. Gas generated during fermentation escapes by forcing its way between the tank wall and the cabbage, or through a plastic tube placed between the cabbage and the cover.

In the United States, the cabbage is allowed to remain in the tanks until at least 1% lactic acid is formed (about 30 days minimum, depending upon the temperature), and is stored beyond this time and until such time as needed

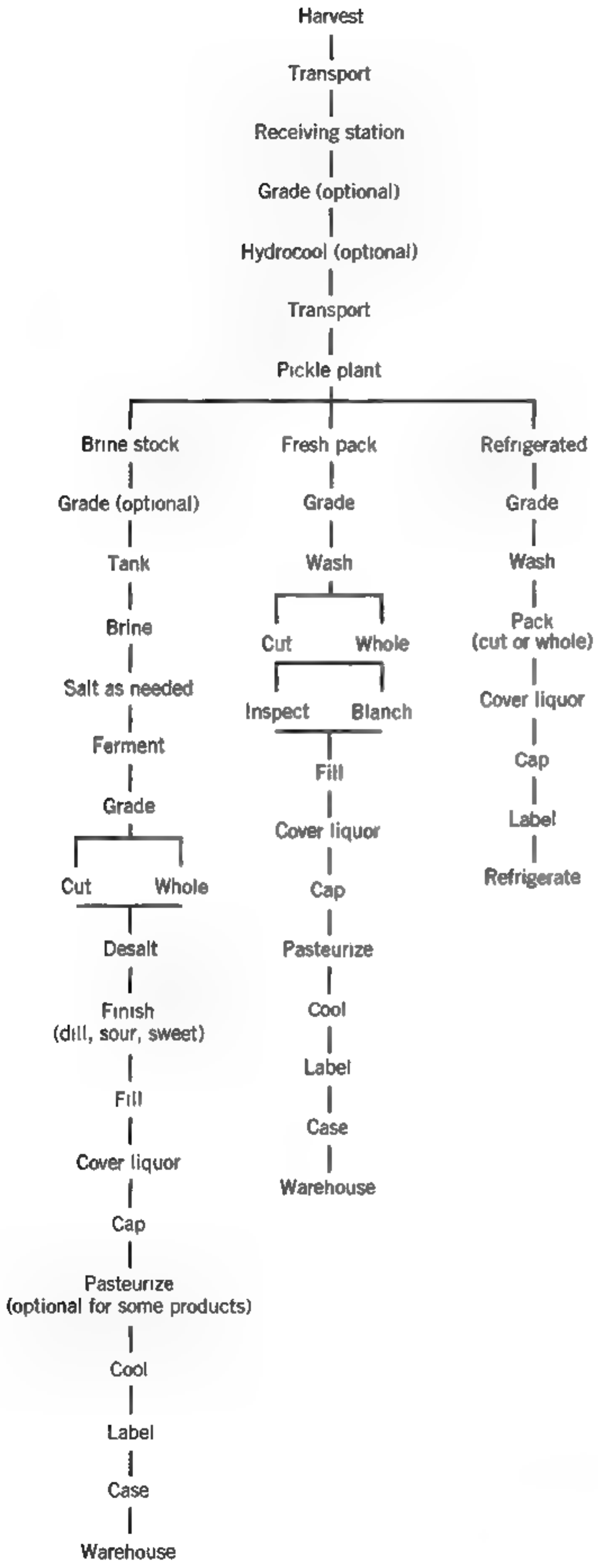

Harvest
Transport
Receiving station
Grade (optional)
Hydrocool (optional)
Transport
Pickle plant
Brine stock
Grade (optional)
Tank
Brine
Salt as needed
Ferment
Grade
Cut
Whole
Desalt
Finish
(dill, sour, sweet)
Fill
Cover liquor
Cap
Pasteurize
(optional for some products)
Cool
Label
Case
Warehouse
Fresh pack
Grade
Wash
Cut
Whole
Inspect
Blanch
Fill
Cover liquor
Cap
Pasteurize
Cool
Label
Case
Warehouse
Refrigerated
Grade
Wash
Pack
(cut or whole)
Cover liquor
Cap
Label
Refrigerate

Figure 1. Flow diagrams for three methods of cucumber processing. *Source:* Ref. 3.

for processing. Thus, the sauerkraut tanks are used for fermentation as well as storage. Although extended periods of holding in the tanks can result in excess acid formation and waste generation due to the need to wash excess acid from the product before canning, this disadvantage is offset by the economic advantages of bulk storage. Also, most U.S. sauerkraut companies specialize in this product only and desire the option of further processing of sauerkraut throughout the year so as to distribute labor and equipment needs. This U.S. procedure differs significantly from that of many European manufacturers who process the sauerkraut into finished products when it reaches the desired level of titratable acidity (calculated as lactic acid, typically 1%). Thus, European manufacturers have greater control over product uniformity, but lose the economic advantages of bulk storage. Also, the European method results in less waste generation since less excess acid is produced.

The sauerkraut is removed pneumatically, by mechanical fork, or by hand from the tanks as needed for processing during the year. The sauerkraut may be packaged in cans, glass, or plastic containers. When packaged in glass or plastic, the product is not heated. Rather, sodium benzoate (0.1%, w/w) and potassium metabisulfite are added as preservatives, and the product is held under refrigeration (5°C).

Canned sauerkraut is preserved by pasteurization without the addition of preservatives. Heating is performed either by steam injection into a thermal screw or by a sauerkraut juice immersion-type cooker. The product is heated to 74 to 82°C for about 3 min and hot-filled into the cans. After closure, the cans are immediately cooled to less than 32°C.

Kimchi

Kimchi is a general term applied to a Korean product made by the lactic acid fermentation of salted vegetables (dry salted or brined) with or without secondary ingredients. Unless otherwise specified, however, kimchi generally means a product made from Chinese cabbage as the primary vegetable with secondary ingredients. Kimchis made from vegetables other than Chinese cabbage as the primary vegetable are specified with a qualifying word, eg, radish kimchi, cucumber kimchi, and green onion kimchi. Both solids and liquids of kimchis are consumed.

Kimchi has been a major table condiment in Korea for about 200 years. Kimchi provided a spicy and flavorful adjunct to a rather narrow choice of foods for Koreans in difficult times, complementing the bland taste of cooked rice.

Winter kimchi has been most traditional and is made between mid-November and early December, depending on the climate of the particular year. It is consumed until the following spring. The fresh ingredients are tightly filled into large earthen jars with earthen lids. The jars are partially buried (80–90% of container depth) under ground and are covered with bundles of rice straw for protection from direct sunlight and the climate. This procedure is still practiced in much of the rural areas and some households in urban areas. The subsurface temperature of the earth during the winter season is fit for slow but excellent fermentation of kimchi and also for the subsequent storage. Overacidification and development of yeasty flavor are the two most important problems in the long-term storage of kimchi. However, as the temperature goes up late in the following spring, the quality of winter kimchi is reduced.

Today, lesser amounts of winter kimchi are made, and Koreans now enjoy freshly fermented kimchi anytime of the year owing to year-round availability of fresh vegetables, general availability of household refrigerators, and distribution of commercial products through efficient cold distribution systems. Also, it is common for Korean families to make small batches of kimchi as needed and to store it under refrigeration for short periods to extend the fresh quality.

Kimchis can be divided into two types simply depending on the way the primary vegetable is salted. Primary vegetables are salted (either by dry salting or brining) and then the liquid is drained off completely before blending with secondary ingredients in the preparation of general kimchis. However, sufficient brine is poured over the packed ingredients to cover whole cucumber fruits or radish roots. Pickles are usually prepared without secondary ingredients.

FERMENTATION AND MICROBIOLOGY

Most vegetable fermentations are the result of growth by naturally occurring lactic acid bacteria present on the raw vegetable and the concentration of salt present, which dictates the types and extent of growth by these bacteria. The concentration of salt used varies among commodities (Table 1) and depends upon the flavor and textural properties desired. Cucumbers tend to soften (enzymatically) more if brined below about 5% NaCl. Fermentation of vegetables occurs in various stages (Table 2). The principal species of lactic acid bacteria and their metabolic end products are summarized in Table 3.

Glucose and fructose are the principle sugars of cucumbers and cabbage. Cabbage also contains low levels of sucrose. The metabolic pathways involved in fermentation of these sugars to end products are illustrated in Figure 2.

Leuconostoc mesenteroides is an important species for initiating the fermentation of sauerkraut and kimchi because of its production of acetic as well as lactic acid and nonacidic end products (ethanol, mannitol), which results in a more flavorful product. However, this species is rather acid sensitive and soon is overtaken by *Lactobacillus plan-*

Table 1. Brining Procedures for Vegetables

	Concentration (%, w/v) of salt		
Method of salting	Fermentation	Storage	Vegetable
Dry salting	1.5–2.5	1.5–2.5	Cabbage
Dry salting	3	3	Kimchi
Brine solution	5–8	8–16	Cucumbers

Note: The concentrations of salt indicated generally are used for the vegetables listed. Wide variations exist in the salt concentration used for peppers, onions, and cauliflower.

Table 2. Stages of Microbial Activities During the Natural Fermentation of Vegetables

Stage	Prevalent microorganisms (conditions)
Initiation of fermentation	Various Gram-positive and Gram-negative bacteria
Primary fermentation	Lactic acid bacteria, yeasts (sufficient acid has been produced to inhibit most bacteria)
Secondary fermentation	Fermentative yeasts (when residual sugars remain and lactic acid bacteria have been inhibited by low pH)
	Spoilage bacteria (degradation of lactic acid when pH is too high and/or salt/acid concentration is too low, eg, propionic acid bacteria, clostridia)
Postfermentation	Open tanks: surface growth of oxidative yeasts, molds, and bacteria
	Anaerobic tanks: none (provided the pH is sufficiently low and salt or acid concentrations are sufficiently high)

Source: Ref. 6.

Table 3. Lactic Acid-Producing Bacteria Involved in Vegetable Fermentations

Genus and species	Fermentation type[a]	Main product (molar ratio)	Configuration of lactate
Streptococcus faecalis	Homofermentative	Lactate	L(+)
Streptococcus lactis	Homofermentative	Lactate	L(+)
Leuconostoc mesenteroides	Heterofermentative	Lactate/acetate/CO_2 (1:1:1)	D(−)
Pediococcus pentosaceus	Homofermentative	lactate	DL, L(+)
Lactobacillus brevis	Heterofermentative	Lactate/acetate/CO_2 (1:1:1)	DL
Lactobacillus plantarum	Homofermentative	Lactate	D(−), L(+), DL
	Heterofermentative[b]	Lactate/acetate (1:1)	D(−), L(+), DL
Lactobacillus bavaricus	Homofermentative	Lactate	L(+)

Source: Ref. 7.

[a] With respect to hexose fermentation

[b] Heterofermentative with respect to pentoses (facultatively heterofermentative).

tarum, which produces mainly lactic acid and can result in a harsh, too-acidic product. However, *L. mesenteroides* (a heterofermentor) produces CO_2 (a gas) that can cause bloater damage (gas pockets) in fermented cucumbers. For this reason, *L. plantarum* or *Pediococcus pentosaceus* (homofermentor of hexoses, ie, produces only lactic acid and no CO_2) is preferred for cucumber fermentations. Fortuitously, the texture of cabbage is retained at relatively low NaCl concentrations (1.5–2.5%), which is conducive to growth by *L. mesenteroides*. The higher salt concentration required for texture retention of fermented cucumber (5–8%) prevents growth by *L. mesenteroides*, but not *L. plantarum*, which is a preferred species to dominate cucumber fermentation because of its acid tolerance and homofermentative metabolism.

NUTRITIVE VALUE

The chief nutritive value of vegetables is in their content of vitamins, which may include important amounts of β-carotene, ascorbic acid, and folic acid, and less important amounts of riboflavin and some of the other B vitamins. Vitamin B_{12} is not present in vegetables. Vegetables used for fermentation are an important source of fiber, but contain low levels of protein and calories.

The effect of brining and fermentation on the nutritive value of vegetables has been reviewed (8–10); however, relatively little information is available on the subject. Lactic acid bacteria conventionally important in vegetable fermentations are nutritionally fastidious and would not be expected to increase essential nutrients during fermentation.

Although microbial action may alter the nutrient content of vegetables during fermentation, other factors may significantly influence the retention of nutrients during storage and further processing. If the vegetables are brined at high levels of salt, large losses in nutrients will result when the vegetables are subsequently desalted before use. From a nutritive standpoint, therefore, it would be preferable to brine vegetables at a low level of salt. Unfortunately, softening and other spoilage problems dictate the use of higher levels of salt in some vegetables than would otherwise be desirable. There is nearly complete loss of ascorbic acid from salt-stock cucumbers, which are stored at 8 to 16% salt, when the cucumbers are desalted to 2 to 4% salt for use in finished products. Fellers (9) reported 86, 82, and 28% losses for vitamin C (ascorbic acid), thiamin, and carotene, respectively, in desalted salt-stock cucumbers; but, in genuine dills, which are not desalted, 33 to 60% vitamin C retention was found. Sauerkraut requires only 2 to 3% salt for preservation and, therefore, is not desalted before use. Thus, vitamin C is largely retained during storage of sauerkraut, but may be diminished during later processing, depending upon exposure to air and the extent of heating.

Ascorbic acid and thiamin are stabilized by acid and exclusion of oxygen. Because fermented vegetables are normally held under such conditions, good retention of these vitamins would be expected. Current nutritional labels on commercial sauerkraut show a usual ascorbic acid content of 50% of the U.S. RDA per 100 g serving.

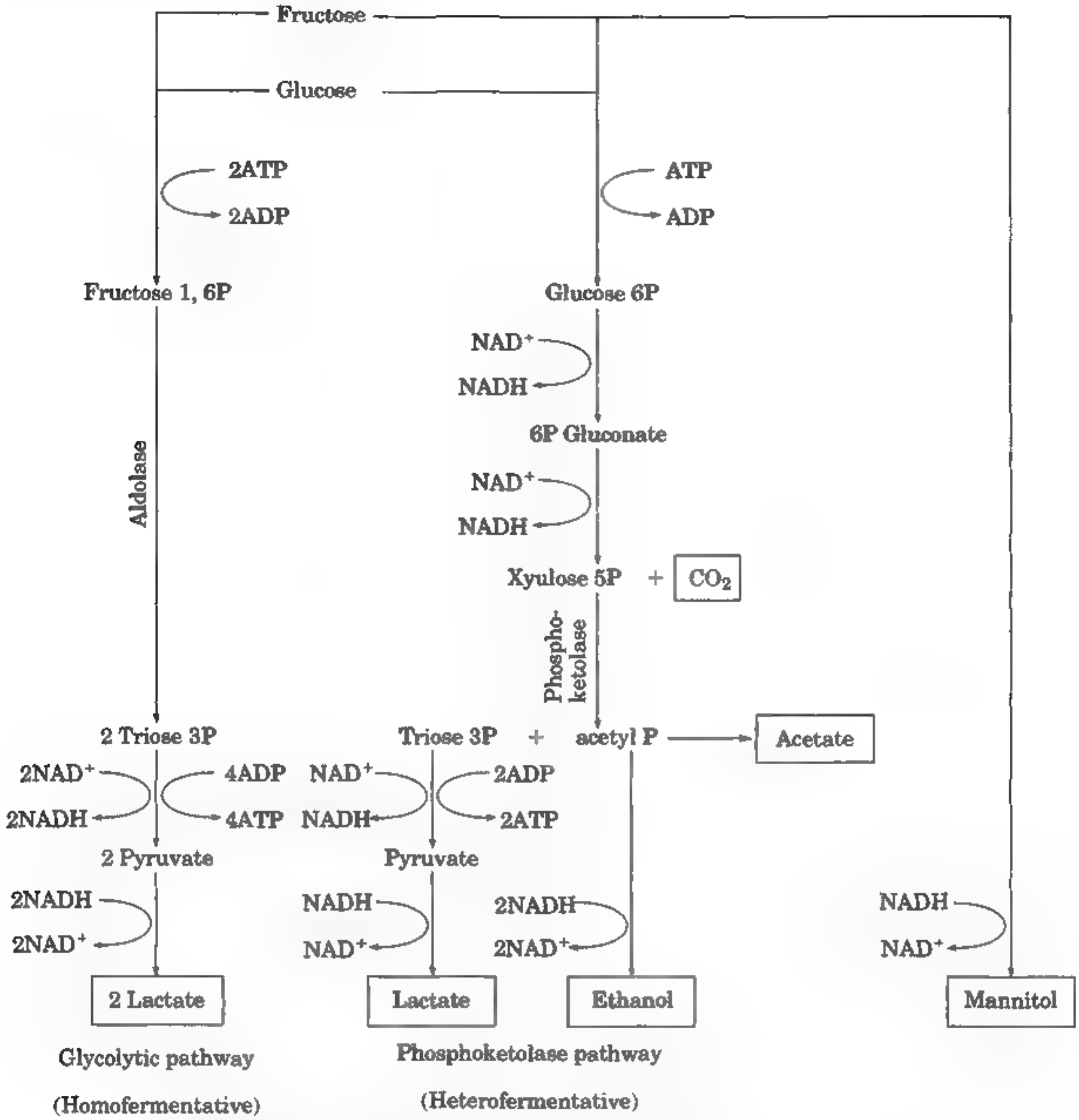

Fructose
Glucose
2ATP
2ADP
ATP
ADP
Fructose 1, 6P
Glucose 6P
NAD⁺
NADH
6P Gluconate
NAD⁺
NADH
Aldolase
Xyulose 5P + CO₂
Phospho-ketolase
2 Triose 3P
Triose 3P + acetyl P
Acetate
2NAD⁺
2NADH
4ADP
4ATP
NAD⁺
NADH
2ADP
2ATP
2 Pyruvate
Pyruvate
2NADH
2NAD⁺
NADH
NAD⁺
2NADH
2NAD⁺
NADH
NAD⁺
2 Lactate
Lactate
Ethanol
Mannitol
Glycolytic pathway
(Homofermentative)
Phosphoketolase pathway
(Heterofermentative)

Figure 2. Major pathways for sugar fermentation by lactic acid bacteria. *Source:* Ref 12.

SHELF LIFE AND BULK STORAGE

The shelf life of pasteurized pickles typically is 12 to 18 months, depending on storage temperature (22°C or below preferred). The shelf life of refrigerated pickles varies from a few weeks (nonacidified type) to several months (acidified type, with preservatives). The shelf life of sauerkraut depends largely on the integrity of the can. With enamel-lined cans, the shelf life typically is 18 to 30 months. Sauerkraut packaged in glass or plastic bags usually contains preservatives such as potassium bisulfite (for maintaining a light color) and sodium benzoate or potassium sorbate (as microbial inhibitors), which results in a shelf life of 8 to 12 months.

Fermented cucumbers held at 8% NaCl or higher before being processed into finished products can be held for a year or longer. The storage life is extended at higher salt concentrations. Sauerkraut is held at relatively low salt concentration (1.5–2.5%) in bulk storage, and typically is held for up to a year (United States) or only for a month or so (Europe).

SAFETY

There are no authenticated reports of pathogenic microorganisms associated with standard commercial pickle products prepared under good manufacturing practices of acid, salt, and sugar content (and combinations thereof) from brined, salted, and pickled vegetable brine-stock, including cucumbers. The Commissioner of the FDA stated that "No instances of illness as the result of contamination of commercially processed fermented foods with *Clostridium botulinum* have been reported in the United States" (11). Essentially the same pattern of consumer safety applies to fresh-pack (pasteurized) pickle products. The pasteurization process calls for the packed product to be acidified at the outset with a sufficient amount of food-grade organic acid (eg, vinegar, acetic acid, or lactic acid) to result in an equilibrated brine product pH of 4.0 or below (preferably 3.8). Vinegar (acetic acid) is usually the acidulant of industry choice for cucumber pickle products. The basic pasteurization procedure, with acidified product heated to an internal temperature of 74°C and held for 15 min, has been used successfully by industry since about 1940. Insufficient acidification of pasteurized pickles can result in butyric-acid-type spoilage, possibly involving public health concerns.

STARTER CULTURES

Currently, most vegetable fermentations rely on the natural microflora, although use of starter cultures has been suggested by various authors. Various reasons have been proposed to explain the lack of commercial use of cultures, including economics, lack of sufficiently unique and valuable properties to justify their use, and the fact that vegetables will undergo a natural lactic fermentation under proper environmental conditions (12). However, recent research with cucumbers, sauerkraut, and olives indicate that use of special cultures for vegetable fermentations may find application in the near future. Examples of novel properties of lactic acid bacteria cultures that make them potentially useful in commercial vegetable fermentations:

- *Inability to degrade malic acid to lactic acid and CO_2.* A culture of *L. plantarum* was developed by mutation and selection for use in cucumber fermentation. Because this culture does not produce CO_2 from malic acid, the natural acid present in cucumbers, it is being evaluated for use to ferment whole cucumbers to avoid the need for purging to prevent bloater damage.
- *Inability to produce biogenic amines.* Biogenic amines such as histamine are formed by decarboxylation of amino acids. Selection of cultures lacking this ability has been considered.
- *Exclusive l(+)-lactic acid formation.* Health concerns have been raised about the production of D(-)-lactic acid (10). Starter cultures that produce only L(+)-lactic acid have been developed.
- *Bacteriocin production.* Cultures of lactic acid bacteria that produce nisin and other bacteriocins have been isolated and may prove useful in inhibiting the growth of undesired bacteria in vegetable fermentations.
- *Bacteriophage resistance.* Bacteriophage sensitivity of starter cultures for use in preparing fermented dairy products is a serious concern. Although bacteriophage problems are not currently apparent with fermented vegetables, the problem may become significant when the use of starter cultures becomes more prominent.

BIBLIOGRAPHY

1. C. S. Pederson, *Microbiology of Food Fermentations*, 2nd ed., AVI Westport, Conn., 1979.
2. C. H. Miller and T. C. Wehner, "Cucumbers," in N. A. M. Eskin, ed., *Quality and Preservation of Vegetables*, CRC Press, Boca Raton, Fla., 1989, pp. 245–264.
3. H. P. Fleming and W R. Moore, "Pickling," in I. A. Wolff, ed., *CRC Handbook of Processing and Utilization in Agriculture*, Vol. II, CRC Press, Boca Raton, Fla., 1983, pp. 397–463.
4. R. J. Monroe et al., "Influence of Various Acidities and Pasteurizing Temperatures on the Keeping Quality of Fresh-Pack Dill Pickles," *Food Technol.* **23**, 71–77 (1969).
5. M. H. Dickson and D. H. Wallace, "Cabbage Breeding," in M. A. Bassett, ed., *Breeding Vegetable Crops*, AVI, Westport, Conn., 1986, pp. 395–432.
6. H P. Fleming, K. H. Kyung, and F. Breidt, "Vegetable Fermentations," in H. J. Rehm and G. Reed, eds., *Biotechnology*, Vol. 9, VCH, New York, 1995, pp. 629–661.
7. O. Kandler, "Carbohydrate Metabolism in Lactic Acid Bacteria," *Antonie van Leeuwenhoek* **49**, 209–224 (1983).
8. I. D. Jones and J. L. Etchells, "Nutritive Value of Brined and Fermented Vegetables," *Am. J. Public Health* **34**, 711–718 (1944).
9. C. R. Fellers, *Effects of Fermentation on Food Nutrients*, in R. S. Harris and H. Von Loesecke, eds., AVI, Westport, Conn., 1960, p. 161.
10. R. F. McFeeters, "Effects of Fermentation on the Nutritional Properties of Food," in E. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., Van Nostrand, New York, 1988, pp. 423–446.
11. FDA, "Acidified Foods and Low-Acid Canned Foods in Hermetically Sealed Containers," *U.S. Food Drug Admin.*, Fed. Reg. **44**, 16204, 1979.
12. H. P. Fleming, R. F. McFeeters, and M. A. Daeschel, "The Lactobacilli, Pediococci, and Leuconostocs: Vegetable Products," in S. E. Gilliland, ed., *Bacterial Starter Cultures for Foods*, CRC Press, Boca Raton, Fla., 1985, pp. 97–118.

GENERAL REFERENCES

S. Davidson et al., *Human Nutrition and Dietetics*, Churchill Livingstone Inc., New York, 1979.

J. L. Etchells, T. A. Bell, and W. R. Moore, Jr., "Refrigerated Dill Pickles—Questions and Answers," *Pickle Pak Science* **5**, 1–20 (1976).

H. P. Fleming and R. F. McFeeters, "Use of Microbial Cultures: Vegetable Products," *Food Technol.* **35**, 84–88 (1981).

H. P. Fleming, R. F. McFeeters, and M. A. Daeschel, "Fermented and Acidified Vegetables," in C. Vanderzant and D. F. Splittstoesser, eds., *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed., American Public Health Association, Washington, D.C., 1992, pp. 929–952.

H. O. Lee and I. W. Yang, "Studies on the Packaging and Preservation of Kimchi," *J. Korean Agricultural Chemical Soc.* **13**, 207–210 (1975).

J. R. Stamer, "Lactic Acid Fermentation of Cabbage and Cucumbers," in H. J. Rehm and G. Reed, eds., *Biotechnology*, Vol. 5, Verlag Chemie, Weinheim, Germany, 1983, pp. 365–378.

J. R. Stamer and B. O. Stoyla, "Stability of Sauerkraut Packaged in Plastic Bags," *J. Food Protection* **41**, 525–529 (1978).

HENRY FLEMING
USDA/ARS
Raleigh, North Carolina

VEGETARIAN AND VEGETARIAN-AWARE EATING TRENDS

Today, more Americans are willing to make meatless choices, at least occasionally, than were willing to do so in the past. Vegetarian menus are more acceptable among shoppers today, and they are seen as a healthful and smart way of eating. Americans are not giving up meat altogether, but they are no longer as loyal to meat on the menu. They are seeking solutions for preventing disease and maintaining daily good health through vegetarian food choices.

Two percent of shoppers say they always maintain a vegetarian diet, and 26% say they usually (7%) or sometimes (19%) do so. This 28% of shoppers drives demand for vegetarian products on grocery shelves, in restaurants, and at school cafeterias.

Growth in the market for vegetarian products in recent years has been largely fueled by vegetarian-aware shoppers; the number of true vegetarian shoppers has remained relatively stable. Not interested in avoiding meat altogether; vegeterian-aware shoppers eat meatless meals more often for health reasons; they seek out fruits, vegetables, and whole grains and adopt meatless burgers, soy hot dogs, and other vegetarian choices.

WHAT VEGETARIAN MEANS

As a dietary habit or lifestyle, vegetarianism includes a spectrum of dietary choices:

- Vegans do not eat any animal-derived products.
- Ovo-vegetarians do not eat meats or dairy products, but do eat eggs.
- Lacto-ovo-vegetarians do not eat meats, but do eat eggs and dairy products.
- Pesco-vegetarians do not eat meats, but do eat eggs, dairy products, and fish.
- Semi-vegetarians do not eat red meats, but do eat eggs, dairy products, fish, and poultry.
- Vegetarian-aware shoppers do not avoid meats, dairy products, or eggs altogether, but are trying to eat meatless meals more often.

A BRIEF HISTORY

Vegetarianism has existed for centuries, often as a tenant of eastern religions and philosophies. In the United States, vegetarianism has Christian roots. Reverend William Metcalfe introduced vegetarianism to the United States in 1817. In 1863, the Seventh-Day Adventist church was founded, and members adopted eating guidelines prohibiting meat, tobacco, alcohol, tea, and coffee consumption. Around the turn of the century, Dr. John Harvey Kellogg, of cereal fame, founded a sanitarium in Battle Creek, Michigan, that advocated vegetarianism and healthy living. During the 1960s, vegetarianism became tied to the counter-culture as environmental issues fueled new interest in vegetarian eating habits. In 1971, Frances Moore Lappé harshly examined the meat industry and the resources necessary to support it in her book, *Diet for a Small Planet*. An immensely popular book, *Diet for a Small Planet* increased awareness of the environmental reasons for becoming a vegetarian among the American public.

NUTRITIONALLY MISUNDERSTOOD

Until recently, the health benefits of vegetarian diets have been largely misunderstood. Just 25 years ago, vegetarian diets were predicted to give rise to osteoporosis, anemia, and protein deficiencies. Vegetarian eating habits are now nutritionally acceptable and are generally recognized as healthy. Although vegetarian once meant counter-culture, vegetarian today is viewed by mainstream consumers as a smart way to eat healthfully. Male and female vegetarians have significantly less occurrence of cancer, heart disease, and obesity.

Once the domain of health food or other specialty brands, major brands such as Green Giant and Stouffer's now offer vegetarian frozen burgers and dinners. Two icons of American culture, Walt Disney World and Denny's, have vegetarian menu options in their restaurants.

VEGETARIAN-AWARE SHOPPERS

Vegetarian-aware shoppers are interested in eating less meat, primarily because of their interest in eating less fat and eating more vegetables, fruits, whole grains, and beans.

This term was coined by HealthFocus, a market research and consulting firm based in Des Moines. HealthFocus has conducted their National Study of Public Attitudes and Actions Toward Shopping and Eating every 2 years since 1990. The national survey is conducted in two stages: telephone recruitment and a mailed questionnaire. More than 300 questions are asked. More than 2000 people participated in 1996 and 1994, and more than 1000 people participated in 1992 and 1990.

The survey objectives are to

- Identify current issues in consumer health and nutrition attitudes and actions, such as avoiding red meats and eating more vegetables.
- Assess trends in consumer priorities regarding eating habits and nutrition issues, from price and convenience to better nutrition.
- Examine where consumers are headed in their behavior toward health and diet.
- Determine what nutritional issues will be most important to shoppers in the near future.

MEAT ON THE MENU

It important to recognize that vegetarian and vegetarian-aware shoppers are not giving up meat altogether; rather, they are viewing meat as a less prominent aspect of their meals. Some shoppers say, "I'm a vegetarian; I only eat chicken and never beef or pork." This is not really being a true vegetarian.

According to the 1996 HealthFocus Study, both vegetarian shoppers and vegetarian-aware shoppers still eat meat on occasion, although vegetarian shoppers are far less likely to eat meat. Fish and seafood are the most widely used meats among vegetarian and vegetarian-aware shoppers, followed by poultry.

Almost one-third (31%) of vegetarian shoppers always or usually avoid dairy products and ingredients; 79% always or usually avoid foods with red meat. Still, only 78% of shoppers who say they always maintain a vegetarian diet eat meatless meals twice a week or more often (Fig. 1).

MARKET SIZE

According to the HealthFocus Survey, almost 30% of American shoppers, shopping for 25.7 million households, are choosing vegetarian or meatless meals. Since 1990, the number of vegetarian shoppers has remained relatively stable, with about 2% of all shoppers always maintaining a vegetarian diet.

The number of shoppers making vegetarian-aware choices is increasing. In 1996, 26% of shoppers maintain a vegetarian diet usually (7%) or sometimes (19%), up from 24% of shoppers in 1994. The number of shoppers maintaining a vegetarian diet at least on occasion increased by 6 percentage points, from 48% in 1994 to 54% in 1996 (Table 1).

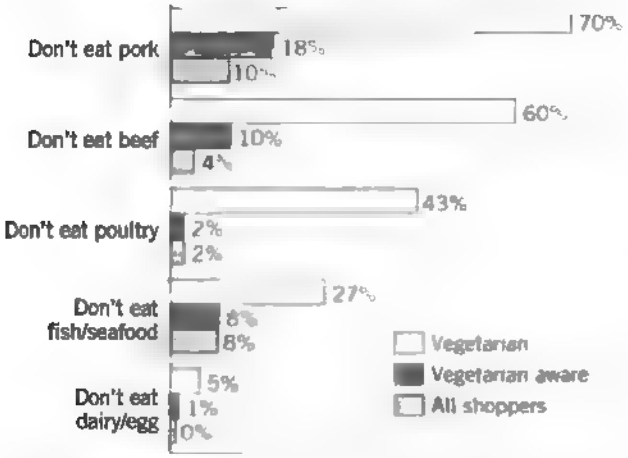


Figure 1. Meat and dairy consumption as reported by respondents.

Table 1. How Many Vegetarians Are There?

	1994 (%)	1996 (%)	No. of households (1996)
Vegans	0.5	0.5	460,000
Vegetarians	1.5	1.5	1,380,000
Vegetarian aware	24	26	23,920,000
Total	*26*	*28*	*25,760,000*

DEMOGRAPHICS OF VEGETARIAN AND VEGETARIAN-AWARE SHOPPERS (TABLE 2)

Men are somewhat more likely to be vegetarian shoppers, and women are more likely to be vegetarian-aware shoppers: 3% of men always maintain a vegetarian diet, compared to 2% of women. Twenty-six percent of women usually or sometimes maintain a vegetarian diet, compared to 21% of men.

Vegetarian shoppers tend to be younger: 21% are 18–29 years old, compared to 12% of shoppers overall in this age group. They are also more likely to have young children at home: 42% have children younger than 2 years of age, and 42% have children 3 to 6 years of age.

In contrast, vegetarian-aware shoppers are older: 26% are 50–64 years old, compared to 23% of all shoppers in this age group; 22% are over age 65, compared to 17% of shoppers overall. Vegetarian-aware shoppers have older children at home: 47% have teenagers, compared to 44% of all shoppers and 42% of vegetarian shoppers.

Shoppers making vegetarian and vegetarian-aware choices are somewhat better educated than other shoppers. Forty percent of vegetarian shoppers and 36% of vegetarian-aware shoppers have at least a college degree; 32% of all shoppers have college degrees.

Vegetarian-aware shoppers live in higher income households than vegetarian shoppers, likely because of their age and experience in the workplace. Vegetarian shoppers have a median household income of $29,000, and vegetarian-aware shoppers have a median household income of $37,800.

Geographically, vegetarian and vegetarian-aware shoppers live in highest concentrations in the midwest region of the United States. Vegetarian shoppers are most likely to live in the North Central (33%), Pacific (21%), Mid-Atlantic (14%), and New England (12%) regions. Vegetarian-aware shoppers are most likely to live in the North Central region (27%).

Compared to the general population, vegetarian shoppers are found in lower concentrations in the South Central (9%) and Mountain (2%) regions. Vegetarian-aware shoppers are found in higher concentrations in the South Atlantic (18%) and South Central (19%) regions.

DIETARY PRIORITIES

The most significant aspect of the vegetarian and vegetarian-aware consumer market is their strong com-

Table 2. Selected Demographics of Vegetarian Shoppers

	Vegetarian	Vegetarian aware	All shoppers
Men	23%	16%	19%
Women	77%	84%	81%
Adult (18–39 years)	42%	29%	35%
Mid-Life (40–55 years)	33%	36%	35%
Prime (56–64 years)	16%	13%	12%
Senior (65+ years)	9%	22%	18%

mitment to healthy eating habits. Healthy eating is more important to vegetarian and vegetarian-aware shoppers, compared to shoppers overall. Their attitudes are extreme and their behavior is more consistent, compared to other shoppers (Fig. 2).

Fifty percent of vegetarian shoppers and 24% of vegetarian-aware shoppers strongly agree that their diet is very important to them, compared to 16% of all shoppers. Fifty-eight percent of vegetarian shoppers and 32% of vegetarian-aware shoppers strongly agree that they think about the healthfulness or nutritional value of what they eat, compared to just 24% of all shoppers.

Thirty-six percent of vegetarian shoppers and 17% of vegetarian-aware shoppers always select foods for healthy reasons. Forty-eight percent of vegetarian and 36% of vegetarian-aware shoppers always read labels on food packages, compared to 27% of all shoppers.

HEART-HEALTHY, LOWFAT, AND LOW-CALORIE DIETS

Eighty percent of vegetarian shoppers and 67% of vegetarian-aware shoppers always or usually maintain a heart-healthy diet, compared to about half of all shoppers who place similar importance on heart-healthy eating habits. Seventy-four percent of vegetarian shoppers and 65% of vegetarian-aware shoppers always or usually maintain a lowfat diet (Fig. 3).

Vegetarian and vegetarian-aware shoppers also put a higher priority on maintaining a low-calorie diet. Sixty percent of vegetarians and 43% of vegetarian-aware shoppers always maintain a low-calorie diet, compared to 26% of shoppers overall.

This emphasis on health, rather than on religious or environmental issues, has meant that many longstanding vegetarian brands and products have had to reformulate and reposition themselves to address heart-healthy, lowfat, low-calorie diets. For many consumers, the simplest way to cut back on fat is to cut back on meats. These choices are preventive measures that are motivated by interest in better future health.

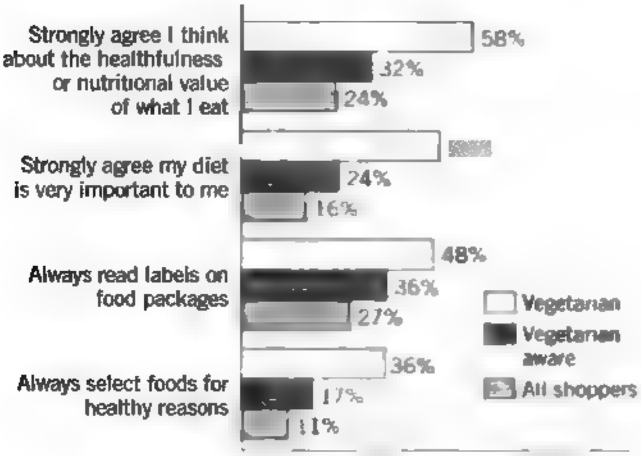


Figure 2. Dietary priorities among respondents.

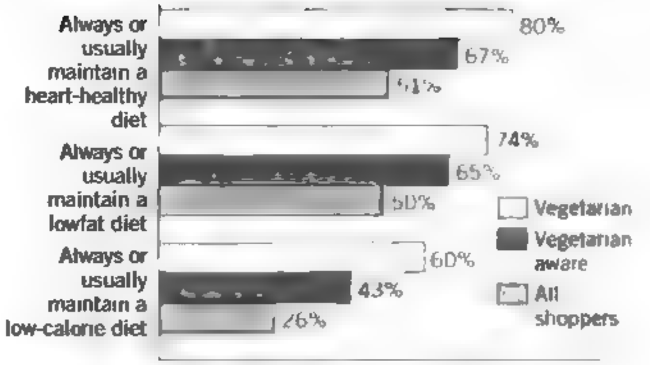


Figure 3. Importance of lowfat eating habits among respondents.

HEALTH ISSUES

Ensuring future good health and managing weight are the primary reasons Vegetarian and Vegetarian Aware Shoppers make healthy choices (Fig. 4). Ensuring future good health is the primary reason for choosing healthy foods for 67% of vegetarian-aware shoppers, 65% of all shoppers, and 56% of vegetarian shoppers.

Although philosophical or spiritual reasons are strong motivators for those strictly vegetarian (18%), the secondary reason vegetarian shoppers (24%) choose healthy foods is to lose weight. Only 10% of vegetarian-aware shoppers and 13% of all shoppers select healthy foods to lose weight.

The secondary reason vegetarian-aware shoppers (16%) choose healthy foods is to control existing health problems.

HEALTH CONCERNS

Vegetarian and vegetarian-aware shoppers have more extreme concerns about future health problems, compared to

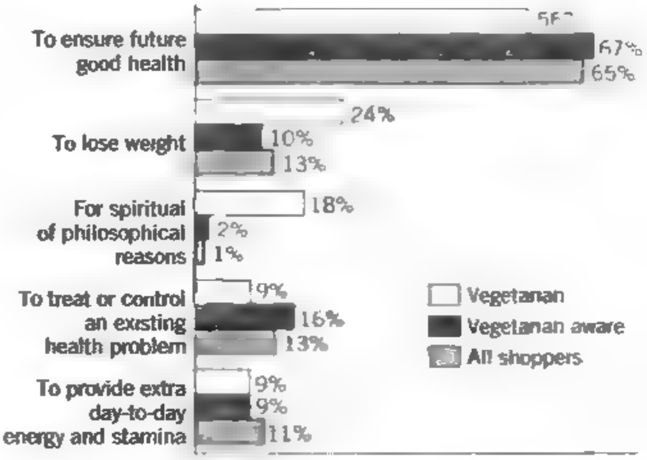


Figure 4. Primary reason for choosing healthy foods.

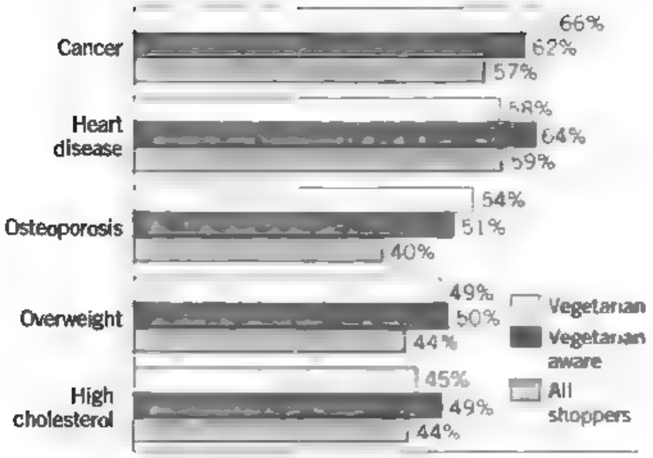


Figure 5. Health issues respondents are extremely or very concerned about.

shoppers overall. Cancer tops the list of health concerns, followed by heart disease, osteoporosis, being overweight, and having high cholesterol (Fig. 5).

Vegetarians have the highest cancer concerns. Sixty-six percent of vegetarians are extremely or very concerned about cancer, compared to 62% of vegetarian-aware shoppers and 57% of shoppers overall. They also express high concerns about osteoporosis and their weight.

Vegetarian-aware shoppers are especially concerned about heart disease and high cholesterol, more so than vegetarians are. Sixty-four percent of vegetarian-aware shoppers are extremely or very concerned about heart disease, compared to 58% of vegetarians and 59% of shoppers overall. Similarly, 49% of vegetarian-aware shoppers are extremely or very concerned about high cholesterol, compared to 45% of vegetarians and 44% of shoppers overall.

HEALTH PROBLEMS

The comparatively low levels of concern about heart disease and high cholesterol among vegetarian shoppers is probably in part a reflection of their better health status (Fig. 6). Sixty-five percent of vegetarian shoppers describe their health as excellent or very good, compared to 53% of vegetarian-aware shoppers and 53% of shoppers overall.

Vegetarian shoppers are significantly less likely to be personally affected by health problems than are other shoppers. Three exceptions are lactose intolerance, diabetes, and arthritis, where vegetarian choices may be helping to manage the health problem once it occurs or is diagnosed. Younger and more attentive to heart-healthy eating habits, vegetarian shoppers are relatively less likely to report being overweight, having high cholesterol levels or hypertension, or having allergies or stress, compared to vegetarian-aware shoppers and shoppers overall.

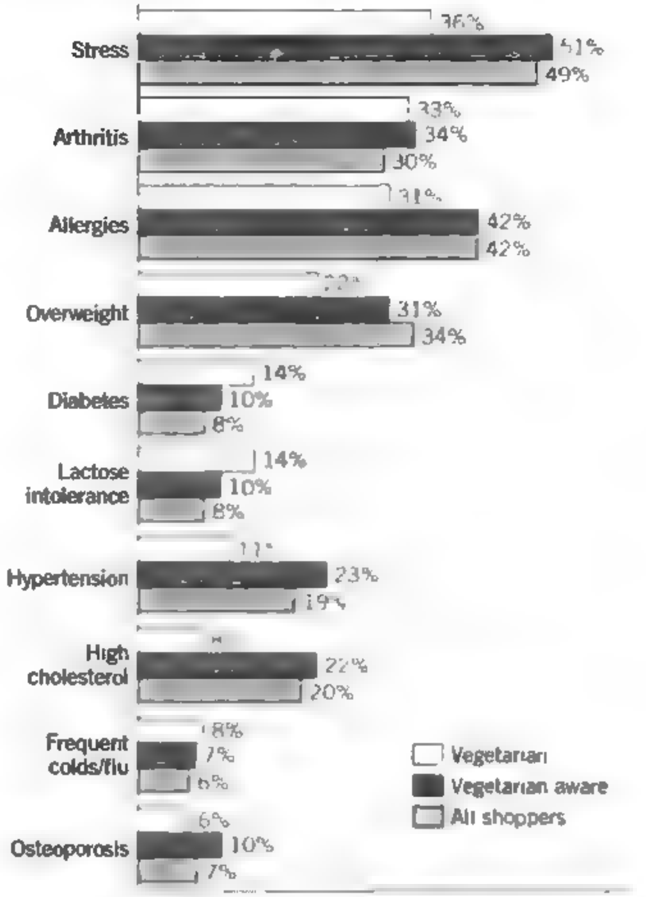


Figure 6. Examples of health problems personally affecting respondents.

Vegetarian-aware shoppers are somewhat more likely than all shoppers to report hypertension or high cholesterol levels, stress, diabetes, arthritis and osteoporosis, but they are less likely than all shoppers to report being overweight. They are equally likely to report allergies.

Stress, arthritis, and allergies are the most common health problems reported by vegetarian shoppers. Thirty-six percent of vegetarian shoppers are affected by stress, 33% have arthritis, and 31% have allergies. Osteoporosis, frequent colds and flus, and high cholesterol levels are the least widespread.

The most common health problems among vegetarian-aware shoppers are stress, allergies, and arthritis. Fifty-one percent of vegetarian-aware shoppers have stress, 42% report allergies, and 34% have arthritis. Frequent colds and flus, diabetes, lactose intolerance, and osteoporosis are their least common health problems.

SHOPPING HABITS

Just as vegetarian and vegetarian-aware shoppers differ in their nutrition attitudes and health priorities, they have different shopping habits (Fig. 7). Both shop at natural

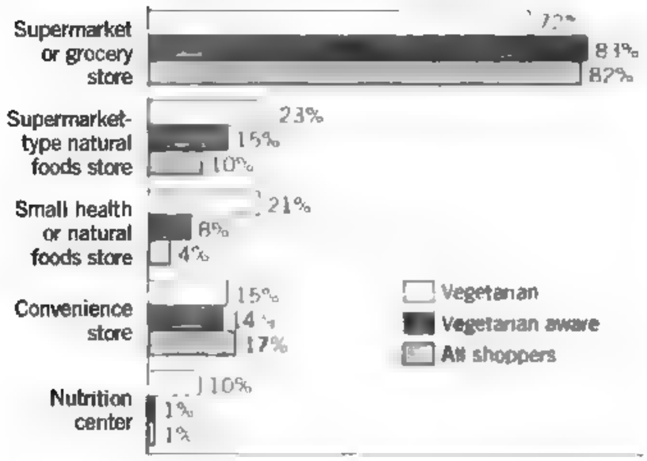


Figure 7. Shopping habits of respondents. Percent of those shopping one or more times per week at various stores.

foods and health food stores more often than shoppers overall, but vegetarian shoppers are much more likely than vegetarian-aware shoppers to go often and to go to nutrition centers. Vegetarian shoppers go to the supermarket less often: 72% visit their supermarket weekly, compared to 83% of vegetarian-aware shoppers and 82% of shoppers overall.

Twenty-three percent of vegetarian shoppers go to a supermarket-type natural foods store weekly, and 21% go to a small health or natural foods store as often. Fifteen percent of vegetarian-aware shoppers go to a supermarket-type natural foods store weekly, and 8% go to a small health or natural foods store. This compares to 10% of all shoppers who go to a supermarket-type natural foods store weekly, and 4% who go to a small health or natural foods store.

Ten percent of vegetarian shoppers frequent nutrition centers (stores specializing in vitamins and supplements) weekly; 1% of vegetarian-aware shoppers and 1% of shoppers overall shop at nutrition centers at least once a week.

Surprisingly, vegetarian shoppers are about as likely as shoppers are overall to shop at a convenience store at least once a week. Fifteen percent of vegetarian shoppers, 14% of vegetarian-aware shoppers, and 17% of all shoppers make the convenience store stop weekly.

PACKAGE INFORMATION

When shopping, vegetarian shoppers are label readers: 79% always or usually read labels on packages. Seventy-six percent of vegetarian-aware shoppers and 66% of all shoppers do the same.

The freshness date is the most important part of the package labeling, followed by the nutrition fact box and ingredient statement. Vegetarian shoppers give extreme importance to this label information. Seventy percent consider freshness dating extremely important, 60% consider the nutrition fact box extremely important, and the same number say the ingredient statement is extremely important.

Freshness dating is extremely important to 60% of vegetarian-aware shoppers and to 58% of shoppers overall. Forty-six percent of vegetarian-aware shoppers and 36% of shoppers overall consider the nutrition fact box extremely important. Forty-three percent of vegetarian-aware shoppers and 33% of shoppers overall consider the ingredient statement extremely important.

Other important label information includes content claims and health claims, a customer service or 800 number, and recipes. Thirty-four percent of vegetarian shoppers consider a customer service number on the label extremely important, compared to 22% of vegetarian-aware shoppers and 17% of all shoppers. Twenty-six percent of vegetarian shoppers like recipes on the package and consider them extremely important. Half as many vegetarian-aware shoppers give recipes this level of importance, and only 11% of shoppers overall do so.

LABEL CLAIMS

Vegetarian shoppers are significantly more interested in and influenced by health claims and content claims than are vegetarian-aware shoppers or shoppers overall. Content claims on labels are extremely important to 43% of vegetarian shoppers and 31% of vegetarian-aware shoppers, compared to only 25% of shoppers overall. Health claims are important to 37% of vegetarian shoppers and 24% of vegetarian-aware shoppers, compared to only 19% of shoppers overall.

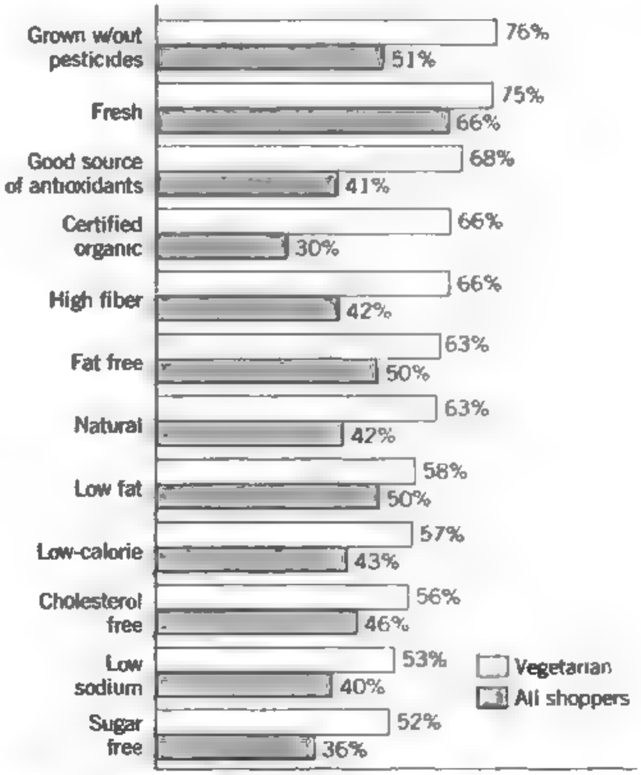


Figure 8. Label claims considered extremely or very important to vegetarian shoppers.

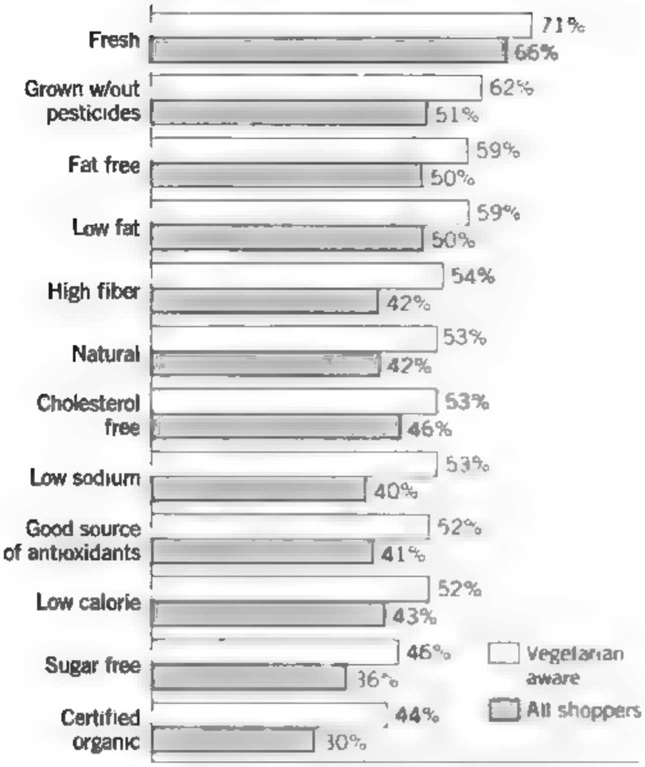


Figure 9. Label claims considered extremely or very important to vegetarian-aware shoppers.

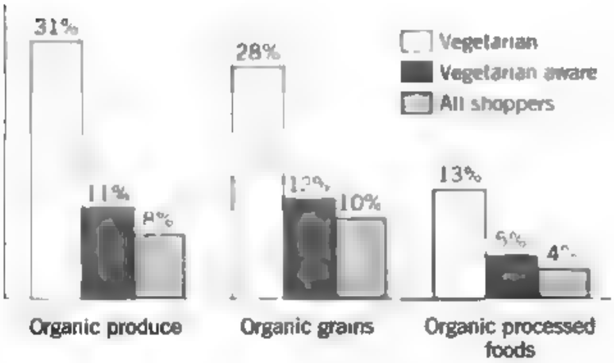


Figure 10. Percent of respondents using organic foods twice a week or more often.

The differing health priorities among vegetarian and vegetarian-aware shoppers are apparent in their label interests (Figs. 8 and 9). Vegetarian shoppers give the most importance to labels reading "grown without pesticides," "fresh," "good source of antioxidants," and "certified organic." Vegetarian-aware shoppers have more mainstream interests, giving the most importance to labels reading "fresh," "grown without pesticides," "fat free," and "low fat."

NATURAL AND ORGANIC

Just as vegetarian and vegetarian-aware shoppers are more likely to shop at health and natural foods stores, they are more likely to look for brands and to support companies that share their social, environmental, and other values. Seventy-nine percent of vegetarian shoppers strongly agree or agree that environmental issues have had an impact on their purchases, compared to 64% of vegetarian-aware shoppers and 51% of shoppers overall. Vegetarian (73%) and vegetarian-aware shoppers (61%) prefer to buy products from companies that support their social, community, or environmental interests.

This social and environmental sense of responsibility is reflected at the checkout counter in organic foods purchases. Thirty-one percent of vegetarian shoppers use organic produce twice a week or more, compared to only 11% of vegetarian-aware shoppers and 8% of all shoppers. Twenty-eight percent of vegetarian shoppers use organic grains twice a week or more, compared to 12% of vegetarian-aware shoppers and 10% of all shoppers. Organic processed foods are used twice a week or more by 13% of vegetarian shoppers, 5% of vegetarian-aware shoppers and 4% of all shoppers.

A GROWING MARKET

The use of vegetarian products and meatless meal options is growing and is expected to continue to grow, driven by consumer interest in healthier eating and scientific evidence supporting the importance of more produce and grains in the diet and less meat. Fifty-four percent of all shoppers in 1996 report they eat a vegetarian diet at least on occasion, up from 48% just 2 years previously. Nine percent of shoppers call themselves vegetarian, although fewer than 2% practice strict vegetarian eating habits including no meat, no eggs, and no dairy products.

In 1996, 40% of all shoppers reported eating meatless meals such as pastas or salads weekly, which is up from 13% in 1990.

Meat substitutes are also becoming more popular with American shoppers. Fifteen percent of all shoppers use these products weekly, up from 9% in 1994 and 7% in 1992.

The weekly use of soy foods such as tempeh or tofu has also increased, from 3% of shoppers in 1992 and 4% in 1994 to 5% in 1996.

Clearly, use of vegetarian products and meatless meal options is growing and will continue to grow. Market growth will be driven by consumer interest in healthier eating and fueled by scientific evidence supporting the importance of more produce and grains in the diet and less meat.

BIBLIOGRAPHY

All data are taken from the 1996 HealthFocus Survey, HealthFocus, Inc., Des Moines, Iowa.

LINDA GILBERT
HealthFocus, Inc.
Des Moines, Iowa

VITAMINS

Vitamins: Survey 2440

Vitamins: Ascorbic Acid 2449

Vitamins: Biotin 2467

Vitamins: Folic Acid 2478

Vitamins: Niacin, Nicotinamide, and Nicotinic Acid 2487

Vitamins: Pantothenic Acid 2496

Vitamins: Pyroxidine (B_6) 2505

Vitamins: Riboflavin (B_2) 2515

Vitamins: Thiamine (B_1) 2527

Vitamins: Vitamin A 2538

Vitamins: Vitamin B_{12} 2551

Vitamins: Vitamin D 2562

Vitamins: Vitamin E 2585

Vitamins: Vitamin K 2592

VITAMINS: SURVEY

Vitamins are specific organic compounds that are essential for normal metabolism. Many participate as cofactors or coenzymes in mammalian biochemical reactions. The common thread for the diverse chemical structures of the vitamins is that they are micronutrients. Micronutrients are compounds that are required in only small amounts and are not synthesized by humans, either at all or, at least, in sufficient quantity for metabolic needs. Vitamins are obtained from the diet or as synthetic preparations used in food fortification or supplements.

The term vitamin is derived from *vitamine*, a word created in 1911 (1) when it was believed that the antiberiberi factor (thiamine) was an amine essential to the maintenance of life (*vita*). Later discoveries showed that not all vitamins are amines. However, a form of the name has remained.

The history of the vitamins can be divided into five major, overlapping periods (2).

PREHISTORY TO CA 1900

In this period, the empirical healing of certain diseases by foods was established. Examples (3) were the treatment of night blindness (vitamin A deficiency) with liver in many cultures over centuries, of beriberi (vitamin B_1 deficiency) by use of unpolished rice by the Japanese navy, of scurvy (vitamin C deficiency) by citrus fruits in the British navy or pine needle extracts by North American natives, and pellagra (niacin deficiency) by a dietary shift away from corn-based foods in many countries. Other, nondietary empirical treatments involved, eg, exposure of children in northern latitudes to sunlight to cure rickets (vitamin D deficiency) (4).

1880–1905

The ability to induce disease states in animals by manipulation of the diet was established in this period. The classical work by Eijkman (5), in which a beriberi-like condition was induced in chickens fed on polished rice, was significant. These findings led to the concept by Hopkins that small amounts of accessory growth factors are necessary for survival and growth.

1900–1972

During this 70 year period, all 13 of the substances now recognized as vitamins were discovered and isolated in pure form. Structure elucidation for each vitamin was completed, as was its total synthesis (Table 1).

1933–PRESENT

The first commercial synthesis of a vitamin occurred in 1933 when the Reichstein approach was employed to manufacture vitamin C (6). All 13 vitamins are available in commercial quantities, and their biological functions have largely been established (7). A list of Nobel prize winners associated with vitamin research is given in Table 2.

1955–PRESENT

The possibility that vitamins might have physiological functions beyond the prevention of deficiency diseases was first recognized in 1955 with the finding (8) that niacin can affect serum cholesterol levels in humans. An explosion of research (9–11) in the intervening years has been aimed at establishing optimal vitamin levels and anticipating the health consequences.

STRUCTURE

Common names, chemical structure, and synonyms for the vitamins are given in Table 3. The names given to the vi-

Table 1. History of the Vitamins

Vitamin	Discovery	Isolation	Chemical structure	Synthesis
A	1909	1931	1931	1947
D	1918	1932	1936	1959
E	1922	1936	1938	1938
K	1929	1939	1939	1939
B_1	1897	1926	1936	1936
B_2	1920	1933	1935	1935
Niacin	1936	1935	1937	1867
B_6	1934	1938	1938	1939
B_{12}	1926	1948	1956	1972
Folic acid	1941	1941	1946	1946
Pantothenic acid	1931	1938	1940	1940
Biotin	1931	1935	1942	1943
C	1912	1928	1933	1933

Source: Ref. 4.

Table 2. Nobel Prizes for Vitamin Research

Year	Recipient	Field	Citation
1928	Adolf Windaus	Chemistry	Research into constitution of steroids and connection with vitamins
1929	Christiaan Eijkman	Medicine, physiology	Discovery of antineuretic vitamins
	Sir Frederick G. Hopkins	Medicine, physiology	Discovery of growth-stimulating vitamin
1934	George R. Minot, William P. Murphy, George H. Whipple	Medicine, physiology	Discoveries concerning liver therapy against anemias
1937	Sir Walter N. Haworth	Chemistry	Research into constitution of carbohydrates and vitamin C
	Paul Karrer	Chemistry	Research into constitution of carotenoids, flavins, and vitamins A and B_2
	Albert Szent-Györgyi	Medicine, physiology	Discoveries in connection with biological combustion processes, with special reference to vitamin C and catalysis of fumaric acid
1938	Richard Kuhn	Chemistry	Work on carotenoids and vitamins
1943	Carl Peter Henrik Dam	Medicine, physiology	Discovery of vitamin K
	Edward A. Doisy	Medicine, physiology	Discovery of chemical nature of vitamin K
1953	Fritz A. Lipmann	Medicine, physiology	Discovery of coenzyme A and its importance for intermediary metabolism
1964	Konrad E. Bloch, Feodor Lynen	Medicine, physiology	Discoveries concerning mechanism and regulation of cholesterol and fatty acid metabolism
	Dorothy C. Hodgkin	Chemistry	Structural determination of vitamin B_{12}
1967	Ragnar A. Granit	Medicine, physiology	Research which illuminated electrical properties of vision by studying wavelength discrimination by eye
	Halden K. Hartine	Medicine, physiology	Research on mechanisms of sight
	George Wald	Medicine, physiology	Research on chemical processes that allow pigments in eye retina to convert light into vision

Source: Ref. 2.

tamins and/or their letter designations do not follow a logical pattern and are of historical significance only. Despite this, the nomenclature is in common use.

Vitamins are classified by their solubility characteristics into fat-soluble and water-soluble groups. The fat-soluble vitamins A, E, D_3 and K result from the isoprenoid biosynthetic pathway. Vitamin A is derived by enzymic cleavage of the symmetrical C_{40} beta-carotene, also known as pro-vitamin A. Vitamins E and K result from condensations of phytyldiphosphate (C_{20}) with aromatic components derived from shikimic acid. Vitamin D results from photochemical ring opening of 7-dehydrocholesterol, itself derived from squalene (C_{30}).

The water-soluble vitamins, B_1, B_2, B_6, B_{12}, niacin, folic acid, pantothenic acid, biotin, and C, have a much less common biosynthetic heritage. Furthermore, multiple biosynthetic paths exist for several of the vitamins. Thus, vitamins B_1, B_2 and folic acid are derived from purines, with components coming from carbohydrates. Portions of vitamins B_1 and folic acid also originate in amino acids. One biosynthesis of niacin and that of vitamin B_6 are derived from glycerin. Acetyl-CoA serves as common precursor, in extremely complex processes, to biotin (via pimelic acid) and vitamin B_{12} (through 5-aminolevulinic acid). Pantothenic acid is constructed from glyoxylic acid and beta-alanine, itself derived from several pathways. Vitamin C, a carbohydrate, is biosynthesized from glucose or galactose (4,12).

VITAMERS

Although the 13 substances described in Table 3 are commonly recognized as the vitamins, significant complexities exist because of the following factors: (*1*) A number of similar chemical structures can exhibit, at least qualitatively, the biological activity of the vitamin; (*2*) The biological activity can vary by organism type, eg, in microorganisms vs humans; (*3*) The physiologically active form of the vitamin may be other than that indicated; for example, several water-soluble vitamins are active in co-enzyme form; (*4*) A metabolite may be the active form of the vitamin, as in the case of 1-α-25-dihydroxy-vitamin D_3; (*5*) Biochemical interconversion of several forms of the vitamins can occur. The best studied example is pyridoxine, pyridoxal, pyridoxamine, and their 5′-phosphates; (*6*) Synthetic commercial forms, often manufactured and sold because of their increased stability, are convertible in the target organism to the vitamin. Esters of α-tocopherol and retinol are the most prominent examples. These compounds are known as vitamers. Table 4 presents a partial list of these vitamers. Specifically segmented are the major commercial forms of the vitamin.

PROPERTIES

Because of their diverse structures, there are few common threads to vitamin chemical properties aside from their fat or water solubility. Of general concern in all applications, however, is vitamin stability. Table 5 provides generic information regarding stability under several conditions. Levels of stability vary greatly and are impacted by acid or base strength, light intensity, etc.

The relative instability of many of the vitamins has led to the creation of derivatives and stabilized product forms capable of withstanding the rigors of food, animal feed, and pharmaceutical process conditions. Depending on the vitamin and application, stabilized forms may involve spray

Table 3. Vitamin Names and Structures

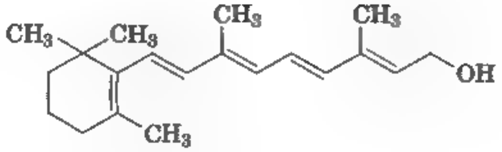
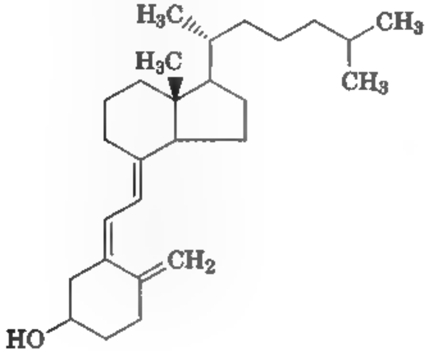
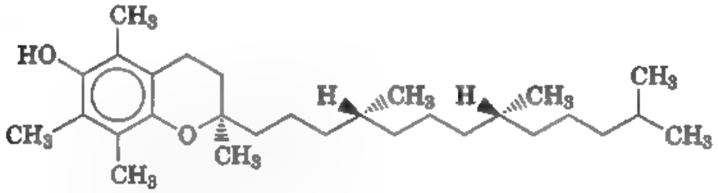
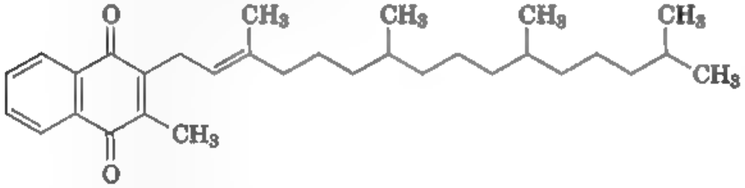
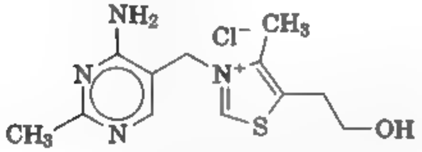
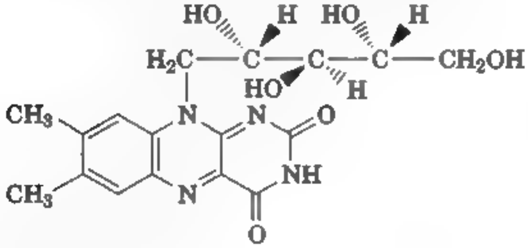
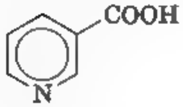
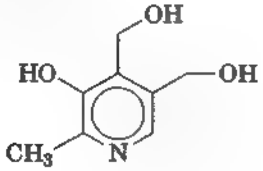
Vitamin	Structure	CAS Registry Number	Common name and synonyms
A		[*68-26-8*]	Retinol, axerophthol
D		[*520-91-2*]	Vitamin D_3, cholecalciferol, antirachitic vitamin
E		[*59-02-9*]	α-Tocopherol, (*RRR*)-α-tocopherol, antisterility vitamin
K		[*84-80-0*]	Vitamin K_1, phylloquinome, coagulation vitamin, antihemorrhagic vitamin, phytonadione
B_1		[*67-03-8*]	Thiamine, aneurin
B_2		[*83-88-5*]	Riboflavin, vitamin G, lactoflavin, hepatoflavin, ovoflavin, verdoflavin
Niacin		[*59-67-6*]	Vitamin B_3, nicotinic acid
B_6		[*58-56-0*]	Pyridoxine, pyridoxol, antiacrodynia factor

Table 3. Vitamin Names and Structures *(continued)*

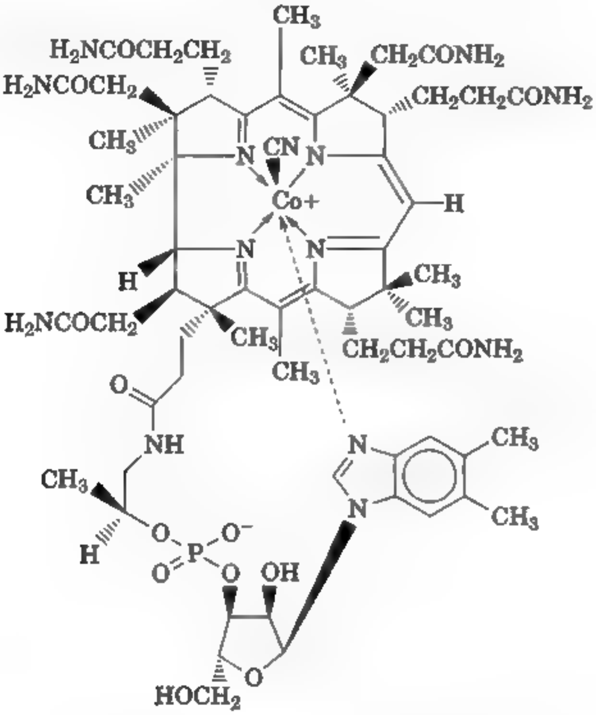
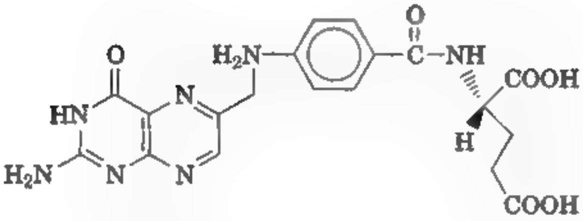
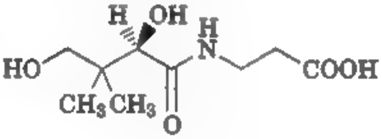
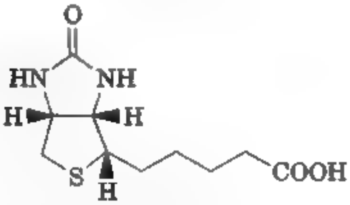
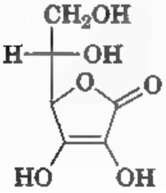
Vitamin	Structure	CAS Registry Number	Common name and synonyms
B_{12}		*[68-19-9]*	Cobalamin, cyanocobalamin
Folic acid		*[59-30-3]*	Folacin, pteroylglutamic acid, antianemia factor, fermentation, *L. casei* factor
Pantothenic acid		*[79-83-4]*	Chick antidermatitis factor
Biotin		*[58-85-5]*	*d*-Biotin, vitamin H, anti-egg-white-injury factor, bios II, coenzyme R
C		*[520-91-2]*	Ascorbic acid, antiscorbutic vitamin

Table 4. Vitamers

Vitamin	Vitamers	Major commercial forms
A	Retinal (vitamin A aldehyde), retinoic acid (vitamin A acid), retinol cis-isomers, β-carotene, other carotenoids	Vitamin A acetate, vitamin A propionate, vitamin A palmitate, vitamin A acid, β-carotene, β-apo-8′-carotenal
D	Ergocalciferol (vitamin D_2), 1-α-25-dihydroxycholecalciferol (calcitriol)	Cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2)
E	(*RRR*)-β-Tocopherol, (*RRR*)-γ-tocopherol, (*RRR*)-δ-tocopherol, 2*R*-α-tocotrienol, 2*R*-β-tocotrienol, 2*R*-γ-tocotrienol, 2*R*-δ-tocotrienol, all-*rac*-α-tocopherol	(*RRR*)-α-Tocopherol, (*RRR*)-α-tocopheryl acetate, all-*rac*-α-tocopherol, all-*rac*-α-tocopheryl acetate, all-*rac*-α-tocopheryl succinate
K	Vitamin K_2 (menaquinone), vitamin K_3 (menadione), 2-(*R,S*)-vitamin K_1 (trans)	2-(*R,S*)-Vitamin K_1 (cis, trans-mixture), menadione sodium bisulfite, menadione dimethylpyrimidinol bisulfite
B_1	Thiamine diphosphate (cocarboxylase), thiamine orthophosphate	Thiamine chloride, thiamine mononitrate, thiamine diphosphate, thiamine disulfide
B_2	Riboflavin mononucleotide (FMN), riboflavin dinucleotide (FAD)	Riboflavin, riboflavin sodium phosphate
Niacin	Niacinamide (nicotinamide), nicotinamide adenine dinucleotide (NAD, coenzyme I), nicotinamide adenine dinucleotide phosphate (NADP, coenzyme II)	Niacin, niacinamide
B_6	Pyridoxine-5′-phosphate, pyridoxal, pyridoxal-5′-phosphate (codecarboxylase), pyridoxamine, pyridoxamine-5′-phosphate	Pyridoxine hydrochloride, pyridoxal-5′-phosphate
B_{12}	Methylcobalamin, adenosyl-cobalamin (coenzyme B_{12}), hydroxocobalamin, aqua-cobalamin chloride	Cyanocobalamin, hydroxocobalamin
Folic acid	Tetrahydrofolic acid, *N*-formyl-tetrahydrofolic acid, pteroic acid, pteroyltriglutamic acid, pteroylhexaglutamic acid	Folic acid
Pantothenic acid	Coenzyme A, pantethein	Calcium pantothenate, *d*-panthenol, *d,l*-panthenol
Biotin	Desthiobiotin	*d*-Biotin
C	Dehydroascorbic acid	Ascorbic acid, ascorbyl palmitate, sodium ascorbate, calcium ascorbate, ascorbyl-2-polyphosphate

Table 5. Vitamin Stability

Vitamin	Oxygen	Light	100°C	Acids[a]	Bases[a]
A	U	U	S	U	S
D	U	U	U	U	U
E	U	U	S	S	S
K	S	U	S	S	U
B_1	U	U	U	S	U
B_2	S	U	U	S	U
Niacin	S	S	S	S	S
B_6	S	U	U	S	S
B_{12}	U	U	U	S	S
Folic acid	U	U	U	U	S
Pantohenic acid	S	S	U	U	U
Biotin	S	S	U	S	S
C	U	U	U	S	U

Note: S = stable; U = unstable.

[a] In absence of oxygen.

drying in a suitable matrix (eg, gelatin), encapsulation (beadletting), or coating with fats or waxes (qv).

ANALYSIS

Analytical methods for the vitamins involve physical and chemical, chromatographic, microbiological, and biological methods. The method of choice for a particular vitamin depends on the substance being measured, as well as its concentration. For example, detection of biotin, folic acid, and vitamin B_{12} is accurately achieved by high pressure liquid chromatography (hplc) or gas—liquid chromatography (glc). However, chromatographic methods often are not sufficiently sensitive for the low levels at which these vitamins usually are found. Microbiological methods, though slower, more expensive, and usually less accurate, are then employed for these low level assays. Biological assays, although extremely significant in the history of the vitamins, are rarely used in the 1990s.

An excellent overview of vitamin analytics is available (13). Specifications for the vitamins are available for food use (14) and pharmaceutical use (15).

PHYSIOLOGICAL FUNCTION

Physiological functions as well as clinical symptoms that occur in humans deficient in specific vitamins are given in Table 6. It is becoming more authenticated that vitamins have additional potential health benefits when administered, via the diet or by supplementation, at levels above those required for obviating deficiency. Although for most vitamins the optimal levels are not yet established, some

Table 6. Vitamin Functions, Clinical Deficiency Symptoms, and Potential Health Benefits

Vitamin	Function	Clinical deficiency symptoms	Potential health benefits
A	Needed for normal vision, reproduction, and maintenance of healthy skin, mucous membranes, bones, red blood cells, cell differentiation, and immune function	Night blindness, xerophthalmia, loss of appetite, increased susceptibility to infections, skin disorders, poor growth, defective reproduction	Regression of precancerous lesions, reduces measle-associated morbidity in children
D	Enhances calcium absorption, regulates calcium and phosphorus metabolism, promotes mineralization of bones, plays role in cell differentiation	Insufficient bone mineralization, skeletal deformities (rickets and osteomalacia)	Protection against certain types of cancer, reduces risk of osteoporosis, psoriasis (topical)
E	Lipid-soluble antioxidant, prevents lipid oxidation of membranes, needed for healthy blood cells and tissues, blocks nitrosamine formation, protects PUFAs from autoxidation, important for normal immune function	Neuromuscular disorders, red blood cell rupture (both uncommon)	Reduces risk of chronic disease (cardiovascular, precancerous lesions, cancer), immunoenhancement, protection from exercise-induced muscle injury, improves metabolic control, reduces risk of complications in diabetes
K	Essential for normal blood clotting, needed for formation and maintenance of healthy bones, essential for formation of osteocalcin (bone protein)	Reduced blood clotting, increased risk for brittle bones	Reduces risk of osteoporosis
B_1	Helps convert carbohydrates from food into energy, required by muscle, nervous system, and brain	Mental confusion, loss of appetite, muscular weakness and paralysis, heart failure (beriberi)	Important role in energy metabolism
B_2	Involved in carbohydrate, protein and fat metabolism	Oral lesions, cracks in corners of mouth, dermatitis	Important role in energy metabolism
Niacin	Involved in carbohydrate, protein, and fat metabolism; at pharmacologic levels used to lower blood cholesterol	Mouth soreness, diarrhea, abdominal pain, skin and nervous system disorders (pellegra)	Reduces risk of atherosclerosis
B_6	Active in protein metabolism, required for bone health, involved in homocysteine metabolism	Dermatitis, anemia, nervous system disorders	Reduces risk of cardiovascular disease (also B_{12} and folic acid), immunoenhancement, reduces risk of osteoporosis, protection against carpal tunnel syndrome
Folic acid	Involved in amino acid interconversions, needed for red blood cell formation and DNA and RNA synthesis, protects against neural tube birth defects, involved in homocysteine metabolism	Anemia, nervous system disorders, gastrointestinal lesions	Reduces risk of cardiovascular disease (also B_6 and B_{12}), certain cancers, and precancerous lesions; birth defect prevention
Pantothenic acid	Active in carbohydrate, protein, and fat metabolism	Abnormal sensations in legs and feet (rare)	
Biotin	Involved in protein, fat, and carbohydrate metabolism	Dermatitis, hair loss	Helps develop healthy nails
C	Helps form and maintain collagen; important for healthy cartilage, bones, teeth, and gums; needed for wound healing; enhances nonheme iron absorption; water-soluble antioxidant protects cells from free-radical damage; blocks nitrosamine formation; increases body's resistance to infection	Swollen or bleeding gums, joint pain, slow wound healing, lowered resistance to infection, scurvy	Protection against chronic diseases (cancer, cardiovascular, eye disease), reduces symptoms of common cold, periodontal disease protection, improves fertility in men, reduces risk of osteoporosis

Table 7. Recommended Dietary Allowances

Category	Age	Vitamin A (μg RE)[a]	Vitamin D (μg)[b]	Vitamin E (mg α-TE)[c]	Vitamin K (μg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)	Folate (μg)	Vitamin C (mg)
Infants	0.0–0.5	375	7.5	3	5	0.3	0.4	5	0.3	0.3	25	30
	0.5–1.0	375	10	4	10	0.4	0.5	6	0.6	0.5	35	35
Children	1–3	400	10	6	15	0.7	0.8	9	1.0	0.7	50	40
	4–6	500	10	7	20	0.9	1.1	12	1.1	1.0	75	45
	7–10	700	10	7	30	1.0	1.2	13	1.4	1.4	100	45
Males	11–14	1000	10	10	45	1.3	1.5	17	1.7	2.0	150	50
	15–18	1000	10	10	65	1.5	1.8	20	2.0	2.0	200	60
	19–24	1000	10	10	70	1.5	1.7	19	2.0	2.0	200	60
	25–50	1000	5	10	80	1.5	1.7	19	2.0	2.0	200	60
	51+	1000	5	10	80	1.2	1.4	15	2.0	2.0	200	60
Females	11–14	800	10	8	45	1.1	1.3	15	1.4	2.0	150	50
	15–18	800	10	8	55	1.1	1.3	15	1.5	2.0	180	60
	19–24	800	10	8	60	1.1	1.3	15	1.6	2.0	180	60
	25–50	800	5	8	65	1.1	1.3	15	1.6	2.0	180	60
	51+	800	5	8	65	1.0	1.2	13	1.6	2.0	180	60
Pregnant lactating		800	10	10	65	1.5	1.6	17	2.2	2.2	400	70
1st six months		1000	10	12	65	1.6	1.8	20	2.1	2.6	280	95
2nd six months		1200	10	11	65	1.6	1.7	20	2.1	2.6	260	90

Source: Ref. 18.

[a] RE = retinol equivalents; 1 RE = 1 μg retinol or 6 μg β-carotene.

[b] As cholecalciferol; 10 μg cholecalciferol = 400 IU vitamin D.

[c] TE = α-Tocopherol equivalents; 1 α-TE = 1 mg (*RRR*)-α-tocopherol.

of the potential health benefits to be derived from vitamins are indicated (16). In one case, the level of scientific proof is such that the U.S. FDA has allowed "a health claim that women who are capable of becoming pregnant and who consume adequate amounts of folate daily during their childbearing years may reduce their risk of having a pregnancy affected by spina bifida or other neural tube defects" (17).

The Recommended Dietary Allowances (RDA) of the National Academy of Sciences are intended as daily intake guidelines for preventing deficiencies and maintaining reasonable reserves among most healthy people. These recommendations, revised every 5 to 10 years, are made by a committee of the U.S. Food and Nutrition Board and are based on scientific information that is available at the time of the review. This includes studies on subjects on deficient diets, nutrient balance studies, measures of tissue saturation, normal intakes of the nutrient, and extrapolation from animal data. The most recent RDAs are given in Table 7 (18). The 1989 RDA has a special requirement of 100 mg of vitamin C for cigarette smokers. Otherwise, there is no recognition of special needs for specific groups such as those using certain drugs chronically, people with disease, or the elderly (eg, over 65 years). Neither does it address the needs for the prevention of any of the chronic diseases.

Because there is less information upon which to base dietary allowances for biotin and pantothenic acid, ranges of intake are provided, as in Table 8.

Along with increasing evidence of health benefits from consumption of vitamins at levels much higher than RDA recommendations comes concern over potential toxicity. This topic has been reviewed (19). Like all chemical substances, a toxic level does exist for each vitamin. Traditionally it has been assumed that all water-soluble vitamins are safe at any level of intake and all fat-soluble vitamins are toxic, especially at intakes more than 10 times the recommended allowances. These assumptions are now known to be incorrect. Very high doses of some water-soluble vitamins, especially niacin and vitamin B_6, are associated with adverse effects. In contrast, evidence indicates that some fat-soluble micronutrients, especially vitamin E, are safe at doses many times higher than recommended levels of intake. Chronic intakes above the RDA for vitamins A and D especially are to be avoided, however.

Table 8. Estimated Safe and Adequate Daily Dietary Intake

Category	Age	Biotin (μg)	Pantothenic acid (mg)
Infants	0–0.5	10	2
	0.5–1	15	3
Children and adolescents	1–3	20	3
	4–6	25	3–4
	7–10	30	4–5
	11+	30–100	4–7
Adults		30–100	4–7

Source: Ref. 18.

PRODUCTION

Preparation of the vitamins in commercial quantities can involve isolation, chemical synthesis, fermentation, and mixed processes, including chemical and fermentation steps. The choice of process is economic, dictated by the need to obtain materials meeting specifications at the lowest cost. Current process technologies (ca 1997) employed for each vitamin are indicated in Table 9.

More than one process is available for some of the vitamins. Further, manufacturers have developed variants of the classical syntheses during optimization. Whereas some of this information is available, as described in the individual sections on vitamins, much is closely held as trade secrets. Judging from the more recent patent literature, the assessment can be made that vitamin production technologies are in general mature. However, the economic value of these products drives continuing research aimed at breakthrough processes. Annual production of vitamins varies greatly, from ca 10 metric tons of vitamin B_{12} to ca 50,000 metric tons of vitamin C.

APPLICATIONS

It is generally assumed that adequate vitamin levels in humans can be obtained through a balanced diet. However, ongoing studies continue to indicate that the majority of the U.S. population is not receiving even the RDA through diet. Supplementary vitamins are thus provided for fortification of foods (20) and as oral or parenteral dosage forms.

The use of vitamins in humans consumes ca 40% of vitamins made worldwide. The majority of the vitamins, particularly in countries outside the United States, are used in animal husbandry. It is well established (21) that vitamins are critical to animal productivity, especially under confined, rapid growth conditions. Newer information (22) has shown that vitamin E added to cattle feed has the additional effect of significantly prolonging beef shelf life in stores. Additional applications of vitamins exist. A small but growing market segment involves cosmetics (qv) (23). The use of the chemical properties of the vitamins, particularly as antioxidants (qv) in foods and, more recently, in plastics (vitamin E (24)), has emerged as a growing trend.

ECONOMIC ASPECTS

Vitamins are sold for direct application to foods and animal feeds. In addition, they are further processed into nutritional supplements. This last market is particularly significant in the United States. In many other countries, vitamins are regulated as drugs, leading to a much lower supplement usage.

It can be estimated that approximately $3,000,000,000 of vitamins were sold in 1996. Market growth is slightly

Table 9. Vitamin Production Processes

Isolation	Chemical synthesis	Fermentation	Synthesis/fermentation
(Vitamin A)	Vitamin A	Vitamin B_2	Vitamin B_2
Vitamin E[a]	Vitamin D	Vitamin B_{12}	Vitamin C
(Vitamin D)	Vitamin E[b]		
(Vitamin K)	Vitamin K		
	Vitamin B_1		
	Niacin		
	Vitamin B_6		
	Folic acid		
	Pantothenic acid		
	Biotin		

Note Parentheses indicate minor processes primarily of historical significance.
[a] (*RRR*)-α-Tocopherol.
[b] All-*rac*-α-tocopherol.

higher than population growth, but varies widely by individual vitamin, geographical area, and/or application. The largest vitamin manufacturer is Hoffmann-La Roche. Other significant producers include BASF, Takeda, Eisai, and Rhône-Poulenc. Additional vitamins are produced in China, Russia and India.

VITAMIN-LIKE COMPOUNDS

Although it is a general consensus that these 13 substances represent the known vitamins, other compounds upon which there is less agreement often enter the vitamin discussion (25). Included in this group are choline, myoinositol (Bios I), vitamin F (essential fatty acids including linoleic acid, γ-linolenic acid, and arachidonic acid), α-lipoic acid, ubiquinones, plastoquinone, vitamin P (bioflavonoids), vitamin L (*o*-aminobenzoic acid), vitamin T (mixture including folic acid and vitamin B_{12}), vitamin U (L-methioninylmethylsulfonium chloride), orotic acid (vitamin B_{13}), and pyrroloquinoline quinone.

ACKNOWLEDGMENTS

The Vitamins articles have been reprinted from the *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 25, John Wiley & Sons, New York, 1998.

BIBLIOGRAPHY

1. C. Funk, *J. Physiol. (London)* **43**, 395 (1911).
2. *Vitamins Basics*, F. Hoffmann-La Roche, Ltd., Basel, Switzerland, 1994, p. i.
3. K. Reese, *Today's Chem. Work* **59** (Oct. 1995).
4. O. Isler and G. Brubacher, *Vitamine I: Fettlösliche Vitamine*, Georg Thieme Verlag, Stuttgart, Germany, 1982.
5. C. Eijkman, *Geweskund Tijdsche Ned. Indie* **30**, 295 (1896).
6. T. Reichstein and A. Grüssner, *Helv. Chim. Acta* **17**, 311 (1934).
7. L. J. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991.
8. R. Altschul, A. Hoffer, and J. D. Stephen, *Arch. Biochem. Biophys.* **54**, 558 (1955).
9. H. E. Sauberlich and L. J. Machlin, eds., *Annal. NY Acad. Sci.* **669** (1992).
10. A. Bendich and C. E. Butterworth, Jr., *Micronutrients in Health and Disease Prevention*, Marcel Dekker, New York, 1991.
11. S. K. Gaby, A. Bendich, V. N. Singh, and L. J. Machlin, *Vitamin Intake and Health: A Scientific Review*, Marcel Dekker, New York, 1991.
12. O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, *Vitamine II: Wasserlösliche Vitamine*, Georg Thiem Verlag, Stuttgart, Germany, 1988.
13. J. Augustin, in *Encyclopedia of Food Technology*, Vol. 4, John Wiley & Sons, Inc., New York, 1991, p. 2697.
14. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981.
15. *The United States Pharmacopeia XXIII* (USP XXIII-NF XVIII), United States Pharmacopeial Convention, Inc., Rockville, Md., 1995.
16. V. Singh, personal communication, Hoffmann-La Roche Inc., Nutley, N.J., 1996.
17. *Fed. Reg.* **59**, 433 (Jan. 4, 1994).
18. Food and Nutrition Board, National Research Council, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
19. *Vitamin Nutrition Information Service Backgrounder*, Vol. 5 (No. 1), Hoffmann-La Roche Inc., Nutley, N.J., 1994.
20. J. Giese, *Food Technol.*, 110 (May 1995).
21. *Vitamin Compendium*, F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland, 1976.
22. J. A. Sherbeck, D. M. Wulf, J. B. Morgan, J. D. Tatum, G. C. Smith, and S. N. Williams, *J. Food Sci.* **60**, 250 (1995).
23. D. Djerassi, *The Role of Vitamins in Skin Care*, Hoffmann-La Roche Inc., Nutley, N.J., 1993
24. S. F. Laermer, S. S. Young, and P. F. Zambetti, *Converting Mag.*, 80 (Feb. 1996).
25. M. M. Cody, in Ref. 7, p. 565.

JOHN W. SCOTT
Hoffmann-La Roche, Inc.

VITAMINS: ASCORBIC ACID

Ascorbic acid [*50-81-1*] (1) (Fig. 1) is the name recognized by the IUPAC-IUB Commission on Biochemical Nomenclature for Vitamin C (1). Other names are: L-ascorbic acid, L-xyloascorbic acid, and L-*threo*-hex-2-enoic acid γ-lactone. The name implies the vitamin's antiscorbutic properties, eg, the prevention and treatment of scurvy. L-Ascorbic acid is widely distributed in plants and animals. The pure vitamin, $C_6H_8O_6$, mol wt 176.13, is a water-soluble, strongly reducing, optically active (chiral) white crystalline substance.

L-Ascorbic acid biosynthesis in plants and animals as well as the chemical synthesis starts from D-glucose. The vitamin and its main derivatives, sodium ascorbate, calcium ascorbate, and ascorbyl palmitate, are officially recognized by regulatory agencies and included in compendia such as the *United States Pharmacopeia / National Formulary (USP / NF)* and the *Food Chemicals Codex (FCC)*.

The most significant chemical characteristic of L-ascorbic acid (1) is its oxidation to dehydro-L-ascorbic acid (L-*threo*-2,3-hexodiulosonic acid γ-lactone) (3) (Fig. 1). Vitamin C is a redox system containing at least three substances: L-ascorbic acid, monodehydro-L-ascorbic acid, and dehydro-L-ascorbic acid. Dehydro-L-ascorbic acid and the intermediate product of the oxidation, the monodehydro-L-ascorbic acid free radical (2), have antiscorbutic activity equal to L-ascorbic acid.

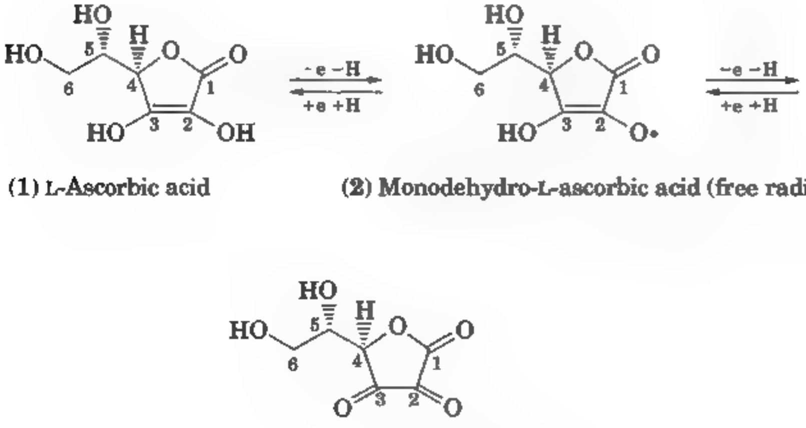
The reversible oxidation of L-ascorbic acid to dehydro-L-ascorbic acid is the basis for its known physiological activities, stabilities, and technical applications (2). The importance of vitamin C in nutrition and the maintenance of good health is well documented. Over 22,000 references relating only to L-ascorbic acid have appeared since 1966.

L-Ascorbic acid was isolated first by Albert Szent-Györgyi in 1930. However, its early history is associated with the etiology, treatment, and prevention of scurvy (3–5). Scurvy is one of the oldest diseases known to humankind. It affected many people in ancient times in Egypt, Greece, and Rome and influenced the course of history. Outbreaks of the disease resulting from inadequate amounts of vitamin C in rations spontaneously ended many military campaigns and long ocean voyages (6). During the Middle Ages, scurvy was endemic in northern Europe, occurring mostly in the winter season when fresh fruits and vegetables were unavailable. By the end of the seventeenth century, scurvy became a severe problem among sailors on long voyages. It was treated as a venereal disease, with disastrous results.

The first clues to the treatment of scurvy occurred in 1535–1536 when Jacques Cartier, on advice from Newfoundland Indians, fed his crew an extract from spruce tree needles to cure an epidemic. Various physicians were recommending the use of citrus fruits to cure scurvy in the mid-sixteenth century. Two hundred years later, in 1753, it was proved by Dr. James Lind, in his famous clinical experiment, that scurvy was associated with diet and caused by lack of fresh vegetables. He also demonstrated that oranges and lemons were the most effective cure against this disease. In 1753, in "*A Treatise on the Scurvy*," Lind published his results and recommendations (7). Forty-two years later, in 1795, the British Navy included lemon juice in seamen's diets, resulting in the familiar nickname "limeys" for British seamen. Evidence has shown that even with undefined scorbutic symptoms, vitamin C levels can be low, and can cause marked diminution in resistance to infections and slow healing of wounds.

Research leading to the discovery of vitamin C began in 1907 when it was observed by Axel Holst and Theodor Fröhlich that guinea pigs were as susceptible to scurvy as humans and that the disease could be produced experimentally in these animals (8). These findings led to the development of an assay for the biological determination of antiscorbutic activity of food products (9).

Between 1910 and 1921, the vitamin was obtained in almost pure form from lemons and some of the physical and chemical properties were determined (10). It was discovered that vitamin C is easily destroyed by oxidation and best protected by reducing agents, and that 2,6-dichlorophenolindophenol is reduced by solutions of the vitamin. Subsequent studies showed that the dichlorophenolindophenol test measured only the vitamin and not dehydroas-



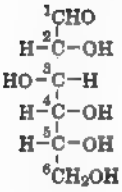
(1) L-Ascorbic acid

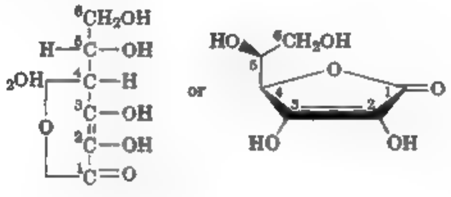
(2) Monodehydro-L-ascorbic acid (free radical)

(3) Dehydro-L-ascorbic acid

Figure 1. Vitamin C redox system.

corbic acid, which also has antiscorbutic activity. The isolation of vitamin C was first accomplished by Szent-Györgyi in 1928 from cabbage, paprika, and the adrenal glands of animals (11). The relationship between vitamin C and the antiscorbutic factor was established in 1932 by Szent-Györgyi and, at the same time, by King and Waugh (12). Also in 1932, the chemical structure of ascorbic acid was determined independently by Haworth and Hirst (13). One year later, in 1933, Reichstein synthesized D-ascorbic acid and L-ascorbic acid (14). L-Ascorbic acid was synthesized from D-glucose (4) because the chiral centers of C-2 and C-3 were in the correct configuration to become C-4 and C-5, respectively, of L-ascorbic acid (15).

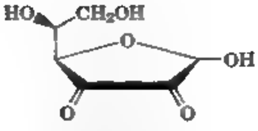

(4) D-Glucose

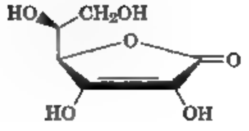

(1) L-Ascorbic acid

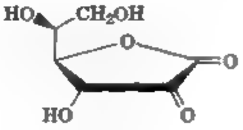
This synthesis was the first step towards industrial vitamin production, which began in 1936. The synthetic product was shown to have the same biological activity as the natural substance. It is reversibly oxidized in the body to dehydro-L-ascorbic acid (3) (L-*threo*-2,3-hexodiulosonic acid γ-lactone), a potent antiscorbutic agent with full vitamin activity. In 1937, Haworth and Szent-Györgyi received the Nobel Prize for their work on vitamin C.

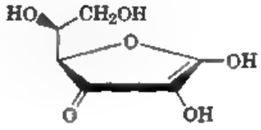
STRUCTURE DETERMINATION

Albert Szent-Györgyi demonstrated that "hexuronic acid," $C_6H_8O_6$, which was first isolated from sources such as cabbage juice, was identical to vitamin C (L-ascorbic acid). Ultraviolet absorption studies carried out in the mid-1930s led to the proposal that L-ascorbic acid was 3-oxo-L-sorbosone (5), which exists in a variety of tautomeric forms, including (6), (7), and L-ascorbic acid (1). The chemical structure of L-ascorbic acid was determined independently by Haworth and Hirst using x-ray crystallographic techniques (13). Soon afterwards, additional data supporting the structure 1 was published by other authors (16–18). Excellent review articles summarizing the early work on vitamin C have appeared (19,20). Years later, with improved x-ray techniques (21,22) and neutron-diffraction methods (23), the structure of L-ascorbic acid was refined (24,25). The five-membered ring containing the enediol group is almost planar. The conformation of the side chain in the crystal is as shown by structure 1. The chiral center at position C-4 has the R- or D-configuration, whereas C-5 has the S- or L-configuration.


(5) 3-oxo-L-sorbosone

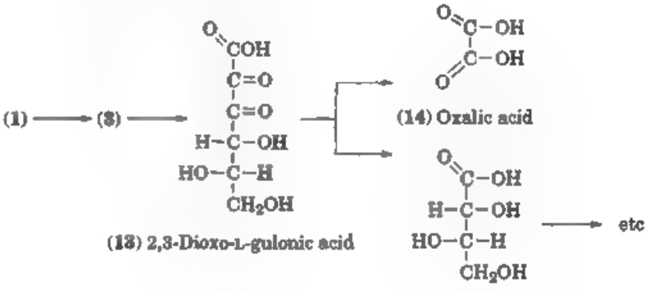

(1)


(6)


(7)

As a result of having two chiral centers, four stereoisomers of ascorbic acid are possible (Table 1) (Fig. 2). Besides L-ascorbic acid (Activity = 1), only D-araboascorbic acid (erythorbic acid (9)) shows vitamin C activity (Activity = 0.025–0.05). The L-ascorbic acid structure (1) in solution and the solid state are almost identical. Ascorbic acid crystallizes in the space group $P2_1$ with four molecules in the unit cell. The crystal data are summarized in Table 2.

Crystalline dehydro-L-ascorbic acid (3) is reported to exist as the dimer (11) (26). In water, it is present as the hemiacetal monomer (12).



PROPERTIES

Physical Properties

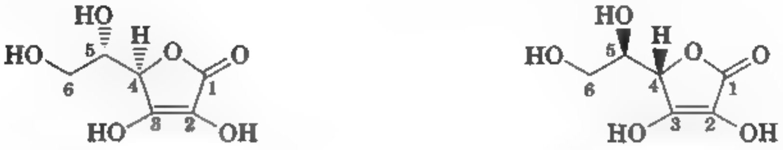
Table 3 contains a summary of the physical properties of L-ascorbic acid. Properties relating to the structure of vitamin C have been reviewed and summarized (32). Stabilization of the molecule is a consequence of delocalization of the π-electrons over the conjugated enediol system. The highly acidic nature of the H-atom on C-3 has been confirmed by neutron diffraction studies (23).

Chemical Properties

The most significant chemical property of L-ascorbic acid is its reversible oxidation to dehydro-L-ascorbic acid. Dehydro-L-ascorbic acid has been prepared by uv irradiation and by oxidation with air and charcoal, halogens, ferric chloride, hydrogen peroxide, 2,6-dichlorophenolindophenol, neutral potassium permanganate, selenium oxide, and many other compounds. Dehydro-L-ascorbic acid has been reduced to L-ascorbic acid by hydrogen iodide, hydro-

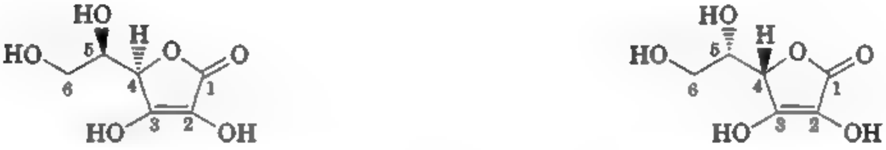
Table 1. Isomeric Ascorbic Acids

Substance	Structure	Mp (°C)	$[\alpha]_D$ (H_2O) (°)	Activity
L-Ascorbic acid	(1)	192	+ 24	1
D-Xyloascorbic acid (D-ascorbic acid, D-*threo*-hex-2-enonic acid γ-lactone)	(8)	192	− 23	0
D-Araboascorbic acid (erythorbic acid, D-*erythro*-hex-2-enonic acid γ-lactone)	(9)	174	− 18.5	0.025–0.05
L-Araboascorbic acid (L-*erythro*-hex-2-enonic acid γ-lactone)	(10)	170	+ 17	0



(1) L-Ascorbic acid

(8) D-Ascorbic acid



(9) D-Araboascorbic acid (erythorbic acid)

(10) L-Araboascorbic acid

Figure 2. Isomers of ascorbic acid.

Table 2. X-Ray Crystal Data of L-Ascorbic Acid

Space group	*P*2$_1$
a	1.7299 nm
b	0.6353 nm
c	0.6411 nm
β	102° 11′
V	0.68859 nm^3
Z	4
d_{calcd}	1.699 g/cm^3
μ(CuK$_\alpha$)	13.9 cm^{-1}

Source: Ref. 24.

gen sulfide, 1,4-dithiothreitol (1,4-dimercapto-2,3-butanediol), and the like (33).

The degradation of L-ascorbic acid in aqueous solution depends on several factors, eg, pH, temperature, presence of oxygen, or metals. Comprehensive reviews of degradation reactions and mechanisms have been published (34–37). In aqueous solution, L-ascorbic acid is more sensitive in the presence of oxygen to bases than to acids. The pH range with the highest stability is between 4 and 6. L-Ascorbic acid is sensitive to heat. In the presence of oxygen and heat, it is oxidized at a rate proportional to the temperature rise. On standing, dehydro-L-ascorbic acid (**3**), the initial oxidation product, undergoes irreversible hydrolysis to 2,3-dioxo-L-gulonic acid (*threo*-2,3-hexodiulosonic acid (**13**)), which is further oxidized to oxalic acid (**14**) and L-threonic acid ((*R*-(*R**,*S**)-2,3,4-trihydroxybutanoic acid)) (**15**) (Fig. 3).

In acidic solution, the degradation results in the formation of furfural, furfuryl alcohol, 2-furoic acid, 3-hydroxyfurfural, furoin, 2-methyl-3,8-dihydroxychroman, ethylglyoxal, and several condensation products (36). Many metals, especially copper, catalyze the oxidation of L-ascorbic acid. Oxalic acid and copper form a chelate complex which prevents the ascorbic acid-copper-complex formation and therefore oxalic acid inhibits effectively the oxidation of L-ascorbic acid, L-Ascorbic acid can also be stabilized with metaphosphoric acid, amino acids, 8-hydroxyquinoline, glycols, sugars, and trichloracetic acid (38). Another catalytic reaction which accounts for loss of L-ascorbic acid occurs with enzymes, eg, L-ascorbic acid oxidase, a copper protein-containing enzyme.

Stability

Ascorbic acid, a white crystalline compound, is very soluble in water and has a sharp, acidic taste. In solution, the vitamin oxidizes on exposure to air, light, and elevated temperatures. Solutions of ascorbic acid turn yellowish, followed by development of a tan color. Ascorbic acid is stable to air when dry but gradually darkens on exposure to light.

SYNTHESIS

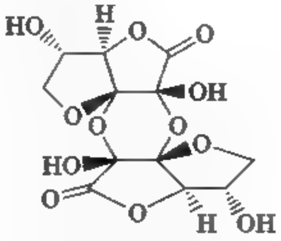
Chemical Synthesis

The first synthesis of ascorbic acid was reported in 1933 by Reichstein and co-workers (14,39–42) (Fig. 4). Similar, independent reports published by Haworth and co-workers followed shortly after this work (13,43–45). L-Xylose (**16**)

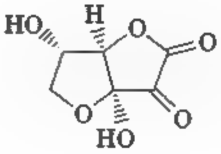
Table 3. Physical Properties of L-Ascorbic Acid

Property	Characteristic(s)	Reference
Appearance	White, odorless, crystalline solid with a sharp acidic taste	
Formula; mol. wt.	$C_6H_8O_6$; 176.13	
Crystalline form	Monoclinic; usually plates, sometimes needles	
Mp °C	190–192 (dec)	27
Density, g/cm^3	1.65	27
Optical rotation	$[\alpha]^{25}$ + 20.5° to + 21.5° (c = 1 in water) $[\alpha]^{23}$ + 48 (c = 1 in methanol)	27
pH		27
5 mg/mL	3	
50 mg/mL	2	
pK_1	4.17	27
pK_2	11.57	27
Redox potential	First stage: E + 0.166 V (pH 4)	27
Solubility, g/mL		27
water	0.33	
95 wt. % ethanol	0.033	
absolute ethanol	0.02	
glycerol USP	0.01	
propylene glycol	0.05	
ether	Insoluble	
chloroform	Insoluble	
benzene	Insoluble	
petroleum ether	Insoluble	
oils	Insoluble	
fats	Insoluble	
fat solvents	Insoluble	
	Spectral properties	
UV	pH 2: E_{max} (1%, 1 cm) 695 at 245 nm (nondissociated form) pH 6.4: E_{max} (1%, 1 cm) 940 at 265 nm (monodissociated form)	28
IR (KBr)	Characteristic wavelengths, cm^{-1} 3455, 3405, 3155 ν OH groups 2570 associated OH groups 1770, 1670 carbonyl lactone 1254 C–O–C lactone 1057 δ OH groups	29
nmr[a]	^{1}H nmr (D_2O) δ 4.97 (d, 1 H, $J_{4,5}$ = 2 Hz, H-4), 4.10 (ddd, 1 H, $J_{5,6}$ = 5 and 7 Hz, $J_{4,5}$ = 2 Hz, H-5), 3.78 (m, 2 H, C-6)	30
	^{13}C nmr (D_2O) δ 174.0 (C-1), 118.8 (C-2), 156.3 (C-3) 77.1 (d, $J_{c\text{-}4,H\text{-}4}$ = 158 Hz, C-4) 69.9 (d, $J_{c\text{-}5,H\text{-}5}$ = 145.0 Hz, C-5), 63.2 (t, $J_{C\text{-}6,H\text{-}6}$ = 145.0Hz, C-6)	31

[a] d = Doublet; ddd = doublet of doublet of doublet, m = multiplet; and t = triplet.



(11) Dehydro-L-ascorbic acid dimer



(12) Dehydro-L-ascorbic acid hemiacetal

Figure 3. Degradation of L-ascorbic acid.

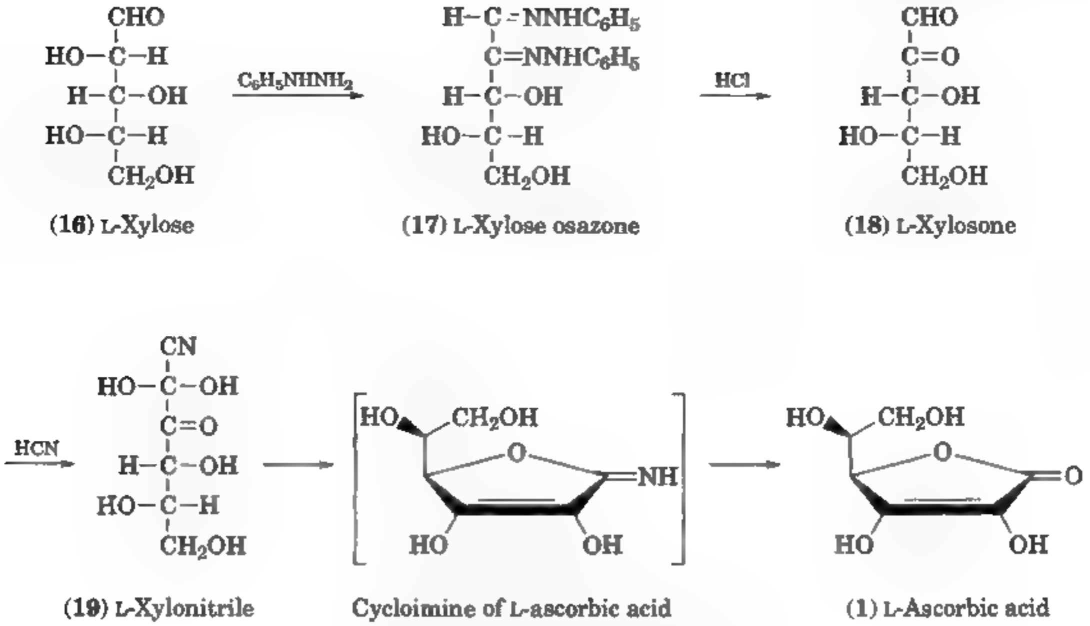


Figure 4. First syntheses of L-ascorbic acid.

was converted by way of its osazone (**17**) into L-xylosone (**18**), which reacted with hydrogen cyanide forming L-xylonitrile (**19**). L-Xylonitrile cyclized under mild conditions to the cycloimine of L-ascorbic acid. Hydrolysis of the cycloimine yielded L-ascorbic acid. The yield for the conversion of L-xylosone to L-ascorbic acid was ca 40%.

The L-xylosone pathway to L-ascorbic acid was never commercialized because L-xylosone was not readily available and was too expensive to prepare. The route, however, was valuable for L-ascorbic acid structure determination and for the preparation of derivatives.

Most current industrial vitamin C production is based on the efficient second synthesis developed by Reichstein and Grüssner in 1934 (15). Various attempts to develop a superior, more economical L-ascorbic acid process have been reported since 1934. These approaches, which have met with little success, are summarized in Crawford's comprehensive review (46). Currently, all chemical syntheses of vitamin C involve modifications of the Reichstein and Grüssner approach (Fig. 5). In the first step, D-glucose (**4**) is catalytically (Ni-catalyst) hydrogenated to D-sorbitol (**20**). Oxidation to L-sorbose (**21**) occurs microbiologically with *Acetobacter suboxydans*. The isolated L-sorbose is reacted with acetone and sulfuric acid to yield 2,3:4,6 diacetone-L-sorbose, (DAS) (2,3:4,6-bis-*O*-isopropylidene-α-L-sorbofuranose) (**22**). The remaining unprotected primary hydroxyl group of DAS is oxidized, either catalytically (O_2, Pd), electrochemically, or by sodium hypochlorite with nickel chloride as catalyst to the corresponding carboxylic acid. The resulting 2,3:4,6-diacetone-2-keto-L-gulonic acid (DAG) (2,3:4,6-bis-*O*-isopropylidene-2-oxo-L-gulonic acid) (**23**) is treated with hydrogen chloride in an inert solvent system. Under these conditions, acetone removal occurs followed by consecutive lactonization and enolization to form L-ascorbic acid (**1**).

Fermentation

Much time and effort has been spent in undertaking to find fermentation processes for vitamin C (47). One such approach is now practiced on an industrial scale, primarily in China. It is not certain, however, whether these processes will ultimately supplant the optimized Reichstein synthesis. One important problem is the instability of ascorbic acid in water in the presence of oxygen; it is thus highly unlikely that direct fermentation to ascorbic acid will be economically viable. The successful approaches to date involve fermentative preparation of an intermediate, which is then converted chemically to ascorbic acid.

L-Sorbose to 2-KGA Fermentation. In China, a variant of the Reichstein-Grüssner synthesis has been developed on an industrial scale (see Fig. 5). L-Sorbose is oxidized directly to 2-ketogulonic acid, (2-KGA) (**24**) in a mixed culture fermentation step (48). Acid-catalyzed lactonization and enolization of 2-KGA produces L-ascorbic acid (**1**).

A Chinese publication (47) with 17 references reviews the use of genetically engineered microorganisms for the production of L-ascorbic acid and its precursor, 2-KGA (49). For example, a 2-keto-L-gulonic acid fermentation process from sorbose has been published with reported yields over 80% (50).

D-Glucose to 2-KGA Fermentation. A different fermentative route to 2-KGA proceeds via 2,5-diketo-L-gluconic acid (51,52). In a two-stage fermentation (Shionogi-Process), D-glucose (**4**) is oxidized to 2,5-diketo-D-gluconic acid (2,5-DKGA) (**25**) with *Erwinia sp.*, followed by stereospecific reduction at C-5 by *Corynebacter sp.*, forming 2-ketogulonic acid (**24**) (Fig. 6). The 2-KGA is rearranged upon treatment with acid to give ascorbic acid. A produc-

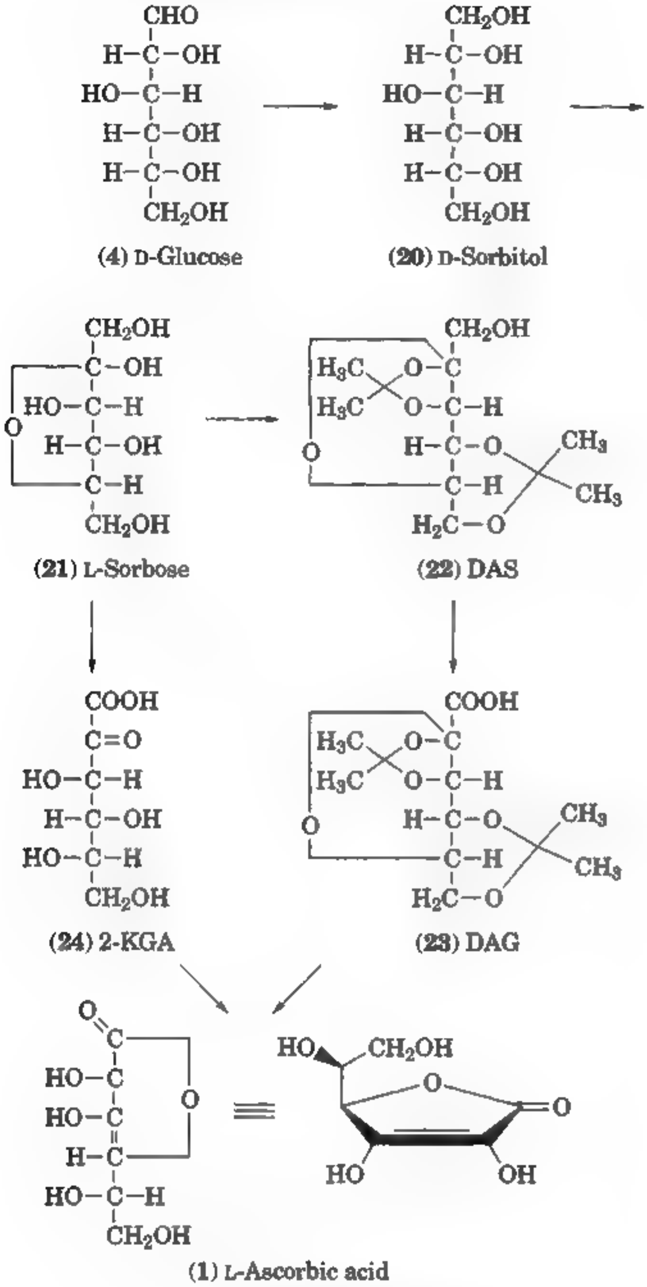


Figure 5. Syntheses of L-ascorbic acid.

tion of ascorbic acid by this route is reportedly being developed, ca 1997. An analogous process using a recombinant strain of *Erwinia citreus* was developed at Biogen (53).

D-Glucose to L-Ascorbic Acid Fermentation. The direct heterotrophic fermentation of D-glucose to L-ascorbic acid with algae is disclosed in a European Patent Application (54). The overall yield of L-ascorbic acid is 4%.

INDUSTRIAL TECHNOLOGY

Vitamin C was the first vitamin to be manufactured by chemical synthesis on an industrial scale. Major suppliers of vitamin C are Hoffmann-La Roche, BASF, Takeda, E. Merck, and various companies in China. Additional production occurs in Eastern Europe and India.

Reichstein and Grüssner's second L-ascorbic acid synthesis became the basis for the industrial vitamin C production. Many chemical and technical modifications have improved the efficiency of each step, enabling this multistep synthesis to remain the principal, most economical process up to 1997 (46). L-Ascorbic acid is produced in large, integrated, automated facilities, involving both continuous and batch operations. The process steps are outlined in Figure 7. Procedures require ca 1.7-kg L-sorbose/kg of L-ascorbic acid with ca 66% overall yield in 1977 (55). Since 1977, further continuous improvement of each vitamin C production step has taken place. Today's overall ascorbic acid yield from L-sorbose is ca 75%. In the mid-1930s, the overall yield from L-sorbose was ca 30%.

The catalytic hydrogenation of D-glucose to D-sorbitol is carried out at elevated temperature and pressure with hydrogen in the presence of nickel catalysts, in both batch and continuous operations, with >97% yield (56,57). The cathodic reduction of D-glucose to L-sorbitol has been practiced (58). D-Mannitol is a by-product (59).

Sterile aqueous D-sorbitol solutions are fermented with *Acetobacter suboxydans* in the presence of large amounts of air to complete the microbiological oxidation. The L-sorbose is isolated by crystallization, filtration, and drying. Various methods for the fermentation of D-sorbitol have been reviewed (60). *Acetobacter suboxydans* is the organism of choice as it gives L-sorbose in >90% yield (61). Large-scale fermentations can be carried out in either batch or continuous modes. In either case, sterility is im-

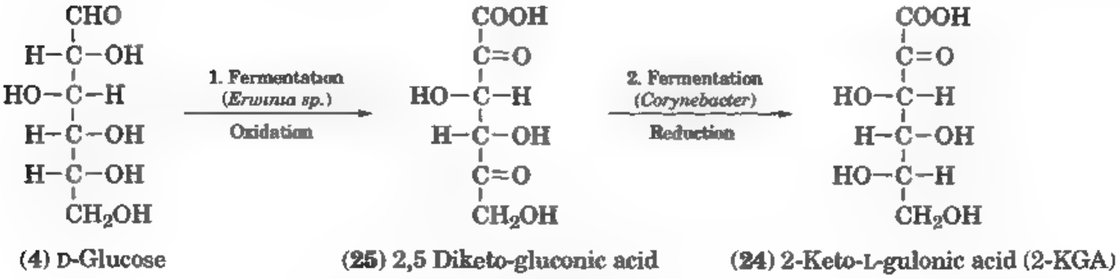


Figure 6. D-Glucose to 2-KGA fermentation.

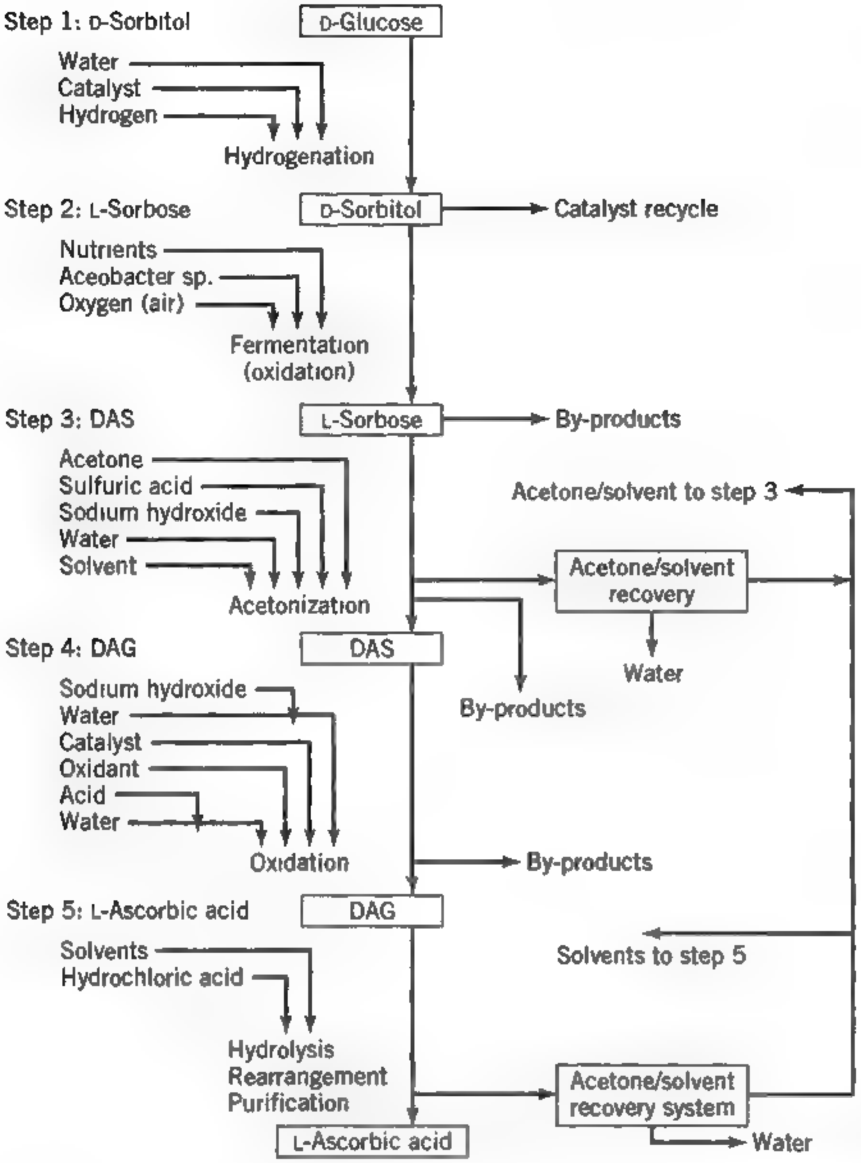

Step 1: D-Sorbitol
D-Glucose
Water
Catalyst
Hydrogen
Hydrogenation
Step 2: L-Sorbose
D-Sorbitol
Catalyst recycle
Nutrients
Aceobacter sp.
Oxygen (air)
Fermentation
(oxidation)
Step 3: DAS
L-Sorbose
By-products
Acetone
Sulfuric acid
Sodium hydroxide
Water
Solvent
Acetonization
Acetone/solvent to step 3
Acetone/solvent recovery
Water
Step 4: DAG
DAS
By-products
Sodium hydroxide
Water
Catalyst
Oxidant
Acid
Water
Oxidation
By-products
Step 5: L-Ascorbic acid
DAG
Solvents to step 5
Solvents
Hydrochloric acid
Hydrolysis
Rearrangement
Purification
Acetone/solvent recovery system
L-Ascorbic acid
Water

Figure 7. L-Ascorbic acid manufactured by Reichstein and Grüssner Synthesis.

portant to prevent contamination, with subsequent loss of product.

In the third step, L-sorbose is reacted with acetone and excess sulfuric acid at low temperatures. The sorbose dissolves on conversion into the 2,3-mono-*O*-isopropylidene-L-sorbose (2,3 monoacetone-L-sorbose) (MAS), and 2,3:4,6-bis-*O*-isopropylidene-α-L-sorbofuranose (2,3:4,6-diacetone-L-sorbose) (DAS). The equilibrium mixture consists of ca 65% DAS and 35% MAS. The sulfuric acid acts as a catalyst and a dehydrating agent. The reaction mixture is worked up by dilution, neutralization, and extraction to separate the DAS from the MAS. MAS is recycled and the mother liquors are distilled to recover acetone and solvents. The original Reichstein and Grüssner process has been optimized over decades by other workers, giving ca 85% yield (55). Other acidic catalysts, besides sulfuric acid, have been investigated, eg, zinc chloride–phosphoric acid, *p*-toluenesulfonic acid, copper(II) sulfate, and cationic exchange resins. Ferric chloride and perchloric acid are active catalysts that give >90% yields of DAS with azeotropic removal of the water (62). Other methods, including use of acetone dimethylacetal as a water removal reagent, have been reported (63). The use of other ketonic and aldehydic protective agents for L-sorbose, eg, cyclohexanone and its derivatives, formaldehyde, benzaldehyde, etc, has also been described (15,64).

2,3:4,6-Diacetone-L-sorbose (DAS) is oxidized at elevated temperatures in dilute sodium hydroxide in the presence of a catalyst (nickel chloride for bleach or palladium on carbon for air) or by electrolytic methods. After completion of the reaction, the mixture is worked up by acidification to 2,3:4,6-bis-*O*-isopropylidene-2-oxo-L-gulonic acid (2,3:4,6-diacetone-2-keto-L-gulonic acid) (DAG), which is isolated through filtration, washing, and drying. With sodium hypochlorite/nickel chloride, the reported DAG yields are >90% (65). The oxidation with air has been reported, and a practical process was developed with palladium–carbon or platinum–carbon as catalyst (66,67).

The electrolytic oxidation with nickel salts as the catalyst has also been published (68).

DAG is treated with ethanol and hydrochloric acid in the presence of inert solvent, eg, chlorinated solvents, hydrocarbons, ketones, etc. The L-ascorbic acid precipitates from the mixture as it forms, minimizing its decomposition (69). Crude L-ascorbic acid is isolated through filtration and purified by recrystallization from water. The pure L-ascorbic acid is isolated, washed with ethanol, and dried. The mother liquor from the recrystallization step is treated in the usual manner to recover the L-ascorbic acid and ethanol contained in it. The crude L-ascorbic acid mother liquor contains solvents and acetone liberated in the DAG hydrolysis. The solvents are recovered by fractional distillation and recycled. Many solvent systems have been reported for the acid-catalyzed conversion of DAG to L-ascorbic acid (46). Rearrangement solvent systems are used which contain only the necessary amount of water required to give >80% yields of high purity crude L-ascorbic acid (70).

The DAG conversion to L-ascorbic acid also can occur by a base-catalyzed mechanism. Methyl 2-oxo-L-gulonate (methyl DAG) is converted, on treatment with sodium methoxide, to sodium-L-ascorbate, which is then acidified to L-ascorbic acid. Various solvent systems have been evaluated and reported (46).

PACKAGING

L-Ascorbic acid is screened or pulverized into a variety of particle sizes. It is usually packaged in 25-kg and 50-kg quantities in standard, polyethylene-lined containers, eg, fiber drums, corrugated boxes, etc. The recommended storage conditions are low humidity and temperatures of ≤23°C.

ENVIRONMENTAL ISSUES

The environmental concerns of an ascorbic acid manufacturing facility are those typical of a chemical processing plant. Its operating design must be patterned to conform to environmental protection regulations. Measures must be taken to contain solvents and to keep emissions within official guidelines. Special condensers, continuous instrumental monitoring, and emergency containment and cleanup systems are required. Wastewater-treatment facilities have to be provided to remove by-product organics and inorganics from effluent streams before disposal. The extent of these treatment facilities depends upon the location of the plant and the local tolerances. Usually, there are secondary treatment facilities for organic removal; at some plant sites, additional treatment may be required to remove inorganic salts.

ECONOMIC ASPECTS

Strong growth in demand during the period 1985–95 from existing and new applications led to firm pricing and expansion of world capacity. Total world demand in 1995 was estimated to be approximately 60,000 metric tons. Balance of capacity and demand at that time resulted in a selling price of $16.00/kg. More recently (ca 1997), prices have dropped to less than $10/kg.

Continuing demand from food, pharmaceutical, animal nutrition, and cosmetic applications will result in growth rates of 3–4% annually throughout the remainder of the 1990s.

SPECIFICATIONS

Specifications for ascorbic acid, sodium ascorbate, calcium ascorbate, and ascorbyl palmitate are found in the *United States Pharmacopeia / National Formulary* (71) and the *Food Chemicals Codex* (72). The official assay for all four compounds is the iodimetric titration with 0.1 *N* iodine solution and starch as the indicator.

ANALYTICAL METHODS

Many different analytical methods have been developed to determine L-ascorbic acid in feed, biological, and pharmaceutical samples. An excellent review article describes the methodology for finding the proper L-ascorbic acid assay method (73). Comprehensive reviews of all analytical methods, including the extraction of ascorbic acid from foods and biological samples, have been published (74). Ascorbic acid has been determined by a variety of methods, including uv absorption; redox and derivatization reactions; electrochemical and enzymatic oxidation reactions; chromatographic, eg, hplc methods; and biological methods with animals. The guinea pig, one of the few animal species requiring ascorbic acid, is used in the bioassay for ascorbic acid activity. The various methods practiced have been described in detail (75).

Because of the time and expense involved, biological assays are used primarily for research purposes. The first chemical method for assaying L-ascorbic acid was the titration with 2,6-dichlorophenolindophenol solution (76). This method is not applicable in the presence of a variety of interfering substances, eg, reduced metal ions, sulfites, tannins, or colored dyes. This 2,6-dichlorophenolindophenol method and other chemical and physiochemical methods are based on the reducing character of L-ascorbic acid (77). Colorimetric reactions with metal ions as well as other redox systems, eg, potassium hexacyanoferrate(III), methylene blue, chloramine, etc, have been used for the assay, but they are unspecific because of interferences from a large number of reducing substances contained in foods and natural products (78). These methods have been used extensively in fish research (79). A specific photometric method for the assay of vitamin C in biological samples is based on the oxidation of ascorbic acid to dehydroascorbic acid with 2,4-dinitrophenylhydrazine (80). In the microfluorometric method, ascorbic acid is oxidized to dehydroascorbic acid in the presence of charcoal. The oxidized form is reacted with *o*-phenylenediamine to produce a fluorescent compound that is detected with an excitation maximum of ca 350 nm and an emission maximum of ca 430 nm (81).

Another method that determines both ascorbic acid and dehydroascorbic acid first reduced the dehydroascorbic acid to ascorbic acid and then retains the ascorbic acid on an anionic Sephadex column (82). The ascorbic acid is oxidized on the column to dehydroascorbic acid by *p*-benzoquinone, which simultaneously elutes the dehydroascorbic acid. The dehydroascorbic acid is reacted with 4-nitro-1,2-phenylenediamine and absorbance of the resulting yellow solution produced is measured at 375 nm.

Chromatographic methods, notably hplc, are available for the simultaneous determination of ascorbic acid as well as dehydroascorbic acid. Some of these methods result in the separation of ascorbic acid from its isomers, eg, erythorbic acid and oxidation products such as diketogulonic acid. Detection has been by fluorescence, uv absorption, or electrochemical methods (83–85). Polarographic methods have been used because of their accuracy and their ease of operation. Ion exclusion (86) and ion suppression (87) chromatography methods have recently been reported. Other methods for ascorbic acid determination include enzymatic, spectroscopic, paper, thin layer, and gas chromatographic methods. Excellent reviews of these methods have been published (73,88,89).

USES

L-Ascorbic acid is used as a micronutrient additive in pharmaceutical, food, feed, and beverage products, as well as in cosmetic applications. The over-the-counter (OTC) vitamin market is strong, growing in demand, and vitamin C is available in the form of pills and tablets to supplement the daily diet to maintain peak physical performance.

Industrial uses of L-ascorbic acid relate to its antioxidant and reducing properties. It is used as an antioxidant in the commercial preparation of beer, fruit juices, cereals, and canned and frozen foods, etc.

A proposal was made to use L-ascorbic acid as an antioxidant replacement for sulfites in the food industries (90,91). Ascorbic acid's antioxidant property also inhibits nitrosamine formation in cured meat. The addition of ascorbic acid in flour improves baking qualities of dough and appearance of baked goods (92). Vitamin C also prevents discoloration of food during cooking and storage. Its fatty acid esters, eg, L-ascorbyl palmitate, are used to stabilize fats and oils (90). Ascorbic acid and its more stable derivatives L-ascorbyl-2-sulfate, ascorbyl 2-monophosphate, etc, are added to fish feed to improve feed utilization and decrease the rate of infection (93–95). The biological and pharmacological activities of L-ascorbyl-2-sulfate have been reviewed (96,97). Ascorbic acid is also used in agriculture as an abscission agent for fruit, in photography as a developing agent, in metallurgy as a reducing agent, in the polymer industry as a catalyst, in cosmetic formulations, in the manufacture of inks, in explosives, and in a variety of other applications (92,98).

In applications where vitamin C activity is unimportant, often D-erythorbic acid (D-araboascorbic acid) can also be used, providing the same antioxidant and reducing properties as L-ascorbic acid.

DERIVATIVES

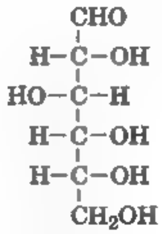
Ascorbic acid has a variety of reactive positions that can be used to synthesize derivatives (99). Various derivatives and analogues have been prepared in attempts to find substances with increased biological activity (100,101).

Only L-ascorbic acid and its salts and C-6-substituted esters have full vitamin activity; sodium L-ascorbate, calcium L-ascorbate, and L-ascorbyl palmitate are commercially significant. L-Ascorbic acid 2-sulfate is bioavailable to fish. 6-Chloro-6-deoxy-L-ascorbic acid was prepared in 1977 and its activity, compared to L-ascorbic acid, is 4/5. Derivatives, eg, 6-deoxy-L-ascorbic acid, L-ascorbic acid 3-*O*-methylether, and 2-amino-2-deoxy-L-ascorbic acid have been prepared; their respective activities compared to L-ascorbic acid are 1/3, 1/25–1/50, and 0. Many more derivatives with and without biological activity have been synthesized (102,103). The highest vitamin C activity correlates with the enediol lactone group, D-configuration for the C-4 hydrogen group, at least a two-carbon substituent on C-4, and L-configuration for the C-5 hydroxyl group. The primary C-6 hydroxyl group has minor impact on the biological activity.

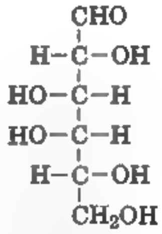
Methods for the preparation of L-ascorbic acids having isotopic C, H, O in various positions have been described and reviewed (104,105). Labeled L-ascorbic acid has played an important role in the elucidation of the metabolic pathway of L-ascorbic acid in plants and animals.

BIOSYNTHESIS

In all plants and most animals, L-ascorbic acid is produced from D-glucose (4) and D-galactose (**26**). Ascorbic acid biosynthesis in animals starts with D-glucose (**4**). In plants, where the biosynthesis is more complicated, there are two postulated biosynthetic pathways for the conversion of D-glucose or D-galactose to ascorbic acid.



(**4**) D-Glucose



(**26**) D-Galactose

Biosynthesis in Animals

Amphibians and reptiles carry the enzyme L-gulono-γ-lactone oxidase which can transform a sugar-like glucose or galactose into ascorbic acid in the kidneys. In mammals (106) and birds (107) this enzyme system has been transferred from the kidneys to the liver. Humans, other primates, guinea pigs, fruit bats, and monkeys from the top of the evolutionary tree, as well as insects and invertebrates from the bottom end of the evolutionary tree, cannot synthesize L-ascorbic acid. Thus, they must consume vitamin C from exogenous sources to survive (108). Animals that are able to produce ascorbic acid do so by the glucuronic acid pathway in the liver or kidneys. The reactions involved in rats are illustrated in Figure 8. In this pathway, the D-glucose chain remains intact, and the C-1 and C-6 of D-glucose become C-6 and C-1, respectively, of L-ascorbic acid as the sequence of carbon-chain numbering is inverted. By measuring the incorporation of ^{14}C from D-glucose-1-^{14}C into urinary L-ascorbic acid, it was determined that the label is found at the C-6 position of L-ascorbic acid (109).

In animals, the glucuronic pathway (Fig. 9) is an important route for glucose utilization leading to the formation of glucuronides and mucopolysaccharides. D-glucose is first phosphorylated to D-glucose-6-phosphate, then isomerized to D-glucose-1-phosphate, which reacts with uridine-triphosphate (UTP) to form UDP-glucose. UDP-glucose is oxidized and hydrolyzed to D-glucuronic acid. D-Glucuronic acid is reduced to L-gulonic acid. L-Gulonic acid lactonizes and forms L-gulono-γ-lactone. Finally, oxidation of this intermediate is carried out by L-gulono-γ-lactone oxidase, the essential oxidizing enzyme, leading to L-ascorbic acid.

D-Galactose may also serve as a precursor of vitamin C because it can be converted to D-glucose. The pathway is important in the metabolism of sugars under normal and diseased conditions and in regulating physiological functions (110). The biosynthesis of L-ascorbic acid is inhibited by deficiencies of certain vitamins, eg, vitamin A, vitamin E, and biotin, but is stimulated by certain drugs, eg, barbiturates, chlorobutanol, aminopyrine, and antipyrine, and by carcinogens, eg, 3-methylcholanthrene and 3,4-benzpyrene (111–113). It has been proposed that the excretion of D-glucaric acid can be used to diagnose both the exposure to the body for foreign substances and the drug metabolic capacity of the liver (114).

Biosynthesis in Plants

As in animals, L-ascorbic acid is also the product of hexose phosphate metabolism in plants, but its biosynthesis is more complicated. There are two biosynthetic pathways for the conversion of D-glucose or D-galactose to L-ascorbic acid in plants (115). The main pathway is postulated as involving retention of configuration by oxidation at C-1 to yield D-gluconic-acid (**27**). Lactonization to yield D-glucono-γ-lactone (**28**) is followed by oxidation at C-2 or C-3 and epimerization at C-5 to afford L-2-oxogulono-γ-lactone (**29**) (Fig. 10). The other biosynthetic pathway from D-glucose and D-galactose may be postulated similarly to Figure 8 to account for configurational inversion (116). The main precursor of L-ascorbic acid is L-galactono-γ-lactone, rather than L-gulono-γ-lactone, which is less active (116,117), and is thought to be epimerized to L-galactono-γ-lactone prior to oxidation to L-ascorbic acid (Fig. 11). Little is known about the functions of ascorbic acid in plants. It is involved in cellular respiration and may contribute to plant growth.

SOURCES OF VITAMIN C

Fruits and vegetables that are good sources of vitamin C include peppers, greens, broccoli, cabbage, spinach, potatoes, tomatoes, strawberries, and citrus products. Meats, fish, poultry, eggs, and dairy products contain small

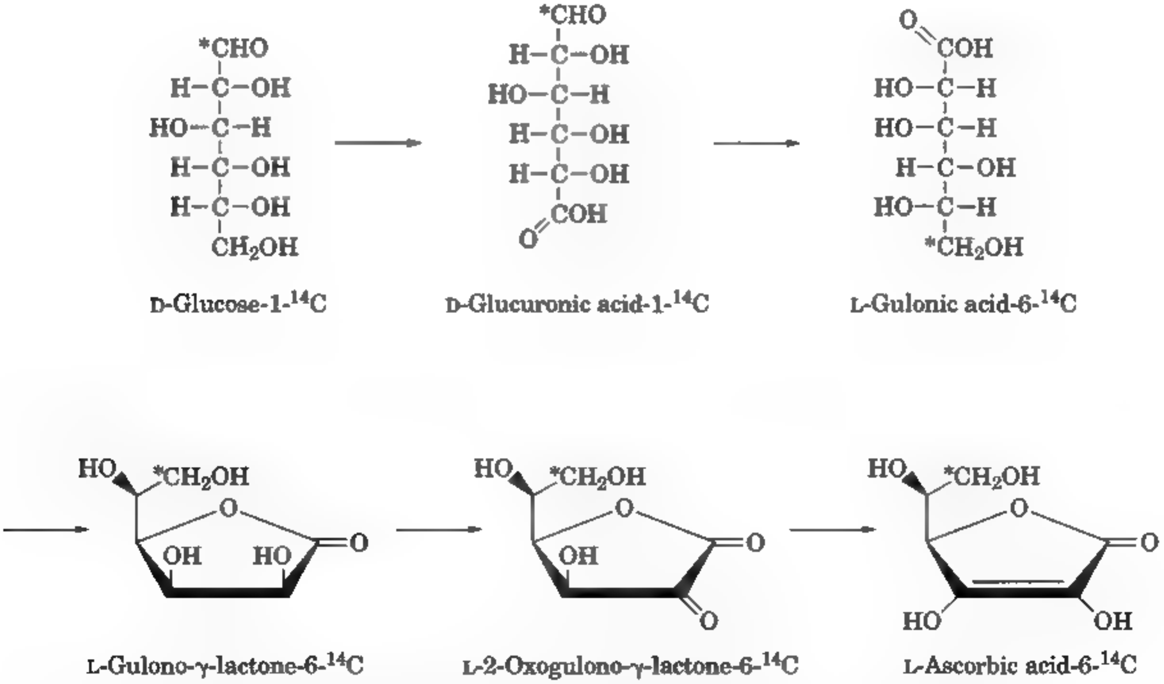


Figure 8. Pathway for the biosynthesis of L-ascorbic acid in rats using C-1-labeled D-glucose; * indicates position of ^{14}C.

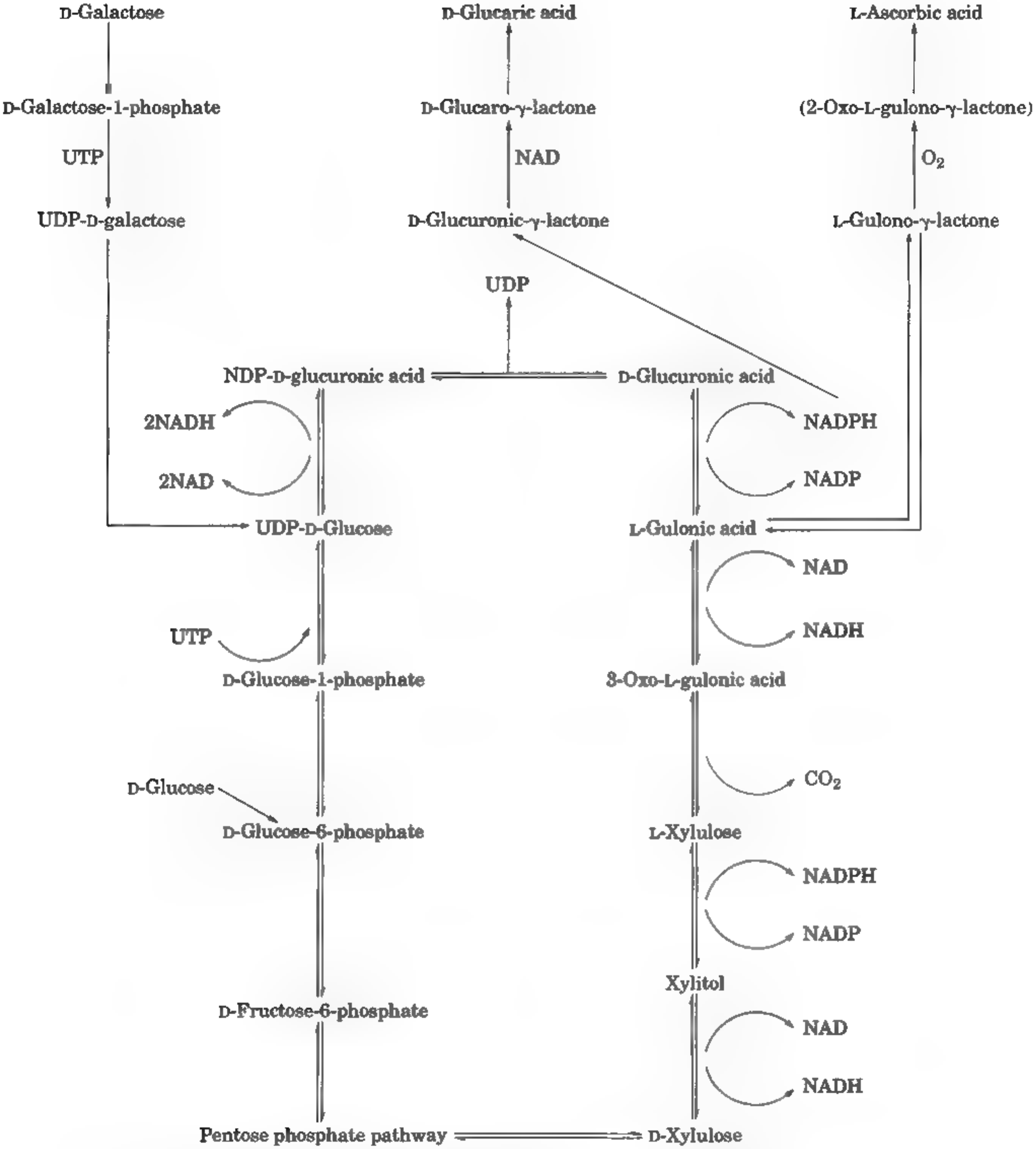
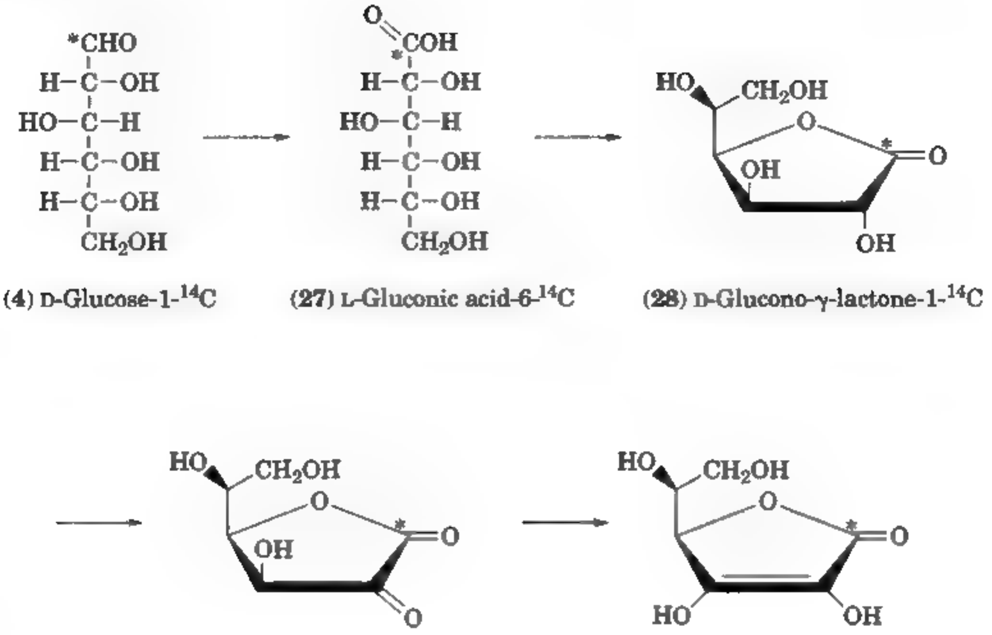

D-Galactose
D-Galactose-1-phosphate
UTP
UDP-D-galactose
D-Glucaric acid
D-Glucaro-γ-lactone
NAD
D-Glucuronic-γ-lactone
L-Ascorbic acid
(2-Oxo-L-gulono-γ-lactone)
O₂
L-Gulono-γ-lactone
UDP
NDP-D-glucuronic acid
D-Glucuronic acid
2NADH
2NAD
NADPH
NADP
UDP-D-Glucose
L-Gulonic acid
NAD
NADH
UTP
D-Glucose-1-phosphate
3-Oxo-L-gulonic acid
CO₂
D-Glucose
D-Glucose-6-phosphate
L-Xylulose
NADPH
NADP
Xylitol
D-Fructose-6-phosphate
NAD
NADH
Pentose phosphate pathway
D-Xylulose

Figure 9. Glucuronic acid pathway. NAD = nicotinamide-adenine dinucleotide; NADH = reduced nicotinamide-adenine dinucleotide; NADP = nicotinamide-adenine dinucleotide phosphate; NADPH = reduced nicotinamide-adenine dinucleotide phosphate; NDP = nucleoside diphosphate; UDP = uridine diphosphate; and UTP = uridine triphosphate.



(**4**) D-Glucose-1-^{14}C (**27**) L-Gluconic acid-6-^{14}C (**28**) D-Glucono-γ-lactone-1-^{14}C

(**29**) L-2-Oxo-gulono-γ-lactone-1-^{14}C (**1**) L-Ascorbic acid-1-^{14}C

Figure 10. Suggested pathway for the biosynthesis of L-ascorbic acid (with retention of configuration) in higher plants based on D-glucose-1-^{14}C experiments; * indicates position of the label.

amounts and grain contains none (118–121). The vitamin C content of some representative foods is listed in Table 4. Potatoes and cabbage have traditionally been the most important sources of vitamin C for the majority of people in the Western World during the winter season.

The contribution of any fruit and vegetable to the vitamin C content of the diet varies depending on climate, soil, and freshness. In actuality, there is a 30% coefficient of variability for ascorbic acid in fresh food products (122). In the case of field-grown spinach, for example, transportation and storage losses have been reported to be as high as 90% (122). During storage after harvesting, the vitamin C content of fruit and vegetables will decrease depending on time and temperature of storage. Ascorbic acid readily oxidizes both enzymatically and chemically on exposure to oxygen. Based on its water solubility, losses are caused through leaching during washing and blanching operations. Heat sterilization of canned foods inactivates completely the enzyme ascorbic acid oxidase which destroys vitamin C. Freezing inactivates the enzymes and is likewise a good method for preserving the vitamin C content of foodstuffs.

Restoration means adding to the food the amount of vitamin C which has been lost during food processing. The term enrichment or fortification is used when a nutrient is added to a food in an amount in excess of that naturally present. Fortification of foods with vitamin C is widely practiced. Technologically, it is important to avoid oxygen, copper, iron, and heat as much as possible to minimize the overages necessary above label claim to ensure compliance throughout the shelf life of the product (123). In canned foods, breakfast drinks, and cereal products (106), overages of 25–50% are commonly used.

PHYSIOLOGY AND BIOCHEMISTRY

Biochemical Functions

Ascorbic acid has various biochemical functions, involving, for example, collagen synthesis, immune function, drug metabolism, folate metabolism, cholesterol catabolism, iron metabolism, and carnitine biosynthesis. Clear-cut evidence for its biochemical role is available only with respect to collagen biosynthesis (hydroxylation of proline and lysine). In addition, ascorbic acid can act as a reducing agent and as an effective antioxidant. Ascorbic acid also interferes with nitrosamine formation by reacting directly with nitrites, and consequently may potentially reduce cancer risk.

Enzymatic Reactions

The polypeptide collagens are the main component of skin and connective tissue, the organic substance of bones and teeth, and the ground substance between cells. The role ascorbic acid plays in collagen formation has been reviewed (124). The synthesis of collagens involves enzymatic hydroxylations of proline and of lysine. The former produces a stable extracellular matrix and the latter is needed for glycosylation and formation of cross-linkages in

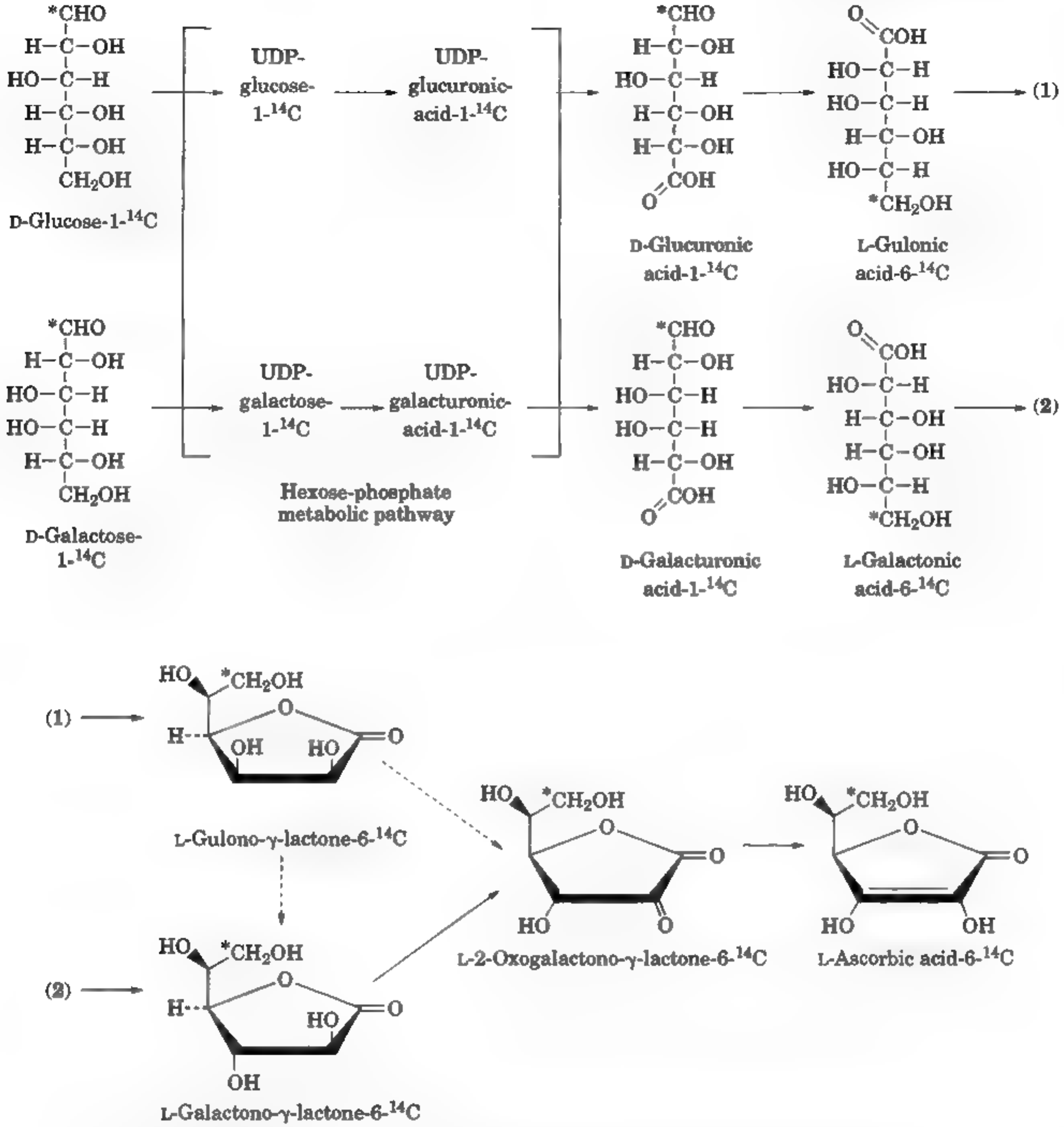

D-Glucose-1-¹⁴C
UDP-glucose-1-¹⁴C
UDP-glucuronic-acid-1-¹⁴C
D-Glucuronic acid-1-¹⁴C
L-Gulonic acid-6-¹⁴C
(1)
D-Galactose-1-¹⁴C
UDP-galactose-1-¹⁴C
UDP-galacturonic-acid-1-¹⁴C
Hexose-phosphate metabolic pathway
D-Galacturonic acid-1-¹⁴C
L-Galactonic acid-6-¹⁴C
(2)
(1)
L-Gulono-γ-lactone-6-¹⁴C
(2)
L-Galactono-γ-lactone-6-¹⁴C
L-2-Oxogalactono-γ-lactone-6-¹⁴C
L-Ascorbic acid-6-¹⁴C

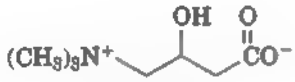
Figure 11. Proposed pathway for the biosynthesis of L-ascorbic acid (with inversion of configuration) in plants using C-1-labeled D-glucose or C-1-labeled D-galactose; * indicates position of the ^{14}C.

the fibers. Vitamin C's role in collagen formation is of importance in wound healing. Intake of 8–50 times the RDA level of 60 mg ascorbic acid per day before and after surgery increases the rate of wound healing considerably (125–127). Many of the clinical signs of scurvy are attributed to defects in collagen synthesis.

Ascorbic acid is involved in carnitine biosynthesis. Carnitine (γ-amino-β-hydroxybutyric acid, trimethylbetaine) (**30**) is a component of heart muscle, skeletal tissue, liver and other tissues. It is involved in the transport of fatty acids into mitochondria, where they are oxidized to provide energy for the cell and animal. It is synthesized in animals from lysine and methionine by two hydroxylases, both containing ferrous iron and L-ascorbic acid. Ascorbic acid donates electrons to the enzymes involved in the metabolism of L-tyrosine, cholesterol, and histamine (**128**).

Table 4. Content of L-Ascorbic Acid in Representative Foods

Food substances	L-Ascorbic acid (mg/100 g)
Meat, fish, and milk	
Beef, pork, fish	≤2
Liver, kidney	10–40
Cow's milk	1–2
Vegetables	
Asparagus	15–30
Brussel sprouts, broccoli	90–150
Cabbage	30–60
Carrots	9
Cauliflower	60–80
Kale	120–180
Leek	15–30
Onion	10–30
Peas, beans	10–30
Parsley	170
Peppers	125–200
Potatoes	10–30
Spinach	50–90
Tomatoes	20–33
Fruit	
Apples	10–30
Bananas	10
Grapefruit	40
Guava	300
Hawthorne berries	160–800
Oranges, lemons	50
Peaches	7–14
Pineapples	17
Rose hips	1000
Strawberries	40–90



(**30**) Carnitine

L-Tyrosine metabolism and catecholamine biosynthesis occur largely in the brain, central nervous tissue, and endocrine system, which have large pools of L-ascorbic acid (128). Catecholamine, a neurotransmitter, is the precursor in the formation of dopamine, which is converted to noradrenaline and adrenaline. The precise role of ascorbic acid has not been completely understood. Ascorbic acid has important biochemical functions with various hydroxylase enzymes in steroid, drug, and lipid metabolism. The cytochrome P-450 oxidase catalyzes the conversion of cholesterol to bile acids and the detoxification process of aromatic drugs and other xenobiotics, eg, carcinogens, pollutants, and pesticides, in the body (129). The effects of L-ascorbic acid on histamine metabolism related to scurvy and anaphylactic shock have been investigated (130). Another cellular reaction involving ascorbic acid is the conversion of folate to tetrahydrofolate. Ascorbic acid has many biochemical functions which affect the immune system of the body (131).

Antioxidant Activity

Ascorbic acid serves as an antioxidant to protect intracellular and extracellular components from free-radical damage. It scavenges free radicals and forms the less reactive ascorbyl radical. The ascorbyl radical can be either reduced to ascorbic acid or oxidized to dehydroascorbic acid (132). Vitamin E is the major fat-soluble antioxidant involved in protecting cells from free-radical impact. During the process of fatty acid oxidation, tocopherol (vitamin E) forms the tocopheroxyl radical. Ascorbic acid has been proven to protect membrane and other hydrophobic compartments from such damage by regenerating the antioxidant form of vitamin E (131). These free-radical scavenging reactions of vitamin C are important in protecting the intracellular and extracellular structure of the lung by quenching free radicals generated by smoke, ozone, and singlet oxygen. The possibility that free-radical damage is also involved in the pathogenesis of HIV and that antioxidants may reduce infection is an area of intense interest (134).

Inhibition of Nitrosamine Formation

Nitrites can react with secondary amines and *N*-substituted amides under the acidic conditions of the stomach to form *N*-nitrosamines and *N*-nitrosamides. These compounds are collectively called *N*-nitroso compounds. There is strong circumstantial evidence that *in vivo* *N*-nitroso compounds production contributes to the etiology of cancer of the stomach (135,136), esophagus (136,137), and nasopharynx (136,138). Ascorbic acid consumption is negatively correlated with the incidence of these cancers, due to ascorbic acid inhibition of *in vivo* *N*-nitroso compound formation (139). The concentration of *N*-nitroso compounds formed in the stomach depends on the nitrate and nitrite intake. Nitrite is part of the preserving process for cured meats. Cigarette smoke contains high levels of nitrite.

Iron Absorption

A very important effect of ascorbic acid is the enhancement of absorption of nonheme iron from foods. Ascorbic acid also enhances the reduction of ferric iron to ferrous iron. This is important both in increasing iron absorption and in its function in many hydroxylation reactions (140,141). In addition, ascorbic acid is involved in iron metabolism. It serves to transfer iron to the liver and to incorporate it into ferritin. Ascorbic acid also forms soluble chelate complexes with iron (142–145). It seems ascorbic acid has no effect on high iron levels found in people with iron overload (146). It is well known, in fact, that ascorbic acid in the presence of iron can exhibit either prooxidant or antioxidant effects, depending on the concentration used (147). The combination of citric acid and ascorbic acid may enhance the iron load in aging populations. Iron overload may be the most important common etiologic factor in the development of heart disease, cancer, diabetes, osteoporosis, arthritis, and possibly other disorders. The synergistic combination of citric acid and ascorbic acid needs further study, particularly because the iron overload produced may be correctable (147).

Deficiency

Scurvy is a vitamin C-specific disease. It is characterized by anemia and alteration of protein metabolism; weakening of collagenous structures in bone, teeth, and connective tissues; swollen, bleeding gums with loss of teeth; fatigue and lethargy; rheumatic pains in the legs and degeneration of the muscles, skin lesions, and capillary weakness, massive hematomas in the thighs; and hemorrhages in many organs, including the eyes. Small (10–60 mg/d) quantities of L-ascorbic acid are sufficient to reverse the trend of both subclinical and clinical scurvy and alleviate their symptoms. Plasma and leukocyte ascorbic acid levels are the most reliable markers of vitamin C intake. Leukocyte levels are more reliable, less sensitive to recent vitamin C intake, and better reflect tissue ascorbate, but require relatively large amounts of blood. Normal leukocyte vitamin C levels are 20–40 $\mu g/10^8$ cells (148). Plasma concentrations of ascorbic acid less than 0.2 mg/dL and leukocyte concentrations below 2 $\mu g/10^8$ cells are seen in scurvy (149). Plasma ascorbic acid levels of 0.5 mg/dL are considered to prevent deficiency symptoms. Normal plasma levels are 0.8–1.4 mg/dL.

Absorption, Transport, and Excretion

The vitamin is absorbed through the mouth, the stomach, and predominantly through the distal portion of the small intestine, and hence, penetrates into the bloodstream. Ascorbic acid is widely distributed to the cells of the body and is mainly present in the white blood cells (leukocytes). The ascorbic acid concentration in these cells is about 150 times its concentration in the plasma (150,151). Dehydroascorbic acid is the main form in the red blood cells (erythrocytes). White blood cells are involved in the destruction of bacteria.

Up to 80% of oral doses of ascorbic acid are absorbed in humans with intakes of less than 0.2 g of vitamin C. Absorption of pharmacological doses ranging from 0.2 g to 12 g results in an inverse relationship, with less than 20% absorption at the higher doses. A single oral dose of 3 g has been reported to approach the absorptive capacity (tissue saturation) of the human intestine. Higher blood levels can be attained by providing multiple divided vitamin C doses per day.

The adrenal glands and pituitary glands have the highest tissue concentration of ascorbic acid. The brain, liver, and spleen, however, represent the largest contribution to the body pool. Plasma and leukocyte ascorbic acid levels decrease with increasing age (152). Elderly people require higher ascorbic acid intakes than children to reach the same plasma and tissue concentration (153).

Ascorbic acid is very soluble in water and mainly excreted in the urine. No ascorbic acid is excreted during vitamin C deficiency. A minimum amount is lost in the feces, even after intake of gram dosages (154).

Mobilization and Metabolism

The total ascorbic acid body pool in healthy adults has been estimated to be approximately 1.5 g, which increases to 2.3–2.8 g with intakes of 200 mg/d (151–158). Depletion of the body pool to 600 mg initiates physiological changes, and signs of clinical scurvy are reported when the body pool falls below 300 mg (149). Approximately 3–4% of the body pool turns over daily, representing 40–60 mg/d of metabolized, or consumed, vitamin C. Smokers have a higher metabolic turnover rate of vitamin C (approximately 100 mg/d) and a lower body pool than nonsmokers, unless compensated through increased daily intakes of vitamin C (159). The metabolism of ascorbic acid varies among different species.

In rats and guinea pigs, respiratory carbon dioxide is the major oxidation product of vitamin C (160). Given to humans in physiological doses, approximately 10% of a dose will be recovered in the urine, and urinary oxalic acid is the predominant metabolite. The formation of urinary metabolites is limited to 30–50 mg/d. Intake of up to 10 g/d ascorbic acid does not result in a considerable increase of urinary metabolites. The vitamin is excreted largely unmetabolized (161). In humans, the metabolites are dehydroascorbic acid, oxalate, 2,3-diketo-L-gulonic acid and derivatives of L-threose and L-threonic acid. L-Ascorbate-2-sulfate has been found in human urine as well (162). The feces are not a significant excretory route unless doses over 1 g are given.

The half-life of ascorbic acid is inversely related to the daily intake and is 13–40 d in humans and 3 d in guinea pigs, which is consistent with the longer time for humans to develop scurvy.

Requirements

The level of ascorbic acid intake required is dependent in part upon the body's handling of the nutrient. The recommendations for the daily intake of vitamin C in various countries range from 30 to 100 mg/d. There is an extensive lack of knowledge about the biochemical and physiological functions of vitamin C. Although as little as 10 mg/d of ascorbic acid can prevent clinical scurvy, this intake is insufficient to maintain an adequate body pool of the vitamin for peak physical and mental health. The RDA (Recommended Dietary Allowances) for vitamin C in the United States is 60 mg for men and women to maintain the body pool (Table 5). Vitamin C levels are higher for pregnant and lactating women to account for losses to the fetus and to breast milk.

The most recent RDA has included a vitamin C recommendation of 100 mg/day for cigarette smokers. An increasing number of investigators have concluded that the current RDA for vitamin C may not be adequate for elderly individuals. Plasma vitamin C level is generally accepted as an indicator of vitamin C status.

A recent study recommended that the current RDA be increased from 60 mg/d to 200 mg. The researchers indicated, however, that vitamin C daily doses above 400 mg have no value (163).

Toxicity

The acceptable daily allowance, which may be ingested without any risk of harm, is 1050 mg for a 70-kg healthy person (20). There is also no evidence in the literature that

Table 5. The 1989 RDA and US RDA for Vitamin C

Group	Age (yr)	Vitamin C (mg)
	RDA	
Infants	0–0.5	30
	0.5–1.0	35
Children	1–3	40
	4–10	45
Males	11–14	50
	15–51 +	60
Females	11–14	50
	15–51 +	60
pregnant		70
lactating		
1st 6 mo		95
2nd 6 mo		90
Cigarette smokers		100
	U.S. RDA	
Infants under 13 mo		35
Children under 4 yr		40
Adults and children over 4 yr		60
Pregnant or lactating women		60

ingestion of up to 10 g vitamin C per day constitutes a serious health risk for humans.

BIBLIOGRAPHY

1. *Biochem. Biophys. Acta* **107**, 4 (1965).
2. W. N. Haworth, *Chem. Ind.* (London) **52**, 482 (1993); W. N. Haworth and E. L. Hirst, *Ergeb. Vitamin-u: Hormonforsch.* **2**, 160 (1939).
3. R. E. Hodges and E. M. Baker, in R. S. Goodhart and M. E. Shils, eds., *Modern Nutrition in Health and Disease*, 5th ed., Lea & Febiger, Philadelphia, Pa., 1973, p. 245; U. Wintermeyer, H. Lahann, and R. Vogel, *Vitamin C: Entdeckung, Identifizierung und Synthese—Heutige Bedeutung in Medizin und Lebensmitteltechnologie*, Deutscher Apotheker Verlag, Stuttgart, Germany, 1981.
4. L. Stone, *The Healing Factor—Vitamin C Against Disease*, Grosset & Dunlap, Inc., New York, 1972, p. 7.
5. I. M. Sharman in G. G. Birch and K. J. Parker, eds., *Vitamin C: Recent Aspects of its Physiological and Technological Importance*, John Wiley & Sons, Inc., New York, 1974, p. 1.
6. A. F. Hess, *Scurvy, Past and Present*, J. B. Lippincott Co., Philadelphia, Pa., 1920.
7. J. Lind, *A Treatise on the Scurvy*, London, 1753 (republished by Edinburgh University Press, Edinburgh, Scotland, 1953).
8. A. Holst and T. Fröhlich, *J. Hyg.* **7**, 634 (1907).
9. H. Chick and E. M. Hume, *J. R. Army Med. Corps* **29**, 121 (1917); *J. Biol. Chem.* **39**, 203 (1919).
10. A. Harden and S. S. Zilva, *Biochem. J.* **12**, 259 (1918); S. S. Zilva and F. M. Wells, *Proc. R. Soc. London Ser. B* **90**, 505 (1917–1919); S. S. Zilva, *Biochem. J.* **21**, 689 (1927).
11. A. Szent-Györgyi, *Biochem. J.* **22**, 1387 (1928); J. L. Svirbely and A. Szent-Györgyi, *Biochem. J.* **26**, 865 (1932).
12. C. G. King and W. A. Waugh, *Science* **75**, 357 (1932).
13. W. N. Haworth and E. L. Hirst, *J. Soc. Chem. Ind.* **52**, 645–646 (1933).
14. T. Reichstein, A. Grüssner, and R. Oppenhauer, *Helv. Chim. Acta* **16**, 1019–1033 (1933).
15. T. Reichstein and A. Grüssner, *Helv. Chim. Acta* **17**, 311 (1934).
16. P. Karrer, K. Schöpp, and F. Zehner, *Helv. Chim. Acta* **16**, 1161–1163 (1933).
17. F. Michael and K. Kraft, Hoppe-Seyler's *Z. Physiol. Chem.* **222**, 235–249 (1933).
18. H. von Euler and C. Martius, *Ann.* **505**, 73–87 (1933).
19. E. L. Hirst, *Fortsch. Chem. Org. Naturst.* **2**, 132–159 (1939).
20. *Wld. Hlth. Org. Techn. Rep Ser.* 1972, No. 505, *Wld. Hlth. Org. Techn. Rep. Ser.* 1974, No. 539.
21. Armour Research Foundation, *Anal. Chem.* **20**, 986–987 (1948).
22. C. von Planta, *Helv. Chim. Acta* **44**, 1444–1446 (1961)
23. J. Hvoslef, *Acta Crystallogr. Sect. B* **24**, 1431–1440 (1968).
24. J. Hvoslef, *Acta Chem. Scand.* **18**, 841–842 (1964).
25. J. Hvoslef, *Acta Crystallogr. Sect. B* **24**, 23–35 (1968).
26. W. Müller-Mulot, Hoppe-Seyler's *Z. Physiol. Chem.* **351**, 52,56 (1970); J. Hvoslef, *Acta Chem. Scand.* **24**, 2238 (1970); *Acta Crystallogr.* **B28**, 916 (1972); J. Hvoslef and B. Pedersen, *Acta Chem. Scand.* **B33**, 503 (1979); *Carbohydr. Res.* **92**, 9 (1981).
27. R. J. Kutsky, *Handbook of Vitamins and Hormones*, Van Nostrand Reinhold Co., Inc., New York, 1973, p. 71; *The Merck Index*, 11th ed., Merck & Co., Inc., Rahway, N.J., 1989, p. 130.
28. J. S. Koevendel, *Nature* **180**, 434 (1957); Y. Ogata and Y. Kosugi, *Tetrahedron* **26**, 4711 (1970).
29. Sadtler Standard Spectra, *Spectrum No. 5424*, Sadtler Research Laboratory, Inc., Philadelphia, Pa., 1972.
30. High Resolution NMR Spectra Catalog, Vol. 2, *Spectrum No. 464*, Varian Assoc., Palo Alto, Calif., 1963.
31. V. F. Johnson and W. C. Jankowski, Carbon-13 NMR Spectra, *Spectrum No. 171*, Wiley-Interscience, New York, 1972; S. Berger, *Tetrahedron* **33**, 1587 (1977); T. Ogama, J. Uzawa, and M. Matsui, *Carbohydr. Res.* **59**, C32 (1977); T. Radford, J. G. Sweeny, and G. A. Jacobucci, *J. Org. Chem.* **44**, 658 (1979).
32. B. M. Tolbert et al., *Ann. N.Y. Acad. Sci.* **258**, 44 (1975); B. M. Tolbert, *Int. J. Vit. Nutr. Res. Suppl.* **19**, 127 (1979).
33. B. Tolbert and J. B. Ward, in *Ascorbic Acid, Chemistry, Metabolism and Uses, Adv. Chem. Sci.* **200**, 101–123 (1982); M. Ohmori, M. Takage, *Agr. Biol. Chem.* **42**, 173–174 (1978).
34. F. E. Huelin, *Food Res.* **18**, 633 (1953).
35. J. Campbell and W. G. Tubb, *Can. J. Res.* **28E**, 19–32 (1950).
36. K. Mikova and J. Davidele, *Chem. Listy* **68**, 715 (1974).
37. F. E. Huelin, *Food Res.* **18**, 633 (1953); J. Campbell and W G. Tubb, *Can. J. Res.* **28E**, 19 (1947).
38. D. W. Bradley, G. Emery, and J. E. Maynard, *Clin. Chim. Acta* **4**, 47–52 (1973).
39. T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta* **16**, 561–565 (1933).
40. T. Reichstein, A. Grüssner, and R. Oppenauer, *Nature (London)* **132**, 280 (1932).
41. T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta* **17**, 510–520 (1934)
42. U.S. Pat. 2,056,126 (Sept. 29, 1936), T. Reichstein (to Hoffmann-La Roche Inc.).
43. W. N. Haworth, *Chem. Ind. (London)* **52**, 482–485 (1933).

44. R. G. Ault et al., *J. Chem. Soc.* 1419–1423 (1933).
45. W. N. Haworth and E. L. Hirst, *Helv. Chim. Acta* **17**, 520–523 (1934).
46. T. C. Crawford and S. A. Crawford, *Adv. Carbohydr. Chem. Biochem.* **37**, 79 (1980).
47. M. Kulhanek, *Adv. Appl. Microbiol.* **12**, 11–33 (1970).
48. G. Xin, *Weishengwuxue Tongbao* **14**, 73 (1987).
49. Eur. Pat. Appl. 278 447 (Prior. Feb. 2, 1987) W. Ning, Z. Tao, C. Wang, S. Wang, Z. Yan, and G. Yin (to Institute of Microbiology Academia Sinica).
50. Eur. Pat. Appl. 221 707 (Prior Oct. 22, 1985) I. Nogami, H. Shirafuji, M. Oka, and T. Yamaguchi (to Takeda Chemical Industries, Ltd.).
51. T. Sonoyama et al., *Appl. Environ. Microbiol.* **43**, 1064 (1982).
52. S. Anderson et al., *Science* **230**, 144–149 (1985).
53. J. F. Grindley, M. A. Payton, H. van do Pol, and K. G. Hardy, *Appl. Environ. Microbiol.* **54**, 1770 (1988).
54. Eur. Pat. Appl. No. 207 763 (1985), T. J. Skatrud and R. J. Huss (to Bio-Technical Resources).
55. I. T. Strukov and N. A. Kopylova, *Farmatsiya* **10**, 8 (1974); *Drug Cosmet. Ind.* **40** (Aug. 1977).
56. W. Ipatiew, *Ber.* **45**, 3218 (1912).
57. L. W. Cover, R. Connor, and H. Adkins, *J. Am. Chem. Soc.* **54**, 1651 (1932).
58. A. T. Kuhn, *J. Appl. Electrochem.* **11**, 261 (1981).
59. W. E. Cake, *J. Am. Chem. Soc.* **44**, 859 (1922).
60. M. Kulhanek, *Adv. Appl. Microbiol.* **12**, 11 (1970).
61. P. A. Wells, J. J. Stubbs, L. B. Lockwood, and E. T. Roe, *Ind. Eng. Chem.* **29**, 1385 (1937); E. J. Fulmer and L. A. Underkofler, Iowa State Coll. *J. Sci.* **21**, 251 (1974).
62. U.S. Pat. 3,622,560 (Nov. 23, 1971), N. Halder, M. J. O'Leary, and N. C. Hindley; U.S. Pat. 3,598,804 (Aug. 10, 1971), N. Halder, N. C. Hindley, G. M. Jaffe, M. J. O'Leary, and P. Weinert; U.S. Pat. 3,607,862 (Sept. 21, 1971), G. M. Jaffe, W. Szkrybalo, and P. Weinert (all to Hoffmann-La Roche Inc.).
63. R. S. Glass, S. Kwoh, and E. P. Oliveto, *Carbohydr. Res.* **26**, 181 (1973).
64. U.S. Pat. 2,039,929 (May 5, 1936), T. Reichstein, U.S. Pat. 2,301,811 (Nov. 10, 1942), T. Reichstein (both to Hoffmann-La Roche Inc.); V. F. Kazimirova, *J. Gen. Chem. (USSR)* **24**, 1559 (1955).
65. J. W. Weijlard, *J. Am. Chem. Soc.* **67**, 1031 (1945); U.S. Pat. 2,367,251 (Jan. 16, 1945), J. Weijlard and J. B. Ziegler (to Merck and Co.); K. Nakagawa, R. Konaka, and T. Nakata, *J. Org. Chem.* **27**, 1597 (1962).
66. L. O. Shnaidman and I. N. Kushehinskaya, *Tr. Vses. Nauchno-Issled. Vitam. Inst.* **8**, 13 (1961).
67. U.S. Pat. 3,832,355 (Aug. 27, 1972), G. M. Jaffe and E. Pleven (to Hoffmann-La Roche Inc.).
68. U.S. Pat. 3,453, 191 (July 1, 1969), G. J. Fröhlich, A. J. Kratavil, and E. Zrike (to Hoffmann-La Roche Inc.); Eur Pats. 40331 (Nov. 25, 1981) and 40709 (Dec. 2, 1981), R. Whittmann, W. Wintermeyer, and J. Butska (to Merck GMBH); P. M. Robertson, P Berg, H. Reimann, K. Schleich, and P. Seiler, *Chimia* **36**, 305 (1982).
69. P. P. Regna and B. P. Caldwell, *J. Am. Chem. Soc.* **66**, 246 (1944).
70. F. Elger, *Festschr. Emil Barell*, 229 (1936); Brit. Pat. 428,815 (May 20, 1935), and Brit. Pat. 466,548 (May 31, 1937), T. Reichstein (to Hoffmann-La Roche, Inc.).
71. *The United States Pharmacopeia / National Formulary (USP XXIII / NFXVIII)*, United States Pharmacopeial Convention, Inc., Rockville, Md., 1995, pp. 130, 245, 1414, 2215.
72. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, pp. 27, 28, 277–278.
73. L. A. Pachla and D. L. Reynolds, *J. Assoc. Off. Anal. Chem.* **68**, 1–12 (1985).
74. J. R. Cooke and R. E. D. Moxon, in *Vitamin C (Ascorbic Acid)*, J. N. Counseil and D. H. Hornig, eds., Applied Science Publishers, London, 1981, pp. 167–198; *Adv. Chem. Ser* **200**, 199 (1982); R. C. Rose and D L. Nahrwold, *Anal. Biochem.* **123**, 389 (1982).
75. C. I. Bliss and P. György, in P. György, ed., *Vitamin Methods*, Vol. II, Academic Press, New York, 1951, pp. 244–261.
76. J. Tillmans, *Z. Unters. Lebensmittel* **54**, 33 (1927).
77. R. Strohecker, *Wiss. Veroff. Dtsch. Ges. Ernahr.* **14**, 157 (1965).
78. T. Tono and S. Fujita, *Agric. Biol. Chem.* **45**, 2947 (1981).
79. K. Dabrowski et al., *J. Aquaculture* **124**, 169–192 (1994)
80. J. H. Roe and C. A. Kuether, *J. Biol. Chem.* **147**, 399–407 (1943).
81. M. J. Deutsch and C. E. Weeks, *J. Assoc. Off. Anal. Chem.* **48**, 1248–1256 (1965).
82. G. Brubacher, W. Müller-Mulot, and D. A. T. Southgate, *Methods for the Determination of Vitamins in Food Recommended by Cost 91*, Elsevier Applied Science, New York, 1985, Chapt. 7.
83. J. Geigert, D. S. Hirano, and S. L. Neidleman, *J. Chromatogr.* **206**, 396–399 (1981).
84. S. Garcia-Castineiras, V. D. Bonnet, R. Figueroa, and M. Miranda, *J. Liq. Chromatogr.* **4**, 1619–1640 (1981).
85. W. Lee, P. Hamernyik, M. Hutchinson, V. A. Raisys, and R. F. Labbe, *Clin. Chem.* **28**, 2165–2169 (1982).
86. H. J. Kim, *J. Assoc. Official Anal. Chem.* **72**, 681–686 (1989).
87. L. L. Lloyd, F. Warner, J. F. Kennedy, and C. A. White, *J. Chromatogr.* **437**, 453–456 (1988).
88. O. Pelletier, in J. Augustin, B. P. Klein, D. A. Becker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, Chapt. 12.
89. M. H. Bui-Nguyen, in A. P. DeLeenheer, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1985, Chapt. 5.
90. B. Borenstein, *Food Tech.* **41**, 98–99 (1987).
91. M. Liao and P. A. Seib, *Food Tech.* **41**, 104–107 (1987).
92. J. C. Bauernfeind, in *Ascorbic Acid: Chemistry, Metabolism, and Uses*, P. A. Seib and B. M. Tolbert, eds., American Chemical Society, Washington, D.C., 1982, pp. 395–497.
93. V. S. Durve and R. T. Lovell, *Can. J. Fish Aquat. Sci.* **39**, 948–951 (1982).
94. M. Matusiewicz, K. Dabrowski, L. Volker, and K. Matusiewicz, *J. Nutr.* **125**, 3055–3061 (1995).
95. R. T. Lovell and G. O. Elnaggar, *Proceedings of the Third International Symposium on Feeding and Nutrition in Fish*, Toba, Japan, (Aug. 28–Sept. 1, 1989), pp. 159–165.
96. G.-O. Tsa, *Sheng Lik'o Hseuh Chin Chan* **12**, 26 (1981).
97. K. Dabrowski, M. Matusiewicz, and J H. Blom, *Aquaculture* **124**, 169–190 (1994).
98. U.S. Pat. No. 4,728,376 (Mar. 1, 1988), E. F. Kurtz (to Golden Powder of Texas, Inc.).

99. O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, *Vitamin II*, Georg Thieme Verlag, Stuttgart, Germany, 1988, pp. 396–444.
100. F. Smith, *Adv. Carbohydr. Chem. Biochem.* **2**, 79 (1946).
101. J. Stanek, M. Cerny, J. Kocourek, and J. Pacak, *The Monosaccharides*, Academic Press, Inc., New York, 1963, p. 735.
102. G. Mohn, in W. Dirscherl and W. Friedrich, eds., *Fermente, Hormone, Vitamine, und die Beziehungen dieser Wirkstoffe sueinander*, 3rd ed., Vol. 3, Pt. 1 (Vitamine), Thieme, Stuttgart, Germany, 1982, pp. 910–913.
103. R. S. Harris, *The Vitamins*, 2nd ed., Vol. 1, Academic Press, New York, London, 1967, 383–385.
104. M. Williams and F. A. Loewus, *Carbohydr. Res.* **63**, 149 (1978).
105. S. L. von Schuching and A. F. Abt, *Methods Enzymol.* **18**, 1 (1970).
106. I. B. Chatterjee, *Science* **82**, 1271–1272 (1973).
107. I. B. Chatterjee, A. K. Majumder, B. K. Nandi, and N. Subramanian, in *Second Conference on Vitamin C*, Vol. 258, C. G. King and J. J. Burns, eds., New York Academy of Sciences, New York, 1975, pp. 24–47.
108. J. J. Burns, *Am. J. Med.* **26**, 740 (1959).
109. F. A. Isherwood, Y. T. Chen, and L. W. Mapson, *Biochem. J.* **56**, 11 (1954); J. J. Burns and C. Evans, *J. Biol. Chem.* **23**, 897 (1956).
110. G. J. Dutton, ed., *Glucuronic Acid-Free and Combined: Chemistry, Biochemistry, Pharmacology and Medicine*, Academic Press, Inc., New York 1966; B. L. Horecker in G. Ritzel and G. Brubacher, eds., *Monosaccharides and Polyols in Nutrition, Therapy and Dietetics*, Hans Huber, Bern, Switzerland, 1967, p. 1.
111. A. H. Conney, G. A. Bray, C. E. Evans, and J. J. Burns, *Ann. N.Y. Acad. Sci.* **92**, 115 (1961).
112. J. J. Burns, C. Evans, and N. Trousof, *J. Biol. Chem.* **227**, 785–794 (1957).
113. F. Horio and A. Yoshida, *J. Nutr* **112**, 416–425 (1982).
114. W. R. F. Notten, Stichting Studentenpers, University of Nijmegen, Nijmegen, The Netherlands, 1975; W. R. F. Notten and P. T. Henderson, *Int. Arch. Occup. Environ. Health* **38**, 197, 209 (1977).
115. F. A. Loewus and M. M. Baig, *Methods Enzymol.* **18**, 22 (1970); F. A. Loewus, G. Wagner, and J. C. Yang, *Ann. N.Y. Acad. Sci.* **258**, 7 (1975); F. A. Loewus in Preiss, ed., *Biochemistry of Plants*, Vol. 3, Academic Press, Inc., New York, 1980, p. 77; C. H. Foyer and B. Halliwell, *Phytochemistry* **16**, 1347 (1977).
116. F. A. Isherwood, Y. T. Chen, and L. W. Mapson, *Biochem. J.* **56**, 1 (1954).
117. M. M. Baig, S. Kelley, and F. A. Loewus, *Plant. Physiol.* **46**, 277 (1970).
118. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, pp. 115–124.
119. W H. Sebrell and R. S Harris, eds., *The Vitamins, Chemistry, Physiology, Pathology, Methods*, 2nd ed., Vol. 1, Academic Press, Inc., New York, 1967, p. 305.
120. J. Marks, *A Guide to the Vitamins, Their Role in Health and Disease*, Medical and Technical Publishing Co., Lancaster, U.K., 1975.
121. S. Nobile and J. M. Woodhill, *Vitamin C: The Mysterious Redox-System—A Trigger of Life*, MTP Press, Boston, Mass., 1981, p. 38.
122. P. A. La Chance and M. C. Fisher, in R. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., Van Nostrand Publishing Co., New York, 1988, p. 506.
123. B. Borenstein, in T. E. Furis, ed., *Handbook of Food Additives*, 2nd ed., CRC Press, Cleveland, Ohio, 1972, pp. 91, 101.
124. B. Peterkofsky and S. Udenfriend, *Proc. Nat. Acad. Sci.* **53**, 335 (1965); M. J. Barnes and E. Kodicek, *Vitam. Horm.* **30**, 1 (1972).
125. A. H. Hunt, *Br. J. Surg.* **28**, 436–461 (1941).
126. S. P. Shukla, *Experientia* **25**, 704 (1969).
127. T. T. Irvin, D. K. Chattopadhyay, and A. Smythe, *Surg. Gynec. Obstet.* **147**, 49–55 (1978).
128. C. J. Bates, in J. N. Counsell and D. H. Hornig, eds., *Vitamin C (Ascorbic Acid)*, Applied Science Publishers, London, 1981.
129. V. G. Zannoni, J. I. Brodfuehrer, R. C. Smart, and R. L. Susick, in J. J. Burns, J. M. Rivers, and L. J. Machlin, eds., *Third Conference on Vitamin C*, N.Y. Academy of Sciences, New York, 1987.
130. W. Dawson and G. B. West, *Br. J. Pharmacol.* **24**, 75 (1965).
131. A. Bendich, *Food Tech.* **41**, 112–114 (1987).
132. A. Bendich, L. J. Machlin, O. Scandurra, G. W. Burton, and D. D. M. Wayner, *Adv. in Free Radical Biol. Med.* **2**, 419–444 (1986).
133. R. E. Beyer, *J. Bioenerget. Biomembr.* **26**, 349–358 1994.
134. R. J. Jariwalla, S. Harakch, in R. R. Watson, ed., *Nutrition and AIDS*, CRC Press, Boca Raton, Fla., 1994, pp. 117–139.
135. S. S. Mirvish, *J. Natl. Cancer Inst.* **71**, 629–647 (1983).
136. P. N. Magee, *Cancer Surv.* **8**, 207–239 (1989).
137. V. M Craddock, *Eur. J. Cancer Prev.* **1**, 89–103 (1992).
138. Y. Zeng and co-workers, in *Cancer Epidemiol. Biomarkers & Prev.* **2**, 195–200 (1993).
139. S. S. Mirvish, *Cancer Res.* (Suppl.), 1948–1951 (Apr. 1, 1994).
140. L. Hallberg, in *Vitamin C (Ascorbic Acid)*, J. N. Counsell and D. H. Hornig, eds., Applied Science Publishers, London, 1981, pp. 49–61.
141. L. Hallberg, M. Brune, and L. Rossander-Hulthen, in J. J. Burns, J. M. Rivers, and L. J. Machlin, eds., *Third Conference on Vitamin C*, Vol. 498, New York Academy of Sciences, New York, 1987, pp. 324–332.
142. E. R. Monsen and J. F. Page, *J. Agric. Food Chem.* **26**, 223–226 (1978).
143. N. W. Solomons and F. E. Viteri, in P. A. Seib and B. M. Tolbert, eds., *Ascorbic Acid: Chemistry, Metabolism, and Uses*, American Chemical Society, Washington, D.C., 1982, pp. 551–569.
144. C. M. Plug, D. Decker, and A. Bult, *Pharm. Weekblad* **16**, 245–248 (1984).
145. J. J. M. Marx and J. Stiekema, *Eur. J. Clin. Pharmacol.* **23**, 335–338 (1982).
146. A. Bendich in L. Packer and J. Fuchs, eds., *Vitamin C in Health and Disease*, J. Marcel Dekker Inc., NY, 1997, pp. 411–416.
147. R. D. Crawford, *Biochem. Molec. Med.* **54**, 1–11 (1995).
148. C. J. Schorah, in T. G. Tayler, ed., *The Importance of Vitamins to Health*, MTP Press, Lancaster, U.K., 1979, pp. 61–72.
149. R. E. Hodges, E. M. Baker, J. Hood, H. E. Sauberlich, and S. C. March, *Am. J. Clin. Nutr.* **22**, 535–548 (1969).
150. R. Anderson, in Ref. 128, pp. 249–272.
151. A. Castelli, G. E. Martorana, E. Meucci, and G. Bonetti, *Acta Vitaminol. Enzymol.* **4**, 189–196 (1982).

152. C. J. Schorah et al., *Am. J. Clin. Nutr.* **34**, 871–876 (1981).
153. P. J. Garry, J. S. Goodwin, W. C. Hunt, and B. A. Gilbert, *Am. J. Clin. Nutr.* **36**, 332–339 (1982).
154. D. Hornig, J. P. Vuileumier, and D. Hartmann, *Intern. J. Vitamin Nutr. Res.* **50**, 309–314 (1980).
155. E. M. Baker, J. C. Saari, and B. M. Tolbert, *Am. J. Clin. Nutr.* **19**, 371–378 (1966).
156. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, and S. C. March, *Am. J. Clin. Nutr.* **22**, 549–558 (1969).
157. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, and E. M. Baker, *Am. J. Clin. Nutr.* **24**, 444–454 (1971).
158. R. E. Hodges, J. Hood, J. Canham, H. E. Sauberlich, and E. M. Baker, *Am. J. Clin. Nutr.* **24**, 432–443 (1971).
159. A. B. Kallner, D. Hartmann, and D. Hornig, *Am. J. Clin. Nutr.* **34**, 1347–1355 (1981).
160. D. Hornig and D. Hartmann, in P. A. Seib and P. M. Tolbert, eds., *Ascorbic Acid: Chemistry, Metabolism and Uses*, American Chemical Society, Washington, D.C., 1982, pp. 293–316.
161. A. Kallner, D. Hartmann, and D. Hornig, *Am. J. Clin. Nutr* **32**, 530–539 (1979).
162. E. M. Baker, D. C. Hammer, S. C. March, B. M. Tolbert, and J. E. Canham, *Science* **173**, 826–827 (1971).
163. M. Levine et al., *Proc. Natl. Acad. Sci. USA* **93**, 3704 (1996).

GENERAL REFERENCES

Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, pp. 115–124.

D. H. Hornig, U. Moser, and B. E. Glatthaar, in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Diseases*, 7th ed., Lea & Febiger, Philadelphia, 1988, pp. 417–435.

R. A. Jacob, J. H. Skala, and S. T. Omave, *Am. J. Clin. Nutrit.* **46**, 818–826 (1987).

R. Marcus and A. M. Coulston, in A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murari, eds., *Goodman and Gilman's Pharmacological Basis of Therapeutics*, 7th ed., Macmillan Publishing Co., New York, 1985, pp. 1151–1573.

U. Moser and A. Bendich, in L. J. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991, pp. 195–232.

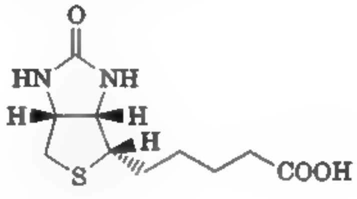
R. L. Pike and M. L. Brown, *Nutrition: An Integrated Approach*, 3rd ed., Macmillan Publishing Co., New York, 1986, pp. 131–136.

H. E. Sauberlich, in M. L. Brown, ed., *Present Knowledge in Nutrition*, 6th ed., International Life Sciences Institute, Nutrition Foundation, Washington, D.C., 1990, pp. 132–141.

VOLKER KUELLMER
Hoffmann-La Roche, Inc.

VITAMINS: BIOTIN

Biotin, [3a*S*-(3a*α*, 4*β*, 6a*α*)]-hexahydro-2-oxo-1*H*-thieno-[3,4-*d*]imidazole-4-pentanoic acid [*58-85-5*] (vitamin H, vitamin B_8, bios IIB, and coenzyme R) (1) is a water-soluble B complex vitamin. The name coenzyme R was coined during work on a protective factor, from the liver, for egg white injury. This protective factor was also called factor S, factor W or vitamin B_W (1–4). Biotin is a complex molecule having three stereocenters. There are eight stereoisomers of biotin; only the naturally occurring one is active in metabolism. The richest sources of biotin are yeast, liver, kidney, egg yolks, pancreas, and milk (5–7). The highest content of biotin in cow's milk occurs early in lactation. Plant materials, such as nuts, seeds, cereals such as oats and bulgar wheat, pollen, molasses, rice, soybeans, mushrooms, fresh vegetables such as cauliflower, split peas, cow peas, and legumes, and some fruits, are also good sources. In addition, small amounts of biotin are found in most fish, eg, mackerel, salmon, and sardines.



(1)

ISOLATION

In 1936, biotin was isolated from egg yolks (8), in 1939 from beef liver (9), and in 1942 from milk concentrates (10). Biotin-producing microorganisms exist in the large bowel but the extent and significance of this internal synthesis is unknown.

BIOCHEMICAL FUNCTION

Biotin forms part of several enzyme systems and is necessary for normal growth and body function. Biotin functions as a cofactor for enzymes involved in carbon dioxide fixation and transfer. These reactions are important in the metabolism of carbohydrates, fats, and proteins, as well as promotion of the synthesis and formation of nicotinic acid, fatty acids, glycogen, and amino acids (5–7). Biotin is absorbed unchanged in the upper part of the small intestine and distributed to all tissues. Highest concentrations are found in the liver and kidneys. Little information is available on the transport and storage of biotin in humans or animals. A biotin level in urine of approximately 160 nmol/24 h or 70 nmol/L, and a circulating level in blood, plasma, or serum of approximately 1500 pmol/L seems to indicate an adequate supply of biotin for humans. However, reported levels for biotin in the blood and urine vary widely and are not a reliable indicator of nutritional status.

NUTRITIONAL REQUIREMENTS

Since exact requirements for biotin are uncertain owing to incomplete knowledge regarding biotin availability from food and a lack of definitive studies concerning biotin requirements, the United States National Research Council has established a safe and adequate daily dietary level of intake for biotin rather than a recommended dietary allowance (RDA). The recommended daily intake of biotin in the United States for all persons ages seven years and older is

152. C. J. Schorah et al., *Am. J. Clin. Nutr.* **34**, 871–876 (1981).
153. P. J. Garry, J. S. Goodwin, W. C. Hunt, and B. A. Gilbert, *Am. J. Clin. Nutr.* **36**, 332–339 (1982).
154. D. Hornig, J. P. Vuileumier, and D. Hartmann, *Intern. J. Vitamin Nutr. Res.* **50**, 309–314 (1980).
155. E. M. Baker, J. C. Saari, and B. M. Tolbert, *Am. J. Clin. Nutr.* **19**, 371–378 (1966).
156. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, and S. C. March, *Am. J. Clin. Nutr.* **22**, 549–558 (1969).
157. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, and E. M. Baker, *Am. J. Clin. Nutr.* **24**, 444–454 (1971).
158. R. E. Hodges, J. Hood, J. Canham, H. E. Sauberlich, and E. M. Baker, *Am. J. Clin. Nutr.* **24**, 432–443 (1971).
159. A. B. Kallner, D. Hartmann, and D. Hornig, *Am. J. Clin. Nutr.* **34**, 1347–1355 (1981).
160. D. Hornig and D. Hartmann, in P. A. Seib and P. M. Tolbert, eds., *Ascorbic Acid: Chemistry, Metabolism and Uses*, American Chemical Society, Washington, D.C., 1982, pp. 293–316.
161. A. Kallner, D. Hartmann, and D. Hornig, *Am. J. Clin. Nutr* **32**, 530–539 (1979).
162. E. M. Baker, D. C. Hammer, S. C. March, B. M. Tolbert, and J. E. Canham, *Science* **173**, 826–827 (1971).
163. M. Levine et al., *Proc. Natl. Acad. Sci. USA* **93**, 3704 (1996).

GENERAL REFERENCES

Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, pp. 115–124.

D. H. Hornig, U. Moser, and B. E. Glatthaar, in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Diseases*, 7th ed., Lea & Febiger, Philadelphia, 1988, pp. 417–435.

R. A. Jacob, J. H. Skala, and S. T. Omave, *Am. J. Clin. Nutrit.* **46**, 818–826 (1987).

R. Marcus and A. M. Coulston, in A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murari, eds., *Goodman and Gilman's Pharmacological Basis of Therapeutics*, 7th ed., Macmillan Publishing Co., New York, 1985, pp. 1151–1573.

U. Moser and A. Bendich, in L. J. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991, pp. 195–232.

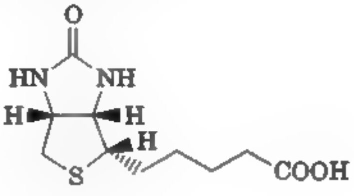
R. L. Pike and M. L. Brown, *Nutrition: An Integrated Approach*, 3rd ed., Macmillan Publishing Co., New York, 1986, pp. 131–136.

H. E. Sauberlich, in M. L. Brown, ed., *Present Knowledge in Nutrition*, 6th ed., International Life Sciences Institute, Nutrition Foundation, Washington, D.C., 1990, pp. 132–141.

VOLKER KUELLMER
Hoffmann-La Roche, Inc.

VITAMINS: BIOTIN

Biotin, [3a*S*-(3a*α*, 4*β*, 6a*α*)]-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid [*58-85-5*] (vitamin H, vitamin B_8, bios IIB, and coenzyme R) (1) is a water-soluble B complex vitamin. The name coenzyme R was coined during work on a protective factor, from the liver, for egg white injury. This protective factor was also called factor S, factor W or vitamin B_W (1–4). Biotin is a complex molecule having three stereocenters. There are eight stereoisomers of biotin; only the naturally occurring one is active in metabolism. The richest sources of biotin are yeast, liver, kidney, egg yolks, pancreas, and milk (5–7). The highest content of biotin in cow's milk occurs early in lactation. Plant materials, such as nuts, seeds, cereals such as oats and bulgar wheat, pollen, molasses, rice, soybeans, mushrooms, fresh vegetables such as cauliflower, split peas, cow peas, and legumes, and some fruits, are also good sources. In addition, small amounts of biotin are found in most fish, eg, mackerel, salmon, and sardines.



(1)

ISOLATION

In 1936, biotin was isolated from egg yolks (8), in 1939 from beef liver (9), and in 1942 from milk concentrates (10). Biotin-producing microorganisms exist in the large bowel but the extent and significance of this internal synthesis is unknown.

BIOCHEMICAL FUNCTION

Biotin forms part of several enzyme systems and is necessary for normal growth and body function. Biotin functions as a cofactor for enzymes involved in carbon dioxide fixation and transfer. These reactions are important in the metabolism of carbohydrates, fats, and proteins, as well as promotion of the synthesis and formation of nicotinic acid, fatty acids, glycogen, and amino acids (5–7). Biotin is absorbed unchanged in the upper part of the small intestine and distributed to all tissues. Highest concentrations are found in the liver and kidneys. Little information is available on the transport and storage of biotin in humans or animals. A biotin level in urine of approximately 160 nmol/24 h or 70 nmol/L, and a circulating level in blood, plasma, or serum of approximately 1500 pmol/L seems to indicate an adequate supply of biotin for humans. However, reported levels for biotin in the blood and urine vary widely and are not a reliable indicator of nutritional status.

NUTRITIONAL REQUIREMENTS

Since exact requirements for biotin are uncertain owing to incomplete knowledge regarding biotin availability from food and a lack of definitive studies concerning biotin requirements, the United States National Research Council has established a safe and adequate daily dietary level of intake for biotin rather than a recommended dietary allowance (RDA). The recommended daily intake of biotin in the United States for all persons ages seven years and older is

30–100 μg/d. In France and South Africa, a recommended daily intake for adults of up to 300 μg/d has been established, whereas in Singapore an intake of up to 400 μg/d is recommended. Diets consisting of a daily biotin intake of 28–42 μg/d are considered adequate. A level of 60 μg/d is sufficient for patients under long-term total parenteral nutrition (fed intravenously). An infant's daily intake ranges from 15–20 μg/d and is acquired mostly through human milk containing 3–20 μg/L, or formulas fortified with biotin. An adequate intake level of 10–30 μ/d for infants and young children is recommended. The safe and adequate levels for daily dietary intake of biotin are listed in Table 1 (11). No side effects have been reported with oral doses of biotin as great as 40 mg or parenteral doses of 5–10 mg/d in infants. No toxicity of biotin has been found (5–7).

PHYSIOLOGICAL SIGNIFICANCE

Animal

Dietary biotin deficiencies are extremely rare, perhaps because of the biosynthesis of biotin by intestinal microorganisms. However, biotin deficiency can be easily induced in most animals by ingestion of large amounts of raw egg white, which contain the biotin binding protein avidin. Avidin has a high affinity for biotin and binds with the ureido group to form a complex that is resistant to digestive enzymes. Biotin deficiency in animals causes a decrease in growth rate, loss of weight, alopecia, scaly dermatitis, hyperkeratosis, achromatrichia, and transverse fissures across the soft sole and cracks in the hard horn of the sole and claw wall. Minute amounts of biotin are known to be adequate to support body functions; therefore, biotin requirements for animals may be covered by the natural content of the feed and by the intestinal biosynthesis of biotin. However, biotin-responsive disease conditions not caused by primary biotin deficiency have been observed. One such condition is the fatty liver and kidney syndrome (FLKS). This syndrome has caused heavy economic losses in commercial broiler flocks. FLKS was found to be the result of a suboptimal biotin content in diet, coupled with other nutritional and environmental factors. Although the symptoms of FLKS are not those of classic biotin deficiency, they can be eliminated by biotin supplements. Supplementation has also been found to reduce the incidence and severity of claw lesions in pigs and weak hoof horn in horses (5–7).

Table 1. 1989 Estimated Safe and Adequate Daily Dietary Intake for Biotin

Group	Age (yr)	Biotin (μg)
Infants	0–0.5	10
	0.5–1.0	15
Children	1–3	20
	4–6	25
	7–10	30
Adolescents and adults	11 +	30–100

Human

Biotin deficiencies in humans are extremely rare. The symptoms of deficiency are anorexia, fatigue, nausea, vomiting, hyperesthesia, glossitis, pallor, mental depression, dry scaly dermatitis, alopecia, and localized parasthesias. Both seborrheic dermatitis and Leiner's disease could be the signs of a biotin deficiency in infants (12). Several studies (5–7) have indicated that an erythematous exfoliative dermatitis is the first clinical sign of a biotin deficiency. Infants under six months of age and people who eat large quantities of raw egg whites are probably the groups most susceptible to biotin deficiency. Another susceptible group would be people who lack biotinidase. Biotinidase is the only enzyme capable of catalyzing the cleavage of biocytin (biotinyl-ϵ-lysine), the bound form of biotin. It has also been postulated that there may be a connection between biotin and the etiology of the sudden infant death syndrome (SIDS), which is a common cause of death in the first year of life. Unless biotin is included in their alimentation, patients receiving long-term parenteral nutrition are susceptible to biotin deficiency. All symptoms can be reversed and conditions corrected with biotin treatment. For adults, biotin dosage levels of 150–300 μg per day would be effective treatment. Finally, no drugs have been found to cause a potential biotin deficiency through short- or long-term use. However, low circulating biotin levels have been observed in heavy smokers, alcoholics, and patients under prolonged treatment with anticonvulsant drugs (5–7).

CHEMICAL AND PHYSICAL PROPERTIES

The empirical formula for biotin, $C_{10}H_{16}N_2O_3S$, was established in 1941 (13). The full structure of biotin was elucidated in 1942 by two independent groups (14–16). The first total chemical synthesis of biotin was achieved by Harris in 1945; this work confirmed the structure of biotin (17). The configuration of *d*-biotin was definitively established in 1956 by x-ray crystallographic analysis (18,19). The physical properties of *d*-biotin are found in Table 2. Chemically pure biotin is stable to air and heat. Biotin is also stable for months in mildly acidic and alkaline solutions; however, alkaline solutions, particularly above pH 9, are the least stable. Although biotin is not affected by reducing agents, it is incompatible with formaldehyde, chloramine T, oxidizing agents such as hydrogen peroxide and potassium permanganate, and strong acids such as nitrous acid (20). In most foodstuffs, biotin is bound to proteins, from which it is released in the intestine by protein hydrolysis and the enzyme biotinidase. Biotin is a highly stable, water-soluble vitamin that is resistant to most processing procedures, long-term storage, and normal cooking heat. Most of the biotin losses during cooking are the result of leaching into the cooking water. On the other hand, canning and food processing cause a moderate reduction in biotin content, owing to decomposition.

CHEMICAL SYNTHESIS

Original Synthesis

The first attempted synthesis of *d*-biotin in 1945 afforded racemic biotin (Fig. 1). In this synthetic pathway, L-

Table 2. Physical Properties of *d*-Biotin

Property	Characteristic
Appearance	Fine long needles
Color	White
Molecular weight	244.31
Molecular formula	$C_{10}H_{16}N_2O_3S$
Elemental analysis, wt %	
carbon	49.16
hydrogen	6.60
nitrogen	11.47
sulfur	13.12
Melting point, °C	232–233
α^{21}, specific optical rotation, degrees	+89–91[a]
Dissociation constant, pK_A	6.3×10^{-6}
Isoelectric point, pH	3.5
Solubility, mg/mL	
H_2O, RT	~0.22[b]
95% alcohol, RT	~0.80
common organic solvents	Insoluble

[a] $c = 1$ in 0.1 *N* NaOH.

[b] Higher in dil alkali.

cysteine [*52-90-4*] (**2**) was converted to the methyl ester [*5472-74-2*] (**3**). An intramolecular Dieckmann condensation, during which stereochemical integrity was lost, was followed by decarboxylation to afford the thiophanone [*57752-72-4*] (**4**). Aldol condensation of the thiophanone with the aldehyde ester [*6026-86-4*] (**5**) afforded the conjugated thiophanone [*85269-47-2*] (**6**). The aldol condensation allowed for attachment of the properly functionalized C-5 side chain in one step, a weakness in many subsequent syntheses. The introduction of the second nitrogen was achieved by converting the keto group to the oxime [*85269-48-3*] (**7**), followed by zinc reduction to the thiophene [*85269-49-4*] (**8**). Stereoselective catalytic hydrogenation of the thiophene double bond gave mainly the thiophane [*78763-60-7*] (**9**), which was easily transformed to biotin. The nonselective catalytic hydrogenation and the epimerization that occurred during the conversion of (**3**) to (**4**) were the major factors leading to formation of racemic biotin (17).

Original Asymmetric Synthesis

The efficient introduction of the three stereocenters in the all-cis configuration was first accomplished in the elegant synthesis developed by Sternbach (21–23) in 1949. This process, the Hoffmann-La Roche industrial synthesis of biotin, is still (ca 1997) the basis of industrial preparations. An improved version of the original Sternbach synthesis is shown in Figure 2 (24). The fixed cis position of the carboxyl groups of the imidazolidine dicarboxylic acid (cycloacid) (**13**) and the stereochemistry of the chiral carbons 4 and 5 of the imidazolidine ring, throughout the synthetic scheme, are established by the starting material, fumaric acid [*110-17-8*] (**10**). Fumaric acid is brominated to dibromosuccinic acid [*608-36-6*] (**11**) followed by reaction with benzylamine to give dibenzylaminosuccinic acid [*55645-40-4*] (**12**). Treatment with phosgene forms the imidazolone ring of cycloacid [*51591-75-4*] (**13**). Cycloacid is dehydrated with acetic anhydride to form cycloanhydride [*56688-83-6*] (**14**). Opening of cycloanhydride with cyclohexanol forms the racemic monoester (**15**). The mixture is resolved by salt formation with (+)-ephedrine [*299-42-3*] (**16**) in high yield. The desired *d*-ephedrine salt undergoes acid cleavage, followed by selective reduction of the ester functionality with lithium borohydride to form, after acidification, the *d*-lactone [*56688-82-5*] (**17**). The undesired *l*-ephedrine salt is recycled via the meso-cycloacid to cycloanhydride. The *d*-lactone, which possesses the desired configuration at two of the stereocenters, is converted to the *d*-thiolactone [*56688-83-6*] (**18**) using potassium thioacetate. The introduction of the C-5 side chain is accomplished by reacting the thiolactone with the Grignard reagent derived from 3-methoxypropyl chloride, followed by dehydration to give the thiophene [*85611-62-7*] (**19**). Stereoselective hydrogenation establishes the third stereocenter and affords the thiophane [*33607-59-9*] (**20**). The thiophane undergoes acid-catalyzed cyclization to form the key intermediate, *l*-thiophanium bromide [*60209-10-1*] (**21**). The last two carbons of the biotin side chain are added by reaction of thiophanium bromide with sodium dimethylmalonate to form the diester [*8554-84-3*] (**22**). Hydrolysis of the ester groups, decarboxylation and debenzylation using strong acid forms homochiral, pure *d*-biotin. Although a number of significant modifications to the Sternbach synthesis have appeared over the last 45 years, it is still the basis of today's industrial preparations.

Synthetic Drawbacks

The major drawbacks in the Sternbach-Goldberg synthesis are the resolution/recycling of the intermediate that leads to *d*-lactone and the multiple manipulations required to add the five-carbon side chain. This sequence is inefficient, bringing with it a net loss of methanol, hydrogen bromide, carbon dioxide, and water that were once part of the molecule. In the resolution of the intermediate that leads to *d*-lactone, 50% of the product is the undesirable isomer, although this material is converted to the cycloacid for recycling. This is inherently inefficient and limits single-run production capacity by at least 50%. Recycling the undesired isomer also requires additional labor.

Industrial Synthetic Improvements

One significant modification of the Sternbach process is the result of work by Sumitomo chemists in 1975, in which the optical resolution–reduction sequence is replaced with a more efficient asymmetric conversion of the *meso*-cycloacid (**13**) to the optically pure *d*-lactone (**17**) (Fig. 3) (25). The cycloacid is reacted with the optically active dihydroxyamine [*2964-48-9*] (**23**) to quantitatively yield the chiral imide [*85317-83-5*] (**24**). Diastereoselective reduction of the pro-*R*-carbonyl using sodium borohydride affords the optically pure hydroxyamide [*85317-84-6*] (**25**) after recrystallization. Acid hydrolysis of the amide then yields the desired *d*-lactone (**17**). A similar approach uses chiral alcohols to form diastereomic half-esters stereoselectivity. These are reduced and directly converted to *d*-lactone (26). In both approaches, the desired diastereomeric half-amide

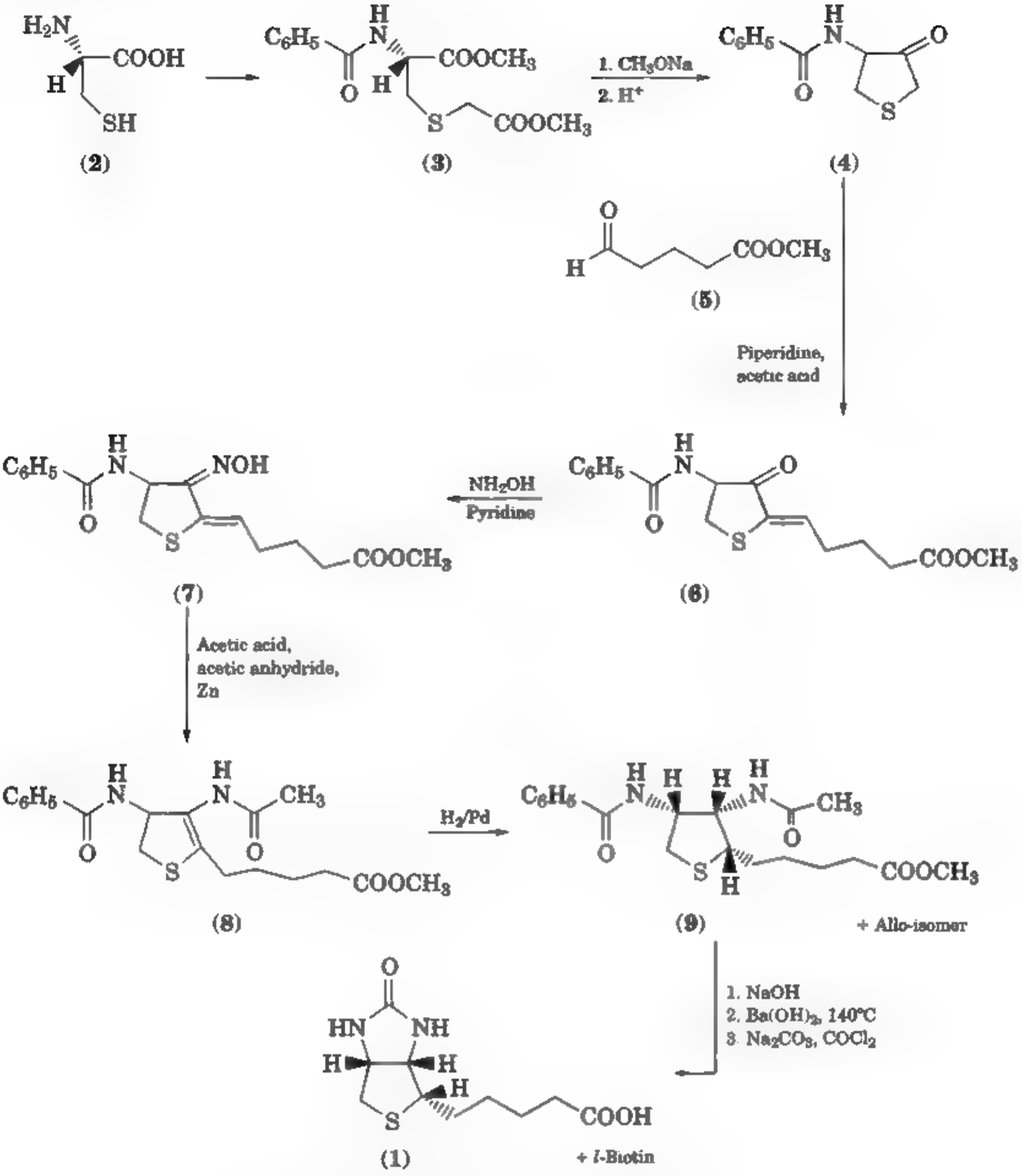


Figure 1. Synthetic pathway for racemic biotin.

or half-ester is formed in excess, thus avoiding the costly resolution step required in the Sternbach synthesis.

Another modification of the Sternbach method involves the direct attachment of the C-5 side chain to thiolactone or a functionalized thiolactone intermediate. One such method, possibly utilized by Sumitomo and reported several times by Lonza (27–29), involves the treatment of the thiolactone (**14**) with the five-carbon Wittig reagent (**26**) to give the olefin (**27**). Hydrogenation of the thiophene (**27**) followed by acid hydrolysis completes the synthesis of *d*-biotin. Another method that directly attaches the five-carbon side chain is patented by Lonza (Fig. 4). This method involves a Wittig reaction of a phosphonium salt derived from the thiolactone (**14**) and a C-5 aldehyde (**30**). The thiolactone (**14**) is reduced to the thiophanol (**28**), which is converted to the Wittig salt (**29**) with Ph_3PH^+ BF_3^-. The ylide (**29**), in the presence of base, undergoes condensation with the aldehyde ester (**30**) to form the thiophene (**27**), which in turn is catalytically hydrogenated to *d*-biotin (**30**).

A final variation of the Sternbach method was reported by Lonza (27–29,31). This method (Fig. 5) not only involves direct attachment of the C-5 side chain to the lactone [*118609-09-9*] (**34**), it also introduces the key stereocenters early in the synthetic pathway via a chiral mono-protected imidazole intermediate [*116291-87-3*] (**32**). Catalytic diastereoselective hydrogenation of the furoimidazole (**32**) affords the *d*-lactone (**34**) as the major product, in 54% yield. The undesired diastereomeric *l*-lactone (**33**) can be easily separated by chromatography or crystallization. In the

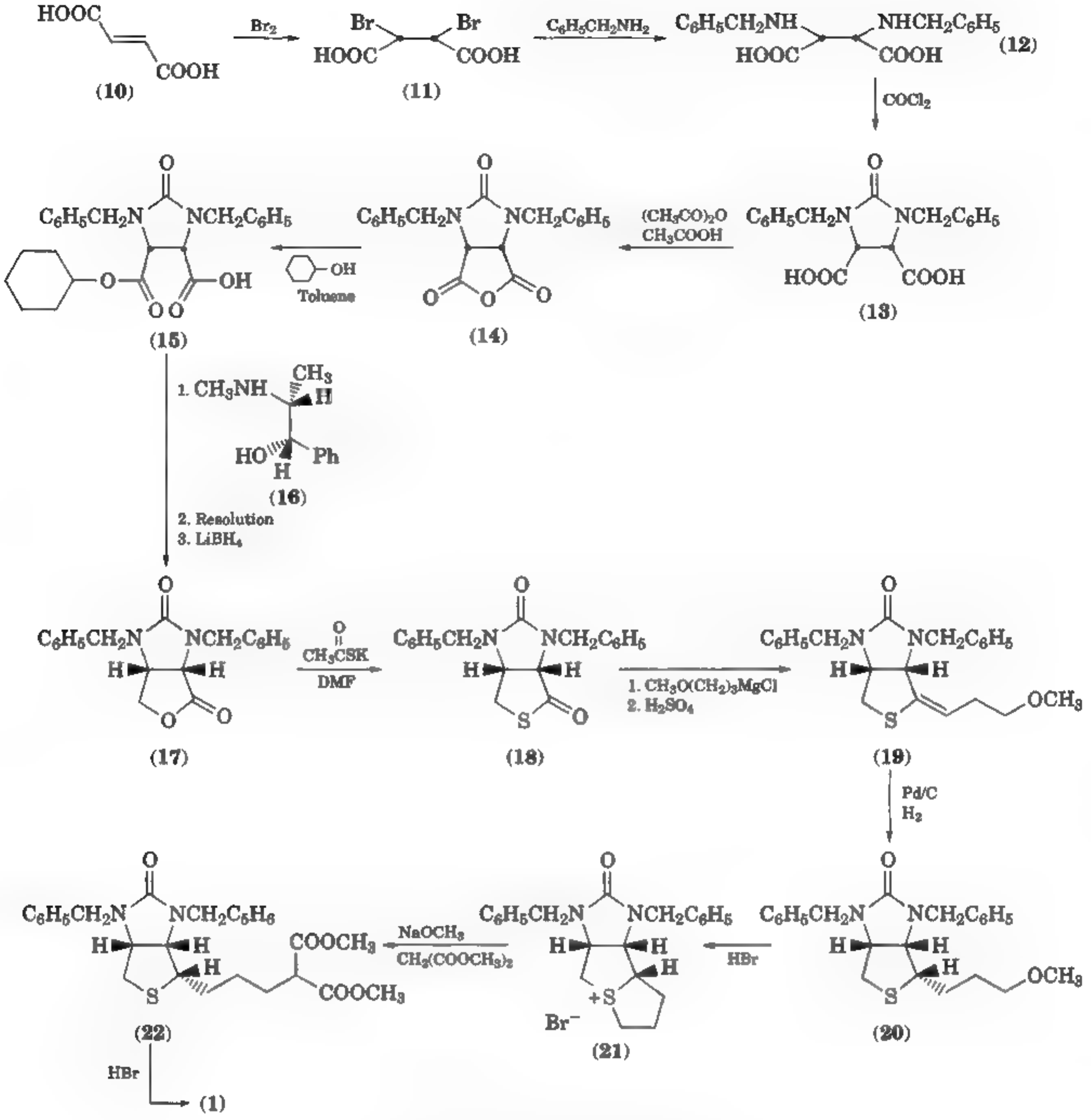

(10)
(11)
(12)
(13)
(14)
(15)
(16)
(17)
(18)
(19)
(20)
(21)
(22)
(1)
Br2
C6H5CH2NH2
COCl2
(CH3CO)2O
CH3COOH
Toluene
1. CH3NH
2. Resolution
3. LiBH4
CH3CSK
DMF
1. CH3O(CH2)3MgCl
2. H2SO4
Pd/C
H2
HBr
NaOCH3
CH2(COOCH3)2
HBr

Figure 2. Synthetic pathway for *d*-biotin (Sternbach synthesis).

step following separation, the *d*-lactone is converted to the thiolactone [*118609-15-7*] (**35**) using a thiocarboxylic acid salt. The thiolactone undergoes a Grignard or Wittig reaction to incorporate the C-5 side chain. The thiophene [*118609-16-8*] (**36**) is then catalytically hydrogenated. This process hydrogenates the carbon–carbon double bond and also removes the chiral protecting group on the nitrogen. This process takes advantage of a short synthetic pathway and the use of a relatively inexpensive starting material. A recent modification of this Lonza process uses a chiral-substituted ferrocenyldisphosphine with a rhodium catalyst to stereoselectively reduce the enamine (**32**) to the *d*-lactone (**34**) in 99% yield. This improvement eliminates the need to separate the undesired *l*-lactone by chromatography or crystallization and increases the yield of the desired lactone from 54 to 99%, making the process extremely efficient (32,33).

Novel Synthetic Methods

More recently, several novel syntheses of *d*-biotin, starting from a variety of chiral starting materials, have been de-

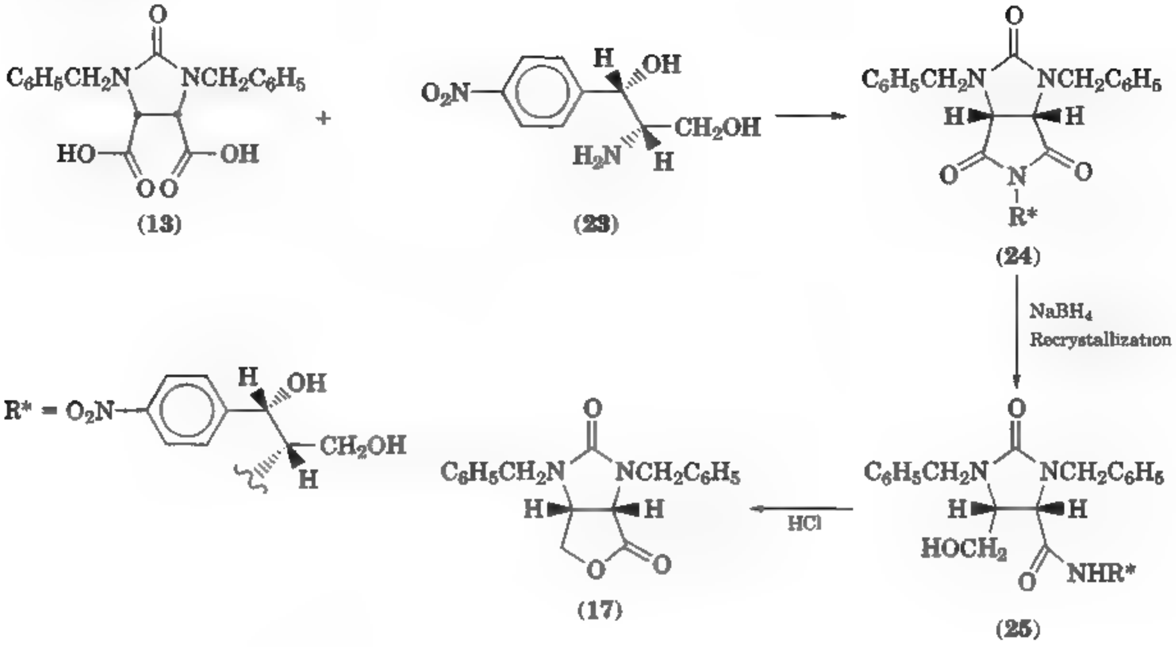


Figure 3. Synthetic pathway for *d*-biotin (Sumitomo synthesis).

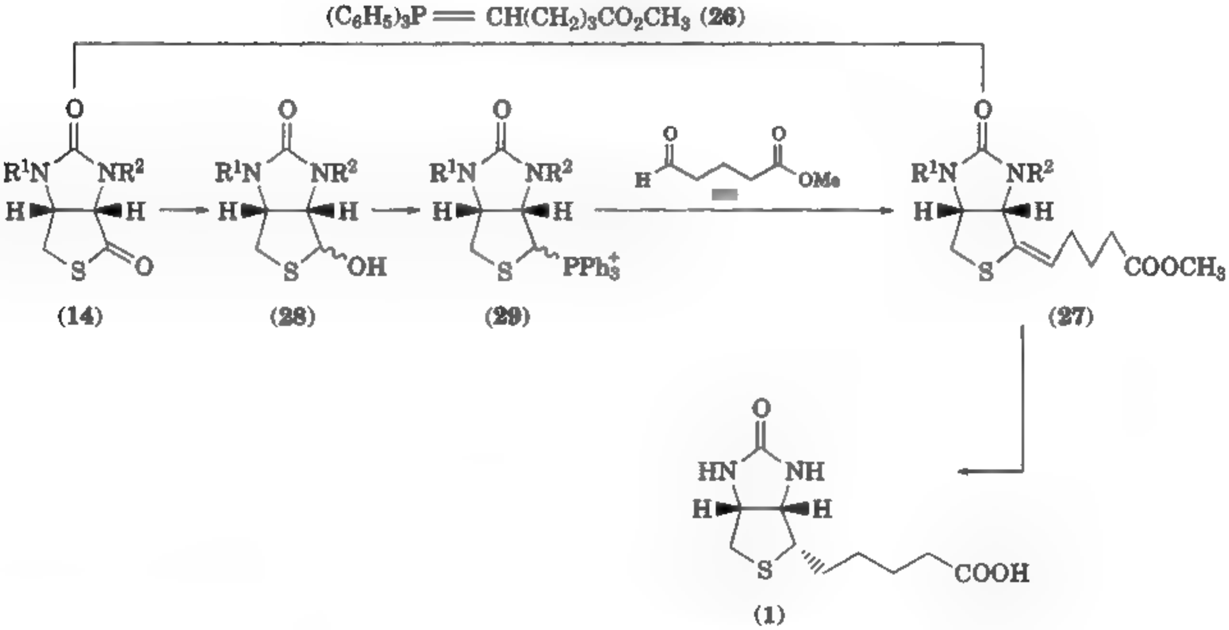


Figure 4. Synthetic pathways for *d*-biotin (Lonza syntheses).

veloped. Seven of these synthetic pathways start from L-cysteine (34–41), two from L-cysteine (42,43), two from D-arabinose (44,45), and one from ribitol (46). Each of these methods has at least one of the following drawbacks: multiple steps with overall low yields; low yield steps associated with cyclization reactions; safety issues associated with reagents such as metal azides, methyl iodide, diazomethane, organoazide reagents, etc; environmental issues with reagents and by products such as triphenyl phosphine oxide and stannane salts; and costs of reagents such as 1,3-dicyclohexylcarbodiimide and trialkyltin hydride. Only one synthetic pathway starts from an achiral starting material, 2,5-dihydrothiophene-1,1-dioxide; this route requires a stereochemical resolution step (47–49). The drawbacks of this pathway are the commercially unavailable starting material and low conversions in several steps.

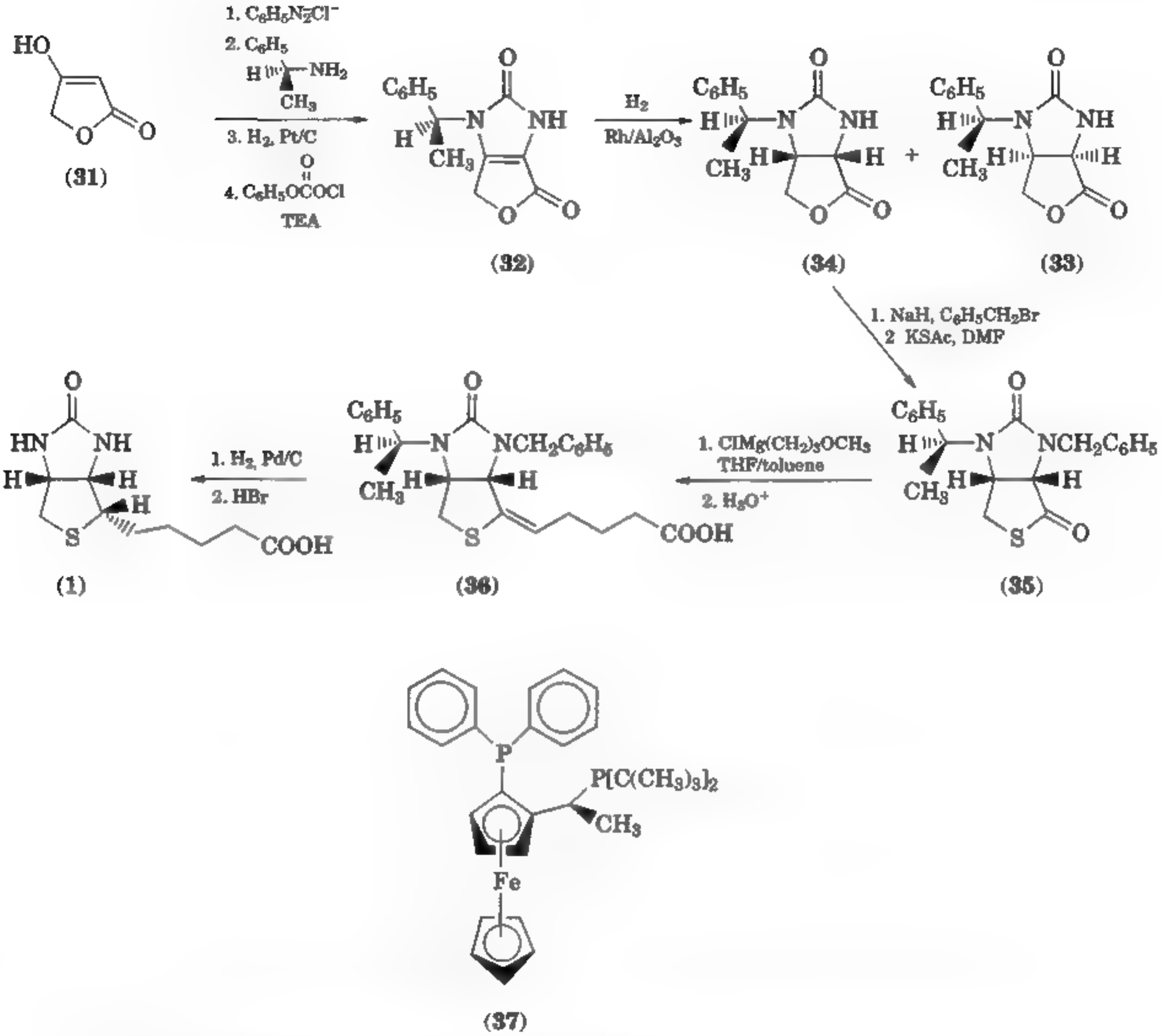


Figure 5. Synthetic pathway for *d*-biotin (Lonza synthesis). An improved process uses the chiral ferrocenyldiphosphine (**37**) to introduce stereospecificity during the hydrogenation of lactone (**32**).

BIOSYNTHESIS

Biotin is produced by a multistep pathway in a variety of fungi, bacteria, and plants (50–56). The established pathway (50,56) in *E. coli* is shown in Figure 6. However, little is known about the initial steps that lead to pimelyl-CoA or of the mechanism of the transformation of desthiobiotin to biotin. Pimelic acid is believed to be the natural precursor of biotin for some microorganisms (51).

Evidence that pimelic acid is a biotin precursor has been found in *Bacillus* species by feeding pimelic acid and observing the concomitant increase in biotin titers. Also, if labeled pimelic acid is used, labeled biotin is formed. In the biosynthetic pathway, pimelic acid [*111-16-0*] (**38**) is converted into pimelyl-CoA (**39**) by the enzyme pimelyl-CoA-synthetase in the presence of ATP and Mg^{2+} at 32°C and pH 7–8 (57). On the other hand, *E. coli* does not seem to rely on pimelic acid as a starting material for biotin synthesis. *E. coli* seems to form pimelyl-CoA by a pathway similar to that of fatty acid and polyketide synthesis (58). Pimelyl-CoA (**39**) is transformed into 7-keto-8-aminopelargonic acid [*4707-58-8*] (7-KAPA) (**40**) by reaction with L-alanine. The reaction requires pyridoxal-5′-phosphate (PLP) as a co-reactant and is catalyzed by the enzyme KAPA-synthetase (59). 7-Keto-8-amino-pelargonic acid (**40**) is converted into 7,8-diamino-pelargonic acid [*157120-40-6*] (DAPA) (**41**) by the enzyme DAPA-aminotransferase, which uses *S*-adenosyl-L-methionine (SAM) instead of a simple amino acid as the amino group donor (60,61). 7,8-Diaminopelargonic acid (**41**) is converted by the enzyme DTB-synthetase to desthiobiotin [*533-48-2*] (**42**). The optimal biological production of desthiobiotin requires ATP, Mg^{2+} and bicarbonate at 50°C and pH 7–8 (62–66). Finally desthiobiotin (**42**) is converted to biotin, supposedly by the enzyme biotin synthetase, which is encoded by the Bio B gene (56).

The conversion of desthiobiotin to biotin occurs in growing as well as resting cells in which the internal source of

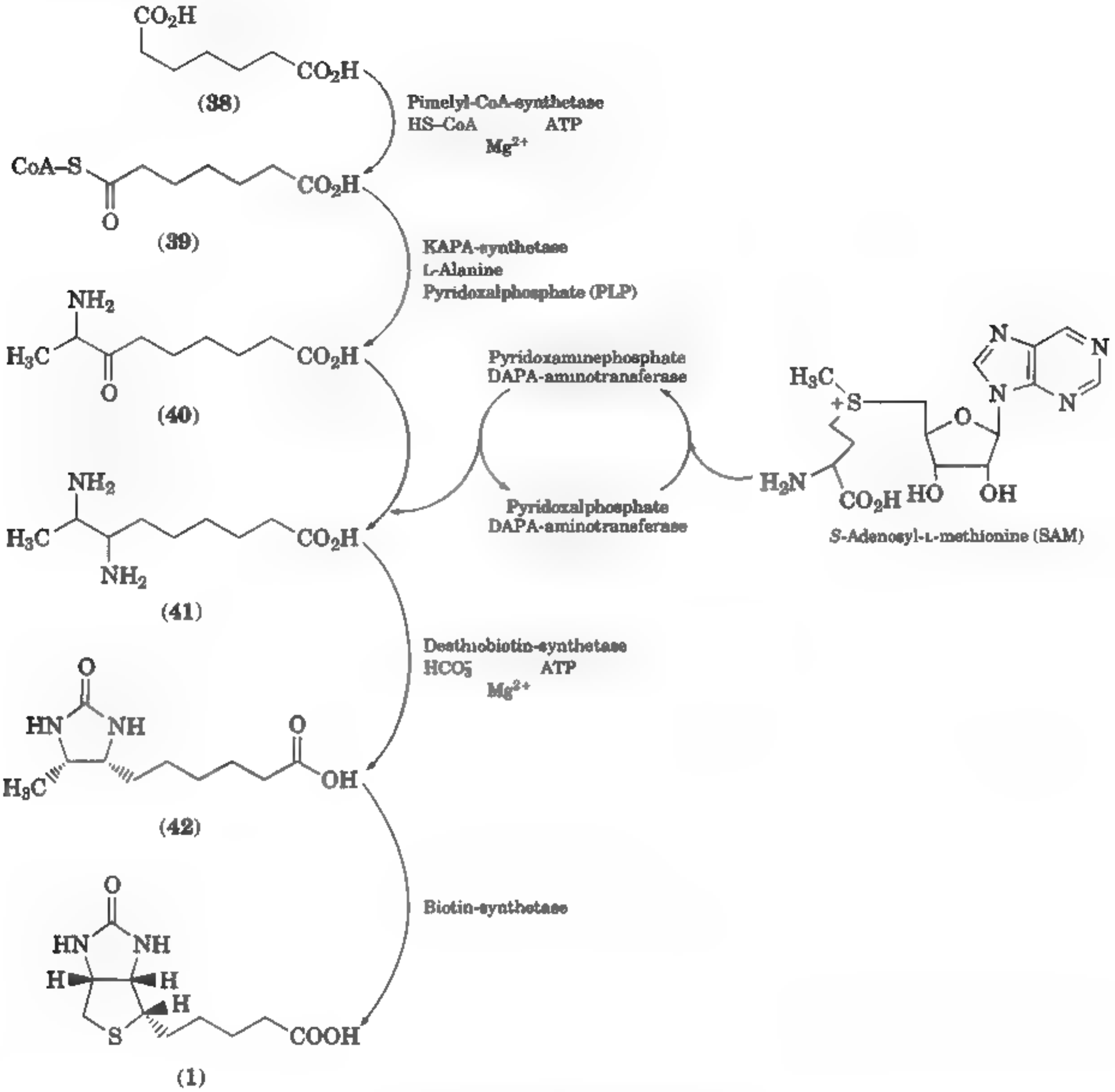

(38)
Pimelyl-CoA-synthetase
HS–CoA
ATP
Mg2+
(39)
KAPA-synthetase
L-Alanine
Pyridoxalphosphate (PLP)
(40)
Pyridoxaminephosphate
DAPA-aminotransferase
Pyridoxalphosphate
DAPA-aminotransferase
S-Adenosyl-L-methionine (SAM)
(41)
Desthiobiotin-synthetase
HCO3−
ATP
Mg2+
(42)
Biotin-synthetase
(1)

Figure 6. Biosynthetic pathway of *d*-biotin.

sulfur is believed to be cysteine or methionine. The introduction of sulfur into desthiobiotin has been investigated by several groups and it is proposed that the sulfur is introduced at C-4 of desthiobiotin with retention of configuration (67–69). The biosynthesis of biotin has led several groups to explore the feasibility of synthesizing biotin on a commercial scale by microorganisms. To date, no commercial scale total synthesis of biotin by fermentation is known. However, several microorganisms and mutant strains have been evaluated (Table 3). One of the problems associated with biotin biosynthesis via fermentation is that total biotin production decreases with increasing biotin concentrations in the fermentation broth. In fact, biotin strongly inhibits all steps of the biosynthesis except the synthesis of pimelyl-CoA. This inhibition by biotin leads to the isolation of several biotin vitamers. A vitamer is a compound, structurally similar to a vitamin, that exhibits varying degrees of vitamin activity. The most important biotin vitamer is desthiobiotin. Inhibition of biotin biosynthesis is the subject of much research.

ANALYTICAL METHODS

Biological Assay

Various analytical methods, including microbiological, biological, chemical, enzymatic and chromatographic assays, have been used to determine biotin levels in food, feed, and body fluids (79–81). The biological assay of biotin is conducted with rats or chicks made biotin-deficient by special diets containing either raw egg whites or avidin. The assay is based on growth response curves since test-animal weight gain is proportional to the logarithm of the biotin dose. A biological assay of biotin is advantageous because

Table 3. Organisms Used for Biotin Fermentation

Organism name	Titer (mg/L)	References
Cornebacteria flavum		70,71
Brevibacterium flavum	0.5[a]	70,72
Serratia marcescens		73–78
	600	73
	500	74
	120	75,78
	83	77
Escherichia coli	2	79,80
Bacillus sphaericus		81–85
	70	81
	20	82
	365	83
Rhodotorula rubra		86
Sporobolomyces roseus		86
Yarrowia lipolytica		86
Candida shehatae		87
Rhizopus delemar	0.6	88,89

no pretreatment of the sample is necessary and the assay measures the amount of biotin available to the test animal. However, biological assays are time-consuming (four weeks to actually run the assay and six to seven weeks to prepare the biotin-deficient animals), costly to run, and require a large number of chicks or rats for accurate results.

Microbiological Assay

An alternative to the biological assay is the microbiological assay, which takes less time, costs less, requires less space, and has greater sensitivity than the biological assay. The typical microorganisms used for a microbiological assay of biotin are *Lactobacillus casei* ATCC 7469, *Neurospora crassa, Ochromonas danica, Saccharomyces cerevisiae* ATCC 7745, and *Lactobacillus plantarum* ATCC 8014. *Lactobacillus plantarum* ATCC 8014 is the test organism employed by most laboratories for biotin assay. Since biotin occurs in both the free and bound forms in nature and *L. plantarum* ATCC 8014 responds only to free biotin, all available biotin must be converted to free biotin prior to the microbiological assay. Bound biotin is usually converted to free biotin by acid hydrolysis with sulfuric acid or by digestion with papain. Hydrochloric acid should not be used in place of sulfuric acid for the acid hydrolysis because it may inactivate biotin. Lipids that stimulate the growth of *L. plantarum* interfere with the assay and have to be removed by filtration of the acid extracts or by preliminary ether extraction prior to the assay. A microbiological assay involves extraction, addition of graded levels of standard and sample to the assay tubes, addition of the medium, sterilization of the assay tubes, inoculation with *L. plantarum* and incubation. After the cell growth stops, the biotin content of the test material is determined by measuring the growth response of the organism (eg, colorimetrically, spectrophotometrically, or titrimetrically with sodium hydroxide) as compared to cell growth with known biotin concentration standards. Microbiological assay of biotin using *L. plantarum* is capable of detecting as little as 0.05 ng of biotin/mL of test solution.

Isotope Dilution Assay

An isotope dilution assay for biotin, based on the high affinity of avidin for the ureido group of biotin, compares the binding of radioactive biotin and nonradioactive biotin with avidin. This method is sensitive to a level of 1–10 ng biotin (82–84), and the radiotracers typically used are [^{14}C]biotin (83), [^{3}H]biotin (84,85) or an ^{125}I-labeled biotin derivative (86). A variation of this approach uses ^{125}I-labeled avidin (87) for the assay.

High-Performance Liquid Chromatography Analysis

The analysis of biotin has also been achieved by high performance liquid chromatography (hplc). Biotin has been analyzed in B-complex tablets, vitamin premixes, and multi-vitamin–multimineral preparations by reverse-phase, high performance liquid chromatographic methods (hplc) using a C^{18} column and uv detection at either 230 nm or 200 nm (88–91). Although this method can detect biotin in vitamin premixes, it is not sensitive enough to determine typical biotin levels in food or feed. Another method, one having greater sensitivity, is a reverse-phase ion-interaction reagent hplc method, with a biotin detection limit of 4 μg. An hplc method with even greater sensitivity involves derivatizing biotin prior to chromatographic separation. This technique can be used for either uv-absorbing derivatives, such as the bromoacetophenone ester (92), or fluorescent derivatives, such as methyl methoxycoumarin ester (92) and anthryldiazomethane ester (93). The biotin detection limit for the bromoacetophenone ester derivative at uv 254 nm was 50 ng. The detection limit for the methyl methoxycoumarin ester at excitation wavelength 360 nm and emission wavelength 410 nm was 5 ng; whereas, the limit of the anthryldiazomethane ester at excitation wavelength at 365 nm and emission wavelength 412 nm was 0.1–10 ng.

Gas Chromatography Analysis

From a sensitivity standpoint, a comparable technique is a gas chromatographic (gc) technique using flame ionization detection. This method has been used to quantify the trimethylsilyl ester derivative of biotin in agricultural premixes and pharmaceutical injectable preparations at detection limits of approximately 0.3 μg (94,95).

SPECIFICATIONS AND PRODUCT FORMS

According to the *Food Chemicals Codex* (96), the biologically active, food-grade form of biotin must have an assay of 97.5%, a melting point in the range of 229–232°C, and a specific rotation at 25°C in 0.1 *N* NaOH in the range of +89–93°. It must also contain less than 3 ppm of arsenic and less than 10 ppm of heavy metals, eg, lead, mercury, and copper. Finally, it must be able to be quantitatively sieved through U.S. Standard Sieves No. 80 using a mechanical shaker. Biotin is listed as GRAS in the *Code of Federal Regulations* (97,98) for use as a nutrient or dietary supplement (21 CFR 182.5159). The specifications for *d*-biotin for pharmaceutical applications are similar to those for food and are listed in the *United States Pharmacopeia*

(99). No specifications for the optically inactive, *d,l*-biotin are given for either food or pharmaceutical applications.

ECONOMIC ASPECTS

The biotin market is divided between agricultural and human use, with ~90% of biotin used in the animal health care market and ~10% for the human nutritional market. The major producers of biotin are Hoffmann-La Roche, Lonza, E. Merck-Darmstadt, Rhône-Poulenc, Sumitomo Pharmaceutical, E. Sung, and Tanabe Seiyaku (100). Worldwide production of biotin in 1994 was approximately 60 metric tons. The list price for pure biotin in 1995 was ~$7.00/g; whereas, the list price for technical feed-grade biotin was ~$5.50/g. Biotin is used in various pharmaceutical, food, and special dietary products, including multivitamin preparations in liquid, tablet, capsule, or powder forms. One of the commercially available products of *d*-biotin is Britrit-1, which is a 1% biotin trituration used in food premixes.

BIBLIOGRAPHY

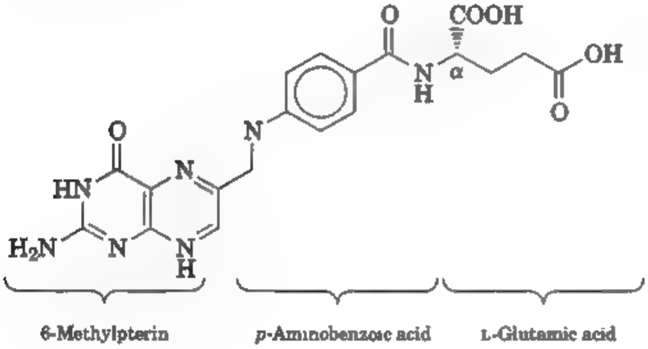
1. P. György and F.-W. Zilliken, in R. Amman and W. Dirscherl, eds., *Fermente, Hormone, Vitamine*, Vol. 31, 3rd ed., Georg Thieme Verlag, Stuttgart, Germany, 1974, p. 766.
2. O. Isler, G. Bracher, S. Ghisla, and B. Kräutler, *Vitamine II. Wasserlösliche Vitamine*, Georg Thieme Verlag, Stuttgart, Germany, 1988, p. 231.
3. P. N. Achuta Murthy and S. P. Mistry, *Prog. Food Nutr. Sci.* **2**, 405 (1977).
4. F. A. Robinson, *The Vitamin Co-Factors of Enzyme Systems*, Pergamon Press, Oxford, U.K., 1966, p. 497.
5. *Encyclopedia of Food Science and Technology*, Pts. 1–8, Vol. 4, John Wiley & Sons, Inc., New York, 1991, p. 2764.
6. J. P. Bonjour, in L. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker Inc., New York, 1991, p. 393.
7. J. Gallagher, *Good Health with Vitamins and Minerals: A Complete Guide to a Lifetime of Safe and Effective Use*, Summit Books, New York, 1990, p. 67.
8. F. Kögl and B. Tönnis, *Z. Physiol. Chem.* **242**, 43 (1936).
9. P. György, R. Kuhn, and E. Lederer, *J Biol. Chem.* **131**, 745 (1939).
10. D. B. Melville, K. Hofmann, E. Hague, and V. du Vigneaud, *J. Biol. Chem.* **142**, 615 (1942).
11. Food and Nutritional Board, National Research Council, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
12. J. P. Bonjour, *Int. J. Vit. Nut. Res.* **47**, 107 (1977).
13. V. du Vigneaud, K. Hofmann, D. B. Melville, and J. R. Rachele, *J. Biol. Chem.* **140**, 763 (1941).
14. V. du Vigneaud et al., *J. Biol. Chem.* **146**, 475 (1942).
15. D. B. Melville, A. W. Moyer, K. Hofmann, and V. du Vigneaud, *J. Biol. Chem.* **146**, 487 (1942).
16. P. György and B. W. Langer, Jr., in W. H. Sebrell, Jr., R. S. Harris, eds., *The Vitamins*, 2nd ed., Academic Press, New York, 1968, p. 261.
17. S. A. Harris et al., *JACS* **67**, 2096 (1945).
18. W. Traub, *Nature* **178**, 649 (1956).
19. J. Trotter and Y. A. Hamilton, *Biochemistry* **5**, 713 (1966).
20. S. Budavari, ed., *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*, 11th ed., Merck & Co., Inc., Rahway, N.J., 1989, p. 192.
21. U.S. Pat. 2,489,232 (Nov. 22, 1949), M. W. Goldberg and L. H. Sternbach (to Hoffmann-La Roche Inc.)
22. U.S. Pat. 2,489,235 (Nov. 22, 1949), M. W. Goldberg and L. H Sternbach (to Hoffmann-La Roche Inc.).
23. U.S. Pat. 2,489,238 (Nov. 22, 1949), M. W. Goldberg and L. H. Sternbach (to Hoffmann-La Roche Inc.).
24. M. Gerecke, J. P. Zimmermann, W. Aschwanden, *Helv. Chim. Acta* **53**, 991 (1970).
25. U.S. Pat. 3,876,656 (Apr. 8, 1975), A. Hisao, A. Yasuhiko, O. Shigeru, and S. Hiroyuki (to Sumitomo Chemical Co., Ltd.); Pat. Appl. E.P.O. 044,158 (Jan. 20, 1982), A. Hisao, A. Yasuhiko, O. Shigeru, and S. Hiroyuki (to Sumitomo Chemical Co., Ltd.).
26. Eur. Pat. Appl. EP 161,580 (Nov. 21, 1985), C. Wehrli and H. Pauling (to F. Hoffmann-La Roche AG).
27. Eur. Pat. Appl. EP 273270-A1 (July 6, 1988), J. McGarrity and L. Tenud (to Lonza AG).
28. Eur. Pat. Appl. EP 270076-A1 (Feb. 12, 1986), M. Eyer, R. Fuchs, D. Laffan, J. F. McGarrity, T. Meul, and L. Tenud (to Lonza AG).
29. U.S. Pat. 4,851,540 (Dec. 1, 1987), J. F. McGarrity, L. Tenud, and T. Meul (to Lonza AG).
30. Eur. Pat. Appl. EP 387,747 (Sept. 19, 1990), M. Eyer (to Lonza AG).
31. U.S. Pat. 4,873,339 (Nov. 30, 1988), J. F. McGarrity, L. Tenud, and T. Meul (to Lonza AG).
32. Eur. Pat. 624587 (Nov. 17, 1994), J. McGarrity, F. Spindler, R. Fuchs, and M. Eyer (to Lonza AG).
33. PCT Int. Appl. WO 94 24,137 (Oct. 27, 1994), M. Eyer and R. E. Merrill (to Lonza AG).
34. U.S. Pat. 4,837,402 (Jan. 6, 1988), E. Poetsch and M. Casutt (to Merck Patent GmbH).
35. E. Poetsch and M. Casutt, *Chimia* **41**, 148 (1987).
36. Ger. Offen. DE 3,926,690 (Feb. 14, 1991), M. Casutt, E. Poetsch, and W. N. Spekamp (to Merck Patent GmbH).
37. F. D. Deroose and P. J. DeClercq, *Tetrahedron Lett.* **34**, 4365 (1993).
38. F. D. Deroose and P. J. DeClercq, *Tetrahedron Lett.* **35**, 2615 (1994).
39. F. D. Deroose and P. J. DeClercq, *J. Org. Chem.* **60**, 321 (1995).
40. T. Fujisawa, M. Nagai, Y. Koike, and M. Shimizu, *J. Org. Chem.* **59**, 5865 (1994).
41. H. L. Lee, E. G. Baggiolini, and M. R. Usković, *Tetrahedron* **43**, 4887 (1987).
42. E. J. Corey and M. M. Mehrotra, *Tetrahedron Lett.* **29**, 57 (1988).
43. T. Ravindranathan, S. V Hiremath, D. R. Reddy, and R. B. Tejwani, *Synth. Commun.* **18**, 1855 (1988).
44. Jpn. Kokai Tokkyo Koho, JP 61254590 (Nov. 12, 1986), T. Kono, Y. Shimakawa, M. Takahashi, T. Horisaki, and S. Masuda (to Teikoku Chemical Industry Co., Ltd.).
45. N. A. Hughes, K.-M. Kuhajda, and D. A. Miljkovic, *Carbohydr. Res.* **257**, 299 (1994).

46. D. A. Miljkovic and S. Velimirovic, *J. Serb. Chem. Soc.* **53**, 37 (1988).
47. H. A. Bates, L. Smilowitz, and J. Lin, *J. Org. Chem.* **50**, 899 (1985).
48. H. A. Bates, L. Smilowitz, and S. B. Rosenblum, *J. Chem. Soc. Chem. Commun.*, 353 (1985).
49. H. A. Bates and S. B. Rosenblum, *J. Org. Chem.* **51**, 3447 (1986).
50. M. Eisenberg, in F. Neidhardt, ed., *Escherichia coli and Samonella typhimzerium Cellular and Molecular Biology*, American Society for Microbiology, New York, 1987, p. 544.
51. R. Gloeckler et al., *Gene* **87**, 63 (1990).
52. Y. Izumi and K. Ogata, *Adv. Appl. Microbiol.* **22**, 145 (1977).
53. P. Baldet, C. Alban, S. Axiotis, and R. Douce, *Arch. Biochem. Biophys.* **303**, 67 (1993).
54. T. Schneider, R. Dinkins, K. Robinson, J. Shellhammer, and D. Meinke, *Dev. Biol.* **131**, 161 (1989).
55. J. Shellhammer and D. Meinke, *Plant Physiol.* **93**, 1162 (1990).
56. M. A. Eisenberg, *Ann. N.Y. Acad. Sci.* **447**, 335 (1985).
57. O. Ploux, P. Soularue, A. Marquet, R. Gloeckler, and Y. Lemoine, *Biochem. J.* **287**, 685 (1992).
58. I. Sanyal, S.-L. Lee, and D. H. Flint, *JACS* **116**, 2637 (1994).
59. M. A. Eisenberg and C. Star, *J. Bacteriol.* **96**, 1291 (1968).
60. G. L. Stoner and M. A. Eisenberg, *J. Biol. Chem.* **250**, 4029 (1975).
61. C. H. Pai, *J. Bacteriol.* **105**, 793 (1971).
62. K. Krell and M. A. Eisenberg, *J. Biol. Chem.* **245**, 6558 (1970).
63. P. Cheeseman and C. H. Pai, *J. Bacteriol.* **104**, 726 (1970).
64. O. Ifuku, J. Kishimoto, S. Haze, M. Yanagi, and S. Fukushima, *Biosci. Biotechnol. Biochem.* **56**, 1780 (1992).
65. R. L. Baxter, A. J. Ramsey, L. A. McIver, and H. C. Baxter, *J. Chem. Soc., Chem. Commun.*, 559 (1994).
66. R. L. Baxter and H. C. Baxter, *J. Chem. Soc., Chem. Commun.*, 759 (1994).
67. F. Frappier, M. Jouany, A. Marquet, A. Olesker, and J.-C. Tabet, *J. Org. Chem.* **47**, 2257 (1982).
68. D. A. Trainer, R. J. Parry, and A. Gutterman, *JACS* **102**, 1467 (1980).
69. L. Even, D. Florentin, and A. Marquet, *Bull. Soc. Chim. Fr.* **127**, 758 (1990).
70. Jpn. Kokai Tokkyo Koho, JP06339371, (Dec. 13, 1994), Y. Yoneda, T. Abe, N. Hara, I. Ohsawa, and A. Fujisawa (to Nippon Zeon Co., Ltd.).
71. I. Ohsawa et al., *J. Ferment. Bioen.* **73**, 121 (1992).
72. Eur. Pat. Appl. EP 316229 (May 17, 1989), S. Haze, O. Ifuku, and J. Kishimoto (to Shiseido Co., Ltd.).
73. Jpn. Kokai Tokkyo Koho, JP 58060996 (Apr. 11, 1983), (to Nippon Zeon Co., Ltd.).
74. Eur. Pat. Appl. EP 375525 (June 27, 1990), R. Gloeckler, D. Speck, J. Sabatie, S. Brown, and Y. Lemoine (to Transgene S. A., Fr.).
75. B. M. Pearson, D. A. MacKenzie, and M. H. J. Keenan, *Lett. Appl. Microbiol.* **2**, 25 (1986).
76. J. C. Du Preez, J. L. F. Kock, A. M. T. Monteiro, and B. A. Prior, *FEMS Microbiol. Lett.* **28**, 271 (1985).
77. Otkrytiya, Izobret., *Prom. Obraztsy, Tovarnye Znaki* (38), 93 (1983); Sov. Pat. SU 1047956 (Oct. 15, 1983), L. I. Vorob'eva, E. V. Shchelokova, and E. S. Naumova (to Moscow State University) (in Russian).
78. V. N. Maksimov, E. V. Shchelokova, and L. I. Vorob'eva, *Prikl. Biokhim. Mikrobiol.* **19**, 353 (1983).
79. I. D. Lumley and P. R. Lawrance, *J. Micronutr. Anal.* **7**, 301 (1991).
80. J. Scheiner, J. Augustin, B. P. Klein, D. A. Becker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, p. 535.
81. F. Frappier and M. Gaudry, in A. P. DeLeenheer, W. E. Lambert, and M. G. M. De Ruyter, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1985, p. 482.
82. Dakshinamurti, A. D. Landman, L. Ramamurti, and R. J. Constable, *Anal. Biochem.* **61**, 225 (1974).
83. R. L. Hood, *J. Sci. Food Agric.* **26**, 1847 (1975).
84. R. Bettenmaier, *Anal. Chim. Acta* **113**, 107 (1980).
85. T. Suormala et al., *Clin. Chim. Acta* **177**, 253 (1988).
86. E. Livaniou, G. R. Evangeliatos, and D. S. Ithakissions, *Clin. Chem.* **33**, 1983 (1987).
87. D. M. Mock and D. B. DuBois, *Anal. Biochem.* **155**, 272 (1986).
88. T. S. Hudson, S. Subramanian, and R. J. Allen, *J. Assoc. Official Anal. Chemists* **67**, 995 (1984).
89. S. L. Crivelli, P. F. Quirk, D. J. Steible, and S. P. Assenza, *Pharm. Res.* **4**, 261 (1987).
90. A. Rizzolo, C. Baldo, and A. Polesello, *J. Chromatogr.* **553**, 187 (1991).
91. M. C. Gennaro, *J. Chromatogr. Sci.* **29**, 410 (1991).
92. P. L. Desbene, S. Coustal, and F. Frappier, *Anal. Biochem.* **128**, 359 (1983).
93. K. Hayakawa and J. Oizumi, *J. Chromatogr.* **413**, 247 (1987).
94. V. Viswanathan, F. P. Mahn, V. S. Venturella, and B. Z. Senkowski, *J. Pharm. Sci.* **59**, 400 (1970).
95. H. Janecke and H. Voege, *Naturwissenschaften* **55**, 447 (1968).
96. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, p. 38.
97. *Evaluation of the Health Aspects of Biotin as a Food Ingredient*, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md., 1978.
98. *Code of Federal Regulations, Title 21, Food and Drugs, Parts 100–199 rev.*, Office of the Federal Register, General Services Administration, U.S. Government Printing Office, Washington, D.C., 1977.
99. *The United States Pharmacopeia XXIII (USP XXIII-NFXVIII)*, United States Pharmacopeial Convention, Inc., Rockville, Md., 1995, p. 206.
100. J. D. Greer and B. Rhomberg, *CEH Marketing Research Report, Animals: Chemical Inputs for Nutrition and Health—Overview*, Chemical Economics Handbook-SRI International, Palo Alto, CA 1995, 201.3001A-B, Aug. 22, 1996.

ROBERT A. OUTTEN
Hoffmann-La Roche, Inc.

VITAMINS: FOLIC ACID

Folic acid [*59-30-3*] (1) belongs to the group of B-vitamins. The term folate is used to designate all members of the family of compounds based on the *N*-[(6-pteridinyl)methyl]-*p*-aminobenzoic acid skeleton conjugated with one or more L-glutamic acid units. In 1930, a dietary factor in yeast and crude liver extract was found to cure megaloblastic anemia in pregnant women (1). Purified folate was isolated by using two bioassay procedures, the microbiological growth assay and the assay for the antianemic factor for chickens (2–4). The growth factor from yeast and the antianemic factor (vitamin B_c) were later shown to be different entities. A crystalline compound was isolated from spinach, which was called folic acid (5,6). It was later shown that several of the above-mentioned factors belonged to the nutritionally and chemically related family of pteroylglutamic acid compounds. The structure of pteroylglutamic acid was elucidated in 1946 (7). The metabolically active forms of folic acid have a reduced pteridine ring and several glutamic acids residues. A detailed account on the discovery and early development of folic acid is available (8).



(1)

OCCURRENCE, SOURCE, AND BIOAVAILABILITY

Good food sources of folate are liver; fresh, dark green, leafy vegetables; beans; wheat germ; and yeasts (qv) (Table 1). Folic acid is synthesized only by microorganisms and plants (10–14). Most dietary folates exist in the polyglutamate form, which are converted to the more readily bioavailable monoglutamate form in the small intestine by the jejunal brush border folate conjugase. Certain foods such as cabbage and legumes contain conjugase inhibitors, which can decrease folate absorption.

Table 1. Select Contributors of Folate in the U.S. Diet

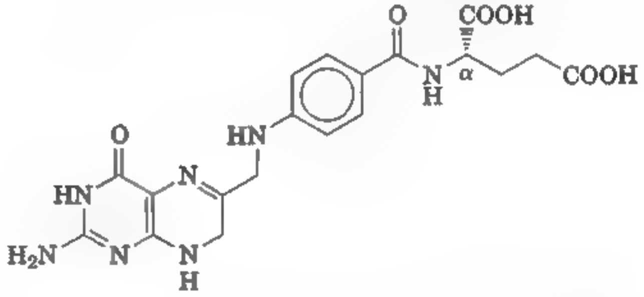
Ranking	Description	Total folate (%)
1	Orange juice	9.7
2	White bread, rolls, crackers	8.6
3	Pinto, navy, other dried beans	7.1
4	Green salad	6.8
5	Cold cereals	5.0
6	Eggs	4.6
9	Liver	3.1
23	Hamburger	1.2
25	Spinach	1.0
30	Green beans	0.8
34	Broccoli	0.7

Source: Ref. 9.

The total folate content of food varies, based on the method of preparation and length of storage (15–17). Different forms of folates occur in nature and the stability or bioavailability of each form varies. Most folates in food are easily oxidizable and therefore are susceptible to oxidation under aerobic conditions during storage and processing. Folic acid (commercial form) has superior bioavailability because it is more readily absorbed when compared to the tri- or heptaconjugates. The factors affecting the bioavailability of food folates are not well understood, but seem to include iron and vitamin *C* (qv) status. Deficiencies of both of these nutrients in humans is associated with impaired utilization of dietary folate. Improved research techniques such as use of radiolabeled folates has provided a powerful tool for determining bioavailability (18). Under fasting conditions, folic acid is almost completely absorbed, whereas only 50–80% of folyl polyglutamate is absorbed, as determined by urinary excretion measurements (19).

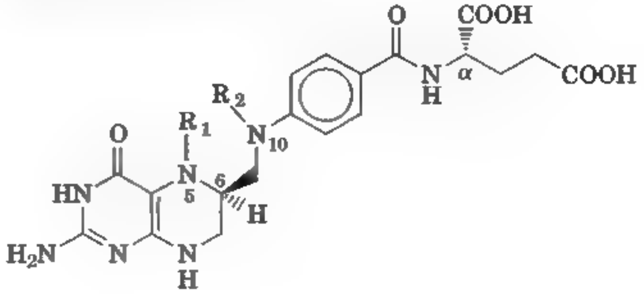
CHEMICAL AND PHYSICAL PROPERTIES

L-Folic acid (1) contains three subunits: 6-methylpterin, *p*-aminobenzoic acid, and L-glutamic acid. The *Chemical Abstracts* name is *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid.



(2)

Enzymatic reduction of folic acid leads to the 7,8-dihydrofolic acid (H_2 folate) (2), a key substance in biosynthesis. Further reduction, catalyzed by the enzyme dihydrofolic acid reductase, provides (6*S*)-5,6,7,8-tetrahydrofolic acid (H_4 folate) (3). The H_4 folate (3) is the key biological intermediate for the formation of other folates (4–8) (Table 2).



(3)

Table 2. H_4 Folate Cofactors

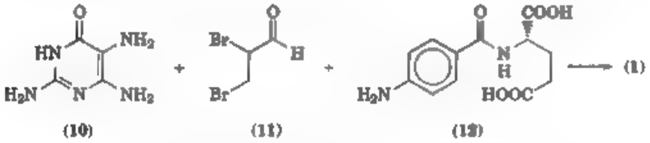
Structure	R_1	R_2	Nomenclautre	Configuration
(3)	H	H	Tetrahydrofolic acid	6*S*, α(*S*)
(4)	CH_3	H	5-Methyltetrahydrofolic acid	6*S*, α(*S*)
(5)	CH_2		5,10-Methylenetetrahydrofolic acid	6*R*, α(*S*)
(6)	HC=O	H	5-Formyltetrahydrofolic acid	6*S*, α(*S*)
(7)	H	HC=O	10-Formyltetrahydrofolic acid	6*R*, α(*S*)
(8)	HC=NH	H	5-Formiminotetrahydrofolic acid	6*S*, α(*S*)
(9)	CH^+		5,10-Methenyltetrahydrofolic acid	6*R*, α(*S*)

Nomenclature and symbols for folic acid assigned based on recommendation published by IUPAC–IUB joint commission on Biochemical Nomenclature 1986 (20,21); see structure (3).

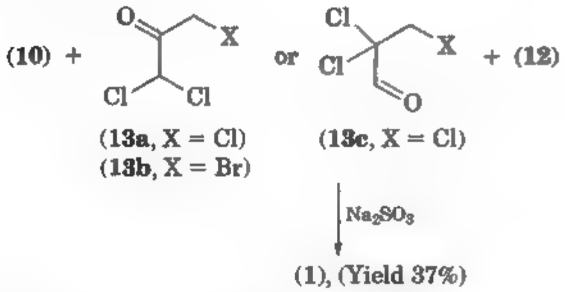
Folic acid (1) is found as yellow, thin platelets which char above 250°C. The uv spectrum of L-folic acid at pH 13 shows absorptions at $\lambda = 256$ nm ($\epsilon = 30{,}000$), 282 nm ($\epsilon = 26{,}000$), and 365 nm ($\epsilon = 9800$). Folic acid has a specific rotation of $[\alpha]_D^{27} = +19.9°$ ($c = 1$, 0.1 *N* NaOH). Solutions of folic acid are stable at room temperature and in the absence of light. It is slightly soluble in aqueous alkali hydroxides and carbonates but is insoluble in cold water, acetone, and chloroform. Table 3 lists some physical properties of selected folic acid derivatives.

SYNTHESIS

The first L-folic acid synthesis was based on the concept of a three-component, one-pot reaction (7,22). Triamino-4(3*H*)-pyrimidinone [*1004-45-7*] (**10**) was reacted simultaneously with C_3-dibromo aldehyde [*5221-17-0*] (**11**) and *p*-amino-benzoyl-L-glutamic acid [*4271-30-1*] (**12**) to yield folic acid (**1**).

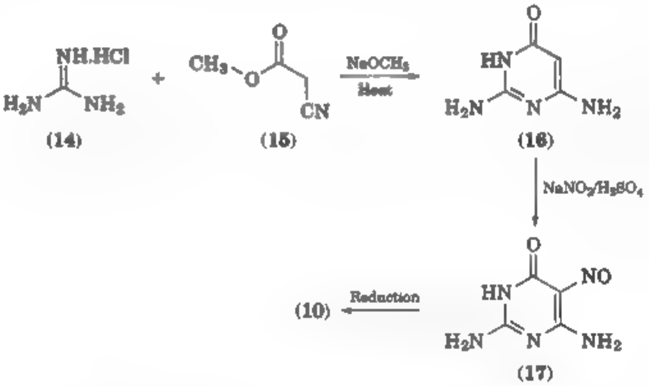


All known commercial syntheses are based on this approach with improvements in preparations of the three components (23). Shortly after the first synthesis, similar methods were published employing other C_3-halo compounds, such as 1,1,3-tribromo-2-propanone, 2,2,3-tribromopropanal (24), 2,2,3-trichloropropanal, and 1,1,3-trichloro-2-propanone (23).

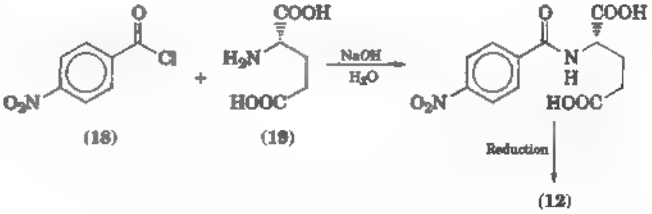


Yields were improved to ≥37% by the addition of sodium sulfite to the reaction mixture. Apart from the sulfite, the C_3-component unit has the greatest influence on the yield of folic acid. The use of nickel(II) chloride as an additive has been claimed to give higher yields (25).

The required triamino-4(3*H*)-pyrimidinone is prepared in three steps starting from guanidine [*50-01-1*] (**14**) (26). Condensation with methylcyanoacetate [*105-34-0*] (**15**) under basic conditions, followed by nitrosation of the intermediate [*56-06-4*] (**16**), gives 2,6-diamino-5-nitrosopyrimidinone [*2387-48-6*] (**17**). Chemical reduction using sodium sulfite (27) or catalytic hydrogenation using Raney nickel (28) furnishes (**10**).



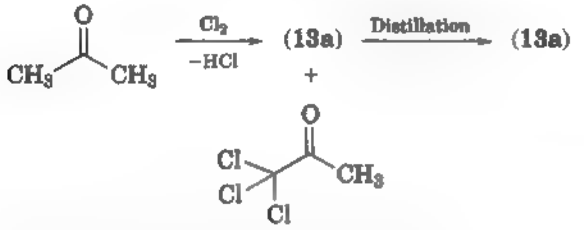
p-Aminobenzoyl-L-glutamic acid (**12**) is obtained by condensation of *p*-nitrobenzoyl chloride [*122-04-3*] (**18**) with L-glutamic acid [*56-86-0*] (**19**) under Schotten-Baumann conditions. This is followed by reduction of the nitro group with either sodium hydrogen sulfide (29) or by electrochemical methods (30).



1,1,3-Trichloroacetone [*921-03-9*] (**13a**) is prepared by chlorination of acetone. The reaction is nonselective and the required compound is isolated by distillation. The selectivity has been improved by catalyzing the reaction with iodine (31).

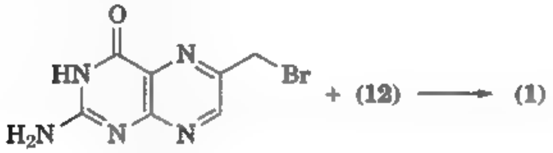
Table 3. Physical Properties of Folic Acid Derivatives

Compound	Structure number	CAS Registry Number	uv λ_{max} (nm)	ϵ	Stability	Molecular formula (mol wt)
Folic acid Pteroylglutamic acid PteGlu	(1)	[*59-30-3*]	(pH 13) 256	30,000	Unstable in alkaline and acidic solutions	$C_{19}H_{19}N_7O_6$ (441.41)
Dihydrofolic acid H_2 Folate	(2)	[*4033-27-6*]	(pH 7.2) 282	28,600	Highly air-sensitive	$C_{19}H_{21}N_7O_6$ (443.42)
Tetrahydrofolic acid H_4 Folate	(3)	[*135-16-0*]	(pH 7.8) 296	28,000	Sensitive to oxygen	$C_{19}H_{23}N_7O_6$ (445.44)
5-Methyltetrahydrofolic acid 5-CH_3-H_4 Folate	(4)	[*134-35-0*]	(pH 7) 290	32,000	Stable to oxygen	$C_{20}H_{25}N_7O_6$ (459.46)
5,10-Methylenehydrofolic acid 5,10-CH_2-H_4 Folate	(5)	[*3432-99-3*]	(pH 7.2) 294	32,000	Sensitive to hydrolysis	$C_{20}H_{23}N_7O_6$ (457.45)
10-Formyltetrahydrofolic acid 10-CHO-H_4 Folate	(6)	[*2800-34-2*]	(pH 7.5) 260	17,000	Quite unstable	$C_{20}H_{23}N_7O_7$ (473.45)
5-Formyltetrahydrofolic acid (6*R*,*S*)-5-CHO-H_4 Folate	(7)	[*58-05-9*]	(pH (13) 282	32,600	Most stable	$C_{20}H_{23}N_7O_7$ (473.45)
(6*S*)-5-CHO-H_4 Folate		[*68538-85-2*]				$C_{20}H_{23}N_7O_7$ (473.45)
5-Formiminotetrahydrofolic acid 5-NHCH-H_4 Folate	(8)	[*2311-81*]	(pH 7) 285	35,400	Hydrolyzed in aqueous solution	$C_{20}H_{24}N_8O_6$ (472.46)
5,10-Methyltetrahydrofolic acid 5,10-CH^+-H_4 Folate	(9)	[*65981-89-7*]	(pH 1) 352	23,900		$C_{20}H_{22}N_7O_6^+$ (cation)



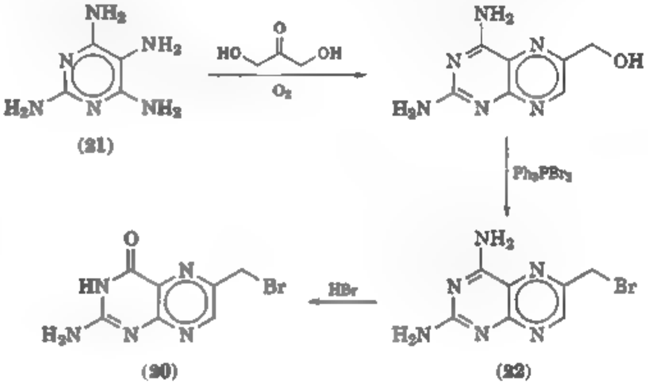
Alternative Approaches for Synthesis of L-Folic Acid

L-Folic acid (**1**) has been prepared in two steps by condensing 6-bromomethylpterin (**20**) with *p*-aminobenzoyl-L-glutamic acid (**12**) in 80% yield (32). Dissolved folic acid further reacts easily with one more equivalent of 6-bromomethylpterin to form the undesired dialkylated aminobenzoyl-L-glutamic acid.

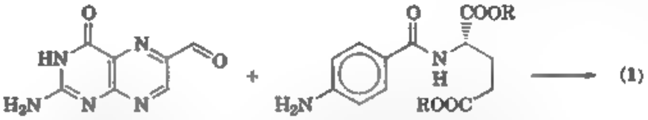


(**20**)

In spite of the good yields of L-folic acid obtained in this reaction, all of the published methods for the synthesis of 6-bromomethylpterin (**20**) are multistep procedures with low overall yields (33–36). For example, the route starting from 2,4,5,6-tetraaminopyrimidine [*5392-28-9*] (**21**) gave 6-bromomethylpterin (**20**) in three steps with an overall yield of only 18% (33,35,36). This synthesis is not economical because the intermediate 6-bromomethyl-2,4-diamino-4-pterin (**22**) has to be deaminated in an additional step to form 6-bromomethylpterin (**20**).

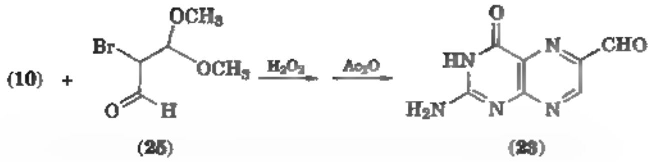


Another viable method for the synthesis of L-folic acid (**1**) starts from 6-formylpterin (**23**). The diester of L-glutamic acid (**24**) is condensed with 6-formylpterin (**23**). Reduction of the Schiff base with sodium borohydride is followed by hydrolysis to yield L-folic acid (37).

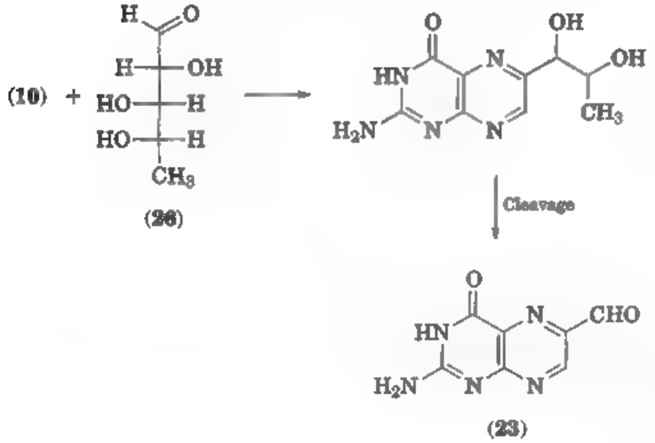


(**23**) (**24**, R = CH_3, C_2H_5)

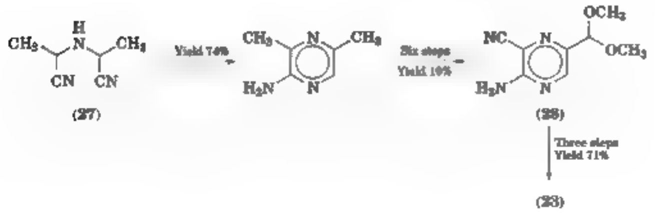
A cost-efficient synthesis of folic acid via Schiff base formation is feasible only if 6-formylpterin (**23**) is readily available. This compound is prepared by the reaction of 2-bromomalondialdehyde dimethylacetal [*59453-00-8*] (**25**) with triaminopyrimidinone (**10**), followed by acetylation and cleavage of the acetal to give compound (**23**) in 51% overall yield (38).


(10)
+
OCH3
Br
OCH3
O
H
H2O2
Ac2O
O
HN
N
CHO
H2N
N
N
(25)
(23)

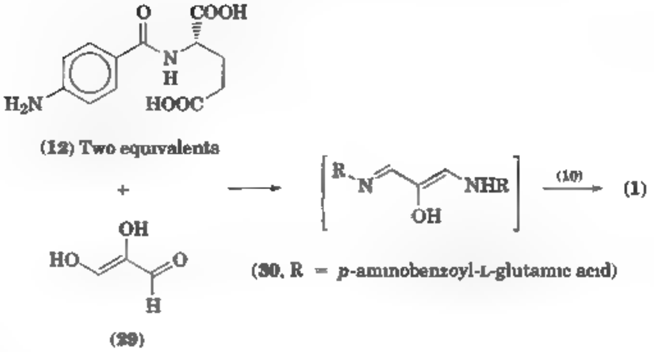
A second approach for the synthesis of 6-formylpterin (**23**) involves the condensation of triaminopyrimidinone (**10**) with 5-deoxy-L-arabinose (**26**). The key diol is obtained in four steps starting from compound (**10**). Cleavage of the diol side chain is achieved either with periodate (39) or with lead(IV) (40) to furnish 6-formylpterin (**23**) in 45% overall yield.


(10)
+
H
O
H
OH
HO
H
HO
H
CH3
(26)
O
OH
HN
N
OH
H2N
N
N
CH3
Cleavage
O
HN
N
CHO
H2N
N
N
(23)

A third approach for the synthesis of 6-formylpterin (**23**) starts from iminodipropionitrile [*2869-25-2*] (**27**). The intermediate pyrazine (**28**) is also prepared starting from chloropyruvaldehyde oxime (41,42). The required formylpterin (**23**) is obtained in three steps in 71% yield, starting from the intermediate pyrazine (**28**). A few other routes for the synthesis of 6-formylpterin (**23**) are described in the literature. All are multistep procedures with only moderate overall yields (43–45).


CH3
H
N
CH3
CN
CN
(27)
Yield 74%
CH3
N
CH3
H2N
N
Six steps
Yield 10%
OCH3
NC
N
OCH3
H2N
N
(28)
Three steps
Yield 71%
(23)

A new variant of the three-component, one-pot synthesis of L-folic acid has been reported by Hoffmann-La Roche Inc


O
COOH
N
H
H2N
HOOC
(12) Two equivalents
+
OH
HO
O
H
(29)
R
N
NHR
OH
(10)
(1)
(30, R = p-aminobenzoyl-L-glutamic acid)

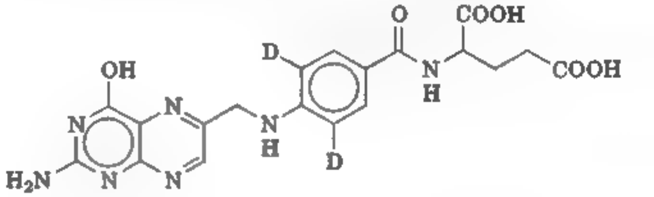
One equivalent of 2-hydroxymalondialdehyde [*497-15-4*] (**29**) is condensed with two equivalents of *p*-aminobenzoyl-L-glutamic acid (**12**). The intermediate dimine (**30**) is treated with one equivalent of triaminopyrimidinone (**10**) to obtain L-folic acid in 84% yield (46).

Fermentation

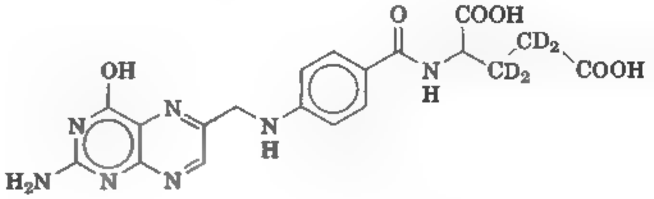
Development of an economically viable production process for folic acid either by genetically engineered microorganisms or by extraction from natural sources is not yet feasible.

LABELED COMPOUNDS

Radiolabeled folate provides a powerful tool for folate bioavailability studies in animals and for diagnostic procedures in humans. Deuteration at the 3- and 5-positions of the central benzene ring of folic acid (**31**) was accomplished by catalytic debromination (47,48) or acid-catalyzed exchange reaction (49). Alternatively, deuterium-labeled folic acid (**32**) was prepared by condensing pteroic acid with commercially available labeled glutamic acid (50).


O
COOH
D
OH
N
COOH
H
N
N
N
H
D
H2N
N
N

(**31**)


O
COOH
CD2
OH
N
CD2
COOH
H
N
N
N
H
H2N
N
N

(**32**)

DERIVATIVES AND ANALOGUES

The metabolically active H_4 folate cofactors (see Table 2) are prepared synthetically as follows. 7,8-Dihydrofolic acid

(**2**) and 5,6,7,8-tetrahydrofolic acid (**3**) are prepared via catalytic hydrogenation of folic acid under controlled reaction conditions (51,52). Optical rotation of (6*S*,*R*)-H_4 folate (**3**) is $[\alpha]_D^{27} = +14.9$Ɖg (0.1 *N* NaOH) and the natural (6*S*)-H_4 folate (**3**) is $[\alpha]_D^{27} = -16.9°$ (0.1 *N* NaOH).

5-Methyltetrahydrofolic acid (5-CH_3-H_4 folate) (**4**) is involved in methionine biosynthesis. Condensation of formaldehyde with H_4 folate (**3**), followed by the reduction of the intermediate 5,10-CH_2-H_4 folate (**5**) with sodium borohydride gave 5-CH_3-H_4 folate (**4**) (53). 5,10-Methylenetetrahydrofolic acid (5,10-CH_2-H_4 folate) (**5**) is a coenzyme in thymidylate biosynthesis; the natural (6*R*)-stereoisomer is prepared by enzymatic reduction of H_2 folate (**2**), followed by condensation with formaldehyde (54).

Formylation of H_4 folate (**3**) or hydrolysis of 5,10-CH^+-H_4 folate (**9**) gives (6*R*,*S*)-5-formyltetrahydrofolic acid (**6**) (5-HCO-H_4 folate) (55). On the other hand, (6*S*)-5-HCO-H_4 folate is obtained by selective crystallization in the form of its calcium salt from the diastereomeric mixture of (6*S*,*R*)-5-HCO-H_4 folate (56). 10-Formyltetrahydrofolic acid (**7**) is a coenzyme in purine synthesis which is synthesized by hydrolysis of 5,10-CH^+-H_4 folate (**9**) or by hydrogenation of 10-CHO-folate (57).

Folic acid analogues containing amino acids other than glutamate, and also folate covalently bound to a protein for the purposes of antibody production, have been prepared (58–60). Methotrexate is an analogue of folic acid that is widely used in cancer chemotherapy (61). Other analogues such as trimethoprim and pyrimethamine are used in the treatment of malaria and protozoal diseases (62). These analogues bind extremely tightly to dihydrofolate reductase.

BIOSYNTHESIS

Folic acid is synthesized both in microorganisms and in plants. Guanosine-5-triphosphate (GTP) (**33**), *p*-aminobenzoic acid (PABA), and L-glutamic acid are the precursors. Reviews are available for details (63,64). The sequence of reactions responsible for the enzymatic conversion of GTP to 7,8-dihydrofolic acid (**2**) is shown (see Scheme 1. In *E. coli*, GTP cyclohydrolase catalyzes the conversion of GTP (**33**) into 7,8-dihydroneopterin triphosphate (**34**) via a three-step sequence. Hydrolysis of the triphosphate group of (**34**) is achieved by a nonspecific pyrophosphatase to afford dihydroneopterin (**35**) (65). The free alcohol (**36**) is obtained by the removal of residual phosphate by an unknown phosphomonoesterase. The dihydroneopterin undergoes a retro-aldol reaction with the elimination of a hydroxy acetaldehyde moiety. Addition of a pyrophosphate group affords hydroxymethyl-7,8-dihydropterin pyrophosphate (**37**). Dihydropteroate synthase catalyzes the condensation of hydroxymethyl-7,8-dihydropteroate pyrophosphate with PABA to furnish 7,8-dihydropteroate (**38**). Finally, L-glutamic acid is condensed with 7,8-dihydropteroate in the presence of dihydrofolate synthetase.

ANALYTICAL METHODS

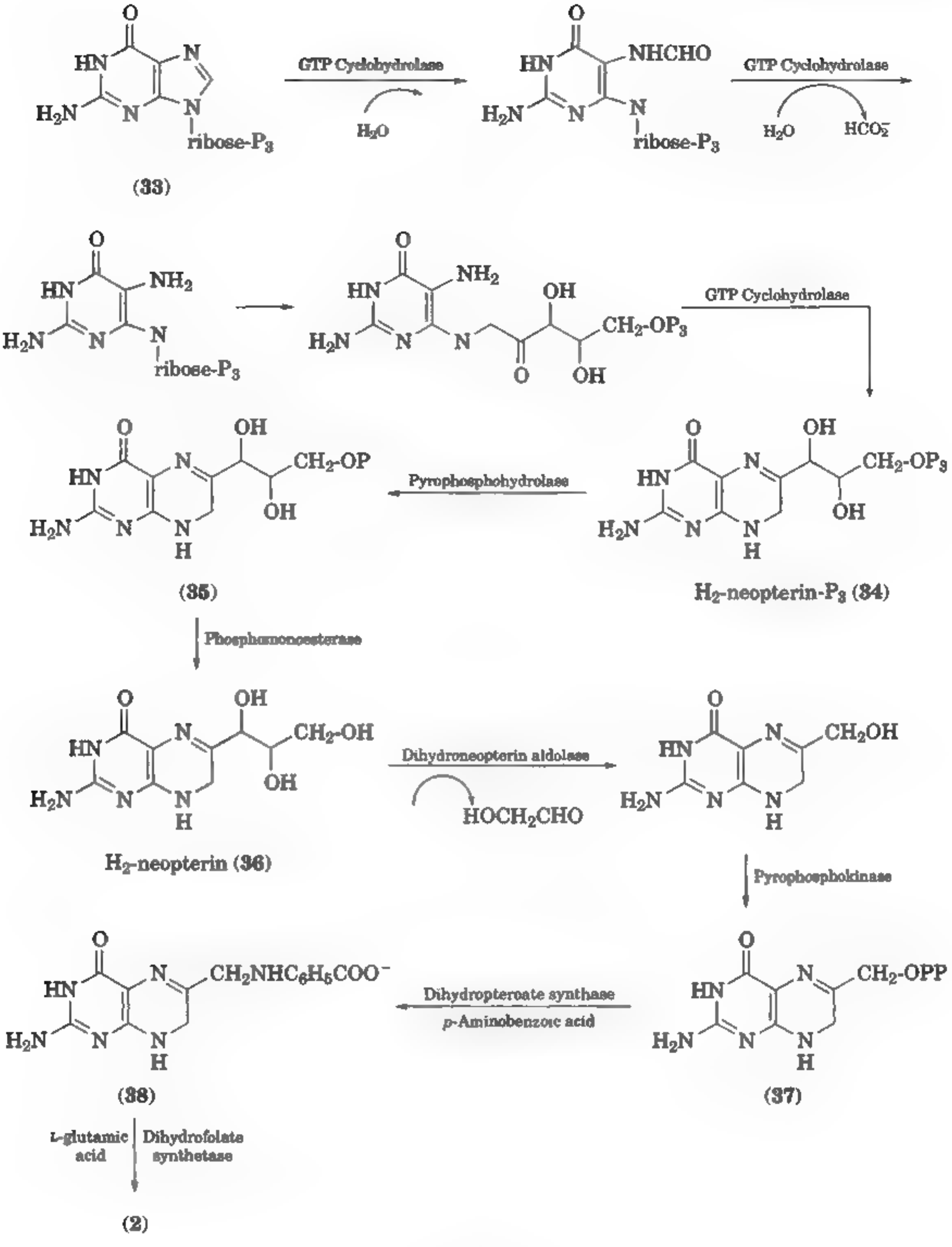
Analysis of folic acid is difficult because most natural folates exist in the polyglutamate form and there is variation in the oxidation state of the single-carbon substituent. Determination of the individual folate vitamers is complicated; assay simplification is achieved by determining folate in the monoglutamyl or diglutamyl forms after enzymatic deconjugation. Methods for determining folic acid in food and feed include biological, microbiological, chemical, chromatographic, and radiometric assays. The microbiological assay using *Lactobacillus casei* is the official method of the Association of Official Analytical Chemists (AOAC). Folyl polyglutamates react very slowly with this organism compared to mono- and diglutamates. As a result, it is required to hydrolyze the polyglutamate chain using γ-glutamylhydrolase prior to microbiological analysis (66). The monoglutamate in the food and feed extract is separated using anion-exchange column chromatography, followed by differential assay of the separated fraction by a microbiological assay.

A radioassay procedure has been developed to determine folic acid in erythrocyte and blood samples. The method is based on competitive protein binding between radiolabeled and unlabeled folate compounds for folic acid binding protein. A very sensitive, nonisotopic microtitration plate, folate-binding protein assay (FBPA) was developed to measure down to the 6 pg level of folyl monoglutamate and vitamin-active folate (67). A hplc method is useful for analysis of high potency premix samples containing folic acid, along with other water-soluble vitamins. Ion-pair reverse-phase columns using uv detection at 280 nm or post-column derivatization techniques have been employed to determine free folic acid. Details on hplc applications are available (68–70).

DEFICIENCY

Folic acid is a precursor of several important enzyme cofactors required for the synthesis of nucleic acids (qv) and the metabolism of certain amino acids. Folic acid deficiency results in an inability to produce deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and certain proteins (qv). Megaloblastic anemia is a common symptom of folate deficiency owing to rapid red blood cell turnover and the high metabolic requirement of hematopoietic tissue. One of the clinical signs of acute folate deficiency includes a red and painful tongue. Vitamin B_{12} and folate share a common metabolic pathway, the methionine synthase reaction. Therefore a differential diagnosis is required to measure folic acid deficiency because both folic acid and vitamin B_{12} deficiency cause megaloblastic anemia. Serum and red blood cell levels of folate are measured to confirm and diagnose the deficiency. Serum folate levels less than 3 ng/mL are diagnostic of a deficiency. Some forms of dietary folic acid are more poorly absorbed by the elderly than by younger individuals. However, vitamin supplements containing the monoglutamate form are well absorbed in all age groups (71).

Folate antagonists (eg, methotrexate and certain antiepileptics) are used in treatment for various diseases, but their administration can lead to a functional folate deficiency. Folate utilization can be impaired by a depletion of


(33)
GTP Cyclohydrolase
H2O
GTP Cyclohydrolase
H2O
HCO2-
GTP Cyclohydrolase
Pyrophosphohydrolase
(35)
H2-neopterin-P3 (34)
Phosphomonoesterase
Dihydroneopterin aldolase
HOCH2CHO
H2-neopterin (36)
Pyrophosphokinase
Dihydropteroate synthase
p-Aminobenzoic acid
(38)
(37)
L-glutamic acid
Dihydrofolate synthetase
(2)

Scheme 1.

zinc. In humans, the intestinal brush border folate conjugase is a zinc metalloenzyme (72). One study indicates that the substantial consumption of alcohol, when combined with an inadequate intake of folate and methionine, may increase the risk of colon cancer (73). Based on this study, it is recommended to avoid excess alcohol consumption and increase folate intake to lower the risk of colon cancer.

Incomplete closure of neural tube during the embryo development in humans can lead to spina bifida. The condition is characterized by an opening in the spinal cord and results in physical disability in a child. Incomplete closure of the skull produces anencephaly. These and similar conditions are collectively called neutral tube defects (NTD). Each year in the United States approximately 2500 infants are born with spina bifida and anencephaly and an estimated 1500 fetuses affected by these birth defects are spontaneously aborted (74). It has been shown that folic acid given at 400 μg/day prevents the recurrence of NTD and that doses of 800 μg/day prevent both the occurrence and recurrence of NTD in the majority of cases. Published studies also indicate that folic acid supplementation taken six weeks before conception may reduce the risk of neural tube defects by at least 50% (74,75). Folic acid may be more effective in reducing neural tube defect incidence than conjugated food folate because free folic acid is more readily absorbed (76). A cost-benefit analysis, based on the U.S. population, of preventable neural tube defects indicates that folic acid fortification of grains in the United States may yield a substantial economic benefit (77). The U.S. Food and Drug Administration (FDA) has amended the standards of identity for several enriched grain products. The agency is requiring that these products be fortified with folic acid at levels ranging from 0.95 to 3.09 mg per kg of product (78). This is the first B-vitamin fortification requirement since 1943 when the U.S. government mandated fortification of flour with niacin, thiamin, and riboflavin. Incidence of cleft lip/cleft palate has been reported in animal studies due to folic acid deficiency. Poor folic acid status has been associated with megaloblastic changes in the cells of the uterine, cervix (79), and intestinal epithelium (80).

Homocysteine arises from dietary methionine. High levels of homocysteine (hyperhomocysteinemia) are a risk factor for occlusive vascular diseases including atherosclerosis and thrombosis (81–84). In a controlled study, serum folate concentrations of ≤9.2 nmol/L were linked with elevated levels of plasma homocysteine. Elevated homocysteine levels have been associated also with ischemic stroke (9). The mechanism by which high levels of homocysteine produce vascular damage are, as of yet, not completely understood. Interaction of homocysteine with platelets or endothelial cells has been proposed as a possible mechanism. Clinically, homocysteine levels can be lowered by administration of vitamin B_6, vitamin B_{12}, and folic acid.

REQUIREMENTS

The amount of folic acid required for daily intake is estimated based on the minimum amount required to maintain a certain level of serum folate. The recommended dietary allowance (RDA) for folic acid accounts for daily losses and makes allowances for variation in individual needs and bioavailability from food sources (85). The U.S. recommended daily allowance for adults is 400 μg and for pregnant women is 800 μg (Table 4).

ANIMAL NUTRITION

To obtain optimal performance of farm animals, folic acid supplementation is required (86) and as is the case with most of the vitamins, the majority of worldwide consumption is as feed supplements. The folic acid requirement for chickens and pigs is about 0.2–0.5 mg of folic acid/kg diet and 0.3 mg/kg diet, respectively. Increased amounts, 0.5–1.0 mg/kg feed for chickens and 0.5–2.0 mg/kg for swine, are recommended under commercial production conditions (87). The degree of intestinal folic acid synthesis and the utilization by the animal dictates the folic acid requirements for monogastric species. Also, the self-synthesis of folacin is dependent on dietary composition (88).

Table 4. RDA and U.S. RDA for Folic Acid

Group	Age	Folic acid (μg)
	RDA	
Infants	0–0.5	25
	0.5–1.0	35
Children	1–3	50
	4–6	75
	7–10	100
Males	11–14	150
	>15–51	200
Females	11–14	150
	15–51	180
Pregnant, lactating		400
1st six months		280
2nd mix months		260
	U.S. RDA	
Infants <13 months		100
Children <4 yr		200
Adults and children >4 yr		400
Pregnant or lactating women		800

Source Ref. 21.

Folacin requirements are related to the type and level of production. The more rapid the growth or production rates, the greater the need for folacin owing to its role in DNA synthesis. In poultry, the requirement for egg hatchability is higher than for production (88). In swine, folic acid supplementation has been shown to increase fertility and growth rates (89).

METABOLISM

The principal function of the folate coenzyme is to carry one-carbon units. Dietary folylpolyglutamate is hydrolyzed to the monoglutamate form by folyl polyglutamate hydrolyases (conjugases) prior to transport across the intestinal mucosa. Intestinal folate absorption occurs in the jejunum. A slightly acidic pH of 6 is optimum for intestinal absorption and transportation into the blood stream (90). It was shown by the competitive inhibition method (91) that a single protein seems to be responsible for transportation of the monoglutamate. Transportation may occur by an anion-exchange mechanism (92). Folate transportation may be partially dependent on sodium ions in the cell medium (93).

Three forms of folate appear to be transported in the blood: folic acid, folate loosely bound to low affinity binder serum proteins (such as albumin, α-macroglobulin, and transferrin), and folate bound to high affinity protein binders. Approximately 5% of total serum folate is being transported by high affinity protein binders but the function of these proteins is not well understood (94,95). Folic acid is stored in folyl polyglutamate form. The liver and other tissues (mitochondria) convert folic acid and methyltetrahydrofolate into folylpolyglutamate by employing polyglutamate synthetase. The total folate pool in adult humans is estimated to be around 5–10 mg, with half of this in the liver; folylpolyglutamate synthetase activity is highest in liver.

The metabolism of folic acid involves reduction of the pterin ring to different forms of tetrahydrofolylglutamate. The reduction is catalyzed by dihydrofolate reductase and NADPH functions as a hydrogen donor. The metabolic roles of the folate coenzymes are to serve as acceptors or donors of one-carbon units in a variety of reactions. These one-carbon units exist in different oxidation states and include methanol, formaldehyde, and formate. The resulting tetrahydrofolylglutamate is an enzyme cofactor in amino acid metabolism and in the biosynthesis of purine and pyrimidines (10,96). The one-carbon unit is attached at either the N-5 or N-10 position. The activated one-carbon unit of 5,10-methylene-H_4 folate (**5**) is a substrate of T-synthase, an important enzyme of growing cells. 5-10-Methylene-H_4 folate (**5**) is reduced to 5-methyl-H_4 folate (**4**) and is used in methionine biosynthesis. Alternatively, it can be oxidized to 10-formyl-H_4 folate (**7**) for use in the purine biosynthetic pathway.

TOXICITY

Folic acid is safe, even at levels of daily oral supplementation up to 5–10 mg (97). Gastrointestinal upset and an altered sleep pattern have been reported at 15 mg/day (98). A high intake of folic acid can mask the clinical signs of pernicious anemia which results from vitamin B_{12} deficiency and recurrence of epilepsy in epileptics treated with drugs with antifolate activity (99). The acute toxicity (LD_{50}) is approximately 500 and 600 mg per kg body weight for rats and mice, respectively (100).

USES

L-Folic acid is available as a crystalline dihydrate containing 8% water. Approximately 80% of the commercial production is consumed for feed enrichment in animal nutrition. Folic acid is being offered by the pharmaceutical industry for therapeutic and prophylactic use. Pharmacological doses of folic acid are commonly used as a rescue dose during cancer chemotherapy, in women using oral contraceptives, and alcoholics. Several studies have provided evidence that multivitamins or folic acid (0.8–4 mg/day) supplementation prevent the majority of neural tube defects (101).

ECONOMIC ASPECTS

The world production of synthetic L-folic acid 1996 was estimated at 400 metric tons per year. The total market is expected to grow with increasing recognition of need, especially during pregnancy and lactation. The principal producers of folic acid are Hoffmann-La Roche, Takeda, Sumika Fine Chemical (previously Yodogawa Pharmaceuticals), Kongo, and three Chinese companies, Changzhou Pharmaceuticals, Changshu Hugang Pharmaceuticals, and Zhejiang Jiangnan Pharmaceuticals. Smaller quantities are also produced by companies in India, China, and Russia. The 1996 sale price varied between $50 to $130/kg.

CONCLUSIONS

All known commercial syntheses of folic acid are based on the three-component process developed in the late 1940s. Industrial production of folic acid by genetically engineered microorganisms or extraction from natural sources is not yet economically viable. The mechanism governing folate turnover and excretion is still poorly understood. Improved research techniques will aid in the development of a better understanding of factors affecting intestinal absorption and *in vivo* kinetics in human beings. Folic acid and multivitamin supplement use are associated with a decreased occurrence of neural tube defects. Abnormalities in homocysteine metabolism are observed in many women who have given birth to children with neural tube defects and in individuals with cardiovascular disease. Folic acid is likely to be involved in overcoming this abnormality. The exact mechanism by which folic acid prevents these diseases is a current active area of research.

BIBLIOGRAPHY

1. L. Wills, P. W. Clutterbuck, and P. D. F. Evans, *Biochem. J.* **31**, 2136 (1937).
2. A. G. Hogan and E. M. Parrott, *J. Biol. Chem.* **132**, 507 (1940).
3. E. E. Snell and W. H. Peterson, *J. Bacteriol.* **39**, 273 (1940).
4. H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Am. Chem. Soc.* **63**, 2284 (1941).
5. S. B. Binkley, O. D. Bird, E. S. Bloom, R. A. Brown, D. G. Calkins, C. J. Campbell, A. D. Emmett, and J. J. Pfiffner, *Science* **100**, 36 (1944).
6. J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and L. B. O'Dell, *J. Am. Chem. Soc.* **68**, 1392 (1946).
7. R. B. Anger et al., *Science* **103**, 667 (1946).
8. A. D. Welch, *Perspect. Biol. Med.* **27**, 64 (1983).
9. W. H. Giles, S. J. Kittner, R. F. Anda, J. B. Croft, and M. L. Casper, *Stroke* **26**, 1166 (1995).
10. W. Friedrich, *Handbuch der vitamine*, Urban & Schwarzenberg, Baltimore, Md., 1987, p. 398.
11. B. Botticher and R. Kluthe, in K. Pietrzik, ed., *Folsäure-Mangel*, W. Zuckschwerdt Verlag, Germany, 1987, p. 15
12. T. Brody, in L. J. Machlin, ed., *Handbook of Vitamins*, Marcel Dekker Inc., New York, 1991, p. 453.
13. L. B. Bailey, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker Inc., New York, 1995, p. 123.
14. K. H. Bässler, E. Grühn, D. Loew, and K. Pietrzik, *Vitamin-Lexikon*, G. Fischer Verlag, New York, 1992, p. 127.
15. J. Leichter, A. F. Landymore, and C. L. Krumdieck, *Am. J. Clin. Nutr.* **32**, 92 (1979).

16. S. P. Rothenberg, M. P. Iqbal, and M. Da Costa, *Anal. Biochem.* **103**, 152 (1980).
17. J. F. Gregory, *Adv. Food Nutr. Res.* **33**, 1 (1989).
18. J. F. Gregory, *Food Technol.* **42**, 230 (1988).
19. H. E. Sauberlich, M. J. Kretsch, J. H. Skala, H. L. Johnson, and P. C. Taylor, *Am. J. Clin. Nutr.* **46**, 1016 (1987); J. F. Gregory, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker Inc., 1995, p. 195.
20. IUPAC-IUB Joint commission on biochemical nomenclature (JCBN), *Eur. J. Biochem.* **168**, 251 (1987); *Pure Appl. Chem.* **59**, 834 (1987).
21. D. Bhatia, in *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, p. 2770.
22. C. W. Waller et al., *J. Am. Chem. Soc.* **70**, 19 (1948).
23. F. Weygand and V. Schmied-Kowarzik, *Chem. Ber.* **82**, 333 (1949).
24. S. Uyeo, S. Mizukami, T. Kubota, and S. Takagi, *J. Am. Chem. Soc.* **72**, 5339 (1950).
25. Jpn. Pat. 7405995 (1974), K. Ito, H. Fukushima, and K. Nakagawa (to Nisshin Flour Milling Co., Ltd.).
26. W. Traube, *Berliner Berichte* **33**, 1371 (1900).
27. Ger. Pat. 3403468 A1 (Aug. 8, 1985), H. Blum and G. Dreesmann (to Federal Republic of Germany).
28. Eur. Pat. 444266 A1 (Sept. 4, 1991), A. Hunds and W. Rogler (to Huls AG).
29. Jpn. Pat. 59204158 (1974) (to Daicel Chem Ind., KK).
30. Ger. Pat. 3419817 A1 (Dec. 6, 1984), K. Yoshida, T. Niinobe, and T. Baba (to Takeda Chemical Industries Ltd.).
31. Ger. Pat. 3605484A (Aug. 8, 1987), W. Deinhammer and H. Petersen (to Walker-Chemie GmbH); Eur. Pat. 394968 A (Oct. 31, 1990), B. D. Dombek and T. T. Wenzel (to Union Carbide Chemical).
32. J. R. Piper, G. S. McCaleb, and J. A. Montgomery, *J. Heterocycl. Chem.* **24**, 279 (1987).
33. C. M. Baugh and E. Shaw, *J. Org. Chem.* **29**, 3610 (1964).
34. Ger. Pat. 2741383 (Feb. 22, 1979), E. Catalucci (to Lonza AG).
35. J. R. Piper and J. A. Montgomery, *J. Org. Chem.* **42**, 208 (1977).
36. J. R. Piper and J. A. Montgomery, *J. Heterocycl. Chem.* **11**, 279 (1974); U.S. Pat. 4,077,957A (Mar. 7, 1978), J. A. Montgomery and J. R. Piper (to U.S. Sec. Dept. Health); U.S. Pat. 4,079,056 (Mar. 13, 1978), J. A. Montgomery and J. R. Piper (to U.S. Sec. Dept. Health).
37. J. H. Bieri and M. Viscontini, *Helv. Chim. Acta* **56**, 2905 (1973); K. Khalifa, P. K. Sengupta, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta* **59**, 242 (1976).
38. M. Sletzinger, D. Reinhold, J. Grier, M. Beachem, and M. Tishler, *J. Am. Chem. Soc.* **77**, 6365 (1955).
39. M. Viscontini and J. H. Bieri, *Helv. Chim. Acta* **54**, 2291 (1971).
40. Swiss Pat. 255409 (1949) (to Hoffmann-La Roche Inc.).
41. Ger. Pat. 3242193 A1 (May 17, 1984), F. Brunnmueller and M. Kroener (to BASF AG).
42. E. C. Taylor and T. Kobayashi, *J. Org. Chem.* **38**, 2817 (1973); E. C. Taylor, R. N. Henrie, and R. C. Portnoy, *J. Org. Chem.* **43**, 736 (1978).
43. B. Schirks, J. H. Bieri, and M. Viscontini, *Helv. Chem. Acta* **68**, 1639 (1985).
44. E. C. Taylor and K. Lenard, *Liebigs. Ann. Chem.* **726**, 100 (1969); E. C. Taylor and D. J. Dumas, *J. Org. Chem.* **46**, 1394 (1981).
45. Ger. Pat. 3242195 A1 (May 17, 1984), F. Brunnmueller and M. Kroener (to BASF AG); Eur. Pat. 175263 A2 (Mar. 26, 1986), H. Leininger, W. Littmann, J. Paust, and W. Trautmann (to BASF AG); Eur. Pat. 175264 A2 (Mar. 26, 1986), H. Leininger, W. Littmann, and J. Paust (to BASF AG).
46. Eur. Pat. Appl. 608 693 A2 (Aug. 3, 1994), C. Wehrli (to Hoffmann-La Roche AG).
47. J. F. Gregory and J. P. Toth, *J. Labelled Comp. Radiopharm.* **25**, 1349 (1988).
48. J. F. Gregory, *J. Agric. Food Chem.* **38**, 1073 (1990).
49. D. L. Hachey, L. Palladino, J. A. Blair, I. H. Rosenberg, and P. D. Klein, *J. Labelled Comp. Radiopharm.* **14**, 479 (1978).
50. J. F. Gregory and J. P. Toth, *Anal. Biochem.* **170**, 94 (1988).
51. P. A. Charlton, D. W. Young, B. Birdsall, J. Feeney, and G. C. K. Roberts, *J. Chem. Soc. Perkin Trans. I*, 1349 (1985).
52. C. M. Tatum, M. G. Fernald, and J. P. Schimel, *Anal. Biochem.* **103**, 255 (1980).
53. J. A. Blair and K. J. Saunders, *Anal. Biochem.* **34**, 376 (1970).
54. C. Zarow, A. M. Pellino, and P. V. Danenberg, *Prep. Biochem.* **12**, 381 (1983).
55. E. Khalifa, A. N. Ganguly, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta* **63**, 2554 (1980).
56. D. B. Cosulich, J. M. Smith, and H. P. Broquist, *J. Am. Chem. Soc.* **74**, 4215 (1952).
57. J. E. Baggott and C. L. Krumdieck, *Biochemistry* **18**, 1036 (1979).
58. C. M. Baugh, J. Stevens, and C. Krumdieck, *Biochim. Biophys. Acta* **212**, 116 (1970).
59. B. L. Hutchings et al., *J. Biol. Chem.* **170**, 323 (1947).
60. J. D. Cook, D. J. Cichowicz, S. George, A. Lawler, and B. Shane, *Biochemistry* **26**, 530 (1987).
61. M. C. Li, R. Hertz, and D. M. Bergenstal, *New Engl. J. Med.* **259**, 66 (1958).
62. J. Burchanall and G. Hitchings, *Mol. Pharmacol.* **1**, 216 (1965).
63. G. M. Brown and H. Williamson, *Adv. Enzymol. Relat. Areas Mol. Biol.* **53**, 345 (1982).
64. G. M. Brown and H. Williamson, in F. C. Neidhart, ed., *Escherichia Coli* and *Salmonella Typhmurium*, Vol. 1, American Society for Microbiology, Washington, D.C., 1987, p. 521.
65. Y. Suzuki and G. M. Brown, *J. Biol. Chem.* **249**, 2405 (1974).
66. J. Kas and J. Cerna, *Methods Enzymol.* **66E**, 443 (1980).
67. P. M. Finglas, R. M. Faulks, and M. R. A. Morgan, *J. Micronutrient Anal.* **4**, 295 (1988).
68. C. L. Krumdieck, T. Tamura, and I. Eto, *Vitamins Horm.* **40**, 45 (1983).
69. G. Brubacher, W. Müller-Mulot, and D. A. T. Southgate, *Methods for Determination of Vitamins in Food*, Elsevier Applied Science Publisher, New York, 1985, p. 158.
70. D. B. McCormick and L. D. Wright, ed., *Methods Enzymol.* **66E**, 429 (1980).
71. H. Baker, S. P. Jaslow, and O. Frank, *J. Am. Geriatr. Soc.* **26**, 218 (1978).
72. S. A. Anderson and J. M. Talbot, *FDA Technical Report* FDA/RF-82/13, Washington, D.C., 1981.
73. E. Giovannucci, E. B. Rimm, A. Ascherio, M. J. Stampfer, G. A. Colditz, and W. C. Willett, *J. Natl. Cancer Inst.* **87**, 265 (1995).

74. *MMWR Morb Mortal Wkly Rep.* **44**, 716 (1995).
75. A. Milunsky, J. Herschel, S. S. Jick, C. L. Bruell, D. S. Maclaughlin, K. J. Rothman, and W. Willett, *J. Am. Med. Assoc.* **262**, 2847 (1989).
76. C. Bower and J. F. Stanley, *Med. J. Austral.* **150**, 613 (1989).
77. P. S. Romano, N. J. Waitzman, R. M. Scheffler, and R. D. Pi, *Am. J. Public Health* **85**, 667 (1995).
78. B. F. Satchell, *FDA Technical Report*, FDA/61RF-8781, Washington, D.C., 1996
79. N. Whitehead, F. Reyner, and J. Lindenbaum, *J. Am. Med. Assoc.* **226**, 1421 (1973).
80. A. Bianchi, D. W. Chipman, A. Dreskin, and N. S. Rosensweig, *New Engl. J. Med.* **282**, 859 (1970).
81. K. S. McCully, *Am. J. Pathol.* **56**, 111 (1969).
82. C. S. Berwanger, J. Y. Jeremy, and G. Stansby, *Br. J. Surg.* **82**, 726 (1995).
83. J. Selhub, P. F. Jacques, A. G. Bostom, R. B. D'Agostino, P. W. Wilson, A. J. Belanger, D. H. O'Leary, P. A. Wolf, E. J. Schaefer, and I. H. Rosenberg, *N. Engl. J. Med.* **332**, 286 (1995).
84. J. L. Mills, J. M. McPartlin, P. N. Kirke, Y. J. Lee, M. R. Conley, D. G. Weir, and J. M. Scott, *Lancet* **345**, 149 (1995).
85. A. F. Subar, G. Block, and L. D. James, *Am. J. Clin. Nutr.* **50**, 508 (1989).
86. *Nutrient Requirements of Poultry*, 9th. ed., National Research Council, National Academy Press, Washington, D.C., 1994; *Nutrient Requirements of Swine*, 9th ed., National Research Council, National Academy Press, Washington, D.C., 1988.
87. L. R. McDowell, *Vitamins in Animal Nutrition; Comparative Aspects to Human Nutrition*, Academic Press, Inc., San Diego, CA, 1989, pp. 298–325.
88. *Vitamin Nutrition for Poultry*, Dept. of Animal Health and Nutrition, Hoffmann-La Roche, Inc., Nutley, N.J., 1991.
89. *Vitamin Nutrition for Swine*, Dept. of Animal Health and Nutrition, Hoffmann-La Roche, Inc., Nutley, N.J., 1991.
90. J. Zimmerman, Z. Gihula, J. Selhub, and I. H. Rosenberg, *Int. J. Vit. Nutr. Res.* **59**, 151 (1989).
91. J. Zimmerman, J. Selhub, and I. H. Rosenberg, *Am. J. Clin. Nutr.* **46**, 518 (1987).
92. C. H. Young, F. M. Sirtnak, and M. Dembo, *J. Membrane Biol.* **79**, 285 (1984).
93. D. W. Horne, W. Y. Briggs, and C. Wagner, *J. Biol. Chem.* **253**, 3529 (1978).
94. G. B. Henderson, *Ann. Rev. Nutr.* **10**, 319 (1990).
95. C. Wagner, *Ann. Rev. Nutr.* **2**, 229 (1982).
96. C. Wagner, in L. B Bailey, ed., *Folate in Health and Disease*, Marcel Dekker, Inc., New York, 1995, p. 23.
97. C. E. Butterworth and T. Tamura, *Am. J. Clin. Nutr.* **50**, 353 (1989).
98. R. Hunter, J. Barnes, H. F. Oakeley, and D. M. Matthews, *Lancet* **1**, 61 (1970).
99. *New Engl. J. Med.* **237**, 713 (1947).
100. A. Hanck, in *Spektrum Vitamine* **42**, 81 (1986).
101. A. F. Czeisal and J. Dudas, *New Engl. J. Med.* **327**, 1832 (1992); MRC Vitamin Study Research Group, *Lancet* **338**, 131 (1991).

GENERAL REFERENCES

J. F. Gregory, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker Inc., New York, 1995, p. 195.

T. Brody, in L. J. Machlin, ed., *Handbook of Vitamins*, Marcel Dekker Inc., New York, 1991, p. 453.

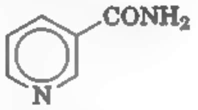
O. Isler and G. Brubacher, in O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, eds., *Vitamine II*, Georg Thieme Verlag, New York, 1982, p. 264.

S. K. Gaby and A. Bendich, in S. K. Gaby, A. Bendich, V. N. Singh, and L. J. Machlin, eds., *Vitamin Intake and Health*, Marcel Dekker Inc., New York, 1991, p. 175.

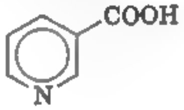
THIMMA R. RAWALPALLY
Hoffmann-La Roche, Inc.

VITAMINS: NIACIN, NICOTINAMIDE, AND NICOTINIC ACID

3-Pyridine carboxamide [*98-92-0*] (nicotinamide) (**1**) and 3-pyridine carboxylic acid [*59-67-6*] (nicotinic acid) (**2**) have a rich history and their early significance stems not from their importance as a vitamin but rather as products derived from the oxidation of nicotine. In 1867, Huber prepared nicotinic acid from the potassium dichromate oxidation of nicotine. Many years later, Engler prepared nicotinamide. Workers at the turn of the twentieth century isolated nicotinic acid from several natural sources. In 1894, Suzuki isolated nicotinic acid from rice bran, and in 1912 Funk isolated the same substance from yeast (1).



(**1**)



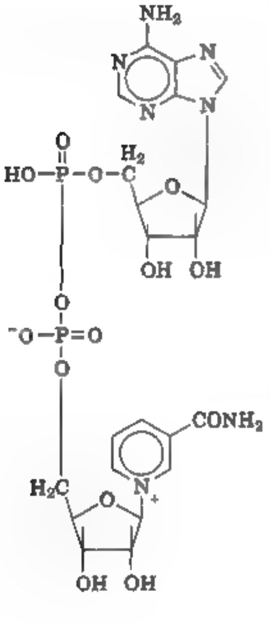
(**2**)

In 1913, Goldberger demonstrated that pellagra was due to a dietary deficiency. Pellagra had been earlier described by Thiery, who had coined the term *mal de la rosa* for this disease. Several decades later, Elvehjem and co-workers isolated nicotinamide from a liver extract and identified it as a pellagra-preventing factor (1).

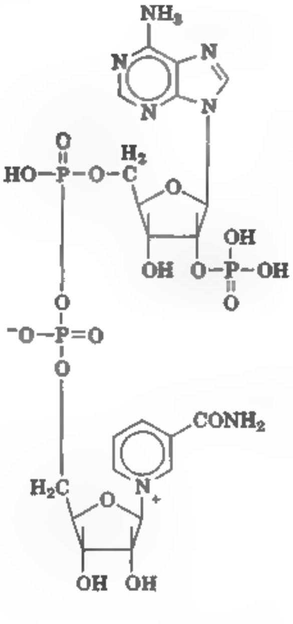
There is considerable confusion regarding the nomenclature of these simple substances. The term niacin is a generic descriptor for pyridine 3-carboxylic acid and derivatives that exhibit the biological activity of nicotinic acid (1). However, niacin and vitamin PP (pellagra preventing) are frequently used interchangeably with nicotinic acid. Vitamin B_3 is often used as a designation for nicotinamide (1). The following is a list of common names for these substances.

Nicotinamide	Nicotinic acid
Aminicotin	Akotin
Benicot	Apelagrin
Delonin amide	Daskil
NAM	Efacin
Niacinamide	Linic
Niavit PP	Niacin
Nicasir	Nicacid
Nicosan 2	Nicangin
Nicovit	Nicolar
Pelmine	Pellagrin
Pelonin amide	Pelonin
Vitamin B	SR 4390
Vitamin B_3	

The biological importance of these compounds stems from their use as cofactors. Both nicotinamide and nicotinic acid are building blocks for coenzyme I (Co I), nicotinamide—adenine dinucleotide (NAD) (3) and coenzyme II (Co II), nicotinamide—adenine dinucleotide phosphate (NADP) (4) (2).



(3)



(4)

CHEMICAL AND PHYSICAL PROPERTIES

Nicotinamide is a colorless, crystalline solid. It is very soluble in water (1 g is soluble in 1 mL of water) and in 95% ethanol (1 g is soluble in 1.5 mL of solvent). The compound is soluble in butanol, amyl alcohol, ethylene glycol, acetone, and chloroform, but is only slightly soluble in ether or benzene. Physical properties are listed in Table 1.

Nicotinic acid is an amphoteric solid with needle-shape crystals. It is less soluble than nicotinamide and its poor solubility in diethyl ether can be used as a basis to separate these compounds. Because nicotinamide has some solubility in ether, extraction of aqueous solutions of the acid and the amide with ether allows for selective extraction of the amide into the organic phase. Table 2 lists the physical properties of this vitamin.

The ring nitrogen of both the amide and the carboxylic acid can be quaternized and oxidized. *N*-methylnicotinate (trigonelline) (5) is an important component of green coffee beans and is converted to nicotinic acid during the roasting process. The reactivity of the 3-substituent parallels standard functional groups. Acid chlorides, esters, amides, and anhydrides have been prepared from nicotinic acid. Both the corresponding aldehyde and alcohol are available from the acid with a variety of reducing agents. Nicotinamide can be converted to nicotinic acid esters, the nitrile and acylamidines by routine methods.

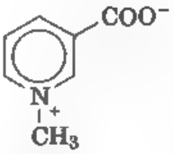
Table 1. Physical Properties of Nicotinamide

Property	Value
Molecular weight	122.12
Melting point, °C	
Stable modification	129–132
Unstable modifications	
I	105
II	110
III	111
IV	113
V	116
Boiling point, °C (0.067 Pa)	150–160
Sublimation range, °C	80–100
True dissociation constants in water, at 20°C	
K_{b1}	2.24×10^{-11}
K_{b2}	3.16×10^{-14}
Specific heat, kJ/(kg·K)[a]	
Solid, 55°C	1.30
65°C	1.34
75°C	1.39
Liquid, 135°C	2.18
Heat of solution in water, kJ/kg[a]	−148
Heat of fusion, kJ/kg[a]	381
Density of melt, at 150°C, g/cm³	1.19

[a] To convert J to cal, multiply by 4.184.

Table 2. Physical Properties of Nicotinic Acid

Property	Value
Molecular weight	123.11
Melting point, °C	236–237
Sublimation range	≥150
Density of crystals, g/cm³	1.473
Dissociation constants in water, at 25°C	
K_a	1.50×10^{-5}
K_b	1.04×10^{-12}
Isoelectric point in water, at 25°C, pH	3.42
pH of saturated aqueous solution	2.7
Solubility, g/L	
Water	
0°C	8.6
38°C	24.7
100°C	97.6
Ethanol, 96%	
0°C	5.7
78°C	76.0
Methanol	
0°C	68.0
62°C	345.0



(5)

SYNTHESIS

Key intermediates in the industrial preparation of both nicotinamide and nicotinic acid are alkyl pyridines (Fig. 1).

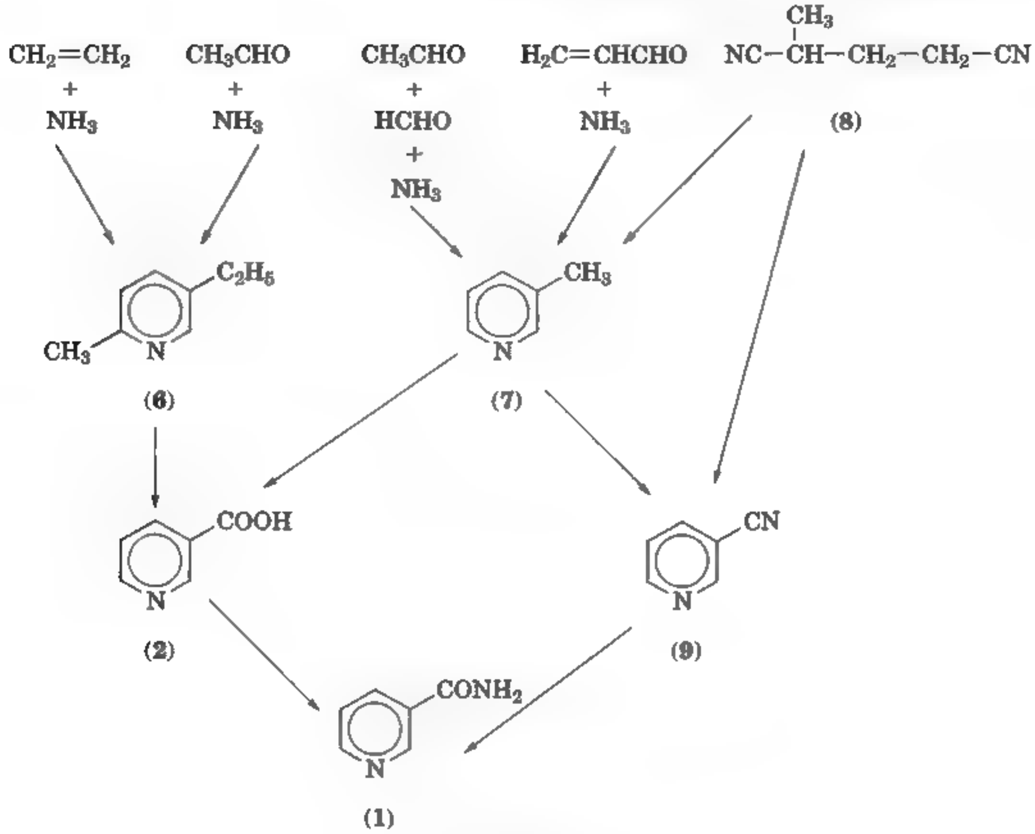


Figure 1. Alkyl pyridines.

2-Methyl-5-ethylpyridine (**6**) is prepared in a liquid-phase process from acetaldehyde. Also, a synthesis starting from ethylene has been reported. Alternatively, 3-methylpyridine (**7**) can be used as starting material for the synthesis of nicotinamide and nicotinic acid and it is derived industrially from acetaldehyde, formaldehyde (qv), and ammonia. Pyridine is the principal product from this route and 3-methylpyridine is obtained as a by-product. Despite this and largely due to the large amount of pyridine produced by this technology, the majority of the 3-methylpyridine feedstock is prepared in this fashion.

3-Methylpyridine can also be prepared by the condensation of acrolein and ammonia. Yields of 40–50% are obtained with pyridine as a by-product. Higher yields have been claimed when both propionaldehyde and acrolein have been used. A recent U.S. patent claims better selectivity if the cyclization is carried out in the presence of zeolites (3).

In an alternative approach, 2-methylglutaronitrile (**8**) is hydrogenated and cyclized to give high yields of 3-methylpyridine. The feedstock for this process is produced as a by-product of the production of adiponitrile. Oxidative cyclization of 2-methylglutaronitrile to nicotinonitrile (**9**) has been described (4).

The alkyl pyridines (**6**) and (**7**) can be transformed either to nicotinic acid or nicotinonitrile. In the case of nicotinic acid, these transformations can occur by either chemical or biological means. From an industrial standpoint, the majority of nicotinic acid is produced by the nitric acid oxidation of 2-methyl-5-ethylpyridine. Although not of industrial significance, the air oxidation has also been reported. Isocinchomeronic acid (**10**) (Fig. 2) is formed as an intermediate.

Although an inherently more efficient process, the direct chemical oxidation of 3-methylpyridine does not have the same commercial significance as the oxidation of 2-methyl-5-ethylpyridine. Liquid-phase oxidation procedures are typically used (5). A Japanese patent describes a procedure that uses no solvent and avoids the use of acetic acid (6). In this procedure, 3-methylpyridine is combined with cobalt acetate, manganese acetate and aqueous hydrobromic acid in an autoclave. The mixture is pressurized to 101.3 kPa (100 atm) with air and allowed to react at 210°C. At a 32% conversion of the picoline, 19% of the acid was obtained. Electrochemical methods have also been described (7).

In more recent work, several groups have reported on fermentative approaches to nicotinic acids from 3-picoline. *Rhodococcus* (8), *Acinetobactorr* (9), and *Pseudomonas* (10) have found utility in this application.

Nicotinonitrile is produced by ammoxidation of alkylpyridines (11–24). A wide variety of different catalysts have been developed for this application. For example, a recent patent describes a process in which 3-methylpyridine is reacted over a molybdenum catalyst supported on silica gel. The catalyst ($PV_4Mo_{12}O_x$) was prepared from

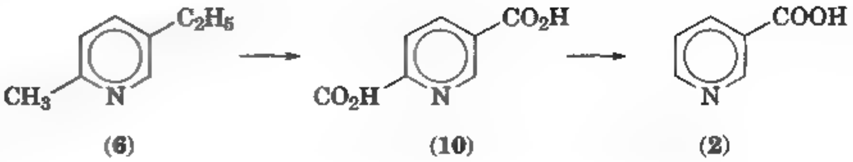


Figure 2. Formation of isocinchomeronic acid (10).

NH_4VO_3, H_3PO_4, and $(NH_4)_5Mo_7O_{24}$. Reaction at 380°C at a residence time of 2.5 seconds gave 95% of nicotinonitrile at a 99% conversion (16).

Conversion of the nitrile to the amide has been achieved by both chemical and biological means. Several patents have described the use of modified Raney nickel catalysts in this application (25,26). Also, alkali metal perborates have demonstrated their utility (27). Typically, the hydrolysis is conducted in the presence of sodium hydroxide (28–31). Owing to the fact that the rate of hydrolysis of the nitrile to the amide is fast as compared to the hydrolysis of the amide to the acid, good yields of the amide are obtained. Other catalysts such as magnesium oxide (32), ammonia (28,29,33), and manganese dioxide (34) have also been employed.

Since the late 1980s, there has been considerable activity in the development of fermentative approaches for the preparation of the amide from the nitrile. Organisms such as *Achromobacter* (35), *Agrobacterium* (36), *Streptomyces* (37), *Rhodococcus* (38,39), and *Cornebacterium* (40) have been described. Purified enzymes in either free (41) or immobilized form (42,43) have also been used in this application.

Nicotinic acid has also been produced by microbial means from the nitrile. *Rhodococcus* (44,45) has frequently been used in this regard. Interestingly, irradiation of a *Corynebacterium* suspension during the fermentation led to higher yields of nicotinic acid (46).

BIOSYNTHESIS

A significant pathway for the formation of nicotinic acid mononucleotides begins with tryptophan (11). In the first step, cleavage of the indole ring is catalyzed by the enzyme L-tryptophan-2,3-dioxygenase. This step is rate determining unless it is hormonally induced by glucocortocoids and even tryptophan itself. The resulting N'-formylkynurenine (**12**) is deformylated to the free aniline derivative, kynurenine (**13**). Hydroxylation to yield (**14**) is followed by oxidative removal of alanine to form 3-hydroxyanthranilic acid (**15**). Oxidative cleavage of the aromatic ring to the semialdehyde (**16**) is followed by dehydration to the important metabolite, quinolinic acid (**17**). Quinolinic acid is decarboxylated with concomitant alkylation at nitrogen to yield nicotinic acid mononucleotide (**18**) (Fig. 3).

The result of this biosynthesis is that the product is nicotinic acid mononucleotide rather than free nicotinic acid. Ingested nicotinic acid is converted to nicotinic acid mononucleotide which, in turn, is converted to nicotinic acid adenine dinucleotide. Nicotinic acid adenine dinucleotide is then converted to nicotinamide adenine dinucleotide. If excess nicotinic acid is ingested, it is metabolized into a series of detoxification products (Fig. 4). Physiological metabolites include N-methylnicotinamide (**19**) and N-methyl-6-pyridone-2-carboxamide (**24**) (1).

Nicotinamide is incorporated into NAD and nicotinamide is the primary circulating form of the vitamin. NAD has two degradative routes: by pyrophosphatase to form AMP and nicotinamide mononucleotide and by hydrolysis to yield nicotinamide adenosine diphosphate ribose.

ANALYTICAL METHODS

Both nicotinic acid and nicotinamide have been assayed by chemical and biological methods. Owing to the fact that niacin is found in many different forms in nature, it is important to indicate the specific analyte in question. For example, if biological assay procedures are used, it is necessary to indicate whether the analysis is to determine the quantity of nicotinic acid or if niacin activity is the desired result of the analysis. If nicotinic acid is desired, then a method specific for nicotinic acid should be used. If quantitation of niacin activity is the desired outcome, then all compounds (bound and unbound) which behave like niacin will assay biologically for this substance (1).

The König reaction (Fig. 5) has been used to determine the amount of nicotinic acid and niacinamide. In this procedure, quaternization of the pyridine nucleus by cyanogen bromide is followed by ring opening to generate the putative dialdehyde intermediate. Reaction of this compound with an appropriate base, such as p-methylaminophenol sulfate (47) or sulfanilic acid (48), generates a dye. The concentration of this dye is determined colorimetrically.

In the case of nicotinamide, the color yield is often low. This problem can be circumvented by either hydrolysis to nicotinic acid or by conversion of the amide to a fluorescent compound. Treatment of nicotinamide with methyl iodide yields the quaternary ammonium salt, N-methyl nicotinamide (5). Reaction of this compound with acetophenone yields a fluorescent adduct (49). Other carbonyl compounds have also been used (50–54).

For more specific analysis, chromatographic methods have been developed. Using reverse-phase columns and uv detection, hplc methods have been applied to the analysis of nicotinic acid and nicotinamide in biological fluids such as blood and urine and in foods such as coffee and meat. Derivatization techniques have also been employed to improve sensitivity (55). For example, the reaction of nicotinic amide with DCCI (N'-dicyclohexyl-O-methoxycoumarin-4-yl)methyl isourea to yield the fluorescent coumarin ester has been reported (56). After separation on a reversed-phase column, detection limits of 10 pmol for nicotinic acid have been reported (57).

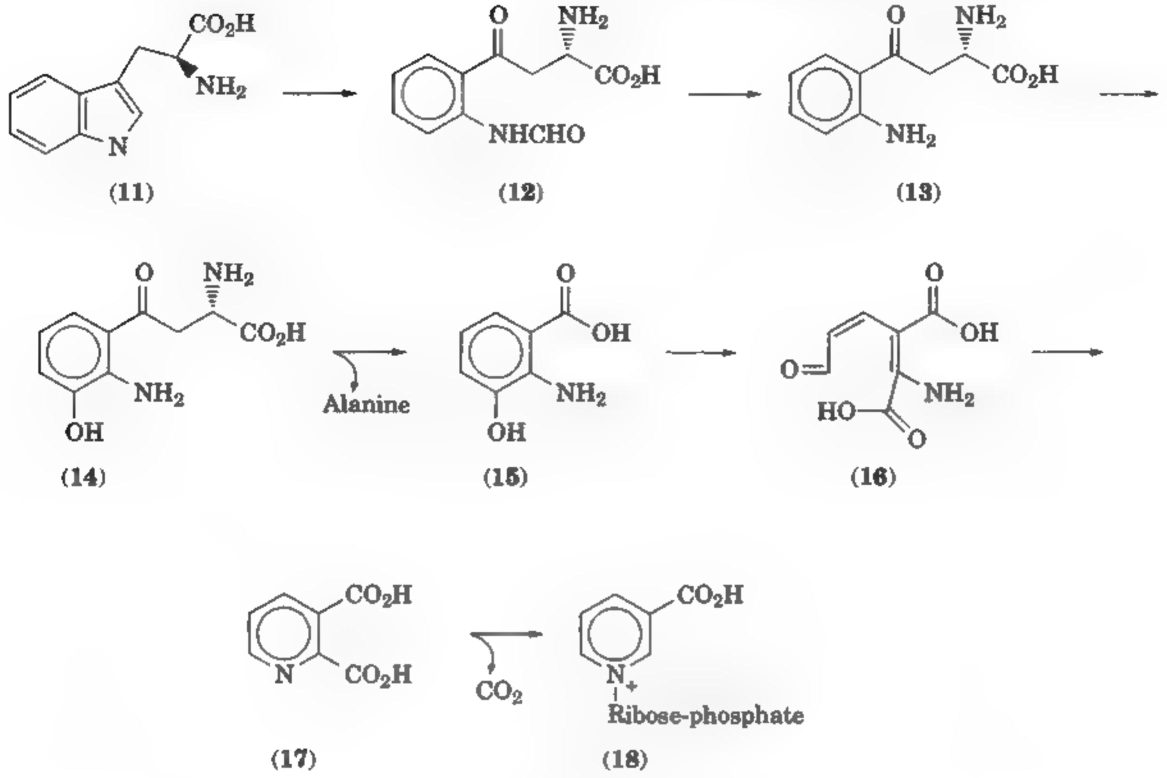


Figure 3. Formation of nicotinic acid mononucleotides.

Owing to poor volatility, derivatization of nicotinic acid and nicotinamide are important techniques in the gc analysis of these substances. For example, a gc procedure has been reported for nicotinamide using a flame ionization detector at detection limits of ~0.2 μg (58). The nonvolatile amide was converted to the nitrile by reaction with heptafluorobutryic anhydride (56). For a related molecule, quinolinic acid, fmol detection limits were claimed for a gc procedure using either packed or capillary columns after derivatization to its hexafluoroisopropyl ester (58).

As with many of the vitamins, biological assays have an important historical role and are widely used. For example, microbiological assays use *Lactobacillus plantarum* ATCC No. 8014 (57,59) or *Lactobacillus arabinosus* (60). These methods are appropriate for both nicotinamide and nicotinic acid. Selective detection of nicotinic acid is possible if *Leuconostoc mesenteroides* ATCC No. 9135 is used as the test organism (61). The use of microbiological assays have been reviewed (62).

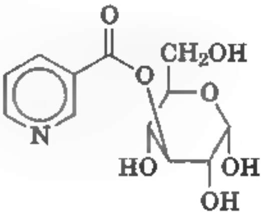
Bioassays procedures have been developed in species such as chicks which have been fed a niacin-deficient diet. Due to the fact that, for example, tryptophan is a biological precursor of niacin, niacin can be produced from other sources (55). As a result, the tryptophan content of the diet has to be monitored carefully for accurate results.

Specifications for niacin and niacinamide for food use are given in the *Food Chemicals Codex* (63) and for pharmaceutical use in the *United States Pharmacopeia* (64). The *Codex* also gives specifications for niacinamide ascorbate.

OCCURRENCE

Nicotinamide and nicotinic acid occur in nature almost exclusively in the bound form. In plants, nicotinic acid is prevalent whereas in animals nicotinamide is the predominant form. This nicotinamide is exclusively in the form of NAD and NADP.

Nicotinic acid is found in plants associated with both peptides and polysaccharides. For example in wheat bran, two forms are described: a peptide with a molecular weight of approximately 12,000 and a carbohydrate complex that is called niacytin. Polysaccharides isolated from wheat bran have been found to contain 1.05% nicotinic acid in bound form. Hydrolysis yielded a fragment identified as *β*-3-*O*-nicotinoyl-D-glucose (**25**.



(25)

From a bioavailability standpoint, the fact that a significant amount of nicotinic acid is in a bound form has important biological consequences. Poor bioavailability

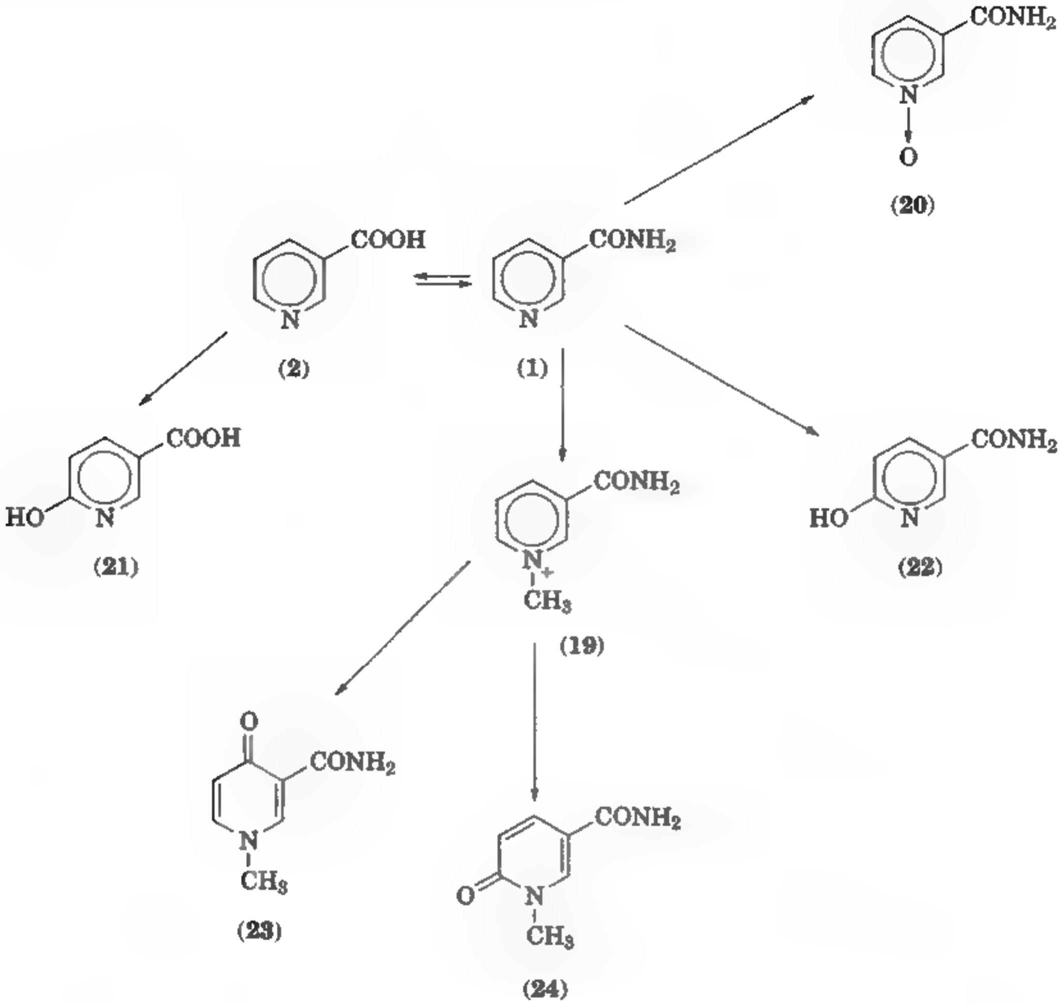

(20)
(2)
(1)
(21)
(22)
(19)
(23)
(24)

Figure 4. Detoxification products as the result of digestion of excess nicotinic acid.

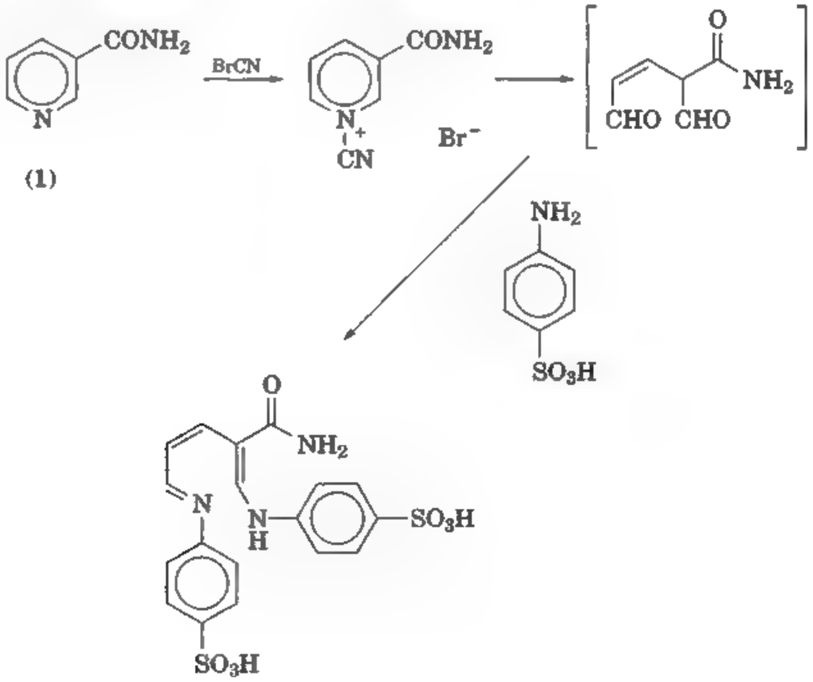


Figure 5. The König reaction.

stems from the fact that the ester linkage is resistance to digestive enzymes. In the case of corn, this condition can be alleviated if corn is pretreated with alkali. This food preparation method is frequently practiced in Mexico for the preparation of tortillas.

Nicotinamide and nicotinic acid are prevalent in many common foodstuffs and are especially concentrated in brewer's yeast, wheat germ and liver. In this regard, tryptophan is considered a provitamin and is assigned a niacin equivalent of 1/60. The following lists the vitamin B_3 content of many common foodstuffs and in Table 3, values of vitamin B_3 content are compared to niacin potential from tryptophan.

Corn, yellow
Corn, gluten feed
Rye
Oats
Milo
Barley
Wheat
Wheat bran
Tapioca flour
Potatoes
Soybean meal
Rapeseed-extract meal
Cottonseed cake
Palm kernel meal
Groundnut cake
Sunflower cake
Blood meal
Meat and bone meal
Fish meal
Skim milk, dried
Torula yeast

Table 3. Nicotinic Acid and Nicotinamide (Vitamin B_3) Content of Foodstuffs (mg/kg)

Foodstuff	Vitamin B_3	Potential from tryptophan
Cow milk, fresh whole summer	0.8	7.8
Cheese, cream cheese	0.8	7.4
Eggs, whole, raw	0.7	36.1
Butter, salted	Trace	Trace
Beef, lean, average, raw	52	43
Beef liver, fresh	178	
Pork, lean, average, raw	62	38
Pork liver, fresh	118	
Corn, whole	12	
Corn, flour	Trace	1
Breakfast cereals, all-bran	490	32
Cornflakes		
Fortified	210	9
Unfortified	6	
Wheat flour		
Whole meal	56	25
White, 72% for bread		
Fortified	20	23
Unfortified	7	
Brown, 85%		
Fortified	42	26
Unfortified	12	
Bread, white	14	16
Macaroni, boiled	3	9
Rice, polished, boiled	3	5
Rice, bran	366–437	
Soybean flour, full fat	20	86
Spaghetti, boiled	3	9
Potatoes, raw	12	5
Yeast. Baker's dry	257	

Source: Ref. 2.

Coffee represents a fertile source of nictonic acid and the average cup of coffee contains between 1–2 mg. The amount of nicotinic acid depends on the roasting conditions (Table 4). During the roasting process, trigonelline (**5**), a plentiful component in green beans is demethylated to nictonic acid (65). The ratio of trigonelline (**5**) to nicotinic acid and nicotinamide is defined as the roasting number.

BIOCHEMICAL FUNCTION

NAD and NADP are required as redox coenzymes by a large number of enzymes and in particular dehydrogenases (Fig. 6). NAD^+ is utilized in the catabolic oxidations of carbohydrates, proteins, and fats, whereas $NADPH_2$ is the coenzyme for anabolic reactions and is used in fats and steroid biosynthesis. $NADP^+$ is also used in the catabolism of carbohydrates (2).

In addition to their *in vivo* function, these cofactors have important *in vitro* functions as co-factors in enzymatic synthesis. Because these cofactors are too expensive to be used in equimolar amounts, many methods have been developed for their regeneration. These include chemical (66), biological (67) and electrochemical (68) methods. Enzymatic regeneration has found particular utility in this application (69).

DEFICIENCY

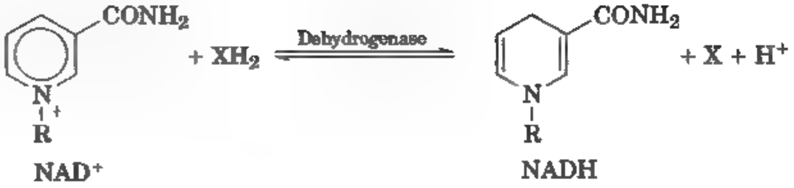
A deficiency of niacin manifests itself in the disease pellagra. The symptoms of pellagra include dermatosis, dementia, and diarrhea. The dermatosis is expressed as lesions. These lesions are most frequently found on the hands, wrists, elbows, face and neck, knees, and the feet. At the onset of the disease, the affected areas resemble sunburn, progressing to keratosis and scaling of pigmented skin. Other symptoms include insomnia, loss of appetite, weight and strength loss, soreness of the tongue and mouth, indigestion, diarrhea, abdominal pain, burning sensations, vertigo, headache, numbness, nervousness, distractability, apprehension, mental confusion, and forgetfulness (1).

A deficiency of niacin also affects the nervous system. Numbness is initially observed and later, paralysis, particularly in the extremities is common. Severe cases are characterized by tremor and a spastic or ataxic gait and are frequently associated with peripheral neuritis. Left untreated, severe thought disorders can ensue (1).

Table 4. Vitamin B_3 Content in Roasting Coffee

Coffee beans	Vitamin B_3 (mg/kg)
Green	20
Medium roasted	80–150
Dark roasted	≤500

Source Ref. 2.



(3)

Figure 6. Coenzymes required for dehydrogenase.

Table 5. RDA and U.S. RDA for Niacin

Category	Niacin (mg NE)
RDA	
Infants	
0.0–0.5 yr	5
0.5–1.0 yr	6
Children	
1–3 yr	9
4–6 yr	12
7–10 yr	13
Males	
11–14 yr	17
15–18 yr	20
19–50 yr	19
>51 yr	15
Females	
11–50 yr	15
>51 yr	13
pregnant	17
lactating	20
U.S. RDA	
Infants and children <4 yr	9
Adults and children >4 yr	20
Pregnant or lactating women	20

REQUIREMENTS

The RDA for niacin is based on the concept that niacin coenzymes participate in respiratory enzyme function and 6.6 niacin equivalents (NE) are needed per intake of 239 kJ (1000 kcal). One NE is equivalent to 1 mg of niacin. Signs of niacin deficiency have been observed when less than 4.9 NE/239 kJ or less than 8.8 NE per day were consumed. Dietary tryptophan is a rich source of niacin and the average diet in the United States contains 500–1000 mg of tryptophan. In addition, the average diet contains approximately 8–17 mg of niacin. In total, these two quantities total 16–34 NE daily. Table 5 lists the RDA and U S. RDA for niacin (69).

SAFETY

Despite structural similarities, the pharmacological consequences of excesses of these substances are quite different. Due to the interest in the effects of nicotinic acid on atherosclerosis, and in particular its use based on its ability to lower serum cholesterol, the toxicity of large doses of nicotinic acid has been evaluated. For example, in a study designed to assess its ability to lower serum cholesterol, only 28% of the patients remained in the study after receiving a large initial dose of 4 g of nicotinic acid due to intolerance at these large doses (70).

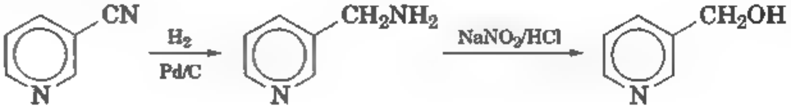
Nicotinamide can also be toxic to cells at concentrations that increase the NAD levels above normal. Individuals consuming nicotinamide at levels of 3 g/d for extended periods (3–36 months) have experienced various side effects such as heartburn, nausea, headaches, hives, fatigue, sore throat, dry hair, and tautness of the face (1).

USES

Both nicotinic acid and nicotinamide have been used in the enrichment of bread, flour, and other grain-derived products. Animal feed is routinely supplemented with nicotinic acid and nicotinamide. Nicotinamide is also used in multivitamin preparations. Nicotinic acid is rarely used in this application. The amide and carboxylic acid have been used as a brightener in electroplating baths and as stabilizer for pigmentation in cured meats.

DERIVATIVES

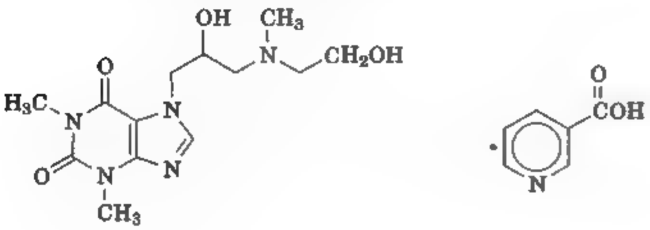
Nicotinyl alcohol (3-pyridinylcarbinol, 3-pyridinemethanol) (**27**) has use as an antilipemic and peripheral vasodilator. It is available from either the reductions of nicotinic acid esters or preferably, the reduction of the ni-



(**9**) (**26**) (**27**)

Figure 7. Formation of nicotinyl alcohol (**27**).

trile to the amine followed by diazotation and nucleophilic displacement. It is frequently administered in the form of the tartrate (Fig. 7). Nicotinic acid is frequently used as a salt in conjunction with basic drugs such as the peripheral vasodilator xanthinol niacinate (**28**). Nicotinic acid and its derivatives have widespread use as antihyperlipidemic agents and peripheral vasodilators (1).



(**28**)

ECONOMIC ASPECTS

U.S. manufacturers of niacin and niacinamide include Nepera, Inc. and Reilly Industries, Inc. U.S. suppliers include BASF Corporation, Hoffmann-La Roche Inc., and Rhône-Poulenc. Western European producers and suppliers include Degussa, Rhône-Poulenc, BASF, Hoffmann-La Roche, and Lonza (71). In 1995, the prices for niacin and nicotinamide were $9.75/kg and $9.25/kg, respectively (72,73).

BIBLIOGRAPHY

1. J. van Eys, in L. Machlin, ed., *Handbook of Vitamins*, Marcel Dekker, New York, 1991, pp. 311–340.
2. D. Bhatia, *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, pp. 2276–2783.
3. U.S. Pat. 5,395,940 (Mar. 7, 1995), P. J. Angevine, C. T. Chu, and T. C. Potter (to Mobil Oil Corp.).
4. U.S. Pat. 4,721,789 (Dec. 6, 1988), R. Dicosimo, J. D. Burrington, and R. K. Grasselli (to Standard Oil Co.).
5. Jpn. Pat. 94-26603 (Feb. 24, 1994), Y. Asamidori, I. Hashiba, and S. Takigawa (to Nissan Chemical Ind Ltd. Japan).
6. World Pat. 9305022 A1 (Mar. 18, 1993), M. Hatanaka and N. Tanaka (to Nissan Chemical Industries, Ltd., Japan).
7. U.S. Pat. 4,482,439 A (Nov. 13, 1984), J. E. Toomey, Jr. (to Reilly Tar and Chemical Corp.).
8. Jpn. Pat. 93-193212 (July 8, 1993), T. Ishikawa, K. Maeda, Y. Mukohara, and T. Kojima (to Nippon Soda Co., Japan).
9. Jpn. Pat. 90-80698 (Mar. 30, 1990), F. C. Fujita (to Nitto Chemical Industry Co., Ltd.).
10. Eur. Pat. 442430 A2 (Aug. 21, 1991), A. Kiener (to Lonza, AG).
11. Eur. Pat. 684074 A1 (Nov. 29, 1995), Y. K. Lee, C. H. Shin, T. S. Chang, D. H. Cho, and D. K. Lee (to Korea Research Institute of Chemical Technology, South Korea).
12. Eur. Pat. 525367 A1 (Feb. 3, 1993), Y. Onda, K. Tsukahara, and N Takahashi (to Mitsubishi Gas Chemical Co.).
13. U.S. Pat. 5,028,713 (July 2, 1991), R. Dicosimo, J. D. Burrington, and R. K. Grasseli (to Standard Oil Co.).
14. U.S. Pat. 4,876,348 (Oct. 24, 1989), R. Dicosimo, J. D. Burrington, and D. D. Suresh (to Standard Oil Co.).
15. Eur. Pat. 339680 A2 (Nov. 2, 1989), M. Saito, K. Tsukahara, K. Yamada, and H. Imai (to Mitsubishi Gas Chemical Co., Inc.).
16. Int. Pat. 163169A (Aug. 20, 1988), B. V. Suvorov and coworkers (to the Institute of Chemical Sciences, Academy of Sciences, Kazakh SSR).
17. Ger. Pat. 241903 A1 (Jan. 7, 1987), B. Luecke and co-workers (to Akademie der Wissenschaften der DDR).
18. Eur. Pat. 253360 A2 (Jan. 20, 1988), S. Shimizu and coworkers (to Koei Chemical Co., Ltd., Japan).
19. Jpn. Pat. 86-154741 (July 1, 1986), N. Abe, M. Dojo, and A. Iguchi (to Koei Chemical Industry Co., Ltd.).
20. Jpn. Pat. 85-169554 (July 30, 1985), H. Sato and K. Hirose (to Sumitomo Chemical Co., Ltd., Japan).
21. U.S. Pat. 4,603,207 A (July 29, 1986), R. Dicosimo, J. D. Burrington, and D. D. Suresh (to Standard Oil Co., Ohio).
22. Eur. Pat. 96-100676 (Jan. 18, 1996), L. V. Hippel, A. Neher, and D. Arntz (to Degussa Aktiengesellschaft, Germany).
23. World Pat. 9532055 (Nov. 30, 1995), D. C. Sembaer, F. A. Ivanovskaya, and E. M. Guseinov (to Lonza Ltd, Switzerland).
24. World Pat. 9532054 (Nov. 30, 1995), B. V. Suvorov, L. A. Stepanova, B. N. Anatoljevna, J. R. Chuck, and D. Pianzola (to Lonza Ltd, Switzerland).
25. Jpn. Pat. 93-206579 (Aug. 20, 1993), H. Hirayama (to Showa Denko KK, Japan).
26. Eur. Pat. 85-306670 (Sept. 19, 1985), S. Asano and J. Kitagawa (to Mitsui Toatsu Chemicals, Inc.).
27. World Pat 9009988 A1 (Sept. 7, 1990), A. McKillop and D. Kemp (to Interlox Chemicals, Ltd.).
28. U.S. Pat. 2,471,518 (May 31, 1949), B. F. Duesel and H. H. Friedman (to Pyridium Co.).
29. U.S. Pat. 4,721,789 (Dec. 6, 1988), R. Dicosimo, J. D. Burrington, and R. K. Grasselli (to Standard Oil Co.).
30. Brit. Pat. Appl. 11357/55 (Aug. 28, 1942), B. F. Duesel and H. H Friedman (to Pyridium Co.).
31. Ger. Offen. Appl. 2,517,054 (Apr. 17, 1975), H. Beschke, H. Friedrich, K.-P. Muller, and G. Schreyer (to Degussa Co.).
32. C. B. Rosas and G. B. Smith, *Chem. Eng. Sci.* **35**, 330 (1975).
33. Brit. Pat. 777,517 (Mar. 28, 1956), E. J. Gasson and D. J. Hadley (to The Distillers Co., Ltd.).
34. U.S. Pat. 4,008,241 (Feb. 15, 1977), A. P. Gelbein and coworkers (to the Lummus Co.).
35. Ger. Pat. 2,1313,8134 (Oct. 19, 1972), L. R. Haefele (to R. J. Reynolds Tobacco Co.).
36. Jpn. Pat. 93-18645 (Feb. 5, 1993), A. Hatamori, M. Shimada, and K. Ishiwatari (to Mitsui Toatsu Chemicals).
37. Eur. Pat. 568072Y A2 (Feb. 27, 1991), Y. Takashima, K. Kumagai, and S. Mitsuda (to Sumitomo Chemicals Co.).
38. Jpn. Pat. 90-80697 (Mar. 30, 1990), F To and C. Fuijita (to Nitto Chemical Industry Co., Ltd.).
39. Jpn. Pat. 89-81137 (Mar. 31, 1989), H. Yamada, T. Nagasawa, and H. Shimizu (to Nitto Chemical Industry Co., Ltd.).
40. Eur. Pat. 204555 A2 (Dec. 10, 1986), K. Kawakami, T Tanabe, and O. Nagano (to Asahi Chemical Industry Co., Ltd.).

41. Jpn. Pat. 82-91408 (May 31, 1982) (to Nitto Chemical Industry Co , Ltd.).
42. Eur. Pat. 579907 A1 (Jan. 26, 1994), K. Yamada, M. Ochiai, Y. Yotsumoto, Y. Morimoto, and Y. Teranishi (to Mitsubishi Kawei Corp., Japan)
43. J. Eyal and M. Charles, *Ann. N.Y. Acad. Sci.* **589**, 678 (1990).
44. Jpn. Pat. 86-109401 (May 15, 1986), K. Kawakami and T. Tanabe (to Technology Research Association for New Application Development for Light-Weight Fractions, Japan).
45. Eur. Pat. 91-102938 (Feb. 27, 1991), H. Yamada, T. Nagasawa, and T. Nakamura (to Nitto Chemical Co., Ltd., Japan).
46. C. D. Mathew, T. Nagasawa, and M. Kobayashi, *Appl. Environ. Microbiol.* **54**, 1030–1032 (1988).
47. Eur. Pat. 86-100305 (Jan. 11, 1986), K. Enomoto, Y. Sato, Y. Nakashima, A. Fujiwara, and T. Doi (to Nitto Chemical Industry Co., Ltd., Japan, Mitsubishi Rayon Co., Ltd.).
48. F. E. Friedemann and E. J. Frazier, *Arch. Biochem.* **26**, 361 (1950).
49. *Official Methods of Analysis*, 8th ed., W. Horwitz, ed., Association of Official Agricultural Chemists, Washington, D.C., 1955, p. 826.
50. B. R. Clark, in D. B. McCormick and L. D. Wright, eds., *Methods in Enzymology*, Vol. 66, Academic Press, Inc., New York, 1980, p. 5.
51. J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.* **167**, 157 (1947).
52. N. Levitas, J. Robinson, F. Rosen, J. W. Huff, and W. A. Perlzweig, *J. Biol. Chem.* **167**, 169 (1947).
53. H. B. Burdch et al., *J. Biol. Chem.* **212**, 897 (1955).
54. K. H. Carpenter and E. Kodicek, *Biochem. J.* **46**, 421 (1950).
55. G. A. Goldsmith and O. N. Miller, in P. György and W. N. Pearson, eds., *The Vitamins*, 2nd ed., Vol. VII, Academic Press, Inc., New York, 1967, p. 137.
56. Y. Tsuruta, K. Kohashi, S. Ishida, and Y. Ohkura, *J. Chromatogr.* **309**, 309 (1984).
57. K. Shibata and T Shimono, in A. D. P. De Leenheer, W. E. Lambert, and H. J. Nelis, eds., *Modern Chromatographic Analysis of Vitamins*, 2nd ed., Marcel Dekker, Inc., New York, 1992, pp. 285–317.
58. A. Tanaka, M Iijima, Y. Kikuchi, Y. Hoshino, and N. Nose, *J. Chromatogr.* **466**, 307 (1989).
59. M. P. Heyes and S. P. Markey, *Anal. Biochem.* **174**, 349 (1988).
60. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C., 1980, pp. 759–761, 763–764.
61. *Methods of Vitamin Assay*, Interscience Publishers, New York, 1966, pp. 172–176.
62 B. C. Johnson, *J. Biol. Chem.* **159**, 227 (1945).
63. M. R. S. Fox and G. M. Briggs, *J. Nutr.* **72**, 243 (1960).
64. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, pp. 205–206.
65. *The United States Pharmacopeia XXII* (USP XXII-NF XVII), United States Pharmacopeial Convention, Inc., Rockville, Md., 1990, pp. 943–944.
66 H. Tagachi, M. Sakaguchi, and Y. Shimabayashi, *Agri. Bio. Chem.* 49, 3467–3471 (1985).
67. R. Ruppert, S Hermann, and E. Steckhan, *J. Chem. Soc. Chem. Comm.*, 1150–1151 (1988).
68. Y. Morita, H. Furui, H. Yoshii, and R. Matsuno, *J. Ferment. Bioeng.* **77**, 13–16 (1994).
69. C. H. Wong and G. Whitesides, *Enzymes in Organic Chemistry*, Pergamon Press, Elsevier Science Ltd., Oxford, U.K., 1994.
70. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, pp. 137–142.
71. H. K. Schock, in W. L. Holmes, L. H. Carlson, and R. Paoletts, eds., *Drugs Affecting Lipid Metabolism*, Plenum Press, New York, 1969, p. 405.
72. *Chemical Economics Handbook*, SRI International, Menlo Park, Calif., July 1995.
73. *Chem. Mark. Rep.* (Mar. 18, 1996).

GENERAL REFERENCES

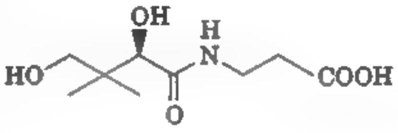
Refs. 2, 3, and 57 are good general references.

O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, "Die Niacin-Gruppe (Vitamin PP)" in *Vitamine II*, Georg Thieme Verlag Stuttgart, New York, 1988, pp. 160–192.

Susan D. Van Arnum
Westfield, New Jersey

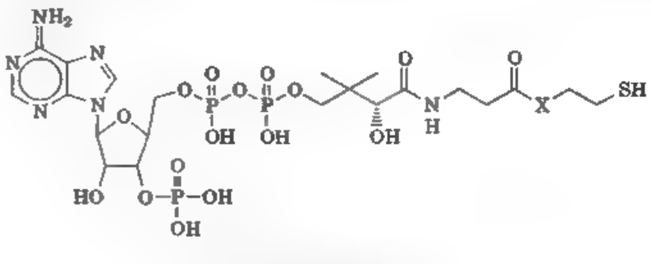
VITAMINS: PANTOTHENIC ACID

(*R*)-Pantothenic acid [*79-83-4*] (**1**) is a member of the B-complex vitamins. It is a water-soluble vitamin and has been designated as vitamin B_5. It was discovered by Williams and co-workers in 1933 by extracting a growth factor for yeast from a wide range of biological tissues (1). Later the growth factor was identified as pantothenic acid (1). Independently two groups, Woolley and co-workers (2) in 1939 and Jukes (3) between 1939 and 1941, identified the liver filtrate factor in rats and antidermatitis factor in chicks. These factors later proved to be identical with pantothenic acid. The total synthesis of (*R*)-pantothenic acid was achieved in 1940 by three groups: Stiller and co-workers (4), Reichstein and Grüssner (5), and Kuhn and Wieland (6).



(**1**)

After a full structural elucidation of coenzyme A in 1953 by Baddiley, it became evident that pantothenic acid is one of the components of coenzyme A (**2**) (7).



(**2a**, X = NH; coenzyme A)
(**2b**, X = S, thio analogue)

41. Jpn. Pat. 82-91408 (May 31, 1982) (to Nitto Chemical Industry Co , Ltd.).
42. Eur. Pat. 579907 A1 (Jan. 26, 1994), K. Yamada, M. Ochiai, Y. Yotsumoto, Y. Morimoto, and Y. Teranishi (to Mitsubishi Kawei Corp., Japan)
43. J. Eyal and M. Charles, *Ann. N.Y. Acad. Sci.* **589**, 678 (1990).
44. Jpn. Pat. 86-109401 (May 15, 1986), K. Kawakami and T. Tanabe (to Technology Research Association for New Application Development for Light-Weight Fractions, Japan).
45. Eur. Pat. 91-102938 (Feb. 27, 1991), H. Yamada, T. Nagasawa, and T. Nakamura (to Nitto Chemical Co., Ltd., Japan).
46. C. D. Mathew, T. Nagasawa, and M. Kobayashi, *Appl. Environ. Microbiol.* **54**, 1030–1032 (1988).
47. Eur. Pat. 86-100305 (Jan. 11, 1986), K. Enomoto, Y. Sato, Y. Nakashima, A. Fujiwara, and T. Doi (to Nitto Chemical Industry Co., Ltd., Japan, Mitsubishi Rayon Co., Ltd.).
48. F. E. Friedemann and E. J. Frazier, *Arch. Biochem.* **26**, 361 (1950).
49. *Official Methods of Analysis*, 8th ed., W. Horwitz, ed., Association of Official Agricultural Chemists, Washington, D.C., 1955, p. 826.
50. B. R. Clark, in D. B. McCormick and L. D. Wright, eds., *Methods in Enzymology*, Vol. 66, Academic Press, Inc., New York, 1980, p. 5.
51. J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.* **167**, 157 (1947).
52. N. Levitas, J. Robinson, F. Rosen, J. W. Huff, and W. A. Perlzweig, *J. Biol. Chem.* **167**, 169 (1947).
53. H. B. Burdch et al., *J. Biol. Chem.* **212**, 897 (1955).
54. K. H. Carpenter and E. Kodicek, *Biochem. J.* **46**, 421 (1950).
55. G. A. Goldsmith and O. N. Miller, in P. György and W. N. Pearson, eds., *The Vitamins*, 2nd ed., Vol. VII, Academic Press, Inc., New York, 1967, p. 137.
56. Y. Tsuruta, K. Kohashi, S. Ishida, and Y. Ohkura, *J. Chromatogr.* **309**, 309 (1984).
57. K. Shibata and T Shimono, in A. D. P. De Leenheer, W. E. Lambert, and H. J. Nelis, eds., *Modern Chromatographic Analysis of Vitamins*, 2nd ed., Marcel Dekker, Inc., New York, 1992, pp. 285–317.
58. A. Tanaka, M Iijima, Y. Kikuchi, Y. Hoshino, and N. Nose, *J. Chromatogr.* **466**, 307 (1989).
59. M. P. Heyes and S. P. Markey, *Anal. Biochem.* **174**, 349 (1988).
60. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C., 1980, pp. 759–761, 763–764.
61. *Methods of Vitamin Assay*, Interscience Publishers, New York, 1966, pp. 172–176.
62 B. C. Johnson, *J. Biol. Chem.* **159**, 227 (1945).
63. M. R. S. Fox and G. M. Briggs, *J. Nutr.* **72**, 243 (1960).
64. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, pp. 205–206.
65. *The United States Pharmacopeia XXII* (USP XXII-NF XVII), United States Pharmacopeial Convention, Inc., Rockville, Md., 1990, pp. 943–944.
66 H. Tagachi, M. Sakaguchi, and Y. Shimabayashi, *Agri. Bio. Chem.* 49, 3467–3471 (1985).
67. R. Ruppert, S Hermann, and E. Steckhan, *J. Chem. Soc. Chem. Comm.*, 1150–1151 (1988).
68. Y. Morita, H. Furui, H. Yoshii, and R. Matsuno, *J. Ferment. Bioeng.* **77**, 13–16 (1994).
69. C. H. Wong and G. Whitesides, *Enzymes in Organic Chemistry*, Pergamon Press, Elsevier Science Ltd., Oxford, U.K., 1994.
70. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, pp. 137–142.
71. H. K. Schock, in W. L. Holmes, L. H. Carlson, and R. Paoletts, eds., *Drugs Affecting Lipid Metabolism*, Plenum Press, New York, 1969, p. 405.
72. *Chemical Economics Handbook*, SRI International, Menlo Park, Calif., July 1995.
73. *Chem. Mark. Rep.* (Mar. 18, 1996).

GENERAL REFERENCES

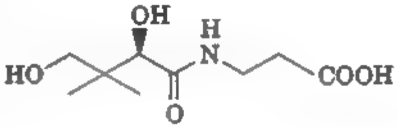
Refs. 2, 3, and 57 are good general references.

O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, "Die Niacin-Gruppe (Vitamin PP)" in *Vitamine II*, Georg Thieme Verlag Stuttgart, New York, 1988, pp. 160–192.

SUSAN D. VAN ARNUM
Westfield, New Jersey

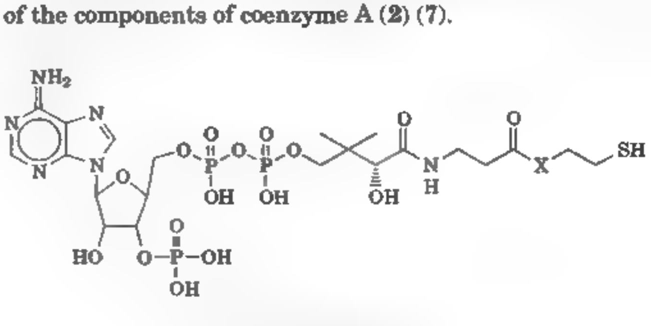
VITAMINS: PANTOTHENIC ACID

(*R*)-Pantothenic acid [*79-83-4*] (1) is a member of the B-complex vitamins. It is a water-soluble vitamin and has been designated as vitamin B_5. It was discovered by Williams and co-workers in 1933 by extracting a growth factor for yeast from a wide range of biological tissues (1). Later the growth factor was identified as pantothenic acid (1). Independently two groups, Woolley and co-workers (2) in 1939 and Jukes (3) between 1939 and 1941, identified the liver filtrate factor in rats and antidermatitis factor in chicks. These factors later proved to be identical with pantothenic acid. The total synthesis of (*R*)-pantothenic acid was achieved in 1940 by three groups: Stiller and co-workers (4), Reichstein and Grüssner (5), and Kuhn and Wieland (6).



(1)

After a full structural elucidation of coenzyme A in 1953 by Baddiley, it became evident that pantothenic acid is one of the components of coenzyme A (**2**) (7).



(**2a**, X = NH; coenzyme A)
(**2b**, X = S, thio analogue)

Biosynthesis of coenzyme A (CoA) in mammalian cells incorporates pantothenic acid. Coenzyme A, an acyl group carrier, is a cofactor for various enzymatic reactions and serves as either a hydrogen donor or an acceptor. Pantothenic acid is also a structural component of acyl carrier protein (ACP). ACP is an essential component of the fatty acid synthetase complex, and is therefore required for fatty acid synthesis. Free pantothenic acid is isolated from liver, and is a pale yellow, viscous, and hygroscopic oil.

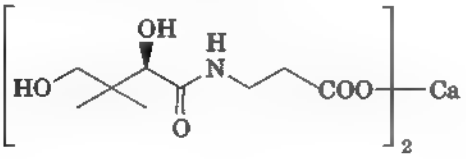
OCCURRENCE, SOURCE, AND BIOAVAILABILITY

Good food sources of pantothenic acid include yeast, chicken, beef, potatoes, oat cereal, vegetables, legumes, and whole grain. Pantothenic acid in foods and feedstuffs is fairly stable to ordinary means of cooking and storage, but appreciable losses have been reported as a result of canning and storage of some foods. Pantothenic acid is synthesized only by plants and microorganisms. Estimation of the dietary intake of pantothenic acid is difficult because the acid exists in the free form and is also incorporated in coenzyme A and fatty acid synthetase. Total pantothenic acid content present in the food is estimated after releasing the bound acid enzymatically (8,9).

Relatively little is known about the bioavailability of pantothenic acid in human beings, and only approximately 50% of pantothenic acid present in the diet is actually absorbed (10). Liver, adrenal glands, kidneys, brain, and testes contain high concentrations of pantothenic acid. In healthy adults, the total amount of pantothenic acid present in whole blood is estimated to be 1 mg/L. A significant (2–7 mg/d) difference is observed between different age-group individuals with respect to pantothenic acid intake and urinary excretion, indicating differences in the rate of metabolism of pantothenic acid.

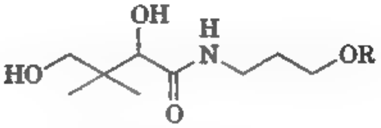
PHYSICAL AND CHEMICAL PROPERTIES

(*R*)-Pantothenic acid (**1**) contains two subunits, (*R*)-pantoic acid and *β*-alanine. The chemical abstract name is *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-*β*-alanine (11). Only (*R*)-pantothenic acid is biologically active. Pantothenic acid is unstable under alkaline or acidic conditions, but is stable under neutral conditions. Pantothenic acid is extremely hygroscopic and there are stability problems associated with the sodium salt of pantothenic acid. The major commercial source of this vitamin is thus the stable calcium salt (**3**) (calcium pantothenate).



(**3**)

Panthenol (**4**) is the reduced form of pantothenic acid and is the pure form most commonly used. The alcohol is more easily absorbed and is converted into the acid *in vivo* (12). Both panthenol and pantyl ether are used in hair care products.



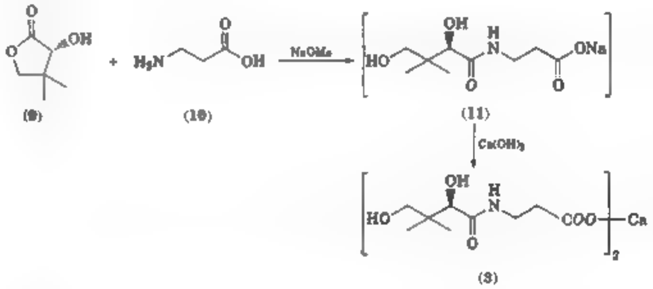
(**4**, R = H)
(**5**, R = C_2H_5)

The biologically active *R*- or *d*-pantothenic acid can be obtained upon hydrolysis of coenzyme A with a combination of two enzymes, alkaline phosphatase and pantotheinase (13) (Fig. 1). The phosphatase catalyzes the selective cleavage of the phosphate bond in coenzyme A to afford adenosin-3′5′-diphosphate (**6**) and 4-phosphopantetheine (**7**). The latter substance is dephosphorylated enzymatically to yield pantetheine (**8**), which is rapidly converted by pantotheinase to pantothenic acid (**1**). Table 1 lists some physical properties of pantothenic acid and its derivatives.

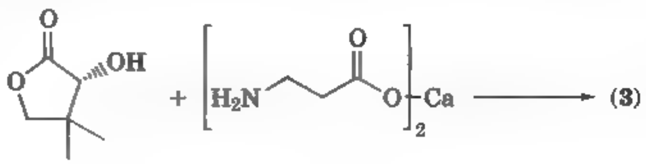
SYNTHESIS

Currently (ca 1997) pantothenic acid is produced mainly by chemical methods. Initial efforts in this area are summarized in Reference 14. Several groups are actively involved in developing syntheses of pantothenic acid or its precursor, (*R*)-pantolactone (**9**) by microbial methods.

(*R*)-Calcium pantothenate (**3**) is prepared by condensing (*R*)-pantolactone (**9**) with *β*-alanine (**10**) in the presence of base, followed by treatment of the sodium salt (**11**) with calcium hydroxide.



An alternative procedure for the preparation of (*R*)-calcium pantothenate (**3**) is to condense (*R*)-pantolactone (**9**) with the preformed calcium salt (**12**) of *β*-alanine (15).



(**9**) (**12**)

Similarly, panthenol (**4**) and pantyl ether (**5**) are prepared by condensing 3-aminopropanol (**13**) and 3-ethoxypropylamine (**14**) with (*R*)-pantolactone (**16,17**).

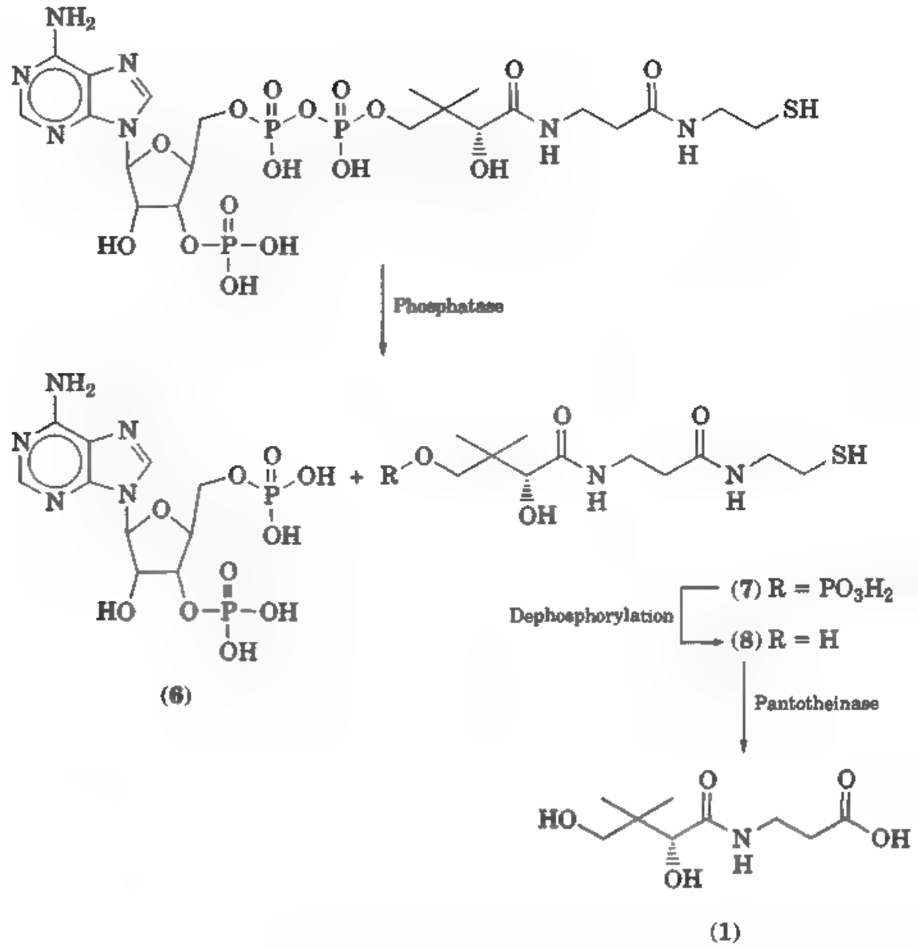
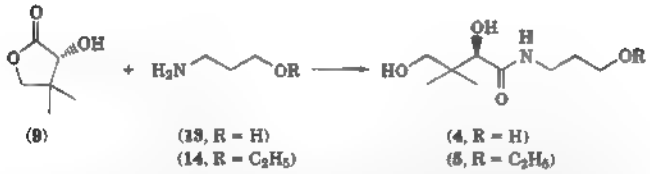
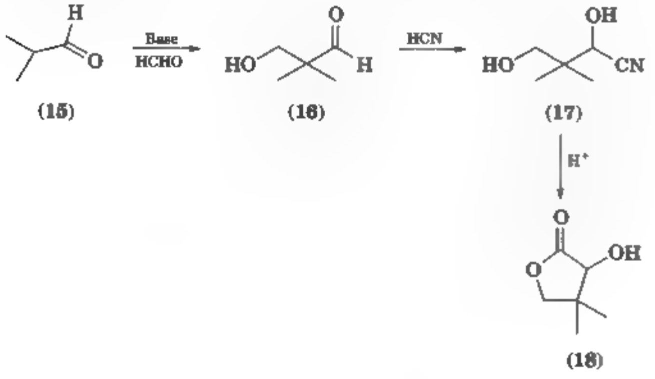

NH2
N
N
N
N
O
O
P
O
P
O
OH
OH
OH
N
H
N
H
SH
HO
O–P–OH
OH
Phosphatase
OH
+
R
SH
(7) R = PO3H2
Dephosphorylation
(8) R = H
(6)
Pantotheinase
HO
N
H
OH
OH
(1)

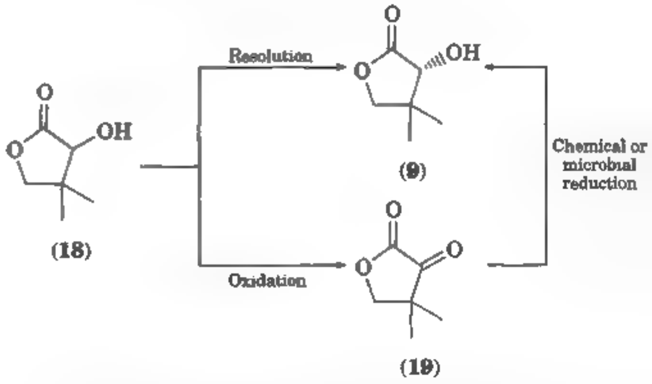
Figure 1. Enzymatic hydrolysis of coenzyme A (**2**).


OH
+ H2N
OR
HO
N
H
OR
(9)
(13, R = H)
(14, R = C2H5)
(4, R = H)
(5, R = C2H5)

Racemic pantolactone is prepared easily by reacting isobutyraldehyde (**15**) with formaldehyde in the presence of a base to yield the intermediate hydroxyaldehyde (**16**). Hydrogen cyanide addition affords the hydroxy cyanohydrin (**17**). Acid-catalyzed hydrolysis and cyclization of the cyanohydrin (**17**) gives (*R,S*)-pantolactone (**18**) in 90% yield (18).


H
O
Base
HCHO
HO
H
HCN
OH
HO
CN
(15)
(16)
(17)
H+
OH
(18)

(*R*)-Pantolactone (**9**) is prepared in either of two ways: by resolution of the (*R,S*)-pantolactone mixture (**18**), or by stereoselective reduction of ketopantolactone (**19**) by chemical or microbial methods (19).


Resolution
OH
(9)
Chemical or microbial reduction
OH
(18)
Oxidation
(19)

Chemical resolution of pantoic acid, readily available from (*R,S*)-pantolactone (**18**), is the most efficient method currently being practiced on an industrial scale. A wide variety of chiral resolving agents are being used, such as (+)-2-aminomethylpinane by BASF (20), 2-benzylamino-1-phenylethanol by Fuji (21), chiral aminoalcohols by Alps and Sumitomo (22,23), (1*R*)-3-endoaminoborneol by Hoff-

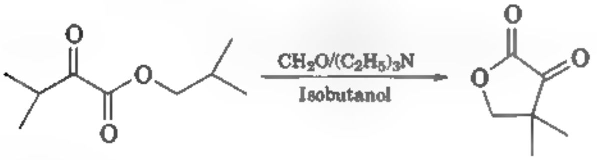
Table 1. Physical Properties of Pantothenic Acid and Derivatives

Compound	CAS registry number	α_D^{20a}	bp/mp (°C)	Molecular formula	Molecular weight
(*R*)-Pantothenic acid	*[79-83-4]*			$C_9H_{17}NO_5$	219 24
(*S*)-Pantothenic acid	*[37138-77-5]*			$C_9H_{17}NO_5$	219.24
(*R*)-Pantothenic acid calcium salt	*[137-08-6]*	+28.2°	195°C	$(C_9H_{16}NO_5)_2Ca$	476.5
(*R*)-Pantothenic acid sodium salt	*[876-81-2]*	+27.7°	171–178°C	$C_9H_{16}NO_5Na$	241.2
(*R*)-Panthenol	*[81-13-0]*	+29.5°	120°C^b	$C_9H_{19}NO_4$	205.25
(*S*)-Panthenol	*[74561-18-5]*	−29.5°	120°C^b	$C_9H_{19}NO_4$	205.25
(*R,S*)-Panthenol	*[16485-10-2]*	0	66–69°C	$C_9H_{19}NO_4$	205.25
(*R,S*)-Ethylpanthenol	*[667-84-5]*	0	210°C	$C_{11}H_{23}NO_4$	233.1
(*R*)-Ethylpanthenol	*[667-83-4]*			$C_{11}H_{23}NO_4$	233.1

[a] C = 5 in H_2O for all values other than 0.
[b] At 0.02 mm Hg.

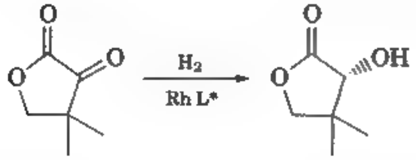
mann-La Roche (24), and 1-ethylbenzylamine by Daicel (25). Other approaches include optical resolution by inclusion crystallization (26) and spontaneous resolution of (*R,S*)-pantolactone by seeding (27). (*R,S*)-Pantolactone can be resolved directly using chiral phase chromatography (28), or after derivatization using optically active acids or isocyanates (29). The sodium salt of pantoic acid is enantioselectively protonated in the presence of (1*S*)-(+)-10-camphorsulfonic acid and then undergoes spontaneous cyclization to (*R*)-pantolactone (**9**) (30).

Ketopantolactone (**19**) is conveniently prepared by oxidation of (*R,S*)-pantolactone (**18**). Various oxidizing agents have been patented for the oxidation of pantolactone, such as MnO_2 (31), DMSO–Ac_2O (32), and hypohalites (33). An improved yield of ketopantolactone (**19**) via electrolytic oxidation of pantolactone with an aqueous solution containing an alkali metal salt was reported (34). Ketopantolactone (**19**) has been prepared in good yield via cyclocondensation of the 2-keto-3-methylbutyrate (**20**) with formaldehyde (35).



(20) **(19)**

Asymmetric hydrogenation of ketopantolactone (**19**) in the presence of chiral dirhodium complexes gave (*R*)-pantolactone (**9**) in high yield and excellent selectivity (36) (Table 2).

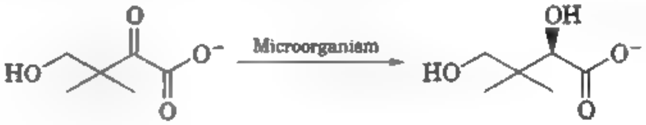


(19) **(9)**

Table 2. Asymmetric Hydrogenation of Ketopantolactone (19)

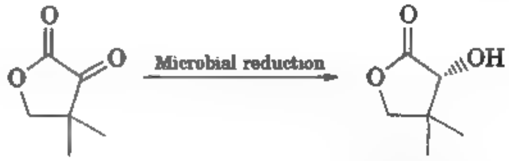
Catalyst/chiral ligand	Isolated yield (%)	ee (%)	Ref.
[Rh(bppm) (1,5-Hexadiene)Cl]	93	56	37
[Rh(bppm) (COD)Cl]	93	86.7	38
Dimethyl-(*R*)-malate	93	>99	39
Di-(μ-carboxylato)bis(aminophosphine-phosphinite) dirhodium complexes	100	91	36

The microbial reduction of ketopantoate (**21**) to (*R*)-pantoate (**22**) has been investigated, employing one or two microorganisms (40–44). Reduction with *Proteus vulgaris* gave (*R*)-pantoate (**22**) in high (460 mmol) concentration, excellent selectivity (97% ee), and 98.5% yield with acceptable productivity numbers. Productivity number (PN) is derived by dividing the concentration in mmol of product formed by the dry weight in kg of the cells, multiplied by the time in hours required for the conversion. Higher PN is favored for production (44) (Table 3).



(21) **(22)**

Reduction of ketopantolactone (**19**) to (*R*)-pantolactone (**9**) also was evaluated using microbes (45–52) (Table 4).



(19) **(9)**

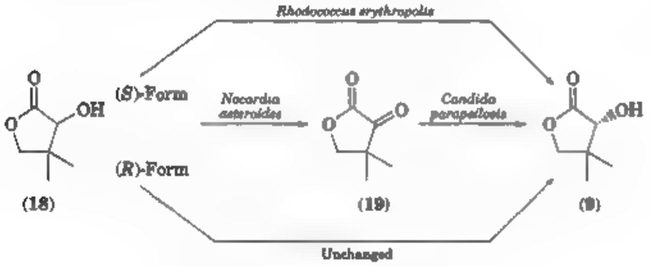
These microbial methods have afforded excellent ee values and yields (40–52). However, owing to low, ie, 30–130, productivity numbers these processes have not yet been commercialized.

Table 3. Microbial Reduction of Ketopantoate (21) to (*R*)-Pantoate (22)

Microorganism	Conversion yield (%)	ee (%)	Final product concentration (mM)	PN[a]	Reference
Rhodotorula erythropolis	90.5	94.4	140		40
Agrobacterium sp. S-242	90	>98	910	120	41
Nocardia asteroides/*Agrobacterium radiobacter*	89	82.8	620	130	42
Candida macedonienis-AK4 4588	97.2	98	80		43
Proteus vulgaris	98.5	>97	460	740	44

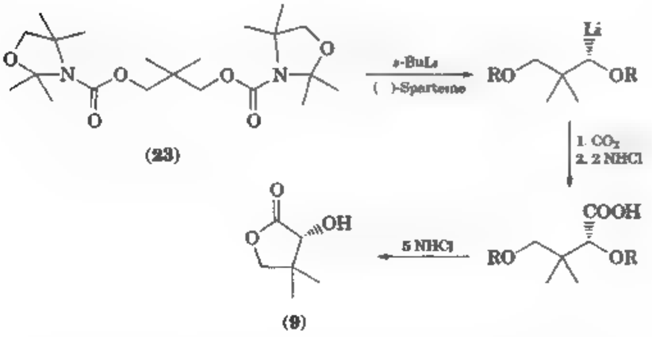
[a] Productivity number (PN) = product in mmol/dry wt of cells in kg x time, h.

In a novel approach, enantiomerically enriched (*R*)-pantolactone (**9**) is obtained in a enzymatic two-step process starting from racemic pantolactone.

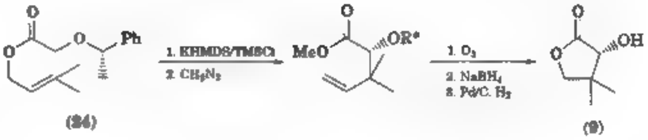


In a first step, *Nocardia asteroides* selectively oxidizes only (*S*)-pantolactone to ketopantolactone (**19**), whereas the (*R*)-pantolactone remains unaffected (47). The accumulated ketopantolactone is stereospecifically reduced to (*R*)-pantolactone in a second step with *Candida parapsilosis* (product concentration 72 g/L, 90% molar yield and 100% ee) (48). Racemic pantolactone can also be converted to (*R*)-pantolactone by one single microbe, ie, *Rhodococcus erythropolis*, by enantioselective oxidation to (*S*)-pantolactone and subsequent stereospecific reduction in 90% yield and 94% ee (product concentration 18 g/L) (40).

Although not of industrial importance, several asymmetric syntheses of (*R*)-pantolactone (**9**) have been developed. Stereoselective abstraction of the *si*-proton of the achiral 1,3-propanediol derivative (**23**) by *sec*-butyllithium-(−)-sparteine, followed by carboxylation and hydrolysis, results in (*R*)-pantolactone in 80% yield and 95% ee (53).

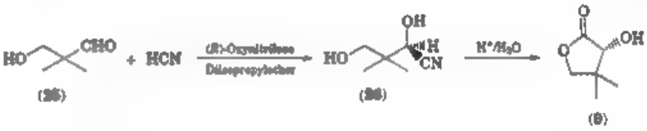


(*R*)-Pantolactone is also prepared in a sequence involving Claisen rearrangement of the chiral glycolate (**24**), although with poor enantioselectivity (54).



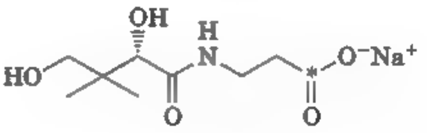
By employing Sharpless epoxidation as a key step, a multistep chemical synthesis of (*R*)-pantolactone has also been reported (55).

Enantioselective addition of hydrogen cyanide to hydroxypivaldehyde (**25**), catalyzed by (*R*)-oxynitrilase, afforded (*R*)-cyanohydrin (**26**) in good optical yield. Acid-catalyzed hydrolysis followed by cyclization resulted in (*R*)-pantolactone in 98% ee and 95% yield after one recrystallization (56).



LABELED COMPOUNDS

Various labeled degradation products of pantothenic acid and coenzyme A are known. Both (1-^{14}C)-sodium pantothenate (**27**) and (*R*)-(1-^{14}C)-panthenol are



(27)

known and are commercially available from New England Nuclear Corporation (NEN, Boston, Mass.). The syntheses of ^{14}C-phosphopantotheine (57) and ^{14}C-labeled coenzyme A (58) have also been reported.

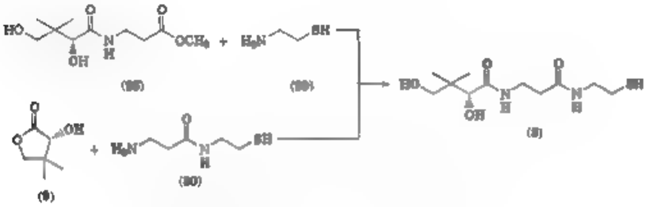
DERIVATIVES AND ANALOGUES

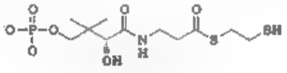
Methyl and diacetyl derivatives of pantothenic acid are prepared via methylation and acetylation of pantothenic acid (59). Pantethein (**8**) is one of the biochemical degradation products derived from coenzyme A. Synthetically, it can be prepared in several different ways, starting from either methyl pantothenate (**28**) or (*R*)-

Table 4. Microbial Reduction of Ketopantolactone (19) to (*R*)-Pantolactone (9)

Microorganism	Conversion yield (%)	ee (%)	Final product concentration (m*M*)	PN	Reference
Saccharomyces cerevisiae	>90	>98	40	30	45
Byssochamys fulva	>90	>96			46
Nocardiaasteroides / candida parapsilosis	>90	>98	390	130	47
Candida parapsilosis	>90	94–98	700		48
Rhodotorulaminuta	80.3	99.7	380		49
Saccharomyces cerevisiae	39	93	20		50
Zygosaccharo-myces	96.8	90			51
Baker's yeast/β-cyclodextrin	39	93			52

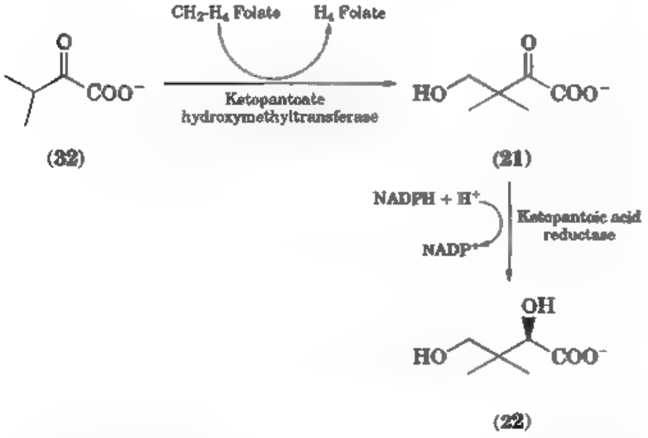
pantolactone. Pantethein (**8**) is prepared either by condensing cysteamine (**29**) with methyl pantothenate (**28**) (60) or by coupling (*R*)-pantolactone (**9**) with 3-amino-*N*-(2-mercaptoethyl)propanamide (**30**) (61). Phosphopantetheine analogue (**31**) was prepared starting from (*R*)-pantothenic acid. Enzymatic condensation of this compound with adenosin-3,5-diphosphate (**6**) gave CoA analogue (**2b**) (62).




(31)

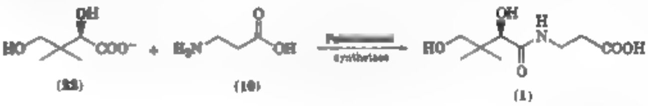
BIOSYNTHESIS

The metabolically active form of pantothenic acid is coenzyme A. Pantothenic acid is produced only by microorganisms, starting from (*R*)-pantoate (**22**) and β-alanine. (*R*)-Pantoate is synthesized by a set of enzymatic reactions, as follows (63,64):


CH₂-H₄ Folate
H₄ Folate
COO⁻
Ketopantoate hydroxymethyltransferase
HO
(32)
(21)
NADPH + H⁺
NADP⁺
Ketopantoic acid reductase
OH
(22)

The conversion of α-ketoisovalerate (**32**) to ketopantoate (**21**) is catalyzed by ketopantonate hydroxymethyltransferase and a cofactor tetrahydrofolate (65). Further reduction of ketopantoate (**21**) to (*R*)-pantoate (**22**) is catalyzed by ketopantoic acid reductase (66).

Aspartic acid decarboxylase catalyzes the decarboxylation of asparatic acid to yield β-alanine (**10**), a precursor for the biosynthesis of pantothenic acid (67). Finally, (*R*)-pantothenic acid is obtained by coupling β-alanine (**10**) with (*R*)-pantoate (**22**) in the presence of pantothenate synthetase:

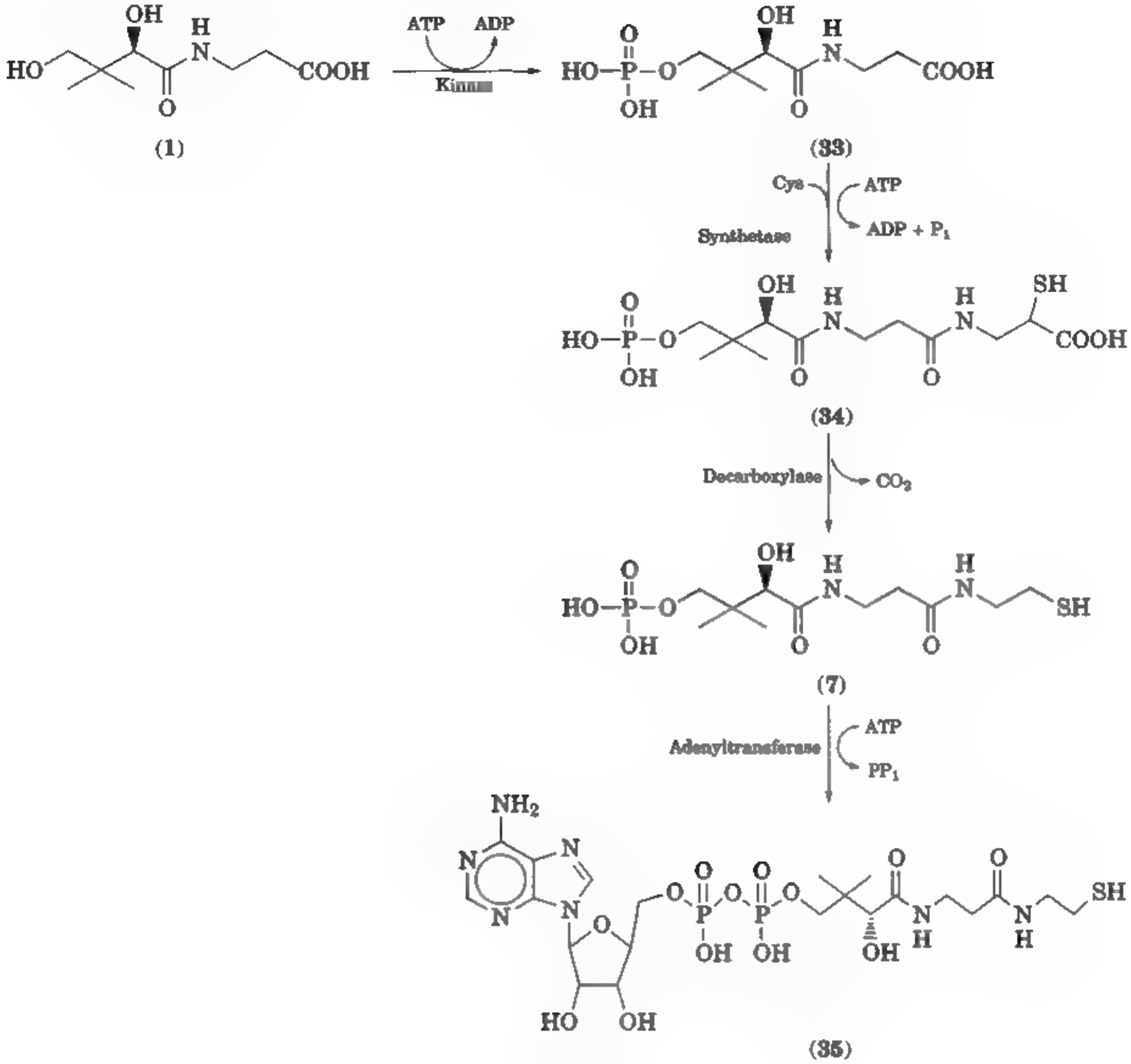


Various pathways have been proposed for the conversion of pantothenic acid to coenzyme A (68,69). The currently accepted pathway involves the following sequence; pantothenate to 4-phosphopantothenic acid (**33**), 4-phosphopantothenyl cysteine (**34**), 4-phosphopantetheine (**7**), dephosphocoenzyme A (**35**), and coenzyme A (**2**). Phosphorylation of pantothenic acid to 4-phosphopantothenic acid is catalyzed by pantothenic kinase in the rate-limiting step in the overall synthesis of coenzyme A (70).

ANALYTICAL METHODS

Chemical, physical, animal, microbiological, and biochemical assays have been used to determine pantothenic acid. Pantothenic acid in foods is found in both the free form and as the vitamin moiety of coenzyme A and phosphopantetheine (see Scheme 1). For most assays, the incorporated pantothenic acid has to be liberated enzymatically. Usually, a combination of pantotheinase and alkaline phosphatase is used to liberate the bound pantothenic acid. The official method for pantothenic acid of the Association of Official Analytical Chemists (AOAC) is the microbiological assay that uses *L. Plantarium* (ATCC 8014) as the test organism (71). Samples are extracted at 121°C at pH 5.6–5.7, proteins are precipitated at pH 4.5, and the resulting clear extracts are adjusted to pH 6.8 prior to assay. This procedure is only suitable to determine calcium pantothenate or other free forms of pantothenic acid.

A radioimmunoassay method has been developed by Wyse and co-workers (72). It is a sensitive method for the determination of small amounts of pantothenic acid in biological fluids. The assay is based on the binding of an en-



Scheme 1.

zyme specific for pantothenic acid. Hemolyzed blood samples are subjected to a double-enzyme treatment with 5 IU of bovine intestinal alkaline phosphatase and 0.1 IU of pantetheinase. After hydrolysis, the clear supernatant extract is analyzed for pantothenate by a radioimmunoassay method. In the case of pharmaceutical preparations, vitamin concentrations are determined by using spectrophotometric and fluorometric methods (73). For spectrophotometric methods, detection is done at 358 nm and for fluorescence, excitation is done at 350 nm and collection at 450 nm. Food pantothenate assays involve hydrolysis of the food by 25% hydrochloric acid in a 95–100°C water bath, extraction of the pantoyl lactone into dichloromethane and analysis by gas chromatography with flame ionization detection (74). Chiral polysiloxane fused silicon capillary columns (XE-60-L-Val-(*S*)- or (*R*)-α-phenylethylamide) have been developed for separation of the (*R*)- and (*S*)-pantothenic acid mixture (75). Recently, clinical separation and simultaneous determination of (*R*)- and (*S*)-pantothenic acids in rat plasma using gc–ms has been described (76). Deproteinization of the plasma samples is done by eluting the plasma sample through an anion-exchange resin using 1 *M* sodium chloride solution. Pantothenic acid is extracted quantitatively with ethyl acetate after acidifying the basic aqueous solution. After esterification of the carboxylic group, each derivative is identified using gs–ms.

DEFICIENCY

A deficiency of pantothenic acid in humans has not been detected because it is widely available in food (77). In the case of prisoners of war in the 1940s, a burning feet syndrome, which was attributed to pantothenic acid deficiency as a result of severe malnutrition, was observed (78). Pantothenic acid deficiency in humans is induced experimentally by feeding a diet deficient in pantothenic acid along with a pantothenic acid antagonist such as ω-methylpantothenic acid. This results in clinical malaise, fatigue, headache, sleep disturbance, nausea, vomiting, and cardiovascular instability. Impaired responses to insulin, histamine, and ACTH (stress hormone) have also been observed. A wide variety of abnormalities such as retarded growth, impaired fertility, gastrointestinal lesions, and adrenal necroses are reported when rats are fed a pantothenic acid-deficient diet (79,80). Chicks on a pantothenic acid-deficient diet develop lesions at the corners of the mouth, swollen eyelids, hemorrhagic cracks on the feet, and listlessness (81).

ABSORPTION

Pantothenic acid occurs in most foods and feedstuffs as CoA and acyl-carrier protein. The utilization of the vitamin depends upon the hydrolytic digestion of these protein complexes to release the free vitamin. Coenzyme A is hydrolyzed in the intestinal lumen before absorption into the cell by a sodium ion-dependent, passive mechanism (82,83). High concentrations of pantothenic acid are found in the heart and kidney, compared to other organs such as the intestine and liver (84). Pantothenic acid utilization in the formation of acetyl CoA is impaired in alcoholics because the ethanol metabolite acetaldehyde inhibits the conversion (85). Erythrocytes, which carry most of the vitamin in the blood, carry it predominantly in the form of CoA. Blood and urinary levels of pantothenic acid are lower for oral contraceptive users than for nonusers (86,87). Absorption is also inhibited in the presence of known antagonists, such as ω-methylpantothenic acid, and to some extent with (*S*)-pantothenic acid. Another factor which influences vitamin absorption is a high fat diet. This condition significantly lowers the utilization of pantothenic acid in the formation of coenzyme A in the liver because of changes in lipid metabolism (88). On the other hand, high levels of protein in the diet may promote better utilization of this vitamin in the synthesis of coenzyme A (89).

NUTRITIONAL REQUIREMENTS

Pantothenic acid is widely distributed in food and because of the lack of conclusive evidence regarding quantitative needs, a recommended dietary allowance (RDA) for pantothenic acid has not been established. In 1989, the Food and Nutrition Board of the United States National Research Council suggested a safe intake of 4–7 mg/d for adults. The provisional allowance for infants is 2–3 mg daily (90).

FUNCTIONS

The most important functions of pantothenic acid are its incorporation in coenzyme A and acyl carrier protein (ACP). Both CoA and ACP/4-phosphopantetheine function metabolically as carriers of acyl groups. Coenzyme A forms high-energy thioester bonds with carboxylic acids. The most important coenzyme is acetyl CoA. Acetic acid is produced during the metabolism of fatty acids, amino acids, or carbohydrates. The active acetate group of acetyl CoA can enter the Krebs cycle and is used in the synthesis of fatty acids or cholesterol. ACP is a component of the fatty acid synthase multienzyme complex. This complex catalyzes several reactions of fatty acid synthesis (condensation and reduction). The nature of the fatty acid synthase complex varies considerably among different species (91).

TOXICITY

Pantothenic acid toxicity has not been reported in humans. Massive doses (10 g/d) in humans have produced mild intestinal distress and diarrhea. Acute toxicity was observed in case of mice and rats by using calcium pantothenate at fairly large doses (92).

USES

The bulk of the industrial supply of the calcium salt of (*R*)-pantothenic acid is used in food and feed enrichment. Food enrichment includes breakfast cereals, beverages, dietetic, and baby foods. Animal feed is fortified with calcium-(*R*)-pantothenate which functions as a growth factor.

Panthenol is used in skin care products. When applied topically to alleviate itching, it helps to keep the skin moist and supple, stimulates cell growth, and accelerates wound healing by increasing the fibroblast content of scar tissue (93,94). It is also used as a moisturizer and conditioner in hair care products. It is believed to protect hair against chemical and mechanical damage caused by perming, coloring, and shampooing (95).

ECONOMIC ASPECTS

The major producers of the calcium salt of pantothenic acid and panthenol are Hoffmann-La Roche, Daiichi, BASF, and Alps. Racemic panthenol is used mainly in hair care products, whereas (*R*)-panthenol is exclusively used in top-of-the-line, more expensive skin and hair care products. The current (1997) price of (*R,S*)-panthenol varies between $12 and $18 per kg, that of D-panthenol varies between $30 and $45 per kg, and that of D-calcium pantothenate (Calpan) varies between $22 and $30 per kg.

FUTURE PROSPECTS

Despite the progress made in the stereoselective synthesis of (*R*)-pantothenic acid since the mid-1980s, the commercial chemical synthesis still involves resolution of racemic pantolactone. Recent (1997) synthetic efforts have been directed toward developing a method for enantioselective synthesis of (*R*)-pantolactone by either chemical or microbial reduction of ketopantolactone. Microbial reduction of ketopantolactone is a promising area for future research.

BIBLIOGRAPHY

1. R. J. Williams, C. M. Lyman, G. H. Goodyear, J. H. Truesdail, and H. Holaday, *J. Am. Chem. Soc.* **55**, 2912 (1933).
2. D. W. Woolley, H. A. Waisman, and C. A. Elvehjem, *J. Biol. Chem.* **129**, 673 (1939).
3. T H. Jukes, *J. Am. Chem. Soc.* **61**, 975 (1939).
4. E. T. Stiller, S. A. Harris, J. Finkelstein, J. C. Keresztesy, and K. Folkers, *J. Am. Chem. Soc.* **62**, 1785 (1940).
5. T. Reichstein and A. Grüssner, *Helv. Chem. Acta* **23**, 650 (1940).
6. R. Kuhn and Th. Wieland, *Chem. Ber.* **73**, 962 (1940).
7. J. Baddiley and E. M. Thain, *J. Chem. Soc.* **3421** (1951); J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 1610 (1953).
8. M. L. Orr, *Home Economic Research Report* No. 36, U.S. Department of Agriculture, Washington, D.C., 1969.
9. J. H. Walsh, B. W. Wyse, and R. G. Hansen, *J. Am. Diet. Assoc.* **78**, 140 (1981).
10. J. B. Tarr, T. Tamura, and E. L. R. Stokstad, *Am. J. Clin. Nutr.* **34**, 1328 (1981).
11. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), *Pure Appl. Chem.* **59**, 834 (1987).
12. J. Marks, *The Vitamins in Health and Disease*, Little, Brown & Co., Boston, MA, 1968, pp. 121–125.
13. D. Novelli, N. O. Kaplan, and F. Lipman, *Fed. Proc.* **9**, 209 (1950).
14. O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, *Vitamine II*, Georg Thieme Verlag, New York, 1988, p. 309
15. E. H. Wilson, J. Weiglard, and M. Tischler, *J. Am. Chem. Soc.* **76**, 5177 (1954); Ger. Pat. DE 2919515 (Nov. 22, 1979), D. Bartoldus and E. Broger (to Hoffmann-La Roche Inc.).
16. O. Schnider in *Festschrift Emil Barell*, Hoffmann-La Roche, Basel, Switzerland, 1946, p. 85; E. E. Snell and W. Shive, *J. Biol. Chem.* **158**, 551 (1945); G. S. Kozlowa, T. I. Erokhina, and V. I. Gunar, *Pharm. Chem. J.* **11**, 505 (1977).
17. J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2803 (1954).
18. Ger. Pat. DE 2758883 (July 5, 1979), H. Distler and W. Goetze (to BASF); E. Glaser, *Herstellung Monatsch. Chem.* **25**, 46 (1904); Jpn. Pat. 55062080 (May 10, 1980) (to UBE Industries Ltd.).
19. S. Shimizu and H. Yamada, in T. O. Baldwin, F. M. Raushel, and A. I. Scott, eds., *Chemical Aspects of Enzyme Biotechnology*, Plenum Press, New York, 1990, p. 151.
20. Ger. Pat. DE 2404305 (Oct. 27, 1983), S. Pfohl and co-workers (to BASF AG).
21. Jpn. Pat. 02240073, (Sept. 25, 1990) S. Takeda, M. Takeda, K. Suzuki, and M. Yuya (to Fuji Chemical Industrial Co., Ltd.).
22. Ger. Pat. DE 2558508, (Sept. 7, 1976) A. Kinugasa, T. Okuda, M. Goto, and M. Saito (to Alps Pharmaceutical Industrial Co., Ltd.).
23. Jpn. Pat. 8115240 (Aug. 10, 1982) (to Sumitomo Chemical Co., Ltd.).
24. C. Fizet, *Helv. Chim. Acta.* **69**, 404 (1986).
25. Jpn. Pat. 8735340, (Aug. 23, 1988) H. Nohira, S. Yoshida, M. Nohira, K. Sato (to Daicel Chemical Industries Ltd.).
26. F. Toda, A. Sato, K. Tanaka, and T. C. W. Mak, *Chem. Lett.*, 873 (1989).
27. I. Masahiro, *Res. Inst. Yakugaku Zasshi* **96**, 71 (1976); Jpn. Pat. 47022220, (June 22, 1972) S. Nabeta, Y. Nakabe, and M. Nagaki (to Daiichi Seiyaku Co., Ltd.).
28. N. Oi, T. Doi, H. Kitahara, and Y. Inda, *J. Chromatogr.* **208**, 404 (1981); W. A. König and U. Sturm, *J. Chromatogr.* **328**, 357 (1985); E. Francotte and D. Lohmann, *Helv. Chim. Acta.* **70**, 1569 (1987); D. W. Armstrong, W. Li, C. Chang, and J. Pitha, *Anal. Chem.* **62**, 914 (1990); W.-Y. Li, H. L. Jin, and D. W. Armstrong, *J. Chromatogr.* **509**, 303 (1990).
29. T. Arai, H. Matsuda, and H. Oizumi, *J. Chromatogr.* **474**, 405 (1989); A. Takasu and K. Ohya, *J. Chromatogr.* **389**, 251 (1987).
30. K. Fuji, M. Node, M. Murata, S. Terada, and K. Hashimoto, *Tetrahedron Lett.* **27**, 5381 (1986)
31. Jpn. Pat. 04095087 A2, (Mar. 27, 1992) N. Kuroda and K. Kashiwa (to Takeda Yakuhin Kogyo K. K.).
32. Jpn. Pat. 04095086 A2, (Mar. 27, 1992) N Kuroda and K. Kashiwa (to Takeda Yakuhin Kogyo K. K.).
33. Jpn. Pat. 04095084 A2, (Mar. 27, 1992) N. Kuroda and K. Kashiwa (to Takeda Yakuhin Kogyo K. K.).
34. Jpn. Pat. 01208488 A2, (Aug. 22, 1989) H. Sato, S. Takeda, and M. Yuya (to Fuji Chemical Industrial Co., Ltd.).
35. DE 3229026 A1, (Aug. 4, 1982) K. Halbritter and M Eggersdorfer (to BASF AG) (Aug. 4, 1982).
36. J.-F. Carpentier, F. Agbossou, and A. Mortreux, *Tetrahedron Asymm.* **6**, 39 (1995).
37. K. Achiwa, T. Kogure, and I. Ojima, *Tetrahedron. Lett.* **18**, 4431 (1977).
38. T. Ojima, T. Kogure, and Y. Yoda, *Org. Synth.* **63**, 18 (1985).
39. I. Ojima, T. Kogure, and T. Terasaki, *J. Org. Chem.* **43**, 3444 (1978).
40. S. Shimizu, S. Hattori, H. Hata, and H. Yamada, *Appl. Environ. Microbiol.* **53**, 519 (1987).
41. M. Kataoka, S. Shimizu, and H. Yamada, *Agric. Biol. Chem.* **54**, 177 (1990).
42. M. Kataoka, S. Shimizu, and H. Yamada, *Recl. Trav. Chim. Pays-Bas.* **110**, 155 (1991).
43. M. Kataoka, S. Shimizu, Y. Doi, and H. Yamada, *Appl. Environ. Microbiol.* **56**, 3595 (1990).
44. R. Eck and H. Simon, *Tetrahedron Asymm.* **5**, 1419 (1994).
45. R. Kuhn and Th. Wieland, *Chem. Ber.* **75**, 121 (1942).
46. R. P. Lanzilotta, D. G. Bradley, and K. M. McDonald, *Appl. Microbiol.* **27**, 130 (1974).
47. S. Shimizu, S. Hattori, H. Hata, and H. Yamada, *Enzyme Microb. Technol.* **9**, 411 (1987).
48. H. Yamada and S. Shimizu in D. S. Clark, D. Estell, and J. Dordick, eds., *Enzyme Engineering*, Vol. 11, The New York Academy of Sciences, New York, 1992, p. 374.
49. S. Shimizu, H. Yamada, H. Hata, T. Morishita, S. Akutsu, and M. Kawamura, *Agric. Biol. Chem.* **51**, 289 (1987).
50. K. Nakamura, S.-I. Kondo, Y. Kawai, and A. Ohno, *Tetrahedron Asymm.* **4**, 1253 (1993).
51. Jpn. Pat. 05227987 A2, (Sept. 7, 1993) K. Sakamoto, H. Yamada, and A. Shimizu (to Fuji Yakuhin Kogyo KK).
52. Jpn. Pat. 06311889 A2, (Apr. 30, 1993) J. Ohno, K. Nakamura, K. Yasushi and K. Shinichi (to Kokai Tokkyo Koho); K. Nakamura, K. Shin-ichi, and O. Atsuyoshi, *Bioorganic. Med. Chem.* **2**, 433 (1994).
53. M. Paetow, H. Ahrens, and D. Hoppe, *Tetrahedron Lett.* **33**, 5323 (1992).
54. J. Kallmerten and T. Gould, *J. Org. Chem.* **51**, 1152 (1986).
55. A. V. Rama Rao, S. Mahender Rao, and G. V. M. Sharma, *Tetrahedron Lett.* **35**, 5735 (1994).
56. F. Effenberger, J. Eichhorn, and J. Roos, *Tetrahedron Asymm.* **6**, 271 (1995).

57. C. J. Chesterton, P. H. W. Butterworth, and J. W. Porter, *Meth. Enzymol.* **18A**, 371 (1970).
58. C. J. Chesterton, P. H. W Butterworth, and J. W. Porter, *Meth. Enzymol.* **18A**, 364 (1970).
59. U.S. Pat. 2441949 (May 25, 1948) S. H. Babcock (to the University of California, Berkeley, Calif.).
60. E E. Snell et al , *J. Am. Chem. Soc.* **72**, 5349 (1950).
61. J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 800 (1952).
62. D. P. Martin and D. G. Drueckhammer, *J. Am. Chem. Soc.* **114**, 7288 (1992).
63. D. S. Vallari and C. O. Rock, *J. Bacteriol.* **162**, 1156 (1985).
64. G. M Brown and J. M. Williamson, *Adv. Enzymol.* **53**, 345 (1982).
65. J. H Teller, S. G. Powers, and E. E. Snell, *J. Biol. Chem.* **251**, 3780 (1976).
66. S. Shimuzu, M. K. Qtaoka, M. C. M. Chunag, and H. Yamandu, *J. Biol. Chem.* **263**, 1207 (1988).
67. J. M. Williamson and G. M. Brown, *J. Biol. Chem.* **254**, 8074 (1979).
68. D. G. Novelli, *Physiol. Rev.* **33**, 523 (1953).
69. Y. Shigeta and M. Shichiri, *J. Vitamin.* **12**, 186 (1966).
70. Y. Abiko, S. Ashida, and M. Shimuzu, *Biochim. Biophys. Acta* **268**, 364 (1972).
71. D. Bhatia, in *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, p. 2783, B. W. Wyse, in J. Augustin, B. P. Klein, D. A. Becker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, Chapt. 16, p. 2783.
72. B. W. Wyse, C. Withwer, and R. G. Hansen, *Clin. Chem.* **25**, 108 (1979).
73. R. B. Roy and A. Buccafuri, *J. Assoc. Off. Anal. Chem.* **61**, 720 (1978).
74. J. Davidek, J. Velisek, J. Cerna, and T. Davidek, *J. of Micronutr. Anal.* **1**, 39 (1985).
75. W. A. König and U. Sturm, *J. Chromatogr.* **328**, 357 (1985).
76. K. Banno, S. Horimoto, and M. Matsuoka, *J. Chromatogr.* **564**, 1 (1991).
77. R. E. Hodges, M. A. Ohlson, and W. B. Bean, *J. Clin. Invest.* **37**, 1642 (1958).
78. M. Glusman, *Am. J. Med.* **3**, 211 (1947).
79. E. Kazuko, N. Kubato, T. Nishigaki, and M. Kikutani, *Chem. Pharm. Bull.* **23**, 1 (1975).
80. M. M. Nelson, F. Van Nouheys, and H. M. Evans, *J. Nutr.* **34**, 189 (1947).
81. D. M. Hegsted, J. J. Oelson, R. C. Mills, C. A. Elvehjem, and E. B Hart, *J. Nutr.* **20**, 599 (1940).
82. G. D. Lopaschuk, M. Michalak, and H. Tsang, *J. Biol. Chem.* **262**, 3615 (1987).
83. K. Shibata, C. J. Gross, and L. M. Henderson, *J. Nutr.* **113**, 2107 (1983).
84. D. K. Reibel, B. W. Wyse, D. A. Berkich, and J. R. Neely, *Am. J. Physiol.* **240**, H 606 (1981).
85. B. R. Eissenstat, B. W. Wyse, and R. G. Hansen, *Am. J. Clin. Nutr.* **44**, 931 (1986).
86. W. B. Bean and R. E. Hodges, *Proc. Soc. Exp. Biol. Med.* **86**, 693 (1954).
87. P. C. Fry, H. M. Fox, and H. G. Tao, *J. Nutr. Sci. Vitaminol.* **22**, 339 (1976).
88. Y. Furukawa and S. Kimura, *J. Vitamin.* **18**, 213 (1972).
89. R. W. Luecke, J. A. Hoefer, and F. Thorp, Jr., *J. Anim. Sci.* **11**, 138 (1952).
90. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
91. G. F. Combs, *The Vitamins*, Academic Press, San Diego, Calif., 1992, Chapt. 15, p. 345.
92. K. Unna and J. C. Greslin, *J. Pharmacol. Exp. Ther.* **73**, 85 (1941).
93. J. F. Grenier, M. Aprahamian, C. Genot, and A. Dentinger, *Acta Vita. et Enzy.* 4, 81 (1982).
94. M. Aprahamian, A. Dentinger, C. Stoch-Damge, J. C. Kouassi, and J. F. Grenier, *Am. J. Clin. Nutr.* **41**, 578 (1985).
95. F. Vaxman et al., *Eur Surg. Res.* **27**, 158 (1995).

GENERAL REFERENCES

H. M. Fox in L. J. Machlin, eds., *Handbook of Vitamins*, Marcel Dekker Inc., New York, 1991, pp. 429–451.

O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, *Vitamine II*, Georg Thieme Verlag, New York, 1982, pp. 309–339

S. Shimizu and Y. Yamada, in T. O. Baldwin, F. M. Raushel, and A. I. Scott, eds., *Chemical Aspects of Enzyme Biotechnology*, Plenum Press, New York, 1990, p. 151.

B. W. Wyse, in J. Augustin, B. P. Klein, D. A. Becker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, Chapt. 16.

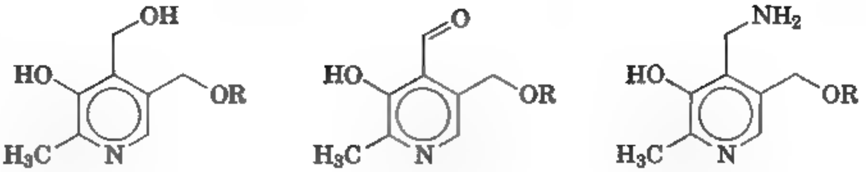
G. F. Combs, *The Vitamins*, Academic Press, San Diego, Calif., 1992, Chapt. 15, pp. 345 ff.

THIMMA R. RAWALPALLY
Hoffmann-La Roche, Inc.

VITAMINS: PYRIDOXINE (B_6)

Pyridoxine [*65-23-6*] (vitamin B_6) is the recommended IUPAC-IUB designation for a group of closely related, biologically interconvertible 3-hydroxy-2-methylpyridines which exhibit the biological activities of pyridoxine in rats (1). This group includes pyridoxine [*65-23-6*] (**1**), pyridoxal [*66-72-8*] (**2**), and pyridoxamine [*85-87-0*] (**3**) and their respective 5′-phosphates (**4–6**). In common usage, though, the term vitamin B_6 most often refers to pyridoxine hydrochloride [*58-56-0*] (**7**), the most important commercial form (Fig. 1). Contrary to previous proposals and explanations, pyridoxine is not to be used as a general name for this group of vitamers. Pyridoxine is the official name of the specific substance 5-hydroxy-6-methyl-3,4-bis(hydroxymethyl)pyridine (**1**) which usually comprises only a fraction of the total amount of vitamin B_6 in natural sources. The older term pyridoxol is seldom used.

Vitamin B_6 is widely distributed in small amounts in the tissues and fluids of nearly all living substances. Bound to enzymes as the active form, pyridoxal phosphate (**2**), it acts as a coenzyme for a number of biochemical conversions of amino acids important to general cellular energy supply, to growth, and to specific organ functions. Deficiency in animals leads to a variety of symptoms ranging from mild (dermatitis, loss of appetite, irritability, and muscular weakness) to serious (anemia, weight loss, and



R			
R = H	(1)	(2)	(3)
R = OPO_3H_2	(4)	(5)	(6)
R = H HCl salt	(7)		

Figure 1. Forms of vitamin B$_6$.

nerve degradation leading to convulsive seizures) and even death. Vitamin B$_6$ requirements depend on protein intake and other factors. Meats, cereals, beans, and nuts are rich sources. Bioavailability and stability vary with the food sources and methods of preparation and storage. As a preventive measure, pyridoxine hydrochloride, the most stable form, is used in food, feed, and nutrient supplements. Pyridoxine is also used therapeutically for treatment of deficiency conditions and other diseases. No natural sources are rich enough, nor is biosynthetic understanding sufficiently advanced, to allow cost-effective production from biological sources. All commercially available pyridoxine hydrochloride is manufactured by chemical processes practiced at fine chemical scale.

Vitamin B$_6$ was found in the 1940s during the unraveling of the mysteries of the nitrogen-base B vitamin complex. A diet-induced pellagra-like condition in rats was shown to be cured by a water-soluble base, later differentiated from the known vitamins B$_1$ and B$_2$ and named vitamin B$_6$ by Gyorgy in 1934. Several research groups undertook isolation and characterization of this substance and five different laboratories announced this achievement in 1938. The chemical structure of pyridoxine, the form first isolated, was correctly identified and reported along with two independent syntheses in 1939 (2,3). Snell showed microorganisms that could not use pyridoxine were satisfied with the natural vitamin B$_6$ extracts. This led to the discovery and synthesis of the vitamers pyridoxal (**2**) and pyridoxamine (**3**) in 1944. Subsequent clinical and biochemical investigations have shown the vitamin B$_6$ complex has therapeutic and prophylactic uses for treatment of a number of diseases. Commercial production of pyridoxine hydrochloride began in the 1940s. Production rose rapidly to more than 3000 metric tons per year worldwide.

CHEMICAL AND PHYSICAL PROPERTIES

Physical and Chemical Characterization

Ultraviolet absorptions of the B$_6$ vitamers vary with pH and the substituent at position 4. As hydroxypyridines, the B$_6$ vitamers show strong fluorescence (excitations at 310–330 nm, emissions at 365–425 nm, depending on structure and pH) which are useful in their detection (4,5). Ir and ^1H, ^{13}C, and ^{15}N nmr show the expected effects of N-protonation, hydrogen bonding, and exchange of the acidic phenolic proton (6–8). In mass spectrometry (ms), normal electron impact ionization is sufficient to observe the molecular ion and an intense peak for a quinone—methide fragment from loss of water at the 4-hydroxymethyl position (9). Thermospray hplc/ms at picogram sample levels showed particularly strong molecular ions for pyridoxine and derivatives (10).

Reactions and Stability

The B$_6$ vitamers show weakly basic and acidic properties (4) and undergo reactions expected of their functionalities (11,12). Acidity constants are reported in Table 1. Pyridoxine gives the characteristic reactions of phenols such as color tests, ether formation, diazo coupling, etc. Ketalization or acetalization of pyridoxine gives the thermodynamically more stable six-membered ring products involving the 3- and 4-positions. Esterification gives first mainly the 4,5-diester, then the triester with excess acylating agent. Pyridoxal and pyridoxamine are phosphorylated on the 5′-position with reagents such as phosphorus pentoxide in phosphoric acid or metaphosphoric acid. Pyridoxine requires prior protection of substituents at positions 3- and 4- as a ketal to allow selectivity. In biological systems, glycosylation occurs at the 5′-hydroxyl.

The B$_6$ vitamers are labile. Pyridoxine is the most stable and pyridoxal the least. The reactions of the substituents at the 4-carbon dominate. On mild oxidation, pyridoxine is converted to pyridoxal (**2**), which is in equilibrium with its hemiketal [*17281-92-4*] (**8**) (13). Air oxidation gives pyridoxic acid [*82-82-6*] (**9**), an intensely blue fluorescent substance of value for quantitation (4). Heating pyridoxine with ammonia converts it to pyridoxamine (**3**) and with alcohols to the 4-ethers (13). Pyridoxine, pyridoxal, and pyridoxamine are relatively stable in acid when cold or heated at 100°C for short periods. Prolonged heating of pyridoxine in hydrochloric acid gives the 4,5-bis-chloromethyl analogue, the 4-chloromethyl group of which is preferentially hydrolyzed. Heating pyridoxine in neutral solution in the absence of good nucleophiles gives dimeric products (14). Some of these reactions are readily rationalized by an intermediate quinone—methide (**10**) (Fig. 2).

Pyridoxal and its 5′-phosphate undergo typical aldehyde reactions, such as aldol condensations, bisulfite addition, etc (4,11,12). Both form imines, assisted by the electronegative 2-methylpyridine ring and the acidic 3-hydroxyl. Extensive kinetic and thermodynamic data on imine formation in model systems are available (4). The epsilon-amino group of a lysine unit is usually involved in the active sites of enzymes but other groups can bind pyr-

Table 1. Properties of Pyridoxine, Pyridoxamine, Pyridoxal, and Derivatives

Substance	Structure number	CAS registry number	Formula	Mol wt	Form	Mp in water (°C)	Solubility (g/100 mL)	p*K* Values
Pyridoxine	(1)	*[65-23-6]*	$C_8H_{11}NO_3$	169.18	Needles	160	Soluble	5.0, 9.0
Pyridoxine HCl	(7)	*[58-56-0]*	$C_8H_{12}ClNO_3$	205.64	Platelets	204–206 dec	22	
Pyridoxine-5′-PO_4	(4)	*[447-05-2]*	$C_8H_{12}NO_6P$	249.16	Needles	212–213 dec	Soluble	5.0, 9.4
Pyridoxamine	(3)	*[85-87-0]*	$C_8H_{12}N_2O_2$	168.20	Crystals	193	Soluble	3.4, 8.1, 10.5
Pyridoxamine di-HCl		*[524-36-7]*	$C_8H_{14}Cl_2N_2O_2$	241.12	Platelets	226–227 dec	50	
Pyridoxamine-5′-PO_4	(6)	*[529-96-4]*	$C_8H_{13}N_2O_5P$	248.18			Soluble	3.5, 5.8, 8.6, 10.8
Pyridoxal	(2)	*[66-72-8]*	$C_8H_9NO_3$	167.16			50	4.2, 8.7, 13
Pyridoxal HCl		*[65-22-5]*	$C_8H_9NO_3$	203.63	Rhombic	173 dec	50	
Pyridoxal-5′-PO_4	(5)	*[54-47-7]*	$C_8H_{10}NO_6P$	247.14			Soluble	3.5, 8.4

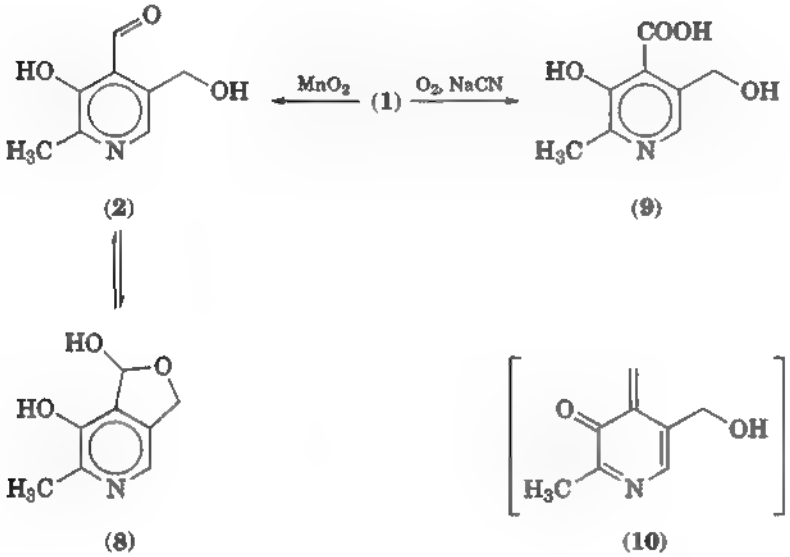


Figure 2. Reactions of pyridoxal (**2**).

idoxal during thermal processing and storage, reducing bioavailability (4,15). Hydroxylamines and hydrazine derivatives, such as isoniazid, react and cause enzyme inhibition (4). Imine formation with pyridoxal is used as a tool for selective modification of proteins in biochemical studies (4).

Reactions of imines and their tautomers are the basis of the important biochemistry of pyridoxal 5′-phosphate (**5**), the coenzyme form for over 100 enzymatic reactions. Reaction with an epsilon-amino group of a lysyl residue and ionic interaction of the 5′-phosphate binds pyridoxal to the enzyme in the resting state. Displacement of lysine by a substrate amino group initiates a sequence of facile equilibrations of the aldimine, ketimine, and enamine forms, along with elimination and addition reactions which rationalize the various conversions and products. Known reactions involving the alpha-, beta-, and gamma-carbons of the amino acid and their substituents include decarboxylations, transaminations, racemizations, aldol and retroaldol reactions, eliminations, and hydration reactions (4) (Fig. 3). Many of these transformations have been modeled *in vitro* in chemical studies. Some of this chemistry can cause covalent bonding of a substrate to other reactive residues in the active site, resulting in irreversible "suicide inactivation" of the enzyme. This is a mechanism of action of some naturally occurring toxins and a protocol in rational drug design (4).

Pyridoxine hydrochloride is stable if kept dry or in acidic solution. Its aqueous solutions may be heated for 30 minutes at 120°C without decomposition (16). Losses have been reported for mixtures of the vitamers stored in solution at room temperature or below unless they are kept acidic (17). Decomposition in foods on processing and storage depends mainly on pH, moisture, and temperature. First-order kinetics are often followed and stabilities can be estimated using Arrhenius calculations (4,18). All B_6 vitamers are susceptible to decomposition by near-uv light to give reddish brown materials, especially in solution at neutral or higher pH. Pyridoxal and pyridoxamine decompose most rapidly, even in acid (18,19). Gamma irradiation of food can also cause losses (20). Hydroxyl radical formed from ascorbate gives the 6-hydroxyl derivative of pyridoxine. This process may occur during the storage of fruits and vegetables containing high ascorbate levels (21,22).

Salts and Derivatives

Generally the B_6 vitamers are high melting crystalline solids that are very soluble in water and insoluble in most other solvents. Properties of the common forms are listed

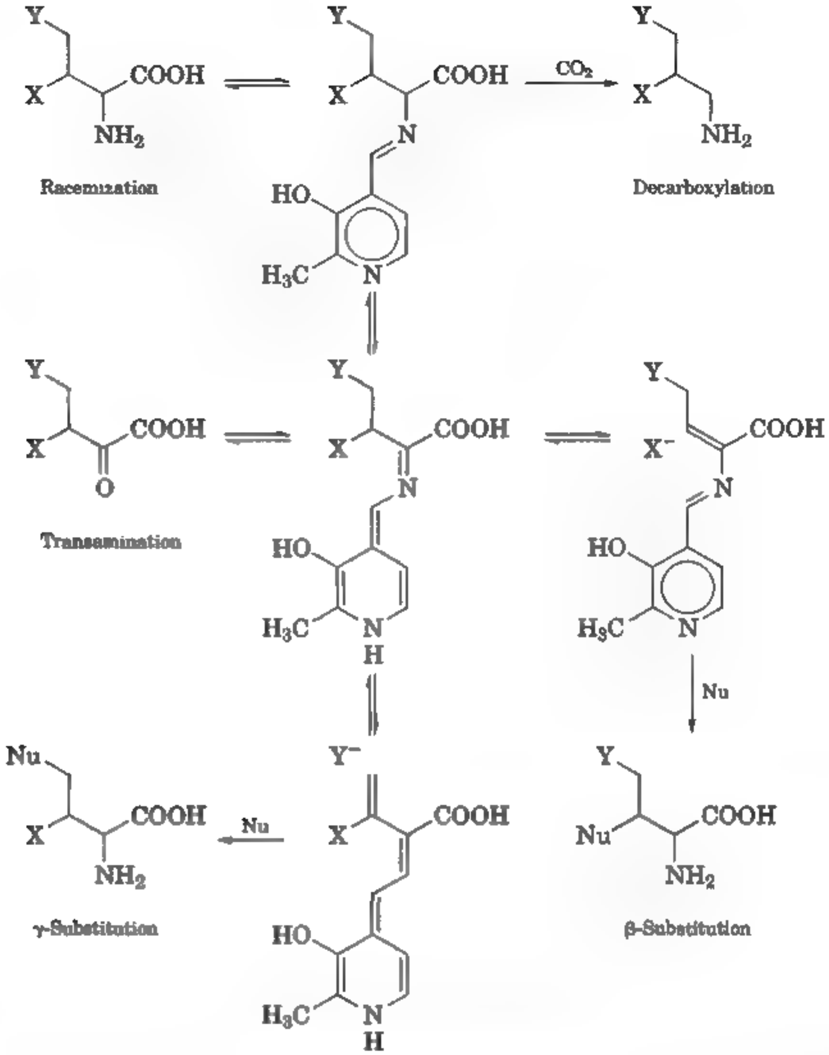


Figure 3. Reactions of imines and their tautomers.

in Table 1. The only commercially important form of vitamin B_6 is pyridoxine hydrochloride (7). This odorless crystalline solid is composed of colorless platelets melting at 204–206°C (with decomposition). In bulk, it appears white and has a density of ~0.4 kg/L. It is very soluble in water (ca 0.22 kg/L at 20°C), soluble in propylene glycol, slightly soluble in acetone and alcohol (ca 0.014 kg/L), and insoluble in most lipophilic solvents. A 10% water solution shows a pH of 3.2. Both the hydrochloride and corresponding free base sublime without decomposition (16).

OCCURRENCE

Vitamin B_6 occurs in the tissues and fluids of virtually all living organisms, attesting to its essential role in amino acid biochemistry. Pyridoxal phosphate bound to protein is the main form in animals. Pyridoxine, pyridoxamine, and their phosphates are the usual forms in plants (23,24). Significant percentages in plants occur as pyridoxine glycosides, which are substantially less bioavailable (23,25,26). Nearly all foods in a typical human diet contain detectable amounts of the B_6 vitamers. Relatively rich sources are edible yeast, meats (especially salmon and calf liver), whole grain cereals, legumes, and nuts (qv). Cheeses with extensive mold growth are also good sources. Broccoli, cauliflower, spinach, corn, potatoes, bananas, and raisins are good sources, but vegetables and fruits in general are not (24) (Table 2).

Table 2. Average Vitamin B_6 Contents of Raw Foods, mg/100 g[a]

Food	Vitamin B_6	Food	Vitamin B_6
Beef liver	0.84	Brown rice	0.55
Beef	0.33	White rice	0.17
Chicken	0.33–0.68	Whole wheat flour	0.34
Pork	0.35	White flour	0.06
Fish	0.17–0.43	Beans	0.56–0.81
Eggs	0.11	Peanuts	0.40
Whole milk	0.04	Corn	0.25
Bananas	0.51	Potatoes	0.25
Raisins	0.24	Vegetables, green	0.15–0.28
Other fruits	0.02–0.07	Tomatoes	0.10

[a] By microbial assay.

As vitamin B_6 is mainly located in the germ and aleurone layer in cereal grains; polishing for the production of flour removes a substantial portion. White bread is therefore a poor source unless fortified. Some nonedible yeasts contain up to 38 mg/100 g dry weight vitamin B_6, the highest level of the natural sources (4,27). As a rule, these amounts are too low for cost-effective isolation.

BIOCHEMICAL AND PHYSIOLOGICAL FUNCTIONS

Because vitamin B_6 is widely distributed in nature, severe deficiency symptoms are seldom observed in humans with adequate diets. However, there is little doubt vitamin B_6 is essential in human and animal nutrition. As enzyme cofactors, pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate are required for important biotransformations of amino acids (4). Decarboxylations of amino acids give rise to a number of important amines, among them the vasodilator histamine, the vasoconstrictor and neurotransmitter serotonin, and the major neurotransmitters gamma-aminobutyric acid, epinephrine, norepinephrine, tyramine, and dopamine. Transamination interconverts specific pairs of amino acids and their corresponding ketoacids and amino groups and aldehydes, key functions in amino acid biosynthesis and catabolism. Racemization provides D-amino acids, constituents of the cell walls of bacteria. Other important transformations requiring pyridoxal include bioconversions of tryptophan (involved in the biosynthesis of the vitamin niacin), serine transhydroxymethylation (a key step in one carbon metabolism leading to nucleic acid synthesis), glycogen phosphorylation (in liver and muscle-maintaining glucose supplies), biosynthesis of delta-aminolevulinic acid (a precursor to heme synthesis), and key steps in the metabolism of sulfur-containing amino acids. Other roles of pyridoxal and its phosphate include binding to hemoglobin (affecting oxygen binding affinity), effects on the conversion of linoleic acid to arachidonic acid, possible effects on cholesterol and fatty acid metabolism, and modulation of steroid action. As a result, vitamin B_6 levels affect gluconeogenisis, nervous system activity, red cell formation and metabolism, immune function, and hormone functions (4,23).

A large variety of biochemical lesions are found in deficiency (23). Depressed antibody, nucleic acid, and protein synthesis are characteristic. Consequences of vitamin B_6 deficiency in humans and many animals include scaling dermatitis (especially around the eyes, nose, and mouth), anemia, loss of appetite, poor growth or weight loss, muscular weakness, central nervous system changes such as irritability or depression, kidney stones, and abnormalities seen by electroencephalography. In severe cases, nerve degradation leading to convulsive seizures and death can occur. Possible vitamin B_6 deficiency must be assessed; estimation of dietary intake is not sufficient. An older method measures the level of pyridoxal-dependent metabolite xanthurenic acid in the urine after ingestion of a large dose of tryptophan. Improved methods measure pyridoxal phosphate in plasma or pyridoxic acid excretion in urine by sensitive chromatographic procedures (23).

Humans cannot biosynthesize vitamin B_6 and must obtain it from dietary sources. Ruminant animals derive some benefit from microfloral supply in the stomach. All animals interconvert the six major forms (**1–6**). Thus, requirements of the enzyme cofactor forms can be maintained by ingestion of pyridoxine only. The bioavailability of vitamin B_6 in foods is still an active research area with some unresolved issues (4,15,23). Reported levels of availability vary widely. Earlier studies suggest processing, storage, and preparation could lead to losses of up to 80% of vitamin B_6, depending on conditions. Irreversible reductive binding to lysyl residues is reported to give inactivity or even antivitamin effects (15,28). One study estimates bioavailability of vitamin B_6 from foods representing the average North American human diet at ca 70–80% (29).

In humans, vitamin B_6 in phosphorylated forms is mostly unavailable until hydrolyzed, then passively absorbed in the small intestine. The free vitamins are interconverted and supplied to the circulation by the liver, then enter cells by simple diffusion and are phosphorylated. A typical adult pool of pyridoxal is estimated to be about ≥25 mg, most of it bound to enzymes in muscle (23). Small amounts of all forms are excreted in the urine. Most is oxidized in the liver to the main urinary metabolite 4-pyridoxic acid (**9**), the corresponding diacid, and ring-cleaved fragments (4,30).

Human requirements for vitamin B_6 parallel protein metabolism, with increased intake of protein requiring higher intake of the vitamin. As little as 0.010–0.015 mg of vitamin B_6 per gram of protein prevents deficiency signs with adequate protein intake. Slightly higher intake maintains acceptable metabolite excretion and plasma vitamin levels. The Recommended Daily Allowance (RDA) for vitamin B_6 assumes consumption of twice the recommended amount of protein (Table 3) (31). Allowances for pregnant and lactating women reflect increased protein needs. Low levels in breast milk place infants at risk for deficiency (4,23).

The typical U.S. daily diet contains 1.1–3.6 mg of vitamin B_6, most coming from meats and vegetables. Poor diets may provide less than half of these amounts and less than the RDA. Some populations require higher amounts: persons with high protein intakes, pregnant and lactating women, users of oral contraceptives, alcoholics, users of drugs which interfere with vitamin B_6 function, and those afflicted with some diseases. Several reviews have examined the relationship of vitamin B_6 and specific diseases in more detail (4,23).

MANUFACTURE

Pyridoxal (**2**) can be prepared by oxidation of pyridoxine (**1**) (13). Pyridoxamine (**3**) can be made by reduction of pyridoxal oxime (32). Only pyridoxine (**1**), the most stable of

Table 3. U.S. RDA for Vitamin B_6

Age	RDA (mg)
Infants and children <4 yr	0.7
Adults and children >4 yr	2.0
Pregnant or lactating women	2.5

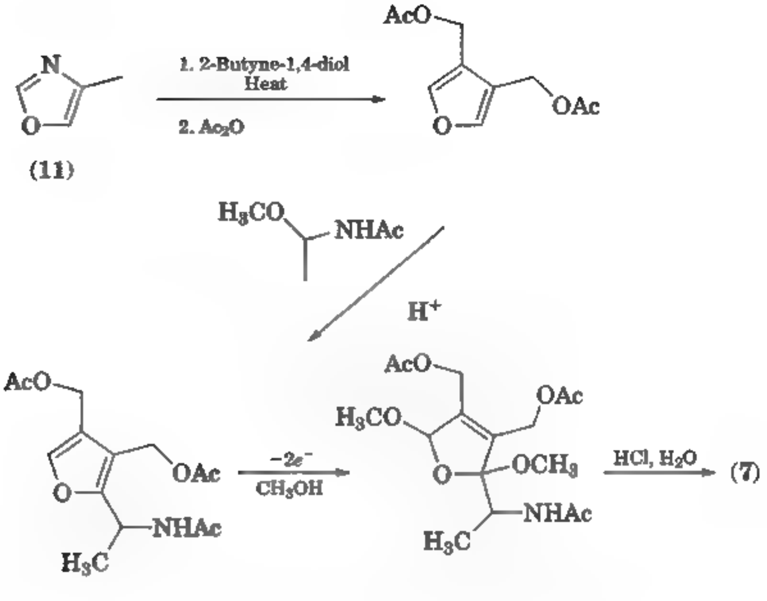


Figure 4. Furan route to pyridoxine.

the three, is manufactured. The earliest syntheses, by degradation of isoquinoline rings, are only of historical interest. The first syntheses to be applied on a large scale relied on classical condensation reactions. Many variations were explored during the 1940s and 1950s and some formed the basis of early production. Such routes required numerous steps giving low overall yields, in part due to difficulties of obtaining the correct oxidation levels of the 4- and 5-substituents. These methods have been reviewed (11,12). In the mid-1950s, electrooxidation of furan derivatives was shown to provide direct precursors to hydroxypyridines (33). In later improvements, cycloaddition of readily available 2-butyne-1,4-diol to 4-methyloxazole [*693-93-6*] (**11**) followed by amidoalkylation conveniently provided the furans (34,35). The overall yields of these linear sequences are modest (Fig. 4).

The current paradigm for B_6 syntheses came from the first report in 1957 of a synthesis of pyridines by cycloaddition reactions of oxazoles (36) (Fig. 5). This was adapted for production of pyridoxine shortly thereafter. Intensive research by Ajinomoto, BASF, Daiichi, Merck, Roche, Takeda, and other companies has resulted in numerous publications and patents describing variations. These routes are convergent, shorter, and of reasonably high throughput.

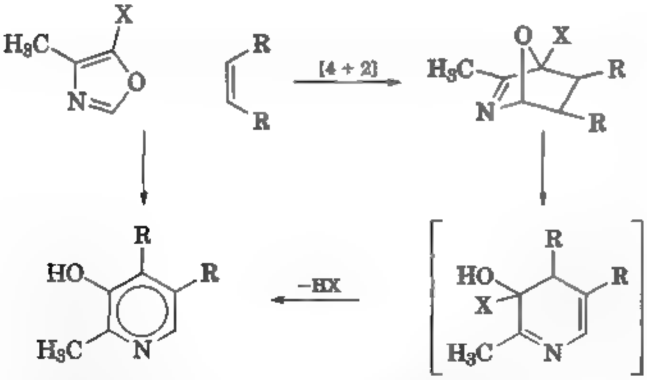


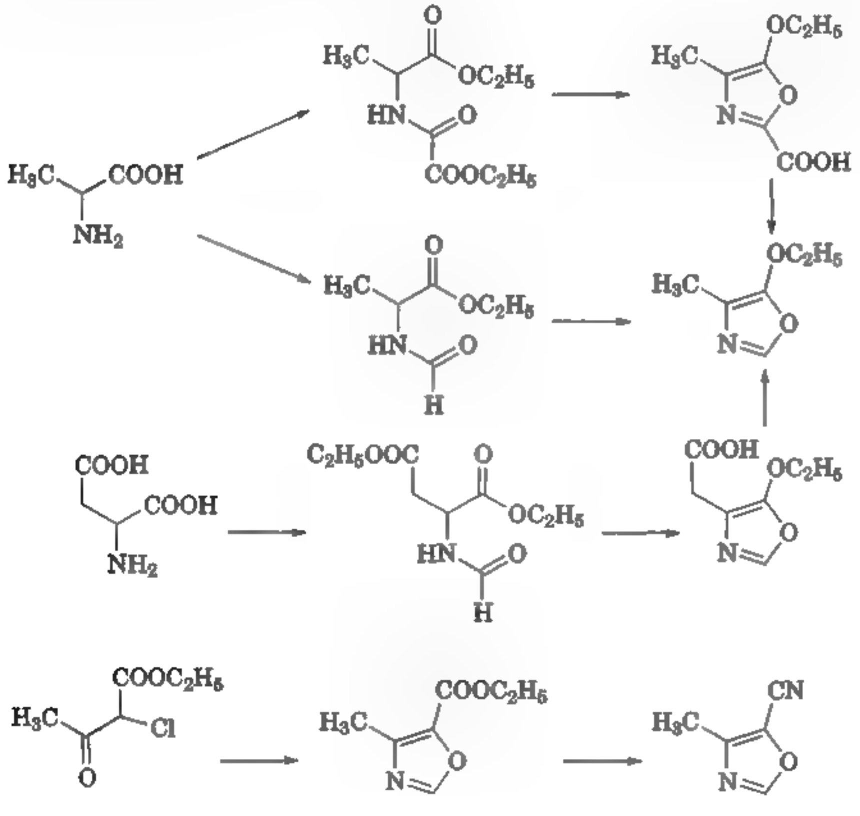
Figure 5. Synthesis of pyridines by cycloaddition reactions of oxazoles.

Substitution on the azadiene and alkene affect reactivity and only certain pairs react efficiently. Electrophilic alkenes and oxazoles bearing donor groups generally allow faster reactions at lower temperatures (37,38). Steric effects play a lesser role. Earlier variations with 4-methyloxazole (**11**) and maleic acid derivatives require the presence of oxidants such as hydrogen peroxide or nitroarenes to aromatize the cycloadducts in order to obtain good yields (11). Subsequent costly reduction steps are also needed to correct the oxidation level of the product side chains. One advantage is the use of leaving groups on one of the cycloaddition partners to facilitate aromatization without loss of the product 3-hydroxyl group. These superfluous groups can be added at the 5-position of the oxazoles or on the alkene.

Oxazoles preferred in practice bear 5-ethoxy- or 5-cyanosubstituents. Other alkoxy (39–42), trimethylsilyloxy (43), alkythio (44), amino (45), and carboalkoxy (46) substituents are reported. Processes using 5-ethoxyoxazoles (**12**), (**13**), and (**14**) have been published (38,47–60). Extra carbons carried by the Takeda, Chinese, and Ajinomoto intermediates are lost as waste by decarboxylation. A process with 5-cyano-4-methyloxazole (**15**) has been disclosed by Roche (59). Reactions of these azadienes with butenediol derivatives, the usual alkene partner, take place at higher temperatures (115–180°C) and require longer reaction times (10–20 h) than those with maleate partners. The ethoxy compound (**12**) reacts under milder conditions and the adduct is isolable. Mild acid converts it to the product and ethanol. With the higher temperatures required of the cyano compound [*1003-52-7*] (**15**), the intermediate cycloadduct is converted directly to the product by elimination of waste hydrogen cyanide. Often the reactions are run with neat liquid reagents having an excess of alkene as solvent. Polar solvents such as sulfolane and *N*-methylpyrrolidone are claimed to be superior for reactions of the ethoxy compound with butenediol (53). Organic acids, phenols, maleic acid derivatives, and inorganic bases are suggested as catalysts (51,52,54,59,61,62) (Fig. 6).

As the alkene partners, (*Z*)-2-butene-1,4-diol acetals, ketals, esters or ethers (11), and a polymer-bound acetal (63) are described. Butenediol may be used if polar solvents are employed (54). Current manufacturers generally use the cyclic acetal [*5417-35-6*] (**16**) of butenediol with isobutyraldehyde, a liquid of conveniently high boiling point that readily allows the product to be deprotected with no waste (Fig. 7). Butenediol is readily and cheaply available from the reaction of acetylene and formaldehyde to 2-butyne-1,4-diol, then catalytic hydrogenation. In an unusual variant of this cycloaddition approach, a leaving group on the alkene facilitates aromatization. Cyclic sulfone [*41409-84-1*] (**17**) paired with 4-methyloxazole (**11**) effectively gives the pyridine nucleus (64,65) taking advantage of the increased reactivity of an electrophilic alkene while maintaining the correct oxidation level of the side chains. Electrophilic dihydrofuran [*332-77-4*] (**18**) reacts efficiently with excess ethoxymethyloxazole (**12**), also at lower temperatures (66).

Ethoxyoxazole (**12**) is constructed from *N*-formyl alanine esters (67) and ethoxyoxazole acid (**13**) from *N*-oxalyl alanine esters (55–58). Alanine is readily available from China by chlorination of propionic acid, then ammonation. Both ethoxyoxazole (**12**) and the acid (**14**) are derived from maleic anhydride by *N*-formyl aspartate esters (52,68). Cyanooxazole (**15**) is derived from ketene by ethyl chloroacetoacetate and formamide (69) or, alternatively, from diketene (70). All routes require dehydration of amides as a critical step. Traditionally these dehydrations have been accomplished with phosphorus pentoxide, phosphoryl chloride, or phosgene; however, more environmentally acceptable procedures use acetic anhydride (71), cyanuryl chloride (72), or gas-phase catalytic dehydration (73). In some cases, the oxazoles are generated *in situ* in the cycloaddition reactions from *N*-formyl compounds by the isonitriles under dehydrating conditions (45,74,75). Other cycloaddition routes include reactions of 1,2,4-triazines with butynediol or enamine derivatives give the pyridoxine nu-



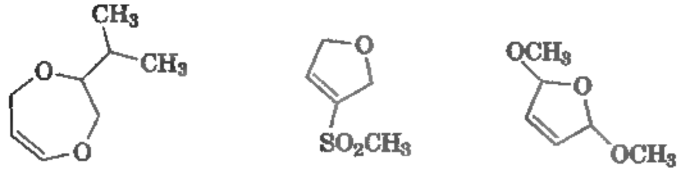
(**13**)

(**12**)

(**14**)

(**15**)

Figure 6. Process chemistry for oxazoles.



(**16**) (**17**) (**18**)

Figure 7. Dienophiles.

cleus (76,77), or a cobalt-catalyzed [2 + 2 + 2] cycloaddition of acetylenic ethers with acetonitrile (78,79) (Fig. 8).

ECONOMIC ASPECTS

Significant producers include Daiichi, Hoffmann-La Roche, Takeda, and several factories in mainland China. Takeda and Daiichi practice processes based on alanine by ethoxyoxazole (**12**), at least one Chinese producer from alanine by oxazole acid (**13**), and Roche from ketene via cyanooxazole (**15**). Production is practiced at many hundreds of metric tons annually by batchwise or semicontinuous operation in automated glass and stainless steel equipment typical of fine chemical manufacture. World production in 1995 was estimated at about 3000 MT with sales prices in the United States in the range of \$22–\$30 per kilogram.

PRODUCT SPECIFICATIONS AND TESTING

Nutrients and diet supplements without claims of therapeutic effects are considered foods, and are thus regulated by the U.S. Food and Drug Administration. These are further subject to specific food regulations. Specifications for pyridoxine hydrochloride (**7**) for foods are given in the *Food Chemicals Codex* (80) and for pharmaceuticals in the *U.S. Pharmacopeia* (81). General test methods have been summarized (82).

SAFETY AND HANDLING

Pyridoxine hydrochloride is typically packaged in standard 20–25 kg polylined fiber cartons or drums. Smaller packing sizes are also available. Pyridoxine hydrochloride is kept under normal dry storage conditions not to exceed 25°C and protected from light. It is listed in the TSCA inventory and material safety data sheets are available from suppliers. Protection against dust exposure to the eyes, skin, or lungs (IOEL of 2.0 mg/m^3 time weighted average) and fire and dust explosion are indicated. NPFA ratings for health, fire, and reactivity are 1, 2, and 1. Neither national nor international transportation is regulated. The U.S. EPA has not established reportable quantities for environmental releases. The acute LD_{50} (rat oral) of 7750 mg/kg classifies this material as practically nontoxic orally (83), Oral doses of 100–300 mg/d (50–150 times the RDA) have been used in therapeutic studies without adverse effects. Excess amounts are readily cleared by the kidney. More recent studies show that long-term consumption of very large doses can lead to sensory nerve damage. Supplements of more than 200 mg/d should be taken only under medical supervision. Intravenous administration is not associated with toxicity (84).

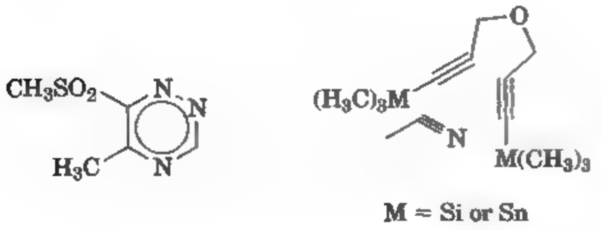


Figure 8. Other cycloaddition routes.

ANALYTICAL METHODS

Determining vitamin B_6 in foods and tissues is complicated by the number of forms, typical low levels, varying degrees of ionization, lability to heat, light, and base, interconversion, and binding to proteins and glucose. Colorimetric, fluorometric, microbiological, animal, and chromatographic methods are used and have been reviewed in depth (5,11,85,86). Few are fully suitable for simultaneous analysis of all six bioactive forms at the typical low levels. Colorimetric assays involve derivatization, are relatively insensitive, and used only for assay of high potency samples such as premixes. Microbiological assays have previously been the most commonly used, especially for foods. The yeast *Saccharomyces uvarum* (ATCC 9080, also known as *S. carlsbergensis* 4228) responds well to pyridoxine and pyridoxal, less to pyridoxamine, and not at all to the phosphate forms. Hydrolysis is first necessary; by the official AOAC method, the matrix is autoclaved with hydrochloric acid (87). Such methods are slow and overestimate bioavailability. Animal assays in vitamin B_6-deficient chicks or rats are time consuming and expensive but have been useful in determining bioavailability of derivatives and related materials.

Increasingly, chromatographic methods are used for their ease, accuracy, and ability to determine all forms simultaneously. Gas-liquid chromatography is hampered by the polarity of the compounds unless silyl or acyl derivatives are used (5,88). The comprehensive method of choice generally is high pressure liquid chromatography (HPLC) (5). Separations by ion exchange or reverse-phase absorption with ion-pair reagents and isocratic or gradient mobile phases are common. Direct uv detection is less sensitive but useful for premixes and vitamin mixtures. Post-column derivatization and fluorescence detection allows measurement to nanogram levels. High performance capillary zone electrophoresis (cze) coupled with fluorescence detection allows measurement to below picogram levels (89). Gc/ms, hplc/ms, and cze/ms hold promise for measurement and identification of femtomole levels of vitamers and metabolites (90–92). Immunoassay (qv) method are being developed (4).

BIOSYNTHESIS

Almost all microorganisms synthesize B_6 vitamers intracellularly at levels of 0.05–0.3 mg/g dry weight of cells and excrete at levels of 0.05–0.5 mg/L. Biosynthesis in *E. coli* is known to be controlled by both feedback inhibition and repression. Some organisms can overproduce, for example *B. subtilis*, up to 2.0–5.0 mg/L, *Flavobaterium sp* 238-7 up to 20 mg/L, and yeast *Pichia guilliermondi* up to 25 mg/L (4,93). The mechanisms of vitamin B_6 biosynthesis are

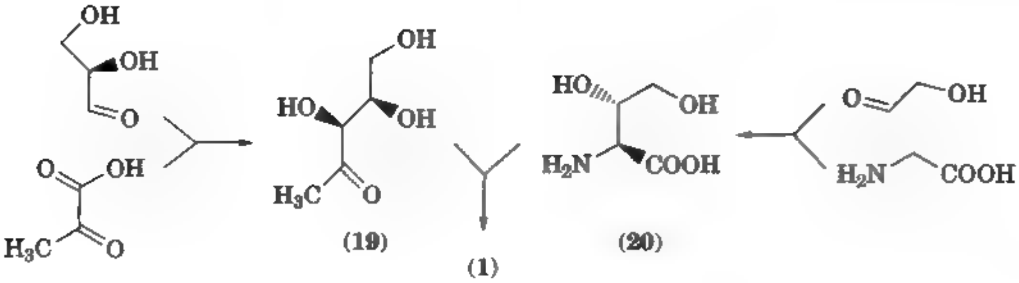


Figure 9. Biosynthetic pathway to pyridoxine.

partly understood. Extensive isotopic label feeding studies with *E. coli* mutants show all carbons are derived from glucose and the nitrogen from glycine. Union of the intermediates 1-deoxy-D-xylulose [*16709-34-9*] (**19**) and 4-hydroxy-L-threonine [*21768-45-6*] (**20**) gives pyridoxine in several steps, only two of which require enzymatic catalysis (Fig. 9) (94). In aerobic bacteria, pyridoxal phosphate is made as needed by the action of oxidase or dehydrogenase enzymes on pyridoxine phosphate or pyridoxamine phosphate (4,93). The pathways in *Flavobacterium* sp 238-7 (95) and in *Saccharomyces cerevisiae* (96) may differ from that in *E. coli* in the early steps. In microorganisms, some genes and related enzymes for biosynthesis have been identified (94,97–99). Significant additional progress will be necessary before cost-effective production of vitamin B_6 by bioprocesses is possible.

ANALOGUES AND ANTAGONISTS

Over 250 analogues of the B_6 vitamers have been reported (11,100). Nearly all have low vitamin B_6 activity and some show antagonism. Among these are the 4-deshydroxy analogue, pyridoxine 4-ethers, and 4-amino-5-hydroxymethyl-2-methylpyrimidine, a biosynthetic precursor to thiamine. Structurally unrelated antagonists include drugs such as isoniazid, cycloserine, and penicillamine, which are known to bind to pyridoxal enzyme active sites (4).

USES

The primary uses of pyridoxine hydrochloride are in multivitamin supplement tablets and for fortification of human food and animal feed, especially for poultry and pigs. Most breakfast cereals and infant formulas in the United States are supplemented. Lesser amounts are used therapeutically to correct deficiencies or to treat specific disorders. Pyridoxine hydrochloride has been used experimentally to treat a variety of conditions with varying degrees of effectiveness (4,23). Pyridoxine hydrochloride is readily incorporated into premixes and foods.

BIBLIOGRAPHY

1. Commission on Biological Nomenclature, *Biochem. J.* **137**, 417 (1974).
2. S. A. Harris and K. Folkers, *J. Am. Chem. Soc.* **61**, 1245 (1939).
3. R. Kuhn et al., *Naturwissenschaften* **27**, 469 (1939).
4. D. Dolphin, R. Poulson, and O. Avramovic, eds., *Coenzymes and Cofactors*, Vols. 1A & 1B, John Wiley & Sons, Inc., New York, 1986.
5. J. Ubbink, in A. P. De Leenheer, W. Lambert, and H. Nelis, eds., *Modern Chromatographic Analysis of Vitamins*, 2nd ed., Marcel Dekker, New York, 1992, Chapt. 10.
6. W. Korytnyk and R. P. Singh, *J. Am. Chem. Soc.* **85**, 2813 (1963).
7. I. D. Spenser et al., *J. Biol. Chem.* **262**, 7463 (1987).
8. A. Lycka and J. Cizmarik, *Pharmazie* **45**, 371 (1990).
9. W. Korytnyk et al., *J. Am. Chem. Soc.* **88**, 1233 (1966).
10. J. Iida and T. Murata, *Anal. Sci.* **6**, 273 (1990).
11. O. Isler, G Brubacher, S. Ghisla, and B. Kraeutler, eds., *Vitamine II*, Verlag, New York, 1981, pp. 193–227.
12. J. M. Osbond, *Vitamins and Hormones* **22**, 367 (1964).
13. K. Folkers et al., *J. Am. Chem. Soc.* **73**, 3430 (1951) and cites therein.
14. S. A. Harris, *J. Am. Chem. Soc.* **63**, 3363 (1941).
15. *Nutr. Rev.* **36**, 346 (1978)
16. *The Merck Index*, 11th ed., Merck & Co., Rahway, N.J., 1989, p. 1269.
17. G. S. Shephard and D. Labadarios, *Clin. Chim. Acta* **60**, 307 (1981).
18. B. Saida and J. Warthesen, *J. Agric. Food Chem.* **31**, 876 (1983).
19. C. Y. W. Ang, *J. Assoc. Official Anal. Chem.* **62**, 1170 (1979).
20. A. R. Forester and I. G. Davidson, *Radiat. Phys. Chem.* **36**, 403 (1990).
21. K. Tadera et al., *Agric. Biol. Chem.* **52**, 2359 (1988).
22. M. L. Hu et al., *Chem. Biol. Interact.* **97**, 63 (1995).
23. J. E. Leklem, in L. J. Machlin, ed., *Handbook of the Vitamins*, 2nd ed., Chapt. 6, Marcel Dekker, New York, 1991.
24. M. L. Orr, *Pantothenic Acid, Vitamin B6 and Vitamin B12 in Foods*, Home Economics Report No. 36, U.S. Dept. of Agriculture, Washington, D.C., 1969.
25. J. F. Gregory and D. B Sartain, *J. Agr. Food Chem.* **39**, 899 (1991).
26. K. Tadera and Y. Naka, *Agr. Biol. Chem.* **55**, 563 (1991).
27. K. D. Lunan and C. A. West, *Arch. Biochem. Biophys.* **101**, 262 (1963).

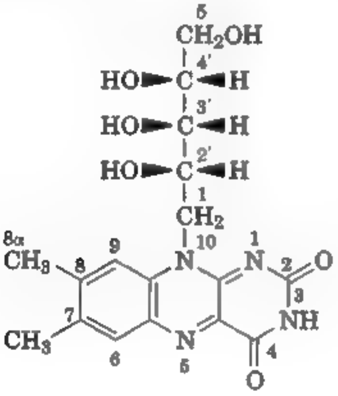
28. J. Gregory et al , *J Food Sci.* **51**, 1345 (1986).
29. J B. Tarr, T Tamura, and E. L. R. Stokstad, *Am. J. Clin. Nutr.* **34**, 1328 (1981).
30. A. H. Merrill and M. J. Henderson, *Ann. N.Y. Acad. Sci.* **585**, 110 (1990).
31. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Daily Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1980, pp. 142–150.
32. P. Karrer et al., *Helv. Chim. Acta* **32**, 1004 (1948).
33. N. Clauson-Kaas et al., *Acta Chem. Scand.* **9**, 1,9,14,17,23 (1955).
34. Ger. Pat. 1,935,009 (Jan. 14, 1971), F. Graf and H. Konig (to BASF).
35. Y. Matsumura et al., *Chem. Lett.*, 1121 (1981).
36. M. Y. Karpeiskii and V. L. Flornet'ev, *Russ. Chem. Rev.* **38**, 540 (1969).
37. I. Turchi and M. J. S. Dewar, *Chem. Rev.* **75**, 388 (1975).
38. G. Y. Kondrat'eva et al., *Dokl. Akad. Nauk SSR* **142**, 593 (1962).
39. T. Naito et al., *Chem. Pharm. Bull.* **13**, 873 (1965).
40. Z. I. Itov et al., *Khim. Farm. Zh.* **13**, 59 (1980).
41. E. E. Harris et al., *Tetrahedron* **23**, 943 (1967).
42. Ger. Pat. 3,029,231 (Nov. 3, 1982), H. R Mueller (to Merck).
43. H. Takagaki et al., *Chem. Lett.*, 183 (1979).
44. Jpn. Pat. 69-23,088 (Oct. 1, 1969), T. Naito, K. Ueno, and T. Miki (to Daiichi Seiyaku Co. Ltd.).
45. Jpn. Pat. 81-128,761 (Oct. 8, 1981) (to Daiichi Seiyaku Co, Ltd.).
46. H. Murakami and M. Iwanami, *Bull. Chem. Soc. Jpn.* **41**, 726 (1968).
47. E. E. Harris et al., *J. Org. Chem.* **27**, 2705 (1962).
48. U.S. Pat. 3,227,721 (May 24, 1965), E. E. Harris, K. Pfister, and R. A. Firestone (to Merck & Co.).
49. Jpn. Pat. 73-30,636 (Sept. 21, 1973), Y. Morita, S. Onishi, and T. Yamagami (to Daiichi Seiyaku Co.).
50. Jpn. Pat. 70-39,259 (Dec. 10, 1970), T. Naito, Y. Morito, and U. Toshihiro (to Daiichi Seiyaku Co.).
51. Jpn. Pat. 72-39,115 (Oct. 3, 1972), T. Miki and T. Matsuo (to Takeda Chemical Industries, Ltd.)
52. Jpn. Pat. 81-99,461 (Aug. 10, 1981) (to Takeda Chemical Industries, Ltd.).
53. T. Miki and T. Matsuo, *Yakugaku Zasshi* **87**, 323 (1967).
54. U.S. Pat. 3,796,720 (Mar. 12, 1974), H. Miki and H. Saikawa (to Takeda Chemical Industries).
55. J. Maeda et al., *Bull Chem Soc. Jpn.* **42**, 1435 (1969).
56. Fr Pat. 1,533,817 (July 19, 1968) (to Ajinomoto Co.).
57. H. Zhou et al., *Zhongguo Yiyao Gongye Zazhi* **25**, 385 (1994).
58. Chin. Pat. 86-101,512 (July 7, 1986), H. Zhou (to Shanghai Industrial Res. Inst.).
59. U.S. Pat. 3,250,778 (Nov. 11, 1962), W. Kimel and W. Leimgruber (to Hoffmann-La Roche Inc.).
60. M. Dumic et al., *Prax. Vet.* **37**, 81 (1989).
61. D. Szlopmek-Nesteruk and co-workers, *Przem. Chem.* **54**, 238 (1975).
62. U.S. Pat. 3,822,274 (July 2, 1974), E. E. Harris and R. Currie (to Merck & Co.).
63. H Ritter and R. Sperber, *Macromolecules* **27**, 5919 (1994).
64. W. Boell and H. Koenig, *Ann. Chem.*, 1657 (1979).
65. U.S. Pat. 3,876,649 (Apr. 8, 1975), W Boell and H. Koenig (to BASF).
66. T. Naito et al., *Tetr. Lett.*, 5767 (1968).
67. P. Karrer and C. Granacher, *Helv. Chem. Acta* **7**, 763 (1924).
68. Jpn. Pat. 68-30,286 (Dec. 26, 1968), T. Kitazawa, T. Matsuyama, K. Kunimitsu, and K. Masuya (to Fujisawa Pharmaceutical Co.).
69. T. Rinderspacher and B. Prijs, *Helv. Chim. Acta* **43**, 1522 (1960).
70. U.S. Pat. 4,026,901 (June 6, 1978), D. L. Coffen (to Hoffmann-La Roche Inc.).
71. U.S. Pat. 4,011,234 (Mar. 8, 1977), H. Hoffmann-Paquotte (to Hoffmann-La Roche Ltd.).
72. Eur. Pat. 612736 (Aug. 31, 1994), W. Bonrath, R. Karge, and H. Pauling (to Hoffmann-La Roche Ltd.).
73. Eur. Pat. 492,233 (July 1, 1992), P. Noesberger (to Hoffmann-La Roche Ltd.).
74. S. Shimada and M. Oki, *Chem. Pharm. Bull.* **32**, 38 (1984).
75. Jpn. Pat. 79-20,493 (July 23, 1979), S. Ohnishi and Y. Morita (to Daiichi Seiyaku Co.).
76. M. Oki and S. Shimada, *Chem. Pharm. Bull.* **35**, 4705 (1987).
77. E. C. Taylor and J. Macor, *J. Org. Chem.* **54**, 1249 (1989).
78. R. E. Geiger et al., *Helv. Chim. Acta* **67**, 1274 (1984).
79. C. A. Parnell and K. P. C. Vollhardt, *Tetr.* **41**, 5791 (1985).
80. Food and Nutrition Board, National Research Council, *Food Chemicals Codex* 3rd ed., National Academy Press, Washington, D.C., 1981, p. 260.
81. United States Pharmacopeial Convention, Inc., *The United States Pharmacopeia XXII* (USP XXII-NF XVII), Rockeville, Md., 1990, p. 1194.
82. H. Y. Aboul-Enein and M. A. Loutfy, in K. Florey, ed., *Analytical Profiles of Drug Substances*, Vol. 13, Academic Press, New York, 1984, p. 447.
83. *Pyridoxine Hydrochloride*, MSDS 11076, Hoffmann-LaRoche Inc., Nutley, N.J., 1997.
84. M. Cohen and A. Bendich, *Toxicol. Lett.* **34**, 129 (1986).
85. M. M Polansky, in J. Augustin, B. P. Klein, D. A. Decker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., Association of Vitamin Chemists, John Wiley & Sons, Inc., New York, 1985, Chapt. 17.
86. J. F. Gregory, *J. Food Comp. Anal.* 1, 105 (1988).
87. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1990, p. 961.15.
88. S. P. Colburn and J. D. Mahuren, *J. Biol. Chem.* **262**, 2642 (1987).
89. Y. Kurosu et al., *Bitamin* **66**, 91 (1992).
90. R. D. Smith et al., *Anal. Chem.* **60**, 436 (1988).
91. R. Benyon et al., *J. Chrom.* **581**, 179 (1992).
92. J. Iida and T. Murata, *Anal. Sci.* **6**, 273 (1990)
93. Y. Tani, in E. Vandamme, ed., *Biotechnology of Vitamins, Pigments and Growth Factors*, Elsevier, New York, 1988, Chapt. 13.
94. R. E. Hill et al., *J. Am. Chem. Soc.* **117**, 1661 (1995).
95. M. Morisaki, *Kurume Igakkai Zasshi* **53**, 649 (1990).
96. K. Tazuya et al., *Biochem. Biophys. Acta* **1244**, 113 (1995).

97. M. Winkler et al., *J. Bacteriol.* **177**, 2804 (1995) and cites therein.
98. M. Winkler et al., *FEMS Microbiol. Lett.* **135**, 275 (1996).
99. T. Copeland et al., *J. Bacteriol.* **174**, 1544 (1992).
100. W. Korytnik and M. Ikawa, in S. P. Colowick and N. O. Kaplan, eds., *Methods in Enzymology*, Vol. 18A, Academic Press, New York, pp. 524–566.

DAVID BURDICK
Hoffmann-La Roche, Inc.

VITAMINS: RIBOFLAVIN (B_2)

Riboflavin [*83-88-5*] (vitamin B_2, vitamin G, lactoflavin, ovoflavin, lyochrome, hepatoflavin, uroflavin) has the chemical name 1-deoxy-1-(3,4-dihydro-7,8-dimethyl-2,4-dioxobenzo[*g*]pteridin-10(2*H*)-yl)-D-ribitol, 7,8-dimethyl-10-D-ribitylisoalloxazine, $C_{17}H_{20}N_4O_6$ (1), mol wt 376.37.

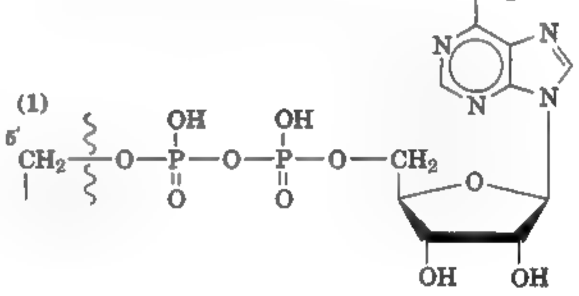


(1)

In 1933, R. Kuhn and his co-workers first isolated riboflavin from eggs in a pure, crystalline state (1), named it ovoflavin, and determined its function as a vitamin (2). At the same time, impure crystalline preparations of riboflavin were isolated from whey and named lyochrome and, later, lactoflavin. Soon thereafter, P. Karrer and his co-workers isolated riboflavin from a wide variety of animal organs and vegetable sources and named it hepatoflavin (3). Ovoflavin from egg, lactoflavin from milk, and hepatoflavin from liver were all subsequently identified as riboflavin. The discovery of the yellow enzyme by Warburg and Christian in 1932 and their description of lumiflavin (4), a photochemical degradation product of riboflavin, were of great use for the elucidation of the chemical structure of riboflavin by Kuhn and his co-workers (5). The structure was confirmed in 1935 by the synthesis by Karrer and his co-workers (6), and Kuhn and his co-workers (7).

For therapeutic use, riboflavin is produced by chemical synthesis, whereas concentrates for poultry and livestock feeds are manufactured by fermentation using microorganisms such as *Ashbya gossypii* and *Eremothecium ashbyii*, which have the capacity to synthesize large quantities of riboflavin.

In the free form, riboflavin occurs in the retina of the eye, in whey, and in urine. Principally, however, riboflavin fulfills its metabolic function in a complex form. In general, riboflavin is converted into flavin mononucleotide (FMN, riboflavin-5′-phosphate) and flavin-adenine dinucleotide (FAD) (**2**), which serve as the prosthetic groups (coenzymes), ie, they combine with specific proteins (apoenzymes) to form flavoenzymes, in a series of oxidation–reduction catalysts widely distributed in nature. In several riboflavin coenzymes, the apoenzyme is covalently attached to $C - 8\alpha$ through a linkage to the nitrogen of a histidine imidazolyl group, to the sulfur of a cysteine residue, or to the oxygen of a tyrosine moiety. Riboflavin is not a nucleotide, since it is derived from D-ribitol rather than D-ribose, and therefore FMN and FAD are not truly nucleotides; yet this designation has been accepted overwhelmingly and continues to be used.



(2)

As a coenzyme component in tissue oxidation–reduction and respiration, riboflavin is distributed in some degree in virtually all naturally occurring foods. Liver, heart, kidney, milk, eggs, lean meats, malted barley, and fresh leafy vegetables are particularly good sources of riboflavin (see Table 1). It does not seem to have long stability in food products (8).

Riboflavin is widely used in the pharmaceutical, food-enrichment, and feed-supplement industries. Riboflavin USP is administered orally in tablets or by injection as an aqueous solution, which may contain nicotinamide or other solubilizers. As a supplement to animal feeds, riboflavin is usually added at concentrations of 2–8 mg/kg, depending on the species and age of the animal.

PROPERTIES

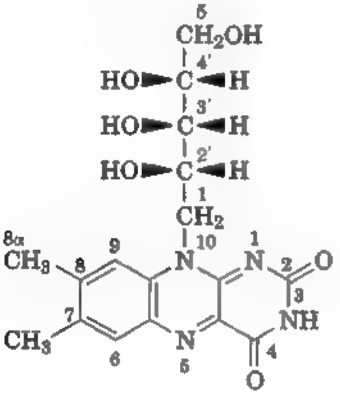
Riboflavin forms fine yellow to orange-yellow needles with a bitter taste from 2 *N* acetic acid, alcohol, water, or pyridine. It melts with decomposition at 278–279°C (darkens at ca 240°C). The solubility of riboflavin in water is 10–13 mg/100 mL at 25–27.5°C, and in absolute ethanol 4.5 mg/100 mL at 27.5°C; it is slightly soluble in amyl alcohol, cyclohexanol, benzyl alcohol, amyl acetate, and phenol, but insoluble in ether, chloroform, acetone, and benzene. It is very soluble in dilute alkali, but these solutions are unstable. Various polymorphic crystalline forms of riboflavin exhibit variations in physical properties. In aqueous nicotinamide solution at pH 5, solubility increases from 0.1 to

97. M. Winkler et al., *J. Bacteriol.* **177**, 2804 (1995) and cites therein.
98. M. Winkler et al., *FEMS Microbiol. Lett.* **135**, 275 (1996).
99. T. Copeland et al., *J. Bacteriol.* **174**, 1544 (1992).
100. W. Korytnik and M. Ikawa, in S. P. Colowick and N. O. Kaplan, eds., *Methods in Enzymology*, Vol. 18A, Academic Press, New York, pp. 524–566.

DAVID BURDICK
Hoffmann-La Roche, Inc.

VITAMINS: RIBOFLAVIN (B_2)

Riboflavin [*83-88-5*] (vitamin B_2, vitamin G, lactoflavin, ovoflavin, lyochrome, hepatoflavin, uroflavin) has the chemical name 1-deoxy-1-(3,4-dihydro-7,8-dimethyl-2,4-dioxobenzo[*g*]pteridin-10(2*H*)-yl)-D-ribitol, 7,8-dimethyl-10-D-ribitylisoalloxazine, $C_{17}H_{20}N_4O_6$ (1), mol wt 376.37.

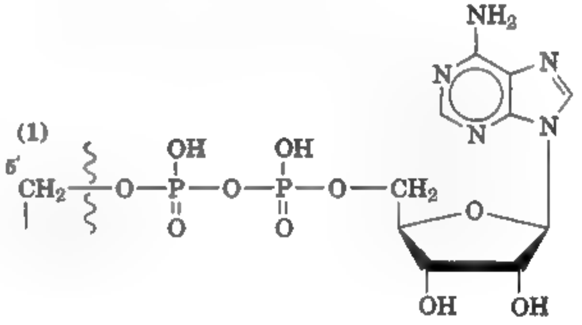


(1)

In 1933, R. Kuhn and his co-workers first isolated riboflavin from eggs in a pure, crystalline state (1), named it ovoflavin, and determined its function as a vitamin (2). At the same time, impure crystalline preparations of riboflavin were isolated from whey and named lyochrome and, later, lactoflavin. Soon thereafter, P. Karrer and his co-workers isolated riboflavin from a wide variety of animal organs and vegetable sources and named it hepatoflavin (3). Ovoflavin from egg, lactoflavin from milk, and hepatoflavin from liver were all subsequently identified as riboflavin. The discovery of the yellow enzyme by Warburg and Christian in 1932 and their description of lumiflavin (4), a photochemical degradation product of riboflavin, were of great use for the elucidation of the chemical structure of riboflavin by Kuhn and his co-workers (5). The structure was confirmed in 1935 by the synthesis by Karrer and his co-workers (6), and Kuhn and his co-workers (7).

For therapeutic use, riboflavin is produced by chemical synthesis, whereas concentrates for poultry and livestock feeds are manufactured by fermentation using microorganisms such as *Ashbya gossypii* and *Eremothecium ashbyii*, which have the capacity to synthesize large quantities of riboflavin.

In the free form, riboflavin occurs in the retina of the eye, in whey, and in urine. Principally, however, riboflavin fulfills its metabolic function in a complex form. In general, riboflavin is converted into flavin mononucleotide (FMN, riboflavin-5′-phosphate) and flavin-adenine dinucleotide (FAD) (**2**), which serve as the prosthetic groups (coenzymes), ie, they combine with specific proteins (apoenzymes) to form flavoenzymes, in a series of oxidation–reduction catalysts widely distributed in nature. In several riboflavin coenzymes, the apoenzyme is covalently attached to $C - 8\alpha$ through a linkage to the nitrogen of a histidine imidazolyl group, to the sulfur of a cysteine residue, or to the oxygen of a tyrosine moiety. Riboflavin is not a nucleotide, since it is derived from D-ribitol rather than D-ribose, and therefore FMN and FAD are not truly nucleotides; yet this designation has been accepted overwhelmingly and continues to be used.



(2)

As a coenzyme component in tissue oxidation–reduction and respiration, riboflavin is distributed in some degree in virtually all naturally occurring foods. Liver, heart, kidney, milk, eggs, lean meats, malted barley, and fresh leafy vegetables are particularly good sources of riboflavin (see Table 1). It does not seem to have long stability in food products (8).

Riboflavin is widely used in the pharmaceutical, food-enrichment, and feed-supplement industries. Riboflavin USP is administered orally in tablets or by injection as an aqueous solution, which may contain nicotinamide or other solubilizers. As a supplement to animal feeds, riboflavin is usually added at concentrations of 2–8 mg/kg, depending on the species and age of the animal.

PROPERTIES

Riboflavin forms fine yellow to orange-yellow needles with a bitter taste from 2 *N* acetic acid, alcohol, water, or pyridine. It melts with decomposition at 278–279°C (darkens at ca 240°C). The solubility of riboflavin in water is 10–13 mg/100 mL at 25–27.5°C, and in absolute ethanol 4.5 mg/100 mL at 27.5°C; it is slightly soluble in amyl alcohol, cyclohexanol, benzyl alcohol, amyl acetate, and phenol, but insoluble in ether, chloroform, acetone, and benzene. It is very soluble in dilute alkali, but these solutions are unstable. Various polymorphic crystalline forms of riboflavin exhibit variations in physical properties. In aqueous nicotinamide solution at pH 5, solubility increases from 0.1 to

Table 1. Riboflavin Content of Various Foods

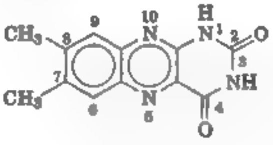
Food	mg/100 g
Fruits	
Apple, raw	0.01
Banana, raw	0.04
Citrus, grapefruit, orange	0.03–0.04
Strawberry	0.03
Vegetables	
Broccoli, raw	0.27
Cabbage, raw	0.05
Fresh green peas	0.14
Mushroom	0.57
Parsley	0.24
Potato, raw	0.03
Sweet corn	0.14
Sweet potato, raw	0.05
Tomato, raw	0.03
Meat	
Beef muscle	0.16–0.32
Pork muscle	0.19–0.33
Chicken muscle	0.10–0.31
Liver, beef, pork	3.00–3.60
Fish	
Cod, haddock, raw	0.17
Salmon, raw	0.17
Salmon, canned	0.12
Tuna, canned	0.13
Whitefish, herring, halibut	0.17–0.29
Grain	
Corn, entire	0.10
Wheat, entire	0.10
Wheat, germ	0.6
Rice, entire	0.06
Rye, entire	0.20
Cereal products	
Refined	
Bread	0.07–0.10
Cereal	0.10–0.15
Soda cracker	0.02–0.10
Whole grain and enriched	
Bread	0.12–0.25
Cereal	0.20–1.25
Dairy products	
Cheese	0.33–0.68
Eggs	0.48
Milk	0.15–0.18

Note: Averages from several sources, often encompassing a wide range of analytical results, should be regarded as working estimates which vary with geography, season, and preparative method.

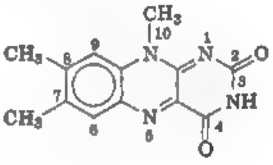
2.5% as the nicotinamide concentration increases from 5 to 50% (9).

In aqueous solution, riboflavin has absorption at ca 220–225, 226, 371, 444 and 475 nm. Neutral aqueous solutions of riboflavin have a greenish-yellow color and an intense yellowish green fluorescence with a maximum at ca 530 nm and a quantum yield of $\Phi_f = 0.25$ at pH 2.6 (10). Fluorescence disappears upon the addition of acid or alkali. The fluorescence is used in quantitative determinations. The optical activity of riboflavin in neutral and acid solutions is $[\alpha]_D^{20} = +56.5 - 59.5°$ (0.5%, dil HCl). In an alkaline solution, it depends upon the concentration, eg, $[\alpha]_D^{25} = -112 - 122°$ (50 mg in 2 mL 0.1 *N* alcoholic NaOH diluted to 10 mL with water). Borate-containing solutions are strongly dextrorotatory, because borate complexes with the ribityl side chain of riboflavin; $[\alpha]_D^{20} = +340°$ (pH 12).

Photochemical decomposition of riboflavin in neutral or acid solution gives lumichrome (3), 7,8-dimethylalloxazine, which was synthesized and characterized by Karrer and his co-workers in 1934 (11). In alkaline solution, the irradiation product is lumiflavin (4), 7,8,10-trimethylisoalloxazine; its uv–vis absorption spectrum resembles that of riboflavin. It was prepared and characterized in 1933 (5). Another photodecomposition product of riboflavin is 7,8-dimethyl-10-formylmethylisoalloxazine (12).



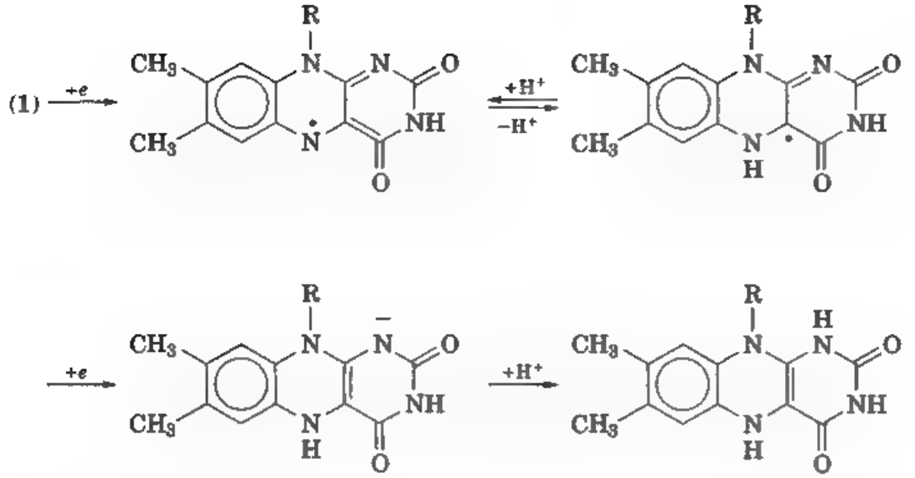
(3)



(4)

Riboflavin is stable against acids, air, and common oxidizing agents such as bromine and nitrous acid (except chromic acid, $KMnO_4$, and potassium persulfate). Upon reduction by conventional agents such as sodium dithionite, $Na_2S_2O_4$, zinc in acidic solution, or catalytically activated hydrogen, riboflavin readily takes up two hydrogen atoms to form the almost colorless 1,5-dihydroriboflavin (5) (Fig. 1), which is reoxidized by shaking with air. This oxidation–reduction system has considerable stability, a normal potential of −0.208 V (referred to as the normal hydrogen electrode), and is probably responsible for the physiological functions of riboflavin. The flavins are reduced to dihydroflavins through intermediate semiquinone radicals (13,14), which have been directly observed by electron spin resonance (esr) (15–18) and electron double resonance (endor) (19).

Riboflavin forms a deep-red silver salt (1). The strong bathochromic shift of the spectra of riboflavin analogues occurring by interaction with Ag^+ can also be obtained with Cu^+ and Hg^{2+} complexes (20). These complexes contain the flavin and the metal ligand anion in a ratio of 1:1. Their color is the result of a charge transfer between the metal and flavin (21). The chelates with Fe(II/III), Mo(V/VI), Cu(I/II), and Ag(I/II) belong to this group; the last two are stable in the presence of water. Another group of metal complexes, radical chelates, are formed with Mn(II), Fe(II),



(5)

R = D-Ribityl

Figure 1. Formation of dihydroriboflavin.

Co(II), Ni(II), Zn(II), and Cd(II); in these cases, the radical character of the ligand is conserved (22).

CHEMICAL SYNTHESIS

In 1935, Karrer (6) and Kuhn (7) each proved independently that riboflavin was 7,8-dimethyl-10-D-ribitylisoalloxazine by total synthesis (see Fig. 2). These syntheses are essentially the same and involve a condensation of 6-D-ribitylamino-3,4-xylidine (**6**) with alloxan (**7**) in acid solution. Boric acid as a catalyst increases the yield considerably (23). The intermediate (**6**) was prepared by a condensation of 6-nitro-3,4-xylidine (**8**) with D-ribose (**9**), followed by catalytic reduction of the riboside (**10**). The yield based on D-ribose was increased (24) by using *N*-D-ribityl-3,4-xylidine (**11**), which was prepared by the condensation of 3,4-xylidine (**12**) with D-ribose (**9**), followed by catalytic reduction. The reduced product (**11**) was coupled with *p*-nitrophenyldiazonium salt to give 4,5-dimethyl-2-*p*-nitrophenylazo-1-D-ribitylaminobenzene (**13**), which was reduced to (**6**) and treated with alloxan (**7**) (25) to give riboflavin. Replacement of (**7**) by 5,5-dichlorobarbituric acid (26), 5,5-dibromobarbituric acid, or 5-bromobarbituric acid (27) in the above syntheses yields riboflavin.

More conveniently, compound (**13**) was directly condensed with barbituric acid (**14**) in acetic acid (28) or in the presence of an acid catalyst in an organic solvent (29). The same azo dye intermediate (**13**) and alloxantin give riboflavin in the presence of palladium on charcoal in alcoholic hydrochloric acid under nitrogen. This reaction may involve the reduction of the azo group to the o-phenylenediamine by the alloxantin, which is dehydrogenated to alloxan (30).

Although it is not suitable for large-scale manufacture, the synthesis of riboflavin from lumazine derivatives is interesting in connection with the biosynthesis of riboflavin (see Fig. 3). Thus, 5-amino-6-D-ribitylaminouracil (**15**) was condensed with a dimeric or trimeric aldol of biacetyl to give riboflavin (**1**) through the formation of intermediary 6,7-dimethyl-8-D-ribityllumazine (**16**) (31). A variation of the above synthesis involves the condensation of monomeric biacetyl and preformed (**16**) prepared by the condensation of (**15**) with biacetyl (32). The condensation of (**15**) with 4,5-dimethyl-1,2-benzoquinone (**17**) is another pathway to riboflavin, although in low yield (33).

Later, a completely different and more convenient synthesis of riboflavin and analogues was developed (34). It consists of the nitrosative cyclization of 6-(*N*-D-ribityl-3,4-xylidino)uracil (**18**), obtained from the condensation of *N*-D-ribityl-3,4-xylidine (**11**) and 6-chlorouracil (**19**), with excess sodium nitrite in acetic acid, or the cyclization of (**18**) with potassium nitrate in acetic in the presence of sulfuric acid, to give riboflavin-5-oxide (**20**) in high yield. Reduction with sodium dithionite gives (**1**). In another synthesis, 5-nitro-6-(*N*-D-ribityl-3,4-xylidino) uracil (**21**), prepared *in situ* from the condensation of 6-chloro-5-nitrouracil (**22**) with *N*-D-ribityl-3,4-xylidine (**11**), was hydrogenated over palladium on charcoal in acetic acid. The filtrate included 5-amino-6-(*N*-D-ribityl-3,4-xylidino)uracil (**23**) and was maintained at room temperature to precipitate (**1**) by autoxidation (35). These two pathways are suitable for the preparation of riboflavin analogues possessing several substituents (Fig. 4).

The chemistry of flavins, including several synthetic methods for the preparation of *N*-D-ribityl-3,4-xylidine (**11**) is reviewed in Reference 36.

MICROBIAL SYNTHESIS

Biosynthetic Mechanism

Riboflavin is produced by many microorganisms, including *Ashbya gossypii*, *Asperigillus* sp, *Eremothecium ashbyii*, *Candida* yeasts, *Debaryomyces* yeasts, *Hansenula* yeasts, *Pichia* yeasts, *Azotobactor* sp, *Clostridium* sp, and *Bacillus* sp.

These organisms have been used frequently in the elucidation of the biosynthetic pathway (37,38). The mecha-

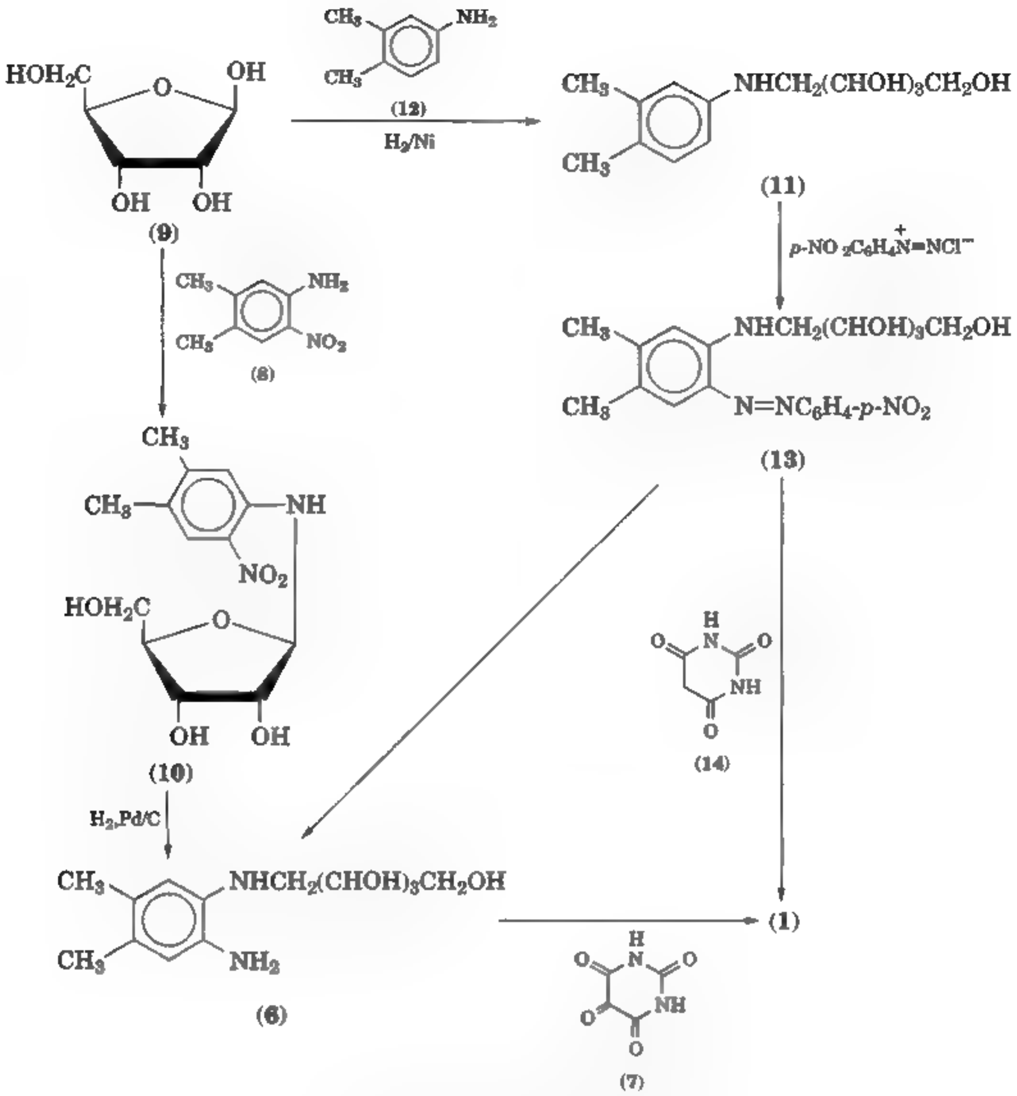

HOH2C
OH
OH OH
(9)
(12)
H2/Ni
NHCH2(CHOH)3CH2OH
(11)
p-NO2C6H4N+=NCl−
N=NC6H4-p-NO2
(13)
(8)
NH
NO2
(10)
H2,Pd/C
(14)
NH2
(6)
(7)
(1)

Figure 2. Syntheses of riboflavin.

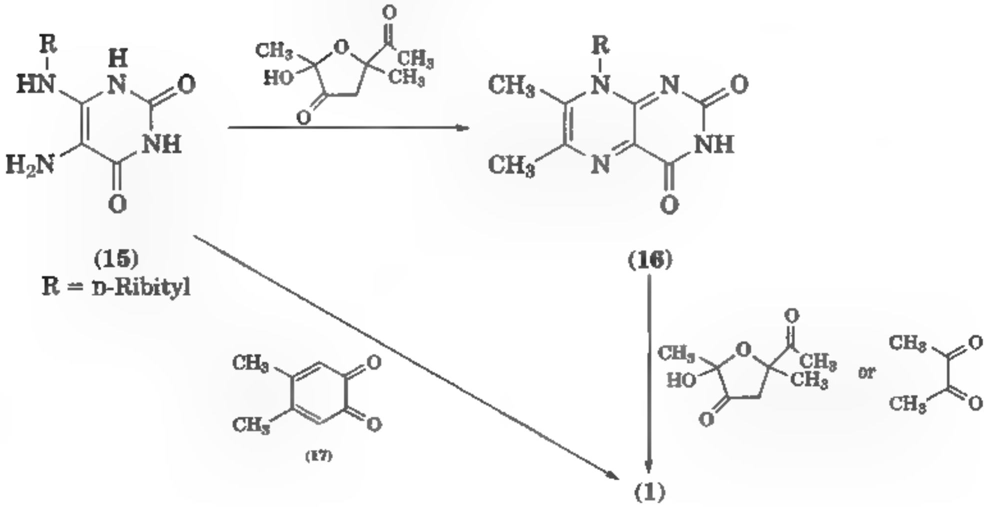
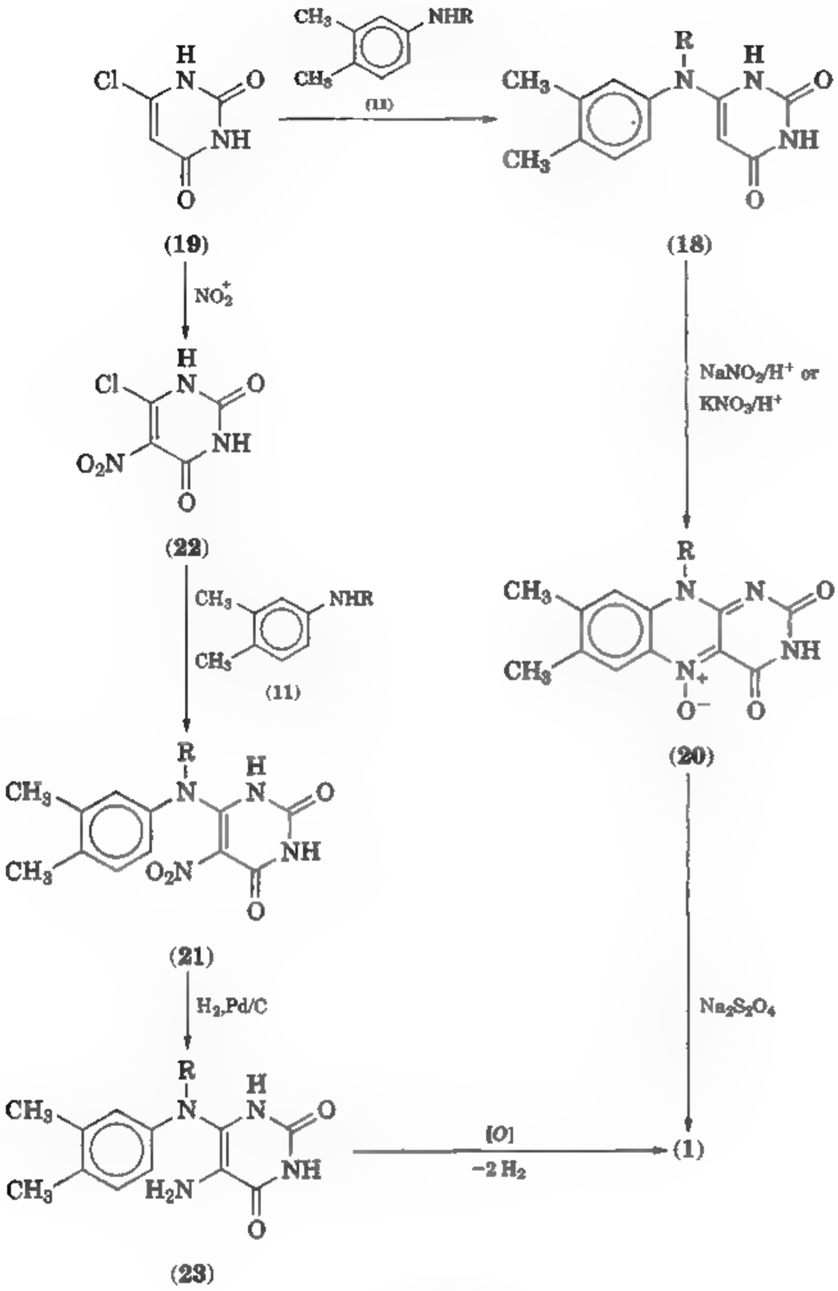

(15)
R = D-Ribityl
(16)
(17)
or
(1)

Figure 3. Syntheses of riboflavin from a lumazine.



R = D-Ribityl

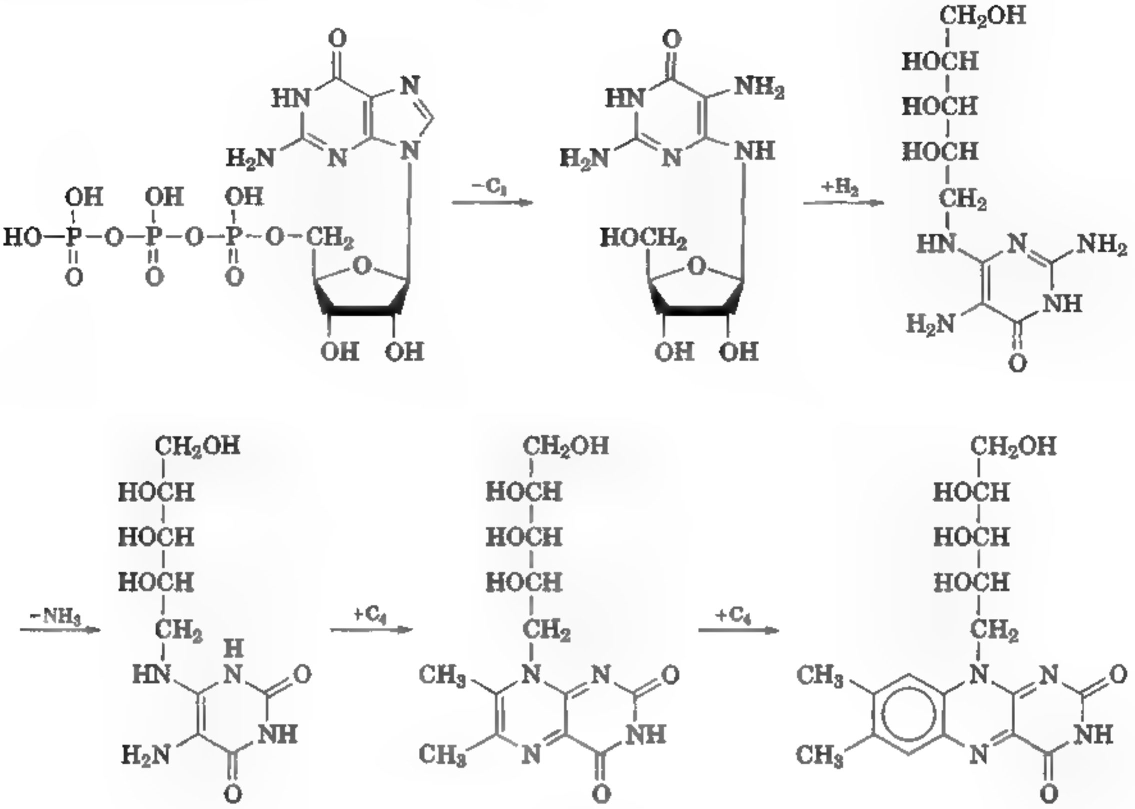
Figure 4. Alternative syntheses of riboflavin.

nism of riboflavin biosynthesis has formally been deduced from data derived from several experiments involving a variety of organisms (Fig. 5). Included are conversion of a purine such as guanosine triphosphate (GTP) to 6,7-dimethyl-8-D-ribityllumazine (**16**) (39), and the conversion of (**16**) to (**1**). This concept of the biochemical formation of riboflavin was verified *in vitro* under nonenzymatic conditions (40).

Fermentative Manufacture

Throughout the years, riboflavin yields obtained by fermentation have been improved to the point of commercial feasibility. Most of the riboflavin thus produced is consumed in the form of crude concentrates for the enrichment of animal feeds. Riboflavin was first produced by fermentation in 1940 from the residue of butanol–acetone fermentation. Several methods were developed for large-scale production (41). A suitable carbohydrate-containing mash is prepared and sterilized, and the pH adjusted to 6–7. The mash is buffered with calcium carbonate, inoculated with *Clostridium acetobutylicum*, and incubated at 37–40°C for 2–3 d. The yield is ca 70 mg riboflavin/L (42).

Most varieties of *Candida* yeasts produce substantial amounts of riboflavin when glucose is the carbon source. Particularly, *Candida guilliermondia* and *Candida flaveri*



(16)

(1)

Figure 5. Biosynthesis pathway to riboflavin.

produce high yields on a simple synthetic medium of low cost. Some modifications employing several *Candida* yeasts have been patented; eg, *C. intermedia var A*, a newly isolated microorganism assimilating lactose and ethanol, gave yields of 49.2 mg riboflavin/L from ethanol in the presence of biotin (43). *Candida T-3*, assimilating methanol, produces riboflavin by this method from 50 g methanol (44). *Candida* bacteria have the advantage of extremely low iron tolerance. Chelating agents, such as 2,2′-dipyridyl, are recommended to control the iron content (45).

Most of the commercial riboflavin production by aerobic fermentation is obtained by biosynthesis with the yeast-like fungus *Eremothecium ashbyii*. Many variations for the production of riboflavin by *E. ashbyii* have been patented. Employing *E. ashbyii* grown on a yeast medium, riboflavin production on an industrial scale is said to have reached 11.6 g/kg from dried powder (46). Riboflavin is also obtained by fermentation with *E. ashbyii* preserved on Difco-treated millet seeds. The culture, kept for 7–8 d at 32°C on the medium containing crude collagen, corn extracts, unrefined plant oil, glucose, KH_3PO_4, trace elements, and H_2O at pH 7–8, gave 4.5–5.0 g/L (47). In another procedure, *E. ashbyii* was cultivated on a culture medium containing sources of assimilable nitrogen, essential minerals, and growth factors, along with sources of assimilable carbohydrates, unsaturated fatty acids, saccharides, and amino acid or their salts. Incubation at 29°C for 6 d on a rotary shaker with aeration gave average yields of 3.8 g/L (48). *Eremothecium ashbyii* grown in a culture medium containing the oil cake obtained after extraction of lipids from the biomass grown on hydrocarbons gives an even better yield (49).

In operations similar to the *E. ashbyii* procedures, the closely related fungus *Ashbya gossypii* gave similar yields. Thus, a yield of 7.3 g/L was obtained with a lyophilized culture in a medium containing fat, leather glue, and corn extracts (50), and 6.420 g/L with bone or hide fat, alone or in a mixture with other plant or animal fats as the carbon source (51). The yield from immersed cultures of *A. gossypii* was increased to 6.93–7.20 g/L by use of waste fats or technical cod-liver oil (52).

Riboflavin is also made by aerobic culturing of *Pichia guilliermondii* on a medium containing n-C_{10}–C_{15} paraffins in a yield of 280.5 mg/L (53). A process employing *Pichia* yeasts, such as *P. miso, P. miso* Mogi, or *P. mogii*, in a medium containing a hydrocarbon as the carbon source, has been patented (54).

Processes employing *Torulopsis xylinus* (55), *Hansenula polymorpha* (56), *Brevibacterium ammoniagenes* (57), *Achromobactor butrii* (58), *Micrococcus lactis* (59), *Streptomyces testaceus* (60), and others have also been patented. These procedures yield, at most, several hundred milligrams of riboflavin per liter.

Manufacturing procedures of riboflavin have also appeared using *Saccharomyces* bacteria, eg, fermentation with a purine-independent *S.* reverse mutant (61) and with *S. cerevisiae* NH-268 (62) produced 2.79 g/L and 4.9 g/L, respectively.

Further efficient fermentative methods for manufacture of riboflavin have been patented; one is culturing *C. famata* by restricting the carbon source uptake rate, thereby restricting growth in a linear manner by restriction of a micronutrient. By this method, productivity was increased to >0.17 g riboflavin/L/h (63). The other method, using *Bacillus subtilis* AJ 12644 low in guanosine monophosphate hydrolase activity, yielded crude riboflavin 0.9 g/L/3 days, when cultured in a medium including soy protein, salts, and amino acids (64).

In recent (ca 1997) years, fermentative manufacturing methods using recombinant microorganisms have been developed. The mutant clone GA18Y8-6#2, prepared by fusion and mutagenation of *C. flaveri* mutants A22 and GA18, produced riboflavin 7.0–7.5 g/L/6 d, when cultivated in 4B medium with a supplement of $FeCl_3$, yeast extracts, peptone, and malt extracts (65). Riboflavin overproducing bacteria prepared by expression of the cloned *rib* operon of *Bacillus subtilis* showed increases in riboflavin manufacture of up to a hundredfold (up to 0.7 g riboflavin/L/48 h). The *rib* operon was cloned as series of overlapping genes using an oligonucleotide derived from the amino acid sequence of the riboflavin synthase β subunit (66). Culturing recombinant *Corynebacterium ammoniagenes* KY13313 harboring gene for at least guanidine triphosphate cyclohydrolase and riboflavin synthase produced riboflavin ~30-fold higher than that with the controlled bacteria (67).

INDUSTRIAL ASPECTS

For the industrial production of riboflavin as pharmaceuticals, the traditional methodology comprising the direct condensation of (**13**) with (**14**) in an acidic medium with continuous optimization of the reaction conditions is still used (28). A great part of riboflavin manufactured by fermentative methods is used for feeds in the form of concentrates. The present world demand of riboflavin may be about 2500 t per year. Of this amount, 60%, 25%, and 15% are used for feeds, pharmaceuticals, and foodstuffs, respectively. The main producers are Hoffmann-La Roche, BASF, Merck & Co., and others.

ANALYTICAL METHODS

Riboflavin can be assayed by chemical, enzymatic, and microbiological methods. The most commonly used chemical method is fluorometry, which involves the measurement of intense yellow-green fluorescence with a maximum at 565 nm in neutral aqueous solutions. The fluorometric determinations of flavins can be carried out by measuring the intensity of either the natural fluorescence of flavins or the fluorescence of lumiflavin formed by the irradiation of flavin in alkaline solution (68). The later development of a laser-fluorescence technique has extended the limits of detection for riboflavin by two orders of magnitude (69,70).

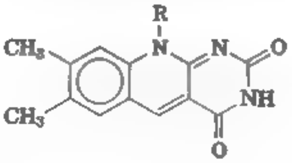
Polarography is applied in the presence of other vitamins, eg, in multivitamin tablets, without separation. The polarography of flavins is reviewed in Reference 71.

The microbial assay is based on the growth of *Lactobacillus casei* in the natural (72) or modified form. The lactic acid formed is titrated or, preferably, the turbidity measured photometrically. In a more sensitive assay, *Leuconostoc mesenteroides* is employed as the assay organism (73). It is 50 times more sensitive than *L. casei* for assaying riboflavin and its analogues (0.1 ng/mL vs 20 ng/mL for *L. casei*). A very useful method for measuring total riboflavin in body fluids and tissues is based on the riboflavin requirement of the protozoan cliate *Tetrahymena pyriformis*, which is sensitive and specific for riboflavin. This method can be applied to large-scale nutrition studies.

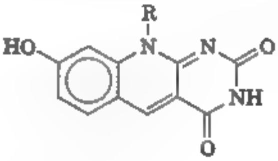
Although riboflavin can be assayed more readily by chemical or microbiological methods than by animal methods, the latter are preferred for nutritional studies and as the basis of other techniques. Such assays depend upon a growth response; the rat or chick is the preferred experimental animal. This method is particularly useful for assaying riboflavin derivatives, since the substituents frequently reduce or eliminate the biological activity.

An enzymatic method for assessing riboflavin deficiency in humans has been developed (74). It is based on the fact that NADPH-dependent glutathione reductase of red cells reflects riboflavin fluctuations.

High pressure liquid chromatography (hplc) has been extensively used for the riboflavin determinations. This method is usually automated and more rapid and sensitive than the microbial method. It has been used in combination with fluorometric detection for the riboflavin assay in foods (75), meat and meat products (76), and enriched and fortified foods (77), as well as in a simple assay for animal tissues (78). A rapid and efficient reversed-phase hplc method is described for the quantitative separation of flavin coenzymes and their structural analogues such as 5-deazaflavin [*19342-73-5*] (**24**) and 8-hydroxy-7-demethyl-5-deazariboflavin [*37333-48-5*] (**25**) (F_{420} chromophore) (79). Comprehensive reviews of the analytical methods for riboflavin are given in References 80 and 81.



(**24**)



(**25**)

R = D-Ribityl

BIOLOGICAL FUNCTION

In biological systems, riboflavin functions almost exclusively in the form of flavo-proteins, in which the FMN or FAD is generally bound as prosthetic group or coenzyme to specific proteins. These enzymes catalyze oxidation-reduction reactions (Table 2). The flavin group of the oxidized coenzyme is reduced chemically or enzymatically to 1,5-dihydroflavin coenzyme, probably in two one-electron steps, each involving the addition of a single electron. Stable semiquinone radicals are formed as intermediates, be-

Table 2. Some Reactions Catalyzed by Flavoproteins

Enzyme	Electron donor	Product	Coenzyme and other components	Electron acceptor
D-Amino acid oxidase	D-Amino acids	α-Keto acids + NH_3	2FAD	$O_2 \rightarrow H_2O_2$
L-Amino acid oxidase (liver)	L-Amino acids	α-Keto acids + NH_3	2FAD	$O_2 \rightarrow H_2O_2$
L-Amino acids oxidase (kidney)	L-Amino acids	α-Keto acids + NH_3	2FMN	$O_2 \rightarrow H_2O_2$
L(+)-Lactate dehydrogenase (yeast)	Lactate	Pyruvate	1FMN; 1heme (*cyt* b_5)	Respiratory chain
Glycolate oxidase	Glycolate	Glyoxylate	FMN	$O_2 \rightarrow H_2O_2$
NADH-Cytochrome *c* reductase	NADH	NAD^+	FMN; 2Mo, NHI	Cytochrome c_{ox} respiratory chain
NADH-Cytochrome b_5 reductase	NADH	NAD^+	FAD; Fe	Cytochrome b_5
Aldehyde oxidase (liver)	Aldehydes	Carboxylic acids	FAD; Fe, Mo	Respiratory chain
α-Glycerol phosphate dehydrogenase	Glycerol 3-phosphate	Dihydroxyacetone phosphate	FAD; Fe	Respiratory chain
Succinate dehydrogenase	Succinate	Fumarate	FAD; Fe, NHI	Respiratory chain
Acyl-CoA(C_6–C_{12}) dehydrogenase	Acyl-CoA	Enoyl-CoA	FAD	Electron-transferring flavoprotein
Nitrate reductase	NADPH	$NADP^+$	FAD; Mo, Fe	Nitrate
Nitrite reductase	NADPH	$NADP^+$	FAD; Mo, Fe	Nitrite
Xanthine oxidase	Xanthine	Uric acid	FAD; Mo, Fe	O_2
Lipoate dehydrogenase	Reduced lipoic acid	Oxidized lipoic acid	2FAD	NAD^+
Dehydroorotate dehydrogenase	Dihydroorotic acid	Orotic acid	2FMN; 2FAD, 4Fe	Undetermined

Source: Ref. 82.

cause the unpaired electron is highly delocalized by the conjugated isoalloxazine structure.

In contrast to the nicotinamide nucleotide dehydrogenases, the prosthetic groups FMN and FAD are firmly associated with the proteins, and the flavin groups are usually only separated from the apoenzyme (protein) by acid treatment in water. However, in several covalently bound flavoproteins, the enzyme and flavin coenzymes are covalently affixed. In these cases, the flavin groups are isolated after the proteolytic digestion of the flavoproteins.

Many flavoproteins react directly with molecular oxygen to produce hydrogen peroxide. Some flavoproteins, such as the flavin-containing monooxygenase, give water instead of hydrogen peroxide. In these cases, one atom of oxygen is introduced into a substrate to undergo hydroxylation, whereas the other oxygen atom is released as water. Several flavoproteins include metal complexes where these reactions take place. A number of reviews of the preparation, properties, and mechanism of action of these enzymes have been published (83,84).

DEFICIENCY, REQUIREMENTS, AND TOXICITY

Riboflavin is essential for mammalian cells. A lack of riboflavin in the human diet causes characterized deficiency syndromes, such as sore throat, hyperemia, cheilosis, angular stomatitis, glossitis (magenta tongue), a generalized seborrheic dermatitis, scrotal and vulval skin changes, and a normocytic anemia. Because riboflavin is essential to the functioning of vitamins B_6 and niacin, some symptoms attributed to riboflavin deficiency are actually due to the failure of systems requiring these other nutrients to operate effectively (85).

The 1989 Recommended Dietary allowances (RDA) of the Food and Nutrition Board (86) are 0.6 mg riboflavin per 239 kJ (1000 kcal) for essentially healthy people of all ages. This leads to the ranging from 0.4 mg/day for early infants to 1.8 mg/day for young males. For elderly people and others whose daily calorie intake may be less than 478 kJ (2000 kcal), a minimum of 1.2 mg/day is recommended. During pregnancy, an additional riboflavin intake of 0.3 mg/day is recommended in view of the increased tissue synthesis for both fetal and maternal development. For the lactating woman, the requirement is assumed to increase by an amount at least equal to that excreted in milk, which has a mean riboflavin content of 35 μg/100 mL. At an average milk production of 750 mL/day and 600 mL/d during the first and second 6 months of lactation, riboflavin secretion is 0.26 mg/d and 0.21 mg/d, respectively. Because the utilization of the additional riboflavin for milk production is assumed to be 70%, and the coefficient of variation of milk production is 12.5%, an additional daily intake of 0.5 mg is recommended for the first 6 mo of lactation and 0.4 mg thereafter.

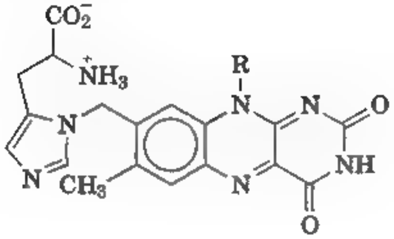
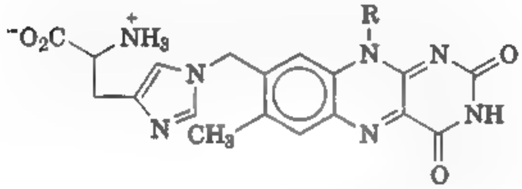
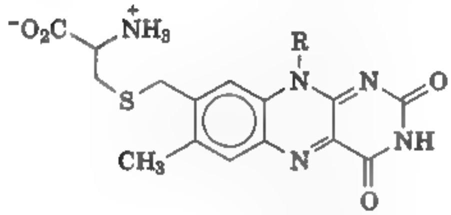
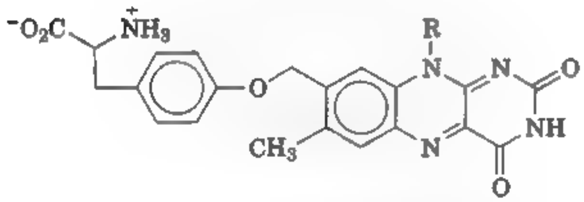
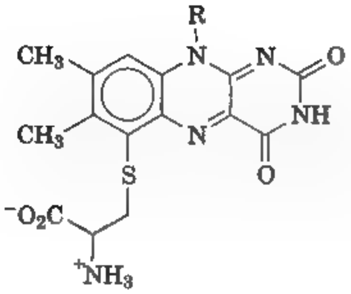
Riboflavin is essentially nontoxic. The LD_{50} values in mice and rats by intraperitoneal injection are 340 mg/kg (87) and 560 mg/kg (88), respectively. The oral administration of 10 g/kg to rats or 2 g/kg to dogs showed no toxic effects (89).

DERIVATIVES

Riboflavin-5′-Phosphate

Riboflavin-5′-phosphate [*146-17-8*] (vitamin B_2 phosphate, flavin mononucleotide, FMN, cytoflav), $C_{17}H_{21}N_4O_9P$, mol wt 456.35, is a microcrystalline yellow solid, mp 195°C, $[\alpha]_D^{23} = +44.5°$ (2% soln in conc HCl) with biological and enzymatic activity. It is prepared by phosphorylation of riboflavin with chlorophosphoric acid (90), pyrophosphoric acid (91), metaphosphoric acid (92), or catechol cyclic phosphate (93). It is soluble in water to the extent of 3 g/100

Table 3. Covalently Bound Flavins

Prosthetic group (R = D-Ribityl)	CAS Registry Number	Formula	Enzyme
 8 α-(N^1-Histidyl)riboflavin	*[58525-92-1]*	$C_{23}H_{27}C_7O_8$	Thiamine dehydrogenase β-Cyclopiazonate oxidocyclase L-Gulono-γ-lactone oxidase L-Galactonolactone oxidase Cholesterol oxidase
 8 α-(N^3-Histidyl)riboflavin	*[37854-44-7]*	$C_{23}H_{27}N_7O_8$	Succinate dehydrogenase Fumarate reductase Salcosine dehydrogenase Salcosine oxidase Dimethylglycine dehydrogenase D-Hydroxynicotine oxidase Choline oxidase D-Gluconate dehydrogenase
 8 α-*S*-Cysteinylriboflavin	*[35836-22-7]*	$C_{20}H_{25}N_5O_8S$	Monoamine oxidase A Monoamine oxidase B *Chlorobium* Cytochrome C_{552} *Chlomatium* cytochrome C_{553}
 8 α-*O*-Tyrosylriboflavin	*[38065-74-6]*	$C_{26}H_{29}N_5O_9$	*p*-Cresol methylhydroxylase
 6-*S*-Cysteinylriboflavin	*[73647-60-6]*	$C_{20}H_{25}N_5O_8S$	Trimethylamine Dehydrogenase Dimethylamine Dehydrogenase

mL at 25°C as the sodium salt but tends to gel. Because of the high sensitivity of FMN to uv, it must be preserved in dark, tight containers.

Flavin mononucleotide was first isolated from the yellow enzyme in yeast by Warburg and Christian in 1932 (4). The yellow enzyme was split into the protein and the yellow prosthetic group (coenzyme) by dialysis under acidic conditions. Flavin mononucleotide was isolated as its crystalline calcium salt and shown to be riboflavin-5′-phosphate; its structure was confirmed by chemical synthesis by Kuhn and Rudy (94). It is commercially available as the monosodium salt dihydrate [*6184-17-4*], with a water solubility of more than 200 times that of riboflavin. It has wide application in multivitamin and B-complex solutions, where it does not require the solubilizers needed for riboflavin.

Riboflavin-5′-Adenosine Diphosphate

Riboflavin-5′-adenosine diphosphate [*146-14-5*] (flavin–adenine dinucleotide, FAD), $C_{27}H_{33}N_9O_{15}P_2$ (**2**), mol wt 785.56, was first isolated in 1938 from the D-amino acid oxidase as its prosthetic group (95), where it was postulated to be flavin–adenine dinucleotide. The structure was established by the first synthesis in Todd's laboratory (96); the monosilver salt of FMN was condensed with 2′,3′-isopropylidene-adenosine-5′-benzylphosphorchloridate, followed by removal of protective groups. It was also synthesized directly from FMN and adenosine-5′-monophosphate (AMP) with di-*p*-tolylcarbodiimide as the condensation agent (97). Another direct synthesis was achieved by dehydration between FMN and AMP with trifluoroacetic acid anhydride (98). A 40% yield was obtained by condensation of adenosine-5′-phosphoramidate and FMN using a mixture of pyridine and *o*-chlorophenol as the solvent (99). Condensation of AMP with FMN in ethoxyacetylene gives a 10–15% yield; by-products such as riboflavin-4′,5′-cyclic phosphate are avoided (100). An efficient procedure was developed, which transforms the free acid of both nucleotides into their tri-*n*-butyl-ammonium salts and uses a reactive carbodiimide for the coupling reaction. The yield of FAD can be as high as 70% (101). In addition to D-amino acid oxidase, FAD is the prosthetic group for the other flavoproteins including glucose oxidase, glycine oxidase, fumarate hydrogenase, histaminase, and xanthine oxidase.

Covalently Bound Flavins

The FAD prosthetic group in mammalian succinate dehydrogenase was found to be covalently affixed to protein at the 8 α-position through the linkage of 3-position of histidine (102,103). Since then, several covalently bound riboflavins (104,105) have been found successively from the enzymes listed in Table 3. The biosynthetic mechanism, however, has not been clarified.

6-Hydroxyriboflavin

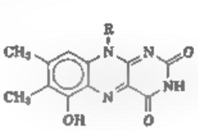
This compound [*86120-61-8*] (**26**) was isolated as a green coenzyme of the NADH dehydrogenase from *Peptostreptococcus elsdenii* and also from glycolate oxidase of porcine liver. It is not fluorescent, and its structure was established by synthesis (106). The 5′-monophosphate serves as a cofactor for glycolate oxidase from pig liver.

8-Nor-8-hydroxyriboflavin

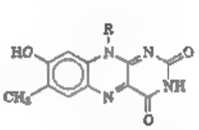
A prosthetic group of red color has been isolated from NADH dehydrogenase of the electron-transferring flavoprotein of *Peptostreptococcus elsdenii*. Its structure [*52134-62-0*] (**27**) has been established as the FAD derivative of 8-hydroxy-7-methylisoalloxazine. Proof has been obtained by the synthesis of 8-hydroxy-7-methylisoalloxazine models and stepwise degradation of the naturally occurring compound (107).

Roseoflavin

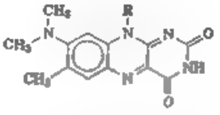
Roseflavin [*51093-55-1*], $C_{18}H_{23}N_5O_6$, (**28**), mol wt 405.41, mp 274–297°C, $[\alpha]_D = -320°$ (0.1*M* NaOH), was isolated from a culture medium of *Streptomyces davawensis* as dark, reddish-brown fine needles (from ethanol); the 8-methyl group of riboflavin is substituted by a dimethylamino group. This structure was confirmed by the synthesis. Roseflavin shows antimicrobial activity against gram-positive bacteria (108).



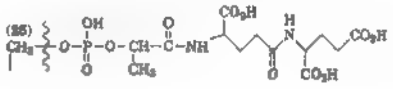
(26)



(27)



(28)



(29)

R = D-Ribityl

5-Deazariboflavin

In 5-deazariboflavin (**24**), the N-5 of riboflavin is replaced by CH; it serves as cofactor for several flavin-catalyzed reactions (109). It was first synthesized in 1970 (110); improved synthetic processes were reported later (111).

A low potential electron carrier, the fluorescent factor F_{420} [*37333-48-5, 64885-97-8*] (**29**) (it absorbs maximally at 420 nm), possessing a 5-deazaflavin moiety, was isolated from methane-producing bacteria (112). F_{420} is an obligate intermediate for passage of an electron from H_2 to $NADP^-$ to generate NADPH. The structure of F_{420} was proposed as an 8-hydroxy-7-demethyl-5-deazaflavin derivative (**25**) (113) and confirmed as compound (**29**) by the total synthesis (114).

BIBLIOGRAPHY

1. R. Kuhn, P. György, and T. Wagner-Jauregg, *Ber Dtsch. Chem. Ges.* **66**, 576 (1933).
2. Ref. 1, **66**, 317 (1933).
3. P. Karrer and K. Schöpp, *Helv. Chem. Acta* **17**, 735, 771 (1934).

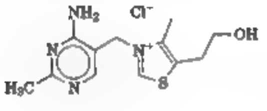
4. O. Warburg and W. Christian, *Biochem. Z.* **254**, 438 (1932); **266**, 377 (1933)
5. R. Kuhn, H. Ruby, and T. Wagner-Jauregg, *Ber. Dtsch. Chem. Ges.* **66**, 1950 (1933).
6. P. Karrer, K. Schöpp, and F. Benz, *Helv. Chim. Acta* **18**, 426, 522 (1935).
7. R. Kuhn, K. Reinemund, H. Kaltschmitt, K. Stöbele, and H. Trischmann, *Naturwissenschaften* **23**, 260 (1935).
8. E. A. Woodcock and J. J. Warthesen, *J. Food Sci.* **47**, 545 (Apr 1982)
9 U.S. Pat. 2,407,412 (Sept. 10, 1946), D. V. Frost (to Abbott Laboratories).
10. A. W. Varnes, R. B. Dodson, and E. L. Wehry, *J. Am. Chem. Soc.* **94**, 946 (1972).
11. P. Karrer, H. Salmon, K. Schöpp, E. Schlittler, and H. Fritsche, *Helv. Chim. Acta* **17**, 1010 (1934).
12. E. C. Smith and D. Metzler, *J. Am. Chem. Soc.* **85**, 3285 (1963).
13. R. Kuhn and T. Wagner-Jauregg, *Ber. Dtsch. Chem. Ges.* **67**, 361 (1934).
14. L. Michaelis, M. P. Schubert, and C. V. Smythe, *J. Biol. Chem.* **116**, 587 (1936).
15. A. Ehrenberg, *Acta Chem. Scand.* **11**, 205 (1957).
16. B. Commoner and B. Lippincott, *Proc. Natl. Acad. Sci. U.S.A.* **44**, 1110 (1958).
17. F. Müller, P. Hemmerich, and A. Ehrenberg in H. Kamin, ed., *Flavins and Flavoproteins*, University Park Press, Baltimore, Md., 1971, p. 107.
18. F. Müller et al., *Eur. J. Biochem.* **116**, 17 (1981).
19. H. Kurreck et al., *J. Am. Chem. Soc.* **106**, 737 (1984).
20. P. Hemmerich, *Experientia* **16**, 534 (1969).
21. P. Bamberg and P. Hemmerich, *Helv. Chim. Acta* **44**, 1001 (1961); K. H. Dubley, A. Ehrenberg, P. Hemmerich, and F. Müller, *Helv. Chim. Acta* **47**, 1354 (1964).
22. A. Ehrenberg and P. Hemmerich, in T. P. Singer, ed., *Biological Oxidations*, Wiley-Interscience, New York, 1968, p. 722.
23. R. Kuhn and F. Weygand, *Ber. Dtsch. Chem. Ges.* **68**, 1282 (1935).
24. P. Karrer and H. F. Meerwein, *Helv. Chim. Acta* **18**, 1130 (1935).
25. Ref. 24, **19**, 264 (1936).
26. M. Tishler, J. W. Wellman, and K. Ladenburg, *J. Am. Chem. Sco.* **67**, 2165 (1945).
27. T. Matsukawa and K. Shirakawa, *Yakugaku Zasshi* **69**, 208 (1949).
28. M. Tishler, K. Pfister, R. D. Babson, K. Ladenburg, and A. J. Fleming, *J. Am. Chem. Soc.* **69**, 1487 (1947).
29 U.S. Pat. 4,673,742 (June 16, 1987), J. Grimmer and H. C. Horn (to BASF AF); Ger. Pat. 3,542,837 (June 11, 1987), J. Grimmer (to BASF AG).
30. F. Bergel, A. Cohen, and J. W. Haworth, *J. Chem. Soc.*, 165 (1945).
31. R. M. Cresswell and H. C. S. Wood, *J. Chem. Soc.*, 4768 (1960).
32. Jpn. Pat. 10,031 (Nov. 13, 1959), A. Masuda (to Takeda Pharmaceutical Industries).
33. J. Davol and D. D Evans, *J. Chem. Soc.*, 5041 (1960); see also R. M. Cresswell, T. Neilson, and H. C. S. Wood, *J. Chem. Soc.*, 477 (1961).
34. F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Am. Chem. Soc.* **98**, 830 (1976).
35. F. Yoneda, Y. Sakuma, and K. Shinozuka, *J. Chem. Soc. Perkin Trans. 1*, 348 (1978).
36. T. Wagner-Jauregg in W. H. Sebrell, Jr., and R. E. Harris, eds., *The Vitamins*, Vol. V, Academic Press, Inc., New York, 1972, p. 19; J. P. Lambooy in R. C. Elderfield, ed., *Heterocyclic Compounds*, Vol. 9, John Wiley & Sons, Inc., New York, 1967, p. 118.
37. G. W. E. Plaut in M. Florkin and E. H. Stotz, eds., *Comprehensive Biochemistry*, Vol. 21, Elsevier Publishing Co., Amsterdam and New York, 1971, p. 11.
38. A. Bacher in F. Müller, ed., *Chemistry and Biochemistry of Flavoenzymes*, Vol. 1, CRC Press, Boca Raton, Fla., 1991, p. 215.
39. M. Mitsuda, K. Nakajima, and T. Nadamoto, *J. Nutr. Sci. Vitaminol.* **22**, 477 (1976), **23**, 71 (1977).
40. T. Rowan and H. C. S. Wood, *J. Chem. Soc.*, 452 (1968); T. Paterson and H. C. S. Wood, *J. Chem. Soc. Perkin Trans. 1*, 1051 (1972).
41. U.S. Pat. 2,202,161 (May 28, 1940), C. S. Miner (to Commercial Solvents Corp.).
42. U.S. Pat. 2,369,680 (Feb. 20, 1945), R. E. Maeda, H. L. Polland, and N. E. Rodgers (to Western Condensing Co.); U.S. Pat. 2,449,144 (Sept. 14, 1948), N. E. Rogers, H. L. Polland, and R. E. Maeda (to Western Condensing Co.).
43. Jpn. Pat. 73 19,958 (June 18, 1973), S. Sugawara and K. Sato (to Nippon Beet Sugar Manufacturing Co.).
44. Jpn. Pat. 76 19,187 (Feb. 16, 1976), Y. Ichida, H. Abe, and A. Aoike (to Kuraray Co.).
45. U.S. Pat. 2,425,280 (Aug 5, 1947), R. Hickey (to Commercial Solvents Corp.).
46. Ger. Pat. 1,936,238 (Jan. 28, 1971), E. M. Dikanskaya and A. A. Balabanova (to All-Union Scientific Research Institute of Protein Biosynthesis).
47. Ger. Pat. 2,028,355 (Jan. 14, 1971), I. Nitelea and co-workers (to Romania Antibiotic Plant).
48. Ger. Pat. 1,767,260 (Aug. 19, 1976), G. M. Miescher (to Commercial Solvents Corp.).
49. USSR Pat. 194,261 (June 15, 1974), E. M. Dikanskaya (to All-Union Scientific-Research Institute of Protein Biosynthesis).
50. Ger. Pat. 2,453,827 (May 15, 1975), T. Slave and co-workers (to Institutul de Cercetari Chimico Farmaceutice).
51. Pol. Pat. 66,611 (Mar. 15, 1973), T. Szczesniak and co-workers (to Instytut Przemyslu Fermentacyjnego).
52. Pol. Pat. 76,481 (Mar. 10, 1975), T. Szezesniak and co-workers (to Instytut Przemyslu Farmaceutycznego).
53. Ger. Pat. 2,037,905 (Feb. 3, 1972), T. Kamikubo and N. Hiroshima (to Kanegafuchi Chemical Industry Co.).
54. U.S. Pat. 3,433,707 (Mar. 18, 1969), T. Matsubayashi and Y. Suzuki (to Dai Nippon Suger Manufacturing Co. and Nitto Physico-Chemical Research Institute).
55. Jpn. Pats. 73 96,790 and 73 96 791 (Dec. 10, 1973), T. Fukukawa, T. Matsuyoshi, and J. Hiratsuka (to Mitsui Petrochemical Industries).
56. Jpn. Pat. 79 80,495 (June 27, 1979), S. Uragami (to Mitsubishi Gas Chemical Co.).
57. Jpn. Pat. 77 110,897 (Sept. 17, 1977), K. Nakayama, K. Araki, and S. Shimojo (to Kyowa Hakko Kogyo Co.).
58. Jpn. Pat. 77 54,094 (May 2, 1977), I. Chibata and co-workers (to Tanabe Seiyaku Co.).
59. USSR Pat. 511,742 (Sept. 25, 1976), T. E. Popova and co-workers (to All-Union Scientific-Research Institute of Protein Biosynthesis).

60. Jpn. Pat. 75 116,690 (Sept. 12, 1975), H. Umezawa and co-workers (to Sanraku-Ocean Co.).
61. Jpn. Kokai Tokkyo Koho Jpn. Pat. 87 25,996 (Feb. 3, 1987), A. Matsuyama and co-workers (to Daicel Chemical Industries, Ltd.).
62. Jpn. Kokai Tokkyo Koho Jpn. Pat. 88 112,996 (May 18, 1988), A. Matsuyama and co-workers (to Daicel Chemical Industries, Ltd.).
63. PCA Int. Appl. WO 9201,060 (Jan. 23, 1992), R. B. Bailey, G. W. Lauderdale, D. L. Heefner, C. A. Weaver, M. J. Yarus, L. A. Burdzinski, and A. Boyte (to Coors Biotechnology, Inc.).
64. Eur. Pat. Appl. EP 531,708 (Mar. 17, 1993), N. Usui, Y. Yamamoto, and T. Nakamatu (to Ajinomoto Co.).
65. Eur. Pat. Appl. EP 231,605 (Aug. 12, 1987), D. L. Heefner, M. Yarus, A. Boyts, and L. Burdzinski (to Adolph Coors Co.).
66. Eur. Pat. Appl EP 405,370 (Jan. 2, 1991), D. B. Perkins, J. K. Pero, and A. Sloma (to Hoffmann-La Roche, F., Co.).
67. Eur. Pat. Appl. EP 604,060 (Jan. 29, 1994), S. Koizumi, Y. Yonetani, and S. Teshiba (to Kyowa Hakko Kogyo Co.).
68. J. Koziol, in D. B. McCormick and L. D. Wright, eds., *Methods in Enzymology*, Vol. 43, Academic Press, Inc., New York, 1971, p. 253.
69. J. H. Richardson in D. B. McCormick and L. D. Wright, eds., *Methods in Enzymology*, Vol. 66, Academic Press, Inc., New York. 1980, p. 416.
70. N. Ishibashi, T. Ogawa, T. Imasaka, and M. Kunitake, *Anal. Chem.* **51**, 2096 (1979).
71. E. Knobloch in Ref. 63, p. 305.
72. E. E. Snell and F. M. Strong, *Ind. Eng. Chem. Anal. Ed.*, 11, 346 (1939).
73. H. A. Kornberg, R. S. Langdon, and V. H. Cheldelin, *Anal. Chem.* **20**, 81 (1948).
74. J. A. Tillotson and E. M. Baker, *Am. J. Clin. Nutr.* **25**, 425 (1972).
75. P. J. Richardson, D. J. Favell, G. C. Gidley, and A. D. Jones, *Proc. Anal. Div. Chem. Soc.* **15**, 53 (1978).
76. C. Y. Wang and F. A. Moseley, *J. Agric. Food Chem.* **28**, 483 (1980).
77. J. F. Kamman, T. P. Labuza, and J. J. Warthesen, *J. Food Sci.* **45**, 1497 (1980).
78. K. Yagi and M. Sato, *Biochem. Int.* **2**, 327 (1981).
79. D. R. Light, C. Walsh, and M. A. Marletta, *Anal. Biochem.* **109**, 87 (1980).
80. W. N. Pearson, in P. György and W. N. Pearson, eds., *The Vitamins*, Vol. VII, Academic Press, Inc., New York, 1967, p. 99.
81. H. Baker and O. Frank in R. S. Rivilin, ed., *Riboflavin*, Plenum Press, New York, 1975, p. 49.
82. E. E. Conn, P. K. Stumpf, G. Bruening, and R. H. Doi, *Outlines of Biochemistry*, 5th ed., John Wiley & Sons, Inc., New York, 1987, p. 207.
83. K. M. Horowitt and L. A. Wittig, in W. H. Sebrell, Jr., and R. E. Harris, eds., *The Vitamins*, Vol. V, Academic Press, Inc., New York, 1972, p. 53; C. A. Hamilton, *Prog. Bioorg. Chem.*, 1, 83 (1971); T. C. Bruice, *Prog. Bioorg. Chem.* 4, 1 (1976); C. Walsh, *Annu. Rev. Biochem.* **47**, 881 (1978); V. Massey and P. Hemmerich in P. D. Boyer, ed., *The Enzymes*, Vol. 12, Academic Press, Inc., New York, 1976, p. 191.
84. F. Müller, ed., *Chemistry and Biochemistry of Flavoenzymes*, Vol. 1, 1991, Vol. 2, 1991; and Vol. 3, 1992 CRC Press, Boca Raton.
85. D. B. McCormick, in M. E. Sils and V. R. Young, eds., *Modern Nutrition in Health and Disease*, Lea and Febiger, Philadelphia, Pa., 1988, p. 362.
86. *Recommended Dietary Allowances*, 10th ed., Food and Nutrition Board, National Research Council, National Academy Press, Washington, D.C., 1989, p. 132.
87. R. Kuhn and P. Boulanger, *Hoppe-Seyler's Z. Physiol. Chem.* **241**, 233 (1936).
88. K. Unna and J. G. Greslin, *J. Pharmacol. Exp. Ther.* **76**, 75 (1942).
89. V. Demole, *Z. Vitaminforsch.* **7**, 138 (1938).
90. U.S. Pats. 2,610,178 and 2,610,179 (Sept. 9, 1952), L. A. Flexser and W. G. Farkas (to Hoffmann-La Roche Inc.).
91. U.S. Pat. 2,535,385 (Dec. 26, 1950), P. J. Breivogel (to White Laboratories).
92. M. Viscontini, C. Ebnother, and P. Karrer, *Helv. Chim. Acta* **35**, 457 (1952); M. Viscontini and co-workers, *Helv. Chem. Acta* **38**, 15 (1955).
93. T. Ukita and K. Nagasawa, *Chem. Pharm. Bull.* **7**, 465 (1959).
94. R. Kuhn and H. Rudy, *Ber. Dtsch. Chem. Ges.* **68**, 353 (1935).
95. O. Warburg and W. Christian, *Biochem. Z.* **296**, 294 (1938); **298**, 150 (1938); O. Warburg, W. Christian, and A. Griese, *Biochem. Z.* **297**, 417 (1938).
96. S. M. H. Christie, G. W. Kenner, and A. R. Todd, *Nature (London)* **170**, 924 (1952); *J. Chem. Soc.*, 46 (1954).
97. F. M. Huennekens and G. L. Kilgour, *J. Am. Chem. Soc.* **77**, 6716 (1955).
98. C. DeLuca and N. O. Kaplan, *J. Biol. Chem.* **223**, 569 (1956).
99. J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.* **80**, 3756 (1958).
100. H. Wassermann and D. Cohen, *Chem. Eng. News*, 47 (1962).
101. F. Cramer and H. Neunhöffer, *Ber. Dtsch. Chem. Ges.* **95**, 1664 (1962).
102. E. B. Kearney and T. P. Singer, *Biochem. Biophys. Acta* **17**, 596 (1955).
103. T. Y. Wang, C. L. Tsuo, and Y. L. Wang, *Sci. Sin.* **5**, 73 (1956).
104. T. P. Singer and D. E. Edmondson in Ref. 64, p. 253 and references cited therein.
105. D. E. Edmondson and R. De Francesco in Ref. 37, p. 73.
106. S. G. Mayhew, C. D. Whitefield, S. Ghisla; and M. Schuman-Jörns, *Eur. J. Biochem.* **44**, 579 (1974).
107. S. Ghisla and S. G. Mayhew, *Eur. J. Biochem.* **63**, 373 (1976).
108. S. Otani, M. Takatsu, M. Nakano, S. Kasai, R. Miura, and K. Matsui, *J. Antibiot.* **27**, 88 (1974); S. Kasai, R. Miura, and K. Matsui, *Bull. Chem. Soc. Jpn.* **48**, 2877 (1975).
109. P. Hemmerich, V. Massey, and H. Fenner, *FEBS Lett.* **84**, 5 (1977).
110. D. E. O'Brien, L. T. Weinstock, and C. C. Cheng, *J. Heterocycl. Chem.* **7**, 99 (1970).
111. F. Yoneda in Ref. 64, p. 267; F. Yoneda and B. Kokel in Ref. 37, p. 121.
112. D. Eirich, G. D. Vogels, and R. S. Wolfe, *Biochemistry* **17**, 4583 (1978).
113. W. T. Ashton, R. D. Brown, F. Jacobson, and C. Walsh, *J. Am. Chem. Soc.* **101**, 4419 (1979).
114. T. Kimachi, M. Kawase, S. Matsuki, K. Tanaka, and F. Yoneda, *J. Chem. Soc., Perkin Trans. 1*, 253 (1990).

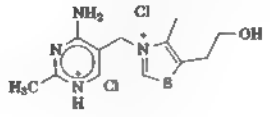
FUMIO YONEDA
Fujimoto Pharmaceutical Corporation

VITAMINS: THIAMINE (B_1)

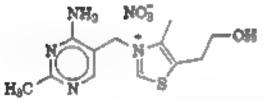
Thiamine *[59-43-8]* is the official IUPAC-IUB name for 3-(4-amino-2-methyl-5-pyrimidinyl) methyl-5-(-2-hydroxyethyl)-4-methylthiazolium chloride, $C_{12}H_{17}$-N_4OSCl (**1**). The names thiamine, thiamin (used in many official and commercial documents), aneurine, and the older term vitamin B_1 are also casually used. These names usually refer to the chloride hydrochloride *[67-03-8]* (**2**), the common biological and commercial form.



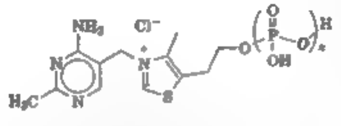
(1)



(2)



(3)



(4, n = 1)
(5, n = 2)
(6, n = 3)

Thiamine is found in varying, low levels as its salts and phosphate esters in the tissues of practically all life forms, where its pyrophosphate *[154-87-0]* (**5**), known as cocarboxylase, plays essential roles in carbohydrate metabolism. Plants and most microorganisms biosynthesize thiamine, but animals are incapable of doing so, require it in their diets in amounts varying with their carbohydrate use, and excrete the excess. Thiamine content of foods varies and can be partially lost during storage or processing, as it is one of the less stable of the vitamins. Thiamine deficiencies can lead to a range of effects, from the malaise and fatigue of a lesser deficiency to the serious disorders of gross deficiency observed for many animal species, such as beriberi, where weight loss, heart disease, and serious neurological degeneration can lead to death. As preventive measures, thiamine is used to enrich foods and feeds and as an ingredient of dietary supplements. Thiamine is also used therapeutically for treatment of specific deficiency conditions. Thiamine was one of the first of the vitamins to be manufactured for commerce. The common commercially available forms of thiamine, the chloride hydrochloride (**2**) and the mononitrate *[532-43-4]* (**3**), are manufactured by chemical processes operated at fine chemical scales. At present, natural sources and bioprocesses are not cost-competitive for bulk production.

The history of thiamine is linked with the disease beriberi, which once caused a great toll of suffering and death in parts of the world where milled or polished rice is a staple of the diet (1). Beriberi was recognized in China as early as 2600 B.C., but the first cure was only demonstrated in 1885. Takaki, then surgeon general of the Japanese navy, eradicated beriberi in the fleet by adding more protein to the sailors' diet of mainly refined rice to achieve a more Western-style diet. By 1901 the Dutch physicians Eijkman and Grijn showed hens kept on a diet of dehusked rice developed a disease similar to human beriberi which could be reversed or prevented by adding rice bran and other nitrogenous materials to their feed. They suggested beriberi was caused not by pathogens or toxins but by the lack of a vitally important food constituent, which was a radical idea at the time. They also showed the preventive factor was leached by water and destroyed by heat. Searches for a specific water-soluble nitrogenous substance led the English chemist Funk in 1911 to coin the term vitamine (vital amine, an amine essential for life), popularizing the concept of deficiency diseases. In 1926, a small, pure crystalline sample of thiamine hydrochloride was painstakingly first isolated from rice bran extracts by Dutch chemists Jansen and Donath. After eight years of effort, in 1932 the German chemist Windaus and his coworkers obtained pure thiamine from yeast extracts and established the correct empirical formula. In 1935–1936, following similarly prolonged isolation efforts, the American chemists Williams and Cline and German chemist Grewe independently proposed the correct structure based on degradation studies. The name thiamine was first used by Williams. Shortly thereafter, the Williams group confirmed the structure by rational synthesis (2), followed closely by two different syntheses by other workers (3–5). Whereas in 1933 Williams had succeeded in isolating only gram quantities from metric tons of rice polishings, a highly enriched source, in 1937 chemists at Merck and Hoffmann-La Roche developed a production level of about 100 kg within a year based on two different, relatively involved chemical syntheses. Demand and production levels have risen steeply since then to their present estimated level of about 3300 t/yr worldwide.

PHYSICAL AND CHEMICAL PROPERTIES

Salt Formation

As a weakly basic pyrimidine and a thiazolium cation, thiamine forms both mono- and dipositive salts, eg, the two commercial forms.

Thiamine chloride hydrochloride *[67-03-8]*, (thiamine hydrochloride) $C_{12}H_{18}N_4OSCl_2$ (**2**), crystallizes as colorless monoclinic needles, mp 248–250°C (with decomposition), which in bulk appear white and have an approximate density of 0.4 kg/L. Several polymorphic crystal forms have been reported. The salt has a characteristic thiazole meatlike odor and a slightly bitter taste. On exposure to air of average humidity, the hydrochloride (**2**) can adsorb up to one mole of water (more typically slightly less, to about 4% by weight), which may be removed by heating to 100°C or by vacuum drying. It is very soluble in water (over 1 kg/L at 25°C), soluble in glycerol (0.056 kg/L), propylene glycol, and methanol, sparingly soluble in 95% ethanol (0.01 kg/L), and practically insoluble in less polar organic solvents. In 1–5% solutions in water it shows a pH of 3–3.5 (6,7).

Thiamine mononitrate *[532-43-4]* (**3**), $C_{12}H_{17}N_5O_4S$, is an apparently white, colorless crystalline solid with a typical odor, melting point of ca 196–200°C (dec.) and an approximate bulk density of 0.5 kg/L. It is much less soluble in water than the hydrochloride (0.027 kg/L at 25°C, 0.030 kg/L at 100°C) and practically nonhygroscopic. Dilute solutions in water show a pH of 6.5–7 (6,8).

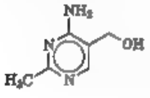
Numerous other salts have been reported in the literature, including some which are insoluble in water.

Physical Chemical Characterization

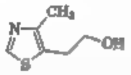
Thiamine, its derivatives, and its degradation products have been fully characterized by spectroscopic methods (9,10). The ultraviolet spectrum of thiamine shows pH-dependent maxima (11). ^{1}H, ^{13}C, and ^{15}N nuclear magnetic resonance spectra show protonation occurs at the 1-nitrogen, and not the 4-amino position (12–14). The ^{1}H spectrum in D_2O shows no resonance for the thiazole 2-hydrogen, as this is acidic and readily exchanged via formation of the thiazole ylid (**13**) an important intermediate in the biochemical functions of thiamine. Recent work has revised the pK_a values for the two ionization reactions to 4.8 and 18 respectively (9,10,15). The mass spectrum of thiamine hydrochloride shows no molecular ion under standard electron impact ionization conditions, but fast atom bombardment and chemical ionization allow observation of both an intense peak for the parent cation and its major fragmentation ion, the pyrimidinylmethyl cation (16).

Reactions and Stability

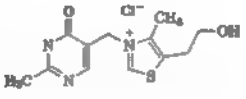
Thiamine hydrochloride is stable as a solid if kept dry. Heating to 100°C for 24 h does not diminish its potency. In solution, its stability depends heavily on conditions of pH, temperature, and oxygen. Aqueous solutions below pH 5 are stable to oxygen, heating, and even autoclaving (11). Heating in water to 140°C under pressure gives decomposition to 4-amino-5-hydroxymethyl-2-methyl-pyrimidine [*73-67-6*] (**7**) and 4-methyl-5-(2-hydroxyethyl)thiazole [*137-00-8*] (**8**) (17). Heating with 20% hydrochloric acid hydrolyzes the 4-amino group to yield oxythiamine [*582-36-5*] (**9**), an antagonist of thiamine (18). Above pH 5, aqueous thiamine is destroyed relatively rapidly by boiling. At pH 7 destruction occurs even at room temperature. Concentrated solutions of thiamine in alcohol when neutralized degrade rapidly at room temperature to liberate thiazole (**8**) and form oligomers of the pyrimidine where the 5-methylene is linked to N-1 of another pyrimidine unit (10,14).



(7)



(8)



(9)

At pH 8 or above, even at room temperature, water solutions of thiamine turn yellow, then fade as a series of reactions ensues (9,10,19). Basification allows intramolecular attack of the 4-amino function on the thiazole ring to generate dihydrothiochrome [*80483-97-2*] (**10**), which eliminates thiolate to generate the salt of the yellow form, 5,6-dihydropyrimido(4,5*d*)pyrimidine [*84825-03-6*] (**11**), as a kinetic product (9,10,19) (Fig. 1). The sequence is reversible. More slowly, under the same conditions, a competing hydrolysis of the thiazolium ring via the pseudobase generates another product, known as thiamine thiol or the thiol form [*554-45-0*] (**12**) as the thermodynamic product. Also present in the system is a small amount of thiamine ylid [*84812-92-0*] (**13**), formed by deprotonation of C-2. The products dihydrothiochrome (**10**), yellow form (**11**), and ylid (**13**) undergo significant and useful chemistry.

The yellow form (**11**) on acidification is converted to the more stable thiol form (**12**). On oxidation, typically with alkaline ferricyanide, yellow form (**11**) is irreversibly converted to thiochrome [*299-35-4*] (**14**), a yellow crystalline compound found naturally in yeast but with no thiamine activity. In solution, thiochrome exhibits an intense blue fluorescence, a property used for the quantitative determination of thiamine.

The thiol form (**12**) undergoes reactions mainly via its *N*-formyl or ene–thiol groups. Heating an aqueous solution of the thiol form (Fig. 2) effects hydrolysis to the diamine 4-amino-5-aminomethyl-2-methyl-pyrimidine [*95-02-3*] (**16**), 5-hydroxy-3-mercaptopentan-2-one [*15678-01-0*] (**17**), and formic acid (20). Neutralization of a solution of the thiolate anion with carbon dioxide gives a fat-soluble basic material [*21682-72-4, 35922-43-1*] (**20**), presumably via dihydrothiochrome (**10**) (21). Acylation of the thiolate occurs on both sulfur and oxygen to give mono- or diacyl thiamines, some of which are interesting fat-soluble depot forms of thiamine.

The thiol form (**12**) is susceptible to oxidation (see Fig. 2). Iodine treatment regenerates thiamine in good yield. Heating an aqueous solution at pH 8 in air gives rise to thiamine disulfide [*67-16-3*] (**21**), thiochrome (**14**), and other products (22). The disulfide is readily reduced to thiamine *in vivo* and is as biologically active. Other mixed disulfides, of interest as fat-soluble forms, are formed from thiamine, possibly via oxidative coupling to the thiol form (**12**).

Whereas a claim of isolation of the thiamine ylid (**13**) has been the subject of controversy (15,23), kinetic and product studies and molecular orbital calculations support the formation and reactivity of a thiamine ylid as an unstable intermediate (15,24–26). Ylid (**13**) is accepted as an intermediate in explanations of the enzymatic and nonenzymatic reactions of thiamine. Among these reactions are the enzymatic oxidative decarboxylation of pyruvic and 2-ketoglutaric acids, the formation of acetoin, the reversible alpha-ketol transfer reactions catalyzed by transketolase, and the nonenzymatic acyloin condensation. According to the thiazolium ylid mechanism, ylid (**13**) reacts reversibly with carbonyl reagents, facilitating aldol–retroaldol and decarboxylation reactions (Fig. 3), via the acyl anion equivalent thiazolium ylids (**23**), the so-called active aldehydes, which are the key intermediates (27). In the specific case of oxidative decarboxylation of pyruvic acid, for example, ylid (**23**) transfers the acyl group to lipoic acid. Other reactions involving thiamine similarly involve nucleophilic additions of a thiazolium ylid to a suitable acceptor. Syntheses of a thiamine–pyruvate adduct (**22**) and a protonated form of its decarboxylation product (**23**) (28), as well as model studies with thiazolium ions (29), further support the thiazolium ylid mechanism. There is still current debate and investigation of the mechanistic details of the involvement of the thiamine ylid (**13**) in biological systems, including the role of the associated metal ions (15,30).

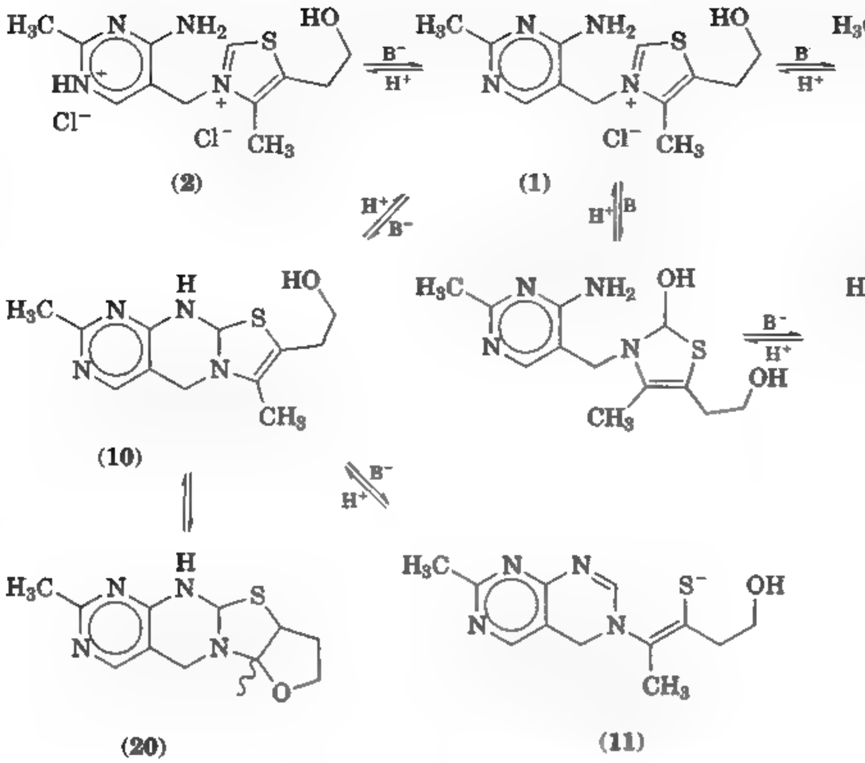

(2)
(1)
(13)
B⁻
H⁺
(10)
(12)
(20)
(11)

Figure 1. Structural changes of thiamine with pH.

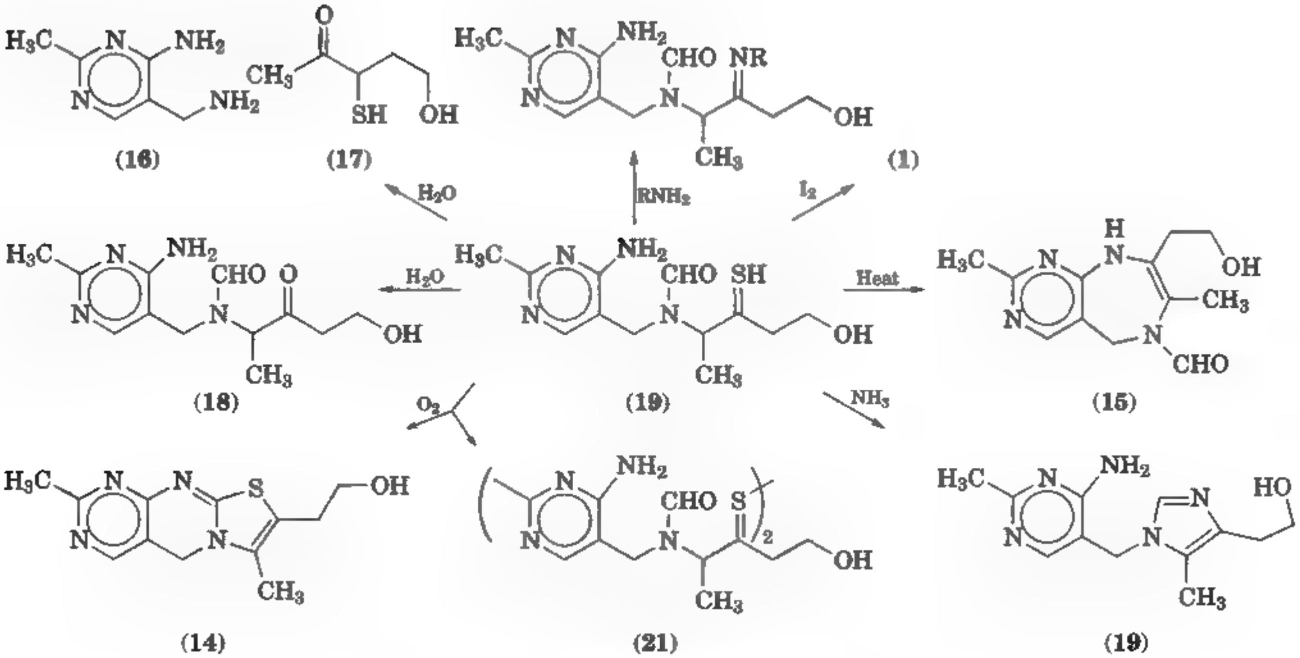

(16)
(17)
(1)
H₂O
RNH₂
I₂
Heat
(18)
(19)
(15)
O₂
NH₃
(14)
(21)
(19)

Figure 2. Reactions of the thiol form (12).

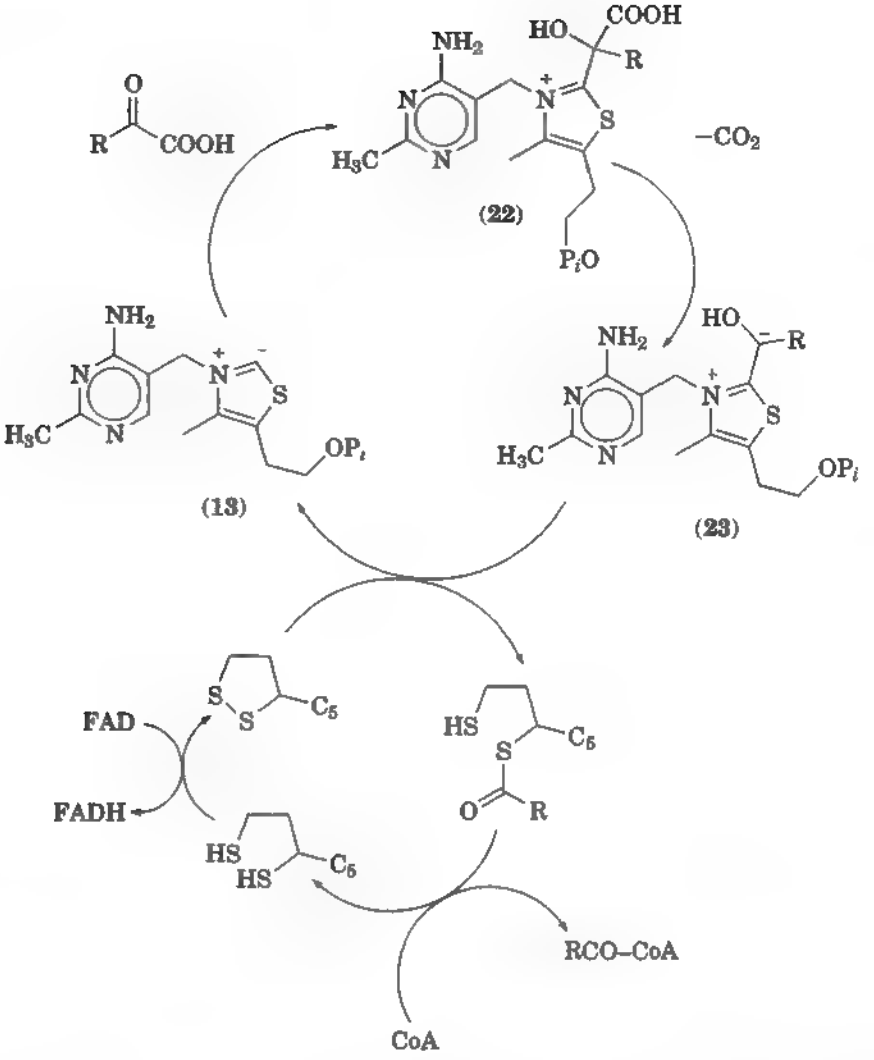
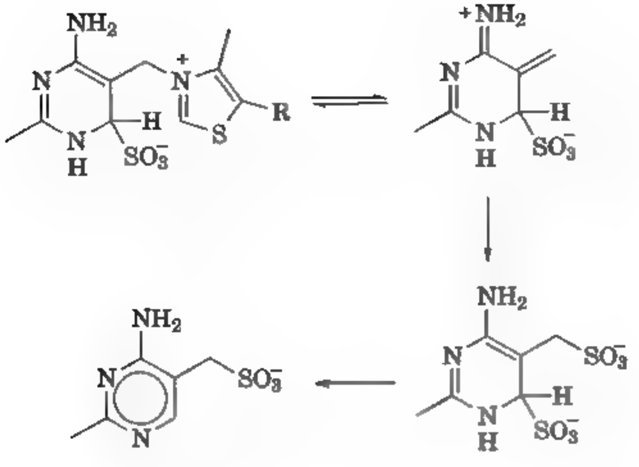


Figure 3. Oxidative decarboxylation of alpha-ketoacids.

Thiamine is susceptible to reaction with various nucleophilic species. During the race for the isolation of thiamine, it was found by chance during attempted stabilization of extracts that thiamine is readily ruptured by sulfite treatment. This occurs slowly at pH 3 and rapidly at pH 5 and above, to give 4-amino-2-methyl-5-pyrimidine-methanesulfonic acid [*108084-76-0*] (**24**) (Fig. 4), with liberation of the thiazole moiety (8) (2,10). Other nucleophilic groups such as pyridines, phenolates, anilines, and azide react similarly in the required presence of sulfite. An addition–elimination mechanism involving sulfite attack on the pyrimidine ring has been elucidated (9,10). Similar reactions are observed in the degradation of thiamine by thiaminases, enzymes found in certain foods and bacteria, including some found in the human intestine (1,10,31). Thiaminase I (EC 2.5.1.2), found in shellfish, ferns, some vegetables and some bacteria, promotes the replacement of the thiazole by other organic bases, including purines. Thiaminase II (EC 3.5.99.2), found mostly in bacteria, catalyzes the cleavage of thiamine into (**7**), the biosynthetic precursor of thiamine and an antagonist of pyridoxine (vitamin B_6). A thiol group at the active site is strongly implicated (11). These enzymes can have strong effects on animals as they promote thiamine deficiency (32). Fortunately, they are thermolabile, and hence a problem mostly in uncooked foods or feeds.

Thermostable substances which inactivate thiamine have been found in a large number of plants and in some animal tissues. Among these are polyphenols such as caffeic acid, tannic acid, hydroxylated derivatives of tyrosine, and some flavonoids (1). Thiamine is susceptible to destruction by x-rays, gamma rays, and ultraviolet light to generate diamine (**16**) by cleavage of the thiazole ring (1). Photochemical and thermal degradation of thiamine gives rise to numerous sulfur-containing heterocycles, some with meaty or bread aromas of interest to the flavor industry (33).

Operations in preparing or preserving food and feeds can sometimes lead to significant losses of thiamine value through leaching, chemical destruction (from high heat, high energy irradiation, or especially alkaline oxidizing conditions), and specific chemical interactions with thiamine-destroying substances. Such losses range in amounts from a few percent to as high as 85%. In many



(24)

Figure 4. Mechanism of nucleophilic degradation.

cases, the destruction of thiamine shows apparent first-order kinetics, and Arrhenius calculations can be used to estimate losses (1,34).

Thiamine forms the expected derivatives of the thiazole alcohol function, such as carboxylic and phosphate esters. Few reactions at the pyrimidine 4-amino function have been reported. Most of the usual conditions used for formation of amides, for example, lead to destruction of the thiazolium ring.

NATURAL OCCURRENCE

Thiamine is widespread in nature, although generally in only relatively minute quantities (1,35). In microorganisms it is found mainly intracellularly, although minute amounts are lost to the natural environment upon cell lysis. In higher plants the most abundant form is free thiamine along with lesser amounts of the phosphate esters (**4–6**). Within the plant, thiamine is unevenly distributed, its amounts and location depending on the life stage. Seeds and uptake from the soil supply the plant until biosynthesis in the leaves begins. Thiamine is then transported from the leaves to the roots where it exhibits hormone-like effects on their growth. Later thiamine is again concentrated in the seeds, especially in the germ and in the pericarpal layers surrounding the starchy areas of the seed (36). As the germ and bran is often removed during processing for aesthetic reasons, highly refined rice or wheat, for example, can have significantly lowered contents of thiamine, and can be a cause of thiamine deficiency when used as staple foods.

In the tissues of animals, most thiamine is found as its phosphorylated esters (**4–6**) and is predominantly bound to enzymes as the pyrophosphate (**5**), the active coenzyme form. As expected for a factor involved in carbohydrate metabolism, the highest concentrations are generally found in organs with high activity, such as the heart, kidney, liver, and brain. In humans this typically amounts to 1–8 μg/g of wet tissue, with lesser amounts in the skeletal muscles (35). A typical healthy human body may contain about 30 mg of thiamine in all forms, about 40–50% of this being in the muscles owing to their bulk. Almost no excess is stored. Normal human blood contains about 90 ng/mL, mostly in the red cells and leukocytes. A value below 40 ng/mL is considered indicative of a possible deficiency. Amounts and proportions in the tissues of other animal species vary widely (31,35).

Good natural human dietary sources of thiamine are unrefined cereal grains, organ meats, pork, legumes, and nuts (Table 1). Oils, fats, and highly refined foods are essentially devoid of thiamine (3,36). Although thiamine is widespread in foods, some can be lost in food preparation and storage. As a result, dietary intake can vary significantly. In most developed countries, foods (typically white rice and white flour) and feeds are supplemented with thiamine and its use in vitamin tablets as dietary supplements is common. Enriched grains, cereals, and baked products contribute about 30–45% of the recommended daily allowance (RDA) for the adult diet in the United States.

BIOCHEMICAL AND PHYSIOLOGICAL FUNCTIONS

Thiamine serves essential functions and its deficiency causes particularly deleterious effects on an organism's energy status and, in higher organisms, its nerve functions. In living systems, the only established biochemically active form is the pyrophosphate (cocarboxylase) (**5**), which plays a vital role in intermediate metabolism as a cofactor for some important enzymatic reactions. Dehydrogenase enzymes require cocarboxylase (**5**) for oxidative decarboxylation of 2-ketoacids, notably pyruvate in glycolysis, 2-oxoglutarate in the citric acid cycle, and other ketoacids from amino acid decarboxylation (1). Transketolase enzymes require cocarboxylase (**5**) for the reversible transfer of alpha-ketols in ketose–aldose transformations important in the production of pentoses for RNA and DNA synthesis (1). Thiamine triphosphate (**6**) occurs in unusually higher concentrations in nerve tissues and the brain and may play an essential role in the stimulation of peripheral nerves (35).

Table 1. Average Thiamine Contents of Foods

Food	Thiamine (μg/100 g)
Wheat germ	2050
Dried brewer's yeast	1820
Soybeans	1300
Pork	600–950
Dried beans and peas	680
Dried milk whey	500
Nuts	300–560
Brown rice	300
Beef liver	300
Potatoes	170
Fish	50–90
Eggs	70
Vegetables (fruit, leaf, stem, root)	60–70
Whole milk	30–70
White rice	50

In humans, thiamine is both actively and passively absorbed to a limited level in the intestines, is transported as the free vitamin, is then taken up in actively metabolizing tissues, and is converted to the phosphate esters via ubiquitous thiamine kinases. During thiamine deficiency all tissues stores are readily mobilized. Because depletion of thiamine levels in erythrocytes parallels that of other tissues, erythrocyte thiamine levels are used to quantitate severity of the deficiency. As deficiency progresses, thiamine becomes indetectable in the urine, the primary excretory route for this vitamin and its metabolites. Six major metabolites, of more than 20 total, have been characterized from human urine, including thiamine fragments (**7**,**8**), and the corresponding carboxylic acids (1,37,38).

The classic pathology resulting from severe thiamine deficiency in humans is called beriberi. Similar conditions have been described for many other animals. Beriberi develops primarily from inadequate intake of thiamine or from ingestion of food containing antithiamine factors and is somewhat rare in developed areas of the world. Less severe thiamine deficiency is more common and is characterized by anorexia and mental disturbances, such as irritability, inattention, memory defects, depression, and insomnia. If a lesser deficiency is left untreated, one of several clinical forms of beriberi develops, the symptoms of which include mental changes, peripheral neuritis, paresthesias, muscle cramps, edema, muscular atrophy, and cardiac failure. The most commonly encountered type of thiamine deficiency in Western countries is associated with alcohol abuse. This is generally thought to result from high intake of empty calories and low intake of nutritionally adequate foods. Other factors which influence thiamine status include general level of muscular activity; dietary practices such as high intake of refined carbohydrates, tea or coffee, or raw seafood; reduced thiamine intake as a result of disease, or parasites or drugs lowering food intake and utilization or thiamine absorption; pregnancy and lactation; heavy smoking; advanced age; genetic background; and stress. In other animals, climate and intestinal microflora also become important, and toxic effects can occur from changes in the microflora (39). Thiamine status has been monitored by blood thiamine levels, thiamine and metabolite levels in the urine, blood pyruvate and lactate levels, and blood transketolase activity (1,37,38).

Thiamine requirements vary and, with a lack of significant storage capability, a constant intake is needed or deficiency can occur relatively quickly. Human recommended daily allowances (RDAs) in the United States are based on calorie intake at the level of 0.50 mg/4184 kJ (1000 kcal) for healthy individuals (Table 2). As little as 0.15–0.20 mg/4184 kJ will prevent deficiency signs but 0.35–0.40 mg/4184 kJ are required to maintain near normal urinary excretion levels and associated enzyme activities. Pregnant and lactating women require higher levels of supplementation. Other countries have set different recommended levels (1,37,38).

Table 2. U.S. RDAs for Thiamine

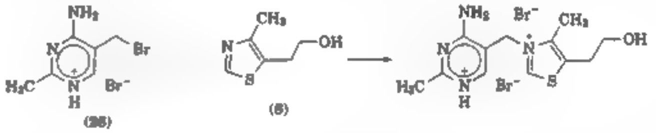
Population segment	RDA (mg)
Infants and children <4 yr	0.7
Adults and children >4 yr	1.5
Pregnant or lactating women	1.7

MANUFACTURE

Chemistry

Isolation of thiamine from natural sources (rice bran, yeast extracts, or wheat germ) is only of historical interest. Production by bioprocesses is not cost-effective at present. All of the thiamine produced worldwide is manufactured by chemical processes operated at moderately large scale. Two major synthetic routes have been used: alkylation of a preformed thiazole, or construction of the thiazolium salt from a pyrimidine carrying the ultimate thiazole nitrogen (9,40). The latter approach is now generally preferred for manufacturing.

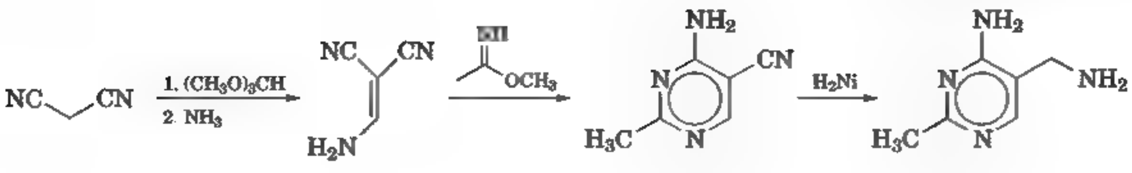
The first approach parallels the known biosynthetic pathway where the alkylating agent is the pyrophosphate ester of alcohol (**7**). Typical of this approach is Williams' first convergent synthesis, which is the basis for the first industrial method developed by Merck & Co. (2,5,41). Synthetic 4-amino-5-bromomethyl-2-methylpyrimidine [*2908-71-6*] (**26**) and thiazole (**8**), or its *O*-acetate, were condensed and then the bromide was replaced by use of silver salts. Numerous variants were published or patented in the 1930s and 1940s. Such methods generally gave only moderate yields, used higher temperatures in polar solvents, required tedious purification of the colored products and are no longer used.



In the second general method, the amine (**16**), known as Grewe diamine, is the paradigm intermediate onto which the thiazolium ring is constructed. Differences occur only in the raw materials and methods used for the manufacture of the diamine. The same end of the linear sequence is universally practiced by all large manufacturers.

The synthesis which forms the basis of production at Hoffmann-La Roche (Fig. 5) proceeds via the pyrimidinenitrile [*698-29-3*] (**27**) made from malononitrile, trimethylorthoformate, ammonia, and acetonitrile (42,43). High pressure catalytic reduction of the nitrile furnishes diamine (**16**). The overall sequence is short, highly efficient, and generates almost no waste. However, malononitrile is a relatively expensive and hazardous three-carbon source.

Other syntheses produce Grewe diamine using inexpensive acrylonitrile and alkyl formates as raw materials. Such routes deliver the pyrimidine bridge carbon at the correct aminomethyl oxidation level without a need for reduction. Thus, in the older method of Shionogi, base-catalyzed acylation of 3-methoxypropanenitrile followed by enolate alkylation and ring closure with two equivalents



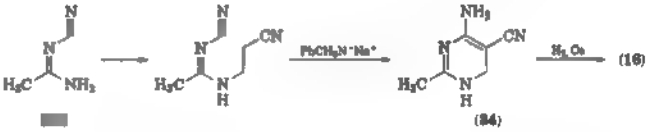
(27) (16)

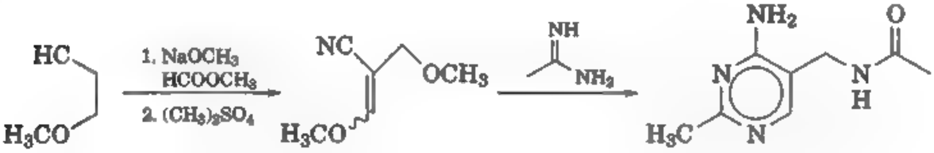
Figure 5. Roche process.

of acetamidine gives the acetyl derivative [*23676-63-3*] (**28**) of Grewe diamine (Fig. 6) (44–46). Alternatively, in a newer method by BASF, acylation of 3-formamidopropanenitrile, followed by ring closure with acetamidine, gives diamine as its formamide [*1886-34-6*] (**29**) (Fig. 7) (47–50). Both the Shionogi and BASF methods are simple technologies based on inexpensive raw materials, but both generate significant levels of salts and organic carbon as wastes.

In the past decade Takeda and Ube, in a joint venture have developed a new process for diamine, also based on acrylonitrile and alkyl formate, but carrying the pyrimidine bridge carbon at the carbonyl level (Fig. 8) (50–55). Metal-catalyzed oxidation of acrylonitrile in methanol generates 3,3-dimethoxypropanenitrile [*57597-62-3*] (**30**) which is acylated with methoxide/carbon monoxide under pressure. Unlike other methods in which the intermediate enolate is alkylated, in this process the enolate is acetalized and the acetal thermally converted to the acetal enol ether [*87466-78-2*] (**31**). Condensation with acetamidine provides the remaining carbons. After hydrolysis of the acetal [*16057-06-0*] (**32**), reductive amination at high pressure with a specialized catalyst provides diamine (**16**) in very high overall yield. The process is operated in a new, large-scale, automated, continuous, technically complex plant in Japan. Advantages include use of inexpensive acrylonitrile and carbon monoxide, avoidance of the costs of an alkylating agent, and consumption of only one equivalent of acetamidine.

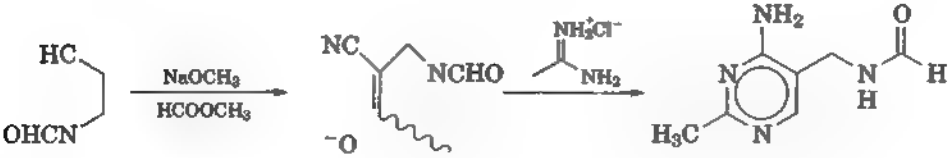
In a much different approach based on cyanamide, acrylonitrile, and acetonitrile, cyanoacetamidine [*56563-07-6*] (**33**) is cyanoethylated and the condensation product [*56563-10-1*] (**34**) is dehydrogenated and hydrogenated directly to Grewe diamine in the presence of Raney cobalt and ammonia (56).





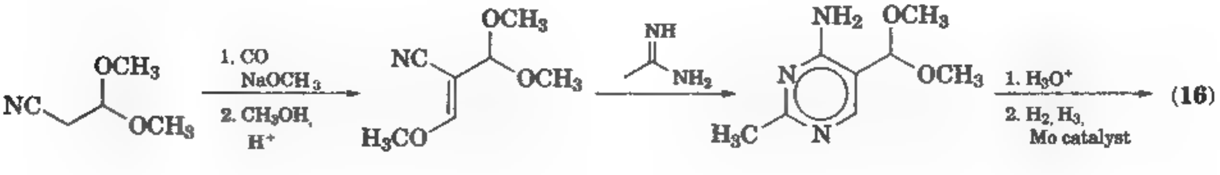
(28)

Figure 6. Shionogi process.



(29)

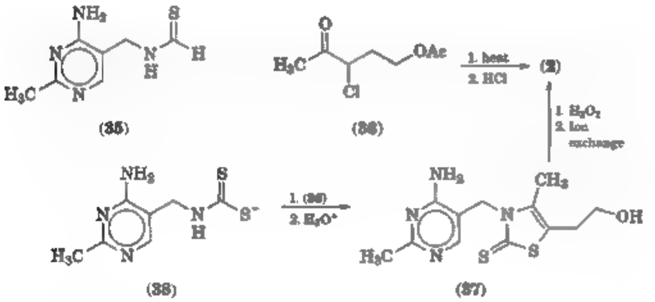
Figure 7. BASF process.



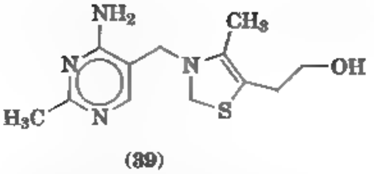
(30) (31) (32)

Figure 8. Takeda–Ube process.

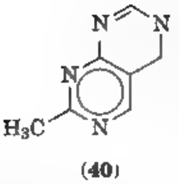
Methods for synthesis of the thiazolium ring also have matured technically based on the cost, throughput, and waste disposal issues of production. In earlier syntheses, the 2-carbon and the sulfur atom were supplied as potassium dithioformate, made from chloroform and potassium sulfide. *N*-(4-Amino-2-methyl-5-pyrimidinyl)-methylthioformamide [*31375-20-9*] (**35**) served as a partner to 5-acetoxy-3-chloropentan-2-one [*119867-66-2*] (**36**) in a classical Hantzch thiazole synthesis (3,57). Such routes suffered from similar limitations to the quaternization routes mentioned before, but were used in commercial production until the discovery of the paradigm intermediate known as thiothiamine [*299-35-4*] (**37**). In the 1940s and 1950s, chemists at Tanabe and Takeda showed that the 2-carbon and the sulfur atom can be supplied very efficiently via carbon disulfide, the extra sulfur atom being readily removed by oxidation in nearly quantitative yield (58,59). Typically, an aqueous solution of diamine and alkali is treated in succession with carbon disulfide to form the dithiocarbamate [*2882-49-7*] (**38**), then with chloroketone (**36**), then acid to form the relatively insoluble, thiothiamine (**37**). Oxidation with hydrogen peroxide forms thiamine sulfate, which is converted by ion exchange (qv) to a solution of the hydrochloride (**2**) which is concentrated, crystallized, and dried. The much less soluble nitrate (**3**) is precipitated from aqueous solution with alkali metal nitrate. The advantages of this approach, ie, a cheap, easily handled sulfur source, very high overall yield and throughput, excellent product color and purity, and the use of water as solvent, have made it the preferred method of manufacture worldwide. Disadvantages include use of chlorinated intermediates, salt load from the sulfate waste stream, and malodorous aqueous waste which must be well contained and treated. This chemistry is practiced by Takeda and Hoffmann-La Roche in automated factories using standard glass and stainless steel fine chemical processing equipment operated continuously at many hundreds of metric tons annually. Production in the several smaller Chinese factories is probably basically similar.



In two unique, convergent approaches to the thiazole ring, other formate synthons are used for the 2-carbon. In one, reaction of formaldehyde with diamine (**16**) and thiol (**17**) gives dihydrothiamine [*959-18-2*] (**39**), which is oxidized to thiamine (60). In another, reaction of diamine (**16**) with orthoformate gives intermediate dihydropyrimidine [*31375-19-6*] (**40**), to which thiol (**17**) is added. Acidic rearrangement gives thiamine in high yield (60).

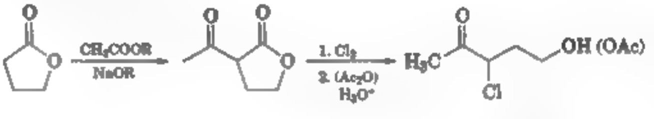


(**39**)



(**40**)

Although numerous other materials have been proposed as carbon sources for the thiazole ring, all manufacturing of thiamine is believed to use 3-chloro-5-acetoxypentan-2-one (**36**) or the corresponding alcohol [*13045-13-1*] (**41**) as intermediates. These are made by chlorination of acetylbutyrolactone, the latter from inexpensive butyrolactone and methyl acetate, generating chlorinated wastes.



(**36**, OAc)
(**41**, OH)

Worldwide production of thiamine was estimated at 3300 t in 1995. The principal suppliers were Hoffmann-La Roche, Takeda, and several Chinese factories. Prices in the United States were in the range of \$20–\$28/kg in 1995.

Product Specifications and Testing

Nutrients and diet supplements without claims are considered foods, and thus are regulated by the U.S. Food and Drug Administration and are further subject to specific food regulations. Specifications for the hydrochloride (**2**) and the mononitrate (**3**) for foods are given in the *Food Chemicals Codex* (62) and for pharmaceuticals in the *U.S. Pharmacopeia* (63). General test methods have been summarized (64).

Safety and Handling

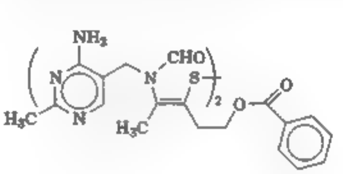
The hydrochloride (**2**) and the nitrate (**3**) are typically packaged in standard 20–25-kg foil or poly-lined cardboard boxes. Smaller packaging sizes are also available. Both hydrochloride (**2**) and nitrate (**3**) are required to be kept under normal cool, dry storage conditions, not to exceed 70°C and protected from light. Both are listed in the TSCA Inventory and material safety data sheets (MSDS) are available from suppliers. Precautions against dust exposure to the eyes, skin, or lungs (IOEL of 3.0 mg/m^3 time weighted average) and against fire and dust explosion are indicated. The NPFA ratings for health, fire, and reactivity for the hydrochloride (**2**) are 1, 2, and 1, respectively, and for the nitrate (**3**) they are 1, 3, and 1. Neither national nor international transportation is regulated. The U.S. EPA has not established reportable quantities for environmental releases (7,8). The acute LD_{50} (rat, oral) of 12,300 mg/kg for the hydrochloride (**2**) and 15,900 for the nitrate (**3**) qualify these materials as relatively harmless orally. There is no evidence of toxicity from thiamine taken orally by humans, even when doses of 500 mg, over 300 times the RDA, are taken daily for a month. Excess thiamine is easily cleared by the kidney (7,8,65). Parenteral doses of the hydrochloride (subcutaneous, intramuscular, or intravenous) have generally been well tolerated up to 100–500 mg with very few toxic effects. Some cases of sensitization or anaphylactic shock on repeated injections have been reported (1).

ANALYTICAL METHODS

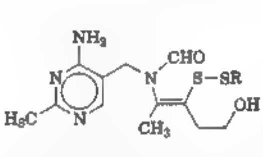
Fluorometric, chromatographic, microbiological, and animal assays have been used for thiamine and its derivatives (66). The most widely used and officially sanctioned method has been the fluorometric assay (67), although high performance liquid chromatography has been increasingly employed (68). In natural materials, thiamine is often present as its phosphate esters and is protein-bound, therefore procedures to free it are necessary steps in most assays. Determination of thiamine by the fluorometric method involves acid extraction, phosphatase hydrolysis, ion exchange to remove interferences, and oxidation with ferricyanide or other reagents to thiochrome, whose fluorescence (excitation 365 nm/emission 435 nm) is measured and compared with a standard (67). Various hplc methods have been developed for quantitation of thiamine and its phosphates in biological matrices to picomole to femtomole levels. Separations are made with reversed-phase, ion-exchange, and specialized straight-phase packings and detection with uv, post-column derivatization—fluorometry, or electrochemical techniques. Alternatively, precolumn derivatization and measurement of thiochrome has been used (68). High performance capillary electrophoresis has been applied (69). Gas chromatography has been used to determine thiamine to picomole level as the thiazole portion following cleavage of thiamine with sulfite (71). Microbiological assays are simple, inexpensive, and sensitive (5–50 ng thiamine). The results agree well with fluorometric methods but can take longer to achieve results. Microorganisms that have been most widely used include *Lactobaccillus fermenti* (ATCC 9338) and *Lactobacillus viridescens* (ATCC 12706), partly because they respond only to intact thiamine and not to its precursors (31). Bioassays in rats based on growth or cure of polyneurititis symptoms are time consuming and expensive but have been historically valuable in determining thiamine availability in foods and thiamine activity of related compounds (31).

FORMS, DERIVATIVES, AND ANALOGUES

The hydrochloride and mononitrate are the only commercial forms approved in the United States. The mono-, di-, and triphosphate esters (**4–6**) are colorless, water-soluble, organic-insoluble, high melting solids found naturally. Although they have been synthesized (9), they have not been produced commercially on large scale. In Japan and Europe, other forms have been approved for human use. Thiamine disulfide (**21**) is somewhat more soluble than thiamine in organic media. This material and its more fat-soluble *O,O*-dibutyrate and *O,O*-dibenzoate [*2667-89-2*] (**42**) have been used therapeutically for treatment of thiamine deficiency (70). Treatment of thiamine with extracts of garlic or other *Allium* species converts it to a lipid-soluble disulfide derivative which is a very physiologically active depot form of thiamine. Thiamine allyl disulfide [*554-44-9*] (allithiamine) (**43**), was the first of several thiamine alkyl disulfides to be used therapeutically for thiamine deficiency, including prosultiamine [59-58-5] (**44**) and fursultiamine [*804-30-8*] (**45**). Because of their greater lipid solubility, they are absorbed and retained more strongly in the body. *In vivo* they are converted back to thiamine. They are of interest almost exclusively in Japan and are not yet approved in the United States (70,71). Sugar derivatives (qv) are claimed to have better taste and no odor (73). In applications where water solubility is detrimental, such as fish feeds with high thiaminase activity, formulations of the hydrochloride or nitrate with a waxy coating are effective.

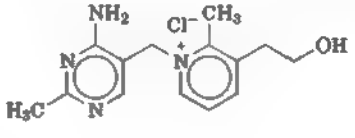


(42)

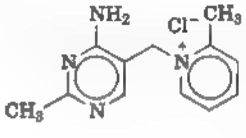


(43), Allyl
(44), Propyl
(45), Tetrahydrofurfuryl

Numerous analogues of thiamine have been synthesized by structural modifications of the pyrimidine or thiazole rings or the bridging atoms (1,9,10). None has been found to exceed the biological activity of thiamine but some act as antagonists. Substitution at the 2-methyl, 4-amino, or 6-position of carbon for ring nitrogens, or of the methylene bridge, results in loss of activity. In the thiazolium ring, the sulfur atom, hydrogen at the 2-position, and the 2-hydroxyethyl group are essential for activity. The pyridine analogue, pyrithiamine [*534-64-5*] (**46**) is the most potent thiamine antagonist known. Some thiamine antagonists were found at low levels to selectively inhibit thiamine uptake by *Coccidia*. A modified thiamine-like molecule, called Amprolium [*121-25-5*] (**47**) was once claimed to be useful as a coccidiostat for poultry (73).



(46)



(47)

BIOSYNTHESIS

Higher plants, most bacteria, and some fungi biosynthesize thiamine. Humans or most other animals cannot, although some of their gut microflora can. Many microbial species are self-sufficient, others can synthesize thiamine if one or both of immediate precursors are available, and still others require the complete substance. Few produce much of an excess over their own requirements. Biosynthesis of thiamine in microorganisms has been reviewed (9,74–76). Media modifications and mutagenesis have achieved small increases of thiamine levels with microorganisms, the highest levels of a few mg/L being found with yeasts (qv) (77). A broader search among many yeast species has reported a *Saccharomyces cerevisiae* strain which accumulates up to 200 mg/L in the culture broth (78).

The pathways for thiamine biosynthesis have been elucidated only partly. Thiamine pyrophosphate is made universally from the precursors 4-amino-5-hydroxymethyl-2-methylpyrimidine pyrophosphate [*841-01-0*] (**48**) and 4-methyl-5-(2-hydroxyethyl)thiazole phosphate [*3269-79-2*] (**49**), but there appear to be different pathways in the earlier steps. In bacteria, the early steps of the pyrimidine biosynthesis are same as those of purine nucleotide biosynthesis, 5-Aminoimidazole ribotide [*41535-66-4*] (AIR) (**50**) appears to be the sole and last common intermediate; ultimately the elements are supplied by glycine, formate, and ribose. AIR is rearranged in a complex manner to the pyrimidine by an as-yet undetermined mechanism. In yeasts, the pathway to the pyrimidine is less well understood and may be different (74–83) (Fig. 9).

In the biosynthesis of the thiazole, cysteine is the common sulfur donor. In yeasts, the C-2 and N may be supplied by glycine, and the remaining carbons by D-ribulose-5-phosphate [*108321-99-9*] (**51**). In anaerobic bacteria, the C-2 and N may be recruited from tyrosine and the carbons from D-1-deoxyxylulose [*16709-34-5*], (**52**), whereas in aerobic bacteria the C-2 and N may be derived from glycine, as in yeasts (74–76,83–86) (see Fig. 9).

Biosynthesis of pyrophosphate (**5**) from pyrimidine phosphate (**48**) and thiazole phosphate (**49**) depends on the activity of five enzymes, four of them kinases (87). In yeasts and many other organisms, including humans, pyrophosphate (**5**) can be obtained from exogenous thiamine in a single step catalyzed by thiamine pyrophosphokinase (88).

A number of the genes involved in the biosynthesis of thiamine in *E. coli* (89–92), *Rhizobium meliloti* (93), *B. subtilis* (94), and *Schizzosaccharomyces pombe* (95,96) have been mapped, cloned, sequenced, and associated with biosynthetic functions. Thiamine biosynthesis is tightly controlled by feedback and repression mechanisms limiting overproduction (97,98). A cost-effective bioprocess for production of thiamine will require significant additional progress.

USES

Most of the thiamine sold worldwide is used for dietary supplements. Primary market areas include the following applications: addition to feed formulations, eg, poultry, pigs, cattle, and fish fortification of refined foods, eg, flours, rice, and cereal products; and incorporation into multivitamins. Small amounts are used in medicine to treat deficiency diseases and other conditions, in agriculture as an additive to fertilizers (qv), and in foods as flavorings. Generally for dry formulations, the less soluble, nonhygroscopic nitrate is preferred. Only the hydrochloride can be used for intravenous purposes. Coated thiamine is used where flavor is a factor.

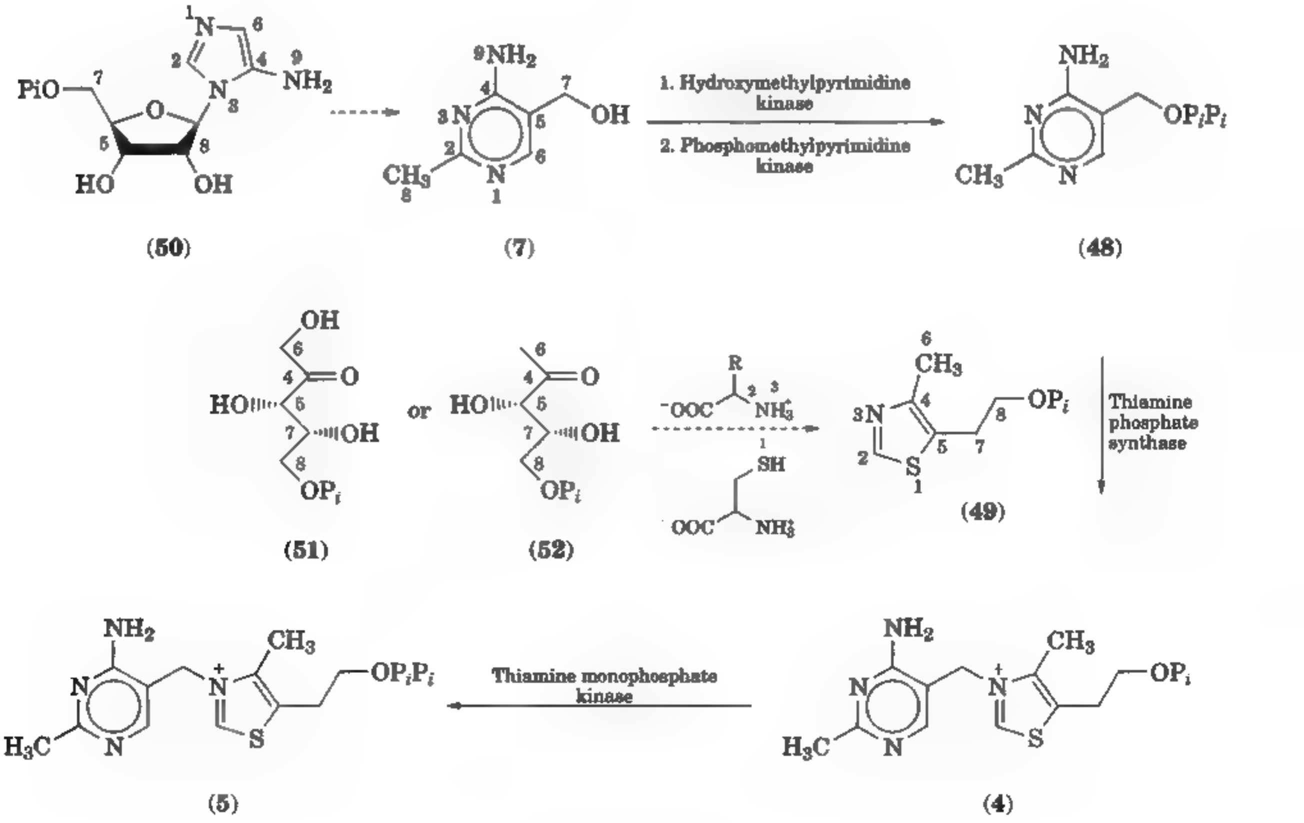

(50)
(7)
1. Hydroxymethylpyrimidine kinase
2. Phosphomethylpyrimidine kinase
(48)
(51)
or
(52)
Thiamine phosphate synthase
(49)
Thiamine monophosphate kinase
(5)
(4)

Figure 9. Biosynthesis of thiamine.

BIBLIOGRAPHY

1. C. J. Gubler, in L. J. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991, pp. 233–280.
2. R. R. Williams and J. K. Cline, *J. Am. Chem. Soc.* **58**, 1504 (1936); *ibid.* **59**, 1052 (1937).
3. A. R. Todd and F. Bergel, *J. Chem. Soc.*, 364 (1936).
4. A. R. Todd and F. Bergel, *J. Chem. Soc.*, 26 (1938).
5. H. Andersag and K. Westphal, *Ber. Dtsch. Chem. Ges.* **70**, 2035 (1937).
6. *The Merck Index*, 11th ed., Merck & Co., Rahway, N.J., 1989, p. 1464.
7. *Thiamine Hydrochloride*, MSDS 3834, Hoffmann-La Roche Inc., Nutley, N.J.
8. *Thiamine Mononitrate*, MSDS 3835, Hoffmann-La Roche Inc., Nutley, N.J.
9. O. Isler, G. Brubacher, S. Ghisla, and B. Kraeutler, *Vitamine II*, Georg Thieme Verlag, New York, 1988, pp. 17–49.
10. J. A. Zoltewicz and G. Uray, *Bioorganic Chem.* **22**, 1–28 (1994) and references therein.
11. J. M. E. H. Chahine and J. E. Dubois, *J. Am. Chem. Soc.* **105**, 2335 (1983).
12. J. D. Roberts et al., *J. Am. Chem. Soc.* **99**, 6423 (1977).
13. A. Lycka and J. Cizmarik, *Pharmazie* **45**, 371 (1990).
14. J. M. E. H. Chahine, *J. Chem. Soc., Perkin Trans. II*, 505 (1990).
15. R. Kluger, *Chem. Rev.* **87**, 863 (1987).
16. R. D. Sedgwick et al., *J. Chem. Soc., Chem. Commun.*, 325 (1987).
17. J. J. Windheuser and T. Higuchi, *J. Pharm. Sci.* **51**, 3545 (1962).
18. T. Matsukawa and S. Yurugi, *Yakugaku Zasshi* **71**, 827 (1951).
19. J. Herrmann et al., *J. Chem. Soc., Perkin Trans. II*, 463 (1995).
20. A. Watanabe and Y. Asahi, *Yakugaku Zasshi* **77**, 153 (1957).
21. A. Takamizawa et al., *Chem. Pharm. Bull.* **19**, 759 (1971).
22. T. Matsukawa and S. Yurugi. *Yakugaku Zasshi* **72**, 1599 (1952).
23. H. Sugimoto et al., *J. Org. Chem.* **55**, 46 (1990).
24. J. Fournier, *Bull. Soc. Chim. Fr.*, 854 (1988).
25. J. M. E. H. Chahine and J. E. Dubois, *J. Chem. Soc., Perkin Trans. II*, 25 (1989).
26. Y.-T. Chen and F. Jordan, *J. Org. Chem.* **56**, 5029 (1988).
27. R. Breslow, *J. Am. Chem. Soc.* **80**, 3719 (1958).
28. J. T. Stiners and M. W. Washabaugh, *Bioorg. Chem.* **18**, 425 (1990).
29. R. Breslow, *Pure Appl. Chem.* **62**, 1859–1866 (1990).
30. M. Louloudi and N. Hadjiliadis, *Coord. Chem. Rev.* **135/136**, 429–468 (1994).
31. W. H. Sebrell and R. S. Harris, eds., *The Vitamins*, 2nd ed., Vol. V, Academic Press, Inc., New York, 1972, p. 98.
32. W. C. Evans, in P. L. Munson, J. Glover, E. Diezfalusy, and R. E. Olson, eds., *Vitamins and Hormones*, Vol. 33, Academic Press, Inc., New York, 1975, pp. 467–504.
33. P. Werkhoff et al., *ACS Symp. Ser.* **564**, 199–223 (1994).
34. L. Mauri et al., *Int. J. Food Sci. Technol.* **24**, 1–9 (1989).
35. M. P. Lamden, in W. H. Sebrell, Jr. and R. S. Harris, eds., *The Vitamins*, 2nd ed., Vol. V, Academic Press, Inc., New York, 1972, pp. 114–120.
36. A. Mozafar, *Plant Soil* **167**, 305–311 (1994).
37. R. E. Davis and G. C. Icke, *Adv. Clin. Chem.* **23**, 93 (1983).
38. P. M. Finglas, *Int. J. Vit. Nutr. Res.* **63**, 270 (1993).
39. J. Harmeyer and U. Kollenkirchen, *Nutr. Res. Rev.* **2**, 201–225 (1989).
40. D. Brown et al., *The Pyrimidines*, 2nd ed., John Wiley & Sons, Inc., New York, 1994.
41. U.S. Pat. 2,235,862 (Mar. 25, 1940), O. Zima (to Merck & Co.).
42. R. Grewe, *Z. Physiol. Chem.* **242**, 89 (1936).
43. Fr. Pat. 831,110 (Aug. 23, 1938), (to Hoffmann-La Roche).
44. A. Takimizawa and K. Hirai, *Chem. Pharm. Bull.* **12**, 393 (1964).
45. H. Miromoto et al., *Chem. Ber.* **106**, 893 (1973).
46. K. Tokuyama et al., *Bull. Chem. Soc. Jap.* **46**, 253 (1973).
47. U.S. Pat. 4,226,799 (Oct. 16, 1978), W. Bewert and W. Littmann (to BASF).
48. Ger. Pat. 3,511,273 (Mar. 28, 1985), W. Ernst and J. Paust (to BASF).
49. Ger. Pat. 3,520,982 (Dec. 18, 1986), H. Kiefer and W. Bewert (to BASF).
50. K. Nishihara et al., *Kagaku Kogaku* **55**, 433 (1991).
51. Eur. Pat. 55,108 (June 30, 1982), K. Matsui et al. (to Ube Industries).
52. Eur. Pat. 279,556 (Aug. 24, 1988), K. Nishihara et al. (to Ube Industries).
53. U.S. Pat. 4,536,577 (Aug. 20, 1985), H. Yoshida and S. Niida (to Ube Industries).
54. Ger. Pat. 3,303,789 (Aug. 11, 1983), H. Yoshida et al. (to Ube Industries).
55. Jpn. Pat. 6,307,869 (July 10, 1988). Y. Miyashiro et al. (to Takeda Chemical Industries).
56. A. Edenhoffer et al., *Helv. Chim. Acta* **58**, 1230 (1975).
57. T. Matsukawa et al., *J. Pharm. Soc. Japan* **63**, 216 (1943).
58. K. Washimi, *J. Pharm. Soc. Japan.* **66**, 62 (1946).
59. T. Matsukawa and S. Hojiro, *J. Pharm. Soc. Japan* **69**, 550 (1949); *ibid.* **70**, 28 (1950); **71**, 667, 720, 1215 (1951).
60. H. Hirano and Y. Yonemitsu, *J. Pharm. Soc. Japan* **76**, 1332 (1956).
61. G. Moine et al., *Helv. Chim. Acta* **73**, 1300 (1990).
62. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, pp. 324–325.
63. *The United States Pharmacopeia XXII* (USP XXII-NF XVII) United States Pharmacopeial Convention, Inc., Rockville, Md., 1990, pp. 1356, 1357–1358.
64. F. J. Al-Shammary et al., *Anal. Profiles Drug Subst.* **18**, 414 (1989).
65. H. E. Samerlich and L. J. Machlin, *Ann. NY Acad. Sci.* **699**, 1 (1992).
66. W. C. Ellefson, in J. Augustine, B. P. Klein, D. A. Becker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, pp. 351–352.

67. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1990, pp. 942.23, 953.17, 957.17, and 986.27.

68. T. Kawasaki, in A. P. Leenheer, W. E. Lambert, and M. G. M. DeRuyter, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1992, pp. 319–354.

69. U. Jegle, *J. Chrom.* **652**, 495–501 (1993).

70. C. Kawasaki, in R. Hamb, I. Wool, and J. Lomaine, eds., *Vitamins and Hormones*, Vol. 21, Academic Press, Orlando, Fla., 1963, pp. 69–111.

71. R. E. Echols et al., *J. Chrom.* **347**, 89–97 (1985).

72. C. E. Mueller et al., *Int. J. Pharm.* **57**, 41–47 (1989).

73. Y. Susuki and K. Uchida, *Biosci. Biotech. Biochem.* **58**, 1273 (1994).

74. W. H. Ott et al., *Poult. Sci.* **44**, 920 (1965).

75. A. Iwashima in E. Vandamme, ed., *Biotechnology of Vitamins, Pigments and Growth Factors*, Elsevier, Amsterdam, the Netherlands, 1988.

76. D. W. Young, *Nat. Prod. Rep.* **3**, 395 (1986).

77. G. M. Brown and J. M. Williamson, in F. C. Neidhardt, ed., *Escherichia coli and salmonella typhimurium*, American Society for Microbiology, Washington, D.C., 1987, p. 521.

78. A. Silhamkeva, *J. Inst. Brew.* **191**, 78 (1985).

79. G. G. Stewart et al., *Can. J. Microbiol.* **38**, 1156 (1992).

80. T. Kozlok and I. D. Spenser, *J. Am. Chem. Soc.* **109**, 4968 (1987).

81. D. M. Downs and L. Peterson, *J. Bacteriol.* **176**, 4858 (1994).

82. N. J. Leonard et al., *Proc. Natl. Acad. Sci.* **85**, 7174 (1988).

83. B. Estramareix et al., *Biochem. Biophys. Acta* **1035**, 154 (1990).

84. K. Tazuya et al., *Biochem. Mol. Biol. Int.* **30**, 893 (1993).

85. K. Tazuya et al., *Biochem. Int.* **10**, 689 (1985).

86. R. H. White and F. B. Rudolph, *Biochem. Biophys. Acta* **542**, 340 (1978).

87. K. Tazuya et al., *Biochem. Int.* **14**, 153 (1987).

88. Y. Kawasaki, *J. Bacteriol.* **175**, 5153 (1993).

89. A. Iwashima et al., *J. Biol. Chem.* **268**, 17440 (1993).

90. T. Nohno, Y. Kasai, and T. Saito, *J. Bacteriol.* **170**, 4097 (1988).

91. N. Imamura and H. Nakayama, *J. Bacteriol.* **151**, 708 (1982).

92. J. Ryals et al., *J. Bacteriol.* **151**, 899 (1982).

93. T. P. Begley et al., *J. Am. Chem. Soc.* **117**, 2351 (1995).

94. H. Brennen et al., *J. Bacteriol.* **167**, 66 (1986).

95. J. A. Hoch et al., in A. L. Sonenshein, J. A. Hoch, and R. Losick, eds., *Bacillus subtilis*, American Society for Microbiology, Washington, D.C., 1993, pp. 425.

96. K. G. Maundrell et al., *Yeast* **10**, 1075 (1994).

97. A. Zurlinden and M. E. Schweingruber, *J. Bacteriol.* **176**, 6631 (1994).

98. A. Iwashima et al., *J. Bacteriol.* **172**, 6145 (1990); *ibid.* **174**, 4701 (1992).

99. A. Schweingruber et al., *Genetics* **130**, 445 (1992).

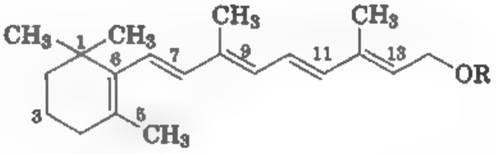
David Burdick
Hoffmann-La Roche, Inc.

VITAMINS: VITAMIN A

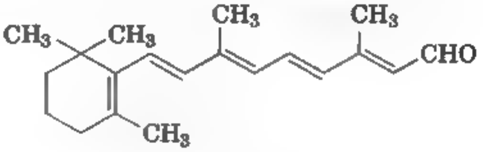
The curative role of the juice of cooked liver extracts in the treatment of night blindness was first recognized and practiced by the Egyptians in ancient times (1). Several millenniums later, in the early part of the twentieth century, many groups identified a lipid-soluble factor found in milk, butter, and egg yolks which was therapeutic for this condition. In 1913, McCollum and Davis coined the term fat-soluble A and subsequently, ascribed the growth-promoting effects of liver extracts to this material (2,3). Later, Osborne and Mendel found that cod liver oil contained an ingredient that was essential for growth promotion in rats (4).

The structure of vitamin A [*11103-57-4*] and some of the important derivatives are shown in Figure 1. The parent structure is all-*trans*-retinol [*68-26-8*] and its IUPAC name is (all-*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol (**1**). The numbering system for vitamin A derivatives parallels the system used for the carotenoids. In older literature, vitamin A compounds are named as derivatives of trimethyl cyclohexene and the side chain is named as a substituent. For retinoic acid derivatives, the carboxyl group is denoted as C-1 and the trimethyl cyclohexane ring as a substituent on C-9. The structures of vitamin A and *β*-carotene were elucidated by Karrer in 1930 and several derivatives of the vitamin were prepared by this group (5,6). In 1935, Wald isolated a substance found in the visual pigments of the eye and was able to show that this material was identical with Karrer's retinaldehyde [*116-31-4*] (**5**) (7).

Vitamin A_2 [*79-80-1*] (**6**) is structurally similar to vitamin A_1 [*68-26-8*] and is also found in fish oils. This compound is important biologically for fish and other lower animals. Interestingly, tadpoles require vitamin A_2 but after metamorphosis require vitamin A_1 (8).



(**1**, R = H)
(**2**, R = CH_3CO)
(**3**, R = $CH_3(CH_2)_{14}CO$)
(**4**, R = CH_3CH_2CO)



(**5**)

Figure 1. Vitamin A and derivatives: retinol (1), retinyl acetate [*127-47-9*] (**2**), retinyl palmitate [*79-81-2*] (**3**), and retinyl propionate [*7069-42-3*] (**4**).

67. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1990, pp. 942.23, 953.17, 957.17, and 986.27.

68. T. Kawasaki, in A. P. Leenheer, W. E. Lambert, and M. G. M. DeRuyter, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1992, pp. 319–354.

69. U. Jegle, *J. Chrom.* **652**, 495–501 (1993).

70. C. Kawasaki, in R. Hamb, I. Wool, and J. Lomaine, eds., *Vitamins and Hormones*, Vol. 21, Academic Press, Orlando, Fla., 1963, pp. 69–111.

71. R. E. Echols et al., *J. Chrom.* **347**, 89–97 (1985).

72. C. E. Mueller et al., *Int. J. Pharm.* **57**, 41–47 (1989).

73. Y. Susuki and K. Uchida, *Biosci. Biotech. Biochem.* **58**, 1273 (1994).

74. W. H. Ott et al., *Poult. Sci.* **44**, 920 (1965).

75. A. Iwashima in E. Vandamme, ed., *Biotechnology of Vitamins, Pigments and Growth Factors*, Elsevier, Amsterdam, the Netherlands, 1988.

76. D. W. Young, *Nat. Prod. Rep.* **3**, 395 (1986).

77. G. M. Brown and J. M. Williamson, in F. C. Neidhardt, ed., *Escherichia coli and salmonella typhimurium*, American Society for Microbiology, Washington, D.C., 1987, p. 521.

78. A. Silhamkeva, *J. Inst. Brew.* **191**, 78 (1985).

79. G. G. Stewart et al., *Can. J. Microbiol.* **38**, 1156 (1992).

80. T. Kozlok and I. D. Spenser, *J. Am. Chem. Soc.* **109**, 4968 (1987).

81. D. M. Downs and L. Peterson, *J. Bacteriol.* **176**, 4858 (1994).

82. N. J. Leonard et al., *Proc. Natl. Acad. Sci.* **85**, 7174 (1988).

83. B. Estramareix et al., *Biochem. Biophys. Acta* **1035**, 154 (1990).

84. K. Tazuya et al., *Biochem. Mol. Biol. Int.* **30**, 893 (1993).

85. K. Tazuya et al., *Biochem. Int.* **10**, 689 (1985).

86. R. H. White and F. B. Rudolph, *Biochem. Biophys. Acta* **542**, 340 (1978).

87. K. Tazuya et al., *Biochem. Int.* **14**, 153 (1987).

88. Y. Kawasaki, *J. Bacteriol.* **175**, 5153 (1993).

89. A. Iwashima et al., *J. Biol. Chem.* **268**, 17440 (1993).

90. T. Nohno, Y. Kasai, and T. Saito, *J. Bacteriol.* **170**, 4097 (1988).

91. N. Imamura and H. Nakayama, *J. Bacteriol.* **151**, 708 (1982).

92. J. Ryals et al., *J. Bacteriol.* **151**, 899 (1982).

93. T. P. Begley et al., *J. Am. Chem. Soc.* **117**, 2351 (1995).

94. H. Brennen et al., *J. Bacteriol.* **167**, 66 (1986).

95. J. A. Hoch et al., in A. L. Sonenshein, J. A. Hoch, and R. Losick, eds., *Bacillus subtilis*, American Society for Microbiology, Washington, D.C., 1993, pp. 425.

96. K. G. Maundrell et al., *Yeast* **10**, 1075 (1994).

97. A. Zurlinden and M. E. Schweingruber, *J. Bacteriol.* **176**, 6631 (1994).

98. A. Iwashima et al., *J. Bacteriol.* **172**, 6145 (1990); *ibid.* **174**, 4701 (1992).

99. A. Schweingruber et al., *Genetics* **130**, 445 (1992).

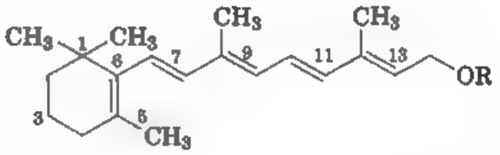
DAVID BURDICK
Hoffmann-La Roche, Inc.

VITAMINS: VITAMIN A

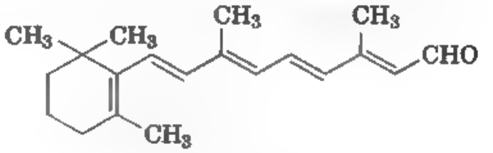
The curative role of the juice of cooked liver extracts in the treatment of night blindness was first recognized and practiced by the Egyptians in ancient times (1). Several millenniums later, in the early part of the twentieth century, many groups identified a lipid-soluble factor found in milk, butter, and egg yolks which was therapeutic for this condition. In 1913, McCollum and Davis coined the term fat-soluble A and subsequently, ascribed the growth-promoting effects of liver extracts to this material (2,3). Later, Osborne and Mendel found that cod liver oil contained an ingredient that was essential for growth promotion in rats (4).

The structure of vitamin A [*11103-57-4*] and some of the important derivatives are shown in Figure 1. The parent structure is all-*trans*-retinol [*68-26-8*] and its IUPAC name is (all-*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol (**1**). The numbering system for vitamin A derivatives parallels the system used for the carotenoids. In older literature, vitamin A compounds are named as derivatives of trimethyl cyclohexene and the side chain is named as a substituent. For retinoic acid derivatives, the carboxyl group is denoted as C-1 and the trimethyl cyclohexane ring as a substituent on C-9. The structures of vitamin A and β-carotene were elucidated by Karrer in 1930 and several derivatives of the vitamin were prepared by this group (5,6). In 1935, Wald isolated a substance found in the visual pigments of the eye and was able to show that this material was identical with Karrer's retinaldehyde [*116-31-4*] (**5**) (7).

Vitamin A_2 [*79-80-1*] (**6**) is structurally similar to vitamin A_1 [*68-26-8*] and is also found in fish oils. This compound is important biologically for fish and other lower animals. Interestingly, tadpoles require vitamin A_2 but after metamorphosis require vitamin A_1 (8).

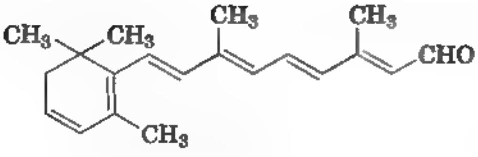


(**1**, R = H)
(**2**, R = CH_3CO)
(**3**, R = $CH_3(CH_2)_{14}CO$)
(**4**, R = CH_3CH_2CO)



(**5**)

Figure 1. Vitamin A and derivatives: retinol (1), retinyl acetate [*127-47-9*] (**2**), retinyl palmitate [*79-81-2*] (**3**), and retinyl propionate [*7069-42-3*] (**4**).

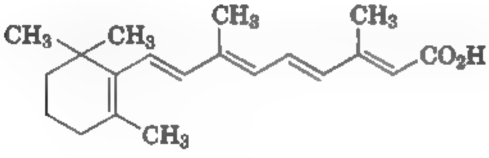


(6)

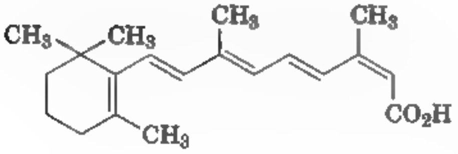
Vitamin A constitutes the most significant sector of the commercial retinoid market and is used primarily in the feed area. In the pharmaceutical area, there are several important therapeutic dermatologic agents which structurally resemble vitamin A and they are depicted in Figure 2. The carotenoids as provitamin A compounds also represent an important commercial class of compounds with β-carotene [*7235-40-7*] (**10**) occupying the central role (Fig. 3) (9).

CHEMICAL AND PHYSICAL PROPERTIES

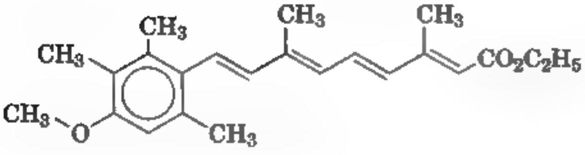
Because of the presence of an extended polyene chain, the chemical and physical properties of the retinoids and carotenoids are dominated by this feature. Vitamin A and related substances are yellow compounds which are unstable in the presence of oxygen and light. This decay can be accelerated by heat and trace metals. Retinol is stable to base but is subject to acid-catalyzed dehydration in the presence of dilute acids to yield anhydrovitamin A [*1224-18-8*] (**16**). Retrovitamin A [*16729-22-9*] (**17**) is obtained by treatment of retinol in the presence of concentrated hydrobromic acid. In the case of retinoic acid and retinal, reisomerization is possible after conversion to appropriate derivatives such as the acid chloride or the hydroquinone adduct. Table 1 lists the physical properties of β-carotene [*7235-40-7*] and vitamin A.



(7)

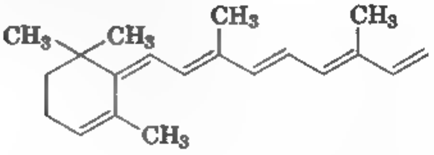


(8)

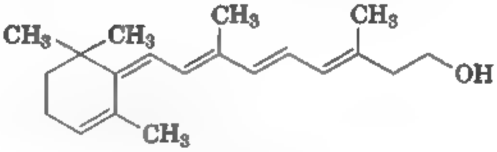


(9)

Figure 2. Commercially important retinoids: retinoic acid [*302-79-4*] (Tretinoin) (**7**), 13-*Z*-retinoic acid [*4759-48-2*] (Isotretinoin) (**8**), and etretinate [*54350-48-0*] (**9**).



(16)

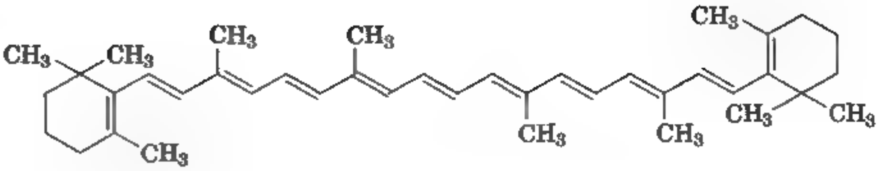


(17)

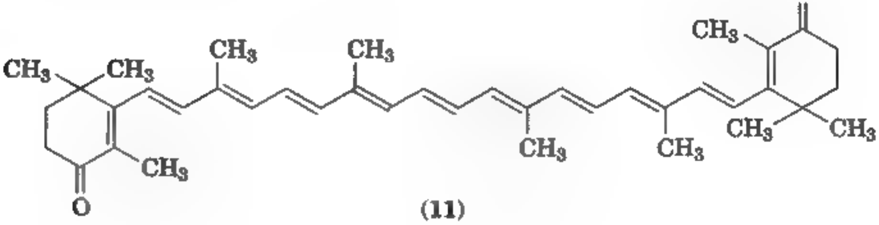
Of the 16 possible geometric isomers of vitamin A, only the all-trans form has full vitamin A activity and these compounds are commonly named according to the older, nonsystematic nomenclature (Table 2). From a biological standpoint, 11-*cis*-retinal plays a critical role in vision (*vide infra*). As earlier described, 13-*cis*-retinoic acid is important as a dermatological agent. Recently, 9-*cis*-retinoic acid has also been shown to be an important biological isomer and has been identified as a novel endogenous hormone in mammalian tissues (11). The other 13 isomers have no biological or commercial significance. Isolation, characterization, and synthesis of these isomers have been reported (12).

The most conspicuous physical feature of the retinoids and of the carotenoids is the uv spectrum. In the case of the carotenoids, this coloration property is one reason for their commercial significance. In general, there are several factors which influence the position of λ_{max}, the intensity of the absorbance, and the degree of fine structure (13). These include the length of the polyene chain, the number of cis double bonds, and end group functionality. For example, all-*trans*-retinal (**5**) has an absorption maximum at 368 nm and a molecular extinction coefficient at 48,000, whereas the 7,9,11,13-cis isomer has values of 308 nm and 15,500. Both hypsochromic shifts and hypochromic effects are observed when the stereochemistry of the double bond is changed from trans to cis. External factors such as solvent, temperature, and molecular environments also influence the uv spectra. A striking example of the latter phenomenon is the observed significant bathochromic shift (ca 150 nm) during the association of a carotenoid with a lipoprotein. From a molecular standpoint, the origins of this effect are not completely understood and this remains an area of active research (14,15).

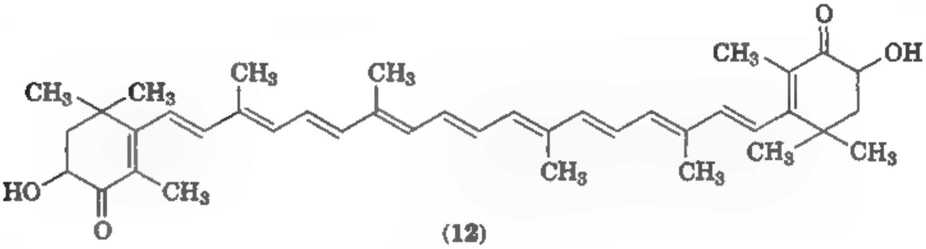
Other spectroscopic methods such as infrared (ir), ^{1}H and ^{13}C nuclear magnetic resonance (nmr), circular dichroism (CD), and mass spectrometry (MS) are invaluable tools for identification and structure elucidation. Nmr spectroscopy allows for geometric assignment of the carbon–carbon double bonds, as well as relative stereo-



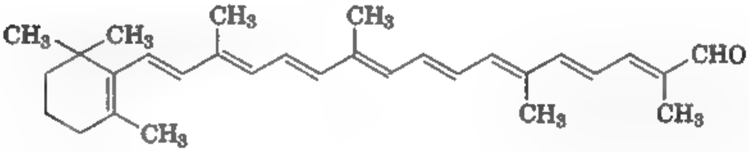
(10)



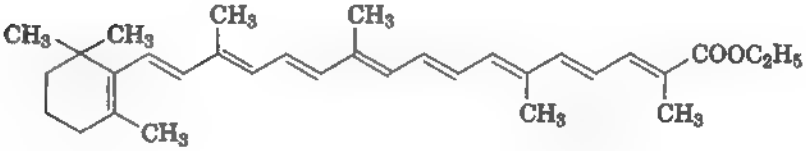
(11)



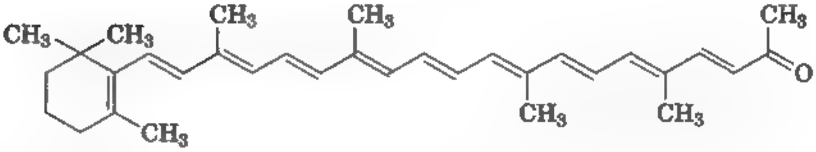
(12)



(13)



(14)



(15)

Figure 3. Commercially important carotenoids: β-carotene (**10**), canthaxanthin [*514-78-3*] (**11**), astaxanthin [*472-61-7*] (**12**), β-apo-8′-carotenal [*1107-26-2*] (**13**), β-apo-8′-carotenoic acid ethyl ester [*1109-11-1*] (**14**), and citranaxanthin [*3604-90-8*] (**15**).

chemistry of ring substituents. These spectroscopic methods coupled with traditional chemical derivatization techniques provide the framework by which new carotenoids are identified and characterized (16,17).

SYNTHESIS

Vitamin A acetate [*11098-51-4*] (**2**) is the commercially significant form of the vitamin and is mainly produced by Hoffmann-La Roche, BASF, and Rhône-Poulenc (Fig. 4). All of these processes have β-ionone (**18**) as their key intermediate and in this regard are based on work performed in the 1940s (18,19). Their differences lie in methodology to this key intermediate as well as in the methodology to elaborate the side chain. A review of the early work is available (20).

In the process practiced by Hoffmann-La Roche, a C_{13} + C_1 + C_6 strategy is employed. In this approach, β-ionone

Table 1. Properties of β-Carotene and Vitamin A Derivatives

Property	Retinol[a]	Retinyl acetate[a]	Retinyl palmitate[a]	Retinyl propionate[b]	β-Carotene[a]
Appearance	Crystalline	Crystalline	Crystalline or amorphous	Oil	Crystalline
Color	Yellow	Yellow	Yellow	Light yellow	Dark red-dark purple
Odor	Faint, hay-like	Faint, hay-like	Faint, hay-like	Slight	Faint, hay-like
Molecular weight	286.46	328.50	534.88	342.50	536.85
Molecular formula	$C_{20}H_{30}O$	$C_{22}H_{32}O_2$	$C_{36}H_{60}O_2$	$C_{23}H_{34}O_2$	$C_{40}H_{56}$
Melting point, °C	63–64	57–59	28–29		180–182
Solubility, g/100 mL					
Water	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Ethanol	Soluble	Soluble	Soluble	Soluble	Slightly soluble
Isopropanol	Soluble	Soluble	Soluble		Slightly soluble
Chloroform	Soluble	Soluble	Soluble		Soluble
Acetone	Soluble	Soluble	Soluble		Slightly soluble
Fats, oils	750	750	750		0.05–0.08
Spectrophotometric properties, nm, max	375[c]	326[c]	325[c]		497, 466[d]
Fluorescence					
Excitation, max, nm	325	325	325		
Emission, max, nm	470	470	470		

Source. [a]Ref. 8 and [b]Ref. 10.

[c] Isopropanol.

[d] Chloroform.

Table 2. Physical Properties of Stereoisomers of Vitamin A

Isomer	Alcohol mp (°C)	λ_{max} (nm)	E_{max}	Vitamin A potency (%)	Aldehyde mp (°C)	λ_{max} (nm)	E_{max}	Vitamin A potency (%)
All-*trans*-Vitamin A	62–64	325	1,832	100	57/65	381	1,530	91
13-*cis*-Vitamin A	58–60	328	1,686	75	77	375	1,250	93
11-*cis*-Vitamin A	Oil	319	1,220	23	63.5–64.7	376.5	878	48
11,13-di-*cis*-Vitamin A	86–88	311	1,024	15	oil	373	700	31
9-*cis*-Vitamin A	81.5–82.5	323	1,477	22	64	373	1,270	19
9,13-di-*cis*-Vitamin A	58–59	324	1,379	24	49/85	368	1,140	17

(18) is subjected to a Darzen's condensation to yield the C_{14} aldehyde [116-31-4] **(19)** (see Fig. 4). Construction of the side chain is completed by a metal acetylide coupling reaction with compound **(20)**. Acetylation, partial reduction of the triple bond, and acid-catalyzed elimination of water completes the synthesis. BASF and Rhône-Poulenc use a different scheme and extend the side chain of β-ionone in an initial step via a Grignard reaction with a metal acetylide. Semihydrogenation yields vinyl β-ionol **(21)** and it is at this point that the approaches diverge. In the Rhône-Poulenc $C_{15} + C_5$ synthesis, the carbon terminus of vinyl β-ionol is activated by conversion to the sulfone **(22)**. In a second step, the anion of the sulfone is reacted with C_5-chloroacetate **(23)** to yield vitamin A acetate. BASF utilizes a Wittig olefination and first prepares the phosphonium salt of the C_{15} unit. Reaction of the salt **(24)** with the C_5 aldehyde **(25)** leads to vitamin A acetate (21).

Vitamin A palmitate [*79-81-2*] **(3)**, a commercially important form of the vitamin, is produced from vitamin A acetate **(2)** via a transesterification reaction with methyl palmitate. Enzymatic preparation of the palmitate from the acetate has also been described (22).

In addition to differences in their methodology to extend the carbon chain, these manufacturers differ in their syntheses of β-ionone. β-Ionone is commercially prepared via an acid-catalyzed rearrangement of pseudoionone **(26)**. This intermediate is manufactured on an industrial scale from either citral **(27)** or dehydro-linalool **(28)** (21) (Fig. 5).

Citral is prepared starting from isobutene and formaldehyde to yield the important C_5 intermediate 3-methylbut-3-enol **(29)**. Pd-catalyzed isomerization affords 3-methylbut-2-enol **(30)**. The second C_5 unit of citral is derived from oxidation of **(30)** to yield 3-methylbut-2-enal **(31)**. Coupling of these two fragments produces the dienol ether **(32)** and this is followed by an elegant double Cope rearrangement (21) (Fig. 6).

The synthesis of dehydro-linalool **(28)** relies on the basic chemicals acetone and acetylene. Addition of a metal acetylide to acetone yields methylbutynol **(33)**. Semihydrogenation affords the alkene **(34)** which is reacted with *i*-propenyl-methyl ether. A Cope rearrangement of the adduct yields methylheptenone **(35)**. Addition of a second mole of metal acetylide to dehydro-linalool **(28)** is followed

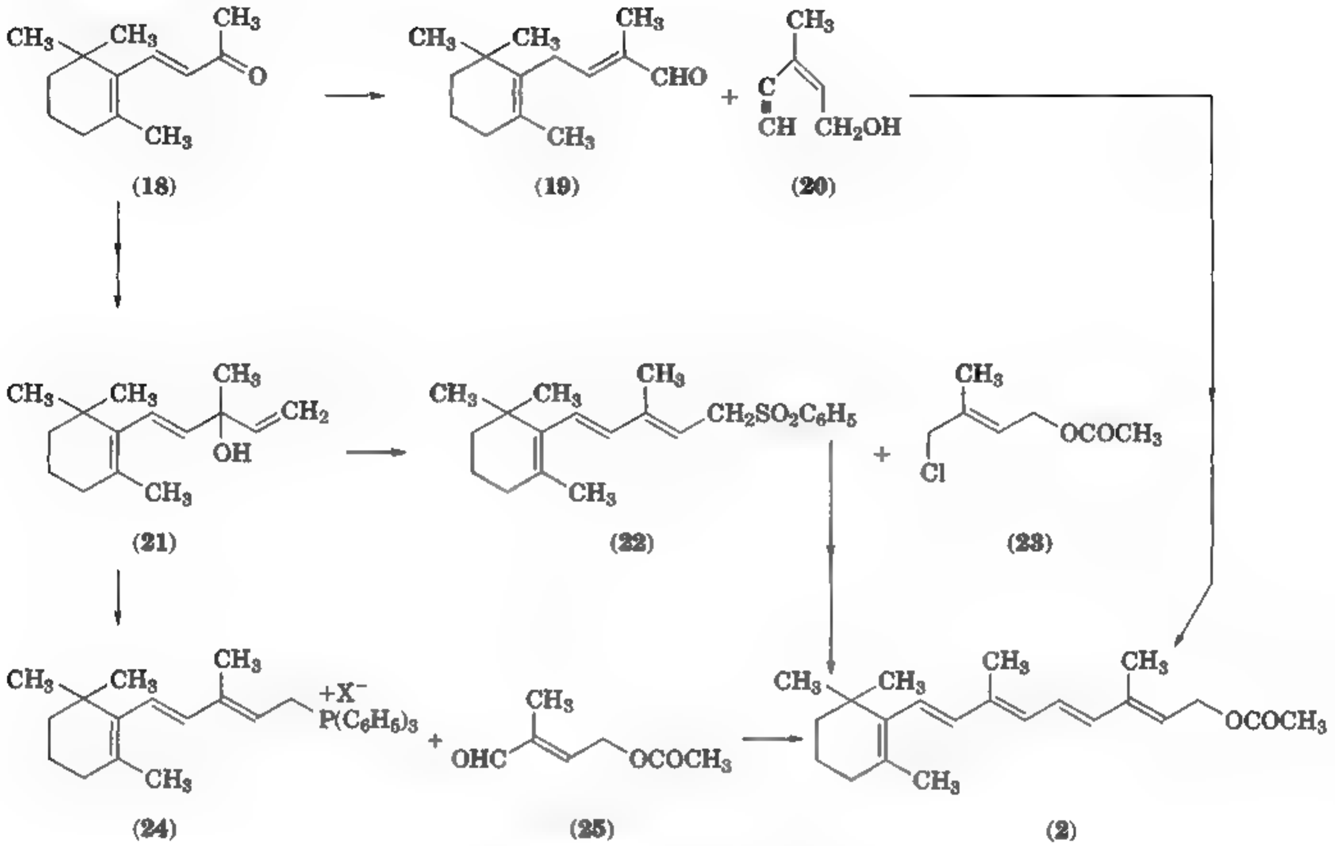


Figure 4. Commercial synthesis of vitamin A acetate (**2**).

by a second Cope rearrangement to yield pseudoionone (**26**) (9,21) (Fig. 7).

In other work, sulfone chemistry plays an integral part of the syntheses of both β-carotene and vitamin A by workers at Kuraray. In this approach, the anion of C_{10} β-cyclogeranyl sulfone (**36**) is condensed with the C_{10} aldehyde (**37**). The resulting β-hydroxy sulfone (**38**) is treated with dihydropyran followed by a double elimination to yield vitamin A acetate. Alternatively, the β-hydroxy sulfone (**38**) can be converted to the δ-halo sulfone (**39**) and a similar double elimination scheme is employed (23,24) (Fig. 8).

Work at Rhône-Poulenc has involved a different approach to retinal and is based on the palladium-catalyzed rearrangement of the mixed carbonate (**41**) to the allenyl enal (**42**). Isomerization of the allene (**42**) to the polyene (**43**) completes the construction of the carbon framework. Acid-catalyzed isomerization yields retinal (**5**). A decided advantage of this route is that no by-products such as triphenylphosphine oxide or sodium phenylsulfinate are formed. However, significant yield improvements would be necessary for this process to compete with the current commercial syntheses (25–27) (Fig. 9).

In contrast to the similarities seen in the majority of the industrial syntheses of vitamin A, substantial diversity is observed in the preparations of the carotenoids. Owing to the fact that all-trans stereochemistry is required in the final product and that many olefination reactions yield the cis product, the ease of isomerization at a given locus on the polyene chain has influenced the choice of building blocks. In addition, technological strengths in the construction of these building blocks as well as synergies with a core olefination strength have played an important role in the choice of synthesis.

Hoffmann-La Roche has produced β-carotene since the 1950s and has relied on core knowledge of vitamin A chemistry for the synthesis of this target. In this approach, a five-carbon homologation of C_{14} vitamin A aldehyde (**19**) is accomplished by successive acetalizations and enol ether condensations to prepare the C_{19} aldehyde (**46**). Metal acetylide coupling with two molecules of aldehyde (**46**) completes construction of the C_{40} carbon framework. Selective reduction of the internal triple bond of (**47**) is followed by dehydration and thermal isomerization to yield β-carotene (21) (Fig. 10).

In the BASF synthesis, a Wittig reaction between two moles of C_{15} phosphonium salt (vitamin A intermediate (**24**) and C_{10} dialdehyde (**48**) is the important synthetic step (9,28,29). Thermal isomerization affords all *trans-β*-carotene (Fig. 11). In an alternative preparation by Roche, vitamin A process streams can be used and in this scheme, retinol is carefully oxidized to retinal, and a second portion is converted to the C_{20} phosphonium salt (**49**). These two halves are united using standard Wittig chemistry (8) (Fig. 12).

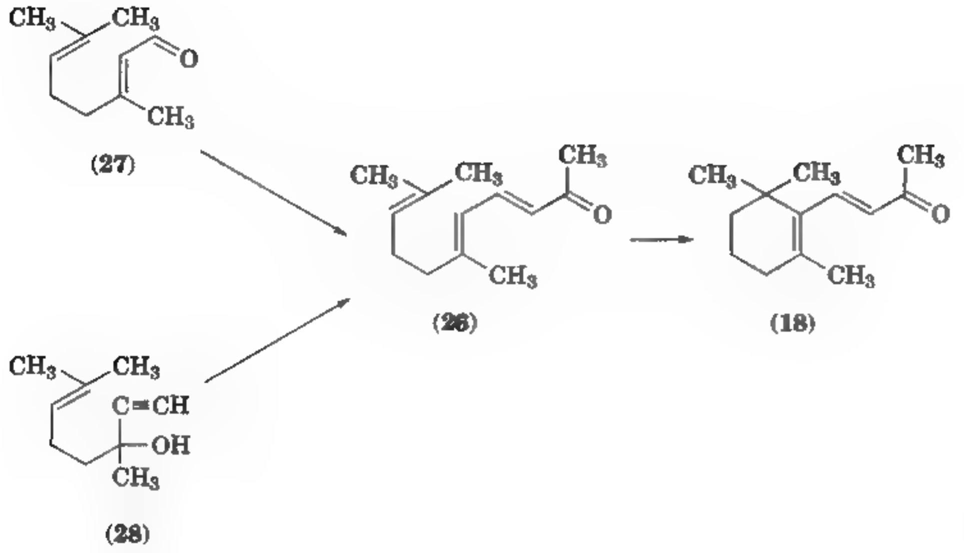


Figure 5. Industrial approaches to β-ionone (18).

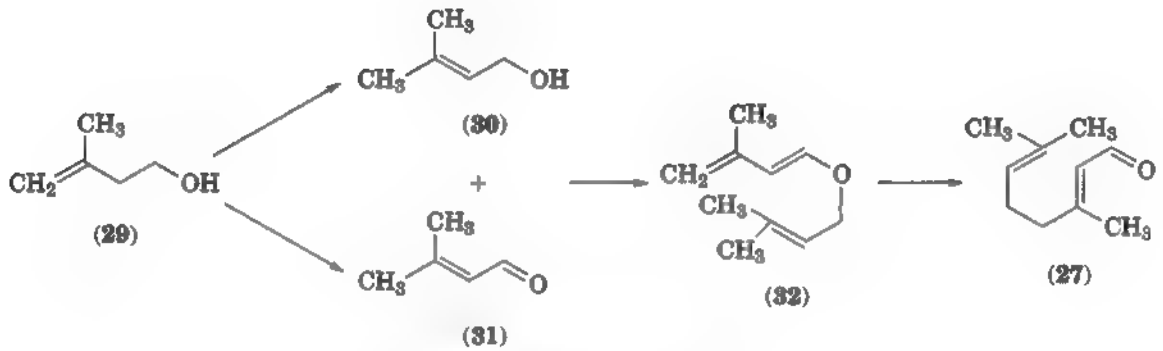


Figure 6. Synthesis of citral (27).

Other approaches to direct C_{20} couplings have been reported (9,30–35). Based on their knowledge of sulfone chemistry, Rhône-Poulenc has patented many syntheses of β-carotene which use this olefination chemistry (36–41). Horner-Emmons chemistry has also been employed for this purpose (42). The synthetic approaches to the carotenoids have been reviewed (43).

Compounds labeled ^{14}C, ^{13}C, ^{3}H, or ^{2}H are extremely important in understanding the absorption, distribution, metabolism, and excretion of these materials in biological systems. The preparation of these materials has been reviewed (44,45) and has been the subject of recent investigations (46).

BIOSYNTHESIS

In nature, vitamin A aldehyde is produced by the oxidative cleavage of β-carotene by 15,15′-β-carotene dioxygenase. Alternatively, retinal is produced by oxidative cleavage of β-carotene to β-apo-8′-carotenal followed by cleavage at the 15,15′-double bond to vitamin A aldehyde (47). Carotenoid biosynthesis and fermentation have been extensively studied both in academic as well as in industrial laboratories. On the commercial side, the focus of these investigations has been to increase fermentation titers by both classical and recombinant means.

The carotenoid skeleton is assembled in a primary step by the coupling of two moles of geranylgeranyl pyrophosphate (**52**) by an enzyme which is encoded by the crt B (carotenogenic) gene. The resulting prephytoene pyrophosphate (**53**) is further transformed to phytoene (**54**) possibly by products also derived from the crt B gene. Phytoene is a common intermediate in carotenoid biosynthesis. For example, in *E. uredovora* phytoene is converted to lycopene (**55**) in sequential dehydration steps. These reactions are catalyzed by an enzyme called phytoene desaturase which is encoded on the gene cluster crt I. Interestingly, only *cis*-phytoene and *trans*-lycopene are detected and it is hypothesized that *trans*-phytoene is formed in a nonenzymatic step. By further biosynthetic transformations, β-carotene (**10**) is produced from lycopene and zeaxanthin (**56**) from β-carotene (Fig. 13) (48).

The majority of industrial research describes classical approaches to yield improvement (49). However, there has been some work using genetically modified organisms. In the case of these recombinant organisms, the carotenoid biosynthetic gene cluster has been expressed in non-

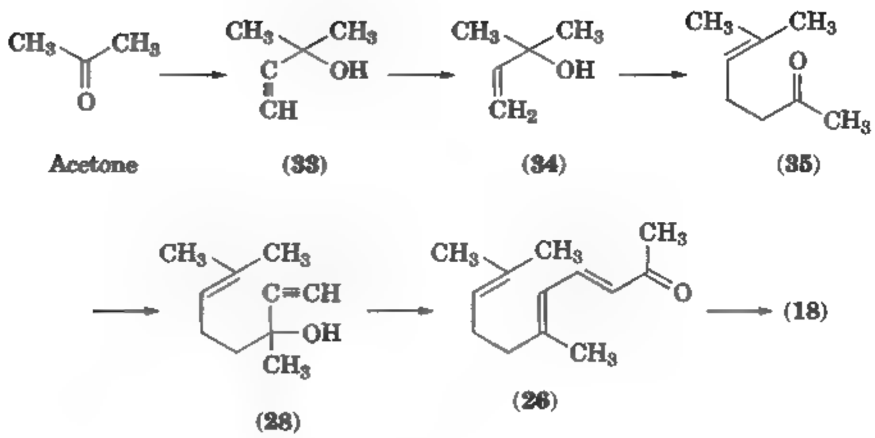


Figure 7. Acetone-based synthesis of β-ionone.

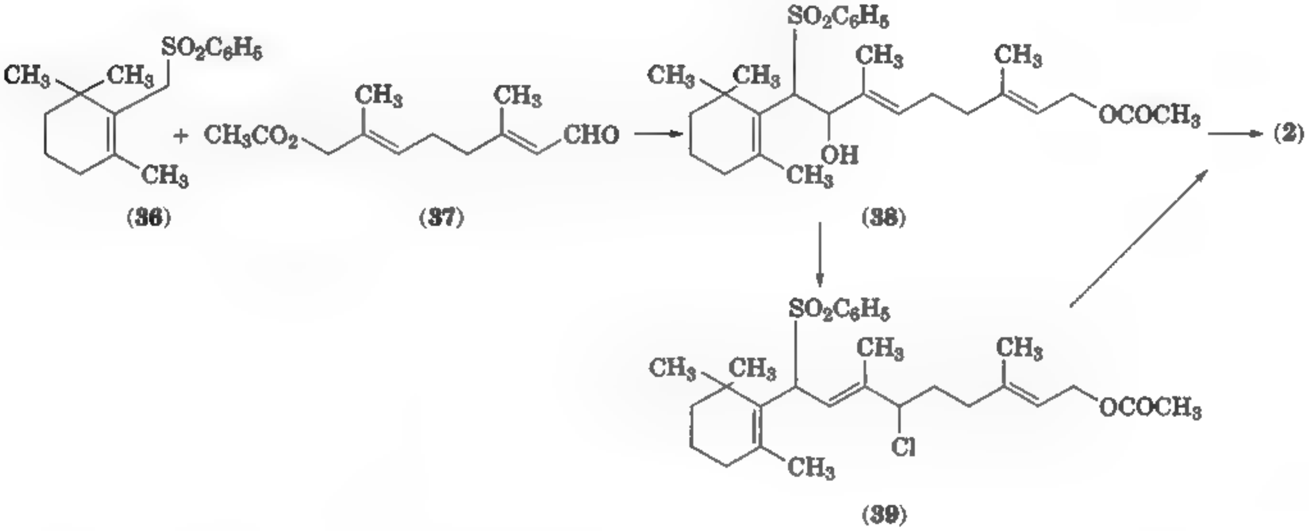


Figure 8. Kuraray synthesis.

carotegenic species such as *E. coli* (50) and *S. cerevisiae* (51).

ANALYTICAL METHODS

Biological, spectroscopic, and chromatographic methods have been used to assay vitamin A and the carotenoids. Biological methods have traditionally been based on the growth response of vitamin A-deficient rats. The utility and shortcomings of this test have been reviewed (52,53). This test has found applicability for analogues of retinol (54,55). Carotenoids which function as provitamin A precursors can also be assayed by this test (56).

Spectroscopic methods such as uv and fluorescence have relied on the polyene chromophore of vitamin A as a basis for analysis. Indirectly, the classical Carr-Price colorimetric test also exploits this feature and measures the amount of a transient blue complex at 620 nm which is formed when vitamin A is dehydrated in the presence of Lewis acids. For uv measurements of retinol, retinyl acetate, and retinyl palmitate, analysis is done at 325 nm. More sensitive measurements can be obtained by fluorescence. Excitation is done at 325 nm and emission at 470 nm. Although useful, all of these methods suffer from the fact that the method is not specific and any compound which has spectral characteristics similar to vitamin A will assay like the vitamin (57).

More specific methods involve chromatographic separation of the retinoids and carotenoids followed by an appropriate detection method. This subject has been reviewed (57). Typically, hplc techniques are used and are coupled with detection by uv. For the retinoids, fluorescent detection is possible and picogram quantities of retinol in plasma have been measured (58–62). These techniques are particularly powerful for the separation of isomers. Owing to the thermal lability of these compounds, gc methods have also been used but to a lesser extent. Recently, the utility of cool-on-column injection methods for these materials has been demonstrated (63).

Owing to the light and air sensitivity of the carotenoids and retinoids, sample handling is a critical issue. It is recommended to conduct extraction of these materials with peroxide-free solvents, to store biological samples at

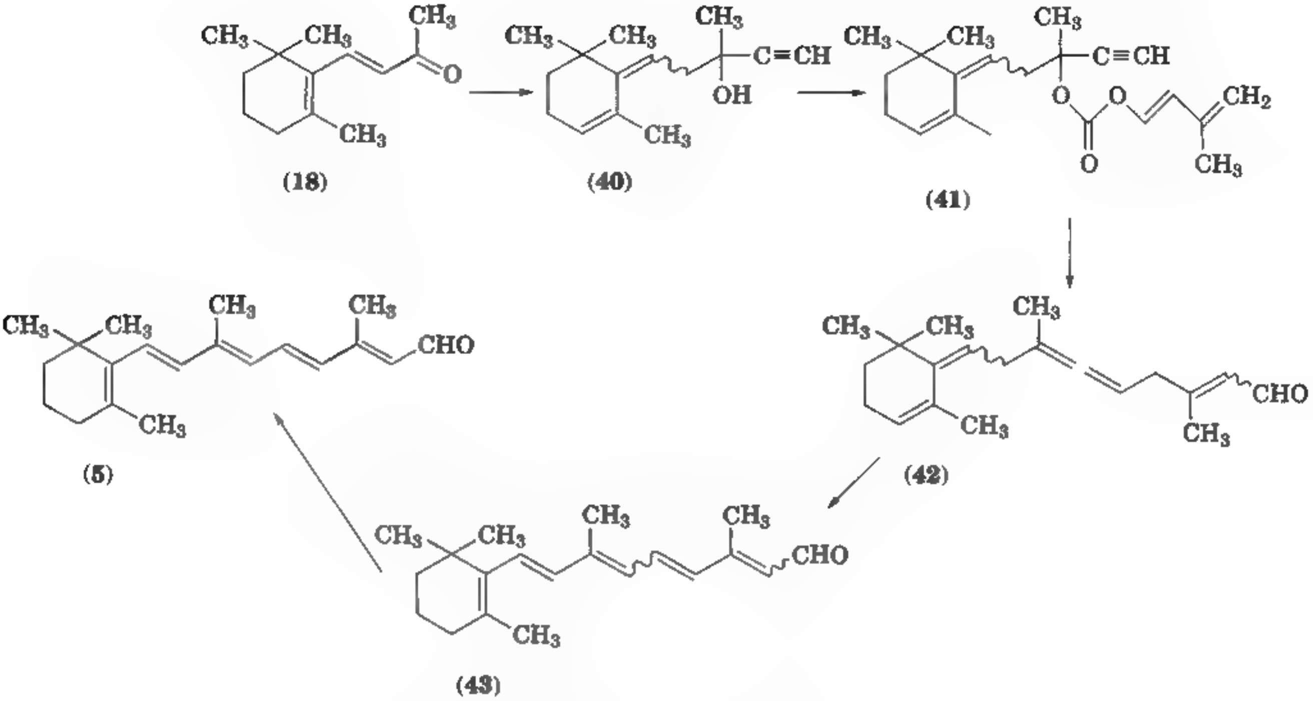


Figure 9. Allene synthesis of vitamin A aldehyde.

−70°C under argon and in the dark, to perform the analysis under yellow light, and to use reference compounds of high purity (57).

In the *United States Pharmacopeia* (USP), vitamin A content is determined by the ratio of the corrected absorbance to the observed absorbance and is not to be less than 0.85 at 325 nm. Total vitamin A content is to be 95% of label claim. For β-carotene, the assay is performed using uv spectroscopy and is determined by the absorbance at 455 nm. The range of the assay should be from 96.0 to 101.0% (64).

OCCURRENCE

Rich sources of vitamin A include dairy products such as milk, cheese, butter, and ice cream. Eggs as well as internal organs such as the liver, kidney, and heart also represent good sources. In addition, fish such as herring, sardines, and tuna, and in particular the liver oil from certain marine organisms, are excellent sources. Because the vitamin A in these food products is derived from dietary carotenoids, vitamin A content can vary considerably. Variation of vitamin A content in food can also result from food processing and in particular, oxidation processes (8).

Fertile sources of carotenoids include carrots and leafy green vegetables such as spinach. Tomatoes contain significant amounts of the red carotenoid, lycopene. Although lycopene has no vitamin A activity, it is a particularly efficient antioxidant. Oxidation of carotenoids to biologically inactive xanthophylls represents an important degradation pathway for these compounds (56).

BIOLOGICAL FUNCTIONS

In humans, vitamin A has important functions in vision, growth, and tissue differentiation. Its role in vision is fairly well understood and involves initial absorption of all-*trans*-retinol by the pigment epithelium cells of the eye. This event is followed by isomerization to 11-*cis*-retinol by all-*trans*-11-*cis*-retinol isomerase. After conversion of 11-*cis*-retinol to 11-*cis*-retinal, reaction with opsin forms rhodopsin. Absorption of light by this pigment generates the visual signal by cis/trans isomerization. After release of *trans*-retinal from the pigment, *trans*-retinal is rapidly reduced to all-*trans*-retinol and the cycle repeats itself. Consequently, vitamin A is not consumed in the process. One feature of this system is that the basic chemistry is common to many photosensitive systems from such diverse species as halophilic bacteria to higher organisms such as vertebrates (65).

As previously described, the biological assay for vitamin A was based on the growth response of rats fed a vitamin A-deficient diet. Vitamin A is necessary for normal bone growth and remodeling and is required for the activity of epiphyseal cartilage. Retinoic acid is the active form of vitamin A for this function and it has been observed that ingestion of retinoic acid restores growth but cannot maintain vision (8). In animals cycled on retinoic acid, ie, fed a retinoic acid-containing diet then deprived of a retinoic acid-containing diet, these animals become sensitive to the removal of retinoic acid. This condition is characterized by a loss in appetite followed by a depression in growth. As a result, loss of appetite is one of the first symptoms of a vitamin A-deficient animal (8).

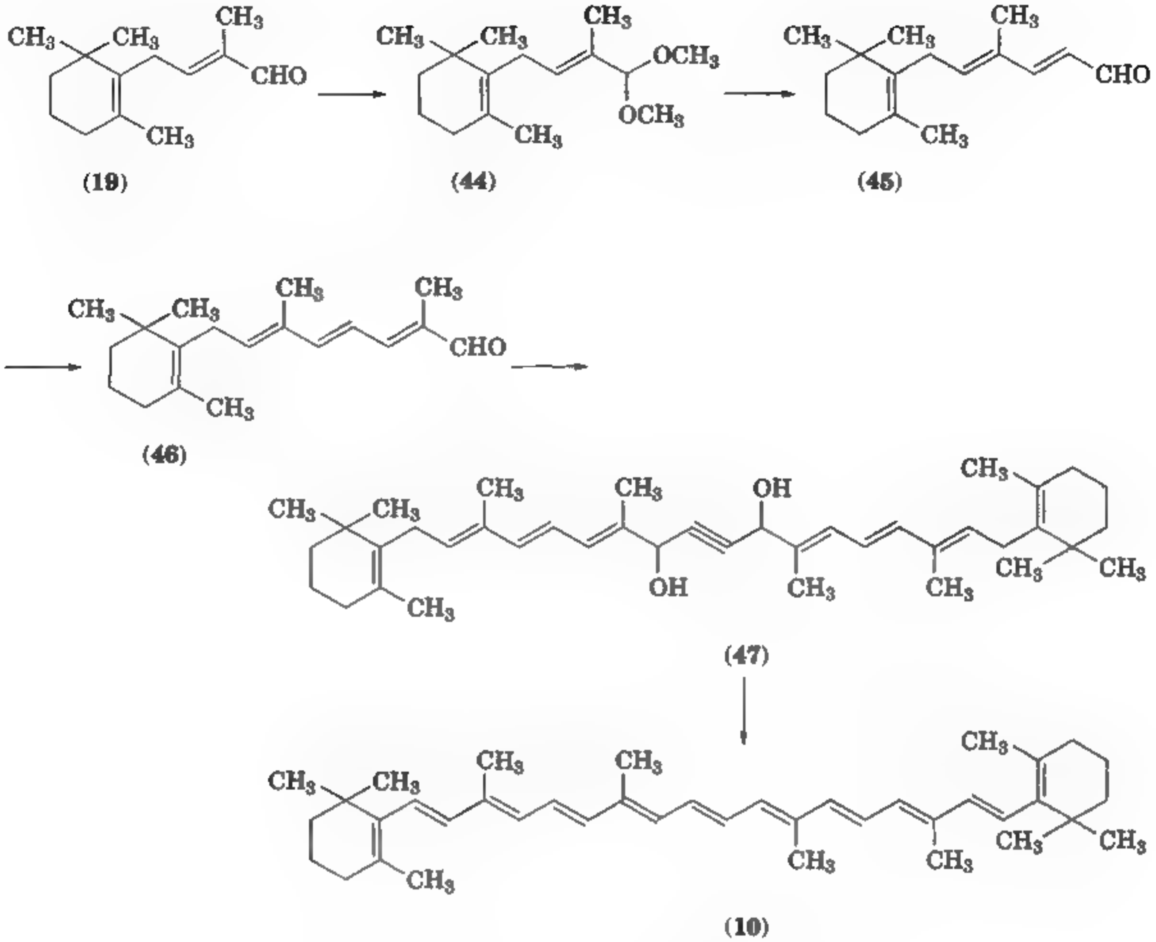


Figure 10. Hoffmann-La Roche synthesis of β-carotene.

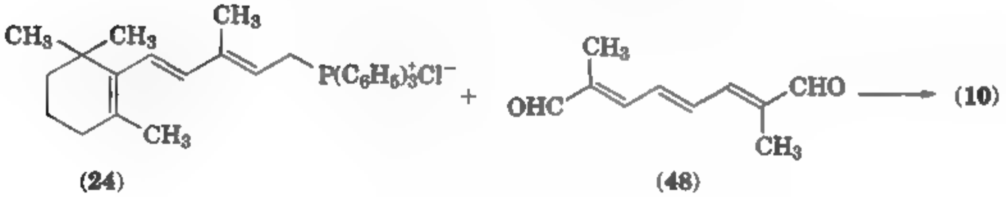


Figure 11. BASF synthesis of β-carotene.

The specific role of vitamin A in tissue differentiation has been an active area of research. The current thinking, developed in 1979, involves initial delivery of retinol by *holo*-RBP (retinol-binding protein) to the cell cytosol (66). Retinol is then ultimately oxidized to retinoic acid and binds to a specific cellular retinoid-binding protein and is transported to the nucleus. Retinoic acid is then transferred to a nuclear retinoic acid receptor (RAR) which enhances the expression of a specific region of the genome. Transcription occurs and new proteins appear during the retinoic acid-induced differentiation of cells (56).

In contrast to vitamin A, the carotenoids are important in both plant and animal kingdoms. In plants, carotenoids are associated with photosynthetic structures. The function of these pigments is twofold: (*1*) the pigments serve as acceptors and act as energy-transfer agents and allow for excitation energy to be transferred from the carotenoid to the porphyrin; (2) the pigments protect the organism from light-induced photooxidative damage. In this regard, protection is achieved by two mechanisms: quenching of the excited triplet state of chlorophyll (itself a producer of singlet oxygen), and quenching of singlet oxygen. In both cases, a triplet excited state of the carotenoid, which decays back to the ground state by a radiationless conversion, is produced (67).

Many carotenoids function in humans as vitamin A precursors; however, not all carotenoids have provitamin A activity (Table 3). Of the biologically active carotenoids, β-

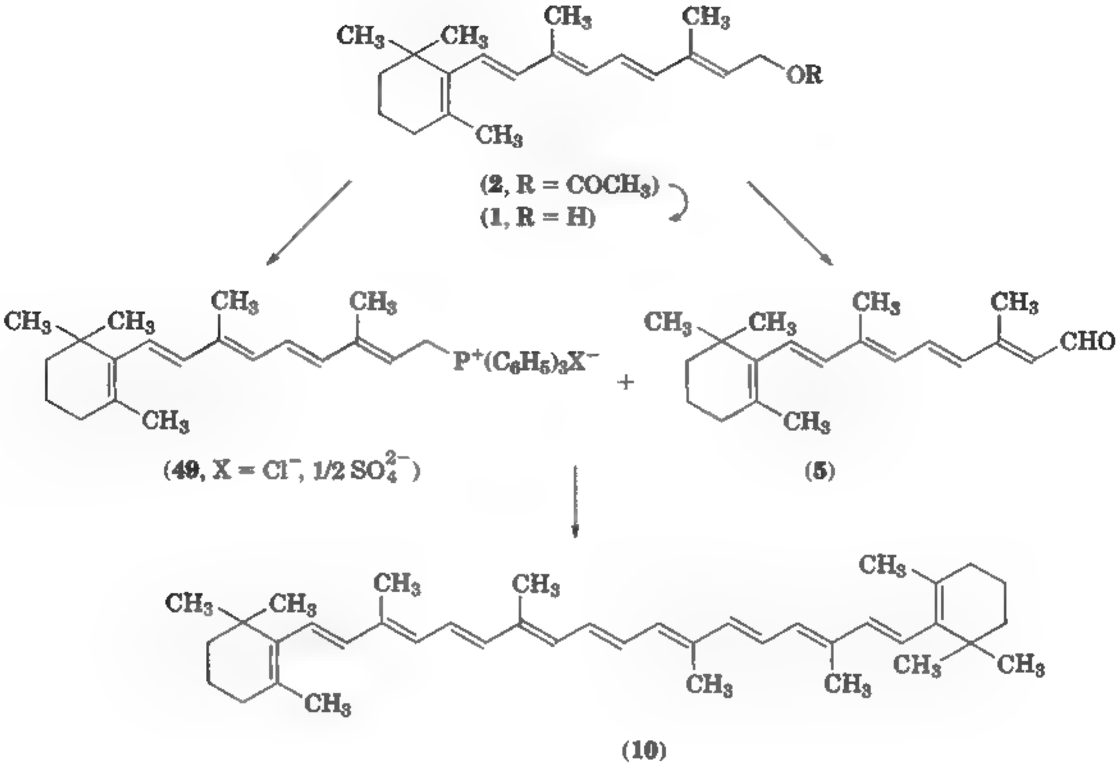


Figure 12. Alternative Roche synthesis of β-carotene.

carotene has the greatest activity. Despite the fact that theoretically one molecule of β-carotene is a biological source of two molecules of vitamin A, this relationship is not observed and 6 μg of β-carotene is equivalent to 1 μg of vitamin A. Although β-carotene and vitamin A have complementary activities, they cannot totally replace each other. Because the conversion of β-carotene to vitamin A is highly regulated, toxic quantities of vitamin A cannot accumulate and β-carotene can be considered as a safe form of vitamin A (8).

Owing to the presence of an extended polyene chain, all carotenoids are effective single-oxygen quenchers and antioxidants. In addition, they can stimulate the immune response and, as a result, may protect against certain forms of cancer. These separate functions, ie, vitamin A equivalents or antioxidants, have allowed for carotenoids to be classified according to whether they are nutritionally or biologically active. For example, β-carotene is both nutritionally and biologically active whereas 14′-β-apocarotenal is nutritionally active but biologically inactive. Lycopene is nutritionally inactive but biologically active, whereas phytoene, the natural precursor to β-carotene, is both nutritionally and biologically inactive (56).

REQUIREMENTS

Animals cannot synthesize vitamin A-active compounds and necessary quantities are obtained by ingestion of vitamin A or by consumption of appropriate provitamin A compounds such as β-carotene. Carotenoids are manufactured exclusively by plants and photosynthetic bacteria. Until the discovery of vitamin A in the purple bacterium *Halobacterium halobium* in the 1970s, vitamin A was thought to be confined to only the animal kingdom (56). Table 4 lists RDA and U.S. RDA for vitamin A (67).

DEFICIENCY

In humans, vitamin A deficiency manifests itself in the following ways: night blindness, xerophthalmia, Bitot's spots, and corneal involvement and ulceration. Changes in the skin have also been observed. Although vitamin A deficiency is seen in adults, the condition is particularly harmful in the very young. Often, this results from malnutrition (56).

On a vitamin A-deficient diet, mucus-secreting tissues become keratinized. This condition tends to occur in the trachea, the skin, the salivary glands, the cornea, and the testes. When this occurs in the cornea, it can be followed by blindness. Vitamin A deficiency is the principal cause of blindness in the very young. This problem is particularly acute in the third world (8).

SAFETY

Vitamin A toxicity can be categorized as either acute or chronic. Acute toxicity results from extremely high doses (≥500,000 IU of vitamin A). In children, approximately half of that amount causes problems. Hypervitaminosis A is characterized by headache, blurred vision, loss of coor-

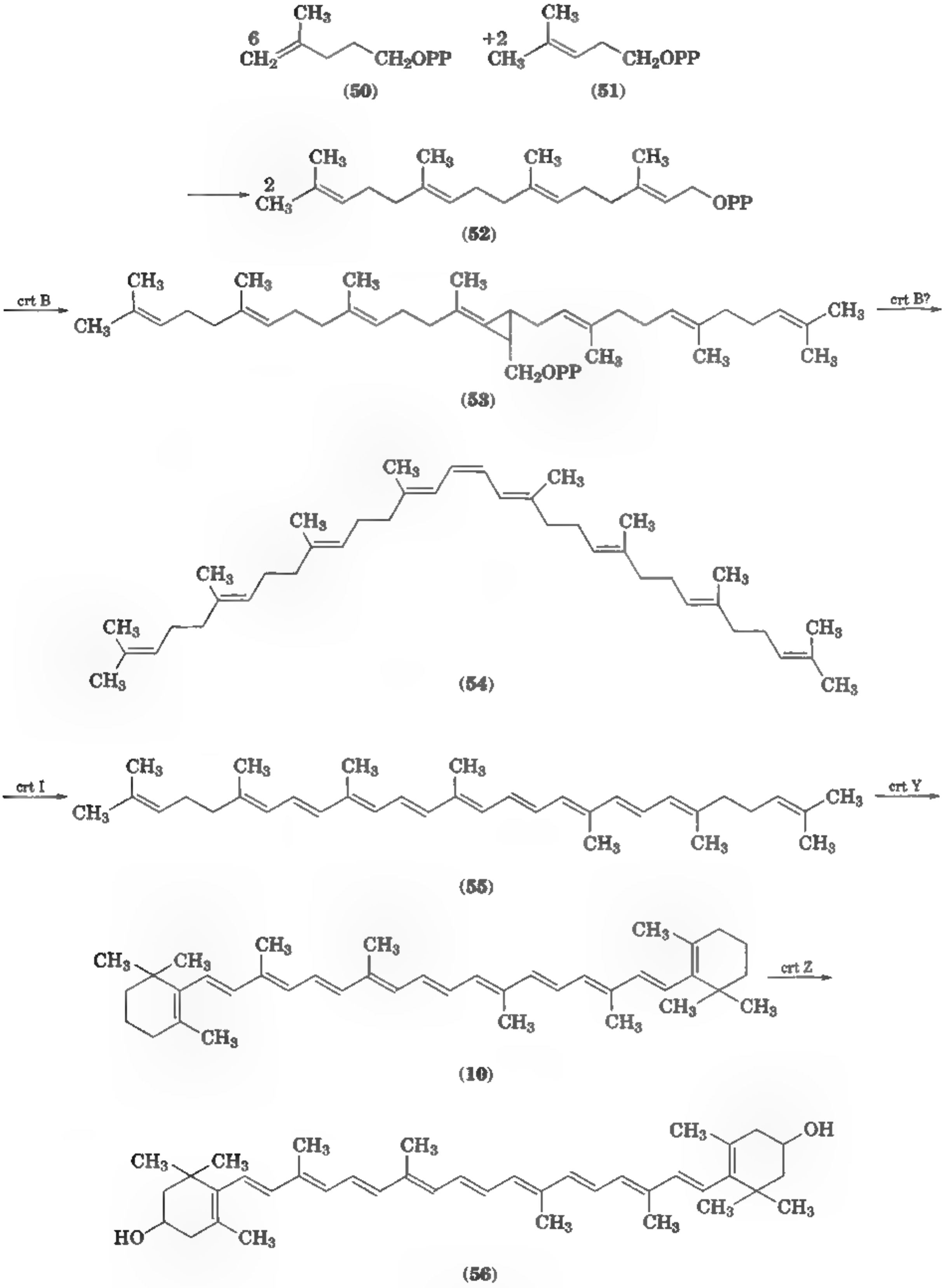

6
CH3
CH2
CH2OPP
(50)
+2
CH3
CH2OPP
(51)
2
CH3
OPP
(52)
crt B
CH2OPP
(53)
crt B?
(54)
crt I
(55)
crt Y
(10)
crt Z
HO
OH
(56)

Figure 13. Biosynthetic transformation of β-carotene.

Table 3. Provitamin A Activity of Selected Carotenoids

Provitamin A activity	No provitamin A activity
β-Carotene	Astaxanthin
α-Carotene	Canthaxanthin
γ-Carotene	Lutein
β-Crypotoxanthin	Lycopene
β-Zeacarotene	Zeaxanthin

Source. Ref. 8.

Table 4. Recommended Daily Allowances of Vitamin A

Sex/age group	1989 RDA (RE)[a]	U.S. RDA (IU)[b]
Infants <12 months	375	1,500
Children		
1–3 yr	400	2,500
4–6 yr	500	5,000
7–10 yr	700	5,000
Males ≥ 11 yr	1,000	5,000
Females ≥11 yr	800	5,000
Additional during pregnancy		+3,000
Additional during lactation		+3,000

Source: Ref. 8.

[a] RE = retinol equivalents (1 RE = 1 μg of all-*trans*-retinol).

[b] IU = international units (1 IU = 0.30 μg of all-*trans*-retinol).

dination, nausea, and peeling and itchy skin. Chronic vitamin A toxicity occurs in adults with long-term intakes of ≥50,000 IU/d. Symptoms include dry hair, hair loss, weakness, headache, bone thickening, enlarged liver and spleen, anemia, abnormal menstrual periods, stiffness, and joint pain. Most of these symptoms are reversible. In animals, extremely high doses of vitamin are teratogenic (56). On the other hand, the carotenoids are generally nontoxic and there have been only a few isolated cases of problems associated with a large daily intake. Tanning pills which contain large doses of canthaxanthin were shown to cause canthaxanthin retinopathy in patients with eryrthropoietic porphyria (68,70).

USES

Vitamin A is generally used in feeds, foods, and pharmaceutical applications; however, the principal use of carotenoids is as a colorant. β-Carotene, for example, is used to color fat products such as margarine, shortening, butter, cheese, egg nog, and ice cream. It is also used in baked goods, pasta products, juices, and beverages. It imparts a natural yellow to orange shade. Conversely, if an orange to reddish orange color is desired, the carotenoid of choice is β-apo-8′-carotenal [*1107-26-2*]. This carotenoid is used to color juices, fruit drinks, soups, jams, jellies, and gelatin (qv). Carotenoids are also added to feed to color poultry, fish, and of egg yolks. In order to guard against oxidative damage during feed processing, these compounds are protected in a gelatin matrix as beadlets.

ECONOMIC ASPECTS

Vitamin A is manufactured by Hoffmann-La Roche (Switzerland), BASF (Germany), and Rhône-Poulenc (France), as well as by some smaller suppliers in India, China, and Russia. The worldwide production is estimated to be 2500 to 3000 metric tons. About three-quarters of this production is for animal feed; the remainder is for food fortification and pharmaceuticals (qv). The main trade names of feed products are Rovimix, Lutavit, and Microvit. Prices depend on application forms and are approximately \$60–\$70/10^9 IU retinol (1995); ie, \$200–\$233/10^9 RE. One IU is equivalent to 0.300 μg of all-*trans*-retinol and 1 RE is equivalent to 1 μg of all-*trans*-retinol.

BIBLIOGRAPHY

1. G. Wolf, *Am. J. Clin. Nutr.* **31**, 290 (1978).
2. E. V. McCollum and M. Davis, *J. Biol. Chem.* **15**, 167 (1913).
3. E. V. McCollum and M. Davis, *J. Biol. Chem.* **23**, 291 (1915).
4. T. B. Osborne and L. B. Mendel, *J. Biol. Chem.* **37**, 187 (1923).
5. P. Karrer, A. Helfensten, H. Wehrli, and A. Wettstein, *Helv. Chim. Acta* **13**, 1084 (1930).
6. P. Karrer, R. Morf, and K. Schopp, *Helv. Chim. Acta* **14**, 1431 (1931).
7. G. Wald, *Nature (London)* **134**, 65 (1934).
8. D. Bhatia, in *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, pp. 2701–2712.
9. J. Paust, *Pure Appl. Chem.* **63**, 45 (1991).
10. Technical data, Hoffmann-La Roche, Inc., Nutley, N.J., 1996.
11. R. A. Heyman, D. J. Mangelsdorf, J. A. Dyck, R. B. Stein, G. Eichelle, R. M. Evans, and C. Thaller, *Cell* **68**, 397 (1992).
12. R. S. H. Liu and A. E. Asato, *Tetrahedron* **40**, 1931 (1984); Y. Bennani, *J. Org. Chem.* **61**, 3542 (1996); A. E. Asato, A. Kini, M. Denney, and R. S. H. Liu, *J. Am. Chem. Soc.* **105**, 2923 (1983); G. Wald, P. K. Brown, R. Hubbard, and W. Oroshnik, *Natl. Acad. Sci. USA* **41**, 438 (1955); G. Wald, P K. Brown, R. Hubbard, and W. Oroshnik, *Natl. Acad. Sci. USA* **42**, 578 (1956); K. Ramamurthy and R. Liu, *Tetrahedron* **31**, 201 (1975); A. E. Asato and R. S. H. Liu, *J. Am. Chem. Soc.* **97**, 4128 (1975); R. S. H. Liu and A. E. Asato, *Methods. Enzymol.* **88**, 506 (1982); A. Kini, H. Matsumoto, and R. S. H. Liu, *J. Am. Chem. Soc.* **107**, 5078 (1979); A. Kini, H. Matsumoto, and R. S. H. Liu, *Biorg. Chem.* **9**, 406 (1980); G. G. Ladson, S. C. Cary, and W. H. Okamura, *J. Am. Chem. Soc.* **102**, 6355 (1980).
13. G. Britton, in G. Britton, S. Liaaen-Jensen, and H. Pfander, eds., *Carotenoids, Vol. 1B: Spectroscopy*, Birkhauseer, Basel, Switzerland, 1995, pp. 13–62.
14. G. Britton, G. Armitt, S. Y. M. Lau, A. K. Patel, and C. S. Shone, in G. Britton and T. W. Goodwin, eds., *Carotenoid Chemistry and Biochemistry*, Pergamon Press, Oxford, U.K., 1984, pp. 237–251.
15. P. Zagalsky, in Ref. 13, *Vol. 1A: Isolation and Analysis*, pp. 287–294.
16. S. Liaaen-Jensen, in Ref. 13, pp. 343–354.
17. C. H. Eugster, in Ref. 15, pp. 343–354.
18. O. Isler, W. Huber, A. Ronoco, and M. Kofler, *Helv. Chim. Acta* **30**, 1911 (1942).
19. O. Isler, A. Ronco, W. Guex, N. C. Hindley, W. Huber, K. Dialer, and M. Kofler, *Helv. Chim. Acta* **32**, 489 (1949).

20. O. Isler, *Pure Appl. Chem.* **51**, 447 (1979).
21. C. Mercier and P. Chabardes, *Pure Appl. Chem.* **66**, 1509 (1994).
22. Jpn. Pat. 86-93840 (1986), Y. Inada (to Bihaman, Hishara).
23. J. Otera, H. Misana, T. Mandai, T. Onishi, S. Suzuki, and Y. Fujita, *Chem. Lett.*, 1883 (1985).
24 J. Otera, H. Misawa, T. Onishi, S. Suzuki, and Y. Fujita, *J. Org Chem.* **51**, 3834 (1986).
25. H. Bienayme, *Tetrahedron Lett.* **35**, 7387 (1994).
26. Ref. 25, p. 7383.
27. H. Bienayme, *Bull. Soc. Chim. Fr.* **132**, 696 (1995).
28. H. Pommer and P. C. Thieme, *Top. Curr. Chem.* **109**, 165 (1983).
29. A. Nurrenbach, J. Paust, H. Pommer, J. Scheider, and B. Schultz, *Liebigs Ann. Chem.*, 1146 (1972).
30. H. J. Bestman, C. Kisielowski, and N. Distler, *Angew. Chem.* **88**, 297 (1976).
31. Ger. Pat. 2,551,914 (1975), H. J. Bestmann and N. Distler (to Hoechst AG).
32. Ger. Pat. 2,702,633 (1977), B. Schulz, J. Paust, and J. M. Schneider (to BASF AG).
33. Jpn. Pat. 76-108106 (1979), M. Mukaiyama and A. Ishida (to Japan); A. Ishida, T. Mukaiyama, *Chem. Lett.*, 1127–1130 (1972).
34. Ger. Pat. 2,641,075 (1977), J. McMurray (to University of California).
35. Ger. Pat. 2,515,011 (1976), J. McMurray (to University of California).
36. Ger. Pat. 2,224,606 (1971), P. Chabardes and J. Marc (to Rhône-Poulenc).
37. Ger. Pat. 2,264,501 (1971), P. Chabardes and M. Julia (to Rhône-Poulenc).
38. Ger. Pat. 2,305,217 (1972), P. Chabardes, M. Julia, and A. Menet (to Rhône-Poulenc).
39. Ger. Pat. 2,355,898 (1972), P. Chabardes, M. Julia, and A. Menet (to Rhône-Poulenc).
40. Ger. Pat. 2,305,267 (1972), P. Chabardes, M. Julia, and A. Menet (to Rhône-Poulenc).
41. Ger. Pat. 2,202,689 (1971), M. Julia (to Rhône-Poulenc).
42. U.S. Pat. 4,916,250 (Apr. 10, 1990), J. H. Babler (to University of Loyola); J. H. Babler and S. A. Schlidt, *Tetrahedron Lett.* **33**, 7697–7700 (1992).
43. K. Bernhard and H. Mayer, *Pure Appl. Chem.* **63**, 35–44 (1991); E. Widmer, *Pure Appl. Chem.* **57**, 741–752 (1985).
44. O. Isler, R. Rüegg, U. Schwieter, and J. Wursch, *Vitam. Horm. (NY)* **18**, 295 (1960).
45. H. H. Kaegi, in M. B. Sporn, A. R. Roberts, and D. S. Goodman, eds., *The Retinoids*, Vol. I, Academic Press, Orlando, Fla., 1984, pp. 147–174.
46. M. F. Boehm et al., *J. Med. Chem.* **37**, 408–414 (1994); I. M. Mishina, E. N. Karnaukhova, V. I. Shvets, and M. G. Vladimirova, *Bioteknologiya*, 13–17 (1993); A. A. Liebman, W. Burger, S. C. Choudhry, and J. Cupano, *Methods Enzymol.* **213**, 42–49 (1992); M. Groesbeek and J. Lugtenberg, *Photochem. Photobiol.* **56**, 903–908 (1992); R. W. Curley, Jr., M. A. Blust, and K. A. Humphries, *J. Labelled Compd. Radiopharm.* **29**, 1331–1335 (1991); E. M. M. Van den Berg, A. Van der Bent, and J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas* **109**, 160–167 (1990); H. Akita, *Chem. Pharm. Bull.* **31**, 1796–1799 (1983); S. C. Welch and J. M. Gruber, *J. Org. Chem.* **47**, 385–389 (1982).
47. J. Ganguly, *Biochemistry of Vitamin A*, CRC Press, Boca Raton, Fla., 1989.
48. N. Misawa, M. Nakagawa, K. Kobayashi, S. Yamano, Y. Izawa, K. Nakamura, and K. Harahima, *J. Bacteriol.* **172**, 6704 (1990).
49. World Pat. 9406918 (1994), A. J. van Ooyen (to Gist-Brocades, Inc.); Eur. Pat. 635576 A1 950125 (1995), T. Kiyota, M. Takaki, A. Tsubokura, and H. Yoneda (to Nippon Oil KK); Eur. Pat. 454024 A 911030 (1991), W. D Prevatt and R. E. Torregrossa (to Phillips Petroleum Co.); World Pat. 9103571 (1991), D. L. Gierhart (to Applied Food BioTechnology, Inc.).
50. Eur. Pat. 107493 900420 (1990), N. Misawa, K. Kobayashi, and K. Nakamura (to Kirin Brewery Co, Ltd.).
51. World Pat. 9113078 A1 91095 (1991), R. L. Ausich, F. L. Brinkhaus, I. Mukharji, J. H. Proffitt, J. G. Yarger, and B. Yen (to Amoco Corp.).
52. N. D. Embree, S. R. Ames, R. W. Lehman, and P. L. Harris, *Meth. Biochem. Anal.* **4**, 43 (1957).
53. P. L. Harris, *Vitam. Horm.* **18**, 341 (1960).
54. P. Tosukhowong and J. A. Olson, *Biochem. Biophys. Acta* **529**, 438 (1978).
55. A. B. Barua and J. A. Olson, *Biochem. J.* **244**, 231 (1987).
56. J. A. Olson, in L. J. Machlin, ed., *The Handbook of Vitamins*, Marcel Dekker, New York, 1991, pp. 1–57.
57. H. C. Furr, A. B. Barua, and J. A. Olson, in A. P. De Leenheer, W. F. Lambert, and H. J. Nelis, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1992, pp. 1–71.
58. R. Schindler, A. Kloop, C. Gorny, and W. Feldheim, *Int. J. Vit. Nutr. Res.* **55**, 25 (1985).
59. A. T. Rhys Williams, *J. Chromatogr.* **341**, 198 (1985).
60. A. J. Speek, C. Wongkham, N. Limratana, W. Saowakontha, and W. H. Schreurs, *Curr. Eye. Res.* **5**, 841 (1986).
61. C. A. Collins and C. K. Chow, *J. Chromatogr.* **317**, 349 (1984).
62. H. K. Biesalski and H. Weiser, *J. Clin. Chem. Clin. Biochem.* **27**, 65 (1989).
63. C. R. Smidt, A. D. Jones, and A. J. Clifford, *J. Chromatogr.* **21**, 434 (1988).
64. *The United States Pharmacopeia XXII* (USP XXII-NF XVII), United States Pharmacopeial Convention, Inc., Rockville, Md., 1990, pp. 156–157, 1451, 1550–1551.
65. C. D. B. Bridges, in Ref. 45, pp. 126–176.
66. F. Chytil and D. Ong, *Fed. Proc.* **38**, 2510 (1979).
67. P. Mathis and C. C. Schenck. in Ref. 14, pp. 339–351.
68. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
69. U. Weber, G. Goerz, and R. Hennekes, *Klin. Monatsbl. Augenheilkd.* **186**, 351 (1985).
70. B. Daicker, K. Schiedt, J. J. Adnet, and P. Bermond, *Graefe's Arch. Clin. Exp. Ophthalmol.* **225**, 189 (1987).

GENERAL REFERENCES

J. Ganguly, *Biochemistry of Vitamin A*, CRC Press, Boca Raton, Fla., 1989.

A. P. De Leenheer, W. F. Lambert, and H. J. Nelis, eds., *Retinoids and Carotenoids* in *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1992.

M. A. Livrea and G. Vidali, eds., *Retinoids: From Basic Science to Clinical Applications*, Birkhauser Verlag, Germany, 1994.
L. J. Machlin, ed., *The Handbook of Vitamins*, Marcel Dekker, New York, 1991.
M. B. Sporn, A. B. Roberts, and D. S. Goodman, eds., *The Retinoids*, Vols. 1 and 2, Academic Press, Inc., Orlando, Fla., 1994.

SUSAN D. VAN ARNUM
Westfield, New Jersey

See also CAROTENOID PIGMENTS.

VITAMINS: VITAMIN B$_{12}$

Vitamin B$_{12}$ [*68-19-9*] (1,2) is the generic name for a closely related group of substances of microbial origin. Although the last of the vitamins to be characterized, its history is a long one, dating from 1824 when Combe (3) proposed the relationship of pernicious anemia, a disease characterized by defective (megoblastic) red blood cell formation, to disorders of the digestive system. Additional study of pernicious anemia, in particular the work of Addison (4), continued for over 100 years before Minot and Murphy (5) reported that a diet containing large quantities of raw liver restored the normal level of red blood cells in patients with pernicious anemia. This clinical breakthrough was based on the findings of Whipple and Robscheit-Robbins (6) that liver was of benefit in regeneration of blood in anemic dogs. For this work, Whipple, Minot, and Murphy were awarded the Nobel prize in medicine and physiology in 1934.

In 1929, Castle (7) tied the work of Combe and Addison with that of Whipple, Minot, and Murphy by proposing that both an extrinsic factor and an intrinsic factor are involved in the control of pernicious anemia. The extrinsic factor, from food, is vitamin B$_{12}$. The intrinsic factor is a specific B$_{12}$-binding protein secreted by the stomach. This protein is required for vitamin B$_{12}$ absorption.

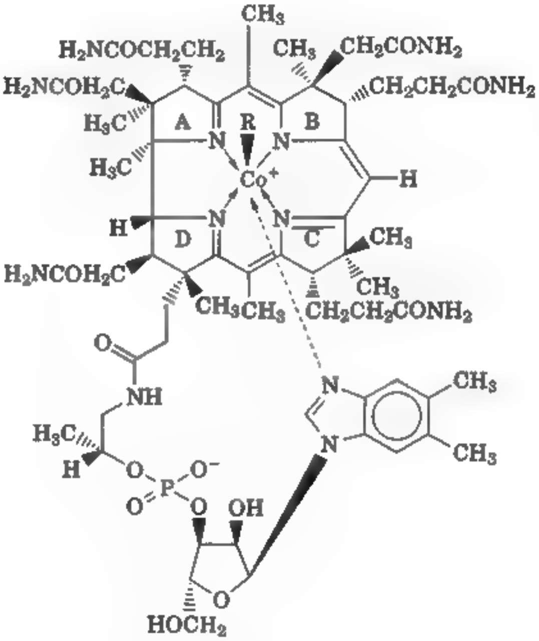
Work for the next 20 years focused on purification of the extrinsic factor from liver. The work was slow and tedious, because fractionation was guided by tests on pernicious anemia patients. The discovery (8) that *Lactobacillus lactis* Dorner requires liver extracts for growth greatly accelerated the isolation process. In 1948, groups at Merck (9) in the United States and Glaxo (10) in England reported isolation of vitamin B$_{12}$ (cyanocobalamin) as a crystalline, red pigment. The clinical efficacy of this material in the treatment of pernicious anemia was rapidly established (11).

Parallel to the activities in the treatment of pernicious anemia were observations in the 1930s that most farm animals had a requirement for an unknown factor beyond the vitamins then known. The lack of this factor became apparent, eg, when chicks or pigs fed a diet with only vegetable protein evidenced slow growth rate and high mortality. It became apparent that the required factor, termed animal protein factor, was present in animal sources such as meat and tissue extracts, milk, whey, and cow manure. Subsequent to its isolation, it was rapidly shown that vitamin B$_{12}$ is the same as animal protein factor.

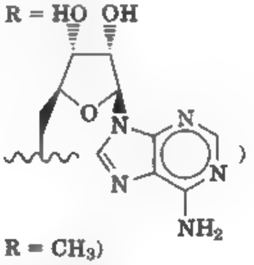
After its separation from liver extracts, vitamin B$_{12}$ was isolated also from cultures of *Streptomyces aureofaciens* (12). All vitamin B$_{12}$ sold commercially is produced by microbial fermentation.

STRUCTURE

The structure of the first isolated vitamin B$_{12}$, cyanocobalamin [*68-19-9* (**1a**) is known to occur only sporadically, at best, in biological systems. Its isolation was an artifact resulting, probably, from use of charcoal containing cyanide in the purification process.



(**1a**, R = CN)
(**1b**, R =



)

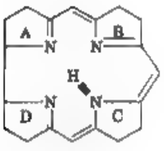
(**1c**, R = CH$_3$)
(**1d**, R = OH)
(**1e**, R = NO$_2$)
(**1f**, R = OH$_2$Cl)

It is recognized that there are several important forms of vitamin B$_{12}$. The active coenzyme forms are adenosylcobalamin [*13870-90-1*] (coenzyme B$_{12}$ (**1b**)) and methylcobalamin [*13422-55-4*] (**1c**). These, along with hydroxocobalamin [*13422-51-0*] (vitamin B$_{12a}$ (**1d**)), are the forms found in humans and other animals. Other forms of interest are nitrocobalamin (vitamin B$_{12c}$ (**1e**)) and aquacobalamine chloride [*13422-52-1*] (vitamin B$_{12b}$ (**1f**)). The primary commercial form is cyanocobalamin, due to its ease of isolation and purification as well as its stability.

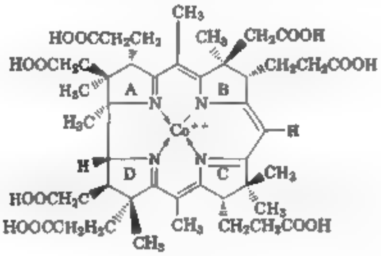
The IUPAC-IUB Commission on Biochemical Nomenclature (13) recommends that the term vitamin B_{12} be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. However, because of its commercial importance, cyanocobalamin is used interchangeably with vitamin B_{12} herein.

Determination of the structure of vitamin B_{12} was a slow, tedious process with the tools available in the 1940s. Despite extensive information gathered by degradation studies, the structures of cyanocobalamin and its coenzyme forms were only established beginning in 1955 by x-ray crystallography (14–16). The value of this work in the study of natural products was recognized by the award to Hodgkin of the Nobel prize in chemistry in 1964.

Vitamin B_{12} belongs to the class of molecules known as corrins. The core corrin structure (2) consists of four linked, partially saturated pyrrole rings. Corrin is a truncated form (no CH_2 between rings A and D) of the more common porphyrin skeleton. The corrin ring in vitamin B_{12} is substituted in a highly regio- and stereospecific manner with eight methyl groups, three acetic acid chains, and four propionic acid chains, as in cobyrinic acid (3). The six conjugated double bonds of the corrin system give octahedral complexes with a number of metals. However, only complexes with cobalt exhibit vitamin B_{12} activity.

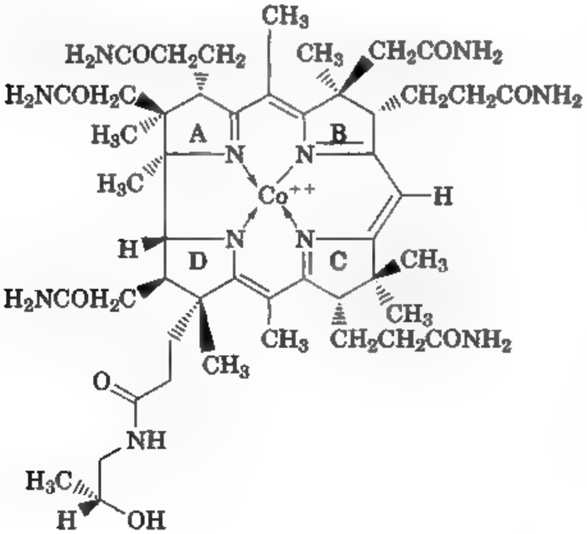


(2)



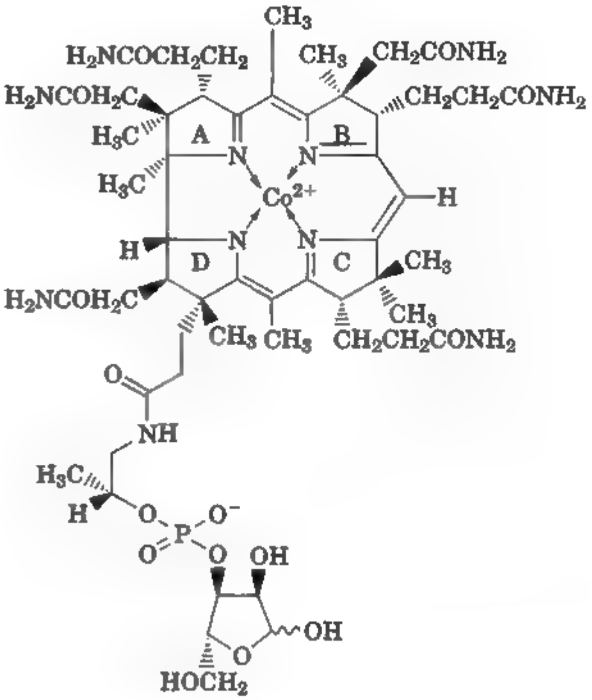
(3)

Cobrynic acid in which the propionic acid is amidated with 1-amino-2-propanol is known as cobinic acid, whereas the compound in which all other acids are primary amides is cobinamide (4).



(4)

Cobinic acid and cobinamide, which are linked to ribose 3-phosphate, are known as cobamic acid and cobamide (5), respectively.



(5)

The cobalt corrin complex is octahedral. The four nitrogens of the corrin ring occupy the four equatorial ligand positions in a virtually planar arrangement. The nucleotide formed from cobamide and 5,6-dimethylbenzimidazole provides a fifth ligand in the alpha, axial position of vitamin B_{12}. The group occupying the sixth, beta-position can vary substantially, as noted in structure (1), and dictates the compound name. Thus, when the ligand is cyanide, the compound name is cyanocobalamin; when methyl, methylcobalamin, etc.

Vitamin B_{12} exists as a neutral complex. The beta-ligand and one nitrogen atom in the corrin ring each contribute a negative charge to Co(III). The nucleotide phosphate contributes the final negative charge. The structure of vitamin B_{12} is highly organized and compact, as dictated by charge neutralization and the ligand sphere of the cobalt atom.

Many analogues of vitamin B_{12} are known. These occur either naturally or are obtained by synthesis, degradation of the vitamin, or directed biosynthesis. Most of these compounds have little or no biological activity in animals. However, many have significant microbiological activity. Among the analogues found are those in which the 5,6-dimethylbenzimidazole is replaced by another (or no) base. These include 2-methyl adenine (Factor A), no base (Factor B), guanine (Factor C), 5-hydroxybenzimidazole (Factor III), 5-methoxybenzimidazole (Factor III_m) and adenine (pseudovitamin B_{12}) (17–20).

OCCURRENCE

For many years, it was thought that the occurrence of vitamin B$_{12}$ was limited to animal tissues and bacteria, with all of the material originating from bacterial sources. More recently, the presence of vitamin B$_{12}$ and/or vitamin B$_{12}$-like activity at low levels in plants has been recognized (21–24). Nonetheless, the largest portion of the vitamin B$_{12}$ requirement is met by consumption of animal tissues and from microorganisms in the animal's digestive tract. Herbivorous animals satisfy their vitamin B$_{12}$ needs by absorption of material produced by rumenal or intestinal flora. In humans and carnivorous/omnivorous animals, intestinal production of vitamin B$_{12}$ occurs but at an insufficient level. As a result, vitamin B$_{12}$ levels are maintained by consumption of foods rich in vitamin B$_{12}$ such as liver, heart, and kidney. Egg yolk and some fish and shellfish are also good sources. Vitamin B$_{12}$ supplements, both oral and parenteral, are also available. Dietary sources of foodstuffs are provided in Table 1 (25–27).

BIOCHEMICAL FUNCTIONS

Methylcobalamin and adenosylcobalamin are the two coenzyme forms of vitamin B$_{12}$ in animals and humans. Each is involved in the catalysis of a specific transformation. In humans, it appears that there are only two enzymes requiring vitamin B$_{12}$ as an essential coenzyme although many other, particularly bacterial enzyme systems also require a vitamin B$_{12}$ coenzyme (2,28).

Adenosylcobalamin (coenzyme B$_{12}$) is required in a number of rearrangement reactions; that occurring in humans is the methylmalonyl-CoA mutase-mediated conversion of (*R*)-methylmalonyl-CoA (**6**) to succinyl-CoA (**7**) (eq. 1). The mechanism of this reaction is poorly understood, although probably free radical in nature (29). The reaction is involved in the catabolism of valine and isoleucine. In bacterial systems, adenosylcobalamin drives many 1,2-migrations of the type exemplified by Scheme 1 (30).

Table 1. Dietary Sources of Vitamin B$_{12}$

>50 μg/100 g	5–50 μg/100 g	<5 μg/100 g
Liver	Liver	Beef (lean)
Lamb	Rabbit	Lamb
Beef	Chicken	Pork
Calf	Kidney	Chicken
Pork	Rabbit	Egg (whole)
Kidney	Beef	Cheese
Lamb	Fish	American
Brain	Sardines	Swiss
Beef	Salmon	Milk (cow)
	Herring	Fish
	Heart	Cod
	Beef	Flounder
	Rabbit	Haddock
	Chicken	Sole
	Egg yolk	Halibut
	Clams	Swordfish
	Oysters	Tuna
	Crabs	Mackerel
		Lobster
		Scallop
		Shrimp
		Green vegetables

Methylcobalamin is involved in a critically important physiological transformation, namely the methylation of homocysteine (**8**) to methionine (**9**) (Scheme 2) catalyzed by N^5-methyltetrahydrofolate homocysteine methyltransferase. The reaction sequence involves transfer of a methyl group first from N^5-methyltetrahydrofolate to cobalamin (yielding methylcobalamin) and thence to homocysteine. Once again, the intimate details of the reaction are not well known (31). Demethylation of tetrahydrofolate to tetrahydrofolic acid is a step in the formation of thymidine phosphate, in turn required for DNA synthesis. In the absence of the enzyme, excess RNA builds up in red blood cells.

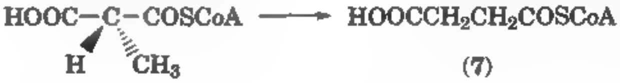
Homocysteine has been identified as an independent risk factor for atherosclerosis (32) and thus metabolic control over homocysteine levels has major health implications.

Deficiency

Macrocytic anemia, megaloblastic anemia, and neurological symptoms characterize vitamin B$_{12}$ deficiency. Alterations in hematopoiesis occur because of the high requirement for vitamin B$_{12}$ for normal DNA replication necessary to sustain the rapid turnover of the erythrocytes. Abnormal DNA replication secondary to vitamin B$_{12}$ deficiency produces a defect in the nuclear maturational process of committed hematopoietic stem cells. As a result, the erythrocytes are either morphologically abnormal or die during development.

Neurological symptoms result from demyelination of the spinal cord and are potentially irreversible. The symptoms and signs characteristic of a vitamin B$_{12}$ deficiency include paresthesis of the hands and feet, decreased deep-tendon reflexes, unsteadiness, and potential psychiatric problems such as moodiness, hallucinations, delusions, and psychosis. Neuropsychiatric disorders sometimes develop independently of the anemia, particularly in elderly patients. Visual loss may develop as a result of optic atrophy.

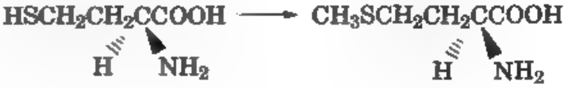
Clinical manifestation of vitamin B$_{12}$ deficiency is usually a result of absence of the gastric absorptive (intrinsic)



$$\mathrm{HOOC{-}\underset{H\quad CH_3}{C}{-}COSCoA \longrightarrow HOOCCH_2CH_2COSCoA}$$

(6) **(7)**

Scheme 1.



$$\mathrm{HSCH_2CH_2\underset{H\quad NH_2}{C}COOH \longrightarrow CH_3SCH_2CH_2\underset{H\quad NH_2}{C}COOH}$$

(8) **(9)**

Scheme 2.

factor. Dietary deficiency of vitamin B_{12} is uncommon and may take 20 to 30 years to develop, even in healthy adults who follow a strict vegetarian regimen. An effective enterohepatic recycling of the vitamin plus small amounts from bacterial sources and other contaminants greatly minimizes the risk of a complete dietary deficiency. Individuals who have a defect in vitamin B_{12} absorption, however, may develop a deficiency within three to seven years.

Dietary deficiency in the absence of absorption defects can be effectively reversed with oral supplementation of 1 μg of vitamin B_{12} daily. If deficiency is related to a defect in vitamin absorption, daily doses of 1 μg administered subcutaneously or intramuscularly are effective (33). However, a single intramuscular dose of 100 μg of cobalamin once per month is adequate in patients with chronic gastric or ileal damage. Larger doses are generally rapidly cleared from the plasma into the urine and are not effective unless the patient demonstrates poor vitamin retention.

Requirement

A daily intake of 1 μg should cover the daily loss of vitamin and maintain an adequate body pool. The RDA (34), however, has been established at 2 μg/day to cover metabolic variation among individuals and to ensure normal serum concentrations and adequate pool sizes (Table 2).

Smaller pool sizes with normal serum B_{12} levels may be maintained with dietary intakes below 1 μg. However, more substantial pool sizes are considered advantageous as protection against the development of pernicious anemia, which may occur in advanced age; achlorhydria becomes more common after age 60, resulting in compromised absorption of vitamin B_{12}.

In general, maternal stores of vitamin B_{12} are considered adequate to meet the demands of pregnancy.

Safety

No toxicity has been associated with acute or chronic intakes of vitamin B_{12} in doses of 100 and 1 mg, respectively. Vitamin B_{12} absorption is both limited and affected by vitamin status. Therefore, absorption is reduced with improved status, lessening the risk of toxicity.

Cobalamin should be administered parenterally by the intramuscular or subcutaneous route. Isolated cases of anaphylaxis have been reported with intravenous administration.

Table 2. The 1989 RDA for Vitamin B_{12}

Group	Age (yrs)	Vitamin B_{12} (μg)
	RDA	
Infants	0–0.5	0.3
	0.5–1.0	0.5
Children	1–3	0.7
	4–6	1.0
	7–10	1.4
Males	11–51+	2.0
Females	11–51+	2.0
	Pregnant	2.0
	Lactating	2.6
	U.S RDI (daily value)	
Infants and children <4 yr		3.0
Adults and children >4 yr		6.0
Pregnant or lactating women		8.0

METABOLISM

Absorption

An absorption mechanism capable of handling between 1.5 to 3.0 μg of vitamin B_{12} is responsible for most of the intestinal absorption. This mechanism includes the binding of vitamin B_{12} to a specific transport protein (intrinsic factor) and the presence of specific membrane-bound receptors on the ileal cell surface in the small intestine. In addition, there is a second mechanism for the absorption of vitamin B_{12} by diffusion. This mechanism can provide a physiologically significant source of the vitamin when delivered in pharmacological dosages.

Food vitamin B_{12} appears to bind to a salivary transport protein referred to as the R-protein, R-binder, or haptocorrin. In the stomach, R-protein and the intrinsic factor competitively bind the vitamin. Release from the R-protein occurs in the small intestine by the action of pancreatic proteases, leading to specific binding to the intrinsic factor. The resultant complex is transported to the ileum where it is bound to a cell surface receptor and enters the intestinal cell. The vitamin B_{12} is then freed from the intrinsic factor and bound to transcobalamin II in the enterocyte. The resulting complex enters the portal circulation.

Transport

Transcobalamin II delivers the absorbed vitamin B_{12} to cells and is the primary plasma vitamin B_{12}-binding transport protein. It is found in plasma, spinal fluid, semen, and extracellular fluid. Many cells, including the bone marrow, reticulocytes, and the placenta, contain surface receptor sites for the transcobalamin II-cobalamin complex.

Other plasma vitamin B_{12} proteins, transcobalamines I and III, appear to have primarily a storage function and only a lesser role in transport.

Tissue Uptake and Storage

Cell surface receptors take up the transcobalamin II-cobalamin complex which is internalized into endosomes. The complex is dissociated and the transcobalamin II released. The mechanism by which cobalamin leaves the endosome is uncertain.

The liver is the principal site of vitamin B_{12} storage, containing between 50 and 99% of the total body pool. The average total body pool is estimated to range between 2.0 and 5.0 mg. Higher storage values reported probably reflect noncobalamin analogues as well as cobalamin. The main storage form of vitamin B_{12} appears to be coenzyme B_{12}. The primary circulating form in the plasma appears to be methylcobalamin.

Metabolism and Mobilization

On entry of vitamin B_{12} into the cell, considerable metabolism of the vitamin takes place. Co(III)cobalamin is reduced to Co(I)cobalamin, which is either methylated to form methylcobalamin or converted to adenosylcobalamin (coenzyme B_{12}). The methylation requires methyl tetrahydrofolate.

Approximately 0.05 to 0.2% of vitamin B_{12} stores are turned over daily, amounting to 0.5–8.0 μg, depending on the body pool size. The half-life of the body pool is estimated to be between 480 and 1360 days with a daily loss of vitamin B_{12} of about 1 μg. Consequently, the daily minimum requirement for vitamin B_{12} is 1 μg. Three micrograms (3.0 μg) of vitamin B_{12} are excreted in the bile each day, but an efficient enterohepatic circulation salvages the vitamin from the bile and other intestinal secretions. This effective recycling of the vitamin contributes to the long half-life. Absence of the intrinsic factor interrupts the enterohepatic circulation. Vitamin B_{12} is not catabolized by the body and is, therefore, excreted unchanged. About one-half of the vitamin is excreted in the urine and the other half in the bile.

PROPERTIES

Table 3 lists some of the physical and chemical properties of vitamin B_{12} (1,35). Crystalline vitamin B_{12} is stable in air and is not affected by moisture. The anhydrous compound, however, is very hygroscopic, and when exposed to moist air may absorb about 12% of water.

Aqueous solutions of vitamin B_{12} at pH 4.0 to 7.0 show no decomposition during extended storage at 25°C. For optimum stability at elevated temperatures, solutions should be adjusted to pH 4.0 to 4.5. Aqueous solutions in this pH range may be autoclaved for 20 minutes at 120°C without significant decomposition.

Redox Reactions

Critical to the function of cobalamins as enzyme co-factors is the ability of the cobalt atom to exist in the Co(III) and Co(I) oxidation states. Chemically, Co(II) forms are also known. Each oxidation state has different ligand-accepting abilities. The chemical (**1a, 1d–1f**) and coenzyme (**1b, 1c**) forms of cobalamin are trivalent. These compounds are readily reduced to Co(II) and Co(I)cobalamins. The redox potentials for the aquacobalamin to Co(II)cobalamin and Co(II)cobalamin to Co(I)cobalamin couples are −0.04 and −0.85 V, respectively (36). Chemical reduction of aquacobalamin to Co(II)cobalamin is effected by thiols or carbon monoxide. Cobalt(II)cobalamin contains a single unpaired electron in the $3d_{z^2}$ orbital of the cobalt ion; one of the two axial positions is unoccupied and thus this material is five-coordinate. Chemically, Co(I)cobalamin is obtained with strong reducing agents, eg, $NaBH_4$ or zinc and NH_4Cl. It is a powerful reducing agent, and reacts rapidly with oxygen and reduces protons to hydrogen. It is therefore unstable in aqueous acid solution, less so in neutral or basic aqueous solution. It is frequently used for the preparation of organocobalamins, eg, adenosylcobalamin and methylcobalamin. Co(I)cobalamin contains two electrons in the $3d_{z^2}$ orbital. It has been postulated that the coordination number for the cobalt is four and that both axial ligands are vacant (37).

Table 3. Physical and Chemical Properties of Vitamin B_{12} (Cyanocobalamin)

Property	Characteristic
Appearance	Crystalline
Color	Dark red
Molecular weight	1355.42
Empirical formula	$C_{63}H_{88}CoN_{14}O_{14}P$
Melting point, C°	Darkens 210–220; does not melt <300
Specific rotation	$\alpha^{23}_{656} = -59 \pm 9°$ (diluted aqueous)
UV Absorption	278,361,550 nm (water)
pH (aq)[a]	Neutral
Solubility %	
H_2O	1.25
alcohol	2.03
acetone	Insoluble
ether	Insoluble

Note: Properties given for the anhydrous compound.

[a] Vitamin B_{12} exhibits maximum stability between pH 4.5 and 5.0.

Exchange of Axial Ligands

Many ligand-exchange reactions involve groups in which the coordination to the metal is through nitrogen (NH_3, N_3^-), oxygen (H_2O, OH^-), sulfur (SH^-, SO_3^{-2}), halogen, or carbon (CN^-, CH_3^-). Important reactions involve displacement of the heterocyclic base from the alpha-coordination position by a solvent, usually water. This displacement occurs in acidic solution and results from the protonation of the heterocyclic base. The protonation is associated with a characteristic change in the spectrum. The pK_a for the base-on/base-off equilibrium depends on the nature of the beta-ligand. Displacement of H_2O, adenosyl, or methyl from cobalt by cyanide has also been studied. In the presence of cyanide ion, aquacobalamin and adenosylcobalamin are converted to cyanocobalamin. In contrast, methylcobalamin and other alkyl corrinoids are stable in the presence of 0.1 *M* cyanide in the dark (37,38). The equilibrium, aquacobalamin ⇌ hydroxocobalamin + H^- (pK_a = 6.9–7.8), is not a pure ligand exchange but only a ligand modification. The cobalt-bound water is acidic and reversibly loses a proton at neutral pH.

Chemical Reactions

A wealth of information exists on the chemistry of vitamin B_{12} (1,39,40). Much of this chemistry was established during the studies leading to structure determination, partial synthesis, and the total synthesis of vitamin B_{12}. Vitamin B_{12} is slowly decomposed by ultraviolet or strong visible light. By controlled irradiation with visible light, cyanide may be selectively liberated from cyanocobalamin without destruction of the cobalamin structure, but long exposure to light results in complete inactivation of the vitamin. Vitamin B_{12} is also inactivated by treatment with strong acids or bases.

One development involves the use of vitamin B_{12} to catalyze chemical, in addition to biochemical processes. Vitamin B_{12} derivatives and B_{12} model compounds (41,42) catalyze the electrochemical reduction of alkyl halides and formation of C-C bonds (43,44), as well as the zinc–acetic acid-promoted reduction of nitriles (45), alpha, beta-unsaturated nitriles (46), alpha, beta-unsaturated carbonyl derivatives and esters (47,48), and olefins (49). It is assumed that these reactions proceed through intermediates containing a Co-C bond which is then reductively cleaved.

ANALYSIS

Specifications

Cyanocobalamin is the commercial form of vitamin B_{12}. It is sold under the following trade names (35):

Anacobin
Antipernicin
Bedoce
Bedodeka
Bedoz
Behepan
Berubi
Berubigen
Betalin-12
Betolvex
Bevatine-12
Bevidox
Bexii
Bexil
Biocobalamine
Biocres
Bitevan
B-Telve
B-Twelv
Byladoce
Claretin-12
Cobalin
Cobamin
Cobamine
Cobione
Covit
Crystamin
Cycobemin
Cycolamin
Cykobeminet
Cytacon
Cytamen
Cytobion
Distivit (B_{12} peptide)
Dobetin
Docemine
Docibin
Docigram
Docivit
Dodecabee
Dodecabee
Dodecavite
Dodex
Ducobee
Duodecibin
Embiol
Emociclina
Eritrone
Erycytol
Erythrotin
Euhaemon
Fresmin
Hemo-B-Doze
Hemomin
Hepagon
Hepavis
Hepcovite
Hydroxamin
Hydroxobase
Macrabin
Megabion (Indian)
Megalovel
Milbedoce
Millevit
Nagravon
Normocytin
Peraemon
Pernaevit
Pernipur
Plecyamin
Poyamin
Redamina
Redisol
Rhodacryst
Rubesol
Rubivitan
Rubramin
Rubripca
Rubrocitol
Sytobex
Vitalt
Vibisone
Virubra
Virubra
Vitarubin
Vita-Rubra
Vitral

Specifications are found in the *Codex* for food use (50) and in the USP (51) for pharmaceutical use.

Analytical Methodology

Vitamin B_{12} can be determined by microbiological, radioisotope dilution, spectrophotometric, chemical, or biological methods employing animals (52–54). Microbiological assays involve the extraction and stabilization of vitamin B_{12} from the food, feed, or pharmaceutical matrix prior to assay. The official method of the AOAC (55) accomplishes this by autoclaving samples in a phosphate–citric acid buffer containing metabisulfite, whereas the British Analytical Methods Committee (56) recommends extraction with aqueous cyanide solution at pH 4.6–5.0 in a boiling water bath. The AOAC extraction procedure was found to yield somewhat higher results than the British Analytical Methods Committee procedure (57).

The AOAC assay is based on the graded growth of the bacterium *Lactobacillus leichmannii* ATCC 7830 in a medium containing all required growth factors but vitamin B_{12}. Results are obtained by determining the transmittance of the sample and standard tubes after 16–24 h incubation at 30–40°C or by titrating the acid produced after 72 h incubation. The British Analytical Methods Committee assay employs the protozoan *Ochromonas malhamensis* ATCC 11532 in an assay based on the graded growth of the protozoan. The assay incubation period varies from 3–6 days and degree of growth is measured turbidimetrically. Although the *L. leichmannii* assay is claimed to be less specific than the *O. malhamensis* assay it has several advantages over the latter. The AOAC procedure has a shorter incubation period and the assay is set up in test tubes, whereas the *O. malhamensis* assay is set up in 25-mL micro-Fernbach flasks, the assay medium is less complex, and samples whose turbidity or color would interfere in a turbidimetric assay can be analyzed by the titrimetric assay. Derivatives of deoxynucleic acid that stimulate the growth of *L. leichmannii* in the absence of vitamin B_{12} can be measured after destroying the vitamin B_{12} by autoclaving the sample at pH 11–12 and determining the residual activity. The bacteria *Lactobacillus lactis* Dorner ATCC 8000 and *Escherichia coli* ATCC 10799 and 14169 as well as the protozoan *Euglena gracilis* ATCC 12716 have been used also for the determination of vitamin B_{12}.

Radioisotope dilution assays are based on the principle of competition between radioactive labeled (^{57}Co) vitamin B_{12} and cobalamins extracted from matrices for binding sites on the intrinsic factor (a glycoprotein). Binding is in proportion to the concentration of the radioactive and nonradioactive B_{12} with the concentration of intrinsic factor as the limiting factor. Free cobalamins are separated from those bound on the intrinsic factor by absorption onto treated charcoal and the amount of free-labeled vitamin B_{12} is determined. Vitamin B_{12} content of the sample is determined from a standard curve. Results obtained by a radioisotopic dilution method are very similar to those obtained by the AOAC microbiological assay (58).

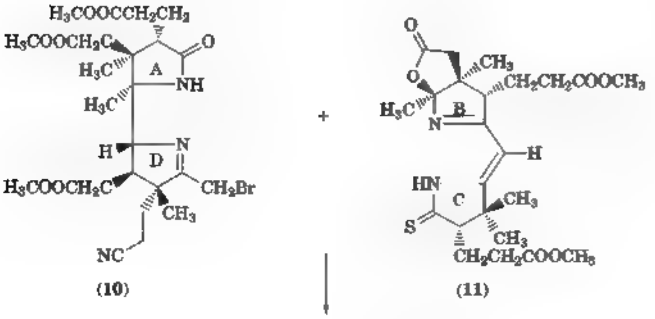
Spectrophotometric determination at 550 nm is relatively insensitive and is useful for the determination of vitamin B_{12} in high potency products such as premixes. Thin-layer chromatography and open-column chromatography have been applied to both the direct assay of cobalamins and to the fractionation and removal of interfering substances from sample extracts prior to microbiological or radioassay. Atomic absorption spectrophotometry of cobalt has been proposed for the determination of vitamin B_{12} in dry feeds. Chemical methods based on the estimation of cyanide or the presence of 5,6-dimethylbenzimidazole in the vitamin B_{12} molecule have not been widely used.

Various aspects of the chromatography of vitamin B_{12} and related corrinoids have been reviewed (59). A high performance liquid chromatographic (hplc) method is reported to require a sample containing 20–100 μg cyanocobalamin and is suitable for premixes, raw material, and pharmaceutical products (60).

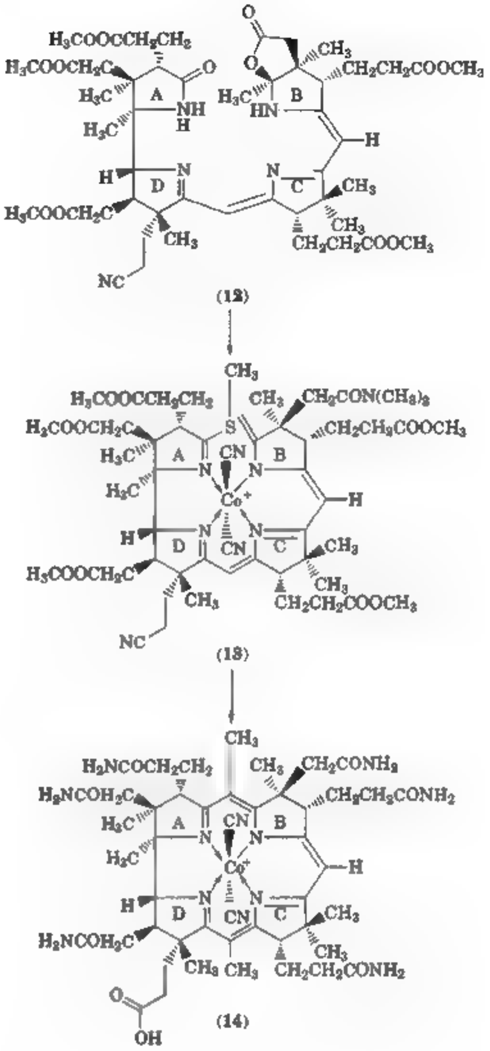
Bioassays are based on the growth response of vitamin-depleted rats or chicks to graded amounts of vitamin B_{12} added in the diet. These assays are not specific for vitamin B_{12} because factors, other than vitamin B_{12} present in biological materials, produce a growth response. Because coenzyme B_{12}, a primary form of natural vitamin B_{12}, is light sensitive, assays should be carried out in subdued light.

SYNTHESIS

The achievements in total synthesis of organic compounds in recent years are perhaps nowhere better illustrated than in the synthesis of vitamin B_{12} by the groups of Woodward (61–63) and Eschenmoser (64–69) in a collaborative effort (70,71). The work has been reviewed (1,72,73). The real value of the synthesis lies in the synthetic methodology developed in the course of the work. During the synthesis of the A–D fragment, the Woodward-Hoffmann rules for the conservation of orbital symmetry (74) were developed. For this work, Hoffmann shared the Nobel prize in chemistry in 1981 with Fukui. Woodward was awarded the Nobel prize in chemistry in 1965 for the synthesis and structure elucidation of natural products, including the work leading up to the synthesis of vitamin B_{12}. Thus, the history of vitamin B_{12} is intimately connected with the awarding of four Nobel prizes to date.



(10) (11)



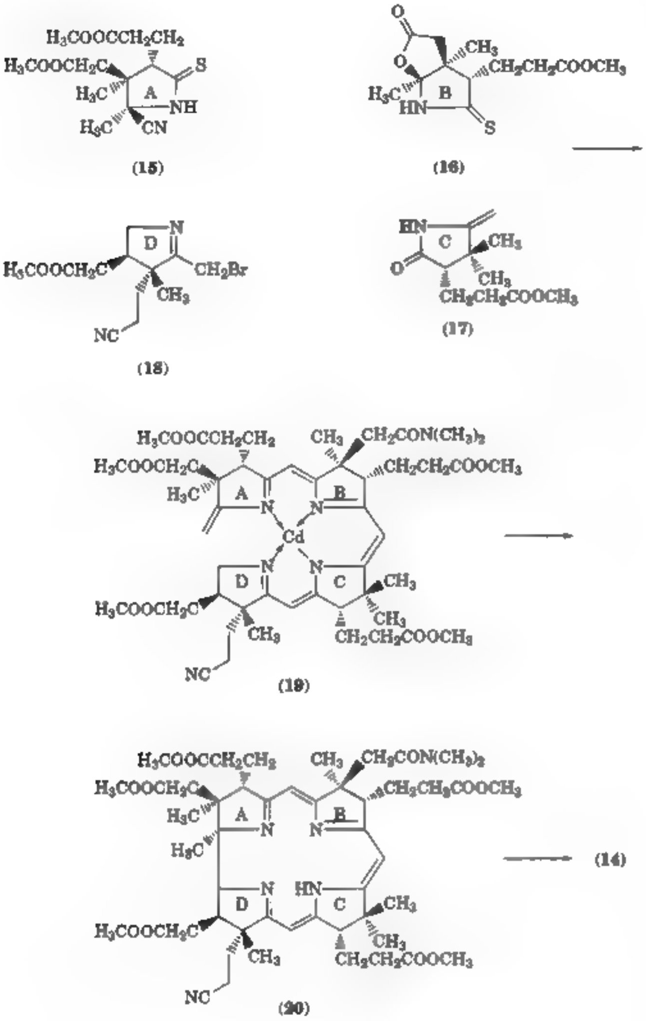
(12)

(13)

(14)

The core of the first synthesis of vitamin B_{12} involved condensation of the A–D ring fragment (**10**) with the B–C fragment (**11**). The former compound was obtained by the Harvard group in ca 35 steps, including a classical resolution, from 3-methoxyaniline and camphor.

The synthesis of the second fragment (**11**) by the group at the ETH in Zurich occurred in 18 steps from camphorquinone (ring C) and *trans*-3-methyl-4-oxopentenoic acid (ring D). Base-catalyzed alkylation of (**11**) with (**10**) yielded a thioiminoether which underwent sulfide contraction upon treatment with acid to give the tetrapyrrole (**12**). Manipulation of substituents and introduction of the cobalt atom gave complex (**13**). The stereo-organizing template effect of the complex allowed base-catalyzed cyclization to complete the corrin ring system. Functional group exchange and addition of the final methyl group then yielded cobyric acid (**14**). This material had previously been converted to vitamin B_{12} (75) and thus its obtention by total synthesis completed the synthesis.



(15) (16) (17) (18) (19) (20)

A second synthesis of cobyric acid (**14**) involves photochemical ring closure of an A–D secocorrinoid. Thus, the Diels-Alder reaction between butadiene and *trans*-3-methyl-4-oxopentenoic acid was used as starting point for all four ring A–D synthons (**15–18**). These were combined in the order B + C → BC + D → BCD + A → ABCD. The resultant cadmium complex (**19**) was photocyclized in buffered acetic acid to give the metal-free corrinoid (**20**). A number of steps were involved in converting this material to cobyric acid (**14**).

The total syntheses have yielded cobyric acid and thence cyanocobalamin. Routes to other cobalamins, eg, methylcobalamin and adenosylcobalamin, are known (76–79). One approach to such compounds involves the oxidative addition of the appropriate alkyl halide (eg, CH_3I to give methylcobalamin) or tosylate (eg, 5′-*p*-tosyladenosine to yield adenosylcobalamine) to cobalt(I)alamine.

The complexity of the vitamin B_{12} molecule makes it extremely unlikely that total synthesis will ever be employed for preparation of commercial quantities.

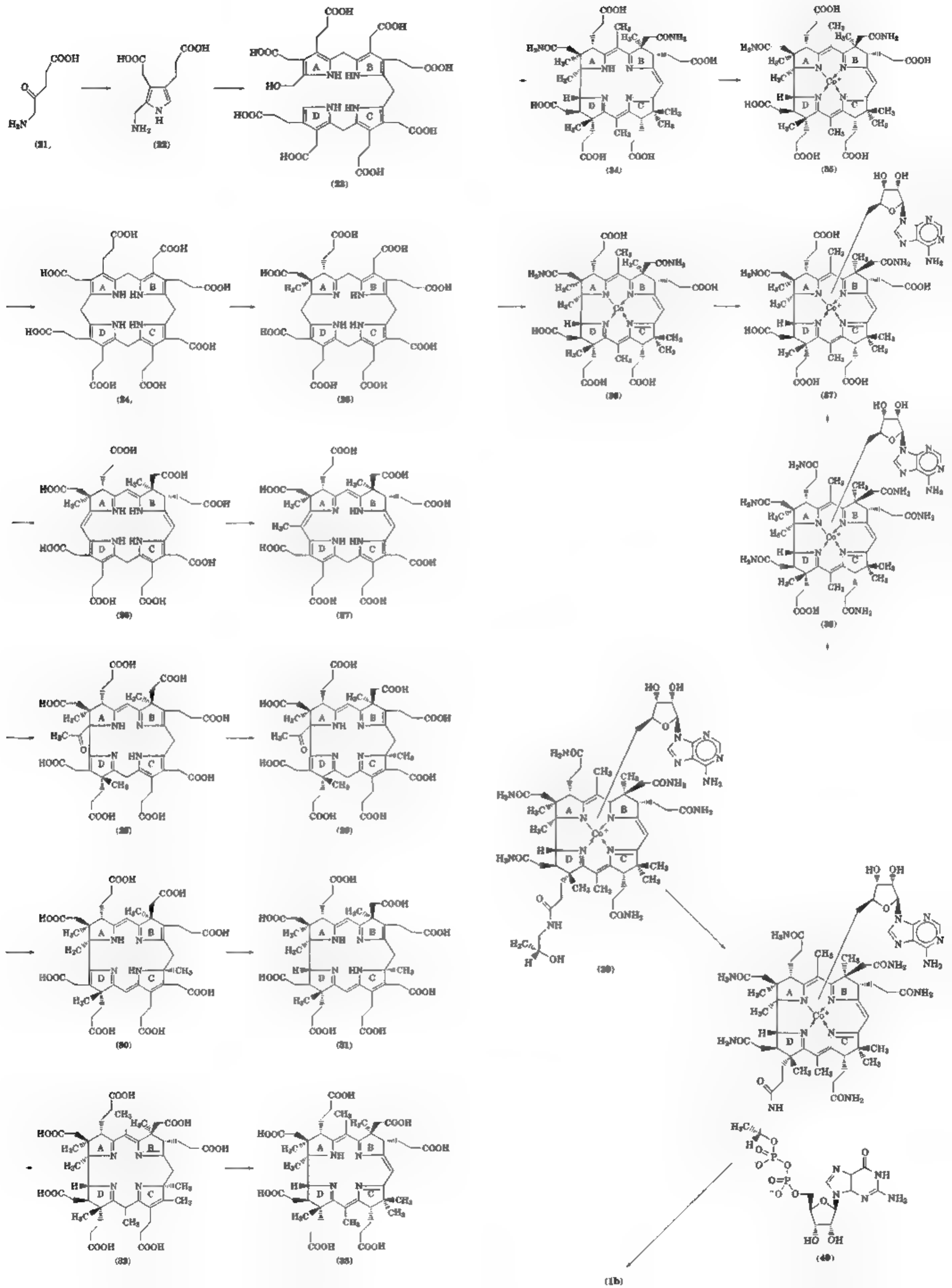
BIOSYNTHESIS

The study of the biosynthesis of vitamin B_{12} is a saga whose resolution, due primarily to Battersby (80–83) and Scott (84,85), required an effort on the same magnitude as the total synthesis. It was only when recent molecular biology tools became available to complement enzymology, isotopic labeling, chemical synthesis, and spectroscopy that solution of this problem became possible.

The biosynthesis of vitamin B_{12} in the species *Pseudomonas dentrificans* has received the most attention due to its importance as a commercial source of the vitamin. The 22 genes involved have been identified and the sequence of the resulting proteins described (83). The sequence is initiated from 5-aminolevulinic acid (**21**) which is biosynthesized from succinyl-CoA (**7**) and glycine. Condensation of two molecules of 5-aminolevulinic acid yields porphobilinogen (**22**) which is tetramerized as a linear, head-to-tail arrangement to give hydroxymethylbilane (**23**). Ring closure and rearrangement (formally on exchange of acetate and propionate substituents in ring C) gives uroporphyrinogen III (uro'gen III) (**24**). Uro'gen III is a key biosynthetic branchpoint, leading not only to vitamin B_{12} but also to chlorophyll and heme.

The sequence to vitamin B_{12} proceeds by the introduction of the eight methyl groups, punctuated by ring contraction to the corrin, an oxidation and reduction, and a methyl migration. Thus, the first three methylations lead stereospecifically to precorrin-1 (**25**) and thence by precorrin-2 (**26**) to precorrin-3A (precorrin-3) (**27**). Oxidation, ring contraction to the corrin, and introduction of the fourth methyl group give precorrin-4 (**28**). Further methylation leads to precorrin-5 (**29**). Loss of the acetyl group created by ring contraction as acetate occurs with methylation to give precorrin-6A (precorrin-6x) (**30**) which is reduced to precorrin 6B (precorrin-6y) (**31**). Decarboxylation of the ring C acetic acid subsequent to addition of the last two methyl groups (precorrin-8x) (**32**) and methyl migration gives hydrogenobyrinic acid (**33**).

Introduction of the cobalt atom into the corrin ring is preceeded by conversion of hydrogenobyrinic acid to the diamide (**34**). The resultant cobalt(II) complex (**35**) is reduced to the cobalt(I) complex (**36**) prior to adenosylation to adenosylcobyrinic acid *a,c*-diamide (**37**). Four of the six remaining carboxylic acids are converted to primary amides (adenosylcobyric acid) (**38**) and the other amidated with (*R*)-1-amino-2-propanol to provide adenosylcobinamide (**39**). Completion of the nucleotide loop involves conversion to the monophosphate followed by reaction with guanosyl triphosphate to give diphosphate (**40**). Reaction with α-ribazole 5′-phosphate, derived biosynthetically in several steps from riboflavin, and dephosphorylation completes the synthesis.


(21)
(22)
(23)
(24)
(25)
(26)
(27)
(28)
(29)
(30)
(31)
(32)
(33)
(34)
(35)
(36)
(37)
(38)
(39)
(40)
(1b)
A
B
C
D
Co⁺

The biosynthetic sequence for other aerobic bacteria appears, where known, to be similar to that in *Pseudomonas dentrificans* although the genes involved, and thus the enzymes, exhibit differences.

In anaerobic (or more correctly, almost anaerobic or microaerophilic) bacteria such as *Propionibacterium shermanii*, a fundamental difference occurs in that the cobalt atom is introduced at a much earlier stage, possibly to precorrin-2 or precorrin-3A.

MANUFACTURE

As noted above, all vitamin B_{12} is produced by microbial fermentation. A partial list of microorganisms which synthesize vitamin B_{12} under appropriate conditions follows. Most strains, in their wild state, produce less than 10 mg/L vitamin B_{12}, although a few approach 40 mg/L. The organisms are both aerobes and anaerobes. The carbon requirements in the fermentations are satisfied from sources as wide ranging as hydrocarbons, methanol, and glucose.

Arthrobacter hyalinus
Bacillus megaterium
Butyribacterium rettgeri
Clostridium sticklandii
Clostridium tetanomorphum
Clostridium thermoaceticum
Corynebacterium and Rhodopseudomonas
Crithidia fasciculata
Methanobacterium arbophilicum
Methanobacterium formicicum
Methanobacterium ruminantium
Methanobacterium thermoautotrophicum
Methanosarcina barkeri
Micromonospora purpurea
Nocardia gardneri
Nocardia rugosa
Propionibacterium arabinosum
Propionibacterium freudenreichii
Propionibacterium pentosaceum
Propionibacterium peterssoni
Propionibacterium shermanii
Propionibacterium technicum
Propionibacterium vannielli
Protaminobacter ruber
Pseudomonas denitrificans
Rhizobium meliloti
Rhodopseudomonas capsulata
Rhodopseudomonas spheroides
Strigomonas oncopelti
Streptomyces aureofaciens
Streptomyces griseus
Streptomyces olivaceus

Commercial Production

Vitamin B_{12}, as cyanocobalamin, is produced by several companies. The market is dominated, however, by two French firms, Rhône-Poulenc and, to a lesser extent, Roussel-Uclaf. Smaller amounts are produced in Japan by Nippon Petrochemical, in Hungary by Medimpex-Richter, and by minor producers in several other countries. Earlier manufacturers, particularly Merck (U.S.) and Glaxo (U.K.), have exited the market. Although estimates vary, it appears that ca 10,000 kg/yr of vitamin B_{12} is produced (1).

The process employed by Rhône-Poulenc for production of vitamin B_{12} has not been revealed. However, from a variety of sources (83,86) it can be inferred that a *Pseudomonas dentrificans* producing over 200 mg/L is employed. The high production is the result of classical mutation as well as (possibly) genetic engineering.

A fermentation such as that of *Pseudomonas dentrificans* typically requires 3–6 days. A submerged culture is employed with glucose, cornsteep liquor and/or yeast extract, and a cobalt source (nitrate or chloride). Other minerals may be required for optimal growth. pH control at 6–7 is usually required and is achieved by ammonium or calcium salts. Under most conditions, adequate 5,6-dimethylbenzimidazole is produced in the fermentation. However, in some circumstances, supplementation may be required.

The fermentation product, which is primarily adenosylcobalamin, is retained to the largest extent within the cell. Centrifugation of the broth yields a sludge which, when dispersed in a minimum of water, water–alcohol, or water–acetone and heated, releases the vitamin into the solution. Addition of cyanide converts the cobalamins into cyanocobalamin which is then extracted from the filtered solution. Many procedures have been reported for this step, including adsorption on charcoal (87), bentonite (88), ion-exchange resins (89–91), or aluminum oxide (92). For elution, water, water–alcohol, organic bases, or hydrochloric acid are used. Chromatography on aluminum oxide and crystallization from methanol–acetone or water–acetone complete the process (93,94).

Market Forms

Vitamin B_{12} is sold almost exclusively as cyanocobalamin. Approximately one-third of the material is for the human pharmaceutical market whereas two-thirds is used in the animal feed market, primarily for poultry and swine. Modest growth in both markets has occurred in the period 1980–1995 and this trend is expected to continue.

In the human market, oral and parenteral dosage forms are prepared from the crystal. However, because of the extremely high potency, more dilute (0.1–10%) forms are available. These include dilutions with mannitol, triturations on dicalcium phosphate or resins, and spray-dried forms. Prices for these forms are driven by that of the crystal, which in early 1996 was ca \$9.50/gram (95). Prices for the vitamin have risen during the first half of the 1990s. However, little growth in price beyond inflation is anticipated.

For animal feed use, vitamin B_{12} is usually provided in a diluted form on a carrier such as calcium carbonate and/or rice hulls. An earlier practice of using a spray-dried fermentation biomass in this application appears to be no longer used.

BIBLIOGRAPHY

1. B. Kräutler, in O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, eds., *Vitamin II· Wasserlösliche Vitamine*, Georg Thieme Verlag, New York, 1988, pp. 340–388.
2. L. Ellenbogen and B. A. Cooper, in L. J. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, Inc., New York, 1991, pp. 491–536.
3. J. S. Combe, *Trans. Med. Chirurg. Soc* (*Edinburg*), 1, 194 (1824).
4. T. Addison, *On the Constitutional and Local Effects of Disease of the Suprarenal Capsules*, Samuel Highley, London, 1855, p. 2.
5. G. R. Minot and W. P. Murphy, *JAMA* 91, 923 (1926).
6. G. H. Whipple, C. W. Hooper, and F. S. Robscheit, *Am. J. Physiol.* 53, 236 (1920).

7. W. B. Castle, *Am. J. Med. Sci.* **178**, 748 (1929).
8. M. S. Shorb and G. M. Briggs, *J. Biol. Chem.* **176**, 1463 (1948).
9. E. L. Rickes, N. G. Brink, F. R. Koniuszy, T. R. Wood, and K. Folkers, *Science* **107**, 396 (1948).
10. E. L. Smith, *Nature (London)* **161**, 638 (1948).
11. R. West, *Science* **107**, 398 (1948).
12. J. V. Pierce, A. C. Page, E. L. R. Stokstad, and T. H. Jukes, *J. Am. Chem. Soc.* **71**, 2952 (1949).
13. "The Nomenclature of Corrinoids (1973 Recommendations)," *Biochemistry* **13**, 1555 (1974).
14. D. C. Hodgkin and co-workers, *Nature (London)* **176**, 325 (1995).
15. P. G. Lenhert and D. C. Hodgkin, *Nature (London)* **192**, 937 (1961).
16. D. C. Hodgkin, *Science* **150**, 979 (1965).
17. J. W. G. Porter, in H. C. Heinrich, ed., *Vitamin B$_{12}$ and Intrinsic Factor 1*, Europäisches Symposion, Hamburg, 1956, F. Enke, Stuttgart, 1957, p. 43.
18. M. E. Coates and S. K. Kon, in Ref. 17, p. 72.
19. H. C. Heinrich, W. Friedrich, E. Gabbe, S. P. Manjrekar, and M. Staak, in *Abstracts of the 5th International Congress on Nutrition*, Washington, D.C., 1960, p. 62.
20. E. L. Smith, *Vitamin B$_{12}$*, Methuen, London, 1965.
21. L. Fries, *Physiol. Plantarum* **15**, 566 (1962).
22. G. G. Laties and C. Hoelle, *Phytochemistry* **6**, 49 (1967).
23. A. P. Petrosyan, L. A. Abramyan, and M. B. Sarkisyan, *Vopr. Mikrobiol.* **4**, 181 (1969).
24. J. M. Poston, *Science* **195**, 301 (1977).
25. L. J. Bogert, G. M. Briggs, and D. H. Calloway, *Nutrition and Physical Fitness*, W. B. Saunders, Philadelphia, Pa., 1973.
26. W. Friedrich, *Vitamin B$_{12}$ und Verwandte Corrinoide*, Georg Thiem, Stuttgart, Germany, 1975, p. 170.
27. J. Marks, *A Guide to the Vitamins, Their Role in Health and Disease*, Medical and Technical Publishing Co., Lancaster, U.K., 1975, p. 118.
28. J. P. Glusker, in G. Litwack, ed., *Vitamins and Hormones*, Vol. 50, Academic Press, Inc., New York, 1995, pp. 1–76.
29. Y. Zhao, P. Such, and J. Rétey, *Angew. Chem. Int. Ed. Engl.* **31**, 215 (1992); M. He and P. Dowd, *J. Am. Chem. Soc.* **118**, 711 (1996).
30. T. C. Stadtman, *Science* **102**, 859 (1971).
31. R. G. Mathews, in R. L. Blakely and S. J. Benkovic, eds., *Folates and Pteridins*, Vol. 1, John Wiley & Sons, Inc., New York, 1984, p. 497.
32. C. S. Berwanger, J. Y. Jeremy, and G. Stansby, *Brit. J. Surgery* **82**, 726 (1995).
33. V. Herbert and N. Coleman, in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Disease*, 7th ed., Lea and Febiger, Philadelphia, Pa., 1988, pp. 388–416.
34. Food and Nutrition Board, National Research Council, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.
35. *The Merck Index*, 11th ed., Merck and Co., Rahway, N.J., 1989, pp. 9921–9922.
36. H. P. C. Hogenkamp, *Am. J. Clin. Nutr.* **33**, 1 (1980).
37. H. P. C. Hogenkamp, in B. M. Babior, ed., *Cobalamin-Biochemistry and Pathophysiology*, John Wiley & Sons, Inc., New York, 1975, p. 21.
38. B. M. Babior and J. S. Krouwer, *CRC Crit. Rev. Biochem.* **6**, 35 (1979).
39. R. Bonnett, in D. Dolphin, ed., *B$_{12}$*, John Wiley & Sons, Inc., New York, 1982, p. 201; J. Halpern, *ibid.*, p. 501; B. T. Golding, *ibid.*, p. 543.
40. E. L. Smith, *Vitamin B$_{12}$*, Methuen, London, 1965.
41. G. N. Schrauzer, *Acc. Chem. Res.* **1**, 97 (1968).
42. D. Dodd and M. D. Johnson, *J. Organomet. Chem.* **52**, 1 (1973).
43. G. Rytz, L. Walder, and R. Scheffold, in B. Zagalak and W. Friedrich, eds., *Vitamin B$_{12}$, Proceedings of the Third European Symposium on Vitamin B$_{12}$, Zurich, Switzerland, 1979*, Walter de Gruyter, Berlin, 1979, p. 173.
44. R. Scheffold, M. Dike, S. Dike, T. Herold, and L. Walder, *J. Am. Chem. Soc.* **102**, 3642 (1980).
45. A. Fischli, *Helv. Chim. Acta* **61**, 2560, 3028 (1978).
46. A. Fischli, *Helv. Chim. Acta* **62**, 882 (1979).
47. A. Fischli and D. Süss, *Helv. Chim. Acta* **62**, 48, 2361 (1979)
48. A. Fischli and J. J. Daly, *Helv. Chim. Acta* **63**, 1628 (1980).
49. A. Fischli and P. M. Müller, *Helv. Chim. Acta* **63**, 529, 1619 (1980).
50. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, p. 343.
51. *The United States Pharmacopeia XXIII* (*USP XXIII–NF XVIII*), United States Pharmacopeial Convention, Inc., Rockville, Md., 1995, pp. 1719–1721.
52. H. B. Chin, J. Augustin, B. P. Klein, D. A. Becker, and P. B. Venugolpal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, Chapt. 19.
53. H. L. Rosenthal, in W. H. Sebrell, Jr. and R. S. Harris, eds., *The Vitamins*, 2nd ed., Vol. II, Academic Press, Inc., New York, 1968, pp. 145–170.
54. H. R. Skeggs, in P. György and W. N. Pearson, eds., *The Vitamins*, 2nd ed., Vol. VII, Academic Press, Inc., New York, 1968, pp. 277–293; H. Baker and O. Frank *ibid.*, pp. 293–301.
55. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1990, pp. 952.20, 986.23.
56. Analytical Methods Committee, *Analyst* **81**, 132 (1956).
57. H. L. Newmark, J. Scheiner, M. Marcus, and M. Prabhudesai, *Am. J. Clin. Nutr.* **29**, 645 (1976).
58. P. J. Casey, K. R. Speckman, F. J. Ebert, and W. E. Hobbs, *J. Assoc. Off. Anal. Chem.* **65**, 85 (1982).
59. J. Lindemans and J. Abels, in A. P. De Leenheer, W. E. Lambert, and M. G. M. De Ruyter, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1985, Chapt. 12.
60. T. S. Hudson, S. Subramanian, and R. J. Allen, *J. Assoc. Off. Anal. Chem.* **67**, 996 (1984).
61. R. B. Woodward, *Pure Appl. Chem.* **17**, 519 (1968).
62. Ref. 61, **25**, 283 (1971).
63. Ref. 61, **33**, 145 (1973).
64. A. Eschenmoser, *Q. Rev. Chem. Soc. London* **24**, 366 (1970).
65. A. Eschenmoser, *Pure Appl. Chem. Suppl.* **2**, 69 (1971).
66. A. Eschenmoser, *Naturwissenschaften* **61**, 513 (1974).
67. A. Eschenmoser, *Chem. Soc. Rev.* **5**, 377 (1976).
68. A. Eschenmoser and C. E. Wintner, *Science* **196**, 1410 (1977).
69. A. Eschenmoser, in Ref. 43, p. 89.
70. J. H. Kreiger, *Chem. Eng. News*, 16 (1973).
71. T. H. Maugh, *Science* **179**, 266 (1973).

72. A. H. Jackson and K. M. Smith, in J. ApSimon, ed., *The Total Synthesis of Natural Products*, Vol. 1, John Wiley & Sons, Inc., New York, 1983, p. 232; *Ibid.*, Vol. 6, 1984, p. 258.
73. N. Anand, J. Bindra, and S. Ranganathan, *Art in Organic Synthesis*, 2nd ed., John Wiley & Sons, Inc., New York, 1988, p. 375.
74. R. B Woodward, *Chem. Soc. Spec. Publ.* **21**, 217 (1967).
75. W. Friedrich, G. Gross, K. Bernhauer, and P. Zeller, *Helv. Chim. Acta* **43**, 704 (1960).
76. E. L. Smith, L. Mervyn, A. W. Johnson, and N. Shaw, *Nature (London)* **194**, 1175 (1962).
77. A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, 4146 (1963).
78. K. Bernhauer and O. Müller, *Biochem., Z.* **336**, 102 (1962).
79. D. Autissier, P. Barthelemy, and L. Penasse, *Bull. Soc. Chim. Fr. Pt. II*, 192 (1980).
80. A. R. Battersby, *Acc. Chem. Res.* **26**, 15 (1993).
81. A. R. Battersby, *Pure Appl. Chem.* **65**, 1113 (1993).
82. A. R. Battersby, *Science* **264**, 1551 (1994).
83. F. Blanche et al., *Angew. Chem. Int. Ed. Engl.* **34**, 383 (1995).
84. A. I. Scott, *Angew. Chem. Int. Ed. Eng.* **32**, 1223 (1993).
85. A. I. Scott, *Tetrahedron* **50**, 13315 (1994).
86. J. Crouzet, B. Cameron, F. Blanche, D. Thibaut, L. Debussche, in R. H. Baltz, G. D. Hegeman, and P. L. Skatrud, eds., *Industrial Microorganisms: Basic and Applied Molecular Genetics*, American Society for Microbiology, Washington, D.C., 1993, p. 195.
87. U.S. Pat. 2,505,053 (Apr. 25, 1950), F. A. Kuehl and L. Chaiet (to Merck & Co., Inc.).
88. U.S. Pat. 2,626,888 (Jan. 27, 1953), S. Kutosh, G. B. Hughey, and R. Malcolmson (to Merck & Co., Inc.).
89. U.S. Pat. 2,628,186 (Feb. 10, 1953), W. Shive (to Research Corp.).
90. Ger. Pat. 953,643 (June 14, 1952), H. M. Shafer and A. J. Holland (to Merck & Co., Inc.).
91. H. Vogelmann and F. Wagner, *J. Chromatogr.* **76**, 359 (1973).
92. Ger. Pat. 1,037,066 (Feb. 12, 1959), K. Bernhauer and W. Friedrich (to Aschaffenburger Zellstoffwerke).
93. U.S. Pat. 2,563,794 (Aug. 7, 1951), E. L. Rickes and T. R. Wood (to Merck & Co., Inc.).
94. U.S. Pat. 2,582,589 (Jan. 15, 1952), H. H. Friecke (to Abbott Laboratories).
95. *Chem. Mark. Rep.* (Feb. 5, 1996).

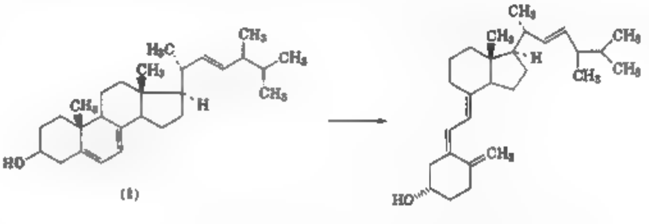
JOHN W. SCOTT
Hoffmann-La Roche, Inc.

VITAMINS: VITAMIN D

Vitamin D [*1406-12-2*] is a material which is formed in the skin of animals upon irradiation by sunlight and serves as a precursor for metabolites which control the animal's calcium homeostasis and act in other hormonal functions. A deficiency of vitamin D can cause rickets, as well as other disease states. This tendency can be a problem wherever animals, including humans, especially infants and children, receive an inadequate amount of sunshine. The latter phenomenon became prevalent with the advent of the industrial revolution, and efforts to cure rickets resulted in the development of commercial sources of vitamin D for supplementation of the diet of livestock, pets, and humans.

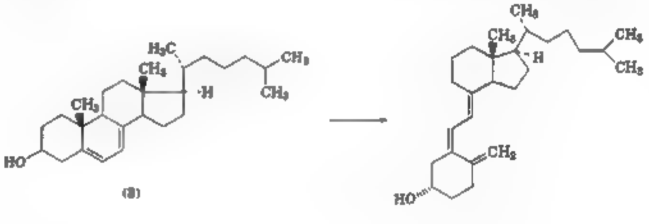
Research conducted during and subsequent to the 1970s revealed that vitamin D is better defined as those natural or synthetic substances that are converted by animals into metabolites that control calcium and phosphorus homeostasis and act in a variety of other hormonal-like functions.

Vitamin D_2 and vitamin D_3 are the two economically important forms of vitamin D. The other D vitamins have relatively little biological activity and are only of historical interest. Vitamin D_2 (ergocalciferol; ercalciol), (5*Z*,7*E*,22*E*)-(3S)-9,10-*seco*-5,7,10(19)-22-ergostatraene-3-ol (**2**), is active in humans and other mammals, although recently (ca 1997) it has been shown to be less active than vitamin D_3 in cattle, swine, and horses. It is relatively inactive in poultry. It is prepared by the uv irradiation of ergosterol (provitamin D_2), (24-methylcholesta-5,7,22-triene-3B-ol) (**1**), a plant sterol.



(1) (2)

Vitamin D_3 (cholecalciferol; calciol), (5*Z*,7*E*)-(3*S*)-9,10-*seco*-5,7-10(19) cholestatriene-3-ol (**4**), is the naturally occurring active material found in all animals. It is produced in the skin by the irradiation of stored 7-dehydrocholesterol (provitamin D_3), cholesta-5,7-diene-3B-ol (**3**).



(3) (4)

NOMENCLATURE

The Vitamin D compounds are steroidal materials and thus are named according to the IUPAC-IUB rules for nomenclature (1) (Table 1). Vitamin D_1 [*520-91-2*] is a mixture of vitamin D_2 and lumisterol.

The common name vitamin D is used throughout the pharmaceutical industry for simplicity. The trivial name calciferol has also been used extensively with the prefix ergo- and chole-, which indicate vitamin D_2 (**2**) and vitamin D_3 (**4**), respectively. Vitamin D_2 was originally named calciferol in 1931 by Angus and his co-workers (2). Historically, a number of substances were referred to as vitamin D and were distinguished from one another by a subscript numeral, eg, vitamin D_2, vitamin D_3, etc.

72. A. H. Jackson and K. M. Smith, in J. ApSimon, ed., *The Total Synthesis of Natural Products*, Vol. 1, John Wiley & Sons, Inc., New York, 1983, p. 232; *Ibid.*, Vol. 6, 1984, p. 258.
73. N. Anand, J. Bindra, and S. Ranganathan, *Art in Organic Synthesis*, 2nd ed., John Wiley & Sons, Inc., New York, 1988, p. 375.
74. R. B Woodward, *Chem. Soc. Spec. Publ.* **21**, 217 (1967).
75. W. Friedrich, G. Gross, K. Bernhauer, and P. Zeller, *Helv. Chim. Acta* **43**, 704 (1960).
76. E. L. Smith, L. Mervyn, A. W. Johnson, and N. Shaw, *Nature (London)* **194**, 1175 (1962).
77. A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, 4146 (1963).
78. K. Bernhauer and O. Müller, *Biochem., Z.* **336**, 102 (1962).
79. D. Autissier, P. Barthelemy, and L. Penasse, *Bull. Soc. Chim. Fr. Pt. II*, 192 (1980).
80. A. R. Battersby, *Acc. Chem. Res.* **26**, 15 (1993).
81. A. R. Battersby, *Pure Appl. Chem.* **65**, 1113 (1993).
82. A. R. Battersby, *Science* **264**, 1551 (1994).
83. F. Blanche et al., *Angew. Chem. Int. Ed. Engl.* **34**, 383 (1995).
84. A. I. Scott, *Angew. Chem. Int. Ed. Eng.* **32**, 1223 (1993).
85. A. I. Scott, *Tetrahedron* **50**, 13315 (1994).
86. J. Crouzet, B. Cameron, F. Blanche, D. Thibaut, L. Debussche, in R. H. Baltz, G. D. Hegeman, and P. L. Skatrud, eds., *Industrial Microorganisms: Basic and Applied Molecular Genetics*, American Society for Microbiology, Washington, D.C., 1993, p. 195.
87. U.S. Pat. 2,505,053 (Apr. 25, 1950), F. A. Kuehl and L. Chaiet (to Merck & Co., Inc.).
88. U.S. Pat. 2,626,888 (Jan. 27, 1953), S. Kutosh, G. B. Hughey, and R. Malcolmson (to Merck & Co., Inc.).
89. U.S. Pat. 2,628,186 (Feb. 10, 1953), W. Shive (to Research Corp.).
90. Ger. Pat. 953,643 (June 14, 1952), H. M. Shafer and A. J. Holland (to Merck & Co., Inc.).
91. H. Vogelmann and F. Wagner, *J. Chromatogr.* **76**, 359 (1973).
92. Ger. Pat. 1,037,066 (Feb. 12, 1959), K. Bernhauer and W. Friedrich (to Aschaffenburger Zellstoffwerke).
93. U.S. Pat. 2,563,794 (Aug. 7, 1951), E. L. Rickes and T. R. Wood (to Merck & Co., Inc.).
94. U.S. Pat. 2,582,589 (Jan. 15, 1952), H. H. Friecke (to Abbott Laboratories).
95. *Chem. Mark. Rep.* (Feb. 5, 1996).

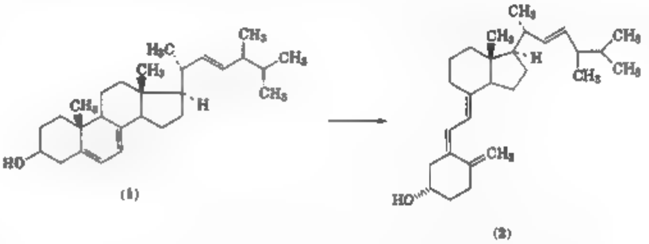
JOHN W. SCOTT
Hoffmann-La Roche, Inc.

VITAMINS: VITAMIN D

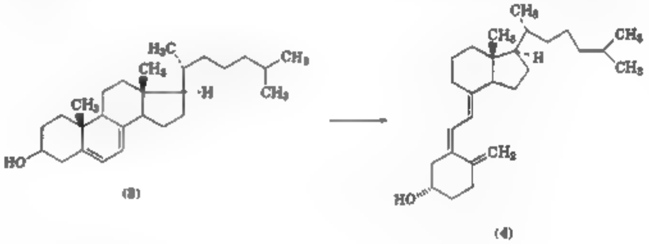
Vitamin D [*1406-12-2*] is a material which is formed in the skin of animals upon irradiation by sunlight and serves as a precursor for metabolites which control the animal's calcium homeostasis and act in other hormonal functions. A deficiency of vitamin D can cause rickets, as well as other disease states. This tendency can be a problem wherever animals, including humans, especially infants and children, receive an inadequate amount of sunshine. The latter phenomenon became prevalent with the advent of the industrial revolution, and efforts to cure rickets resulted in the development of commercial sources of vitamin D for supplementation of the diet of livestock, pets, and humans.

Research conducted during and subsequent to the 1970s revealed that vitamin D is better defined as those natural or synthetic substances that are converted by animals into metabolites that control calcium and phosphorus homeostasis and act in a variety of other hormonal-like functions.

Vitamin D_2 and vitamin D_3 are the two economically important forms of vitamin D. The other D vitamins have relatively little biological activity and are only of historical interest. Vitamin D_2 (ergocalciferol; ercalciol), (5*Z*,7*E*,22*E*)-(3S)-9,10-*seco*-5,7,10(19)-22-ergostatraene-3-ol (**2**), is active in humans and other mammals, although recently (ca 1997) it has been shown to be less active than vitamin D_3 in cattle, swine, and horses. It is relatively inactive in poultry. It is prepared by the uv irradiation of ergosterol (provitamin D_2), (24-methylcholesta-5,7,22-triene-3B-ol) (**1**), a plant sterol.



Vitamin D_3 (cholecalciferol; calciol), (5*Z*,7*E*)-(3*S*)-9,10-seco-5,7-10(19) cholestatriene-3-ol (**4**), is the naturally occurring active material found in all animals. It is produced in the skin by the irradiation of stored 7-dehydrocholesterol (provitamin D_3), cholesta-5,7-diene-3B-ol (**3**).



NOMENCLATURE

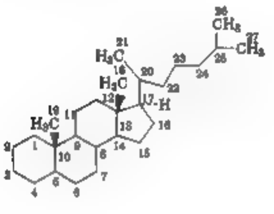
The Vitamin D compounds are steroidal materials and thus are named according to the IUPAC-IUB rules for nomenclature (1) (Table 1). Vitamin D_1 [*520-91-2*] is a mixture of vitamin D_2 and lumisterol.

The common name vitamin D is used throughout the pharmaceutical industry for simplicity. The trivial name calciferol has also been used extensively with the prefix ergo- and chole-, which indicate vitamin D_2 (**2**) and vitamin D_3 (**4**), respectively. Vitamin D_2 was originally named calciferol in 1931 by Angus and his co-workers (2). Historically, a number of substances were referred to as vitamin D and were distinguished from one another by a subscript numeral, eg, vitamin D_2, vitamin D_3, etc.

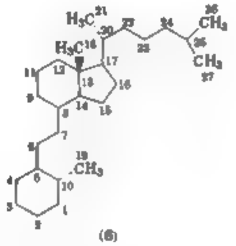
Table 1. Vitamin D Substances

Common name	CAS registry number	Provitamin	Trivial name	IUPAC-IUB name
Vitamin D_2	*[50-14-6]*	Ergosterol	Ergocalciferol	9,10-*seco*-5,7,10(19),22-Ergostatetraen-3β-ol
Vitamin D_3	*[67-97-0]*	7-Dehydrocholesterol	Cholecalciferol	9,10-*seco*-5,7,10(19),Cholestatrien-3β-ol
Vitamin D_4	*[511-28-4]*	22,23-Dihydroergosterol		24-Methyl-9,10-*seco*-5,7,10(19)-cholestatrien-3β-ol
Vitamin D_5	*[71761-06-3]*	7-Dehydrositosterol	Sitocalciferol	2,4-Ethyl-9,10-*seco*-5,7,10(19)-cholestatrien-3β-ol
Vitamin D_6	*[481-19-6]*	7-Dehydrostigmasterol		24-Ethyl-9,10-*seco*-5,7,10(19)-22-ergo-statetraen-3β-ol
Vitamin D_7	*[20304-51-2]*	7-Dehydrocampesterol		2,4-Methyl-9,10-*seco*-5,7,10(19)-cholestatrien-3β-ol

The vitamins D are 9,10-secosteroids, that is, steroid molecules with an opened 9,10 bond of the B-ring. The relationship between the provitamin steroid (perhydro-1,2-cyclopentanophenanthrene ring system) and the 9,10-secosteroid nucleus is shown in structures (**5**) and (**6**), cholestane and 9,10-secocholestane (calcitane), respectively.



(5)



(6)

In 1981, the IUPAC-IUB Joint Commission on Biochemical Nomenclature proposed that there be a set of trivial names for the important vitamin D compounds, including calciol *[67-97-0]* for vitamin D, calcidiol *[19356-17-3]* for 25-hydroxy-vitamin D_3, and calcitriol *[32222-06-3]* for 1α,25-dihydroxy-vitamin D_3. This nomenclature has met with varying degrees of acceptance, as has the proposal to use calcine *[69662-75-5]* (deoxy-vitamin D_2) and ercalcine *[68323-40-0]* (deoxy-vitamin D_3) to name the triene hydrocarbon structure for 9,10-*seco*-cholesta-5,7,10(19)-triene and 9,10-*seco*-ergosta-5,7,10(19),22-tetraene, respectively. In systematic nomenclature, calcitane can be used for the basic 27-carbon skeleton instead of 9,10-secocholestane.

ISOLATION AND STRUCTURE DETERMINATION

The isolation and structure elucidation of vitamin D is closely related to the efforts to understand and cure rickets and related bone diseases. The advent of the use of soft coal, the migration of people to cities, and the tendency of people and animals to spend less time in sunshine caused a decline in the ability of populations to synthesize sufficient quantities of vitamin D_3. This led to the increased incidence of rickets, beginning around the mid-1600s (3).

The bone disease rickets in children, and a similar condition in adult known as osteomalacia, is characterized by the body's inability to calcify the collagen matrix of growing bone, resulting in wide epiphyseal plates and large areas of uncalcified bone called osteoid. The resultant lack of rigidity of bones leads to the ends becoming twisted and bent, particularly in long bones. The ribs develop a bumpy and uneven texture known as rosary ribs and the legs become bowed. Also, the cranium becomes soft and misshapen. In adults, no long-bone growth occurs, but new bone which is being continually remodeled, activates cells to resorb bone, followed by osteoblast-mediated bone-growth replacement (4,5). Metabolites of vitamin D act in the body to modulate these activities and maintain strong bone structure.

Although there is evidence that rickets was manifest in humans as early as 800 B.C., it was not until 1645 that it was first described (6). The progress of finding a cure for rickets was relatively slow until the late 1800s, when a sufficient number of scientific developments began to allow workers to unravel the difficult puzzle of vitamin D metabolism. Tarret in 1889 had isolated ergosterol from ergot of rye and demonstrated that it was different from cholesterol. The similarity of the structures, however, led to difficulty in the structural elucidation of the vitamin D molecule. Mellanby (7) demonstrated the lack of a dietary component could be used to develop rickets and was able to raise D-deficient animals. This allowed research aimed at finding the antirachitic factor to be carried out.

In 1919, Huldschinski (8) realized that uv light cured rickets and impacted on its etiology. The uv light and cod liver oil were found to be useful in the treatment of the disease, and irradiation of food produced the same effect as irradiation of the animal. The link between irradiation and plant materials led to the conclusion that ergosterol was an antirachitic substance, and an extensive effort was made to characterize the chemistry of irradiated ergosterol.

Windaus in 1933 derived the skeletal structure of vitamin D_2. He found the elemental formula to be $C_{28}H_{44}O$. The side chain was unequivocally characterized by x-ray crystallography later, in 1948. Reindel and Kipphan (9) in 1932 ozonized the side chain and elucidated the C_{22} double bond. The reaction to form a Diels Alder adduct with maleic anhydride and the typical diene uv spectrum led to the conclusion there was a diene in the B ring. In 1934 Fernholz and Chakravorty (10) determined the provitamin had a 3β-hydroxy group because the molecule could be precipitated with digitonin, a reaction characteristic of the 3β-ol functionality in steroids.

Perbenzoic acid gave a doubly unsaturated triol monobenzoate. Only two hydroxyl groups could be acetylated, and one was tertiary. The saturated triol reacted with lead tetracetate to give an α glycol. When reacted with chromic acid, it gave a hydroxy lactone. From these observations, Windaus and Grundmann (11) described the correct structure for ergosterol (**1**).

The observation that the uv spectrum of provitamin D changed with uv irradiation and also produced antirachitic activity led to the conclusion that vitamin D was derived from the provitamin. Windaus found the vitamin D_2 formula $C_{28}H_{44}O$ to be isomeric with the provitamins.

Bunal in 1932 used x-ray crystallography to demonstrate that many of the preparations described in the literature were mixtures and that vitamin D_2 was one crystal structure.

Calciferol, when hydrogenated catalytically, took up 4 moles of hydrogen and gave a compound with the empirical formula $C_{28}H_{52}O$. Sodium in ethanol reduction gave a dihydroproduct which reacted with 3 moles of perbenzoic acid, thus demonstrating the derivative to have three double bonds.

Ozonolysis of vitamin D_2 gave 2,3-dimethylbutanol, showing the side chain to contain the 22 double bond and a C_{24} methyl group.

Vitamin D_2 reacted with maleic anhydride to give a mono Diels-Alder adduct, which hydrolyzed to yield a dicarboxylic acid. Acetylation of the alcohols, esterification of carboxylic acids, and hydrogenation gave a compound which, when ozonized, gave a saturated ketone, $C_{19}H_{34}O$. This molecule contains the C and D ring and side chain from vitamin D_2, indicating that the B ring must have been opened by photolytic cleavage of the C_9–C_{10} bond (12). The vitamin D_2 molecule was thus shown to be a tricyclic material with four double bonds.

Windaus and Boch (13) isolated and characterized 7-dehydrocholesterol in 1937 from pig skin. They showed, further, that vitamin D_3 could be generated from the provitamin by uv irradiation.

Irradiated ergosterol was found not to be as antirachitic in the chick as in the rat, whereas the chick could be protected by direct irradiation. The provitamin in cholesterol was shown not to be ergosterol. Rygh (14) in 1935 found that 1 rat unit of cod liver oil was 100 times more potent in chicks than 1 rat unit of vitamin D_2. Brockmann (15) in 1936, prepared the pure crystalline 3,5-dinitrobenzoate derivative of vitamin D_3 obtained from tuna liver oil (subsequently demonstrated in other species like halibut and blue fin tuna). This material was shown to be identical to the dinitrobenzoate derivative of vitamin D_3 obtained by Windaus from the irradiation of 7-dehydrocholesterol. Thus, the chemical identity of vitamin D_2 and D_3 were well established. A more detailed description of the events leading to the structure elucidation of the vitamin Ds can be found in Reference 6.

OCCURRENCE

The provitamins, precursors of the vitamin Ds, are distributed widely in nature, whereas the vitamins themselves are less prevalent. The amounts of provitamins D_2 and D_3 in various plants and animals are listed in Table 2.

Fish-liver oil, liver, milk, and eggs are good natural sources of the D_3 vitamin. Most milk sold in the United States is fortified with manufactured vitamin D. Fish oil is the only commercial source of natural vitamin D_3, and the content of the vitamin varies according to species as well as geographically, ie, Atlantic cod contain 100 IU/g where IU (International Unit) = 0.025 μg of vitamin D_3, whereas oriental tuna (*Percomorpli*) contain 45,000 IU/g of oil.

Vitamin D_3 rarely occurs in plants. However, *Solanum glaucophyllum, Solanum malacoxylon, Cestrum diurnum,* and *Trinetum flavescens* have been shown to contain water-soluble glycosides of vitamin D analogues with 1α,25-dihydroxy-vitamin D activity (16–22). The vitamin D content in various plant and animal materials is shown in Table 3. Vitamin D_3 occurs naturally in all animals (24).

CHEMICAL AND PHYSICAL PROPERTIES

Provitamin

The chemistry of the D vitamins is intimately involved with that of their precursors, the provitamins. The manufacture of the vitamins and their derivatives usually involves the synthesis of the provitamins, from which the vitamin is then generated by uv irradiation. The chemical and physical properties of the provitamins are discussed below, followed by the properties of the vitamins.

3β-Hydroxy steroids which contain the 5,7-diene system and can be activated with uv light to produce vitamin D compounds are called provitamins. The two most important provitamins are ergosterol (**1**) and 7-dehydrocholesterol (**3**). They are produced in plants and animals, respectively, and 7-dehydrocholesterol is produced synthetically on a commercial scale. Small amounts of hydroxylated derivatives of the provitamins have been synthesized in efforts to prepare the metabolites of vitamin D, but these products do not occur naturally. The provitamins do not possess physiological activities, with the exception that provitamin D_3 is found in the skin of animals and acts as a precursor to vitamin D_3, and synthetic dihydroxalated analogues of pro- and previtamins have been found to have selective activity towards nuclear and nonnuclear receptors (24–27).

Provitamin D_2

Ergosterol is isolated exclusively from plant sources. The commercial product is ca 90–100% pure and often contains up to 5 wt % of 5,6-dihydroergosterol. Usually, the isolation of provitamin D_2 from natural sources involves the isolation of the total sterol content, followed by the separation of the provitamin from the other sterols. The isolation of the sterol fraction involves extraction of the total fat component, its saponification, and then reextraction of the unsaponifiable portion with an ether. The sterols are in the unsaponifiable portion. Another method is the saponification of the total material, followed by isolation of the nonsaponifiable fraction. Separation of the sterols from the unsaponifiable fraction is done by crystallization from a suitable solvent, eg, acetone or alcohol. Ethylene dichloride, alone or mixed with methanol, has been used commercially for recrystallization. In the case of yeasts, it is particularly difficult to remove the ergosterol by simple extraction, thereby obtaining only ca 25% recovery. Industri-

Table 2. Occurrence of the Provitamins D in Selected Plants and Animals, Parts per Thousand of Total Sterol

Source	Amount	Source	Amount
Cottonseed oil	28	Gallstones, man	0.25
Rye grass	15	*Mytilus edulis,* sea mussel	100
Wheat germ oil	10	*Modiolus demissus,* ribbed mussel	370
Carrot	1.7	*Ostrea virginica,* oyster	80
Cabbage	0.5	*Ostrea edulis,* oyster	34
Skin, pig	46	*Asterias rubens,* starfish	3.8
Skin, chicken feet	25	Common sponges	20
Skin, rat	19	Common coral	10
Skin, mouse	9	*Aspergillus niger,* mold	1,000
Skin, calf	7	*Cortinellus shiitake,* mushroom	1,000
Skin, human infant	1.5	*Claviceps purpurea,* ergot	9,000
Skin, human adult	4.2	*Saccharomyces cerevisiae,* yeast	800
Liver, Japanese tuna	11	*Penicillium puberculum,* mold	1,000
Liver, Atlantic cod	4.4	*Fucus vesiculosus,* alga, seaweed	0.8
Liver, shark	1.0	*Tubifex* sp, waterworm	210
Liver, halibut	0.6	*Bumbricus terrestris,* earthworm	170
Liver, tuna	1.0	*Tenebrio molitor,* mealworm	120
Eggs, Chinese duck	60	*Gyronomus,* sp, goat	61
Eggs, cod (roe)	5.5	*Cancer pagurus,* common crab	15
Eggs, hen	1.6	*Dephnia* sp, water flea	7.5
Wool fat, sheep	3.9	*Musca domestica,* housefly	7.0
Milk, cow	2.3	*Crangon vulgaris,* shrimp	3.8
Pancreas, beef	1.8	*Homarus vulgaris,* lobster	2.5
Spinal cord, beef	1.2	*Helix pomatia,* edible snail	97
Blood serum, cow	0.5	*Arion empiricorum,* slug, red road snail	220
Herring oil	0.5	*Littorina littorea,* periwinkle	170
Heart, calf	0.32	*Septa* sp, cuttlefish	12

Table 3. Distribution of Vitamin D Activity

Sample	Amount[a]
Phytoplankton	0
Sargassum (a gulfweed)	Some activity
Clover hay	
Sun-cured	Slight
Dark-cured	0
Mushrooms (*Agaricus campestris*)	0.21 IU/g
Milk (unfortified)	
Winter (bovine)	5.3 IU/L
Summer (bovine)	53 IU/L
Milk (human)	63 IU/L
Milk colostrum (human)	315–635 IU/L
Egg yolk	150–400 IU/g
Butter	4–8 IU/g
Fish-liver oils	50–45,000 IU/g

Source. Ref 23.

[a] IU (International Unit) = 0.025 μg of vitamin D_3.

ally, therefore, the ergosterol is obtained by preliminary digestion with hot alkalies or with amines (28–33). Variations of the isolation procedure have been developed. For example, after saponification, the fatty acids may be precipitated as calcium salts, which tend to absorb the sterols. The latter are then recovered from the dried precipitate by solvent extraction.

Provitamin D_3

Provitamin D_3 is made from cholesterol, and its commercial production begins with the isolation of cholesterol from one of its natural sources. Cholesterol occurs in many animals, and is generally extracted from wool grease obtained by washing wool after it is sheared from sheep. This grease is a mixture of fatty-acid esters, which contain ca 15 wt % cholesterol. The alcohol fraction is obtained after saponification, and the cholesterol is separated, usually by complexation with zinc chloride, followed by decomplexation and crystallization. Cholesterol can also be extracted from the spinal cords and brains of animals, especially cattle, and from fish oils.

Cholesterol (**7**) is converted to 7-dehydrocholesterol (**3**) (see Fig. 1). This process usually involves the Ziegler allylic bromination of the 7 position followed by dehydrobromination (34). Esterification of the cholesterol (**8**) is necessary to prevent oxidation of the 3β-alcohol by the brominating agent. Allylic bromination may be accomplished with a variety of brominating agents, eg, *N*-bromosuccinimide, *N*-bromophthalimide, or preferably 5,5-dimethyl-1,3-dibromohydantoin (35). Bromine in carbon disulfide can be used if the free-radical bromination is photocatalyzed (36). A mixture of 7α-(**9**) and 7β-bromo cholesteryl esters (**10**) is obtained and treated with an appropriate base to dehydrohalogenate the molecule and give the 7-dehydrocholesteryl ester (**11**) (37,38). Proper conditions for this reaction are necessary to generate a high yield of the desired 7-dehydro product instead of the undesired cholesta-4,6-dien-3β-ol ester (**12**), which is formed as a by-product. Various reagents can be used to perform the dehydrohalogenation. Trimethyl phosphite or pyridine bases, particularly trimethylpyridine, have been used; the symmetrical collidine is the reagent of choice (35,38,39). *t*-Butylammonium fluo-

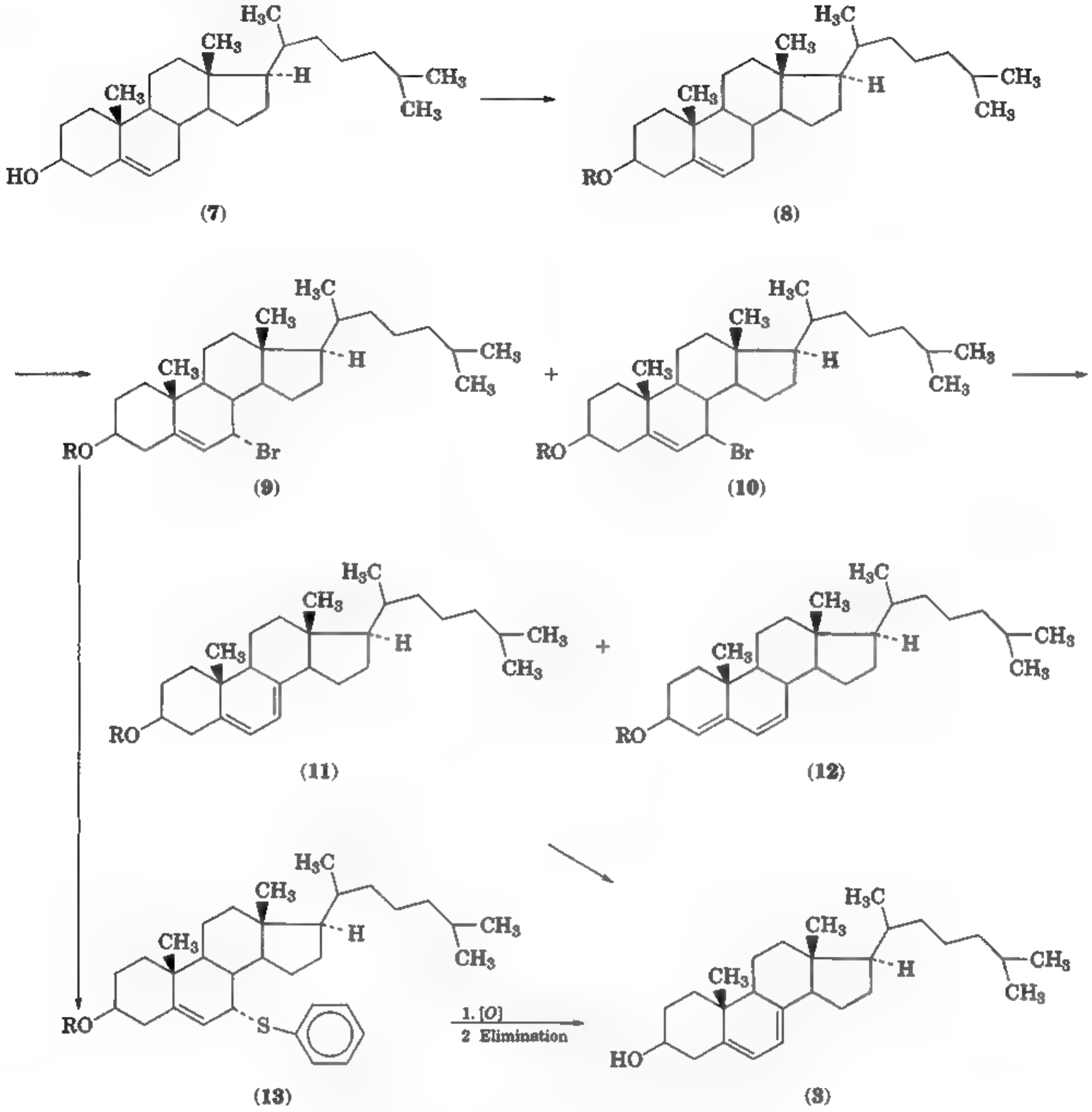


Figure 1. Conversion of cholesterol to 7-dehydrocholesterol.

ride has also been used to improve the yield of high quality 5,7-diene (40).

The 7α-bromo steroid (**9**) can also be treated with sodium phenyl selenolate (41). The resultant 7β-phenyl selenide (**13**) can be oxidized and the corresponding phenyl selenoxide eliminated to form the 7-dehydrocholesteryl ester (**11**).

7-Dehydrocholesterol has also been made from cholesterol by the Windaus procedure (Fig. 2); the 3,7-dibenzoate (**16**) is obtained (via (**14**) and (**15**) by oxidation and reduction), which undergoes thermal elimination to give the 7-dehydrocholesteryl benzoate (**11**) (42–44). However, the yields are substantially lower than those achieved by the bromination–dehydrobromination method.

7-Tosylhydrazone and 7-phenyl sulfoxide groups have also been introduced into cholesterol and eliminated to prepare the 5,7-diene (45,46). The method of choice is the allylic bromination–dehydrobromination procedures, and the commercial yields in converting cholesterol to 7-dehydrocholesterol are in the range of 35–50%.

Vitamin D

The irradiation of the provitamins to produce vitamin D as well as several isomeric substances was first studied with

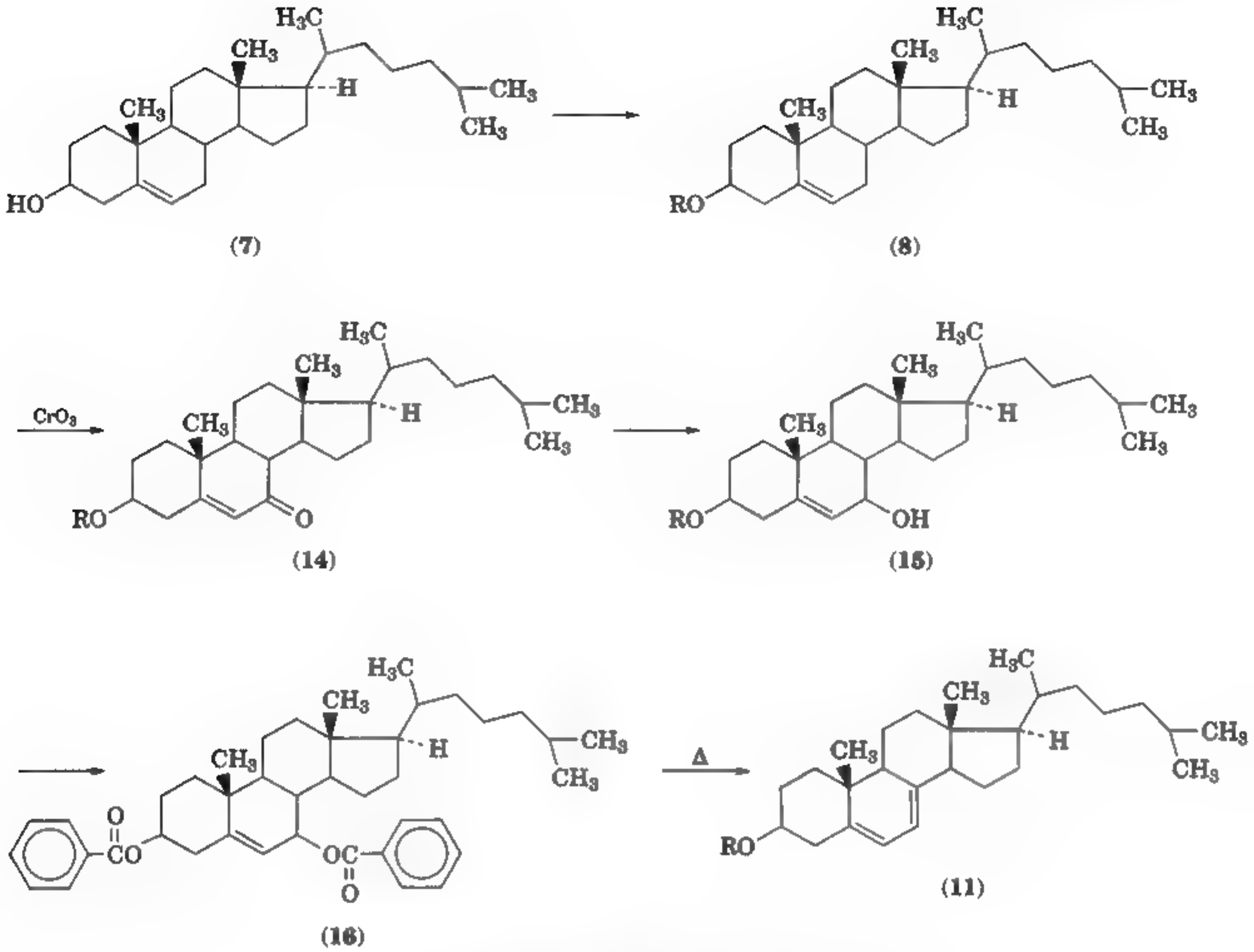


Figure 2. The Windaus procedure. *Source:* Ref. 42.

ergosterol. The chemistry is identical for the vitamin D_3 series and yields analogous isomers. In 1932, a scheme for the irradiation of ergosterol leading to vitamin D_2 was proposed (45). Twenty years later, the mechanism of the irradiation of the provitamins to vitamin D and its photoisomers was further elucidated (46,47). More recently, Jacobs (48) has reviewed the photochemistry. A number of products associated with the irradiation process are shown in Figure 3. The geometry and electronic characteristics of these molecules have been well established by x-ray crystallographic analysis and valence force-field calculations (50–52). The irradiation process, which converts 7-dehydrocholesterol to vitamin D also occurs in the skin of animals if sufficient sunlight is available. The photochemical and thermal isomerizations occur during the generation of vitamin D *in vivo* as well as during its synthetic photochemical preparation (24,53,54). The initial step involves ring opening of the B-ring of the sterol by ultraviolet activation of the conjugated diene. The absorbance of uv energy activates the molecule, and the $\pi \rightarrow \pi^*$ excitation (absorption, 250–310 nm; $\lambda_{max} = 291$ nm, $\epsilon = 12{,}000$) results in the opening of the 9,10 bond and the formation of the (*Z*)-hexadiene, previtamin D_3 (R) [*1173-13-3*] (**17**) or previtamin D_2 (R′) [*21307-015-1*] (**18**). The uv irradiation of 7-dehydrocholesterol or ergosterol results in the steady diminution in concentration of the provitamin, initially giving rise to predominantly previtamin D. The pre- levels reach a maximum as the provitamin level drops below ca 10%. The concentration of the previtamin then falls as it is converted to tachysterol and lumisterol, which increase in concentration with continued irradiation (see Fig. 4). Temperature, frequency of light, time of irradiation, and concentration of substrate all affect the ratio of products. Previtamin D undergoes thermal equilibration to vitamin D (**2**) or (**4**), *cis*-vitamin D_2 [*50-14-6*] and *cis*-vitamin D_3 [*67-97-0*], respectively. The conversion of previtamin D (**18**) or (**17**) at temperatures of ≤80°C by thermal isomerization to give the cis vitamin (ergocalciferol) (**2**) or cholecalciferol (**4**) involves an equilibrium, as indicated in Table 4 (55,56).

The equilibrium composition is normally ca 80% vitamin D and 20% previtamin D. This reaction is an intramolecular [1–7] H sigmatropic shift and occurs through a rigid cyclic transition state (57).

Additionally, the $\pi \rightarrow \pi^*$ excitation of previtamin D can result in ring closure back to the provitamin (**1**) [*57-87-4*] or (**3**) [*434-16-2*] or to lumisterol$_2$ [*474-69-1*] (**19**) or lumi-

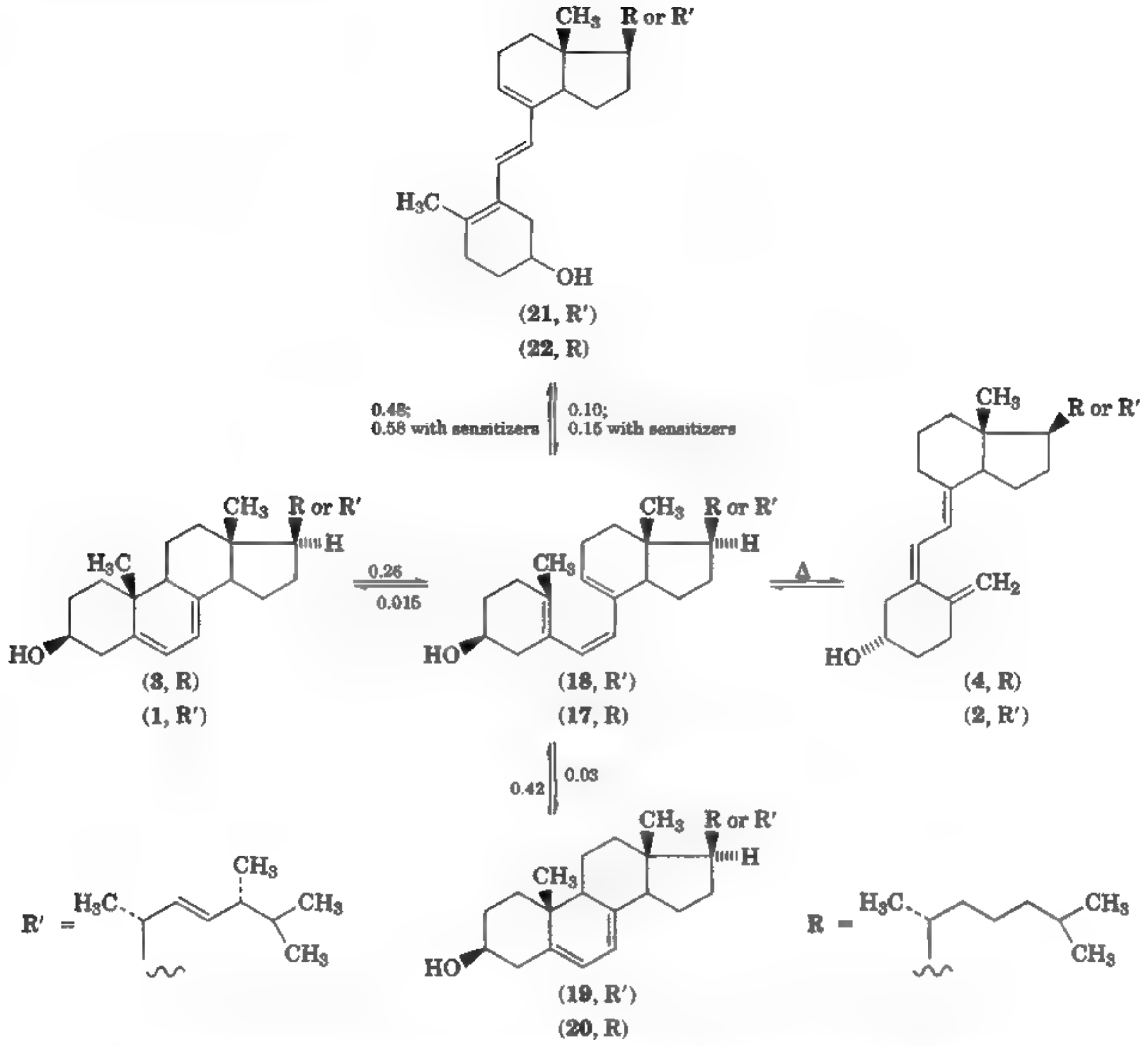


Figure 3. Photochemical and thermal isomerization products of vitamin D manufacture (49). The quantum yields of the reactions are listed beside the arrows for the given reactions.

sterol$_3$ [*5226-01-7*] (**20**), which have the 9β,10α configuration. This excitation can also exhibit (Z) $\rightleftharpoons$ (E) photoisomerization to the 6,7-(E)-isomer, tachysterol$_2$ [*115-61-7*] (**21**) or tachysterol$_3$ [*63902-44-3*] (**22**) (58,52).

Other photoinduced cyclization reactions can occur by conrotatory bond formation to give the 9β,10β-antiisomers, isopyrocalciferol$_2$ [*474-70-4*] (**23**) or isopyrocalciferol$_3$ [*10346-44-8*] (**24**) (Fig. 5), whereas thermal cyclization at >100°C leads to the two 9,10-syn isomers, (9α,10α)-pyrocalciferol (**27**) [*128-27-8*] or (**28**) [*10346-43-7*] by a disrotatory bond formation mechanism (47). Ultraviolet overirradiation leads to photopyro- (**29**) [*41411-05-6*] or (30) and photoisopyrocalciferols (**25**) [*26241-65-6*] or (**26**) [*85354-28-5*], respectively and to the formation of suprasterols of the type shown in structure (**37**) (59,60) (Fig. 6). Prolonged irradiation of the mixture of isomers can also lead to toxisterols of the type shown in structures (**38**) and (**39**), where R = D$_2$ and R′ = D$_3$ side chains (see Fig. 3)

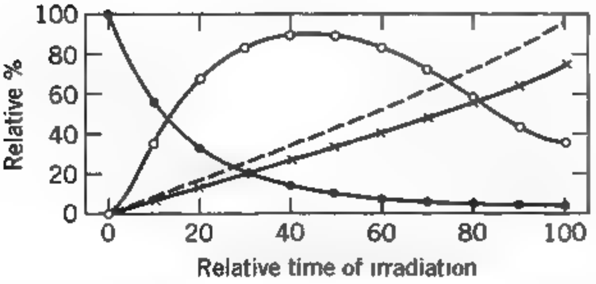


Figure 4. Time course for uv-irradiation of 7-dehydrocholesterol (●). (○), previtamin D; (×), lumisterol; (—), tachysterol.

Table 4. Inverconversion Time of Previtamin D_3 and Vitamin D_3 at 20°C, and 40°C

	Vitamin D_3 from previtamin D_3			Previtamin D_3 from vitamin D_3	
Formation (%)	Days at −20°C	Days at 20°C	Hours at 40°C	Days at 20°C	Hours at 40°C
2	27	0.2	0.4	2.2	3.7
5	68	0.4	1.1	8.2	11.2
7	96	0.5	1.5		18.6
10	140	0.8	2.3		43.3
20	269	1.6	4.8		
30	474	2.6	7.8		
40	681	3.8	11.3		
50	926	5.2	15.6		
60	1230	7.0	21.2		
70	1628	9.5	29 2		
80	2204	13.3	43.4		
90	2910	23 3			

Source: Ref. 55.

(61–63). More than 20 members of this type of substance have been identified. There is little evidence that these materials are toxic; however, their nomenclature leads to some misunderstanding. These compounds generally show little if any biological activity and have not been found *in vivo* (15,63–66). Normal irradiation conditions for the production of vitamin D include sufficiently low temperatures so that these products do not form, and they are not found in normal commercial samples of D_3 resins.

The irradiation of calciferol in the presence of iodine leads to the formation of 5,6-*trans*- vitamin D_2 [*14449-19-5*] (**31**) or D_3 [*22350-41-0*] (**32**) (67,68). 5,6-*trans*-Vitamin D as well as vitamin D (**2**) or (**4**) can be converted to isovitamin D by treatment with mineral or Lewis acids. Isocalciferol (**35**) [*469-05-6*] or (**36**) [*42607-12-5*] also forms upon heating of 5,6-*trans*-vitamin D. Isotachysterol (**33**) [*469-06-7*] or (**34**) [*22350-43-2*] forms from isocalciferol or vitamin D upon treatment with acid, and its production appears to be the result of sequential formation of *trans*- and isocalciferol from calciferol. These reactions are the basis of the antimony trichloride test for vitamin D (69–72).

Commercially, the irradiation of the 5,7-diene provitamin to make vitamin D must be performed under conditions that optimize the production of the previtamin while avoiding the development of the unwanted isomers. The optimization is achieved by controlling the extent of irradiation, as well as the wavelength of the light source. The best frequency for the irradiation to form previtamin is 295 nm (64–66). The unwanted conversion of previtamin to tachysterol is favored when 254 nm light is used. Sensitized irradiation, eg, with fluorenone, has been used to favor the reverse, triplet-state conversion of tachysterol to previtamin D (73,74).

The molecular extinction coefficients (at various wavelengths) of the four main components of the irradiation are shown in Table 5. The absorption of light above 300 nm is favored by tachysterol. A yield of 83% of the previtamin at 95% conversion of 7-dehydrocholesterol can be obtained by irradiation first at 254 nm, followed by reirradiation at 350 nm with a yttrium aluminum garnet (YAG) laser to convert tachysterol to previtamin D. A similar approach with laser irradiation at 248 nm (KrF) and 337 nm (N_2) has also been described (76).

The irradiation of the provitamin has been achieved using the acetate and benzoate esters, although the free alcohol form of the provitamin is usually used (77).

The physical properties of the provitamins and vitamins D_2 and D_3 are listed in Table 6. The values are listed for the pure substances. The D vitamins are fat-soluble and, as such, are hydrophobic.

SHIPPING AND HANDLING

Vitamin D and its products are sensitive to uv light, heat, air, and mineral acids. Its sensitivity to these conditions is exaggerated by the presence of heavy-metal ions, eg, iron. Care should be taken to store and ship vitamin D and its various product forms so that exposure to these conditions is minimized. Pharmaceutical grade vitamin D_3 is supplied in the pure crystalline form. This is used in vitamin D as well as multivitamin preparations. Commercial sources of feed-grade vitamin D are usually the vitamin D_3 resin stabilized by spray- or roll-drying a starch or gelatin suspension of the vitamin. These products should be stored in a cool, dry place in opaque, hermetically-sealed containers under nitrogen. Vitamin D is generally recognized as safe when used in accordance with good manufacturing or feeding practices (79).

Shipping vitamin D in crystalline or resin form should be done in containers marked appropriately to indicate the material is toxic by DOT standards. Its proper DOT labeling is DOT Hazard Class 6.1, poisonous. Waste material should be burned or placed in an appropriate landfill.

The provitamins are also unstable to heat and light. They generally should be stored in a dark, cool, dry place. The provitamin is more stable if shipped with 10–15 wt % methanol rather than in a dry form.

ANALYTICAL AND TEST METHODS

The development of reliable uv analysis permitted the dependable detection and assay of the provitamins and vitamins. Prior to this, the Lieberman-Bouchard chemical test was used, but the color reaction gave many false positives and was relatively inaccurate.

In 1949 the World Health Organization adopted the biological activity of 1 mg of an oil solution containing 0.025 μg of crystalline D_3 as the analytical standard for vitamin D_3. This standard was discontinued in 1972. USP uses crystalline cholecalciferol as a standard (80). Samples of reference standard may be purchased from U.S. Pharmacopeial Convention, Inc., Reference Standards Order Department, 12601, Twinbrook Parkway. Rockville, Maryland 20852. One international unit of vitamin D activity is that activity demonstrated by 0.025 μg of pure crystalline *cis*-vitamin D_3. One gram of vitamin D_3 is equivalent to 40

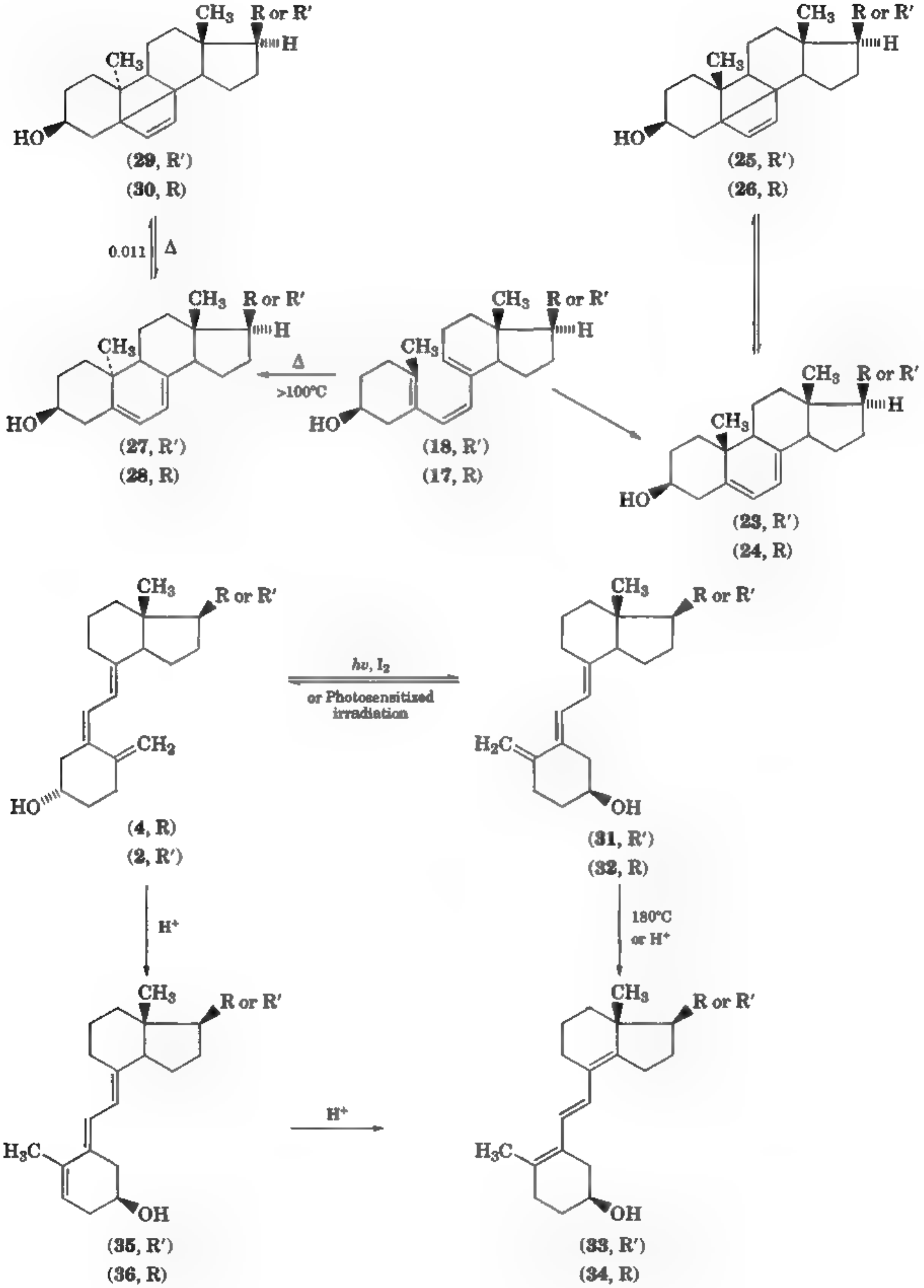
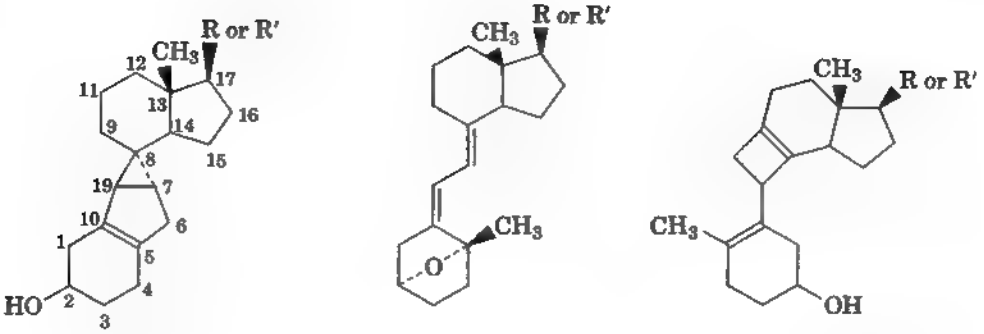

CH3
R or R′
H
CH3
HO
(29, R′)
(30, R)
CH3
R or R′
H
CH3
HO
(25, R′)
(26, R)
0.011
Δ
CH3
R or R′
H
CH3
HO
(27, R′)
(28, R)
Δ
>100°C
CH3
R or R′
H
CH3
HO
(18, R′)
(17, R)
CH3
R or R′
H
CH3
HO
(23, R′)
(24, R)
CH3
R or R′
CH2
HO
(4, R)
(2, R′)
hν, I2
or Photosensitized irradiation
CH3
R or R′
H2C
OH
(31, R′)
(32, R)
H+
180°C
or H+
CH3
R or R′
H3C
OH
(35, R′)
(36, R)
H+
CH3
R or R′
H3C
OH
(33, R′)
(34, R)

Figure 5. Products of the isomerization of pre- and *cis*-vitamin D.



(37) (38) (39)

Figure 6. Products of overirradition of vitamin D: suprasterol II (37), toxisterol-E (38), and toxisterol E_1 (39).

Table 5. Molecular Extinction Coefficients of Irradiation Products

λ (nm)	7-Dehydrocholesterol	Previtamin D_3	Tachysterol$_3$	Lumisterol$_3$
254	4,500	725	11,450	4,130
300	1,250	930	11,250	1,320
330	25	105	2,940	30
340	20	40	242	25
350	10	25	100	20

Source: Ref. 75.

× 10^6 IU or USP units. The international chick unit (ICU) is identical to the USP unit.

USP also issues vitamin D_3 capsules for AOAC determination in rats and an oil solution for the vitamin D_3 AOAC determination in chicks. Historically, the following units (shown with their approximate international unit equivalence) have been used but are currently abandoned: 1 clinical unit = 12–17 IU; 1 biological unit = 0.125 IU; 1 protection unit = 0.125 IU; 1 Laquer unit = 0.14 IU; 1 Poulson unit = 0.2 IU; 1 Steenbach unit = 3 IU. The MRC, ICU, and Coward units all approximated the international unit and are also no longer in common use.

The standard chemical and biological methods of analysis are those accepted by the *United States Pharmacopeia XXIII* as well as the ones accepted by the AOAC in 1995 (81–84). The USP method involves saponification of the sample (dry concentrate, premix, powder, capsule, tablet, or aqueous suspension) with aqueous alcoholic KOH; solvent extraction; solvent removal; chromatographic separation of vitamin D from extraneous ingredients; and colormetric determination with antimony trichloride and comparison with a solution of USP cholecalciferol reference standard.

The AOAC (978.42) recognizes a similar procedure, except that the unsaponifiable material is treated with maleic anhydride to remove the trans-isomer which may possibly be present (83). The antimony trichloride colorimetric assay is performed on the trans-isomer-free material. This procedure cannot be used to distinguish certain inactive isomers, eg, isotachysterol; if present, these are included in the result, giving rise to a falsely high analysis. A test must therefore be performed to check for the presence of isotachysterol.

Preferably, high pressure liquid chromatography (hplc) is used to separate the active pre- and cis-isomers of vitamin D_3 from other isomers and allows their analysis by comparison with the chromatograph of a sample of pure reference *cis*-vitamin D_3, which is equilibrated to a mixture of pre- and cis-isomers (82,84,85). This method is more sensitive and provides information on isomer distribution as well as the active pre- and cis-isomer content of a vitamin D sample. It is applicable to most forms of vitamin D, including the more dilute formulations, ie, multivitamin preparations containing at least 1 IU/g (AOAC Methods 979.24; 980.26; 981.17; 982.29; 985.27) (82). The practical problem of isolation of the vitamin material from interfering and extraneous components is the limiting factor in the assay of low level formulations.

A number of methods have been developed for the chromatographic separation of vitamin D and related substances and can be found in Table 7.

Biological Assay

The USP and AOAC recognize a biological method for the determination of vitamin D. The rat line test, however, is slow, expensive and not as accurate as the chemical or chromatographic methods. Rachitic rats are fed diets containing the vitamin D sample. This test measures bone growth on the proximal end of the tibia or distal end of the ulna, which is visualized by staining with silver nitrate. This test is not applicable to products offered for poultry

Table 6. Physical Properties of Provitamins and Vitamins D_2 and D_3

	Substance			
Properties	7-Dehydrocholesterol	Ergosterol	Vitamin D_2 (ergocalciferol)	Vitamin D_3 (cholecalciferol)
Melting point, °C	150–151	165	115–118	84–85
Color and form	Solvated plates from ether–methanol	Hydrated plates from alcohol; needles from acetone	Colorless prisms from acetone	Fine colorless needles from dilute acetone
CAS Registry Number	*[434-16-2]*	*[57-87-4]*	*[50-14-6]*	*[67-97-0]*
Optical rotation (α_D^{20}),°				
Acetone			82.6	83.3
Ethanol			103	105–112
Chloroform	−113.6	−135	52	51.9
Ether			91.2	
Petroleum ether			33.3	
Benzene	−127.1			
Coefficient of rotation per °C in alcohol			0.515	
UV max, nm	282	281.5	264.5	264.5
Specific absorption, E_{max} at 1% conc	308		458.9 ± 7.5	473.2 ± 7.8
Potency[a] IU/g			40×10^6	40×10^6
Biological activity			in mammals	in mammals and birds
Chicken efficacy, %			8–10[b]	100
Solubility, g/100 mL				
Acetone at 7°C			7	
Acetone at 26°C			25	
Absolute ethanol	sl sol		28	
At 26°C		0.15		
At Δ°C		2.2		
Ethyl acetate at 26°C			31	
Water	Insol	Insol	Insol	Insol

[a] The international standard for vitamin D is an oil solution of activated 7-dehydrocholesterol (3). The IU is the biological activity of 0.025 μg of pure cholecalciferol.

[b] Studies have claimed an efficacy as high as 10% (77).

feeding. The AOAC recognizes another procedure which measures the vitamin D sample activity in increasing bone ash of growing chicks compared to the activity of a USP cholecalciferol reference standard. It too is slow, expensive, and gives variable results.

Physical Methods

Vitamins D_2 and D_3 exhibit uv absorption curves that have a maximum at 264 nm and an E_{max} (absorbance) of 450–490 at 1% concentration (Table 8). The various isomers of vitamin D exhibit characteristically different uv absorption curves. Mixtures of the isomers are difficult to distinguish. However, when chromatographically separated by hplc, the peaks can be identified by stop-flow techniques based on uv absorption scanning or by photodiodearray spectroscopy. The combination of elution time and characteristic uv absorption curves can be used to identify the isomers present in a sample of vitamin D.

Infrared and nmr spectroscopy have been used to help distinguish between vitamins D_2 and D_3 (87–89). X-ray crystallographic techniques are used to determine the vitamin D structure, and gas chromatography also is a method for assaying vitamin D (49,90–95).

Provitamin D

The molecular extinction coefficient of 7-dehydrocholesterol at 282 nm is 11,300 and is used as a measure of 7-dehydro isomer content of the provitamin (96,97). High pressure liquid chromatography can also be used to analyze the provitamins. There are a variety of chemicals that show characteristic colors when reacted with the provitamins. Some of these are listed in Table 9.

The extremely low levels of vitamin D and its metabolites in biological systems make it very difficult to assay these products by traditional methods. Calcium-binding protein is not found in the intestinal mucosa of vitamin D-deficient animals. It is synthesized only in response to the presence of a material with vitamin D activity. Thus, using antiserum specific to intestinal calcium-binding protein, a radioimmunodiffusion assay (98) conducted on homogenates of intestinal mucosa of chicks fed the test material for 7–10 d allows assay of the material for vitamin D activity down to 1–200 IU. The development of this technique has allowed research to be conducted since the mid-1970s to elucidate the vitamin D metabolism and biochemistry and is now used routinely to assay vitamin D-active materials in clinical as well as research samples (6,40,51,53,55).

SYNTHESIS

Manufacture

Most of the vitamin D produced in the world is made by the photochemical conversion of 7-dehydrocholesterol. Er-

Table 7. Methods for Chromatographic Separation of Vitamin D and Related Steroid

Method	Comments
	Paper chromatography
Quinoline-impregnated paper	
Reversed-phase paper	
Column chromatography	
Alumina	
Floridin	
Celite	Useful for multivitamin tablets
Silicic acid	Useful for metabolites
Factice	Can resolve vitamin D_2 and vitamin D_3
Sephadex LH-20	Highly useful for metabolites
	High-pressure liquid chromatography
Zorbax-Sil support	Separates 24(*R*), 25-dihydroxy-vitamin D_3 from 24(*S*), 25-dihydroxy-vitamin D_3 Useful for metabolites Assay of 25-hydroxy-vitamin D_3
ODS[a]-Permaphase	Useful for vitamin D metabolites
Silica gel	Commercial vitamin D_3 assay
	Thin-layer chromatography
Silicic acid	Effective for irradiation mixtures
Silica gel G	Two-dimensional
	Gas chromatography
	Separates trimethylsilyl ethers of vitamin D_3 Cyclizes vitamin D_3 Useful for 25-hydroxy-vitamin D_3 Useful for multivitamin tablets Useful for vitamin D_2 in milk

Source: Ref 86.

[a] ODS = octadecyl (C_{18}) silane.

Table 8. Approximate UV Absorbance of 7-Dehydrocholesterol, Pre- and *cis*-Vitamin D

	(E 1% 1 cm)		
Absorbance at μm	7-Dehydrocholesterol	Previtamin D	*cis*-Vitamin D
230	35	190	250
235	40	170	280
240	50	210	325
245	60	235	360
250	85	250	400
255	120	260	430
260	150	270	460
265	200	265	470
270	270	250	450
273	282		
275	250	220	420
277	240		
280	290	180	340
282	293		
285	240	150	300
290	155	100	180
295	170	70	120
300	70	50	80

gosterol is not used as extensively as it once was, because it offers no real price advantage and, upon irradiation, it gives vitamin D_2, which has been shown to be less active in many species. The pig, chicken, cow, and horse have been shown to discriminate against vitamin D_2 (99). Irradiation of 7-dehydrocholesterol or ergosterol is carried out by dissolving the steroid in an appropriate solvent, eg, peroxide-free diethyl ether. Solvents such as methanol, cyclohexane, and dioxane have also been used. The cooled solution is pumped through uv-transparent quartz reactors which permit the light from high pressure mercury lamps to impinge upon the solution (Fig. 7). The solution is recycled until the desired degree of irradiation has been achieved. This results in a mixture of unreacted 7-dehydrosterol, previtamin D, vitamin D, and irradiation by-products.

Among the light sources used for irradiation are carbon arcs, metal-corded carbon rod, magnesium arcs, and mercury-vapor lamps; the high pressure mercury lamp is most widely used. Higher yields and more favorable isomer distribution can be achieved if the frequency of light is kept at 275–300 nm. Several arrangements of the components of the simple reactor shown are possible and have been used. An important feature is a cooling jacket which controls the high temperature (ca 800°C) of the mercury-vapor lamps. Water solutions for cooling may contain salts for screening frequencies of light. Light below 275 nm can be filtered by aromatic compounds, as well as by a 5-wt % lead acetate or other inorganic salt solution. Glass filters can also be used as screens for frequencies which are outside the chemical filter ranges. Photosensitizers, eg, eosin, erythrosin, dibromodinitrofluorescein, and others, have been suggested to limit light frequencies and improve the isomer distribution.

The vitamin D resin is stabilized against oxidation by the addition of ≤ 1 wt % butylated hydroxyanisole or butylated hydroxytoluene.

The solvent is then evaporated, and the unconverted sterol is recovered by precipitation from an appropriate solvent, eg, alcohol. The recovered sterol is reused in subsequent irradiations. The solvent is then evaporated to yield vitamin D resin. The resin is a pale yellow-to-amber oil that flows freely when hot and becomes a brittle glass when cold; the activity of commercial resin is $20–30 \times 10^6$ IU/g. The resin is formulated without further purification for use in animal feeds. Vitamin D can be crystallized to give the USP product from a mixture of hydrocarbon solvent and aliphatic nitrile, eg, benzene and acetonitrile, or from methyl formate (100,101). Chemical complexation has also been used for purification.

In 1938, it was estimated that 7.5×10^{13} quanta of light were required to convert ergosterol to 1 USP unit of vitamin D_2 (102). The value was later determined to be 9.3×10^{13} quanta.

A flow diagram for the D_3 manufacturing process is shown in Figure 8. First, ether solution containing 7-

Table 9. Chemical Test Methods for Provitamin D

Name of reaction	Components	Results	Interpretation
Revised Salkowski reaction	$CHCl_3$ + H_2SO_4 (conc)	Deep red acid layer	Differentiates from sterols lacking conjugated diene (red color in $CHCl_3$ layer) acid gives green fluorescence
Lieberman-Burchard reaction	$CHCl_3$; acetic acid—H_2SO_4 added dropwise	Red color develops and changes to blue-violet to green	Can be quantitative; acts similarly, but red color lasts longer
Tortelli-Jaffé reaction	Acetic acid + 2 wt % Br_2 in $CHCl_3$	Green	Sterols with ditertiary double bonds; vitamin D and compounds that give similar bonds upon isomerization or reaction
Rosenheim reaction	$CHCl_3$ + trichloroacetic acid in H_2O	Red color develops and changes to light blue	
Rosenheim reaction	$CHCl_3$ + lead tetraacetate in CH_3COOH is added; then trichloroacetic acid is added	Green fluorescence	Not given by esters of provitamin D; can be used to distinguish between provitamin and provitamin ester; sensitive to 0.1 μg and is quantitative
Chloral hydrate	Mixture of crystalline provitamins and chloral hydrate heated slowly; melts at 50°C	Color develops and changes red to green to deep blue	Other sterols, eg, cholesterol, do not react to give color
Antimony trichloride reaction	$CHCl_3$ + $SbCl_3$	Red color	
Chugaev reaction	Glacial acetic acid plus acetyl chloride and zinc chloride heated to boiling	Eosin-red greenish yellow fluorescence	1:800,000 sensitivity

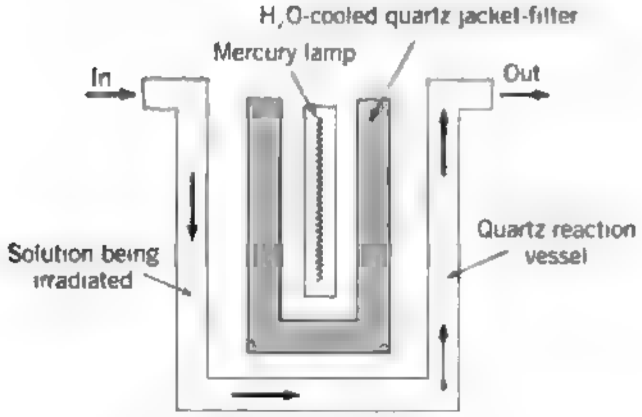
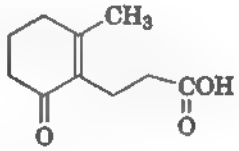


Figure 7. Ultraviolet-light-transparent quartz reactor for sterol irradiation.

dehydrocholesterol is recirculated through a quartz uv reactor, and the ether is distilled off. Methanol is added to the 7-dehydrocholesterol–vitamin D_3 mixture, and the remaining ether is azeotroped. The resulting solution is transferred to a crystallizer, and the 7-dehydrocholesterol is crystallized and recovered by filtration. The methanol is distilled, and the vitamin D_3 resin is heated to isomerize the pre-vitamin D to the cis-vitamin D isomer. Vitamin D_3 resin is then packaged for shipment.

Total Synthesis

Poor yields encountered during the manufacture of vitamin D stimulated early attempts to synthesize vitamin D. In 1959 Inhoffen synthesized vitamin D_3 from 3-methyl-2-(2-carboxyethyl)-2-cyclohexenone (40), using the Wittig reaction extensively (103).



(40)

5,6-*trans*-Vitamin D_3 was prepared, followed by photochemical isomerization into vitamin D_3. Direct preparation of the previtamin and vitamins D is described in References 104 and 105.

The discovery that vitamin D_3 was metabolized to biologically active derivatives led to a significant effort to prepare 25-hydroxy vitamin D_3 and, subsequently, the 1α-hydroxy and 1,25 dihydroxy derivatives. Initial attempts centered around modification of steroidal precursors, which were then converted to the D derivatives by conventional means.

Chemical syntheses of 25-hydroxy-vitamin D_3 were achieved by several groups of researchers (106–112). Many of these syntheses depended on the availability of the precursor 25-oxo-27-norcholesterol. Grignard reaction followed by introduction of the 7-dehydro function and irradiation allows 25-hydroxy-vitamin D_3 to be isolated (109). Fucosterol (stigmasta-5,24(28)-dien-3β-ol) as well as bile acids, pregnenolone, and desmosterol have also been used

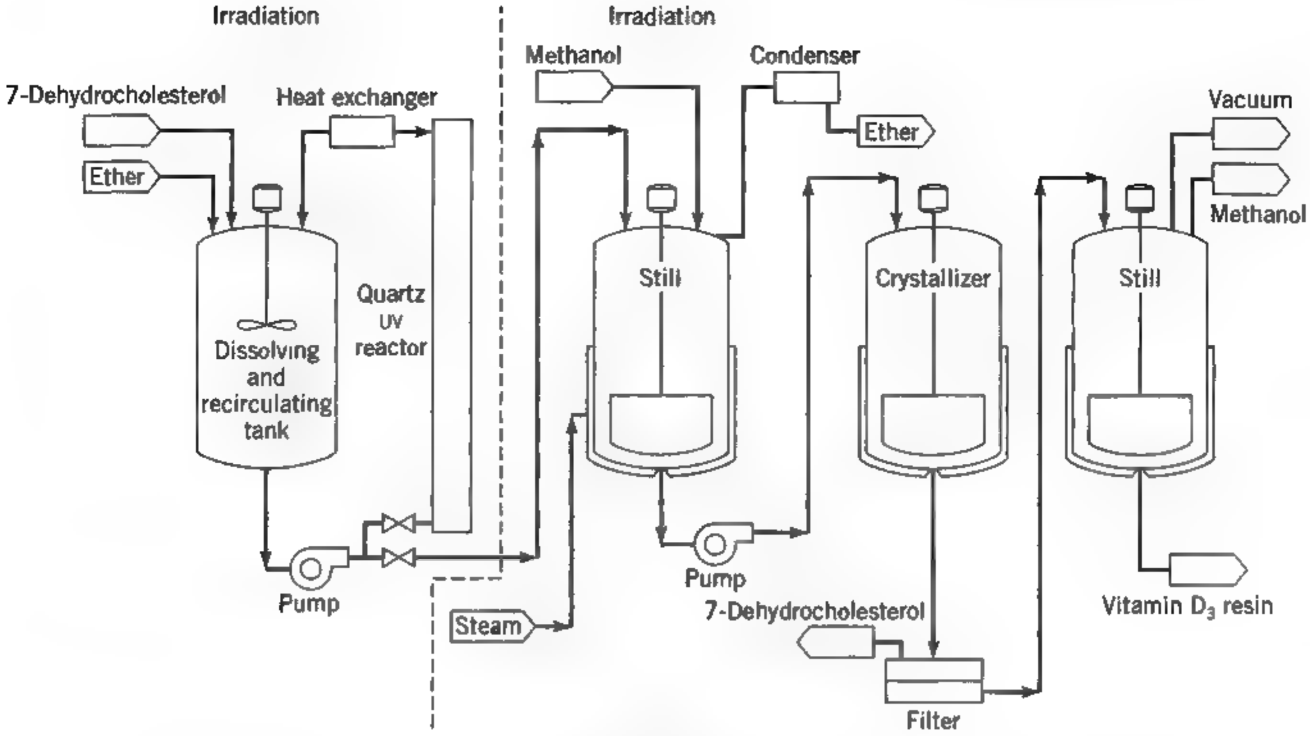


Figure 8. Vitamin D irradiation process.

as starting materials for the synthesis of 25-hydroxy intermediates. 24,25-Dihydroxy-vitamin D_3 [*40013-87-4*] was isolated and chemically characterized in 1972 (113). The first synthesis of this important D_3 catabolite was of a racemic mixture, and it was followed by the stereospecific syntheses of the two epimers (114–123). The biosynthesized material migrates exclusively with the synthetic 24(*R*) epimer.

The yield of the first chemical synthesis of 1,25-dihydroxy-vitamin D_3 was $<0.005\%$ (124). A key intermediate compound was 1,25-dihydroxy-cholesterol (109,125–130).

Subsequent synthesis of Vitamin D metabolites involved oxidative degradation of the vitamin D molecule to obtain the C- and D-ring portion with the intact side chain. Recombination of this molecule with an appropriate structure containing the A-ring was then carried out by a Wittig-type condensation.

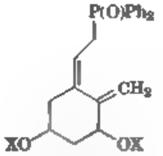
The chemical syntheses of 1,24(*R*),25-trihydroxy-vitamin D_3 [*56142-94-0*] and 1,24(*S*),25-trihydroxy-vitamin D [*56142-95-1*] were reported (131,132) in 1975. The chemical synthesis of 25,26-dihydroxy-vitamin D_3 [*29261-12-9*] has also been described, and it has been determined that the biologically occurring epimer is 25(*R*),26-dihydroxy-vitamin D_3 (117,133–135). The 23,25-dihydroxy-24-oxo metabolite has been isolated (136) as well. 1α-Hydroxycalcitroic acid (1-hydroxy-24-nor-9,10-secochola-5,7-10(19)-trien-23-oic acid) [*71204-89-2*], 25-hydroxy-26,23-lactone vitamin D_3 [*71203-34-6*], and homocalcitroic acid (9,10-secohola-5,7,10(19)-trien-24-oic acid) have also been reported, and the biological activity of these metabolites has been studied (137). The synthetic chemistry associated with these and other metabolites of vitamin D_2 and vitamin D_3 is described in References 6, 40, and 138, and more recently in References 16, 51, 55, 139–141.

The discovery that vitamin D metabolites play a much larger biochemical role than just maintaining calcium homeostasis has stimulated a number of groups around the world to develop more economical chemical syntheses for the vitamin D metabolites and analogues, which might be useful in studying and treating D_3-related diseases and conditions. Many of these methods are reviewed in References 139 and 140.

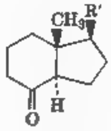
The most useful synthetic routes include the following pathways to the vitamin D structures and their derivatives:

Photochemical ring opening of 7-dehydrocholesterol derivatives which have ring A or the side chain modified (142,143).

A phosphine oxide of type (**41**) can be coupled with Grundman's ketone (**42**) to produce the D_3 skeleton (105,144–151).



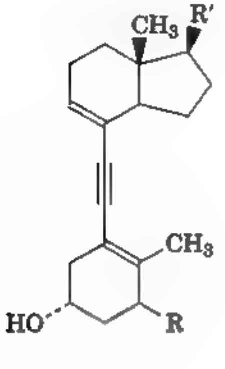
(**41**, X = a protective group)



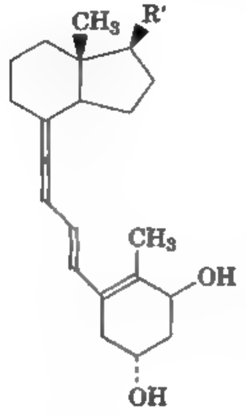
(**42**, R′ = cholesterol side chain or derivative)

Synthetic dienynes like (**43**) are semihydrogenated to form previtamin D and are then rearranged to the D structure (152–155).

Vinyl allenes (**44**) are rearranged with heat or metal catalysis and photosensitized isomerization to produce the vitamin D triene (156–160).



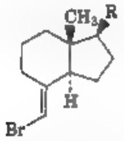
(**43**, R = H, OH)



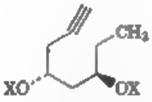
(**44**)

R′ = cholesterol side chain or derivative

Intermolecular cross-coupling of (**45**) with a synthetic alkyne structure like (**46**) leads to the D_3 skeleton (161,162).



(**45**, R = cholesterol side chain or derivative)



(**46**, X = protective group)

Direct modification of vitamin D_2 or D_3 or its metabolites to give derivatives by a variety of synthetic methods has also been used extensively (163–171).

Whereas these preparations are extremely useful for obtaining sufficient quantities of the D_3 metabolites and analogues for study and as possible therapeutic treatments, their cost is high compared to the cost of manufacture of vitamin D_3. For this reason, the metabolites are unlikely to be successful in replacing vitamin D_3 as an ingredient in animal or human nutrition.

BIOCHEMISTRY

Biochemistry

Vitamin D is introduced into the bloodstream either from the skin after natural synthesis by the irradiation of 7-dehydrocholesterol stored in the epidermis (172) or by ingestion and absorption of vitamin D_2 or vitamin D_3 through the gut wall (40). Between 60 and 80% of the vitamin introduced in the blood is taken up by the liver, where cholecalciferol is transferred from chylomicrons to a vitamin D-binding protein (DBP), an α-globulin specific for vitamin D and its metabolites but one which does not bind with previtamin D in the skin (173). Cholecalciferol is hydroxylated in the liver at the C-25 position (51,141,174). This hydroxylation occurs in the endoplasmic reticulum and requires NADPH, a flavoprotein, cytochrome P-450, Mg^{2+}, and O_2 (175). 25-Hydroxylation also occurs in intestinal homogenates of chicks (176), but does not appear to occur outside the liver in mammals (177). 25-Hydroxycholecalciferol is the main circulating form of vitamin D_3, and its normal concentration level is 15–47 ng/mL (178).

25-Hydroxy vitamin D pools in the blood and is transported on DBP to the kidney, where further hydroxylation takes place at C-1 or C-24 in response to calcium levels. 1-Hydroxylation occurs primarily in the kidney mitochondria and is catalyzed by a mixed-function monooxygenase with a specific cytochrome P-450 (52,179,180). 1α- and 24-Hydroxylation of 25-hydroxycholecalciferol has also been shown to take place in the placenta of pregnant mammals and in bone cells, as well as in the epidermis. Low phosphate levels also stimulate 1,25-dihydroxycholecalciferol production, which in turn stimulates intestinal calcium as well as phosphorus absorption. It also mobilizes these minerals from bone and decreases their kidney excretion. Together with PTH, calcitriol also stimulates renal reabsorption of the calcium and phosphorus by the proximal tubules (51,141,181–183).

Vitamin D receptors have been identified in intestine, bone, and kidney. Receptors have also been shown, to a lesser degree, to be present in the pituitary, brain, skin, reproductive organs, and cells of the immune system, suggesting vitamin D may play a broader role in controlling cellular proliferation and differentiation (184). The nuclear receptor (genomic) for 1,25-dihydroxy vitamin D_3 has been shown to be a member of the super-family of transactivating regulators of gene transcription similar to the receptors of other steroid hormones (185). Nongenomic activation by 1,25-dihydroxy vitamin D_3 on the cellular and subcellular level has been observed and is believed to be responsible for calcium transport in tissue known as transcaltachia (186,187).

Further side-chain oxidation of vitamin D_3 metabolites may be necessary for phosphate transport (188,189). 24,25-Dihydroxycholecalciferol is produced by kidney mitochondria, but can also be produced elsewhere (190,191). 1α,24,25-Trihydroxy-vitamin D_3 stimulates intestinal calcium mobilization to the extent of ca 60% of the 1α,25 activity and has 10% of the 1α,25 activity in causing bone resorption (192). It is less active in chicks and is excreted.

More than 20 other, naturally occurring metabolites of vitamin D have been isolated and characterized, and many derivatives have been synthesized. Their function is the subject of continuing research (16,51,141,162).

Although it is being found that vitamin D metabolites play a role in many different biological functions, metabolism primarily occurs to maintain the calcium homeostasis of the body. When calcium serum levels fall below the normal range, 1α,25 -dihydroxy-vitamin D_3 is made; when calcium levels are at or above this level, 24,25-dihydroxycholecalciferol is made, and 1α-hydroxylase activity is discontinued. The calcium homeostasis mechanism involves a hypocalcemic stimulus, which induces the secretion of parathyroid hormone. This causes phosphate diuresis in the kidney, which stimulates the 1α-hydroxylase activity and causes the hydroxylation of 25-hydroxy-vitamin D to 1α,25-dihydroxycholecalciferol. Parathyroid hormone and 1,25-dihydroxycholecalciferol act at the bone site cooperatively to stimulate calcium mobilization from the bone. Calcium blood levels are also influenced by the effects of the metabolite on intestinal absorption and renal resorption.

Interaction of vitamin D and its metabolites with sex hormones has been demonstrated, particularly in birds in which the egg-laying functions combine calcium needs and reproductive activity. The metabolites of vitamin D behave as hormones. As such, they play an active role in the endocrine system, along with other hormones, to maintain the various body functions. Several biological influences of metabolites of vitamin D have been studied, including effects related to cancer (193–197), skin diseases (198–201), immunomodulatory effects (202,203), and Alzheimer's disease (204–206) (Fig. 9).

The metabolism of vitamin D_2 follows a pathway similar to that described for Vitamin D_3.

Vitamin D_3 Deficiency

Vitamin D_3 deficiency is uncommon in normal adults. However, when it does occur, it can be serious, particularly in pregnant women. Some vitamin D_3 deficiency can occur because of a large reduction of fat intake, which decreases D_3 absorption. Strict vegetarians also risk reduced vitamin D_3 intake. Premature infants and elderly people who are exposed to minimal sunlight and consume little vitamin D_3 also have a reduced capacity to metabolize D_3 and can develop vitamin D_3 deficiency.

Clinical stresses which interfere with vitamin D_3 metabolism, can result in calcium deficiency leading to osteomalacia and osteoporosis (secondary vitamin D deficiency). These stresses include: intestinal malabsorption (lack of bile salts); stomach bypass surgery; obstructive jaundice; alcoholism; liver or kidney failure decreasing hydroxylation of vitamin D_3 to active forms; inborn error of metabolism; and use of anticonverdiants that may lead to increased D_3 requirement.

People who experience the exclusion of sunlight (living in northern climates; cultures where apparel limits sunlight), as well as infants and children that are confined to bed or limited outdoor activity because of weather or illness, also can show a tendency towards vitamin D_3 deficiency.

Vitamin D deficiency in animals may be caused by the fact that the vitamin is not available to the livestock. Modern animal husbandry subjects animals to total confinement with little or no exposure to sunlight. This mandates that they be given vitamin D-fortified diets. The vitamin is sensitive to oxidation, heat, light, and minerals, and significant losses may occur in the fortified feed unless the product is adequately protected. Mycotoxins in feeds also interfere with utilization of vitamin D in feeds (207–209).

Symptoms of D_3 deficiency in animals include poor appetite, stunted growth, and weight loss; increased incidence of irritability and convulsions (tetany); some growth abnormalities; decreased egg production in poultry with reduced hatchability and thin eggshell quality; and birth of weak, dead, or deformed offspring in other animals.

For a more detailed description of symptoms, see References 4,5,210, and 211.

Dietary Requirements

Vitamin D is essential for growth and maintenance of good health. According to the National Research Council (NRC), the vitamin D requirement for optimum health is ca 400 IU/*d* in humans, regardless of age. This amount of vitamin D gives ample protection from rickets, provided a sufficient amount of the other essential nutrients, including calcium and phosphorus, is supplied (212). Recommended NRC amounts of vitamin D per kilogram of feed for various species are as follows: starting and growing chicks, 200 IU; laying and breeding hens, 500 IU; turkeys, 1100 IU; ducks, 200 IU; quail, 480–900 IU; geese, 200 IU; and swine 125–220 IU. Calves require 600 IU per 100 kg of body weight (213–215). Practical feeding levels are somewhat higher, in order to assure delivery of the vitamin to the animals.

The present (ca 1997) maximum safe level of vitamin D_3 for long-term feeding in most species is four to ten times the NRC dietary requirements. Short-term (<60 d), most species can tolerate 100 times their apparent dietary requirements (210).

Animals exposed to sunlight for extended lengths of time do not require substantial dietary vitamin D. Current livestock management practices place an emphasis on high productivity, and most feed manufacturers recommend vitamin D supplementation of diets. Recommendations for practical levels of vitamin D in feeds for various animals, as recommended by feed manufacturers, are listed in Table 10.

Historically, rickets prevention or cure was used to evaluate adequate vitamin D_3 nutrient levels. More recently, in the absence of uv light, Edwards (216) found different vitamin D_3 levels were required for the optimization of the various effects of vitamin D_3 in poultry, ie, 275 IU/kg for growth, 503 IU/kg for bone ash, 552 IU/kg for blood plasma calcium, and 904 IU/kg for rickets prevention.

Toxicity

Vitamin D toxicity was known as early as the year 1429 (217). Accidental toxicity has been reported in monkeys, dogs, horses, pigs, chinchillas, and humans, and particularly in cattle when extremely high doses of vitamin D have been used to treat milk fever.

Vitamin D intoxication causes 25-hydroxy vitamin D_3 blood levels to go from a normal of 30–50 ng/mL to 200–400 ng/mL. At this high level, the metabolite can compete with 1α-25-dihydroxy vitamin D_3 for receptors in the intestine and bone and induce effects usually attributed to the dihydroxy vitamin D_3. Thus, 25-hydroxy vitamin D_3 is believed to be the critical factor in vitamin D intoxication. Vitamin D_2 is metabolized slower than vitamin D_3 and thus appears to be less toxic (218).

The overall effect in most animals is to stimulate intestinal absorption of calcium with a concomitant increase in

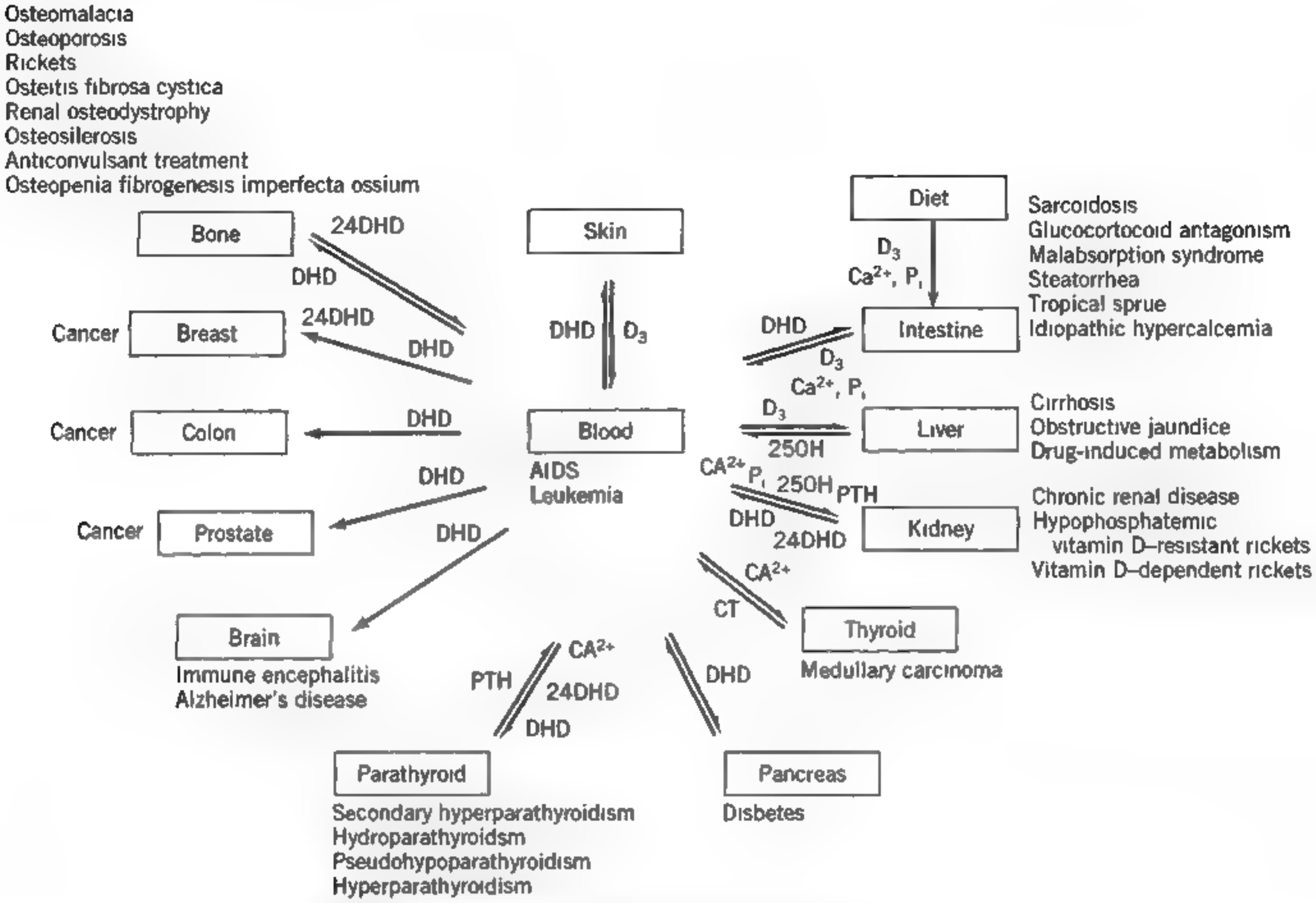

Osteomalacia
Osteoporosis
Rickets
Osteitis fibrosa cystica
Renal osteodystrophy
Osteosilerosis
Anticonvulsant treatment
Osteopenia fibrogenesis imperfecta ossium
Bone
24DHD
DHD
Skin
Diet
Sarcoidosis
Glucocortocoid antagonism
Malabsorption syndrome
Steatorrhea
Tropical sprue
Idiopathic hypercalcemia
D3
Ca2+, Pi
Cancer
Breast
24DHD
DHD
DHD
D3
DHD
Intestine
D3
Ca2+, Pi
Cancer
Colon
DHD
Blood
D3
Liver
250H
Cirrhosis
Obstructive jaundice
Drug-induced metabolism
AIDS
Leukemia
DHD
CA2+ Pi
250H
PTH
Chronic renal disease
Hypophosphatemic vitamin D–resistant rickets
Vitamin D–dependent rickets
Cancer
Prostate
DHD
DHD
24DHD
Kidney
CA2+
CT
Thyroid
Medullary carcinoma
Brain
Immune encephalitis
Alzheimer's disease
CA2+
PTH
24DHD
DHD
DHD
Parathyroid
Secondary hyperparathyroidism
Hydroparathyroidsm
Pseudohypoparathyroidism
Hyperparathyroidism
Pancreas
Disbetes

Figure 9. Human disease states in which vitamin D has been implicated. D_3 = vitamin D_3; DHD = 1a,25-dihydroxyvitamin D_3; 24DHD = 24(*R*),25-dihydroxyvitamin D_3; 25OH = 25-hydroxyvitamin D_3; PTH = parathyroid hormone; CT = calcitonin; P_i = inorganic phosphorus.

serum calcium and a reduction in parathyroid hormone (PTH). Modest hypercalcemia allows the glomerular filtration rate to remain stable and hypercalciuria to occur because of increased filtered load of calcium and reduction of tubular resorption of calcium with reduced PTH. However, with further increases in serum calcium, the glomerular filtration rate decreases, resulting in an even more rapid increase in serum calcium and the subsequent fall in urinary calcium.

Polyuria, along with vomiting can cause extracellular fluid to be reduced, contributing to further renal function disruption. Renal dysfunction then, becomes the major contributor to loss of calcium homeostasis, and the resulting hypercalcemia during vitamin D intoxication. Loss of appetite, gastrointestinal disturbances, head and joint pain, and muscle weakness are typical clinical symptoms. Death from hypervitaminosis D is usually caused by renal failure. Cardiovascular and kidney mineralization results. Respiratory tract mineralization has also been observed, as well as calcification in the salivary gland. The severity of effects of toxic levels of vitamin D depend on the dose, functional state of the kidneys, composition of the diet, as well as differences in toxicology of the various species (210).

Vitamin D withdrawal is an obvious treatment for D toxicity (219). However, because of the 5–7 d half-life of plasma vitamin D and 20–30 d half-life of 25-hydroxy vitamin D, it may not be immediately successful. A prompt reduction in dietary calcium is also indicated to reduce hypercalcemia. Sodium phytate can aid in reducing intestinal calcium transport. Calcitonin glucagon and glucocorticoid therapy have also been reported to reduce serum calcium resulting from D intoxication (210).

Vitamin D_3 is nonirritating to rabbit eye and dermal tests. It has an oral LD_{50} of 35–42 mg/kg in rats; 42 mg/kg in mice (136 mg/kg IP), and 4–80 mg/kg in dogs (10 mg/kg IP and 5 mg/kg IM and IV). It is nontoxic on inhalation.

The metabolites of vitamin D are usually more toxic than the vitamin because the feedback mechanisms that regulate vitamin D concentrations are circumvented. 25-Hydroxycholecalciferol has a one-hundredfold increase in toxicity over vitamin D_3 when fed to chicks (220) and 1α,25-dihydroxy vitamin D_3 is several times more toxic than the 25-hydroxy analogue. Vitamin D_2 seems to have less toxicity than vitamin D_3, a circumstance which is believed to be caused by the more efficient elimination of 25-hydroxy and the 1α,25-dihydroxy vitamin D_2 from the ani-

Table 10. Practical Feeding Levels of Vitamin D, 10^6 IU/t

Animal	Amount
Poultry	
Chickens	
Broilers	2–4
Replacement birds	1–3
Layers	1–3
Breeding hens	2–4
Turkeys	
Starting	3–5
Growing	2–4
Breeding	3–5
Ducks	
Market	1–3
Breeding	2–4
Swine	
Prestarter (to 10 kg)	2–3
Starter (10–35 kg)	1–2
Growing-finishing (35 kg to market)	1–2
Gestation	1–2
Lactation	2–3
Boars	2–3
Fish	
Trout	1–2
Catfish	1–2
Dairy cattle	
Calf starter	1–2
Calf milk replacer	2–4
Replacement heifers	1–2
Dry cows	1–2
Lactating cows	2–4
Bulls	2–4
Beef cattle	
Calf starter	1–2
Replacement heifers	1–2
Feedlot	2–3
Dry pregnant cows	1–2
Lactating cows	1–2
Bulls	1–2
Sheep	
Fattening lambs	2–3
Breeding	1–2
Other	
Dogs	5–1
Cats	1–2
Horses	1–2

mals. Estimated safe upper dietary levels are given in Table 11.

Disease States

Rickets is the most common disease associated with vitamin D deficiency. Many other disease states have been shown to be related to vitamin D. These can involve a lack of the vitamin, deficient synthesis of the metabolites from the vitamin, deficient control mechanisms, or defective organ receptors. The control of calcium and phosphorus is essential in the maintenance of normal cellular biochemistry, eg, muscle contraction, nerve conduction, and enzyme function. The vitamin D metabolites also have a function in cell proliferation. They interact with other factors and receptors to regulate gene transcription.

Table 11. Estimated Safe Upper Dietary Levels

Species	NRC recommended dietary daily requirement (IU/kg)	Exposure, multiple of daily requirement	
		Long-term (<60 d)	Short-term (>60 d)
Chicken	200	200	14
Quail	1,200	100	4
Turkey	900	100	3.9
Cow	300	83	3.9
Catfish	1,000		20
Trout	1,800		555
Horse	400		5.5
Sheep	275	91	8
Pig	220	150	10

Source Ref. 210.

In the treatment of diseases where the metabolites are not being delivered to the system, synthetic metabolites or active analogues have been successfully administered. Vitamin D_3 metabolites have been successfully used for treatment of milk fever in cattle, turkey leg weakness, plaque psoriasis, and osteoporosis and renal osteodystrophy in humans. Many of these clinical studies are outlined in References 6, 16, 40, 51, and 141. The vitamin D receptor complex is a member of the gene superfamily of transcriptional activators, and 1,25 dihydroxy vitamin D is thus supportive of selective cell differentiation. In addition to mineral homeostasis mediated in the intestine, kidney, and bone, the metabolite acts on the immune system, β-cells of the pancreas (insulin secretion), cerebellum, and hypothalamus.

Vitamin D metabolites may therefore play an active role in diseases related to these functions, ie, leukemia, cancer (breast, colon, prostate), and autoimmune diseases (AIDS, immune encephalitis, and diabetes) (51,141,193–197,202,203).

ECONOMIC ASPECTS

Vitamin D is available in a variety of forms. Cod-liver oil and percomorph-liver oil historically were good sources of vitamin D. Recent cost increases of these materials have caused a decline in their market position. Cod-liver oil sold for \$0.40–0.45/L in 1970 and as high as \$1.45/L in 1979 and \$3.43/L in 1996. The prices of the cod-liver oils and of vitamin D_2 and vitamin D_3 from 1955 to 1995 are shown in Table 12.

Vitamin D_3 is produced as a resin (20–30 × 10^6 IU/g) which sold in 1980 for ca \$1.02/10^6 IU. The 1979 prices of

Table 12. Price History of Vitamin D Materials

	Source	Vitamin D	
Year	Cod-liver oil, $/L	Ergocalciferol (crystalline vitamin D_2), $/g	Cholecalciferol D_3 ($/g)[a]
1955	1.51	0.60	0.045
1960	1.40	0.54	0.045
1965	1.71	0.48	0.045
1970	1.70	0.48	0.045
1975	5.50	0.63	0.045
1980	8.00	0.63	0.045
1985	7.25	[b]	24[c]
1990	13.00	[b]	24[c]
1995	13.00	[b]	24[c]

Source: Ref. 221

[a] 850,000 IU/g unless otherwise indicated.

[b] D_2 costs were not quoted after 1983.

[c] 40×10^6 IU/g.

vitamin D were $24.25/$10^9$ IU ($9.70/kg of product at 400,000 IU/g) (222). In the United States in 1979, $$3.6 \times 10^6$ worth of vitamin D was sold. The 1996 price for vitamin D_3 products was $13.50–14.00/g for resin and $24.00/g (223) for crystalline material. Vitamin D_3 feed-grade formulated products sell for approximately $0.03–0.04/$10^6$ units of activity.

Estimates of world demand in 1979 were as high as 1300×10^{12} IU of vitamin D_3. This was divided into thirds for Europe, the United States, and the rest of the world, respectively. Of this demand, 90% was estimated for animal-feed fortification and 100% for food and pharmaceutical uses. It is estimated that the demand will be 1500–1600 $\times 10^{12}$ IU in 1997 for animal usage and 100×10^{12} IU for human use. The United States will require approximately 500 TU (1 Trillion Units = 25 kg *cis*-vitamin D_3 or 17 t of resin) for animal use and 30 TU (approximately 1 t of crystalline *cis*-vitamin D_3) for human use. This represents approximately 50 t of vitamin D_3 resin/yr for animal use worldwide and about 2.5 t of crystalline vitamin D_3 for human use. A substantial proportion of the vitamin D_3 is imported, and with all uses included, it is estimated that 80–90% of the sales are of vitamin D_3.

The vitamin D_2 volume is estimated at only 1200 kg/yr, exclusive of Chinese sources.

USES

Most of the vitamin D sold is synthetic. Vitamin D_2 as a concentrate or in microcrystalline forms is used in many pharmaceutical preparations, although vitamin D_3 is preferred by many manufacturers and consumers. Vitamin D_2 in the form of irradiated yeast has been used as a feed supplement for cattle, swine, and dogs, but its use has declined in favor of Vitamin D_3. In the United States, swine usage accounts for 13% and poultry for 41% of vitamin D consumption in animal agriculture. The beef and dairy industries account for 44% of vitamin D consumption, of which 22% is by dairy calves. The remaining 1% of total consumption of D vitamins is largely in prepared pet foods. European usage in 1994 was estimated at: 58% cattle, 19% swine, 20% poultry, and 3% other. Crystalline vitamin D_3 is used for medicinal preparations and formulations in the pharmaceutical industry, as well as for the fortification of fresh and evaporated milk and nonfat dry milks. Preparations based on the use of the resin are less expensive than those using crystalline vitamin D. Essentially all milk produced in the United States is fortified with vitamin D_3. Cereals and margarine are also fortified with vitamin D_3. The vitamin must be diluted to a proper dosage form, and many supplement preparations are marketed, eg, tonics, drops, capsules, and tablets; oil-based injectables are also available. Combination formulations are used widely, particularly with vitamin A for humans and with vitamins A and E for animals. Preemulsified products are used for increased bioavailability. Water-miscible formulations have also been developed (224). Solutions of vitamin D in oil or in oil-on-dry carriers, eg, corn or flour, are used in animal feeds. These diets contain high levels of minerals, and the vitamin D formulation is not stable unless it is protected. Several stable forms are patented and sold commercially; they include beadlets or powders of dry suspensions in gelatin, carbohydrates, wax, and cellulose derivatives (89,225–228).

Animal uses employ resin in various stabilized forms at levels of 200,000; 400,000; 500,000; and 1×10^6 units of D_3 per gram. Combination products containing A and D are also available, with 650,000 units of vitamin A and 325,000 units vitamin D per gram of product being the most common dosage form.

Approximately 200 kg/yr of Vitamin D_3 formulations are also marketed as rat poisons. The metabolites of vitamin D_3 and synthetic derivatives are being used or developed for treatment of osteoporosis, skin psoriasis, and other diseases in humans. 1α-Hydroxy vitamin D_3 is being used for milk fever in cows, and 25-hydroxy vitamin D_3 has been proposed for eggshell thickness in poultry and is being marketed as an animal dietary nutritional supplement.

BIBLIOGRAPHY

1. P. Karlson et al., IUPAC-IUB Joint Commission on Biochemical Nomenclature. "Nomenclature of Vitamin D Recommendations 1981," *Eur. J. Biochem.* **124**, 223 (1982).

2. T. C. Angus et al., *Proc. R. Soc. London, Ser. B* **105**, 340 (1931).
3. W. F. Loomis, *Sci. Am.* **223**, 77 (1970).
4. H. M. Frost, *Bone Dynamics in Osteoporosis and Osteomalacia*, Surgery Monograph Series, Charles C. Thomas, Publisher, Springfield, Ill., 1966.
5. H. F. DeLuca in R. B. Alfin-Slater and D. Kratchevsky, eds., *Nutrition and the Adult: Micronutrients*, Plenum Press, New York, 1980, p. 207.
6. A. W. Norman, *Vitamin D—The Calcium Homeostatic Steroid Hormone*, Academic Press, Inc., New York, 1979, p. 3.
7. E. Mellanby, *J. Physiol. (London)* **52**, L 111 (1919); *Lancet* **196**, 407 (1919).
8. K. Huldschinski, *Dtsch. Med. Wochenschr.* **45**, 712 (1919).
9. F. Reindel and H. J. Kipphan, *Justus Liebigs Ann. Chem.* **493**, 181 (1932).
10. E. Fernholz and P. N. Chakravorty, *Ber. Deutsch. Chem. Ger. A.* **67**, 2021 (1934).
11. A. Windaus and H. Grundmann, *Justus Liebigs Ann. Chem.* **524**, 295 (1936).
12. Ref. 6, pp. 39–48.
13. A. Windaus and F. Bock, *Z. Physiol. Chem. Hoppe-Seglers* **245**, 168 (1937).
14. O. Rygh, *Nature (London)* **136**, 396 (1935).
15. H. Brockmann et al., *Z. Physiol. Chem. Hoppe-Seglers* **241**, 104 (1936); **245**, 96 (1937).
16. A. W. Norman, K. Schaefer, H.-G. Grigoleit, D. V. Herath, eds., *Vitamin D. Chemical, Biochemical and Clinical Update, Proceedings of the Sixth Workshop on Vitamin D*, Merano, Italy, March, 1985, Walter de Gruyter, Berlin, 1985, p. 55.
17. R. H. Wasserman, J. D. Henion, M. R. Haussler, and T. A. McCain, *Science* **194**, 853 (1976).
18. M. Peterlik et al., *Biochem. Biophys. Res. Commun.* **70**, 797 (1976).
19. J. P. Simonit, K. M. L. Morris, and J. C. Collins, *J. Endocrinol.* **68**, 18 (1976).
20. H. Zucker and W. A. Rambeck, *Centralbl. Veterinaermed.* **28**, 436 (1981).
21. W. A. Rambeck et al., *Z. Pflanzenphysiol* **104**, 9 (1981).
22. R. H. Wasserman et al., *Nutr. Rev.* **33**, 1 (1975); *J. Nutr.* **106**, 457 (1976); *Biochem. Biophys. Res. Commun.* **62**, 85 (1975).
23. Ref. 6, p. 49.
24. M. F. Hollick in Ref. 16, p. 219.
25. A. W. Norman et al., *J. Biol. Chem.*, 13811–13819 (1994).
26. M. C. Dormanen et al., *Biochem. Biophys. Res. Comm.* **201**, 394–401 (1994)
27. I. Nemere et al., *J. Biol. Chem.* **269**, 23750–23756 (1994).
28. U.S. Pat. 2,395,115 (Feb. 19, 1946), K. J. Goering (to Anheuser-Busch, Inc.).
29. U.S. Pat. 2,874,171 (Feb. 17, 1959), H. A. Nelson (to Upjohn Co.).
30. U.S. Pat. 3,006,932 (Oct. 31, 1961), J. Green, S. A. Price, and E. E. Edwin (to Vitamins, Ltd.).
31. U.S. Pat. 2,794,035 (May 28, 1957), O. Hummel (to Zellstaff-Fabrik Waldhof).
32. U.S. Pat. 2,865,934 (Dec. 23, 1958), R. A. Fisher (to Biofermentation Corp.).
33. Ger. Pat. 1,252,674 (Oct. 26, 1967), K. Petzoldt, K. Klieslich, and H. J. Koch (to Schering A.G.).
34. K. Ziegler, *Ann.* **551**, 80 (1942).
35. S. Bernstein, L. J. Benovi, L. Dorfman, K. S. Sax, and Y. Subbarow, *J. Org. Chem.* 14, 433 (1949); U.S. Pat. 2,498,390 (Feb. 21, 1950), S. Bernstein and K. J. Sax (to American Cyanamid Co.).
36. U.S. Pat. 2,446,091 (May 4, 1968), J. Van Der Vliet and W Stevens (to Hartford National Bank and Trust Co.).
37. H. Schaltagger, *Helv. Chim. Acta* **33**, 2101 (1950).
38. D. N. A. Holwerda, Doctoral Dissertation, Imperial University Leyden, 1970.
39. F. Hunziker and F. X. Mullner, *Helm. Chim. Acta* **41**, 70 (1958).
40. A. W. Norman, K. Schaefer, D. V. Herrath, and H. G. Grigoleit, eds., *Vitamin D· Chemical, Biochemical, and Clinical Endocrinology of Calcium Metabolism, Proceedings of the Fifth Workshop on Vitamin D*, Williamsburg, Va., Feb. 1982, Walter de Gruyter, Berlin, 1982, p. 1133.
41. W. G. Salmond, M. A. Serta, A. M. Cain, and M. C. Sobala, *Tetrahedron Lett.* **20**, 1683 (1977).
42. U.S. Pat. 2,098,984 (Nov. 16, 1937), A. Windaus and F. Schenck (to Winthrop Chem. Co., Inc.).
43. L. E. Fieser, *J. Am. Chem. Soc.* **75**, 4394 (1953).
44. U.S. Pat. 2,505,646 (Apr. 25, 1950), W. E. Meuly (to E. I. du Pont de Nemours & Co., Inc.).
45. A. Windaus, F. VonWeder, A. Luttringhaus, and E. Fernholz, *Ann.* **499**, 188 (1932).
46. L. Velluz, G. Amiard, and B. Goffinet, *Bull. Soc. Chim. France* **22**, 1341 (1955).
47. E. Havinga, R. J. DeKoch, and M. Rappoldt, *Tetrahedron* **11**, 276 (1960).
48. H. J. C. Jacobs, *Pure & Appl. Chem.* **67**, 63 (1995).
49. J. G. Bell and A. A. Christie, *Analyst* **99**, 385 (1974).
50. P. B. Braun, J. Hornstra, C. Knoblee, E. W. M. Rutten, and C. Romer, *Acta Crystallogr.* **B29**, 463 (1973).
51. E. Havinga, *Experimentia* **29**, 1181 (1973).
52. A. W Norman, R. Bouillon, M. Thomasset, eds., *Vitamin D. A Pluripotent Steroid Hormone: Structural Studies, Molecular Endocrinology and Clinical Applications. Proceedings of the Ninth Workshop on Vitamin D*, Orlando, Florida, May 1994, Walter de Gruyter, Berlin, 1994, p. 89.
53. J. I. Pedersen, J. G. Ghazarian, N. R. Orme-Johnson, and H. F. DeLuca, *J. Biol. Chem.* **251**, 3933 (1976).
54. A. W. Norman et al., eds., *Vitamin D, Basic Research and its Clinical Application, Proceedings of the Fourth Workshop on Vitamin D*, Berlin, Feb. 1979, Walter de Gruyter, Berlin, 1979, p. 173.
55. K. H. Hanewald, M. P. Rappoldt, and J. R. Roborgh, *Rec Trav. Chim. Pays-Bas* **80**, 1003 (1961).
56. A. W. Norman, K. Schefer, H.-G. Grigoleit, D. Herrath, eds., *Vitamin D, Molecular, Cellular and Clinical Endocrinology Proceedings of the Seventh Workshop on Vitamin D*, April, 1988, Walter de Gruyter, Berlin, 1988, p. 83.
57. A. Verloop, A. L. Koevoet, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **76**, 689 (1957).
58. A. L. Hoevoet, A. Verloop, and E. Havinga, *Rec. Trav. Chim.* **74**, 788 (1955).

59. W. G. Dauben et al., *J. Am. Chem. Soc.* **80**, 4117 (1958).
60. W. H. Okamura, M. L. Hammond, A. J. C. Jacobs, and J. Von Thiegil, *Tetrahedron Lett.* **52**, 4807 (1976).
61. E. Havinga, *Chimia* **30**, 27 (1976); D. H. R. Barton and coworkers, *Chem. Comm.*, 65 (1976).
62. F. Boosman, H. J. C. Jacobs, E. Havinga, and A. Van den Gen, *Tetrahedron Lett.* **7**, 427 (1975).
63. A. W. Norman et al., eds., *Vitamin D Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism; Proceedings of the Third Workshop on Vitamin D*, Asilomar, Pacific Grove, Calif., Jan. 1977, Walter de Gruyter, Berlin, 1977, p. 15.
64. S. Kobayshi and M. Yasumura, *J. Nutr. Sci. Vitaminol.* **19**, 123 (1973); S. Kobayashi et al., *J. Nutr. Sci. Vitaminol.* **26**, 545 (1980).
65. D. H. R. Barton, R. H. Heese, M. M. Pecket, and E. Rizzardo, *J. Am. Chem. Soc.* **95**, 2748 (1973).
66. K. Pfoertner and J. D. Weber, *Helv. Chim. Acta* **55**, 921, 937 (1972).
67. A. Verloop, A. L. Koevoet, R. Van Moorselase, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **78**, 1004 (1959).
68. A. Verloop, A. L. Koevoet, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **74**, 1125 (1955).
69. T. Kobagashi, *J. Vitaminol. (Jpn).* **13**, 255 (1967).
70. H. H. Inhoffen, G. Quinkert, J. H. Hess, and H. M. Erdman, *Chem. Ber.* **89**, 2273 (1956).
71. C. H. Nields, W. C. Russell, and A. Zimmerli, *J. Biol. Chem.* **136**, 73 (1940).
72. J. B. Wilkie, S. W. Jones, and O. L. Kline, *J. Amer. Pharm. Assoc. Sci. Ed.* **47**, 395 (1958).
73. S. C. Eyley and D. H. Williams, *J. Chem. Soc. Chem. Comm.*, 858 (1975).
74. E. C. Snoeren, M. R. Daha, J. Lugtenburg, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **89**, 261 (1970).
75. W. G. Dauben and R. B. Phillips, *J. Am. Chem. Soc.* **104**, 355 (1982).
76. V. Malatesta, C. Willis, and P. A. Hackett, *J. Am. Chem. Soc.* **103**, 6781 (1981).
77. U.S. Pat. 3,661,939 (May 9, 1972), M. Toyoda, Y. Tawara (to Nisshin Flour Milling Co., Ltd.).
78. P. S. Chen and H. B. Bosman, *J. Nutr.* **83**, 133 (1964).
79. Code of Federal Regulations, Title 21-Food and Drugs, Part 582.5923, p. 541, April 1, 1995.
80. *Chron. WHO* **3**, 747 (1965).
81. *The United States Pharmacopeia 23 (USP 23-NF 18)*, The United States Pharmacopeial Convention, Inc., Rockville, Md., 1995.
82. P. Cunniff, ed., *Official Methods of Analysis of the International Association of Official Analytical Chemists*, 16th ed., Washington, D.C., 1995.
83. F. Mulder, *J. Assoc. Off. Anal. Chem.* **60**, 989 (1977).
84. H. Hofsass, N. J. Alicino, A. L. Hirsch, L. Amieka, and L. D. Smith, *J. Assoc. Off. Anal. Chem.* **61**, 774 (1978).
85. F. Mulder et al., *J. Assoc. Off. Anal. Chem.* **64**, 61 (1981); **65**, 1228 (1982); **68**, 822 (1985).
86. Ref. 6, p. 64.
87. J. Carol, *J. Pharm. Sci.* **50**, 451 (1961).
88. W. W. Morris, Jr., J. B. Wilkie, S. W. Jones, and L. Friedman, *Anal. Chem.* **34**, 381 (1962).
89. R. Strohecker and H. M. Henning, *Vitamin Assay-Tested Methods*, CRC Press, Cleveland, Ohio, 1966, p. 281.
90. U.S. Pats. 2,777,797 and 2,777,798 (Jan. 15, 1977), M. Hochberg and M. L. MacMillan (to Nopco Chemical Co.).
91. J. R. Evans, *Clin. Chim. Acta* **42**, 167 (1972).
92. T. K. Murray, K. C. Day, and E. Kodicek, *Biochem. J.* **98**, 29P (1966).
93. L. V. Aviolo and S. W. Lee, *Anal. Biochem.* **16**, 193 (1966).
94. D. Sklan, P. Budowski, and M. Katz, *Anal. Biochem.* **56**, 606 (1973).
95. D. O. Edlund and F. A. Filippini, *J. Assoc. Anal. Chem.* **56**, 1374 (1973).
96. T. G. Hogness, A. E. Sidwell, Jr., and F. P. Zscheile, Jr., *J. Biol. Chem.* **120**, 239 (1937).
97. W. Huber, G. W. Ewing, and J. Kriger, *J. Am. Chem. Soc.* **67**, 609 (1945).
98. R. H. Wasserman et al., *Vitam. Horm. (N.Y.)* **32**, 299 (1974).
99. R. L. Horst et al., Ref. 55, p. 93.
100. U.S. Pat. 3,334,118 (Aug. 1, 1967), K. Schaff, S. Schmuhler, and H. C. Klein (to Nopco Chemical Co.).
101. U.S. Pat. 3,665,020 (May 23, 1972), R. Marbet (to Hoffmann-LaRoche, Inc.).
102. R. S. Harris, J. W. M. Bunker, and L. M. Moser, *J. Am. Chem. Soc.* **60**, 2579 (1938).
103. H. H. Inhoffen et al., *Chem. Ber.* **91**, 2309 (1958); H. H. Inhoffen et al., *Chem. Ber.* **92**, 1564 (1959).
104. T. M. Dawson, J. Dixon, P. S. Littlewood, B. Lythgoe, and A. K. Saksena, *J. Chem. Soc. C*, 2960 (1971).
105. B. Lythgoe, M. E. N. Nambudiry, and J. Tideswell, *Tetrahedron Lett.* **31**, 3685 (1977).
106. J. A. Campbell, D. M. Squires, and J. C. Babcock, *Steroids* **13**, 567 (1969).
107. S. J. Halkes and N. P. VanVliet, *Rec. Trav. Chim. Pays-Bas* **88**, (1969).
108. J. W. Blunt and H. F. DeLuca, *Biochemistry* **8**, 671 (1969).
109. M. Morisaki, J. Rubio-Lightbourn, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 457 (1973).
110. J. J. Partridge, S. Faber, and M. R. Uskokovic, *Helv. Chim. Acta* **57**, 764 (1974).
111. U.S. Pat. 4,172,076 (Apr. 4, 1977), A. Hirsch and J. Pikl (to Diamond Shamrock, subsequently assigned to A. L. Labs, Jan. 1, 1982).
112. U.S. Pat. 4,226,770 (Oct. 7, 1980), E. Kaiser.
113. M. H. Holick et al., *Biochemistry* **11**, 4251 (1972).
114. Y. Tanaka, H. Frank, H. F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistry* **14**, 3293 (1975).
115. Y. Tanaka, H. F. DeLuca, N. Ikekawa, M. Morisaki, and N. Koizumi, *Arch. Biochem. Biophys.* **170**, 620 (1975).
116. H.-Y. Lam, H. K. Schnoes, H. F. DeLuca, and T. C. Chen, *Biochemistry* **12**, 4851 (1973).
117. M. Seki, J. Rubio-Lightbourn, J. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 2783 (1973).
118. J. Redel, P. Bell, F. Delbarre, and E. Kodicek, *C. R. Acad. Sci.* **278**, 529 (1974).
119. J. Redel et al., *J. Steroid Biochem.* **6**, 117 (1975).
120. N. Ikekawa, M. Morisaki, N. Koizumi, Y. Kato, and T. Takeshita, *Chem. Pharm. Bull.* **23**, 695 (1975).

121. G. Milhaud, M.-L. Labat, J. Redel, *C. R. Acad. Sci.* **279**, 827 (1974).
122. M. Seki, N. Koizumi, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, 15 (1975).
123. J. J. Partridge, V. Toome, and M. R. Uskokovic, *J. Am. Chem. Soc.* **98**, 3739 (1976).
124. E. J. Semmler, M. F. Holick, H. K. Schnoes, and H. F. DeLuca, *Tetrahedron Lett.*, 4147 (1972).
125. K. Ochi et al., *J. Chem. Soc. Perkin I*, 165 (1979).
126. T. A. Narwid, J. F. Blunt, J. A. Iacobelli, and M. R. Uskokovic, *Helv. Chim. Acta* **57**, 781 (1974).
127. J. Rubio-Lightbourn, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 1854 (1973).
128. M. Morisaki, K. Bannai, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 1853 (1973).
129. M. Morisaki, J. Rubio-Lightbourn, N. Ikekawa, and T. Takeshita, *Chem. Pharm. Bull.* **21**, 2568 (1973).
130. H. E. Paaren, D. E. Hamer, H. K. Schnoes, and H. F. DeLuca, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2080 (1978).
131. N. Ikekawa et al., *Biochem. Biophys. Res. Commun.* **62**, 485 (1975).
132. J. J. Partridge, S. J. Shivey, E. G. Baggiolini, B. Hennessy, and M. Uskokovic, in A. W. Norman and co-eds., *Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism*, Walter de Gruyter, Berlin, 1977, p. 47.
133. H.-Y. Lam, H. K. Schnoes, and H. F. DeLuca, *Steroids* **25**, 247 (1975).
134. J. Redel, P. Bell, F. Delbarre, and E. Kodicek, *C. R. Acad. Sci.* **276**, 2907 (1973).
135. J. Redel et al., *Steroids* **24**, 463 (1974); J. Redel, N. Bazely, Y. Tanaka, and H. F. DeLuca, *FEBS Lett.* **94**, 228 (1978).
136. A. W. Norman et al., *Biochemistry* **22**, 1798 (1983).
137. H. F. DeLuca and H. K. Schnoes, in A. W. Norman and co-eds., *Vitamin D. Recent Basic Advances and Their Clinical Application*, Walter de Gruyter, Elmsford, N.Y., 1979.
138. D. E. M. Lawson, *Vitamin D*, Academic Press, Inc., New York, 1978.
139. H. Dai and G. H. Posner, *Synthesis*, 1383 (1994).
140. W. H. Okamura and C. D. Zhu, *Chemical Reviews* **95**, 1877 (1995).
141. A. W Norman, R. Bouillon, and M. Thomasset, eds., *Vitamin D, Structure, Function Analysis and Clinical Application, Proceedings of the Eighth Workshop on Vitamin D*, Paris, July 5, 1991, Walter de Gruyter, Berlin, 1991.
142. D. H. R. Barton, R. H. Hesse, M. M. Pechet, and E. Rizzardo, *J. Chem. Soc., Chem. Commun.*, 203–204 (1974).
143. D. H. R. Barton, R. H. Hesse, M. Pechet, and E. Rizzardo, *J. Am. Chem. Soc.* **95**, 2748 (1973).
144. B. Lythgoe et al., *Tetrahedron Lett.*, 3863–3866 (1975).
145. B. Lythgoe et al., *J. Chem. Soc., Perkin Trans.* 1, 590–595 (1978).
146. B. Lythgoe, T. A. Moran, M. E. N. Nambudiry, and S. Ruston, *J. Chem. Soc., Perkin Trans.* 1, 2386–2390 (1976).
147. B. Lythgoe et al., *J. Chem. Soc., Perkin Trans.* 1, 387–395 (1978).
148. J. V. Frosch, I. T. Harrison, B. Lythgoe, A. K. Saksena, *J. Chem. Soc., Perkin Trans.* 1, 2005–2009 (1974).
149. E. G. Baggiolini et al., *J. Org. Chem.* **51**, 3098–3108 (1986).
150. J. DeSchrijver and P. J. De Clercq, *Tetrahedron Lett.* **34**, 4369 (1993).
151. G. H. Posner and C. M. Kinter, *J. Org. Chem.* **55**, 3967–3969 (1990).
152. L. Castedo, J. L. Mascarenas, A. Mourino, and L. A. Sarandeses, *Tetrahedron Lett.* **29**, 1203–1206 (1988).
153. L. Castedo, A. Mourino, and L. A. Sarandeses, *Tetrahedron Lett.* **29**, 1203–1206 (1988).
154. S. A. Barrack, R. A. Gibbs, and W. J. Okamura, *J. Org Chem.* **53**, 1790–1796 (1988).
155. J. M. Aurrecoechea and W. H. Okamura, *Tetrahedron Lett.* **28**, 4947–4950 (1987).
156. W H. Okamura, J. M. Aurrecoechea, R. A. Gibbs, and A. W. Norman, *J. Org. Chem.* **54**, 4072–4083 (1989).
157. E. M. VanAlstyne, A. W. Norman, and W. H. Okamura, *J Am. Chem. Soc.* **116**, 6207–6216 (1994).
158. M. L. Hammond et al., *J. Am. Chem. Soc.* **100**, 4907 (1978).
159 P. Condran et al., *J. Am. Chem. Soc.* **102**, 6259 (1980).
160. W. H. Okamura et al., in Ref. 53, pp. 5–12.
161. B M Trost, J. Dumas, and M. Villa, *J. Am. Chem. Soc.* **114**, 9836–9845 (1992).
162. K. Nagasawa, Y. Zako, H. Ishihara, and I. Shimizu, *Tetrahedron Lett.* **32**, 4937–4940 (1991).
163. M. Sheves and Y. Mazur, *J. Am. Chem. Soc.* **97**, 6249–6250 (1975).
164. H. E. Paaren, H. F. DeLuca, and H. K. Schnoes, *J. Org. Chem.* **45**, 3253–3258 (1980).
165. M Kabat et al., *Tetrahedron Lett.* **32**, 2343–2346 (1991).
166 S. R. Wilson, A. M. Venkatesan, C. E. Augelli-Szafran, and A. Yasmin, *Tetrahedron Lett.* **32**, 2339–2342 (1991).
167. D. R. Andrews et al., *J. Org. Chem.* **51**, 1635–1637 (1986).
168. H. H Inhoffen, G. Quinkert, and S. Schutz, *Chem. Ber.* **90**, 1283–1286 (1957).
169 H. H. Inhoffen, G. Quinkert, H.-J. Hess, and H. Hirschfeld, *Chem. Ber.* **90**, 2544–2553 (1957).
170. D. R. Andrews, D. H. R. Barton, R. H. Hesse, and M. M. Pechet, *J. Org. Chem.* **51**, 4819–4828 (1986).
171. L. Vanmaele, P. J. De Clercq, and M. Vandewalle, *Tetrahedron Lett.* **41**, 141–144 (1985).
172. M. F. Holick, *Am. J. Clin. Nutr.* **60**, 619–630 (1994).
173. M. F. Holick et al., *Endocrinology* **135**, 655 (1994).
174. G. Ponchon, A. L. Kennan, and H. F. DeLuca, *J. Clin. Invest.* **48**, 1273 (1969).
175. T. C. Madhok and H. F. DeLuca, *Biochem. J.* **184**, 491 (1979); E. Axen, T. Bergman, and K. Wikvall, *J. Steroid Biochem. Mol. Biol.* **51**, 97 (1994).
176. S. A. Holick, M F. Holick, T. E. Tavela, H. K. Schnoes, and H. F. DeLuca, *J. Biol. Chem.* **251**, 397 (1976).
177. E. B. Olson, Jr., J. C. Knutson, M. H. Bhattacharyya, and H. F. DeLuca, *J. Clin. Invest.* **57**, 1213 (1976).
178. J. A. Eisman, R. M. Shepard, and H. F. DeLuca, *Anal. Biochem.* **80**, 198 (1977).
179. D. R. Fraser and E. Kodicek, *Nature (London)* **228**, 764 (1970).

180. J. G. Ghazarian, C R. Jefcoate, J. C. Knutson, W. H. Orme-Johnson, and H. F. DeLuca, *J. Biol. Chem.* **249**, 3026 (1974).

181. L. T. Boyle, L. Miravet, R. W. Gray, M. F. Holick, and H. F. DeLuca, *Endocrinology* **90**, 605 (1972).

182. T. C. Chen, L. Castillo, M. Korycka-Dahl, and H. F. DeLuca, *J. Nutr.* **104**, 1056 (1974)

183 H. F. DeLuca et al., in C. Hansch, P. G. Sammes, and J. B. Taylor, eds., *Comprehensive Medicinal Chemistry*, Vol. 3, Pergamon Press, Oxford, U.K., 1991, p. 1129.

184. A. W. Norman et al., *J. Steroid Biochem. Mol. Biol.* **41**, 231 (1992).

185 K. E. Lowe, A. C. Maiyor, and B. W Norman, *Crit. Rev. Eukar. Gene. Exp.* **2**, 65–109 (1992).

186. D. T. Baran, *J. Cell. Biochem.* **270**, 303 (1994).

187 D. W. Beno et al., *J. Biol. Chem.* **270**, 3542 (1995).

188. R. Kumar, D. Harnden, and H. F. DeLuca, *Biochemistry* **15**, 2420 (1976).

189 D. Harnden, R. Kumar, M. F. Holick, and H. F. DeLuca, *Science* **193**, 493 (1976).

190. M. F. Holick et al., *Biochemistry* **11**, 4251 (1972).

191. Y. Tanaka, L. Castillo, H. F. DeLuca, and N. Ikekawa, *J. Biol. Chem.* **252**, 1421 (1977).

192. M. F. Holick et al., *J. Biol. Chem.* **248**, 6691 (1973).

193. J. N. M. Heersche and J. A. Kanis, eds., *Bone and Mineral Research*, Vol. 8, Elsevier Science B.V., Amsterdam, the Netherlands, 1994, p. 45.

194. M. Gross et al., *J. Bone Miner. Res* **1**, 457 (1986).

195. T. V. Wijngaarden et al., *Cancer Res.* **54**, 5711 (1994).

196. R. J. Skowronski, D. M. Pechl, and D. Feldman, *Endocrinology* **136**, 20 (1995).

197. M. Inaba et al., *Blood* **82**, 53 (1993).

198. J. A. MacLaughlin et al., *Proc. Natl. Acad. Sci.* **82**, 5409 (1985).

199. S. Morimoto et al., *Calcif. Tissue Int.* **39**, 209 (1986).

200. S. Morimoto et al., *Calcif. Tissue Int.* **38**, 119 (1986).

201. K. J. Kragballe, *J. Cell. Biochem.* **49**, 46 (1992).

202. J. M. Lemire, *J. Cell. Biochem.* **49**, 26 (1992).

203. E. P. Amento, *Steroids* **49**, 55 (1987).

204. M. S. Saporito et al., *Brain Res.* **633**, 189 (1994).

205. M. S. Saporito et al., *Exp. Neurol.* **123**, 295 (1993).

206. S. Carswell, *Exp. Neurol.* **124**, 36 (1993).

207. H. Kohler et al., *Zentralbl. Veterinaermed. Reihe B.* **25**, 89 (1978).

208. F. H. Bird, *Poult. Sci.* **57**, 1293 (1978).

209. B. Jedek et al., *Zentralbl. Veterinaermed. Reihe B.* **25**, 29 (1978).

210. *Vitamin Tolerances of Animals*, National Research Council, National Academy Press, Washington, D.C., 1987, p. 10.

211. Y. H. Hull, *Encyclopedia of Food Science and Technology*, Vol. 4, John Wiley & Sons, Inc., New York, 1991, Pts. 1–8, p. 2713.

212. *Recommended Dietary Allowances*, 7th ed., National Academy of Science, National Research Council, Washington, D.C., 1968, p. 1964.

213. *Nutrient Requirements of Poultry*, 9th ed., National Academy of Science, National Research Council, Washington, D.C., 1994, p. 15.

214. *Nutritent Requirements of Swine*, 9th ed., National Academy of Science, National Research Council, Washington, D.C., 1988, p. 35.

215. *Nutritional Requirements of Dairy Cattle*, 3rd ed., National Academy of Science, National Research Council, Washington, D.C., 1966, p. 1349.

216. H. Edwards, "Factors Influencing Leg Disorders in Broilers," p. 21 in (Proceedings of the Maryland Nutrition Conference, March 23, 1995. University of Maryland, College Park, Maryland).

217 W Putscher, *Z. Kinderheilkd.* **48**, 269 (1929).

218. D. O. Harrington and E. H. Page, *J. Am. Vet. Med. Assoc.* **182**, 1358 (1983).

219. K. Diem and C. Lentner, eds., *Scientific Tables*, Ciba Geigy, Ltd., Basel, Switzerland, 1971, p. 464.

220. R. L. Morrissey et al., *J. Nutr.* **107**, 1027 (1977).

221. *Oil, Paint and Drug Reporter* **167**, No. 2, p. 23 (1955) and subsequent issues.

222. *Chemical Economics Handbook*, Stanford Research Institute, Menlo Park, Calif., 1980.

223. *Chem. Mktg. Rep.* **251**, No. 5, p. 39 (1997).

224. U.S. Pat. 2,417,299 (Mar. 11, 1947), L. Freedman and E. Green (to U.S. Vitamin Corp.).

225. U.S. Pat. 2,702,262 (Feb. 15, 1955), Burley and A. E. Timrech (to Charles Pfizer and Co., Inc.).

226. U.S. Pat. 2,827,452 (Mar. 18, 1978), H. Schlenk, D. M. Sand, and J. A. Tillotson (to University of Minnesota).

227. U.S. Pat. 3,067,104 (Dec. 4, 1962), M. Hochberg and C. Ely (to Nopco Chemical Co.).

228. U.S. Pat. 3,143,475 (Aug. 4, 1964), A. Koff and R. F. Widmer (to Hoffmann-LaRoche, Inc.).

GENERAL REFERENCES

Refs. 5, 6, 16, 51, 62, and 141 are also general references.

C. E. Bells, in W. H. Sebrell and R. S. Harris, eds., *The Vitamins*, 1st ed., Vol. 2, Academic Press, New York, 1954, p. 132.

G. F. Combs and H. F. DeLuca, *The Vitamins: Fundamental Aspects in Nutrition and Health*, Academic Press, Inc., 1992, p. 151.

E. D. Collins and A. W. Norman, in L. J. Macklin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991, Chapt. 2, p. 59.

H. F. DeLuca in R. B. Olfin-Slater and D. Kritchevsky, eds., *Nutrition and the Adult: Micronutrients*, Plenum Press, New York, 1986, p. 205.

W. Friedrich, *Vitamins*, Walter de Gruyter, New York, 1988, p. 141.

A. W. Norman, in M. Brown, ed., *Present Knowledge in Nutrition*, 6th ed., International Life Sciences Institute–Nutrition Foundation, Washington, D.C., 1990, Chapt. 12, p. 108.

Arnold L. Hirsch
Alpharma, Inc.

VITAMINS: VITAMIN E

Vitamin E was first described in 1922 and the name was originally applied to a material found in vegetable oils. This material was found to be essential for fertility in rats. It was not until the early 1980s that symptoms of vitamin E deficiency in humans were recognized. Early work on the natural distribution, isolation, and identification can be attributed to Evans, Burr, and Emerson (University of California) and Mattill and Olcott (University of Iowa). Subsequently a group of substances (Fig. 1), which fall into either the family of tocopherols or tocotrienols, were found to act like vitamin E (1–4). The structure of *α*-tocopherol was determined by degradation studies in 1938 (5).

CHEMICAL, BIOLOGICAL, AND PHYSICAL PROPERTIES

The structures (see Fig. 1) of all vitamin E compounds are characterized by a 6-chromanol ring with a C_{16} side chain. As a result of three asymmetric carbons, there are eight possible optical isomers. However, it is the configuration around the 2-position on the ring which determines, to the largest extent, biological activity (6). Natural vitamin E is characterized by all R stereochemistry. The tocopherols are distinguished by a saturated phytol side chain and the tocotrienols are characterized by an unsaturated side chain. The isomers within the two families differ by the extent and position of the methylation on the ring. Although tocopherols and tocotrienols are chemically related, they have varying degrees of vitamin E activity depending on their structure and stereochemistry (Table 1).

The biological activity of various vitamin E forms was established by the fetal resorption assay in rats and is assumed to be applicable to humans. The results of some human studies may indicate that the ratio of 1.36 underestimates the biological activity of the *RRR* form relative to the *all-rac* form of *α*-tocopheryl acetate (10–12).

α-Tocopherol and *α*-tocopheryl acetate are viscous oils. The fat solubility of *α*-tocopherol and *α*-tocopheryl acetate is characteristic of the family of compounds. Both are readily soluble in ethanol, chloroform, and acetone, but are insoluble in water. Other physical properties of *α*-tocopherols and their acetate esters are given in Table 2.

Although they are generally stable to heat and alkali, and acids in the absence of oxygen, free tocopherols and tocotrienols can be oxidized by atmospheric oxygen in the presence of light, unsaturated fatty acids, heat, or metal ions. The reported oxidation products of *α*-tocopherol are shown in Figure 2. These products can be readily synthesized by oxidizing *α*-tocopherol with such reagents as nitric acid, silver nitrate, ferric chloride, or benzoyl peroxide. As

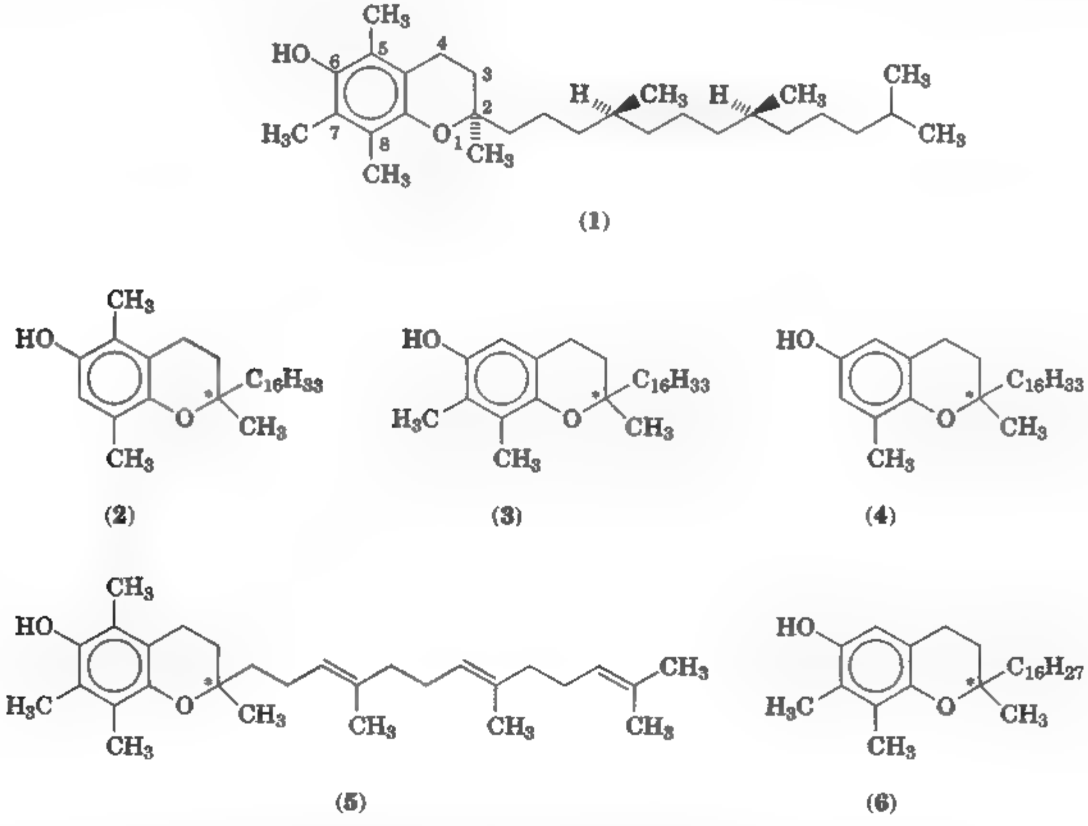


Figure 1. The four naturally occurring tocopherols (*α*-tocopherol, *RRR* [*59-02-9*]/*all-rac* [*2074-53-5*] (**1**); *β*-tocopherol [*148-03-8*] (**2**); *γ*-tocopherol [*54-28-4*] (**3**); *δ*-tocopherol [*119-13-1*] (**4**)), *α*-tocotrienol [*1721-51-3*] (**5**), and *β*-tocotrienol [*14101-61-2*] (**6**) where asterisks denote asymmetric centers and the absolute configuration of *RRR*-*α*-tocopherol is shown.

Table 1. Relative Activity of Vitamin E Forms

Substance	Vitamin E activity
all-rac-α-Tocopheryl acetate	1.00
RRR-α-Tocopheryl acetate	1.36
all-rac-α-Tocopheryl acid succinate	0.89
RRR-α-Tocopheryl acid succinate	1.21
all-rac-α-Tocopherol	1.10
RRR-α-Tocopherol	1.49
β-Tocopherol	0.50
γ-Tocopherol	0.35
α-Tocotrienol	0.30
γ-Tocotrienol	0.10

Source: Refs. 7–9.

a result of the ease of oxidation, significant amounts of vitamin E (tocopherols) may be lost during food processing (qv). The severity of the processing, ie, cooking, canning, and baking, determines the amount of tocopherol remaining in foods and edible oils. Storage conditions can also impact on the losses of vitamin E in foods and animal feeds. The stability of α-tocopherol is significantly enhanced by esterification. Although the acetate ester does not have any inherent antioxidant activity, it is bioactive and is the commercially important form because of its greater stability.

NATURAL OCCURRENCES AND SOURCES

Unesterified tocopherols are found in a variety of foods: however, concentration and isomer distribution of tocopherols vary greatly with source. Typically, meat, fish, and dairy contain <40 mg/100 g of total tocopherols. Almost all (>75%) of this is α-tocopherol for most sources in this group. The variation in the content of meat and dairy products can be related to the content of the food ingested by the animal. A strong seasonal variation can also be observed. Vegetable oils contain significant levels of γ-, β-, and δ-tocopherol, along with α-tocopherol (Table 3).

Vegetable oils, typically soybean, are important feedstocks for the commercial production of the *RRR* forms of vitamin E.

SYNTHESIS

Although all four tocopherols have been synthesized as their *all-rac* forms, the commercially significant form of tocopherol is *all-rac*-α-tocopheryl acetate. The commercial processes in use are based on the work reported by several groups in 1938 (15–17). These processes utilize a Friedel-Crafts-type condensation of 2,3,5-trimethylhydroquinone with either phytol (16), a phytyl halide (7,16,17), or phytadiene (7). The principal synthesis (Fig. 3) in current commercial use involves condensation of 2,3,5-trimethylhydroquinone (**13**) with synthetic isophytol (14) in an inert solvent, such as benzene or hexane, with an acid catalyst, such as zinc chloride, boron trifluoride, or orthoboric acid/oxalic acid (7,8,18) to give the *all-rac*-α-tocopherol (**15a**). Free tocopherol is protected as its acetate ester (**15b**) by reaction with acetic anhydride. Purification of tocopheryl acetate is readily accomplished by high vacuum molecular distillation and rectification (<1 mm Hg) to achieve the required USP standard.

Trimethylhydroquinone can be synthesized from various isomeric trimethylphenols such as 2,4,6- or 2,3,6-trimethylphenol (Fig. 4). When starting with 2,3,6-trimethylphenol (**16**), this can be accomplished by catalytic oxidation using oxygen and cupric halide catalysts in a solvent such as water-alcohol, 2-methoxyethanol, acetone, or *N,N*-dimethylformamide (19,20). The resulting benzoquinone (**17**) can then be hydrogenated catalytically using Pd or Pt in an inert solvent, such as methanol, isobutanol, or toluene (21) to yield 2,3,5-trimethylhydroquinone (**13**). When 2,4,6-trimethylphenol (**18**) is used, 4-hydroxy-2,4,6-trimethyl-2,5-cyclohexadien-1-one (**19**) is obtained by oxidation with sodium hypochlorite (22) or oxygen and base (23). This compound can then be rearranged by base to trimethylhydroquinone (22,23). The trimethylphenols can be obtained by methylation of phenol.

The isophytol side chain can be synthesized from pseudoionone (Fig. 5) using chemistry similar to that used in the vitamin A synthesis (9). Hydrogenation of pseudoionone (**20**) yields hexahydropseudoionone (**21**) which can be reacted with a metal acetylide to give the acetylenic alcohol (**22**). Rearrangement of the adduct of (**22**) with isopropenylmethyl ether yields, initially, the allenic ketone (**23**) which is further transformed to the C_{18}-ketone (**24**). After reduction of (**24**), the saturated ketone (**25**) is treated with a second mole of metal acetylide. The acetylenic alcohol (**26**) formed is then partially hydrogenated to give isophytol (**14**).

Although the eight stereoisomers of α-tocopherol have been synthesized (24), these preparations are at present

Table 2. Physical Properties of Tocopherols

Property	*all-rac*-α-Tocopherol	(*RRR*)-α-Tocopherol	*all-rac*-α-Tocopheryl Acetate	*RRR*α-Tocopheryl Acetate
CAS Registry Number	*[2074-53-5]*	*[59-02-9]*	*[7695-91-2]*	*[58-95-7]*
Color	←— colorless to pale yellow —→			
Form	←— viscous oil —→			
Boiling point, °C	200–220 (0.1 mm)		224 (0.3 mm)	
sp gr_{25}^{25},	0.947–0.958	0.950–0.964	0.950–0.964	
n_D^{20}	1.5030–1.5070	1.4940–1.4985	1.4940–1.4985	
Molecular weight	430.69	430.69	472.73	472.73
UV absorption				
maxima, nm	292–294	292–294	285.5	285.5
$E_{1\,\mathrm{cm}}^{1\%}$ (ethanol)	71–76	71–76	40–44	40–44

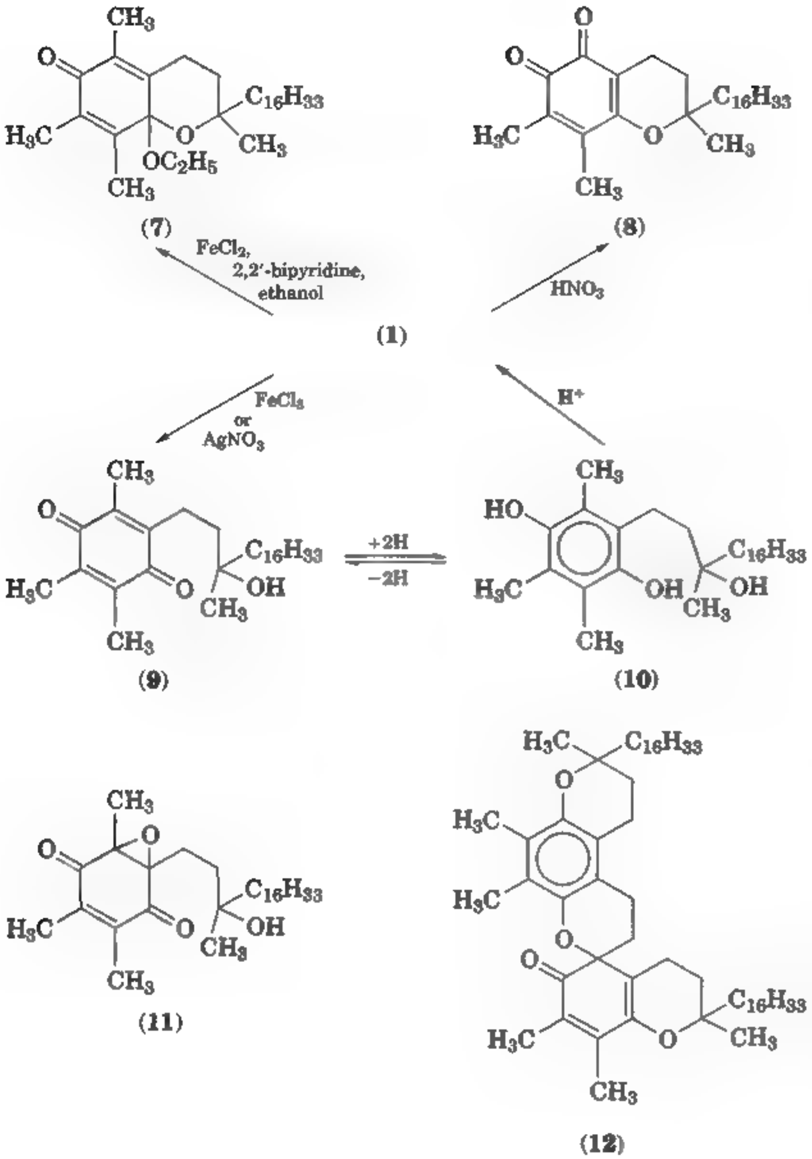
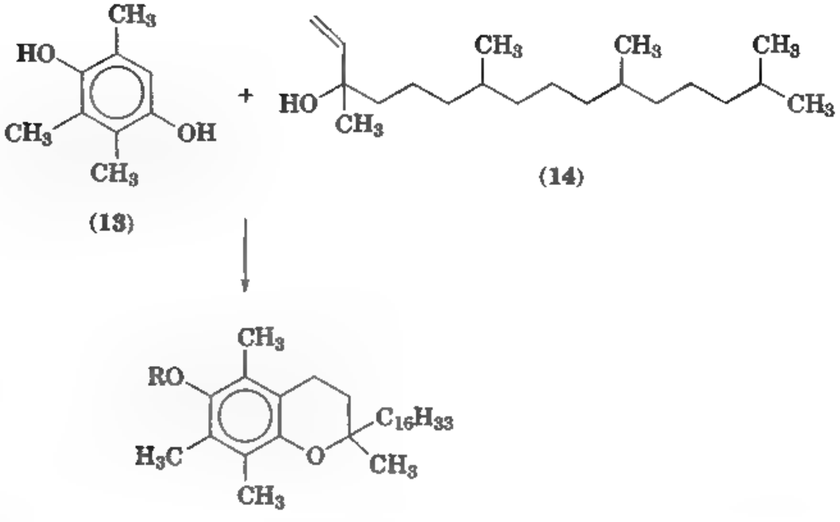

(7)
(8)
FeCl$_2$, 2,2′-bipyridine, ethanol
HNO$_3$
(1)
FeCl$_3$ or AgNO$_3$
H$^+$
+2H
−2H
(9)
(10)
(11)
(12)

Figure 2. Oxidation products of α-tocopherol: tocopherethoxide [*511-72-8*] (**7**), tocored [*17111-16-9*] (**8**), α-tocoquinone [*7559-04-8*] (**9**), α-tocohydroquinone [*14745-36-9*] (**10**), α-tocoquinone-2,3-oxide [*35499-91-3*] (**11**), and α-tocopherol dimer [*1604-73-5*] (**12**).

Table 3. Vitamin E Content of Common Oils, mg/100 g

Oil source	Total tocopherols	α-Tocopherol	γ-Tocopherol	β-Tocopherol	δ-Tocopherol
Soybean	14	8–10	59		26
Corn	24	10–16	60	5	2
Safflower	41	40	17		1
Canola	23	19	43		
Cottonseed	48	39–44	39		
Sunflower	49	49	5		0.8
Coconut	1	0.5–1			0.6
Palm	29	20–26	32		7
Olive	10	5–10			
Peanut		13	22		2
Rapeseed		18	17		1
Wheatgerm		133	26	71	27

Source: Refs. 13 and 14.


(13) (14)

(15a, R = H)
(15b, R = CH_3CO)

Figure 3. Synthesis via trimethylhydroquinone [*700-13-0*] (TMHQ) (**13**) and isophytol [*505-32-8*] (**14**) of α-tocopherol (**15a**) and α-tocopherol acetate (**15b**).

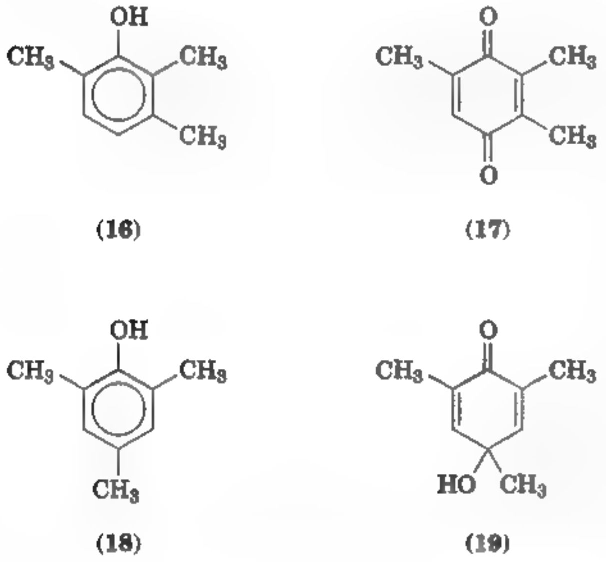

(16) (17) (18) (19)

Figure 4. Starting materials and intermediates in the TMHQ syntheses: 2,3,6-trimethylphenol [*2416-94-6*] (**16**), 2,3,5-trimethylquinone [*935-92-2*] (**17**), 2,4,6-trimethylphenol [*527-60-6*] (**18**), and 4-hydroxy-2,4,6-trimethyl-2,5-cyclohexadien-1-one [*28750-52-9*] (**19**).

only of academic interest. However, *RRR*-α-tocopherol and the other natural forms of vitamin E can be isolated from deodorizer distillates produced as by-products of vegetable oil processing. This requires a series of steps built around key molecular distillations which are required to increase the purity of the *RRR*-α-tocopherol and mixed tocopherol fractions for use in commercial preparations. A typical process would involve a distillation of alkali-treated soybean oil at high vacuum (<1 mm Hg) in a continuous molecular still. This minimizes thermal degradation. The distillate, which contains α-, γ-, and δ-tocopherols, is then cooled to low temperature (~ −10°C) in a solvent to remove impurities such as sterols. The other impurities, such as fatty acids, can be removed by saponification. Further molecular distillation is required to produce a fraction containing high levels (≥60%) of tocopherols. The sterols and fatty acids can be sold.

The yield of the more active *RRR*-α-tocopherol can be improved by selective methylation of the other tocopherol isomers or by hydrogenation of α-tocotrienol (25,26). Methylation can be accomplished by several processes, such as simultaneous haloalkylation and reduction with an aldehyde and a hydrogen halide in the presence of stannous chloride (27), aminoalkylation with ammonia or amines and an aldehyde such as paraformaldehyde followed by catalytic reduction (28), or via formylation with formaldehyde followed by catalytic reduction (29).

The *all-rac* forms of β-, γ-, and δ-tocopherols can be synthesized using the same condensation reaction as used for *all-rac*-α-tocopherol. To synthesize *all-rac*-β-tocopherol, 2,5-dimethylhydroquinone instead of trimethylhydroquinone is condensed with isophytol. For *all-rac*-γ- and δ-tocopherol, 2,3-dimethylhydroquinone and methylhydroquinone are used, respectively.

Although apparently not commercially important, fermentation (qv) processes have been reported for the production of tocopherols. *Aspergillus niger* (30), *Lactobacter* (31), *Euglena gracilis Z.* (32,33), and *Mycobacterium* (34) have been shown to produce (*RRR*)-α-tocopherol. In the case of *Euglena*, titers of 140–180 mg/L have been reported.

PHYSIOLOGICAL EFFECTS AND REQUIREMENTS

The symptoms of vitamin E deficiency in animals are numerous and vary from species to species (13). Although the deficiency of the vitamin can affect different tissue types such as reproductive, gastrointestinal, vascular, neural,

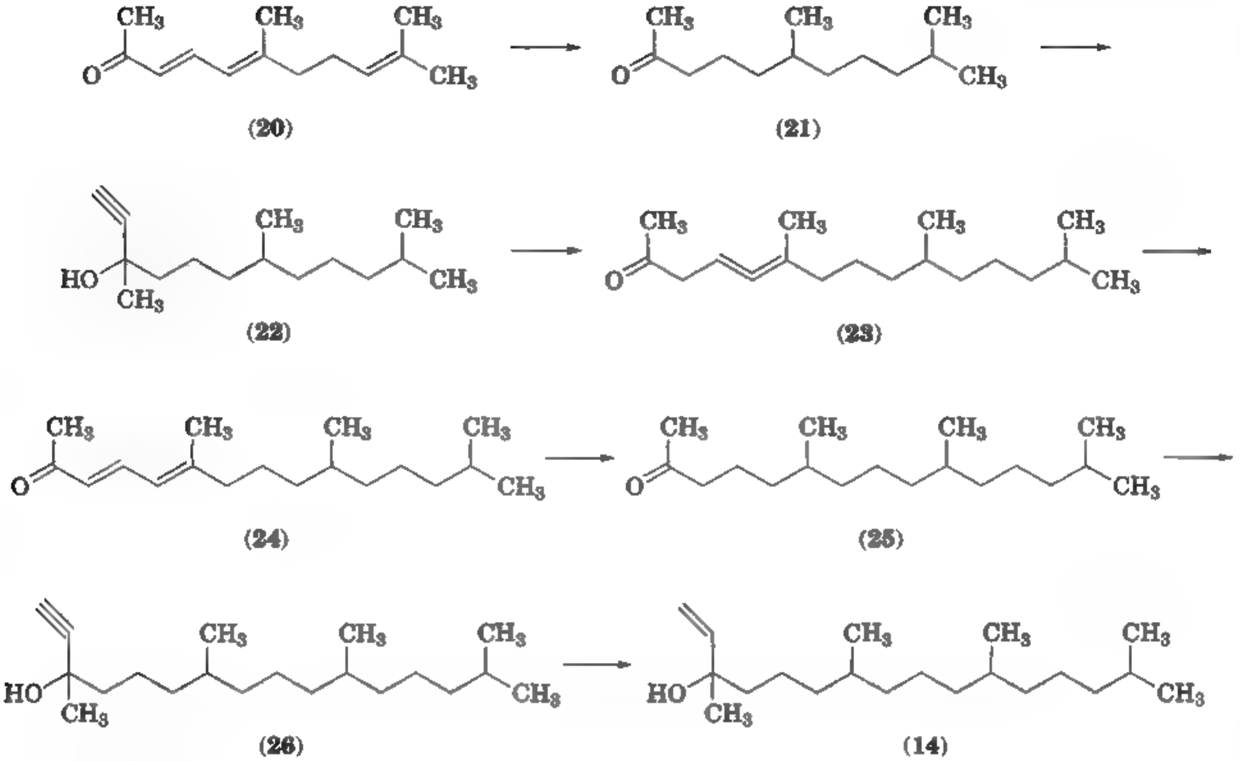


Figure 5. Synthesis of isophytol (**14**): pseudoionone [*141-10-6*] (**20**), hexahydropseudoionone [*1604-34-8*] (**21**), C_{15}-acetylenic alcohol [*1604-35-9*] (**22**), C_{18}-allenic ketone [*16647-10-2*] (**23**), C_{18}-diene ketone [*1604-32-6*] (**24**), C_{18}-saturated ketone [*16825-16-4*] (**25**), and C_{20}-acetylenic alcohol [*29171-23-1*] (**26**).

hepatic, and optic in a variety of species such as pigs, rats, mice, dogs, cats, chickens, turkeys, monkeys, and sheep, it is generally found that necrotizing myopathy is relatively common to most species. In humans, vitamin E deficiency can result from poor fat absorption in adults and children. Infants, especially those with low birth weights, typically have a vitamin E deficiency which can easily be corrected by supplements. This deficiency can lead to symptoms such as hemolytic anemia, reduction in red blood cell lifetimes, retinopathy, and neuromuscular disorders.

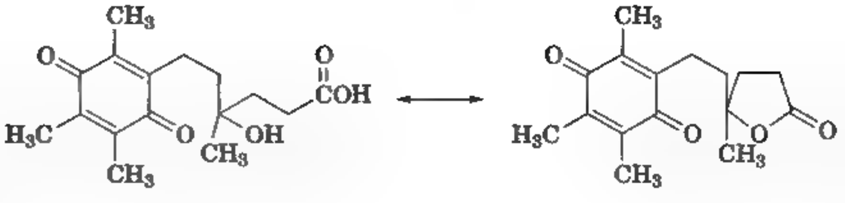
Vitamin E can also act as an antioxidant (qv) in animals and humans alone or in combination with vitamin C (qv). Both are good free-radical scavengers with the vitamin C acting to preserve the levels of vitamin E (35). Vitamin E in turn can preserve the levels of vitamin A in animals (13). It has been shown that vitamin E reduces the incidence of cardiovascular disease (36–39). This most likely results from the antioxidant property of the vitamin which inhibits the oxidation of low density lipoproteins (LDLs) (40–42). The formation of the oxidized LDLs is considered important in decreasing the incidence of cardiovascular disease (43).

The recommended daily allowance for vitamin E ranges from 10 International Units (1 IU = 1 mg *all-rac-α*-tocopheryl acetate) for children under 4 years of age to 30 IU for adults and children over 4 years of age. This should be adequate to prevent vitamin E deficiency in humans. High levels enhance immune responses in both animals and humans. Requirements for animals vary from 3 USP units/kg diet for hamsters to 70 IU/kg diet for cats (13). The complete metabolism of vitamin E in animals or humans is not known. The primary excreted breakdown products of α-tocopherol in the body are gluconurides of tocopheronic acid (**27**) (Fig. 6). These are derived from the primary metabolite α-tocopheryl quinone (**9**) (see Fig. 2) (44,45).

Vitamin E is considered nontoxic at levels up to 3200 mg/day. It has not been found to be teratogenic, mutagenic, or carcinogenic at doses below 1 g/kg of body weight. Vitamin E can heighten the effect of vitamin K deficiency (coagulation defect) or anticoagulation therapy. A recent study, however, indicates that it may be the oxidation product α-tocoquinone that causes the effect (46).

ECONOMIC ASPECTS

World production in the late 1990s of both natural and synthetic forms of vitamin E is estimated at 22,000 metric tons and growth is expected to keep pace with increasing need. The 1993 U.S. production was 14,096 metric tons (47) with an additional 1080 metric tons from imports. The principal U.S. producers of the natural form are Eastman Chemical Company, Archer Daniels Midland Company, and Henkel, and of synthetic vitamin E, Hoffmann-La Roche and BASF. International producers include Hoffmann-La Roche, BASF, Eisai, and Rhône-Poulenc.



(27) (28)

Figure 6. α-Tocopheronic acid [*1948-76-1*] (**27**) and its lactone, α-tocopheronolactone [*3121-68-4*] (**28**).

The price of synthetic vitamin E decreased steadily until about 1990 when prices began to increase. The price of the natural form, although higher than the synthetic form, has approximately paralleled the synthetic form (Table 4).

ANALYTICAL METHODS AND SPECIFICATIONS

Specifications and standards for various vitamin E forms and preparations for use in pharmaceutical applications are given in the *United States Pharmacopeia* (52). All products should contain not less than 96.0% or more than 102.0% of the appropriate form. The products must be labeled to indicate both the chemical and stereochemical forms contained in the product.

Label claims for tocopherol levels in preparations can be based on milligrams or International Units. Only the *RRR* or *all-rac*-α-tocopherol and its esters can be claimed. No vitamin E activity can be claimed for the tocotrienols and the non-α-tocopherols. International Units are also used in some reference books and compendia, eg, *Food Chemicals Codex* (40,53), which is of particular importance for specifications for food fortification.

All formulations of vitamin E must show low acidity, and contain not more than 0.004% heavy metals (reported as Pb) and not more than 10 ppm Pb. Formulations which contain (*RRR*)-α-tocopherol must have a specific rotation of +24° for the oxidation product with alkaline potassium ferricyanide.

Analysis of vitamin E can be done by a variety of methods depending on the form and level present and the preparation in which the form is found (54). For pure or highly concentrated forms, this is accomplished by reaction with Emmerie-Engel reagent (2,2′-bipyridine (α,α′-dipyridyl) and ferric chloride) to give a red color. This color results from the combination of bipyridine with ferrous ions formed from the reduction of the ferric ions by the tocopherol and is directly proportional to the amount of tocopherol present. Analysis of α-tocopherol in forms which contain low levels, such as vegetable oils, foods, or feeds, is difficult because the colorimetric method is nonspecific and significant sample preparation is involved to remove interferences such as other tocopherols, tocotrienols, and β-carotene. The AOAC Official Methods of Analysis describes a packed column gas chromatographic method for the analysis of tocopherol isomers in mixed concentrates (41). This method separates α- and δ-tocopherols as discrete peaks, but β- and γ-tocopherols elute as a combined peak.

Numerous high pressure liquid chromatographic techniques have been reported for specific sample forms: vegetable oils (55,56), animal feeds (57,58), sera (59,60), plasma (61,62), foods (63,64), and tissues (63). Some of the methods require a saponification step to remove fats, to release tocopherols from cells, and/or to free tocopherols from their esters. All require an extraction step to remove the tocopherols from the sample matrix. The methods include both normal and reverse-phase hplc with either uv absorbance or fluorescence detection. Application of supercritical fluid (qv) chromatography has been reported for analysis of tocopherols in marine oils (65).

Bioassay Method

A modification of the Evans resorption–gestation method can be used to determine α-tocopherol activity in supplements. Although the method is time-consuming, when carefully performed, it can produce reasonably accurate results. The method requires raising female rats on a vitamin E-free diet and mating them with normal males. Unless a vitamin E supplement containing more than 0.3–1.0 mg (depends on methodology) of α-tocopherol is administered in the first 10–12 days of pregnancy, the embryos die and are reabsorbed without apparent harm to the mother. If the test supplement contains greater than the threshold dose of α-tocopherol, the pregnancy proceeds normally.

APPLICATIONS AND PRODUCT FORMS

Both α-tocopherol and its esters are constituents of multivitamin and single-dose nutrient capsules or liquid dietary supplements. Supplements for human use range from a few milligrams in multivitamin preparations to 500–

Table 4. Bulk Prices of Pharmaceutical-Grade α-Tocopheryl Acetate, $/kg

Product	1948	1951	1954	1960	1970	1982	1991	1994	1995
(*RRR*)-form	250	250	185	122	68	68	57	59	59
all rac-form	750	350	136	90	50	27	23	34	34

Source. Refs. 48–51.

1000 mg in single-dose supplements. Specialty items, such as ointments, creams, salves, and suppositories containing vitamin E provide other outlets for α-tocopherol. Tocopherols have significant application in cosmetics (qv) and dermatology. Tocopherols can be used in topical cream or oil forms to treat chronic skin diseases (66), reduce scarring in wound healing (67), reduce inflammation (68), and protect against uv radiation (69). Tocopherols are also incorporated into cosmetic formulations to reduce nitrosamine and nitrosamide formation from amines and amides also present in the formulation (70).

Animal feeds consume approximately 40% of the commercial production of α-tocopherol acetate. Although tocopherol is normally found in feed, the poultry, beef and dairy cattle, lamb, and swine industries use vitamin E in supplements and concentrates to replace tocopherol lost during feed processing and storage. The acetate ester is added to the feeds as either a dry, granular, free-flowing, nondusting powder containing 44,000–276,000 units per kilogram (20,000–125,000 units per pound) or as a high potency oil concentrate (23–100% α-tocopheryl acetate).

Foods are not typically fortified with vitamin E because of the lack of signs of deficiency in the general population. Fortification does occur for infant formulas as a supplement and cereals to replace processing losses. Tocopherols are finding additional applications as antioxidants in foods as the less expensive butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) used are being prohibited in more and more countries. Foods such as lards and citrus oils which do not contain appreciable levels of natural tocopherols can be protected with α-tocopherol at 0.02% levels (weight based on lipid phase) (9). The meat from turkeys and chickens is less prone to rancidity when refrigerated or frozen if the animal's diet contains vitamin E. The meat from pigs fed vitamin E supplements also has better storage (71) and color (72) stability than meat from pigs with normal diets. It has also been reported that veal has better storage stability (73) and beef has better color stability (74) when vitamin E supplements are used in the animal feeds. Tocopherol can be used in bacon and similar foods to prevent the formation of nitrosamines. The traditional antioxidants, such as BHT and phosphites, used in high density polyethylene (HDPE) and polypropylene (PP), can be replaced by *all-rac-α*-tocopherol (75–77).

BIBLIOGRAPHY

1. J. F. Pennock, F. W Hemming, and J. D. Kerr, *Biophys. Res. Commun.* **17**, 542 (1964).
2. International Union of Nutritional Sciences, *Nutr. Abstr. Rev.* **48**, 831 (1978).
3. R. H. Bunnell, J Keating, A. Quaresimo, and G. K. Parman, *Am. J Clin. Nutr.* **17**, 1 (1965).
4. J. G. Bieri and R. P. Evarts, *J. Am. Diet. Assoc.* **62**, 147 (1973).
5. E. Fernholz, *J. Am. Chem. Soc.* **60**, 1741 (1938).
6. H. Weiser and M. Vecchi, *Int. J. Vitam. Nutr. Res.* **52**, 351–370 (1982).
7. S. Kasparak, in L. J. Machlin, ed., *Vitamin E: A Comprehensive Treatise*, Marcel Dekker, Inc., New York, 1980, pp. 7–65.
8. S. Kijima, in M. Mino, H. Nakamura, A. I. Diplock, and H. J. Kayden, eds., *Vitamin E: Its Usefulness in Health and Curing Diseases*, Japan Societies Press, Tokyo, Japan, and S. Karger AG, Basel, Switzerland, 1993, p. 7.
9. D. Bhatia et al., "Vitamins," in *Encyclopedia of Food Technology*, Vol. 4, parts 1–8, John Wiley & Sons, Inc., New York, 1991.
10. G. W. Burton and K. U. Ingold, in L. Packer and G. Fuchs, eds., *Vitamin E in Health and Disease*, Marcel Dekker, Inc., New York, 1993, pp. 329–344.
11. R. V. Acuff, S. S. Thedford, N. N. Hidiroglou, A. M. Papas, and T. Odom, *Am. J. Clin. Nutr.* **60**, 397–402 (1994).
12. H. J. Kayden and M. G. Traber, *T. Lipid Res.* **34**, 343–358 (1993).
13. L. J. Machlin, in L. Machlin, ed., *Handbook of Vitamins*, Marcel Dekker, Inc., New York, 1990, pp. 99–144.
14. H. T. Slover, *Lipids* **6**, 291–296 (1971).
15. P. Karrer, H. Fritzsche, B H. Ringier, and H. Solomon, *Helv. Chim. Acta* **21**, 520 (1938).
16. F. Bergel, A. Jacob, A. R. Todd, and T. S. Work, *Nature* **142**, 36 (1938).
17. L. I. Smith, H. E. Ungnade, and W. W. Prichard, *Science (Washington, D.C.)* **88**, 37 (1938).
18. Ger. Pat. 92-4208477 (1993), P. Grafen, H. Kiefer, and H. Jaedicke (to BASF AG).
19. U.S. Pat. 5,041,572 (Aug. 20, 1991), U. Hearcher, B. Jessel, B. Bockstiegel, P. Grafen, and H. Laas (to BASF AG).
20. Ger. Pat. 2221624 (Nov. 1972), W. Brenner (to Hoffmann-LaRoche AG).
21. Eur. Pat. Appl. 87-115070 (1988), T. Yui and A. Ito (to Mitsubishi Gas Co. Inc.).
22. Jpn. Pat. 62,263,136(86-104088) (1987), T. Kunitomi and H. Tamai (to Kuraray Co. Ltd.).
23. Y. Ichikawa, Y. Yamanaka, N. Suzuki, T. Naruchi, O. Kobayashi, and H. Tsuruta, *Ind. Eng. Chem. Prod. Res. Dev.* **18**, 373–375 (1979).
24. N. Cohen, C. G. Scott, C. Neukom, R. J. Lopresti, G. Weber, and G. Saucy, *Helv. Chim Acta* **64**, 1158 (1981).
25. J. F. Pennock, F. W. Hemming, and J. D. Kerr, *Biochem. Biophys. Res. Commun.* **17**, 542 (1964).
26. H. May, P. Schindl, R. Ruegg, and O. Isler, *Helv. Chim. Acta* **46**, 963 (1963).
27. U.S. Pat. 2,486,539 (Nov. 1, 1949), L. Weisler (to Distillation Products).
28. U.S. Pat. 2,519,863 (Aug. 22, 1950), L. Weisler (to Eastman Kodak Co.).
29. U.S. Pat. 2,592,628 (Apr. 15, 1952), L. Weisler (to Eastman Kodak Co.).
30. Y. Kawai, M. Otaka, M. Kakio, Y. Oeda, N. Inoue, and H. Shinano, *Hokkaido Daigaku Suisangakubu Kenkyo Iho* **45**, 26–31 (1994).
31. H. Ariga, M. Okazaki, and M. Yamada, *Rakuna Kagaku, Shokuhin no Kenkyu* **41** (1992).
32. Y. Tani and S. Osuka, *Agric. Biol. Chem.* **53**, 2313–2318 (1989).
33. Y. Tani and H. Tsumura, *Agric. Biol. Chem.* **53**, 302–315 (1989).
34. Jpn. Pat. 57,163,490(82,163,490) (Oct. 1982), (to Shiseido Co., Ltd.).
35. E. R. Pacht, H. Kaseki, J. R. Mohammed, D. G. Cornwell, and W. B. Davis, *J. Clin. Invest.* **77**, 789 (1986)

36. N. G. Stephens, A. Parsons, P. M. Schofield, F. Kelly, K. Cheeseman, M. J. Mitchinson, and M. J. Brown, *Lancet* **347**, 781–786 (1996).
37. M. J. Stampfer, C. H. Hennekens, J. E. Manson, G. A. Colditz, B. Rosner, and W. C. Willett, *N. Eng. J. Med.* **328**, 1444–1449 (1993)
38. E. B. Rimm, M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz, and W. C. Willett, *N. Eng. J. Med.* **328**, 1450–1456 (1993).
39. K. G. Losonczy, T. B. Harris, and R. J. Havlik, *Am. J. Clin. Nutr.* **64**, 190–196 (1996).
40. H. M G. Princen, G. Poppel, C. Vogelzang, R. Buytenhek, and F. J. Kok, *Arterioscler. Thromb.* **12**, 554–562 (1992).
41. H. Esterbauer, M. Dieber-Rotheneder, M. Striegel, and G. Waeg, *Am. J. Clin. Nutr.* **53**, 314s–321s (1991).
42. I. Jialal, C. J. Fuller, and B. A. Huet, *Arterioscler Thromb. Vasc. Biol.* **15**, 190–198 (1995).
43. D. Steinberg, *Circulation* **84**, 1420–1425 (1991).
44. C. Drevon, in Ref. 4, pp. 75–76.
45. C. K. Chow, *World Rev. Nutr. Diet.* **45**, 133–166 (1985).
46. P. Dowd and Z. B. Zheng, *Proc. Natl. Acad. Sci. USA* **92**, 8171–8175 (1995).
47. Synthetic Organic Chemicals, *United States Production and Sales, 1993*, USITC Publication 2810, U.S. Government Printing Office, Washington, D.C., 1994, pp. 3–191.
48. *Chem. Mark. Rep.* **221**, 47 (1982).
49. *Chem. Mark. Rep.* **240**, 32 (1991).
50. *Chem. Mark. Rep.* **245**, 31 (1994).
51. *Chem. Mark. Rep.* **247**, 45 (1995).
52. *The United States Pharmacopeia XXIII (USP XXIII-NF XVIII)*, The United States Pharmacopeia Convention, Inc., Rockville, Md., 1995, p. 1631.
53. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, p. 330.
54. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1990, pp. 1070–1079.
55. K. Abe, Y. Yuguchi, and G. Katsui, *J. Nutr. Sci. Vitaminol.* **21**, 183–188 (1975).
56. E. Guzman-Contreras and F. C. Strong III, *J. Agric. Food Chem.* **30**, 1109–1112 (1982).
57. H. Cohan and M. R. LaPointe, *J. Assoc. Off. Anal. Chem.* **63**, 1254–1257 (1980).
58. C. H. McMurray and W. J. Blanchflower, *J. Chrom.* **176**, 488–492 (1979).
59. L. Jansson, B. Nilsson, and R. Lingren, *J. Chrom.* **181**, 242–247 (1980).
60. W. J. Driskell, J. Neese, C. C. Bryant, and M. M. Bashor, *J. Chrom.* **231**, 439–444 (1982).
61. C. H. McMurray and W. J. Blanchflower, *J. Chrom.* **178**, 525–531 (1979).
62. J. Lehman and H. Martin, *Clin. Chem.* **28**, 1784–1787 (1982).
63. J. N. Thompson and G. Hatina, *J. Liq. Chrom.* **2**, 327–344 (1979).
64. U. Manz and K. Philipp, *Int. J. Vit. Nutr. Res.* **51**, 342–348 (1981).
65. A. Staby, C. Borch-Jensen, S. Balchen, and J. Mollerup, *Chromatographia* **39**, 697–705 (1994).
66. W. Nikolowski, in *Vitamins* (3) Editones (Roche), F. Hoffmann-LaRoche & Co. Ltd., Basle, Switzerland, (1973).
67. M. Ehrlich, H. Traver, and T. Hunt, *Annal. Surg.* **175** (Feb. 1972), pp. 31–36.
68. M. Kamimura, *J. Vitaminol.* **18**, 201–209 (1972).
69. W. A. Pryor, *Free Radicals in Biology*, Academic Press, Inc., New York, 1976, Chapt. 1.
70. W. Mergens and E. DeRiter, *Cosmetic Technol.* (Jan. 1980).
71. F. J. Monahan, D. J. Buckley, P. A. Morrissey, P. B. Lynch, and J. I. Gray, *Food Sci. Nutr.* **42F**, 203 (1990).
72. A. Asgher, J. I. Gray, A. M. Booser, E. A. Gomaa, M M. Abonzied, and E. R. Miller, *J. Sci. Food Agric.* **57**, 31 (1991).
73. R. Ellis, W. I. Kimato, J. Bitman, and L. F. Edmonson, *J. Am. Chem. Soc.* **51**, 4 (1974).
74. F. B. Shorland, J. O. Igene, A. M. Pearson, J. W McGuffy, and A. E. Aldridge, *J. Agric. Food Chem.* **29**, 863 (1981).
75. D. Burdick, S. Laermer, S. Young, and P. Zambetti, "A New Primary Antioxidant System for Polyolefins," presented at *Additives '95*, Clearwater, Fla., Feb. 22–24, 1995.
76. S. F. Laermer and P F. Zambetti, *J. Plast. Film Sheet.* **8**, 228–248 (1992).
77. Y. C. Ho, K. L. Yam, S. S. Young, and P. F. Zambetti, *J. Plast. Film Sheet.* **10**, 194–212 (1994).

GENERAL REFERENCES

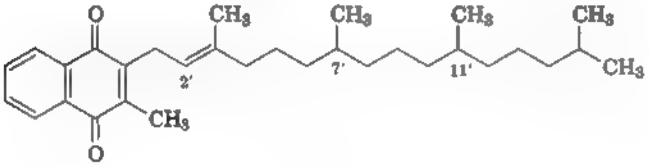
L. J. Machlin, ed., *Vitamin E: A Comprehensive Treatise*, Marcel Dekker, Inc., New York, 1980.

ROBERT CASANI
Hoffmann-La Roche, Inc.

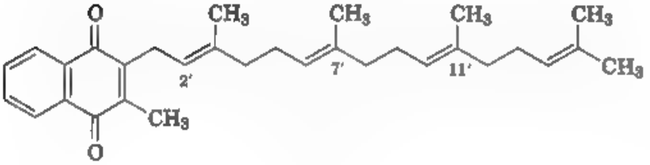
VITAMINS: VITAMIN K

Vitamin K represents a class of substances which contain the 2-methyl-1,4-naphthoquinone moiety and are characterized by their antihemorraghic properties. Vitamin K was first discovered by Dam in 1929 during experiments with chicks on a lipid-deficient diet (1). Dam coined the term *Koagulations vitamin* to describe this new substance (2,3). In the late 1930s, several groups identified the causative agent responsible for the antihemorraghic properties of alfalfa (4–7). Structural determination of vitamin K_1 (**1**) was the result of work from several laboratories (8,9). Stereochemical assignment came much later from Mayer (10). Additional studies isolated and characterized related materials. Vitamin K_2 (**2**) was first found in decaying fish meal (9); it is a crystalline compound and is distinguished by its repeating isoprenyl units. Vitamin K_3 *[58-27-5]* (**3**) is the simplest form of the vitamin. The chemical name and common names for some important forms of the vitamin follow.

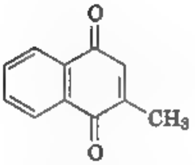
Name	Vitamin K_1
CAS Registry Number	*[84-80-0]*
Chemical name	1,4-Naphthalenedione, 2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-, [*R*-[*R**,*R**-(*E*)]]-
Common name	Phylloquinone, phytomenadione, phytonadione
Name	Vitamin $K_{2(20)}$
CAS Registry Number	*[863-61-6]*
Chemical name	1,4-Naphthalenedione, 2-methyl-3-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl)-, (*E*,*E*,*E*)-
Common name	Menaquinone 4, menaquinone K4, MK-4
Name	Vitamin K_3
CAS Registry Number	*[58-27-5]*
Chemical name	1,4-Naphthalenedione, 2-methyl
Common name	Menadione; menaquinone-0; synkay



(1)



(2)



(3)

The K vitamins are named after the original vitamin and the number of carbon atoms in the side chain. Using this convention, vitamin $K_{2(20)}$ is so named because it contains 20 carbon atoms in the chain. In the biological literature, vitamin K_2 is frequently referred to as menaquinone and is further designated by the number of isoprene units in the side chain. For example, vitamin $K_{2(20)}$ is also called menaquinone-4 or MK-4. Vitamin K_3 is also referred to as menadione or MK-0.

Vitamin K is typically found in green, leafy vegetables such as cabbage, broccoli, and spinach at levels of 95–200 μg/100 g of fresh vegetables. Cauliflower at a level of 136 μg/100 g also represents an excellent source of dietary vitamin K. Additionally, animal sources such as liver and eggs provide good sources of vitamin K (11).

CHEMICAL/PHYSICAL PROPERTIES

Vitamin K compounds are yellow solids or viscous liquids. The natural form of vitamin K_1 is a single diastereoisomer with 2′(*E*), 7′(*R*), 11′(*R*) stereochemistry. The predominant commercial form of vitamin K_1 is the racemate and a 2′(*E*)/(*Z*) mixture. Table 1 lists some physical and spectral properties of vitamin K_1.

Vitamin K_1 is insoluble in water and is soluble in 70% alcohol, chloroform, petroleum ether, benzene, and hexane. Vitamin K_1 is stable in air but should be protected from light. Although unstable in alkali, the vitamin is stable in acidic medium. Its facile decomposition in basic solution forms the basis of the Dam-Karrer color test.

Early structural determination lends insight into the chemical reactivity of vitamin K_1. Catalytic hydrogenation of vitamin K_1 consumes four moles of hydrogen and affords a colorless compound. Because complete hydrogenation of a 1,4-naphthoquinone structure consumes three molecules of hydrogen, consumption of the fourth mole indicates unsaturation in the side chain. Reductive acetylation of vitamin K_1 affords the diacetate of dihydrovitamin K_1. This behavior further supports the conclusions that vitamin K_1 contains a naphthoquinone group (13).

In addition to its reactivity toward reducing agents, vitamin K_1 is also reactive toward oxidizing agents. Chromic acid oxidation of vitamin K_1 gives rise to two principal products, 2-methyl-1,4-naphthoquinone-3-acetic acid and phthalic acid. By comparison with known compounds, it has been concluded that the benzene ring is unsubstituted and the substitution pattern on the naphthoquinone ring is 2,3-dialkyl (8,14–16).

ANALYTICAL METHODS

The classical method for the determination of vitamin K is based on the clotting time of a vitamin K-deficient chick. It is relatively easy to produce a hemorraghic state in chicks (17). Vitamin K-deficient rats have also been used for this assay (18). Owing to the development of modern chromatographic techniques, this method of analysis has been supplanted by other methodology.

There are several comprehensive reviews of analytical methods for vitamin K (19,20). Owing to the presence of a naphthoquinone nucleus, the majority of analytical methods use this structural feature as a basis for analysis. Several identity tests such as its reaction with sodium bisulfite or its uv spectrum exploit this characteristic. Although not specific, titrimetric, polarographic, and potentiometric methods have also been used (20).

Chromatographic methods including thin-layer, hplc, and gc methods have been developed. In addition to developments in the types of columns and eluents for hplc applications, a significant amount of work has been done in the kinds of detection methods for the vitamin. These detection methods include direct detection by uv, fluorescence after post-column reduction of the quinone to the hydroquinone, and electrochemical detection. Quantitative gc methods have been developed for the vitamin but have found limited applications. However, gc methods coupled

Table 1. Physical and Spectral Properties of Vitamin K_1

Item	Vitamin K_1	Vitamin $K_{2(30)}$	Vitamin $K_{2(35)}$	Vitamin K_3
Color	Yellow, Viscous oil	Yellow crystals	Yellow crystals	Bright yellow crystals
Melting point, °C	−20	50	54	105–107
Molecular weight	450.68	580.9	649.02	172.17
Molecular formula	$C_{31}H_{46}O_2$	$C_{41}H_{56}O_2$	$C_{46}H_{64}O_2$	$C_{11}H_8O_2$
Spectrophotometric data, λ_{max}, nm	242, 248, 260, 269, 325	243, 248, 261, 270, 325	243, 248, 261, 270, 325	244
ϵ, petroleum ether	396, 419, 383, 387, 68	304, 320, 290, 292, 53	278, 195, 266, 267, 48	1150 (Hexane)

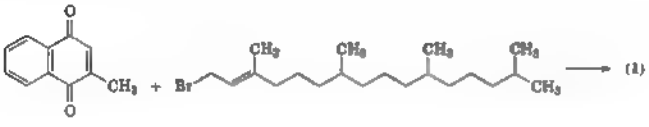
Source: Ref. 12.

with highly sensitive detection methods such as gc/ms do represent a powerful analytical tool (20).

As described in the USP, phytonadione is a mixture of the cis- and trans-isomers of vitamin K_1. This mixture should not contain more than 103% and not less than 97.0% of total vitamin K content. The amount of the cis-isomer is also specified and is not to exceed 21%. In addition to the pure substance, the USP also describes methods for the analysis of parental as well as tableted forms of the vitamin (21).

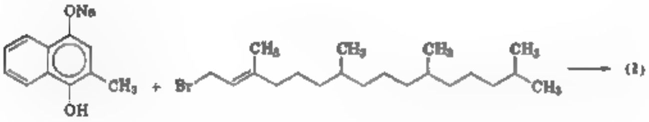
SYNTHESIS

The first synthesis of vitamin K_1 was reported by several workers in the late 1930s and the synthetic approaches have been reviewed (22). Vitamin K_1 was prepared by the reaction of menadione with phytyl bromide in the presence of zinc (23).

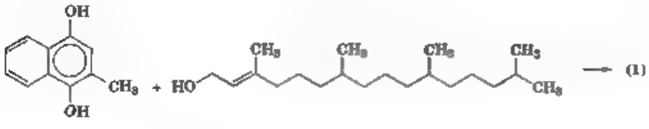


(3)

Vitamin K_1 has been synthesized from the condensation of the monosodium salt of menadiol with phytyl bromide (24).

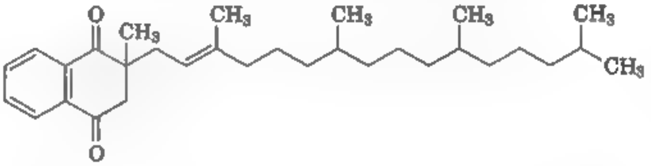


In methodology developed by Fieser, unprotected menadiol (5) was condensed with natural phytol using oxalic acid as catalyst (25). Oxidation of the hydroquinone to the naphthoquinone yielded vitamin K_1. A similar approach has been reported (26). The commercial synthesis of vitamin K_1 is largely based on the above with some important improvements from Roche (27) and Merck (28).



(5)

A significant improvement in this technology was the discovery that BF_3-etherate could be substituted as a condensation catalyst in place of oxalic acid (qv). Owing to the fact that oxalate esters of isophytol did not form, this substitution reduced the formation of undesired phytadiene by-products. A second notable advance was the use of 1-acetyl-2-methyl-1,4-naphthoquinone as starting material for the condensation. In addition to phytadiene, a significant by-product is 2-methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone. The use of the monoprotected compound eliminates the formation of this impurity. Moreover, in the earlier process, the sensitivity of the unprotected compound toward air oxidation necessitated a reduction before purification by base extraction of the crude condensation mixture. The acetylated compound is not labile toward air oxidation. As a result, the reduction step is not warranted and its inherent complications are avoided.



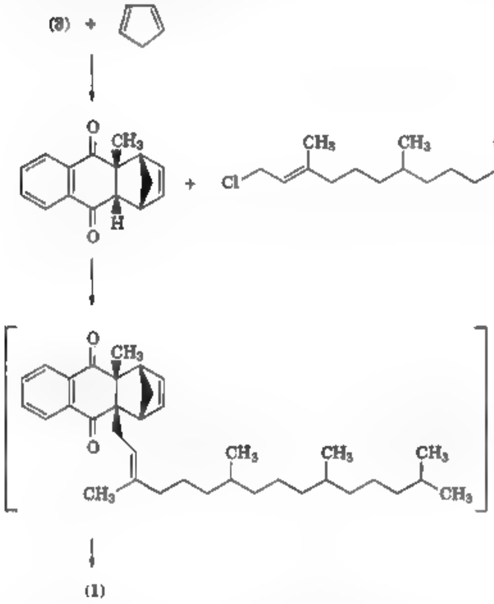
As practiced by Hoffmann-La Roche, the commercial synthesis of vitamin K_1 is outlined inFigure 1. Oxidation of 2-methylnaphthalene (4) yields menadione (3). Catalytic reduction to the naphthohydroquinone (5) is followed by reaction with a benzoating reagent to yield the bisbenzoate (6). Selective deprotection yields the less hindered benzoate (7). Condensation of isophytol (8) (see VITAMINS: VITAMIN E) with (7) under acid-catalyzed conditions yields the coupled product (9). Saponification followed by an air oxidation yields vitamin K_1 (1) (29).

Although the industrial synthesis of vitamin K_1 remains largely unchanged from its early beginnings, significant effort has been devoted to improvements in the condensation step, the oxidation of dihydrovitamin to vitamin K_1, and in economical approaches to vitamin K_3 (*vide infra*). Also, several chemical and biochemical alternatives to vitamin K_1 have been developed.

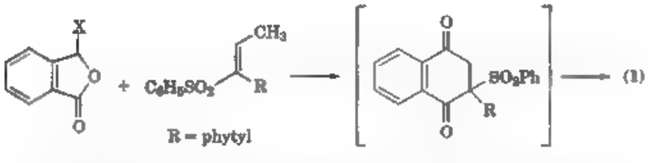
A Japanese patent has claimed improvements in the direct condensation of menadione with phytyl chloride in the presence of a reducing metal such as zinc or iron powder (30). Tin chloride has been reported to be a useful catalyst for this condensation (31,32)

In a patent assigned to Mitsubishi, air oxidation is carried out in the presence of copper salts to avoid the formation of complicating impurities in the oxidation of dihydrovitamin K_1 to vitamin K_1 (33). In other work, high yields of vitamin K_1 were obtained by performing the oxidation in an alkali medium (34). High purity vitamin K_1 can also be obtained by an oxidation in dimethyl sulfoxide (35).

In a novel approach to vitamin K_1, Hoffmann-La Roche has exploited the potential acidity at C-3 as a means to attach the side chain of vitamin K_1 (36). Menadione was reacted with cyclopentadiene to yield the Diels-Alder adduct. The adduct is treated with base and alkylated at C-3 with phytyl chloride. A retro Diels-Alder reaction yields vitamin K_1. Process improvements in this basic methodology have been claimed by Japanese workers (37).



Although not of industrial importance, many organometallic approaches have been developed (38). A one-pot synthesis of vitamin K_1 has been described and is based on the anionic [4 + 2] cycloaddition of three-substituted isobenzofuranones to 1-phytyl-1-(phenylsulfonyl)propene. Owing to the rather mild chemical conditions, the (*E*)-stereochemistry is retained (39).



Although the predominant commercial form of vitamin K_1 is the racemate, natural (2′(*E*), 7′(*R*), 11′(*R*))-vitamin K_1 is accessible either from a natural source or from condensation with natural phytol. In the curative blood clotting test, these compounds have been shown to have nearly equivalent biopotencies. The synthesis and spectral properties of all four stereoisomers of (*E*)-vitamin K_1 has been described and their biopotencies determined. Using natural vitamin K_1 as a standard, the relative activities were found to be 1.0, 0.93, 1.19, and 0.99 for 2′(*E*), 7′(*R*), 11′(*R*); 2′(*E*), 7′(*R*), 11(*S*′); 2′(*E*), 7′(*S*), 11′(*S*); and 2′(*E*), 7′(*S*), 11′(*R*), respectively. These differences were not considered significant and it was concluded that these compounds have identical activities (40).

Aside from chemical methods, several patents have appeared on the biochemical production of natural vitamin K_1 from callus tissue cultures (41). In addition, a patent has appeared which describes the concentration and purification of natural vitamin K_1 from deodorizer distillates (42). The biosynthesis of vitamin K_1 and vitamin K_2 has been reviewed (43).

VITAMIN K_2

As compared to vitamin K_1, vitamin K_2 is relatively unimportant industrially with only a few producers, such as Teikoku Kagaku Sangyo and Eisai, and is dominated by the manufacture of vitamin $K_{2(20)}$. The industrial synthesis parallels that of vitamin K_1 and involves as a key step alkylation of monosubstituted menadione with an appropriate (all-*E*) reagent (44,45). Several academic syntheses have been described (46–49).

In contrast to vitamin K_1, there has been considerably more activity on fermentative approaches to vitamin K_2 (50). The biosynthetic pathway to vitamin K_2 is analogous to that of vitamin K_1 except that poly(prenylpyrophosphates) are the reactive alkylating agent (51,52). Menaquinones of varying chain lengths from C_5 to C_{65} have been isolated from bacteria. The most common forms are vitamin $K_{2(35)}$, $K_{2(40)}$, and $K_{2(45)}$. A significant amount of $K_{2(20)}$ was observed in a *Flavobacterium* fermentation and was increased by the use of a mutant resistant to usnic acid (53). In other work, production of vitamin K_2 was enhanced by the use of 1-hydroxy-2-naphthoate (54). Many Japanese patents have appeared that describe production of menaquinones from bacteria (55).

VITAMIN K_3

Industrially, vitamin K_3 is prepared from the chromic acid oxidation of 2-methylnaphthalene (56). Although the yields are low, the process is economical owing to the low cost and availability of the starting material and the oxidizing agent. However, the process is complicated by the formation of isomeric 6-methyl-1,4-naphthoquinone. As a result, efforts have been directed to develop process technology to facilitate the separation of the isomeric naphthoquinone and to improve selectivity of the oxidation.

A process has been disclosed in which the mixture of naphthoquinones is reacted with a diene such as butadiene. Owing to the fact that the undesired product is an unsubstituted naphthoquinone, this dieneophile readily reacts to form a Diels-Alder adduct. By appropriate control of reaction parameters, little reaction is observed with the substituted naphthoquinone. Differential solubility of the adduct and vitamin K_3 allows for a facile separation (57,58).

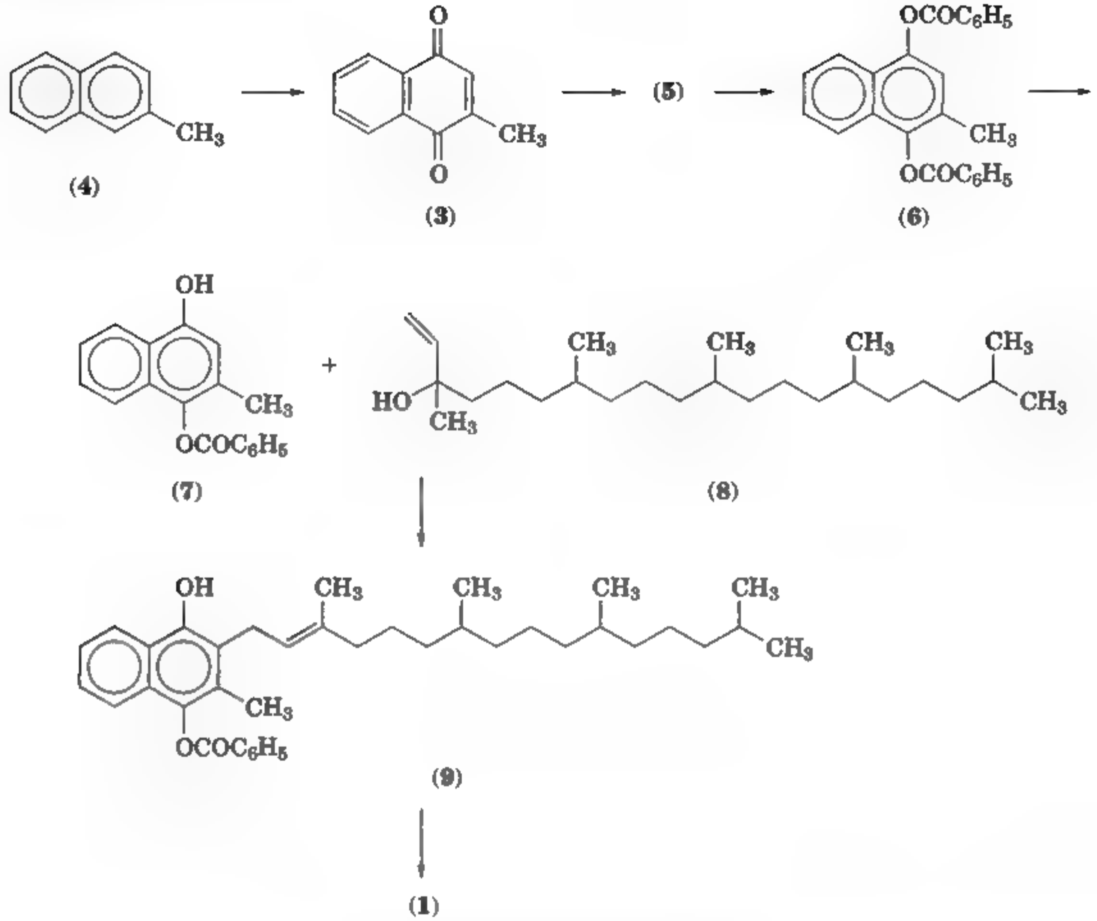
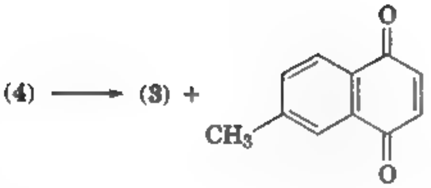


Figure 1. Synthesis of vitamin K_1.

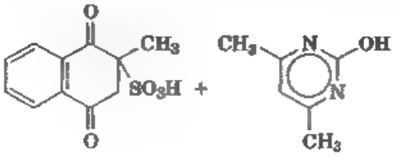


In an alternative approach, the desired 2-methyl-1,4-naphthoquinone is derivatized to form a water-soluble and commercially important sodium bisulfite adduct. Extraction of the organic phase with water separates the isomeric quinones (59).

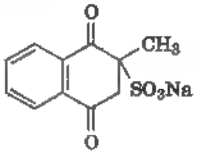
In order to circumvent this problem, there has been significant activity directed toward the search for a less environmentally toxic and more selective oxidizing agent than chromium. For example, Hoechst has patented a process which uses organorhenium compounds. At a 75% conversion, a mixture of 86% of 2-methyl-1,4-naphthoquinone and 14% 6-methyl-1,4-naphthoquinone was obtained (60). Ceric sulfate (61) and electrochemistry (62,63) have also been used.

In a biotechnology-based approach, Japanese workers have reported on the microbial conversion of 2-methylnaphthalene to both 2-methyl-1-naphthol and menadione by *Rhodococcus* (64). The intermediate 2-methyl-1-naphthol can readily be converted to menadione by a variety of oxidizing agents such as heteropoly acids (65) and copper chloride (66). A review of reagents for oxidizing 2-methylnaphthalene and naphthol is available (67).

In addition to its industrial importance as an intermediate in the synthesis of vitamin K_1, menadione, or more specifically, salts of its bisulfite adduct, are important commodities in the feed industry and are used as stabilized forms in this application. Commercially significant forms are menadione dimethyl pyrimidinol (MPB) (**10**) and menadione sodium bisulfite (MSB) (**11**). MSB is sold primarily as its sodium bisulfite complex. The influence of feed processing, ie, pelleting, on the stability of these forms has been investigated (68). The biological availabilities and stability of these commercial sources has been determined (69,70).



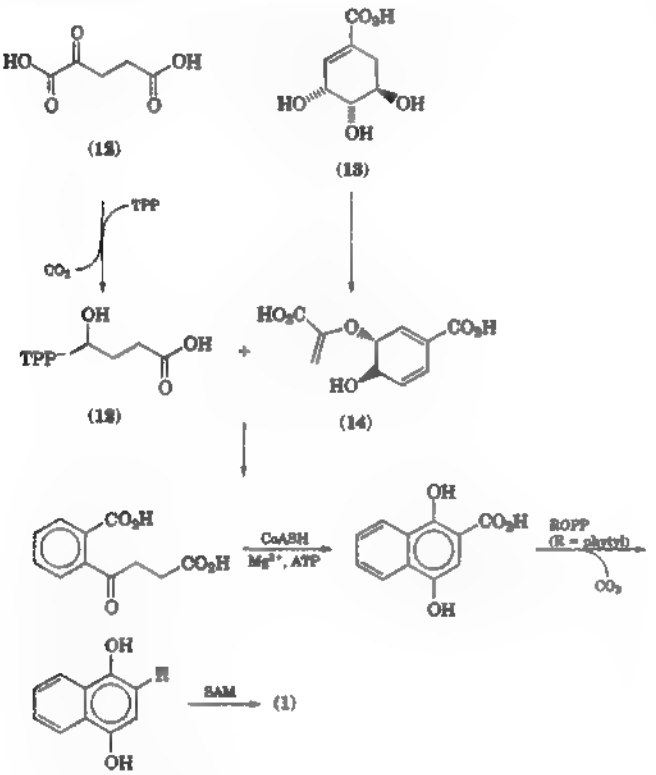
(10)



(11)

BIOSYNTHESIS

Animals cannot synthesize the naphthoquinone ring of vitamin K, but necessary quantities are obtained by ingestion and from manufacture by intestinal flora. In plants and bacteria, the desired naphthoquinone ring is synthesized from 2-oxoglutaric acid (**12**) and shikimic acid (**13**) (71,72). Chorismic acid (**14**) reacts with a putative succinic semialdehyde TPP anion to form *o*-succinyl benzoic acid (73,74). In a second step, *ortho*-succinyl benzoic acid is converted to the key intermediate, 1,4-dihydroxy-2-naphthoic acid. Prenylation with phytyl pyrophosphate is followed by decarboxylation and methylation to complete the biosynthesis (75).



ECONOMIC ASPECTS

The total market for vitamin K_1 is relatively small and the principal producer of vitamin K_1 is Hoffmann-La Roche. Nisshin Flour Milling Company is the predominant manufacturer for the optically active form of vitamin K_1. Total world market for vitamin K_3 is 1500 t with Vanetta Company as the dominant manufacturer. The list price for vitamin K USP grade is \$3.75–\$4.05/g. For the 1% spray dried formulated product, the price ranges from \$76–\$79/kg. The majority of vitamin K_1 is sold to the pharmaceutical industry, whereas vitamin K_3 is consumed primarily by the feed industry.

REQUIREMENTS

Owing to the ubiquitous natural occurrence of vitamin K and its production by intestinal bacteria, vitamin K deficiencies are rare. However, they can be caused by certain antibiotics (qv) coupled with a reduced dietary intake. Newborn infants who do not possess the necessary intestinal bacterial population are at danger for vitamin K deficiency. As a result, vitamin K injections are routinely given to the newborn.

Table 2 lists the Recommended Dietary Allowances (RDA) for vitamin K. Although manufacture by intestinal bacteria represents a significant source of plasma menaquinone concentrations, reliance on this source alone is not sufficient to maintain healthy concentrations of menaquinone. Consequently, dietary supplementation is necessary (76).

BIOCHEMISTRY

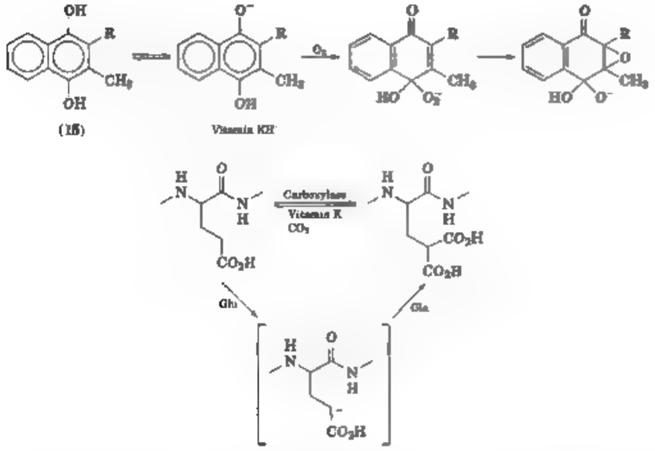
As a result of empirical findings that alfalfa and other plants can alleviate a hemorrhagic situation in chicks, work was initiated to identify and isolate the causative agent. Concurrent to these activities was the observation that prothrombin levels in chicks given certain leafy green vegetables were enhanced. Studies on the role of vitamin K in blood clotting and more specifically, in the conversion of fibrinogen to fibrin, have led to the conclusion that the important function of vitamin K in the clotting mechanism is to act as a cofactor in the biosynthesis of the active form of prothrombin (11).

Work in the mid-1970s demonstrated that the vitamin K-dependent step in prothrombin synthesis was the conversion of glutamyl residues to γ-carboxyglutamyl residues. Subsequent studies more clearly defined the role of vitamin K in this conversion and have led to the current theory that the vitamin K-dependent carboxylation reaction is essentially a two-step process which first involves generation of a carbanion at the γ-position of the glutamyl (Gla) residue. This event is coupled with the epoxidation of the reduced form of vitamin K and in a subsequent step, the carbanion is carboxylated (77–80). Studies have provided thermochemical confirmation for the mechanism of vitamin K and have shown the oxidation of vitamin KH_2

Table 2. RDA Requirements for Vitamin K

Category	Age	Vitamin K (μg)
Infants	0.0–0.5	5
	0.5–1.0	10
Children	1–3	15
	4–6	20
	7–10	30
Males	11–14	45
	15–18	65
	19–24	70
	25–50	80
	>51	80
Females	11–14	45
	15–18	55
	19–24	60
	25–50	60
	>51	60
U.S. RDA	None established	

(15) can produce a base of sufficient strength to deprotonate the γ-position of the glutamate (81–83).



Although the role of vitamin K in blood clotting has clearly been demonstrated and involves carboxylation of multiple proteins in addition to prothrombin and includes factor VII, factor IX, and factor X, proteins containing Gla residues have been found in other tissues. For example, in 1975, Hauschka isolated an EDTA-soluble protein fraction of chick bones and identified the presence of Gla (84). Additional work sequenced the protein which was called bone Gla protein or osteocalcin (85). The properties of the protein as well as its function in bone mineralization have been extensively studied (86,87). However, its specific function is not completely understood. In addition, vitamin K-dependent carboxylase activity has been observed in cell cultures. For example, a vitamin K-dependent protein has been identified in a screen for growth arrest specific gene products. This protein, Gas6, has been identified as a ligand for tyrosine kinase (88). This observation has suggested that vitamin K may have a more general metabolic role (89–94).

CONCLUSIONS

Despite the fact that commercial synthesis in the 1990s of vitamin K has largely remained unchanged from its early roots, there has been significant activity in the area of process improvements to the basic approach. Also, several biotechnological systems have been described and this may be a future research area. A thorough understanding of the role of vitamin K in biological systems will continue as an active research area.

BIBLIOGRAPHY

1. H. Dam, *Biochem. Z.* **215**, 475 (1929).
2. H. Dam, *Biochem. J.* **29**, 1273 (1935).
3. H. Dam, *Nature*, **135**, 652 (1935).
4. H. J. Almquist, *J Biol. Chem.* **114**, 241 (1936).
5. H. J. Almquist, *J. Biol. Chem.* **115**, 587 (1936).
6. H. Dam and F. Schonheyder, *Biochem. J.* **30**, 897 (1936).
7. S. A. Thayer, D. W. MacCorquodale, S. B. Binkley, and E. A. Doisy, *Science* **88**, 243 (1938).
8. D. W. MacCorquodale, L. C. Cheney, S. B. Binkley, W. F. Holcomb, R. W. McKee, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.* **131**, 357 (1939).
9. D. W. MacCorquodale, S. B. Binkley, R. W. McKee, S. A. Thayer, and E. A. Doisy, *Proc. Soc. Exptl. Biol. Med.* **40**, 482 (1939).
10. H. Mayer, U. Gloor, O. Isler, R. Ruegg, and O. Wiss, *Helv. Chim. Acta* **47**, 221 (1964).
11. J. W. Suttie, in L. J. Machlin, ed., *The Handbook of Vitamins*, Marcel Dekker, Inc., New York, 1991, pp 145–169.
12. *Merck Index*, 11th ed., Merck & Co., Rahway, N.J., 1989, pp. 1580–1581.
13. R. W. McKee, S. B. Binkley, D. W. MacCorquodale, S. A. Thayer, and E. A. Doisy, *J. Am. Chem. Soc.* **61**, 1295 (1939).
14. S. B. Binkley, D. W. MacCorquodale, L. C. Cheney, S. A. Thayer, R. W. McKee, and E A. Doisy, *J. Am. Chem. Soc.* **61**, 1612 (1939).
15. D. W. MacCorquodale, S. B. Binkley, S. A. Thayer, and E. A. Doisy, *J. Am. Chem. Soc.* **61**, 1928 (1939).
16. S. B. Binkley, L. C. Cheney, W. F. Holcomb, R. W. McKee, S. A. Thayer, D. W. MacCorquodale, and E. A. Doisy, *J. Am. Chem. Soc.* **61**, 2558 (1939).
17. H. Dam and E. Sondegarrd, in P. György and W. N. Pearson, eds., *The Vitamins*, 2nd ed., Vol. VI, Academic Press, Inc., New York, 1967, pp. 245–260.
18. J. T. Matshirer and W. V. Taggart, *J. Nutr* **94**, 52 (1968).
19. M. M. A. Hussan, J. S. Mossa, and A. H. U. K. Taragan, *Anal. Profiles Drug Subst.* **17**, 449–531 (1988).
20. W. E. Lambert and A. P. De Leenheer, in A. P. De Leenheer, W. F. Lambert, and H. J. Nelis, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1992, pp. 197–233.
21. *The United States Pharmacopeia XXII* (USP XXII-NF XVII), United States Pharmacopeial Convention, Inc., Rockville, Md., 1990, pp. 1081–1082.
22. A. Ruettimann, *Chimia* **40**, 290 (1986); S. Yamada, T. Takeshita, and J. Tanaka, *Yuki Gosei Kagaku Kyokai Shi* **40**, 268 (1982).
23. H. J. Almquist and A. A. Klose, *J. Am. Chem. Soc.* **61**, 2557 (1939); H. J. Almquist and A. A. Klose, *J. Biol. Chem.* **132**, 469 (1940).
24. S. B. Binkley, L. C. Cheney, W. F Holocomb, M. W. McKee, S. A. Thayer, D. W. MacCorquodale, and E. A. Doisy, *J. Am. Chem. Soc.* **61**, 2553 (1939).
25. L. F. Fieser, *J. Am. Chem. Soc.* **61**, 3467 (1939).
26. P. Karrer, H. Simon, and E Zbinden, *Helv. Chem. Acta* **27**, 317 (1944).
27. O. Isler and K. Doebel, *Helv. Chim. Acta* **37**, 225 (1954).
28. R. Hirschmann, R. Miller, and N. L. Wendler, *J. Am. Chem. Soc.* **76**, 4592 (1954).
29 D. Bhatia, in *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, pp. 2727–2732.
30. Jpn. Pat. 76-154299 (1979), Y. Tachibana (to Nisshin Flour Milling Co.).
31. Jpn. Pat. 661212 (1971), S. Kitamura, S. Matsuyama, and M. Morioka (to Teikoku Chemical Industry).
32. Jpn. Pat. 670414 (1970), S. Kitamura, Y. Fujita, and M. Morioka (to Teikoku Chemical Industry).
33. Jpn. Pat. 95-218011 (1989), A. Shinno, I. Teraro, T. Matsuda, and I. Tadashi (to Mitsubishi Gas Chemical Co., Inc.).

34. Jpn. Pat. 77-69415 (1979), S. Himoto, K. Miyahara, and Y. Yoshino (to Nisshin Flour Milling Co., Ltd.).

35. Jpn. Pat. 680309 (1971), S. Kitamura, Y. Fujita, and M. Morioka (to Teikoku Chemical Industry, Inc.).

36. Eur. Pat. 84-108332 (1985), A. Ruettimann and G. Buechi (to Hoffmann-La Roche Inc.).

37. Eur. Pat. 94-102979 (1994), K. Hamamura, T. Iwana, C. Seki, and M. Konishi (to Eisai Chemical Co., Ltd.).

38. S. Araki, T. Sato, H. Miyagawa, and Y. Bukugan, *Bull. Chem. Soc. Jpn.* **57**, 3523 (1984); Y. Naruta, *J. Org Chem.* **45**, 4097 (1980); B. L. Chenard, M. J. Manning, P. W. Reynolds, and J. W. Swenton, *J. Org. Chem.* **45**, 378 (1980); K. H. Doetz and I. Pruskil, *J. Organomet. Chem.* **209**, C4 (1981); Ger. Pat. 2909091 (1980), K. H. Doetz (to Hoffmann-La Roche); Y. Tachibana, *Chem. Lett.*, 901 (1977); P. W. Raynolds, M. J. Manning, and J. S. Swenton, *J. Chem. Soc. Chem. Comm.*, 499 (1977); C. D. Synder and H. Rapport, *J. Am. Chem. Soc.* **96**, 8046 (1974).

39. H. Tso and Y. J. Chen, *J. Chem. Res.* (*S*), **3**, 104 (1995).

40. R. Schmid, S. Antoulus, A. Rüttimann, M. Schmid, M. Vecchi, and H. Weiser, *Helv. Chim. Acta* **73**, 1276 (1990).

41. Jpn. Pat. 87-287021 (1990), T. Fujita and S. Takato (to Snow Brand Milk Products Co., Ltd.); Jpn. Pat. 87-287022 (1990), T. Fujita, H. Hatamoto, and S. Takato (to Snow Brand Milk Products Co., Ltd.).

42. Jpn. Pat. 85-90577 (1987), K. Kuihara and Y. Takagi (to Nisshin Oil Mills, Ltd.).

43. E. Leistner, *Recent Adv. Phytochem.* **20**, 243 (1986).

44. Jpn. Pat. 67-67366 (1973), S. Kitamura, M. Morioka, H. Tono, and Y. Fujita (to Teikoku Chemical Industry Co., Ltd.).

45. Jpn. Pat. 86-308171 (1989), T. Ichino, K. Nakano, T. Udagawa, Y. Kusaba, T. Muramatsu, and A. Katayama (to Eisai Chemical Co., Ltd.).

46. B. Liu, L. Gu, and J. Zhang, *Recl. Trav. Chim. Pays-Bas* **110**, 99 (1991).

47. M. Inoue, T. Uragaki, and S. Enomoto, *Chem. Lett.* **12**, 2075 (1986).

48. Y. Naruta, *J. Am. Chem. Soc.* **102**, 3774 (1980).

49. K. Sato, S. Inoue, and K. Saito, *J. Chem. Soc., Perkin Trans. I* **20**, 2289 (1973).

50. D. H. L. Bishop and H. H. King, *Biochem. J.* **85**, 550 (1962); D. H. L. Bishop, K. P. Pandya, and H. K. King, *Biochem. J.* **83**, 606 (1962); D. R. Threfall, *Vitam. Horm. (NY)*, **29**, 155 (1971).

51. H. Taber, "Vitamin K Metab.—Vitamin K-Dependent Proteins," in J. W. Suttie, ed., *Proc. Steenbock Symp. 8th*, University Park Press, Baltimore, Md., 1980, for a review of function of Vitamin K_2.

52. H. Taguchi and Y. Tani, *Microb. Util. Renewable Res.* **8**, 350 (1993).

53. Y. Tani, S. Asahi, and H. Yamada, *J. Nutr. Sci. Vitaminol.* **32**, 137 (1986).

54. Y. Tani, S. Asahi, and H. Yamada, *J. Ferment. Technol.* **62**, 321 (1984).

55 Jpn. Pat. 87-29722 (1988), Y Tani (to Mitsubishi Gas Chemical Co., Inc.); Jpn. Pat. 86-237128 (1988), S. Uragami, Y. Tani, and Y. Tokunaga (to Mitsubishi Gas Chemical Co., Inc.); Jpn. Pat. 86-41840 (1988), Y. Tani (to Mitsubishi Gas Chemical Co., Inc.); Jpn. Pat. 85-55458 (1987), Y. Tani (to Mitsubishi Gas Chemical Co., Inc).

56. Jpn. Pat. 92-72044 (1995), S. Nakoyooji (Daiwa Kasei KK).

57. U.S. Pat. 5,329,026 (July 12, 1994), N. Sugishiwa, N. Ikeda, and Y. Fujii (to Nippon Shokubui Kajaka Kogyo Co., Ltd.).

58. Eur. Pat. 490-122643 (1991), N. Sugishiwa, N. Ikeda, Y. Fujii, R. Aoki, and Y. Hatta (to Nippon Shokuba).

59. Jpn. Pat. 85-252445 (1986), T. Komatsui and T. Sumino (to Kawasaki Kasei Kogyo).

60. Eur. Pat. 665209 A1 950802 (1995), W A. Hermann, J. D. Galamba Correia, R. W. Fischer, W. Adam, J. Lin, R. C. Saha-Moeller, and M. Shimizu (to Hoechst AG).

61. World Pat. 91-EP1958, H. M. Becher (1992) (to Shell International Research).

62. World Pat. 91-US3623 (1991), J. E. Toomey (Reilly Industries, Inc.).

63. S. Ito, Y. Karob, M. Iwata, K. Susuki, and A. Okika, *J. Appl. Electrochem.* **23**, 671 (1993).

64. Jpn. Pat. 92-178171 (1994), S. Tanyoshi (Kawasaki Steel Co.).

65. K. I. Matveev, E. G. Zhizhina, and V. F. Odyakov, *React. Kinet. Catal. Lett.* **55**, 47 (1995); R. Neumann and M. de la Vega, *J. Mol. Catal.* **84**, 93 (1993); K. I. Matveev, E. G. Zhizhina, V. F. Odyakov, and V. N. Parmon, *Izv Akad. Nauk. Ser. Khim.* **7**, 1208 (1994).

66. Jpn. Pat. 92-352595 (1994), K. Nakao and H. Tamui (Kurary Co.); Jpn. Pat. 87-247363 (1989), N. Hirose and S. Kijima (Eisai Co., Ltd.).

67. U. M. Azizov and L. I. Leonteva, *Zhim. Farm. Zh.* **23**, 1488 (1989).

68. I. Nir, I. Kafri, and R. Cohen, *Poultry Sci.* **57**, 206 (1978).

69. P. W. Waldroup, W. D Bussel, A. B. Burke, and Z. B. Johnson, *Arkansas Farm Res.* **26**, 11 (1977).

70. P. W. Waldroup, W. D. Bussell, A. B. Burke, Z. B. Johnson, *Animal Feed Sci. Technol.* **3**, 329 (1978).

71. R. Bentley, *Pure Appl. Chem.* **41**, 47 (1975).

72. R. Bentley and R. Megananthan, *Microbial. Rev.* **46**, 241 (1978).

73. I. M. Campbell, *Tetrahedron Lett.* **10**, 4777 (1969).

74. J. Soll and G. Schultz, *Biochem. Biophys. Res. Comm.* **99**, 907 (1981).

75. O. Samuel and R. Azerad, *FEBS Lett.* **2**, 336 (1969).

76. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, D.C., 1989.

77. J. Stenfo, *J. Biol. Chem.* **249**, 5527 (1974).

78. J. Stenflo, P. Fernlund, W. Egan, and P. Roepstorff, *Proc. Natl. Acad. Sci. USA* **71**, 2730 (1974).

79. G. L. Nelsestuen, T. H. Zytkovicz, and J. B. Howard, *J. Biol. Chem.* **249**, 6347 (1974).

80. S. Magnusson, L. Sottrup-Jensen, T. E. Petersen, H. R. Morris, and A. Dell, *FEBS Lett.* **44**, 189 (1974).

81. P. Dowd, S. W. Ham, and S. J. Geib, *J. Am. Chem. Soc.* **113**, 7734 (1991).

82. R. A. Flowers, II., S. Naganmathan, P. Dowd, E. M. Arnett, and S. W. Ham, *J. Am. Chem. Soc.* **115**, 9409 (1993).

83. E. A. Arnett, P. Dowd, R. A. Flowers, II., S. W. Ham, S. Naganmathan, *J. Am. Chem. Soc.* **114**, 9209 (1992).

84. P. V. Hauscka, J. B. Lian, and P. M. Gallop, *Proc. Natl. Acad. Sci. USA* **72**, 3925 (1975).

85. P. A. Price, A. S. Ofsuka, J. W. Poser, J. Kiristaposis, and N. Raman, *Proc. Natl. Acad. Sci. USA* **73**, 1447 (1976).

86. P. A. Price, J. W. Poser, and N. Raman, *Proc. Natl. Acad. Sci. USA* **73**, 3374 (1976).

87. J. B. Lian, P. V. Hauschksa, and P. M. Gallop, *Fed. Proc.* **37**, 2615 (1978).
88. T. N. Stitt et al., *Cell* **80**, 661 (1995).
89. J. B. Lianm, R. J. Levy, J. T. Levy, and P. A. Friedmann, in F. L. Siegel, E. Carafoli, R. H. Kretsinger, D. H. MacLennan, and R. H. Wassermann, eds., *Calcium-Binding Proteins: Structure and Function*, Elsevier North-Holland, New York, 1980, p. 449.
90. R. J. Lecvy, C. Gundberg, and R. Scheinmann, *Artherosclerosis* **46**, 49 (1983).
91. J. A. Helpern, S. J. McGee, and J. M. Riddle, *Henry Ford Hosp. Med. J.* **30**, 152 (1982).
92. J. W. Suttie, in A. T. Diplock, ed., *The Fat Soluble Vitamins*, William Heienemann, Ltd., London, 1985, p. 225.
93. C. Vermeer, *Haemostatsis* **16**, 239 (1986).
94. M. C. Roncaglioni, A. P. B Dalessandro, B. Casali, C. Vermeer, and M. B. Donati, *Haemostatis* **16**, 295 (1986)

GENERAL REFERENCES

O. Isler and G. Brubacher, *Vitamine I*, Georg Thieme Verlag, New York, 1982, pp. 152–176.

W. E. Lambert and A. P. De Leenheer, in A. P. De Leenheer, W. F. Lambert, and H. J. Nelis, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1992, pp. 197–233.

S. Patai, ed., *The Chemistry of Quinonoid Compounds*, Parts 1 and 2, John Wiley & Sons, Inc., New York, 1974.

S. Patai and Z. Rappoport, eds., *The Chemistry of Quinonoid Compounds*, Vol. 2, Parts 1 and 2, John Wiley and Sons, Ltd., Chichester, U K., 1988.

J. W. Suttie, in L. J. Machlin, ed., *The Handbook of Vitamins*, Marcel Dekker, Inc., New York, 1991.

Susan D. Van Arnum
Westfield, New Jersey

WASTE MANAGEMENT: ANIMAL PROCESSING

This section examines waste management from the standpoint of three animal-based food categories: dairy processing, egg processing, and meat and poultry processing. Waste management for an animal-based processing facility is essential because waste streams impact a plant's ability to operate within the state or local community. Permits to discharge wastewater under the National Pollution Discharge Elimination System (NPDES) (1) are restrictive for parameters such as biological oxygen demand (BOD), suspended solids, fats, oils, and greases (FOG), and ammonia. If compliance within permit limitations is not achieved, fines and civil penalties are triggered. Similar penalties can occur when biosolids are taken to landfills that are regulated under the Resource Conservation and Recovery Act (RCRA) (2). Restrictions on material composition determine the method of disposal, site, and quantity of biosolids that can be applied to land or buried in public landfills.

DAIRY-PROCESSING-RELATED WASTE MANAGEMENT

Figures 1 to 4 present utility input and waste output process schemes for fluid milk, cottage cheese, other cultured dairy products, and ice cream and frozen desserts (3–5).

Overview of Practices Contributing to Waste Generation

Table 1 presents a comparison of waste stream characteristics and coefficients for a 1000-kg milk throughput. Depending on the product being processed, the waste stream characteristics vary widely. Viscosity, product solute content, and time and temperature factors influence the waste stream's organic strength (3,4). Cleaning and sanitizing processes as well as product and water losses influence the waste stream's volume. The values presented reflect the milk handling and process practices that influence the final waste stream's organic composition and water volume.

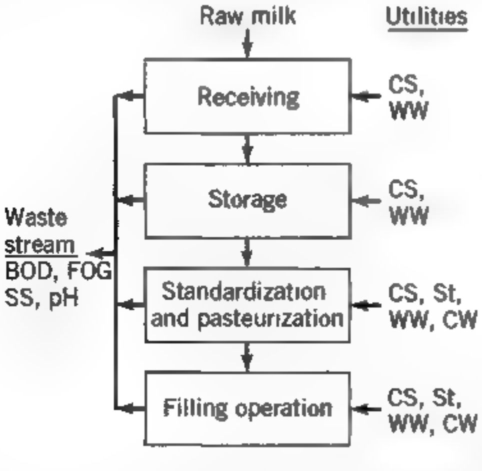

Raw milk
Utilities
Receiving
CS, WW
Storage
CS, WW
Waste stream
BOD, FOG
SS, pH
Standardization and pasteurization
CS, St, WW, CW
Filling operation
CS, St, WW, CW

Figure 1. Fluid milk processing scheme, utility inputs and waste outputs. CS, cleaning and sanitizing; St, steam; WW, wash water; CW, cooling water; SS, suspended solids; BOD, biological oxygen demand; FOG, fats, oils, and greases.

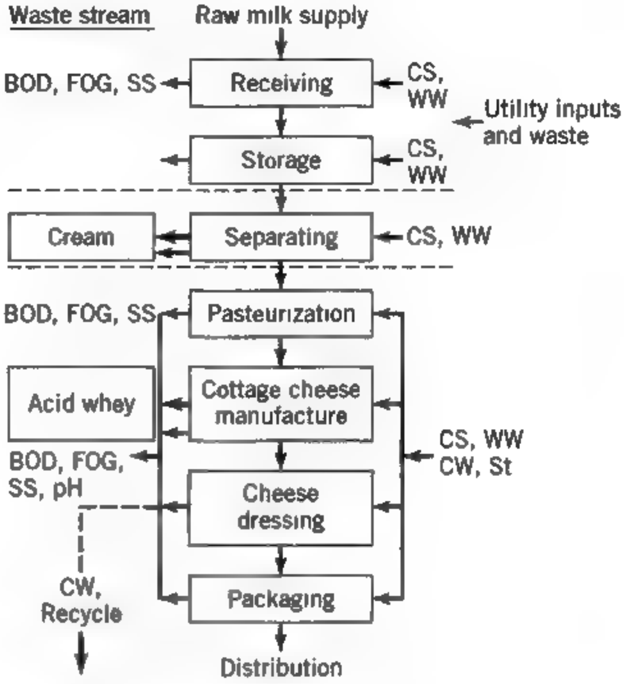

Waste stream
Raw milk supply
BOD, FOG, SS
Receiving
CS, WW
Utility inputs and waste
Storage
CS, WW
Cream
Separating
CS, WW
BOD, FOG, SS
Pasteurization
Acid whey
Cottage cheese manufacture
CS, WW
CW, St
BOD, FOG, SS, pH
Cheese dressing
CW, Recycle
Packaging
Distribution

Figure 2. Cottage cheese process scheme, utility inputs and outputs. Abbreviations defined in Figure 1.

In addition to the process practices adopted by the manufacturing operation, there are operational factors that contribute to waste generation (4,6,7). These include absence of salvage or recovery protocols when storage tanks, pipelines, and equipment are cleaned; loss of sealing integrity of pipelines, homogenizers, and valves as a result of poor maintenance; malfunction of equipment; leakage of product from damaged containers; failure to properly handle returned products; failure to isolate whey and curd wash water; inefficient use of water; and absence of recovery protocols for milk solids derived from rinses or products lost from pasteurization start-up and product changeover. Consistent day-to-day management of these areas is essential for minimizing pollutant releases and excessive hydraulic loads to the waste stream and the environment. The implementation of a sound management program can achieve up to a 50% reduction in the original waste load (8). The values given in Table 1 are approximated from both literature and field experience data and should be used only for estimating volume and waste parameters (3,5,6). These values should not be used for the design of a waste pretreatment or treatment system. Actual field measurements by qualified environmental design engineers should be used for such applications.

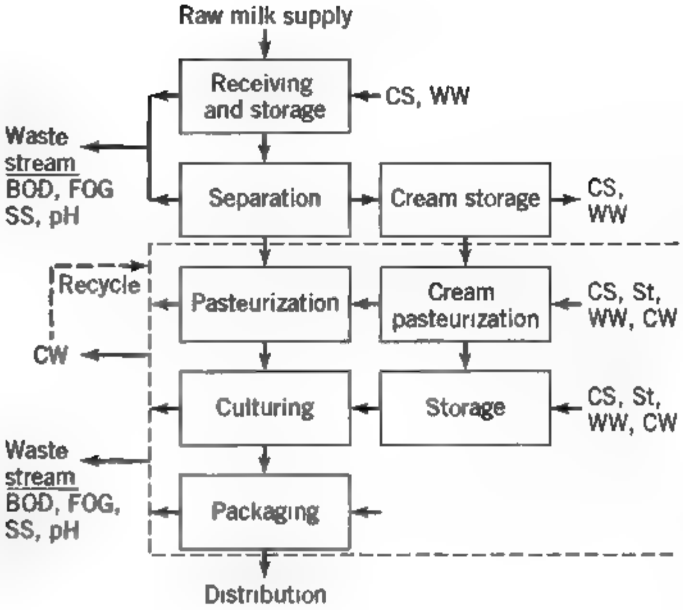

Raw milk supply
Receiving and storage
CS, WW
Waste stream
BOD, FOG
SS, pH
Separation
Cream storage
CS, WW
Recycle
Pasteurization
Cream pasteurization
CS, St, WW, CW
CW
Culturing
Storage
CS, St, WW, CW
Waste stream
BOD, FOG, SS, pH
Packaging
Distribution

Figure 3. Dairy cultured product process scheme, utility inputs and waste outputs. Abbreviations defined in Figure 1.

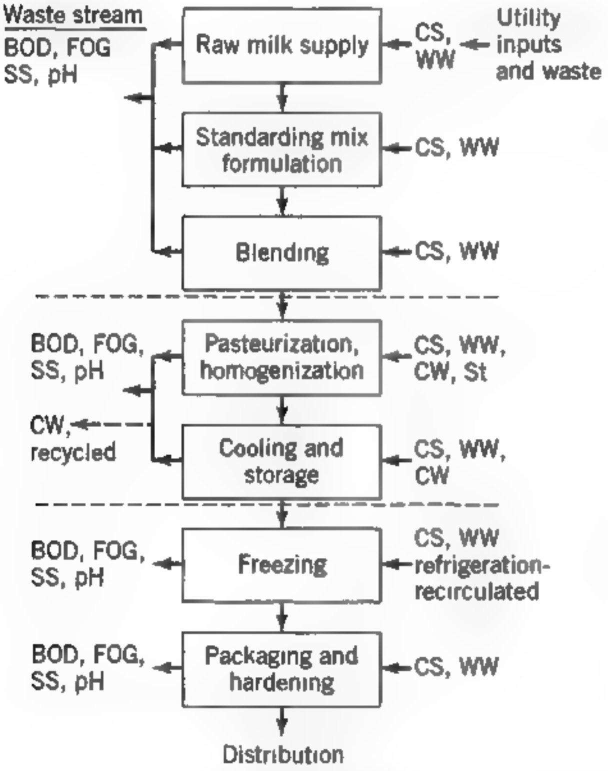

Waste stream
BOD, FOG
SS, pH
Raw milk supply
CS, WW
Utility inputs and waste
Standarding mix formulation
CS, WW
Blending
CS, WW
BOD, FOG, SS, pH
Pasteurization, homogenization
CS, WW, CW, St
CW, recycled
Cooling and storage
CS, WW, CW
BOD, FOG, SS, pH
Freezing
CS, WW refrigeration-recirculated
BOD, FOG, SS, pH
Packaging and hardening
CS, WW
Distribution

Figure 4. Ice cream and frozen dessert process scheme, utility inputs and waste outputs. Abbreviations defined in Figure 1.

Point Sources for Waste Generation

As fluid milk is handled and processed, the major point source for organic loading and water usage is pasteurization. Pasteurization includes three steps: heat exchange, separation, and homogenization. Approximately 30% of the total biological oxygen demand (BOD) and 50% of the waste stream volume can be attributed to this operation. Milk receiving and storage account for approximately another 30% of the BOD and waste stream volume (3,5–7). Figure 1 identifies the key point sources for the fluid milk process.

The process scheme for cottage cheese is presented in Figure 2. The most important management consideration is the handling and disposal of acid whey (9,10). For purposes of this overview, it is assumed that the initial whey discharge is collected and not discharged to the dairy waste stream. The major point source for BOD is the curd washing step, which usually requires three wash cycles in which the rinses are discharged to the drain (3,5–7). Not surprising, this step accounts for 53% and 86% of the total BOD and water volume, respectively, released to the waste stream. Pasteurization contributes 25% of the total BOD and 4% of the water volume to the waste stream.

Figure 3 presents the flow scheme for other cultured dairy products. During the manufacture of cultured dairy products such as buttermilk, yogurt, and sour cream, the respective product's viscosity becomes a contributing factor to the quantity of BOD discharged to the waste stream. In most cases, milk receiving, storage, separation, blending, and pasteurization yield about the same BOD and wastewater volume values for a defined quantity of milk throughput (3,5–7). Collectively, these operations contribute 59% of the total BOD and 65% of the wastewater to the waste stream. However, 36% of the BOD and 43% of the wastewater come specifically from the pasteurization process. In contrast, viscosity and organic composition of the respective cultured products become evident as the *culturing phase* of manufacture is compared. Sour cream has the higher contribution, representing 18% of the total BOD generated, and buttermilk and yogurt contribute 14% and 16%, respectively.

Figure 4 presents the process flow scheme for ice cream and frozen desserts. It is important to remove product residue from pipelines and freezing equipment before clean-

Table 1. Waste Load Coefficients Based on 1000-kg Milk Throughput

Waste stream characteristics	Fluid milk processing	Cultured products processing	Cottage cheese	Ice cream
Water Usage (kL)	0.10–5.40	Approx. 1.16	0.80–12.40	0.80–5.60
Average (1000 L)	3.25	—	6.00	2.80
BOD (kg)	—	Buttermilk, 2.15	1.30–71.20, whey included	1.90–20.40
	0.20–7.80	Yogurt, 2.20	23.0, whey excluded	
		Sour cream, 2.25	23.0, whey excluded	
Average kg SS	4.20	—		5.76
approx. kg as % of BOD value	50%	35%	35%	25%
pH	7.6–11.5	4.1	4.4	5.8–6.8
Viscosity (cp)	2.2	Buttermilk, 500 Yogurt, 9000 Sour cream, 10,000	Whey, 1.3	Mix, 35 Frozen, 121

Source: Refs. 3, 5, and 6

up to minimize the discharge of this material to the waste stream (4). The combination of high viscosity and low temperature has a major influence on the amount of product residual in the equipment (6). The two major point source contributors to the waste stream are pasteurization and freezing (3–5,7). Pasteurization accounts for 27.5% and 43.5% of the total BOD and wastewater volume, respectively. The freezing step contributes 52% of the total BOD and 30% of the waste stream hydraulic load.

Waste Recovery, Disposal, and Treatment

The ideal situation is to reuse any milk, cream, or product recovered from the process equipment (4). This requires strict sanitary practices to avoid environmental contamination. In all cases, the recovered material must pass through the pasteurizer before it enters the final product package. Recovery practices must be approved by the state agency responsible for enforcement of dairy-related regulations.

In the cheese manufacturing sector, sweet whey is recovered and used to make whey powder, whey proteins, lactose, and delactosed whey powder (9,10). Membrane technologies are applied extensively for whey protein recovery and whey demineralization. Whey is also used extensively as a milk replacer for veal calf operations.

The more common practice throughout the dairy industry is to salvage, pool, and isolate recovered whey and dairy products for use as animal feed (9,11). Costs for handling, storing, and transporting these products to a pet food factory or to a farm are shared by the milk processor and the factory or farmer.

Land application is usually the last option for disposal of salvaged whey and dairy products (3,4,10,12). Application rates per acre and fertilizer value such as nitrogen and phosphates are important considerations when this method is used. Whey, dairy products with milkfat, and curd wash water that is high in sodium chloride present specific challenges because of the influence on the soil's pH, percolation, and flocculation properties (12). When land application is used, the salvaged materials must be "soil injected" to prevent pollutant runoff and odor releases into the atmosphere.

Figure 5 presents the possible treatment steps for dairy wastewater (6,11–13). The primary treatment may exist either as a pretreatment step or as the first phase of a waste treatment system. Pretreatment addresses a number of potential biological problems, such as sudden releases of extreme hydraulic volumes, excessive organic loads, or dramatic shifts in alkalinity or acidity. In cases where high concentrations of fats and proteins are present, physical removal is necessary in order to reduce the BOD strength of the waste stream. Pretreatment is a necessary management intervention to adjust the waste stream's characteristics to domestic strength so it is amenable to the subsequent treatment process. Generally, sewer use ordinances require industrial discharges to be at domestic wastewater strengths, or the industry is levied a costly surcharge.

The waste treatment process system is designed to achieve performance standards that meet limitations defined by the discharge permit. The waste treatment system may incorporate any of the unit operations identified in Figure 5. Generally, the process system selected is based mainly on organic assimilation by an active biological mass. Chemically oriented processes also can be incorporated into the waste treatment scheme. Waste treatment systems usually have four functional units of operation: (*1*) settling whereby fats and heavy particles are removed from the waste stream; (*2*) biological assimilation; (*3*) clarification whereby biologically active solids are recovered and recycled, and the treated water is discharged to a final treatment phase; and (*4*) polishing, which could involve a filtration process and disinfecting (13). Discharge standards to a receiving stream have a limit of 10 mg/L BOD, 10 mg/L suspended solids, 5 mg/L nitrogen as NH_3, and a pH value within a 6–9 range.

EGG-PROCESSING-RELATED WASTE MANAGEMENT

Figure 6 depicts an overview of an egg production operation (14). This section discusses the management of the processing plant wastes for shell eggs and liquid eggs.

Overview of Practices Contributing to Waste Generation

Major factors that influence the level of egg loss to the processing waste stream are strength of eggshell, egg-handling practices, and operation of washer equipment

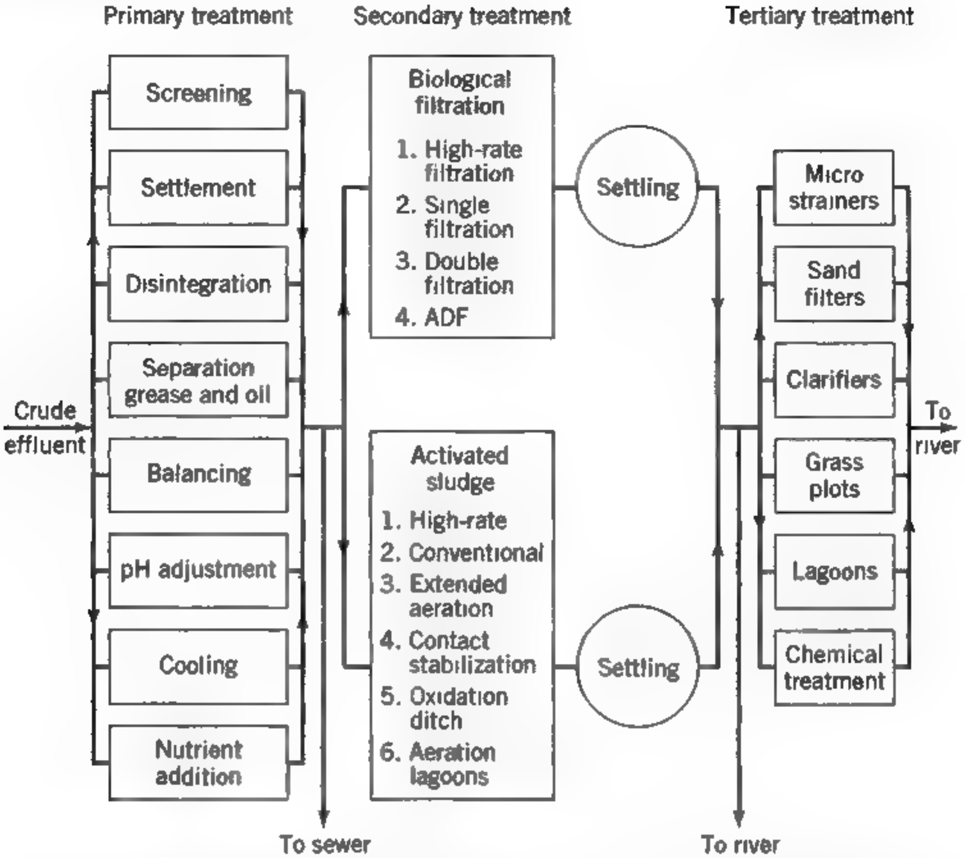

Primary treatment
Secondary treatment
Tertiary treatment
Crude effluent
Screening
Settlement
Disintegration
Separation grease and oil
Balancing
pH adjustment
Cooling
Nutrient addition
Biological filtration
1. High-rate filtration
2. Single filtration
3. Double filtration
4. ADF
Activated sludge
1. High-rate
2. Conventional
3. Extended aeration
4. Contact stabilization
5. Oxidation ditch
6. Aeration lagoons
Settling
Settling
Micro strainers
Sand filters
Clarifiers
Grass plots
Lagoons
Chemical treatment
To river
To sewer
To river

Figure 5. Possible treatment steps for dairy-food plant wastewaters. ADF, air dissolved flotation. *Source:* Ref. 12.

(14–16). Regarding the shell issue, two notable factors are the age of the laying hen and the extreme temperatures under which eggs are produced. The major egg-handling practices that generate waste are abusive handling during transportation from farm to processing facility and rough handling of the filler flats while the flats are being loaded to the egg washer conveyor. Equipment operational errors contribute to waste generation if wash water temperatures exceed 10°C above egg temperatures; wash water is discharged directly to the waste stream instead of being recycled; eggs are dropped when there is poor contact of suction cups with the eggshell surface during loading; or scrubber brushes exert too much pressure on the shell surface during the cleaning process.

Point Sources for Waste Generation

During shell egg washing, candling, and sizing, the washing and clean-up operations are the major point sources for waste generation. Incidental waste is generated from broken eggs that drop to the floor during handling and inspection. About 5% of the total shell egg production is lost either when it becomes inedible or when it enters the waste stream. Approximately 76% of the U.S. shell egg production goes directly to the retail marketplace (17). The remaining percentage goes to egg-breaking operations for processing as liquid egg-derived products. An estimated 7% of liquid products either becomes inedible or is lost to the waste stream (18). In both shell egg and egg-breaking plants, the quantity of waste produced is *directly* proportional to the volume handled and the handling procedures practiced. Industrial plant waste surveys demonstrate that a well-managed liquid egg processing facility generates about 0.018 kg of BOD per kg of liquid egg throughput (15,19,20). Regarding water usage, each dozen of shell eggs washed generates approximately 2.5 L of wastewater (15,19,20).

For egg-breaking operations, the following management interventions can help to reduce product loss and waste (15,19,20): implement an efficient collection protocol for discarded eggs and shells with attached albumen; recover egg solids from storage units before rinsing and before the initial flush from blend tanks and the pasteurizer; eliminate storage vat spillovers; reduce the length of product lines; reduce water usage during plant clean-up; and dispose of on land the overflow and sump discharges from the egg-washing operation.

Waste Recovery, Disposal, and Treatment

Waste recovery usually involves the collection of cracked, broken, or leaking eggs during candling and the collection of broken shell residuals from liquid egg-derived products (21). The use of an apron underneath conveyors and egg-breaking equipment can be another means of salvaging egg solids. Egg materials collected can be centrifuged to

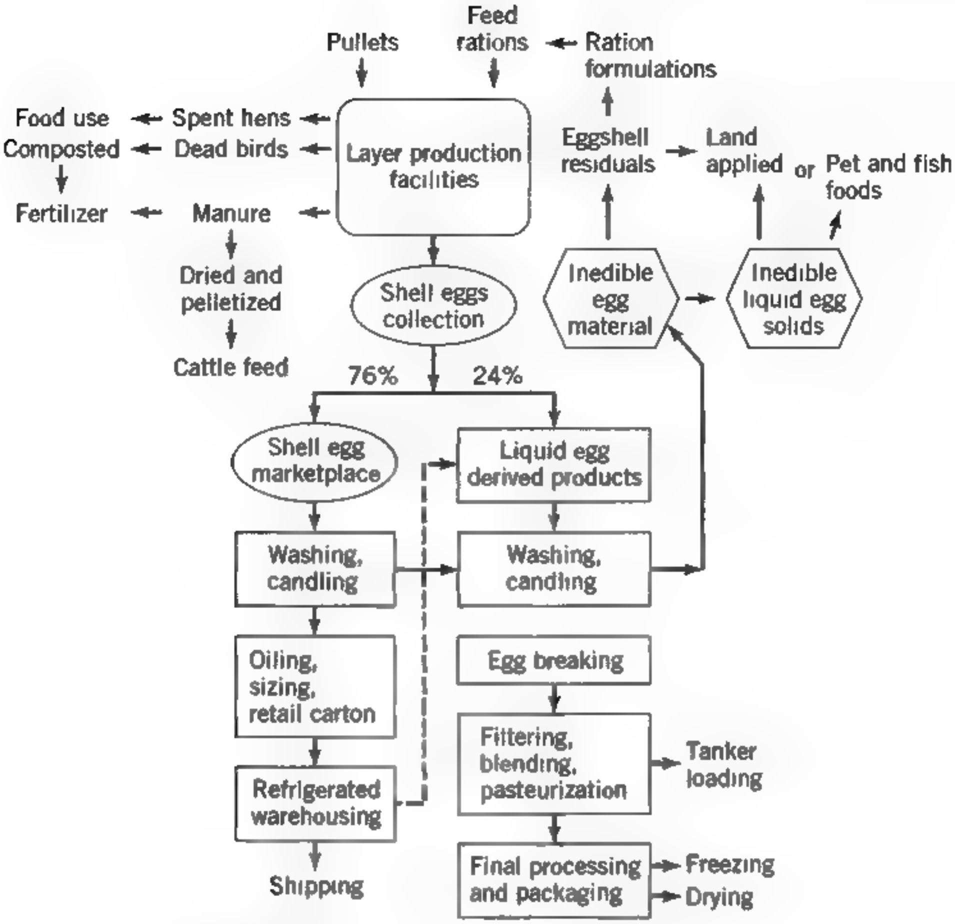

Pullets
Feed rations
Ration formulations
Food use
Spent hens
Composted
Dead birds
Fertilizer
Manure
Layer production facilities
Eggshell residuals
Land applied
or
Pet and fish foods
Dried and pelletized
Cattle feed
Shell eggs collection
Inedible egg material
Inedible liquid egg solids
76%
24%
Shell egg marketplace
Liquid egg derived products
Washing, candling
Washing, candling
Oiling, sizing, retail carton
Egg breaking
Filtering, blending, pasteurization
Tanker loading
Refrigerated warehousing
Shipping
Final processing and packaging
Freezing
Drying

Figure 6. Material flow for an egg production breaking operation.

separate and collect the egg solids away from the shell material. Egg solids can be used in pet food formulations or as fish food. The shell portion of the egg can be used as a calcium-rich source for animal rations (22). Egg solids or shells require sterilization when used in feed applications. The shell material also has been used for industrial applications (23,24).

For egg-breaking facilities located in rural settings, most waste streams are applied to the land as fertilizer (15,25,26). On the other hand, many facilities in rural and urban locations discharge to a publicly owned treatment works (POTW) and must comply with limitations set forth by permit. A common permit issue is that egg-breaking facilities can be major contributors (over 25%) to the POTW's BOD and hydraulic inputs. In small waste treatment operations where a trickling filter system is used, it is not uncommon to encounter poor BOD removal performance and odor problems. This is because of the proteinaceous nature of the egg solids, which apply a high BOD loading rate to the system. Additionally, egg solids contain high quantities of sulfur-containing amino acids that ultimately develop into nuisance odors created by biomass activity and oxygen-deficient conditions.

To address permit limitations and to lower sewer surcharges, a pretreatment system could be installed at the egg-handling facility (12,13,27). This system would be designed to first average out the hydraulic flow to a constant flow rate. The second design feature would be to settle and float out suspended particles that are collected and disposed of to a POTW biosolids digester or on the land. A third design element would be to incorporate a dissolved air floatation (DAF) unit into the system to remove the colloidal materials that again are disposed of to a biosolids digester system or land. The fourth option would be to operate an anaerobic process to reduce the suspended solids and to have a subsequent discharge to the POTW. Depending on the overall influence of the egg solids and quantity of cleaning agents used on the pH of the waste stream, it may be necessary to provide an acid addition to maintain the pH value between 6 and 9.

Egg-handling and processing plants in rural locations generally are required to treat wastewater before disposal (12,13,19,20,28,29). Most plants adopt an aeration lagoon-polishing pond system and discharge to a receiving stream. This system is economical to maintain and does an adequate job of removing egg wastes from the aquatic environment. However, it has its share of operational problems, such as inadequate mixing and oxygen transfer, nuisance odors caused by biosolids accumulation that lead to an anaerobic condition, and increased biological activity due to atmospheric temperature changes, specifically during the summer months. Incorporating a pretreatment–treatment system is the best strategy. In the pretreatment phase, emphasis should be placed on suspended solids removal using either a DAF unit or an anaerobic process. For the aeration lagoon-polishing pond treatment, a supplemental filtering unit should be employed and a disinfectant added to meet the low discharge standards. Nitrogen content in the discharge stream also can become a regulatory issue if adequate denitrification is not achieved in the polishing pond.

MEAT- AND POULTRY-PROCESSING-RELATED WASTE MANAGEMENT

Figures 7 and 8 depict overviews of the slaughtering steps for beef and poultry, respectively (11). Meat is processed into human foods (edible meat) and pet foods and animal feed (inedible meat) (30). However, when the United Kingdom and Europe experienced an outbreak of bovine spongiform encephalopathy (BSE) in the late 1990s, strict regulations were adopted to disrupt possible BSE transmittal pathways. For example, materials containing nerve tissues are prohibited for food use. Bovine meat cannot be used in beef livestock feed, and inedible meat from sheep has been excluded as a source for animal feed.

Overview of Practices Contributing to Waste Generation

Factors that influence the ultimate composition of processing wastes are sizes and species of animals; quantity of daily throughput; water usage; sanitation and housekeeping; and method of product conveyance. Typical waste stream characteristics for beef and broiler processing activities are given in Table 2.

Point Sources for Waste Generation

The largest amount of waste in meat and poultry processing is generated at the abattoir or slaughter phase (Figs. 7 and 8). Common forms of waste are manure, paunch, hair, feathers, grit, blood, and body fluids.

In the case of beef livestock, approximately 60% of the animal's live weight either is rendered or enters the waste stream (33). Paunch contributes to the waste stream up to 5.5 kg of BOD/1000 kg live body weight at kill (LWK). Blood accounts for 33 kg of BOD/1000 kg LWK in the absence of a blood collection protocol versus the more common loss of 6.6 kg, with blood collection. Washing the carcass contributes the highest water usage rate at 350 gal/min.

For poultry, the following BOD waste loads are generated, expressed as kilogram units per 1000 birds processed (34): bleeding, 2.35; scalder, 0.98; feather, 0.94; eviscer, 1.68; offal, 1.16; washing, 1.06; prechiller, 0.96; and chiller, 0.76. The feather operation utilizes the largest amount of water at 21.45 gal/min.

Beef wholesale cutting operations contribute the least to the waste stream. However, waste materials common to this operation are meat and fat trimmings and bone dust. These wastes add another 0.3 to 3.3 kg of BOD to the waste stream per 1000 kg LWK processed.

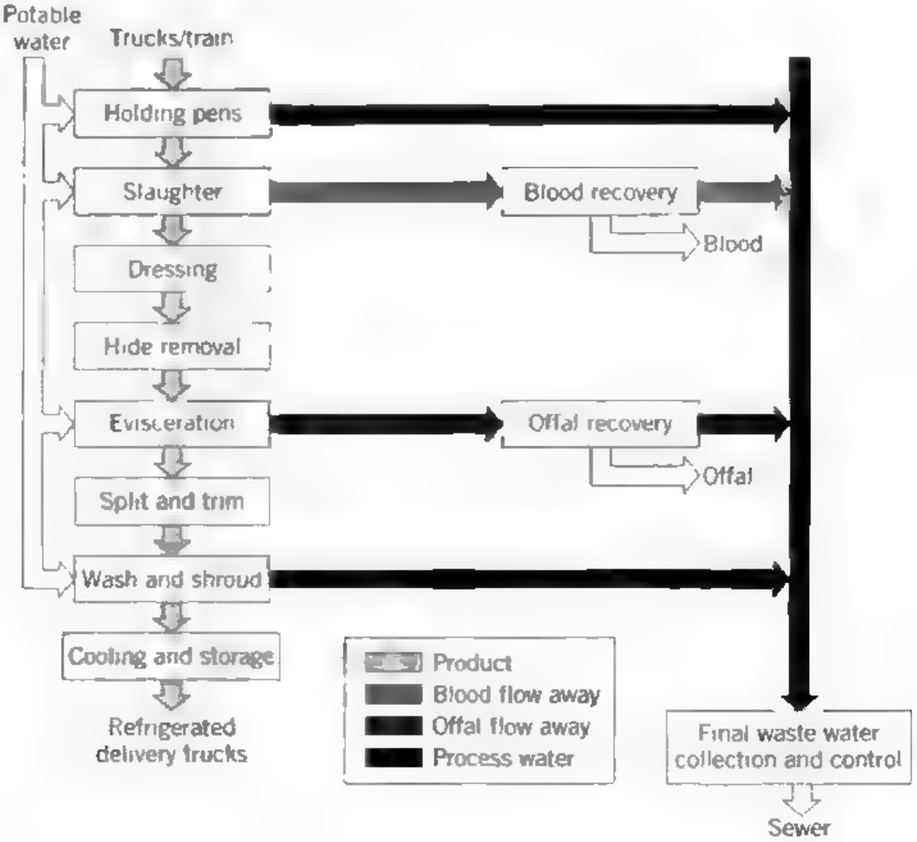


Figure 7. Process flow diagram for beef slaughtering operation.

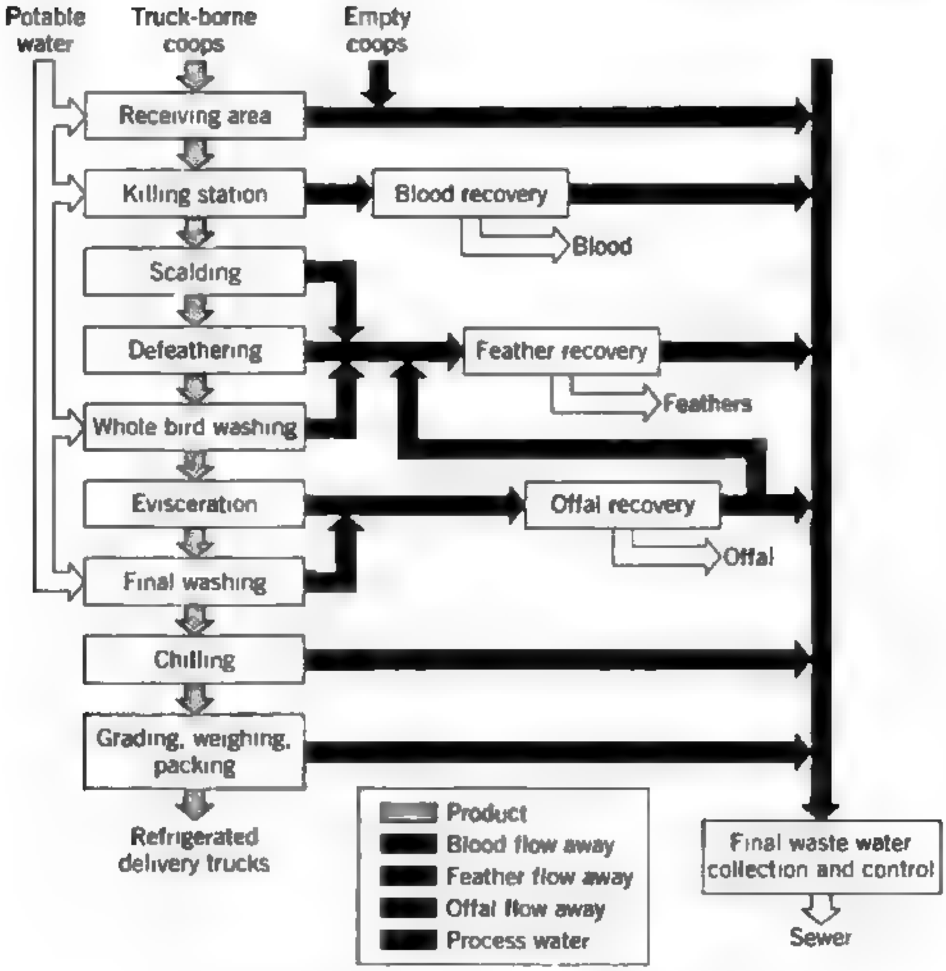

Potable water
Truck-borne coops
Empty coops
Receiving area
Killing station
Blood recovery
Blood
Scalding
Defeathering
Feather recovery
Feathers
Whole bird washing
Evisceration
Offal recovery
Offal
Final washing
Chilling
Grading, weighing, packing
Refrigerated delivery trucks
Product
Blood flow away
Feather flow away
Offal flow away
Process water
Final waste water collection and control
Sewer

Figure 8. Process flow diagram for poultry processing operation.

Table 2. Waste Characteristics for a Beef and Poultry Processing Operation

	Waste parameter contributed (lb/1000 animals)			
Animal type	BOD	TSS	FOG	Water (gal/animal)
Beef (31)	6710	6860	440	350
Poultry (32)	49	57	8	5.8

Waste Recovery, Disposal, and Treatment

Common by-products recovered from processing operations are blood, hide, bone, offal, and feathers. For example, blood is used commercially for microbial growth media, extraction of animal albumin, animal feed protein, and iron and nutrients for specific plants such as roses. Inedible meat materials are salvaged for use in pet and animal foods or sold for rendering purposes (30).

In addition to recovery of animal by-products, efforts are made to remove meat cutting and processing debris from the waste stream. For example, it is a common practice to remove debris by placing screens into the drain wells that collect wastewater from conveyance equipment, clean-up, and sanitation activities. This collected material usually is disposed of to a landfill operation.

Further removal of suspended solids takes place in the pretreatment phase of the wastewater disposal operation. However, this presents a major challenge to the meat and poultry processing industries (11,35). Inadequately designed facilities, frequently with an under-capacity throughput, are common problems for these industries. Screens, sedimentation, floatation, and physicochemical methods are used to reduce the colloidal content in the discharged waste stream (12,13). However, in spite of the removal of suspended solids, considerable soluble BOD remains in the waste stream and must be treated further to remove the organic pollutants (2,11,33,34,36,37).

For ammonia control, blood and paunch collection practices have the greatest influence on nitrogen content in the waste stream. To address ammonia control, processing plants have adopted practices such as ammonia stripping (38), land application (11–13,33,34,36,39,40), deep-ditch aeration, and breakpoint chlorination (41).

Because of the high protein and fat content in the meat and poultry waste streams, it has been found that the use of an anaerobic-aerobic lagoon-polishing pond treatment sequence provides an effective means to remove organic pollutants (11–13,33,34,42,43). Attention must be given, however, to a routine biosolids removal protocol from the aerobic lagoon and polishing pond since solids accumulation can lead to odor and operational problems for these systems. For waste streams discharged to the POTW, the waste treatment process of choice is the activated sludge system.

BIBLIOGRAPHY

1. 40 *CFR* Parts 122, 405 and 432, U.S. Environmental Protection Agency, Washington, D.C., 1998.
2. 40 *CFR* Parts 243, 257, 258, and 503, U.S. Environmental Protection Agency, Washington, D.C., 1998.
3. R. E. Carawan, J. V. Chambers, and R. R. Zall, *Water and Wastewater Management in Food Processing, Dairy Spin Off Manual*, Extension Special Report No. AM-18B, North Carolina Agricultural Extension Service, Raleigh, N. Car., 1979.
4. W. J. Harper, J. L. Blaisdell, and J. Grosshopf, *Dairy Food Plant Wastes and Waste Treatment Practices*, U.S. Environmental Protection Agency, Washington, D.C. 1971.
5. *Development document for effluent limitations guidelines and new source performance standards for the dairy product processing point source category*, EPA-440/1-74-02/2, U.S. Environmental Protection Agency, Washington, D.C., 1974.
6. W. J. Harper, and J. L. Blaisdell, "State-of-the art of dairy food plant wastes and waste Treatment," *Proc. 2nd Food Wastes Symp.*, Denver, Colo., 1971, pp. 509–545.
7. *An Industrial Waste Guide to the Milk Processing Industry*, Public Health Services Publication No. 298, U.S. Department of Health, Education, and Welfare, Washington, D.C., 1963.
8. W. J. Harper, R. E. Carawan, and M. E. Parkin, *Wastewater Management Control Handbook for the Dairy Industry*, U.S. Environmental Protection Agency, Washington, D.C., 1984.
9. H. W. Moller, "Whey Utilization Systems," paper presented at the American Cultured Dairy Products Institute, San Antonio, Texas, 1981.
10. R. R. Zall, "Cost Effective Disposal of Whey," *Dairy Ind. Intl.* 30–39 (1980).
11. *Waste Management and Utilization in Food Production and Processing*, Task Force Report No. 124, Council for Agricultural Science and Technology, Ames, Iowa, 1995.
12. R. E. Carawan, J. V. Chambers, and R. R. Zall, *Water and Wastewater Management in Food Processing, Wastewater Treatment Spin Off Manual*, Extension Special Report No. AM-18J, North Carolina Agricultural Extension Service, Raleigh, N. Car., 1979.
13. Metcalf and Eddy, *Wastewater Engineering: Treatment, Disposal, Reuse*, 2nd ed., McGraw-Hill, New York, 1979.
14. O. J. Cotterill, "Egg-Products Industry," in W. J. Stadelman and O. J. Cotterill, eds., *Egg Science and Technology*, 4th ed., Harworth Press, Inc., New York, 1995, pp. 221–230.
15. R. E. Carawan, J. V. Chambers, and R. R. Zall, *Water and Wastewater Management in Food Processing, Egg Spin Off Manual*, Extension Special Report No. AM-18H, North Carolina Agricultural Extension Service, Raleigh, N. Car., 1979.
16. W J. Stadelman, "The preservation of quality in shell eggs," in W. J. Stadelman and D. J. Cotterill, eds., *Egg Science and Technology*, 4th ed., Harworth Press, Inc., New York, 1995, pp. 67–80.
17. *Annual Agricultural Statistics*, National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C., 1997.
18. O. J. Cotterill and L. E. McBee, "Egg-Breaking," in W. J. Stadelman and O. J. Cotterill, eds., *Egg Science and Technology*, 4th ed., Harworth Press, Inc., New York, 1995, pp. 231–264.
19. W. J. Jewell et al., "Egg Breaking and Processing Waste Control and Treatment," EPA-660/2-75-019, U.S. Environmental Protection Agency, Corvallis, Oregon, 1975.
20. W. J. Jewell et al., "Egg Breaking and Processing Waste Control and Treatment," *Proc. 6th Natl. Symp. Food Processing Wastes*, EPA-600/2-76-224, December, 1976.
21. C. L. Barth, J. Brock, and R. Stovall, "Waste and Resource Management for a 720,000-Layer Complex," *Proc. 6th Intl. Symp. Agricultural and Food Processing Wastes*, Chicago, Ill., December 17–18, 1990, pp 33–40.
22. C. Tadtiyanant, J. J. Lyons, and J. M. Vandepopuliere, "Extrusion Processing used to Convert Dead Poultry, Feathers, Egg-Shells, Hatchery Waste, and Mechanically Deboned Residue into Feedstuffs for Poultry," *Poultry Sci.* 72, 1515–1527 (1993).
23. J. M. Vandepopuliere et al., "Elimination of Pollutants by Utilization of Egg Breaking Plant Shell-Waste," EPA-600/2-78-044 U.S. Environmental Protection Agency, 1978.
24. G. W. Fronning and D. Bergquist, "Utilization of Inedible Egg-Shells and Technical Egg White Using Extrusion Technology," *Poultry Sci.* **69**, 2051–2053 (1990).
25. L. E. Carr, "Utilization of Poultry Solid Waste on Crops," *Proc. Natl. Poultry Waste Management Symp.*, Columbus, Ohio, April 18–19, 1988, pp. 133–140.
26. B. W. Jones, and G. Petitout, "In-House Sludge Management for Poultry Waste Utilizing Land Application: A Case Study," *Proc. 1989 Food Processing Waste Conf.*, Georgia Technology Research Institute, Atlanta, Ga., 1989.
27. D. E. Totzke, "Anaerobic Treatment Technology of Food Processing Wastes: A Review of Operating Systems," *Proc. 1987 Food Processing Waste Conf.*, Georgia Technology Research Institute, Atlanta, Ga., 1987.
28. J. C. Barker, P. W. Westerman, and L. M. Safley, "Lagoon Design and Management for Layer Waste Treatment and Storage," *Proc. Natl Poultry Waste Management Symp.*, Columbus, Ohio, April 18–19, 1988, pp. 26–33.
29. J. C. H. Shih, "Anaerobic Digestion of Poultry Waste and By-product Utilization," *Proc. Natl. Poultry Waste Management Symp.*, Columbus, Ohio, April 18–19, 1988, pp. 45–50.
30. A. N. Battacharya and J C. Taylor, "Recycling Animal Waste as a Feedstuff: A Review," *J. Animal. Sci.* 41, 1438–1457 (1975).
31. T. W. Stebor et al., "Case history: Anaerobic Treatment at Packerland Packing," *Proc. 1989 Food Processing Waste Conf.*, Georgia Technology Research Institute, Atlanta, Ga., 1989.
32. G. E. Valentine, C. C. Ross, and S. R. Harper, *High Rate Anaerobic Fixed-Film Reactor for the Food Processing Industry*, Final Report N 400-960 and A-4817, Georgia Technology Research Institute, Atlanta, Ga., 1988.
33. R. E. Carawan, J. V. Chambers, and R. R. Zall, *Water and Wastewater Management in Food Processing, Meat Spin Off Manual*, Extension Special Report No. AM-18C, North Carolina Agricultural Extension Service, Raleigh, N. Car., 1979, pp. 91–98.
34. R. E. Carawan, J. V. Chambers, and R. R. Zall, *Water and Wastewater Management in Food Processing, Poultry Spin Off Manual*, Extension Special Report No. AM-18D, North Carolina Agricultural Extension Service, Raleigh, N. Car., 1979.
35. M. C. Rooney and M. H. Wu, "Joint Treatment of Meat-Packing and Municipal Wastewater by Full-Scale AWT Facilities," *Proc. 36th Industrial Waste Conf.*, Ann Arbor Science Publishers, Ann Arbor, Mich., 1981, pp. 301–310.

36. R. E. Carawan, "Water Conservation and Waste Reduction for the Poultry Processing Industries," *Proc. Clean Water Act and the Poultry Industries*, Washington, D.C., 1989.

37. F. C. Blanc, J. C. O'Shaughnessy, and S. H. Corr, "Treatment of Beef Slaughtering and Processing Wastewaters Using Rotating Biological Contractors," *Proc. 38th Industrial Waste Conf.*, Ann Arbor, Mich., May 5, 1983, pp. 1133–1140.

38. Griggs, W. E., Damned if you do and damned if you don't, an ammonia discharge at a rendering plant. *Proceedings, 1989 Food Processing Waste Conference*, Georgia Technology Research Institute, Atlanta, 1989.

39. L. E. Carr et al., "Land Disposal of Dissolved Air Flotation Sludge from Poultry Processing," *Trans. Am. Soc. Agric. Eng.* **31**, 463–469 (1988)

40. O. C. Pancorbo and H. M. Barnhart, "Microbiological Characterization of Raw and Treated Poultry Processing Wastewater and Sludge: Preliminary Assessment of Suitability for Land Application," *Proc. 1989 Food Processing Waste Conf.*, Georgia Technology Research Institute, Atlanta, Ga., 1989.

41. Harper, S. R., R. Wallace, T. D. Shadburn, T. L. King, J. N. Mullis and J. T. Cook. Ammonia stripping and chlorination for nitrogen removal from poultry processing wastewater. Final Report. Project N 400-950. Environmental Sciences and Technology Division, Georgia Technology Research Institute, Atlanta, 1989.

42. *"Design of Anaerobic Lagoons for Animal Waste Management,"* Standards, EP403.2, American Society of Agricultural Engineers, St. Joseph, Mich., 1993. pp. 543–557.

43. E. R. Collins, "Lagoon Treatment of Poultry Wastes," *Proc. National Poultry Waste Management Symp.*, Raleigh, North Carolina, October 3–4, 1990, pp. 266–277.

James V. Chambers
Purdue University
West Lafayette, Indiana

WASTE MANAGEMENT: FRUITS AND VEGETABLES

Large quantities of liquid and solid wastes are produced annually in the processing of many different types of fruits and vegetables. According to Rose and coworkers (1), over 80 billion gallons of wastewater and nearly 9 million tons of solid residues are generated annually by the fruit and vegetable-processing industry in the United States (Table 1). Of all the residues generated by the food-processing industry, fruits and vegetables together account for 93% (2). The four crops processed in the largest quantities are citrus, corn, tomatoes, and white potatoes. These crops account for 67% of raw tonnage, 61% of wastewater, 45% of organic load expressed as the biochemical oxygen demand (BOD), 61% of suspended solids, and 71% of solid residues (1). The BOD value of a typical domestic sewage is usually about 250 mg/L. In general, the fruit and vegetable–processing industry produces a liquid waste approximately 10 times the BOD value of typical domestic sewage (3). The wastewater must be properly treated before it can be discharged to a stream or other water body. According to Hudson's survey results (2), nearly all the liquid waste is disposed of in water (stream, lake, bay, or ocean) and in public waste-treatment systems. Approximately 79% of the fruit and vegetable solid residues is used for animal feed and the remaining 21% is handled as waste (2).

For the fruit and vegetable–processing industry to remain competitive, it is important that both liquid and solid wastes be properly managed. The objective of this article is to discuss the sources and characteristics of fruit and vegetable–processing wastes, to describe the in-plant waste-reduction practices, and to review the current developments in the processes for waste treatment and for recovery of useful by-products from the wastes.

SOURCES AND CHARACTERISTICS OF FRUIT AND VEGETABLE WASTES

The major contributing point sources for fruit-processing wastewater are washing, peeling, sorting and slicing, cooking, can cooling, and plant cleanup. Table 2 shows the estimated average volumes of wastewater if only fresh water is used in all the processing steps in the fruit-processing plant. If efforts are made to reuse water, the volumes of wastewater can be significantly reduced. For example, the cooling water can be reused for the can-cooling operation or washing the raw product. Reductions in the volume of fresh water consumed will result in corresponding reductions in the volume of wastewater to be treated (4). In vegetable processing, the major sources of wastewater include washing, grading (sorting), fluming, peeling, blanching, can cooling, and plant cleanup. The volumes of wastewater generated in the processing of vegetables can be significantly reduced if the water is reused in the plant according to the counterflow water reuse system (4). In this system, fresh water is used in the final step, collected and reused in a previous step, and recollected and reused in this manner one or several times. The water always flows countercurrent to the product. However, sufficient chlorine must be added to reduce bacterial contamination of the reused water used in each step. Most organic loads in the wastewater originate in the peeling and blanching operations at the fruit and vegetable–processing plants. For example, peeling and blanching contribute more than 80% of the BOD discharged from a beet-processing plant. Surplus brines generated in sauerkraut fermentation represent nearly 20% of the raw cabbage used (Table 3), and they have a BOD value of as much as 65,000 mg/L (Table 4). The soaking operations used in the processing of sour cherries and baked beans are a significant source of high BOD wastewater (7). The major sources of solid wastes from fruit and vegetable–processing plants are sorting, cutting, slicing, peeling, pulping, and pressing. In sauerkraut production, for example, trim losses represent over 35 kg per 100 kg of raw cabbage processed (Table 3). Fruit pomace is a waste product of the fruit juice industry, representing 10 to 20% of the fresh weight of the raw material. The strength and pH of fruit and vegetable–processing wastewater vary widely with the type of raw product being processed (Table 4). Some of the variations in strength and pH reflect differences in the processing methods used in individual plants. For example, the waste generated in the fermentation of cabbage to sauerkraut has a pH value of as low as 3.5 and its BOD value is as high as 65,000 mg/L (6).

Table 1. Wastes from Canned and Frozen Fruits and Vegetables

	Raw tons (1,000 tons)	Wastewater (million gal)	BOD (million lb)	Suspended solids (million lb)	Solids residuals (1000 tons)
Apple	1,000	5,000	40	5	320
Apricot	120	600	7	1	21
Cherry	190	400	4	1	27
Citrus	7,800	23,000	31	55	3,390
Peach	1,100	4,400	66	11	270
Pear	400	1,600	28	8	120
Pineapple	1,000	500	20	8	450
Other fruit	400	3,200	8	4	80
Asparagus	120	1,200	1	1	45
Bean, lima	120	1,100	3	10	10
Bean, snap	630	2,800	19	3	130
Beet	270	1,100	40	14	110
Carrot	280	1,100	15	11	150
Corn	2,500	4,500	62	25	1,660
Pea	580	2,900	29	6	78
Pumpkin, squash	220	700	18	3	150
Sauerkraut	230	100	3	1	75
Spinach, gr.	240	2,200	6	2	37
Sweet potato	150	1,000	30	12	(in other vegetable)
Tomato	5,000	10,000	60	20	400
Other vegetables	1,300	5,200	80	40	500
White potato	2,400	9,600	190	120	910
Total	*26,400*	*82,000*	*760*	*360*	*8,940*

Source: Ref. 1.

Table 2. Sources and Volumes of Wastewater from Fruit-Processing Steps

Operation	Wastewater Gal/h	Wastewater Gal/ton	Percentage of total flow
Peeling	1,200	48	2
Spray washing	11,000	385	17
Sorting, slicing, etc	3,000	120	5
Exhausting of cans	1,200	48	2
Processing	600	24	1
Cooling cans	24,000	945	37
Plant cleaning	21,000	840	33
Box washing	1,900	70	3

Source Ref. 3.

Table 3. Material Balance of Sauerkraut Production

	Tons
Raw cabbage	100.00
Solid wastes (trim loss)	35.30
Shredded cabbage in vat	64.70
Salt added	1.70
Liquid wastes	
Early brine	11.00
Late brine	8.50
Total	*19.50*
Yield of finished product	46.90

Source: Ref. 5.

Table 4. Strength and pH of Fruit and Vegetable–Processing Wastes

Product	COD (mg/L)	BOD (mg/L)	Mean (BOD/COD)	pH
Apple	395–37,000	240–19,000	0.55	4.1–8.2
Beets	445–13,240	530–6,400	0.57	5.6–11.9
Carrots	1,750–2,910	817–1,927	0.52	7.4–10.6
Cherries	1,200–3,795	660–1,900	0.53	5.0–7.9
Corn	3,400–10,100	1,587–5,341	0.50	4.8–7.6
Green beans	78–2,200	43–1,400	0.55	6.3–8.3
Peas	723–2,284	337–1,350	0.61	4.9–9.2
Sauerkraut	470–65,000	300–41,000	0.66	3.6–6.8
Tomatoes	652–2,305	454–1,575	0.72	5.6–10.8
Wax beans	193–597	55–323	0.58	6.5–8.2
Wine	495–12,200	363–7,645	0.60	3.1–9.2

Source: Ref. 6.

At the other extreme, certain waste effluents are strongly alkaline because of the presence of lye-peeling liquors or alkaline cleaning compounds. Waste effluents with high and low pH values present problems for waste treatment. For optimal biological treatment, the pH of wastewater must be adjusted to between 6.0 and 9.0.

Waste Reduction

The pollution loads at the fruit and vegetable–processing plants can be reduced through the development of improved processing methods or through in-plant treatment and reuse.

Process Modification. Most pollution loads are generated in the wet peeling of fruits and vegetables. The conventional wet peeling process, for example, produces 10,200 lb of total solids/day, whereas the dry-caustic peeling process produces only about 1,050 lb of total solids/day (Table 5). The dry-peeling process also reduces the BOD load from 2,670 to 190 lb/day, representing a reduction of more than 90% (4). The conventional water- or steam-blanching process is also a major source of pollution loads. It inactivates enzymes and removes gases from the plant tissues but also leaches out organic matter. To minimize both the hydraulic and organic loads, a hot gas–blanching method has been developed for vegetable processing. Hot gas blanching has been reported to reduce the volume of vegetable processing wastewater, BOD, and suspended solids (SS), by as much as 99.9% (4).

Other water-conservation and waste-reduction techniques are available. These include the use of air cooling after blanching, the installation of low-volume, high-pressure cleaning systems, the substitution of mechanical conveyors for flumes, the use of automatic shutoff valves on water hoses, the separation of can cooling water from composite waste flow, the countercurrent reuse of wash/flume/cooling water, the separation of low- and high-strength waste streams, and use of air flotation units to remove suspended debris from raw crop materials (8).

In-Plant Treatment and Reuse. An important treatment system has been developed for reduction of the volume of water consumption at the fruit and vegetable–processing plants (4). This in-plant system consists of coagulation, sedimentation, and filtration. Chemicals such as alum and polymers are added to the screened wastewater to facilitate coagulation. Coagulated solids or sludge is discharged to a solid waste–disposal area and the clarified water is passed through activated carbon filters. The filtered effluent is chlorinated and reused for initial product washing, for product washing after blanching, and for carrying out solid wastes to the screening area. This system can remove all the suspended solids, including bacteria (4).

Waste Utilization

Fruit and vegetable–processing residues are rich in biodegradable organic matter, and disposal of these waste materials may pose many environmental pollution problems. Fortunately many opportunities exist for converting them into a wide variety of valuable by-products. Some examples follow below.

Animal Feed. Most fruit and vegetable–processing solid residues are presently used for animal feed (2). They may be fed fresh, as silage, or as dried waste solids. Citrus-, pineapple-, and potato-processing residues are almost entirely fed to animals. Other fruit and vegetable residues, however, are not widely used in animal feed rations because they are available only during the short processing [illegible]

Natural Food Ingredients. Natural food ingredients can be isolated from fruit and vegetable–processing residues. Anthocyanins, tartrates, and grape-seed oil have been directly extracted from grape pomace (9). Carotenoids can be produced from sauerkraut brine by fermentation with the yeast *Rhodotortula rubra* (10). Apple- and citrus-processing solid wastes can serve as a good source of raw material for commercial production of pectins. Attempts are now being made to isolate health-promoting dietary fibers and other phytochemicals from fruit and vegetable residues. For example, a product with a dietary fiber content of over 56% has been produced from apple residues by Three Top Inc., Selah, Washington (7).

Biofuels. Biofuels have been generated from fruit and vegetable–processing residues by fermentation. Methane production is a two-stage process. The substrate is first converted to organic acids by acid-producing bacteria. The organic acids are then fermented anaerobically by methane-producing bacteria to methane. Capital and operating costs of methane production are dependent on the digester size, raw material, and operating conditions. The fermentation efficiency increases with decreased substrate particle size. Nearly 80% of the organic matter in apple pomace, for example, can be readily converted to methane (7). Ethanol can be directly produced from fruit-processing residues by yeast fermentation (7,9). Starch and cellulosic waste materials, however, must be hydrolyzed first to glucose before ethanol fermentation. For example, the ethanol yields from different grape pomace samples under solid-state fermentation conditions range from 68 to 86% (Table 6).

Citric Acid. Citric acid is a commercially important organic acid that has been produced from fruit and vegetable–processing residues by *Aspergillus niger* (7,9).

Table 5. Water Usage and Properties of Wastewater in Two Different Peeling Operations of Beets

Measurement	Conventional peeling	Dry-caustic peeling
Raw beets, input, ton/day	80	80
Water flow rate, gal/day	48,000	12,000
Total solids, lb/day	10,200	1,050
SS, lb/day	340	40
BOD, lb/day	2,670	190

Source: Ref. 4.

Table 6. Ethanol Production from Grape Pomace under Solid-State Fermentation Conditions

			Yeast count, CFU/g		Sugar content g/kg		Ethanol content, g/kg		
Variety	pH	Moisture, %	0 h	96 h	0 h	96 h	0 h	96 h	Yield[a] %
			Vinifera						
Chardonnay	3.7	74.5	5 × 10^3	3 × 10^7	125.0	32.5	0.4	40.8	86
Riesling	3.6	65.5	1 × 10^5	7 × 10^7	137.5	7.5	0.2	54.2	82
			Geneva hybrid						
Cayuga white	3.6	64.3	5 × 10^6	2 × 10^7	97.5	9.4	2.9	40.6	86
			French hybrid						
Aurora	3.6	64.5	3 × 10^5	2 × 10^7	70.0	15.6	2.5	24.6	80
Baco noir	3.3	57.0	7 × 10^4	1 × 10^5	121.2	12.5	0.4	42.7	76
Ravat	3.6	62.0	3 × 10^6	8 × 10^4	156.2	41.2	2.6	49.8	80
Red hybrid[b]	3.3	57.0	3 × 10^6	8 × 10^7	83.8	11.6	0.9	31.4	82
Seyval	3.8	68.4	9 × 10^5	4 × 10^7	137.5	13.0	3.8	58.0	86
Ventura	3.5	64.3	4 × 10^5	1 × 10^7	115.0	15.3	0.4	43.1	84
Verdelet	3.6	70.6	1 × 10^7	8 × 10^6	106.2	9.4	7.2	48.0	82
			Labrusca						
Cascade	3.7	60.9	7 × 10^6	7 × 10^7	103.8	18.1	0.4	38.4	86
Concord									
Cold-pressed	3.3	66.7	2 × 10^4	3 × 10^7	58.1	10.5	0.9	20.8	82
Hot-pressed	3.3	54.8	1 × 10^5	8 × 10^7	103.8	20.9	0.6	32.0	74
Delaware	3.7	57.9	4 × 10^6	5 × 10^7	118.8	17.2	3.3	44.7	80
Diamond	3.5	68.1	5 × 10^4	2 × 10^7	76.2	38.1	15.0	28.4	68
Dutchess	3.6	64.9	5 × 10^6	3 × 10^7	130.0	7.2	0.4	46.3	72
Ives	3.5	56.7	5 × 10^4	1 × 10^5	87.5	12.5	0.8	32.2	82
Niagara	3.5	65.9	9 × 10^3	5 × 10^5	85.0	24.4	0.4	26.8	86

[a] Yield, % = Actual ethanol produced/Theoretical ethanol from sugar consumed × 100.

[b] Includes deChaunac, Rougeon, Chelois, Rosette.

The yields of citric acid from apple pomace, corncobs, kiwi peel, and grape pomace are about 320, 250, 230, and 270 g/kg dry matter, respectively.

Single-Cell Protein. Single-cell protein can be produced from fruit and vegetable–processing residues by fermentation. Hang (11) has described the processes for single-cell protein production from the waste effluents generated in the processing of cabbage, corn, baked beans, and potatoes. The processes not only produce single-cell protein but also reduce the BOD value by as much as 90%.

Enzymes. Enzymes of commercial importance can be directly extracted from fruit and vegetable–processing residues. Bromalain is a protease, for example, that has been commercially produced from pineapple stem (Sigma Chemical Company, St. Louis, Mo.). The enzyme has a variety of food-processing applications. Attempts have been made to utilize the fruit and vegetable–processing residues as a substrate for fermentative production of industrial enzymes by food-grade microorganisms. Sauerkraut waste, for example, has been found to be a favorable substrate for production of yeast enzymes such as polygalacturonase, invertase, diacetyl reductase, and beta-glucosidase (12).

Waste Treatment and Disposal. Liquid wastes generated in the processing of fruit and vegetables contain principally biodegradable organic matter in soluble and suspended form. The waste effluents are generally deficient in both nitrogen and phosphorus. For optimum biological treatment, it is essential to add inorganic nitrogen and phosphorus to give a BOD-to-nitrogen-to-phosphorus ratio of 100:5:1. Several waste-treatment processes have been used to make fruit and vegetable–waste effluents suitable for discharge. Aerated lagoons are the most widely used to treat the wastewater (4). In aerated lagoons, oxygen is added by either mechanical agitation or compressed-air diffusion. For solid separation, lagoons in series are used. The activated sludge process consists of a bioreactor that provides an environment for converting the BOD to microbial cells under aerobic conditions and a clarifier where microbial cells or sludge is settled. The settled sludge may be returned to the reactor or wasted. This system has been used to remove 80 to 99% of BOD depending on the waste composition, loading rate, and other operating conditions. The wastewater can also be treated in an anaerobic digester to produce methane. In pray irrigation, the wastewater is screened and sprinkled on land to simulate rainfall. Parameters that affect the system are the waste characteristics (solids, BOD, pH, salts, etc), site, and discharge limits. The organic loading rates range from 36 to 108 lb of BOD/acre/day and are dependent on the filtration and percolation capacity of the soil at the treatment site (4).

Most solid wastes are presently disposed of on land either by filling or by spreading (4). In land filling, solid wastes are dumped into an excavated pit and covered with soil. A crop is maintained to use the waste solids, minimize erosion, and enhance the appearance of the landfill site. The loading rates are about 5 to 50 tons of dry solids per acre per year (4). The leachates from the landfill sites are odorous and rich in organic matter. They must be properly treated before discharge to avoid environmental and public-health problems. In land speading, solid wastes are spread on land in thin layers and allowed to remain on the surfaces long enough to dry before they are tilled into soils. The loading rates by this method are about 3 to 5 tons of dry solids/acre/year (4).

Biotechnology and Waste Management. Research is needed to reduce the pollution loads generated in the processing of fruits and vegetables through genetic engineering of raw products and through development of new or modified processing methods. Fruits and vegetables can be genetically modified to have desirable processing characteristics such as increased solids content, uniform ripening, and size. For example, new hybrids of cabbage with a higher solids content have been developed for sauerkraut production. The genetically modified cabbage can increase the product yield with an accompanying decrease in the production of brine (13). The surplus brine generated in sauerkraut fermentation has been a serious pollution problem for kraut processors (5). To reduce the pollution loads at the processing plant, it is necessary to develop new processing techniques for processing raw materials at the harvest location. For the unavoidable processing wastes, new biotechnological processes should be developed to convert them into protein, fuels, biochemicals, or other valuable products with an accompanying decrease in the pollution loads. For example, to maximize the production of value-added products from grape pomace and to minimize the waste disposal problems, it is necessary to use several technologies in the following sequences (Fig. 1):

- Removal of seeds from the pomace for oil recovery
- Fermentation of the pomace minus seeds to ethanol or citric acid
- Extraction of anthocyanins and tartrates from spent pomace
- Anaerobic decomposition of the remainder for methane geneation (9)

CONCLUSION

Large quantities of both liquid and solid wastes are produced annually by the fruit and vegetable–processing industry. These waste materials contain principally biodegradable organic matter, and disposal of them can pose many environmental-pollution problems. Factors affecting the costs of waste treatment and disposal are the volume or hydraulic load and the strength or organic load. The waste load at the processing plant can be reduced significantly through the use of modified processing methods and through in-plant treatment and reuse. A number of waste-treatment processes are available to remove the BOD and SS from the processing waste effluents. Biological treatment systems are widely used to make the wastewater suitable for discharge. Land irrigation of wastewater is used in rural areas. Most solid wastes are disposed of by returning them to the harvest fields for land treatment. The key to minimizing the disposal cost is to remove excessive moisture from the wastes. Many opportunities exist for better utilization of fruit and vegetable–processing wastes. A variety of processes have been developed for converting the waste materials into natural food ingredients, enzymes, fuels, and other valuable by-products.

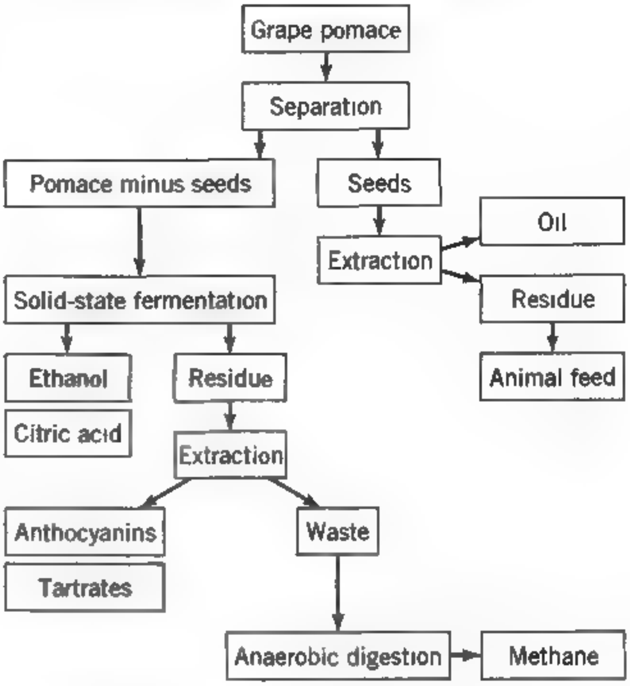


Figure 1. Resource recovery from grape pomace flow diagram. *Source:* Ref. 9.

BIBLIOGRAPHY

1. W. W. Rose et al., "Production and Disposal Practices for Liquid Wastes from Canning and Freezing Fruits and Vegetables," in *Proc. 2nd Natl. Symp. Food Processing Wastes*, Oregon State University, Corvallis, Or., 1971, pp. 109–127.
2. H. T. Hudson, "Solid Waste Management in the Food Processing Industry," in *Proc. 2nd Natl. Symp. Food Processing Wastes*, Oregon State University Corvallis, Or., 1971, pp. 637–654.
3. *Fruit Processing* Industry, U.S. Department of Health, Education, and Welfare, PHS Pub. No. 952, Washington, D.C., 1962.
4. U.S. Environmental Protection Agency, *Pollution Abatement in the Fruit and Vegetable Industry*, EPA-625/3-77-0007, Washington, D.C. 1977.
5. Y. D. Hang et al., "Wastes Generated in the Manufacture of Sauerkraut," *J. Milk Food Technol.* **35**, 432–435 (1972).
6. D. F. Spittstoesser and D. L. Downing, "Analysis of Effluents from Fruit and Vegetable Processing Factories," *New York State Agric. Exp. Sta. Res. Circ.* **17** (1969).

7. Y. D. Hang and R. H. Walter, "Treatment and Utilization of Apple Processing Wastes," in D. L. Downing, ed., *Processed Apple Products*, AVI Publishing Co., Westport, Conn., 1989, pp. 365–377.
8. *Waste Management and Utilization in Food Production and Processing*, Task Force Report No. 124, Council for Agricultural Science and Technology, Ames, Iowa, 1995.
9. Y. D. Hang, "Recovery of Food Ingredients from Grape Pomace," *Process Biochem.* **23**, 2–4 (1988).
10. C. T. Shih and Y. D. Hang, "Production of Carotenoids by *Rhodotorula rubra* from Sauerkraut Brine," *Food Sci. Technol.* **29**, 570–572 (1996).
11. Y. D Hang, "Production of Single Cell Protein from Food Processing Wastes" in J. H. Green and A. Kramer, eds., Food Processing waste management, AVI Publishing Co., Westport, Conn., 1979, pp. 442–455
12. S. L. Sim and Y. D. Hang, "Sauerkraut Brine: A Potential Substrate for Production of Yeast β-Glucosidase," *Food Sci. Technol.* **29**, 365–367 (1966).
13. J. R. Stamer and M. H. Dickson, "High Solids Cabbage for Sauerkraut Production: Its Effect Upon Brine Reduction and Yield of Product," *J. Milk Food Technol.* **38**, 688–690 (1975).

YONG D. HANG
Cornell University
Geneva, New York

WATER ACTIVITY: FOOD TEXTURE

FOOD TEXTURE

The textural properties of a food may be defined as that group of physical characteristics that arise from the structural elements of a food, are sensed by the feeling of touch, are related to the deformation, disintegration, and the flow of the food under a force and are measured objectively by functions of mass, time, and distance (1). The term textural properties is preferred over the word texture because texture implies a one dimensional property (eg, pH) while texture is multi-dimensional in nature and cannot be completely described by a single parameter (2). Because of the long time required to measure all the textural properties of a food the most common practice is to measure only one or a few of its most dominant textural properties.

A number of test principles are used for instrumental measurement of textural properties including force, time, distance, work, and rate of flow. Force measurements are the most common and can be classified according to the geometry of the test apparatus as puncture, extrusion, cutting shear, compression (single and multiple), bending—snapping, and torque (1).

Since texture is primarily a sensory perception (3), instrumental measurements need to be calibrated against the human palate. Sensory testing ranges from describing a single texture note up to a complete texture profile analysis that generally describes 15–30 texture notes depending on the food (4,5). Some of the most widely used sensory texture descriptors are firm, crisp, chewy, creamy, sticky, gummy, viscous, fibrous, granular, flakey, pulpy, moist, and greasy (2).

A promising hybrid sensory-instrumental technique is the use of a multiple-point sheet sensor (6–8). This comprises a thin flexible plastic film about 5–6 cm square on which are printed two series of stripes perpendicular to each other using electrically conductive ink. The resistivity of the ink is directly proportional to the force applied to it. When placed in the mouth, the pressure can be measured at many points over time, showing the distribution of forces rather than an average force. Although in an early stage of development, the technique shows great promise. For example, in testing crackers equilibrated to A_w levels of 0.14, 0.36, 0.63, and 0.80, the force-deformation curves detected by the multiple-point sheet sensor in the mouth closely matched force-deformation curves obtained with a compression test conducted in a universal testing machine (6).

WATER ACTIVITY

Water activity is defined as the ratio of the vapor pressure of water in a food to the vapor pressure of pure water at the same temperature:

$$A_w = \frac{P}{P_0}$$

where A_w is water activity, P is vapor pressure of water in the food, and P_0 is vapor pressure of pure water at the same temperature. Since solutes in food depress the water vapor pressure, P will always be less than P_0. Therefore A_w always has a value between 0 and 1.0, where 0 represents the total absence of water and 1.0 represents pure water. The effects of chemical composition on A_w and more rigorous definitions of A_w involving thermodynamic concepts are given elsewhere in this volume. However, the definition given above is adequate for the purposes of this article.

The older literature uses the term equilibrium relative humidity (ERH), which is the relative humidity of the air surrounding a food when the water vapor pressure in the air is the same as in the food (equilibrium state). Since relative humidity is expressed on a percentage basis, the old literature values for ERH can be easily transformed into A_w values by dividing by 100: $A_w = \text{ERH}/100$. For example, if the ERH = 65%, then the $A_w = 65/100 = 0.65$.

The concept of water activity in food has many useful applications. A major application is to determine whether a food will absorb or lose water in a given environment. It is well known that liquid water always flows spontaneously from a high-pressure area to a low-pressure area (ie, downhill). Although invisible macroscopically, water vapor also flows spontaneously from high-pressure regions (high A_w) to low-pressure regions (low A_w). A food with $A_w = 0.60$ placed in air at 40% relative humidity (RH) (ie, $A_w = 0.40$) will give up water vapor to the air until the water activity in the food equals that in the air. In moving air at constant 40% RH the food will eventually reach $A_w = 0.40$; in a confined space with no change in the air the equilibrium A_w will be somewhere between 0.40 and 0.60. If the food at $A_w = 0.60$ is placed in air at 80% RH ($A_w = 0.80$) it will absorb water vapor from the air until equilibrium is reached at a level higher than A_w 0.60.

7. Y. D. Hang and R. H. Walter, "Treatment and Utilization of Apple Processing Wastes," in D. L. Downing, ed., *Processed Apple Products*, AVI Publishing Co., Westport, Conn., 1989, pp. 365–377.
8. *Waste Management and Utilization in Food Production and Processing*, Task Force Report No. 124, Council for Agricultural Science and Technology, Ames, Iowa, 1995.
9. Y. D. Hang, "Recovery of Food Ingredients from Grape Pomace," *Process Biochem.* **23**, 2–4 (1988).
10. C. T. Shih and Y. D. Hang, "Production of Carotenoids by *Rhodotorula rubra* from Sauerkraut Brine," *Food Sci. Technol.* **29**, 570–572 (1996).
11. Y. D. Hang, "Production of Single Cell Protein from Food Processing Wastes" in J. H. Green and A. Kramer, eds., Food Processing waste management, AVI Publishing Co., Westport, Conn., 1979, pp. 442–455
12. S. L. Sim and Y. D. Hang, "Sauerkraut Brine: A Potential Substrate for Production of Yeast β-Glucosidase," *Food Sci. Technol.* **29**, 365–367 (1966).
13. J. R. Stamer and M. H. Dickson, "High Solids Cabbage for Sauerkraut Production: Its Effect Upon Brine Reduction and Yield of Product," *J. Milk Food Technol.* **38**, 688–690 (1975).

YONG D. HANG
Cornell University
Geneva, New York

WATER ACTIVITY: FOOD TEXTURE

FOOD TEXTURE

The textural properties of a food may be defined as that group of physical characteristics that arise from the structural elements of a food, are sensed by the feeling of touch, are related to the deformation, disintegration, and the flow of the food under a force and are measured objectively by functions of mass, time, and distance (1). The term textural properties is preferred over the word texture because texture implies a one dimensional property (eg, pH) while texture is multi-dimensional in nature and cannot be completely described by a single parameter (2). Because of the long time required to measure all the textural properties of a food the most common practice is to measure only one or a few of its most dominant textural properties.

A number of test principles are used for instrumental measurement of textural properties including force, time, distance, work, and rate of flow. Force measurements are the most common and can be classified according to the geometry of the test apparatus as puncture, extrusion, cutting shear, compression (single and multiple), bending—snapping, and torque (1).

Since texture is primarily a sensory perception (3), instrumental measurements need to be calibrated against the human palate. Sensory testing ranges from describing a single texture note up to a complete texture profile analysis that generally describes 15–30 texture notes depending on the food (4,5). Some of the most widely used sensory texture descriptors are firm, crisp, chewy, creamy, sticky, gummy, viscous, fibrous, granular, flakey, pulpy, moist, and greasy (2).

A promising hybrid sensory-instrumental technique is the use of a multiple-point sheet sensor (6–8). This comprises a thin flexible plastic film about 5–6 cm square on which are printed two series of stripes perpendicular to each other using electrically conductive ink. The resistivity of the ink is directly proportional to the force applied to it. When placed in the mouth, the pressure can be measured at many points over time, showing the distribution of forces rather than an average force. Although in an early stage of development, the technique shows great promise. For example, in testing crackers equilibrated to A_w levels of 0.14, 0.36, 0.63, and 0.80, the force-deformation curves detected by the multiple-point sheet sensor in the mouth closely matched force-deformation curves obtained with a compression test conducted in a universal testing machine (6).

WATER ACTIVITY

Water activity is defined as the ratio of the vapor pressure of water in a food to the vapor pressure of pure water at the same temperature:

$$A_w = \frac{P}{P_0}$$

where A_w is water activity, P is vapor pressure of water in the food, and P_0 is vapor pressure of pure water at the same temperature. Since solutes in food depress the water vapor pressure, P will always be less than P_0. Therefore A_w always has a value between 0 and 1.0, where 0 represents the total absence of water and 1.0 represents pure water. The effects of chemical composition on A_w and more rigorous definitions of A_w involving thermodynamic concepts are given elsewhere in this volume. However, the definition given above is adequate for the purposes of this article.

The older literature uses the term equilibrium relative humidity (ERH), which is the relative humidity of the air surrounding a food when the water vapor pressure in the air is the same as in the food (equilibrium state). Since relative humidity is expressed on a percentage basis, the old literature values for ERH can be easily transformed into A_w values by dividing by 100: $A_w = \text{ERH}/100$. For example, if the ERH = 65%, then the $A_w = 65/100 = 0.65$.

The concept of water activity in food has many useful applications. A major application is to determine whether a food will absorb or lose water in a given environment. It is well known that liquid water always flows spontaneously from a high-pressure area to a low-pressure area (ie, downhill). Although invisible macroscopically, water vapor also flows spontaneously from high-pressure regions (high A_w) to low-pressure regions (low A_w). A food with $A_w = 0.60$ placed in air at 40% relative humidity (RH) (ie, $A_w = 0.40$) will give up water vapor to the air until the water activity in the food equals that in the air. In moving air at constant 40% RH the food will eventually reach $A_w = 0.40$; in a confined space with no change in the air the equilibrium A_w will be somewhere between 0.40 and 0.60. If the food at $A_w = 0.60$ is placed in air at 80% RH ($A_w = 0.80$) it will absorb water vapor from the air until equilibrium is reached at a level higher than A_w 0.60.

Figure 1 shows schematically the similarity between the flow of liquid water and the flow of water vapor. In both cases the spontaneous flow is always from the high-pressure region to the low pressure region. While most graphs plot water activity across the abscissa (horizontal axis), it conceptually helps to understand water activity if one imagines it on a vertical axis as shown in Figure 1. This rendition makes it easy to understand why the flow of water vapor is always from high A_w to low A_w. When two foods with different water activities are enclosed in the same airtight container but not in contact, an invisible transfer of water in the form of vapor will occur. Water vapor will migrate from the food with a high A_w into the air and then be absorbed from the air by the food with low A_w. Both foods and the air will eventually equilibrate at equal A_w levels. The only way to prevent this spontaneous transfer of water vapor is to place a barrier around the food that retards the rate of transfer of the vapor. The degree of resistance to the passage of water vapor is an important criterion in the selection of packaging materials for those foods whose quality will deteriorate if they pick up or lose water to the atmosphere during storage.

Fresh fruits and vegetables have a high water content, usually in the 80–90% range, and a water activity of about 0.99. They would quickly lose moisture to the air and become wilted, limp, and dry and lose their crispness if it were not for the skin, which acts as a barrier to impede the loss of water vapor. Many fruits and vegetables secrete a wax on the skin (cuticular wax), which makes the skin a more effective barrier to dehydration. Some commodities such as apples, oranges, cucumbers, winter tomatoes, and rutabagas have additional wax added to the skin to further impede loss of water vapor and extend their storage life. The waxing process also increases the gloss of the skin imparting a shiny appearance that makes it look more attractive. Fruits and vegetables lose water rapidly in the air when the skin is punctured or broken. For this reason, care must be taken not to break the skin of those items that will be stored for any length of time.

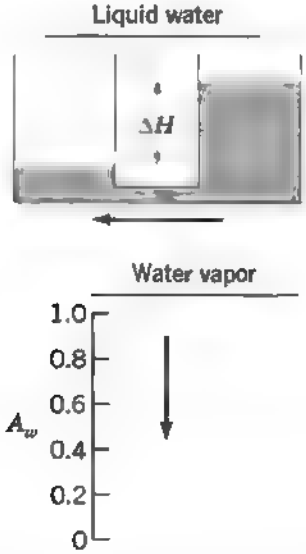


Figure 1. Top: The flow of liquid water is always from high-pressure to low-pressure regions at a rate proportional to the pressure difference *ΔH*. Bottom: Similarly the flow of water vapor is always from the high-A_w to the low-A_w region.

GENERAL CONSIDERATIONS

It has been known for many years that foods may pick up or lose moisture from the air during storage and that these changes can affect the texture. For example, in 1945 Woodroofe and co-workers (9) demonstrated that raw unshelled peanuts stored at 80% RH maintained a good texture for 60 days; they became clammy after 90 days and soggy after 360 days. Peanuts stored at 65% RH maintained a good texture for 360 days. At 50% RH they maintained a good texture for 120 days, became dry after 180 days, and become slightly hard after 240 days. Shelled raw peanuts stored under the same conditions exhibited the same texture changes as did the unshelled ones when stored at 80 and 65% RH. Although these early results were more descriptive than quantitative, they clearly showed that losing moisture and gaining moisture can cause loss of textural quality. When moisture is lost, the peanuts become brittle or dry. When moisture is gained, they become soggy or clammy.

SORPTION ISOTHERMS

The relationship between percentage water content and water activity is complex. The A_w level almost always increases with increasing water content over the full A_w range, but in a nonlinear fashion. A plot of water content versus A_w usually takes the form of an S-shaped curve (see Fig. 2). These curves are determined experimentally. A food product is equlibrated at various A_w levels by exposing it to a series of atmospheres of constant RH until it reaches constant weight, and then the water content is measured by chemical analysis. Plotting these data points on a graph and connecting the points gives rise to curves

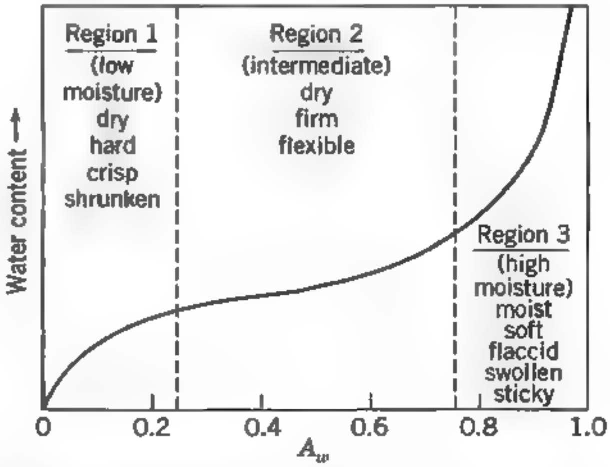


Figure 2. Food textures as a function of localized water sorption isotherms. *Source:* Ref. 10.

similar to those in Figure 2. Some researchers do not explore the full A_w range but present data over a limited range of A_w. In these cases the typical S-shaped curve may not be evident. When these experiments are performed at constant temperature, the graphs are called water sorption isotherms, when the food is gaining water and water desorption isotherms, when it is losing water. An excellent compilation of water sorption isotherms for many foods has been published (11). Note that in Figure 2 the slope of the water sorption isotherm is always positive, ie, an increase in water activity is always accompanied by an increase in percentage water content, although the rate of increase (ie, slope) varies across the A_w spectrum. There are occasional exceptions to this rule that water content always increases with increasing A_w. Some of these will be discussed under the section on sugars below.

A typical water sorption isotherm may be divided into three regions, each with characteristic textures (12). Region 1 is the lowest moisture level. Here the moisture is bound tightly to the hydrophilic constituents in the food. It is present at first in a monomolecular layer and then in multiple molecular layers. Typical textures in this region are hard, dry, crisp, brittle, and shrunken. In the intermediate moisture region 2 the moisture level is less tightly bound as additional molecular layers of water are absorbed into the food. This region is characterized by a shallower slope than regions 1 and 3. Dry, firm, flexible, and leathery are typical textures found in this region. Region 3 is the high-moisture region. A large increase in water content causes a relatively small increase in A_w. The water is probably held by the weak forces of capillary absorption. Typical textures in region 3 are moist, soft, flaccid, limp, tender, swollen, and sticky. A number of mathematical models have been developed that attempt to describe moisture sorption isotherms. A recent publication provides an up-to-date introduction to this literature (13).

Most foods are consumed at high A_w levels because people like food to have a moist and tender texture. Ready-to-eat foods with a low A_w are generally snack foods such as potato chips and crackers. These products must absorb saliva and soften rapidly during mastication to be acceptable. Foods that remain dry during mastication are not relished. This preference for foods with a moist and tender texture conflicts with their keeping properties. Molds will grow on the food with A_w above about 0.7, yeast grows at A_w above about 0.8, and bacteria above about 0.9. Although refrigeration inhibits the rate of growth of spoilage microorganisms and freezing, thermal sterilization or preservatives prevent microbial growth, the major volume of the present food supply is kept at $A_w < 0.7$ for long-term storage. Cereal grains, seeds such as beans and nuts, honey, dried fish, fruit, milk, and vegetables must be brought to $A_w < 0.7$ to prevent microbial growth. A large part of food processing and preparation is designed to convert a microbiologically stable low A_w food with unacceptable texture into another form that is texturally desirable, but renders the food subject to microbial spoilage. Spaghetti and other pasta shapes, rice, and bean seeds are stored at ambient temperature in the home and are moistened and softened during preparation in the kitchen. After preparation they must be promptly consumed or refrigerated. Grains of wheat are converted into bread, cake, and similar tender-textured foods in readiness for consumption. Grains of barley are converted into beer. At the present time few foods are known to possess a tender texture at A_w low enough to prevent microbial growth. The goal of producing intermediate-moisture foods with a tender moist texture, acceptable flavor, and long shelf life has proved difficult, as many research laboratories will attest.

The relationship between water activity and texture is specific to each kind of food. These will now be discussed on an item-by-item basis.

APPLES

Bourne measured texture profile parameters of fresh apple and dehydrated apple equilibrated to A_w levels of 0.85, 0.75, 0.65, 0.55, 0.44, 0.33, 0.23, 0.12, and 0.01 (14). Cubes 10 mm on each side were cut from large pieces of apple after equilibration to the appropriate water activity level and subjected to 90% compression two times in a universal testing machine at a speed of 50 mm/min. The detailed procedure for instrumental texture profile analysis has been given (15,16). Six representative force—time curves generated by the Instron are shown in Figure 3. In each graph the left-hand curve represents the first compression and the right-hand curve, the second compression. In no case did the force—time plot fall below zero to a negative value which indicates zero adhesiveness at all A_w levels. The changes in textural properties of apple will be first described in qualitative terms referring to Figure 3 and then in quantitative terms referring to Figure 4. The curve for fresh apple with a calculated $A_w = 0.99$ is shown in the top right-hand side of Figure 3. The initial steep linear slope indicates that the apple is firm with low deformability. The sharp reduction in force that follows indicates sudden failure and that the apple is fracturable. The force continues to rise with continued compression reaching a maximum at the end of the compression; this force peak is defined as hardness. The force quickly falls to zero during decompression. In the second compression cycle the force rises smoothly to a peak with no sudden drops in force during the compression. The area under the compression curves is a measure of the work required to compress the apple. The second compression requires much less work than the first compression. The ratio of area under second compression curve to area under first compression curve is defined as cohesiveness. It is low for fresh apple. The horizontal distance between a perpendicular dropped from the force peak on the second compression curve and the point where the force curve moves off the baseline represents the height the apple recovered between the first and second compressions and is defined as springiness. It is low for fresh apple.

The curve for apple at $A_w = 0.85$ is shown in the top left-hand side of Figure 3. The shape of the first compression curve is greatly different from that for fresh apple. There is no fracturability peak. The initial slope is low, indicating that the rigidity has been lost and the deformability has become high. The maximum force (hardness) is about the same as for fresh apple at about 100 N. The area

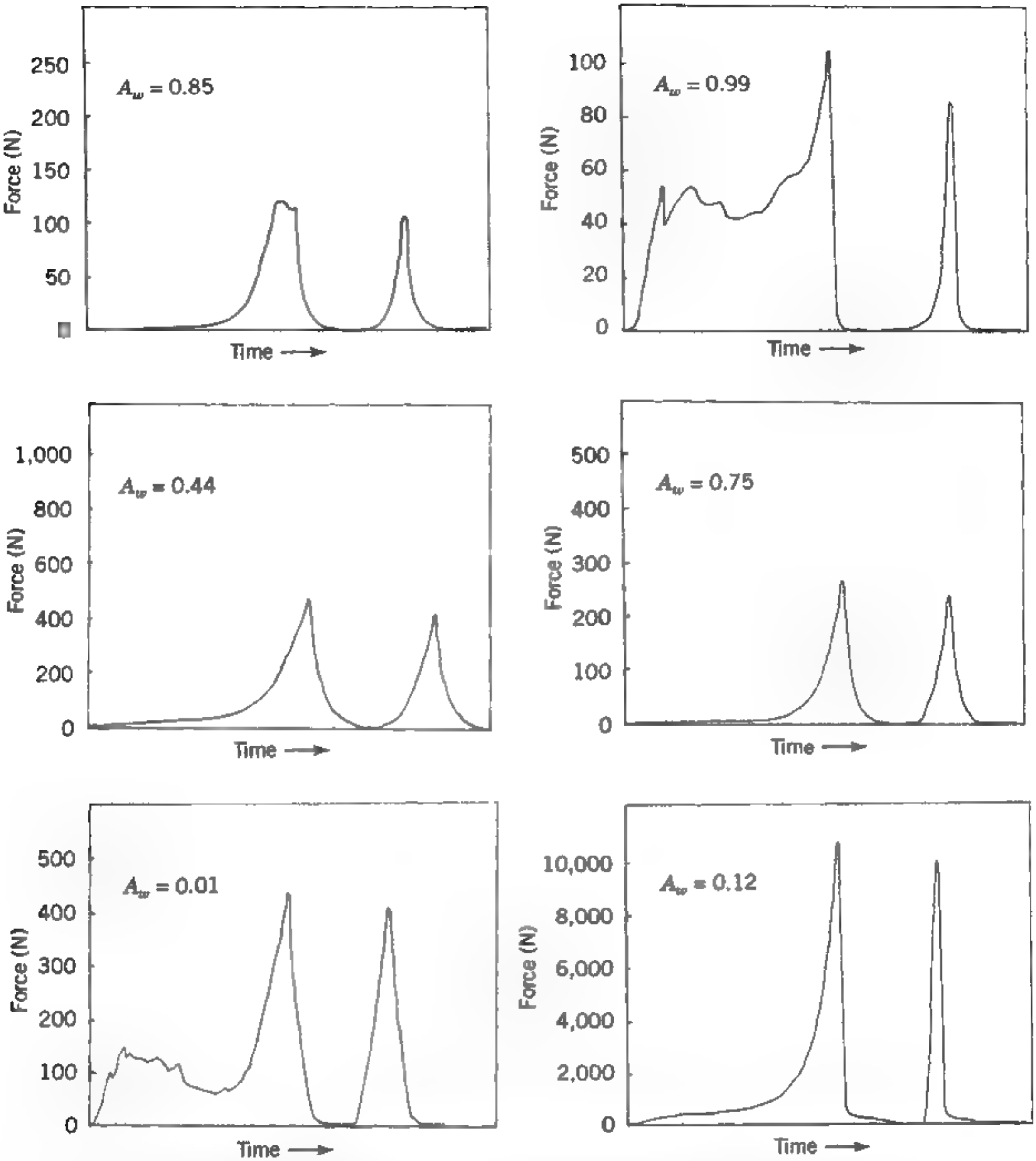


Figure 3. Typical force–time curves traced from Instron charts of texture profile analyses (two 90% compressions) on apple tissue dried to various water activity levels; note the differences in the force scales on the ordinates. *Source:* Ref. 14.

under the force curve in the first compression is much smaller than for the fresh apple, while the area under the second compression curve is about the same. This leads to an increased value of cohesiveness. There is little change in springiness. The Texture Profile Analysis (TPA) curve for apple at A_w levels 0.75, 0.65, 0.55, 0.44. 0.33, and 0.23 are qualitatively similar and are represented by the two curves with $A_w = 0.75$ and 0.44 in the center of Figure 3. They are characterized by no fracturability, high deformability (low initial slope), and high cohesiveness. The hardness increases as the A_w is lowered. Note that the force scales in Figure 3 are increasing. The shape of these TPA curves resembles those for meat. Sensorial evaluation showed that the dominant textural characteristics are chewiness, toughness, and sponginess somewhat like that of meat. The texture profile at $A_w = 0.12$ is similar to those for higher A_w levels except that cohesiveness and springiness are lower and hardness are extraordinarily high. It is

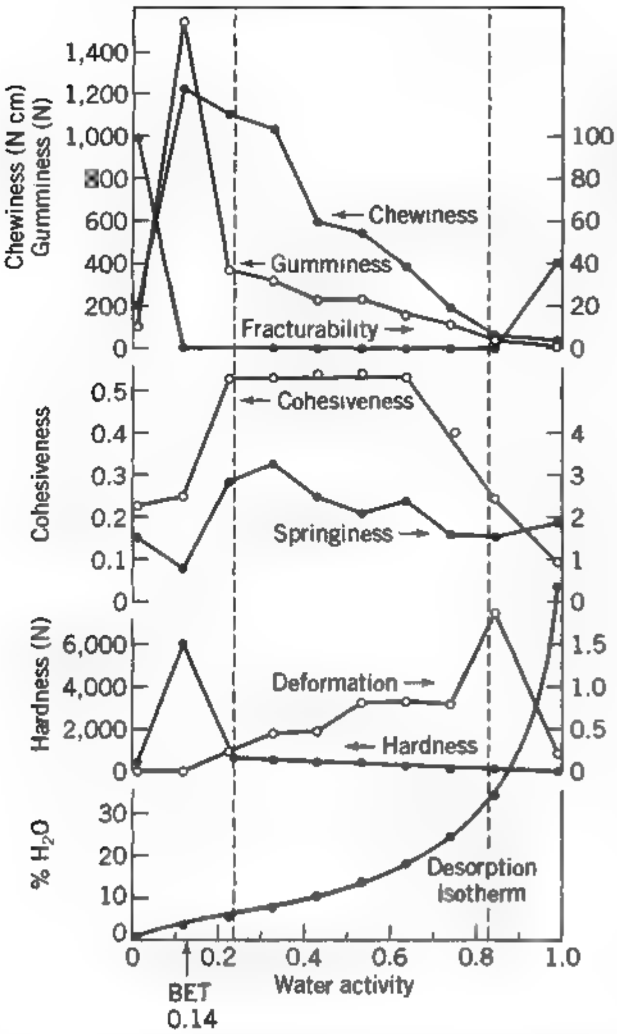


Figure 4. Moisture sorption isotherm and texture profile parameters of apple flesh equilibrated to various A_w levels. *Source:* Ref. 10.

remarkable that a little 1-cm cube of apple requires a force as high as 10,000 N (~1/2 ton!) to compress it by 90%.

There is an abrupt change in texture profile as A_w is reduced below 0.12 as shown in the lower left-hand graph in Figure 3. The fracturability peak reappears, the hardness drops and the shape of the curve resembles that of fresh apple but with higher fracturability and hardness.

The changes in texture profile parameters with decreasing moisture are consolidated in Figure 4. The desorption isotherm for apple as shown at the bottom of Figure 4 is consistent with desorption isotherms for apples and apple products that have been reported by others (11). The texture—A_w curves can be divided into three distinct stages during desorption of water. In the first stage the moisture content drops from 87 to 35% and A_w from 0.99 to 0.85. This causes total loss of the turgor pressure and fracturability of the fresh apple, a great increase in deformability and cohesiveness and minor changes in hardness, springiness, gumminess, and chewiness.

In the second stage the moisture content drops from 35 to 5% and the A_w from 0.85 to 0.23. This causes a progressive increase in hardness, springiness, gumminess, and chewiness and a decrease in deformation. Cohesiveness increases from $A_w = 0.85$ to $A_w = 0.65$ and then remains at a high level from $A_w = 0.65$ to 0.23. This increase in hardness as apple is dried was confirmed by a later study reporting the cutting-shear force of dehydrated apple over the range A_w 0.77 to 0.29 (17).

The third stage is at a water content below 5% and $A_w < 0.23$ and is characterized by rapid seesaw changes in textural properties. Hardness and gumminess increase rapidly from $A_w = 0.23$–0.12 and decline just as sharply between $A_w = 0.12$ and 0.01. Chewiness falls rapidly between $A_w = 0.12$ and 0.01. Springiness and cohesiveness decrease sharply between $A_w = 0.23$ and 0.12. Fracturability is zero at $A_w = 0.23$ and 0.12 and reappears at $A_w = 0.01$ with a value of about 100 N, which is more than twice the fracturability value of 40 N for fresh apple. At the lowest A_w level (0.01) the textural parameters resemble that of fresh apple but with much higher hardness and fracturability. At all A_w levels between 0.12 and 0.99 the texture is tough, leathery, deformable, and chewy with zero fracturability and crispness.

The Brunauer–Emett–Teller (BET) value is the A_w level at which one complete monolayer of water molecules covers all the hydrophilic sites in the food. For apple the BET was calculated to be at $A_w = 0.14$, which is about the middle of the third stage. The transition from the multilayer to a monolayer of absorbed water and from a monolayer to almost zero water content is associated with major textural changes in apple flesh.

[illegible]

Kapsalis, from the U.S. Army Natick Laboratories in Massachusetts, published some of the earliest and most comprehensive studies on texture and water activity in beef (18–20). He cooked beef semimembranous muscle to 63°C and, when cooled, cut it into 12-mm-thick slices which were frozen, freeze-dessicated over silica gel at 0.3 mmHg pressure, and then remoistened to a series of higher moisture levels. After equilibration the texture was measured in the standard 10-blade test cell of the Food Technology Corp. texture press (Kramer shear press). The cutting-shear force increased as the A_w was raised from 0 up to 0.8 and then decreased sharply at A_w levels above 0.8 (Fig. 5). There was considerable variation in the absolute values of the texture press force from one animal to another as demonstrated by the two curves in Figure 5, but the peak force was always around $A_w = 0.8$.

In another experiment beef semimembranosus muscle was cooked to 63°C and when cold cut into 12-mm-thick slices, freeze-dried, and then remoistened to a series of moisture levels. 38-mm-diameter disks were cut out and compressed 25% two times between extensive flat surfaces in a universal testing machine. This is less destructive than the texture press described above. The following mechanical properties were measured from this test:

Secant modulus—the ratio of stress to strain at 3.5% compression. This parameter is now called modulus of deformability.

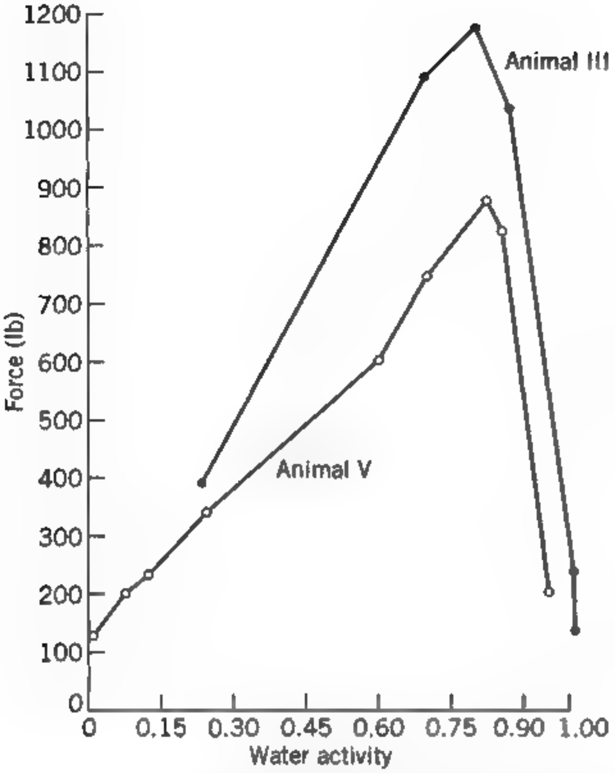


Figure 5. Effect of A_w on maximum cutting–extrusion force of precooked, freeze-desiccated beef that has been remoistened. *Source:* Ref. 19.

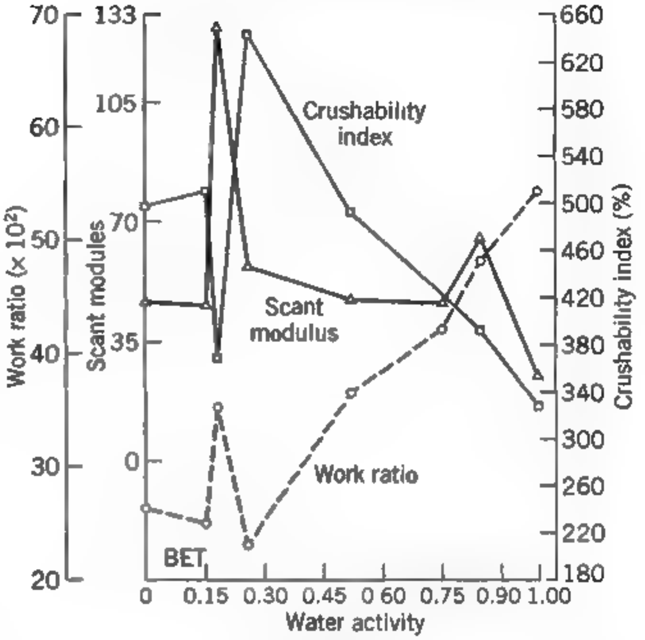


Figure 6. Effect of A_w on mechanical properties of precooked, freeze-dried beef that has been remoistened. *Source:* Ref. 19.

Degree of elasticity—the ratio of the elastic deformation to the sum of the elastic and plastic deformations derived from the load–unload curve of the first compression cycle.

Toughness—work per unit volume to compress the sample 25%. It is derived from the area under the curve up to 25% compression.

Crushability index—The ratio of nonrecoverable to recoverable work in the first compression. It is defined as (area under compression portion minus area under decompression portion of a curve)/(area under decompression portion of the curve).

Work ratio—the ratio of the area under the curve of the second compression to the area under the curve of the first compression. This value is similar to the cohesiveness parameter of the instrumental texture profile analysis.

The changes in work ratio, secant modulus, and crushability index are shown in Figure 6. There was little change in these three properties as the freeze-dried beef absorbed moisture from $A_w = 0$ to 0.15. Rapid seesaw changes occurred between $A_w = 0.15$ and 0.30, which spans the BET monolayer $A_w = 0.16$. Work ratio and secant modulus rapidly increased and just as rapidly decreased while the crushability index rapidly decreased and just as rapidly increased again. The crushability index steadily decreased and the work ratio increased as the A_w was raised above 0.3 while the secant modulus showed a downward trend. The changes in secant modulus and work ratio are plotted again in Figure 7 but with both moisture content and A_w shown on the abscissa, and the A_w scale is nonlinear. Degree of elasticity and toughness are also shown in Figure 7. Elasticity follows a similar trend to work ratio and toughness is similar to secant modulus. Both of these properties change rapidly near the BET level of A_w. The texture press force shown in Figure 5 is often considered as an index of toughness of meat, and it peaks at $A_w = 0.8$ and shows no rapid changes near the BET A_w level. In contrast, the toughness curve shown in Figure 7 peaks at $A_w \sim 0.2$. The difference between texture press force and toughness is probably a result of the texture press being a highly destructive test while the toughness value is obtained by a different test that is approaching a nondestructive mode.

Figure 7 also shows the differential entropy change $\overline{-S^\circ}$ with changing moisture content. It was pointed out that the sorption of water could cause two opposing trends: (a) $\overline{-S^\circ}$ increases due to an increase in crystallization or similar effect or (b) $\overline{-S^\circ}$ decreases due to increased movement of the polymer network chains, unfolding of peptide chains or local solubilization (19). The $\overline{-S^\circ}$ curve in Figure 7 first decreases sharply, then increases sharply, and then alternately decreases and increases to a lesser extent with increasing A_w. Complex changes must be occurring in this system to account for this unusual $\overline{-S^\circ}$ curve.

Other workers rapidly equilibrated cubes of freeze-dried beef at 0, 20, 40, 60, 80, and 100% RH and measured hardness and chewiness by a 35–40% compression of the cubes in a universal testing machine (21). Although plagued by variations in the operating conditions of the freeze-drier a statistical fit of the pooled data showed that

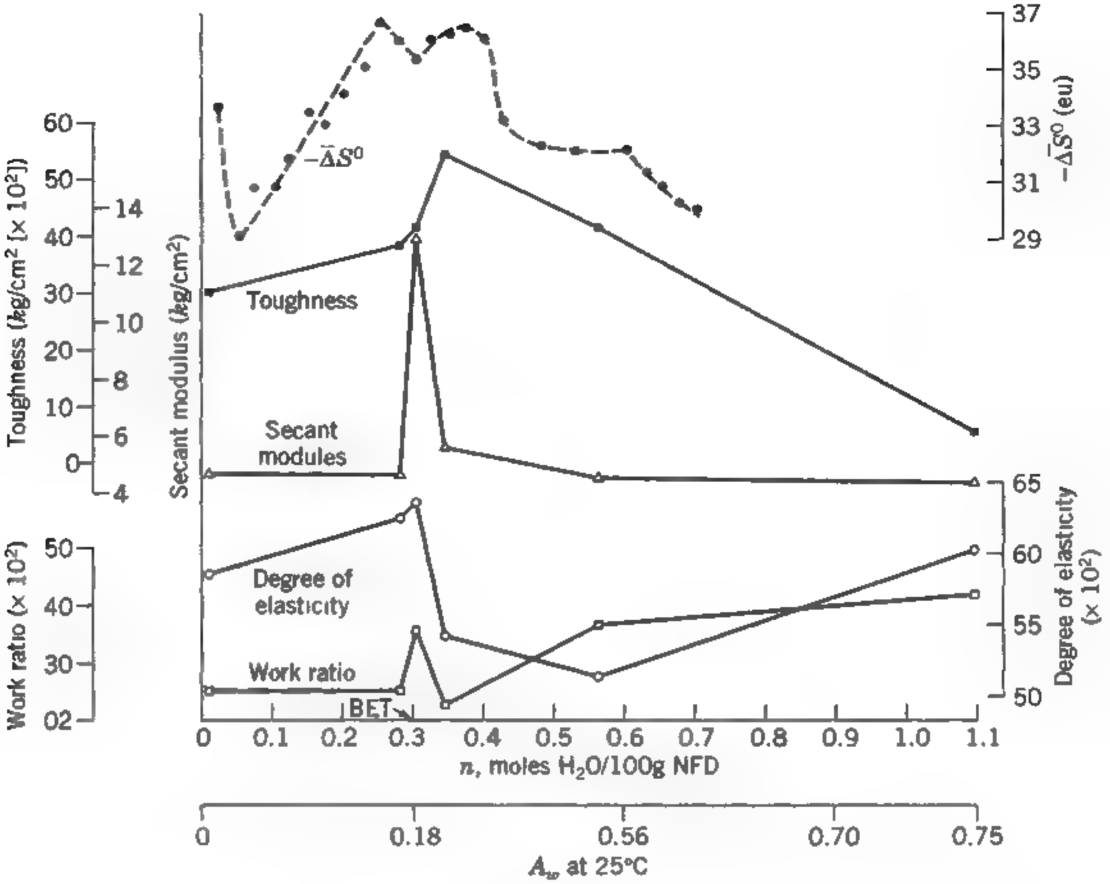


Figure 7. Relationships between textural properties measured by compression testing and standard differential entropy of water vapor sorption in precooked, freeze-dried beef as it is moistened (NFD = nonfat dry solids). *Source:* Ref. 19.

both hardness and chewiness increased from $A_w = 0$ to 0.4 and decreased from A_w 0.4 to 1.0. In a subsequent study (22) the same group reported that after 6-month storage both hardness and chewiness increased over the full A_w range (Fig. 8) with no maximum at $A_w = 0.4$.

Another group in England prepared intermediate moisture beef by immersing slices cut from longissimus dorsi muscle of one steer in a solution of 9.5% NaCl and 39.4% glycerol, cooking, and then storing at 38°C for up to 12 weeks under aerobic and anaerobic conditions (23). The samples equilibrated at $A_w = 0.83$ and 42% H_2O. The Warner–Bratzler shear values declined during storage. (This test is widely used as an index of toughness of meat.) An instrumental texture profile analysis was used to obtain four additional texture parameters: (*1*) hardness, which declined; (*2*) elasticity, which declined after 6 weeks and then increased up to 12 weeks; (*3*) cohesiveness, which followed the same down–up trend as elasticity and (*4*) adhesion, which decreased. These workers attributed the discrepancies between their results and the results discussed in the previous paragraph to traces of impurity in the glycerol reacting with the proteins in the beef.

It is evident from the differing reports between different researchers on beef texture that the relationships between A_w and texture of beef is not clear. It is well known that a number of chemical and biochemical changes can occur in stored foods at all levels of A_w. Perhaps the time in storage has as great an effect on textural properties as the actual A_w level. It is noteworthy that one group of investigators working with beef (18–20) and another group working with apples (14) both found dramatic changes in textural properties around BET A_w level.

CEREAL GRAINS

Rice

Single grains of rice of the cultivar IR8 fashioned into little cylinders and equilibrated to various moisture levels were compressed to failure in a universal testing machine (24). Three compression speeds (2.54, 0.508, 0.127 mm/min) and five test temperatures (25, 36, 47, 58, 69°C) were used. Although the absolute values of failure stress changed with different compression speeds and temperatures, the same trend of decreasing strength with increasing moisture content was shown for all experimental conditions. The data for 36°C and 2.54 mm/min compression speed (plotted in Fig. 9) are typical of the trends for all the other

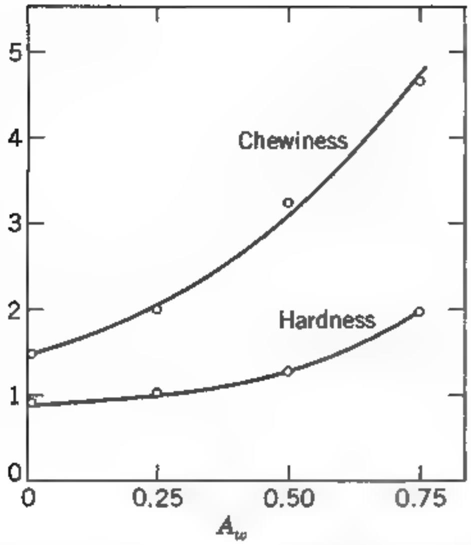


Figure 8. Effect of A_w on hardness and chewiness of freeze-dried beef after 6-month storage at 38°C. *Source:* Plotted from data in Ref. 21.

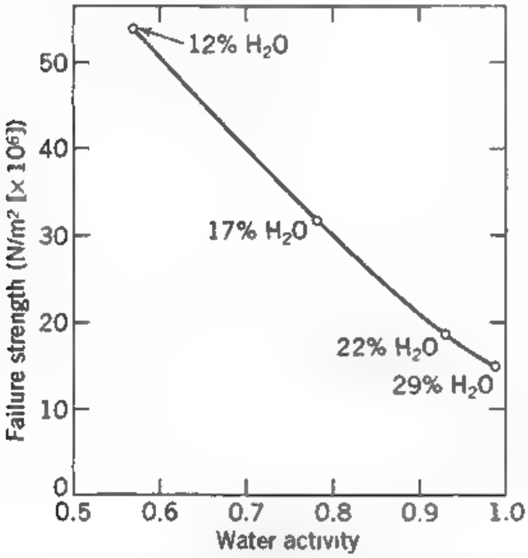


Figure 9. Failure strength of dehulled rice grains compressed 0.254 cm/min in a universal testing machine at 36°C. *Source:* Plotted from data in Ref. 24.

data. These researchers expressed moisture content on a percentage dry-matter basis. A water sorption isotherm for rice (25) was used to convert these data to an A_w basis for illustration in Figure 9. There is a fourfold decrease in failure strength as the moisture content of the whole rice grain increases from 12% H_2O (0.57 A_w) to 29% H_2O (0.93 A_w). In a later report, the same authors (26) showed that the uniaxial compression modulus and relaxation modulus, Young's modulus (E), shear modulus (G), and bulk modulus (K) of rice kernels all decreased with increasing moisture content. However, Poisson's ratio (μ) was practically unchanged.

Wheat

The apparent modulus of elasticity of whole grains of wheat of the Talent variety over a wide range of moisture has been measured (27). One interesting feature of this work was that the modulus of elasticity was measured after both sorption and desorption to different water activity levels to determine whether any hysteresis effects were present. One-half of the grain at 16% H_2O (dry-matter basis) was dried under vacuum with no heating to 5% H_2O. The other half was mixed with sufficient water containing dye to raise the moisture level to 33%. Blends of the dried grain and moistened grain were made, stored in closed containers, and slowly and continuously mixed for 3 weeks until the moisture in the grains equilibrated.

Whole grains of wheat were compressed at 0.5 mm/min in a universal testing machine, and the apparent modulus of elasticity was calculated from the slope of the initial linear section of the force deformation curve. The Hertz equation was used to correct for differences in dimensions between individual kernels. The uncolored grains were known to have absorbed water to reach their equilibrium water content, and the colored grains were known to have desorbed water to reach the same equilibrium water content (27). Two plots of percentage water (dry-matter basis) versus water activity were obtained: an absorption curve and a desorption curve. The absorption curve lay below the desorption curve.

Figure 10 shows that for both absorption and desorption the elastic modulus decreases rapidly with increasing A_w and moisture content. There is about a sevenfold decrease in the modulus over the moisture level range studied. In

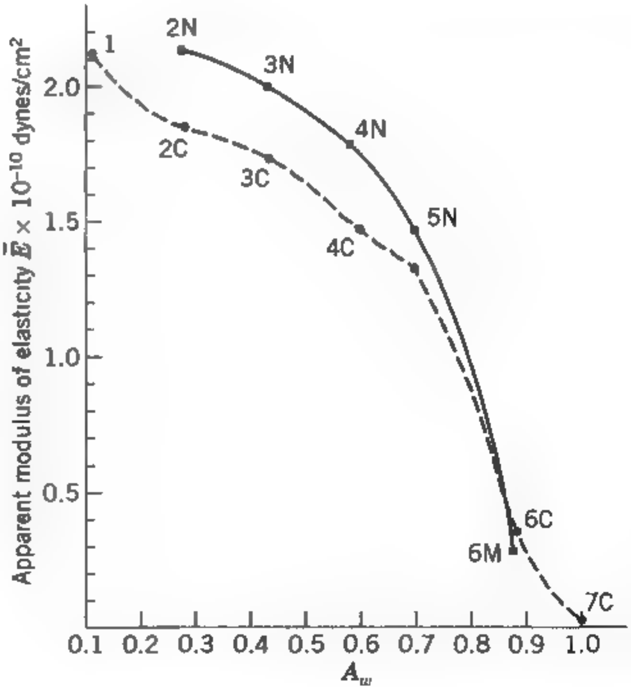


Figure 10. Apparent modulus of elasticity ($E \times 10^{10}$ dyn/cm²) of wheat grains versus A_w; (—) absorption curve; (—) desorption curve. *Source:* Ref. 27.

the 0.2–0.6 A_w range the apparent modulus of elasticity for desorbing grain was about 13% less than that for absorbing grains at the same A_w level. The steep decrease in modulus of wheat grains with increasing moisture level is similar to the decrease in failure strength of rice grains discussed above (24) and in Figure 9.

A Canadian group measured physical properties of wheat grains equilibrated to 9.5, 11.0, 12.5, 14.0, and 15.5% H_2O, which corresponds to a range of 0.44–0.75 A_w (28). Three kinds of wheat—hard red spring, soft white, and durum—were studied and six different parameters were measured:

1. Wheat hardness index (WHI) which is the maximum torque produced by grinding in a two step Brabender hardness tester (BHT) divided by the percentage yield of flour.
2. Wheat hardness index measured in a one-step BHT.
3. Time (in seconds) to grind a standard sample of wheat into flour in a two-step BHT.
4. Pearling resistance index (PRI), which is the weight in grams after pearling 20 g of wheat grains for 20 s in a Strong Scott barley pearler.
5. Torque needed to grind grain in a two-step BHT.
6. Particle size index (PSI), which is the percent of flour passing through a 177-μm sieve after grinding in a two-step BHT.

The results are shown in Figure 11. The torque and both WHIs decreased with increasing moisture content (A,B,E). The time to grind (C) and the pearling resistance index (D) increased. Particle size (F) is practically constant, indicating that the locus of the failure planes in the wheat kernels is unaffected over this moisture level range.

Corn

The stress-relaxation properties of yellow dent corn were measured over the A_w 0.12–0.86 (H_2O 9.7–26% d.b.) (29) and temperature range 25–100°C. The relaxation modulus decreased with increasing A_w. Although the decrease was unidirectional, there was a transition between A_w 0.55 and 0.76; the corn kernels were brittle below A_w 0.55 and ductile above A_w 0.76. The same group found a similar brittle-to-ductile transition in durum semolina at about the same moisture content.

CEREAL-BASED FOODS

Changes in textural quality of a limited number of processed cereal foods have been measured over a range of water activities. Sensory and physical textural parameters of three commercially available snack foods pre-equilibrated to water activity levels A_w = 0, 0.549 and 0.653 have been measured (30). These data are summarized in Table 1. In every case sensory crispness and sensory crunchiness declined with increasing A_w levels while compression force and compression increased. The same trends were noted for saltine crackers equilibrated at A_w = 0, 0.44 and 0.653 (30).

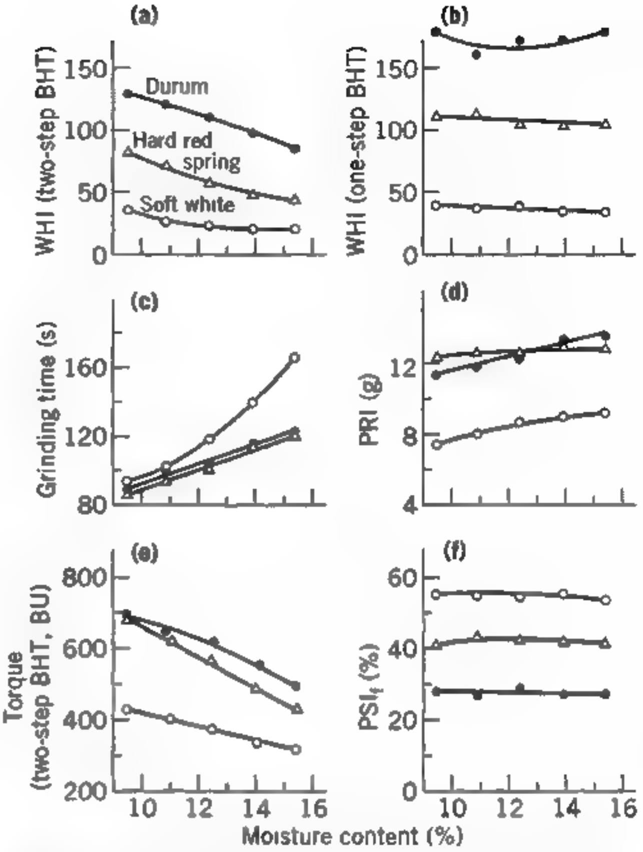


Figure 11. Hardness indices for three classes of wheat as a function of moisture content equivalent to A_w = 0.44–0.75; details of the six tests are given in the text. *Source:* Ref. 28.

Another group measured the sensory crispness of four commercial snack foods at A_w = 0–0.75 (30). Saltines, potato chips, puffed corn curls, and popcorn each lost their sensory crispness approximately linearly with increasing A_w. The puffed corn curl had almost zero crispness at A_w = 0.52, saltines and potato chips had zero crispness at A_w = 0.75, and popcorn was still slightly crisp at A_w = 0.75. Figure 12 shows the crispness versus water activity curve for popcorn. Hedonic scores declined in a similar fashion to crispness scores. These workers also reported that the initial slope of the force–distance compression curve for saltine crackers decreased with increasing water activity.

A group in France measured sensory crispness and crushing strength of breakfast cereals over the range A_w = 0.84 (32). The sensory crispness of corn flakes and rice crispies declined slowly from A_w = 0.53 then rapidly from A_w = 0.53 to 0.71. Crispness was very low at A_w > 0.71. The crispness curve for corn flakes is shown in the right-hand side of Figure 13. The crushing force for these breakfast cereals showed a remarkably similar pattern to the crispness curve (left-hand side of Fig. 13). It decreased slowly from A_w = 0 to 0.53 and then rapidly above A_w = 0.53.

Another group in Massachusetts (33) observed the characteristic sigmoid shape of the above data from France and

Table 1. Sensory and Physical Properties of Three Snack Foods

	Crunch twist[a]			Potato chips[b]			Rippled potato chips[c]		
Property	$A_w = 0$	$A_w = 0.549$	$A_w = 0.653$	$A_w = 0$	$A_w = 0.549$	$A_w = 0.653$	$A_w = 0$	$A_w = 0.549$	$A_w = 0.653$
Sensory crispness[d]	10.7	7.7	6.7	10.7	9.0	6.0	10.0	6.7	5.0
Sensory crunchiness[d]	9.7	6.7	5.7	11.3	8.7	6.3	10.0	7.0	5.0
Compression force, N	92	326	537	846	1042	1224	415	592	754
Compression work,[e] mJ	345	1057	1598	2630	2972	3537	566	796	1045

[a] Keebler and Co., Chicago.
[b] O'Grady's, Frito-Lay, Dallas.
[c] Pringles, Procter and Gamble, Cincinnati.
[d] Trained panel using a 0–13-point scale.
[e] Units of product compressed under a standard Food Technology texture press 10-blade plunger mounted in a universal testing machine (selected data from Ref. 30).

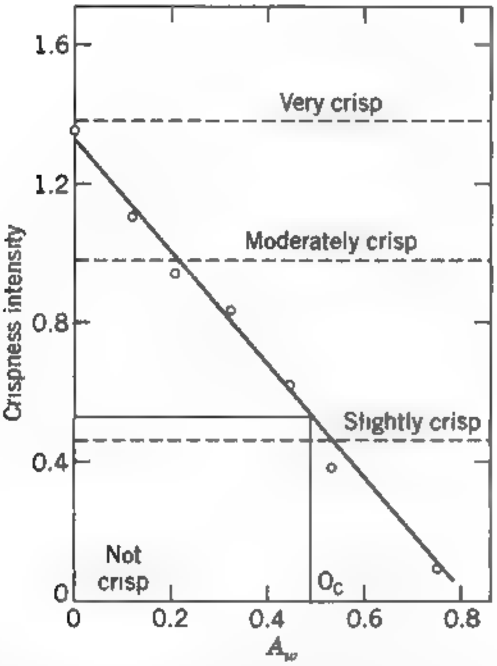


Figure 12. Sensory crispness intensity of popcorn vs A_w. *Source:* Ref. 31.

showed that it fitted the Fermi function with a correlation coefficient of $r^2 \geqq 0.99$:

$$Y(A_w) = Y_0/\{1 + \exp[(A_w - A_{wc})/b]\}$$

where $Y(A_w)$ is the sensory score at a given A_w level and Y_0 the sensory score of the cereal in the dry glassy state, A_w is the water activity of the sample, A_{wc} the critical water activity representing the inflection point in $Y(A_w)$, and b a constant representing the steepness of $Y(A_w)$ in the water-activity region where much of the plasticization occurs.

The same group (34) showed that the above equation fitted the force to compress other brittle cellular cereal foods with correlation coefficients $r^2 \geqq 0.955$ for cheese balls, cheese puffs, French bread croutons, and pumpernickel croutons and r^2 values from 0.79 to 1.00 for the acoustic and mechanical signatures of the same products (35). They also showed (36) r^2 values of the Fermi equation of $r^2 \geqq 0.988$ for almonds and hazelnuts and $r^2 \geqq 0.99$ for kidney beans and chick peas.

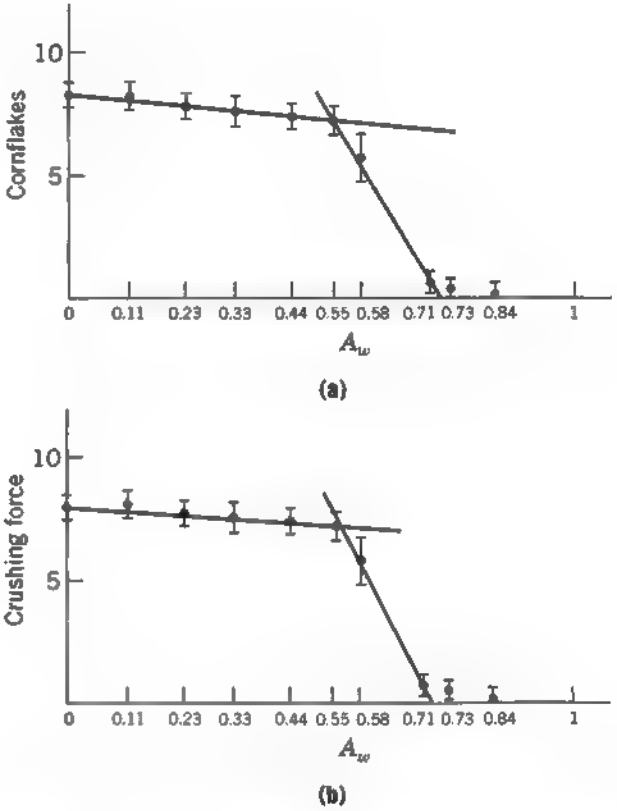


Figure 13. (a) Sensory crispness of cornflakes vs A_w; (b) crushing force of cornflakes vs A_w. *Source.* Ref. 32.

The effect of moisture content on mechanical and textural properties of two cornmeal extrudates has been measured (36). Yellow cornmeal was passed through a Brabender one inch diameter single screw extruder fitted with a 5-mm-diameter die. Sample A was prepared with a feed moisture content of 25%, barrel temperature 60°C in zone I and 80°C in zone 2 and a screw speed of 120 rpm. The extrudate had a solid spaghetti like structure, a bulk density of 1.25 g/mL, and a mean diameter of 7.4 mm. Sample B was prepared with a feed moisture content of 50%, barrel temperatures 120°C in zone 1 and 180°C in zone 2, and a screw speed of 180 rpm. There were many tiny pores in the extrudate due to the violent puffing. The bulk density was 0.914 g/mL and mean diameter, 6.3 mm. After extrusion the samples were equilibrated at moisture contents ranging from 3.4 to 31.3% and compressed 30% two times in a universal testing machine. Figure 14 shows the compressive strength (maximum force per initial unit area) as a function of moisture content. For the high-density extrudate A, the strength increases from 3.9 to 8.9% moisture, remains level from 8.9 to 15.3% moisture and declines again at moisture levels above 15.3%. The low-density extrudate B shows a similar pattern except that the strength is about half that of sample A. The values for stiffness and fracturability show an inverted U shape similar to compressive strength. The values for springiness increase and for relaxation modulus decrease monotonically with increasing moisture content. The relaxation time for both samples declines as the moisture rises from 4.7 to 15.7% and increases at moisture levels above 15.7%.

The breaking strength of freshly baked sugar snap cookies after equilibration for 5 days in atmospheres ranging from 11 to 93% RH was measured with a single-blade compression bar mounted in a Food Technology Corp. texture press (38). The results are shown in Figure 15. The breaking strength of cookies prepared from hard wheat flour (low quality) was always less than for cookies made from soft wheat flour (high quality). The breaking strength decreased with increasing A_w with the fastest rate of increase occurring between $A_w = 0.11$ and 0.33. A parallel trend was found for tenderness of these cookies (38).

The texture of puffed whole-grain brown rice cakes equilibrated to A_w levels from $A_w = 0.23$–0.84 was measured by means of a punch-and-die compression test performed in an Instron (39). Crispness decreased with increasing A_w reaching zero at $A_w = 0.57$. Hardness increased from $A_w = 0.23$–0.65 and decreased from 0.65 to 0.84. Rice cakes with A_w between 0.23 and 0.44 were crisp, low in hardness, and required less work to snap.

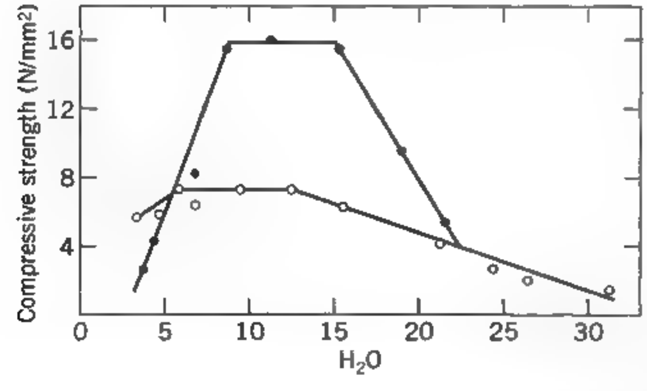


Figure 14. Compressive strength of two cornmeal extrudates as a function of water content. ● high-density sample; ○, low-density sample. *Source:* Plotted from data in Ref. 37.

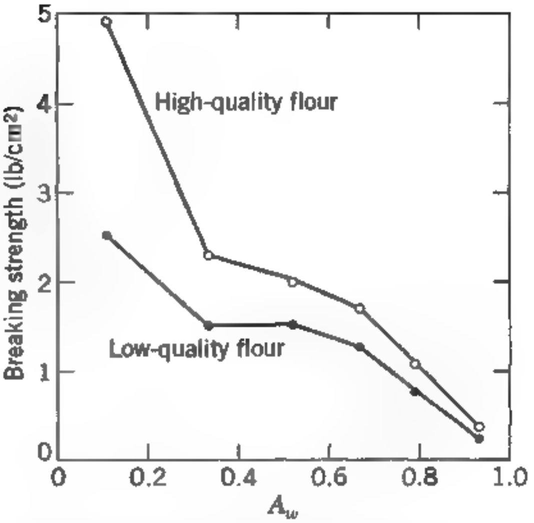


Figure 15. Breaking strength of sugar-snap cookies made from high-quality cookie flour (soft red winter wheat) and a low-quality cookie flour (hard red winter wheat). *Source:* Plotted from data in Ref. 38.

The texture of a breakfast cereal made from barley flakes was studied over the A_w range 0–0.96 (40) using three-point bending and punch-and-die compression tests. In the bend test, the fracture force and maximum fiber stress increased more than threefold as A_w increased from 0 to 0.67 and then decreased above $A_w = 0.67$, reaching a low figure at $A_w = 0.87$. In the punch-and-die test, deformation increased from $A_w = 0$–0.32, remained reasonably constant from $A_w = 0.34$–0.75, and increased rapidly from $A_w = 0.75$–0.87; the modulus of elasticity declined slowly from $A_w = 0$–0.75 and then declined rapidly above $A_w = 0.75$, giving a curve similar to that shown for breakfast cereal flakes made from corn and rice in (32).

POWDERS

It has been observed that the cohesiveness of finely powdered foods increases with increasing moisture content (41). Food powders with a higher moisture content had a lower bulk density, lower relaxation constant, and higher compressibility than did low-moisture powders (Table 2). When a cohesive powder is poured into a container, it forms an open structure supported by interparticle forces and contains many voids, imparting a low bulk density, highly compressible, mechanically weak structure. A noncohesive power contains smaller voids created by random orientation of the particles. After compression the cohesive power has a higher tensile strength than the low-moisture powder that results from the greater interparticle attractive forces and a greater number and area of contact points between its particles (42).

Table 2. Some Physical Characteristics of Food Powders

Materials[a]	Moisture (%)	Bulk density (g/mL)	Compressibility	Relaxation constant
Baby formula	dry	0.49	6.4	2.6
Baby formula	1.0	0.46	8.1	1.5
Bran	2.8	0.55	5.0	2.0
Bran	5.4	0.42	6.7	1.4
Onion powder	0.7	0.66	8.6	2.0
Onion powder	1.2	0.55	9.5	1.4
Onion powder	2.3	0.50	8.1	1.0
Sucrose	dry	0.77	4.5	4.0
Sucrose	0.17	0.71	6.0	2.5

Source: Ref. 41.

[a] All material had a particle size − 100 + 200 mesh.

It has also been reported that the rate of caking of onion powder with a moisture content of 4–5% (which is equivalent to A_w = 0.30–0.35) increased rapidly with increasing temperature (43). At 15°C there was no tendency for the onion powder to cake after 6 months of storage. At 25°C agglomerates formed in 7–10 days, at 30°C caking occurred in 7–10 days, and at 35°C caking occurred in 3 days. With onion powder of 7% moisture no anticaking agents were successful while at 3% moisture the powder remained free-flowing even without caking agents after 30 days at 35°C. Since increasing the temperature increases the A_w of foods held at constant moisture content it seems likely that the caking of onion powder is directly related to its water activity.

A group on Brazil studied caking phenomena in tropical fruit powders (44). They found that the degree of caking of freeze-dried guava powder increased steadily but slowly from about A_w 0.3 to 0.6. From A_w 0.6 to 0.85 the degree of caking increased very rapidly with or without anticaking agents present. These authors also showed that at high temperatures (above about 40°C) caking always occurred regardless of the water activity in the powder and they point out that other workers have also had similar results at high temperatures.

SUGARS

Dry sugar can exist in two forms. One is as a glassy amorphous solid. Fine powders of glassy sugars are usually free-flowing and not sticky. The other form is crystalline, which is thermodynamically more stable than the glassy state but is often sticky. Under suitable conditions the glassy form spontaneously changes to the crystalline form. Each form has its own characteristic moisture sorption isotherm with the curve for the crystalline sugar lying well below that for the glassy sugar. When molten sugars are rapidly cooled, or sugar solutions are sprayed-dried or freeze-dried, they solidify in the glassy form, which is a metastable state. When this dry glassy sugar is exposed to increasing relative humidity atmospheres, it absorbs moistures and begins to form a conventional S-shaped water sorption isotherm. However, when the A_w rises sufficiently, the glassy sugar spontaneously changes into the crystalline form, which has an entirely different water sorption isotherm and a sharp negative slope appears in the water sorption isotherm (Fig. 16 and Ref. 41). This is an example of an exception to the rule discussed earlier (beginning of section on sorption isotherms) that A_w always increases with increasing moisture content. It is caused by change in state of the sugar.

Moisture content of powdered sugars exposed to various relative humidities at 25°C for up to 3 years has been measured (46). The data for sucrose are plotted in Figure 17 using a logarithmic time scale. At 4.6 and 11.8% RH the powders absorbed a little moisture as they reached equilibrium and then the moisture level remained constant for 1,000 days. At 24% RH the moisture content was level for about 80 days and then declined because the sucrose began to crystallize. At 28.2% RH the moisture level began to fall after about 10 days. At 33.6% RH it began to fall immediately, indicating that conversion of the sugar into the crystallized form began immediately. The crystalline sugar caked badly even though it had a much lower moisture content than the glassy sugar. Those authors obtained similar results with amorphous glucose powder (42). This result has been confirmed by others (47) and also has been shown to occur with lactose (45). The addition of gums and stabilizers to powdered sucrose delayed the crystallization process and loss of moisture but had a negligible effect on the moisture content in the sugar after crystallization was complete (48).

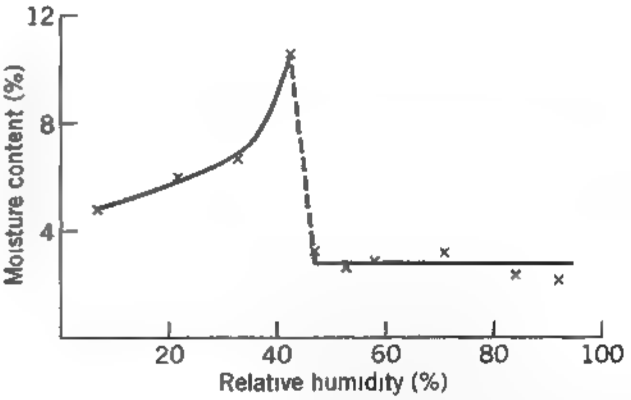


Figure 16. Absorption moisture isotherm for lactose at 25°C; on left-hand side, lactose is in glassy state; on right-hand side, it is in a crystalline state. *Source:* Ref. 45.

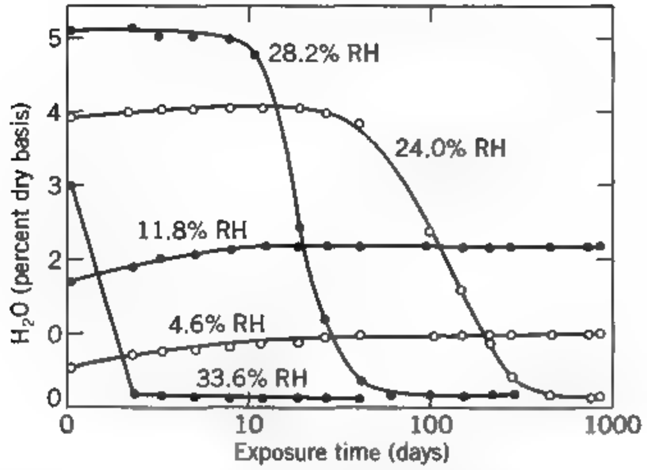


Figure 17. Changes in moisture content of amorphous sucrose powder stored in atmospheres of various relative humidities (RH) at 25°C. *Source:* Ref. 10.

The fact that crystallized sugars equilibrate at a lower moisture content than glassy sugars or sugars in solution is the basis of a clever process to impart shelf-stable contrasting textures to cookies (49). Fresh home-baked cookies are prepared with sucrose and are characterized by a crisp, firm texture on the outside and a soft, moist, chewy texture on the inside because the outside of the cookie is dry while the inside of the cookie is still moist when the cookies are removed from the oven. The moisture begins to migrate from the moist interior to the dry exterior of the cookie as soon as it is taken from the oven until there is uniform A_w throughout the cookie. The sucrose crystallizes, the cookie becomes uniformly dry and hard throughout and the highly relished contrasting textures of the freshly baked cookie are rapidly lost. Home-baked cookies lose their desirable dichotomous texture within a day or two. Traditional store-bought cookies are either hard and dry throughout, or soft and moist throughout because the A_w of all parts of the cookie equilibrates during distribution.

The new process uses two doughs, an outside dough that is extruded around an inside dough with different formulation (49). The inside dough contains a high proportion of crystallization-resistant sugars such as corn syrup or high-fructose corn syrup. During baking and storage these sugars do not crystallize. The outside dough has about the same proportions of flour, shortening, and sugar as the inside dough, but the sugar is mostly sucrose, which crystallizes easily. During the first few days after baking the A_w equilibrates throughout the cookie, the sucrose in the outer part crystallizes and releases most of the water it has absorbed. The center part of the cookie absorbs this moisture because its sugars do not crystallize. Moisture equilibrium is reached throughout the cookie after about 2 weeks of storage at $A_w \approx 0.45$–0.55 but the center of the cookie has a moisture content 2–4% higher than the outside. The result is a cookie that is dry and crisp on the outside and moist and soft on the inside similar to fresh home-baked cookies. The texture and moisture differential is shelf stable and is maintained for many months, unlike fresh home-baked cookies, which lose their dichotomous texture within a few days.

MISCELLANEOUS FOODS

Some reports relating changes in texture with changes in water activity use such a narrow A_w range or so few A_w levels that one cannot confidently predict the textural properties over a wide range of A_w levels. Such reports will not be discussed here. Other researchers vary the method of processing or the formulation in so many ways (eg, water content, fat content, protein content, and salt content of sausages) that one cannot find any meaningful relationship between textural properties and water activity. Reports of this kind will not be discussed here because they throw no useful insights into texture—A_w relationships.

Walnuts

Some physical properties of shelled walnut kernels have been studied over the range $A_w = 0.1$–0.85 (50) using three test procedures:

1. Breakage. Cans of unbroken kernels were dropped 915 mm two times onto a concrete floor. The broken kernels were separated and weighed. The percentage of broken kernels decreased continuously with increasing A_w (see Fig. 18), indicating that the walnut meats became less brittle and more difficult to fracture as the moisture content increased.
2. Abrasion. 250-g lots of kernels were placed in 1-L tin cans and vibrated at 7 Hz and 5-mm amplitude for 15 min, and then the kernels were emptied into a tray and the chaff brushed off and weighed. The amount of chaff lost decreased rapidly from $A_w = 0.1$ to 0.55 and very rapidly from $A_w = 0.55$ to 0.75.
3. Pellicle content (skin). An estimate of the amount of walnut meat in the chaff was made on the basis of its fat content and subtracted from the value for the chaff to give the pellicle content. It followed a similar decreasing pattern as the chaff (Fig. 18).

Freeze-Dried Foods

Freeze-dried foods constitute a large portion of the food supply of astronauts because this reduces the weight of food that must be lifted into orbit. A modified texture profile analysis on bite-sized freeze-dried beef, chicken and cheese sandwiches, and chicken bites has been reported (18). Hardness (maximum force on the first bite) and cohesiveness (ratio of work done in second compression/work done in first compression) increased with increasing A_w for all four items (see Fig. 19). The crushability index (which is explained in the section on beef above) decreased for the beef and the chicken sandwiches and the chicken bites. For the cheese sandwich it increased from $A_w = 0$ to 0.5 and then decreased (Fig. 19).

Xixona Turrón

The effect of water activity on the textural properties of *xixona turrón*, a typical Spanish confectionery product

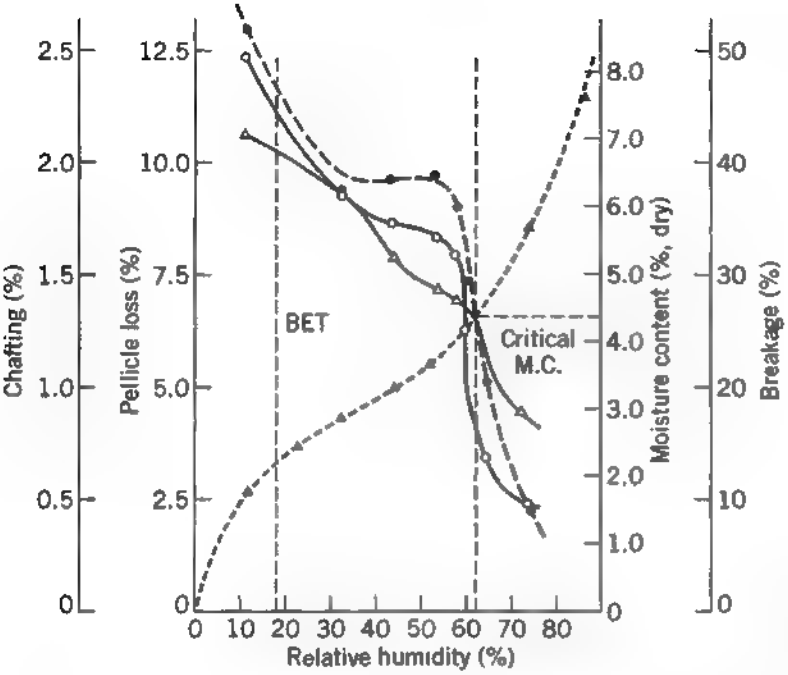


Figure 18. Moisture sorption isotherm and effect of A_w of walnut kernels on pellicle loss, chaffing, and kernel breakage. *Source:* Ref. 50.

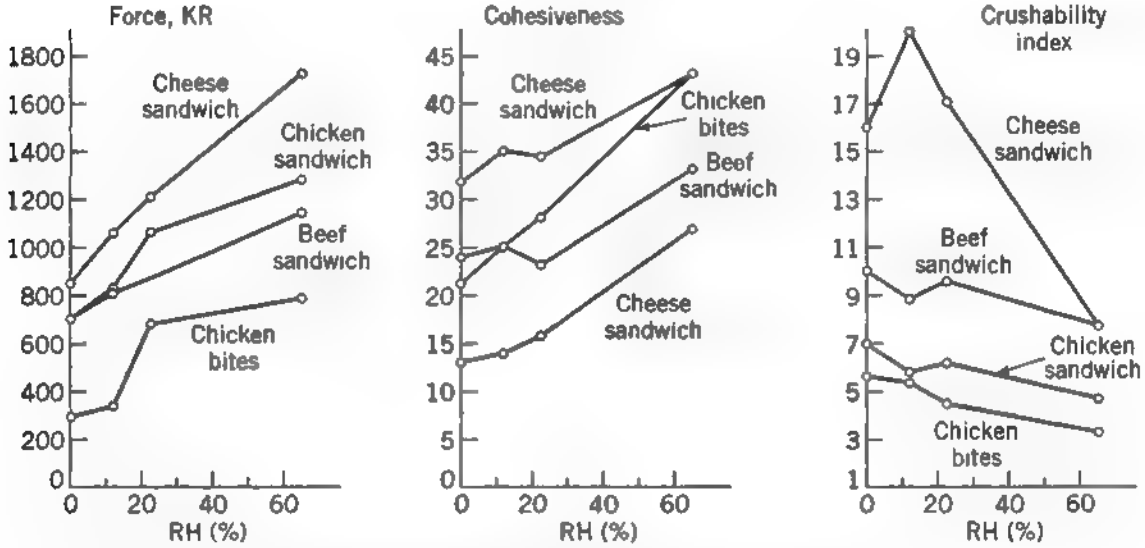


Figure 19. Hardness, cohesiveness, and crushability index vs relative humidity for four dehydrated space foods. *Source:* Ref. 15.

made from toasted almonds, sugar, inverted sugar, honey, and a little egg white is reported (51). The texture profile analysis (TPA) was measured at ~25°C. Hardness and chewiness increased sharply from $A_w = 0.11$ to $A_w = 0.22$, decreased sharply from $A_w = 0.22$ to $A_w = 0.33$, and then continued to decrease at a slower rate from $A_w = 0.33$ to $A_w = 0.75$ while springiness (elasticity) and cohesiveness increased slowly from $A_w = 0.11$ to $A_w = 0.43$ and steeply from $A_w = 0.43$ to $A_w = 0.75$. The glass-transition temperature (T_g) was 24°C at $A_w = 0.11$ and dropped steadily to −46°C at $A_w = 0.75$. Fracturability was only seen in samples with a moisture content lower than the critical water content (CWC), the level at which the product changes from the rubbery state to the glassy state.

Although these authors did not elaborate the point, their data suggest that the textural properties of a food with moisture content near the CWC can change rapidly when the temperature is changed a few degrees to take it through the T_g region. Such a change could be expected to make the food brittle, fracturable, and crisp when cooled below the T_g, changing to leathery and chewy when warmed above the T_g. This data indicates the promising potential for studies involving T_g, A_w, and texture near ambient temperatures.

Fruit Bar

A Canadian group studied the change in cutting force of restructured fruit bars manufactured from apple puree, apple concentrate, water, maltodextrin, low-methoxyl pectin, citric acid, sodium citrate, and $CaHPO_4$ (52). The cutting force using a single blade with a 3 × 70-mm face increased 100-fold from $A_w = 0.86$ to $A_w = 0.33$ and then fell sharply from $A_w = 0.33$ down to $A_w = 0.30$ due to the change from cutting failure to brittle fracture. The general pattern of hardness change was similar to that found with dried apple discussed earlier (14), but the 100-fold increase in the formulated bar was much greater than the 6-fold increase found in dehydrated apple. The authors attribute the difference to the fruit bar being subjected to a cutting type of test and the apple to a uniaxial compression test. Another notable difference between these reports is that the apple test used 10-mm cubes for all A_w levels, whereas the fruit bars had a thickness of 5–6 mm at $A_w = 0.87$, which decreased with decreasing A_w until a thickness of only 1.5 mm was reached at $A_w = 0.3$. Another difference is that the fruit bars appeared to be case hardened at the lowest A_w levels.

Lempuk

This is a traditional semimoist product in Malaysia and nearby countries. It is made by cooking the flesh of durian fruit with sugar until the desired consistency is attained (53). TPA tests on lempuk over the range $A_w = 0.57$–0.84 (26–50% H_2O d.b) showed no fracturability and no change in cohesiveness and springiness over this range of water activity. Hardness and chewiness decreased and adhesiveness increased as A_w increased. There was a sharp change in slope, but not direction, for hardness, chewiness, and cohesiveness at $A_w = 0.75$ (33.6% H_2O), which was attributed to the glass transition for this product at about 33% H_2O and 25°C. However, the T_g for this product was not reported.

Agricultural Commodities

The physical properties of nonfood agricultural commodities such as seeds, hay, and tobacco leaf are often affected by water activity. For example, the breaking force in compression and deformation-to-break of bean seeds both increased as A_w increased from 0.35 to 0.65 (54). With soybean seeds, fewer split seeds and a higher germination was found as the moisture content was increased from 7.2 to 16.2% when the seeds were impacted against a steel plate, but the reverse effect was found when the soybean seeds were impacted against a hard polyurethane plate (55). See also Refs. 6 and 29.

This subject will not be pursued further because these are not foods. The four examples cited above should be sufficient to acquaint the reader with the fact that changing the A_w often has a profound effect on physical properties of nonfood agricultural commodities.

SUMMARY

The results discussed above do not paint a clear unequivocal of the relationships between A_w and texture. There are a number of reasons for this complex behavior.

1. Complex Texture. Every food possesses diverse textural properties that act independently with changing A_w. Over a given A_w range different textural properties in the same food may increase, decrease, or remain steady (Figs. 4, 6, 11, 19).
2. Water Activity Range. Some textural properties change in slope but not in direction with changing A_w (Figs. 8–10, 12, 13, 18). Very rapid changes in textural properties can occur near the BET monolayer (Figs. 4, 6, 7).
3. Previous history of sample treatment. A food that has desorbed water to reach a given A_w may be quantitatively different in texture than when it has absorbed moisture to reach the same A_w (Fig. 10). The time a food is stored can affect its textural properties because of chemical reactions that are occurring in it (22). Polymers such as proteins and complex carbohydrates can undergo complex molecular changes such as crystallization, cross-linking, folding, and unfolding, which can affect the texture.
4. Quality of ingredients. Different quality flours produce cookies with different breaking strengths (Fig. 15). Impurities in ingredients can cause anomalous trends in texture of beef (22).
5. Phase changes. When sugars change from the amorphous into the crystalline form, there are changes in the texture of the pure sugars (16, 17) and in products with a high sugar content (49).
6. Processing conditions. Extruded corn meals at the same A_w manifested substantial differences in textural quality because the different processing conditions in the extruder caused wide variations in structure (14 and Ref. 32).
7. Structure. Native foods such as fish, fruit, meat, and vegetables have a nonhomogeneous cellular structure that is substantially retained during preservation and storage. Some cellular regions may be affected by changes in moisture level more than other regions, eg, fiber bundles vs parenchyma cells in vegetables. The texture of fabricated foods such as cookies, crackers, and sausages can be affected by changes in formulation. For example, changing the fat content (which is hydrophobic and essentially inert to changes in moisture content) can affect the structure and the manner in which overall texture responds to changes in A_w.
8. Fundamentals. Theories such as water acting as a plasticizer in food and of glass transitions in food polymers are being developed that may eventually explain fundamental relationships between A_w and textural properties of foods (26,35,39,56). In the meantime we have to continue to gather empirical data for each food for each A_w level. This information will support or discredit the fundamental theories that are presently under development.

BIBLIOGRAPHY

1. M. C. Bourne, *Food Texture and Viscosity*, Academic Press, New York, 1982.

2. A. S. Szczesniak, "Classification of Textural Characteristics," *Journal of Food Science* **28**, 385–389 (1963).
3. A. S. Szczesniak, "Consumer Awareness of Texture and of Other Food Attributes," *Journal of Texture Studies* **2**, 196–200 (1971).
4. A. S. Szczesniak, "Correlating Sensory with Instrumental Texture Measurements—an Overview of Recent Developments," *Journal of Texture Studies* **18**, 1–15 (1987).
5. M. C. Bourne, A. M. R. Sandoval, M. Villalobos, and T. S. Buckle, "Training a Sensory Texture Profile Panel and Development of Standard Rating Scales in Colombia," *Journal of Texture Studies* **6**, 43–52 (1975).
6. K. Kohyama, M. Nishi, and T. Suzuki, "Measuring Texture of Crackers with a Multiple Point Sheet Sensor," *Journal of Food Science* **62**, 922–925 (1997).
7. K. Kohyama et al., "Evaluation of Texture of Cooked Rice Using Electromyography," *Journal of Texture Studies* **29**, 101–113 (1998).
8. W. E. Brown et al., "Use of Combined Electromyography and Kinesthesiology during Mastication to Chart the Oral Breakdown of Foodstuffs, Relevance to Measurement of Food Texture," *Journal of Texture Studies* **29**, 145–167 (1998).
9. J. G. Woodroofe, S. R. Cecil, and H. H. Thompson, *The Effects of Moisture on Peanuts and Peanut Products*, Georgia Experiment Station, Bulletin 241, May 1945.
10. M. C. Bourne, "Effects of Water Activity on Textural Properties of Food," in L. B. Rockland and L. A. Beuchat, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, New York, 1987, pp. 75–99.
11. H. A. Iglesias and J. Chirife, *Handbook of Food Isotherms: Water Sorption Parameters for Food and Food Components*, Academic Press, New York, 1982.
12. L. B. Rockland, "Water Activity and Storage Stability," *Food Technology* **23**, 11–21 (1969).
13. P. P. Lewicki, "A Three Parameter Equation for Food Moisture Sorption Isotherms," *Journal of Food Process Engineering* **21**, 127–144 (1998).
14. M. C. Bourne, "Effect of Water Activity on Texture Profile Parameters of Apple Flesh," *Journal of Texture Studies* **17**, 233–260 (1986).
15. M. C. Bourne, *Food Texture and Viscosity*, Academic Press, New York, 1982, pp. 114–117.
16. M. C. Bourne, "Texture Profile Analysis," *Food Technology* **32**(7), 62–66, 72 (1978).
17. T. Beveridge and S. E. Weintraub, "Effect of Blanching Pretreatment on Color and Texture of Apple Slices at Various Water Activities," *Food Research International* **28**, 83–86 (1995).
18. J. G. Kapsalis, B. Drake, and B. Johansson, "Textural Properties of Dehydrated Foods. Relationships with the Thermodynamics of Water Vapor Sorption," *Journal of Texture Studies* **1**, 285–308 (1970).
19. J. G. Kapsalis, J. E. Walker, and M. Wolf, "A Physicochemical Study of the Mechanical Properties of Low and Intermediate Moisture Foods," *Journal of Texture Studies* **1**, 464–483 (1970).
20. J. G. Kapsalis, "The Influence of Water on Textural Parameters in Foods at Intermediate Moisture Levels," R. B. Duckworth, ed., *Water Relations in Foods*, Academic Press, New York, 1975, pp. 627–637
21. G. A. Reidy and D. R. Heldman, "Measurement of Texture Parameters of Freeze-Dried Beef," *Journal of Texture Studies* **3**, 218–226 (1972).
22. D. R. Heldman, G. A. Reidy, and M. P. Palnitkar, "Texture Stability during Storage of Freeze-Dried Beef at Low and Intermediate Moisture Contents," *Journal of Food Science* **38**, 282 (1973).
23. D. A. Ledward, S. K. Lynn, and J. R. Mitchell, "Textural Changes in Intermediate Moisture Meat during Storage at 38°C," *Journal of Texture Studies* **12**, 173–184 (1981).
24. P. K. Chattopadhyay, J. R. Hammerle, and D. D. Hamann, "Time, Temperature and Moisture Effects on the Failure Strength of Rice," *Cereal Foods World* **24**, 514–516 (1979).
25. J. T. Hogan and M. L. Karon, "Hygroscopic Equilibria of Rough Rice at Elevated Temperatures," *Journal of Agricultural and Food Chemistry* **3**, 385 (1955).
26. P. K. Chattopadhyay and D. D. Haman, "The Rheological Properties of Rice Grain," *Journal of Food Processing Engineering* **17**, 1–17 (1994).
27. J. L. Multon, H. Bizot, J. L. Doublier, J. Lefebre, and D. C. Abbott, "Effects of Water Activity and Sorption Hysteresis on Rheological Behavior of wheat Kernels," in L. B. Rockland and G. F. Stewart, eds., *Water Activity: Influences on Food Quality*, Academic Press, New York, 1981, pp. 180–197.
28. W. Obuchowski and N. Boshuk, "Wheat Hardness: Comparison of Methods of Evaluation," *Cereal Chemistry* **57**, 421–425 (1980).
29. K. K. Waanen and M. R. Okos, "Stress Relaxation Properties of Yellow-Dent Corn Under Uniaxial Loading," *Transactions of the American Society of Agricultural Engineers* **35**, 1249–1258 (1992).
30. S. K. Seymour and D. D. Hamann, "Crispness and Crunchiness in Low Moisture Foods," Ph.D. dissertation, North Carolina State University at Raleigh, 1985.
31. E. E. Katz and T. P. Labuza, "Effect of Water Activity on the Sensory Crispness and Mechanical Deformation of Snack Food Products," *Journal of Food Science* **46**, 403–409 (1981).
32. F. Sauvageot and G. Blond, "Effect of Water Activity on Crispness of Breakfast Cereals," *Journal of Texture Studies* **22**, 423–442 (1991).
33. M. Peleg, "A Mathematical Model of Crunchiness/Crispiness Loss in Breakfast Cereals," *Journal of Texture Studies* **25**, 403–410 (1994).
34. M. Harris and M. Peleg, "Patterns of Changes in Brittle Cellular Cereal Foods Caused by Moisture Sorption," *Cereal Chemistry* **73**, 225–231 (1996).
35. R. Tesch, M. D. Normand, and M. Peleg, "Comparison of the Acoustic and Mechanical Signature of Two Cellular Crunchy Cereal Foods at Various Water Activity Levels," *Journal of Science of Food and Agriculture* **70**, 347–354 (1996).
36. A. Borges and M. Peleg, "Effect of Water Activity on the Mechanical Properties of Selected Legumes and Nuts," *Journal of Science of Food and Agriculture* **75**, 463–471 (1997).
37. G. W. Halek, S. W. Paik, and K. C. B. Chang, "The Effect of Moisture Content on Mechanical Properties and Texture Profile Parameters of Corn Meal Extrudates," *Journal of Texture Studies* **20**, 43–55 (1989).
38. M. E. Zabik, S. G. Fierke, and D. K. Bristol, "Humidity Effects on Textural Characteristics of Sugar-Snap Cookies," *Cereal Chemistry* **56**, 29–32 (1979).
39. F. Hsieh et al., "Effects of Water Activity on Textural Characteristics of Puffed Rice Cake," *Lebensmittel-Wissenschaft und Technologie* **23**, 471–473 (1990).
40. C. K. Mok et al., "Effects of Water Activity on Crispness and Brittleness, and Determination of Shelf-life of Barley Flake," *Korean Journal of Food Science and Technology* **13**, 289–298 (1981).

41. R. Moyreyra and M. Peleg, "Compressive Deformation Patterns of Selected Food Powders," *Journal of Food Science* **45**, 864–868 (1980).
42. M. Peleg, "The Role of Water in the Rheology of Hygroscopic Food Powders," in D Simatos and J. L. Multon, eds., *Properties of Water in Foods*, Martinus Nijoff Publishers Dordrecht, 1985, pp. 393–404.
43. Y. Peleg and C. H. Mannheim, "Caking of Onion Powder," *Journal of Food Technology* **4**, 157–160 (1969).
44. J. Cal-Vidal, R. F. DeCarvalho, and S. C. S. Santos, "Caking Phenomena in Tropical Fruit Powders," in M. La Maguer and P. Jelen, eds., *Food Engineering and Process Calculations*, Vol. 1, Elsevier Applied Science Publishers, London, 1986, pp. 483–497.
45. S. Warburton and S. W. Pixton, "The Moisture Relations of Spray Dried Skimmed Milk," *Journal of Stored Products Research* **14**, 143–158 (1978).
46. B. Makower and W. B. Dye, "Equilibrium Moisture Content and Crystallization of Amorphous Sucrose and Glucose," *Journal of Agricultural and Food Chemistry* **4**, 72–77 (1956).
47. H. A. Iglesias, J. Chirife, and J. L. Lombardi, "Comparison of Water Vapour Sorption by Sugar Beet Root Components," *Journal of Food Technology* **10**, 385–391 (1975).
48. H. A. Iglesias and J. Chirife, "Delayed Crystallization of Amorphous Sucrose in Humidified Freeze-Dried Model Systems," *Journal of Food Technology* **13**, 137–144 (1978).
49. C. A. Hong and W. J. Brabbs, "Doughs and Cookies Providing Storage-Stable Texture Variability," U.S. Patent 4,455,333 issued June 1984 (to The Procter and Gamble Co.).
50. P. Veeraju, J. Hemuvathy, and J. V. Prabbhakar, "Influence of Water Activity on Pellicle Chaffing, Color and Breakage of Walnut (*Juglans regia*) Kernels," *Journal of Food Processing Preservation* **2**, 21–31 (1978).
51. N. Martinez and A. Chiralt, "Glass Transition and Texture in a Typical Spanish Confectionary Product Xixona Turrón," *Journal of Texture Studies* **26**, 653–663 (1995).
52. S. R. Owen, M. A. Tung, and T. D. Durance, "Cutting Resistance of a Restructured Fruit Bar as Influenced by Water Activity," *Journal of Texture Studies* **22**, 191–199 (1991).
53. C. C. Seow, "Lempuk, A Traditional Malaysian Intermediate Durian Product," *ASEAN Food Journal* **9**, 127–131 (1994).
54. A. P. M. Bay, M. C. Bourne, and A. G. Taylor, "Effect of Moisture Content on Compressive Strength of Whole Snap Bean (*Phaseolus vulgaris* L.) seeds and Separated Cotyledons," *International Journal of Food Science and Technology* **31**, 327–331 (1996).
55. M. D. Evans, R. G. Holmes, and M. B. McDonald, "Impact Damage to Soybean Seed as Affected by Surface Hardness and Seed Orientation," *Transactions of American Society of Agricultural Engineers* **33**, 234–239 (1990).
56. G. Roudat C. Dacremont, and M. LeMeste, "Influence of Water on the Crispness of Cereal-Based Foods: Acoustic, Mechanical and Sensory Studies," *Journal of Texture Studies* **29**, 199–213 (1998).

GENERAL REFERENCES

J. M. V. Blanshard and P. Lillford, eds., *Food Structure and Behavior*, Academic Press, New York, 1987

J. M. V. Blanshard and J. R. Mitchell, eds., *Food Structure—Its Creation and Evaluation*, Butterworths, London, 1988.

M. C. Bourne, *Food Texture and Viscosity, Concept and Measurement*, Academic Press, New York, 1982 (reprinted 1994).

D. Holcomb and M. Kalab, eds., *Studies of Food Microstructure*, Scanning Electron Microscopy Inc., AMF O'Hare, Ill., 1981.

M. Peleg and E. B. Bagley, eds., *Physical Properties of Foods*, AVI Publishing Co., Westport, Conn., 1983.

M. A. Rao and S. S. H. Rizvi, eds., *Engineering Properties of Foods*, Marcel Dekker, New York, 1986.

L. B. Rockland and L. R. Beuchat, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, New York, 1987.

L. B. Rockland and G. F. Stewart, eds., *Water Activity. Influences on Food Quality*, Academic Press, New York, 1981.

A. J. Rosenthal, ed., *Food Texture: Measurement and Perception*, Aspen Publishers, Gaithersburg, Md., 1999.

C. C. Seow, ed., *Food Preservation by Moisture Control*, Elsevier Science Publishing Co., New York, 1988.

P. Sherman, ed., *Food Texture and Rheology*, Academic Press, London, 1979,

D. Simatos and J. L. Multon, eds., *Properties of Water in Foods*, Martinus Nijhoff, Dordrecht, 1985.

J. A. Troller and J. H. B. Christian, *Water Activity and Food*, Academic Press, New York, 1978.

MALCOLM C. BOURNE
Cornell University
Geneva, New York

WATER ACTIVITY: GOOD MANUFACTURING PRACTICE

Water activity (a_w) is a measure of the free moisture in a product. It is defined as the quotient of the water vapor pressure of the substance (p) divided by the vapor pressure of pure water (p_0) at the same temperature; $a_w = p/p_0$. The amount of free moisture is of critical importance in supporting the growth of microorganisms. The primary public health concern associated with improperly processed low-acid foods is the possibility of a foodborne intoxication due to botulinum toxin.

The current good manufacturing practice (GMP) regulation for thermally processed low-acid foods packaged in hermetically sealed containers, Title 21 Code of Federal Regulations, Part 113 (21 CFR 113), establishes the specific level of a_w and pH to define the low-acid foods. Low-acid foods means any foods, other than alcoholic beverages, with a finished equilibrium pH >4.6 and an a_w >0.85. Commonly recognized low-acid canned foods (LACFs) with water activity level near 1.0 depend on thermal destruction of spores of pathogenic bacteria for safety. Water-activity-controlled LACFs are protected by achieving a safe a_w level, which is unfavorable for the germination and outgrowth of spores having public health significance. The application of mild heat treatment is intended to eliminate vegetative forms of microorganisms.

Control of water activity may be achieved through the proper design of product formulation. The rate of destruction of microorganisms when subject to heat is believed to be logarithmic in order. Knowledge of a_w control, thermal processing, and GMP requirements enable us to study this unique method of food preservation.

41. R. Moyreyra and M. Peleg, "Compressive Deformation Patterns of Selected Food Powders," *Journal of Food Science* **45**, 864–868 (1980).
42. M Peleg, "The Role of Water in the Rheology of Hygroscopic Food Powders," in D Simatos and J. L. Multon, eds., *Properties of Water in Foods*, Martinus Nijoff Publishers Dordrecht, 1985, pp. 393–404.
43. Y. Peleg and C. H. Mannheim, "Caking of Onion Powder," *Journal of Food Technology* **4**, 157–160 (1969).
44. J. Cal-Vidal, R. F. DeCarvalho, and S. C. S. Santos, "Caking Phenomena in Tropical Fruit Powders," in M. La Maguer and P. Jelen, eds., *Food Engineering and Process Calculations*, Vol. 1, Elsevier Applied Science Publishers, London, 1986, pp. 483–497.
45. S. Warburton and S. W. Pixton, "The Moisture Relations of Spray Dried Skimmed Milk," *Journal of Stored Products Research* **14**, 143–158 (1978).
46. B. Makower and W. B. Dye, "Equilibrium Moisture Content and Crystallization of Amorphous Sucrose and Glucose," *Journal of Agricultural and Food Chemistry* **4**, 72–77 (1956).
47. H. A. Iglesias, J. Chirife, and J. L. Lombardi, "Comparison of Water Vapour Sorption by Sugar Beet Root Components," *Journal of Food Technology* **10**, 385–391 (1975).
48. H. A. Iglesias and J. Chirife, "Delayed Crystallization of Amorphous Sucrose in Humidified Freeze-Dried Model Systems," *Journal of Food Technology* **13**, 137–144 (1978).
49. C. A. Hong and W. J. Brabbs, "Doughs and Cookies Providing Storage-Stable Texture Variability," U.S. Patent 4,455,333 issued June 1984 (to The Procter and Gamble Co.).
50. P. Veeraju, J. Hemuvathy, and J. V. Prabbhakar, "Influence of Water Activity on Pellicle Chaffing, Color and Breakage of Walnut (*Juglans regia*) Kernels," *Journal of Food Processing Preservation* **2**, 21–31 (1978).
51. N. Martinez and A. Chiralt, "Glass Transition and Texture in a Typical Spanish Confectionary Product Xixona Turrón," *Journal of Texture Studies* **26**, 653–663 (1995).
52. S. R. Owen, M. A. Tung, and T. D. Durance, "Cutting Resistance of a Restructured Fruit Bar as Influenced by Water Activity," *Journal of Texture Studies* **22**, 191–199 (1991).
53. C. C. Seow, "Lempuk, A Traditional Malaysian Intermediate Durian Product," *ASEAN Food Journal* **9**, 127–131 (1994).
54. A. P. M. Bay, M. C. Bourne, and A. G. Taylor, "Effect of Moisture Content on Compressive Strength of Whole Snap Bean (*Phaseolus vulgaris* L.) seeds and Separated Cotyledons," *International Journal of Food Science and Technology* **31**, 327–331 (1996).
55. M. D. Evans, R. G. Holmes, and M. B. McDonald, "Impact Damage to Soybean Seed as Affected by Surface Hardness and Seed Orientation," *Transactions of American Society of Agricultural Engineers* **33**, 234–239 (1990).
56. G. Roudat C. Dacremont, and M. LeMeste, "Influence of Water on the Crispness of Cereal-Based Foods: Acoustic, Mechanical and Sensory Studies," *Journal of Texture Studies* **29**, 199–213 (1998).

GENERAL REFERENCES

J. M. V. Blanshard and P. Lillford, eds., *Food Structure and Behavior*, Academic Press, New York, 1987

J. M. V. Blanshard and J. R. Mitchell, eds., *Food Structure—Its Creation and Evaluation*, Butterworths, London, 1988.

M. C. Bourne, *Food Texture and Viscosity, Concept and Measurement*, Academic Press, New York, 1982 (reprinted 1994).

D. Holcomb and M. Kalab, eds., *Studies of Food Microstructure*, Scanning Electron Microscopy Inc., AMF O'Hare, Ill., 1981.

M. Peleg and E. B. Bagley, eds., *Physical Properties of Foods*, AVI Publishing Co., Westport, Conn., 1983.

M. A. Rao and S. S. H. Rizvi, eds., *Engineering Properties of Foods*, Marcel Dekker, New York, 1986.

L. B. Rockland and L. R. Beuchat, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, New York, 1987.

L. B. Rockland and G. F. Stewart, eds., *Water Activity. Influences on Food Quality*, Academic Press, New York, 1981.

A. J. Rosenthal, ed., *Food Texture: Measurement and Perception*, Aspen Publishers, Gaithersburg, Md., 1999.

C. C. Seow, ed., *Food Preservation by Moisture Control*, Elsevier Science Publishing Co., New York, 1988.

P. Sherman, ed., *Food Texture and Rheology*, Academic Press, London, 1979,

D. Simatos and J. L. Multon, eds., *Properties of Water in Foods*, Martinus Nijhoff, Dordrecht, 1985.

J. A. Troller and J. H. B. Christian, *Water Activity and Food*, Academic Press, New York, 1978.

MALCOLM C. BOURNE
Cornell University
Geneva, New York

WATER ACTIVITY: GOOD MANUFACTURING PRACTICE

Water activity (a_w) is a measure of the free moisture in a product. It is defined as the quotient of the water vapor pressure of the substance (p) divided by the vapor pressure of pure water (p_0) at the same temperature; $a_w = p/p_0$. The amount of free moisture is of critical importance in supporting the growth of microorganisms. The primary public health concern associated with improperly processed low-acid foods is the possibility of a foodborne intoxication due to botulinum toxin.

The current good manufacturing practice (GMP) regulation for thermally processed low-acid foods packaged in hermetically sealed containers, Title 21 Code of Federal Regulations, Part 113 (21 CFR 113), establishes the specific level of a_w and pH to define the low-acid foods. Low-acid foods means any foods, other than alcoholic beverages, with a finished equilibrium pH >4.6 and an a_w >0.85. Commonly recognized low-acid canned foods (LACFs) with water activity level near 1.0 depend on thermal destruction of spores of pathogenic bacteria for safety. Water-activity-controlled LACFs are protected by achieving a safe a_w level, which is unfavorable for the germination and outgrowth of spores having public health significance. The application of mild heat treatment is intended to eliminate vegetative forms of microorganisms.

Control of water activity may be achieved through the proper design of product formulation. The rate of destruction of microorganisms when subject to heat is believed to be logarithmic in order. Knowledge of a_w control, thermal processing, and GMP requirements enable us to study this unique method of food preservation.

WATER ACTIVITY AND CONTROL OF MICROBIAL GROWTH

The purposes of food preservation are to extend shelf life, prevent microbial spoilage, and destroy potentially harmful microorganisms. The growth of microorganisms is inhibited at lower water activity levels. In the a_w range below 1.0 to 0.93, all known foodborne bacterial pathogens can grow in the upper part of this range. At a_w, 0.93 to 0.86, the only foodborne bacterial pathogen that can grow and cause an outbreak is *Staphylococcus aureus*. Under anaerobic conditions this nonsporulating bacteria is inhibited at an a_w level of 0.91, but aerobically it is inhibited at 0.86. Scientific evidence showed that the decimal reduction time *D* for microorganisms is greater at lower water activity levels. In one investigation, the minimum water activity for the growth of spores and toxin production was found to be 0.95 for *Clostridium botulinum* types A and B, and 0.97 for type E, at the optimum growth temperature (30–40°C) and pH (7.0). Another work showed that the minimum a_w for supporting the growth of botulinum types A and B was at 0.94 (1).

Thermal processing of commonly canned low-acid foods resembles wet heat sterilization ($a_w = 1.0$), whereas water-activity-controlled low-acid canned foods resemble dry-heat sterilization in that the water activity level inside the sealed container will be less than 1.0 during and after sterilization. Scientific evidence reveals that greater thermal resistance of microorganisms occurs when a reduced water activity level is encountered. In other words, the heating rate of an a_w-controlled low-acid canned food is lower than that of a wet low-acid food ($a_w = 1.0$).

A published paper showed that when a_w decreases from 1.0 to 0.90, and 0.80, resembling dry-heat sterilization, the decimal reduction time *D* increases from 7-fold and 33-fold, respectively. The resistance of bacterial spores to thermal kill increases with the decrease in a_w levels consistent with those for most a_w-controlled low-acid foods. The *D* value of *C. botulinum* spores increases with an increase in pH; the effect is greatest at lower processing temperatures (2). The synergistic effects of low a_w, pH, F^{z}_{T}, and preservatives have clearly been proved to be effective against the growth of microorganisms. Generally, a_w-controlled foods contain large amounts of solutes and their a_w levels are between 0.8 and 0.95. Intermediate-moisture (IM) foods with $a_w = <0.85$ are not covered by 21 CFR 113. The microbial resistance to thermal kill will be different from that of wet-heat sterilization. For example, the $D_{65°\text{C}}$ values of *S. aureus* in skim milk, with 10 and 57% sugar, are 4.88 and 22.35, respectively. Although microorganisms show a greater resistance to thermal kill when at lower a_w levels, the addition of humectant(s) such as a high concentration of sugar, salt, or a combination of the two, for example, have proved to be effective in the preservation of foods. Thus, the growth of microorganisms in foods may be prevented by a_w control.

USDA's Agricultural Research Service developed a pathogen-modeling program (PMP) to predict the growth characteristics of various bacteria in food systems. This PMP is based on the equations derived by response surface analysis of growth data fitted using the Gompertz function in conjunction with nonlinear regression analysis. The program considers effects and interactions of sodium chloride (or a_w), acidity (or pH), temperature, under aerobic and anaerobic conditions, and so on. Evaluation of the models has indicated that they provide useful estimates of the microorganisms' growth characteristics in food systems.

ESTABLISHING SCHEDULED PROCESSES

Many factors influence the intrinsic characteristics and the microbial stability of foods. The five most important factors governing the growth of microorganisms in processed foods are a_w, pH, container seal integrity, temperature of storage and distribution, and thermal processing or equivalent treatment (F_0).

The thermal death time of 2.45 min with a *z* value of 17.6 for the 60 billion *C. botulinum* spores in pH 7.0 phosphate buffer when heated at 250°F (3) is generally accepted as a guide in process computations. The correlations between the spore resistance in phosphate and in a canned food may be expressed by phosphate factor (= resistance in food/resistance in standard phosphate buffer). The use of the food phosphate factor is a mechanism for adjusting the minimum public health sterilization value for a specific product. Perkins (4) indicated that many commercial processes are still computed on the basis of Esty and Meyer's classic *C. botulinum* resistance values, but there are many occasions when an F_0 of 5 to 7 min and even higher are used to achieve the commercial sterility cook for low-acid canned foods.

Alstrand and Ecklund proposed a guide for the selection of F_0 in relation to pH and carbohydrate content of canned foods (5). Many calculated F_0 values of successful commercial processes were presented, and most of those were greater than 3.0 min. Pflug and coworkers (6) developed a model showing that at pH 6.0, 5.5, 5.0, and 4.6, the calculated F_0 value for public health processes were 3.0, 2.3, 1.6, and 1.2, respectively. Pflug (7) pointed out that when designing a low-acid canned food–sterilization process, three preservation conditions must be considered: (*1*) public health ($F_0 = 3.0$), (*2*) nonpathogenic mesophiles ($F_0 = 5–7$), and (*3*) thermophiles ($F_0 = 5–7$). However, if the product is expected to be stored and distributed at elevated temperatures, then F_0 should be 15 to 21. Low-acid foods packed in hermetically sealed containers are commonly processed in a retort to render the foods commercially sterile. As pointed out earlier, a minimum public health process of $F_0 = 3.0$ min or greater is usually called for. However, commercial sterility may also be achieved through inhibition instead of destruction of spores. To achieve this goal, factors including the intended level of preservation (or length of shelf life), the processing system, the characteristic of the food, the container type and size to be processed, and the expected commercial production conditions are to be considered.

The severe retorting process may be detrimental to the quality of certain foods heated by conduction. Reduced thermal processing requirements may be possible in that a scheduled process may include a proper combination of safe a_w level, a pasteurization process, and other treatment(s). The proper combination treatments would render the food commercially sterile. One investigator (8) pointed out that the canned cured meats with suitable amount of salt and nitrite are processed to achieve an F_0 value generally in the order of 0.05 to 0.6, but it may vary from 0 to 1.5. This reduced thermal treatment would also provide an immediate benefit of energy savings.

Safe a_w level is a level of water activity low enough to prevent the growth of undesirable microorganisms in the finished product under the intended conditions of manufacturing, storage, and distribution. The maximum safe a_w level is an a_w that will be considered safe for a food if adequate data are available that demonstrate that the food at or below the given a_w will not support the growth of undesirable microorganisms.

FDA Title 21 CFR 113.83 specifies that scheduled processes for low-acid foods shall be established by qualified persons having expert knowledge of thermal processing requirements for low-acid foods in hermetically sealed containers and having adequate facilities for making such determinations (9).

FDA GMP REGULATIONS GOVERNING THE PROCESSING REQUIREMENT

Canning is the most economical and widely used method of food preservation. Commercial sterility must be achieved for all thermally processed low-acid food packaged in hermetically sealed containers, including those low-acid intermediate-moisture foods. As defined by FDA's GMP regulation 21 CFR 113.3(e), commercial sterility of thermally processed food means the condition achieved (9):

1. By the application of heat which renders the food free of
    a. Microorganisms capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution; and
    b. Viable microorganisms (including spores) of public health significance; or
2. By the control of water activity and the application of heat, which renders the food free of microorganisms capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution.

Water activity is one of the two critical parameters used to determine the applicability of FDA's GMP regulations, 21 CFR 108, 110, 113, and 114. Figure 1 shows the type of foods and the applicable GMP regulations based on product a_w and pH levels.

Title 21 CFR 108.35 specifies that processors must register and file their scheduled processes with the FDA. The scheduled processes are reviewed for their adequacy for public health protection. When a process is considered potentially inadequate, follow-up action is generally required. Request for process establishment information, including the microbiological basis of a scheduled process, has been the course of action most often recommended. Of the possible concerns are processes based on the lowest product sterilization values (<3 min) (10). The type of information needed by the FDA to evaluate the adequacy of a process for a water-activity-controlled low-acid food is specific under the following three conditions:

1. When the filed $a_w <= 0.85$ and the data appear reliable, the product in question is not covered by the LACF (low-acid food) regulations.
2. When $a_w <= 0.85$ to 0.90, the production in question is covered by the regulations 21 CFR 108.35 and 113, and it is necessary for the processor to register and file the scheduled process. The filing should include the critical maximum a_w necessary to control germination and outgrowth of *C. botulinum* spores and the critical thermal treatment necessary to destroy vegetative cells of pathogenic bacteria as well as prevent spoilage.
3. When the $a_w <= 0.90$, the product is covered by the regulations 21 CFR 108.35
4. and 113. The processor must register and file the scheduled process and will be requested to provide appropriate microbiological supporting data demonstrating process adequacy, in addition to the critical factors already mentioned for a_w-controlled products. The process-supporting data are to be obtained and reviewed and must be acceptable before the process filing forms are accepted for filing.

Title 21 CFR 113.40(i) requires that critical factors specified in the scheduled process, such as the a_w used in conjunction with thermal processing, shall be measured with instruments having the accuracy and dependability adequate to ensure that the requirements of the scheduled process are met. Official method of analysis for a_w has been adopted by the Association of Official Agricultural Chemists (AOAC) as the official final action method (Ref. 11).

Food preservation based on the principle of hurdle technology is recognized. 21 CFR 113 81(f) states that "when a low-acid food requires different solute to permit safe processing at low temperatures, such as in boiling water, there shall be careful supervision to ensure that the equilibrium water activity of the finished product meets that of the scheduled process." Specific parts and paragraphs of applicable GMP regulations from Title 21 of the Code of Federal Regulations delineating a_w in relation to control measures and safety requirements are:

1. 21 CFR 110: Current GMP in manufacturing, packing, or holding human food; 21 CFR 110.3(n), 110.3(r), 110.40(f), 110.80(b)(2), 110.80(b)(4), and 110.80(b)(14)
2. 21 CFR 113: Thermally processed low-acid foods packaged in hermetically sealed containers; 21 CFR 113.3(e)(ii), 113.3(n), 113.3(w), 113.10, 113.40(i), 113.81(f), 113.83, 113.89, 113.100(a)(6)

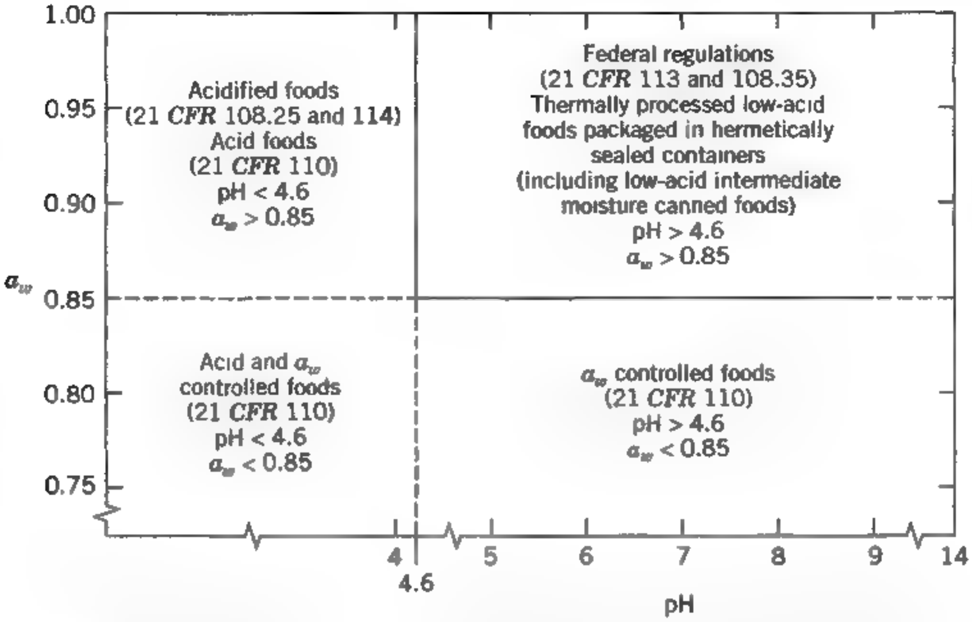


Figure 1. Applicability of the Food and Drug Administration's good manufacturing practice regulations.

3. 21 CFR 114: Acidified foods; 21 CFR 114.3(b), 114.3(d), and 114.3(h).

HUMECTANTS AND THEIR REGULATORY USE

Manipulation of moisture as a means of extending shelf life in perishable foods can be accomplished through either dehydration or a_w control. Dehydration and a_w control may be viewed as two different technologies. Dehydration is essentially direct removal of moisture, whereas a_w control is the addition of humectant(s) to bind or reduce free moisture, and not necessarily for the removal of free moisture from foods. Foods with the same amount of moisture could exhibit different levels of a_w. Foods with a higher amount of moisture content could be adjusted to exhibit a lower level of a_w than those with less moisture content. Formulated foods and foods preserved by the addition of salt, sugar, and glycerol are typical examples. According to a summation of experimental work over many years, it is generally conceded that 50% sugar or 10% salt is completely inhibitory to outgrowth of the *C. botulinum* spores of the proteolytic types A and B (12). These concentrations of sugar and salt correspond to the same a_w of 0.935. Through the use of salt, sugar, glycerol, and other humectants to control a_w levels, many low-acid foods are now processed at low temperatures. Most intermediate-moisture low-acid foods contain a large amount of solutes or humectants. Typical intermediate-moisture low-acid foods include salt-cured meat and meat products, salted fish and fish products, soup bases, specialty sauces, syrups, some preserves, puddings, some preserved vegetables, processed cheese products, and some snack foods.

Some humectants and their CFR-specified uses are summarized as follows:

1. Sucrose, 21 CFR 184.1854 and 582.1
2. Salt, 21 CFR 182.1 and 582.1
3. Glycerin, 21 CFR 182.1320 and 582.1320
4. Sodium nitrite, 21 CFR 172.175, 181.34, and 573.700
5. Potassium sorbate, 21 CFR 182.3640
6. Sorbitol, 21 CFR 184.1835
7. Potassium chloride, 21 CFR 184.1622
8. Propylene glycol, 21 CFR 184.1666 and 582.1666
9. Propylparaben, 21 CFR 184.1670
10. Lactic acid, 21 CFR 184.1061 and 582.1061
11. Invert sugar, 21 CFR 184.1859
12. High-fructose corn syrup, 21 CFR 184.1866
13. Modified food starch, 21 CFR 172.892

WATER ACTIVITY DETERMINATIONS

There are many ways to estimate the water activity of foods. The principles governing water activity determinations may be illustrated as follows:

$$a_w = p/p_o \quad \text{(by definition)} \quad (1)$$

$$= n_o/(n_o + n_s) \quad \text{(Raoult's law)} \quad (2)$$

$$= (a_w)_1\,(a_w)_2\,(a_w)_3 \ldots \quad \text{Gibbs-Duhem} \quad (3)$$

$$= \text{ERH}/100 \quad \text{by thermodynamic principle} \quad (4)$$

where ERH is the equilibrium relative humidity. Equation 1 is the basis of the vapor pressure manometric method, in which the vapor pressure in the evacuated headspace above the food sample is determined. This provides a direct measurement of the vapor pressure.

Equation 2 is often used in work in formulating the intermediate-moisture (IM) foods and in predicting their a_w. The biochemical potential of the idealized solutions obeys Raoult's law. Most nonelectrolytes are also adequately represented by Raoult's law up to several molal concentrations. A modified equation, $a_w = n_o/(n_o + 1.5n_s)$ was found to fit experimental values up to high concentrations of sugars.

Equation 3 is commonly used in formulating the IM foods and in predicting their a_w. This equation (13) is very useful for aqueous systems involving more than two solutes. It is assumed that in a food system each a_w-lowering component behaves independently. The final a_w is a product of each component $(a_w)_n$, based on each solute being dissolved in all the water in the system.

Equation 4 is the basis for water activity measurement by hygrometers and isopiestic equilibrium methods. According to the thermodynamic principle, when at equilibrium, the activity of a water vapor equals the activity of the corresponding aqueous phase in a closed system. Therefore, the measurement of the ERH of a food is a measure of the water activity of that food, and a_w is numerically equal to the ERH of that food expressed as a decimal fraction; a_w = ERH/100.

DISCLOSURE STATEMENT

Instruments and methods for water activity determinations are detailed in the 1995 (16th) edition of AOAC Official Methods of Analysis, Method 978.18.

ACKNOWLEDGMENTS

This article was written by the author in his private capacity. No official support or endorsement by the U.S. Food and Drug Administration is intended or should be inferred.

BIBLIOGRAPHY

1. D. A. Kautter et al., "The Detection, Identification, and Isolation of *C. botulinum*," in M. Herzberg, ed., *Toxic Microorganisms*, Government Printing Office, Washington, D.C., 1970.
2. H. Xezones and I. J. Hutchings, "Thermal Resistance of*C botulinum* (62A) Spores as Effected by Fundamental Food Constituents," *Food Technol.* **19**, 113–115 (1965).
3. I. J. Esty and K. F. Meyer, "The Heat Resistance of the Spores of *C. botulinum* and Allied Anaerobes, XI," *J. Infectious Dis.* **31**, 650 (1922).
4. W. E. Perkins, "Prevention of Botulism by Thermal Processing," in *Book Botulism*, U.S. Department of Health, Education, and Welfare, Public Health Service Publication No. 999-FP-1.
5. D. V. Alstrand and O. F. Ecklund, "The Mechanics and Interpretation of Heat Penetration Tests in Canned Foods," *Food Technol.* **6**, 185 (1952).
6. I. J. Pflug, T. E. Odlaug, and R. Christensen, "Computing a Minimum Public Health Sterilizing Value for Food with pH Values from 4.6 to 6.0," *J. Food Protection* **48**, 848–850 (1985).
7. I. J. Pflug, "Factors Important in Determining the Heat Process Value, F_t, for Low-Acid Canned Foods," *J. Food Protection* **50**, 528–530 (1987).
8. A. H. W. Hauschild, "*Clostridium botulinum*," in M. P. Doyle, ed., *Foodborne Bacterial Pathogens*, Marcel Dekker, New York, 1989.
9. U.S. Food and Drug Administration, Title 21 *Code of Federal Regulations*, Parts 108, 110, 113, 114, 121, 172, 175, 182, and 184, U.S. Government Printing Office, Washington, D.C., 1990.
10. R. T. Mulvaney, "Regulatory Review of Scheduled Thermal Process," *Food Technol.* **78**, 73–75 (1978).
11. Official Methods of Analysis, 15th ed., Method 978.18, Association of Official Analytical Chemists, Arlington, Va., 1990.
12. C. F. Schmidt, "Spores of *C. botulinum*: Formation, Resistance, Germination," in *Book Botulism*, U.S. Department of Health, Education, and Welfare, Public Health Service Publication No. 999-FP-1.
13. K. Ross, "Estimation of Water Activity in Intermediate Moisture Foods," *Food Technol.* **29**, 26–28 (1975).

GENERAL REFERENCES

A. C. Baird-Parker and B. Freame, "Combined Effect of Water Activity, pH and Temperature on the Growth of *Clostridium botulinum* from Spore and Vegetative Cell Inocula," *J. Appl. Bacteriol.* **30**, 420–429 (1967).

M. R. Johnston and R. C. Lin, "FDA Good Manufacturing Practice Regulations," Food Quality **3**, 109–118 (1980).

M. R. Johnston and R. C. Lin, "FDA Views on the Importance of a_w in Good Manufacturing Practice," in L. B. Rockland and L. R. Beuchat, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, New York, 1987.

M. R. Johnston, R. C. Lin, and F. A. Philips, "FDA Views on the Importance of pH in Good Manufacturing Practice," *Food Product Devel.* **11**, 75–77 (1977).

R. C. Lin, P. H. King, and M. R. Johnston, "Examination of Containers for Integrity," *FDA Bacteriological Analytical Manual*, 6th ed., Association of Official Analytical Chemists, Arlington, Va., 1985.

W. G. Murrel and M. J. Scott, "The Heat Resistance in Bacteria Spores at Various Water Activities," *J. General Microbiol.* **43**, 411–425 (1966).

I. J. Pflug and T. E. Odlaug, "Review of z and F values Used to Ensure the Safety of Low-Acid Canned Food," *Food Technol.* **32**, 63–70 (1978).

C. R. Stumbo, K. S. Purohit, and T. V. Ramakrishnan, "Thermal Process Lethality Guide for Low-Acid Foods in Metal Containers," *J. Food Sci.* **40** (1975).

J. A. Troller, "Effect of Water Activity on Enterotoxin B Production and Growth of *Staphylococcus aureus*," *Appl. Microbiol.* **21**, 435–439 (1971).

RONG C. LIN
U.S. Food and Drug Administration
Washington, D.C.

WATER ACTIVITY: MICROBIOLOGY

The influence of water activity (a_w) on food product quality and stability began to receive considerable attention during the early 1950s. Such interest was promoted by inconsistent empirical observations of total moisture content and product stability. Microbiologists were among the first to recognize that a_w rather than total moisture content controlled microbiological growth, death, survival, sporulation, and toxin production by diverse microorganisms. Between the 1970s and 1980s, this group of scientists focused their interest on the influence of a_w by studying how microorganisms respond under different conditions of temperature, pH, and a_w (1).

DEFINITION OF WATER ACTIVITY

Water activity as a broad thermodynamic concept is defined as a relation of fugacities between liquid and vapor phases of a confined system while it is in equilibrium. A review of basic and applied thermodynamics is required to understand the a_w concept (2). Assuming that fugacity is a function of pressure and that the correction factor for vapor pressure is the same for an aqueous solution and pure water, then the fugacity ratio can be replaced by the pressure ratio (3):

$$a_w = P_w/P_w^\circ = \mathrm{RHE}/100$$

where P_w is the vapor pressure of water in equilibrium with a food, P_w° is the vapor pressure of pure water at the same temperature of the food, and RHE is the relative humidity in equilibrium. Therefore, the vapor pressure of water in equilibrium with a food can be measured or related with the RHE. However, it should be taken into account that a_w is defined only for a system that presents a thermodynamic equilibrium, and its value is applicable only for specific conditions of temperature and pressure.

Many multicomponent food systems constituted by two or more phases (solid, liquid, aqueous, and oil) do not present equilibrium from one phase to the other (4). For this reason, a_w may not be an adequate thermodynamic parameter. Franks (5) proposed that the presence of hysteresis in food sorption isotherms (Fig. 1) may imply the presence of nonreversible equilibrium. These food systems are not really stable, but are in a pseudoequilibrium or metastable state. Thus, in these cases, the term water activity is not applied; instead, relative vapor pressure or relative humidity is used. However, when hysteresis is not shown, vapor pressure can be a measure of a_w.

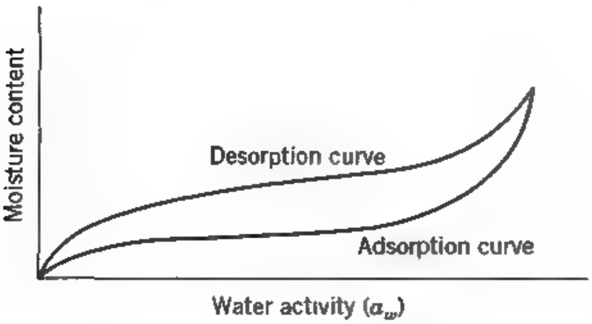


Figure 1. Typical sorption isotherm showing hysteresis.

WATER ACTIVITY AS AN INDICATOR OF MICROBIAL SAFETY AND SHELF LIFE

The influence of food water content on perishability has been known since ancient times. Almost every primitive culture found a convenient way to reduce food water content to a level that would protect against microbial spoilage (eg, drying, salting, adding sugars, concentrating). However, the occasional failure of these preservation techniques produced predictable results: salted products turned red because of the development of halophilic bacteria, sugar-preserved foods were fermented by osmophilic yeasts, and dried products were occasionally attacked by xerophilic molds (6). Also discovered was that food water content did not fully define microbial stability, because dried milk would rapidly spoil if it contained about 12% water, whereas cereals would remain fully sound at about 14% and dried fruits even at 18%. A better quantitative approach to define the influence of water on microbial response in foods was introduced by Scott (7) with the concept of a_w.

Considering a_w in relation to microbial stability, the minimum a_w values that permit microbial growth for different types of microorganisms are of considerable concern. Table 1 presents the a_w and microbial spoilage of foods with a classification of microorganisms as osmosensitive or osmotolerant. Corry (8), Beuchat (9,10), and Gould (11) generated extensive tables with minimum a_w values for the growth and toxin production of several pathogenic and spoilage microorganisms. Table 2 shows minimal a_w for the growth of foodborne bacterial pathogens at their optimum pH and temperature. With the exception of *Staphylococcus aureus*, for which a minimum a_w of 0.86 is needed to avoid growth under aerobic conditions, the growth of all other pathogens can be inhibited by a reduction in a_w to approximately 0.92. The major findings of Corry (8), Beuchat (9,10), and Gould (11) can be summarized as follows:

- At $a_w < 0.90$ to 0.92, most pathogenic bacteria are usually inhibited, except *Staphylococcus aureus*, which can grow at a_w 0.86.
- Minimum a_w for growth is always equal or lower than minimum a_w for toxin production.
- The minimum a_w for growth depends on the solute used to control a_w, the so-called solute effect.

Water activity is a major factor governing the microbial responses in foods. The concept of a_w has been successfully applied to the water relations in microorganisms because measured values of the food media in which they are immersed generally correlate well with the potential for growth and metabolic activity. Ecological factors and their interactions control the microbial responses in foods that determine what growth may occur. The combination of the

Table 1. Wateer Activity and Food Microbial Spoilage

Range of a_w	Microorganisms inhibited	Examples of foods
1.00–0.95	Some yeasts, Gram-negative rods, bacterial spores	Foods containing 40% sucrose or 7% salt
0.95–0.91	Most cocci, lactobacilli, vegetative cells of bacilli, some molds	Foods containing 50% sucrose or 12% salt
0.94	Growth and toxin production by all types of *Clostridium botulinum*	Meats packaged under anaerobic conditions
0.91–0.87	Most yeasts	Foods containing 65% sucrose or 15% salt
0.8–0.80	*Staphylococcus aureus*, most molds	Flour, rice, etc. containing 15–17% water
0.86	Aerobic growth of *Staphylococcus aureus*	Mushrooms
0.80–0.75	Most halophilic bacteria	Foods with 26% salt
0.80	Production of micotoxins	
0.75–0.65	Xerophilic molds	Foods containing less than 10% water
0.68	Practical limit for fungi	
0.65–0.60	Osmophilic yeasts	Confectionery products, fruits containing 15–20% water

food a_w and pH frequently determine whether bacterial or fungal growth may take place, and the type of these species that will develop is often determined by temperature, gas atmosphere, redox potential, nutritional status, physical states, antimicrobial substances, and chemical preservatives (12).

The predominant microbial flora of a particular food (Figs. 2 and 3) can be determined by the interaction between its a_w and pH. Although bacteria are the major spoilage organisms in high pH (>4.6) and a_w (0.980–0.999) foods, yeasts and molds will dominate when the pH is below 4.0. In high a_w foods with a pH below 4.0, such as fruit juices and yogurt, yeasts as well as some lactic acid bacteria are most likely to be dominant. Some filamentous fungi also compete well in high a_w–low pH environments. The majority of yeasts and molds that cause spoilage are nonxerophilic, growing well at high a_w (>0.99) but also at a_w values below 0.90. In sugar-rich environments where bacteria do not seem to adapt, yeasts and molds take over as the major spoilage organisms. However, in foods such as meat with neutral pH and high a_w, bacteria dominate even though they may not be capable of growing in the food at a_w values below 0.95. Bacteria rarely spoil foods with a_w values below 0.85, but brines and salted foods may be spoiled by moderate and extreme halophilic bacteria (12).

The a_w concept has assisted food scientists and microbiologists in their efforts to predict the onset of food spoil-

Table 2. Minimal a_w for the Growth of Foodborne Bacterial Pathogens Under Optimum pH and Temperature Conditions

Bacteria	a_w
Campylobacter jejuni	0.990
Aeromonas hydrophila	0.970
Clostridium botulinum E	0.965
Clostridium botulinum G	0.965
Shigella spp.	0.960
Yersinia enterocolitica	0.960
Clostridium perfringens	0.945
Clostridium botulinum A & B	0.940
Salmonella spp.	0.940
Escherichia coli	0.935
Vibrio parahaemolyticus	0.932
Bacillus cereus	0.930
Listeria monocytogenes	0.920
Staphylococcus aureus (anaerobic)	0.910
Staphylococcus aureus (aerobic)	0.860

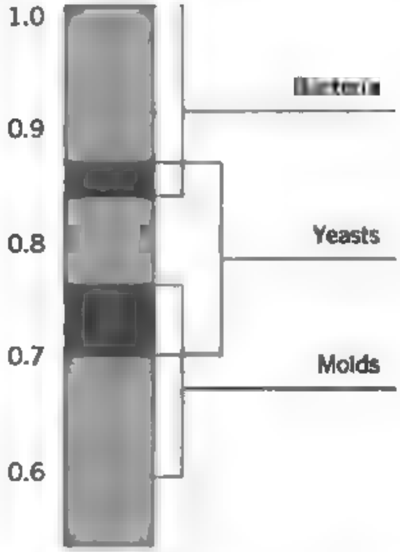


Figure 2. Effects of a_w on the growth of bacteria yeasts and mold microorganisms.

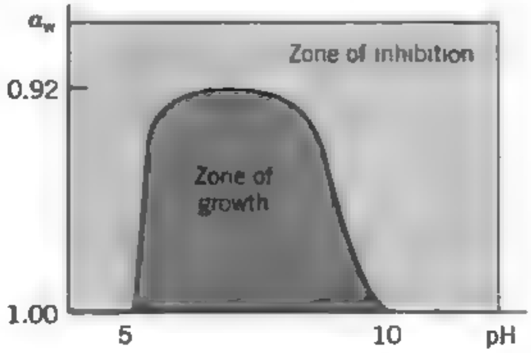


Figure 3. Interacting effects of pH and a_w on the growth of selected microorganisms.

age and identify and control foodborne disease hazards that may exist in various food products (13). However, the a_w of a medium is not the only determining factor regulating biological response because the nature of the a_w controlling solute in the so-called solute effect occasionally plays a role as well (14). Consequently, the concept of a_w in foods as a determinant of microbial growth has been challenged (13). The occurrence of specific effects from different solutes already posed questions about the role of a_w as a credible measure of physiological viability because solute effects can mislead the prediction of microbial growth based on measured levels of a_w (9,13). During the 1980s, an approach that focused attention on the dynamic aspects of food systems of the low to intermediate moisture type that are nonequilibrium systems also received considerable attention (13,15). This perspective, which addresses the importance of maintaining foods in kinetically metastable, dynamically constrained glassy states, has incorporated the theoretical bases of fundamental structure and property principles from the field of synthetic polymer science, which includes the innovative concepts of water dynamics and glass dynamics in what has been called food polymer science (15). In many foods and biological materials, the solids are in an amorphous metastable state that is very sensitive to changes in moisture content and temperature (15). This amorphous matrix may exist either as a very viscous glass or a more liquidlike rubbery structure. The characteristic temperature (T_g) at which the glass–rubber transition occurs has been proposed as a physicochemical parameter that can determine product properties such as stability and safety better than a_w (15). However, the food science polymer approach may not constitute a better alternative to the concept of a_w as a predictor of microbial growth in foods despite the limitations of the a_w concept and possible influences of nonequilibrium situations (13).

OSMOTIC STRESS AND OSMOREGULATION IN MICROORGANISMS

Changes in medium osmolality (decreasing a_w) lead to a passive flow of water across microbial cell membranes, which results in a loss of turgor. The universal cell mechanism to overcome the initial loss following a hyperosmotic shock is the accumulation of cytoplasmatic solute(s) that increase the internal osmotic pressure, which in turn can restore turgor (11,16). There is great variation in the type of solutes accumulated by various microorganisms (eg, cations, amino and imino acids, amines, quaternary amines, polyols, sugars), but all exhibit a number of common properties, such as allowance of continued activity of cytoplasmic enzymes at lower a_w than the external solutes commonly present in foods and solubility to high concentrations.

Control of microbial growth in foods by a_w modifications can be achieved by two different strategies:

1. Using a_w as the only stress factor and therefore reducing it to very low levels (ie, fully dehydrated foods of $a_w < 0.6$).
2. Using other stress factors in combination with the low a_w (hurdle approach) that reduce the amount of energy available for osmoregulation, or impairing some other stresses that increase energy consumption (ie, intermediate moisture or high a_w foods).

WATER ACTIVITY IN DETECTION AND ENUMERATION OF MICROORGANISMS

All the ecological factors that are determinants of microbial response cannot always be considered in the microbial examination of foods. As discussed earlier, both the a_w and pH of a food substrate have a major influence on the type of microflora capable of colonizing and causing spoilage or foodborne diseases. These are facts that need consideration for reliable isolation and enumeration of significant microorganisms. The majority of nonxerophilic fungi that cause spoilage in moisture-rich foods can be detected, without special considerations, on general-purpose enumeration media such as acidified potato dextrose agar, malt extract agar, or dichloran rose bengal chloramphenicol (DRBC) agar (17). Conversely, the consideration of product a_w in intermediate and low moisture foods, such as syrups, confections, fruit concentrates, honey, dried fruits, salted dry fish and meat, grains, legumes, nuts, or spices, is of major relevance. Such products have characteristic mycoflora that are determined by factors such as a_w, type of solutes present (sugar or salt), and storage conditions, such as temperature, CO_2 concentration, and available oxygen (12). Because many fungi species require reduced a_w media; they are not capable of growth at a_w 0.99 and may be underestimated or undetected when traditional methods and media are used (ie, general plate enumeration in acidified potato dextrose agar, malt extract agar, or DRBC agar) (18).

Xerophilic fungi have been described as capable of growth below a_w 0.85. Under at least one set of environmental conditions they include osmophilic yeasts and xerophilic filamentous fungi: *Debaryomyces hansenii, Zygosaccharomyces bailii*, and *Z. rouxii* among the yeasts and several fungi species of *Aspergillus* (moderate xerophilic: *A. restrictus* series and *A. glaucus* series or *Eurotium*) and *Penicillium* as well as *Chrysosporium, Eremascus, Paecelomyces, Wallemia, Xeromyces, Basipetospora, Polypaecylum, Geomyces* and *Monascus*. Among the fastidious extreme xerophiles that require reduced a_w to grow are *Chrysosporium farinicola, C. fastidium, C. inops, C. xerophilium, Eremascus albus, E. fertilis* and *Xeromyces bisporus*; *Basipetospora halophila* and *Polypaecilum pisce* are halophilic molds. The inadequacy of media with high a_w to support the growth of xerophilic fungi is magnified when foods under examination contain inhibitory chemicals or have additional stress factors of a physical nature that affect the ability of cells to grow on enumeration media (18).

Among the common media for detection or enumeration of fungi in intermediate and low moisture foods is dichloran 18% glycerol (DG18) agar (a_w 0.95, pH 6.5), developed by Hocking and Pitt (17) for enumeration of moderately xerophilic molds in commodities such as grains, flours, nuts, and spices. The presence of dichloran in this media restricts the growth of rapidly growing mucoraceous species such as *Eurotium* spp. that tend to spread over the plates and obscure the growth, detection, and recovery of slower growing xerophiles, although this recovery is present in all but fastidious xerophiles. To overcome this problem, the addition of nontoxic chemicals (Triton X-301 or iprodione) into DG18 to enhance the case of counting colonies formed by the maximum number of viable propagate of moderately xerophilic molds present in intermediate moisture and dehydrated foods has been investigated. DRBC and DG18 are presently recommended as general purpose isolation and enumeration media that discriminate among foods depending on their a_w values (>0.90); DG18 for instance, is less suitable for fresh fruits and vegetables than DRBC.

To analyze foods with lower a_w, the major selective principles should be high concentrations of carbohydrates or sodium chloride. Malt salt agar (MSA) has long been used for enumerating molds in flour, but has recently been replaced by the more efficient glycerol-containing medium DG18. Other media are glucose citric acid tryptone (50 GCT) agar (pH 4.0) for enumeration of sugar-tolerant yeast in concentrated orange juice; Scarr's osmophilic wort (SOW) agar for xerophilic yeasts in high sugar products; a series of malt extract glucose (MYG) agar up to 60% glucose (a_w 0.85); malt extract yeast 70% glucose fructose (MY70GF) (a_w 0.76) for isolation of extreme xerophiles that may be accompanied by *Eurotium* spp.; and malt extract-yeast extract 5% salt 12% glucose agar (a_w 0.96) (18). In bacteriology, there are relatively few uses for salt-based media of reduced a_w, but because salt is an important component of selective enrichment broths and plating identification media, these are used to detect and enumerate salt-requiring or salt-tolerant bacterium such as many *Vibrio* species that have a physiological requirement for NaCl.

It is important to consider the composition and process history of reduced a_w foods when selecting methods for mycological analysis (18), cell adaptation to low a_w can occur because tolerance to low a_w can be gained or lost in the presence of various concentrations of solute. Therefore, the state of microbial cell adaptation will influence its ability to develop colonies on various enumeration media. As for diluents, peptone (0.1%) water and 0.05 to 0.1 M potassium phosphate buffers are common for general yeast and mold enumeration. Diluents containing a solute are recommended for analyzing intermediate and low-moisture foods for xerophilic fungi in order to minimize osmotic shock to fungal cells when making serial dilutions before plating. However, if cells are stressed, the use of diluents with reduced a_w can be critical. Rehydration of low a_w foods before homogenizing in diluent and plating is expected to enhance the resuscitation of injured cells (18).

The choice of solutes used to achieve the desired a_w value in diluents and detection or enumeration media depends on the major solute present in the food to be analyzed. As such, there continues to be a need for improved and new media that will enable a more rapid and accurate assessment of foods in which a_w plays a significant role as a determinant of microbial growth.

INFLUENCE OF WATER ACTIVITY ON MICROBIAL DEATH AND SURVIVAL

When a microorganism is transferred to a new environment, it either survives or dies depending on its ability to adapt. A generalized growth response of a microbial vegetative cell subjected to a reduced a_w is presented in Figure 4. Other than the presence of nutrients, the most important factors for growth and toxin production are temperature, a_w, pH, and oxygen. The basis for survival and death of microorganisms as influenced by a_w is complex. Several intrinsic and extrinsic factors differing within food types and processes and among types of microorganisms may affect this relation. Temperature, oxygen, and chemical and other physical treatments are some extrinsic factors that influence microbial spoilage in foods and the a_w-microorganism response. The use of combinations of extrinsic and intrinsic factors together with lowered a_w levels are common in the food industry. Generally, as the minimal a_w for growth of a microorganism is approached, changes in other environmental factors will have a greater impact on death or survival.

The effects of temperature on the survival of microorganisms are widely documented; the heat resistance of vegetative cells and spores as influenced by a_w is probably the most extensively studied area in terms of microbial inactivation. In general, vegetative cells and fungi spores are more resistant as the a_w of a heating menstruum is reduced. The type of solute used to adjust a_w to some value may result in significant differences in the heat resistance of a given microorganism. Small molecular weight compounds such as sodium chloride and glycerol have been shown to decrease heat resistance in *Salmonella* strains, whereas larger molecular weight solutes such as sucrose exerted a more protective effect against heat inactivation.

WATER ACTIVITY MEASUREMENT

The demand for quality control in the food industry necessitates accurate, fast, and convenient methods of measuring a_w. This is particularly true for food in which a_w is a controlling factor of microbial growth. This section pres-

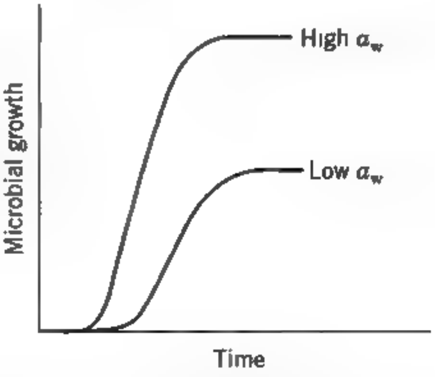


Figure 4. Generalized microbial growth response as affected by a_w.

Table 3. Relative Humidities Generated by the Saturated Solutions of Different Salts

Salt	10°C	15°C	20°C	25°C	30°C
Lithium bromide	7.1	6.9	6.6	6.4	6.2
Sodium hydroxide	—	9.6	8.9	8.2	7.6
Lithium chloride	11.3	11.3	11.3	11.3	11.3
Potassium acetate	23.5	23.5	23.0	22.5	22.0
Magnesium chloride	33.5	33.0	33.0	33.0	32.5
Potassium carbonate	44.0	43.5	43.0	43.0	43.0
Sodium bromide	60.0	59.0	58.0	57.5	56.5
Copper chloride	68.0	68.0	68.0	67.5	67.0
Potassium iodide	72.0	71.0	70.0	69.0	68.0
Sodium chloride	76.0	75.5	75.5	75.5	75.0
Ammonia sulfate	81.0	80.5	80.5	80.0	80.0
Potassium chloride	87.0	86.0	85.0	84.5	84.0
Sodium benzoate	88.0	88.0	88.0	88.0	88.0
Potassium nitrate	95.5	95.0	94.0	93.0	92.0
Potassium sulfate	98.0	98.0	97.5	97.0	97.0

Source: Ref. 21.

ents some of the available methods used to measure a_w in foods (19–21).

Vapor Pressure Measurement

Based on the definition of a_w, food is placed under vacuum conditions until it reaches equilibrium (at controlled temperature) with the surrounding atmosphere of known and constant relative humidity (Table 3), and then the vapor pressure of the atmosphere that is in equilibrium with the sample is measured with a manometer or pressure transducer. Therefore, the vapor pressure could be a direct measure of the a_w. The disadvantage is that the time needed to reach equilibrium is long, making this method inadequate for quick routine analysis.

Depression of the Freezing Point

This colligative property can be expressed by the Robinson and Stokes equation (3), which is valid for liquid foods of $a_w > 0.97$, although it has been acceptable for values as low as 0.80.

Dew Point Hygrometer

This method, which can measure a wide range of a_w at different temperatures, is based on the condensation of water vapor on the surface of a mirror that is cooled to a photoelectrically detected dew temperature of the atmosphere generated by the studied sample. Until recently, a_w meters based on the chilled mirror technique (dew point) were the only ones to feature measuring times of only a few minutes per product sample.

Thermocouple Psychrometer

This apparatus measures a_w by taking into account a wet bulb temperature, which is the temperature an air volume would have if it were cooled to saturation at constant pressure by evaporating water into it. The a_w measurement is based on relative humidity values that are a measure of the difference between dry and wet bulb temperatures.

Electric Hygrometers

The performance of this hygrometer is based on the use of hygrosensors formed by an electrical wire covered by a high hygroscopic salt such as lithium chloride. When the salt absorbs the water vapor released by the sample, a change on the conductance of the wire is provoked.

Filament Hygrometer

This low-price instrument uses a synthetic fiber that shrinks when exposed to high relative humidity. The dimensional modification is recorded and related to the sample a_w. This hygrometer is affected in an important way by temperature changes and the presence of volatiles because it has a low sensitivity, although it is functional within the 0.70–0.95 a_w range.

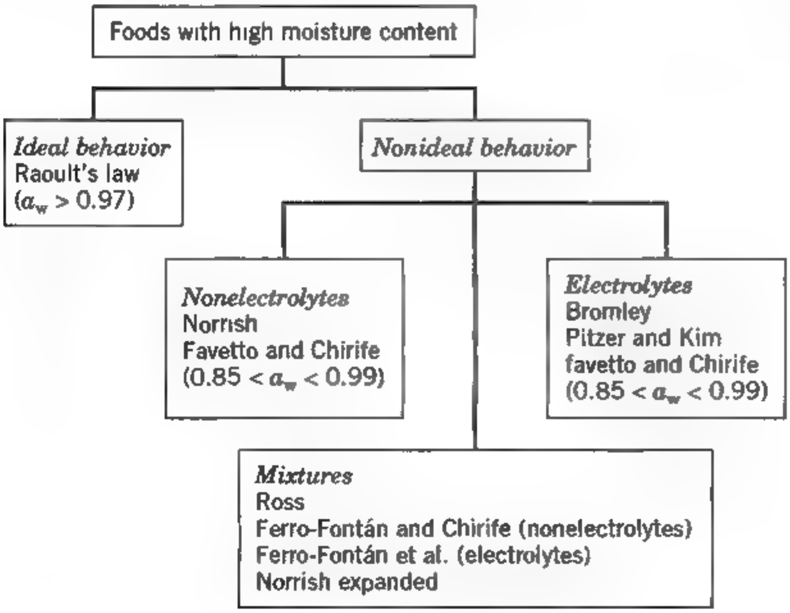


Figure 5. van den Berg's scheme to predict a_w.

PREDICTION OF WATER ACTIVITY

van den Berg and Bruin (4), Chirife (21), and Roa and Tapia (22) have presented a series of comprehensive analyses of the procedures traditionally used to calculate or predict a_w. Each discusses the applicability of diverse theoretical and empirical models on foods, including descriptive examples. Figure 5 summarizes several of these methods.

FINAL REMARKS

Even though there are restrictions and problems associated with use of the a_w term in some food products, its measurement or prediction continues to be useful in process design, food formulation, and the selection of storage conditions. Water activity is an important factor in food microbial responses because their relation to a_w has significant impact and practical implications in the food industry. Some of the areas where a_w has practical applications are in the storage of raw materials, quality of foods, and shelf life of foods stored under various conditions. It is clear that a_w plays a significant role in the survival and death of microorganisms in foods and is also an important influence on microbial response when combined with other intrinsic and extrinsic preservation factors.

BIBLIOGRAPHY

1. L. M. Lenovich, "Survival and Death of Microorganisms as Influenced by Water Activity," in L. B. Rockland and L. R. Beuchat, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, New York, 1987, pp. 119–136
2. J. Welti and F. T. Vergara, "Actividad de Agua: Concepto y Aplicación en Alimentos con Alto Contenido de Humedad," in J. M. Aguilera, ed., *Temas en Tecnologia de Alimentos*, CYTED-Instituto Politécnico Nacional, México, DF, 1997.
3. R. A. Robinson and R. M. Stokes, *Electrolyte Solutions*, 2nd ed., Butterworths Scientific Publishers, London, 1965.
4. C. van den Berg and S. Bruin, "Water Activity and its Estimation in Food Systems: Theoretical Aspects," in L. B. Rockland and G. F. Stewart, eds., *Water Activity: Influences on Food Quality*, Academic Press, New York, 1981.
5. F. Franks, "Water Activity· A Credible Measure of Food Safety and Quality?," *Trends in Food Sci.* **2**, 68–72 (1991).
6. D. A. A. Mossel, "Water and Microorganisms in Foods—A Synthesis," in R. B. Duckworth, ed., *Water Relations in Foods*, Academic Press, New York, 1975.
7. W. J. Scott, "Water Relations of Food Spoilage Microorganisms," *Advances in Food Res.* **7**, 83–127 (1957).
8. J. E. L. Corry, "The Water Relations and Heat Resistance of Microorganisms," *Progress Industrial Microbiol.* **12**, 73–80 (1973).
9. L. R. Beuchat, "Influence of Water Activity on Growth, Metabolic Activities and Survival of Yeasts and Molds," *J. Food Protection* **46**, 135–140 (1983)
10. L. R. Beuchat, "Influence of Water Activity on Sporulation, Germination, Outgrowth, and Toxin Production," in L. B. Rockland and L. R. Beuchat, eds., *Water Activity: Theory and Applications to Food*, L. B. Marcel Dekker, New York, 1987.
11. G. W. Gould, "Drying, Raised Osmotic Pressure and Low Water Activity," in G. W. Gould, ed., *Mechanisms of Action of Food Preservation Procedures*, Elsevier Applied Science, New York, 1989.
12. A. D. Hocking and J. H. B. Christian, "Microbial Ecology Interactions in the Processing of Foods," in G. V. Barbosa-Cánovas and J. Welti-Chanes, eds., *Food Preservation by Moisture Control, Fundamentals and Applications*, Technomic Publishing, Lancaster, Pa., 1996.
13. J. Chirife and M. P. Buera, "Water Activity and Glass Dynamics, and the Control of Microbiological Growth in Foods," *Crit. Rev. Food Sci. Nutrition* **36**, 465–512 (1996).
14. J. H. B. Christian, "Specific Solute Effects on Microbial Water Relations," in L. B. Rockland and J. F Stewart, eds., *Water Activity: Influences on Food Quality*, Academic Press, New York 1981.
15. L. Slade and H. Levine, "Beyond Water Activity: Recent Advances Based on an Alternative Approach to the Assessment of Food Quality," *Crit. Rev. Food Sci. Nutrition* **30**, 115–360 (1991).
16. C. Gutiérrez, T. Abee, and I. R. Booth, "Physiology of the Osmotic Stress Response in Microorganisms," *Int. J. Food Microbiol.* **28**, 233–244 (1996).
17. A. D. Hocking and J. I. Pitt. "Media and Methods for Enumeration of Microorganisms with Consideration of Water Activity," in L. B. Rockland and L. R. Beuchat, eds., *Water Activity: Theory and Applications to Food*, Marcel Dekker, New York, 1987.
18. L. R. Beuchat, "Detection and Enumeration of Microorganisms in Hurdle Technology Foods, with Particular Consideration of Foods with Reduced Water Activity," in G. V. Barbosa-Cánovas and J. Welti-Chanes, eds., *Food Preservation by Moisture Control, Fundamentals and Applications*, Technomic Publishing, Lancaster, Pa., 1996, pp. 603–612.
19. J. A. Troller and J. H. B. Christian, *Water Activity and Food*, Academic Press, New York, 1978.
20. L. B. Rockland and S. K. Nishi, "Influence of Water Activity on Food Product Quality and Stability," *Food Technol.* **34**(4), 42–51, 59 (1980).
21. J. Chirife, "An Update on Water Activity Measurements and Prediction in Intermediate and High Moisture Foods: The Role of Some Nonequilibrium Situations," in G. V. Barbosa-Cánovas and J. Welti-Chanes, eds., *Food Preservation by Moisture Control. Fundamentals and Applications*, Technomic Publishing, Lancaster, Pa., 1996, pp. 169–189.
22. V. Roa and M. S. Tapia, "Estimating Water Activity in Systems Containing Multiple Solutes Based on Solute Properties," *J. Food Sci.* **63**, 559–564 (1998).

A. LÓPEZ-MALO
University of las Américas
Puebla, México

M. S. TAPIA
Central University of Venezuela
Caracas, Venezuela

S. M. ALZAMORA
University of Buenos Aires
Buenos Aires, Argentina

J. WELTI-CHANES
University of las Américas
Puebla, México

M. M. GÓNGORA-NIETO
Washington State University
Pullman, Washington

G. V. BARBOSA-CÁNOVAS
Washington State University
Pullman, Washington

WHEAT SCIENCE AND TECHNOLOGY

References to wheat are worldwide, from the beginnings of recorded time (1–14). Ancient Chinese writings describe the growing of wheat 2,700 years before Christ, and even today wheat is considered a sacred crop in some parts of China.

Theophrastos, a Greek, wrote in 300 B.C. of the many different kinds of wheat grown along the Mediterranean Sea. Written records, works of art, and the excavation of ancient cities show the progressive advancement of the art of milling and baking in Greece and Rome and through the Middle Ages.

No one knows where the wheat plant originated, although it was cultivated where modern humans supposedly first appeared—in southwestern Asia. As early as 10,000–15,000 B.C. humans probably used wheat as food. In 1948 archaeologists from the University of Chicago uncovered an ancient village in Iraq, established 6,700 years before. In the ruins they found two different kinds of wheat similar to those grown today.

Most wheaten foods are made from flour and most of our flour is used by commercial bakers. Over 40 million loaves of bread are sold daily in the United States, and some bakers offer as many as 200 different products—loaves of bread of various shape, crust and texture, or rolls, buns, cookies, crackers, cakes, and pastries.

White flour is also found in the kitchens of homes and restaurants. It is used to prepare home-baked products; as a thickening agent in gravies, sauces, soups, puddings; and in the filling of pies and cakes.

Among the most popular forms of wheat are the macaroni foods—spaghetti, macaroni, and noodles. The basic ingredient of the best macaroni foods is obtained from a hard, amber-colored wheat called durum, grown especially for the macaroni market. The durum wheat is milled with special equipment into a golden-toned, coarse product called semolina, or into granulars that contain a higher percentage of flour, or into flour itself (15).

Macaroni foods made from durum wheat are superior because of their desirable yellow-amber color and nutty flavor, and because they hold their shape and firm texture when cooked.

Many canned, refrigerated, frozen, dried, or ready-to-eat packaged foods contain some wheat or are flour-based: crackers, cookies, snack food items, complete meals; frozen casseroles, soups, prepared sauces, dressings, and gravies.

Breakfast cereals include puffed wheat, wheat flakes, shredded wheat, and bran flakes in sugar-coated, flavored, or plain ready-to-eat form; other cereals are wheat meal, malted breakfast food, and farina—to be cooked and served hot.

By-products of wheat milling are used in rations for cattle, poultry, and other animals. Wheat starches are used industrially, and when priced advantageously, may be substituted for other starches, as in paper sizing, laundry starch, marshmallows, or other products (16–18).

WHEAT

Botanically, wheat belongs to the grass family Gramineae and the genus Triticum. The 14 species of wheat are (1) *Triticum aegilopoides* (wild einkorn), (2) *T. monococcum* (einkorn), (3) *T. dicoccoides* (wild emmer), (4) *T. dicoccum* (emmer), (5) *T. durum* (macaroni wheat), (6) *T. persicum* (Persian wheat), (7) *T. turgidum* (rivet wheat), (8) *T. polonicum* (Polish wheat), (9) *T. timopheevi* (which has no common name), (10) *T. aestivum* (common wheat), (11) *T. sphaerococcum* (shot wheat), (12) *T. compactum* (club wheat), (13) *T. spelta* (spelt), and (14) *T. macha* (macha wheat). The first two species have 7 chromosomes; the following seven species, 14 chromosomes; the last five species, 21 chromosomes (1).

All common wheat such as hard, red winter and spring, soft, red and white wheats, and the club wheats are included in the *Triticum aestivum* classification. Wheat is also classified in several other ways. Some of the factors involved are winter or spring habit of growth, time needed to mature, height, stem leaf, spike, glume, awn or beard, kernel characteristics, and yield.

The common ancestor of all wheats is believed to be wild einkorn (one-seed), which cross-bred with an unknown grass to produce our 7-chromosome wheat, einkorn. Kernels of both the wild and cultivated einkorn were found in the excavated ruins of a village known as Jarmo, dating from 6700 B.C. It is situated in the upper reaches of the Tigris-Euphrates basin in southwestern Asia, called the Fertile Crescent, the presumed birthplace of modern civilization. Einkorn wheats still grow in the Middle East.

The 14- and 21-chromosome species of wheats were believed to have developed as natural hybrids of the original einkorn and possibly emmer. Of the 14 species of wheat, only three—common, club, and durum—account for 90% of all wheat grown in the world today (1).

At the turn of the century, plant scientists began to produce new varieties of wheats artificially from parents selected for desirable traits: greater yield, resistance to diseases and insect infestation, a shorter growing season, better milling and baking qualities, and shorter straw to reduce the chance that wind and weather might flatten the wheat and make harvesting more difficult. Most of the varieties of wheat now grown commercially in the United States were unknown even 10 years ago. While about 30,000 wheat varieties belonging to 14 species are grown throughout the world, only about 1,000 varieties are of commercial significance (1).

PRODUCTION, TRADE, AND USES

Wheat is cultivated in most countries on all continents. World wheat production, consumption, and net exports (imports) are summarized in Table 1.

The top five wheat producing countries are the former Soviet Union, the People's Republic of China, the United States, India, and Canada. Of these five countries, only the United States and Canada grow more wheat than they use and export to other countries. The other three nations are large wheat importers. Some of the top wheat customers of the United States have been China, India, the former Soviet Union, Japan, and Brazil.

A nation of one billion people, China is traditionally regarded as a rice-eating nation. But China grows almost as

Table 1. Wheat: World Production, Consumption, and Net Exports, 1985/1986 (million metric tons)[a]

Country	Production	Consumption	Net exports
	Major exporters		
United States	64.68	30.21	32.53
Canada	23.50	5.50	17.50
Australia	17.00	3.10	15.20
EC-10	70.07	53.07	14.30
Argentina	11.50	4.85	6.70
Turkey	13.00	13.70	−0.15
	Major importers		
USSR	83.00	100.00	−19.00
China	87.00	94.00	−7.00
Eastern Europe	38.98	38.97	−0.21
Other Western Europe	9.57	9.68	−0.29
Brazil	2.20	6.40	−4.40
Mexico	4.40	4.50	−0.30
Other Latin America	.05	2.72	2.69
Japan	.79	6.35	−5.50
India	45.00	43.00	1.40
South Korea	.02	2.37	−2.30
Indonesia	0	1.37	−1.15
Other Asia	17.56	25.21	−8.14
Egypt	1.85	8.45	−6.70
Morocco	1.83	4.14	−2.30
Other northern Africa–Mideast	11.83	25.47	−14.23
Other Africa	3.58	9.48	−5.85
Residual	2.43	10.34	−3.92
Total world	*509.84*	*502.88*	

Source: Grain and Food Division, USDA.

[a] Trade on July–June years.

much wheat as the United States and buys and uses more wheat than any other country in the world. Each person in China on the average consumes 180 lb of wheat every year, mostly in the form of noodles. The average American eats only about 116 lb of wheat flour per year in all types of wheat-based products. Some nations have much higher per capita consumption, up to 300 lb of wheat per year per person (1,19,20).

Wheat is grown in most of the 50 states of the United States. The kind of wheat grown and quantity vary widely from one region to another. In total over 200 varieties are grown annually. Winter wheats are planted in the fall. After the grasslike seedlings emerge from the ground, they lie dormant during the winter. They come up again in the spring, ripen, and are harvested in early summer. Spring wheats are planted in the spring and harvested in late summer. Spring wheats grow best in the northern areas of the United States where the summers are not too hot for the young plants. Winter wheats grow best in areas of the country where the winters are not too harsh for the young plants.

The many varieties of winter and spring wheat are grouped into five official classes. The class a variety fits into is determined by the hardness, the color of its kernels, and its planting time. Each class of wheat has its own relatively uniform characteristics, including those related to milling, baking, or other food uses (1,19,20). Protein range and flour use of major wheat classes are listed in Figure 1 (21).

Hard red winter (HRW) is an important bread wheat that accounts for more than 40% of the United States' wheat crop and wheat exports. This fall-seeded wheat is produced in the Great Plains, which extend from the Mississippi River west to the Rocky Mountains, and from the Dakotas and Montana south to Texas. Significant quantities are also produced in California. HRW has moderately high protein content, usually averaging 11–12%, and good milling and baking characteristics.

Hard red spring (HRS), another important bread wheat, has the highest protein content, usually 13–14%, in addition to good milling and baking characteristics. This spring-seeded wheat is primarily grown in the north central United States—North Dakota, South Dakota, Minnesota, and Montana. HRS constitutes about 15% of U.S. wheat exports. Subclasses based on the dark, hard, and vitreous (DHV) content, include Dark Northern Spring, Northern Spring, and Red Spring.

White wheat (WW) is a preferred wheat for noodles, flat breads, and bakery products other than loaf bread. WW, which includes both fall and spring-seeded varieties, is grown mainly in the Pacific Northwest. This low-protein wheat, usually about 10%, comprises about 15% of U.S. wheat exports, destined primarily for East Asia and the Middle East. Subclasses include hard white, soft white, western white, and white club.

Soft red winter (SRW), which is grown in the eastern third of the United States, is a high-yielding wheat, but relatively low in protein, usually about 10%. SRW best provides flour for cakes, pastries, quick breads, crackers, and snack foods. This fall-seeded wheat constitutes about one-quarter of U.S. wheat exports.

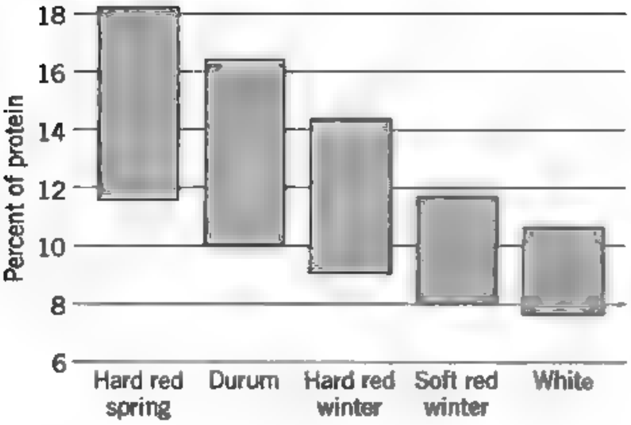
Durum, the hardest of all U.S. wheats, provides semolina for spaghetti, macaroni, and other pasta products. This spring-seeded wheat is grown primarily in the same northern areas as hard red spring, but small winter sown quantities are also grown in Arizona and California. Durum represents about 5% percent of total U.S. wheat exports. Subclasses are hard amber durum, amber durum, and durum.

Grades and grade requirements for all U.S. wheat classes are given in Table 2. Table 3 compares some important quality characteristics of the five classes. Soft white samples are listed separately for the East (SWE) and West (SWW) and club wheats. Values of the total domestic consumption of wheat products as foods in the United States are listed in Table 4.

GROSS COMPOSITION

The chemical composition of wheat, in common with that of other cereal grains, varies widely, for it is influenced by genetic, soil, and climatic factors. Variations are encountered in the relative amounts of proteins, lipids, carbohydrates, pigments, vitamins, ash, and mineral elements.

Wheat is characterized by relatively low protein and high carbohydrate levels; the carbohydrates consist essen-



Floor uses:
- Used to blend with weaker wheats for bread flour
- Whole wheat bread, hearth breads
- Egg noodles (U.S.), macaroni and other alimentary pastes
- White bakers' bread, bakers' rolls
- Waffles, muffins, quick yeast breads, all-purpose flour
- Noodles (Oriental) kitchen cakes and crackers, pie crust, doughnuts, and cookies, foam cakes, very rich layer cakes

Figure 1. Protein range and flour uses of major wheat classes. Flour uses are listed according to the approximate level of protein required for specified wheat products. Durum is not traded on the basis of protein content. *Source:* Ref. 21.

Table 2. Official U.S. Standards for Grain (USDA)

	Minimum limits of		Maximum limits of							
	Test weight per bushel (lb)							Wheat of other classes[a]	
	Hard red spring wheat or white club wheat[b]	All other classes and subclasses	Heat-damaged kernels (%)	Damaged kernels, total (%)[c]	Foreign material	Shrunken and broken kernels (%)	Defects, total (%)[d]	Contrasting classes (%)	Wheat of other classes (%)[e]
U.S. no. 1	58.0	60.0	0.2	2.0	0.5	3.0	3.0	1.0	3.0
U.S. no. 2	57.0	58.0	0.2	4.0	1.0	5.0	5.0	2.0	5.0
U.S. no. 3	55.0	56.0	0.5	7.0	2.0	8.0	8.0	3.0	10.0
U.S. no. 4	53.0	54.0	1.0	10.0	3.0	12.0	12.0	10.0	10.0
U.S. no. 5	50.0	51.0	3.0	15.0	5.0	20.0	20.0	10.0	10.0
U.S. sample grade[f]									

[a] Unclassed wheat of any grade may contain not more than 10% of wheat of other classes.
[b] These requirements also apply when Hard Red Spring wheat or White Club wheat predominate in a sample of mixed wheat.
[c] Includes heat-damaged kernels.
[d] Defects (total) include damaged kernels (total), foreign material, and shrunken and broken kernels. The sum of these three factors may not exceed the limit for defects.
[e] Includes contrasting classes.
[f] U.S. sample grade shall be wheat that (a) does not meet the requirements for U.S. grades 1, 2, 3, 4, or 5; or (b) contains 8 or more stones, 2 or more pieces of glass, 3 or more crotalaria seeds (*Crotalaria* spp.), 2 or more castor beans (*Ricinus communis*), 4 or more particles of an unknown foreign substance(s) or a commonly recognized harmful or toxic substance(s), or 2 or more rodent pellets, bird droppings, or equivalent quantity of other animal filth per 1,000 g of wheat; or (c) has a musty, sour, or commercially objectionable foreign odor (except smut or galic odor); or (d) is heating or otherwise of distinctly low quality.

tially of starch (≥90%), dextrins, pentosans, and sugars. The various components are not uniformly distributed in the different kernel structures. Composition of different parts of the wheat kernel is shown in Table 5 (2). The hulls and bran are high in cellulose, pentosans, and ash; the germ is high in lipid, protein, sugar, and ash. The endosperm consists primarily of starch and is lower in protein content than the germ and, in some cereals, than the bran. It is also low in crude fat and ash. In milling, the hulls, germ, and bran are removed. The composition of the main anatomical parts of wheat and of flours that differ in extraction rate are compared in Table 6. The values in the table are representative averages. Analyses of ash, protein, crude fat, crude fiber, and starch of whole wheat and mill streams are summarized in Table 7.

Starch

The starch content of wheat flour is, in general, inversely related to protein content. In flours below 80% extraction, the starch content ranges from about 65–70% (on an as-is moisture basis) and the proportion of linear (amylose) to branched (amylopectin) fractions in starch is about 1:3.

Proteins

The protein content of wheat is an important index of its quality for the manufacture of different food products and is influenced by climate, weather, soil, and the variety of grain (25,26). The breadmaking potential of bread-wheat is largely associated with the quantity and quality of its protein. The quantity of protein is influenced to a large

Table 3. Mean Values of Studied Parameters in Wheats

Parameter	HRW	HRS	SRW	SWE	SWW	CLB	DUR
Test weight, lb/bu	60.9	61.4	61.9	61.8	61.0	60.7	61.8
1,000-kernel weight, g	28.0	32.1	33.3	37.8	36.0	29.1	39.3
Wheat							
Density, g/mL	1.4594	1.4605	1.4252	1.4224	1.4417	1.4451	1.4583
Ash, %	1.57	1.56	1.48	1.49	1.46	1.34	1.59
Protein, %	11.7	13.7	9.7	9.3	10.0	10.5	13.4
Flour yield, %	72.9	75.2	71.8	69.6	72.1	72.7	70 4
Mill rating (hardness)	6.1	7.3	3.3	1.9	3.2	3.4	9.5
Starch damage, %	7.0	7.2	4.4	4.2	5.0	4.4	13.8
Particle size index, %	28.0	26.5	41.7	41.3	36.6	38.4	15.7
Flour protein, %[a]	10.7	13.1	8.6	8.2	8.9	9.4	12.6
Bake absorption, %	63.9	67.5					
Mixograph development time, min	3.7	3.6	3.3	3.0	2.2	2.0	2.8
Loaf volume, mL	879	985					
Crumb grain[b]	3.7	2.5					
Specific loaf volume, mL/1% protein	58.7	56.0					
Cookie diameter, cm			9.5	9.5	9.2	9.4	

Source: Ref. 22.

[a] 14% moisture basis, $N \times 5.7$.

[b] The higher the number, the harder the wheat. Average = normal hard-wheat hardness, 6–8; S/A = softer than average, 5, Q-S = too soft to be milled as hard wheat, 3, 4, U-S = satisfactory for use with hard-wheat blends, 1, 2, Q-U = harder than normal hard wheat, 9,10. The higher the score, the poorer the crumb grain. On a scale of 1–10, 1 = satisfactory, 10 = very unsatisfactory.

Table 4. Value of Total Domestic Consumption of Grain Products, 1983[a]

Product	Value of consumption (million U.S. $)
Baked goods[b]	26,052.9
Bread and rolls	9,354.1
White bread	6,494.7
Other bread	1,595.7
Rolls	1,263.8
Crackers, cookies[c]	10,498.1
Sweet goods[d]	6,200.6
Frozen and refrigerated baked goods	1,868.6
Cereal, flour, pasta[e]	8,390.5
Cereals[f]	4,170.6
Flour[g]	2,141.3
Pasta[h]	1,526.4
Macaroni	373.9
Noodles	273.9
Spaghetti	480.4
Total	*36,312.0*

Source. Ref 23.

[a] All consumption, whether at home, in restaurants, or institutions, in terms of retail store valuation.

[b] Total minus cereal, flour, pasta, frozen and refrigerated baked goods.

[c] Includes snacks (chips, pretzels, popcorn).

[d] Cakes, pastries, doughnuts, pies, and in-store bakery.

[e] Includes rice.

[f] Cold and hot cereals, hominy grits, infant cereals.

[g] Cake flour, corn meal, family flour, pancake and waffle mixes, and prepared mixes, including cakes and frosting.

[h] Includes canned pasta.

extent by environmental factors; the quality of protein is heritable. In wheat varieties grown under comparable environmental conditions, a high-quality wheat produces good bread over a fairly wide range of protein levels, whereas a low-quality wheat produces relatively poor-quality bread even when its protein content is high. For many years it has been believed that differences in protein content among varieties of wheat grown under comparable conditions are small compared with differences due to environment. In recent years, however, it has been shown that the protein content of wheat can be greatly increased by selective breeding.

The proteins in wheat (Fig. 2) include water-soluble proteins (albumins), salt-soluble proteins (globulins), alcohol-soluble proteins (prolamins or gliadins), and acid-and alkali-soluble proteins (glutelins). When water is added, the wheat endosperm proteins (gliadin and glutenin) form a tenacious colloidal complex known as gluten, which is responsible for the superiority of wheat over the other cereals for the manufacture of leavened products. Gluten is responsible for the retention of carbon dioxide produced by yeast or chemical leavening agents.

As a class, cereal proteins are not as high in biological value as are the proteins from certain legumes, nuts, or animal products (28). The limiting amino acid in wheat endosperm proteins is lysine. Although biological values of the proteins of entire cereal grains are greater than those of the refined mill products, which consist chiefly of the endosperm, many diets normally include animal products as well as cereal products. With mixed diets, different proteins tend to supplement each other, and thus cereals can be important and valuable sources of amino acids for the synthesis of body proteins.

Lipids

Total wheat flour lipids (ca 1.4–2.0%) contain about equal amounts of nonpolar and polar components. Triglycerides are a major component of nonpolar lipids; digalactosyldiglycerides, of glycolipids; and lysophosphatidylcholines and phosphatidylcholines, of phospholipids. Wheat germ is an abundant source of α-tocopherol, as vitamin E (28).

Table 5. Composition of Different Parts of the Wheat Kernel

Kernel tissue[a]	Crude protein (%)	Lipid (%)	Starch (%)	Reducing sugars (%)	Pentosans (%)	Cellulose (%)	Ash (%)
Whole kernel	12.0	1.8	58.5	2.0	6.6	2.3	1.8
Pericarp	7.5	0.0	0.0	0.0	34.5	38.0	5.0
Testa and hyaline	15.5	0.0	0.0	0.0	50.5	11.0	8.0
Aleurone layer	24.0	8.0	0.0	0.0	38.5	3.5	11.0
Outer endosperm	16.0	2.2	62.7	1.6	1.4	0.3	0.8
Inner endosperm	7.9	1.6	71.7	1.6	1.4	0.3	0.5
Embryo and scutellum	26.0	10.0	0.0	26.0	6.5	2.0	4.5

Source: Ref. 2.

[a] Protein content calculated by nitrogen × 5.83; moisture content, 15%.

Table 6. Weight, Ash, Protein, Lipid, and Crude Fiber Contents of Main Anatomical Parts of the Wheat Kernel and of Flours Varying in Milling Extraction Rate[a]

	Wheat kernel fractions (%)						
Parameter	Pericarp	Aleurone layer	Starchy endosperm	Germ	Milling extraction		
Weight	9	8	80	3	75	85	100
Ash	3	16	0.5	5	0.5	1	1.5
Protein	5	18	10	26	11	12	12
Lipid	1	9	1	10	1	1.5	2
Crude fiber	21	7	>0.5	3	>0.5	0.5	2

Source: Ref. 24.

[a] Expressed on a 14% moisture basis.

Table 7. Gross Chemical Composition of Wheat and Mill Streams[a]

	Yield (%)	Ash (%)	Protein, $N \times 5.7$ (%)	Crude fat (%)	Crude fiber (%)	Starch (%)
Wheat	100	1.2–1.7	9.2–13.8	1.1–1.9	1.7–2.6	54.1–61.8
Flour	72.6–77.0	0.35–0.42	8.6–13.0	0.8–1.0	—	64.3–73.7
Germ	0.6–1.1	3.5–4.3	21.7–24.5	6.3–10.6	2.8–4.0	14 0–23.9
Red dog	1.4–4.7	1 5–2.7	12.7–15.2	2.3–4.7	1.2–3.2	37.0–47.8
Shorts	6.6–8.9	3.1–4.3	13.8–16.5	3.7–6.3	5.6–7.2	15.9–21.7
Bran	12.5–16.9	4.7–7.1	12.1–15.4	3.0–4.2	9.2–11.6	4.6–7.2

Source: Ref. 24.

[a] Expressed on a 14% moisture basis.

Vitamins

Wheat has a relatively high content of thiamine and niacin compared to other cereal grains. It is low in riboflavin and contains no vitamin A or C (28).

MILLING

Conventional, Modern Milling

Before wheat can be used in the production of most foods, it must undergo several mechanical and chemical changes. The first change involves milling of wheat into flour (1,25). The steps involved in wheat-to-flour production are wheat selection, blending, cleaning, conditioning, milling, and maturing. The endosperm, which forms about 83% of the kernel, is the source of white flour and contains 70–75% of the kernel's protein. The bran, forming about 14% of the kernel, is included in whole wheat flour, but is more often removed and used in animal or poultry feed. Because the cellulosic material of the bran cannot be digested and tends to accelerate the passage of food through the human digestive tract, the total nutritive contribution of whole wheat flour is less than that found in enriched white flour products. The germ, forming about 3% of the kernel, is the embryo or sprouting tissue of the seed. It is usually separated out because it contains oil, which limits the keeping quality of flours. Although the germ is available as human food, it is usually added to animal or poultry feed. The modern milling process is depicted in a diagrammatic form in Figure 3 (1).

The mill flow diagram begins with a separator, where the wheat first passes through a vibrating screen that removes straw and other coarse materials, and then over a second screen through which drop small foreign materials like seeds. An aspirator lifts off lighter impurities in the wheat. After the aspirator, wheat moves into a disk separator, consisting of disks revolving on a horizontal axis. The disk surfaces are indented to catch individual grains of wheat but reject larger or smaller material. The blades push the wheat from one end of the machine to the other. The revolving disks discharge the wheat into a hopper or

Table 5. Composition of Different Parts of the Wheat Kernel

Kernel tissue[a]	Crude protein (%)	Lipid (%)	Starch (%)	Reducing sugars (%)	Pentosans (%)	Cellulose (%)	Ash (%)
Whole kernel	12.0	1.8	58.5	2.0	6.6	2.3	1.8
Pericarp	7.5	0.0	0.0	0.0	34.5	38.0	5.0
Testa and hyaline	15.5	0.0	0.0	0.0	50.5	11.0	8.0
Aleurone layer	24.0	8.0	0.0	0.0	38.5	3.5	11.0
Outer endosperm	16.0	2.2	62.7	1.6	1.4	0.3	0.8
Inner endosperm	7.9	1.6	71.7	1.6	1.4	0.3	0.5
Embryo and scutellum	26.0	10.0	0.0	26.0	6.5	2.0	4.5

Source: Ref. 2.

[a] Protein content calculated by nitrogen × 5.83; moisture content, 15%.

Table 6. Weight, Ash, Protein, Lipid, and Crude Fiber Contents of Main Anatomical Parts of the Wheat Kernel and of Flours Varying in Milling Extraction Rate[a]

	Wheat kernel fractions (%)						
Parameter	Pericarp	Aleurone layer	Starchy endosperm	Germ	Milling extraction		
Weight	9	8	80	3	75	85	100
Ash	3	16	0.5	5	0.5	1	1.5
Protein	5	18	10	26	11	12	12
Lipid	1	9	1	10	1	1.5	2
Crude fiber	21	7	>0.5	3	>0.5	0.5	2

Source: Ref. 24.

[a] Expressed on a 14% moisture basis.

Table 7. Gross Chemical Composition of Wheat and Mill Streams[a]

	Yield (%)	Ash (%)	Protein, $N \times 5.7$ (%)	Crude fat (%)	Crude fiber (%)	Starch (%)
Wheat	100	1.2–1.7	9.2–13.8	1.1–1.9	1.7–2.6	54.1–61.8
Flour	72.6–77.0	0.35–0.42	8.6–13.0	0.8–1.0	—	64.3–73.7
Germ	0.6–1.1	3.5–4.3	21.7–24.5	6.3–10.6	2.8–4.0	14.0–23.9
Red dog	1.4–4.7	1.5–2.7	12.7–15.2	2.3–4.7	1.2–3.2	37.0–47.8
Shorts	6.6–8.9	3.1–4.3	13.8–16.5	3.7–6.3	5.6–7.2	15.9–21.7
Bran	12.5–16.9	4.7–7.1	12.1–15.4	3.0–4.2	9.2–11.6	4.6–7.2

Source: Ref. 24.

[a] Expressed on a 14% moisture basis.

Vitamins

Wheat has a relatively high content of thiamine and niacin compared to other cereal grains. It is low in riboflavin and contains no vitamin A or C (28).

MILLING

Conventional, Modern Milling

Before wheat can be used in the production of most foods, it must undergo several mechanical and chemical changes. The first change involves milling of wheat into flour (1,25). The steps involved in wheat-to-flour production are wheat selection, blending, cleaning, conditioning, milling, and maturing. The endosperm, which forms about 83% of the kernel, is the source of white flour and contains 70–75% of the kernel's protein. The bran, forming about 14% of the kernel, is included in whole wheat flour, but is more often removed and used in animal or poultry feed. Because the cellulosic material of the bran cannot be digested and tends to accelerate the passage of food through the human digestive tract, the total nutritive contribution of whole wheat flour is less than that found in enriched white flour products. The germ, forming about 3% of the kernel, is the embryo or sprouting tissue of the seed. It is usually separated out because it contains oil, which limits the keeping quality of flours. Although the germ is available as human food, it is usually added to animal or poultry feed. The modern milling process is depicted in a diagrammatic form in Figure 3 (1).

The mill flow diagram begins with a separator, where the wheat first passes through a vibrating screen that removes straw and other coarse materials, and then over a second screen through which drop small foreign materials like seeds. An aspirator lifts off lighter impurities in the wheat. After the aspirator, wheat moves into a disk separator, consisting of disks revolving on a horizontal axis. The disk surfaces are indented to catch individual grains of wheat but reject larger or smaller material. The blades push the wheat from one end of the machine to the other. The revolving disks discharge the wheat into a hopper or

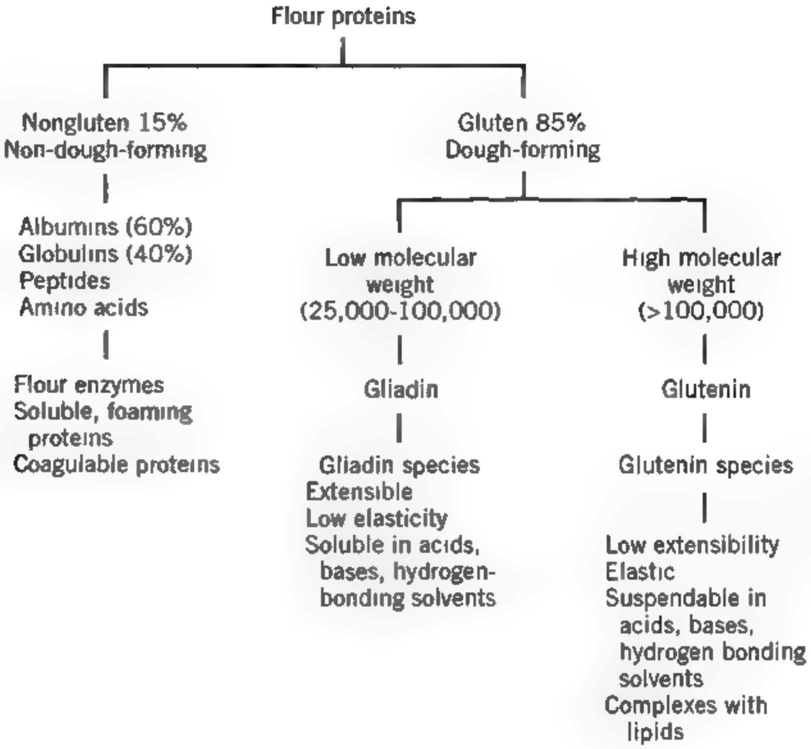

Flour proteins
Nongluten 15%
Non-dough-forming
Gluten 85%
Dough-forming
Albumins (60%)
Globulins (40%)
Peptides
Amino acids
Low molecular weight (25,000-100,000)
High molecular weight (>100,000)
Flour enzymes
Soluble, foaming proteins
Coagulable proteins
Gliadin
Glutenin
Gliadin species
Extensible
Low elasticity
Soluble in acids, bases, hydrogen-bonding solvents
Glutenin species
Low extensibility
Elastic
Suspendable in acids, bases, hydrogen bonding solvents
Complexes with lipids

Figure 2. Schematic presentation of the main protein fractions of wheat flour. *Source:* Ref. 27.

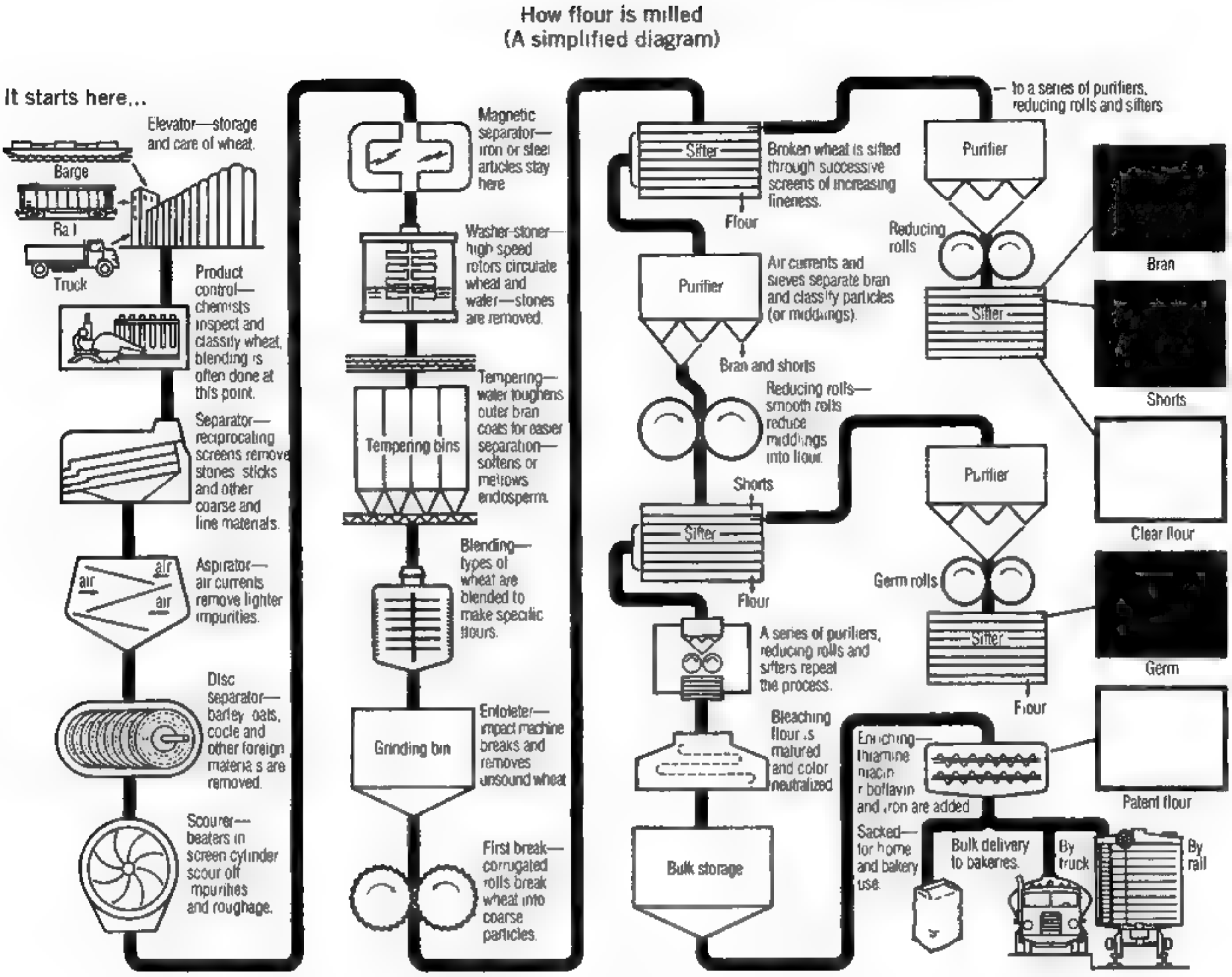
into the continuing stream. The wheat then moves into a scourer—a machine in which beaters attached to a central shaft throw the wheat against a surrounding drum. Scourers may be either horizontal or upright, with or without brushes, and adjusted for mild, medium, or hard scouring. Air currents carry off the dust and loosened particles of bran coating.

The stream of wheat next passes over a magnetic separator that pulls out iron and steel particles. A washer–stoner may be the next piece of equipment. High-speed rotors spin the wheat in a water bath. Excess water is thrown out by centrifugal force. Stones drop to the bottom and are removed. Lighter materials float off, leaving only the clean wheat. In the production lines of some mills, a dry stoner is also used. The wheat passes over an inclined, vibrating table that pushes stones and heavy material away from the lighter wheat, discharged separately.

The clean wheat is then tempered before the start of grinding; in the process moisture is added. Tempering aids the separation of the bran from the endosperm and helps provide a constant, controlled amount of moisture and temperature throughout the milling process. The percentage of moisture, length of conditioning, and temperature are the three important factors in tempering, with different requirements in soft, medium, and hard wheats (1). Some water for tempering may be added, dampening the wheat; the wheat may be washed until a certain amount of moisture is absorbed; or the wheat may be subjected to steam under low pressure. The dampened wheat is held in a bin for a prescribed period—usually 8–24 h, depending on the type of wheat. The outer layers of the wheat berry tend to be brittle, and tempering toughens the bran coat for better separation of the endosperm. Within the kernel, tempering also mellows the endosperm so that the floury particles break more freely in milling. When the moisture content is properly dispersed in the wheat for efficient milling, up to approximately 16%, the grain is passed through an Entoleter scourer–aspirator as a final step in cleaning. Disks revolving at high speed in the scourer–aspirator throw the wheat against fingerlike pins. The impact cracks unsound kernels (including insect-damaged), which are rejected. The sound wheat flows to a grinding bin or hopper from which it is fed in a continuous metered stream into the mill itself (1).

The first-break rolls of a mill are corrugated rather than smooth like the reduction rolls that reduce the particles of endosperm further along in the process. The rollers are paired and rotate inward against each other and at different speeds. The clearance between rollers and the pressure as well as the speed of each separate roller, can be adjusted. At each breaking step, the miller selects the milling surface and the corrugations; the speed of and interrelation between the rollers depend on the type and condition of the wheat.

The next major step introduces the broken-ground particles of wheat and bran into a sifter where they are shaken through a series of bolting cloths or screens to separate the larger from the smaller particles. The fractions, and particles of endosperm graded by size are carried to purifiers. In a purifier, a controlled flow of air lifts off bran particles while bolting cloth at the same time separates and grades coarser fractions by size and quality. Four or five additional break rolls, with successively finer corrugations and each followed by a sifter, are usually used to rework the coarse stocks from the sifters and reduce the wheat particles to granular middlings as free from bran as possible. Germ


How flour is milled
(A simplified diagram)
It starts here...
Elevator—storage and care of wheat.
Barge
Rail
Truck
Product control—chemists inspect and classify wheat, blending is often done at this point.
Separator—reciprocating screens remove stones, sticks and other coarse and fine materials.
Aspirator—air currents remove lighter impurities.
air
air
air
Disc separator—barley, oats, cockle and other foreign materials are removed.
Scourer—beaters in screen cylinder scour off impurities and roughage.
Magnetic separator—iron or steel articles stay here
Washer-stoner—high speed rotors circulate wheat and water—stones are removed.
Tempering bins
Tempering—water toughens outer bran coats for easier separation—softens or mellows endosperm.
Blending—types of wheat are blended to make specific flours.
Grinding bin
Entoleter—impact machine breaks and removes unsound wheat
First break—corrugated rolls break wheat into coarse particles.
Sifter
Broken wheat is sifted through successive screens of increasing fineness.
Flour
Purifier
Air currents and sieves separate bran and classify particles (or middlings).
Bran and shorts
Reducing rolls—smooth rolls reduce middlings into flour.
Shorts
Sifter
Flour
A series of purifiers, reducing rolls and sifters repeat the process.
Bleaching flour is matured and color neutralized
Bulk storage
to a series of purifiers, reducing rolls and sifters
Purifier
Reducing rolls
Sifter
Bran
Shorts
Purifier
Clear flour
Germ rolls
Sifter
Germ
Flour
Enriching—thiamine niacin riboflavin and iron are added
Patent flour
Sacked—for home and bakery use.
Bulk delivery to bakeries.
By truck
By rail

Note: This chart is greatly simplified. The sequence, number and complexity of different operations vary in different mills.

Figure 3. Milling process of flour. *Source:* Wheat Flour Institute, Chicago, Ill.

particles, being somewhat plastic, are flattened by passage through the smooth reduction rolls and can be separated. The reduction rolls reduce the purified, granular middlings or farina to flour.

The process is repeated until the maximum amount of flour is separated, consisting of at least 72% of the wheat. This flour is made up of the various grades shown in Figure 4 (29). The remaining percentage of the wheat berry is classified as millfeed. The flour can be classified in several ways. Straight flour is all the flour produced, with various streams of flour mixed into one. Flour emerges at a number of points in the milling process, with the purified middlings yielding the extrashort or patent or bread flour. In hard wheat mills, as much as 75–80% may be run together as first clear or split into fancy clear and second clear.

In a soft wheat mill, 40–60% of the fancy patent may be taken off separately, leaving about 55% of the remaining flour to be classified as fancy clear. The chart (Fig. 4) is a generalization rather than an exact description of the yield of and single mill from a particular kind of wheat. It shows how the various streams of flour may be classified, starting with fancy patent, through short, medium, and long patent flours, leaving less and less to be classified as clear flour. The extrashort or fancy patent is the finest, with grades dropping down the scale to clears (30,31).

Toward the end of the millstream, the finished flour flows through a device that releases a bleaching–maturing agent in measured amounts. For bakery customers, the finished flour flows into hoppers for bulk storage, since most bakers add their own form of the enrichment formula to dough—a combination of thiamine, niacin, riboflavin, and iron. For packing as family flour, the enrichment ingredients are added in another mixing machine as the flour flows to the packing room. If the flour is self-rising, a leavening agent and salt are also added.

In milling of durum wheat for the macaroni trade, special equipment is required, especially additional purifiers to separate the bran from the semolina, a coarse granula-

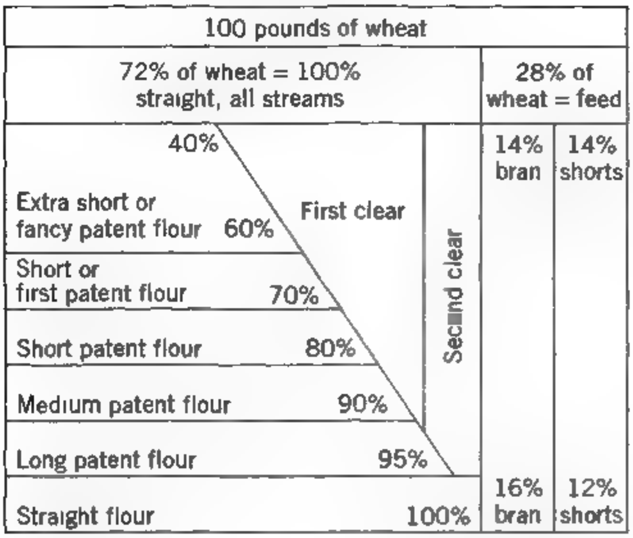


Figure 4. Grades of flour. *Source:* Ref. 29.

tion of the endosperm (15). By federal definition, semolina is prepared by grinding and bolting durum wheat, separating the bran and germ, to produce a granular product with no more than 3% flour. Durum millers also make granulars, or a coarse product with greater amounts of flour; or they grind the wheat into flour for special use in macaroni products, particularly in noodles.

New Milling Developments

In the early 1960s it was found that wheat could be ground to a very fine flour by impact milling and that the product could be further separated into products varying widely in protein. Finished flour from a conventional roller mill is further reduced in particle size in a highspeed grinder, an impact or pin mill. Disintegration of the flour particles takes place as they strike one another, the surface of the rotors, and the pins. The flour is fractured in granular form rather than pressed and broken as in a roller system. The reground flour contains a mixture of relatively coarse endosperm chunks, intermediate fragments, and fines.

The reground flour is channeled to a classifier, where swirling air funnels the larger particles down and away while the smaller fines are lifted up and separated. Repeating the process as shown in Figure 5 (1), 20–30% of the flour is separated into a low-protein product suitable for cakes and pastries; 5–15% makes up the fine fraction, containing 15–22% protein.

The high-protein flour may be used to fortify or blend with other flours. Recombining some of the high-protein fraction with the coarse portions permits a miller to tailor a flour of protein value to a buyer's specifications.

Fine grinding and air classification make possible the production of some cake flour from hard wheat and some bread flour or high-protein fractions from soft wheat. Application of the process theoretically frees the miller from dependence on different wheats, either hard or soft, that change each crop year. The problem is how to market the larger volume of low-protein or starch fractions at prices adequate to justify the installation and operation of the special equipment (1).

Bleaching and Maturing Agents

Flour is often bleached, both for use by bakers and for the family flour trade. Bleaching improves the color of the flour, and some bleaching agents mature the flour and condition the gluten, improving baking quality. Flour fresh from the mill may be aged in storage or treated with a bleaching-maturing agent to improve baking quality. In aged, unbleached flour or in treated flour, the proteins are slightly oxidized by oxygen from air. The oxidation of flour makes gluten stronger or more elastic and produces better baking results. Cookie, pie crust, and cracker flours usually perform better when no bleach is used.

Dough handling properties can be modified beneficially, or adversely, by the addition of minute amounts of reducing agents (such as cysteine). In addition, the performance of a flour can be improved significantly by the addition of certain oxidizing agents. A list of oxidizing and reducing agents used to improve the baking quality of wheat flour is given in Table 8.

FLOUR TYPES

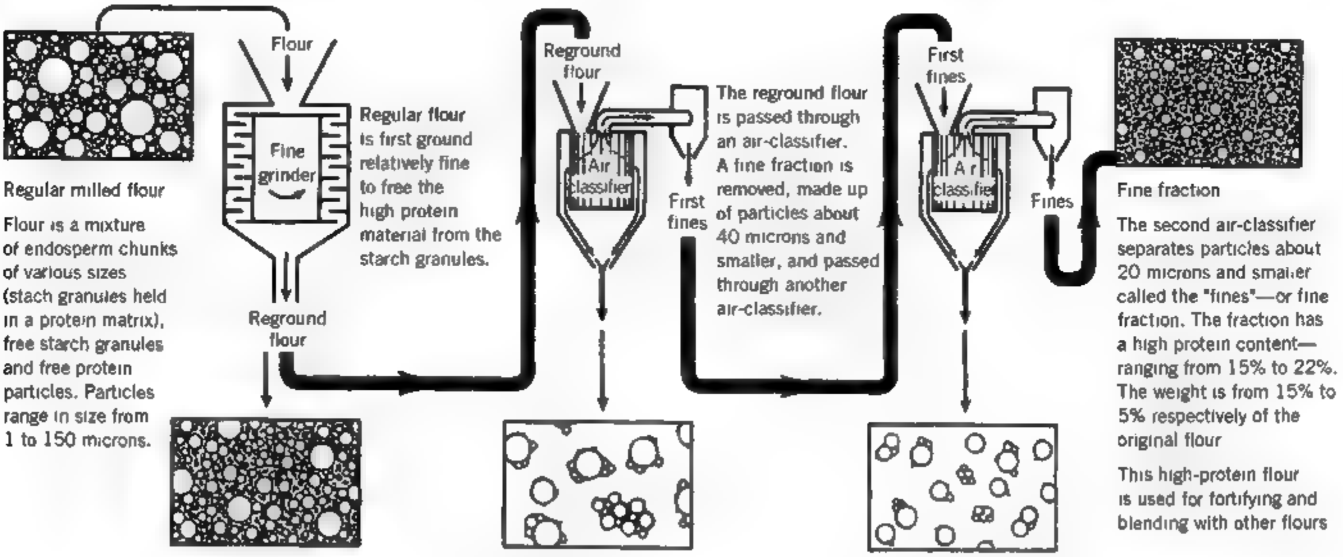
Hard-wheat flours are usually higher in protein than are soft-wheat flours. They may be milled from either winter or spring wheat varieties. Those with highest protein content, characterized by their capacity to develop the strongest gluten, are used in commercial bread production where doughs must withstand the rigors of machine handling. Other hard-wheat flours with more mellow gluten, easier to develop in kneading by hand, are packed as family flour, all-purpose flour, and self-rising flour. The protein in hard spring wheat flour runs from 11 to 16%; in hard winter wheat flour from 10–14% (19,20).

Soft-wheat flours are sold for general family use, as biscuit or cake flours, and for the commercial production of crackers, pretzels, cakes, cookies, and pastry. The protein in soft wheat flour runs from 7 to 10%. There are differences in appearance, texture, and absorption capacity between hard- and soft-wheat flour subjected to the same milling procedures. Hard-wheat flour falls into separate particles if shaken in the hand whereas, soft-wheat flour tends to clump and hold its shape if pressed together. Hard-wheat flour feels slightly coarse and granular when rubbed between the fingers; soft-wheat flour feels soft and smooth. Hard-wheat flour absorbs more liquid than does soft-wheat flour. Consequently, many recipes recommend a variable measure of either flour or liquid to achieve a desired consistency.

Whole wheat flour, according to FDA specifications, is a coarse-textured flour ground from the entire wheat kernel. It contains the bran, germ, and endosperm. The presence of bran reduces gluten development. Baked products made from whole wheat flour tend to be heavier and denser than those made from white flour. Whole wheat flour is rich in B-complex vitamins, vitamin E, fat, protein, and contains more trace minerals and dietary fiber than does white flour. In most recipes, whole wheat flour can be mixed half

How flour is fractioned

Notes:
- Schematic drawings of flour are approximately 100 times actual size
- Flour particles measured in microns–1 micrin = 1/25,000 of an inch
- White areas in circles indicate starch granules
- Black portions in schematics indicate protein



Regular milled flour

Flour is a mixture of endosperm chunks of various sizes (stach granules held in a protein matrix), free starch granules and free protein particles. Particles range in size from 1 to 150 microns.

Regular flour is first ground relatively fine to free the high protein material from the starch granules.

Reground flour

Reground flour is a mixture of course endosperm chunks, intermediate size fragments and "fines." This flour is now ready for separation.

The reground flour is passed through an air-classifier. A fine fraction is removed, made up of particles about 40 microns and smaller, and passed through another air-classifier.

Endosperm chunks

Coarse chunks are separated from the "first fines." The protein content of these chunks is near that of the original flour. THis portion is about 65% of the total flour wewnght and may be reground or used for various purposes.

Intermediate fraction

The protein content of this starchy fraction is well below that of the original flour. The intermediate fraction is about 20% to 30% of the total flour weight and has some uses in applications with low protein requirements

Fine fraction

The second air-classifier separates particles about 20 microns and smaller called the "fines"—or fine fraction. The fraction has a high protein content—ranging from 15% to 22%. The weight is from 15% to 5% respectively of the original flour

This high-protein flour is used for fortifying and blending with other flours

Note: This is a generalization of the fractionation process. The number and sequences of operations vary with different types of flour made from various types of wheat and for the specific uses of the flour.

Figure 5. How flour is fractionated. *Source:* Wheat Flour Institute, Chicago, Ill.

and half with white flour for a satisfactory product. Graham flour is synonymous with whole wheat flour, named after a physician, Dr. Sylvester Graham, who advocated the use of whole wheat flour in the first half of the nineteenth century (19,20).

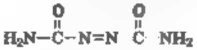
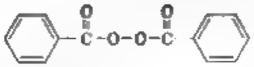
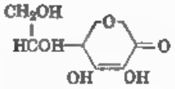
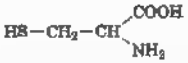
According to FDA standards, wheat flour must be milled from cleaned wheat, essentially free of bran and germ, and be ground to such a degree that the product will pass through a sieve having 65 openings per lineal inch (or 4,225 openings per square inch). The moisture content must not exceed 15%.

All-purpose flours are designed for home baking of a wide range of products—yeast breads, quick breads, cakes, cookies, and pastries. Such flours may be made from low-protein hard wheat, from soft or intermediate wheats, or from blends of hard and soft wheat designed to achieve mellow gluten a homemaker can manipulate, and tenderness of structure in the final baked product. All-purpose flour, sometimes called general-purpose or family flour, is most commonly used for home baking. Most modern recipes for home baking are designed for use with all-purpose flour.

Self-rising flours are all-purpose flours to which leavening agents and salt have been added. The leavening agents used are sodium bicarbonate and an acid-reacting substance, monocalcium phosphate, sodium acid pyrophosphate, sodium aluminum phosphate, or a combination of these acids. The sodium bicarbonate and the acid ingredient react in the presence of liquid to release carbon dioxide. The leavening agent and salt are added in amounts sufficient to produce not less than 0.5% of their combined weight of carbon dioxide. Their combined weight must not exceed 4.5 parts per hundred parts of flour. Phosphated flour is all-purpose flour to which the acid-reacting ingredient, monocalcium phosphate, has been added in a quantity of not more than 0.75% of the weight of the finished phosphated flour. It assists in stabilizing gluten and helps nourish yeast. Bromated flour contains potassium bromate in a quantity not exceeding 50 ppm. The bromate has an oxidizing effect that improves the baking qualities of the flour. Such flour must be labeled as bromated.

Cake flours are milled from low-protein soft wheat especially suitable for baking cakes and pastries or from low-protein fractions derived in the milling process. Cake

Table 8. Flour Treatment Agents[a]

	Compound							
	Bromate	Iodate	Anodicarbonamide	Chlorine dioxide	Chlorine	Benzoyl peroxide	L-Ascorbic acid	L-Cysteine
Structure	$KBrO_2$	KIO3	 $H_2N-\overset{\overset{O}{\|}}{C}-N=N-\overset{\overset{O}{\|}}{C}-NH_2$	ClO_2	Cl_2			
Form	Powder	Powder	Powder	Gas	Gas	Powder	Powder	Powder
Introduced	1916	1916	1962 patent	1948	1912	1921	1935	1962 patent
Regulations								
Flour	50 ppm max. U.S. whole wheat, 75 ppm max.	Not allowed	45 ppm max.	Sufficient for bleaching	Sufficient for bleaching	U.S.—sufficient for bleaching Canada—150 ppm max	200 ppm max.	U.S. not allowed; Canada—90 ppm max.
Bread	U.S.—75 ppm max. (see below) Can.—100 ppm max. (see below)	45 ppm max. (see below)	U.S. and Canada, 45 ppm max.				U.S.—no limit; Canada—200 ppm max.	U.S. and Canada—90 ppm max.
Labeling	Potassium bromate or bromated flour	Potassium iodate	Bleached flour	Bleached flour	Bleached flour	Bleached flour	Ascorbic acid added as a dough conditioner	L-Cysteine
Used in	Bread flour and dough	Bread dough	Bread flour and dough	Bread flour	Cake flour	Bread and cake flour	Bread flour and dough	Flour (Canada only), miscellaneous doughs
Action	Oxidant—improver	Oxidant—improver, mix reducer	Oxidant—improver, maturing	Oxidant—maturing, bleaching	Oxidant—maturing, bleaching	Oxidant—bleaching	Reductant—oxidant—mix reducer, improver	Reductant—mix reducer, relaxer
Reaction rate	Slow	Fast	Fast	Fast	Fast	1–3 days	Medium	Fast
Reaction product	Bromides	Iodides	Biurea	Chlorides	Chlorides	Benzoic acid		
Usage rates, [redacted]								
flour, normal	15	0	5	15	120	50	70	30
total. range	10–75	10–20	2–20		25–500	30–100	50–100	15–70
Overtreatment effects	Open grain holes Keyholing Incorrect volume	Low volume Weak, sticky doughs	Dry, bucky dough; low volume; poor grain; crust checking	Poor volume; poor grain	Low volume, dense crumb	None	None	Weak, sticky doughs; poor grain and texture
Testing								
Flour, spot	AACC 48-02		No	No	No	AACC 48-06B	Yes—good	Yes—fair
Flour, quantitative	AACC 48-42	Yes—difficult	No	pH used	Flour color	Yes	No	
Bread	Very difficult	Difficult	No	No	No	Benzoic acid—hard	No	No
Pennwalt products	Bromolux No—D-Lay		Maturox Dependox	Dyox	Flour Chlorination systems	Novadelox Zerolux	No—D-Lay AA-25	No—D-Lay Assist

Source. Courtesy: Pennwalt, Buffalo, NY.

[a] These oxidating–reducing agents are used to improve the baking quality of wheat flour. They are added at the flour mill, the bakery, or both. Having the proper oxidation treatment is critical, it ranks second only to protein quality and quantity in determining flour quality.

Government regulations on bakery use are as follows: United States—potassium bromate, calcium bromate, potassium iodate, calcium iodate, and calcium peroxide, or any combination of these, cannot exceed 75 ppm. This includes any bromate added to the flour. Canada—potassium bromate, calcium peroxide, ammonium persulfate and potassium persulfate, or any combination of these, cannot exceed 100 ppm. This includes any bromate added to the flour. Calcium iodate, potassium iodate or any combination of iodates cannot exceed 45 ppm.

flours are usually not enriched, but are bleached. The sale of cake flours declined during the 1950s as packaged cake mixes became more popular. Instantized, instant blending, or quick-mixing flour is a granular or more dispersible type of product for home use. It is free-pouring like salt, and dust-free compared to regular flour. It eliminates the need for sifting, since it does not pack down in the package and since it pours right through a screen or sieve. Granular flour instantly disperses in cold liquid rather than balling or lumping as does regular flour. The granular flour is produced by special processes of grinding and bolting, or from regular flour subjected to a controlled amount of finely dispersed moisture that causes the flour to clump or agglomerate. It is then dried to a normal moisture level (19,20). Gluten flour is milled to have a high wheat gluten and a low starch content and is used primarily by bakers for dietetic breads, or mixing with other flours of a lower protein content.

Semolina is the coarsely ground endosperm of durum wheat. High in protein, it is used by American and Italian manufacturers for high-quality pasta products such as macaroni and spaghetti. In Africa and Latin America it is also used for a dish called couscous. Durum flour, a by-product in the production of semolina, is used to make commercial American noodles. Farina is the coarsely ground endosperm of hard wheats. It is the prime ingredient in many American breakfast cereals. It is also used by manufacturers for inexpensive pasta.

The following is a list of additional basic wheat products (20):

1. The *wheat berry* is another name for the wheat kernel. Wheat berries can be used to grow sprouts which are then added to salads or baked goods. The cooked whole kernel can be used as a meat extender, breakfast cereal, or substitute for beans in chili, salads, and baked dishes.
2. *Bulgur* is processed by soaking and cooking the whole wheat kernel, drying it, and then removing 5% of the bran and cracking the remaining kernel into small pieces. Bulgur absorbs twice its volume in water and can be used in place of rice in some recipes.
3. *Cracked wheat* is similar in nutrition and texture to bulgur. It is the whole kernel broken into small pieces, but has not been precooked. Cracked wheat can be added to baked goods for a nutty flavor and crunchy texture or cooked as hot cereal.
4. *Wheat germ* is often added to baked goods, casseroles, and even beverages to improve the nutritional value and give a nutty, crunchy texture.
5. *Bran* makes a nonnutritive addition to baked goods for additional dietary fiber.
6. *Commercial cereals* can be prepared from wheat and eaten as snacks, breakfast cereals, or added to baked products. Ready-to-eat cereals can also be used as coatings for meats, toppings for casseroles, and extenders for meat loaves and casseroles. A variety of ready-to-eat wheat cereals are available. The wheat may be shredded, puffed, flaked, or rolled. The bran may be in the form of flakes or granules. Bran cereals are a nutritious addition for muffins, breads, biscuits, and other baked goods providing an excellent source of fiber in the diet (19,20).

BIBLIOGRAPHY

1. *From Wheat to Flour*, Wheat Flour Institute, Chicago, Ill., 1965.
2. W. R. Akroyd and J. Doughty, *Wheat in Human Nutrition*, FAO Nutritional Studies No. 23, FAO, Rome, 1970.
3. H. Hanson, N. E. Borlaug, and R. G. Anderson, *Wheat in the Third World*, Westview Press, Boulder, Colo., 1982.
4. D. W. Kent-Jones and A. J. Amos, *Modern Cereal Chemistry*, 6th ed., Food Trade Press, London, 1967.
5. Y. Pomeranz, *Modern Cereal Science and Technology*, VCH Publishers, New York, 1987.
6. Y. Pomeranz, ed., *Wheat Chemistry and Technology*, 3rd ed., Vol. 2, American Association of Cereal Chemists, St. Paul, Minn., 1988.
7. Y. Pomeranz, ed., *Advances in Cereal Science and Technology*, Vols. I–IX, American Association of Cereal Chemists, St. Paul, Minn., 1976–1988.
8. E. G. Heyne, ed., *Wheat and Wheat Improvement*, 2nd ed., American Society of Agronomy, Madison, Wisc., 1987.
9. G. E. Inglett, ed., *Wheat: Production and Utilization*, AVI Publishing Co., Westport, Conn., 1973
10. I. D. Morton, ed., *Cereals in a European Context*, VCH Publishers, New York, 1987.
11. R. C. Hoseney, *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1986.
12. N. L. Kent, *Technology of Cereals with Special Reference to Wheat*, Pergamon Press, Oxford, 1960.
13. Y. Pomeranz, ed., *Wheat is Unique*, American Association of Cereal Chemists, St. Paul, Minn., 1989.
14. J. Storck and W. D. Teague, *A History of Milling, Flour for Man's Bread*, University of Minnesota Press, Minneapolis, 1952.
15. G. Fabriani and C. Lintas, eds., *Durum Wheat, Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minn., 1988.
16. Y. Pomeranz, ed., *Industrial Uses of Cereals*, American Association of Cereal Chemists, St. Paul, Minn., 1973.
17. Y. Pomeranz, *Functional Properties of Food Components*, Academic Press, Orlando, Fla., 1985.
18. Y. Pomeranz and L. Munck, eds., *Cereals: A Renewable Resource, Theory and Practice*, American Association of Cereal Chemists, St. Paul, Minn., 1981.
19. *From Flour to Bread*, Wheat Flour Institute, Chicago, Ill., 1971.
20. *Nature's Best Wheat Foods*, Wheat Foods Council, Washington, D.C., 1984.
21. W. G. Heid, *U.S. Wheat Industry*, Agricultural Economics Report No. 432, U.S. Department of Agricultural Economics, Statistics and Cooperative Service, Washington, D.C., 1979.
22. Y. Pomeranz and co-workers, "Hardness and Functional (Bread and Cookie-making) Properties of U.S. Wheats," *Cereal Foods World* 33, 297–304 (1988).
23. National Food Review, "1985 Wheat Facts," *The Wheat Grower* 8, 28 (1985).

24. Y. Pomeranz and M. M. MacMasters, "Structure and Composition of the Wheat Kernel," *Baker's Digest* **42**, 24–26, 28, 29, 32 (1968).
25. Y. Pomeranz, "From Wheat to Bread, a Biochemical Study," *American Scientist* **61**, 683–691 (1973).
26. J. Holas and J. Kratochvil, eds., *Progress in Cereal Chemistry and Technology*, 2 vols., Elsevier Science Publishers, Amsterdam, 1983.
27. J. Holme, "A Review of Wheat Flour Proteins, and Their Functional Properties," *Bakers' Digest* **40**, 38 (1966).
28. Y. Pomeranz, ed., *Cereals' 78: Better Nutrition for the Worlds' Millions*, American Association of Cereal Chemists, St. Paul, Minn., 1978.
29. C. O Swanson, *Wheat and Flour Quality*, Burgess Publishing Co., Minneapolis, Minn., 1938.
30. W. T. Yamazaki and C. T. Greenwood, eds., *Soft Wheat. Production, Milling, Uses*, American Association of Cereal Chemists, St. Paul, Minn., 1981.
31. J H. Scott, *Flour Milling Process*, 2nd ed., Chapman and Hall, London, 1951.

Y. POMERANZ
Washington State University
Pullman, Washington

See also CEREALS SCIENCE AND TECHNOLOGY.

WHEY: COMPOSITION, PROPERTIES, PROCESSING, AND USES

In the dairy industry, the term whey denotes the greenish translucent liquid that separates from clotted milk during manufacture of cheese or industrial casein. More generally, residual streams from liquid fractionation processes of other food systems (especially of other protein solutions) have been referred to as whey on occasion, as in oilseed protein recovery processes (1) or in alkali extraction of protein from meat boning waste (2). This article is concerned solely with the traditional dairy whey and similar wheylike by-products of the modern dairy processing industry, using cow's milk as the principal raw material. With certain minor allowances for compositional differences, much of the information presented here is also relevant for whey resulting from processing of milk of other milking animals such as goats, buffaloes, and sheep.

TYPES AND FORMS OF WHEY

The traditional whey is produced as a result of processes aimed at recovering casein, the principal protein of milk. Separation of casein from the rest of the milk (as in cheese making or production of industrial casein and caseinates) is usually accomplished by acidification to pH 4.5–4.8 or through the action of rennet, a casein-coagulating enzyme preparation. In acid coagulation, the pH is lowered either by microbial fermentation of the milk sugar lactose into lactic acid or by direct addition of a mineral (phosphoric, hydrochloric, sulfuric, etc) or an organic (lactic, citric) acids. The fermentation route is most often used in the production of cottage cheese, quarg, and other fresh cheeses, and the direct acidification is typical for production of industrial casein and caseinate products; in both cases, the resulting whey is referred to as acid whey. In contrast, sweet wheys are obtained in the manufacture of most hard and semihard cheeses, including cheddar, Swiss, Gouda, and mozzarella, for which the rennet coagulation principle is employed as well as in the production of industrial rennet casein. Because enzymatic clotting of milk by rennet occurs at pH 6.0 or higher, the lactic acid content of freshly obtained sweet whey is low but may increase quickly if subsequent bacterial fermentation is not controlled by rapid pasteurization and/or by deep cooling. Approximately 9 L of whey will be obtained from 10 L of milk for every kg of cheese produced; this general ratio will vary somewhat depending on the type of cheese, fat content of the raw milk used, and other factors. In high-moisture fresh cheeses such as cottage cheese (where a portion of the original raw milk is returned to the cheese as cream dressing), the ratio may be as low as 6:1.

Either sweet or acid wheys can exist in various forms, depending primarily on whether any moisture has been removed in subsequent processing. Because whey contains on the average about 93–94% water, it is often desirable to remove some or most of its water for further handling. Application of common food processing operations such as evaporation or drying results in concentrated, semisolid, or dried forms of whey, the principal difference being the residual moisture content (Table 1). Liquid whey forms may be further processed into many additional modified whey products useful as ingredients for other food processors by reverse osmosis (RO), ultrafiltration (UF), electrodialysis (ED), ion exchange (IE), lactose hydrolysis (LH), and other modern unit operations now available to the whey processing industry.

OTHER BY-PRODUCTS OF MILK AND WHEY PROCESSING

The use of UF for cheese making and for further whey processing results in a wheylike liquid waste stream referred to as UF permeate. Although in principle UF permeate is similar to whey in terms of its physical state and volume, the main compositional difference between the permeate and the traditional whey is due to the almost complete protein recovery in the UF retentate that leaves the permeate virtually protein-free (Table 1). Minor compositional differences are normally observed between permeates from UF of milk and of the two types of whey, primarily because of the variations in the mineral fraction.

COMPOSITION AND PROPERTIES

Composition of Unprocessed Whey

Whey is a multicomponent solution of various water-soluble milk constituents in water; the dry matter of whey consists primarily of carbohydrate (lactose), protein (several chemically different whey proteins), and various minerals. Fat content of the freshly separated liquid whey may

Table 1. Proximate Composition Ranges for Major Whey Forms

Whey form	Total solids (%)	Total protein (%) (N × 6.38)	Lactose (%)	Minerals (% ash)
Liquid, unprocessed	6.0–7.0	0.4–0.8	4.4–4.9	0.5–0.8
Concentrated (RO)	20–25	1.2–3.0	14–20	1.0–3.0
Concentrated (evaporator)	40–60	4.0–8.0	26–48	3–8
Dried	96–97	10–13	70–75	7–12
Liquid UF Permeate	4.0–5.5	0.1–0.3	3.9–4.8	0.3–0.8

Note: After fat recovery by centrifugation, common for many types of cheese whey (eg, cheddar). Fat content of cottage cheese whey is negligible due to the use of skim milk. RO, reverse osmosis, UF, ultrafiltration.
Source: Orientation data based on various sources, including Refs. 3 and 4.

be up to 0.5–1% depending on the type of milk used and the efficiency of the cheese-making operation. However, the valuable butterfat is usually recovered by whey centrifugation and returned to the cheese or processed into whey butter. Thus, the fat content usually reported for the various types of whey is typically below 0.1 %. Fat content of most acid wheys is negligible because skim milk is used in the manufacture of these cheeses. The proximate composition of various wheys may show significant variations due to the pretreatment of the cheesemilk (heating, centrifugation, homogenization); the processing differences characteristic for the various cheeses (cultures used, mechanical handling, use of processing aids such as the yellow color, use of membrane processes); and the whey handling and pretreatment processes (pasteurization, preconcentration, recovery of casein fines). Average compositional data for cheese wheys of the sweet and acid types are shown in Table 2.

Lactose, the principal component of the whey apart from water, constitutes about 4.4–4.9% of the whey "as is" depending on the whey type. Lower lactose content is usually found in the acid wheys due to the preceding fermentation process in which some of the lactose is converted to lactic acid. The most valuable whey component is the whey protein, constituting approximately 0.7% of the whey (about 9–11% of the dry matter). In addition, whey may contain about 0.2–0.3% of nitrogenous matter denoted as protein and reported as total whey protein (N × 6.38).

Table 2. Average Composition Data for Sweet and Acid Types of Whey

Component	Sweet whey (pH 5.9–6.4)	g/L	Acid whey (pH 4.6–4.8)
Total solids		63.0–70.0	
Protein (N × 6.38)	6.0–8.0		6.0–7.0
Lactose	46.0–52.0		44.0–46.0
Fat	0.2–1.0		0.1–0.5
Calcium	0.4–0.6		1.2–1.6
Magnesium	0.08		0.11
Phosphate	1.0–3.0		2.0–4.5
Citrate	1.2–1.7		0.2–1.0
Lactate[a]	2.0		6.4
Sodium		0.4–0.5	
Potassium		1.4–1.6	
Chloride		1.0–1.2	

[a]No lactate in uncultured rennet or mineral acid whey.
Source: From various sources including Refs. 5 and 6.

Whey protein is neither acid nor rennet coagulable and thus, when the classical cheese-making processes are used, the whey protein will pass from milk into the whey of either type. Finding suitable technological alternatives allowing recovery of whey protein in the cheese is one of the major concerns of current cheese research. Because about 50–60% of the whey proteins can be coagulated by heat, high heating of milk is now practiced for some types of fresh, acid coagulated cheese such as quarg (7) or cottage cheese where the quality is not greatly compromised and the diminished rennet clottability is not a major problem. As a result, whey obtained from such cheese-making operations will contain mainly the heat noncoagulable whey proteins, and its total protein content will be substantially lower, often around 0.4% (8). Table 3 shows the main components of the whey protein fraction of cow's milk, together with their relative content and heat stability characteristics.

After lactose and whey protein, minerals constitute the third major component group of whey dry matter. The mineral composition shows the greatest variations between the two types of whey, together with pH and the lactic acid content. Although the overall compositional values for each of the two types are dependent on many factors related to the cheese-making process, the main differences between the two types of wheys reflect the different casein coagulation pathways. In addition to lower pH and higher lactic acid (and correspondingly lower lactose) content, the acid wheys show substantially higher calcium and phosphorus contents caused by the solubilization of the calcium–phosphate complex of the casein micelle at the acid pH range, used for the acid coagulation of the casein. In contrast, the calcium removal from the casein micelle does not occur as a result of the rennet clotting at pH 6.0 or higher; thus, much of the milk calcium is retained in the cheese rather than being lost in the sweet whey. The differences in acidity as well as the higher calcium content of the acid whey appear to be the main reasons for variations in physicochemical properties of the two whey types, including the substantially lower heat stabilities of the dairy systems containing added UF retentates from acid wheys in comparison to sweet wheys (11); on the contrary, in the acidic pH range below 3.9, high-calcium containing acid whey appears to be more resistant against heat-induced instability (12).

Composition of Modified Whey Products

Using some of the typical whey processing unit operations such as UF, ED, or IE, numerous modified whey products

Table 3. Composition and Thermal Characteristics of the Whey Protein Fraction

Protein	Approx. content (g/L whey)	Total whey protein (%)	Denaturation temperature (°C)	Heat precipitation stability
α-Lactalbumin	0.6–1.7	20	61	Quite stable[a]
β-Lactoglobulin	2.0–4.0	55	82	Labile
Serum albumin	0.2–04	5	66	Very labile
Immunoglobulins	0.5–1.0	8	72	Extremely labile
Proteose-peptones	0.2–0.4	12		Heat stable
Other (caseins glycoproteins)	0.1	1		

[a] Due to its renaturation characteristics and despite its low denaturation temperature.
Source. Adapted from various sources, including Refs. 5, 6, 9, and 10.

Table 4. Characteristic Composition of Major Types of Modified Whey Products

Product	Total protein (%) (N × 6.38)	Lactose (%)	Minerals (%)	Fat (%)
Liquid WPC (10% TS)	3.3	5.5	0.8	0.3
Dried WPC (35% protein)	35.0	50.0	7.2	2.1
Dried WPC (80% protein)	81.0	3.5	3.1	7.2
Dried WPI (90% protein)	90.0	2.0	4.0	1.0
Lactose, edible grade	0.1	99.0	0.2	0.1
Whey powder				
Demineralized (70%)	13.7	75.7	3.5	0.8
(90%)	15.0	83.0	1.0	0.9
Lactose-reduced demin	30.5	51.5	7.8	2.0
Regular	12.5	73.5	8.5	0.8

Note: WPC, whey protein concentrate; WPI, whey protein isolate; TS, total solids.
Source: Refs. 3, 6, 13, and product specifications of various manufacturers.

may be obtained. Table 4 lists some of these products and gives their basic composition. Because most of these whey modification processes are capable of handling both sweet and acid wheys, the compositional differences of the final products often reflect the processing techniques used rather than the type of whey. Most of the modified whey products available on the market today are manufactured by various fractionation processes aimed at separation of the undesirable components and thereby enrichment of the target substance. The main exception is the enzymatically modified whey syrup where the constituent disaccharide lactose is converted to two monosaccharides, glucose and galactose, thus leaving the proximate composition virtually unchanged. Similar modifications involving enzymatic breakdown of the whey proteins are also of interest (14), one of the main industrial applications being the production of hypoallergenic baby formula (15).

Table 5. Some Physicochemical Properties of Whey in Comparison to Skim Milk (Orientation Data)

Property	Whey	Skim milk	Ref.
Viscosity (mPa s)	1.2	1.5	16
Surface tension (dyn/cm)	42	48	16,17
Freezing point (°C)	−0.500/−0.600[a]	−0.520	16,18,19
Stability against heat coagulation (standard pH)	Unstable	Stable	16
Stability against acid coagulation (no heat)	Stable	Unstable	16

[a] Depending on type of whey.

Properties of Whey and Whey Components

Although whey is a rather dilute aqueous solution of lactose, whey protein, and some minerals, some physicochemical properties of unmodified wheys as determined by the individual system components are similar to those of water, but others differ rather substantially. As seen in Table 5, some of the physical properties (such as viscosity or surface tension) are very similar to skim milk, which can be expected from the compositional similarities. Freezing points of wheys and UF permeates can vary rather substantially depending on the type (19). The main difference between whey and skim milk is in the protein fraction, which accounts for a major difference in heat stability of the two systems. In the absence of casein, whey protein will coagulate and precipitate from the solution upon heating above the whey protein denaturation temperature, which varies depending on the type of protein (Table 3) and the heating time. As a general guideline, heating to more than 70°C will cause precipitation in whey systems above pH 3.9, the severity of the phenomenon being dependent on the heating time, temperature, and calcium content. Complete precipitation of the heat denaturable whey protein will occur upon heating to at least 90°C with holding for several minutes. Whey systems containing little calcium (such as rennet whey, decalcified acid whey, or solutions of whey protein isolates) will show increased turbidity but little or no precipitation upon heating (20). At pH lower than 3.7–3.9 depending on the calcium content, whey proteins become highly resistant to heat-induced precipitation; even severe heating above 90°C for 20 min or more will not result in protein precipitation or turbidity increase (12,21). Heating of sweet or acid whey systems will enhance the already high foamability of whey (22), probably due to the effect of heat on some of the heat-noncoagulable whey protein fractions.

Limited solubility and low sweetness are the most important properties of the main whey component, lactose, influencing many whey processing operations. Because the solubility of lactose is only about 20 g/100 g water at room temperature and 60 g/100 g water at 60°C, as compared with about 188 g/100 g and 235 g/100 g water for sucrose at the same conditions (23), whey concentration to more than about 36–38% total solids (TS) will result in formation of crystalline lactose. This is the principle of the lactose manufacturing process as well as the reason for specific processing steps aimed at avoiding formation of large lactose crystals in manufacture of dried whey, the Norwegian whey cheese mysost, table spreads based on whey (3,24,25), and other products containing high concentrations of lactose. To avoid the lactose crystal formation and/or to increase the sweetness of lactose-containing foods if desired, the lactose may be hydrolyzed to its two constituent monosaccharides, glucose and galactose. The hydrolysis may be accomplished enzymatically using either free or immobilized lactase (β-galactosidase, EC.3.2.1.23), by chemical hydrolysis through high temperature-low pH processes, or by sonicated or otherwise disrupted bacteria (26).

The main differences in physicochemical properties of sweet and acid wheys are due to the varying content of minerals and acids. Calcium appears to be particularly important in terms of whey protein heat stability, and sodium and potassium are primarily responsible for the saltiness and, together with lactose, for the osmolality of the whey (18). The mineral content of whey can be modified by ion exchange, electrodialysis, or certain specialized membrane processes (eg, nanofiltration).

Optical properties of the whey and wheylike systems are influenced by the content and type of protein present and by the most abundant whey vitamin, the riboflavin. The greenish color of most traditional whey systems is caused by the water-soluble and heat stable riboflavin. However, riboflavin is sensitive to light as well as to ionizing radiation treatments, and whey systems exposed to these conditions will show fading of the green color (27). As a small molecule, riboflavin will be retained by RO but not by most UF membranes, so both wheys as well as UF permeates of milk or whey processing have the same color character. Processing of whey on a metallic UF membrane cast within a porous carbon tube resulted in adsorption of riboflavin on the carbon support (28); the affinity of riboflavin for various adsorbents as crystalline lactose or carbon has been known for some time (29). The turbidity of most industrially produced wheys is due to the casein fines (30). Properly clarified wheys are virtually transparent and, in visual appearance, not substantially different from good quality UF permeates. Turbidity measurements can be used as indication of the heat effects in various whey systems; heating will cause aggregation of the whey protein into larger clusters that will change the optical character of the heated whey system even if no precipitation will result. Yellow discoloration of whey may occur after prolonged heating, especially in concentrated whey systems, because of the Maillard nonenzymatic browning reaction between the lactose and whey proteins. Yellowing may be also caused by the use of the water soluble annato color in the cheese-making process.

INDUSTRIAL PROCESSING OF WHEY

Traditionally, whey has been known as a troublesome byproduct of cheese manufacturing and, because of its very high biological oxygen demand (BOD) (about 40,000 ppm), as one of the strongest industrial wastewater pollutants of any kind (31). The opportunities for using whey as a valuable resource have been recognized for some time, but so far, very few countries (eg, the Netherlands) have established a record of nearly complete utilization of all available whey. However, the question of whey utilization as opposed to whey disposal is becoming increasingly important in view of more stringent ecological constraints worldwide. Intensified research activities have resulted in many new approaches to industrial whey processing, and the value of cheese whey as a raw material for various consumer products or industrial food ingredients is now being recognized. Some of the largest whey processing facilities in the world are capable of handling in excess of 10^7 L of whey daily and may rely on supplies of raw or RO preconcentrated whey from cheese manufacturers several hundred kilometers away. The main whey disposal problem continues to be the acid whey from cottage cheese and other fresh cheese products, because the resulting amount of whey is not large enough to warrant separate whey processing facility. One of the possible solutions for these processors could be the production of high-value consumer products based on whey, such as whey beverages and whey cheeses.

Consumer Whey Products

Whey cheeses and whey drinks are traditional foods. The use of whey as a beverage has been recorded in history since the time of Hippocrates (32), and traditional whey cheeses such as ricotta and mysost (24,33–35) have been popular in various parts of the world for centuries. These products usually require simple processing facilities and thus, in their traditional versions, may be suitable for small whey processors supplying primarily local markets. However, neither whey cheeses nor whey beverages (with a few exceptions) have been particularly successful on large scale, possibly because of the limited consumer appeal outside of the traditional producing countries.

Whey Cheeses. There are two types of whey cheese: the Norwegian whey cheese mysost (also known as gjetost, primost, brunost, or Gudbrandsdalsost) and the Italian whey cheese ricotta. Figures 1 and 2 show schematically the basic processing steps used in production of these cheeses. The technology for mysost production is based on evaporation of water from the whole whey, followed by solidification of the evaporated mass under carefully controlled

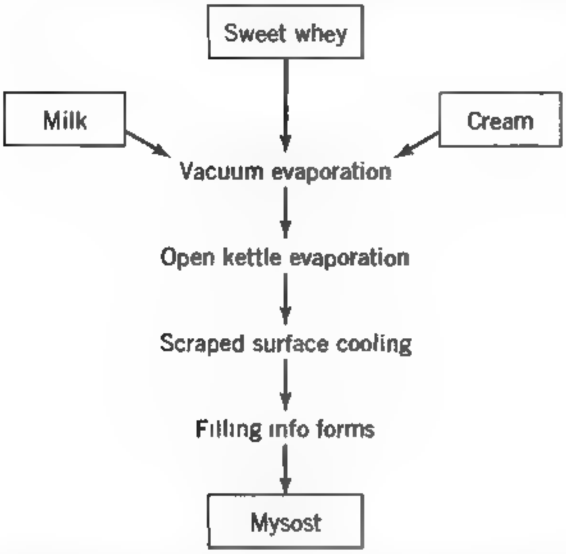


Figure 1. Main technological steps in the production of mysost whey cheese.

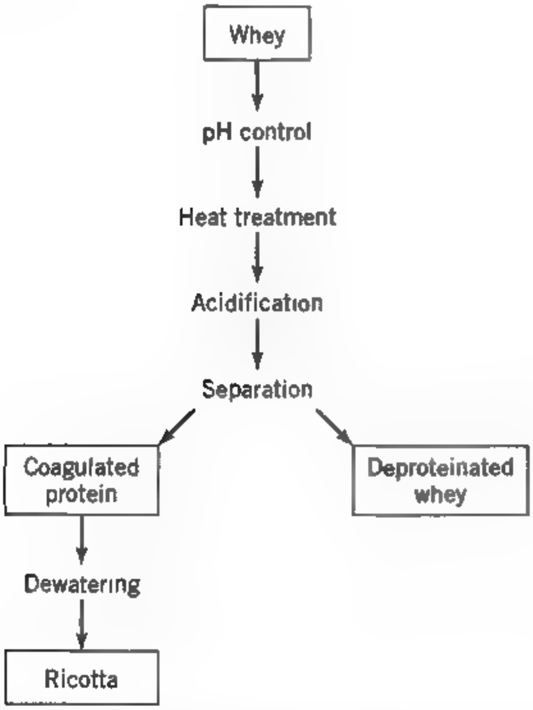


Figure 2. Main technological steps in the production of ricotta whey cheese.

Table 6. Illustrative Compositional Data for Ricotta- and Mysost-Type Whey Cheeses

	Ricotta type		Mysost type	
Component	Fresh	Pressed	Regular	Spread
Total solids (%)	25	40	80	66
Protein (%)	12	19	11.5	7.5
Carbohydrate (%)[a]	4	5	36	46[b]
Fat (%)	7	10	30	7.5

[a] Lactose only.
[b] May include additional carbohydrate sweeteners.
Source. Refs. 24, and 34–36.

conditions to avoid growth of lactose crystals beyond sizes that would be recognized as grittiness upon product consumption (24). In contrast, the ricotta-type whey cheese is based on the recovery of the whey protein coagulum obtained by high heating of the whey raw material, often containing additional skim milk. As a result, the composition of the two products differs rather substantially, as shown in Table 6. Because all traditional milk-based cheeses are manufactured by retaining casein and removing whey proteins and lactose, describing mysost or ricotta as a cheese may be a misnomer, although allowed for in the modified FAO definition of cheese (36). The character of the protein-based ricotta is closer to the traditional cheese than the mysost, in which lactose is the most abundant component. From the standpoint of whey disposal, the mysost technology is much more suitable because it leaves no residue, whereas heat precipitation of protein from whey still results in a partially deproteinated whey stream almost as high in the BOD as the original whey. The BOD content of the deproteinated whey is dependent primarily on lactose, which is not recovered by the ricotta process. More details about the whey cheese manufacture, properties, and uses may be found elsewhere (33,35,36).

Whey Beverages. Incorporation of whey into various beverages and developments of whey drinks using numerous technological approaches have been attempted by large number of researchers. Extensive literature reviews exist (32,33,37) summarizing many of these attempts. However, there are still only a few truly successful whey beverages that can be found on market shelves in various countries. Virtually no products have established any major international reputation. Table 7 gives examples of whey beverage products known to have enjoyed some commercial success, as an illustration of the types of beverages that may be produced from whey. Figure 3 shows diagrammatically the main technological pathways available for

Table 7. Examples of Commercially Available Whey Beverage Products, 1983–1988

Product type	Commercial name	Country
Whey with fruit juices	Djoez	Netherlands
	Taksi	Netherlands
	Big M	Germany
	Mango-Molke mix	Germany
	Latella	Australia
	Hedelmatarha	Finland
	Nature's Wonder	Sweden, Canada, Germany
Drinkable yogurt with whey	Yor	Netherlands
	Interlac	Belgium
Soft drink (carbonated)	Rivella	Switzerland, Japan

Source: Ref. 33.

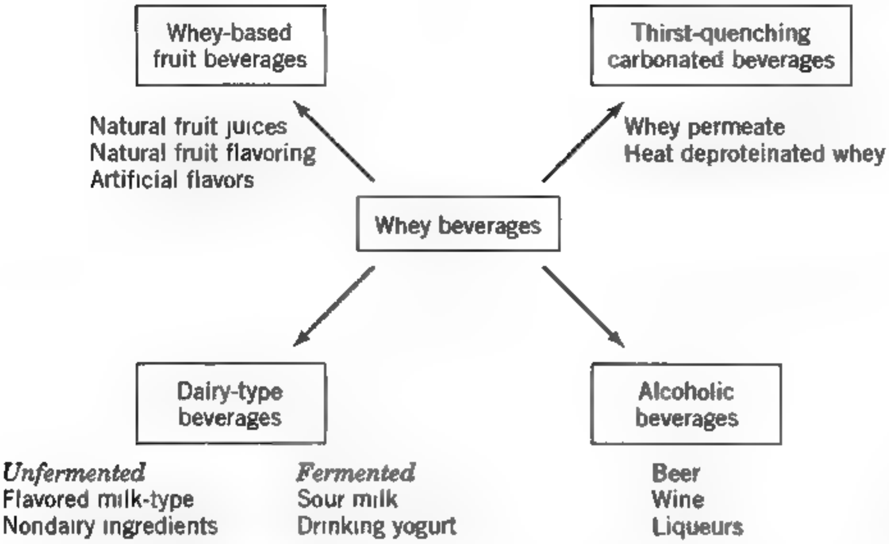


Figure 3. Alternative technological pathways for production of whey-based beverages.

production of a whey beverage. The most often used approach appears to be mixing whey with fruit juices, thereby combining the nutritional benefits of high-quality whey protein, calcium, and riboflavin from whey with the traditionally recognized source of various vitamins, the fruit. Nationally successful products of this type include the Taksi and Djoez drinks marketed in the Netherlands, the Austrian Latella, or the Finnish Hedelmatarha. However, frequent market failures of the less successful products in this category indicate that the seemingly simple technology of mixing cannot be applied without careful product and market development research. A typical quality problem found often in these products is a sediment formation, usually caused by the heat-labile whey proteins and their interactions with some of the fruit components (as pectins) even below the pH range of 3.9–3.7 where whey protein should be heat-stable (38). Similarly, drinkable-yogurt type products have been developed and, in view of the currently increasing popularity of yogurt-based drinks, should represent another avenue of success for further product development. Contrary to the precipitation in the fruit-juice-based products, the pectin can be used to stabilize these casein-containing beverages as in other fermented dairy products (39). A third type of whey beverage that has been successfully marketed is the clear product based on whey UF permeate or heat-deproteinated whey. In fact, the main representative of this approach, the Swiss product Rivella, is the longest existing, and the most successful, of all whey beverage products. Its popularity on the Swiss beverage market rivals that of Coca-Cola. This carbonated product, made by mixing the clear deproteinated whey serum with a flavor extract of various herbs, represents a general thirst-quenching beverage rather than a nutritious, dairy-based product such as the fruit-juice-based or the yogurt-based drinks. In this sense, the use of whey for Rivella production is similar to other industrial uses of whey, since the Rivella is manufactured by a specialized beverage company rather than a dairy processor.

Industrial Whey Products

Processing of whey into various industrial products has become the most attractive alternative to whey disposal. This includes the drying of whey, which may have been viewed earlier as a process merely reducing the disposal problem, since utilization of the dry whey often has been unprofitable. Significant research efforts worldwide have resulted in many new alternative technological approaches and uses for the industrial whey products, and the whey processing industry is today one of the most rapidly developing segments within the dairy field. In addition to the traditional dairy products made from whey (concentrated or dried whey, lactose, whey butter, partially delactosed whey, and heat-precipitated whey protein), many new whey-based products are now available as ingredients for the food industry; these include whey protein concentrates (WPC), isolates (WPI), or fractions (WPF); blends of whey or whey protein with other nondairy sources; demineralized whey; lactose hydrolyzed whey products; fractionated and enzymatically modified whey protein products for specialized food, pharmaceutical, and other nonfood uses; and various products of the whey fermentation processes, including biomass, food-grade, or fuel alcohol and methane. Some of the representative industrial whey products are listed in Table 4.

Whey Protein Products. The most valuable component of whey is the whey protein. The oldest process for production of a WPC consists of heating of whey and recovering the heat-precipitable whey proteins as an insoluble high-protein product (usually referred to as traditional lactalbumin). In the modern whey processing industry, various membrane-based separation technologies are being used to produce WPCs and WPIs with much improved functional properties, in particular regarding good water solubility. The predominant technology for production of modern WPCs is UF, which is often combined with diafiltration to remove additional lactose and ash and thereby increase the protein content of the final product. The typical protein content of the ordinary WPC, produced by simple UF as a skim milk replacement, is about 35%, but products with up to 80% protein are now being produced for many food ingredient uses. WPIs with protein content over 90% are also available for specialized applications. The modern WPCs and WPIs have some important functional properties differentiating them from other food protein ingredients, such as high solubility (including solubility in acidic systems), foaming properties, and heat coagulability combined with high water binding in the heat-denatured state. Because whey protein from cow's milk is similar to human whey protein, one of the main applications of the WPCs and WPIs is in the production of baby formulas. Enzymatic modification and/or fractionation techniques to separate the individual whey proteins are being developed to counteract the possible allergenicity of the most abundant whey protein, the β-lactoglobulin (15), and to maximize the functionality of the individual whey protein fractions (10,40). Other technological alternatives that have been suggested for whey protein recovery include the use of chemical precipitants, especially various biopolymers as pectin, carrageenan, or carboxymethylcellulose, as well as ion exchange resins or gel filtration (4,10). In general, whey protein is considered to be nutritionally one of the best proteins for human food use, and its versatile functional properties make it a valuable ingredient for the modern food industry.

Lactose Products. Because lactose is the predominant whey component, any protein recovery operation not accompanied by some lactose processing alternative will result in a large lactose disposal problem. Traditionally, lactose has been recovered as an article of commerce by crystallization from highly concentrated whey (typically to 65–70% TS), with or without prior whey protein removal. Crystallizing lactose from unmodified whey will result in partially delactosed whey residue that can be dried to obtain a product with sufficiently high protein content to be valuable as a skim milk replacement in certain applications. When the whey protein is removed before the crystallization process, either by heat precipitation or by UF, yield of both products can be maximized and the rate of lactose crystallization optimized (23), but the residual mother liquor from the lactose crystallization process could constitute a disposal problem. However, lactose-producing plants are increasingly using UF permeate as the raw material for lactose manufacture (41). Lactose of edible grade or better can be produced also by spray drying of highly purified deproteinated and demineralized whey. Because the world market for lactose is rather static, new opportunities for lactose utilization would have to be found if lactose production were to increase substantially.

Hydrolysis of lactose into its two monosaccharide constituents, glucose and galactose, will result in increased sweetness of the mixture as well as reduced lactose crystallization problems in concentrated food systems (as ice cream). Sweetening syrups can be produced by lactose hydrolysis in purified lactose streams for use in canning, as brewing adjuncts, or for other applications. Lactose hydrolysis in whey or whey UF permeate for use in manufacture of whey drinks will decrease the caloric content of the final product as well as the required sweetener addition when using some of the high potency noncaloric sweeteners (42). Lactose can be further modified chemically to obtain useful food or industrial ingredients such as lactitol, lactulose, or lactosyl urea (43); however, the raw material for these lactose derivatives is usually the isolated lactose rather than the whole whey.

Whole Whey Products. The most common industrial whey product is spray-dried whey and its numerous variants. Whey drying is not an easy process because of the high lactose content and, in the case of acid whey, the high content of the nonvolatile lactic acid. For a typical spray-drying operation, the whey is first concentrated by multistage vacuum evaporation to more than 50% TS. Preconcentration by RO is often practiced to increase the capacity of the evaporator and/or to bring the raw material from a distant supplier to the central drying facility. To produce a nonhygroscopic whey powder, the lactose should be left to crystallize for at least 24 h before the spray-drying operation, otherwise noncrystalline, unstable, and highly hygroscopic lactose glass will be formed in the drying process. Sometimes, the whey proteins are also purposely heat denatured before the spray drying to minimize a potential for protein deposition on the heat-transfer surfaces, resulting in processing difficulties. As a result, the hygroscopicity, solubility, and other functional properties of whey powders may differ substantially depending on the processing conditions. Other drying procedures traditional for the dairy industry, such as drum drying, are seldom used because of the great operational difficulties and the inferior quality of the final product.

In addition to regular whey powders, products with modified lactose, protein, or mineral content can be produced using lactose crystallization, membrane processing, demineralization by IE or ED, or blending the liquid whey with other nondairy protein sources, including soy, pea, and other vegetable proteins as well as bran or other food materials. A U.S. patent (44) claims that wet grinding of dry cereal or oilseed protein sources (soy, rapeseed, oats) in whey or whey UF permeate followed by spray drying of the blends will result in improved functional properties, especially higher resistance toward lipid rancidity development in the dry product. Other whole whey products include sweetened and/or lactose-hydrolyzed concentrated whey as an in-house ingredient in ice cream and other dairy products and highly concentrated whey in the form of semisolid lick-blocks for cattle feeding purposes.

Fermentation Products from Whey. Because lactose is fermentable by certain microorganisms, whey has been a traditional substrate for fermentation processes, including the production of antibiotics, lactic acid, or biomass. However, these established processes of the past have been replaced to a large degree by more economical alternatives, such as direct chemical synthesis. Recent developments of modern processing approaches as well as the preference of natural over synthetic ingredients may revitalize the interest in various fermentation processes based on whey. Production of lactic acid is one of the examples where modern technology (45) and the back-to-nature philosophy may make the traditional fermentation route successful again.

Likewise, production of food-grade alcohol from whey for use in dairy-based liqueurs have been successfully developed in Ireland (46). In New Zealand and other countries, whey fermentation into industrial alcohol in large commercial installations appears to be profitable (47,48). Use of liquid whey medium for bulk propagation of lactic starter cultures for in-house use is well established in many dairy processing plants. Further fermentation of whey for various whey-based beverages is a natural possibility, including production of whey wine or whey beer, even though lactose is not fermentable by many common yeasts without special pretreatments. Alcoholic whey-based beverages have been described in literature (32,37), but their commercial success is not well documented apart from the manufacture of whey beer in Germany during the period of scarcity in the World War II. In the nonfood area, whey has been used as a substrate for biomass production by yeast fermentation (the wheast process), for bacterial fermentation into lactic acid with continuous neutralization to produce fermented ammoniated concentrated whey (FACW) for ruminant feeding (49), or as a source of biogas generated in anaerobic waste treatment installations (50). However, these processes may be considered more as alternatives for whey disposal rather than profitable opportunities for manufacture of industrial whey products.

Whey Disposal and On-Farm Utilization

Although whey has become much too valuable to be disposed of on a large scale, there are still many smaller and isolated cheese manufacturing operations where whey disposal can be a major economical and/or ecological burden. Where industrial whey processing or manufacture of high-value consumer products is not feasible, other methods of whey disposal must be selected, based mainly on the associated cost. The down-the-drain approach is certainly the most wasteful although not always the most expensive alternative, especially when municipal or existing industrial waste treatment facilities are located nearby and the volume of whey to be discarded is not great. Spraying the whey onto fields is still being practiced by many isolated cheesemakers in rural areas, sometimes relying on sophisticated approaches with time-controlled application schedules to maximize the potential fertilizer value and to minimize soil salinity and acidity problems inherent in these techniques (51). Direct feeding of whey to agricultural animals, especially pigs, appears to be one of the most profitable on-farm uses of whey (52). When the whey feeding is combined with methane production from the animal excreta for use as a fuel in a cheese factory located nearby, a truly ecologically enclosed industrial system can be developed, as evidenced by several commercial installations in Switzerland.

NUTRITIVE VALUE AND USES OF WHEY

As a by-product of dairy processing, cheese whey contains about 50% of the nutrients found in milk. The most valuable whey components from a nutritional standpoint are the whey proteins, the water-soluble vitamins (especially riboflavin), and some of the nutritionally important minerals. Acid whey is a particularly good source of calcium; its content approaches that of regular cow's milk. Whey protein from either sweet or acid whey is one of the best proteins for human food use because of its balanced amino acid profile and superior digestibility (53). Dried cheese whey composition can be compared to that of regular wheat flour (31) in terms of the proximate composition and some of the micronutrients. In addition to the unquestionable value of the whey protein, calcium, and riboflavin, some sources also emphasize the beneficial effects of the high (L+) lactic acid content of whey produced with certain dairy cultures (53).

The use of many industrial whey products as ingredients in various foods is based not only on the nutritional qualities but also on the desirable functionality of some of the products. With the high lactose and protein content, dried whey is an ideal substrate for the nonenzymatic Maillard browning reaction in foods where such color development is desired, as in breaded meat or fish products or in toasted bread. Lack of sweetness in lactose is advantageous in many applications of whole or modified whey ingredients, especially when used in meat products, processed cheese foods, gravies, soups, sauces, salad dressings, etc. Whey protein is ideally suited for fortification of various foods both because of its nutritional quality and its functional attributes, including bland taste compatible with most food flavors and solubility in aqueous food systems (54). Many additional uses of whole whey and whey-based traditional or modern food ingredients have been suggested in various reviews (4,55–57). Inclusion of whey protein in cheese, either by varying the cheese processing technology or by reincorporation of the protein recovered from the whey, is one of the important research subjects concerning whey at the present time. Several alternative technological pathways can be used for incorporation of the heated whey protein precipitate into fresh cheeses such as quarg and quarg-based food products (7), in which the cooked flavor and quality impairment, often encountered in traditional cheeses, need not be a problem. This option is especially attractive for fresh cheese plants where whey from production of other cheese varieties is available.

Although food use of whey and whey-based products is the most desirable whey disposal alternative, various nonfood uses of whey also have been proposed, including incorporation into foamed insulation materials for the construction industry or use as a binder for iron ore pellets (58). Enzymatic modifications of whey proteins for use in the pharmaceutical industry may follow the successes predicted for the casein (14). Rapid advances in biotechnology will likely discover new opportunities for conversion of whey into valuable industrial food or nonfood materials such as biosorbents, biopolymers, stabilizer gums, flavoring or coloring compounds, bacteriocins or fat replacers such as the Simplesse developed from whey protein and egg white (59). With its high lactose content, surplus dried whey could be used as a quick source of energy in international hunger-relief programs; however, the prevalence of lactose intolerance in most third world countries makes such use difficult without careful development of foods that can be tolerated by the lactose malabsorbers. Incorporation of whey into high-solid indigenous foods should be considered because these foods appear to be better tolerated by lactose malabsorbers than more liquid products (60,61).

The current interest in functional foods and nutraceutical products (62) has opened yet another major avenue for utilization of whey as a raw material in production of special dietary aids and nutritional supplements. Whey protein isolates have become the most valued protein source for body-building formulas and similar high protein preparations (63) used by active athletes following intensive training regimes. Some of the minor whey proteins, such as lactoferrin, lactoperoxidase, or the various immunoglobulins, are rapidly becoming prized commodities for both food and nonfood uses, including personal hygiene products such as mouthwashes or skin creams (64); even the whey minerals are being marketed as natural food ingredients (62). A whey-protein-based dietary supplement has been patented (65) and is being promoted under the trade name Immunocal on the basis of its alleged stimulatory effect on immune functions through increased production of intracellular glutathione (66). The ultimate success of these and other proposed novel whey protein nutraceutical applications may depend on the proved physiological functionality, yet to be documented in humans in many cases, as well as on economically feasible utilization or disposal of the remaining whey constituents, especially lactose. In this regard, the rapidly increasing commercial interest in prebiotics of dairy origin, such as galactooligosaccharides, lactosucrose, and lactulose, for use in bifidobacteria-containing dairy foods may hold promise. Additional physiologically functional effects of some of these oligosaccharides, currently being investigated with animal models, include calcium absorption and reduction of the risk of colon cancer (67).

The possibilities for treating whey as an opportunity rather than a problem have increased dramatically in the recent years. In some dairy plants recently installed, all whey produced in cheesemaking is completely used on site in a variety of integrated processes, leaving no residue to dispose of. With continued technological developments, the emerging scarcity of inexpensive dairy ingredient sources, and the increasing emphasis on ecologically responsible industrial processes, the image of whey is rapidly changing from that of a bothersome by-product to a highly prized resource.

BIBLIOGRAPHY

1. J. T. Lawhon, L. J. Manak, and E. W. Lusas, "An Improved Process for Isolation of Glandless Cottonseed Protein Using Industrial Membrane Systems," *J. Food Sci.* **45**, 197 (1980).
2. P. Jelen, M. Earle, and W. Edwardson, "Recovery of Meat Protein from Alkaline Extracts of Beef Bones," *J. Food Sci.* **44**, 327 (1979).
3. G. Kjaergaard-Jensen and J. K. Oxlund, "Concentration and Drying of Whey and Permeates," in *Trends in Utilization of Whey and Whey Derivatives*, International Dairy Federation, Bull. 233, Brussels, Belgium, 1988, p. 21.
4. D. M. Irvine and A. R. Hill, "Cheese Technology," in M. Moo Young, ed., *Comprehensive Biotechnology*, Vol. 3, Pergamon Press, Toronto, Canada, 1985, p. 523.
5. K. R. Marshall, "Industrial Isolation of Milk Proteins: Whey Proteins," in P. F. Fox, ed., *Developments in Dairy Chemistry 1*, Elsevier, London, 1982, p. 340.
6. K. R. Marshall and W. J. Harper, "Whey Protein Concentrates," in *Trends in Utilization of Whey and Whey Derivatives*, IDF Bull. 233, Brussels, Belgium, 1988, p. 21.
7. P. Jelen and A. Renz-Schauen, "Quarg Manufacturing Innovations and Their Effect on Quality, Nutritive Value and Consumer Acceptance," *Food Technol.* **43**, 74 (1989)
8. H. Sheth et al., "Yield, Sensory Properties and Nutritive Qualities of Quarg Produced from Lactose—Hydrolyzed and High Heated Milk," *J. Dairy Sci.* **71**, 2891 (1988).
9. W. N. Eigel et al., "Nomenclature of Proteins of Cow's Milk: Fifth Revision," *J. Dairy Sci.* **67**, 1599 (1984).
10. J. L. Maubois et al., "Industrial Fractionation of Main Whey Proteins," in *Trends in Whey Utilization*, IDF Bull. 212, Brussels, Belgium, 1988, p. 150.
11. P. Jelen, W. Buchheim, and K.-H. Peters, "Heat Stability and Use of Milk with Modified Casein: Whey Protein Content in Yogurt and Cultured Milk Products," *Milchwissenschaft* **42**, 418 (1987).
12. J. Patocka, A. Renz-Schauen, and P. Jelen, "Protein Coagulation in Sweet and Acid Whey Upon Heating in Highly Acidic Conditions," *Milchwissenschaft* **41**, 490 (1986).
13. F. M. Driessen and M. G. van den Berg, "New Developments in Whey Drinks," in *Special Addresses at IDF Annual Sessions*, International Dairy Federation, Bull. 250, Brussels, Belgium, 1990, p. 11.
14. J. L. Maubois and J. Leonil, "Peptides du fait a activite biologique," *Le Lait* **69**, 245 (1989).
15. R. Jost, J. C. Monti, and J. J. Pahud, "Whey Protein Allergenicity and its Reduction by Technological Means," *Food Technol.* **41**, 118 (1987).
16. J. Sherbon, "Physical Properties of Milk," in N. P. Wong et al., eds., *Fundamentals of Dairy Chemistry*, 3rd ed., Van Nostrand Reinhold, New York, 1988, p. 426.
17. D. Roehl and P. Jelen, "Surface Tension of Whey and Whey Derivatives," *J. Dairy Sci.* **71**, 3167 (1988).
18. P. Jelen, "Physico-Chemical Properties of Milk and Whey in Membrane Processing," *J. Dairy Sci.* **62**, 1343 (1979).
19. W. Rattray and P. Jelen, "Protein Standardization of Milk and Dairy Products," *Trends in Food Sci. Technol.* **7**, 227–233 (1996).
20. M. Britten et al., "Composite Blends from Heat-Denatured and Undenatured Whey Protein-Emulsifying Properties," *Int. Dairy J.* **4**, 25–36 (1994).
21. P. Jelen and W. Buchheim, "Stability of Whey Protein Upon Heating in Highly Acidic Conditions," *Milchwissenschaft* **39**, 215 (1984).
22. P. Jelen, "Whipping Studies with Partially Delactosed Cheese Whey," *J. Dairy Sci.* **56**, 1505 (1973).
23. P. Jelen and S. T. Coulter, "Effects of Supersaturation and Temperature on the Growth of Lactose Crystals," *J. Food Sci.* **38**, 1182 (1973).
24. P. Jelen and W. Buchheim, "Norwegian Whey Cheese," *Food Technol.* **30**, 62 (1976).
25. P. Jelen, "Reprocessing of Whey and Other Dairy Wastes for Use as Food Ingredients," *Food Technol.* **37**, 81 (1983).

26. P. Jelen, "Lactose Hydrolysis Using Sonicated Dairy Cultures," in *Lactose Hydrolysis*, International Dairy Federation, Bull. 289, Brussels, Belgium, 1993, pp. 54–56.
27 T. H. Jones and P. Jelen, "Low Dose Gamma-Irradiation of Camembert, Cottage Cheese and Cottage Cheese Whey," *Milchwissenschaft* **43**, 233 (1989).
28. N. Dunford and P. Jelen, "Adsorption of Riboflavin by the CARBOSEP Ultrafiltration System," *8th World Congress of Food Sci. Technol.*, Toronto, Canada, September 29–October 4, 1991.
29. A. Leviton, "Adsorption of Riboflavin by Lactose," *Ind. Eng. Chem.* **36**, 744 (1944).
30 J. Patocka and P. Jelen, "Rapid Clarification of Cottage Cheese Whey by Centrifugation and Its Control by Measurement of Absorbance," *Milchwissenschaft* **44**, 501 (1989).
31. P. Jelen, "Industrial Whey Processing Technology: An Overview," *Agric. Food Chem.* **27**, 658 (1979).
32. V. H. Holsinger, L. P. Posati, and E. D. De Vilbiss, "Whey Beverages: A Review," *J. Dairy Sci.* **57**, 849 (1974).
33. P. Jelen, "Whey Cheeses and Beverages," in J. G. Zadow, ed., *Whey and Lactose Processing*, Elsevier, London, 1991, pp. 147–194.
34. H. W. Modler, "Development of a Continuous Process for the Production of Ricotta Cheese," *J. Dairy Sci.* **71**, 2003 (1988).
35. F. V. Kosikowski and V. V. Mistry, *Cheese and Fermented Milk Foods*, F. V. Kosikowski L.L.C., Westport, Conn., 1997.
36. R. Scott, *Cheesemaking Practice*, Elsevier, London, 1986, pp. 24, 316, 382.
37. E. F. Kravchenko, "Whey Beverages," in *Trends in Utilization of Whey and Whey Derivatives*, International Dairy Federation, Bull. 233, Brussels, Belgium, 1988, p. 61.
38. K. Devkota and P. Jelen, "Pectin–Whey Protein Interactions in Cottage Cheese Whey," *Posters and Brief Communications of the 23rd Int. Dairy Congress*, Montreal, Canada, October 8–12, 1990.
39. H. Trapp, *Technological Approaches in the Development of a Whey-Based Yogurt Beverage*, M.S. Thesis, University of Alberta, Edmonton, Canada, 1990.
40. R. J. Pearce, "Fractionation of Whey Proteins," in *Trends in Whey Utilization*, International Dairy Federation, Bull 212, Brussels, Belgium, 1987, p. 150.
41. A. K. Keller, "Permeate Utilization," in *Proc. Dairy Products Technical Conf.*, Madison, Wisc., April 25–26, 1990, p. 101.
42. C. Beukema and P. Jelen, "High Potency Sweeteners in Formation of Whey Beverages," *Milchwissenschaft* **45**, 576 (1990).
43. R. A. Visser, M. J. van den Bos, and W. P. Ferguson, "Lactose and Its Chemical Derivatives," in *Trends in Utilization of Whey and Whey Derivatives*, International Dairy Federation, Bull. 233, Brussels, Belgium, 1988, p. 33.
44. U.S. Pat. 4,968,521 (1990), P. Melnychyn.
45. J. R. Rosenau, G. Colon, and T. C. Wang, "Lactic Acid Production from Whey via Electrodialysis," in M. LeMaguer and P. Jelen, eds., *Food Engineering and Process Applications*, Vol. 2, Elsevier, London. 1986, p. 245.
46. J. A. Barry, "Alcohol Production from Cheese Whey," *Dairy Ind. Int.*, 19–21 (1982).
47. J. G. Zadow, "Fermentation of Whey and Permeate," in *Trends in Utilization of Whey and Whey Derivatives*, International Dairy Federation, Bull. 233, Brussels, Belgium, 1988, p. 53.
48. A. J. Mawson, "Bioconversions for Whey Utilization and Waste Abatement," *Bioresource Technol.* **47**, 195–203 (1994).
49. F. W. Juengst, "Use of Total Whey Constituents—Animal Feed," *J. Dairy Sci.* **62**, 106 (1979).
50. "South Caernarvon Creamery Converts Whey into Energy," *Dairy Ind. Int.* **49**, 16 (1984).
51. J. B. Bradford, D. B. Galpin, and M. F. Parkin, "Utilization of Whey as a Fertilizer Replacement for Dairy Pasture," *New Zealand J. Dairy Sci. Technol.* **21**, 65 (1986).
52. H. W. Modler et al., "Economic and Technical Aspects of Feeding Whey to Livestock," *J. Dairy Sci.* **63**, 838 (1980).
53. E. Renner, *Milk and Dairy Products in Human Nutrition*, Volkswirtschaftlicher Verlag, Munich, Germany, 1983.
54. A. Renz-Schauen and E. Renner, "Fortification of Nondairy Foods with Dairy Ingredients," *Food Technol.* **41**, 122 (1987).
55. T. Sienkiewicz and C. L. Riedel, *Whey and Whey Utilization*, Verlag T. Mann, Gelsenkirchen-Buer, Germany, 1990.
56. F. V. Kosikowski, "Whey Utilization and Whey Products," *J. Dairy Sci.* **62**, 1149 (1979).
57. B. P. Robinson, J. L. Short, and K. R. Marshall, "Traditional Lactalbumin—Manufacture, Properties and Uses," *New Zealand J. Dairy Sci. Technol.* **11**, 114 (1976).
58. J. V. Chambers and A. Ferretti, "Industrial Applications of Whey/Lactose," *J. Dairy Sci.* **62**, 112 (1979).
59. N. S. Singer, "Simplesse—All Natural Fat Substitute and the Dairy Industry," in *Proc. Dairy Products Technical Conf.*, Madison, Wisc., April 25–26, 1990 p. 85.
60. N. Shah and P. Jelen, "Lactose Absorption by Post-Weaning Rats from Yogurt, Quarg and Guarg Whey," *J. Dairy Sci.* **74**, 1512 (1991).
61. N. P. Shah, R. N. Fedorak, and P. Jelen, "Food Consistency Effects of Quarg in Lactose Malabsorption," *Int. Dairy J.* **2**, 257–269 (1992).
62. P. Jelen and S. Lutz, "Functional Milk and Dairy Products," in G. Mazza, ed., *Functional Foods—Biochemical and Processing Aspects*, Technomics Publishing Co., Lancaster, Pa., 1998, pp. 357–380.
63. F. Nettl, "The Shocking Truth About Whey Protein," *Muscle Media 2000*, 72–77 (1995).
64. G. O. Regester et al., "Bioactive Factors in Milk—Natural and Induced," in R. A. S. Welch et al., eds., *Milk Composition, Production and Biotechnology*, CAB International, New York, 1997, pp. 119–132.
65. Can. Pat. 1,338,682 (October 29, 1996), G. Bounous, P. Gold, and P. L. Kongshavn.
66. G. Bounous and P. Gold, "The Biological Activity of Undenatured Dietary Whey Proteins—Role of Glutathione," *Clin. Invest. Med.* **14**, 296–309 (1991).
67 R. Tanaka and K. Matsumoto, "Recent Progress on Prebiotics in Japan, Including Galactooligosaccharides," Int. Dairy Fed. Bulletin 336, Brussels, Belgium, 1998, pp. 21–27

P. Jelen
University of Alberta
Edmonton, Alberta
Canada

See also Dairy ingredients for foods.

WINE

INTRODUCTION

The word wine was first used for the fermented product of grapes, although fermented juices of many fruits (apples, berries, peaches, and even herbs) are now called wine (usually peach wine, etc). Wine has a long association with human artistic, cultural, and religious activities. The qualities of wine were praised in pre-Christian as well as post-Christian times. Addition of herbs and spices was common, to provide distinct flavors (and mask off-flavors) and to induce supposedly medicinal and aphrodisiac properties. The religious and allegorical significance of wine developed very early and has been utilized by many religions, including Christianity.

Grape cultivation first began around 6000 years ago. Although many of the wines were poor by modern standards, a developing devotion and taste for wine (beginning with the Roman era) encouraged improvement in grape and wine production to the point where the regional quality of special wines became recognized. In contrast to the invading Romans, who brought grapevines with them, the Moslems, in later years, virtually destroyed the wine industry of the countries they conquered. From then and throughout the Middle Ages, in central Europe monasteries maintained vineyards as a source of wine for religious ceremonies. By the seventeenth century, the coopers built more and better casks and barrels so that wines could be safely aged for years. At the same time, glass was strengthened when coal (instead of charcoal) was used in its manufacture. This permitted bottling of sparkling wines. The introduction of cork extended the time for which bottled wines could be stored. By the nineteenth century, the scientific revolution, as exemplified by the work of Pasteur and others, revolutionized the wine industry: the role of yeasts and bacteria and methods for their control changed the wine industry from a cottage industry of chance to a controlled business. Mechanization of operations in the vineyard and winery followed. Although producing quality wines is still something of an art, today's wine industry makes some of the best wine ever produced, with minimum spoilage and with much less human labor.

REGULATIONS AND APPELLATIONS

Wine is the fermented product of the fruit of several species of *Vitis*, mainly of cultivars of *Vitis vinifera*. The United States's definition of wine is contained in the U.S. Internal Revenue Code. Table and sparkling wines (<14.1% alcohol) are distinguished from dessert and flavored (vermouth, etc) wines (>14.0% alcohol) and from wine coolers (<7% alcohol). Each country has its own definitions, and the European Community (EC) has regulations for trade among its members and for imports from nonmember countries.

In Germany, the eastern United States, and parts of France, sugar, grape concentrate, or reduced musts (crushed grapes) may be added when the grapes do not ripen sufficiently *and* when local laws approve it. Generally addition of water and sugar is forbidden. Limits on chlorides, sulfates, lead, arsenic, volatile acidity (mainly acetic acid), and sulfur dioxide have been established by federal regulations (such as national public-health authorities). Because a small percentage of asthmatics are extremely sensitive to sulfur dioxide, U.S. government regulations require a warning about sulfites on wine labels.

Appellations of origin are used in many countries. In general, these delimit the region, the varieties of grapes used, maximum yield per hectare, and often the wine practices and in some cases (eg, Portugal [for Port], France, Germany, and South Africa) require sensory evaluation of each wine to qualify for a regional designation. Typically, use of the name of a specific vineyard on a wine label implies that the wine is of higher quality than those labeled for larger districts. In Germany, quality is also related to ripeness. The higher-quality wines are made with no sugar addition. Whereas France, Germany, Spain, and Italy have had protected geographical appellations for many years, it was not until 1978 that appellation procedures were instituted in the United States. In contrast to European laws, U.S. regulations define the boundaries of the delimited viticultural area and limit neither the variety of grapes grown nor the types of wine produced. In most countries (including the United States), where the appellation does not regulate which varieties may be planted, wines are labeled with the name of the grape variety from which it is made, hence the term varietal wines.

VITICULTURE (THE SCIENCE OF GRAPE GROWING)

V. vinifera, the main genus and species of grapes used for wine, produces the best grapes in temperate climates with mild winters and dry summers; vines are killed by very cold winters and are susceptible to mold, mildew, and diseases in humid summer regions. *V. vinifera* vines have no resistance to the root louse, *Phylloxera*, which virtually destroyed the wine-making industry in France and California in the late 1800s. Other species, such as *V. labrusca* (Concord grape), are resistant to the pest. Today *V. vinifera* vines in France and California are usually grafted to *Phylloxera*-resistant rootstocks.

Grape leaves appear in early spring, with grape flowering occurring about 45 days later. The small, hard berries continue to expand for 2 months until ripening is initiated (known as *veraison*). From this time on, the grape begins to color and soften and the sugar (glucose and fructose) rapidly increases. The concentrations of tartaric and malic acids, the major organic acids, peak at veraison. With the onset of ripening the acid level decreases as the berry expands, and in hot climates, the malic acid can decrease rapidly.

The maximum sugar accumulated by the grape during ripening depends on the coolness or warmth of the region where they are grown, on the variety of grape, and on other viticultural factors such as the crop level (amount of fruit per vine). For most varieties, 15 to 25% sugar is reached, but late-harvest fruit (such as those used for sauternes from France or *Beerenauslese* or *Trockenbeerenauslese* from Germany) may have 30 to 40% sugar. (High humidity during the final ripening of the grapes may result in infection with the mold *Botrytis cinerea*. This fungus loosens the skin and permits rapid loss of moisture and shriveling of the berry, resulting in a high sugar content and producing a honeylike flavor).

The pH and the concentration of acids, measured as titratable acidity, are further indicators of suitability of grapes for harvesting. During ripening, if malic acid decreases greatly, the grapes may have a high pH (pH ≥4). Addition of tartaric acid to crushed grapes is sometimes necessary to lower the pH in order to reduce susceptibility to microbial spoilage. In contrast, in cool-climate regions, the acidity may be so high at harvest that processing steps may be needed to reduce the acidity. Normal grape juice has pH values from 3.0 to 3.6 and with titratable acidities of 0.6 to 1.5% (calculated as tartaric acid). Under these conditions undesirable microorganisms grow slowly, if at all, whereas desirable yeasts grow well.

Although only a small amount of nitrogenous materials (0.3–1.0%) are found in mature grapes, they are significant for yeast and malolactic bacteria nutrition as well as for flavor development. With very low levels of nitrogen, the fermentation may proceed very slowly and may stop before the sugar is completely fermented, producing a "stuck" fermentation. However, nitrogen levels in the grape can be controlled by appropriate viticultural practices, such as fertilization or reduction in crop level.

Anthocyanins, the colored pigments of red grapes, are located in the epidermal (outer) cells of the skin, although a few varieties of grapes have pigments in the pulp as well. Tannins (phenolic compounds) are located primarily in grape skins and seeds. The tannins, which are found in higher concentrations in red wines, have some positive health benefits as antioxidants. Recently, evidence has been presented that drinking moderate amounts of red wine lowers susceptibility to heart disease and exerts anticarcinogenic effects.

MICROORGANISMS AND FERMENTATION

Wines are normally fermented with the yeast *Saccharomyces cerevisiae*, although *S. bayanus* and *S. oviformis* are sometimes used. The primary or alcoholic fermentation is the process of conversion of the grape sugars, glucose and fructose, to ethanol and carbon dioxide. In addition to *Saccharomyces*, other wild yeasts, such as *Kloeckera apiculata* and *Metschnikowia pulcherima*, can be found on the fruit and participate in the early stages of fermentation. However, these latter yeasts can impart compounds that alter the aroma of the wine.

Yeast is present at high enough concentrations on the grape skins and in the winery to initiate fermentation. However, pure strains of yeast cultures are often added to assure the completion of fermentation by *Saccharomyces*. To inhibit growth of the wild yeasts and other undesirable microorganisms, 25 to 75 mg/L sulfur dioxide (SO_2) is added in most white wine fermentations and frequently in reds.

In addition to yeast, bacteria are also associated with the wine-making process. Although lactic acid bacteria are found on the fruit and are present in the winery, a bacteria inoculum is usually added at the later stages of the alcoholic fermentation to ensure completion of the secondary or malolactic fermentation. During this secondary fermentation, malic acid from the grape is converted to lactic acid, carbon dioxide, and other compounds. These malolactic (ML) bacteria also produce diacetyl, which imparts the characteristic buttery aroma found in many wines. ML bacteria, of which the most frequently found is *Oenococcus oeni*, are very sensitive to SO_2; consequently, lower levels of SO_2 are used when the ML fermentation is desired.

Unlike many other products, no harmful bacteria or molds grow on grapes or in wine. However, spoilage organisms can be found in fermentations and in finished wine. The acetic acid bacteria that are inhibited by SO_2 can produce high levels of acetic acid in the presence of air, which is desirable only in production of wine vinegar. Once acetic acid is formed, the yeast and other microorganisms can convert it to ethyl acetate, which contributes a solvent odor similar to that of nail polish remover. The yeast *Brettanomyces* can contribute distinctive aromas ranging from barnyard, horsey, leathery, phenolic, and fecal to mousy. Although these "spoilage" organisms may enhance the complexity of wine aroma at low concentrations, they often result in "off-odors" at higher concentrations.

WINE MAKING

Still Wines

The basic steps used in making wine are very similar for small producers of the highest-quality wines, large producers of inexpensive wines, and home wine makers, although the size and sophistication of the equipment varies enormously. Grapes are first crushed in a stainless-steel crusher-destemmer. These operate at a high speed, separating the stems by centrifugal force and crushing the grapes with revolving paddles. The crushed grapes (called must) fall through holes in the cylinder as the stems are ejected.

Must pumps transfer the crushed white grapes to presses and the crushed red grapes to fermentors, with some exceptions. White wines are made from white grapes or by pressing crushed red grapes immediately after crushing. If the skins are rapidly separated from the juice, light or pink-colored musts can be produced from red grapes. This process is used to make the so-called blush wines such as White Zinfandel that comes from the red Zinfandel grapes; similarly, blanc de noir sparkling wines are made from the red Pinot noir grape by pressing rapidly so that minimum pigment is extracted.

The old-fashioned basket press is still found, but the new and larger wineries often use the Willmes press, which has an inflatable bag inside a revolving tank. As the bag is inflated, the musts are pressed against a perforated cylinder and the juice flows out to the bottom of the press. Some hydraulic presses are horizontally operated, with pressure exerted from both ends. The fermentors are usually tanks made of stainless steel that has the advantage of being chemically inert, thermodynamically uniform, and easy to clean. The fermentors are often jacketed to permit computerized control of temperature by the circulation of coolants. The size of the fermenting tanks varies with the winery from 500 gal to over 100,000 gal.

Sulfur dioxide (50–75 mg/L) may be added to white musts, which are then often centrifuged or settled overnight to clarify the juice before transfer to the fermentor, at which point a yeast inoculum is added to the white musts. In the case of red grapes, yeast and sulfur dioxide are added to the crushed grapes after they are pumped into the fermentation tank.

Fermenting tanks of white wines are usually maintained at 10 to 15°C, with the fermentation taking from 3 to 4 weeks. Red musts are fermented warmer than white, typically at temperatures between 18 and 27°C, so that fermentation is completed in 3 to 10 days.

For red musts, the skins rise to the surface, forming a cap during fermentation due to the evolution of carbon dioxide gas. To help extract the pigments from the skins, the fermenting must at the bottom of the tank is pumped over and sprayed onto the floating cap. Depending on the style of red wine being made, red musts can undergo an extended fermentation on the skins up to 4 weeks before they are pressed and then fermented to dryness (a level of sugar below 0.5%). Longer contact with the skins provides greater extraction of anthocyanin pigments and phenolic material, which have a direct effect on the sensory properties of wine. Accordingly, these wines are intended to be aged before they reach their maximum quality. The wine made with shorter skin-contact time is lighter in color and less bitter and astringent so that it can be consumed without aging.

When the fermentations have reached dryness, the wine is transferred from the fermentor to clean stainless-steel tanks for clarification or to wood barrels for aging. Wines that will not be exposed to oak cooperage are clarified by settling and transferring (racking) the clear wine to other tanks. To prevent formation of crystals of potassium acid tartrate (cream of tartar) in the consumer's refrigerator, wines are cold stabilized. The wine is stored at −4°C and the precipitated tartrate crystals are removed by filtration. Before bottling, the wine is fined by addition of an agent to clarify the wine by adsorbing and removing suspended or dissolved material. The most common fining agent for white wines is bentonite (a clay), whereas reds are fined with a variety of agents, including egg whites and gelatin. Other fining materials include isinglass, casein, polyvinyl-polypyrrolidone (PVPP), and silica suspensions.

Most red and some white wines are stored in oak barrels to extract flavor components from the oak. Wines are transferred to 50-gal oak barrels where they are held for periods from 3 months to 2 years. During this aging period, the level of wine in the barrels is frequently topped off to prevent exposure to air. After oak storage, the wines are fined and cold stabilized.

Before bottling, wine is usually filtered to be brilliantly clear. If the wine contains more than 0.7% sugar, it is microbially unstable. In this case a fine-mesh or sterile filtration is also used to remove microorganisms and prevent spoilage in the bottle. On automated bottling lines, the filtered wine is filled into bottles, corks are inserted, labels are attached, and the bottles are put in cases.

Sparkling Wines

Wines with a visible excess of carbon dioxide (CO_2) are called sparkling wines. In the United States a sparkling wine is one that contains a barely perceptible level of CO_2 (above 3.9 g/L at 15.7°C), although the typical level of carbon dioxide is much higher: 4 to 6 atm pressure. The most famous sparkling wine is Champagne, produced in the Champagne region of France, where it was first commercialized. Depending on local regulations, sparkling wines are also labeled *Sekt* (Germany), *cava* (Spain), or just sparkling. The two primary methods of production are bottle fermentation (*méthode champenoise*) and closed-tank fermentation (*Charmat* or *Tank*). Chardonnay, Pinot noir and Meunier wines are used for the premium sparkling wines, but other varieties, including Muscat and Riesling, are also used. The base wines are blended and yeast and sugar are added (24 g sugar per liter will produce a pressure of 6 atm). The resulting mixture of wine, sugar, and yeast is transferred to bottles (750 mL) or to stainless-steel pressure tanks for the secondary (alcoholic) fermentation.

The tank process is much cheaper to use, and fermentation is generally completed in less than 1 month. At that time the wine is filtered under pressure, sugar is added to achieve a desired level of sweetness, and the wine is filled into bottles. In contrast, although the *méthode champenoise* fermentation is completed in the filled bottles between 3 and 6 months, the wines are typically held in contact with the yeast for a minimum of 3 years. The bottles are intermittently jostled to force the yeast deposit to settle in the neck of the bottle. To remove the yeast deposit from the bottle, the neck of the bottle is frozen. When the cap is removed, the pressure in the bottle pushes out the frozen yeast deposit. The bottle is immediately filled with more of the same wine, and the dosage (sugar in wine or brandy) is added to achieve the desired sweetness before the bottle is closed with a cork.

SENSORY PROPERTIES

The appeal of wines is due to the complex flavors that are often difficult to describe without resorting to poetic images. Wine flavor is an overall perception, resulting from the integration of appearance, aroma, taste, and mouthfeel. Although wine aromas are often very complex, with little effort they can be described in terms that are precise and can be understood by others. For example, the wine aroma wheel (WAW) in Figure 1 lists terms that describe the aroma notes most often perceived in table wines. The flavor perceived in the mouth is primarily aroma, plus sweet, tart, or bitter tastes and an astringent mouth-feel. Flavors can be informally described using the WAW terminology or more formally profiled by trained judges using descriptive analysis to provide a "photograph of wine flavor."

The purpose of the WAW is to facilitate communication about wine flavor by providing a standard terminology. These terms are precise and not hedonic or judgmental. "Floral" is a general but analytical descriptive term, whereas "fragrant," "elegant," or "harmonious" are either

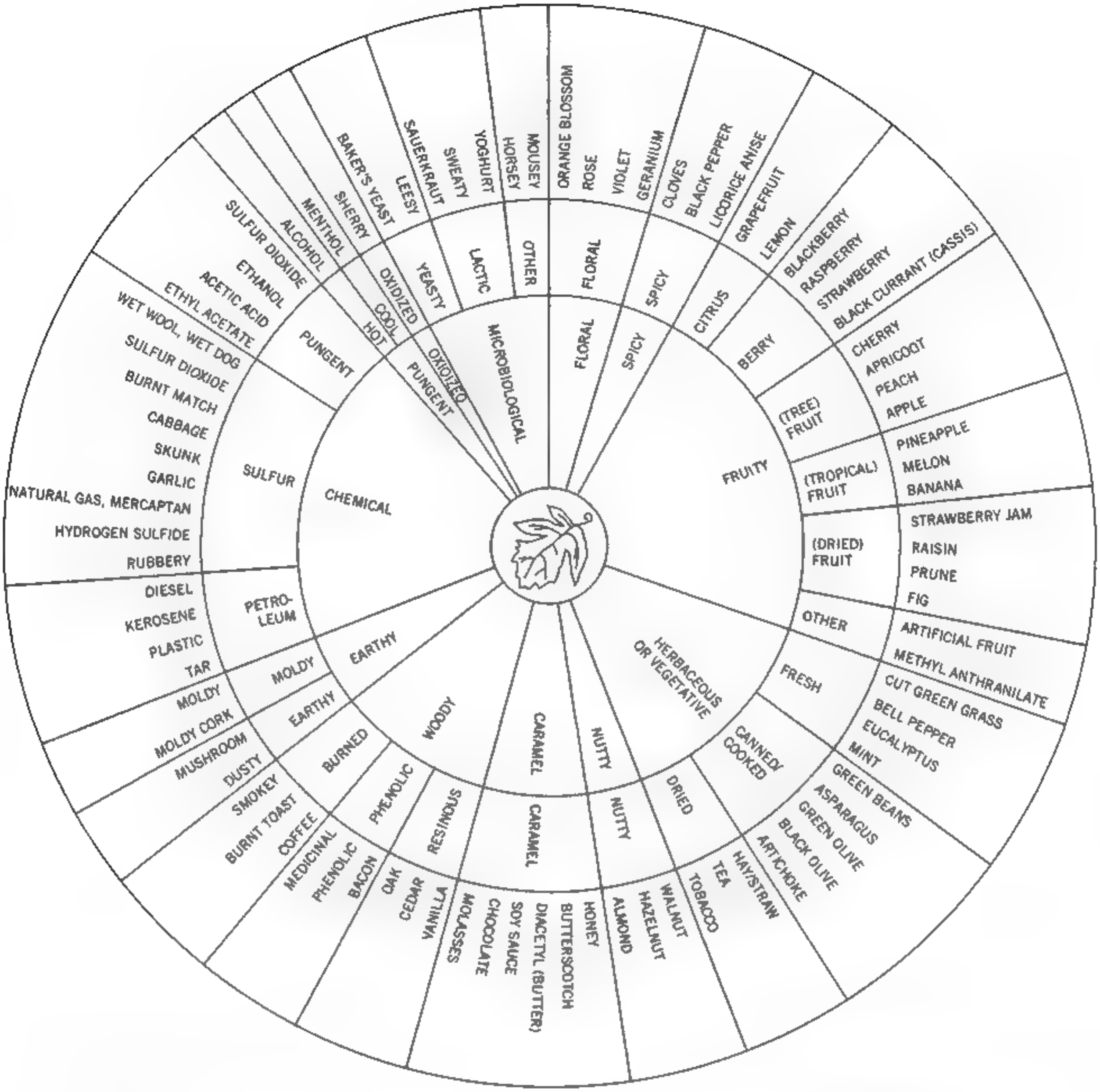

MICROBIOLOGICAL
FLORAL
SPICY
FRUITY
HERBACEOUS OR VEGETATIVE
NUTTY
CARAMEL
WOODY
EARTHY
CHEMICAL
PUNGENT
OXIDIZED
OTHER
LACTIC
YEASTY
OXIDIZED
COOL
HOT
PUNGENT
FLORAL
SPICY
CITRUS
BERRY
(TREE) FRUIT
(TROPICAL) FRUIT
(DRIED) FRUIT
OTHER
FRESH
CANNED/ COOKED
DRIED
NUTTY
CARAMEL
RESINOUS
PHENOLIC
BURNED
EARTHY
MOLDY
PETRO-LEUM
SULFUR
MOUSEY
HORSEY
YOGHURT
SWEATY
SAUERKRAUT
LEESY
BAKER'S YEAST
SHERRY
MENTHOL
ALCOHOL
SULFUR DIOXIDE
ETHANOL
ACETIC ACID
ETHYL ACETATE
ORANGE BLOSSOM
ROSE
VIOLET
GERANIUM
CLOVES
BLACK PEPPER
LICORICE ANISE
GRAPEFRUIT
LEMON
BLACKBERRY
RASPBERRY
STRAWBERRY
BLACK CURRANT (CASSIS)
CHERRY
APRICOT
PEACH
APPLE
PINEAPPLE
MELON
BANANA
STRAWBERRY JAM
RAISIN
PRUNE
FIG
ARTIFICIAL FRUIT
METHYL ANTHRANILATE
CUT GREEN GRASS
BELL PEPPER
EUCALYPTUS
MINT
GREEN BEANS
ASPARAGUS
GREEN OLIVE
BLACK OLIVE
ARTICHOKE
HAY/STRAW
TEA
TOBACCO
WALNUT
HAZELNUT
ALMOND
HONEY
BUTTERSCOTCH
DIACETYL (BUTTER)
SOY SAUCE
CHOCOLATE
MOLASSES
VANILLA
CEDAR
OAK
BACON
PHENOLIC
MEDICINAL
COFFEE
BURNT TOAST
SMOKEY
DUSTY
MUSHROOM
MOLDY CORK
MOLDY
TAR
PLASTIC
KEROSENE
DIESEL
RUBBERY
HYDROGEN SULFIDE
NATURAL GAS, MERCAPTAN
GARLIC
SKUNK
CABBAGE
BURNT MATCH
SULFUR DIOXIDE
WET WOOL, WET DOG

Figure 1. The wine aroma wheel presents descriptive terms for aroma notes that are often perceived in the aromas of table wines. General terms are located on the inside tier, with the most specific terms located on the outside tier. *Source:* Copyright 1990, A. C. Noble.

imprecise and vague (fragrant) or hedonic and judgmental (elegant and harmonious).

The center of the wheel has very general terms, whereas more specific terms are in the outer tiers. These terms are not the only ones that can be used to describe wines, but they represent aroma notes that are often encountered. The aromas for these descriptive terms can be defined by physical standards. For example, a small piece of bell pepper, a grapefruit section, or a few drops of vanilla extract can be presented to define "bell pepper," "grapefruit," or "vanilla," respectively.

The flavor of wine is influenced by the grape variety, the climate and location in which the grapes are grown, viticultural practices, yeast and malolactic fermentations, skin-contact time, storage in oak cooperage, and aging. The characteristic aromas of many of the major *V. vinifera* cultivars used for making premium wines throughout the world arise from the grape. Zinfandel grapes and wines typically have black pepper and berry flavors, whereas red wines from Burgundy (made from Pinot noir grapes) and Pinot noir varietal wines from other locations are distinctive for their (straw)berry or cherry aromas. Red wines from Bordeaux and unblended red wines made from the same varieties (Cabernet Sauvignon, Merlot, and Cabernet Franc) typically have berry, black pepper, and herbaceous/vegetative aromas such as that of a green bell pepper or asparagus. The white varietal, Sauvignon blanc, is similarly characterized by the bell pepper note but also by many fruity attributes such as pineapple, peach, and apricot, which are found in most white wine varietals. Gewürztraminer, Riesling, and Muscat grapes and their respective wines are characterized by floral, citrus, and apricot notes.

Chardonnay is a white varietal that is noted for its citrus, pineapple, peach, and apricot aromas. However, it is more typically identified with aromas arising from winemaking practices. Chardonnay is sometimes fermented in oak and usually aged in oak barrels. Exposure to oak imparts spicy, clove, and vanilla aromas. If the barrels were heavily toasted or charred when coopered, then toasted/smoky aromas may also be perceived. Malolactic fermentation is encouraged in most Chardonnays, thereby infusing a distinct buttery aroma. Most red varietal wines, including reds from Bordeaux and Burgundy, undergo malolactic fermentation and aging in oak. Thus buttery, spicy, and vanilla notes are perceived in addition to the aromas coming from the grape.

Immediately after fermentation, wines tend to be very fruity, because high concentrations of fruity esters are formed in both red and white wines by yeast during the primary fermentation. However, these fruity notes diminish fairly rapidly and other very complex flavors develop during the aging process. Slow aging in oak cooperage or in the bottle results in the development of very complex aromas in red wines in which leather, tobacco, soy, and other related characteristics can be perceived. White wines are usually not aged as long as reds but can also develop complex aromas when stored at cool temperatures.

In sparkling wine, flavor is influenced most strongly by the time in which the wine is held in contact with the yeast. In the tank process, the wine is in contact with the yeast for only a few weeks, and the resulting sparkling wine is very fruity. In the bottle-fermented sparkling wines, the wines are usually not disgorged until after 3 years. The best sparkling wines are often disgorged after 10 years on the yeast. The extended contact with the yeast produces distinctive flavors through the slow breakdown (autolysis) of the yeast. Thus the *méthodé champenoise* wines are less fruity and have very complex flavors, such as caramel, malted, and nutty.

Although this objective approach describes wines well, it does not provide an overall preference or quality rating. Quality is a subjective and individual response, varying from person to person because of different experiences, expectations, and preferences. As a consequence, even wine experts will have differences of opinion about wine quality. Wine consumers also vary considerably in their preference ratings in that many factors other than wine flavor influence their purchasing behavior and quality judgments. Despite this, sales of many wines are influenced by the medals awarded at wine shows or by comments from wine critics.

Quality of wine is not directly related to price, which is influenced primarily by the cost of grapes and winemaking practices. The highest-quality grapes are grown in cooler climates at lower crop yields on very expensive land. Oak barrels are expensive and their maintenance is labor-intensive. Aging wines in the barrel or bottle represents a capital investment in time and assets.

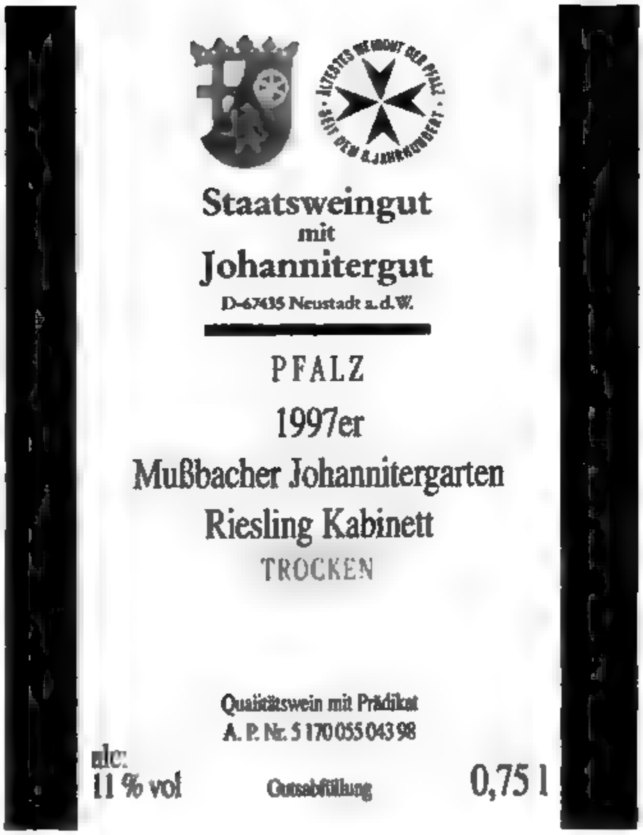


Figure 2. German white wine (Riesling) label. *Source:* SLFA Neustadt, Neustadt Germany, 1998.

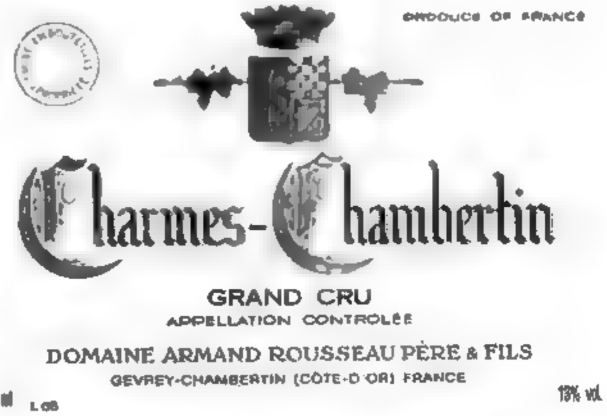


Figure 3. A red wine (Pinot noir) label from the Burgundy region of France. *Source:* University of California, Davis, Department of Viticulture and Enology Archives, 1998.

With each wine-tasting experience, consumers learn more about wines and develop their own preferences and expectations, which may change over time. In the end, each person is the ultimate judge of his or her own preference and wine quality.

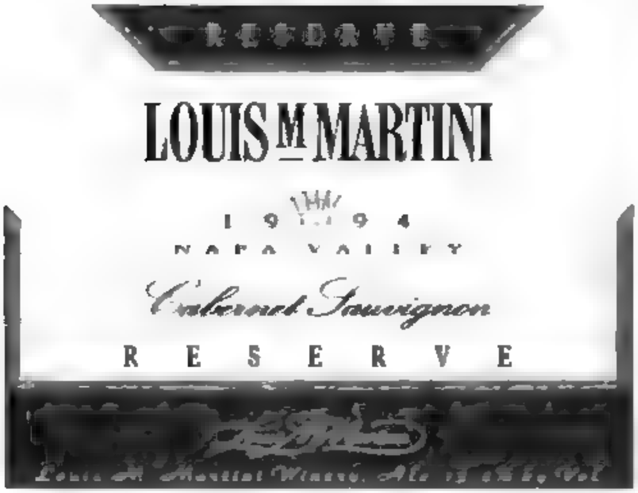


Figure 4. Cabernet Sauvignon wine label from Napa Valley, California. *Source:* Louis M. Martini Winery, St. Helena, California, 1998.

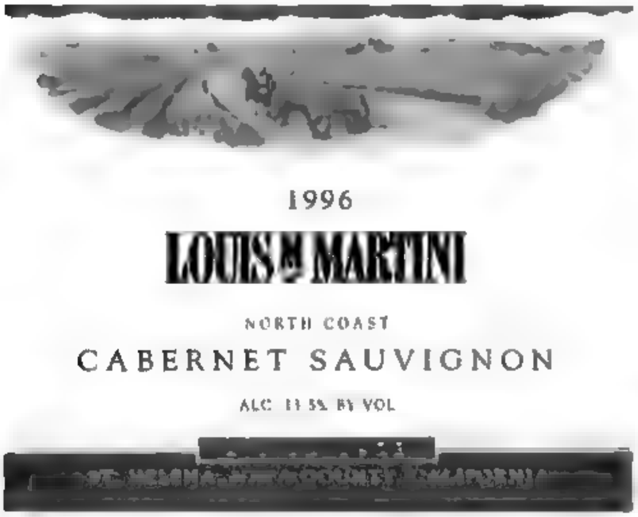


Figure 5. Cabernet Sauvignon wine label from Napa Valley, California, indicating grapes were harvested from the North Coast American Viticultural Area (AVA). *Source:* Louis M. Martini Winery, St. Helena, California, 1998.

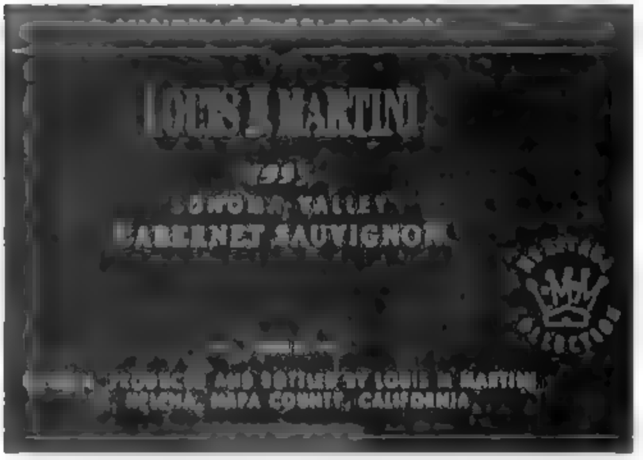


Figure 6. Cabernet Sauvignon wine label from Napa Valley, California, indicating a special vineyard as the source of the grapes. *Source:* Louis M. Martini Winery, St. Helena, California, 1998.

Table 1. Per Capita Wine Consumption by Country, 1970, 1980, and 1996

	L/gal		
1996 rank per capita	1996	1980	1970
1. Italy	61.1/16.2	79.8/21.2	110.8/29.3
2. France	59.9/15.8	90.9/24.0	108.2/28.6
3. Portugal	54.9/14.5	28.8/7.6	23.1/6.1
4. Luxembourg	53.6/14.2	N.A.	N.A.
5. Switzerland	41.1/10.9	47 0/12.4	38.9/10.3
6. Argentina	39.5/10.4	76.2/20.1	91.7/24.2
7. Slovenia	38.8/10.3	N.A.	N.A.
8. Spain	37.4/9.9	59.9/15.8	61.4/16.2
9. Austria	33.1/8.7	35.5/9.4	37.8/10
10. Romania	31.3/8.3	N.A.	N.A.
11. Greece	30.1/17.9	44.9/11.9	39.9/10.6
⋮			
15. Germany	22.9/6.1	25.5/6.7	16.2/4.3
16. Belgium	20.0/5.3	N.A.	N.A.
17. Australia	18.0/4.8	17.4/4.6	8.5/2.3
18. Chile	16.2/4.3	50.2/13.3	43.9/11.6
⋮			
25. New Zealand	10.5/2.8	4.38/1.2	2.34/0.62
26. U.S.A.	9.03/2.4	8.0/2.1	4.9/1.3
27. South Africa	8.99/2.37	9.1/2.4	11.2/3.0

Source: Ref. 1, provided by Wine Institute, San Francisco, 1998.

READING WINE LABELS

Wine labels from different countries contain different information because of variation in legal regulations and appellations of origin (France, *Appellation d'Origine Contrôlée*; Spain, *Denominacion de Origen*; Italy, *Denominazione di Origine*). The first label (Fig. 2) is typical of those from Germany. *PFALZ* refers to the region, the Palatinate or Rheinpfalz, which is the second-largest wine region in Germany. The grapes for this wine were harvested in 1997 from the Johannitergarten vineyard in the town of Mussbach. The winery is a state winery (Staatsweingut). At least 85% of the grapes are Riesling. *Qualitätswein mit Prädikat* (QmP) means that it is a "quality wine with special distinction" and did not have any sugar added. *Kabinett* means the grapes achieved a minimum required level of sugar. (If the grapes had been harvested at least 7 days later and were riper, the wine could have been labeled *Spätlese*). *TROCKEN* means the wine is dry (<9.0 g per liter sugar). The *A.P. Nr . . .* is the *Amtliche Prüfungsnummer* (State Institute quality control number), which identifies the details of the testing for chemical and sensory defects. The wine is 11% alcohol by volume and the bottle contains 750 mL (or 0.75 liter).

The second label (Fig. 3) is from a wine from the Burgundy region in France. The wine is a *Grand Cru* or "great growth," which is the highest-quality classification in the

Table 2. Leading Wine Producers by Country, 1996

	Hectoliters (in millions)	Gallons (in millions)
1. France	59.65	1576
2. Italy	59	1559
3. Spain	32.68	863
4. U.S.A.	21.95	580
5. Argentina	12.68	335
6. S. Africa	10	264
7. Portugal	9.53	252
8. Germany	8.3	219
9. Romania	7.66	202
10. Australia	6.78	179
11. China	4.3	114
12. Hungary	4.19	111
13. Greece	4.11	109
14. Chile	3.82	101
15. Yugoslavia	3.49	92

Source. Ref. 1, Wine Institute, San Francisco, 1998.

Table 3. Wine Consumption in the United States, Selected Years

	Total wine per capita[a] (gal)	Total wine (in million gals)	Total table wine (in million gals[b])
1997	1.95	523	462
1996	1.9	505	443
1995	1.79	469	408
1994	1.77	459	395
1993	1.74	449	381
1992	1.87	476	405
1991	1.85	466	394
1990	2.05	509	423
1989	2.11	524	432
1988	2.24	551	457
1987	2.39	581	481
1986	2.43	587	487
1985	2.43	580	378
1984	2.34	555	401
1983	2.25	528	402
1982	2.22	514	397
1981	2.2	506	387
1980	2.11	480	360
1970	1.31	267	133
1960	0.91	163	53
1950	0.93	140	36
1940	0.68	90	27

[a] All wine types, including sparkling wine, dessert wine, vermouth, other special natural wine, and table wine. Based on resident population. Per capita consumption will be higher if based on legal drinking age population.

[b] Because of changes in reporting, these numbers include all still wines not over 14% alcohol.

Note: To convert gallons to liters multiply by 3.785.

Source: Wine Institute, San Francisco, 1998.

Table 4. Wine Production by State, 1995–1997 (in Thousands of Gallons)

State	1995	1996	1997[a]	Rank 1997	% total 1997
AZ	N.A.	50	N.A.	N.A.	N.A.
AR	147	N.A.	N.A.	N.A.	N.A.
CA	397,042	418,376	422,560	1	90.57
CO	N.A.	147	110	17	0.02
CT	N.A.	64	50	20	0.01
FL	1,026	612	640	11	0.14
GA	1,241	1,163	1,120	7	0.24
ID	363	301	N.A.	N.A.	N.A.
KY	201	697	820	10	0.18
MD	N.A.	57	70	19	0.02
MA	50	68	90	18	0.02
MI	273	N.A.	N.A.	N.A.	N.A.
NC	176	190	210	14	0.05
NJ	742	843	840	9	0.18
NM	85	86	140	16	0.03
NY	21,096	25,157	23,180	2	4.97
OH	1,339	843	1,130	6	0.24
OR	1,586	1,544	1,860	4	0.4
PA	317	402	410	12	0.09
TN	132	130	150	15	0.03
TX	763	902	980	8	0.21
VT	N.A.	1,384	1,550	5	0.33
VA	391	292	320	13	0.07
WA	8,660	5,596	5,200	3	1.11
WI	119	N.A.	10	21	0
Other	1,292	1,178	5,100	N.A.	1.09
Total	435,749	458,904	461,440		100

[a] May through December are estimated.

Note Based on volumes removed from fermenters.

Source: Ref. 2.

Burgundy area. Grand Cru wines can be named using only the name of the winery on the label, here *Charmes—Chambertin*. The regulations that determine the variety of grape (Pinot noir) and regulate specific viticultural and enological practices are defined by this appellation of origin (*Appellation Contrôlée*). The winery is located in the village Gevrey–Chambertin (in the Côte d'Or region). The producer's name appears above the village name.

The third label (Fig. 4) comes from the Louis M. Martini Winery in St. Helena, California. The U.S. wine appellation of origin defines the geographical boundaries of American Viticultural Areas (AVA), which, unlike in the European system, are not an indication of the wine quality. By California law, 100% of the grapes must be grown in California if any California place name appears on the label. A minimum of 85% of the grapes used in making this wine must come from the AVA, Napa Valley. To be bottled as a cabernet sauvignon varietal wine, a minimum of 75% cabernet sauvignon grapes must have been used. To be vintage labeled, 95% of the wine must have come from grapes harvested in the vintage year, 1994. The word "Reserve" has no legal meaning in the United States but implies that the wine maker has used his best grapes and aged the wines longer before release.

The fourth label (Fig. 5) comes from the Louis M. Martini Winery in St. Helena, California. A minimum of 85% of the grapes used in making this wine must come from the AVA, North Coast.

The fifth label (Fig. 6) comes from the Louis M. Martini Winery in St. Helena, California. The vineyard designation here reflects that the winery chose to bottle the wine made from this particular vineyard separately and does not imply that this vineyard is an AVA on its own.

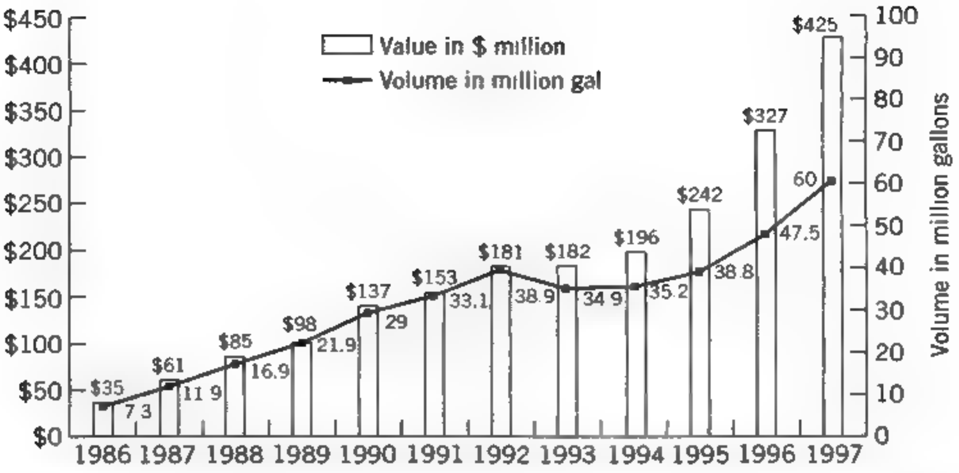


Figure 7. Dollar value (in millions US$) and volume (in millions of gallons) of U.S. exports from 1986 to 1997. In 1992, there was a decrease in the volume of U.S. wine exports. The price per gallon held steady at about $5/gal from 1986 to 1992 and then began a steady increase to approximately $7/gal in 1997.

Table 5. Commercially Produced Wines Entering U.S. Distribution Channels, Calendar Year Average (in Thousands of Gallons)

	Domestic Production		Imports		Total	
Type	1985–1986	1995–1996	1985–1986	1995–1996	1985–1986	1995–1996
Table	267,088	317,277	97,765	68,267	364,853	385,544
Sparkling	30,171	22,247	15,029	8,038	45,200	30,285
Dessert	31,259	18,034	3,380	2,473	34,639	20,507
Vermouth	3,804	2,411	2,845	1,823	6,649	4,234
Other spec (not over 14%)	12,793	17,431	2,603	491	15,396	17,922
Total	*345,115*	*377,400*	*121,622*	*81,092*	*466,737*	*458,492*

Source Gomberg, Fredrikson & Associates from reports of the California State Board of Equalization, Bureau of Alcohol, Tobacco and Firearms, U.S. Treasury Dept., Bureau of Census, U.S. Dept. of Commerce. Data provided by Wine Institute, San Francisco, 1998.

PRODUCTION AND CONSUMPTION STATISTICS

Per capita consumption of wine in the United States is low (Table 1) compared to the other major wine-producing nations. The United States, in 1996, was fourth in world production (Table 2) but 26th in per capita consumption. In addition to the top ten consuming countries, Table 2 lists other countries that are both major wine producers and low per capita consumers. Overall, the global per capita consumption has decreased sharply since the 1980s, especially in France and Italy, who still, nonetheless, retain their prominent positions as the leading consumers and producers of wine. U.S. consumption declined by 20% from the mid-1980s high of 2.43 gallons per person annually to 1.95 gallons per capita in 1997 (Table 3).

Ninety one percent (410 million gallons) of the wine produced in the United States comes from California, which accounts for 71% of U.S. retail sales ($11.4 billion). Considerable development of the wine industry in other states occurred during the decade from 1980 to 1990. Consequently, increased plantings outside California, as well as shipment of California grapes and grape concentrate to other states for the purposes of wine production, catalyzed industry expansion into approximately 40 states. Table 4 lists the top 25 states in wine production from 1995 to 1997.

As one of the world's leading producers, the United States has witnessed a steady increase in the export of its wines (Figure 7). Despite the millions of gallons produced for both internal consumption and exportation, approximately 18% of the wine on the American market is imported (Table 5). However, the amount of imported wines in all categories (table, sparkling, dessert, etc) has declined by 33%, whereas domestic production of table and other special wines has increased by 20% and the domestic sparkling, dessert, and vermouth production has decreased by 23%.

BIBLIOGRAPHY

1. *International Review*, Vol. 71, Office International de la Vigne et Vin, Paris, 1998.
2. *U.S. Wine Stats* 2–16, Bureau of Alcohol, Tobacco and Firearms, Washington, D.C., 1997.

GENERAL REFERENCES

R. B Boulton et al., *Principles and Practices of Winemaking*, Chapman and Hall, New York, 1996.

B. G. Coombe and P. R. Dry, eds., *Viticulture; Vol. 1 Resources; Vol. 2 Practices*, Winetitles, Adelaide, Australia, 1988.

K. C. Fugelsang, *Wine Microbiology*, Chapman and Hall, New York, 1997.

K. C. Fugelsang, B. H. Gump, and F. S. Nury, *Wine Analysis and Production*, Chapman and Hall, New York, 1996.

R. Jackson, *Wine Science: Principles and Applications*, Academic Press, New York, 1994.

H. Johnson, *The World Atlas of Wine*, 4th ed., Simon & Schuster, New York, 1994

H. Johnson, *Hugh Johnson's Modern Encyclopedia of Wine*, 4th ed., Simon & Schuster, New York, 1998.

S. Koplan, B. H. Smith, and M. A. Weiss, *Exploring Wine. The Culinary Institute of America's Complete Guide to Wines of the World*, Van Nostrand Reinhold, New York, 1996.

C. S. Ough, *Winemaking Basics*, Food Products Press, New York, 1991.

C. S. Ough and M. A. Amerine, *Methods for Analysis of Musts and Wines*, 2nd ed., John Wiley and Sons, New York, 1988.

C. A. Rice Evans and L. Packer, eds., *Flavonoids in Health and Disease*, Marcel Dekker, New York, 1998.

J. Robinson, ed., *The Oxford Companion to Wine*, Oxford University Press, New York, 1994.

A. C. NOBLE
J. F. PFEIFFER
University of California
Davis, California

GENETIC ENGINEERING: ANIMALS

Animals in many capacities have been important components of human enterprise since prehistory, as food, shelter, transport, work, companionship, and, in more recent times, to determine safety and efficacy of therapeutics and as models to study disease. The development of the capacity to modify animals at the molecular level has expanded their roles, especially in the latter areas, and has added a new dimension to this compendium, "molecular pharming," the production of valuable products in milk.

For most of our long intertwined history, animals' most consistent contribution has been within the agricultural arena. With increased social awareness, we have begun to question and debate their role in this capacity, and with the advent of genetic engineering this has taken on a new level of complexity. This paper will provide a reasonable overview of the development and use of transgenic animals and will touch on the ethical and societal implications but will leave a more expanded analysis of these issues to more qualified reviewers.

New technologies have been applied in agriculture and food production as they evolved. Genetic engineering through the application of recombinant DNA methods and the use of DNA typing to aid traditional breeding are the new technologies currently having the greatest impact. In the latter half of the twentieth century, major improvements in agricultural productivity have been largely based on selective breeding programs for plants and animals; intensive use of chemical fertilizers, pesticides, and herbicides; advanced equipment developments; and widespread irrigation programs. This has been a very successful model at raising productivity, yet these improvements have brought corresponding problems of increasing uniformity in the genetic base of crop plants and domestic animals, pests resistant to chemical pesticides, adverse impacts on environmental quality, and capital-intensive production. In addition, from a global perspective, these advances have been the prerogative of more affluent regions. Farmers in developing countries have not had access to many of these technologies and capital-intensive methods of production. The emerging biotechnology revolution is stimulating hope that it will provide the basis for more sustainable agriculture.

The new aspects of biotechnology differ from previous agricultural technologies in a number of ways that are likely to be of significance. First, biotechnology can enhance productivity and product quality with minimal increase in production costs by providing tools to more effectively develop and incorporate desired characteristics of plants and animals. Second, biotechnology can provide more effective mechanisms for improving animal health. Third, biotechnology has the potential to conserve natural resources and improve environmental quality by reducing the dependency of chemical inputs through the development of natural fertilizers and of pest-resistant plants and by contributing to the functioning of biocontrol systems. Finally, biotechnology can provide modified organisms to degrade wastes and pollutants and for biomass conversion to reduce dependency on nonrenewable resources.

In agriculture, biotechnology in the form of recombinant DNA technology is a powerful assistant in traditional plant and animal breeding. Traditional breeding programs are time-consuming, labor-intensive, and limited to transfers of genes between closely related species. In addition, because the breeder has no control at the genome level, many undesirable traits can also be incorporated, such as increased disease susceptibility and slower growth. Recombinant DNA technology permits the precise and predictable manipulation of genes. Single traits can be modified much more quickly than was possible using traditional selection and breeding methods alone. Because of the capability of moving genes between species, desirable traits from one organism can be transferred to another.

There is no evidence that rDNA techniques or rDNA-modified organisms pose any unique or unforeseen environmental or health hazards. In fact, a National Research Council study found that "as the molecular methods are more specific, users of these methods will be more certain about the traits they introduce into plants." Greater certainty means greater precision in safety assessments.

In a world whose population is increasing at a rate that threatens to confirm Malthus's worst predictions, it is hard to envisage feeding and sustaining these numbers in a livable environment without the use of biotechnology. It is difficult to imagine a "promising alternative" to biotechnology and industrial agriculture that will sustain such numbers without catastrophic consequences.

The application of recombinant DNA technology to the modification of organisms was first demonstrated in 1972 when Paul Berg of Stanford used enzymes to paste two DNA strands together to form a hybrid circular molecule. This was the first recombinant DNA molecule. The following year Stanley Cohen and Annie Chang of Stanford University and Herbert Boyer of UCSF "spliced" sections of two different plasmids (an extra-chromosomal piece of DNA) cut with the same restriction enzyme, creating a plasmid with dual antibiotic resistance. They then transformed bacteria with the plasmid, thereby producing the first recombinant DNA organism.

Since that time, rapid advances have been made in the application of rDNA techniques to increasingly complex organisms, from bacteria and yeasts to plants and mammalian species. Transgenic animals have tremendous potential to act as valuable research tools in the agricultural and biological sciences. They can be modified specifically to address scientific questions that were previously difficult if not impossible to determine.

Though the first directed "engineering" of animals was selection of animals with desirable traits for breeding purposes, there is no doubt that the first scientific contribution to reproductive physiology in animals was the successful attempt to culture and transfer embryos in 1891. The development of artificial insemination helped with the costs and control of breeding, but the first technological shift came with Gurdon's 1970 transfer of a nucleus of a somatic adult frog cell into an enucleated frog ovum and the birth of viable tadpoles. This experiment was of limited success in that none of the tadpoles developed into adult frogs. In 1977 Gurdon expanded the field further through the transfer of messenger RNA (mRNA) and DNA into toad (*Xenopus*) embryos where he observed that the transferred nucleic acids were expressed (turned on) (1). Also in the 1970s, Ralph Brinster developed a now-common technique used to inject stem cells into embryos. When these embryos became adults, they produced offspring carrying the genes of the original cells. In 1982, Brinster with his colleagues gained further renown by transferring genes for rat growth hormone into mice under the control of a mouse liver-specific promoter and producing mice that grew into "supermice"—twice their normal size (2).

During the two years 1980 and 1981, there were several reported successes at gene transfer and the development of transgenic mice. Gordon and Ruddle first coined the term transgenic to describe animals carrying exogenous genes integrated into their genome (3). Since that time this definition has been extended to include animals that result from the molecular manipulation of endogenous genomic DNA, including all techniques from DNA microinjection to embryonic stem (ES) cell transfer and "knock-out" mouse production.

Notwithstanding the advent of successful nuclear transfer technology with the dawn of Dolly (the cloned sheep), the most widely used technique for the production of transgenic animals including mice is by microinjection of DNA into the pronucleus of a recently fertilized egg. Using various transgenic tools such as antisense technology (putting a reverse copy to switch off expression), it is now possible to add a new gene to the genome, increase the level of expression or change the tissue specificity of expression of a gene, and decrease the level of synthesis of a specific protein. An additional factor added by the new nuclear transfer technology is the capability of removing or altering an existing gene via homologous recombination. See Table 1 for different mechanisms of creating transgenic animals.

Since Palmiter's mouse, transgenic technology has been applied to several species, including agricultural species such as sheep, cattle, goats, pigs, poultry, and fish (Table 2). The applications for transgenic animal research fall broadly into two distinct areas, namely, medical and agricultural applications. The recent focus on developing animals as bioreactors to produce valuable proteins in their milk can be cataloged under both areas. Underlying each of these, of course, is a more fundamental application, that is, the use of those techniques as tools to ascertain the molecular and physiological bases of gene expression and animal development. This understanding can then lead to the creation of techniques to modify development pathways.

Table 1. Transgenic Technologies

Integration of retroviral vectors into an early embryo
Injection of DNA into the pronucleus of a newly fertilized egg
Incorporation of genetically manipulated embryonic stem cells into an early embryo
Incorporation of genetically manipulated primordial germ cells into an early embryo
Sperm delivery
Nuclear transplantation
Microprojectile injection

Table 2. Examples of Species to Which Transgenic Technology Has Been Applied

Mammals	Birds	Fish
Mice	Chickens	Salmon
Rats	Japanese quail	Trout
Rabbits		Talapia
Cattle		Carp
Pigs		Channel catfish
Sheep		Medaka
Goats		Zebrafish
		Loach
		Goldfish
		Northern Pike

Source: Ref. 4.

As noted by Pinkert and Murray, there are still fundamental limitations to the widespread use of transgenic technology in all animals except the mouse. Limitations include (*1*) lack of knowledge concerning the genetic basis of factors limiting production traits, (*2*) identification of tissue and developmentally specific regulatory sequences for use in developing gene constructs and expression vectors and in gene targeting, and (*3*) establishment of novel methods to increase efficiency of transgenic animal production. In medicine no doubt the most versatile animal model has been the mouse, and this especially applies because the technology has been perfected to customize the engineering of mice. Up until recently the principal advantage that the mouse held over all other species was the ability to isolate with relative ease ES cells, which remain pluripotent and amenable to engineering. ES cells are derived from the inner cell mass of the blastocyst formed during early embryogenesis. Distinguished from all other stem cells, they are pluripotent, able to develop into virtually any and all cells and tissues in the body; and, consistent with their expression of telomerase, self-renewing, a potentially limitless source of cells. One of the areas in which the mouse has been supreme is the ability, using these ES cells, to target with great specificity regions within chromosomes via what is termed homologous recombination. Using this method, researchers can (*1*) incorporate a novel foreign gene into a mouse genome, (*2*) modify an endogenous gene, or (*3*) delete a portion of a specific endogenous gene, creating a "loss-of-function" mutant, termed a "knock-out" mouse, from which to study phenotypic effects of inactivating genes. This flexibility makes the knock-out mouse a really good model from which to study disease and development in humans.

Up until November 1998, isolating ES cells in other mammals proved elusive, but in a milestone paper in the November 5, 1998, issue of *Science*, James A. Thomson, a developmental biologist at the University of Wisconsin–Madison, reported the first successful isolation, derivation, and maintenance of a culture of human ES cells (hES cells) (5). Obviously this is not the focus of this chapter, but it is interesting to note as an aside the leap made from mouse to man. As Thomson himself put it, these cells are different from all other human stem cells isolated to date, and as the source of all cell types, they hold great promise for use in transplantation medicine, drug discovery and development, and the study of human developmental biology.

The other great advance in the field of reproductive biology and methods for genetic engineering was Ian Wilmut's landmark work using nuclear transfer technology to generate the lambs Morag and Megan, reported in 1996 (from an embryonic cell nuclei), and the truly groundbreaking work of creating Dolly from an adult cell nucleus, reported in February 1997 (6). With the birth of Dolly, the sheep, Wilmut and his colleagues at the Roslin Institute demonstrated for the first time that the nucleus of an adult cell can be transferred to an enucleated egg to create cloned offspring. This technology supports the production of genetically identical and genetically modified animals. Thus, the successful "cloning" of Dolly has captured the imagination of researchers around the world. This technological breakthrough should play a significant role in the development of new procedures for genetic engineering in a number of mammalian species. It should be noted that nuclear cloning, with nuclei obtained from either mammalian stem cells or differentiated "adult" cells, is an especially important development for transgenic animal research. Since that time the clones have been arriving rapidly, with specific advances made by a Japanese group who used cumulus cells rather than fibroblasts to clone calves. They found that the percentage of cultured, reconstructed eggs that developed into blastocysts was 49% for cumulus cells and 23% for oviductal cells. These rates are higher than the 12% previously reported for transfer of nuclei from bovine fetal fibroblasts. Following on the heels of Dolly, Polly and Molly became the first genetically engineered transgenic sheep produced through nuclear transfer technology. Polly and Molly were engineered to produce human factor IX (for hemophiliacs) by transfer of nuclei from transfected fetal fibroblasts. Until then germline-competent transgenics had been produced in mammalian species, other than mice, only using DNA microinjection.

DNA microinjection has not been a very efficient mechanism to produce transgenic mammals. However, in November 1998, a team of Wisconsin researchers reported a nearly 100% efficient method for generating transgenic cattle. The established method of cattle transgenes involves injecting DNA into the pronuclei of a fertilized egg or zygote. In contrast, the Wisconsin team injected a replication-defective retroviral vector into the perivitelline space of an unfertilized oocyte. The perivitelline space is the region between the oocyte membrane and the protective coating surrounding the oocyte known as the zona pellucida.

In addition to ES cells, other sources of donor nuclei for nuclear transfer might be used such as embryonic cell lines, primordial germ cells, or spermatogonia to produce offspring. The utility of ES cells or related methodologies to provide efficient and targeted *in vivo* genetic manipulations offer the prospects of profoundly useful animal models for biomedical, biological, and agricultural applications. The road to such success has been most challenging, but recent developments in this field are extremely encouraging.

From Table 3 it can be seen that the development of transgenic animals has tremendous potential in medical research and therapeutic applications, including the creation of models to study disease, early development, aging, and specific gene function and the production of valuable proteins in milk (Tables 4 and 5). For example, pigs have been engineered as large-animal models for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. A novel use also includes the creation of engineered animals where the surface antigens of the organs, such as the heart, have been altered so that they can be used for transplantation without rejection by the recipient because the latter's immune system will not see the transplants as foreign.

AGRICULTURAL APPLICATIONS

Since 1985, transgenic farm animals harboring growth-related gene constructs have been created, although ideal growth phenotypes were not achieved because of an inability to coordinately regulate either gene expression or the ensuing cascade of endocrine events.

Using DNA microinjection, the types of genes and regulatory sequences introduced into livestock species become important considerations. To date, for agricultural species, the types of transgenes used fall into two main types, those encoding growth factors and those encoding proteins for expression in the mammary gland.

The work with growth factors was carried out in an attempt to alter the efficiency of meat production and alter the partitioning of nutrient resources toward increased lean production. In addition to the work with livestock transgenic for growth factor, considerable effort has been directed toward increasing the efficiency of wool growth in Australian sheep by insertion of the two bacterial or yeast genes required for sheep to synthesize *de novo* the sulfur amino acid cysteine.

Milk is one of the principal targets for engineering productivity traits. This includes altering the properties or proportions of caseins, lactose, or butterfat in milk of transgenic cattle and goats. Another focus is engineering

Table 3. Use of Transgenic Animals in Medicine

Animal models to study
Disease—cancer, cystic fibrosis, bovine spongiform encephalopathy (BSE), sickle cell anemia
Development—embryology, aging
Epigenetic changes—imprinting and telomere shortening
Gene targeting—gene function, gene therapy
"Pharming"—producing valuable proteins in milk
Xenotransplants—immunocompatible organs for transplantation from animals

Table 4. Human Proteins in Animals

Protein	Use	Animal(s)
α-1-anti-protease inhibitor	α-1-antitrypsin deeficiency	Goat
α-1-antitrypsin	Anti-inflammatory	Goat, sheep
Antithrombin III	Sepsis and disseminated intravascular coagulation (DIC)	Goat
Collagen	Burns, bone fracture, incontinence	Cow
Factor VII and IX	Hemophilia	Sheep, pig
Fibrinogen	"Fibrin glue," burns, surgery localized chemotherapeutic drug delivery	Pig, sheep
Human fertility hormones	Infertility, contraceptive vaccines	Goat, cow
Human hemoglobin	Blood replacement for transfusion	Pig
Human serum albumin	Burns, shock, trauma, surgery	Goat, cow
Lactoferrin	Bacterial gastrointestinal infection	Cow
LAtPA	Venous stasis ulcers	Goat
Monoclonal antibodies	Colon cancer	Goat
Tissue plasminogen activator	Heart attacks, deep vein thrombosis, pulomary embolism	Goat

Source: Table modified from GEN.

Table 5. Transgenic Animals and Protein Yields, Estimated Herd Size for Selected Proteins Produced in the Milk of Transgenic Animals

Protein	Estimated annual need (kg)	Estimated herd size	Animal
α-1-antitrypsin	5,000	33,000	Sheep
		42,000	Goat
Factor IX	2	13	Sheep
		22	Pig
Human serum albumin	1,000	300	Cow
		8,300	Goat

Note: The number of lactating animals may be calculated as (expression in g/L) × (annual milk production in L) × (purification efficiency)

Table 6. Use of Transgenic Animals in Agriculture

To create tissue-specific gene expression
To study development
To improve productivity
To increase disease resistance
To improve meat and milk quality
To produce valuable proteins in milk, blood, or urine
To preserve rare or threatened animals
To cross species barriers

for enhanced resistance to viral and bacterial diseases, including development of "constitutive immunity," or germline transmission of specific, modified antibody genes. Castilla et al. reported in the April 1998 issue of *Nature Biotechnology* the generation of transgenic mice that secrete virus-neutralizing antibodies in their milk (7). These antibodies were directed against transmissible gastroenteritis coronavirus (TGEV). TGEV infection is an important disease in swine that causes a mortality close to 100% in 3-week-old piglets and severe diarrhea in young pigs. Therefore, the development of transgenic sows that synthesize virus-neutralizing antibodies in their milk could reduce the serious effects resulting from TGEV infection in newborn swine.

Work on the directed expression of new proteins with pharmaceutical value to the mammary gland of cattle, goats, pigs, and sheep has been more successful. A number of pharmaceutically important proteins have been expressed in the mammary gland (see Table 4).

The production of transgenic farm animals is costly and time-consuming (Table 6). In mouse experiments, less than 2 months is required from the time the construct is ready for microinjection through weaning of baby mice. In contrast, for pig experiments, 1 month to a year is required for a sufficient number of DNA injections and recipient transfers to ensure the likelihood of success. In addition, the time frame from birth of a founder transgenic animal to the establishment of lines can be 1 to 2 years for pigs, sheep, and goats to 4 to 5 years for cattle. Neal First, UW-Madison, has come up with an ingenious mechanism for testing the feasibility of developing specific transgenics before heading down the expensive road of genetically engineering an entire animal. He first does a test run by using replication-defective retrovirus vectors to transiently express gene constructs in the mammary gland of animals. He has used an alpha virus containing a human growth hormone gene to produce human growth hormone in the milk of a goat. However, the transformed cells are sloughed off over time and the process needs to be repeated, but it does establish whether or not the construct will work.

Transgenic techniques have been developed for other vertebrate species in addition to mammals, including poultry and fish. In both instances, genetic selection is an exceedingly slow process. Because DNA microinjection into pronuclei of embryonic cells in poultry is not feasible, transfection using similar retroviruses to that used by Neal First has prevailed. As described, methods have included transfection of genes into cells of embryonic blastoderm, insertion of genes using replication-competent retroviruses, the use of replication-defective retroviruses, and sperm-mediated gene transfer. Although the last method has come under critical dispute, the other methods have led to the development of experimental models.

In contrast to poultry studies, work with fish has moved ahead with far greater speed. The principal area of research has focused on growth performance, and initial transgenic growth hormone (GH) fish models have demonstrated accelerated and beneficial phenotypes. DNA microinjection methods have propelled the many studies reported and have been most effective due to the relative ease of working with fish embryos. Bob Devlin's group in Vancouver has demonstrated extraordinary growth rate in coho salmon that were transformed with a growth hormone from sockeye salmon. The transgenics achieve up to 11 times the size of their littermates within 6 months, reaching maturity in about half the time. Interestingly, this dramatic effect is observed only in feeding pens where the transgenics' ferocious appetites demand constant feeding. If the fish are left to their own devices and must forage for themselves, they appear to be outcompeted by their smarter siblings.

DOMESTIC ANIMALS AS BIOREACTORS

The second general area of interest has been the development of lines of transgenic domestic animals for use as bioreactors. One of the main targets of these so-called "gene pharming" efforts has involved attempts to direct expression of transgenes encoding biologically active human proteins. In such a strategy, the goal is to recover large quantities of functional proteins that have therapeutic value from serum, urine, or the milk of lactating females. To date, expression of foreign genes encoding lysozyme, α-1-antitrypsin, tissue plaminogen activator, clotting factor IX, and protein C were successfully targeted to the mammary glands of goats, sheep, cattle, or a combination of these swine, (Tables 4 and 5). Similarly, lines of transgenic pigs and mice have been created that produce human hemoglobin or specific circulating immunoglobulins. The ultimate goal of these efforts is to harvest proteins from the serum of transgenic animals for use as important constituents of blood transfusion substitutes, or for use in diagnostic testing.

THE FUTURE

There is no question that the use of transgenic animals in medical research has already made contributions in helping us understand physiological processes. It shows much promise for the future in providing tools to elucidate the mechanisms of development, disease, and aging; to develop effective therapeutics, including gene therapy, and effective approaches to disease prevention and health maintenance; and to generate animals for the production of valuable proteins and modified organs for xenotransplantation. The recent acquisition of Roslin Bio-Med by Geron juxtaposes three of the major advances in this field in the last 2 years, namely, nuclear transfer technology; pluripotent stem cell, and telomerase, which allows cells to remain viable. The combined technologies are expected to enhance and accelerate the development of new transplantation therapies for numerous degenerative diseases such as diabetes, Parkinson's disease, cancer, and heart disease.

On the agriculture end, however, while transgenic animal technology continues to open new and unexplored frontiers, it also raises questions concerning regulatory and commercialization issues as demonstrated by molecular farming efforts. A number of major regulatory and public perception hurdles exist that may affect the time to commercialization of transgenic animals. These include safety issues related to food produced from genetically engineered animals and ethical considerations such as animal welfare. So the commercialization of genetically engineered animals in agriculture will have several hurdles to overcome on the way to market, including technical, regulatory, and consumer perception. Considering the long and winding path that genetically modified crops took on the road to acceptance, it is expected that for animals the path will be even more convoluted and fraught with obstacles. The potential is so great, however, that I remain optimistic that the future looks bright.

BIBLIOGRAPHY

1. J. B. Gurdon, "Nuclear Transplantation and Gene Injection in Amphibia," *Brookhave Symposia in Biology* **29**, 106–115 (1977).
2. R. D. Palmiter et al., "Dramatic Growth of Mice that Develop From Eggs Microinjected with Metallothionein-Growth Hormone Fusion Genes," *Nature* **300**, 611–615 (1982).
3. J. W. Gordon and F H. Ruddle, "Integration and Stable Germline Transmission of Genes Injected into Mouse Pronuclei," *Science* **214**, 1244–1246 (1981).
4. C. A. Pinkert and J. D Murray, "Transgenic Farm Animals," in J. D. Murray et al., eds., *Transgenic Animals in Agriculture*, CABI, London, 1999, pp. 1–18.
5. J. A. Thomson et al., "Embryonic Stem Cell Lines Derived from Human Blastocysts," *Science* **282**, 1145–1147 (1998).
6. I. Wilmut et al., "Viable Offspring Derived from Fetal and Adult Mammalian Cells," *Nature* **385**, 810–813 (1997).
7. J. Castilla et al., "Engineering Passive Immunity in Transgenic Mice Secreting Virus-Neutralizing Antibodies in Milk," *Nature Biotech.* **16**, 349–354 (1998).

GENERAL REFERENCES

R. L. Brinster, "Stem Cells and Transgenic Mice in the Study of Development," *Int. J. Dev. Biol.* **37**, 89–99 (1993).

M. R. Cappechi, "Altering the Genome by Homologous Recombination," *Science* **244**, 1288–1292 (1989).

A. W. S. Chan et al., "Transgenic Cattle Produced by Reverse-Transcribed Gene Transfer in Oocytes," *Proc. Natl. Acad. Sci.* **95**, 14028–14033 (1998).

R. A. Dunham, and R. H. Devlin, "Comparison of Traditional Breeding and Transgenesis in Farmed Fish with Implications for Growth Enhancement and Fitness," in J. D. Murray et al., eds., *Transgenic Animals in Agriculture*, CABI, London, 1999, pp. 209–230.

R. E. Hammer et al., "Production of Transgenic Rabbits, Sheep and Pigs by Microinjection," *Nature* **315**, 680–683 (1985).

Y. Kato et al., "Eight Calves Cloned from Somatic Cells of a Single Adult," *Science* **282**, 2095–2098 (1998).

E. A. Maga and J. D. Murray, "Mammary Gland Expression of Transgenes and the Potential for Altering the Properties of Milk," *Bio/Technol.* **13**, 1452–1457 (1995).

M. M. Perry and H. M. Sang, "Transgenesis in Chickens," *Transgenic Res.* **2**, 125–133 (1993).

R. M. Petters et al., "Genetically Engineered Large Animal Model for Studying Cone Photoreceptor Survival and Degeneration in Retinitis Pigmentosa," *Nature Biotechnol.* **15**, 965–970 (1997).

C A. Pinkert, "Transgenic Pig Models for Xenotransplantation," *Xeno* **2**, 10–15 (1994).

C. A. Pinkert, "The History and Theory of Transgenic Animals," *Lab Anim.* **26**, 29–34 (1997).

C. A. Pinkert, M. H. Irwin, and R. J. Moffatt, "Transgenic Animal Modeling," in R. A. Meyers, ed., *Encyclopedia of Molecular Biology and Molecular Medicine*, Vol. 6, VCH Publishers, New York, 1997, pp 63–74

V. G. Pursel and C. E Rexroad, Jr., "Status of Research with Transgenic Farm Animals," *J. Anim. Sci.* **71** (Suppl. 3), 10–19 (1993).

V. G. Pursel et al., "Genetic Engineering of Livestock," *Science* **244**, 1281–1288 (1989).

G E Rogers, "Improvement of Wool Production through Genetic Engineering," *Trends Biotechnol.* **8**, 6–11 (1990).

A. E. Schnieke et al., "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts," *Science* **278**, 2130–2133 (1998).

R. M. Shuman, "Production of Transgenic Birds," *Experientia* **47**, 897–905 (1991)

K. R. Thomas and M. R. Capecchi, "Site-Directed Mutagenesis by Gene-Targeting in Mouse Embryo-Derived Stem Cells," *Cell* **51**, 503–512 (1987).

R. J Wall, H. W Hawk, and N. Nel, "Making Transgenic Livestock: Genetic Engineering on a Large Scale," *J. Cellular Biochem.* **49**, 113–120 (1992).

S. A. Wood et al., "Simple and Efficient Production of Embryonic Stem Cell–Embryo Chimeras by Coculture," *Proc. Natl. Acad. Sci.* **90**, 4582–4585 (1993).

MARTINA MCGLOUGHLIN
University of California
Davis, California

NUTS

Since ancient times, nuts and seeds have been a staple in many healthy diets. Worldwide, these foods have been consumed in main dishes, in desserts, and alone as snacks. Nuts contain a variety of nutrients and are important in a healthy diet, especially for those who are consuming mainly plant-based diets.

Nuts are classified as either tree nuts or peanuts. Tree nuts are actually a one-seeded fruit in a hard shell. Tree nuts include cashews, almonds, hazelnuts, Brazil nuts, macadamia nuts, pecans, pine nuts, walnuts, and pistachio nuts.

WHERE THE SCIENCE BEGAN

Many large population studies show that the evidence linking frequent peanut and nut consumption to a 25 to 50% reduced risk of heart disease is strong. A landmark study in 1992 at Loma Linda University examined the diets of approximately 27,000 Seventh-Day Adventists. This study was one of the first to suggest that nut eaters are a healthy group. Researchers looked at the relationship between 65 different foods and coronary heart disease. Out of all the foods studied, nuts by far had the strongest protective effect on the risk of having a heart attack or dying of heart disease. People eating nuts more than 5 times a week decreased their risk of heart disease by over 50%; those eating nuts 1 to 4 times a week decreased their risk by 27% (1). A further diet analysis showed that 32% of the nuts consumed by study participants were peanuts.

Participants enjoyed a wide variety of nuts, including peanuts, almonds, and walnuts. They ate them out of hand as a snack, in fruit shakes, and cooked in food. Interestingly, the participants who consumed nuts were significantly thinner than the rest of the group. Though this does not prove that eating nuts makes us thinner, it shows that it does not necessarily make us obese.

This study was validated by the Iowa Women's Study, which looked at the eating habits of 34,000 women. Over a 5-year period, the researchers found an inverse relationship between nut consumption and death from heart disease. The scientists determined that women who ate nuts were only 40% as likely to die from heart disease as those who never ate nuts (2).

The DASH diet, an eating plan that has been clinically proven to lower blood pressure in hypertensive men and women, may also be effective in preventing hypertension, according to a study in the *New England Journal of Medicine* (3). Although the DASH diet is an entire eating plan, a significant element is that it recommends eating 4 to 5 servings from the nuts, seeds, and legumes group each week.

Another study, in Australia, found that people who consumed diets rich in monounsaturated fats, mainly from peanuts, experienced a larger decline in LDL cholesterol as compared to the low-fat diet. Instead of raising triglyceride levels as on the low-fat diet, the peanut-enriched-diet eaters lowered their triglyceride levels (4).

Researchers note the favorable fatty acid profile of peanuts and nuts—low in saturated fat and high in monounsaturated fats (MUFA) and polyunsaturated fats—as one possible explanation for their protection against heart disease

More recent research has further confirmed the relationship between consuming nuts and preventing heart disease. In the Nurses' Health Study published in the November 1998 *British Medical Journal*, researchers at Harvard School of Public Health found that "frequent consumption of peanuts and nuts was associated with a lowered risk of coronary heart disease (CHD)" (5). This is one of the most prestigious research studies, looking at the dietary habits of more than 86,000 female nurses. Those who ate more than 5 servings of nuts, peanuts, and peanut butter each week decreased their risk of heart disease by about one-third, compared to women who rarely or never ate nuts.

Preliminary results of the Physicians' Health Study, presented at the American Heart Association 1998 Scientific Sessions, found similar results. In a group of 22,000 male physicians, the risk of cardiac death and sudden death decreased as nut consumption increased (6).

Different types of clinical studies using a variety of nuts, such as peanuts, macadamia nuts, and walnuts, demonstrate that consumption of nuts can lower LDL cholesterol levels and possibly reduce the risks contributing to both fatal and nonfatal coronary heart disease (2,7–10).

PEANUTS IN A WEIGHT-LOSS DIET

Penny Kris-Etherton, professor of nutrition at Pennsylvania State University, found that a weight-loss diet high in MUFA provided by peanuts and peanut butter not only lowered cholesterol but also helped subjects lose weight. Participants on both low-fat (less than 20% calories from fat) and higher-fat (35% of calories from fat, which was mainly monounsaturated) diets lost an average of 2 lb per week (11). Both diets lowered total and LDL cholesterol, known risk factors for cardiovascular disease.

In this study, the healthy MUFA was added to the diets by using peanut butter instead of butter and jam on bagels and toast; snacking on peanuts instead of pretzels, cookies, or crackers, using peanut butter in sandwiches instead of lean luncheon meats; and using peanut-based dressing with salads and vegetables.

According to Kris-Etherton, "Consumers should understand that 'fat-free' does not always translate into weight loss and that healthy diets can include favorite foods, such as peanuts and peanut butter, while promoting weight loss and weight maintenance."

NUTS HELP PEOPLE STICK TO WEIGHT-LOSS DIETS

A real-life example of the benefits of adding peanuts and peanut butter to a healthy diet is a study conducted by Frank Sacks and Kathy McManus at Harvard Medical School and Brigham and Women's Hospital in Boston. Almost 3 times as many people were able to stick to a higher-fat diet that included peanuts and peanut butter during an 18-month free-living weight-loss study (12). One hundred one overweight men and women were assigned to either of two weight-loss diets: (*1*) a low-fat diet and (2) a higher MUFA "Mediterranean-style" diet.

After 18 months, the researchers found that subjects on the Mediterranean-style diet fared better throughout the study period. Those on the higher-fat Mediterranean-style diet were able to lose an average of 11 lb each and maintain their weight loss, whereas those following the low-fat diet gained almost half of the lost weight back, resulting in only a 6-lb weight loss. In addition, more than 80% of the participants on the low-fat diet dropped out of the study compared to fewer than half (46%) of the subjects on the higher-fat diet (12).

The Mediterranean-style diet included foods high in heart-healthy MUFA such as peanuts, peanut butter, peanut oil, nuts, avocados, and olive oil on a daily basis. When allowed to choose their own MUFA-rich foods, subjects consistently chose peanuts, peanut butter, and mixed nuts, favorite foods that used to be on a dieter's "forbidden" list.

Interestingly, those on the higher-fat diet ate more vegetables, fiber, and protein. This may be due to the fact that Mediterranean dishes typically consist of many vegetables and whole grains, sprinkled with peanuts and nuts or drizzled with peanut or olive oil to emphasize palatability.

McManus, manager of clinical nutrition at Brigham and Women's Hospital, adds, "As obesity becomes an epidemic in America, it is more important than ever to identify eating patterns that can not only promote weight loss, but help sustain that weight loss for a lifetime. Patients were delighted to be able to eat peanuts and peanut butter again" (12).

PEANUTS AND NUTS SATISFY HUNGER

Rick Mattes, a satiety expert at Purdue University, examined why peanuts and peanut butter may help people stick to weight-loss programs. Mattes and collaborator Corinna Lermer found that when 500 cal of peanuts were added to participants' regular diets, substituted in the diet for other fat, or eaten freely, the results were the same—the men and women automatically compensated for most of the additional calories, and they spontaneously commented on the high satiety of the peanuts (13). Those who either added peanuts to their regular diets or substituted peanuts for other fats had the added benefit of significantly lowering their triglyceride levels (TG), a known risk factor for cardiovascular disease (CVD).

Mattes notes, "Regular consumption of small amounts of peanuts does not necessarily lead to weight gain and may contribute to a healthy, more satisfying diet" (13).

NUT AND PEANUT EATERS HAVE BETTER BODY PROFILES

A study conducted by Brenda Eissenstat at Penn State found that the body mass index (BMI), a measure health professionals use to evaluate body weight, of peanut eaters was more favorable than that of non–peanut eaters (14). In addition, peanut eaters consistently tend to have higher levels of key nutrients and overall healthier diets than their non–peanut eating counterparts.

Additionally, a number of studies in the United States, Israel, and Australia have demonstrated the positive effects of almonds and other nuts on heart disease and cardiovascular disease risk factors. When suggesting that nuts can be a useful food for the population in terms of cardiovascular health, a common question is whether they will make people fat. In a growing body of evidence on the subject, it is interesting to note that in the large population studies, frequent consumers of nuts were more successful at maintaining their body weight than those who never ate nuts. Also, in studies investigating almonds' effects on blood cholesterol, participants consumed up to 3 oz of almonds a day, yet no one gained weight, despite the higher fat intake (15).

PEANUTS AND NUTS DECREASE THE RISK OF A SECOND HEART ATTACK

Lisa Brown at Harvard School of Public Health examined the eating patterns of participants in the Cholesterol and Recurrent Events (CARE) trial. People who ate 2 or more servings of peanuts or nuts a week decreased their risk of having another heart attack by 25% (16). Brown found that a healthy diet that includes peanuts and nuts can have benefits above and beyond those provided by drugs alone. This study gives further credibility to the diet and disease prevention link.

NUTRIENTS RECENTLY FOUND IN PEANUTS AND NUTS

Researchers at the United States Department of Agriculture (USDA) Agricultural Research Service at North Carolina State University have identified resveratrol, the phytochemical in red wine, in peanuts (17). The "French paradox" associates resveratrol's presence in red wine with reduced cardiovascular disease and refers to the fact that, despite consuming a relatively high-fat diet, the French have surprisingly low rates of heart disease.

Peanuts also contain other plant chemicals, such as phytosterols and isoflavones. A study at the State University of New York at Buffalo identified an important phytochemical, beta-sitosterol (SIT), in peanuts and peanut products. SIT has previously been shown to inhibit cancer growth, especially prostate cancer. Atif Awad examined the SIT content of peanuts, peanut butter, peanut flour, and peanut oil and found that the content varied from 44 mg SIT/100 g (peanut flour) to 191 mg SIT/100 g (unrefined peanut oil). Snack peanuts contain 160 mg SIT/100 g and peanut butter contains approximately 120 mg SIT/100 g (18). The amount of protective SIT in unrefined peanut oil is comparable to that of soybean oil (183 mg SIT/100 g).

In addition to phytochemicals, peanuts contain significant amounts of heart-healthy MUFA, plant protein, fiber, magnesium, and vitamin E, all of which may contribute to their healthfulness. Many hard-to-get nutrients such as copper, phosphorus, potassium, and zinc are also found in peanuts and peanut butter. All of these, plus the many plant sterols and phytochemicals that are still being discovered in peanuts, make them a complex food with many health benefits.

PEANUTS

Though many people think of peanuts as nuts, they are actually legumes and belong to the *Leguminosae* family. They are used in diets and cuisines as nuts, but their physical structure and nutritional benefits more closely resemble those of other legumes, such as beans.

History

The peanut, though grown in tropical and subtropical regions throughout the world, is native to the Western Hemisphere. It probably originated in South America and spread throughout the New World as Spanish explorers discovered the peanut's versatility. When the Spaniards returned to Europe, peanuts went with them. Later, traders were responsible for spreading peanuts to Asia and Africa. The peanut made its way back to North America during the slave trading period. Although there were some commercial peanut farms in the United States during the 1700s and 1800s, peanuts were not extensively grown. This lack of interest in peanut farming is attributed to the fact that the peanut was regarded as food for the poor and to the slow, difficult growing and harvesting techniques. Until the Civil War, the peanut remained a regional food associated with the southern United States.

After the Civil War, the demand for peanuts increased rapidly. By the end of the nineteenth century, the development of equipment for production, harvesting, and shelling peanuts, as well as processing techniques, contributed to the expansion of the peanut industry. The new twentieth-century labor-saving equipment resulted in a rapid demand for peanut oil, roasted and salted peanuts, peanut butter, and confections.

Also associated with the expansion of the peanut industry is the research of George Washington Carver at Tuskegee Institute in Alabama at the turn of the century. The talented botanist recognized the intrinsic value of the peanut as a cash crop. Carver proposed that peanuts be planted as a rotation crop in the southeastern cotton-growing areas where the boll weevil threatened the region's agricultural base. Not only did Carver contribute to changing the face of southern farming, he also developed more than 300 uses for peanuts, mostly for industrial purposes.

Early Legislative Action

As peanut consumption continued to rise, the U.S. government instituted programs in 1934 to regulate the acreage, production, and price of this food item. Federal government production controls were lifted during World War II to meet the heavy demand for fats and oils required for the U.S. war effort. Controls were reestablished in 1949, and in 1977 a two-tier price-support system was initiated. This system has subsequently been revised, most recently by the 1995 Farm Bill.

Peanut Types and Production

Seven states account for approximately 98% of all peanuts grown in the United States: Georgia (37.7%) grows the major proportion of all peanuts, followed by Texas (23.2%), Alabama (10.5%), North Carolina (9.3%), Florida (6.4%), Virginia (5.4%), and Oklahoma (5.2%). There are approximately 40,000 peanut farms in the major producing regions. (Percentages are based on 1997 production of quota and nonquota peanuts.) The peanut-growing regions of the United States have direct access to port facilities of the Atlantic Ocean and the Gulf of Mexico. The United States produces four basic varieties of peanuts: runner, Virginia, Spanish, and Valencia. Each type is distinctive in size and flavor.

Runners

Runners have become the dominant type due to the introduction in the early 1970s of a new runner variety, the Florunner, which was responsible for a spectacular increase in peanut yields. Runners have rapidly gained wide acceptance because of their attractive kernel size range; a high proportion of runners are used for peanut butter. Runners, grown mainly in Georgia, Alabama, Florida, Texas, and Oklahoma, account for 73% of total U.S. production.

Virginia

Virginia have the largest kernels and account for most of the peanuts roasted and eaten as in-shells. When shelled, the kernels are sold as salted peanuts. Virginias are grown mainly in southeastern Virginia, northeastern North Carolina, and western Texas. Virginia-type peanuts account for about 22% of total U.S. production.

Spanish

Spanish-type peanuts have smaller kernels covered with a reddish-brown skin. They are used predominantly in peanut candy, although significant quantities are also used for salted nuts and peanut butter. They have a higher oil content than the other types of peanuts, which is advantageous when crushing for oil. Grown primarily in Oklahoma and Texas, Spanish-type peanuts account for 4% of U.S. production.

Valencia

Valencias usually have three or more small kernels to a pod. They are very sweet peanuts and are usually roasted and sold in the shell; they are also excellent for fresh use as boiled peanuts. Because of the greater demand for other varieties, Valencias account for less than 1% of U.S. production and are grown mainly in New Mexico.

Growing

Peanuts are the seeds of an annual legume that grows close to the ground and produces its fruit below the soil surface. U.S. peanuts are planted after the last frost in April or May when soil temperatures reach 65 to 70°F (20°C). Preplanting tillage ensures a rich, well-prepared seedbed. Seeds are planted about 2 in. (5 cm) deep, one every 2 to 4 in. (5–10 cm) in the Southeast, and 4 to 6 in. (10–15 cm) in the Virginia–Carolina area, in rows about 3 ft (1 m) apart. The row spacing is determined to a large extent by the type of planting and harvesting equipment utilized.

Cultivating

Peanuts may be cultivated up to three times, depending on the region, to control weeds and grasses. A climate with approximately 200 frost-free days (175 for Spanish peanuts) is ideal for a good crop. Warm weather conditions, coupled with rich, sandy soil, will result in the appearance of peanut leaves 10 to 14 days after the first planting. Farmers generally follow a 3-yr rotation pattern with cotton, corn, or small grains planted on the same acreage in intervening years to prevent disease. In addition, many farmers are using irrigation in an effort to reduce crop stress and thereby enhance opportunities for the production of high-quality peanuts.

Harvesting

The peanut-harvesting process occurs in two stages. Digging, the first stage, begins when about 70% of the pods have reached maturity. At optimum soil moisture, a digger proceeds along the rows of peanut plants, driving a horizontal blade 4 to 6 in. (10–15 cm) under the soil. The digger loosens the plant and cuts the taproot. A shaker lifts the plant from the soil, gently shakes the soil from the peanuts, and inverts the plant, exposing the pods to the sun in a windrow. The peanuts are now ready for the second phase of the harvest—curing. After they cure in the field for 2 or 3 days, a combine separates the pods from the vines, placing the peanut pods into a hopper on the top of the machine. The vine is returned to the field to improve the soil fertility or baled into hay for livestock feed. Freshly dug peanuts are then placed into drying wagons for further curing, with forced hot air slowly circulating through the wagons. In this final stage of the curing process, moisture content is reduced to 8 to 10% for safe storage.

Shelling and Grading

After proper curing, farmers' stock peanuts (harvested peanuts that have not been shelled, cleaned, or crushed) are inspected and graded to establish the quality and value of the product. The inspection process determines the overall quality and on-farm value of the shelled product for commercial sales and price-support loans.

The inspection and grading of peanuts by the Agricultural Marketing Service (AMS) of the USDA occur at buying stations or shelling plants usually located within a few miles of where the peanuts have been harvested. A pneumatic sampler withdraws a representative quantity of peanuts from the drying wagon, and from this sample the USDA inspector determines the meat content, size of pods, damaged kernels, foreign material, and kernel moisture content. Once the grade is established, the loan value is determined from USDA price-support schedules.

Peanuts are separated into three classifications at this farmers' stock marketing and grading stage: segregation I, segregation II, or segregation III. These classifications based on USDA grades are mainly concerned with the amount and type of damage in the kernels. Peanut shelters can buy only segregation I for use in edible products. Those peanuts not classified as segregation I are crushed for oil.

Segregation 1 peanuts move on to the shelling process, where they are first cleaned; stones, soil, bits of vines, and other foreign material are removed. The cleaned peanuts move by conveyor belt through shelling machines in which the peanuts are forced through perforated grates that separate the kernels from the hulls. Shakers separate the kernels and the pods. The kernels are then passed over the various screens where they are sorted by size into market grades. The edible nuts are individually inspected with electronic eyes that eliminate discolored or defective nuts as well as any remaining foreign material.

In-shell peanuts are usually produced from large Virginia-type peanuts or Valencias that have been grown in light-colored soil. Very immature and lightweight pods are removed by vacuum. The largest remaining pods are separated into size categories by screens. Stems are removed and any remaining immature pods are removed by specific gravity. Dark or damaged pods are then removed by electronic eyes so that only the most mature, brightest pods remain.

Manufacturing

Unlike in other countries where the end products are peanut oil, cake, and meal, the prime market for U.S. peanuts is edible consumption. Only 15% of U.S. production is normally crushed for oil. U.S. per capita consumption is approximately 6.5 lb per person (excluding crushing).

Peanut Butter

Peanut butter is one of America's favorite foods. Found in about 75% of American homes, peanut butter is considered by many to be a staple like bread and milk. By law, in the U.S., any product labeled "peanut butter" must be at least 90% peanuts. The remaining 10% may include salt, sweetener, and stabilizers that prevent the peanut oil from separating and rising to the top. About 40% of the U.S. peanut crop is used to make peanut butter. The peanuts are roasted and cooled, then the skins are removed. The kernels are inspected again and then ground, usually through two grinding stages, to produce a smooth, even-textured butter. The peanut butter is heated to about 170°F during grinding. After the stabilizer is added, the peanut butter is rapidly cooled to 120°F or below. This crystallizes the stabilizer, thus trapping the peanut oil that was released by the grinding. Peanut granules can be added at this point to make crunchy peanut butter.

Snack Peanuts

Americans consume more than 300 million lb of snack peanuts each year. Most snacking peanuts are shelled and blanched (to remove the skins) before roasting. Peanuts can be oil-roasted in continuous cookers that take a steady stream of peanuts through hot oil for about 5 min or dry-roasted in a large oven with dry, hot forced air. About 10% of the U.S. crop is sold as in-shell peanuts—usually the large Virginia and long Valencia types. The peanuts are conveyed over sizing screens that let the smaller pods fall through for shelling. In this way, only the largest pods are sold as in-shell peanuts. The peanuts are then roasted and packaged for sale. In-shell peanuts often are roasted with salt and occasionally with spicy seasonings.

Confectionery

The confectionery industry uses about 25% of the U.S. crop to make candy. Five of the eight top-selling chocolate confections contain peanuts or peanut butter. Peanut butter and chocolate seem to be one of America's favorite pairs.

Peanut Oil

Peanut kernels range in oil content from about 43 to 54%, depending on the variety of peanut and seasonal growing conditions. Peanuts supply one-sixth of the world's vegetable oil. Oil is extracted from shelled and crushed peanuts by one or a combination of the following methods: hydraulic pressing, expeller pressing, and solvent extraction. Peanut oil is an excellent-quality cooking oil with a high smoke point (440°F) and neutral flavor and odor. It allows food to cook very quickly with a crisp coating and little oil absorption. Peanut oil is liquid at room temperature. Highly aromatic 100% peanut oil and peanut extract are high-value products with a strong roasted peanut flavor and nut aroma. These products have applications in flavor compounds, confections, sauces, and baked goods.

Peanut Flour

Roasted and naturally processed to obtain a strong roasted peanut flavor, partially defatted peanut flour works well as a fat binder in confectionery products or to add flavor and extend shelf life. Peanut flour is also high in protein (40–45%).

Export Markets

World peanut exports are approximately 1.3 million t (shelled basis). The major suppliers to the export market are the United States, China, and Argentina. Although U.S. peanuts represent approximately 10% of world peanut production, the United States has become one of the leading world exporters, accounting for about one-fourth of world peanut trade. Other producers such as India, Vietnam, and several African countries, periodically enter the export market, depending on their crop quality and world market demand.

Sixty percent of U.S. raw peanut exports are destined for the European Union (EU). The major markets for peanuts within the EU are the United Kingdom, the Netherlands (which serves as the primary port of entry for peanuts), and Germany.

Demand in Europe for peanuts has been steady, although competition within a dynamic snack market has put considerable pressure on peanuts to compete with a growing range of products (potato chips, extruded products, pretzel sticks, etc). In addition, quality standards and import requirements continue to tighten, requiring the implementation of improved monitoring and quality-control standards at origin.

Exports of processed peanuts and specialty peanut products have steadily increased, representing approximately 25% of total U.S. peanut exports by value. The largest U.S. export market for processed peanut butter is Saudi Arabia, followed by Canada, Japan, Germany, and Korea. Major snack nut markets are the Netherlands, Spain, the United Kingdom, France, and Germany.

U.S. Quality Control and Research

Consumers throughout the world are concerned about consistently obtaining flavorful, wholesome peanuts that are uniform in size and free from foreign material and contamination. The U.S. peanut industry continues to invest heavily in plant modernization and the latest designs in automated cleaning and sorting equipment to ensure that all buyers receive the best possible product. U.S. government inspectors monitor processing at each stage of the peanut's journey from the farm to the manufacturer—and to the grocery shelf in the case of domestic production.

Marketing Agreement

In 1965, the AMS/USDA, the Food and Drug Administration, and the U.S. peanut industry initiated a cooperative quality-control program to prevent aflatoxin-contaminated peanuts from entering food channels. Under the provisions of the agreement, peanuts are subject to strict quality standards that are enforced by USDA and federal and state inspectors. These inspectors supervise, inspect, and grade peanuts from delivery at buying points to shipment from shelling plants. Strictly enforced government regulations ensure that U.S. peanuts are of consistently high quality. In addition, comprehensive lot identification systems enable peanuts to be tracked throughout their various stages of processing until final delivery to a domestic processor or upon export.

Industry Research

Due to the emphasis on production of edible peanuts, both the government and the U.S. industry allocate a considerable amount of time and money to peanut research to produce a high-quality food item. Government- and industry-funded peanut research provides farmers with the latest information on improving quality and yields. Since the late 1950s, peanut yields have tripled as a result of new varieties, advanced agronomic technology, irrigation, and totally mechanized operations.

CASHEWS

The cashew fruit consists of two distinct parts: a fleshy stalk in the form of a pear—also called the cashew apple—with a brilliant yellow or red skin that can measure from 5 to 10 cm; and a gray-brown-colored nut (the cashew) in the shape of a kidney, which hangs from the lower end of the stalk, or apple. The nut section of the cashew fruit is very rich in carbohydrates and vitamin A.

Cashews are believed to have originated in the northeast of Brazil, near the equator. It is likely that Spanish sailors first introduced the cashew to Central America in the sixteenth century. Later, Portuguese colonists brought cashews to territories in East Africa (Mozambique) and India (Goa), where its cultivation extended to Indonesia and the Philippines. Today the principal cashew-producing countries are Brazil, India, and Mozambique. Juices, syrups, preserves, wine, and liquors are obtained from the stalk or apple. However, the main commercial use is the cashew nut itself. Cashews are marketed in the shelled, roasted, and salted forms for use as a snack and as an ingredient (delicacies, chocolate, etc).

ALMONDS

Unlike other flowering fruit trees that bear edible fruit, the almond tree's "pearl" is the delicious nut found inside the fruit.

History

The exact ancestry of almonds is unknown, but they are thought to have originated in China and central Asia. Explorers ate almonds while traveling the Silk Road between Asia and the Mediterranean. Before long, almond trees flourished in the Mediterranean, especially in Spain and Italy. The almond tree was brought to California from Spain in the mid-1700s by the Franciscan padres. The moist, cool weather of the coastal missions, however, did not provide optimum growing conditions. It wasn't until the following century that trees were successfully planted inland. By the 1870s, research and crossbreeding had developed several of today's prominent almond varieties. By the turn of the twentieth century, the almond industry was firmly established in the Sacramento and San Joaquin areas of California's great Central Valley.

Throughout history, almonds have maintained religious, ethnic, and social significance. The Bible's Book of Numbers tells the story of Aaron's rod that blossomed and bore almonds, giving the almond the symbolism of divine approval. They were a prized ingredient in breads served to Egypt's pharaohs. The Romans showered newlyweds with almonds as a fertility charm. Today, Americans give guests at weddings a bag of sugared almonds, representing children, happiness, romance, good health, and fortune. In Sweden, cinnamon-flavored rice pudding with an almond hidden inside is a Christmas custom. Find it, and good fortune is yours for a year.

Marketing

The principal almond-producing countries are the United States, Spain, Italy, Portugal, Morocco, Tunisia, and Turkey. Almonds are marketed in various forms: in-shell, shelled, blanched, slivered, chopped, flour, roasted, sweetened, and salted. They are used as a snack, in confectionery (marzipan, *turron*, nougat, etc), in food products (almond milk, ice cream, chocolate), in culinary recipes, and also as a cosmetic base.

Growing

In the fall, flower parts begin to develop on the edges of the growing bud. By mid-December, pollen grains are present. The tiny bud remains dormant until early January, when it grows rapidly. A good chill during November and December followed by a warmer January and February coax the first almond tree blossoms from their buds. Because the almond tree is not self-pollinating, at least two different varieties of trees are necessary for a productive orchard. Bees pollinate alternating rows of almonds varieties. From February onward, orchards should be frost-free, having mild temperatures (55–60°F) and minimal rain so blossoms can flourish and bees can do their job.

After the petals drop and the trees have leafed out, the first signs of the fuzzy gray-green fruit appear. The hull continues to harden and mature, and in July it begins to split open. Between mid-August and late October, the split widens, exposing the shell, which allows the kernel (nut) to dry. The whole nut and stem finally separate and, shortly before harvest, the hull opens completely.

Harvest

State-of-the-art technology is used to ensure the highest-quality almonds. California's growing and sanitary standards lead the world, both in the field and in the almond-processing plant. To prepare for harvest, orchard floors are swept and cleared. Mechanical tree shakers knock unshelled nuts to the ground, where they are allowed to dry before they are swept into rows and picked up by machine. Finally, they are transported to carts and towed to the huller.

Packaging

At the processing plant, a random sample of almond shells are cracked open and the nuts inside are graded according to size and quality. Almonds are inspected to make sure they are whole, clean; well dried; and virtually free from decay, rancidity, insects, foreign matter, mold, and any kind of breakage or blemish. Almonds are then processed and packed to specification in an assortment of sizes and shapes. Stored properly at 40°F with low humidity, almonds have a shelf life of up to 3 years.

RESEARCH

Vitamin E (technically called tocopherol) is a strong antioxidant that has been associated with reduced risks of developing prostate cancer and coronary heart disease. The term vitamin E, however, does not refer to a single compound. Rather it refers to a category of compounds with similar, but not identical, biological functions. Stone performed a chemical analysis of vitamin E as it appears in almonds. He found that almonds are an excellent source of natural alpha-tocopherol, the most biologically active form of the various vitamin E compounds. A single 1-oz serving of almonds supplies approximately 10 IU (66%) of the recommended dietary allowance (RDA) for men and 83% of the RDA for women. Almonds also contain gamma-tocopherol, which may participate in neutralizing destructive chemicals purported to be involved in the development of degenerative diseases.

BRAZIL NUTS

Brazil nuts are the large seeds of an enormous evergreen tree of the Amazon district, *Bertholletia excelsa*. This tree can reach a height of more than 40 m, with a trunk diameter of nearly 2.5 m. Its round fruits, with a dark brown color, can weigh more than 1.5 kg each and resemble large coconuts with a woody shell.

The first historical reference to Brazil nuts dates from 1569, when a Spanish colonial official collected thousands of these nuts for his tired and hungry troops. Understandably, the soldiers recovered quickly, as Brazil nuts contain a high energy level, with high contents of digestible fats, calcium, phosporous, potassium, and vitamin B.

Growing Regions

Brazil, Peru, Bolivia, Colombia, Venezuela, and the Guianas are presently the principal Brazil nut–producing countries.

Marketing

Brazil nuts are marketed in in-shell and shelled form and are eaten raw, roasted, and salted as well as in ice creams, chocolate, and bakery and confectionery products.

Health

Brazil nuts contain a notably high amount of bioavailable selenium. Earlier research has shown that selenium supplementation can suppress some forms of cancer growth in different animal tumor models (19).

HAZELNUTS

Hazelnuts grow in clusters on the hazel tree in temperate zones around the world. The fuzzy outer husk opens as the nut ripens, revealing a hard, smooth shell.

History

One of the oldest agricultural crops, hazelnuts are believed to have originated in Asia, then extended into Europe. Today, the principal hazelnut-producing countries are Turkey, Spain, Italy, and the United States.

Since prehistoric times, proven by fossils found in Mesolithic and Neolithic sites in Sweden, Denmark, and Germany, the hazelnut formed part of primitive human diet. The origin of the hazelnut seems to be in Asia; from there it extended to Europe, where it constitutes one of the oldest agricultural cultivations. Several hazelnut varieties exist in the world, the most important being the European variety, *Corylus avellana L.* The hazelnut was present in Greek and Roman mythology and in the Bible, mentioned for its extraordinary nutritional and healing values and even as a tool for finding buried treasures and subterranean streams of water.

Marketing

Also known as filberts and cobnuts, these sweet, rich, grape-size nuts are marketed in in-shell and shelled forms, roasted or natural, and are used mostly in the baking and confectionery industries and for the preparation of food products such as chocolate, ice cream, and nougat. However, they can also be used in salads and main dishes or as snacks. Hazelnuts have been around for over 4,000 years. The hazelnut today is recognized by professional chefs, knowledgeable home cooks, and leading food processors around the world for its unique nutty-sweet flavor, rich, "creamy" texture; versatility; and consistent high quality. Hazelnuts are grown and processed using a combination of ancient artistry and the latest technology, then made available in either a natural or a dry-roasted state in a variety of forms for consumer, food service, or food-product manufacturing needs.

Growing Regions

Presently the principal hazelnut-producing countries are Turkey, Spain, Italy, France, and the United States. Hazelnuts are grown in the world's temperate zones. Two regions in particular stand out. Turkey is by far the world's largest hazelnut producer, accounting for more than three-quarters of annual worldwide production. The hazelnut has been an agricultural mainstay along the rugged and beautiful Black Sea coast for more than 2,000 years. Turkey's ancient hazelnut tradition today combines a rich heritage with twenty-first-century technology. Oregon is America's hazelnut capital, where rich valley soil, cool ocean breezes, and gentle Pacific Northwest winters offer an ideal climate for the hazel tree.

Harvest

Although hazel trees bloom in the middle of winter, their nuts are typically harvested between late summer and early autumn, when the mature nut turns from bright green to shades of hazel. Then the nuts are cracked and processed in either natural or dry-roasted form. The process of dry roasting gives hazelnuts a crunchier texture and a richer, more intense flavor.

Hazelnut Products

Hazelnuts can be processed into fine particles and are useful in adding texture and flavor to a dish. They can be used to replace added fat; as a binding agent for tempuras, to encrust fish, to thicken pies, or to dredge foods for sautéing or baking. They can also be used in cake batters, breads, fillings, and extruded snacks. Hazelnut butter is made of hazelnuts processed to a rich, buttery consistency. It can stand alone in a recipe or can be used with additional forms of hazelnuts for maximum flavor impact. Hazelnut paste is essentially hazelnut butter with added natural sweeteners. Hazelnut oil is a fragrant, full-flavored oil pressed from hazelnuts and can be used in salad dressings and sautéed foods.

Health

Ancient lore has it that hazelnuts held the cure for everything from baldness to stomachaches. Modern science has shown that although those claims may be somewhat overstated, hazelnuts are a good source of vital nutrients. High in dietary fiber, calcium, magnesium, potassium, and vitamin E, and one of the best nut sources of MUFA, hazelnuts are also an excellent source of protein. What's more, hazelnuts, like other plant foods, are naturally cholesterol-free. Mounting scientific evidence suggests that MUFA may work to lower low-density lipoprotein (LDL) cholesterol levels (the so-called "bad" cholesterol), thereby reducing the risk of coronary heart disease (CHD) (20). New research shows that the antioxidant vitamin E could play a role in preventing certain kinds of cancer and CHD (21–23).

Storage and Handling

Store hazelnuts at no more than 40°F for up to one full year. Allow the hazelnuts to warm to room temperature in an unopened container before using. Hazelnuts can also be stored frozen at 27°F or lower for up to 2 yr. Again, for optimum product performance, allow hazelnuts to warm to room temperature in an unopened container, in a well-ventilated area away from odor-producing substances. Hazelnut oil is best stored under refrigeration to prevent rancidity.

Quality and Sanitation Standards

Hazelnuts are grown and processed under exacting sanitary conditions. They meet or exceed quality and sanitation standards set by USDA and the Canadian Food Inspection Agency. Hazelnut equivalencies are as follows (24):

1 lb in-shell hazelnuts = 1 1/2 cups hazelnut kernels
1 lb (shelled) hazelnut kernels = 3 1/4 cups
1 cup hazelnut kernels = 1 1/8 cups large diced
1 cup hazelnut kernels = 1 1/4 cups small diced
1 cup hazelnut kernels = 1 1/3 cups meal

MACADAMIA NUTS

The macadamia is one of the youngest of the edible tree nuts; its commercial cultivation dates from 1858. The fruit consists of a fleshy husk that covers a spherical seed protected by a hard and durable shell. Inside this shell is the macadamia nut, with an exquisite flavor that is sometimes compared with that of a superfine hazelnut. In 1882 the macadamia was introduced to Hawaii, one of the principal production areas along with Australia and New Zealand. Although still little known to the consumer, the macadamia is, together with the pistachio and the pine kernels, one of the world's most expensive edible nuts. Very rich in calories and proteins, the macadamia is always marketed in shelled form and is used mostly as a snack, in confectionery, and for the elaboration of chocolate.

Though most people associate the macadamia with Hawaii, the nut is native to Australia. (In fact, it is the only commercial food crop that is native to Australia.) The colonization of Australia by the British began in 1788 with the establishment of a penal colony at Botany Bay. But it was not until 1875 that the recorded history of the macadamia began. Ferdinand Von Muller, Royal Botanist at Melbourne, and Walter Hill, director of the Botany Garden at Brisbane, were botanizing in the forest along the Pine River in the Moreton Bay district of Queensland. They discovered a species of tree in the family *Proteaceae* previously unknown to European and American botanists. This species did not fit into any previously established genera in that family, so in 1858 Muller established a new genus, Macadamia, named in honor of his friend John Macadam, secretary of the Philosophical Institute of Victoria.

Of course, the British were not the first inhabitants of Australia. At the time of their arrival Australia was inhabited by a primitive people generally referred to as aborigines whose population numbered around 300,000. Their food consisted mainly of fish, shellfish, turtle eggs, grubs of certain tree bark insects, kangaroo, koala, wombat, bandicoot, and other small animals and birds plus yarns and grass seeds. However, during the months of fall and winter (March to June), they would come from far and near to congregate on the eastern slopes of the Great Divide Range. Here they would feast on the seeds of two kinds of trees that were abundant in the area. One tree they called *BunyaBunya*, and the other they called *kindalKindal*. The former we now know botanically as *Araucaria bidwillii*, the latter as the macadamia.

The *Macadamia* genus consists of at least 10 species, but only two of those produce edible nuts, *integrifolia* and heritage with twenty-first-century technology. Oregon is America's hazelnut capital, where rich valley soil, cool ocean breezes, and gentle Pacific Northwest winters offer an ideal climate for the hazel tree.

Harvest

Although hazel trees bloom in the middle of winter, their nuts are typically harvested between late summer and early autumn, when the mature nut turns from bright green to shades of hazel. Then the nuts are cracked and processed in either natural or dry-roasted form. The process of dry roasting gives hazelnuts a crunchier texture and a richer, more intense flavor.

Hazelnut Products

Hazelnuts can be processed into fine particles and are useful in adding texture and flavor to a dish. They can be used to replace added fat; as a binding agent for tempuras, to encrust fish, to thicken pies, or to dredge foods for sautéing or baking. They can also be used in cake batters, breads, fillings, and extruded snacks. Hazelnut butter is made of hazelnuts processed to a rich, buttery consistency. It can stand alone in a recipe or can be used with additional forms of hazelnuts for maximum flavor impact. Hazelnut paste is essentially hazelnut butter with added natural sweeteners. Hazelnut oil is a fragrant, full-flavored oil pressed from hazelnuts and can be used in salad dressings and sautéed foods.

Health

Ancient lore has it that hazelnuts held the cure for everything from baldness to stomachaches. Modern science has shown that although those claims may be somewhat overstated, hazelnuts are a good source of vital nutrients. High in dietary fiber, calcium, magnesium, potassium, and vitamin E, and one of the best nut sources of MUFA, hazelnuts are also an excellent source of protein. What's more, hazelnuts, like other plant foods, are naturally cholesterol-free. Mounting scientific evidence suggests that MUFA may work to lower low-density lipoprotein (LDL) cholesterol levels (the so-called "bad" cholesterol), thereby reducing the risk of coronary heart disease (CHD) (20). New research shows that the antioxidant vitamin E could play a role in preventing certain kinds of cancer and CHD (21–23).

Storage and Handling

Store hazelnuts at no more than 40°F for up to one full year. Allow the hazelnuts to warm to room temperature in an unopened container before using. Hazelnuts can also be stored frozen at 27°F or lower for up to 2 yr. Again, for optimum product performance, allow hazelnuts to warm to room temperature in an unopened container, in a well-ventilated area away from odor-producing substances. Hazelnut oil is best stored under refrigeration to prevent rancidity.

Quality and Sanitation Standards

Hazelnuts are grown and processed under exacting sanitary conditions. They meet or exceed quality and sanitation standards set by USDA and the Canadian Food Inspection Agency. Hazelnut equivalencies are as follows (24):

1 lb in-shell hazelnuts = 1 1/2 cups hazelnut kernels
1 lb (shelled) hazelnut kernels = 3 1/4 cups
1 cup hazelnut kernels = 1 1/8 cups large diced
1 cup hazelnut kernels = 1 1/4 cups small diced
1 cup hazelnut kernels = 1 1/3 cups meal

MACADAMIA NUTS

The macadamia is one of the youngest of the edible tree nuts; its commercial cultivation dates from 1858. The fruit consists of a fleshy husk that covers a spherical seed protected by a hard and durable shell. Inside this shell is the macadamia nut, with an exquisite flavor that is sometimes compared with that of a superfine hazelnut. In 1882 the macadamia was introduced to Hawaii, one of the principal production areas along with Australia and New Zealand. Although still little known to the consumer, the macadamia is, together with the pistachio and the pine kernels, one of the world's most expensive edible nuts. Very rich in calories and proteins, the macadamia is always marketed in shelled form and is used mostly as a snack, in confectionery, and for the elaboration of chocolate.

Though most people associate the macadamia with Hawaii, the nut is native to Australia. (In fact, it is the only commercial food crop that is native to Australia.) The colonization of Australia by the British began in 1788 with the establishment of a penal colony at Botany Bay. But it was not until 1875 that the recorded history of the macadamia began. Ferdinand Von Muller, Royal Botanist at Melbourne, and Walter Hill, director of the Botany Garden at Brisbane, were botanizing in the forest along the Pine River in the Moreton Bay district of Queensland. They discovered a species of tree in the family *Proteaceae* previously unknown to European and American botanists. This species did not fit into any previously established genera in that family, so in 1858 Muller established a new genus, Macadamia, named in honor of his friend John Macadam, secretary of the Philosophical Institute of Victoria.

Of course, the British were not the first inhabitants of Australia. At the time of their arrival Australia was inhabited by a primitive people generally referred to as aborigines whose population numbered around 300,000. Their food consisted mainly of fish, shellfish, turtle eggs, grubs of certain tree bark insects, kangaroo, koala, wombat, bandicoot, and other small animals and birds plus yarns and grass seeds. However, during the months of fall and winter (March to June), they would come from far and near to congregate on the eastern slopes of the Great Divide Range. Here they would feast on the seeds of two kinds of trees that were abundant in the area. One tree they called *BunyaBunya*, and the other they called *kindalKindal*. The former we now know botanically as *Araucaria bidwillii*, the latter as the macadamia.

The *Macadamia* genus consists of at least 10 species, but only two of those produce edible nuts, *integrifolia* and *tetraphylla*. The macadamia was introduced into California in late 1877 by C. H. Dwinelle of the University of California at Berkeley. He obtained seeds from Australia and planted several seedlings along Strawberry Creek on the Berkeley campus, and at least one of those trees is still growing in its original site. The next year the macadamia was introduced into Hawaii by Walter H. Perves, manager of the Honokoa Sugar Company at Haina, Hawaii. As of 1995 Hawaii had 22,000 acres planted in macadamias.

The first large planting of macadamias occurred in 1890 on the Frederickson estate at Rouse Mills, New South Wales. They planted around 250 trees as a source of nuts for the family. Many of those trees still exist and are still producing a good crop of nuts. Around 1910, two nurserymen, Ernest Braunton and Charles Knowlton, started selling seedling trees in southern California. But not until 1946 did a commercial planting finally occur in California. Robert W. Todd planted 2 acres of seedling trees on his property on Grandview Street in Oceanside. Several years later those trees were grafted, and today, more than 100 of them are still there and still producing. Macadamias are grown commercially in Hawaii, Australia, Malawi, Kenya, South Africa, Israel, Costa Rica, Guatemala, Mexico, Brazil, and many other tropical and subtropical regions, including Florida. The macadamia is grown in California from San Luis Obispo south to the Mexican border, west of the mountains.

The primary limiting factor in growing macadamias is temperature. Macadamia trees can take temperatures as low as 28°F for up to about 4 hours, and they start to get stressed around 105°F. Southern California has about 2,500 acres planted to the macadamia. Commercially, only *M. integrifolia* and *M. tetraphylla* and their hybrids are important. They are very similar to each other and botanically very closely related to a third species, *M. ternifolia F. Muell*, which produces a small, bitter kernel unsuitable as a table nut.

M. integrifolia is commonly referred to as the smooth-shell species. The fruit consists of a white kernel, high in oil content (72% oil and 4% sugar when dry), very uniform, and of excellent quality. It is enclosed in a round, hard shell about 1 in. (2.5 cm) in diameter surrounded by a smooth, bright green pericarp (husk). The leaves are stiff, oblong to lanceolate, 4 to 10 in. (10–25 cm) long in nodal whorls of three (rarely four), and either light green or bronze when young. The small, perfect white flowers are borne in racemes 4 to 8 in. (10–20 cm) long. Only a few flowers in a raceme will set fruit.

M. tetraphylla is called the rough-shelled species because of the pebbliness of the shell's surface. The husk is somewhat spindle shaped; grayish green; and covered with a dense, white pubescence. Kernels have a grayish base and are darker in color and more variable in quality than those of *M. integrifolia*. The oil content averages 67% in the dry nut, with 6 to 8% sugar. The leaves are characteristically sessile and serrated along the margins, in whorls of four at the nodes. The flowers are pink and in racemes 6 to 18 in. (15–45 cm) long. Cultivars that are hybrids of the two species possess characteristics of both, and the quality of their nuts compares favorably with that of *M. integrifolia*. The trees of both species are tall and spreading, reaching 60 ft (20 m) or more in height. The wood is hard and brittle; the exposed bark sunscalds very easily.

Cultivation and Production

There are approximately 40 described cultivars, most of them in Australia. The following varieties have been used for commercial plantings in Hawaii: Keauhou, Ikaika, Kakea, and Keaau. Kakea, however, is no longer propagated for commercial use. In California, Elimba, a *M. tetraphylla* cultivar, is also considered of commercial value. Beaumont, a productive hybrid cultivar, is also recommended for home plantings. With good care, cultivars of *M. integrifolia* begin producing 5 yr after planting, but appreciable yields are obtained only after the 8th year. A productive variety will bear as many as 150 lb of in-shell nuts per tree.

Before large-scale plantings are attempted, cultivars should be tested locally. In Hawaii, *M. integrifolia* cultivars are more productive on *M. tetraphylla* rootstocks in soils where they are not likely to suffer from iron deficiency. Where this deficiency may be a problem, *M. integrifolia* seedlings should be used as a rootstock. The season of production in Florida for *M. integrifolia* runs from July through November. Very few commercial cultivars have been tested in this state, and more information is needed to make specific recommendations.

Climate

Macadamias are well adapted to warm, subtropical conditions. Mature trees can withstand winter temperatures of as low as 25 to 26°F (3–5°C) for short periods with minor damage to the foliage. However, young trees and foliage are very tender and are killed by temperatures very near freezing. Temperatures below 28°F (−2°C) cause damage to flowers and young fruit and reduce production. In the tropics, macadamias are better adapted to medium elevations of 2100 to 3600 ft (700–1200 m), but in Hawaii, macadamias are not planted commercially above 2500 ft (800 m). Although the plant is quite resistant to drought, supplemental irrigation is very important, particularly during the flowering and fruit-setting season. Severe moisture stress results in considerable drop of young fruit.

Soils and Fertilizers

Macadamias are not demanding for soil fertility, but they do require good drainage. They also need relatively higher amounts of phosphorous in the fertilizer than other fruit crops, particularly when the trees are young.

Pests and Diseases

Leaves are occasionally infested by thrips and mites, which may become serious in large plantings. Green stinkbugs cause considerable damage at times by injuring very young fruit when the shell is still soft. Rats, squirrels, and nut borers also cause substantial losses if unchecked. Anthracnose (*Colletotrichum* spp.) attacks leaves and the husks of immature nuts. Diseased nuts do not drop when mature and usually spoil while still attached. *Phytophthora cinnamoni*, which causes root rot in avocados, produces a trunk canker in macadamia that may kill young seedlings. Fortunately, the tree is qute resistant to root rot caused by this fungus.

Harvesting and Processing

Mature nuts fall to the ground and have to be gathered manually every week to prevent spoilage, particularly from molds. Husking is done soon after harvesting and before the nuts are mechanically cracked. They are air dried at temperatures not higher than 110°F. The moisture content of the kernel is reduced to less than 1.5% to prevent development of off-flavors after roasting (either dry roasting or in a refined coconut oil at 275°F for 12–15 min).

Uses

Macadamias at present command premium prices because the demand is greater than current production. They are ordinarily offered on the gourmet shelves of supermarkets as salted nuts packed in glass jars. The largest use, however, is in confections. Only whole nuts are packed in jars.

PECANS

This native American tree nut is a member of the hickory family. Long before the arrival of the Europeans to the New World, pecans were part of the diet of the Indian tribes of the central and southern regions of the United States. The pecan slightly resembles the walnut but is longer in shape and has a smoother shell and a higher proportion of kernel in its shell (40–60%). The pecan nut is extraordinarily rich in fats and calories. The principal pecan-producing countries are the United States, Mexico, Australia, and Israel. Pecans are marketed in in-shell and shelled forms and can be eaten raw or roasted. They are used in the bakery, confectionery, and dairy industries and in chocolate and ice creams. Pecans are also added to cereals, breads, pastries and cookies, salads, and main dishes and are eaten as toppings on desserts and as a snack. The wood of the pecan tree is highly appreciated for its timber and is often used as decorative paneling.

History

Pecans can be traced back to the sixteenth century. The only major tree nut that grows naturally in North America, the pecan is considered the most valuable North American nut species. The name pecan is a Native American word of Algonquian origin that was used to describe all nuts requiring a stone to crack and a Natchez Indian word for the pecan plant. Originating in central and eastern North America and the river valleys of Mexico, pecans were widely used by pre-Colonial residents. Pecans were favored because they were accessible to waterways, easier to shell than other North American nut species, and great tasting.

The pecan's normal environment is the floodplains along the Mississippi, Ohio, Missouri, and Red rivers and along many of the large rivers in central Texas and northeastern Mexico. Due to its ready availability, many Native American tribes in the United States and Mexico used the wild pecan as a major food source during autumn. The kernel was used to produce a creamy fluid for cooking and drinking. It is speculated that the nut was used to produce a fermented intoxicating drink called *Powcohicora* (this was also the source of the name hickory). Because the pecan was a major food and trade item, it is said that the pecan tree was cultivated by Native Americans. It is reported that the Creek tribe possessed an "ancient cultivated field" of pecans.

Planting

One of the first known cultivated pecan tree plantings appears to have been accomplished in the late 1600s or early 1700s by Spanish colonists and Franciscans in northern Mexico. These plantings are documented to around 1711, about 70 yr before the first recorded planting by U.S. colonists. The first pecan planting in the United States was in Long Island, New York, in 1772. By the late 1700s nuts from the northern range reached the English portion of the Atlantic seaboard and were planted in the gardens of easterners such as George Washington (1775) and Thomas Jefferson (1779). Settlers were also planting pecans in community gardens along the Gulf Coast at this time.

Economics

In the late 1770s the French and Spanish colonists settling along the Gulf of Mexico realized the economic potential of pecans. By 1802 the French were exporting pecans to the West Indies. It is speculated that the nuts were exported to the West Indies and Spain earlier by Spanish colonists in northern Mexico.

Marketing

In 1805 it was advertised in London that the pecan was "a tree meriting attention as a cultivated crop." Because of its location near the mouth of the Mississippi, New Orleans became of great importance in the marketing of pecans. The city had a natural market and avenue for redistribution of nuts to other parts of the United States and the world. This market stimulated local interest in planting orchards, which led to the adaptation of vegetative propagation techniques and demand for trees that produced superior nuts. At least 15 commercial cultivars had developed in Louisiana by the end of the nineteenth century. In San Antonio, Texas, the wild pecan harvest was more valuable than row crops such as cotton. During the 1700s and the early 1800s the pecan nut had become an item of commerce for the American colonists, and the pecan industry had begun.

Because pecan groves (trees established by natural forces) and orchards (planted by farmers) consisted of diverse nuts of various sizes, shapes, shell characteristics, flavor, fruiting age, and ripening dates, a premium market for large, thin-shelled pecans was created. With such a variability in trees, there was the occasional discovery of a wild tree with unusually large, thin-shelled nuts, which were in high demand by customers. In 1822 Abner Landrum of South Carolina discovered a pecan-budding technique. This system provided a way to graft (unite with a growing plant by placing in close contact) plants derived from superior wild selections. However, this invention was lost or overlooked until the 1880s.

Grafting Pecans

In Louisiana in 1846 an African-American slave gardener, Antoine, successfully propagated pecan by grafting a superior wild pecan to seedling pecan stocks. This clone was named "Centennial" because it won the prize as best pecan exhibited at the Philadelphia Centennial Exposition in 1876. This planting, which became 126 "Centennial" trees, was the first planting of improved pecans. The successful use of grafting techniques led to grafted orchards of superior genotypes and was a milestone for the pecan industry. The adoption of these techniques was slow and had little commercial impact until the 1880s, when Louisiana and Texas nurserymen learned of pecan grafting and began propagation on a commercial level. This was the start of a booming pecan growing and shelling industry.

Pecan Timeline (25,26)

- American Indians utilized and cultivated wild pecans—1500s
- Spanish colonists cultivated orchards—late 1600s–early 1700s
- English settlers planted pecan trees—1700s
- George Washington planted pecan trees—1775
- Thomas Jefferson planted pecan trees—1779
- Economic potential for pecans realized—1700s
- Pecans exported by the French to the West Indies—1802
- Pecan budding technique discovered—1822
- Successful grafting of the pecan tree—1846
- First planting of improved pecans—1876
- Commercial propagation of pecans begins—1880s

Pecan production has steadily increased in the United States since 1925, rising from 2.2 million lb in 1920 to 338.1 million lb in 1998. Before 1920 the few pecans that were consumed were hand shelled by consumers.

With the development of commercial shelling equipment, the pecan industry began to grow. In the early 1920s pecan processing was developed along with equipment used for sizing, separation of faulty nut meats and shells, cracking, grading of meats, drying, and packaging. The use of pecans was increased by the improvement of storage life through controlling temperature, relative humidity, air circulation, and storage atmospheres, as well as improved packaging. Since 1948 more than 80% of the pecans sold have been shelled before being marketed. Some shelling plants operate year-round, whereas others are seasonal (fall months). Many pecan plants have a capacity of 1 to 30 tons per day. Large plants can have as many as 14 cracking machines with a capacity of 150,000 lb a day and 30 million lb seasonally Plants usually have a cold-storage facility, some capable of holding several million pounds of unshelled and shelled nuts.

Growing Regions

Not surprisingly, most shelling plants are located in the southeastern and southwestern United States, where the majority of pecans are grown. The leading production states (alphabetically) are Alabama, Arizona, Arkansas, California, Florida, Georgia, Kansas, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, and Texas. Significant quantities of pecans also are produced in Mexico's states of Chihuahua, Coahuila, Durango, Nuevo Leon, and Sonora. Modest quantities are produced in Australia, Israel, Peru, and South Africa.

Technology

Machinery used in shelling plants includes (*1*) grading and sizing machinery equipped with screens and blowers for removal of faulty nuts and foreign materials and segregating nuts into sizes, (*2*) machines with vats or tanks for sterilizing and conditioning pecans for shelling, (*3*) cracking machines (called crackers) for cracking the shell, (*4*) shellers for separating shells from the meat, (*5*) screens for separation of halves and broken pieces, (*6*) dryers to remove excess moisture from meats, (*7*) grading belts or tables for handpicking of dried meats, and (*8*) electric eyes for sorting shell and foreign matter from the meats.

Shelling pecans reduces their weight from 50% to 65% and their volume approximately by half. To prevent the shattering of pecan meat, the nuts are moistened, or conditioned, before cracking. Two methods are used to accomplish this. The cold-water method soaks the pecans for as long as 8 h in water with chlorine. The pecans are then drained for 16 to 24 h in sacks, vats, or barrels for cracking within the next 24 h. Using the steam-pressure method, pecans are exposed to hot water or steam pressure for 6 to 8 min, then cooled and held for 30 to 60 min. This method is faster but can cause discoloration of the kernels.

The nuts are cracked by a machine applying force to the ends of the nuts. The cracked nuts are then placed on a conveyer that moves them to the sheller machines. Meats and shells are separated by a series of shaker screens. Shelled pecans are then separated into grades by the halve-size or pieces. The sizing is done as meats pass over rapidly vibrating machines with holes of different sizes (halves come in 8 sizes, based on the number per pound). Once shelled, the pecan meats contain from 7 to 9% moisture. For quality the moisture is reduced to 4% or lower. This is done by rapidly circulating dry air through the pecan meats. The meats are then ready for storage.

Storage

In-shell pecans are put in refrigerated storage as soon as possible after harvest. Refrigerated storage is necessary not only to maintain freshness and shelf-life, but also to avoid insect infestation during the warm-weather months. Freezing pecans from season to season is an excellent method for storage. Even at temperatures as low as −170°F (solid carbon dioxide), there is no seepage of oil or decrease in the nut quality.

Shelled pecans are packed in vacuum-packed cans, jars, glass, cellophane bags, and poly-lined boxes to protect against high humidities, foreign flavors, oxidation, insects, rodents, and light. After being packaged, the nuts are stored or shipped to retail, food-service, or commercial markets. There are five U.S. grades for shelled pecans; U.S. No. 1 halves, U.S. Commercial halves, U.S. No. 1 pieces, U.S. Commercial pieces, and Unclassified.

PINE NUTS

The pine tree is one of the most familiar trees in both Europe and North America, but it is mainly in the Mediterranean area that it obtains its highest importance with regard to production and consumption. The nuts are found inside the pinecone.

The pine kernel, or pine nut, is an edible nut with an exquisite flavor and high protein content. The Roman legions carried pine kernels as provisions, and all over Europe it is used as a culinary ingredient in the preparation of meat, fish, and vegetable dishes, as well as in the confectionery industry in chocolates and other delicacies.

Growing Regions

Spain, Italy, China, Portugal, and Turkey are the principal producing countries of pine kernels. Also called Indian nut, piñon, pinoli, and pignolia, the pine nut is marketed in shelled form.

Varieties

There are two main varieties. The Mediterranean or Italian pine nut is from the stone pine. It is torpedo shaped, has a light delicate flavor, and is the more expensive of the two. The stronger-flavored Chinese pine nut is shaped like a squat triangle. Its pungent flavor can easily overpower some foods.

PISTACHIO

With an antiquity of more than 10,000 years, the pistachio is one of the oldest edible nuts on earth. Originating from Southeast Asia, Asia Minor, Pakistan, and India, the pistachio belongs, like the cashew, to the family of Anacardiaceae. The fruit of the pistachio differs from all the other nuts because of its characteristic green color and the semi-opening of the shell, which in Iran is called the "smiling pistachio." This singular morphology makes the pistachio the only edible nut that does not need to be shelled for roasting and salting.

The pistachio has a delicious flavor and a high nutritional value, being very rich in proteins and vitamins. The principal pistachio-producing countries are Iran, Turkey, the United States, Greece, and Italy. Pistachios are always marketed in their in-shell form, roasted and salted, but they can be purchased shelled. They are mainly used as snack and confectionery and used in ice creams.

History

The history of pistachios includes royalty, perseverance, and pride. Pistachios date back to the Holy Lands of the Middle East, where they grew wild in high desert regions. Legend has it that lovers met beneath the trees to hear the pistachios crack open on moonlit nights for the promise of good fortune. A rare delicacy, pistachios were a favorite of the Queen of Sheba, who hoarded the entire Assyrian supply for herself and her court. The royal nut was imported by American traders in the 1880s, primarily for U.S. citizens of Middle Eastern descent. Some 50 years later, pistachios became a popular snack food, introduced in vending machines.

Storage

Pistachios are excellent keepers when stored properly. To keep them at their freshest, store pistachios in a refrigerated, airtight container, or keep them in the freezer for long-term storage. (A temperature of 32–40°F should be maintained with a relative humidity of 70%.) To restore crispness to pistachios that have lost their crunch, toast the nut meats at 200°F for 10 to 15 min. Any pistachios stored in bulk containers should be rotated frequently to maintain freshness. Kept in the refrigerator or freezer, pistachios can be stored for as long as a year.

Shelling

To remove the skin from nut meats by blanching, drop shelled nuts into boiling water and let them soak off the heat for about 1 min. Drain and rub the pistachios with a clean towel. To dry, spread on a large baking sheet in an oven preheated to 300°F for 10 to 15 min. The nut meat can also be removed by toasting. Spread the shelled nuts in one layer on a baking sheet. Bake in an oven preheated to 400°F for 4 to 5 min, watching carefully that they do not burn. Remove and let cool, then rub off the skins.

Red Color

The first pistachios available to consumers were imported from the Middle East. Importers dyed the shells red, both to disguise staining from antiquated harvesting methods and to make pistachios stand out among other nuts in vending machines.

Pistachios in California

Until the 1970s, there was no domestic pistachio industry in the United States. California harvested its first commercial crop in 1976. The entry of California pistachios into the marketplace made available nuts with clean, naturally tan shells. California's Kerman variety is also larger in size and a more vibrant green color.

Availability

Pistachios are harvested in September, but sufficient supply and state-of-the-art storage systems allow the industry to provide pistachios throughout the year.

Exports

Through the U.S. Foreign Agricultural Service, California pistachios are exported to the following markets: Japan, Korea, China, Malaysia, the Philippines, Thailand, Indonesia, Canada, and the United Kingdom.

California pistachio exports exceeded 41 million lb during the 1996–1997 crop year and were expected to exceed 60 million lb during the 1997–1998 crop year. The 1997 harvest produced a record 179.5 million lb. Monthly shipment and inventory records as reported by industry processors are available.

WALNUTS

Consumed since prehistoric times, the walnut has various origins, in East Asia, southeastern Europe, and North and South America. There exist more than 15 varieties of walnuts, but the two most popular are the English (also called Persian) walnut and the black walnut. A close relative is the butternut, also referred to as the white walnut. By far the most delicious and commercially important variety is the English walnut. The Greeks gave it the name *kara* (head), because of its resemblance to the human skull and brain. This similarity is the source of medieval beliefs of the walnut's healing properties for any kind of headache.

Production

Today, the world production of walnuts is spread over the United States, France, China, India, Turkey, Italy, and Chile. Walnuts are marketed in shelled and in-shell forms and are primarily used as a snack, in desserts and confectionery, and for the preparation of several food products. Consumers and professionals enjoy adding walnuts to various culinary recipes including salads, pasta, main dishes, and baked goods.

History

Walnuts have been recognized as one of the oldest tree foods known to humankind, dating back to about 7000 B.C. Considered food for the gods in the early days of Rome, walnuts were named *Juglans regia* in honor of Jupiter. Today they are commonly called English walnuts, in reference to the English merchant marines whose ships once transported the product for trade to ports around the world. Historians prefer the name Persian walnuts, referring to Persia, the birthplace of walnuts. With its mild climate and deep, fertile soils, California provides ideal growing conditions for the California walnut, producing 98% of the total U.S. commercial crop and accounting for two-thirds of the world's trade.

The Franciscans are credited with bringing walnuts to California from Spain or Mexico. The first commercial planting began in 1867 when Joseph Sexton, an orchardist and nurseryman in the Santa Barbara County town of Goleta, planted English walnuts. For several years, walnuts were predominantly planted in the southern areas of California, accounting for 65% of all bearing acreage. Some 70 years after Sexton's first planting, the center of California walnut production moved north to the Stockton area. Better growing areas, improved irrigation, and better pest-control methods in the north resulted in greater yields, which gradually increase each year.

Coloring

California walnuts come in a spectrum of color shades ranging from extra light to amber. Color is caused naturally by a number of factors including the time of harvest, location of the tree in the orchard, variety, and probably factors we do not yet understand. Darkness of walnuts is not a defect. In fact, in parts of the world and in many industries, darker walnuts are prized because they normally have a stronger walnut taste. At the same time, lighter walnuts are always prized by bakers around the world as decoration for cakes and pastries.

NUTS IN DIET PATTERNS

Nuts are a good fit in a healthy diet. They help achieve optimal levels of protein, vitamin E, fiber, zinc, copper, and iron in the diet. Substituting nuts in place of saturated fat in any diet can also improve the ratio of unsaturated fats to saturated fats.

Because nuts are consumed in a variety of ways—in confections and baked goods, on salads and vegetables, in main dishes, or simply as a snack food—it is easy to incorporate them into the diet every day. In the USDA Food Guide Pyramid, nuts are found in the meat and legume category because of their high protein content. Nuts are a great source of protein for many people, especially those who choose not to eat animal meat.

MEDITERRANEAN DIET PYRAMID

The Mediterranean-style diet is based on the dietary traditions of the people of the many regions bordering the Mediterranean Sea, including Greece, southern Italy, Spain, southern France, Tunisia, Lebanon, and Morocco. People in this region eat an abundance of fruits, vegetables, nuts and legumes, and whole grains; some dairy products, fish, and poultry; and very small amounts of meats. The main source of fat, which usually constitutes 35 to 40% of calories, is MUFA from olive oil and nuts and omega-3 fat from fish (26). In addition, a Mediterranean-style diet includes moderate amounts of alcohol, particularly wine, which has been associated with a reduced risk of cardiovascular disease and seems to be compatible with a healthy lifestyle (27).

Mediterranean diets have long been known for their delicious tastes and flavors. Currently, the many health benefits of Mediterranean-style eating are being realized. In the Mediterranean Diet Pyramid, nuts are on the same level as fruits, vegetables, beans, and legumes. They fall closer to the bottom of the pyramid, meaning they should be eaten more often.

PEANUTS AND NUTS IN WORLD CUISINES

Peanuts have been used in healthy traditional cuisines around the world. Asian, Mediterranean, African, and Latin American people have included peanuts in their cooking for centuries. Stir-fries with peanuts, soups and satays, salads and vegetable dishes, sweets and confections—the list goes on. As nutrition scientists are unlocking more of the health benefits of peanuts, chefs are finding more and more ways to include them in healthy cuisines.

ALLERGIES

Although milk and eggs are the most common food allergens, a peanut or tree nut allergy can be among the most severe. A food allergy is an immunological response in which the protein from a specific food triggers a reaction that results in the release of massive amounts of histamines and other mediators. Symptoms that can occur from this reaction include rash, cough, hives, asthma, swelling, difficulty in breathing, cramps, vomiting, and a fall in blood pressure. The most severe reaction is called anaphylaxis and can result in shock or death. Some individuals are so allergic that a very small amount of the allergen can trigger a severe response. The best protection is avoidance. In addition, the allergic individual should carry an epi-pen, which contains medicine that can relieve even the most severe symptoms until the individual can reach a hospital.

The exact number of food allergy sufferers is unknown, but it is estimated that 1 to 2% of the population is affected by some food allergy (28). Though individuals who experience food allergies as children often outgrow them, peanut, tree nut, and shellfish allergies tend to be lifelong. Researchers, with the help of industry, are working toward treatments to prevent or decrease the symptoms of serious food allergies.

BIBLIOGRAPHY

1. G. Fraser et al., "A Possible Effect of Nut Consumption on Risk of Coronary Heart Disease," *Arch. Internal Med.* **152**, 1416–1424 (1992).
2. R. J. Prineas et al., "Letter to the Editor," *New Eng. J. Med.* **329**, 359 (1993).
3. L. J. Appel et al., "A Clinical Trial of the Effects of Dietary Patterns on Blood Pressure," *New Eng. J. Med.* **336**, 1117–1123 (1997).
4. D. M. Colquhoun et al., "Comparison of the Effects of a High-Fat Diet Enriched with Peanuts and a Low-Fat Diet on Blood Lipid Profiles," American Heart Association, 1996, p. 733 (abstract).
5. F. B. Hu et al., "Frequent Nut Consumption and Risk of Coronary Heart Disease in Women: Prospective Cohort Study," *Br. Medical J.* **317**, 1341–1345 (1998).
6. C. M. Albert et al., "Nut Consumption and the Risk of Sudden and Total Cardiac Death in the Physicians Health Study," American Heart Association, 1998 (abstract).
7. M. Abbey et al., "Partial Replacement of Saturated Fatty Acids with Almonds or Walnuts Lowers Total Plasma Cholesterol and Low-Density-Lipoprotein Cholesterol," *Amer. J. Clin. Nutrition* **59**, 995–999 (1994).
8. J. D. Curb et al., "Comparison of Lipid Levels in Humans on a Macadamia Nut Based High Monounsaturated Fat Diet and a High Fat Typical American Diet," *American Heart Association Scientific Conference on Efficacy of Hypocholesterolemic Dietary Interventions*, 1995, pp. 3–5.
9. J. Sabate et al., "Effect of Walnuts on Serum Lipid Levels and Blood Pressure in Normal Men," *New Eng. J. Med.* **328**, 603–607 (1993).
10. M. L. Dreher, C. Maher, and P. Kearney, "The Traditional and Emerging Role of Nuts in Healthful Diets," *Nutrition Rev.* **54**, 241–245 (1996).
11. T. A. Pearson et al., "Weight Loss and Weight Maintenance: Effects of High MUFA vs Low Fat Diets on Plasma Lipids and Lipoproteins," *Experimental Biology '99*, April 19, 1999.
12. K. McManus, L. Antinoro, and F. M. Sacks, "Weight Reduction: A Comparison of a High Unsaturated Fat Diet with Nuts Versus a Low-Fat Diet," *Experimental Biology '99*, April 19, 1999.
13. C. M. Lermer and R. M. Mattes, "Effects of Chronic Peanut Consumption on Body Weight and Serum Lipid Levels in Humans," *Experimental Biology '99*, April 19, 1999.
14. B. Eissenstat et al., "Impact of Consuming Peanuts and Peanuts Products on Energy and Nutrient Intakes of American Adults," *Experimental Biology '99*, April 19, 1999.
15. G. A. Spiller et al., "Nuts and Plasma Lipids: An Almond-based Diet Lowers LDL-C While Preserving HDL-C," *J. Amer. College of Nutrition* **17**, 285–290 (1998).
16. L. Brown et al., "Nut Consumption and Risk of Recurrent Coronary Heart Disease," *Experimental Biology '99*, April 19, 1999.
17. T. H. Sanders and R. W. McMichael, "Occurrence of Resveratrol in Edible Peanuts," *American Oil Chemists Society Meeting*, Las Vegas, Nev., 1998.
18. A. B. Awad et al., "Anticancer Properties," *Experimental Biology '99*, April 19, 1999.
19. C. Ip and D. J. Lisk, "Characterization of Tissue Selenium Profiles and Anticarcinogenic Responses in Rats Fed Natural Sources of Selenium-Rich Products," *Carcinogenesis* **15**, 573–576 (1994).
20. F. B. Hu et al., "Dietary Fat Intake and the Risk of Coronary Heart Disease in Women," *New Eng. J. Med.* **337**, 1491–1499 (1997).
21. L. H. Kushi et al., "Dietary Antioxidant Vitamins and Death from Coronary Heart Disease in Post-Menopausal Women," *New Eng. J. Med.* **334**, 1156–1162 (1996).
22. E. Rimm et al., "Vitamin E Consumption and the Risk of Coronary Heart Disease in Men," *New Eng. J. Med.* **328**, 1450–1456 (1993).
23. M. J. Stampfer et al., "Vitamin E Consumption and the Risk of Coronary Heart Disease in Women," *New Eng. J. Med.* **328**, 1444–1449 (1993).
24. The Hazelnut Council Web site, URL: *http://www.hazelnutcouncil.org*.
25. National Pecan Sheller's Association Web site, URL: *http://www.ilovepecans.org*.
26. A. Keys, *Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease*, Harvard University Press, Cambridge, Mass., 1980.
27. E. B. Rimm and R. C. Ellison, "Alcohol in the Mediterranean Diet," *Amer. J. Clinical Nutrition* **61**, 1378S–1382S (1995).
28. Food Allergy Network Web site, URL: *http://www.foodallergy.org*.

GENERAL REFERENCES

American Peanut Council Web site, URL: *http://www.peanutsusa.org*.

International Tree Nut Council Web site, URL: *http://www.nuthealth.org*.
Macadamia Society of Hawaii Web site, URL: *http://www.hmna.org*.

Patricia Kearney
PMK Associates
Arlington, Virginia

POSTHARVEST INTEGRATED PEST MANAGEMENT

Effective management of pests within systems where food is produced, processed, and distributed is critical for maintaining an abundant, affordable, and safe food supply. More than 100,000 pest species are known to cause losses in crop and livestock production and in the systems designed for processing and distributing food commodities (1). It has been estimated that at least one-third of the food supply potentially available to the population of the United States is lost on an annual basis as a result of pest infestations during production and after harvest. In addition, nearly $8.5 billion is spent annually on chemical pesticides applied in agriculture and industry as farmers and processors attempt to reduce losses (2). In food production and processing systems, the primary approach to pest control has been one of eradication, with the objective of total elimination of pest populations (3). This approach has resulted from concerns, arising foremost in production of fresh fruit and vegetables, that any pest injury would cause these commodities to become aesthetically unacceptable to consumers (4). Also, these commodities have such high value that it is considered unreasonable to risk the possibility of reduced yields or grades of produce (5). Demands for eradication of pests have resulted in excessive reliance on chemical pesticides to the extent that applications often been made according to schedules without assessment of pest infestation levels.

Gradual changes in approaches to pest control are now occurring in food production, processing, and distribution systems. Whereas once little consideration was given to alternatives to chemical pesticides, greater interest now exists for implementation of controls that are less threatening to the environment and nontarget species (6,7). Integrated pest management (IPM) concepts are gradually being incorporated into the systems that supply food for the world's population. With acceptance of principles of IPM has come willingness on the part of farmers and processors to implement control programs that are aimed not at eradication of pests, but at reduction of infestations and close monitoring of the benefits vs costs of control measures.

The goal of this article is to review general concepts that are basic to IPM as developed in the production agriculture venue and to provide an overview of the current issues, methodologies, and practical concerns relevant to pest management in the postharvest food industry. The topics included pertain to management of insects or other arthropod pests that infest durable commodities such as grains, nuts and dried foods, and all the processed food products derived from these commodities. Pest issues related to postharvest handling of fresh commodities are discussed briefly. Canned or otherwise processed fruits and vegetables and prevention of microbial contamination are discussed in other articles.

WHAT IS IPM?

IPM is a systematic approach to pest regulation that emphasizes increased sampling to assess pest infestation levels and promote improved decision making so that control costs can be reduced and social, economic, and environmental benefits can be maximized (8). From the standpoint of benefit vs cost in IPM, the basic goal of control programs is defined by the concept of economic injury levels. The economic injury level (EIL) is defined as the pest infestation level at which the loss due to pest is equal to the cost of available control measures. The EIL concept is used as the basis for determining economic thresholds (often called action thresholds), defined as the level of infestation at which the *potential* loss due to a pest infestation *exceeds* the cost of an available control measure. In decision making regarding chemical pesticide applications, the economic return from use of a pesticide is maximized when the application is made at the time the pest population reaches the economic threshold level. Implementation of IPM has typically resulted in reduction of purchased inputs, such as chemical pesticides, because of more effective assessment of pest infestation levels and well-defined criteria for determining when controls are warranted. In crop production systems, programs involving entomologists, plant pathologists, weed scientists, and economists have yielded cost-efficient tactics for suppressing pests while limiting contamination of the environment and the harvested food commodities. IPM concepts are now emphasized for postharvest systems using benefit-vs-cost principles to guide pest control decisions in storage and distribution systems, just as has occurred in field settings. Considering ecological, as well as economic concerns, IPM is regarded as an essential approach to preserving a safe food supply and healthy environment, while keeping U.S. agriculture competitive and profitable.

Explaining the context in which the terms *pest* and *management* are used can be quite helpful to understanding concepts of IPM. The term pest has historically referred to insects, weeds, plant pathogens, and rodents that compete for resources valued by humans in production, processing, and distribution systems for agricultural commodities. Thus, species attain pest status in the context of their association with plant and animal species used by humans as sources of food and fiber. The abundance of these species, and hence their importance as pests, is often enhanced in modern production and distribution systems for agricultural commodities because these systems result in abundant habitats and vast supplies of resources unlike anything that occurs in natural ecosystems.

IPM makes use of decision-making processes needed to produce commodities in carefully planned systems intended to keep pest populations from reaching economically damaging levels, while maintaining profitability of the enterprise with limited adverse environmental and social effects. The process of managing pests has significantly broadened in definition and scope during the past 30 years from unilateral controls applied against single species; to integrated controls employing multiple tactics against single species; to IPM programs designed to regulate insect pests, pathogens, and weeds in production systems; and to quite broad concepts of biointensive IPM (1) and integrated resource management (9). There is a substantial need for increased research and implementation efforts to keep pace with the theoretical development of management concepts. Practical aspects of implementation have tended to lag far behind IPM theory (1).

DEVELOPMENT OF IPM PROGRAMS

Basic changes in decision-making processes are important to development of effective IPM programs. In the past, eradication of pests was often the primary objective; now programs must address ecological, economic, and health-related concerns in conjunction with acceptable levels of pest regulation as defined by the EIL concept. IPM programs require time and expertise devoted to assessment of problems and decision making to gain maximum returns on inputs such as chemical pesticides. Significant resources must be committed to training of personnel or hiring consultants who have the necessary expertise to accurately monitor production fields and postharvest facilities for the presence of pest infestations and decide on appropriate management options. Before IPM programs were developed, applications of pesticides were routinely made according to schedules as insurance or prophylactic treatments. However, to address current economic, environmental, and social concerns, IPM programs require a greater time commitment for comprehensive assessment of pest infestation levels to ensure that control options are employed in a judicious, as opposed to routine, manner.

Food processors and distributors must realize that most pest management decisions have consequences far beyond the time and location that pests must be controlled, a lesson learned in field settings during the past 30 years. Comprehensive plans for pest management greatly improve effectiveness, profitability, and safety of pest control efforts over what can be achieved with piecemeal approaches. Important keys to effective, profitable, and environmentally safe pest management are careful monitoring of pest populations, complete records of infestation levels and controls applied, and comprehensive benefit-vs-cost analyses. To provide necessary training and technology, IPM programs must be supported by multidisciplinary research and extension efforts through the USDA and land grant universities and working with the food industry. A broad range of control options available for incorporation into IPM programs now exists.

1. *Biological controls*. Natural enemies such as parasites, predators, pathogens, and competitors of pest species in applied control programs.
2. *Cultural controls*. Methods such as tillage, host plant resistance, and crop rotation to reduce pest infestations in production systems.
3. *Chemical controls*. Chemical toxicants, repellents, protectants, growth regulators, germination inhibitors that are used to regulate populations of insect pests, plant pathogens, and weeds.
4. *Mechanical and physical controls*. Approaches that have greatest application in postharvest pest management include heat and cold treatments, sanitation, and protective packaging.
5. *Legislative controls*. Imposition of inspection and quarantine regulations to prevent importation and spread of introduced pests in living plants and animals or in harvested commodities.

IPM programs combine these control options to achieve the safest and most cost-effective regulation of all types of pests in food production, processing, and distribution systems.

SETTING THE STAGE FOR IPM: THE PESTICIDE ERA

Throughout most of the history of agriculture, a lack of highly effective, unilateral control measures (such as modern-day chemical pesticides) resulted in the use of various combinations of controls (eg, integrated control) to reduce losses caused by pest species. Controls included a variety of cultural measures such as cultivation and crop rotation; removal of insects and weeds from crops by hand; and application of inorganic pesticides containing active ingredients such as sulfur, lime, and arsenic. It was not until the modern insecticide era after World War II that use of highly effective compounds such as DDT and BHC (benzene hexachloride) introduced a new philosophy of pest control. Within a few years of their first applications, these organochlorine insecticides were used extensively to eradicate pest populations. Little concern was given to the potential for deleterious consequences to nontarget species, hazards to farm workers, or harmful residues in human food. The ready availability and high degree of effectiveness of these pesticides resulted in reliance on them as a unilateral control. By 1966, nearly 150 million pounds of insecticides were being applied per year in the United States, most of which was used in crop production (10). Although insecticides were the first of the chemical pesticides to be used in such vast quantities, research efforts led to the discovery of phytotoxic chemicals that were used as herbicides. Herbicide use peaked at more than 450 million pounds per year in 1982 (10). The increase in the quantity of fungicides applied has been much more modest, from 21 million pounds in 1966 to 37 million pounds in 1992 (2).

The availability of highly effective chemical insecticides has certainly been an important factor contributing to the significant reduction in incidence of arthropod-borne diseases in humans and domesticated animals. Chemical pesticides contributed to the 230% increase in agriculture productivity in the United States from 1947 to 1986 (11). Ease of application and the relatively low cost of these broad-spectrum pesticides have resulted in their tremendously high levels of use in food production and processing systems. By 1970, insecticide use in cotton in the United States had exceeded 70 million pounds per year. By 1985, herbicide use in corn had reached 250 million pounds per year (10).

The extensive reliance on chemical pesticides has led to serious problems. Questions relating to continued use of these compounds continue to be asked and have resulted in the cancellation of registrations for many uses of these products. In extreme instances, as many as 40 to 60 applications of pesticides have been made per year in cotton (12), with the result being serious issues of environmental pollution and mortality of nontarget species. The following are among the problems resulting from excessive use of chemical pesticides.

- Environmental contamination where pesticides in soils have entered surface and groundwater, threatening the safety of water supplies for human and animal consumption (2,10).
- Increasing human health risks resulting from exposure to residues in food and water supplies, particularly relating to the potential of some pesticides to cause cancer, disrupt the endocrine system, and interrupt normal development of the central nervous system in children (2). Despite cancellation of registrations for nearly all organochlorine insecticides in the U.S., residues of these compounds are still commonly detected in food products because of their use in other countries and long life in the environment.
- Threats to nontarget organisms such as wildlife species in both terrestrial and aquatic environments (2).
- Mortality of beneficial parasitic, predatory, and pathogenic species that often serve important roles in regulating pest populations (2,13). Destruction of beneficial organisms contributes to problems of pest resurgence and outbreaks of secondary pest species.
- Development of resistance in pest species to chemical pesticides. This is a growing problem for all types of pests worldwide, with more than 600 arthropod species, more than 100 species of plant pathogens, and more than 100 species of weeds exhibiting resistance to insecticides, fungicides, and herbicides, respectively, by 1996 (14).
- Increasing costs associated with intensive usage of chemical pesticides in crop production (10).

The increased urgency to find solutions to these problems has resulted in the passage of several major pieces of legislation by the U.S. government. The Federal Environmental Pesticide Control Act of 1972, comprised of extensive amendments to the Federal Insecticide, Fungicide, and Rodenticide Act of 1947, and the Food Quality Protection Act of 1996 will continue to have great impacts or availability and use patterns for chemical pesticides in the future. These problems have also generated great emphases for research and extension efforts to develop and employ alternative controls.

SETTING THE STAGE FOR IPM: ENTERING THE IPM ERA

Although environmental contamination and food safety concerns have resulted in some increased interest in development of integrated control programs to reduce reliance on chemical pesticides, the major impetus for reduction in their usage has resulted from political activism. Rachel Carson's *Silent Spring* (15) was a catalyst that resulted in a strong negative reaction by the general public to the extensive use of chemical pesticides, especially the organochlorine insecticides such as DDT. The public reaction to threats of environmental pollution and contamination of the food supply by pesticide residues (16) resulted in cancellation of all registrations for most organochlorine insecticides. Although insecticides belonging to the organophosphate and carbamate classes replaced the organochlorine compounds for most uses, growing support among farmers and consumers for integrated control programs has resulted in reduced reliance on these chemical insecticides and a movement from an industrial to an ecological model of pest management (17).

Public support for the IPM concept has led to gradual development of a philosophy that integrates pest control tactics and crop management from both agronomic and economic standpoints (18). From an original, relatively narrow focus on field-crop production (19), application of principles of IPM has now attained a much broader focus to include processing and distribution systems for food commodities. In relation to crop production, application of IPM provides a basis for continued profitable production of food and fiber commodities with much less threat of environmental degradation or harmful residues in food. Employing principles of IPM in processing and distribution of commodities is an additional, important step in maintaining a safe and wholesome food supply.

FOOD SAFETY AND IPM

Many countries maintain strict guidelines for the wholesomeness and safety of food products sold to end-user consumers. Food products must be free of contamination by harmful chemical residues and biological organisms, such as insects or microorganisms. They must also comply with standards of purity, composition, and preparation that are specified by regulatory agencies (see the article FOOD REGULATIONS: INTERNATIONAL, CODEX ALIMENTARIUS). The food safety challenge for industry requires delivery of products that are free of pest contamination while also containing pesticide residues that are below legal minimum levels.

Pests, Pesticides, and Regulations

Hundreds of species of insects and mites are known to infest grain and other raw commodities in storage, and many of these organisms infest grain-based food products after processing and packaging. Pest infestation in raw grain can begin a cascade of problems with serious economic and food safety implications. Infestation of bulk-stored grains by insects can result in weight loss of the grain, as well as, increased heating and moisture levels that facilitate growth of molds and spread of mycotoxins. The end result of these combined infestations by insects and molds is that grain may be rendered unusable (20).

Most insects infesting grain being prepared for milling can be removed with grain-cleaning equipment before grinding into flour; however, eggs of some species may survive cleaning and contribute to subsequent infestations in processed foods. Additionally, insect larvae and pupae inside grain kernels cannot be removed, but are milled with the flour and remain as fragmentary contaminants. Insect fragments, rodent hair, feces, and other foreign material are classified as filth that must be kept below legal threshold levels. Though not toxic, insect fragments can have direct human health effects as allergens for some persons (21). Contamination by insect-related filth signals an overall low level of quality and wholesomeness of food products.

Chemical insecticides are used in two principle ways to limit infestation of stored grains by insects. Insecticides in the form of residual protectants are used as grain enters storage facilities to prevent infestation during storage (see the article GRAINS AND PROTECTANTS). Fumigant insecticides are used to eliminate insect infestations that have developed after commodities are placed in storage. Fumigants such as phosphine and methyl bromide usually leave no detectable residue that may contaminate food. For this reason, fumigants are not considered significant threats to food safety; however, the lack of residual effects with these chemicals means that they do not protect grain from reinfestation after treatment. By contrast, residual protectant insecticides, which are used to treat raw grain and are applied throughout the food system to treat structures and equipment where foods are processed and packaged foods are stored, may pose problems through contamination by residues. Insecticide residues can remain in stored grain for months or years; chemical degradation is slow in the storage environment because of low light or darkness, stable or gradually changing temperatures, and relatively low moisture levels. Milling and, to a greater extent, baking or cooking cause substantial degradation of residues. However, some residues may still remain as the original chemical used in treatment or in the form of degradation products (22).

Pesticide residues in food pose both proved and suspected health risks to humans, and they generate much public concern and governmental regulatory action throughout the world (see the article PESTICIDE RESIDUES IN FOOD). The commonly used residual insecticides applied in grain storage facilities belong to the organophosphate and pyrethroid chemical classes. In the United States, no pyrethroids and relatively few organophosphate insecticides are registered for application directly to grain. However, additional organophosphates and certain pyrethroids can be used in structural surface treatments (see the article GRAINS AND PROTECTANTS). The Codex Alimentarius Commission has established international guidelines for permissible residues of organophosphates and pyrethroids in both raw grain and processed food products. Individual governmental regulatory agencies in most countries have generally concurred with these guidelines by passing appropriate regulations for enforcement.

Recent surveys of U.S. stored grain for detection of pesticide residues have not revealed levels above established tolerances, but the majority of samples contained detectable residues of organophosphates (Table 1). In spite of evidence for compliance by the food industry with established tolerance levels for pesticide residues, consumer advocacy organizations have called for stricter tolerance guidelines. The Food Quality Protection Act (FQPA) passed by the U.S. Congress in 1996 mandates a reassessment of all existing pesticide registrations with strict attention to human safety. The FQPA emphasizes accumulated risk resulting from exposure to classes of chemical pesticides, not just to individual active ingredients. For example, all organophosphates used in a variety of applications to food commodities, inside homes, on lawns, and in public areas will be considered together in estimating accumulated exposure. Risks will be assessed based on the most sensitive members of the population, among those being infants and children. Residues in food appear to constitute the highest exposure risks because they result in direct ingestion of pesticides.

Table 1. Detection of Insecticide Residues in Wheat Samples from Grain Elevators in Major Wheat-Producing Regions of the United States

Insecticide	Percentage of samples with detectable levels in a given year[a] 1995 (600)	1996 (340)	1997 (623)
Carbaryl	0.5	0.3	0.3
Chlorpyrifos	19.5	14.4	6.4
Chlorpyrifos methyl[b]	54.2	73.2	55.6
Dichlorvos	0	0	0
Malathion[b]	71.0	70.3	68.2
Methoxyhlor	1.0	4.7	5.2
Methyl parathion	0	0.3	0.2

Source. Data compiled from Refs. 23–25.

[a] Number in parentheses refers to the number of samples analyzed in a given year.

[b] Registered by U.S. Environmental Protection Agency for direct admixture to grain as a protectant; other insecticides residues presumably resulted from other applications.

Organophosphate insecticides have been targeted as a class for initial review and potential elimination by the U.S. Environmental Protection Agency because of their activity as neurotoxins and their potential as endocrine disruptors (26). Cancellation of registrations for organophosphates currently applied to grain and used throughout the food industry would create a great need for effective controls as replacements.

The fumigant methyl bromide is a fast-acting, residue-free chemical that is used routinely in flour mills, food processing facilities, and warehouses to control insect infestations. It has perhaps been the most effective insecticide used in the value-added food industry, but registrations for this product are slated for cancellation. Because methyl bromide is an ozone-depleting substance and may pose a threat to the protective atmospheric ozone layer around the earth, its production and application are scheduled to cease in the United States by 2005 as mandated by the Clean Air Act. Other industrialized and developing countries worldwide are eliminating or severely reducing the use of methyl bromide over the next several decades in compliance with an international agreement known as the Montreal Protocol (27). In this case, an environmental rather than a food safety concern is fueling the campaign for cancellation of an effective and residue-free insecticide for the food industry. This cancellation could possibly lead to increased use of residual insecticides. Ideal substitutes for methyl bromide have not been found, but much research and discussion are aimed at developing safe alternatives for the food industry. One alternative that may become quite important is IPM, an approach that could keep chemical insecticide usage at a minimum while utilizing a variety of alternative controls to limit pest populations.

The Case for IPM in the Food Industry

It seems apparent that the impact of more stringent regulations on pesticide use in the food industry, in combination with growing consumer demands for more wholesome and pesticide-free foods, must result in more extensive adoption of IPM practices. Although purchase contract specifications for grain commonly stipulate that there can be no detectable pesticide residues, they also stipulate that the grain be of relatively high quality with little insect damage (28). In addition, the tolerance levels for insect contaminants in value-added, finished food products are even lower than those for raw commodities, essentially a zero tolerance. For the future, it is clear that raw commodities destined for use in food products must be kept in good condition by combined action of several preventative measures employed through an IPM program. Similarly, pest-free, finished products in warehouses and retail outlets that are at risk for reinfestation must be protected until purchased by the consumer.

Managers at all levels of the food processing and marketing continuum, from bulk storage of commodities to retail outlets of high-value, finished products, must adopt IPM strategies that include sanitation, prevention, effective monitoring, and informed decision making regarding pest suppression tactics. Informed decision making based on cost-vs-benefit analyses is the cornerstone of IPM and should guide pest control decisions at all levels of the food industry. The EIL concept explained earlier in this article must become the basis for these decisions. Because the relative value of products varies greatly through the different levels of the food industry, so also will the economic thresholds used to guide control decisions.

IPM FOR FRESH COMMODITIES WITH ZERO PEST TOLERANCE

Fresh fruits and vegetables are sold as soon after harvest as possible, and there is typically little time or need for postharvest IPM. However, IPM is practiced during production of these commodities to prevent damage, and those involved with postharvest handling must be concerned with quality preservation. Only undamaged, blemish-free fruits and vegetables are selected for the fresh table market. Producers must avoid damage that is evident in an exterior examination and also eliminate insects confined within tissues of harvested produce. These pests are not only serious problems for the domestic market, but in the export market they are often the targets of legal quarantines enforced to prevent the spread of pest species.

Fumigants such as methyl bromide and ethylene dibromide were once commonly used to ensure that fresh fruits and vegetables for export were pest-free (29), but these chemicals are now being replaced by nonchemical methods. Safe and effective physical controls are being used commercially, such as cold treatment to eliminate larvae of codling moths from apples and vapor heat treatment to kill fruit flies infesting papayas (30). Ionizing radiation is an effective treatment for killing immature insect life stages inside fruits and vegetables while maintaining product quality (31). However, radiation has only limited commercial adoption for use with fresh produce because of serious opposition in some locations by consumers who reject irradiated food. Research on controlled atmospheres using high carbon dioxide concentrations or low oxygen levels shows promise for controlling postharvest pests of fresh commodities, but practical limitations in maintaining proper treatment conditions has hindered commercial adoption of these methods (32).

POSTHARVEST IPM FOR RAW, DURABLE COMMODITIES

Although IPM methods discussed here address stored grain almost exclusively, the same concepts and methods apply to other durable commodities such as legumes, nuts, dried fruits, and other dried commodities that can be stored in bulk. Cereal and feed grains comprise the most important basic inputs to the world food supply, and because of their seasonal growth patterns, they must be safely stored and maintained for year-round consumption.

Grain Storage: Pest Prevention and Sanitation

Insect pest problems in stored grain can be prevented or delayed by limiting access to the storage facility. With exception of a few species that may infest ripening grain to a limited extent before harvest, stored grain insects do not infest crops in the field and normally do not enter storage facilities with the new harvest (33). Insects that become pests in storage facilities must have dried grains or other appropriate food on which to feed and reproduce. Typically, the source of insects infesting a newly stored commodity is from populations already infesting some previously stored material. A common source of infestation is from residues of grain in empty storage structures (bins or silos), harvesting and conveying equipment, and transportation vehicles. Grain storage structures and grain-handling machines must be thoroughly cleaned of residual grain before a subsequent crop is stored. If grain is stored in an insect-free structure then the only source of insects to infest grain is by immigration into the structure. Every effort must be made to repair leaks and close openings in grain bins and silos to prevent insect entry. Complete exclusion of insects from grain storages is extremely difficult, but minimizing entry points does reduce potential routes of infestation. Once storage structures are cleaned and sealed, surfaces should be treated with a residual spray of a registered insecticide to provide protection against insects that may remain hidden within the structure or that may immigrate into the bin soon after grain is stored.

Monitoring Storage Conditions

Insect population growth in grain is dependent primarily on temperature of the air and/or grain, moisture content of the grain, and the quality of the grain as a food source (33). Because most grains and grain products are nutritionally adequate, unless they have been severely degraded by microbial agents, food quality is rarely limiting for insects infesting stored grain. Temperature and grain moisture may vary greatly among storage situations and are important for insect survival and population growth. Biological factors, such as development time from egg to adult, survival rates, and the number of eggs produced by females all attain maximum levels within optimal ranges of temperatures and grain moisture. Typically, these life history variables decrease at temperatures and moisture contents either above or below the optimal zone. Insects that feed as larvae inside grain kernels are more sensitive to grain moisture content than are the external feeders. Optimal grain moisture levels for growth and survival of internal grain feeders ranges from 12 to 15%. Optimal temperatures for growth and development of stored grain insects are between 25 and 32°C. Most species experience mortality or reduced population growth at prolonged periods above 40°C and cease development below 15°C (34). It is clear, therefore, that insect population growth can be slowed by maintaining grain in a cool, dry condition.

For effective stored-grain IPM, it is essential to have the best information possible about the current status of the commodity being managed and the potential pest populations at any time. As grain enters the storage facility, it should be characterized for quality factors such as bulk density, percent composition comprised by broken kernels and foreign material, and moisture content. Information on initial moisture content is critical because high-moisture grain may need to be stored separately, mechanically dried, or later blended with drier grain to attain a composite grain mass that is safe for storage.

The storage structure should be equipped with thermocouple cables or similar devices to monitor grain temperature at various locations throughout the grain mass during the storage period. Temperature cables are typically hung from the roof of a storage structure and extend vertically through the grain mass. Thermocouples are positioned on cables at a spacing of 2 to 3 m from bottom to top of the mass, and a series of cables should be deployed at 5 to 10 m intervals so that the grain mass is evenly monitored. Temperatures from each monitoring point should be recorded on a regular basis, ideally weekly or biweekly, and any abrupt increases in temperature over a short period of time should be investigated by sampling the grain mass. Temperature cables are useful in detecting hot spots where moisture, mold growth, and insect infestation may have begun. Temperature monitoring is also important to check the progress of grain cooling during aeration.

The species and numbers of insects in stored grain can best be monitored by direct and systematic sampling of the grain and by use of traps. Direct grain sampling is the best monitoring approach, but it is labor intensive and requires a certain level of skill. Managers of grain storage facilities can easily learn the required methods and implement grain sampling for insects as part of their regular commodity management routine.

Samples can be obtained to a depth of 1 to 2 m by using a spear-shaped sampler to withdraw grain from the mass, or sampling may be done as grain is moved from bin to bin within a facility using a cup-type (pelican) sampler (Fig. 1). Increasing the number of samples enhances the level of accuracy achieved in estimating insect population density (35). For example, five 1-kg samples must be taken from each 27 metric tons (1000 bushels) of grain to determine with 90% accuracy if a grain mass contains as many as two insects per kg, which is a common economic threshold for control decisions. Grain samples must be processed with an appropriate sieve or sifting device to remove insects for identification and counting. Grain probe traps are also available that capture insects as they move through a grain mass (Fig. 1). These traps are effective for detection of insects at low population densities (36), but the data obtained are not easily converted to numbers of insects per unit of grain volume.

Decision Making for Insect Control

Correct interpretation of insect sampling data is important for making control decisions in stored grain IPM. As indicated earlier, decisions should be based on cost-vs-benefit analyses that incorporate assessments of the quality parameters. Raw grain is marketed based on quality, which is quantified by a numerical grade in the United States. The highest quality grain, in addition to having a high bulk density (test weight) and little or no foreign material, contains no live insects and has no measurable damage from insects. Damaged kernels result from feeding within by species such as weevils in the genus *Sitophilus* and the lesser grain borer, *Rhyzopertha dominica*, that chew holes through kernels. If one or more individuals of these species are found in a grain sample or probe trap sample, then more extensive sampling should be conducted. Discovery of damage by internal feeders may warrant a fumigation treatment to eliminate the infestation and prevent additional damage.

The presence of large numbers of external feeding insects, which cause no direct grain damage, may be revealed in grain or trap samples, but they can be tolerated until the time of sale as long as grain quality, temperature, and moisture remain acceptable. Live external feeding insects can be eliminated with an effective fumigation before grain is transported. It should be noted that many international markets will not tolerate even dead insects in grain, so treatment decisions must be adjusted accordingly.

Nonchemical Controls for Stored Grain Pests

Effective IPM programs for stored grain involve a variety of approaches intended to prevent the occurrence of damaging pest infestations. Sanitation was discussed earlier as a preventative measure used before grain enters the storage facility. Among the most effective nonchemical means for pest prevention or suppression after grain is in storage is use of ambient air cooling with aeration. This is achieved with air flow from fans mounted at the base of the storage structure that either blow air into the bottom of the mass and force it upward or draw air through roof vents downward through the grain mass. Effective aeration cooling occurs only when the outside air temperature is below the temperature of the grain. Thermostatically

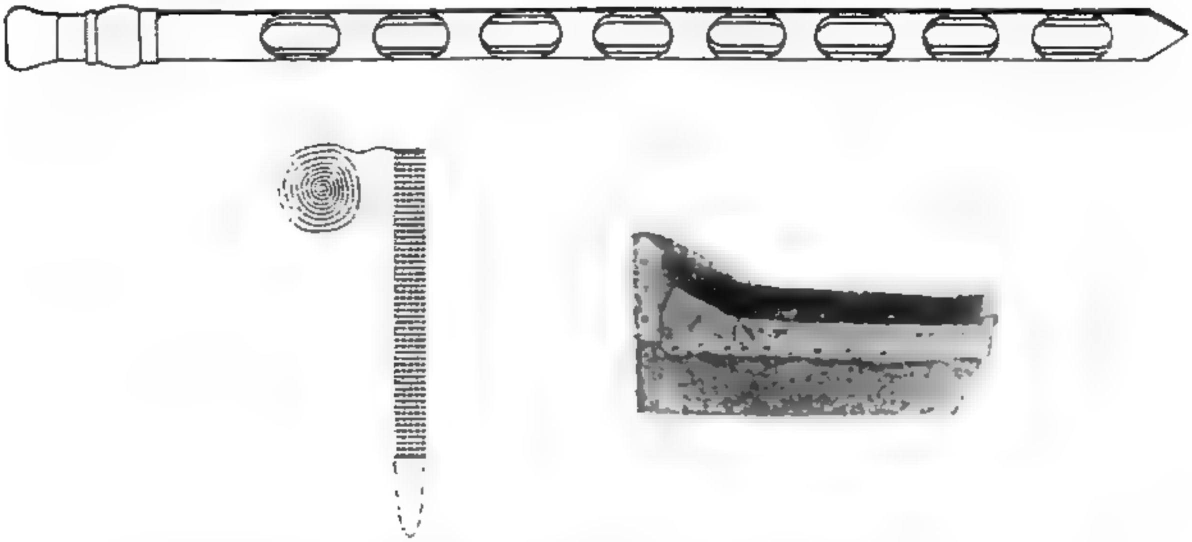


Figure 1. Devices used to sample grain for insect population monitoring. (a) A spear-shaped grain trier to extract a sample of grain from bulk storage. (b) An insect probe trap that is inserted into a grain mass; insects moving through the grain pass through the holes in the trap and fall into the collection tip. (c) A pelican sampler used to collect a sample of flowing grain as it falls vertically.

controlled fans can be used to ensure that they operate at the proper ambient temperatures (37). Because insect developmental rates are directly influenced by temperature, lowering the temperature of the grain mass below the optimum level for insect growth (below 15°C for most species) can significantly reduce population increase and the potential for damage. Low grain temperatures are achieved most efficiently in temperate regions when seasonal changes from summer to fall result in lower ambient air temperatures. However, even if grain cannot be cooled below 15°C immediately, any level of cooling can be effective in reducing rates of insect population increase (33).

Simulation models of temperature-dependent insect population growth rates have been developed for the most damaging stored grain pests. These models verify the benefits of aeration for suppression of pest populations (Fig. 2). Although costs of aeration depend on local electricity costs, in most cases expenditures are justified based on reductions in insect infestations that can be achieved (38).

Biological controls may provide additional nonchemical methods for pest control in stored grain. The community of insect species that infests stored grain includes those that consume the grain itself, those that feed on molds that infect the grain, and parasitoids and predators that feed on other insects. Studies have been published in which experimental populations of grain pests have been reduced by addition of natural enemies to storage containers (39). A recent study conducted in grain bins has validated the efficacy of introducing parasitic wasps to suppress populations of the lesser grain borer (40). The objective of these studies has been to identify beneficial species that can maintain pest populations below the EIL. Although some companies mass produce and sell parasitoids and predators of stored grain pests, the use of beneficial insects in grain storage facilities has not yet been widely adopted.

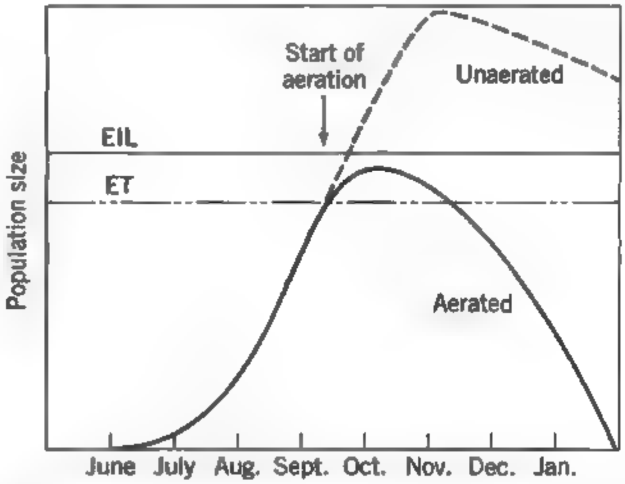


Figure 2. Conceptual plot of change in the size of a grain insect population, with and without aeration to cool grain. ET, economic threshold; EIL, economic injury level.

Chemical Insecticides for Stored Grain Pests

When alternative nonchemical controls fail to maintain insect pest populations below EILs, chemical insecticides remain the most effective tools for pest suppression in stored grain. Fumigant insecticides are ideal for bulk-stored commodities because they are released as gases that penetrate the commodity in place. Phosphine gas, or hydrogen phosphide, is the most commonly used fumigant for stored grain worldwide (41). Phosphine formulated as pellets or tablets remains a relatively inexpensive control costing a few cents per bushel (38). Phosphine is liberated from pellets or tablets of aluminum or magnesium phosphide upon reaction with water in the air. Because phosphine has a density close to that of air, it penetrates bulk commodities well. It must be stated, however, that this same low-density property can be a detriment because it allows phosphine to dissipate easily if structures are not sealed well. Aluminum phosphide pellets are typically added to grain that is being transferred from one storage structure to another or to a transport vehicle, allowing even distribution throughout the grain mass.

Recirculation methods have been developed that allow adequate penetration by the gas even when pellets are applied just to the top of a large grain mass and/or to a bottom access via the aeration plenum. Phosphine accumulated in the headspace above the grain is drawn through a pipe in the roof of the structure and reintroduced at the bottom of the mass, where it travels upward through the mass and attains even distribution (42). Phosphine is relatively slow acting, and several days exposure at optimum temperatures may be required to produce insect mortality. Long exposure time for bulk grain is usually not a problem because considerable time is available during storage or transport. Treatment times may be reduced by use of phosphine gas dissolved in carbon dioxide and contained in gas cylinders now becoming available in some countries. This formulation also provides a means for rapid, even distribution of gas throughout a storage structure.

Grain protectants are among the most commonly used controls for insects in stored grain. These residual insecticides are applied to grain at the time it initially goes into storage to provide protection against infestations for several months. Although more expensive than phosphine on a per bushel basis, protectants are the preferred control if a high risk of infestation exists. Among the disadvantages of grain protectants, as mentioned earlier, is the potential for residues to remain in the grain at the time of sale. Also, as the time grain is kept in storage increases, the possibility exists that the insecticide may lose effectiveness. There is also evidence that some species are not effectively controlled because of the development of resistance to the insecticides (43). Malathion, for example, remains a commonly used grain protectant in the United States, though it is well established that many populations of the common stored grain insects have evolved resistance to this compound (43). Because of these problems and the growing expense of protectant applications, the future of this approach for control of stored grain insects is in doubt. In addition, the use of protectants is not consistent with principles of IPM because they are often applied with little assessment of potential pest infestation levels. It is possible that use of insecticides as preventative measures will be restricted to applications as residual sprays in empty structures as part of a comprehensive sanitation program.

Diatomaceous earth is a naturally occurring absorbent dust that poses no chemical residue or food safety problems. Insects are naturally protected from dehydration by waxes deposited on the surface of the exoskeleton. Diatomaceous earth kills insects that contact it by absorbing and removing waxes from the exoskeleton, and death occurs due to desiccation (44). However, diatomaceous earth is as expensive to use as synthetic chemical insecticides, and it reduces the bulk density and the value of grain with which it is thoroughly admixed. Additionally, because its effectiveness is yet to be well established, the future contribution of diatomaceous earth to IPM in stored products is not clear.

IPM FOR FOOD PROCESSING

The information in this section relates to any facility in which a raw commodity such as grain is processed in any way to make a more valuable product; such a product may be ready for the consumer market or it may be an intermediate product that is processed further at another facility. Thus, food processing here refers to activities of flour mills, bakeries, pet food plants, breakfast cereal manufacturers, snack food plants, chocolate factories, and confectioneries.

Stored-product insects that infest food processing facilities are essentially the same species that infest raw grain, but the species composition and their relative importance is different. Internal grain feeding insects such as weevils and borers are not as important in processing environments because the concern for grain damage is no longer an issue. Species considered secondary in importance in raw grain because they feed only on broken kernels, such as flour beetles, sawtoothed grain beetles, and the Indianmeal moth, are considered of primary importance in food processing because they can easily infest milled products and contribute to problems with contaminants. For example, the Association of Operative Millers has listed the red flour beetle as the most important insect pest of flour mills because of its ability to survive and reproduce in flour. This species is also difficult to control in flour mills (45). By comparison, the red flour beetle, though common in stored grain, is rarely considered more than a nuisance. Also important is the consideration that once grain is milled into flour, its value increases greatly, and EILs for pests decrease accordingly.

Sanitation and Prevention

Food processing facilities must devote personnel and other resources to sanitation, pest monitoring, and pest control if they are to maintain a level of cleanliness and product quality that is demanded by consumers and mandated by governmental regulations. As with stored grain, preventive measures are basic to pest management programs. For flour mills and other processing plants, the sanitation routine should include daily or twice daily cleaning of equipment and floors where flour dust can accumulate (46). Use of compressed air to blow dust from machines and floors is a popular cleaning tool, but it should used sparingly so that dust is not simply blown into hard-to-clean areas. Food debris that accumulates in these areas can support residual populations of pests that constantly contribute to infestation of the product. Sweeping is effective when combined with vacuum cleaning. Problem areas for dust accumulation should be identified and regularly targeted with vacuum cleaning. Spills need to be cleaned immediately and broken packages discarded. Broken windows, window screens, and other structural components should be repaired promptly to limit entry of food pests into a facility. Rotation of raw and finished stock to utilize older products first represents a simple preventive measure to avoid the opportunity for growth of pest populations. Common-sense approaches to sanitation and prevention need to be adopted, but in many facilities these simple measures are often overlooked in favor of increased production.

Monitoring for Pest Infestations

Active efforts at pest detection and monitoring should be incorporated into a regular routine with sanitation and prevention efforts of food plants. Direct sampling of products and residues from the production line can alert managers to the presence of incipient pest problems (46). As dust and siftings from different points in the milling process are cleaned up, these materials should be carefully inspected daily for the presence of insects. Similarly, floor sweepings and the residue from machinery clean-outs must be inspected. Boots of elevator legs and the bottom trays of vertical conveyor systems contain spaces where food and pests can accumulate, and they should be cleaned and inspected regularly. Insect traps baited with attractants for certain species, or designed to capture any insects present, can be used throughout a facility to determine the presence, relative abundance, and location of pests (47). Traps baited with pheromones are highly sensitive to the presence of the target species for the attractant. By deploying traps in a regular gridlike arrangement throughout facilities, managers can identify areas with the highest likelihood of insect infestation and concentrate monitoring and control efforts in these areas. Monitoring data from traps or product and residue samples must be documented and the records maintained over time, so that trends in pest numbers can be observed and used in IPM decisions.

Controlling Pests Without Methyl Bromide

Methyl bromide has been used effectively for many years to suppress pest populations in structures such as flour mills and food processing plants (48). Grain millers and other processors consider this chemical to be an essential component of their pest control and sanitation programs. Methyl bromide will be unavailable for most uses in the United States and in most other industrialized countries beginning in 2005. No fumigant gas is available that can directly replace methyl bromide for rapid, effective insect control without the potential harmful residues in food products. Phosphine is registered for use in structures containing food and leaves no harmful residues on food. However, phosphine often requires several days to achieve effective insect control, compared to several hours required with methyl bromide. Large flour mills and similar plants continue production 7 days weekly and cannot operate profitably with the long shutdowns that would be required for phosphine fumigation. Another concern of many food processors when considering fumigation with phosphine is that copper and other metal components of machinery can be corroded by phosphine at high humidities and warm temperatures (49). Electrical wiring, computers, microprocessors, and other sensitive electronic equipment may be put at risk when phosphine is used in flour mills or food processing plants. One innovative application method for phosphine as a substitute for methyl bromide is use of relatively low doses with elevated temperatures and the addition of carbon dioxide. This method may increase activity of the insecticide and reduce potential damage to machines by shorter exposure times, especially in areas where sensitive electronic equipment is housed (50,51). The presence of carbon dioxide and higher temperatures apparently increases respiration rates of insects and enhances mortality.

Various other physical and chemical methods have been proposed as alternatives to use of methyl bromide for pest control in food processing; however, none can be considered an effective replacement in terms of efficiency and effectiveness (52). Controlled atmospheres with high carbon dioxide or low oxygen concentrations have been proposed but are not practical or cost effective for food processing facilities because of the need for enormous amounts of gases released in structures that cannot be sealed tightly. Heat treatment, or heat sterilization, of entire facilities has been attempted with some success; however, for effective insect control it is necessary to maintain the temperature at approximately 50°C for 24 to 36 h (53). A major problem with heat treatment is the difficulty in attaining temperatures lethal to insects inside walls and machines and close to floors in basements.

Numerous residual insecticides have been proposed as replacements for methyl bromide. Their effectiveness is limited, however, because they often cannot be applied in all areas where control is needed because of risks of contamination of food products by chemical residues. Consequently, control must be attained through residual activity on treated surfaces where insecticide residues are not likely to contaminate food products with the hope that insects may be controlled through contact with treated surfaces (43). Also being evaluated in flour mills is a combination of elevated temperatures with a thorough dusting of diatomaceous earth (54). This method also has limitations for control of insects harbored deep within structures. It seems apparent that when registrations for methyl bromide are finally cancelled, food processing facilities will be forced to develop more intensified efforts at prevention, vigilant monitoring, and specialized mitigation measures to replace the current unilateral applications of methyl bromide for pest control.

IPM FOR VALUE-ADDED FOOD PRODUCTS

At the end of the food processing continuum are the high-value, packaged products ready for retail marketing to consumers. After leaving a processing facility, these products are typically transported to regional wholesale distribution centers where they may be stored for periods ranging from a few days to many months. Although ownership of these products may reside with retail marketers, problems with pest infestations during storage and even at retail outlets are often blamed on the food processors. Regardless of who takes the responsibility for protection of these products, substantial investments in preventive measures must be made to protect finished products from infestation.

Internal corporate regulation of finished product quality must be maintained through a system of regular inspections and business-partner accountability. As a first avoid the opportunity for growth of pest populations. Common-sense approaches to sanitation and prevention need to be adopted, but in many facilities these simple measures are often overlooked in favor of increased production.

Monitoring for Pest Infestations

Active efforts at pest detection and monitoring should be incorporated into a regular routine with sanitation and prevention efforts of food plants. Direct sampling of products and residues from the production line can alert managers to the presence of incipient pest problems (46). As dust and siftings from different points in the milling process are cleaned up, these materials should be carefully inspected daily for the presence of insects. Similarly, floor sweepings and the residue from machinery clean-outs must be inspected. Boots of elevator legs and the bottom trays of vertical conveyor systems contain spaces where food and pests can accumulate, and they should be cleaned and inspected regularly. Insect traps baited with attractants for certain species, or designed to capture any insects present, can be used throughout a facility to determine the presence, relative abundance, and location of pests (47). Traps baited with pheromones are highly sensitive to the presence of the target species for the attractant. By deploying traps in a regular gridlike arrangement throughout facilities, managers can identify areas with the highest likelihood of insect infestation and concentrate monitoring and control efforts in these areas. Monitoring data from traps or product and residue samples must be documented and the records maintained over time, so that trends in pest numbers can be observed and used in IPM decisions.

Controlling Pests Without Methyl Bromide

Methyl bromide has been used effectively for many years to suppress pest populations in structures such as flour mills and food processing plants (48). Grain millers and other processors consider this chemical to be an essential component of their pest control and sanitation programs. Methyl bromide will be unavailable for most uses in the United States and in most other industrialized countries beginning in 2005. No fumigant gas is available that can directly replace methyl bromide for rapid, effective insect control without the potential harmful residues in food products. Phosphine is registered for use in structures containing food and leaves no harmful residues on food. However, phosphine often requires several days to achieve effective insect control, compared to several hours required with methyl bromide. Large flour mills and similar plants continue production 7 days weekly and cannot operate profitably with the long shutdowns that would be required for phosphine fumigation. Another concern of many food processors when considering fumigation with phosphine is that copper and other metal components of machinery can be corroded by phosphine at high humidities and warm temperatures (49). Electrical wiring, computers, microprocessors, and other sensitive electronic equipment may be put at risk when phosphine is used in flour mills or food processing plants. One innovative application method for phosphine as a substitute for methyl bromide is use of relatively low doses with elevated temperatures and the addition of carbon dioxide. This method may increase activity of the insecticide and reduce potential damage to machines by shorter exposure times, especially in areas where sensitive electronic equipment is housed (50,51). The presence of carbon dioxide and higher temperatures apparently increases respiration rates of insects and enhances mortality.

Various other physical and chemical methods have been proposed as alternatives to use of methyl bromide for pest control in food processing; however, none can be considered an effective replacement in terms of efficiency and effectiveness (52). Controlled atmospheres with high carbon dioxide or low oxygen concentrations have been proposed but are not practical or cost effective for food processing facilities because of the need for enormous amounts of gases released in structures that cannot be sealed tightly. Heat treatment, or heat sterilization, of entire facilities has been attempted with some success; however, for effective insect control it is necessary to maintain the temperature at approximately 50°C for 24 to 36 h (53). A major problem with heat treatment is the difficulty in attaining temperatures lethal to insects inside walls and machines and close to floors in basements.

Numerous residual insecticides have been proposed as replacements for methyl bromide. Their effectiveness is limited, however, because they often cannot be applied in all areas where control is needed because of risks of contamination of food products by chemical residues. Consequently, control must be attained through residual activity on treated surfaces where insecticide residues are not likely to contaminate food products with the hope that insects may be controlled through contact with treated surfaces (43). Also being evaluated in flour mills is a combination of elevated temperatures with a thorough dusting of diatomaceous earth (54). This method also has limitations for control of insects harbored deep within structures. It seems apparent that when registrations for methyl bromide are finally cancelled, food processing facilities will be forced to develop more intensified efforts at prevention, vigilant monitoring, and specialized mitigation measures to replace the current unilateral applications of methyl bromide for pest control.

IPM FOR VALUE-ADDED FOOD PRODUCTS

At the end of the food processing continuum are the high-value, packaged products ready for retail marketing to consumers. After leaving a processing facility, these products are typically transported to regional wholesale distribution centers where they may be stored for periods ranging from a few days to many months. Although ownership of these products may reside with retail marketers, problems with pest infestations during storage and even at retail outlets are often blamed on the food processors. Regardless of who takes the responsibility for protection of these products, substantial investments in preventive measures must be made to protect finished products from infestation.

Internal corporate regulation of finished product quality must be maintained through a system of regular inspections and business-partner accountability. As a first step, conscientious food companies should set rigorous, internal standards and conduct sanitation audits on their own facilities and those of suppliers to ensure that their standards and those imposed through governmental regulations are met. Sanitation standards or specific good manufacturing practices (GMPs) are sometimes outlined in supplier contracts, placing suppliers at risk of losing business should their facilities fail to pass sanitation audits (55). All facilities should be inspected through the auditing system, including mills, processing plants, warehouses, and retail outlets.

Companies that produce value-added products can take several measures to prevent pest infestations through protective food packaging and even by engineering modifications to buildings. Many types of food packaging are at risk for penetration by insects; those comprised of paper and cellophane are least resistant. Polypropylene and similar polymer films provide some additional protection against boring and chewing insects, and their resistance to penetration improves with film thickness (56). The greatest protection is afforded by glass and metal containers. Certain food-safe, natural insect repellents have recently been approved by EPA for use in food packages to deter insect infestation, but the effectiveness of these agents has not been proved.

Structural design features for production facilities, warehouses, and retail outlets can be improved to enhance sanitation and pest control (57). Cracks, crevices, and other unnecessary openings in walls and floors should be sealed. Wall-to-floor junctions should be protected by concave molded tile to prevent harborage of pests, and wood-to-wood joints should be avoided or appropriately sealed. Voids in double-wall construction should be eliminated in new structures because they may allow accumulation of food debris and provide excellent harborage for pests. Physical separation of raw commodity storage areas, processing areas, and warehouses will help to prevent movement of pests from raw grain storage to high-value processed foods. Building exteriors should be free from vegetation, soil, and product spills. Exterior lights should not be mounted over doors or windows to avoid attracting insects to these openings at night. Rather, lights should be positioned away from the building and illuminate the structure from a distance.

ADOPTING IPM: CHALLENGE AND NECESSITY

The postharvest food industry faces serious challenges in pest management as a result of the attractiveness of food products to pests; greater public demands for safe, high-quality food products; and increasing regulatory constraints on use of chemical insecticides. It is apparent that the industry will no longer be able to rely on the quick fix provided by chemical pesticides such as methyl bromide. The industry must develop and adopt IPM programs to meet consumer demands and comply with governmental regulations. Fundamental components of the IPM programs for the food industry must include preventing infestations wherever possible through sanitation and pest exclusion by sealing of structures. Also, consistent monitoring programs and judicious decision making to guide the use of chemical pesticides will be critical. Alternatives to chemical pesticides, such as physical controls and biological control agents, must be used when possible. Thus, IPM in the food industry will be both preventive and responsive (58).

Decision-making parameters are not as well defined in the postharvest food industry as in production agriculture, but EILs and economic thresholds must be established for all key pests. Adoption of IPM practices is being encouraged through incentives at various levels in the industry. Some retailers are putting IPM labeling on products and report a favorable public response to these products. A positive public perception of IPM coupled with a desire to reduce pesticide inputs is giving momentum to the drive for adoption of IPM. Despite adoption of IPM, there are many unknowns regarding the safety and quality of the food supply with fewer pesticide inputs. Increased adoption of hazard analysis critical control points (HACCP) and related protocols will facilitate co-adoption of IPM for insect pests and should help ensure safety and quality. HACCP programs are customized for each food plant and are typically targeted at reducing risk from microbial contamination by detecting and identifying the potential for quality problems in a process or location, the so-called critical control points (see the article HAZARD ANALYSIS AND CRITICAL CONTROL POINTS [HACCP]). Flour mills also have HACCP plans centered around contamination (46), even though these facilities have fewer microbial problems compared to those with wet processes such as meat and dairy plants. Increased adoption of HACCP by the food industry has fostered a heightened sense of awareness for contamination and pest problems by facility managers. It should be possible for raw commodity and processed food managers to develop IPM programs modeled after a HACCP protocol. Such HACCP-based IPM would be custom designed for each facility and would identify key locations or activities that need to be addressed on a routine basis in order to prevent or mitigate pest problems.

BIBLIOGRAPHY

1. C. O. Knowles, "The Pesticide Revolution," *Proceedings of Pesticides. Risks, Management, Alternatives*, Virginia Polytechnic and State University, Blacksburg, Va., June 8–10, 1988.
2. C. M. Benbrook, *Pest Management at the Crossroads*, Consumers Union, Yonkers, N.Y., 1996.
3. D. J. Sissons and G. M. Telling, "Agricultural Chemicals," in S. R. Tannenbaum, ed., *Nutritional and Safety Aspects of Food Processing*, Marcel Dekker, New York, 1979, pp. 295–367.
4. G. F. Stewart and M. A. Amerine, *Introduction to Food Science and Technology*, Academic Press, Orlando, Fla., 1973.
5. G. W. Ware and J. P. McMullum, *Producing Vegetable Crops*, Interstate Printers and Publishers, Danville, Ill., 1980.
6. J. M. Luna and G. J. House, "Pest Management in Sustainable Agricultural Systems," in C. A. Edwards et al., eds., *Sustainable Agricultural Systems*, Soil and Water Conservation Society, Ankeny, Iowa, 1990, pp. 157–173.
7. R. Frisbie and J. Magaro, "IPM in the Southwest," *Proc. Amer. Chem. Soc. Meet.*, Point Clear, Ga., August, 1989.

8. E. G. Rajotte et al., *The National Evaluation of Extension's Integrated Pest Management (IPM) Programs*, Publication No. 491-010, Virginia Cooperative Extension Service, Blacksburg, Virginia, 1987.
9. C. J. Scifres, "Decision-Analysis Approach to Brush Management Planning for Integrated Range Resources Management," *Journal of Range Management* **40**, 482–490 (1988).
10. National Research Council, *Alternative Agriculture*, National Academy Press, Washington, D.C., 1989.
11. P. I. Szmedra, "Pesticide Use in Agriculture," in D. Pimentel ed., *Handbook of Pest Management in Agriculture*, Vol. 1, CRC Press, Boca Raton, Fla., 1991, pp. 649–677.
12 W. H. Luckmann and R. L. Metcalf, "The Pest Management Concept," in R. L. Metcalf and W. H. Luckmann, eds., *Introduction to Insect Pest Management*, Wiley, New York, 1982, pp. 1–31.
13. B. Croft, "Pesticide Effects on Arthropod Natural Enemies: A Database Summary," in *Arthropod Biological Control Agents and Pesticides*, Wiley, New York, 1990, pp. 17–46.
14. T. M. Brown, "Applications of Molecular Genetics in Combatting Pesticide Resistance, An Overview," in T. M. Brown, ed., *Molecular Genetics and Evolution of Pesticide Resistance*, American Chemical Society, Washington, D.C., 1996, pp. 1–8.
15. R. Carson, *Silent Spring*, Fawcett, Greenwich, Conn., 1962.
16. Food and Drug Administration, *Residues in Food; 1988*, Washington, D.C., 1988.
17. R. Levins, "Perspectives in Integrated Pest Management: From an Industrial to an Ecological Model of Pest Management," in M. Kogan, ed., *Ecological Theory and Integrated Pest Management Practice*, Wiley, New York, 1986, pp. 1–17.
18. V. M. Stern et al., "The Integrated Control Concept," *Hilgardia* **29**, 81–101 (1959).
19. B. A. Croft, "Integrated Pest Management: The Agricultural Rationale," in R. E. Frisbee and P. L. Adkisson, eds., *Integrated Pest Management on Major Agricultural Systems*, Texas A&M University, College Station, Tex., 1985, pp. 712–728.
20. D. S. Jayas, N. D. G. White and W. Muir, eds., *Stored Grain Ecosystems*, Marcel Dekker, New York, 1995.
21. J. K. Phillips and W. E. Burkholder, "Health Hazards of Insects and Mites in Food," in F. J. Bauer, ed., *Insect Management for Food Storage and Processing*, American Association of Cereal Chemists, St. Paul, Minn., 1984, pp. 279–290.
22. J. T Snelson, *Grain Protectants*, Australian Center for International Agricultural Research, Canberra, Australia, 1987.
23. U.S. Department of Agriculture, *Pesticide Data Program Annual Summary Calendar Year 1995*, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D.C., 1997
24. U.S. Department of Agriculture *Pesticide Data Program Annual Summary Calendar Year 1996*, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D.C., 1998.
25. U.S. Department of Agriculture, *Pesticide Data Program Annual Summary Calendar Year 1997*, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D.C., 1998.
26. L. Keith, *Environmental Endocrine Disruptors: A Handbook of Property Data*, Wiley, New York, 1997.
27. N. Price, "Methyl Bromide in Perspective," in C. H. Bell, N. Price, and B. Chakrabarti, eds., *The Methyl Bromide Issue*, Wiley, Chichester, U.K., 1996, pp. 1–26.
28. P. Kenkel et al., "Biological Preservation of Grain Quality: Losses in Storage and Handling," in J. L. Steele and O. K. Chung, eds., *Proceedings of the International Wheat Quality Conference*, Grain Industry Alliance, Manhattan, Kans., 1997, pp. 385–389.
29. J. D. Stark, "Chemical Fumigants," in R. E. Paull and J. W. Armstrong, eds., *Insect Pests and Fresh Horticultural Products: Treatments and Responses*, CAB International, Wallingford, U.K., 1994, pp. 69–84.
30. J. W. Armstrong, "Heat and Cold Treatments," in R. E Paull and J. W. Armstrong, eds., *Insect Pests and Fresh Horticultural Products: Treatments and Responses*, CAB International, Wallingford, U.K., 1994, pp. 103–119.
31. J. L. Nation and A. K. Burditt, Jr., "Irradiation," in R. E. Paull and J. W. Armstrong, eds., *Insect Pests and Fresh Horticultural Products: Treatments and Responses*, CAB International, Wallingford, U.K., 1994, pp. 85–102.
32. G. J. Hallman, "Controlled Atmospheres," in R. E. Paull and J. W. Armstrong, eds., *Insect Pests and Fresh Horticultural Products: Treatments and Responses*, CAB International, Wallingford, U.K., 1994, pp. 121–136.
33. D. W. Hagstrum, P. W Flinn, and R. W. Howard, "Ecology," in B. Subramanyam and D. W. Hagstrum, eds., *Integrated Management of Insects in Stored Products*, Marcel Dekker, New York, 1996, pp. 71–134.
34. P. G. Fields, "The Control of Stored-Product Insects with Extreme Temperatures," *Journal of Stored Product Research* **28**, 89–118 (1992).
35. G. W. Cuperus and R. C. Berberet, "Training Specialists in Sampling Procedures," in L. P. Pedigo and D. G. Buntin, eds., *Handbook of Sampling Methods for Arthropods in Agriculture*, CRC Press, Boca Raton, Fla., 1994, pp. 669–681.
36. B. Subramanyam and D. W. Hagstrum, "Sampling," in B. Subramanyam and D. W. Hagstrum, eds., *Integrated Management of Insects in Stored Products*, Marcel Dekker, New York, 1996, pp. 135–193.
37. R. T. Noyes, G. W. Cuperus, and P. Kenkel, "Using Controlled Aeration for Insect and Mould Management in the South-Western United States," in E. Highley et al., eds., *Proceedings of the Sixth International Working Conference on Stored-Product Protection*, CABI Press, Canberra, Australia, 1994, pp. 323–334.
38. P. Kenkel et al., *Current Management Practices and Impact of Pesticide Loss in the Hard Red Wheat Post-Harvest System*, Circular E-930, Oklahoma Cooperative Extension Service, Stillwater, Okla., 1993.
39. J. H. Brower et al., "Biological Control," in B. Subramanyam and D. W. Hagstrum, eds., *Integrated Management of Insects in Stored Products*, Marcel Dekker, New York, 1996, pp. 223–286.
40. P. W. Flinn and D. W. Hagstrum, "Suppression of Beetles in Stored Wheat by Augmentative Releases of Parasitic Wasps," *Environmental Entomology* **25**, 505–511 (1996).
41. V. E. Walter, "Fumigation in the Food Industry," in J. R. Gorham, ed., *Ecology and Management of Food Industry Pests*, FDA Technical Bulletin No. 4, Association of Official Analytical Chemists Arlington, Va. 1991, pp. 441–457.
42. R. T. Noyes and P. Kenkel, "Closed-Loop Fumigation Systems in the South-Western United States," in E. Highley et al., eds., *Proceedings of the Sixth International Working Conference on Stored-Product Protection*, CABI Press, Canberra, Australia 1994, pp. 335–341.
43. F. H. Arthur, "Grain Protectants: Current Status and Prospects for the Future," *Journal of Stored Products Research* **32**, 293–302 (1996).

44. Z. Korunic, "Diatomaceous Earths, A Group of Natural Insecticides," *Journal of Stored Products Research* **34**, 87–97 (1998).
45. D. L. Faustini, "How to Use *Tribolium* Pheromone Traps," *Association of Operative Millers Bulletin* (Leawood, Kansas), 5715–5717 (1990).
46. R. Mills and J. Pederson, *A Flour Mill Sanitation Manual*, Eagan Press., St. Paul, Minn., 1990.
47. T. W. Phillips, "Semiochemicals of Stored-Product Insects: Research and Applications," *Journal of Stored Product Research* **33**, 17–30 (1997).
48. B. Chakrabarti, "Methyl Bromide in Storage Practice and Quarantine," in C. H. Bell, N. Price, and B. Chakrabarti, eds., *The Methyl Bromide Issue*, Wiley, Chichester, U.K., 1996, pp. 237–274.
49. E. J. Bond, T. Dumas, and S. Hobbs "Corrosion of Metals by the Fumigant Phosphine," *Journal of Stored Product Research* **20**, 57–63 (1984).
50. D. K. Mueller, "A New Method of Using Low Levels of Phosphine in Combination with Heat and Carbon Dioxide," in E. Highley et al., eds., *Proceedings of the Sixth International Working Conference on Stored-Product Protection*, CABI Press, Canberra, Australia 1994, pp. 123–125.
51. U.S. Pat. 5,403,597 (April 4, 1995), D. K. Mueller.
52. P. C. Annis and C. J. Waterford, "Alternatives-Chemicals," in C. H. Bell, N. Price, and B. Chakrabarti, eds., *The Methyl Bromide Issue*, Wiley, Chichester, U.K., 1996, pp. 275–321.
53. K. O. Sheppard, "Heat Sterilization (Superheating) as a Control for Stored-Grain Pests in a Food Plant," in F. J. Bauer, ed., *Insect Management for Food Storage and Processing*, American Association of Cereal Chemists, St. Paul, Minn., 1984, pp. 193–200.
54. P. G. Fields, A. Dowdy, and M. Marcotte, *Structural Pest Control: The Use of an Enhanced Diatomaceous Earth Product Combined with Heat Treatment for the Control of Insect Pests in Food Processing Facilities*, Agriculture Canada Publication, Ottawa, Ontario, 1997.
55. R. L. Hohman, "Food Industry Self-Inspection," in J. R. Gorham, ed., *Ecology and Management of Food Industry Pests*, FDA Technical Bulletin No. 4, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 519–527.
56. H. A. Highland, "Protecting Packages against Insects," in J. R. Gorham, ed., *Ecology and Management of Food Industry Pests*, FDA Technical Bulletin No. 4, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 345–350.
57. H G. Scott, "Design and Construction: Building Out Pests," in J. R. Gorham, ed., *Ecology and Management of Food Industry Pests*, FDA Technical Bulletin No. 4, Association of Official Analytical Chemists, Arlington, Va., 1991, pp. 331–343.
58. D. W. Hagstrum and P. W. Flinn, "Integrated Pest Management," in B. Subramanyam and D. W. Hagstrum, eds., *Integrated Management of Insects in Stored Products*, Marcel Dekker, New York, 1996, pp. 399–408.

THOMAS W. PHILLIPS
RICHARD C. BERBERET
GERRIT W. CUPERUS
Oklahoma State University
Stillwater, Oklahoma

UNITED STATES FOOD MARKETING SYSTEM

The United States Food Marketing System connects roughly two and half million ranchers, farmers, and fishers to more than 270 million domestic consumers and a much larger and growing number of consumers worldwide. The primary role of the marketing system is to coordinate the vast array of economic activity involved in transporting and transforming producers' products to consumers—when, where, and how they prefer them. This coordination relies on economic markets, legal contracts, and direct ownership of various operations within the vertical marketing system to give order to the vertical flow of food products. Although the exact marketing channels used vary by product, the general vertical flow is depicted in Figure 1.

Consumers are the focal point of the marketing system because their demands are what everyone else attempts to profit from. It is this interplay of what consumers are willing (based on their needs, wants, and whims) and able (based on their incomes and prices) to buy and what sellers are willing and able to supply at a profit to themselves that makes the U.S. food marketing system an economic marvel. Americans today purchase essentially all their food from others—expending $561 million in 1997, a record amount for domestically produced farm foods (Fig. 2). Nearly 80% of that amount goes to pay the marketing bill to cover the costs of all the activities that lie between the primary producers and the ultimate consumers in the vertical system. The farm value share of food expenditures fell to a record low of 21% in 1997 (Fig. 3). In 1952, U.S. consumers spent $51 million and farmers received 40% of those expenditures. Over time, farmers have dramatically increased productivity with improved technology and spe-

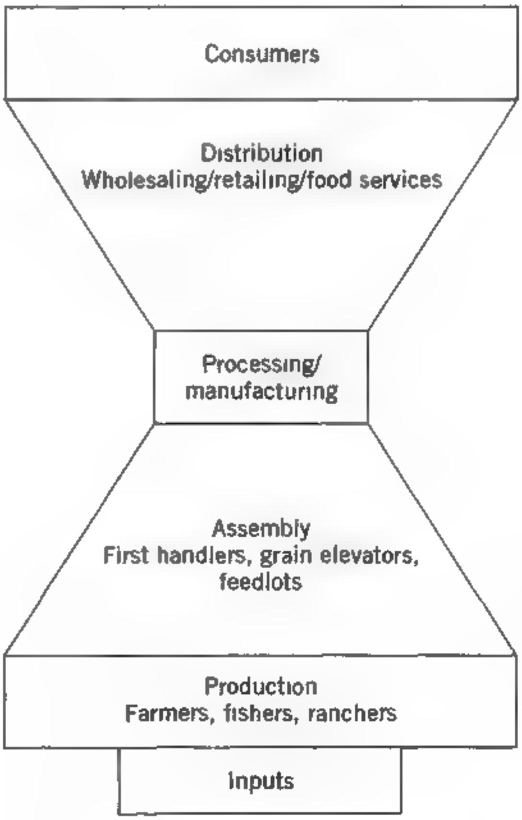

Consumers
Distribution
Wholesaling/retailing/food services
Processing/
manufacturing
Assembly
First handlers, grain elevators, feedlots
Production
Farmers, fishers, ranchers
Inputs

Figure 1. The vertical food marketing system.

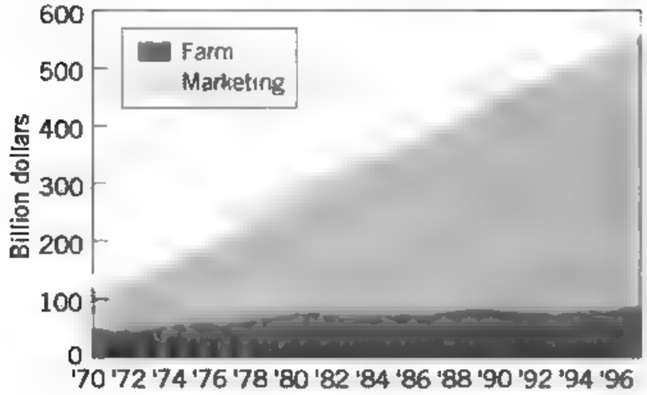


Figure 2. The distribution of food expenditures between farming and marketing, 1970–1997. The marketing bill is 79% of 1997 food expenditures. *Source:* Ref. 1.

cialization and have seen their share of the consumers' food dollar fall. Others, the so-called middlemen, have not only taken on the tasks abandoned by the producers but have specialized in providing all the marketing utilities to the eventual consumer by getting the right product (form utility), to the right place (space utility), at the right time (time utility), and at the right price (possession utility). It is these utilities that have increased the price of marketing bill over time as consumers demand more and more of these services.

AN OVERVIEW OF TRENDS

An overview should begin with consumers, the ultimate and somewhat elusive target of the entire marketing system. Then the focus shifts to the starting point of the vertical system to examine primary agriculture (and to a certain extent the input sector), and then in turn food processing, food wholesaling and retailing, and food service.

Consumer demands do not stand still, but change along with their preferences and incomes. Consumption patterns have seen some dramatic shifts over time, as shown in Figure 4. In beverages alone huge changes have occurred since 1970. Coffee consumption has fallen 32% despite the emergence of gourmet coffee houses. The only growth segments are in the smaller specialty-coffee beverages that include flavorings. Milk consumption is also down 23%, and only the no-fat and lowfat segments show increases. Calcium-fortified products have eliminated one of milk's traditional advantages; even orange juice companies suggest consumers substitute orange juice for milk. The big winner in the beverage aisle is soft drinks, up about 118%. The new bottled water market, flavored and unflavored, continues to grow as consumers consider tap water inferior (Table 1).

Consumption of eggs and red meat has also declined, 23% and 16% respectively, whereas poultry has increased dramatically and rivals beef as the leading meat consumed in the U.S. on a trimmed and boneless basis (3). The success of the broiler industry accounts for the majority of the poultry consumed. Fish consumption is up 24% since 1974, but remains a small part of the consumers' meat diet despite its known health benefits. Cheese consumption had the largest increase, up 146% since 1970. Americans have increased their consumption of fruits and vegetables 24%, but also had similar increases in fats and oils and caloric sweeteners. In addition, candy consumption reached a record 25 pounds per capita in 1997.

Much public and private effort has been directed at improving the diet of Americans. The Federal Food Guide

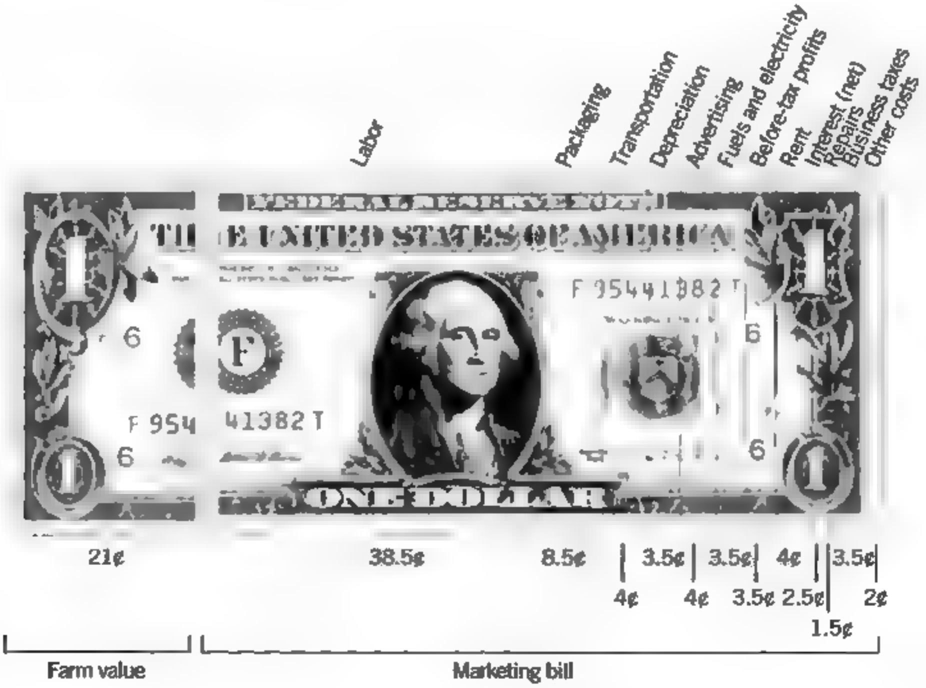


Figure 3. What a U.S. dollar spent for food paid in 1997. *Source:* Ref. 1.

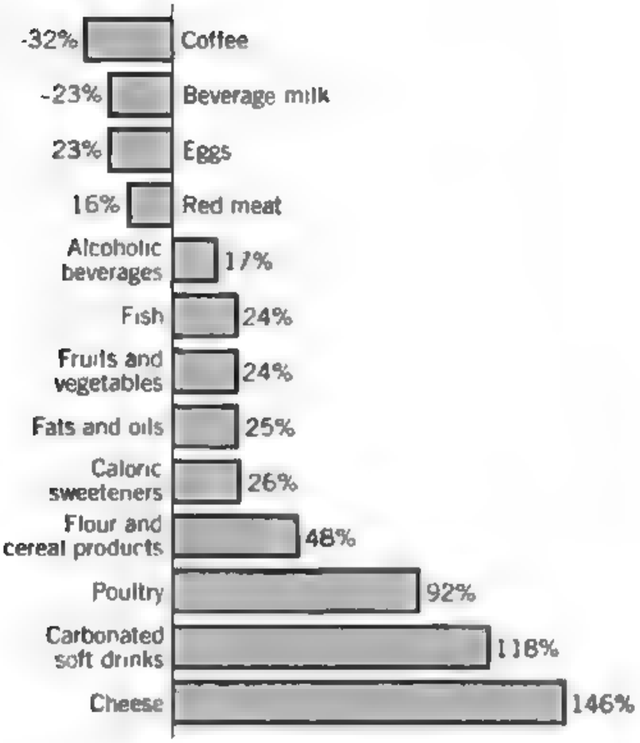


Figure 4. Changes in U.S. per capita food consumption, 1970–1997. *Source:* Ref. 2.

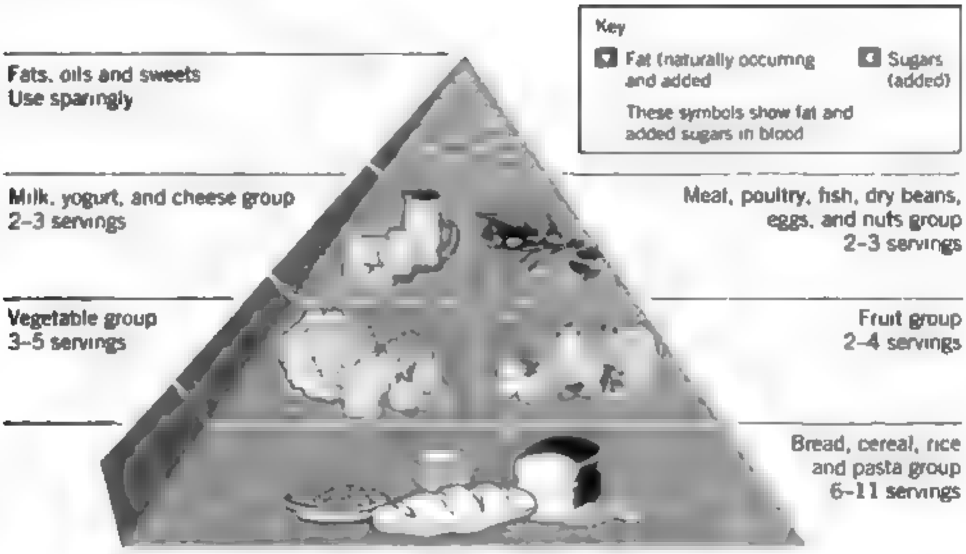
Table 1. Major Foods: U.S. Per Capita Food Supply

Food	1970	1980	1997
	Pounds		
Total meats[a]	177.3	179.6	192.0
Beef	79.6	72.1	63.8
Pork	48.0	52.1	45.6
Veal	2.0	1.3	.9
Lamb and mutton	2.1	1.0	.8
Chicken	27.4	32.7	51.2
Turkey	6.4	8.1	14.8
Fish and shellfish	11.7	12.4	14.7
Eggs (number)	308.9	271.1	238.4
Cheese[b]	11.4	17.5	28.0
Ice cream	17.8	17.5	16.2
Lowfat ice cream	7.7	7.1	7.9
Fluid cream products	5.2	5.6	9.1
All dairy products[c]	563.8	543.2	579.8
Added fats and oils	52.6	57.2	65.6
Peanuts and tree nuts[d]	7.2	6.6	8.0
Fruit and vegetables[e]	573.2	598.8	710.8
Fruit	237.7	262.4	294.7
Vegetables	335.4	336.5	416.0
Caloric sweeteners[f]	122.3	123.0	154.1
Sucrose	101.8	83.6	66.5
Corn sweeteners	19.1	38.2	86.2
Grain products[g]	135.6	144.7	200.1
Wheat flour	110.9	116.9	149.7
Rice	6.7	9.4	19.5
Corn products	11.1	12.9	23.1
	Gallons		
Beverage milks	31.3	27.6	24.0
Whole	24.8	16.5	8.2
Lower fat and skim	5.8	10.5	15.5
Coffee	33.4	26.7	23.5
Tea	6.8	7.3	7.4
Carbonated soft drinks	24.3	35.1	53.0
Fruit juices	5.7	7.4	9.2
Bottled water	N.A.	2.4	13.1
Beer	18.5	24.3	23.9
Wine	1.3	2.0	2.0
Distilled spirits	1.8	2.0	1.2

Source: Ref. 2.
Note: N.A., not available
[a] Boneless, trimmed weight.
[b] Excludes full-skim American, cottage, pot, and baker's cheese.
[c] Milk equivalent, milkfat basis.
[d] Shelled basis.
[e] Farm-weight, excludes wine grapes.
[f] Dry basis. Includes honey and edible syrups.
[g] Consumption of items at the processing level (excludes quantities used in alcoholic beverages and corn sweeteners).

Pyramid was developed as a simple guide to assist consumers in improving their nutrition (Fig. 5). The pyramid was controversial because industry groups positioned themselves to maximize benefit or minimize damage from the new recommendations. A study of American diets found some improvements, but some concerns as well. For example, consumption of fruits and vegetables did reach a record amount in 1996, but the use of caloric sweeteners also was a record because "on average, people consume too many servings of added fats and sugars and too few servings of fruits, vegetables, dairy products, lean meats, and foods made from whole grains" (5). The increased popularity of dining out has also contributed to nutrition concerns because consumers tend to eat more healthful foods at home than away from home (6).

Reasons for the changes in consumption can be grouped into three general areas: demographics, economics, and an explosion in available information (7). American households have changed tremendously since 1950. Total food consumption in an affluent society is linked to population because the amount of food consumed per person has not risen much, although more calories are being consumed. The U.S. population does continue to grow, roughly at 0.7% per year, driven by increased life expectancies, immigration, and fertility. In 1999, there were about 270 million Americans, but the census projects a population of 400 million by 2050. The U.S. population is aging; in 1996, 12.8% of the population was 65 and over, and it is projected to reach 20% in 2050 (8). Older people have different food interests and needs, and the marketing system will respond to this major change. Although the average age is increasing, the proportion of young people is also increasing. In addition, the population is also becoming more ethnically diverse; by 2050 non-Hispanic whites will make up 53% of the population, down from 74% in 1996. African-Americans will increase only slightly from 11.5 to 13.6%, and Asians will increase from 1.5 to 9.6%. The majority of the population will be over 45 or under 18 by 2010, with nearly 50% of children belonging to an ethnic minority (9). The increased diversity should be reflected in the food sys-


Key
Fat (naturally occurring and added)
Sugars (added)
These symbols show fat and added sugars in blood
Fats, oils and sweets
Use sparingly
Milk, yogurt, and cheese group
2–3 servings
Meat, poultry, fish, dry beans, eggs, and nuts group
2–3 servings
Vegetable group
3–5 servings
Fruit group
2–4 servings
Bread, cereal, rice and pasta group
6–11 servings

Source: U.S. Department of Agriculture/U.S. Department of Health and Human Services

Figure 5. Food guide pyramid. *Source:* Ref. 4.

tem. People's food choices are related to their cultural backgrounds, including religious beliefs, and as the population continues to diversify the food brought to the American table will reflect this diversity. Additionally, most Americans, especially younger people, will begin to experiment with the new ethnic foods.

The typical U.S. household has become smaller, older, and more likely to be a single-parent home. In 1955, 23% of households were a married couple, with two or more children under the age of 18, and the wife not in the labor force (7). By 1992, only 7% of American households fit that description. In 1960, 9.8% of households were single-parent households, and in 1995, the number increased to 27% (10). In 1960, 18.6% of married women with children under the age of 6 years old were in the labor force. By 1997, the percent was up to 63.6% (11). By 1996, nearly 60% of households with children under the age of 7 had personal computers (10). Such households have become very busy places, and the eating habits have changed accordingly. Children often prepare their own meals, usually by microwaving a ready-to-heat-and-eat meal.

Household economics influence consumer demands as relative prices change and as incomes rise allowing greater spending and increasing the value of leisure time. Rising incomes allow consumers to indulge more of their wants. Americans, on average, now spend less of their disposable income on food than any other country in the world, and the average percentage fell to a record low of 10.7% in 1997 (Table 2). This figure is both a statement about America's wealth and its efficient food system.

Averages, however, conceal the diversity that exists among U.S. households. Households with after-tax income of $5,000 to under $10,000, spend 34.2% of their after-tax income on food, whereas the food share for households with $70,000 and higher after-tax income was only 8.7% (2). The average U.S. household income has been flat for 15 years, resulting in a reduction in the number of middle-class households and an increased number of affluent and low-income households (9). Some fear a permanent underclass, left behind by a modern information driven economy, and a growing affluent segment whose wealth allows dramatic lifestyle changes.

Whereas much of modern business marketing focused on the mass market and the efficiencies from the one-size-fits-all approach, today mass markets are segmenting, allowing customized marketing to cater to the needs of the various segments—from those with higher incomes to those with lower incomes; from those who stress health issues to those who prefer taste and convenience. Such segments are still large, and combined with new technologies and increased incomes, firms can pursue a strategy of mass customization where they attempt to retain the efficiencies of mass marketing while providing consumers with products better suited to their preferences. Dual-earning households have more money than time, and such consumers demand more convenient foods, higher quality foods, and other timesaving services. Affluent households now hire people to shop, cook, and clean for them. Internet shopping has exploded, yet firms find profits elusive to date. Firms seek to profit from saving consumers time by dramatically altering the way they shop for food. As one founder put it, "There is money to be made simplifying people's hectic lives" (12). Also, with or without Internet shopping but certainly more so with it, home delivery has returned.

For the middle classes, income growth has stalled, and the number of hours worked per week now exceeds 40 hours. Leisure time is short until retirement, but people are retiring earlier and living longer, which greatly increases the leisure time of retirees. Women have less leisure time than men because they still do 86% of the cooking and 91% of the shopping (7). Such time pressure adds stress to busy lives and has consumers looking for relief from timesaving products.

Table 2. Food Expenditurs As a Share of Disposable Personal Income, 1967–1997

	Disposal personal income	Food at home		Food away from home		Total	
Year	Billions of dollars	Billions of dollars	Percent	Billions of dollars	Percent	Billions of dollars	Percent
1967	562.1	60.3	10.7	19.8	3.5	80.1	14.2
1972	855.3	84.4	9.9	31.3	3.7	115.8	13.5
1977	1,401.4	131.8	9.4	58.5	4.2	190.3	13.6
1982	2,347.3	198.4	8.5	104.5	4.5	302.9	12.9
1987	3,363.1	249.0	7.4	146.3	4.3	395.3	11.8
1992	4,626.7	321.6	7.0	192.0	4.2	513.6	11.1
1997	5,885.2	390.3	6.6	239.1	4.1	629.4	10.7

Source: Ref 2.

In single-parent households, there is not the benefit of two wage earners, but the time pressure can be even more severe. Adults increasingly view dinner as an impulse purchase. One study found 71% of adults do not plan their weekday dinner before 4 p.m., regardless of income level, marital status, or education (13). A Nabisco study put the figure at 61%, but found working parents are denied the fulfillment factor of doing something for their families (14). Those in single-parent households seldom have the luxury of using increased incomes to buy services to lessen the strain of their busy schedules, so they turn to convenient, yet inexpensive foods.

The explosion of studies relating health to diet have affected consumer demands. Although many such studies are controversial and are often sensationalized, the media cover them because there is great interest by consumers. Everyone wants to feel and look better, to live longer. Although some consumers have become jaded to the conflicting news, the information still moves markets. The markets for healthful products, including the explosion of nutraceuticals and functional foods, cater to these consumer desires. Consumers still do not want to exert too much effort to achieve dramatic results. The long list of diet and exercise books and the increasing proportion of Americans who are overweight (now a majority) suggest that there is a desire but also a lack of will.

As American households embraced the economic tenets of specialization and gains from trade, there has been some rethinking of the resulting lifestyle. The additional family income gained from dual wage earners is used to buy time-saving items and prepared meals. Dining out is no longer a luxury to many Americans but is done to save the meal preparation time. The dining experience itself must not take too long. Drive-up, fast-food windows proliferate because consumers cannot afford the time to have a full-service dinner experience, and they certainly lack the time to make traditional meals at home. The division of labor and both sexes in the paid labor force can lead to frustration, guilt, and even boredom. People long to do the things that their jobs prevent them from doing. Gardening and cooking, once dreaded chores, are considered enjoyable, but constrained by the lack of time. A recent study found only 13% of adults dislike cooking; 37% actively enjoy it and 50% do not mind it (15). Cooking is second only to pet care as the most enjoyable household task. Cooking has regained some popularity; 43% of adults indicated they often cook in their leisure time, which is up from 36% in 1991. Interest in food is up; the number of food magazines and cooking shows on cable TV is at an all-time high. Consumers, however, are left unable to find the time and energy to try their new ideas. The same study found 30% of adults always willing to try new or unusual flavors, with 21% having a strong interest in foods from other countries. Younger consumers were more likely to try something new.

The implications for the food marketing system are clear. As we move from a mass market where everyone eats similar foods to segmented markets with greater choices, firms will face new opportunities and challenges. Some consumers will avoid bioengineered foods; others will embrace it as science solving important problems. The controversies will be hotly debated, and food choice will contain plenty of political overtones. This is nothing new: the Pilgrims clashed over whether the making of a minced pie was a violation of their prohibitions against Catholic-style symbolism (16). Marketing firms strive to give consumers what they want, but firms often avoid full disclosure. "A 1997 survey of 1,000 U.S. consumers found that 93% wanted bio-engineered food to be labeled—presumably because many would avoid it" (17). Firms, however, do not want to label such foods, because they fear consumers are either ill-informed or merely paranoid about such tampering with foods. Instead, firms stress the advantages bio-engineered food can offer—characteristics such as improved taste, reduced spoilage, and enhanced visual appeal, all factors consumers prefer. As technology advances, consumer sovereignty in the marketplace will be tested.

There is increasing demand for value-added products. Rising household incomes allow greater purchasing of value-added products, where the value can be in convenience, healthfulness, and quality. The aging baby boomers and other health-conscious consumers will seek more functional foods and nutraceuticals. Time-pressed consumers of all income levels will be searching for quick meals, and families will seek meals that children enjoy, thus lessening the stress involved in family dining. Taste remains paramount, and most foods must pass the taste test, even if they offer nutrition and convenience, especially in the away-from-home segment. McDonald's reduced-fat burger did not survive the consumer taste test.

Consumers will also respond to environmental concerns, so-called green marketing. However, again a discrepancy exists between one's desires and one's actual behavior. One study that tracks the attitudes of college freshmen found less interest in programs to clean up the environment in 1997 than in the early 1970s (18). Fast-paced lifestyles and demands for fresh produce year round lead to an increased use of transportation and packaging. Consumers face numerous conflicting decisions in balancing their views with their purchases. In addition to green marketing, consumers will respond to products where the added value is not product specific but combines nonfood attributes with the food item, such as products with "save the rainforest" and "save the family farm" themes. Consumers now realize that voting with their pocketbooks can also signal their views on the world in which they wish to live.

The U.S. food marketing system will continue its growing involvement in international trade. Immigration and increased interest in new foods will bring more global trade. Also, the demand for fresh produce year round will increase shipments from the southern hemisphere. Because industry growth is largely constrained by population, U.S. firms will continue to search for growth in overseas markets.

PRODUCTION

Farming still has the greatest number of businesses in any of the vertical stages in the food system, but consolidation continues. Farming once employed the largest number of people in the food system, but machines have replaced people, and it is now the food service sector that employs the most people (Table 3). The number of farms has fallen in every census year, the latest 1997 census shows 1.9 million farms, accounting for under 2% of the U.S. population, a record low number (Table 4). The other major trend is toward greater dispersion between the very small farm and the very large. In 1992, nearly half the farms had sales of under $10,000 but accounted for less than 2% of total farm sales, whereas just over 2% of the farms had sales over $500,000 and did nearly 50% of total sales. There is also growing diversity among regions of the country and by crop speciality. California is the largest agricultural state in terms of dollar value, and the top 10 states accounted for nearly 53% of farm value in 1997 (Table 5). California is an amazing state because it is more commonly associated with nonagricultural images, such as Hollywood, yet it has almost twice the farm value of Texas, the second largest agricultural state. The other leading farm states, like Iowa and Nebraska, are often associated with agriculture and rural communities.

Increasingly, producers must align their production choices with consumer demands, rather than rely on others in the marketing system, mainly processors, to alter the primary foodstuffs to match consumer demands. Processors aware of what their consumers are willing and able to buy now ask producers to deliver raw products best suited to these demands. For example, a potato chip company that wants potatoes specially tailored for the chip making process will invest in research and technology to develop the best chipping potato and seek growers who will grow these potatoes under a legal contract. Even food service companies need to align their needs with producers to ensure a good match between consumer demand and product supply. For example, KFC (formerly Kentucky Fried Chicken) contracts with processors to supply the birds best suited to their cooking procedure.

This increased vertical coordination between farmers and processors has become known as the industrialization of agriculture (26). Farmers continue to specialize in a narrower slice of the food marketing system. Great efficiencies have been gained from advanced technology and special-

Table 3. Food System Employment, 1950–1997 (thousands)

Year	Agriculture	Manufacturing[a]	Wholesaling	Retailing	Food Service
1950	7160	1626	N.A.	1231	N.A.
1960	5458	1571	N.A.	1649	N.A.
1982	3364	1676	674	2348	4666
1992	3295	1541	812	2969	6548
1997	3399	1602	N.A.	3147	7636

[a] Includes tobacco manufacturing.

Note. N.A., not available.

Source Refs. 19–24

Table 4. Number of Food-System Business Establishments, 1972–1997

Year	Number of farms	Number of manufacturing establishments	Number of wholesale establishments	Number of retail establishments	Number of food-service establishments
1972	2,869,710	28,103	60,363	173,084	287,250
1977	2,455,830	26,656	59,473	171,592	308,614
1982	2,400,550	22,130	58,766	176,219	319,873
1987	2,176,110	20,201	42,075	190,706	391,303
1992	1,925,300	20,798	42,874	180,568	433,608
1997	1,911,859	20,913	N.A.	174,284	N.A.

N.A., not available.

Source: Refs. 19–22

ized equipment. Inputs previously supplied on the farm are now purchased from the input sector. Dramatic growth has occurred in the chemical businesses that supply fertilizer, pesticides, and insecticides to farmers. Private seed companies have largely replaced public research, and under protection provided by the 1972 Plant Varieties Protection Act, they have invested heavily in patentable seeds. Given the protection to intellectual property rights, these companies now sell designer seeds with favorable characteristics. Others view this as dangerous tampering with the public's genetic seed stock that leaves farmers dependent on the seed companies that hold the patents to seeds engineered unable to reproduce.

The seed companies continue to consolidate into ever larger, worldwide firms. DuPont's announcement to buy the remaining 80% of Pioneer Hi-Bred International makes this a dominant two-firm industry, with DuPont and Monsanto controlling half of the U.S. soybean seed market and over half of the corn seed market—the two biggest crops (27). In the smaller produce seed markets, such as lettuce, tomatoes, and cucumbers, similar consolidation has occurred. A Mexican company has quietly amassed a dominant share in many of these markets; for example, it holds a 55% share of the U.S. lettuce seeds used by commercial farmers (17). It has linked with Monsanto to alter its lettuce seeds, making them immune to Monsanto's Roundup herbicide used by farmers to kill unwanted vegetation. Many consumers would love nonbrowning lettuce, attractively priced, but others view this technology as dangerous, sending them to the organic lettuce aisle.

PROCESSING AND MANUFACTURING

The processing stage has the fewest number of establishments in the vertical food system, but the processor/food manufacturer is often consider the most powerful, influential firm in the system—the marketing channel leader. These are the food firms the world knows by name: Philip Morris, Coca-Cola, Cargill, Kellogg's, and so on (Table 6). About 80% of all raw domestic food products pass through this stage; only produce and eggs avoid processing because they only require minimal market preparation services such as cleaning, sorting, and packaging (29). Processors and manufacturers, hereafter referred to as processors, add the form utility to the raw agricultural products and have invested heavily in market research to understand consumer demands. They buy or contract from farmers who have been advised (including through price signals) or legally bound to supply raw foodstuffs with desired characteristics for transforming into the products consumers eventually buy. In terms of value added, the economist's preferred measure of economic size, processing once contributed a smaller portion of value added to the GDP than farming, but since the 1950s, it has exceeded farming's value added and remained behind retailing and wholesaling and food service (Table 7).

The location decisions made by food processors involve a calculated tradeoff between processing costs, including input costs, and the costs of delivering their finished products to consumers. Since most of the country's consumers live near the coasts and most of the raw agricultural foodstuffs come from the middle of the country, the location decision is not always obvious. Over time, with modern transportation and refrigeration technologies, the balance has shifted to locating where the inputs are produced rather than where the people live. California is in the unique situation of being both the number 1 farm state and the number 1 food processing state by far (Table 5). It has both the agricultural commodities and the population. States such as Nebraska and Kansas, 4th and 5th in farm value, rank 24th and 27th, respectively, in processing. Overall, there is a strong association between farm value rank and food processing rank, with a simple correlation coefficient of 0.75. In certain crops it is even more pronounced, such as wine or broilers. Broiler processors prefer to locate within a 25-mile radius of where their chickens are raised to market weight, and the leading states in both production and processing closely follow a geographical pattern known as the broiler belt (3).

The largest food processors among the roughly 16,000 companies involved in food processing are huge, both in absolute terms and relative to the others. The largest 100 food and tobacco processors accounted for nearly 80% of the value added in 1995 (Fig. 6), almost doubling their share since 1954. The top 100 is itself skewed toward the very large, with the top 20 firms accounting for over 50% of total value added in 1995, more than doubling its 1967 share (Fig. 7). The remaining 80 firms among the top 100 firms actually lost share over the last 30 years. The sector is best described by a big–small model, where extremely large firms control leading positions in most markets and smaller companies, including start-ups, operate in a competitive fringe trying to serve a particular market niche or develop a new idea. The large companies know that if a new idea turns promising they can buy the entire company after the start-up has borne much of the risk.

The previous figures refer to overall size, or what economists call aggregate concentration, but market performance hinges on market concentration—the extent of market power held by leading firms in a well-defined economic market. Market power is what enables a firm to enhance prices to buyers, extract price reductions from its product suppliers, and subdue rivals. Although market definition is a complex task, it can be approximated by the census four-digit industry group, the four-digit SIC. The food and tobacco processing sector had 52 such industries in 1992, most of which remain too broadly defined, certainly so on the input side because substitution opportunities are much greater in consumption than in production (33). Although there are no monopolies and several industries are what economists call workably competitive (where the four largest firms have a combined market share of 40% or less), most have become oligopolies (Table 8). In oligopolies, firms get some of the advantages of market power without government regulation that would come if they were monopolies (34). Over time, most of these four-digit industries have lost companies, averaging a 25.5% reduction in company counts, and have increased in concentration as measured by the four-firm concentration ratio, CR4, which in-

Table 5. Ranking of States by Value of Agricultural Products Sold, (1997) and Value-Added in Food Manufacturing, (1996)

	Rank		1997 value of agricultural products sold			1996 value added in manufacturing			
State	Agriculture	Food manufacturing	$ million	Percent of U.S. total	Cumulative percent	Food manufacturing ($ million)	Percent of U.S. total	Total manufacturing ($ million)	Food manufacturing as percent of state's total
California	1	1	23,032	11.7	11 7	20,265	11.3	188,805	10.7
Texas	2	3	13,767	7.0	18.7	11,778	6.6	116,631	10.1
Iowa	3	10	11,948	6.1	24.8	6,367	3.6	27,021	23.6
Nebraska	4	24	9,832	5.0	29.8	2,549	1.4	9,218	27.6
Kansas	5	27	9,207	4.7	34.4	2,136	1.2	18,820	11.4
Illinois	6	2	8,556	4.3	38.8	12,602	7.1	92,011	13.7
Minnesota	7	15	8,290	4.2	43.0	5,023	2.8	34,716	14.5
North Carolina	8	12	7,677	3.9	46.9	5,144	2.9	76,475	6.7
Florida	9	14	6,005	3.1	49.9	5,071	2.8	38,621	13 1
Wisconsin	10	8	5,580	2.8	52.8	6,379	3.6	53,619	11.9
Arkansas	11	20	5,480	2.8	55.6	3,542	2.0	18,512	19.1
Missouri	12	9	5,368	2.7	58.3	6,377	3.6	40,208	15.9
Indiana	13	17	5,230	2.7	60.9	4,304	2.4	61,896	7.0
Georgia	14	6	4,993	2.5	63.5	6,796	3.8	51,753	13.1
Washington	15	19	4,768	2.4	65.9	3,582	2.0	31,929	11.2
Ohio	16	4	4,684	2.4	68.3	9,786	5.5	105,497	9.3
Colorado	17	21	4,534	2.3	70.6	2,919	1.6	19,215	15.2
Oklahoma	18	32	4,146	2.1	72.7	1,396	0.8	15,875	8.8
Pennsylvania	19	5	3,998	2.0	74.7	9,386	5.3	82,922	11.3
South Dakota	20	39	3,570	1.8	76.5	674	0.4	3,974	17.0
Michigan	21	11	3,568	1.8	78.3	5,149	2.9	85,688	6.0
Idaho	22	31	3,346	1.7	80.0	1,467	0.8	7,977	18.4
Mississippi	23	29	3,127	1.6	81.6	1,823	1.0	17,295	10.5
Alabama	24	30	3,099	1.6	83.2	1,551	0.9	27,451	5.7

Kentucky	25	23	3,064	1.6	84.8	2,685	1.5	35,040	7.7
Oregon	26	28	2,969	1.5	86.3	2,064	1.2	21,838	9.5
North Dakota	27	41	2,869	1.5	87.7	537	0.3	1,808	29.7
New York	28	7	2,835	1.4	89.2	6,419	3.6	90,665	7.1
Virginia	29	13	2,344	1.2	90.4	5,090	2.8	42,519	12.0
Tennessee	30	16	2,178	1.1	91.5	4,632	2.6	42,288	11.0
Louisiana	31	25	2,031	1.0	92.5	2,435	1.4	25,125	9.7
Arizona	32	33	1,903	1.0	93.5	1,365	0.8	22,850	6.0
Montana	33	49	1,871	1.0	94.4	165	0.1	1,707	9.7
New Mexico	34	45	1,618	0.8	95.2	333	0.2	11,745	2.8
South Carolina	35	34	1,588	0.8	96.0	1,312	0.7	30,769	4.3
Maryland	36	22	1,312	0.7	96.7	2,729	1.5	17,455	15 6
Wyoming	37	50	899	0.5	97.2	148	0.1	[illegible]	14.8
Utah	38	35	877	0.4	97.6	1,032	0.6	11,239	9.2
New Jersey	39	18	697	0.4	98.0	4,136	2.3	49,995	8.3
Delaware	40	37	691	0.4	98.3	920	0.5	5,791	15.9
Hawaii	41	42	497	0.3	98.6	533	0.3	1,609	33.2
Vermont	42	46	476	0.2	98.8	320	0.2	3,986	8.0
Massachusetts	43	26	454	0.2	99.0	2,272	1.3	44,047	5.2
West Virginia	44	47	447	0.2	99.3	257	0.1	8,965	2.9
Maine	45	40	439	0.2	99.5	541	0 3	6,675	8.1
Connecticut	46	36	422	0.2	99.7	947	0.5	24,772	3.8
Nevada	47	44	357	0.2	[illegible]	397	0.2	3,275	12.1
New Hampshire	48	43	149	0.1	100.0	478	0.3	10,815	4.4
Rhode Island	49	48	48	0.0	100.0	249	0.1	5,407	4.6
Alaska	50	38	25	0.0	100.0	683	0.4	1,470	46.5
Total			*196,865*	*100.0*		*178,742*	*100.0*	*1,748,981*	*10.2*

Source: Refs. 24 and 25.

Table 6. The Top 25 Food Processing Companies, 1998

		Millions $		
Rank	Company	Food sales	Total sales	Percent food
1	Philip Morris	31,527	71,592	44
2	Conagra	28,840	28,840	100
3	Cargill	21,400	51,000	42
4	Pepsico	20,917	20,917	100
5	Coca-Cola	18,800	18,868	100
6	Archer Daniels Midland	16,109	16,109	100
7	Mars	14,000	14,000	100
8	IBP	13,259	13,259	100
9	Anheurser-Busch	12,832	12,832	100
10	Sara Lee	10,800	20,000	54
11	H.J. Heinz	9,209	9,209	100
12	Nabisco	8,734	8,734	100
13	Bestfoods	8,400	8,400	100
14	Nestle USA	7,800	7,800	100
15	Dairy Farmers of America	7,000	7,000	100
16	Kellogg	6,830	6,830	100
17	Campbell Soup	6,696	6,696	100
18	Pillsbury	6,500	6,500	100
19	Tyson Foods	6,356	6,356	100
20	General Mills	6,033	6,033	100
21	Quaker Oats	5,010	5,010	100
22	Proctor and Gamble	4,376	37,154	12
23	Dole Food	4,336	4,336	100
24	Hershey Foods	4,300	4,300	100
25	Land O'Lakes	4,195	4,195	100

Source: Ref. 28.

creased on average from 43.9 in 1967 to 53.3 in 1992, the last year data are available.

The immense size of processors has always concerned farmers, who fear the processors would exploit their bargaining power and pay farmers less than fair market value for their crops. Such fears led to agricultural cooperatives and the Capper-Volstead Act of 1922. Both economic theory and empirical studies (35) conclude that open membership cooperatives can negate market power imperfections and hence benefit both farmers and consumers. In Table 8, the share of an industry's shipments controlled for by the 100 largest cooperatives ranges from a high of 63% in the butter industry to several industries without any cooperatives, and averages 5.4% for all of food and tobacco processing (36).

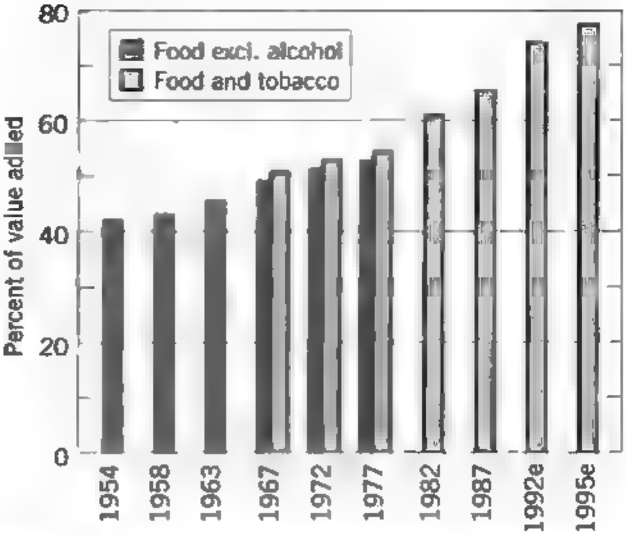


Figure 6. Largest 100 food manufacturing companies, census years 1954–1995. e, estimated. *Source:* Ref. 30; 1992 and 1995 were estimated from trade sources.

Food processors have long used branded products and a pull marketing strategy where they create consumer demand for their products, so retailers are obliged to carry the products or lose sales. New products with strong media advertising support, especially television, are central to this strategy. Other marketing strategies (eg, coupons) are often correlated with new products and advertising efforts (29). New product introductions rose from 4540 items in 1983 to a high of nearly 17,000 in 1995 and fell back to 12,400 items in 1997 (Table 9). Most, by far the majority, do not represent truly new products but variations on existing products. Nevertheless, most new products fail in the marketplace, underscoring both the difficulty of knowing what the consumer wants and the wastefulness of new product launches. Even Coca-Cola, with its huge marketing muscle, misfired with New Coke. Consumers loudly voiced their preference for the original; hence, we now have Coke Classic and New Coke, with the former far outselling the newer product.

Table 7. Value Added by Industry Sector, Selected Years (in Billions of Dollars)

Industry sector	1947	1972	1982	1987	1996
Farming	19.5	22.0	48.1	51.0	95.0
Processing and manufacturing	11.3	30.6	65.0	85.7	131.0
Retailing and wholesaling	30.0	37.9	87.0	108.3	148.0
Transportation	15.3	6.5	16.9	20.5	24.0
Eating and drinking places	6.0	18.5	46.8	63.5	169.0
Other supporting sectors[a]	—	49.9	121.6	165.3	226.0
Total sector value added	82.1	165.4	385.4	494.3	793.0
U.S. gross national product	252.0	1,212.8	3,166.0	4,526.7	7,636.0
Total sector divided by GNP	32.6%	13.6%	12.2%	10.9%	10.4%

Source: Refs 11, 29, 31, and 32.

[a] Includes auxiliary activities such as packaging.

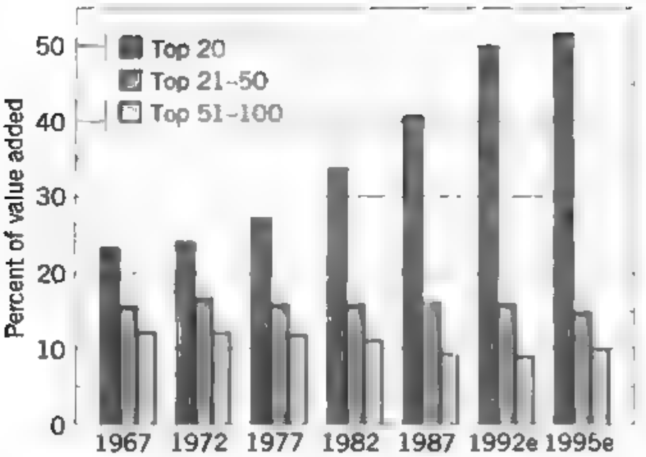


Figure 7. Increasing dominance by the top 20 among the largest 100 food and tobacco manufacturing companies, census years 1967–1995. e, estimated. *Source:* Ref. 30; 1992 and 1995 were estimated from trade sources.

Food processors outspend all other stages of the vertical marketing system advertising their products. In 1997, they accounted for over 65% of all media advertising in the food system (Table 10). Only restaurants, especially fast food, also spend large amounts on advertising, with a 27% share of the total. The bulk of the $55 million spent in farm-related expenditures was for farm chemicals and pest controls that were advertised by the large chemical companies targeting farmers as consumers of their chemical products. Media advertising accounts for somewhere between a fourth and a third of marketing dollars spent by processors. Within the food system, advertising created and maintained for product differentiation provides protection from new entrants and inroads from smaller rivals. Oligopolists often prefer to compete with their rivals in nonprice ways. Advertising and new product rivalry are perfectly suited to this strategy because they allow competition among the few in a manner that collectively erects barriers to entry to others not involved in the marketing fray. The cola wars are often mentioned as an example of the intense competition in the soft drink industry, but a former president of Pepsi once commented that such struggles did not involve "some gladiatorial contest where one of us has to leave on a stretcher. We're both winning" (28, p. 112).

Media advertising is not for the cash-starved start-up company, but it is standard operating procedure among the largest food and tobacco processors. Philip Morris, the number one food and tobacco advertiser, spent over a billion dollars promoting its brands, which include Miller beer, Marlboro cigarettes, Kraft cheese, General Foods Post cereals, Oscar Mayer hot dogs, and several other major brands. Of the 16,000 food and tobacco processing firms, the top 100 advertisers accounted for 96.4% of media advertising, and the top 8 alone accounted for over 50% in 1992 (Table 11). The vast majority of the remaining 15,900 food firms use media advertising minimally or resort to other marketing tactics. Because advertising-created-and-maintained product differentiation is the major advantage in the food system, these firms are at disadvantage and are left to compete primarily on price and efficiency and the rare, truly new product that captures consumer interest. The concentration of advertising expenditures has risen sharply from 1967 to 1992, the last year the data were analyzed, with the four largest advertisers increasing their share from 19.4% in 1967 to 36.9% in 1992.

FOOD WHOLESALING, RETAILING, AND SERVICE

Wholesalers distribute the food products from points of production and processing to the retail stores and food service establishments located in every local community. Food wholesalers are classified into three general categories: (*1*) merchant wholesalers who take title of the products they distribute, (*2*) manufacturers' sales branches and offices that are the wholesaling arm of food processors but report their activities as wholesaling as long as the facility is located at a different address than the processor's location, and (*3*) agents, brokers, and commission merchants who do not take title of the products they distribute but work on commissions from the manufacturers whose products they represent. Merchant wholesalers account for the largest slice of the wholesaling function, with over 77% of the wholesale establishments and roughly 55% of total wholesaling sales in 1992 (39). The other two categories share the remainder, with manufacturers' sales branches and offices being slightly larger than the agents, brokers, and commission merchants.

In general, wholesalers are challenged by the processors' own sales forces on the one side and by retailers who self-supply on the other side. Nearly half of all retail food sales were from retailers who have integrated backward and provide their own wholesaling functions. Major wholesalers have integrated forward and own retail stores, but most remain as merely affiliated with retailers, either in a voluntary or a cooperative format.

Wholesalers must convince processors that they offer a more profitable method for distributing the processors' products than could be done through their own sales force. In addition, they must convince retailers that self-supply is less profitable than specializing in retailing and using a wholesaler to supply the products. Wholesalers work closely with both processors and retailers to coordinate product flows to benefit both parties while profiting from their activities and market expertise as well. They understand the local retail market and can use their personnel efficiently to represent processors' products and still provide retailer with supply savings, management, and merchandising services that only the largest retailers would find profitable to provide for themselves. One major service merchant wholesalers provide retailers is a line of private label products—products that they arrange for processors to pack and label with the wholesalers' label—that often sell at prices beneath the national brands and still offer a higher margin to the retailer (eg, IGA, President's Choice, and World Classics).

The wholesaling sector continues to consolidate, with larger firms gaining a larger share of the overall business.

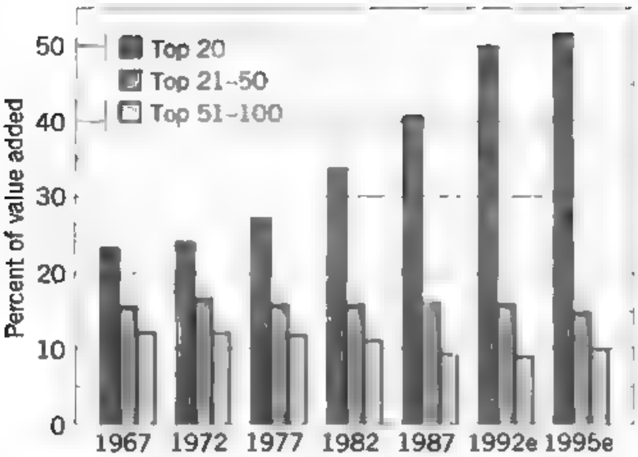


Figure 7. Increasing dominance by the top 20 among the largest 100 food and tobacco manufacturing companies, census years 1967–1995. e, estimated. *Source:* Ref. 30; 1992 and 1995 were estimated from trade sources.

Food processors outspend all other stages of the vertical marketing system advertising their products. In 1997, they accounted for over 65% of all media advertising in the food system (Table 10). Only restaurants, especially fast food, also spend large amounts on advertising, with a 27% share of the total. The bulk of the $55 million spent in farm-related expenditures was for farm chemicals and pest controls that were advertised by the large chemical companies targeting farmers as consumers of their chemical products. Media advertising accounts for somewhere between a fourth and a third of marketing dollars spent by processors. Within the food system, advertising created and maintained for product differentiation provides protection from new entrants and inroads from smaller rivals. Oligopolists often prefer to compete with their rivals in nonprice ways. Advertising and new product rivalry are perfectly suited to this strategy because they allow competition among the few in a manner that collectively erects barriers to entry to others not involved in the marketing fray. The cola wars are often mentioned as an example of the intense competition in the soft drink industry, but a former president of Pepsi once commented that such struggles did not involve "some gladiatorial contest where one of us has to leave on a stretcher. We're both winning" (28, p. 112).

Media advertising is not for the cash-starved start-up company, but it is standard operating procedure among the largest food and tobacco processors. Philip Morris, the number one food and tobacco advertiser, spent over a billion dollars promoting its brands, which include Miller beer, Marlboro cigarettes, Kraft cheese, General Foods Post cereals, Oscar Mayer hot dogs, and several other major brands. Of the 16,000 food and tobacco processing firms, the top 100 advertisers accounted for 96.4% of media advertising, and the top 8 alone accounted for over 50% in 1992 (Table 11). The vast majority of the remaining 15,900 food firms use media advertising minimally or resort to other marketing tactics. Because advertising-created-and-maintained product differentiation is the major advantage in the food system, these firms are at disadvantage and are left to compete primarily on price and efficiency and the rare, truly new product that captures consumer interest. The concentration of advertising expenditures has risen sharply from 1967 to 1992, the last year the data were analyzed, with the four largest advertisers increasing their share from 19.4% in 1967 to 36.9% in 1992.

FOOD WHOLESALING, RETAILING, AND SERVICE

Wholesalers distribute the food products from points of production and processing to the retail stores and food service establishments located in every local community. Food wholesalers are classified into three general categories: (*1*) merchant wholesalers who take title of the products they distribute, (*2*) manufacturers' sales branches and offices that are the wholesaling arm of food processors but report their activities as wholesaling as long as the facility is located at a different address than the processor's location, and (*3*) agents, brokers, and commission merchants who do not take title of the products they distribute but work on commissions from the manufacturers whose products they represent. Merchant wholesalers account for the largest slice of the wholesaling function, with over 77% of the wholesale establishments and roughly 55% of total wholesaling sales in 1992 (39). The other two categories share the remainder, with manufacturers' sales branches and offices being slightly larger than the agents, brokers, and commission merchants.

In general, wholesalers are challenged by the processors' own sales forces on the one side and by retailers who self-supply on the other side. Nearly half of all retail food sales were from retailers who have integrated backward and provide their own wholesaling functions. Major wholesalers have integrated forward and own retail stores, but most remain as merely affiliated with retailers, either in a voluntary or a cooperative format.

Wholesalers must convince processors that they offer a more profitable method for distributing the processors' products than could be done through their own sales force. In addition, they must convince retailers that self-supply is less profitable than specializing in retailing and using a wholesaler to supply the products. Wholesalers work closely with both processors and retailers to coordinate product flows to benefit both parties while profiting from their activities and market expertise as well. They understand the local retail market and can use their personnel efficiently to represent processors' products and still provide retailer with supply savings, management, and merchandising services that only the largest retailers would find profitable to provide for themselves. One major service merchant wholesalers provide retailers is a line of private label products—products that they arrange for processors to pack and label with the wholesalers' label—that often sell at prices beneath the national brands and still offer a higher margin to the retailer (eg, IGA, President's Choice, and World Classics).

The wholesaling sector continues to consolidate, with larger firms gaining a larger share of the overall business.

Table 8. Concentration in Food and Tobacco Processing Industries, 1967 to 1992

SIC	Name	Concentration-CR4 1967	Concentration-CR4 1987	Concentration-CR4 1992	Change 1967–1987	Change 1987–1998	Number of companies 1967	Number of companies 1987	Number of companies 1992	% Change 1967–1987	% Change 1987–1998	VA/VS	Ag input share	Co-op VS share
20–21	All food and tobacco products[a]	51	66	75	15	9	26958	15790	16151	−41.4	2.3	38.8	—	5.4
2011	Meat packing plant products	26	32	50	6	18	2529	1328	1296	−47.5	−2.4	11.6	75.9	0.1
2013	Sausages and prepared meats	15	26	25	11	−1	1294	1207	1128	−6.7	−6.5	26.9	0.0	0.1
2015	Poultry and egg processing	15	28	34	13	6	709	284	373	−59.9	31.3	27.6	68.5	5.0
2021	Butter	15	40	49	25	9	510	44	31	−91.4	−29.5	9.4	19.1	62.8
2022	Cheese, natural and processed	44	43	42	−1	−1	891	508	418	−43.0	−17.7	20.2	47.2	23.4
2023	Condensed and evaporated milk	41	45	43	4	−2	179	124	153	−30.7	23.4	40.8	36.1	27.1
2024	Ice cream and ices	33	25	24	−8	−1	713	469	411	−34.2	−12.4	32.4	7.2	6.0
2026	Fluid milk	22	21	22	−1	1	2988	652	525	−78.2	−19.5	26.4	56.4	17.2
2032	Canned specialities	69	59	69	−10	10	150	183	200	22.0	9.3	49.6	5.7	0.5
2033	Canned fruits and vegetables	22	29	27	7	−2	930	462	502	−50.3	8.7	45.8	28.0	13.7
2034	Dehydrated fruits, vegetables, soups	32	39	39	7	0	134	107	124	−20.1	15.9	51.2	15.0	14.2
2035	Pickles, sauces, salad dressings	33	43	41	10	−2	479	344	332	−28.2	−3.5	50.4	10.3	1.8
2037	Frozen fruits and vegetables[b]		31	28	2	−3		194	182	42.6	−6.2	45.2	46.2	8.4
2038	Frozen specialities[b]		43	40	5	−3		244	308	−37.1	26.2	49.9	5.9	0.2
2041	Flour and other grain mill products	30	44	56	14	12	438	237	230	−45.9	−3.0	26.8	70.1	1.0
2043	Cereal breakfast foods	88	87	85	−1	−2	30	33	42	10.0	27.3	74.7	8.7	0.0
2044	Milled rice and by-products	45	56	50	11	−6	54	48	44	−11.1	−8.3	38.0	88.2	44.5
2045	Prepared flour mixes and refrigerated doughs	63	43	39	−20	−4	126	120	156	−4.8	30 0	48.7	0.0	0.0
2046	Wet corn milling	68	74	73	6	−1	32	31	28	−3.1	−9.7	43.3	53.3	0.0
2047	Dog, cat, and other pet food	46	61	58	15	−3		130	102	−11.6	−21 5	54.1	7.0	0.2
2048	Prepared feeds, n.e.c.[b,c]	22	20	23	−2	3		1182	1161	−25.1	−1.8	22.7	16.0	4.2
2051	Bread, cake, and related products	26	34	34	8	0	3445	1948	2180	−43.5	11.9	64.9	0.0	0.2
2052	Cookies and crackers	59	58	56	−1	−2	286	316	374	10.5	18.4	65.0	0.0	0.0
2053	Frozen bakery products		59	45		−14		103	160		55.3	51.6	0.0	0.0
2061	Sugar cane mill products	43	48	52	5	4	61	31	37	−49.2	19.4	40.7	81.3	10.7
2062	Refined cane sugar	59	87	85	28	−2	22	14	12	−36.4	−14.3	18.1	0.0	15.5
2063	Refined beet sugar	66	72	71	6	−1	15	14	13	6.7	−7.1	33.5	75.3	29.3
2064	Candy and confectionary[c]		45	45		0			705			55.0	1.9	0.7

2066	Chocolate and cocoa products		69	75		6		173	146		−15.6	46.6	0.3 d	0.0
2067	Chewing gum[e]	86	96	96	10	0	19	8	8	57.9	0.0	68.8	0.0	0.0
2068	Nuts and seeds		43	42		−1		79	102		29.1	39.8	35.5	25.9
2074	Cottonseed oil mill products	42	43	62	1	19	91	31	22	−65.9	−29.0	22.7	67.6	16.0
2075	Soybean oil mill products	55	71	71	16	0	60	47	42	−21.7	−10.6	11.1	76.0	16.8
2076	Vegetable oil mill products, n.e.c.	56	74	89	18	15	34	20	18	−41.2	−10.0	19.2	70.8	4.3
2077	Animal and marine fats and oils	28	35	37	7	2	477	194	159	−59.3	18.0	42.7	0.0	1.5
2079	Shortening and cooking oils	43	45	35	2	−10	63	67	72	6.3	7.5	30.4	0.0	4.3
2082	Malt beverages	40	87	90	47	3	125	101	160	−19.2	58.4	53.5	1.9	0.0
2083	Malt and malt byproducts	39	64	65	25	1	32	15	16	−53.1	6.7	28.9	85.4	0.0
2084	Wines, brandy, and brandy spirits	48	37	54	−11	17	175	469	514	168.0	9.6	43.0	27.0	2.5
2085	Distilled liquor, except brandy	54	53	62	−1	9	70	48	43	−31.4	−10.4	59.5	2.0	0.1
2086	Bottled and canned soft drinks	13	30	37	17	7	3057	846	637	−72.3	−24.7	38.5	0.0	4.1
2087	Flavoring extracts and syrups n.e.c.	67	65	69	−2	4	401	245	264	−38.9	7.8	70.6	0.0	0.3
2091	Canned and cured seafood inc soup	44	26	29	−18	3	268	153	144	−42.9	−5.9	36.9	0.0 d	0.0
2092	Fresh or frozen packaged fish	26	18	19	−8	1		579	600		3.6	26.8	0.0 d	0.0
2095	Roasted coffee	53	66	66	13	0	206	110	134	−46.6	21.8	40.5	0.0 d	0.6
2096	Potato chips and similar products	41	62	70	21	8		277	333		20.2	65.5	19.4	0.0
2097	Manufactured ice	33	19	24	−14	5	688	503	513	−26.9	2.0	70.0	0.0	0.0
2098	Macaroni and spaghetti	34	73	78	39	5	190	196	182	3.2	−7.1	58.6	0.0	0.0
2099	Food preparations, n.e.c.	23	26	22	3	−4	1824	1510	1644	−17.2	8.9	52.4	8.3	0.6
2111	Cigarettes	81	92	93	11	1	8	9	8	12.5	−11.1	74.7	2.3	0.0
2121	Cigars	59	73	74	14	1	126	16	25	−87.3	56.3	55.5	4.7	0.0
2131	Chewing, smoking tobacco, snuff	51	85	87	34	2	41	23	23	−43.9	0.0	71.1	4.2	0.0
2141	Tobacco stemming and redrying	63	66	72	3	6	54	62	32	14.8	−48.4	19.0	49.5	0.0
Means for SIC 20–21		43.9	51.1	53.3	7.5	2.1				−25.5	3.0	42.8	24.1	6.9

Source. Ref. 30.

Note: CR4s aree from four-digit industry data, where available, or else 4-digit product class data from Rogers. VA/VS is the ratio of value added to the value-of-shipments, percent, 1987 data. Ag input share is the percentage of total cost of materials accounted for by agricultural inputs, 1987 data. Co-op VS share is the 1987 estimated percent of value-of-shipments accounted for by the 100 largest agricultural marketing cooperatives.

[a] For SIC 20 + 21, the concentration data are the percent of the sector's value added held by the top 100 food and tobacco companies.

[b] The changes are from 1972, not 1967.

[c] In 1992, SIC 2067, chewing gum, was combined with SIC 2064. The 1992 data for SIC 2067 are estimated by Rogers.

[d] Cocoa, coffee, and fish inputs were ignored.

[e] 1967 CR4 is estimated.

Table 9. New Food Product Introductions, 1983–1997

Food category	1983	1985	1987	1989	1991	1993	1995	1997
Baby foods	24	14	10	53	95	7	61	53
Bakery foods	515	553	931	1,155	1,631	1,420	1,855	1,200
Baking ingredients	134	142	157	233	335	383	577	422
Beverages	506	625	832	913	1,367	1,842	2,854	1,606
Breakfast cereals	34	56	92	118	104	99	128	83
Condiments	906	1,146	1,367	1,355	1,885	2,043	2,462	2,505
Candy, gum, and snacks	775	904	1,145	1,701	2,787	3,147	3,698	2,631
Dairy products	486	671	1,132	1,348	1,111	1,099	1,614	862
Desserts	37	62	56	69	124	158	125	109
Entrées	319	409	691	694	808	631	748	629
Fruits and vegetables	126	195	185	214	356	407	545	405
Pet foods	62	103	82	126	202	276	174	251
Processed meats	348	383	581	509	798	453	790	672
Side dishes	133	187	435	489	530	680	940	678
Soups	135	167	170	215	265	248	292	292
Total food	*4,540*	*5,617*	*7,866*	*9,192*	*12,398*	*12,893*	*16,863*	*12,398*

Source. Refs. 9 and 37.

Table 10. Total Measured Media U.S. Advertising Spending by Category, 1997 and 1996

	Millions $		%
Category	1997	1996	Change
Total farm-related advertising	55.4	49.2	12.6
Food and food products	3,361.6	3,209.6	4.7
Beverages	1,320.7	1,324.5	−0.3
Beer, wine, and liquor	1,089.2	1,019.5	6.8
Candy and snacks	1,094.4	965.7	13.3
Pet foods and supplies	360.1	317.1	13.6
Cigarettes, cigars, and tobacco	455.1	488.3	−6.8
Total food and tobacco processing	*7,681.1*	*7,324.7*	*4.9*
Restaurants and fast food	3,147.1	2,960.8	6.3
Food and liquor retail	859.6	804.1	6.9
Food and liquor-direct mail	19.8	14.2	39.4
Total food and tobacco related	*11,763.0*	*11,153.0*	*5.5*
Total—U.S. media advertising	*73,214.7*	*66,711.0*	*9.7*

Source: Ref. 38.

Table 11. Concentration of Advertising Expenditures in Food and Tobacco Processing, 1967–1992

	1967 (%)	1982 (%)	1987 (%)	1992 (%)
Top 4	19.4	26.8	32.8	36.9
Top 8	29.9	39.3	47.3	51.0
Top 20	53.4	65.7	72.1	75.3
Top 50	78.1	88.7	90.6	91.1
Top 100	90.5	95.6	96.2	96.4

Note Excludes advertising by associations, boards, and governments.
Source. Ref. 30.

Also, retailers continue to explore backward integration, and the larger processors often prefer to have their own sales force manage the distribution of their products in all markets where they have a sufficient volume to justify the expense. By 1992, the four largest wholesalers controlled 11.2% of wholesaling sales, up from 6.5% in 1982, and the 20 largest held a 25.2% share in 1992, up from 16.9% in 1982 (Table 12). The two largest wholesalers, Supervalu and Flemming Cos., are nearly of equal size and much larger than the other wholesalers. Both have invested in retail stores, and although wholesaling accounted for about three-fourths of their total sales, it has declined and their retailing sales have increased. The five largest wholesalers are listed in Table 13.

FOOD RETAILING AND FOOD SERVICE

Americans spent over $700 billion on food in 1997, including imports and fish, with food at home accounting for 55% of the total and the away-from-home market holding a 45%

Table 12. Aggregate Concentration of Largest Wholesale Firms, Grocery Stores, and Restaurants, 1982–1992

	Percent of total sales		
	1982	1987	1992
	Wholesale firms		
4 largest	6.5	7.9	11.2
8 largest	10.1	12.2	16.9
20 largest	16.9	20.9	25.2
50 largest	27.0	32.5	35.2
	Grocery stores		
4 largest	16.4	17.4	16.1
8 largest	24.1	26.5	25.3
20 largest	35.6	37.2	37.6
50 largest	44.7	47.7	49.9
	Restaurants		
4 largest	5.6	6.9	7.8
8 largest	9.0	10.3	10.6
20 largest	12.6	14.4	15.2
50 largest	16.1	18.1	18.9

Source: Refs. 20 and 21.

Table 13. Top Five Food Wholesalers, 1998

Wholesaler	Total sales (millions $)	No. of shores	Private label share (%)
Supervalu	16,552	4,400	10
Fleming Cos.	16,487	3,500 +	15
Nash Finch Co.	4,451	12,000	12
Wakefern Food Corp.	4,300	185	30
C&S Wholesale Grocers	3,400	1,250	10

Source: Ref. 40.

share (Table 14). This 55/45 percentage split of total food expenditures has remained roughly constant since 1988, but it is up substantially from the 75/25 percentage split in 1954 (42). Retail food stores have realized that they must compete with the away-from-home firms if they are to continue to grow as a food supplier. They have countered this trend toward away-from-home dining by offering ever more prepared foods from in-store delis to entire ready-to-serve meals prepared in their stores. No one has solved this marketing puzzle completely, but everyone is trying as they respond to time-pressed but more affluent shoppers seeking a better solution to their food needs and desires.

Food retailing is constantly changing. Store formats come and go as retailers strive to achieve greater efficiencies and a competitive advantage. A store's meat section was once the featured department to differentiate a store, but today a well-stocked, attractively displayed produce section is what consumers value most. Prices are always among the leading factors consumers mention when asked about why they shop at particular stores, but convenience and quality are also primary considerations. Affluent shoppers are willing and able to pay more for time saved. Home delivery, a retail method long thought dead, has returned, and in 1997 consumers spent $10.3 billion for food home delivery and mail order, up over three times in 10 years (43). It now accounts for 2.6% of food-at-home expenditures and 1.5% of total food expenditures. The proliferation of Internet shopping sites adds interest in this format; the best known Internet retailer, Amazon.com, has invested in an Internet grocery venture (44).

Retail food stores can be arrayed along two dimensions, variety and price, to capture most of the variation found in store formats today (Fig. 8). In the middle at the origin is the conventional supermarket, with a full line of groceries, meat, produce, service deli and bakery and with general merchandise and health and beauty aids, which account for 6% to 8% of store sales. Alternative formats move out from the origin by adding or subtracting the number of products carried and by featuring lower or higher prices. The traditional mom-and-pop corner store continues to lose share but has not been eliminated. Nontraditional formats, such as wholesale clubs (eg, Costco and Sam's) and Supercenters (eg, WalMart, Kmart, Fred Myer) have increased their share of grocery volume (defined as items commonly found in a traditional channel) to roughly 10% (Table 15) (39). Newer formats that operate in the traditional channel, such as Superstores and Food Drug Combos, account for about two-thirds of store sales. The conventional supermarket's share of total sales peaked at 70% in 1965 and has fallen to just under 25% in 1995.

Table 14. Expenditures for Food, 1977–1997

	Millions $			Percent of total	
Year	Food at home[a]	Food away from home[b]	Total (millions $)	Home	Away
1977	137,332	84,835	222,167	61.81	38.19
1982	205,583	139,776	345,359	59.53	40.47
1987	254,629	198,926	453,555	56.14	43.86
1992	326,456	264,908	591,364	55.20	44.80
1997	394,593	320,275	714,868	55.20	44.80

Source: Ref. 41.

[a] Food for off-premise use.

[b] Meals and snacks.

Overall or aggregate concentration in food retailing has not shown as much consolidation as found in the other sectors (Table 12) until recently. By 1992, the four largest food grocery retailers held 16.1% of total sales, down a point from 1987 and essentially unchanged since 1982. Among the top 20 (and 50), concentration did increase, but not dramatically, from 35.6% (44.7%) in 1982 to 37.6% (49.9%) in 1992 for the top 20 (top 50). This stability was sharply broken in the 1990s as the 10 largest chains increased their share of sales to 60% from 35% in 1995 (46). The five leading food retailers doubled their national share to 40% in 1998 from 20% in 1993 (Table 16).

Overall concentration is less interesting to economists than market concentration, and in food retailing, the geographic size of the market is local rather than national. Most economists use the Metropolitan Statistical Area (MSA) as the more appropriate geographic scope, but unfortunately the U.S. government does not publish concentration data at this level unless requested to perform a special tabulation, for which they charge. Such tabulations have been done in past census years. Alternatively, a trade publication does collect and publish data at the MSA level. When examined at the MSA level, food retailing markets are commonly oligopolies, with a CR4 of 50% or more (39). These data are consistent with a more thorough analysis of concentration in U.S. grocery MSAs done with special tabulations of the census data (48). Franklin and Cotterill found average market concentration did increase in the 1980s, with smaller markets more concentrated than larger markets on average.

The only challenger to the processor for control of the vertical marketing channel is the modern retailer. Retailers face consumers daily and have begun to harness the power of information generated by their checkout scanners. No longer does the retailer turn to the processor's personnel to explain what consumers are buying because they now control the most valuable asset in market research—data on consumers and their purchases. With the use of frequent shopper cards and other incentives, retailers can link demographic data to food sales in ways only dreamed about a decade ago. They are still learning to exploit this valuable information, which processors now must buy from retailers. No longer are they unsure of what sells and which products generate the highest profits. In Eu-

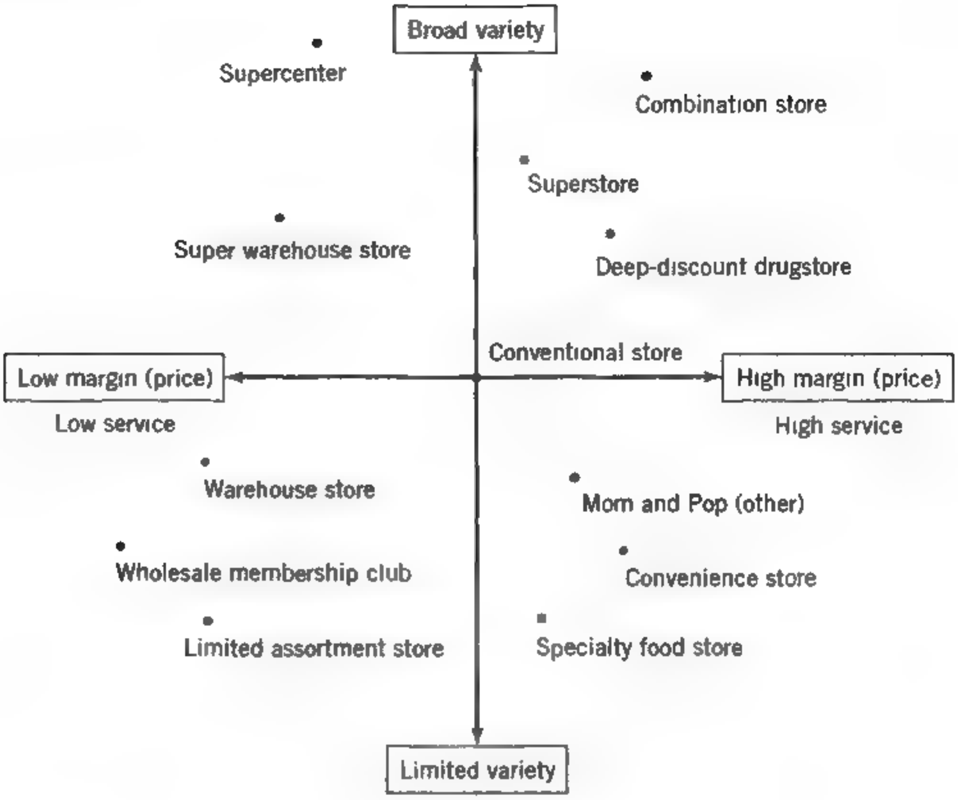

Broad variety
Supercenter
Combination store
Superstore
Super warehouse store
Deep-discount drugstore
Conventional store
Low margin (price)
Low service
High margin (price)
High service
Warehouse store
Mom and Pop (other)
Wholesale membership club
Convenience store
Limited assortment store
Specialty food store
Limited variety

Figure 8. Food retail formats.
Source: Refs. 39 and 45.

Table 15. Store Format and All Commodity Volume (ACV) Share Trends

	1993		1994		1995	
	% of stores	% of ACV	% of stores	% of ACV	% of stores	% of ACV
			Traditional channel[a]			
Conventional	9.3	26.1	9.5	25.6	9.5	24.3
Superstore	3.8	22.4	4.1	23.3	4.3	24.2
Food drug combo	1.3	10.2	1.5	11.2	1.7	12.3
Warehouse store	1.5	6.5	1.3	5.4	1.1	4.8
Super warehouse	0.3	3.4	0.3	3.5	0.3	3.7
Limited assortment	0.4	0.6	0.6	0.6	0.6	0.6
Convenience (trad)[a]	30.2	6.6	30.3	6.4	29.6	5.9
Convenience (petro)[a]	20.8	3.6	22.2	3.7	22.9	3.8
Other	31.3	11.8	29.1	11.4	28.6	12.1
Subtotal	98.9	91.2	98.8	91.1	98.8	91.6
			Nontraditional channel[b]			
Hypermarket	0.0	0.2	0.0	0.1	0.0	0.1
Wholesale club	0.4	5.6	0.4	4.8	0.4	4.7
Mini club	0.1	0.3	0.1	0.3	0.1	0.3
Supercenter	0.2	1.5	0.2	2.0	0.3	2.5
Deep discounter	0.4	1.2	0.4	1.7	0.3	0.8
Subtotal	1.1	8.8	1.2	8.9	1.2	8.4
Total	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*

Source. Ref. 39

[a] Merchandise sales only (non-gas).

[b] Reflects share adjustments for items not commonly found in traditional grocery channel.

Table 16. Top Five Food Retailers, 1998

Rank	Company	Sales in billions $[a]	Market share (%)
1	Kroger	43.0	10
2	Wal-Mart[b]	39.0	9
3	Albertson's	36.0	9
4	Safeway	27.1	7
5	Ahold USA	22.2	5

Source: Ref. 47.

[a] Sales figures are based on fiscal year, and include mergers through 2/28/99.

[b] Includes grocery sales by Wal-Mart Supercenters and Sam's Clubs.

rope, the food retailer has long been the channel leader, and experts are predicting it to emerge as the leader in America as well. In terms of profitability, food retailers average only a little over 1% after-tax profit as percent of sales, whereas food manufacturers average at least four times that (Table 17). To compare these two vertical stages based on sales is misleading, and a more accurate comparison uses after-tax returns to stockholder equity. By this measure, manufactures and retailers both have averaged just over a 16% rate of return since 1980.

Today's consumer is shopping more for complete meals rather than meal ingredients to be prepared at home, even if such preparation is minimal. Food service outlets have long catered to this demand. Food service includes both commercial (eg, restaurants) and noncommercial (eg, school cafeterias, vending machines, hospitals) outlets, with the commercial outlets accounting for about 80% of the total sales volume. Consumers believe they have greater choice in selecting their food in the commercial sector. Currently, many noncommercial food service outlets are being replaced by commercial vendors.

Although food service has not reached an even split of the consumers' food dollar with food at home, it has been the growth segment of the retail food business for at least 40 years. Rising incomes, smaller households, younger ages, and time-pressed shoppers all lead to greater interest in dining out. The time pressure and younger ages along with a car culture drove the fast-food segment from a 4% share of the away-from-home market in 1954 to 33% in 1996 (42). The rise of McDonald's alone charts the success of this segment.

Table 17. Profit Margins of Food Manufacturers and Retail Food Chains, Industry Averages, 1981–1997

	After-tax profits as a percentage of			
	Food manufacturers		Retail food chains	
Year and quarter	Sales	Stockholder equity	Sales	Stockholder equity
1981	3.1	13.6	1.0	13.9
1983	3.3	13.3	1.1	13.6
1987	4.6	17.5	0.9	12.8
1989	4.2	17.1	0.8	20.7
1991	4.8	17.5	1.1	18.8
1993	3.7	13.5	0.8	11.7
1995	5.5	18 5	1.5	21.3
1997	5.6	19.8	1.6	17.4

Source Ref. 49.

Overall concentration is not high in food service, but like food retailing it is a local market rather than a national one. If all restaurants are combined, the largest four held only an 8% share and the largest 20 only a 15% share in 1992 (Table 12). Within local markets, the shares would be much higher, but no data are available, and most markets have several competing outlets. The overall concentration is highest in the fast-food segment, with fast-food burger restaurants having the greatest concentration. McDonald's held a 42% national share in 1997 (Table 18), and the top 5 chains held a 87.6% share. The pizza segment is not as highly concentrated, with the largest firm, Pizza Hut, holding a 22.6% national share in 1997 (Table 19). The largest chains control a larger share of the advertising expenditures in food service than they do in sales, especially so in the pizza segment.

Food retailers have responded to the loss of sales to the away from home market and now offer complete meals, called home replacement meals (HMR), and consumers show increased interest in these products. There has been some blurring of the lines between supermarkets and food service, fast-food restaurants now reside within retail stores and food retailers offer take-home meals such as fully cooked pizza and complete meals prepared while con-

Table 18. Top 10 Fast-Food Burger Restaurants, 1997

Rank	Brand	Share of market (%)	Share of media advertising (%)
1	McDonald's	42.2	41.8
2	Burger King	21.4	30.6
3	Wendy's	11.9	12.4
4	Hardee's	8.8	5.8
5	Jack in the Box	3.3	3.7
Total top 5 share		*87.6*	*94.4*
Total market		*$40,600.0*	*$1,380.9*

Source Ref. 50.

Note. Dollars are in millions. Measured media from Competitive Media Reporting. Sales from Technomic. BK sales include major franchisors, Carrols, and AmeriKing, Sydran and Quality. Wendy's includes DavCo. Hardee's includes Boddie-Noell and the Advantica Hardee's franchises acquired in April. Hardee's media estimate.

Table 19. Top 5 Pizza Chains, 1997

Rank	Brand	Share of market (%)	Share of media advertising (%)
1	Pizza Hut	22.6	42.2
2	Domino's Pizza	11.4	35 1
3	Little Caesars	8.2	10.4
4	Papa John's	4.0	4.8
5	Chuck E. Cheese's	2.0	4.8
Total top 5 share		*48.2*	*97.3*
Total market in dollars (millions)		*$21,700.0*	*$349 2*

Source Ref. 50.

Note: Measured media from Competitive Media Reporting. Sales from Technomic. Pizza Hut also includes franchisor NPC.

sumers shop for other groceries. Fast-food outlets are still the primary source for takeout food, with a 41% share in 1997 (Table 20). Other restaurants hold a 21% share, but supermarkets have increased their share to 22% from 12% a year earlier. If consumers remain satisfied with these meals and retailers can eventually profit from supplying them, then supermarkets will share in the growth of the takeout market. Busy people have less interest in dining out because it adds rather than detracts from daily stress. Such consumers are eager to take fully prepared meals home to be eaten in the comfort and privacy of their own homes.

FUTURE FORCES FOR CONTINUAL CHANGE

General

The U.S. food marketing system is a mature sector, but one that is constantly changing. To predict the characteristics of the food industry in the twenty-first century requires both the cautious extrapolation of the economic trends and insightful consideration of the potential impact from the most significant forces exerting pressure on the U.S. food marketing system.

Powerful forces—new technologies, industrialization and consolidation, globalization, and the changing consumer—will alter the future food system. The changing U.S. consumer was discussed earlier along with the implications for the food marketing system. Forecasting is always uncertain, and it is difficult to project quantitatively the impact these areas of change might have on the food system. It is impossible to predict the yet-to-be discovered information or technology, for example, that might alter the course of events in a monumental fashion, yet it must be expected that such discoveries can occur. Each individual firm's success, if not survival, and the development of the industry will be shaped by the industry's resilience and willingness to accept and adapt—indeed, to take advantage—of changes.

Technological Change

Much of the early development of the U.S. food system was catalyzed by the development and introduction of new technologies, especially in production and processing. Significant food science innovations from 1939 to 1989 include aseptic processing and packaging, minimum safe canning processes for vegetables, the microwave oven, frozen concentrated citrus juices, controlled atmosphere packaging for fruits and vegetables, freeze drying, frozen meals, the concept of water activity, food fortification, and ultrahigh temperature processing of milk and other products (51). Entire businesses have been developed on the basis of such technologies.

Table 20. Shoppers' Primary Source of Takeout Food (%)

	1997	1996	Point change
Fast-food restaurant	41	48	−7
Restaurant	21	25	−4
Supermarket	22	12	10
Deli/pizza, bagel, coffee shop	5	4	1
Gourmet/specialty store	7	3	4
Convenience store	1	1	0
Other/don't know	3	6	−3

Source: Ref. 40.

In the latter half of the twentieth century, food companies, in particular processors, concentrated most of their research efforts on the development and proliferation of new products rather than development of technology. Most of these new products do not represent great technological change. In fact, by most measures, the food industry has not positioned itself for significant internal technology development. Research and development investments are low compared to other industries. Public investment in food research for postproduction industries is low, and food-industry employment of engineers and scientists is considerably lower than other industries (52). Still, productivity in the food industry has grown at an annual rate of 3% since 1963, largely due to borrowing technologies from other industries.

A more complicated research effort, but still related to new product development, has been the search for fat alternatives and to offer fat-free versions of popular products. However, because animal and plant fats are difficult to duplicate in the lab, they have had limited success. Procter & Gamble's Olestra is best known and is being used commercially by companies, such as Pepsi Co's Frito Lay in their reduced-fat chips. Consumer acceptance is still unclear despite heavy promotional spending. Some consumer groups are strongly opposed to products using such alternative ingredients and have sought increased labeling of possible harmful effects and even the outright banning of such products. In addition, consumers show signs that their fat-avoidance days are waning.

Not all the technological effort in the last half of the century has been directed to minor tinkering with consumer products, and several new technologies have brought, or promise to bring, developments to rival any in our past history. Separation techniques allow basic commodities to be fragmented efficiently into components for restructuring or incorporation into other foods or for removal of undesirable compounds. Corn wet milling, introduced in the United States in the mid-twentieth century, is an example. The new separation technologies include passive membrane filtration (reverse osmosis, ultrafiltration, and microfiltration) and supercritical fluid extraction. These technologies have already achieved commercial success in the food industry.

Information processing and automation have had dramatic impacts on the entire food system as more efficient, productive methods replace outdated methods. Farmers can match fertilizer and pesticide applications to each acre's needs guided by on-board computers running advanced geographic information systems. Farm managers can harvest vast amounts of information and use it to improve operations from record keeping to computer-controlled nurseries for plants or animals. At the processing level, computer-aided measurement devices can efficiently evaluate product quality and pay producers accordingly. Computer-integrated manufacturing, which integrates order entry, scheduling, operations, inventory control, and other operations management activities, has been implemented in many facilities. At the retail level, information processing, including the development of direct product profitability strategies, has helped shift the balance of power in the food system from processors to retailers. New information processing hardware and software, coupled to on-line sensing and control devices, have encouraged increased vertical coordination in the industry and strategic alliances by linking retail activity with distribution, processing, and, perhaps, production. Retailers' scanning information has altered how consumer research is done, giving the retailer control of the best marketing information ever assembled.

The growth of the Internet and the growing comfort level with on-line shopping has led many visionaries to invest in Internet grocery businesses, some with home delivery. No company has managed to profit from these investments to date, but the appeal brings new investors and firms on a continual basis. Far more impressive results have been with company-to-company Internet sales, estimated to be five times the consumer retail total. Forrester Research, a market research firm, predicts on-line business-to-business sales will grow from $43 billion in 1998 to $1.3 trillion, or 9.4% of such sales within four years (53). The Internet will continue as a major force in altering businesses, especially in retailing.

Biotechnology and the related technologies, often called bioengineering or genetic engineering, have brought controversy and promise but few profits to date. Chemical companies and other input supply firms view biotechnology as the future and have several products now in wide use. Monsanto has been a pioneer in these developments. Production agriculture has seen the development of crops with unique characteristics, including disease and pest resistance and improved yield and quality. A gene, patented in 1998 and now purchased by Monsanto, causes genetically engineered crops to produce sterile seeds (54). Animal agriculture has adopted growth regulators, such as bovine growth hormone. Genetic manipulation of animals, including cloning, seeks commercial application. The lines between food firms and pharmaceutical companies continue to blur as these new products emerge. These new advances have raised both hopes of a better world and concerns that science is out of control and requires regulation before ethical lapses or mistakes result in unforeseen disasters.

The new processing technologies have provided more efficient means of converting commodities into high-quality foods and also have enhanced the consolidation of the entire food system. These new larger operations, from the biotech firms that supply the growth hormones to the mega-hog farms to the slaughtering firms that transform the animals into value-added products ready for the retailer's meat case, have their critics who charge that any benefits from these changes have been at an unacceptable cost in terms of environmental hazards, untold future dangers to health, and a decline in food safety. Massive meat recalls in the late 1990s and other food safety problems from salad bars to organic fruit drinks have damaged consumers' confidence in the safety of their food supply. Business and government have responded with programs such as hazard analysis critical control points (HACCP) and by allowing greater use of food irradiation to kill harmful bacteria (55). The use of irradiation in the United States has been limited by consumer resistance over its safety, but given the routine occurrence of *Listeria*, *Salmonella*, and other bacteria outbreaks, consumers may accept this technology, which has been used in other countries (56). Others argue that the problems were created by technology in the first place and that a return to more traditional agricultural methods will eliminate the problems without resulting to technological fixes that may entail unknown repercussions.

History teaches that attempts to stifle technology are largely unsuccessful. Consumers must recognize that technological change itself will be shaped by the marketplace. Technologies will be developed and successfully introduced only when an economic advantage is afforded or a need in the marketplace is met and when consumers are willing to accept them or are passive and apathetic regarding their use. If consumers refuse to accept certain technologies, then businesses will abandon them or public officials will ban or regulate them. The Europeans have held firm to their disapproval of genetically-altered foods and their refusal to accept U.S. hormone-enhanced beef, but Americans appear more diverse in their views. Such consumer diversity leads to a variety of market niches from small organic farms to factory farming enhanced by biotechnology. Perhaps a huge event will grab attention and galvanize consumers against bioengineered foods. Alternatively, consumers may find their fears will fade as the products deliver promised benefits without side effects. The concern over bovine growth hormone in the milk supply did not result in any decline in consumption (57), yet an organic milk market has evolved during the same period (58).

Globalization

The global economy has had a huge impact on countries around the world. Trade has been embraced by even the most protectionist countries, and trading groups continue to form, from NAFTA to the European Union. The United States, with its abundant resources and huge size, began its international participation as primarily an exporter of surplus agricultural products. In the 1990s, however, the U.S. trade in high-value products, such as speciality products (fruit, nuts, breeder livestock, and processed foods) and dairy products, beverages, and prepared foods, overtook exports of bulk agricultural commodities, exceeding $30 billion in 1996 (Fig. 9). Additionally, Americans became major importers of agricultural products, going well beyond those products that cannot be grown profitably in the United States, such as cocoa, coffee, bananas, and palm oil. In 1996, the United States imported $33.6 billion, up from only $4 billion in 1959, on agricultural goods, with the vast majority being products that are grown in the U.S., such as meat, dairy, fruits, and vegetables (42). Some of this growth stems from foreign suppliers catering to U.S. consumer demand for fresh fruit and vegetables regardless of the season, but other imports represent lower priced foreign goods competing with domestic supplies. Overall, the United States maintains a trade surplus in agricultural products, some $22 billion in 1997 (11).

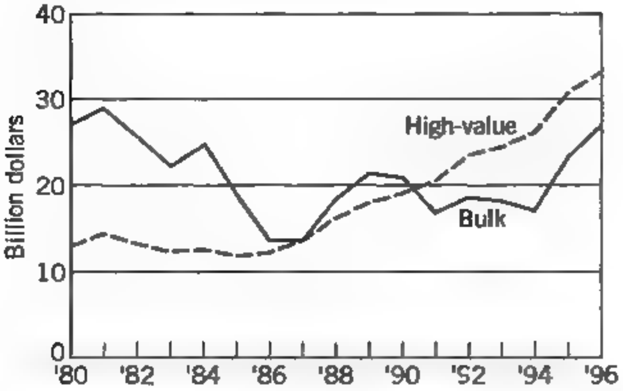


Figure 9. U.S. high-value and bulk agricultural exports, 1980–1996. *Source:* Ref. 42.

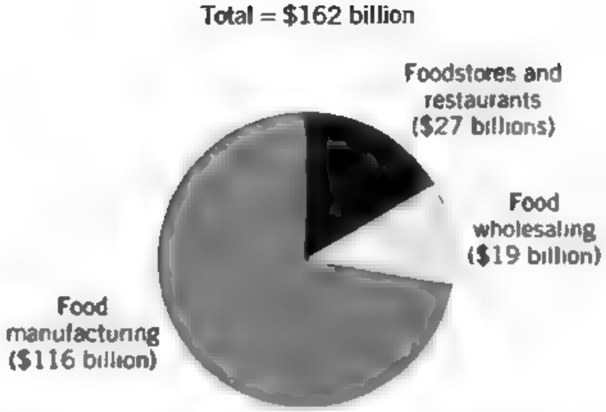


Figure 11. Estimated sales of foreign affiliates of U.S. food marketing firms, 1996. Largest share of sales came from food processing. *Source:* Ref. 32.

The United States is the world's leading importer and exporter of processed foods. Some of our leading domestic brands are also leading brands around the world, such as Coca-Cola soft drinks, Marlboro cigarettes, and Kellogg's cereals. Likewise, many popular brands are imported, such as Heineken beer and Perrier water. In 1996, the U.S. exported $30.1 billion of processed foods and imported $27.8 billion, leaving a surplus of $2.3 billion. Except in 1990, the United States has had a trade surplus in processed foods in the 1990s.

Actual international trade data fail to capture the full globalization of U.S. food marketing firms. U.S. food processing firms often prefer to invest in local facilities or in licensing agreements in a host country rather than export finished products from the U.S. Such foreign direct investment accounts for almost four times the value of sales than from exporting (Fig. 10). In 1996, U.S. food processing firms had estimated sales of $116 billion from their foreign affiliates (Fig. 11). In food retailing and food service, traditional exports are not possible, yet foreign direct investment allows transporting a firm's system and reputation to a foreign country. McDonald's operates in over 110 countries. The estimated sales of foreign affiliates of U.S. food stores and restaurants reached $27 billion in 1996. Foreign direct investment offers firms several advantages over exporting from lower transportation costs to greater understanding of the consumers and familiarity with local government officials. It also avoids many tariffs and other barriers to trade, while retaining the brand name of the U.S. parent company.

Foreign direct investment also flows from abroad to the United States, with several leading firms, such as Nestle in food processing, Loblaw in food wholesaling and retailing, Ahold (owner of several food retailers including Stop & Shop and Giant Food Stores), and Diageo PLC (owner of Burger King) in food service. In 1996, the estimated sales of U.S. affiliates of foreign food marketing firms was $152 billion (Fig. 12), with roughly 77% of that coming form an even split between food processing ($59 billion) and food retailing ($58 billion).

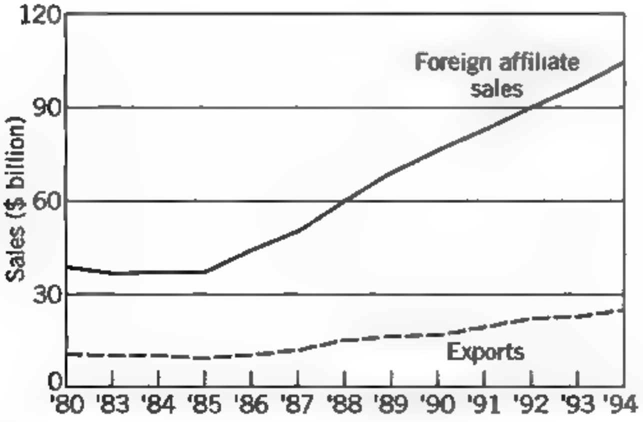


Figure 10. United States processed food exports and sales of U.S.-owned foreign affiliates, 1982–1994. *Source:* Ref. 42.

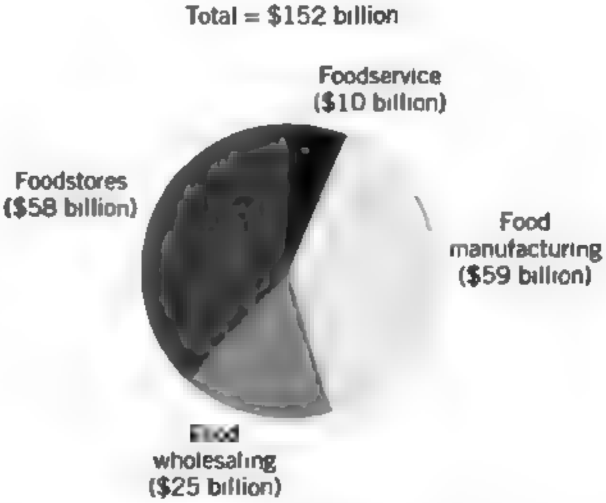


Figure 12. Estimated sales of U.S. affiliates of foreign marketing firms, 1996. Largest share of sales came from foodstores and processing. *Source:* Ref. 32.

The international flow of products and the mixing of cultures will continue as transportation improves and communication links increase around the globe. Jumbo jets now fly frozen fish from Taiwan to New York and fresh fruits and vegetables from Chile to the United States. Increased consumer diversity and rising incomes will encourage these flows. International trade agreements, such as GATT, also reduce the barriers to trade. The World Trade Organization (WTO) seeks to resolve trade disputes before they erupt into trade wars. Nevertheless, strategic moves and counter moves by countries and powerful firms will continue to seek an advantage. The current U.S.-Europe trade dispute over bananas and hormone-treated meat are suggestive of future squabbles, yet pose little threat to reversing the increased flow of goods and services across national borders. Many of the same issues exist in domestic trade as well because consumers vary on their acceptance of biotechnology and other practices that affect the food supply, such as pesticide residues.

Industrialization and Consolidation

American agriculture continues to consolidate and industrialize as consumers splinter into more segments and technology allows catering to the diversity of demands. Economic markets can be incredibly efficient in making sense out of the economic chaos involved in moving products from production to consumption because they summarize the information contained in buyers' demands and sellers' supplies. In the real world, however, traditional agricultural markets are not as perfect as the economist's model suggests; they miss opportunities to link producers and processors in more profitable arrangements. For example, major chicken processors have been fully integrated by ownership from the hatchery to the processing plant for decades. They merely hire contract growers to raise the birds to market weight without ever transferring ownership. They even supply the feed and other inputs required for the growout operation. Other industries have turned to legal contracts to secure input supplies tailored to their operations (eg, vegetables for processing) rather than using markets or ownership of the farms (59). Farmers benefit in lowering their risks and processors are assured of supplies with appropriate features. Several major processors have entered strategic alliances with growers where they contract for character-specific raw products in a relationship that all parties expect to be ongoing (60).

Economists understand the benefits of these nonmarket transactions, but also the costs. As more product volume moves through nonmarket methods, the less is known about true product values because key economic information summarized by price becomes more difficult to discover. To date, most of these nonmarket arrangements involve linking the processing and production stages of the marketing system. However, other stages have established nonmarket coordination in what has been termed supply chain management. Large retailers now contract for much of the produce they sell rather than buy their produce at the various regional markets. Much of the industrialization has featured improved information, tailored inputs, and reduced cost of production and processing. Consumer concerns arise from whether there will be sufficient competition to force such efficiencies to be passed on as lower prices and rural communities wrestle with major issues resulting from factory farms that reduce the number of family farms and add to environmental concerns. Even producers who entered these contracts worry whether they will receive fair prices for their products once the marketplace is removed or diminished.

All stages of the vertical system are becoming more concentrated as larger operations increase their size. At the same time, there is increased diversity as the larger firms get larger and the number of smaller firms increases. It is the middle-sized firm that is most endangered by the consolidation movement. Whether in farming or retailing, as the largest firms increase their share of the sector's output, a growing number of smaller firms emerge in the cracks and eddies left behind by the larger firms.

Food and tobacco processing has seen the most dramatic consolidation in this century. Merger patterns have followed the four great merger movements of the general economy. The first major merger wave occurred around the turn of the century and created some of the famous trusts that antitrust legislation was suppose to prevent (61). For example, American Tobacco and General Mills were formed during this merger wave. The next wave came during the 1920s when companies such as General Foods were being formed through merger. The third merger wave was in the 1960s and was characterized by amazing conglomerates being formed as unrelated firms sought management synergies, such as ITT buying Continental Baking. The fourth merger movement came in the late 1970s and 1980s and was a wild period of leveraged buyouts and hostile takeovers funded with questionable, often illegal, financial instruments. Food companies were at the forefront of these mergers with record-setting deals, such as the $25 million leveraged buyout of RJR Nabisco. The largest food and tobacco processor, Philip Morris, is essentially a case history in a merger-built business. Starting from its dominant position in cigarettes, Philip Morris purchased such already huge companies as Miller Brewing, General Foods (who already had bought Oscar Mayer), and Kraft Foods. Few American shoppers now know the parent company of the branded goods they bring home from the supermarket.

It appears that we are in the midst of a fifth merger wave, and again the food businesses are major players (Fig. 13). Most of the food-related mergers involve food processing and retailing firms, including some the largest mergers in history, but increasingly mergers in food service and wholesaling are commonplace. Wholesalers are increasing their ownership of retailers as they seek to survive in the modern food system. Some of the failures of the previous merger wave are being undone as firms now seek brands from other firms as they selectively add and subtract from their portfolio of brands. Others merely purchase firms whose brands fit well with their current offerings. This current merger wave is more horizontal in nature as processing firms seek merger partners among current rivals. Gone are the wild conglomerate mergers as firms now seek to consolidate their hold on leading positions in markets where they currently hold a strong position. Some economists have become concerned about the

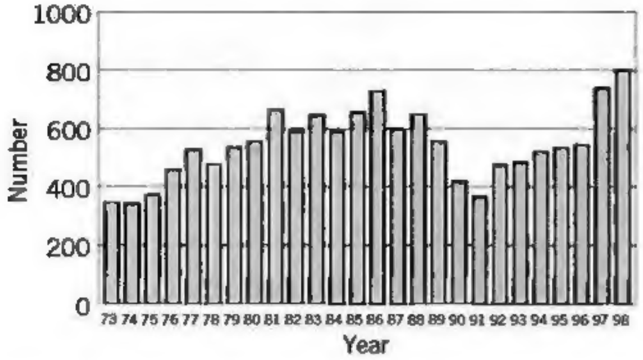


Figure 13. Food mergers and acquisitions, 1973–1998. *Source:* Ref. 62.

Table 21. Average Hourly Earnings of Production and Nonsupervisory Employees of Food Industries, 1977–1997 (Dollars Per Hour)

Year	Manufacturing food and kindred products	Wholesale trade groceries, and related products	Food stores	Eating and drinking places
1977	5.37	5.43	4.77	2.93
1982	7.92	8.25	7.22	4.09
1987	8.93	9.53	6.95	4.42
1992	10.20	11.09	7.56	5.29
1997	11.49	12.87	8.59	6.05

Source: Ref. 63.

growing concentration and march toward oligopoly in almost every market. There is little evidence of any positive benefits from such mergers outside of the stock market evaluation of these firms. The stock market rewards downsizing as a cost efficiency, and increased market share enhances profitability potential through uncontested price increases. The firm defenses of increased efficiencies and productivity gains have been overshadowed by a reduction in research and development and diminished employee morale. Professional organizations and associations, such as the Institute of Food Technologists and agricultural associations, have called the U.S. government to increase their oversight of this consolidation. Without public policy changes these consolidations will continue for several reasons: (*1*) the food industry is more predictable and less cyclical than most industries; (*2*) food companies generally generate high cash flows; (*3*) purchase of brands, often available through food company buyouts, is particularly attractive; and (*4*) international exchange rates and the stable political environment make the assets of U.S. food companies especially attractive to foreign companies looking to increase penetration into U.S. markets.

The Changing Consumer

It is appropriate to end this overview of the U.S. food system where we began—with the consumer—and to examine the changes that will challenge current firms and provide opportunities to new businesses. Consumer demand, in fact, will be the principal source of growth and development in the food industry in the future. Economists do not forecast significant changes in total caloric intake, although it has drifted upward slightly over time, but they do anticipate consumer demand to be affected by increases and demographic changes in the U.S. population, shifts in income distribution, and changes in consumer preferences. Increases in total consumer demand can be forecasted fairly accurately based on Census Bureau projections of the U.S. populations. These projections have been made, but generally they are based on the premise that consumers of certain age and income groups in the future will exhibit the same preference as persons of similar age and income groups in today's population. This premise is not universally accepted by marketers. In fact, changes in preference are almost certain to occur. Some preference changes will arise from continued immigration and assimilation of new cultural values. Others will arise from changes in household patterns. Most, however, will arise from poorly understood social phenomena. The interplay between technology and consumers will be critical to the future food system.

The workforce experiences the same changes, especially with respect to declining numbers of potential workers in the age groups normally associated with entry-level jobs. Wage rates in many food-related industries are low (Table 21), and firms have difficulty attracting workers. Some agricultural production will be forced offshore because of shortages of people willing and able to do manual labor for low wages. Another shift has been and will be the increasing assimilation of women, minorities, and immigrants into the workforce, especially in upper management positions. The changing workforce composition will place special demands on human resource management and development in the coming years, especially in the food industry, where a high percentage of entry-level workers are employed.

The increased diversity of the American consumer along with the diversity tapped through globalization provides many opportunities for businesses. Marketers no longer have to use the mass marketing methods that view all consumers as similar. They can target those consumers that they can best serve—from vegetarians who prefer organic foods to cigarette smokers who prefer red meat meals without the vegetables. The world is a fascinating place with a wide array of consumer preferences, and the food system has always reflected the entire distribution of humankind, making it an exciting place for career opportunities.

BIBLIOGRAPHY

1. U.S. Department of Agriculture, *1998 Agricultural Fact Book*, URL: *http://www.usda.gov* (last accessed Sept. 19, 1999).
2. J. J. Putnam and J. E. Allshouse, *Food Consumption, Prices, and Expenditures, 1970–97*, Statistical Bulletin No. 965, Food and Rural Economics Division, Economic Research Service, U.S. Department of Agriculture, Washington, D.C., 1999.
3. R. T. Rogers, "Broilers: Differentiating a Commodity," in L. L. Duetsch, ed., *Industry Studies*, 2nd ed., M. E. Sharp, Armonk, N.Y., 1998, pp. 65–100.

4. *Food Guide Pyramid Information*, U.S. Department of Agriculture and U.S. Department of Health and Human Services, URL: *http://www.nal.usda.gov/fnic/Fpyr/pyramid.html* (last accessed Sept. 19, 1999).
5. L. S. Kantor, *A Dietary Assessment of the U.S. Food Supply: Comparing Per Capita Food Consumption with Food Guide Pyramid Serving Recommendations*, Agricultural Economic Report No. 772, Food and Rural Economics Division, Economic Research Service, U.S. Department of Agriculture, Washington, D.C., 1998.
6. B.-H. Lin, E. Frazao, and J. Guthrie, *Away-from-Home Foods Increasingly Important to Quality of American Diet*, Agriculture Information Bulletin No. 749, U.S. Department of Agriculture, Washington, D.C., 1999.
7. J. Kinsey, "Changes in Food Consumption from Mass Market to Niche Markets," in Lyle P. Schertz and Lynn M. Daft, eds., *Food and Agricultural Markets: The Quiet Revolution*, NPA Report No. 270, Economic Research Service, U.S. Department of Agriculture, Washington, D.C., 1994, pp. 19–43.
8. *Population Trends 1996–2050*, The Food Institute, Fair Lawn, N.J., 1996.
9. "Publisher Categorizes Shoppers Six Ways," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., February 15, 1999, p. 3.
10. M. Beck, "Next Population Bulge Shows Its Muscle," *Wall Street Journal*, February 3, 1997.
11. "Labor Force, Employment, and Earnings," *Statistical Abstract of the United States: 1998*, U.S. Department of Commerce, Bureau of the Census, Washington, D.C., September 25, 1998.
12. "These Grocery-Delivery Services Might Even Do Windows," *Wall Street Journal*, February 12, 1998.
13. "What's For Dinner? Ask Me Later," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., March 9, 1998, p. 1.
14. "Nabisco Dumping Grey Poupon 'Pardon Me' Ads," *Wall Street Journal*, April 6, 1998.
15. "Americans Do Cook," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., February 15, 1999, p. 2.
16. J. Krasner, "Pilgrim Dessert Returns, but Who Will Give Thanks?" *Wall Street Journal*, March 17, 1999.
17. J. Friedland and S. Kilman, "As Geneticists Develop an Appetite for Greens, Mr. Romo Flourishes," *Wall Street Journal*, January 28, 1999.
18. "Greed Is Good," *The Economist*, January 17, 1998, p. 26.
19. U.S. Department of Commerce, Bureau of the Census, *Statistical Abstract of the United States*, U.S. Government Printing Office, Washington, D.C., 1952–1998.
20. U.S. Department of Commerce, Bureau of the Census, *Census of Retail Trade*, U.S. Government Printing Office, Washington, D.C., 1972, 1977, 1982, 1987, 1992.
21. U.S. Department of Commerce, Bureau of the Census, *Census of Wholesale Trade*, U.S. Government Printing Office, Washington, D.C., 1972, 1977, 1982, 1987, 1992.
22. U.S. Department of Commerce, Bureau of the Census, *Census of Manufacturers*, U.S. Government Printing Office, Washington, D.C., 1972, 1977, 1982, 1987, 1992.
23. U.S. Department of Commerce, Bureau of the Census, *Economic Census*, U.S. Government Printing Office, Washington, D.C., 1972, 1977, 1982, 1987, 1992.
24. U.S. Department of Agriculture, *1997 Census of Agriculture*, U.S. Government Printing Office, Washington, D.C., 1997.
25. U.S. Department of Commerce, Bureau of the Census, *1996 Annual Survey of Manufacturers*, U.S. Government Printing Office, Washington, D.C., 1996.
26. J. S. Royer and R. T. Rogers, eds., *The Industrialization of Agriculture: Vertical Coordination in the U.S. Food System*, Ashgate, Aldershot, United Kingdom, 1998.
27. S. Warren and S. Kilman, "DuPont Co. Lands Huge Biotech Prize," *Wall Street Journal*, March 16, 1999.
28. M. E. Kuhn, "The 1998 Top 100 Food Companies," *Food Processing*, December 1998, pp. 19–44.
29. J. M. Connor et al., *The Food Manufacturing Industries*, Lexington Books, Lexington, Mass., 1985.
30. R. T. Rogers, "The Role of Cooperatives in Increasingly Concentrated Markets: Reaction," in M. Cook et al., eds., *Cooperatives: Their Importance in the Future Food and Agricultural System*, National Council of Farmer Cooperatives and The Food and Agricultural Marketing Consortium, Washington, D.C., 1997, pp. 49–65.
31. U.S. Department of Agriculture, *Food Marketing Review*, Commodity Economics Division, Economic Research Division, Agricultural Economics Report No. 614, 1989.
32. A. E. Gallo, *The Food Marketing System in 1996*, Agricultural Information Bulletin No. 743, U.S. Department of Agriculture, Washington, D.C., 1998.
33. R. T. Rogers and R. J. Sexton, "Assessing the Importance of Oligopsony Power in Agricultural Markets," *American Journal of Agricultural Economics* **76**, 1143–1150 (1994).
34. G. P. Zachary, "Many Industries Are Congealing into Lineup of Few Dominant Giants," *Wall Street Journal*, March 8, 1999.
35. R. T. Rogers and L. M. Petraglia, "Agricultural Cooperatives in Food Manufacturing: Implications for Market Performance," *Journal of Agricultural Cooperation* **9**, 1994, 1–12 (1994).
36. R. J. Sexton, "The Role of Cooperatives in Increasingly Concentrated Agricultural Markets," in Michael Cook et al., eds., *Cooperatives: Their Importance in the Future Food and Agricultural System*, National Council of Farmers Cooperatives, Washington, D.C., 1997, pp. 31–47.
37. "Gorman's New Product News," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., January 21, 1989, p. 7.
38. Advertising Age Dataplace, URL: *http://adage.com/dataplace/archives/dp265.html* (last accessed March 25, 1999).
39. J. J. Belonax, Jr., *Food Marketing*, Simon and Schuster Custom Publishing, Needham Heights, Mass., 1999.
40. *Food Industry Review, 1998*, The Food Institute, Fair Lawn, N.J., 1998, p. 116.
41. A. E. Gallo, *Food Industry Review, 1999*, Economic Research Center, U.S. Department of Agriculture, Washington, D.C., 1999.
42. K. L. Lipton, W. Edmondson, and A. Manchester, *The Food and Fiber System: Contributing to the U.S. and World Economies*, Agriculture Information Bulletin No. 742, USDA Economic Research Service, Washington, D.C., 1998.
43. "Food Sales Top $10 Billion Mark," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., February 8, 1999, p. 1.
44. G. Anders, "Amazon.com Buys 35% Stake of Seattle Online Grocery Firm," *Wall Street Journal*, May 18, 1999.
45. B. W. Marion, *The Organization of the U.S. Food System*, Lexington Books, D.C. Heath, Lexington, Mass., 1986.
46. "Nash Finch Does More Than Gnash Its Teeth," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., April 5, 1999, p. 2.
47. "Five Retailers Now Account for 40% of Grocery Sales," *Food Institute Report*, The Food Institute, Fair Lawn, N.J., August 9, 1999, p. 1.

48. A. W. Franklin and R. W. Cotterill, *An Analysis of Local Market Concentration Levels and Trends in the U.S. Grocery Retailing Industry*, Food Marketing Policy Center, Research Report No. 19, May 1993.
49. U.S. Department of Commerce, "Profit Margins of Food Manufactures and Retailers," URL: *http://www.econ.ag.gov/briefing/foodmark/cost/data/profit.htm* (last accessed March 15, 1999).
50. Advertising Age, URL: *http://adage.com/dataplace/archives/dp240.html* (last accessed March 25, 1999).
51. Institute of Food Technologists, "Top Ten Food Science Innovations, 1939–1989," *Food Technol.* **43**(9), 308 (1989).
52. Research Committee, Institute of Food Technologists, "The Growth and Impact of the Food Processing Industry: "A Summary Report," *Food Technol.* **42**(5), 95–110 (1988).
53. "B-to-B On-Line Sales Dwarf Retail," *Food Institute Report*, April 26, 1999, p. 1.
54. *Food Regulation Weekly*, April 26, 1999, p. 16.
55. F. M. Biddle, "Titan Adapts Defense Technology to Food Irradiation," *Wall Street Journal*, May 13, 1999.
56. C. M. Bruhn and H. G. Shultz, "Consumer Awareness and Outlook for Acceptance of Food Irradiation," *Food Technol.* **43**(7), 93–94 (1988).
57. L. Aldrich and N. Blisard, *Consumer Acceptance of Biotechnology: Lessons from the rbST Experience*, Agriculture Information Bulletin No. 747-01, USDA, Economic Research Service, 1998.
58. "Organic Milk Making a Splash," *Food Institute Report*, March 22, 1999, p. 2.
59. M. Drabenstott, "Industrialization: Steady Current of Tidal Wave?," *Choices*, Fourth Quarter, 4–8 (1994).
60. E. van Duren, W. Howard, and H. McKay, "Forging Vertical Strategic Alliances," *Choices*, Second Quarter, 81–83 (1995).
61. J. M. Connor and F. E. Geithman, "Mergers in the Food Industries," *Choices*, Second Quarter, 1988.
62. *Food Business Mergers and Acquisitions*, The Food Institute, Fair Lawn, N.J., 1982–1998.
63. U.S. Department of Labor, "Employment and Earnings, March 1997," URL: *http://www.econ.ag.gov/briefing/foodmark/cost/data/bill/wages.htm* (last accessed March 15, 1999).

RICHARD T. ROGERS
University of Massachusetts
Amherst, Massachusetts